DISEASES OF THE

Kidney & Urinary Tract

DISEASES OF THE Kidney & Urinary Tract

Eighth Edition

Volume 1

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DEDICATION

Carl William Gottschalk, MD was a man for all seasons—brilliant scholar, committed mentor of students, dedicated citizen of the University of North Carolina and the broader academic community, and a gentleman in every sense of the word. Carl was a native Virginian whose southern manners and warm demeanor emanated a personal charm to his friends, colleagues, and students. He graduated Phi Beta Kappa from Roanoke College in 1942 and received an Honorary Doctor of Science from that institution in 1966. An Alpha Omega Alpha graduate from the University of Virginia School of Medicine, Carl received his training in internal medicine at the Massachusetts General Hospital and his fellowship in Cardiology at the University of North Carolina. For the next 40 years (1952–1992), his loyalty and many talents were an integral part of the University of North Carolina, which accounted for the respect and affection that he received from his many colleagues and friends. He was the Kenan Professor of Medicine and Physiology from 1969 until his untimely death on October 15, 1997, in Chapel Hill, North Carolina.

Carl's scientific contributions were recognized by his election to the National Academy of Science. He was president of the American Society of Nephrology from 1976 until 1977 and was a Councilor of the International Society of Nephrology (ISN). Carl founded the History of Medicine Commission of the ISN and the ISN Archives in Amsterdam, *The Netherlands*, which are appropriately named the Carl W. Gottschalk Archives of the ISN. Among many honors, awards, and distinguished named lectureships, Carl received the Homer W. Smith Award from the New York Heart Association and the David H. Hume Award from the National Kidney Foundation. The American Physiological Society has established the Carl W. Gottschalk Distinguished Lectureship in Renal Physiology, and the University of North Carolina has inaugurated the Carl W. Gottschalk Lectureship in the Basic Sciences.

The written persona of Carl W. Gottschalk—scientist, medical historian, lepidopterist who has a butterfly (*Strymon cecrops Gottshalki*) named after him, recipient of many honors and awards—can only project a very modest picture of this Renaissance man. His kindness and consideration for others was unparalleled. It was my honor to have worked with him on three editions of *Diseases of the Kidney* and to dedicate this current edition to his memory.

Robert W. Schrier, MD

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PREFACE

The recent advances in all aspects of our knowledge of the kidney and its diseases mandate a new edition of *Diseases of the Kidney & Urinary Tract*. As in previous editions, a group of international experts was assembled to present this information in a comprehensive, authoritative, concise, and readily accessible fashion. The chapters have been extensively revised and updated.

Nephrology is a discipline that combines the basic and clinical sciences. Successful integration of this knowledge is the goal of this eighth edition. The 14 sections of the three volume book are actually individual texts which can stand on their own. Moreover, a unique feature of the book is a comprehensive inclusion of diseases of the urinary tract as well as the kidney.

The first section presents an overall view of the structural, physiologic, and biochemical aspects of the kidney. This section incorporates the latest developments in cellular and molecular biology, emphasizing the most current information and concepts on cell signaling, receptors, and ion channels. The subsequent 13 sections are disease-oriented, with each section beginning with a pathophysiology chapter. The goal of *Diseases of the Kidney & Urinary Tract* is to publish the most comprehensive material for practicing and academic physicians caring for patients with renal, hypertension, and urological diseases. The 14 sections of the book cover 104 chapters and are summarized as follows:

I. **Biochemical, Structural, and Functional Correlations in the Kidney** includes structural, hemodynamic, hormonal, ion transport and metabolic functions in nine chapters.
II. **Clinical Evaluation** is covered in six chapters on urinalysis, laboratory evaluation, urography, tomography, and angiography, with indications and interpretations for renal biopsy.
III. **Hereditary Diseases,** in five chapters, covers genetic mechanisms, medullary cystic and sponge disorders, polycystic kidney disease, Alport syndrome, Fabry disease, and nail-patella syndrome, as well as isolated renal tubular disorders.
IV. **Urological Diseases of the Genitourinary Tract** are described in six chapters, including congenital abnormalities, urinary tract obstruction, renal calculi, reflux nephropathy, and prostatic and micturition disorders.
V. **Neoplasms of the Genitourinary Tract** are addressed in five chapters covering molecular mechanisms in malignancy, testicular carcinoma, prostate and bladder cancer, and primary neoplasms of the kidney and renal pelvis.
VI. **Infections of the Urinary Tract and the Kidney** are contained in seven chapters, including host defense mechanisms; urinary bacterial infections, including tuberculosis and fungal infections; renal abscesses; and cystitis.
VII. **Acute Renal Failure** is described in 11 chapters, including the pathophysiology of renal cell ischemia and nephrotoxic injury, acute tubular necrosis, acute interstitial nephritis, and acute nephrotoxic renal disease.
VIII. **Hypertension** and its renal manifestations are covered in seven chapters, which include pathophysiology, renal vascular and endocrine-related hypertension, as well as hypertension in pregnancy and in diabetes.
IX. **Glomerular, Interstitial, and Vascular Renal Diseases** are discussed in 16 chapters, including collagen vascular diseases, chronic interstitial nephritis, primary glomerulonephritides, and vasculitides.
X. **Systemic Diseases of the Kidney** are covered in eight chapters, including diabetes, hepatorenal syndrome, sickle cell disease, gout, myeloma/amyloidosis, and tropical diseases.
XI. **Disorders of Electrolyte, Water, and Acid–Base** are covered in ten chapters, including SIADH, central and nephrogenic diabetes insipidus, cardiac failure, cirrhosis, and the nephrotic syndrome.
XII. **Uremic Syndrome** covers pathophysiology, anemia, osteodystrophy, the nervous system, cardiovascular complications and metabolic and endocrine dysfunctions in six chapters.
XIII. **Management of End-Stage Renal Disease** by transplantation, peritoneal dialysis and hemodialysis, including complications outcomes, and is discussed in four chapters.
XIV. **Nutrition, Drugs, and the Kidney** are covered in four chapters, including protein and caloric dietary issues, as well as drug dosing recommendations in renal failure.

I would like to thank our authoritative and remarkably talented contributing authors whose dedication to nephrology is unmatched.

Robert W. Schrier, MD

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DISEASES OF THE

Kidney & Urinary Tract

SECTION I: BIOCHEMICAL, STRUCTURAL, AND FUNCTIONAL CORRELATIONS IN THE KIDNEY

CHAPTER 1 ■ STRUCTURAL–FUNCTIONAL RELATIONSHIPS IN THE KIDNEY

ROBERT F. REILLY, JR., RUTH ELLEN BULGER, AND WILHELM KRIZ

STRUCTURE–FUNCTION CORRELATIONS ALONG THE RENAL TUBULE

The kidney functions as it does, in large part, because of its architecture. In no instance is this more evident than in the urinary concentrating mechanism, where the complex nephron and vascular interrelationships permit the coordinated function of different nephron and vascular elements into countercurrent multiplication and exchange processes. A recent proliferation of detailed structural, biochemical, and functional information has led to an appreciation of other structural–functional relationships that are relevant to solute and water handling by the kidney. Moreover, it has become evident that an understanding of the pathological developments in kidney diseases is only possible on the basis of a thorough knowledge of kidney structure.

The purpose of this chapter is to review some of the recent findings, with special emphasis on structural–functional relationships that can be assembled to enhance our understanding of overall renal function; therefore, this chapter is divided into two parts. The first part considers the structural and functional interrelationships of each morphologic segment of the urinary tubule, stressing the unique characteristics of each segment. The second part discusses structure and function in terms of more general mechanisms used by several segments of the renal tubule to accomplish specific functions, such as ion or water transport.

Form of the Human Kidney

Human kidneys are paired, bean-shaped organs situated in a retroperitoneal position on the posterior aspect of the abdominal cavity, on either side of the vertebral column against the psoas major muscle. A fibrous capsule located within the perirenal adipose tissue and surrounded by perirenal fascia surrounds each kidney. The lateral border of each kidney is convex. The kidneys of an adult man weigh approximately 120 to 170 g each and measure roughly 11 × 6 × 2.5 cm; those of an adult woman weigh slightly less and are somewhat smaller. In both men and women, total kidney mass best correlates with body surface area.

The concave medial margin has a slitlike aperture, called the *renal hilum*. Branches of the renal artery, vein, nerves, lymphatics, and the expanded pelvis of the ureter pass through the hilum. The hilum communicates with a flattened space within the kidney, called the *renal sinus*. Within this space, the renal pelvis branches into major and minor calyces.

Sections through the kidney reveal the cortex and medulla (Fig. 1-1). The human kidney is a multilobar organ containing 4 to 18 (average, eight) pyramids of medullary substance (1) and is situated so that their bases are adjacent to the cortex. The darker red cortical substance covers the base of each medullary pyramid like the cap of an acorn. During fetal life, the kidney surface is demarcated by clefts that gradually disappear in the normal adult kidney. The apex of each medullary pyramid (called the *papilla*) extends into the renal sinus and is capped by a funnel-shaped, minor calyx. The minor calyces receive the urine that is released from the kidney into the extrarenal collecting system. A lobe of the kidney is composed of the conical medullary pyramid and the surrounding cortical substance. During fetal development, some lobes may fuse and calyces are remodeled so that the mature kidney has fewer calyces and papillae than the original number of papilla anlagen (1); one calyx may drain a fused papilla developed from up to four anlagen, predominantly at the kidney poles. Striated elements called *medullary rays* extend peripherally at intervals from the bases of the medullary pyramids and penetrate into the cortex. These rays resemble the medulla in structure, although they extend deeply into the cortex; they are part of the cortex. The rest of the cortex is called the *cortical labyrinth*.

The medulla can be subdivided further either grossly or microscopically (Fig. 1-2). The medulla has an outer zone that is adjacent to the cortex and an inner zone that includes the papilla. The outer zone is subdivided into an inner and outer stripe. This zonation is important, because it represents the location and orientation of the various segments of the renal tubules within the kidney.

The relative volumes occupied by the cortex, outer medulla, and inner medulla are 70%, 27%, and 3%, respectively (2) in humans. The relative thicknesses vary considerably among mammalian species.

Renal (Uriniferous) Tubules

Human renal morphology resulted from a long evolutionary process in which animals adapted to many changing environmental conditions. The three sequential types of kidneys that evolved were the pronephros, mesonephros, and metanephros. The urogenital system of each human embryo repeats this evolutionary process. The pronephros develops first, but degenerates before attaining any functional capacity.

The mesonephric kidney functions for a short period *in utero,* but it also degenerates, with the notable exception of the part of the mesonephric tubules that form a portion of the excurrent duct system of the male reproductive tract. The metanephric kidney forms last and eventually becomes the functional kidney of the human. The metanephric kidney is well suited to the human condition because of its efficient filtering device and its complex tubule, which allows for the production of not only dilute urine but also concentrated urine. This process occurs only in mammals and birds. Although it is well suited for maintaining homeostasis, the mammalian kidney is an inefficient organ for the elimination of salt and water. In humans, 180 L of fluid are filtered into the tubular lumen every

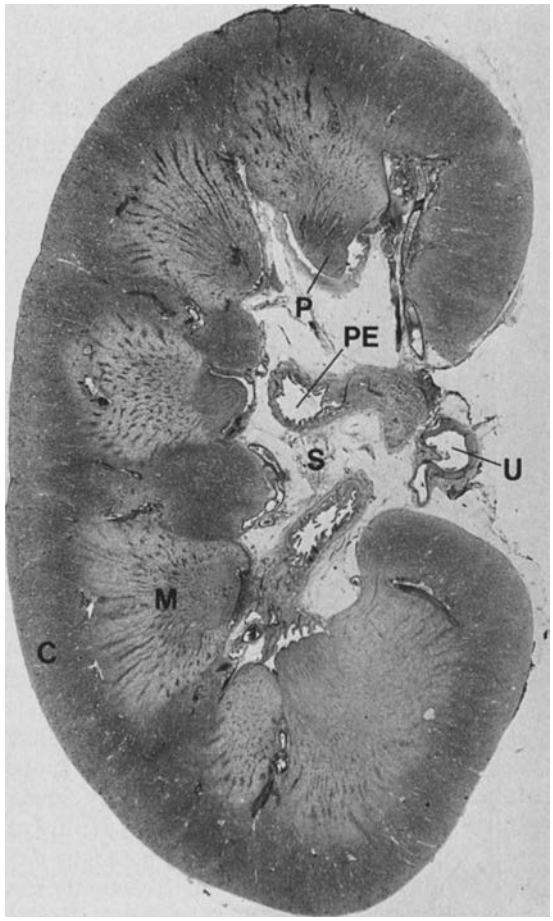


FIGURE 1-1. Gross anatomic appearance of a human kidney. A paraffin section through the whole kidney shows elements of the internal structure: *C*, cortex; *M*, medulla; *P*, papilla projecting into a minor renal calyx; *PE*, pelvis; *S*, sinus; *U*, ureter.

24 hours, of which approximately 178 L must be returned to the blood.

Each human kidney contains approximately one million functional units, called *nephrons* (with considerable interindividual variation) (3). Each nephron is made up of a renal corpuscle (*glomerulus*) and a complex tubular portion, which drain into a unifying tubular system called the *collecting duct system*. Both kinds of tubules represent the renal (or uriniferous) tubules.

The nephrons are derived from the metanephric blastema, the collecting ducts from the urethral bud. A connecting tubule lies between the nephron and collecting ducts. At present, there is controversy as to whether the connecting tubule is derived from the metanephric blastema (4–6) or the ureteric bud (7). As is discussed in the next section, the connecting tubule has marked morphologic similarities to the cortical collecting duct (CCD).

The segmentation of the renal tubule then includes the following regions (8).

The Nephron

I. Renal corpuscle (most of which is called glomerulus)
   A. Bowman's capsule
   B. Glomerular tuft
II. Proximal tubule
   A. Convoluted part (pars convoluta) consists of P_1 and the first part of P_2 (PCT)
   B. Straight part (pars recta) consists of the last part of P_2 and all of P_3 (PST)
III. Thin limbs of the loop of Henle (intermediate tubule)
   A. Thin descending part of short-looped nephrons (SDTL)
   B. Upper thin descending part of long-looped nephrons (LDTL up)
   C. Lower thin descending part of long-looped nephrons (LDTL lp)
   D. Ascending thin part of long-looped nephrons (ATL)
IV. Distal tubule
   A. Straight part (pars recta)
      1. Medullary thick ascending limb (MTAL), which includes regions located within the inner stripe and outer stripe of the medulla
      2. Cortical thick ascending limb (CTAL), which includes the part ascending through the cortex, the macula densa (MD), and the post macula densa segment
   B. Convoluted part (pars convoluta) (DCT)

The Collecting Duct System

I. Connecting tubule (CNT)
II. Cortical collecting duct (or tubule) (CCD)
III. Outer medullary-collecting duct (or tubule) (OMCD)
IV. Inner medullary-collecting duct (or tubule) including the papillary collecting ducts (also called *the ducts of Bellini*) (IMCD)

Nephrons lie in characteristic positions (Fig. 1-2), with the renal corpuscles and proximal convoluted segments in the cortex (Figs. 1-3; 1-4). The straight part of the proximal tubule, the thin limb segments, and the straight part of the distal tubule form the loop of Henle, which enters a medullary ray of the cortex and extends into the medulla, where it bends, returning to the cortex by means of the same medullary ray. The loops of juxtamedullary nephrons directly connect the outer stripe of the medulla without ever being contained in a medullary ray. As the straight part of the distal tubule returns to the cortex, it passes by the renal corpuscle from which the nephron originated, forming the macula densa; then, after a short post-macula densa segment, it continues as the distal convoluted tubule within the cortex.

The morphology of the nephron varies with the position of the renal corpuscle in the cortex. Each nephron is classified as superficial, midcortical, or juxtamedullary, according to the position of its renal corpuscle within the respective regions of the cortex (Fig. 1-2) and the pattern of efferent vessel formation (9–11). A given segment tends to occupy a specific region of the kidney, which gives rise to the gross zonation referred to in the preceding. In the human kidney, superficial nephrons empty singly into a terminal collecting duct, whereas several juxtamedullary nephrons empty into an arched tubular portion (arcade) that courses peripherally in the cortex before it turns to enter a medullary ray. Most midcortical nephrons from humans empty individually as well (5,12). As known from the study of several species (rats and rabbits), an arcade is established by the connecting tubule epithelium; data from studies in humans are not available.

Nephrons also are classified as short or long looped according to the location of the position where their loops of Henle turn within the kidney. Short-looped nephrons arise from renal corpuscles located in superficial and midcortical regions and have loops of Henle that turn within the outer medulla. In humans, some superficial nephrons may have loops within the cortex itself. Short-looped nephrons have short, thin limb segments that occur only along the descending limb. Long-looped nephrons have loops of Henle that turn within the inner medulla and have thin limb segments in both descending

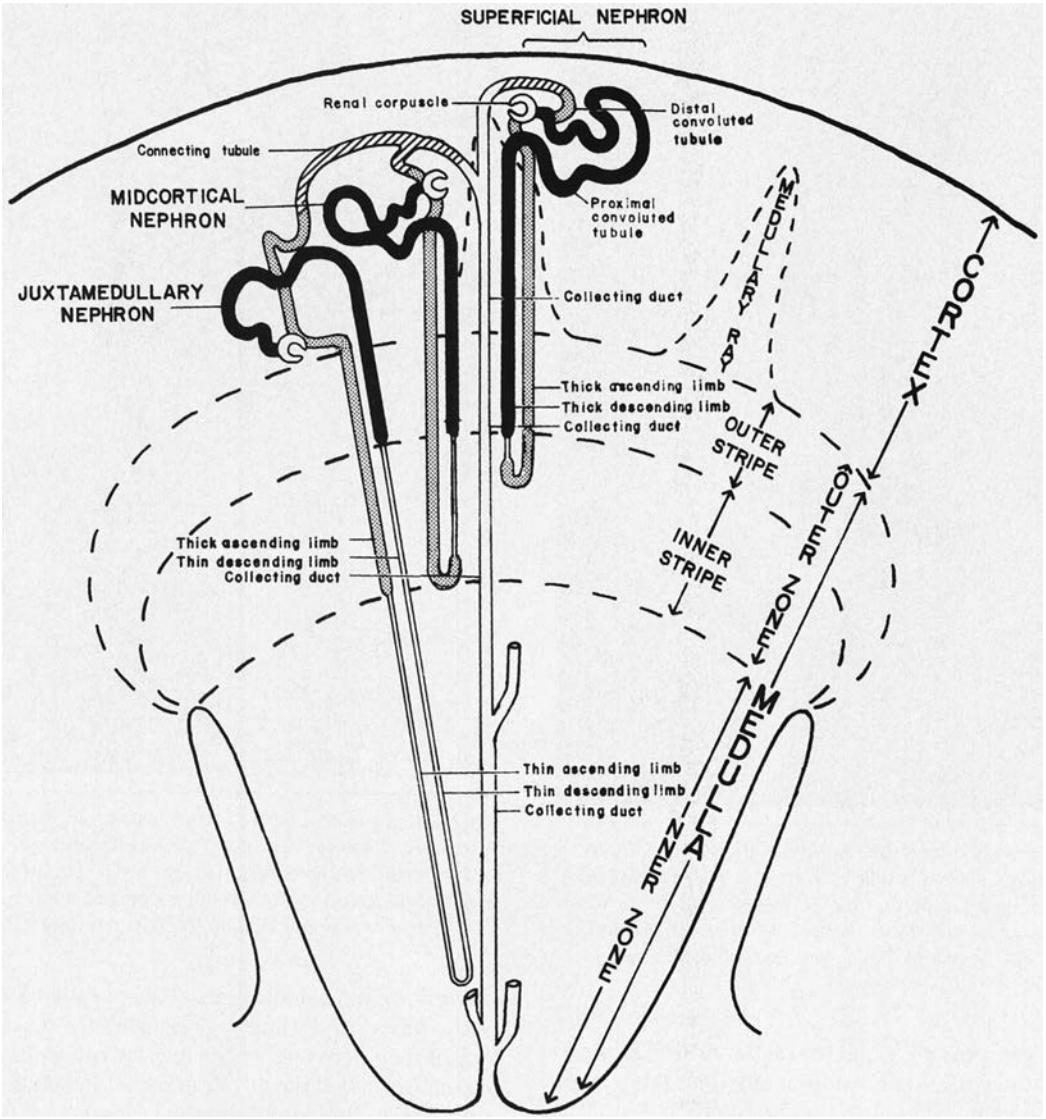


FIGURE 1-2. The relationship of nephron segments to zones of the kidney.

and ascending limbs. Although most species have both long- and short-looped nephrons, some species, such as dogs and cats, have only long ones (13), whereas other species, such as beavers, have only short ones (14,15). In human kidneys, the ratio of short- to long-looped nephrons is 6:1 to 7:1.

The Renal Corpuscle

The renal corpuscle (first segment of the nephron) is the site at which an ultrafiltrate of the blood is produced (Figs. 1-5). The filtrate moves from the capillary lumen into Bowman's space. This flow is influenced by the following factors: renal blood flow; the oncotic and hydrostatic pressures in the capillaries and in Bowman's space; the size, shape, and charge of plasma molecules; and the various morphologic components of the wall separating the capillary lumen from Bowman's space. The filtrate contains only barely detectable quantities of plasma proteins (16). The filtration barrier increasingly restricts the passage of larger molecules, with very little filtration of molecules larger than albumin (70 kDa) (17).

The renal corpuscle consists of Bowman's capsule and the glomerular tuft. The latter is made up of capillaries, derived from the afferent arteriole, their supporting cells, and an envelope consisting of the glomerular basement membrane (GBM) and the visceral (podocyte) layer of Bowman's capsule (Fig. 1-5). At the vascular pole, the visceral epithelium becomes the parietal epithelium, which then transforms into the proximal tubule epithelium at the urinary pole (Fig. 1-5). The space between both layers is the urinary space (Bowman's space).

The human renal corpuscle is roughly ovoid and approximately 150 to 240 μm in diameter. The juxtamedullary glomeruli are larger in size (18). The term *glomerulus* is widely used to refer to the entire renal corpuscle. The renal corpuscle without the parietal epithelial cells is referred to as the glomerular tuft. The afferent arteriole enters the renal corpuscle at the vascular pole, where it divides into several primary branches that each ramify to form a network of anastomosing capillaries, called a *lobule.* The lobule has a supporting region called the *mesangium* (Figs. 1-6; 1-7). All lobules together establish the tuft; the converging mesangial regions are called the *glomerular stalk,* by which the tuft is connected to the extraglomerular mesangium (see Juxtaglomerular Apparatus). The capillaries coalesce toward the center of the capillary tuft to form the efferent arteriole, which runs through the stalk and exits from the vascular pole. The efferent arteriole again breaks up to form a

FIGURE 1-3. Scanning electron micrograph of the cortex. Convoluted tubules are shown, along with renal corpuscles, some of which contain a glomerular tuft (*GT*) and some from which the tuft is removed (*arrow*). A cortical radial artery (*A*) and vein (*V*) are also apparent. Note the thin wall of the vein. (Magnification ×140.)

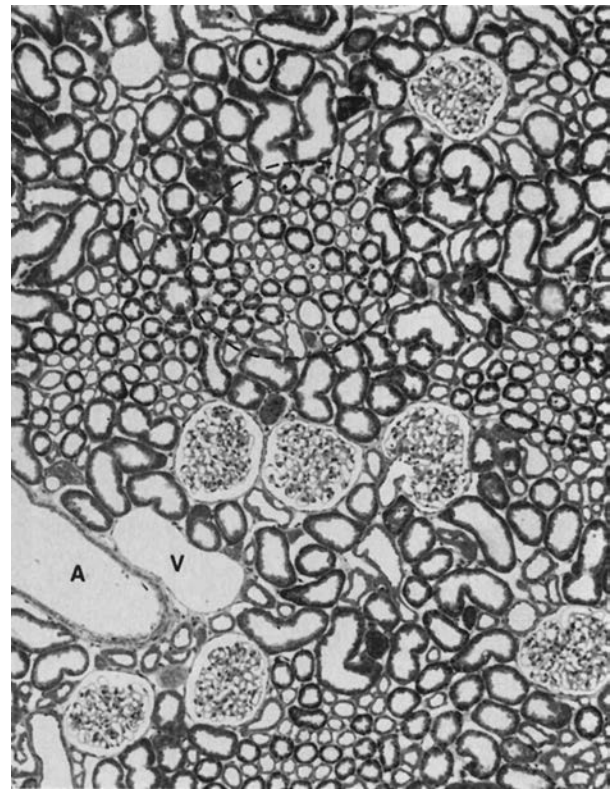


FIGURE 1-4. Light micrograph of renal cortex (rat). Cortical radial vessels (*A*, artery; *V*, vein), glomeruli, and convoluted tubules make up the cortical labyrinth. The straight tubular portions are found in the medullary rays of the cortex (one medullary ray is delineated by the hatched line). (Magnification ×80.)

second capillary network, which surrounds the tubules and is called the peritubular capillary network.

The renal corpuscle, therefore, consists of the following parts: (a) the parietal epithelium, (b) the visceral epithelium (podocytes), (c) the endothelial cells lining the capillaries, (d) the glomerular basement membrane (GBM), and (e) the intraglomerular mesangial cells and matrix. In the rat, the ratio of the number of endothelial cells to mesangial cells to visceral epithelial cells is 3:2:1 (19).

The Visceral Epithelium of Bowman's Capsule

The visceral epithelial cells (nowadays generally called *podocytes*) are octopus-shaped cells that reside in Bowman's space being attached to the GBM only by way of their processes (see below). This shape was well described by Zimmermann (20) and is seen to advantage in scanning electron micrographs (SEM) (see later, Fig. 1-10). The exact details of cell shape differ depending on the species being studied (21,22). As a consequence of the high degree of differentiation, podocytes, like neurons, are unable of regenerative cell replication in the adult. Thus, podocytes that were lost, for any reason, can not be replaced by new ones (23–25). At least no proliferation was documented in a wide variety of glomerular diseases associated with podocyte loss. On the other hand, in glomerular diseases based on dedifferentiation of podocytes (collapsing glomerulopathy), a vivid proliferation of the dedifferentiated cells advances the disease (26).

The cell bodies give rise to large primary processes that may branch another time, finally splitting apart into terminal processes, called foot processes (as seen from SEM pictures "finger processes" would be a better word) or pedicels (Figs. 1-6; 1-8 through 1-11, and 1-13). The foot processes are anchored within the GBM to a depth of about 40 to 60 nm. The foot processes interdigitate in a complicated manner with those from adjacent cells to form an elaborate layer of small processes along the glomerular basement membrane. This interdigitation results in the formation of an extensive series of narrow slits between the foot processes, which provide a long extracellular path for filtration of water and solutes (Fig. 1-10). In transmission electron micrographs, these slits are bridged by a layer of extracellular material (4 to 6 nm thick) called the *filtration-slit membrane* (Figs. 1-9; 1-13). If tannic acid is added to the fixative solution, a highly ordered isoporous substructure is revealed in *en face* views of the filtration-slit membrane (27). Staggered rodlike units project from the podocyte plasmalemma and connect to a central linear bar. These rodlike units delineate rectangular pores 4 × 14 nm within the slit membrane (i.e., which approximate the size of an albumin molecule).

Generally, the slit diaphragm is considered as an adherens-like intercellular junction (28). Intensive research in recent years has uncovered several transmembrane proteins that participate in the formation of the slit membrane (Fig. 1-11)—including nephrin (29), Neph1 (30), P-cadherin (28), and FAT (31). Other molecules, such as ZO1 (32), Podocin (33), CD2AP (34), and catenins mediate the connection to the actin cytoskeleton (see below). Nephrin is a member of the immunoglobin superfamily (IgCAM); its gene, NPHS1, has been identified as the gene whose mutations cause congenital nephritic syndrome of the Finnish type (29). In addition to its role as a structural component, nephrin acts as a signaling

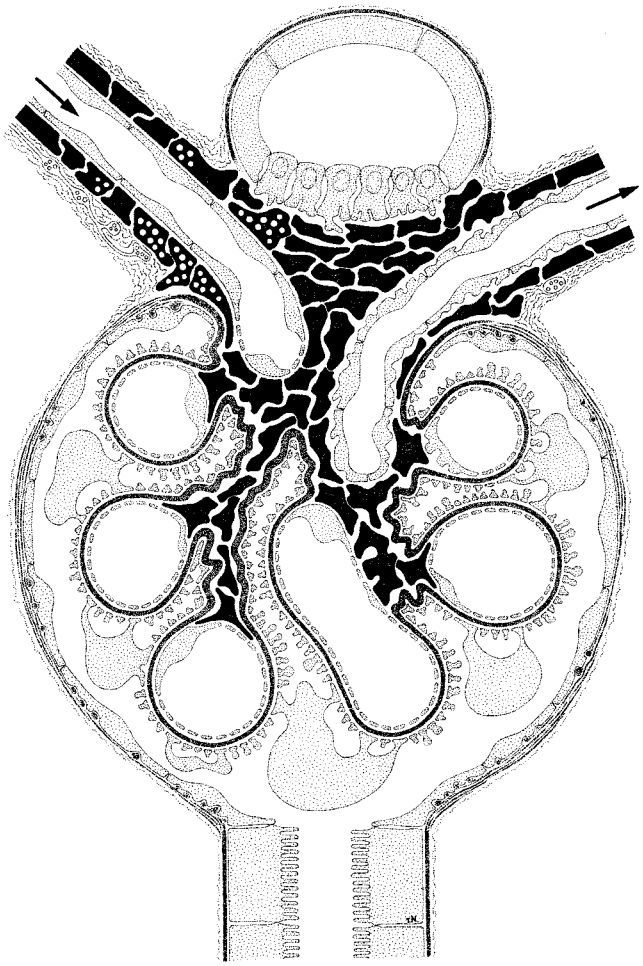


FIGURE 1-5. Schematic of a longitudinal section through a glomerulus and juxtaglomerular apparatus (*JGA*). The direction of blood flow in the glomerular arterioles is indicated by arrows. The capillary network, together with the mesangium, is enclosed in a common compartment bounded by the glomerular basement membrane (*GBM*) (*shown in dark gray*). The outer aspect of the GBM is covered by the glomerular visceral epithelium (podocytes). Note that there is no basement membrane at the interface between the capillary endothelium and the mesangium. At the vascular pole, the visceral epithelium, together with the GBM, is reflected into the parietal epithelium of Bowman's capsule, which, at the urinary pole, passes over into the epithelium of the proximal tubule. The JGA consists of the macula densa of the distal tubule, the extraglomerular mesangium (which is continuous between both arterioles and continues via the glomerular stalk into the intraglomerular mesangium), and the granular cells within the afferent arterioles. All cells that have been suggested to be of smooth muscle origin are shown in black. Note the sympathetic nerve terminals at the afferent arteriole. (From: Kriz W, Sakai T, Hosser H. Morphological aspects of glomerular function. In: Davison AM, ed. *Nephrology*, Vol. 1. Proceedings of X International Congress of Nephrology, London, 1987. London: Bailliere Tindall, 1988:3, with permission.)

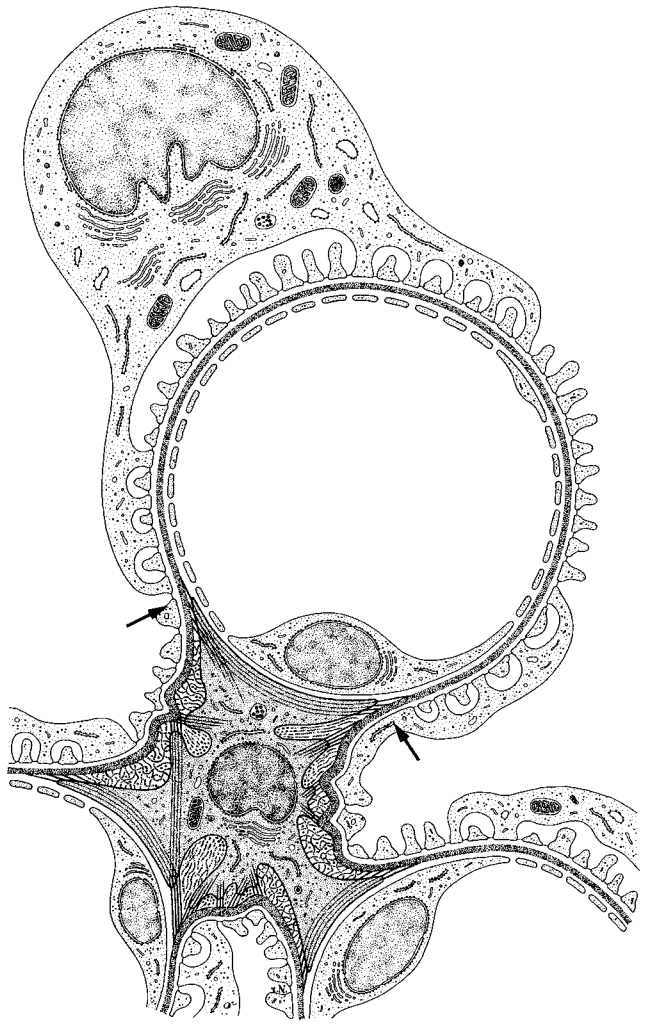


FIGURE 1-6. Schematic of a cross section of a glomerular capillary and its relationships to the mesangium. The capillary is made up of a fenestrated epithelium. The peripheral part of the endothelium tubule is surrounded by the GBM (*shown in dark gray*), which, at mesangial angles (*arrows*), deviates from a pericapillary course and covers the mesangium. The outer aspect of the GBM is covered by the interdigitating pattern of podocyte foot processes. In the center, a mesangial cell is shown; its many processes contain microfilament bundles and extend toward the GBM, to which they are connected. The mesangial matrix contains an interwoven network of microfibrils. (From: Venkatachalam MA, Kriz W. Anatomy of the kidney. In: Heptinstall R, ed. *Pathology of the kidney*, 4th ed. Boston: Little, Brown, 1991, with permission.)

molecule that can activate MAP kinase cascades (35). Neph1 is considered as a ligand for nephrin. Podocin belongs to the raft-associated stomatin family, whose gene, NPHS2, is mutated in a subgroup of patients with autosomol-recessive-steroid-resistent nephrotic syndrome (33). These patients show disease onset in early childhood and rapid progression to end-stage renal failure. Podocin interacts with nephrin and CD2AP (36). FAT is a novel member of the cadherin superfamily with 34 tandem cadherinlike extracellular repeats and a molecular weight of 516 KDa (37). Because FAT has a huge extracellular domain, it is speculated that it dominates the molecular structure of the slit membrane (31); the FAT mutant mouse fails to develop a slit membrane (38). P-cadherin (28) is thought to mediate with its intracellular domain the linkage to β- and γ-catenin, a complex which then connects to the actin cytoskeleton via α-catenin and α-actinin. Taken together, many components of the slit membrane are known, but an integrative model of its substructure including all components is thus far lacking.

The cell body of podocytes contains a large nucleus that tends to be indented in the region of the large Golgi-apparatus. Furthermore, it houses abundant rough-surface endoplasmic reticulum; individual cisternae, generally arranged in one complex per cell, are widened and filled with fine-granular material of varying electrondensity; their relevance is unknown. In addition to the synthesis of membrane proteins necessary to

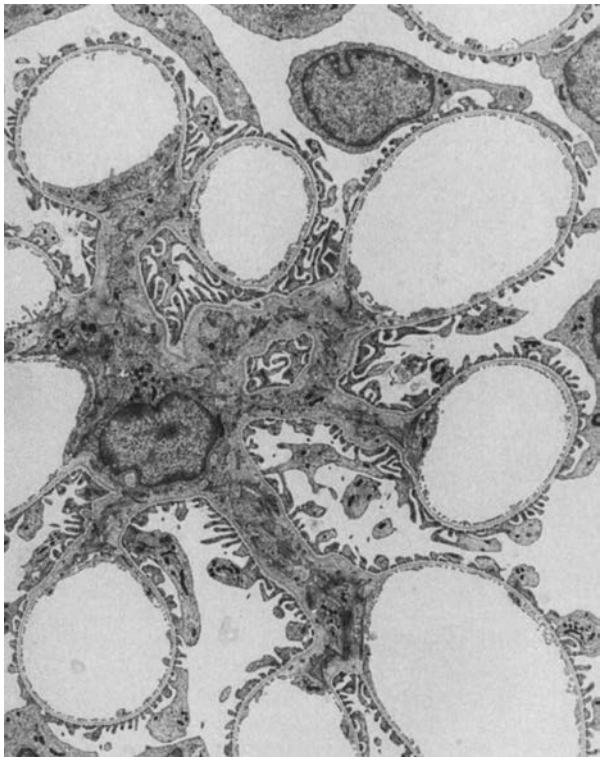


FIGURE 1-7. Transmission electron micrograph of a rat glomerular lobule. Glomerular capillaries and the glomerular mesangium occupy a common compartment enclosed by the GBM. The mesangial cell body (*in the center*) gives rise to many processes that fill (together with the mesangial matrix) radial arms that extend to the peripherally located capillaries. The outer aspect of the GBM is covered by podocytes. (Magnification ×3500.)

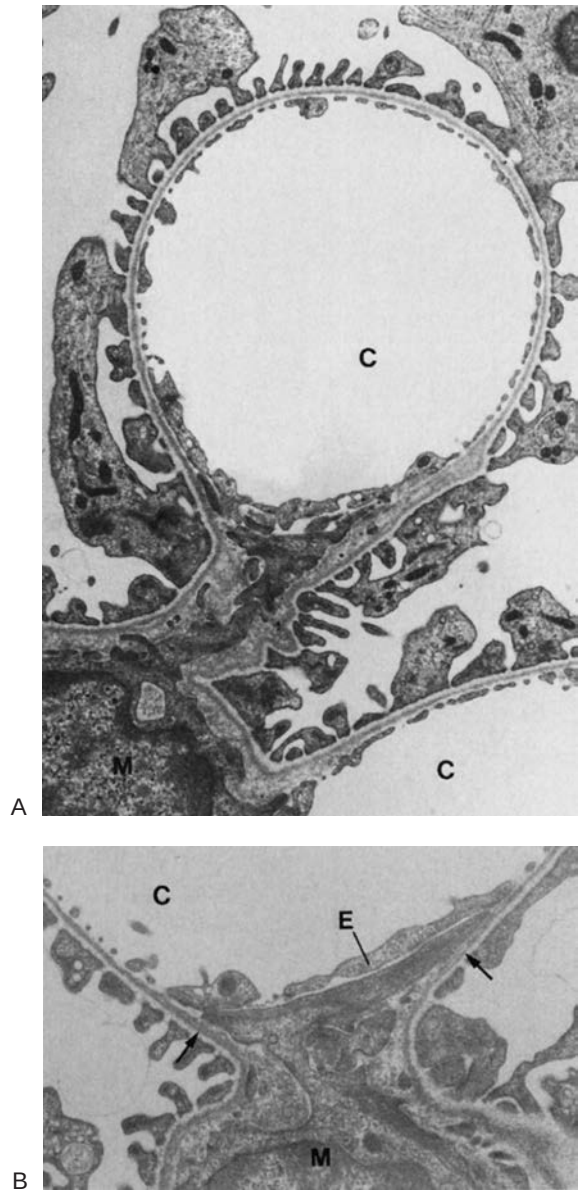


FIGURE 1-8. Transmission electron micrographs of glomerular capillaries (*C*) and associated mesangium. **A:** A mesangial cell body (*M*) gives rise to cell processes that extend to peripherally located capillaries. Note that there is no basement membrane at the interface between the capillary endothelium and the mesangium. (Magnification × 13,000.) **B:** Capillary mesangium interface. Beneath the endothelium (*E*), tonguelike mesangial cell processes run toward both opposing turning points of the GBM (*arrows*). They contain microfilament bundles that obviously interconnect the GBM of both mesangial angles. (Magification ×24,000.)

supply the huge surface of their processes, podocytes in the adult synthesize and secrete all components of the GBM (39) (see below). On the other hand, abundant multivesicular bodies (predominantly found in the large cell processes) demonstrate strong catabolic activity in podocytes.

Podocytes contain a well-developed cytoskeleton that accounts for the unique shape of the cells and the maintenance of the processes. In the cell body and the primary processes, microtubules and intermediate filaments, such as vimentin and desmin, dominate, whereas microfilaments are densely accumulated in the foot processes. Here, they are part of a complex contractile apparatus (40). The microfilaments form loop-shaped bundles, with their limbs running along the longitudinal axis of the foot processes. The bends of these loops are located centrally at the transition to the primary processes and are probably connected to the microtubules by the microtubule-associated protein τ (41). Peripherally, the actin bundles appear to be anchored in the dense cytoplasm associated with the cell membrane of the soles of foot processes.

Specific transmembrane matrix receptors anchor the podocyte foot processes to the GBM. Two systems are so far known. The first is a specific integrin heterodimer consisting of $\alpha_3\beta_1$ integrins. Within the GBM, these integrins bind to collagen type IV, fibronectin and laminin 11 (42,43). Second, a dystroglycan complex connects the intracellular molecule utrophin to laminin 11, agrin and perlecan in the GBM (44,45). Both, integrins and dystroglycans are coupled via adapter molecules (paxillin, vinculin, α-actinin a.o.) to the podocyte cytoskeleton, allowing outside-in and inside-out signaling as well as transmission of mechanical force in both directions.

In addition to the actin cytoskeleton within the foot processes, a subplasmalemmal actin system is found in podocytes (40). This actin network is connected to the transmembrane sialoprotein podocalyxin, that represents the major protein of the negatively charged surface coat of podocytes (46,47). The cell coat has the characteristics of a glycocalyx that contains sialic acid. A decrease in the content of sialic acid is associated with a podocyte foot-process effacement and results in protein leakage through the filter; this has been shown under a great variety of circumstances (48–52).

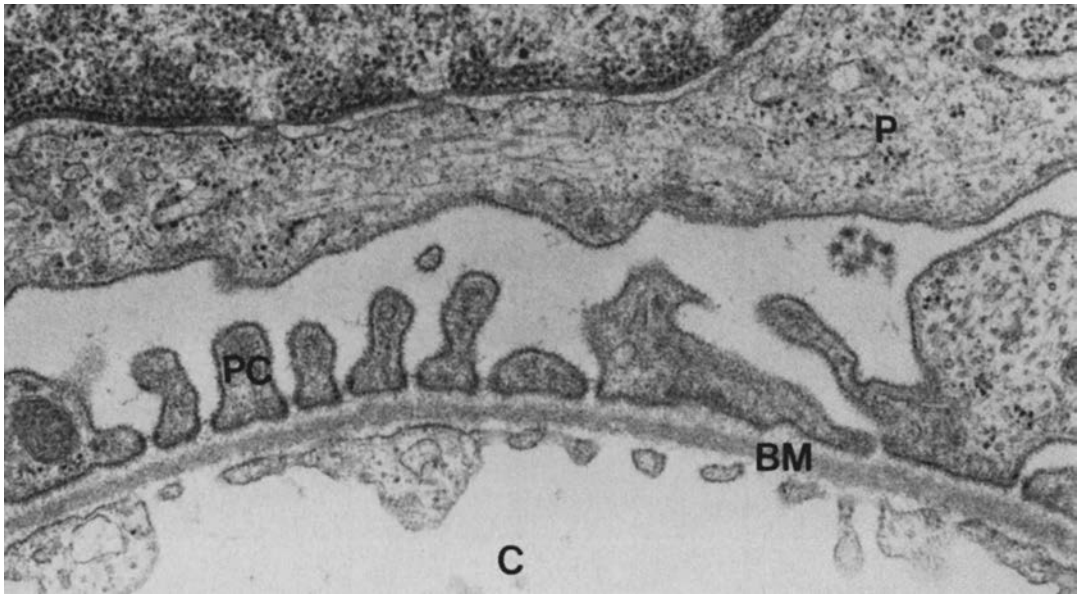


FIGURE 1-9. Transmission electron micrograph showing the podocyte (*P*), pedicels (*PC*) near the basement membrane (*BM*), and the endothelial cells lining the capillary (*C*). (Magnification ×34,000.)

A huge body of data has been accumulated in recent years concerning the inventory of receptors and signaling processes starting from them in podocytes. cGMP signaling (stimulated by ANP, BNP, and CNP, as well as by NO), cAMP signaling (stimulated by prostaglandin E_2, dopamine, isoproterenol, PTH/PTHrP), and Ca^{2+} signaling (stimulated by a huge number of ligands, including angiotensin II, acetylcholine, PGF_2, AVP, ATP, endothelin, histamine, a.o.) have been identified (for details see [53]. The major target of this signaling orchestra is the cytoskeleton, the concrete effects of which, however, are poorly understood. Other receptors, such as for C3b (54), Heymann's antigen (55), TGFβ (56,57), FGF2 (58) and various other cytokines and chemokines have been shown to be involved in the development of podocyte diseases (for details see reference 53).

Endothelium

The endothelium consists of a simple squamous layer of fenestrated cells with the cell nuclei generally located near the axial region of the capillary loop (Fig. 1-6). The fenestrated regions (Fig. 1-12), which compose roughly 55% of the surface area (59), have a thin layer of cytoplasm (about 50 nm thick) penetrated by numerous fenestrae of round, oval, or irregular shape

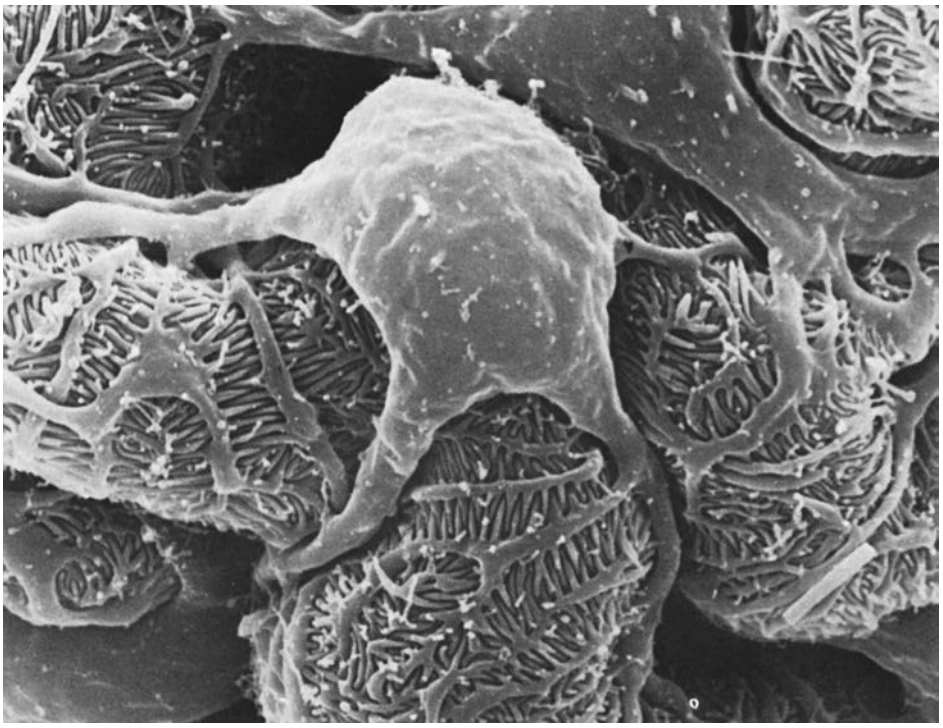


FIGURE 1-10. Scanning electron micrograph showing the elaborate cell shape of rat podocytes. (Magnification ×5,900.)

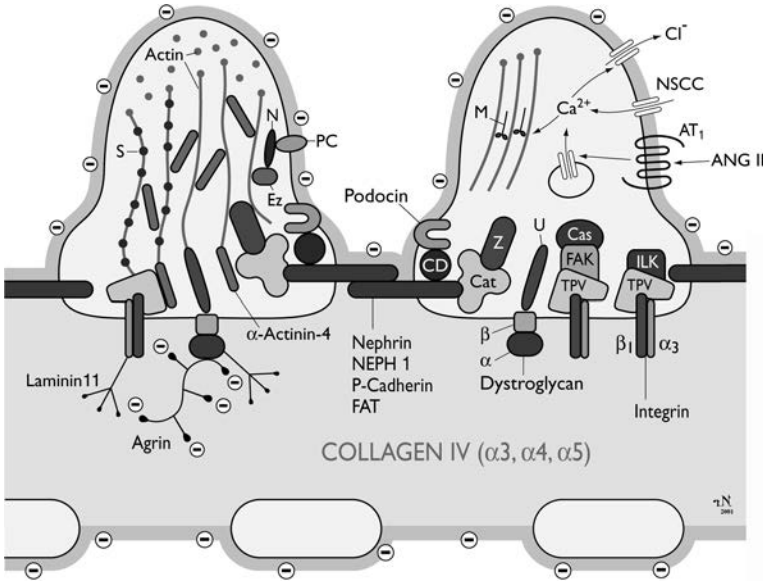


FIGURE 1-11. Schematic drawing of the molecular equipment of podocyte foot processes *Cas*, p130Cas; *Cat*, catenins; *CD*, CD2-associated protein; *Ez*, ezrin; *FAK*, focal adhesion kinase; *ILK*, integrin-linked kinase; *M*, myosin; *N*, NHERF2; *NSCC*, nonselective cation channel; *PC*, podocalyxin; *S*, synaptopodin; *TPV*, talin-paxilin-vinculin; *U*, utrophin; Z, ZO-1. See text for further explanations. (From: Endlich K, Kriz W, Witzgall R; Update in podocyte biology. *Curr Opin Nephrol Hypertens* 2001: 10:331, with permission.)

and varying sizes. The total area occupied by fenestrae accounts for 13% of the capillary surface. The fenestrated regions outline the pericapillary portions of the glomerular basement membrane, but also may be found adjacent to the mesangium (Fig. 1-8). The fenestrae have a diameter of 50 to 100 nm (thus they are larger than endothelial fenestrae elsewhere in the body) and generally are not bridged by diaphragms. Fenestrae with diaphragms are found only in the outflow segment of the efferent arteriole (60). Nonfenestrated regions are generally seen over nuclei and mesangial cell regions. Human glomerular endothelial cells also are fenestrated (61,62). A cell coat that is rich with polyanionic glycoproteins, including podocalyxin, covers the endothelial surface (63–65) and appears to fill the fenstrae like "sieve plugs" (66).

As elsewhere in the body, glomerular endothelial cells are active participants in processes controlling coagulation,

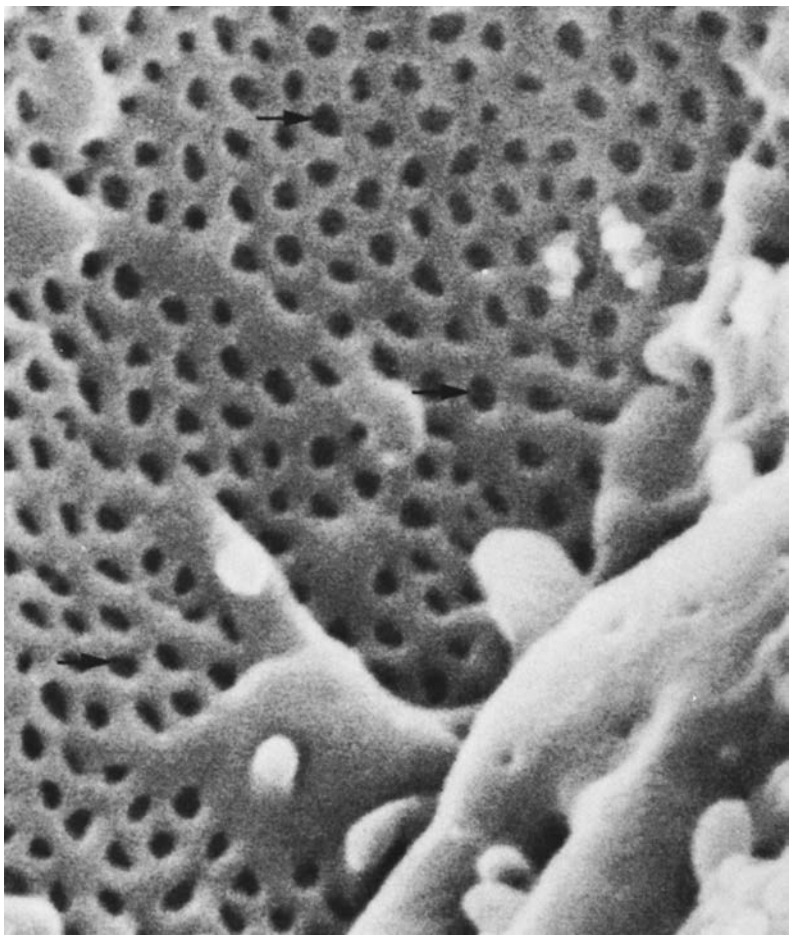


FIGURE 1-12. Scanning electron micrograph of a sectioned capillary loop showing the pores (*arrows*) of the endothelium. (Magnification ×50,400.)

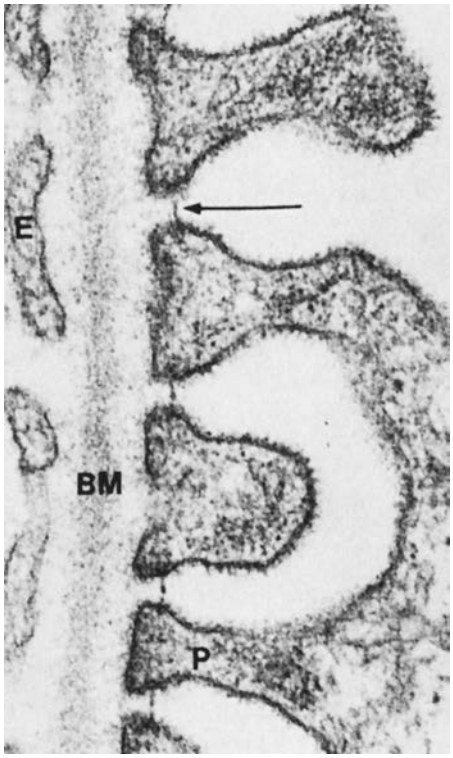


FIGURE 1-13. Transmission electron micrograph from a rat renal corpuscle showing the endothelial lining (*E*), the basement membrane (*BM*), and the pedicels (*P*). The filtration-slit membrane (*arrow*) bridges the pedicels. (Magnification ×23,600.)

inflammation, and immune processes. Renal endothelial cells express surface antigens of the class 2 histocompatibility complex. Like platelets, glomerular endothelial cells contain components of the coagulation pathway and are capable of binding factors IXa and Xa and synthesize, release, and bind von Willebrand factor (factor VIII) (62). Glomerular endothelial cells synthesize and release endothelin-1 and endothelium-derived relaxing factor (EDRF) (67). Glomerular endothelial cells have receptors for vascular endothelial growth factor (VEGF) and angiopoetin I that are produced by podocytes (68,69). The signaling axis via VEGF appears to have a major relevance for the maintenance of the glomerular tuft.

Glomerular Basement Membrane

The glomerular basement membrane (GBM) covers the capillary loops except in the axial region, where it is reflected over the mesangium to the next capillary loop, accompanied by the layer of foot processes (Figs. 1-6 through 1-8). The endothelial cells do not have a separate basement membrane; thus, the endothelial cells directly abut the mesangium. In adult humans, the basement membrane has a mean diameter of 320 to 340 μm (70). It is thinner in young children and most laboratory animals (71).

The basement membrane is composed of three layers: an outer, less dense subepithelial layer, the *lamina rara externa;* a central, electron-dense layer, the *lamina densa;* and an inner subendothelial layer, the *lamina rara interna,* which is continuous with the mesangial matrix (Figs. 1-9; 1-13).

During glomerulogenesis, the GBM is generated as two separate layers produced by glomerular endothelial and epithelial cells. The two sheets are fused together to form the mature GBM (72–75). In the adult, the GBM is subject to a continues turnover (76–79), but few details are known so far about these processes. Podocytes appear to be the dominant cell type to synthesize and probably to degrade the GBM. Podocytes are alone capable to synthesize all the components of the GBM (39,80,81); glomerular endothelial cells and also mesangial cells may contribute to the formation of the GBM (82). It is less clear how the GBM degrades. In recent years, several extracellular matrix degrading enzymes have been described being produced by podocytes and mesangial cells (83–85). The relevance of these enzymes for the turnover of the GBM remains to be established.

The GBM is generally considered as a hydrated meshwork consisting of collagen type IV, laminin, entactin/nidogen, and sulfated proteoglycans including agrin, and perlecan (39, 86–90). Models of the ultramicroscopic structure of the basement membrane picture the GBM as a mat of collagen type IV. Monomers of type IV collagen consist of a 400-μm triple helix that, at its carboxy-terminal end, has a large noncollagenous globular domain called NC 1. At the amino-terminus the helix possesses a 60-μm triple helical rod, the 7S domain. Interactions between the 7S domain and the NC1 domain allow collagen type IV monomers to form tetramers that, by lateral association of triple helical strands, assemble into a three-dimensional network (91,92). Laminin forms a second network that is superimposed to the collagenous network. Laminin is a noncollagenous glycoprotein consisting of three polypeptide chains, two of which are glycosylated and cross-linked by disulfide bridges (86,93,94). Laminin bind to specific sites on the polymerized network of type IV collagen as well as the basal endothelial and epithelial integrins (see the preceding). The α-5-, β-2-, γ-1-laminin chains are assembled to form the GBM-specific heterotrimeric laminin 11 (42). This combined network of collagen type IV and laminin provides mechanical stability to the basement membrane and serves as a basic structure on which other matrix components attach.

The proteoglycans of the basement membrane consist of core proteins and covalently bound glycosaminoglycans, which are concentrated in the laminae rarae internae and externae, where they have been referred to as anionic sites and can be localized with cationic probes (95). The major proteoglycans of the GBM are of the heparan sulfate type; the most prominent is agrin (87), but perlecan also has been shown to occur in the GBM (39). Digestion of these molecules with heparinase leads to a dramatic increase in the permeability of the basement membrane to anionic native ferritin used as a probe (63).

Mesangium

The mesangium consists of mesangial cells that are embedded in a mesangial matrix. The term *mesangium* was introduced by Zimmermann in 1929 (20) to describe the cells that form the stalk of the glomerulus and the axes of its lobules. Glomerular capillaries pursue a tortuous, highly anastomosing course around the mesangial axes. Together with the capillaries, the mesangium occupies the space inside the GBM, frequently termed the "endocapillary region." Topographically, the mesangium can be subdivided into a juxtacapillary region, where it abuts the capillary endothelium, and more centrally located axial regions, which are bound by the perimesangial GBM (Figs. 1-5 through 1-7) (96). The glomerular mesangium is continuous with the extraglomerular mesangium (Polkissen or lacis cells) along the glomerular stalk (Fig. 1-5). Both intraglomerular and extraglomerular mesangial cells have many similarities.

Mesangial cells are quite irregular in shape, with many cytoplasmic processes extending from the cell body toward the GBM. They have structural characteristics similar to those of smooth muscle cells in that they contain many bundles of microfilaments (especially within the cell processes) and peripheral dense bodies. Actin, myosin, and α-actin have been

shown by immunocytochemistry to be contained in mesangial cells (97,98). The relevance of mesangial cell contractility is discussed in the following.

The processes of mesangial cells extend toward the GBM, to which they are attached either directly or by the interposition of extracellular bundles of microfibrils (Figs. 1-6; 1-9). The GBM has to be considered as the effector structure of mesangial contractility (96,99). Connections between mesangial cells and the GBM are especially prominent in the juxtacapillary region. At this site, tonguelike mesangial cell processes (packed with microfilament bundles) run underneath the capillary endothelium toward the turning points (mesangial angles) of the GBM, where they are anchored. Generally, two of these processes interconnect the GBM from two opposing mesangial angles (Fig. 1-8). In the axial mesangial region, contractile filament bundles are predominantly found within the numerous fingerlike projections of mesangial cells. These microprojections also run toward the GBM and are anchored to it. As in the juxtacapillary region, these microfilament bundles interconnect opposing parts of the GBM (96).

The mesangial matrix fills the highly irregular spaces between mesangial cells and the perimesangial part of the GBM. In immunocytochemical studies, collagen types IV and V, heparan sulfate proteoglycan, fibronectin, laminin, and entactin have been localized within the mesangial matrix (90,100,101). Among these components, fibronectin is the most abundant and has been shown to be associated with microfibrils (100,102). Fibrillin 1 and other specific elastic fiber proteins been detected in the glomerular mesangium and have been shown to be produced by mesangial cells (103,104).

In specimens prepared for transmission electron microscopy according to routine methods, the mesangial matrix appears as basement membranelike material, albeit more fibrillar in character than the basement membrane proper (105). In specimens prepared by a technique that avoids osmium tetroxide and uses tannic acid for staining, the mesangial matrix is seen to contain a dense network of microfibrils (96,106). Microfibrils are noncollagenous, nonbranching hollow structures of indeterminate length that are about 15μm thick (107). Within the mesangium, microfibrils form a three-dimensional network that establishes a solid base of contact between mesangial cells and the GBM, fettering the GBM to mesangial cells. Distinct bundles of microfibrils may be regarded as "microtendons" that allow the transmission of contractile force of mesangial cells to specific sites of the GBM (96,106). The functional relevance of this system is discussed below.

The relevance of mesangial cells in phagocytosis is well documented. Mesangial cells are able to ingest particular tracers as well as macromolecules, such as thorotrast (108), ferritin (105), and aggregated proteins, as well as immune complexes (109). An increased uptake of such materials by mesangial cells has been noted in proteinuric states (105,110). It appears, however, that mesangial cells proper (i.e., mesangial cells that have contractile properties) are not primarily phagocytotic. A small subpopulation (3% to 7%) of cells in this region has been recognized as bone marrow-derived; they represent macrophages that have taken up residence in the mesangium (111).

Supportive Functions of the Mesangium and Podocytes

The glomerular tuft is constantly exposed to comparably high intraglomerular pressures within glomerular capillaries and mesangium. The high intraglomerular pressures challenge not only the glomerular capillaries themselves but also the folding pattern of the glomerular tuft. Increased pressures lead to the loss of the folding pattern and to dilation of the glomerular capillaries. Therefore, we have to ask what are the specific structures and mechanisms that counteract the expansile forces in the glomerular tuft. To answer this question we have to distinguish between the structures and mechanisms maintaining (i) the folding pattern of the glomerular tuft and those maintaining (ii) the width of glomerular capillaries.

The folding pattern of the glomerular tuft is primarily sustained by the mesangium (96,99,112). Mesangial cells are connected to the GBM by their contractile proceses; by centripetal contractions they maintain the infoldings of the GBM, thereby allowing for the capillaries to arrange in the peripheral expansion of the GBM. This supporting role of mesangial cells is best illustrated under circumstances with loss of mesangial cells, such as Thy-1 nephritis (113). Under those circumstances the folding pattern of the GBM is progressively lost, finally resulting in mesangial aneurysms. Podocytes clearly contribute to maintenance of the folding pattern by specific cell processes that interconnect opposing parts of the GBM from outside within the niches of the infoldings. This function is also best illustrated under circumstances with loss of mesangial support: podocytes are capable of maintaining a high degree of the GBM folding pattern for 2 to 4 days after which they obviously fail, and mesangial aneurysms become prominent (113).

The width of glomerular capillaries, in the long run, is probably controlled by growth processes accounting for differently sized capillaries. The width of a given capillary, in an acute situation being exposed to changes in blood pressure, appears to be stabilized by the GBM, which is a strong elastic structure (114) and, together with the mesangial cell bridges (see above), capable of developing wall tension (112,115). In addition, the tensile strength of the GBM is reinforced by podocytes. Podocytes are a kind of pericytes; their foot processes represent a unique type of pericyte process which, like elsewhere in the body, counteract the dilation of the vessel. Podocyte processes are firmly attached to the underlying GBM (see above); their cytoskeletal tonus counteracts the elastic extension of the GBM. In this function, podocytes cannot be replaced by any other cell; failure in this function will lead to capillary dilation.

Glomerular Filtration Barrier

The essential components of the glomerular filtration barrier are the endothelium (Fig. 1-13), which is perforated by large open pores, the extracellular matrix feltwork of the GBM membrane, and the slit diaphragms between the podocyte foot processes. When compared with the barrier established in capillaries elsewhere in the body, a glomerular filtration barrier is quite different in two respects: its permeability to water, small solutes, and ions is extremely high, whereas its permeability to plasma proteins the size of albumin and larger is very low.

The high hydraulic permeability is rooted in the fact that filtration occurs along extracellular routes. All components of this route—the endothelial pores, the highly hydrated GBM, and the slit membrane—can be expected to be quite permeable to water and small solutes. Drummond and Deen (116) have calculated the hydraulic conductance of the individual layers. According to this calculation, the hydraulic resistance of the endothelium is negligible. The GBM and the filtration slits each make up roughly one-half of the total hydraulic resistance of the filtration barrier.

Any decrease in the length of the filtration slit and thus in slit area, as in experimental and clinical glomerulopathies along with footprocess effacement, correlates with the decrease in the ultrafiltration coefficient K_f (117,118). A model simulating those conditions showed, along with a decrease in slit area, its relevance in determining increases in flow resistance. The decrease in filtration slit area caused the average path length for the filtrate, through the basement membrane, to increase, thereby explaining the overall decreased hydraulic permeability (119).

On the basis of evidence of contractility of mesangial cells exposed to vasoconstrictor stimuli in culture (120,121), of

dimensional changes observed in intact glomeruli *ex vivo* (122,123), and of changes in ultrafiltration coefficient K_f found *in vivo* in response to vasoactive substances (124,125), some researchers have concluded that mesangial cells contract *in situ* and that this contraction alters glomerular filtration dynamics by decreasing filtration surface area.

Other considerations speak against this possibility. The geometric arrangement of the mesangial contractile apparatus (Fig. 1-6), however, does not seem to be compatible with the previously mentioned sequence of actions. Shortening of the mesangial cell processes connecting opposing angles would only bring the angles closer together, compressing the mesangial capillary interface, but leaving peripheral capillary wall area (filtration area) unaltered. In addition, with regard to the contractility of mesangial cells *ex vivo* (in culture as well as preparations of whole glomeruli), it should be remembered that mesangial contraction in these cases is not opposed by intercapillary hydrostatic pressure as it is *in situ*. These considerations, together with the absence of measurable changes in glomerular tuft dimensions in morphometric studies (126,127), as well as in response to vasoactive substances *in vivo* (128), have led to the suggestion (112) that the mechanical action of the mesangial cell contraction is essentially static in nature, developing from tension that serves to counteract expansile forces on the tuft without inducing significant changes in capillary dimension. If mesangial contractility acutely alters the glomerular ultrafiltration coefficient K_f, it is, therefore, probably not because of an acute change in filtration surface area.

The low permeability of the glomerular filtration barrier to plasma proteins is still poorly understood. Several points seem to be relevant. First, there is no vesicular transport of proteins through this barrier as in most other capillaries elsewhere in the body. The barrier function of the glomerular filter for macromolecules is quite specific and is selective for size, shape, and charge (129–131).

In early extensive studies, Farquhar and associates (129,132,133), as well as Rennke and associates (134), used tracers, such as ferritin and dextrans of different sizes and charge, to elucidate the role of the various layers in determining the selectivity of this filtration barrier. When their results are summarized, it appears that the basement membrane may be the major barrier to anionic substances, whereas the most restrictive part for uncharged and cationic substances may be the slit diaphragm. Uncharged macromolecules up to an effective radius of 1.8 μm pass freely through the filter. Larger compounds are more and more restricted (indicated by their fractional clearances, which progressively decrease) and are totally restricted at effective radii of more than 4.0 μm. The *effective radius* is an empiric value, measured in artificial membranes, that takes into account the shape of micromolecules and also attributes a radius to nonspherical molecules. Plasma albumin has an effective radius of 3.6 μm; without the repulsion because of the negative charge, plasma albumin would pass through the filter in considerable amounts (135).

Thus, since these early studies, the glomerular barrier has been generally suggested to contain a size- and a chargerestrictive element. Despite more than 30 years of intensive research, the principles of the glomerular barrier function are still controversial and poorly understood—a situation that gives room even to hypotheses claiming that the glomerular barrier itself does not have any restrictive properties to macromolecules, such as albumin (136–138).

Summarizing the present status of knowledge, accumulating evidence suggest that the GBM has little relevance in size restriction, but the slit membrane is the decisive size-restrictive element within the glomerular barrier (139). The current strong engagement in elucidating the molecular architecture of the slit membrane promises to enlighten the porous pattern of the slit membrane and, hopefully, explain its size restrictive property. Regarding the charge selectivity, it appears that the proximal portion of the barrier, above all the endothelium, are most important (66,140–142). Furthermore, prevention of albumin from entering the filter is also dependant on normal hemodynamic conditions in glomerular capillaries, as first shown by Ryan and Karnovsky (143).

Proximal Tubule

At the urinary pole of the glomerulus, the flat parietal epithelium of Bowman's capsule transforms abruptly into the high epithelium of the proximal tubule. In some species (rabbits) (144), a neck segment is found interposed between the glomerulus and the proximal tubule; short neck segments also may be seen in humans (71). In contrast, in mice, the proximal tubule epithelium generally begins deep within the Bowman's capsule.

The proximal tubule is composed of segments that have differing morphology, functional relevance, and vulnerability to toxins (Figs. 1-14–1-18). The two segments most frequently identified are the proximal convoluted portion (pars convoluta) occupying the cortical labyrinth (Figs. 1-14–1-17), and the straight portion (pars recta) in the medullary rays of the cortex and the outer stripe of the outer medulla (Fig. 1-18). Further subdivision on structural criteria results into three segments: P_1, P_2, and P_3 according to Jacobsen and Jorgensen (145), and S_1, S_2, and S_3 according to Maunsbach (146,147). S_1, or P_1, corresponds to the first segment of the pars convoluta and lies exclusively in the cortical labyrinth; S_2, or P_2, corresponds to the remainder of the convoluted segment and the beginning of the pars recta, with the first part occupying the cortical labyrinth and the remainder occupying the medullary ray; and S_3, or P_3, corresponds to the remaining part of the pars recta located primarily in the outer stripe of the outer zone of the renal medulla. The transition from P_1 to P_2 is gradual, whereas the transition from P_2 to P_3 is generally abrupt, except in rabbits (148).

FIGURE 1-14. Transmission electron micrograph of a proximal tubule (P_1 segment) of the rat. Note the apical brush border, the vesicular zone of the apical cytoplasm, and the basal zone of interdigitating cell processes filled with mitochondria. (Magnification ×2,300.)

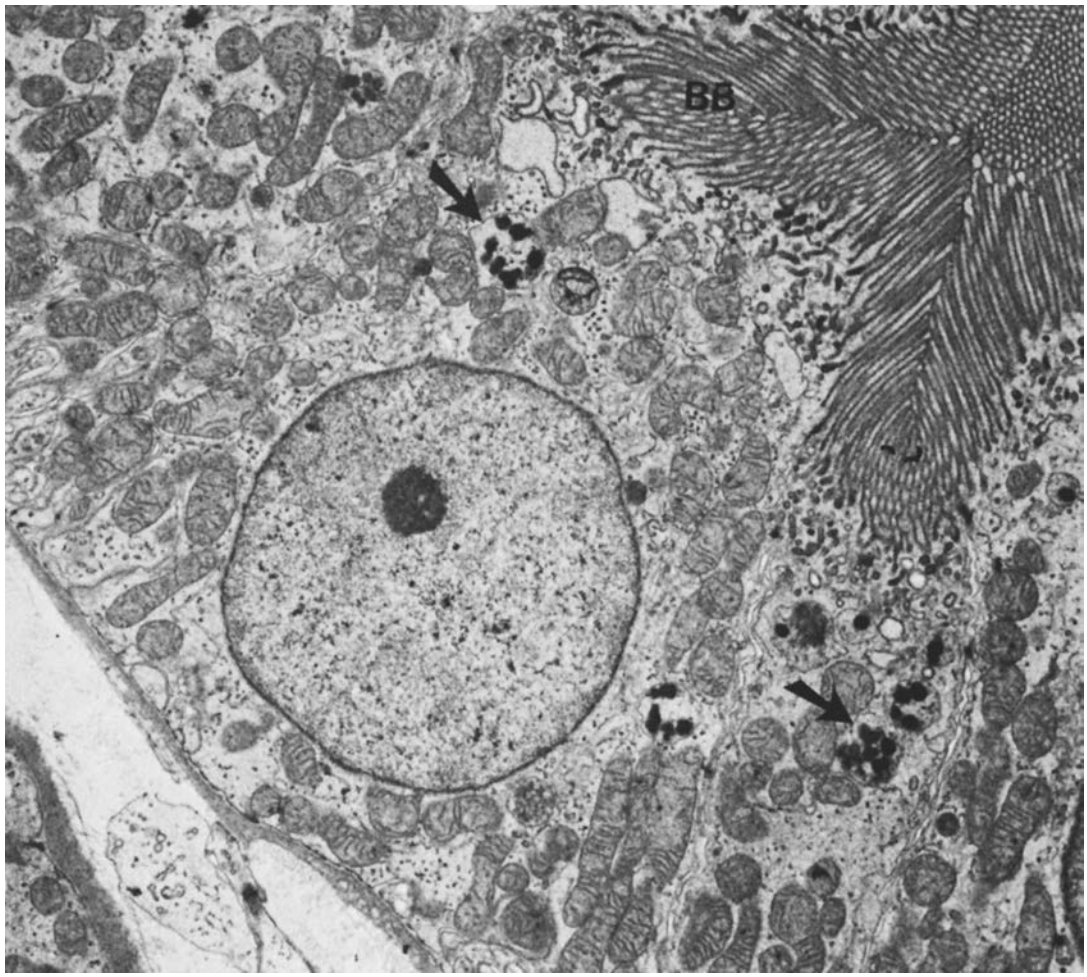


FIGURE 1-15. Transmission electron micrograph of a human proximal convoluted tubule showing the brush border (*BB*) and apical condensing vacuoles containing small dense bodies (*arrows*). (Magnification ×7,800.)

To confuse matters further, the morphology of the subdivisions differs not only from each other but also among species, including mice (149), rats (146), rhesus monkeys (150), rabbits (148), and dogs (151). For a detailed description of a particular species, the reader should consult the original studies. Segmentation of human proximal tubules has not been studied as recently or thoroughly because of a lack of availability of well-fixed normal human kidney and, therefore, has been divided only into convoluted and straight regions (152).

In general, cells of the P_1 region are tall, have a well-developed apical microvillus border, an elaborate cell shape with well-developed lateral interdigitating processes containing abundant, large mitochondria, and a well-developed endocytic apparatus (Fig. 1-16). P_2 cells decrease in cell height from those seen in P_1, have a shorter microvillus border, and are less elaborately shaped cells with smaller mitochondria (Fig. 1-17). The P_3 cells are more cuboidal in shape (less elaborate) and their microvillus border is generally less elaborate. The cells of rats are an exception, having a well-developed brush border of very long microvilli (Fig. 1-18) (146). This cytologic segmentation of the proximal tubule is maintained in superficial, midcortical, and juxtamedullary nephrons.

Proximal Convoluted Tubule (P_1 and Part of P_2)

The proximal convoluted tubule is the longest and largest segment of the mammalian nephron. The tubule is lined by cells that have an elaborate cell shape based on extensive basolateral interdigitations (Figs. 1-19,1–20,1-22), a well-developed microvillus border, a prominent intracellular digestive tract (endocytic apparatus and lysosomes (Fig. 1-15), and numerous large peroxisomes (microbodies). The single ovoid nucleus lies in the middle to basal region of the cytoplasm.

Cell Shape and Mitochondria. The cells are characterized by an extensive system of lateral cell processes that interdigitate with lateral processes from adjacent cells (Figs. 1-19; 1-20; 1-22). These lateral processes can extend the entire height of the epithelium, especially in the P_1 segment, but become more elaborate toward the basal regions of the lateral surface (Fig. 1-16). The complex shape of these cells serves to increase the lateral cell surface area manyfold, which provides a greater area for transporters, above all of the Na^+-K^+-ATPase. These lateral extensions usually contain one or two layers of mitochondria (Fig. 1-20). The mitochondria are long, narrow rods that branch and double back on themselves. They are oriented perpendicular to the basement membrane and lie adjacent to the cell membrane, to which they presumably supply energy for transport processes.

The lateral extensions and their mitochondria cause the pattern of basal striations that are typical for numerous transporting cells. The lateral processes of proximal convoluted tubule cells in humans are not as elaborate as those seen in

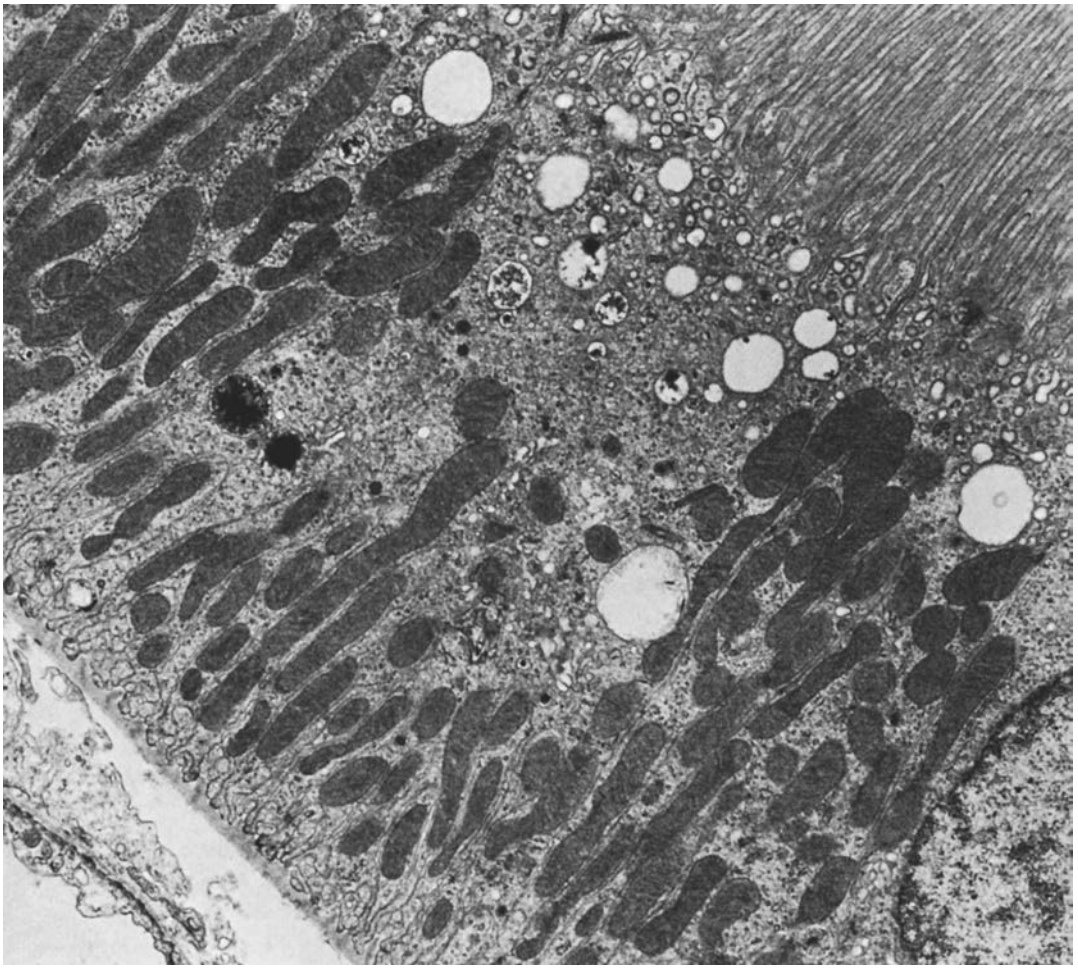


FIGURE 1-16. Transmission electron micrograph of a P_1 segment from a rat kidney. (Magnification ×8,000.)

rats (compare Figs. 1-15 and 1-16). In rabbits, the lateral cell surface is increased 20 times over that of the basal cell surface (153). The lateral extensions also establish a complex and extensive surface labyrinth of lateral intercellular spaces ("basal labyrinth").

Cell Junctions. The proximal convoluted tubular cells have an apical junctional complex that consists of a shallow, beltlike, tight junction next to the tubular lumen; a deeper, beltlike intermediate junction (the *zonula adherens*); and only small and infrequently seen desmosomes. The proximal tubular tight junctions are shallow and consist of only one or two lines of fusion of the outer leaflet of the cell membrane (Fig. 1-21). Freeze-fracture studies of normal proximal tight junctions, however, reveal focal discontinuities. During volume expansion, striking increases in the length of discontinuities were found (154). Transmission micrographs also show multiple areas of nonfusion of tight junctions associated with renal venous constriction and increased ureteral pressure (155). These sites of nonfusion may explain the increased permeability seen in these situations. Proximal tubular cells are electrically coupled by gap junctions (Fig. 1-21) (156).

Microvilli. A layer of slender (approximately 80 to 90 μm), finger-shaped processes extends into the tubular lumen, forming the *microvillus,* or *brush border.* The length and number of processes vary with the segment and species. The luminal surface of the pars convoluta is increased 40 times in rats (147). The luminal surface is increased 36 times in the pars convoluta and 15 times in the pars recta in rabbits (153). Each microvillus has a core of microfilaments that extend into the apical cytoplasm connecting them to the cytoskeleton.

Cytoplasm. The cells contain a very prominent vacuolar apparatus (see below), a Golgi apparatus, free ribosomes, and cisternae of rough and smooth surface endoplasmic reticulum. The latter forms specialized cisternae, called the perimembranous cisternal system, near the lateral cell membranes (71). In addition, the cells contain many peroxisomes that frequently have eyecatching shapes (157). Peroxisomes are limited by a single membrane and contain a dense matrix, frequently exhibiting crystalloid nucleoids (Fig. 1-17); platelike inclusions at the peroxisomal edge, which are called *marginal plates,* are also frequently encountered. Peroxisomes are invariably wrapped by elements of the smooth-surfaced endoplasmic reticulum. In the kidney, large peroxisomes are exclusively found in the proximal tubule, most frequently in the P_3 segments (157); all other nephron portions contain only microperoxisomes. Peroxisomes contain enzymes from a primitive respiratory chain in which oxidases produce hydrogen peroxide, which is, in turn, destroyed by the catalase; hence the name *peroxisome* (158). Peroxisomes participate in the breakdown of very-long-chain fatty acids by lipid β-oxidation; in addition, they may play a protective role by destroying hydrogen peroxide produced by free radicals.

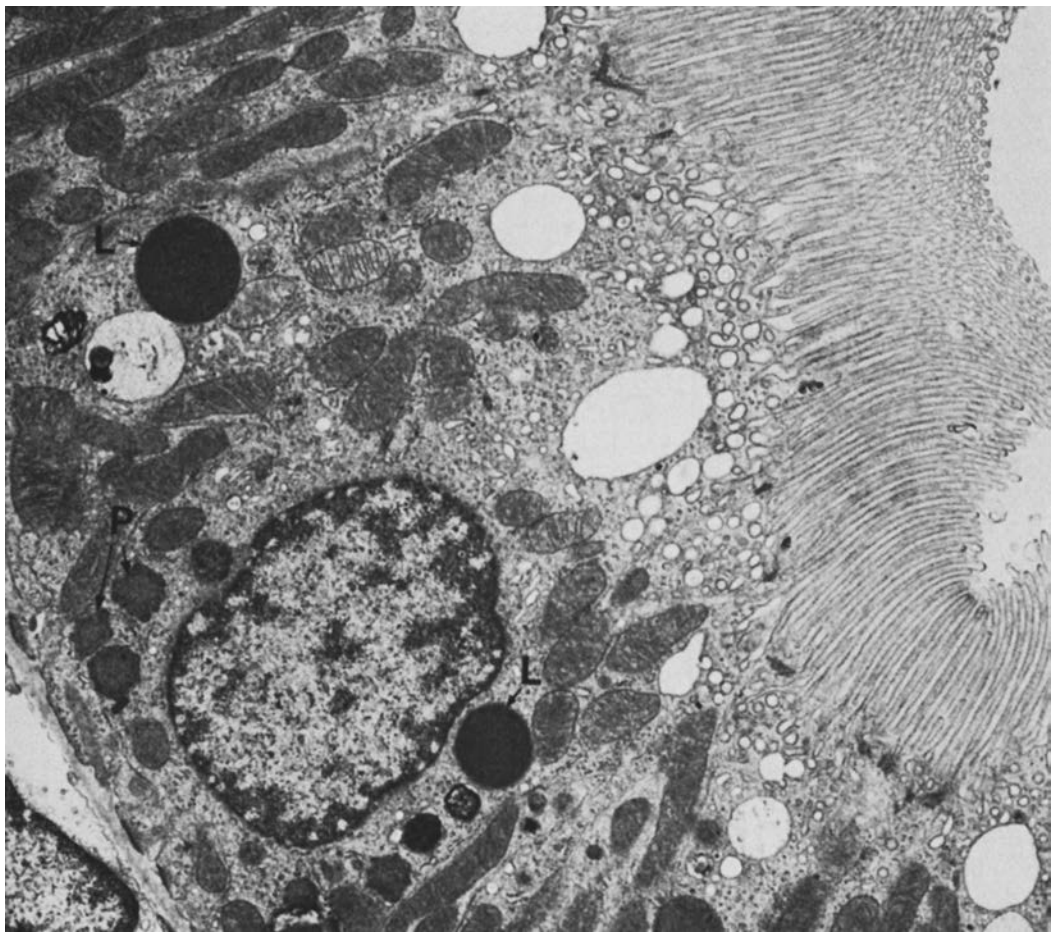


FIGURE 1-17. Transmission electron micrograph of a P_2 segment from a rat kidney. Note the large dense lysosomes (*L*) and peroxisomes (*P*). (Magnification ×10,000.)

Straight Part of the Proximal Tubule (Last Part of P_2 and All of P_3)

The straight part of the proximal tubule (pars recta) begins in the medullary ray and penetrates into the outer stripe of the outer zone of the medulla (Fig. 1-18). The proximal pars recta converts into the thin limb near the junction of the inner and outer stripe. In rats, the pars recta includes the final region of both P_2 and P_3 (159), and the transition between the two segments occurs at various levels in the medullary ray. This region is marked by a sudden increase in microvillar length, a decrease in endocytic apparatus, and a decrease in interdigitation between adjacent cells. The microvilli of the pars recta cells decrease in height and number in rabbits (148) and humans (152) but remain high in rats (Fig. 1-18).

In general, the P_3 pars recta cells have been described as lower in height with a less elaborate shape. In humans, the pars recta cells have a convex apical surface, and some lipid droplets are found in the basal cytoplasm. The mitochondria are fewer and no longer closely applied to the cell membrane. The lysosomes are smaller and the Golgi and endocytic apparatuses are less well developed. Peroxisomes are more numerous in the pars recta (71,152). The tight junctions of P_3 can be more complex in shape, consisting of several junctional strands in rats, dogs, and cats (160).

Structure–Function Correlations

The functional relevance of the proximal tubule is manifold and quantitatively enormous. It reabsorbs about 70% of filtered Na^+, Cl^-, and water, 95% of bicarbonate, 60% of Ca^{2+}, and sodium phosphate. Reabsorption of the filtered glucose is complete, whereas of amino acids is almost complete. This occurs either by transcellular transport via specific channels and transporters in both membranes (water, sodium, phosphate, glucose, amino acids, and bicarbonate in a specific enzyme-mediated mode) or predominantly by paracellular transport through the leaky tight junctions (Ca^{2+}, Cl^-). Furthermore, filtered macromolecules undergo reuptake by a prominently developed endocytotic apparatus. In addition, the proximal tubule (predominantly S_3 segments) secretes organic cations and anions, which constitute an extraordinarily diverse array of compounds of physiologic, pharmacologic, and toxicologic importance. The transepithelial transport involves separate entry steps at the basolateral membrane and exit steps at the luminal membrane with specific transporters at both sites (161–164). Other potentially toxic xenobiotic compounds are metabolized within the well-developed smooth endoplasmic reticulum of S_3 segments (165).

Unique to the proximal tubule is the reabsorption and degradation of filtered protein and peptides (Figs. 1-15; 1-16). As discussed above, all proteins smaller than albumin leak through the glomerular filter; even albumin, in small amounts, is always contained in the filtrate. All these diverse macromolecules are taken up by proximal tubule cells via receptor-mediated endocytosis and degraded in lysosomes. In proteinuric states, this function is greatly enhanced. The uptake and digestion include the following steps (166,167):

1. Binding of the filtered protein to two multiligand receptors, megalin and cubulin, which are densely accumulated within

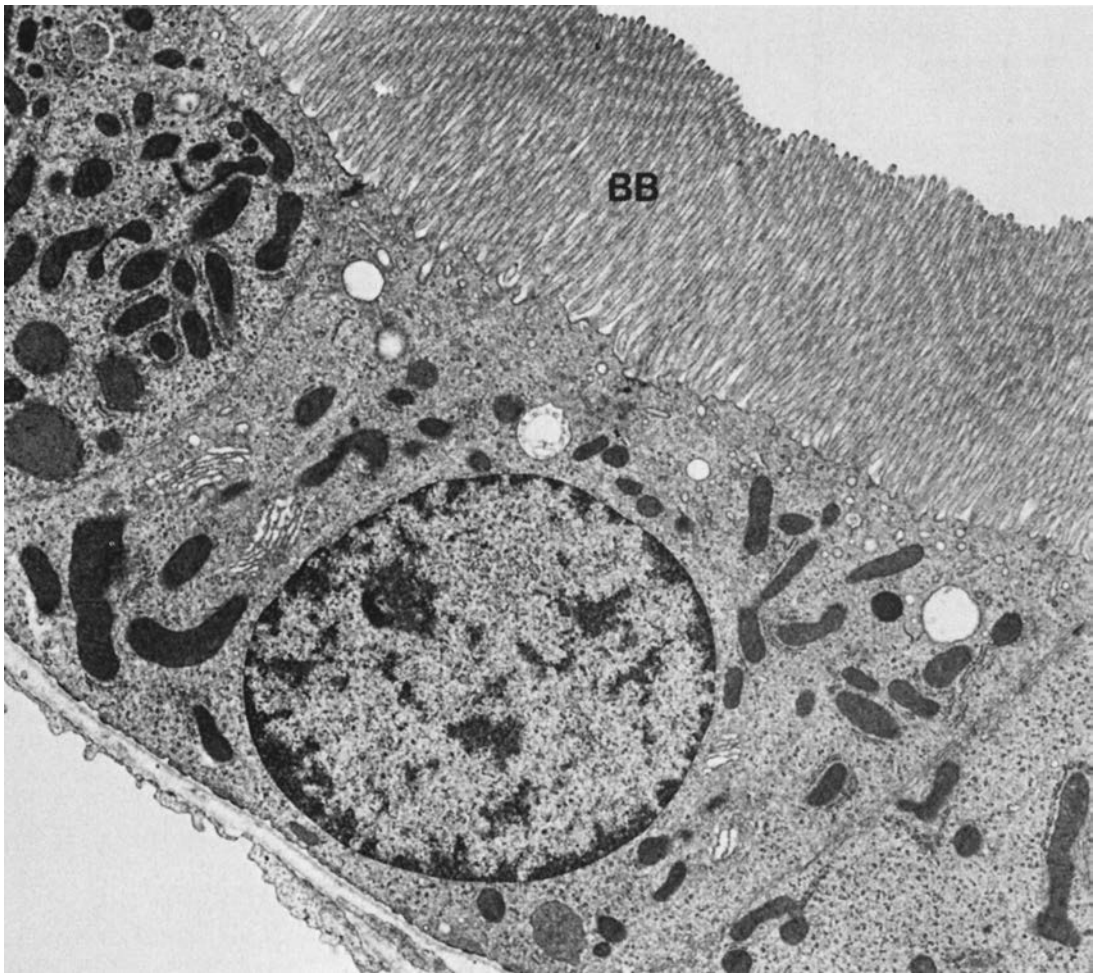


FIGURE 1-18. Transmission electron micrograph of a P_3 segment from a rat kidney showing the extensive microvillus border (*BB*) and more simple cell shape of this segment. (Magnification ×8,000.)

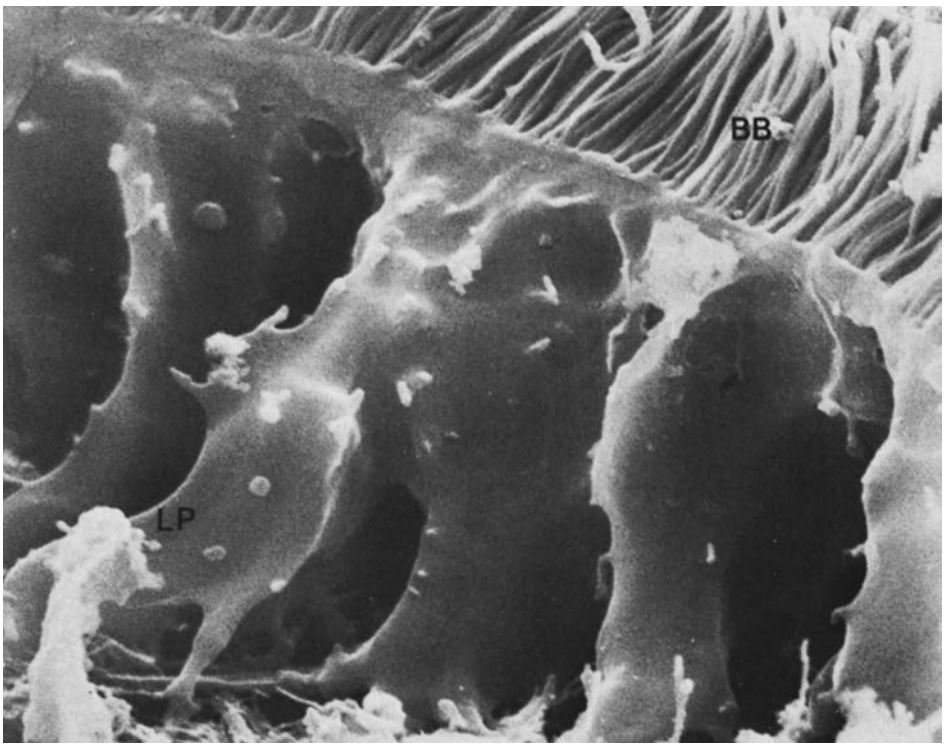


FIGURE 1-19. Scanning electron micrograph of a rat proximal tubule epithelial cell showing the apical brush border (*BB*) and the lateral processes (*LP*). (Magnification ×14,700.)

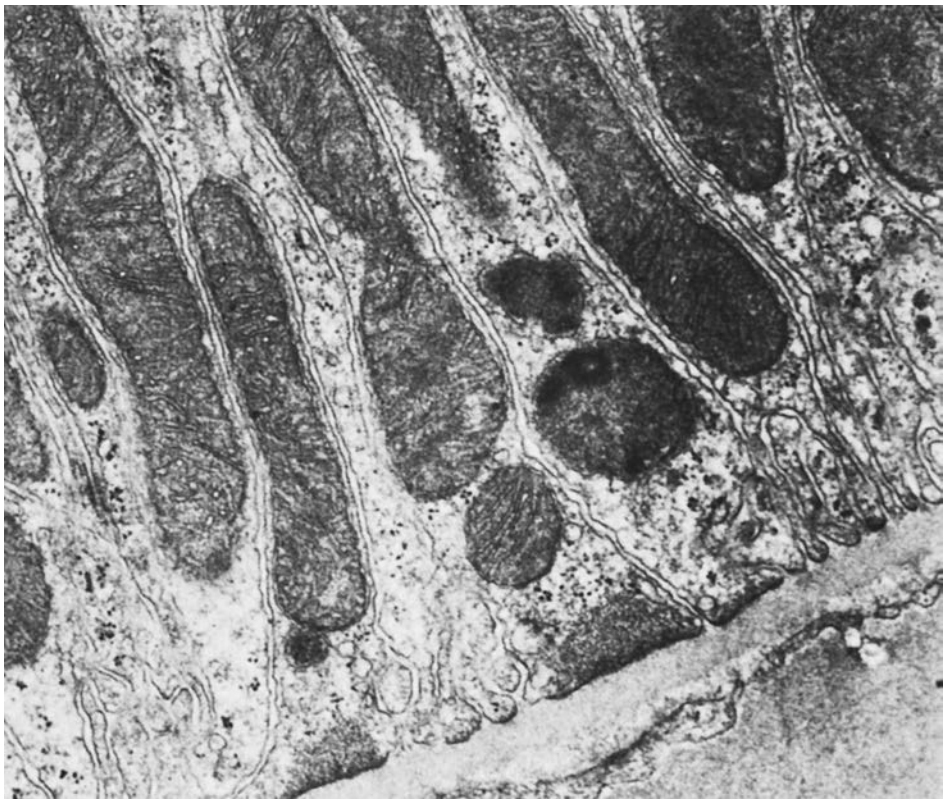


FIGURE 1-20. Transmission electron micrograph of the basal cytoplasm from a rat proximal tubular cell. (Magnification ×20,600.)

clathrin-coated pits in the intermicrovillar areas of the apical membrane.

Megalin is a 600-kDa transmembrane protein belonging to the LDL-receptor family (see reviews [166,167]). The extracellular domain contains four clusters of cysteine-rich, complement-type repeats, constituting the ligand-binding regions. Cubulin is a 416-kDa peripheral membrane protein identical to the intrinsic factorvitamin B_{12} receptor, known from the small intestine. It contains 27 CUB domains, which are responsible for the ability to interact with a great variety of ligands.

2. Small apical vesicals pinch off from the tubular invagination at the intermicrovillar areas to ferry the protein to the next component.
3. Large apical vacuoles located in the apical cytoplasm are formed by fusion of small vesicles representing the early endocytotic compartment.
4. Condensing vacuoles form in which the protein is condensed. These vacuoles move basally in the cell and acquire hydrolytic enzymes by fusion either with primary or secondary lysosomes. The proteins sequestered within lysosomes appear to be broken down into amino acids that are reused by the cell.
5. Already sequestered in the early endosomal compartment, the receptors are concentrated in dense, apical tubules by which they are returned to the apical plasma membrane.

This process of megalin-mediated internalization appears to be of even much wider relevance. As mentioned above, the proximal tubule reabsorbs phosphate, representing a key element in phosphate homeostasis. Depending on the amount of sodium phosphate cotransporters present in the brush border cell membrane, the reabsorption varies. Internalization of the phosphate transporter type II (e.g., PTH induced to downregulate reabsorption) occurs via megalin-mediated endocytosis (168,169). The proximal tubule has also endocrine relevance. In response to stimulation by PTH, it produces, by adding a second hydroxyl group, the active vitamin D_3, calcitriol.

Thin Limbs of the Loop of Henle (Intermediate Tubule)

Thin limbs can be short, occurring only along the descending limb, or they can be long, reaching varying distances into the inner medulla. In the long-looped variety, thin limb segments compose part of both the descending and ascending limbs. Four types of thin limb segments are routinely identified: (a) descending thin limbs of short-looped nephrons (SDTL) (Figs. 1-23–1-25), (b) upper portions of descending thin limbs of long-looped nephrons (LDTL up), (c) lower portions of descending thin limbs of long-looped nephrons (LDTL lp), and (d) ascending thin limbs of long-looped nephrons (ATL) (Figs. 1-24; 1-25). This pattern has been observed in rat (170,171), mouse (172), syric hamster (173), the desert rodent *Psammomys obesus* (174,175), and the rabbit (148). Thin limbs have not been studied as carefully in humans as in several other animal species (13,176,177). An additional subsegment of thin limbs has been identified in chinchillas (178).

Surprisingly, as seen by light microscopy, these simple looking epithelia are strikingly different from each other—not only the ascending from the descending limbs but, most remarkably, the descending limbs of short from those of long loops. Furthermore, within the descending segments, the proximal portion, although structurally not different from the distal portion, express a different pattern of transporters than the distal portion. Even islets or interposed limb pieces of functionally different cells are encountered within an otherwise homogenous thin limb segment (179,180). Beyond all these heterogeneities, there are prominent differences among species. This situation may explain the doggedly persistent discussion about the integrated function of thin limbs in the urine-concentrating process; a generally accepted concept of how the final concentration of the urine in the inner medulla occurs is lacking.

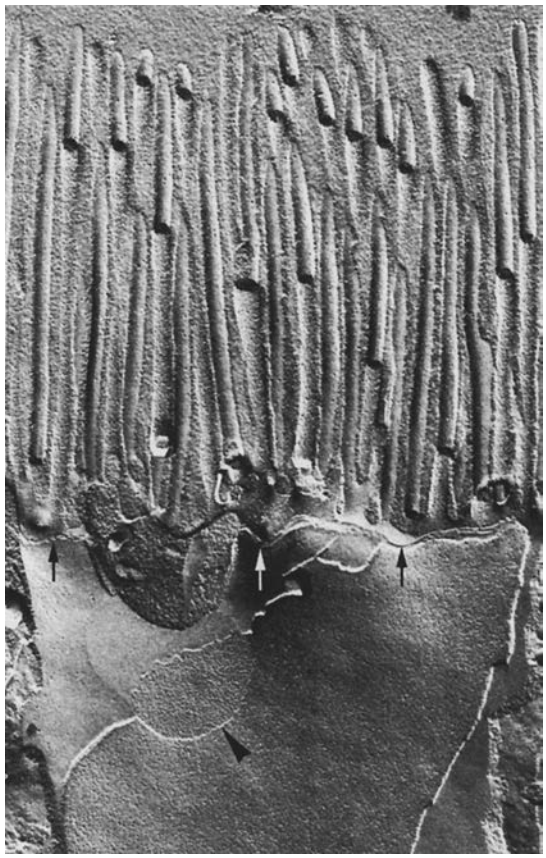


FIGURE 1-21. Freeze-fracture electron micrograph of the apical part of proximal tubule cells (rabbit) showing the shallow tight junction (*arrows*) and a gap junction (*arrowhead*). (Magnification ×37,000.) (From: Kriz W, Kaissling B, Schiller A, et al. Morphologische merkmale transportie-render epithelien. *Klin. Wochenschr* 1979;57:967, with permission.)

The type-1 epithelium lining (SDTL) is composed of flat, noninterdigitating cells, joined by tight junctions that consist of several anastomosing strands. Cell organelles are exceedingly sparse. Functionally, this segment contains aquaporin 1 (AQP1) and the urea transporter UT-A2 in its membranes (181,182,183,184). Thus, it is water and urea permeable. However, these properties are unequally distributed; within the proximal part, the water permeability is high, whereas, within the distal part, the urea permeability is high (181,183,185). In species with complex vascular bundles (rat, mouse a.o.), the SDTLs lie within vascular bundles (177); in these surroundings, the thin limbs are in an ideal position to recycle urea from the ascending vasa recta into the short-loop nephrons.

The descending limbs of long loops (LDTL) are generally much larger in diameter and have a thicker epithelium than those of short loops (Fig. 1-25). Moreover, these thin limbs segments are heterogenous; those of the longest long loops begin in the inner stripe as a much thicker tubule than those of shorter long loops. The character of the epithelium gradually changes as the limbs descend toward and into the inner medulla. The subdivision of the long descending thin limbs into an upper part (type-2 epithelium) and a lower part (type-3 epithelium) is an approximation and reflects the gradual change between the two epithelia. Moreover, this process of epithelial transformation appears to be related to the length of each loop. It occurs earlier and more quickly in short long loops and is delayed in the longest long loops (148,149,171,172,177,179,186).

Furthermore, considerable interspecies differences, particularly prominent in type 2 epithelia, complicate the situation. Two patterns of type-2 epithelium may be distinguished (177,187). In one group of species (mouse, rat, *Psamomys* a.o.) (149,174,175,188,189) the type-2 epithelium is characterized by an extremely high degree of cellular interdigitation and a shallow tight junction consisting of only one junctional strand pointing to prominent paracellular transports. In addition, the epithelium has numerous apical microvilli, considerable numbers of mitochondria, and exhibits a high expression of Na^+-K^+ ATPase (190,191). In a second group of species (rabbit, minipig, and, possibly, man) (192,193), the type-2 epithelium is much more simply organized. The prominent paracellular pathway is lacking; the cells do not interdigitate and are joined by deep tight junctions. In other respects, however, the epithelia are similar in the two groups: numerous luminal microvilli, many mitochondria, and a dense assembly of intramembrane particles in luminal and basolateral membranes are present in both groups. The high density of intramembrane particles may, at least, be partially due to the high density of aquapotin1 (AQP1) channels in both membranes; corresponding to the decrease of particle density along its descending course. The density of AQP1 channels also decreases and finally terminates completely (185,194,195). Since structurally a clear cut border between the upper and the lower segment of the LDTL cannot be defined, it may be reasonable to discriminate both segments by the absence of AQP1 in the lower segments (179,185,194).

Type-3 epithelium (found in LDTL lp) is comparably simple; interspecies differences are no longer prominent. The epithelium consists of flat, noninterdigitating cells, joined by tight junctions of intermediate apico-basal depth (171); it

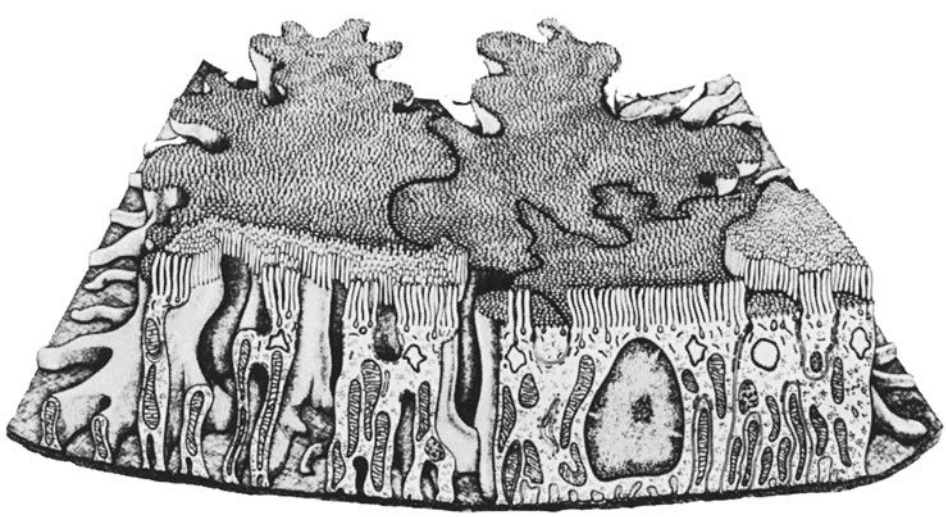


FIGURE 1-22. Diagram of a proximal convoluted tubular cell showing the elaborate shape of these cells. Some interdigitating processes extend the full height of the cells. The apical and basal cytoplasmic regions also have smaller, more elaborate interdigitating processes. (From: Bulger RE. The shape of rat kidney tubular cells. *Am J Anal* 1965;116:237, with permission.)

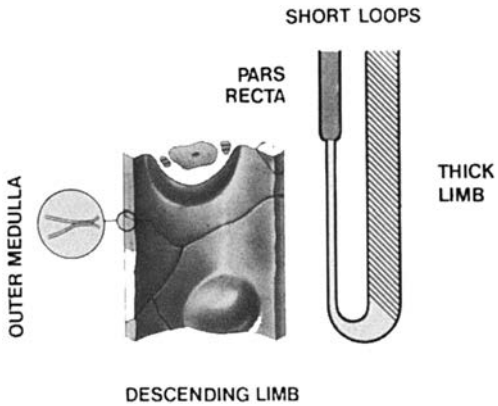


FIGURE 1-23. Diagrammatic representation of thin limb structure in short loops of Henle showing the shapes of constituent cells and the morphology of the occluding junctions (*inset*). (From: Schwartz MM, Venkatachalam MA. Structural differences in thin limbs of Henle: physiological implications. *Kidney Int* 1974;6:193, with permission.)

lacks AQP1 and may, accordingly, have a very low water permeability. Regarding the permeability to urea and the distribution of the urea transporter, UT-A, conflicting data are published, especially when comparing data from different species (180,181,194,196–198).

The ascending thin limb (ATL) is present only in long-loop nephrons and is uniformly organized among mammals. Generally the transition from the type-3 epithelium of the DTL to the type-4 epithelium of the ATL occurs in a short, but fairly constant, distance before the bend [("prebend segment"; (170,175,179)]. Therefore, functionally, the entire bend should be regarded as part of the ATL. The type-4 epithelium is characterized by very flat but heavily interdigitating cells joined by shallow tight junctions, consisting of only one, but prominent, junctional strand. This leaky organization of the paracellular pathways correspond with functional studies (199,200), which all demonstrate that the ATLs are highly permeable for ions.

The change from the type-3 epitheliumto the type-4 epithelium coincides with the full disappearance of the urea transporter UT-A and the abrupt beginning of the expression of the chloride channel ClC-K1 (179,194); aquaporins are also lacking. Thus, the ATL is water and urea impermeable, but highly permeable for Cl^- and also Na^+.

In humans (201,202) dogs (151), and minipigs (193), a gradual transition is seen from the thin limb to the ascending thick limb; however, an abrupt transition is seen in most other species (148,203,204).

The Distal Tubule Overview

The distal tubule of mammalian kidneys has been defined in many and conflicting ways. For morphologists, the distal tubule is divided into several serial segments with differing locations in the kidney and of varying ultrastructural patterns (205) (Fig. 1-2). The first morphologic segment of the distal tubule for long-looped nephrons begins at the boundary between the inner zone of the medulla and the inner stripe of the outer zone of the medulla. At this boundary, the cells lining the ascending thin limbs of long-looped nephrons increase in height, forming the medullary thick ascending limb (MTAL). (The MTAL of short-looped nephrons are encompassed within the inner stripe of the outer zone in which the descending thin limb of short-looped nephrons converts into a descending thick limb, which then makes a hairpin turn and ascends as an MTAL.) The MTAL limb traverses the inner stripe of the outer zone of the medulla and continues toward the cortex through the outer stripe of the outer zone of the medulla. The MTAL then enters the cortex, ascending within the medullary ray as the cortical thick ascending limb (CTAL). These two segments, the MTAL and CTAL are also called the straight part of the distal tubule. The CTAL then leaves the medullary ray and enters the pars convoluta of the cortex, running between the afferent and efferent arteriole of the renal corpuscle from which that tubule was derived. A plaque of taller, but narrower, cells is found in the wall of the CTAL in this region; this plaque of cells forms the macula densa (JGA). In humans, the cells of the MTAL are

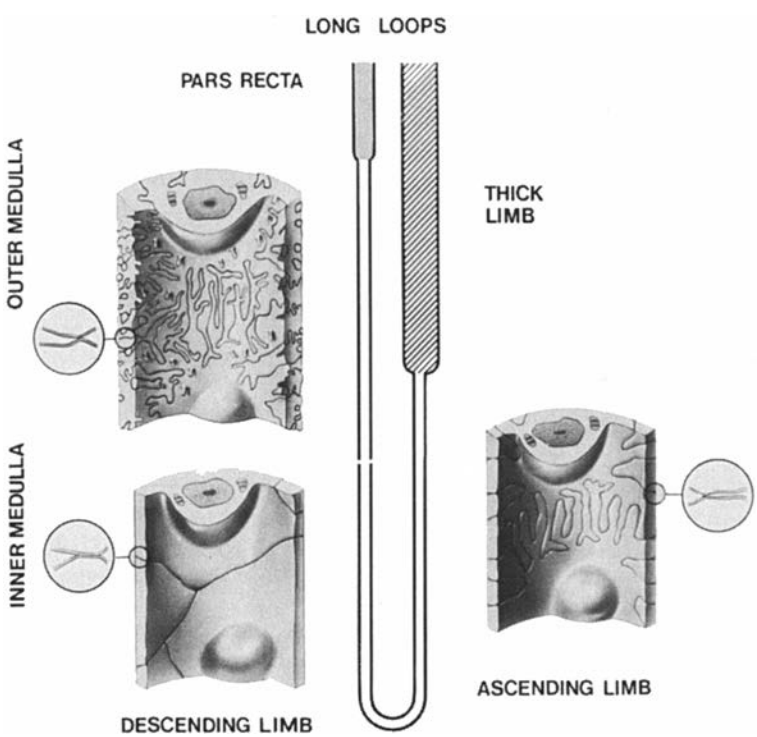


FIGURE 1-24. Diagrammatic representation of thin limb structure in long loops of Henle, showing the shapes of constituent cells and the morphology of the occluding junctions (*inset*). (From: Schwartz MM, Venkatachalam MA. Structural differences in thin limbs of Henle: physiologic implications. *Kidney Int* 1974;6:193, with permission.)

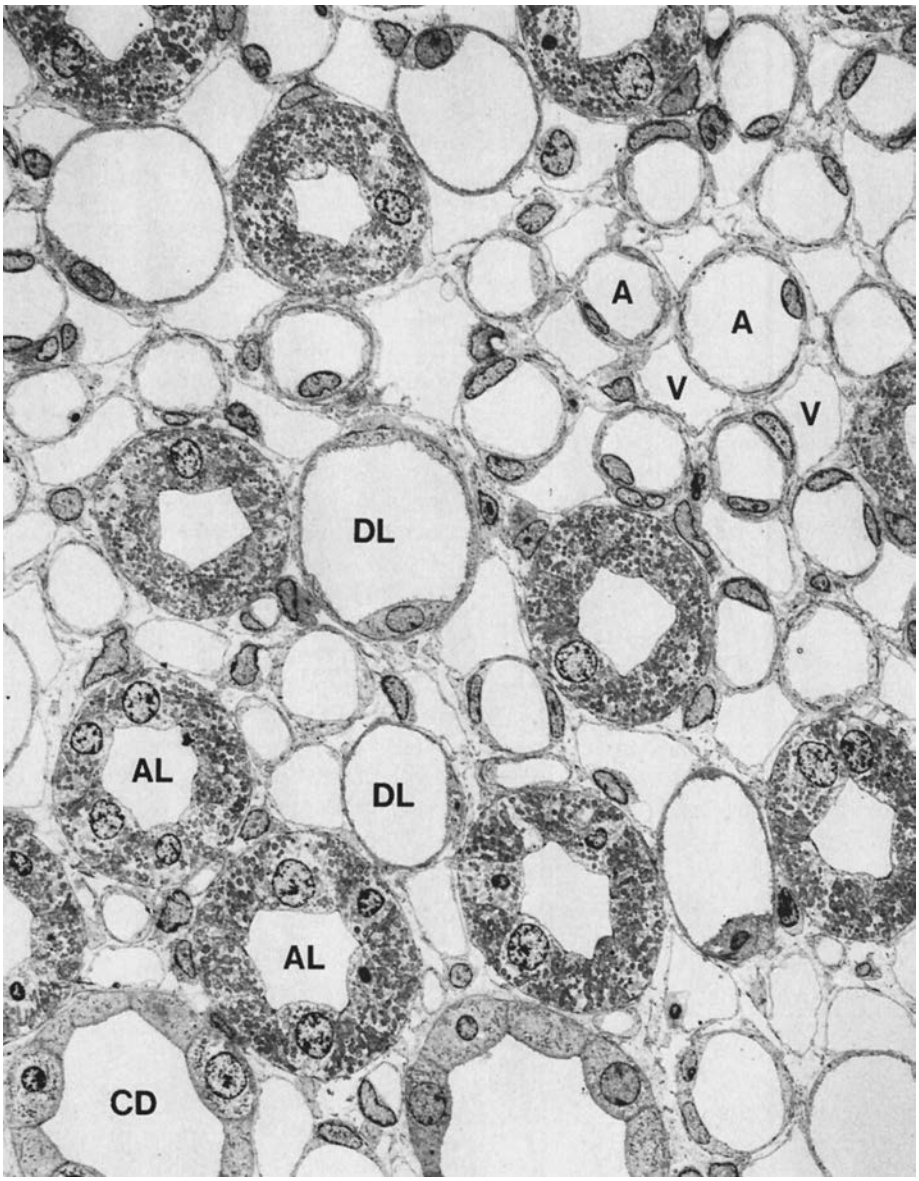


FIGURE 1-25. Low-power electron micrograph of a cross section through the inner stripe (rabbit). A vascular bundle with arterial (*A*) and venous (*V*) vasa recta is surrounded by descending thin limbs (*DL*; the small profiles belong to short loops and the large profiles to long loops), and thick ascending limbs (*AL*) and collecting ducts (*CD*); note the dense pattern of capillaries between the tubules. (Magnification ×900.)

not as elaborately shaped as those described in the majority of laboratory animals that have been studied (202).

After a short post macula densa region of the CTAL, the distal tubule becomes more convoluted and it is now lined by epithelial cells that increase in height from the CTAL, which forms the distal convoluted tubule (DCT) (Fig. 1-27) (204,206). In rabbits, the DCT contains one type of cells known as distal convoluted tubular cells (148). In this species, the DCT is abruptly replaced by the connecting tubule (CNT). In other species, such as rats and mice (207), humans (208), or minipigs (193), the transition is more gradual with intermixing of cells so that in the late distal convoluted tubule of these species connecting tubule cells and intercalated cells (IC) begin to dominate, although DCT cells and principal cells of the collecting duct also can be identified. This segment is then defined as the CNT. The CNT of superficial nephrons begin as unbranched segments, each emptying into a collecting duct (some investigators call the first part of the collecting duct the initial collecting duct). In midcortical and juxtamedullary nephrons, the change from the DCT to the CNT occurs a few cells before the fusion of the two tubules (148). These CNT segments join to form branching connecting tubule segments called *arcades*, which arch upward in the cortex and then empty into a CCD (7). The number of each type of CNT architecture varies with the species.

Medullary Thick Ascending Limb

The MTAL is lined by cells of one type that has extensive basal interdigitating processes filling the basal three-fourths of the cytoplasm (see Fig. 1-26). The larger cell processes contain large mitochondria that are elongated on an axis perpendicular to the tubular basement membrane. In addition to their normal contents, the mitochondria contain prominent intramitochondrial granules and occasional filamentous bodies (209). The epithelium lining the MTAL is approximately 7 μm in height (203,210).

The apical surface of the MTAL cells does not have an elaborate shape in rabbits (148); it is more elaborate in rats (211). Tisher and associates (203,206,210), using scanning electron microscopy, have described two surface configurations of the MTAL cells, with variations in apical cell outlines and in the number of apical microplications. Some cells have smooth apical surfaces with a few microprojections, whereas others have a rough surface with numerous microprojections. The

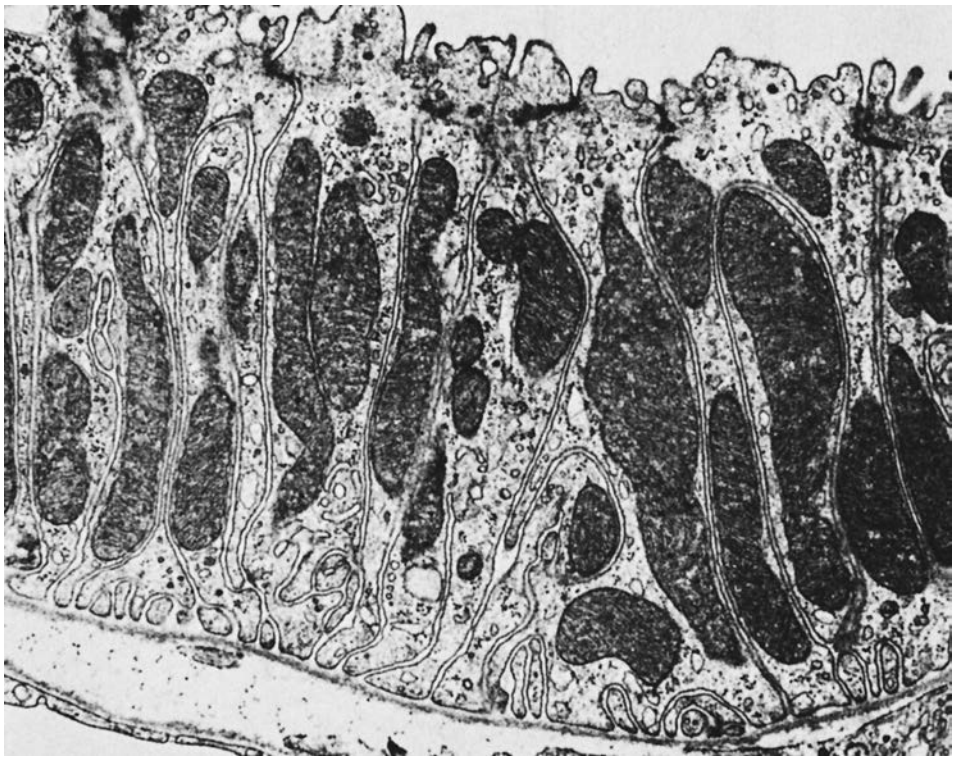


FIGURE 1-26. Transmission electron micrograph of the pars recta of the distal tubule from a rat showing the interdigitating cellular processes. (Magnification ×15,000.)

MTAL cells have both extensive invaginations of the basolateral plasma membrane, as well as a large lateral interdigitating process of adjacent cells (210). Since a main function of the TAL is the reabsorption of sodium and chloride from the tubular lumen to the interstitium, these basolateral processes have Na^+-K^+-ATPase activity (212). In addition, they have an apical bumetanide-sensitive sodium-potassium-2 chloride cotransporter called NKCC2 located in the apical membrane (see the discussion of the membrane amplification principle later in this chapter). The MTAL demonstrates a low permeability for water so the active transport of the sodium ions out of the basolateral membranes of the cells in exchange for potassium ions participates in forming the hypertonicity of the medullary interstitium. The apical cytoplasm contains a variable number of vesicles and a prominent Golgi apparatus. Cisternae of rough-surfaced endoplasmic reticulum can be seen throughout the cytoplasm. The tight junction is of low to intermediate apical–basal depth, consisting of several parallel

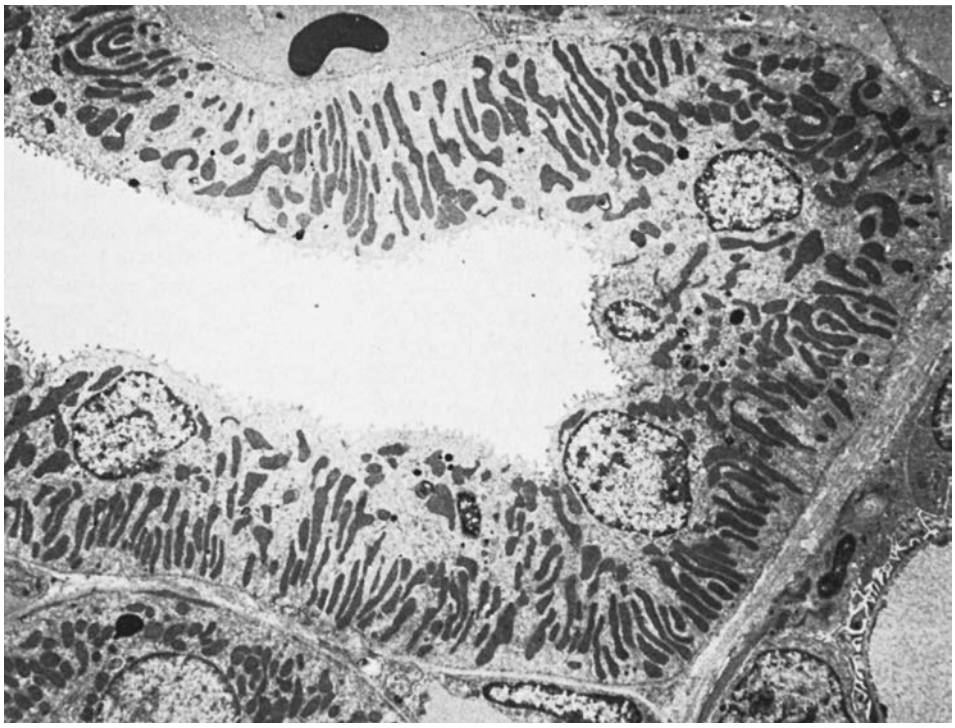


FIGURE 1-27. Transmission electron micrograph from a rat distal convoluted tubule showing the numerous mitochondria within interdigitating processes. (Magnification ×2 ,850.)

strands (213). These tight junctions appear to be related to the relative impermeability to water (see discussion later in this chapter of tight junction structure and their role in transepithelial solute and water transport). As the MTAL traverses the outer stripe in rats, it decreases in cell height, but retains its prominent lateral interdigitations. In rabbits, the apical surface becomes more elaborate in this region (148).

The administration of vasopressin (antidiuretic hormone) to rats that have hereditary diabetes insipidus causes hypertrophy of the MTAL in Brattleboro rats (214). This effect can also be shown by water restriction in normal rats (215) and after high protein intake (216,217).

Cortical Thick Ascending Limb

The cells of the CTAL are lower in height than those of the MTAL in the rat, measuring about 5 μm in height (211) and 2 μm in the rabbit (2). The cells still have prominent basolateral interdigitating cell projections, which increase the basolateral cell membrane approximately 10-fold over the apical or basal cell surface (218). These interdigitating processes are more prominent in a circumferential direction (218). Mitochondria are still prominent in the basolateral cell processes, but somewhat smaller than those of the MTAL. The apical cell border becomes more tortuous in this segment, having more prominent invaginations of the entire lateral cell margins, including the apical region of the cell (148), and has more microvilli along its surface (203). Tamm–Horsfall glycoprotein has been identified covering the plasma membrane in the TAL of rat (219,220).

The MTAL and CTAL differ from each other physiologically, especially in hormone responsiveness (221,222). The MTAL has a high density of Na^+-K^+-ATPase (223). Hebert et al. (212) have shown in mice that antidiuretic hormone (ADH) increases the transepithelial voltage and net chloride reabsorption in the medullary, but not the cortical, region of the ascending thick segment. The ascending thick region functions in flow-dependent absorption of NaCl mediated by the furosemide (bumetanide) -sensitive cotransport NKCC2 (224,225). Nielsen et al. (226) and Obermuller et al. (227) using immunohistochemistry, have demonstrated bumetanide-sensitive Na-K-2Cl labeling in the apical plasma membrane and the subapical intracellular vesicles of MTAL and CTAL in the rat and rabbit. The apical plasma membrane of the macula densa region also had distinct labeling consistent with a role in tubuloglomerular feedback.

The TAL in the inner stripe has a greater reabsorptive capacity for NaCl than the cortical segment (228), but the cortical segment can maintain a higher concentration gradient (229) (see the discussion of Na^+-K^+-ATPase and the basolateral membrane area later in this chapter). The sodium chloride reabsorbed by the TAL contributes to the hypertonicity of the medullary interstitium. As the CTAL ascends toward the renal corpuscle from which it was derived, there is an increase in apical plasma membrane surface area, both by an increase in apical microvilli and by an increase in lateral cell margins (211).

The MD region is formed by a plaque of cells in the wall of the CTAL (see Fig. 1-35), where the ascending thick tubule runs between the afferent and efferent arterioles of the renal corpuscle from which the tubule is derived. A group of extraglomerular mesangial cells (lacis cells, Polkissen cells) fills the cone-shaped area formed by those three structures (230). These four elements, the afferent and efferent arterioles, the extraglomerular mesangium, and the macula densa constitute the juxtaglomerular apparatus, which will be discussed separately in more detail later in this chapter.

Distal Convoluted Tubule

The DCT (distal pars convoluta) is shorter than the proximal convolution, being about 1.2 mm in length in the rat (207). Therefore, fewer profiles are seen in sections of the renal cortex. The DCT begins with a rather marked increase in the height of the lining cells from those of the TAL, although the cells are similar to those lining the TAL. In the rabbit, DCT cells are 3 to 4 times taller in the DCT than the cells lining the CTAL (204). The DCT tubule has a variable diameter and contains one type of cell with more nuclear profiles than are seen in the proximal convoluted tubule (Fig. 1-27). The DCT extends from the CTAL to the CNT. The cells lining the DCT have short, bulbous luminal microvilli, but no regular brush border. The microvilli are more numerous than seen on the TAL cells, the CNT cells, or the principal cells of the CCD (231). In humans, small lipid droplets are seen in the cytoplasm (202). The endocytic apparatus is not well developed, but some vacuoles and numerous small vesicles are seen in the apical region. Dorup (231) describes four types of vesicles in these cells: intermediate vesicles (80 to200 μm), which are generally uncoated; small vesicles with a mean diameter of 50 μm that are continuous with these intermediate vesicles; large vesicles (>200 μm); and tubular profiles (these being less frequent in DCT cells). Some rough-surfaced endoplasmic reticulum and lysosomes are also present in this segment. The cell nuclei occupy an apical position, because the basal two thirds of the cytoplasm is filled with extensive lateral interdigitating processes surrounded by basolateral cell membranes. These basolateral processes are filled with large elongated mitochondria that have their longitudinal axis perpendicular to the basement membrane. The presence of the large mitochondria lying adjacent to the basolateral cell membranes is consistent with the need for adenosine triphosphate (ATP) for the continued active reabsorption of solute that occurs in this tubule (see Fig. 1-27). The volume of mitochondria in the DCT cells is larger than in the CNT cells or cortical collecting duct (CCT) cells (231). An increase delivery of sodium to the DCT brings about an increase in the volume of the cell, in the mitochondrial volume, and in the proliferation of the basolateral membranes in the cells of the DCT, as well as in the CNT cells and the principal cells of the collecting ducts (232–234).

The apical membrane of the DCT cells has numerous small microprojections. The tight junctions between the cells of the distal tubule are elaborate and are composed of multiple lines of membrane fusion (235), a characteristic that correlates with the ability of the cells to maintain a large electrochemical gradient. The DCT reabsorbs NaCl against a steep chemical gradient and, as expected for such a sodium-absorbing epithelium, contains abundant Na^+-K^+-ATPase on its basal lateral cell membrane (223,236,237). DCT proliferation and basolateral membrane amplification occur when active sodium reabsorption increases (238,239). Kaissling et al. (240) increased the NaCl load to this segment by administering furosemide and demonstrated a marked adaptive increase in the basolateral membrane in the DCT.

In rabbits, an abrupt transition is seen from the distal tubule to the connecting piece of the nephrons (148). The situation is less clear in other species, in which the transition does not appear to be either as abrupt or as completely studied. Recent studies by Biner et al. (241) used immunohistochemistry to localize the various transport systems along the human cortical distal nephron. The DCT demonstrates luminal thiazide-sensitive sodium-chloride cotransporter (NCC). The NCC overlaps with epithelial sodium chloride channels (ENaC) for a short region at the end of the DCT. IC were interspersed among the DCT cells near the end of the DCT. (For more detailed information, see recent reviews from the groups of Kaissling [241,242] and Bachmann [243]).

The Connecting Tubule

The CNT forms the next region of the distal nephron and lies between the DCT and the collecting duct system. At the present time, the CNT may be best classified as part of the DCT, since it appears to be derived from the metanephric blastema (4). Peter (7) believed that the arcades arise from the ureteric bud, while Oliver (5), Potter (244), and, more recently, Neiss (4), all believe that the metanephric blastema is the correct source. However, Howie et al. (245), using immunohistological methods with substances related to the ABO blood groups, various cytokines, and Tamm–Horsfall protein, found that the ureteric bud and the connecting piece express the same antigens. The morphology of the CNT cells appears to have features of both the DCT cells, such as some degree of lateral interdigitations that contain mitochondria, but also has features of the CCD cells, such as, the presence of more true basal infoldings (Fig. 1-28). Because of the intermixing of DCT cells, CNT cells, IC, and principal cells of the CCD in some species, such as rats and mice, there is a gradual transition from the DCT to the CNT in these species (203,207). Neiss (4) demonstrated that IC (also called dark cells) in the CNT arose from the metanephric blastema, while the IC found in the collecting duct arose from the ureteric bud. Kim et al. (246) using specific antibodies to carbonic anhydrase II, H^+-ATPase, and band-3 protein, demonstrated that IC appeared simultaneously in both the CNT and the medullary collecting duct. These IC differentiated from separate foci, one in the nephron (developing from the metanephric blastema) and one in the collecting duct (developing from the ureteric bud). When first identified, cells with distinct apical staining for H^+-ATPase (presumed to by type A intercalated cells), as well as cells with distinct basolateral H^+-ATPase labeling (presumed to be type B intercalated cells), were observed seemingly being developed from two embryologically distinct parts of the kidney. Some of these IC in certain locations subsequently disappeared (246).

FIGURE 1-28. Light micrograph of the renal cortex (rabbit) showing a connecting tubule composed of connecting tubule cells (*1*) and intercalated cells (*2*). (Magnification ×900.) (From: Kaissling B, Zürich, with permission.)

The CNT of superficial nephrons are short and drain individually into the collecting duct (some classify the point of junction as the initial collecting duct). CNTs of juxtamedullary and some midcortical nephrons generally form arched collecting ducts in many species. The arched duct starts deep within the cortex with the conversion and confluence of several DCT into the arcade, and then ascends, while collecting parts of other nephrons, before the duct turns, and enters a medullary ray. This arched duct results from an early embryonic type of nephron induction (4,7). The number of nephrons that empty directly into the CCD, compared with the number that enter first into an arched tubule, varies with the species (5). Both types occur in humans.

Two types of cells generally line the CNT, although intermixing of these two cell types with cells of other cell types, such as the DCT cells and the principal cells of the CD, are seen in some species. The two main types of cells seen are CNT and IC cells (Fig. 1-28). The CNT cells appear to be characterized mainly by extensive true infoldings of the basal cell membrane, which can extend quite deeply into the cytoplasm. However, basolateral interdigitations have also been described, but are less pronounced than seen in DCT cells (231). In rabbits, the plicated membranes can reach the apical cytoplasm (148). Stanton et al. (234) demonstrated a striking increase in basolateral membranes in the rat CNT and the initial CCD of potassium-adapted animals, indicating that potassium is secreted by the CNT cells, as well as the principal cells of the (initial) collecting duct. In this study, no changes were seen in the IC. This suggests that potassium is secreted by the CNT cell and the principal cell of the initial collecting duct (234,247).

The basolateral membranes of CNT cells are partially separated by mitochondria, which are smaller, more randomly distributed, and less numerous than those found in the DCT. The volume density of mitochondria in rat CNT cells was significantly lower than in the DCT cells (231). This arrangement differs from principal cells of the collecting duct, in which the mitochondria are found mainly above the basal infoldings, not among the basolateral membranes. Microvilli on the apical surface tend to be slender and infrequent. Apical vesicles were about as frequent as seen in the DCT cells (231). Mitochondria, the nucleus, and other cell organelles fill the apical cytoplasm. The CNT cells appear to exist in most species, including rats, mice (202,207), and humans (202).

Biner et al. (241) demonstrated an epithelial sodium channel ENaC along the entire CNT in humans. The major part of the CNT also coexpresses aquaporin 2 with the ENaC. IC were identified interspersed among the CNT cells in the human. Loffing and Kaissling (248) reviewed the transport pathways along several mammalian distal nephrons and showed that ENaC was present along the CNT from the rabbit, rat, mouse, and human. Frindt et al. (249), using patch clamp techniques in rat kidney tubule segments, demonstrated that the CNT could reabsorb sodium at a rate 10 times higher than that of the CCT. Using immunogold labeling and electron microscopy, aquaporin 2 (apical), 3, and 4 (basolateral) were all shown to be colocalized in CNT cells of rat (250).

The CNT cell displays an amplification of basal cell membrane in rats (234) and rabbits (239) in situations in which there is a low Na^+ and high K^+ intake. This effect is axial along the tubule, being greatest at the early segment of the CNT and

decreasing along its length (239). The axial change in structure is paralleled by similar changes in Na^+-K^+-ATPase (251).

The second cell type is the IC (or dark cells). Three types of IC have been described: type A involved with the secretion of protons into the lumen; type B involved with the secretion of bicarbonate into the tubular lumen; and non-A–non-B, which may be able to secrete both protons and bicarbonate into the tubular lumen. IC will be discussed under the collecting ducts, since these cells comprise such an important number of the cells lining the tubule in that region. Electrophysiologic studies in rabbits suggest that approximately 98% of the IC in the connecting tubule are the HCO_3^--secreting Btype (252).

The Collecting Ducts

The collecting ducts extend from the CNT through the medullary rays as CCDs. They then cross the outer and inner stripe of the outer medulla as well as the inner medulla, to empty their contents at the tip of the renal papillae. They include the CCDs (including the initial collecting ducts), the outer medullary collecting ducts (OMCDs), and the inner medullary collecting ducts (IMCDs).

The collecting ducts are the final regulators of fluid and electrolyte balance, playing roles in the handling of Na^+, Cl^-, K^+, and acid and base. Although there has been great emphasis on the role of the collecting ducts as the main controllers of urinary sodium and potassium excretion, Meneton et al. (253) stress the pivotal role played by both the late part of the DCT and the CNT , especially in situations which prevail in our current environment, in which the dietary sodium intake is high and the potassium intake is low. They propose that a large proportion of the aldosterone-regulated sodium reabsorption and potassium secretion occurs before the tubular fluid reaches the collecting duct. The difference between the function of the late DCT and the CNT compared to the collecting duct seems to be more quantitative than qualitative, with a large proportion of the aldosterone-regulated sodium reabsorption and potassium secretion being done in the late DCT and CNT. The collecting duct would function mainly when the requirement for sodium and water conservation is maximal and the upstream segments are overloaded by diet or some genetic defect.

The collecting ducts are lined by two types of cells: collecting duct principal (light) cells and intercalated (dark) cells (Figs. 1-29–1-30). About 30% of CCD cells are IC in rat (254). Kaissling and Kriz (148) estimate that rabbits have 33% IC in the CCD and 50% in the outer medullary collecting duct. About one-third of the cells lining the outer medullary collecting duct in the rat are IC (255). The number of IC decreases as the collecting duct descends into the medulla and are absent below the first portion of the IMCD. The principal cells of the collecting duct gradually change in morphology as they descend toward the papilla. They increase in cell height and have more complex tight junctions, while the amount of basal infoldings and the number of mitochondria decrease. Fusions of the collecting ducts occur in the inner renal medulla to form the large papillary collecting ducts (ducts of Bellini). These large collecting ducts exit at the papillary tip in an area known as the *area cribrosa*.

The principal cells of the collecting duct in the cortex are cuboidal in humans (208) and low cuboidal in rats (see Fig. 1-29). They form the most numerous cell type. They have a simple cell shape, with fairly straight lateral cell borders that have small interlocking projections. Their pale-staining cytoplasm contains a few small, oval, randomly oriented mitochondria and other organelles, and the nucleus is situated in the middle to upper one-half of the cell in the cortex. The luminal surface is generally smooth with a few short microvilli and a single cilium. The basal surface is characterized by true basal infoldings,

FIGURE 1-29. Transmission electron micrograph of the epithelium of a cortical collecting duct (rat) showing a collecting duct cell (principal cell) (*above*) and an intercalated cell (A-type) (*below*). Note the basal infoldings in the collecting duct cell and the apical vesicles in the intercalated cell. (Magnification ×5,000.)

with few lateral interdigitations. The amplification factor of basolateral membranes was significantly lower than in the CNT cells (231). Because these infoldings are short and closely spaced, they do not have mitochondria lying between them. The mitochondria are located mainly above the infoldings and in the apical cytoplasm, which contained few intermediate and small vesicles, tubular profiles and large vesicles (231). The tight junctions are deep (213,256), and the apical surface has a prominent glycocalyx (257).

Conditions that increase potassium secretion, such as potassium adaptation or high endogenous or exogenous mineralocorticoid levels, bring about dramatic increases in these basal cell membrane infoldings (239,252,258,259). For example, striking increases in the basolateral membranes of the

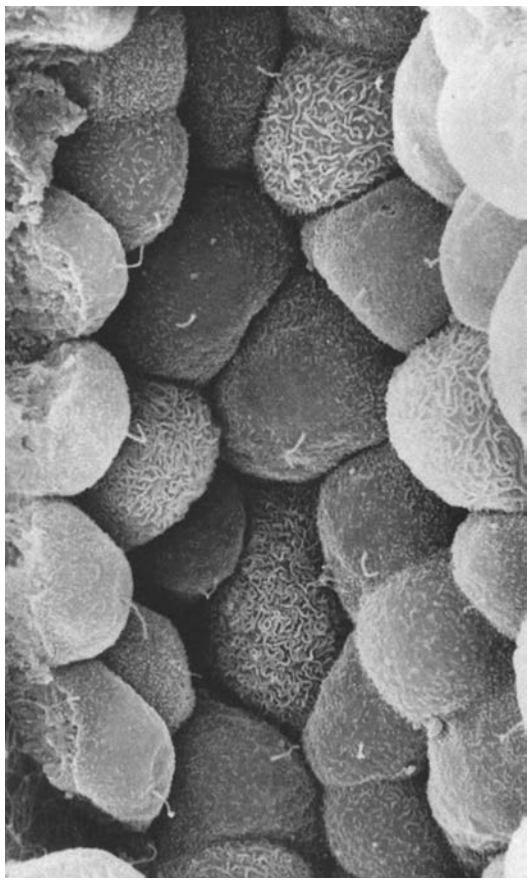


FIGURE 1-30. Scanning electron micrograph of a cortical collecting duct (rat) showing the apical aspect of collecting duct cells (with an apical cilium) and intercalated cells (with apical microfolds). (Magnification ×2,400.)

principal cells of the initial segment of the collecting duct have been demonstrated in potassium-adapted animals (234). In addition, the principal cells in rats showed a 35% decrease in the basolateral membranes in adrenalectomized animals, that was restored to control levels by the administration of physiologic amounts of aldosterone, but not of glucocorticoids (260). In addition, increasing the dose of aldosterone over control levels caused an increase in the basolateral membranes by 111% compared with controls. They did not note changes in the luminal membranes of the principal cells (260). These studies provide evidence that principal cells in the collecting duct are involved with potassium secretion.

The collecting duct responds to ADH by an increase in water permeability. The principal cells are vasopressin sensitive and show dramatic increases in water permeability of the apical membrane. Sun et al. (261) have demonstrated direct evidence that AQP2 on principal cells is located in clathrin-coated pits that recycle between the plasma membrane and intracellular vesicles in response to the availability of ADH. The mechanisms underlying these changes are discussed in detail later in this chapter.

Biner et al. (241) using immunohistochemical localization techniques on human kidney, demonstrated the presence of an amiloride-sensitive epithelial sodium channel (ENaC) and AQP2 activity on collecting duct principal cells. Loffing et al. (262) using immunohistochemistry in rabbit kidney cortex, demonstrated that ENaC is found in the CNT cells and the CCD cells. The ENaC shifted from the apical membrane in the upstream CNT cells to a cytoplasmic location downstream in the CNT and CCD cells. In the rabbit, the AQP2 was seen only on the CCD cells. The apical membranes of the collecting duct principal cells of human contain ENaC and AQP2, while the basal membranes contain Na^+-K^+-ATPase and aquaporins 3 (AQP3) and 4 (AQP4); hence, the CCD plays a critical role in the concentration of urine. The CCD also responds to the mineralocorticoid aldosterone (263).

The collecting duct cells undergoes gradual, although considerable, changes from the cortex downstream to the upper one-third of the inner medulla (148,208). The cells of the outer medulla include principal cells similar to those seen in the cortex, however, the cells become taller with decreasing concentrations of several cellular organelles and basal infoldings. IC similar to type A are found in the outer medulla. From the deeper cortical levels downward into the inner medullary region, the basal labyrinth of the principal cells continues to decrease gradually, with a steeper reduction within the outer stripe (148,205,208). The number of mitochondria also continues to decrease, whereas lysosomal elements and apical-coated vesicles seem to increase. The density of the cytoskeletal network lying under the apical cell membrane becomes more prominent and the tight junctional belt becomes deeper (148).

From the second one-third of the inner medulla, collecting duct cell size increases steeply. These tall IMCD cells are distinct from collecting duct cells upstream according to several criteria (203,210,264) (Fig. 1-31). Their luminal membrane is covered by numerous stubby microvilli and lacks the central cilium. The lateral intercellular spaces are more extensively developed and are prominent by their dense assembly of microvilli and microfolds projecting from the lateral cell membranes. A prominent feature of principal cells of the last one-third of IMCDs is the expression of the ADH-sensitive urea transporter UT-A1 (183,265). In most other respects, IMCD cells resemble the other collecting duct cells. The morphology of the various regions of the renal tubule are summarized in Figure 1-33.

Intercalated Cells

Intercalated (IC) cells have long been identified in CCDs in a variety of species such as rats (203,207,254,266–270), mice (254,271), humans (208,241), and rabbits (148,238,272–275) (Figs. 1-29; 1-32). It has become obvious that IC are not only found in the CCD but, depending on the species, in the latter region of the DCT in humans (208,241,270) and rat (207,231,255,269), in the connecting tubule in rabbits (148,238) and humans (241), in the CCD (as listed above), in the outer medullary collecting ducts in rats (255), and in the upper part of the inner medullary cells in rats and rabbits (148). IC constitute about 37 to 40% of the cells in CCD in rats and rabbits (255,271,275). Kaissling and Kriz (148) estimate that rabbits have 33% IC in the CCD and 50% in the outer medullary collecting duct, while Hansen et al. (255) estimate that 36% to 40% IC are present in the outer medullary collecting ducts in rat. IC show a slow decrease in the upper part of the IMCD in rats (275).

IC cells (Fig. 1-32) are sometimes called dark cells because their cytoplasm stains more densely. They exhibit significant structural heterogeneity even within a single segment of the collecting duct. IC can also be distinguished from principal cells by differences in cell shape, cytochemical staining, and uptake of the pH-sensitive fluoroprobe, 2′,7′-bis(carboxyethyl)—5,6-carboxyfluorescein (BCECF). IC have a more circular, rather than hexagonal, profile that bulges into the tubule lumen when observed in isolated perfused tubules by means of interference-contrast optics. In rabbits, IC can be identified by positive staining with peanut lectin (276,277) and by luminal uptake of acetoxymethyl BCECF (277). The apical surface of the intercalated cell is frequently adorned by luminal extensions, which

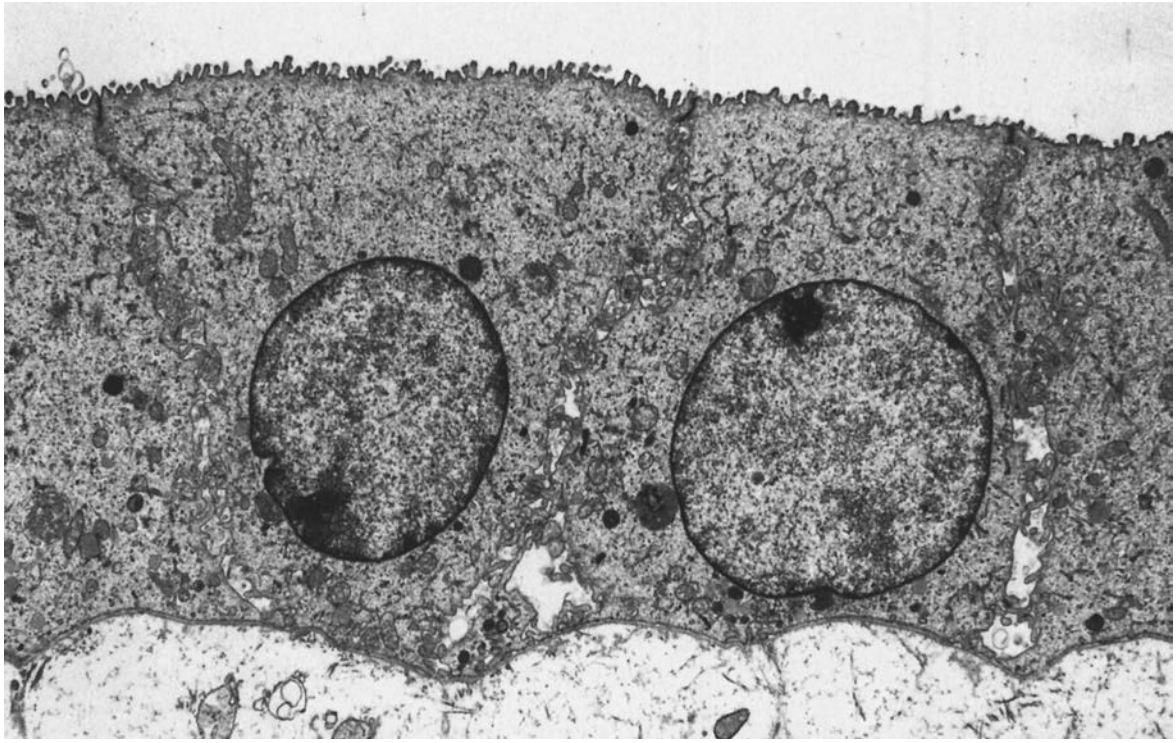


FIGURE 1-31. Transmission electron micrograph of the inner medullary collecting duct epithelium (rat) showing the high inner medullary collecting duct cells with many stubby microvilli of the luminal membrane and prominent lateral intercellular spaces filled with lateral microfolds. (Magnification ×15,500.)

include microvilli and microridges (called *microplicae*). Basal membrane infoldings resemble those of the collecting duct cell.

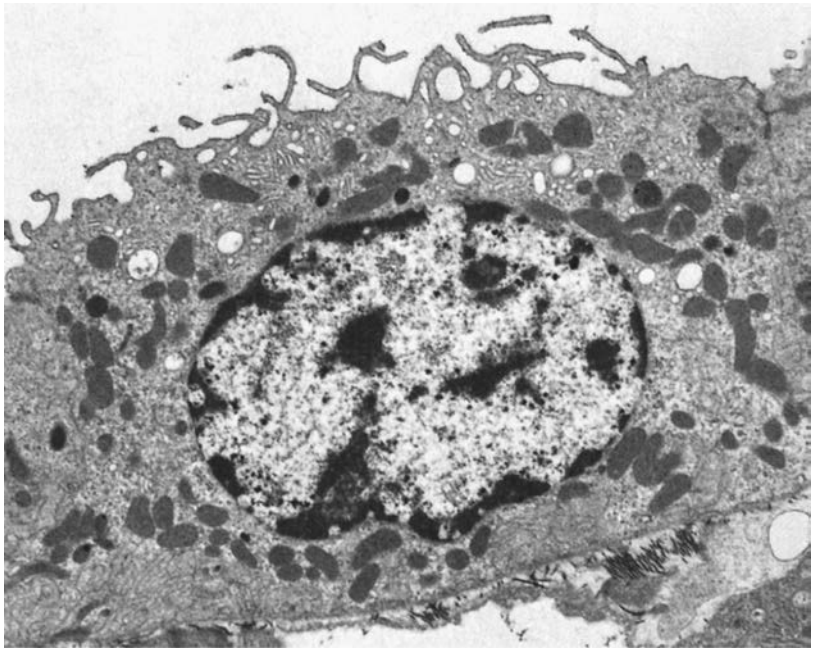
Three types of IC have been defined morphologically and by immunohistochemical staining, including: type A, type B, and non-A–non-B (254,271). However, there is a striking difference in the number and distribution of the various types of IC from animal to animal and among the nephron segments of a particular species (254). Carbonic anhydrase II immunoreactivity was seen in all IC , but type A stained more intensely than type B. The immunostaining in type-A cells was pronounced in the apical cytoplasm and apical microprojections. In type-B cells, the staining was more diffuse throughout the cytoplasm. In the non-A–non-B cells, the staining was also diffuse (268).

Type-A IC tend to have a more circular apical cell profile (269), a centrally placed nucleus, prominent apical microvilli and microplicae extending from the apical plasma membrane, and prominent apical cytoplasmic tubulovesicular profiles (see Fig. 1-32A). Mitochondria are numerous and can be located above the nucleus, as well as between the nucleus and the basal membrane infoldings. Using freeze-fracture techniques, there are rod-shaped particles and studs present on the cytoplasmic face of the apical plasmalemma and on the tubulovesicular profiles in the IC of the CCD and the outer medullary collecting ducts (271,278,279). H^+-ATPase is expressed on the apical plasma membrane and in the cytoplasmic tubulovesicular and vesicular profiles of type-A IC. (See review of renal vacuolar H^+-ATPase [280].)

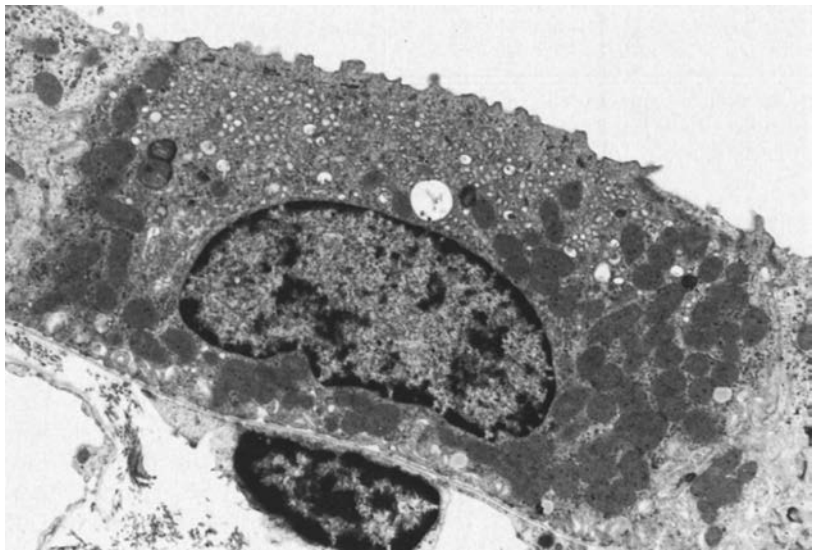
Verlander et al. (269) showed that IC in animals with respiratory acidosis show a striking increase in apical microprojections and tubulovesicular profiles as well as an increase in surface density of the apical plasma membrane. However, no changes were seen with the number of IC. When there was a stimulation of bicarbonate secretion in rats, Verlander and associates (279) documented a withdrawal of the marker for H^+-ATPase from the apical plasma membrane with its storage in apical cytoplasmic vesicles in the IC in both the CCD and the OMCD. H^+-ATPase activity appeared to be inserted into the basal plasma membrane of type-B IC(279).

Luminal and/or tubulovesicular membranes exhibit two specific types of particles. Large club-shaped particles called "studs" have been observed on the cytoplasmic surface of the tubulovesicular structures on the cytoplasmic side of the apical plasma membrane; the rapid-freeze, deep-etch technique shows 10–μm spherical structures composed of multiple subunits and arranged in paracrystalline hexagonal arrays (281). Brown et al. (282) have presented evidence that the studlike material coating the vesicles contains cytoplasmic domains of the proton-pumping H^+-ATPase. On the basis of both morphologic characteristics and immunocytochemistry, the structures appear to be (or related to) the vacuolar-type H^+-ATPase (267,282). In addition, freeze-fracture studies have shown the presence of rod-shaped particles in vesicles and cell surface membranes (283), which may form a component of this H^+-ATPase. Changes in membrane structure have been shown to be related to proton secretion (238,251). When H^+ secretion is stimulated, cytoplasmic vesicles bearing rod-shaped intramembranous particles (IMP) fuse with the apical plasma membrane, inserting IMP's into the membrane.

This supports the idea that type-A IC demonstrate net excretion of protons into the tubular lumen that is accomplished by the vacuolar H^+-ATPase located in the apical plasma membrane and apical tubulovesicular profiles (254,267,271, 279,284,285). For this process, it has been suggested that the hydrogen ions are produced by cytosolic carbonic anhydrase in CNT cells and CCD cells of the mouse and rat (254,268,271,285,286) and human (241,287,288). The bicarbonate that is generated is released by a band 3-like Cl^-/HCO_3^- exchanger AE1, located in the basolateral plasma membrane, into the interstitium at the base of the cell.



A



B

FIGURE 1-32. Transmission electron micrographs of intercalated cells (rat). **A:** Type A exhibiting apical microfolds and flat vesicles in the apical cytoplasm. (Magnification ×6,800.) **B:** Type B showing a rather smooth apical surface, many small round vesicles, and mitochondria located predominantly in the lateral and basal parts of the cell. (Magnification ×6,800.)

Medullary IC from rats fed a diet with a high K^+ content had a small luminal membrane area and a cell apex with numerous vesicles. The ingestion of a low K^+ diet led to an increased luminal membrane area with few apical vesicles (258). It has, therefore, been postulated that IC function in potassium reabsorption.

A similar increase in the apical plasma membrane with a decrease in tubulovesicular profiles was seen in IC of the outer medulla in chronic metabolic acidosis (289) and acute respiratory acidosis in rats (290). In respiratory acidosis, a marked increase in apical microprojections was seen as was an increase in the surface density of the apical membrane of type-A cells. No changes were seen with type-B cells in rat CCD (269). Madsen and Tisher (203) postulated that hydrogen ion pumps located in the apical vesicles had been inserted into the apical cell membrane by vesicle fusion with the apical membrane.

Type-B IC are present in the CCD and the CT of rats (266,268,269,291), mice (254,271), and rabbits (220,272, 273,292). The type-B cell (Fig. 1-32B) has an angular outline (269) and a relatively smooth apical plasma membrane with short sparse microvilli (without rod-shaped studs in the apical membrane) (271). The apical membrane generally lacks studs, but the basolateral membrane exhibits studs (269). The nucleus lies in an eccentric position and the numerous small mitochondria are densely packed and are concentrated at the basal part of the cytoplasm. The cytoplasm and the organelles stain more densely in light and electron microscopy. Cytoplasmic vesicles (mostly noncoated) are seen throughout the cell cytoplasm. In the type-B cells, the H^+-ATPase is expressed in the basolateral plasma membrane and in the cytoplasmic vesicles throughout the cells (267,271,282,291,293–296). Using freeze-fracture techniques, rod-shaped rectangular particles are found on the basal membranes in these cells and not on the apical plasma membrane (267,291). The basal hydrogen ion secretion is thought to mediate HCO_3^- secretion into the luminal fluid by an apical Cl^-/HCO_3^- exchanger different from AE1 (254,273).

One candidate for this anion exchanger is pendrin, a Na^+-independent Cl^-/HCO_3^- exchanger. Quentin et al. (297) have shown that the Cl^-/HCO_3^- exchanger pendrin in rat kidney is

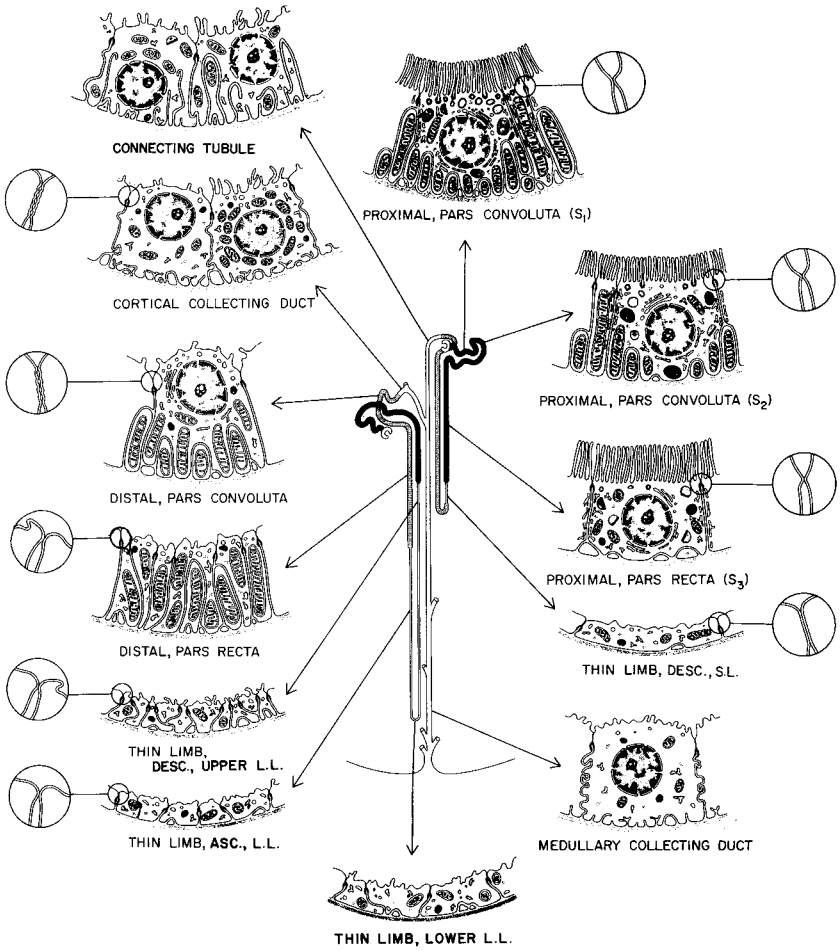


FIGURE 1-33. Summary diagram, showing cells from the various regions of the urinary tubule.

specifically regulated in response to chloride balance independent of sodium and acid/base balance. Using a mouse model, Wall et al. (298) demonstrated that pendrin protein was localized in the apical cytoplasmic vesicles in both type B and non-A–non-B IC from a subset of cells in the DCT, the type-B cells in the CNT, and the CCD. Pendrin mRNA was expressed mainly in the cortex. Pendrin immunoreactivity was highest in the apical cytoplasmic vesicles, although there was little immunogold staining along the apical plasma membrane of type-B IC, but non-A–non-B IC had intense pendrin immunoreactivity along the apical plasma membrane. Kim et al. (299) using immunoelectron microscopy demonstrated pendrin in both the apical plasma membrane and intracellular vesicles throughout the cell. Kim et al. (268) found the carbonic anhydrase activity more diffuse in the cytoplasm of type-B than Type-A cells.

The third type of IC is non-A–non-B cells that have been identified in the CT and the CCD of rats (254,266,268,300) and mice (254,266,271). Non-A–non-B cells have vacuolar type H^+-ATPase in both apical plasma membranes and apical vesicles, but do not have the basolateral band 3-like immunoreactivity of AE1 (254). In rabbits and mice, most non-A–non-B cells in the collecting duct have an electroneutral Na-independent Cl^-/HCO_3^- exchanger in the apical membrane (254). Pendrin immunoreactivity is intense along the apical membrane, as well as being present in apical vesicles in some cells in the DCT, in the CNT, and CT (298). Since both pendrin immunoreactivity and pendrin-mediated HCO_3^- secretion are present in the apical plasma membrane and apical intracellular vesicles in type-B and non-A–non-B IC, Kim et al. (299) suggest that HCO_3^- secretion could be regulated by trafficking of pendrin between the two membraneous compartments. In addition, since non-A–non B cells seem capable of both apical HCO_3^- and H^+ secretion, the simultaneous secretion of both HCO_3^- in exchange for Cl^- and proton secretion mediated by electrogenic H^+-ATPase, would cause chloride reabsorption with no change in acid/base status (299).

The Juxtaglomerular Apparatus

At the vascular pole of the renal corpuscle, the macula densa region of the CTAL comes into close proximity to the efferent and afferent arterioles and a group of cells called the extraglomerular mesangium (301,302). The juxtaglomerular apparatus consists of four parts (Figs. 1-5; 1-34): a plaque of cells in the wall of the CTAL of the distal nephron in the region of the vascular pole of a renal corpuscle, called the "macula densa"; the termination of the afferent arteriole as it enters this renal corpuscle; the initiation of the efferent arteriole as it exits from the same renal corpuscle; and a cone-shaped region of extraglomerular mesangial cells (also called the lacis, Polkissen, or Goomaghtigh cells) lying in the space between the macula densa and the two arterioles. This area receives a rich supply of sympathetic nerve endings.

In the human kidney, 40% of the basal lamina region of the macula densa lies adjacent to the base of the cone-shaped extraglomerular mesanium, 10% of the macula densa basal lamina is in contact with the afferent arteriole, and 5% is in contact with the efferent arteriole (299).

The MD consists of the cells in the wall of the CTAL of the distal tubule, which lies adjacent to the glomerular vascular pole. The macula densa appears as a dense spot upon

FIGURE 1-34. Light micrograph of a renal corpuscle with both urinary and vascular poles in the section. The juxtaglomerular apparatus contains the macula densa (*MD*), the two arterioles (*A*), and the extraglomerular mesangium (between the *A*'s). The urinary pole (*UP*) is also apparent. (Magnification ×13,000.) (From: Dobyan D, with permission.)

hematoxylin and eosin staining, because the cells are narrow, and the nuclei are close together. MD cells (Fig. 1-35) are not interdigitated with each other by large lateral cell processes, as in other regions of the distal tubule. In contrast, the lateral intercellular spaces between MD cells extend very straight in an apical–basal direction; their width appears to vary according to function (303). The mitochondria are shorter and more randomly arranged. The Golgi apparatus lies on the basal side of the nucleus. The basal aspect of the macula densa touches the extraglomerular mesangium. Additional, but variable, contacts are found with the efferent as the afferent arterioles (see review [13]). The most conspicuous difference of MD cells to any other cells of the nephron is the occurrence of nitric oxide synthase I (Fig. 1-37C) (304,305). A prominent feature of the macula densa (MD)is also the expression of cyclooxygenase-2 (306).

Modified smooth muscle cells in the wall of the afferent arterioles, called granular cells (formerly also called juxtaglomerular cells), contain specific membrane-bound granules (Fig. 1-36). *In situ* hybridization (307) and immunocytochemistry (308) have shown that these cells synthesize renin (Fig. 1-37A) that is then stored in granular form (Figs. 1-36B and 1-37B). The granules stain with the Bowie method and have a positive periodic acid-Schiff reaction. Like other smooth muscle cells, the juxtaglomerular cells also contain intracellular filaments and dense bodies, but they have more cisternae of rough-surfaced endoplasmic reticulum, a large Golgi apparatus, and mature and immature secretory granules in their cytoplasm, consistent with the ability of these cells to synthesize small peptides. The immature granules appear to have a paracrystalline structure (71,309). The secretory product is released by exocytosis into the extracellular space within or surrounding the wall of the arteriole (310,311). Processes of the juxtaglomerular cells contact the surrounding cells as well as the endothelial cells by means of gap junctions (312).

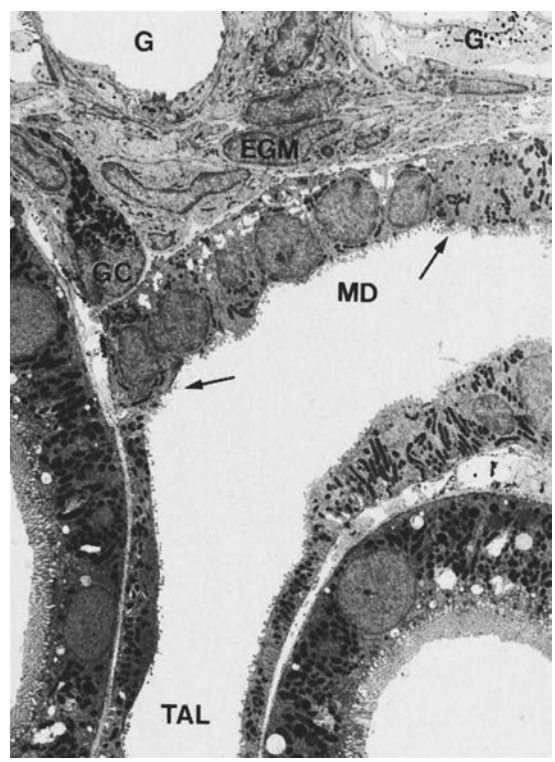


FIGURE 1-35. Transmission electron micrograph of the juxtaglomerular apparatus (rat). The transition of the thick ascending limb (*TAL*) into the macula densa (*MD*) is seen; the basal aspect of the macula densa abuts the extraglomerular mesangium (*EGM*) and also a granular cell (*GC*). *G*, glomerulus. (Magnification ×1,900.)

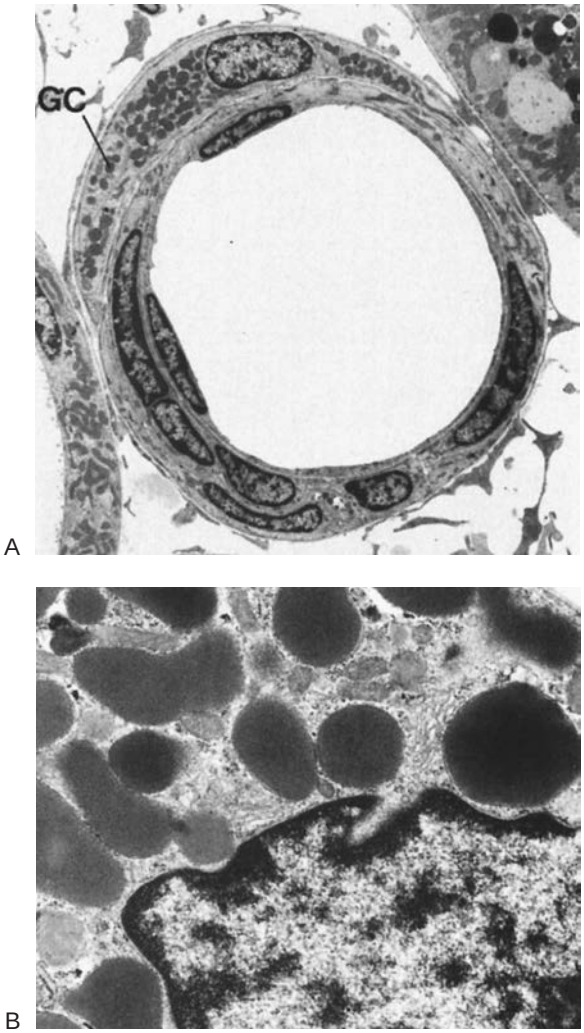


FIGURE 1-36. Transmission electron micrograph (rat). **A:** An afferent arteriole containing in its wall a granular cell (*GC*). (Magnification ×2,100.) **B:** Part of a granular cell containing in its cytoplasm specific membrane-bound granules. (Magnification ×14,500.)

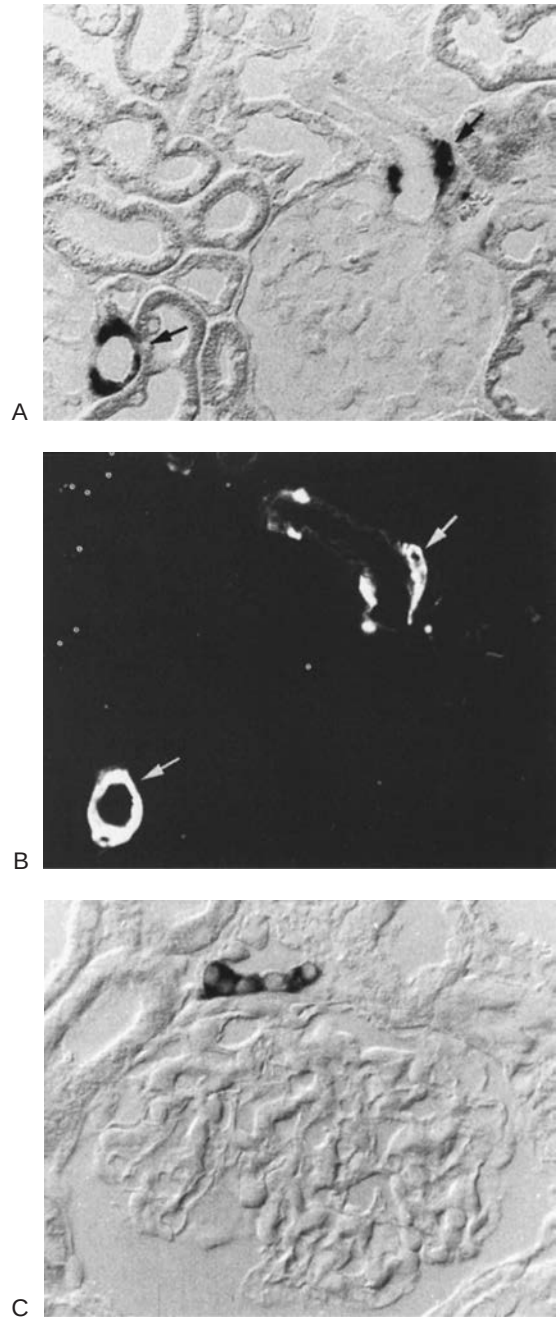


FIGURE 1-37. Rat kidney containing granular cells in afferent arterioles (*arrows*) of two glomeruli. The two pictures show that renin is synthesized (A) and stored (B) in the same cells (same section). **A:** *In situ* hybridization using a 330-bp rat renin riboprobe (*cRNA*) labeled with digoxygenin (detection system: alkaline phosphatase). **B:** Immunocytochemistry using a rabbit polyclonal antirat renin antibody (detection system: Texas red coupled second antibody). (From: Bachmann S, Heidelberg, with permission.) **C:** NADPH diaphorese reaction showing positivity of exclusively macula densa cells reflecting activity of nitric oxide synthase. (From: Bosse H-M, Heidelberg, with permission.)

The extraglomerular mesangium (301) (Goormaghtigh cells, polar cushion, Polkissen cells, and lacis cells) fills the area between the afferent and efferent arterioles and the macula densa (see Figs. 1-5; 1-35). It is composed of nongranulated cells that are continuous with granular cells and smooth muscle cells of the arteriolar walls and with the intraglomerular mesangial cells (301). The extraglomerular mesangial cells are "flatly pressed" cylinders with both ends splitting into a group of parallel processes. These extraglomerular mesangial cells are surrounded by abundant extracellular matrix. Numerous gap junctions occur between the processes of the same cell, with adjacent smooth muscle cells, with granular cells, and at the renal hilus with intraglomerular mesangial cells (230,313,314).

An additional cell type, called the peripolar cell, has been described at the glomerular vascular pole of a wide range of animals, including humans (311); they are regularly found in sheep, but are rare in most other species. They are in a position encircling the origin of the glomerular tuft, with one surface flattened along the capsular basement membrane and the other surface exposed to the urinary space. Thus, they are always separated from "classic" components of the juxtaglomerular apparatus by the glomerular basement membrane; their relevance is unknown. The initmate and systematic juxtaposition of tubular and vascular cells within the JGA has given rise to early speculations about a functional connection in which a signal related to the composition of the tubular fluid at the macula densa affects glomerular vascular tone and the glomerular filtration rate (315). It has now become clear that the JGA serves two different functions: it regulates the flow resistance of afferent arterioles in the so-called tubuloglomerular feedback mechanism and it participates in the control of renin synthesis and

release from granular cells in the afferent arteriole (316). Researchers originally assumed that the two responses might be related to each other in that renin released from the granular cells not only has systematic relevance, but locally triggers the formation of angiotensin II and thus is responsible for the afferent vasoconstriction as well; however, it now appears that the final activation of smooth muscle and granular effector cells occurs through largely independent pathways. Renin release from granular cells is the major source of systemic angiotensin II and thus plays an essential role in controlling extracellular volume and blood pressure, whereas the vasoconstriction of the afferent arteriole locally serves to modulate the filtration of this nephron.

For both mechanisms, it is well established that a change in NaCl concentration in the tubular fluid at the MD causes a graded release of mediators that reach their target by diffusion, thus acting in a paracrine fashion. Note that the extraglomerular mesangium that mediates the contact between the MD and the effector cells is not vascularized, so that the build up of any paracrine agent would not be perturbed by blood flow.

With respect to renin release, the most likely paracrine mediators of this process are prostaglandin E_2 and nitric oxide (305,317,318). With respect to the vasoconstrictor response purinergic mediators,, either ATP or adenosine, as first suggested by Oswald and colleagues in 1980 (319)–appear to play the major role (316,320,321). For an up-to-date discussion of the function of the JGA, see the reviews by Schnermann und Levine (316), Persson and colleagues (322), and Komlosi et al. (323).

Renal Blood Vessels

The renal arteries arise from the lateral region of the abdominal aorta at the level of the first and second lumbar vertebrae. Each artery divides into an anterior and posterior division before traversing the renal hilus. These divisions usually form a total of five segmental branches. The anterior division gives rise to the upper, middle, and lower segmental arteries, whereas the posterior division becomes the posterior segmental artery. The apical segmental artery can arise from either division. The segmental arteries give rise to interlobar arteries within the renal sinus. The interlobar arteries enter the renal columns adjacent to the renal pyramids.

The intrarenal microvaculature has been extensively studied by several groups (10,11,148,324); a basic pattern is established throughout the mammalian kidneys that may be described as follows. At the corticomedullary junction, the interlobar artery branches into several arcuate arteries that arch across the base of the renal pyramid (Fig. 1-38). The arcuate arteries give rise to interlobular arteries (cortical radial arteries) that course peripherally, between the medullary rays, and, thus, within the cortical labyrinth. The interlobular arteries also branch, and the branches give rise to afferent arterioles that supply the renal corpuscles.

Glomerular capillaries are derived from the afferent arteriole, which—strictly at the entrance level—divides into several (two to five) primary capillary branches (325–327).

Each of these branches gives rise to an anastomosing capillary network that runs toward the urinary pole and then turns back toward the vascular pole. Thereby, the glomerular tuft is subdivided into several lobules, each of which contains an afferent and efferent capillary portion. The lobules are not strictly separated from each other; some anastomoses between lobules occur. The capillaries converge to form the more centrally located efferent arteriole, which is already established inside the glomerular tuft. Thus, the efferent arteriole has a significant intraglomerular segment that runs through the glomerular stalk (Fig. 1-5) (327). At this site a mesangial layer surrounds

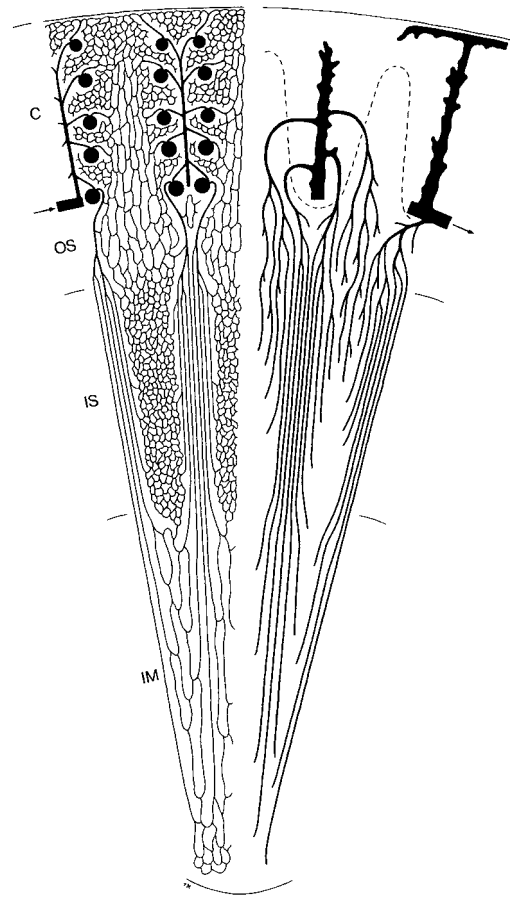


FIGURE 1-38. A basic pattern of renal microvasculature. *C*, cortex; *OS*, outer stripe; *IS*, inner stripe; *IM*, inner medulla. The left panel shows the arterial vessels and capillaries. An arcuate artery (*arrow*) gives rise to cortical radial arteries from which the glomerular afferent arterioles originate. Efferent arterioles of juxtamedullary glomeruli descend into the medulla and divide into the descending vasa recta, which, together with ascending vasa recta, form the vascular bundles. At intervals, descending vasa recta leave the bundles to feed the adjacent capillaries. The right panel shows the venous vessels. The cortical radial veins start within the superficial cortex; in the human kidney, some of them start as stellate veins on the surface of the kidney (shown on the right side). They all drain into arcuate veins. The venous drainage of the medulla is carried out by venous vasa recta; those from the inner medulla all traverse the inner stripe within the vascular bundles, whereas most of the venous vasa recta from the inner stripe ascend outside the bundles. After traversing the outer stripe as wide tortuous channels, the ascending vasa recta drain into arcuate or cortical radial veins. (From: Rollhäuser H, Kriz W. Das Gefäss-system der Rattenniere. *Z Zellforsch* 1964;64:381, with permission.)

the vessel. After leaving the glomerulus, the efferent arteriole is reestablished as a proper arteriole.

The efferent arterioles of superficial (or subcapsular) glomeruli perfuse convoluted tubules through long pathways extending to the kidney surface or through intermediate branches near the renal corpuscle. In the midcortex, the efferents either branch near the glomerulus and perfuse convoluted tubules in that region or extend directly to the long meshed network of the medullary ray. Efferent arterioles from juxtamedullary nephrons extend downward to form the descending vasa recta (Fig. 1-38), and occasional branches to regions between the bundles. The early divisions give rise to capillaries located in the outer stripe of the outer medulla. The descending vasa recta then descend within the vascular bundles to the inner stripe and inner medulla (Fig. 1-39) (10). The medulla is

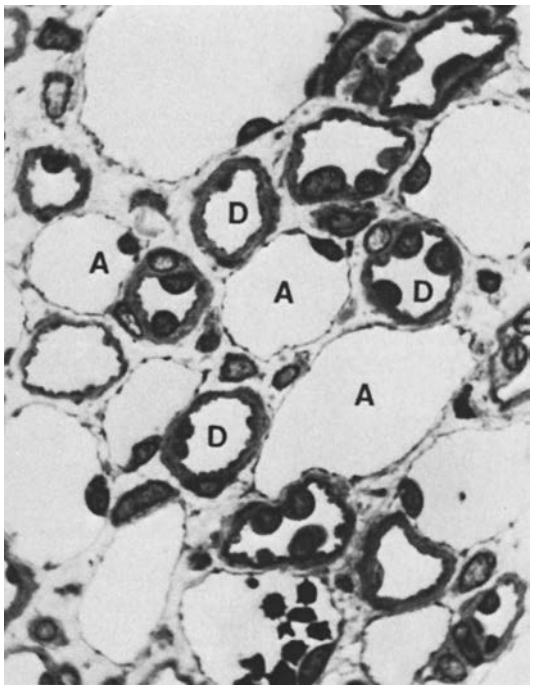


FIGURE 1-39. Light micrograph of a vasa recta bundle, showing descending (*D*) and ascending (*A*) vessels. (Magnification ×950.)

drained by venous (ascending) vasa recta, which lie adjacent to descending vasa recta, forming the vascular bundles that function as a countercurrent exchanger. Venous vasa recta that drain the inner medulla remain in the vascular bundles through the inner and outer stripe region. The venous vessels that drain the region of the upper and middle inner stripe ascend within the interbundle region to the outer stripe, there joining the vessels leaving the bundles to form together the major part of the blood supply to this region by a network of venous vessels. The density of capillaries that are derived directly from efferent arterioles is debated (328). Finally, the ascending vasa recta empty into arcuate or interlobular veins (Fig. 1-38).

The venous system is more irregular. Small venules start near the renal capsule and run centrally as the interlobular veins (cortical radial veins), receiving tributaries from the cortical peritubular capillary network (Fig. 1-38). The interlobular veins empty into arcuate veins lying adjacent to the arcuate arteries. The arcuate veins receive blood from the venous vasa recta, as described in the preceding. Interlobar veins form by confluence of arcuate veins and the latter finally form the renal vein. In contrast to the arcuate arteries, which are terminal arteries, the arcuate veins form true anastomosing arches at the corticomedullary border.

The morphology of the descending and ascending parts of the vasa recta differs markedly (Fig. 1-39) (177,329,330). The descending vasa recta are lined by a continuous nonfenestrated endothelium, with cells oriented longitudinally along the cell axis, forming 10 to 20 cell profiles in a single cross section (177,330). The endothelium contains the urea transporter UT-B1 (183). Pericytes are seen encircling the descending vasa recta, but they become less frequent as the vessels descend into the inner medulla, where these vessels finally convert into capillaries. The capillaries and the ascending vasa recta are lined by a thin fenestrated epithelium. The fenestrae are similar to those seen in peritubular capillaries, as well as in most regions of the body, being 40 to 70 μm in diameter (177) and bridged by a thin diaphragm. Uniquely, fenestrated endothelium can line quite large vessels in the kidney.

Lymphatic vessels are seen only in the cortex accompanying the arteries within the periarterial tissue sheaths (see below). The medulla has no lymphatic drainage (331).

Interstitium

The interstitium of the kidney comprises the extravascular intertubular spaces of the renal parenchyma, with their attendant cellular elements and extracellular substances (332). It is bounded on all sides by tubular and vascular basement membranes. The lymphatics are considered as part of the interstitium.

In functional studies, the interstitial volume of the kidney has been estimated to amount to 13% of the total kidney volume, whereas stereologically derived values for the cell-free interstitial space of the cortex and outer medulla of the rat range between 3% and 5% (333–335). Thus, the functional interstitium includes more than just the peritubular spaces; the prominent periarterial connective tissue sheaths (see the following) may, in fact, account for one-half of the entire interstitial volume (336).

In the *cortex*, the peritubular interstitium is distinguished from the periarterial connective tissue (Fig. 1-40). The *peritubular interstitium* is subdivided into a narrow and wide part (337), the former accounting for 0.6% of the cortical volume and the latter for 3.4%. The narrow interstitium is the space in which the outer surface of a capillary directly abuts a neighboring tubule. This applies to 54% to 67% (337) of the cortical peritubular capillary surface, whereas only 26% of the tubular surface is directly adjacent to peritubular capillaries (338). Thus, most of the exchanges among tubules and vessels have to pass through a wide interstitial compartment.

The *periarterial connective tissue* forms a fluid-rich loose connective tissue sheath that surrounds the intrarenal arteries and contains the lymphatic vessels of the kidney (Figs. 1-40; 1-41) (331,336,339). The periarterial lymphatic sheath extends along the intrarenal arteries as far as the afferent arteriole, where it becomes quite attenuated. It is particularly well developed around the arcuate and cortical radial arteries.

The lymphatic capillaries begin within these sheaths; lymphatics do not, in general, penetrate the renal parenchyma proper and are not found in the medulla (331,340). The lymphatic vessels converge along with the intrarenal arteries to emerge at the renal hilus. The peritubular interstitium of the cortex freely communicates and drains into the periarterial tissue sheaths. Within the sheaths, fluid and solutes gradually may enter the lymphatic vessels as they converge toward the hilus (Fig. 1-41). In addition to lymphatic drainage, the periarterial connective tissue sheaths probably participate in the intrarenal distribution of renin and angiotensin and in the intrarenal movement of lymphocytes, macrophages, and similar cells (339). It also contains the renal nerves.

In the *medulla,* three types of interstitial spaces can be distinguished (332), roughly corresponding to that of the outer stripe/vascular bundle, that of the interbundle region of the inner stripe, and that of the *inner medulla.* The relative interstitial volume exhibits a pronounced axial gradient from cortex to medulla. The outer stripe has a very narrow, sparse interstitium, occupying 3% to 5% of outer stripe volume (341). The interstitial volume of the interbundle region of the inner stripe is somewhat greater (10% in rats). The most distinctive type of regional interstitium is that of the inner medulla. Here, the interstitium constitutes a much larger part of the total tissue volume (30% to 40%) (334,341), and particular fibroblasts are found in this region.

The following types of interstitial cells have been distinguished in the kidney (332,342–345).

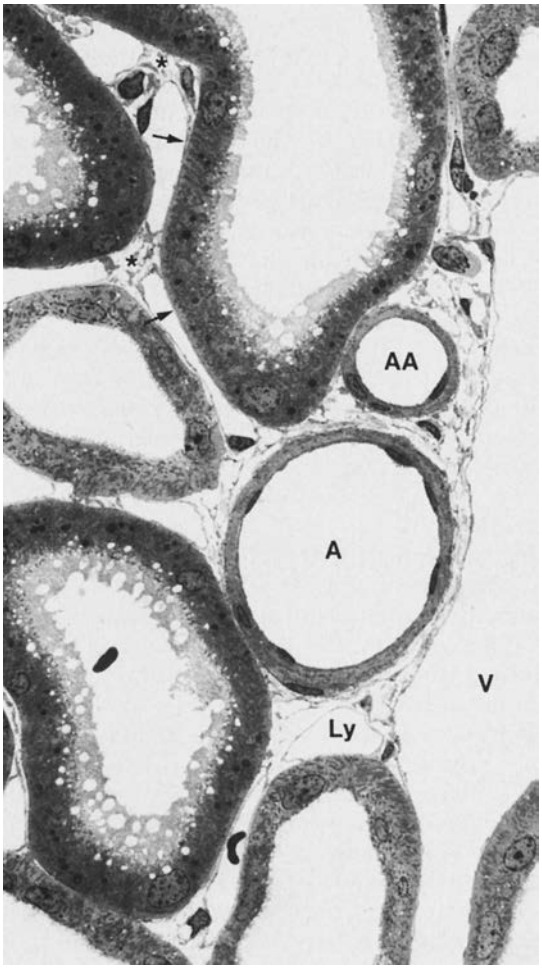


FIGURE 1-40. Low-power electron micrograph of a cross section through the renal cortex (rat). A cortical radial artery (*A*) and vein (*V*), an afferent arteriole (*AA*), and several tubular profiles are seen. The arteries are surrounded by the loose connective tissue sheath that contains the intrarenal lymphatics (*Ly*); the interstitial spaces of this sheath are continuous with the peritubular interstitium, which includes wide (*stars*) and narrow (*arrows*) portions. (Magnification ×1,000.)

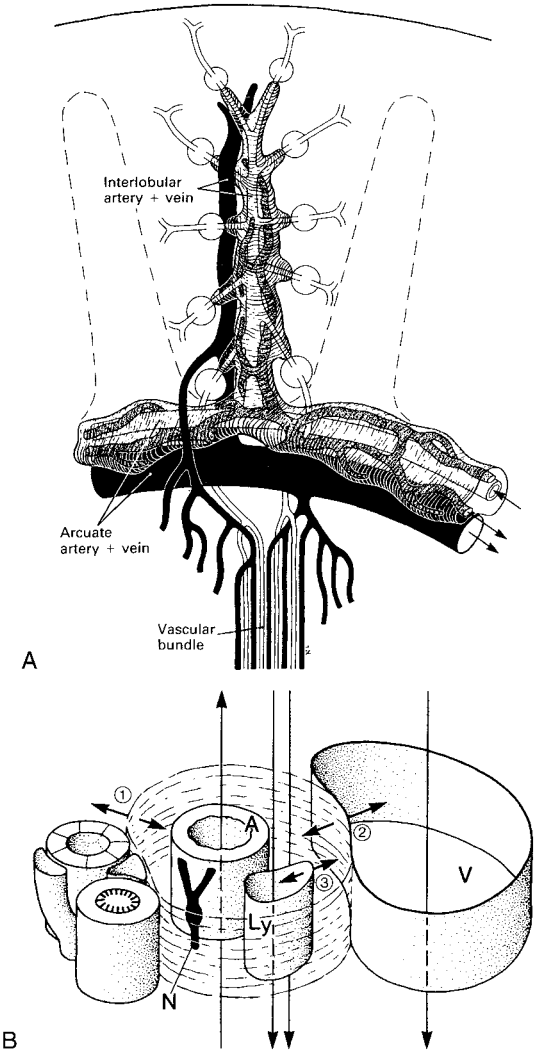


FIGURE 1-41. Schematics showing (**A**) the distribution and (**B**) the relationships of periarterial connective tissue sheath. The periarterial sheath is schematically indicated as a wide "stocking" drawn over the intrarenal arteries. In reality, there is no limiting membrane between the sheath and the surrounding interstitium. The lymphatics (*stippled area*) originate and travel within the periarterial sheath. The medullary rays are indicated by a broken line. The traverse section in **B** shows possible relationships between the sheath and the surrounding structures (*double-headed arrows*): (*1*) with the peritubular interstitium, (*2*) with the accompanying vein (*V*), and (*3*) with lymphatics (*Ly*). The single-headed arrows indicate the flow of the respective fluid. *N*, nerve. (From: Kriz W. A periarterial pathway for intrarenal distribution of renin. *Kidney Int* 1987;31:551, with permission.)

The *fibroblasts* of the cortex and outer medulla are extensively branched, with long, often sheetlike processes (Fig. 1-42) (346). They contain an abundant rough endoplasmic reticulum. Mitochondria, Golgi complexes, lysosomes, and microfilament bundles are regularly encountered. The lipid-laden cells of the inner medulla represent a local variety of fibroblasts (see below). The fibroblasts cells are responsible for the production and degradation of extracellular material, fibers, and ground substance. Fibroblasts of the renal cortex contain the enzyme ecto-5-nucleotidase (347,348), which can generate adenosine within the cortical interstitium. In addition, a subfraction of cortical interstitial cells synthesize erythropoietin (349–351).

In the inner medulla, the fibroblasts contain numerous homogenous osmiophilic lipid droplets. Hence, they are generally called *lipid-laden interstitial cells* (Fig. 1-43) (342,344,352). These star-shaped cells interconnect loops of Henle and vasa recta, spanning these axial structures like the rungs of a ladder. They increase in number toward the tip of the papilla and have an abundant rough endoplasmic reticulum, with cisternae that are often dilated and filled with flocculent material. A cytoskeleton is especially well developed in their most peripheral cell processes. These cells possess receptors for angiotensin II and bradykinin (353,354). They are responsible for the production of extracellular fibers and ground substance, including the abundant glycosaminoglycans and hyaluronic acid of the inner medulla (355).

The lipid-laden interstitial cells participate in producing the medullary prostaglandins. The lipid droplets of these cells contain polyunsaturated fatty acids that appear to be precursors of prostaglandins and other lipid-derived hormones (353,354). The cells produce vaso-depressing lipids, in particular, prostaglandin E_2 (356,357).

The second abundant cell type in the renal interstitium are *dendritic cells* (Fig. 1-42). They originate from the bone marrow and, as in other organs, are subject to a vivid turnover (358). In the kidney, dendritic cells are found in the interstitium throughout the cortex and the outer medulla (345,346). They enter the interstitium from the blood, reside for some days

FIGURE 1-42. Transmission electron micrograph of interstitial cells in the cortical peritubular interstitium. Two types are seen: fibroblasts (*1*) with many processes and a dendritic cell (*2*). *C*, capillary. (Magnification ×5,800.)

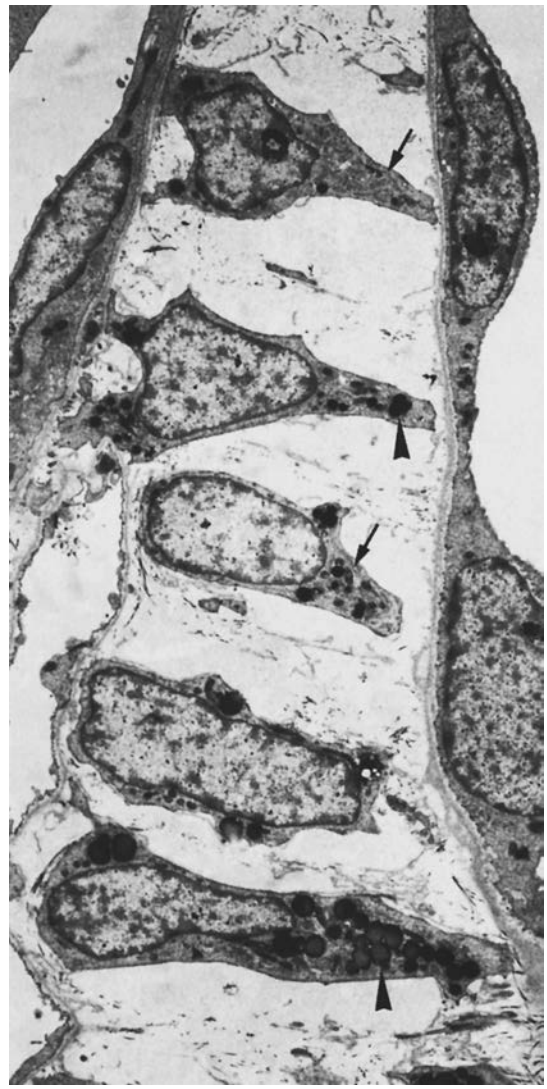


FIGURE 1-43. Transmission electron micrograph of a longitudinal section of the inner medulla (rat) showing lipid-laden interstitial cells arranged like the rungs of a ladder between parallel running tubes or vessels. Note the numerous lipid droplets (*arrowheads*) and the prominent endoplasmic reticulum (*arrows*). (Magnification ×3,300.)

in interstitial spaces, and leave the interstitium with the lymph flow.

The *extracellular components* of the interstitium form a matrix that may be thought of as a hydrated gel of ground substance within a fibrillar reticulum (332). Several fibers make up the interstitial reticulum. Collagen fibers (types I, III, and VI) are present in the matrix, both in isolation and in bundles. Type I collagen forms typical cross-banded fibers, generally more than 30 μm in diameter. Type III fibers (10 to 40 μm in diameter) and type VI fibers (6 to 10 μm in diameter) are often seen associated with type I fibers. In addition, unbanded microfibrils with a diameter of 15 to 30 μm and an electron-lucent core have been described (332,343).

Myofibroblasts are absent from healthy kidneys. They differ from fibroblasts by their high production of extracellular matrix (359) and expression of vimentin, as well as α-smooth muscle actin (αSMA). The accumulation of myofibroblasts in a diseased kidney is generally suggested to occur by transformation of fibroblasts into myofibroblasts in response to stimuli from locally produced cytokines (359,360). An alternative hypothesis postulates that myofibroblasts may develop by "epithelialmesenchymal transition" (EMT) from tubular cells (361); the evidence for this origin is not yet conclusive.

Macrophages (histiocytes) are also rarely found in the healthy kidney, except for the periarterial tissue sheaths (345) (Fig. 1-42). These round cells demonstrate primary and secondary lysosomes and characteristic surface folds. Along with interstitial affections, the number of macrophages may dramatically rise.

STRUCTURE–FUNCTION RELATIONSHIPS WITHIN THE RENAL MEDULLA

During phylogeny, the renal medulla has developed in response to the necessity to conserve water by excreting a concentrated urine (18). Loops of Henle, collecting ducts, and a specific blood supply through vascular bundles have developed into a complex structural system that accounts for this function. Whereas the mechanisms to produce a concentrated urine up to a concentration of about 600 mOsmol/L in the outer medulla are fairly well known, the mechanisms underlying the final urine concentration in the inner medulla are still poorly understood.

Within the outer medulla the reabsorption of NaCl from the MTAL represents the driving force to produce a corticomedullary osmotic gradient, that provokes the osmotic water withdrawal from the collecting duct passing through this region. As will be discussed in more detail later in this chapter, ADH initiates the insertion of AQP2 channels into the apical plasma membrane of collecting duct principal cells. Together with the constitutive AQP3 and AQP4 channels in the basolateral membrane of these cells, this allows osmotic water withdrawal into the hypertonic interstitium of the outer medulla (182). The reabsorbed water is brought back into the systemic circulation by venous vasa recta (2).

The final step of the urine concentrating process in the inner medulla is basically identical in as far as water is reabsorbed by osmosis through ADH-stimulated water-permeable collecting ducts into an hypertonic interstitium. However, the mechanisms for creating the osmotic gradient in the inner medulla up to 1200 mOsmol/L in humans and much higher in many rodent species are much more complex than in the outer medulla and are insufficiently understood. A driving force like the sodium pump in MTALs in the outer medulla is lacking. This has led to several "passive" models (362,363), which take into account the specific transport properties of thin limbs, collecting ducts, and vasa recta to elucidate, by mathematical modeling, how part of the osmotic energy produced by the function of MTALs in the outer medulla may be transferred into the inner medulla, leading there to an osmotic gradient toward the tip of the papilla. A review of these models is far beyond the scope of this chapter. From a structural point of view, two features appear most relevant.

1. **The shape of the inner medulla.** The inner medulla has a particular shape, tapering from a broad basis to a tiny papilla. The mass of the inner medulla is, therefore, unevenly distributed along the longitudal axis. A reconstruction study in the rat (2,364,365) has shown that the inner medulla is shaped like a mushroom, consisting of a broad head and a thin stalk. Calculations in the model have shown that the first one-half of the inner medulla comprises roughly 80% of the total inner medullary volume and, consequently, only 20% are left for the second–the papillary half. Thus, the decrease of the mass in the inner medulla along the longitudal and axis is almost exponential. This shape perfectly reflects what happens with the structures within the inner medulla: loops of Henle, collecting ducts, and vasa recta all decrease rapidly in number from the base to the tip of the papilla (364,365). It has been calculated that of an estimated 10,000 long loops entering the inner medulla at its base, only about 1,500 reach the papillary one-half of the inner medulla (2). Thus, the majority of thin limbs, the "short" long thin limbs, turn back shortly after entering the inner medulla, a smaller, but still substantial, number of thin limbs reach the middle part of the inner medulla, and only a small population of "long" long loops really reach the papilla.

   This has led to the proposal (366) and, later, mathematical models (194,367) that this arrangement might account for a cascadelike transport of solutes toward the tip of the papilla. The large fraction of "short" long loops, by some way or another, manages the transport of solutes into the first one-third of the inner medulla, the intermediate fraction of long loops brings a proportion of these solutes further down into the middle part, and finally the small fraction of "long" long loops completes the transport of a still much smaller solute fraction down into the papilla.
2. **Structural arrangements in the renal medulla that allow the recycling of urea into the inner medulla via short loops of Henle.** The descending thin limbs of short loops in the outer medulla are arranged in a pattern that allows the shifting of urea from the venous blood coming up with the venous vasa recta from the inner medulla into tubular fluid of descending thin limbs of short loops. This possibility is perfectly developed in the complex vascular bundles in the outer medulla of rodents with high urine-concentrating abilities (149,177,187,188). Within these vascular bundles, the SDTLs are arranged in a countercurrent fashion with ascending vasa recta coming up from the inner medulla. Thus urea, by countercurrent exchange, may directly enter the descending thin limbs through the urea transporter UT-A2 (183,265)—(the fenestrated endothelium of venous vasa recta may readily be expected to be highly permeable for urea). In a renal medulla with "simple" vascular bundles (as they are found in most species), countercurrent exchange of urea first occurs from ascending to descending vasa recta (which contain the urea transporter UT-B1), which, afterward, will deliver their blood to the capillary plexus of the interbundle region surrounding the thin limbs of short loops on their descending direction. Thus, even if probably less effectively, urea from the inner medulla has access to the SDTLs (183,187,368,369).

Starting with the MTAL, thus abruptly at the end of the ATL, the uriniferous tubule is impermeable to urea until the terminal collecting duct in the inner medulla, which, starting abruptly, expresses the ADH-dependent urea transporter UT-A1 (183,265). Because of water reabsorption upstream from this segment, urea becomes concentrated within the tubular urine to levels always higher than the adjacent interstitium. In the terminal CD segment, a major part of this urea diffuses into the papillary interstitium and where it mixes with other solutes (NaCl), contributing to the osmotic driving force that reclaims water from the collecting duct. The locus of urea delivery in the papilla, i.e., at the "ultimate bend" of the complex countercurrent exchange system of the inner medulla, is optimal, since (1) by countercurrent exchange in vasa recta and thin limbs, urea is largely trapped and distributed in a longitudinal gradient within the inner medulla and (2) the fraction of urea that escapes the inner medulla is subject to recycling via short loops back into the inner medulla. Thus, this latter fraction of urea is available another time within the papillary interstitium ready to withdraw and/or to balance water reabsorption from the collecting duct. Recycling is not simply trapping, since this fraction of urea on its nephron way back into the inner medulla is concentrated a second time, essentially by the work of the sodium pump in the MTALs. In essence, urea recycling by short loops (not by long loops) represents an effective transport of osmotic energy from the outer into the inner medulla.

Whether these mechanisms are sufficient to build an osmotic gradient up to several osmoles in desert rodents, or whether there are additional sources complementing the solute gradients (e.g., continuous production of osmotic active substances) is presently unknown. Even if this question does not belong to the most urgent problems in medicine, it represents a highly challenging biological enigma.

SOME STRUCTURE–FUNCTION RELATIONSHIPS AND PRINCIPLES

Tight Junction Structure and Role in Transepithelial Solute and Water Transport

General Considerations

Among the many functions performed by renal epithelia, the most characteristic and important is the directed transport of water and solutes that ultimately transforms the glomerular

ultrafiltrate into urine. The mere aligning of cells into arrays that form the tube-shaped nephron is not sufficient to give direction to the flow of water and solutes across these epithelial structures. Indeed, certain characteristics have evolved that provide for highly specialized, directed transport. The two most important of these characteristics are the polarization of each cell's plasma membrane into apical and basolateral sides, both structurally and functionally, and the joining of adjacent cells by highly specialized structures, known as *junctional complexes*. These latter structures completely surround cells near their apical surfaces and, in general, consist of three components aligned in series: the tight junction (*zonula occludens*), an intermediate junction (*zonula adherens*), and desmosomes (*macula adherens*). In addition, gap junctions (channels for cell-to-cell communication) have been observed in a more basal position in the proximal tubule. The junctional complex not only helps in holding the cells together and in separating the luminal and basolateral components of the plasma membrane, but also allows the real or potential development of a space between it and the basement membrane—the *lateral intercellular space*. Thus, as illustrated in Figures 1-16, 1-19, and 1-31, solutes and water may be transported through cells, called the *transcellular transport route* between cells, called the *paracellular transport route*, or both.

Tight junctions are the most important structure in determining the transport characteristics (e.g., electrical resistance, ion selectivity, and water permeability) of the paracellular transport pathway. In addition, tight junctions appear to form a diffusion barrier in the plane of the membrane, thereby helping to maintain the selective compositional characteristics that serve to differentiate apical from basolateral membranes (e.g., differences in lipids and membrane proteins; see Simons [371] for a review). Within this structure, the cell membranes of opposing cells join in specific, limited areas that encircle the cells, and on freeze-fracture replicas these areas of cell fusion appear as "strands" about 10 μm wide (see Fig. 1-21). Earlier ultrastructural studies (213,283,372–374) suggested that these strands consisted of an array of globular structures (either protein [375] or lipid [376,377] in nature), and that the strandlike appearance resulted from cross-linking of these globular subunits with glutaraldehyde during fixation. Recent studies employing quick-freeze methods, however, have demonstrated that the strands are continuous and that the "particles" were an artifact resulting from slow freezing (378,379).

Both lipid and protein compositions have been proposed for the continuous strands forming tight junctions (378) (see Simons [371] for a review). The lipid model proposed by Kachar and Reese (378) has been challenged by the demonstration that the tight junction structure is preserved after exposure to detergents that would be expected to disrupt lipids (379). In contrast, inhibitors of protein synthesis appear to block the normal development of tight junctions (380). Specific support for a protein model of tight junction structure has come from the isolation of two proteins that are specifically associated with the tight junctions of epithelia (ZO-1 [381], a 225-k Daprotein, and cingulin [382], a heat-stable acidic protein with an elongated shape and containing 108-kDa and 140kDa molecular masses). Recent studies of MDCK cells have provided evidence for critical roles of both extracellular and intracellular calcium in the formation of tight junctions (see Stuart et al. [383] for a review). Moreover, protein kinase C-dependent phosphorylation processes appear to be important in the assembly of proteins comprising these tight junctions (384).

The tight junction complex can be divided into three components: (i) integral tight junction proteins; (ii) plaque proteins that link integral proteins to the actin cytoskeleton; and (iii) proteins that interact with plaque proteins to regulate a variety of cellular functions (385). The integral membrane proteins include the occludins, claudins, and the junction adhesion molecule. Occludins are targets for a number of protein and tyrosine kinases. Their over-expression increases transepithelial resistance, but their exact function remains unknown. The claudin family currently has at least 24 members. They comprise short C- and N-termini, four membrane-spanning domains, and large first and second extracellular loops with a very broad range of isoelectric points. Junction adhesion molecule is a member of the immunoglobulin superfamily. It is expressed in both endothelial and epithelial cells and facilitates the migration of mononuclear cells into tissues.

Farquhar and Palade (235) performed the first detailed and systematic electron microscopic study of these cell-to-cell attachments and, using electron-dense protein tracers, concluded that tight junctions located at the apical region of the lateral intercellular space provided a tight seal between the apical and basal sides of epithelia for large molecules. Around the same time, a leak pathway was being observed in certain epithelia by physiologists (386–388). This pathway was subsequently shown both morphologically, using the electron-dense tracer colloidal lanthanum (389,390), and electrically, using voltage-scanning electrodes (391), to involve the junctional complexes. These studies led to the functional classification of epithelia as either "tight" or "leaky" (392) and demonstrated unequivocally that the so-called tight junctions were not always impermeable to ions or small solutes.

In general, tight junctions can be classified morphologically according to their depth, total number of strands, density of strands (number of strands per unit of length of tight junction depth), and the length around cells per unit of surface area of the epithelium. The functional significance of these structural features was first put forth by Claude and Goodenough (393), who observed an approximate direct relation between the number of junctional strands and the transepithelial electrical resistance and, by inference, ionic permeability. In other words, low-resistance, or electrically leaky, epithelia contain one to a few strands, and high-resistance, or electrically tight, epithelia contain many strands. Although this view has been challenged by some investigators (394) with respect to certain epithelia, the general correlation between complexity of junctional structure and electrical resistance or ionic permeability appears to be valid (395), at least for mammalian renal epithelia, particularly if junctional length and depth, strand density, or both are considered (160,372,373,396). The potential importance of tight junctional length becomes apparent if one considers that tight junctional strands contain a relatively fixed number of permeability sites per unit of length; that is, the density of a permeability site is constant from one nephron segment to another. For this circumstance, extensive interdigitation of cells with each other would produce a many-fold increase in junctional length per unit of surface area of the epithelium and, thereby, the contribution of the paracellular pathway to the total transepithelial permeability (cellular and paracellular) would be increased. Thus, the combination of a shallow junctional depth and extensive cellular interdigitation may be the morphologic hallmark of a leaky epithelium. These tight junctional morphologic features appear to correlate with the high ionic permeabilities or low electrical resistances of thin descending limbs (397–399), thin ascending limbs (400–403), and thick ascending limbs (212,404–406).

The functional significance of the aforementioned morphologic characteristics of tight junctions in determining differences in transepithelial water permeability is less well understood. It is clear, however, that a large paracellular ion leakiness (or low electrical resistance) is not necessarily linked with a high water permeability of junctional complexes in a particular epithelium. A good example of this occurrence is the

thick ascending limb of the loop of Henle, where these parameters appear to be completely dissociated; that is, the junctional complexes of this nephron segment are electrically leaky, but watertight (212). A single junctional strand as seen in the thin ascending limb may be sufficient to impart water permeability to a nephron segment (373,407). It should be noted in this regard that the water permeability of junctional complexes in any segment of the nephron is not known with certainty, even for the proximal tubule, which has a very high transepithelial water permeability. Although the presence of a significant solvent drag effect (see the following discussion) on NaCl absorption has suggested that the water permeability of junctional complexes in proximal tubules may be high (408), this view has been challenged (409).

The details of the role (or roles) that tight junctions play in determining solute and water transepithelial transport are not established unequivocally for most nephron segments. However, several generalizations are instructive. First, concentration differences that develop as a result of active transcellular transport events (pump) tend to be passively dissipated through tight junctions (leak). Axial heterogeneity in the pump and leak rates for solutes along certain segments of the nephron also appear to be particularly important in accomplishing the formidable task of reabsorbing almost completely large quantities of these solutes. For example, active transepithelial transport of glycine or glucose tends to decrease as one proceeds down the proximal tubule. For example, Barfuss and Schafer (410,411) have confirmed that, in isolated perfused rabbit tubules, the maximal rates of active transport of these solutes decrease from S_1 to the S_3 segment and, in addition, these workers demonstrated that the decreases in active transport are paralleled by reductions in the apparent passive permeability coefficients for these solutes. Presumably, these passive permeability properties are attributable primarily to the paracellular pathway. Thus, the high-capacity, low-affinity active transport system in the S_1 segment of the proximal tubule allows a large quantity of glucose or glycine to be rapidly absorbed. However, because the high paracellular permeability permits a large leak of solute back into the lumen, this segment cannot completely reabsorb these solutes. On the other hand, the S_3 segment can reduce the luminal glucose or glycine concentration to a very low level by virtue of its low-capacity but high-affinity active solute transport system, coupled with a very low paracellular permeability (low leak rate) for these solutes. In addition, Burg (412) and Knepper and Burg (413) have stressed that the maximal diluting capacity of the nephron may be fixed because of a similar series arrangement of different capacity, active transport systems for NaCl absorption and paracellular permeabilities for Na^+ and Cl^- (pumpleak rates) in cortical and medullary portions of the thick ascending limb. Reeves and co-workers (414) have modeled the diluting power of the thick ascending link based on the different pump-leak characteristics of the medullary and cortical thick ascending limbs.

A second way in which the paracellular pathway may be involved in transepithelial transport is providing a route for the movement of electrolytes that are driven by transepithelial voltages developed by rheogenic transport processes. For example, recent studies assessing the mechanism of NaCl absorption in the thick ascending limb (415,416) have suggested that the lumen-positive transepithelial voltage drives 50% of the total transepithelial sodium absorption through the cation-selective paracellular route. Furthermore, most of the transepithelial transport of divalent cations (e.g., calcium and magnesium) in the thick ascending limb also may be accounted for by movement through the paracellular route, again being driven by the lumen-positive transepithelial voltage (417). Wittner et al. (418) provided evidence for regulation of the paracellular permeability to divalent cations by parathyroid hormone, suggesting that hormone-activated intracellular signaling events (e.g., cyclic adenosine monophosphate [cAMP] may alter tight junctional permeability [see next section]).

Until recently it was assumed that electrolytes moved through the paracellular pathway nonspecifically. The recent cloning of paracellin-1 by Simon et al. (419) suggests, for the first time, however, that conductance through this pathway is selective. The authors studied a group of patients with familial hypomagnesemia, hypercalciuria, and nephrocalcinosis. Other features of this disorder include urinary tract infection, kidney stones, hyperuricemia, and ocular abnormalities. Positional cloning techniques were employed to identify the novel defective gene (paracellin-1). The protein contains 305 amino acids and four transmembrane domains. The first extracellular loop is highly negatively charged and may contribute to the cation selectivity. Paracellin-1 was immunolocalized to the tight junction of the thick ascending limb, the region of the nephron where 67% of magnesium is reabsorbed. Sequence homology shows that paracellin-1 is a member of the claudin gene family. The PDZ binding motif of the claudins interacts with ZO-1, -2, and -3, other members of the tight junction complex.

Paracellin-1 mutation in humans results in renal magnesium and calcium wasting. These results suggest that paracellin-1 plays a role in selective paracellular magnesium conductance. Definitive proof of this will await expression studies of the protein. Paracellin-1 may form a selective pore through which magnesium and calcium are conducted. Recently, a group of 11 families was identified with idiopathic hypercalciuria secondary to a mutation in paracellin-1 (claudin-16). These patients had childhood-limited hypercalciuria and preserved glomerular filtration rates. The mutations in these families was in a PDZ-binding domain that inhibited binding to ZO-1 and resulted in lysosomal mislocalization. This finding provides further evidence that paracellin-1 plays a role in paracellular calcium transport. Previous models of the tight junction as a simple seal that either opens or closes to allow the flow of solutes must be modified to one that includes ion-selective proteins (420). Given the large size of the claudin gene family, it is reasonable to assume that other claudins may act as selective pores for other ions. This is supported by recent studies that show that over-expression of claudin-4 in MDCK cells decreases paracellular conductance because of decreased permeability of sodium across the tight junction (421).

Finally, water flow through the paracellular route may be influenced by the paracellular permeability to solutes or, conversely, the water permeability of tight junctions could influence the flow of charged or uncharged solutes through the tight junctions by the "solvent drag" effect. For example, the differing reflection coefficients for solutes across the paracellular pathway might produce an effective osmotic gradient across the S_3 segment of the proximal tubule sufficient to drive a significant fraction of water absorption (see Schafer [422] for review).

Control of Paracellular Permeability

Little is established about whether physiologic stimuli can regulate the permeability characteristics of tight junctions (423). It is clear, given the importance of the paracellular pathway in solute and water transport across epithelia (see the previous discussion), that changes in the functional characteristics of tight junctions could profoundly influence transepithelial transport in the nephron. Several recent studies in both renal and nonrenal epithelia support, at least in general terms, a role for the paracellular pathway in the regulation of transepithelial transport. Nigam and co-workers (383,384) have established the critical role of intracellular signaling events (e.g., Ca^{2+} and

protein kinase C) in tight junctional formation and stability in MDCK cells. In the *Necturus* gallbladder, cAMP decreases tight junction ionic permeability in a rapid, reversible fashion, and freeze-fracture studies have shown that this effect is associated with an increase both in the number of tight junction strands and in the depth of the junctions (424,425). Furthermore, the observations that raising intracellular Ca^{2+} (425) and applying drugs that modify cytoskeletal elements (426) also result in similar structural and functional changes in the *Necturus* gallbladder suggest that cAMP may exert its action by modifying these cytoskeletal elements. Cyclic AMP is also capable of modifying the permselectivity characteristics of the intestine of winter flounder, *Pseudopleuronectes americanus*, by increasing the permeability to Cl^- (427,428). Whether this effect involves a structural change in tight junction morphology is unknown. In addition, Mazoriegos et al. (429) have demonstrated in rats that when parotid glands are stimulated with isoproterenol, there is an associated transient increase in junctional permeability.

Several investigators have provided evidence indicating that cAMP may similarly modulate tight junctional permeability in the proximal nephron and have suggested that this effect of cAMP may play a role in the response of this nephron segment to parathyroid hormone. It is well recognized that parathyroid hormone inhibits volume absorption in the proximal nephron (430,431) and that this effect can be mimicked by cAMP (431,432). Furthermore, Lorentz (433) has argued from *in vivo* studies utilizing microinjection of cAMP into rats that this effect might involve an alteration in the paracellular permeability, characteristic of the proximal nephron. Indeed, *in vitro* studies of Jacobson (431) on superficial proximal convoluted tubules of rabbits confirmed that cAMP was capable of altering junctional permeability in this nephron segment. Thus, the functional characteristics of the paracellular pathway can be modified in the proximal nephron by acute administration of cAMP and this effect may account, in large part, for the parathyroid hormone (PTH)-mediated decline in volume absorption in this nephron segment. This notion is further supported by the alteration of paracellular divalent cation permeability by PTH in CTAL of mice (418).

Cytoskeletal proteins appear to be involved in determining tight junctional permeability and may be involved in the aforementioned effects of cAMP. Actin filaments were demonstrated to be linked to the intermediate junction by an adhesion plaque containing the actin-binding proteins α-actinin and vinculin (434). Agents that disrupt these cytoskeletal elements can alter both the structure and function of tight junctions (426,435), suggesting that tight junctions and intermediate junctions are interconnected both structurally and functionally (436).

Extracellular calcium concentration can also affect the integrity of junctional complexes. Lowering the calcium concentration opens junctional complexes and lowers the transepithelial resistance (437). Gumbiner and Simons (436) have suggested that this is an indirect effect of calcium mediated by uvomorulin (L–CAM), a cell adhesion protein located in the intermediate junction or basolateral membrane of epithelial cells (437). Calcium-dependent conformational changes in this protein may be important in the assembly of the intermediate junction and thereby affect tight-junction structure and function (436).

Finally, it has been recognized for some time that apical (luminal) hypertonicity can disrupt junctional complexes in tight epithelia, leading to increases in electrolyte and nonelectrolyte permeabilities (388). These functional changes appear to be associated with blistering within tight junctions (438). Similar functional and morphologic changes also have been seen in electrically leaky renal epithelia, such as the amphibian proximal tubule (439) and mammalian MTAL (440). Although these conditions are probably not physiologic, changes in paracellular permeability, presumably related to increases in hydrostatic pressure, have been demonstrated in certain conditions, such as volume expansion and ureteral or venous occlusion (441–443).

Structural and Biochemical Aspects of Transcellular Transport Processes in the Nephron

The Membrane Amplification Principle

As discussed in the preceding section, the paracellular route participates in the transport of ions and solutes across renal tubular epithelia in a variety of ways; however, all these paracellular transport processes ultimately depend on the transepithelial electrochemical gradients for these species developed by cellular transport events. The transport of solutes and water through cells is limited by three barriers in series: the apical plasma membrane, the cytosol, and the basolateral plasma membrane. Transport through the cytoplasm is generally considered to be diffusional in nature and the rate-limiting steps for solute and water transport in most nephron segments appear to reside at the apical and basolateral membranes. Thus, our current thinking regarding transcellular solute transport in the nephron is based on the double-membrane model of Koefoed-Johnsen and Ussing (444). Differences in the solute and water permeabilities of these two membranes, related to differences in lipid composition or the quantity and type of integral transport proteins spanning the lipid bilayer matrix, or both, provide for the vectoral (secretion or absorptive) transport of matter through these cells. In general, the transport characteristics of each of these plasma membranes could be altered by changing the type, function, or number of carrier, channel, enzyme, or receptor proteins contained in the membrane. If the density of transport proteins in a given membrane is relatively constant, an increase in their number would require an associated increase in membrane area. For this circumstance, differences in the transcellular transport capacities among individual nephron segments or between different functional states in a single nephron segment would be paralleled by differences in their apical or basolateral membrane area. This may be referred to as the *membrane amplification principle*.

Although modest amplifications of plasma membranes can be produced by increasing the diameters or lengths of nephron segments, or both, a much greater increase in membrane area is achieved by the folding of these membranes, with relative preservation of tubule dimensions. In apical membranes, these foldings can take the form of a highly organized and complex assembly of thin fingerlike microvilli (e.g. the brush border of the proximal nephron) or lesswell-organized microvilli and microfolds, called *microplicae*. In basolateral cell membranes, amplification is achieved both by basal infoldings of various depth and by lateral membrane foldings that result in cellular interdigitations.

Quantitative micromethodologies for evaluation of morphologic, physiologic, and biochemical differences at the single nephron or cellular level have provided valuable insights into the relations between structure and function in the nephron. Studies utilizing these methods have provided general support for the membrane amplification principle, both in comparing the intrinsic function of different nephron segments and in the functional adaptation of renal tubular epithelia that exist beyond the macula densa (the distal convoluted tubule,

connecting tubule, and collecting tubule) to alterations in salt intake or H^+ ion balance.

Structure-Function Relationships in Na^+ and K^+ Transport

Na^+-K^+-ATPase and Basolateral Membrane Area

It is generally accepted that Na^+-K^+-ATPase is the enzymatic equivalent of the Na^+-K^+ pump, which is responsible for transport of Na^+ out of, and of K^+ into, cells across plasma membranes. Both the enzyme and cation countertransport pump are characterized by the hydrolysis of adenosine triphosphate (ATP), the requirement for both Na^+ and K^+, but on opposite sides of the membrane, and specific inhibition with digitalis glycosides, for example, ouabain.

The mammalian kidney is rich in this enzyme and renal medullary tissue, in particular, has served as a major source for purification of Na^+-K^+-ATPase (445–447). The purified renal medullary enzyme has one of the highest specific activities of any Na^+-K^+-ATPase (10,000 ATP min/molecule) (445–447). Freeze-fracture studies of Na^+-K^+-ATPase-containing membranes from the outer medulla of pigs were consistent with biochemical studies suggesting that renal protein exists in a dimeric form (448): two α and two β subunits, each of these units being approximately 100 kDa and 40 kDa, respectively (i.e., total molecular weight of about 280 kDa) (447,449). Recently, a gamma subunit (molecular weight 10 kDa) was described that modulates the kinetic behavior of the pump (450). The hydrolysis of one molecule of ATP appears to energize the countertransport of three Na^+ ions and two K^+ ions across plasma membranes against their respective electrochemical gradients. Given this stoichiometry, the process would result in the net transport of charge across a membrane, that is, it would be electrogenic. Ouabain binds specifically to the α subunit and can be used to quantitate the amount of functional enzyme, since one molecule of this agent binds to one functional unit of Na^+-K^+-ATPase.

The activity of this enzyme has been determined in individual segments of nephrons from rabbits (223,451–455), rats (452,456,457), and mice (452,458). These studies have added considerably both to our appreciation of the axial heterogeneity of salt transport along the nephron and to our understanding of the regulation and adaptation of salt transport in individual nephron segments. Biochemical (236,459), autoradiographical (460), and immunological (461,462) studies have clearly localized this enzyme to the basolateral plasma membrane of renal tubule epithelial cells. As a consequence, Na^+ transport may be directed from the luminal to the serosal side of the renal tubule if the apical membrane is rendered permeable to Na^+ by inserting some facilitating transport mechanism for this ion (444). Moreover, since Na^+-K^+-ATPase also transports K^+ into cells, this cation may be secreted to a variable extent, depending on the relative permeabilities of apical and basolateral membranes to K^+.

Katz and co-workers (452) have summarized the activity profiles of Na^+-K^+-ATPase along rabbit, rat, and mouse nephrons, and these data are shown in Figure 1-44. Qualitatively similar profiles were found by others (451,457). Although the activity of the enzyme varies somewhat from one study to another depending, at least in part, on the method used for enzyme activity measurement, the relative distribution of enzyme is virtually identical, even among the different species. The general distribution of the enzyme shown in Figure 1-44, when coupled with the density of individual tubule segments in the various regions of the kidney, explains why Na^+-K^+-ATPase activity is highest in outer medullary and lowest in papillary regions of the kidney.

Epstein and co-workers (463,464) have provided compelling evidence that Na^+-K^+-ATPase directly mediates at least 50% of the total Na^+ absorbed by the mammalian nephron. The high activities of Na^+-K^+-ATPasein proximal and distal convoluted tubules and in the medullary and cortical thick ascending limbs (see Fig. 1-44) are particularly striking and correspond to the nephron segments where the bulk of Na^+ reabsorption occurs. Such comparisons have suggested that the level of Na^+-K^+-ATPase activity may correlate with the rate of active Na^+ absorption along the nephron. This correlation has been shown most clearly in rabbits by Garg et al. (451). Their data, summarized in Figure 1-45, demonstrate a direct relation between Na^+ transport rates and Na^+-K^+-ATPase activities in nephron segments of this species. This result, when coupled with the general observation that ouabain completely inhibits active Na^+ absorption in these segments, provides strong evidence that Na^+-K^+-ATPase is the active Na^+ transporter in the kidney. Because Na^+-K^+-ATPase is involved in the translocation of both Na^+ and K^+, it would be reasonable to infer that this enzyme complex might also play a part in regulating renal K^+ excretion. Indeed, this appears to be the case. Potassium

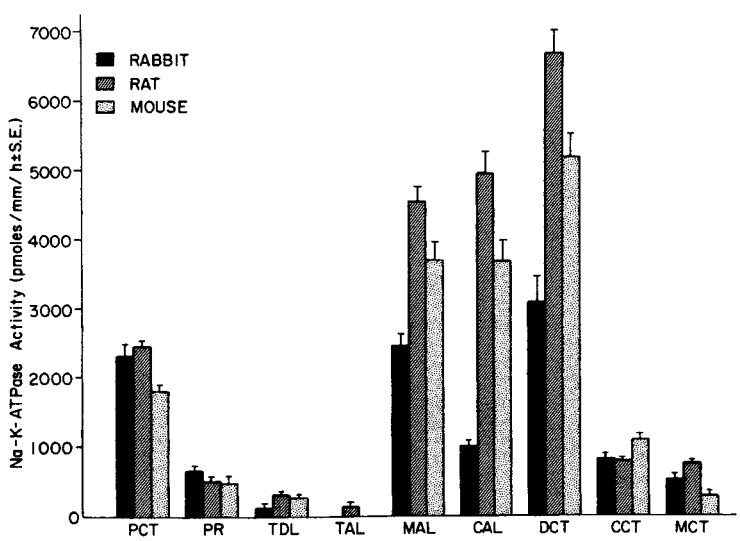


FIGURE 1-44. Na^+-K^+-ATPase activity profile in rabbit, rat, and mouse nephron. *PCT*, proximal convoluted tubule; *PR*, pars recta; *TDL*, thin descending limb; *TAL*, thin ascending limb; *MAL*, medullary thick ascending limb; *CAL*, cortical thick ascending limb; *DCT*, distal convoluted tubule; *CCT*, cortical collecting tubule; *MCT*, medullary collecting tubule. (From: Katz AI, Doucet A, Morel F. Na–K-ATPase activity along the rabbit, rat, and mouse nephron. *Am J Physiol* 1979;237:F114, with permission.)

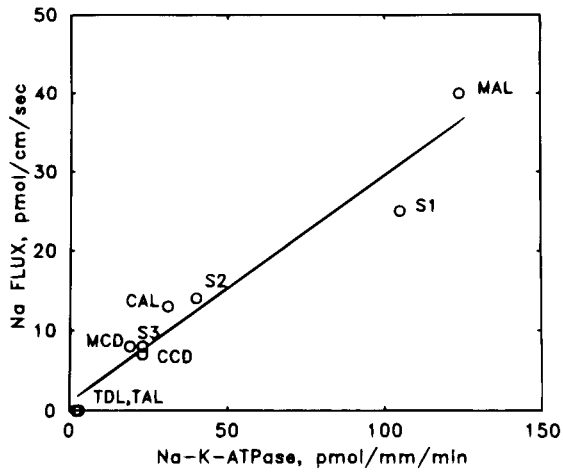


FIGURE 1-45. Relation between net sodium absorption and Na^+-K^+-ATPase activity in individual rabbit nephron segments. *TDL*, thin descending limb; *TAL*, ascending limb; *MAL*, medullary thick ascending limb; *CAL*, cortical thick ascending limb; *CCD*, cortical collecting duct; *MCD*, medullary collecting duct. (From: Garg L, Knepper M, Burg M. Mineralocorticoid effects on Na^+-K^+-ATPase in individual nephron segments. *Am J Physiol* 1981;240:F536, with permission.)

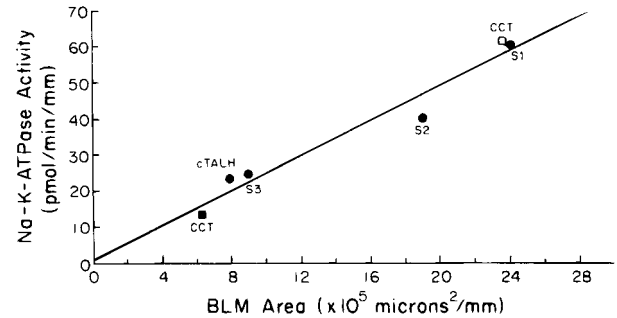


FIGURE 1-46. Relation between Na^+-K^+-ATPase activity and basolateral membrane area in individual nephron segments. The open square represents the CCT from animals treated with a low-sodium diet, DOCA, or both. (From: Gibson M, Kumaratilake J, Cleary E. The protein components of the 12-nanometer microfibrils of elastic and nonelastic tissues. *J Biol Chem* 1989;264:4590; Nielsen S. Renal aquaporins: an overview. *BJU International* 200;90:1; Bouby N, Trinh-Trang-Tan M, Laouari D, et al. Role of the urinary concentrating process in the renal effects of high protein intake. *Kidney Int* 1988;34:4; Bankir L, Kriz W. Adaptation of the kidney to protein intake and to urine concentrating activity: similar consequences in health and CFR. *Kidney Int* 1995;47:7; Muto S, Yasoshima K, Yoshitomi K, et al., Electrophysiological identification of α- and β-intercalated cells and their distribution along the rabbit distal nephron segments. *J Clin Invest* 1990;86:1829; Verlander J, Madsen K, Tisher C. Axial distribution of band 3-positive intercalated cells in the collecting duct of control and ammonium chlorid-loaded rabbits. *Kidney Int* 1996;57:S137; Citi S, Sabanay H, Jakes R, et al. Cingulin, a new peripheral component of tight junctions. *Nature [London]* 1988;333:272; Ussing H, Windhager E. Nature of shunt path and active sodium transport path through frog skin epithelium. *Acta Physiol Scand* 1964;61:484; Machen T, Erlij D, Wooding F. Permeable junctional complexes. The movement of lanthanum across rabbit gallbladder and intestine. *J Cell Biol* 1972;54:302. S_1, S_2, and S_3, proximal tubule segments 1, 2, and 3; *CCT*, cortical collecting tubule; *cTALH*, cortical thick ascending limb of the loop of Henle.

loading induces increases in Na^+-K^+-ATPase activity from both cortical and medullary regions of the kidneys in rats (465,466). Doucet and Katz (458) localized these increases in enzyme activity in mice to the cortical and medullary collecting ducts, segments that are responsible for enhanced potassium secretion during potassium adaptation in rats (467).

The profile of Na^+-K^+-ATPase activity depicted in Figure 1-44 could result from differences either in the enzyme-turnover rates or in the number of catalytic units, or possibly both. Mernissi and Doucet (223) have recently distinguished between these possibilities by comparing ouabain binding and Na^+-K^+-ATPase activity along the nephron. Since the profiles of pump activity and the number of catalytic units were similar—that is, pump activity was proportional to the number of catalytic units in all segments of the nephron that were evaluated—these authors concluded that the maximal turnover rate of Na^+-K^+-ATPase was virtually identical among the various nephron segments. In other words, an increase in Na^+-K^+-ATPase activity in one nephron segment compared with another would be due to an increase in the number of functional Na^+-K^+-ATPase units. Given this latter finding, by using the membrane amplification principle, we would argue that the level of Na^+-K^+-ATPase activity—and therefore the rate of Na^+ absorption—and the amount of basolateral membrane area in the various nephron segments should be parallel. The relation between Na^+-K^+-ATPase activity and basolateral membrane area for a number of nephron segments from rabbits is shown in Figure 1-46. Assuming that differences in Na^+-K^+-ATPase activity relate mainly to changes in the number of pump units, then the data in Figure 1-46 indicate that there is a direct relation between basolateral membrane surface area and the number of Na^+-K^+-ATPase units.

Direct relations between Na^+-K^+-ATPase activity and net fluid absorption or basolateral membrane area also have been observed in developing renal proximal tubules. Developmental increases in the reabsorptive capacity of the proximal tubule are accompanied by growth of the basal and lateral cell membranes and are paralleled by increases in Na^+-K^+-ATPase activity (468–472). Moreover, premature development of these physiologic, biochemical, and ultrastructural changes can be induced by pharmacologic treatment with adrenocorticotropic hormone (ACTH) or glucocorticoids (473).

Finally, the profiles of oxygen consumption (QO_2), cellular ATP content, and mitochondrial density along the nephron are strikingly similar to those of Na^+-K^+-ATPase activity. The similarity in all of the profiles is consistent with the dependence of Na^+-K^+-ATPase activity on an adequate supply of ATP resulting from aerobic glycolysis.

Relations among Na^+-K^+-ATPase, Basolateral Membrane Area, and Salt Transport in the Distal Nephron: Effect of Corticosteroids

In distal nephron segments beyond the macula densa (including the distal convoluted tubule, the connecting tubule, and the collecting tubules), four morphologically distinct cell types (DCT cells [see Fig. 1-27], CT cells [see Fig. 1-28], principal cells [see Fig. 1-29], and IC [see Figs. 1-29 and 1-32]) were identified. These morphologic distinctions are paralleled by heterogeneity in both function (474–477) and hormone action (221,432) along the distal nephron.

In three of the cell types found in the distal nephron (DCT, CT, and collecting duct cell), basolateral membrane area and Na^+-K^+-ATPase activity can be altered dramatically by various dietary manipulations, administration of corticosteroids, or both (238,263). Nowhere is this more evident than in principal cells located in the cortical collecting tubule (CCT). The original observations of Gross et al. (478) in rabbits that long-term doses of deoxycorticosterone acetate (DOCA) increased the voltage across the CCT but not the DCT have been confirmed and extended by O'Neil and Helman (479), Schwartz and Burg (480), and Koeppen et al. (481). These latter

groups demonstrated that chronic elevation of plasma mineralocorticoid levels in rabbits, either by feeding animals a diet low in Na^+ and high in K^+ or by administering DOCA to animals on a standard diet, caused marked increases in the rates of Na^+ absorption and K^+ secretion, as well as in the conductive pathways for these ions in apical plasma membranes.

Several investigators have evaluated the role of Na^+-K^+-ATPase in the DOCA-induced changes in Na^+ and K^+ transport rates in the CCT. Garg et al. (451), LeHir et al. (251), and, more recently, O'Neil and Dubinsky (482) demonstrated a doubling in the Na^+-K^+-ATPase activity in this nephron segment with either DOCA or low Na^+, high K^+ diets. Indeed, Garg et al. (451) showed a direct correlation between the rates of Na^+ absorption, previously obtained by Schwartz and Burg (480), and their Na^+-K^+-ATPase activities in CCT from rabbits fed a low-salt diet or given DOCA. This correlation was similar to that shown by these workers in nephron segments in general (see Fig. 1-45). The mineralocorticoid specificity of these changes in cation transport and Na^+-K^+-ATPase activity not only was supported by the localization of aldosterone-binding sites in the CCT (209,395–397), but also was confirmed by the lack of increases in Na^+-K^+-ATPase by the glucocorticoids, dexamethasone, or corticosterone (398). Moreover, Marver and co-workers (263) have also shown that mineralocorticoids, but not glucocorticoids, were able to increase the activity of citrate synthase, a probable marker for mineralocorticoid activity, in the CCT. All these observations fit the general model for aldosterone action in epithelia, in which this steroid enhances both the entry and exit steps for Na^+ and K^+ in apical and basolateral plasma membranes.

In addition to these well-described physiologic and biochemical changes, the membrane amplification principle would suggest that modulation of Na^+ and K^+ in the diet or administration of DOCA also should be associated with marked alterations in the area of the basolateral membranes. Wade et al. (259) were the first to demonstrate clear increases in basolateral membrane area in the CCT in rabbits given DOCA long term. These morphologic changes were restricted to principal cells, suggesting that this cell type may be the one specifically involved in the effect of DOCA on Na^+ and K^+ transport in the collecting duct.

Basolateral membrane amplification in principal cells, but not IC from both mammalian and amphibian (401) collecting ducts, has also been observed after chronic exposure to a high K^+ diet (213,216,402), and these morphologic changes are associated with increases in Na^+-K^+-ATPase activity (373). Moreover, a similar ultrastructural change also has been noted in another condition associated with a high rate of K^+ excretion. Zalups and co-workers (483) found that in rats subjected to a 75% nephrectomy, the area of basolateral membranes of principal cells from initial collecting tubules had increased more than twofold 10 days after the procedure. Since this morphologic alteration was restricted to principal cells, these investigators argued that the enhanced K^+ excretion associated with partial nephrectomy was mediated, at least in part, by the increase in basolateral membrane area.

The role of mineralocorticoids in these functional and structural changes in the collecting duct, associated with potassium adaptation, remains uncertain (210,381,404). Although aldosterone appears to contribute to the phenomenon of potassium adaptation, it is less clear whether physiologically elevated levels of this hormone are required. Recent studies in rats have indicated that physiologically high levels of aldosterone may (405) or may not (406) be required for potassium adaptation. Finally, it should be noted that a recent morphologic study in rats has indicated that a different mechanism may contribute to potassium adaptation in papillary collecting ducts (216). During potassium adaptation, a 50% increase in apical membrane area occurs in this nephron segment, while the basolateral membrane area remains unchanged. The physiologic significance of this structural change is unknown.

Kaissling et al. (232,239,240) have extended these morphologic and biochemical observations to include the DCT and the CT in rabbits. These investigators demonstrated basolateral membrane amplification in DCT cells after chronic intake of a high Na^+, low K^+ diet, an effect that was unaltered by exogenous administration of deoxycorticosterone. None of the other cell types in the distal nephron were affected by this diet. In addition, a low Na^+, high K^+ diet, with or without aldosterone synthesis inhibition with potassium canrenoate, had no effect on the DCT cell. The absence of any effect of this latter diet on DCT cells is in marked contrast to the stimulating effect of this dietary manipulation on basolateral membrane amplification—and Na^+-K^+-ATPase in principal cells (see the previous discussion in this section). Although a high Na^+, low K^+ diet markedly increased the basolateral membrane area in the DCT cells in rabbits, LeHir et al. (251) found no augmentation of Na^+-K^+-ATPase activity. However, in a later study, Mernissi and co-workers (368) observed a nearly twofold increase in Na^+-K^+-ATPase activity in this latter segment from DOCA-treated rabbits. Thus, it is probable that basolateral membrane area and Na^+-K^+-ATPase activity also parallel each other in DCT cells. Because neither increases or decreases in K^+ intake alone have any effect on distal convoluted cells in rats (213), Kaissling (203) suggested that the basolateral membrane amplification—and probably also the increase in Na^+-K^+-ATPase observed by Mernissi et al. (223)—was a response to the chronic high Na^+ intake.

The morphologic and biochemical responses of the connecting tubule cells to a low Na^+, high K^+ intake in both rabbits (402) and rats (213) are similar to those observed in the CCT. Clear increases occur in both Na^+-K^+-ATPase activity and basolateral membrane area. These responses appear to be, at least in part, as a result of mineralocorticoid effects, because administration of large doses of DOCA to animals fed a high Na^+, low K^+ diet also resulted in basolateral membrane amplification in these cells.

Although all the studies cited tend to provide general support for the membrane amplification principle as it relates to Na^+-K^+-ATPase activity, the specificity of this membrane response may be questioned. In other words, is the functional number of other enzyme units also increased by the DOCA- or diet-mediated increases in basolateral membrane area? The view that this dramatic change in membrane area is indeed specific for Na^+-K^+-ATPase has been strengthened by the observations of Mernissi et al. (368). These workers demonstrated that the DOCA-mediated increase in basolateral membrane area in CCT was specific for Na^+-K^+-ATPase, since adenylate cyclase activity, another enzyme complex localized to the basolateral membrane of this nephron segment, was unchanged by this steroid. Finally, the similarity in morphologic and enzymatic changes associated with DOCA treatment or a low Na^+, high K^+ diet, or both, suggests that Na^+ absorption and K^+ secretion in the distal nephron may be localized primarily to the CT and collecting duct cells.

Relations among Na^+-K^+-ATPase, Basolateral Membrane Area, and Salt Transport in the Distal Nephron: The Effect of Diuretics

Kaissling et al. (484) treated rats with a continuous infusion of furosemide and noted an increase in the height (60%) and length (12%) of the DCT. Electron microscopy revealed both an increase in the size of the cells and an increase in the basolateral cell membrane area (12%) compared to controls. The increase in basolateral membrane area in DCT cells was not paralleled by a similar increase in luminal membrane area. The number of apical microvilli per unit tubular length was similar

compared to controls. These changes developed over the very short period of the study (7 days). The authors speculated that the chronic increase in solute load stimulated NaCl transport of DCT cells that caused them to hypertrophy. As described above, these authors had previously shown similar changes in the DCT of rabbits on a chronic high-Na, low-K diet. This study demonstrated that changes in the transport activity of one segment can alter the function of downstream segments and that tubular fluid composition plays a role in the regulation of transport function.

Ellison and co-workers (485) studied the effects of changes in solute delivery on distal nephron morphology in three groups of animals: group 1, high NaCl diet; group 2, low NaCl diet; and group 3, continuous furosemide infusion (485). Free-flow micropuncture, *in vivo* microperfusion, and morphometry were performed in each group of animals. Dietary NaCl restriction did not affect NaCl delivery to the early DCT, but markedly increased transport capacity. Chronic extracellular fluid (ECF) volume depletion increases the ability of the DCT to reabsorb Na and Cl. Under these circumstances, ion transport capacity increases without structural alteration. Although transport capacity is increased in this setting, the delivery of Na and Cl is not, and there is no change in morphology. Chronic furosemide infusion increased NaCl delivery to the DCT and increased the rate of NaCl transport in this segment. In contrast to groups 1 and 2, only rats in group 3 exhibited hypertrophy of the DCT (increase in fractional volume of 100%). These results are consistent with the hypothesis of Kaissling and Stanton (484) that hypertrophy results from increased rates of transcellular transport.

Morsing et al. (486) administered hydrochlorothiazide to rats via osmotic minipumps for 10 to 14 days. NaCl transport capacity of the DCT was reduced. The number of thiazide-sensitive NaCl cotransporters as measured by [^{3}H]metolazone binding was higher in the thiazide-treated animals than in controls. Loffing (487) found that thiazide treatment of rats provoked apoptosis in the DCT. Metolazone or hydrochlorothiazide was infused continuously for 3 days via osmotic minipump. Thiazide-treated animals showed apoptosis and reduced expression of calbindin D_{28K} and Ca^{2+}-ATPase. The thiazide-sensitive NaCl cotransporter was no longer expressed in the apical membrane, but was localized to the basal one-half of the cell and to small intracellular vesicles. These effects were not seen in more distal parts of the DCT. Northern analysis revealed a marked decrease in the thiazide-sensitive NaCl cotransporter transcript. Focal inflammatory infiltrates surrounded these damaged DCT cells. The authors speculated that reduced Na entry either directly or indirectly was responsible for the abnormalities. These results are in contrast to the paucity of reports of adverse structural effects of thiazides in humans. Bachmann, Reilly, and Ellison (488) showed that the DCT can be molecularly subdivided into two segments: DCT-1 and DCT-2. In DCT-1, the only Na entry pathway is via the thiazide-sensitive NaCl cotransporter. DCT-2 expresses both the thiazide-sensitive NaCl cotransporter and ENaC, as well as 11β-OH steroid dehydrogenase and the Na/Ca exchanger. DCT-2 has molecular features of the early DCT and the CT. We have shown that this segment is much larger in humans than in rats. If this lesion is due to blockade of the sole Na entry pathway in DCT-1 the relatively longer length of this segment in humans compared to rats may be the reason why humans tolerate thiazides better in the long-term.

Responses of Intercalated Cells to Potassium Restriction: Apical Membrane Changes

Potassium depletion appears to be associated with morphologic responses that are restricted to IC. Oliver et al. (489) originally proposed that the population of IC was labile and that the number of IC varied with K^+ balance. Indeed, several experimental maneuvers have been suggested to change the relative frequency of IC (see Richet and Hagege [490] for a review). Several groups of workers have recently reexamined this issue with respect to the collecting ducts of rats. Although the studies of Evan et al. (491) suggested a reciprocal relationship between the number of IC and K^+ intake, other workers (234,283,492) did not detect any significant change in the population of these cells, regardless of the K^+ content of the diet. The weight of current evidence appears to favor the view that IC form a distinct and fixed cell population, which is seen from the CT to the outer medullary collecting duct, at least in rats.

Giebisch and co-workers (234,283) have suggested that earlier studies, relying mainly or exclusively on light microscopy for identification of IC (or dark cells), may have led to some confusion. Some cells, having characteristics of both principal and IC, have been identified under a variety of experimental conditions ("intermediate cells" (452,493)). These observations have been used to suggest that IC may represent only a specific functional state of principal cells. Alternatively, Stetson et al. (283) suggested that the total number of IC is not altered by potassium depletion, but that apparent changes in the frequency of these cells reported in earlier studies reflected only an alteration in cell structure within a fixed cell population. Specifically, these workers (283), using freeze-fracture techniques, were able to distinguish two different forms of the IC (possibly similar to dark and gray cells [494]) in outer medullary ducts of rats. Also, they noted that potassium depletion was associated with an increase in luminal membrane surface area, a decrease in apical cytoplasmic vesicles, and an increase in the density of rod-shaped particles in luminal membranes of these cells.

The morphologic changes seen in IC during potassium depletion are strikingly similar to those reported by Madsen and Tisher (289,290) during acute respiratory or chronic metabolic acidosis (see next section). Since potassium depletion in rats is associated with chronic metabolic alkalosis and an increase in ammonia excretion by the distal nephron (495), the changes in potassium depletion could be related to the alterations in acid-base balance rather than to the potassium depletion per se. Further studies will be required to distinguish the relative importance of these two physiologic factors.

In contrast to the observations of Stetson et al. (283) in the medullary collecting duct in rats (cited previously), Stanton et al. (234) did not find any morphologic alterations in IC of the *initial* collecting tubule after potassium depletion in this same species. These discrepant findings are consistent, however, with the studies of Toback et al. (496), who noted that only IC in the medulla were affected by potassium depletion. Taken together, these studies indicate that the apical membrane area of IC, at least in the medullary collecting duct, is increased during potassium depletion. Since this medullary nephron segment is involved in the enhanced potassium reabsorption that occurs during potassium depletion, this structural change may be related to an increase in apical membrane potassium transport units in accord with the membrane amplification principle.

Heterogeneity in Na^+-K^+-ATPase Activity and Basolateral Membrane Area in Thin Limbs

Morphologic studies of thin limbs of the loops of Henle have shown that this region of the nephron exhibits considerable morphologic heterogeneity in several mammalian species, including rats (171), mice (172), and rabbits (497). In particular, the descending thin limb of juxtamedullary nephrons in the outer medulla of mice and rats is characterized by extensive cell-to-cell interdigitations of basolateral membrane, shallow tight junctions, and a large junctional length per unit of surface area. In contrast, there is little amplification of basolateral membrane area in this same segment in rabbits.

Recent ultrastructural studies on the cytochemical localization of Na^+-K^+-ATPase in the medulla (498) have shown that this enzyme is present on basolateral membranes of descending thin limbs in the outer medulla of rats (190,191), but not rabbits. No other thin limb segments in either species exhibited cytochemical reactivity for this enzyme. These studies have suggested to some investigators that this particular segment of the descending limb might be involved in active ion transport, since the sensitivity of the cytochemical reaction is generally considered such that positive reactivity is demonstrable only in segments of the nephron that transport large quantities of salt. Ernst and Schreiber (191) have proposed that this segment of the descending limb in rats may possess an active NaCl secretory mechanism that would increase salt entry into the descending portion of the loop of Henle, thereby enhancing countercurrent multiplication. Accordingly, the observed differences between rabbits and rats in Na^+-K^+-ATPase activity and basolateral membrane area of outer medullary thin descending limbs may reflect the greater concentrating ability of the latter species.

Another possible role for the large amount of Na^+-K^+-ATPase in this segment of the nephron has been proposed by Jamison and colleagues (499,500). These researchers observed that the delivery rate of K^+ to the end of the descending limb of long-looped nephrons in the rat was equal to the filtered load, suggesting that there was considerable secretion (active or passive) of this ion into loop urine somewhere proximally. Although net K^+ secretion has been demonstrated in the S_3 segment of the proximal tubule of both superficial and juxtamedullary nephrons (501,502), Jamison et al. (513) have argued that this may not be sufficient to account completely for the rates of K^+ delivery observed *in vivo*. However, this latter argument depends on the concentration of K^+ in medullary interstitial fluid. The results of these functional studies suggest that the outer medullary thin descending limb may be involved in the secretion of K^+ into the urine, a possibility consistent with the unique biochemical and ultrastructural features of this thin limb segment.

Structure-Function Relationships: Trafficking of Membrane Vesicles and Alterations in Transport Processes

Newly synthesized proteins are transported to plasma membranes by a means of small transport vesicles and this process involves budding and docking steps as vesicles move from donor to acceptor membranes along the intracellular transport pathway (503). Guanosine triphosphate (GTP)-binding proteins have recently been established as playing important roles in the regulation of vesicular transport (504). It is now clear that similar vesicular trafficking processes—by the movement of transporter-containing vesicles into and out of apical membranes—are involved in the regulation of certain transport processes in renal tubular epithelial cells.

Vesicular Trafficking and Proton Transport. Morphologic, biochemical, and physiologic studies of turtle and toad bladders have added considerably to our current understanding of proton secretion by the mammalian collecting duct. The capacity of these bladders to secrete protons is related to the number of cells containing carbonic anhydrase (505), which may exist in two functional states that can be distinguished morphologically on the basis of their apical surface area and surface topography (506–508). In the hydrogen-secreting state, these cells have large apical membrane surfaces that are folded to form microplicae; within 2 hours after inhibition of proton secretion, the surface area of these cells is dramatically reduced, and the surface topography changes from microplicae to microvilli (506,509).

A proton-translocating ATPase (H^+-ATPase) appears to be responsible for H^+ secretion in the turtle bladder (510), and recent studies have suggested that this proton pump is inserted into the apical membrane by an exocytic process in response to stimuli that enhance net acid secretion (511–513). The membranes containing proton pumps are thought to reside in vesicles that are located in the cytoplasm immediately below the apical surface, and stimulation of acid secretion results in insertion of these vesicles into the apical membrane by a cytoskeletal-dependent process (525). This vesicle-insertion process then results in the amplification of the apical membrane surface area. This general mechanism has the advantage of providing a pool of transport units (pumps) that can be activated rapidly by an appropriate stimulus and, in general terms, is similar to the shuttle mechanism proposed by Wade et al. (513) for the insertion of water transport units (presumably channels) into the apical membranes of ADH-responsive epithelia.

The secretion of protons in the mammalian collecting duct, like that in the turtle bladder, appears to be mediated by an H^+-ATPase (511,512,514), and this process is facilitated by carbonic anhydrase and, hence, can be inhibited by acetazolamide. Humbert et al. (515) first noted that rod-shaped particles were present in IC, but not in principal cells of the mammalian collecting duct. The studies of Madsen and Tisher (289,290) suggest that the H^+ secretory mechanism (i.e., H^+-ATPase) may reside on subapical vesicles in the IC of the outer medullary collecting duct and be transported from the cytosolic, subapical tubulovesicular pool to the apical surface in response to acidosis. These workers have demonstrated small coated vesicles, resembling clathrin vesicles, involved in absorptive endocytosis and secretion, budding off Golgi cisternae and fusing with subapical vesicles.

These vesicles in the IC do not contain clathrin (in contrast to the vesicles-containing water channels; see next section), but are "coated" with the cytoplasmic domains of proton pumps (516,517,518). The increase in apical membrane surface density and decrease in the number of subapical vesicles during acute respiratory (290) and chronic metabolic (289) acidosis is consistent with their proposed cycling of vesicles containing the H^+-transporting units to the apical membrane. The general mechanism appears to be quite similar to that proposed for the stimulation of proton secretion in the turtle bladder (519–521); see Brown (516) for a review of the recycling mechanism). Thus, the cycling of cytosolic vesicles containing specific transport proteins appears to represent one way of rapidly altering the H^+ transport function in a number of epithelia.

Vesicular Trafficking and Water Transport: ADH-Mediated Cycling of Cytosolic Vesicles into Apical Membranes. ADH regulates water reabsorption across mammalian collecting ducts, as well as across ADH-responsive anuran epithelia (frog skin and toad urinary bladder), by increasing the water permeability of the luminal membranes of these epithelia (212,522,523). Observations on rabbit CCT perfused *in vitro* suggest that the major route for water permeation across luminal membranes of this nephron segment, either with or without ADH, is through small aqueous channels that span the lipid bilayer core of these plasma membranes. The effect of ADH is to increase the number of these small aqueous channels in apical plasma membranes, thereby increasing its water permeability (212,524,525). Morphologic observations on the response of the CCD to ADH have confirmed that the rate-limiting site for water permeation, and thus the final site of action of ADH, is at the luminal membrane (526–528). Using transmission electron micrographs of tubules fixed at various stages in the ADH response, several researchers (526,528) have shown that, in the presence of a

lumen—to–bath osmotic flow, there is an increase in the apex—to-base cell height, intracellular vacuolation, and distention of lateral intercellular spaces in this nephron segment. Both principal and IC demonstrated these ADH-associated morphologic changes. Similar morphologic changes are also seen in the papillary collecting ducts of rats in going from water diuresis to antidiuresis (529).

Kirk et al. (523) determined the temporal sequence of the structural changes associated with ADH-induced osmotic water flow in single CCT segments, using differential-interference contrast microscopy. These investigators demonstrated that (i) the time courses of ADH-mediated transepithelial osmotic water flow and the increases in the volumes of cells and lateral intercellular spaces were similar and (ii) that these morphologic changes were both reversible and seen only in the presence of a lumen—to-bath osmotic gradient (i.e., when there was transepithelial water flow). Furthermore, cytoplasmic vacuoles formed only during the latter stages of the osmotic response, as well as after the increases in cell volume and lateral intercellular space dilation, and their formation continued after this transport (and morphologic) response had reached a steady state. Kirk et al. (525) interpreted these results as indicating that transcellular water flow in the CCT follows two parallel pathways: one that does not require cytosolic vacuoles and rapidly empties into lateral intercellular spaces, and another that involves these large cytosolic vacuoles and is much slower. These conclusions are in general agreement with the observation of Andreoli and coworkers (525,526) that there may exist a significant cellular (cytosolic) constraint on water diffusion in this nephron segment.

Over the past decade, ultrastructural studies of ADH-responsive anuran epithelia (principally toad and frog urinary bladders), using freeze-fracture techniques, have led to the appreciation that a number of ultrastructural changes occur in the apical membranes of granular cells in association with the application of ADH to the serosal membrane. The freeze-fracture technique physically splits membranes, presumably between the bilayer leaflets, to expose any particles embedded in the membrane. These particles are thought to represent membrane proteins. Chevalier et al. (527) were the first to report an aggregation of intramembranous particles in the apical membrane in frog urinary bladders treated with oxytocin. Kachadorian et al. (528) reported similar apical membrane aggregates in toad urinary bladder exposed to ADH, and demonstrated that the aggregates are organized in very discrete rows.

Much evidence has accumulated demonstrating that these aggregates are associated with the ADH-induced increase in apical membrane water permeability (see various authors [509,526,527] for reviews). The appearance of aggregates depends on the interstitial application of ADH (524,525,528–533), cAMP (532,533), dehydration, or all of these, but not hydration. It is independent of an imposed osmotic gradient (532,534,535) and it can be inhibited by drugs, such as methohexital, which selectively inhibits the ADH-induced increase in water flow, but not by phloretin, which inhibits ADH-induced urea, but not water movement (534,535).

Although the number or cumulative area, or both, of aggregates has been correlated with the level of ADH-induced osmotic water flow in many circumstances (529–534), under certain conditions, the correlation is less consistent. When the time course of aggregate accumulation in toad urinary bladder was studied at short intervals after the addition of ADH, aggregate accumulation was maximal before osmotic water flow peaked (535), and when ADH-stimulated water flow declined after 60 minutes of hormone exposure, the aggregate number did not (376). However, this dissociation between ADH-induced intramembranous particle aggregation and net water flow may not necessarily mean a lack of association between aggregation and the water permeability of apical membrane. Recent observations in toad urinary bladders with the use of cytochalasin B, a toxin that prevents polymerization of intracellular actin filaments (microfilaments), indicate that this toxin inhibits the maximal increase in osmotic water flow induced by ADH, but does not prevent the ADH-induced increase in ^3H_2O diffusion. In association with these effects, the toxin produces marked granular cell swelling and the formation of large intracellular water-filled vacuoles, or "lakes" (536,537). These studies suggest that cytochalasin B may inhibit water flow by disrupting the cellular or cytosolic pathway of water movement and not by decreasing the water permeability of the apical membrane.

In the absence of ADH stimulation, similar aggregates have been identified in both toad (533,537) and frog urinary bladders in vesicles beneath the apical membranes of granular cells. After the addition of ADH, the number of aggregate-containing vesicles decreases markedly and occasionally these vesicles have been seen fusing with apical membranes (521,538). The frequency of these fusion events, seen on freeze-fracture sections of apical membranes in response to ADH, appears to correlate with the accumulation of aggregates, and this observation has led to the hypothesis that the water permeation sites are"shuttled"on the membranes of these vesicles to the apical membrane under the influence of ADH (513). Indeed, an increase in apical membrane capacitance, presumably indicative of an increase in apical membrane area, has recently been demonstrated in ADH-treated urinary bladders of toads (539,540). However, it is unlikely that fusion of these aggregate-containing vesicles with the apical membrane can account entirely for the large increase in apical membrane capacitance. Furthermore, the surface topography of granular cells changes from broad ridgelike villous structures to fine microvillus structures in the presence of ADH and this surface change could produce the large increase in membrane surface area and capacitance. Since the ADH-induced increase in water flow can be dissociated from this change in the appearance of the apical membrane surface, the relation between these physiologic and morphologic hormone effects is not clear.

Structural studies performed in mammalian collecting ducts have demonstrated apical intramembranous particle aggregates in early (541,542), but not late (543), papillary-collecting ducts of rats and in outer medullary and CCT of rabbits. The aggregates are confined to the apical membranes of principal cells (544). The particle aggregates in these nephron segments are similar to, but not identical with, those of anuran epithelia (543,544). Brown and co-workers (see Brown [516] for review) have presented compelling evidence that clathrin-coated vesicles participate in water-channel recycling in the mammalian collecting duct and that vasopressin stimulates the appearance of these "coated" vesicles. In addition, Edwards and Harmanci (542) have shown that in Brattleboro rats with central diabetes insipidus, apical membrane particle clusters did not parallel urine osmolality in dehydrated rats in the absence of exogenous ADH. These apical membrane aggregates appear to be related to the establishment of ADH-dependent antidiuresis (542). Verkman and colleagues (545) have demonstrated that endosomes in collecting tubule cells from Brattleboro rats contain vasopressin-sensitive water channels.

Eleven different waterchannel-forming intrinsic membrane proteins have been identified (AQPs 0 to 10; [546–548] for reviews) following the initial identification of CHIP28 by Agre et al. (AQP 1 [547]. They fall into two major groups: (i) the orthodox aquaporins that are water selective and include AQPs 0, 2, 4, and 5; and (ii) the multifunctional aquaglyceroporins that transport water, glycerol and other small solutes, and include AQPs 3, 7, and 9. AQP 2 has now been identified as the ADH-sensitive water channel present in apical membranes of the collecting duct (549–551), and the human gene for this water channel is on chromosome 12q13. AQP 3 is ADH insensitive and appears to provide for the high intrinsic water permeability

of the basolateral membrane of the collecting duct (552–555). AQP 4 is expressed in the basolateral membrane of the IMCD and is responsible for most of the basolateral membrane water permeability in this segment (556). AQP 5 is expressed in the apical membrane of type I pneumocytes (557). AQP 6 is found in IC, but has very low intrinsic water permeability (558). AQP 7 is expressed in the S3 segment of the proximal tubule (559).

The phenotypical analysis of aquaporin knockout mice has provided important new insights into the functional roles of aquaporins in kidney. AQP 1 knockout decreases transepithelial water permeability in the proximal tubule (S2 segment) fivefold (560). End proximal tubular sampling by free-flow micropuncture shows a 50% reduction in isosmolar fluid reabsorption. Single nephron GFR falls due to a stimulation of tubuloglomerular feedback and distal fluid delivery is normal. Urine output in these animals is increased as a result of an inability to maintain a hypertonic interstitium because of a lack of expression of AQP 1 in the thin descending limb of Henle. Freeze-fracture studies of these animals showed that AQP 1 is responsible for the particles observed in the cell membrane of the thin descending limb (561). AQP 3 knockout mice are polyuric and polydipsic and are able to generate a partially concentrated urine in response to exogenous vasopressin or water deprivation (562). The countercurrent exchange system is largely intact. The nephrogenic diabetes insipidus in these animals is related to a threefold decrease in the water permeability of the basolateral membrane of the CCD, as well as a downregulation of AQP 2. AQP 4 knockout decreases water permeability of the IMCD fourfold and causes a mild defect in urinary concentrating ability (563). Further studies establishes that AQP 4 is the "orthogonal array protein (OAP)" (564), which are square arrays of intramembrane particles that have been observed by freeze-fracture electron microscopy in the basolateral cell membrane of the collecting duct and a variety of other cell types. AQP 4 is highly expressed in astroglial cells at the blood–brain and brain–cerebrospinal fluid barriers. These animals are resistant to brain edema that occurs with acute water intoxication and ischemic stroke (565).

SUMMARY

Morphologic, biochemical, and physiologic investigations have made us aware of the great diversity (heterogeneity) of structure and function along the nephron. Combining these methods, as in analysis of the morphologic changes associated with specific alterations in conditions (e.g., diet, drugs, and hormones), can provide powerful insights into the specific roles of both individual nephron segments and of cell types within a single region of the nephron. There is overwhelming evidence that many, if not all, nephron segments adapt to changes in environmental conditions and that these adaptations involve alterations not only in transport processes, but also in cell morphology and biochemistry. Thus, it is important to view the structure of nephron segments in a dynamic, not in a static, sense. We have attempted to illustrate some of the more important or well-recognized structural-functional relationships in the nephron. There seems to be little doubt that many more such relations will become evident in the future and thereby contribute greatly to our understanding of the complex function of the nephron.

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CHAPTER 2 ■ RENAL CIRCULATION AND GLOMERULAR HEMODYNAMICS

WILLIAM J. ARENDSHORST AND L. GABRIEL NAVAR

The capability of the kidneys to achieve their sophisticated homeostatic function is optimized by an intricate microvascular system that adjusts vascular resistance to maintain an appropriate intrarenal hemodynamic environment. The intrinsic and extrinsic renal regulatory mechanisms are responsible for controlling the one-fifth of the cardiac output that circulates through the kidneys. Essentially all the renal blood flow (RBF) traverses through the glomerular capillaries, where about 20% of the plasma is filtered. The complex glomerular filtration apparatus is truly unique in having both a very high hydraulic conductivity and a remarkably low permeability to proteins. One major function of the renal vasculature is to regulate the intraglomerular forces so that an adequate, yet not excessive, volume is filtered into the urinary tubules. In this chapter, we discuss the characteristics of the filtering and the reabsorbing microcirculatory structures in the normal kidney. Particular emphasis is placed on the dynamic interactions among the intrarenal paracrine and extrarenal homeostatic mechanisms that help to regulate these processes. To allow a better appreciation of basic mechanisms, some structural relationships and fundamental concepts related to vascular smooth muscle, endothelial cells, and other components of the renal microvascular network are also discussed (1–3).

MAGNITUDE OF RENAL BLOOD FLOW (RBF) AND GLOMERULAR FILTRATION RATE

The multiple intrarenal parallel arteriolar pathways provide the kidneys with a very low vascular resistance. They normally receive about 20% of the cardiac output. This amounts to a blood flow of 1,000 to 1,200 mL/min in a 70- to 75-kg person. RBF is even more impressive when considered per unit of kidney weight, as the kidneys account for only 0.5% of the total body weight, or about 300 g. Thus, blood flow per gram of kidney weight is about 4 mL/min (Table 2-1), which is 5 to 50 times greater than the flow through other organs and circulatory beds. Based on a total of 1 million glomeruli in each kidney or a glomerular density of 7,000 glomeruli/g, the average blood flow per glomerulus is 570 nL/min. This large flow, coupled with the maintenance of a high hydrostatic pressure in glomerular capillaries, allows the filtration of about 20% of the plasma, which amounts to an average glomerular filtration rate (GFR) of 120 mL/min, or 170 L/day (4–9).

The extraordinarily high RBF is in marked excess of that simply required to provide the renal parenchyma with adequate supplies of oxygen and nutrients. For this reason, it is generally recognized that RBF is regulated primarily to maintain the glomerular and peritubular intrarenal hemodynamic environments at levels compatible with optimum delivery of filtrate to the nephrons and appropriate reabsorption of fluid back into the vascular system.

Relationship of Renal Blood Flow to Oxygen Consumption

While oxygen utilization is not a major determinant of RBF, oxygen consumption by the kidneys is still quite high, because of the very high metabolic activity of the tubules. Over 99% of the filtered fluid, electrolytes, and essential organic nutrients are normally reabsorbed by the tubules and returned to the circulation via the peritubular capillaries. The tubular reabsorptive processes depend either directly or indirectly on the integrity of the epithelial transport systems, in particular, the energy requiring Na-K-ATPase. These tubular enzyme systems account for the majority of the oxygen consumption.

RBF is about 400 mL/min per 100 g of tissue and the arteriovenous oxygen difference is relatively low—on the order of only 1 to 2 mL/dL of blood. Thus, oxygen consumption by the kidney is about 8 ml O_2/min or 400 μM O_2/min per 100 g, which amounts to 6% to 8% of the whole body oxygen consumption. This level of oxygen utilization is relatively constant and is not reduced by moderate hypoxemia. Under physiological conditions, there is a generally consistent relationship between RBF and renal oxygen consumption. However, this relationship is a consequence of the associated changes in GFR and filtered sodium load, reflecting a direct causal relationship between tubular sodium reabsorption and oxygen consumption, as shown in Figure 2-1. The rate of actively transported sodium appears to be the primary determinant of the rate of oxygen consumption. About 20% of the consumption, or approximately 100 μM O_2/min per 100 g of kidney, is used for basal metabolic purposes and continues even in the absence of filtration. Above this basal rate, there is a linear relationship. Approximately 27 to 35 mEq of sodium is reabsorbed per millimole of oxygen consumed, depending on the contribution of passive transport via paracellular pathways, which may be in the order of 40%.

The balance of oxygen consumption for sodium reabsorption and oxygen delivery is reflected by the tissue pressure of O_2 (PO_2). A cortical-medullary gradient of oxygenation exists in the kidney. In the superficial cortex, PO_2 in efferent arterioles is 40 to 45 mm Hg, with values of 40 mm Hg recorded in other cortical structures (proximal tubule, distal tubule, superficial cortical tissue), and 30 mm Hg in the deep cortex 30 mm Hg. The fact that PO_2 in the renal vein exceeds that of any site in the cortex indicates precapillary shunting of O_2 from artery to vein. Renal medullary PO_2 is about 20 mm Hg, with cells functioning in a climate of constant relative hypoxia (10–12).

In the renal medullary microcirculation, the net amount of O_2 reabsorbed from vasa recta into the interstitium is about 10^{-6} mol/s, i.e., significantly lower than estimated medullary oxygen requirements based on active sodium reabsorption. Low inner medullary PO_2 results from the countercurrent arrangement of vasa recta and high vascular permeability to O_2, as well as high metabolic needs. Diffusional shunting of O_2 between descending and ascending vasa recta explains why

TABLE 2-1

RENAL HEMODYNAMIC FUNCTION IN HUMANS

	Total	Per kilogram body weight	Per gram kidney weight
Renal blood flow	1,200 mL/min	17 mL/min	4 mL/min
Renal plasma flow	670 mL/min	9.6 mL/min	2.2 mL/min
Glomerular filtration rate	130 mL/min	1.9 mL/min	0.45 mL/min
Number of glomeruli	2 million	28,500	7,000
Oxygen consumption	1,200 μM O_2/min	17 μM O_2/min	4 μM O_2/min

a 20-mm Hg decrease in initial PO_2 at the corticomedullary border only leads to a small drop in PO_2 at the papillary tip PO_2 (<2 mm Hg with baseline parameter values). On the other hand, small decreases in medullary blood flow, hematocrit, and O_2 consumption by tubules markedly reduce interstitial PO_2. Without erythrocytes, papillary tip PO_2 cannot be maintained above 10 mm Hg, even when oxygen consumption is zero. An increase in medullary blood flow during water diuresis improves medullary O_2 delivery.

The renal medulla normally functions in an hypoxic environment. Tissue hypoxia impacts on oxygen-regulated genes and leads to the renal production of adrenomedullin and erythropoietin. Hypoxia-inducible factor-1α (HIF-1α) is a transcription factor that regulates the oxygen-dependent expression of many genes. This transcription factor may contribute to gene expression in renal medullary cells that function normally under hypoxic conditions. In this regard, the classic loop diuretic, furosemide markedly increases renal medullary PO_2 levels (from 20 to 50 mm Hg) in association inhibited reabsorption along the ascending limb of Henle's loop and a reduction in HIF-1α.

The efficiency of the coupling between tubular transport and O_2 consumption is modified by paracrine/autocrine factors. For example, nitric oxide (NO) normally suppresses O_2 consumption by epithelial mitochondria. Inhibition of NO synthesis increases oxygen extraction and O_2 consumption and reduces the efficiency of sodium transport. Regulation of renal O_2 consumption by NO may become impaired during oxidative stress when superoxide production is excessive. Oxidative stress and decreased availability/activity of NO can lead to reduced intrarenal PO_2 due to enhanced O_2 usage (QO_2) relative to tubular sodium transport.

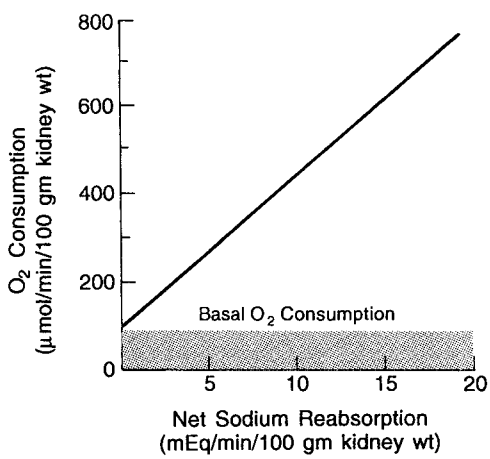

FIGURE 2-1. Relationship between tubular sodium reabsorption and oxygen consumption by the kidney. The primary determinant of oxygen consumption above basal levels is the rate of active sodium transport. (From: Gotshall RW, Miles DS, Sexson WR. Renal oxygen delivery and consumption during progressive hypoxemia in the anesthetized dog. *Proc Soc Exp Biol Med* 1983;174:363; Ostensen J, Stokke ES. Energy requirement of sodium reabsorption in the thick ascending limb of Henle's loop in the dog kidney: effects of bumetanide and ouabain. *Acta Physiol Scand* 1996;157:275.)

Endothelial-dependent NO plays a role in the regulation of renal O_2 consumption in normal kidney. Baseline cortical O_2 consumption is about 600 nmol/min per g tissue. Stimulation of NO production by bradykinin or administration of a NO donor reduces O_2 consumption 25% to 30% in the renal cortex and 30% in the medulla. Superoxide scavenging of NO attenuates the stimulatory effect of bradykinin or NO donor (11,13–17).

CHARACTERISTICS OF THE CONTRACTILE PROCESS

Structural-Functional Aspects

There are important interactions among various cell types in the microvasculature that determine the caliber of the small resistance vessels. The complex vasculature of the kidney (Fig. 2-2) allows the fine regulation of the intrarenal hemodynamic environment. Smooth muscle cells surround all vascular structures from the main renal artery to the individual afferent arterioles. The preglomerular vasculature also has extensive innervation and responds to renal nerve stimulation and many different hormonal, paracrine, and physical stimuli. Nevertheless, most of the fine control of preglomerular resistance occurs in the small-diameter afferent arterioles. The afferent arterioles vary in length and in the angle at which they branch from the interlobular arteries; those in juxtamedullary portions branch at a much sharper angle. In addition, smooth muscle cells of the afferent arterioles are modified as the vessels approach the glomerulus. The proximal portions of afferent arterioles possess typical elongated smooth muscle cells, while cells closer to the glomerulus are more rounded and many possess granules (5,7,18).

As is shown in Figure 2-3, the magnitude of the pressure drop within the arterial tree is relatively small up to the terminal segments of the afferent arterioles. About 70% of the preglomerular pressure drop occurs in the terminal portion of the afferent arteriole. Some studies in rats suggest that the arteries and larger arterioles leading to the superficial nephrons contribute more to this pressure drop. In any event, the pressure drop up to the glomerular capillary tuft is much lower than in other vascular beds. This allows a high hydrostatic pressure in the glomerular capillaries, which is much greater than the plasma colloid osmotic pressure and is thus responsible for ultrafiltration of fluid into Bowman's space (5,7,18–22).

The terminal portion of the afferent arterioles contains the modified granular epithelioid cells that form part of the juxtaglomerular apparatus. The granules contain renin and other

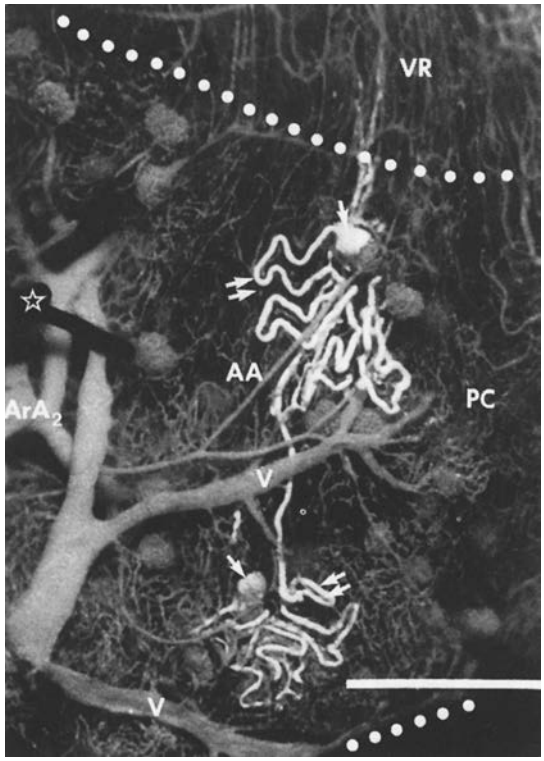

FIGURE 2-2. Photograph of renal microcirculation showing branching of afferent arterioles from arcuate arteries, glomerular capillary tufts, efferent arterioles, peritubular capillaries, initial portions of vasa recta, and venous system. The vessels are filled with dark elastic polymer (Microfill) and two tubules are filled with light polymer, showing Bowman's capsule (*single arrow*) and proximal tubules (*double arrow*) and parts of loop of Henle. *AA*, afferent arteriole; ArA_2, arcuate artery; *PC*, peritubular capillary; *V*, venule; *VR*, vasa recta. (From: Casellas D, Navar LG. *In vitro* perfusion of juxtamedullary nephrons in rats. *Am J Physiol* 1984;15:F349, with permission.)

components of the renin–angiotensin system, and the extent of granulation varies inversely with sodium intake. There is a reciprocal relationship between the amount of renin and actin and, it has been suggested, that granular cells have only residual contractile capability. As is shown in Figure 2-4, the juxtaglomerular granular cells are adjacent to the macula densa (MD) segment at the end of the ascending loop of Henle and they are associated with the nongranular extraglomerular mesangial cells that fill the area between the afferent and efferent arterioles. The appearance of the MD cells with large nuclei along with their close apposition to the glomerular vessels serve as the morphologic basis for the concepts that alterations in flow or composition of the tubular fluid generate signals that are transmitted to the afferent arteriole or juxtaglomerular cells to control vascular tone and renin release (1,14,23–28).

As an afferent arteriole approaches a glomerulus, the muscle cells surrounding the vessel intermingle with the extraglomerular mesangial cells and the intraglomerular mesangial cells. As it enters the glomerulus, the arteriole expands into a manifold lined by endothelial cells, which, in turn, gives rise to a series of glomerular capillary loops. The loops subdivide further into a branching system of exchange channels. Finally, the channels coalesce into a small number of terminal capillaries, which join to form the efferent arteriole. Greater structural detail regarding the glomerular capillaries that subserve the filtration process is provided in Chapter 1, Structural–Functional Relationships in the Kidney.

The efferent arterioles originate deep within the glomeruli and vary with regard to length, diameter, and density of smooth muscle cells. In the outer cortex, these vessels are relatively short, have a smaller diameter, and have a less well-developed muscular wall than efferent arterioles in deeper cortical regions. Many of the smooth muscle cells of the superficial efferent arterioles resemble pericytes that often extend onto the peritubular capillaries. In the midcortex, the efferent arterioles are usually longer and have a greater degree of smooth muscle development. In the deeper portion of the cortex, the efferent arterioles are more variable in length. Some efferent arterioles of

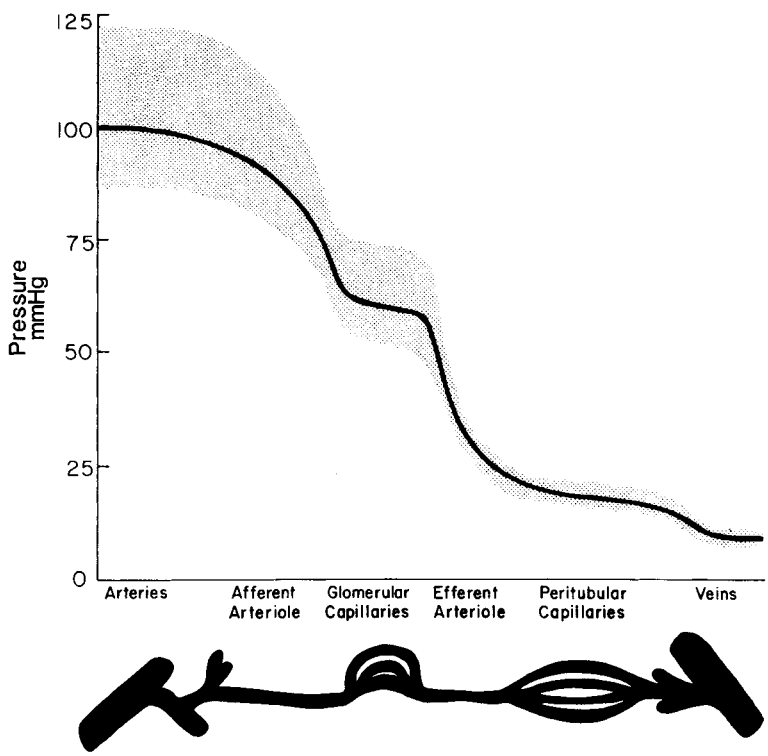

FIGURE 2-3. Representative pressure profile along renal microvasculature in normal kidney. The segments are depicted at the bottom of the graph and the range of ideal pulse pressures is represented by the stippled area.

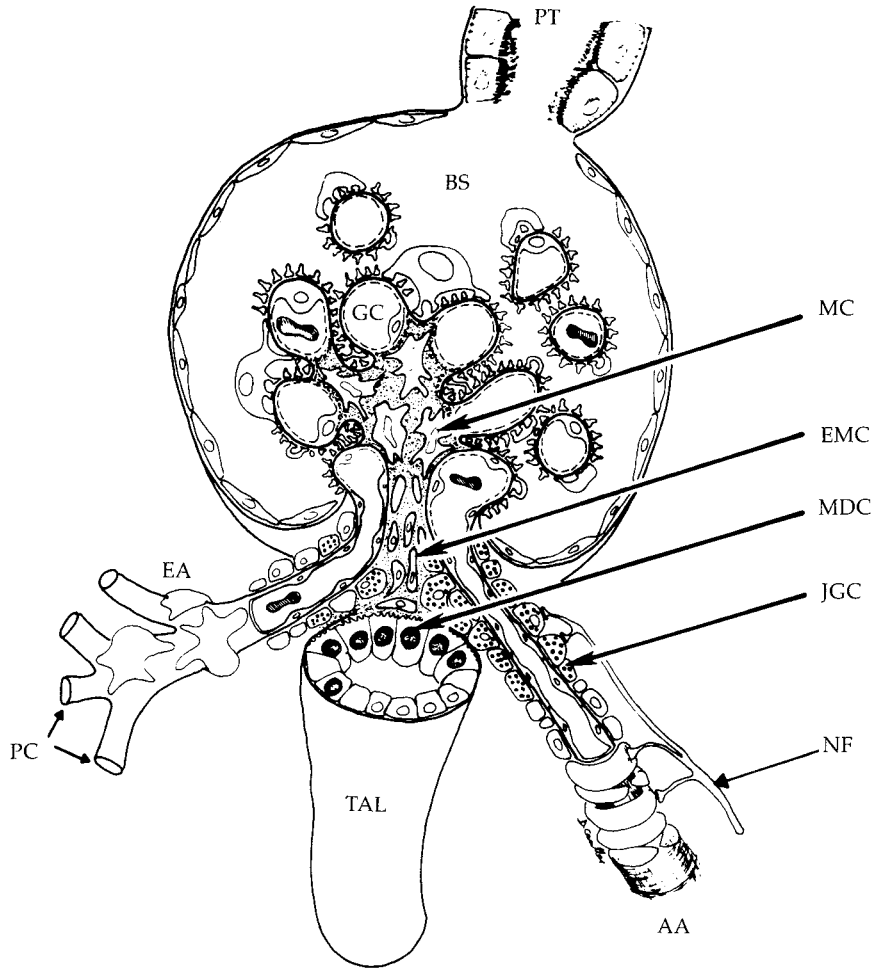

FIGURE 2-4. Drawing of glomerulus and juxtaglomerular complex consisting of afferent arteriole (*AA*) with the granular cells (*JGC*) of the juxtaglomerular apparatus, the extraglomerular mesangial cells (*EMC*), the macula densa (*MDC*) segment of the ascending loop of Henle, and the efferent arteriole (*EA*). Also shown are the thick ascending limb (*TAL*), proximal tubule (*PT*), Bowman's space (*BS*), glomerular capillaries (*GC*), peritubular capillaries (*PC*), mesangial cells (*MC*), and nerve fibers (*NF*). (Drawing from Casellas D, with permission.)

juxtamedullary nephrons give rise to typical cortical peritubular capillary systems, while others are much longer and descend toward the medulla. These two distinct capillary networks arising from the efferent arterioles subserve the reabsorptive functions of the cortex and medulla, respectively, and may be subject to independent regulation. At the corticomedullary border, the efferent arterioles break up into vascular bundles that branch into numerous descending vasa recta. Vasa recta branch to form a capillary plexus at each level within the medulla. There are three distinct capillary plexi, with the densest found in the inner stripe of the outer medulla. The ascending vasa recta are morphologically distinct from the descending vasa recta and ascend within vascular bundles to drain into the arcuate veins. The ascending vasa recta are more numerous and have a highly fenestrated, thin endothelium, while the descending vasa recta have a continuous thick endothelium. These anatomic differences suggest that the ascending vasa recta have a much greater permeability to macromolecules than the descending vasa recta (5,6,18,29–33).

Each resistance segment contributes to the regulation of glomerular blood flow and pressure in a unique manner because the glomerular capillary is "nested" between the afferent and efferent arterioles. Although a more quantitative analysis of their respective roles is presented in a following section, it should be appreciated that alterations in preglomerular arteriolar resistance produce changes in glomerular blood flow, pressure, and GFR, which are directionally similar. In contrast, changes in efferent arteriolar resistance cause more complex GFR responses because glomerular pressure and blood flow change in opposite directions. The maintenance of appropriate efferent arteriolar tone serves to keep glomerular capillary pressure sufficiently high to provide an adequate hydrostatic pressure for filtration. The efferent arterioles also are responsible for the marked decrease in pressure at the peritubular capillaries, which allows the reabsorptive force of the plasma colloid osmotic pressure to predominate (5,7–9,18,34).

There are important regional differences in the circulation within the kidney, which may have considerable functional significance. The relative distribution of glomerular and postglomerular blood flow is depicted in Figure 2-5. Glomerular blood flow is proportional to the size of the glomeruli. The larger deep juxtamedullary nephrons have higher flows than the superficial or midcortical glomeruli. With regard to the postglomerular blood flow, about 85% of the total RBF is distributed to the cortical peritubular capillaries, while 15% goes to the medullary region. Blood flow throughout the cortex is much higher than in the medulla and is higher in the outer cortex than in the inner cortex. Overall cortical blood flow averages 4 to 6 mL/min per gram of tissue. Medullary blood flow ranges from 2.0 to 3.5 mL/min per gram in the outer medulla to much lower values of approximately 0.2 to 1.0 mL/min per gram of tissue in the inner medulla and papilla. Regional mean transit times of intravascular indicators are 1 to 3 s for the cortex, 4 to 6 s for the outer medulla, and 10 to 30 s for the inner medulla the outer medulla, and 10 to 30 s for the inner medulla (5,7,29,32,35,36).

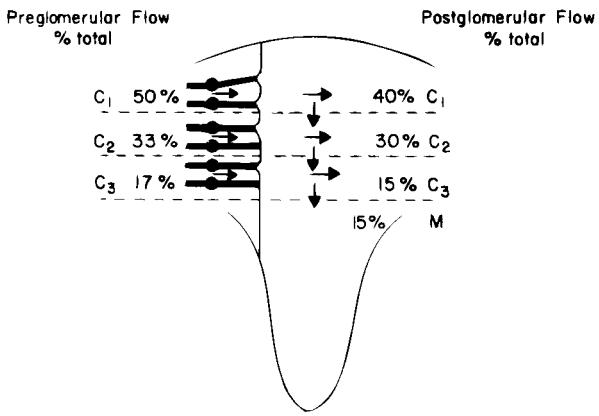

FIGURE 2-5. Distribution of glomerular and postglomerular flow and of cortical (C_1, C_2, C_3) and medullary (M) blood flow. The distribution of glomerular flow is expressed as a percentage of total blood flow; preglomerular flow is presented on the left, and postglomerular flow on the right. The deep cortical flow is subdivided to account for medullary flow distribution. As noted from the arrows, there is a general shift of the postglomerular blood flow toward deeper areas. (From: Navar LG, Evan AP, Rosivall L. Microcirculation of the kidneys. In: Mortillaro NA, ed. *The physiology and pharmacology of the microcirculation*. New York: Academic, 1983:354, with permission.)

Receptors and Receptor Activation

Contractile responses at various sites along the vascular network have different functional characteristics, depending on expression of receptor populations and/or activation mechanisms. The actions of circulating hormones and neural stimuli combined with local paracrine factors from endothelial and epithelial cells are expressed through different effector mechanisms to provide a highly integrated regulation of the renal microcirculation and the interstitial environment. Many vasoactive agents interact with membrane receptors on the vascular smooth muscle, endothelial, and mesangial cells.

Membrane receptors can be subdivided into three main groups in which the receptor is coupled to a guanine nucleotide-binding protein (G protein), regulates enzyme activity, or actively serves as part of an ion channel. Examples of the latter two groups are the atrial natriuretic peptide (ANP) receptor, guanylate cyclase, in vascular smooth muscle and the nicotinic–acetylcholine receptor that directly activates a cation channel at the neuromuscular junction. Almost all known vasoactive agents affect vasomotor tone via receptor coupling to G proteins. G protein-coupled receptors appear to share several common structural features. A single gene product has seven transmembrane domains with three extracellular and three intracellular loops. The extracellular loops act in concert with the transmembrane domains to bind the agonist. The intracellular loops function to activate a G protein. G proteins are heterotrimeric proteins consisting of α, β, and γ subunits. The α subunit of a G protein is unique for each receptor and is responsible for generating a specific intracellular signal(s). The β and γ subunits share a high degree of homology among G proteins; together they function to modulate the ability of the α subunit to generate the signal.

The general mechanism of action of G proteins involves a conformational change in the G protein following agonist binding to a membrane receptor, which, in turn, enables guanosine triphosphate (GTP) to replace guanosine diphosphate (GDP) on the α subunit. The G_α-GTP complex then dissociates from the β and γ subunits and interacts with an effector such as an enzyme or a channel. The intrinsic GTPase activity of the α subunit then hydrolyzes GTP to GDP, and the α-GDP complex reassociates with the β and γ subunits, which terminates the response. G proteins linked to adenylate cyclase are classified as $G\alpha_s$ or $G\alpha_i$, depending on whether the effect on adenylate cyclase and cAMP generation is stimulatory or inhibitory. Other G proteins, for example, $G\alpha q_{11/12}$ activate membrane-bound phospholipase C or activate membrane calcium channels, or both. An example of multiple effects an agonist can produce depending on receptor coupling to different G proteins (e.g., $G\alpha q_{11/12}$) is provided by norepinephrine. The binding of norepinephrine to an α_2-adrenoceptor inhibits adenylate cyclase, reduces the formation of cAMP, and attenuates activity of protein kinase A, whereas binding to a β_1- or β_2-adrenoceptor activates adenylate cyclase, increases cAMP generation, and enhances protein kinase A activity. In addition, norepinephrine can bind to an α_1-adrenoceptor and activate a $G\alpha_q$protein, which is coupled to phospholipase C, leading to formation of inositol trisphosphate (IP_3) and release of calcium from the sarcoplasmic reticulum (37–45).

Regulation of Microvascular Contractility

Changes in vascular perfusion are ultimately mediated by smooth muscle cell contraction or relaxation, which elicit a change in vessel radius and in vascular resistance. Multiple steps and enzymatic cascades are involved in the contractile process and many of these mechanisms can interact with one another to modulate the contractile response. A pivotal step in mediating the contractile response in vascular smooth muscle cells is an increase in the cytosolic concentration of free ionized calcium (Ca^{2+}) above its very low basal value of 10^{-7} M. (This is approximately 0.01% of the ionic calcium levels of the plasma and extracellular fluid, 1 mM.)

As shown in Figure 2-5, cytosolic Ca^{2+} binds with calmodulin. The Ca^{2+}-calmodulin complex activates myosin light chain kinase, leading to phosphorylation of myosin light chains, which interact with actin and adenosine triphosphate (ATP) to elicit tension development. Relaxation occurs as a consequence of removal or sequestration of Ca^{2+} from the cytosol and/or myosin dephosphorylation, which can result from alterations in the activity of several constituents of the cascade to alter the Ca^{2+} sensitivity of phosphorylation. Because there are multiple steps involved, many of the intracellular mechanisms can influence the magnitude of the contractile response to a specific stimulus. For example, both cAMP and cyclic guanosine monophosphate (cGMP)-dependent protein kinases may phosphorylate myosin light chain kinase and decrease the calcium sensitivity of myosin light chain phosphorylation. They might also lower intracellular Ca^{2+} levels by activating calcium extrusion mechanisms, stimulating reuptake into intracellular stores or inhibiting IP_3-mediated release of stored Ca^{2+}. Other protein kinases, such as phospholipid-dependent protein kinase C (PKC), exert the opposite effect and enhance the Ca^{2+} sensitivity of the contractile response. Decreases in Ca^{2+} signaling occur following dissociation of these ligands from cell surface receptors, receptor inactivation or internalization or as a consequence of membrane hyperpolarization, such as occurs when membrane potassium channels are activated (46–57).

The small monomeric GTPase Rho A affects contraction of VSMC by increasing Ca^{2+} sensitivity of the contractile apparatus. Rho A interacts with Rho kinase that phosphorylates the same site on MLC as MLC-kinase. Rho-kinase may also increase myosin phosphorylation by inhibiting myosin phosphatase and activation of phospholipase D. In glomerular arterioles, many vasoconstrictor G protein-coupled receptors (e.g., AT_1, ET_A, TP) signal through Gq and Gi proteins to activate the Rho/Rho kinase pathway. In addition to increasing Ca^{2+} sensitivity, Rho GTPases stimulate Ca^{2+} signaling by activating PLCβ isozymes. Rho signaling may be involved in

stretch-induced mechanotransduction, and some of the vasoconstriction produced by superoxide anion is mediated by Rho/Rho kinase. Rho/Rho kinase mediates basal myogenic and Ang II-induced tone in the afferent, but not the efferent arteriole. Some vasodilators appear to exert their actions by inhibiting Rho/Rho kinase. For example, NO induces vasodilation through cGMP-dependent protein kinase inhibition of RhoA (58–64).

Various hormones and drugs activate plasma membrane receptors to induce contraction by eliciting an increase in cytosolic Ca^{2+}. Cytosolic Ca^{2+} is increased through a combination of calcium entry from the extracellular environment and mobilization of calcium from internal stores. Ca^{2+} can be released from intracellular stores via two sets of receptors/release channels, activated by IP_3 or by a ryanodine-like ligand. It appears that Ca^{2+} is released from a common sarcoplasmic reticular pool, with uptake mediated by a common sarcoplasmic endoplasmic reticular Ca^{2+} ATPase (SERCA). Many vasoconstrictor agents increase intracellular calcium by activating a guanine nucleotide-binding protein (G protein) following ligand–receptor interaction. G protein stimulation results in phospholipase C activation and hydrolysis of membrane-bound phosphatidylinositol 4,5-diphosphate (PIP_2) into two pivotal intracellular messengers, IP_3 and 1,2-diacylglycerol (DAG). The soluble IP_3 binds to an IP_3 receptor, located on the sarcoplasmic reticulum, leading to activation of calcium channels and release of calcium into the cytoplasm. The lipophilic DAG remains within the membrane environment and activates one of the PKC isoforms that can phosphorylate various regulatory proteins. DAG may also be generated by the actions of phospholipase D on phosphatidylcholine that is not accompanied by concurrent IP_3 generation and the associated increase in intracellular calcium. Agents that activate PKC, such as phorbol esters, induce slowly developing, sustained contractions or enhance contractile responsiveness to other stimuli (2,46–49,54,65–68).

Activation of cell surface G protein-coupled receptors may also lead to stimulation of plasma membrane-bound cADP-ribosyl cyclase to convert the substrate nicotinamide adenine dinucleotide (NAD+) to adenosine 5′-cyclic diphosphate-ribose (cADP-ribose), a potent (nM range) Ca^{2+} mobilizing agent acting on the RyR to trigger Ca^{2+} release from the sarcoplasmic reticulum. cADP-ribose is a calmodulin-dependent Ca^{2+}-mobilizing second messenger system that acts independently of IP_3, with RyR Ca^{2+} release reinforced by IP_3-mediated Ca^{2+} mobilization. The RyR is extremely sensitive to $(Ca^{2+})_i$ and exhibits Ca^{2+}-induced Ca^{2+} release (CICR), a form of autopotentiation. Concentrations of Ca^{2+} over a wide range (200 to 1000 nM) enhance the open probability of RyR, which contrasts with a narrower range (150 to 250 nM) for the IP_3R (69–71).

Calcium entry can occur through a variety of pathways, including voltage-operated channels (VOC) that are activated upon membrane depolarization and voltage-independent receptor- and store-operated channels (ROC and SOC). Membrane depolarization downstream of G protein-coupled receptors activation is thought to result from activation of Cl^- channels or inactivation of K^+ channels, but the precise mechanism by which this occurs is not well-defined. Voltage-independent ROC and SOC also contribute to agonist-induced Ca^{2+} entry, although these Ca^{2+} entry mechanisms have not been thoroughly examined in preglomerular vessels and efferent arterioles. ROC are activated downstream of cell surface receptors, independent of Ca^{2+} mobilization, via signaling mechanisms involving DAG and calmodulin, and possible other intermediates. SOC are known to contribute to agonist-induced Ca^{2+} entry in renal and other vascular smooth muscle cells; they are activated by an unknown mechanism after depletion of Ca^{2+} stores (72–75).

Among the identified families of transient receptor potential (TRP) proteins, the canonical TRP (TRPC) family (TRPC1–TRPC7) is thought to be involved in Ca^{2+} entry in vascular smooth muscle cells, most likely functioning as voltage-independent SOC and/or ROC. TRPC1 proteins in vascular smooth muscle cells tend to form tetrameric channels in association with TRPC4 or TRPC5. In general, TRPC3, -6, and -7 appear to be activated by diacylglycerol independent of store depletion. TRPC6 is an essential component of α_1-adrenoceptor-activated cation channels in portal venous smooth muscle cells. Preglomerular arterioles have a predominance of TRPC3 and -6 subunits that may comprise SOC and/or ROC channels in these vessels (73,74,76–80).

Several different potassium channels have been identified; the most prominent are Ca^{2+}-activated and ATP-dependent potassium channels, which mediate relaxation by hyperpolarizing the cell membrane and reducing Ca^{2+} entry through voltage-gated calcium channels. Importantly, increases in intracellular ATP inhibit potassium channels, thus causing depolarization. Channel activity is reduced by intracellular ATP concentrations normally present, suggesting that the activity of this channel is quite low in normal cells and may serve a primary protective role during depletion of energy reserves (54,81–83).

Two major classes of well-recognized G protein-dependent receptors participate in the regulation of vascular tone through activation or inhibition of adenylate cyclase (Fig. 2-6). Ligand–receptor complexes that activate a stimulatory G protein (G_s) increase intracellular levels of cAMP and thus contribute to relaxation. cAMP activates protein kinase A (PKA), which has multiple actions, including phosphorylation of myosin light chain kinase, reducing its efficacy. As mentioned earlier, cAMP also may activate Ca^{2+} translocation mechanisms that increase extrusion of Ca^{2+} out of the cell or return Ca^{2+} to the sarcoplasmic reticulum or inhibit IP_3-mediated mobilization of Ca^{2+} from sarcoplasmic reticulum stores. Drugs or agents that increase cAMP produce increases in RBF and GFR. Small amounts of such ligands can buffer the action of vasoconstrictor agents without affecting baseline vascular tone. In contrast, ligand–receptor complexes coupled to the inhibitory G protein ($G\alpha_i$) reduce cAMP levels and cause greater contraction for a given level of cytosolic Ca^{2+} (46,53, 67,84,85).

Another family of receptors operates through G protein-dependent activation of guanylate cyclase, generation of cGMP, and activation of protein kinase to mediate vasodilation. In addition, there are at least two major guanylate cyclase activators that are not G protein dependent. Nitric oxide (NO), derived from endothelial cells, directly interacts with soluble guanylate cyclase. ANP also directly activates particulate guanylate cyclase. The mechanisms mediating cGMP-dependent vasorelaxation are thought to be similar, in part, to those utilized by cAMP. cGMP-dependent kinases lead to inhibition of voltage-gated Ca^{2+} channels, activation of a sodium/calcium exchanger, stimulation of Ca^{2+}-ATPase, inhibition of IP_3 formation, and phosphorylation of phospholamban, resulting in increased Ca^{2+}-ATPase activity in the sarcoplasmic reticulum. cGMP also may stimulate Ca^{2+}-activated potassium channels, which leads to hyperpolarization (86–88).

The arachidonic acid pathways constitute another intracellular signaling system. Increased cytosolic Ca^{2+} can activate phospholipase A_2 and release arachidonic acid from membrane phospholipids, resulting in production of various metabolites that can lead to vasodilation or vasoconstriction. Arachidonic acid metabolites exert effects through multiple pathways, including cAMP, cytosolic calcium, and inhibition of potassium channels. Arachidonic acid itself may increase Ca^{2+} entry via a noncapacitative Ca^{2+} entry channel. These will be discussed in detail in a later segment of the chapter (89–91).

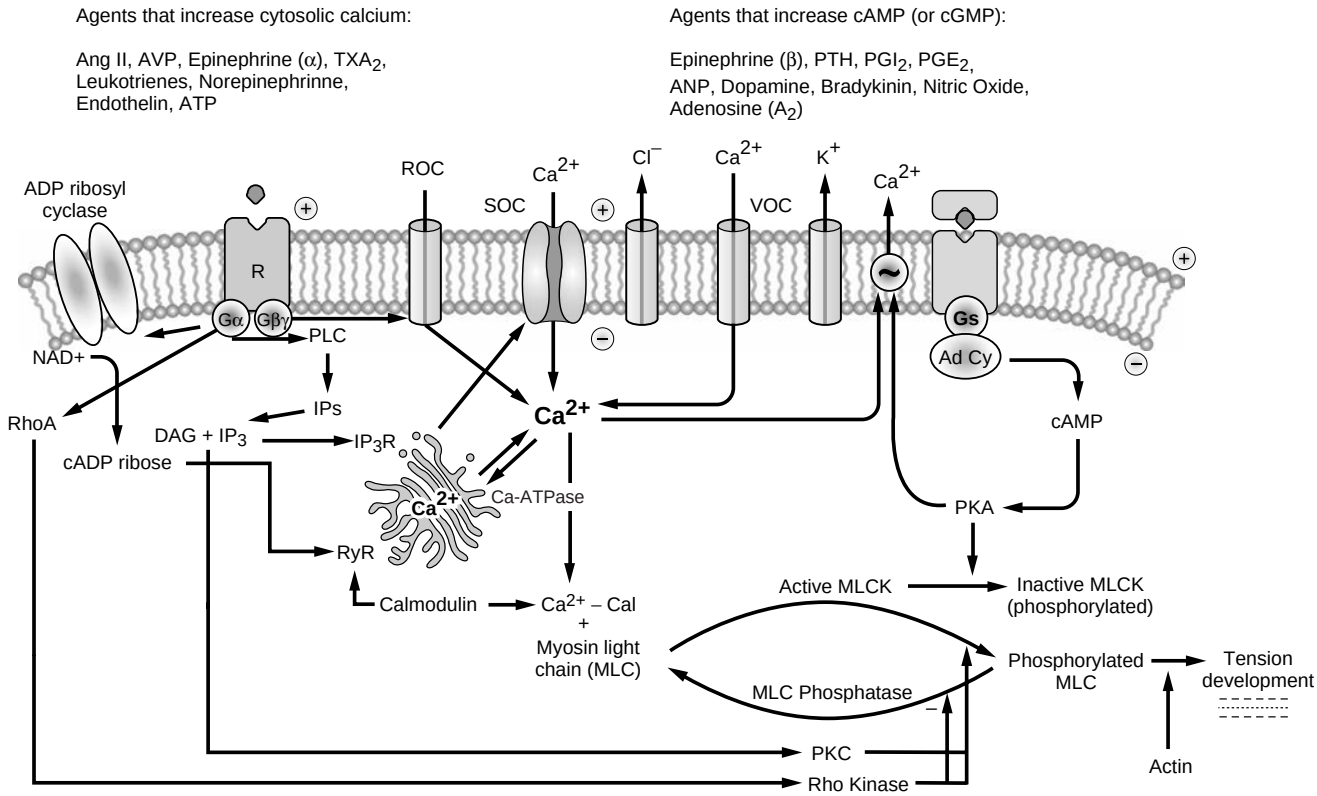

FIGURE 2-6. Primary intracellular signaling systems of smooth muscle cell or mesangial cell contraction, with effects of various hormones and vasoactive agents on the two major types of receptor systems. For ease of presentation, only two receptor mechanisms are depicted, but each agent acts on its own receptor system.

Differences in cellular sites and mechanisms of smooth muscle activation may be partially responsible for the large variety of renal hemodynamic responses produced by different vasoactive agents. There is a major difference in the mechanisms leading to Ca^{2+} activation in the vascular smooth muscle of the afferent and efferent arterioles. Preglomerular vessels have a strong dependence on L-type voltage-gated Ca^{2+} channels, whereas their influence is not readily apparent in efferent arterioles. Antagonists of Ca^{2+} influx through L-type, dihydropyridine-sensitive Ca^{2+} channels selectively block agonist-induced constriction of the preglomerular vessels, including the afferent arteriole, without affecting efferent arteriolar contraction. This is the case for Ang II, norepinephrine, endothelin, 5-hydroxytryptamine, and potassium chloride-induced depolarization (constriction). Agents such as nifedipine, diltiazem, and verapamil primarily cause afferent vasodilation and impair autoregulatory responses to changes in renal perfusion pressure and tubuloglomerular feedback responses. In contrast, T-type Ca^{2+} channels are active at both afferent and efferent arteriolar sites and can influence vascular responsiveness. T-type Ca^{2+} channel blockers vasodilate afferent and efferent arterioles and prevent contractile responses to various stimuli. In afferent arterioles, T-type channels may act cooperatively with L-type channels to bring about membrane depolarization and calcium entry. A primary action on the preglomerular vasculature also explains the large increases in GFR and glomerular capillary pressure produced by calcium entry blockers as well as inhibition of the tubuloglomerular feedback system. An example of a hormone that exerts its effects through different mechanisms is Ang II. Its effects are mediated by at least two mechanisms. Afferent arteriolar responses are highly dependent on Ca^{2+} entry via L-type channels, whereas the efferent arteriolar response appears to be influenced by Ca^{2+} mobilization and Ca^{2+} entry via T-type channels and through SOC in the absence of any entry through voltage-gated L-type channels. Clearly, the mechanisms of activation, Ca^{2+} entry and mobilization, and signal transduction differ in the various microvessels in the renal circulation (2,5,65,84,92–94).

In addition to the smooth muscle cells of the resistance vessels, the mesangial cells within the glomerular tufts also possess contractile capability, which may contribute not only to the regulation of blood flow through the glomerulus, but also to the filtering capacity. Decreases in the filtration coefficient may occur as a consequence of mesangial cell contraction. Glomeruli contain specific receptors for many different hormones and also are the site of synthesis of vasoactive hormones. Specific glomerular receptors have been demonstrated for Ang II, arginine vasopressin, bradykinin, dopamine, parathyroid hormone, histamine, norepinephrine, prostaglandins (PGs) (PGE_2, PGI_2, and thromboxane), leukotrienes, serotonin, ANP, and endothelin. Receptor binding alters Ca^{2+} permeability, intracellular Ca^{2+} mobilization, hormone-sensitive adenylate, or guanylate cyclase, or all of these. Agonists that directly induce an increase in cytosolic Ca^{2+}, such as Ang II, endothelin, norepinephrine, and vasopressin, cause mesangial cell contraction (Fig. 2-6). However, β-adrenergic agonists, some prostaglandins (PGE_2 and PGI_2), parathyroid hormone (PTH), and histamine increase cAMP in isolated glomeruli and cultured mesangial cells, an effect that would be expected to oppose contraction. Indeed, PTH does not induce contraction of isolated mesangial cells, and PGE_2 and PGI_2 directly oppose the effects of Ang II. Agents that increase cyclic AMP in the

juxtaglomerular cells stimulate renin release, which could increase the local production of Ang II (7,95–98).

The contractile state of mesangial cells may influence flow dynamics within the glomerulus and the glomerular filtration coefficient (K_f). Mesangial contraction is postulated to reduce the K_f by decreasing the radius of capillaries or the surface area available for filtration, or both. Many agents, including Ang II and vasopressin, can reduce K_f. In addition, low K_f values have been observed during sodium depletion when plasma and local concentrations of endogenous Ang II are elevated. These responses are thought to reflect specific receptor-mediated effects on mesangial cells, since the response can be reversed by selective receptor antagonists *in vivo* and in cultured mesangial cells. However, podyocytes are closely associated with mesangial cells *in vivo* and also respond to Ang II suggesting that part of the actions of Ang II or K_f could be due to responses by podocytes. The roles of mesangial cell receptors for many of these other agents remain unclear, but they may exert stabilizing effects on mesangial cell contraction and counteract the influence of excessive levels of vasoconstrictor agents or serve metabolic functions. The structural mechanism by which mesangial cell contraction actually alters K_f remains unclear (5,7,8,30,99–101).

Interactions between Endothelial Cells and Smooth Muscle

The vasculature is lined with a continuous layer of endothelial cells, which serve many functions, including its role as a diffusion barrier and the prevention of vascular thrombosis. Endothelial cells are dynamic metabolic units having membrane receptors and membrane-bound enzymes, which allow them to respond to and contribute to changes in the concentration of circulating humoral agents. Membrane-bound ectoenzymes form or degrade circulating vasoactive substances, such as, Ang II (angiotensin-converting enzyme, or ACE), ET-1 (endothelin-converting enzyme), bradykinin (kininase II), adenonucleotides (three ectonucleotidases convert ATP, adenosine diphosphate [ADP], AMP), and endothelin (metalopeptidase).

The vascular endothelium serves an important paracrine role (Fig. 2-7). Endothelial cells directly participate in contractile and dilator mechanisms by responding to a variety of stimuli and by forming potent vasoactive substances, which act locally to modulate tone of adjacent smooth muscle cells. General classes consist of endothelium-derived relaxing factors (EDRF) and contracting factors (EDCF). Specific examples of relaxing factors are NO, PGE_2 and PGI_2 (prostacyclin), carbon monoxide (CO), and a hyperpolarizing factor that has been linked to the cytochrome P450 pathway, most likely an epoxyeicosatrinoic acid (EET) that causes renal vasodilation (90). Examples of endothelium-derived contracting factors include endothelin, thromboxane, angiotensin, and oxygen-free radicals. These autacoids can act on vascular smooth muscle cells to modify vasomotor tone, modulate the proliferative state of smooth muscle cells, and provide a balance between antioxidant defense mechanisms and excess generation of oxygen-derived free radicals. Thus, the endothelial cells are intimately involved in the control of the microcirculation.

One of the most studied interactions between endothelial cells and smooth muscle cells involves the ability of the endothelium to modify the vascular responses to acetylcholine, which is a powerful vasodilator *in vivo* and also in isolated smooth muscle preparations that have an intact endothelium. However, when applied to smooth muscle preparations whose endothelium has been removed, acetylcholine induces vasoconstriction by acting directly on the muscarinc receptors of the smooth muscle cells. Many other substances have now been shown to stimulate the release of endothelium-derived vasoactive factors. Considerable recent emphasis has been placed on one of the major relaxing factors, namely nitric oxite (NO) derived from L-arginine. These paracrine systems will be discussed in later sections (98,102–112).

Other Functions

Another important function of endothelial cells is the conversion of the inactive decapeptide, Ang I, to the active vasoconstrictor octapeptide, Ang II, by endothelial-bound ACE. Localization of ACE in preglomerular vessels allows the conversion of systemically delivered Ang I to Ang II, which can then induce vasoconstriction locally or in downstream segments (25,113).

Endothelial cells have a remarkable capability to transport substances across their layers through a variety of mechanisms. One of the most impressive features of endothelial cell layers is their ability to form fenestrations, which serve as extracellular channels among the endothelial cells. This feature occurs predominantly in capillary structures having large rates of transcapillary volume flux. Both glomerular and peritubular capillary systems have fenestrations. The glomerular capillaries have abundant, well-rounded fenestrations that are 50 to 100 nm in diameter and lack a diaphragm. These fenestrations constitute

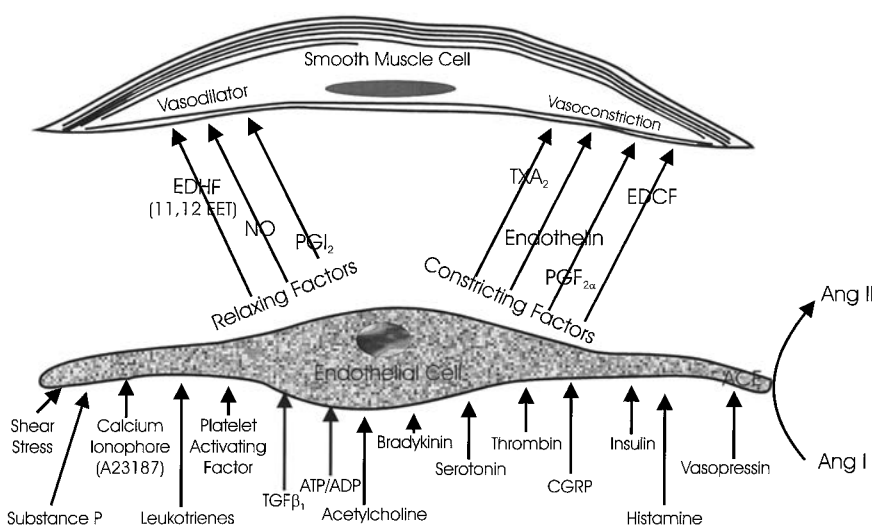

FIGURE 2-7. Interaction of endothelial cells with smooth muscle or mesangial cells. Agents that are known to influence EDRF or nitric oxide (NO) production by endothelial cells are shown. Endothelial cells also produce several other vasoconstrictor and vasodilator agents, as shown in the figure and described in the text.

pathways for the large volume of plasma filtrate that continuously traverses from the glomerular capillaries into Bowman's space. While it is not clear whether subtle changes in the size of the fenestrations can contribute to the regulation of the hydraulic conductivity of the glomerular capillary barrier, it is apparent that their integrity is essential for the maintenance of glomerular filtration (5–7,14,18).

The fenestrations of the peritubular capillaries are bridged by a thin diaphragm and are smaller in diameter (20 nm). Considering the total number of capillaries, there is much more peritubular than glomerular capillary surface area. However, since the overall reabsorptive rate by the peritubular capillaries is nearly equal to the GFR, the average hydraulic conductivity of the peritubular capillaries per unit of surface area is estimated to be less than that of the glomerular capillaries (5,114,115). Fenestrations also exist in the terminal segments of afferent arterioles, and they may provide a pathway for renin entry into the circulation from the juxtaglomerular granular cells (116).

TRANSCAPILLARY EXCHANGE IN RENAL MICROCIRCULATION

Forces Governing Ultrafiltration at the Glomerulus

Bulk movement of fluid across the capillary membranes of the renal microcirculation is passive in nature, driven by an imbalance of physical forces. As blood flows from the afferent arterioles into the glomerular capillary tufts, the high hydrostatic pressure predominates over the counteracting forces caused by Bowman's space hydrostatic pressure and plasma colloid osmotic pressure. Therefore, fluid is driven from the glomerular capillaries through the endothelial fenestrations, across the basement membrane, and between the podocyte foot processes into Bowman's space. This movement of fluid can be described quantitatively by the Starling filtration–reabsorption principle, which is based on the premises that (i) water and solutes flow through extracellular channels or pathways and (ii) the diameters of these channels are large with respect to water molecules, hydrated ions, and solutes of low-molecular weight, such as urea, glucose, and amino acids. Thus, except for the larger solutes, mainly plasma proteins, that approach or exceed the size of the channels, the filtrate is translocated without substantive compositional alterations. Detailed consideration of the morphologic basis of the glomerular filtration barriers is provided in Chapter 1, Structural–Functional Relationships in the Kidney.

The physical forces acting across the glomerular membrane are glomerular capillary pressure (P_g), Bowman's space pressure (P_B), glomerular plasma colloid osmotic pressure (π_g), and colloid osmotic pressure of filtrate in Bowman's space (π_B). The filtering capacity of the filtration barrier is expressed as the glomerular filtration coefficient (K_f), which is the product of the hydraulic conductivity of the glomerular membrane (L_p) and the total filtering surface area (S_f). Since the net forces change as fluid is filtered along the length of the glomerular capillaries, total GFR can be expressed by the equation:

$$GFR = K_f \int_0^1 [(P_{g(x)} - P_B) - \sigma(\pi_{g(x)} - \pi_B)]\, dx \qquad [1]$$

where x represents the normalized length of the glomerular capillaries, with *0* designating the afferent end and *1* designating the efferent end; σ is the reflection coefficient, which has a range of 0 to 1 (119). When σ is 1, proteins are completely "reflected" by the capillary wall, and the colloid osmotic pressure is maximally effective. Normal glomerular capillaries are extremely efficient in restricting the passage of macromolecules, and the amount of protein present in the normal filtrate in Bowman's space is less than 0.01% of the plasma protein. For practical considerations, σ has a value of unity and the colloid osmotic pressure in Bowman's space (π_B) is less than 1 mm Hg. Thus, the effective colloid osmotic pressure is equivalent to that of the plasma in the glomerular capillaries (π_g). As is shown in Figure 2-8, this value increases progressively along the length of the capillaries as a function of the relative volume of protein-free fluid that is filtered. Because colloid osmotic pressure is the major force retarding glomerular filtration, filtration is greatest in the initial segments of the glomerular capillaries and decreases progressively (5,7,8,117).

The exact hydrostatic pressure drop along the glomerular capillaries is uncertain because experimental assessment is not possible. Nevertheless, there are abundant parallel capillaries that collectively have a large cross-sectional area relative to that of the afferent and efferent arterioles; thus, the hydrostatic pressure drop along the glomerular capillaries is probably small as compared with the pressure drops across the afferent and efferent arterioles. Computations based on the number and dimensions of the glomerular capillaries yield estimates that

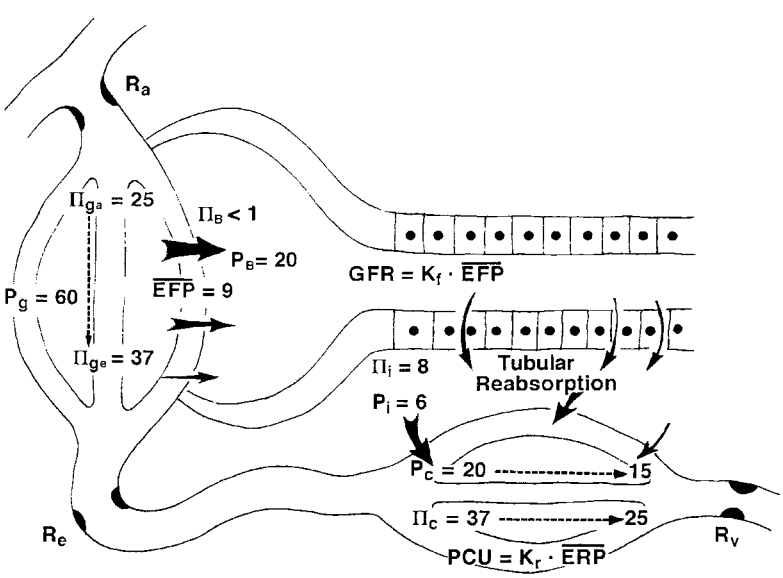

FIGURE 2-8. Schematic diagram of the forces responsible for filtration of fluid from the glomerular capillaries and reabsorption of fluid into the peritubular capillaries. The values are considered representative of forces in humans.

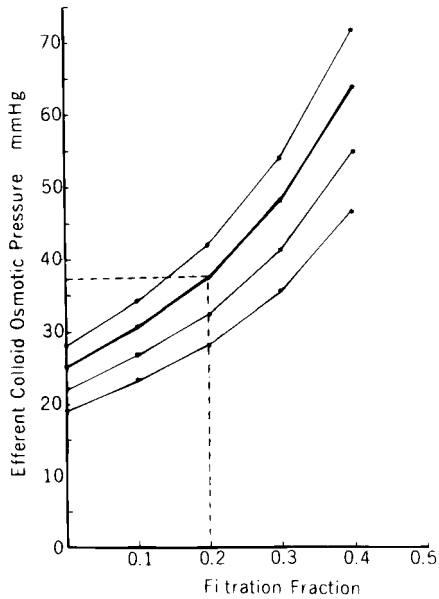

FIGURE 2-9. Nomogram relating the efferent colloid osmotic pressure to the initial plasma colloid osmotic pressure and the filtration fraction. Normal afferent colloid osmotic pressure, 25 mm Hg, is indicated by the thicker curve. An example of how to estimate efferent colloid osmotic pressure for any given filtration fraction and plasma colloid osmotic pressure is shown by the dashed lines.

are in the range of 1 to 4 mm Hg. Thus, P_g is usually treated as a constant value. With these simplifying assumptions and the use of average values for hydrostatic and colloid osmotic pressures in glomerular capillaries, the more commonly used formulation for GFR results:

$$GFR = K_f(P_g - P_B - \pi_g) \quad [2]$$

The net, or mean, effective filtration pressure (EFP) is calculated as

$$EFP = P_g - P_B - \pi_g \quad [3]$$

The increase in plasma protein concentration is a direct function of the filtration fraction, defined as the quotient of GFR and renal plasma flow. Because of the nonlinear relationship between plasma protein concentration and colloid osmotic pressure, the rate of increase in colloid osmotic pressure from the afferent to the efferent arteriole increases progressively (Fig. 2-9). Empirically derived relationships allow the prediction of colloid osmotic pressure (π) from the total plasma protein concentration (C) when the albumin-to-globulin (A/G) ratio is known. The commonly used Landis–Pappenheimer relationship

$$\pi = 2.1\ C + 0.16\ C^2 + 0.009\ C^3 \quad [4]$$

applies to an A/G ratio of about 1.2, which is considered normal for humans.

The efferent arteriolar colloid osmotic pressure is determined by the initial plasma value and the filtration fraction. The nomogram in Figure 2-9 allows estimation of the efferent arteriolar colloid osmotic pressure and is independent of A/G ratios. For example, at a normal filtration fraction of 0.20 and normal plasma colloid osmotic pressure of 25 mm Hg, the predicted value for efferent colloid osmotic pressure is 37 mm Hg.

The hydrostatic pressure in Bowman's space (P_B) in humans is uncertain. In laboratory animals, P_B is similar to proximal tubular pressure, which ranges from 11 to 15 mm Hg in rats and from 18 to 22 mm Hg in dogs. Proximal tubular pressure is also slightly higher than the pressure in adjacent peritubular capillaries. Peritubular capillary pressure has not been measured directly in humans, but it can be estimated from intrarenal venous pressure measurements obtained by retrograde passage of a renal vein catheter. Values obtained in humans are 20 to 25 mm Hg and provide reasonable estimates of proximal tubular pressure. This pressure plus an average efferent colloid osmotic pressure of 37 mm Hg provides a minimal glomerular pressure in humans in the range of 57 to 62 mm Hg; actual values are higher to the extent that there is net filtration pressure at the terminal end of the glomerular capillaries.

Micropuncture studies in animals also indicate that glomerular pressure is 50 to 60 mm Hg and approximately 40 mm Hg greater than the opposing hydrostatic pressure in Bowman's space. From this difference in transglomerular capillary hydrostatic pressure, it can be calculated that EFP ranges from 15 mm Hg at the afferent end of the glomerular capillaries to about 3 mm Hg at the efferent end, yielding an average EFP of 9 mm Hg (Fig. 2-8). Using this value and one of 120 mL/min for total GFR, a K_f of 13 mL/min/mm Hg for the total nephron population is calculated. Assuming there are 2 million nephrons in both human kidneys, the K_f for a single glomerulus is approximately 6 to 7 nL/min/mm Hg. This value generally agrees with micropuncture measurements, which indicate that K_f for an individual glomerulus is 4 to 5 nL/min/mm Hg in dogs and 2 to 5 nL/min/mm Hg in rats. The large variation in K_f among rats is due in part to differences observed among different strains.

The filtration process can operate under one of two conditions. The first condition is the case described above, in which filtration continues throughout the entire length of the glomerular capillaries and a finite positive EFP remains at the efferent end of the glomerular capillaries. This pattern of disequilibrium is shown by the solid lines in the left panel of Figure 2-10. The second condition occurs when the increase in colloid osmotic pressure is so rapid that the forces favoring and opposing filtration become equal at some point within the capillary system, a condition termed *filtration pressure equilibrium* (Fig. 2-10, solid lines in right panel). Under equilibrium conditions, the latter part of the available filtering surface area is not utilized and becomes a functional reserve. Studies in some strains of rats have suggested that the normal condition is one of filtration equilibrium. Data from studies of other strains of rats and dogs indicate that, under normal circumstances, glomerular capillary hydrostatic pressure is sufficiently high and the K_f is sufficiently low to prevent the achievement of filtration equilibrium within the glomerular capillaries.

A physiological consequence of the equilibrium or disequilibrium of filtration pressures is the influence of plasma flow on GFR. Using a mathematical model presented later, the specific effect of plasma flow can be predicted for both conditions when the transcapillary hydrostatic pressure gradient is kept constant. As shown by the dashed line in Figure 2-10 (right panel), an increase in plasma flow to a system in filtration equilibrium diminishes the rate of increase of colloid osmotic pressure along the length of the glomerular capillaries. The EFP is not dissipated as quickly, and the point of equilibration of hydrostatic and colloid osmotic forces is moved distally, which, in effect, results in recruitment of additional filtering surface area (S_f) and an increase in the functional K_f. Consequently, increases in plasma flow can increase the GFR proportionately even when glomerular capillary pressure is unchanged. In the case of filtration pressure disequilibrium, increases in plasma flow increase GFR only modestly as a consequence of a reduced colloid osmotic pressure profile, and there is no net recruitment of previously unused surface area (see Fig. 2-10, dashed lines in left panel). Thus, the magnitude of a selective plasma flow effect is smaller during filtration pressure disequilibrium than

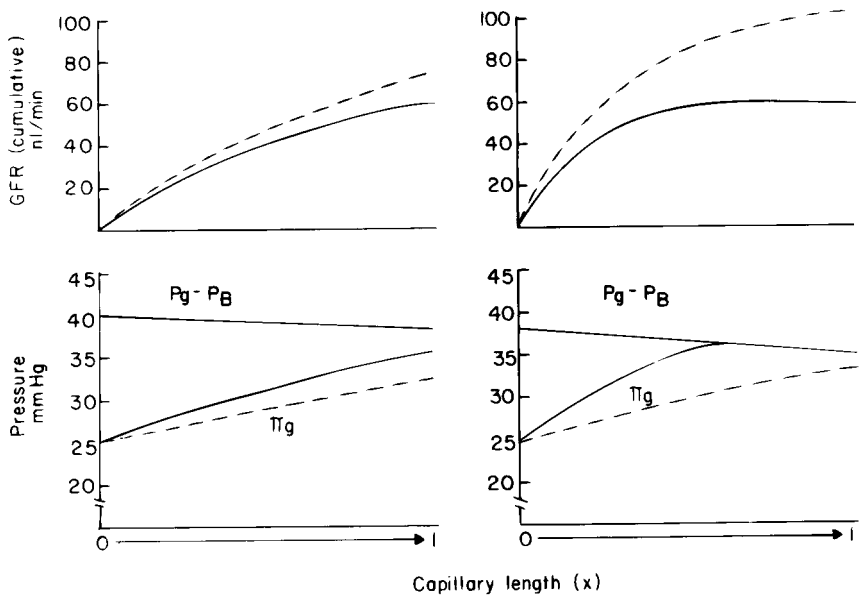

FIGURE 2-10. Comparison of filtration dynamics in conditions of filtration equilibrium (*right*) and disequilibrium when filtration occurs throughout the length of capillary (*left*). The lower panels represent the changes in the transcapillary hydrostatic pressure gradient ($\pi_g - P_B$) and the colloid osmotic pressure (π_g), and the upper panels represent the cumulative GFR along the length of the glomerular capillary. The dashed lines indicate the changes occurring in response to doubling of plasma flow under both conditions.

during equilibrium. In humans, the low filtration fraction and the relative lack of plasma flow dependence of GFR suggest that the filtration process continues throughout the entire length of the glomerular capillaries, i.e., disequilibrium, as shown in the left panel of Figure 2-9 (5,7–9,117–120).

Glomerular Permeability to Macromolecules

Experiments examining the filterability of test molecules of different sizes, shapes, and charges have been used to characterize the hydrodynamic properties of the filtration barrier. A sieving coefficient (σ), or fractional clearance of a test molecule, is obtained relative to that of a freely filtered reference molecule, such as inulin. Accurate determinations can be made when both substances enter the urine by means of filtration and are not subjected to tubular reabsorption or secretion. Such data have been fitted to various theoretic models based on limiting membrane structures, consisting of an impermeable matrix that is perforated with cylindrical pores, rectangular slit-like openings, or a meshwork of fibrous or granular gel-like structures. Evaluation of molecular sieving or steric restriction in each model, however, is based on the principle of geometric exclusion of large solute molecules from a portion of the membrane that is accessible to water and small solutes. In essence, the larger molecules that approach or exceed the effective size of the channels are restricted or "sieved." Conceptually, the simplest model that is applicable to the glomerular barrier consists of a size-discriminating membrane with a large population of fluid-filled cylindrical pores of about 5 nm in radius, which totals approximately 5% of the total surface area. There may also be a very small population of much larger pores.

Passage of macromolecules across capillary membranes is dependent on several factors in addition to the effective radius. These factors include the electrical charge and the structural conformation and rigidity of the molecule. As shown in Figure 2-10, the glomerular sieving coefficient or fractional clearance (usually determined as C_D/C_{IN}) of graded sizes of electrically neutral dextran molecules declines progressively as effective radius and molecular weight increase. Water, electrolytes, and other small, uncharged solute molecules with an effective Stokes–Einstein radius of less than 1.8 nm are freely permeant. As the effective radius increases, there is a progressive restriction. The fractional clearance of macromolecules the size of IgG (5 nm) is essentially zero. For the same equivalent radius, the fractional clearances of albumin (3.6 nm) and negatively charged dextran sulfate are considerably lower than the clearances of uncharged molecules. In addition, polycationic macromolecules are filtered more readily than neutral molecules. These differences in transport of electrically charged macromolecules are due to the membrane-bound polyanionic glycoproteins that are rich in sialic acid and heparan sulfate residues, which set up a negative electrostatic field that repels polyanions. These are associated with the glycoprotein coat that covers the endothelial fenestrations, the basement membrane, and the epithelial cells. Partial loss of these anionic sites on the glomerular capillary wall can lead to albuminuria in the absence of any gross structural abnormalities and in cases of mild glomerulonephritis. Such a loss has been induced experimentally by neutralization of the electrostatic barrier with the polycation protamine. In more severe glomerular injury-associated proteinuria, a larger fraction of the filtrate appears to pass through a population of large-diameter, nonselective pores.

In addition to size and charge, molecular configuration influences the sieving coefficient (Fig. 2-11). Rigid or globular molecules such as horseradish peroxidase or ficoll have lower sieving coefficients for any given molecular size than neutral dextran polymers with highly deformable linear structures. Thus, it is likely that the curve for neutral dextrans in Figure 2-11 overestimates the true permeability characteristics of more rigid, globular-structured macromolecules, such as, plasma proteins. Because shape, flexibility, and deformability contribute to the quantitative relationship between molecular size and transglomerular solute flux, it is difficult to establish the true dimensions of the extracellular channels. Data currently available indicate that the effective radius of the channels in the glomerular membrane is in the range of 4.5 to 6 nm (7,18,121–123).

Most studies involving quantitative consideration of macromolecular passage through capillary membranes have relied on the thermodynamic approach developed by Kedem and Katchelsky (124). Derivations for solute flux (J_s) across a constraining membrane include a convection term, which is the solute flux that occurs as a consequence of the bulk volume flow (J_v), and a diffusion flux, which is a function of the concentration gradient of the solute. In its most elementary form,

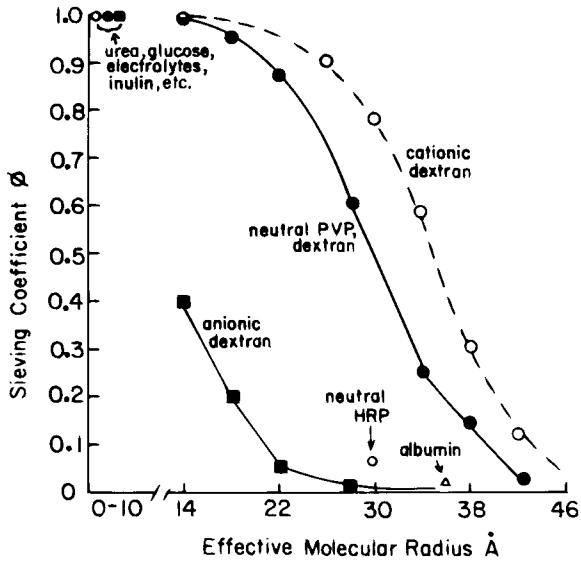

FIGURE 2-11. Representative sieving curves for several test molecules in the glomerular circulation. The curve representing neutral molecules is based on data obtained with use of polyvinylpyrrolidone and neutral dextran. The curves for anionic and cationic molecules are based on studies with charged dextrans. Also shown are the sieving values for neutral horseradish peroxidase (neutral HRP) and for albumin. The smaller molecules are shown to have a sieving coefficient of 1.0. (From: Navar LG, Bell PD, Evan AP. The regulation of glomerular filtration rate in mammalian kidneys. In: Andreoli TE, Hoffman JF, Fanestill DD, eds. *Physiology of membrane disorders,* 2nd ed. New York: Plenum, 1986;637; Maddox DA, Deen WM, Brenner BM, Glomerular filtration. In: Maddox DA, Deen WM, Brenner BM, eds. *Handbook of physiology*, Vol. 1. New York: American Physiological Society, 1992:545; Deen WM, Bridges CR, Brenner BM. Biophysical basis of glomerular permselectivity. *J Membr Biol* 1983;71:1; Hostetter T, Rosenberg ME, Renal hemodynamics and permselectivity. *J Am Soc Nephrol* 1990;1:S55; Adal Y, Pratt L, Gomper WD. Transglomerular transport of DEAE dextran in the isolated perfused kidney. *Microcirculation* 1994;1:169; Ohlson M, Haraldsson B. Glomerular size and charge selectivity in the rat as revealed by FITC-Ficoll and albumin. *Am J Physiol Renal Physiol* 2000;279:F84.)

solute flux due to convection is

$$J_s = J_v C_s (1 - \sigma) \quad [5]$$

and solute flux due to diffusion is

$$J_s = PS(\Delta C_s) \quad [6]$$

where J_V is the volume flow (in this case the GFR), and C_σ is the average concentration across the membrane, s is the reflection coefficient, previously discussed, ΔC_S is the concentration difference across the capillary wall, and PS is the diffusional, permeability surface-area product coefficient. With small uncharged molecules, such as glucose, σ approaches zero and, thus, glucose flux is simply defined by the product of GFR and the plasma glucose concentration. For very large molecules that are restricted with almost complete efficiency, σ approaches 1 and, thus, solute flux due to convection is negligible. The most relevant example is for plasma albumin. Using a value of 1 to 3 mg/dL for albumin concentration in early tubular fluid and a systemic plasma albumin concentration of 3,600 mg/dL, σ is greater than 0.99. Furthermore, the PS coefficient is so low (0.001 mL/min) that solute flux due to diffusion also approaches zero. These quantitative considerations also highlight the difficulty in attempting to evaluate mechanisms of proteinuria. Theoretically, protein passage across the glomerular membrane could increase more than 100-fold, which could be accounted for by a change in σ from 0.99 to 0.95. Such small changes in membrane permeability would not be expected to be associated with discernible morphologic changes (5,124,125).

Hemodynamics in Peritubular Capillaries and Role in Fluid Reabsorption

Virtually all of the peritubular capillary network stems from efferent arterioles. About 85% of the postglomerular blood flow is distributed to peritubular capillaries in the cortex, and the remaining 15% goes to the medulla and papilla (Fig. 2-5). The overall density of peritubular capillaries and the total surface area are considerably greater than those of glomerular capillaries. The peritubular capillary wall consists of a thin fenestrated endothelial layer covered by a thin basement membrane. Per unit of surface area, it has a lower hydraulic conductivity and a slightly higher permeability to large molecules than the glomerular wall.

In a manner analogous to the process of filtration, the peritubular capillary reabsorption (PR) of fluid that is reabsorbed by the renal tubules is determined by the imbalance of hydrostatic and osmotic forces between the interstitial space and adjacent peritubular capillaries. If one considers the forces responsible for reabsorption into the capillaries, then

$$PR = K_r[(\pi_c - \pi_i) - (P_c - P_i)] \quad [7]$$

where K_r is the reabsorptive coefficient, π_c and π_i represent the average colloid osmotic pressures in the capillaries and in the interstitial fluid, and P_c and P_i represent the corresponding hydrostatic pressures.

As plasma emerges from the glomerular capillaries, it has a colloid osmotic pressure of 35 to 37 mm Hg (Fig. 2-8). Furthermore, the hydrostatic pressure drops about 40 mm Hg along the efferent arteriole (Fig. 2-3), yielding an initial peritubular capillary pressure of about 20 mm Hg. With regard to the interstitial compartment, estimates of π_i and P_i are about 6 to 8 mm Hg and tend to cancel each other out. Thus, the mean effective reabsorption force is 15 mm Hg at the beginning of the peritubular capillary bed. As fluid is reabsorbed into the capillaries, the plasma proteins are diluted and the colloid osmotic pressure progressively decreases to the original value enterng the kidney. There is also a small, but measurable, progressive decline in capillary hydrostatic pressure. Thus, there is an effective reabsorptive force over the entire length of the peritubular capillaries, which varies from about 15 mm Hg to about 8 mm Hg (Fig. 2-8). The hydraulic reabsorptive coefficient, K_f, for the peritubular capillary is about 9 to 10 mL/min/mm Hg, which is slightly lower, overall, than the glomerular K_f. This suggests a lower hydraulic conductivity because of the larger surface area of the peritubular capillaries.

With regard to macromolecular permeability, the situation existing in the peritubular circulation contrasts with that in the glomerulus, because the convective component is directed inward in association with the continuous fluid reabsorption. Thus, the loss of macromolecules from the postglomerular circulation occurs as a consequence of diffusion of macromolecules from the plasma into the interstitial compartment. Although it is known that significant amounts of protein accumulate in the interstitium, the actual permeability may still be quite low, because of the low removal rate. Some studies have indicated that the postglomerular circulation constrains molecules that can readily pass through the glomerular membrane. There also may be a small population of pores with diameters greater than 5 nm. Nevertheless, most of the channels appear to have a high degree of efficiency in restricting albumin and other plasma proteins, so their reflection coefficients are very close to 1. This occurrence is owed, in part, to an

electrostatic barrier similar to that found in the glomerular capillaries such that negatively charged macromolecules permeate slower than neutral molecules of the same size. Thus, plasma proteins exert almost their full osmotic pressure across the peritubular capillaries. In spite of these high reflection coefficients, the concentration of albumin in renal lymph, and presumably in the interstitial fluid, is about one-fourth that in systemic plasma. Although this concentration seems rather high, it should be noted that lymph flow is very low, and less than 1% of net protein is lost from the plasma flowing through the peritubular capillaries.

Lymphatic capillaries, primarily distributed throughout the cortex, are very permeable to protein and fluid. They serve to return the proteins that leak out of the peritubular capillaries back to the circulation, and it is usually assumed that the protein concentration in the lymph reflects the protein concentration in the interstitial fluid. The normal renal lymph flow in humans is estimated to be about 2 to 5 mL/min, or less than 1% of the plasma flow. Lymph flow is increased by elevations in interstitial hydrostatic pressure, such as those accompanying diuretic states, ureteral obstruction, or increases in renal venous pressure (5,6,126,127).

Capillary Uptake by Vasa Recta

Efferent arterioles of juxtamedullary nephrons provide most of the vascular supply to the outer medulla. These efferent arterioles branch into long-looped capillaries, termed *vasa recta*, which descend into the medulla in vascular bundles. The vasa recta bundles are intimately associated with, and surrounded by, concentric rings of loops of Henle and collecting ducts. The medullary circulation has the important function of removing water and solutes reabsorbed from descending and ascending loops of Henle and collecting ducts without disrupting the large longitudinal osmotic gradients that exist in the inner medulla. This delicate balance is achieved by virtue of the low blood flow and an efficient countercurrent diffusion of fluid and small molecular weight solutes, which occur because of the specialized structure of the hairpin-shaped parallel loops of the descending and ascending vasa recta. The end result is passive equilibration and shunting of fluid across the vasa recta, from descending to ascending limbs, and trapping of solute at the bends. Because the descending vasa recta have a continuous thick endothelium, albeit aquaporin 1 (AQP1) channels allow water efflux. Because protein permeability is low, the high plasma protein concentration of the efferent arteriolar blood is preserved. In contrast, the ascending vasa recta have a highly fenestrated thin endothelium, which greatly facilitates passive reabsorption. The ascending vasa recta also have a higher permeability to protein. However, the hydrostatic pressure in the ascending vasa recta is relatively low, about 10 mm Hg, and probably not much higher than interstitial hydrostatic pressure. In the face of a very small outward hydrostatic pressure gradient, the transcapillary colloid osmotic pressure gradient provides an important reabsorptive force, favoring capillary fluid uptake throughout these specialized capillaries. Importantly, the vasa recta also possess pericytes that provide contractile capability and a means to locally regulate blood flow (5,29,30,32,36,128).

Quantitative Analysis of Filtration and Reabsorption Dynamics

The mechanisms regulating GFR involve complex interactions among the individual determinants of filtration rate. To reach a better understanding of the singular effects of each determinant, one can examine the theoretical influence of selective changes in an idealized situation where the other determinants are held constant. Such theoretical predictions can be made from the simple mathematical model shown in Figure 2-12, which analyzes fluid flow dynamics along the length of a single filtering capillary and the resistances of the afferent and efferent arterioles (5,9,18,117,129).

This model can be used to analyze the effects on GFR of singular perturbations, such as changes in transcapillary hydrostatic pressure gradient, systemic plasma protein concentration, glomerular plasma flow, and the filtration coefficient. As shown in Figure 2-13 (panel A), changes in the transcapillary hydrostatic pressure difference produce striking responses in GFR. An increase of 10% causes a greater relative increase in EFP leading to an increase in GFR of 19%. GFR is inversely related to plasma colloid osmotic pressure; as can be seen in panel B, an increase of plasma protein concentration of 10% reduces GFR by 25%. The influences of changes in K_f and in plasma flow on GFR are more complex because they affect the rate of rise of plasma colloid osmotic pressure along the capillary and thus EFP. Panel C in Figure 2-13 shows that GFR is affected more by decreases than increases in K_f. The reduced effect of increases in K_f above the normal values reflects the achievement of filtration-pressure equilibrium. Once filtration equilibrium is reached, further increases in K_f enhance ultrafiltration in early portions of the capillary, which causes protein concentration to increase more rapidly. However, this effect is offset by the fact that the colloid osmotic pressure equilibrates with the hydrostatic pressure difference at a more proximal site along the capillary. Thus, the mean EFP and total GFR remain the same, although the locus of equilibrium is shifted to an earlier site.

Panel D of Figure 2-13 demonstrates that the effects of glomerular plasma flow on GFR are also nonlinear. In the absence of changes in the other determinants, GFR increases only modestly with increases in plasma flow. On the other hand, decreases in plasma flow below 200 nl/min produce roughly proportional decrements in GFR. This increase in sensitivity is again due to the attainment of filtration equilibrium at the lower values of plasma flow. Glomerular plasma flow exerts

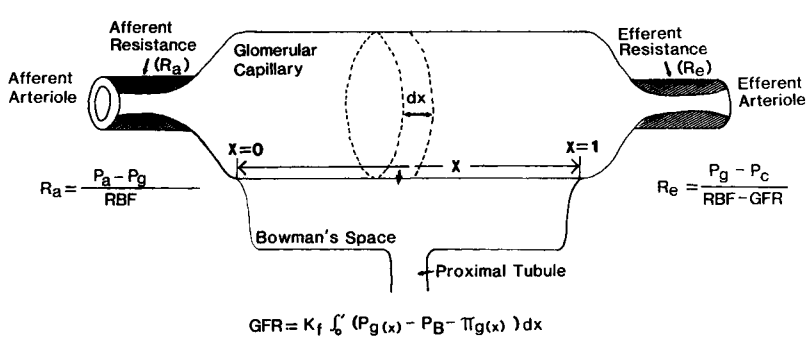

FIGURE 2-12. "Single capillary" model of glomerular filtration dynamics and afferent (R_a) and efferent (R_e) resistances.

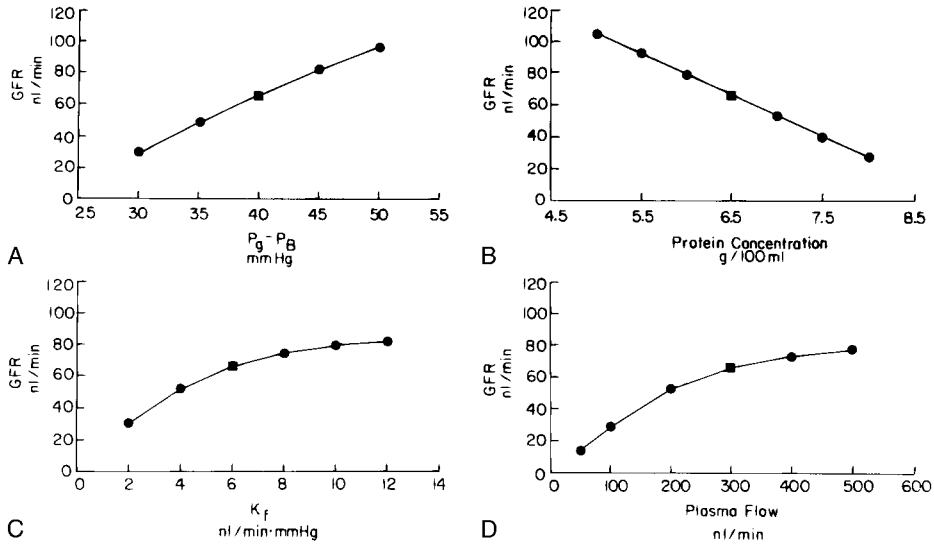

FIGURE 2-13. Theoretical effects of singular perturbations in (**A**) transcapillary hydrostatic pressure gradient ($P_g - P_B$), (**B**) plasma protein concentration, (**C**) the filtration coefficient (K_f), and (**D**) plasma flow at entry to glomeruli. For these simulations, control values (*squares*) estimated to be representative of single nephron function in humans were used: GFR, 65 nL/min; plasma flow, 300 nL/min; ($P_g - P_B$), 40 mm Hg; plasma protein concentration, 6.5 g/dL.

these effects by modifying the intraglomerular profile of colloid osmotic pressure and thus mean EFP. The effects of increases in plasma flow on the system during filtration equilibrium and not in equilibrium were discussed earlier and are shown in Figure 2-10. Changes in plasma flow have a relatively small effect on the colloid osmotic pressure profile during filtration disequilibrium (Fig. 2-10). A decrease in plasma flow increases the fraction of plasma being filtered per unit of capillary length in proximal segments. As a result, the rate of rise of colloid osmotic pressure is increased progressively. During filtration pressure equilibrium, GFR is highly plasma flow-dependent. Thus, there are two major functional consequences of filtration pressure equilibrium. GFR is insensitive to increases in K_f and is strongly influenced by changes in plasma flow. In contrast, GFR is directly responsive to K_f and is less plasma flow-dependent under disequilibrium conditions. In either situation, glomerular capillary pressure is quantitatively a much more powerful determinant of GFR than plasma flow.

A mathematical analysis that is of more physiological relevance involves an integrated consideration of changes in preglomerular and efferent arteriolar resistance on glomerular dynamics. Presented in Figure 2-14 are the predicted effects of constriction and dilation of either afferent or efferent arterioles under idealized conditions, when the other resistance, as well

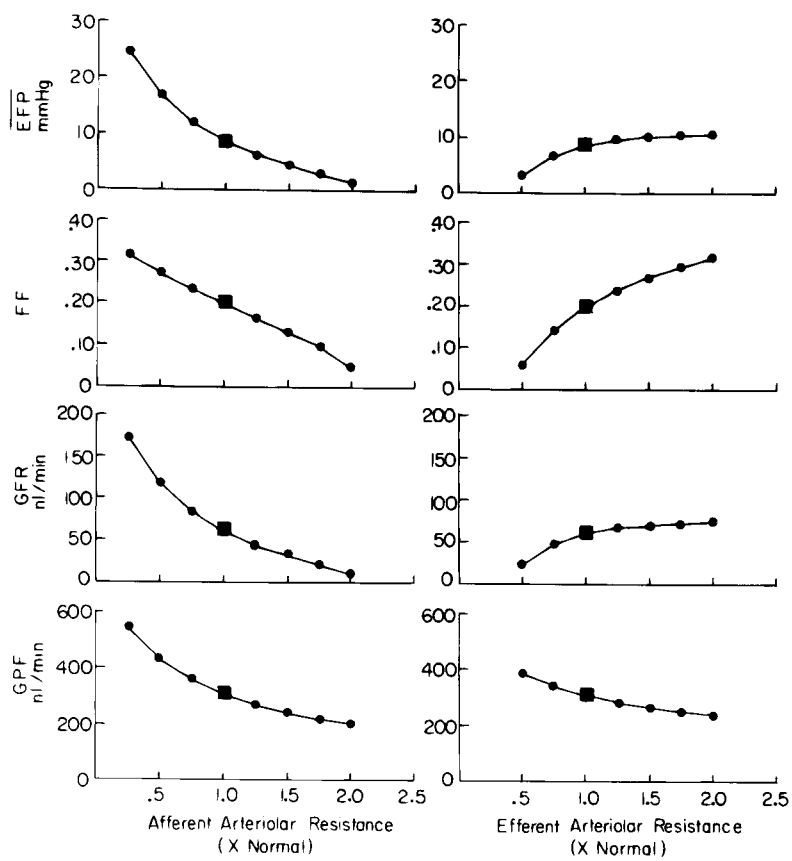

FIGURE 2-14. Effects of increases and decreases in afferent and efferent arteriolar resistance on glomerular filtration rate (*GFR*), glomerular plasma flow (*GPF*), filtration fraction (*FF*), and mean effective filtration pressure (*EFP*).

as other inputs, are maintained at normal values. A selective increase in afferent resistance reduces plasma flow and hydrostatic pressure in glomerular and peritubular capillaries. GFR decreases proportionately more than plasma flow and thus the filtration fraction falls. In contrast, an increase in efferent arteriolar resistance reduces plasma flow but increases glomerular pressure. GFR initially increases slightly but quickly reaches a plateau. The plateau region of mean EFP is because of the counteracting effects of the increases in glomerular capillary and colloid osmotic pressure. From these quantitative considerations, it is apparent that the preglomerular resistance is ideally suited to control GFR. Efferent arteriolar resistance contributes to subtle alterations in GFR, but exerts major effects on the dynamics of the postglomerular circulation. It is important to emphasize that changes in filtration fraction alone cannot be used to determine the localization of resistance changes to a specific manipulation, condition, or drug. For example, combined increases in afferent and efferent resistances reduce the plasma flow proportionately more than the GFR, and the filtration fraction increases. Likewise, combined decreases in afferent and efferent arteriolar resistances increases blood flow proportionately more than GFR, and filtration fraction falls (129). These changes in filtration fraction have often been interpreted incorrectly as being indicative of selective change in efferent arteriolar resistance.

Control of Glomerular Dynamics by Regulation of Filtration Coefficient

In addition to regulation of capillary flow and pressure by resistance changes of the preglomerular and postglomerular vascular segments, K_f and K_r may be subjected to regulatory influences. Alterations in the size of the capillaries or closure of a fraction of the capillaries may reduce the available filtering surface area and thus influence K_f. The hydraulic conductivity may be altered by adjustments in the size and number of endothelial fenestrations, the thickness or permeability of the basement membrane, and the number or structural configuration of the slit pores between the foot processes. Recent attention has been focused on the role of the podocytes in control of GFR (130). Changes in any of these properties could be manifested as changes in K_f.

Animal studies suggest that vasoconstrictor hormones and some vasodilators are capable of reducing K_f. K_f may also be reduced drastically in disease states that involve sclerosis of glomerular capillaries or thickening of the basement the basement membrane. K_f may be increased slightly in certain circumstances, such as increased plasma colloid osmotic pressure. Differences in basal K_f are reported among different species and different strains and colonies of rats. Agents such as Ang II and catecholamines can decrease K_f. Blockade of the vascular effects of Ang II with the structural analog, saralasin, negates the K_f lowering effect of prostaglandins E_2 and I_2 and PTH, but it has no effect on changes in K_f produced by vasopressin. In addition, several vasodilators (acetylcholine, bradykinin, and histamine) have been shown to decrease K_f in rats through mechanisms that are not clear. While the vasodilator actions are now known to be because of NO release, it is not apparent how this would decrease K_f. In contrast to the effects observed in rats, vasodilator agents do not affect K_f appreciably in dogs. The reason for this apparent species difference is not known. Nevertheless, it seems clear that a variety of humoral agents, including recently discovered endothelial-derived nitric oxide, endothelin, and leukotrienes, can influence K_f and may influence filtration dynamics. The intrarenal effects of some of these hormones may be secondary to stimulation of glomerular synthesis or release of other vasoactive agents, including prostaglandins, leukotrienes, and Ang II (5,7–9,18,99,130–135).

With regard to the postglomerular vasculature, the major regulator of hydrostatic pressure in the peritubular capillaries is the efferent arteriolar resistance. For a given flow, an increase in efferent arteriolar resistance increases the pressure drop along this vessel and thus reduces the pressure in peritubular capillaries. An increase in downstream resistance because of venous obstruction or elevated tubular pressure increases hydrostatic pressure in the capillaries. Interstitial hydrostatic pressure tends to change in the same direction as pressure in the peritubular capillaries. Colloid osmotic pressure of blood entering the peritubular capillaries is primarily regulated by the filtration fraction. A higher colloid osmotic pressure exerts a greater reabsorptive force in the postglomerular circulation. The colloid osmotic pressure in interstitial fluid is determined by a balance of protein entry from circulating plasma and protein exit by means of the lymphatic circulation. In general, stimuli promoting efferent arteriolar constriction reduce hydrostatic pressure in peritubular capillaries and increase efferent arteriolar colloid osmotic pressure, changes that favor increased fluid reabsorption from the renal interstitium into the peritubular capillaries. Vasodilating stimuli have the opposite effects and are often accompanied by natriuretic and diuretic responses. In all cases, however, there is a very intimate coupling between the rate of fluid reabsorption from the tubules into the interstitium and fluid reabsorption from the interstitial compartment into peritubular capillaries (114,115,126,127).

REGULATION OF RENAL HEMODYNAMICS

The high sensitivity of glomerular and peritubular capillary dynamics to variations in the intrarenal pressures and flows emphasizes the importance of regulatory mechanisms that maintain the intrarenal hemodynamic environment. Overall control is shared by several mechanisms that exert specific effects on various segments of the renal vasculature. Some of these mechanisms are intrinsic to the kidney, while others depend on extrarenal signals mediated by neural or hormonal stimuli.

Mechanisms of Autoregulation

Intrinsic paracrine signals can adjust intrarenal vascular resistance in response to a variety of extrarenal perturbations. The alterations in vascular resistance serve to counter the effect of the extrarenal disturbance so that RBF and GFR are stabilized. The most widely studied manifestation of these intrinsic mechanisms is the phenomenon of renal autoregulation. In response to alterations in renal arterial pressure over a wide range, the kidney adjusts its vascular resistance in order to maintain, or "autoregulate," RBF. This range encompasses the physiologically relevant arterial pressures, both above and below normal. In response to reductions in arterial pressure, which may occur during situations, such as sleep or recumbency, intrarenal mechanisms decrease renal vascular resistance to maintain RBF and GFR at optimum levels. Likewise, increases in arterial pressure, which might occur during exercise or acute episodes of stress, elicit signals that increase vascular resistance and thus maintain RBF and GFR at or near control levels. In addition to RBF and GFR, the microvascular and tubular pressures exhibit autoregulatory behavior. Since glomerular pressure and GFR are autoregulated, it is generally agreed that the predominant adjustments of vascular resistance are localized to the preglomerular arterioles. Figure 2-15 shows representative relationships between the renal

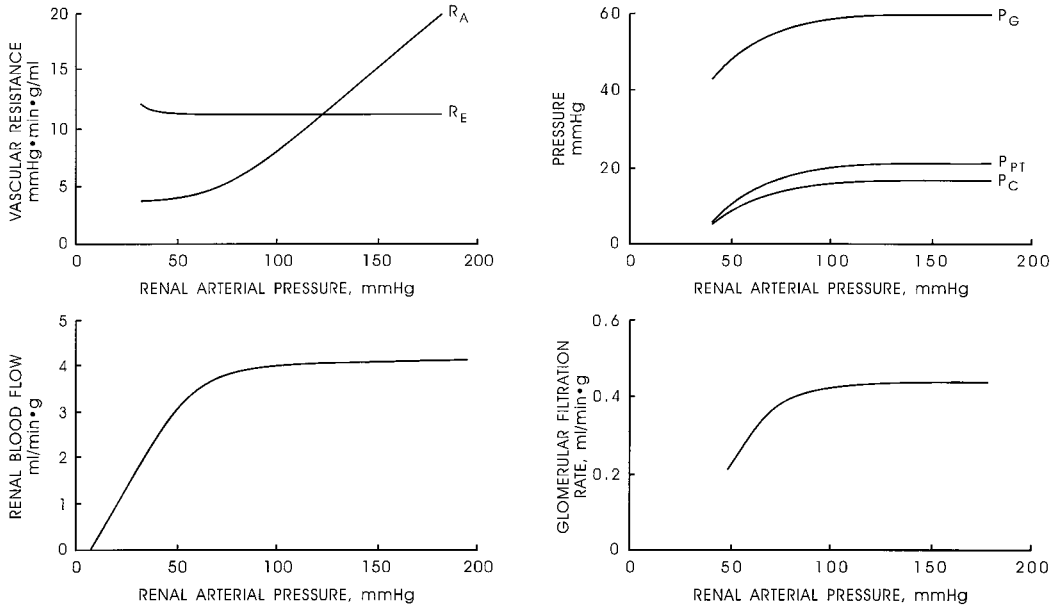

FIGURE 2-15. Representative relationships between renal arterial pressure and renal blood flow (*lower panel*), glomerular filtration rate (*middle panel*), and afferent arteriolar vascular resistance (*upper panel*). R_a afferent arteriolar resistance; R_e, efferent arteriolar resistance; R_v, capillary and venous resistance.

arterial pressure and RBF, GFR, and segmental vascular resistances. The responses of vascular resistance to changes in perfusion pressure represent the most commonly investigated aspect of the renal autoregulatory mechanism, but other stimuli, such as increases in ureteral or renal venous pressure or changes in plasma colloid osmotic pressure, may elicit autoregulatory responses. In most cases, the response serves a negative feedback function to counteract the effect of the disturbance and restore RBF or GFR back toward normal (2,5,7,18,20, 136–139).

The autoregulatory component of the vasculature is activated by voltage-dependent Ca^{2+} channels and is sensitive to the organic Ca^{2+} channel blockers. Much of the research oriented toward understanding this basic response is focused on the mechanisms by which messages are initiated, transmitted to, and received by the smooth muscle cells to effect the requisite alterations in vascular resistance. The two basic mechanisms that contribute to the autoregulation phenomenon are the myogenic mechanism and the tubuloglomerular feedback mechanism (2,5,137,138,140–143).

The myogenic mechanism responds to a distending force on the vessel wall caused by an increase in arterial pressure. The actual distending force can be calculated from the law of Laplace, which relates tangential wall tension (T) to the inner radius of the vessel (r) and the transmural pressure difference,

$$T = r\ (P_a - P_i) \qquad [8]$$

where P_a is intraarteriolar hydrostatic pressure and P_i is the interstitial fluid pressure. When the transmural pressure difference increases, wall tension is increased, which leads to constriction and a reduction of the radius, so that tension returns toward normal. A myogenic response occurs in preglomerular vessels, but not postglomerular efferent arterioles. This may be due to the differential activating mechanisms in these vessels, since efferent arterioles do not normally have functional L-type Ca^{2+} channels. In addition to the afferent arteriole, the arcuate and interlobular arteries also display myogenic responses. Although a large vessel, such as the arcuate artery, may respond to pressure, its contribution to total resistance is quite small. Thus, the preglomerular arteriolar network has the ability to respond to extrinsic physical or mechanical disturbances by a myogenic response. This is also supported by the fact that the initial autoregulatory adjustments in vascular resistance occur so rapidly (a few seconds) that they are difficult to explain except by a direct local vascular mechanism. Such a rapid response is thought to buffer the glomerular capillaries and the tubular network from sudden changes in arterial pressure and protect from high systolic pressures, especially at higher frequencies (2,137,138,140–145).

Contraction associated with an increase in intraluminal pressure is associated with cell membrane depolarization and increased Ca^{2+} entry through voltage-gated L-type Ca^{2+} channels. This conclusion is supported by the ability of L-type channel blockers to inhibit the myogenic response and the stretch-activated Ca^{2+} channels in the preglomerular microcirculation. The inositol phosphate cascade may increase levels of IP_3 and DAG and activate PKC. Inhibition of PKC attenuates the autoregulatory constrictor response to a pressure increase. Subconstrictor concentrations of either Ang II or ET-1 may potentiate myogenic contraction of afferent arterioles. Nitric oxide attenuates the rate and strength of the myogenic response. Although endothelial cells play a role in the rate of autoregulation, stretch-induced, steady-state myogenic tone is observed in vessels without a functional endothelium (2,137,138,140–142,144,146–148).

Additional theories to explain the autoregulatory phenomenon evolved because of the recognition that slower-acting hemodynamic mechanisms have to be responsive to the metabolic demands of tubular transport. The existence of structures within the kidney that seem ideally suited to act as communication links between the distal tubular segments and the vasculature provide the morphologic basis for the tubuloglomerular feedback hypothesis. Indeed, the unique morphology of the juxtaglomerular complex (Fig. 2-4) suggests that the MD senses some aspect of fluid composition at the start of the distal tubule and transmits signals to the adjacent afferent arterioles. Thus, the juxtaglomerular complex provides the anatomic basis for a negative feedback mechanism, operating in each nephron, that maintains balance between the hemodynamic inputs that control GFR and filtered load and the

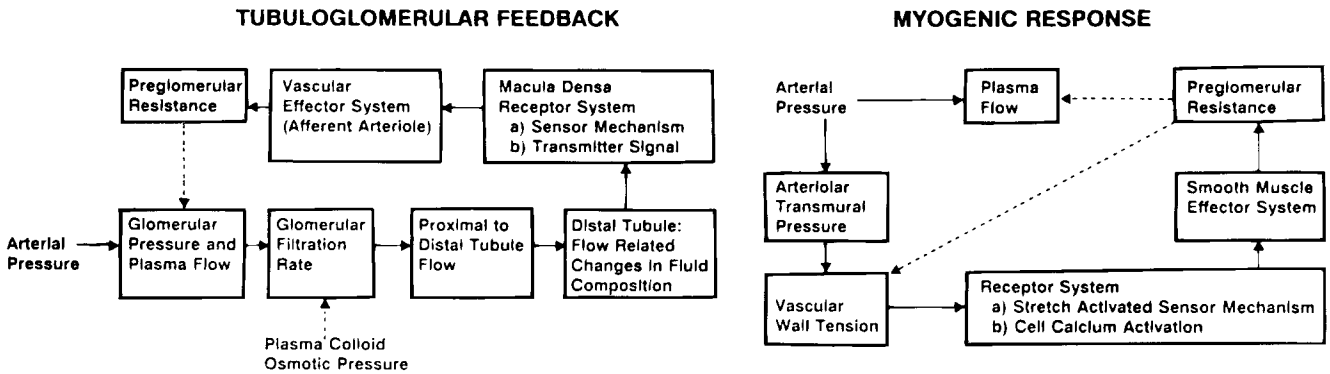

FIGURE 2-16. Macula densa tubuloglomerular feedback hypothesis (*left panel*) and myogenic response (*right panel*) as mechanisms to explain renal autoregulation. Solid lines indicate direct relations; dashed lines indicate inverse effects.

metabolically determined reabsorptive function of the tubules (1,5,18,20,24,27,28,94,136,138,141,147,149–151).

The general scheme for explaining autoregulation according to the tubuloglomerular feedback mechanism is shown in the left panel of Figure 2-16. For example, an increase in arterial pressure initially increases RBF, glomerular pressure, and GFR. The increased filtered load increases fluid and solute delivery from the proximal convoluted tubule into the loop of Henle. Such an effect leads to flow-dependent increases in the sodium chloride concentration and osmolality of the tubular fluid in the ascending loop of Henle. The MD cells sense the increased tubular fluid NaCl or total solute concentration and transmit vasoconstrictor signals to the afferent arterioles, thus restoring RBF and GFR to preexisting levels. Conversely, a decrease in arterial pressure causes a reduction in tubular fluid flow that elicits dilation of the afferent arterioles. The tubuloglomerular feedback mechanism also helps to explain vascular responses that occur when the solute load to the distal nephron changes as a consequence of changes in tubular reabsorptive function, such as those during pharmacologically induced changes in proximal reabsorption rate (2,5,27,152).

There are many examples of physiological responses that are consistent with the tubuloglomerular feedback concept. Elevations in plasma colloid osmotic pressure, which decrease the effective filtration pressure and, thus, GFR, lead to decreases in afferent arteriolar resistance resulting in elevations in glomerular pressure sufficient to counteract the increases in colloid osmotic pressure. Studies at the single nephron level, using micropuncture techniques, indicate that the maintenance of flow to the distal nephron is requisite for the full manifestation of autoregulation of GFR. As is shown in Figure 2-17, autoregulation of single nephron GFR in response to acute changes in arterial pressure is highly efficient when tubular fluid flow to the distal nephron is maintained, but it is significantly impaired when flow past MD cells is interrupted. Similar responses have been reported for glomerular capillary pressure. Nevertheless, the impairment in GFR autoregulation is not as great as would be predicted for a fully passive mechanism, so it seems that the tubuloglomerular feedback mechanism works in concert with the myogenic mechanism to yield the highly efficient autoregulation characteristic of the renal circulation. Recent studies suggest that the interactions are synergistic in that the presence

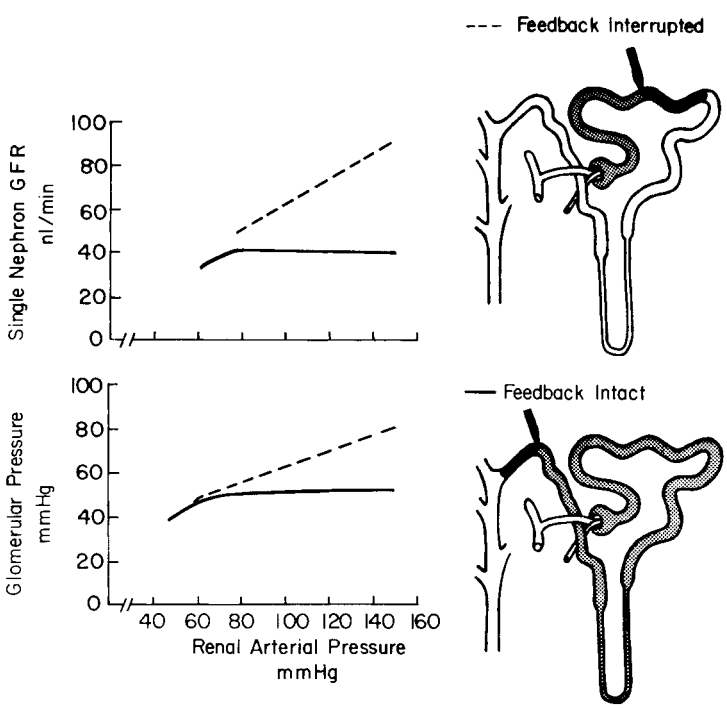

FIGURE 2-17. Responses of single nephron GFR and glomerular pressure to changes in arterial pressure during conditions of intact flow to the MD (*solid lines*) and during interrupted flow conditions (*dashed lines*). (From: Navar LG. Renal autoregulation: perspectives from whole kidney and single nephron studies. *Am J Physiol* 1978;234:F357, with permission.)

of active MD signals augments the sensitivity of the myogenic response. The afferent arteriole is the effector limb of both mechanisms and blockade of Ca^{2+} entry through L-type channels inhibits both the myogenic and tubuloglomerular feedback responses. The highly localized myogenic component can respond very quickly to a pressure stimulus. The tubuloglomerular feedback loop is more complex, involving multiple structures and cell types, and its response to a pressure change being transmitted along the tubule is slower, on the order of 15 s. The relative importance of these two systems may vary according to experimental conditions. A comprehensive quantitative analysis of the dynamic control of the renal circulation has been recently published (2,5,20,27,137,138,140–142,151).

Increases in flow through the loop of Henle elicit constriction of afferent arterioles with consequent reductions in glomerular pressure and filtration rate of the same nephron. These responses are represented in Figure 2-18. Note that the responses are nonlinear, with the most sensitive region in the physiological range of tubular flow. Another important feature is that the reactivity or sensitivity of the tubuloglomerular feedback mechanism can be modified by a variety of paracrine agents, hormones, and pharmacologic agents. Increased sensitivity is generally associated with extracellular fluid volume contraction and reduced responsiveness has been observed during expansion of extracellular fluid volume (2,150,151).

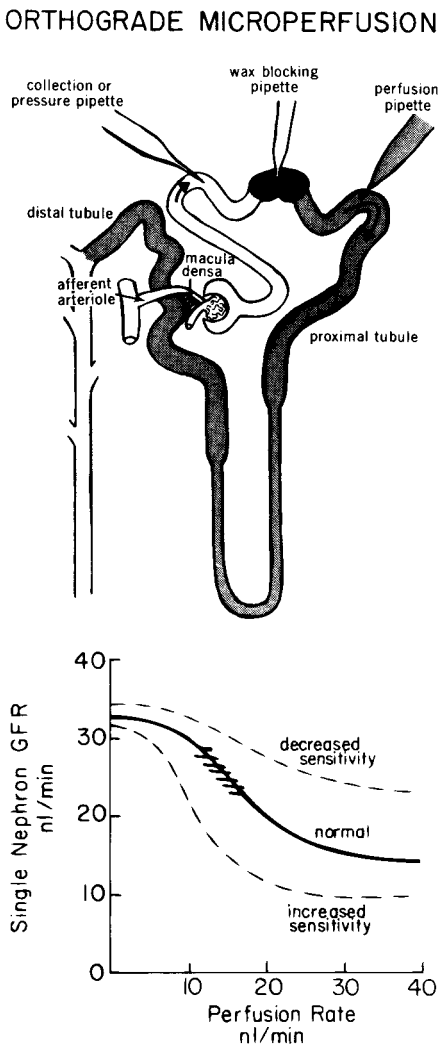

FIGURE 2-18. Relationships between the perfusion rate into a late proximal tubule and single nephron GFR. Dashed lines show responses during conditions of decreased and increased feedback sensitivity. Stippled area shows physiological range of single nephron GFR as a function of late proximal flow rate. The technique used to obtain the feedback responses is illustrated in the top panel.

The MD sensing segment is located at the end of the ascending loop of Henle. The ascending limb of the loop of Henle is virtually impermeable to water and has a powerful sodium chloride transport mechanism that results in the delivery of a hypotonic fluid to the MD cells. The nature of the transport processes of the thick ascending limb is discussed in detail in Chapter 4, Tubular Sodium Transport. In essence, increases in fluid delivery from the proximal tubule lead to progressive increases in distal flow, sodium chloride concentration, and osmolality. This coupling between fluid flow through the ascending limb and tubular fluid solute concentration at the MD provides the means by which volume delivery out of the proximal tubule is sensed. The signal sensed by MD cells may be a specific constituent of tubular fluid, such as, sodium or chloride or total solute concentration (5,18,24,27,147,151).The cellular mechanisms responsible for transmitting signals have remained an intriguing issue. MD cells possess a Na-K-2Cl cotransporter, sensitive to the diuretic furosemide, that is present in the thick ascending loop of Henle. This cotransporter must be functional in order for tubuloglomerular feedback signals to be transmitted, but it may not be the actual mechanism that activates intracellular signals, which appear to involve increases in intracellular Ca^{2+} ions and increases in Ca^{2+} entry from the basolateral side of the MD cells. Increases in cytosolic Ca^{2+} concentration occur when MD sodium chloride transport is increased. The mechanisms by which cytosolic Ca^{2+} concentrations are related to the formation and release of vasoactive mediators of tubuloglomerular feedback signals remain unclear. Calcium changes can be counteracted by elevations in cAMP. These intracellular ionic changes signal the MD cells to form and secrete constrictor and dilator substances that influence vascular contraction as a function of MD transport. Nevertheless, the actual final effector messenger between the MD cells and the afferent arteriole(s) remains under investigation. Early studies fostered the belief that the final effector signal was locally formed Ang II. However, numerous findings have established that the changes in the activity of the renin–angiotensin system modulate the sensitivity of the feedback response but do not directly mediate tubuloglomerular feedback signals. The effector agent is thought to operate primarily by activating voltage-dependent calcium channels in the afferent arteriole and, perhaps, the interlobular artery. Attractive candidates that have been recently considered, include purinergic agents, such as, adenosine and ATP or arachidonic acid metabolites. Several recent studies have provided new evidence supporting the hypothesis that increased tubuloglomerular feedback-dependent signals elicit increased secretion of ATP by MD cells, which cause afferent arteriolar constriction via activation of P2 receptors, and increases in calcium entry via voltage-dependent calcium channels. Alternative views suggests that the ATP is metabolized to adenosine, which is then the active metabolite that elicits vasoconstriction or that an arachidonic acid metabolite, such as, 20-HETE participates in mediating tubuloglomerular feedback-dependent vasoconstriction (1,2,5,24, 27,147,149–164).

Modulation of Tubuloglomerular Feedback Activity by Vasoactive Agents

MD cells also signal the juxtaglomerular cells to vary renin synthesis and release. Mechanisms of renin release are discussed in the section on the renin–angiotensin system in this chapter.

In brief, the directional changes in renin release and angiotensin formation are opposite to those that are required to mediate tubuloglomerular feedback. For example, high tubular flows and elevated luminal NaCl concentrations are associated with reduced renin release, when tubuloglomerular feedback-mediates afferent arteriolar constriction. Nevertheless, Ang II exerts an important role in modulating tubuloglomerular feedback activity during changes in salt diet, extracellular fluid volume, and renal perfusion pressure. Tubuloglomerular feedback is absent in mice lacking AT_{1A} receptors, although the renal vasculature is capable of responding to Ang II. Tubuloglomerular feedback is nonresponsive in animals unable to produce Ang II, because angiotensin-converting enzyme is mutated (25–27,113,149–151).

The neuronal nitric oxide synthase (NOS) isoform is localized in MD cells. MD cells have potent mechanisms for NO synthesis, which contributes to the modulation of tubuloglomerular feedback signals. Blockade of NO synthesis leads to augmentation of tubuloglomerular feedback responses, while enhanced NOS levels attenuate the vasoconstrictor response to increased distal nephron flow rate. Salt uptake across the luminal membrane by a furosemide-sensitive Na-K-2Cl transporter may link increases in cellular cAMP and calcium to NO production. Recent studies have indicated that NO-dependent vasodilatory signals are activated during marked increases in flow to the MD in order to buffer against excess tubuloglomerular feedback-mediated vasoconstriction. Under certain conditions, oxygen radicals generated in the vicinity of the JGA can act to scavenge NO, limiting MD NO signaling and, thereby, produce vasoconstrictionlike effects, enhancing tubuloglomerular feedback activity. Normal tubuloglomerular feedback is found in gene knockout animals lacking function of neuronal NOS (149,165–168).

Arachidonic acid metabolites also may modulate tubuloglomerular feedback activity and interact with other vasoactive modulators. Cyclooxygenase 2 (COX-2) has been localized to MD cells and surrounding ascending loop of Henle cells; PGE_2 release from MD cells has been reported. A COX-2 metabolite attenuates the vasoconstrictor autoregulatory, tubuloglomerular feedback-mediated, response of the afferent arteriole to an increase in arterial pressure. Such a dilator agent also appears to contribute to MD production of NO, which can also inhibit afferent arteriolar responses to pressure. Recent evidence suggests that thromboxane A_2 and cytochrome P450 metabolites, such as, 20-HETE, are also involved in the constrictor limb of tubuloglomerular feedback. However, gene targeting rendering the thromboxane receptor nonfunctional has no effect on tubuloglomerular feedback activity (27,149,158,169–172).

In addition to paracrine influences, interstitial physical forces have been proposed to modulate tubuloglomerular feedback activity. Changes in interstitial fluid pressure or volume may exert independent effects on tubuloglomerular feedback reactivity. Moreover, renal epithelial cells seem to produce undefined natural modulator factors during chronic salt loading and natriuresis produced by ANP. For example, stronger tubuloglomerular feedback responses are observed during these natriuretic conditions when native tubular fluid is presented to the macula densa region, compared with artificial tubular fluid (150,173).

The Renin-Angiotensin System

Formation of Ang II

Consideration of the role of the renin–angiotensin system in the control of renal hemodynamics is complicated in that Ang II acts as both a circulating hormone and a locally generated paracrine or autocrine agent. Renin is a proteolytic enzyme that cleaves Ang I from renin substrate (angiotensinogen), which is formed primarily by the liver, but also in the kidney. Renin is synthesized primarily in granular epithelioid cells of the juxtaglomerular apparatus and is most likely secreted into the surrounding interstitium; renin is also formed in proximal tubule cells and principal cells of connecting tubule and collecting duct segments. Substrate availability in the plasma and intrarenal angiotensinogen levels are less than are required to produce maximum reaction velocity, so alterations in renin substrate contribute to the regulation of Ang I production. The inactive decapeptide is then cleaved by angiotensin converting enzyme (ACE) to form the active octapeptide. There are abundant amounts of endothelium-bound ACE in the lung and almost all other tissues. However, most of the Ang II in the systemic arterial blood is formed by the lungs. The major sites of ACE localization in the kidney are on the luminal surface of the endothelial cells lining the arteries and arterioles, in particular, the afferent arterioles, but also the efferent arterioles and the glomerular capillaries and on the brush border and basolateral membranes of the proximal tubule; ACE is also present on the lumen of collecting duct segments. Functional data suggest there is also extravascular ACE in the interstitial compartment (25,27,113,174–178). A different ACE, termed ACE2, has been recently identified and characterized. It cleaves a single amino acid from Ang I to form Ang 1–9, and from Ang II to form Ang 1–7 (Fig. 2-19) (179).

Ang II can be delivered to renal vascular receptors as a circulating hormone or it may be formed locally from systemically delivered Ang I by endothelial cell-bound ACE. About 20% of the circulating Ang I is converted to vasoactive Ang II in the kidney. Ang II also may be formed in the interstitial fluid from Ang I, generated as a consequence of enhanced renin release or from Ang I that diffuses from the peritubular capillary structures into the interstitium. At present, the relative roles of these two sources of Ang II are uncertain. Because the renal tissue Ang II levels are higher than that of arterial blood, significant amounts of Ang II are generated intrarenally. In addition, Ang I and II may be formed within the juxtaglomerular cells and coreleased with renin to act on adjacent glomerular arterioles. High concentrations of Ang II also exist in proximal tubular fluid, indicating formation by proximal tubular cells. Ang II derived from proximal tubular cells also may have vascular effects after traversing the interstitium (25,27,113,174–176,180).

Renin Production and Release

As Figure 2-19 shows, renin is released in response to a variety of stimuli, including decreases in sodium intake, contraction of extracellular fluid volume or blood volume, increases in sympathetic renal nerve activity, decreases in sodium load to the MD, and decreases in renal arterial perfusion pressure. Ang II, vasopressin, endothelin, nitric oxide, prostaglandins, and potassium also influence renin release directly. The final effector mechanism appears to involve changes in the calcium and cAMP concentrations in juxtaglomerular cells. The calcium mechanism is unusual for exocytosis of secretory granules in that a decrease in cytosolic calcium functions as a stimulator of renin release. In certain conditions, renin release may be associated with an increase in cellular cAMP levels; however, this occurrence also may be associated with a reciprocal decrease in cytosolic calcium concentration as the final common pathway. Cytosolic levels of cAMP are controlled by synthesis via adenylate cyclase and hydrolysis by cyclic nucleotide phosphodiesterases (PDE). PDE_3 is the major isoform localized to afferent arterioles and recent evidence indicates pharmacologic inhibition of PDE_3 increases cAMP, basal renin secretion, and enhances the renin secretory response to β-adrenergic stimulation in animals and humans. cAMP stimulates exocytosis

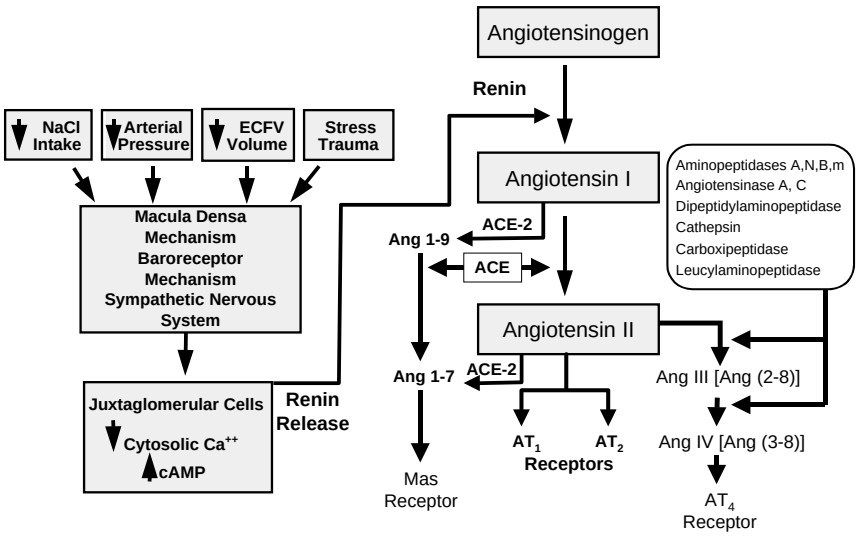

FIGURE 2-19. Schematic representation of the renin–angiotensin system and the mechanisms of renin release.

of secretory granules in the juxtaglomerular cells as does a β-adrenoceptor agonist and cell shrinkage with high extracellular osmolality. Juxtaglomerular cells have Ca^{2+}-sensitive voltage-gated K channels (BK_{Ca}) that are activated by cAMP to hyperpolarize cells from −32 mV to −48 mV. Vasoconstrictor G protein-coupled receptor agonists inhibit renin release by activating PLC and mobilizing Ca^{2+} that activated Ca^{2+} entry through store-operated cation channels (SOC). Whether or not L-type Ca^{2+} channels are present in juxtaglomerular granular cells is controversial (25–27,174–176,180–183).

There are several first-messenger systems that send signals to regulate cAMP or Ca^{2+} in juxtaglomerular cells to regulate renin release. These are discussed briefly below and in more detail in Chapter 9, Hormones and the Kidney.

Sympathetic Nervous System and Catecholamines. The cells of the juxtaglomerular apparatus are richly innervated and respond to renal nerve stimulation and to circulating catecholamines. There are both direct and indirect effects. Subtle increases in renal nerve traffic or circulating epinephrine activate β_1-adrenoreceptors on juxtaglomerular cells and, by means of increased cAMP, enhance renin release. Strong renal nerve activity causes substantial reductions in RBF and GFR through activation of α-adrenergic receptors and subsequent indirect stimulation of renin release, secondary to intrarenal baroreceptor and MD stimuli (184).

Macula Densa. MD cells detect decreases in the sodium chloride load or concentration emerging form the ascending loop of Henle and send signals to the juxtaglomerular cells to increase renin secretion rate. While the sensing mechanism involving furosemide-sensitive luminal uptake of sodium chloride is not completely understood, evidence suggests that adenosine can inhibit renin release; its precursor, ATP, is secreted by MD cells. Circumstances that result in extracellular volume depletion or sodium deprivation stimulate renin release, at least in part, by a macula densa mechanism. Reduced flow to the MD also leads to PGE_2 release that stimulates renin release (26,27, 113,172).

Renal Vascular Baroreceptor. Decreases in renal afferent arteriolar pressure directly increase renin release, independent of the renal nerves and the macula densa mechanism. The juxtaglomerular cells appear to be directly sensitive to stretch, such that decreased wall tension decreases cell calcium entry and cytosolic calcium levels, while the opposite occurs at elevated arterial pressures. Under some circumstances, the same extrinsic disturbance may influence both the MD and the vascular baroreceptor mechanism to increase renin release, but the vascular receptor system can act independently (25,113).

Other Factors. Renin secretion is inhibited by elevated plasma concentrations of Ang II, vasopressin, adenosine, thromboxane A_2, and potassium. The effects of Ang II and other vasoconstrictors appear to be a direct consequence of an end-product inhibition on the juxtaglomerular cells. PGE_2 and PGI_2 can stimulate renin release through direct effects, which may be related to stimulation of cellular cAMP levels. Reduced NaCl reabsorption by MD cells stimulates renin secretion from juxtaglomerular cells. Stimulatory prostaglandins derived from COX-2 in MD cells are critically involved in distal tubular NaCl control of renin release. Atrial natriuretic peptide (ANP) increases cellular cGMP production and inhibits renin release. Endothelium-derived factors also appear to modulate renin release, with NO and ET-1 stimulating renin release (25,181,183–190).

Interaction with Nitric Oxide (NO)

COX-2, neuronal NO synthase (nNOS), and renin synthesis often change in parallel to changes in salt diet and alterations in distal tubular NaCl concentration, and their products may mutually determine synthesis and activity of these enzymes. nNOS and COX-2 are coexpressed in MD cells, and the expression of both enzymes is stimulated in high-renin states. Parallel changes are observed during chronic changes in salt diet, with low salt stimulating nNOS, COX-2, and renin secretion as compared to inhibition occurring on chronic high salt diet. NO derived from nNOS may exert a stimulatory role on COX-2 expression to produce PGE_2/PGI_2, strong stimulants to renin release from juxtaglomerular granular cells by acting on EP_4 and IP receptors. PGE_2 may exert short-loop feedback to inhibit nNOS expression. In this regard, eNOS-derived NO may, more importantly, impact on renin expression than nNOS-derived NO. In some situations, NO appears to play a more indirect permissive role, permitting the MD pathway of renin secretion to function normally. NO stimulates renin release by cGMP-inhibiting PDE_3 to increase cell concentrations of cAMP by reducing cAMP breakdown in juxtaglomerular cells, with activation of cAMP-sensitive protein kinase (PKA) (189,191–194).

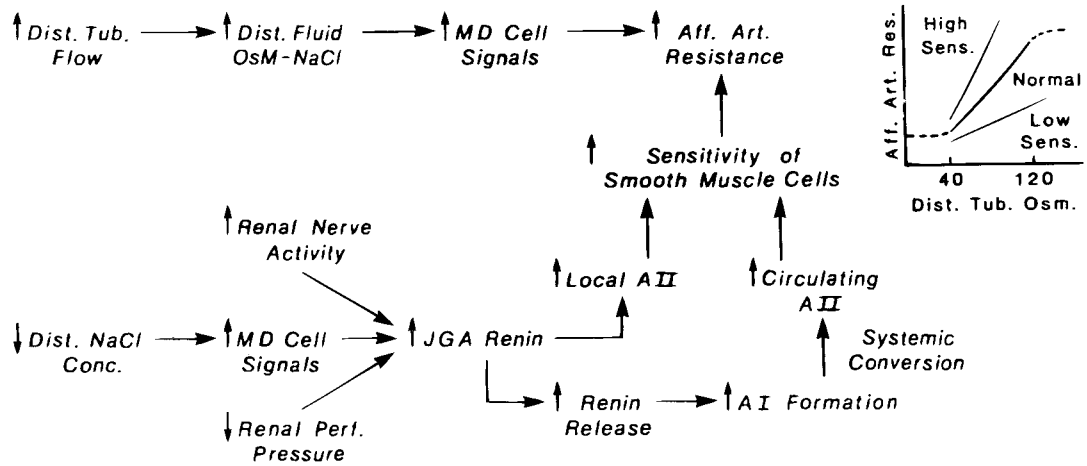

FIGURE 2-20. Modulator hypothesis for postulated interactions between the tubuloglomerular feedback mechanism and the intrarenal renin–angiotensin system. Flow-related changes in tubular fluid concentration can elicit signals from macula densa (MD) cells to vascular contractile cells independent of angiotensin levels. Changes in angiotensin II concentration influence the sensitivity or responsiveness of MD cells or of vascular smooth muscle cells to the signals coming from the MD cells.

Although many different types of stimuli increase the activity of the renin–angiotensin system, most are related to circumstances that compromise body fluid volume homeostasis. Thus, stimulation of renin release and activation of Ang II-dependent mechanisms help to minimize renal fluid and sodium losses and to maintain extracellular fluid volume and arterial blood pressure. The myriad of actions exerted by Ang II all seem to be homeostatically appropriate to achieve this end. Only the renal vascular actions of angiotensin will be covered in this chapter, but it should be pointed out that Ang II is also a potent stimulator of aldosterone release and can directly enhance proximal tubular sodium reabsorption rate and function in Henle's loop and distal nephron segments. It also has important effects on the central nervous system, stimulating thirst, vasopressin release, and sympathetic nerve activity (25,113,176).

There are several peptidases that act on angiotensinogen to form other biologically active peptides. Angiotensin with amino acids 1 to 7 is formed by neutral endopeptidase, but appears to have only slight effects on the renal vasculature under physiological conditions. Elevated levels of Ang 1 to 7 occur during ACE inhibition and may dilate renal and nonrenal vascular beds. Ang III (angiotensin with amino acids 2 to 8) has actions similar to that of Ang II, which can be blocked by Ang II receptor antagonists. Ang IV (angiotensin with amino acids 3 to 8), a hexapeptide, is reported to produce vasodilation by release of NO or prostanoids from endothelial cells by acting on a specific AT_4 (fourth subtype of angiotensin) receptor. A recently described enzyme, ACE-2, forms Ang (1 to 9) from Ang I and Ang (1 to 7) from Ang II. Thus, ACE-2 can produce more Ang (1 to 7) from Ang II and, in this manner, reduces the available Ang II (179,195–199).

It should be emphasized that the MD has distinct mechanisms for renin release and the tubuloglomerular feedback system. The tubuloglomerular feedback mechanism sends a vasoconstrictor signal to the afferent arteriole in response to increases in distal flow and the accompanying increases in solute concentration. Under these conditions, the macula densa mechanism decreases renin release and local Ang II activity, which is opposite to that required for Ang II to mediate the tubuloglomerular feedback-induced contraction of afferent arterioles. Thus, Ang II clearly does not mediate tubuloglomerular feedback responses; it does, however, modulate the sensitivity of the feedback mechanism. When tissue Ang II levels are increased, smooth muscle responsiveness is augmented. In contrast, when Ang II levels are suppressed, whether in response to physiological manipulations or as a consequence of pharmacologic blockade, the sensitivity of the tubuloglomerular feedback system is attenuated. Interestingly, sensitivity can be restored by administration of Ang II, but not norepinephrine. In addition to directly affecting the afferent arteriole, Ang II affects tubuloglomerular feedback activity by altering tubular reabsorption and fluid delivery to the MD. The vascular and tubular effects work in concert to reduce GFR when sodium excretion is reduced, as observed during volume contraction. As previously mentioned (Fig. 2-18), changes in tubuloglomerular feedback responsiveness during altered volume states may be largely due to changes in tissue levels of Ang II. The interactions between the renin–angiotensin system and the tubuloglomerular feedback mechanism are illustrated in Figure 2-20 (5,113,149,175,176,197).

Angiotensin Receptors

Several subtypes of receptors for Ang II have been identified in the renal circulation. *In utero*, animals have a larger population of AT_2 than AT_1 receptors, which decreases progressively after birth; in adult life, the major subtype on vascular smooth muscle cells is the AT_1 receptor. The AT_1 receptor is present on preglomerular arteries and arterioles, juxtaglomerular cells, glomerular mesangial cells, efferent arterioles, and vasa recta bundles of the inner medullary stripe. Humans appear to have one AT_1 receptor, whereas rodents have two, termed AT_{1A} and AT_{1B}, which are 94% homologous. Currently available pharmacologic antagonists of AT_1 receptors, such as candesartan, losartan, and valsartan, do not distinguish between the two AT_1 subtypes. Almost all of the vasoconstrictor actions of Ang II on the renal vasculature under physiological conditions are mediated by AT_1. Both the AT_{1A} and AT_{1B} receptor mediate Ang II-induced calcium signaling in smooth muscle cells and renal vasoconstriction. Evidence for the role of AT_{1B} receptors derives from mice lacking a functional AT_{1A} receptor (69,133,176,195,200–207).

The AT_1 receptor is primarily coupled to the GTP-binding protein $G\alpha q_{11/12}$, which activation leads to triggering of several signaling pathways, including stimulation of phospholipase $C\beta 1$. Receptor activation of protein tyrosine kinases leads to somewhat slower stimulation of phospholipase $C\gamma$. Phospolipases D and A2 may also be activated, favoring release of DAG

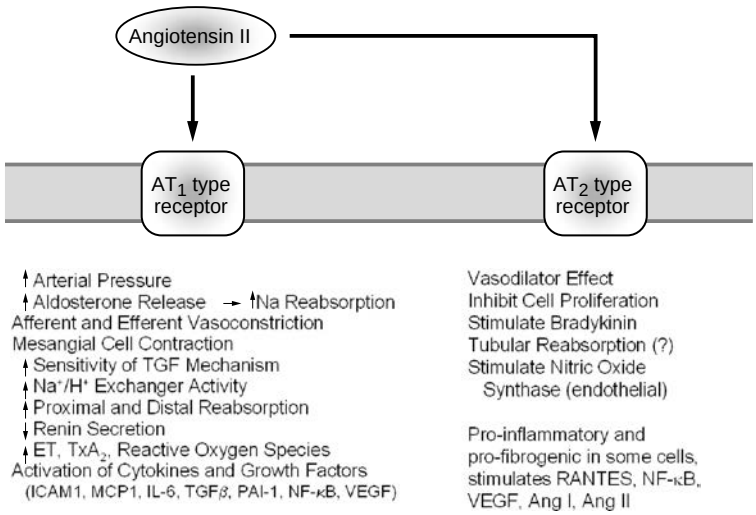

FIGURE 2-21. Multiple actions of angiotensin II on renal function mediated by AT_1 and AT_2 receptors.

and phosphatidic acid from phosphotidylcholine and arachidonic acid, respectively. The PLCs act on membrane-bound phosphoinositides to yield DAG and IP3. DAG stimulates protein kinase C, while IP_3 diffuses through the cytosol to active IP_3-sensitive receptors on membranes of the sarcoplasmic reticulum, triggering Ca^{2+} release to the cytosol. The mobilized Ca^{2+} seems to trigger a complex array of events. Calcium-sensitive chloride channels may be stimulated to promote chloride efflux and, thereby, depolarization of the plasma membrane. Such a signal will activate voltage-gated L-type calcium channels to allow influx down a very steep gradient, from 1 to 2 mM in the extracellular fluid to 100 to 200 nM in the cytosol. Calcium release from internal stores also signals SOC to open and allow further Ca^{2+} entry. AT_1 receptor activation may also trigger voltage-dependent Ca^{2+} channels independently of Ca^{2+} release from intracellular stores. As discussed earlier, afferent arteriolar contraction induced by Ang II is dependent on calcium entry through voltage-gated channels, whereas efferent arterioles are not affected by L-type channel blockers, but are influenced by T-type channel blockers (5,66,69,133,157,176,208).

The AT_2 receptor is characterized by a high affinity to the nonpeptide antagonists PD123319 and CGP 42112 and has a sequence 33% identical to that of the AT_1 receptor. Its high expression in fetal tissue suggests a role in embryonic development. This receptor has a typical pattern of seven transmembrane domains and is also coupled to a G protein. Recent studies suggest that AT_2 receptors may exert important modulatory actions partially counteracting the effects caused by AT_1 receptor activation. AT_2 receptor activation has been shown to increase bradykinin and NO levels leading to increases in cGMP and vasodilation. AT_2 receptor activity may be upregulated during chronic states of salt deprivation. AT_3 and AT_4 receptors may be selective for angiotensin with amino acids 1 to 7 and Ang IV (angiotensin with amino acids 3 to 8), although they appear to play a minor role in the regulation of renal hemodynamics (5,197,203,209,210).

Ang II receptors are regulated in response to different physiological conditions. It is of interest to note that glomerular and vascular receptors are regulated differently from proximal tubular receptors. A low-salt diet and high levels of Ang II lead to downregulation of arteriolar and mesangial cell receptors, but upregulation of tubular receptors (200,211).

Actions of Ang II on the Renal Microvasculature

At high concentrations, Ang II elicits dose-dependent AT_1-mediated decreases in RBF and GFR. The decreases in GFR are often smaller than the decreases in RBF, such that filtration fraction increases. The increased vascular resistance is due to a combination of afferent and efferent arteriolar constriction. Glomerular capillary pressure is well maintained and, at higher doses, the glomerular filtration coefficient is reduced. Ang II produces more pronounced vasoconstriction when endogenous levels are low, presumably because of receptor upregulation, and when vasodilator prostaglandins are blocked by cyclooxygenase inhibitors. Larger Ang II effects are noted after endothelial production of NO is blocked. As mentioned earlier, Ang II can potentiate tubuloglomerular feedback-mediated changes in preglomerular vascular tone. The multiple effects of Ang II are illustrated in Figure 2-21. In addition to these effects, Ang II influences the medullary circulation, even at concentrations lower than those required to elicit overall vasoconstriction in the cortex. This notion is supported by the finding that the Ang II receptor density is much higher in the medullary vessels and interstitial cells than in the postglomerular cortical vasculature (5,9,113,119,133,176,210,212).

The effects of endogenous Ang II have been determined using angiotensin-receptor antagonists or ACE inhibitors in states when the prevailing Ang II levels are high. In sodium-restricted animals, RBF is increased after AT_1 receptor blockade, while the GFR responses are smaller and more variable; filtration fraction usually declines. Renin inhibition produces similar results and combined renin and angiotensin inhibition causes decreases in renal vascular resistance and increases in GFR. Activation of the AT_1 receptor is primarily responsible for the renal vascular effects of the endogenous Ang II on afferent and efferent arterioles and on K_f. Whole-kidney autoregulation is not affected by AT_1 receptor blockade or ACE inhibition, although the contribution of the tubuloglomerular feedback mechanism may be reduced, and the plateau of the autoregulation relationship is elevated (5,9,113,119,133,176,213–216).

Endothelium-Derived Vasoactive Factors

Nitric Oxide

Endothelial cells release nitric oxide (NO) in response to many signals, including mechanical shear stress, hormonal, and chemical, that increase cytosolic Ca^{2+}. Nitric oxide is formed by vascular endothelial cells from the amino acid L-arginine via NO synthase (NOS), which is a soluble NADPH- and Ca^{2+}-calmodulin-dependent citrulline-forming enzyme that requires two cosubstrates (oxygen and NADPH) and four cofactors (heme, flavin mononucleotide, flavin adenine dinucleotide,

and H_4 biopterine). As shown in Figure 2-7, several agents are thought to induce their vascular effects via endothelial cell eNOS activation and release of NO. These include agents that stimulate the M_1-muscarinic receptor (acetylcholine), B_2-kinin receptor, H_1-histamine receptor, α_2-adrenoceptor, purinergic receptors (ATP, ADP), substance P, ET_B receptor, leukotriene D_4, thromboxane A_2, 5-hydroxytryptamine (5-HT_1)-serotoninergic receptor, and thrombin. The rate of renal production of NO is higher in the medulla than in the cortex. Nitric oxide and nitrosamines have short biologic half-lives of less than 10 s, which is especially shortened in oxygenated solutions in the presence of the superoxide anion O_2^-, by a mechanism involving production of NO_3^- from NO (82,102–106,131,132,165,166,217–224).

Basal release of NO tonically maintains a low renal vascular resistance at rest, acting to buffer vasoconstriction produced by Ang II, ET-1, and catecholamines. Acute inhibition of basal NO production causes renal vasoconstriction, with decreases of 25% to 35% in RBF and reductions in cGMP levels in the interstitium and urine. GFR responses are smaller and tend to be unchanged or decrease about 10%. Thus, filtration fraction is increased. Basal NO dilates both the preglomerular vasculature (arcuate and interlobular arteries, afferent arterioles) and efferent arterioles. NO also appears to contribute to increased or maintained glomerular filtration coefficient. Endothelial factors may modulate pressure-dependent responses of vascular segments exhibiting autoregulatory behavior. Note, however, that NO does not fit a mediator role in this scheme, because increased pressure and shear stress cause increased production of NO, a vasodilator, at the same time the responsive vascular elements exhibit vasoconstriction. NO inhibition results in renal vasoconstriction, but that steady-state autoregulatory responses to changes in perfusion pressure are unaffected and remain highly efficient (82,110,131,217–219, 225–229).

NO-induced renal vasodilation is mediated by cGMP-dependent and perhaps a cGMP-independent system. The latter appears to involve cGMP cross-talk with the cAMP pathway, with cGMP inhibiting cAMP breakdown by PDE_3, more so than activation of cGMP-protein kinase activity. Part of the dilatory response to NO may be mediated through reduced production of 20-HETE (91,103,106,131,182,221–224,230,231).

Although basal RBF is markedly reduced by inhibition of NO synthesis, steady-state autoregulatory adjustments in vascular resistance are not impaired in response to changes in perfusion pressure. Thus, NO influences a component of vascular resistance distinct from the autoregulatory component(s). Stimulation of cGMP by ANP dilates the preglomerular resistance vessels, but does not affect steady-state autoregulatory adjustments in renal vascular resistance. On the other hand, NO may influence the rapidity of the autoregulatory adjustments in resistance. Inhibition of NOS leads to greater relative decreases in medullary blood flow than in cortical blood flow. NO blockade usually reduces renin release and responsiveness to reductions in perfusion pressure (5,82,185,218 219,229,232–238).

Endothelial NO synthase is localized to the endothelial cells all along the renal vasculature (interlobular arteries, afferent and efferent arterioles, glomerular capillaries, and vasa recta). Targeting of eNOS to specialized plasma membrane invaginations termed *calveolae* is required for maximal eNOS activity. Neuronal NO synthase (nNOS) is present primarily in epithelial cells (thick ascending limb of Henle's loop, MD, and collecting duct). An inducible form of NO synthase is normally quiescent, but is capable of producing large amounts of NO in vascular smooth cells and mesangial cells. An endogenous inhibitor of NOS is asymmetric dimethylarginine (ADMA), which is normally inactivated by NG-NG-dimethylarginine dimethylaminohydrolase (DDAH) to form L-citrulline. DDAH expression is found at the same sites as NO synthase (109,165,182,231,239).

Endothelin

Endothelin refers to a family of long-acting vasoconstrictor peptides that act locally as paracrine hormones. ET-1, -2, and -3, each a 21 amino-acid peptide, are constitutively released. ET-1 is the major form synthesized and secreted by endothelial cells of the preglomerular vasculature and vasa recta and by medullary collecting ducts. Endothelin-converting enzyme (ECE) is the main enzyme responsible for the genesis of ET-1 from prepro-ET-1; chymase and matrix metalloproteinase II are also involved in the production of ET intermediates. ECE is pivotal in the genesis of ET-1. Ang II is a potent stimulus of ET-1 production. Other simulants include bradykinin, ATP, platelet-activating factor (PAF), thrombin, and shear stress. Neutral endopeptidase 24 to 11 degrades and inactivates ET-1. The two known receptor types, ET_A and ET_B, are G protein coupled and lead to IP_3 and cADP ribose formation, PKC activation, and calcium mobilization, in addition to calcium entry. ET_A receptors are predominantly found on vascular smooth muscle cells. ET_B receptors are localized on endothelial and tubular cells, as well as vascular smooth muscle cells. The afferent arteriolar constriction produced by ET-1, like that caused by Ang II, is dependent, in part, on calcium entry through voltage-dependent channels. The action of endothelin on efferent arterioles appears to depend exclusively on mobilization of intracellular calcium and/or entry through voltage-independent cation channels. The highest concentrations of ET-1 in the body exist in the renal medulla, where it is synthesized by collecting duct cells and acts in a paracrine/autocrine manner mediating natriuretic and diuretic effects through ET_B receptors in a nitric oxide-dependent manner. In addition, ET-1 has inotropic, chemotactic, and mitogenic properties. Overall, ET-1 increases blood pressure and vascular tone (240–243).

The vascular actions of ET-1 reflect a combination of vasoconstrictor ET_A and ET_B receptors on smooth muscle cells and vasodilator ET_B receptors on endothelial cells. ET-1 reduces RBF because of smooth muscle $ET_A + ET_B$ receptor stimulation in combination with buffering effects of endothelial ET_B receptors that are mediated by NO and an ill-defined mechanism. In contrast, stimulation of ET_B receptors alone elicits renal vasoconstriction. Thus, sole ET_B receptor activation exerts a net renal vasoconstriction, whereas a net dilator action is observed when ET_B is costimulated with ET_A receptors. Such opposing actions indicate more complex than additive interaction between ET receptor subtypes. Endothelial ET_B receptors in the kidney and lung also seem to function as clearance receptors, effectively reducing the local concentration of ET-1 and thereby attenuating vasoconstriction (244–246).

Exogenous ET-1 reduces glomerular blood flow by stimulating both ET_A and ET_B receptors to cause constriction of arcuate and interlobular arteries and afferent and efferent arterioles. Glomerular capillary pressure is relatively well maintained in the presence of reduced RBF. The decrease in GFR is primarily mediated by reductions in plasma flow and K_f. The reduction in K_f appears to be mediated by secondary release of Ang II, eicosanoids, or neurotransmitters. The preglomerular vasculature has equal proportions of ET_A and ET_B receptors as compared to a ratio of 2:1 on their smooth muscle cells devoid of endothelial cells.

Under basal conditions, ET-1 exerts dual actions on the vasculature that are equal and opposite. ET_A receptor blockade produces renal vasodilation because of a 5% to 10% increase in RBF, whereas ET_B receptor antagonism leads to constriction of 5% to 10%. Although ET-1 contributes to basal vascular tone, it does not interfere with renal autoregulatory mechanisms (135,240,243,245–256).

ET_A and ET_B receptors stimulate cytosolic Ca^{2+} concentration in vascular smooth muscle cells of preglomerular arteries/arterioles through a combination of mobilization and entry pathways. Low concentrations of ET-1 may preferentially activate Ca^{2+} entry channels, with higher concentrations mobilizing intracellular Ca^{2+} via the PLC–IP_3R and cADPR–RyR pathways. Calcium channel blockers reduce the amount and duration of ET-1-induced renal vasoconstriction. The afferent arteriolar constriction produced by ET-1, similar to that caused by Ang II, is dependent, in part, on Ca^{2+} entry through voltage-dependent L-type channels. The action of ET-1 on efferent arterioles appears to depend exclusively on mobilization of intracellular Ca^{2+}. ET-1 inhibits renin synthesis and release by ET_A and ET_B receptor stimulation of cytosolic Ca^{2+} in juxtaglomerular granular cells. Both receptors mediate ET-1 stimulation of adrenal aldosterone synthase gene to favor aldosterone secretion (240,249,257,258).

ET-1 acts on glomerular mesangial cells to cause contraction and stimulation of mitogenesis. High concentrations of ET-1 that reduce sodium excretion increase plasma renin activity, presumably via the macula densa mechanism. In addition, ET-1 may affect neurotransmission, eicosanoid synthesis, and ANP synthesis and release. Activation of endothelin receptors causes release of eicosanoids and NO from endothelial cells and ANP from myocytes (240,240,259).

Heme Oxygenase and Carbon Monoxide

A heme oxygenase pathway appears to be involved in the pathogenesis of cell injury in atherosclerosis and progressive renal disease; its role in physiological regulation in health is less clear. For example, oxidized LDL induces heme oxygenase (HO), a microsomal enzyme that is involved in heme detoxification and is a major endogenous source of carbon monoxide. Activation of heme oxidase has an antioxident effect by degrading the heme moiety of heme-containing proteins, such as nitric oxide synthase, COX2, and cytochrome P450 monoxygenase. It is proposed that CO produced from endogenous heme oxygenase present in large arteries and small arterioles is a potent vasodilator. Heme oxygenase catalyzes the metabolism of heme to biliverdin, free iron, and carbon monoxide. Carbon monoxide induces arteriolar dilation via activation of soluble guanylate cyclase, an effect reversed by inhibition of Ca^{2+}-activated K^+ channels. The HO substrates heme–L-lysinate and –L-arginate also produces dilation that is blocked by inhibition of K^+ channels or by chromium mesophorphyrine, an inhibitor of HO. Recent evidence implicates some vasoconstriction actions of CO on vascular endothelium indirectly caused by inhibition of nitric oxide synthase (260,261).

Arachidonic Acid Metabolites

Renal prostaglandins, or eicosanoids, are biologically active fatty acid products of arachidonic acid that contribute to the regulation of renal hemodynamics. They are synthesized intracellularly and immediately released to act locally on the renal vasculature as paracrine/autocrine agents. Free intracellular arachidonic acid can be metabolized on demand via one of three major enzymatic pathways: cyclooxygenase, lipoxygenase, or cytochrome P450 monooxygenase. Depending on cell types, net production of the various metabolites may cause vasoconstriction under some conditions and vasodilation during others. The multiple products of this cascade are depicted in Figure 2-22. Phospholipase A_2 catalyzes formation of arachidonic acid, an unsaturated 20-carbon fatty acid from membrane phospholipids.

A key regulatory, rate-limiting step for prostaglandin synthesis is the conversion of arachidonic acid to prostaglandin G_2/H_2 by cyclooxygenase (COX). There are two major COX enzymes. COX-1, a constitutive enzyme, is found in renal

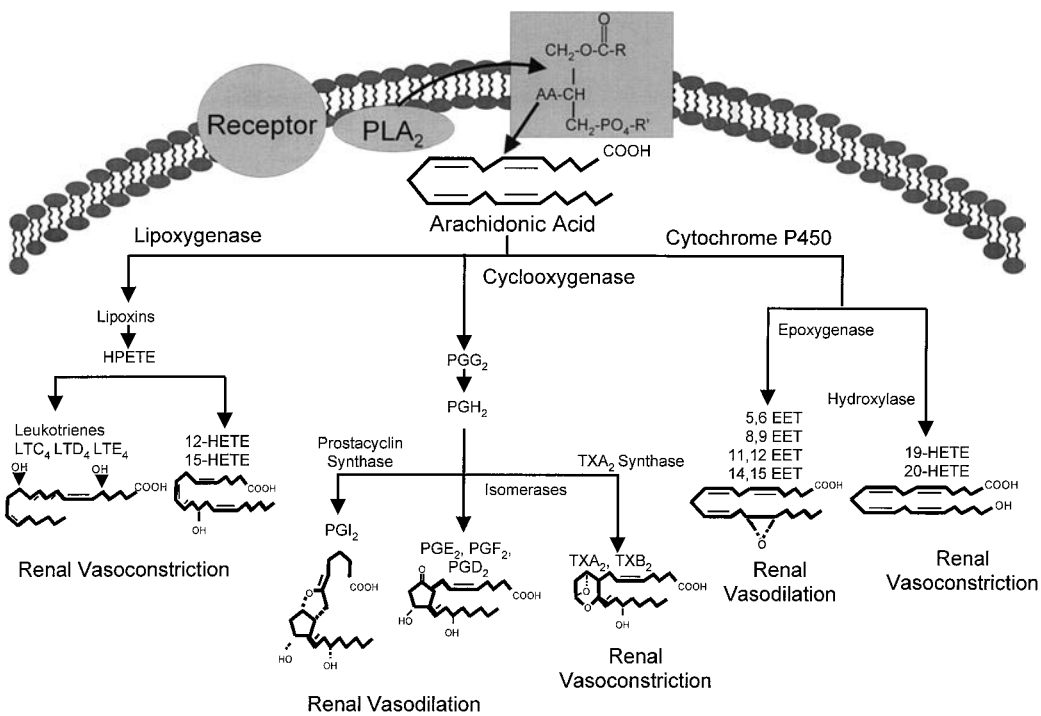

FIGURE 2-22. Three major pathways of eicosanoid synthesis from arachidonic acid involving cyclooxygenase, lipoxygenase, and cytochrome P450 monooxygenase enzyme systems. Note that only the configuration of the cyclopentane ring is shown. *LT*, leukotriene. (From: Navar LG, Inscho EW, Majid DSA, et al. Paracrine regulation of the renal microcirculation. *Physiol Rev* 1996;76:425, with permission.)

arteries and arterioles, glomeruli, cortical and medullary collecting ducts, and medullary interstitial cells. COX-2 is a regulated constitutive enzyme that is important in development and in adult animals is commonly activated by growth factors, inflammatory states, glucocorticoids, and low-salt diet. Renal COX-2 mRNA is localized primarily to cortical thick ascending limb epithelial cells, including the MD region and medullary interstitial cells, with greater amounts in the papilla than the cortex. COX-2 mRNA is noticeably absent from arterioles, glomeruli, and cortical or medullary collecting ducts sites of COX-1 expression; COX-2 also is expressed in endothelial cells of medullary vasa recta. COX-2 expression in thick ascending limb cells and MD cells varies with salt diet, increasing during chronic sodium restriction and decreasing during sodium loading. COX-2 expression in medullary interstitial cells is induced by water deprivation and high interstitial osmolality, high levels of Ang II and AVP, as well as growth factors and cytokines through MAP kinase pathways (262,263). Leukotrienes are synthesized by another major pathway involving the enzyme lipoxygenase. Vasoactive metabolites are also formed via the cytochrome P450 monooxygenase pathway. Medullary tubular and interstitial cells have a larger synthetic capacity than the vasculature in the cortex. There are multiple sites of arachidonic acid metabolism in the renal vasculature. These sites include arterial and arteriolar smooth muscle, endothelial cells, and glomerular mesangial and epithelial cells. Endothelial cells produce PGE_2 and PGI_2, while thromboxane A_2 seems to derive from vascular smooth muscle cells and mesangial cells (89–91,95,264–266).

Prostaglandins

General stimuli for renal prostaglandin synthesis are renal vasoconstriction and states of volume depletion and hypoperfusion. Anesthesia, surgery, and associated stress may exacerbate prostaglandin production. The diuretics ethacrynic acid and furosemide also stimulate renal release of prostaglandins. Many vasoactive receptor agonists stimulate phospholipases that promote release of arachidonic acid from membrane phospholipids. Stimulation of PGE_2 and PGI_2 production by Ang II is well characterized. In turn, PGE_2 and PGI_2 usually buffer the vasoconstriction elicited by Ang II and stimulate renin release from juxtaglomerular cells. Other stimulants include ET_A receptor agonists. Vasodilators, such as acetylcholine and bradykinin, stimulate production of PGE_2 or PGI_2, as well as NO. In addition, acetylcholine may stimulate production of thromboxane A_2. The vasoactive peptides Ang II, endothelin, vasopressin, acetylcholine, and bradykinin increase availability of the substrate, arachidonic acid, secondary to membrane receptor-mediated calcium influx and activation of phospholipase A_2. Phospholipase A_2 may be activated by increased activity of the calcium–calmodulin complex, increased production of DAG, or phospholipase C-mediated phosphorylation of lipocortin, a membrane-bound enzyme that normally inhibits phospholipase A_2. Thus, there is a common pathway by which many vasoconstrictor agents can increase production of COX-derived prostaglandins, primarily vasodilatory PGE_2 and PGI_2, which, in turn, can counteract vasoconstriction. α-Adrenergic neurotransmission can be prejunctionally and postjunctionally inhibited by prostaglandins (95,100,262,264,265).

Four forms of PGE_2 receptors (termed EP_1 through EP_4) have been identified and cloned. The EP_4 receptor, coupled via G proteins, to generate cAMP and protein kinase A, predominates along the preglomerular vasculature and mediates the principle vasodilator actions of PGE_2. Similar actions are exerted by the single IP receptor for PGI_2 (prostacyclin). Vasodilatory prostaglandins, produced by endothelium of glomerular arterioles, and mesangial cells exert net effects to stimulate adenylate cyclase and the formation of cAMP and activation of protein kinase A. Low concentrations of the prostaglandins PGE_2 and PGI_2 normally formed by the arterial vasculature appear to exert their physiological effects by attenuating the actions of vasoconstrictor, with larger amounts acting as vasodilators that increase RBF.

The buffering action of PGE_2 and PGI_2 in the preglomerular vasculature is primarily mediated by the ability of cAMP and protein kinase A to inhibit IP_3-induced release of Ca^{2+} from internal stores. A low density of vasocontrictor receptor (EP_1 or EP_3) may counteract some of the net dilation. The EP_1 receptor increases calcium mobilization. The EP_3 receptor inhibits production of cAMP via a pertussis toxin-sensitive G_i protein. EP_2 receptors, which act through a G_s protein to increase cAMP in tubules, appear to be absent from the renal vasculature under normal conditions. Arachidonic acid dilates the isolated interlobular artery and afferent arteriole; a smaller response occurs in efferent arterioles. Intrarenal infusions of arachidonic acid, PGE_2, PGI_2, or PGD_2 increase RBF and reduce renal resistance without affecting GFR. $PGF_{2\alpha}$ has little or no effect on the renal circulation. Local administration of small amounts of PGE_2 or its stable analog causes renal vasodilation and attenuates the constrictor effects of Ang II, thromboxane A_2, and norepinephrine. PGE_2 and PGI_2 dilate both afferent and efferent arterioles such that glomerular capillary pressure is constant when the stimulatory effects of the prostaglandins on angiotensin formation are blocked. However, application of PGE_2 from the interstitial side causes vasoconstriction of juxtamedullary nephrons, a response due to subsequent metabolism to a vasoconstrictor agent or activation of EP_1 or EP_3 receptors (9,52,85,261,264,265, 267–274).

Endogenous prostaglandins regulate RBF and GFR by direct effects on vascular smooth muscle and indirectly by modification of the action of other hormones or neural stimuli. The vasodilator prostaglandins serve an important protective function and homeostatically balance the hemodynamic effects of vasoconstrictor substances. Studies of inhibition of COX activity indicate that the major function of COX-derived prostaglandins is to attenuate the influence of vasoconstrictor substances during activation of the renin–angiotensin system, the sympathetic nervous system, or both. These counteracting effects provide a balance between the vascular effects of Ang II and those of prostaglandins during variations in plasma volume and sodium intake. As is shown in Figure 2-23, blockade of the compensatory dilator action of prostaglandins promotes vasoconstriction when the renin–angiotensin and sympathetic nervous systems are stimulated, such as during sodium depletion. Prostaglandins also antagonize the tendency for high concentrations of vasopressin to produce renal vasoconstriction. In contrast, in a healthy, unstressed individual with a normal plasma volume, inhibition of prostaglandin synthesis has little or no effect on RBF and GFR (Fig. 2-23). Basic autoregulatory efficiency of whole-kidney RBF and GFR remains high even during blockade of renal prostaglandin synthesis via the COX pathway. Inhibition of COX-2 produces renal vasoconstriction that may be greater in the medulla than in the cortex. During afferent arteriolar constriction induced by tubuloglomerular feedback, COX-2 generates vasodilatory metabolites in response to increased nNOS activity that attenuate the strength of feedback-mediated vasoconstriction (168,169,263, 273–275).

Renin release is increased by arachidonic acid, endoperoxides, PGE_2, and PGI_2. PGE_2 and PGI_2 act on their respective EP_4 and IP receptors on juxtaglomerular granular cells to stimulate renin release by stimulating adenylate cyclase activity and producing cAMP. COX inhibitors reduce renin release under basal, as well as during stimulated, conditions. α-adrenergic neurotransmission can be inhibited prejunctionally and postjunctionally by prostaglandins (25,187,190).

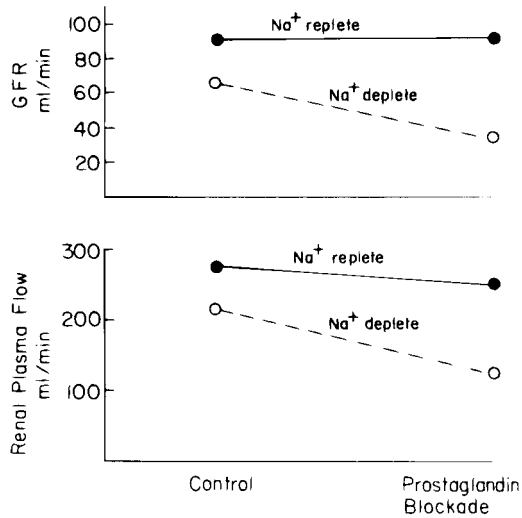

FIGURE 2-23. RBF and GFR responses to prostaglandin blockade produced by inhibition of cyclooxygenase (indomethacin) in conscious dogs maintained on a normal salt diet (*solid lines*) or on a low-sodium diet (*dashed lines*). (Data from De Forrest JM, Davis JO, Freeman RH, et al. Effects of indomethacin and meclofenamate on renin release and renal hemodynamic function during chronic sodium depletion in conscious dogs. *Circ Res* 1980;47:99.)

The COX-2 metabolite PGE_2 plays a key role in linking NaCl control of macula densa signaling to juxtaglomerular cells to regulate renin release and to afferent arterioles to regulate tubuloglomerular feedback. COX-2 activity in the MD increases in response to a salt-deficient diet and to a loop diuretic, such as furosemide, with increases in PGE_2 production and renin release. nNOS in the same cells may serve as an upstream intermediate responding to salt transport. High levels of nNOS and COX-2 parallel each other and inhibition of nNOS causes a fall in COX-2 expression and an uncoupling to transport. On the other hand, high renin states limit COX-2 expression in the cortical thick ascending limb and MD cells via Ang II and AT_1 receptors; ACE inhibition has the opposite effect. Recruitment of renin-containing cells retrograde along the afferent arteriole during chronic salt restriction or ACE inhibition appears to be COX-2 dependent as renin is restricted to juxtaglomerular cells during these stimuli in COX-2 deficient mice. Plasma renin activity is reduced in COX-2 null mice and also in nNOS-deficient mice. Inhibition of COX-2 produces renal vasoconstriction that may be greater in the medulla than the cortex (169,183,190,262, 275,276).

In disease states, endogenous vasodilator prostaglandins serve a protective role in maintaining renal function by means of their effects on vascular resistance, GFR, and renin release. Administration of nonsteroidal, anti-inflammatory drugs to patients with clinical disorders, such as, advanced hepatic cirrhosis, severe congestive heart failure, and sodium depletion often produces deleterious effects, with reductions in renal perfusion and GFR. In other pathophysiological conditions, there may be enhanced production of thromboxane A_2, which may contribute to deterioration of renal function. In certain settings, some of the vasoconstriction produced by Ang II may be mediated by thromboxane A_2. Administration of a stable thromboxane-receptor agonist reduces RBF and GFR by causing afferent and efferent arteriolar constriction. K_f appears to be unaffected, although it is noteworthy that thromboxane A_2 causes contraction of isolated glomeruli and cultured mesangial cells. As mentioned in the section on tubuloglomerular feedback, although very little thromboxane A_2 is produced under normal conditions, thromboxane plays a modulatory role in the preglomerular vasoconstriction associated with activation of the tubuloglomerular feedback mechanism and the vasoconstrictor response to chronic Ang II infusion. The thromboxane A_2–PGH_2 receptor is coupled by the G_q protein to activation of phospholipase C, increased levels of cytosolic calcium, and possible inhibition of adenylate cyclase. The renal vasoconstriction produced by thromboxane A_2 is primarily mediated by Ca^{2+} influx. Chronic activation of PKC appears to reduce the number of thromboxane A_2 receptors (95, 277–283).

Leukotrienes

Lipoxygenase enzymes convert arachidonic acid to leukotrienes (LTs), HETEs, and lipoxins (see Fig. 2-22). The major lipoxygenase products are monohydroxyeicosatetraenoic acids—12-HETE and 15-HETE—generated by glomeruli, mesangial cells, renal cortical tubules, and vascular tissues. Leukotrienes (LTB_4, LTC_4, LTD_4, and LTE_4) are hydroperoxy fatty acid products of the intermediate 5-hydroperoxyeicosatetraenoic acid (HPETE). Stereoisomers of 12-HETE are produced by different enzymatic pathways. The 12(S)-HETE isomer is predominantly produced in the cortex; however, the 12(*R*)-HETE is a primary biologically active metabolite of the cytochrome P450 pathway. Vascular generation of 12-HETE is increased in pathological conditions (90,266,281,284).

Receptors for LTC_4 and LTD_4 have been identified on preglomerular arteries, glomeruli and cultured mesangial cells, and the efferent arteriole. Infusion of LTC_4 or LTD_4 causes renal vasoconstriction, reduces both GFR and filtration fraction, and activates the renin–angiotensin system. Leukotriene C_4 receptors are linked to ion channels, but specific mechanisms are not known. Some of the leukotriene actions may be mediated by endothelial cells. Leukotriene D_4 is known to release NO in addition to activating a pertussis toxin-sensitive G protein. Lipoxin A_4 increases renal plasma flow and GFR, with a small reduction in K_f. Interestingly, the effects are COX-dependent and can be completely reversed by inhibition of this enzyme. On the other hand, the ability of lipoxin B_4 to decrease RBF and GFR are independent of COX activity (90,134,266,281,284).

Cytochrome P450 Metabolites

Vasoactive metabolites of the cytochrome P450 pathway are produced by vascular smooth muscle cells, endothelial cells, renal tubular cells, and glomeruli. Arachidonic acid is oxygenated via NADPH-dependent microsomal monooxygenases. As is shown in Figure 2-22, epoxygenase enzymes are responsible for production of epoxides or epoxyeicosatrienoic acids (EETs) (e.g., 11,12-EET and 14,15-EET). A second cytochrome P450 pathway involves ω-hydroxylase formation of 19- and 20-HETEs (hydroxyeicosatetraenoic acids). The major metabolites of this pathway seem to be EETs in the cortex and HETE in the medulla. Salt diet, Ang II and other hormones, and various pathophysiological settings alter renal cytochrome P450 metabolism (90,91,261,269,281,285,286).

Cytochrome P450 ω-hydroxylase metabolites, in particular, 20-HETE, may participate in regulation of cortical and whole-kidney blood flow. Studies in dogs and rabbits have failed to show an effect of cytochrome P450 inhibition of autoregulation and of pressure natriuresis. In isolated rat vessels, transmural pressure stimulates ω-hydroxylase activity to produce 20-HETE, which is thought to participate in myogenic vasoconstriction. Cytochrome P450-4A-derived eicosanoids may also participate in the renal hemodynamic effects of Ang II and endothelin. 20-HETE is thought to cause vasoconstriction by inhibition of tonically active potassium channels, thereby

causing depolarization and activation of voltage-gated Ca^{2+} channels. Also suggested is a dependency on COX activity and production of thromboxane (90,91,261,269,285,286).

Epoxygenase metabolites elicit variable vascular responses. 11,12-EET is an endothelial-derived factor, distinct from NO, which can produce vasodilation mediated by membrane hyperpolarization, following activation of cAMP/protein kinase A and of K channels. 5,6-EET or 8,9-EET induces vasoconstriction with a decrease in GFR; however, COX inhibition changes the renal response to vasodilation and an increase in GFR. The renal vasodilation induced by administration of 5,6-EET is mediated by the cyclooxygenase products PGE_2 and PGI_2. Inhibition of epoxygenase activity does not affect blood flow autoregulation (89–91,146,158,261,285–287).

Isoprostanes, such as 8-epi-$PGF_{2\alpha}$, are vasoconstrictor metabolites related to prostaglandins. They are stable products of lipid peroxidation of arachidonic acid, formed in and released from cell membranes, and are excreted in urine. Isoprostane production is stimulated by peroxynitrate, a reactive oxygen species resulting from NO scavenging by superoxide anion (89,288).

Products of the cytochrome P450 pathway potentiate control of preglomerular vasomotor tone by the juxtaglomerular apparatus. Inhibition of P450 metabolites blunts tubuloglomerular feedback activity, while luminal perfusion of 20-HETE restores tubuloglomerular feedback responses (5,91,158,280,282,289–291).

Kallikrein–Kinin System

Plasma and glandular kallikreins are functionally different serine protease enzymes that act on kininogens (inactive α_2-glycoproteins) to form the biologically active nonapeptide bradykinin and the decapeptide lysyl-bradykinin (kallidin). It is unlikely that circulating kinins affect the renal resistance vessels, because they are rapidly inactivated enzymatically by endothelial-bound kininase (ACE). Within the kidney, glandular kallikrein (active and inactive forms) and its substrate, kininogen, are located predominantly in the cortex, in association with the granular portion of the distal convoluted and cortical collecting tubules (i.e., connecting tubule cells), which appear to make contact with the afferent arteriole of the parent glomerulus. Synthesis and release of kallikrein into the tubular fluid and interstitium can be stimulated by prostaglandins, mineralocorticoids, Ang II, increased renal perfusion pressure, and several diuretic drugs. The glomerulus and cells of the distal nephron segments contain two major types of specific membrane receptors for kinins. Bradykinin B_2 receptors on vascular endothelial cells are involved in vascular responses because they mediate the stimulation of NO and prostaglandin synthesis by kinins (292–295).

Infusion of bradykinin or kallidin elicits renal vasodilation that is characterized by a larger increase in RBF than GFR and a natriuresis and diuresis. Exogenous kinins also produce independent stimulation of prostaglandin formation (PGE_2 and PGI_2) and renin release. Kinin-induced vasodilation, however, is similar in the presence and absence of inhibition of prostaglandin synthesis. Bradykinin infusions also reduce the vasoconstrictor responses to norepinephrine or Ang II, an effect that may be, in part, prostaglandin-dependent. Studies on isolated renal arteries demonstrate that bradykinin has potent endothelium-dependent relaxant effects that are mediated by NO; the effects even persist when COX and prostaglandin synthesis are blocked. The decline in glomerular K_f contrasts with the well-known effect of kinins to increase capillary permeability in other tissues. While the vasodilator actions are known to be a result of NO release, it is possible that K_f is reduced by a direct effect independent of NO. Additional effects include enhanced conversion of inactive to active renin and presynaptic inhibition of adrenergic neurotransmitter release. Most of the vascular *in vivo* effects appear to be largely due to NO release from endothelial cells. Vasodilatory prostaglandins may also be released, as well as an ill-defined hyperpolarizing factor(s), related to EETs, released by the cytochrome P450 system. Isolated vessels and cultured mesangial cells devoid of endothelium exhibit B_2-receptor-dependent constrictor responses to bradykinin. Signal transduction appears to involve a pertussis toxin-insensitive G protein, a PKC pathway, increased cytosolic calcium, and arachidonic acid metabolites (5,9,102,215,216,296–298).

It has been proposed that the kallikrein–kinin system is an important intrarenal modulator of prostaglandin synthesis and renin–angiotensin activity. Kinins stimulate renin release by acting on juxtaglomerular cells. It is noteworthy that a major route of kinin inactivation is mediated by the cortical enzyme kininase II, a peptidyl dipeptidase, which is also responsible for conversion of Ang I to II (ACE). Drugs that inhibit this common enzyme may exert hemodynamic effects not only by blocking the formation of Ang II but also by augmenting kinin concentration. Kinins are also inactivated by a neutral endopeptidase in the vascular endothelium and proximal tubular brush border (293–295).

Early studies evaluated endogenous kinin activity, using infusion of bradykinin–binding antibodies, suppression of renal kallikrein activity with the serine protease inhibitor aprotinin, and pharmacologic inhibition of kininase II. The results suggested that locally formed kinins may attenuate renal vasoconstriction in other states associated with vasoconstriction. Further understanding has been gained recently by experiments employing more specific receptor antagonists. Use of a specific B_2 receptor antagonist reveals that basal kinin levels do not appear to contribute appreciably to the regulation of renal function during normal conditions. However, when their levels are elevated as during sodium restriction and volume depletion, kinins act as vasodilators in a manner similar to the prostanoids, which buffer the renal vasoconstriction associated with elevated local levels of Ang II, norepinephrine, and vasopressin. The B_2 receptor density in glomeruli is reduced by a low-sodium diet and water deprivation. Studies also show that renal kinins are degraded by a neutral endopeptidase in addition to kininase II. Combined blockade of both degrading enzymes leads to increases in RBF and GFR in association with increased urinary excretion of kinins. Kinins may participate in the autoregulation of GFR and the tubuloglomerular feedback mechanism, although RBF autoregulation is not affected by kinin receptor blockade. Kinins may exert larger vasodilatory effects in the medulla than in the cortex (216, 297–304).

Purinergic Actions on the Renal Microcirculation

Adenosine and ATP

Adenosine has received considerable attention as a possible regulator of renal hemodynamics and of renin release. It is proposed that GFR and filtered sodium load are coupled to tubular transport capacity and oxygen consumption via hydrolysis of ATP and resultant adenosine production. Adenosine and other adenine nucleotides may eventually end up in interstitial fluid in the extracellular compartment. ATP is released from cells through membrane channels and coreleased from nerve terminals with transmitter. In this fashion, purine-based substances can act as paracrine agents to influence the renal microcirculation (5,82,157,305–308).

Purinergic Receptors

Renal purinoceptors display different sensitivities for ATP, ADP, AMP, and adenosine. P_1 purinoceptors respond primarily to adenosine and sometimes to AMP, but are relatively insensitive to ADP and ATP. Extracellular ATP predominantly activates P_2 purinoceptors. There are at least two subtypes of adenosine-responsive (A) P_1 receptors in the renal vasculature. P_1–A_1 receptors are coupled to a pertussis toxin-sensitive G_i protein that inhibits adenylate cyclase and cAMP production. Stimulation of A_1 receptors decreases GFR and RBF. Local application of an A_1 receptor agonist causes constriction of both the preglomerular and postglomerular microvessels. A_2 receptors mediate vasodilation, stimulating adenylate cyclase through a G_s protein. Afferent and efferent arterioles dilate in response to an A_2 receptor agonist (5,305–307,309).

P_2 receptors, present on endothelial and vascular smooth muscle cells, have a greater affinity for ATP and ADP than for adenosine or AMP. Interstitial fluid ATP concentrations are sufficiently high to play a role in regulating the vascular resistance changes responsible for autoregulation and tubuloglomerular feedback. The receptor subtype P_{2X} increases cytosolic calcium concentration by increasing calcium influx through voltage-gated and receptor-operated calcium channels. Low-frequency stimulation of renal sympathetic nerves causes renal vasoconstriction that is mediated, in part, by a nonadrenergic component. P_{2X} receptors are thought to mediate the nonadrenergic component, presumably responding to ATP coreleased with neurotransmitter. P_{2Y} receptor activation increases cytosolic calcium concentration via the phospholipase C–IP_3 cascade. Endothelial cells have a high concentration of P_{2Y} receptors and their activation by ATP leads to vasorelaxation mediated by NO or PGI_2 or both. However, ATP causes rapid, marked constriction of the afferent arteriole when NO synthesis is inhibited. The P_{2U} purinoceptor is termed a nucleotide or 5′-nucleotide receptor because it responds to all nucleotides with a similar potency of ATP and uridine triphosphate. P_{2U} receptor activation increases cytosolic calcium by a G protein stimulating the phospholipase C pathway (5,157,305,309).

Adenosine

Intrarenal administration of adenosine produces a biphasic renal response. Resistance vessels respond initially with transient vasoconstriction, mediated by P_1–A_1 receptors, followed by gradual vasodilation, mediated in part by P_1–A_2 receptors. Decreases in GFR and filtration fraction reflect increased preglomerular vascular resistance and reduced efferent arteriolar resistance. In isolated preparations, adenosine constricts both afferent and efferent arterioles. The extent to which endogenous adenosine serves as an important regulator of renal hemodynamics is uncertain. Blockade of A_1 receptors decreases afferent arteriolar resistance and K_f in anesthetized animals. Adenosine does not appear to be essential for autoregulation. Interstitial fluid concentrations of adenosine are unchanged over the autoregulatory range of arterial pressure and administration of adenosine-receptor antagonists does not impair autoregulatory capability. Nevertheless, adenosine-receptor blockade reduces tubuloglomerular feedback control of preglomerular resistance in some, but not all, situations. Mice that do not have functional A_1 receptors exhibit marked impairment of tubuloglomerular feedback responses, suggesting a critical mediator role of adenosine. However, unopposed A_2 receptor activation may result in substantial vasodilation (5,82,157,160,161,163,212,306,308,310–314).

Although adenosine and Ang II can act independently of each other, adenosine may act synergistically with angiotensin in certain situations. The vasoconstriction because of adenosine during high renin states is dependent on endogenous Ang II. Adenosine causes larger long-term decreases in GFR and filtration fraction in sodium-depleted animals with increased renin–angiotensin activity than in animals consuming a normal salt diet. On the other hand, Ang II-induced vasoconstriction occurs independently of an interaction with adenosine receptors (163,315).

It has been proposed that salt transport by the ascending limb of Henle's loop or MD cells requires metabolic energy and utilization of ATP that is linked to control of preglomerular vascular resistance through the tubuloglomerular feedback system. As transport increases, more adenosine is liberated within cells and available to diffuse to the afferent arteriole, where it elicits vasoconstriction to reduce blood flow and glomerular capillary pressure. Support is based on findings that luminal perfusion of A_1 adenosine-receptor agonists causes larger feedback-induced vasoconstrictor changes in glomerular capillary pressure. Attenuated feedback-mediated responses of the afferent arteriole are observed when an adenosine antagonist is added to either luminal fluid or peritubular capillary blood. The effects are insensitive to the salt transport inhibitor furosemide, indicating P_1–A_1 adenosine receptor-mediated effects by acting on either the MD or vascular smooth muscle cells (312,313,315–317).

Adenosine participates in the regulation of renin secretion. Adenosine infusion inhibits renin release *in vivo*. The mechanism involves adenosine receptors on juxtaglomerular cells, with A_1 and A_2 receptors having opposite effects. Renin release is inhibited by A_1 purinergic receptors coupled to a G_i protein and stimulated by an A_2 receptor agonist. As a second communication link, adenosine may affect renin release by acting on MD cells. Evidence for a tonic inhibitory effect has been observed in isolated afferent arterioles with and without MD cells attached (186,188,317).

ATP

P_2 receptors appear to be involved in mediating autoregulatory adjustments in renal vascular resistance and tubuloglomerular feedback responses. Extracellular ATP, at high levels that saturate P_2 purinoceptors, causes renal vasoconstriction and impairs the autoregulatory ability of the vasculature to constrict further in response to increases in arterial pressure. The effect on autoregulation appears to be specific to ATP, as similar studies show that RBF is not impaired when the vasoconstrictor is norepinephrine.

Renal arterial infusion of ATP increases RBF. The renal vasodilation is mediated by endothelial release of NO and is converted to vasoconstriction during NO synthesis inhibition, suggesting the involvement of more than one receptor subtype. P_2 receptors are present on vascular endothelium, with P_{2Y} receptors being responsible for NO-dependent vasodilation. P_{2X} receptors on vascular smooth muscle cells produce vasoconstriction. In considering a paracrine role for ATP, it seems likely that endogenous ATP reaches vascular smooth muscle cells from the interstitium. The vasoconstriction is mediated by Ca^{2+} mobilization and entry through L-type Ca^{2+} channels. ATP also stimulates renin secretion; the effect is mediated by P_{2Y} receptor stimulation and production of NO (5,153,157,226,309,318–321).

ATP acts on P_2 purinoceptors to selectively constrict afferent arterioles in a direct and sustained manner. This is consistent with autoradiographic and immunohistochemical evidence of abundant P_{2x} receptors limited to the preglomerular vasculature, excluding efferent arterioles. Autoregulatory-mediated afferent arteriolar constriction is prevented by P_2 receptor saturation or desensitization by high arterial concentrations of ATP. Tubuloglomerular responses also are markedly blunted during peritubular capillary perfusion with saturating doses of ATP or slowly metabolizable analogs. P_2 receptor blockade prevents autoregulatory responses of afferent arterioles. Mice lacking

functional P_{2x} receptors have blunted autoregulation and loss of tubuloglomerular feedback. MD cells secrete ATP through a basolateral maxichloride channel that is permeable to ATP in response to increases in luminal NaCl concentratioin. Although MD cells have abundant mitochondria, they have low levels of Na^+-K^+-ATPase, making them a good candidate for a source of extracellular ATP (24,159,226,318–320).

Renal interstitial fluid concentrations of ATP measured by microdialysis are closely associated with autoregulatory and tubuloglomerular feedback-mediated changes in renal vascular resistance. A direct relationship is observed when tubuloglomerular feedback is stimulated by increased distal fluid delivery because of carbonic anhydrase inhibition of proximal tubular reabsorption and when tubuloglomerular feedback is inhibited by the transport inhibitor furosemide (5,322).

Sympathetic Nervous System and Catecholamines

The renal vasculature is richly innervated with postganglionic adrenergic fibers originating from sympathetic celiac and aorticorenal plexi that receive inputs from the sixth thoracic through the second lumbar spinal nerves. All arterial segments of the vasculature and the large veins are extensively innervated with neuroeffector junctions containing norepinephrine. A heavy concentration appears in subadventitial layers of arcuate arteries, with notable innervation of smooth muscle cells of both the afferent and efferent arterioles and also of juxtaglomerular cells. The efferent innervation is predominantly adrenergic, although some nerve endings are reported to contain dopamine and neuropeptide Y. ATP may be coreleased with norepinephrine from some nerve terminals. Three types of α-adrenoceptors are found on renal resistance vessels, with relative amounts of α_{1D} (65%), α_{1A} (25%), and α_{1B} (10%). α_{1A} and α_{1D} increase cytosolic Ca^{2+} concentration and elicit vasoconstriction in preglomerular arterioles. The functional role of α_{1B} receptors is less clear (323–329).

Renal nerves are divided into two types, with type I terminating on afferent arterioles, while type II innervate both afferent and efferent arterioles. Type II nerves are immunoreactive for neuropeptide Y, whereas type I is not. Electrical stimulation of the greater splanchnic or renal efferent nerves produces frequency-dependent renal vasoconstriction that is abolished by α_1-adrenergic receptor antagonists. The vasoconstrictor responses are elicited in the medullary as well as the cortical circulation. The increased vascular resistance in the cortex is due to constriction of preglomerular and efferent arteriolar segments. As is shown in Figure 2-24, low levels of stimulation cause equivalent constriction of both afferent and efferent arterioles such that glomerular pressure is unchanged. Similar results are obtained in isolated afferent and efferent arterioles when α_1-adrenoceptors are stimulated. Higher frequencies of nerve stimulation produce predominant constriction of preglomerular vessels and thus reduce glomerular capillary pressure and GFR. Intense stimulation at 10 Hz produces complete glomerular ischemia (184,327,330–332).

In addition to a direct effect on vascular smooth muscle, the renal efferent nerves exert indirect hemodynamic effects as a consequence of stimulation of the constrictors angiotensin and endothelin and the formation of dilator prostaglandins and nitric oxide. Renal nerve stimulation elicits an increase in renin release from juxtaglomerular cells primarily by activation of β_1-adrenoceptors. Prostaglandin synthesis is enhanced by activation of phospholipase A_2 and augmented availability of arachidonic acid. The vasoconstriction caused by moderate renal nerve stimulation is reduced in the presence of Ang II blockade, suggesting that part of the effect is mediated by

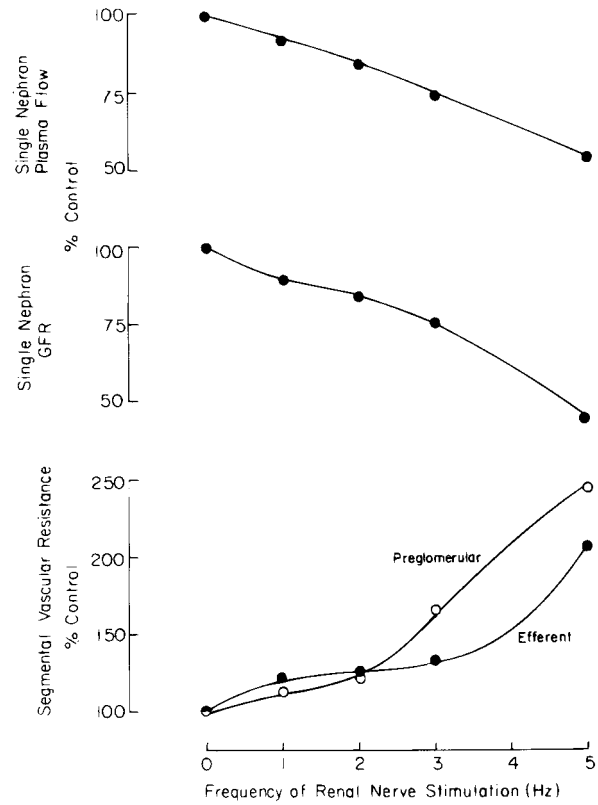

FIGURE 2-24. Effect of electrical stimulation of renal efferent nerves at 1 to 5 Hz on single nephron GFR, plasma flow, and resistance of preglomerular vessels and efferent arterioles. (Data from: Kon V. Neural control of renal circulation. *Miner Electrolyte Metab* 1989;15:33; Hermansson K, Larson M, Kallskog O, et al. Influence of renal nerve activity on arteriolar resistance, ultrafiltration dynamics and fluid reabsorption. *Pflugers Arch* 1981;389:85.)

increased intrarenal Ang II formation because of neural stimulation of renin. On the other hand, vasodilatory prostaglandins buffer a significant fraction of the vasoconstriction elicited by nerve stimulation as evidenced by much larger changes in GFR, RBF, and vascular resistance during inhibition of prostaglandin synthesis. The effect of renal nerve stimulation on K_f is controversial. Some investigators report a reduction; others find that K_f is normally unaffected by nerve stimulation but that a reduction of K_f is evident during inhibition of prostaglandin synthesis (331,332).

Infusions of norepinephrine, epinephrine, or α_1-adrenergic agonists produce similar dose-related effects on the renal microcirculation. Studies on individual vessels indicate that norepinephrine constricts the interlobular artery and the afferent and efferent arterioles. Total renal vascular resistance responses to norepinephrine are substantially attenuated by Ca^{2+} channel blockers and by inhibition of IP_3-medicated release of stored Ca^{2+}. Calcium responses of the afferent arteriole to α-adrenoceptor stimulation are mediated by mobilization from internal stores in combination with Ca^{2+} entry through voltage-gated L-type channels and channels insensitive to dihydropyridine Ca^{2+} channel blockers. The latter includes receptor-operated Ca^{2+} channels. Efferent arteriolar responses to norepinephrine appear to be independent of L-type channels (7,66,72,184,328,332,333).

The renal nerves are not required for efficient autoregulation of RBF and GFR or for the operation of the tubuloglomerular-feedback mechanism. Under unstressed conditions, while basal efferent sympathetic tone is low, neither acute nor chronic

renal denervation affects RBF or GFR. In general, physiological reflex activation of renal sympathetic nerves has less influence on renal hemodynamics than does experimental electrical stimulation at supramaximal voltage. Moderate increases of about 50% in renal nerve activity that are induced reflexively by high- or low-pressure receptors fail to alter renal hemodynamics, although this level of stimulation is capable of affecting tubular sodium reabsorption and renal release of renin and prostaglandins. Neurally induced vasoconstriction is more readily demonstrable during more stressful states, such as during dehydration, blood volume contraction, hemorrhage, and congestive heart failure. Pronounced activation of renal efferent nerve activity (+500%) produced by auditory or emotional stimuli causes intense renal vasoconstriction (5,7,18,184,325,326).

The renal nerves also mediate renal vasoconstriction during arterial hypoxemia produced by acutely lowering inspired oxygen tension to 60 mm Hg. The increased renal vascular resistance is prevented by renal denervation or denervation of systemic baroreceptors and chemoreceptors. Hypercapnic acidosis associated with an increase in PCO_2 from 25 to 70 mm Hg also activates the renal efferent nerves and denervation studies indicate that the neurally mediated renal vasoconstriction is effectively counteracted, at least in part, by enhanced synthesis of vasodilatory prostaglandins (334).

The hemodynamic effects of catecholamines can be modulated by a complex interaction with the renin–angiotensin and prostaglandin systems. Nerve stimulation, circulating catecholamines, and adrenergic-receptor agonists stimulate renal release of renin and prostaglandins. In turn, Ang II and prostaglandins are capable of acting presynaptically to stimulate and inhibit, respectively, norepinephrine release, in addition to exerting direct effects on vascular smooth muscle (184,332).

Afferent renal nerves also may serve important functions in neurohumoral control of arterial pressure, vasopressin release, and renal excretion of sodium and water. Afferent nerve endings contain neuropeptides, such as calcitonin gene-related peptide, substance P, and vasoactive intestinal peptide. Both mechanoreceptors and chemoreceptors have been identified within the kidney and afferent nerve traffic can elicit renorenal reflexes. Renal mechanoreceptors respond to changes in arterial and renal venous pressure. Chemoreceptors sense the composition of pelvic fluid and also are responsive to renal ischemia. Two examples of excitatory renorenal reflexes that affect renal hemodynamics are neurally mediated ipsilateral vasoconstriction during increased renal venous pressure and contralateral renal vasoconstriction following a unilateral increase in ureteral pressure (184,324,326,327,335–337).

Although cholinesterase-containing fibers and β-adrenergic sites have been identified histologically in the renal cortex, there is little functional evidence for neurogenic renal vasodilation mediated by acetylcholine or β-adrenoceptors. Neural release of acetylcholine, however, may exert a small indirect effect at presynaptic sites to inhibit norepinephrine release. Infusions of exogenous acetylcholine increase RBF and reduce renal vascular resistance, while GFR is unaffected. The decline in total resistance is due to parallel reductions in afferent and efferent arteriolar resistance. Acetylcholine relaxes isolated preparations of the interlobular artery and the afferent and efferent arterioles. As pointed out earlier, however, the full vasodilatory effect of acetylcholine requires an intact endothelium and is primarily mediated through release of NO and may also involve release of prostanoids (7,9,102,139).

Dopamine, a sympathomimetic amine precursor of norepinephrine, is another neurotransmitter capable of regulating renal hemodynamics, renin secretion, and sodium excretion. Histofluorescent evidence suggests dopaminergic innervation of the cortical vessels, primarily the glomerular vascular poles. However, dopamine synthesized from L-dopa by proximal tubular cells appears to be the major contributor to urinary excretion of dopamine and its metabolites. Two types of specific dopamine-binding sites, DA_1 and DA_2, are present in the kidney. The DA_1 receptor is a postsynaptic receptor located on vascular smooth muscle cells and tubular cells, but apparently absent from glomeruli. DA_2 receptors can be either presynaptic or postsynaptic and are located in glomeruli as well as vessels and tubules in both the cortex and medulla (184,327,338–340).

Although specific receptor blockers abolish renal effects produced by exogenous dopamine, such antagonists do not consistently affect basal renal hemodynamics. Further, prejunctional stimulation of dopamine receptors during moderate levels of renal efferent nerve activity has little influence on renal vascular resistance. Small amounts of dopamine or a DA_1 receptor agonist dilate the renal vasculature. Responses include an increase in RBF and a decrease in filtration fraction; GFR and glomerular capillary pressure are unaffected. Most of the dopamine-induced renal vasodilation is mediated by DA_1 receptors, which are coupled to adenylate cyclase and lead to increased production of cellular cAMP. Dopamine and DA_1 receptor agonists relax afferent and efferent arterioles and interlobular and arcuate arteries. The DA_1-induced dilation of the preglomerular vasculature does not impair autoregulatory adjustments in vascular resistance to changes in perfusion pressure. DA_1-receptor stimulation attenuates tubuloglomerular feedback responsiveness. The vasodilation elicited by dopamine is more pronounced after denervation or -pharmacologic blockade of α-adrenoceptors, suggesting that presynaptic DA_2-dopamine receptors augment norepinephrine release from nerve terminals. DA_2-receptor stimulation is associated with increases in GFR and attenuation of Ang II-induced contraction of glomerular mesangial cells (27,184,327,338,340–342).

Neuropeptide Y (NPY) is another postulated neurotransmitter. Nerve endings containing immunoreactive neuropeptide are localized along the interlobular and arcuate arteries extending to glomerular arterioles, innervating both afferent and efferent arterioles. A cotransmitter of the renal sympathetic nerves, NPY is coreleased with and may potentiate the vascular pressor effects of norepinephrine and ATP. The kidney expresses NPY receptors, which can also be activated by peptide YY (PYY), a circulating hormone released from gastrointestinal cells. Via the Y1 receptor, NPY and PYY produce potent renal vasoconstriction. Administered NPY constricts both the afferent and efferent arterioles. Nerves innervating the efferent arteriole appear to contain more immunoreactive NPY than those to the afferent arteriole. NPY acts prejunctionally to inhibit noradrenaline release via Y2 receptors. Despite marked reductions in RBF, systemic NPY infusion elicits a diuresis and natriuresis that is mediated in part by bradykinin. NPY antagonists enhance basal RBF, but do not alter basal urinary excretion. The vasoconstriction appears to be mediated by a calcium entry step, much like angiotensin (307,330,343–345).

Atrial Natriuretic Peptide

Atrial natriuretic peptide (ANP) is a 28-amino acid peptide family involved in the physiological regulation of renal function and sodium excretion. A high molecular-weight precursor of ANP is constitutively synthesized in cardiocytes of the atria. ANP is released into the circulation as a function of atrial volume or distention in association with changes in sodium and water balance. In addition to ANP, related peptides include brain and renal natriuretic peptides (urodilatin). Recent studies implicate renal conversion of ANP to urodilatin, which is closely related to sodium excretion under a variety of conditions (345,346).

ANP receptors are concentrated in glomerular capillaries and the collecting duct; they are also present along cortical arterioles and medullary arterioles and vasa recta; cell types include the collecting duct, vascular smooth muscle, endothelial, and mesangial cells. There are three subtypes of natriuretic peptide receptors termed NPR-A, NPR-B, and NPR-C. The A- and B-type receptors include a cytoplasmic catalytic domain and the active particulate guanylate cyclase, exerting physiological effects by increasing cell cGMP. The functional ANP receptor is associated with particulate guanylate cyclase, which elicits physiological effects by increasing cell cGMP. ANP reduces calcium influx and calcium mobilization in cultured mesangial cells. ANP may buffer the action of vasoconstrictors, such as, angiotensin and norepinephrine, via an interaction of cGMP with intracellular Ca^{2+}, perhaps secondary to stimulation of calcium efflux. Renal vascular endothelial cells also have biologically silent, NPR-C "clearance" receptors, which remove ANP from the circulation with no apparent role in signal transduction or guanylate cyclase activity. It is reported that NPR-C may cause venodilation, but the mechanism is not clear. The ratio of biologically active to silent receptors is about 1:4 under normal conditions (87,345,347–350).

ANP is a rapid-acting, potent natriuretic and diuretic substance that is also capable of lowering arterial pressure by direct vasodilatory effects on the systemic vasculature and reducing cardiac output. Physiological concentrations of ANP can inhibit tubular reabsorption without altering RBF, GFR, or the filtered sodium load. Medullary blood flow is increased during administration of ANP, but the effect seems to be secondary to the ANP-induced natriuresis. Several tubular sites have been evaluated, including an amiloride-sensitive sodium channel in the collecting duct and an angiotensin-sensitive exchanger in the proximal tubule. ANP has a direct inhibitory action on renin release in cultured juxtaglomerular cells that is mediated by cGMP (350–352).

The renal vascular effects of ANP are mediated by NPR-A and NPR-B receptors. Activation of NPR-A receptors dilates preglomerular resistance vessels, including arcuate and interlobular arteries and afferent arteriole, as well as efferent arterioles. The NPR-B receptor contributions to dilation of the preglomerular vasculature. The reported effect of ANP on K_f is variable. Although glomerular capillary pressure is increased, GFR is usually unchanged, presumably because of a decrease in K_f. The preglomerular vasodilation produced by ANP does not impair autoregulation of either RBF or GFR. ANP appears to have both indirect and direct effects on the tubuloglomerular feedback mechanism. ANP markedly inhibits tubuloglomerular feedback control of glomerular hemodynamics when feedback activity is evaluated by perfusion of Henle's loop with artificial fluid. However, less marked inhibition is observed when natural tubular fluid is allowed to reach the MD segment, presumably because of the presence of an unknown factor that increases feedback sensitivity and overcomes some of the ANP-induced inhibition. Studies on mice with under- and overexpression of the NPR-A receptor highlight the important role ANP has in mediating the renal vascular and tubular changes resulting from blood volume expansion. Overexpression of NPR-A receptors is associated with enhanced renal vasodilatory and natriuretic responses, whereas the responses are markedly attenuated in mice lacking NPR-A receptors (87,173,351–360).

Other Vasoactive Agents

Histamine

Histamine is a naturally occurring biogenic amine that may play important physiological or pathophysiological roles in modulating renal hemodynamics. The glomeruli appear to be the major site of histamine formation within the kidney, containing large amounts of the synthetic enzyme histidine decarboxylase. Glomeruli incubated with histidine accumulate histamine in amounts that are sufficient to alter cAMP and cGMP. In addition, endothelial cells have the capacity to synthesize histamine. Glomeruli and tubules contain the enzymes for intrarenal catabolism of histamine (361).

Infusion of histamine or histamine agonists produces renal vasodilation, while GFR remains relatively constant. Micropuncture studies in rats indicate that the vasodilation is mediated by a larger reduction in preglomerular than efferent arteriolar resistance. An increase in glomerular capillary pressure is offset by a reduction in K_f. These effects contrast with the marked increase in filtration coefficient and membrane permeability produced by histamine in nonrenal capillaries. Histamine also may stimulate renin release, at least transiently, by a prostaglandin-dependent mechanism (261,362).

The renal microcirculation contains two classes of membrane receptors for histamine. In rats, the hemodynamic effects of histamine can be reversed completely by a H_1-receptor antagonist, whereas a H_2-receptor antagonist has no effect. The functional role of H_2 receptors that have been identified in isolated rat glomeruli remains to be determined. Both receptor subtypes appear to mediate hemodynamic responses in the kidney in dogs. Vasodilation is produced by agonists of either H_1 or H_2 receptors, and antagonists of both receptors partially block the renal vasodilation induced by histamine. Binding to vascular receptors may mediate smooth muscle relaxation by stimulation of cAMP formation. Endothelial-derived relaxing factor, NO, renal prostaglandins, and inhibition of adrenergic neurotransmission also may contribute to the histamine-induced vasodilation. Histamine stimulates renin release through activation of H_2 receptors (25,261,361,362).

In normal kidneys, intrarenally formed histamine appears to exert little effect on vascular tone under basal conditions, as evidenced by the lack of response to H_1- and H_2-receptor antagonists. While it has been suggested that histamine H_1 antagonists inhibit renal autoregulation, the mechanism remains uncertain (362).

Vasopressin

In addition to its antidiuretic properties, arginine vasopressin (AVP) is a potent vasoconstrictor. Elevated levels of AVP are associated with renal vasoconstriction and, in particular, reductions in medullary vasa recta blood flow. The plasma concentration of vasopressin (antidiuretic hormone) is directly related to plasma osmolality and is inversely related to blood volume and pressure. Recent evidence indicates that vasopressin is capable of producing effects on renal function by binding to two types of membrane receptors. The well-known osmoregulatory function of low plasma concentrations of vasopressin is mediated through a V_2 tubular membrane receptor, which stimulates cAMP to alter water permeability and sodium reabsorption. Vasopressin also exerts important pressor effects that contribute to the maintenance of arterial pressure during conditions of chronic water deprivation, graded hemorrhage, and possibly in various forms of hypertension (363).

The vascular effects of vasopressin are due to activation of V_1 receptors. The V_{1A} receptor on vascular smooth muscle cells has seven transmembrane spanning domains and is coupled to G_q protein that leads to increased IP_3 production and PKC activation. Cytosolic Ca^{2+} is increased by a combination of release from sarcoplasmic reticulum and entry through voltage-gated Ca^{2+} channels and SOC. Arachidonic acid metabolites may be involved in modulating Ca^{2+} entry channels. The V_{1A} receptor also may mediate vasopressin-induced NO

release. Desensitization of the V_1 receptor and reduced receptor density are related to PKC-mediated phosphorylation. Vasopressin interacts with the renal arachidonic acid and the renin–angiotensin systems. Vasopressin can stimulate renal release of PGE_2 *in vivo*. *In vitro* prostaglandin production is increased by vasopressin–receptor interaction with either V_1 or V_2 receptors on medullary interstitial cells or glomerular mesangial cells. Vascular smooth muscle V_{1A} receptor activation evokes intracellular events that include stimulation of phospholipase A_2 (5,36,69,72,95,363–368).

The role of AVP in the physiological regulation of the renal circulation is uncertain. Supramaximal concentrations of vasopressin are known to increase systemic arterial blood pressure by increasing total peripheral resistance. In this regard, the cortical renal vasculature appears to be relatively insensitive to vasopressin. Administration of antagonists for the V_1, V_2, or both receptors failed to affect whole-kidney hemodynamics in conscious, unstressed animals. As will be discussed in the section on medullary circulation, however, endogenous AVP appears to reduce medullary blood flow via activation of V_1 receptors (32,363,366,368–371).

Pharmacologic blockade of the vascular effects of endogenous vasopressin in water-deprived animals does not affect renal vascular resistance, GFR, or K_f. The GFR and RBF are usually unaffected, while the expected antidiuretic or diuretic responses of vasopressin agonists and antagonists, respectively, are clearly demonstrable. In rats with reduced vasopressin levels, infusion of nonpressor doses of exogenous vasopressin does not alter GFR or RBF or single nephron function. The vasoconstrictor effect of higher vasopressin concentrations on the renal vasculature is more readily apparent during prostaglandin inhibition. The K_f lowering effect of vasopressin may be mediated by a direct action on mesangial cells. It was found, however, that K_f could be normalized by combined blockade of the vascular action of both vasopressin and angiotensin, whereas selective blockade of either peptide was ineffective (72,364, 369–371).

Adrenomedullin

Adrenomedullin (AM), α- and β-calcitonin gene-related peptide (CGRP), calcitonin (CT), and amylin are homologous polypeptides with overlapping biological actions, such as, vasodilation, inhibition of bone resorption, and antiproliferative activity. Adrenomedullin is a potent renal vasodilating and natriuretic peptide (52 amino acids with a disulfide ring) that is made in the kidney as well as the adrenal gland. mRNA for adrenomedullin and its receptor are colocalized to renal vessels, glomeruli, and inner medullary collecting ducts. The proximal tubule is abundant in adrenomedullin mRNA, whereas the greatest amounts of receptor mRNA are in the papilla. Changes in salt diet do not appear to change expression of the peptide or its receptor in either the cortex or medulla. This hormone/paracrine agent increases RBF and sodium excretion without affecting GFR. Natriuretic potency increases during inhibition of neutral endopeptidase, an enzyme that cleaves endogenous peptides with a disulfide ring, such as, adrenomedullin and ANP.

Adrenomedullin produces vasodilation by cAMP/PKA signaling and by stimulating endothelial NO production, leading to activation of ATP-sensitive K channels and Ca-dependent K channels and hyperpolarization of vascular smooth muscle cells. Adrenomedullin increases renin release via increasing cAMP in juxtaglomerular granular cells. CGRP exerts NO-dependent as well as cAMP/PKA vasodilation. Administration of adrenomedullin or CGRP increase RBF and to a lesser extent GFR; both are natriuretic and diuretic. Adrenomedullin and CGRP dilate afferent arterioles and blunt Ang II-induced renal vasoconstriction (372,373–379).

Reactive Oxygen Species

Small amounts of reactive oxygen species (ROS) are constantly produced by aerobic metabolism in a finely controlled fashion in almost all cells. They have important roles in signal transduction in vascular cells under normal physiological conditions. Markedly elevated levels of ROS in pathophysiological states initiate and amplify deleterious events, such as, lipid oxidation and tissue/DNA damage associated with glomerular inflammation and proteinuria, atherosclerosis, and vascular hypertrophy, and vasoconstriction in hypertension. ROS are products of the partial reduction of oxygen, generated by enzymatic and nonenzymatic reactions at cell membranes and within cells. Common oxidative enzymes include nicotinamide adenine dinucleotide/reduced nicotinamide adenine dinucleotide phosphate (NADH/NADPH) oxidases, cyclooxygenase, cytochrome P450, xanthine oxidase, and glucose oxidase. The reduction of molecular oxygen ($O_2 + e^-$) produces superoxide anion ($\bullet O_2^-$), which is normally balanced by its degradation. Superoxide dismutases catalyze the dismutation of superoxide anion to hydrogen peroxide (H_2O_2) that has oxidizing potential; it may be subsequently neutralized by glutathione peroxidases and catalase. Antioxidative enzymes such as superoxide dismutase, catalase, and glutathione peroxidase normally act to protect against oxidative stress. Vitamins A, E, and C and bilirubin may act as scavengers of ROS (289,380–385).

As intracellular signals, ROS may activate or inactivate redox-sensitive protein kinases and phosphatases to modulate receptor phosphorylation and transcription factors. Hydrogen peroxide plays a central role in signal transduction, in growth, and, perhaps, vasomotor tone. Normally, there is a fine balance between activities of oxidative and antioxidant enzymes, optimizing NO activity and minimizing superoxide anion generation. Hydrogen peroxide has been proposed to function as the counterpart to nitric oxide and its antiproliferative and vasodilatory actions. H_2O_2 is more stable than superoxide anion, is uncharged, and is freely diffusible among and within cells. A major source of H_2O_2 is superoxide anion generated by NAD(P)H oxidases. ROS also appear to play a role in tyrosine phosphorylation of PKC, lipid peroxidation, and p53-mediated apoptosis. Targets of H_2O_2 include tyrosine kinases and phosphatases. Shear stress stimulates NADH oxidase activity in endothelial cells. In vascular smooth muscle cells, growth factors and Ang II stimulate ERK1/2, p38 MAPK and Akt through increases in H_2O_2 and NAD(P)H activation. Although little is known about the influence of H_2O_2 on vasomotor tone, H_2O_2 is involved in tyrosine phosphorylation of protein kinase C and after catalase reacts with H_2O_2, it can activate guanylate cyclase and perhaps affect COX activity. Long-term effects of H_2O_2 on vascular reactivity are clearly evident in terms of growth-promoting effects and vascular remodeling.

In the kidney, oxygen radicals are formed in arteries and arterioles, glomeruli, and juxtaglomerular cells endowed with oxidases, such as, NADPH oxidase, nitric oxide synthase, and cyclooxygenase. Ang II and other peptide ligands (e.g., ET-1 and TxA_2) can rapidly stimulate NAD(P)H oxidase to produce $\bullet O_2^-$. Tonic levels of $\bullet O_2^-$ regulate renal vascular resistance. Enhanced basal $\bullet O_2^-$ activity by acute inhibition of superoxide dismutase (SOD, diethyldithiocarbamate) reduces total renal, cortical and medullary blood flow by 25%. The vasoconstriction is greater when the buffering afforded by NO is removed by NOS inhibition. Acute infusion of Ang II produces more pronounced renal vasoconstriction when NAD(P)H

oxidase is intact, as contrasted with transgenic animals with the subunit gp91Phox mutated and nonfunctional. Oxygen radicals antagonize vasodilation normally produced by NO, reducing effective levels of NO to favor vasoconstriction during a variety of conditions, including reperfusion injury, chronic infusion of Ang II, and hypertension. Reactive oxygen intermediates act directly on smooth muscle cells to increase cytosolic calcium and elicit contraction. Superoxide is thought to elicit increases in $(Ca^{2+})_i$ and cause constriction by activation of the cell membrane enzyme cADP ribosyl cyclase to produce cADP ribose, a ligand for the RyR on the sarcoplasmic reticulum that sensitizes the RyR to Ca^{2+} and triggers calcium-induced calcium release. H_2O_2 appears to exert concentration-dependent biphasic effects on arteries/arterioles, constricting at low concentrations ($<50\mu$M), with higher concentrations ($>200\ \mu$M) eliciting transient constriction followed by sustained dilation. Most of H_2O_2 actions are on K channels and membrane potential. Low H_2O_2 concentrations inhibit inward-rectifying K^+ currents to depolarize the plasma membrane, whereas higher H_2O_2 concentrations increased the amplitude of the hyperpolarizing outward K^+ current through KCa channels. The constrictor phase appears to be mediated by endothelium-independent TxA_2. Renal vasoconstriction due to reactive oxygen species can be reversed by administration of superoxide dismutase. Ang II stimulates production of reactive oxygen species and appears to inhibit neutralization; ACE inhibitors have been shown to induce superoxide dismutase, catalase, and glutathione peroxidase in renal tissues. Macula densa feedback control of afferent arteriolar resistance at high tubular flow rates is determined by an autocrine balance of vasodilator NO generated by nNOS and by vasoconstrictor $\bullet O_2^-$ generated by NAD(P)H in MD cells. When nNOS is inhibited, $\bullet O_2^-$-mediated vasoconstriction enhances tubuloglomerular feedback. On the other hand, tubuloglomerular feedback is attenuated by the dilator NO when $\bullet O_2^-$ concentration is reduced by superoxide dismutase. In addition, $\bullet O_2^-$ produced by MD cells may cause afferent arteriolar constriction by a direct paracrine action above its ability to scavenge and reduce the activity of NO. Tubuloglomerular feedback is exaggerated by nNOS inhibition at high tubular flows; administration of a superoxide oxide dismutase attenuates maximum feedback responses in healthy kidneys (16,107,289,374,383, 385–393).

An alternative pathway for superoxide anion is to react rapidly and irreversibly with NO to form peroxynitrite (ONOO-), which clearly limits the half-life, diffusion distance, and biological activity of NO. Peroxynitrite can oxidize arachidonic acid to preferentially form the vasoconstrictor 8-iso-$PGF_{2\alpha}$ (isoprostane), with smaller amounts of isothromboxane and isoleukotrienes. This isoprostane activates either the thromboxane A2 receptor or a unique receptor on smooth muscle cells. Isoprostanes appear to increase endothelin release. Peroxynitrite may react with thiols (e.g., glutiathione) to produce NO and a nitrothiol (e.g., *S*-nitrogluthione), resulting in vasodilation. Chronic exposure to high levels of Ang II stimulates isoprostane production by the kidney. Oxidative stress is capable of modulating the strength of tubuloglomerular feedback. Superoxide production in the interstitium scavenges NO and thereby impacts on signal transduction between MD and afferent arteriolar smooth muscle cells. Exaggerated tubuloglomerular feedback activity in models of hypertension is normalized by administration of a mimetic of superoxide dismutase, which reduces urinary excretion of isoprostanes.

Recent studies suggest that oxygen radicals contribute as signaling molecules to the regulation of renal cortical and medullary microvascular function under normal conditions, as well as in pathophysiological conditions. (107,383–385,387, 389–395).

Medullary Circulation

The vasoactive responses that have been described for the cortical circulation generally occur in the vasculature of the renal medulla. Because of limitations in methodology, it has been difficult to ascribe specific roles to the various extrinsic and intrarenal mechanisms in the regulation of medullary blood flow. Nevertheless, it is recognized that because of the countercurrent exchange system in the vasa recta, maintenance of low, well-controlled medullary blood flow is extremely important for efficient operation of the mechanisms responsible for urinary concentration and dilution (5,32,36).

It is generally accepted that there is an inverse relation between medullary blood flow and urinary osmolality; high urinary osmolality is associated with low medullary perfusion and blood flow is increased during water diuresis. Since physiological concentrations of vasopressin do not affect total RBF, it has been proposed that vasopressin has a predominant effect on the juxtamedullary circulation to reduce blood flow in deep cortical nephrons and in the medulla. Small amounts of vasopressin and a vasopressin V_1 receptor agonist are reported to reduce medullary blood flow without altering total RBF. Likewise, increased plasma vasopressin associated with water restriction reduces medullary blood flow via V_1 receptor stimulation without affecting cortical perfusion. This effect would modify the Starling physical forces in the vasa recta to be more conducive to fluid uptake. There is some evidence that exogenous vasopressin predominantly decreases RBF in the inner cortex and juxtamedullary areas. This effect is apparently independent of endogenous prostaglandins. Recent studies that directly assess the influence of vasopressin showed reduction in both ascending and descending vasa recta blood flows, which could be blocked by the vascular antagonist of vasopressin (36,371,396).

The inner medullary circulation appears to be autoregulated, but the range of arterial pressure is somewhat narrower than observed for the cortex and is more subject to changes in hydration state. Studies utilizing videomicroscopy or laser Doppler flowmetry in rats show good autoregulation over pressures ranging from 85 to 105 mm Hg to 125 mm Hg. A wider range of arterial pressure is noted for autoregulation of medullary blood flow in dogs. Other investigators report a narrower range of approximately 100 to 125 mm Hg, with less efficient autoregulation during extracellular fluid volume expansion at a time when cortical and whole-kidney blood flow autoregulation are efficient and unaffected by volume expansion. In addition to animal studies, autoregulation has been evaluated in other preparations. The blood-perfused juxtamedullary nephrovascular unit exhibits autoregulation of blood flow when pressure is varied between 100 and 150 mm Hg (5,32,36,73,74,138,366–368,397–399).

Recent evidence obtained with videomicroscopy and laser Doppler flowmetry indicates that various agents added locally to the medullary interstitium through implanted matrices can selectively influence the medullary circulation without affecting perfusion in the cortex or at the whole-kidney level. Examples of agents that increase inner medullary perfusion include NO, kinins, prostaglandins, adenosine, and inhibitors of dihydropyridine voltage-sensitive calcium channels. Vasoconstrictor stimuli include Ang II and arginine vasopressin and endothelin. While vasa recta vessels are encircled by smooth musclelike pericytes and focal constriction has been observed, most vasoactive agents seem to alter perfusion by influencing the contractile state of the major resistance vessels: afferent and efferent arterioles in juxtamedullary nephrons. Recent studies suggest a positive correlation among changes in inner medullary blood flow, interstitial hydrostatic pressure, and sodium excretion produced by various receptor

antagonists or synthesis stimulants and inhibitors. The mechanisms are thought to be central to the genesis and maintenance of hypertension (5,32,36,73,74,366,396,398–403).

Prostaglandins have been implicated in the regulation of medullary blood flow. Potential vascular target sites include not only glomerular arterioles of juxtamedullary nephrons, but also vasa recta encircled by pericytes in the prostaglandin-rich inner medulla. Several reports indicate that infusion of prostaglandins or arachidonic acid increases inner cortical and juxtamedullary blood flow in association with a reduction in papillary tonicity. There is also evidence that inhibition of prostaglandin synthesis reduces papillary blood flow. Inhibition of COX reduces blood flow in vasa recta to a similar amount in both control animals and animals subjected to water diuresis, indicating an influence of medullary prostaglandins independent of vasopressin effects (5,32,36,73,367,368,398,399,401–403).

Ang II also may have an important role in the selective control of medullary hemodynamics. Recent studies have discovered a high concentration of Ang II receptors in the medulla, probably associated with the vasculature and interstitial cells. In addition, it appears that Ang II can decrease medullary blood flow at low doses that do not affect total RBF. Furthermore, in physiological or pathophysiological conditions associated with a highly activated renin-angiotensin system, there is a reduced papillary blood flow that can be ameliorated by decreasing Ang II levels or by administering pharmacologic antagonists. However, ACE inhibitors increase medullary blood flow primarily by their action to increase medullary concentrations of kinins. As observed for the cortex, vasodilator prostaglandins and NO can modulate the vasoconstrictor influence of Ang II (32,73,74,204,307,384,396,399–403).

Recent studies suggest that ANP and endogenous kinins may exert larger vasodilatory effects in the medulla than in the cortex. It is of interest that ANP affects tubular transport in the medulla and that the ANP-induced natriuresis occurs before an increase in medullary blood flow (5,74,352,355,356,402).

ADAPTATION OF RENAL HEMODYNAMICS TO ALTERED PHYSIOLOGICAL STATES

Changes in Salt Intake

In a normal person, the kidneys respond rapidly to wide fluctuations in the level of sodium chloride intake and accompanying changes in extracellular fluid volume. To a large extent, the requisite changes in sodium excretion are affected by changes in tubular reabsorption rate, which can adjust fractional sodium excretion from less than 0.1% to 3% to 5% or greater. Nevertheless, there are important renal hemodynamic adjustments that contribute to this capability (210,404–410).

As pointed out in the section on the renin–angiotensin system, a close inverse relationship exists between the level of sodium intake and the activity of the renin–angiotensin system. This relationship is striking in that it is particularly steep during low levels of sodium intake. Under these conditions of low sodium intake or extracellular volume depletion, the influence of the renin–angiotensin system on renal hemodynamics is greatly increased. This influence is illustrated by the marked responses in renal vascular resistance to the administration of antagonists of the renin–angiotensin system. As Figure 2-25 shows, when sodium-depleted animals are given ACE inhibitors, they exhibit a much greater increase in RBF and GFR. These responses occur although arterial pressure decreases more in the sodium-restricted animals; thus, they indicate that

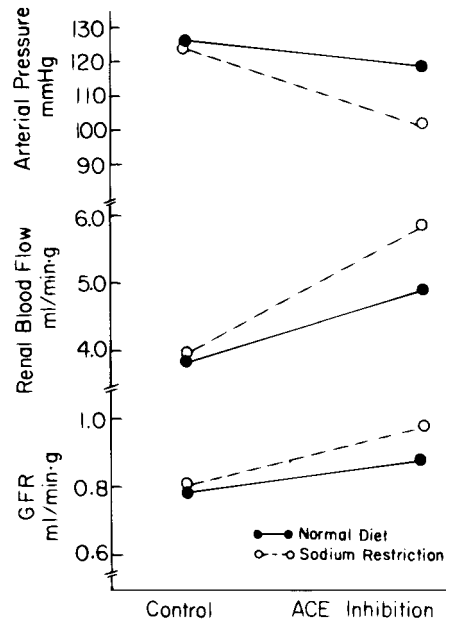

FIGURE 2-25. Comparison of renal hemodynamic and arterial pressure responses to angiotensin converting enzyme (ACE) inhibition in sodium-replete (*solid lines*) and sodium-depleted (*dashed lines*) anesthetized dogs. (Based on data from: Rosivall L, Youngblood P, Navar G. Renal autoregulatory efficiency during angiotensin-converting enzyme inhibition in dogs on a low sodium diet. *Renal Physiol* 1986;9:18.)

the prevailing influence being exerted on renal hemodynamic function by the renin–angiotensin system is much greater during states of sodium depletion than in the sodium–replete condition. With blockade of the renin–angiotensin system, there are roughly equivalent decreases in both preglomerular and postglomerular resistance. The increases in GFR following angiotensin blockade also may be caused, in part, by restoration of K_f. With regard to intrarenal blood flow distribution, a high-salt diet appears to predominantly increase cortical blood flow. The increase may be related to decreased renin release, because cortical blood flow is unchanged when plasma concentration of Ang II is kept constant (7,18,113,119, 384).

Although the Ang II-dependent influence on renal hemodynamics is increased in sodium depletion, it is intriguing that the differences in the steady-state RBF and GFR levels are relatively small. Studies conducted in humans have evaluated the effects of variations in sodium intake, from 10 to 1,500 mEq/day, on renal function. Despite these extreme variations, GFR exhibited a slight trend with a positive influence of salt intake. Renal plasma flow was even less responsive. Because the RBF and GFR values do not differ very much in a setting where the angiotensin-dependent influence on renal hemodynamics is increased, other intrarenal hormonal systems are concomitantly stimulated to counter the constrictive effects of the elevated Ang II levels existing in states of sodium depletion. In particular, vasodilatory prostaglandins derived from ubiqitous COX are greatly increased in situations in which angiotensin levels are augmented. These prostaglandins, in particular, PGI_2 and PGE_2, counteract the vasoconstrictor effects of angiotensin and thus allow the maintenance of near-normal RBF and GFR values. In such a setting, the renal hemodynamic status is much more dependent on intrarenal prostaglandin synthesis, with GFR and RBF decreasing markedly following administration of COX inhibitors (Fig. 2-23). Low salt intake also increases the interstitial kinin levels, suggesting that the vasodilatory action of bradykinin may contribute to counteracting the constrictor effects of Ang II. Nitric oxide synthesis plays an important role

in renal adaption to increased sodium intake. In healthy humans, increased NO production is responsible for increases in RBF and sodium excretion during a high-sodium diet. Inhibition of NO production in low-salt subjects has no effect on RBF or sodium excretion. Infusion of L-arginine to increase renal production of NO during changes in salt intake in humans increases sodium excretion during high-salt diet and reduces sodium excretion during low-salt intake. The excretory responses are most likely because of changes in reabsorption in proximal and distal nephron segments as assessed by lithium and sodium clearances (405–412).

Volume contraction and sodium depletion also activate the sympathetic nervous system and increase plasma vasopressin concentrations. Although the relative importance of the various neural and humoral influences may vary with the degree and duration of volume contraction, each of these factors is known to augment the production of vasodilatory prostaglandins that, in turn, act to buffer the primary rise in renal vascular resistance. Thus, it can be appreciated that renal hemodynamic function becomes more dependent on the hormonal and neural regulators, in particular, the renin–angiotensin system and the renal prostaglandins, as the level of sodium intake is reduced. As sodium chloride intake is progressively increased, this vulnerability is diminished, and there is less dependency on these hormonal systems although basal levels of RBF and GFR are altered to a relatively small extent. Presumably, as the hormonal influences diminish, the contribution of the intrinsic control mechanisms becomes the dominant regulatory influence (210,404–413).

Hydration and Volume Status

Dehydration produced by water deprivation is generally not associated with marked alterations in renal hemodynamics, although it may sensitize the renal circulation to anesthesia and surgery. Studies in conscious humans and dogs indicate that RBF is unaffected by water restriction. In addition, acute administration of vasopressin to conscious rats that are deficient in vasopressin (Brattleboro Diabetes Insipidus rats) failed to change GFR or renal plasma flow. Thus, changes in vasopressin (ADH) levels may not directly influence total RBF and GFR to a significant extent, although they may alter medullary blood flow, as discussed in an earlier section (36,369,414).

The reductions in RBF and GFR observed in anesthetized water-deprived rats are apparently not directly caused by the elevated vasopressin levels, since administration of vasopressin antagonists does not restore renal hemodynamic function. In contrast, blockade of Ang II was found to nearly restore GFR, RBF, K_f, and glomerular capillary pressure to normal. Similar responses have been observed in water-deprived rats following acute renal denervation, regardless of whether or not the vascular effects of Ang II were blocked with a competitive antagonist. Thus, the elevated activity of the renal efferent sympathetic nerves is the primary factor responsible for the renal vasoconstriction and serves as the stimulus for renin release during water deprivation. During water loading, GFR and RBF are essentially normal. All the determinants of filtration rate, including preglomerular and efferent arteriolar resistances, are within the normal range. Inhibition of prostaglandin synthesis does not have significant effects on RBF and renal vascular resistance under conditions of overhydration (72,364,369,412–415).

Relatively mild, nonhypotensive hemorrhage does not elicit significant changes in RBF or GFR. Modest reductions in GFR and RBF usually accompany hemorrhage that causes a 5% to 15% reduction in mean arterial pressure. More severe hemorrhagic hypotension that results in as much as a 30% reduction in arterial pressure causes pronounced renal ischemia. The renin–angiotensin system, the renal efferent adrenergic nerves, and circulating catecholamines are major factors in renal vasoconstriction. Also, 10- to 20-fold increases in plasma vasopressin have been noted during hemorrhagic hypotension, but, as noted above, the renal hemodynamic effects of the elevated vasopressin levels may be minor. As under other circumstances, vasodilator prostaglandins act to attenuate some of the vasoconstriction, since the renal ischemia is more pronounced after inhibition of prostaglandin synthesis (415,416).

Modification in Dietary Protein Intake

It has long been recognized that variations in the protein diet and plasma amino acid concentrations can have significant effects on the renal circulation. A diet that is rich in protein is a general characteristic of modern Western society. Consumption of protein in excess of 1 g/kg per day is usually associated with renal vasodilation in animals and humans. In dogs, consumption of a high-protein meal leads to increases in RBF and GFR that are maximal at 3 to 6 hours and then progressively return to normal by 24 hours. The effect of protein feeding on renal function in humans is less marked than that in dogs. Short-term intravenous infusion of casein produces renal vasodilation that is sustained for up to 8 hours although the blood amino acid concentrations rapidly return to preexisting levels after the infusion is stopped. Various combinations of amino acids usually produce renal vasodilation and increase GFR; the changes are rapid in onset and reversible. It has been noted that only amino acids that are metabolized dilate the renal vasculature, while nonmetabolized amino acids do not affect RBF (417–420).

The mechanism(s) responsible for the relaxation of vascular smooth muscle with a high-protein diet remains unclear. Urea, the principal end product of protein metabolism, has virtually no effect on renal hemodynamics. A significant portion of the renal vasodilation and impaired ability to autoregulate RBF appears to be mediated by prostaglandins. Prostaglandin synthesis is stimulated by a high-protein diet and cyclooxygenase inhibition results in a reduction in RBF and GFR and restoration of RBF autoregulation. Recent studies also have suggested that increased NO production may mediate some of the renal vasodilation occurring in response to amino acid infusions and high-protein intake (420–425).

The systemic mediators of the protein- and amino acid-induced hyperemia and hyperfiltration remain unclear. Glucagon, secretin, and growth hormones—peptides that are released with increased dietary intake of protein and infusion of amino acids—may be involved in some of the changes in renal hemodynamics. Glucagon is a unique vasodilator in that both RBF and GFR increase during intrarenal infusion. Secretin also is a vasodilator, but GFR is relatively unaffected and, in contrast to most other renal vasodilators, does not produce natriuresis or an increase in interstitial hydrostatic pressure. Plasma volume changes probably do not contribute to the renal hemodynamic alterations. Recent micropuncture studies indicate that the increases in GFR and plasma flow during acute infusion of amino acids occur even in the absence of a contribution of the tubuloglomerular feedback mechanism (418–420,425–430).

It is also well established that long-term consumption of a low-protein diet is associated with renal vasoconstriction. Animals fed a normocaloric, low-protein diet have a reduced RBF and GFR with increased resistance of preglomerular and efferent arterioles. The glomerular filtration coefficient is reduced, as is glomerular cross-sectional surface area. Some of the vasoconstriction may be mediated by the renin–angiotensin system, although plasma volume appears to be normal (421,422,426).

CONCLUSION

As is evident from the previous discussion, there are many exciting issues concerning the area of renal hemodynamics and the multiple control mechanisms that are under active investigation. The modern technological developments have allowed a more detailed and direct evaluation of the characteristics of specific segments of the renal microvasculature and of the various membrane and cellular mechanisms mediating differential responses. Direct assessment of responses of individual arterioles has allowed the clarification of long-standing controversies. In addition, developments related to interactions between endothelial cells and vascular smooth muscle cells are now receiving considerable attention from investigators studying the renal circulation, as has been the case for interactions between distal tubular MD cells and vascular cells of the afferent arteriole and glomerulus. This has led to exciting new concepts with far-reaching implications. In addition, many of these integrative mechanisms are now being addressed in terms of dynamic as well as steady-state characteristics. This combination of new developments has provided the impetus for renewed interest in the area of renal hemodynamics and the interactions with other intrarenal systems. These new investigations should result in a much better appreciation of the exact mechanisms that regulate renal microvascular contractility and reactivity.

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CHAPTER 3 ■ REGULATION OF WATER BALANCE: URINE CONCENTRATION AND DILUTION

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INTRODUCTION

The volume and composition of body fluids are regulated through independent, but coordinated, homeostatic mechanisms that control solute and water excretion. Both the volume (50% to 60% of body weight) and the osmolality (280 to 295 mOsm/kg) of body water are maintained within a narrow range in health, despite large variations in water and solute intake. This remarkable day-to-day constancy results from a dynamic balance between intake and output. Water intake from all sources (liquids, water in solid foods, and metabolic water of oxidation) is typically 1.5 to 3.0 L per day in normal persons, but these limits are not fixed and may be greatly exceeded. The chief avenues of water loss are urine losses, evaporation, sweating, and losses through feces.

Of all these routes of excretion, only the kidney is responsive to homeostatic mechanisms governing the volume and composition of body water. The magnitude of evaporative losses is determined by body temperature and surface area, the frequency and depth of ventilation, and environmental factors, such as ambient temperature and humidity. Sweat volume is determined by the requirements of temperature regulation. Gastrointestinal losses are ordinarily small but may become important when function is disturbed. Daily urine volume, on the other hand, may vary between extremes of 0.5 and 15.0 L or more, depending on the requirements for water balance. In dehydration, once the kidney's capacity to conserve water is taxed to the limit, a sensation of thirst is activated, causing water intake to be increased.

The control of water balance requires the intervention of hypothalamic centers that influence both water intake and urinary excretion. Over the past 10 years, studies have underscored an essential role of renal water channels, aquaporins (AQP), in this process. This was based on the pioneering discovery of aquaporin membrane water channels by Agre et al. (1–3). This answered a longstanding biophysical question of how water crosses biological membranes specifically, and provided insight, at the molecular level, into the fundamental physiology of water balance and the pathophysiology of water balance disorders. Out of eleven mammalian aquaporin isoforms, at least six are known to be present in the kidney at distinct sites along the nephron and collecting duct (Table 3-1) (4,5). AQP1 is extremely abundant in the proximal tubule and descending thin limb where it represents the chief site for proximal nephron water reabsorption (6). It is also present in the descending vasa recta (7,8). AQP2 (9) is abundant in the collecting duct principal cells and is the chief target for the regulation of collecting duct water reabsorption by vasopressin (10–13). Acute regulation involves vasopressin-induced trafficking of AQP2 between an intracellular reservoir in vesicles and the apical plasma membrane (12). In addition, AQP2 is involved in chronic/adaptational control of body water balance, which is achieved through regulation of AQP2 expression (14). Importantly, multiple studies have now underscored a critical role of AQP2 in several inherited and acquired water balance disorders (Table 3-2). This includes inherited forms of nephrogenic diabetes insipidus, acquired states of nephrogenic diabetes insipidus, and other diseases associated with urinary concentrating defects where AQP2 expression and targeting is affected. Conversely, AQP2 expression and targeting appears to be increased in some conditions with water retention, such as congestive heart failure (15–19). AQP3 (20,21) and AQP4 (22,23) are basolateral water channels located in the kidney collecting duct and represent exit pathways for water reabsorbed via AQP2 (24–26). Similar to AQP2, the expression of AQP3 is also regulated by vasopressin (24). Several additional AQPs have been identified in kidney. AQP6 is expressed in collecting duct intercalated cells in an entirely intracellular distribution (27–29). AQP7 (30) is expressed in the brush border of segment-3 proximal tubules and may be involved in proximal tubule water reabsorption (31,32). AQP8 (33,34) and AQP11 (35,36) are also expressed in kidney, but little is known about their physiologic function. In this chapter, we focus mainly on the role of collecting duct AQPs in water balance regulation and in the pathophysiology of water-balance disorders.

STRUCTURAL ORGANIZATION OF THE KIDNEY

The ability of the kidney to concentrate and dilute the urine depends on a specialized organization of renal tubule and vascular elements in the various regions of the kidney. The major arrangement of nephrons and collecting ducts in these regions is summarized in Figure 3-1.

Two populations of nephrons merge to form a common collecting duct system (see Fig. 3-1). These two populations are defined by the length of their loops of Henle. One population (long-looped nephrons) has loops that bend at various levels of the inner medulla. In rats and many other species, more than 70% bend in the outer one-half of the inner medulla and progressively fewer loops extend to successively deeper levels of the inner medulla. The other population (short-looped nephrons) has loops that bend in the outer medulla. In some mammalian species, a small fraction of the short-looped nephrons have their bends in the medullary rays. These are termed "cortical nephrons." The loops of Henle receive the effluent from the proximal convoluted tubules. They carry tubule fluid into and out of the renal medulla, establishing countercurrent flow between the two limbs of the loop.

Several discrete nephron segments compose the loop of Henle, as described in Figure 3-1. The descending part of the loop consists of the S2 proximal straight tubules in the

TABLE 3-1

DISTRIBUTION OF AQUAPORINS IN KIDNEY

	Localization in kidney	Subcellular distribution	Regulation	Localization extrarenal
Aquaporin 1	Proximal tubules	APM/BLM descending thin limbs	–	Multiple org.
Aquaporin 2	Collecting duct principal cells	APM VES	+++	Testis
Aquaporin 3	Collecting duct principal cells	BLM	+	Multiple org.
Aquaporin 4	Medullary collecting duct principal cells	BLM	–	Brain and Multiple org.
Aquaporin 6	Intercalated cells	VES Proximal tubule?	+	?
Aquaporin 7	Proximal tubule (straight)	APM	?	Testis, adipocyte
Aquaporin 8	Proximal tubule, collecting duct	VES	?	Testis, epididymis pancreas, liver, colon, heart, placenta
Aquaporin 10	Proximal tubule, collecting duct	?	?	Testis, thymus, liver, intestine, brain, lung
Aquaporin 11	Proximal tubule	VES	?	

APM, apical plasma membrane; *BLM*, basolateral plasma memrane; *VES*, intracellular vesicles.

TABLE 3-2

CONDITIONS ASSOCIATED WITH ALTERED ABUNDANCE AND/OR TARGETING OF AQP2

Reduced abundance of AQP2	Increased abundance of AQP2
Conditions demonstrating reduced AQP2 abundance and polyuria	*Conditions demonstrating increased AQP2 abundance with expansion of extracellular fluid volume*
Genetic defects	- Vasopressin infusion (SIADH)
- Brattleboro rats (Central DI)	- Hepatic cirrhosis (CCL_4-Induced, noncompensated)?
- DI +/+ Severe mice (low cAMP)	- Congestive heart failure
- AQP2 mutants (human)	- Pregnancy
- V_2-receptor variants (human)*	*Conditions demonstrating increased AQP2 abundance with poluria*
Acquired NDI (rat models)	Osmotic diuresis (diabetes mellitus in rat)
-Lithium	
- Hypokalemia	
- Hypercalcemia	
- Postobstructive NDI	
- bilateral	
- unilateral	
Water loading (compulsive water drinking)	
Chronic renal failure (5/6 nephrectomy model)	
Ischemia or cisplatin-induced acute renal failure (polyuric phase in rat model)	
Calcium channel blocker (nifedipine) treatment (rat model)	
Age-induced NDI**	
Conditions demonstrating reduced AQP2 abundance and altered urinary concentration without polyuria	
Nephrotic syndrome models (rat models)	
- PAN-induced	
- Adriamycin-induced	
- $HgCl_2$-induced	
Hepatic cirrhosis (CBDL, compensated)	
Ischemia-induced acute renal failure (oliguric phase in rat model)	
Low protein diet (urinary concentrating defect without polyuria)	

CBDL, common bile duct ligation; *CCl_4*, carbon tetrachloride; *DI*, diabetes insipidus; *DM*, diabetes mellitus; *NDI*, nephrogenic diabetes insipidus; *PAN*, puromycin aminonucleoside; *SIADH*, syndrome of inappropriate secretion of antidiuretic hormone.
*Reduced V_2-receptor density has profound effect on the AQP2 targeting and expression.
**Mild increase in urine production rates.

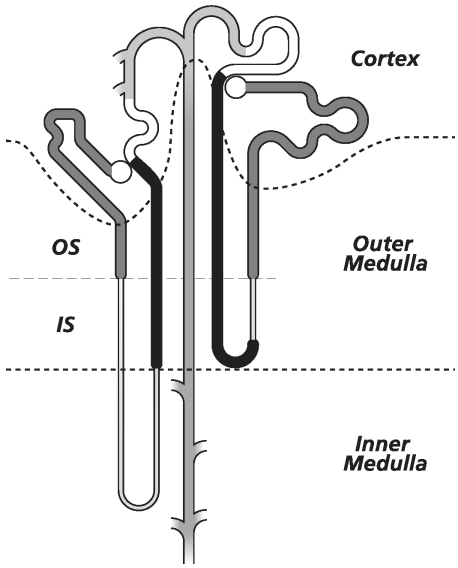

FIGURE 3-1. Mammalian renal structure. Major regions of the kidney are shown on the left. The major portions of the nephron are proximal tubules (*hatched*), thin limbs of loop of Henle (*single line*), thick ascending limbs of loops of Henle (*solid*), distal convoluted tubules (*stipled*), and the collecting ducts system (*open*). (From: Knepper MA, Stephenson JL: Urinary concentrating and diluting processes. In: Andreoli TE, Fanestil DD, Hoffman JF, Schultz SG, eds. *Physiology of membrane disorders*, 2nd ed. New York: Plenum, 1986:713, with permission.)

medullary rays, the S3 proximal straight tubule in the outer stripe of the outer medulla, and the thin descending limbs in the inner stripe of the outer and inner medulla. The descending limbs of short loops of Henle (SDL) differ structurally and functionally from descending limbs of long loops of Henle (LDL) (37–40). In addition, long-looped descending limbs in the outer medulla (LDLOM) differ morphologically and functionally from long-looped descending limbs in the inner medulla (LDLIM) (38,41–46). The transition from the LDLOM to the LDLIM is gradual; it often occurs a considerable distance into the inner medulla (46). In the inner medulla, the immediate prebend region of the thin descending limb has morphologic characteristics similar to those of the thin ascending limb, in some species (46,47), but appears to have unique morphologic and functional properties in the chinchilla (41,42,48) and *Psammomys* (49). Recent studies have also defined an axial heterogeneity in the cell composition and expression of channels (50,51). The ascending part of the loop of Henle consists of the ascending thin limbs (which are present only in long loops), the medullary thick ascending limbs in the inner stripe of the outer medulla, and the cortical thick ascending limbs in the medullary rays. The thick ascending limb of the outer stripe of the outer medulla is morphologically (52,53) and functionally (54,55) similar to the cortical thick ascending limb.

After exiting the loop of Henle, tubule fluid enters the distal convoluted tubules (DCT) in the cortical labyrinth. Several distal tubules merge to form a connecting tubule arcade in most mammalian species (56). The arcades ascend upward through the cortical labyrinth in association with the interlobular arteries and veins (45). The connecting tubule cells of the arcades express both AQP2 (the vasopressin-regulated water channel) and the V2-vasopressin receptor (57), suggesting that, like the collecting ducts, the arcades are sites of regulated water absorption. The arcades deliver their tubule fluid to initial collecting tubules in the superficial cortex. Finally, the initial collecting tubules join to form a cortical collecting duct, usually in the superficial part of the medullary rays. The initial collecting tubule is morphologically similar to the cortical collecting duct (58). In rats, mice, and rabbits, five or six nephrons combine at the level of the arcades and initial collecting tubules to form a single cortical collecting duct (45,59,60).

The collecting duct system spans all the regions of the kidney between the superficial cortex and the tip of the inner medulla (see Fig. 3-1). The collecting ducts are arrayed parallel to the loop of Henle in the medulla and medullary rays. Like the loop of Henle, the collecting duct system is composed of several morphologically discrete tubule segments. The cortical collecting duct (CCD), the outer stripe portion of the outer medullary collecting duct, the inner stripe portion of the outer medullary collecting duct, the initial part of the inner medullary collecting duct (IMCD), and the terminal part of the IMCD have all been shown to have distinct morphologic and functional characteristics (58,61–63). The collecting ducts descend straight through the medullary rays and outer medulla without joining other collecting ducts. However, repeated joinings occur in the inner medulla, which results in a progressive reduction in the number of IMCDs toward the renal papillary tip. This reduction in the number of collecting ducts combined with the progressive reduction in the number of loops of Henle reaching successive levels of the inner medulla accounts for the tapered structure of the renal papilla.

The major blood vessels that carry blood into and out of the renal medulla are called the *vasa recta*. The descending vasa recta receive blood from efferent arterioles of juxtamedullary nephrons (64). The descending vasa recta supply blood to the capillary plexuses at each level of the medulla. The capillary plexus in the outer medulla is much more dense and much better perfused than the plexus in the inner medulla (64,65). Blood from the capillary plexus of the inner medulla feeds into ascending vasa recta. Ascending vasa recta are never formed directly from descending vasa recta (64,65). Ascending vasa recta from the inner medulla traverse the outer stripe of the outer medulla in close physical association with the descending vasa recta in vascular bundles. These vascular bundles vary in size and complexity among the various species (56,66). In some species, the vascular bundles contain only descending and ascending vasa recta; in others, descending thin limbs of short loops of Henle are interspersed with vasa recta (67). The capillary plexus of the outer medulla is drained by vasa recta that ascend through the outer stripe of the outer medulla separate from the descending vasa recta (68).

The counterflow arrangement of vasa recta in the medulla promotes countercurrent exchange of solutes and water. The exchange is abetted by the presence of AQP1 water channels (7,8) and specialized urea transporters (69,70) in the endothelial cells of the descending portion. Countercurrent exchange provides a means of reducing the effective blood flow to the medulla while maintaining a high absolute perfusion rate (71). The low effective blood flow that results from countercurrent exchange is thought to be important to the preservation of solute concentration gradients in the medullary tissue.

In contrast to the medulla, the cortical labyrinth has a high effective blood flow. The rapid vascular perfusion to this region promotes the speedy return of solutes and water absorbed from the nehron to the general circulation. The rapid perfusion is thought to maintain the interstitial concentrations of most solutes at levels close to those in the peripheral plasma. The medullary rays of the cortex have a capillary plexus that is considerably sparser than that of the cortical labyrinth (72). Consequently, the effective blood flow to the medullary rays has been postulated to be lower than that of the cortical labyrinth (73).

The renal medullary interstitium is a complex space that contains fluid, microfibrils, extracellular matrix, and interstitial cells (74). In the outer medulla and the outer portion of the inner medulla, the interstitium is relatively small in volume (59),

which may be important in limiting diffusion of solutes along the medullary axis. The interstitial space is much more prevalent in the inner one-half of the inner medulla (59). A gelatinous matrix found in this region contains large amounts of acid mucopolysaccharides, chiefly highly polymerized hyaluronic acid (75). This gelatinous matrix is thought to limit lateral diffusion of solutes and lateral flow of water in the inner medullary interstitium, perhaps permitting local differences in composition transverse to the medullary axis (68). Interstitial cells express COX-2 and are involved in the regulation of collecting duct water metabolism (76,77).

AQUAPORINS

Identification of Aquaporins

The molecular identity of membrane water channels long remained elusive until the pioneering discovery of AQP1 by Agre and colleagues (5,78). A 28 kDa polypeptide was partially copurified with a rhesus antigen protein (2,3) and found to exist as an oligomeric protein with the physical characteristics of a tetramer. The N-terminal amino acid sequence was identified, which subsequently allowed cDNA cloning (2). *Xenopus laevis* oocytes injected with cRNA for AQP1 exhibited remarkably high osmotic water permeability causing the cells to swell rapidly and burst in hypotonic buffer (2). To further establish the role of AQP1 as a molecular water channel, a series of studies were performed on purified AQP1 reconstituted in liposomes. The result confirmed that AQP1 is a molecular water channel (79). This laid the groundwork for the identification of a number of related channels by homology cloning and other means, which has led to the discovery of a family of water channels, the aquaporins. Studies over the past 10 years have led to the understanding that AQPs play essential roles in transmembrane and transepithelial water transport in the kidney and elsewhere and are critical in renal regulation of body water balance and in many water-balance disorders (4).

Aquaporins in Kidney

Absorption of water out of the renal tubule depends on the osmotic driving force for water reabsorption and the equilibration of water across the tubular epithelium (80). The driving force is established, at least in part, by active Na^+ transport. Moreover, the generation of a hypertonic medullary interstitium results as a consequence of countercurrent multiplication. This requires active transport and low water permeability in some kidney tubule segments, whereas in other segments there is a need for high water permeability (either constitutive or regulated). A series of studies over the past 10 years has made it clear that osmotic water transport across the tubule epithelium is chiefly dependent on aquaporin water channels. The distribution of aquaporins in kidney is listed in Table 3-1.

AQP1

The archetypal member of the aquaporin family, AQP1 (2,3,81) is highly abundant in the proximal tubule and descending thin limb (Fig. 3-2) (4,71) and it constitutes almost 3% of total membrane protein in the kidney (6). AQP1 is absent in other nephron segments and the collecting duct (6) and is thus exclusively expressed in segments of the kidney nephron, which are constitutively, and highly, water permeable, and is not involved in the vasopressin regulation of kidney water transport. Immunoelectron microscopical analysis has documented that

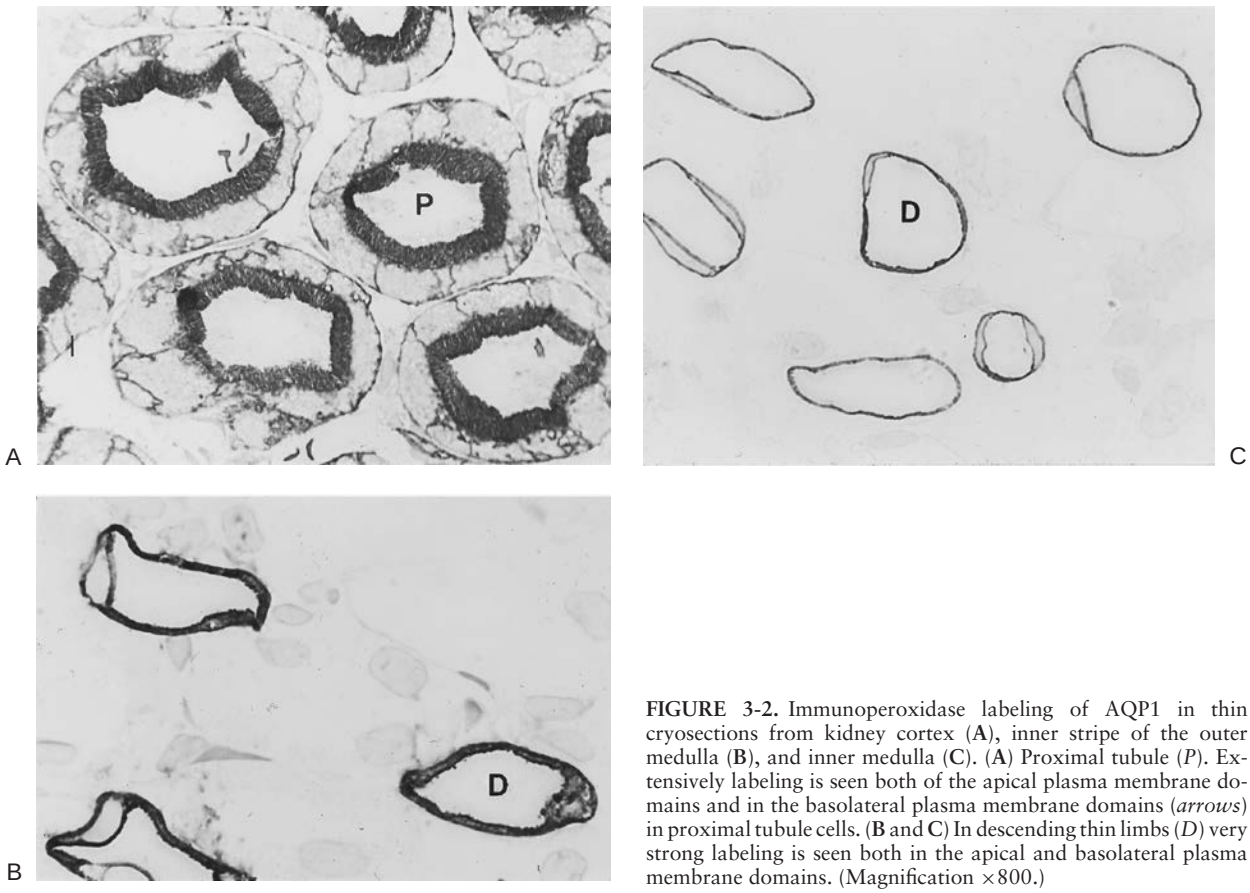

FIGURE 3-2. Immunoperoxidase labeling of AQP1 in thin cryosections from kidney cortex (**A**), inner stripe of the outer medulla (**B**), and inner medulla (**C**). (**A**) Proximal tubule (*P*). Extensively labeling is seen both of the apical plasma membrane domains and in the basolateral plasma membrane domains (*arrows*) in proximal tubule cells. (**B** and **C**) In descending thin limbs (*D*) very strong labeling is seen both in the apical and basolateral plasma membrane domains. (Magnification ×800.)

FIGURE 3-3. Immunoelectron microscopical localization of AQP1 in proximal tubule cell (cryosubstituted and low-temperature Lowicryl HM20 embedded tissue). AQP1 is extremely abundant in the apical plasma membrane of the brush border. (Magnification ×70,000.)

AQP1 is abundant in both apical and basolateral plasma membranes in proximal tubules (Fig. 3-3) and descending thin limbs (Fig. 3-4) consistent with a role for AQP1 in the movement of water across both surfaces of the cells (6).

Immunocytochemistry (6,7,82) revealed that AQP1 immunolabeling is especially abundant in segment 2 and 3 of the proximal tubule, although significant levels are also present in segments 1. Importantly, there is an axial heterogeneity and segment-to-segment differences in the expression levels of AQP1 in the descending thin-limb segments (6,7). This heterogeneity parallels closely the water permeability characteristics in the different segments, established using isolated perfused descending thin limbs (41,42,48). The short loop descending limb also appears to be heterogenous with greater labeling in the proximal than in the distal part. Thus, the abundance of AQP1 parallels the differences in the osmotic water permeability, consistent with the hypothesis that AQP1 is providing the main route for water movement across the tubule wall. Recently, it has been demonstrated that within the descending thin limbs, there are areas devoid of AQP1 and the distal part of the thin descending limb is also devoid of AQP1 (50,51,83).

The biophysical properties, the overall extraordinary abundance of AQP1 in the proximal tubule and descending thin limb, combined with it expression levels in different segments of the descending thin limb, strongly supported the view that AQP1 is essential for proximal nephron water handling and urinary concentration (4,80). The critical role of AQP1 in urinary concentration was confirmed in humans lacking AQP1 (84) and in transgenic knockout mice lacking AQP1 (85–87).

Humans have been identified who totally lack the AQP1 protein (84). Studies have indicated that these Co null individuals have a urinary-concentrating defect in response to vasopressin or water deprivation.

The AQP1 deficient mice were severely polyuric (87) and were unable to concentrate urine to more than approximately 700 mOsm/kg H_2O, even in response to water deprivation, during which they become rapidly dehydrated: plasma osmolalities increased dramatically up to 400 to 500 mOsm/kg H_2O. Thus, AQP1 is required for the formation of concentrated urine. It is presumed that lack of AQP1 undermines the countercurrent multiplication process, which depends on the rapid equilibration of water across the descending thin limb of Henle's loop. A potential defect in descending vasa recta may also be of importance. Subsequent studies have demonstrated that the osmotic water permeability of perfused proximal tubules isolated from AQP1 knockout mice were only

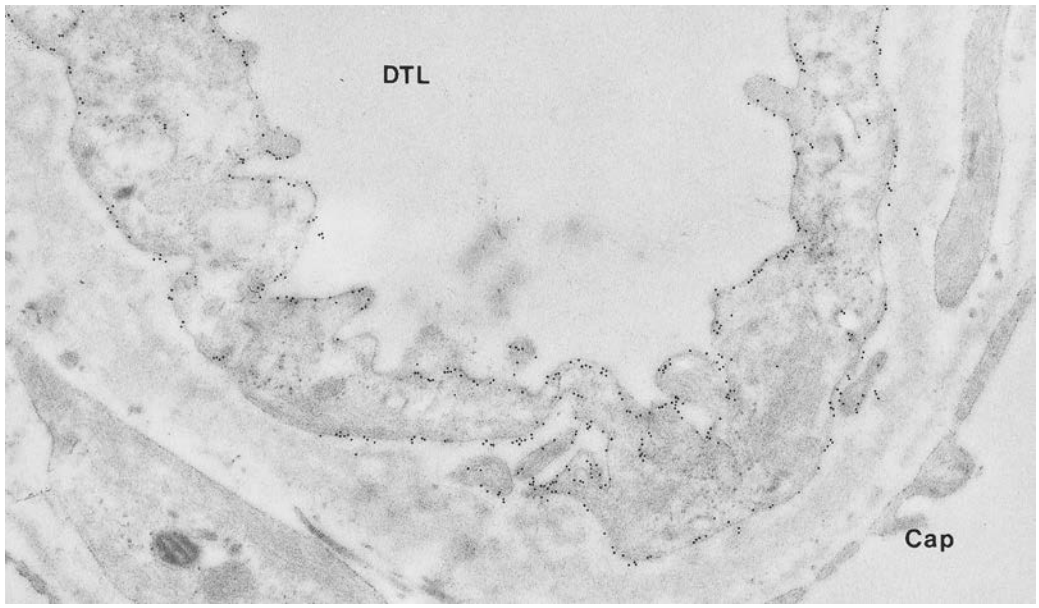

FIGURE 3-4. Immunoelectron microscopical localization of AQP1 in descending thin limbs (DTL) of the loop of Henle (cryosubstituted and low-temperature Lowicryl HM20 embedded tissue). AQP1 is extremely abundant in both the apical and basolateral plasma membrane. Lumen and basement membrane. A capillary (*CAP*) is indicated. (Magnification ×35,000.)

one-fifth of the permeabilities in proximal tubules dissected from kidneys of normal mice (86). Chou and colleagues (85) also demonstrated that the osmotic water permeability of descending thin limb (dissected from kidneys of AQP1 deficient animals) is reduced by 90%. These studies in AQP1 deficient mice and the subsequent studies in AQP1 deficient humans not only indicate a major importance of AQP1 for water reabsorption in the proximal nephron, but also provide strong evidence that the major pathway for water reabsorption in the proximal tubule and descending thin limbs is transcellular (via AQP1) and not paracellular. Additional support for a critical role of AQP1 for osmotic equilibration across the tubular epithelium came from free-flow micropuncture experiments in AQP1 deficient mice by Schnermann and colleagues (88). They determined that the osmolality difference between the plasma and the kidney proximal tubule luminal fluid is excessively increased in the AQP1 deficient mice compared to control mice, strongly supporting the view that AQP1 is important for efficient water transport across the tubular epithelium.

In addition to its presence in the proximal tubule and descending thin limb, AQP1 is also expressed in the descending vasa recta of rat kidney but not in the ascending vasa recta (7). Functional studies by Pallone and collegues (89) using isolated perfused descending vasa recta revealed a marked sensitivity for mercurials on the transendothelial water permeability, confirming that AQP1, at this site, may play a significant role for water transport. This was later also confirmed by Pallone and colleagues (89), using isolated perfused descending vasa recta from wild type mice and from mice lacking AQP1. Water equilibration across the vasa recta may also play a critical role in avoiding the disruption of the osmotic gradient established by the countercurrent exchanger.

AQP2

AQP2 (9) is abundant in the apical plasma membrane and apical vesicles in the collecting duct principal cells (11) (see Figs. 3-5 and 3-6) and in CTs (57,90). In addition to its presence in the apical plasma membrane and intracellular vesicles (Fig. 3-7), some AQP2 immunostaining has also been found to be associated with the basolateral plasma membrane, especially in the inner medullary collecting duct principal cell and in the CT (see Fig. 3-7C) (11,91,92).

AQP2 is the primary target for vasopressin regulation of collecting duct water permeability. This conclusion was solidly established in studies showing (i) the cellular and subcellular distribution (9,11) (ii) a direct correlation between AQP2 expression and collecting duct water permeability in rats (93); (iii) a direct correlation between the osmotic water permeability and AQP2 levels in the apical plasma membrane of collecting duct principal cells in isolated perfused collecting ducts (12) and in whole-animal experiments (only of the onset phase [10,13,91]); and (iv) in studies demonstrating that humans with mutations in the AQP2 gene (94) or rats with 95% reduction in AQP2 expression (95) have profound nephrogenic diabetes insipidus (NDI). Transgene mice with knock in of a nonfunctional mutant AQP2 (Verkman) or in AQP2 total knock-out mice (using the flx/CRE approach) (96) also revealed severe polyuria, hydronephrosis, and die within 2 weeks because of dehydration. Recently, it has become clear that there are very significant axial heterogeneity in AQP2 distribution and in regulation of AQP2 in different segments (92). That the connecting tubule and the CT both play very significant roles for vasopressin-regulated water reabsorption was confirmed in transgene mice that express AQP2 in the connecting tubule, but not in the CT (AQP2 flx/flx mice crossed with CRE mice with CRE expression driven by the connecting tubule-promoter Hoxb7 [96]).

AQP3 and AQP4

AQP3 and AQP4 are expressed in cells in a wide range of organs (see, for example, references 97 through 103), including in the collecting duct principal cells (20,21,24–26,97,104,105). AQP4 is especially abundant in the brain and is thought to play a role in brain water metabolism (106–119). In the CT, AQP4 and AQP3, as well, are abundant in the basolateral plasma membranes (Figs. 3-5 and 3-6), and represent potential exit pathways from the cell for water entering via AQP2. There is some heterogeneity in the segmental and subcellular localization of these two aquaporins in kidney collecting duct. AQP3 is very abundant in connecting tubule, cortical, and outer and inner medullary collecting duct (24,25). In contrast, AQP4 is mainly abundant in the inner medulla, although some expression is also noted in the more proximal segments (25). At the subcellular level, AQP3 is abundant in both the basal and in the lateral plasma membranes of collecting duct principal cells. In contrast, AQP4 is mainly present in the basal plasma membrane of collecting duct principal cells in rat. In mice, AQP4 was also present in the collecting duct principal cells. Interestingly, expression has also been reported in the basolateral plasma membranes of proximal tubule S3 segments (120). This was corroborated by freeze-fracture electron microscopy revealing orthogonal arrays of intramembrane particles (OAPs; known to represent AQP4) on the basolateral membranes of the S3 segment in normal mice, but lack of AQP4 immunostaining and OAPs in collecting duct and proximal tubule in AQP4 knockout mice (121).

A number of studies have also focused on examining whether the expression of AQP3 and AQP4 are regulated, and whether this correlates with changes in renal water-balance regulation. In brief, it has been demonstrated that the expression of AQP3 is regulated by changes in vasopressin levels (26). Brattleboro rats, which lack endogenous vasopressin, have lower expression levels of AQP3 than do Long-Evans rats (the parent strain of the Brattleboros, which have normal vasopressin expression). Long-term vasopressin treatment of Brattleboro rats (using implantable osmotic minipumps to deliver vasopressin for 5 days), results in a significant increase in AQP3 expression, in parallel with an increase in AQP2 expression. Thirsting of normal rats is also associated with increased AQP3 expression (26). In contrast AQP4 appears not be regulated or at least not as markedly as AQP2 and AQP3. Long-term vasopressin infusion did not affect AQP4 expression.

To obtain further information about the physiological role of AQP3 and AQP4, gene knock-out mice have been produced (121–124). Although such studies are complicated by potential interfering compensatory mechanisms during fetal and postnatal development, the phenotyping of these gene knock-out mice have been very informative. Transgenic knockout mice lacking AQP4 showed a mild urinary-concentrating defect (123) and studies using isolated perfused collecting ducts from inner medulla (IMCDs) from AQP4-lacking mice revealed a fourfold reduction in the vasopressin-stimulated osmotic water permeability (122). This indicates that AQP4 may be responsible for a substantial fraction of the basolateral membrane water movement in IMCDs under maximal vasopressin stimulation. The lower abundance of AQP4, together with higher abundance of AQP3 in cortical and outer medullary collecting duct, raises the possibility that AQP3 may play a more significant role at these more proximal segments of the collecting duct. AQP3 knock-out mice revealed a marked urinary concentrating defect with very severe polyuria (124). After deamino-8-D-arginine vasopressin (dDAVP) administration or water deprivation, the AQP3-deficient mice were able to concentrate their urine partially to approximately 30% of that in wild-type mice. Osmotic water permeability of dissected, nonperfused cortical collecting duct measured by an optical method was reduced in IMCDs

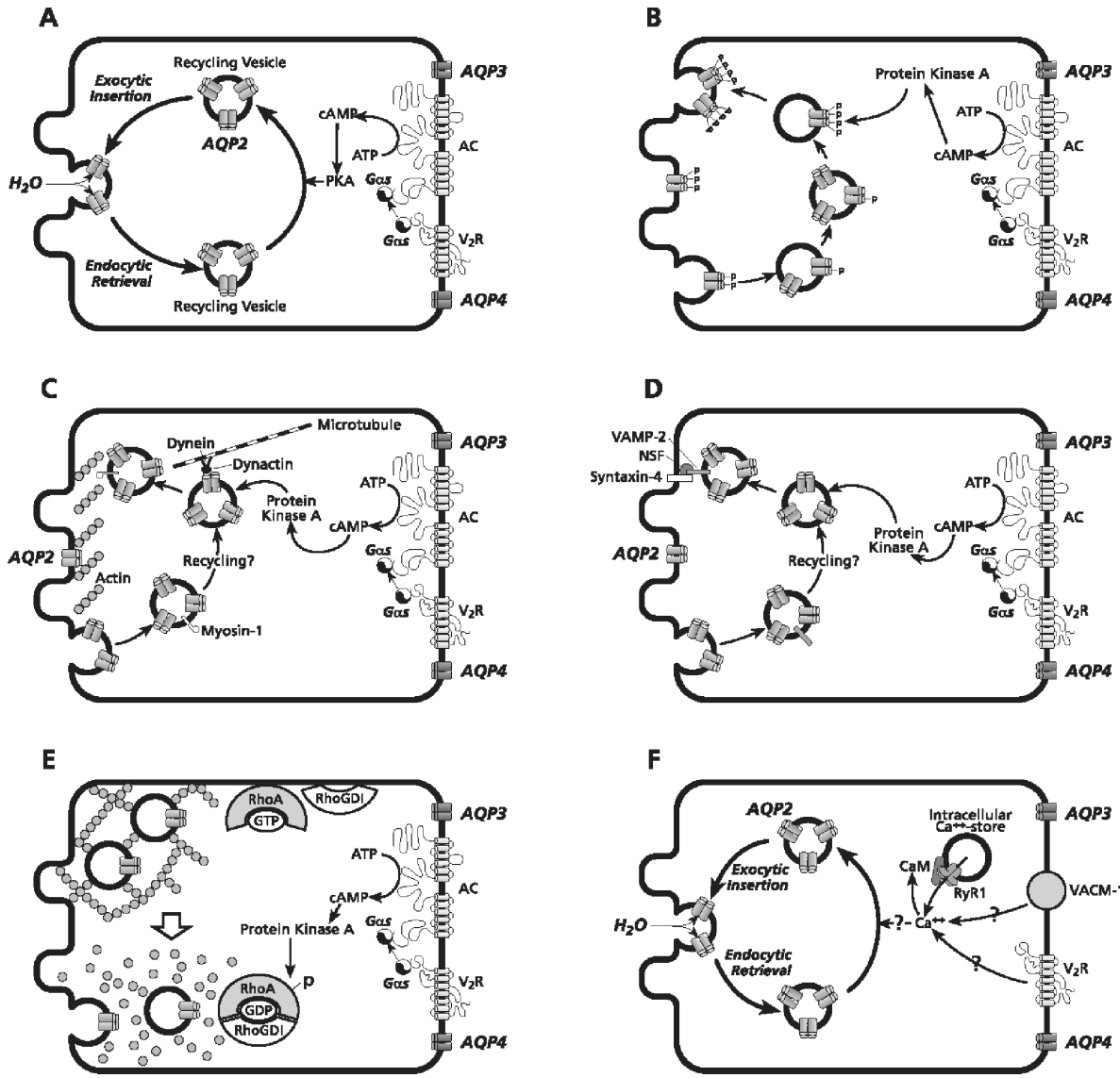

FIGURE 3-5. Overview of vasopressin-controlled short-term regulation of AQP2 trafficking in AQP2-containing collecting duct cell. Signaling cascades and molecular apparatus involved in vasopressin regulation of AQP2 trafficking are shown. **A.** Vasopressin binding to the G protein–linked V2 receptor stimulates adenylyl cyclase leading to elevated cAMP levels and activation of protein kinase A. AQP2 is subsequently translocated to the apical plasma membrane. **B.** Role of AQP2 phosphorylation in AQP2 recruitment to the plasmamembrane. Protein kinase A phosphorylates AQP2 monomers and phosphorylation of at least three of four AQP2 monomers in an AQP2 tetramer is associated with translocation to the plasma membrane. It is currently unknown if dephosphorylation of AQP2 is necessary for endocytosis of AQP2. **C.** Overview of cytoskeletal elements, which may be involved in AQP2 trafficking. AQP2-containing vesicles may be transported along microtubules by dynein/dynactin. The cortical actin web may act as a barrier to fusion with the plasmamembrane. **D.** Vesicle targeting receptors and AQP2 trafficking. A number of vesicle-targeting receptors, for example, SNARE proteins have been localized to the AQP2-containing collecting duct cells and cultured cells. The exact role of these remains to be established. **E.** Changes in the actin cytoskeleton associated with AQP2 trafficking to the plasma membrane. Inactivation of RhoA by phosphorylation and increased formation of RhoARhoGDI complexes seem to control the dissociation of actin fibers seen after vasopressin stimulation. **F.** Intracellular calcium signalling and AQP2 trafficking. Increases in intracellular Ca^{2+} concentration may arise from stimulation of the V2 receptor. The existence and potential role of other receptors and pathways, for example, VACM-1, in Ca^{2+} mobilization is still uncertain. The downstream targets of the calcium signal are unknown and conflicting data exist on the importance of a rise in intracellular Ca^{2+} for the hydroosmotic response to vasopressin. Vasopressin–V2 receptors (*V_2R*), adenylyl cyclase (*AC*), cyclic AMP (*cAMP*), guanosine diphosphate (*GDP*), and protein kinase A (*PKA*), vasopressin-activated Ca^{2+} mobilizing (VACM-1) receptor, vesicle associated membrane protein type 2 (VAMP-2), N-ethylmaleimide-sensitive fusion protein (NSF).

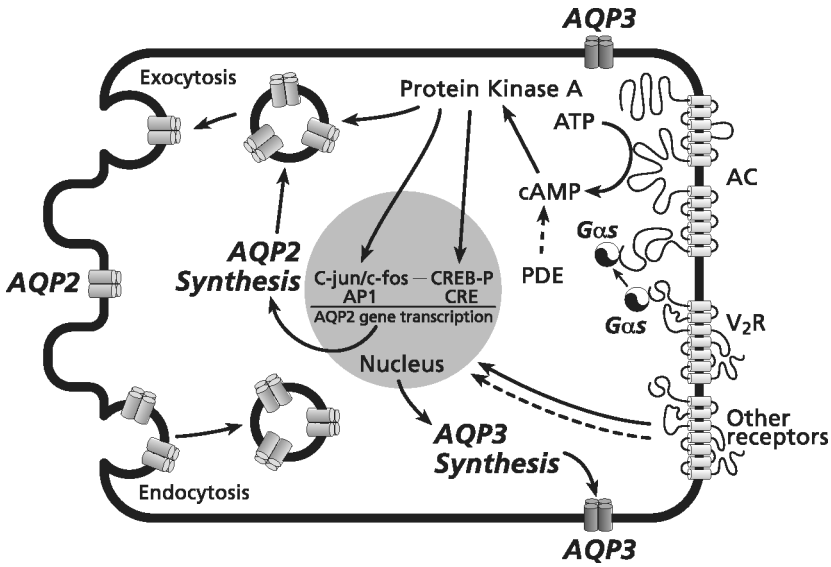

FIGURE 3-6. Regulation of AQP2 trafficking and expression in collecting duct principal cells. Vasopressin (*AVP*) acts on V2 receptors (V_2R) in the basolateral plasma membrane. Through the GTP-binding protein (*GS*), adenylyl cyclase (*AC*) is activated, which accelerates the production of cyclic AMP (*cAMP*) from ATP. cAMP then binds to the regulatory subunit of protein kinase A (*PKA*), which activates catalytic subunit of PKA. PKA phosphorylates AQP2 in intracellular vesicles and possible other cytosolic or membrane proteins. Specifically cAMP participates in the long-term regulation of AQP2 by increasing the levels of the catalytic subunit of PKA in the nuclei, which is thought to phosphorylate transcription factors, such as cyclic AMP responsive element binding protein (CREB-P) and C-Jun/c-Fos. Binding of these factors are thought to increase gene transcription of AQP2 resulting in synthesis of AQP2 protein, which, in turn, enters the regulated trafficking system. In parallel, AQP3 and AQP4 synthesis and trafficking to the basolateral plasma membrane takes place.

from AQP3 knock-out mice. One complicating factor is that in the AQP3 knock-out mice, AQP2 expression in the CCD is also reduced extensively raising the possibility that part of the polyuria in these AQP3 knock-out mice may be caused by the reduced expression of AQP2. Further studies (125) revealed that AQP3 null mice, in addition to exhibiting severe polyuria and polydipsia, exhibit increased urinary sodium excretion. In AQP3 null mice, there was a marked downregulation of AQP2 and pAQP2 both in connecting tubule and CCD. Moreover, AQP4 was virtually absent in the CNT and in the CCD in AQP3 null mice and, in contrast to rat, there is virtually no basolateral AQP2 in mice (both transgenic and normal mice). This, therefore, demonstrated that no basolateral AQPs are expressed in CNT and CCD of AQP3 null mice. However, in the medullary collecting ducts, there was no difference in the expression levels and subcellular localization of AQP2, pAQP2, and AQP4 between AQP3 +/− and AQP3 null mice. Moreover, a striking reduction in the immunolabeling of α_1 subunit of Na,K-ATPase was observed in CCD in AQP3 null mice, while medullary collecting duct exhibited normal labeling. Immunolabeling of all ENaC subunits in the collecting duct was unchanged. Although it cannot be ruled out that the changes are secondary to polyuria, the results raises the possibility that the severe urinary-concentrating defect in AQP3

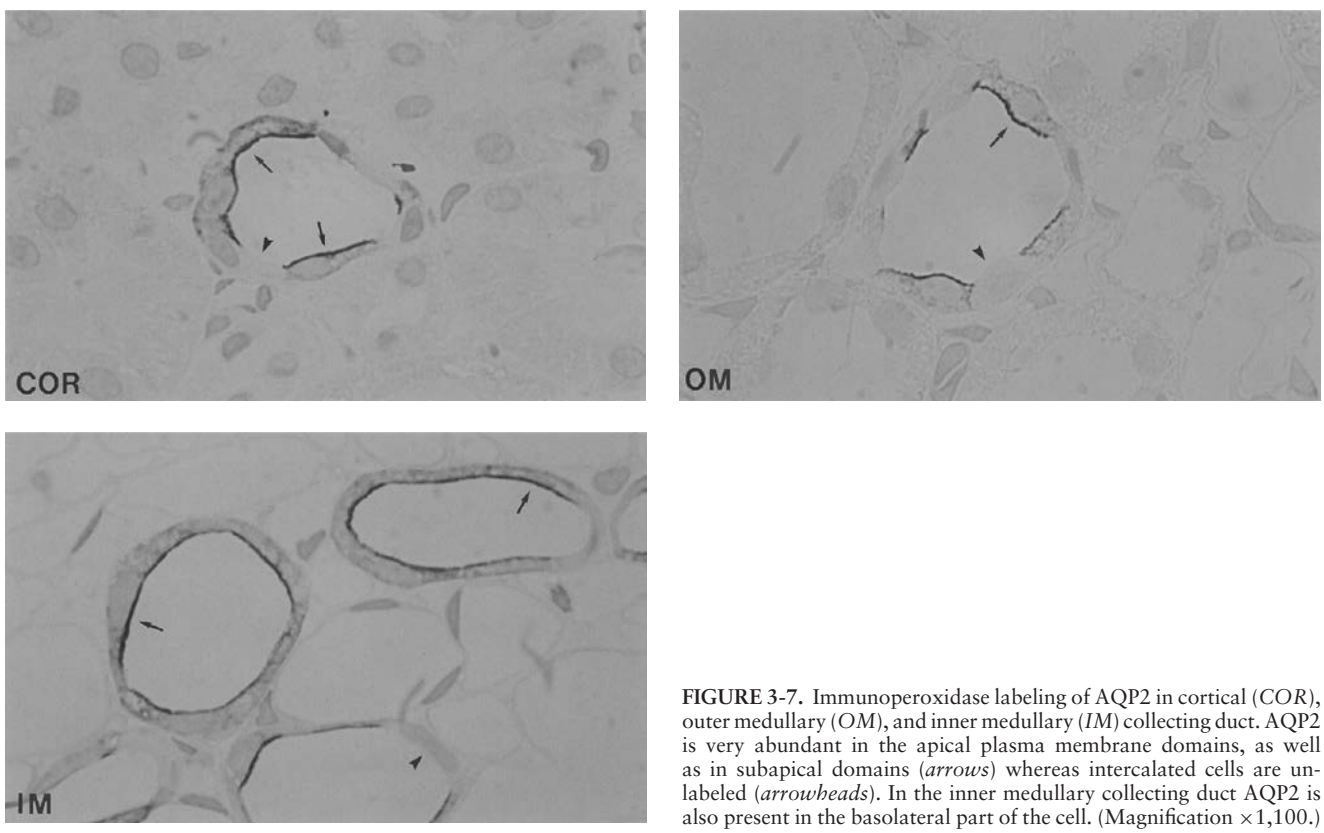

FIGURE 3-7. Immunoperoxidase labeling of AQP2 in cortical (*COR*), outer medullary (*OM*), and inner medullary (*IM*) collecting duct. AQP2 is very abundant in the apical plasma membrane domains, as well as in subapical domains (*arrows*) whereas intercalated cells are unlabeled (*arrowheads*). In the inner medullary collecting duct AQP2 is also present in the basolateral part of the cell. (Magnification ×1,100.)

null mice may, in part, be caused by the reduced expression of AQP2, pAQP2, and AQP4 in CNT and CCD, while the increased urinary sodium excretion may, in part, be accounted for by reduced Na,K-ATPase in CCD in AQP3 null mice. Of interest, is that copper and nickel has also been found to inhibit AQP3 (126,127). AQP1 null mice also have changes in sodium transporter expression (likely to be involved in the compensation to the severe polyuria [128]), and it is very likely that cell type or segment-specific gene knock-out approaches (like CT-specific connecting tubule-sparing AQP2 gene knock out [96]) will be of importance in future studies.

AQP6

Four additional aquaporin cDNAs have been identified in the kidney. One of these is AQP6. Initially, a cDNA from rat kidney (WCH3) and the human homolog (hKID) was identified, and when expressing these in *Xenopus laevis* oocytes they increased the osmotic water permeability slightly, but unsuccessful attempts to raise antibodies led the authors to conclude that the protein is not immunogenic (129,130). The International Human Genome Nomenclature Committee adopted the name aquaporin (AQP) as the referencing system (131). AQP6 was designated for rat WCH3 and human hKID (132), although the cellular locations were not established at the time. While searching for new AQPs in kidney by polymerase chain reaction (PCR), a rat cDNA clone was isolated encoding a water-channel protein (AQP6), which is closely related to rat WCH3 and human hKID. Production of antibodies against AQP6 allowed the definition of the cellular and subcellular localization of AQP6 in rat kidney. AQP6 was expressed in CT intercalated cells in cortical, outer medullary, and inner medullary collecting duct (27). The presence in CT intercalated cells in the outer and inner medulla allowed the conclusion that AQP6 was present in Type-A intercalated cells and in the Type-A-like intercalated cell found in the inner medullary collecting duct (this has later been confirmed and extended [29]). A major surprise was that AQP6 was almost exclusively found associated with intracellular vesicles (27), with an almost complete absence of AQP6 immunogold labeling in the plasma membranes (27,28). Thus AQP6 appears, at least in rat kidney, to be an exclusively intracellular water (and ion) channel with unique biophysical characteristics (28,133–135). Its physiologic role remains elusive despite analyses of AQP6 gene knock-out mice (Yasui et al., unpublished data, 2003).

AQP7 to 11

AQP7 is highly abundant in spermatocytes (30,136–138), but it may also be present in other tissues, such as muscle cells (139). Studies using antibodies to rat or mouse AQP7 have revealed that AQP7 is expressed in the proximal tubule brush border (BB) especially in the segment-3 proximal tubule (31,32) (Table 3-1). Recent studies have indicated that AQP7 may play a role for glycerol metabolism by showing adaptation to fasting by glycerol transport through AQP7 in adipose tissue (140) and AQP7 gene knock-out mice have shown increased glycerol excretion in urine (141).

AQP8 (33,142) is expressed in several tissues, including liver, gall bladder, other parts of the digestive system, spinal cord, and kidney (143–149). Based on biophysical analyses, it may be speculated that, in liver, AQP8 may be involved in transport of ammonia (150), although this remains to be clarified. Phenotyping of AQP8 null mice did not reveal any renal deficiencies (151). AQP9 is also expressed in the liver, but not in kidney (152–154). AQP10 (155) has not been identified in kidney, but in other tissues (156). AQP11 apparently is expressed in the proximal tubule and transgene mice lacking AQP11 develop cystic kidney disease (35,36).

RENAL REGULATION OF BODY WATER BALANCE

Vasopressin and Regulation of Vasopressin Secretion

Neuroendocrine control of body fluid metabolism is very complex and involve the complex and integrated action of various hormones, such as antidiuretic hormone vasopressin (ADH), atrial natriuetic peptide (ANP), angtiotensin II, and others (157). Antidiuretic hormones are phylogenetically ancient neuropeptides that have been found in all vertebrate species in which they have been sought. Three naturally occurring peptides with antidiuretic properties have been identified (158). Arginine vasopressin is the ADH of humans and most other mammals, with the notable exception of members of the pig family, in which lysine vasopressin is found. In birds and all lower vertebrates, the ADH equivalent is arginine vasotocin. All three hormones are nonapeptides, possessing a ring structure formed by a disulfide bridge between cysteine units at positions 1 and 6, a tripeptide side chain in which the terminal glycine is amidated, and a basic amino acid, arginine or lysine, at position 8. Structurally similar peptides that lack any of these three features have little or no antidiuretic action.

The main, but not the sole, function of vasopressin in mammals is to control water balance by making it possible for the kidney to vary urine volume and concentration over wide limits. The hormone is also a potent vasoconstrictor; it stimulates glycogenolysis in hepatocytes, and it may facilitate or directly stimulate adrenocortioctropin hormone (ACTH) release from the anterior pituitary (159). It also is secreted into ventricular cerebrospinal fluid and appears to function intracerebrally as a neurotransmitter or neuromodulator, facilitating memory consolidation (160,161). The effects of disturbances in vasopressin synthesis and release are most visible, however, in the resulting disorder of renal function.

Vasopressin is synthesized in cell bodies of neurons that are widely distributed in the hypothalamus, but the hormone that is released in response to the homeostatic requirements of water balance originates chiefly in magnocellular neurons of the supraoptic and paraventricular nuclei (162). Axons of these neurons travel through the pituitary stalk to terminate in the posterior lobe of the pituitary gland. Hormone biosynthesis begins with the formation of a protein of approximately 20,000 Da of molecular mass that incorporates both vasopressin and its associated carrier protein, neurophysin II (163–165). This prohormone travels down the axon, undergoing partial degradation while in transit. Finally, vasopressin (1,100 Da) is stored as a stoichiometric (1:1) complex with its neurophysin (approximately 10,000 Da), in secretory granules near the nerve terminals in the neurohypophysis. On stimulation of the neuron, the terminal membrane of its axon is depolarized, and vasopressin and neurophysin are secreted together into the circulation by a process of exocytosis. The complex separates after discharge, allowing the hormone to circulate free. Injury to any part of this system—to the hypothalamic nuclei, to the posterior pituitary, or to the neural pathway between these areas—may interfere with the release of vasopressin.

Osmotic Control of Vasopressin Secretion

Vasopressin secretion is subject to modulation by a variety of factors, but the most important determinant of hormone release is, without doubt, osmolality. The classic work on the control of ADH secretion was performed by Verney (166). He demonstrated that antidiuresis could be evoked in dogs by

injecting hypertonic solutions of sodium chloride, sodium sulfate, or sucrose into the carotid artery in amounts too small to have an effect when given intravenously. Urea, to which most cell membranes are somewhat permeable, had no effect. Verney postulated that an increase in the effective extracellular fluid osmolality causes water withdrawal and shrinkage of osmoreceptors located in some unknown portion of the brain that is perfused by the internal carotid, and that this, in turn, leads to ADH release. The exact location and nature of the osmoreceptors remain uncertain, but the best available evidence suggests that they are neurons that reside in the anterior hypothalamus, in close proximity to, but anatomically distinct from, the supraoptic nuclei (167,168).

Robertson and colleagues (169–172), using a sensitive and specific radioimmunoassay for vasopressin, have refined and extended Verney's observations. They found that the plasma vasopressin concentrations is suppressed to very low or undetectable levels when the plasma osmolality is less than a threshold value and that it rises in more or less linear fashion as osmolality is increased above the threshold. Both the threshold and the sensitivity of the response to changes in osmolality may vary between persons and at different times in the same individual, but circulating vasopressin usually becomes undetectable in humans when the plasma osmolality is reduced below about 280 mOsm/kg. Above this value there is a highly significant correlation between plasma osmolality (POsm) and vasopressin concentration (PAVP).

Although the plasma vasopressin concentration rises apparently without limit as plasma osmolality increases, no further increase in urine concentration occurs beyond a limiting value, which is about 1,200 mOsm/kg in young adults, but declines slowly with aging (173). This concentration is reached at a plasma vasopressin level of approximately 5 pg/ml, which corresponds to a plasma osmolality of only about 295; thus, the entire range of the renal response, from a maximum volume of dilute urine to a small volume of highly concentrated urine, is evoked by changes in plasma osmolality of 15 mOsm/kg or less. Most persons with free access to water will maintain a plasma osmolality somewhere near the middle of the dose-response range; thus, the urine, although hypertonic, is not maximally concentrated, and its osmolality can be changed in either direction in response to a change in plasma osmolality. Since the onset of action of vasopressin occurs within minutes and its half-life in plasma is only about 5 minutes, the effects of osmotic stimuli (water ingestion or deprivation, solute ingestion or infusion) are rapidly translated into changes in water excretion. In addition, long-term changes in water intake modify maximal concentrating capacity of the kidney (174–176). Thus, for example, a sustained reduction in water intake over several days results in an increase in the urinary osmolality attained in response to an injection of ADH (176).

Nonosmotic Factors Regulating ADH Release

Many nonosmotic stimuli also can influence vasopressin release, including changes in blood volume and blood pressure, nausea, pain, anxiety, and other forms of emotional stress, and numerous drugs. In most instances, osmoregulation of vasopressin release still can be demonstrated, and the effect of most nonosmotic stimuli, including volume disturbances, appears to be to alter the set point and, at times, the sensitivity of the osmoreceptor mechanism (171).

Bleeding, sequestration or redistribution of blood, or loss of fluid by sweating, diarrhea, or vomiting may result in both thirst and vasopressin release. Inadequate delivery of blood to the arterial circulation, as in congestive heart failure (CHF) or high-output states associated with liver disease, also may be associated with increased ADH secretion. Conversely, expansion of the extracellular fluid by infusion of isotonic saline may suppress release of the hormone. This volume-sensitive regulation appears to be mediated by neural pathways that are activated by distention of the left atrium and by baroreceptors in the arch of the aorta and the carotid sinuses (177–179). Afferent impulses arising from these loci appear to exert tonic inhibition of ADH secretion and the secretory response to volume depletion results from a loss of this inhibition. The ADH release mechanism is much less sensitive to small changes in blood volume than comparable percentage changes in osmolality (Fig. 3-4). The relationship to volume depletion is nonlinear, however, and with large decreases in blood volume, in excess of 15%, volume may become the controlling determinant of ADH secretion (180).

Since angiotensin II levels are characteristically high in states of volume depletion, a role for this hormone in the modulation of ADH secretion has been suggested. Indeed, intracerebral infusion of angiotensin (181) or intravenous administration of pressor doses (182) results in a modest increase in ADH secretion, but the effect is relatively small and a role for angiotensin in the control of ADH release is not nearly as well established as it is in genesis of thirst.

Nausea, whether occurring in the course of illness or induced by motion sickness or drugs, is a powerful stimulus to vasopressin secretion (161,183). Very high levels of circulating hormone are commonly observed, even when the nausea is transient and unaccompanied by hemodynamic disturbances. The effect of nausea is blunted, but not abolished, in persons undergoing water loading. Since many disease states and drugs, as well as emotional disturbances, can stimulate the emetic center, this effect provides a possible final common pathway for the induction of ADH release in a variety of otherwise unrelated conditions.

A number of pharmacologic agents influence the release of vasopressin. Although some may directly stimulate or inhibit secretion, it is probable that most act indirectly, either through effects on blood pressure or other cardiovascular parameters or by means of stimulation or suppression of the emetic center. General anesthetics and barbiturates, especially the oxybarbiturates, appear to stimulate the release of ADH. Nicotine is a potent stimulus to vasopressin release in nonsmokers, who may also experience nausea and vasomotor disturbances, but experienced smokers may be unresponsive (46). Vasoactive drugs, especially those, such as isoproterenol, that stimulate β-adrenergic receptors, appear to induce vasopressin release by lowering blood pressure (184). Among the drugs that suppress vasopressin release are norepinephrine, which acts directly by raising arterial pressure, and dopamine antagonists, such as haloperidol and promethazine, which probably act by suppressing the emetic center (170,185). The capacity of ethanol to suppress the release of vasopressin and to induce a water diuresis is legendary; however, ethanol is not effective in the presence of a powerful counterstimulus, such as dehydration, marked hyperosmolality, or blood loss, and it may even stimulate ADH release if nausea and vomiting occur.

The release of vasopressin is influenced by neural impulses that arise from cerebral cortical areas. Pain, anxiety, and other forms of emotional stress can cause release of vasopressin, and such factors may play a role in the retention of water that occurs in severely ill patients, especially in postoperative situations in which the problem may be compounded by a variety of pharmacologic agents.

Importantly, vasopressin release also exhibits a cirrhadian rhythm with enhanced secretion during night (186). A fraction of children suffering from bedwetting showed a blunted cirrhadian rhythm of vasopressin secretion indicating that this is a pathophysiologic factor (187). Studies have also demonstrated a blunted cirrhadian rhythm in elderly persons (188).

This potentially may aggravate or lead to nocturia, especially in association with heart failure.

Acute Regulation of Collecting Duct Water Permeability

Vasopressin Regulation of AQP2 Trafficking

The final concentration of the urine depends on the medullary osmotic gradient built up by the loop of Henle, and the water permeability of the collecting ducts carrying the urine through the cortex and medulla. Collecting duct water permeability is regulated by vasopressin, and it has been suspected for many years on the basis of indirect biophysical evidence that the vasopressin-induced increase in permeability depended on the appearance of specific water channels in the apical plasma membrane of the ADH-responsive cells.

Much of the early work on vasopressin action was done in amphibian skin or bladder, which are functional analogs of the kidney collecting duct (189–191). These studies by Wade and colleagues (192) led them to propose the "membrane shuttle hypothesis," which proposed that water channels were stored in vesicles and inserted exocytically into the apical plasma membrane in response to vasopressin. The identification of the aquaporins (AQP) and, subsequently, AQP2, later shown to be the predominant vasopressin-regulated water channel (for recent review see [4]), made it possible to prepare antibodies and investigate the effects of vasopressin in mammalian collecting ducts directly. AQP2 is present in the apical and subapical parts of collecting duct principal cells, and immunoelectron microscopy (see Fig. 3-8) showed that AQP2 is very abundant both in the apical plasma membrane and in small subapical vesicles (11).

Water flow out of the collecting duct is determined by the apical plasma membrane of the collecting duct cells, which provides the rate-limiting barrier to water movement (193). *In vitro* and *in vivo* studies have now underscored the importance of regulated trafficking of AQP2 as the predominant mechanism for vasopressin to acutely regulate collecting duct water reabsorption (see Fig. 3-5A-F). *In vitro* studies using isolated perfused tubules allowed a direct analysis of both the on-set and off-set responses to vasopressin. In this study it was demonstrated that changes in AQP2 labeling density of the apical plasma membrane correlated closely with the water permeability in the same tubules, while there were reciprocal changes in the intracellular labeling for AQP2 (12). *In vivo* studies using normal rats or vasopressin-deficient Brattleboro rats also showed a marked increase in apical plasma membrane labeling of AQP2 in response to vasopressin or dDAVP treatment (10,13,91). The off-set response has been examined *in vivo* using acute treatment of rats with vasopressin–V2-receptor antagonist (194,195) or acute water loading (to reduce endogenous vasopressin levels [196]). These treatments (both reducing vasopressin action) resulted in a prominent internalization of AQP2 from the apical plasma membrane to small intracellular vesicles further underscoring the role of AQP2 trafficking in the regulation of collecting duct water permeability. Regulation of the water conductance of AQP2 appears not to be important (197,198).

Recycling of AQP2: Regulation of AQP2 Endocytosis and Exocytosis. Several groups have now successfully reconstituted the AQP2 delivery system using cultured cells transfected with either wild-type AQP2 or AQP2 tagged with a marker protein or a fluorescent protein (199–203). The studies have recently been reviewed (204), and the following is based on this review. Using such cultured cells stably transfected with AQP2, the authors have shown shuttling of AQP2 from vesicles to the plasma membrane, albeit in some cases to the basolateral membrane, as well as retrieval and subsequent trafficking back to the surface upon repeated stimulation. This recycling of AQP2 also occurs in LLC-PK1 cells in the continued presence of cycloheximide preventing *de novo* AQP2 synthesis. Inhibiting endocytosis, by depleting the membrane of cholesterol using methyl-β-cyclodextrin, resulted in accumulation of cmyc-tagged AQP2 in the plasma membrane domain within 15 minutes, indicating a rapid turnover of plasma-membrane AQP2 (205). Methyl-β-cyclodextrin treatment even lead to the accumulation of AQP2 S256A mutant molecules (205),

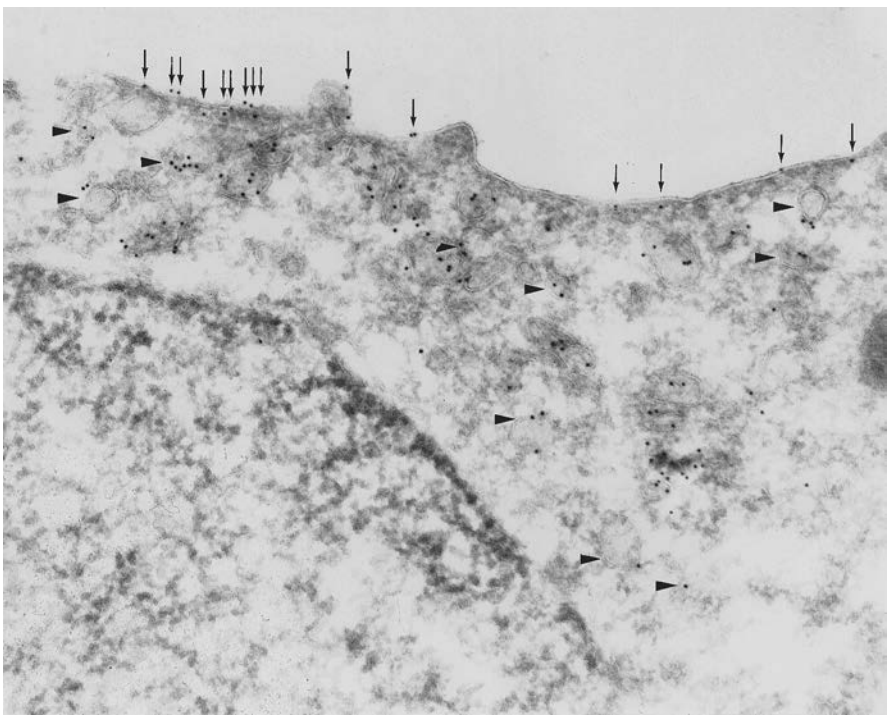

FIGURE 3-8. Immunoelectron microscopical labeling of AQP2 in ultrathin cryosection of inner medullary collecting duct. AQP2 is abundant both in the apical plasma membrane (*arrows*) and in small subapical vesicles (*arrowheads*). (Magnification ×50,000.)

which, in other cell cultures, have been found not to appear in the plasma membrane (201). This, together with the evidence by Valenti and co-workers (206) showing that okadaic acid treatment-induced AQP2 plasma membrane insertion in the presence of the protein kinase A (PKA) blocker H-89, provides evidence for a phosphorylation-independent plasma membrane accumulation of AQP2 and, thus, the existence of constitutive recycling/trafficking of AQP2, as well as S256A-AQP2.

It remains unsettled if both the exocytic and the endocytic step are subject to regulation. Mathematical modeling (207) provided support of this possibility. Recent data, indeed, support the possibility that regulation of the endocytic step may be of importance. Brown and co-workers have proposed that the constitutive recycling (i.e., nonstimulated) may be enough to drive sufficient AQP2 to the plasma membrane and that the vasopressin-induced accumulation in the plasma membrane could potentially be due to inhibition of AQP2 endocytosis after vasopressin stimulation (205). Other studies also support regulation of exocytosis. Changes in membrane capacitance in primary cultured IMCD cells were determined, and it was shown that vasopressin and cAMP generated an increase in the number of exocytotic step-changes in capacitance, which indicates increased exocytosis (208). It is likely that both stimulation of AQP2 exocytosis and inhibition of AQP2 endocytosis may be involved in the vasopressin response. The relative roles of changes in these processes still remain to be established and, thus, additional studies are needed to establish whether vasopressin (*in vivo*) stimulate exocytosis or inhibit endocytosis of AQP2. Furthermore, it is, at present, still unknown if AQP2 is constitutively recycled in the collecting duct principal cells *in vivo*.

Involvement of the Cytoskeleton and Ca^{2+}. The coordinated delivery of AQP2-bearing vesicles to the apical part of the cell appears to depend on the translocation of the vesicles along the cytoskeletal elements. In particular, the microtubular network has been implicated in this process, since chemical disruption of microtubules inhibits the increase in permeability both in the toad bladder and in the mammalian collecting duct (209,210). Recently, it has been shown that dynein is present in the kidney of several mammalian species (for references see [211]), and that both dynein, and dynactin, a protein complex believed to mediate the interaction of dynein with vesicles, associate with AQP2-bearing vesicles (211). Furthermore, both vanadate, a rather nonspecific inhibitor of ATPases, and EHNA, a relatively specific inhibitor of dynein, inhibit the antidiuretic response in toad bladder (212,213), and this has recently also been demonstrated in kidney collecting duct (214). Thus, it seems likely that dynein may drive the microtubule-dependent delivery of AQP2 bearing vesicles toward the apical plasma membrane.

Actin filaments also appear to be involved in the hydrosmotic response (215–220). Recently, evidence was provided that the myosin light chain kinase (MLCK) pathway, through calmodulin-mediated calcium activation of MLCK, leads to phosphorylation of MLCK and nonmuscle myosin 2 motor activity (221). Studies in isolated perfused rat IMCD's showed the MLCK inhibitors ML-7 and ML-9 reduce vasopressin-induced increase in IMCD water permeability (221). The results indicate that MLCK may be a downstream target for the vasopressin-induced Ca^{2+} signal. However, it may not be completely ruled out that the inhibitor concentrations might potentially also inhibit PKA and, thus, affect AQP2 trafficking through the cAMP pathway. Recently, the calcium-activated nonmuscle myosin 2b and the unconventional myosin Va and Vb isoforms have been localized to AQP2-containing collecting duct cells by immunohistochemistry and immunoblotting (222). Further studies are warranted to further investigate the potential role of myosins in AQP2 trafficking.

Role of Ca^{2+}. The intracellular Ca^{2+} concentration has been shown to increase upon stimulation of isolated and perfused rat inner medullary collecting ducts with vasopressin or dDAVP (223). These observations have been followed by a number of studies of the role of the calcium signal in isolated IMCDs and in cell cultures. In isolated perfused IMCDs, the calcium signal was not associated with an increase in IP_3 or activation of PKC (224). Vasopressin or 8,4-chlorophenylthio-cAMP also induced a marked increase in intracellular calcium in isolated perfused IMCD and BABTAAM blocked the rise in intracellular calcium and co-contaminantly lead to inhibition of the vasopressin-induced increase in tubular water permeability (225). Moreover, blocking calmodulin with the inhibitors W7 or trifluoperazine also inhibited the effect of vasopressin on tubular water permeability and inhibited the vasopressin-induced AQP2 plasma membrane targeting in IMCD cell primary cultures (225). Removing calcium from both bath and lumen in isolated perfused tubules did not affect the vasopressin-induced calcium signal, indicating that calcium was released from an intracellular source. This was further supported by the observations that ryanodine inhibited the calcium signal in perfused IMCDs and inhibited AQP2 accumulation in the plasma membrane in IMCD cell primary cultures. In addition, RyR1 ryanodine receptors were localized to rat IMCD by immunohistochemistry (225).

Ryanodine receptors are generally believed to mediate a positive feedback and release Ca^{2+} from intracellular stores in response to an initial rise in intracellular calcium (Fig. 3-5). Thus, it is likely that another mechanism is responsible for the initialization of the rise in intracellular Ca^{2+}. Further studies revealed that vasopressin or dDAVP elicited oscillations in intracellular calcium concentration in isolated perfused rat IMCD (226). In agreement with the results from Chou et al., BABTAAM and ryanodine inhibited the vasopressin-induced calcium increase. The results on isolated perfused IMCDs support the view that an intracellular increase in calcium concentration is an obligate component of the vasopressin response, leading to increased AQP2 abundance in the apical plasma membrane. It should be mentioned that studies on cultured IMCD cells failed to support this view (208) and further studies are warranted.

Signaling elicited by stimulation by dDAVP have, in general, been considered to be the result of V2-receptor stimulation, since dDAVP has been considered a V2-receptor specific agonist. This notion is based on the fact that dDAVP does not stimulate the V1a receptor, which is a linked to phospholipase C-mediated calcium signaling. However, dDAVP has been shown also to agonize the V1b receptor (227) and this has been proposed to explain the reports (228) of dual-signaling (calcium and cAMP) pathways for the V2 receptor in IMCD (229).

Vesicle Targeting Receptors. Vasopressin-stimulated apical membrane accumulation of AQP2 vesicles involves several steps (see recent review [204]). In brief, (i) translocation of vesicles from a diffuse distribution throughout the cell to the apical region of the cell, (ii) translocation of AQP2 across the apical part of the cell composed of a dense filamentous actin network, (iii) priming of vesicles for docking, (iv) docking of vesicles, and (v) fusion of vesicles with the apical plasma membrane. Theoretically, each of these steps could be target for regulation by vasopressin (230). In the final three steps in AQP2 vesicle trafficking—vesicle priming, docking with the plasma membrane, and fusion with the plasma membrane—substantial evidence point to a role of vesicle targeting receptors, the so-called "SNARE" proteins. These proteins have been identified chiefly in the context of regulated exocytosis of synaptic vesicles (231,232). The SNARE proteins have been proposed to mediate specific interaction between given vesicle

and its target membrane with which it is destined to fuse. One class of targeting receptors in the vesicles (v-SNARES) has been called "VAMPs" (or "synaptobrevins"). Another class of targeting receptor found predominantly in the target membrane (t-SNARES) is the syntaxins. The third family of SNARE proteins is the SNAP-25-like proteins. These were initially thought to function as t-SNAREs, but are now recognized to reside in translocating vesicles as well as the target membrane. Members of all three of these families have been found in collecting duct cells with a subcellular localization consistent with a role in AQP2 vesicle exocytosis. First, VAMP-2 has been shown to be present in AQP2 vesicles and to be virtually absent from the apical membrane of principal cells of the inner medullary collecting ducts (233). Similar evidence for an intracellular localization of VAMP-2 in collecting duct cells has been provided in other studies (234–236). Second, syntaxin-4 has been localized to the apical plasma membrane of collecting duct principal cells (236). Finally, SNAP-23 has been found to be present in both the apical plasma membrane of principal cells and in AQP2 vesicles (237). These three proteins may potentially form a complex with the *N*-ethylmaleimide-sensitive factor (NSF). Studies in AQP2-transfected cells and by use of proteases have provided functional evidence for SNAREs in AQP2 trafficking (238).

Signaling Pathways Involved in Collecting Duct Water Permeability

The signal transduction pathways have been described thoroughly in previous reviews (230). Cyclic AMP (cAMP) levels in collecting duct principal cells are increased by binding of vasopressin to V2 receptors (239,240). The synthesis of cAMP by adenylate cyclase is stimulated by a V2 receptor-coupled heterotrimeric GTP-binding protein, G_s. G_s interconverts between an inactive GDP-bound form and an active GTP-bound form, and the vasopressin–V2 receptor complex catalyzes the exchange of GTP for bound GDP on the α subunit of G_s. This causes release of the α subunit, $G_{s\alpha}$-GTP, which, subsequently, binds to adenylate cyclase, thereby increasing cAMP production (Fig. 3-5A). Protein kinase A (PKA) is a multimeric protein, which is activated by cAMP, and consists in its inactive state of two catalytic subunits and two regulatory subunits. When cAMP binds to the regulatory subunits, these dissociate from the catalytic subunits, resulting in activation of the kinase activity of the catalytic subunits.

AQP2 contains a consensus site for PKA phosphorylation (RRQS) in the cytoplasmic COOH terminus at serine 256 (9). Recent studies using ^{32}P labeling or using an antibody specific for phosphorylated AQP2 (see below) showed a very rapid phosphorylation of AQP2 (within 1 min) in response to vasopressin treatment of slices of the kidney papilla (241). This agrees well with the time course of vasopressin-stimulated water permeability of kidney collecting ducts (242). It was recently demonstrated that vasopressin or forskolin treatment failed to induce translocation of AQP2 when serine-256 was substituted by an alanine (S256A) in contrast to a significant regulated trafficking of wild-type AQP2 in LLC-PK1 cells (201,243). Thus, these studies indicate a specific role of PKA-induced phosphorylation of AQP2 for regulated trafficking. Recent studies using antibodies that specifically recognize phosphorylated AQP2 are consistent with this view (244). However, recent evidence suggests that at least three of the subunits in each tetramer need to be phosphorylated in order to be transported to the plasma membrane (Fig. 3-7) (245). Studies have also pointed to a critical role of calcium (see above) and of cGMP (246), which may be of importance in understanding the effect of ANP. Other hormones may also influence AQP2 expression and trafficking, for example, insulin, PGE2, dopamine, and others (247,248).

Long-Term Regulation of Urinary Concentration

It has been known for many years that chronically water-loaded patients, such as those with compulsive polydipsia, have a markedly impaired maximal urinary concentrating capacity (174). Conversely, prolonged dehydration or fluid restriction resulted in an increased maximal concentrating capacity (175,176). These results have been confirmed in rat models (11,91,93,230,249,250). Such studies have demonstrated that the total amount of AQP2 present in the kidney also decreases during over-hydration and increases after dehydration. There is a rapid change in AQP2 mRNA expression, followed more slowly by protein levels (104,251–253), suggesting that the long-term alterations in concentrating capacity are due to changes in total AQP2 protein levels, which are caused in turn by changes in gene transcription.

Water Intake and Thirst

Thirst is a subjective sensory impression that activates the desire to drink water. In normal persons, a significant water deficit manifests itself promptly by thirst and, if access is unrestricted, water intake increases and continues until thirst is abated and the deficit is corrected. Thirst is not the sole determinant of water intake and, in fact, it appears to be a dominant driving force in the maintenance of water balance only under exceptional circumstances. Water intake of most terrestrial mammals, including humans, is usually greater than the minimum required for the maintenance of water balance. Therefore, urine is usually less than maximally concentrated. A definite diurnal variation in the water intake is also observed, since most drinking occurs intermittently at specific times of day, usually in association with eating (254).

Hyperosmolar Thirst

An increase in the effective osmolality of the extracellular fluid may be produced either by water deprivation or by administration of a hypertonic solution of a solute, such as sodium chloride, that is effectively excluded from the intracellular compartment. In both cases, cellular dehydration is produced and, if the increase in osmolality is sufficiently large, a sensation of thirst is experienced. Since urea freely traverses cell membranes, it does not alter the effective osmolality of the extracellular fluid and an increase in urea concentration, as in acute or chronic renal failure, does not cause cell shrinkage or directly stimulate the sensation of thirst. It is thus reasonable to suggest that the mechanism for this type of dipsogenic stimulation is dehydration of osmoreceptors that activate neural pathways, leading to the sensation of thirst. Hypothalamic centers that respond to locally produced increases in sodium concentration, osmolality, or both, and lead to drinking have been described (255,256). These osmoreceptors appear to be discrete from those responsible for osmotic control of antidiuretic hormone (ADH) release (167). Thirst and antidiuresis, ordinarily simultaneous responses to hyperosmolality, may, on occasion, be dissociated in disease states, while normal responses to isotonic volume contraction are retained.

The effective osmolality of the body fluid is narrowly regulated in health within an average range of approximately 285 to 290 mOsm/kg. In this range, a healthy person does not experience thirst and water balance in the face of small variations in osmolality is maintained by appropriate changes in urine volumes and concentration. The urine volume, in turn, is modulated by changes in the circulating level of ADH, which is delicately tuned to plasma osmolality (see the following

discussion). When the plasma osmolality reaches approximately 295 mOsm/kg, the circulation level of ADH is sufficient to cause excretion of a minimal volume of maximally concentrated urine. It is at this point, when no further renal compensation is possible, that thirst first appears (170) (Fig. 3-1). The sensation of thirst does not abate if dehydration is sustained, but remains a powerful drive until the hyperosmolar state is corrected.

Regulation of AQP2 and AQP3 Expression

There is good evidence that expression of AQP3, the major basolateral water channel in the cortical and outer medullary collecting duct is regulated by vasopressin (24). In contrast, the expression of AQP4, the major basolateral water channel in the inner medullary collecting duct, does not appear to be regulated (25,26,257), although one study found parallel changes in AQP2, 3, and 4 mRNA with changes in hydration (258). The expression of the major water channel found in the proximal tubule, AQP1, also appears to be unaffected by changes in hydration status (25,26,257), although levels of AQP1 are markedly reduced in some pathologic conditions (259–261), as discussed below.

Signaling Pathways Involved in Regulation of AQP2 Expression

There is clear evidence that vasopressin stimulates AQP2 expression. Brattleboro rats lack endogenous vasopressin and are profoundly polyuric (262). AQP2 expression is about two to one-third of that found in their parent strain (Long Evans) (93,263,264). Chronic infusion of vasopressin via osmotic minipumps for 5 days both reversed their polyuria and increased their AQP2 expression to that of the normal animals (93). As described above, regulation of both trafficking and expression of AQP2 is regulated by vasopressin (Fig. 3-6). Recent studies have indicated that higher cAMP levels are necessary to stimulate trafficking than to induce AQP2 expression (264).

Chronic vasopressin infusion also increases AQP2 expression in normal rats (265), while administration of V2 receptor antagonists decreases it (195,266), albeit only by about 50%. Experiments with diabetes insipidis (DI) +/+ mice, a strain with an activating mutation of cAMP phosphodiesterase (267) show severely reduced levels of AQP2, suggesting that cAMP acts as the second messenger for vasopressin stimulation of AQP2 expression, as well as shuttling (268). Consistent with this finding, there is known to be a cAMP-responsive element in the 5′-flanking region of the AQP2 gene (269–272).

Other factors than vasopressin are important for controlling AQP2 expression. Evidence for this comes from a variety of experiments, which show that AQP2 expression can be altered independent of the activity of vasopressin. One of the most clear-cut involved the so-called vasopressin-escape phenomenon (251,265,273). In these experiments, rats were given a chronic vasopressin infusion, but then given a water load mixed with their diet. This is accompanied by a striking fall in renal AQP2 levels, despite the continued high level of circulating vasopressin. This provided support to earlier evidence for a non-vasopressin-mediated effect on AQP2 levels, which was based on experiments with a lithium-induced nephrogenic DI (NDI) model (95,257). In these experiments, lithium produced a profound (>90%) downregulation of AQP2, in parallel with a massive polyuria. Treatment of lithium-NDI rats with high doses of dDAVP led to a marginal increase in AQP2 expression, whereas dehydration for 2 days caused a fivefold increase in AQP2 expression. Since the reduction of AQP2 expression caused by lithium treatment is much greater than can be obtained by the use of V2 receptor blockers, it seems likely that the downregulation of AQP2 expression during lithium treatment also appears to be partly via a vasopressin-independent pathway. Further evidence for this comes from the observation that lithium treatment also causes a profound downregulation of AQP2 in Brattleboro rats (264).

A range of other factors might provide stimuli for increased or decreased AQP2 expression, but these remain to be determined. One possible signal would be tubular flow itself, either by some mechanical process, or by altering the concentration of solute delivered to the tubule. However, the osmotic diuresis (glycosuria) seen with streptozotocin-induced diabetes mellitus is associated with a modest increase in AQP2 rather than a decrease, as seen in NDI models (274). In this case, urine osmolality remains high (about 1000 mOsm/Kg H_2O), because of the glucose, but tubular flow is markedly increased. Furthermore, furosemide, a loop diuretic, had no significant effect on AQP2 expression, despite causing a profound polyuria (266,275). This latter treatment will also result in washout of the medullary osmotic gradient, since loop diuretics inhibit salt uptake in the thick ascending limb of Henle's loop. Thus, the absence of any alteration in AQP2 levels implies that interstitial tonicity is not a major determinant of AQP2 expression in the inner medulla. However, a hypertonicity responsive element has been described in the 5′-flanking region of the AQP2 gene (276), and recent results using primary cultured papillary collecting duct cells revealed increased AQP2 expression in response to extracellular hypertonicity, indicating that regulation of AQP2 expression may indeed be regulated, in part, in response to changes in interstitial osmolality (277). Recent studies have demonstrated in cultured cells natively expressing AQP2 (277) that in collecting duct principal cells: (i) a short-term increase of extracellular osmolarity decreases AQP2 expression through inhibition of AQP2 gene transcription; (ii) a long-term increase of extracellular tonicity, but not osmolarity, enhances AQP2 expression via stimulation of AQP2 gene transcription; and (iii) long-term hypertonicity and PKA increases AQP2 expression through synergistic, but independent, mechanisms.

AQP2 expression may also provide the collecting duct cells with an enhanced ability for rapid activation of regulatory volume decrease mechanisms (278).

A surprising element concerns the role of food intake on AQP2 expression and urinary concentration. It was recently demonstrated that AQP2 expression is acutely reduced and urine output temporarily increased over the first 24 hours of starvation and, conversely, an increase is seen in AQP2 expression and reduced urinary output in response to prolonged fasting (279). Similar observations have also been observed in humans (280).

MECHANISMS OF URINE DILUTION AND CONCENTRATION VIA COUNTERCURRENT MULTIPLICATION

In this section we describe the basic mechanisms behind urine dilution and countercurrent multiplication for regulation of urinary concentration. The roles of AQP in these processes will be delineated, whereas it is outside the scope of this chapter to review the sodium and solute channels and transporters involved in these processes. The sites of tubule fluid concentration and dilution along the mammalian nephron have been demonstrated by micropuncture studies in rats and other rodents. These results are summarized in Figure 3-9.

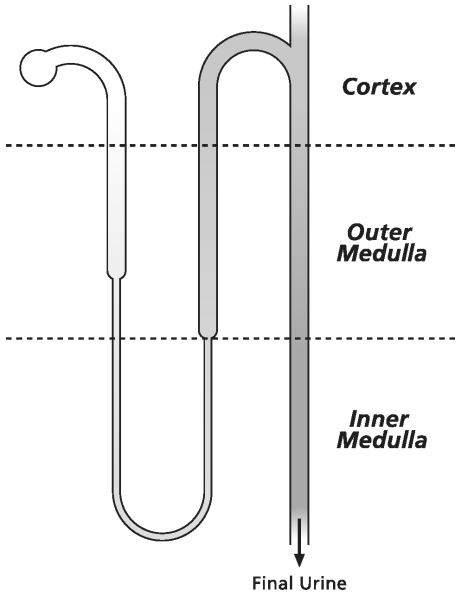

FIGURE 3-9. Typical osmolarities (in mOsm/kg H_2O) found in various sites along the renal tubule of a rat kidney. Fluid in the proximal tubule is always isosmotic with plasma (290 mOsm/kg H_2O). Fluid emerging from the loop of Henle (entering the early distal tubule) is always hypotonic. Osmolality in the distal tubule increases to plasma level only during antidiuresis. Final urine is hypertonic when the circulating vasopressin level is high and hypotonic when the vasopressin level is low. A high osmolality is always maintained in the loop of Henle and vasa recta. During antidiuresis, osmolalities in all inner medullary structures are nearly equal. Osmolalities are somewhat attenuated in the loop of Henle during water diuresis (not shown). (Based on micropuncture studies by Wirz H. Der osmotische Druck in den corticalin Tubuli der Ratten niere. *Helv Physiol Pharmacol Acta* 1956;14:353; Gottschalk CW, Mylle M. Micropuncture study of the mammalian urinary concentrating mechanism: evidence for the countercurrent hypothesis. *Am J Physiol* 1959;196:927; Jamison RL, Buerkert J, Lacy F. A micropuncture study of collecting tubule function in rats with hereditary diabetes insipidus. *J Clin Invest* 1971;50:2444, with permission.)

Mechanism of Tubule Fluid Dilution

Micropuncture measurements in rats have revealed that the hypotonicity of the fluid in the early distal tubule is chiefly due to a low luminal NaCl concentration (281). In theory, a low NaCl concentration could result from active NaCl absorption from the loop of Henle or water secretion into the loop. However, micropuncture studies using inulin as a volume marker demonstrated net water absorption from the superficial loop of Henle during antidiuresis (282), which rules out the possibility of water secretion as a mechanism of tubule fluid dilution.

Studies of isolated perfused thick ascending limbs of loops of Henle from rabbits have directly demonstrated the mechanism of dilution (283,284). NaCl is rapidly absorbed by active transport; this lowers the luminal NaCl concentration and osmolality to levels less than those in the peritubular fluid. The osmotic water permeability is low, which prevents dissipation of the transepithelial osmolality gradient by water fluxes. Consistent with this, no AQPs have been found to be expressed in the TAL.

The distal convoluted tubule (DCT), a short renal tubule segment that follows the cortical thick ascending limb, probably also contributes to renal tubule diluting ability. Like the thick ascending limb, it carries out active NaCl absorption at a relatively rapid rate (285). Limited studies indicate that water permeability is low (286). Indeed, thus far, none of the known AQP water channels has been localized to the DCT (287), further supporting the view that its epithelial water permeability is quite low. Thus, luminal dilution in the DCT may be achieved by a mechanism similar to that in the cortical thick ascending limb.

The hypotonicity of tubule fluid is maintained throughout the distal tubule and collecting duct system during water diuresis, abetted by the low osmotic water permeability of the collecting ducts when circulating levels of vasopressin are low. Although the dilute state is sustained in the collecting ducts, the solute composition of the tubule fluid is modified in the collecting duct system, chiefly by Na^+ absorption and K^+ secretion. Active NaCl reabsorption by the collecting ducts is presumably responsible for the further dilution of the collecting duct fluid beyond that achieved in the thick ascending limbs (288).

Mechanism of Tubule Fluid Concentration

During antidiuresis, extensive net water absorption occurs between the late distal tubule and the final urine (i.e., in the collecting ducts) (282). Measurements along the IMCDs of antidiuretic hamsters directly demonstrated that water is absorbed in excess of solutes, with a resulting rise in osmolality along the collecting ducts toward the papillary tip (289). Thus, the collecting duct fluid is concentrated chiefly by water absorption.

The osmotic driving force for water absorption along the collecting ducts is present because of the existence of an axial osmolality gradient in the medullary tissue, with the highest degree of hypertonicity at the papillary tip. Such an osmolality gradient was initially demonstrated in a classic study by Wirz and colleagues (290). These investigators used an ingenious microcryoscopic method to measure the osmolality in the lumens of individual renal tubules in tissue slices from quick-frozen rat kidneys. The measurements revealed that in antidiuretic rats, there was a continuous osmolality gradient throughout the medullary axis, including both the outer medulla and the inner medulla, with the highest osmolality in the deepest part of the inner medulla, the papillary tip. Furthermore, in the medulla, the osmolality was about as high in the large tubules (presumably collecting ducts) as in the small tubules (presumably loops of Henle); this demonstrates that the high tissue osmolality was not simply a manifestation of a high osmolality in a single structure, namely, the collecting duct. Consistent with this view, Wirz (291) demonstrated by micropuncture that the osmolality of vasa recta blood, sampled from near the papillary tip in antidiuretic hamsters, was virtually equal to that of the final urine. Later, Gottschalk and Mylle (292), using micropuncture in antidiuretic hamsters, confirmed that the osmolality of the fluid in the loops of Henle, the vasa recta, and the collecting ducts was approximately the same, in support of the view that the collecting duct fluid is concentrated by osmotic equilibration with a hypertonic medullary interstitium. Subsequently, it was demonstrated in studies *in vitro* that collecting ducts have a high water permeability in the presence of vasopressin (293,294), as is required for osmotic equilibration.

The axial osmolality gradient in the renal medulla is made up of gradients of several individual solutes. However, the principal solutes responsible for the osmolality gradient are NaCl and urea (295,296).

Role of Vasopressin

Water excretion is regulated by vasopressin largely as a result of its effect on the water permeability of the collecting ducts. When the water permeability is low in collecting ducts, because of a low circulating level of vasopressin, relatively little water is absorbed in the collecting ducts. The dilute fluid exiting the

loops of Henle remains dilute as it passes through the collecting duct system, yielding a large volume of hypotonic urine. When the water permeability of the collecting ducts is high because of a high circulating level of vasopressin, water is rapidly reabsorbed along the collecting duct system by osmosis, drawn by the osmolality gradient between the lumen and the peritubular interstitium. The osmolality of the final urine approaches that of the inner medullary interstitium, which results in a small volume of hypertonic urine.

Micropuncture studies have demonstrated that the late distal tubule is the earliest site along the renal tubule at which water absorption increases during antidiuresis (291). Recognizing that the distal tubule, as defined by micropuncturists is heterogeneous, Woodhall and Tisher (297) showed that only the late-portion distal tubule responds to vasopressin, corresponding to the initial collecting tubule. In addition, recent studies have demonstrated that both AQP2 and the V2-vasopressin receptor are expressed in the connecting segment (57), suggesting that it is a site at which vasopressin regulates water permeability. In contrast, AQP2 expression was not observed in the DCT. Thus, among the segments making up the portion of the distal tubule accessible by cortical micropuncture (DCT, connecting segment, and initial collecting tubule), only the connecting segment and the initial collecting tubule exhibit vasopressin-regulated water transport.

The amount of water absorption in the connecting segment and initial collecting tubule required to raise tubule fluid to isotonicity is considerably greater than the additional amount required to concentrate the urine to greater than the osmolality of plasma in the medullary portion of the collecting duct system (73). Consequently, most of the water reabsorbed from the collecting duct system during antidiuresis enters the cortical labyrinth where the effective blood flow is high enough to return the reabsorbed water to the general circulation without diluting the interstitium. If such a large amount of water were absorbed along the medullary collecting ducts, it would be expected to have a significant dilutional effect on the medullary interstitium and impair concentrating ability.

During water diuresis, a corticomedullary osmolality gradient persists, although it is attenuated (296,298,299). In the absence of vasopressin, the water permeability of the collecting ducts is low, but not zero. Consequently, some water is absorbed by the collecting ducts during water diuresis. Most of the water absorption occurs from the terminal part of IMCDs, where the transepithelial osmolality gradient is highest and the basal water permeability is also highest (62,249). In fact, more water is absorbed from the terminal collecting ducts during water diuresis than during antidiuresis because of a much larger transepithelial osmolality gradient (300). A high rate of water absorption from the IMCDs is thought to contribute to the reduction of the medullary interstitial osmolality during water diuresis by its dilutional effect. The decrease in inner medullary tissue osmolality during water diuresis results largely from an increase in tissue water content (301,302) associated with the higher rate of water absorption from the collecting ducts, although reductions in the quantities of urea and NaCl in the medullary tissue have also been documented.

Aside from its effect on the water permeability in the collecting duct system, vasopressin has additional effects that are important to the overall function of the concentrating mechanism. Vasopressin increases the urea permeability in the terminal part of the IMCD (294,303–305), thereby regulating the delivery of urea to the inner medullary interstitium. Vasopressin increases the rate of active NaCl absorption in the medullary thick ascending limb of the loops of Henle (306–308), enhancing countercurrent multiplication, the process responsible for the corticomedullary NaCl gradient. Vasopressin also causes a sustained increase in the rates of active NaCl absorption and fluid absorption in the rat cortical collecting duct (309–311) and distal tubule (presumably the initial collecting tubule [312]). This effect helps shift water absorption to the earliest parts of the collecting duct system during antidiuresis, which maintains the bulk of water absorption in the renal cortex where the rapid blood flow can return it to the general circulation. Finally, vasopressin increases the rate of K^+ secretion in the rat cortical collecting duct (311) and distal tubule (312,313). Field et al. (313) have postulated that this action balances the decrease in K^+ secretion that would occur because of the decreased rate of tubule fluid flow during antidiuresis. Thus, stimulation of K^+ secretion by vasopressin would tend to maintain a nearly constant rate of K^+ secretion in the early part of the collecting duct system and help stabilize the excretion of K^+ in the urine when water excretion changes.

Countercurrent Multiplication

The process of urine concentration consists of two relatively independent components: (i) countercurrent multiplication, which generates a hypertonic medullary interstitium, and (ii) osmotic equilibration of the tubule fluid in the medullary collecting ducts with the hypertonic medullary interstitium to form a hypertonic final urine. In this section, we discuss countercurrent multiplication.

The concept of countercurrent multiplication originally evolved from a consideration of industrial processes that separate and concentrate economically useful products (e.g., countercurrent extraction and distillation). In these processes, a single stage (given the appropriate energy input) is capable of modest concentration of one component. However, the effect of a single stage ("single effect") can be multiplied by successive applications of the effect. Werner Kuhn and his colleagues (314–316) used this concept to provide an explanation for the corticomedullary osmolality gradient in the renal medulla.

Kuhn and colleagues recognized that it was a simple matter to extend the countercurrent multiplier scheme involving several discrete stages to a continuous-flow scheme in which discrete stages are replaced by ascending and descending streams whose interaction is distributed uniformly throughout their lengths. A small concentration difference between the counterflowing descending and ascending streams could result in a large axial concentration gradient. The development of the concept by Kuhn and colleagues (314,316) that such a continuous countercurrent scheme could explain urine concentration was a landmark event in renal physiology.

A continuous countercurrent multiplier is capable of producing a small volume of concentrated output, which, in theory, could be withdrawn from the bend of the hairpin loop. However, Hargitay and Kuhn (314) recognized that a more realistic scheme would include a third tube (a collecting duct) that equilibrates osmotically with the loop fluid to produce a concentrated output. Such a scheme has the advantage that it can concentrate solutes in the collecting duct other than those responsible for the axial osmolality gradient in the loop. The volume flow into the collecting duct must be considerably less than in the loop for a significant overall concentrating effect to be maintained.

It is now generally accepted that the axial osmolality gradient in the outer medulla is generated by countercurrent multiplication driven by active NaCl transport in the thick ascending limbs. However, the explanation for the axial osmolality gradient in the inner medulla is not yet known. The inner medullary ascending limb (thin ascending limb) has little or no capacity for active NaCl transport (294,317–319); this apparently rules out a countercurrent multiplication process identical to that in the outer medulla. Several other theories have been proposed to explain the axial solute concentration gradient in the inner medulla, none of which has become generally accepted.

URINARY CONCENTRATING DEFECTS

There are a variety of disorders in which renal water handling is disturbed. Some of these disorders are owing to primary renal disorders, while others reflect changes in other organs or systems. However, the renal handling of water is considered abnormal in all of them, since there is a breakdown of the body's normal homeostatic mechanisms. Over the past decade, the role of changes in the expression and/or function of AQPs has been investigated in a range of these conditions, including genetic or acquired defects showing a decreased renal responsiveness to vasopressin (acquired nephrogenic diabetes insipidus), and conditions in which there is an inappropriate retention of water. The importance of AQP playing an essential role for regulation of renal water balance has been established (see Table 3-2).

INHERITED CENTRAL DIABETES INSIPIDUS AND NEUROGENIC DIABETES INSIPIDUS

There are two significant inherited forms of diabetes insipidus (DI): central and nephrogenic. In central (or neurogenic) DI, there is a defect of vasopressin production. CDI is rarely hereditary in man, usually occurring as a consequence of head trauma or diseases in the hypothalamus or pituitary gland. However, the Brattleboro rat provides an excellent model of this condition. These animals have a total or near-total lack of vasopressin production (320). Consequently, Brattleboro rats have substantially decreased expression levels of vasopressin-regulated AQP2 than those in the parent strain (Long Evans), and the deficit was reversed by chronic vasopressin infusion, suggesting that patients lacking vasopressin are likely to have decreased AQP2 expression (93). The subsequent work showing that expression of AQP3 is also regulated by vasopressin implies that the expression levels of these water channels will also be decreased in patients with CDI. The most important denominator is the absence of AQP2 trafficking to the apical membrane. These deficits are likely to be the main causes of the polyuria from which these patients suffer, which will be reversed by the treatment given for their condition (desmopressin).

The second form of DI is called nephrogenic DI (NDI) and is caused by the inability of the kidney to respond to vasopressin stimulation. The most frequent hereditary cause is a mutation of the V2 receptor, making the collecting duct cells insensitive to vasopressin. This gene is found on the X chromosome in man and shows the classic expected pattern, with males affected much more often while females are usually asymptomatic carriers, because they have inherited a normal gene from their fathers. Although there is no direct evidence, it is likely that this form of NDI will be associated with decreased expression of AQP2, since the cells are unable to respond to circulating vasopressin. This will compound the lack of AQP2 trafficking. Consistent with this, urinary AQP2 levels are very low in patients with X-linked NDI (321,322). However, since the amount of AQP2 in the urine appears to be determined largely by the response of the collecting duct cells to vasopressin (323) rather than their content of AQP2, the data must be interpreted with caution with respect to predicting AQP2 expression levels. Rather, this reflects the absence or decrease of AQP2 trafficking and, hence, less urinary excretion.

In approximately 95% of the cases, congenital NDI (CNDI) is X-linked and caused by mutations in the AVPR2 gene (324). Mutations in the AQP2 water channel gene account for the remaining 5% of cases of CNDI. The V2-receptor protein consists of 371 amino acid residues and comprises seven transmembrane, four extracellular, and four cytoplasmatic domains (325–327). The gene encoding the protein is located on the X-chromosome (Xp28) (328) and comprises three exons and two introns. More than 155 mutations in the vasopressin receptor gene have been recognized to cause CNDI. Functional analysis has been carried out for more than 79 (329) of these mutations. The analyses have revealed four different types of mutant receptors. The most common being impaired intracellular trafficking, which is seen in up to 70% of cases (329). The remaining types include reduced ligand binding capacity, failure to generate cAMP, and defects in the synthesis of stable mRNA (330). Of the 156 known mutations, only 5 cause partial CNDI (D85N, R104C, G201D, P322S, and S329R).

Less commonly (about 5% to 10% of hereditary NDI), there is a genetic defect in the AQP2 gene. In most cases, this results in an autosomal recessive condition (94,331), although a few cases have been reported that show an autosomal dominant inheritance (332). It is thought that the recessive forms on NDI are due to mutations in which either the mutant protein is unable to form tetramers with the normal form, or in which the normal protein functions normally. In contrast, in the dominant cases, it has been shown that heterotetramers between the mutant and normal forms can be created, but that they are unable to travel to the plasma membrane (333). Thus, the mutant protein can prevent the function of the normal form.

Of the 19 mutations so far reported, about one-half are found in the B or E loops of the protein, where it is likely that they will destroy the pore function of the molecule. The remainder are scattered throughout the molecule, although three occur together on the C loop. This is consistent with a previous report suggesting that this region of the molecule is closely associated with the channel (334).

Most mutations in AQP2 result in impaired trafficking to the plasma membrane, although some of the mutants will act as functional water channels (333,335). This has led to the idea that it may be possible to treat patients with such mutations by using chemical chaperones to increase the number of channels, which the cell can package successfully and transport to the surface. Such methods have been used successfully in cultured cells transfected with mutant AQPs (336), but much work remains to be done before such methods are available clinically.

The literature on pharmaceutical treatment of CNDI is scarce and the studies available are based upon small numbers of patients and are not randomized controlled trials. Furthermore, as no single treatment modality has been shown to be effective, various combinations of therapies have been proposed. There is still no combination of treatments that has been shown superior to others. A basic principle in reducing urine output is salt restriction combined with hydrochlorothiazide diuretics (2 to 3 mg/kg/Da in two daily doses). The suggested mechanism behind this treatment is compensatory increased proximal sodium reabsorption secondary to a negative sodium balance and volume contraction. This regimen, however, is associated with significant compliance problems because of the difficulties to comply with severe salt restriction, especially in children. Further addition of amiloride (0.3 mg/kg/Da or 20 mg/1.73 m^2/Da in three daily doses) to this regimen has been advocated by some (337) with the proposed benefits of an additive effect on diuresis and a reduced need for potassium supplements. To this regimen, prostaglandin synthetase inhibitors, such as indomethacin, has been shown to further increase efficacy in terms of diuresis (338,339).

Recent information indicated that cGMP mediated trafficking of AQP2 (246). Moreover, dDAVP treatment of Brattleboro rats caused a marked redistribution of phosphorylated AQP2 to the apical plasma membrane, which is in agreement with an important role of PKA phosphorylation in this trafficking (244). Conversely, treatment with V2-receptor antagonist

induced a marked decrease in expression of phosphorylated AQP2 (244) likely to be because of either reduced PKA activity and/or increased dephosphorylation of AQP2, for example, by increased phosphatase activity. In addition, it is now known that AQP2 is trafficked in response to an elevation of cGMP, which is stimulated by NO (246).

An interesting form of NDI with no known human parallel occurs in the DI +/+ severe mouse. These animals have an activating mutation of cAMP-phosphodiesterase, which breaks down cAMP in the collecting duct principal cells. As a consequence, these animals are unable to increase the intracellular cAMP levels in response to vasopressin and, hence, are unable to establish an antidiuretic response compared with controls. They have greatly reduced levels of AQP2 protein and mRNA, as well as AQP3 protein (268), consistent with the hypothesis that the regulation of the expression of these water channel proteins is at least partly driven by vasopressin acting via the production of cAMP.

Numerous other genetic defects are associated with urinary concentrating defects which are secondary to the failure of other transport processes. For example, Bartter's syndrome and Gitelman's syndromes are both caused by defective NaCl uptake in the thick ascending limb and DCT, respectively (340–343). Both conditions cause hypokalemia, which is known to be associated with NDI. Impaired reabsorption of sodium and chloride in the loop of Henle will also cause a urinary concentrating defect because of a reduced medullary osmotic gradient. Although humans lacking functional AQP1 do not have any gross functional disturbance (84), the results from AQP1 knockout mice suggests that during dehydration, a concentrating defect was revealed because of impaired function of the loop of Henle caused by reduced water permeability in the descending limb (85). Water handling by the vasa recta is also impaired (89,344), which will result in the carriage of more water down into the inner medulla and the washout of more salt. Both of these events will further impair the countercurrent multiplication process and, hence, prevent the formation of concentrated urine. Presumably, water reabsorption in the proximal tubule is also less efficient, since equilibration of water across the tubule epithelium will be slower in the absence of AQP1. Defects in other proximal tubule transporters (Fanconi syndrome) can also cause modest polyuria, but the effects of such changes on AQP expression and function have not yet been reported.

ACQUIRED NEPHROGENIC DIABETES INSIPIDUS

Acquired NDI is the consequence of numerous conditions (Table 3-2), characterized by an increased urine output and reduced urine osmolality, despite elevated levels of AVP. The role of vasopressin-regulated AQP2 has been established in a number of rat models with NDI. In many of these conditions, the kidney is unable to handle water because of an impaired responsiveness to vasopressin. In addition to NDI, a few other serious conditions are associated with reduced AQP2 levels (see Table 3-2).

Lithium-Induced Nephrogenic Diabetes Insipidus

Lithium has widely been used for treating bipolar affective disorders in human patients (345). However, lithium treatment is associated with a variety of renal side effects, including nephrogenic diabetes insipidus (NDI, i.e., a pronounced vasopressin-resistant polyuria and inability to concentrate urine) (95,257,346), increased urinary sodium excretion (347), and distal renal tubular acidosis (348). Patients who have been treated with lithium manifest a slow recovery of urinary-concentrating ability when treatment is discontinued. It is estimated that 1 in 1000 of the population receive lithium, and roughly 20% to 30% of these patients develop serious side effects, including polyuria (346,349). AQP2 and AQP3 levels were progressively reduced to approximately 5% of levels in control rats after 4 weeks of lithium treatment (95,257). The downregulation of AQP2 was paralleled by a progressive development of severe polyuria (95,257). Quantitative immunoelectron microscopy of AQP2 labeling in the inner medullary collecting duct principal cells showed that there was a reduction of AQP2 in the apical plasma membrane, as well as in the basolateral plasma membrane and intracellular vesicles. The reduced AQP3 expression was also demonstrated by immunocytochemistry (257). Thus, downregulation of both AQP2 and AQP3 appears to play a significant role for the development of lithium-induced polyuria. Reduction in AQP2 (and AQP3) expression may be induced by a lithium-dependent impairment in the production of cAMP in collecting duct principal cells (346), indicating that inhibition of cAMP production may, in part, be responsible for the reduction in AQP2 expression, as well as the inhibition of targeting to the plasma membrane in response to lithium treatment. This is consistent with the presence of a cAMP-responsive element in the 5′-untranslated region of the AQP2 gene (271,272) and with the demonstration that mice with inherently low cAMP levels have low expression of AQP2 (DI +/+ severe mouse) (268).

Lithium treatment is also associated with a concomitant increase in urinary sodium excretion, which is likely to play a role in the polyuria. A recent study demonstrated that chronic lithium treatment induces a marked decrease in protein abundance of epithelial sodium channel β- and γ-ENaC in the cortex and outer medulla, whereas the other renal sodium transporters upstream from the connecting tubule are unchanged (347). This was also revealed by immunocytochemistry showing an almost complete absence of β-ENaC and γ-ENaC labeling in cortical and outer medullary collecting duct. The results suggest a reduced responsiveness to aldosterone and vasopressin in these specific renal tubule segments and the dysregulation of ENaC subunits is likely to play a role in the development of natriuresis and partly in the decreased urinary concentrating ability in rats with lithium-induced NDI.

Hypokalemia- and Hypercalcemia-Induced Nephrogenic Diabetes Insipidus

It is known that both hypokalemia and hypercalcemia, clinically common electrolyte abnormalities, are associated with polyuria because of a vasopressin-resistant urinary concentrating defect. Recently, part of the underlying molecular defects involved in the development of the polyuria was described. Potassium-deficient diet for in rats for 4 days induced a significant hypokalemia that was associated with downregulation of AQP2 expression (275,350,351) and polyuria. Moreover, hypercalcemia induced in rats by oral treatment with dihydrotachysterol for 7 days produced a urinary concentrating defect and polyuria, which was also associated with downregulaion of AQP2 (350,351). Thus, both hypokalemia and hypercalcemia are associated with downregulation of AQP2 expression and immunolocalization studies of AQP2 demonstrated similar features. In addition to the downregulation of AQP2, expression of Na-K-2Cl cotransporter in the thick ascending limb was decreased in both conditions (352,353), suggesting that reduced sodium and chloride reabsorption in the thick ascending limb and, hence, decreased medullary hyperosmolality could also

partly contribute to the polyuria and decreased urinary concentration in hypokalemia and hypercalcemia.

Urinary Tract Obstruction-Induced Nephrogenic Diabetes Insipidus

Urinary tract obstruction is a serious clinical condition seen both in children and adults, associated with complex changes in renal function involving marked alterations in both glomerular and tubular function. Bilateral urinary tract obstruction (BUO) may result in long-term impairment in the ability to concentrate urine (354). BUO for 24 hours in rats is associated with markedly reduced expression of AQP2, -3, -4, and -1 (261,355,356). In addition, BUO is associated with marked downregulation of key sodium transporters and urea transporters (357,358). Following release of the obstruction, there is a marked polyuria during which period AQP2 and -3 levels remain downregulated up to 2 weeks after release, providing an explanation at the molecular level for the observed postobstructive polyuria. Moreover, it was demonstrated that AQP1 expression levels were long-term downregulated up to 30 days after release of obstruction in whole-kidney samples, indicating that reduced AQP1 expression contributes to the long-term reduced urinary-concentrating capacity seen after release of BUO. Changes in AQP1 expression in the descending limb of Henle's loop (DTL) may directly affect urinary-concentrating capacity and, importantly, AQP1 expression levels were significantly downregulated 14 days after release of BUO (355). These findings are consistent with the demonstration that AQP1 knock-out mice have a severe urinary-concentrating defect, a decreased transepithelial water permeability in the proximal tubule (PT) and DTL of Henle's loop, and defective fluid absorption (85–87). In a number of studies, BUO has been demonstrated to be associated with COX-2 induction and cellular infiltration of the renal medulla (359). Using specific COX-2 inhibition to rats subjected to BUO, it was demonstrated that this treatment prevents downregulation of AQP2 and several sodium transporters located to the proximal tubule and MTAL (359). Moreover, specific inhibition of the AT1 receptor to rats subjected to BUO prevented downregulation of $NaPi_2$ in the PT, Na-K-2Cl in the MTAL, and AQP2 in the collecting duct 3 days after release of BUO (360), confirming that the renin–angiotensin system plays an important role for the pathophysiologic changes in urinary tract obstruction.

In contrast to BUO conditions, unilateral ureteral obstruction is not associated with changes in the absolute excretion of sodium and water, since the nonobstructed kidney compensates for the reduced ability of the obstructed kidney to excrete solutes. In order to examine whether the previously identified reduction in the expression of aquaporins and sodium transporters in response to bilateral ureteral obstruction was caused by local factors (increased tissue pressure, changes in renal hemodynamics or parenchymal biochemistry) or systemic changes in the animal, experiments were performed to investigate the effects of unilateral ureteral obstruction for 24 hours (357,361). These studies demonstrated a profound downregulation of AQP1, -2, -3, -4, and pAQP2 levels in the obstructed kidney and a moderate reduction in AQP1 in the nonobstructed kidney suggesting that 1ocal factors play a major role, whereas systemic factors may induce a change in the nonobstructed kidney. Consistent with this, urine production was increased by 150% from the nonobstructed kidney. Additional experiments revealed that changes in AQP2 expression are reciprocal to the changes in solute-free water clearance demonstrating a functional association between these two parameters. The decrease in AQP2 expression in the nonobstructed kidney may participate in the increased urine output by decreasing solute-free water reabsorption, thus compensating for the loss of excretion from the obstructed kidney. These results support the view that local factors play an important role in the downregulation of AQP2 expression during obstruction, but the signals leading to this decrease remain to be determined. Moreover, the present studies demonstrated marked downregulation of key sodium transporters in the obstructed kidney coinciding with a marked reduction in the ability to reabsorb sodium. Thus these studies demonstrate a functional association between renal handling of water and sodium and the molecular protein changes along the nephron.

URINARY-CONCENTRATING DEFECTS IN RENAL FAILURE

Ischemia-Induced Acute Renal Failure

Renal failure, both acute and chronic, is associated with polyuria and a urinary-concentrating defect and, in both cases, there is a wide range of glomerular and tubular abnormalities that contribute to the overall renal dysfunction. Ischemia and reperfusion (I/R)-induced experimental acute renal failure (ARF) in rats is a model that is widely used. In this model, there are structural alterations in renal tubule, in association with an impaired urinary concentration. The proximal tubule (S3 segment) and thick ascending limb are known to be main sites of ischemic injury (362,363).

ARF is associated with defects both in collecting duct water reabsorption and proximal tubule water reabsorption, as well as defects in solute handling (362,364–366). In an isolated tubule microperfusion study of the rabbit kidney, it was observed that water reabsorption in the proximal tubule and cortical collecting duct was significantly reduced following ischemia (366). There are no differences in either basal or vasopressin-induced cAMP levels in outer or inner medulla in rats with ARF compared to sham-operated rats (367), supporting the view that there are defects in collecting duct water reabsorption. It is also well known that kidneys subjected to ischemic injury are unable to establish or maintain a high medullary solute content (368). Consistent with this observation, a decreased ability of the thick ascending limb to lower perfusate chloride ion concentration was observed (366). These studies, therefore, suggest that there are defects in both collecting duct water permeability and countercurrent multiplication in response to the ischemic damage.

Consistent with these findings, it has been demonstrated that AQP2 and -3 levels in the collecting duct, as well as AQP1 levels in the proximal tubule, are significantly reduced in response to ARF (259,369). The decreased levels of aquaporins were associated with impaired urinary concentration in rats with both oliguric or nonoliguric ARF.The reduced expression of AQP1-3 and the reduced urinary concentration capacity was significantly prevented by cotreatment with α-melanocyte-stimulating hormone (α-MSH), which is an anti-inflammatory cytokine that inhibits both neutrophil and nitric oxide pathways (259). This suggests that decreased levels of AQPs in both the proximal tubule and collecting duct in postischemic kidneys may play a significant role in the impaired urinary concentration. Recently it has also been demonstrated that hemorrhagic shock-induced ARF is associated with decreased expression of collecting duct water channel AQP2 and -3 (370). Moreover, erythropoietin treatment (single or combined with α-MSH) in rats with I/R-induced ARF, which is known to prevent caspase-3, -8, and -9 activation *in vivo* and reduces apoptotic cell death (371), prevents or reduces the urinary-concentrating defects and downregulation of AQPs expression levels (372).

Chronic Renal Failure

Patients with advanced chronic renal failure (CRF) have urine that remains hypotonic to plasma despite the administration of supramaximal doses of vasopressin (373). This vasopressin-resistant hyposthenuria specifically implies abnormalities in collecting duct water reabsorption in CRF patients. Consistently, Fine et al. (374) observed that isolated and perfused cortical collecting ducts dissected from remnant kidneys of severely uremic rabbits exhibited a significantly decreased water flux and adenylate cyclase activity in the response to vasopressin. Importantly, they demonstrated that the 8-bromo-cAMP failed to induce a normal hydrosmotic response in cortical collecting duct from remnant kidneys (374). The impaired urinary concentrating ability may, at least partly, be caused by impairment of vasopressin-stimulated water reabsorption in the collecting duct in CRF (375,376). As an extension of these observations, RT-PCR of total RNA from the inner medulla of CRF rat kidneys revealed virtual absence of V2 receptor mRNA (377). Thus, these studies provide firm evidence for significant defects in the collecting duct water permeability. Consistent with these observations, it has shown both decreased collecting duct water channel AQP2 and -3 expression and a vasopressin-resistant downregulation of AQP2 in a 5/6 nephrectomy-induced CRF rat model (260). In addition to the downregulation of AQPs in the collecting duct, a study showed markedly decreased expression of urea transporters UT-A1, UT-A2, and UT-B1 in the renal medulla of CRF rats (378). This could also partly contribute to the decreased urinary concentration in CRF.

STATES OF WATER RETENTION

Chronic Heart Failure

Severe chronic heart failure is characterized by defects in the renal handling of water and sodium resulting in extracellular fluid expansion and hyponatremia. An increased baroreceptor-mediated vasopressin release is believed to play a critical role for the renal water retention contributing to the development of hyponatremia. Two studies have looked at changes in renal AQP expression in rats with congestive heart failure (CHF) induced by ligation of the left coronary artery (15,16) to test if upregulation of AQP2 expression and targeting may play a role in the development of CHF. The studies demonstrated that the renal water retention in severe congestive heart failure in rats is associated with an upregulation of AQP2 expression levels in the collecting duct principal cells and a marked redistribution of AQP2 in the collecting duct principal cells with most channels located to the apical plasma membrane (15,16). Rats with severe heart failure had significantly elevated left ventricular end-diastolic pressures (LVEDP) compared with Sham-operated animals (26.9 ± 3.2 vs. 4.1 ± 0.3 mm Hg) and had reduced plasma sodium concentrations (15). These results may provide an explanation at the molecular level for excess solute-free water retention in severe CHF and for the development of hyponatremia.

It was demonstrated that plasma AVP levels are progressively increased following the severity of NYHA class in patients with congestive heart failure. Urinary excretion of AQP2 is correlated with plasma AVP levels, suggesting that increased urinary excretion of AQP2 is dependent on baroreceptor-mediated release of AVP in patients with CHF (379). In CHF, arterial underfilling, caused by decreased cardiac output or peripheral arterial vasodilation, leads to activation of the sympathetic nervous system and renin–angiotensin–aldosterone system in addition to the nonosmotic vasopressin release (18). A recent study demonstrated an effect of angiotensin II AT1 receptor blocker losartan treatment in CHF rats, which inhibits increased sodium reabsorption in the PT and Na-K-2Cl cotransporter in the thick ascending limb of Henle's loop and water reabsorption through AQP2 in the collecting ducts (380). Consistent with this interpretation, angiotensin II AT1 receptor blockade in dDAVP-treated rats is associated with decreased urine concentration and decreased AQP2 expression, compared with dDAVP-treated rats (381).

Hepatic Cirrhosis

Hepatic cirrhosis is another serious chronic condition associated with water retention. Several studies have examined the changes of renal AQPs expression in rats with cirrhosis induced by common bile duct ligation (CBDL) (382–384). The rats displayed impaired vasopressin-regulated water reabsorption despite normal plasma vasopressin levels. This was demonstrated by an impaired effect of a vasopressin V2-receptor antagonist OPC 31260 in cirrhotic rats. Consistent with this observation, semiquantitative immunoblotting showed a significant decrease of AQP2 expression in rats with hepatic cirrhosis (382,383). In addition, the expression levels of AQP3 and -4 were downregulated in CBDL rats, which may predict a reduced water permeability of the collecting duct in this model (382). In contrast, Fujita et al. (253) demonstrated that hepatic cirrhosis induced by intraperitoneal administration of carbon tetrachloride (CCl_4) was associated with a significant increase in both AQP2 protein levels and AQP2 mRNA expression. Interestingly, AQP2 mRNA levels correlated with the amount of ascites, suggesting that AQP2 may play a role in the abnormal water retention, followed by the development of ascites in hepatic cirrhosis (385). Another study using a model of CCl_4-induced cirrhosis demonstrated an increased

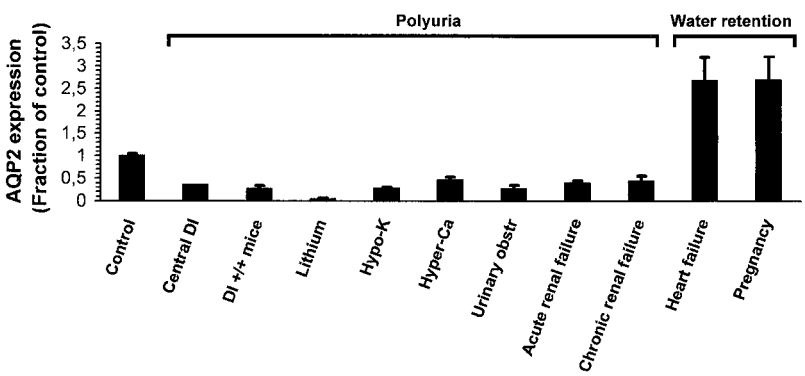

FIGURE 3-10. Changes in AQP2 expression seen in association with different water balance disorders. Levels are expressed as a percentage of control levels (*leftmost bar*). AQP2 expression is reduced, sometimes dramatically, in a wide range of hereditary and acquired forms of diabetes insipidus characterized by different degrees of polyuria. Conversely, congestive heart failure and pregnancy are conditions associated with increased expression of AQP2 levels and excessive water retention.

trafficking of AQP2 to the apical plasma membrane in parallel to the increase of plasma vasopressin level (386).

Although the differences between experimental groups induced by CBDL and CCl_4 remains to be determined, it is known that the dysregulation of body water balance depends on the severity of cirrhosis (387,388). CBDL results in a compensated cirrhosis characterized by peripheral vasodilation and increased cardiac output, whereas cirrhosis induced by chronic CCl_4 administration is associated with the late decompensated state of liver cirrhosis characterized by sodium retention, edema, and ascites (387). Thus, the downregulation of AQP2 observed in milder form of cirrhosis (i.e., a compensated stage without water retention) may represent a compensatory mechanism to prevent development of water retention. In contrast, the increased levels of vasopressin seen in severe "noncompensated" cirrhosis with ascites may induce an inappropriate upregulation of AQP2 that would, in turn, participate in the development of water retention.

Experimental Nephrotic Syndrome

The nephrotic syndrome is characterized by extracellular volume expansion with increased renal sodium and water reabsorption. The mechanisms of sodium and water retention can be expected to be associated with dysregulation of renal AQPs and sodium transporters. *In vivo* micropuncture studies in the unilateral model of puromycin aminonucleoside (PAN)-induced nephrotic syndrome have shown that sodium reabsorption is specifically increased in the collecting duct (389). Subsequently, a few studies have demonstrated that sodium retention in rats with PAN-induced nephrotic syndrome is correlated with increased activity and expression of Na,K-ATPase in the cortical collecting duct (390,391). Furthermore, rats with PAN-induced nephrotic syndrome have increased or sustained expression of ENaC subunits, along with an increased apical targeting of ENaC subunits in the late distal convoluted tubule, connecting tubule and collecting duct segments (392). This suggests that increased abundance and apical targeting of ENaC subunits in the distal nephron and collecting duct are likely to play a role in the development of sodium retention associated with PAN-induced nephrotic syndrome.

In contrast to CHF and liver cirrhosis, a marked downregulation of AQP2 and -3 expression was demonstrated in rats with PAN- and adriamycin-induced nephrotic syndrome (393,394). This reduced expression of collecting duct water channels could represent a physiologically appropriate response to extracellular volume expansion. The signal transduction involved in this process is not clear, but circulating vasopressin levels are high in rats with PAN-induced nephrotic syndrome. Thus, the marked downregulation of AQP2 in experimental nephrotic syndrome is similar to the downregulation of AQP2 in water-loaded dDAVP-treated rats, which would be an escape from the action of vasopressin (251,265).

Vasopressin Escape

Recently, Ecelbarger and colleagues (265) examined the mechanisms behind vasopressin escape, a conditions where the normal hydrosmotic response to vasopressin is suspended to prevent water intoxication. Rats were infused with dDAVP in osmotic minipumps that were either forced water loaded or allowed free access to water. Despite the fact that all rats had the same levels of circulating dDAVP (i.e., clamping of plasma antidiuretic hormone levels), the water-loaded rats had a dramatic downregulation of AQP2 and developed polyuria, compared to the antidiuretic control rats. This downregulation of AQP2 may, therefore, represent a physiologically appropriate way to reduce the capacity to reabsorb water (265). The signaling transduction pathways involved in the altered long-term regulation of AQP2 during vasopressin escape are at present unknown, but the study strongly suggest that a vasopressin-independent signaling pathway may be involved. Thus, the existence and potential importance of a vasopressin-independent signaling pathway (95) has gained considerable support (265).

Regulation of AQP2 by Female Sex Hormones

Pregnancy is characterized by a 30% to 50% increase in extracellular fluid, plasma, and blood volume in different mammalian species, including humans and rats (395). Other characteristic changes during pregnancy are arterial vasodilatation together with sodium and water retention and a decrease in plasma osmolality both in rats and humans. In parallel with the arterial vasodilation the renin–angiotensin–aldosterone system is activated and it has been suggested that the decrease in plasma osmolality is caused by resetting of the threshold for vasopressin secretion during pregnancy (396). Therefore, the hypothesis that AQP2 expression could be increased during pregnancy was tested in rats on days 7, 14, and 20 of pregnancy (397). Plasma osmolality, sodium, and body weight were consistently lower in pregnant rats and associated with this AQP2 mRNA and AQP2 levels significantly increased during pregnancy. Administration of the V2-receptor agonist suppressed the increase in both AQP2 mRNA and AQP2 despite normal levels of plasma vasopressin (397). Thus, this study suggests that the upregulation of AQP2 contributes to water retention during pregnancy in part through a V2 receptor-mediated effect, but AVP-independent factors (such as oxytocin) may also be important for this upregulation (398).

Urinary Biomarkers in Diseases with Urinary-Concentrating Defects

Urine provides an alternative to blood, plasma, and tissue as a potential source of disease biomarkers in kidney diseases and conditions associated with altered kidney function (399,400). Moreover, these diseases are often characterized by urinary excretion of distinct renal and extrarenal proteins and one urinary biomarker already exploited in clinical studies is AQP2. A number of studies have demonstrated that the response of urinary AQP2 excretion to dDAVP infusion is an index of vasopressin action on the kidney (322,323,401,402). Urinary excretion was increased during dehydration or vasopressin treatment and increased after hydration (323); CDI patients treated with vasopressin had increased urinary AQP2 excretion (321). Furthermore, it was shown that the amount of daily excretion of AQP2 in the urine was the same in men and women and urinary AQP2 content was not affected by age of the subjects (401). Importantly, urinary AQP2 showed a positive correlation with urine osmolality (401). The fraction of AQP2 excreted in the urine compared with whole kidney content was determined in rats and approximately 3% to 4% of AQP2 in the kidney was excreted daily (323,401). Thirsting or vasopressin treatment markedly increased urinary AQP2 excretion, whereas water loading suppressed AQP2 excretion supporting the view that urinary AQP2 excretion closely parallels changes in vasopressin action (323). There is strong evidence that urinary AQP2 excretion is mediated by an apical pathway, which is tightly regulated by vasopressin, since AQP3 located to the basolateral plasma membrane is undetectable (323). In a recent study using immunoelectron microscopy and nanospray liquid chromatography-tandem MS (LC-MS/MS) analysis of urinary membrane proteins, it was demonstrated that AQP2 and other

apical plasma membrane proteins are excreted through the process of exosome formation (403). Importantly, this proteomic analysis demonstrated that in addition to AQP2 there are numerous other proteins excreted in urine from volunteers, giving promise that proteomic analysis of urinary exosomes can provide a tool for early detection of renal disease. Numerous studies have suggested that urinary AQP2 excretion may be a valuable marker in clinical conditions with altered water balance. In a recent study where patients with CHF were treated with the V2-receptor antagonist (VPA-985), urinary AQP2 excretion was dramatically decreased in a dose-dependent manner (404). This decrease correlated with increased urinary excretion of solute-free water, supporting a functional associated between AQP2 levels and water reabsorption at the collecting duct level. Future studies will determine whether urinary AQP2 excretion will be a useful parameter.

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CHAPTER 4 ■ TUBULAR SODIUM TRANSPORT

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Sodium is the primary extracellular cation and, as such, is of critical importance to maintenance of extracellular fluid volume. The kidney is the dominant organ regulating the excretion of sodium. Each day, roughly 25,000 mEq of sodium are filtered through the glomeruli; from this quantity, almost ten times the total exchangeable sodium in the body, the kidneys typically absorb over 99%. A remarkable feature of the sodium absorptive process is the precision with which the excretion of the final 1% of the filtered sodium load is regulated. An individual consuming a typical diet containing 6 g of sodium will excrete 260 mEq of sodium per day; the same individual, when placed on a 2-g sodium-restricted diet, will promptly reduce sodium excretion to 87 mEq per day. Thus, the fraction of the filtered sodium load absorbed by the kidney changes from 99.0%, on a standard diet, to 99.6% on a sodium-restricted diet. This small change, however, is equivalent to the addition or removal of over 1 L per day to extracellular fluid volume. Stated simply, the kidneys absorb large amounts of filtered sodium with remarkably precise control.

The ability of the kidneys to absorb large amounts of sodium with exquisite control relies on the sequential actions of various segments of the nephron, each with highly specialized transport capabilities. Figure 4-1 provides an overview of sodium transport along the nephron. In general, the absolute rates of sodium reabsorption are greatest in the proximal tubule and fall as the tubular fluid proceeds from proximal to distal segments. At the same time, the ability to transport sodium against steep tubular fluid to blood gradients and the relative ability to regulate sodium transport increase along the nephron. For example, the proximal tubule reabsorbs the bulk (60% to 70%) of the filtered sodium load, but, as will be detailed later, does so against trivial electrochemical gradients. Moreover, the ability to alter sodium transport in the proximal tubule, in relative terms, is rather limited, usually varying by less than 15% to 20%. The collecting duct, in contrast, reabsorbs only a minor fraction (~2% to 4%) of the filtered sodium load. However, the collecting duct can transport sodium against a large electrochemical gradient to produce urine, which is almost sodium-free (<10 mEq/L). In addition, the rate of sodium transport in the collecting duct can vary over a wide range (10-fold) in response to physiologic stimuli. It is this sequential action of the different nephron segments that permits both high rates of sodium transport (proximal segments) and highly regulated sodium transport (distal segments).

Substantial progress has been made over the past decade in identifying the proteins that mediate sodium transport in each nephron segment and in defining the interactions and regulation of these proteins within each segment. Many of the sodium transport proteins discussed in this chapter have been linked to specific genetic disorders (Table 4-1). Given the primacy of renal sodium transport to the control of extracellular fluid volume, it is not surprising that the majority of these genetic disorders are characterized by either hypo- or hypertension. Some of these disorders, the underlying genetic defects, and their functional and clinical consequences are presented in Table 4-1. An updated and curated database of these and other genetic disorders is maintained at the Online Mendelian Inheritance in Man Web site (www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM).

This chapter considers the transepithelial transport of sodium by the various nephron segments. The discussion of each nephron segment begins with a description of the general features of sodium transport in that segment along with pertinent structure–function relations. The mechanism of sodium transport is then considered on a cellular or subcellular level, with emphasis on recent electrophysiological, biochemical, and molecular findings. Finally, each section includes a consideration of the factors that regulate sodium transport in the individual segments.

PRINCIPLES OF MEMBRANE TRANSPORT

This section describes some of the physical principles that underlie the movement of ions across individual membranes and epithelia. This discussion is not intended to be an extended treatment of the thermodynamic aspects of membrane transport processes; readers interested in such a quantitative approach to the subject are directed to other sources (1).

Diffusion Processes

Solute transport across membranes may occur by diffusion or convection, or by a mediated process. Diffusion is the random Brownian motion of a molecule with respect to adjacent molecules and occurs as the consequence of thermal energy (2). Since the diffusional movement of an individual molecule is random, the net transfer of molecules across a membrane, that is, the translation of the random diffusion of individual molecules to the vectorial movement of an ensemble of molecules, is a statistical process, which depends on the presence of a concentration gradient across a membrane. Thus, the concentration gradient represents the driving force for net transport.

For charged solutes, the driving force for transport is the sum of the chemical and electrical potential gradients. The Nernst equation describes the equilibrium condition for a membrane permeable only to a single ionic species:

$$V_m = V_2 - V_1 = -RT/ZF \ ln \ C_2/C_1 \qquad [1]$$

where R is the gas constant, T is the absolute temperature, Z is the valence of the solute, F is Faraday's constant, and C and V are concentration and electrical potential terms, respectively. At equilibrium, then, the voltage (V_m) across an ideally selective membrane is defined by the concentrations of the permeant ion on both sides of the membrane, C_2 and C_1,

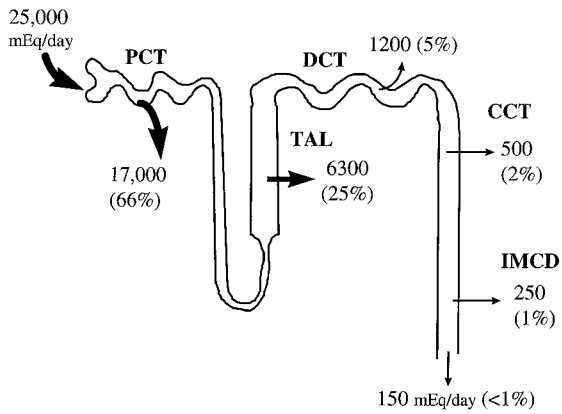

FIGURE 4-1. Contribution of various nephron segments to sodium transport.

respectively. For systems containing more than one permeant ion, the equilibrium voltage can be described by the Goldman–Hodgkin–Katz (GHK) equation (3,4):

$$V_m = -\ RT/F\ ln\ [(P_{Na}C^2{}_{Na} + P_K C^2{}_K + P_{Cl}\ C^1{}_{Cl})/ (P_{Na}\ C^1{}_{Na} + P_K\ C^1{}_K + P_{Cl}\ C^2{}_{Cl})] \quad [2]$$

where P_x is the permeability of the respective solutes, in this case, sodium, potassium, and chloride. Thus, in a system containing multiple charged solutes, the transmembrane voltage is a function of the concentrations and permeabilities of each solute.

Convective Processes

Convection is the vectorial movement of an ensemble of adjacent molecules with respect to an external geometric coordinate and is driven by an externally imposed force, for example, hydrostatic pressure. Examples of convective transport include glomerular filtration and solvent drag, a process in which solute molecules exhibit movement which is coupled to water movement.

Bulk water flow may be driven by hydrostatic pressure or osmotic pressure. The familiar Starling equation

$$J_v = K(\Delta P - \Delta\pi) \quad [3]$$

describes net volume flow (J_v) in response to hydrostatic (ΔP) and osmotic ($\Delta\pi$) pressure differences. The equivalence of osmotic and hydrostatic pressure is explicit in the Starling equation. The degree to which a solute exerts an osmotic pressure depends on the degree to which it permeates membranes. The ratio of the observed osmotic pressure to that predicted if a solute were excluded absolutely from a membrane is termed the reflection coefficient, σ:

$$\sigma = \Delta\pi_{obs}/\Delta\pi_{theoretical} \quad [4]$$

For impermeant solutes, $\sigma = 1$; for highly permeable solutes, σ approaches 0.

For solutes with $\sigma < 1$, transmembrane solute flux will be accelerated in the direction of volume flow. This acceleration is known as the *solvent drag* phenomenon (5). Thus, the net passive flux of a permeable solute across a membrane may be driven both by diffusion and by entrainment with solvent flow, that is, by solvent drag.

Facilitated Diffusion

Biological membranes are composed primarily of lipids arranged in a bilayer configuration. Since the permeability of many hydrophilic solutes through lipid membranes is rather low, membranes contain proteins, which facilitate the transport of certain solutes. These kinds of transport proteins, which are often termed *carriers*, have a high degree of specificity for the transported solute. The limited number of transporters gives rise to a saturation phenomenon as the solute concentration is increased. An example of carrier-mediated facilitated diffusion is the entry of glucose into renal tubular cells across the basolateral membrane mediated by the hexose transporter, GLUT-1 (6). The movement of ions through specialized pathways known as ion channels represents another form of facilitated diffusion. In this case, integral membrane proteins, usually containing several membrane-spanning domains, form pores in cell membranes through which ions can move. Ion channels are characterized by a high degree of specificity for the ions being transported and by the very high rates of transport effected by each channel protein. Facilitated diffusion mechanisms, like enzymes, serve only to accelerate the rate of transport, but do not affect the equilibrium distribution of solutes. In other words, facilitated diffusion, like simple diffusion and convection, is a passive, or dissipative, process.

Active Transport Processes

Active transport is a special case of facilitated transport in which chemical bond energy is supplied to the transport process so that the final distribution of the solute is remote from equilibrium. The coupling of solute transport to the energy source can take two forms. In primary active transport, solute transport is coupled directly to an energy-yielding reaction.

The most widely recognized example of primary active transport is the transport of Na^+ and K^+ by the $Na^+ + K^+$–ATPase. This enzyme, often referred to as the "sodium pump," couples the extrusion of cellular sodium to cellular potassium uptake (7). In renal tubules, this enzyme is localized to the basolateral membrane (8,9). In Figure 4-2 is illustrated the distribution of $Na^+ + K^+$–ATPase activity in various nephron segments. In general, segments, which maintain high rates of active sodium transport, have high $Na^+ + K^+$–ATPase activity.

The hydrolysis of each ATP molecule ordinarily pumps three sodium ions out of the cell coupled to two potassium ions moving inward (10–12). Therefore, the pump is electrogenic. The $Na^+ + K^+$–ATPase is responsible for maintaining the cell sodium activity at a low level, which, in turn, provides the energy for the sodium-coupled transport of many other solutes. Thus, inhibition of $Na^+ + K^+$–ATPase either by peritubular ouabain addition or by peritubular potassium removal causes a significant rise in the cell sodium activity (13–15).

The affinity constant (K_m) of the pump for intracellular sodium, about 15 to 30 mM (16,17), is similar to the intracellular sodium activity measured in proximal tubule cells (13,15, 18). Therefore, the pump is unsaturated with respect to sodium and pump activity is sensitive to changes in the intracellular sodium concentration activity. When the intracellular sodium concentration in the proximal tubule is varied, the transepithelial water and sodium fluxes vary proportionately (19).

In secondary active transport, the movement of a solute uphill against its electrochemical gradient is energized by the movement of another solute down its own gradient (20). Sodium, because of its steep favorable electrochemical gradient maintained by the sodium pump, often participates in the transport of other solutes, either in the same (cotransport or symport) or opposite (countertransport or antiport) direction. Thus, by coupling solute transport with sodium movement into cells, cellular metabolic energy generated by the $Na^+ + K^+$–ATPase is stored in the form of a sodium concentration gradient, analogous to a battery, and then dissipated in the

TABLE 4-1

INHERITED DISORDERS OF RENAL SODIUM TRANSPORT

Disorder	Affected gene product	OMIM #	Nephron distribution	Functional consequences	Clinical features
Hypotensive disorders					
Bartter, Type 1 (antenatal)	NKCC2 (SLC12A1)	600839	TAL	Decreased Na:K:2Cl cotransport	Hypotension, hypokalemia, metabolic alkalosis
Bartter, Type 2 (antenatal)	ROMK (KCNJ1)	600359	TAL, CCD	Decreased apical K^+ recycling	Same as above
Bartter, Type 3 (infantile)	ClC-Kb	602023	TAL	Decreased basolateral Cl^- flux	Hypokalemia, alkalosis, hyperreninemia
Bartter, Type 4	barttin	606412	tAL, TAL, inner ear	Decreased basolateral Cl^- flux	As above, with sensorineural deafness
Gitelman	NCCT (SCL12A3)	600968	DCT	Decreased NaCl cotransport	Hypokalemia, metabolic alkalosis, hypocalciuria
Pseudohypoaldosteronism type 1 (autosomal recessive)	ENaC subunits (SCNN1A, B, G)	264350	Collecting duct	Decreased amiloride-sensitive sodium transport	Hypotension, hyperkalemia, metabolic acidosis
Pseudohypoaldosteronism type 1 (autosomal dominant)	Mineralocorticoid receptor (NR3C2)	177735	Collecting duct	Decreased response to mineralocorticoids	Hypotension, hyperkalemia (less severe than recessive)
Hypertensive disorders					
Liddle	β or γ ENaC (SCNN1B, SCNNIG)	177200	Collecting duct	Increased cell surface expression and activity of ENaC channels	Hypertension, hypokalemia, metabolic alkalosis, responsive to amiloride
Pseudohypoaldosteronism type 2 (Gordon)	WNK1 or WNK4	145260	DCT, collecting duct	Increased NaCl cotransport, increased paracellular Cl^- permeability	Hypertension, hyperkalemia, metabolic acidosis, responsive to thiazide diuretics
Apparent Mineralocorticoid Excess	11β-HSD2	218030	DCT, collecting duct	Decreased oxidation of glucocorticoids causing activation of mineralocorticoid receptor	Hypertension, hypokalemia, metabolic alkalosis, responsive to dexamethasone
Other disorders					
Renal glucosuria	SGLT2 (SLC5A2)	233100	Proximal tubule	Decreased sodium-coupled glucose absorption	Decreased threshold for glucosuria
Proximal RTA	NBC1 (SLC4A4)	603345	Proximal tubule	Decreased sodium-coupled HCO_3^- transport across basolateral membrane	Metabolic acidosis, ocular abnormalities, mental retardation

TAL, thick ascending limb; *tAL*, thin ascending limb; *DCT*, distal convoluted tubule; *OMIM #*; accession number for Online Mendelian Inheritance in Man. Updated references available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM.

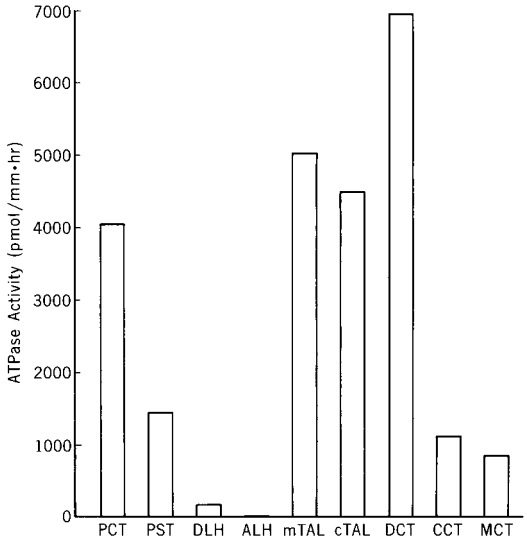

FIGURE 4-2. Distribution of $Na^+ + K^+$-ATPase activity along the mammalian nephron. *PCT*, proximal convoluted tubule; *PST*, proximal straight tubule; *DLH*, descending thin limb of Henle; *ALH*, ascending thin limb of Henle; *mTAL*, medullary thick ascending limb; *cTAL*, cortical thick ascending limb; *DCT*, distal convoluted tubule; *CCT*, cortical collecting tubule; *MCT*, medullary collecting tubule. (From: Garg, LC, Narang N, Wingo CS. Glucocorticoid effects on Na-K-ATPase in rabbit nephron segments. *Am J Physiol* 1985;248:F487; Katz AI, Doucet A, Morel F. Na-K-ATPase activity along the rabbit, rat, and mouse nephron. *Am J Physiol* 1979;237:F114; Garg LC, Knepper MA, Burg MB. Mineralocorticoid effects on Na-K-ATPase in individual nephron segments. *Am J Physiol* 1981;240:F536; Schmidt U, Dubach UC. Activity of ($Na^+ + K^+$) stimulated adenosine triphosphatase in the rat nephron. *Pflugers Arch* 1969;306:219, with permission.)

transport of a variety of different solutes. Some examples of symport processes include sodium–glucose and sodium–amino acid cotransport; sodium–proton and sodium–calcium exchange are two examples of countertransport processes.

PROXIMAL TUBULE

General Features

The proximal tubule is the major site for sodium absorption within the kidney and serves two major purposes. First, the proximal tubule protects the extracellular fluid volume by reclaiming the bulk, approximately 60% to 80%, of the glomerular filtrate (21–23). This equates with the absorption of the entire extracellular fluid volume once every $2^1/_2$ hours. Second, the absorption of sodium in the proximal tubule provides, by way of coupled processes, the driving force for absorption of other solutes, such as bicarbonate, glucose, and amino acids.

Under most circumstances, fluid at any given point along the proximal tubule has virtually the same sodium concentration and osmolality as plasma (21,23). The isosmotic nature of proximal tubule fluid absorption derives from the high water permeability of this segment (24,25), which effectively clamps the osmolality of the tubular fluid at that of plasma. Although sodium transport in the proximal tubule occurs in the absence of large electrical or chemical gradients, several lines of evidence indicate that the bulk of sodium absorption in the proximal tubule involves active transport. For example, under the appropriate experimental conditions, sodium can be shown to be reabsorbed against both concentration (23,26,27) and electrical (28–30) gradients. In addition, fluid absorption and sodium transport cease when $Na^+ + K^+$–ATPase activity is inhibited or when cell metabolism is slowed (31–33).

Not all proximal sodium absorption, however, involves primary active transport. Rather, a significant amount of proximal sodium transport also occurs passively (34–36). In the late convoluted and straight tubules (S2 and S3 segments), sodium diffuses passively out of the tubule driven by the lumen-positive electrical potential difference in those segments. This potential difference, as will be discussed later, derives from a chloride concentration gradient across the tubule wall. Even in this case, however, it is the active transport of sodium in upstream portions of the proximal nephron that ultimately accounts for these gradients and potentials.

Nephron Heterogeneity

Analyses of proximal tubular sodium transport are complicated by two factors: a nonhomogeneous nephron population and axial changes in fluid composition. As reviewed in Chapter 1 of this volume, there is considerable axial heterogeneity of both morphologic and functional characteristics of the proximal tubule. The cells of the earliest part of the proximal tubule, the S1 segment, have extensive basal interdigitations, numerous mitochondria, and a well-developed luminal brush border (37). The S3 segment, in contrast, consists of flatter cells with fewer mitochondria, lower brush borders, and much less extensive basolateral membranes than S1 cells (37). Normalized to tubule length, the basolateral membrane area and $Na^+ + K^+$–ATPase activity of the S3 straight segment are only 25% of that for the S1 segments (38–40). As might be expected on the basis of these observations, the net rates of the Na^+ and fluid transport in the S3 straight segment are, in general, lower than in the S1 convoluted tubule (41–45).

Proximal nephron segments also differ with respect to their transport properties according to their location within the kidney. Thus, juxtamedullary convoluted tubules have higher rates of volume and bicarbonate absorption than their superficial counterparts (42,46), although this disparity has not been noted between juxtamedullary and superficial straight segments (47).

Glomerular ultrafiltrate, which enters the early proximal tubule, also undergoes axial change in its composition. Figure 4-3 illustrates that the chloride concentration rises, while the bicarbonate concentration falls, early in the proximal tubule (48). These changes occur as a consequence of the preferential absorption of sodium bicarbonate over sodium chloride in this segment (48–50). Glucose, amino acids, and other organic compounds are also absorbed avidly in association with sodium in the early proximal tubule, so that their concentrations in luminal fluids approach zero in spite of net water reabsorption in this segment (48,51).

This coupling of sodium absorption to the absorption of bicarbonate, glucose, and amino acids is a prominent property of the proximal convoluted tubule. Omission of glucose and amino acids from luminal fluids reduces both the potential difference and the volume absorptive rate (46,49,52,53). In the straight segment, on the other hand, deletion of glucose and alanine has no effect either on the potential difference or on the fluid absorptive rate (47,54,55), although deletion of all organic solutes does reduce volume absorption by 50% (56). The rates of transport of glucose, amino acids, phosphate, and sodium in the early proximal convoluted tubule exceed those in the proximal straight tubule (41,43–45,57). As a correlate, the rate of volume absorption is two to three times higher in the convoluted than in the straight tubule. These rates correlate well, as noted above, with the relative basolateral membrane areas of the respective segments (40).

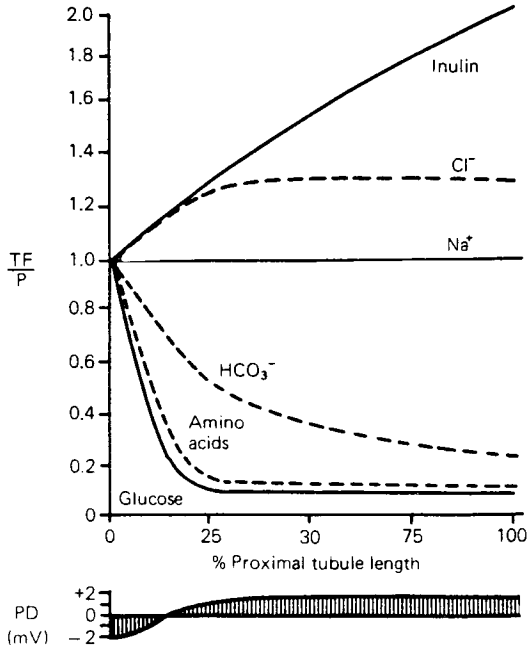

FIGURE 4-3. Profile of transepithelial voltage and solute concentrations along the mammalian proximal tubule. (From: Rector FC, Jr. Sodium, bicarbonate, and chloride absorption by the proximal tubule. *Am J Physiol* 1983;244:F461, with permission.)

Electrophysiology of the Proximal Tubule

Transepithelial Potential Difference

The electrogenic nature of the transport of sodium coupled to glucose and amino acids creates a lumen-negative transepithelial electrical potential difference in the early proximal convoluted tubule. The deletion of glucose and alanine from the luminal fluid reduces the potential difference from about –5.0 mV, lumen-negative, nearly to zero (46,49,53,58). Free-flow micropuncture studies indicate that this transepithelial potential difference becomes lumen-positive (+2 to 4 mV) after the first millimeter of proximal tubule (30,52), at a point where the tubular fluid to plasma chloride concentration ratio is approximately 1.3 (52,59). This lumen-positive voltage is probably a diffusion potential arising from the chloride concentration gradient.

Electrical Resistance

The proximal tubule is a classic example of an electrically leaky epithelium. The electrical resistance of the mammalian proximal tubule is remarkably low. The reported values for mammalian proximal tubule resistance are in the range 5 to 10 Ωcm^2 (29,60–62).

Obviously, dissipative transepithelial currents might traverse paracellular or transcellular pathways. In order to know the relative and absolute currents crossing cellular and paracellular pathways, it is necessary to know R_e, the transepithelial resistance, R_c, the cellular resistance, and R_s, the paracellular resistance. Only a few measurements of cell resistance of mammalian tubules are available (60,63,64). However, from these measurements, it seems clear that the total cellular resistance (i.e., the sum of the apical and basolateral resistance) is 20- to 70-fold greater than the transepithelial resistance. Thus, it is probable that the predominant route for passive ion flows in the proximal tubule involves the paracellular pathway.

Ionic Selectivity

The initial convolution of the superficial proximal tubule is sodium selective; thereafter, the superficial convoluted and straight tubules are chloride selective (54,58). In contrast, juxtamedullary proximal tubules are sodium selective throughout their course (58). Since the chloride concentration rises as fluid flows along the convoluted tubule (Fig. 4-3), oppositely directed gradients for chloride and bicarbonate in late convoluted and straight segments give rise to a lumen-positive transepithelial potential difference (48). The latter indicates a higher permeability for chloride than bicarbonate. Values of P_{HCO_3}/P_{Cl} appreciably less than one have been found in all proximal tubule segments, both superficial and juxtamedullary (42,65–67).

MECHANISMS OF SODIUM REABSORPTION

Apical Membrane Sodium Entry

Two general classes of sodium entry mechanisms have been described for the apical membranes of proximal tubular cells. Sodium entry may be coupled to the movement of other solutes, such as glucose, chloride, or protons; or sodium may enter independently. In either case, the driving force for sodium entry is the steep electrochemical gradient favoring sodium influx.

In mammalian proximal tubules, the intracellular sodium activity ranges from 15 to 35 mM (17,68,69). In the amphibian proximal tubule, the cell sodium activity measured by intracellular electrodes is 20 to 30 mM, with a total electrochemical driving force of approximately 100 mV directed inwardly for sodium (13,15,18,70). The entry of sodium into cells appears to be rate-limiting for transepithelial sodium transport. Amphotericin B, a polyene antibiotic, increases the permeability of the luminal membrane to sodium and causes a large rise in net sodium absorption (19,71).

Na^+–H^+ Exchange

Directly coupled Na^+–H^+ exchange in the proximal tubular brush border (72) is responsible for most proton secretion (73) and for a large fraction of sodium reabsorption in the proximal tubule. The role of Na^+–H^+ exchange in renal acidification is discussed in Chapter 6 of this volume. The mechanism whereby Na^+–H^+ exchange effects Na^+ reabsorption is presented in Figure 4-4. Briefly, entry of Na^+ is coupled to extrusion of a proton into the lumen. The proton titrates a filtered HCO_3^-

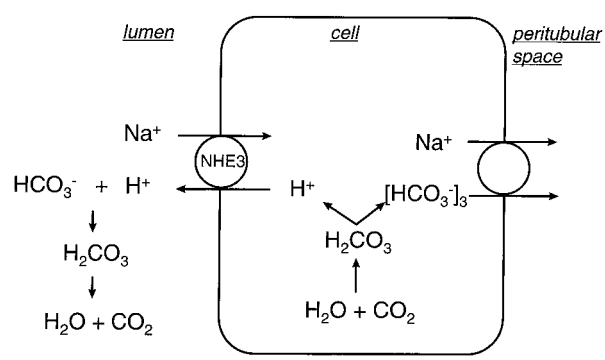

FIGURE 4-4. Scheme of $NaHCO_3$ transport mediated by Na^+–H^+ exchange. See text for explanation.

molecule to form carbonic acid. Carbonic acid subsequently is dehydrated to CO_2 in a reaction catalyzed by carbonic anhydrase in the brush border membrane. Within the cell, the reverse process occurs: Carbonic acid formed by the hydration of CO_2 dissociates into H^+ and HCO_3^-. The H^+ is extruded into the lumen by Na^+-H^+ exchange to repeat another cycle while the HCO_3^- is transported into the blood via a $1Na^+:3HCO_3^-$ cotransport process. Because 1 $Na^+/3HCO_3^-$ cotransport is electrogenic, carrying two net negative charges out of the cell, it can be driven by the inside-negative cell membrane potential, although both Na^+ and HCO_3^- are moving against concentration gradients. A number of $Na^+:HCO_3^-$ cotransporters have been cloned (74). One of these, NBC1 (encoded by SLC4A4) is localized to the basolateral membrane of the S1 and S2 segments of the proximal tubule (75) and likely accounts for basolateral HCO_3^- transport in these segments. As illustrated in Figure 4-4, the net result of these steps is the reabsorption of Na^+ and HCO_3^- from the luminal fluid. As is discussed in Chapter 6, this process accounts for the bulk of HCO_3^- reabsorption and about 20% of Na^+ reabsorption in the proximal tubule.

The mammalian Na^+-H^+ exchanger is electroneutral with a stoichiometry of one proton for one sodium (72,76,77). The exchanger is reversibly inhibited by high concentrations of amiloride (78) and is stimulated by intracellular acidosis. The stimulatory effect of internal protons, mediated by an internal activator site rather than increased occupancy of the internal transport site (79), helps to account for the increase in Na^+-H^+ exchange in response to intracellular acidosis (80,81).

Studies indicate that the apical and basolateral membranes of kidney cells contain different forms of Na^+/H^+ exchangers with different affinities for amiloride (82,83). The apical Na^+/H^+ exchanger is involved in urinary acidification and has a low affinity for amiloride, while the basolateral exchanger is presumed to serve a "housekeeping" role and has a high affinity for amiloride. An Na^+/H^+ exchanger was first cloned from human genomic DNA (84). The clone NHE1 encodes a 99 kDa protein, which contains 10 membrane-spanning regions. The sensitivity of NHE1 to amiloride (84) and the basolateral localization (84a) suggest that it represents the "housekeeping" Na^+/H^+ exchanger.

Other isoforms of the Na^+/H^+ exchanger have also been cloned (85). One of these, NHE3, has been shown to be located in the brush border membrane of proximal tubule cells (86,87). Mice, which lack the NHE3 exchanger, have been produced by genetic "knock out" of the *NHE3* gene. Micropuncture studies demonstrate that the rates of sodium and bicarbonate transport in the proximal tubules of these mice are markedly reduced (88,89). In addition, pharmacologic inhibitors of NHE3 reduce proximal tubule sodium reabsorption by about one-third (90). However, a significant, albeit reduced, rate of amiloride-sensitive bicarbonate transport still persists in the proximal tubules of NHE3 knock-out mice (91). Together, these observations indicate that the NHE3 isoform of Na^+-H^+ exchange is responsible for much, but not all, proximal tubular acidification and coupled NaCl absorption (*vida infra*). Another NHE isoform, NHE8, is also expressed in the apical membranes of cortical tubules and may contribute to these processes (92).

Sodium–Glucose Cotransport

The coupling of proximal tubular glucose and amino acid absorption to Na^+ absorption has already been mentioned. Electrophysiological studies in the rat, rabbit, and frog kidney all show that, on addition of glucose to luminal fluids, apical membranes depolarize (93,94). The depolarization occurs because the Na^+-glucose transporter, unlike the Na^+/H^+ exchanger, transfers a positive charge into the cell and, thus, is electrogenic. The Na^+-glucose transporter is specific for the D-stereoisomers of glucose, galactose, and α-methyl-D-glucoside (95–98). Unlike the Na^+/H^+ exchanger, the Na^+-glucose cotransporter has little affinity for cations other than sodium (95). Phlorizin inhibits Na^+-glucose cotransport by competing with glucose for its binding site (99).

The rate of glucose transport by the early proximal tubule is greater than in late proximal segments (41,43,45). Barfuss and Schafer (41), studying perfused rabbit proximal tubules, and Turner and Moran (100), studying rabbit brush border membrane vesicles, found axial differences in the affinities of the Na^+-glucose carrier for glucose. In the proximal straight tubule, the K_m for D-glucose is 5 to 20 times lower than in the proximal convoluted tubule. Moreover, the transporter in the early proximal tubule (cortical origin) has a 1:1 sodium to glucose stoichiometry (100), whereas the transporter in the straight segment (medullary origin) has a 2:1 stoichiometry (101). By coupling the energy from two sodium ions moving down their electrochemical gradient to the transport of each glucose molecule, the medullary transporter is able to establish a much greater cellular to extracellular glucose concentration ratio than a 1:1 Na^+:glucose transporter (51). The 2 Na^+:1 glucose transporter is, therefore, well suited to the straight segment, where tubular fluid glucose concentrations have already been reduced by glucose absorption in the convoluted tubule.

Hediger et al. (102,103) cloned a Na^+-glucose cotransporter (SGLT-1) from rabbit jejunum and human intestine. The cDNA codes for proteins of 662 and 664 amino acids, respectively, and a molecular weight of 73 kDa. Analysis of kidney mRNA, using the intestinal Na^+ glucose cotransporter cDNA as a probe, indicates that the kidney contains a transporter identical to that in the intestine (104). Western and Northern blot analysis indicates that SGLT-1 is located predominantly in the outer medulla (105). When expressed in *Xenopus* oocytes, SGLT-1 mediates high affinity Na–glucose cotransport with a sodium-to-glucose coupling ratio of 2:1 (106). A second sodium–glucose cotransporter, SGLT-2, has been cloned from human kidney (107). This protein shares 59% homology to SGLT-1. However, when expressed in *Xenopus* oocytes, SGLT-2 mediates low-affinity Na–glucose cotransport with a sodium–to-glucose coupling ratio of 1:1. Moreover, *in situ* hybridization revealed high levels of SGLT-2 message in the S1 segment of the proximal tubule (108,109). Thus, it appears that SGLT-2 may represent the low-affinity, high-capacity sodium–glucose cotransporter in the early proximal tubule, while SGLT-1 may represent the high-affinity, low-capacity transporter of the proximal straight tubule (109).

Mutations in SGLT-2 form the basis for renal glucosuria (Table 4-1), an inherited condition characterized by a lowered threshold for tubular reabsorption of glucose (110). In contrast, the dominant clinical manifestations of inactivating mutations of SGLT-1 (111) relate to the failure to absorb sugars in the intestinal tract (glucose–galactose malabsorption). These findings suggest that SGLT-2 plays a much more significant role, quantitatively, than SGLT-1 in proximal tubule glucose reabsorption.

Sodium–Amino Acid Cotransport

The proximal tubule reabsorbs amino acids from the tubular fluid. The accumulation of amino acids in the cell at a higher concentration than in the tubular fluid places an active transport step at the luminal membrane (54,57). Samarzija and Frömter (93), using double-perfusion micropuncture techniques and electrophysiologic measurements, examined the kinetics of amino acid reabsorption in the rat proximal tubule. They observed a depolarization of the luminal membrane

during amino acid transport and, from competition studies, were able to identify five classes of amino acid transporters in the luminal membrane.

Both sodium-dependent (112–115) and -independent (116,117) amino acid uptake pathways have been characterized in the kidney. Neutral amino acid transport appears to involve at least three separate transport systems (118), one which transports all neutral amino acids, one specific for imino acids, and one for the β-amino acids. Glycine may also have a specific transporter (119). The acidic and basic amino acid groups each have their own transport system (120,121). At least one amino acid transporter, a sodium-independent transporter for neutral and dibasic amino acids, has been cloned from the kidney (116,117,122,123).

NaCl Transport

Two basic mechanisms account for sodium chloride reabsorption in the proximal tubule. In simple electrogenic Na^+ entry, sodium is transported actively through the cell, thereby creating a lumen-negative potential difference. Chloride reabsorption then proceeds through the paracellular pathway driven by the lumen-negative potential difference. In electrically neutral NaCl transport, both Na^+ and Cl^- move through the cell at equal rates, such that no transepithelial potential and, hence, no driving force for paracellular Cl^- movement is generated.

Neutral NaCl Transport

Several lines of evidence indicate that a sizable fraction of proximal NaCl transport is transcellular and electroneutral (124). First, by virtue of the coupling of Cl^- entry to apical Na^+ entry, the intracellular Cl^- activity of proximal tubule cells is greater than predicted from an equilibrium distribution (125–128). Second, Cl^- absorption persists even when the driving force for passive, paracellular movement is abolished (129–132). Conversely, Cl^- reabsorption is inhibited by cyanide in the absence of any change in the passive driving forces for Cl^- movement (129,132). Finally, luminal application of SITS (133), an anion-exchange inhibitor, or removal of chloride from the tubule perfusate (134), reduces net sodium reabsorption. It is also important to note that the rate of transcellular, electroneutral NaCl transport is much higher in the rat proximal tubule (129) than in the rabbit proximal tubule (130).

In principle, electroneutral NaCl transport across the apical membrane of proximal tubule cells could occur as directly coupled NaCl cotransport or as parallel Na^+/H^+ and Cl^-/base exchangers. There is no good evidence for the former process in the mammalian proximal tubule (124). However, considerable evidence supports the view that electroneutral NaCl transport in the proximal tubule involves parallel exchangers. The coupling of Na^+ absorption to Cl^- absorption in the latter instance occurs because of the relation between cell pH and concentration of base within the cell. With reference to Figure 4-5, the extrusion of H^+ in exchange for Na^+ results in the liberation of base for participation in Cl^-–base exchange. The uphill entry of Cl, then, is indirectly coupled to the downhill entry of Na^+, because both are coupled to the transport of an acid–base pair. The model illustrated in Figure 4-5 utilizes formate–Cl^- exchange as the anionic component of electroneutral NaCl transport.

As indicated previously, there is abundant evidence for an Na^+/H^+ exchanger in proximal tubule brush border membranes. With respect to NaCl transport, inhibition of Na^+-H^+ exchange by high concentrations of amiloride (135,136) or more specific inhibitors of NHE3 (90) results in a dramatic fall in transcellular NaCl transport. Likewise, genetic knock out of the NHE3 Na^+/H^+ exchanger also reduces NaCl and fluid

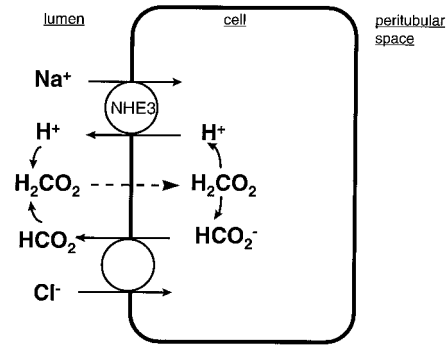

FIGURE 4-5. Scheme of neutral NaCl transport mediated by the parallel action of Na^+-H^+ exchange and formate–Cl^- exchange. Formate (HCO) combines with H^+ in the tubular lumen to form formic acid (H_2CO_2), which re-enters the cell by nonionic diffusion. A similar scheme applies for oxalate–Cl^- exchange.

reabsorption in the proximal tubule (88,89). Several Cl^-/base exchangers have been implicated in NaCl transport. Recent interest has focused on the role of Cl^-–formate (HCO_2^-) and Cl^-–oxalate ($C_2O_4^{2-}$) exchange in NaCl transport.

A Cl^-/formate exchanger is present in brush border membrane vesicles (137,138). A role for Cl^-–formate exchange in neutral NaCl transport is suggested by the finding that the addition of formate to the luminal perfusate increases the rate of NaCl reabsorption in rabbit and rat proximal tubules (139,140). As depicted in Figure 4-5, formate is presumed to leave the cell in exchange for Cl^-. The secreted formate then combines with a proton, which was transported by the Na^+/H^+ exchanger to form formic acid. The formic acid then re-enters the cell by nonionic diffusion and dissociates to supply substrate for the continuation of both exchange processes. A protein capable of mediating Cl^-–formate exchange, CFEX (SLC26A6), a homolog of pendrin, is present in apical membranes of the proximal tubule (141).

Cl^-–oxalate ($C_2O_4^{2-}$) exchange has also been demonstrated in brush border membrane vesicles (142). It has been suggested that Cl^-–oxalate exchange may mediate neutral NaCl transport in a manner analogous to that described for Cl^-–formate exchange. In this regard, the addition of only 5 μm oxalate to the luminal and capillary perfusates resulted in a 57% increase in the rate of NaCl reabsorption in the rat proximal tubule (140). It has also been suggested that NaCl absorption proceeds via the operation of three parallel transporters: Na^+/sulfate cotransport, sulfate–oxalate exchange and Cl^-–oxalate exchange (143). Indeed, the CFEX protein (*vide supra*), in addition to Cl^-–formate exchange, is also able to mediate Cl^-–oxalate, oxalate–formate, oxalate–oxalate, and oxalate–sulfate exchange (144).

Other sodium-dependent transport processes have been described in the apical membrane of the proximal tubule. These other processes, which do not contribute significantly to sodium reabsorption, because of the low concentrations of substrate present, are listed in Table 4-2.

Simple Electrogenic Na^+ Entry

The classic Ussing model for salt reabsorption involves passive entry of sodium across apical membranes and extrusion across the basolateral membrane by $Na^+ + K^+$–ATPase. A problem in assessing the contributions of rheogenic (electrogenic) processes to sodium transport in the proximal tubule is the presence of other mechanisms of sodium entry. However, when the contributions of Na^+-H^+ exchange and sodium cotransport to net Na^+ absorption are minimized by deleting glucose,

TABLE 4-2

NA^+-COUPLED TRANSPORT PROCESSES IN THE PROXIMAL TUBULE APICAL MEMBRANE

Process	Ref.
D-Glucose	(51,95,704)
L-Amino acids	(93,705)
L-Lactate	(706,707)
Protons	(72,77)
Inorganic phosphate	(208)
Sulfate	(185,708)
Dicarboxylic acids	(708,709)
Tricarboxylic acids	(708,710)
Adenosine	(711)

amino acids, and bicarbonate from the perfusate, a fraction of fluid absorption in isolated perfused straight segments persists and the transepithelial potential is –1.0 mV (56). These results indicate that, in the proximal straight tubule, simple rheogenic sodium transport constitutes one of the mechanisms for sodium absorption. A conductive sodium pathway has been demonstrated in brush border membrane vesicles (77). Unlike the sodium channel found in the distal nephron segments, the sodium channel in the proximal tubule is not blocked by amiloride (77).

In the proximal convoluted tubule, however, deletion of glucose, bicarbonate, and amino acids completely abolishes fluid absorption (42,52,58). Consequently, simple rheogenic proximal sodium transport may be limited to straight segments.

Passive NaCl Absorption

The rise in tubular fluid chloride concentration, and the attendant lumen-positive voltage (Fig. 4-3), provides a mechanism for passive NaCl absorption in late regions of the proximal nephron. In the chloride-selective superficial pars recta, approximately one-third of net NaCl absorption can be accounted for by this mechanism (35,36).

Basolateral Membrane

The proximal tubule, particularly the S1 segment, possesses a high activity of $Na^+ + K^+$–ATPase activity in the basolateral membrane. The $Na^+ + K^+$–ATPase pumps Na^+, which entered cells apically, across basolateral membranes. In other words, the pump keeps the cell sodium activity low and maintains the electrochemical gradient for sodium entry across the apical membrane. Consequently, inhibition of $Na^+ + K^+$–ATPase activity with ouabain decreases transepithelial sodium reabsorption and increases the intracellular sodium activity in the proximal tubule (13,16).

Sodium also exits across the basolateral membrane in concert with bicarbonate. Studies in intact tubules and in membrane vesicles have demonstrated an electrogenic, stilbene-sensitive Na-HCO_3 cotransporter in the basolateral membrane of rat and rabbit proximal tubules (145–148). The cotransporter transfers two net negative charges across the basolateral membrane. The stoichiometry of this process has been determined to be 1 Na^+: 1 HCO_3^-:1 CO_3^{2-} (or SO_3^{2-}) (149,150). Thus, this transport moiety for Na^+ extrusion, $[Na^+(HCO_3^-)_3]^{2-}$ is electronegative, and the lumen-negative cell interior provides a major driving for Na^+ extrusion. It is believed that this cotransport process accounts for the basolateral transport of most of the bicarbonate reclaimed from the

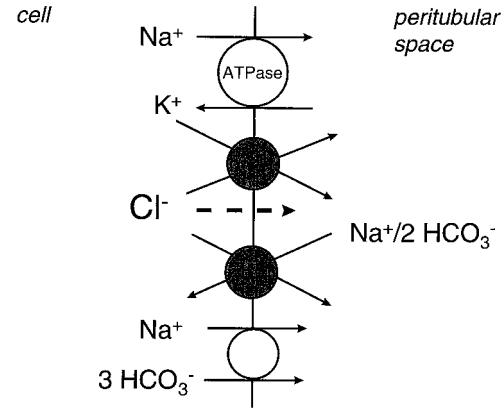

FIGURE 4-6. Transport pathways for Na^+ and Cl^- absorption across the basolateral membrane of proximal tubular cells. Cl^- can leave the cell via KCl cotransport, Na^+–$2HCO_3^-$ –Cl^- exchange, and Cl^- channels (minor). Na^+ exits via the $Na^+ + K^+$–ATPase and Na^+-$3(HCO_3^-)$ cotransport.

luminal fluid (149). Several sodium bicarbonate cotransporters (NBC) have been cloned, and at least three are expressed in the kidney (151). Mutations in one of these, NBC-1, cause proximal renal tubular acidosis (Table 4-1) (152). Presumably, NBC-1 is a major route for basolateral HCO_3^- efflux in the proximal tubule.

The pathways for Cl^- exit across the basolateral membrane are less well defined. Several pathways for Cl^- exit across the basolateral membrane have been proposed: Cl^- channels, KCl cotransport, and $Na^+(HCO_3^-)_2$–Cl^- exchange (Fig. 4-6). It appears that a basolateral Cl^- conductance does not participate in transcellular Cl^- transport (153). Studies of convoluted proximal tubule segments from either rabbit (126,154) or rat (155) using intracellular microelectrodes have indicated that the proximal tubule cell has a very low chloride conductance. Similar conclusions regarding the basolateral membrane have been reached in the rabbit proximal straight tubule (PST) (128,155,156). It has been argued from such data that, in normal PCT (126,154) and PST (128,155,156) segments, conductive Cl^- efflux across basolateral membranes plays a minor role in NaCl absorption. Under hypotonic conditions, however, cell swelling dramatically increases the basolateral membrane Cl^- conductance (157,158).

Since the chemical gradient for K^+ to leave cells (~10:1) exceeds the chemical gradient for Cl^- entry (~3:1), KCl cotransport can mediate basolateral Cl^- exit from proximal tubule cells. Ion-selective microelectrode studies have demonstrated KCl cotransport in basolateral membranes of rabbit proximal tubule cells (126,128). KCl cotransport has also been demonstrated in basolateral membrane vesicles (130), although this is not a consistent finding (137,159).

Stilbene-sensitive, sodium-dependent Cl^-–HCO_3^- exchange has been demonstrated in rat (160) and rabbit (161,162) proximal tubules. In this case, the entry of 1 Na^+ and 2 HCO_3^- across the basolateral membrane is coupled to the efflux of Cl^-. The Na^+ and HCO_3 which enter the cell are thought to be recycled through the $[Na^+(HCO_3^-)_3]^{2-}$ cotransporter (see above). Indeed, Ishibashi et al. (161) estimate that $Na^+(HCO_3^-)_2$–Cl^- exchange accounts for much more Cl^- movement than KCl transport. Sodium-independent Cl^-–HCO_3^- exchange is also present in the basement membrane of proximal tubules (160–162). However, under physiologic conditions, this process mediates net Cl^- influx and does not contribute to net NaCl absorption.

CONTROL OF PROXIMAL TUBULAR SODIUM REABSORPTION

Glomerulotubular Balance (GTB)

The proximal tubule responds to an increase in glomerular filtration with an increase in the absolute rate of fluid absorption (APR) such that variations in the fractional proximal fluid absorption are minimized. This phenomenon is termed glomerulotubular balance (GTB). The efficiency of GTB, that is, the extent to which APR/GFR remains constant, is subject to physiologic and pathologic control (163). The prime factor modulating GTB *in vivo* is the effective circulating volume. Thus, at a constant or near-constant GFR, volume expansion and volume contraction decrease and increase, respectively, the absolute rate of proximal sodium absorption. In other words, volume expansion and volume contraction reset GTB upward and downward, respectively (164,165). This section considers some of the factors that modulate proximal sodium absorption.

Peritubular capillary oncotic pressure is one of the factors regulating the rate of salt and water absorption from the proximal tubule (166–168). The oncotic pressure of the peritubular proteins favors movement of fluid across the basement membrane, while capillary hydrostatic pressure retards this movement. Thus, at a given renal blood flow, an increase in the glomerular filtration rate and, hence, the filtration fraction, will cause an increase in the oncotic pressure in the postglomerular peritubular capillaries. At constant single nephron glomerular filtration rates (SNGFR), perfusion of efferent capillaries with hypooncotic fluids decreases the absolute rate of proximal fluid absorption, while perfusion of the capillaries with hyperoncotic fluids increases proximal absorption (166). Similarly, increasing single nephron GFR (SNGFR) by infusion of colloid-free solutions causes a minimal increase in the APR, while increases in SNGFR following plasma infusions are associated with greater increases in proximal absorption (167). That is, during colloid-free volume expansion, when postglomerular oncotic pressure is reduced, glomerulotubular balance is disrupted, but is preserved during volume expansion with protein-containing solutions (166). The effects of the peritubular protein concentration can also be demonstrated in isolated perfused tubules (169–172).

It is not precisely clear how the peritubular protein concentration modulates proximal fluid absorption. The effect is not simply because of the oncotic pressure exerted by the proteins, since changes in absorption do not occur when active transport is inhibited or from comparable changes in the transtubular hydrostatic pressure (171). It has been suggested that the peritubular protein may have some direct effect on transcellular sodium transport (173), or may affect the back-leak of sodium through the paracellular pathway (172,174–176).

Luminal factors also contribute to glomerulotubular balance (177). The flow dependence of proximal absorption has been investigated in the convoluted (24,178) and straight segments (25,179) of isolated, perfused rabbit nephrons. The key observations are: (a) volume absorptive rate is clearly dependent on the perfusion rate, (b) flow dependence persists in the absence of active transport when anion gradients are present, and (c) flow dependence is abolished in the absence of active transport and anion gradients.

An explanation (179,180) for these results lies in a consideration of axial versus radial changes in fluid composition along the tubule. The rate of passive sodium absorption in the late proximal nephron is dependent on the magnitude of the chloride gradient between the lumen and bath. At low axial perfusion rates, the radial chloride gradient tends to dissipate as a function of distance along the tubule, but this dissipation is minimized at higher axial perfusion rates. At infinitely high axial flow rates, the composition of the luminal fluid remains constant, and the full chloride gradient is expressed radially across the entire length of the tubule. Hence, the integrated driving force for passive NaCl absorption increases with the rate of tubule perfusion. In addition, the availability of solutes, such as glucose, amino acids, and bicarbonate is also partly responsible for the flow-rate dependence of proximal fluid absorption (181,182).

Catecholamines

Renal denervation results in a depression of proximal tubule sodium and fluid absorption (183–185). Radioligand-binding studies and molecular probes have identified both α and β-adrenergic receptors in the proximal tubule (186–189). The rate of salt and water reabsorption in the proximal tubule is stimulated by α- and β-adrenergic agonists (190–192). α-adrenergic agonists increase apical Na^+ entry via stimulation of Na^+-H^+ exchange (188). α-adrenergic agonists also increase basolateral Na^+ efflux via $Na^+ + K^+$–ATPase activity in rat proximal tubules by a pathway that involves the activation of the calcium-dependent protein phosphatase, calcineurin (193). The effects of β-adrenergic agonists on $Na^+ + K^+$–ATPase activity are less clear. In one study, β_2-adrenergic agonists increased $Na^+ + K^+$–ATPase activity via protein kinase C (PKC) (194). In contrast, Aperia et al. (195,196) found that β-adrenergic agonists, acting via protein kinase A (PKA) (196), inhibit $Na^+ + K^+$–ATPase ATPase activity (195).

Dopamine, produced by proximal tubular cells (197,198), inhibits sodium reabsorption in the proximal tubule (199). Dopamine, via binding to DA-1 and DA-2 receptors (200) results in an inhibition of $Na^+ + K^+$–ATPase activity (201). The decrease in $Na^+ + K^+$–ATPase activity results from the phosphorylation of a protein, DARPP-32, which modulates the phosphorylation state and activity of $Na^+ + K^+$–ATPase (202). The sensitivity of proximal tubule cells to dopamine may be regulated by the recruitment of cytoplasmic dopamine receptors into the plasma membrane (203,204).

Parathyroid Hormone

Parathyroid hormone (PTH) produces a 30% to 50% reduction in proximal tubular sodium and phosphate absorption (205–207). The hormone activates adenylate cyclase, thereby stimulating formation of cyclic adenosine monophosphate (cAMP) (208,209). Parathyroid hormone and cAMP inhibit Na^+-H^+ exchange in the luminal membrane of rat (210) and rabbit (211) proximal tubules, and in cultured mammalian proximal tubular cells (212–214). Inhibitors of calmodulin block the effect of cAMP on volume absorption, suggesting that calmodulin may be required for the expression of cAMP-induced transport changes in the proximal tubule (215). In a series of studies, Weinman et al. (216) reconstituted the brush border Na^+/H^+ exchanger into liposomes and demonstrated that phosphorylation by cAMP-dependent protein kinase inhibits Na^+-H^+ exchange activity (217) by interaction with a regulatory subunit, termed NHERF (218), which is associated with the exchanger (219–221). In the absence of NHERF, cAMP does not inhibit the exchange activity of NHE3. However, when NHERF and NHE3 are expressed together, cAMP inhibits Na^+-H^+ exchange (222). In the same system, calmodulin-dependent protein kinase II inhibits Na^+-H^+ exchange activity, while PKC stimulates the exchanger (223).

NHE3 is directly phosphorylated by several protein kinases. However, the functional significance of direct phosphorylation of NHE3 is not clear (224). In addition to the acute inhibition of Na^+-H^+ exchange activity via NHERF, long-term exposure to PTH may also decrease Na^+-H^+ exchange activity through internalization of NHE3 (225).

Angiotensin II

Systemic administration of low doses of angiotensin II (Ang II) inhibits the excretion of sodium (226), while inhibitors of Ang II increase sodium excretion (227,228). Systemic administration of Ang II causes changes in renal blood flow, aldosterone secretion, filtration fraction, and catecholamine release from renal sympathetic nerve endings (229). Studies utilizing *in vivo* microperfusion (230) and *in vitro* tubule perfusion (231) have examined more directly the effect of Ang II on proximal tubule sodium transport. Low concentrations of Ang II (10^{-12} to 10^{-10} M) cause an increase in proximal tubule fluid reabsorption, and higher doses ($10^{-7}$to 10^{-6} M) depress fluid absorption. Studies have demonstrated a stimulatory effect of Ang II on the rate of Na^+-H^+ exchange in cultured proximal tubule cells (232,233), in isolated perfused rabbit proximal tubules (234), and in isolated proximal tubular cells in suspension (235). The effects of Ang II are mediated by the AT1 subtype of Ang II receptors present in both the luminal and basolateral membranes of the proximal tubule (236). The physiologic effects of Ang II may involve the coupling of these receptors to both phospholipase A_2 and G proteins (237).

Thyroid Hormone

On a clinical level, hypothyroidism is associated with a decreased cardiac output, renal blood flow, and glomerular filtration rate (GFR). Clearance studies in hypothyroid rats have documented decreases in GFR, renal sodium reabsorption, and renal $Na^+ + K^+$–ATPase activity (238). These changes are reversible after thyroid hormone replacement (239).

It now appears that thyroid hormone exerts direct effects to stimulate proximal tubular salt and fluid reabsorption (240). It has been proposed that the proximal event in thyroid hormone-induced stimulation of transport is an increased permeability of the basolateral membrane to potassium (240). Studies in cultured kidney cells, however, are consistent with a direct stimulation of Na^+-H^+ exchange by thyroid hormone (241), perhaps because of an increase in the transcription of NHE3 (242).

Corticosteroids

Two major types of high-affinity cytoplasmic steroid receptors have been identified in the kidney: Type I, which mediates mineralocorticoid effects, and Type II, which binds glucocorticoids (243). Mineralocorticoid binding is absent in the proximal tubules (244,245). Accordingly, no effect of mineralocorticoids on proximal tubular sodium reabsorption has been demonstrated (246). Bilateral adrenalectomy, however, does result in a fall in the $Na^+ + K^+$–ATPase activity in the proximal tubule, which can be reversed by dexamethasone treatment (247). This has been taken as evidence for Type-II receptors in the proximal tubule. The physiologic significance of glucocorticoid binding is not certain, although glucocorticoids increase brush border Na^+-H^+ exchange activity in adrenalectomized rats (248) and cultured kidney epithelial cells (249). Dexamethasone also inhibits sodium–phosphate cotransport in cultured proximal tubular cells (250). This effect appears to be mediated through activation of PKC.

Nitric Oxide

The inducible and endothelial forms of nitric oxide synthase (NOS) are expressed in the proximal tubule (251). Although basal production of nitric oxide (NO) by the proximal tubule is low, the rate of production can be increased dramatically by lipopolysaccharide (LPS) and cytokines. Moreover, even in basal conditions, the proximal tubule may be affected by NO produced by adjacent cells, such as endothelium or other nephron segments. The overall effect of NO on proximal tubule sodium transport is unclear (252). *In vivo* studies using either genetic knockouts of NOS (253,254) or pharmacologic inhibitors of NOS (255,256) have yielded conflicting results. *In vitro*, NO decreased Na^+-H^+ exchange and $Na^+ + K^+$–ATPase activity in cultured proximal tubule cells (257,258).

MECHANISM OF ISOTONIC FLUID ABSORPTION

Proximal tubule water absorption is coupled tightly to solute absorption, since the measured osmolality of the tubular fluid is, within experimental error, identical to plasma. Three general mechanisms of solute–solvent coupling have been suggested to account for this isotonic absorption: lateral interspace hypertonicity, effective osmotic gradients because of different reflection coefficients for solutes in the tubular and peritubular fluids, and luminal fluid hypotonicity.

The standing gradient theory argues that active transport of salt into lateral intercellular space raises the osmolality of the space, thus providing an osmotic gradient for fluid transport from lumen to interspace (259,260). The tight junctions in this model are presumed to be impermeable to water, so that the osmotic flow of water from the cell into the hypertonic interspace raises the hydrostatic pressure in this compartment and forces fluid across the basement membrane.

An alternative explanation (179,180) proposes that an effective osmotic driving force for fluid absorption can exist between solutions of identical osmolalities if the reflection coefficients of the membrane for the solutes in the solutions differ. Specifically, the elevated tubular fluid-to-plasma chloride concentration found in the late proximal convolution and in the pars recta provides an effective osmotic driving force for fluid absorption since σHCO exceeds σCl. That is, the bicarbonate in the peritubular fluid is a more "effective" osmotic agent than is the chloride in the tubular fluid, and, thus, results in net water flow out of the tubule. While this mechanism may be applicable to the proximal straight tubule, the reflection coefficients for NaCl and $NaHCO_3$ measured across the rabbit proximal convoluted tubule are virtually identical (261,262), so that oppositely directed Cl- and HCO_3^- gradients may make a negligible contribution to fluid absorption in convoluted segments.

Finally, because of the high osmotic water permeability of the proximal tubule, only small degrees of absolute luminal hypotonicity are needed to provide a sufficient driving force to account for the observed rates of fluid reabsorption (35). Experimental evidence supports the view that absolute luminal hypotonicity is a significant driving force for fluid reabsorption in the proximal tubule. Thus, when proximal tubules are perfused and bathed by symmetric NaCl solutions, the luminal fluid becomes slightly hypotonic (50,263). The development of luminal hypotonicity can be amplified by maneuvers that decrease the water permeability of the proximal tubule. As discussed in Chapter 3, AQP1 is a water channel protein with abundant expression in the proximal tubule. In knock-out mice lacking functional AQP1 (264), the osmolality of tubular fluid at the end of the proximal tubule is significantly lower than in

normal mice (265). Implicit in the argument that the luminal fluid becomes hypotonic is that the resorbate must be hypertonic. Thus, when isolated proximal tubules are perfused under oil, the resorbate osmolality of droplets is 10 to 20 mOsm/L higher than that of the tubular fluid (266); the degree of resorbate hypertonicity correlates with the rate of volume reabsorption by the tubules (267).

THE LOOP OF HENLE

The dissociation of salt and water absorption by the loop of Henle is ultimately responsible for the capacity of the kidney, either to concentrate or to dilute the urine. The active absorption of NaCl in the water-impermeable thick ascending limb of Henle (TALH) serves both to dilute the urine and to supply the energy for the "single effect" of countercurrent multiplication. A functionally similar segment, known as the diluting segment, is found in amphibians and teleosts (268).

The mammalian loop of Henle contains the descending thin limb, the ascending thin limb, and the thick ascending limb. The loop of Henle absorbs about 25% to 40% of the filtered sodium load (21,269,270). Furthermore, the fluid leaving the loop is dilute, indicating that more sodium chloride is absorbed in the loop than water.

SALT TRANSPORT BY THE THIN DESCENDING AND THIN ASCENDING SEGMENTS

According to the passive models for urinary concentration (271,272), the thin descending limb should have a very high water permeability, such that the tubular fluid is concentrated by water abstraction rather than solute entry. *In vitro* microperfusion studies, with one exception (273), have confirmed that both the upper and lower portions of mammalian descending limbs are very permeable to water (274–278) (Table 4-3). The passive models also require that the thin ascending limb be rather impermeable to water, highly permeable to sodium chloride, and only modestly permeable to urea. As indicated in Table 4-3, *in vitro* microperfusion studies of thin ascending limb segments have demonstrated that these requirements are, in fact, satisfied.

The permeability of thin descending limb segments to sodium and chloride has been measured in hamster, rats, and rabbits. In hamsters and rats, the upper portion of long-looped descending limbs has a higher sodium permeability and higher P_{Na}/P_{Cl} ratio than do descending limbs of short-looped nephrons (274,275). In contrast, there is little difference in P_{Na}/P_{Cl} between long- and short-loop nephrons in rabbits (279). These results are consistent with the morphologic evidence of greater heterogeneity in rats and hamsters than in rabbits. The pathways for transepithelial movement of sodium and chloride in the descending limb are not defined. Lopes et al. (280) studied the mechanism of cell volume regulation in rabbit descending limb cells and concluded that the basolateral membrane contains Cl^- and K^+ channels and a KCl cotransport process.

The formation of dilute urine begins in the thin ascending limb of Henle. Fluid from the thin ascending limb is more dilute than fluid obtained from the descending limb at the same level (281,282). The decrease in osmolality is due primarily to a fall in the NaCl content of the luminal fluid. The mechanism for NaCl transport across the thin ascending limb epithelium is incompletely understood. Measurements of salt dilution potentials in microperfused thin ascending limb segments reveal them to be chloride selective with P_{Cl}/P_{Na} ratios of 2.2 to 3.5 in rats and hamsters (283). Segments perfused and bathed with symmetric solutions do not generate a spontaneous transepithelial voltage (283–286) and do not show net transport of solute (284). These observations, together with the very low activity of $Na^+ + K^+$–ATPase in this segment (287), have been interpreted to indicate that salt transport *in vivo* results from passive electrodiffusion rather than active transport. Although driven by passive electrochemical gradients, Cl^- movement across the TAL proceeds through a transcellular, and regulated, pathway. $^{36}Cl^-$ flux ratios and salt dilution voltages indicate that the Cl^- pathway discriminates among anions and is saturable (283,285). Reduction of the Cl^- concentration of the basolateral solution causes a spikelike depolarization of the basolateral cell membrane (288). Likewise, reductions of the luminal perfusate Cl^- concentration caused a depolarization of the apical membrane. These results are consistent with the presence of conductive pathways for Cl^- in both the apical and basolateral membranes of TAL cells (288). The basolateral Cl^- conductance is inhibited at low pH (288), by low intracellular Ca^{2+} concentrations (289), and by compounds known to block Cl^- channels in other tissues (290).

TABLE 4-3

PERMEABILITY PROPERTIES OF THIN LIMB OF HENLE SEGMENTS

	P_f (10^{-3} cm/sec)	P_{Na} (10^{-5} cm/sec)	P_{Cl} (10^{-5} cm/sec)	P_{Na}/P_{Cl} SDL	P_{Na}/P_{Cl} LDL_u	P_{urea} (10^{-5} cm/sec)
Descending limb						
Rabbit	240–250	1.61	—	0.75	0.76	1.5
Rat	227	34–47	—	0.61	5.0	
Hamster						
SDL	285	4.2	1.3	0.68	—	7.4
LDL_u	403	45.6	4.2	—	4–6	1.5
Ascending limb						
Rabbit	0	25.5	117	0.29		6.7
Rat	2.5	67.9	183.7	0.43		23.0
Hamster	3	87.6	196	0.47		18.5

P_f, osmotic water permeability; *SDL*, short-loop descending limb; LDL_u, upper portion of long-loop descending limb; P_{Na}, P_{Cl} determined from isotope flux measurements. P_{Na}/P_{Cl} determined from salt dilution voltages. Data from Refs. (275–279,283,712,713).

A chloride channel cloned from the renal medulla, ClC-Ka, may represent the major Cl^- channel in the thin ascending limb (291). This channel, which belongs to the ClC family of Cl channels, is expressed exclusively within the kidney and has been localized by immunohistochemistry to both the apical and basolateral membranes of the thin ascending limb of Henle (292). The expression of ClC-Ka is increased by dehydration (291,293). The initial reports of the functional characteristics of ClC-Ka showed some similarities with the Cl conductance observed in isolated thin ascending limb segments, suggesting that this channel formed the primary Cl conductance in this segment (291). Genetic knockout of the ClC-Ka gene in mice produced a urinary-concentrating defect confirming the role of passive NaCl transport in the thin ascending limb in the urinary-concentrating mechanism (294).

NaCl ABSORPTION IN THE THICK ASCENDING LIMB

General Features

Rocha and Kokko (295) and Burg and Green (296) first demonstrated the salient characteristics of salt absorption in rabbit medullary and cortical TAL segments, respectively. First, net salt absorption resulted in a lumen-positive transepithelial voltage (V_e, mV), which could be abolished by furosemide, and in dilution of the luminal fluid. Second, the transport of Cl^- under these circumstances occurred against both electrical and chemical gradients and, hence, involved an active transport process; however, the nature of the active process was not specified at that time. Third, both net chloride absorption and the transepithelial voltage depended on $Na^+ + K^+$–ATPase activity, present in large amounts along the basolateral membrane of this segment (287). A final curious feature of the TAL is that this segment is a hybrid epithelium possessing a very low permeability to water, yet a high ionic conductance. The ionic conductance determined from the fluxes of $^{22}Na^+$ and $^{36}Cl^-$ is approximately 20 to 50 mS/cm^2 and is cation-selective (P_{Na}/P_{Cl} = 2 to 6). This high electrical conductance is unusual among epithelia with low water permeabilities.

A low permeability to water is required for the TAL to function as a diluting segment. In fact, the apical cell membrane constitutes the major barrier to transcellular water flow in this segment (297–299). Note that the water permeability of the paracellular pathway must also be low in order to achieve dilution of the luminal fluid.

Studies of the electrophysiologic (Table 4-4) and biochemical properties of intact isolated perfused thick limb segments and of apical and basolateral membranes of thick limb cells have provided insights into the specific transport mechanisms involved in salt absorption and the origin of the lumen-positive transepithelial voltage in this nephron segment (298–308). A model for salt absorption by the TAL, which integrates the results of these studies, is shown in Figure 4-7.

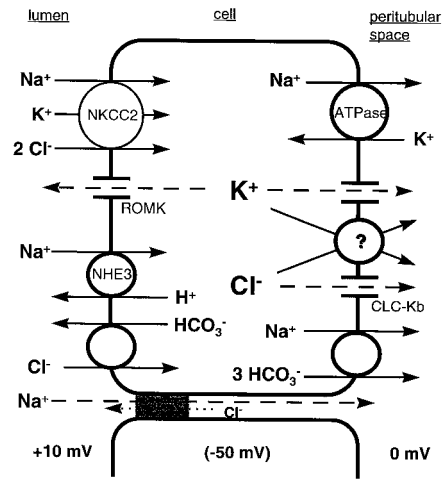

FIGURE 4-7. A model depicting the major elements of the mechanism of NaCl absorption by the thick ascending limb. Dashed lines indicate passive ion movements down electrochemical gradients.

According to the model shown in Figure 4-7, net Cl^- absorption by the TAL is a secondary active transport process. Luminal Cl^- entry into the cell is mediated by an electroneutral Na^+:K^+:2 Cl^- cotransport process driven by the favorable electrochemical gradient for sodium entry. More specifically, the net driving force for the entry of Cl^- into the cell is determined by the sum of the chemical gradients for Na^+, K^+, and Cl^-. Unfortunately, the intracellular activities of all three of these ions have not been determined in the mammalian TAL. However, in the diluting segment of *Amphiuma*, these measurements (309–311) confirm that there is a favorable gradient for Na^+:K^+:2 Cl^- entry into cells and that the gradient for Na^+ accounts for the majority of this favorable integrated chemical gradient (309). Since the Na^+ gradient is maintained by the continuous operation of the basolateral membrane $Na^+ + K^+$–ATPase pump, apical entry of Cl^- via the cotransporter ultimately depends on the operation of the basolateral $Na^+ + K^+$–ATPase. Accordingly, maneuvers that inhibit ATPase activity, such as removal of K^+ from, or addition of ouabain to, peritubular solutions leads to dissipation of the Na^+ gradient and subsequent inhibition of apical membrane Cl^- entry (295,298).

TABLE 4-4

BASAL ELECTROPHYSIOLOGICAL PARAMETERS OF THICK ASCENDING LIMB SEGMENTS

	V_e (mV)	G_e (mS/cm^2)	G_c (mS/cm^2)	G_s (mS/cm^2)	V_a (mV)	V_{bl} (mV)	R_a/R_b	Ref.
Rabbit cTAL	4–8	33	12	21	76	– 69	2.0	(305)
Mouse mTAL	3–7	70–100	45–50	40–60	55.4	– 50.7	1.2	(312,318,398)
Mouse cTAL	7–14	88	39	49				(714)
Hamster mTAL	4.0	934				– 72		(715)

V_e, transepithelial voltage; G_e, transepithelial conductance; G_c, transcellular conductance; G_s, paracellular conductance; V_a, apical membrane voltage; V_{bl}, basolateral membrane voltage; R_a/R_b, apical to basolateral membrane resistance.

In contrast to the electroneutral entry of Cl^- across the apical membrane, the majority of Cl^- efflux across the basolateral membrane proceeds through conductive pathways (308,312,313). A favorable electrochemical gradient for Cl^- efflux through dissipative pathways has been demonstrated by Greger et al. (303) in the rabbit cTAL. In this study, an intracellular Cl^-activity of 22mM, measured using single-barrelled Cl^--selective microelectrodes, was substantially above the equilibrium value (5 mM) predicted from the intracellular voltage. Intracellular Cl^- is maintained at concentrations above electrochemical equilibrium by the continued entry of Cl^- via the apical Na^+:K^+:2 Cl^- cotransporter. Blocking Cl^- entry through this pathway with furosemide, or substitution of extracellular Cl^- by gluconate, caused the intracellular Cl^- activity to fall to a value close to its equilibrium (303). Measurements of intracellular Cl^- activity in *Amphiuma*-diluting segments using double-barreled microelectrodes have also documented a favorable electrochemical gradient for basolateral Cl^- efflux under transporting conditions (309).

In addition to electrodiffusive efflux of Cl^- across the basolateral membrane, a component of electroneutral KCl cotransport has been proposed in some species (306). As will be discussed below, the evidence for KCl cotransport is strongest in the low basolateral conductance cells of the *Amphiuma* diluting segment (314,315).

Also, according to the model in Figure 4-7, the potassium that enters TAL cells via the Na^+:K^+:2 Cl^- cotransporter recycles, to a large extent, across the apical membrane by way of a K^{+-} conductive pathway. This apical K^+ recycling serves several purposes. First, it ensures a continued supply of luminal potassium in order to sustain Na^+:K^+:2 Cl^- cotransport. Without recycling, the luminal K^+ concentration would fall rapidly as a consequence of K^+ entry via Na^+:K^+:2 Cl^- cotransport and would limit net NaCl absorption. Second, the apical membrane potassium current provides a pathway for net potassium secretion by the TAL. In mouse mTAL, for example, the rate of K^+ secretion amounts to about 10% of the rate of net Cl^- absorption (308). K^+ secretion in this segment is an active process, ultimately driven by the $Na^+ + K^+$–ATPase, proceeding in the face of a lumen-positive transepithelial potential. Third, under open circuit conditions, the transcellular and paracellular pathways form a current loop in which the currents traversing the two pathways are of equal size, but opposite direction. The potassium current from cell to lumen polarizes the lumen and causes an equivalent current to flow from lumen to bath through the paracellular pathway (305,316). Since the paracellular pathway is cation-selective ($P_{Na}/P_{Cl} = 2$ to 6), the majority of the current through the paracellular pathway is carried by sodium moving from the lumen to the interstitium. This paracellular absorption of sodium increases the efficiency of sodium transport by the TAL (317). With reference to Figure 4-7, for each Na^+ transported through the cell-and requiring utilization of ATP one Na^+ is transported through the paracellular pathway without any additional expenditure of energy. Finally, the apical K^+ current satisfies the continuity requirement imposed by a high degree of conductive Cl^- efflux across basolateral membranes (312,318).

A small component of sodium transport by the TAL is accounted for by sodium bicarbonate absorption (319). In the rat TAL, the rate of sodium bicarbonate absorption is roughly 5% to 10% of that for sodium chloride absorption. Sodium bicarbonate absorption is thought to be mediated by an apical membrane amiloride-sensitive Na^+/H^+ exchanger and a basolateral membrane electrogenic 3 $Na^+/(HCO_3^-)$ cotransporter (319,320).

The following sections will describe the individual components of the mechanism for TAL salt transport (Fig. 4-7) in greater detail.

Apical Na^+:K^+:2Cl^- Cotransport

Studies of Cl^- transport across apical membranes of intact TAL segments (298,301,308) and in isolated membrane vesicle preparations (321) established that the predominant mode for Cl^- entry into the TAL cell is via a Na^+:K^+:2 Cl^- cotransporter. A characteristic feature of this transporter is its sensitivity to inhibition by furosemide, bumetanide, and other 5-sulfamoylbenzoic acid derivatives (322). Measurement of isotope flux into TAL cells or membrane vesicles prepared from the inner stripe of outer medulla yielded a stoichiometry of 1 Na^+:1 K^+:2 Cl^- cotransport (321,323,324). K^+-independent NaCl cotransport has also been described under certain conditions (325,326).

The proteins that mediate Na^+:K^+:2 Cl^- cotransport have been cloned. An absorptive form of Na:K:2 Cl cotransporter, referred to as BSC1 or NKCC2, was initially cloned by Gamba et al. (327) based on sequence homology to the thiazide-sensitive Na:Cl cotransporter (see below). A second Na^+:K^+:2 Cl^- cotransporter, NKCC1, was cloned by Payne et al (328). Several lines of evidence indicate that NKCC2 (BSC1) is the protein that mediates apical salt entry in the thick ascending limb. First, *in situ* hybridization and single-nephron reverse transcriptase polymerase chain reaction (RT-PCR) studies demonstrated expression of NKCC2 primarily in the MTAL and CTAL (327,329,330). Moreover, immunohistochemical studies using antibodies to NKCC2 indicate that the protein is localized to the apical membrane of the MTAL and CTAL (329). The most compelling evidence, however, is the identification of inactivating mutations in the NKCC2 gene in some patients with Bartter syndrome (Table 4-1). This syndrome, characterized by hypokalemia, metabolic alkalosis, hyperaldosteronism, and normal blood pressure, results from a defect in salt absorption by the TAL. Simon et al. (331) first reported the linkage of Bartter syndrome to mutations in the NKCC2 (BSC1) gene. Subsequently, a large number of different NKCC2 mutations have been found in families with Bartter syndrome (332,333). These results provide strong support for the conclusion that NKCC2 is responsible for apical Na^+:K^+:2 Cl^- cotransport in the thick ascending limb.

The NKCC2 cDNA encodes a protein containing ~1,100 amino acids and having a predicted molecular weight of 115 to 120 kDa (327). Six isoforms of NKCC2 have been identified, which are the result of alternative splicing in the 5′ and 3′ regions of the NKCC2 gene. Three 5′ splice products, termed A, B, and F, were reported by Payne and Forbush (334). These variants differ only in a 96-bp region, which encodes the amino acids forming the second transmembrane domain. The isoforms show differential expression within the thick ascending limb. Using RT-PCR, Yang et al. (330) examined the distribution of NKCC2 isoforms in single-nephron segments. The A isoform was found in both the cortical and medullary TAL, the B isoform was restricted to the cortical TAL, while the F isoform was present in the medullary, but not cortical, TAL and, to a lesser extent, in the outer medullary collecting duct. All three products are capable of mediating Na^+:K^+:2 Cl^- cotransport. However, the isoforms have different transport properties that may have physiologic relevance. Specifically, the A and B isoforms have higher affinities for Na^+, K^+, and Cl^- than the F isoform. The F isoform appears to have a greater transport capacity than the other isoforms (335). Thus, the presence of the F isoform in the medullary thick ascending limb could account for the observed high rates of NaCl transport relative to the cortical segment (Table 4-4), while the expression of the A and B isoforms in the cortical thick ascending limb may subserve the ability to achieve lower luminal NaCl concentrations in that segment. Alternative splicing at the 3′ end of NKCC2 produces additional isoforms with either long (C9) or short (C4)

carboxy termini (336). Under isotonic conditions, the truncated (C4) isoforms do not mediate Na:K:2 Cl cotransport and inhibit the transport activity of the full-length (C9) isoforms when the two are coexpressed (337). This inhibition is relieved by cAMP (337). Under hypotonic conditions, however, the C4 isoforms mediate K^+-independent NaCl cotransport, which is stimulated by cAMP (338) and may account for the K^+ independent NaCl transport noted in earlier studies (325,326).

The inhibition of C9 NKCC2 isoforms by C4 isoforms suggests a physical interaction between these proteins. Biochemical studies have recently established that NKCC2 exists as a dimer (339). Thus, it is possible that different combinations of NKCC2 isoforms within the dimer could produce transporters with a wide variety of functional properties. Moreover, the subunit composition of the dimers may be a point of physiologic regulation.

Apical K^+ Conductance

An important feature of the luminal membrane of the TAL and nonmammalian diluting segments is a barium-inhibitable potassium conductance (305,308,340). This apical membrane K^+ conductance allows K^+ to be recycled from the cell back into the luminal fluid to support further NaCl absorption via the NaCl cotransporter. Using measured values of intracellular potassium activity (rabbit cTAL [341] and *Amphiuma* [310]), apical membrane conductance, and intracellular voltage, it can be shown that the measured apical membrane conductance is sufficient to provide for the recycling of all of the potassium uptake via the Na^+:K^+:2 Cl^- cotransporter.

Three types of potassium channels have been characterized in the apical membrane of the thick ascending limb: a high-conductance (150 pS) calcium-activated K^+ channel, which does not contribute to net K^+ transport (342), an intermediate-conductance (70 pS) K^+ channel (343,344), and a low-conductance (30 pS) K^+ channel (344,345). The latter two channels account for the majority of the apical K^+ conductance and K^+ recycling (346). Both the intermediate- and the low-conductance channels are inhibited by intracellular ATP. This inhibition by ATP is reversible and is dependent on the ratio of ATP to ADP. These properties would render the channels sensitive to changes in cell metabolism. For example, during an increase in transcellular salt absorption, ATP would be consumed and ADP produced. The fall in the ATP/ADP ratio, then, might activate apical K^+ channels providing more K^+ recycling to support further salt absorption. The activities of both the intermediate and low conductance channels are increased by high dietary potassium (347).

Ho et al. (348) cloned an ATP-dependent potassium channel from rat kidney. This channel, termed ROMK, is the prototype for a large family of inward-rectifying K channels (Kir channels) having a peculiar architecture, featuring two transmembrane-spanning domains with an intervening pore-forming region (Fig. 4-8). The properties of the ROMK channels, their regulation by intracellular pH (349), ATP, phosphorylation (350), and dietary K^+, and their localization (351–353) all suggest that ROMK is the low-conductance K^+ channel of the thick ascending limb (346). Further support for this view comes from studies of ROMK knockout mice. Specifically, no low-conductance K^+ channels were detected by patch-clamp analysis in ROMK(−/−) mice, while channels in heterozygous ROMK(+/−) mice were detected at a rate approximately one-half that of wild-type ROMK(+/+) mice (354). Complicating this interpretation is the observation that ROMK(−/−) mice also lack intermediate conductance K^+ channels in the thick ascending limb (347). It has been proposed that the intermediate-conductance channel may be a heteromeric protein containing ROMK and other, as yet, unidentified subunits.

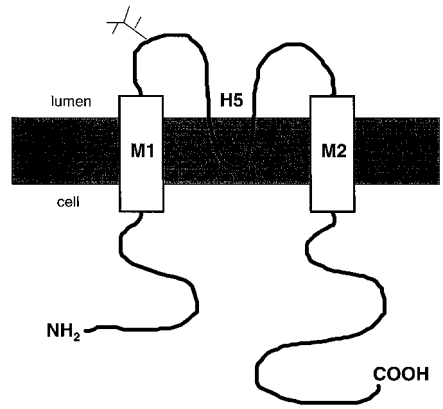

FIGURE 4-8. A schematic representation of the proposed topology of the ROMK (Kir 1.1) potassium channel. The pore-forming region, H5, is flanked by two transmembrane domains. The H5 region is highly conserved within the Kir family. Based on Ho K, Nichols CG, Lederer WJ, et al. Cloning and expression of an inwardly rectifying ATP-regulated potassium channel. *Nature (London)* 1993;362:31.

The notion that ROMK is the predominant channel responsible for apical K^+ recycling in the thick ascending limb is also supported by the finding of mutations in the ROMK gene in some families with Bartter syndrome (333,355–358). At least some of these mutations have been confirmed to result in defective K^+-channel function (359). As noted, Bartter syndrome results from a defect in thick ascending limb salt transport. Thus, the presence of ROMK mutations as a cause of Bartter syndrome indicates the important role of ROMK in net salt absorption by the thick ascending limb (Table 4-1).

Basolateral Membrane Cl^- Transport

Cl^- exit across the basolateral membrane of TAL cells is largely conductive, proceeding down its electrochemical gradient through Cl^--selective channels in the basolateral membrane (298,306,360). Paulais and Teulon (361) detected a 40 pS anion-selective channel ($P_{Cl}/P_{Na} = 20$) in the basolateral membrane of collagenase treated mouse cortical TAL segments. The current-voltage relations of the channel were linear in both the cell-attached and excised configurations. The open probability of the channel in the cell-attached state was voltage-dependent, increasing as the membrane was depolarized. Greger et al. (362) described a Cl^- channel in the basolateral membrane of rat TAL segments. This channel also had a conductance of about 40 pS, but rather than having a linear I-V relation, this channel exhibited outward rectification. The open probability increases with depolarization in both the cell-attached and excised-patch configuration. A low-conductance Cl^- channel (8 to 10 pS) having linear I-V relations has also been detected in the basolateral membrane of TAL cells (363,364). The activity of this channel was increased following incubation with cAMP-dependent protein kinase and ATP (364).

Chloride channel activity was also demonstrated when vesicles from rabbit outer medulla were incorporated into planar lipid bilayers (365). These channels were anion-selective ($P_{Cl}/P_K = 10$) and had a single-channel conductance of 80 to 90 pS in 320 mM KCl solution. The open probability of this channel was voltage-dependent, increasing activity with depolarizing voltages. These channels are also seen in vesicles made from highly purified suspensions of mouse thick ascending limb (366) and from cultured mouse medullary thick limb cells (367), confirming their origin as the thick ascending limb.

A unique feature of these medullary chloride channels is the dependence of channel activity on the chloride activity of the solutions bathing the lipid bilayer (367,368). Increases in the Cl^- concentration of the extracellular solution over the range 50 to 300 mM produced almost linear increases in channel open probability. In contrast, the Cl^- concentration dependence of the intracellular surface of the channel was most pronounced in the range of 0 to –50 mM Cl^- and was almost saturated at 50 mM Cl^-. Dependence of channel activity on extracellular Cl^- has also been shown for certain members of the ClC family of Cl^- channels (369). As will be discussed, it is believed that the basolateral Cl^- channel of the TAL is a member of this family of channels.

A chloride channel, termed ClC-0, was cloned from the electric organ of the *Torpedo* ray by Jentsch et al. (370). Based on homology to the ClC-0 channel, Uchida et al. (291), Adachi et al. (371), and Kieferle et al. (372) reported the cloning of two chloride channels, ClC-K1 and ClC-K2 expressed exclusively within the rat kidney. In the human, the two corresponding channels are denoted hCLC-Ka and hCLC-Kb and are located contiguously on chromosome 1 (373). Because of the high degree of sequence similarity between hCLC-Ka and hCLC-Kb, it is not certain which of the human channels correspond to CLC-K1 versus CLC-K2, although the distribution of ClC-K2 along the nephron most closely matches that of hClC-Kb (374). Zimniak et al. (375) have cloned a cDNA from rabbit renal outer medulla, which they named rbClC-Ka, that shares 80% homology to the rat CLC-K1 and CLC-K2. The distribution of rbClC-Ka along the nephron resembles that of CLC-K2 rather than ClC-K1 (375,376). Immunologic and molecular evidence now indicates that CLC-K2 (and the human homolog hCLC-Kb) is the channel that mediates chloride efflux across the basolateral membrane of the thick ascending limb (293,371,375–377). Indeed, mutations in the human homolog of CLC-K2 (hCLC-Kb) have been identified in patients with neonatal Bartter syndrome (378,379). Thus, as was the case for the NKCC2 and ROMK proteins, linkage of ClC-K2 (hClC-Kb) to Bartter syndrome establishes the importance of its gene product in transepithelial NaCl transport in the thick ascending limb (Table 4-1).

Some of the difficulty in assigning a role for CLC-K2/hCLC-Kb in thick ascending limb Cl^- transport was the inability to achieve functional expression in heterologous systems. This was recently shown to be because of the requirement of an additional subunit for channel activity. This subunit, named barttin, was originally identified by positional cloning of the BSND gene, which is responsible for a form of Bartter's syndrome (Table 4-1), accompanied by sensorineural deafness (380). By *in situ* hybridization (380) and immunohistochemistry (381), barttin is located in the basolateral membranes of the thin and thick ascending limb and also in the stria vascularis of the inner ear, where it is believed to play a role in K^+ secretion into endolymph. Although expression of ClC-Kb or barttin alone in *Xenopus* oocytes produces no significant conductance, their coexpression results in a large Cl^- conductance (381,382). Barttin is believed to act, at least in part, by increasing the cell surface expression of ClC-Kb (381,382). The mutations of barttin identified in Bartter's patients generally, although not always, impair the ability of barttin to produce a Cl^- conductance when expressed with ClC-Kb (381,382).

Some uncertainty remains regarding the role of basolateral electrochemical KCl symport in net Cl^- efflux across that membrane. In the rabbit cortical TAL, Greger and Schlatter (306) concluded that KCl symport accounted for about one-third of basolateral Cl^- efflux. This conclusion is based upon the following observations: An increase in the K^+ concentration or decrease in the Cl^- concentration of the basolateral solution depolarized the basolateral membrane; bath barium depolarized the basolateral membrane and abolished the K^+ induced changes in V_{bl}; barium had no discernible effect on the transepithelial resistance or fractional resistance of the basolateral membrane. The lack of an effect of barium on resistance persuaded the investigators to propose that a barium-sensitive KCl cotransporter was present (306). A cloned KCl cotransporter, KCC4, is present in the basolateral membrane of the thick ascending limb (383). However, its physiologic role in thick ascending limb function is not known.

Synchronous Na^+–H^+:Cl^-–HCO_3^- Exchange

Friedman and Andreoli (384) found that net Cl^- absorption and the transepithelial voltage were doubled when CO_2 and HCO_3^- were added to the external solutions bathing cTAL segments. Since the (CO_2 + HCO_3^-)-stimulated rate of NaCl absorption did not result in net CO_2 transport and could be abolished by the lipophilic carbonic anhydrase inhibitor ethoxyzolamide or by the luminal addition of the anion-exchange inhibitor SITS or DIDS, it was proposed that the apical membrane of the mouse cTAL contains parallel, near-synchronous Na^+/H^+:Cl^-/HCO_3^- exchangers in addition to a Na^+:K^+:2Cl^- cotransporter. Addition of CO_2 and HCO_3^- to the bathing solutions has no effect on net NaCl transport in either the rabbit cTAL or the mouse mTAL. Both the rat and mouse mTAL do contain Na^+/H^+ exchangers in their apical membranes. However, in these segments, Na^+–H^+ exchange plays a role in net HCO_3^- transport and cell pH regulation rather than transcellular NaCl absorption (319,385). The NHE_3 isoform of the Na^+/H^+ exchanger is the major isoform expressed in the apical membrane of the thick ascending limb (386).

REGULATION OF SALT ABSORPTION IN THE TAL

The rate of salt absorption by the thick ascending limb is modulated by at least two general classes of factors: physical factors, such as luminal flow rate and the composition and osmolality of luminal and peritubular fluids; and hormones, such as vasopressin and glucagon, which exert their effects through interactions with specific cellular receptor proteins. The regulation of TAL salt transport is characterized by considerable interspecies variation as well as intranephron heterogeneity. A good example of both species and intranephronal heterogeneity is the effect of antidiuretic hormone (ADH) vasopressin on TAL transport.

In Table 4-5 are summarized the effects of ADH on salt and water transport in segments of the mouse, rabbit, and rat TAL perfused *in vitro*. The cellular effects of ADH in this segment are mediated by cAMP. Thus, ADH stimulates adenylate cyclase activity in the TAL (209,387), and cAMP analogs (298) and forskolin (388) mimic the effects of ADH on transport in this segment. ADH increases the transcellular electrical conductance of the mouse mTAL and this conductance increase is a major element in the mechanism for the hormone-dependent increase in the rate of net salt absorption (307,308). The available evidence suggests that both the apical and basolateral membrane conductances are increased by ADH. In apical membranes, ADH increases conductance by increasing the functional number of K^+ channels (312); this increase occurs even when net salt absorption is abolished by furosemide. ADH increases apical NaCl entry acutely via activation of NKCC2 (318) and chronically by increasing the abundance of NKCC2 (389).

The predominant portion of the ADH-induced increase in cellular conductance is accounted for by an increase in the basolateral membrane Cl^- conductance (312,318). Two

TABLE 4-5

EFFECTS OF ADH ON NaCl TRANSPORT BY THICK ASCENDING LIMB SEGMENTS

Species	ADH	V_e (mV)	J_{NaCl} (pmol/sec/cm^2)	Ref.
Mouse	–	5	2600	(298,308,716,717)
	+	11	10,800	(298,308,716,717)
Rat	–	2.4–3.3	4825	(717–719)
	+	3.6–4.7	7770	(717–719)
Rabbit	–	3–7	6400	(295,717)
	+	3–7	Unchanged	(295,717)

mechanisms have been suggested for the hormone-dependent increase in basolateral Cl^- conductance. Schlatter and Greger (313) have proposed that the ADH-induced increase in intracellular cAMP results in a direct increase in chloride channel activity. Such a mechanism has been amply demonstrated in Cl^--secreting epithelial, such as the trachea and intestine.

Alternatively, ADH might enhance Cl^- conductance indirectly by increasing apical membrane Cl^- entry (312,318). According to this proposal, an ADH-dependent activation of apical membrane Na^+:K^+:2 Cl^- cotransporter and K^+ channels leads to an increase in intracellular Cl^- concentration. Since the activity of basolateral Cl^- channels is exquisitely sensitive to changes in intracellular Cl^- concentration (365,367,368), this increase will translate into an increase in the basolateral membrane Cl^- conductance.

Prostaglandins

Prostaglandin 1 (PGE_2), the major product of prostaglandin synthesis in the renal medulla, participates in a local negative-feedback system that modulates the rate of NaCl absorption by the TAL. PGE_2resulted in a 50% reduction in ADH-stimulated net Cl^- absorption in isolated perfused mammalian mTAL segments, but had no effect in the absence of ADH (388,390). It is likely that PGE_2 inhibits ADH-stimulated generation of cAMP in the mTAL by activating an inhibitory G protein, G_i (388,391). Although interstitial cells are a major source of PGE_2 production in the renal medulla, thick ascending limb cells can metabolize arachidonic acid through at least two pathways. Escalante et al. (392) demonstrated that purified medullary thick ascending limb cells produce 20-hydroxyeicosatetraenoic acid (20-HETE) via the cytochrome P450 enzyme, ω-hydroxylase. 20-HETE was subsequently shown to inhibit NaCl transport in the thick ascending limb at steps, which include the apical K^+ channel (393) and the Na^+:K^+:2 Cl^- cotransporter (392). Thick ascending limb cells, particularly in the macula densa (MD), also express COX-2 (394). COX-2 expression in these cells may be coupled to renin secretion. Thus, COX-2 expression is increased by salt restriction, diuretics, and, in Bartter syndrome, all conditions characterized by hyperreninemia (395). Moreover, COX-2 inhibitors reduce renin secretion in these settings (396,397).

Osmolality

Peritubular osmolality is a third factor regulating mTAL salt absorption. In isolated mouse and rabbit TAL segments, increases in peritubular osmolality rapidly and reversibly inhibit the ADH-stimulated rate of net Cl^- absorption (299,398). Molony and Andreoli (398) determined that hypertonicity inhibits the basolateral membrane chloride conductance. This inhibition of transcellular salt absorption occurs at a locus beyond the generation of cAMP, since supramaximal concentrations of either ADH or cAMP are unable to reverse the hypertonicity-mediated effect (299). Thus, increasing the absolute magnitude of interstitial osmolality provides a negative feedback signal, which can reduce ADH-dependent salt absorption by the mTALH. A scheme integrating the effects of ADH, PGE2 and peritubular osmolality on urinary concentration is presented in Figure 4-9.

Hypercalcemia

Hypercalcemia often results in an ADH-resistant urinary concentrating defect, that is, nephrogenic diabetes insipidus (399). At least part of this concentrating defect results from the inhibition of ADH-stimulated cAMP production in the TAL by calcium (400). Preincubation of tubule segments with pertussis toxin abolishes the effect of hypercalcemia on cAMP generation, indicating that the inhibition of cAMP generation is

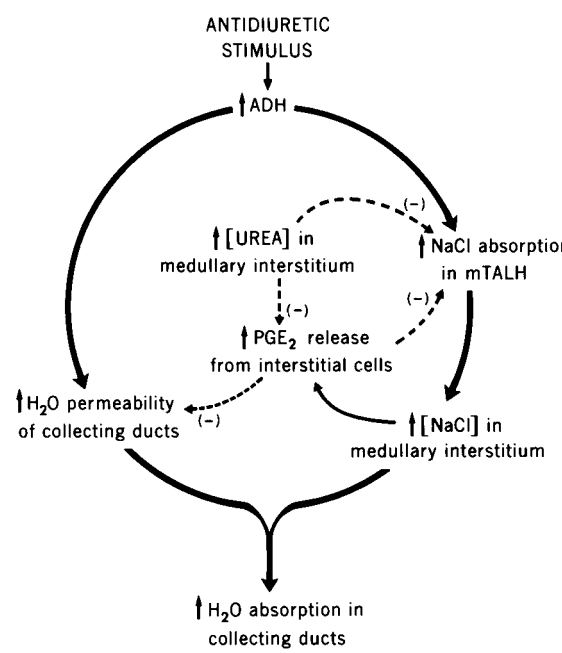

FIGURE 4-9. Interrelations of antidiuretic hormone (*ADH*), prostaglandin E_2 (*PGE_2*), and interstitial osmolality on NaCl absorption by medullary TAL and on water absorption from collecting ducts. Dashed lines indicate negative feedback loops. (From: Hebert SC, Andreoli TE. Control of NaCl transport in the thick ascending limb. *Am J Physiol* 1984;246:F745, with permission.)

mediated through activation of G_i(401). The effects of hypercalcemia are mediated by a G protein-coupled calcium-sensing receptor (CaSR) present on the basolateral membrane of TAL cells (402,403). Activation of this receptor also inhibits the activity of the 70 pS apical K channel via the production of 20-HETE, a P450 metabolite of arachidonic acid (404,405). Even changes in serum calcium within the physiologic range can alter NaCl absorption via the CaSR (406). This may help to explain the hypotensive effect of high calcium intake in salt-sensitive hypertensive individuals (407).

Modulation of NaCl Absorption by Other Peptide Hormones

In addition to ADH, a number of other peptide hormones stimulate adenylate cyclase activity in the TAL. In the mouse and rat, glucagon stimulates NaCl reabsorption and increases the transepithelial potential in isolated microperfused mTAL segments (408,409). Calcitonin and PTH stimulate sodium transport in the cortical, but not medullary, portions of the TAL (409).

Angiotensin II (Ang II) receptors are present in the thick ascending limb (410). Ang II has been reported to both stimulate and inhibit sodium transport in the thick ascending limb (411,412). Chronic infusion of Ang II increased the abundance of NKCC2 in rat outer medulla by 87% (413). In a model of heart failure, in which Ang II levels are elevated, NKCC2 expression was also increased and this increase could be prevented by treatment with an angiotensin receptor antagonist (414).

Adrenergic Agents

β-Agonist-sensitive adenylate cyclase activity is present in the rat, but not rabbit TAL (209,415). Likewise, β-adrenoceptors have been detected along the rat TAL by autoradiographic localization (416,417). The physiologic effects of adrenergic agents have been tested in micropuncture and in *in vitro* microperfusion studies. DiBona and Sawin (418) demonstrated an enhancement of loop NaCl absorption during low-frequency renal nerve stimulation. Acute renal denervation, on the other hand, depressed NaCl absorption by the loop of Henle (419).

Micromolar concentrations of isoproterenol stimulate net Cl^- absorption by *in vitro* perfused mouse medullary and cortical TAL (420). The effects of isoproterenol on NaCl absorption in these segments can be blocked by propranolol.

Mineralocorticoids

Several lines of evidence suggest that mineralocorticoids influence NaCl transport in the TAL (421). First, clearance studies indicate that aldosterone increases free water clearance in adrenalectomized animals (422), consistent with an increase in NaCl absorption by the TAL. Second, nuclear mineralocorticoid receptors are present in both the medullary and cortical portions of the TAL of rat and rabbit (423,424). 11β-Hydroxysteroid dehydrogenase, the enzyme which confers mineralocorticoid specificity to cells, has been detected in the TAL using enzymatic assays (425) and by immunohistochemistry (426). Third, aldosterone appears to modulate the activity of certain transport related enzymes in the TAL, such as $Na^+ + K^+$–ATPase (427,428) and citrate synthase (428). In the adrenal-intact mouse, pharmacologic doses of mineralocorticoid increased as $Na^+ + K^+$–ATPase activity of mTAL segments by 25% (429). Other studies, however, have failed to show an effect of mineralocorticoids on as $Na^+ + K^+$–ATPase activity in the TAL (430–432).

Finally, *in vivo* and *in vitro* microperfusion studies have demonstrated effects of aldosterone on TAL sodium transport. In an *in vivo* microperfusion study of superficial loop segments, Stanton (433) showed that adrenalectomy inhibited loop of Henle sodium absorption by 33%. Aldosterone, but not dexamethasone, increased sodium transport to control levels. Work and Jamison (434) demonstrated, using *in vitro* perfusion, that the rate of salt absorption and the transepithelial voltage in TAL segments dissected from adrenalectomized rats were reduced by 50% compared to tubules from adrenal intact rats. Moreover, administration of aldosterone to the adrenalectomized rats returned salt absorption and transepithelial voltage back to control values. In the rat medullary TAL, adrenalectomy also decreases $NaHCO_3$ absorption by 33% (435). Taken together, these data indicate that aldosterone stimulates sodium absorption by the mammalian TAL. In contrast, recent studies failed to show an effect of aldosterone on the abundance of NKCC2 in rat outer medulla (436) or in NKCC2 activity *in vitro* (437).

Nitric Oxide

Acute administration of nitric oxide donor or L-arginine, the substrate for NOS, decreases NaCl absorption in isolated perfused thick ascending limb segments (252,438). The effect of L-arginine on NaCl transport can be blocked by L-NAME, and inhibitor of nitric oxide synthase (NOS), indicating that endogenous production of NO mediates the effect of L-arginine. The inhibitory effect of NO on net NaCl absorption appears to involve, at least in part, inhibition of NKCC2 activity (439). The thick ascending limb expresses all three isoforms of NOS (440). Plato et al. (441) used mice deficient in the various NOS isoforms in determining that the effect of L-arginine is mediated by e-NOS rather than i-NOS or n-NOS. In contrast to its effect on NaCl absorption, NO stimulates $NaHCO_3^-$ absorption in the thick ascending limb (412). Finally, although short-term exposure to NO inhibits NaCl absorption, chronic exposure increases NKCC2 expression (442), which could translate into increased NaCl absorption.

Sodium Balance

Dietary sodium restriction in rats results in a transient decrease in NKCC2 expression (443) while high sodium intake has no major effect on NKCC2 expression (444). Chronic treatment with furosemide in conjunction with a high-sodium diet increases NKCC2 expression (445). The latter phenomenon may account for the development of diuretic resistance and the interdose rebound in sodium absorption in patients chronically treated with loop diuretics.

THE DISTAL NEPHRON

Anatomical Considerations

The distal nephron may be divided into three segments: the distal convoluted tubule (DCT), the connecting tubule (CNT), and the collecting duct. While these segments are clearly delineated in the rabbit (446), in many species the transition between contiguous segments is gradual (447). Therefore, distal tubule segments are most accurately defined by their respective cell types: the DCT cell, found only in the DCT; the CNT cell,

found only in the CNT; and the principal cell, found in cortical collecting tubules and in medullary collecting tubules.

Na^+ Transport in Distal Convoluted Tubule and Connecting Segment

General Characteristics

Much of the information regarding salt transport in the distal tubule has derived from micropuncture studies which did not distinguish between the early and late distal tubule. For this reason, the DCT and the connecting segment will be discussed together. The DCT absorbs roughly 10% of the filtered sodium load (448–450). Fluid enters the DCT with a sodium concentration of 25 to 30 mM, but salt is added along the initial 20% of the DCT, so that the sodium concentration averages 50 mM at a point 200 to 300 μm from the MD (451). From there, tubular sodium concentration decreases along the DCT to a value of approximately 30 mM at the end (449,452). Tubular fluid to plasma sodium ratios as low as 0.10 have been observed during stationary microperfusion (270,452,453). This finding, together with the presence of the lumen-negative potential difference (see below), establishes clearly the active nature of sodium absorption in this segment.

Sodium absorption by the DCT is load-dependent. That is, over a wide range of delivery rates, the proportion of sodium absorbed by the DCT remains constant at 80% (449). At high tubular fluid flow rates, the fall in luminal sodium concentration along the tubule is attenuated; thus more sodium is available to distal sodium absorptive sites at high than at low flow rates.

Sodium absorption in the rat DCT has been reported to be 25 to 30 pEq/mm^2/sec; that is, about one-third of the rate occurring in the PCT (454). However, rates comparable to those in the PCT have been reported in isolated perfused DCT (455–457).

Electrophysiologic Considerations

The transepithelial voltage in the earliest loops of the DCT, measured with fine-tip electrodes, ranges from –9 to –19 mV, lumen-negative (458,459). Small lumen-positive voltages, +3.7 to 5.7 mV, have been measured both in micropuncture experiments, by using low-resistance micropuncture electrodes (460,461), and in isolated, perfused early DCT segments (462). The variation in lumen voltages may reflect differences in the experimental conditions under which the measurements were made. When the composition of the luminal perfusate resembled distal tubular fluid, that is, having a low NaCl concentration (see above), the lumen voltage tended to be slightly positive whereas when tubules were perfused with solutions resembling plasma, that is, high NaCl concentration, the lumen voltage was negative (460). The lumen negative potential measured with high NaCl solutions was abolished by luminal amiloride, an inhibitor of epithelial sodium channels. The positive voltage under *in vivo* conditions is a salt dilution potential arising from the differential permeability of the DCT to Na and Cl, while the lumen negative potential under symmetric solutions reflects active Na^+ reabsorption via amiloride-sensitive sodium channels (see below).

The transepithelial electrical potential in the late DCT of rats measured *in vivo* ranges from –37 to –60 mV (463). Values in isolated rabbit DCT and CNT segments perfused with symmetric solutions are less negative, –5 to –30 mV (462,464–467). The voltage can be inhibited by peritubular ouabain or luminal amiloride (466,468), and is quite sensitive to changes in perfusion pressure and flow rate, decreasing with high pressures or flow rates (464).

The transepithelial resistance of the rat DCT decreases from 337 Ω-cm^2 in the early DCT to 135 Ω-cm^2 in the late DCT (469,470). Somewhat lower electrical resistances have been found in isolated perfused rabbit DCT (466,467) and CNT (471). The passive ionic permeability determined by single-ion dilution voltages (472) reveals the following sequence: $K^+ > Na^+ > Cl^-$.

Intracellular microelectrode analysis of rabbit DCT cells has yielded conflicting results. Yoshitomi et al. (466) found evidence for both K^+ and Na^+ conductive pathways in the apical membrane and K^+ and Cl^- conductive pathways in the basolateral membrane. The apical membrane accounted for 80% of the total cellular resistance. In contrast, Velazquez et al. (473,474) found the apical membrane to comprise over 99% of the cellular resistance and could not detect any Na^+ or K^+ conductances in that membrane. Perhaps some of this discrepancy could have arisen from the examination of different portions of the DCT in the two laboratories. Ellison et al. (457) have demonstrated that sodium reabsorption in the early DCT is largely mediated by a thiazide-sensitive, neutral NaCl cotransporter, while sodium absorption in the late DCT involves an amiloride sensitive electrogenic pathway. Thus, Yoshitomi et al. (466) may have described the late DCT and Velazquez et al. (473,474) the early DCT.

Mechanism of Na^+ Absorption

The available evidence permits the delineation of certain facets of Na^+ absorption in the DCT. A general model for these mechanisms is presented in Figure 4-10. Cardinal among these are the following characteristics.

Basolateral Electrogenic Na^+ Pump. The voltage (V_{bl}) across the basolateral membrane of the *Amphiuma* (475) and rabbit (466,467,474) DCT is –60 to –90 mV. A reduction in the luminal sodium concentration causes V_{bl} to depolarize, while increases in the sodium concentration hyperpolarize V_{bl} (475,476). In addition, V_{bl} depolarizes after ouabain

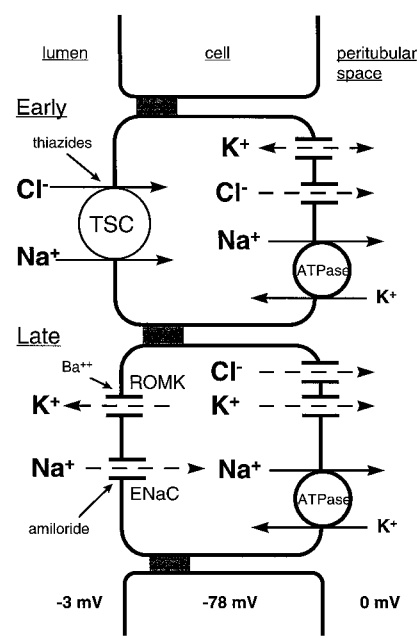

FIGURE 4-10. Model of NaCl absorption by cells of the early and late DCT. The early DCT cell possesses a thiazide-sensitive, electroneutral NaCl cotransporter. The late DCT cell resembles the principal cell of the CCD.

treatment (477). These observations are consistent with the notion that apical sodium entry stimulates the electrogenic $Na^+ + K^+$–ATPase system in the basolateral membrane.

Apical NaCl Cotransport. The absorption of sodium and chloride in the early DCT are coupled (473). The coupling of chloride to sodium entry provides a mechanism to maintain the intracellular Cl^- activity above its electrochemical equilibrium (478). The early distal tubule is the site of action of thiazide diuretics (455,457,465). Autoradiographic studies (479) and immunocytochemical studies (480) have demonstrated thiazide-binding sites in the apical membranes of DCT and connecting tubule cells.

A thiazide-sensitive neutral NaCl cotransporter (TSC) was cloned from the flounder urinary bladder using an expression-cloning strategy (481). A homologous transporter, Na:Cl cotransporter (NCC) (SLC12A3) was subsequently cloned from the mammalian kidney (327). The transporter shares considerable sequence homology to the bumetanide-sensitive Na^+:K^+:2 Cl^- cotransporter (NKCC2) present in the TAL (327,334), yet exhibits markedly different inhibitor sensitivity and ionic requirements compared to the latter transporter. NCC transports NaCl with a 1:1 stoichiometry, is K-independent, and is inhibited by thiazide diuretics (482). The expression of NCC within the kidney has been determined by *in situ* hybridization (483,484), RT-PCR of single-nephron segments (330), and by immunohistochemistry (480,483,485–487). The results indicate that NCC is expressed in the apical membrane of DCT cells and extends, in most species, into the connecting segment. In addition, mutations in the NCC gene have been linked to the pathogenesis of Gitelman syndrome (333,488–491). This syndrome resembles Bartter syndrome (hypokalemia, alkalosis, sodium-wasting), except that urinary calcium excretion is reduced in Gitelman syndrome and is elevated in most cases of Bartter syndrome. Thus, physiologically, Gitelman syndrome mimics the effects of thiazide diuretics. The Gitelman mutations have little effect on the functional and biochemical properties of NCC protein, but markedly reduce its cell surface expression (492,493). That mutations in the NCC gene are seen in Gitelman syndrome provides further support for the view that the NCC gene product is the thiazide-sensitive cotransporter of the DCT. Studies of another inherited disorder of distal sodium transport, Gordon's syndrome, have yielded additional insights into the regulation of NCC function. Gordon's syndrome, or pseudohypoaldosteronism type II (PHAII) is the phenotypic opposite of Gitelman syndrome and is characterized by hypertension, hyperkalemia, and metabolic acidosis (Table 4-1). The disorder is largely corrected by thiazide diuretics. These features suggested that an increase in NCC activity may be involved in the pathogenesis of PHAII. Positional cloning demonstrated that PHAII is caused by mutations in either of two serine-threonine kinases, WNK1 and WNK4 (494). Subsequent studies have shown that WNK4 normally inhibits NCC-mediated NaCl transport, likely by reducing cell surface expression of NCC (495,496). Mutant WNK4, in contrast, is unable to inhibit NCC activity and results in higher rates of NaCl transport (495,496). WNK4 also affects the activity of apical ROMK channels (497) and the Cl^- permeability of the paracellular pathway (498). The latter observation supports the speculation, made over 20 years ago by Schambelan et al. (499), that an increased paracellular Cl^- permeability was responsible for the hyperkalemia and metabolic acidosis in PHAII. The mechanism whereby WNK1 produces PHAII is less well defined. Yang et al. (495) found that WNK1 does not directly affect NCC activity, but modulates the inhibition of NCC by WNK4. They propose that mutations, which increase the activity of WNK1, prevent WNK4 from inhibiting NCC-mediated transport.

In addition to thiazide-sensitive NaCl cotransport, a component of electroneutral NaCl absorption may be mediated by parallel actions of Na^+/H^+ and Cl^- organic anion exchangers. The net effect of synchronous Na^+/H^+ and Cl^- base exchange, as discussed for the proximal tubule (Fig. 4-5), is the reabsorption of NaCl with recycling of the organic anion. In this regard, the addition of either formate or oxalate to the perfusate of microperfused rat distal tubules stimulated chloride transport (140). Moreover, the formate- or oxalate-stimulated component of Cl^- transport was not inhibited by thiazide diuretics. The protein(s), which mediates the Cl^- anion exchange, is not known. CFEX, which may mediate proximal tubule Cl^- anion exchange, is not expressed in the distal tubule (141). It is interesting, though, that WNK4 inhibits CFEX activity (497). Perhaps the distal tubule Cl^- anion exchanger is also regulated by WNK4.

Apical Conductive Sodium Channels. The entry of sodium into the *Amphiuma* distal tubular cell (476) and late rat DCT cell (456) is inhibited by amiloride, a sodium channel blocker. A sodium channel in the apical membrane would serve to depolarize the membrane and create the observed lumen-negative transepithelial potential. This transepithelial voltage, in turn, is a driving force for passive chloride reabsorption. Sodium channel subunits have been found by immunolocalization in the late DCT in mouse and rat kidney (500,501), but not human kidney (487).

Regulation of NaCl transport in the DCT

Na^+ Delivery. NaCl reabsorption in the DCT is dependent on the delivered load of NaCl (449). The DCT responds to chronic increases in the delivery of NaCl with an increase in the capacity for NaCl transport (450,502,503), as well as marked ultrastructural changes in the DCT cell. These morphologic changes include an increase in the size of the DCT cell, an increase in the basolateral membrane surface area, and in increase in the size of mitochondria (450,504). Accompanying the functional and morphologic changes are an increase in $Na^+ + K^+$–ATPase activity and an increase in thiazide-binding sites (505,506). These effects appear to result from an increase in sodium entry into the DCT cell rather than the increase in distal NaCl delivery or changes in plasma aldosterone or ADH levels, which occur with chronic furosemide treatment. Inhibition of NaCl entry into DCT cells with chronic thiazide treatment resulted in a loss of cell height, loss of normal polarity, and apoptosis of the DCT cells (507). The cellular mechanisms whereby NaCl entry affects transport function and morphology are not known.

Dietary Na^+. Studies in rats and rabbits have yielded conflicting results regarding the effects of increased dietary sodium on DCT morphology and sodium transport. In rats, no consistent effect of a high-sodium diet on either cell morphology, transport rates, or thiazide receptor density could be demonstrated (450,505,508). Rats fed a low-sodium diet (443) or treated with thiazide diuretics (445), however, demonstrated a significant increase in NCC expression, which may have been because of stimulation of mineralocorticoid secretion (436). In contrast, rabbits fed a high- sodium diet developed hypertrophy of DCT cells, an increased rate of DCT sodium reabsorption, and an increase in $Na^+ + K^+$–ATPase activity (509,510).

Steroid Hormones. Currently available evidence suggests that adrenal steroid hormones regulate sodium transport in the DCT. The presence of both mineralocorticoid and glucocorticoid receptors in the DCT has been demonstrated by immunohistochemistry and by hormone binding (423,511–515). In addition, adrenalectomy resulted in a decrease in $Na^+ + K^+$–ATPase activity in the DCT (430). The $Na^+ + K^+$–ATPase

activity could be restored by replacement doses of glucocorticoids (247,430,516), but not by mineralocorticoids (430,431,516). Microperfusion studies of superficial distal tubules (containing both DCT and CNT), however, demonstrated an increase in sodium transport in animals receiving aldosterone infusions (433,517). Both the thiazide- and thiazide-insensitive components of sodium transport were increased by aldosterone (517). The former may reflect neutral NaCl cotransport in the DCT, while the latter reflects electrogenic sodium absorption in the late DCT or CNT. Aldosterone infusion also resulted in an increase in thiazide-binding sites in the renal cortex, as determined by [^{3}H]metolazone binding, an increase in the natriuretic response to thiazide diuretics, and a large increase in NCC protein (517–519). These findings establish NCC as an aldosterone-regulated transporter. By combining immunohistochemical and *in situ* hybridization techniques, Bostonjoglo et al. (515) determined that DCT cells coexpress NCC, mineralocorticoid receptors, and 11β-hydroxysteroid dehydrogenase type 2, an enzyme typically found in mineralocorticoid target sites. Thus, DCT cells, particularly those in the late portions of the DCT, express the key elements required for selective mineralocorticoid actions.

As just mentioned, glucocorticoids increase $Na^+ + K^+$–ATPase activity following adrenalectomy in the DCT (247,430,516). This effect was not blocked by spironolactone, a mineralocorticoid receptor antagonist, suggesting that glucocorticoids were acting via glucocorticoid receptors rather than mineralocorticoid receptors (516). In addition, dexamethasone infusions increased thiazide-sensitive NaCl transport and [^{3}H]metolazone binding sites in adrenalectomized rats (517,518). Nonetheless, the role of glucocorticoids in the physiologic regulation of sodium transport in the DCT remains unclear.

Gonadal steroid hormones may also influence NaCl transport in the DCT. Chen et al. (520) reported gender differences in the density of thiazide receptors and in the natriuretic response to thiazides in rats. Female rats had higher levels of thiazide-binding sites in the renal cortex than males. The levels in females fell following ovariectomy, while levels rose in males following orchiectomy. Moreover, the increase in urinary sodium excretion caused by thiazides was greater in females than in males, suggesting that the differences in thiazide-binding sites were reflective of differences in thiazide-sensitive salt transport *in vivo*. Likewise, using antibodies against NCC, Verlander et al. (486) found that estrogen treatment increased NCC expression in the DCT. These results are consistent with the view that male sex hormones, for example, testosterone, may downregulate NCC expression and salt transport, while estrogens increase NCC expression and salt transport in the DCT. The authors are not aware of gender differences in the response of humans to thiazide diuretics.

Na^+ TRANSPORT IN THE CORTICAL COLLECTING DUCT

General Considerations

The transport processes in the collecting duct are responsible for the final adjustments in urinary composition. The collecting duct is a major locus of action of mineralocorticoid hormones and plays a major role in potassium homeostasis and acid-base balance. Quantitatively, it is a minor site of sodium absorption, reclaiming only about 2% of the filtered sodium load (521).

Electrophysiologic Aspects

The electrophysiologic and transport properties of the CCD are summarized in Table 4-6. The transepithelial voltage in the cortical collecting duct (CCD) varies widely, but generally in a range from +10 to–100 mV (455,463,522–524). This variability is largely the result of differences in the mineralocorticoid status of animals. The voltages in isolated human CCD segments averaged +6.8 mV, lumen-positive (525). The lumen-negative voltage in other mammalian CCD segments is abolished by ouabain (522,526), luminal amiloride (527–529), and luminal sodium deletion (526,528,530). The lumen-negative voltage is increased by a high-K^+, low-Na^+ diet, and mineralocorticoid treatment (464,523,527,530,531).

The reported values for transepithelial resistance vary from 30 Ω-cm^2 to 2700 Ω-cm^2 (532–535). The apical membrane contains a sodium and a potassium conductance, as shown by the effects of luminal amiloride and luminal barium, respectively, on transepithelial resistance (524,535–537). The basolateral membrane is conductive to K^+ and, at least in rabbits, Cl^- (535,538–540).

Mechanisms of Salt Absorption in Collecting Ducts

A proposed model for sodium absorption and potassium secretion in the CCD is presented in Figure 4-11. As indicated

TABLE 4-6

EFFECTS OF MINERALOCORTICOIDS ON THE ELECTROPHYSIOLOGIC CHARACTERISTICS OF THE RABBIT CCD[a]

	V_T	V_b	G_a Na	G_a K	G_b K	G_b Cl	Ipump (μA/cm^2)
	(mV)		(mS/cm^2)		(mS/cm^2)		
Low ALDO	– 2	– 64	0.2	1.4	3.4 (total)		9
Control	– 7	– 80	0.7	5	1.0	4.2	40
High ALDO	– 34	– 100	1.7	7.6	3.7	10.5	195

[a]Studies performed using *in vitro* perfused CCD segments obtained from adrenalectomized rabbits (low ALDO); adrenal intact rabbits (control); and rabbits treated with mineralocorticoid (DOCA) for 9 to 16 days (high ALDO). V_T, V_b, transepithelial and basolateral membrane voltage. G_aNa; G_a K and G_bNa,G_bK, conductances of the apical and basolateral membranes to the indicated ions. Ipump, the current generated by $Na^+ + K^+$–ATPase. Data from (524,539,615,720).

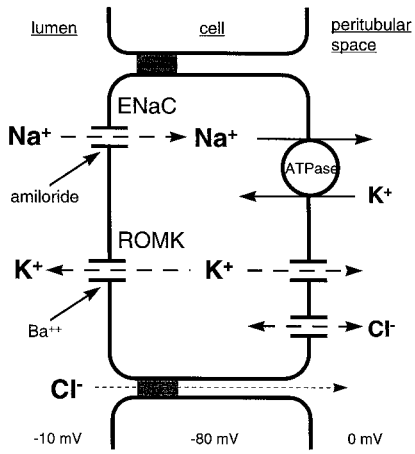

FIGURE 4-11. Model of salt transport by the principal cell of the cortical collecting duct. Apical Na^+ entry proceeds via amiloride-sensitive Na^+ channels (ENaC). Apical K^+ channels (ROMK) mediate K^+ secretion by this segment. Cl^- absorption is driven by the lumen-negative voltage through the paracellular pathway.

above, apical membranes of principal cells of the CCD possess conductive pathways for sodium and for potassium (530,536,537,541). Sodium enters principal cells through sodium channels in the apical membrane, along its electrochemical gradient. Sodium is then pumped across the basolateral membrane by $Na^+ + K^+$–ATPase in exchange for potassium. The sodium current across the apical membrane depolarizes the latter, so that the cellular potassium is above its equilibrium concentration, and, hence, leaves the cell through the conductive pathway in the apical membrane (542).

Apical Sodium Channels

The conductive entry of sodium depolarizes the apical cell membrane relative to the basolateral membrane, resulting in a lumen-negative transepithelial voltage. This lumen-negative voltage, in turn, provides the driving force for Cl^- reabsorption through the paracellular pathway.

Patch-clamp studies of collecting duct cells and A6 cells, from amphibian urinary bladder, have provided some details regarding the electrophysiologic properties and regulation of the apical membrane sodium channels (543–551). The amiloride-sensitive sodium channel in these cells has a single-channel conductance of 4 to 5 pS and is highly selective for sodium over potassium ($P_{Na}/P_K>20$). This channel exhibits rather slow gating, with openings and closings lasting several seconds (545,549). Amiloride causes a flickering block of the channel at submicromolar concentrations (545,549,552), that is, much lower than required to inhibit Na^+–H^+ exchange.

An amiloride-sensitive sodium channel has been cloned (553,554). The channel, termed ENaC, consists of three homologous subunits (α, β, and γ) (553). The subunits share a common structure consisting of two transmembrane domains, intracellular N- and C-termini, and a large extracellular loop (555,556). While the α subunit of ENaC can form sodium channels on its own (554), the coexpression of all three subunits dramatically increases the membrane sodium conductance (553). The native channel appears to be a heterotetramer consisting of two α, one β and one γ subunit (557–559). The single channel properties of the expressed channel (i.e., single-channel conductance, voltage dependence, gating kinetics, and inhibitor sensitivity) all closely resemble those of the 4 to 5 pS highly selective channel studied in native tissues (553,557). That ENaC actually is the sodium channel, which is responsible for Na^+ reabsorption in the collecting duct, has been confirmed by two experiments of nature (Table 4-1). Specifically, mutations in the ENaC gene are responsible for Liddle syndrome, an autosomal dominant form of hypertension, and pseudohypoaldosteronism type 1, an autosomal recessive form of salt wasting (560,561). In Liddle syndrome, mutations in the ENaC subunits result in an increase in amiloride-sensitive Na^+ channel activity with a consequent increase in sodium reabsorption and volume-mediated hypertension (562–564). Most Liddle mutations occur in the cytoplasmic C-termini of the γ (SCNN1G) and β (SCNN1B) subunits (561,562). These mutations affect a conserved PY motif in the C-terminus, which is necessary for interaction with Nedd4-2 and subsequent internalization and degradation of ENaC (565). As a result of the mutation, excessive ENaC accumulates in the cell membrane, leading to increased sodium absorption. A mutation in the extracellular domain of the γ subunit was found in one family with Liddle syndrome (566). This mutation increased the activity of ENaC channels without affecting cell surface expression. Pseudohypoaldosteronism type I (PHAI), the clinical opposite of Liddle syndrome, is caused by homozygous inactivating mutations in the ENaC channel, resulting in a syndrome of sodium-wasting, hypotension, and hyperkalemia (567,568). The majority of mutations causing PHA-1 are frameshift or nonsense mutations which result in truncated, nonfunctional ENaC proteins (560).

Apical Electroneutral NaCl Transport. In the rat, a portion of Na^+ entry across the apical membrane of principal cells may be mediated by a neutral, thiazide-sensitive NaCl cotransporter (569). Specifically, in perfused rat CCD segments, luminal hydrochlorothiazide inhibited Na^+ and Cl^- absorption without changing the transepithelial voltage (569). Similarly, amiloride, which completely blocks Na^+ transport in the rabbit CCD, inhibited Na^+ transport in the rat by only 50%. Finally, the effects of amiloride and hydrochlorothiazide were additive. Thus, the rat CCD may possess two parallel transport pathways for Na^+: an electrogenic pathway involving amiloride-sensitive Na^+ channels and a thiazide-sensitive neutral NaCl cotransport pathway. However, these results contrast with the studies of Schafer and colleagues (570–573) in rat CCD, which provided no evidence for thiazide-sensitive NaCl cotransport. Likewise, the cloned thiazide-sensitive cotransporter (NCC) has not been detected in the collecting duct using either RT-PCR, *in situ* hybridization, or immunohistochemistry (330,483,485,487,515). The reasons for these contradictory findings are not known.

Control of Na^+ Absorption in the CCD

Aldosterone. Aldosterone is one of the most important regulators of Na^+ transport in the collecting duct, where it increases the rates of Na^+ absorption and K^+ secretion (524,527,531,539,571,574). The major target site for mineralocorticoid effects is the principal cell of the CCD (574), although actions in the DCT have also been documented (see above). Mineralocorticoid effects are produced by the binding of either mineralocorticoids or glucocorticoids (575) to Type-I steroid receptors found predominantly in the CCD (423,511,513,576). In addition, the binding of glucocorticoids to Type-II receptors can also produce mineralocorticoid responses (575). This lack of specificity is because of two factors: first, Type-I (mineralocorticoid) receptors do not discriminate between aldosterone and glucocorticoids (577) so that either class of steroids can bind to, and activate, the receptor. Second, the DNA binding domains of Type-I and Type-II receptors are highly conserved such that both receptors can activate many of the same genes (578). The specificity for mineralocorticoids *in vivo* is provided by the selective degradation of glucocorticoids, but not mineralocorticoids, by the enzyme 11 β-hydroxysteroid

dehydrogenase (577). 11β-Hydroxysteroid dehydrogenase activity is high in CCD segments (425,579,580). The type 2 isoform of 11β-hydroxysteroid dehydrogenase (581–583), specifically, is expressed almost exclusively by aldosterone target cells, including the principal cell of the cortical collecting duct (426,584,585). Illustrating the important role of this enzyme in regulating access of hormones to the mineralocorticoid receptor, genetic deficiency of this enzyme produces a syndrome, Apparent Mineralocorticoid Excess, resembling hyperaldosteronism (hypertension, hypokalemia, metabolic alkalosis), except that aldosterone levels are low (586–588). The clinical manifestations result from the stimulation of mineralocorticoid (Type-I) receptors by circulating glucocorticoids.

In isolated perfused CCDs from mineralocorticoid treated rabbits, there is an increase in both the Na^+ and K^+ conductance of the apical membrane and an increase in basolateral $Na^+ + K^+$–ATPase activity (524,589,590). The functional changes after aldosterone treatment are accompanied by morphologic changes in principal cells. The basolateral membrane length of principal cells falls by 35% after adrenalectomy, and administration of aldosterone, but not dexamethasone, restores the membrane length to control levels (591).

An early effect of aldosterone, occurring within a few hours of exposure, is an increase in the sodium permeability of the apical membrane of the CCD principal cell. Results from electrophysiologic (592–594) and immunologic (595,596) studies support the view that the early aldosterone-induced increase in apical sodium permeability is because of the activation of quiescent Na^+ channels rather than the synthesis and/or insertion of new Na^+ channels into the membrane. Several pathways have been implicated in the aldosterone-mediated increase in Na^+ channel activity. An important downstream mediator of aldosterone is Sgk, serum and glucocorticoid-regulated kinase (597,598). Sgk, a serine and threonine kinase, is expressed in the thick ascending limb, DCT, connecting segment, and cortical collecting tubules (599). Aldosterone increases the transcription of Sgk *in vitro* (597,598), although the effects of aldosterone on Sgk protein expression *in vivo* are minor (599). Coexpression of Sgk with ENaC results in markedly greater sodium currents than seen with ENaC alone (597,598,600). Sgk is required for the stimulation of sodium transport by aldosterone both *in vitro* (600) and *in vivo* (601). Sgk increases sodium currents by increasing cell surface expression of ENaC (602) and also increasing the open probability of individual ENaC channels (603). The effect of Sgk on cell surface expression of ENaC may be mediated via Nedd4-2. Specifically, Nedd4-2 is a substrate for Sgk. Upon phosphorylation by Sgk, the affinity of Nedd4-2 for the PY domains of ENaC is diminished (604). In addition, Sgk itself contains a PY motif and might compete with ENaC for Nedd4-2 binding. As discussed earlier, binding of Nedd4-2 leads to the internalization and degradation of ENaC channels. Accordingly, Sgk may increase cell surface ENaC by decreasing Nedd4-2 binding and/or availability. Methylation of proteins and lipids is increased by aldosterone. It has been suspected for almost two decades that methylation may mediate the effects of aldosterone on Na^+ transport (605). Recent work supports this view. As reviewed recently (606), aldosterone increases the activity of a number of cellular methyltransferase enzymes. Moreover, the β subunit of ENaC itself is a substrate for methylation, and, when methylated, exhibits increased Na^+ transport activity (607,608). The effects of aldosterone on Na^+ channel activity may also involve small GTP-binding proteins, for example, *Ras* (609), and phosphatidylinositol 3-kinase (610). Sgk may be a downstream mediator of phosphoinositide-3-kinase (611).

Somewhat after the increase in apical membrane Na^+ conductance occurs, the basolateral membrane $Na^+ + K^+$–ATPase activity and pump current increase (539). This increase is due initially to an effect of increased cell sodium activity on existing pump units (612,613). Later, aldosterone induces synthesis of additional $Na^+ + K^+$–ATPase pump units (614). There is evidence that increased apical membrane Na^+ entry may modulate the late synthesis of $Na^+ + K^+$–ATPase. Inhibition of sodium entry by amiloride markedly reduced the aldosterone-induced increase in $Na^+ + K^+$–ATPase activity (589,590,615,616). Aldosterone also exerts an additional, late effect on the amiloride-sensitive Na^+ conductance. Patch-clamp studies in rats exposed to high levels of aldosterone for several days demonstrated a large increase in the amiloride-sensitive whole-cell Na^+ conductance (617). The increase in whole-cell conductance correlated with an increase in the number of active Na^+ channels in the apical membrane (548). In contrast, the single-channel conductance and open probability of individual Na^+ channels was not affected by aldosterone (548). Northern blot (618–621) and Western blot analysis (436) has shown an increase in ENaC mRNA and protein levels in aldosterone-treated tissues suggesting that the synthesis of new ENaC channels may contribute to the late aldosterone-induced increase in Na^+ conductance. Sgk, in addition to increasing cell surface expression of ENaC, also regulates the transcription of ENaC subunits (622).

The pump current associated with the increased rate of $Na^+ + K^+$—ATPase activity in aldosterone-treated tubules hyperpolarizes the basolateral membrane to the degree that potassium entry, rather than efflux, occurs through basolateral membrane potassium channels (539,542). The basolateral K^+ influx provides K^+ for secretion into the tubular lumen.

ADH. Exposure of rat CCD segments *in vitro* to antidiuretic hormone results in a sustained stimulation of Na^+ absorption (572). ADH increases the transepithelial potential, depolarizes the apical membrane, and increases the conductance of the tubule and, more specifically, of the apical membrane of principal cells (540). These changes are entirely reversed by luminal amiloride indicating that ADH increases the apical membrane sodium conductance of the principal cell. These effects of ADH are mediated intracellularly by cAMP (623). Moreover, the effects of ADH on Na^+ transport in the rat CCD are enhanced by prior treatment of the animals with mineralocorticoids (572,624). In contrast, in rabbit CCD, ADH produces only a transient stimulation, or even inhibition, of Na^+ transport and this effect is lost after treatment with mineralocorticoids (625–627). The transient response to ADH in the rabbit is not due to downregulation of ADH-stimulated cAMP generation as the response to cAMP analogs is also transient (628). Rather, the transient response may be due to a PKC-mediated inhibition of Na^+ transport. According to this view, the initial increase in apical Na^+ entry induced by ADH leads to an increased intracellular $[Na^+]$ with a consequent rise in intracellular $[Ca^{2+}]$ via basolateral Na^+–Ca^{2+} exchange (629). The increase in $[Ca^{2+}]$ then activates PKC with the subsequent inhibition of further apical Na^+ entry. Supporting this notion are studies showing a transient increase in intracellular $[Na^+]$ following ADH exposure (628), and that the stimulation of Na^+ transport by ADH in CCD cells is sustained, rather than transient, in the presence of a low calcium bath (629) or after downregulation of PKC (630). As will be discussed below, PGE_2 inhibits Na^+ transport in the rabbit CCD. Since ADH stimulates prostaglandin synthesis in renal tissue, it has been suggested that PGE_2 may be responsible for the eventual decline in transport after the transient stimulation by ADH observed in the rabbit. In this regard, pretreatment of CCD segments with indomethacin to inhibit PG synthesis prevented the late inhibition of Na^+ transport following ADH exposure (631). PGE_2 also inhibits ADH-mediated water transport in the CCD (632,633). However, the effects of PGE_2 on ADH-induced Na^+ transport and water transport appear to be mediated via different pathways (634). The inhibition of water transport by

PGE_2 can be traced to a pertussin–toxin-sensitive inhibition of ADH-stimulated cAMP production (633,635). The effects of PGE_2 on ADH modulation of Na^+ transport, however, are not mediated via changes in cAMP but, rather, via an increase in intracellular $[Ca^{2+}]$(631). In the rat, which has a sustained response to ADH (636), PGE_2 does not inhibit sodium transport by the CCD (637).

While both ADH and aldosterone increase the apical membrane sodium conductance, their mechanisms of action are different. In contrast to aldosterone, which activates quiescent channels, ADH, via cAMP, leads to the insertion of additional Na^+ channels into the apical membrane (550,638). In addition, cAMP, via PKA-mediated phosphorylation also directly stimulates the activity of sodium channels already present in the apical membrane (639–641). Long-term exposure to ADH may also increase the capacity of the collecting duct for Na^+ transport by increasing the expression of both $Na^+ + K^+$–ATPase and amiloride-sensitive Na^+ channels (389,642).

Other Agents. *Bradykinin* is produced in the connecting duct (643,644) and binds to specific receptors in the CCD (645,646). Intrarenal infusion of bradykinin produces a diuresis. However, the evidence that bradykinin effects Na^+ transport in the CCD is equivocal. Bradykinin has been reported to reduce Na^+ absorption in rat CCD segments (647,648). Since sodium transport fell in the absence of any change in transepithelial voltage, it was proposed that this agent inhibits neutral NaCl cotransport. However, other laboratories have found no effect of bradykinin on Na^+ transport in rat CCD (570,649).

As will be discussed below, the major site of action of *atrial natriuretic peptide* (ANP) is the inner medullary collecting duct. ANP stimulates cGMP production in the CCD (650), and inhibits the hydroosmotic actions of ADH in the CCD (651,652). In addition, Nonoguchi et al. (652) reported that ANP reduced Na^+ transport in the rat CCD by 90%. The inhibition of Na^+ transport occurred without a change in the transepithelial voltage (652), suggesting that ANP inhibited electroneutral NaCl cotransport. However, Rouch et al. (570) and Schlatter et al. (649) found no effect of either ANP or cGMP analogs on Na^+ transport in the rat CCD. There is no obvious explanation for these discrepancies.

α2-adrenergic agonists inhibit sodium reabsorption in the rat CCD (570). This inhibition is associated with an increase in the apical membrane resistance reflecting a decrease in sodium movement through amiloride-sensitive sodium channels (570). These changes appear to result from an inhibition of adenylate cyclase by α2 agonists (653). In the rabbit CCD, clonidine, an α2-adrenergic agonist, does not inhibit sodium transport (637) or adenylate cyclase activity (653).

β-Adrenergic Agonists. β-adrenergic agonists reduce the transepithelial voltage in the CCD (654,655). However, in spite of their effects on transepithelial voltage, these agents produce no change in net Na^+ transport. Rather, the effects of β-adrenergic agents on transepithelial voltage may be due to stimulation of electrogenic H^+ secretion by α-intercalated cells (656).

Prostaglandin E_2 exerts diuretic and natriuretic effects on the kidney. Part of this action is mediated by an inhibition of Na^+ absorption in the CCD (657). Application of PGE_2 to the basolateral surface of perfused rabbit CCD segments reversibly inhibits the negative transepithelial voltage and net sodium absorption (631,657,658). The effect of PGE_2 on sodium transport is pertussis toxin-insensitive, is coupled to a rise in intracellular $[Ca^{2+}]$, and is dependent on activation of PKC (631,634). Four PGE_2 receptor subtypes, designated EP1, EP2, EP3, and EP4, have been characterized. EP1 and EP3 were identified in the CCD of human kidney by *in situ* hybridization (659). Studies using receptor subtype-specific agonists and antagonists suggest that the EP1 receptor mediates PGE_2-dependent inhibition of Na^+ transport in the CCD (660). In contrast to the inhibitory effect of basolateral PGE_2 on Na^+ transport, luminal PGE_2 increases transepithelial voltage (and presumably Na^+ absorption) (661,662). The effects of luminal PGE_2 may be mediated via the EP4 receptor (661).

Epidermal growth factor (EGF) reduces Na^+ absorption in rabbit CCD by about 50% (663,664). The electrophysiologic effects of EGF have been examined in isolated perfused CCD segments (665) and in cultured CCD cells (666). In both cases, EGF reduces the transepithelial voltage, hyperpolarizes the apical cell membrane, increases the transepithelial resistance, and increases the resistance of the apical membrane (665,666). These effects are consistent with an inhibition of apical electrogenic Na^+ entry via the amiloride-sensitive Na^+ channel.

Nitric oxide decreases Na^+ transport in rat CCD segments by 40% to 80% (667). The addition of nitric oxide to tubules decreased the intracellular $[Na^+]$, but did not affect the activity of basolateral $Na^+ + K^+$–ATPase, suggesting that the primary effect of nitric oxide was to inhibit apical Na^+ entry. NO donors increased the activity of basolateral K^+ channels in rat CCD (668), but the physiologic significance of this effect is uncertain.

Dopamine inhibits ADH-dependent Na^+ transport and transepithelial voltage in rat CCD segments (669). The actions of dopamine are believed to be mediated via the D4 receptor subtype.

Angiotensin II (AII) stimulates sodium channel activity in rabbit and mouse cortical collecting ducts (670). Chronic AII infusion also increases the abundance of the α subunit of ENaC, the rate-limiting subunit for ENaC assembly (671). Both the acute effects of Ang II on ENaC activity and the chronic effects on ENaC abundance are mediated by the AT1 receptor (670,671).

Na^+ TRANSPORT IN THE OUTER MEDULLARY COLLECTING DUCT

The transport properties of the outer medullary collecting duct (OMCD) have been studied by *in vitro* perfusion of isolated tubule segments (Table 4-7). The functional properties of the OMCD differ depending on the location of the segment within the outer medulla. Segments within the outer stripe of the outer medulla ($OMCD_o$) exhibit electrophysiologic properties resembling the cortical collecting duct; that is, a lumen negative transepithelial voltage and electrogenic apical Na^+ entry (672). Compared to the CCD, the $OMCD_o$ displays a less negative transepithelial voltage, much lower ionic permeabilities, and a lower rate of active reabsorption of Na^+ (673). Electrophysiologic studies have shown that as the collecting duct descends into the medulla principal cells, which mediate Na^+ and K^+ transport in the CCD (see above), are replaced by cells, and whose electrical properties are similar to intercalated cells of the CCD, that is, the apical membrane lacks a demonstrable Na^+ or K^+ conductance (674). Within the inner stripe of outer medulla ($OMCD_i$), principal cells are virtually absent and no net Na^+ absorption occurs (673,675,676).

Na^+ TRANSPORT IN THE INNER MEDULLARY COLLECTING DUCT

Mechanism of Na^+ Transport

The analysis of salt transport by the IMCD has been confounded by problems of axial tubule heterogeneity, species variability, and differences in experimental approach. Based

TABLE 4-7

ELECTROPHYSIOLOGICAL AND TRANSPORT PROPERTIES OF THE RABBIT OMCD

	V_t (mV)	R_t (Ωcm^2)	V_{bl} (mV)	fRa	J_{Na}	J_{Cl} (pmol/mm/min)
Outer OMCD	– 2–11	233–272	–65	0.81	7.9	– 1.4
(references)	(672,673,675,721)	(672,722)	(672,722)	(672,722)	(673,675)	(673,675)
Inner OMCD	+ 2– + 48	294–534	– 24–36	0.96–0.99	1.5	– 9.8
(references)	(673–676,723–725)	(674,723,724)	(674,723,724,726)	(674,723,724)	(673,675)	(673,675,726)

From: Koeppen BM, Stanton BA. Sodium chloride transport—Distal nephron. In: Seldin DW, Giebisch G, eds. *The kidney: physiology and pathophysiology,* 2nd ed. New York: Raven Press, 1992:2003.

on morphologic factors, the IMCD has been divided into three subsegments: $IMCD_1$, $IMCD_2$, and $IMCD_3$ (677). This morphologic heterogeneity is paralleled, to some extent, by functional heterogeneity. For example, the urea permeability of $IMCD_1$ is low, and not affected by ADH, while $IMCD_2$ and $IMCD_3$ segments have a higher basal urea permeability, which is increased further by ADH (678). Further complicating the analysis is the observation that similar subsegments from different species exhibit different properties relating to salt transport (679,680). Finally, for unknown reasons, studies examining IMCD function *in vivo*, for example, by microcatheterization or micropuncture, have yielded markedly different results than have *in vitro* studies of isolated perfused tubules. For example, *in vivo* microcatheterization studies and microperfusion studies have demonstrated that the IMCD reabsorbs about 80% of the sodium delivered to it (681,682) while, with one exception (683), little sodium transport occurs in *in vitro* perfused IMCD tubules (680,684).

Electrophysiologic studies of the IMCD (Table 4-8) have found that the transepithelial voltage is generally in the range of 0 to – 5 mV (lumen-negative) and that the transepithelial resistance is in the range of 40 to 100 ohm cm^2 (679,680,683,684). Limited data regarding the conductive properties of IMCD cells are available. Stanton performed microelectrode impalements of IMCD segments from rat (680). The apical membrane constituted the major cellular resistance (Fra = 0.92). Luminal application of amiloride resulted in an increase in the apical membrane voltage and resistance and a fall in the transepithelial voltage. These results, and others (681,685,686), are consistent with the presence of an amiloride-sensitive sodium conductance in the apical membrane of IMCD cells. Patch-clamp studies of cultured rat IMCD cells indicate that Na^+ entry is mediated by a 20 to 30 pS amiloride-sensitive, nonselective, cGMP-gated cation channel in the apical cell membrane (686,687). The basolateral membrane of IMCD cells contains $Na^+ + K^+$–ATPase, a potassium conductance and a bicarbonate conductance (679,680).

The results discussed above can be combined into a tentative model for sodium transport in the IMCD (Fig. 4-12). The model is essentially that described by Koefoed-Johnsen and Ussing for electrogenic sodium transport in the frog skin (688). Sodium entry across the apical membrane occurs down its steep electrochemical gradient through amiloride-sensitive Na^+ channels. Sodium is extruded across the basolateral membrane by the $Na^+ + K^+$–ATPase, thereby maintaining a low intracellular Na^+ concentration. The basolateral membrane K^+ conductance serves to recycle the K^+, which enters on the $Na^+ + K^+$–ATPase. The K^+ conductance also hyperpolarizes the cell thereby favoring Na^+ entry across the apical membrane.

The evidence for additional electroneutral Na^+ entry pathways in IMCD cells is controversial. Furosemide and thiazide diuretics both inhibit a portion of Na^+ absorption by IMCD segments *in vivo* (689,690). In addition, a $Na^+/K^+/2\ Cl^-$ cotransporter may be present in isolated rat papillary collecting duct cells (691). Rocha et al. (692) also have presented data that support the presence of a $Na^+:K^+:2\ Cl^-$ cotransporter in the basolateral membrane of terminal IMCD segments (IMCD2,3). A $Na^+:K^+:2\ Cl^-$ cotransporter has been cloned from cultured IMCD cells (693). This cotransporter represents the "secretory" isoform of the $Na^+:K^+:2\ Cl^-$ cotransporter (NKCC1) rather than the absorptive isoform present in the apical membrane of the thick ascending limb. This cotransporter appears to participate in NaCl secretion rather than reabsorption. Further work is required to determine what role, if any, electroneutral Na^+ entry pathways play in net Na^+ absorption by the IMCD.

Regulation of Na^+ Transport

The IMCD appears to be the major target site for the potent diuretic hormone, *atrial natriuretic peptide* (ANP) (681,694). This hormone, working through cGMP (695), inhibits sodium entry via apical membrane Na^+ channels. In

TABLE 4-8

ELECTROPHYSIOLOGICAL AND TRANSPORT PROPERTIES OF THE RAT IMCD

	V_t (mV)	R_t ($\Omega \cdot cm^2$)	V_{bl} (mV)	fRa	J_{Na} (pmol/mm/min)	J_{Cl}
Initial IMCD	– 2ω–0	73	– 51	0.94	10	
(references)	(680,727–729)	(680)	(680)	(680)	(680)	
Terminal IMCD	0	148	– 81	0.99	54–92	72
(references)	(683,700,728)	(656)	(656)	(656)	(683,700)	(700)

From: Koeppen BM, Stanton BA. Sodium chloride transport—Distal nephron. In: Seldin DW, Giebisch G. eds. *The kidney: physiology and pathophysiology,* 2nd ed. New York: Raven Press, 1992:2003.

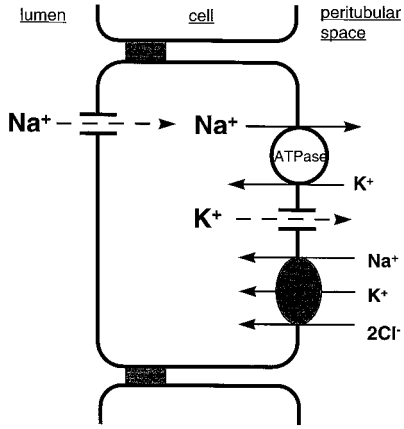

FIGURE 4-12. A model of Na^+ transport in the inner medullary collecting duct. Apical Na^+ entry proceeds via a nonselective amiloride-sensitive cation channel. Basolateral Na^+:K^+:2 Cl^- cotransport may be involved in Na^+ secretion.

suspensions of rabbit IMCD cells, ANP inhibited O_2 consumption (696) and inhibited conductive $^{22}Na^+$ uptake (697). Light et al. (686,698,699) have examined in detail the effects of ANP on an amiloride-sensitive cation channel in cultured inner medullary cells. In cell-attached patches, both ANP and dibutyryl cGMP inhibited the activity of the cation channel (699). In excised patches, cGMP also inhibited the channel. The inhibition by cGMP involves at least two pathways, a phosphorylation-independent pathway and a pathway involving cGMP-dependent protein kinase (698). The latter pathway also involves a pertussis toxin-sensitive G protein.

Rocha et al. (692) have suggested that, in addition to inhibiting Na^+ reabsorption, ANP stimulates Na^+ secretion in the IMCD. In isolated perfused IMCD segments, ANP increased the bath to lumen flux rate of Na^+ and Cl^-. This increase was inhibited by peritubular furosemide and by omission of either counterion, leading the authors to propose that ANP stimulated Na^+ secretion via a basolateral membrane $Na^+/K^+/2\ Cl^-$ cotransporter (700). Sands et al. (684), however, found no effect of ANP on the Na^+ permeability of the rat IMCD.

In one study, *antidiuretic hormone* stimulated sodium absorption by terminal IMCD segments perfused *in vitro*. The stimulation was blocked by luminal amiloride and mediated through a V_1 receptor mechanism (683). Sands et al. (684), however, found no effect of ADH on either transepithelial voltage, transepithelial resistance or on the chloride permeability of rat IMCD segments. If ADH stimulates conductive sodium entry, a decrease in the transepithelial voltage and resistance should have been observed. Likewise, Light et al. (686) failed to find an effect of ADH or cAMP on the amiloride-sensitive cation channel in apical membranes of cultured IMCD cells.

Micropuncture studies by Ullrich et al. (701) demonstrated that mineralocorticoids increased net Na^+ absorption in the terminal IMCD. This effect was attributed to a decrease in the passive Na^+ permeability of the tubule leading to a decrease in back-leak of NaCl into the lumen. In support of this hypothesis, Sands et al. (684) have confirmed that mineralocorticoids increase the transepithelial resistance and reduce the passive NaCl permeability of the rat IMCD. Mineralocorticoids may also increase active Na^+ reabsorption in the IMCD. For example, in a recent study of cultured rat IMCD cells, aldosterone produced a three- to sevenfold stimulation of electrogenic Na^+ transport (702). Likewise, chronic mineralocorticoid exposure *in vivo* increased the activity of $Na^+ + K^+$–ATPase in the IMCD (703).

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CHAPTER 5 ■ TUBULAR POTASSIUM TRANSPORT

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Potassium (K) is the most abundant intracellular ion in mammals, and a variety of fundamental cell processes are critically dependent on its concentration, and its distribution across cell membranes. The kidney is the main organ involved in the long-term regulation of total body K. Protein synthesis requires a high K concentration in the cytosol. Electrical properties in both excitable (nerve, muscle) and nonexcitable tissues (transporting epithelia) depend on the difference in K concentration that most cells maintain across their plasma membranes. Regulation of cell volume depends, in large part, on the effective osmolality of intracellular fluid contributed by cellular K (1). Metabolic processes are affected by changes in cytosolic hydrogen ion activity (2) and, thus, indirectly by changes in cellular K, which tend to modify intracellular acidity.

Extracellular K concentration must be maintained within a narrow range (3.5 to 5.0 mEq/L) since both hypokalemia and hyperkalemia are associated with significant cardiac rhythm disturbances. This balance not only depends on total body K but also, and more importantly, on factors that affect K distribution across cell membranes

Several overlapping regulatory mechanisms serve these important functions both by maintaining total body K content (50 to 55 mmol per kg body weight) and by partitioning K between extracellular and intracellular fluid (3). A healthy steady state requires maintenance of a continuing balance between intake and excretion of K. These rates are approximately 100 mmol per day in humans and could vary by up to 50% depending on one's diet. Since extracellular fluid contains only approximately 2% of total body K, the daily intake of K could double plasma K levels, if not for the ability of cells to take up K rapidly and continuously. Because of the efficiency of cell uptake, plasma K concentration is normally kept quite constant in the range from 3.5 to 5.0 mM (4–7). Most of the 98% of body K that is inside cells is in skeletal muscle. Regulation of this cellular uptake of circulating K (acute K balance) is an important component of the extrarenal mechanisms contributing to control of plasma K concentration.

Variations in K intake are matched within hours to days by parallel adjustments in K excretion (chronic K balance). The kidney is largely responsible for the adaptive variation in excretion that is required to maintain K balance. While the colon is also able to excrete K and to respond to stimuli calling for greater or lesser excretion (8,9), it usually contributes less than 10% of the total excreted. Regulation of external K balance is primarily a function of the kidney, which excretes most of the K gained each day from food.

To accomplish excretion of this variable quantity of K, the kidney must extract K from blood plasma in which K circulates at a relatively low concentration (3.5 to 5.5 mM). Even though dietary K intake is approximately equal to sodium (Na) intake (80 to 120 mmol per day), the concentration of K in plasma and, therefore, the rate at which K is filtered by glomeruli, is only 1/30th that of Na. The glomerular filtration rate (GFR) of normal kidneys is high enough, however, that K could conceivably be excreted by filtration alone. Because two human kidneys together filter approximately 180 L per day, filtration of plasma containing K at 4 mM could remove up to 720 mmol per day. However, if the GFR were reduced to 10% to 15% of normal, as it may be in a variety of disease states, filtration alone would not be able to keep up with the normal dietary intake. Even if the GFR were reduced only to 50% of normal, it is unlikely that filtration alone could suffice, because not all filtered K can escape reabsorption. Further, even when the GFR is normal, an excretion mechanism relying solely on filtration would have a limited capacity for adaptive increase and could not achieve the 20-fold increase in K excretion that has been observed in animals exposed to increased intake of K by diets high in K, or by parenteral infusions containing K.

Clearance studies conducted in the 1940s provided the first evidence that the kidney possesses secretory mechanisms enabling it to transfer K from plasma to tubule fluid (10–12). Subsequent studies using micropuncture, microperfusion cellular, and molecular biology methods have contributed much to our understanding of renal K handling.

An overview of the renal processes involved in K excretion is shown in Figure 5-1. Proximal nephron segments between the glomerulus and the distal convoluted tubule (DCT) reabsorb a rather fixed fraction (80% to 90%) of the filtered K, whereas distal tubules and collecting ducts remove a variable quantity of K from plasma and secrete it into tubule fluid (13–17). This secretory transfer of K is accomplished by certain specialized cells in distal tubules and collecting ducts. Although these cells constitute only a minute fraction of the body's intracellular fluid volume, many of the mechanisms in use throughout the body to shift K from plasma into cells also operate in these cells of the distal nephron. Other cells in distal tubules and collecting ducts are able to reabsorb K and contribute to conservation of K.

Thus, in terms of body fluid compartments, K enters intestinal cells, is transferred to other cells by circulating in plasma at a low concentration, and is removed from the body by specialized kidney cells. By varying the rate, and even the direction, of net K transport, cells of the distal nephron are able to respond homeostatically to changes in dietary K intake or to changes in extracellular K caused by other gains (parenteral administration, release from cellular pools) or losses (from the gastrointestinal tract or skin). In people ingesting K at 80 to 120 mmol per day, the usual rate of K excretion in the postabsorptive period between meals is approximately 10% of the rate of K filtration. In the hours after meals, or ingestion or infusion of supplementary K salts, the rate of urinary excretion of K can increase greatly to approach or even exceed the rate at which it is filtered. The increment in K excretion can be attributed to an increase in the quantity secreted by distal nephron segments.

Increased secretion may not entirely account for increases in K excretion in all cases. Reduction in reabsorption may be important in some circumstances. Conversely, decreased secretion is not the only mechanism for conservation of K. If K intake is

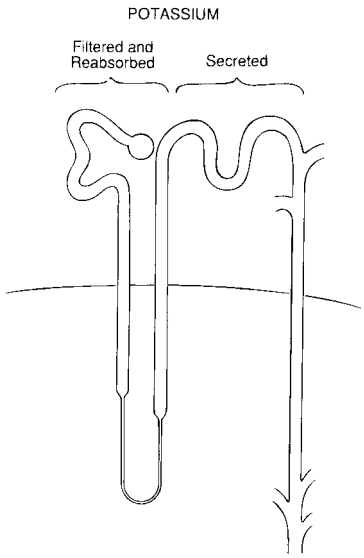

FIGURE 5-1. Simplified picture of the nephron unit responsible for K excretion. Most of the filtered K is reabsorbed along the proximal tubule and loop of Henle. Excreted K is largely accounted for by secretion into the distal tubule and collecting duct.

reduced or eliminated (or if the body is depleted of K by nonrenal losses), urinary K excretion declines as the deficit in total body K increases, both because of decreased K secretion and because specialized cells in the distal nephron increase their rate of K reabsorption. Within a few days, K excretion can be reduced to very low levels, particularly in otherwise healthy subjects (18–20) . Potassium conservation is less complete in many clinical situations when subjects are ill or stressed (21,22).

FACTORS AFFECTING POTASSIUM EXCRETION

Multiple factors operate simultaneously to regulate renal K excretion thereby providing both flexibility and a reserve capacity to maintain acute and chronic K balance. At one level, one can identify changes in the state of other body systems result in changes in K excretion. Next, one can describe local factors capable of modifying renal transport mechanisms and influenced by extrarenal perturbations.

Systemic variables, and the local factors they influence at the level of the distal nephron, are summarized in Table 5-1. In brief, the main points emphasized in this table are the following:

1. Increases in *K intake* and *body K* content tend to raise the concentration of K in plasma and cell water (23). Increases in plasma K concentration affect the kidney directly (24,25), and stimulate the adrenal glands to secrete aldosterone (26). Decreases in K intake or losses of K that exceed intake tend to reduce total body K content and have opposite effects on plasma K concentration and aldosterone secretion. Potassium loading results in increased K excretion (27,28), and several of the factors outlined in Table 5-1 are changed at the same time. A clear demonstration that plasma K concentration is a variable affecting renal K excretion was provided in experiments using adrenalectomized dogs (25).
2. Increases in *Na intake,* either because of high dietary intake of salt or because of parenteral infusions of saline solutions, tend to expand *extracellular fluid volume.* An enhancing effect of extracellular fluid volume on K excretion has been inferred from a variety of experiments (29). Infusion of solutions containing sodium chloride, sodium bicarbonate, or sodium sulfate sufficient to more than double Na excretion, also increases renal K excretion without increasing plasma K concentration, and presumably without increasing aldosterone concentration or changing acid–base balance (30). Renal effects of extracellular fluid volume expansion lead to decreased aldosterone secretion because of suppression of the renin–angiotensin system, and to increased Na excretion and urine flow because of reduced reabsorption of Na and water. In the renal tubules, increased flow rate of tubule fluid, as discussed later, acts to stimulate K excretion. The increases in tubule fluid flow rate that occur when extracellular fluid volume is expanded can stimulate K secretion and may be the major factor accounting for the association between increased Na excretion and increased K excretion. Directionally similar changes in Na and K excretion are seen not only when extracellular fluid volume is expanded, but after the administration of *diuretic drugs.* Agents with different tubular sites of action such as acetazolamide, furosemide, and chlorothiazide are known to increase K excretion. As is discussed subsequently, diuretic drugs probably have both renal and systemic effects. Renal actions include their ability to increase tubule fluid flow rate and their effects on transport mechanisms that result in increased K secretion. Diuretics also have systemic effects, including increased aldosterone levels and acid–base changes.
3. Primary changes in the function of cells of the zona glomerulosa of the adrenal cortex can modify K excretion. Hypersecretion of *aldosterone* leads to K depletion and hypokalemia. Adrenal insufficiency is associated with hyperkalemia. Separate effects of circulating aldosterone on renal K excretion were observed in another series of experiments that related rates of K excretion to varying rates of aldosterone infusion (31). These experiments indicate a direct dependence of K excretion on aldosterone infusion rate and provide support for the long-standing belief that mineralocorticoid hormones promote K excretion (32). Aldosterone acts on cells of the distal nephron to increase Na absorption and K secretion. *Glucocorticoid* hormones (cortisol, corticosterone) also promote K excretion, although they do not appear to act directly on cells in the distal nephron.
4. Changes in *acid–base balance* influence K excretion by modifying several local factors. Not only do gains or losses of acid or alkali alter plasma hydrogen ion and bicarbonate concentrations, but they modify proximal fluid and

TABLE 5-1

MODIFIERS OF RENAL EXCRETION AND DISTAL SECRETION OF POTASSIUM

Systemic influences affecting renal K excretion	Local factors affecting distal K secretion
K intake	$[K^+]$: plasma, cell, tubule fluid
Body K	Aldosterone
Na intake	Flow rate of tubule fluid
Extracellular fluid volume	
Diuretic drugs	Diuretic drugs
Adrenal cortex	Aldosterone, cortisol
Acid–base balance	$[H^+]$, $[HCO_3^-]$: plasma, tubule fluid
Nonchloride anion (A^-)	$[Cl^-]$, $[A^-]$ %AQ: tubule fluid

electrolyte transport processes and, thus, affect tubule fluid flow rate. Changes in acid–base balance can alter renal K excretion (33). In general, acute metabolic *alkalosis* stimulates K excretion, and acute metabolic *acidosis* inhibits K excretion (34). Changes in extracellular proton and bicarbonate concentrations appear to affect intracellular K activity and K transport mechanisms influenced by cellular K.

5. Increases in plasma concentration of either bicarbonate (HCO_3^-) or other *nonchloride anions* (A^-), such as sulfate, phosphate, nitrate, hippurate, or ferrocyanide, tend to promote increases in K excretion (35,36). As noted previously, K excretion accelerates when solutions containing Na salts are infused systemically. When the salt is NaCl, extracellular volume expansion leads to changes in proximal fluid reabsorption that increase the rate of flow of distal tubule fluid. When the salt is $NaHCO_3$, the change in local acid–base conditions appears to provide an additional stimulus to K excretion. When nonchloride anions such as sulfate, phosphate, gluconate, nitrate, or ferrocyanide predominate in these infusions, K excretion may be increased even more than it is when NaCl solutions are infused, and without changes in blood acidity (28,37–43). Cells of the distal tubule respond to changes in the anion composition of distal tubule fluid; increased rates of K secretion are seen when luminal Cl concentrations are low (36,44).

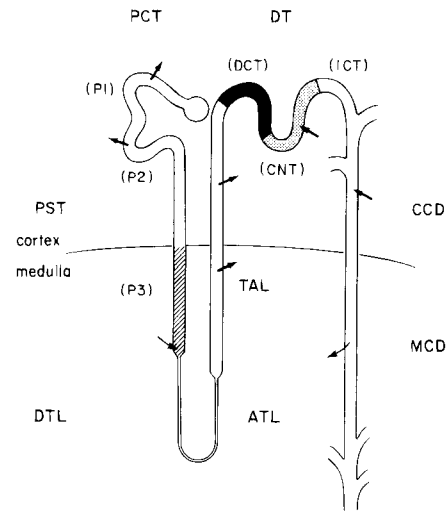

FIGURE 5-2. More complete description of K transport along the nephron. *Thick arrows* indicate uphill transport; *thin arrows* indicate downhill leaks. Major segments are identified as follows: *PCT*, proximal convoluted tubule; *PST*, proximal straight tubule; *DTL*, descending thin limb; *ATL*, ascending thin limb; *TAL*, thick ascending limb; *DT*, distal tubule; *CCD*, cortical collecting duct; *MCD*, medullary collecting duct. Subsegments of the proximal tubule are indicated by (*P1*), (*P2*), and (*P3*). Subsegments of the distal tubule are the distal convoluted tubule (*DCT*), connecting tubule (*CNT*), and the initial collecting tubule (*ICT*). Potassium is reabsorbed from the proximal tubule, the thick ascending limb, and the outer medullary collecting duct. Potassium is secreted into the proximal straight tubule or descending thin limb, the distal convoluted tubule, the connecting tubule, and the cortical collecting duct.

POTASSIUM HANDLING BY INDIVIDUAL NEPHRON SEGMENTS

The simple view, that filtered K is largely reabsorbed by proximal nephron segments and that excreted K is secreted by distal segments, provides a useful framework for integrating information about renal handling of K, but a closer look at the cytologically distinct subdivisions of the nephron (45) and the way each handles K reveals a more complicated picture (Fig. 5-2). Potassium is not reabsorbed from all tubule segments proximal to the secretory sites in the distal tubules and collecting ducts. The rather constant fractional delivery of K (10% to 20% of the filtered quantity) that has been found by collecting and analyzing samples of fluid from the earliest portion of the superficial distal tubule accessible to micropuncture (29,46,47) is probably achieved by a sequence of reabsorption, secretion, and reabsorption as the glomerular filtrate travels through successive segments of the proximal tubule and the loop of Henle (15–17).

Glomerulus

Potassium ions that are free in plasma water pass across the glomerular capillary membrane in the filtrate with little hindrance. Because nonfilterable proteins in plasma may bind a small fraction of K (48), and because their net negative charge causes the distribution of small ions to tend toward the Donnan equilibrium, K concentration in glomerular filtrate tends to be somewhat lower than in plasma water. However, because the bulk of proteins occupies a sufficiently large fraction of the volume of plasma, the concentration of K in plasma is approximately 6% lower than in plasma water. Thus, because these factors tend to cancel each other, the concentrations of K in glomerular filtrate and in plasma, or serum, are approximately equal. Although variations in GFR do cause proportional variations in the rate of K filtration, they do not usually result in large changes in K excretion, because mechanisms promoting glomerulotubular balance tend to stabilize the rate of K delivery out of the proximal tubule. In the 1950s, when renal clearance methods were first applied systematically to the study of K regulation by the kidney, it was observed that rates of glomerular filtration and final K excretion could be varied independently of one another (28,49).

Proximal Tubule

Potassium is reabsorbed from proximal convoluted tubules of both superficial and deep nephrons. Decisive information about the direction and magnitude of K transport processes along the nephron was obtained in the 1960s, when micropuncture techniques were applied to the study of intact kidneys in anesthetized animals (46,47,50–52). In these experiments, samples of freely flowing tubule fluid were collected at successive sites along the nephron while inulin was infused intravenously. Measurement of K and inulin concentrations in samples of tubule fluid and plasma enabled calculation of the fractional delivery of K to the site of collection. Collections from superficial proximal tubules showed that 30% to 50% of filtered K reaches the last accessible surface segment of the proximal convoluted tubule (46,47,50–52). Collections from proximal sites closer to the glomerulus showed that reabsorption of K begins in the most proximal portion of this nephron segment (48). The reabsorption of K, like that of Na, usually proceeds without a large concentration difference developing across the proximal tubule wall. Thus, roughly similar fractions of water, Na, and K are absorbed as fluid flows along the proximal tubule.

The proximal tubule appears to be sufficiently permeable to K to allow rather modest passive driving forces to effect a substantial fraction of absorptive transport. *In vivo* microperfusion experiments show that proximal K transport is very

sensitive to changes in luminal K concentration and transepithelial voltage. The apparent K permeability is higher in the proximal tubule than in the distal tubule and the dependence of K transport on the transepithelial electrochemical potential difference evident in these experiments is consistent with a mechanism involving diffusive movement through a single barrier: presumably the intercellular junctions. Movement of K through the junctions between cells appears to be importantly affected by the concurrent rate of net fluid transport. In microperfusion experiments, it was observed that addition of mannitol to a perfusion solution otherwise resembling fluid reaching the late proximal tubule caused net fluid transport to decrease and then reverse direction (53). Stopping net fluid absorption slowed, but did not stop Na absorption. In contrast, the direction of net K transport reversed from net absorption to net secretion when fluid absorption went to zero. When the direction of net fluid transport was reversed and fluid entered the tubule, the rate of K secretion increased (53). This dependence of K transport on net fluid transport may indicate direct coupling through the same pathway: a solvent drag mechanism. Alternatively, it may be that fluid absorption raises K concentration immediately adjacent to the apical membrane of proximal cells and thus creates a concentration difference favoring K absorption by diffusion. A few studies have provided suggestive evidence that proximal K transport may be able to proceed uphill (54–57).

Loop of Henle

As the proximal straight tubule enters the outer medulla, the direction of K transport reverses and K is secreted into the third proximal segment (P3 or S3) and into the thin descending limb of the loop of Henle. Higher K concentrations are attained in loops of deeper nephrons that penetrate further into the inner medulla. Potassium is then reabsorbed by ascending limbs. Active K absorption has been identified in thick ascending limbs (TAL), and net reabsorption probably occurs in both medullary and cortical TAL of both deep and superficial nephrons.

Free-flow micropuncture studies in the 1960s showed net reabsorption of K between the last accessible segment of surface proximal tubules and the first accessible segment of surface distal tubules (46,47). As shown in Figure 5-2, this portion of the nephron comprises several morphologically and functionally different segments: the second and third segments of the proximal tubule (P2 and P3), the thin descending and thin ascending limbs, the medullary and cortical TAL, and the beginning of the DCT. The contributions of each of these segments to overall K reabsorption cannot be assessed separately by sampling only surface segments of proximal and distal tubules. Collection of fluid from surface distal tubules has shown that K concentration is reduced in passing through the loop of Henle. Under normal nondiuretic conditions, the K concentration in fluid emerging from the loop is lower than in plasma (46,47).

In contrast, collection of fluid from the tips of the loops of Henle of deep nephrons (accessible on the surface of the exposed renal papilla) has shown that K concentration is elevated above plasma levels midway along the loop (55,58). deRouffignac and Morel (55) suggested that K is added to tubule fluid along the descending limb and that the source of this K is absorption from the ascending limb and collecting duct. Fluid near the tip of the papilla could have as much as 10 times the K concentration of systemic plasma (59). In normal rats, the rate of K delivery to the end of the descending limb of deep nephrons was equal to the rate of K filtration (59), and in rats either fed a high-K diet (60) or infused with K acutely (61), the rate of K delivery exceeded the rate of filtration. If K is also secreted into the proximal straight tubule of superficial nephrons (62,63), and if the delivery of K to the DCT of deep nephrons is substantially less than the filtered quantity, then in both populations of nephrons K must be reabsorbed by ascending limbs. These observations reveal that K is recycled from the ascending limb and collecting duct to the descending limb and the medullary interstitium.

Studies with isolated TAL perfused *in vitro* show that these segments are capable of absorbing K actively (64,65). Thus, K is trapped in the medulla by countercurrent exchange between the ascending and descending limbs of the loop of Henle. The TAL also absorbs Na and Cl. The overall capacity for Na and Cl absorption is quite high because the TAL extends for several millimeters through the outer medulla and cortex. Because the TAL epithelium has a relatively low permeability to water, absorption of Na, K, and Cl leads to substantial reduction in the concentration of these ions and to dilution of tubule fluid. The class of diuretic drugs known as loop diuretics decrease Na, K, and Cl absorption in the TAL by inhibiting the Na-K-2Cl cotransporter. The drugs act on the ion transport mechanism in the apical membrane of TAL cells after gaining entrance to the lumen by way of secretion in the proximal tubule. Loop diuretics can reverse the direction of K transport in the TAL from net reabsorption to net secretion.

Distal Tubule and Collecting Duct

Anatomic and Functional Subdivisions

The portion of the nephron beyond the macula densa (MD) segment, where each ascending limb contacts its parent glomerulus, comprises several segments that are cytologically distinguishable. The nephron region extending from the MD to the first confluence with another nephron has been called the *distal tubule* (or *distal convolution*) (29,47,66–68). The term *distal tubule* is not a formally accepted anatomic designation, but it is particularly useful in discussing micropuncture experiments because most of this segment is accessible at the surface of rat kidneys and its limits can be clearly defined by microdissection and measurement (68,69). This chapter uses *distal tubule* in this way. It is necessary, however, to be aware that the anatomic segment between the MD and the first confluence has several cytologically distinct subdivisions.

The first segment of the distal tubule, the DCT, extends approximately 1 mm beyond the MD and is functionally distinct from the TAL segment. Sometimes referred to as the *early* distal tubule, the DCT has been identified as a site for secretion of K (44,70). By cytologic criteria, the DCT occupies only the more proximal one-half or so of the distal tubule (71–74). Another segment, the connecting tubule (CNT), is interposed between the DCT and the first confluence with another tubule. The CNT, and the segment following it, the cortical collecting duct (CCD), are the major sites of K secretion. In superficial nephrons, cells characteristic of the CCD form the wall of the last segment of the distal tubule before it joins with another tubule. This terminal portion of the distal tubule has also been termed the *initial collecting tubule* (ICT).

In deeper nephrons, the CNTs frequently join to form arcades before flowing into the collecting duct. Potassium is secreted into the collecting duct throughout the cortex and probably in the outer stripe of the outer medulla as well (75,76). In the inner stripe of the outer medulla, K reabsorption appears once again and contributes to K accumulation in the medullary interstitium. Both secretion and reabsorption of K have been described along the terminal portions of the inner medullary collecting duct (58,77–79).

The DCT differs from both the preceding TAL and the subsequent CNT and ICT (80). The DCT secretes K, but not as rapidly as the subsequent segments. The DCT usually receives fluid with a low K concentration from the TAL. Potassium

secretion into this segment is favored by the concentration difference between plasma and tubule fluid, but can proceed even when tubule fluid and plasma concentrations are equal (44,70). Under conditions of K depletion, the DCT is able to maintain the low K concentration characteristic of fluid emerging from the TAL. Fluid entering the DCT is also dilute: Na and Cl concentrations are less than one-third of their levels in plasma. The water permeability of the DCT is low, like that of the TAL, and is not influenced by vasopressin. The transepithelial voltage recorded in this segment is less lumen negative than that found in the next most distal segments (81–83). The DCT is able to absorb Na and Cl; however, the reabsorptive mechanism may not be as powerful or efficient as that of the TAL. When the DCT does absorb Na and Cl, it serves to maintain the low luminal Na and Cl concentrations achieved by the TAL (84). The DCT is the site of the primary action of the thiazide class of diuretic drugs (85–93). A DCT localization of the thiazide-sensitive transporter has been confirmed by *in situ* hybridization in rat, rabbit, and human kidney. Probes directed against the cloned Na-Cl cotransporter protein identified cells expressing the transporter. In both rat and rabbit, the signal is restricted to the DCT; in human kidney, some signal extends into the first portion of the CNT.

The CNT normally secretes K at a higher rate, because the transepithelial voltage becomes more lumen negative along the CNT (81,83), Na is reabsorbed against both electrical and chemical gradients. The transepithelial voltage is not affected by mineralocorticoid administration (73,94). Cl absorption can be demonstrated to proceed uphill under some circumstances (69). β-Adrenergic agonists and parathyroid hormone (PTH) stimulate adenylate cyclase in the CNT (73,95). Vasopressin does not stimulate adenylate cyclase or increase water permeability. In superficial nephrons, the CNT is less than 1 mm long; in deep nephrons, the CNT's frequently join to form arcades that may be several millimeters long. A morphologic indication that CNT cells contribute to K secretion comes from studies of Stanton et al. (96) and Kaissling (97,98), who observed that the height and basolateral membrane area of CNT cells are increased by feeding rabbits diets low in Na and high in K.

The CCD begins in superficial nephrons some distance before the confluence of two distal tubules. This portion is accessible to *in vivo* micropuncture and has been called the *ICT.* The CCD continues with similar morphology into the outer stripe of the outer medulla. The ICT has been investigated (frequently along with the CNT) in micropuncture studies of surface nephrons. The unbranched portion of the CCD that runs in medullary rays through the inner cortex has been studied by perfusing isolated tubules *in vitro* (99–104). The CCD reabsorbs Na and Cl, secretes K, and exhibits a lumen-negative transepithelial voltage. (105,106). The principal cells in this segment respond to vasopressin and aldosterone (95,107,108). Na entry into principal cells is blocked by amiloride and triamterene, compounds called *K-sparing diuretics,* because they increase Na excretion modestly, but reduce K excretion (109,110). A minority cell type, α-intercalated (A-type) cells, secretes hydrogen ions and appears to be capable of K reabsorption (111–114).

Factors Affecting Distal Potassium Secretion

Renal excretion of K is influenced by several local factors (Table 5-1) that individually affect cells of the distal tubule and CCD. Potassium secretion by the distal tubule is altered by changes in the concentration of several ions in plasma and tubule fluid, by the flow rate of tubule fluid, and by circulating hormones, including aldosterone and vasopressin.

The multitude of influences on distal cells that modify K secretion can be divided into two categories: luminal factors and peritubular factors (Table 5-2). The group of luminal factors includes both the flow rate of tubule fluid and the concentrations of K, Na, Cl, and other substances in tubule fluid that influence K transport across the apical membrane of distal cells.

TABLE 5-2

FACTORS AFFECTING POTASSIUM SECRETION

Luminal	Peritubular
$[K^+]$	$[K^+]$
Flow rate	Aldosterone
$[Na^+]$	$[H^+]$, HCO_3^-
$[Cl^-]$, $[A^-]$	Vasopressin
$[H^+]$, $[HCO_3^-]$	
Inhibitors	
Amiloride	Spironolactone
Triamterene	
Ca^{2+}	
Thiazide	
Ba^{2+}	

Tubule Fluid $[K^+]$. Net K transport is very dependent on the luminal K concentration (115). The rising concentration of K in tubule fluid resulting from secretion of K into the tubule acts to dissipate the driving force responsible for diffusion of K across the apical membrane of distal cells. Under normal circumstances, the maximum concentration that the K secretory mechanisms can develop is approximately 7- to 10-fold greater than the plasma K concentration.

Tubule Fluid Flow Rate. The effects of the volume flow rate of tubule fluid on distal K secretion were examined separately from the effects of changes in tubule fluid composition by means of *in vivo* microperfusion (115). Because individual tubules were perfused in otherwise normal animals, systemic factors such as plasma K concentration, aldosterone levels, and acid–base balance were unchanged when either tubule flow rate or tubule fluid composition was varied. When the tubule perfusion rate was increased, K secretion by the distal tubule increased. The lumen-negative transepithelial voltage did not increase when the perfusion rate was increased (115,116). At low flow rates, secretion of K causes the concentration of K in luminal fluid to increase from the initially low values established by absorption along the ascending limb to values approaching the maximum possible concentration gradient. At higher flow rates, K secretion does not raise the luminal K concentration to the same extent. Thus, increasing the flow rate of distal tubule fluid acts to decrease the average luminal K concentration. The effect of increased flow rate to stimulate K secretion occurs, in part, because of the flow-dependent decrease in luminal K concentration. Aditionally, changes in flow rate have been shown to activate ENaC, which could result in increased Na absorption, with a concomitant increase in K secretion (117). The activity of a large voltage- and calcium-activated K (Maxi-K) channel is also dependent on flow rate, and K secretion could occur through this channel in high luminal flow states (118). In the CCD of the rabbit, increases in luminal flow rate above 5 nL per minute appear to have little effect on K secretion (119), although the normal rates of flow are very likely lower in the collecting duct than in the distal tubule. As is true for a variety of other flow-dependent transport processes (120), it is to be expected that the rate of K secretion plateaus above some rate of flow and further increases in flow will have little stimulatory effect (121,122).

Tubule Fluid [Na^+]. Increases in luminal Na concentration can cause distal K secretion to increase. Systemic infusions that expand extracellular fluid volume and increase Na and K excretion also increase early distal Na concentration. *In vivo* microperfusion experiments have been performed in which the perfusion rate was kept constant and the concentration of Na in the perfusion fluid was varied between 3 and 95 mM (123). Potassium and chloride concentrations in perfusion fluids were kept constant. Potassium secretion by the distal tubule was sensitive to changes in luminal Na concentration only in the range below 35 mM. Luminal Na concentration is not likely to be a major regulatory factor, at least in segments of the distal tubule. This is so because the concentration of Na does not usually fall below 35 mM. A mathematical model of the CCD has been developed (124,125) that takes into account cellular heterogeneity and many epithelial and cell membrane parameters, including luminal Na concentration, flow rate, and net K secretion. This model also predicts a rather constant rate of K secretion over a wide range of luminal Na concentration. Along the CCD, however, it is possible that Na concentration may fall below 30 to 40 mM and thus become a factor limiting K excretion.

Tubule Fluid [Cl^-] and A^-. Decreases in luminal Cl concentration from normal levels of 25 to 35 mM to values less than 10 mM are associated with increases in distal K secretion. Chloride concentrations below 5 mM have been observed in tubule fluid samples collected from distal tubules of rats being infused with sodium sulfate solution. *In vivo* microperfusion experiments have also shown that when all Cl in perfusion fluid was replaced with another anion, such as sulfate, bicarbonate, or gluconate, K secretion by the distal tubule increased (36,126). The increase in K secretion in these experiments occurred without increases in luminal Na concentration, without changes in tubule fluid flow rate, and without changes in transepithelial voltage. The effect requires that luminal Cl concentration be reduced below 5 to 10 mM.

Tubule Fluid [H^+] and [HCO_3^-]. Increased acidity of luminal fluid has been observed to decrease K secretion in some studies. In cortical collecting tubules perfused *in vitro,* lowering the luminal pH from 7.4 to 6.8 decreased net K secretion and increased the lumen-negative transepithelial voltage (127). Evidence indicates that this response to increased luminal acidity involves a decrease in apical membrane K conductance (128). Both acid luminal pH and luminal barium depolarize the apical membrane and block the ability of changes in luminal K concentration to effect changes in apical membrane voltage. Whether the effect of increased luminal acidity occurred through an interaction at the apical surface or through a change in cell pH was not evaluated in these studies. Little or no effect of decreasing luminal pH from 7.8 to 6.5 was seen during *in vivo* perfusion of distal tubules in rats (129).

Inhibitors

Potassium secretion is affected by at least two different classes of inhibitors that act when present in luminal fluid. One group of inhibitors, the *K-sparing diuretics* (amiloride, triamterene), affects Na channels in the apical membrane of certain distal cells. These compounds inhibit both Na absorption and K secretion by the distal tubule and the CCD. They act by blocking channels through which Na diffuses from the lumen into the cells (128). As a result of this blockade of diffusion of Na ions, the voltage across the apical membranes of distal cells is increased (cell more negative with respect to the lumen), causing a decrease in the lumen-negative transepithelial voltage (lumen less negative with respect to peritubular fluid) (130). The hyperpolarization of the apical membrane voltage decreases the electrochemical driving force, and, thus, retards diffusion of K from cell to lumen. An important side effect of the antibacterial drug, trimethoprim, also appears to be inhibition of Na channels and a decrease of K secretion in the distal tubule as it is being excreted (131,132).

Calcium ions can also inhibit K secretion (133). In distal tubules of rats perfused *in vivo,* increasing the luminal Ca^{2+} concentration to 0.8 mM maximally inhibited K secretion and reduced the transepithelial voltage (133). This concentration is near the upper limit of the luminal Ca^{2+} concentration found in normal animals and suggests a possible regulatory role for changes in tubule fluid Ca^{2+} on K secretion. The effect of luminal Ca^{2+} is similar to the effect of amiloride. Calcium ions and amiloride interact (113) and, when present together in high enough concentrations, can abolish the lumen-negative voltage and K secretion. Decreasing the concentration of Ca^{2+} alone at maximal inhibitory concentrations of amiloride results in an incomplete inhibition of the lumen-negative voltage and K secretion.

A second type of inhibitory action is exemplified by *barium.* Millimolar concentrations of Ba^{2+} in luminal fluid also decrease Na absorption and K secretion (126,134–136). The mechanism, however, contrasts with that of amiloride in that Ba^{2+} blocks channels through which K diffuses. This action tends to depolarize the apical membranes of distal cells (and to increase the lumen-negative transepithelial voltage). Barium ions indirectly affects distal Na absorption because the change in membrane voltage opposes diffusion of Na from lumen to cell.

The group of peritubular factors includes several constituents of plasma and interstitial fluid: K, hydrogen ions, bicarbonate, and hormones.

Plasma [K^+]. Increases in the plasma *K concentration* and the circulating level of *aldosterone* increase renal K excretion by stimulating distal K secretion. In experiments using *in vivo* perfusion of surface distal tubules in rat kidneys, different levels of plasma K concentration were established by infusing K over a 4-hour period (137). Because the tubules were perfused by delivering an artificial solution through a micropipette, entering concentrations of K, Na, and Cl and the flow rate of tubule fluid could be kept constant. Plasma aldosterone was 600 pM when plasma K was 4.2 mM, and the hormone levels increased two- to threefold as plasma K was elevated. Potassium secretion into these perfused tubules increased as both plasma K and aldosterone increased and plateaued above a plasma K of 6 mM (137). Thus, modest increases in plasma K (and secondary increases in aldosterone levels) are adequate to stimulate distal K secretion maximally.

Aldosterone. It has also been possible to examine separately the effects of changes in plasma K concentration and aldosterone levels (138–140). Adrenalectomized rats were maintained for several days by infusing "basal" quantities of aldosterone and dexamethasone. Then, solutions containing either vehicle or additional aldosterone were infused with either a Ringer's solution or a KCl solution. When aldosterone was kept constant, at either rate of administration, increasing plasma K concentration from 5 to 7 mM stimulated distal K secretion. Conversely, when the plasma K concentration was kept constant, at either normal or elevated levels, infusion of aldosterone for 2 hours increased the rate of distal K secretion. In another group of animals, acute infusion of additional dexamethasone was without effect on the rate of distal K secretion (139), although it did increase the rate of excretion in the final urine, as others have observed (141,142). Spironolactone blocks the binding of aldosterone to mineralocorticoid receptors, and thus inhibits aldosterone actions on transepithelial voltage and K secretion (143).

Plasma [H^+] and [HCO_3^-]. The effects of changes in plasma acidity on K secretion by the distal tubule have also been studied by *in vivo* microperfusion (129). In these experiments, the flow rate and solute composition of luminal fluid were kept constant. Metabolic acidosis, produced by gavage of 3 to 4 mmol NH_4Cl 1 hour before the measurements, caused distal K secretion to decrease. Metabolic alkalosis, produced by intravenous infusion of 3 to 4 mmol sodium bicarbonate 1 hour before the measurements, caused distal K secretion to increase (129).

Vasopressin. It has recently been recognized that circulating levels of vasopressin can also affect K secretion (140,144–146). *In vivo* microperfusion experiments have shown that systemic infusion of vasopressin stimulates secretion of K into perfused distal tubules (140). It appears that when vasopressin acts to increase the water permeability of apical membranes by a process involving incorporation of water channels from vesicular stores in the cytoplasm (147), it also causes Na and K conductance to increase (145,148). This effect is rapid and may be transient (149). Potassium secretion is stimulated both because the depolarization of the apical membrane increases the electrochemical driving force favoring diffusion of K into the tubule lumen (145) and because the density of K channels is increased (148). When vasopressin stimulates Na and Cl absorption by medullary TAL, it also increases K conductance (150).

CELL MECHANISMS OF POTASSIUM TRANSPORT

The walls of renal tubules consist of a single layer of epithelial cells surrounded by a basement membrane. The cells are polarized, with apical and basolateral domains separated by junctional complexes. Transport proteins integrated into these membranes in contact with either luminal fluid or interstitial fluid determine the functional contributions of different cell types along the nephron. In renal tubule cells, as in other cells in the body, steady-state levels of cell K depend on the balance between influx and outflow. Potassium ions are actively taken up from interstitial fluid by the adenosine triphosphatase (ATPase)-driven Na/K exchange pump located in the basolateral membranes. Potassium is able to leak out of renal tubule cells across basolateral membranes, and in some tubule cells, across the apical membranes as well. These leak pathways include K channels, which permit diffusion of K ions, and K-Cl cotransporters, which provide an electroneutral pathway. Outward diffusion of K through K channels in the basolateral membrane is largely responsible for the inside-negative voltage across this barrier.

Potassium Channels

Potassium (K) channels are integral membrane proteins that mediate passive flow of K across cell membranes via a highly selective, aqueous pore. K channels represent the largest and most diverse class of ion channels as revealed by molecular studies and by numerous electrophysiologic studies documenting the existence of a wide array of K currents in virtually all cells examined to date (151). While the use of the patch-clamp method led to rapid progress with regard to the kinetic and pharmacologic properties, and the cellular and tissue distribution of K currents, biochemical and structural information lagged behind until 1987. Conventional biochemical approaches failed to elucidate the structure of K channels, principally, because of a lack of high-affinity probes and because of the fact that these proteins are expressed in very low amounts and are not readily amenable to large-scale purification. A major breakthrough in understanding the molecular structure of K channels was achieved in 1987. Electrophysiological studies of *Drosophila melanogaster* mutants (*Shaker*) that exhibited leg shaking when exposed to ether, a volatile anesthetic, indicated the *Shaker* locus might encode a structural component of a voltage-dependent K (K_v) channel. A combination of mutation mapping and chromosome walking was used to successfully identify the *Shaker* gene (147,152–154). Expression of the *Shaker* gene product in *Xenopus* oocytes confirmed it mediated K_v currents (155). Three additional *Drosophila* K channel genes (*Shal, Shaw,* and *Shab*) were isolated by homology cloning (156), and the first mammalian K channel was isolated by homology cloning in 1988 (157). Subsequently, homologs of each *Drosophila* gene have been identified in mammals either by homology or expression cloning. Three other major classes of K channels have been isolated, namely the calcium-activated K channels (158), the inward rectifiers (159) and the two-pore (2P) channels (160,161). The 2P channels appear to be the most diverse group of K channels with more than 50 members identified to date.

All K channels mediate the rapid (more than 1 million ions per second) and selective transport of potassium. Prior to the cloning of the first K channel, a fairly comprehensive and rather accurate view of the pore and the selectivity filter was constructed based on the available electrophysiological data (162). Site-directed mutagenesis experiments of known K channels proteins (163–168) have confirmed the model. Direct and structural data provided further details about the pore (169–171). The essential components of the pore are two membrane segments linked by a stretch of approximately 25 amino acids, hydrophobic enough to partly penetrate the lipid bilayer, and also containing a highly conserved, signature sequence: TMxTVGYG. This sequence, along with the last membrane segment (M2 and S6 for the 2-TM and 6-TM class), form the lining of the pore and determine permeation and selectivity. The pore has a wide extracellular vestibule that narrows abruptly to about 3 Å to form the selectivity filter. This filter provides three binding pockets in which a dehydrated K ion fits better than a Na ion. Potassium ions are arranged in a single file and in close enough proximity that a high dissociation rate is driven by electrostatic repulsion. This model may not accurately reflect the more complex structure of the 6-TM K channels, since in these larger channels, other segments also modify pore function (170,172).

If deduced amino acids sequences and secondary structure information are used to construct a structural classification of potassium channels, three broad classes emerge from such analysis. The first consists of channels with six or seven transmembrane segments and one pore region, and includes the *Shaker*-related K_v channels (Fig. 5-3), KvLQT1 and Ca^{+2}-activated K channels (K_{Ca}). The inward rectifiers have two transmembrane segments and one pore, and include ATP-sensitive channels and channels such as ROMK. The newest and most diverse group is comprise of K channels with four transmembrane segments and two pores (160,161). These channels appear to mediate background, leak K currents in a variety of cells, including renal cells.

The properties observed for cloned α subunits very rarely match currents known to exist in native tissues. This is usually because the pore-forming α subunits interact with accessory proteins that modify their properties, and often modulate the levels of protein expression. K_v channels are complexes consisting of four α subunits that all contribute to form the pore and β subunits that modulate channel function in variety of ways. The tetrameric nature of K channels has been clearly proved (173–178). A number of β subunits have been characterized so far. The Kvβ genes encode soluble proteins (367 to 404 amino acids), related to the NAD(P)H-dependent oxireductase

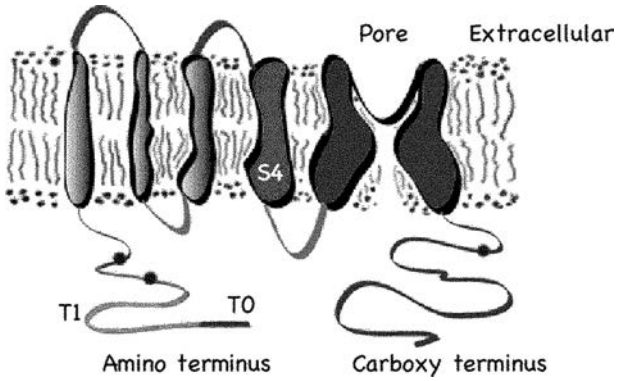

FIGURE 5-3. Schematic representation of the α subunit of Kv1.3, a voltage-gated, K_v channel with six transmembrane segments; *S4* represents the voltage sensor; *T0*, domain that regulates channel abundance at the plasma membrane; *T1*, tetramerization domain, which modulates hetero- and homomultimeric assembly. (From: Desir, GV. Kv1.3 potassium channel blockade as an approach to insulin resistance. *Expert Opin Ther Targets*. 2005;94:571, with permission.)

superfamily, that interact with many Kvα subunits (179). The KChIP genes encode calcium sensors that modify the expression level and kinetic properties of Kv4.2 and Kv4.3 (180). The KChAP proteins act as chaperone for Kv proteins, such as Kv1.3, Kv2.1, Kv2.2, and Kv4.3 (181). The MinK related proteins (MiRPs) also interact with K_v channels (182–186). *KCNA4B* encodes a soluble protein (141 amino acids) with no amino acid homology to known β subunits, but with limited structural similarity to the NAD(P)H-dependent oxireductase superfamily. It increases KCNA10 current expression by at least 2.8-fold and also alters its sensitivity to cAMP (187). The calcium sensitivity of the Maxi-K channel is modulated by a β subunit (188). The inward rectifiers also interact with specific β subunits (sulfonylurea receptor or SUR) that confer sensitivity to inhibition by sulfonylureas (189).

Specific Functions of K Channels

Regulation of Membrane Voltage

Electrogenic transporters exist in the proximal tubule and their activities result in significant cell membrane voltage depolarization (190–194). For instance, proximal tubule cells can depolarize by 10 to 15 mV when electrogenic transport processes such as Na–glucose or Na–amino acid transport are activated. Since depolarization of the cell membrane results in decreased in solute uptake, mechanisms that restore the membrane potential are critical. Voltage- gated potassium channels are well suited for that task since, when activated by membrane depolarization, they allow K efflux and cell membrane hyperpolarization. Several K_v channels have been detected at the apical membrane of the proximal tubule, by patch clamp, and immunocytochemistry. The most commonly reported K_v channel belongs to the Maxi-K class, since it is also activated by calcium and has a large single-channel conductance (194). Northern blotting, *in situ* hybridization, and immunocytochemistry clearly indicate the presence of at least two other types of voltage-gated potassium channels, namely, KCNQ1 and KCNA10. KCNQ1, a 6-TM channel, forms a small conductance (2 to 10 pS) K_v channel and is thought to exist in kidney as a heteromultimeric complex with KCNE1 (MinK), a protein with a single transmembrane segment that is highly expressed at the apical membrane of the proximal tubule. Both proteins are also abundantly expressed in kidney and recent data indicate that KCNQ1/KCNE1 may play an important role in the regulation of sodium-coupled glucose and amino acid uptake (195). KCNE1-deficient mice exhibit a reduction in glucose and amino acid uptake by the proximal tubule However, these data do not exclude the possibility that additional K_v channels, such as Maxi-K and KCNA10 (196), also participate in stabilizing proximal tubule membrane potential during electrogenic Na uptake.

K Recycling

Leakage of K out of tubule cells plays an important role in the regulation of cell K concentration and in the process of K secretion (197–201). Under steady-state conditions, the rate of active K uptake is equal to the passive leak of K. If the stoichiometry of Na/K exchange (three Na exchanged for two K) does not vary, stimulation of the active pump must be matched by an increase in the K leak. For example, with large increases in transepithelial net Na transport, both K uptake and apical and basolateral K conductance must also increase. The converse is also true. For cells to maintain normal K levels when pump activity is reduced, the passive leak of K ions must be proportionately decreased. This occurs, for example, in damaged kidneys and has been observed in slices of renal cortex or in preparations of collapsed tubules (199,202–209).

Possible explanations for this functional coupling between K uptake by Na/K exchange and K exit through leak pathways include mediation by cytosolic ATP levels, cytosolic Na concentration, and basolateral membrane voltage. Increases in Na/K-ATPase activity are expected to consume ATP. Because cytosolic ATP reduces conductance of apical K channels in several tissues (210,211), decreases in ATP would favor increased K leak when K uptake is increased. Similarly, increases in Na/K exchange are expected to decrease the cellular Na concentration (197,200). Because calcium extrusion from these cells is accomplished by a basolateral Na/Ca exchanger, a reduction in cellular Na would favor increased Ca^{2+} extrusion and reduction of cellular Ca^{2+}. Because cellular Ca^{2+} reduces the activity of apical and basolateral K channels (212), decreases in Ca^{2+} favor increased K leak when K uptake is increased. A third explanation for the coupling between K uptake and K leak recognizes that increases in Na/K exchange increase the cell negative basolateral membrane voltage. Because basolateral K channels open when basolateral voltage is increased (hyperpolarization) (213), increases in Na/K exchange should be accompanied by voltage-induced increases in K conductance (214).

The predominant *basolateral K channels* observed in patch-clamp studies are selective for K, have a relatively small conductance (30 pS), are moderately inwardly rectifying, have a high open probability (0.9), and are, in general, insensitive to ATP. These channels are activated by cyclic guanosine monophosphate and nitric oxide and are inhibited by increased cell H^+ concentration, by millimolar concentrations of Mg, and Ca^{2+}. Thus, these channels are similar to the K_{IR} channels identified in brain and other tissues, but differ from the apical K secretory channel in displaying stronger inward rectification and being insensitive to ATP and glibenclamide. Structurally, basolateral K channels may, like apical secretory K channels, belong to the family of K_{IR} proteins, but basolateral K channels appear to represent K_{IR} 6.1. (215). This protein, like ROMK, has two transmembrane segments and a hydrophobic pore region, and when coexpressed with the sulfonylurea receptor (SUR2A/2B) in *Xenopus* oocytes, it mediates an ATP-sensitive K current that closely resembles the native basolateral K current, with sensitivity to inhibition by both taurine and glibenclamide.

K Secretion

Observations from a number of studies (102,135,148,212, 216–228) indicate that the *secretory K channels* in apical

membranes of distal cells are highly selective for K, have a relatively small conductance (10 to 40 pS), are weakly inwardly rectifying, have a high open probability (0.9), and are insensitive to membrane voltage. These channels are activated by protein kinase A (PKA) and are inhibited by increased cell H^+ concentration, by millimolar concentrations of Mg-ATP, and by protein kinase C (PKC) or calcium/calmodulin kinase. Thus, these channels are similar to the ATP-sensitive K channels (K_{ATP}) in heart and other tissues (229). Structurally, secretory K channels appear to belong to the family of inwardly rectifying K channel proteins (K_{IR}) discovered by Ho et al. (159) in rat kidney outer medulla and called ROMK (K_{IR} 1.1). Three isoforms of this protein have been identified with somewhat different distributions along the distal nephron (230–234). Important features of this protein are two transmembrane segments and a hydrophobic pore region (analogous to the pore region of the voltage-gated channel) that forms a pore when four subunits associate in the membrane. It is likely that, *in vivo*, the active channel is a heteromultimeric complex containing a sulfonylurea receptor molecule (SUR) (235). ROMK channel activity is regulated in a complex manner by a variety of factors (151). Cytosolic acidification inhibits and alkalinization stimulates channel activity (236). Expression levels and channel function are modulated by several kinases and phosphatase. Serine-threonine kinases, such as PKA prevent channel run-down excised patches (237) by phosphorylating Ser-44, Ser-219, and Ser-313 in ROMK1 (233). The with-no-lysine kinase, WNK4, has been shown to modulate the expression of ROMK at the plasma membrane by stimulating protein endocytosis and, thereby, decreasing channel activity. Interestingly, WNK4's action on ROMK appears to be independent of phosphorylation (238). Mutations in either WNK1 or WNK4 cause pseudohypoaldosteroninsm type II (Gordon syndrome), which is characterized by hypertension and hyperkalemia (239–241). Aldosterone increases serum glucocorticoid-activated kinase (SGK) levels, which, in turn, phosphorylates ROMK1 at Ser-44, causing a shift in acid sensitivity and an increase in channel activity (242). SGK1 action on ROMK requires the presence of the NHERF-2, a regulatory factor of the Na^+/H^+ (242). Unlike PKA and SGK, PKC inhibits ROMK channel activity through a phosphatidylinositol 4,5-bisphosphate-dependent mechanism (243). ROMK channel activity is linked to changes in dietary K intake (244,245). Increased K load stimulates ROMK channel activity and reduced K intake does the converse. Protein tyrosine kinase and phosphatases appear to play a critical role in this process, by regulating the endocytic recycling of ROMK channels at the plasma membrane. ROMK channel activity is also inhibited by arachidonic acid (246) and activated by phosphatidylinositol phosphates (PIP_2) (247).

Genes coding for voltage-gated K channel (K_V) proteins have been cloned from renal libraries, cell cultures, and microdissected CCDs (196,248–250). Structurally, these channel proteins are in the gene family identified in the mutant *Drosophila, Shaker*, which has members extensively studied in excitable cells. The measured intracellular potential across the luminal membrane in cell of the distal part of the CCD average –20 mV and most K_v channels would be active at these potentials. In the inner medulla, extracellular K can reach levels greater than 40 mM as a result of K being trapped in the tubule by the well-documented process of K recycling. The resting membrane potential is close to the reversal potential for K (–90 mV), since K channels constitute the major ionic pathway of unstimulated cells. Therefore, increasing extracellular K from 4 to 30 mM will cause the resting membrane potential to change from approximately -90 mV to -20 mV. This degree of depolarization would be sufficient to activate many K_v channels. There are functional data attesting to the expression of K_v channels in the renal medulla (250). While several Shaker-related gene are detected in kidney, Kv1.2 and Kv1.3 appear to be preferentially localized at the distal tubule (249,251,252). Several recent findings support the notion that Kv1.3 participates in distal tubular Na reabsorption and K secretion. Indeed, K_v currents resembling those mediated by Kv1.3 have been recorded at the apical membranes of the CCD; Kv1.3 protein is expressed at the luminal membrane of principal cells of the CCD. Kv1.3 is activated by serumglucocorticoid-activated kinase (SGK), an important mediator of aldosterone action at the distal tubule (253). These observations suggest that Kv1.3 may link aldosterone-stimulated Na reabsorption and K secretion and could contribute to the maintenance of membrane potential.

Maxi-K channels are believed to play a significant role, not only in the regulation of the volume of renal cells (254–257), but also in renal solute transport. Large (100 to 250 pS) Ca^{2+}-activated K currents have been detected at the apical membrane of the principal cells of the CCD (256). While their contribution to K secretion was deemed uncertain in light of their very low open probability, recent studies suggest an important role for these K channels in flow-dependent potassium secretion both in the connecting tubule (258) and in the CCD (259). Microperfusion data indicate that flow-stimulated net K secretion occurs through a charybdotoxin (CTX) sensitive pathway. Maxi-K channels are excellent candidates for mediating this flux, since they are expressed at the apical membrane of the CCD and since, unlike ROMK (believed to mediate the bulk of K secretion by principal cells), they are exquisitely sensitive to inhibition by CTX. Maxi-K channels require both Ca^{2+} and membrane depolarization in order to be activated, and it was therefore postulated that the increased flow rate might stimulate a stretch-activated calcium channel, which would, in turn, mediate the necessary increase in intracellular Ca^{2+}. It is also possible that other CTX-sensitive pathways contribute to flow-dependent potassium secretion. Overall, these studies have provided a clearer understanding of the processes involved in distal tubular K secretion and it appears that ROMK primarily mediates baseline K secretion, while Maxi-K channels and possibly other K_v channels, facilitate flow-dependent K secretion.

KCl COTRANSPORTERS

KCl cotransporters (KCC) belong to the electroneutral cation–chloride-coupled cotransporter gene family (SLC12), which is subdivided into two main subfamilies, and contains nine members to (260). The first KCC gene was isolated by homology cloning (261) and three additional genes have been identified. The KCC1-4 genes encode integral membrane proteins with 12 membrane-spanning domains. Gene expression is not tissue specific except for KCC2, which is predominantly found in neurons. Expression of these proteins in *Xenopus* oocytes reveals swelling-activated K fluxes. KCC4 is highly expressed in kidney and gene inactivation in mice leads to renal tubular acidosis, which is thought to reflect defective acid secretion by α-intercalated cells (262). KCC proteins could mediate renal K secretion if luminal chloride concentration fell to levels sufficiently low to generate a favorable chemical gradient for chloride efflux.

Proximal Tubule Cells

Even though a large fraction of filtered K is reabsorbed by the proximal convoluted and straight tubules, the mechanisms responsible for transporting K in these segments remain incompletely understood. A component of active K reabsorptive transport likely exists in early segments of the proximal convoluted tubule, where the transepithelial voltage is lumen negative (263). In several studies, the luminal K concentration has been

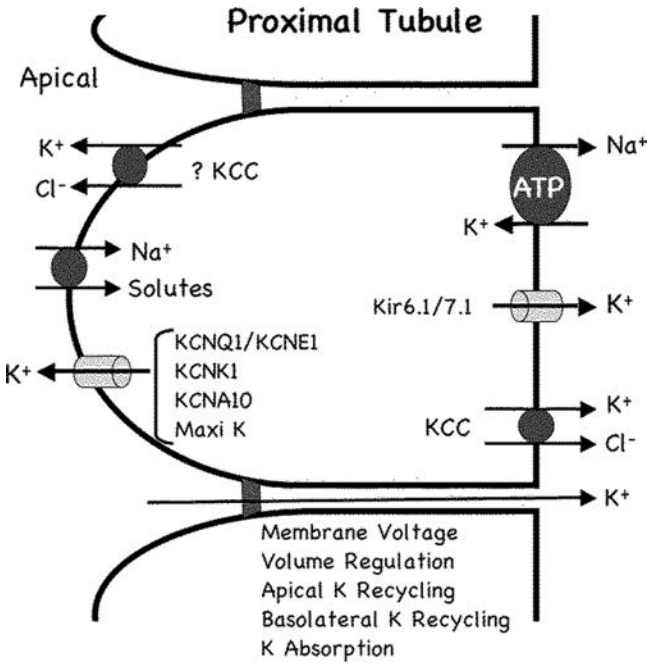

FIGURE 5-4. Proximal tubule cell. Both transcellular and paracellular pathways for K absorption are shown. Sodium enters these cells by several cotransport mechanisms not shown here. The Na/K-adenosine triphosphatase pump on the basal and lateral membranes raises cellular K and lowers the K concentration in the intercellular spaces. Basolateral K exit pathways include both K channels and K-Cl cotransporters. The transepithelial voltage reverses direction from lumen negative in P1 to lumen positive in P2 and P3. The molecular identity of the known K channels and transporters are specified. *KCC*, KCl cotransporter; *KCNQ1, KCNA10*, voltage-gated K channels; *Maxi-K*, voltage- and calcium-gated K channel; *KCNK1*, two-pore K channel; *Kir 6.1/7.1*, inward rectifier K channels.

found to be lower than in plasma (54). Potassium absorption in these circumstances is uphill. An active uptake mechanism has not been identified in the apical membrane of proximal tubule cells. An alternative explanation (54) is that the basolateral Na/K-ATPase may provide the necessary driving force (Fig. 5-4). Na/K pumps located in lateral cell membranes along the intercellular spaces would be expected to reduce the K concentration in the paracellular compartment. Thus, although the luminal K concentration is somewhat lower than the peritubular K concentration, there still could be a downhill gradient for K across the junctional complex from lumen to intercellular space. Potassium could continue to move from the intercellular space to the interstitial space driven by bulk flow of water. Water moves by osmosis from lumen to cell across the apical membrane and then from cell to interspace across the lateral membrane (264).

These considerations should not be taken to mean that active K reabsorption is the only mechanism of transport in the proximal convoluted tubule. There is also evidence that passive K reabsorption, particularly in the later part of the proximal convoluted tubule, is functionally important. First the lumen-positive transepithelial voltage in the later part of the proximal convoluted tubule provides a favorable driving force for net K reabsorption. It is likely that K movement driven by the transepithelial voltage occurs through the paracellular shunt pathway. Second, K movement is affected by net fluid movement. Experiments using *in vivo* microperfusion (53,265–267) have shown that the direction of net K transport depends on that of net fluid movement when the latter was changed by varying the tubule fluid osmolarity (addition of mannitol or raffinose). Previous observations had shown that maneuvers inhibiting proximal Na and water transport (extracellular volume expansion) also block K reabsorption. This effect is consistent with the notion that Na-dependent fluid reabsorption entrains K ions.

Thick Ascending Limb Cells

Most information about the functional characteristics of these cells has come from studies using *in vitro* perfusion of isolated segments of mammalian TAL and the early distal tubule of the frog and the congo eel (*Amphiuma means*) (64,268–274). The larger amphibian cells are more amenable to direct impalement with microelectrodes, and, therefore, have allowed insight into the transport mechanisms serving to control K transport in these nephron segments. Cell culture of TAL cells has also provided access for studies of ion transport mechanisms at the cellular level (275–277).

Potassium reabsorption by the TAL depends on the three-ion cotransporter situated in the apical membrane of TAL cells (Fig. 5-5). Potassium transport in this segment usually contributes to reabsorption of filtered K ions. Potassium is carried from lumen to cell by the Na-K-2Cl cotransporter and leaks back to the lumen through apical K^+ channels (274,278–281). This recycling across the apical membrane means that K facilitates reabsorption of Na and Cl, that inhibition of the cotransport mechanism can lead to net K secretion, and that inhibition of the K conductance can reduce absorption of Na and Cl (270).

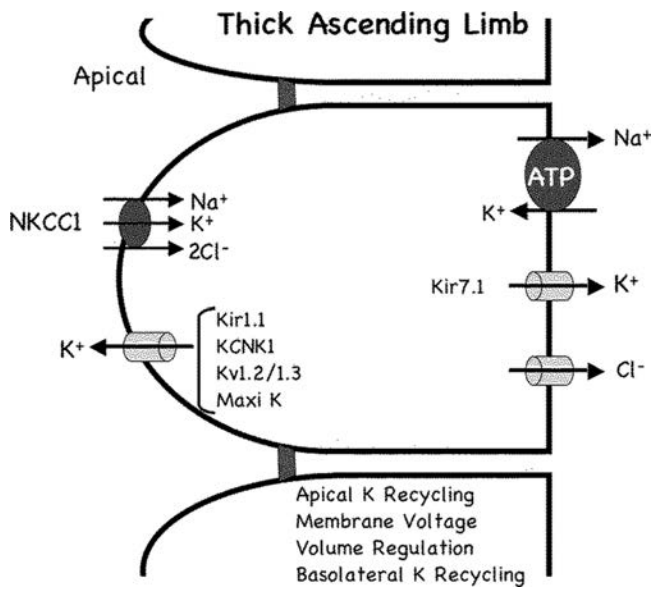

FIGURE 5-5. Thick ascending limb cell. Potassium can be absorbed by the Na-K-2Cl cotransporter (inhibited by furosemide) and secreted by diffusion through K channels in the apical membrane. The Na/K-adenosine triphosphatase pump on the basal and lateral membranes raises cellular K and lowers cellular Na concentrations. Basolateral K and Cl exit pathways include both K and Cl channels and K-Cl cotransporters. The lumen-positive transepithelial voltage provides a driving force for cation transport through the paracellular pathway. The molecular identity of the known K channels and transporters are specified. *KCC*, KCl cotransporter; *NKCC1*, Na/K/2Cl cotransporter; *Kv1.2, Kv1.3*, voltage-gated K channels; *Maxi-K*, voltage- and calcium-gated K channel; *KCNK1*, two-pore K channel; *Kir7.1*, *ROMK*, inward rectifier K channels.

Both an intermediate- (70 pS) and a low-conductance (30 pS) K channel have been identified in the apical membrane of the mammalian TAL (274,281,282). In the rat, both are present, whereas in the rabbit, the intermediate-conductance channel appears to be absent. These two channels are similar in that the open probability is very high (0.8 to 0.9) and is decreased by millimolar ATP and barium, and that both channels are stimulated by low adenosine diphosphate (ADP)/ATP ratios. However, they differ in that the small-conductance channel is not inhibited by tetraethylammonium (TEA), quinidine, or millimolar calcium and is sensitive to glyburide, an inhibitor of K_{ATP} channels. Both channels are present at approximately the same density. The properties of these native channels bear a strong resemblance to the properties of the ROMK channel. The physiologic roles of the individual apical K channels have not been identified with certainty.

The basolateral Na/K exchange pump provides the primary driving force for net K reabsorption. Na/K exchange reduces cell Na and generates a steep Na gradient across the apical membrane. This gradient provides the energy for the coupled entry of Na, K, and 2Cl ions. The Na-K-2Cl cotransporter was cloned (283,284) and is the site of action of the potent loop diuretics. The apical membrane permits diffusion of K ions (273,285). The K concentration gradient across the apical membrane favors diffusion of K from cell to lumen and allows recycling of K ions back to the tubule lumen. This back-diffusion provides a continuous luminal supply of K ions for cotransport with Na and Cl at a high rate. Without this apical membrane recycling of K, the low luminal K concentration (1 to 3 mM) would limit the capacity for absorption of Na and Cl. A subgroup of patients with Bartter's syndrome provides an example of the consequences when this process of apical K recycling is absent (286). In these patients, a mutation in the ROMK gene, leading to a lack of apical K channels, was identified as the cause of the disease. A persistent failure of Na and Cl absorption by the TAL results in extracellular fluid volume depletion, normal blood pressure, and the characteristic hypokalemic metabolic alkalosis of these patients. Apical K channels are also responsible for net K secretion under those conditions in which the activity of the reabsorptive cotransport mechanism has been impaired. For example, net reabsorption of K can be changed to net secretion after administration of loop diuretics (furosemide, bumetanide, piretanide, muzolamine, ethacrynic acid) or replacement of luminal Cl with non-chloride anions (287–289).

The basolateral membrane permits diffusion of both K and Cl. A Cl conductance dominates (278,290–292), although K-Cl and K-HCO_3 cotransport pathways have been identified in the basolateral membrane (293). By itself, the Cl concentration gradient across the basolateral membrane favors diffusion of Cl from interstitium into the cell. Thus, ion fluxes through both apical K channels and basolateral Cl channels generate diffusion potentials oriented with the cell interior negative to the extracellular fluids. However, because the K concentration gradient across the apical membrane exceeds the Cl concentration gradient across the basolateral membrane, the voltage across the apical membrane exceeds the voltage across the basolateral membrane (278,290). This difference between membrane voltages creates the lumen-positive transepithelial voltage and provides the driving force for passive absorption of Na and divalent cations through the intercellular shunt pathway (294). Thus, a component of K reabsorption could also occur passively through the intercellular pathway. Chloride is able to diffuse from cell to interstitium because the cell-negative voltage across the basolateral membrane exceeds the Cl equilibrium potential. It is kept above its electrochemical equilibrium concentration by secondary active transport from the lumen into the cell through the Na-K-2Cl cotransporter.

In addition to conductive mechanisms for K and Cl exit across the basolateral membrane of the TAL cell, an electroneutral pathway for K and Cl fluxes has also been postulated (290). A gene encoding a K/Cl cotransporter protein has been cloned (171). This cotransporter (KCC) is a member of a large cation-chloride cotransporter family that includes the thiazide-sensitive cotransporter and the furosemide-sensitive three-ion cotransporter. To date, four KCC genes have been identified (295,296). The KCC4 isoform appears to be expressed in the basolateral membrane of TAL cells (257).

Net reabsorption of Na, K, and Cl and the lumen-positive voltage are abolished in the absence from the lumen of any of the three ions (270,280,297). In addition, luminal application of furosemide eliminates Na, K, and Cl absorption and the transepithelial voltage (298). Interference with the cotransport system, either by luminal ion substitution or by administration of furosemide, also affects cellular ion activities (567). Inhibition of the apical Na-K-2Cl transporter lowers cellular Cl and Na activities, supporting the view that the cotransport system is the main pathway for entry of Na and Cl ions into the cell. Loop diuretics not only inhibit Na and Cl absorption, but induce net K secretion (285). These effects are explained as the consequence of inhibition of Na-K-2Cl cotransport and continued leak of K through K^+ channels in the apical membrane, resulting in the appearance of K secretion (289,299–301). Apical Na/H exchange provides a second pathway for Na to enter TAL cells and is independent of luminal K.

Evidence that a small conductance K channel is present in the TAL (and in the CCD principal cell; see later) and is involved in apical K recycling and salt absorption comes from experiments using the sulfonylurea, glyburide (302). Sulfonylureas block channels expressed in oocytes (K_{ATP}, ROMK) and native channels in patches of rat TAL cells (234,281,303–305). Clearance experiments in awake rats show an increase in Na excretion with glyburide infusion without increases in K excretion (302). The data are consistent with this agent blocking apical K channels in both the TAL and CCD principal cells. Na-K-2Cl turnover in the TAL is decreased because of impaired apical K recycling, and increased K secretion downstream is prevented because of a direct block of the secretory K channel (302).

A gene encoding a calcium receptor protein was cloned from bovine parathyroid (306,307). This calcium-sensing molecule is thought to bind calcium external to the cell and initiate through G protein coupling a cascade of intracellular events. Phosphoinositol turnover is increased, calcium is released, and other pathways are activated. These signals ultimately lead to a decrease in PTH secretion by the cell (306,307). A renal form of this calcium-sensing protein has now been found in cortex and medulla by *in situ* hybridization. The distribution is consistent with localization in the TAL (308). The role of the calcium sensor may be important in regulating calcium transport by the TAL in familial hypocalciuric hypercalcemia (309). It may also prove to be important in the regulation of K transport and Na and Cl transport responsible for dilution and concentration of the urine.

Systemic influences on K excretion appear to affect TAL cells, although clear evidence that these changes play a regulatory role is not in hand. Potassium depletion, for example after ingestion of a low-K diet, appears to enhance K absorption (113,310). The apical membrane K conductance is increased by aldosterone, vasopressin, and alkalosis (150,311–313). On the other hand, K conductance is reduced by acidification of the intracellular and extracellular fluids (314,315). Either lowering bicarbonate or elevating PCO_2 reduced the K conductance of the apical membrane. This effect is consistent with the K-sparing effect of acidosis as demonstrated in perfusion studies of the distal tubule (129).

In some species, a mechanism that exchanges luminal Na for cytosolic hydrogen ions is located in the apical membrane and contributes to raising intracellular pH (316,317). In amphibians, aldosterone has been found to enhance apical K conductance and net K secretion secondarily by activating apical Na/H exchange and by alkalinizing the cell (311,318,319). Amiloride, when administered in doses high enough to inhibit Na/H exchange, decreases K secretion by acidifying the cell. Loop diuretics may act secondarily to increase K secretion (in addition, to the direct effect described previously) by lowering cell Na influx through the Na-K-2Cl cotransport mechanism, lowering cell Na concentration, increasing the driving force for Na/H exchange, raising cell pH, and thus enhancing apical K conductance (311). Increased luminal acidification caused by loop diuretics could also be related to the stimulation of Na/H exchange affected by the decrease in cell Na.

Distal Convoluted Tubule Cells

The DCT is a cytologically homogeneous segment (72,74) that begins a short distance beyond the MD region and extends to the CNT. The border between the DCT and the CNT is abrupt in some species (rabbit) and gradual in others (rat, human, and mouse) (72,74,97,320,321). The cells are notably larger than the TAL cells and have prominent basolateral infoldings packed with elongated mitochondria. The DCT epithelium has been studied in a number of different species using both *in vivo* and *in vitro* techniques (44,80,86,88,91,322–324).

Most of the *in vivo* data on K transport by the DCT have been obtained by applying microperfusion methods in rat kidneys. The DCT does secrete K (44,70); however, compared with the next segments downstream (ICT and CCD), it makes a smaller contribution to final K excretion under most circumstances.

Although some observations indicate that K and Na transport by the DCT occurs by conductive mechanisms (channels) (80,232,324–330), most available data demonstrate electroneutral processes for apical K, Na, and Cl transport (80,320,321) (Fig. 5-6). A reexamination of the transepithelial voltage along the rabbit distal tubule perfused *in vitro* (330) suggests that the voltage of the DCT segment is zero and not lumen negative as has been reported previously. In support of primarily electroneutral processes in the DCT, the perfused tubules showed no response to luminal amiloride or barium. These observations (330) argue against the presence of functional amiloride-sensitive Na channels or barium-sensitive K channels. Molecular and immunohistochemical techniques have also been used to study the expression of the epithelial Na channel protein (ENaC) in the DCT of rats and rabbits. Although the ENaC α subunit was found to be expressed, neither the β nor the γ subunit is expressed by the DCT (325,326,328,330). Even though the potassium channel protein ROMK is expressed at the apical membrane of the DCT, most physiologic data suggest that this channel is not active in the DCT under normal conditions (80,232,327,330). Results obtained from DCT cells grown in culture or from DCT cell lines differ fundamentally from data obtained *in vivo* or from perfused tubules. In general, DCT cells in culture exhibit a lumen-negative voltage, express amiloride-sensitive sodium channels, and secrete K through a barium-sensitive pathway (323,331). Potassium secretion by the DCT *in vivo* is stimulated by low luminal Cl concentrations and by high luminal Na concentrations. When luminal Cl was lowered to less than 10 mM, the rate of K secretion by the DCT was doubled (44). Because this change in K secretion occurred without a change in transepithelial voltage, it appears that an electroneutral mechanism for K flux from cell to lumen coupled to Cl was present in the apical membrane of the rat DCT.

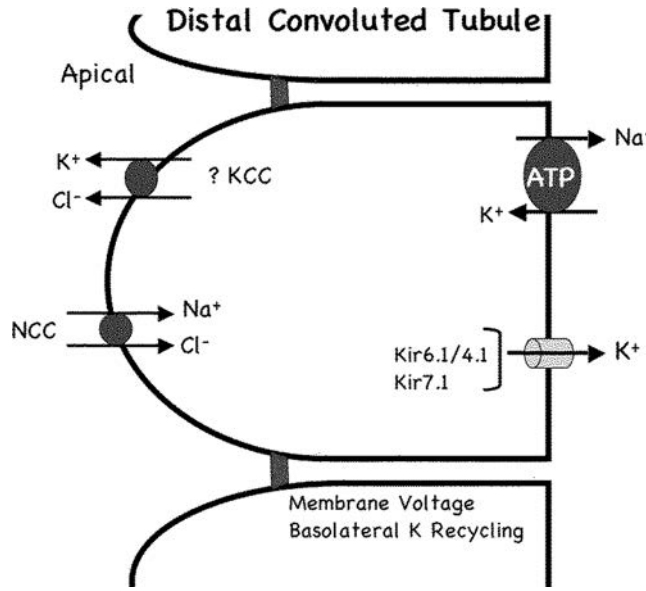

FIGURE 5-6. Distal convoluted tubule cell. Major transport pathways in the apical membrane are electroneutral cotransporters. The Na-Cl cotransporter is sensitive to thiazide diuretics. The K-Cl cotransporter is sensitive to cell , luminal Cl concentrations. Basolateral K exit pathways include both K channels, K-Cl cotransporters. The molecular identity of the known K channels, transporters are specified. *KCC*, KCl cotransporter; *NCC*, Na/Cl cotransporter; *Kir6.1/4.1, Kir 7.1*, inward rectifier K channels.

It has been shown previously that when luminal Cl is kept above 70 mM, increasing the luminal Na concentration above 40 mM did not affect K secretion by distal tubules (123). However, when luminal Na was raised above 40 mM, while the luminal Cl concentration was simultaneously kept below 10 mM, K secretion by the DCT was increased (36). The stimulation of K secretion by raising luminal Na concentration in the presence of low luminal Cl concentrations appears to be a unique response of the DCT (36). To date, there is no evidence that an Na concentration above 40 mM stimulates K secretion in segments distal to the DCT.

Additional evidence that supports a role for the DCT in K secretion and suggests that this segment is a site of Na-dependent K secretion (K secretion stimulated by high luminal Na while luminal Cl is low) comes from the use of specific inhibitors of Na transport by DCT cells. Thiazide diuretics are known to inhibit Na and Cl absorption by the rat DCT (86,87,89). A gene encoding an Na-Cl cotransporter protein of the kidney was cloned from kidney (283) and its expression was localized to the DCT (320,321). Thiazide diuretics also inhibit K secretion when luminal Na concentration is high and Cl concentration is low. To explain the Na dependence of K secretion in thiazide-sensitive cells, Velázquez and co-workers (332) have postulated that an absorptive Na-Cl cotransport mechanism and a secretory K-Cl cotransport mechanism operate in parallel in the apical membrane of DCT cells (332). In this view, increasing the luminal Na concentration increases Na-Cl cotransport, raises cellular Cl, and, thus, drives increased K secretion through K-Cl cotransport. Thus, in conditions, such as intravenous infusion of sodium sulfate solution, which are known to cause high distal concentrations of Na and low concentrations of Cl, renal K excretion may be increased because of enhanced K secretion by DCT cells equipped with apical K-Cl cotransporters.

To account for the largely electroneutral secretion of K and reabsorption of Cl by the DCT, pathways for electroneutral K and Cl exit from the cell must be postulated. Because Cl

is absorbed at approximately the same rate as Na and only a small fraction of K exchanged for Na across the basolateral membrane is secreted, the exit step for K and Cl likely occurs across the basolateral membrane. Evidence that the cytosolic K concentration in the DCT is not above electrochemical equilibrium argues for the presence of a basolateral K-Cl cotransporter in addition to K channels. This electroneutral K-Cl pathway would permit the Cl entering across the apical membrane through the Na-Cl cotransporter, and the K entering across the basolateral membrane through the Na/K-ATPase, to exit the cell and enter the blood.

It is not established whether the apical Cl-dependent K secretion by the DCT is mediated by one of the four currently identified KCC isoforms (295). Recent data, however, indicate that KCC4 is expressed in the basolateral membrane of most cells of the nephron, including those of the DCT (296).

Cortical Collecting Duct Cells

Two of the cell types resident in the CCD—principal cells and α-intercalated cells (A-type)—both appear to be involved in regulating renal K excretion (97,137,333) (Fig. 5-7). The *principal* cells are the most numerous cells in the ICT and the CCD. Principal cells are able to secrete K (15–17,36,46,47,65,101, 103,104,119,137,301,334–344) and respond to the most important regulatory influences (74,97,98,100,333,345–350).

Some caution is required in interpreting experimental observations bearing on distal K secretion. Most information about the *in vivo* behavior of distal cells under a variety of physiologic circumstances has come from micropuncture and microperfusion studies of surface nephrons in kidneys of anesthetized rats. Most information about cellular mechanisms responsible for secretory transport of K is based on *in vitro* electrophysiologic measurements carried out on single collecting ducts isolated from rabbit kidneys and individually perfused (128,336,351). With both approaches, measurements represent the integrated transport activity of the different cell types making up the tubule segment. Despite these limitations, some transport properties of the cells involved in K secretion have become apparent.

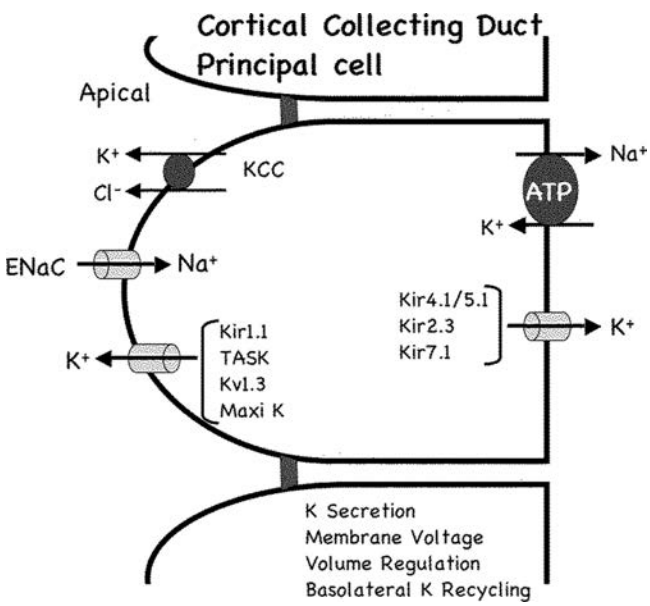

FIGURE 5-7. Cortical collecting duct cells. Principal cells have two K-secretory pathways in the apical membrane: channels through which K can diffuse (blocked by barium), and a K-Cl cotransport mechanism. Na enters these cells by conductive channels (blocked by amiloride). Mineralocorticoids stimulate Na and K transport by these cells. Basolateral K exit pathways include both K channels and K-Cl cotransporters. The molecular identity of the known K channels and transporters are specified. *KCC*, KCl cotransporter; *ENaC*, epithelial sodium channel; *Kv1.3*, voltage-gated K channels; *Maxi-K*: voltage- and calcium-gated K channels; *TASK*, two-pore K channel; *Kir1.1, Kir2.3, Kir4.1/5.1, Kir7.1*, inward rectifier K channels.

In principal cells, an ATPase-coupled Na/K exchange pump is located in the basolateral membrane and is responsible for K uptake. The pump also provides for coupling between Na reabsorption and K secretion. From the rapid depolarization of the basolateral membrane voltage caused by addition of ouabain to the peritubular bathing solution, it has been concluded that the Na/K exchange pump operates in an electrogenic fashion (336,351,352). As in other cells, three Na ions are extruded as two K ions are taken up. Basolateral membranes possess channels that permit K to diffuse back to the interstitium. The Na/K pump responds to several factors known to modulate net K transport normally changes in plasma K, acid–base disturbances, and variations in circulating levels of mineralocorticoid hormones (20,336,351,353–360). Stimulation of K secretion by mineralocorticoid hormones alters basolateral membrane components: activation of the Na/K pump, increased rate of electrogenic cation exchange, and hyperpolarization of the cell-negative basolateral membrane voltage. This increase in membrane voltage is large enough to provide an additional inward-directed driving force to transfer K ions passively from the interstitium into the cell. Mineralocorticoid treatment also increases the K permeability of the basolateral membrane (358).

The *apical membrane* of principal cells has two K permeation pathways that are important for K secretion: K channels and K-Cl cotransport. In functioning tubules, K channels are seen as a K conductance that permits K ions to diffuse along a favorable electrochemical potential gradient from the cell into the lumen. The electrochemical driving force develops because the apical membrane also has a conductive pathway for Na. Diffusion of Na ions into the cells through Na channels causes the cell-negative voltage across the apical membrane to be smaller than the voltage across the basolateral membrane. This asymmetric electrical polarization of the apical and basolateral membranes accounts for the lumen-negative transepithelial voltage and favors net movement of K from blood to lumen (128,336).

At least two types of K channels are present in the apical membrane of principal cells in the CCD. One of these, the secretory K channel, has a relatively small conductance but a high open probability, and is inhibited by increasing cytosolic concentrations of H^+ or Ca^{2+}. A second type, the Maxi-K channel, is likely involved in the regulation of cell volume. This channel has a large conductance, but a low open probability. Increasing cytosolic Ca^{2+} concentrations activate this channel.

Several intracellular messenger systems have been shown to be involved in regulating channel activity and have been reviewed (361). Cyclic adenosine monophosphate-dependent PKA stimulates channel activity (148,362). Calcium-dependent systems, such as PKC and Ca/calmodulin-dependent protein kinase II and arachidonic acid inhibit channel activity (212,363,364). In general, these second messenger systems are also involved in the coupling of transporters and cell membranes to the cytoskeleton (365,366), the integrity of which is required to maintain K channel activity in the rat cortical collecting tubule (367). Sensitivity of K channels to cell acidity may explain stimulation of K excretion in association with metabolic alkalosis or increased rates of bicarbonate excretion (368,369). Evidence also points to angiotensin II as an inhibitor of potassium secretion by the late distal tubule (370).

Changes in the apical membrane voltage and, therefore, in the transepithelial voltage, affect K secretion. For example, imposition of an external current changes the transepithelial voltage and alters the distribution of K across the epithelium of the late distal tubule (371). The effect of amiloride to inhibit K secretion is associated with a reduction of the lumen-negative transepithelial voltage (372) that is caused by hyperpolarization of the apical membrane. This hyperpolarization is the consequence of amiloride blockade of Na channels in the apical membrane (336,372–374). Additional agents that inhibit K secretion by blocking the apical Na channel and reducing the lumen-negative voltage are luminal calcium ions and cationic antibiotics, such as trimethoprim and pentamidine (131–133,265,375). The action of mineralocorticoid hormones to stimulate K secretion by principal cells of the collecting duct, in part, depends on depolarization of the apical membrane as a consequence of increasing apical Na conductance (336). It has been proposed that acid–base changes affect Cl permeability, with consequent effects on apical membrane and transepithelial voltage (376). It is clear from these considerations that both the K conductance and the electrochemical driving force acting on K ions are important factors controlling K secretion through this pathway.

A second K permeation pathway has been identified in microperfusion studies of superficial distal tubules in the rat. As in the DCT, a neutral cotransport mechanism involving K and Cl movement from cell to lumen contributes to net K secretion (35,111,114,547). The key evidence for a K-Cl cotransport mechanism is that K secretion into the distal tubule is stimulated when the lumen is perfused with a solution having a low Cl concentration (332,377). This stimulation of K transport occurs without changes in the transepithelial voltage across the distal tubule wall, and persists in the presence of barium (126,377). Measurements of unidirectional K fluxes suggest that the K-Cl cotransport mechanism is located in the apical membrane (134). It is not known whether this apical K-Cl cotransporter is the same gene expressed by the DCT cell or whether still other KCC genes remain to be identified.

Changes in K transport, induced either by changes in dietary K intake or by mineralocorticoid administration, are accompanied by alterations in cell ultrastructure. The basolateral membrane area of *principal* cells is increased after a prolonged period of dietary K loading. This basolateral membrane amplification occurs in the initial collecting duct (137,378) and the cortical and medullary collecting ducts (346,347,349,350). Measurements showing enhanced Na/K-ATPase activity correlate well with the amplification of the basolateral surface area in collecting duct principal cells (137,348,353,356,379–381).

Ingestion of a high-K diet stimulates the release of aldosterone (137), and this hormone contributes to morphologic changes seen in principal cells (137,382–385). Morphometric analysis of the area of the basolateral membrane of principal cells showed significant reduction after adrenalectomy and a large increase with aldosterone treatment. Dexamethasone had no effect (378,384). It was concluded that mineralocorticoids, but not glucocorticoids, modulate the basolateral membrane area and the K transport capacity of these cells.

Mineralocorticoids exert regulatory effects in all distal nephron segments, including the cortical TAL, the DCT, the CCD, and the outer medullary collecting duct (345,386–390). Like other steroid hormones, aldosterone and other mineralocorticoids enter the cell and bind to specific receptors (mineralocorticoid receptor [MR]) that are in or near the nucleus. Mineralocorticoids have a much lower affinity for glucocorticoid receptors (GR), which also are found in these distal cells (391). In the collecting duct, it appears that MR and GR are primarily expressed in principal cells. However, β-intercalated cells (B-type) have also been shown to possess MR (392,393). The hormone–receptor complex then enters the nucleus, binds to DNA, and activates transcription of messenger RNA for a number of proteins that act on basolateral and apical transport mechanisms (107,394,395). Aldosterone is able to accomplish this activation of new protein synthesis, although it circulates at a concentration of only 100 to 300 pM, which is approximately 1,000 times less than the concentration of glucocorticoids, primarily cortisol. Because aldosterone and cortisol have approximately equal affinity for MR, it would appear that complete occupation of MR by glucocorticoids would prevent variations in circulating aldosterone levels from being recognized (396). This does not happen because the same distal cells that possess MR also are equipped with 11α-hydroxysteroid dehydrogenase (11HSD), which converts glucocorticoids (cortisol and corticosterone) into inactive metabolites (cortisone and 11-dehydrocorticosterone) that do not bind to MR or GR. Several isoforms of 11HSD have been identified. One of these is kidney-specific and is located in the distal nephron (392,397–401). Thus, in aldosterone target cells, 11HSD "protects" MR from occupation by glucocorticoids and makes the receptors available to mineralocorticoids (aldosterone or deoxycorticosterone). It is now recognized that the effect of licorice to mimic mineralocorticoid action (salt retention, K loss, hypertension, and hypokalemia) is a consequence of inhibition by its metabolite (glycyrrhetinic acid) of 11HSD. Loss of the enzyme activity in principal cells permits glucocorticoids to bind MR and induce the synthesis of new proteins that stimulate Na absorption and K secretion (402,403). The density of MR is greater in CCD cells than in TAL cells (393).

Surface amplification of the basolateral cell membrane of principal cells also occurs in animals in which the total number of nephrons has been reduced severely by unilateral nephrectomy plus ligation of three-fourths of the arterial branches to the remaining kidney (404). In this situation, K balance is maintained by a dramatic enhancement of distal K secretion by each of the remaining nephrons (108,118,259,405). Again, a clear increase of the basolateral surface area of principal cells of the initial collecting ducts was noted (404).

Similar, but not identical, morphologic changes have been observed in CNT cells. In both rats (349) and rabbits (98), prolonged feeding of high-K diets has been observed to increase the basolateral membrane area of CNT cells. In contrast to principal cells, however, CNT cells, located in the midportion of distal tubules and in the arcades of deeper nephrons, are not altered structurally by administration of mineralocorticoid. It appears that amplification of basolateral membrane area in these cells is more dependent on dietary K than on circulating aldosterone. Also, the much greater increase in basolateral membrane area produced by feeding a high-K diet is not reduced by antagonists of aldosterone (98).

A growing body of evidence suggests that α-intercalated (A-type) cells may function to reabsorb K (Fig. 5-8). The existence of an active transport mechanism responsible for K reabsorption has been postulated to account for results of micropuncture experiments performed in animals pretreated to promote renal K conservation. The distribution of K ions across the epithelium of the superficial distal tubule under these circumstances does not conform to that expected from the electrical driving force. During experimental K depletion (29,406), K secretion can be completely abolished at a time when a modest lumen-negative potential transepithelial voltage persists. Because the passive driving force favors K secretion, it appears that active reabsorption of K is present. Intercalated cells scattered among the principal cells of the CCD and also among the cells of the distal tubule (infrequently in the DCT, and more commonly in the CNT and initial collecting duct) (71,72,74) may be responsible for K reabsorption and hydrogen ion secretion. Two types of intercalated cells are present: A-type cells secrete protons; B-type cells secrete bicarbonate. Careful morphologic studies of the apical membrane of intercalated cells in

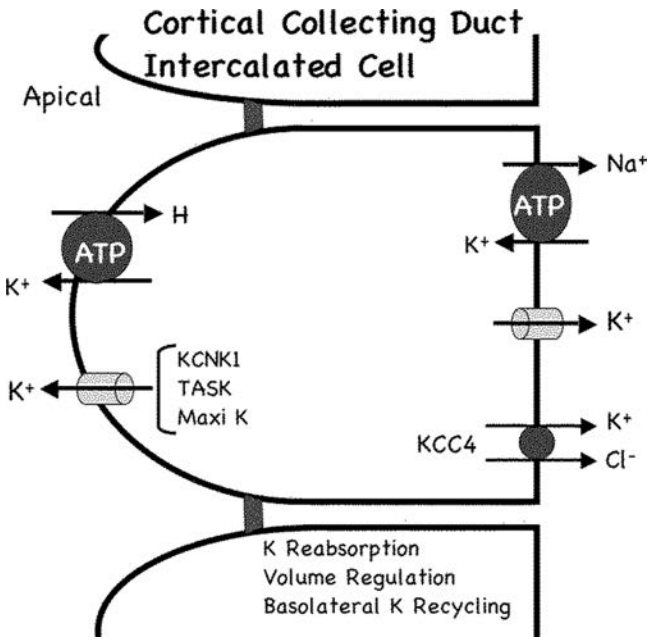

FIGURE 5-8. α-intercalated cells (A-type) secrete protons across the apical membrane by means of an H/K-adenosine triphosphatase pump that actively reabsorbs K. The molecular identity of the known K channels and transporters are specified. *KCC4*, KCl cotransporter; *Maxi-K*, voltage- and calcium-gated K channel; *TASK, KCNK1*, two-pore K channel.

K-depleted rats have identified structural changes. These observations point to the intercalated rather than the principal cells as the site of the postulated mechanism for K reabsorption. Intercalated cells have also been seen to undergo marked morphologic amplification of their apical membranes after acidification. These changes indicate that K reabsorption and hydrogen ion secretion may be functionally linked (407). Intercalated cells differ from principal cells in two important ways: less Na/K-ATPase activity and no conductive ion channels in the apical membrane. An H/K exchange pump in the apical membrane effects proton secretion.

Two types of H/K-ATPase have been identified in kidney: a form expressed in the gastric parietal cells (408,409) and a form expressed in the distal colon (410,411), also classified as nongastric. The distribution in kidney and the function of these two forms of the H/K-ATPase and their variants has been reviewed (356,405,412–416). Omeprazole and Sch-28080, inhibitors of the gastric-type H/K-ATPase, reduce both hydrogen ion secretion and K absorption by the renal distal tubule, the CCD, and the outer medullary collecting duct (99,113,114,356,405,415,417–423). The gastric-type H/K-ATPase appears to be expressed constitutively by intercalated cells throughout the entire distal tubule and all along the collecting duct (416,424,425), whereas the colonic form, although it is expressed, is not active under normal physiologic circumstances. H/K-ATPase activity undergoes adaptive regulation during dietary potassium deprivation, NaCl depletion, and acidotic conditions (416,424,425).

In contrast to the changes observed in CCD principal cells and in CNT cells, no significant change in basolateral membrane area occurs in intercalated cells during either diet or mineralocorticoid-induced stimulation of K secretion (137,333,349,378,384). The density of MR is lower in intercalated cells compared with principal cells (393). In the outer medullary collecting duct, the apical membrane of intercalated cells does change in response to increased K intake. In K-depleted animals, the surface area of the apical membrane is increased by insertion of vesicles containing rod-shaped particles into the apical membrane (349,350). These particles have also been seen in the membranes of a subapical pool of vesicles that diminishes simultaneously with the appearance of the rod-shaped particles in the apical membrane.

Outer Medullary Collecting Duct Cells

Cells in the inner stripe of the outer medulla differ from cells in the cortical and papillary collecting ducts. Studies using *in vitro* perfusion of this segment have failed to demonstrate K secretion (101,351). The epithelium is permeable to K, however, and K diffuses readily in the direction of an applied transepithelial electrochemical driving force. *In vivo,* it is likely that this driving force is oriented from lumen to interstitium, because K secretion in the CCD raises the luminal K concentration and because the electrogenic proton pump (H-ATPase) in the apical membrane of these cells generates a lumen positive transepithelial voltage. Potassium reabsorption by this segment delivers K to the medullary interstitial fluid (as does K reabsorption by the TAL) and thus contributes to the phenomenon of medullary K recycling (59,101,426).

Medullary K recycling helps to promote renal K excretion because it establishes conditions that make it possible for the papillary collecting duct to convey K out of the kidney. Potassium is one of several constituents of plasma that depend on the kidney for excretion, that circulate at low concentrations, and that are secreted by cells of the renal tubules. Ammonium, protons, and a variety of organic solutes also can be included in this class. Beyond their need to be secreted, these solutes pose still another interesting challenge to the kidney. Because the rate of urine flow is determined mainly by considerations of water balance, a constant rate of K excretion can be expected to be associated with a wide range of urinary concentrations of K. Not only does K have to be concentrated in tubule fluid at the site at which it is secreted, but when urine flow rate is low and water reabsorption is nearly complete, the concentration of K in urine has to be raised to levels that can be 100 times higher than the K concentration in plasma. It appears that the countercurrent flow system of the renal medulla contributes importantly to this achievement. When rats are deprived of dietary K for a few days and are permitted free access to water, urinary K concentrations are 20 to 30 mM, and medullary K concentrations are 5 to 10 mM. When rats are continuously ingesting diets with moderate or high K content, and are permitted free access to water, urinary K concentrations are 200 to 300 mM and medullary K concentrations are 35 to 40 mM (60). Aside from the need for a change in distal K secretion sufficient to maintain the rate of K excretion at levels more than 10-fold higher than when rats are deprived of K (427), there is also a need for the medullary collecting duct to maintain tubule fluid K concentrations in excess of 200 mM so that K secreted into distal segments in the cortex is not lost as tubule fluid flows through the medullary collecting ducts. The increase in interstitial K that follows an increase in K intake serves to permit the urine K concentration to rise to the high levels necessary to allow the ingested K to be excreted in relatively small urine volumes. Thus, as K secretion by cortical segments increases, some of the secreted K is lost to the medullary interstitium. Enough of this K is, however, trapped in the medulla by being secreted into the descending limb and then reabsorbed from the ascending limb of the loop of Henle so that the K concentration in the medullary interstitium increases. By acting as a countercurrent exchanger for K, the loop of Henle builds up the medullary K concentration to a level sufficient to limit K reabsorption from the papillary collecting duct.

INTERACTIONS AMONG REGULATORY FACTORS

Because multiple factors and several transport sites are able to respond to influences originating outside the kidney, K excretion is best understood as the net effect of cooperating or competing mechanisms. In some situations, the individual regulatory factors may act in concert, whereas in other cases they may change in directions that tend to oppose one another. The complexity of the integrated action of the several factors precludes straightforward predictions about the ultimate effect that a seemingly simple perturbation may have.

An example of cooperative action is seen in the homeostatic renal response to a high-K intake (a meal or an infusion of a K-containing solution). The increase in plasma K concentration promotes K uptake by K-secreting cells. It also stimulates the adrenal gland to secrete aldosterone that, in turn, increases K uptake from interstitium to cell and K diffusion from cell to lumen in the distal nephron. Increased plasma K concentration also inhibits Na and fluid reabsorption by the proximal tubule (428,429) and therefore increases the flow rate into the distal nephron. The concerted action of these multiple regulatory mechanisms has the appearance of a system developed to defend against the danger of hyperkalemia.

The renal response to K loading becomes greater if the increased intake of K is prolonged. After several days of a high-K intake, distal K secretory capacity is enhanced so that, at a given plasma K concentration, renal K excretion is increased (68,430,431). This adaptive change in distal nephron function confers a degree of tolerance to progressively larger K loads. Prolonged elevation of plasma K, aldosterone, and distal flow result in increased density of basolateral Na/K pumps, and increased density of apical K channels in principal cells. Amplification of the basolateral membrane is apparent after 1 to 2 days of increased K intake.

In contrast, the regulatory factors may not act cooperatively in certain situations involving K depletion. The renal handling of K in these circumstances is nonhomeostatic for K balance, and can be considered to be in the pathophysiologic realm. Although simple reduction in K intake would tend to have effects opposite to those of K loading, if K intake is being restricted because a person has just undergone an abdominal operation, stress-related stimulation of aldosterone secretion may put high aldosterone levels in competition with low plasma K levels and cause the rate of K excretion to exceed the rate of intake. Further, if saline infusions are maintaining extracellular fluid volume and urine flow (432–434), a high flow rate of tubule fluid may further compromise the ability of the kidney to retain K.

Another example of competition among the regulatory factors arises in circumstances involving depletion of extracellular fluid volume. Renal mechanisms that act to defend the circulation have competing effects on renal K handling. Whereas enhanced proximal fluid reabsorption decreases distal flow rate and tends to diminish K excretion, the simultaneous stimulation of the renin–angiotensin–aldosterone system acts to promote K excretion. The result in this situation might be that K balance is unaffected. However, if a low-salt intake is combined with administration of a loop diuretic, such as furosemide, the combined increase in distal flow rate and the elevation of aldosterone result in increased K excretion and negative K balance (435). Similarly, after deprivation of water, competition among regulatory factors may also act to stabilize K excretion. The K-retaining influence of low luminal flow rate is opposed by the stimulatory influence of increased circulating vasopressin.

Finally, in some acid–base disturbances the net result of competing influences on K secretion may cause the kidney to excrete K at rates exceeding K intake and thus to deplete body K. In metabolic alkalosis, plasma K concentration tends to be low, but cell uptake of K is enhanced in both renal and nonrenal tissues. Aldosterone levels are elevated in alkalotic states associated with volume depletion and also tend to promote K secretion. Increased distal delivery of filtered bicarbonate tends to enhance K secretion by two mechanisms: It may be associated with increased distal flow, and high luminal bicarbonate concentrations permit distal Cl concentrations to fall to low levels. Increases in cell pH act to open apical membrane K channels and to increase channel density.

In acute metabolic acidosis, decreased uptake of K by distal cells and inhibition of secretory K channels tend to reduce K secretion. If the acidosis is prolonged, however, proximal fluid absorption is increasingly inhibited and distal flow rate begins to increase. The increase in distal flow may be the reason that K secretion is sufficiently increased to cause K wasting when acidemia is prolonged (22,436).

It is important to recognize that renal K excretion has multiple determinants and that they may not always be responding only to changes in K balance. Knowledge about all of the renal mechanisms participating in the regulation of K excretion should enable us to predict whether and how well the kidney can adapt to disturbances of K homeostasis.

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CHAPTER 6 ■ RENAL ACID–BASE TRANSPORT

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Because many processes in the body depend on the pH of ambient fluid, it is important to maintain intracellular and extracellular pH within narrow limits. In this chapter, we discuss the mechanisms that contribute to this function.

ACID–BASE CHEMISTRY

pH is the negative logarithm of H^+ concentration. An H^+ ion is a hydrogen atom that has lost its only electron and is in essence equivalent to a proton. Because of the equilibrium between H^+ and OH^-,

$$H_2O \longleftrightarrow H^+ + OH^- \quad [1]$$

H^+ concentration is directly affected by OH^- concentration. The product of the concentrations of H^+ and OH^- is a constant. Thus, addition of OH^- to a solution causes the reaction shown in Equation 1 to move to the left, decreasing the H^+ concentration. At 25°C, the product of $[H^+]$ and $[OH^-]$ (ion product constant) is 10^{-14}. Neutrality—when $[H^+] = [OH^-]$—therefore occurs when $[H^+] = 10^{-7}$ M. Equivalently, the sum of pH and pOH is 14. At 37°C, the ion product constant is approximately 13.6, making neutral pH 6.8.

An acid is defined as any substance that donates an H^+, whereas a base is an H^+ acceptor. Acids and bases exist as conjugate pairs according to the reaction:

$$HA \longleftrightarrow H^+ + A^- \quad [2]$$

where HA is the acid and A^- is the base. Each acid–base pair can be described by an equilibrium equation:

$$K = \frac{[H^+][A^-]}{[HA]} \quad [3]$$

where K is the dissociation constant for the acid HA. From this equation, it can be seen that when $[HA] = [A^-]$, $[H^+] = K$. And, when $[H^+] = K$, 50% of the acid is in the undissociated form (HA) and 50% is in the dissociated form ($H^+ + A^-$). When these values are converted to logarithms, Equation 3 can be rewritten in the Henderson–Hasselbalch form as:

$$pH = pK + \log \frac{[A^-]}{[HA]} \quad [4]$$

When pH = p*K*, the concentration of the acid (HA) is equal to the concentration of its conjugate base (A^-) and the log (1) = 0. Because all acid–base pairs are in equilibrium with $[H^+]$, they are all in equilibrium with each other in solution.

A strong acid is an acid with a very low p*K* that donates essentially all of its H^+ at physiologic pH. Conversely, a strong base has a very high p*K* and is essentially totally protonated at physiologic pH. Strong acids include HCl and H_2SO_4; strong bases include NaOH and KOH. Weak acids and bases have p*K*s closer to 7, between 3 and 11, and thus have variable degrees of protonation. Examples of weak acids include acetic acid, lactic acid, and butyric acid; an example of a weak base is ammonia.

The ability of an acid–base pair to accept or donate protons to maintain pH constant is referred to as *buffering,* and the acid–base pairs are referred to as *buffers*. For any given acid–base pair, its *buffering power* depends on its ability to donate or accept H^+ at the ambient pH. *Closed buffers* are buffers whose total concentration is fixed within a solution, for example phosphate and hemoglobin. If H^+ is added to a solution containing a closed buffer, the increase in free H^+ concentration causes the reaction shown in Equation 2 to shift to the left, decreasing the concentration of A^- and increasing the concentration of HA. The result is a minimal change in the concentration of free H^+ and thus, a minimal change in pH. Conversely, addition of OH^- ions to the solution would cause a decrease in H^+ concentration, which would then cause the reaction to move to the right, once again minimizing any change in free H^+ or OH^- concentrations or pH. Closed buffers are most potent when pH is equal to their p*K*. Thus, at pH 7.4, the most potent closed buffers have a p*K* close to 7.4. Among naturally occurring substances, the compounds with p*K*s closest to 7.4 are phosphate and imidazole. Imidazole is the buffer component of histidine, making histidine-rich proteins excellent buffers. Hemoglobin is histidine rich, and thus, a physiologically important buffer.

Open buffers are distinct from closed buffers in that their total concentration in a compartment is not fixed. This usually occurs because one component of the buffer pair is freely permeable across membranes. Thus, rather than the total buffer concentration being fixed, only the concentration of the permeable component is fixed. Consider an acid–base pair similar to that presented in Equation 2. If the concentration of HA is constant, then the concentration of A^- will vary as a function of pH. The net result is that addition of H^+ causes the concentration of A^- to go down and addition of OH^- causes the concentration of A^- to go up. However, the concentration of HA remains constant because HA can freely move in and out of the compartment. The major difference between an open and closed buffer is that whereas closed buffers are most potent at their pK, the buffer capacity of open-buffer systems increases with increasing concentrations of A^-. For an in-depth review of buffer chemistry, the interested reader is referred to Roos and Boron (1).

The most physiologically important open buffer system is the bicarbonate buffer system

$$CO_2 + H_2O \longleftrightarrow H_2CO_3 \longleftrightarrow H^+ + HCO_3^- \quad [5]$$

Since metabolically produced CO_2 can be removed (or retained) from (in) the body by the lungs, the plasma HCO_3^- concentration will vary as a function of added H^+ or OH^-.

Extrarenal Acid–Base Metabolism

As noted, an acid is defined as a proton donor and a base is defined as a proton acceptor. However, it is important to understand that substances may donate protons physiologically through their metabolism, whereas they would not donate

protons in a beaker. Nevertheless, because the net effect is donation of a proton, these substances should be considered physiologic acids. Conversely, other moieties accept protons in metabolism and, thus, in effect, are bases. An example of this is sodium lactate, which is added to peritoneal dialysis fluid to provide alkali. The lactic acid–lactate conjugate pair has a p*K* of approximately 3.9. Thus, in solution at pH 7.4, approximately 3 in 10,000 lactate molecules exist in the form of lactic acid and 9,997 exist as lactate (Eq. 4). This means that addition of 1 mmol of sodium lactate to a solution at pH 7.4 would cause the formation of only 0.3 μmol of lactic acid, leading to buffering of only 0.3 μmol of H^+. Thus, although sodium lactate has the potential to serve as a base or proton acceptor, it quantitatively does this only to a minimal extent in a solution at pH 7.4. However, if a subject ingests sodium lactate, it is almost totally metabolized to either CO_2 and water or glucose, both of which require the consumption of 1 mol of H^+ ions per mol of lactate metabolized. The net result is that in the body sodium lactate functions as a potent alkali because of its metabolism.

To determine whether a compound will serve as an acid or alkali, a few general rules can be used. All carbon-containing molecules that can be metabolized are metabolized to CO_2 and water or glucose, both of which have no net charge. Compounds containing nitrogen and carbon are metabolized to urea, which again has no net charge. Because of conservation of charge, if before metabolism compounds have a net positive charge, such as a lysine and arginine residue, they must donate H^+ ions in metabolism and thus serve as acids. Conversely, if before metabolism compounds have a net negative charge, such as citrate, other organic anions, or proteins that contain glutamate or aspartate residues, they must accept H^+ ions in metabolism and thus serve as alkali. In addition, compounds that contain sulfur or phosphate residues are metabolized to sulfuric acid or phosphoric acid, respectively, and thus donate protons at physiological pHs, because the p*K*s of sulfuric acid are <1.0 and 2.0 and the p*K*s of phosphoric acid 2.2, 7.2, and 12.2. Carnivorous (meat containing) diets are rich in sulfur and nucleic acids, which contain phosphate, causing these diets to result in acid generation when metabolized. Vegetarian diets tend to contain more organic anions, causing them to lead to alkali generation when metabolized.

ROLE OF THE KIDNEY IN ACID–BASE BALANCE

The extrarenal generation of acid by metabolism is referred to as *net acid production*. If a subject ingests an acid-generating diet, net acid production is positive, whereas if the subject ingests an alkali-generating diet, net acid production is negative. Since most individuals consume meat-containing diets, to maintain acid–base balance, the kidney must excrete an amount of acid, referred to as *net acid excretion,* equal to net acid production. Any discrepancy between net acid production and renal net acid excretion leads to an acid–base imbalance, which eventually results in abnormalities in blood pH and HCO_3^- concentration.

The renal glomerulus and tubules function in an integrated manner to maintain acid–base balance. The glomerulus can be considered an alkali-excreting segment of the nephron. It filters an amount of HCO_3^- equal to the product of the glomerular filtration rate (GFR) and the plasma HCO_3^- concentration. Thus, in an average adult with a GFR of 180 L per day and a plasma HCO_3^- concentration of 24 mEq per L, the glomerulus filters 4,320 mEq of HCO_3^- per day. This is an enormous amount of HCO_3^- and loss (excretion) of any significant fraction of this filtered HCO_3^- would lead to a severe metabolic acidosis. Thus,

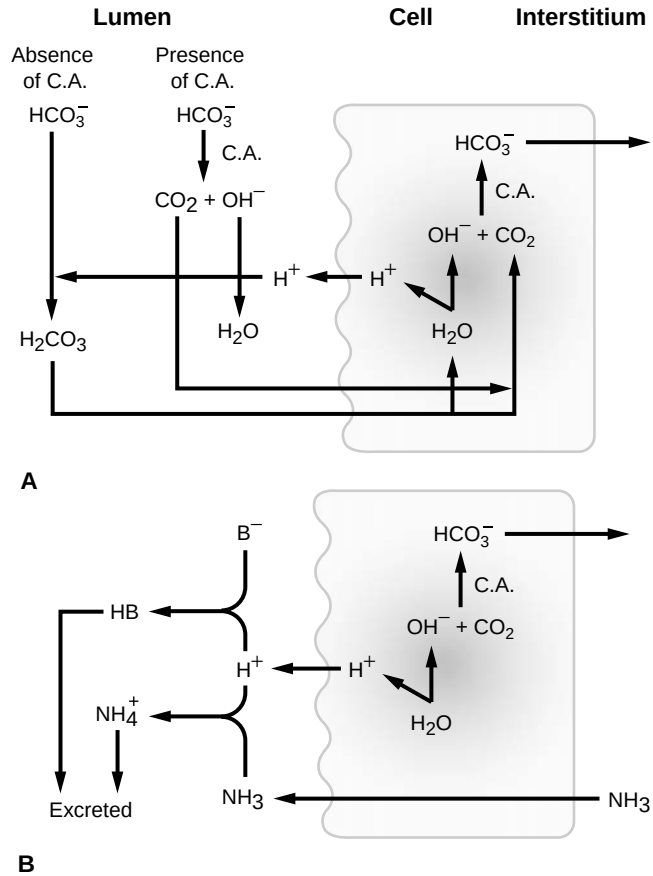

FIGURE 6-1. A: Bicarbonate reclamation. **B:** Bicarbonate regeneration. C.A., carbonic anhydrase.

the first function of the renal tubules in acid–base regulation is the *reclamation* of filtered HCO_3^-. The renal tubules are very adapt at this process and reabsorb most of the filtered HCO_3^-, preventing its loss from the body.

In Figure 6-1A a renal tubule cell model of HCO_3^- reclamation is shown. HCO_3^- reabsorption is mediated by the active secretion of H^+ into the luminal fluid where it reacts with OH^- to form H_2O (2,3). Consumption of the OH^- ion causes HCO_3^- to dissociate into CO_2 and OH^-. This latter reaction is catalyzed by carbonic anhydrase, an enzyme located on the luminal side of the apical membrane in many nephron segments. In nephron segments, where the luminal fluid is not exposed to carbonic anhydrase, or in the presence of carbonic anhydrase inhibitors, H^+ reacts with HCO_3^- to form H_2CO_3 (Fig. 6-1A). Because H_2CO_3 converts to CO_2 and H_2O slowly in the absence of carbonic anhydrase, H_2CO_3 concentrations increase in the luminal fluid, allowing H_2CO_3 to diffuse into the cell (carbonic acid recycling) (4,5). In the tubule cell, H^+ and HCO_3^- are generated from CO_2 and water. The H^+ ions are actively transported into the luminal compartment, whereas HCO_3^- exits the basolateral membrane. The net result is the removal of a HCO_3^- ion from the luminal fluid and the addition of a HCO_3^- molecule to the blood. The secreted H^+ ion in essence recycles across the apical membrane mediating the reclamation of filtered HCO_3^-.

The second function of the renal tubules is to regenerate the HCO_3^- consumed by extrarenal acid generation. As described previously, carnivorous (meat-containing) diets lead to acid generation when metabolized, and thus, to positive rates of net acid production. The generated H^+ added to the

extracellular fluid reacts with HCO_3^- according to the reaction shown in Equation 5 (reaction moves to the left). Thus, each milliequivalent of acid generated by metabolism leads to loss of 1 mEq of HCO_3^- from the extracellular fluid. Regeneration of HCO_3^- by the kidney refers to the excretion of H^+ causing the reaction shown in Equation 5 to move in reverse (to the right), leading to the reappearance of one HCO_3^- molecule for every H^+ excreted. In that regeneration of HCO_3^- consumed in metabolism requires the generation of new HCO_3^- by the kidney, the secreted H^+ cannot be buffered by HCO_3^- in the lumen, as was done in the HCO_3^- reclamation process. As shown in Figure 6-1B, secreted H^+ ions involved in HCO_3^- regeneration are buffered by titratable buffers (B^-) and NH_3 in the luminal fluid (see below).

When an acid-generating diet is consumed, maintenance of acid–base balance requires the kidney to reclaim most of the filtered HCO_3^- and to excrete additional H^+ ions equivalent in amount to net acid production and any excreted HCO_3^-. In contrast, when an alkali-generating diet is ingested, acid–base balance is maintained by the failure of renal tubules to reclaim all of the filtered HCO_3^- and to stop generating new HCO_3^-.

ROLE OF BUFFERS IN RENAL NET ACID EXCRETION

A typical American diet generates approximately 70 mEq of acid per day. For the kidney to excrete 70 mEq of acid per day in a urine volume of approximately 2 L, a luminal pH of 1.5 would be required in the terminal nephron segment (collecting tubule). Because renal H^+-secretory mechanisms cannot generate such steep pH gradients between the cell (pH ~7.3) and lumen, excretion of 70 mEq of acid per day requires that most of this acid be buffered in the luminal fluid. In general, the buffers responsible are the NH_3–NH_4^+ and titratable buffer, such as HPO_4^{2-}–$H_2PO_4^-$, systems. Physiologically, the most significant of these is the NH_3–NH_4^+ system.

Ammonia

Most ammonia is synthesized in the renal proximal tubule cell by the sequential metabolism of glutamine (6,7). As shown in Figure 6-2, glutamine is transported into the proximal tubule cell across both the apical and basolateral membranes. It is then transported into mitochondria, where it is deaminated by glutaminase to glutamate, generating an NH_4^+ molecule. Glutamate is then deaminated to α-ketoglutarate, yielding a second NH_4^+ molecule. The α-ketoglutarate molecule is then metabolized in the tricarboxylic acid (Krebs) cycle to CO_2 and H_2O. In addition to forming CO_2, water, and glucose, these reactions generate two NH_4^+ and two HCO_3^- molecules per glutamine molecule metabolized. In this respect, glutamine metabolism in the proximal tubule does not lead to the generation of net acid or base. Nevertheless, it provides an important first step in HCO_3^- regeneration.

Ammonia Transport in the Nephron

To contribute to acidification, NH_3–NH_4^+ synthesized in the proximal tubule cell must be delivered to the terminal nephron segments (Fig. 6-3). Transport of NH_3–NH_4^+ can occur by one of two general mechanisms, nonionic diffusion (NH_3) or ionic transport (NH_4^+). Nonionic diffusion refers to the free diffusion of NH_3 across plasma membranes. If NH_3 is highly permeable across biologic membranes, it rapidly diffuses from a compartment of higher pH to a compartment of lower pH. In these areas of lower pH, there is rapid conversion of NH_3 to NH_4^+ (pK NH_3 = 9.3), maintaining the gradient for NH_3 diffusion into that compartment. The net result is that nonionic diffusion leads to the accumulation of NH_3–NH_4^+ in acidic compartments. Ammonia can also cross membranes in the form of NH_4^+ by diffusing across tight junctions or being transported on a number of potassium transporters or the proximal tubule apical membrane Na-H antiporter (as Na^+–NH_4^+ exchange) (8). Although, in general, NH_3 has a higher membrane permeability than NH_4^+, the concentration of NH_4^+ exceeds that of NH_3 by approximately 100-fold at pH 7.4. Thus, the relative permeabilities of the two moieties will determine whether NH_3–NH_4^+ crosses membranes by nonionic NH_3 diffusion or ionic NH_4^+ transport. If NH_3 permeability exceeds that of NH_4^+ by 100-fold, approximately equal contributions of nonionic and ionic transport can be anticipated. Some membranes, such as the apical membrane of the thick ascending limb, are highly impermeable to NH_3. Thus, only the NH_4^+ moiety is transported in this nephron segment.

As described previously, the proximal tubule cell generates equal amounts of NH_4^+ and HCO_3^- from glutamine metabolism. To accomplish urinary acidification, NH_4^+ must

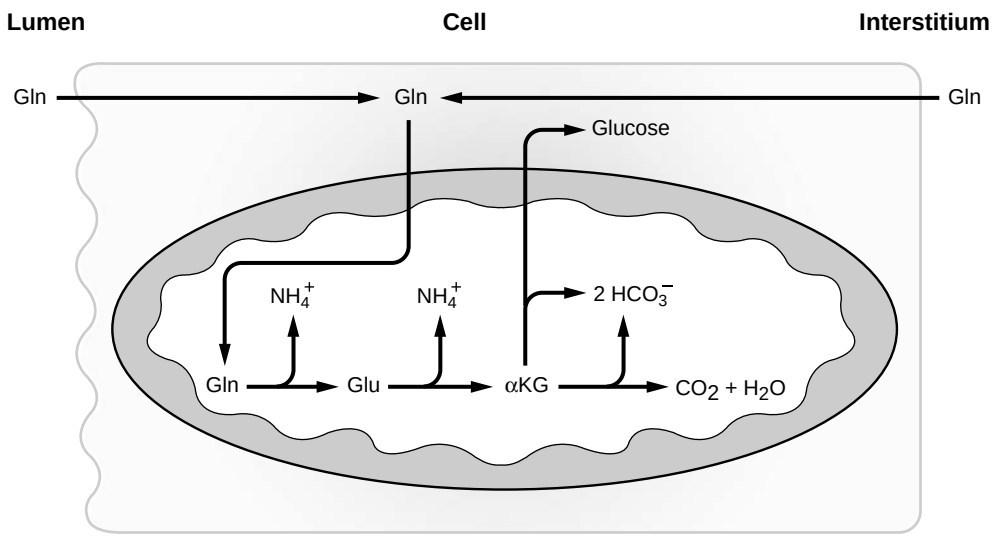

FIGURE 6-2. Ammonia synthesis in the proximal tubule. *Gln*, glutamine; *Glu*, glutamate.

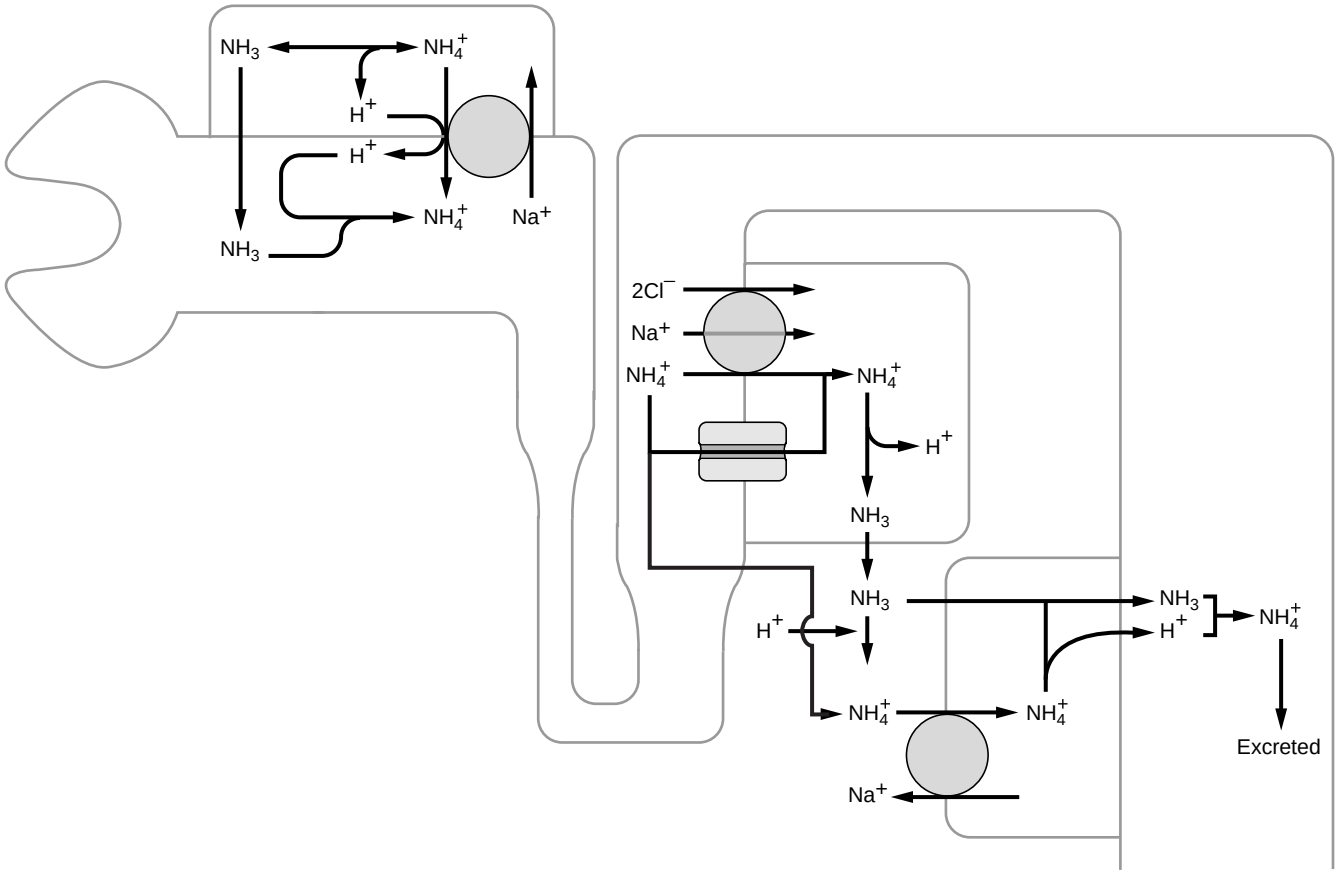

FIGURE 6-3. Ammonia transport along the nephron.

be carried into the urine, while HCO_3^- is returned to the blood. In the proximal tubule cell, HCO_3^- exits across the basolateral membrane on the Na-HCO_3-CO_3 cotransporter (see later). NH_3–NH_4^+ can be transported into the proximal tubule lumen by two mechanisms. First, because proximal tubule luminal pH is lower than cell pH and the apical membrane NH_3 permeability is high (6.2×10^{-2} cm per second) (9), nonionic diffusion of NH_3 into the luminal compartment is significant, where it is rapidly converted to NH_4^+. In addition, the proximal tubule apical membrane Na-H antiporter can transport NH_4^+ (9,10), suggesting a role for ionic transport. The relative contributions of these two processes have been addressed by examining the effect of Na-H antiporter inhibition on apical membrane NH_3–NH_4^+ transport. Although some disagreement exists, important roles for both pathways have been proposed (11,12). However, the Na-H antiporter actually contributes to both processes—ionic transport of NH_4^+ and luminal acidification facilitating nonionic diffusion of NH_3 (see below)—making the apical membrane Na-H antiporter a key player in NH_3–NH_4^+ transport. All of the ammonia that appears in the urine is already present in the luminal fluid by the end of the proximal tubule (8).

Transport mechanisms for NH_3–NH_4^+ in more distal segments serve the purpose of carrying the NH_3–NH_4^+ from the proximal tubule lumen into the urine in a regulated fashion. The next major site of transport is the thick ascending limb, where luminal NH_3–NH_4^+ is transported into the medullary interstitium. The purpose of this transport is not completely settled. One explanation is that if NH_3–NH_4^+ were "allowed" to return to the renal cortical distal nephron, it would diffuse back into the bloodstream and, thus, lost for renal acidification; transport out of the luminal fluid and into the medullary interstitium prevents this sequence of events. A second possible explanation is that NH_3–NH_4^+ is transported out of the luminal fluid so that it can reenter into the collecting tubule in a regulated manner.

As mentioned above, the apical membrane of the thick ascending limb is highly impermeable to NH_3, eliminating nonionic diffusion as a transport mechanism for moving NH_3-NH_4^+ into the medullary interstitium (13). However, three mechanisms exist for ionic transport of NH_4^+, all tied to NaCl transport in this nephron segment (14,15). First, NH_4^+ is transported across the apical membrane on the Na-K-2Cl cotransporter in place of K^+. Second, NH_4^+ is transported across the apical membrane K channel. Last, NH_4^+ can diffuse out of the lumen across the tight junction driven by the lumen-positive voltage. NH_4^+ that enters the thick ascending limb cell can exit the basolateral membrane by nonionic diffusion driven by cell-to-interstitial NH_3 concentration gradient. Once in the interstitium, the NH_3 will again pick up an H^+, forming NH_4^+. Ionic diffusion of NH_4^+ may also contribute to basolateral membrane transport. Furosemide, which inhibits the Na-K-2Cl cotransporter and secondarily inhibits the lumen-positive voltage, significantly inhibits all three mechanisms of thick ascending limb NH_3–NH_4^+ absorption.

The net result of these mechanisms is the accumulation of NH_3–NH_4^+ in the renal medulla. As with any substance that is added to the medullary interstitium, the countercurrent system concentrates the solute toward the tip of the medulla and preserves the cortical-to-medullary gradient. Blood in the descending vasa recta has lower NH_3–NH_4^+ concentrations than the surrounding interstitium, causing net entry. Conversely, blood in the ascending vasa recta has a higher NH_3–NH_4^+ concentration than the surrounding interstitium, causing net efflux. In patients with an intact urinary concentrating system and medullary architecture, the net result is a steep gradient, with highest concentrations of NH_3–NH_4^+ in the inner medullary interstitium.

The last step for NH_3–NH_4^+ transport is transport from the medullary interstitium into the lumen of the collecting tubule (Fig. 6-3). Both the apical and basolateral membranes of the medullary collecting tubule are permeable to NH_3 and, thus, nonionic diffusion of NH_3occurs, driven by the low intraluminal pH of the collecting tubule generated by α-intercalated and terminal collecting tubule cell H^+ secretion (16). In addition, NH_4^+ can be transported from the medullary interstitium into the collecting tubule cell on the Na-K-ATPase (17). Once inside the cell, the dissociation of NH_4^+ into a NH_3 molecule and H^+ ion provides a source of H^+ for transport into the luminal fluid by the vacuolar H-ATPase. H^+ secreted into the luminal fluid will combine with secreted NH_3 to form NH_4^+, maintaining the gradient for nonionic NH_3 diffusion into the luminal compartment. Thus, the final transport of NH_3–NH_4^+ into the lumen of the collecting tubule and ultimately into the urine is regulated by two determinants: the medullary interstitial NH_3–NH_4^+ concentration and luminal pH. Medullary interstitial NH_3–NH_4^+ concentrations are determined by the rate of ammonia synthesis in the proximal tubule, the efficiency of ammonia transport into the lumen of the proximal tubule and, subsequently, into the medullary interstitium in the thick ascending limb, and the efficiency of countercurrent trapping of ammonia in the medullary interstitium. Indirectly, this concentration is also determined by rates of glutamine synthesis in muscle and liver, which are required for proximal tubule ammonia synthesis. Acidification of the luminal fluid is determined by a series of processes occurring throughout the nephron, described later in this chapter.

Titratable Acid

Titratable acid refers to closed buffers that are filtered at the glomerulus and then titrated to more acid forms in the tubule lumen. These buffers are most effective if their p*K* is between 5.0 and 7.4, the pH range of the luminal fluid. The most abundant of these buffers is the phosphate buffer pair, $H_2PO_4^-$–HPO_4^{2-}, which has a p*K* of 6.9. In general, the amount of acid excreted as titratable acid can be predicted from the urine pH. The excretion of phosphate, the major titratable buffer, is usually regulated in accord with phosphate homeostasis. Phosphate excretion does increase in metabolic acidosis; however, the magnitude of the increase is limited by plasma phosphate concentration.

Net Acid Excretion

Based on the foregoing, renal net acid excretion is calculated as follows:

$$\text{Renal net acid excretion} = (U_{NH_4^+} V) + (U_{TA} V) - (U_{HCO_3^-} V) \quad [6]$$

where V is the urinary volume per 24 hours and U refers to the concentration of the moiety listed in the subscript. All three components of renal net acid excretion are determined by renal tubular H^+ secretion into the luminal fluid. Thus, increases in the rate of H^+ secretion will increase urinary ammonium and titratable acid excretion and lower urinary HCO_3^- excretion.

MECHANISMS OF RENAL TUBULAR H^+– HCO_3^- TRANSPORT

Transepithelial transport of any solute involves two components—a transcellular and a paracellular component. Paracellular transport occurs around cells and is always passive. Thus, the magnitude of paracellular transport depends on the relevant chemical and electrical gradients and on the permeability of the tight junction. As is reviewed later, the only site where paracellular transport is likely to be of physiologic significance for acid–base balance is the proximal tubule. Transcellular transport involves a number of processes occurring in series. A solute must diffuse to the cell, be transported across one membrane, diffuse across the cytoplasm, be transported across the second membrane, and then diffuse away from the cell. Most research thus far has attempted to identify the mechanisms responsible for transport of H^+ and HCO_3^- across each of the plasma membranes. Because cell voltage in most cells is −30 to −80 mV, there is a significant electrical driving force attracting H^+ into the cell, or similarly, driving OH^- out of the cell. In addition, H^+ secreted from cell to lumen is frequently being secreted into a more acid compartment than the cell cytoplasm. The net result is that H^+ transport out of the cell is an active process, whereas transport of base out of the cell can occur passively. A number of transport mechanisms have been identified that mediate active H^+ and passive alkali extrusion.

Active Acid Extruders

In general, active acid extrusion occurs by one of two processes, *primary* or *secondary active transport*. Primary active transport is the process whereby metabolism is directly coupled to a transport mechanism. Secondary active transport is the process whereby a transporter coupled to metabolism generates a concentration gradient for a solute, and that solute then provides a driving force for a cotransported solute leading to the uphill transport of the second solute—in this case, H^+ or HCO_3^-.

Two mechanisms have been identified in the nephron for primary active H^+ extrusion (Table 6-1). In both cases, transport of H^+ is directly coupled to the metabolism of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and inorganic phosphate (P_i). These transporters are thus referred to as *ATPases*. The first of these is the vacuolar H-ATPase, which is a multisubunit protein complex that drives transport of H^+ out of the cell (18). These transporters are referred to as *vacuolar* because they have been identified in a number of intracellular compartments. Vacuolar H-ATPases are electrogenic, carrying a positive current. Bafilomycin A_1 is a relatively specific inhibitor of vacuolar H-ATPases and has been used as a probe to determine the role of this transporter in H^+ transepithelial transport. Vacuolar H-ATPases mediate H^+ secretion into the luminal fluid in the proximal tubule cell and the α-intercalated cell in the collecting tubule and may also mediate H^+ transport across the basolateral membrane into the interstitium in the β-intercalated cell in the collecting tubule (see later) (19–23).

TABLE 6-1

ACTIVE ACID EXTRUDERS

Transporters	Location	Inhibitor
Primary active		
Vacuolar H-ATPase	PT, CT	Bafilomycin A
H-K-ATPase	CT	SCH 28080
Secondary active		
Na-H antiporter	PT, TAL, DCT,CT	Amiloride

CT, collecting tubule; *DCT*, distal convoluted tubule; *PT*, proximal tubule; *TAL*, thick ascending limb.

More recently, an H-K-ATPase has been identified that also mediates H^+ transport in the collecting tubule. This transporter is electroneutral, mediating H^+ secretion into the lumen (out of the cell) in exchange for K^+ entry into the cell. These ATPases are part of a family of P-type ATPases that have a number of characteristics in common. They are all coupled to ATP metabolism, have a phosphorylated high-energy intermediate, and possess α and β subunits. Examples of other members of this family include the Na-K-ATPase and the Ca-ATPase. An H-K-ATPase was first identified and cloned in the stomach, where it provides the mechanism for gastric acid secretion (24). A second isoform has been identified in the colon and a third isoform in toad bladder (25,26). Both the gastric and colonic isoforms are expressed in the renal collecting tubule. SCH 28080 is a relatively specific inhibitor of H-K-ATPases and has been used as a probe to determine the role this transporter plays in H^+ and K^+ transepithelial transport. H-K-ATPases have been shown to mediate H^+- HCO_3^- transport in the collecting tubule (21,27–30), but only the colonic isoform increases its activity in potassium depletion.

The only secondary active H^+ transporter identified in the kidney is the Na-H antiporter (Table 6-1). This transporter uses the low intracellular Na^+ concentration established by the Na-K-ATPase to drive the countertransport of H^+. An approximately 10-fold Na^+ gradient exists between the luminal fluid and the intracellular cytoplasm, which is sufficient to drive the extrusion of H^+ from the cell to the luminal or interstitial compartment against its electrochemical gradient. The Na-H antiporter has been cloned, and eight isoforms identified. NHE-1 is the ubiquitous Na-H antiporter present in almost all cells (31). This transporter probably mediates housekeeping functions, such as cell pH defense, growth, cell volume defense, and signaling. NHE-1 is expressed in the kidney on basolateral membranes in the proximal tubule, thick ascending limb, distal convoluted tubule (DCT), and in connecting tubule (CNT) and principal cells of the collecting tubule (32). NHE-3 is the predominent isoform on the apical membrane, where it mediates H^+ secretion in the S_1 and S_2 proximal tubule and thick ascending limb (33–36). Recently, the NHE-8 isoform has been immunolocalized to the apical membrane of all segments of the proximal tubule, with the highest expression in the S3 proximal tubule of the outer stripe of the outer medulla. Its role in renal acidification has not been determined (37,38). On the apical membrane of the distal convoluted tubule, the NHE2 isoform is expressed and mediates H^+ secretion (39–41). All Na-H antiporters contain an amino-terminal hydrophobic domain that includes 10 to 12 membrane-spanning domains and mediates transport and a carboxy-terminal hydrophilic domain that is located in the cytoplasm and mediates regulation of transporter activity. Amiloride, amiloride analogs, such as ethylisopropyl amiloride, and HOE694 serve as potent inhibitors of the various Na-H antiporter isoforms and have been used as probes for examining the role of specific isoforms in the various nephron segments.

Another secondary active transporter that has been identified in vertebrate cells, an Na(HCO_3)-Cl exchanger, has been found in the proximal tubule, where it likely contributes to basolateral membrane Cl^- efflux (42,43).

Base Extruders

In principle, base efflux from the cell could be mediated by a simple H^+, OH^-, or HCO_3^- channel. Such a passive channel would allow the acid–base moiety to move passively, leading to net base efflux. Such transport mechanisms, however, have not been found. Rather, two transporters have been identified that mediate passive base efflux along the nephron.

TABLE 6-2

BASE EXTRUDERS

Transporters	Location	Inhibitor
Cl-HCO_3 exchanger	PT, TAL, CT	SITS, DIDS
Na-HCO_3-CO_3	PT, TAL, CT	SITS, DIDS

CT, collecting tubule; *PT*, proximal tubule; *TAL*, thick ascending limb; *SITS*, 4-Acetamido-4′-isothiocyanostilbene-2,2′-disulfonate; *DIDS*, diisothiocyano-tostilbene-2,2′-disulfonic acid.

An electroneutral Cl–HCO_3 exchanger carries HCO_3^- out of and Cl^- into the cell across the basolateral membrane in the S3 segment of the proximal tubule, thick ascending limb, and collecting tubule (Table 6-2). Although Cl^- is near electrochemical equilibrium in most cells, HCO_3^- is significantly above electrochemical equilibrium. A number of Cl–HCO_3 exchangers have now been cloned. The first of these is the band 3 Cl–HCO_3 exchanger of red blood cells, now referred to as AE1 (anion exchanger 1) (44). A truncated form of AE1, in which the most amino-terminal amino acids are missing, mediates base efflux in the α-intercalated cell of the collecting tubule (45,46). This truncation is due to use of an alternate promoter in renal cells compared with red blood cells (47). AE2 and AE3 mRNA are also expressed in the kidney (48,49). AE2 is expressed on the basolateral membrane of the thick ascending limb, but the specific site of AE3 expression has not been identified. All of the AE gene products have an amino-terminal cytoplasmic domain that interacts with the cytoskeleton and a carboxy-terminal transmembrane domain that mediates transport.

The second transporter that mediates base efflux is the sodium bicarbonate cotransporter (NBC). Four NBC isoforms have been identified. NBC1 is present on the basolateral membrane of the proximal tubule and is electrogenic, as it carries the equivalent of three base molecules with one Na^+ ion (Na^+-HCO_3^--CO_3^{2-}). NBC1 plays a significant role in proximal tubule basolateral membrane base efflux, where it mediates about 80% of renal bicarbonate reabsorption (50–53). NBC2 and -3 are electroneutral isoforms. Using various antibodies one or the other has been shown to be expressed on the basolateral membrane in the thick ascending limb, in the inner medulla, and along the collecting tubule, including the intercalated cells (54–56). NBC4 has been shown to function in both the electrogenic and electroneutral mode and to be expressed in the kidney. Its functional mode and localization await further studies. All of the HCO_3^- transporters described in this section are inhibited by disulfonic stilbenes, such as SITS and DIDS, although their sensitivity to these agents varies.

Transport Mechanisms Along the Nephron

Proximal Tubule

HCO_3^- is freely filtered across the glomerulus, such that the HCO_3^- concentration in Bowman's space is in Donnan equilibrium with that in plasma. The proximal tubule is responsible for reabsorption of 70% to 90% of filtered HCO_3^-, leaving the tubule fluid at the end of the proximal tubule with a HCO_3^- concentration of 5 to 10 mEq per L and a pH of 6.6 to 6.9. The proximal tubule is divided into an early segment, S_1, a midsegment, S_2, and a terminal segment, S_3. The rate of HCO_3^- reabsorption is highest in S_1, decreases in S_2, and is lowest in S_3.

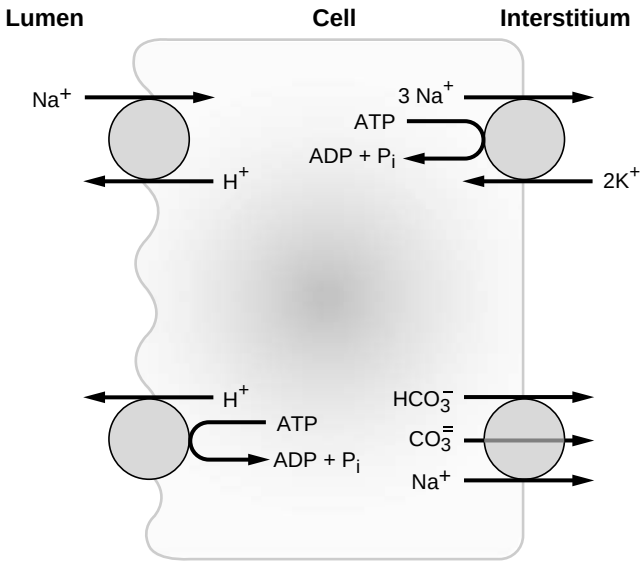

FIGURE 6-4. H^+- HCO_3^- transport in the proximal tubule.

The specific mechanisms responsible for H^+– HCO_3^- transport in the proximal tubule are shown in Figure 6-4. As mentioned above, both an Na-H antiporter (NHE3 in S_1 and S_2, and NHE8 in S_3) and a vacuolar H-ATPase are expressed on the apical membrane of the proximal tubule, where they mediate H^+ secretion from the cell into the luminal fluid (19,57,58). Although inhibitable by amiloride and its analogs, NHE3 is more resistant to these agents than other Na-H antiporter isoforms (59). Using both inhibitors and NHE-3 knockout mice, it has been demonstrated that approximately two-thirds of H^+ secretion is mediated by the apical membrane Na-H antiporter, whereas one-third is mediated by the vacuolar H-ATPase (57,58,60). The relative proportions transported by these two carriers, however, may vary among the S_1, S_2, and S_3 segments.

HCO_3^- generated in the proximal tubule cell exits passively across the basolateral membrane Na^+-HCO_3^--CO_3^{2-} cotransporter encoded by NBC1, which as mentioned above transports the equivalent of three HCO_3^- ions out with every turnover (50–53,61–63). Because this transporter carries a net of two negative charges, the negative cell voltage provides a strong, favorable driving force for base efflux. In addition, this transporter carries a Na^+ out of the cell, which in essence amounts to energetically "free" transport of Na^+ in that ATP is not required. Na^+ ions are also transported across the basolateral membrane on the Na-K-ATPase, which transports three Na^+ out of and two K^+ into the cell, but consumes ATP. These two mechanisms of Na^+ transport maintain the low cell Na^+ concentration that provides the driving force for the apical membrane Na-H antiporter and for other Na^+-coupled apical membrane transport mechanisms in the proximal tubule.

The net result of these transcellular processes is the active reabsorption of luminal HCO_3^- and acidification of the luminal fluid. This creates a driving force for passive paracellular diffusion of base into and acid out of the lumen. The most significant passive paracellular flux is that of HCO_3^- (64). On the basis of measured values of paracellular HCO_3^- permeability, it has been predicted that the rate of passive paracellular HCO_3^- back-diffusion at the end of the rat proximal tubule is 250% of the measured rates of net HCO_3^- absorption (64). Thus, because the paracellular pathway in the proximal tubule is very permeable, net HCO_3^- absorption is significantly lower than active H^+ secretion at the end of the proximal tubule. Changes in the rates of active H^+ secretion or passive HCO_3^- back-diffusion have significant effects on net rates of transport in the late proximal tubule, and thus have significant effects on end proximal tubule luminal pH or HCO_3^- concentration and the distal delivery of HCO_3^-. In most cases, regulation of proximal tubule acidification is because of regulation of transcellular fluxes. However, in at least one circumstance—changes in extracellular fluid volume—regulation is mediated in part through modulation of the paracellular pathway fluxes (65).

As noted previously, in addition to reabsorbing HCO_3^-, the proximal tubule plays a key role in providing the NH_3 needed for HCO_3^- regeneration. As mentioned above, ammonia synthesis in the kidney occurs in the proximal tubule and all of the ammonia present in the urine is already present in the luminal fluid by the end of the proximal tubule (7,8). The proximal tubule also plays a significant role in the titration of titratable buffers. Because the pH of the end proximal tubule lumen is below the p*K* of phosphate (6,9), a significant fraction of filtered phosphate is already titrated to its acid form by the end of the proximal tubule.

Loop of Henle

Fluid leaving the loop of Henle has a pH and HCO_3^- concentration similar to those of fluid entering the loop of Henle. However, because volume is reabsorbed in the descending limb of the loop of Henle, maintenance of the same HCO_3^- concentration requires reabsorption of 50% to 70% of the HCO_3^- delivered out of the proximal tubule. The major site of this HCO_3^- reabsorption occurs in the thick ascending limb of the loop of Henle (66). In Figure 6-5, a model for HCO_3^- reabsorption in this segment is shown. Apical membrane H^+ secretion is mediated by the NHE3 isoform of the Na-H antiporter family, similar to the proximal tubule (36,66). Also, similar to the proximal tubule, the low cellular Na^+ concentration, maintained by the basolateral membrane Na-K-ATPase, provides the primary driving force for the Na-H antiporter. Both AE1 (chloride–bicarbonate exchange) and NBC2 or -3 (sodium–bicarbonate cotransport) are expressed on the basolateral membrane, where they mediate bicarbonate (base) exit across the basolateral membrane (54–56).

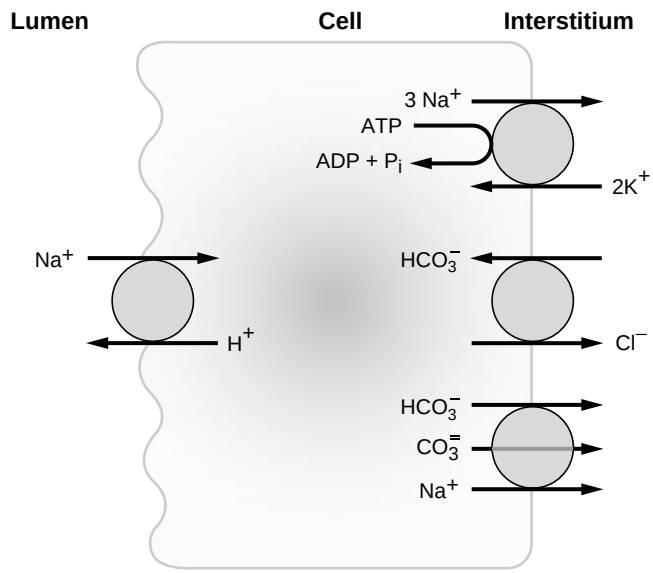

FIGURE 6-5. H^+- HCO_3^- transport in the thick ascending limb.

Distal Nephron

The distal nephron is responsible for the final acidification of the urine. This involves reabsorption of any HCO_3^- remaining in the luminal fluid, the final titration of titratable buffers, and, most importantly, buffering H^+ secreted in the process of HCO_3^- regeneration by trapping ammonium in the lumen. Conversely, when the kidney is required to excrete alkali, the distal nephron is able to secrete HCO_3^-. To accomplish these goals, the distal nephron includes a number of different segments, each of which has somewhat different transport characteristics. The distal nephron is defined as beginning at the macula densa (MD). After the MD is the distal convoluted tubule (DCT), a short segment that exhibits a small amount of amiloride-sensitive HCO_3^- reabsorption, mediated by an apical membrane Na-H antiporter isoform NHE2 (21,39–41).

Subsequent segments, including the connecting tubule, initial cortical collecting tubule, and cortical collecting tubule, all possess intercalated cells that mediate H^+- HCO_3^- transport. As shown in Figure 6-6, intercalated cells exist in two forms, an α-intercalated cell that secretes H^+ into the luminal fluid and a β-intercalated cell that secretes HCO_3^- into the luminal fluid.

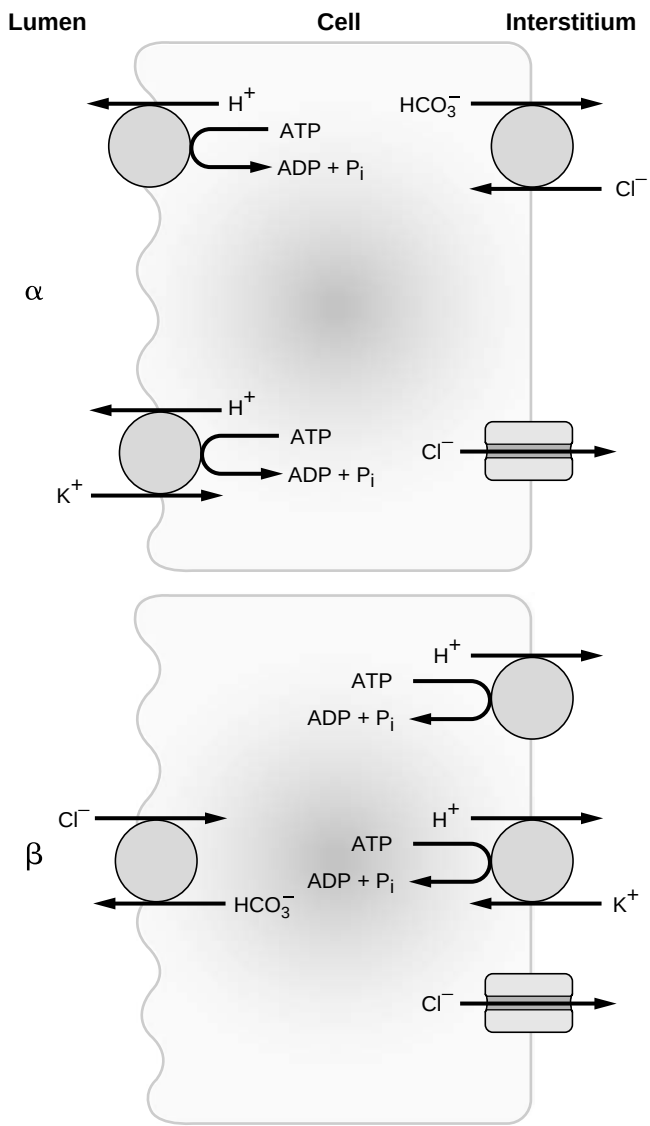

FIGURE 6-6. H^+- HCO_3^- transport in the collecting duct: α- and β-intercalated cells.

In the α-intercalated cell, H^+ ion secretion from cell to lumen is mediated by two ATP-dependent H^+ pumps, a vacuolar H-ATPase and an H-K-ATPase. Evidence in favor of a role for a vacuolar H-ATPase includes (a) labeling of these cells with antibodies generated against subunits of the vacuolar H-ATPase (19,20), (b) inhibition of H^+ secretion by bafilomycin A_1, an inhibitor of vacuolar H-ATPases (21,22), and (c) the electrogenicity of acidification in this nephron segment (67). With regard to the latter, luminal acidification is associated with a lumen-positive voltage. Generation of a transepithelial voltage requires electrogenic or conductive processes on both apical and basolateral membranes. Because the intercalated cell does not possess significant apical membrane conductances (68,69), electrogenic acidification requires an electrogenic H^+ pump.

Substantial evidence also exists for a role of an electroneutral H-K-ATPase. This includes (a) labeling of α-intercalated cells with antibodies against the gastric H-K-ATPase (27), (b) inhibition of H^+ transport by SCH 28080, a specific inhibitor of the gastric H-K-ATPase (21,29,30), and (c) inhibition of K^+ absorption by SCH 28080 and omeprazole (other H-K-ATPase inhibitors) (28,70).

Most likely, both the vacuolar H-ATPase and the H-K-ATPase contribute to H^+ secretion, their relative contributions being species- and nephron segment-dependent. The H-K-ATPase may play a more significant role in K depletion, when its expression is significantly increased to mediate K reabsorption and minimize K loss. An apical membrane Na-H antiporter is not present in these cells. Teleologically, this makes good sense for two reasons. First, such a transporter would have difficulty establishing the low luminal pH required in the collecting tubule lumen to trap ammonia and titrate remaining titratable buffers. Second, luminal Na^+ concentration is highly variable and potentially very low in the distal nephron, making it risky to rely on the lumen-to-cell Na gradient as the primary driving force for H^+ secretion.

HCO_3^- generated in the α-intercalated cell (Fig. 6-1B) exits the basolateral membrane on a Cl^-- HCO_3^- exchanger. This Cl^-–HCO_3^- exchanger is of the AE1 type and, as described above, represents a truncated form of the red cell AE1 Cl^--HCO_3^- exchanger (45,46,71,72). Truncation is accomplished with an alternate promoter, leading to a mRNA lacking the first three exons of the red cell mRNA (47). Because transport is mediated by the carboxy-terminal portion of this protein, the net result is a protein with similar transport characteristics, but altered cytoskeletal interactions. Cl^- enters the cell in exchange for HCO_3^- and exits the basolateral membrane through a Cl^- conductance (68,69).

The cortical nephron segments are unique in possessing a cell that can secrete HCO_3^- into the luminal fluid. HCO_3^- secretion is mediated by the β-intercalated cell by the active secretion of H^+ across the basolateral membrane into the interstitium (Fig. 6-6). As with the α-intercalated cell, H^+ secretion is mediated by both a vacuolar H-ATPase and an H-K-ATPase (29). While an Na-H antiporter is present on the basolateral membrane of these cells, current evidence suggests that it does not play a role in transcellular transport by these cells (73).

HCO_3^- generated in the β-intercalated cell exits from the cell into the luminal fluid on a Cl^-–HCO_3^- exchanger. This Cl^-–HCO_3^- exchanger is not AE1. Although AE2 and AE3 are expressed in the kidney, there is no convincing evidence that either of these isoforms encodes the apical membrane Cl^-–HCO_3^- exchanger of β-intercalated cells. Cl^- that enters the cell in exchange for HCO_3^- exits the cell through a basolateral membrane Cl^- conductance. This process does not generate a transepithelial voltage, because there is no net transcellular current.

The ability to secrete HCO_3^- disappears in the outer medullary collecting tubule. Here, H^+ ion secretion is mediated by cells functionally similar to the α-intercalated cells described previously. In some cases, these cells are indeed α-intercalated cells, whereas, in other cases, these cells are functionally similar, but morphologically distinct. The outer medullary collecting tubule inner stripe appears to be a high-capacity H^+-secreting segment. The inner medullary collecting tubule has been difficult to study because of the difficulty in isolating this nephron segment for tubular perfusion. Nevertheless, evidence suggests a mechanism of H^+ secretion similar to that described for the α-intercalated cell. This is probably the key segment responsible for the final acidification of the tubular fluid to the urinary pH.

REGULATION OF RENAL ACIDIFICATION

The precise regulation of renal acidification involves regulation of transport in each of the nephron segments described previously. In the following sections, we describe some of the most important forms of regulation of H^+–HCO_3^- transport.

Luminal HCO_3^- Delivery

Perhaps one of the most important forms of regulation of tubular H^+ secretion is that of glomerulotubular balance. Changes in GFR lead to large changes in the filtered load of HCO_3^-. It is essential that the tubules respond with parallel changes in the rate of H^+ ion secretion to avoid inappropriate HCO_3^- excretion or retention and the development of a significant metabolic acidosis or alkalosis, respectively.

Increases in GFR lead to increases in luminal flow rate. These increases in luminal flow rate must then signal the tubules to secrete H^+ at more rapid rates. If the tubular H^+ secretion rate is fixed, while there is an increase in luminal flow rate, the net result is that a lower fraction of delivered HCO_3^- is reabsorbed and luminal HCO_3^- concentration and pH fall at a slower rate. This more alkaline luminal pH stimulates apical membrane H^+ transporters (59). Although this effect of luminal flow rate on the axial luminal pH profile contributes to the flow dependence of HCO_3^- absorption, it cannot account for it entirely (74,75).

The above scenario suggests that luminal flow rate can directly regulate the rate of proximal tubule H^+ secretion. This effect was demonstrated directly in a study in which the rate of the apical membrane Na-H antiporter was measured in a microperfused tubule and found to be stimulated by increases in perfusion rate in the absence of changes in luminal composition (76). The mechanism responsible for this "direct" effect of flow rate is not resolved. Flow-dependent mixing in a luminal diffusion barrier (unstirred layer effect) or an effect on the cytoskeleton is possible.

In addition to these acute effects, chronic changes in GFR are associated with chronic changes in the capacity for H^+ secretion. Removal of one kidney or feeding a high-protein diet results in increased GFR and growth in the remaining renal tissue. When studied functionally, the renal proximal tubule demonstrates increased rates of transepithelial H^+ secretion, and parallel increases in the activities of the apical membrane Na-H antiporter and the basolateral membrane Na-HCO_3-CO_3 cotransporter (77). These effects can be demonstrated in the absence of changes in luminal flow rate, and thus represent adaptations in tubular function. All of the aforementioned effects have been demonstrated in the proximal tubule, but probably extend along the nephron.

Plasma pH

In general, decreases in plasma pH stimulate renal acidification and increases in plasma pH inhibit renal acidification. When this occurs in response to changes in pCO_2, it is considered a metabolic compensation for a respiratory acid–base disorder. When it occurs in response to changes in plasma HCO_3^- concentration, it represents a metabolic correction of a metabolic acid–base abnormality. In general, changes in plasma pH lead to changes in renal interstitial pH that affect the driving force for basolateral base efflux. Thus, acidosis enhances basolateral HCO_3^- efflux, and alkalosis inhibits basolateral membrane HCO_3^- efflux. The net result is that in acidosis, cell pH decreases, whereas in alkalosis it increases (78). This change in cell pH then changes the driving force that defines the rate of apical membrane H^+ ion secretion, with decreases in cell pH stimulating H^+ secretion and increases in cell pH, inhibiting H^+ secretion.

Although such simple kinetic mechanisms probably contribute, the mechanisms of regulation are significantly more complex. Decreases in intracellular pH allosterically activate the Na-H antiporter (79). This means that intracellular acidification increases the antiporter rate to an extent greater than would be predicted from a simple kinetic model. Intracellular acidification also stimulates exocytic insertion of membrane containing H^+ transporters into the apical membrane (80,81). This mechanism allows a latent pool of reserve H^+ ion transporters to be incorporated into the apical membrane, which can then enhance the rate of H^+ transport. Such regulation by acidosis has been demonstrated in the proximal tubule and α-intercalated cells.

Long-term changes in blood pH also lead to long-term adaptations in tubular function. In the renal proximal tubule, chronic acidosis, both metabolic and respiratory, leads to adaptations in proximal tubule function that enhance the capacity for H^+ secretion and increase ammonia synthesis (82,83). The increased capacity for H^+ secretion is mediated by parallel increases in the activities of the apical membrane Na-H antiporter and the basolateral membrane Na-HCO_3-CO_3 cotransporter (84–87).

These transporter adaptations represent a direct effect of pH in that they can be induced by incubating cultured proximal tubule cells in acid media (88). With regard to the apical membrane Na-H antiporter, enhanced activity is mediated by trafficking of NHE-3 to the apical membrane, and increasing whole cell NHE-3 abundance (89). Endothelin-1 (ET-1) also increases NHE-3 activity by causing trafficking of NHE-3 to the apical membrane (90). We have demonstrated that metabolic acidosis stimulates renal proximal tubule ET-1 expression (91). ET-1 then binds to the endothelin-B (ET_B) receptor, activation of which leads to NHE3 phosphorylation and exocytic insertion of the protein into the apical membrane (90,92). Studies in ET_B receptor knock-out mice demonstrate that the ET-1/ET_B signaling pathway mediates acid stimulation of NHE3 activity (93,94). Acid feeding to the knock-out mice fails to increase apical membrane NHE3 activity despite the development of a more severe metabolic acidosis compared to wild-type mice. Additional studies have identified a concensus sequence (KXXXVPKXXXV) in the second intracellular loop of the ET_B receptor responsible for ET-1/ET_B stimulation of NHE3 activity (95).

Recent studies have shown that the acid-activated signaling pathway that stimulates proximal tubule Na/H antiporter activity involves the nonreceptor tyrosine kinases Pyk2 and c-Src, mitogen-activated kinases ERK1 and 2, and the immediate early genes *c-fos* and *c-Jun* (96–98). Pyk2, c-Src, and ERK1 and 2 appear to be upstream of ET-1/ET_B in the acid-activated signaling pathway in that their inhibition prevents

acid stimulation of NHE3 activity, but not ET-1/ET_B stimulation of NHE3 activity (Li et al., unpublished observations). On the other hand, RhoA appears to be downstream of ET-1/ET_B because inhibition of the kinase blocks both acid and ET-1/ET_B stimulation of NHE3 activity (99) (Li et al., unpublished observations).

In the thick ascending limb, chronic metabolic acidosis causes adaptive increases in the rate of H^+ secretion and NH_4^+ absorption (100). These are associated with adaptive increases in NHE-3 activity and protein and mRNA abundances (101). In the cortical collecting tubule, long-term increases in dietary acid lead to an increased capacity for H^+ secretion into the lumen, whereas long-term increases in dietary alkali lead to an increased capacity for HCO_3^- secretion (102). As described above, in this nephron segment H^+ secretion is mediated by the α-intercalated cells, while HCO_3^- secretion is mediated by the β-intercalated cells. This effect appears to be mediated by changes in the relative number of α- and β-intercalated cells (103,104). During metabolic acidosis the number of β-intercalated cells decreases, while the number of α-intercalated cells increases without a change in the total number of intercalated cells (105,106). Although the specific cellular mechanisms responsible remain to be elucidated, recent evidence suggests that an extracellular protein hensin may be involved in the "switch" between the predominant intercalated cell type (107). Hensin is deposited in the extracellular matrix of H^+-secreting intercalated cells following acid incubation and anti-hensin antibodies block the conversion of intercalated cells from the β to α phenotype, and prevent the collecting tubule from converting from a HCO_3^- secreting to H^+-secreting mode (108).

Alternately, it is possible that the switch from a HCO_3^- secreting to H^+-secreting epithelium may be no more than exocytotic insertion of H-ATPase (H^+ secretion) transporters into the apical membrane of the α-intercalated cells and endocytic retrival of the Cl–HCO_3^- (HCO_3^- secretion) exchanger from the apical membrane of the β-intercalated cells.

Enhanced collecting tubule H^+ secretion in acidosis requires enhanced availability of ammonia. To accomplish this, as mentioned above, acidosis stimulates ammoniagenesis in the proximal tubule. The effect of an acute acidosis is mediated by allosteric activation of α-ketoglutarate dehydrogenase (109). Chronic metabolic acidosis leads to adaptive increases in the activities of glutaminase, glutamate dehydrogenase, and phosphoenolpyruvate carboxykinase (6). In all cases, increases in activity are associated with increases in the abundance of their respective mRNA (110–112). Citrate is an organic anion whose excretion in the urine is equivalent to excretion of HCO_3^-. In chronic acidosis, the proximal tubule enhances citrate reabsorption and metabolism (113). This is of significant clinical relevance because hypocitraturia in chronic acidosis predisposes to nephrolithiasis and nephrocalcinosis.

K^+ Deficiency

Chronic K^+ deficiency leads to a number of adaptations in H^+ ion secretion that are similar to those present in chronic metabolic acidosis. Thus, in the renal proximal tubule, chronic K^+ deficiency leads to an increased capacity for transepithelial H^+ secretion, parallel increases in the activities of the apical membrane Na-H antiporter and the basolateral membrane Na-HCO_3-CO_3 cotransporter (114), increases in citrate absorption and metabolism (113), and increases in activities of the same ammoniagenic enzymes stimulated by chronic metabolic acidosis (115). The similarity between these two responses probably resides in the fact that chronic K^+ deficiency causes intracellular acidification in the kidney (116). Incubation of cultured kidney cells in low K^+ media leads to cell acidification and increases in apical Na/H antiporter activity (117). It is not clear, however, how chronic K^+ deficiency causes intracellular acidification.

In the collecting tubule, chronic K^+ deficiency increases the capacity for H^+ secretion. Like chronic metabolic acidosis, chronic K^+ deficiency leads to exocytotic insertion of H^+ pumps into the apical membrane of H^+-secreting cells and increases the activity of the H-K-ATPase (21,118). The latter effect would enhance H^+ ion secretion while, at the same time, enhancing K^+ absorption in the distal nephron. Stimulation of renal acidification processes along the nephron is responsible for the fact that K^+ deficiency is associated with a chronic metabolic alkalosis.

Hyperkalemia appears to have the opposite effect on the kidney. This is most evident in regard to ammoniagenesis. Hyperkalemia inhibits proximal tubule ammoniagenesis, which contributes to the acidification defect in hyperkalemic renal tubular acidosis (115). In addition, hyperkalemia inhibits NH_4^+ reabsorption in the thick ascending limb, which may contribute to low NH_3 levels in the renal medulla, and, thus, limited capacity to buffer H^+ secreted in the collecting tubule (14). The consequence of this scenario would be decreased HCO_3^- regeneration and the generation of a metabolic acidosis when ingesting an acid-generating diet.

Mineralocorticoids and Distal Na^+ Delivery

The hormone that most potently regulates renal acidification is aldosterone. Aldosterone interacts with mineralocorticoid receptors present in the distal nephron and stimulates acidification by a number of different mechanisms. First, aldosterone can directly stimulate the rate of H^+ ion transport (119,120). This is mediated by parallel increases in the activities of the apical membrane H^+ pump and the basolateral membrane Cl^--HCO_3^- exchanger (121). The specific H^+ pump activated is the vacuolar H^+ pump (122). Second, aldosterone indirectly increases the rate of H^+ secretion by increasing Na^+ reabsorption in the CCT, which secondarily increases luminal electronegativity. The increased luminal electronegativity enhances the driving force for H^+ ion secretion, which is electrogenic and voltage sensitive (123).

Increases in distal Na^+ delivery can also increase electrogenic Na^+ reabsorption in the CCT, which will stimulate H^+ secretion by the mechanism described above. Clearance studies have consistently shown that maneuvers that increase distal Na^+ delivery in the presence of high aldosterone levels profoundly stimulate renal acidification. Confirmation that the two stimuli are additive has been shown in clearance studies that have demonstrated no effect of aldosterone on renal acidification when distal Na^+ delivery is low (124). This observation suggests that the indirect effect of aldosterone on renal acidification, mediated through changes in Na^+ transport, is probably the dominant effect.

Peptide Hormones

A large number of peptide hormones regulate H^+ transport along the nephron, and thus, regulate renal acidification. These are summarized in Table 6-3. In the renal proximal tubule, parathyroid hormone and dopamine inhibit HCO_3^- absorption and apical membrane Na-H antiporter activity (125–128). Conversely, angiotensin II, endothelin, and catecholamines (interacting with α-adrenergic receptors) stimulate HCO_3^- reabsorption and apical membrane Na-H antiporter activity (129–134). The signaling pathways mediating these effects are being actively pursued. cAMP appears to play a key role in that the aforementioned hormones that inhibit the Na-H antiporter also activate adenylyl cyclase; in contrast, the hormones

TABLE 6-3

PEPTIDE HORMONE REGULATION: CHANGES IN NET H^+ SECRETION

	Proximal tubule	Thick ascending limb	Collecting duct
Dopamine	↓	—	—
Parathyroid hormone	↓	↓	↑
Angiotensin II	↑	—	—
Endothelin	↑	—	—
Catecholamines			
α^2	↑	—	—
β	—	—	↓
Vasopressin	—	↓	↑
Glucagon	—	↓	↓

that stimulate Na-H antiporter activity inhibit adenylyl cyclase (128,129,134–136). cAMP–dependent PKA directly phosphorylates and inhibits NHE-3, an effect that requires the scaffolding protein, NHERF, and involves endocytic retrival of NHE3 via clathrin-coated vesicles (137–144). As mentioned above, RhoA activity is required for endothelin-1 stimulation of proximal tubule Na-H antiporter activity.

In the thick ascending limb, hormones that activate adenylyl cyclase, including parathyroid hormone, vasopressin, and glucagon, all inhibit HCO_3^- absorption (145).

In the cortical collecting tubule, isoproterenol interacting with β-adrenergic receptors stimulates HCO_3^- secretion (73). This effect is also probably mediated by cAMP. Parathyroid hormone has been shown to increase distal H^+ secretion indirectly by inhibiting proximal tubule phosphate absorption and enhancing distal phosphate delivery (146). Phosphate can function as a nonreabsorbable anion and buffer, enhancing distal H^+ secretion. Vasopressin increases net H^+ secretion, whereas glucagon inhibits net H^+ secretion in the distal nephron (147,148).

Effective Arterial Volume

Effective arterial volume is an important regulator of renal acidification. In most cases, metabolic alkalosis is maintained by decreases in effective arterial volume. This is due, in part, to volume-induced decreases in GFR (leading to a decrease in filtered HCO_3^- load) (149). In addition, a decreased effective arterial volume increases renal acidification by (a) decreasing paracellular HCO_3^- permeability in the proximal tubule, secondarily inhibiting HCO_3^- back-leak (65); (b) decreasing distal delivery of Cl^-, which could inhibit HCO_3^- secretion in the cortical collecting tubule (by limiting the amount of apical membrane Cl^-- HCO_3^- exchange); and (c) increasing the levels of hormones, such as aldosterone and angiotensin II, and renal nerve activity, all of which stimulate HCO_3^- reabsorption and regeneration and ammoniagenesis.

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CHAPTER 7 ■ RENAL METABOLISM

ANTON C. SCHOOLWERTH AND SUSAN R. DIGIOVANNI

KIDNEY FUNCTION IN PERSPECTIVE

Kidneys play a principal role in the conservation of many substances essential to the body, the secretion of several physiologically active compounds, and the maintenance of acid–base balance by producing ammonia. Compared with other organs, the kidney demonstrates unusual features with respect to oxygen consumption (QO_2), renal blood flow (RBF), and arteriovenous oxygen extraction. The high rate of QO_2, exceeded only by that of the heart (1), is indicative of the importance of mitochondrial metabolism in the kidney. A greater density of mitochondria has been demonstrated in certain nephron segments, especially in the proximal convoluted tubule (PCT), the proximal straight tubule (PST), and the thick ascending limb (TAL) of Henle's loop (2).

The kidney transports a large number of organic and nonorganic solutes and water. Although Na^+ transport is considered to be the most important from a quantitative point of view, a variety of other solutes are translocated by primary or secondary active transport mechanisms. Transport by these systems requires the generation of energy in the form of adenosine triphosphate (ATP) produced in the mitochondria by the process of oxidative phosphorylation; ATP is also formed directly by enzymatic steps in major pathways of substrate metabolism, such as glycolysis. Synthesis of ATP by this means is termed *substrate-level phosphorylation* and accounts for only a small amount of ATP produced in the cell. Examples of substrate-level phosphorylation include two specific reactions, phosphoglycerate kinase and pyruvate kinase, yielding ATP in the metabolism of glucose to pyruvate. In addition, in mitochondria, guanosine triphosphate is formed and the energy is transferred to ATP in the production of succinate from succinyl-coenzyme A (CoA).

Numerous metabolic reactions yield nicotinamide-adenine dinucleotide hydrogenase (NADH) and ultimately ATP. Net ATP generation or consumption is affected by the metabolic pathways and substrates used (Table 7-1). For example, fatty acid oxidation is the most efficient source of ATP, yielding 129 molecules of ATP from the complete oxidation of 1 molecule of palmitate. Conversely, the formation of glucose from noncarbohydrate precursors is an energy-consuming process; conversion of two molecules of lactate to one molecule of glucose uses the energy stored in six molecules of ATP.

Substrate Compartmentation

Substrates are delivered to the kidney in the plasma and may be exposed to the luminal membrane of renal cells after glomerular filtration or secretion. In addition, substrates may be exposed to the basolateral membrane in the postglomerular circulation. Substrate concentrations differ from extracellular fluid to cell cytosol and mitochondrial matrix space. In addition, it is likely that different concentrations of metabolites exist in different regions of the kidney. For example, as shown in Figure 7-1, O_2 gradients clearly exist with a high O_2 tension in the renal cortex and a very low tension in the medulla (3). Oxygen tension thus decreases as a function of distance from the cortical surface of the kidney. Data have been accumulated to indicate that microcompartmentation of substances may also occur within given cells. For example, Jones (4,5) has demonstrated O_2 and metabolite gradients in renal cells. The significance of these gradients with respect to control of metabolism and susceptibility to injury requires further investigation. However, with regard to compartmentation, it is clear that several potential sites of metabolic regulation exist. This could be in transport steps across the cell and mitochondrial membranes or transport across other organelle membranes. Enzymatic steps in the cytosol and mitochondria may be regulatory, as may the delivery of substrates—that is, substrate concentrations may affect metabolic rate.

Renal Fuels

The preferred substrates used to supply energy needs vary from organ to organ. For example, the brain uses glucose almost exclusively, whereas the heart uses a mixture of substrates that includes glucose, fatty acids, lactate, and ketone bodies (6). Attempts to determine the kidney's preference for substrates have been impeded by the problem imposed by the very high rate of RBF, which supplies the energy needs of the kidney with extraction of only a small fraction of delivered substrate. This high RBF rate, as well as the need to extract numerous substrates rather than a single one, leads to a situation in which a very small arteriovenous difference is present across the kidney for any given compound (7). This small difference complicates accurate estimation of fractional substrate extraction, which is necessary to determine unequivocally the metabolic fuels of the kidney. The situation is made even more complex by the fact that the substrates used may vary under different circumstances, such as metabolic acidosis, and the fact that metabolism in the kidney is heterogeneous. Data indicate that substrate utilization may vary among different regions of the kidney, such as the proximal tubule (8–13) and TAL of Henle's loop (9,12,14).

Substrate Utilization in the Kidney

Given the difficulties in making accurate measurements of small arteriovenous metabolite differences, a number of studies have been reported that indicate consistent net extraction by the kidney of free fatty acids (FFA), glutamine, lactate, and citrate. These compounds are present in sufficiently high concentration in the blood to be considered significant energy sources for the kidney (7,15). Elhamri et al. (16) have reevaluated renal substrate uptake and utilization in rats. In fed rats, these workers found net uptake of glucose, lactate, fatty acids, ketone bodies, citrate, and glycerol; in starved rats, uptake of fatty acids, ketones, glutamine, and glycerol increased, whereas lactate utilization was reduced. Other substances have also been

TABLE 7-1

ADENOSINE TRIPHOSPHATE SYNTHESIS (+) OR UTILIZATION (−) BY MAJOR PATHWAYS OF RENAL METABOLISM

Reaction	Net ATP
1 palmitate → $16CO_2$	+129
1 glucose → $6CO_2$	+36
1 glutamine → $5CO_2$	+27
1 lactate → $3CO_2$	+18
2 glutamine → glucose + $4CO^2$	+14
2 lactate → 1 glucose	−6

ATP, adenosine triphosphate.
(Adapted from: Simpson DP. Renal metabolism. In: Schrier RW, Gottschalk CW, eds. *Diseases of the kidney.* Boston/Toronto: Little, Brown, 1988:241, with permission.)

demonstrated to be extracted, but are present in much lower concentrations, or data differ from study to study. These substrates include glycine, proline, citrulline, β-hydroxybutyrate, pyruvate, and glycerol. As mentioned previously, the substrate used preferentially by the kidney may vary with the metabolic state, although, in general, substrate utilization appears to be directly proportional to the plasma concentration of the substrate in question. As is elaborated later, in metabolic acidosis, for example, clear evidence has been obtained indicating that the preferred renal substrate changes from lactate to glutamine (17). In chronic metabolic acidosis, increased renal glutamine extraction provides sufficient amounts of this substrate for the accelerated rate of ammonium formation by the PCT.

Metabolite Transport: Specific Carriers

Mitochondrial Membrane Transporters

Although the inner mitochondrial membrane is permeable to O_2, water, and CO_2, it is not permeable to most other metabolites, because of their highly hydrophilic nature. If the translocation of these substances for respiration and for ATP formation is to proceed efficiently, specialized carriers are required (Table 7-2). At least 12 specific transporters are recognized on the inner mitochondrial membrane (18–20); most of these carriers transport anions and they can be classified into several categories. For example, some transporters or carriers are electroneutral such that movement of negatively charged metabolites is compensated by movement of a proton. Examples of such carriers include the phosphate, glutamate, and pyruvate transporters. Other transporters are electroneutral such that charges are compensated by the countertransport of another anion. These carriers operate so that one of the metabolites transported is exchanged for another physiologic substrate of this carrier. Examples of such transporters include the dicarboxylate transporter (transporting phosphate, malate, succinate, and oxaloacetate), the α-ketoglutarate (α-KG) carrier, and the tricarboxylate carrier (transporting citrate, isocitrate, phosphoenolpyruvate, malate, and succinate). Other electroneutral carriers also exist, such as the carnitine and glutamine carrier. Finally, there are electrophoretic carriers: one substrate is transported in exchange for another substrate, with net transfer of charge as well. Examples of these electrophoretic transporters include the glutamate-aspartate and adenine nucleotide carriers. Although 12 carriers are known to exist, it is not clear that all transporters are present in all mitochondria. However, many have been identified in renal mitochondria and are thought to contribute to the regulation of metabolism (20). The possible rate-determining role of metabolite transporters has generated considerable controversy, particularly with respect to the adenine nucleotide carrier (18). No consensus has been reached as to whether the transport of adenosine diphosphate (ADP), in exchange for ATP, regulates energy metabolism in oxidative tissues. Further work should clarify the role of carriers in the regulation of metabolism.

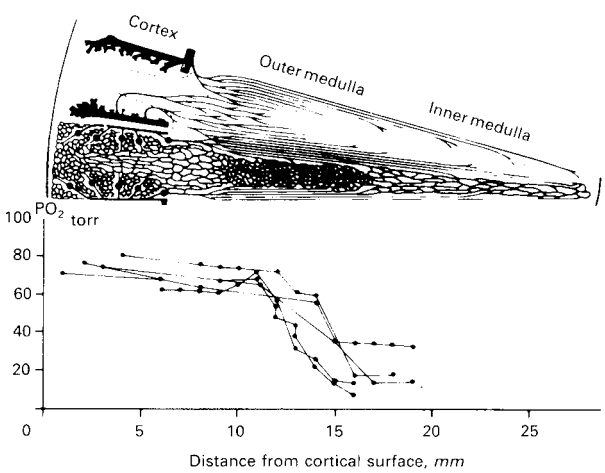

FIGURE 7-1. Oxygen gradient between renal cortex and medulla. Oxygen tensions measured in different parts of the kidney are shown with the vascular pattern superimposed above. A sharp drop in oxygen tension occurs between outer and inner medulla. (From: Thurau K, Levine A. The renal circulation. In: Rouiller C, Muller AF, eds. *The kidney: morphology, biochemistry, physiology.* New York: Academic Press, 1971:1, with permission.)

TABLE 7-2

MITOCHONDRIAL SUBSTRATES AND THEIR CARRIERS

Substrate	Carrier
Glutamate	Glutamate-hydroxyl
Glutamate	Glutamate-aspartate
Aspartate	Glutamate-aspartate
Glutamine	Glutamine
α-Ketoglutarate	α-Ketoglutarate
Pyruvate	Pyruvate-hydroxyl
Citrate	Tricarboxylate
Isocitrate	Tricarboxylate
Aconitate	Tricarboxylate
Phosphoenolpyruvate	Tricarboxylate
Malate	α-Ketoglutarate
Malate	Tricarboxylate
Malate	Dicarboxylate
Succinate	Dicarboxylate
Phosphate	Dicarboxylate
Phosphate	Phosphate-hydroxyl
Carnitine	Carnitine
Acylcarnitine	Carnitine
Adenosine triphosphate	Adenine nucleotide
Adenosine diphosphate	Adenine nucleotide

(From: Simpson DP. Mitochondrial transport function and renal metabolism. *Kidney Int* 1983;23:785, with permission.)

Cell Membrane Transporters

Data have also been obtained to indicate that specific carrier mechanisms exist for the uptake of metabolites into renal cells. These include carriers for monocarboxylic, dicarboxylic, and tricarboxylic acids. For example, transporters of citric acid cycle metabolites have been identified (21–30). Similar, although not identical, carriers appear to be present on the basolateral and luminal membranes (23). It is not clear whether these metabolite carriers on the plasma membranes are rate determining for the metabolism of the citric acid cycle anions.

RENAL OXYGEN CONSUMPTION

Renal QO_2 accounts for approximately 7% of whole-body O_2 uptake. The delivery of O_2 and other substrates is not limited by the perfusion rate, because RBF is high. The arteriovenous O_2 gradient is relatively small in the kidney, in contrast to heart, brain, or skeletal muscle; it remains almost unchanged when RBF is altered. This indicates that most renal tissue is kept under relatively high O_2 tension because arterial O_2 extraction is lower in kidney than any other major organ (1). However, in several mammalian species, a significantly higher O_2 tension is consistently observed in the renal cortex than in the medulla. As illustrated in Figure 7-1, the O_2 tension in the cortex is high, but in the medulla, where blood supply is restricted, tension drops to a much lower level. The countercurrent arrangement of the vasa recta is also thought to restrict O_2 delivery to the medulla. The decrease in the O_2 tension explains why this region of the kidney depends on anaerobic metabolism for a significant fraction of its energy supply (7). Investigators have shown that tubular metabolism is a major determinant of intrarenal oxygenation and that medullary reabsorptive work is, at least in part, responsible for hypoxia observed in this region of the kidney under basal conditions (31). For example, loop diuretics (e.g., furosemide, bumetanide) selectively increased the availability of O_2 in the medulla, improving oxygenation and, consequently, elevating O_2 tension in this region of the kidney. Because furosemide was also shown to reduce medullary blood flow, it appears that loop diuretics may improve oxygenation by reducing tubular QO_2 necessary for reabsorptive ion transport in the medulla. Brezis et al. (32) showed that acute hypotension is associated with a decrease in cortical blood flow, but medullary blood flow either stays the same or is increased. In addition, sodium transport due to reduced glomerular filtration rate (GFR), increased distal delivery, and direct inhibition of transport of Na^+ is reduced under these conditions. The end result is increased medullary O_2 tension (32).

Two vasoactive substances, adenosine and nitric oxide (NO), have been implicated in playing a role in controlling renal QO_2. Adenosine alters medullary O_2 balance by increasing medullary blood flow at the same time it reduces GFR. In addition, it is possible that adenosine directly inhibits active sodium transport (33). Adenosine is thought to be protective for ischemic injury in the kidney. Nitric oxide has also been shown to alter renal QO_2 by direct inhibition of mitochondrial respiration (34,35). Because NO synthesis is stimulated by hypoxia, it may be instrumental in renal hypoxic injury. Thus, medullary O_2 availability increases during renal hypoperfusion and decreases during renal vasodilation (32).

In contrast to heart or skeletal muscle—tissues involved in measurable external work—the extensive renal QO_2 rate is consistent with a high rate of internal work performed by this organ (1,36). For example, under physiologic conditions, the osmolality (and volume) of incoming and outgoing blood is almost unchanged by the kidney. Therefore, the renal osmotic work is considered to be internal work dedicated to the predominant function of the kidney—active reabsorption of solute.

Components of Renal QO_2

Renal QO_2 can be separated into two different components: (a) basal QO_2, reflecting oxygen requirements associated with energy-dependent processes other than active Na^+ transport; and (b) suprabasal QO_2, which is related to the amount of Na^+ actively reabsorbed by renal tubular cells. In the intact mammalian kidney, the basal QO_2 was estimated to be 3% to 18% of the total oxygen consumption (37,38). This value is severalfold higher than that obtained for skin or resting muscle. In general, it has been assumed that the basal rate of renal QO_2 is uniform and not influenced by the rate of Na^+ reabsorption. However, this assumption is difficult to validate because the fraction of Na^+ reabsorbed by passive transport processes can vary among different nephron segments (39,40). In contrast to the distal nephron, where most of the Na^+ reabsorption is active (39,41), a significant fraction of Na^+ transported in the proximal tubule is reabsorbed by passive diffusion not requiring energy transfer from ATP (39). However, the kidney needs energy (reflected by basal QO_2) to perform the work that is unrelated to active Na^+ transport, such as synthetic functions and metabolic interconversions, but that may vary under different conditions. The major fraction of the renal suprabasal QO_2 is coupled to active tubular Na^+ transport; for each mole of O_2 consumed, approximately 28 mol of Na^+ is taken up by the kidney. Therefore, suprabasal rates of QO_2 could be determined mainly by measuring Na^+ reabsorption rates.

In the remnant kidney model, in which both morphologic and functional alterations occur, QO_2 is markedly increased when normalized for Na^+ transport, compared with QO_2 in normal kidney (42). However, QO_2 is identical in remnant and normal kidneys when expressed per gram of kidney weight; the basal QO_2 is also not different in remnant and control kidneys when normalized to kidney weight (43). This increased rate of QO_2 per remaining nephron could be a cause of tubulointerstitial injury; the enhanced renal metabolic activity ("hypermetabolism") and elevated QO_2 may be associated with enhanced oxygen radical generation, which could lead to renal cell damage.

Mitochondrial Oxidative Phosphorylation

Synthesis of ATP by oxidative phosphorylation supplies most of the energy for the kidney. This is a complex process localized in mitochondria (44,45). Oxidative phosphorylation is normally coupled to the electron transport system, generating ATP from the oxidation of NADH (or FADH). The rate of this process can be determined experimentally by measuring QO_2 in the presence of ADP. If an excess of substrate and O_2 is available, respiration continues until all ADP is converted to ATP, at which point the respiration rate, that is, the utilization of O_2, abruptly decreases to a low resting level. Therefore, the level of ADP or its uptake by mitochondria, in addition to the availability of substrates, may be a rate-determining component. As accepted by many investigators, the respiratory chain responsible for the oxidation of NADH, for the generation of a proton gradient across the inner mitochondrial membrane, and for QO_2 contains three sites of phosphorylation. These sites are located between NADH and cytochrome b, cytochrome b and c, and cytochrome c and O_2, respectively. In the kidney, oxidation of cytochromes could be modulated by different factors. For example, in the outer stripe, this process is predominantly influenced by outer medullary blood flow; however, in the inner stripe, the rate of QO_2 plays a regulatory role (46).

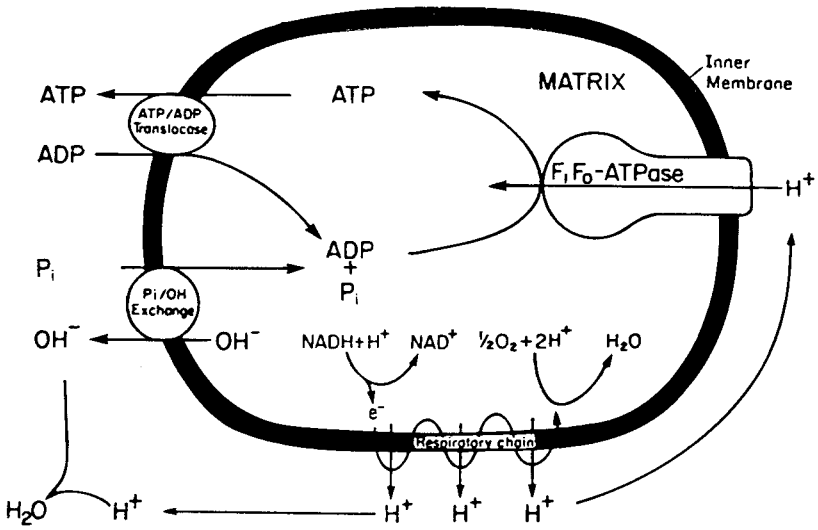

FIGURE 7-2. Basic elements participating in oxidative phosphorylation in the mitochondrion. Adenosine diphosphate (*ADP*) and inorganic phosphate (P_i) enter in exchange for adenosine triphosphate (*ATP*) and OH^-, respectively. Electrons (e^-) are transferred by the respiratory chain from nicotinamide-adenine dinucleotide hydrogenase (NADH) to O_2. The extrusion of H^+ by the respiratory chain generates a transmembrane proton gradient, which drives H^+ entry through the F_1F_0-ATPase to generate ATP. The newly synthesized ATP exits to the cytosol in exchange for ADP. (From: Gullans SR, Hebert SC. Metabolic basis of ion transport. In: Brenner BM, Rector FC Jr, eds. *The kidney.* Philadelphia: Saunders, 1991:76, with permission.)

At each step of the respiratory chain, two electrons are transferred and one molecule of ATP is synthesized from ADP and inorganic phosphate (P_i). The relationship between these processes, described in the so-called *chemiosmotic hypothesis,* is illustrated in Figures 7-2 and 7-3. Mitchell's chemiosmotic theory is widely accepted to describe the mechanism coupling the synthesis of ATP to a spatially arranged electron transport system in the inner mitochondrial membrane. This membrane is impermeable to passively transported small charged molecules, including H^+ and organic anions. However, an active H^+ pump is linked to components of the electron transport chain, such as cytochromes. When NADH enters the sequence of reactions, two electrons from NADH are transferred to half-O_2. Each oxygen atom with two extra electrons accepts two protons from the environment and a molecule of water is formed. The dissipated energy is then used to activate the H^+ pump, which extrudes H^+ into the cytoplasm and leaves OH^- in the mitochondrial matrix. This cyclical process creates an H^+ gradient across the inner mitochondrial membrane and also stimulates a flow of electric current passing the membrane. Consequently, the mitochondrial interior is alkaline and negatively charged. The H^+ may then be transported back into the matrix by an active process involving the ATP synthase (or ATPase) that is located in the inner mitochondrial membrane. The energy of the mitochondrial H^+ gradient and membrane potential, combined into a proton-motive force $\Delta\mu H^+$, initiates a reaction of two protons with one of the oxygens. In this reaction, water is eliminated, the phosphate group joins ADP to form ATP, and the released energy is stored in the high-energy bond of ATP. In summary, oxidation of 1 mol of NADH generates 3 mol of ATP, and one atom of oxygen is consumed. The electron transport chain must cycle twice to pass along two electrons to the atom of oxygen. In this entire process, only two components are consumed: the metabolite (fuel) and oxygen.

Renal Na^+ Transport and QO_2

As mentioned earlier, oxidative metabolism serves as the major energy source for active renal Na^+ transport (37,47). In tubules from adult animals, transcellular active Na^+ reabsorption accounts for one-third of the total, whereas two-thirds occurs passively through the paracellular pathway driven by hydrostatic and oncotic forces (one-third), and by cell-generated effective osmotic and ionic gradients (one-third). In the kidney, the energy cost of active Na^+ movement can be assessed from the ratio of parallel changes in the rate of Na^+ reabsorption (ΔTNa^+) and QO_2(ΔQO_2). The ratio of active to

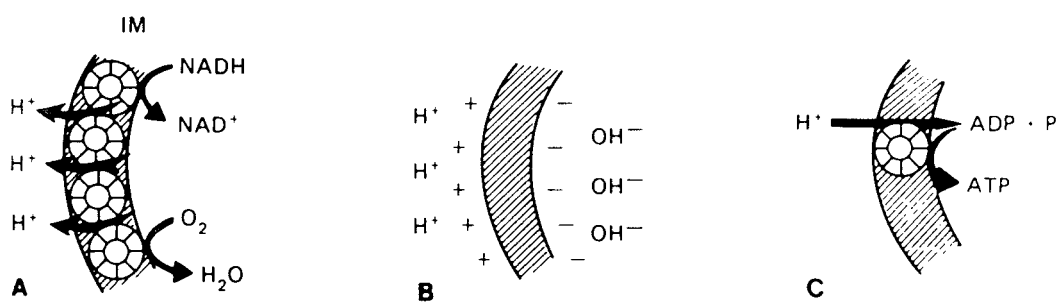

FIGURE 7-3. The development of an H^+ gradient (ΔpH) and its coupling to adenosine triphosphate (*ATP*) synthesis across the inner mitochondrial membrane (*IMM*), as described by the chemiosmotic hypothesis. **A:** During the course of nicotinamide-adenine dinucleotide hydrogenase (*NADH*) oxidation to water, H^+ is actively pumped out of mitochondria by components of the electron transport chain located in the IMM. **B:** As a result of separation of H^+ and OH^- across the IMM, a proton-motive force ($\Delta\mu H^+$) develops: This force consists of a membrane potential with mitochondrial interior negative and a ΔpH (interior alkaline). **C:** $\Delta\mu H^+$ provides energy to drive a membrane ATPase to synthesize ATP from adenosine diphosphate (*ADP*) and phosphate in the matrix. (From: Simpson DP. Mitochondrial transport function and renal metabolism. *Kidney Int* 1983;23:785, with permission.)

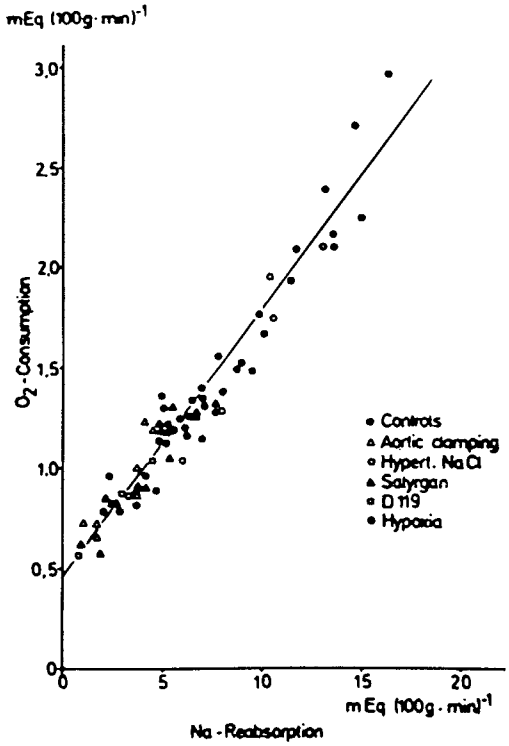

FIGURE 7-4. Relationship between renal oxygen consumption (QO_2) and sodium reabsorption (T_{Na^+}) *in vivo* under various conditions. $\Delta T_{Na^+}/\Delta QO_2$ is 30 mEq Na^+/mmol oxygen and is calculated from the inverse slope of the regression line. The *y* intercept indicates the basal QO_2, which is not associated with Na^+ transport. (From Deetjen P. Measurement of metabolism during renal work. *Int J Biochem* 1980;12:243, with permission.)

passive component of Na^+ transport varies considerably in different nephron segments, because two-thirds of the Na^+ is reabsorbed passively and energy is not required; the mature proximal tubule has a high molar ratio of Na^+ and QO_2. The relationship between ΔT_{Na+} and ΔQO_2 is shown in Figure 7-4. $\Delta T_{Na+}\Delta QO_2$ is the most frequently used indicator of the efficiency of Na^+ transport (48). Extrapolation of the regression line to the ordinate, where ΔTNa^+ equals zero, allows an estimation of basal renal QO_2, and the slope reflects a correlation between Na^+ transport and QO_2 over a wide range of transport rates under various conditions. $\Delta T_{Na+}\Delta QO_2$ for the whole kidney gives only an average value, however, and assumes no change in the passive component of Na^+ movement. Based on the assumption that 6 mol of ATP is produced per 1 mol of oxygen consumed, and that the Na^+–K^+–ATPase is able to transport 3 mol of Na^+ per 1 mol of ATP (49–53), the estimated maximal value of $\Delta TNa^+\Delta QO_2$ is 18 (54–59). However, several investigators observed that in kidney *in vivo* this ratio exceeds the theoretic value and is also higher than the value reported for simple epithelial tissues (e.g., frog skin) (60–62). In addition, the ratio of active to passive Na^+ transport varies considerably in different nephron segments. The renal $\Delta T_{Na+}\Delta QO_2$ ratio ranges from 21 to 30 (54–59). These higher values in the kidney could be explained by a significant contribution of the passive component of Na^+ reabsorption; it has been calculated that approximately 30% of all Na^+ taken up by the kidney occurs through a passive mechanism (39–41). If the correction for this significant passive component of Na^+ reabsorption in the kidney is applied, the $\Delta T_{Na+}\Delta QO_2$ ratio is reduced to approximately 18 and equals the estimated value (41,63). These observations suggest that renal Na^+ transport and QO_2 are altered concurrently. For example, in diabetic rats, cortical tubular Na^+ reabsorption is significantly increased; both total renal QO_2 and QO_2 in cortical tissue, which consists of proximal tubules, are also significantly higher (64). Therefore, the question arose as to how these two processes are coupled. At the cellular level, O_2 is consumed in the inner mitochondrial membrane, but active Na^+ extrusion by the Na^+–K^+ pump takes place at the basolateral cell membrane. Under conditions of reduced Na^+ extrusion and, consequently, decreased activity of Na^+–K^+-ATPase, utilization of ATP and formation of ADP is limited by this enzyme. Therefore, the lowered cytoplasmic ADP level may lead to a reduction of both mitochondrial ATP synthesis and QO_2 in tightly coupled mitochondria. This prediction has been confirmed experimentally in rabbit cortical tubules: inhibition or stimulation of Na^+ transport alters cellular ATP/ADP ratio (65).

QO_2 Required by Na^+-K^+ Pump

The ouabain-inhibitable fraction of QO_2 provides an accurate measurement of the amount of aerobic ATP synthesis that is coupled to active renal Na^+ transport (Fig. 7-5). Therefore, this portion of QO_2 reflects the fraction of O_2 consumption used by the Na^+-K^+ pump. The distribution of ouabain-sensitive QO_2 along the nephron is correlated with Na^+-K^+-ATPase activity, with the highest ouabain-inhibitable fraction present in the proximal tubule, the TAL of Henle's loop, and the distal convoluted tubule (DCT); most of the active Na^+ reabsorption occurs in these regions of the kidney. In diabetic rats, the elevation of total tissue QO_2 was caused entirely by an increase in the Na^+-K^+-ATPase–dependent component of QO_2. It was concluded that diabetes is associated with increased renal O_2 metabolism secondary to the increase in coupled Na^+ reabsorption by the Na^+-glucose cotransporter and Na^+-K^+-ATPase (64).

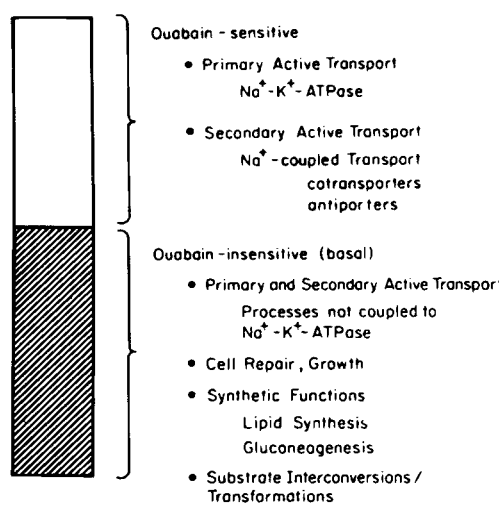

FIGURE 7-5. Components of oxygen consumption (QO_2). QO_2 is often distinguished as ouabain-sensitive or ouabain-insensitive to assess the energy dependence of Na^+ transport in renal cells. This shows the types of adenosine triphosphate (ATP) utilization processes that contribute to these two components of QO_2. The bar represents total QO_2; the hatched region, the basal component of QO_2 (ouabain insensitive); and the clear portion, the component of QO_2 related to the active Na^+ transport process. (From: Gullans SR, Hebert SC. Metabolic basis of ion transport. In: Brenner BM, Rector FC Jr, eds. *The kidney.* Philadelphia: Saunders, 1991:76, with permission.)

ROLE OF CITRIC ACID CYCLE IN RENAL METABOLISM

Translocation of Intermediates

Metabolism of citric acid cycle intermediates and pyruvate depends on rapid movement of these substances in and out of the mitochondria, where the enzymes of the citric acid cycle and pyruvate metabolism are located. One of the best-studied citric acid cycle intermediates with regard to metabolic regulation is citrate. Characteristics of citrate handling by the kidney are shown in Figure 7-6. The percentage of the filtered load of citrate that is excreted varies in different species. In humans, excretion of 30% to 35% of the filtered load has been observed, whereas it is 3% to 7% in rats and less than 1% in dogs (66). Because the amount of citrate extracted by the kidney exceeds that which can be accounted for by filtration, peritubular uptake of this metabolite likely occurs. Citrate, as well as α-KG, is transported into the cell and mitochondria, where oxidation to CO_2 or conversion to glucose ultimately begins (66). Considerable attention has been given to the renal excretion of citrate, because urinary citrate may be an endogenous inhibitor of stone formation. Of particular interest with regard to citrate is the influence of acid–base alterations on its clearance and metabolism. In metabolic alkalosis, citrate excretion increases, whereas it decreases in metabolic acidosis (66). There are multiple mechanisms by which this is thought to occur. Simpson postulated that the uptake and metabolism of citrate by mitochondria was regulated by changes in pH across the mitochondrial membrane (67). Citrate is reabsorbed by the brush border membrane of the proximal tubule via a dicarboxylic acid transporter. Decreased intraluminal pH results in a greater quantity of citrate present in the dicarboxylic form instead of the more abundant tricarboxylic form, thus increasing its transport into the cells of the proximal tubule (68). In addition, the mRNA and the protein abundance for the dicarboxylic acid transporter is increased by chronic metabolic acidosis (69). Recently, the importance of a cytosolic enzyme, adenosine triphosphate citrate lyase, in the development of hypocitraturia during chronic metabolic acidosis has been recognized (70). At present, it is uncertain whether pH_i, extracellular pH (pH_e), or alterations in the ΔpH_m are the regulatory factors in the metabolism and excretion of citrate.

Although the metabolism of α-KG is also increased in acidosis, it is not likely to be due to acceleration of α-KG transporter activity. In fact, studies with phthalonic acid, a specific inhibitor of the α-KG transporter, have indicated that the carrier is not the rate-determining factor in the mitochondrial metabolism of α-KG (71). Rather, the activation of α-KG dehydrogenase (α-KGDH) by H^+ ion is most likely to be the major factor stimulating α-KG metabolism in acidosis (72–74).

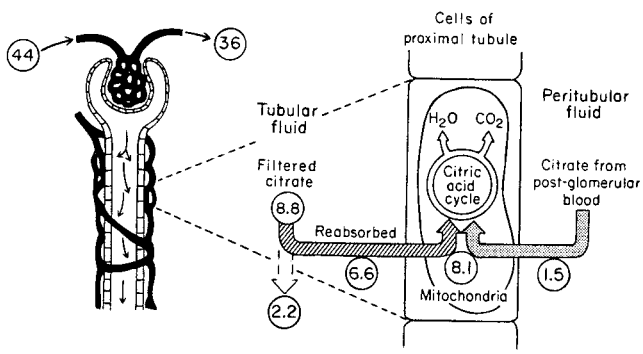

FIGURE 7-6. Characteristics of citrate handling by the nephron. Circled numbers are values for human kidney in micromoles per minute. (From: Nieth H, Schollmeyer P. Substrate utilization of the human kidney. *Nature (London)* 1966;209:1244; Simpson DP. Citrate excretion: a window on renal metabolism. *Am J Physiol* 1983;244:F223, figure reprinted with permission.)

Metabolism of Lactate and Pyruvate in the Kidney

Lactate is extracted from the blood by the kidney, but at endogenous levels little net pyruvate removal occurs (15,75–79). Lactate extraction has been demonstrated to vary directly with plasma lactate levels (75,76,78). The fate of extracted lactate is twofold: It may be converted to glucose, a process that occurs exclusively in the PCT, and it may serve as a substrate for oxidation after it is converted to pyruvate (17,76). This process seems to occur in both PCT and TAL of Henle's loop. The data indicate that as plasma lactate levels rise, an increasing proportion of lactate is converted to glucose (75,76). These results also suggest that oxidation of lactate is not changed markedly by alterations in substrate delivery; increasing lactate uptake provides fuel for glucose synthesis in PCT. Lactate extraction and metabolism are both affected by acid–base state, however. For example, in metabolic alkalosis, lactate is the primary renal fuel, whereas in metabolic acidosis, the utilization of lactate decreases as glutamine metabolism increases (17). Thus, the changes in lactate are opposite to those seen with glutamine uptake.

Pyruvate extraction also varies with changes in concentration of this metabolite in the blood (78). Although no net pyruvate removal by dog kidney occurs under physiologic conditions, when the level of pyruvate in the blood is raised, net renal uptake of this compound develops. Pyruvate oxidation requires entry of pyruvate into the mitochondria and subsequent metabolism by one of several enzymes, of which pyruvate dehydrogenase (PDH) appears to be critical. In fact, PDH seems to be a rate-determining enzyme for both glucose and lactate oxidation. This enzyme is regulated by multiple allosteric substrate and end-product regulators as well as covalent modification by an enzymatic phosphorylation–dephosphorylation cycle (80–83). Increases in the mitochondrial ratio of ATP/ADP, NADH/NAD, and acetyl-CoA/CoA lead to inactivation of PDH, whereas high pyruvate levels and elevation in mitochondrial free Ca^{2+} increase the activity of this enzyme (80,82). These mechanisms explain the effects of fatty acids, acetate, ketone bodies, and α-KG in inactivating PDH (84,85) and also account for a decrease in lactate oxidation in the presence of ketone bodies (86), fatty acids (13,87,88), and proline in cortical tubule suspensions. As mentioned previously, the activity of intramitochondrial enzymes is closely linked to the transport of metabolites into the matrix space. With regard to PDH, a close relationship between pyruvate, tricarboxylate, and dicarboxylate anion transport has been demonstrated (89). The localization of PDH along the nephron has not been reported (83). It is predicted that PDH activity parallels the mitochondrial density in different nephron segments (83,90).

Glucose Metabolism in the Kidney

The liver and kidney are capable of both producing and consuming glucose. Because of the dual capacity of glycolysis and gluconeogenesis, it is difficult to demonstrate net uptake or release of glucose across the kidneys from humans, rodents, or dogs under physiologic conditions. In certain circumstances, net glucose production can be demonstrated, as illustrated later.

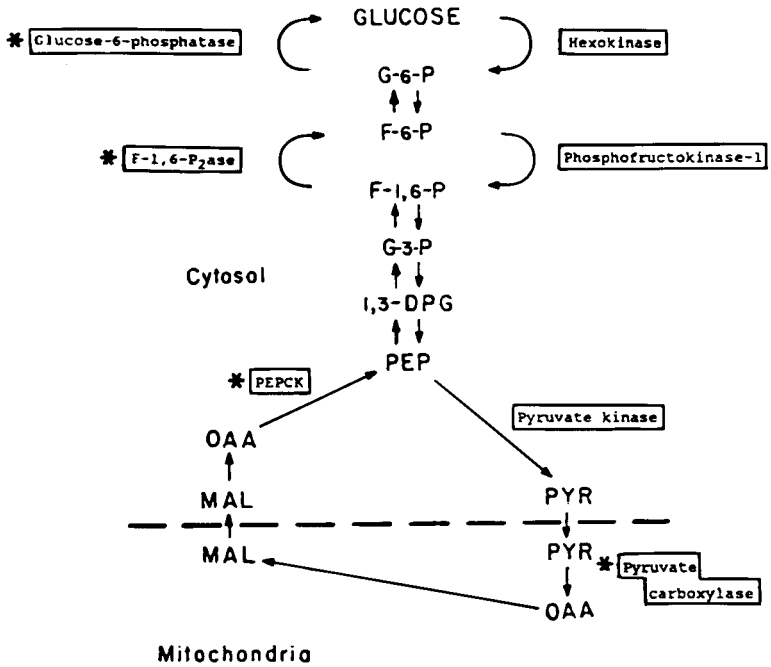

FIGURE 7-7. Metabolic pathways of gluconeogenesis and glycolysis in kidney. Asterisks denote enzymes unique to gluconeogenesis: *1,3-DPG*, 1,3-diphosphoglycerate; *F-1,6-P*, fructose-1, 6-bisphosphate; *F-1,6-P$_2$ase*, fructose-1,6-bis-phosphatase; *F-6-P*, fructose 6-phosphate; *G-3-P*, glycerol 3-phosphate; *G-6-P*, glucose 6-phosphate; *MAL*, malate; *OAA*, oxaloacetate; *PEP*, phosphoenolpyruvate; *PYR*, pyruvate. (From: Schoolwerth AC, Smith BC, Culpepper RM. Renal glucogenesis. *Miner Electrolyte Metab* 1988;14:347, with permission.)

The pathways and enzymes of glycolysis and gluconeogenesis share many enzymatic steps (Fig. 7-7). However, key regulatory steps are distinct in these two processes. For example, in the case of glycolysis, the unique and potential flux-generating steps are hexokinase, phosphofructokinase, and pyruvate kinase (7,83,91). The reverse steps unique to gluconeogenesis involve glucose-6-phosphatase, fructose bisphosphatase, and phosphoenolpyruvate carboxykinase (PEPCK). The coexistence of glucose-consuming and glucose-producing pathways in the same cells would suggest the presence of so-called *futile cycles*, of which numerous examples exist in other tissues. However, with regard to the kidney, evidence has accumulated indicating separation of the metabolic processes responsible for glucose utilization and production. These studies indicated that glucose utilization occurs predominantly in the medulla, whereas glucose production is confined to the cortex (7,90,91). More recent studies have allowed more quantitative measurements of enzyme activities and flux rates at the single-nephron level, which, in turn, has provided a more precise definition of the distribution of these processes. As mentioned later, there has been renewed interest in estimating the contribution of the kidney to body glucose homeostasis. A variety of techniques have been developed and used. These have provided different results and interpretations. As a result, this area is in a state of flux without clear resolution.

Glycolysis and Glucose Oxidation

By measurement of the activities of enzymes unique to glycolysis, it was demonstrated that the PCT had the lowest glycolytic capacity (92–95). Conversely, highest activities were observed in the TAL of Henle's loop and in the collecting tubule (95,96). The data on enzyme distribution are compatible with more recent observations measuring lactate formation from glucose in single microdissected tubule segments from rats (97). These data indicated that lactate formation was very low and even undetectable in the PCT and PST, whereas substantial rates of lactate production were observed in medullary and cortical TAL segments, DCT, and cortical collecting tubules (CCT). Thus, the combined data from measurements of enzyme activities and lactate production from glucose indicate that glycolysis is limited to the TAL and more distal nephron segments. Presumably, in deep medullary segments of the kidney, where the PO_2 is quite low, anaerobic metabolism is more likely to occur. Conversely, as is discussed later, aerobic metabolism occurs in cortical structures, such as PCT and PST. Aerobic oxidation of glucose and lactate requires the conversion of glucose to pyruvate in the cytosol, with subsequent entry of the pyruvate into the mitochondria and oxidation to CO_2 and water. The rate-determining factor in this process is PDH. This enzyme is highest in activity in the outer medulla and lowest in the papilla (90). PDH activity has not been quantitated in single-nephron segments. In general, its activity is thought to follow the distribution of mitochondrial density along the nephron, which suggests high activity in DCT, cortical TAL, and PCT (81–84). Subsequent oxidation to CO_2 and water involves citric acid cycle enzymes and the electron transport chain. The product of the PDH reaction, acetyl-CoA, combines with oxaloacetate to form citrate. Although PCT is rich in mitochondria and contains the enzymes of the citric acid cycle, evidence indicates that little glucose oxidation occurs in PCT, presumably because of low content of hexokinase, phosphofructokinase, and, probably, pyruvate kinase (83).

The energetics of anaerobic glycolysis are quite different from those of glucose oxidation. The conversion of glucose to pyruvate in cytosol yields two molecules of ATP for each molecule of glucose used for the reduction of two molecules of NAD to NADH. Further conversion of pyruvate to lactate regenerates NAD from NADH. In contrast, under aerobic conditions, pyruvate is transported into the mitochondria, and 18 molecules of ATP are produced by the oxidation of 1 pyruvate molecule to CO_2 and water. Because 2 pyruvate molecules result from the metabolism of 1 molecule of glucose, the complete oxidation of each glucose molecule yields 36 molecules of ATP (7).

Flux through the glycolytic pathway can be regulated by the activity of the rate-determining enzymes, substrate supply, and other factors, including ions such as H^+ (activation at alkaline pH), cytosolic energy state, and other intermediates reviewed previously (83,91,98).

Gluconeogenesis

In Vivo Gluconeogenesis

As mentioned previously, it is difficult to demonstrate either net utilization or production of glucose by the mammalian kidney under physiologic conditions. However, under certain circumstances, net production has been demonstrated. These conditions include prolonged fasting, metabolic acidosis, diabetes mellitus, and hydrocortisone administration (99,100). Under extreme circumstances, such as prolonged starvation, the kidneys may produce up to 50% of body glucose (79). Studies using a sensitive isotopic dilution technique have confirmed that in intact rats, the kidneys provide approximately one-fourth of the glucose synthesized in the fed state (100).

Substantial renal glucose production has been demonstrated in the postabsorptive state in dogs (101). A series of papers by Gerich and collaborators (102–105) have refocused interest on the role of the kidney in gluconeogenesis. Using combined isotopic and balance techniques, these investigators have suggested that renal glucose production accounts for 25% of systemic glucose formation in humans. Moreover, they have obtained evidence in humans for stimulation of renal glucose production by epinephrine. Their studies used a combination of renal balance and isotopic ([^{3}H]glucose and [^{14}C]glutamine) techniques. However, there results were not confirmed by Eckberg et al. (106), who calculated the renal contribution to total glucose production using [^{13}C] enrichments of glucose and concluded that the kidney contributes only 5% to blood glucose homeostasis in postabsorptive humans. With a prolonged fast of 60 hours, glucose production by the kidney may account for 20% to 25% of whole-body glucose turnover. Using renal arteriovenous glucose differences and [6-$^2H_2$]glucose, Cersosimo and colleagues (107) showed that renal glucose production was increased during insulin-induced hypoglycemia. When plasma glucose was prevented from changing, insulin was found to lead to a decrease in renal glucose production (108).

The differences in results obtained from various laboratories may relate in part to the different techniques used to estimate renal glucose production *in vivo*. The combined arteriovenous balance and labeled-glucose techniques depend on the accuracy in measuring small differences in arterial and venous glucose concentrations and labeled glucose-specific activities. Several authors have addressed the methods for measuring gluconeogenesis *in vivo* and concluded that the ^2H_2O technique is the most practical and reliable (109–113).

In Vitro Gluconeogenesis

Measurements of gluconeogenic enzyme activities and glucose formation in single nephron segments indicate that gluconeogenesis occurs exclusively in the PCT and PST (83,114). A variety of metabolites have been demonstrated to be efficient substrates for glucose production in PCT and PST. These include lactate, pyruvate, α-KG, and glutamine. For glucose synthesis, energy is consumed rather than released in glycolysis. Glucose formation from the aforementioned substrates requires the formation of oxaloacetate as an intermediate. Subsequent conversion of two molecules of oxaloacetate to one of glucose consumes two molecules of ATP, with the conversion of two molecules of NADH to NAD. Coordination of both mitochondrial and cytoplasmic enzyme steps is required to form glucose in these nephron segments from the substrates mentioned above (7).

Regulation of Renal Glucose Formation

Considerable interest has been generated in understanding the regulation of renal gluconeogenesis (Fig. 7-8). Parallels exist

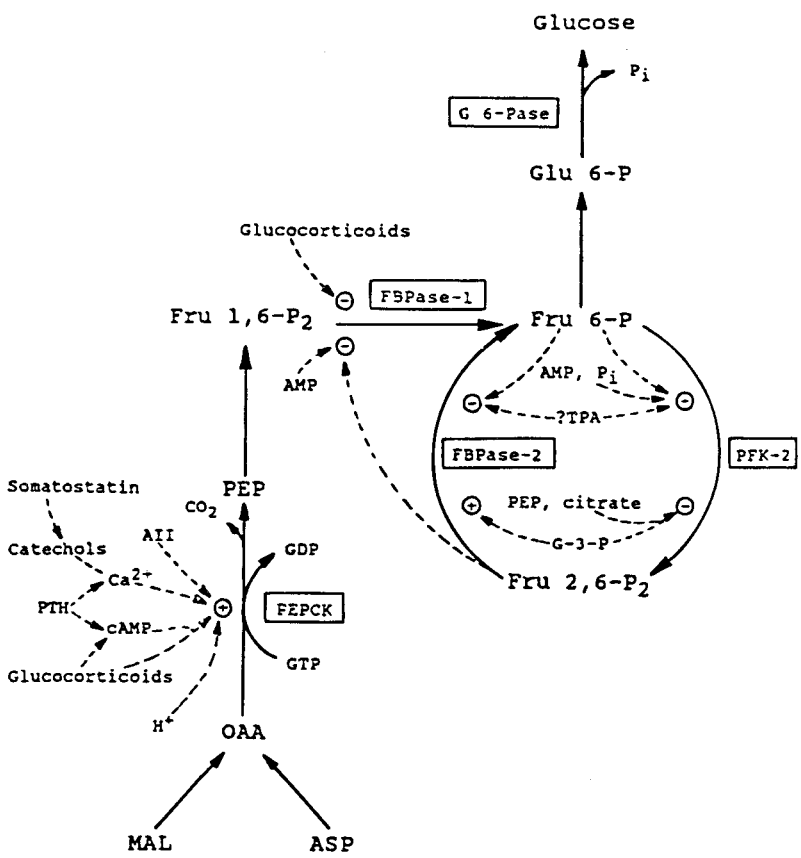

FIGURE 7-8. Proposed regulatory factors in renal gluconeogenesis. Stimulatory factors are indicated by (+), and inhibitory factors, by (–). *AII*, angiotensin II; *ASP*, aspartate; *catechols*, catecholamines; *FBPase-1*, fructose-1,6-bisphosphatase-1; *FBPase-2*, fructose-1,6- bisphosphatase-2; *Fru G-P*, fructose 6-phosphate; *G-3-P*, glycerol 3-phosphate; *G 6-Pase*, glucose 6-phosphatase; *Glu 6-P*, glucose 6-phosphate; *MAL*, malate; *OAA*, oxaloacetate; *PEP*, phosphoenolpyruvate; *TPA*, phorbol ester. (From: Schoolwerth AC, Smith BC, Culpepper RM. Renal glucogenesis. *Miner Electrolyte Metab* 1988;14:347, with permission.)

between regulation of hepatic and renal gluconeogenesis, although several important differences have been observed. For example, in kidney, but not in liver, metabolic and respiratory acidosis lead to an increase in gluconeogenesis (115–118). Similarly, the regulation of renal gluconeogenesis in acute and chronic metabolic acidosis may be different (119,120), as has been noted with respect to ammoniagenesis from glutamine and glutamate. In acute metabolic acidosis, an increase in glutamine flux through mitochondrial pathways occurs, including an increase in α-KGDH flux* (83,91,121). In contrast, in chronic acidosis, at least in rats, in addition to induction of phosphate-dependent glutaminase (PDG) and glutamate dehydrogenase (GDH) (122,123), an earlier induction of cytosolic PEPCK (115) occurs because of an increased amount of PEPCK messenger RNA (mRNA) (124).

Phosphoenolpyruvate carboxykinase plays a unique rate-determining role in glucose production. The enzyme is found in liver, kidney cortex, adipose tissue, and jejunum (125). The enzyme is limited to the cytoplasm in the rat kidney, whereas in other species, including dogs, it is also located in renal mitochondria (see Tannen [126]).

Cytoplasmic PEPCK is expressed in a highly organ-specific manner and responds in both liver and kidney to fasting, glucocorticoids, and cyclic adenosine monophosphate (cAMP) (127–129). Insulin suppresses hepatic, but not renal PEPCK (127). In contrast, metabolic acidosis induces renal, but not liver PEPCK (130,131).

The PEPCK gene is 6.0 kb in length and codes an mRNA of 2.8 kb (132,133). Hormonal responsiveness resides in the 5′-flanking region; eight protein-binding domains have been identified in the promoter region and the proteins binding to these areas have been identified; the major regulatory domains have also been characterized (see Patel et al. [134]).

Acidosis, glucocorticoids, and cAMP increase the renal PEPCK transcription rate (127–131). In kidney, PEPCK mRNA increases within the first half-day of onset of metabolic acidosis, followed by elevated enzyme synthesis and activity (127,128,135–138). Hwang and Curthoys (124,137) reported a sixfold increase in PEPCK mRNA in rat kidney cortex 8 hours after the onset of NH_4Cl acidosis (Fig. 7-9); nuclear run-on experiments indicated that this induction of PEPCK mRNA was due to an accelerated rate of transcription. Recent studies demonstrated that PEPCK mRNA increased 17-fold 10 hours after induction of NH_4Cl acidosis (138). *In situ* hybridization demonstrated that the pattern of PEPCK mRNA changed markedly (Fig. 7-10); PEPCK mRNA was limited to the medullary rays in controls, a finding consistent with localization of the enzyme in the S_3 segment of the PCT. After the onset of acidosis, PEPCK mRNA was seen throughout the cortex, so that by 10 hours it was present in many more tubular cells, a finding consistent with a stimulated transcription rate in metabolic acidosis. LLC-PK_1F^+ cells, gluconeogenic mutants of LLC-PK_1 cells (porcine-derived cells with characteristics of proximal tubule), respond by increasing NH_4^+ synthesis when incubated in acidified medium (139–141). The mechanism by which acidosis (or acid pH) stimulates PEPCK expression has not been delineated. Evidence for promotor elements that may mediate the increase in PEPCK expression to acid pH has been presented (142,143). Cissell and Chalkley have presented data that the proximal promoter region (−400/+1) and a unique site at +1,900 bp are required for the kidney-specific expression and pH responsiveness of PEPCK mRNA (144). Feifel et al. (145) have presented data that the signal that mediates the change in pH may be through a specific MAP kinase pathway.

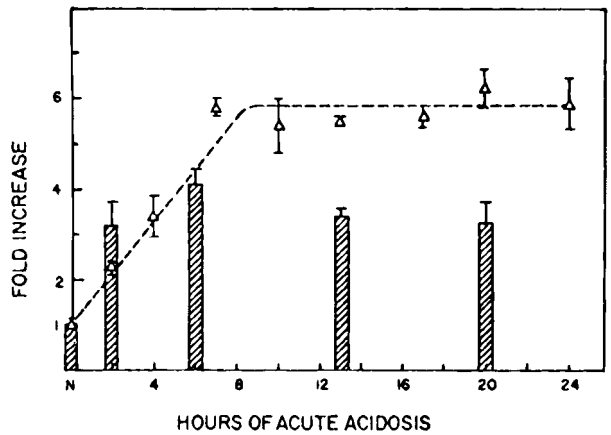

FIGURE 7-9. Comparison of the rates of phosphoenolpyruvate carboxykinase (*PCK*) gene transcription and the level of total PCK mRNA. The relative rates of transcription of the PCK gene (*hatched bars*) and the relative levels of PCK mRNA ($\Delta = \Delta$) were determined with rat renal nuclei and total RNA isolated from normal rats (*N*) and from rats at various times after onset of acute acidosis. All data were calculated as specific hybridization relative to that of β-actin. Each point is the mean $\pm$SD of the values obtained from at least three rats. (From: Hwang JJ, Curthoys NP. Effect of acute alterations in acid-base balance on rat renal glutaminase and phosphoenolpyruvate carboxykinase gene expression. *J Biol Chem* 1991;266:9392, with permission.)

Initially, the close correlation between the response of glucose and ammonium production in metabolic acidosis suggested dual regulation or even regulation of one process by the other; more recent evidence has indicated that this may not be the case. Although the two processes respond similarly to alteration in acid–base homeostasis, an increase in glucose production is not obligatory for the accelerated rate of ammonium production that occurs.

Other ions have been demonstrated to have an affect on renal gluconeogenesis. Most notably, changes in free Ca^{2+} concentration are stimulatory (146). In fact, the response to increased cytosolic free Ca^{2+} levels may underlie some of the hormonal effects that have been observed. For example, α-adrenergic agonists, angiotensin II, parathyroid hormone, dopamine, glucocorticosteroids, and somatostatin stimulate, whereas insulin inhibits renal gluconeogenesis (146–152). Parathyroid hormone may work through cAMP as a second messenger, whereas mobilization of cytosolic Ca^{2+} is probably involved in α-agonist and angiotensin II stimulation through activation of the phosphatidylinositide pathways (91,153). The mechanisms by which Ca^{2+} and cAMP affect gluconeogenesis remain to be fully elucidated. However, with regard to Ca^{2+}, it may involve activation of several intramitochondrial enzymes (i.e., PDH, α-KGDH, and isocitrate dehydrogenase) (154). High, as opposed to low, protein feeding also stimulates renal gluconeogenesis; this effect probably relates to the increased H^+ load obligated by high protein feeding (155). Allosteric regulation of enzymes, as might occur through fructose 2,6-bisphosphate, may prove significant with respect to the control of renal gluconeogenesis (156,157).

Relation to Ion Transport

Several laboratories have investigated the potential interrelationships of gluconeogenesis and ion transport. For example,

*The term *flux* is used to indicate "traffic" through a metabolic step, estimated in intact cells or organelles under circumstances that simulate physiologic conditions (e.g., substrate concentrations). In contrast, *enzyme activity* refers to measurements of the catalytic effect of extracted enzyme obtained under optimum conditions.

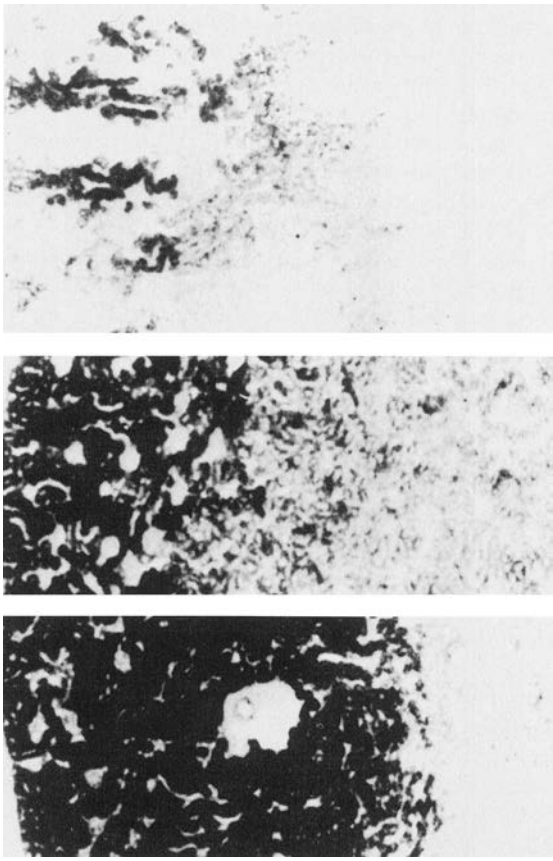

FIGURE 7-10. Effect of NH_4Cl acidosis on patterns of mRNAs in rat kidney. mRNAs for phosphoenolpyruvate carboxykinase (*PEPCK*) were determined by *in situ* hybridization in rat kidney at various times after induction of NH_4Cl acidosis. Panels depict patterns from 0 (*control*), 4, and 10 hours (*top to bottom*). The renal capsule is oriented to the left of each panel. Bar, 2 mm. PEPCK mRNA was confined to the kidney cortex with localization predominantly in the medullary rays. After induction of acidosis, the pattern of PEPCK mRNA was visible throughout much of the cortex by 4 and 10 hours; virtually the entire superficial cortex contained PEPCK mRNA. (From: Schoolwerth AC, deBoer PAJ, Moorman AFM, et al. Changes in mRNAs for enzymes of glutamine metabolism in kidney and liver during ammonium chloride acidosis. *Am J Physiol* 1994;269:F400, with permission.)

Silva et al. (158) have theorized that gluconeogenesis and active Na^+ transport, which are both energy-consuming processes of the PCT, might be related in an inverse fashion based on competition of both processes for a limited pool of cellular ATP. However, subsequent studies have failed to demonstrate that the two processes are dependent on each other because no fixed relationship has been found between gluconeogenesis and Na^+ transport (159). Other studies have evaluated the relationship of gluconeogenesis with phosphate transport, but, as with Na^+, the bulk of data indicate that there is no relationship between these two processes (160,161). It is intriguing that the PCT and PST both produce glucose and participate in transcellular glucose transport. No relationship between the metabolic and transport functions has been demonstrated.

RENAL AMMONIAGENESIS

The kidney plays a primary role in acid–base homeostasis (120,162) by maintaining the bicarbonate (HCO_3^-) content in extracellular fluid. This, in turn, involves the reclamation of filtered HCO_3^- and the synthesis of HCO_3^- molecules consumed in the buffering of H^+ ions. Synthesis of HCO_3^- is closely related to the metabolism of glutamine and synthesis of ammonium by the proximal tubule. This section emphasizes regulation of glutamine metabolism and the renal production of ammonium. The transport of ammonium is covered in other sections of this volume.

Both the excretion of ammonium and the metabolism in kidney cortex of the carbon skeleton of glutamine, in the form of α-KG, are necessary for HCO_3^- synthesis (162,163). The excretion of ammonium is critical to prevent its addition to renal venous blood and incorporation into urea by the liver, because ureagenesis, an HCO_3^--consuming process, would be counterproductive for the needs of HCO_3^- synthesis by the kidney (162–166). The metabolism of the glutamine carbon skeleton, either to glucose or CO_2 and water, is also critical, because two H^+ ions are consumed per mol of glutamine used (162,163). A distinctive feature of renal metabolism is that the kidney is capable of increasing ammonium synthesis in response to metabolic acidosis, as well as several other stimuli. This increase in ammonium production is recognized as an important renal adaptive process.

A controversy has arisen concerning the primacy of the kidney or the liver in acid–base homeostasis. It has been emphasized that urea synthesis involves utilization of HCO_3^-, whereas the end products of amino acid metabolism are NH_4^+ and HCO_3^- (164,167). Thus, it has been suggested that urea synthesis protects the body from the development of metabolic alkalosis (167,168). However, there remains an abundance of data indicating a substantial role for the kidney in the maintenance of plasma HCO_3^-. A view has emerged that the kidney plays the primary role in acid–base homeostasis, but the liver can contribute by virtue of the HCO_3^- consumption involved in urea generation (162,164). Thus, renal synthesis and excretion of ammonium, and the metabolism of α-KG are critical. As is mentioned later, it is this metabolism, rather than the simple combination of $NH_3 + H^+$ in the tubular lumen, that accounts for H^+ consumption and HCO_3^- generation in kidney. A comprehensive quantitative analysis of amino acid oxidation and gluconeogenesis has been performed by Jungas et al. (169). This analysis suggests that amino acid oxidation cannot be simply divided into a phase releasing HCO_3^- and NH_4^+ followed by a phase incorporating these compounds into neutral urea. The investigators emphasize that there is no intermediate stage in which there is a true threat of alkalosis. Nevertheless, studies indicate that the predominant effect of acidosis on hepatic amino acid metabolism is to inhibit amino acid transport into the liver, diverting amino acids to other organs, such as the kidney (170,171).

Effect of pH on Renal Ammonium Synthesis

In metabolic acidosis, the rate of ammonium synthesis in the proximal tubule may increase severalfold. The initial studies providing evidence for increased ammoniagenesis were performed *in vivo* with measurements of urinary ammonium excretion (172). Ammonium excretion is not equivalent to ammonium synthesis. Ammonium produced in the kidney can either be excreted into the urine or pass into the renal venous circulation. It is known that the distribution of ammonium between these two compartments is affected by several factors, the most important of which is the pH of the tubular fluid and urine (pH_u). A greater proportion of ammonium is excreted at acid than at alkaline pH_u. At any given pH_u, urine ammonium content was greater in chronic metabolic acidosis than under control conditions (172). More recently, measurements of total ammonium synthesis in kidneys of dogs, rats, and humans confirmed that enhanced ammonium production occurs

in metabolic acidosis. The time course of the kidney response to an acid load has been well documented in several species (120,162,173). During the first day after administration of an acid load, ammonium excretion rises progressively until a new steady state of acid–base balance is achieved, usually between the third and fifth day (173). At this point, ammoniagenesis remains at the elevated level as long as the acid load is continued. After cessation of the acid load, there is a gradual reduction in renal ammonium production and excretion until the control level has been reached.

Other forms of acidosis may also be associated with an increase in ammonium synthesis. In acute respiratory acidosis, renal ammoniagenesis is increased and reaches levels comparable with those seen in chronic metabolic acidosis (77). However, in chronic respiratory acidosis, data differ with respect to the response of ammonium synthesis (174,175). Some data on dogs suggest that ammonium excretion increases in the chronic stages of respiratory acidosis. Studies in rats suggest that there is no response to chronic hypercapnia, as opposed to an acute elevation in P_{CO_2}.

Other Conditions Affecting Ammoniagenesis

In addition to metabolic and respiratory acidosis, renal ammonium synthesis is increased in potassium depletion in both humans and animals, and the magnitude of the response may be greater than in metabolic acidosis (126,176). The stimulus for enhanced ammonium production under these conditions has not been elucidated. Some data are consistent with the observation that in potassium depletion, pH_i is decreased in kidney as it is in skeletal muscle (177). If this is the case, the intracellular acidosis may provide a stimulus for accelerated ammonium synthesis. However, other data are not compatible with this explanation, at least in the chronic state (178), and further work is necessary to determine the mechanisms involved in enhanced ammoniagenesis in potassium depletion. Exposure of perfused mouse proximal tubules to a low potassium concentration in the bath increased ammonium production, but a decreased luminal potassium concentration had no stimulatory effect on ammonium formation (179).

Whereas potassium depletion may stimulate ammonium synthesis, an increase in the prevailing potassium concentration in body fluids may have the opposite affect (180–182). Hyperkalemia may contribute to the development of metabolic acidosis by virtue of an inhibition of glutamine metabolism. The precise mechanism by which this occurs has not been elucidated, but the observation has been repeatedly made.

Glutamine as a Source for Renal Ammonium Synthesis

Glutamine, the major precursor for renal ammonium, can provide two molecules of ammonium for each molecule metabolized. In normal humans, arteriovenous glutamine differences are typically 10% of the arterial level, representing an extraction rate of approximately 50 μmol/min (7). Formation of ammonium from all the amide and amine nitrogens of glutamine should yield an ammonium production rate of 100 μmol/min. However, 70 μmol/min is produced, indicating that only a portion of the glutamate formed from glutamine is fully metabolized to ammonium. Under physiologic conditions, glutamine can account for ammonium synthesis in dog kidney (183), but no net glutamine extraction has been demonstrated in rats and the source of renal ammonium production has not been identified (184,185). Other amino acids, such as proline, glycine, and citrulline, are also taken up by the kidney and could contribute to the formation of ammonium (186). As metabolic acidosis develops, the extraction of glutamine by human, dog, and rat kidney increases without a significant increase in extraction of other amino acids. Plasma concentrations of glutamine do not change in any consistent pattern, indicating that glutamine delivery to the kidney is unchanged in acidosis. This also indicates that an increase in extrarenal glutamine production or a decrease in utilization occurs, diverting a greater amount of glutamine to the renal circulation (7). Because of the difficulties in measuring ammonium production very early in acidosis, the time after onset of acidosis at which enhanced ammonium synthesis begins is not known with certainty. It has been clearly demonstrated in humans that renal ammonium production is increased by 50% within 24 hours after an acid load (186). However, glutamine extraction does not change during this time. This in turn suggests that (a) other potential sources of ammonium exist, such as glycine or proline, both of which exhibit enhanced extraction in acute metabolic acidosis in humans; or (b) glutamine is more efficiently metabolized to yield two ammonium molecules from both the amine and amide nitrogens as acidosis is initiated. The latter conclusion is supported by *in vitro* evidence, reviewed later.

Despite the foregoing, it has been demonstrated clearly that glutamine is the only amino acid extracted in sufficient quantities to account for all of ammonium synthesized by the kidney in chronic metabolic acidosis.

Pathways of Glutamine Metabolism

Although there are several possible pathways by which glutamine may be metabolized to yield ammonium in kidney cortex, it is generally accepted that the mitochondrial PDG–GDH pathway is the predominant one (120,163,187) (Fig. 7-11). PDG and GDH, the enzymes that constitute this pathway, are located in the mitochondrial matrix in both DCT and PCT in rats (188,189). However, in chronic metabolic acidosis, the activities of both enzymes increase substantially only in the S_1 and S_2 segments of the PCT (188,189). These findings correlate well with measurements of ammonium production in nephron segments microdissected from acidotic rats and incubated *in vitro* with glutamine (190,191).

Regulatory Sites of Glutamine Metabolism

Data indicate that regulation of glutamine metabolism is different in acute, as opposed to chronic, acidosis and that numerous potential sites of control may all be operative. A tendency to extrapolate results from one species to another has confounded a full understanding of the regulation of renal ammoniagenesis. However, several control sites probably exist. The importance of these sites may vary in different species (192,193).

Transport of Amino Acids Across Mitochondrial Membrane

An abundance of data have indicated the complexity surrounding glutamine and glutamate metabolism in rat kidney cortex. These data have emphasized the close relationship between transport of metabolites across the inner mitochondrial membrane and flux through enzymes located in the matrix space (19,20,67,193).

Glutamate Transport

There are several transporters that regulate glutamate transport across the inner mitochondrial membrane in kidney. These transporters, which are also found in mitochondria from other tissues, include the glutamate hydroxyl transporter and the glutamate–aspartate antiporter (19,67). The electroneutral

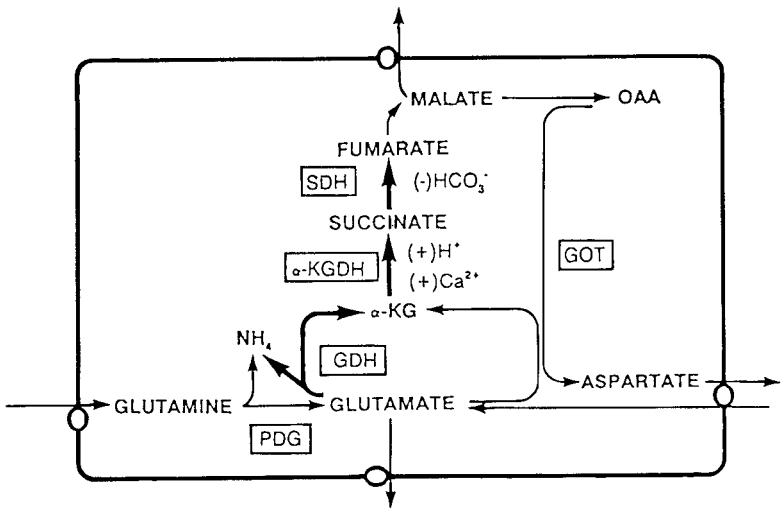

FIGURE 7-11. Intramitochondrial pathway for renal glutamine metabolism. Abbreviated terms enclosed in boxes refer to the enzymes glutaminase (*PDG*), glutamate dehydrogenase (*GDH*), glutamate oxaloacetate transaminase (*GOT* [aspartate aminotransferase]), α-ketoglutarate dehydrogenase (*α-KGDH*), succinate dehydrogenase (*SDH*), and oxaloacetate (*OAA*). Transporters are denoted by ovals on the membrane *(clockwise, beginning at left):* the glutamine transporter, dicarboxylate carrier, the glutamate-aspartate antiporter, and the glutamate-hydroxyl carrier. (+) denotes stimulation; (–), inhibition. *Dark arrows* refer to sites activated at acid medium pH. (From: Schoolwerth AC, Gesek FA. Intramitochondrial pH and ammonium production in rat and dog kidney cortex. *Miner Electrolyte Metab* 1990;16:264, with permission.)

glutamate hydroxyl carrier transports glutamate with H^+; glutamate entering mitochondria on this carrier is functionally linked to metabolism by GDH. This transporter has been shown to be relatively inactive in kidney compared with liver (194,195). Moreover, in contrast to liver, its activity measured in isolated mitochondria is not increased on medium acidification (194,195). In fact, the data indicate that an insignificant amount of glutamate enters kidney mitochondria on the glutamate hydroxyl carrier, resulting in substantial glutamate deamination by GDH (195–197). Only glutamate formed from glutamine in the matrix space can be deaminated to a significant degree under physiologic conditions (197). Although glutamate uptake on this carrier is very slow in kidney, the glutamate hydroxyl transporter may exert some effect on matrix glutamate content and, hence, GDH flux because efflux of glutamate from the matrix space on this carrier is reduced in acidic medium, as might occur in metabolic acidosis (198). The decrease in glutamate efflux under these conditions could contribute to accelerated GDH flux. The resultant increase in matrix glutamate concentration, coupled with a decrease in α-KG content, would increase the matrix ratio of glutamate/α-KG and drive the GDH reaction in the direction of increased glutamate deamination (73).

The other glutamate transporter is an electrogenic glutamate–aspartate countertransporter (199). Glutamate entering mitochondria in exchange for aspartate is transaminated by mitochondrial aspartate aminotransferase (AST) in a reaction that does not result in ammonium formation. However, because α-KG is one of the products of the AST reaction, this carrier can affect ammonium formation. Increased glutamate–aspartate carrier activity, with resultant increased AST flux, can reduce ammonium formation consequent to α-KG inhibition of GDH flux. The response of the glutamate–aspartate carrier to acute acidosis *in vivo* is not known, but it is likely that its activity is reduced and, together with a stimulation of α-KGDH flux, this transport system may contribute to the observed stimulation in GDH flux.

Glutamine Transport

Adam and Simpson (200) postulated a major role for the transport of glutamine across the inner mitochondrial membrane as a rate-controlling step in glutamine metabolism in acidosis. Their studies, and those of others, however, could not clearly distinguish activation of a transporter from that of the enzyme (201–205). Studies performed with rat kidney submitochondrial particles, which are largely free of contaminating enzyme, have suggested that an increase in glutamine uptake occurs when medium pH is decreased from 7.4 to 7.0 (206,207). These findings are compatible with activation of the glutamine transporter in acute acidosis. Data on submitochondrial particles suggest that a decrease in medium pH reduces the K_m of the transporter for glutamine (206). At present, the role of activation of glutamine transport in acute acidosis remains to be confirmed. The data also suggest that glutamine transport may be of particular importance in dogs.

Flux Through Enzymes in Acute Metabolic Acidosis

In response to an acute acid load, marked changes in kidney cortex metabolite levels have been observed (121,183). Of these changes, the most consistent has been a decrease in renal α-KG content of as much as 50% (183). Lowry and Ross (72) first demonstrated that H^+ ion activates renal cortex α-KGDH by decreasing the K_m of the enzyme for α-KG. These findings, subsequently confirmed (208,209), provide an explanation for the observed increase in α-KGDH flux and α-KG oxidation by intact kidney cortex mitochondria at acid pH (73,74). The stimulatory effect of H^+ ion on α-KGDH flux leads to a decrease in α-KG content in the matrix, with a resultant increase in ammonium formation by GDH (73). *In vitro* studies suggest that accelerated flux through GDH is the first event leading to enhanced ammonium synthesis at acid pH (210). Results from several laboratories indicate that acute acidosis in rats stimulates flux through α-KGDH and increases ammonium formation by the GDH reaction. These findings imply that in acute acidosis *in vivo* glutamate deamination precedes an increase in glutamine deamination by PDG.

Nissim et al. (211,212) have demonstrated in cultured LLC-PK_1 cells that the intensity of acidosis affects the pathways of ammonium synthesis from glutamine. Ammonium formation derived from [2-^{15}N]glutamine was increased at medium pH 7.0, compared with 7.4, indicating accelerated GDH flux. When pH was decreased to 6.8, glutamine utilization derived from [5-^{15}N]glutamine was stimulated further, indicating increased glutaminase flux.

The cumulative available data indicate that an early event in acute acidosis is activation of α-KGDH flux consequent to an increase in H^+ activity. Because α-KGDH is a mitochondrial enzyme, it should respond *in situ* to altered H^+ activity in the mitochondrial matrix space. To interpret the significance of these findings, further data were needed to estimate the change in pH in intact PCT in response to altered pH_c. There is

TABLE 7-3

EFFECT OF MEDIUM pH CHANGES ON CYTOSOLIC AND MITOCHONDRIAL pH AND THE pH GRADIENT ACROSS THE INNER MITOCHONDRIAL MEMBRANE

	pH_e[a]	pH_i[a]	pH_m[a]	ΔpH_m
Non-HCO_3^-(7)[b]	7.03 ± 0.01	6.89 ± 0.03	7.65 ± 0.03	0.78 ± 0.09
	7.41 ± 0.01	7.08 ± 0.02	7.96 ± 0.08	0.88 ± 0.09
	7.70 ± 0.01	7.30 ± 0.03	8.37 ± 0.03	1.15 ± 0.03
HCO_3^-(12)[b]	7.04 ± 0.01	6.91 ± 0.02	7.51 ± 0.08	0.62 ± 0.03
	7.44 ± 0.01	7.10 ± 0.03	7.78 ± 0.06	0.68 ± 0.08
	7.73 ± 0.01	7.28 ± 0.02	8.08 ± 8.08	0.79 ± 0.05

[a]Cytosolic pH (pH_i) and mitochondrial pH (pH_m) were determined at different medium pH (pH_e) values in intact rat renal cortical tubules. Values are means ± SEM.
[b]Number of experiments is shown in parentheses. Experiments were performed with nonbicarbonate (non-HCO_3^-)- or bicarbonate (HCO_3^-)-buffered media. The non-HCO_3^- buffer contained HEPES.
(From: Schoolwerth AC. Regulation of renal ammoniagenesis in metabolic acidosis. *Kidney Int* 1991;40:961, with permission.)

abundant evidence to suggest that the matrix space is alkaline with respect to the cytosol, but the response of mitochondrial matrix pH (pH_m) to a decrease in pH_i is not clear. Studies with isolated mitochondria have suggested that as medium pH (pH_e) is reduced, the ΔpH_m increases, reflecting buffering power of the matrix space with little change in pH_m upon medium acidification (20,67,213). However, more recent studies performed *in vitro* in suspensions of proximal tubules suggest that this may not be the case, at least in rat kidney (214). When a fluorescent probe, 2′,7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF) was used to estimate pH_i in combination with the organic anion 5,5-dimethyloxazolidine-2,4-dione (DMO), it was possible to obtain both parameters (i.e., pH_i and pH_m) in intact tubular segments as a function of altered pH_e. From the DMO distribution and pH_i determinations with BCECF, pH_m can be calculated if the volume occupied by the mitochondrial matrix in the cell is known; this has been estimated to be 20% in rats and 28% in dogs (211,212). With use of this technique, the data indicate a linear relationship between pH_e and pH_i, as well as between pH_i and pH_m (Table 7-3). At comparable pH_e, no difference in pH_i was noted with either an HCO_3^- or non-HCO_3^- buffer. However, pH_m was approximately 0.2 pH units lower at any pH_e value, when tubules were incubated in an HCO_3^--containing buffer (Krebs–Henseleit buffer) than in a non-HCO_3^- buffer (e.g., HEPES) (214,216,217). These observations indicate that in intact rat proximal nephron segments, a decrease in pH_m could potentially explain the acceleration in α-KGDH flux that occurs in response to a decrease in pH_e. However, the decreases in pH_m, although related linearly to pH_e and pH_i, are relatively small. It remains uncertain whether this change in pH_m is sufficient to explain fully the activation of α-KGDH or whether concentrations of other ions known to activate α-KGDH, such as Ca^{2+}, change in the matrix space in acute acidosis (200–205). Studies with isolated rat kidney mitochondria indicate that a reduction in medium pH results in an increase in mitochondrial-free Ca^{2+} content under conditions in which medium Ca^{2+} concentration (simulating intracellular free Ca^{2+} level) is less than 160 nM (208).

An additional site of potential regulation of ammoniagenesis is succinate dehydrogenase (SDH). The activity of this enzyme has been shown to be inhibited competitively by HCO_3^- *in vitro* (218,219). Whether this effect is present *in vivo* and contributes to the regulation of glutamine metabolism remains to be determined. However, this HCO_3^--sensitive enzyme could be the regulatory site responsible for the lack of increase in ammonium synthesis in chronic, as opposed to acute, respiratory acidosis in rats (175). In chronic respiratory acidosis, HCO_3^- concentration is elevated reflecting enhanced ammonium formation early in the course of hypercapnia. The rise in HCO_3^- concentration, by inhibiting SDH, could offset any stimulatory effect of increased H^+ level on glutamine metabolism.

Ammonium Production in Chronic Metabolic Acidosis

A correlation has been demonstrated between the increase in ammonium excretion and activities of PDG and GDH in rat kidney cortex in chronic metabolic acidosis (126). The increase in enzyme activities was found to occur exclusively in the S_1 and S_2 segments of PCT (188,220,221). The marked elevation in PDG activity was shown to be due to an increased synthesis of the enzyme (222,223). These findings suggested that stimulated enzyme synthesis may be critical to the enhanced rate of ammonium formation in metabolic acidosis in rats. However, accelerated ammonium production was still observed when an inhibitor of protein synthesis, actinomycin D, was administered (224); these studies were taken to indicate that the synthesis of PDG and GDH was not rate determining for the metabolism of glutamine. The role of enzyme synthesis remains to be determined, at least in the rat.

Phosphate-Dependent Glutaminase

Data have demonstrated that an increase in mRNA for PDG precedes the increase in assayable enzyme activity in rat PCT and in cultured renal cells; PDG mRNA levels rise within 1 day of initiation of acidosis (137,138,225). Rat mRNA for PDG increases sevenfold 10 hours after acute acid loading and is associated with increased expression in renal cortex, as demonstrated by *in situ* hybridization (138). Nuclear run-on assays indicate that transcription rates of the PDG gene are not affected by acid loading (225). These findings in rat kidney suggest that the increase in PDG mRNA levels results from increased mRNA stability in acidosis, consistent with results obtained in cultured renal LLC-PK_1-F^+ cells, suggesting that increases in PDG mRNA may be mediated by changes in both H^+ and HCO_3^- ion activity (144). Indeed, a pH-responsive stability element in the 3′-untranslated region of rat renal PDG mRNA and a specific mRNA-binding protein have been identified (226–229). The regulation of PDG expression is being studied using gluconeogenic LLC-PK_1-$FBPase^+$ cells (230).

Glutamate Dehydrogenase

Rat kidney GDH activity increases by the seventh day of metabolic acidosis (220,231). The increases are not demonstrable by the fourth day, at least in microdissected S_1 segments (232). After NH_4Cl loading, renal cortex GDH mRNA increase two- to threefold within 10 to 24 hours (138,233,234). *In situ* hybridization was consistent with increased expression and suggested recruitment of additional cells by the tenth hour of acidosis (138). Data has been presented which indicate that an increase in stability in the GDH mRNA is sufficient to explain the increased levels of mRNA within the kidney after NH_4CL loading (233). The pH response element found in PDG is also present in GDH and the same mRNA-binding protein has been found to bind to this mRNA as well (235).

One of the major differences between rat and dog kidney is that no increase in PDG or GDH activities has been found in dogs, although they respond to chronic metabolic acidosis with an accelerated rate of ammonium production and excretion (236). These findings suggest that the stimulation of ammoniagenesis in dog kidney does not occur because of an increased amount of PDG and GDH. Studies have been performed in intact animals and kidneys as well as in isolated organelles to deduce the site at which ammoniagenesis is accelerated in acidosis. Studies with isolated rat kidney cortex mitochondria and tubules have demonstrated augmented flux through both PDG and GDH, which accounts for the increased ammonium synthesis in chronic metabolic acidosis (196,231). The precise stimulus and mechanisms accounting for the augmented flux rates have not been determined. Evidence supports an increase in the V_{max} of the mitochondrial glutamine transporter in metabolic acidosis (200,204,205), suggesting recruitment of more transporters or unmasking of existing transport sites (67). However, these findings are not supported by kinetic analysis using submitochondrial particles from acidotic rats, which demonstrated a slight decrease in the K_m for glutamine, but no increase in V_{max} of glutamine transport (207). If the glutamine transporter is exposed to saturating concentrations of glutamine under physiologic conditions, an increase in V_{max} would be expected to be regulatory; if not, then a decrease in K_m would be more likely to affect flux rate significantly. Clarification is required in further studies. Because an increase in GDH flux accounts for up to 50% of the rise of ammonium formation (231), other factors besides the uptake of glutamine and flux through PDG are involved. With respect to dog kidney, the studies of Vinay et al. (237) have suggested activation of flux through both PDG and GDH in metabolic acidosis. However, *in vitro* studies with isolated mitochondria from kidney cortex of acidotic dogs have demonstrated accelerated flux through PDG, but not GDH, which accounts for the increased ammonium production (215,238).

Compensatory Role of Renal Ammonium Production

It is likely that in a variety of clinical conditions associated with increased H^+ activity in body fluids, the enhanced rate of renal ammoniagenesis plays an important physiologic role. For example, in diabetic ketoacidosis, the ketoacidosis of fasting, hyperchloremic metabolic acidosis secondary to diarrhea, and other circumstances, accelerated rates of glutamine metabolism and ammonium formation are important compensatory responses exerted by the kidney. The increased excretion of ammonium together with the metabolism of α-KG generates new HCO_3^- to replenish that consumed by the acid load (162). Although full restoration of this condition requires a reduction in H^+ ion entry into body fluids, morbidity is reduced markedly by virtue of this compensatory response. By the same token, a variety of clinical disturbances can be attributed to abnormalities in renal ammonium formation.

Ammoniagenesis in Chronic Renal Failure

It is well recognized that with the progressive reduction in the number of nephrons that occurs with progressive renal insufficiency, metabolic acidosis ensues. Studies in remnant kidney from rats and dogs and in humans suggest that an enhanced rate of ammonium production and excretion per nephron occurs as renal mass decreases (173,239,240). This increased ammonium formation per nephron retards the development of metabolic acidosis. However, as nephron mass becomes limiting, metabolic acidosis develops. Of interest are studies that suggest that the adaptive response of the kidney, namely increased ammoniagenesis per nephron, may have adverse consequences. Nath and colleagues (241) have demonstrated that NH_3 can activate the alternate complement pathway by amidation and that this activation may lead to tissue injury in the form of tubulointerstitial disease. It has been argued that the enhanced metabolism per nephron in chronic renal disease may have deleterious consequences (42,43). However, Throssell et al. (242) found no evidence that metabolic acidosis causes or exacerbates chronic renal injury in rats, arguing against a role for ammonia in renal tissue injury.

Ammoniagenesis and Renal Hypertrophy

Renal cell hypertrophy occurs in response to metabolic acidosis and has been postulated to be related to the increased ammoniagenesis consequent to acidosis. Data indicate that two mechanisms are involved (243). One involves a cell cycle-dependent mechanism. The other mechanism involves regulation of protein degradation by lysosomal enzymes. The latter is based on findings suggesting that the hypertrophy is mediated by weak base effects of ammonium, allowing it to enter and alkalinize acid vesicular compartments (244). Ling and associates (245, 246) postulate that renal hypertrophy is caused by a reduction of protein degradation, owing, in turn, to decreased activities of cathepsin B and L + B in the absence of enhanced protein synthesis, resulting from alkalinization of lysosomes in NH_4Cl-induced acidosis.

RENAL METABOLITE LEVELS IN RESPONSE TO ACID–BASE BALANCE CHANGES

Measurements of amino acids and citric acid cycle intermediates have indicated that the levels of these metabolites in renal cortex are considerably greater than their concentration in the plasma (7). The concentration gradient for metabolites between the cell and plasma is maintained by metabolite transporters in the cell membrane of cortical tubules. Profound and abrupt alterations in metabolite levels occur in response to alterations in acid–base state (7). These changes have been investigated to provide insight into possible regulatory steps that are affected by disturbances in acid–base balance. By evaluating the changes in metabolite level in a given pathophysiologic state, such as metabolic acidosis, compared with control states, the point at which a crossover occurs can be evaluated; the crossover suggests the localization of a regulatory site. In acute metabolic acidosis, for example, the levels of glutamine, glutamate, and α-KG decreased rapidly, whereas these levels are either unchanged or increased in metabolic alkalosis. In dog and rat kidney tissue, malate levels are unchanged or decreased only later in the course of metabolic acidosis, suggesting that a regulatory step between α-KG and malate, such as α-KGDH, may be a rate-determining step for metabolic activation (183). In fact, these changes led to the identification of the role of α-KGDH in the response to acute acidification. However, others have observed that malate levels decreased

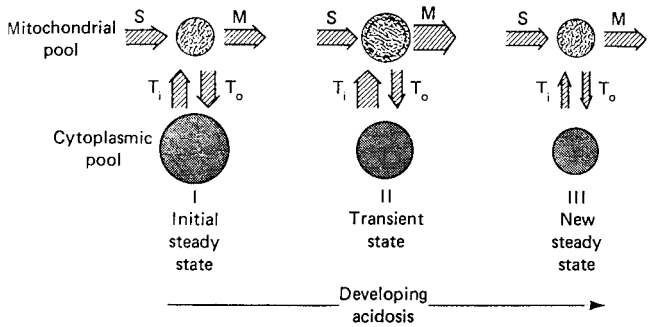

FIGURE 7-12. Proposed role of changes in mitochondrial anion transport in causing alterations in renal cortex substrate levels in response to an acute metabolic acidosis. The area of a circle depicts the size of the cytoplasmic or mitochondrial pool of a substrate. Changes in cytoplasmic pH and HCO_3^- concentration lead to changes in rates of substrate transport in or out of mitochondria as shown by the *arrows*. *M*, mitochondrial metabolism; *S*, mitochondrial synthesis; T_i, rate of transport into mitochondria; T_o, rate of transport out of mitochondria. (From: Simpson DP, Hager SR. pH and bicarbonate effects on mitochondrial anion accumulation: possible mechanism for changes in renal metabolite levels in acute acid-base disturbances. *J Clin Invest* 1979;63:704, with permission.)

promptly in metabolic acidosis, an observation that led to the formulation of a more general hypothesis with respect to the renal metabolic response to metabolic acidosis (74). This hypothesis, illustrated in Figure 7-12, suggests acceleration of metabolite transport into mitochondria in metabolic acidosis. This in turn may be due to an increase in the ΔpH_m across the inner mitochondrial membrane (a decrease in pH_i with no or less change in pH_m would lead to an increase in the ΔpH_m) (217). The increased transport of metabolites into mitochondria would lead to an accelerated rate of flux through the citric acid cycle and the oxidative phosphorylation pathway. The net effect would be a translocation of metabolites from the cytosol to the mitochondria and a reduction in total tissue metabolite levels. To the extent that the metabolite content of most substances is greater in the cytosol than in the mitochondria, this sequence could explain the observation that total tissue metabolite levels in renal cortex decrease in response to metabolic acidosis (67). Evidence in support of this hypothesis has come from studies with isolated renal cortex mitochondria demonstrating increased matrix accumulation of citric acid cycle intermediates (in the presence of metabolism inhibitors such as rotenone) (217). More data are required to validate this hypothesis as a generalized mechanism leading to altered renal metabolism in acid–base disturbances.

More data are required to confirm the response of pH_m and the ΔpH_m to perturbations in pH_e and pH_i in the intact cell; additional studies are needed to evaluate metabolite levels as a function of altered pH_e in different cellular compartments. Data concerning α-KG reported by Guder and Purschel (247) have suggested that α-KG decreases in both the cytosol and mitochondria at acid pH_e. It is likely that the changes in metabolite level may vary in different species, such as rats and dogs. Nissim and colleagues (248,249) used gas chromatography–magnetic resonance spectroscopy or [^{13}C]nuclear magnetic resonance in renal tubules to reevaluate potential metabolic sites of control in rat kidney. Their data indicate diminished flux through citrate synthase and increased flux through PDG, GDH, α-KGDH, SDH, malate dehydrogenase, and pyruvate carboxylase in chronic metabolic acidosis. The authors suggest that in chronic metabolic acidosis, decreased citrate synthase flux and increased α-KGDH flux result in accelerated flux through PDG and GDH (248,249).

FATTY ACIDS AS THE MAJOR RENAL FUEL

Fatty acids are highly efficient sources of energy. In kidney cortex (13) and outer medulla (250), fat is an important fuel for respiration, but in the inner medulla, carbohydrates play a major role in this process. Theoretically, the amount of O_2 consumed in renal fatty acid metabolism can vary from 20% to 90% (15,231,251,252). Although FFA are considered as the major renal fuel, not all FFA taken up by the kidney are completely oxidized, at least not immediately.

Almost 3% of the total mass of the kidney is composed of lipids, which are important components of membrane structure (phospholipids), valuable energy stores, and substrates for renal metabolic demands (neutral lipids), such as oxidative metabolism. Lipids can also be released from the kidney to be used in the synthesis of lipoproteins (253). In humans, approximately one-half of neutral lipids are represented by triglycerides (triacylglycerols), one of the more important vehicles for fatty acid transport (251–256). Under specific conditions, kidney tissue may supply triglycerides, phospholipids, cholesterol esters, and probably cholesterol or diglycerides to the blood pool (253). The predominant fatty acids in kidney triglycerides are oleate, palmitate, and linoleate (257). The remaining fraction of neutral lipids is represented by cholesterol, cholesterol esters, diglycerides, and monoglycerides. In addition, renal tissue has the ability to metabolize glycerol rapidly, but relatively little is known concerning the metabolic fates of circulating triglycerides (258).

Transport and Metabolism

In vivo, FFA are taken up by the kidney in linear correlation with the arterial concentration entering renal tubules from the basolateral side (258–261). They are either oxidized to CO_2 and water or released from the kidney in alternate forms, such as triglycerides or other products of renal metabolism. Net FFA uptake from blood was confirmed by the studies of intact human and dog kidney. Because of insolubility of long-chain fatty acids, they are carried in the blood primarily bound to albumin. Processes involved in the cellular uptake of albumin-bound ligands are not well understood. However, experiments on basolateral membrane vesicles isolated from rat renal cortex suggest that in kidney the translocation of fatty acids derived from albumin–fatty acid complexes occurs only in the presence of high external ion concentration (e.g., NaCl) (261).

The biosynthesis and degradation of fatty acids occur by separate pathways and in different cellular compartments; biosynthesis is localized in the cytoplasm whereas degradation, in the process of β-oxidation, takes place in mitochondria. The separation of these two processes permits them to occur simultaneously. The possible pathways for fatty acid metabolism (i.e., oxidation and incorporation into lipids) are illustrated in Figure 7-13.

In the catabolic pathway of β-oxidation, two-carbon configurations are sequentially removed, starting from the carboxyl-terminal end. In the cytoplasm, fatty acids first must be converted to their CoA derivatives. Subsequent steps take place in the mitochondria. However, the mitochondrial membrane is impermeable to fatty acids as well as to their CoA derivatives. To be transported through the mitochondrial membrane, cytosolic fatty acyl-CoA must be transesterified to the carnitine derivative (Fig. 7-14); the mitochondrial membrane requires an exchange of the CoA group for carnitine to form an acylcarnitine. The entire process, starting with plasma FFA, activation in the cytoplasm, transport into the mitochondrion,

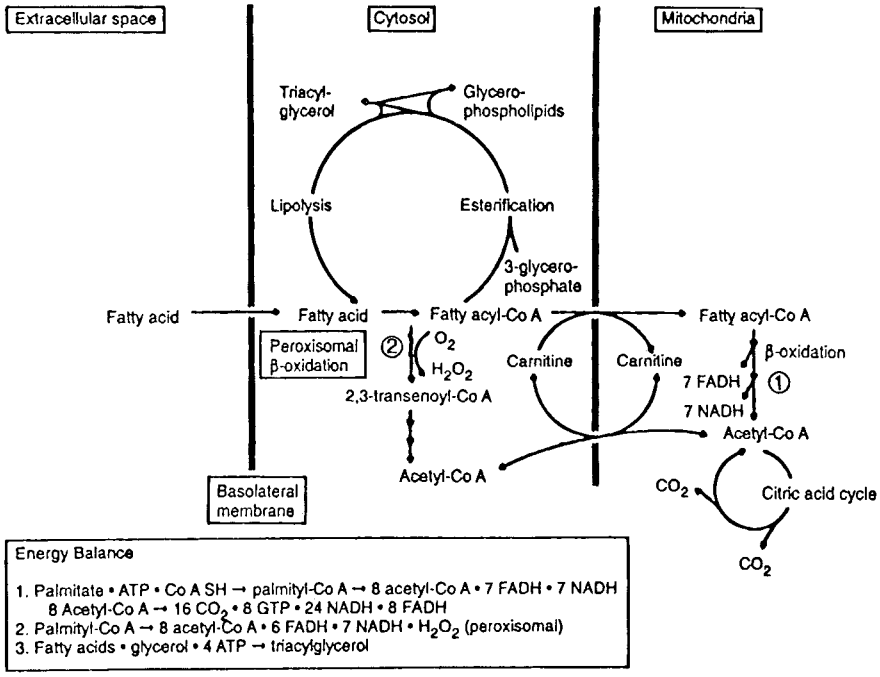

FIGURE 7-13. Pathways of cellular fatty acid metabolism: (*1*) hydroxyacyl-CoA dehydrogenase (mitochondrial), and (*2*) fatty acyl-CoA oxidase (peroxisomal). (From: Wirthensohn G, Guder WG. Renal substrate metabolism. *Physiol Rev* 1986;66:469, with permission.)

and β-oxidation inside the mitochondrion, is outlined in Figure 7-14. β-Oxidation of fatty acids yields acetoacetyl-CoA and two molecules of acetyl-CoA; acetyl-CoA is incorporated in the citric acid cycle. In the cytosol, fatty acyl-CoA either initiates peroxisomal β-oxidation (of long-chain fatty acids) or is transacylated to 3-glycerophosphate and then incorporated into lipids. The biosynthesis and degradation of fatty acids begins and ends with the same compound—acetyl-CoA.

The oxidative pathway of fatty acids is probably saturated at physiologic concentrations of fatty acids (37,253). If taken up by the kidney in excess of tubular energy needs, FFA can be esterified and then accumulated in the nephron as triacylglycerols, mainly in the proximal and outer medullary tubules (253,262). In diabetic ketoacidosis or prolonged starvation, when plasma FFA concentration is significantly elevated, increased rates of renal extraction and esterification to triacylglycerol can be detected (253,263). The elevated fatty acid uptake may negatively influence extraction of other key renal substrates such as glutamine (264). Glucose cannot be produced directly from fatty acids; however, increased uptake of FFA can change the direction of metabolism of substrates like lactate and glutamine from oxidative pathways to gluconeogenesis (7). In the diabetic state, gluconeogenesis is stimulated by changes of the acid–base status. Furthermore, under these conditions mitochondrial glucose oxidation is decreased by inhibition of PDH activity through a preferential oxidation of fatty acids and ketone bodies (262).

Although all parts of the kidney are able to synthesize triglycerides, cortex displays a higher rate of synthesis than medulla. In cortex, the glycerol backbone of triglyceride is contributed from gluconeogenic substrates, such as lactate or glutamine, but in medulla glucose provides the source for glycerol.

Distribution in the Nephron

Fatty acids are metabolized by all regions of the kidney (250). Oxidation of fatty acids to acetyl-CoA is mediated by the process of β-oxidation, which, in kidney, is localized in both the mitochondria and peroxisomes. The rate of β-oxidation decreases from the cortex toward the papillary structures (263). The profile of mitochondrial and peroxisomal β-oxidation was obtained by determinations of the activity of β-hydroxyacyl-CoA

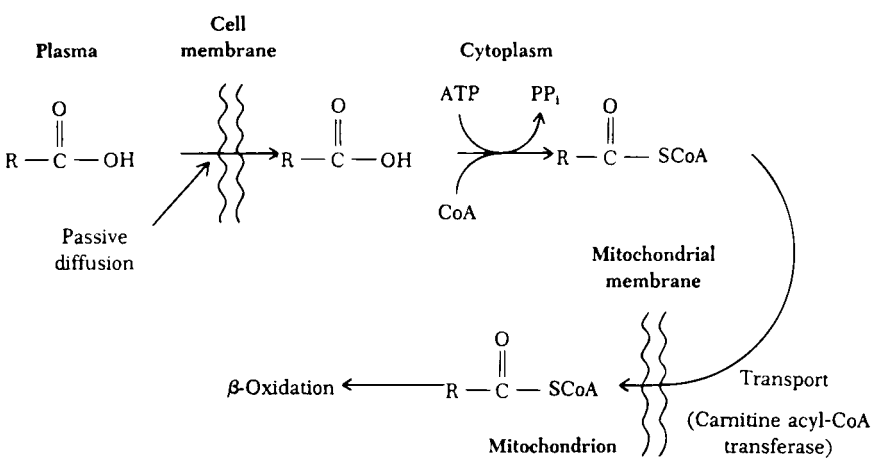

FIGURE 7-14. Passage of fatty acids through various membranes. (From: Orten JM, Neuhaus OW. Lipid metabolism. In: Lotz E, ed. *Human biochemistry.* St. Louis: CV Mosby, 1982, with permission.)

dehydrogenase and fatty acyl-CoA oxidase, respectively (265,266). The activity of β-hydroxyacyl-CoA dehydrogenase was found to be proportional to the density of mitochondria (114,267). The highest activity of this enzyme is in DCT; it is high in both the PCT and TAL and relatively low in glomerulus and CCT (268). On the other hand, peroxisomal fatty acyl-CoA oxidase activity is high in the cortical portion of the PST and parallels the distribution of peroxisomes (266,267). In addition, all nephron structures are able to participate in the esterifying pathway of triacylglycerol synthesis from exogenous fatty acids (269); lipid droplets, consisting largely of triglycerides, were found in renal cortex and medulla (253).

Fatty Acids Energize Tubular Na^+ Transport

Many years ago, endogenous lipids were suggested as an energy source for electrolyte transport in the kidney. Maximal renal Na^+ reabsorption requires both glucose and endogenous fatty acids (270); at least a portion of the energy necessary for Na^+ transport is supplemented by the oxidation of fatty acids (271). An inhibitor of mitochondrial β-oxidation of long-chain fatty acids, α-bromopalmitate, has a significant diminishing effect on renal Na^+ reabsorption (272–274). In addition, a decrease in Na^+ reabsorption and suppressed O_2 uptake was shown in the presence of 2-tetradecylglycidic acid, a specific inhibitor of acylcarnitine transferase-I (271). However, the metabolic inhibitors may have more than one site of action. Nevertheless, the rates of Na^+ transport and FFA utilization are positively correlated, confirming the supportive role of the fatty acid oxidation in the process of active Na^+ movement in renal tubules (258).

KETONE BODY TURNOVER IN THE KIDNEY

The kidney plays an important role in ketone body turnover (275,276). Although other tissues are capable of producing small amounts of ketones, the liver is regarded practically as the sole site of ketogenesis. Ketone bodies are formed from acetyl-CoA and acetoacetyl-CoA (products of fatty acid oxidation) by 3-hydroxy-3-methylglutaryl-CoA synthetase; this rate-determining enzyme is localized almost exclusively in liver. The ketones synthesized in the liver, acetoacetate, β-hydroxybutyrate, and acetone, enter the bloodstream and are transported to extrahepatic tissues. In kidney, only negligible *de novo* synthesis of ketone body from long-chain fatty acids occurs (13).

Reabsorption and Excretion: Ketosis

The reabsorption of β-hydroxybutyrate and acetoacetate by the kidney is essentially complete at normal arterial plasma ketoacid levels of 0.1 to 0.2 mM (277). Thus, ketoacids are virtually absent from human urine under physiologic conditions. However, under conditions in which ketone bodies are formed in increasing amounts, that is, in excess of the ability of the body to use them (ketosis), the kidney contributes significantly to overall ketone body turnover. Renal tissue has the ability to extract, oxidize, esterify, and excrete ketone bodies in proportion to the filtered load. The filtered load of ketoacids increases in parallel with the ketonemia as long as the GFR remains at near-normal levels (278). Renal ketone body uptake follows its arterial concentration (76,279–281). Net reabsorption of ketoacids by renal tubules could be described by the simultaneous operation of both saturable and nonsaturable transport pathways. Saturation of the uptake of β-hydroxybutyrate and acetoacetate in isolated rat kidney cortex tubules was observed at concentrations higher than 5 mM (8,282). During the ketoacidosis of starvation (276) or uncontrolled diabetes mellitus (283), the arterial concentration of β-hydroxybutyrate and acetoacetate increases 50- to 100-fold. The urinary excretion rate of ketones is accelerated in response to the elevated filtered load of ketoacids, as supported by studies in rats. The excess of ketone bodies above the renal threshold is excreted in the urine. The increased excretion of ketone bodies may reflect the saturation of both a reabsorptive component that has relatively low capacity and also the altered function of nonsaturable component of the ketone transport system. Modifications of those transport pathways affect the rate of reabsorption of the tubular loads of ketoacids (278,284,285).

In diabetes, ketone bodies have a nitrogen-sparing effect; renal ammonium formation from glutamine is stimulated in metabolic acidosis. This increase may be partially blunted by a suppressant effect of ketone bodies (262).

Metabolic Pathways

The renal metabolism of ketone bodies is shown in Figure 7-15. All the major reactions involved in the metabolism of ketones are found in the mitochondrial matrix (276). β-Hydroxybutyrate is oxidized to acetoacetate in the presence of β-hydroxybutyrate dehydrogenase. Acetoacetate is then reactivated to acetoacetyl-CoA, which is split into acetyl-CoA by a specific thiolase (286). Acetyl-CoA enters the citric acid cycle to be oxidized to CO_2. Therefore, ketone body metabolism in the kidney, as in the liver, is directly coupled to the citric acid cycle by the CoA transferase reaction (8,83).

In addition, acetyl units derived from ketone bodies may be used for the acetylation reactions in the kidney. Transacetylation of free carnitine formed in the kidney as well as carnitine reabsorbed from the glomerular filtrate to acetylcarnitine may occur in the proximal tubule (287). Under ketotic conditions a

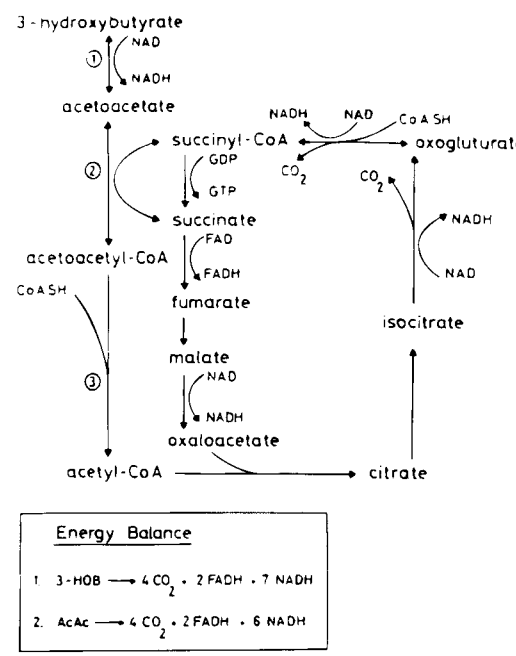

FIGURE 7-15. Oxidative metabolism of ketone bodies: (*1*) 3-hydroxybutyrate dehydrogenase, (*2*) 3-oxoacid-CoA transferase, and (*3*) acetoacetyl-CoA thiolase. (From: Wirthensohn G, Guder WG. Renal substrate metabolism. *Physiol Rev* 1986;66:469, with permission.)

higher ratio of acetylcarnitine–carnitine was found in urine, indicating that besides pyruvate and acetate, ketone bodies serve as a major source of acetyl-CoA for the acetylation reaction. Similar to other tissues, the kidney has the ability to use ketone bodies as an energy source. In studies with kidney slices, acetoacetate was found as a source of up to 80% of renal energy requirements (13).

Data obtained on renal brush-border membrane vesicles provide evidence for direct coupling between the β-hydroxybutyrate level and the rate of Na^+ influx (278); this cotransport system appears to be electrogenic in rabbits (288), but electroneutral in rats (289).

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CHAPTER 8 ■ RENAL TRANSPORT OF ORGANIC IONS AND URIC ACID

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All animal species produce endogenous organic compounds and are exposed to environmental xenobiotics of natural or man-made origin, including drugs. Many of these compounds are weak acids or bases, ionized at physiological pH or becoming ionized after biotransformation. The metabolism of drugs and xenobiotics into more hydrophylic metabolites is essential for the elimination of these compounds from the body. Drug biotransformation reactions involve oxidation reactions classified as phase I reactions (*N*-dealkylation, *O*-dealkylation, hydroxylation, deamination) and/or phase II conjugation reactions with endogenously derived glucuronic acid, sulfate, glutathione, acetate, and amino acids. The kidney is endowed with transport mechanisms allowing rapid excretion of these ionized compounds, a property generally useful for toxic products, but sometimes undesirable for drugs. Some compounds are almost completely removed during a single pass through the kidney such as *p*-aminohippurate (PAH). In general, metabolites are more polar than the parent molecules, and excreted more efficiently, but there are exceptions to this rule (1). For compounds that are extensively bound to plasma proteins, their excretion by the kidney is due more to tubular secretion than to filtration.

Xenobiotics are secreted by two renal transport systems. One is responsible for the secretion of organic anions, for which PAH is the typical substrate. The other one is responsible for the secretion of organic cations, for which tetraethylammonium (TEA) or N_1-methylnicotinamide (NMN) are the typical substrates. Each system includes several subsystems with affinity for different substrates (2,3). Substrates for both transport systems share general properties of molecular size, hydrophobicity, and degree of ionization, but the molecular structure required is rather unspecific (4). Recently, the application of molecular biological techniques allowed the identification of a number of transporters with affinities for substrates known to be secreted and reabsorbed by the organic anion or organic cation transport systems (5). Many aspects related to the contribution of these cloned transporters in the renal secretion or reabsorption of organic anions or cations have not yet been fully determined.

RENAL EXCRETION OF ORGANIC ANIONS AND CATIONS

In this chapter, we will first summarize the general characteristics common to the renal excretion of organic anions and cations. The specificity of substrates and membrane mechanisms for organic anion and cation transport will then be described. Finally, the role of organic ion transports in renal drug interactions and their use in renal function evaluation are discussed briefly. Because the kidney plays an essential role in the regulation of uricemia, the renal excretion of urate is discussed later in this chapter under "Uric Acid." With few exceptions, our description is limited to renal excretion and membrane mechanisms in humans and other mammals, although comparative studies in lower animal species contributed much to our present understanding of transport mechanisms. For these topics, the reader is referred to specific reviews (6–8).

Our knowledge of the renal transport of organic ions mainly comes from animal studies and investigations in humans being limited to clearance measurements. Our understanding of the renal excretion of organic ions is further complicated by important species differences, making extrapolation from laboratory animals to humans often difficult.

Secretion

Our understanding of the mechanisms of secretion of organic ions derives essentially from investigations of a few transported compounds, considered as prototypes for other secreted organic ions. For organic anions the "classic" substrate is PAH, and for organic cations typical compounds are TEA and NMN. These latter molecules are efficiently secreted and reabsorbed in negligible amounts. They are extracted up to 90% or more in a single pass through the kidney. Furthermore, they are not synthesized by the kidney and are generally not, or only marginally, metabolized. Some exceptions exist. Guinea pigs, pigs, or rabbits metabolize PAH and NMN to such an extent that the fractional excretion of PAH or NMN is below unity. *Necturus* and garter snakes do not secrete but extensively reabsorb PAH and NMN, respectively (9). In mammals, secreted organic ions are extracted from the arterial blood less efficiently than PAH, TEA, or NMN, because of their lower affinity for the transporters, and/or because of the occurrence of back-diffusion from lumen into interstitium. It is generally agreed that plasma protein binding is not rate limiting for tubular secretion of organic ions with a high affinity for the transporter (e.g., hydrochlorothiazide, flumethiazide), whereas tubular secretion is reduced for low-affinity compounds (e.g., phenol red).

Secretion of organic ions is a saturable process and can be competitively inhibited. Stop-flow techniques, as well as single tubule micropuncture and microperfusion experiments, have shown that the proximal tubule is the main site for the secretion and reabsorption of organic anion and cation. Facilitated transport of organic compounds in other tubular segments has been reported (10,11), but is of minor importance. Secretion is not uniform along the proximal tubule and may differ between superficial and juxtamedullary nephrons. This heterogeneity of secretion, varying among species and substrates, may correspond to differences in the density of the transporter molecules along the proximal tubule. As an example of such differences, the S_2 segment in rabbits secretes organic anions (PAH and urate) at a higher rate than S_1 and S_3 segments, while in pigs, the S_1 segment appears to secrete more than the S_2 and S_3 segments (6). Since secretion involves basolateral and apical

transport in series, the intracellular concentration of the substrate depends not only on basolateral transport, but also on the rate of efflux at the apical membrane. A low efflux rate of the secreted substrate may lead to its intracellular accumulation and cell toxicity, as observed for cephaloridine and vancomycin in rabbit proximal tubules (12,13). The cellular concentration of organic ions during *in vivo* and *in vitro* secretion or reabsorption can be monitored by fluorescence microscopy (14,15).

Reabsorption

Tubular reabsorption of organic acids or bases usually proceeds through passive back-diffusion of their undissociated forms. Thus, reabsorption depends on the liposolubility of the compound, its pK_a, the pH of the urine, and the contact time with the epithelium (i.e., the tubular flow rate). Alkalinization of urine efficiently decreases reabsorption and enhances renal excretion of organic acids, such as salicylic acid and probenecid, whereas urine acidification decreases reabsorption and enhances excretion of organic bases (triamterene and procaine). Reabsorption can completely offset net secretion and reduce urinary excretion nearly to nil. Other compounds are reabsorbed by a facilitated process, showing competitive inhibition and saturation, and thus may undergo bidirectional transport by facilitated mechanism.

Substrate Recognition

Most studies conducted to determine whether a xenobiotic or a natural compound is substrate for a specific renal transporter are based essentially on the ability of the substrate to compete for transport of prototypical substrates (e.g., PAH for organic anions, and TEA or NMN for organic cations), and not necessarily on its own ability to be transported. Substrate uptake has been investigated in renal cortical slices, in suspension of proximal cells, in cells of perfused or nonperfused proximal tubules, in rat proximal tubule *in situ*, or in membrane vesicles purified from renal cortex (3,16,17). These competition studies demonstrate the affinity of the substrate for the carrier system, but do not establish the transport of the substrate per se. Indeed, inhibitors with high affinity are usually not transported; an inverse relationship has been repeatedly observed between the potency of an inhibitor and its rate of transport (18–22). Renal cell monolayers grown on permeable supports provide a good tool to investigate transepithelial transport. Two established cell lines with proximal tubule transport characteristics—LLC-PK1 and OK cells from pig and opossum kidney, respectively—have been used for organic ion transport studies. The organic cation transport system is functional in both cell lines (23), whereas PAH transport is present in OK cells (24), but not in LLC-PK1. Cell lines originating from human proximal tubule have been described, but their ability to transport organic ions in the secretory direction has not been investigated (25,26). Today a number of transporters have been cloned. Their transport characteristics have been defined when expressed in the *Xenopus* oocyte expression system, or other cell systems, such as HeLa, COS-7 cells, human embryonic kidney cells (HEK293), or MDCK cells (5).

ORGANIC ANIONS

The proximal tubule transports a variety of endogenous and exogenous organic anions. Endogenous organic anions mostly reabsorbed include aliphatic mono- (e.g., lactate, pyruvate), di-, or tricarboxylates (oxalate, citrate, succinate, α-ketoglutarate), vitamins (folate, ascorbate), waste products (urate), prostaglandins, and second messengers (cAMP). The majority of the exogenous organic anions including *p*-aminohippurate (PAH), β-lactam antibiotics, antiviral drugs (AZT), nonsteroidal anti-inflammatory drugs, diuretics, and angiotensin-converting enzyme (ACE) inhibitors, are secreted into the proximal tubular lumen. This secretory transport system is highly efficient and exhibits a broad substrate specificity. Net secretion of organic anions requires the uptake across the basolateral membrane, and the release of the organic anion across the luminal membrane into the tubule lumen. The PAH concentration in the tubule lumen exceeds that in the blood, indicating that this uphill secretory transport requires energy (27).

Membrane Mechanisms for PAH Secretion

The characteristics of PAH transport mechanisms and a detailed discussion of pioneer work have been presented by Pritchard and Miller (3). We will thus limit our review to the main features of PAH transport mechanisms.

Influx across the basolateral membrane of proximal tubules constitutes the first step of secretion. The uptake of negatively charged organic compounds occurs against an inside negative electrical potential difference and, in some instances, against a concentration gradient. This uphill active transport requires energy. In contrast, efflux from cell to lumen across the luminal membrane takes place along the electrochemical gradient and does not necessitate a direct energy expenditure (7). The concentrative uptake of substrates in the proximal cells by the PAH transporter contributes, at least in part, to the nephrotoxicity of cephalosporin antibiotics (13), antiviral nucleotide analogs (cidovir, adefovir) (28), ochratoxin A (29), and some glutathione and cysteine conjugates (30). The nephrotoxicity of these compounds can be reduced by the administration of probenecid, which inhibits their cellular uptake.

Basolateral Transport Mechanisms

The energy dependent uphill transport of PAH across the basolateral membrane of the proximal tubule requires the cooperation of three transporters, the Na/K-ATPase, the sodium-dicarboxylate cotransporter (NaDC3), and the organic anion transporter 1 (OAT1) (see Fig. 8.1) (31,32). The Na^+ pump maintains an inwardly directed Na^+ gradient using ATP hydrolysis. The NaDC3 symporter utilizes the energy provided by inwardly directed Na^+ gradient, by coupling the transport of 3 Na^+ ions with one molecule of α-ketoglutarate in order to accumulate α-ketoglutarate into the cell above its electrochemical equilibrium. The intracellular accumulated α-ketoglutarate then exchanges with extracellular PAH via the OAT1 transporter.

The NaDC-3 transports endogenous di- and tricarboxylates, such as oxaloacetate, fumarate, succinate, and α-ketoglutarate, which are intermediates of the Krebs cycle, and constitute substrates for the energy metabolism of the cell (oxalate is a substrate of the NaDC-3 transporter, but is not involved in the cell energy metabolism) (7). The NaDC-3 transporter has a relatively narrow substrate specificity and does not transport xenobiotics, but, by allowing the intracellular accumulation of α-ketoglutarate, it contributes in driving a variety of exogenous organic anions into the cell via the OAT1 transporter. The intracellular accumulation of α-ketoglutarate can reach more than 600 μM in rat cortical kidney slices for an extracellular concentration of 10 μM. In dogs, the intracellular concentration of α-ketoglutarate is about five to ten times higher than in plasma and thus should generally not be rate limiting for basolateral anion exchange (33). This uphill transport of α-ketoglutarate requires the symport of one molecule of

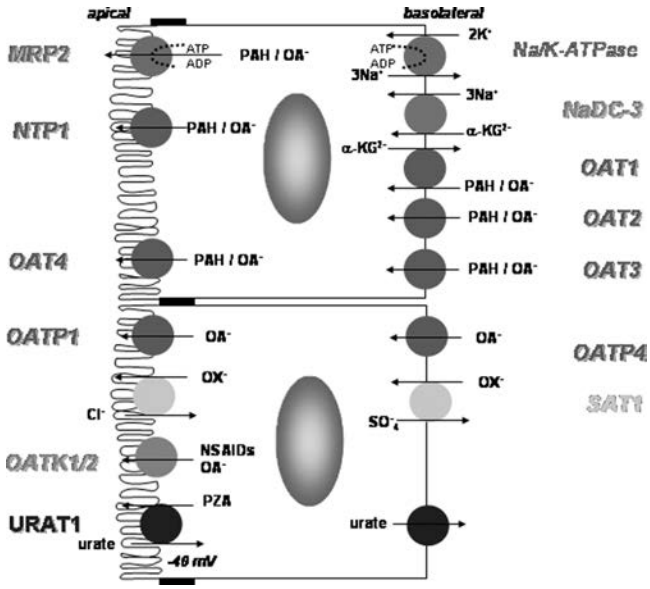

FIGURE 8-1. Organic anion transporters in the proximal tubule. *Upper cell*: transporters involved in the secretion of organic anions (OA^-) and PAH. The first step of PAH secretion is the uptake of PAH across the basolateral membrane in exchange with α-ketoglurate via OAT1 transporter. α-Ketoglurate is recycled back into the cell via the cotransporter NaDC-3. Additional basolateral transporters OAT2 and OAT3 allow entry of PAH into the cells, but do not function as anion exchangers. The exit of PAH into the tubule lumen involves the uniporters OAT4 and MRP2, as well as the P-glycoprotein MRP2 (multidrug resistance). *Lower cell*: organic anion transporters that do not transport PAH, but are involved in the secretion of other organic anions (OA^-), such as nonsteroidal anti-inflammatory drugs (NSAIDs) These transporters include basolateral OATP4 and the apical OATP1, OATK1, and OATK2. Transport of oxalate (OX^-) involves the basolateral SAT1 and, at the apical membrane, an exchanger with Cl^- that has not yet been identified at the molecular level. Urate transport in human mainly involves reabsorption; the apical URAT1 anion exchanger with affinity to pyrazinoate (*PZA*) allows urate uptake into the cell and a basolateral electrogenic-facilitated diffusion that still remains to be identified is responsible for urate exit from the cell.

α-ketoglutarate together with 3 Na ions in order to overcome the large electrochemical gradient across the basolateral membrane. The NaDC-3 protein is expressed essentially in the kidney and immunolabeling showed the presence of NaDC-3 protein in the S3 segment of the proximal tubule (34). The NaDC-3 cotransporter shares with the OAT transporters a common secondary structure prediction that includes 12 transmembrane segments, although they do not belong to the same gene family (35,36).

Molecular Identification of the Basolateral p-Aminohippurate Transporter

OAT1 was first cloned from the rat kidney and identified as an organic anion transporter (37–39). Several orthologs of the rat OAT1 have been isolated from human, rabbit, pig, flounder, and *Caenorhabitis elegans* (8). The mammalian orthologs show ~80% identity at the amino acid level, whereas a 20% and 50% identity with the human OAT was found between the flounder and *C. elegans,* respectively. In the human, four splice variants have been identified with comparable PAH transport capacities (40).

Immunohistochemistry experiments in the rat kidney revealed that the rat OAT is restricted to the basolateral membrane of S2 segments of proximal tubules, although weaker expression has been reported in the S1 and S3 segments (41–43). These findings are consistent with previous physiologic studies showing the highest transport rate of PAH in the S2 segment. When expressed in cell systems (*Xenopus* oocytes, HeLa cells, COS cells), OAT1 functions as a PAH–α-ketoglutarate exchanger, and demonstrates (like the PAH basolateral transport mechanism), a broad specificity for substrate binding, including nonsteroidal anti-inflammatory drugs (NSAIDs). The OAT1 from rat or human expressed in heterologous cell systems shows a very broad substrate affinity (7) that includes prostaglandin-E_2 (PGE_2), cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), urate, α-ketoglutarate, salicylate, acetylsalicylate, indomethacin, and estradiol 17β-D-glucuronide, and β-lactam antibiotics, indicating that OAT1 is a polyspecific transporter with affinity for multiple chemically unrelated compounds.

Substrate Requirements for the Basolateral "p-Aminohippurate Transport Mechanism"

The characteristics required for a substrate to be secreted by the PAH transport system have been intensively investigated. In particular, Ullrich studied all categories of compounds susceptible to undergo secretion in rat proximal tubule *in situ* by measuring their capacity to inhibit the basolateral influx of PAH (17). In general, affinity of a substrate for the PAH transporter necessitates one hydrophobic moiety between 0.4 and 1.0 Å in length and a full or partial electronegative charge. However, some nonionized compounds can also interact if they are hydrophobic enough and form multiple H^+ bonds with the carrier (e.g., steroid hormones, anilin-derived analgesics). Substrates with high affinity have two negative charges 0.6 to 0.7 nm apart (e.g., benzene-1,4-disulfonate). Altogether, substrate binding to the PAH transporter necessitates hydrophobic interactions and the formation of multiple H^+ bonds. The question remains as to whether the cloned OAT1 is functionally identical to the PAH transporter in intact proximal tubule. Despite the lack of a precise comparison of the kinetics for both the *in vivo* and *in vitro* PAH transport systems, there are some clear correspondence between both systems regarding their interaction with aliphatic dicarboxylate and their comparable low affinity for xenobiotics including β-lactams, NSAIDs, urate, and cyclosporin. Thus, it is likely that PAH transport across the basolateral membrane of the proximal tubule occurs via the OAT1 transporter.

OAT2 and OAT3

The OAT2 was cloned from a rat cDNA library. Rat and human OAT2 transcripts are more abundant in the liver than in the kidney. In this latter organ, OAT2 expression at the protein level differs between rats and human. There are sex differences in the tissue expression profile of OAT2, where female rat and mouse have higher levels of OAT2 expression in the kidney compared to males (44,45). In rats, the OAT2 transporter was found in the thick ascending limb of Henle's loop and at the luminal membrane of the cortical collecting duct (CCT). In humans, OAT2 is found to be restricted to the basolateral membrane of the proximal tubule and, therefore, may contribute to the secretion of endogenous or exogenous organic anions (46) (Fig. 8.1).

When expressed in *Xenopus* oocytes, the OAT2 transports a variety of substrates including acetylsalicylate, PAH, and methotrexate, as well as α-ketoglutarate, PGE_2, and PGF_2. Inhibition studies showed that NSAIDS (diclofenac, ibuprofen), antibiotics (penicillin, rifampicin, and tetracycline), and diuretics (bumetanide) interact with OAT2. In contrast to OAT1, OAT2 does not seem to exchange extracellular organic anions with intracellular α-ketoglutarate to drive substrates for

OAT2 across the basolateral membrane. The energetic basis for the OAT2-mediated transport has not yet been determined and, therefore, its contribution in the energy-dependent uphill secretion of organic anions in the proximal tubule remains unclear.

The OAT3 isoform was first cloned from the rat kidney (47). Northern blot analysis revealed that OAT3 is most heavily expressed in the liver and a weaker signal is detected in the kidney and brain (45,47). Interestingly, in the human kidney, the mRNA level of OAT3 is nearly three times higher than that of OAT1. In the human kidney, immunolabeling reveals the presence of OAT3 at the basolateral membrane of the three segments of the proximal tubule and also in the thick ascending limb, and the aldosterone-sensitive distal nephron.

When studied in heterologous cell expression systems, OAT3 transports PAH, but with a lower affinity than OAT1 (7,8). The rOAT3 also transports penicillin, estrone sulfate, ochratoxin A, pravastatin, and the hOAT3 AZT, methotrexate, PGE2, salicylate, valacyclovir. Unexpectedly, human and rat OAT3 transport the weak base cimetidine (pK_a 6.9), suggesting that the selectivity of the OAT3 for the transport of organic anions is not as exclusive as it is for OAT1. Site-directed mutagenesis studies are consistent with the presence of two substrate- binding sites in OAT3, one for the transport of PAH and one for hydrophobic organic anions that exhibit affinity for cimetidine (48). Like OAT2, OAT3 shows important functional differences with respect to OAT1. Neither unlabeled estrone sulfate nor PAH are able to trans-stimulate the uptake of radiolabeled PAH or estrone sulfate by rat or human OAT3, suggesting that OAT3 does not act as a anion exchanger (47). Assuming that OAT3 acts as a uniporter for organic anions, it is not clear how this transporter will overcome the large energy barrier due to the inside negative membrane potential of the cell. It remains that in kidney cortex slices from OAT3 knockout mice, the PAH and estrone sulfate uptake is considerably reduced, indicating a significant contribution of OAT3 in organic anion transport in the proximal tubule. Recent evidence showing that the uptake of PAH and estrone sulfate can be stimulated in oocyte expressing OAT3 after preloading the eggs with glutarate, suggest that OAT3 can indeed operate as an organic anion/dicarboxylate exchanger. Taken together, these observations are consistent with a major contribution of OAT3 in the secretory process of organic anions in the proximal tubule. Finally, OAT3 shows affinity for a variety of acidic neurotransmitter metabolites which are removed from the cerebrospinal fluid by a PAH-inhibitable transport process (49,50).

Brush-Border Membrane Mechanisms

In contrast to the basolateral membrane transport system, little is known about the transport of organic anion and the efflux of PAH across the luminal membrane of proximal tubule cells. To complicate our understanding of the classical secretory pathway in the human kidney proximal tubule, the mechanisms involved in PAH efflux from cell to lumen differ among species. Two distinct transport systems have been identified from brush-border membrane vesicle experiments: a facilitated electrogenic diffusion (or electrogenic pathway) and a carrier-mediated anion exchanger. The former is present in rats, rabbits, and pigs; data are lacking for dogs (3). The anion exchanger, on the other hand, has been identified in dogs and rats, but not in rabbits and pigs. This transporter accepts inorganic anions (Cl^-, HCO_3^-, OH^-) and several organic anions, including uric acid and α-ketoglutarate. In contrast to the PAH uptake across the basolateral membrane, the transport in brush border membrane vesicles does not proceed through a coupling of sodium–α-ketoglutarate symport and α-ketoglutarate–PAH exchange. This is likely explained by a lower affinity of α-ketoglutarate ($\gg$1 mM) for the luminal than for the basolateral anion exchanger. In humans and bovines, PAH apical transport occurs through a PAH anion exchanger that accepts only α-ketoglutarate; it is similar to the PAH–α-ketoglutarate basolateral mechanism. Both apical and basolateral exchangers of the bovine proximal tubule have similar affinities for PAH and for inhibition by probenecid (51).

The respective contribution of the facilitated electrogenic diffusion pathway and the PAH anion exchanger were investigated *in situ* in rat tubular microperfusion experiments. Both transport systems appeared to be not operating. Indeed, an increase in the apical membrane potential and the addition of cold PAH in the tubular lumen did not stimulate PAH transport from cell to lumen (52). In fact, the PAH anion exchanger found only in urate-reabsorbing mammalian species, might be responsible for the minor PAH-reabsorptive transport observed in dogs (53).

Ullrich et al. (17) determined the characteristics required for a substrate to interact with the apical step of PAH secretion. The substrate requirement for binding to the apical PAH transporter appears similar to that required for basolateral transport. As for basolateral transport, both the hydrophobicity and the electronic properties are important determinants for affinity. The relative affinity of various monosubstituted benzoates, benzoylglycines (hippurates), and phenylacetylglycines for the apical and the basolateral transport of PAH was compared in membrane vesicle experiments (54,55). In general, the inhibitory potency of substrates for the apical membrane transport was lower than the one for the basolateral membrane transport.

Molecular Identification of Putative Apical *p*-Aminohippurate Transporter NPT1

NPT1 or NaPi1 was cloned and initially identified as a sodium–phosphate cotransporter (56). This protein has 6 to 8 putative transmembrane segments and does not share any homology with the members of the OAT family. It was then recognized that NaPi1 or NPT1 was not playing an important role in maintenance of phosphate homeostasis and that NPT1 transports organic anions, in particular PAH (Fig. 8.1) (57,58). The binding sites for phosphate and organic anions appear to be distinct. Using cell suspensions of human embryonic kidney cells (HEK293) transfected with NPT1, Uchino and colleagues demonstrated a cellular uptake of PAH, uric acid, benzylpenicillin, faropenem, and estradiol β-glucuronide. The cellular accumulation of PAH was inhibited by various organic anions, including probenecid, benzylpenicillin, and indomethacin. Because the transport of PAH was strongly inhibited by chloride (an extracellular ion), the authors suggested that *in situ* the transporter could act from cell to lumen. NPT1 did not demonstrate PAH anion exchange. Consequently, the authors suggested that NPT1 might be the PAH voltage-sensitive pathway. However, in human brush-border membrane vesicles, it was demonstrated that PAH is transported by an anion exchanger specific for α-ketoglutarate and PAH and not by a voltage-sensitive pathway (59). The renal functional role of human NPT1 still remains unclear.

Multidrug Resistance Associated Protein

Another candidate for the apical transport of PAH secretion is the multidrug resistance associated protein 2 (MRP2), an adenosine triphosphate (ATP)-dependent export pump characterized by its apical localization in polarized cells such as hepatocytes (60). MRP2 is a member of the ATP-binding cassette (ABC) superfamily of transport proteins. In the kidney, MRP2 has been localized at the apical membrane of human and rat proximal tubules (Fig. 8.1) (61). The substrates for this ATP-dependent primary active pump are conjugates of

lipophilic xenobiotics or conjugates of endogenous compounds with glutathione (glutathione *S*-conjugate leukotriene C_4), glucuronate (17β-glucuronosyl estradiol), estradiol, or lipophilic anions, as well as folate and methotrexate (5,62). Recently, PAH and ochratoxin were also identified as substrates of human MRP2 (61). MRP2 thus appears to be responsible for the apical step of PAH and a broad number of organic anions. The functionality of MRP2 was investigated in proximal tubules of killfish. It appears that the apical secretory step of fluorescein–methotrexate, phenol red, carboxyfluorescein, and lucifer yellow, is through MRP2 (15,63). The presence of an active step for organic anion transport through the apical membrane is surprising, considering that the basolateral uptake of organic anions is active and that the concentration in the proximal cells is high, a passive diffusion pathway would be sufficient to drive the anions into the lumen. The respective roles of NPT1 and MRP2 in the apical efflux of PAH and other organic anions, in different animal species, need to be determined *in vivo*.

OAT4

OAT4 is expressed in the kidney, but at the mRNA level its expression is lower than for OAT1 and OAT3 (42). In the human kidney, OAT4 seems to be restricted at the luminal membrane of the proximal tubule (40) (Fig. 8.1). OAT4 transports PAH, but with a low affinity ($\gg$1 mM), which makes it quite unlikely that OAT4 represents the major pathway for PAH secretion in the proximal tubule (64). In addition, OAT4 mediates estrone sulfate transport, has affinity for ochratoxin A, PGE_2, and probenecid, but its role in organic anion handling in the proximal tubule remains uncertain.

Tubular Transport of *p*-Aminohippurate: An Integrated View

The energy source for PAH transport in intact cells is provided by the low intracellular sodium concentration achieved by Na-K–ATPase activity. The transmembrane sodium gradient serves as the energy source to drive α-ketoglutarate intracellularly from interstitium as well as from lumen, since membranes of both sides possess an α-ketoglutarate–sodium cotransport (Fig. 8-1). The α-ketoglutarate–PAH exchange at the basolateral membrane, through the OAT, allows PAH, in turn, to concentrate intracellularly by a tertiary active transport. Thus, the central role of α-ketoglutarate in generating intracellular accumulation of PAH as the first step to secretion is now well established. Earlier reports stating that PAH transport depends on extracellular sodium can be explained by the sodium–α-ketoglutarate cotransport. Similarly, the modulatory effects of metabolic intermediates (e.g., lactate, acetate, pyruvate, and succinate) on PAH transport, as described in older studies, result from the metabolic conversion of these intermediates into α-ketoglutarate.

Intracellular traffic of secreted anions appears more complex than originally thought and might proceed through accumulation into intracellular organelles (65,66). The apical membrane may represent the limiting step for the secretion of organic anions into the tubule lumen. The transport mechanisms across the apical membrane are still uncertain. They may include a PAH anion exchanger accepting different organic or inorganic anions, a PAH–α-ketoglutarate exchanger, as proposed in humans and bovines, and a potential regulated channel, as observed in rats, which might be NTP1, or an ATP-dependent export pump (MRP2).

ORGANIC ANION TRANSPORTERS DISTINCT FROM THE *p*-AMINOHIPPURATE TRANSPORTER

Organic Anion Secreted by the Sulfate–Anion Exchange Mechanism

Oxalate

Oxalate is the most common constituent of renal stones and is secreted by a transport mechanism different from that of PAH. In healthy humans, oxalate plasma concentration averages 2 μM and its fractional excretion ranges between 1% and 2% (67). In rabbits, oxalate secretion is somewhat larger in the S_1 and S_2 segments than in the S_3 proximal segment (68), a pattern different from that of PAH. In the basolateral membrane, oxalate (a divalent ion) exchanges with sulfate, or bicarbonate (69), whereas in the brush-border membrane, oxalate transport occurs by two anion-exchange mechanisms: an electroneutral exchange with one sulfate, two chloride or two formate ions, or an electrogenic exchange with one chloride or one formate ion (70,71) (Fig. 8.1). Studies in rats confirmed the physiologic role of these exchangers in the renal transport of oxalate (72). The basolateral sulfate–anion exchanger (SAT1) has been cloned (73). It is restricted to the basolateral membrane, demonstrating that the apical and the basolateral oxalate–sulfate exchangers represent two distinct mechanisms. The apical oxalate–chloride exchangers differ from the PAH–anion exchanger and from the formate–chloride exchanger (70,74). In contrast to PAH, oxalate is transported by the LLC-PK1 cell line (75).

Earlier experimental studies reported net tubular secretion of a biphosphonate, etidronate (76). Similar findings in rats have been reported for new biphosphonates, such as alendronate. The secretion of biphosphonates remains unaltered by any of the classic organic ion- transport substrates, such as probenecid, quinine, cimetidine, PAH, or NMN. Alendronate secretion, however, can be reduced by etidronate, pointing to a potential transport system specific for biphosphonates. Conceivably, this class of compounds might also be transported by the sulfate carrier (77).

Organic Anion Transporters without Affinity for p-Aminohippurate

A number of transport molecules have been cloned from different tissues and identified in the renal proximal tubule. These transporters, with no affinity for PAH, may contribute to the apical step of organic anion secretion.

OATP

The OATP family of transporters refers to "organic anion transporting polypeptides," which have been studied for their roles in mediating Na^+-independent transport of bile salts, bromosulfophthalein, and conjugated and unconjugated steroid hormones in the liver (8). The S_3 segment of proximal tubule expresses OATP1 (Fig. 8.1). Although hepatic OATP1 is expressed at the basolateral membrane (blood side) of hepatocytes, in the kidney it is localized at the apical membrane (78). In the kidney, by contrast to the liver, the OATP1 mRNA is strongly regulated by androgens and, to a weaker extent, by estrogens. OATP1 might play a role in the renal excretion of estrogens. The OATP2 transports digoxin in the liver and, recently, OATP4 was isolated, and shown to be expressed at the basolateral membrane of the kidney proximal tubule (79). A

homolog of OATP1, OATP3,was isolated from a rat retina and found to be expressed specifically in the retina and in the kidney. It transports taurocholate as well as thyroid hormones (T_3 and T_4) (80).

OATK1 and OATK2

OATK1 and OATK2 are organic anion transporters specific to the kidney and also belong to the organic anion -ransporting polypeptide family (81) (Fig 8.1). In rats, OATK1 is essentially expressed in the apical membrane of proximal straight tubules. When expressed in MDCK cells, OATK1 mediates both uptake and efflux of methotrexate through the apical membrane. Folate is also a substrate, whereas taurocholate is not (82). NSAIDs (indomethacin, ketoprofen, ibuprofen, flufenamate, phenylbutazone) inhibit methotrexate uptake mediated by OATK1. The OATK1 transporter appears to be a site for methotrexate and NSAID interaction (83).

OATK2 is expressed in the whole convoluted proximal tubule and in the collecting duct. When transfected in MDCK cells, OATK2 mediates the apical transport of methotrexate, folate, taurocholate, and PGE_2. In *cis* inhibition studies, steroids, bile acid analogs, and cardiac glycosides were shown to have a high affinity for OATK2, suggesting that it participates to the apical transport of hydrophobic anionic compounds in the kidney (81).

The molecular basis of the renal handling of endogenous and xenobiotic anions is far from being elucidated. The complexity of this organic anion transport system is illustrated by the number of transporters identified by expression cloning (Fig. 8.1). The situation is complicated by the fact that a substrate may exhibit affinities for different transporters. The OAT1 transporter is the most likely candidate for the basolateral step of the PAH secretion in the mammalian proximal tubule. The molecular identification of the apical transporter for PAH remains to be established. Several putative organic anion transporters have been identified, but their apical localization in the proximal tubule remains to be further demonstrated. Finally, the contribution of these apical organic anion transporters in the net renal secretion can now be investigated by gene targeting in mouse models.

ORGANIC CATIONS

Studies in renal handling of organic cations (OC) *in vivo* have been limited by their toxicity at concentrations used for clearance measurements. Their renal handling has, therefore, been studied initially in chicken with the Sperber technique (84). Micropuncture and microperfusion studies then identified the proximal tubule as the primary site of OC secretion. Renal proximal cell lines, LLC-PK1 and OK, grown as monolayers on porous filters, have been further used to investigate mechanisms involved in the renal epithelial transport of organic cations, such as TEA, NMN, cimetidine, procainamide, and nicotine (85–87) from the basolateral to the apical side. Differences in the affinity of different substrates for the secretory transport pathway pointed to the existence of multiple carriers for which substrates have overlapping affinities (85,88). The cloning of organic cation transporters and functional studies in *Xenopus* oocytes, HeLa cells, and human embryonic cell lines expressing these transport molecules set the molecular basis of the secretion of OC in the proximal tubule.

The organic cation secretory system mediates the transport of various endogenous molecules and xenobiotics of widely different molecular structures. Among the cations transported by the secretory mechanism, a large diversity exists; as for organic anions, there are no strict and specific structural requirements. The low selectivity of the organic cation transporter is reflected by the interaction of the basolateral carrier with most nitrogen-containing substrates (23). An extensive list of cationic drugs susceptible to interact with the organic cation transporter has been published by Ullrich (89). With the exception of choline, organic cations show limited reabsorption by facilitated mechanisms. At normal plasma concentrations (~20 μM), choline is extensively reabsorbed by the mammalian and chicken kidney and, at higher plasma concentrations, choline is secreted (84). The apical step of choline reabsorption is electrogenic and specific for choline (90,91).

Membrane Mechanisms for Tetraethylammonium and N_1-Methylnicotinamide Secretion

Mechanisms of organic cation transport have been reviewed in detail by Pritchard and Miller, and, more recently, by Koepsell and co-workers (2,88) and by Wright and Dantzler (204). Only the relevant features will be presented here.

The entry step of organic cations across the basolateral membrane involves an electrogenic transport driven by the intracellular negative potential (facilitated diffusion) (Fig. 8.2). The Nernst equation predicts at equilibrium, an intracellular concentration of monovalent organic cations up to a 10- to 15-fold higher level than in the extracellular milieu. In most experiments with cortical slices (from rabbits, dogs, rats, and mice),

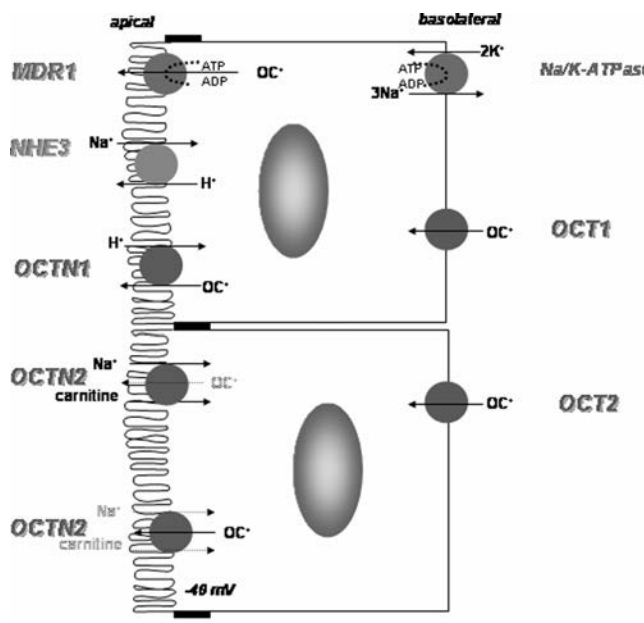

FIGURE 8-2. Organic cation transporters in the renal proximal tubule *Upper cell:* Transporters involved in the secretion of organic cations (OC^+). The entry step of OC^+ into the cell across the basolateral membrane involves OCT1 and OCT2 uniporters. The efflux of OC^+ across the apical membrane occurs in exchange with H^+ via the OCTN1 transporter. Intracellular H^+, driven by the OCTN1 are recycled back into the tubule lumen via the NHE3 Na^+/H^+ exchanger. The ATP-dependent MDR1 transporter also allows secretion of OC^+ across the apical membrane into the tubule lumen. *Lower cell:* The uniporter OCTN2 constitutes an electrogenic diffusion pathway for OC^+ across the apical membrane. The OCTN2 can operate in two modes, as a uniporter or as a Na^+/carnitine cotransport.

such a level of intracellular accumulation could not be observed. By contrast, in isolated nonperfused proximal tubules from rabbits, the intracellular/extracellular concentration ratio could exceed 100 for TEA and about 20 for NMN (3). The maintenance of very high intra- and extracellular concentration ratios has been interpreted as the result of the contribution of an active transport mechanism, in addition to the facilitated diffusion driven by the membrane negative potential. However, it should be pointed out that intracellular TEA is sequestered in cytoplasmic organelles. Furthermore, it has been demonstrated that endosomal membrane vesicles isolated from rat renal cortex can accumulate TEA by an ATP-dependent mechanism (92). It is thus conceivable that the favorable transmembrane potential may represent the principal or single driving force required for cellular uptake of organic cations.

Basolateral Transport Mechanisms

Transport of organic cations at the basolateral membrane occurs by a voltage-sensitive pathway (electrogenic pathway) and, in some cases, also by an organic cation exchanger. Experiments with rabbit basolateral membranes have shown that the transport of TEA, NMN, and procainamide is electrogenic and that the transport of radioactively labeled TEA, in basolateral membrane vesicles or in nonperfused isolated rabbit proximal tubules loaded with TEA, is *trans*-stimulated with choline, mepiperphenidol (MPP^+), or cold TEA (3,20).

In cimetidine-loaded nonperfused rabbit-isolated proximal tubules, the efflux of cimetidine is *trans*-stimulated by external TEA, quinine, procainamide, NMN, and choline (72), suggesting the presence of an organic cation exchanger. This transport system could well represent an alternative mode of transport by the electrogenic facilitated diffusion. Limited data are available for transport in basolateral membrane vesicles of dogs and rats in which only self-exchange of NMN (93) and TEA (94) were investigated. No uptake stimulation was observed in either case. The presence of an organic cation exchange mechanism cannot be firmly excluded in dogs, rats, and mice, based on experiments in cortical slices from these species.

Substrate Requirements for the Basolateral Organic Cation Transport Mechanism

Extensive studies have been conducted in rat proximal tubules *in situ* to define the molecular requirements of substrates for binding to the basolateral transporter of organic cations (17). The substrate affinity depends on both hydrophobicity and basicity (i.e., electron-donating, nucleophilic properties). Cationic substrates form ion pairs with the corresponding ionized groups within the carrier, but noncationic compounds might also act as substrates if they can bind to the transporter through multiple hydrogen bonds (e.g., steroids or cyclophosphamide). The role of hydrophobicity is illustrated by a series of alkylammonium compounds for which an increase in affinity is correlated with the length of the alkyl chain and hydrophobicity without change in charge. The importance of charge negativity is shown by the direct relationship among pK_a of heterocyclic pyridine, quinoline, and isoquinoline analogs and their affinity for binding to the carrier (17). Ionic interaction between substrate and carrier is not required, as illustrated by steroids, which are devoid of nitrogen atoms, but which bear many hydroxyl groups that form hydrogen bonds with the carrier. These findings generally agree with observations on substrate affinities evaluated by the ability of different compounds to inhibit TEA or NMN uptake in renal cortical slices from rats, dogs, and rabbits, or to reduce NMN secretion in chickens, and with data on the isolated rabbit proximal tubule (23).

Molecular Identification of the Basolateral Organic Cation Transporter

Expression cloning allowed the identification of the primary structure of a polyspecific organic cation transporter (rOCT1) from a rat kidney cortex cDNA library (Fig. 8.2) (88). The hydrophobicity profile of the OCT1 is consistent with the presence of 12 transmembrane segments and makes it a member of the solute carrier superfamily (SLC22A). OCT1 is heavily expressed in the kidney cortex and in the liver (95,96). Immunohistochemistry shows that OCT1 is restricted to the basolateral membrane of hepatocytes and proximal tubular cells, in particular, along the early S1 segment (88). Functional expression in *Xenopus* oocytes indicated that this protein shares many characteristics with the basolateral transporter of organic cations in renal proximal tubules with respect to the range and structure of transported compounds and the affinities of substrates and antagonists. The transport mediated by this protein is electrogenic and remains unaltered by an outwardly oriented proton or sodium gradient, properties consistent with the known characteristic of the basolateral secretory mechanism for organic cations. The characterization of rOCT1 was followed by the isolation of OCT1 orthologs and the identification of a rat and human isoform OCT2 (97). Human OCT2 and rat OCT2 are strongly expressed in the kidney, in particular, in the basolateral membrane of the proximal tubule, but not in the liver. The functional characteristics of the OCT2 are qualitatively similar to OCT1; the transport is electrogenic and supports organic cation exchange.

Functional studies in *Xenopus* oocytes, human embryonic kidney cells (HEK 293), and HeLa cells demonstrated that OCT1 and OCT2 of various species transport small organic cations, such as TEA, NMN, 1-methyl-4-phenylpyridinium, choline, dopamine, serotonin, and histamine (88). As expected for the renal basolateral OCT, TEA transport by *Xenopus* oocytes expressing rOCT2 and rOCT1 (98) was inhibited by a broad number of cationic compounds, such as cimetidine, guanidine, choline, decynium 22, 1-methyl-4-phenylpyridinium, procainamide, NMN, quinidine, and nicotine. The human immunodeficiency virus (HIV) protease inhibitors, indinavir, nelfinavir, ritonavir, and saquinavir inhibit TEA transport by hOCT1, but they are probably not transported (99). Inhibitor potency for OCT1 and OCT2 varies with species. Human OCT1 generally interacts with the *n*-tetraalkylammonium compounds with a lower affinity than that of rats, mice, or rabbits (100). The expression levels of rOCT2 mRNA and protein in males are much higher than in females (101). This might correlate with the higher transport of TEA in male basolateral membrane vesicles and cortical slices. Because no gender differences were observed for rOCT1 expression in the kidney, the role of rOCT2 in basolateral transport of organic cations appears dominant.

Brush-Border Membrane Mechanisms

In all species investigated thus far—rats, dogs, rabbits, pigs, and humans—TEA and NMN efflux across the luminal membrane involves a carrier-mediated exchange with protons, which represents the rate-limiting step for secretion of organic cations. In rabbits, amiloride, cimetidine, morphine, and procainamide (3), and in rats, disopyramide (102), are transported by such an electroneutral proton exchanger. Acridine orange (103), gentamicin, and verapamil (104) can *trans*-stimulate and *cis*-inhibit NMN transport in brush-border membrane vesicles and are therefore substrates for the proton–NMN exchanger. The apical Na/H exchanger NHE3 plays a crucial role in the secretion of organic cations by maintaining an inwardly directed proton gradient. Depending on the proton gradient across the luminal membrane, the organic cation proton exchanger

can transport organic cations against a concentration gradient.

Several carriers appear to be involved in the transport of organic cations, which show overlapping affinities for different substrates. Thus, H^+-stimulated guanidine uptake in brush-border membrane vesicles is only minimally inhibited by TEA, NMN, and choline, whereas amiloride, clonidine, imipramine, and harmaline are more potent inhibitors (105). Furthermore, species differences in organic cation secretion may reflect the involvement of different carrier molecules in brush-border membranes. Cephalexin, for instance, shows an affinity for the NMN or TEA transporter in human brush-border membranes (102), while in rats it has no affinity for the TEA transport mechanism. In snakes, TEA is secreted and NMN reabsorbed (9).

The role of the proton organic cation exchanger has been confirmed in LLC-PK1 cells grown on porous support, where basolateral to apical transport of TEA was enhanced by acidification of the apical side (106). Similar enhancement of procainamide and cimetidine transport was observed in OK or LLC-PK1 cell monolayers (86). However, because nonionic back-diffusion of weak bases is decreased by apical acidification, the relative role played by proton organic cation exchange versus nonionic diffusion in the overall transport stimulation by luminal protons cannot be assessed precisely (87).

A number of currently used drugs inhibit NMN or TEA transport in brush-border membrane vesicles. In dogs, quinidine, trimethoprim, cimetidine, famotidine, quinine, amiloride, procainamide, and nizatidine were shown, in decreasing order of inhibitory potencies, to reduce NMN transport (107). In rats, the potency sequence for inhibition was cimetidine, famotidine, and ranitidine, which was about 50 times less potent than cimetidine (108). In humans, these drugs may interact in the renal excretion of other drugs (see also below).

Substrate Requirements for the Apical Organic Cation Transport Mechanism

The structural properties required for apical transport have been investigated *in situ* in rats and in rabbit brush-border membrane vesicles (109). Hydrophobicity is the principal determinant of substrate binding, as illustrated by the correlation between an increase in hydrophobicity and an increase in inhibitory potency of the inhibitors. Hydrophobicity appears to play a more important role for the apical than the basolateral transport system. For example, the apparent affinity for the apical transporter of quinine, TEA, choline, guanidine, and NMN, which are all small, lipophilic molecules, is about 20 times lower than for the basolateral transporter. In contrast, cimetidine and larger hydrophobic compounds such as tetrahexylammonium have a higher apparent affinity for apical than basolateral transporters (three to ten times larger than compounds of smaller molecular size) (17). The cyanine analog, decynium 22, has one of the highest affinities for interacting with MPP^+ luminal efflux in rats. In LLC-PK1 cell monolayers, decynium 22 inhibits radioactive TEA uptake at a concentration 100 times lower than that of cold TEA. At the same concentration ratio, decynium does not inhibit the basolateral uptake of TEA transport.

Enantiomers of some chiral organic cations differ with respect to their affinities for the transporter and their ability to inhibit NMN or TEA uptake by brush-border membrane vesicles (107,109). For example, stereoselectivity was observed for the enantiomers of verapamil, norepinephrine, and epinephrine. No differences were observed in inhibition of TEA uptake between the enantiomer of bupivacaine, or for the optical isomers quinine and quinidine, suggesting that transport stereoselectivity might occur when the center of chirality is close to cationic function of the substrate (110).

Molecular Characteristics of the Apical Organic Cation Transporters

A few transport mechanisms have been identified in the kidney by expression cloning. They might be involved in the apical step of organic cation secretion, although at present their function *in situ* has not been recognized (see Fig. 8.2).

OCTN1 and OCTN2

OCTN1 was first cloned from a fetal human liver cDNA library and showed an approximately 30% homology with the OCT1. The hydrophobicity profile of the OCTN1 amino acid sequence is compatible with 12 transmembrane segments (111,112). The tissue distribution of OCTN1 differs between human and rat. At the mRNA level, OCTN1 expression is low in the human kidney (42), whereas in mouse and rat the largest expression is found in the kidney, intestine, lung, heart, and brain (96). At the protein level, expression of rOCTN1 is found in the straight portion of the proximal tubule and also in the distal tubule. There is no direct evidence for an apical membrane localization of the OCTN1, but the functional characteristics of OCTN1 suggest that it might be involved in the apical step of organic cation secretion.

When expressed in human embryonic kidney cells (HEK293) and *Xenopus* oocytes, human OCTN1 mediates a saturable and pH-dependent transport of TEA; extracellular acidification inhibits OCTN1-mediated transport. In HEK cells expressing hOCTN1, an inwardly directed proton gradient stimulates TEA efflux from the cell, supporting an electroneutral proton OC^+/H^+ exchange mechanism. The transport of TEA was observed to be bidirectional and inhibited by various organic cations, such as choline, clonidine, cimetidine, quinidine, and verapamil, and by zwitterionic compounds such as L-carnitine, cephaloridine, and levofloxacin. The transport of a few of these inhibitors, quinidine, verapamil, and L-carnitine, was demonstrated (113). In summary, OCTN1 is a multispecific, bidirectional, and pH-dependent organic cation transporter that is probably energized by a proton antiport mechanism.

OCTN2 shares with OCT1 a 30% sequence identity. In the kidney, OCTN2 is expressed predominantly in cells of proximal and distal tubules, as well as in glomeruli. OCTN2 has the same functional characteristics as OCTN1, but the substrate affinities for the transporter differ (114). In addition to its ability to transport organic cations, the human, rat, and mouse OCTN2 also functions as a Na^+-dependent L-carnitine transporter with affinities to other zwitterions, such as cephalosporins that contain a quaternary nitrogen (115). Interestingly, mutations in the gene encoding OCTN2 result in a failure to reabsorb carnitine in the proximal tubule and a systemic carnitine deficiency. Site-directed mutagenesis experiments provided evidence that the transport sites for organic cations and for carnitine are distinct (116,117). OCTN2-mediated transport of TEA is independent of Na^+. Thus, OCTN2 can transport substrates in both Na^+-dependent and -independent modes, but both modes carry net charges. OCTN2 thus might play a role in organic cation secretion, and in the reabsorption of carnitine by a sodium-carnitine cotransport. *In vivo,* cephaloridine was reported to increase the fractional excretion of carnitine by interfering with its reabsorption (115). The anionic cephalosporins are not substrates for OCTN2, but they are substrates for the peptide transporters, PEPT1 and PEPT2 (5). Inversely, the cephalosporins, which have affinity for OCTN2, are not substrate of the peptide transporters.

MDR1

The MDR protein, as well as the MRP proteins, are members of the ABC (ATP-binding cassette) superfamily of transport

proteins, which is one of the largest and most diverse family of proteins that mediate transport of solutes across biological membranes (118). MDR1, also called P-glycoprotein (Pgp) is expressed in the kidney, liver, and intestine, where it facilitates secretion or limits absorption of xenobiotics (111). The MDR1-mediated transport is coupled to ATP hydrolysis. There is functional evidence that the ATP-dependent export pump MDR1, characterized by its apical localization in epithelial cells, plays a role in the apical step of the renal secretion of organic cation. Localized at the apical membrane of proximal tubules, MDR extrudes many cationic hydrophobic compounds, for example, vinblastine, vincristine, colchicine, daunomycin, verapamil, and doxorubicin from the cells (119–122). When expressed in MDCK cells, MDR1 mediates the secretion of daunomycin, vinblastine, and cimetidine from the basolateral to the apical side (123). Secretion mediated by P-glycoprotein was demonstrated in killfish, in which uphill transport of the fluorescent derivatives of rapamycin and cyclosporin A into the urinary space of intact proximal tubule was observed (124). P-glycoproteins transport TEA and vinblastine, but not in exchange with luminal protons. Photoaffinity-labeling studies suggest that the Pgp and the exchanger are different transport proteins (125). Examples of compounds transported in kidney cell lines by both the Pgp and the organic cation transporter include daunomycin, colchicine, verapamil, quinidine, cimetidine, and vinblastine (123). The MDR1 gene is constitutively expressed in LLC-PK1 cells and encodes low levels of Pgp in plasma membranes and in acidic particles (probably Golgi apparatus). In LLC-PK1 cell suspension, doxorubicin efflux and uptake by acidic particles are mediated by Pgp. An intracellular redistribution of doxorubicin occurs, depending on the rate of cell efflux, which is characteristic of a doxorubicin-resistant tumor.

THE ORGANIC ION TRANSPORTER GENE FAMILIES

The functional interactions between organic anions and cations for renal transporters may be related to common structural features between OAT and OCT transporters. Indeed, members of the OAT and OCT transporter families show homologies at the amino acid level and share a common membrane topology with 12 putative membrane-spanning segments suggesting that OAT and OCT belong to the same transporter gene family (8).

The sulfate–oxalate–bicarbonate anion exchanger (SAT-1) shares no homology with the members of the OAT family (73). Interestingly, SAT-1 shares a significant homology with a transmembrane protein designated pendrin, which, when mutated, causes a form of a hereditary deafness (126). Pendrin was shown to mediate chloride–formate exchange when expressed in *Xenopus* oocytes (127). Prestin, a motor protein in the cochlear outer cells also shows significant homology with SAT-1 (128). These membrane proteins exhibit 11 putative transmembrane segments with an intracellular N-terminus. These membrane proteins seem to have diverse physiologic functions and may belong to the same gene family.

The Na-dependent dicarboxylate cotransporters (NaDC) represent a distinct gene family that includes at least three members and possibly more remaining to be identified. The current models of the secondary structure of the NaDC family also predict 11 transmembrane segments and an extracellular *N*-glycosylated carboxy terminus (7).

The structural similarities existing between OAT/OCT, the SAT-1, and NaDC remain to be determined, in particular, their membrane topology or their ligand-binding sites. Knowledge about their structure/function will allow researchers to determine whether these organic ion transporters may belong to a unique and large gene superfamily.

Besides these organic ion exchangers or cotransporters, ATP-binding cassette (ABC) transport proteins (MDR1 and MRP2) are present in the kidney, as mentioned before, and are likely involved in the transport of organic cations and anions.

DRUG INTERACTIONS AND OTHER CLINICAL ASPECTS OF RENAL ORGANIC ION TRANSPORT

Because secretion takes places through facilitated mechanisms and the number of carrier molecules is limited, secretion is subject to competition between substrates. Drug interactions for renal excretion might, therefore, be expected. In fact, such interactions appear generally of limited clinical relevance, except in a few situations, which are discussed in more detail in the following paragraphs. It must be pointed out that kinetic drug interactions may occur concomitantly at intestinal, liver, and/or renal transport systems.

Cardiac Glycosides

Excretion of digoxin is primarily renal, by glomerular filtration and tubular secretion and reabsorption. Competition studies have shown that the "classic" anion or cation transport systems are not involved, although the role of a basolateral transporter (OATP4C1) in cellular uptake of digoxin has been recently suggested (79). The secretory process as studied *in vivo* and in a renal epithelium *in vitro* is carried out by the apical membrane P-glycoprotein (MDR). It is clinically well known that several drugs (most notably quinidine, verapamil, nifedipine, propofenone, spironolactone, amiodarone, ritonavir [129], clarithromycin [130]) reduce the renal (tubular) clearance of digoxin and increase the plasma concentration and toxic risks of the cardiac glycoside (131–133). Accordingly, these interactions may be explained by a competition at the secretory step controlled by P-glycoprotein at the luminal membrane. Such a possibility has received experimental support for several of these compounds (120,121,134,135).

Antibiotic and Antiviral Drugs

The central role of the secretory anion pathway in the renal clearance of β-lactam antibiotics has been well documented. Probenecid reduces the excretion of β-lactam compounds, a property that has been used to prolong the plasma half-life and maintain the therapeutic levels of penicillins. The uptake at the basolateral membrane is mediated by the OAT1 transporter (136). As well, uptake at the basolateral membrane of cephalosporins leading to high intracellular concentrations is a major contributing factor for their renal toxicity, particularly if the extrusion rate at the apical membrane is low. Toxicity can be reduced by concomitant administration of probenecid, which inhibits basolateral uptake. It is worth noting, however, that cell accumulation is necessary but not sufficient for cytotoxicity, as shown by cephalexin, which has a low nephrotoxic potential despite marked cortical accumulation (13). Human pharmakinetic studies have shown inhibitory effects by probenecid on renal tubular secretion of ziduvudine, cidofovir, ganciclovir, and by zidovudine or ganciclovir (137).

H_2-Receptor Antagonists

Interactions of cimetidine and other H_2-receptor antagonists with the renal secretion of several drugs have been repeatedly described and comprehensively listed. Thus, cimetidine inhibits renal secretion of procainamide in humans and prolongs its elimination half-life (138,139). Similar inhibitory effects have been shown on creatinine, ranitidine, and many other cationic compounds (140). Conversely, in human volunteers, probenecid reduces the renal excretion of cimetidine (141) and famotidine (142). These observations in humans agree with the experimental data discussed previously, characterizing cimetidine and other H_2-receptor antagonists as bisubstrates.

Antineoplastic Drugs

Methotrexate toxicity is increased by concomitant administration of NSAIDs, such as ibuprofen, salicylates, and flurbiprofen (143). NSAIDs and methotrexate are substrates for the basolateral PAH transporter (OAT) (144) and the apical transporter OATK1 (83). Competition between NSAIDs and methotrexate for transport by OATK1 has been reported,and inhibition of the basolateral uptake of methotrexate by NSAIDs was observed in rabbit kidney slices (145). Recent studies using mouse tubular cells expressing human OAT's conclude that methotrexate transport and interactions with NSAIDs occur at basolateral hOAT1 and hOAT3 and apical hOAT4 transporters (146).

Cisplatin appears to be transported basolaterally by the anion-transport system, because coadministration of probenecid reduces the nephrotoxicity of this chemotherapeutic agent in humans (147). It has been proposed that platinum, like other nephrotoxic metal ions, such as mercury and potassium dichromate, is taken up by tubular cells as sulfhydryl conjugate through a probenecid-sensitive pathway (148). However, cisplatin might also belong to the group of bisubstrates, because quinidine, cimetidine, and ranitidine inhibits its net secretion flux in the dog kidney (149). Furthermore, cisplatin-induced toxicity to renal MDCK might result from intracellular transport through basolateral OCTs (150).

Other Drugs

In healthy volunteers, gemfibrozil entails an increase in exposure (AUC) to simultaneously administered pravastatin, an interaction that appears to be, in part, related to a transport protein at the renal level (151).

Renal Clearance of Organic Anions or Cations to Estimate Renal Function

Estimation of renal plasma flow (RPF) can be derived from the renal clearance value of any compound that is extensively extracted by a single pass through the kidney and completely excreted into the urine. Measurements of PAH clearance at plasma concentrations below transport saturation have been used for decades to determine RPF in subjects with normal renal function. It bears repeating, however, that PAH extraction is markedly reduced in chronic renal disease and its determination by renal venous sampling remains necessary for estimating RPF, since PAH clearance in these conditions is likely to underestimate true RPF (152).

Despite its utility, the use of PAH suffers from the fact that the exogenous marker must be infused. Hippurate, the physiologic analog of PAH, has also been used. However, because its endogenous plasma concentration is low, an exogenous supply is also required (153). It has therefore been proposed to resort to the clearance of an endogenous cationic compound, N^1-NMN, as an alternative to PAH and hippurate for the determination of RPF. Studies in humans have used endogenous NMN clearance as a test for early detection of tubular functional alteration of toxic origin. Thus, reduced NMN clearance during cyclosporin therapy despite normalization of RPF and glomerular filtration rate (GFR) has been interpreted as indicating a persisting toxic tubular effect of the drug (154).

An overview of various techniques used for estimating renal elimination pathways in clinical settings has been published (155).

URIC ACID

In mammals, uric acid (2,6,8-trihydroxypurine) derives exclusively from the degradation of purines, while in other vertebrates it is generated by the catabolism of both purines and amino acids. In most mammals, uric acid is further catabolized into allantoin through the activity of urate oxidase. In humanoid primates, however, uric acid is the final product of purine metabolism, as a consequence of a mutation in the uricase gene resulting in a loss of its enzymatic activity (156,157). It has been argued that the loss of uricase activity may provide an evolutionary advantage, since uric acid has powerful free-radical scavenger properties and may thus exert cell-protective effects, although its persistence entails the occurrence of gout and its attendant complications (158,159).

From a teleologic perspective, a waste product should be easily eliminated from the body. Human kidneys reabsorb a large proportion of filtered urate (160), higher than in most mammals investigated so far, with the exception of the chimpanzee and the *Cebus* monkey. This concomitant loss of uricase activity and extensive renal reabsorption result in higher uratemia in humans than in other mammals, with values close to the limit of solubility in plasma (161). The low solubility of uric acid entirely explains its physiopathologic and toxicologic effects.

Physicochemical Characteristics, Plasma Levels, and Overall Renal Handling of Uric Acid

Uric acid is a trioxypurine with oxygen in positions 2, 6, and 8 of the oxypurine ring. It is a weak acid with a pK_a of 5.75 (–NH position 9) and 10.3 (–NH position 3). Depending on the pH of physiologic fluids, uric acid is present as the mono urate sodium salt and/or the undissociated acid. In plasma (37°C, pH 7.4), uric acid is almost entirely (98%) present as monosodium urate salt. Plasma levels vary throughout life, being rather high in full-term neonates (310 μM), and decreasing (to 140 μM) after a few days, as a result of increased uricosuria. At later stages, urate plasma levels increase in children of both sexes, with a larger rise in males than in females at puberty (161,162). Urate plasma values in postmenopausal women increase and reach the levels of males.

These variations of plasma urate concentrations are related to differences in renal fractional excretion, demonstrating the role of the kidneys in the regulation of uricemia. In healthy humans, the fractional excretion of urate is slightly higher in females (12%) than in males (8%), and is greater in children (15% to 30%) than in adults. The role of estrogens on the renal excretion of urate is controversial (161,163).

The role of genetic factors in the control of renal urate clearance is important, as shown by ethnic variations in fractional

urate excretion (164,165). In addition, hypouricemia may be associated with loss of function mutation of the urate transporter hURAT1 and cases of familial juvenile hyperuricemic nephropathy because of failure to excrete uric acid have been described (166,167).

Renal Excretion of Urate

In healthy humans, net tubular urate transport results in an overall renal reabsorption of urate, leading to a fractional excretion of urate averaging 10%. The most effective uricosuric drugs increase this value from 30% to 50% (160,168). Despite the predominance of reabsorption in the overall renal handling of urate, it has long been established that tubular transport in the proximal tubule is bidirectional. Thus, Gutman et al. (169) demonstrated that, in a few patients with reduced GFR, reabsorption was converted to secretion in the presence of an urate load, an osmotic diuresis, and sulfinpyrazone administration; under these conditions fractional excretion of urate reaches about 110%. An unidirectional secretory transport of radiolabeled urate has also been shown in humans with normal renal function.

Changes in extracellular fluid volume (ECFV) are among the most important factors modulating urate excretion. Thus, ECFV contraction, frequently resulting from the use of diuretics, decreases the fractional excretion of urate. The mechanism of this effect remains unknown, but most likely results from enhanced proximal urate reabsorption. In such conditions, reabsorption of sodium and many other solutes (HCO_3^-, phosphate, glucose) is also increased (170). Although urate reabsorption is not directly coupled with sodium (59,171), it might be stimulated indirectly by cotransport of sodium with other anions having affinity for the urate–anion exchanger responsible for urate reabsorption (172). A decrease in fractional excretion of urate, together with an increase in sodium reabsorption, is observed in diabetes insipidus, hypertension, during angiotensin infusion, and in hyperinsulinemia within physiologic range.

In contrast, expansion of ECFV by isotonic or hypertonic saline enhances the fractional excretion of urate, an effect independent of the increase in urine flow, which as such have little influence on urate excretion. An increase in effective arterial circulatory volume probably plays a more important role in such a context and may, at least, in part, be responsible for the hypouricemia observed in inappropriate secretion of arginine vasopressin (AVP) (173).

Fractional urate excretion is little affected by urinary pH changes, while it is reduced by respiratory or metabolic acidosis. This decrease in urate excretion is mediated, to a large extent, by the enhanced production of lactic, β-hydroxybutyric, and acetoacetic acids, which interfere with urate transport, as discussed later in this chapter. Any condition favoring overproduction of lactic acid or ketone bodies (e.g., diabetic ketoacidosis, starvation, weight loss in obesity, physical exercise, or inherited fructose intolerance) will induce uric acid retention, resulting, in part, from interference with tubular transport of urate.

Unilateral nephrectomy in humans almost doubles fractional urate excretion from the remaining kidney during the first postoperative weeks, allowing the maintenance of a normal urate balance and uricemia. Later, fractional excretion returns to preoperative values in parallel with compensatory hypertrophy and GFR adaptation (174). An increase in fractional urate excretion is also observed in chronic renal failure, in which it may reach values up to 85% when GFR is reduced to about 10% of normal. Although the mechanism of this increase is unknown, uricosuric metabolites accumulated during renal failure may play a role (161,164).

A number of compounds may increase or decrease urate excretion, with corresponding changes in plasma urate. Probenecid, sulfinpyrazone, and benzbromarone, which have been used to treat hyperuricemia (168), generally increase fractional urate excretion to about 30%. Salicylates are also uricosuric at high doses, but antiuricosuric at low doses (175). This so-called paradoxical effect on urate excretion can be observed also, although to a lesser extent, with other uricosuric agents. The majority of diuretic drugs, inducing urate retention by ECFV contraction, may also interfere directly with urate transport (176). Because such effects are usually undesirable, diuretics with uricosuric properties, such as tienilic acid and indacrinone have been developed, but the former proved to be hepatotoxic. Losartan, an antagonist of angiotensin II receptors, has a strong uricosuric effect (177).

The tuberculostatic drugs, ethambutol and pyrazinamide, markedly decrease the renal excretion of urate and, thus, induce hyperuricemia. The traditional view has been that urate retention because of pyrazinamide results from the action of a metabolite, pyrazinoic acid (PZA), which inhibits urate tubular secretion. The frequently used "pyrazinamide test," is based on this putative inhibitory action on urate secretion. It is used to estimate the "secretory" component of renal urate excretion (178); the component of excreted urate suppressed by administration of large doses of pyrazinamide (2 to 3 g) is considered to be the fraction of excreted urate, which is secreted. However, studies on the membrane mechanisms involved in urate transport indicate that the low fractional urate excretion measured during a pyrazinamide test (typically about 1%) most likely relates to a stimulatory effect of PZA on urate reabsorption (160). These observations on the stimulatory effect of pyrazinamide on urate reabsorption are difficult to reconcile with the principles of the "four-component model" of human urate excretion. In this model urate excretion results sequentially from ultrafiltration, followed by a near complete proximal reabsorption, then secretion, and finally a postsecretory reabsorption (179). The problem with this model is that the tubular secretion of urate was assessed using pyrazinamide, which turns out to affect mainly urate reabsorption (160). Thus, the contribution of secretory component of urate in the overall urate excretion in human remains to be clearly established.

Cyclosporin immunosuppressive treatment induces hyperuricemia, which might result from a decrease in fractional urate excretion (180), although a decrease in GFR might also be implicated. Reduced fractional excretion of urate appears to be responsible for the hyperuricemia observed in some cases of chronic lead exposure (181).

Tubular Transport of Urate

The lack of availability of an appropriate animal model made it difficult to understand the renal handling of urate in humans. Clearance experiments have shown surprisingly large differences between various mammalian species; some secrete urate across the whole range of plasma urate concentrations, some reabsorb urate at low plasma concentrations and secrete it at higher levels, and others reabsorb urate even at high plasma levels (182). The reabsorptive capacity of *Cebus* monkeys is close to that of humans, whereas the pig secretes urate as efficiently as PAH. In addition, interindividual variability in fractional excretion of urate is large within members of a single species. While dogs usually have net urate reabsorption, a genetic defect of urate cellular transport in Dalmatian coach hounds leads to net secretion (183). In spite of differences between humans and other mammals in renal urate handling, useful information on the tubular handling of uric acid has been obtained by functional studies at the single nephron level in experimental animals. In all mammals investigated thus far

(rats, dogs, pigs, Dalmatian coach hounds, rabbits, and *Cebus* monkeys), the proximal tubule has emerged as the site of urate secretion and the main segment involved in urate reabsorption. Urate transport can proceed in a secretory as well as in a reabsorptive direction in the same segment of the proximal tubule (184).

Although dogs and rats reabsorb urate, their use in pharmacologic studies to test for drug effects upon urate transport is limited, since they do not respond well to uricosuric or antiuricosuric compounds (185). Mice appear slightly more sensitive to uricosuric agents. Only *Cebus* monkeys and chimpanzees respond well to drugs affecting urate transport, although marked differences in pharmacologic effects between the two species have occasionally been reported (186).

In urate-secreting species, such as pigs and Dalmatian coach hounds, the drugs known to be uricosuric in humans are antiuricosuric, while PZA (a metabolite of pyrazinamide), which, in humans, is highly antiuricosuric, has no obvious effects in secreting species (185). These species differences limit the usefulness of animal models for pharmacologic studies of urate transport.

Membrane Mechanisms for Tubular Transport in Nonhuman Mammals

Comparative studies between various mammalian species, predominantly reabsorbing or secreting urate, and humans have provided insights into the mechanisms of renal urate transport. More recently, several urate transporter genes have been cloned, allowing a more specific analysis of renal urate transport.

Brush-Border Membrane

In "urate reabsorbing" species, such as dogs and rats, apical transport of urate, as shown by studies on isolated membrane vesicles, is mediated by the anion exchanger URAT1 (187). URAT1 is expressed almost exclusively in the kidney and immunohistochemistry shows specific labeling at the apical membrane of the proximal tubule (166). When expressed in *Xenopus* oocytes, URAT1 transports urate in an electroneutral way in exchange with Cl, lactate, nicotinate or PZA, indicating that URAT1 likely represents a major pathway for urate reabsorption in the proximal tubule. Xanthine and hypoxanthine are not substrates for the rat urate anion exchanger (188), while uricosuric drugs, notably benzbromarone, sulfinpyrazone, and probenecid, are potent inhibitors of the exchanger (189). In "urate-secreting" species, such as pigs and rabbits, no urate anion exchanger could be demonstrated (190,191). In these species, the apical urate transport occurs through a voltage-sensitive pathway (electrogenic pathway) shared with PAH, which allows urate secretion along the electrical potential between cell interior and tubular lumen.

In rat and rabbit brush-border membrane vesicles, a voltage-sensitive transport mechanism unmasked by exposure to trace amounts of copper has been described (192); it may correspond to a different transporter. This putative transporter has many affinities similar to the hepatic peroxisomal enzyme uricase (193), and has been cloned (194).

It thus appears that an important difference in brush-border membrane transport properties between "urate reabsorbers" and "urate secretors" lies in the presence of an apical urate anion exchanger allowing bidirectional transport of urate in urate reabsorbers, while in urate secretors the activity of the voltage-dependent transport mechanism results in urate secretion, because of cell electronegativity.

Basolateral Membrane

In urate-reabsorber species (rat), urate transport across the basolateral membrane occurs through a voltage-sensitive pathway. Cell electronegativity allows the exit of intracellular urate into the interstitium (195). The existence of an additional basolateral chloride–urate exchanger has been suggested (196), but not confirmed (195).

In pigs, which are urate secretors, urate is transported at the basolateral side by an anion exchanger with affinity for α-ketoglutarate. This transport system is similar to, but not identical with, that described for PAH secretion (197). It allows uphill transport of urate (see description of PAH transport given previously in this chapter), as evidenced by *in vitro* experiments showing urate accumulation into proximal tubular cells of urate secretors—rabbits, pigs, guinea pigs, and bovine species (184,198). Recent studies in rabbits provide evidence that basolateral rbOAT3 transporter is involved in the secondary active transport of urate in exchange with dicarboxylates and other substrates, and represents the first step in urate tubular secretion (199). No concentrative uptake of urate could be found in proximal cells of urate reabsorbers.

In summary, urate reabsorption involves an apical urate transport (URAT1) into proximal cells in exchange for intracellular anions. Urate then exits into the peritubular interstitium by facilitated diffusion along the electrochemical gradient. For urate secretion, urate enters the cell through a basolateral urate–dicarboxylate exchange (OAT3), and exits the cell across the apical membrane by facilitated diffusion.

Membrane Mechanisms for Tubular Transport of Urate in Humans

Urate transport was investigated in human brush-border membrane vesicles (160,172). These studies show the presence of a voltage-sensitive pathway (electrogenic pathway) together with one, or maybe two, urate anion exchangers, which exchange urate with many monovalent anions. In contrast to dogs and rats, hydroxyl ions and PAH are not substrates for human urate anion exchanger(s).

Uricosuric drugs or compounds, such as orotate, *cis*-inhibit urate exchange with lactate or chloride, according to the following potency order: benzbromarone > losartan > orotic acid > probenecid. Benzbromarone is 50 times more potent than probenecid (200). Pyrazinoate (an antiuricosuric compound) inhibits urate chloride exchange. When loaded in brush-border membrane vesicles, orotate and pyrazinoate *trans*-stimulate urate uptake. However, when sodium is present in the uptake medium, pyrazinoate, but not orotate, *cis*-stimulates urate uptake. Pyrazinoate enters the membrane vesicles by sodium cotransport and then drives urate into the membrane vesicles by anion exchange. Orotate is not cotransported with sodium. The potency of uricosuric drugs and pyrazinoate for inhibiting urate transport by the voltage-sensitive pathway is lower than for inhibiting the urate anion exchangers.

Recently, a human renal urate anion exchanger (hURAT1), localized at the luminal membrane of proximal tubules, has been identified and its gene cloned (166). It shows pharmacologic properties consistent with those described in human brush-border vesicle studies (160), namely, inhibition of urate uptake by uricosuric drugs, independent of membrane potential. This transporter is likely involved in proximal tubule urate reabsorption in humans and probably contributes to the control of uricemia. The important role of hURAT1 in the setting the plasma level of urate is further established by the identification of loss of function mutations of hURAT1 that are associated with hypouricemia (166).

Another apical membrane urate transport protein has been described (UAT, galectin 9), which would function as an ion channel and display electrogenic properties (201). A transporter from the MDR family (human MRP4) has recently been proposed as a cell-to-lumen urate efflux pump. It is described functionally as an unidirectional transporter, with multiple allosteric binding sites with affinites for cGMP, cAMP, and methotrexate (202). Human OAT1 has also been shown to transport urate (203). The transporter is essentially expressed and functional at the basolateral membrane. It is not a Cl^- urate exchanger and is inhibited by PAH and uricosuric compounds. Its precise functional role(s) in urate handling by human kidneys remains to be characterized.

Functional Role of Urate Membrane Transport Mechanisms in Humans

Urate reabsorption in the proximal tubule, mediated by hOAT1 exchanger, critically depends on the concentration gradient across the apical membrane of the exchangeable anions with affinities with the transporter. Accordingly, an increase in the intracellular concentration of substrates for the urate exchanger hURAT1 will stimulate net urate reabsorption. The decreased uricosuria observed in hyperlactacidemia and diabetic ketoacidosis can be explained by an increase in intracellular concentrations of acetoacetate and β-hydroxybutyrate that increases the energy for urate reabsorption via the hOTA1 exchanger. The strong decrease in urate excretion following pyrazinamide administration is explained by the increase in intracellular pyrazinoate concentration resulting from the activity of the sodium–pyrazinoate cotransport; intracellular pyrazinoate is then available to drive luminal urate into the cell via the hURAT1 transporter which has a high affinity for pyrazinoate. This mechanism likely accounts for the anti-uricosuric effect of pyrazinamide. Uricosuric drugs in the lumen of the proximal tubule may either inhibit urate reabsorption by binding to hURAT1 or stimulate urate secretion by exchanging with intracellular urate. This latter case is true for orotate, which is not transported into cells by sodium cotransport.

Urate transport in humans across the basolateral membrane is relatively inefficient in the secretory direction because of the presence of a facilitated diffusion pathway and the lack of urate transporters to overcome electronegativity inside the cell. The predominance of urate reabsorption in the proximal tubule is confirmed by genetic studies showing that loss of function mutations of the apical hURAT1 transporter is associated with hypouricemia and not hyperuricemia (166). It remains possible that intracellular urate can be recycled back into the tubule lumen, depending on the presence of exchangeable anions in the tubule lumen with affinity for the hURAT1 transporter. This back-recycling of urate across the apical membrane may account for the relatively small component of urate secretion in the proximal tubule. In any case, in view of recent genetic and transport studies studies in human kidney, the classical "four-component model" describing urate excretion in human needs to be revisited.

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CHAPTER 9 ■ HORMONES AND THE KIDNEY

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An intricate network of circulating and locally produced hormones regulates glomerular and tubular functions of the kidney. These hormones comprise a chemically heterogeneous group that includes proteins, peptides, lipids, nucleosides, and amino acid-derived molecules. Not only is the kidney a target of hormone action, but it also synthesizes, modifies, and secretes hormones that influence nonrenal functions, such as erythropoiesis and bone metabolism. Furthermore, the kidney plays an important role in the clearance and inactivation of several peptide and protein hormones.

This chapter is a synopsis of the physiologic actions of hormones that modulate renal function. Where appropriate, recent advances in molecular biology that have further elucidated the physiology of these hormones are emphasized. In particular, genetically designed animals in which a hormone is either overexpressed (transgenic) or eliminated (gene knockout) have served as a powerful tool to dissect the often complex and overlapping actions of hormones in the kidney (1). Renal production and metabolism of hormones that act elsewhere in the body will be discussed briefly. Cellular mechanisms of hormone action and receptor-mediated signal transduction have been reviewed in several excellent publications (2–6).

HORMONAL MODULATION OF NEPHRON FUNCTION: AN OVERVIEW

Modulation of Glomerular Filtration Rate

The major physiologic determinants of single nephron glomerular filtration rate (GFR) are glomerular plasma flow (Q_A), glomerular transcapillary hydraulic pressure (P_{GC}), and the ultrafiltration coefficient (K_f) (7). These variables are determined, in part, by the contractile state of the afferent arteriole, efferent arteriole, and mesangial cells. K_f also varies with alterations in the hydraulic permeability of the capillary filtration barrier, which consists of endothelial cells, visceral epithelial cells, and the glomerular basement membrane. By binding to specific receptors on cellular and structural components of the glomerulus, circulating and locally produced hormones influence one or more of the physiologic determinants of GFR. Figure 9-1 summarizes the effects of different hormones on pregomerular, glomerular, and postglomerular contractility. Because the same substance can act at different sites in the glomerular unit, the net effect on GFR will depend on whether its actions are antagonistic or complementary. For example, atrial natriuretic peptide (ANP) decreases afferent arteriolar resistance, while increasing efferent arteriolar resistance, resulting in an augmented P_{GC} and a rise in GFR (8). Under certain conditions, ANP also increases K_f, which, in turn, contributes to the enhancement of GFR (9). On the other hand, Ang II-mediated constriction of both afferent and efferent arterioles results in opposite effects on glomerular plasma flow and P_{GC} and, therefore, no change in single nephron GFR (10). Regulation of glomerular hemodynamics is further complicated by multiple interactions between hormones in the kidney. For example, infusion of Ang II along with a cyclooxygenase inhibitor causes a significant decrease in single nephron GFR, suggesting that endogenous prostaglandin production antagonizes the glomerular effects of Ang II (11). The net effects of renally relevant hormones on GFR are discussed in more detail later in this chapter.

Modulation of Tubule Water and Electrolyte Transport

Renal tubule cells express receptors for many circulating and locally synthesized hormones. The effects of a particular hormone on water or electrolyte transport are partly determined by the differential distribution of its receptor on functionally specialized segments of the renal tubule. For example, arginine vasopressin (AVP) binds almost exclusively to principal cells in the collecting tubule (CT), where it influences the physiologic functions of this segment, primarily water and urea absorption (12). Parathyroid hormone (PTH), on the other hand, exerts its biologic actions on renal tubule segments proximal to the collecting duct, where it modulates calcium, phosphate, magnesium, sodium, and bicarbonate transport (13). Table 9-1 summarizes the net effects of renally relevant hormones on water and solute handling in different tubule segments. Each hormone is discussed in detail in the sections that follow. As in the glomerulus, hormones may modulate their own actions by altering the production of counterregulatory hormones. For example, AVP induces local synthesis of prostaglandin E (PGE), which opposes the effect of AVP on water permeability in the CT (14).

Because of the significant overlap in mechanisms and sites of action of renally relevant hormones, it is impossible to group them under a rigid functional classification. Moreover, it is difficult to categorically divide these hormones according to site of production, because components of certain hormonal systems, such as the PTH–vitamin D and renin–angiotensin axes, are synthesized in a coordinated fashion by renal and nonrenal tissues. Although the order in which hormones are discussed in this chapter does not follow a specific pattern, we have attempted to group together hormones with related physiologic functions.

ARGININE VASOPRESSIN

The primary physiologic role of AVP, or antidiuretic hormone (ADH), is maintenance of normal plasma osmolality through modulation of water excretion by the kidney. Its other

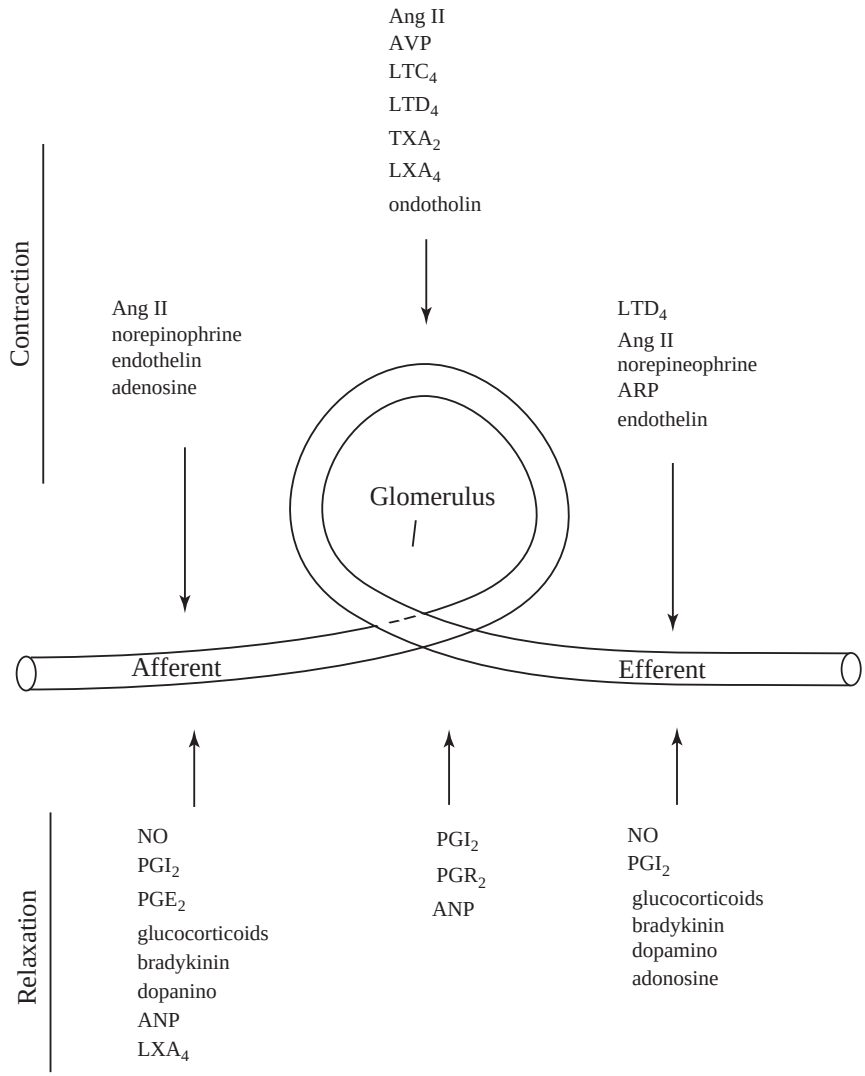

FIGURE 9-1. Hormones regulating glomerular, afferent, and efferent arteriolar contractility. Glucocorticoid action may be pharmacologic and indirect. The precise glomerular site of action for some vasoactive hormones and autacoids, such as nitric oxide (NO) (renal vasodilator) and thromboxane A_2 (TXA_2) (renal vasoconstrictor), are not known. *Ang II*, angiotensin II; *AVP*, vasopressin; *ANP*, atrial natriuretic peptide; *LXA_4*, leukotriene A_4; *LTC_4*, leukotriene C_4; *LTD_4*, leukotriene D_4; *PGI_2*, prostacyclin; *PGE_2*, prostaglandin E_2.

physiologic functions possibly include regulation of blood pressure and the response to stress.

Synthesis and Secretion of Arginine Vasopressin

AVP is a 9-amino acid neuropeptide synthesized by magnocellular neurons in the paraventricular and supraoptic nuclei of the hypothalamus (15) and circulating in the plasma nearly in an unbound form. It is packaged into secretory granules in neuronal bodies and transported in the axons to the posterior pituitary (neurohypophysis) (15). The most sensitive stimulus for secretion of AVP into the bloodstream is increased plasma osmolality. As little as 1% change in plasma osmolality leads to a change in AVP concentration that is sufficient to modify renal water excretion (16). AVP secretion is almost completely suppressed when plasma osmolality decreases below an average of 280 mOsm/kg of water in humans. "Osmoreceptor" neurons that respond to changes in plasma osmotic pressure by varying their intracellular water content have been identified in the anterior hypothalamus (16). Secretion of AVP is also influenced by alterations in intravascular volume and blood pressure, sensed by baroreceptors located in the heart, aortic arch, and carotid sinus (16). Whereas a 5% to 8% decrease in blood volume or systemic arterial pressure has little effect, further hemodynamic compromise leads to a steep increase in circulating AVP levels. Significant reductions (10% to 30%) in circulatory arterial volume or blood pressure can override osmoregulation and result in markedly increased AVP levels in the face of decreased plasma osmolality (17). Other less potent stimuli for AVP secretion include fever, emesis (18), and oropharyngeal osmoreceptors (19).

In addition to the posterior pituitary, AVP synthesis has been detected in the pancreas, adrenal gland, ovary, testis, and regions of the brain (20). Its physiologic function in these sites, however, is not known.

Physiologic Actions of Arginine Vasopressin

AVP exerts its biologic actions by binding to specific receptors on the cell surface. Three types of AVP receptors have been identified: V_{1a}(vascular /hepatic), V_{1b}(anterior pituitary), and V_2 (kidneys) (21). V_{1a} receptors have been located by radioautographic studies at the level of: the thin descending limb of short-loop nephron and inner medullary collecting duct (IMCD), cortical collecting duct (CCD), medullary and cortical thick ascending limbs (MAL and CAL) and outer and inner medullary collecting ducts (OMCD and IMCD), specifically at the luminal aspect of the principal and intercalated cells in the

TABLE 9-1

HORMONAL MODULATION OF TUBULAR TRANSPORT[a]

	Hormones with major effects		
	Reabsorption	**Stimulatory**	**Inhibitory**
Proximal tubule	Na	Ang II, catecholamines, insulin	Dopamine, PTH
	P_i	IGF-1	PTH
	HCO_3	Ang II	PTH
	Ca		PTH
TALH	Na	AVP,[b] catecholamines	PGE
	Ca	PTH, calcitonin, glucagon	
	Mg		AVP, PTH, calcitonin, glucagon
DCT	P_i		PTH
	Ca	PTH	
CCD	H_2O	AVP	PGE, bradykinin, ANP, α-adrenergic agents
	Na	Aldosterone	ANP, PGE, EGF
IMCD	Urea	AVP	
SECRETION			
CCD	K	AVP, aldosterone	β_1 agonists
	H	Aldosterone	

[a] *TALH*, thick ascending limb of the loop of Henle; *DCT*, distal convoluted tubule; *CCD*, cortical collecting duct; *IMCD*, inner medullary collecting duct; *Ang II*, angiotensin II; *IGF*, insulinlike growth factor; *AVP*, arginine vasopressin; *PTH*, parathyroid hormone; *PGE*, prostaglandin E; *EGF*, epidermal growth factor.
[b] Unlikely in humans.

collecting duct (22). They are linked to G_{plc}, a phospholipase C-coupled G protein, which mediates intracellular production of 1,2-diacylglycerol (DAG), and inositol 1,4,5-triphosphate (IP_3). DAG stimulates protein kinase C (PKC), while IP_3 increases cytosolic Ca^{2+}, thus initiating the second-messenger cascade responsible for the cellular actions of AVPs. Biologic effects of AVP mediated by the V_{1a} receptor include platelet aggregation and increased glycogenolysis and gluconeogenesis in the liver (21,23). In addition, high circulating levels of AVP cause arteriolar vasoconstriction by binding to V_{1a} receptors on vascular smooth muscle cells (24). The V_{1b} receptor, also coupled to G_{plc}, is present on neurons in the anterior pituitary (adenohypophysis) and is thought to mediate AVP-induced corticotropin secretion (21,25). Their presence and role at the level of the medulla in rat kidney are not yet clear (26). V_2 receptors, on the other hand, are heavily expressed in cortical, outer medullary, and inner medullary collecting duct (CCD, OMCD, and IMCD) and weakly expressed in inner medullary thin limb and medullary and cortical thick ascending limbs (MAL and CAL), especially at the basolateral membrane of the thick ascending limbs and principal cells in CCD, OMCD, and IMCD (22). They are coupled to an adenylate cyclase stimulatory G protein (G_s) (21). Binding of AVP to V_2 receptors on renal epithelial cells results in increased cyclic adenosine monophosphate (cAMP) levels, activation of PKA, insertion of water channels into the apical membrane, and antidiuresis (12).

Renal Actions of Arginine Vasopressin

Water Channels

Depending on the species, AVP can modulate either solute transport or water transport in the kidney (27). However, the principal effect of AVP in the human kidney is increased water permeability throughout the collecting tubule (12,28). The discovery of the family of aquaporin water channels (AQP1, -2, and -3) was fundamental to our understanding of the mechanism by which vasopressin can increase water permeability in the mammalian kidney. Peter Agre (29) has recently reviewed the different aspects of water channels in the kidney in the *Journal of the American Society of Nephrology*.

AQP1 is constitutively expressed in apical and basolateral membrane domains of proximal tubule and thin descending limb cells. In the collecting duct, water moves across the membrane of principal cells, which express at least three AQP subtypes (AQP2, AQP3, and AQP4) (22,30). After AVP binds to V_2 receptors on the basolateral membrane of principal cells and terminal IMCD cells, preformed water channels located in subapical vesicles are inserted into the apical membrane, thus increasing water permeability (12,28). This increased permeability to water is seen within minutes of AVP binding to the V_2 receptor and can be sustained with chronic infusion (31). The downstream mechanism for vasopressin signaling has been described as phosphorylation of myosin light chain (MLC) by AVP-dependent calcium/calmodulin-dependent myosin light chain (MLCK) (32). In order to increase the number of functional channels in the apical membrane, PKA-dependent V_2–stimulated phosphorylation of AQP2 at Ser-256 acutely leads to intracellular trafficking of subapical vesicles containing the preformed AQP2 channels to the apical membrane (33,34). While "short-term" (over minutes) regulation of collecting duct water permeability involves the insertion of preformed water channels, "long-term" (hours to days) regulation of collecting duct permeability involves an increased production of AQP2 channels by the cell by inducing genes VIP17/MAL (vesicle integral protein/myelin and lymphocyte), annexin II, mitochondrial ATP synthase, α-tubulin, and stimulatory GTP-binding proteins, believed to affect apical sorting or delivery of AQP2 (35). Chronic elevation of AVP induced by either water

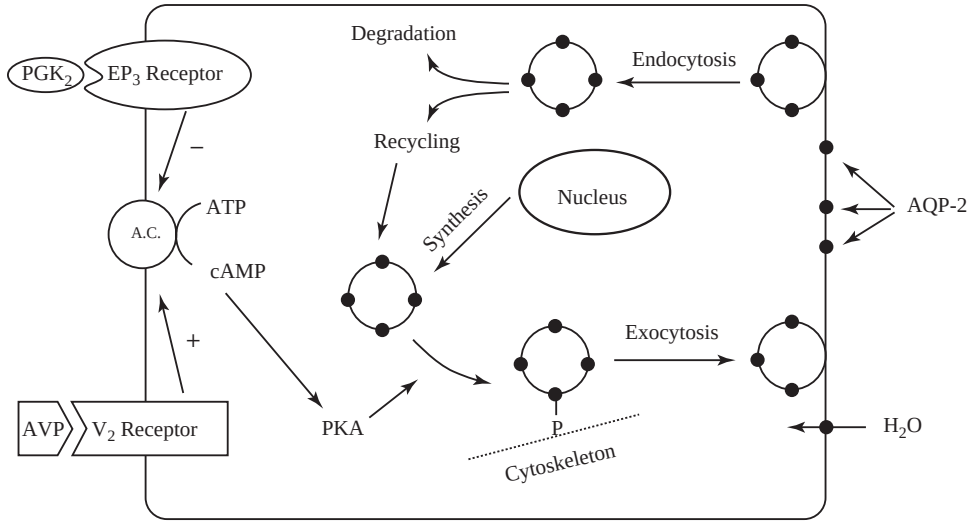

FIGURE 9-2. Schematic diagram of proposed mechanism of arginine vasopressin (AVP) action on the collecting tubule. AVP binds to receptors on the basolateral membrane that activate adenylate cyclase, thereby increasing cyclic adenosine monophosphate (cAMP) and PKA activity. AVP also stimulates local prostaglandin production. Prostaglandin E_2, α_2-adrenergic agonists, kinins (in part through prostaglandins), atrial natriuretic peptides (ANP), hypercalcemia, and hypokalemia all blunt the cAMP response to AVP. In contrast, adrenal steroids potentiate AVP action by inhibiting phosphodiesterase activity and possibly by inhibiting prostaglandins. Increased activity of protein kinase A probably leads to phosphorylation of the AQP2 channels and the subsequent transfer of preformed vesicles that contain functional AQP2 channels to the apical membrane. Downregulation of water transport occurs with endocytosis of membrane vesicles that contain functional AQP2. (From: Nielson S, Kwon T, Christensen B, et al. Physiology and pathophysiology of renal aquaporins. *J Am Soc Nephrol* 1999;10:647, with permission.)

deprivation or AVP infusion in laboratory animals leads to both an increased AQP2 and -3 messenger RNA (mRNA), and a marked increase in the amount of AQP2 and 3, but not AQP4 protein levels (22,36,37). The proposed mechanism of AVP action on collecting duct cells is illustrated in (Fig. 9-2). Hormones, pharmacologic agents, and ions that modulate the antidiuretic action of AVP are also summarized in (Fig. 9-2) (14,38). Oxytocin increases osmotic water permeability in isolated perfused rat at specific binding sites of IMCD, thin limb of Henle's loop, the densa (MD), and paraglomerular structure, as proved by binding of AVP to oxytocin receptors with high–affinity (22). Metabolic acidosis increases circulating and brain levels of AVP with increase of water reabsorption by a fourfold upregulation of AQP2(39).

Urea Transporters

As reviewed by Sands (40), in addition to its antidiuretic effect, AVP regulates urea permeability in the collecting duct (41). The ability of AVP to enhance urea permeability in IMCD was postulated by Morgan and co-workers in 1968 (42), and later confirmed in isolated perfused rat IMCD (43–45). Physiologic data indicate that urea is transported by both facilitated and active transporter proteins. In 1993, You and colleagues (46) cloned from the rabbit inner medulla the complementary DNA (cDNA) for UT-2, a facilitated urea transporter. Northern blot analysis of mRNA using the UT-2 cDNA probe confirmed the presence of two isoforms, UT-1 and UT-2, in the terminal part of rabbit IMCD (46). These isoforms were later named UT-A1 and UT-A2 to distinguish their gene product from that of the erythrocyte urea transporter (47). Subsequently, a human homolog was cloned that shared 90% sequence homology to UT-A2 (48). Differential expression in the kidney of UT-A1 and UT-A2 was identified with UT-A1 being primarily expressed in the IMCD in intracellular vesicles, as well as at the apical membrane (22), while UT-A2 is primarily expressed in the thin descending limb of the loop of Henle, as localized by Northern blot and immunochemistry studies. A novel UT-B was located in descending vasa recta irrigating the inner medulla (49). Expression studies in *Xenopus* oocytes indicate that UT-A2 does not acutely appear to be regulated by cAMP and, therefore, by vasopressin (46), however, immunoblotting indicates that vasopressin analogs and hyperosmolality can chronically upregulate UT-A2 expression (50,51). The UT-A1 isoform is regulated by vasopressin by its phosphorylation and is the predominant transcript in the terminal IMCD (52). Recently, two other isoforms of UT-A have been identified in the rat inner medulla: UT-A3 and UT-A4 (is a spliced variant of the UT-A gene) that has the same subcellular and axial distribution as UT-A1 in intracellular membrane and apical region of terminal portions of rat IMCD (53), which may also be regulated by vasopressin (54). It is believed that AVP-stimulated urea transport results in increased medullary tonicity, which allows formation of more concentrated urine with upregulation of AQP2 and AQP3 expression and AQP2 membrane targeting (55). Insulin and AVP modulate AQP2 expression through different mechanisms, but in synergy. Insulin itself has been shown to increase water reabsorption in proximal tubules, increase water permeability in inner medullary collecting duct (56), and increase AVP-dependent AQP2 expression by increasing AQP2 mRNA levels in a MAP kinase and IP3 kinase-dependent manner (57).

Sodium Channels

In rat kidney, AVP has also been shown to increase NaCl transport in the medullary thick ascending limb of Henle's loop and in the cortical collecting duct, thus further enhancing maximal urine concentration (58,59) by stimulation of ENaC by cAMP production via V_2 receptors, mainly β and γ subunits in long-term regulation (22,60). AVP directly stimulates K secretion in

the collecting duct, an effect that may be important in maintaining potassium balance during antidiuresis (59,61). In cases of hyponatremia, like in SIADH, aldosterone increases sodium absorption, mediated by hypoosmotic activation of voltage-dependent or stretch-induced activation of calcium channels (62). Finally, AVP could also influence proton secretion in the CT (63). It is unclear whether these mechanisms are physiologically important in the human kidney as studies have failed to demonstrate AVP-stimulated cAMP activity in the human thick ascending limb of the loop of Henle (64).

Interaction between Arginine Vasopressin and Atrial Natriuretic Peptide

It has been shown that in the central nervous center AVP stimulates ANP, which, in turn, exhibits a negative feedback on AVP. In the kidney, ANP inhibit the effects of AVP on water and ion transport (22).

Action on Smooth Muscle

Vargiu et al. (65) have demonstrated a contractile action of vasopressin on renal minor calyces and pelvic smooth muscles, via V_1 receptors present at the level of the smooth muscle cells versus a relaxation effect via V_2 receptors (66), mainly through intracellular movement of calcium.

Action on Vascular Tone

In vitro, AVP is a potent vasoconstrictor of afferent, efferent, and arcuate intrarenal arterioles modulated by NO production of endothelial origin, but not by cytochrome P450 or cyclooxygenase derivatives (66).

Transgenic Mouse Models of Arginine Vasopressin Expression

Both the physiologic function of AVP and the regulation of AVP synthesis have been studied in animals. Transgenic mice provide a useful tool to study *in vivo* regulation of gene expression under more pathological conditions (67). Majzoub and colleagues (68) (as reported by Grant et al.) have developed a line of transgenic mice that express AVP in a tissue-specific manner, have appropriate osmotic regulation of transgenic vasopressin mRNA, and have normal water metabolism. Despite increased hypothalamic levels of AVP, both heterozygous and homozygous transgenic mice had normal AVP levels in the posterior pituitary. Plasma AVP levels, however, were increased three- to fourfold in homozygous mice, but basal water homeostasis remained normal. The latter observation suggests that renal resistance to AVP could have developed. Modification of the 5′-and 3′-flanking sequences of the transgene may be useful for further studies of the physiologic importance of genetic elements that regulate AVP gene expression (67).

Studies in Brattleboro rats have demonstrated the stimulatory effect of chronic infusion of AVP, leading to a threefold increase in AQP2 expression at day 14 to 28 (69) and restoration of nephron heterogeneity, an effect reflecting glomerular size, proximal tubule length, and single nephron filtration rate. It has also been shown by Zhang et al. (70) that AVP stimulates indirectly COX-2 expression through activation of both V_1 and V_2 receptors, thereby modulating medullary electrolyte tonicity.

In these knock-out rats, the induction of diabetes mellitus increases AQP2 and NaCl transporters independently of AVP, probably via oxytocin action (71).

AVP modulates expression of renal nNOS (neuronal NOS) through V_2 receptors, but not eNOS (endothelial NOS); nNOS has a similar distribution throughout the kidney as water channels, related to the hydration status of the rat (72).

Other Protein Activation

dDAVP infusion induces the expression of multiples proteins in renal medulla of Brattleboro rats: β-arrestin, neurofibromin, casein kinase II β, 11β-HSD2 (11-β-hydroxysteroid dehydrogenase type 2), Wilms' tumor protein, *c-fos* and *c-jun*, synaptotagmin (73), and calcyclin (74).

THE RENIN-ANGIOTENSIN SYSTEM

In 1898, Tigerstedt and Bergman (75) demonstrated that intravenous injection of a crude renal extract causes a slowly developing and sustained increase in blood pressure. They called the active agent "renin." Renin was later identified as a proteolytic enzyme that acts on a plasma constituent to produce a potent pressor substance, "angiotensin" (76). Subsequent research characterized the components of the renin–angiotensin system and elucidated its important role in the regulation of blood pressure, renal hemodynamics, and fluid and electrolyte homeostasis (Fig. 9-3).

Regulation of Renin Production and Secretion

Renin is produced and stored in granular juxtaglomerular cells, which are modified smooth muscle cells found in the media of afferent arterioles (77–79). Genomic analysis of the renin gene identified a single locus in humans and rats, but mice have two renin genes, designated *Ren-1* and *Ren-2* (77). These genes correspond to the renin produced in mouse submandibular gland and kidney, respectively. Renin is synthesized in an inactive precursor form, preprorenin. Cleavage of the signal peptide from the carboxyl terminal of preprorenin results in prorenin, which is also biologically inactive. Subsequent glycosylation and proteolytic cleavage leads to formation of renin, a 37 to 40 kDa proteolytic enzyme. Both prorenin and renin are secreted from juxtaglomerular cells. Because prorenin is the major circulating form, it is postulated that significant conversion of prorenin to renin follows secretion. Prorenin-activating enzymes have been localized to neutrophils, endothelial cells, and the kidney (77). In addition to juxtaglomerular cells, renin production has also been detected in the submandibular gland, liver, brain, prostate, testis, ovary, spleen, pituitary, thymus, and lung (77). Circulating renin, however, appears to be derived entirely from the kidney.

Stimulation of renin release by juxtaglomerular cells is mediated by increased intracellular cAMP, while a rise in cytosolic free calcium is inhibitory (80). Physiologic regulators of renin secretion include the urinary NaCl concentration sensed by MD cells in the distal tubule, activity of the sympathetic nervous system, alterations in intrarenal perfusion pressure, and endocrine and paracrine hormones and growth factors. Decreased NaCl delivery to MD cells stimulates renin secretion, whereas increased urinary NaCl exerts an opposite effect (81). Schlatter and co-workers (82) demonstrate that changes in luminal Cl concentration alter the rate of Na-K-2Cl transport in macula densa cells (82). The precise mechanism by which variation in the activity of this transporter translates to a signal that regulates renin release by adjacent juxtaglomerular granular cells is not entirely clear. Postulated mediators include

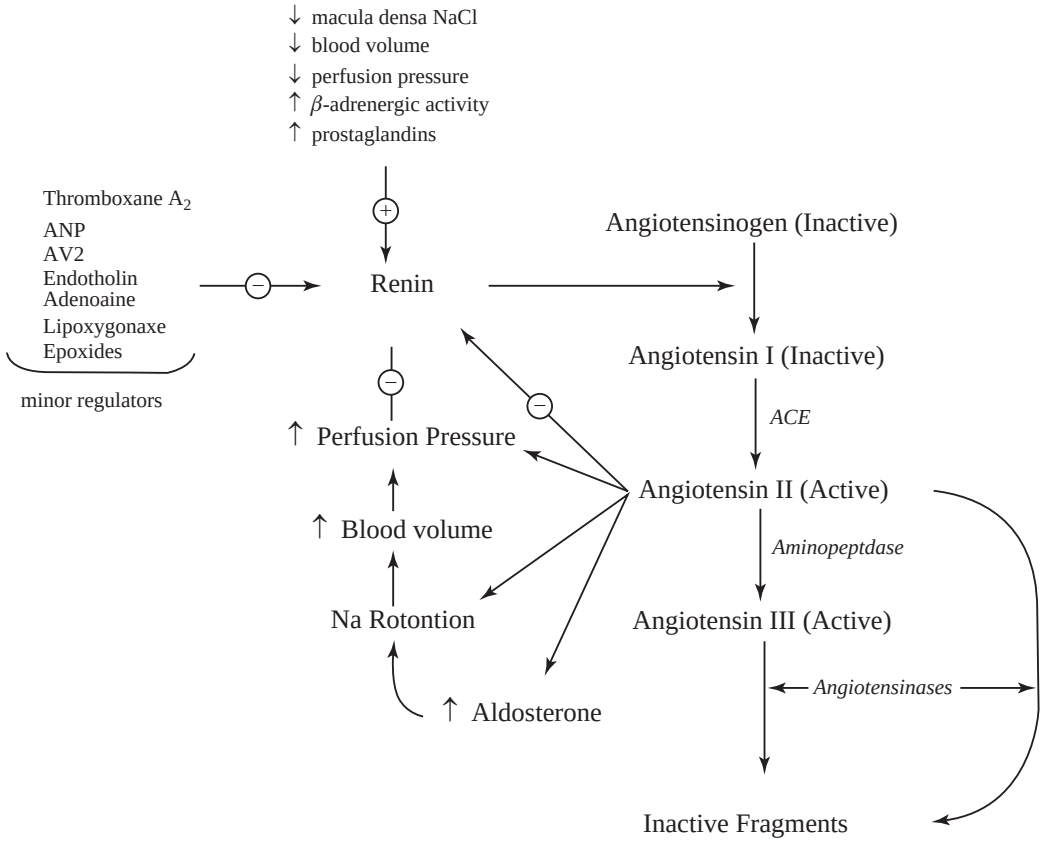

FIGURE 9-3. Components of the renin–angiotensin system. The major feedback inhibitory loop for renin release is via production of angiotensin II.

adenosine, which inhibits renin secretion via activation of A_1 receptors on juxtaglomerular cells, and alterations in interstitial osmolality, which may affect renin secretion directly (81). Experimental evidence also suggests that nitric oxide (NO) produced by MD cells and endothelial cells regulates renin secretion (81,83).

Intraluminal conversion of angiotensin I (Ang I) to angiotensin II (Ang II) can occur in the cortical collecting duct, resulting in enhanced apical sodium entry (84).

Ang II generation in mesangial cells (MC) is increased by high-glucose (HG) exposure resulting from an increase in intracellular renin activity mediated by at least three factors: a time-dependent stimulation of (pro) renin gene transcription, a reduction in prorenin enzyme secretion, and an increased rate of conversion of prorenin to active renin, probably mediated by cathepsin B. The consistent upregulation of ACE mRNA suggests that, besides renin, ACE is directly involved in the increased mesangial Ang II generation induced by high glucose (85). On the other hand, Ang II regulates glucose transporter (GLUT1) gene expression (86).

The importance of renal sympathetic innervation in controlling renin secretion is well recognized (87). Stimulation of postjunctional β-adrenergic receptors increases renin release. The role of α-adrenergic receptors, on the other hand, is controversial (87). Ample evidence suggests that dopamine stimulates renin secretion by direct activation of DA_1 receptors on juxtaglomerular cells (87,88).

Renin release responds inversely to changes in renal perfusion pressure (79). Elevation of intrarenal arterial pressure inhibits renin release and induces a "pressure" natriuresis. At least two mechanisms have been postulated. Increased afferent arteriolar wall tension secondary to increased renal perfusion elevates intracellular calcium in juxtaglomerular cells and inhibits renin secretion (79,80). Increased perfusion pressure also stimulates NO production and release by endothelial cells. NO, in turn, suppresses renin secretion (81,89). Conversely, decreased renal perfusion results in increased production of prostacyclin (prostaglandin I_2), which enhances renin release (90).

Mechanical strain leads to upregulation of the AT1R and increased Ang II production in conditionally immortalized podocytes. The resulting activation of a local tissue angiotensin system leads to an increase in podocyte apoptosis, mainly in an AT1R-mediated fashion (91).

Several endocrine and paracrine hormones regulate renin secretion by the kidney. ANP has been shown to inhibit renin release from isolated juxtaglomerular cells (92). Other inhibitory hormones include AVP, endothelin, and adenosine (A_1-receptor agonists) (79,81). Regulation of renin secretion by AngII is probably the most physiologically relevant (93). Ang II inhibits renin secretion and renin–gene expression in a negative feedback loop. Treatment of transgenic mice bearing the human renin gene with an angiotensin-converting enzyme (ACE) inhibitor increases renin expression in the kidney by five- to tenfold (94). Similarly, ACE inhibition in rats augments renal renin mRNA expression, an effect that is reversed by infusion of Ang II (95). The effects of AngII are believed to be direct and not dependent on changes in renal hemodynamics or tubular transport. Arachidonic acid metabolites produced in the kidney also play an important role in renin secretion (81). Intrarenal infusion of arachidonic acid increases (and indomethacin decreases) plasma renin activity in rabbits (96). Several studies have since confirmed that prostaglandins of the I series are potent stimulators of renin secretion (81,90). On the other hand, lipoxygenase products of arachidonic acid metabolism (12-HPETE, 15-HPETE, and 12-HETE) and cytochrome

P450-mediated epoxides (14,15-epoxyeicosatrienoic acid) have been shown to inhibit renin release in renal cortical slices (97,98).

Biologic Actions of Angiotensin II

Secretion of renin into the circulation, as in situations of decreased intravascular arterial volume, initiates an enzymatic cascade that leads to the production of Ang II (77) (Fig. 9-3). Angiotensinogen, derived from hepatocytes, is a 55–65 kDa globular glycoprotein, which is cleaved by renin to form the decapeptide Ang I. ACE then converts Ang I to the biologically active octapeptide Ang II. ACE is a dipeptidyl carboxypeptidase present in nearly all mammalian tissues and body fluids (77). Endothelial cells constitute a major source of ACE activity. In plasma, all conversion of Ang I to Ang II occurs by the activity of ACE; species variation has not been reported. However, non–ACE-dependent pathways exist at the tissue level and have species variation. In humans, tissue activity of chymase can allow for the local formation of Ang II in the heart, arteries, and kidney. In rats and rabbits, tissue activity of chymase is associated with the local degradation (instead of formation) of Ang II. Therefore, one must carefully evaluate experimental animal data when pharmacologic blockade of the renin–angiotensin system is used (99).

Circulating Ang II exerts its biologic effects by binding to specific receptors on the cell surface (77,100). On the basis of pharmacologic, physiologic, and biochemical evidence, two subtypes of angiotensin II (AT_2) receptors have been identified. AT_1 receptors bind Ang II with higher affinity than angiotensin III (Ang III) and are selectively blocked by the biphenylimidazole compound losartan. AT_2 receptors bind Ang II and III with similar affinity and are selectively blocked by tetrahydroimidazopyridines, such as PD123177 (101).

Megalin is an abundant membrane protein heavily involved in receptor-mediated endocytosis. Megalin is a receptor for Ang II and Ang II internalization in some tissues is megalin-dependent. Megalin may play a role in regulating proximal tubule AngII levels (102).

AT_1 receptors have been shown to mediate many of the functions of Ang II in the regulation of blood volume: cell contraction, cell proliferation, aldosterone secretion, pressor and tachycardic responses, increased thirst, and hypertension secondary to renal artery stenosis. In the rodent, two isoforms of AT_1 receptors exist: AT_{1A}(nephron) and AT_{1B}(glomerulus), while in the human there is only the AT_1 isoform.

There is evidence for the existence of other angiotensin receptors that mediate the renal effects of Ang (1–7) and angiotensin IV (Ang IV) (103). AT_1 receptors are positively coupled to phospholipase C and mitogen-activated protein kinases (PI3 and MAP) and negatively to adenylate cyclase (101,104,105). Stimulation of the AT_1 receptor in murine mesangial cells results in activation of the EGF receptor with subsequent signaling through PI3 kinase and MAP kinase, thereby regulating TGF-β mRNA levels. These data suggest that AT_1 receptor signaling pathways through EGFR may serve as a therapeutic target to inhibit the development of chronic kidney disease (106). The AT_1 receptor has widespread expression in the adult and is found in the kidney, adrenal gland, heart, and brain. In the kidney, AT_1 receptors are found in the glomeruli, proximal tubule brush border and basolateral membranes, thick ascending loop, proximal convoluted tubule, renal vasculature, the proximal and distal nephron segments, and in both cortical and medullary regions (104,107). In addition to its effects on the maintenance of blood pressure, AT_1 receptors may play a role in embryonic nephrogenesis. Blockage of the renin-angiotensin system with ACE inhibitors or AT_1 inhibitors results in abnormal renal development that is characterized by both papillary and tubular atrophy and by interstitial fibrosis and infiltration. In addition, knockout mice lacking both the AT_{1A} and AT_{1B} receptor have similar renal abnormalities (108). Ang II stimulates carbon monoxide (CO) production and release in isolated, perfused rat kidneys, linked to the activation of AT_1 receptors and involves PKC activation and upregulation of renal heme oxygenase-1 isoform (HO-1), but not HO-2 protein expression. Upregulation of renal HO-1 and CO release are protagonic events in a counterregulatory mechanism that attenuates Ang II-induced renal vasoconstriction (109). Liu and Persson have found that Ang II increased Ca^{2+} and stimulated NO production in rabbit MD cells from the basolateral and luminal sides through AT_1 receptors (110).

The predominant physiologic role of the AT_2 receptor is to initiate vasodilation and natriuresis as a counterregulatory response to the vasoconstriction caused by activation of the AT_1 receptor (111). Other functions are: antigrowth, antihypertrophic, and proapoptotic (112). This has been most clearly demonstrated in AT_2 receptor knockout mice that have slightly elevated blood pressure in the basal state, but have an exaggerated increase in blood pressure in response to Ang II infusion compared to wild-type mice (113,114). The intracellular signaling pathways coupled to the AT_2 receptor are unclear. However, there is evidence that the AT_2 receptor may be coupled to the production of a variety of renal vasodilator substances, counterregulating the pressor effects of Ang II via AT_1 receptors (112). The most likely candidates are bradykinin and nitric oxide (NO)-stimulated cyclic guanosine monophosphate (cGMP) (115,116), but other candidates include products of cyclooxygenase, such as PGE_2 and PGI_2 (111). The AT_2 receptor is expressed predominantly in fetal tissues, but in almost all tissues there is postnatal downregulation. AT_2 receptor mRNA is expressed in the fetal and neonatal rat kidney, but disappears after the neonatal period and is not expressed in the normal adult. While the AT_2 receptor mRNA is not found in the adult kidney, both immunohistochemistry and Western blot analysis have detected AT_2 receptor protein in the adult kidney (117). In the adult kidney, AT_2 II receptors are present on glomeruli, cortical tubules, and interstitial cells (100,117). It was thought that AT_2 receptors might play a role in the development of the kidney and urinary tract given the high levels of expression in the fetus. However, while early studies of AT_2 receptor knockout mice showed no gross morphologic abnormalities of the kidney (113,114), a more recent study has demonstrated the presence of increased numbers of congenital anomalies of the urinary tract (118). The AT_2 receptor may play a role in the formation of some congenital abnormalities of the urinary tract or may be involved in the pathophysiologic response to ureteral obstruction by protecting against the formation of interstitial fibrosis (118,119). Ang II modulates the over-expression of AT_2 receptors in renal ablation experiments through its own AT_2 receptor and functional expression of this effect may represent a counterregulatory mechanism to modulate the renal damage induced by renal ablation (120).

The predominant function of the renin-angiotensin system is regulation of vascular tone and renal salt excretion in response to changes in the volume of extracellular fluid or blood pressure. Ang II represents the effector limb of this hormonal system, acting on several organs, including the vascular system, heart, adrenal glands, central nervous system, and kidneys (Fig. 9-4).

Vascular Actions of Angiotensin II

Through direct action on smooth muscle cells, Ang II significantly increases arteriolar resistance in renal, mesenteric, dermal, coronary, and cerebral vascular beds (121). Skeletal muscle and pulmonary vessels, on the other hand, are not affected because of Ang II-stimulated production of vasodilatory

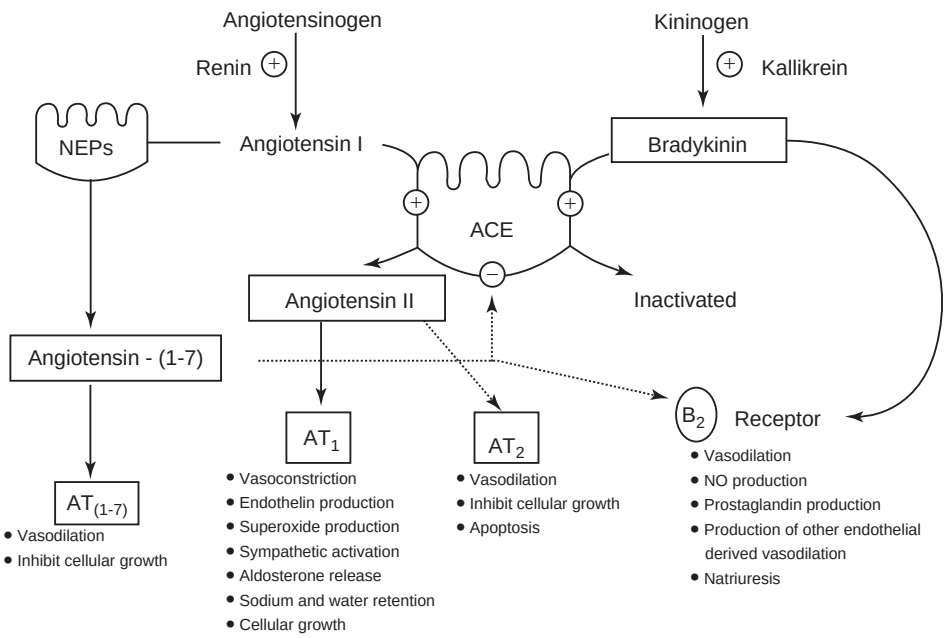

FIGURE 9-4. The renin–angiotensin and kallikrein-kinin systems. *ACE*, angiotensin-converting enzyme; *AT_1*, angiotensin II type 1; *AT(1-7)*, angiotensin 1-7; *AT_2*, angiotensin II type 2; *NEPs*, neutral endopeptidase inhibitors; NO, nitric oxide. (From: Pepine CJ, Handberg EM. The vascular biology of hypertension and atherosclerosis and intervention with calcium antagonists and angiotensin-converting enzyme inhibitors. *Clin Cardiol* 2001;24(Suppl V):1, with permission.)

prostaglandins by endothelial and smooth muscle cells in these vascular beds (122,123). Ang II exerts indirect pressor effects via the central and peripheral nervous systems. Its effects on the central nervous system include increased sympathetic discharge and decreased vagal tone (124). Peripherally, Ang II augments the vasoconstrictive response to renal nerve stimulation in dogs (125) and its inhibition attenuates the pressor response to norepinephrine in humans (126). Experimental data suggest the presence of a local renin–angiotensin system in the vasculature that contributes to the regulation of vascular tone (77).

Endothelin through endothelin-A (ET-A) receptors contributes substantially to the systemic and renal vasoconstriction of low-dose exogenous Ang II in healthy humans (127).

Growth-Promoting Actions of Angiotensin II

A more recently recognized function of Ang II is its growth-promoting effects in smooth muscles of the vasculature, heart, and the kidney. Ang II has been shown to induce hypertrophy and mitogenesis in cultured vascular smooth muscles (128,129). This effect is at least, in part, mediated through autocrine production of growth factors such as platelet-derived growth factor (PDGF) and transforming growth factor-*beta* (TGFβ) (130). Ang II induces a rapid increase in vascular endothelial growth factor (VEGF) protein expression at the initiation phase of translation of VEGF mRNA in renal epithelial cells. Regulation of VEGF translation by Ang II represents a novel pathway of renal response to injury (131). Ang II and hyperglycemia stimulate the TGF-β 1 gene activation through the same PKC- and p38 MAPK-dependent pathways in mesangial cells (132).

Upregulation of AngRem104, a novel gene upregulated in mesangial cells, induced by Ang II was time-dependent and was dose-dependently blocked by the angiotensin II type-1 receptor antagonist, losartan, associated with fibronectin expression and glomerular sclerosis (133). There is *in vivo* evidence that injured glomeruli are sensitive to local tissue actions of Ang II, which promote proliferation and matrix accumulation within the glomerulus (134).

Blockade of Ang II raises PO_2 in the interstitial microvascular compartment of the normal rat kidney. This effect may contribute to the renoprotective action of ACE inhibitors and Ang II receptor antagonists in slowing the progression of chronic renal diseases (135).

In tubular epithelial cells, Ang II upregulates macrophage migration inhibitory factor (MIF) mRNA production and MIF protein secretion. Ang II may promote accumulation and activation of interstitial leukocytes via induction of MIF synthesis and secretion in renal tubular epithelial cells. This may be an important mechanism by which Ang II mediates renal injury (136).

Some studies suggest that the renin–angiotensin system contributes to neointimal formation and restenosis after angioplasty (77). Ang II has direct inotropic, chronotropic, mitogenic, and hypertrophic effects on isolated atria and ventricles (77). Amelioration of hypertensive cardiomyopathy by ACE inhibitors suggests that the renin–angiotensin system play a role in cardiac hypertrophy (77). Ang II also modulates mesangial cell growth, as discussed in the next section, and induces proximal tubular cell hypertrophy in humans, effectively inhibited by irbesartan, an Ang II receptor antagonist (137).

On the contrary, Ang II promoted podocyte apoptosis in a time- and dose-dependent manner through stimulation of p38MAPK and inhibition of JNK (both are MAPK subtypes) (138) and, using the same protein kinase, Ang II-induced activation of latent TGF-β_1 via thrombospondin-1 (139); it also induces glomerular cell apoptosis by a mechanism involving TGF-β_1 expression (140).

Ang II regulates renal parathyroid hormone-related protein (PTHrP), a vasodilator and mitogenic agent upregulated in kidney injury,and its type-I receptor (PTH1R) system via AT_1 receptors (141).

Renal Actions of Angiotensin II

Ang II serves at least three important functions in the kidney: autoregulation of GFR, reduction of salt excretion through direct and indirect actions on renal tubule cells, and growth modulation in renal cells expressing AT_1 receptors.

In conditions of decreased renal blood flow, GFR is preserved at nearly constant value over a wide range of perfusion pressures. This phenomenon is known as *autoregulation* of GFR. At lower levels of renal perfusion pressures, Ang II contributes to this phenomenon (142). Micropuncture studies have shown that Ang II infusion preferentially vasoconstricts

efferent arterioles counteracted by endothelial-derived NO (143), while leaving afferent arteriolar resistance unaltered (10) and constricting descending vasa recta (DVR) through Ca^{2+} signaling in pericytes (144). The disproportionate increase in postglomerular resistance results in a marked increase in P_{GC}, ultrafiltration pressure,and filtration fraction, thus preserving GFR in the face of declining renal plasma flow (RPF). The selectivity of the vasoconstrictive action of Ang II for the efferent arteriole results from stimulation of vasodilatory prostacyclin synthesis by the afferent arteriole (11). In fact, Ang II increases both afferent and efferent resistance in the presence of a cyclooxygenase inhibitor (11). Under certain pathophysiologic conditions, afferent arteriolar constriction predominates, leading to a decrease in both RPF and GFR (145). Deep nephrons have higher postglomerular Ang II tone and also higher Ang II sensitivity than superficial nephrons. The better preserved GFR in deep cortex during Ang II action may contribute toward maintaining the renal-concentrating ability by providing NaCl for reabsorption by the ascending limb of the loop of Henle (146).

In addition to its vascular effects, Ang II induces mesangial cell contraction, which leads to decreased K_f *in vivo* (147,148). This effect, however, is attenuated by the concomitant production of prostaglandins by mesangial cells (149).

Physiologic concentrations of Ang II (10^{-12} to 10^{-10} M) stimulate proximal tubule NaCl and $NaHCO_3$ absorption through both indirect and direct effects on the proximal tubule (93,150). Indirectly, Ang II stimulates ion transport in the proximal tubule by changing the peritubular milieu. Ang II can both decrease peritubular capillary hydrostatic pressure and increase peritubular oncotic pressure, resulting in an increased driving force for ion reabsorption. Directly, Ang II can stimulate transport in the proximal tubule through interaction with the AT_1 receptors found on both the apical and basolateral membranes of the tubule cells (151,152). It also stimulates calcineurin phosphatase activity in proximal tubule epithelial cells through a mechanism involving AT_1 receptor-mediated tyrosine phosphorylation of the PLC isoform, both linked to sodium transport in the proximal tubule (153). Specifically, Ang II stimulates the activity of apical membrane Na–H antiporters and basolateral membrane Na–HCO_3–CO_2 cotransporters (154) and the activity of Na–K-ATPase by changing phosphorylation and conformation of Na–K-ATPase (155). The net effect is increased proximal tubule reclamation of Na and HCO_3. Denervation of the proximal tubule results in attenuation of the Ang II-stimulated NaCl, but not $NaHCO_3$ absorption, suggesting that Ang II enhances proximal Na transport indirectly by increasing presynaptic catecholamine release (152). Of note is that supraphysiologic concentrations of Ang II (10^{-9} to 10^{-7} M) inhibit NaCl and water reabsorption in the proximal tubule (93) and also inhibit Na–glucose cotransporter translocation by inactivation of PKA and decrease of PI3-kinase activity mediated through the AT_1 receptor (156,157). Intraluminal conversion of Ang I to Ang II can occur in the cortical collecting duct, resulting in enhanced apical sodium entry (158). The AT_2 receptor regulates ENaC abundance, consistent with a role for Ang II in regulation of collecting duct function via AT_1receptors (159,160).

On the other hand, Ang II added to the luminal fluid, stimulates ammonia production and secretion to a greater extent in S3 proximal tubule cells segments from acidotic mice. These findings suggest that S3 segments, in the presence of Ang II, can contribute to the enhanced renal excretion that occurs with acid loading (161).

It was demonstrated in rats that the effects of prolonged Ang II to cause hypertension by probable interaction with the endothelin system (162), renal vasoconstriction, renal cortical hypoxia, and reduced efficiency of oxygen usage for Na^+ transport, activation of NADPH oxidase, increased expression of p22 (phox), and reduced expression of EC-SOD can be ascribed to superoxide anion O^{2-}generation (163) that might reduce the local bioavailability of NO (164).

Ang II increases proximal tubule phosphate absorption by direct stimulation of Na/P_i cotransport activity as a result of increase in the expression of brush-border membrane NaPi-IIa protein level and that stimulation is most likely mediated by posttranscriptional mechanisms (165).

Ang II increases basolateral K-channel activity via the stimulation of AT_1 receptors and the stimulatory effect of Ang II is mediated by a NO-dependent cGMP pathway (166).

Other effects of Ang II on proximal tubule cells include enhanced gluconeogenesis and ammonia production (93). The effects of Ang II on distal tubule transport of Na and K are mediated through aldosterone release (167). In addition to proximal tubule epithelial cells, vasa recta, and outer medullary vascular bundles express high density of AT_1 receptors (100). ACE inhibitors increase descending vasa recta blood flow, while Ang II infusion markedly decreases medullary blood flow in rats (168). It is postulated that Ang II influences urinary dilution and concentration by modulating blood flow to the medulla.

Ang II induces hypertrophy of proximal tubule epithelial cells *in vitro* (169). It also exerts similar growth-promoting effects on mesangial cells (170). The signaling mechanism by which Ang II exerts this effect is not precisely known, but p27Kip1 is required for Ang II-induced hypertrophy (171). Downstream potential targets of Ang II are the extracellular signal-regulated kinases 1 and 2 (ERK1/ERK2) and Ang II activates ERK1/ERK2 via the AT_1 receptor (172). Studies have shown that connective tissue growth factor (CTGF) might be an important mediator of Ang II-induced renal hypertrophy, which suggests that inhibiting the production of CTGF might be the new strategy in early prevention of renal fibrosis (173). Ang II can stimulate human kidney fibroblast proliferation and enhance the expression of interleukin-6 of KFB. These findings suggest that Ang II might play a part in the mechanisms for modulating tubulointerstitial changes and inducing renal fibrosis (174).

These observations suggest a potential role for Ang II in mediating renal hypertrophic responses to nephron loss following acute ischemic or toxic injury or uninephrectomy.

Previous *in vivo* studies in cardiomyopathic hamsters suggested that the expression of vasopressin (AVP) V_2 mRNA is upregulated by Ang II. Ang II caused a significant increase in the AVP V_2 mRNA in a dose-dependent manner mediated by PKA, while PKC suppresses the expression of V_2 mRNA in the IMCD of the rat kidney (175).

Ang II decreases AT_1 expression in normotensive, but increases it in hypertensive rats. In addition, Ang II decreases D_3 expression to a greater extent in hypertensive than in normotensive rats. Aberrant interactions between D_3 and AT_1 receptors may play a role in the pathogenesis of hypertension (176).

Extrarenal Actions of Angiotensin II

Ang II exerts its actions on other organs, like the adrenals and the brain, and regulates Na, water, and K homeostasis per se.

At the level of the adrenals, it stimulates aldosterone synthesis and secretion (167). Acute angiotensin administration stimulates the activity of 11β-HSD type 2 in human kidney and exerts a dual effect on the mineral corticoid receptor (i.e., an indirect agonistic effect by increasing aldosterone availability and a direct or indirect antagonistic effect by stimulation of renal 11β HSD type 2 activity) (177).

At the CNS level, it stimulates thirst and salt appetite (178,179) and may increase secretion of vasopressin and oxytocin from the posterior pituitary, and adrenocorticotropic hormone (ACTH), prolactin, and luteinizing hormone from the

anterior pituitary (180). Physiological changes in the activity of the RAS produced by alterations in dietary sodium intake regulate the contribution of endogenous Ang II of brain origin in the modulation of arterial baroreflex regulation of renal SNA (181). Although a direct effect of the renal nerves on sodium excretion is clearly present, the antidiuresis and antinatriuresis phenomena observed during renal activation is further supported by a neurally mediated increase in systemic Ang II (182).

Angiotensin II and Tubular Development

Throughout nephrogenesis, AT_2 receptors are expressed in undifferentiated mesenchyme and are downregulated after birth. AT_1 receptors initially exhibit a widespread localization in the nephrogenic cortex and developing glomeruli, proximal tubules, and vessels. They then become more abundant during maturation of the kidney.

Blocking of RAS with ACE inhibitors or AT receptor blockers from postnatal day 3 onward results in severe renal abnormalities that are characterized by papillary atrophy, tubular atrophy , tubulointerstitial inflammation, and thickening of intrarenal arteries, mostly critical when drugs are administered in rats at E15-20.

Angiotensinogen and ACE knockout mice develop renal phenotypes that are identical to those obtained by pharmacologic inhibition of RAS.

Ang II induces expression of homeobox genes, like *HOX 2.3*, in proximal tubular cells, and promotes proliferation of tubular cells derived from the thick ascending limb of the loop of Henle, mediated by AT_1 receptors, versus apoptosis through AT_2 receptors.

Ang II upregulates TGF-β, bone morphogenic protein (BMP-7), and VEGF (183).

The Intrarenal Renin–Angiotensin System

Endogenous renin–angiotensin systems have been identified in several organs, including the heart, vasculature, brain, and adrenals (184). In addition, all components of the renin–angiotensin system are expressed in the adult kidney (93,150).

Compared with plasma levels, the Ang II tissue contents are much higher despite suppression of renin secretion and release (107). Renin is principally produced by juxtaglomerular cells of the distal afferent arteriole, but has been shown to be expressed in the proximal tubule cells (107), whereas its substrate, angiotensinogen, is expressed by proximal tubule cells. ACE activity in the kidney has also been localized to the proximal tubule, with the highest concentration present on the brush border. Several studies have provided evidence for production of Ang II by the kidney, mainly concentrated in the proximal tubules (107), suggesting that the intrarenal renin-angiotensin system is, indeed, functional (150). Some investigators have proposed that this local system plays a role in proximal tubule NaCl and HCO_3 absorption, pathogenesis of essential hypertension, and expression of the phenotype of autosomal dominant polycystic kidney disease (150). Regulation of renal Ang II production independent of the systemic renin–angiotensin system has not been definitively demonstrated. Circulating Ang II stimulates renal angiotensinogen mRNA production and intact urine angiotensinogen suggests its presence along the whole nephron and that renin and ACE activity are available all through the nephron (107).

Endogenous Ang II in both peritubular blood and luminal fluid is important for maximal expression of the stimulatory influence of this peptide on proximal tubule fluid uptake (185). Physiological concentrations of Ang II can induce apoptosis of human renal proximal tubule epithelial cells. This effect is mediated via AT_2 receptors (186).

Ang II-dependent hypertension results in elevated intrarenal Ang II and angiotensinogen levels, reflected by increased U (AGT), which does not occur in an Ang II-independent hypertensive model (187).

Renal degradation of AngII is constitutively high, is unaffected by chronic levels of arterial blood pressure, and is independent of long-term changes in levels (188).

Low-density lipoproteins (LDL) increases Ang II production by mesangial cells, which, in turn, results in increased O_2 production, cell proliferation, and hypertrophy, these effects of Ang II being mediated by the AT_1 receptor (189).

Angiotensin (1 to 7)

The heptapeptide Ang (1to 7) is currently considered one of the biologically active end products of the RAS. It is formed from Ang I or Ang II in the kidney and heart by the action of tissue peptidases. Once formed it is rapidly hydrolyzed by ACE; in conditions of ACE inhibition or AT_1 receptor antagonism, its concentration may increase severalfold. There are two major interactions between Ang (1 to 7) and bradykinin (BK): potentiation of BK by Ang (1–7) and mediation of vascular actions of Ang (1–7) by kinins, both mechanisms involved in the cardioprotective effects of ACE inhibitors.

At the kidney level, its proposed role is natriuresis and diuresis as opposed to Ang II, an effect blocked by AT_1 receptor antagonist like losartan.

In the vascular bed, it antagonizes the vascular effect of Ang II by acting as a competitive antagonist to AT_1 receptors (103).

The Renin–Angiotensin System in Transgenic Rats

Transgenic animals provide the opportunity to study effects of hormone over-expression on physiologic parameters or pathologic phenotypes. The importance of the renin-angiotensin system in systemic blood pressure control led to interest in its possible contribution to the pathogenesis of hypertension. Mullins and co-workers (190,191) introduced the mouse *Ren-2* gene into normotensive rats, thus creating a transgenic strain that expresses high levels of Ren-2 mRNA in the adrenal glands and, to a much lesser extent, in the kidneys. In these rats, fulminant hypertension developed between 5 and 10 weeks of age. The hypertensive phenotype was dependent on Ang II because treatment with low-dose ACE inhibitors or with Ang II antagonists normalized blood pressure (192). Moreover, male rats had significantly higher blood pressures than females, possibly because of the stimulatory effect of androgens on tissue renin–angiotensin systems (193). This observation is consistent with epidemiological data demonstrating a higher prevalence of hypertension among men. Despite severe hypertension in Ren-2 transgenic rats, the systemic renin-angiotensin system was not stimulated and plasma levels of active renin, angiotensinogen, Ang I, and AngII were lower than those in control animals (190). In contrast, plasma concentration of prorenin was dramatically elevated, and it derived mainly from the adrenal glands. The fact that adrenalectomy normalized blood pressure in the Ren-2 transgenic animals led to the conclusion that a stimulated local adrenal renin–angiotensin system rather than systemically elevated renin levels contributed to hypertension in these animals (191).

Because activation of the renin-angiotensin system has been implicated in the progression of chronic renal disease

(194), Ganten and colleagues (191,195) studied progression of glomerular sclerosis after subtotal nephrectomy in Ren-2 transgenic rats. Compared to pressure-matched spontaneously hypertensive rats, the transgenic animals had significant acceleration of glomerulosclerosis, suggesting a pathogenic role for the intrarenal renin-angiotensin system in progression of renal failure.

Rats have been bred that are transgenic for either the human renin or angiotensinogen genes (191,195). Although these animals had high circulating levels of either renin or angiotensinogen, their Ang II concentrations were normal, and they remained normotensive. These negative findings can be explained by the species specificity of renin–angiotensinogen interaction. Human renin does not act on rat angiotensinogen, and human angiotensinogen does not serve as a substrate for rat renin (191). Similar observations were made in mice transgenic for human renin (196). In contrast to the single human transgenic mice, double transgenic mice (dTGR) for both the human angiotensin and renin genes develop moderately severe hypertension and die of end-organ cardiac and renal disease (197). Fibrinoid necrosis of vessel walls, thrombi formation in small vessels, and infiltration of organs characterize the end-organ damage by monocytes and macrophages (197,198). These organs have increased vascular expression of adhesion molecules: ICAM-1, VCAM-1, PAI-1, and integrins that localize to areas of morphologic damage. Treatment of the dTGR mice with inhibitors to ACE, renin, or AT_1 can lower blood pressure and markedly reduce organ damage (198). Recently a role for Ang II-stimulated reactive oxygen species (ROS) in the pathogenesis has been postulated. Treatment of dTGR rats with pyrrolidine dithiocarbamate (PDTC), an antioxidant, reduced the severity of Ang II-mediated end-organ damage in these animals. It is postulated that the protective mechanism is via inhibition of nuclear factor-γB (NF-γB) (199).

The data from transgenic animals, therefore, suggest that in the setting of high renin–angiotensin levels, direct vascular damage can occur leading to end-organ damage, characterized by inflammatory infiltrates and fibrinoid necrosis of vessels. This process may be mediated by both the upregulation of adhesion molecules and by increased production of ROS (197).

Physiologic Consequences of Disruption of Angiotensin II Receptor Genes (AT_1 and AT_2 Gene-Knockout Mice)

To examine the physiologic functions of the AT_1 receptor, Ito and associates (200) disrupted the mouse gene that encodes this receptor in embryonic stem cells by homologous recombination. Mice homozygous for the disrupted gene do not express AT_1 receptors and have undetectable AT_1 receptor-specific Ang II binding in their kidneys. In addition, pressor responses to infused Ang II are virtually absent. Compared to wild-type controls, systolic blood pressure is reduced by 24 mm Hg in homozygous AT_1 knockout mice. This study demonstrates that AT_1 receptors mediate the pressor functions of Ang II and that intact expression of AT_1 receptors is required for maintenance of normal blood pressure. Furthermore, AT_1 knockout mice have polyuria, which is thought to result from the dysmorphogenesis of the renal medulla observed in these mice. Afferent arteriole vasoconstrictor responses to Ang II are mediated by AT_{1A} and AT_{1B} receptors, whereas efferent arteriolar vasoconstrictor responses to Ang II are mediated by only AT_{1A} receptors in the mouse kidney (201).

In contrast, genetically engineered mice that are homozygous for the disrupted AT_2 receptor gene have normal baseline blood pressure (113,114). Interestingly, these mice have increased pressor response to Ang II injection, suggesting that the AT_2 receptor may play a role in modulating the cardiovascular actions of Ang II. Early studies suggested that there were no histomorphologic abnormalities observed in either the kidneys or the cardiovascular system of AT_2 receptor gene-knockout mice (113,114). However, a more recent study has shown an increase in the rate of congenital abnormalities (118). Furthermore, while there are no differences in baseline water intake in the AT_2 knockout mice compared to wild type mice, the AT_2 knockout mice had impaired water intake in response to water deprivation, perhaps suggesting a decrease in thirst (113,114).

Angiotensinogen Gene-Knockout Mice

Angiotensinogen-deficient mice generated by homologous recombination in mouse embryonic stem cells do not produce angiotensinogen in the liver, resulting in the complete loss of plasma-immunoreactive Ang I (202). The systolic blood pressure of homozygous mutant mice is approximately 35 mm Hg lower than that of wild-type. This profound hypotension in angiotensinogen-deficient mice demonstrates that the renin–angiotensin system is essential for maintaining normal blood pressure.

ATRIAL NATRIURETIC PEPTIDE

Electron microscopic studies conducted by Kisch in 1956 (203) and by Jamieson and Palade in 1964 (204) revealed the presence of secretory granules of unknown function in the atrial myocytes of mammalian hearts. A relationship between the number of atrial granules and perturbations of water and sodium homeostasis was later observed in laboratory animals (205,206). In 1981, de Bold and colleagues (207) demonstrated that injection of crude rat atrial, but not ventricular, extracts into anesthetized rats induced a more than 30-fold increase in urinary sodium excretion, a 10-fold rise in urine volume, and a sustained decrease in systemic blood pressure. Further biochemical studies localized the natriuretic activity to the atrial granules described two decades earlier and led to the isolation and characterization of atrial natriuretic peptide/cardiodilatin ANP (208,209).

Molecular and Biochemical Properties of Atrial Natriuretic Peptide

The cDNA for human ANP was isolated in 1984, and, shortly afterward, the gene was localized to the short arm of chromosome 1 (210,211). The chromosomal gene consists of three exons and two introns encoding for a mature mRNA transcript approximately 900 bases long (211).

Translation of human ANP mRNA results in a 151-amino acid preprohormone (212). Pro-ANP, a 126-residue molecule, is formed after cleavage of the signal peptide sequence of prepro-ANP and represents the major storage form of the hormone in atrial granules (213). The circulating, biologically active form of ANP, often referred to as AN_{99-126} or ANP_{1-28}, is a peptide comprising the 28 carboxy-terminal amino acids of the parent molecule (208,212). The proteolytic mechanism that releases ANP_{99-126} from the amino-terminal fragment of the prohormone is not known. The amino acid sequence of ANP_{99-126} is highly conserved among mammalian species (212). A disulfide bond between cysteine residues 105 and 121 gives ANP_{99-126} its ring structure, which is essential for biologic activity (214).

Pro-ANP (1–30), (31–67), and (68–98) are secreted from the heart and circulate in the plasma. Pro-ANP (1–30) and

(31–67) increase sodium and water excretion and binding sites have been found in the proximal tubules and collecting ducts. Pro-ANP (31–67) inhibits the Na^+-K^+pump at the medullary collecting duct through a prostaglandin-dependent mechanism and no effect on cGMP production. Pro-ANP (1–30) infusion in rats clearly increases urine output, sodium, and potassium excretion, the mechanism of which still needs to be elucidated (215).

Secretion and Physiologic Regulation of Atrial Natriuretic Peptide$_{99-126}$

Cardiac atria contain the highest concentrations of ANP and serve as the major source of circulating hormone (212). ANP is present in much lower concentrations in other tissues, such as the brain, spinal cord, pituitary gland, adrenal medulla, and kidney. Although these organs do not seem to contribute to circulating levels of the hormone, local regulatory functions of ANP are possible.

ANP_{99-126} secretion from cardiomyocytes occurs largely in response to atrial stretch resulting from increased atrial transmural pressure (216). Physiologic stimuli for the release of $ANP_{99-126,}$ include acute salt and volume loading, supine posture (head-down tilt), and head-out water immersion (216–218). An increased rate of atrial contraction has also been shown to stimulate ANP_{99-126} secretion (219,220). Whether this occurs by a mechanism independent of changes in atrial transmural pressure remains controversial (216,221). Elevated plasma ANP_{99-126} levels measured during exercise appear to be secondary to both increased heart rate and atrial distention (218). Ang II, vasopressin, epinephrine, and phenylephrine stimulate ANP_{99-126} secretion from the heart largely because of their systemic vasopressor effects (222). On the other hand, glucocorticoids and endothelin raise ANP_{99-126} levels possibly by acting directly on atrial myocytes (223, 224). Physiologic and pathologic conditions in which elevated plasma levels of ANP_{99-126} have been detected are summarized in Table 9-2 (225–240). In many of these pathologic situations, chronic hyperosmolality (241) increased plasma volume, resulting in elevated atrial transmural pressure. Paradoxically, ANP_{99-126} secretion is inhibited during cardiac tamponade, suggesting that increased intraatrial pressure per se without myocyte stretch does not stimulate ANP_{99-126} release (242,243).

The local synthesis of natriuretic peptides is increased in the kidney and in the vasculature in obstructive uropathy (244). The activation of renin-angiotensin system on low sodium intake antagonizes the biological effect of ANP by interfering in the intracellular metabolism of cGMP (245).

Physiologic Actions of Atrial Natriuretic Peptide$_{99-126}$

ANP_{99-126} exerts its biologic effects by binding to specific cell-surface receptors present in many tissues, including the heart, blood vessels, kidneys, lungs, adrenals, and central nervous system (216). Type-I membrane proteins have been identified as receptors of natriuretic peptides, which are characterized by an intracellular domain of guanylyl cyclase. Three subtypes ANP receptors (NPR) have been identified (246–248) NPR-A and –B, has intrinsic guanylate cyclase activity that catalyzes production of 3′, 5′-cyclic GMP (cGMP) after ligand binding. cGMP then serves as an intracellular second messenger that mediates the biologic activities of ANP_{99-126}. NPR-B appears to have 50-fold higher affinity for a related natriuretic factor originally purified from porcine brain, known as C-type natriuretic peptide (CNP) (249). RNP-C, previously known as the (clearance) receptor, is devoid of guanylate cyclase activity and, therefore, does not confer biologic activity and is thought to mediate clearance of circulating ANP along with metalloendoprotease (E.C.3.4.24.11) (248) and of other related hormones, such as brain natriuretic peptide (BNP) (250). Selective down-regulation of NPR-C in the kidney in response to dietary salt supplementation may contribute to local elevation in ANP levels and may be functionally significant in attenuating the development of salt-sensitive hypertension (251).

It is of interest that hemodynamic and neuroendocrine factors regulate the density of ANP receptors in the kidney (252), like a decrease in ANP receptorsdensity in diabetic rats with significant increase in plasma ANP levels (253).

The major sites of action of ANP_{99-126} are the kidneys, adrenal glands, and vascular smooth muscle (254). Short-term administration of ANP_{99-126} in laboratory animals and in humans induces pronounced natriuresis, diuresis, alteration in renal hemodynamics and tubular function, suppression of renin release, inhibition of aldosterone secretion by the adrenal glands, and decreased vasomotor tone, resulting in transient drop in systemic blood pressure. From these actions it has been postulated that ANP plays an important physiologic role in protecting against extracellular volume overload (255) (Fig. 9-5).

TABLE 9-2

CONDITIONS ASSOCIATED WITH INCREASED LEVELS OF CIRCULATING ATRIAL NATRIURETIC PEPTIDE

Physiologic	Reference nos.	Pathologic	Reference nos.
Acute volume expansion	(140–142)	Congestive heart failure	(153,154)
Supine posture	(140–142)	Atrial tachycardias	(140,149,150)
Head-out water immersion	(140–142)	Myocardial ischemia	(155)
Mineralocorticoid escape	(151)	Acute and chronic renal failure	(156,157)
Exercise	(142)	Postobstructive diuresis	(158)
Neonatal period	(152)	Nephrotic syndrome (subset)	(159)
		Cirrhosis with ascites	(160)
		Severe hypertension	(161,162)
		Primary hyperaldosteronism	(161)
		Hypoxia	(162)
		Other (SIADH[a] myxedema)	(163)

[a] *SIADH*, syndrome of inappropriate antidiuretic hormone secretion.

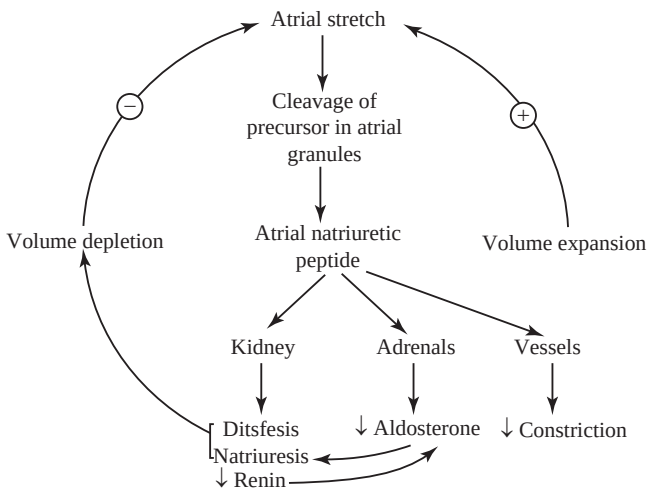

FIGURE 9-5. Regulation of atrial natriuretic peptide (*ANP*) secretion and major sites of action. Action on the kidney causes immediate salt and water excretion. Adrenal mechanism effects a delayed, but more sustained increase in salt and water excretion.

Renal Actions of Atrial Natriuretic Peptide$_{99-126}$

ANP-induced increase in the GFR is well established (256–258). ANP$_{99-126}$ efferent arteriolar resistance, resulting in increased P_{GC} and filtration fraction (8). In addition, ANP$_{99-126}$ relaxes mesangial cells *in vitro*, suggesting that it can increase filtration area by cGMP generation in podocytes (259) and K_f *in vivo* (260). Indeed, when baseline K_f is low, as in water-deprived animals, ANP$_{99-126}$ enhances GFR mainly by increasing K_f (9). The effect of ANP on renal blood flow (RBF) is variable (252). If preexisting vascular constriction is present in isolated perfused kidney, ANP tends to vasodilate renal vessels and increase RBF (261). In whole animals, however, ANP$_{99-126}$ infusion causes either a decline or no change in RBF (262). The effects of ANP$_{99-126}$ on RBF are influenced by its systemic actions on blood pressure and the renin-angiotensin system. ANP seems to increase NO production at both renal and cardiac levels furthermore explaining its natriuretic and diuretic effects (263). Finally, ANP has been reported to induce redistribution of blood flow from the cortex to the medulla and to increase vasa recta flow, leading to dissipation of the medullary solute gradient (264,265).

In acute renal failure, the effect of ANP is not energy conservative as occurs with furosemide, the former increasing tubular sodium reabsorption by 9% and renal oxygen consumption by 26%, while the later has opposite effects (266).

Several studies provide evidence that ANP$_{99-126}$ induces natriuresis and diuresis by mechanisms independent of increased GFR (216,252). ANP$_{99-126}$ has both direct and indirect effects on tubular transport of Na, chloride (267), and water. In the proximal tubule, ANP$_{99-126}$ antagonizes Ang II-induced Na reabsorption (268) and, along with endothelin-3, inhibits the sodium-glucose transporter (269) and, like urodilatin, inhibits Na^+-ATPase activity (270). In the IMCD, it directly inhibits Na transport by binding to ANP-R1 receptors and influencing amiloride-sensitive Na channels and the activity of apical Na-K-2Cl cotransporters (271–273). Other mechanisms by which ANP$_{99-126}$ induces natriuresis and diuresis, include suppression of renin and aldosterone release (274,275), inhibition of the tubular actions of AVP (276), and dissipation of the medullary solute gradient (264,265) by impairing the increase in intracellular Ca^{2+} concentration, ANP blocks both the stimulatory and inhibitory effects of AVP on Na^+-dependent pHi recovery (277).

Nonrenal Actions of Atrial Natriuretic Peptide$_{99-126}$

ANP$_{99-126}$ has several important cardiovascular effects, which include venodilation, arterial vasorelaxation, decreased cardiac filling pressures, and decreased heart rate (216). The net effect is reduced systemic pressure secondary to the decline in peripheral vascular resistance and cardiac output. As discussed earlier, ANP$_{99-126}$ suppresses renin and aldosterone release and antagonizes the actions of Ang II. It is postulated that the hemodynamic and endocrine actions of ANP$_{99-126}$ together protect against hypervolemia and hypertension (255,278). In addition to its hemodynamic effects, ANP$_{99-126}$ contributes to volume homeostasis by inhibiting thirst, salt appetite, and AVP secretion (216). These central nervous system effects are possibly due to paracrine actions of locally produced ANP$_{99-126}$. ANP$_{99-126}$ has also been shown to inhibit proliferation of endothelial, mesangial, and smooth muscle cells (279). The physiologic significance of the antimitotic effect is not known. It also inhibits mesangial cell migration induced by oxidized LDL probably through a cGMP-dependent process (280).

ANP improves pulmonary gas exchange by reducing extravascular lung water and pulmonary arterial pressure, possibly independently from natriuresis/diuresis in oleic acid-induced pulmonary edema (281).

Atrial Natriuretic Peptide Transgenic Mice

The long-term, physiologic role of ANP in regulating extracellular volume and blood pressure has been difficult to evaluate because of the temporal limitations of infusion studies and the short half-life of the molecule *in vivo* (282,283). Field et al. (282–284) have produced transgenic mice through genetic engineering that overexpresses pro-ANP in hepatocytes and thus has chronically elevated plasma levels of the prohormone. These transgenic animals have a hypotensive phenotype (20 to 30 mm Hg lower than control littermates) without compensatory tachycardia. GFR remained normal despite hypotension. Moreover, significant diuresis or natriuresis during steady state was not detected. Contrary to observations made after short-term infusion of ANP$_{99-126}$, plasma renin activity also did not change while aldosterone levels were elevated. In summary, this transgenic model suggests that compensatory mechanisms neutralize the natriuretic and diuretic effects of ANP despite continued secretion of the hormone. Vasodilation and hypotension, however, persist. A transgenic mouse that exhibits chronically high plasma concentration of ANP$_{99-126}$ is currently under study. Preliminary data indicate that its phenotype is similar to the pro-ANP transgenic mouse (282).

Physiologic Consequences of Interrupting the Atrial Natriuretic Peptide Pathway

Disruption of the gene that encodes pro-ANP results in knockout mice that lack expression of ANP. Homozygous ANP knockout mice fed a standard diet have both mildly elevated blood pressure (average increase of 8 mm Hg) and cardiac hypertrophy compared to wild-type mice. This suggests that ANP has a physiologic role in maintaining the normotensive state. This hypertension appears to be sensitive to dietary salt intake as feeding the homozygous ANP knockout mice a diet with an intermediate salt content (2%) would further increase blood

pressure by an average of 20 mm Hg compared to wild-type mice (285). If the ANP-R1 receptor, the one that is coupled to guanylyl cyclase, is the receptor that has biologic activity to ANP, then one would predict that knockout mice without ANP-R1 activity (known as GC-A null mice) would have a phenotype similar to that of the pro-ANP knockout mice. However, they do not. While the GC-A null mice have both elevated blood pressure and cardiac hypertrophy, which is similar to pro-ANP knockout mice, the GC-A null mice have a salt-insensitive form of hypertension (286). It is unclear why there should be a difference in the phenotype of these two types of knockout mice. It is possible that other guanylyl cyclase receptors, such as guanylyl cyclase C, can help regulate blood pressure in the face of changes in dietary salt changes and compensate for the lack of GC-A receptors (287).

Atrial Natriuretic Peptide-Related Peptides (149,212)

BNP is a 32-amino acid peptide with structural homology to ANP_{99-126}. Although originally isolated from porcine brain (288), it is also secreted by cardiac ventricles and, to a lesser extent, from atria. The biologic effects of BNP infusion are similar to those of ANP_{99-126}. Unlike ANP, BNP secretion seems to be constitutive and unrelated to myocyte stretch. The kidney-specific degradation of ANP provides a mechanism for preferential regulation of kidney function by BNP, independent of peripheral ANP concentration (289). In 2001, oral human-BNP was approved for clinical use for acute decompensation of heart failure (248) by the Food and Drug Administration (FDA). In 1990, another homologous peptide, CNP, was isolated from porcine brain (290). CNP is produced in the brain, where it achieves concentrations much higher than those of ANP and BNP. In contrast, circulating levels of CNP are lower. CNP lacks natriuretic, diuretic, and hypotensive effects and probably acts in a paracrine fashion in the central nervous system. The physiologic significance of BNP and CNP is unclear at present.

Gene Therapy with Implanted Human ANP

Animal studies have showed the usefulness of encapsulated ANP gene transfected cells as a new tool for ANP gene delivery in studying renovacsular hypertension and cardiovascular diseases with possible implication for future therapy (291).

URODILATIN OR RENAL NATRIURETIC PEPTIDE

Urodilatin is best described as a paracrine renal natriuretic peptide (RNP) (292). It was first isolated from human urine in 1988 (293). Its amino acid sequence is identical to $ANP_{99-126,}$ except for an additional four amino acids at the amino terminal. Despite its high degree of homology to $ANP_{99-126,}$ specific anti-human RNP polyclonal antibody has been generated and RNP levels can be measured by radioimmunoassay (294). To date, RNP has not been detected in the circulation and the kidney is presumed to be its site of synthesis and action (292).

RNP binds to ANP receptors in the kidney and stimulates cGMP production (295). Its renal actions parallel those of ANP_{99-126} and include more potent hyperfiltration, diuresis, and natriuresis (248,296,297). RNP, like ANP_{99-126}, inhibits sodium uptake by inner medullary duct cells by inhibiting entry of Na through apical sodium channels (298). It appears that the natriuretic effect of RNP is more potent than that of ANP, possibly because it is resistant to degradation by renal cortical metalloendopeptidase (292,299). Systemic infusion of RNP results in effects similar to those of ANP_{99-126}. These include a decrease in mean arterial pressure, right atrial pressure, stroke volume, and cardiac output (297,300,301). The vascular effects of RNP, however, seem to be less potent than those of ANP_{99-126} (296).

Several physiologic studies suggest that RNP functions as a paracrine hormone that regulates renal Na excretion. Drummer and co-workers (302) demonstrated in the human that urinary excretion of RNP, but not plasma ANP concentration, correlates with circadian variation in sodium excretion over a 9-day period. Moreover, acute infusion of normal saline in healthy subjects, balloon dilatation of left atrium, and water immersion induce a significant increase in urinary RNP (303,304,248).

Furthermore, in a model of an isolated perfused rat kidney, increasing renal perfusion pressure increased clearance (production) of RNP while inducing a natriuresis (305). The signaling mechanism for RNP release has not yet been clarified. It is possible that increased cell wall stress may lead to release of RNP by RNP-producing cells (306). Provided in sterile vials for injection, Urodilatin acetate may have a role in combating Na-retentive states observed during congestive heart failure, decompensated liver cirrhosis, nephrotic syndrome, and acute renal failure following major surgeries and organ transplantation (248,292) along with other effects in immune defense and erectile dysfunction (248).

GUANYLIN AND UROGUANYLIN

Guanylin and uroguanylin are cyclic guanosine 3′-5′-monophosphate (cGMP)-regulating agonists isolated in 1994, respectively, from rat intestine and human/opossum urine that appear to have natriuretic properties (307,308). They are part of the "guanylin family peptides" that includes guanylin, uroguanylin, uroguanylin/guanylinlike, and bacterial heat-stable enterotoxin. In human and mouse, earlier studies reported that both genes for guanylin and uroguanylin are located close to each other on chromosomes 1 and 4, respectively, near the ANP *A* and *B* genes and probably arising from an ancestral uroguanylin/guanylinlike gene (309). Molecular cloning of guanylin cDNAs reveals that the bioactive 15-amino acid peptide is a fragment of the COOH-terminal region of preproguanylin, which is approximately 115 amino acids in length. Guanylin can be found in the colon of humans and in the adrenal gland, kidney, and uterus of rats (310,311). Similarly, molecular cloning of uroguanylin cDNAs reveals that the bioactive 16-amino acid peptide is a fragment of the COOH-terminal region of preprouroguanylin that is approximately 110 amino acids in length. Bioactive uroguanylin is probably released by the activity of chymotrypsin. Preproguanylin and preprouroguanylin probably derived from a common precursor gene, as they share approximately 35% homology (312). Bioactive uroguanylin can be found in the urine at higher concentrations than guanylin, suggesting that uroguanylin may be a hormonal link between the intestine and the kidney (313). Both uroguanylin, prouroguanylin, and proguanylin peptides have been shown to circulate in the plasma of man and other animals.

Guanylin and uroguanylin bind to guanylate cyclase-C receptors, which are of three types (A, B, and C) and elevate intracellular cGMP levels (307,314). Guanylate cyclase C receptors have been isolated in the medulla of the human kidney (315). In the isolated perfused rat kidney, uroguanylin, guanylin, and ST peptides stimulate the urinary excretion of sodium, chloride, potassium, and water both *in vivo* and *ex vivo* in the perfused kidney (313).

Uroguanylin produces either hyperpolarization or depolarization of the renal cell membrane, whereas guanylin and

ST cause depolarization of the membrane potential. Also invoked is the regulation of two different classes of potassium channels in the human tubule kidney cell model (IHKE-1) by uroguanylin, guanylin, and ST through two different mechanisms involving cGMP-dependent and -independent pathways. In humans, elevated urinary levels of uroguanylin correlates with renal failure, high dietary salt intake (315), and severe nephrotic syndrome (316). In the nephrotic patients, it appears that a state of relative uroguanylin resistance, similar to ANP resistance, is present.

It is thought that uroguanylin acts as "intestinal natriuretic peptide," as evidenced in knocked-out animals unable to excrete an oral salt load (312).

CORTICOSTEROIDS

Corticosteroids are steroid hormones synthesized by the adrenal cortex. On the basis of their physiologic functions, corticosteroids are traditionally divided into two groups, glucocorticoids (cortisone and cortisol) and mineralocorticoids (aldosterone) (317), based on their potency in electrolyte and metabolism regulation. Corticosterone and 11-deoxycorticosterone, the immediate precursors of aldosterone, are present in the circulation, but have very mild mineralocorticoid activities.

Both glucocorticoids and mineralocorticoids modulate renal function. Both corticosteroid hormones bind to intracellular receptors, the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR). Both are part of the nuclear receptor family that also includes receptors for steroid and thyroid hormones, vitamin D_3, and retinoic acids and these receptors translocate to the nucleus after ligand binding (318).

Aldosterone-sensitive tissues include the distal parts of the nephron (distal tubule, connecting tubule, and all along the collecting duct), the surface epithelium of the distal colon (where it increases sodium absorption and potassium excretion) and salivary and sweat glands and other specific nuclear-binding sites for aldosterone in the thick ascending limb of Henle's loop. All MR-expressing tissues also express GR. In the kidney, evidence exists for distribution of the GR receptors along the whole parts of the nephron, except for the proximal tubule. No evidence exists for glucocorticoid role in the colon (319–321).

MR has the same affinity for both aldosterone and glucocorticoids. Indeed, glucocorticoids concentration in plasma is 100- to 1000-fold higher than aldosterone and only 10% of it circulates as free, while all circulating aldosterone is free. MR selectivity exists to prevent complete occupancy of the MR receptor by glucocorticoids at physiologic concentrations. This is mainly mediated by the colocalization of the 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2) enzyme in the distal nephron, along with MR. This enzyme transforms glucocorticoids (cortisol in humans) into metabolites (cortisone) that have weak affinity to MR (318, 321–323). The major role of 11β-HSD2 is highlighted in clinical situations, like in the syndrome of apparent mineralocorticoid excess where it is inactivated or after ingestion of excessive amounts of licorice where glycyrrhetinic acid, a derivative of licorice, has been well described as an inhibitor of 11β-HSD2 (324). AVP stimulates 11β-HSD2 (318), while progesterone and its metabolites inhibit its activity (325).

MR displays different properties, depending on the nature of the ligand, the latter affecting its conformation and nuclear translocation. It is well known that aldosterone–MR complex is more stable than the cortisol–MR complex and MR stability is variable: highest for aldosterone and deoxycorticosterone and lowest for corticosterone, cortexolone, and cortisol. The opposite is true for the glucocorticoid receptor interaction with different mentioned ligands (321).

Glucocorticoid receptors (GR) have approximately equal affinities for aldosterone and endogenous glucocorticoids, but have the highest affinity for dexamethasone, a synthetic glucocorticoid (318). Because mineralocorticoids circulate at much lower concentrations than glucocorticoids, significant binding of mineralocorticoids to GR does not occur under physiologic conditions.

Recent evidence also suggests that there is a nonclassical membrane-bound aldosterone receptor (reviewed in ref. 326). These data come from kinetic studies that demonstrate saturable, radiolabeled binding of aldosterone to cell surface membranes that have kinetics compatible with physiologic activity (327,328). In some cell lines, aldosterone can have very rapid physiologic effects that are not blocked by inhibitors of cell transcription and translation. For instance, in human mononuclear leukocytes, aldosterone can stimulate release of IP_3 or calcium within 30 seconds of exposure (329,330). These membrane-bound receptors may explain some of the effects of aldosterone that occur prior to gene transcription, such as early stimulation of sodium reabsorption (331) or early stimulation of salt intake (332), possibly via phospholipase C/PKC signaling pathways.

Glucocorticoid Actions in the Kidney

Glucocorticoids appear to be important for the normal maintenance of GFR (333). In both adrenalectomized animals and in humans with adrenal insufficiency, GFR is reduced compared to controls. Adrenalectomized rats given physiologic doses of glucocorticoids regain a normal GFR (334). Furthermore, short-term administration of pharmacologic doses of glucocorticoids has been reported to increase inulin clearance in both normal animals and humans (335,336). Micropuncture studies in normal rats indicate that glucocorticoids enhance GFR by increasing glomerular plasma flow (333,337). The latter results from selective vasodilation of both afferent and efferent renal arterioles (337). The mechanisms by which glucocorticoids alter the glomerular microcirculation remain obscure. Because amino acid infusion causes similar glomerular hemodynamic changes, Baylis and colleagues suggest that glucocorticoids may increase GFR through their effects on catabolism of proteins to free amino acids (333).

Glucocorticoid actions on the proximal tubule, which lacks classical GR expression, include enhancement of gluconeogenesis, ammoniagenesis, and Na reabsorption (338), a part of the nephron that lacks the classical GR. A beta isoform of the GR might mediate these actions; the latter form is not recognized by the GR antibodies classically used in experiments. Lag in ammonium excretion resulting in acid retention is well described in subjects in a glucocorticoid-depleted state (339). In adrenalectomized animals, glucocorticoid replacement restores proximal tubule ammoniagenesis and the ability of the kidney to respond to the chronic phase of acidosis (340). Furthermore, in whole animals glucocorticoid excess accelerates renal base generation, resulting in metabolic alkalosis (341). Glucocorticoids regulate ammoniagenesis possibly through altering glutamine uptake and metabolism by proximal tubule cells (341). Glucocorticoids increase proximal tubule Na reabsorption by at least two mechanisms: enhanced Na–K-ATPase activity and Na–H exchange (342,343). Several experiments suggest that glucocorticoids inhibit Na-dependent phosphate and sulfate reabsorption in the proximal tubule (338). These observations are supported by clinical reports of phosphaturia and lower serum phosphate levels in patients with Cushing's disease and subjects given high doses of glucocorticoids (344).

Both patients with Addison's disease and adrenalectomized animals have decreased urinary concentrating ability (345). This defect is due in part to reduction in RBF, GFR, and

hydroosmotic permeability of the collecting tubule (346). In addition, adrenal corticosteroids contribute to urinary concentration by stimulating Na, K, and HCO_3 transport in the thick ascending limb of Henle's loop (347). It is not entirely clear whether glucocorticoids, mineralocorticoids, or both, mediate the effects of corticosteroids on renal-concentrating mechanisms.

Mineralocorticoid Actions in the Kidney

Ang II, high serum K^+ levels, and ACTH stimulate aldosterone secretion from the adrenal gland (348). ANP and dopamine, on the other hand, suppress aldosterone secretion. Dietary sodium also modulates aldosterone release through its effects on the renin-angiotensin system. The major action of aldosterone in the kidney is regulation of Na, K, and H handling by the distal part of the nephron. Mineralocorticoid deficiency is associated with volume depletion, hyperkalemia, and mild metabolic acidosis. Conversely, mineralocorticoid excess leads to Na retention, hypokalemia, and metabolic alkalosis.

One of the best-documented functions of aldosterone is its ability to increase Na reabsorption in the distal tubule and collecting duct (349–351). The rate-limiting step to sodium reabsorption across tight epithelia is the permeability of the apical membrane of the transporting cell. Aldosterone increases apical Na permeability of tight epithelia, such as those found in the mammalian distal tubule and descending colon, by increasing the activity of the amiloride-sensitive epithelial sodium channel (ENaC). The effect of aldosterone in distal colon and other epithelia is characterized by a latent period of approximately 60 minutes, followed by an increase in ENaC activity with a half-maximal value of 3 hours (352). Recently, the three subunits (alpha, beta, and gamma) of the ENaC have been cloned. Based on coexpression studies in *Xenopus* oocytes, it is believed that the three subunits assemble into a complex heterooligomer forming the amiloride-sensitive pore (353–355). It was thought that regulation of channel activity could be at the transcriptional level, however, induction of channel mRNA lags behind the transcription-dependent increase in channel activity and different organs have differential regulation of Na channel subunit transcription. In the kidney, there is preferential upregulation of the alpha subunit. Currently, it is not clear what regulatory proteins are controlling early activity of the ENaC. Possibilities include K-Ras2A and the serine/threonine kinase sgk (356). Aldosterone also enhances Na reabsorption by increasing Na–K-ATPase activity in basolateral membranes of principal cells in mammalian collecting duct and distal tubule (351). Studies in toad bladder and mammalian nephron suggest that aldosterone upregulates Na–K-ATPase activity by at least three mechanisms: increased Na influx due to opening of amiloride-sensitive Na channels, induction of Na–K-ATPase subunit expression at the gene level, and induction of intracellular alkalosis, which occurs in tissues that contain aldosterone-sensitive Na/H exchangers (357). Other hormones modulate aldosterone's action on Na transport; for example, ANP is inhibitory and vasopressin is stimulatory (358) (Table 9-3).

Hypersecretion of endogenous mineralocorticoids or the administration of mineralocorticoids leads to transient sodium retention followed by a return to Na balance within a few days (359). The return to Na balance despite elevation of circulating mineralocorticoid levels is referred to as aldosterone or mineralocorticoid "escape." During mineralocorticoid escape, increased Na reabsorption by the distal tubule and collecting duct remains unchanged, but is offset by decreased Na reabsorption in other nephron segments (358,360). The latter results from increased renal arterial pressure and elevated plasma ANP levels, both of which suppress proximal tubule transport of Na. Mineralocorticoid escape is also, in part, mediated by decreased Na and water reabsorption in the loop of Henle (360). Other factors, such as TGF-β and interleukin-1 (IL-1), may play a role in regulation of mineralocorticoid escape. These factors have been recently found to inhibit the action of aldosterone on the cells of the IMCD (361).

TABLE 9-3

RENAL ACTIONS OF ADENOSINE

Function	Effect	Receptor
Renal blood flow	Transient ↓	A_1
	Delayed slight ↑	A_2
GFR	↓↓	A_1 and A_2
Renin production	↓↓	A_1
	↑	A_2
Erythropoietin production	↓	A_1
	↑	A_2
Sodium excretion	↑↑	A_2
	↓	A_1
GMC production and proliferation	↓	A_2B

Mineralocorticoids are the predominant hormonal influence on K secretion by principal cells of the collecting duct and connecting segment of the distal tubule (361,362). Although mineralocorticoids always increase K secretion by these nephron segments, this does not necessarily translate into a kaliuresis, because of the strong dependence of K excretion on distal Na delivery and urinary flow rate (363). For example, in conditions of decreased Na delivery and urinary flow to the distal nephron, the kaliuretic effect of aldosterone is either diminished or abolished. The mechanisms by which aldosterone stimulates K secretion by principal cells overlap with those responsible for its Na-retaining action. Aldosterone-induced Na influx through the apical membrane leads to the generation of a lumen-negative potential difference that favors K secretion (350,351). In addition, while mineralocorticoids do not increase the density of active K channels in the apical membrane, they increase the conductance of apical and basolateral K channels independent of Na flux (351,364). Physiologically, it is difficult to understand how one hormone can regulate the concentration of two different solutes that have varying levels of dietary intake. Recent patch-clamp experiments have demonstrated that other nonaldosterone circulating factors exist that can regulate K channel activity. Infusion of aldosterone by osmotic minipump will increase the density of EnaC, but not of K channels (364). However, an increase in dietary K does increase the K-channel density (365). Currently, it is unknown what other circulating factor controls K secretion.

The role of mineralocorticoids in regulation of renal acidification is supported by several clinical observations. Syndromes of aldosterone deficiency are associated with metabolic acidosis because of reduced urinary acid excretion, while mineralocorticoid excess results in metabolic alkalosis. Aldosterone enhances urinary acidification through direct actions on epithelial cells in the collecting duct and indirectly by influencing various intrarenal and extrarenal factors (366). Aldosterone increases H secretion by type-A intercalated cells in the collecting duct via two mechanisms: direct stimulation of the proton pump (H-translocating ATPase), and indirectly by stimulating Na influx, which creates a lumen-negative potential difference (366). As with K, the overall effect of aldosterone on renal acid excretion

depends on Na delivery to the distal part of the nephron. Reduction of Na transport in the collecting duct, because of either decreased Na delivery or inhibition of distal Na reabsorption by amiloride, significantly attenuates the effect of aldosterone on net H excretion (367). Aldosterone regulates the expression of aquaporins, AQP3 with no effect on type 1 and 2, in the collecting duct, independently of Na intake (368).

Lessons Learned from Knockout Mice

The construction of mice that genetically lack the mineralocorticoid receptor (MR) demonstrates that lack of the MR cannot be fully compensated for by the glucocorticoid receptor. The MR is not crucial for prenatal development, but does appear to be important for the adaptation to an environment that has limited salt supply. These mice die very early after birth with hyponatremia, hyperkalemia, and elevated levels of renin, Ang II, and aldosterone. These mice can be "rescued" by NaCl supplementation. Hopefully, these knockout mice can be used to characterize the differences between aldosterone's classical genomic signaling pathways and other nongenomic receptor pathways (369). GR-lacking mice die a few hours after birth because of lung immaturity and hypoglycemia, clearly an outcome not compensated by the presence of mineralocorticoid receptors (321).

Knockout mice have also been generated for the various subunits of the aldosterone stimulated amiloride-sensitive ENaC. All three ENaC subunits appear to be critical for survival, as knockout of any one of the subunits creates a lethal mutation. It is interesting to note that β-subunit knockout mice appear to die mainly from respiratory problems, suggesting a defect in fluid movement in the lung, while α- and α-knockout mice appear to die mainly from electrolyte abnormalities associated with sodium wasting (370).

CATECHOLAMINES

Catecholamines play an important role in the regulation of RBF, GFR, renin secretion, and tubular transport. The endogenous catecholamines that act on the kidney are epinephrine, norepinephrine, and dopamine. These catecholamines derive from renal efferent nerves (norepinephrine and, to a lesser extent, dopamine), from the circulation (epinephrine and norepinephrine), from the adrenal medulla (epinephrine), and from renal proximal tubule cells (dopamine) via α_1 α_2, β_1, and β_2 receptors for epinephrine and norepinephrine and D_1- and D_2-like receptors for dopamine (371–375) (Fig. 9-6).

Effects of α-Adrenergic Stimulation in the Kidney

Both α_1- and α_2-adrenergic receptors are present in the renal cortex (376,377). Adrenergic stimulation causes renal vasoconstriction (increased afferent and efferent arteriolar resistance) by activating α_1 receptors on vascular smooth muscle cells (378). α_1-Mediated renal vasoconstriction results in decreased RBF and GFR. In the proximal convoluted tubule, where α_1- and α_2-adrenergic receptors are expressed in high density, norepinephrine increases Na and water reabsorption, in part, by stimulation of Na^+–K^+-ATPase activity (377,379,380). This activity is partially dependent on the cosecretion of neuropeptide Y that acts to synergize the stimulatory α-adrenergic effects of norepinephrine and to antagonize the inhibitory β-adrenergic effects. This is demonstrated by the fact that norepinephrine alone does not affect Na^+–K^+-ATPase activity in the proximal convoluted tubule unless neuropeptide Y or other β-adrenergic inhibitors are present (381). Moreover, administration of α_2-adrenergic antagonists to humans or laboratory animals with Na retention results in natriuresis and diuresis (377). In isolated rat and rabbit proximal convoluted tubule cells, α_1 and α_2 agonists stimulate Na–H exchange, the overall effect of which is enhanced Na and fluid absorption (382,383). Experimental evidence also indicates that α-adrenergic activation increases Na and fluid absorption in the thick ascending limb of the loop of Henle. Using *in vivo* microperfusion technique, DiBona and Sawin (384) demonstrate that renal nerve excitation stimulates NaCl reabsorption in the loop of Henle. α_1-antagonism with prazosin attenuates this effect. α_2-receptor activation also enhances NaCl reabsorption in the thick ascending

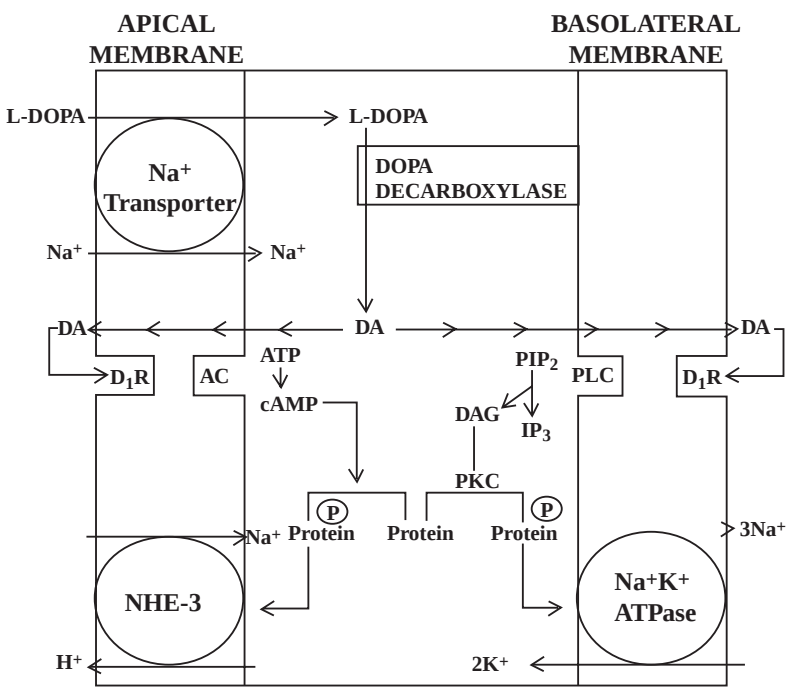

FIGURE 9-6. Schematic depiction of dopamine formation and cell-signaling mechanisms activating sodium transport across the proximal renal tubule cells. *DA*, dopamine; *D_1R*, dopamine D_1 receptor; *PLC*, phospholipase C; *DA*, diacylglycerol; *AC*, adenylyl cyclase.

limb, as shown in experiments using isolated perfused rat kidneys (385). In the collecting duct, Krothapalli and Suki (386) report that α_2 agonists inhibit vasopressin-stimulated water reabsorption by inhibiting adenylate cyclase activity (386). Other investigators, however, challenge this observation (387).

Effects of β-Adrenergic Stimulation in the Kidney

β-adrenergic receptors have been identified in the glomerulus, juxtaglomerular apparatus, thick ascending limb of loop of Henle, distal convoluted tubule, and collecting duct (387,388). β_1 stimulation enhances renin release from the juxtaglomerular cells of the afferent arterioles. Otherwise, there are few β receptors in renal vessels. Although β receptors have not been localized to the proximal tubule, physiologic studies suggest that β-adrenergic stimulation increases Na and fluid transport in this nephron segment independently of enhanced renin secretion and angiotensin II production (387). In the thick ascending limb, β-adrenergic receptor activation stimulates cAMP production and NaCl reabsorption (387). β agonists also increase Cl–HCO_3 exchange and H–K-ATPase activity in the collecting duct (377). The latter effect results in enhanced K reabsorption by type-A intercalated cells (and an apparent decrease in K secretion) (377). A potential mechanism of action involves β-adrenergic stimulation of cAMP production and subsequent conversion to adenosine. Mi and Jackson (388) demonstrate in the isolated perfused rat kidney that β-adrenergic stimulation via isoproteronol increases the secretion rate of adenosine. This effect can be blocked by inhibitors to both β-adrenergic receptors (propranolol) or by inhibitors to ecto-5′-nucleotidase. As will be discussed later in this chapter, adenosine can affect NaCl reabsorption by stimulating sodium reabsorption in the distal tubule of the nephron.

Dopamine Synthesis and Action in the Kidney

Although dopamine-containing nerve endings are present within the kidney, their functional role remains to be determined (389). It is well established, on the other hand, that dopamine is synthesized by proximal tubule cells and that it acts locally to modulate Na excretion (390). Filtered L-dopa enters proximal renal tubule cells via a Na cotransporter in the apical membrane, where it is enzymatically decarboxylated to dopamine by aromatic amino acid decarboxylase (AADC) to generate dopamine. High-salt intake increases AADC activity and dopamine synthesis in proximal tubule possibly by enhancing Na-coupled uptake of L-dopa (390,391). However, regulation of intrarenal dopamine concentrations can occur at several levels: synthesis, storage, or degradation. AADC enzyme activity is increased twofold during high-salt intake; inhibition of AADC prevents the natriuresis generated by a high-salt diet. Similarly, monoamine oxidase A and catechol-*o*-methyltransferase (COMT) is involved in the degradation of dopamine and inhibition of this enzyme can result in increased natriuresis (392).

Dopamine receptors are members of the G protein-coupled superfamily of heptahelical receptors. At least five receptors have been identified, subclassified into D_1- and D_2-like subfamilies. Both of the cloned members of the D_1-like receptor group (D_1 and D_5, also known as D_{1A} and D_{1B} in rodents) are coupled with the stimulating G protein, $G_{s\alpha}$, and stimulate adenylyl cyclase. All three of the cloned D_2-like receptors (D_2, D_3, and D_4) are associated with the inhibitory G protein, G_i/G_0, and inhibit adenylyl cyclase. Both families of receptors are expressed in the kidney at the postjunctional sites. D_1 and probably D_5 are localized in the smooth muscle layer of renal arterioles, juxtaglomerular cells, proximal tubules, and cortical collecting duct. The D_3 receptor is present in arterioles, glomeruli, proximal tubules, medullary thick ascending loop of Henle, and the collecting duct. The D_4 receptor is localized in the cortical collecting duct (372,393,394).

Dopamine produced in the kidney plays a central role in the regulation of Na excretion. In animals, during normal sodium balance, endogenous intrarenal dopamine is a major physiological regulator of urine sodium excretion with no influence on GFR or RBF (293). The major signaling mechanism by which dopamine induces natriuresis is by inhibiting Na^+–K^+-ATPase in all the segments of the nephron via D_1-like receptors; D_2-like receptors stimulate this enzyme. Dopamine inhibits sodium entry into the proximal cell by inhibiting the Na^+/H^+exchanger and a Na^+/P^-cotransporter. Thereafter, both PKA and C inhibit Na^+–K^+-ATPase by three mechanisms: stimulation of PLA_2 activity, generation of eicosanoids, such as 20-HETE (hydroxyecosatetraenoicacid), and phosphorylation of the catalytic subunit of Na^+–K^+-ATPase (393,396). This has been shown in isolated perfused tubule segments and in renal epithelial cell lines (390,397).

The end result of Na^+–K-ATPase inhibition is a dopamine-induced natriuresis. Several investigators suggest that the role of dopamine is to counterbalance the effects of antinatriuretic factors in the kidney (390,392). Interestingly, Kuchel and Kuchel (398) pointed out that dopamine is the predominant catecholamine in fish, in which salt excretion is a priority. On the other hand, norepinephrine predominates in terrestrial animals, in which salt retention is essential for survival. In addition to inhibition of Na–K-ATPase activity, other studies suggest that dopamine suppresses Na–phosphate cotransporter and that it antagonizes the stimulatory effect of Ang II on Na–H exchange in cortical brush-border membranes mediated by cAMP and PKA (399,400) and stimulates renin synthesis in cultured rat juxtaglomerular cells (395). Studies in humans have shown that dopamine does not induce natriuresis in Na-depleted subjects and that its natriuretic effect is more pronounced during conditions of volume expansion (401).

In the whole kidney, dopamine increases RBF and GFR through its D_1 receptor-mediated vasodilatory effects (390, 401). Supraphysiologic concentrations of dopamine, however, stimulate α-adrenergic receptors, which lead to vasoconstriction and decreased RBF. The natriuretic and vasodilating effects of dopamine have earned it a therapeutic role in patients with volume expansion, particularly when administered in low doses that do not activate adrenergic receptors. A recent meta-analysis (402), however, showed by pooled analysis no effect of low-dose dopamine (≤ 5 μg/kg/min) on mortality rates or on the need for renal replacement therapy in patients presenting with acute renal failure. No statistical difference in adverse side effects was noted as compared to control groups. The effect on renal function was most favorable during the first day of infusion, that is, increased urine output by 24%, decreased creatinine level, and improved creatinine clearance–benefits that disappear on the second and third days of therapy.

Moreover, dysfunction of the renal dopamine system has been postulated to contribute to the pathogenesis of systemic hypertension (390). Results from at least two studies suggest that defects in renal generation of dopamine are common in patients with essential hypertension (403,404).

The ability of the D_1 receptor to induce both natriuresis and vasodilation makes D_1 agonists, such as fenoldopam, potential therapeutic agents for the treatment of both hypertensive urgencies and acute renal failure. In healthy normotensive volunteers, fenoldopam has been shown to significantly increase renal plasma flow, while only minimally reducing

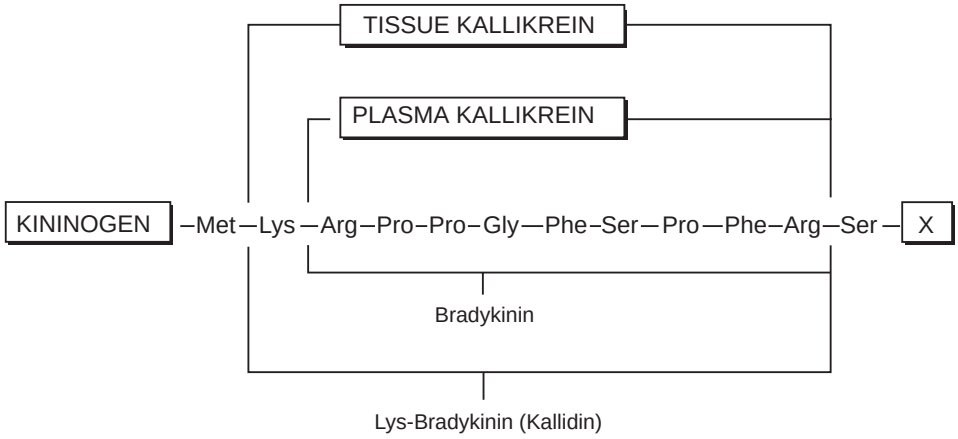

FIGURE 9-7. Release of kinins from kininogen by kallikreins. *Ser-X*, extended sequence in the light chain of kininogen. (From: Erdos EG. Kinins, the long march—a personal view. *Cardiovasc Res* 2002;54:485, with permission.)

systemic blood pressure (405). In people with hypertensive urgencies, fenoldopam has been shown to reduce systemic blood pressure by 23%, while increasing natriuresis by 200%, diuresis by 46%, and renal blood flow by 42% (406). While the selective increase in renal plasma flow could be advantageous in the treatment of certain forms of acute renal failure, further studies must be done to define the specific utility of fenoldopam in this setting.

Physiologic Significance of Renal Innervation

Using electron microscopy and immunohistochemical techniques, Barajas and Mueller (407,408) demonstrate nerve endings in direct contact with the juxtaglomerular apparatus and basement membrane of tubule segments involved in Na and water absorption: the proximal tubule, thick ascending limb of Henle's loop, distal tubule, and collecting duct. Nerve endings have also been localized to the renal, arcuate, and interlobar arteries. Studies by Kopp DiBona (409) have determined that renal innervation is extensively adrenergic and that the end result of renal nerve stimulation is Na and water retention. Low-frequency stimulation increases renin secretion by activating β_1 receptors (410), while higher-frequency stimulation increases Na reabsorption by activating α_1 receptors on tubule epithelial cells. Renal vasoconstriction, mediated by vascular α_1 receptors, occurs at even higher frequencies. The antinatriuretic and vasoconstrictive effects of excessive renal nerve discharge is well recognized in pathophysiologic conditions, such as anesthesia, surgical stress, trauma, congestive heart failure, liver cirrhosis, and the nephrotic syndrome. In addition, intact renal innervation is necessary for normal diuretic and natriuretic response to an acute intravenous volume load (409). Finally, renorenal reflexes, mediated by the renal nerves, coordinate the functions of the two kidneys (411).

THE RENAL KALLIKREIN–KININ SYSTEM

Kinins are vasoactive peptides that possibly play a role in the modulation of salt and water excretion by the kidney and in the control of blood pressure (412,413). The kallikrein-kinin system consists of four components: kallikreins, kininogens, kinins, and kininases (414). Kallikreins are serine proteases that act on kininogens to generate the biologically active kinins (potent vasodilators).

The kallikrein–kinin cascade is a complex one: Prokallikrein of plasma or tissue, after it is activated to kallikrein, releases from kininogen bradykinin (BK) or Lys–BK (Fig. 9-7). The two peptides act on their B_2 receptor. They are metabolized by ACE (angiotensin I converting enzyme) or kininase II by the release of C-terminal Phe-Arg or by carboxypeptidases N or M which cleave off C-terminal Arg only (Fig. 9-8). The resulting des-Arg-kinin then acts on a receptor different from B_2, called B_1. The two receptors are both G protein-coupled, seven-transmembrane, heptahelical receptors on plasma membrane, but otherwise, they differ. While B_2 is ubiquitous, B_1 is mainly expressed after induction by endotoxin, cytokines, ischemia, and other noxious stimuli (415).

Carboxypeptidase-type enzymes are called kininase I and the other one that released the dipeptide, kininase II. We know now that this terminology represents two groups of enzymes, which cleave either at Phe^8 -Arg^9 or Pro^7 -Phe^8 bond of BK. The metallocarboxypeptidases N and M and a serine carboxypeptidase, cathepsin A, or deamidase, form the first group, while

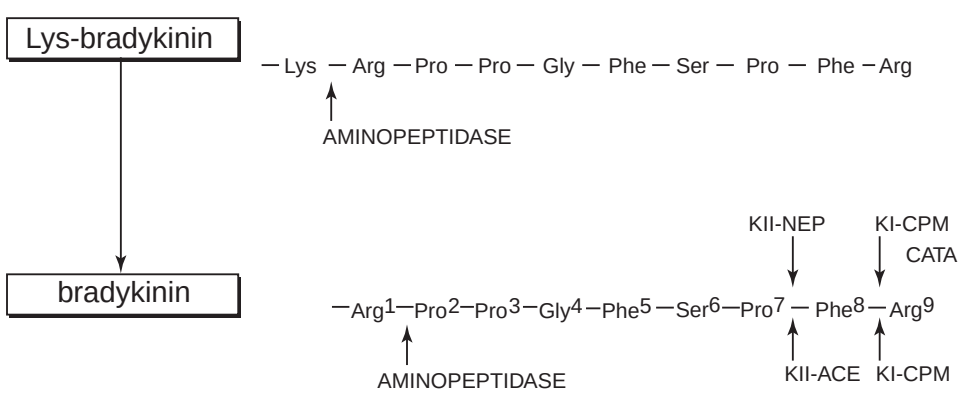

FIGURE 9-8. Cleavage sites of Lys–bradykinin (kallidin) and bradykinin. Conversion from ligand to B_2 receptor to agonist of B_1 receptor. *KI*, kininase I; *KII*, kininase II; *NEP*, neutral endopeptidase 24.11, neprilysin; *CPN*, carboxypeptidase N; *CPM*, carboxypeptidase M; *ACE*, angiotensin I-converting enzyme; *CATA*, cathepsin A deamidase, lysosomal protective protein. (From: Erdos EG. Kinins, the long march—a personal view. *Cardiovasc Res* 2002; 54:485, with permission.)

ACE and neprilysin (neutral peptidase 24.11) belong to the second group (Fig. 9-8) (415).

Renal kallikrein activity is predominantly found in the cortex, where it has been detected in glomeruli, proximal tubule, and distal tubule. The majority of kallikrein found in the urine is of the tissue form and is secreted by the kidney. In addition to the presence of kininases in the vascular endothelium and renal tubule cells, the proximal tubule brush border contains endopeptidase II (enkephalase), which also inactivates kallidin and bradykinin (416). Aprotinin, a polypeptide purified from the lung, inhibits the activity of renal and other tissue kallikreins (413). The molecular biology of the kallikrein–kinin system is reviewed elsewhere (417,418). The volume of research in kinins increased exponentially with time, partially because of the clinical application of ACE inhibitors, and to the manifold direct and mostly indirect effects of BK and Lys–BK or kallidin. These include release of NO, endothelium-derived hyperpolarizing factor (EDHF), norepinephrine, prostaglandins, substance P, cytokines, and tissue plasminogen activator. All of these are, in addition to the direct spasmogenic or algogenic actions of kinins, the consequence of the initial activation of a BK receptor, frequently the B_2 one (415).

Renal Actions of Kinins

Experimental evidence suggests that kinins regulate renal blood flow and renal excretion of Na and water (412,413). Acute infusion of bradykinin (BK) into the renal artery dilates both pre- and postglomerular arterioles. The net effect is increased RBF, without a change in GFR (419). Acute infusion of BK also induces significant natriuresis and diuresis in the absence of GFR alteration (419). In experiments in which BK was administered for several days, the acute rise in salt and water excretion was not sustained, while renal vasodilation persisted (419). However, a sustained increase in renal function and urine flow rate can occur when the human kallikrein gene is overexpressed by adenoviral transfection in a rat model of human renovascular hypertension (420). Although these studies suggest a natriuretic role for BK, they do not elucidate the physiologic role of the renal kallikrein–kinin system. Several groups have attempted to block BK action *in vivo* in order to determine these physiologic functions. Inhibition of endogenous BK by means of specific antibodies or aprotinin has been shown to blunt the natriuretic and diuretic effect of saline infusion (413,421). Siragy demonstrated that infusion of a BK antagonist into the renal arteries of dogs on a low-Na diet causes antidiuresis and a significant decrease in the fractional excretion of Na (422). There were no changes in GFR, plasma aldosterone concentration, plasma renin activity, or systemic arterial pressure during intrarenal administration of the antagonist. Although lower doses did not affect estimated RPF, a higher dose caused a significant decrease. These results suggest that endogenous kinins can act as natriuretic substances.

The mechanism by which kinins induce natriuresis remains a matter of debate. Several studies suggest that kinins directly regulate epithelial transport of ions and water (413). The renal target of these actions is most likely the collecting duct, which, in rabbits, has a high density of specific BK-binding sites (413,423). In addition, renal kallikrein and kininogen have been localized to the distal tubule, connecting segment, and collecting duct, confirming the presence of a complete kallikrein–kinin axis in the distal part of the nephron (413,424). The complex interactions of kinins with other hormonal systems, however, make it difficult to discern whether their renal effects are, indeed, direct.

Studies on the role of the renal kallikrein–kinin system (KKS), using congenitally kininogen-deficient Brown-Norway Katholiek rats and also BK B_2 receptor knockout mice, revealed that this system starts to function and to induce natriuresis and diuresis when sodium accumulates in the body as a result of excess sodium intake or aldosterone release, for example, by angiotensin II. Thus, it is hypothesized that the system works as a safety valve for sodium accumulation. The large numbers of studies on hypertensive animal models and on essential hypertensive patients, particularly those with salt sensitivity, indicate a tendency toward the reduced excretion of urinary kallikrein, although this reduction is modified by potassium intake and impaired renal function (425).

Reduced KKS activity may contribute to the establishment of a pathophysiologic state characterized by unopposed hyperactivity of the renin–angiotensin system, resulting in salt retention (426). It has been shown that this reduced activity of the KKS predominates over renin–angiotensin system overactivity in all conditions of sodium balance in essential hypertensives and family-related hypertension (427).

Kinins activate phospholipase A_2, resulting in increased prostaglandin synthesis (414). In a rat model of unilateral ureteral obstruction, Kopp and Smith (428) demonstrate that indomethacin abolished BK-induced natriuresis and diuresis in the contralateral kidney (428). Lysyl-BK (kallidin) has been shown to inhibit the hydroosmotic action of vasopressin on isolated rabbit cortical collecting duct (429). On the other hand, BK stimulates vasopressin release when infused into the renal artery, but not when administered systemically, suggesting that this effect is mediated by afferent renal nerves (430).

Products of the KKS may modulate cell growth. Experimental evidence suggests that while under certain conditions, *in vitro* BK may inhibit growth of normal renal fibroblasts (431,432), it can also stimulate the growth of fibroblasts, mesangial cells, and arterial smooth muscle cells in other conditions (433). Over-expression in hypertensive Dahl salt-sensitive (DSS) rats of the human kallikrein gene by adenoviral delivery results in reversal of the changes associated with hypertensive nephrosclerosis (433).

Finally, recent evidence suggests that BK participates in the regulation of neonatal glomerular function and acts as a growth regulator during renal development. An intact KKS is necessary for the normal functional development of the kidney (434). During nephrogenesis, tubular and glomerular growth and differentiation, and acquisition of specialized functions, are coordinated in time and space with renal vasculogenesis, glomerulogenesis, and regional hemodynamic changes. The end result ensures that tubular structure and function are tightly coordinated with glomerular filtration during normal kidney development. To achieve this delicate task of glomerulotubular balance, the developing kidney produces growth factors and vasoactive hormones that act in a paracrine manner to regulate nephrovascular growth, differentiation, and physiological functions. One such paracrine system is the KKS, which generates BK. BK activates B_2R to regulate renal blood flow and salt and water excretion. The developing kidney expresses an endogenous KKS. Gene-targeting studies indicate that the fetal KKS plays an important role in the maintenance of terminal epithelial cell differentiation (435).

The Kallikrein–Kinin System in Transgenic Mice

Transgenic mice that overexpress either the human tissue kallikrein gene or the human bradykinin B_2 receptor (G proteincoupled receptor) are hypotensive compared to wild-type littermates. Furthermore, renal function in the human bradykinin B_2-receptor transgenic mice is increased as demonstrated by increased RBF, GFR, and urine flow. This increase in renal function is associated with an increase in urinary

excretion of nitric oxide, cGMP, and cAMP. These results suggest that signal-transduction pathways mediated by either cAMP, cGMP or both are involved in blood pressure reduction and enhanced renal function in human BK B_2 transgenic mice (436).

Furthermore, enhanced kallikrein–kinin levels, after gene transfer, exerted beneficial effects in protection against cardiac remodeling, renal injuries, restenosis, cerebral infarction, and neurological deficits in normotensive animal models without hemodynamic effects, indicating direct actions of kallikrein, independent of its ability to lower blood pressure. These kallikrein effects were mediated by the kinin B_2 receptor. Moreover, kallikrein–kinin had pleiotropic effects in inhibiting apoptosis, inflammation, hypertrophy and fibrosis, and promoting angiogenesis and neurogenesis in the heart, kidney, brain, and blood vessel. Exogenous administration of kallikrein also led to increased NO/cGMP and cAMP levels and reduced NAD(P)H oxidase activation and inflammatory cytokine levels. These results indicate a novel role of kallikrein–kinin through the kinin B_2 receptor as an antioxidant and antiinflammatory agent in protection against stroke, cardiovascular, and renal disease, and may uncover new drug targets for the prevention and treatment of heart failure, vascular injury, end-stage renal disease, and stroke in humans (435).

This was also shown in other studies that demonstrated that tissue kallikrein protects against renal fibrosis in hypertensive DSS rats through increased nitric oxide bioavailability and suppression of oxidative stress and TGF-β expression (437).

Role of Kallikrein–Kinin System in Diabetic Nephropathy

Diabetes alters the activity of the KKS and these alterations contribute to the development of diabetic nephropathy (438). Glomerular kinin receptors are induced by diabetes and this may contribute to the development of glomerular injury and to the development of microvascular complications of diabetes (439). BK regulates the expression of CTGF (connective tissue growth factor), TGF-βRII (transforming growth factor-β), and collagen I in MC (mesangial cells) and provide a mechanistic pathway through which B2KR (BK B_2 receptors) activation may contribute to the development of diabetic nephropathy (438). Also Christopher et al. (440) demonstrated that glucose by itself regulates the expression of B_2 receptors in vascular smooth muscle cells.

ADENOSINE

Adenosine, a purine nucleoside, is a paracrine hormone that regulates cellular and physiologic functions in many tissues (441,442). The classical paradigm is that three major pathways produce adenosine.

The first mechanism is the intracellular pathway. Intracellular generation of adenosine results from the action of 5′-nucleotidase on adenosine monophosphate (AMP) during hypoxia (sequential dephosphorylation of intracellular ATP to adenosine).

In cells that rapidly consume ATP, enhanced utilization of ATP increases the intracellular production rate of adenosine via the intracellular breakdown of ATP to adenosine. In the kidney there are two prime examples of the intracellular ATP pathway. Increased delivery of Na^+ to the thick ascending limb of Henle stimulates Na–K^+-ATPase activity in the basolateral membrane of epithelial cells through the increased flux of Na^+ across the luminal membrane. Oxygen availability in the renal medulla is marginally adequate; consequently, increased Na^+–K^+-ATPase activity may deplete ATP levels and lead to dephosphorylation of adenine nucleotides to form adenosine. Reactive ischemia is a second example of the intracellular ATP pathway in the kidney. A short period of ischemia in the kidney triggers a brief increase in renal vascular resistance, a phenomenon known as *reactive ischemia*. Renal ischemia activates the intracellular ATP pathway of adenosine production and adenosine causes reactive ischemia via activation of A_1 receptors in the preglomerular microvessels (443).

Adenosine, produced intracellularly, can traverse cell membranes by facilitated diffusion and function in a paracrine or autocrine fashion.

The extracellular ATP pathway is yet another mechanism of adenosine production in the kidney. Extracellular production of adenosine from AMP is possible because of the presence of ecto-5′-nucleotidase on the surface of many cell types. Release of adenine nucleotides into the extracellular compartment from renal sympathetic nerve terminals, intrarenal platelets, renal endothelial cells, renal vascular smooth muscle cells, and/or renal epithelial cells exposes extracellular ATP to ecto-ATPases, ecto-ADPases, and ecto-5′-nucleotidases, and these enzymes metabolize adenine nucleotides to adenosine (443). In the kidney, ecto-5′-nucleotidase activity is expressed on tubular luminal membranes, fibroblasts, and mesangial cells and is believed to be the major source of renal adenosine (444). Extracellular ATP and adenosine would have to reach the afferent arteriole via the interstitium (445).

When oxygen supply is adequate, enzymatic hydrolysis of *S*-adenosyl homocysteine to L-homocysteine and adenosine constitutes the major production pathway. It is the transmethylation pathway of adenosine production. Approximately one-third of the adenosine release to the extracellular space by cardiomyocytes is through the transmethylation pathway, but the importance of this pathway in the kidney is unclear (443).

Purinoceptors or adenosine receptors, also called P_1 receptors, are classic G protein-coupled receptors with four known subtypes receptors (A_1, A_2A, A_2B, and A_3 receptors) (443,445). A_1 receptors have a high affinity for adenosine, whereas the affinity of A_2A receptors for adenosine is approximately threefold less compared with that of A_1 receptors. A_2B and A_3 receptors are low-affinity adenosine receptors (443, 445–447).

A_1 receptors evoke vasoconstriction by inhibiting adenylate cyclase activity (via activation of G_i and G_o), thereby reducing cAMP generation in vascular smooth muscle and signaling (443). In renal epithelial cells and isolated rabbit afferent arterioles, A_1 receptor activation also appears to stimulate phospholipase C activity. A_2A and A_2B receptors produce vasodilation by stimulating adenylate cyclase activity to increase cAMP generation; A_2A signal by engaging G_s, G_{olf}, and p21ras and A_2B receptors are also known to stimulate phospholipase C via G_q. A_3 receptors are thought to exert their physiologic effects through activation of calcium signaling pathways (phospholipase via G_q families) and perhaps inhibition of cAMP accumulation (via G_i), but their role in regulating the renal microvascular and epithelial function has not been extensively examined (443,445).

P_2 receptors were originally described by as purinergic receptors, reflecting the idea that they responded to ATP released as a neurotransmitter from peripheral sympathetic nerves. Currently P_2 receptors are divided into two distinct families, P_2X and P_2Y (445).

Distinct genes code for each receptor family and there are marked differences in the structural and signal-transduction characteristics between the two families. Adenosine regulates a wide array of physiologic functions, including cardiac rate and contractility, vascular smooth muscle tone, neurotransmitter release, lipolysis, leukocyte function, platelet function, and renal hemodynamics and electrolyte transport (446,448,449).

The following discussion focuses on the renal actions of adenosine.

The Renal Adenosine System

Adenosine is produced in the kidney and acts in an autocrine or paracrine fashion (444,446). Both high-affinity A_1 receptors and low-affinity A_2 receptors are widely distributed throughout the renal vasculature and the nephron (442,446). The renal effects of adenosine are diverse and include alterations in RBF, GFR, hormone production, neurotransmitter release, and tubular absorption (Table 9-3).

Infusion of adenosine into the renal artery of animals results in transient reduction of RBF secondary to A_1 receptor-mediated afferent arteriolar vasoconstriction, followed by a delayed A_2 receptor-mediated postglomerular vasodilation and return of RBF toward normal (450–452).

It is conceivable, although unproved, that A_1AR are present in endothelial cells along the renal vasculature and that adenosine causes the release of NO and perhaps other endothelial vasodilators when administered from the vascular, but not from the interstitial aspect, of the vessel. The resulting A_1AR-induced constriction would, therefore, be blunted by endothelial factors only when adenosine is given intravascularly. In a study in dogs, the administration of nitric oxide synthase (NOS) inhibitors caused a marked augmentation in the constrictor response of renal blood flow to bolus injections of adenosine, while the dilator effect of the A_2 agonist CGS-21680 was unaffected, indicating that adenosine may cause NOS activation through an A_1AR-mediated mechanism. It is now well recognized that the majority of vasodilator agents act by binding to their receptors on endothelial cells and by eliciting the generation and release of endothelial relaxing factors, most notably NO, endothelial hyperpolarizing factor, and prostaglandins. However, in a number of studies, adenosine appears to augment NOS activity and NO release through an A_2AR-mediated process, an action that would enhance the dilator component rather than diminish the constrictor component of the adenosine actions. In addition, adenosine has also been reported to dilate rabbit renal arteries through an endothelial relaxing factor that does not appear to be NO. Finally, adenosine has been shown to consistently stimulate the production of NO in cultured endothelial cells, usually through an A_2AR-dependent mechanism. Thus, in addition to the possible blunting of A_1AR-induced vasoconstriction as discussed above, endothelial dilator factors generated in response to A_2AR activation may enhance renal vasodilatation, thereby contributing to the waning renal constriction in the kidney during intravenous administration (452).

On the other hand, adenosine induces a sustained decrease in GFR secondary to reduced P_{GC} (453). Adenosine infusion in humans results in an insignificant increase in RBF and a significant, moderate decrease in GFR (454,455). It is postulated that adenosine-mediated reduction in GFR constitutes the underlying mechanism of tubuloglomerular feedback (456,457). The hypothesis states that increased solute delivery to the macula densa (MD) stimulates sodium transport, resulting in ATP hydrolysis and generation of adenosine. Adenosine, in turn, completes the feedback loop by decreasing GFR and normalizing solute delivery to the distal nephron. The hypothesis is supported by experiments demonstrating that A_1 receptor blockade inhibits tubuloglomerular feedback (458,459,460).

As summarized in Figure 9-9, A_1 receptors importantly regulate renal function. Infusion of A_1 receptor agonists into the renal interstitium diminishes blood flow to both superficial and deep nephrons. In the outer cortex, A_1 receptor-induced vasoconstriction is most likely caused by contraction of preglomerular, rather than postglomerular, microvessels; however, in juxtaglomerular nephrons, A_1 receptors mediate vasoconstriction by contracting preglomerular microvessels, efferent arterioles, and outer medullary descending vasa recta. Ang II strongly enhances A_1 receptor-induced preglomerular vasoconstriction. Contraction of preglomerular microvascular smooth muscle cells by A_1 receptors underlies the mediator role of adenosine in tubuloglomerular feedback, explains the ability of adenosine to decrease glomerular filtration rate, and potentiates postjunctional vasoconstrictor responses to renal sympathetic neurotransmission in the kidney (443).

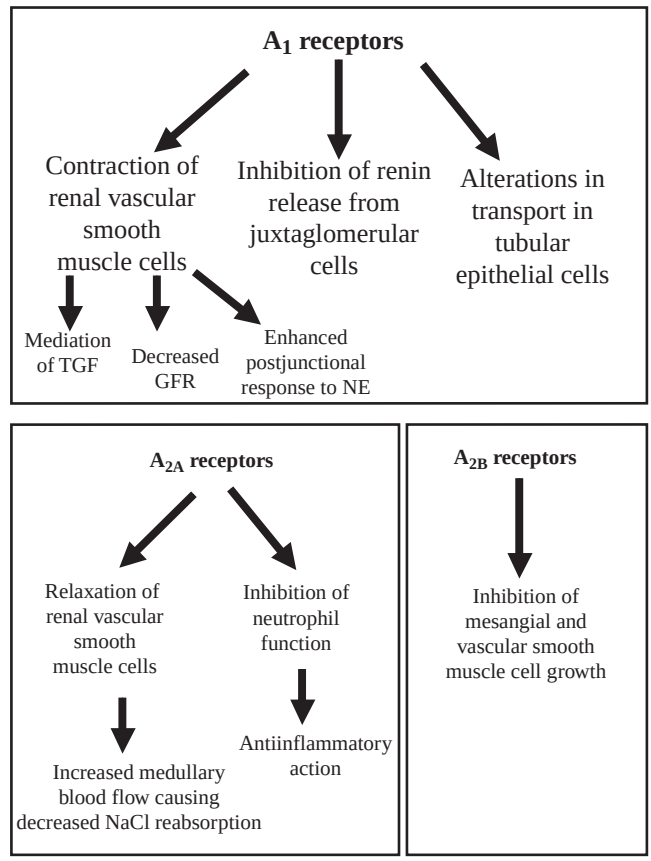

FIGURE 9-9. Regulation of renal function by adenosine. *TGF*, tubuloglomerular feedback; *GFR*, glomerular filtration rate; *NE*, norepinephrine. (From: Jackson EK, Raghvendra DK. The extracellular cyclic AMP-adenosine pathway in renal physiology. *Annu Rev Physiol* 2004;66:571, with permission.)

A_1 receptors also influence renin release. Several studies strongly indicate that adenosine suppresses renin release by the kidney (446). In sodium-depleted animals, renin release is inhibited by maneuvers that increase renal adenosine production (461,462), an effect that results from direct action of adenosine on renin-producing cells (463). Furthermore, inhibition of renin release is most likely mediated by binding of adenosine to high-affinity A_1 receptors (464). In this regard, A_1 receptors restrain renin release responses, a theory known as the *adenosine-brake hypothesis*. Importantly, A_1 receptors are coupled to G_i and, therefore, inhibit adenylyl cyclase. Because stimulation of renin release from juxtaglomerular cells by many stimuli involves activation of adenylyl cyclase, activation of juxtaglomerular A_1 receptors attenuates renin release and antagonism of renal A_1 receptors increases renin release (443). In contrast, recent data demonstrate that agonists selective for the low-affinity A_2 receptor stimulate rather than suppress renin release, particularly when administered in high doses (446,465). This suggests that adenosine regulates renin

release by exerting either an inhibitory or stimulatory effect, depending on its local concentration.

The extracellular cAMP–adenosine pathway may also regulate glomerular mesangial cell (GMC) production and proliferation. Exogenous and endogenous adenosine and adenosine analogs with A_2B receptor agonist activity attenuate vascular smooth muscle cell proliferation and collagen and protein synthesis. Activation of A_2B receptors attenuates proliferation of, and extracellular matrix production by, mesangial and vascular smooth muscle cells (Fig. 9-9). It is likely, therefore, that A_2B receptors importantly influence renal structure and function. Studies with a number of adenosine receptor agonists and antagonists and with antisense oligodeoxynucleotides against the A_2B receptor indicate that the growth inhibitory effects of adenosine on vascular smooth muscle cells are mediated by A_2B receptors (443).

Adenosine also plays a similar regulatory role in erythropoietin production: A_1 receptor stimulation inhibits, while A_2 receptor stimulation enhances, erythropoietin synthesis by renal cells (466).

Activation of A_1 receptors on sympathetic neurons in the kidney causes presynaptic inhibition of norepinephrine release (467). Postjunctionally, however, adenosine seems to enhance sensitivity to norepinephrine (467). Because renal denervation does not alter adenosine-induced changes in RBF and GFR, the hemodynamic actions of adenosine in the kidney are most likely independent of its effect on neurotransmitter release (446).

Selective A_2A receptor agonists augment renal blood flow, a response caused by vasodilation of the medullary renal microcirculation (Fig. 9-9). In contrast to A_1 receptors, which directly stimulate Na^+ reabsorption by increasing epithelial transport mechanisms, A_2A receptors indirectly reduce Na^+ reabsorption by enhancing renal medullary blood flow. Importantly, activation of A_2A receptors in microvessels of juxtaglomerular nephrons and in the medullary microcirculation enhances medullary blood flow, thus altering peritubular forces that modulate Na^+ reabsorption. The net result is an increase in Na^+ excretion (443).

A_2A receptors also exert important antiinflammatory actions that may protect the kidneys from injury (Fig. 9-9). Activation of A_2A receptors strongly inhibits neutrophil endothelial cell interactions *in vitro*, and, *in vivo*, A_2A receptor activation reduces neutrophil–endothelial interactions following ischemia/reperfusion injury. Selective activation of A_2A receptors markedly decreases the renal infiltration of neutrophils and attenuates renal dysfunction in kidneys subjected to ischemia/reperfusion injury (443).

The effect of adenosine on renal tubular transport is complex. Adenosine-induced decrease in solute excretion, particularly sodium, has been generally attributed to the concomitant reduction in GFR and urine flow. The presence of A_1 and A_2 receptors on renal epithelial cells, however, suggests that adenosine may have direct effects on tubular transport (446). In rats, for example, infusing adenosine or adenosine receptor agonists in a dose that does not alter systemic blood pressure, RBF, or GFR has been shown to induce both sodium and water retention (468,469). This effect was independent of renal enervation, at least for A_1-specific agonists, and suggested a direct tubular effect of adenosine on A_1 receptors to stimulate sodium reabsorption (469). Studies conducted on isolated collecting tubule segments and on cells cultured from the thick ascending limb of the loop of Henle suggest that A_1-receptor activation inhibits vasopressin-induced cAMP formation, while A_2 agonists enhance adenylate cyclase activity (470). Coulson and colleagues (471) have demonstrated that A_1-receptor activation stimulates Na-coupled phosphate and glucose transport in cultured opossum kidney epithelial cells. Ma and Ling (472) have demonstrated in A6 cells, a cultured amphibian epithelial model of DCCT cells, that application of luminal adenosine can stimulate Na reabsorption via activation of A_1 receptor. Further studies, however, are needed to determine whether adenosine has a direct *in vivo* effect on Na transport by human renal tubule cells.

The Role of Adenosine in Renal Physiology and Pathophysiology

At present, the physiologic role of adenosine in the kidney is not clear. The complexity arises, in part, from the fact that the net effect of adenosine depends on its local concentration and on the receptor type that is activated. Furthermore, certain actions of adenosine, such as vasoconstriction of the renal afferent arteriole, are either dependent on or significantly enhanced by angiotensin II (446,473).

There is abundant evidence to show that a reduction in ambient Ang II levels and the prevention of AngII formation and action cause a marked attenuation of the vasoconstrictor response of the intact kidney to adenosine. Conversely, an elevation of ambient Ang II concentrations enhances the constrictor effect of A_1AR activation by adenosine or A_1AR-specific ligands. Nevertheless, it is not clear that differences in ambient Ang II levels can explain the different responses of intact kidneys and isolated preparations. One would expect ambient Ang II concentrations to be lower in the artificial environment and adenosine responses should, therefore, be blunted, the opposite of what is actually observed (452).

As mentioned previously, an important physiologic function of adenosine could be mediation of tubuloglomerular feedback (456–458). Pathophysiologic conditions associated with increased renal production of adenosine include acute renal ischemia, myoglobinuric acute renal failure, and mercuric chloride-induced acute renal failure (474–476). Recently, Nishiyama and associates (445,477) demonstrated that hypoxia-induced renal vasoconstriction was associated with elevated interstitial adenosine levels and could be blocked by adenosine A_1-receptor antagonists. It is postulated that adenosine plays a role in balancing oxygen supply and demand during renal hypoxia by regulating RBF, GFR, renin secretion, and solute transport (456). A_1 receptors may also be involved in pathophysiological mechanisms of drug-induced nephrotoxicity. For example, selective antagonism of A_1 receptors attenuates nephropathy caused by such nephrotoxins as cisplatin, gentamicin, cephaloridine, glycerol, and radiocontrast media (443).

PANCREATOHEPATORENAL EXTRACELLULAR cAMP–ADENOSINE PATHWAY

In addition to an autocrine/paracrine role in the kidney, the extracellular cAMP-adenosine pathway (Fig. 9-10) may also function as an endocrine system by which the pancreas and liver regulate renal function. The pancreas releases glucagon directly into the portal circulation in response to appropriate stimuli, thus avoiding dilution of glucagon and maximizing the concentration of glucagon delivered to the hepatocytes. Glucagon released from the pancreas into the portal circulation stimulates hepatic adenylyl cyclase, which results in secretion of cAMP by hepatocytes into the venous circulation. Liver-derived cAMP circulates to the kidney where it is filtered into the proximal tubule. As water is extracted from the proximal tubules, cAMP becomes concentrated. Because proximal tubules can metabolize cAMP to adenosine, this process would proceed to generate adenosine. Adenosine would then engage A_1 adenosine receptors in epithelial cells to enhance electrolyte transport. It is important to note that, in contrast to cAMP,

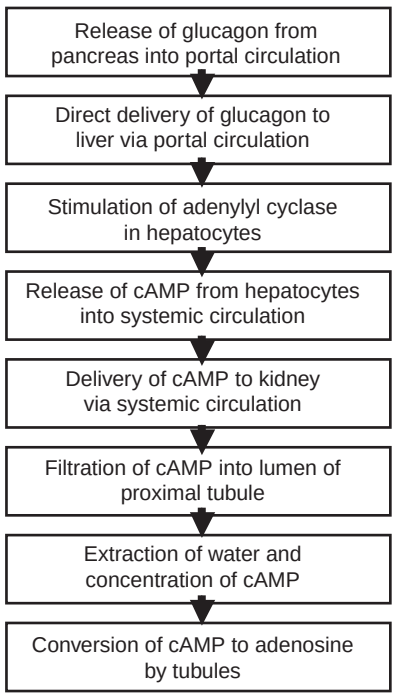

FIGURE 9-10. Pancreatohepatorenal extracellular cAMP-adenosine pathway. cAMP, cyclic adenosine monophosphate. (From: Jackson EK, Raghvendra DK. The extracellular cyclic AMP-adenosine pathway in renal physiology. *Annu Rev Physiol* 2004;66:571, with permission.)

which is stable in blood, adenosine has a half-life in human blood of less than 1 second. Inasmuch as the liver is the largest organ in the body, about 1.5 kg in a normal adult, the liver has the capacity to release significant amounts of cAMP into the hepatic vein. Freshly isolated, proximal convoluted tubules readily convert cAMP into adenosine in a concentration-and time-dependent manner (443).

The most recent studies strongly suggest that an autocrine/paracrine extracellular cAMP-adenosine pathway, in fact, does not participate in the regulation of Na^+ transport by proximal epithelial cells. It appears that the pancreatohepatorenal extracellular cAMP pathway is more important in modulating proximal tubular function. Whereas intrarenal infusions of glucagons do not reduce Na^+ excretion, intraportal infusions of glucagon in sheep cause a marked antidiuresis. Although speculative, it is conceivable that the pancreatohepatorenal cAMP–adenosine pathway participates in physiological adjustments of renal transport, as well as in pathophysiological processes. In normal mammals, both hypoglycemia and exercise are powerful stimulants to glucagon release. Activation of the pancreatohepatorenal cAMP–adenosine pathway by glucagon in response to hypoglycemia might increase Na^+ glucose symport in proximal tubules and, thus, increase the efficiency of glucose transport, an adaptive mechanism to combat hypoglycemia. Activation of the pancreatohepatorenal cAMP-adenosine pathway by glucagon during exercise might enhance Na^+ transport in the proximal tubules and thus increase the efficiency of Na^+ reabsorption, an adaptive mechanism to avoid volume depletion during sustained physical exertion. The pancreatohepatorenal cAMP-adenosine pathway might be overly activated in the metabolic syndrome characterized by obesity, insulin resistance, hyperlipidemia, and hypertension. Although oral glucose normally strongly inhibits glucagon secretion by the pancreas, in animals and people with the metabolic syndrome, an oral glucose challenge markedly stimulates pancreatic glucagon secretion by approximately 200%. If the pancreatohepatorenal cAMP–adenosine pathway exists, each time such a patient ingests a high-carbohydrate meal, the renal tubules would be exposed to a wave of excess adenosine production. Because adenosine causes increased reabsorption of Na^+ and vasoconstriction of the preglomerular microcirculation, this could contribute to the pathophysiology of hypertension in the metabolic syndrome. Importantly, adenosine receptors also inhibit lipolysis in fat cells and may reduce insulin sensitivity in skeletal muscle. Therefore, if adenosine is synthesized by adipocytes and skeletal muscle from liver-derived cAMP, adenosine could be a common denominator linking obesity, insulin resistance, and hypertension. However, at this time, both the physiological and pathophysiological roles of the putative pancreatohepatorenal cAMP-adenosine pathway are speculative. In the proximal tubule, the pancreatohepatorenal extracellular cAMP pathway appears to be more important than the autocrine/paracrine extracellular cAMP-adenosine pathway in determining the exposure of proximal tubules to adenosine. In the collecting duct, the opposite may be true (443).

PARATHYROID HORMONE AND PARATHYROID HORMONE-RELATED PEPTIDE

In response to low levels of extracellular Ca, parathyroid glands secrete parathyroid hormone (PTH), an 84-amino acid polypeptide hormone (478). PTH-related peptide (PTHrP) was first identified as a cause of humoral hypercalcemia of malignancy and is secreted predominately as a 141-amino acid peptide. While PTHrP may have a physiologic role in skeletal muscle development, it appears to play a predominately pathophysiologic role in the adult. PTH and PTHrP have sequence homology in the first 13 amino acids (479). Increased circulating PTH or PTHrP leads to mobilization of Ca from bone, enhancement of Ca reabsorption in the renal tubule, and increased production of 1,25-dihydroxyvitamin D_3 ($1,25[OH]_2D_3$) by proximal tubule cells. $1,25(OH)_2D_3$, in turn, increases Ca absorption by the intestine and possibly Ca reabsorption by the kidney. The combined actions of PTH and $1,25(OH)_2D_3$ result in normalization of the extracellular Ca concentration. Although its main physiologic role is to maintain normal Ca homeostasis, PTH exerts other biologic effects in the body that could be of importance in pathologic conditions, such as uremia (480). In addition to its regulatory actions on calcium balance, PTH can regulate phosphorous balance by inhibiting its reabsorption in the proximal and distal tubules of the nephron.

PTH synthesis and secretion by the parathyroid gland is tightly regulated (478). While a decreased extracellular Ca level stimulates PTH synthesis and secretion, increased extracellular levels of either Ca or $1,25(OH)_2D_3$ are inhibitory. The extracellular phosphorous level regulates PTH production directly at a posttranscriptional level (481) and indirectly by altering circulating Ca and $1,25(OH)_2D_3$ concentrations. Increased serum phosphorous secondary to renal insufficiency, for example, decreases Ca concentration and $1,25(OH)_2D_3$ production, leading to stimulation of PTH release, but can itself increase PTH level without alterations in ionized calcium or serum $1,25(OH)_2D_3$ levels (482,483).

Two types of receptors exist for PTH and PTHrP. Type-1 PTH receptors (PTH1R) bind PTH and PTHrP with high affinity. Binding to PTH1R occurs in the 15- to 34-amino acid region of PTH and PTHrP. It is interesting that these two peptides bind with almost equal affinity and yet do not share sequence homology in this region. The PTH1R mediates the biologic activity of PTH and PTHrP whereas Type2 PTH receptors (PTH2R), which share 51% homology with PTH1Rs Type-2 receptors, can bind PTH but they do not bind PTHrP with high affinity. PTH2Rs are expressed in only a few tissues; their biologic significance is unknown (479).

The remainder of this section will focus on the renal actions of PTH. The effect of PTH on other organs and on the immune system is discussed elsewhere (480).

Renal Actions of Parathyroid Hormone

PTH receptors and PTH-sensitive adenylate cyclase have been identified in glomeruli and basolateral membranes of epithelial cells in the proximal tubule, thick ascending limb of Henle's loop, and distal convoluted tubule (DCT) (13,484). PTH has three major effects on the kidney: It increases Ca reabsorption, inhibits P reabsorption, and stimulates synthesis of the most active form of vitamin D, $1,25(OH)_2D_3$. Its other actions on the kidney include modulation of GFR, gluconeogenesis, magnesium reabsorption, and acid-base handling.

PTH decreases renal Ca excretion through multiple mechanisms. Ichikawa and co-workers (485) demonstrate that PTH infusion in rats decreases GFR by reducing K_f. A decreased GFR leads to decreased filtered load of Ca and, therefore, Ca excretion. PTH also enhances tubular Ca reabsorption by stimulating active Ca transport in the thick ascending limb of Henle's loop and distal tubule (486,487). The effect of PTH on Ca transport in the proximal tubule varies and is probably related to Na and water reabsorption in this nephron segment.

PTH causes phosphaturia primarily by inhibiting phosphate transport in the proximal tubule, specifically by inhibiting sodium-phosphate (Na–P) cotransport (488). Two different renal Na–P cotransporters have been identified and have been termed type 1 (Npt1) and type 2 (Npt2). Npt2 is a target for regulation by PTH and decreases Na–P transport by endocytic retrieval and lysosomal degradation of the Npt2 protein (489). This endocytic retrieval can occur either from proximal tubules exposed to apical or basolateral PTH and can signal via either the cAMP–PKA or the PLC–PKC pathway (490). Mice that are Npt2 null have profound phosphate wasting (491). Npt2-null mice are resistant to further phosphaturic effects from exogenous PTH (492). α_2-Adrenergic receptor stimulation blunts the phosphaturic response to PTH (493). PTH-stimulated synthesis of $1,25(OH)_2D_3$ in the proximal tubule is discussed in the next section of this chapter.

Although no single hormone has been specifically shown to regulate Mg homeostasis, PTH appears to increase Mg reabsorption in the kidney (494). PTH also plays a role in acid–base homeostasis by enhancing urinary acid excretion (495). Although PTH inhibits bicarbonate reabsorption in the proximal tubule, it indirectly stimulates distal hydrogen ion secretion and titratable acid excretion by increasing phosphate delivery to the distal nephron. PTH has also been shown to enhance proximal tubular gluconeogenesis (496) and renin secretion by juxtaglomerular cells (497).

VITAMIN D

Along with PTH, vitamin D plays a central role in calcium and phosphate homeostasis. The active form of vitamin D, $1,25(OH)_2D$, is a steroid molecule synthesized in the body from either vitamin D_3 (cholecalciferol) or vitamin D_2 (ergocalciferol) (498–500). Vitamin D_3 is present in the diet (animal sources, mainly fish oil) and is also synthesized by the skin from 7-dehydrocholesterol upon exposure to ultraviolet light. Vitamin D_2 is available only from dietary sources (plants, yeast, and fungi). The first step in the biosynthesis of $1,25(OH)_2D$ is conversion of vitamin D_2 or D_3 to the inactive intermediary form 25(OH)D through the action of hepatic microsomal and mitochondrial vitamin D–25-hydroxylase. Subsequently, 25(OH)D is hydroxylated exclusively in the proximal tubule of the kidney by the mitochondrial 25(OH)D–1 α-hydroxylase (501). 1α-Hydroxylation is the rate-limiting step in the formation of the most abundant active metabolite $1,25(OH)_2D_3$ (499,500,502). This step is tightly regulated through multiple feedback mechanisms (500,502) (Fig. 9-11). An increase in PTH levels, secondary to decreased serum Ca, stimulates 1α-hydroxylase activity in the kidney. A low serum phosphorous or $1,25(OH)_2D_3$ concentration also activates 1α-hydroxylase, while elevated $1,25(OH)_2D_3$ levels are inhibitory. Other less

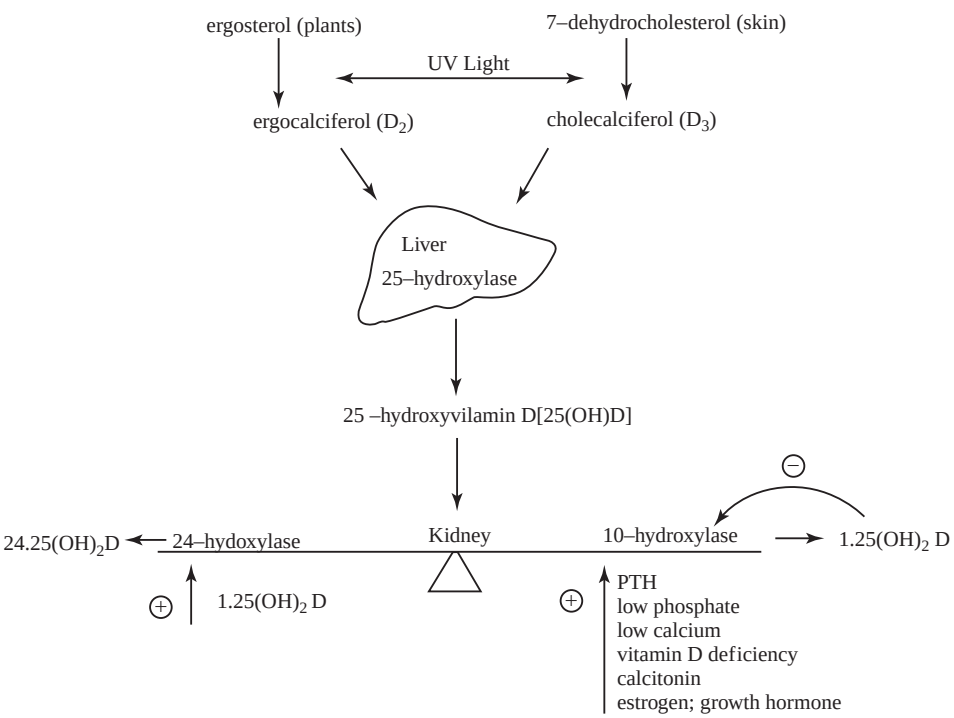

FIGURE 9-11. Major pathways of vitamin D metabolism. Regulation occurs primarily at the 1α-hydroxylase enzyme in the kidney, which controls the rate of synthesis of the most active calcemic metabolite, 1,25-dihydroxyvitamin D [$1,25(OH)_2D$]. When plasma concentrations of $1,25(OH)_2D$ are high, it feeds back to inhibit its own synthesis and favors production of 24,25-dihydroxyvitamin D [$24,25(OH)_2D$].

established stimulators of 1α-hydroxylase activity include calcitonin, growth hormone, insulin, insulin-like growth factor, estrogen, and prolactin (499,500,502).

In addition to the kidney, extrarenal sites of 1α-hydroxylase activity have been identified (503). These include macrophages, keratinocytes, hepatocytes, and human placenta, as well as skeletal muscle cells (504) and various bone cell preparations (505,506). Extrarenal 1α-hydroxylase is regulated independently from the renal enzyme, and $1,25(OH)_2D_3$ produced by these cells most likely functions in an autocrine or paracrine manner. Extrarenal production of $1,25(OH)_2D_3$ can lead to hypercalcemia in certain pathologic situations, as has been described in an anephric patient with active sarcoidosis (503,507) or in patients with lymphoma (508).

The first step in the inactivation and catabolism of vitamin D is hydroxylation of 25(OH)D and $1,25(OH)_2D$ by 24-hydroxylase, which is present in the kidney, intestine, and several other tissues that possess $1,25(OH)_2D_3$ receptors (501,502). Importantly, kidney 24-hydroxylase and 1α-hydroxylase are reciprocally regulated (501,502,509). $1,25(OH)_2D_3$ and hypophosphatimia increase 24-hydroxylase activity while vitamin D deficiency and PTH (via CAMP) suppress it (509).

$1,25(OH)_2D_3$ exerts its biologic actions by binding to intracellular vitamin D receptors (VDR), which in the unliganded form are located in the cytosolic and nuclear compartments (510). Like other members of the steroid thyroid family of receptors, the liganded form of VDR functions as a transcription factor in the nucleus (511). The VDR–$1,25(OH)_2D_3$ complex regulates transcription of more than 60 genes by interacting with DNA sequences known as vitamin D response elements. Central to its role in Ca homeostasis, $1,25(OH)_2D_3$ induces the transcription of genes coding for Ca-binding proteins (512). Vitamin D-dependent Ca-binding proteins (CaBP-Ds) are found in high concentrations in Ca-transporting tissues, such as the kidney, intestine, and placenta. CaBP-Ds bind calcium with high affinity and are associated with Ca transport in these organs. In order to identify other physiologically significant genes that are regulated by vitamin D, the technique of subtractive hybridization was used on mRNA isolated from vitamin D-replete and vitamin D-deficient chicks. This technique identified the upregulation of five nuclear-encoded genes and the downregulation of seven mitochondrially encoded transcripts in the avian kidney (513). Hopefully, this technique will lead to the identification of other physiologically important pathways that are regulated by vitamin D.

Another potential regulatory site for vitamin D signaling, other than the synthesis and degradation of $1,25(OH)_2D_3$, is by regulation of VDR expression. Receptor regulation has been shown to be physiologically important, as $1,25(OH)_2D_3$ signaling is dependent on both the number and occupancy of VDRs on the cell surface membrane (514,515).

Physiologic Actions of $1,25(OH)_2D_3$

$1,25(OH)_2D_3$ participates in a hormonal system that tightly regulates the extracellular Ca concentration (512). A decline in serum Ca stimulates PTH release, which acts on the kidney to increase production of $1,25(OH)_2D_3$. $1,25(OH)_2D_3$, in turn, stimulates intestinal absorption of Ca and decreases its excretion by the kidneys. $1,25(OH)_2D_3$ also acts on bone to stimulate Ca mobilization (516). Normalization of serum Ca shuts off this cascade by suppressing 1α-hydroxylase activity in the kidney and release of PTH from the parathyroid glands. Increased $1,25(OH)_2D_3$ also contributes to turning off Ca-correcting mechanisms by inhibiting its own production and that of PTH (517). In addition to its effects on Ca homeostasis, $1,25(OH)_2D_3$ also enhances phosphate absorption in the intestine and kidney.

The presence of vitamin D receptors on monocytes/macrophages and activated lymphocytes suggests that $1,25(OH)_2D_3$ plays a role in regulating the functions of these cells (518). *In vitro,* $1,25(OH)_2D_3$ has been shown to inhibit lymphocyte proliferation, interleukin-2 production, and the synthesis of several monocyte-derived cytokines. These effects, however, vary between different experimental systems, and their physiologic significance remains unclear.

Effects of $1,25(OH)_2D_3$ on Renal Handling of Calcium and Phosphorus

About 50% to 60% of filtered Ca is reabsorbed in the proximal tubule. This process is Na-dependent, and the majority of Ca is reabsorbed via a paracellular pathway (519,520). Approximately 20% of filtered Ca is reabsorbed in Henle's loop, 10% to 15% in the distal tubule, and 5% in the collecting duct. Unlike the proximal tubule, Ca reabsorption in the distal tubule appears to be Na-independent and occurs against steep chemical and electrical gradients (519). In addition to vitamin D receptors, distal tubule epithelial cells contain CaBP-Ds and ATP-dependent plasma membrane Ca pumps (521). It has been postulated, therefore, that $1,25(OH)_2D_3$ enhances renal Ca reabsorption by direct action on the distal tubule in a manner analogous to stimulation of Ca absorption by intestinal cells (512). Several experimental studies support this hypothesis: Concentrations of CaBP-Ds and Ca transport rates in renal cells are increased by vitamin D, while vitamin D deficiency abolishes CaBP-D synthesis and decreases Ca absorption (512). Experimental studies also suggest a role for $1,25(OH)_2D_3$ in phosphate handling by the kidney. In isolated perfused proximal tubule segments, low concentrations of $1,25(OH)_2D_3$ antagonize the phosphaturic action of PTH (521). In rats in which vitamin D deficiency was induced, but the diet was manipulated to maintain normocalcemia, normophosphatemia, and normal PTH levels, $1,25(OH)_2D_3$ stimulated tubular reabsorption of phosphate (522). It was thought that $1,25(OH)_2D_3$ regulates phosphate reabsorption by direct modulation of Na–P cotransport in renal tubule cells (523). However, recent data on rats subjected to low-phosphate diet suggest that the Na-P cotransport in the kidney cannot be explained by the 1,25(OH)2D–VDR axis (524).

In patients with renal failure requiring dialysis, serum phosphate levels increase as a result of the decreased capacity of the kidney to excrete phosphate (decreased clearance and decreased response to the phosphaturic effect of PTH); the elevated serum phosphate inhibits formation of $1,25(OH)_2D_3$ and leads to decreased serum calcium levels and to increased PTH secretion (secondary hyperparathyroidism). PTH would lead to worsening of hyperphosphatemia with more phosphate released from bone than excreted in the kidney with diminished PTH-sensitivity, ultimately leading to renal osteodystrophy and possible calcium phosphate precipitation in tissues and vessels with an associated increase in cardiovascular events. The use of active vitamin D analogs in an attempt to remedy secondary hyperparathyroidism may increase calcium while worsening hyperphosphatemia therefore increasing the Ca × P product; that is, making CaP precipitation more likely. Phosphate binders can help decrease serum phosphate levels.

A new set of agents have been approved in this setting (e.g., Cinacalcet). They are called calcimimetics, because they bind to the calcium-sensing receptor in the parathyroid gland and increase its sensitivity to calcium, therefore counteracting the excessive PTH secretion in a dose-dependent manner and decreasing the Ca × P product (525).

Fibroblast Growth Factor 23

Fibroblast growth factor 23 (FGF23) is a phosphaturic substance recently discovered in the human circulation that decreases serum phosphate and 1α-hydroxylase levels by a mechanism that is VDR independent, and induces 24-hydroxylase activity, therefore reducing $1,25(OH)_2D_3$ by a VDR-mediated mechanism (526). It has been implicated in the pathogenesis of X-linked hypophosphatemic rickets/osteomalacia (XLH), tumor-induced osteomalacia (TIO), and autosomal-dominant hypophosphatemic rickets/osteomalacia (ADHR) entities with common features, including hypophosphatemia because of renal phosphate wasting and impaired mineralization of bone with normal serum calcium and PTH (527,528).

EICOSANOIDS

The eicosanoids are a group of locally acting hormones or autacoids that are derived from dietary polyunsaturated fatty acids. In humans, arachidonic acid, an essential fatty acid esterified into cellular membrane phospholipids, is the most abundant and important precursor. After deesterification by phospholipases, free arachidonic acid may either rapidly reesterify into membrane lipids, avidly bind intracellular proteins, or undergo enzymatic oxygenation to yield the various biologically active molecules referred to as eicosanoids. The type of product formed depends on the enzymes involved in the oxygenation process (529) (Fig. 9-12). Oxygenation of arachidonic acid by cyclooxygenase results in prostaglandin and thromboxane (TX) synthesis. Oxygenation by lipoxygenase generates hydroxyeicosatetraenoic acids and leukotrienes. These two major enzymatic pathways are all expressed in the kidney (530,531). The specific nature of the products generated varies with both cell type and initial stimulus for arachidonic acid release. Eicosanoids have diverse biologic effects in the kidney, the significance of which will be discussed below.

PROSTAGLANDINS

Prostaglandins (PGs) are a unique group of cyclic fatty acids with diverse biologic effects that are produced throughout the body. The kidney is a major site of PG production, metabolism, and action (530,531). PGs are important modulators of renal function in both physiologic and pathophysiologic settings. The spectrum of their effects in the kidney encompasses modulation of RBF, GFR, salt and water transport, and the release of renal hormones. It is within the setting of compromised renal status that maintenance of renal function is most dependent on PGs. Under these circumstances, inhibition of PG synthesis with nonsteroidal antiinflammatory drugs (NSAIDs) is likely to impair renal function.

Biosynthesis and Metabolism

Arachidonic acid (eicosatetraenoic acid) is the major substrate for the synthesis of PGs in humans. The initial step is catalyzed by cyclooxygenase (COX). For a description of the biosynthetic pathways leading to the different types of PGs and thromboxanes, or collectively prostanoids, the reader is referred to an excellent review by Claria and Arroyo (532) (Fig. 9-12).

The rate of PG production is dependent on the release of free arachidonic acid from tissue stores by phospholipase A_2 (PLA_2). Arachidonate tissue stores vary with dietary intake of essential fatty acids and can be depleted when intake is deficient (533). Fish-oil diets (rich in omega-3 polyunsaturated fatty acids) will compete for the arachidonate oxidation process and inhibit formation of active products (534). Several renal pathophysiologic states such as glomerulonephritis and ureteral obstruction are associated with increased prostanoid production (535–537). Table 9-4 lists several modulators of the key steps involved in PG synthesis.

The COX pathway is the major pathway for arachidonic acid metabolism in the kidney (530,531). In both animals and humans, two separate COX enzymes have been identified that

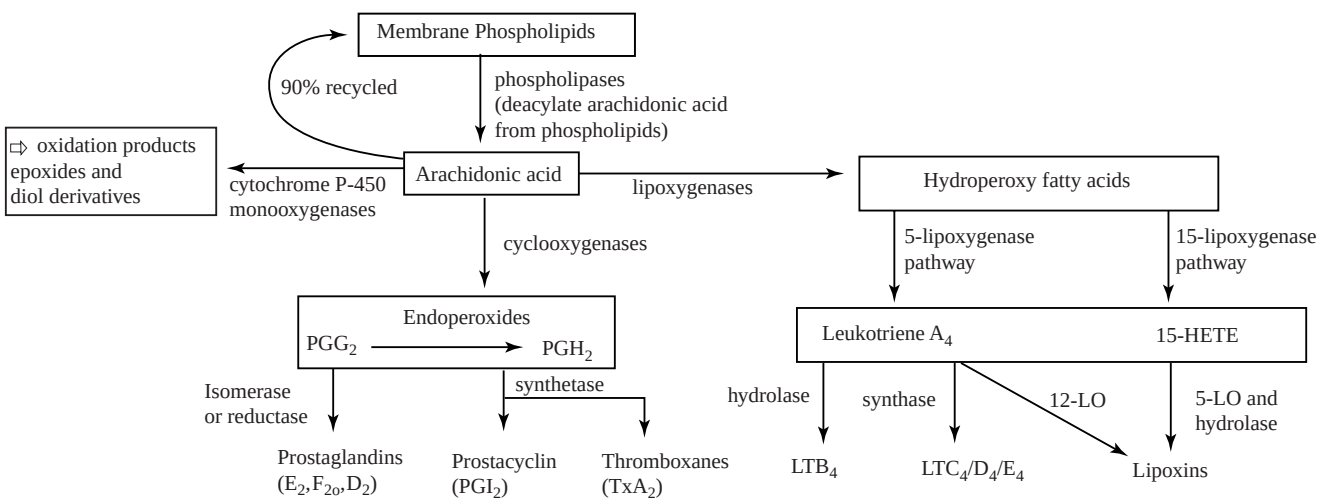

FIGURE 9-12. Renal eicosanoid synthesis. The oxygenated products of arachidonic-acid (eicosatetraenoic-acid) metabolism are referred to as eicosanoids. These include lipoxygenase, cytochrome P450 monooxygenase, and cyclooxygenase products. The lipoxygenase pathway yields hydroxy fatty acids and leukotrienes. The cytochrome P450 monooxygenase pathway yields ω-oxidation products and diol derivatives. The cyclooxygenase pathway yields the prostanoids, which include the prostaglandins (PGE_2, PGF_2, PGD_2, and prostacyclin) and thromboxane. The most important prostaglandins are dienoic (i.e., possessing two double bonds outside the ring structure; hence, the subscript 2.)

TABLE 9-4

MODULATORS OF PROSTAGLANDIN SYNTHESIS[a,b]

Modulator	Site of action
Promoters	
Angiotensin II	PLA_2
AVP	PLA_2
Bradykinin	PLA_2
Norepinephrine	PLA_2
PAF	PLA_2
Interleukin-1	PLA_2 and COX
TNF-α	PLA_2
PDGF	COX
EGF	COX
Calcium	PLA_2
Diabetes	PLA_2
Ischemia	PLA_2
Chronic AVP therapy	COX
Ureteral obstruction	COX and TX synthase
Venous obstruction	COX
Glomerulonephritis	COX
Nephrotic syndrome	TX synthase
Inhibitors	
Glucocorticolds	PLA_2 and COX
Potassium	PLA_2
Urea	PLA_2
Mepacrine	PLA_2
NSAIDs	COX

[a] *AVP*, arginine vasopressin; *PAF*, platelet-activating factor; *TNF-α*, tumor necrosis factor-α; *PDGF*, platelet-derived growth factor; *EGF*, epidermal growth factor; *NSAIDs*, nonsteroidal antiinflammatory drugs; *PLA*, phospholipase A; *COX*, cyclooxygenase; *TX*, thromboxane.
[b] Note that hormones are important physiologic modulators of prostaglandin production.

are encoded by two separate genes: *COX-1* (538) and *COX-2* (539). The human COX-1 enzyme is constitutively present in the renal vasculature (arterial and arteriolar endothelial cells, mesangial cells) (531,532,540–542), glomerular epithelial cells (543), renal interstitial cells (532,544), along most segments of the tubule, although in markedly varying concentrations (545,546,547) and in the medullary and papillary collecting ducts in humans, monkeys, dogs, rabbits, and rats (532). The COX-2 enzyme first thought to be only inducible in inflammatory responses, was found to be constitutive in the kidney (548). Its expression is consistently focal and limited to the MD of the juxtaglomerular apparatus, epithelial cells of the thick ascending limb, and papillary interstitial cells of rats, rabbits, and dogs. In the adult human kidney, expression of the COX-2 protein has been observed in endothelial and smooth muscle cells of arteries and veins, and intraglomerularly in podocytes, but not in MD cells (532).

Biologic Actions of Cyclooxygenase Products

PGs have diverse actions, in part related to their site of synthesis and the cells on which they act mostly in a paracrine fashion since they have a short half-life (Tables 9-5 and 9-6). Their principal physiologic role is mediation and/or modulation of hormone action at these locations (531,532,541,549,550). Thus, cortical production by arterioles and glomeruli is related to regulation of RBF, GFR, and renin release. Other cortical sites of PG production affect ammoniagenesis (551) and Ca and P transport (552). Medullary PG production is directed to regulating vasa recta blood flow, tubular Na and chloride transport, and the response of the collecting duct to AVP. Inhibition of COX activity in the absence of exogenous administration or endogenous release of hormones such as Ang II, norepinephrine, or AVP has little effect on renal functional parameters (553). Once their local release is enhanced, COX products may themselves stimulate the local generation of other hormones. PG-stimulated renin release is an example of this mode of action (549). Under pathophysiologic conditions, such as inflammatory injury, local release of prostanoids may mediate some of the functional derangements that characterize these conditions (535–537).

Prostanoids act through specific and distinct receptors (551). The cDNA for numerous prostanoid receptors, including receptors for TXA_2 (555,556), $PGF_{2\alpha}$ (557), and PGE_2 (558–561), have now been cloned and sequenced. All these receptors are members of the G protein-coupled family of receptors. Multiple subtypes of each of these prostanoid receptors may exist, as in the case with the PGE_2 receptor (EP receptor), thus explaining the apparently contrasting effects mediated by PGE_2 on smooth muscle and collecting duct permeability to water (562). The differential sensitivity of tissues to several structural PGE analogs has led to the identification of at least four distinct EP receptors: the two vasodilator receptors, EP_2 and EP_4, and the two vasoconstrictor receptors, EP_1 and EP_3. As noted, the EP receptors are members of the G protein-coupled family of receptors (563). EP_1 receptors signal mainly by IP_3-mediated increased intracellular Ca^{2+} (564–566). In contrast, the vasodilator receptors, EP_2 and EP_4, signal through increased cAMP (567–569). EP_3 receptors constrict smooth muscle probably by inhibiting cAMP generation via a pertussis toxin-sensitive, G_i-coupled mechanism (570,571). In mesangial cells, the $PGF_{2\alpha}$ receptor (FP receptor) seems to be coupled to increased intracellular Ca^{2+}. At higher concentrations, $PGF_{2\alpha}$ also stimulates EP receptors (572,573). The TXA_2 receptor (TP receptor) appears to signal via phosphatidylinositol hydrolysis, leading to increased intracellular Ca^{2+} (555). There is pharmacologic evidence for existence of TP receptors in the glomerulus (572). The PGI_2 receptor (IP receptor) signals via stimulation of cAMP generation (574). PGI_2 has been demonstrated to play an important vasodilator role in the glomerular microvasculature, where the effects of PGI_2 and PGE_2 to stimulate cAMP generation were distinct and additive (575).

Renal Hemodynamics

There are some species differences in the renal actions of PGs, and this must be taken into account when extrapolating data from animals to humans. While acute inhibition of PG synthesis does not change arterial pressure in normal circumstances, it does produce both an increase in renal vascular resistance and a decrease in sodium and water excretion (576–578). In general, PGE_2 and PGI_2 are vasodilators in most species, while TXA_2, $PGF_{2\alpha}$, and PGE_2 (in certain circumstances) are vasoconstrictors (579,580). PGE_2 relaxes rat and rabbit afferent arterioles (581). PGI_2 is a potent vasodilator in both humans and dogs (582,583). It is also a highly potent relaxant of rabbit afferent, efferent, and interlobular arteriolar smooth muscle (580), but has little intrinsic vasoactive properties in rat kidney (584). $PGF_{2\alpha}$ is without effect on any arterial segment in rabbit kidney (581), but is a mild vasoconstrictor in dogs (583). TXA_2 analogs exert constrictor effects on rat arteriolar smooth muscle and cause renal vasoconstriction accompanied by a severe reduction in filtration fraction, suggesting a predominant preglomerular action (585,586). The contribution of these

TABLE 9-5

RENAL ACTIONS OF EICOSANOIDS[a]

	Product pathway		
Action	**Cyclooxygenase**	**P450 Epoxygenase**	**Lipoxygenase**
Vascular			
Constriction	TXA_2	5,6-EET	LTD_4, LTC_4
		20-HETE	LXA_4, LXB_4
Dilation	PGE_2, PGI_2	5,6-EET	LXA_4, cyclooxygenase-dependent
Mesangial			
Contraction	TXA_2, $PGF_{2\alpha}$	LTD_4, LTC_4	
Relaxation	PGE_2, PGI_2		
Mitogenic	$PGF_{2\alpha}$, TXA_2		LTC_4
Antimitogenic	PGI_2, PGE_2		
Na transport			
Inhibition	PGE_2	5,6-EET	
Na–K-ATPase			
Inhibition	PGE_2	11,12-DHT	
Stimulation		19-HETE	
Water transport			
Inhibition	PGE_2	EETs, DHTs	

[a] *TXA*, thromboxane A; *PGE*, prostaglandin E; *PGI*, prostaglandin I; *PGF*, prostaglandin F; *EET*, epoxyeicosatrienoic acid; *HETE*, hydroxyeicosatetraenoic acid; *DHT*, dihydrotestosterone; *LTD*, leukotriene D; *LTC*, leukotriene C; *LXA*, lipoxin A; *LXB*, lipoxin B. See text for references.

vasoactive properties of COX products to the regulation of renal vascular tone under normal physiologic conditions is probably minimal (587–590). This is best exemplified by the minimal change or absence of change in RBF and GFR in euvolemic rats or humans following COX inhibition (97,98,100) or selective antagonism of TXA_2 synthesis (587) or actions (585).

In contrast, the local release of vasodilator PGs (PGE_2 and PGI_2) in response to renal vasoconstrictors plays an important role in maintaining RBF and GFR. There is compelling evidence indicating that mesangial cell synthesis and release of PGE_2 and PGI_2 modulate the constrictor actions of angiotensin II, norepinephrine, and AVP (531,532,572,591). Activation of the renin–angiotensin and sympathetic nervous systems leading to enhanced release of angiotensin, catecholamines, and AVP occurs in conditions, such as hemorrhage, volume depletion, general anesthesia, cirrhosis, and cardiac failure. While serving to maintain the systemic blood pressure, these hormones constrict mesangial cells and glomerular arterioles. Fortunately, their enhancement of renal PG release locally opposes their constrictor effects. The vasodilatory action of PGs on the afferent arteriole serves to maintain renal perfusion, whereas their relaxant effects on mesangial cells maintains the effective surface area for filtration (579). Inhibition of PG generation in these circumstances is associated with a dramatic fall in RBF and GFR (591–593). Vasodilator PGs, in particular PGI_2, may also counteract the vasoconstrictor responses to calcium in human subjects (594).

In addition to modulating the effects of vasoconstrictors, endogenous PGs mediate the actions of some vasodilator agents. These include a role for PGI_2 in mediating the vasorelaxant actions of dopamine (595) and magnesium (596) in humans, and of hydralazine (594) and epidermal growth factor (598) in dogs. PGs may also mediate the renal vasodilatory response to a protein meal in humans (599). Conversely, TXA_2, whose synthesis is increased in experimental glomerular immune injury and ureteral obstruction, may cause glomerular contraction (535,600).

Solute Excretion

Infusion of arachidonic acid or the COX products PGE_2 or PGI_2 directly into the renal artery results in natriuresis (601,602). While hemodynamic changes probably contribute, the natriuresis is largely a direct tubular phenomenon originating in the distal nephron (602). PGE_2 has mild or no effects on Na transport in the proximal tubule and most segments of the ascending limb of Henle, with the exception of the medullary thick ascending limb in some species (603). This lack of effect is in keeping with both the low rates of PG production and the low density of PG receptors in these nephron segments (546,547). Under normal circumstances, inhibition of COX does not result in alteration of Na delivery out of the loop of Henle to the early distal tubule (604). PGE_2, however, has significant effects on Na transport in the collecting duct, where it inhibits transepithelial Na transport (601,603). In fact, in most mammalian species, the collecting ducts are the major nephron segments responsible for PG synthesis (546,547) and, along with the medullary thick ascending limb, express the majority of receptors for PGE_2 in the kidney (605,606).

There is evidence that PGE_2 exerts its inhibitory effect on rabbit cortical collecting duct Na transport by two mechanisms. The first involves inhibiting principal cell basolateral Na–K-ATPase activity (607,608) and the second by directly decreasing the open probability of the apical amiloride-sensitive Na channels (609). PGE_2 utilizes multiple signal transduction pathways in the cortical collecting duct. These include increase of intracellular Ca (602), activation of PKC (609), and modulation of cAMP levels (610, 611). While modulation of cAMP

TABLE 9-6

RENAL ACTIONS OF THE DIFFERENT PROSTANOIDS[a]

Mediator	Main source	Primary effects
PGE_2	Tubular epithelial cells Interstitial cells	Renal vasodilation Relaxation mesangial cells Modulation of glomerular capillary ultrafiltration coefficient Simulates rennin release from juxtaglomerular apparatus Antagonizes hydroosmotic effect of ADH in collecting tubular epithelial cells Inhibits sodium chloride reabsorption Mediates renal response to loop diuretics
PGI_2	Vascular and glomerular endothelial cells	Renal vasodilation Relaxation of mesangial cells Stimulates rennin release from juxta glomerular apparatus
$PGF_{2\alpha}$	Mesangial cells Glomeruli	Contracts smooth muscle
TXA_2	Glomeruli	Contracts smooth muscle Contracts mesangial cells

[a] *TXA*, thromboxane A; *PGE*, prostaglandin E; *PGI*, prostaglandin I; *PGF*, prostaglandin F.

levels appears to mediate the effects of PGE_2 on water transport in the cortical collecting duct, it is less clear that PGE_2 affects Na transport via modulation of cAMP levels (612). The inhibitory effects of PGE_2 on Na transport in the thick ascending limb probably involve inhibition of adenyl cyclase activation (613). PGE_2 also blocks the phosphaturic action of PTH in the proximal tubule (614), an effect probably mediated by blocking the activation of adenyl cyclase by PTH (615). These effects are probably mediated by the G_i-coupled EP_3 receptor, which is expressed in the thick ascending limb (616–618). Consistent with this possibility, Good (619,620) has demonstrated modulation of ion transport by PGE_2 in the rat thick ascending limb by a pertussis toxin-sensitive mechanism. However, these effects possibly also involve PKC activation, suggesting the involvement of other receptors such as EP_2 and EP_4.

Na loading is associated with an increase in urinary PG excretion, yet PGs are not important in regulation of Na balance in normal euvolemic subjects. In circumstances associated with Na retention and compromised renal function, PGs play a significant role. Inhibition of their synthesis or blocking their effects in such conditions is associated with Na retention (592). Administration of furosemide *in vivo* is associated with increased PG excretion, which is, in part,mediated by a direct action on tubular cells (592,621). Administration of NSAIDs diminishes the natriuretic action of furosemide and other loop diuretics, suggesting a role for PGs in mediating the action of these agents. This cannot be the sole mechanism, however, for the natriuretic response to diuretics outlasts the increase in PG excretion.

Water Excretion

PGs, especially PGE_2, affect water transport in the collecting duct in many ways. Although the direct effect of either PGE_1 or PGE_2 is to stimulate basal hydraulic conductivity of isolated perfused collecting tubules, PGs of the E series blunt the hydraulic conductivity response of the collecting duct to AVP (622,623). In fact, *in vivo* infusions of arachidonic acid or PGE_2 induce a water diuresis, while inhibition of PG synthesis potentiates the urinary hyperosmolality caused by AVP (624). However, in the absence of vasopressin, basolateral PGE_2 actually increases osmotic water reabsorption (625,626). These effects on water conductivity in the collecting ducts have been explained by changes in cyclic AMP accumulation. AVP mediates the increase in water conductivity in the collecting duct through increased cyclic AMP generation. Studies on the effect of PGE_2 on cyclic AMP metabolism in this nephron segment demonstrated that PGE_2 could both stimulate basal cyclic AMP generation and suppress AVP-stimulated cyclic AMP generation (610,611). The inhibitory effects of PGE_2 on AVP-stimulated cyclic AMP generation and water conductivity in the collecting duct are probably mediated through the EP_3 receptor via the pertussis-toxin sensitive inhibitory guanine nucleotide binding protein, G_i (611,627). In addition to affecting water flow via modulation of cyclic AMP levels, PGE_2 has been shown to inhibit AVP-induced water conductivity by activation of protein kinase C and elevation of intracellular calcium (562,623).

Application of basolateral PGE_2 probably increases water absorption in the collecting duct by stimulating cAMP production (625,626). The EP_4 receptor, which is found on the epithelial cells of the ureter, bladder, and collecting duct, is coupled to the G_s-stimulated cAMP signaling pathway (568,628). This suggests that an EP_4 receptor mediates cAMP-stimulated water absorption in the collecting duct.

Another facet of the PGE_2–AVP interaction is that AVP acutely stimulates endogenous PGE_2 production by the collecting duct. This effect has been demonstrated in rats (629,631) and the current consensus is that it also occurs in humans (630,631). It also suggests that PGE_2 participates in a negative feedback loop, whereby endogenous PGE_2 production dampens the action of AVP. In agreement with a functional role for the increase in urinary PGE_2 production seen during AVP infusions are numerous observations of enhanced renal concentrating ability in animals or humans pretreated with inhibitors of PG production (632). Given that the concentration of AVP needed to stimulate PGE_2 production is 10 to 100 times the concentration needed to maximally stimulate water

conductivity, it is controversial whether AVP plays a physiologic role in PGE_2 generation (633). At these concentrations, AVP has been shown to acutely increase intracellular calcium and activate protein kinase C, via activation of phospholipase C (633,634).

Other Effects on Renal Function

Extensive evidence supports the capacity of PGs, particularly PGE_2 and PGI_2, to release renin (635,636). In contrast, TXA_2 exerts a negative effect on renin release (637). Inhibition of COX activity reduces plasma renin activity, suggesting that the predominant influence of prostanoids is stimulatory. PG-mediated renin release is independent of β-adrenergic mechanisms (636). Conflicting evidence exists regarding involvement of prostanoids, particularly TXA_2, in the mediation of tubuloglomerular feedback responses (638).

Role of Prostaglandins in Disease States

PGs, through their vasodilator effects, play a salutary role in maintaining RBF and GFR in several prerenal conditions such as hemorrhage, septic shock, cirrhosis, and low cardiac-output states. Studies in patients with congestive heart failure have confirmed that enhanced PG synthesis is crucial in protecting kidneys from the effects of elevated vasoconstrictor levels in these patients (639). Renal artery stenosis is another condition associated with increased ipsilateral renal PG secretion (640) that may locally act to enhance renal perfusion. Administration of COX inhibitors in these settings with renal hypoperfusion is associated with adverse effects on RBF and GFR (640,641).

With regard to intrinsic renal diseases, COX products have been implicated in modulating or mediating renal injury (or both). After experimental reduction of renal mass, glomerular synthesis and urinary excretion of prostanoids per remaining nephron increases severalfold and probably contributes to the compensatory hypertrophy that follows renal ablation (642–645). In this setting, nonselective inhibition of COX activity reduces nephron perfusion and glomerular capillary permeability, implying a predominantly beneficial role for vasodilator PGs (643,644). In this model of reduction of renal mass, increased expression of COX-2 has been described (548). In contrast, selective inhibition of TXA_2 synthesis is associated with an increase in GFR, lessening of proteinuria, and preservation of renal histology (644,645).

Enhanced TXA_2 production has been implicated in the pathophysiology of the intense vasoconstriction that characterizes the obstructed kidney (646,647) and in mediating the decrease in RBF and GFR that occurs in the early phase of nephrotoxic serum nephritis (535,536). In patients with lupus nephritis, an inverse relation between TXA_2 biosynthesis and GFR has been proposed (648). In this setting, renal function improved after short-term therapy with a TX receptor antagonist, but not with aspirin (648,649). In addition, administration of TXA_2 synthesis inhibitors or receptor antagonists has been associated with improved renal function in animals with allograft rejection and cyclosporin toxicity (579).

The role of COX products in mediating diabetic nephropathy remains controversial. Vasodilator PGs may contribute to the hyperfiltration that occurs in early stages of diabetic nephropathy, whereas TXA_2 may play a role in the subsequent development of albuminuria and basement membrane changes (650). A role for decreased PGI_2 synthesis in type IV renal tubular acidosis associated with diabetes mellitus has also been suggested (651). A recent study has demonstrated an increased renal production of TXA_2 and PGI_2 in type II diabetes, suggesting a role for these compounds in the pathogenesis of diabetic nephropathy (652). Diminished vasodilator renal PG synthesis has also been implicated in the pathogenesis of the severe Na retention that occurs in patients with the hepatorenal syndrome (653). Pregnancy is associated with increased glomerular synthesis and urinary excretion of PGE_2, $PGF_{2\alpha}$, and PGI_2 (654). Augmented renal vasodilator PG production does not appear to regulate GFR and RBF in normal pregnancy; however, diminished synthesis of PGI_2 has been demonstrated in human (655) and animal models (656) with pregnancy-induced hypertension. A beneficial effect of reducing TXA_2 generation, while preserving PGI_2 synthesis, by low-dose (60 to 100 mg/day) aspirin therapy has been proposed in patients at risk for pregnancy-induced hypertension (657,658). In patients with hypertension, COX inhibition by NSAIDs is associated with increased salt retention and resistance to the diuretic action of thiazides and furosemide (659,660). Short-term use of some NSAIDs was found to increase the mean arterial pressure of hypertensive patients (661). On the other hand, attempts to treat hypertension with PG analogs have generally been disappointing (662).

Finally, chronic inhibition of COX by regular use of NSAIDs leads to gastrointestinal toxicity and may increase the risk of chronic renal disease, especially in older patients and patients with heart disease (663,664). Selective COX-2 inhibitors have been developed and have been shown to spare gastric PG production. These nontraditional COX-2 selective antiinflammatory agents might have represented a significant advance for the treatment of acute and chronic inflammatory disorders (665,666), however, the safety of their use has been placed into question after two large prospective cohorts in elderly patients showed an increased risk of cardiovascular events in patients on selective COX-2 inhibitors versus those on NSAIDs (667,668).

LIPOXYGENASE PRODUCTS

Biosynthesis and Metabolism

Lipoxygenases (LOs) comprise a family of enzymes capable of mediating selective lipid oxidation (669). Enzymatic lipoxygenation of arachidonic acid leads to the generation of leukotrienes (LTs), lipoxins (LXs), and hydroxyeicosatetraenoic acids (HETEs). Formation of these compounds is initiated by 5-, 12-, or 15-lipoxygenase, whereby a hydroperoxy group is introduced onto arachidonic acid at carbon-5, carbon-12, or carbon-15, respectively, to yield the corresponding 5-, 12-, or 15-hydroperoxytetraenoic acid (HPETE). HPETEs are unstable compounds that are transformed into the corresponding 5-, 12-, and 15-HETE, which, in turn, undergo enzymatic modification leading to the generation of the various LTs and LXs. The 5-lipoxygenase pathway is a major route of arachidonic acid metabolism in the polymorphonuclear cells and macrophages leading to the formation of 5-HETE and LTs (670–672). 5-Lipoxygenase requires activation by a cell membrane-bound protein called the 5-lipoxygenase-activating protein (FLAP) (673). The 15-lipoxygenase enzyme catalyzes the production of 15-HETE and initiates another major pathway of arachidonic acid metabolism in leukocytes. In activated neutrophils and macrophages, sequential lipoxygenation of arachidonic acid at carbons- 15 and -5 yields trihydroxy derivatives, the LXs.

Biologic Effects of Lipoxygenase Products

Products of lipoxygenase are classically proinflammatory, however, recently it has been demonstrated that lipoxins,

15-HPETE and 15-HETE may exhibit antiinflammatory activity (674).

The LTs are potent proinflammatory molecules. LTB_4 has minimal spasmogenic properties, but is the most potent chemotactic substance yet described for polymorphonuclear cells, and promotes their activation and adhesion to the endothelium (670). It has no significant effects on renal hemodynamics in normal animals, but amplifies glomerular inflammation and proteinuria in animals with glomerulonephritic injury (675). The peptidyl LTs contract vascular, pulmonary, and gastrointestinal smooth muscle and increase vascular permeability to macromolecules (670). LTC_4 and LTD_4 exert potent effects on glomerular hemodynamics. In rats, systemic administration of LTC_4 leads to reduction in RBF and GFR (676). Similarly, infusion of either LTC_4 or LTD_4 in the isolated perfused kidney results in dramatic increase in renal vascular resistance and reduction in GFR (677). LTD_4 mediates these effects by causing a significant increase in efferent arteriolar resistance, leading to a fall in glomerular plasma flow rate (Q_A), and a rise in glomerular capillary hydraulic pressure (P_{GC}). In addition, it markedly reduces the glomerular capillary ultrafiltration coefficient (K_f) and, therefore, its overall effect is to decrease single nephron GFR (678). LTC_4 and LTD_4 contract mesangial cells (679,680) and LTD_4 stimulates neutrophil adhesion to these cells (681). In both rats and humans, specific mesangial cell LTD_4 receptors have been identified. Intracellular signaling for LTD_4 in these cells involves receptor-activated phosphatidylinositol diphosphate (PIP_2) hydrolysis, release of inositol phosphates, and increased intracellular calcium concentrations (682,683).

LXA_4 attenuates LTB_4-induced neutrophil chemotaxis and inhibits natural killer cell cytotoxicity (670,672). The effects of LXA_4 are mediated primarily by functional high-affinity LXA_4 receptors (684). In rat glomerular mesangial cells, LXA_4 competes with LTD_4 at a common receptor whereby LXA_4 mediates partial agonist–antagonist effects (685). Different lipoxins display distinct effects on renal hemodynamics (672,686,687). In rats, LXA_4 causes a selective decrease in afferent arteriolar resistance, thereby increasing RBF, glomerular capillary pressure, and GFR. The LXA_4-induced increase in GFR, however, is partially offset by its mild effect in decreasing K_f (685,687). The vasodilator actions of LXA_4 are mediated by prostaglandins.

Role of Lipoxygenase Products in Kidney Disease

LTs are increasingly recognized as major mediators of glomerular hemodynamic and structural deterioration during the early phases of experimentally induced glomerulonephritis (686, 688,689).

Mesangial cell (MC) proliferation is a central event in the pathogenesis of glomerulonephritis. LTD_4-induced proliferation of mesangial cells is modulated by LXA_4. McMahon et al. (690) demonstrate that LXA_4 inhibits PDGF- and LTD_4-stimulated proliferation through modulation of platelet-derived growth factor receptor beta (PDGFR-β) activation. Specifically, they demonstrate that LTD_4 transactivates PDGFR-β, a process associated with *c-src* recruitment and *ras* activation (690).

Increased glomerular generation of LTB_4 and peptidyl-LTs has been demonstrated in several models of glomerular injury (686,688,689). LTB_4 probably worsens glomerular injury by augmenting leukocyte recruitment and activation and the peptidyl LTs, by depressing K_f and GFR (675-681). Selective blockade of the 5-lipoxygenase pathway, in the course of glomerular injury, is associated with significant amelioration of the deterioration of renal hemodynamic and structural parameters (691,692). In addition, dietary deprivation of essential fatty acids, which results in arachidonic acid and eicosanoid deficiency, confers protection against the histopathologic and the functional consequences of immune-initiated injury in the glomerulus (693). In human glomerulonephritis, 5-lipoxygenase and 5-LO-activating protein (FLAP) mRNA expression have been detected in kidney biopsy specimens from patients with immunoglobulin A (IgA) nephropathy and mesangial proliferative glomerulonephritis and were associated with a clinically worse renal status (694). Urinary LTE_4 levels are also elevated in patients with active systemic lupus erythematosus (695). A pathophysiologic role for LTs has also been described in experimental acute allograft rejection (696), cyclosporin toxicity, and acute ureteral obstruction (689). LXA_4 and 15-*S*-HETE are also generated during experimental glomerular injury and may exert salutary effects on glomerular function by antagonizing the proinflammatory actions of LTs (686,697–700). In animals with experimental glomerulonephritis, 5-lipoxygenase inhibition results in marked reduction in proteinuria and preservation of GFR (688,701). The binding of 5-lipoxygenase to FLAP is a prerequisite for subsequent formation of leukotrienes from arachidonic acid (702). The use of a FLAP antagonist has been shown to reduce proteinuria and restore glomerular size selectivity in human glomerulonephritis (703).

Diabetic nephropathy (DN) is characterized by mesangial cell (MC) hypertrophy and progressive accumulation of glomerular extracellular matrix (ECM). It was reported that 12/15-lipoxygenase (12/15-LO) expression is increased in high-glucose (HG)-stimulated MC and in experimental DN (704–706).

Hashimoto et al. (707) demonstrated that exogenous 12(*S*)-hydroxyeicosatetraenoic acids (HETE) enhanced the IL-1β-stimulated nitrite production and iNOS expression. On the other hand, the COX inhibitors, indomethacin and NS-398, had little effect on nitrite production or iNOS expression. These results suggest that LOX products play important roles in the regulation of stimulus-induced NO production in VSMCs.

The role of 5-lipoxygenase (5-LOX) in the pathophysiology of renal ischemia/reperfusion (I/R) injury is not known. Patel et al. (708) proposed, on one hand, that endogenous 5-LOX metabolites enhance the degree of renal injury, dysfunction, and inflammation caused by I/R of the kidney by promoting the expression of adhesion molecules. On the other hand, inhibitors of 5-LOX might be useful in the treatment of conditions associated with I/R of the kidney (708).

ENDOTHELIN

Endothelin (ET), originally isolated from vascular endothelial cells, is the most potent and long-lasting vasoconstrictor yet found (709). Its role, however, extends beyond being a vasoconstrictor to include effects on cell growth and proliferation, ion transport, eicosanoid synthesis, renin and ANP release, as well as a host of other actions (710–712). The kidney is an important site of ET production and expresses a high density of ET receptors (613). Endothelin may, therefore, act in an autocrine and paracrine manner to influence renal hemodynamics, tubular function, and mesangial cell biology.

Biochemistry, Synthesis, and Receptor Biology

The term *endothelin* refers to a family of homologous 21-amino acid vasoconstrictor peptides found in three distinct isoforms: ET-1, ET-2, and ET-3. In humans, ET isoforms are encoded by three separate genes located on chromosomes 6, 1, and 20, respectively (713–715). The initial ET peptide translation product is a large (approximately 200 amino

acids) isopeptide-specific prohormone named preproendothelin. Posttranslational processing of this prohormone to mature ET requires two steps. The first involves its proteolytic cleavage by dibasic pair-specific endopeptidases on Lys-Arg and Arg-Arg pairs, which respectively flank the N- and C-terminals of the preproendothelin molecule, to yield an intermediate 38- or 39-amino acid proET polypeptide. The subsequent step is accomplished by proteolytic cleavage of proET between Trp^{21} and Val^{22} by a putative "endothelin-converting enzyme" (716,717). All ET isopeptides have a hairpin loop configuration structure imparted by two intrachain disulfide bonds bridging amino acid residues 1 through 15 and 3 through 11, the reduction of which leads to a twofold loss of biologic activity (718). The three ET isoforms are highly homologous in their amino acid sequences and tertiary structure to certain scorpion and snake venoms, the sarafotoxins, which suggests common genetic evolutionary origins (719). While all isoforms of ET are potent vasoconstrictors, there are significant cell- and tissue-specific differences in the secretion of, and biologic responses to, different isoforms (713,720).

Seven isoforms of ECEs have been identified: ECE-1a, ECE-1b, ECE-1c, ECE-1d, ECE-2a, ECE-2b, and ECE-3 (715). Both ECE-1 and ECE-2 cleave big ET-1 more efficiently than either big ET-2 or big ET-3. ECE-1 is expressed ubiquitously with highest expression in endothelium, lung, ovary, testis, and adrenal medulla, while ECE-2 is expressed in neural tissues. ECE-3, which selectively cleaves ET-3, has been found in the bovine iris (721). ECE was located at the cell surface and on intracellular vesicles (722). ECE-1 also hydrolyzes BK, substance P, and insulin (715). Not all the enzymes responsible for the final step of posttranslational processing of ET-1 have been identified. However, proteases besides ECE-1 and ECE-2 are involved in the synthesis of ET-1. Recently, several inhibitors specific for the ET-converting enzyme have been reported.

Initial studies identified ET on the basis of its release from large-vessel endothelial cells. Since then, ET immunoreactivity has been detected in the kidney, spleen, skeletal muscle, and lung (723). In the kidney, the arcuate arteries, veins, glomerular arterioles, and capillaries are a rich source of ET (724). In the glomerulus, there is evidence for ET secretion by mesangial, endothelial, and epithelial cells (712). In the rest of the nephron, the IMCD has been demonstrated to be a major site of ET-1 and ET-3 production (712,725–727).

Normally, blood vessels produce very little ET, and the normal circulating level of ET is extremely low (728). Secretion of ET by endothelial cells is controlled at the level of transcription and these cells do not store ET for future release (716,729). Thus ET-1 probably acts primarily as a paracrine/autocrine mediator and not as a circulating hormone (715). ET peptide secretion is upregulated by various humoral mediators, such as thrombin, BK, insulin, Ang II, AVP, endotoxin, IL-1, TGF-β, and TNF (730,731).

These mediators may be responsible for the increase in ET observed in various pathophysiologic states. Hypoxia is also an important stimulus for ET production (732). In the kidney, increasing osmolarity serves as a stimulus for tubular production of ET (733,734). On the other hand, NO, ANP, and prostacyclin exert inhibitory influences on ET synthesis and release (735–737). In summary, mesangial cells ET-1 release is under complex regulation, with vasoconstrictor, profibrotic, inflammatory, and proliferative agents augmenting its release, whereas vasorelaxant agents tend to inhibit its production (738).

Endothelin acts via specific G protein-coupled receptors that have been identified in a variety of tissues (739). Three receptor subtypes have been cloned: The ET-A receptor, believed to be involved in vasoconstrictive amnd proliferative responses to ET-1 binds with the following affinity: ET-1>ET-2>ET-3 (715); ET-B receptor recognizes all three ET isoforms with equal affinity, its activation induces transient vasidilation, and the recently cloned ET-C receptor binds ET-3 selectively (712,739–741). Both ET-A and ET-B receptors are expressed on vascular smooth muscle. ET-B receptors decrease vasoconstriction by release of endothelial-relaxing factors; these receptors are also expressed by macrophages and platelets (715).

The kidney expresses abundant mRNA transcripts for ET-A and ET-B receptors (712,739,742,743). Expression of ET receptors is especially prominent in the renal artery, glomerular arterioles, endothelium, and mesangium, vasa recta bundles, and collecting duct (710,712,739). Both receptor subtypes are expressed in the glomerulus. Vascular smooth muscle cells of the arcuate arteries and the renal medullary interstitial cells display ET-A receptors. Epithelial cells of the cortical, inner medullary, and outer medullary collecting ducts have ET-B receptors (712).

Activation of the ET receptor on vascular smooth muscle cells leads to:

1. Activation of phospholipase C with formation of inositol triphosphate and diacylglycerol
2. Elevation of free intracellular Ca, which is thought to mediate the contractile response to ET (744–746).
3. Induction of the mitogen-activated protein kinase cascade (715).

ET increases free intracellular Ca by release of Ca^{2+} from intracellular stores via phosphoinositide-mediated mechanisms or by influx of extracellular Ca (or both) because of activation of cell membrane, voltage-dependent, dihydropyridine-sensitive Ca channels (745,746). Calcium-channel blockers inhibit ET-induced vasoconstriction in smaller blood vessels, where the contribution of extracellular Ca is important, such as intramyocardial coronary arteries (747). Endothelial cells can express ET-B receptors linked to formation of NO and prostacyclin and mediate endothelium-dependent vasorelaxation (748). In nephron segments and other renal structures, ET mediates its effects via a multiplicity of intracellular signal-transduction pathways that involve phospholipase activation, tyrosine phosphorylation of proteins, and elevation of intracellular free calcium (749).

Biologic Effects of Endothelin in the Kidney

Endothelin is a potent renal vasoconstrictor, as much as 30-fold more potent in this regard than Ang II (709–712,745–750). In the isolated perfused kidney, ET-1 administration reduces GFR and causes a dose-dependent increase in renal vascular resistance. In whole animals, systemic ET infusion induces a decline in cortical and medullary blood flow, GFR, and urine volume (710–712,745,750). The direct effects of ET-1 on preglomerular and postglomerular resistances are quantitatively similar at lower doses, so the glomerular transcapillary hydraulic pressure and GFR are maintained (750,751). However, at higher doses, a greater increase in preglomerular resistance occurs that, in addition to a decrease in glomerular capillary ultrafiltration coefficient (K_f), leads to a decline in GFR (745,750,751). Both ET-1 and ET-2 are equally potent in constricting microdissected glomerular arterioles, whereas ET-3 is considerably less potent (653). Dihydropyridines inhibit ET-mediated vasoconstriction exclusively in the afferent arterioles (752). Renal hemodynamics may also be influenced by indirect effects of ET, such as modulation of arachidonic acid metabolism and renin release (751,753). Local generation of PGs such as $PGF_{2\alpha}$ may mediate some of the vasoconstrictor effects of ET (753). ET-1 directly inhibits renin release, but the net renin secretory response *in vivo* varies with the ET-1 dose, as well as with the state of activation of intrarenal baroreceptors and the MD-mediated pathway (751).

Despite compromise of RBF and GFR, infusion of nonpressor doses of ET in animals is associated with an increase in urinary flow and Na excretion (710,711,745). In addition, studies in the isolated perfused kidney have shown that ET increases Na excretion despite a dramatic decline in GFR (509,711). These effects on Na and water balance are largely due to the ability of ET to reduce Na–K-ATPase activity and reversibly inhibit AVP-stimulated cAMP generation and water transport in the IMCD (509,711,754). Current evidence supports a physiologic role for ET in regulating Na and water transport in the IMCD in laboratory animals. In this regard, increasing the osmolality of the culture medium of IMCD cells with either NaCl or mannitol, but not urea, causes a dose-dependent reduction in ET-1 release. Moreover, ET-1 mRNA is lower in the medulla of dehydrated rats than in salt-loaded rats, supporting the hypothesis that extracellular osmolality may physiologically regulate ET production by IMCD cells (733). Because the human inner medulla contains a high concentration of ET receptors (742), coupled with the recent demonstration of ET production by human IMCD cells (726), it is conceivable that a similar physiologic role for intrarenal ET may also be operative in humans. ET also affects Na balance indirectly by stimulating release of ANP (755). ET-induced increases in urinary Na excretion in animals are markedly less distinct after pretreatment with ANP antibodies (756). Sonntag et al. (757) showed that IMCD ET-1 increases AVP V_2 receptor expression and these changes are mediated by the ET(B) receptor (757).

In summary, endothelin produced by the collecting duct promotes diuresis under physiologic circumstances (758) and induces naturesis and diuresis through interference with sodium absorption in the proximal convoluted tubules (759).

ET is well known to induce contraction of mesangial cells in culture and results of micropuncture experiments demonstrate that ET-1 directly reduces the coefficient of ultrafiltration (745). ET is also recognized as a growth factor with mitogenic effects on mesangial cells in culture, inducing changes in mesangial cell phenotype and gene expression (710–712,739).

Abundant evidence points to a pivotal role for ET-1 in the biology and, particularly, the pathology of the renal mesangium. The peptide is produced by mesangial cells (MC) and can, in turn, act on MCs to elicit proliferation, hypertrophy, contraction, and/or extracellular matrix accumulation. These effects are mediated in large part through activation of ET-A and particularly involve PKC and MAPK. Excessive ET-1 production by, and action on, MCs is of pathogenic importance in glomerular damage in animal models of GN, diabetes, and hypertension (Fig. 9-13) (738).

Pathophysiologic Significance of Endothelin in Kidney Disease

Declining renal function is associated with an increase in plasma ET levels (760). Patients undergoing hemodialysis have higher ET levels than do either patients undergoing peritoneal dialysis or uremic patients who are not dialyzed (761). Erythropoietin administration as well as acute volume contraction during hemodialysis may contribute to the elevation of ET-1 level in patients undergoing hemodialysis (762,763). Several disease states, such as hypertension, atherosclerosis, cardiogenic shock, congestive heart failure, immunosuppression with cyclosporin administration, and endotoxemia have also been associated in some (but not all) studies (760) with increased plasma ET levels (710–712,764,765). While the significance of elevated plasma levels of a primarily autocrine or paracrine hormone, such as ET remains questionable at present, ET-secreting hemangioendotheliomas produce marked hypertension (766).

A pathophysiologic role for ET has been reported in several conditions affecting the kidney (711,712,765–777). Urinary ET-1 excretion increases in patients with several forms of chronic progressive glomerulopathies (767). Mesangial cells (MC) ET-1 production is increased in rat models of immune complex glomerular neuropathy (GN), nephrotoxic serum nephritis, and mesangial proliferative GN. In addition, MC ET-1 production is augmented in human systemic lupus erythematosis and urinary ET-1 excretion is proportional to the severity of human mesangial proliferative GN (738).

There is abundant evidence that ET-1 increases MC proliferation and matrix accumulation (Fig. 9-2). ET-1 may further stimulate inflammation because it elevates human MC production of TNF, ICAM-1, and VCAM-1. Furthermore, ET-1 reduces TNF-mediated apoptosis in MCs (via induction of COX-2), potentially causing additional cell accumulation (738).

Although there is no direct evidence *in vivo* that MC ET-1 production is increased in DN, there are *in vitro* data suggesting that MC ET-1 synthesis is increased by hyperglycemia (738). Studies suggest that MC ET-1 production is enhanced in diabetic nephropathy and that excessive ET-1 action in the diabetic glomerulus can cause enhanced matrix accumulation, proteinuria, and reduced GFR (738). Endothelin is involved in the progression of diabetic nephropathy in OLETF rats (Otsuka Long Evans Tokushima Fatty), an animal model of type II diabetes and an endothelin type A antagonist is promising for the treatment of diabetic nephropathy (778).

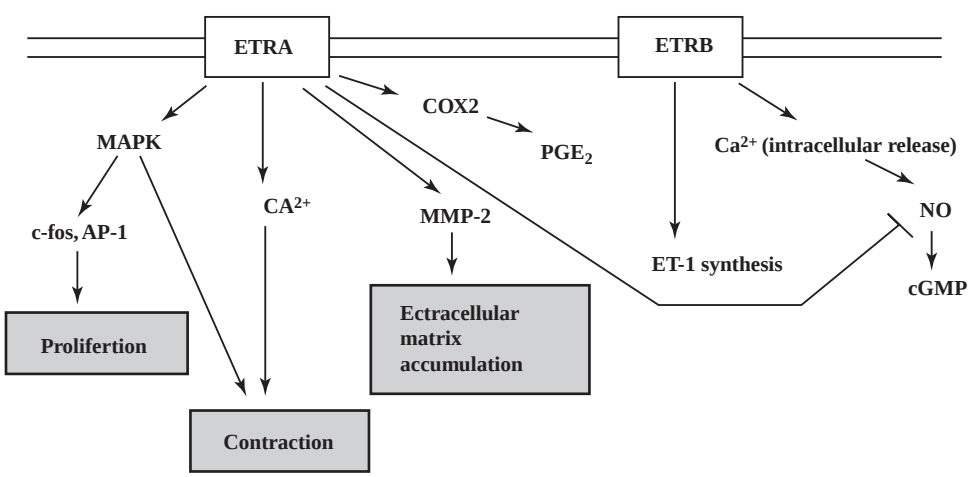

FIGURE 9-13. Schematic representation of biological effects mediated by endothelin receptor A (*ETRA*) and endothelin receptor B (*ETRB*) in mesangial cells. The net effect of ETRA activation is mesangial cell contraction, extracellular matrix accumulation, and proliferation. The net effect of activation of ETRB tends to be vasorelaxant as well as causing autostimulation of ET-1 production. *AP-1*, activator protein-1; *MMP-2*, matrix metalloproteinase-2. (From: Sorokin A, Kohan DE. Physiology and pathology of endothelin-1 in renal mesangium. *Am J Physiol Renal Physiol* 2003;285:579, with permission.)

In animals subjected to surgical reduction of renal mass, ET-1 gene expression increases in parallel with proteinuria and glomerulosclerosis (768). Renal ischemia is a potent stimulus for ET-1 production (769). In models of ischemic renal injury, ET-neutralizing antibodies as well as ET-A receptor antagonists attenuate the decline in renal functional and structural parameters (770,771). ET-1 is upregulated after renal ischemia/reperfusion injury and may contribute to the injury by prolonging vasoconstriction. It is known that ET-A-selective, but not ET-B-selective, antagonist is protective against renal ischemia/reperfusion injury (779). Both exogenous and endogenous nitric oxide have protective effects against ischemia/reperfusion-induced renal dysfunction and tissue damage, probably through the suppression of ET-1 overproduction in postischemic kidneys (780). ET-1 aggravates cell damage through the activation of Na/Ca exchanger and suggests that Ca overload via Na/Ca exchanger plays a critical role in hypoxia/reoxygenation-induced renal tubular injury (781). Radiocontrast stimulates ET synthesis by endothelial cells in culture and increases plasma and urinary ET levels in animals (712,772,773). Cyclosporin infusion causes a transient rise in ET plasma levels in animals (690). ET antiserum and ET receptor antagonism partially ameliorate the renal hypoperfusion and hypofiltration that follow intravenous cyclosporin administration (775,776). Patients with the hepatorenal syndrome have significantly elevated plasma ET-1 and ET-3 levels that may in part be due to release of ET by the kidney (777). The use of ET receptor antagonists suggests that ET may play pathophysiologic role in other conditions, such as the rat remnant kidney model (782,783) and the uninephrectomized, stroke-prone, spontaneously hypertensive rat on a high-salt diet (784). Moreover, administration of an ET-A/ET-B receptor antagonist to patients with chronic renal failure reduced blood pressure and renal vascular resistance (785).

More direct evidence for the role of ET-1 in renal damage derives from studies using transgenic mice. Animals overexpressing the human ET-1 promoter form more ET-1 in their kidneys and develop renal lesions, despite exhibiting no increase in systemic blood pressure (786). Furthermore, rats transgenic for the human ET-2 gene are normotensive, but are characterized phenotypically by renal lesions similar to those seen in rats with a remnant kidney (787).

In summary, ET is a potent vasoconstrictor peptide. In the kidney, it reduces RBF and GFR, contracts mesangial cells, and may function as a paracrine–autocrine factor in modulation of sodium and water balance. It is a potential mediator of growth and proliferative changes within the kidney. It is thought to play a pathophysiologic role in a number of kidney diseases. ET receptor antagonists may prove to be beneficial in certain conditions.

NITRIC OXIDE

Nitric oxide (NO) is a paracrine mediator with a wide spectrum of physiological actions, including the control of vascular tone, antithrombotic actions, cell cycle regulation, neurotransmission, signal transduction, and inflammation. NO is synthesized during conversion of its physiological precursor L-arginine (L-Arg) to L-citrulline (Fig. 9-14) (788,789). This reaction is catalyzed by a family of enzymes known as NO synthases (NOS) (788,790–792). Three NOS isoforms (neuronal [nNOS, NOS1], inducible [iNOS, NOS2], and endotelial [eNOS, NOS3]) have been identified in mammalian tissues. Neuronal nitric oxide synthase (nNOS) was first found in the neuronal cells of the brain, but is also constitutively expressed in the kidney and may be of physiologic importance (791). Inducible NOS (iNOS) is expressed upon activation of macrophages and other cells. The third isozyme is found in endothelial cells (eNOS) and, like nNOS, is constitutive in nature (792,793). NO produced from endothelial cells (eNOS-derived) is important as a tonic vasodilator and inhibitor of platelet aggregation and adhesion. Endothelial-derived NO also prevents polymorphonuclear neutrophil adhesion by interfering with CD11/CD18 adhesion molecules (793). NO produced from iNOS is probably more important in host defense and chronic inflammatory response (707). In these settings, NO leads to the formation of highly reactive species, most notably peroxynitrite ($ONOO^-$). Other cytotoxic actions of NO, when released in tissues rather than into the bloodstream, include destruction of iron–sulfur clusters, thiol nitrosation, mitochondrial damage, and nitration of protein tyrosine residues.

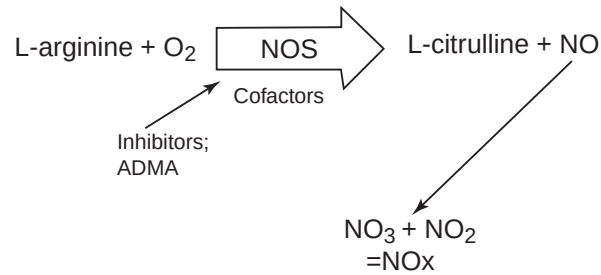

FIGURE 9-14. Simplified scheme showing the biosynthetic pathway for nitric oxide (*NO*) production *in vivo*. (From: Baylis C. Changes in renal hemodynamics and structure in the aging kidney; sexual dimorphism and the nitric oxide system. *Exp Gerontol* 2005;40:271, with permission.)

nNOS and eNOS are traditionally viewed as constitutive enzymes, with a limited tissue distribution, exhibiting intracellular Ca^{2+}-calmodulin dependency, although Ca^{2+}-independent activation has been described (see below). nNOS and eNOS are responsible for producing NO for a variety of physiological purposes. In contrast, iNOS is an inducible enzyme, expressed in all nucleated cells that generate large bursts of NO in response to immunological and certain nonimmunological stimuli. iNOS is usually described as Ca^{2+}-independent, although the presence of Ca^{2+} enhances iNOS activity. Effects of NO are typically mediated by activation of soluble guanylate cyclase, resulting in increased levels of cGMP (788).

It is evident that net NO deficiency could occur by a number of mechanisms. For example, reductions in substrate availability (L-arginine) either ingested or from the endogenous source (kidney), could lead to NO deficiency. Increases in endogenous NOS inhibitors, such as methylated arginine analogs, including asymmetric dimethyl arginine (ADMA), will reduce NOS activity. A decreased abundance or activity of the NOS enzymes, because of either reduction in protein content, reduced availability of essential cofactors or high oxidant state would also lead to NO deficiency. Any of these events either alone or acting together, would reduce the total amount of NO generated. Other, more downstream events could reduce NO-dependent activity after NO formation, for example, by increased NO inactivation with superoxide anion, by decreasing the access of NO to its target tissue (as occurs with a deposition of advanced glycosylated end products) and/or alteration in the NO receptors and their transduction mechanisms (789).

Renal Action of Nitric Oxide

NO acts as a potent modulator of renal function. The three NOS isoforms have been described in normal kidneys. All three isoforms are expressed in the medulla and medullary NO production exceeds that in the cortex (788). nNOS, the constitutive "neuronal" form, has been localized in the MD of both the mouse and rat kidney (788,795). eNOS is found

predominantly in renal vasculature, whereas iNOS immunoreactivity has been shown in the preglomerular portion of the afferent arteriole (796) and in glomerular mesangial cells. Isolated rat proximal tubule and inner medullary collecting duct cells express iNOS following stimulation with TNF-α and IFN-γ (797).

NO controls both afferent and efferent vascular tone, the ultrafiltration coefficient, and medullary blood flow, with preferential action on the afferent arteriole. In addition, NO has natriuretic effect (788). Liang and Knox and Ortiz and Garvin (798) indicate that there is evidence that NO reduces sodium reabsorption in all tubular segments, including the proximal tubule, thick ascending limb, distal tubules, and cortical collecting ducts.

Studies at the Single Nephron Level

Nitric oxide has been shown to participate in the regulation of renal hemodynamics. The administration of a competitive inhibitor of NO production, L-NMMA, to normal rats causes dramatic glomerular hemodynamic changes, including reduced single nephron plasma flow, augmented afferent and efferent arteriolar resistances, decreased ultrafiltration coefficient, and increased glomerular capillary pressure (799–802). Chronic oral supplementation with an L-arginine inhibitor in rats caused proteinuria, increased glomerular capillary pressure, and glomerular hemodynamic changes as described previously (801). These observations suggest that NO might be an important regulator of glomerular capillary pressure and that its dysregulation might be involved in the development of glomerular sclerosis through increases in glomerular capillary pressure.

Dietary supplementation with L-arginine ameliorates the progression of renal disease in rats with subtotal nephrectomy (802). Recent findings suggest that L-arginine supplementation prevents the progression of glomerular sclerosis in subtotal nephrectomized rats, at least in part because of its inhibitory effects on the development of glomerular hypertension (803). Although endothelial cells are widely viewed as the major source of NO, emerging evidence demonstrates that NO is also produced in various other tissues and cells, including macrophages (804), hepatocytes (805), Kupffer cells (806), fibroblasts (807), vascular smooth muscle cells (808,809), and mesangial cells (810). In contrast to the NO synthase in endothelial cells, which is Ca^2-calmodulin-dependent and constitutively expressed, some of these cells have been shown to possess NO synthases that are distinct from the constitutive enzyme in endothelial cells. This type of enzyme is Ca^{+2}-calmodulin-independent and not constitutively expressed, but is induced by several stimuli, including LPS, TNF, IL-1, and interferon C (811). The inducible NO synthase was also identified in endothelial cells (812,813).

Intrarenal Angiotensin II–Nitric Oxide Interactions

There is no doubt that hypertension and diabetes are major risk factors for the progression of renal disease and that interruption of the RAS (renin angiotensin system) is renoprotective. In particular, the balance of interactions between Ang II and NO is a key factor in determining whether injury or protection of target organs ensues (814).

Ang II is a potent vasoconstrictor with growth-promoting properties. Chronic overactivation of the renal RAS contributes to the development and maintenance of renal hypertension and subsequent renal injury. In the kidney, as elsewhere, NO serves in an opposing role to Ang II, functioning as a vasodilator, with antigrowth-promoting effects. NO may also serve an important role in reducing endothelial dysfunction by modulating adhesive interactions between leukocytes, platelets, and vascular endothelium. In contrast to Ang II, NO is produced continuously and imparts a constant vasodilatory effect. The endothelium is a site for the final step in the production of both Ang II and NO and is thus a major site for their interactions (814).

The endothelium and vascular smooth muscle cells produce O_2 in response to various stimuli, including oxidized lipoproteins, hyperglycemia, ischemia, and Ang II; Ang II also acts directly on mesangial cells, further stimulating the synthesis of O_2. Conditions that produce increased levels of O_2 serve to dramatically shorten the bioactivity of NO or even transform it into a toxic metabolite. Furthermore, Ang II promotes the production of O_2 and Ang II synthesis is increased in the kidney when there is a deficiency of NO. Once initiated, these events create a cycle that continues to increase Ang II levels in the glomerular circulation and to raise glomerular capillary pressure (814).

Chronic inhibition of NO synthesis has been shown to induce both renal and cardiac injury. A number of studies have suggested that impairment in the expression of NO in the glomerulus may be an important signal for increased mesangial matrix protein synthesis, whereas the presence of NO can inhibit TGF-1 and collagen synthesis in mesangial cells. A locally activated renal RAS, in conjunction with increased TGF-1 expression, was shown to be a major feature of renal injury in rats with chronic inhibition of NO synthase (814).

Both exogenous and endogenous nitric oxide have protective effects against ischemia/reperfusion-induced renal dysfunction and tissue damage, probably through the suppression of endothelin-1 overproduction in postischemic kidneys (815).

Therapeutic Implications

Not only do ACE inhibitors decrease Ang II synthesis, they also prevent the degradation of bradykinin, which is an important physiologic molecule involved in the release of NO. With ARBs, blocking the AT_1 receptor favors the synthesis of NO and stimulates factors that allow NO to be biologically more active (814).

nNOS is abundantly expressed in MD cells, and its expression is stimulated in various high-renin states including salt restriction, administration of loop diuretics, or inhibition of the renin–angiotensin system, some of the same interventions that augment COX-2 expression. Direct evidence in support of MD NOS, presumably nNOS, acting as a positive regulator of renin secretion came from studies in the *in vitro* perfused juxtaglomerular apparatus (JGA). In this preparation, administration of L-arginine-stimulated renin secretion and NOS blockers almost completely abolished the stimulation of renin secretion by low levels of NaCl. Results from studies in which NOS inhibitors were administered to whole animals are somewhat less consistent; however, possibly owing to the complexities that arise because of the multiple NOS isoforms and cellular sources (816).

Studies have also demonstrated that tubuloglomerular feedback control of afferent arteriolar resistance is influenced by MD NO production, thereby enhancing autoregulation of renal blood flow, GFR, and renin secretion (798).

Effects of Diabetes on the Renal Nitric Oxide System *in Vivo*

Diabetes mellitus is associated with increased incidence of cardiovascular complications. Lack of NO production may exacerbate these complications. Diabetes triggers mechanisms that

in parallel enhance and suppress NO bioavailability in the kidney. It was hypothesized that during the early phases of nephropathy, the balance between these two opposing forces is shifted toward NO. This plays a role in the development of characteristic hemodynamic changes and may contribute to consequent structural alterations in glomeruli. Both eNOS and nNOS can contribute to altered NO production. As the duration of exposure to the diabetic milieu increases, factors that suppress NO bioavailability gradually prevail. Increasing accumulation of advanced glycosylated products (AGE) may be one of the culprits in this process. In addition, this balance is continuously modified by actual metabolic control and the degree of insulinopenia (788).

Nitric Oxide and Aging

There is substantial evidence that chronic NO deficiency occurs in various types of chronic renal disease (CRD) and may contribute to progressive loss of function and evolving structural damage. There is also significant evidence to suggest that NO deficiency develops during normal aging. Endothelial NO-dependent vasodilation is impaired with advancing age in normal human subjects and rats (789). There is clearly a reduction in endothelial NO activity with age, leading to endothelial dysfunction. In fact, oral arginine supplementation can reverse the endothelial dysfunction in the aged, suggesting that a defect in NO production occurs even in healthy aging subjects (789).

There is also a reported sexual dimorphism in the NO system, with premenopausal women making more total NO than males and a greater abundance of the constitutive NO synthases (NOS) in young adult female versus male rat kidney. In addition, estrogen exerts marked stimulatory actions on endothelial NO at both genomic and nongenomic levels and, in addition to antioxidant actions of estrogens, may prolong the active life of NO (789). NO is closely related to suppressive effect of E2-β on renal ET-1 overproduction in acute renal failure rats and this suppression is probably involved in the beneficial effect of E2-β on ischemia/reperfusion-induced renal injury (817).

Estrogen also stimulates nNOS at the gene level and may inhibit iNOS expression, although this is controversial. The situation with androgens is also variable with testosterone-induced inhibition of NO-dependent vasodilation being reported in some parts of the circulation and stimulation in others. Regional variations in the effect of testosterone on the nNOS abundance and activity have also been reported. In contrast to the inhibitory actions of testosterone in some vascular beds, the "female" androgen DHEA is stimulatory on NO (789).

Finally, Beltowski et al. (818) investigated the effect of leptin on systemic NO production, arterial pressure, renal hemodynamics, and renal excretory function in the rat and showed that leptin stimulates systemic NO production, but has no effect on arterial pressure and renal hemodynamics (818).

ERYTHROPOIETIN

Erythropoietin (EPO) is a glycoprotein hormone that acts on the bone marrow to stimulate red blood cell production (819). The kidneys produce 85% to 90% of circulating erythropoietin in adults and the liver accounts for the remainder (819,820). The liver is the major source of erythropoietin in the fetus (820). *In situ* hybridization studies performed in anemic or hypoxic animals and erythropoietin-transgenic mice demonstrated that EPO is synthesized by peritubular cells of the renal cortex, particularly at the corticomedullary junction (820–823). Immunohistochemical characterization by light and electron microscopy showed that these cells are fibroblastlike type I interstitial cells (823). Other tissues were also found to produce EPO; EPO receptors (members of the cytokine receptor superfamily) were localized in different parts of the kidney, as well as in the central nervous system (CNS), endothelial cells, solid tumors, liver, and uterus (824).

The main stimulus for erythropoietin production and secretion is decreased oxygen supply to renal tissue (820,825). Renal hypoxia most commonly results from anemia or reduced oxygen pressure in arterial blood (hypoxemia). It triggers a cascade of reactions mostly mediated by the so-called hypoxia-inducible factors (HIF), which activate a wide set of genes that appear to be involved in protecting the kidney from hypoxia. One such gene is the *EPO* gene (826). This autocrine/paracrine secretion of EPO seemingly helps by decreasing apoptosis and cell necrosis in mesangial and tubular cells cells subjected to hypoxic (ischemia reflow injury) stress and toxic insult (*cis*-platinum), suggesting a potential therapeutic use of EPO or EPO analogs in tubular necrosis (827–829).

Conversely, when the kidney is hyperoxygenated, as occurs after red cell transfusion, EPO production is reduced. DNA sequences involved in regulation of transcription of the human EPO gene have been characterized (820). In addition to modulation by oxygen availability, EPO production is influenced by several cytokines. IL-1 and TNF-α were shown to inhibit EPO mRNA levels and EPO formation in human hepatoma cell cultures and to reduce EPO production in isolated perfused rat kidneys (830). Secretion of these cytokines by macrophages could contribute to defective EPO production and anemia in infectious or inflammatory diseases.

In healthy male volunteers, Ang II injection increased EPO secretion in a dose-dependent manner. This effect was neutralized when a selective AT_1 receptor blocker was administered, inferring that the stimulation of EPO by Ang II is probably via AT_1 receptors (831).

On the other hand, cyanate, a compound that could spontaneously form from urea, reacts with EPO leading to carbamylated EPO (C-EPO), an EPO protein with reduced activity. In renal insufficiency, cyanate can reach levels high enough to reduce the activity of both endogenous and exogenous EPO. This might explain one mechanism of suboptimal responses to recombinant EPO in patients with ESRD on inadequate dialysis. It might also explain the superiority of continuous peritoneal dialysis over hemodialysis in terms of decreasing BUN and, at the same time, improving anemia (832).

In addition to the effects of EPO on bone marrow cells, it has been postulated that erythropoietin has antinatriuretic action, which could account for the worsening hypertension observed in some patients receiving recombinant EPO. In one study in isolated Wistar rat kidneys, EPO decreased Na excretion, possibly by increasing Ang II production (833). These results, however, remain inconclusive.

INSULIN

Besides inhibiting gluconeogenesis in the proximal tubule, insulin exerts a significant vasodilatory effect on the kidney, thus increasing renal plasma flow; this effect appears to be mediated by PGs and partially counteracts the vasoconstrictor effect of Ang II (834).

In contrast, insulin appears to enhance the contractile effect of Ang II on mesangial cells (835).

Insulin also potentiates AVP action in terms of water reabsorption at the collecting ducts by increasing the gene transcription of Aquaporin-2 (AQP2) (836).

C-peptide

C-peptide or connecting peptide, for a long time thought of as an inert by product of the conversion of proinsulin into insulin, has revealed multiple biological roles by partially reversing or preventing complications of insulin-dependent diabetes (837); its administration in physiologic concentrations to diabetic rats activated pathways involved in cellular proliferation, and limited or prevented the glomerular hypertrophy and the mesangial matrix expansion seen in the posthyperfiltration phase of early diabetic nephropathy (838).

RENAL DEGRADATION OF HORMONES

All peptide hormones are extracted by the kidney, which, on average, removes between 16% and 40% of peptide hormone entering the renal circulation (839). In exceptional cases, the kinins for example, more than 90% is removed during a single passage. The removal of biologically active and inactive peptides from the renal circulation occurs predominantly by glomerular filtration. The rate of filtration is influenced by the size, shape, and charge of the molecule. After filtration, peptide hormones are degraded in the proximal tubule via two mechanisms. Larger peptides, such as insulin, require absorption by epithelial cells and degradation in lysosomes or endosomes. Smaller peptides, such as bradykinin, angiotensin, and ANP, are degraded by hydrolysis on the brush border. Other peptide hormones may follow both routes of degradation AVP being one example. Hormone metabolism by the proximal tubule epithelium is highly efficient; urinary hormone excretion accounts for less than 1% to 2% of the filtered load.

In most cases, the liver contributes significantly to peptide hormone metabolism. A few hormones, however, undergo negligible hepatic extraction and the kidneys are their predominant site of degradation (839). Examples of these hormones are calcitonin, the amino-terminal fragment of PTH, and the C-peptide of proinsulin. Complete removal of insulin is also dependent on intact renal function (840). Glomerular filtration accounts for approximately 60% of all the insulin removed by the kidney and the balance is extracted from the peritubular circulation. Uptake and degradation occurs largely by receptor-mediated endocytosis of insulin on the basolateral membrane of tubule epithelial cells (841). One possible etiology of hypoglycemia in diabetic patients on insulin or insulin secretagogue therapy is decreased renal degradation of insulin secondary to decreased renal function (842,843).

The kidneys contribute significantly to disposal of glycoprotein hormones, such as erythropoietin, follicle-stimulating hormone, and luteinizing hormone (752,753). With the exception of vitamin D, the kidney plays only a minor role in the metabolsim of steroid hormones.

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SECTION II
CLINICAL EVALUATION

CHAPTER 10 ■ URINALYSIS

MELANIE S. KIM AND HOWARD L. CORWIN

The examination of urine, or uroscopy, is among the oldest tests in medicine, dating back to Babylonian physicians more than 6,000 years ago. Over subsequent centuries, repeated references to the importance of the study of urine as a diagnostic aid can be found in the writings of early physicians, including Hippocrates and Galen. By medieval times, however, uroscopy was not the sole province of the physician; "uromancy" was widely and profitably practiced by charlatans to prognosticate (1). The progression from these medieval "pisse-prophets" to modern urinalysis was rooted in physicians' understanding of the association of renal disease and function with the examination and analysis of urine in the 18th and 19th centuries. Richard Bright recognized the association of "red particles" in the urine with pathologic changes in the kidney in his classical description of acute glomerulonephritis (1827). Bright observed the presence of heat-coagulable urinary protein in patients with renal failure in 1836. Similarly, in his 1844 textbook, *Urinary Deposits,* Golding Bird (2) described many findings in the urine sediment that are still valid today, including the first use of the term "casts." During the same period, urine chemical analyses for glucose, bile acid, protein, and blood were being developed (3). However, these tests were time consuming and needed large amounts of laboratory space and equipment. In the late 1800s, urinary test strips were developed that fixated reagents onto filter paper, which allowed bedside testing of urine. The modern use of commercially available dipsticks was launched in 1956 with the use of a specific enzyme (glucose oxidase) that improved sensitivity and specificity (3). Currently, new applications and innovations continue, with automation of urinary strips testing, immunologic testing for specific proteins, and increasingly sophisticated microscopy.

Today, even with the explosion in knowledge of renal disease and the accompanying sophistication of techniques to study these processes, "simple" urinalysis remains a cornerstone for the evaluation of the kidney. The examination of urine is still the indispensable first step for most, if not all, clinicians in approaching the patient with suspected kidney disease.

Traditionally, the complete examination of urine has been divided into a macroscopic and microscopic evaluation. The macroscopic analysis of urine includes assessment of its physical characteristics (appearance, odor, specific gravity) and chemical analysis. Today, the "routine" chemical analysis of urine is usually performed using urine dipsticks (4,5). These commercially available test strips contain absorbent pads impregnated with chemical compounds that bind to the substances to be measured. The chemical reaction that follows causes an associated indicator dye to change color. This color change is then compared to a known standard. The result is a rapid semiquantitative measurement of the constituents of interest. Therefore, urine can be screened for a range of substances quickly and easily using dipsticks (Table 10-1). It is important to stress that the information obtained is qualitative or semiquantitative. Accurate measurement of the renal excretion of a substance requires a timed specimen and usually more sophisticated chemical analysis (5). A variety of substances or changes in the character of the urine can interfere with the individual urine dipstick tests. Microscopic analysis of the constituents of urine is performed on either an unspun specimen or, more usually, the sediment from a centrifuged urine specimen. In this chapter, we review the components and clinical significance of the routine urinalysis.

URINE COLLECTION

In general, a "clean catch" urine sample should be obtained. In males, the foreskin should be retracted and the glans penis cleansed. Similarly, in females, the labia should be separated and the area of the labia and urethral meatus cleansed. A midstream clean catch urine sample is preferred. This specimen is less likely to be contaminated by contents of the urethra or vagina and, consequently, is a clearer reflection of the kidney. It is also the sample that is most useful for urine culture. Bladder catheterization is generally reserved for situations where adequate urine cannot be obtained easily by the clean catch method. Suprapubic bladder aspiration is a useful procedure in infants, especially for cultures.

The most information is obtained from an overnight, or "first-void," urine sample. This is usually a concentrated specimen; therefore, it allows assessment of concentrating ability. In addition, cells and casts tend to be preserved by the acid urine often found in a first-void sample. Urine should be examined as soon as possible after voiding (within 2 hours). Over time, the physical and chemical composition of urine change and the elements in the sediment (cells, casts, and crystals) deteriorate, making interpretation difficult. Therefore, transport, storage, and preservation of urine are critical considerations. Urine must reach the laboratory rapidly; once there, it must be promptly analyzed or preserved. In the short term, refrigeration slows the deterioration of urine; however, there is no good substitute for immediate examination.

MACROSCOPIC URINALYSIS

Appearance

Normal freshly voided urine is clear. The color of urine can vary from colorless to pale yellow to amber. The concentration of the pigment urochrome is responsible for the color of normal urine. Colorless urine tends to be very dilute. Turbid urine may result from precipitation of urate or phosphate crystals, especially if the urine is refrigerated. The urine often will clear again as the crystals dissolve with rewarming. Cloudy urine also may occur with leukocytes or bacteria.

Abnormally colored urine does not necessarily indicate disease. A variety of foods and pharmacologic agents can cause wide color variation in normal urine (4,5). Some conditions associated with abnormal urine color include: *red to red–brown:* free hemoglobin, myoglobin, blood or porphyria; *brown:* alkaptonuria; *white:* protein; *orange:* bile pigment, pyridium; *black:* melanin; *yellow–green:* urine bile pigments, *Pseudomonas*

TABLE 10-1

COMMON URINE DIPSTICK SCREENING TESTS

pH
Specific gravity
Glucose
Ketones
Protein
Hemoglobin (RBCs)
Bilirubin
Urobilinogen
Nitrites (bacteria)
Leukocyte esterase (WBCs)[a]

[a] *WBCs*, white blood cells.

bacteriuria (6,7). Further investigation is indicated if an abnormal color is noted (8).

Odor

Normal urine has a characteristic mild odor caused by excreted esters (4). Fresh-void urine may have a somewhat stronger odor, because of its concentration. Urine allowed to stand for long periods of time acquires a particularly pungent odor, as does urine from patients with urinary-tract infections. Certain foods, asparagus in particular, impart a distinctive odor to urine. In general, the odor of urine is of limited clinical importance.

Specific Gravity

Specific gravity measures relative density by comparing the weight of urine to that of distilled water in a constant volume. The specific gravity depends on both the weight and number of particles in solution. Specific gravity and osmolality (which depends on number of particles alone) correlate well in normal urine. Specific gravity is a convenient and rapid measure of osmolality and, hence, urine concentration. The specific gravity of urine ranges from 1.001 to 1.035, although it ranges from 1.008 to 1.030 under normal conditions (4,5). Specific gravity of distilled water is 1.000 and that of normal plasma is 1.010. A high specific gravity (greater than 1.020) indicates concentrated urine, whereas a low specific gravity (less than 1.005) indicates dilute urine.

There are several ways specific gravity can be measured. The oldest method is by measuring the buoyancy of a hydrometer (weighted flotation device) in urine; however, reproducibility is poor and inaccurate. Most laboratories use a refractometer that measures the refractive index and reflects the quantity of solids in a liquid (4,5). However, if substances (e.g., glucose, protein, and radiographic contrast agents) of higher molecular weight than the normal urine constituents are present in the urine, there is a disproportionate rise in the urine specific gravity as compared to urine osmolality as measured by refractometer or hygrometer. Under these conditions, urine specific gravity does not accurately reflect urine concentration. Often the specific gravity in these settings is outside of the normal physiologic range (greater than 1.040). The measurement of specific gravity by urine dipsticks is dependent on ionic concentration in urine. Ionic concentration and urine osmolality are linearly related. The dipstick pad for specific gravity contains a polyionic polymer that binds hydrogen ions, which causes a change in the dissociation constant detected by a pH indicator dye (9). Specific gravity measurements by dipstick can be pH dependent, with falsely elevated values at urine pH less than 6 and falsely lower values at urine pH greater than 7. Dipstick measurements of specific gravity are not affected by the presence of albumin, glucose, or x-ray contrast media (10).

Urine pH

The pH of urine can fluctuate between 4.5 and 8.0. In general, normal urine has a pH between 5.0 and 6.0, reflecting the usual obligate excretion of acids generated from metabolism. The pH measurement obtained with a dipstick is sufficiently accurate in most situations. Urine pH is measured by the double indicators methyl red and bromthymol blue, which give a broad range of colors at different pH. If a more accurate measurement is required, urine should be collected under oil (to prevent CO_2 escape) and measured with a glass pH electrode. The urine pH tends to be lower in the first-void specimen and rise after meals. If urine is left at room temperature, the pH becomes more alkaline, because of the breakdown of urea by bacteria, which liberates ammonia. Urine pH can aid in diagnosing systemic acid–base disorders in conjunction with other laboratory tests.

Protein

In the past, it was commonly thought that there was no protein in normal urine. This misconception resulted from an inability to detect urinary protein because of the insensitivity of available tests. Protein is normally found in the urine, with a 24-hour protein excretion of up to 150 mg/day. In general, this corresponds to a concentration of approximately 10 mg/dL. In normal urine, 60% of the proteins originate from the plasma, and the remaining 40% originate from the kidney and the urogenital tract. There are over 32 different plasma proteins; albumin is the predominant protein. Tamm–Horsfall mucoprotein is the predominant protein derived from the kidney. IgA and urokinase are also secreted in the renal tubule; therefore, normal urinary protein composition is approximately 40% albumin, 40% Tamm–Horsfall protein, 15% immunoglobulins, and the remaining 5% other plasma proteins.

Methods for Measuring Urinary Protein

There are many methods available to measure urinary protein. The method chosen depends on the sensitivity required, the need for quantification, reproducibility, accuracy, cost, and ease of performing the test.

Qualitative Colorimetric Test Strips. Urine is most often screened by dipstick. The dipstick pad for protein contains the pH-sensitive colorimetric indicator tetrabromophenol in a citric acid buffer. Urinary proteins bind to the indicator, causing a color change. The results are graded from negative to trace to 4+, reflecting the respective protein concentration. The lower limit for protein detection by dipstick is 10 mg/dL. A trace result corresponds to 15 to 30 mg/dL, whereas 4+ indicates greater than 2,000 mg/dL. The urine dipstick is most sensitive for negatively charged proteins, such as albumin, and often is insensitive to other proteins, such as the positively charged immunoglobulin light chains found in multiple myeloma. False-positive changes may be seen if the urine is very alkaline or if there is contamination with bacteria, blood, quaternary ammonium compounds, or chlorhexidine (a skin cleanser) (11–13). Consistency of results reporting is highly variable. The result depends on a color change that may not always be clear-cut.

Interpretation of color change is subjective, and leads to inconsistencies that are dependent on the operator (14,15).

Other Methods

Other methods to quantitate total protein concentration depend on precipitation of protein. The most common agent is sulfosalicylic acid, which is added to a measured volume of the sample urine. The resulting turbidity is measured by a photometer or nephelometer and is compared to a set of standards. Results may range from 0 to 4+, corresponding to less than 10 mg/dL to greater than 500 mg/dL. Trichloroacetic acid also can be used. Immunoglobulin light chains are detected by this method. False-positive results can occur if the urine contains tolbutamide, radiocontrast agents, or high levels of cephalosporin, penicillin, or sulfonamide derivatives (12, 16–18).

Tests that use specific antisera to individual proteins for accurate determination of urinary concentration for the tested proteins include radioimmunoassay (RIA), radial immunodiffusion, immunoelectrophoresis, enzyme-linked immunosorbent assay (ELISA), and immunonephelometry. The method of choice for the detection of immunoglobulin light chains in urine is immunoelectrophoresis of a concentrated aliquot from a 24-hour urine collection in patients with multiple myeloma (4,5).

It should be stressed that the determination of urine protein concentration in a random urine sample by dipstick (or turbidity) does not necessarily directly predict 24-hour protein excretion. It is not a quantitative test of proteinuria. Significant proteinuria, for example, could be missed by dipstick screening if urine flow is high, resulting in a high urine volume and low protein concentration. On the other hand, a concentrated urine specimen could lead to an overestimation of the degree of proteinuria. At times, it is necessary to quantify protein excretion; determining the 24-hour protein excretion is clinically useful. However, the largest cause of error in quantifying protein excretion is inaccurate 24-hour collection. The adequacy of the 24-hour collection can be judged by concomitant measure of urine creatinine excretion; nevertheless, this still leads to time-consuming, burdensome, and often unpredictable quantification of urinary protein excretion. It has been shown that the ratio of protein-to-creatinine in a single-voided random urine sample can be used to estimate total protein excretion. A normal ratio is less than 0.1 to 0.2 (100 to 200 mg of protein and 1000 mg of creatinine in 24 hours). A ratio of 1.0 correlates with a protein excretion of 1.0 g/day, whereas a ratio of greater than 3.5 suggests nephrotic range proteinuria (19,20).

Microalbuminuria

Microalbuminuria refers to elevated urine albumin excretion that is below the level of detection (30 to 300 mg/24 hours) by routine urine protein dipstick test. Persistent rates of urine albumin excretion above 20 μg/min are predictive of subsequent diabetic nephropathy and other chronic renal diseases (21–29). Currently, the American Diabetes Association and the National Kidney Foundation recommend screening for microalbuminuria by either determining the rate of albumin excretion using a timed-urine collection (greater than 20 μg/min) or measuring the albumin-to-creatinine ratio in random, spot collection (greater than 2.5 mg/mmol or 30 mg/g) (30,31). Albumin concentration in commercial laboratories can be measured by a variety of methods based on the use of specific antisera, including radioimmunoassay, immunoturbidimetric assay, ELISA, and immunonephelometry assays (21–24). However, as clinical evaluation of microalbuminuria has become increasingly important, there has been a push to make screening more rapid and readily available. Urine dipsticks have been developed to detect levels of albumin greater than 20 mg/L. Micral-Test II (Boehringer-Mannheim, Mannheim, Germany) has been one of the most extensively studied test strips, with a sensitivity of 64% to 97% and a specificity of 70% to 98% when compared to either radioimmunoassay or nephelometric assay (32–35). The test strip, which is a series of reagent pads, is based on an ELISA assay where albumin in the urine binds to a conjugate of antibodies linked to the enzyme β-galactosidase. Clinitek (Bayer Corp., Elkhart, Indiana), a new desktop assay using a reagent strip that tests for both albumin and creatinine, was recently developed. The test utilizes sulfonephthalein dye, binding for albumin and the peroxidaselike activity for creatinine. The color changes on the separate reagent pads are then read on a bench- top analyzer (36–39). Study sample sizes are small, but suggest that this system may provide a semiquantitative assessment for microalbuminuria. Studies have suggested that urinary albumin concentration may be underestimated by immunoassays. However, use of high-performance liquid chromatography, which measures total albumin (immunoreactive plus -unreactive), permits earlier detection of microalbuminuria (40).

Glucose

The renal threshold for glucose is a serum concentration of 180 mg/dL, although this may vary among individuals. Glucose appears in the urine if the concentration of glucose in serum exceeds the renal threshold. Glucose is detected in the urine by the use of a dipstick employing glucose oxidase. This enzyme is specific for glucose; therefore, it does not detect other reducing substances, such as other sugars (e.g., lactose and fructose). These dipsticks detect glycosuria in concentrations ranging from 50 mg/dL to over 1,000 mg/dL. False-negative results can be seen if ascorbic acid or aspirin is present in the urine. False-positive results can be seen with hydrogen peroxide.

Ketones

The urine dipstick for ketones uses a nitroprusside reagent. Ketones found in urine are acetoacetic acid, acetone, and β-hydroxybutyric acid. Both acetoacetic acid and acetone react with nitroprusside; however, β-hydroxybutyric acid is not detected. Concentrations of ketones in urine as low as 5 mg/dL can be detected by dipstick testing. Ketones are most commonly found in the urine of patients with diabetes, starvation (including dieting and anorexia), and severe dehydration. False-positive results can be seen with ascorbic acid, phenazopyridine, and free sulfhydride compounds, such as *N*-acetylcysteine (7). In addition, the ketone portion of multiple-test strip dipsticks is more sensitive to moisture than other test pads.

Bilirubin

Conjugated bilirubin is water soluble; therefore, it appears in the urine. On the other hand, unconjugated bilirubin is bound to albumin and, hence, water insoluble; therefore, it is not usually found in the urine. Dipsticks containing pads embedded with an aniline dye can detect bilirubin. The test is sensitive to below 0.05 mg/dL. Rapid testing of urine is important for detecting bilirubin because bilirubin in urine breaks down, especially after exposure to light. False-negative results also can be obtained if there are large amounts of ascorbic acid or nitrites present in urine. False-positive results can be obtained with contamination with stool or in the presence of chlorpromazine. Detection of urinary bilirubin is not a valuable screening

tool for liver disease, because serum bilirubin provides more useful information.

Urobilinogen

Urobilinogen is produced from conjugated bilirubin that is metabolized by intestinal bacteria. Urobilinogen is reabsorbed into the bloodstream and subsequently appears in the urine. The dipstick test is based on Ehrlich's reagent. The test must be performed quickly because urobilinogen is rapidly oxidized to nondetectable urobilin when exposed to air. Conditions associated with high levels of bilirubin also result in the appearance of urobilinogen in the urine, unless there is an obstruction preventing bilirubin from reaching the intestine from the biliary tract. In these latter conditions, bilirubin, but not urobilinogen, can be detected in the urine. The rapid degradation of urobilinogen makes urinary urobilinogen a less valuable clinical screening test.

Nitrite and Leukocyte Esterase

Screening for urinary tract infections by urine dipstick tests (nitrite and leukocyte esterase) has been shown to be effective for symptomatic and high-risk patients. The nitrite test is based on bacteria reducing nitrates to nitrites in the urine, which is not normally found. Nitrites react with *p*-arsenilic acid, forming a diazonium compound that reacts with a colorimetric indicator. It is a qualitative test with either a positive or negative result. A positive test indicates greater than 10 organisms/mL. False-negative results are obtained if the bacteria present in urine do not produce nitrites (e.g., *Streptococcus faecalis, Neisseria gonorrhoeae,* and *Mycobacterium tuberculosis*) and yeast infections. High levels of ascorbic acid also produce a false-negative result. In addition, low urinary retention may also give false-negative results, because it takes up to 4 hours for the conversion of nitrate to nitrite. Nitrites also degrade with prolonged storage.

The leukocyte esterase test is based on the release of leukocyte esterase from lysed neutrophils. The leukocyte esterase reacts with an ester on the reagent strip, producing a 3-hydroxy-5-phenyl pyrrole, which, in turn, reacts with a diazo salt, causing a color change. The color produced reflects the number of neutrophils present in the urine. The test is usually positive if greater than 5 white blood cells (WBCs)/hpf are present. Intact neutrophils are not necessary for a positive test to occur; leukocyte esterase (from lysed neutrophils) can be detected in the urine, even when no WBCs can be identified in the sediment by microscopy. False-positive results can occur with vaginal contamination. The leukocyte esterase reagent performs well when compared to microscopy (41–45). Other cells found in the urine (e.g., red blood cells [RBCs]epithelial) do not contain leukocyte esterase and, therefore, do not cause a reaction.

As noted, these tests alone or in combination have been used in screening for urinary tract infection. A range of sensitivities and specificities has been reported for each test alone, paired in conjunction (both tests positive), or in disconjunction (either test positive) in a number of clinical settings (46–54). In addition, meta-analysis has been performed in adults (55) and more recently in children (56), which corroborates the use of nitrite and leukocyte esterase tests in combination to screen for urinary tract infection. This approach results in ranges of sensitivity of 78% to 97%, and specificity of 75% to 98%. The negative predictive value was 97.5%, which suggests that if both tests are negative the likelihood of a positive urine culture is small. Nitrite testing alone gives less satisfactory results, with sensitivity of 27% to 54% and specificity of 94% to 99%.

Blood and Hemoglobin

Occult blood can be detected in urine by the use of dipsticks impregnated with ortho-toluidine and organic peroxidase. Hemoglobin, either free in urine or from RBCs in urine, catalyzes an oxidation reaction, which results in a color change. Intact RBCs hemolyze on the reagent strip, liberating hemoglobin and initiating the described reaction, which produces a colored "dot." On the other hand, free hemoglobin results in a uniform color on the reagent pad.

The urine dipstick is very sensitive for detecting even small numbers of RBCs in the urine (57–59). The sensitivity for greater than 3 RBCs/hpf ranges from 80% to 95%, with a specificity of 95% to 99%. In fact, RBCs can be detected in 40% to 60% of samples containing 1 to 3 RBCs/hpf. The ease and reliability of the dipstick has led to the suggestion that it be utilized as a screening test for asymptomatic populations (60–64); however, the cost-effectiveness of this approach is yet to be conclusively demonstrated (65–72).

As noted, the presence of free hemoglobin in the urine (e.g., transfusion reaction) gives a false-positive dipstick result for RBCs. Similarly, myoglobin in the urine (e.g., rhabdomyolysis) also yields a positive dipstick result. The presence of a positive dipstick test for occult blood with absent RBCs on microscopic examination of the sediment is important evidence for the presence of pigmenturia (either hemoglobinuria or myoglobinuria). Oxidizing contaminants in the urine or Betadine (povidone iodine) may cause a false-positive result. Ascorbic acid may cause a false-negative result.

MICROSCOPIC URINALYSIS

Microscopic examination of the urine sediment (or occasionally unspun urine) generally follows macroscopic urinalysis. It has been suggested that microscopic analysis should be deferred if the macroscopic analysis (dipstick) is negative (45,73). However, the clinical situation and patient population should determine the extent of analysis undertaken. Microscopic examination of the sediment often can provide very useful information.

The urine sediment is examined microscopically for the presence of cells, casts, and crystals. Normal urine contains small numbers of all these elements, which can be identified in the sediment (Table 10-2). Abnormal urine sediment contains either constituents of normal urine in abnormally high numbers, or constituents that are not usually present in normal urine in any number. In the following discussion we review the individual constituents of "normal" and "abnormal" urine, which can be identified by microscopic examination of the urine sediment.

Preparation of the Urine Sediment

Prior to any discussion of the individual elements that can be identified in the urine sediment, it is important to briefly review the technical aspects of preparing the urine sediment for examination. The importance of standardization of technique and quality assurance has been stressed (73). The important steps include: centrifugation, resuspension of sediment, slide preparation, and microscopic examination. Adherence to proper technique assures accurate and reproducible sediment analysis.

In brief, a 10-mL volume of urine should be centrifuged for 5 minutes at approximately 2,000 rpm (1,500 to 2,500 rpm). The supernatant is carefully decanted and the sediment resuspended by gentle agitation. This is often done in a constant

TABLE 10-2

URINE SEDIMENT

	Normal	Abnormal
Cells		
RBC[a]	3–5/hpf	>5/hpf
WBC[a]	3–5/hpf	>5/hpf
Epithelial	0–2/hpf	>5/hpf
Oval fat body	−	+
Casts		
Hyaline	Few	Many
Granular	Few	Many
RBC[a]	−	+
WBC[a]	−	+
Epithelial	−	+
Pigment	−	+
Fatty	−	+
Waxy	−	+

[a] *RBC*, red blood cells; *WBC*, white blood cells.

(e.g., 1 mL) volume. However, a concentrated specimen facilitates identification of the constituents present in the sediment. A "drop" is pipetted onto a slide and a coverslip placed. Commercial systems are available that ensure that a constant sediment volume is transferred to the slide (5,73).

Collection of urine samples also may affect the microscopic analysis. Skin flora or vaginal contamination significantly alters the results of microscopic examination. A midstream clean-catch specimen should be collected and the patient instructed to retract the foreskin or labia. A first-morning void, which is usually more acidic, tends to preserve both cellular elements and casts. Urine needs to be examined as quickly as possible before deterioration of casts and cells. Refrigeration slows lysis, but may cause precipitation of phosphates and urates, making it difficult to evaluate the sediment.

Examination Techniques

The urine sediment (or unspun urine) is routinely examined using bright-field microscopy under both low (×100 magnification) and high power (×400 magnification). Either stained or unstained sediment can be examined. Often stains are used for special circumstances (e.g., Wright's or Hansel's stain for eosinophils in acute interstitial nephritis; see the following). The use of polarizing light to examine the sediment also is common and particularly helpful for identifying crystals and fats in the urine (73).

Phase-contrast microscopy often is utilized for routine urine examination (74). This technique may facilitate the identification of the formed elements in the sediment. It has been particularly useful in identifying the source of RBCs detected in the urine (see the following). Even greater detail can be provided by examination of the sediment using interference contrast microscopy (75); however, this is not routinely available.

A variety of other more sophisticated techniques of urine examination have been reported over the last two decades. Most of these, however, have not found their way into routine clinical practice, but remain primarily research tools. Both scanning electron microscopy (SEM) and transmission electron microscopy (TEM) have provided much basic information regarding the composition and origin of casts in the urine (76–78). It has been suggested that TEM also can provide useful diagnostic and prognostic information in acute renal injury (75,79), urinary viral and parasitic infections (80–82), and urogenital carcinoma (83). Similarly, immunofluorescence and monoclonal antibodies have been used to identify the cells and casts in the urine sediment in a range of renal diseases (84). Immunologic techniques, including monoclonal antibodies, also have identified enzymes, tubular antigens in renal diseases, viruses, and malignant cells that appear in the urine (85).

Cells

Red Blood Cells (RBCs)

It is generally accepted that "normal" individuals (i.e., those without any apparent renal or urologic disease) occasionally excrete RBCs in the urine. However, the upper limit of normal for RBCs in the urine still remains unclear. Addis (86) was the first to look at this issue in a 1926 study of RBC excretion in "normal" medical students. He found up to 425,000 RBCs in a 12-hour urine collection. Others have reported excretion rates of up to 600,000 in normal individuals (87). This roughly corresponds to 5,000 RBCs/mL (88) or 2 RBCs/hpf upon examination of the urinary sediment (89).

It needs to be stressed that significant urologic disease may still be present with normal levels of RBC excretion. It is clear that there is overlap, regardless of the criterion chosen for dividing normal from abnormal numbers of RBCs in urine. Most accept 3 to 5 RBCs/hpf as a reasonable dividing line between normal and abnormal. From a practical standpoint, this is the limit of sensitivity for RBCs in the urine dipstick, which is becoming the main screening method for the presence of hematuria. Estimates of the prevalence of microscopic hematuria vary from 2.5% to 13%, depending on population and the criterion for hematuria selected (62,63,66,90–94).

Distinguishing between hematuria of glomerular origin and hematuria originating from the lower urinary tract is of obvious importance when evaluating the patient with microscopic hematuria (Figs. 10-1 and 10-2). Fairley and Birch (95), using phase-contrast microscopy, were the first to note that RBCs of glomerular origin were dysmorphic in contrast to RBCs of nonglomerular origin. Although these findings have been confirmed (96–99), other investigators could not demonstrate either a high sensitivity or specificity with this technique (100–102). Significant discrepancies among observer interpretations of cell morphology were noted. Other approaches have been developed based on the observation that RBCs of glomerular origin have a lower mean corpular volume (MCV) than RBCs of nonglomerular origin (103). Coulter counter analysis of urine RBCs can be used to make this distinction (104). Recently, a newly developed automated urinary flow cytometer was able to differentiate between glomerular and nonglomerular hematuria with a sensitivity of 90.3% to 100% and specificity of 86.6% to 92.5% (105,106). RBC casts and/or significant proteinuria also suggest a glomerular source for the RBCs.

White Blood Cells (WBCs)

Leukocytes are commonly observed in normal urine. Up to 400,000 cells may be excreted per hour in normal urine, corresponding to greater than 10 WBCs/mL in an unspun urine sample (107). Leukocyte excretion, however, is most often determined by counting cells/hpf in the sediment. The detection of 1 WBC/hpf in the sediment is approximately 5 WBC/mL in unspun urine (108). In general, 5 WBCs/hpf is considered the upper limit for normal urine (46,109,110). The leukocyte

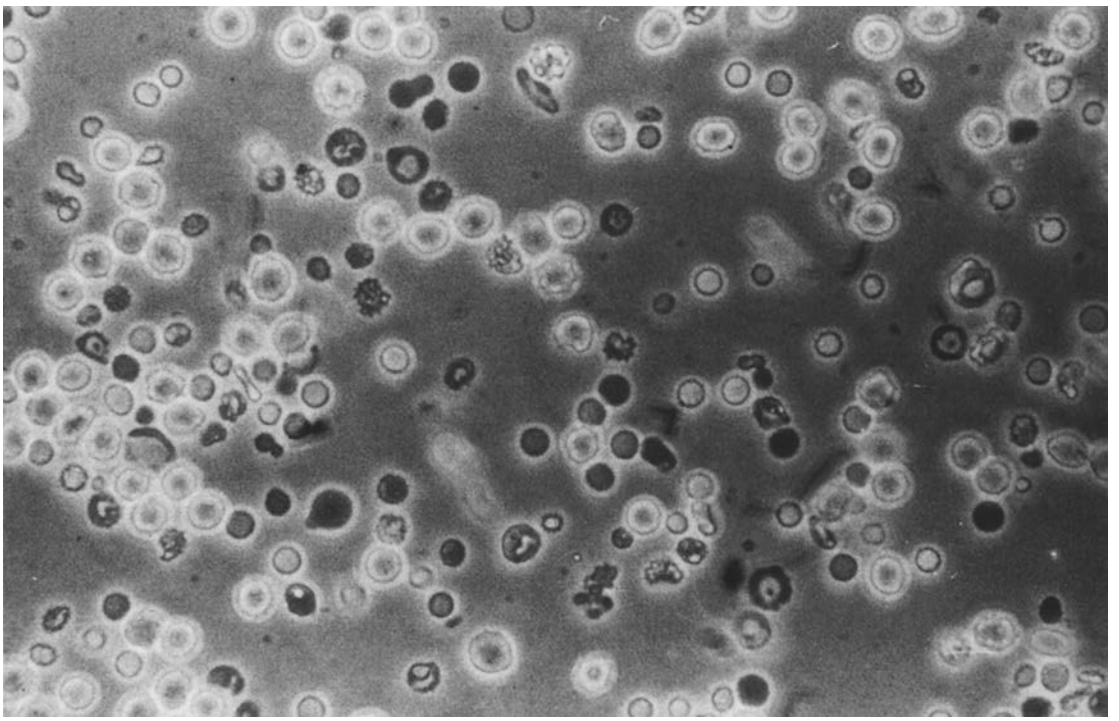

FIGURE 10-1. Urine from a patient in relapse with mesangial immunoglobulin A glomerulonephritis, showing a population of well-hemoglobinated cells of similar size and shape, as well as dysmorphic red cells typically seen in glomerulonephritis. The coexistence of a nonglomerular source of bleeding would need to be considered in such a case, but this "mixed" pattern is commonly encountered when the erythrocyte count is high in this form of glomerulonephritis.

esterase reagent strip (see the preceding text) is sensitive for greater than 5 WBCs/hpf in the sediment, making it a reasonable screening test for pyuria. The presence of large numbers of leukocytes implies inflammation (i.e., interstitial nephritis or infection). Infection is the most common cause of pyuria; most patients with infection have pyuria (111).

The polymorphonuclear neutrophil is the predominant leukocyte seen in the urine, particularly in normal urine; however, at times other leukocytes may be found (Fig. 10-3). The presence of eosinophils has been suggested as a sign of acute interstitial nephritis (112–114). Eosinophils can be detected in the urine using either Wright's or Hansel's stain. However,

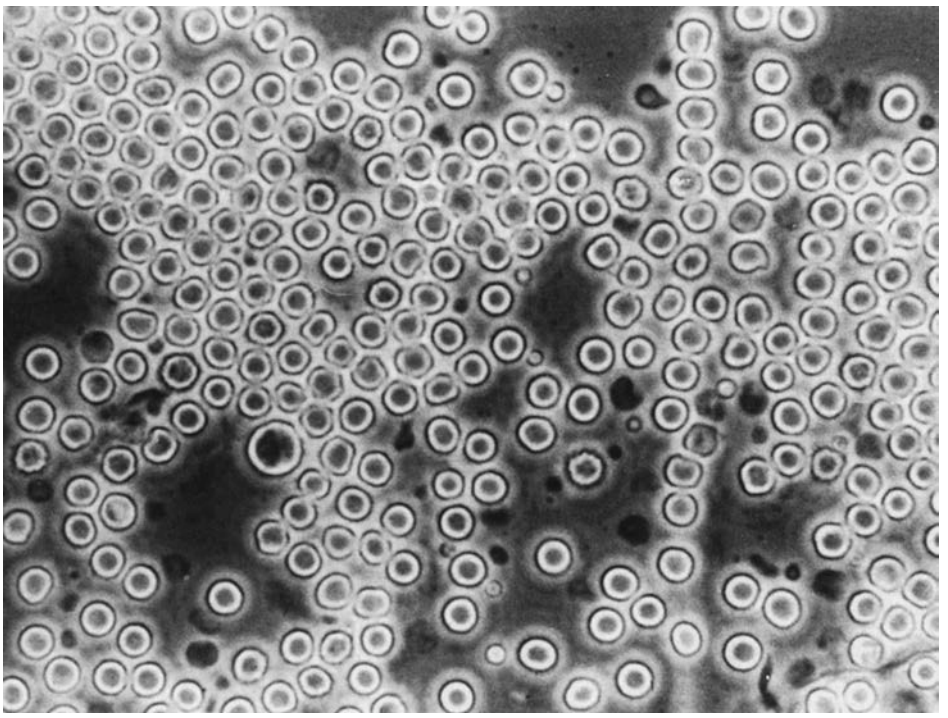

FIGURE 10-2. Nonglomerular bleeding, showing a very similar well-hemoglobinated population of erythrocytes. Tumors, stones, menstrual contamination, or blood added artificially by a psychiatrically disturbed patient would give this picture.

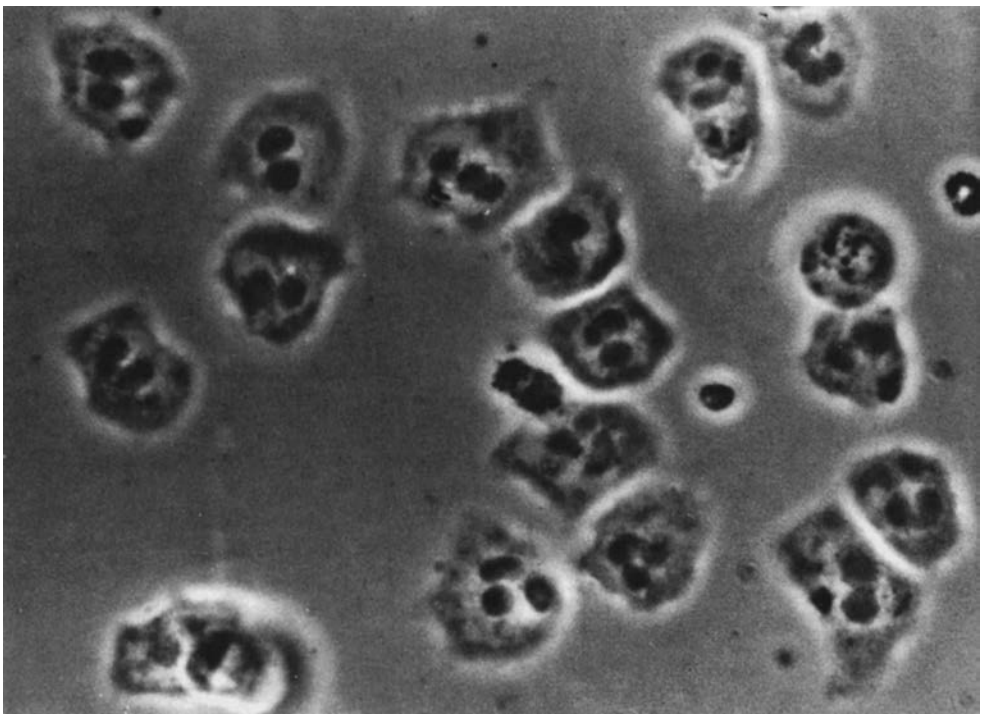

FIGURE 10-3. The lobed nuclei of the leukocytes, clearly seen on phase-contrast microscopy.

Hansel's is much more sensitive than Wright's stain for detecting eosinophils in urine (113,114). Urine eosinophils are best reported as a percentage of cells counted in the sediment (usually 100 to 500 WBCs total counted). Greater than 1% is considered positive; the sensitivity, specificity, and predictive value positive and negative for interstitial nephritis have been reported as 63%, 93%, 50%, and 98%, respectively (114). The higher the percentage of eosinophils in urine (e.g., greater than 5%), the more predictive the finding is of acute interstitial nephritis (112). Urine eosinophils in low numbers (less than 1%) may be seen with a variety of other clinical conditions, urinary tract infection, in particular (112–114).

Epithelial Cells

Three types of epithelial cells appear in the urine: renal tubular, transitional, and squamous. These cells appear in small numbers in normal urine, less than 3 to 5/hpf, or approximately 68,000 to 78,000/hour in women and men, respectively (range 25,000 to 200,000 cells/hour) (115,116). The reason for the higher excretion rate in men is not clear, although differences in renal mass have been suggested (116).

Renal tubular cells are mononuclear cells that derive from the nephron (Fig. 10-4). Usually these are easily distinguished from leukocytes. Papanicolaou stain can be used to distinguish tubular cells from other mononuclear cells (5). Increased numbers of urinary renal tubular cells appear in clinical conditions where there is tubular damage (e.g., acute tubular necrosis, nephrotoxin injury, or transplant rejection). Tubular cells that have absorbed lipids that are visible in the cytoplasm are called oval fat bodies (Fig. 10-5). These are often seen associated with nephrotic syndrome. If the fat droplets contain large amounts of cholesterol, a "Maltese cross" pattern may be seen when the sediment is examined with polarized light (117).

Transitional epithelial cells are derived from the renal pelvis, ureters, or bladder. Increased numbers of these cells may be seen with inflammatory conditions of the ureters or bladder. Cytologic examination is required to look for evidence of transitional cell carcinoma if large numbers of transitional cells appear in the urine.

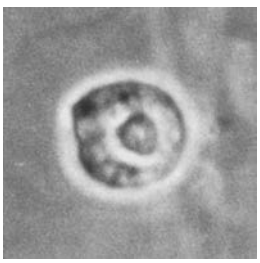

FIGURE 10-4. Renal tubular epithelial cell with the single nucleus, clearly shown on phase-contrast microscopy.

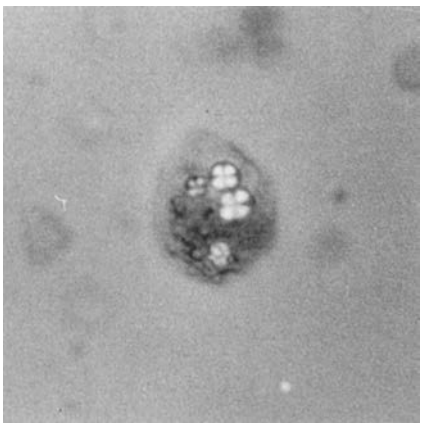

FIGURE 10-5. An oval fat body viewed under polarized light. The dark lipid material filling most of the cell is seen clearly, as are the classic "Maltese crosses."

Squamous epithelial cells are large, easily identifiable cells derived from the female and terminal male urethra. These cells are most often seen in the urine of females because of vaginal contamination of the urine.

Casts

Casts are formed in the renal tubule as a consequence of the precipitation of Tamm–Horsfall protein. Tamm–Horsfall protein, which is the most abundant protein in normal urine, is a glycoprotein excreted by the renal tubule (118). The function of this protein is not completely clear, although it may have immunoregulatory actions. However, it is the major constituent of urinary casts. Conditions that facilitate polymerization of Tamm–Horsfall protein (e.g., calcium, sodium, albumin, radiocontrast media, Bence Jones proteins, and dehydration) also lead to increased cast formation. An acid, concentrated urine tends to favor cast formation as well. The gel of the Tamm–Horsfall protein takes the shape of the lumen of the renal tubule from which it originates. Material in the tubule at the time the cast is formed (cells, debris, crystals, and proteins) may be associated with the cast and results in the variety of casts that appear in the urine in different disease states (see the following).

Small numbers (on average 2,000 to 10,000/day) of hyaline and granular casts (the vast majority of which are hyaline) can be seen in normal urine (86,115,119). Increased numbers of these casts, cylindruria, are not necessarily pathologic. They may be excreted in increased numbers in association with stress, strenuous exercise, or dehydration; however, the increase in cast excretion disappears within 24 to 48 hours after resolution of these conditions (120,121). Fever and diuretics also cause a transient increase in hyaline and granular cast excretion (122,123). Although hyaline and granular casts may not always indicate pathology, all other casts are abnormal and suggest renal disease. The individual casts that may be seen in urine are described below.

Hyaline Casts

These are acellular casts composed of Tamm–Horsfall protein alone. They form in the distal tubules or collecting ducts. As noted, hyaline casts appear in small numbers in normal urine. The detection of increased numbers of hyaline casts does not necessarily imply underlying disease, because stress, exercise, fever, diuretics, and dehydration result in a transient increase in hyaline cast excretion (120–123). Hyaline casts appear alone (or with occasional granular casts) in these circumstances. Large numbers of hyaline casts also are seen in disease states, however, virtually always in association with other abnormalities in the urine, such as cellular casts, cells, and protein, and so on. Casts may be much wider (four to five times) than normal when formed in damaged, dilated, or hypertrophied nephrons or collecting ducts. These "broad casts" indicate severe renal disease and often are detected in patients with chronic renal failure.

Granular Casts

The presence of granules in the basic cast protein matrix leads to the designation of a granular cast. Granular casts are seen in small numbers (significantly less than hyaline casts) in normal urine, but are very common in disease states. These granules result either from the aggregation of proteins or cellular debris in the Tamm–Horsfall matrix (77,78,124). In fine granular cast, the granules are small and regular. Cell injury and death can lead to large granules of variable size and shape, forming a coarse granular cast. The appearance of large numbers of deeply pigmented granular "muddy-brown" casts in the urine is the characteristic urine sediment finding in acute tubular necrosis.

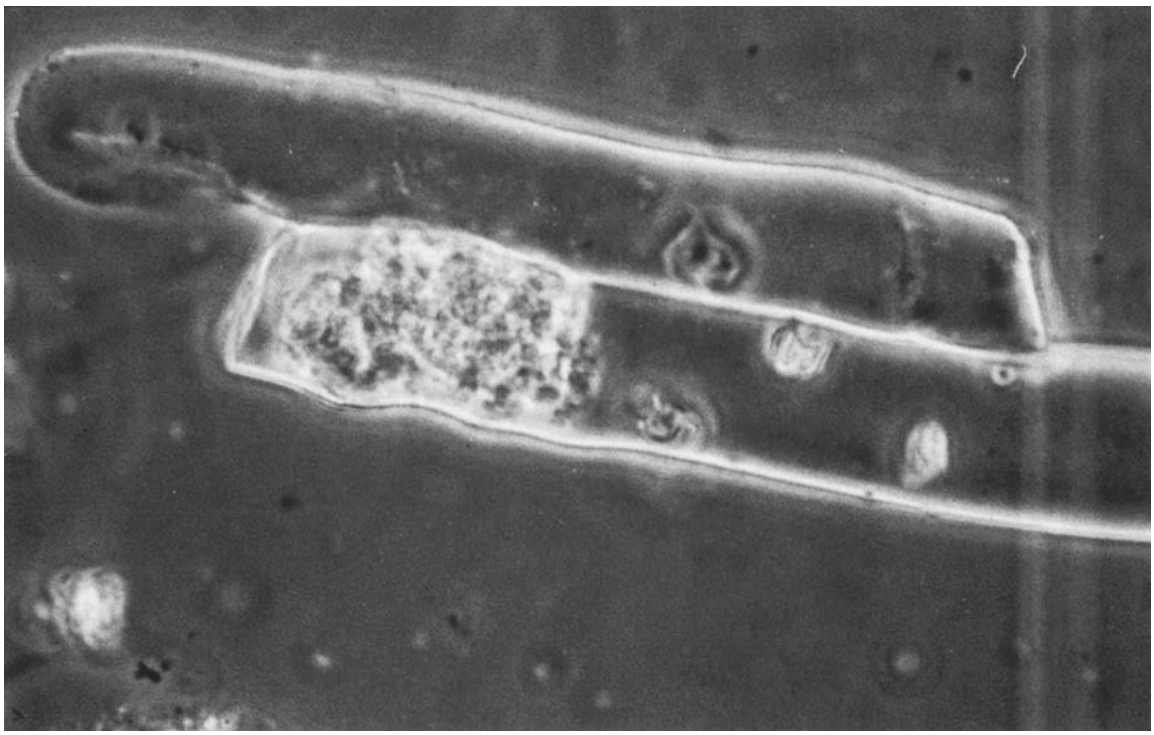

FIGURE 10-6. A waxy cast, showing very sharply defined edges. A clump of disintegrating cells is seen at one end.

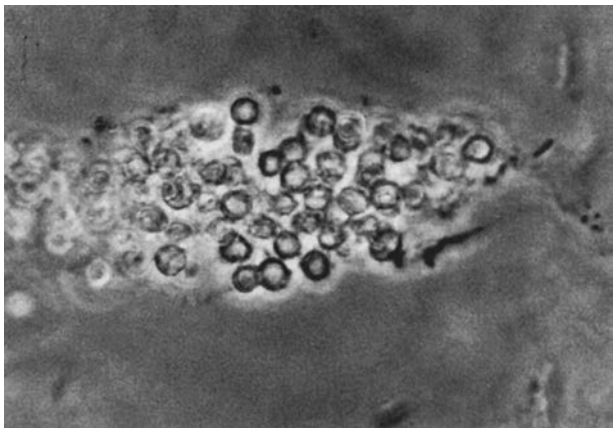

FIGURE 10-7. An erythrocyte cast in an acid urine composed of red blood cells from which much of the hemoglobin has disappeared.

Fine granular casts tend to be seen in normal urine. Both fine and coarse granular casts are seen in disease. The distinction between fine and coarse granular casts is of little clinical importance. Coarse granular casts are thought to represent an early stage of degeneration of cellular casts. Ultimately, further degeneration can result in the formation of waxy casts.

Waxy Casts

These are highly refractile, smooth, homogeneous, blunt-ended casts (Fig. 10-6). Occasionally, a few granules or some cells or cell debris may be contained. It has been suggested that they represent the final phase of granular cast degeneration, but their origin is not completely clear (77). They often appear as broad casts and, as such, are called renal failure casts. Waxy casts can indicate advanced renal disease and, thus, when detected in the urine in large numbers are suggestive of a poor prognosis.

Red Blood Cell Casts

An RBC cast is formed as a result of RBCs adhering to or embedding in a hyaline cast (77). The RBC can enter the tubule either through the glomerular basement membrane or the tubular basement membrane. The former route is predominant in glomerular disease, whereas the latter is the route responsible for the RBC casts occasionally seen associated with renal infarction or pyelonephritis. The RBCs in the casts may be intact or show varying degrees of disruption and degeneration (Fig. 10-7). There is almost always an associated hematuria. The RBCs seen may show dysmorphic features, suggesting a glomerular origin for the cells (95). The detection of RBC casts requires careful searching of the sediment, because they may be sparse even in the presence of significant glomerular disease. The identification of even one RBC cast is essentially pathognomonic for glomerular disease.

Pigment Casts

If the disruption or degeneration in a RBC cast becomes severe, the RBC membranes can become indistinct, resulting in an orange–red granular-appearing, hemoglobin cast. Similar appearing casts can be seen with myoglobinuria. Pigmented casts containing bilirubin or (more rarely) melanin also occasionally can be seen in the sediment.

White Blood Cell Casts

White blood cell casts contain leukocytes, usually neutrophils, in a hyaline cast matrix (77). The WBCs enter the tubule via the tubular basement membrane and then become incorporated into the cast. The detection of WBC casts usually suggests interstitial nephritis or pyelonephritis. However, glomerular nephritis may result in WBC casts, on occasion, if associated with significant neutrophil infiltration.

Renal Tubular Epithelial Cell Casts

These casts are formed from renal tubular cells that slough off and are, in turn, embedded in the hyaline matrix (77). They can be seen associated with any renal tubular injury; however, they are commonly seen with acute tubular necrosis. It is often difficult to distinguish between a WBC and a renal tubular cast.

Fatty Casts

Fats may be seen in the urine as free globules, oval fat bodies, or fatty casts. Fatty casts contain fats as free globules or contained within embedded tubular epithelial cells (oval fat bodies). If cholesterol or cholesterol esters are present, examination with polarized light may show a characteristic "Maltese cross" pattern. Neutral fats, on the other hand, stain with Sudan III or oil red O dye. The finding of lipiduria is most commonly associated with the presence of nephrotic syndrome. It is also a "classic" finding of the fat embolism syndrome and occasionally is seen following severe long-bone fractures.

Bacterial and Fungal Casts

Bacteria and fungi may be observed within the matrix of a cast (125). They often appear as granules in a cast; thus, the frequent diagnosis of a granular cast. There are often associated with WBC casts and these microorganisms may be present in WBC

FIGURE 10-8. Two conglomerations of uric acid crystals. The typical shape of the single crystals can be seen in those protruding from the sides of the conglomerations.

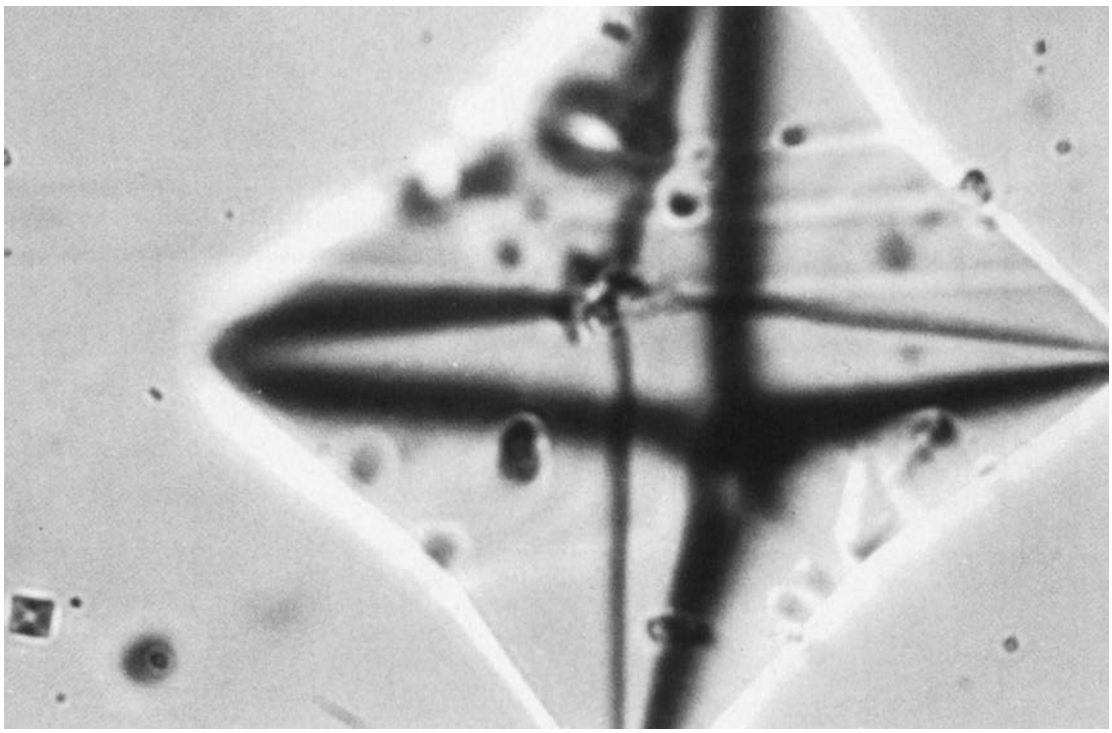

FIGURE 10-9. A very large calcium oxalate crystal. Its large size is obvious when this crystal is compared with the small calcium oxalate crystal (*bottom left corner*).

casts as well. The finding of bacterial or fungal casts strongly suggests renal infection.

Crystals

Crystals are a common finding in the urine, particularly if urine is allowed to stand before examination (Figs. 10-8 and 10-9). The type of crystal observed, in large part, reflects the urine pH as well as the constituents of the urine. In general, identification of crystals in the urine is of limited clinical importance. The crystals that can be seen in normal urine are listed in Table 10-3. The type of crystal observed in stone disease may be a clue to the origin of the stone (Figs. 10-10 and 10-11). Similarly, large numbers of calcium oxalate crystals found in the urine are associated with ethylene glycol overdose and often are an important clue to the early clinical diagnosis of this toxicity. Crystals that are always abnormal if detected include: cystine, tyrosine, leucine, sulfonamides, ampicillin, radiologic dye, and indinavir (4,126).

TABLE 10-3

CRYSTALS IN NORMAL URINE

	pH	
	Acid	Alkaline
Amorphous urate	+	
Uric acid	+	
Calcium oxalate	+	
Amorphous phosphate	+	
Triple phosphate		+
Ammonium biurate		+
Calcium carbonate		+

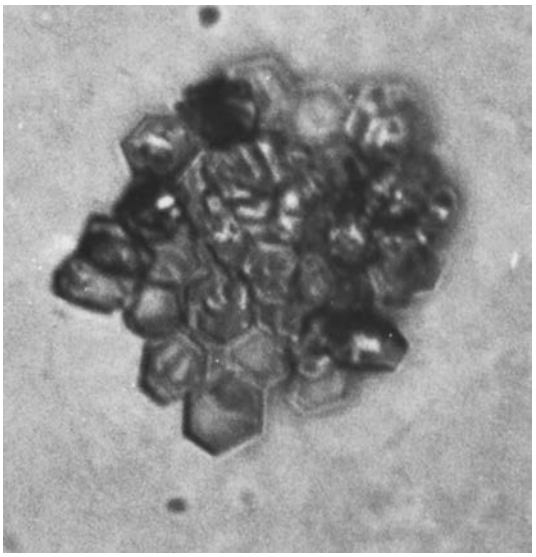

FIGURE 10-10. A conglomeration of cystine crystals from a patient with cystine stones. The typical benzene rings can be seen along the edges.

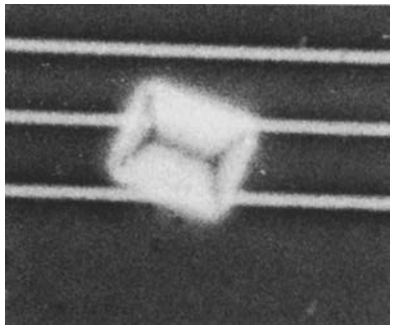

FIGURE 10-11. A triple phosphate crystal with a typical "coffin-lid" appearance, commonly seen in association with infection stones.

Automated Systems

Although manual urinalysis continues to be an important diagnostic tool, concerns about quality assurance, standardization, and costs remain. Interoperator variability for both interpretation of microscopic and macroscopic analyses have been reported (14,15,127,128). In addition, labor costs for microscopic examination have led many laboratories only to perform manual microscopic examination if there is an abnormality in the macroscopic (dipstick) examination. This has led to the development of fully automated systems for examination of the microscopic sediment by flow cytometer (e.g., Sysmex UF-100, TOA Medical Electronics, Kobe, Japan). The Sysmex UF-100 analyzer stains DNA of cells and membranes of the formed elements in the urine. The sample then passes as a laminar flow through a laser beam, and light scattering, fluorescence, and impedance are measured. Simultaneous measurements using manual microscopy show linear correlation of 0.83 for RBCs, 0.934 for leukocytes, 0.88 for epithelial cells, and 0.4 for casts (129). Currently, several reports suggest that the automated flow cytometer would improve standardization of microscopic examination, as well as considerable savings in time and labor costs (129–134). The mean time for an automated urinalysis using the recently introduced Sysmex UF-50 was 72 seconds as compared to a mean time of 330 seconds with a manual urinalysis system (135). Algorithms have been developed to select which urine samples require subsequent microscopic review after automated urinalysis (136). In addition, an automated test stripping analyzer also has been developed with photometric measurement of the reagent field for RBCs, WBCs, glucose, protein, urobilinogen, bilirubin, nitrite, ketones, pH, and specific gravity (137). Over the last several years, several novel approaches have been applied to urinalysis, which has involved the application of DNA and RNA analysis of urine sediment (138). These techniques have been used to detect a variety or renal and urologic disease including malignancy.

CONCLUSION

Our knowledge of renal disease and the constituents of urine have expanded dramatically. This in no small measure is a result of the increasing sophistication of the diagnostic techniques available. Although these new techniques have kept the study of urine a dynamic field, the "technology" required for the basic examination of the urine by the clinician, has remained quite simple (Table 10-4). Virtually all of the clinically relevant information still can be obtained from the urine with this basic approach. It is interesting that, in the face of all the rapid changes over the years, routine urinalysis still remains as important to the clinician today as it was to Richard Bright.

TABLE 10-4

APPROACH TO ROUTINE URINALYSIS

Macroscopic examination
Physical characteristics
Color
Odor
Specific gravity
Chemical analysis—dipstick screen
Glucose
Ketone
Protein
Hemoglobin
Bilirubin/urobilinogen
Nitrite
Leukocyte esterase
Microscopic analysis (question if screen positive)
Bright-field, low and high power
Phase contrast (if available)
Polarized light (if available)
Do not culture if nitrite and leukocyte esterase are negative.

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CHAPTER 11 ■ LABORATORY EVALUATION OF KIDNEY FUNCTION

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The kidney performs numerous specialized functions in an effort to maintain constancy of the internal composition of the body fluids. These functions include excretion of waste products, regulation of extracellular fluid volume and composition, production and catabolism of hormones, and regulation of acid-base balance. The normal kidney can compensate for wide variations in intake and in extrarenal loss of fluid and electrolytes. In this chapter, we focus on the laboratory evaluation of kidney functions that are of greatest importance in the clinical practice of medicine. We begin with a discussion of glomerular filtration as an index of overall kidney function and proteinuria as a marker of kidney damage. In particular, we emphasize their use in the detection of chronic kidney disease and measuring its progression over time. Tests of urine concentration and dilution are also discussed in this chapter as urine specific gravity is routinely reported as a component of the urine dipstick examination. In some conditions, abnormalities in concentration and dilution of the urine may be a marker of kidney disease. Other components of the urine dipstick examination, such as hematuria, are discussed in other chapters.

GLOMERULAR FILTRATION: DETERMINANTS AND MEASUREMENT

Normal Glomerular Filtration

The human kidney contains approximately 1 million glomeruli (1,2), each of which is approximately 150 to 200 μm in diameter. The total surface area provided for filtration approximates 1 square meter (3). Approximately 180 L per day (or 125 mL/minute) of tubular fluid are produced from the rich renal plasma flow by the process of ultrafiltration. Glomerular filtration, driven by the high hydrostatic pressure across the glomerular capillaries, is facilitated by a hydraulic permeability of the glomerular capillary wall that is one to two orders of magnitude greater than other capillaries (4).

The glomerular filtration barrier is both size and charge-dependent. Substances with molecular weights lower than 10,000 daltons cross the glomerular capillary wall as easily as water and electrolytes (5–7). Micropuncture sampling of glomerular filtrate in amphibians and mammals shows the filtrate to be identical in nonprotein composition to plasma, with electrolyte concentrations conforming to the Gibbs-Donnan relationship (6,8). As discussed later, plasma proteins are excluded from the filtrate as a consequence of the unique structure of the glomerular capillary wall.

Determinants of the Glomerular Filtration Rate

In principle, the rate of glomerular filtration (GFR) is dependent on the number of nephrons (N) and the single-nephron glomerular filtration rate (SNGFR), as described below:

$$\mathrm{GFR} = \mathrm{N} \times \mathrm{SNGFR} \qquad [1]$$

In normal individuals, regulation of GFR occurs via regulation of SNGFR. In patients with kidney disease, in whom the nephron number may be reduced, regulation of SNGFR remains important in modulating GFR. SNGFR is determined by two major factors. The first factor is the net ultrafiltration pressure (P_{UF}), determined by the difference between the net transcapillary hydraulic pressure (ΔP) favoring filtration and the net oncotic pressure ($\Delta\pi$) opposing filtration. ΔP is determined by the difference between the glomerular capillary hydraulic pressure (P_{GC}) and that in the earliest proximal tubule (Pt). $\Delta\pi$ is determined by the glomerular oncotic pressure alone as the ultrafiltrate is virtually protein free. The second factor, K_f, describes the surface area and permeability characteristics of the glomerular ultrafiltration barrier. This relationship can be expressed by the equation:

$$\mathrm{SNGFR} = K_f(\Delta P - \Delta\pi) \qquad [2]$$

Absent from this equation is the renal plasma flow rate. Alterations in renal plasma flow affect SNGFR largely by affecting $\Delta\pi$. Changes in determinants of SNGFR as plasma traverses the glomerular capillary are demonstrated in Figure 11-1. For a detailed analysis of these determinants and the multiple factors that result in the regulation of glomerular filtration, the reader is directed to Chapter 2.

Normal Range and Variability of GFR

The glomerular filtration rate cannot be measured directly. Instead, as discussed later, it is estimated from the urinary clearance of an ideal filtration marker, such as inulin. When measured repeatedly in a single individual, under constant conditions and according to a standard protocol, the glomerular filtration rate is remarkably constant. Homer Smith measured the inulin clearance in one "hospitalized but otherwise normal subject" 15 times during 1 year; the range was 113 to 137 mL/minute with a mean of 122 mL/minute (9). However, variation among individuals is quite large, and normal values show considerable spread. As discussed later, the major causes of variability in healthy individuals are age, gender, and body size. Hence, measured values of GFR are typically adjusted for body size (surface area) and compared to normative values for

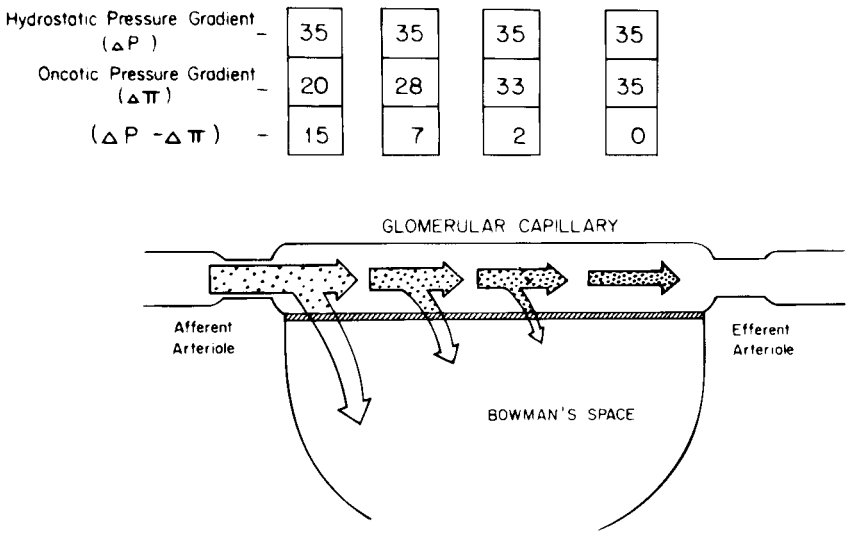

FIGURE 11-1. The changes in hydrostatic and oncotic pressures that occur as plasma traverses the glomerular capillary. As water is filtered without protein, the oncotic pressure gradually rises, decreasing the net pressure favoring filtration. The pressure favoring filtration falls toward zero and filtration stops in this model before the plasma reaches the efferent arteriole. (From: Deen WM, Robertson CR, Brenner BM. Glomerular ultrafiltration. *Fed Proc* 1974;33:14, with permission.)

age and gender (Fig. 11-2) (10). Even after elimination of these sources of variation, important variability remains. A compilation of inulin clearance measurements in hydrated young adults (adjusted to a standard body surface area of 1.73 m^2) shows the mean value in men to be 131 mL/minute with a coefficient of variation (CV) (defined as the standard deviation divided by the mean) of 18%, and the mean value in women to be 120 mL/minute, with a CV of 14% (9,10). The following sections discuss causes of normal variation. These same factors also contribute to variation in GFR in patients with kidney disease.

Age, Gender, and Body Size

The surface area adjustment was first introduced to minimize variability in urea clearance results among normal adults and children (11–13). Based on the relationship of GFR to glomerular surface area, it is not surprising that the level of GFR is related to kidney size, which in turn, is related to body surface area and metabolic activity (14). Measured values for GFR are conventionally factored by 1.73 m^2, the mean surface area of men and women 25 years of age. Nonetheless, as described earlier, surface-area adjusted values for GFR are approximately 8% higher in young men than in women of the same age.

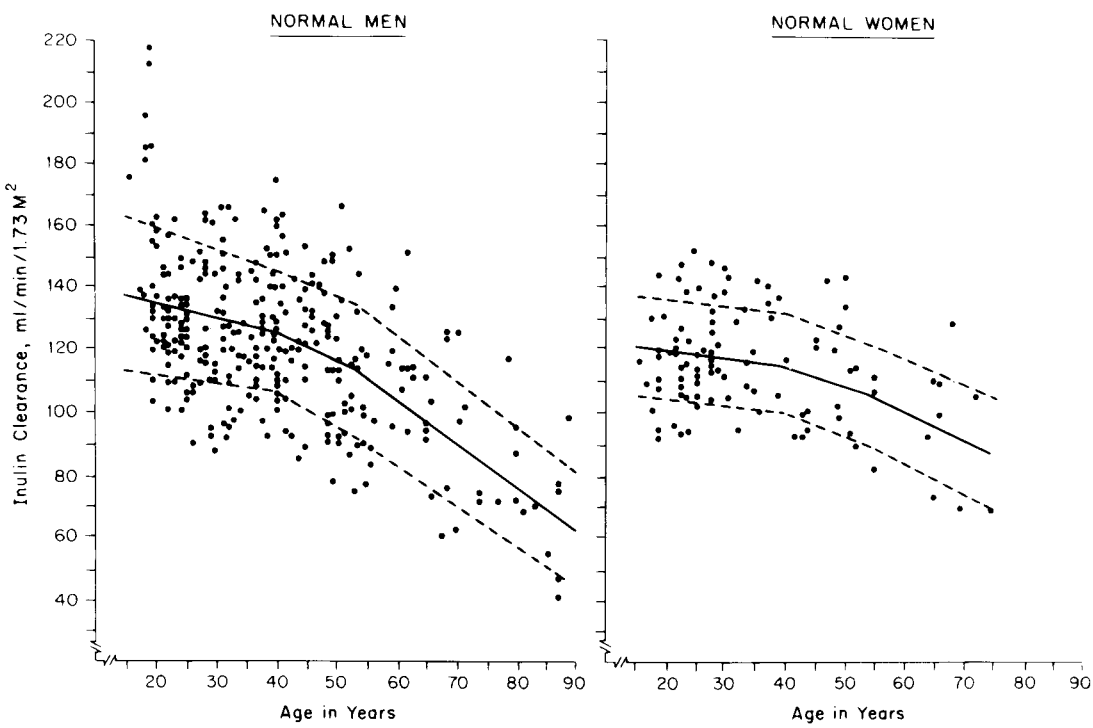

FIGURE 11-2. Normal values for glomerular filtration rate, adjusted for body surface area, in men and women of various ages. (From: Wesson LG, ed. *Physiology of the human kidney.* New York: Grune & Stratton, 1969, with permission.)

The surface area adjustment is not appropriate for newborns, whose adjusted GFR is less than 50% of the value achieved at approximately 1 year of age (15,16). More recent studies strongly suggest that in newborns, GFR should be expressed in mL/minute/kg, with the normal value being 0.6 to 1.6 mL/minute/kg. Such an approach reduces the apparent variation in measured GFR more than 10-fold (17). Over age 1 to 2 years, however, GFR values in normal children, adjusted to 1.73 m^2, are the same as those for young adults.

Both cross-sectional and longitudinal studies in normal men demonstrate an age-related decline in GFR of approximately 10 mL/minute/1.73 m^2 per decade after the age of 30 years (10,18–21). Thus, during the 50 years from age 30 to 80, normal GFR declines by almost 40% from approximately 130 to 80 mL/minute/1.73 m^2. Cross-sectional studies in normal women indicate roughly similar results, but comparable longitudinal studies have not been performed. This age-related decline in GFR is consistent with the anatomic observation that the number of glomeruli in the normal human kidney declines with age; in the sixth and seventh decades, the number of glomeruli is less than one-half the number present in young adults (1).

Pregnancy

Marked increases in GFR occur during pregnancy; elevations as much as 50% occur during the first trimester, and these high levels persist until term (22–25). These increments in GFR are associated with an increase in renal plasma flow and relatively constant filtration fraction throughout most of pregnancy, reflecting hemodynamic consequences of widespread vasodilatation. Late in pregnancy, it appears that hyperfiltration becomes dependent on reduced plasma oncotic pressure. This change persists in very early postpartum, but the GFR returns to normal in the first 4 to 8 weeks following the end of pregnancy (25,26).

Interestingly, pregnancy-induced hyperfiltration also occurs in women with preexisting chronic kidney disease (27). This observation suggests that the physiologic vasodilatation of pregnancy can further augment the single-nephron hyperperfusion and hyperfiltration associated with chronic kidney disease. However, this phenomenon may be restricted to women with only mild reduction in GFR. Improvement of GFR was not observed in one study of 23 women with chronic kidney disease and pre-pregnancy serum creatinine levels greater than 1.4 mg/dL (28).

Protein Intake

The effect of protein intake to modulate GFR in experimental animals was recognized more than 50 years ago (29,30). It is now clear that these effects occur in humans, although the magnitude of the effect varies among studies (31). Important causes of variation include the duration of protein feeding (habitual protein intake vs. meat meals or amino acid infusions), the type of protein (animal vs. vegetable or soya protein sources; essential vs. nonessential amino acids), and the filtration marker used to measure GFR (inulin vs. creatinine).

In a classic study, Pullman et al. placed healthy humans on low (0.1 to 0.4 g/kg/day), medium (1.0 to 1.4 g/kg/day) and high (2.6 g/kg/day) protein diets for 2 weeks. Compared to the low protein diet, inulin clearance increased after ingestion of the medium and high protein diets by 9% and 22%, respectively. These changes were accompanied by parallel changes in renal plasma flow, indicating a hemodynamic basis for the changes in GFR. A longer period of habituation may have greater effects on GFR. Similarly, in patients with chronic malnutrition, inulin clearance was 27% to 64% lower than after repletion of nutritional status (32–35), and returned to near normal values only after 1 month of refeeding. In addition, malnourished patients had smaller kidneys, suggesting that differences in kidney function were due to structural as well as hemodynamic alterations (32). Increases in GFR and kidney size in association with increased protein intake have been noted in diverse clinical circumstances, such as in patients receiving total parenteral nutrition and in insulin-dependent diabetic patients with poor metabolic control (36). Some studies suggest a greater response to animal than vegetable protein in habitual diets as well as in response to protein loads (37–39).

After a meat meal, GFR, renal plasma flow, and splanchnic blood flow rise within an hour and remain elevated for several hours (40). In humans, the increment in inulin clearance is about 10% (41,42), and appears to be less than the increment in creatinine clearance (31). Nonessential amino acids are more potent than essential amino acids in inducing the postprandial rise in GFR, and branched-chain amino acids appear to have little or no effect.

It had been proposed that protein-induced hyperfiltration represents "renal reserve capacity," which is lost prior to the reduction in baseline GFR associated with kidney disease (43). However, it has now been shown conclusively that changes in GFR occur in response to changes in habitual protein intake or meat meals in patients with kidney disease and reduced GFR (42,44–46) This is consistent with studies in animals with experimental kidney diseases, which show that changes in protein intake further modulate the determinants of single-nephron GFR. In particular, a high protein diet raises the already increased glomerular plasma flow and transcapillary hydrostatic pressure gradient (47,48). Thus, similar to pregnancy-induced hyperfiltration, protein-induced hyperfiltration augments the hyperperfusion and hyperfiltration of chronic kidney disease.

Diurnal Variation

A normal diurnal variation in filtration rate occurs, with 10% higher values occurring in the afternoon than in the middle of the night (49). In large part, the diurnal variation is thought to be related to variation in protein intake during the day (10,43). Possibly, diurnal variation may also be related to transient reductions in GFR associated with exercise. Indeed, a decrease of 40% or more is seen with severe exertion (10,50,51). However, diurnal variation is also observed in quadriplegics (52), arguing against physical activity as the sole cause of diurnal variation. Possibly, diurnal variation may also reflect variation in hydration. GFR increases with overhydration and decreases with water restriction. However, the changes are small except when gross disturbances in fluid balance occur.

Antihypertensive Therapy

The level of GFR remains relatively constant throughout a wide range of blood pressure. Nonetheless, antihypertensive therapy can be associated with reductions in GFR, due, in part, to the effect of lowering blood pressure and, in part, to specific effects of classes of antihypertensive agents. Indeed, marked reduction in GFR can complicate treatment in patients with severe hypertension and acute or chronic kidney disease (53), which is an effect thought to be due to the loss or reset of autoregulation due to sclerosis of the renal vasculature from hypertensive injury (54). In normal individuals and in patients with kidney disease, GFR is transiently reduced by a variety of antihypertensive agents, including diuretics, beta-blockers, central alpha-2 agonists, and peripheral alpha blockers (55). In contrast, angiotensin converting enzyme (ACE) inhibitors, calcium channel blockers, and directly acting vasodilators do not regularly lower GFR, although, reduction in GFR after beginning ACE inhibitors has been noted in some patients with chronic kidney disease (56). In general, persistent effects have not been noted. However, a large study in patients with chronic kidney disease and well-controlled hypertension showed persistent small (less

than 5 mL/minute), but significant, reductions in GFR associated with the use of ACE inhibitors as well as diuretics and beta-blockers (57). In addition, after controlling for the effect of these classes of antihypertensive agents, a small effect of lowering blood pressure remained. Because the effects of the various classes of medications and of lowering blood pressure appear to be independent, a clinically significant reduction in GFR could occur in patients with chronic kidney disease undergoing treatment with multiple antihypertensive agents.

Measurement of the Glomerular Filtration Rate

Clearance

The "gold standard" for the measurement of GFR is the urinary clearance of inulin. The term *clearance* was introduced into kidney physiology by Van Slyke and his colleagues in reference to studies of the excretion of urea in 1929 (12). Two years later, Jollife and Smith extended the use of the term to the *excretion of creatinine* and later to the excretion of many other substances (58). In the many decades since these pioneering studies, the concept of clearance has maintained its primacy as the cornerstone of our understanding of the measurement of glomerular filtration.

The clearance of a substance is defined as the rate at which it is cleared from the plasma per unit concentration. The clearance of substance "x" (C_x) is given in the following equation:

$$C_x = A_x/P_x \quad [3]$$

where A_x is the amount of x eliminated from the plasma, P_x is the average plasma concentration. Hence, C_x is expressed in units of volume per time. The value for clearance does not represent an actual volume, but a virtual volume of plasma that is completely cleared of the substance per unit of time, without reference to the route of elimination. The value for clearance is related to the efficiency of elimination; the greater the rate of elimination, the higher the clearance.

Relationship of GFR to Urinary Clearance

For a substance that is cleared by urinary excretion, the clearance formula may be rewritten as follows:

$$C_x = U_x \times V/P_x \quad [4]$$

where U_x is the urinary concentration of x and V is the urine flow rate. The term $U_x \times V$ is defined as the urinary excretion rate of x. If substance x is filtered freely across the glomerular capillary walls and excreted only by glomerular filtration, then the rate of filtration is equal to the rate of urinary excretion:

$$GFR \times P_x = U_x \times V \quad [5]$$

where the term $GFR \times P_x$ is defined as the filtered load of x. By substitution into Equation 2:

$$C_x = GFR \quad [6]$$

Hence, substance x would be defined as an "ideal filtration marker" whose urinary clearance could be used to measure GFR.

However, if substance x is also reabsorbed or secreted by the renal tubules, then the following equations apply:

$$U_x \times V = GFR \times P_x - TR_x + TS_x \quad [7]$$

$$GFR = (U_x \times V - TR_x + TS_x)/P_x \quad [8]$$

$$GFR = C_x - TR_x/P_x + TS_x/P_x \quad [9]$$

where TR_x and TS_x are the rates of tubular reabsorption and secretion of x, respectively, and TR_x/P_x and TS_x/P_x are the clearances of substance x due to reabsorption (C_{TRx}) and secretion (C_{TSx}), respectively. In this case, the rate of urinary excretion ($U_x \times V$) does not equal the filtered load ($GFR \times P_x$), and clearance does not equal GFR. Therefore, the value for urinary clearance of x (C_x) is determined not only by the rate of glomerular filtration, but also by the mechanism of excretion by the kidney. For substances that are filtered and secreted, clearance exceeds GFR, and for substances that are filtered and reabsorbed, clearance is less than GFR.

Relationship of GFR to the Plasma Solute Concentrations

An important concept for the following discussion is the steady state of solute balance. A steady state with regard to substance x is achieved when the rate of generation in body fluids (either from endogenous production or exogenous intake) is constant and equal to its rate of elimination from body fluids (either from excretion or metabolism). Therefore, in the steady state, the plasma concentration of substance x is constant. Furthermore, if the elimination of substance x is solely by excretion in the urine, the following equation applies:

$$G_x = U_x \times V \quad [10]$$

where G_x is the rate of generation of x. An important corollary is that, in the steady state, the rate of generation can be assessed from the urinary excretion rate.

For an ideal filtration marker, the steady state plasma concentration is determined by the GFR and the rate of generation or urinary excretion.

$$P_x = G_x/GFR = U_x \times V/GFR \quad [11]$$

Hence, P_x is inversely related to GFR, and GFR can be assessed from the value for P_x if generation or urinary excretion is known.

$$GFR = G_x/P_x = U_x \times V/P_x \quad [12]$$

In principle, if generation and urinary excretion are not known, but can be inferred from known demographic and clinical variables, then GFR can be estimated from the value for P_x and these variables. Estimating equations for GFR use demographic and clinical variables as observed surrogates for these unmeasured physiological factors.

$$G_x \sim X + Y + Z \quad [13]$$

Where X, Y, and Z are numerical values for clinical and demographic variables related to generation of the filtration marker (Px). Therefore,

$$GFR\ estimate = (a\,X + b\,Y + c\,Z)/a\,P_x \quad [14]$$

where and a, b, c, and d are coefficients relating P_x and other variables to measured GFR. Estimating equations for GFR are often written as a regression equation in the logarithmic scale and, therefore, have the appearance of

$$\log GFR\ estimate = a\,P_x + b\,X + c\,Y + d\,Z + \varepsilon \quad [15]$$

$$GFR\ estimate = [P_x]^a \times X^b \times Y^c \times Z^d \times \varepsilon \quad [16]$$

where a is a negative coefficient to account for the inverse relationship; and ε is the error based on uncertainty due to measurement, biological variability, and statistical techniques used to derive the coefficients. The development and validation of accurate and generalizable estimating equations for widespread clinical use requires strict adherence to epidemiological and statistical principles (59). Specific estimating equations for GFR are discussed in more detail later in the chapter.

Inulin Clearance as a Measure of GFR

The requirements for an ideal filtration marker, as outlined by Smith (9), include the following:

1. It is freely filtered at the glomerulus. It passes from the glomerular capillary blood into Bowman's space unhindered by its size, charge, or binding to plasma proteins.
2. It is not altered during its passage through the nephron. It is not reabsorbed, secreted, synthesized, or metabolized by the tubules.
3. It is physiologically inert and does not alter the function of the kidney.

Inulin, a 5,200-dalton, inert, uncharged polymer of fructose, meets these criteria, and it remains the standard for experimental and clinical measurement of GFR (9,60,61). The conclusion that inulin is freely filtered and is neither secreted nor reabsorbed in the normal kidney was originally based on indirect evidence, but a large body of direct micropuncture observations have verified this assumption (6,62–65). Similar evidence is not available, however, in all experimental kidney diseases. For example, in several models of acute kidney failure with extensive tubular basement membrane damage, leakage of inulin across the tubules is readily demonstrated (66,67). In such situations, of course, the urinary excretion of inulin is less than the filtered load, and inulin clearance is not a reliable measure of GFR.

Although the measurement of inulin clearance is a highly accurate and reproducible means of estimating GFR, there are several disadvantages that make it impractical for clinical use. First, the classical method includes measurement under fasting conditions in the morning, a continuous intravenous infusion, multiple clearance periods requiring repetitive blood and urine collections over 3 hours, oral water loading to stimulate diuresis, bladder catheterization to assure complete urine collection, and careful timing of blood sampling at the midpoint of the urine collection. Period-to-period variability in GFR (intratest variation; expressed as CV) is approximately 10%. Intratest variation may reflect incomplete bladder emptying and is often used to judge the quality of a urinary clearance study (68). However, one recent study has shown that the precision of GFR determinations is only weakly affected by intratest variability (69), probably because averaging over several clearance periods minimizes error due to incomplete bladder emptying. In a study in normal individuals using the classical method of inulin clearance, the CV for repeated measurements within an individual (intertest CV) was 7.5% (70). These estimates of measurement error are probably lower than would be observed in most clinical settings. Second, inulin is difficult to dissolve in aqueous solutions, difficult to measure, and is in short supply. Because of these disadvantages, clinical assessment of GFR uses other filtration markers and clearance methods.

We will first discuss endogenous filtration markers (creatinine, urea, and cystatin C), because of their widespread clinical use. Endogenous filtration markers have the advantage that clearance can be computed from urine collections and blood sampling under usual clinical conditions without the need for administration of the marker. Then, we will discuss alternative exogenous filtration markers and alternative clearance methods designed for their use.

CREATININE AS A FILTRATION MARKER

Assumptions

The use of creatinine clearance (C_{cr}) as an index of GFR rests on the assumption that creatinine is an ideal filtration marker; hence, creatinine clearance would equal GFR. Therefore,

$$GFR = C_{cr} = U_{cr} \times V/P_{cr} \quad [17]$$

where C_{cr} is creatinine clearance, $U_{cr} \times V$ is the urinary excretion rate of creatinine, and P_{cr} is the plasma concentration. The use of serum (or plasma) creatinine as an index of GFR is based on the additional assumption that creatinine is excreted only by the kidney and that the patient is in a steady state of creatinine balance:

$$G_{cr} = U_{cr} \times V \quad [18]$$

where G_{cr} is creatinine generation rate. Under these conditions, the serum level (P_{cr}) would be inversely related to GFR, as follows:

$$P_{cr} = G_{cr}/GFR = U_{cr} \times V/GFR \quad [19]$$

The normal level of GFR is sufficient to maintain a low concentration of creatinine in serum, approximately 0.8 to 1.2 mg/dL. Figure 11-3 showns hypothetical changes in creatinine generation (production), excretion, balance, and serum concentration following a 50% decrement in GFR. A new steady state would be reached when the serum concentration rises sufficiently so that the filtered load, and hence the urinary excretion rate, again equals the generation rate. In the new steady state, the reduced level of GFR would be reflected by a reciprocal increase in the serum concentration (Fig. 11-3).

In the steady state, therefore, changes in serum creatinine would reflect changes in GFR. Because of their reciprocal relationship, a large change in GFR would be required to raise serum creatinine from the normal to the elevated range. However, once serum creatinine is elevated, even small changes in GFR would raise it considerably more. Expression of the serum

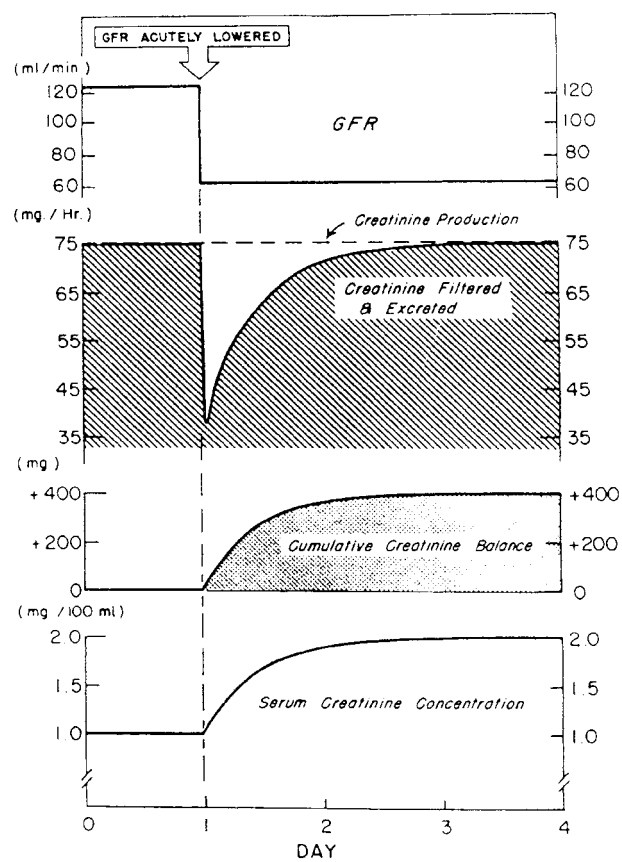

FIGURE 11-3. Effect of a sudden decrease in glomerular filtration rate on creatinine excretion, production, balance, and serum creatinine concentration. (From: Kassirer JP. Clinical evaluation of kidney function—glomerular function. *N Engl J Med* 1971;285:385, with permission.)

TABLE 11-1

CLINICAL CONDITIONS THAT CAUSE ERRORS IN THE ESTIMATION OF GFR FROM MEASUREMENT OF CREATININE CLEARANCE OR SERUM CREATININE

Condition	Effect on C_{cr}	Effect on P_{cr}	Comment
Plasma Ketosis	None	Increase	Interference with the picric acid assay for creatinine
Medications			
Certain cephalosporins or flucytosine	None	Increase	Interference with the picric acid and iminohydrolase assays for creatinine, respectively
Cimetidine or trimethoprim	Decrease	Increase	Inhibition of tubular secretion of creatinine
Dietary Protein			
Ingesting cooked meat	Increase	Increase	Transient increase in GFR and creatinine generation
Restriction of dietary protein	Decrease	Decrease	Sustained decrease in GFR and creatinine generation
Muscle Change			
Vigorous prolonged exercise	Decrease	Increase	Transient decrease in GFR and increase in muscle creatinine generation
Muscle wasting	None	Decrease	Decrease in muscle creatinine generation
Muscle growth	None	Decrease	Increase in muscle creatinine generation
Kidney Disease[a]	Increase	Decrease	Decrease in GFR, but stimulation of tubular secretion of creatinine, and possible decrease in creatinine generation

C_{cr}, creatinine clearance; *P_{cr}*, serum creatinine; *GFR*, glomerular filtration rate.
[a]Effects on C_{cr} and P_{cr} relative to effects on GFR (i.e., C_{cr} is higher than expected and P_{cr} is lower than expected for the reduction in GFR; see text).
(From: Levey AS. Clinical evolution of renal function. In: Greenberg A, ed. *Primer of kidney diseases,* San Diego: Academic Press, 1998:23.)

creatinine level as its reciprocal ($1/P_{cr}$) would more clearly reflect the magnitude of the decline in GFR.

Unfortunately, as described later, neither of the two assumptions described earlier is strictly correct, and in some circumstances, serious errors can result from estimation of GFR from creatinine clearance or serum creatinine (Table 11-1). The sources of these errors can best be appreciated from an examination of the kidney handling, metabolism, and measurement of creatinine. As described later, estimating equations account for some of these factors and thereby provide a more accurate estimate of GFR than either creatinine clearance or serum creatinine alone.

Kidney Handling of Creatinine

Creatinine as a Filtration Marker

Creatinine is small (molecular weight 113 daltons, molecular radius 0.3 nm) and not bound to plasma proteins; hence, it passes freely through the glomerular capillary wall into the Bowman's space. However, it is also secreted by the tubules, probably by the same pathway used for other organic cations (71). Therefore, creatinine is excreted not only by glomerular filtration, but also by tubular secretion

$$U_{cr} \times V = GFR \times P_{cr} + TS_{cr} \quad [20]$$

where TS_{cr} is the rate of tubular secretion. Consequently, it is not an ideal filtration marker. The true relationship between creatinine clearance and GFR is as follows

$$C_{cr} = GFR + TS_{cr}/P_{cr} \quad [21]$$

where TS_{cr}/P_{cr} is the clearance of creatinine due to tubular secretion (C_{TScr}). Thus, at all levels of GFR, creatinine clearance exceeds GFR by an amount equal to the clearance of creatinine due to tubular secretion.

Tubular Secretion of Creatinine

Creatinine secretion was recognized long ago (72), and has been re-emphasized in the modern era (73). Nonetheless, it has not been widely recognized as a limitation to the estimation of GFR from creatinine clearance; the major reason is related to the method of measurement of serum creatinine used in the past. As discussed later, the classical method, the Jaffe reaction, uses a colorimetric reaction that detects both creatinine and a number of noncreatinine chromogens in serum, but not in urine. Thus, the serum "chromogen creatinine" exceeds the true serum creatinine measured by more-accurate methods, and using the "chromogen creatinine" to calculate creatinine clearance leads to a systematic underestimation of the true value. On the other hand, because of tubular secretion, the true creatinine clearance exceeds GFR. The net result was that estimated creatinine clearance deviated little from GFR in normal individuals. With the introduction of more accurate methods to measure serum creatinine, the discrepancy between creatinine clearance and GFR has become more apparent. To limit this, clinical laboratories sometimes calibrate the serum results to maintain the relationship between creatinine clearance and GFR.

The level of serum creatinine in the low range is overestimated when older assays for serum creatinine are used. On average, creatinine secretion in normal individuals was observed to account for 5% to 10% of the excreted creatinine. Hence, creatinine clearance exceeded GFR by approximately 10 mL/minute/1.73 m^2. However, with the newer assays, creatinine secretion can exceed GFR by much larger amounts. This has not been well quantified. Most studies find greater creatinine secretion in patients with reduced GFR, which leads to a clear disparity between creatinine clearance and GFR (74). Moreover, the magnitude of creatinine secretion is variable among individuals and over time. Only some of the factors responsible for this variability are known. The level of GFR appears to be a major determinant (Fig. 11-4) (73). The mean difference between C_{cr} and GFR (the clearance due to tubular secretion) within the range of GFR from 40 to 80 mL/minute/

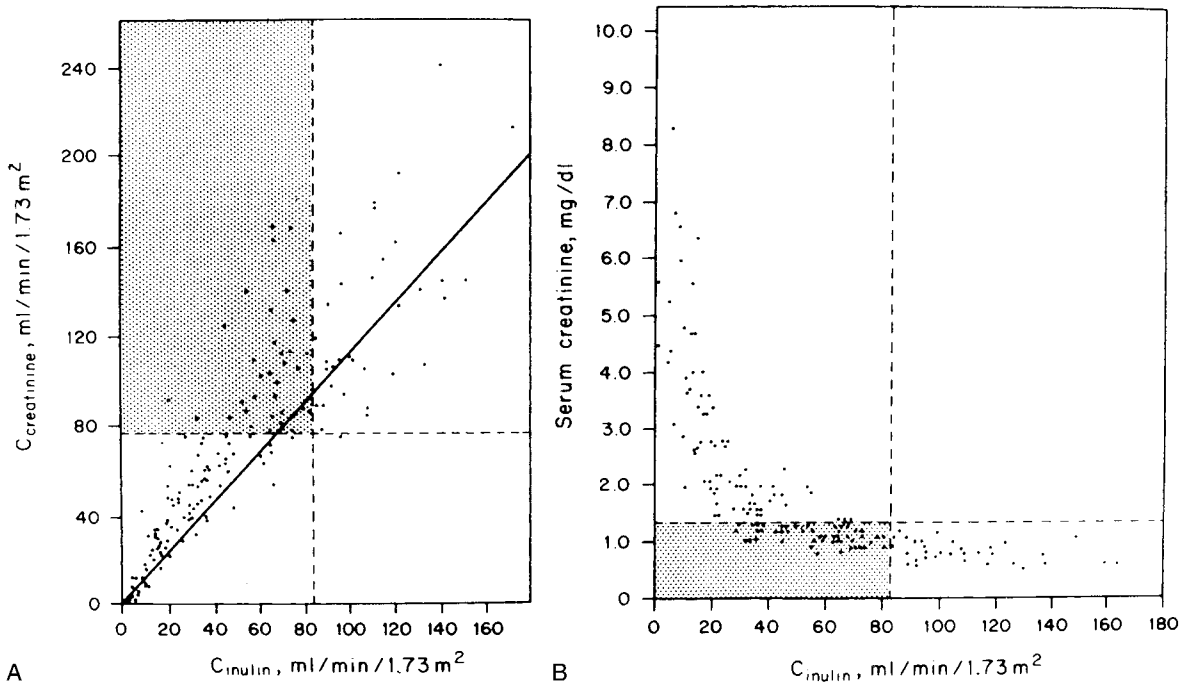

FIGURE 11-4. Relationship of creatinine clearance (**A**) and serum creatinine (**B**) to glomerular filtration rate (GFR) (inulin clearance) in patients with chronic kidney disease. *Horizontal lines* indicate lower limit of normal for creatinine clearance (**A**) and upper limit of serum creatinine (**B**). *Vertical lines* represent lower limit of normal for GFR. *Diagonal line* (**A**) is the line of identity. *Shaded areas* indicate the proportion of patients in whom the level of creatinine clearance or serum creatinine is normal despite reduced GFR. (From: Shemesh O, Golbetz H, Kriss JP, et al. Limitations of creatinine as a filtration marker in glomerulopathic patients. *Kidney Int* 1985;28:830; with permission.)

1.73 m^2 is approximately 35 mL/minute/1.73 m^2 and lower at lower GFR.

Other factors determining the magnitude of creatinine secretion are the type of kidney disease and the quantity of dietary protein intake. Patients with polycystic kidney disease and tubulointerstitial diseases have lower mean values for creatinine clearance due to secretion than patients with glomerular diseases and other diseases (44), perhaps reflecting more serious tubular injury and limitation of tubular secretion. On the other hand, higher protein intake is associated with higher mean values for creatinine clearance due to secretion (44), perhaps due to stimulation of secretion due to protein ingestion. This finding may account for the greater effect of protein loads on creatinine clearance compared to GFR (31).

Several commonly used medications, including cimetidine and trimethoprim (75), competitively inhibit creatinine secretion, thereby reducing creatinine clearance and raising the serum creatinine concentration, despite no effect on GFR. Clinically, it can be difficult to distinguish a rise in serum creatinine due to drug-induced inhibition of creatinine secretion from a decline in GFR. A clue to inhibition of creatinine secretion is that urea clearance and blood urea nitrogen concentration remain normal.

Some investigators have proposed using cimetidine to inhibit creatinine secretion during creatinine clearance measurements, thereby permitting a more accurate assessment of GFR (76,77). However, complete inhibition of creatinine secretion may require prolonged high dose cimetidine therapy (78). Variable inhibition of tubular secretion by cimetidine makes interpretation of the test difficult.

Tubular Reabsorption of Creatinine

To a limited extent, creatinine may also be reabsorbed by the tubules. Studies in normal animals and humans with very low urine flow rates (79–81), and in patients with decompensated congestive heart failure or uncontrolled diabetes mellitus (82–86) have demonstrated a ratio of clearances of creatinine and inulin <1.0. Reabsorption of creatinine may be due to its passive back-diffusion from the lumen to blood because of the high tubular creatinine concentration that occurs during low urine flow. Based on the clearance ratios observed in these studies, the maximum effect of creatinine reabsorption probably would be a 5% to 10% decrease in creatinine clearance.

Creatinine Metabolism

Generation

Creatinine is distributed throughout total body water. It is generated in muscle from the nonenzymatic conversion of creatine and phosphocreatine (Fig. 11-5). Approximately 98% of the total creatine pool is contained in muscle and about 1.6% to 1.7% per day is converted to creatinine (87). For example, in an individual with a total creatine pool of 100 g, creatinine generation would be 1.6 to 1.7 g/day. Thus, creatinine generation is proportional to muscle mass, which can be estimated from age, gender, and body size. Based on five reports containing data on 1,100 healthy individuals and patients without renal or hepatic disease, Walser derived the following equations

$$U_{cr} \times V = 28.2 - 0.172 \times \text{age (men)} \quad [22]$$

$$U_{cr} \times V = 21.9 - 0.115 \times \text{age (women)} \quad [23]$$

where creatinine excretion (given in mg/kg/day) is assumed to equal creatinine generation and age is given in years. These equations do not take into account racial and ethnic differences in muscle mass. African American (black) males and females have higher muscle mass, and consequently higher creatinine

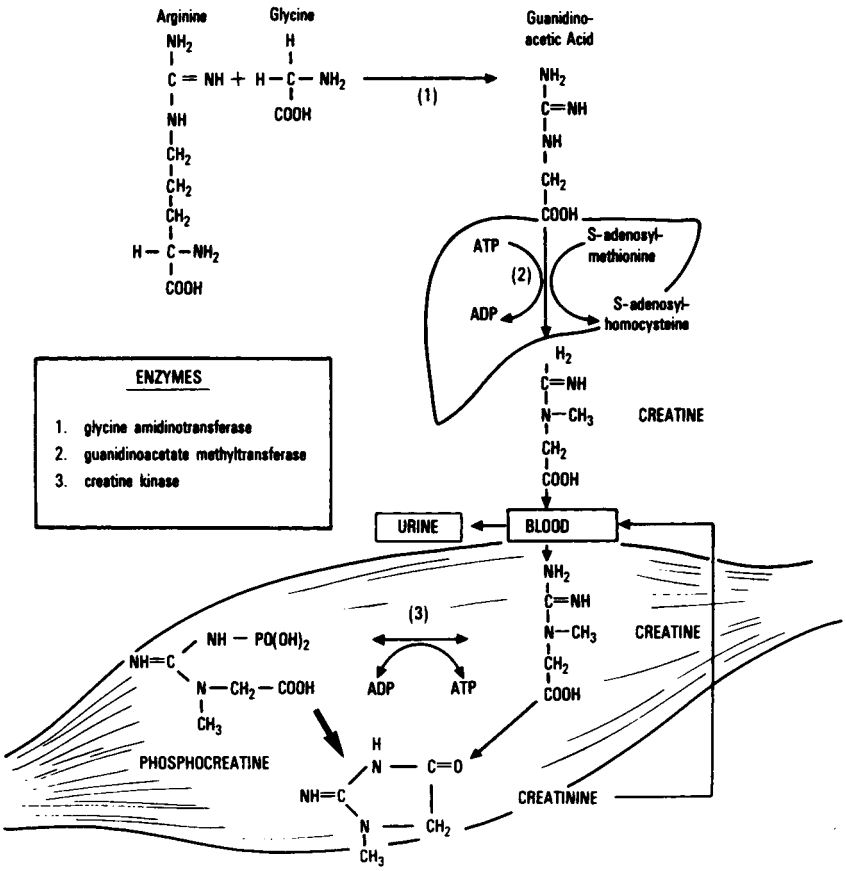

FIGURE 11-5. Pathways of creatinine metabolism. (From: Heymsfield SB, Arteaga C, McManus C, et al. Measurement of muscle mass in humans: validity of the 24-hour urinary creatinine method. *Am J Clin Nutr* 1983;37:478, with permission.)

excretion than their European American (white) counterparts (88–92)

The relationship of creatinine generation to age, gender, and body weight is affected by muscle mass and diet. Muscle wasting is associated with a decreased creatine pool, which leads to decreased creatinine generation and excretion (93–96). However, some muscle diseases are associated with increased creatine turnover (94), which in principle could transiently increase creatinine generation and excretion. Reduction in dietary protein causes a decrease in the creatine pool by 5% to 15%, which is probably due to the reduction of the availability of creatine precursors, arginine, and glycine (87,97). Of greater importance is the effect of creatine in the diet. Creatine is contained largely in meat; uncooked lean beef contains about 3.5 to 5 mg of creatine per g (98,99). Elimination of creatine from the diet decreases urinary creatinine excretion by as much as 30% (97,100,101). Conversely, ingesting a creatine supplement increases the size of the creatine pool and increases creatinine excretion (97,102–104). Meat intake also affects creatinine generation and excretion independent of its effect on the creatine pool. During cooking, a variable amount (18% to 65%) of the creatine in meat is converted to creatinine, which is absorbed from the gastrointestinal tract. Therefore, following ingestion of cooked meat, there is a sudden transient increase in the serum creatinine concentration and urinary creatinine excretion. These findings are not observed when a similar quantity of uncooked meat is ingested (105,106).

Extrarenal Elimination

Extrarenal loss of creatinine is not detectable in normal individuals, but may account for up to 68% of daily creatinine generation in patients with severe decrease in GFR. One likely, but still not established, mechanism is degradation of creatinine within the intestinal lumen by microorganisms due to induction of the enzyme *creatininase* (107–111).

Thus, in patients with kidney disease, creatinine excretion underestimates creatinine generation:

$$U_{cr} \times V = G_{cr} - E_{cr} \qquad [24]$$

where E_{cr} is the rate of elimination of creatinine by extrarenal routes.

Creatinine Clearance as an Index of Kidney Function

Most commonly, creatinine clearance is calculated from the creatinine excretion rate in a 24-hour urine collection and the single measurement of serum creatinine, assuming a steady state. The urine collection is performed at home. At the onset of the collection period, the patient is instructed to empty the bladder and discard the urine. During the collection period, all subsequent urine is saved. At the end of the period, the patient is asked to void completely and to add this last specimen to the urine collection. Shortly thereafter, a blood sample is obtained for the measurement of serum creatinine. Although this method appears straightforward and simple, a number of factors can give rise to erroneous measurements of creatinine clearance.

The most common problem rests with inaccuracies in the process of urine collection. Collecting a 24-hour urine sample is inconvenient for patients who are not at home during the day. Also, incomplete urine collections can result from misunderstanding by the patient or personnel of the timing directions, such as omitting urine specimens during the interval or incompletely emptying the bladder at the start or end of the collection period. At first glance, it might appear that the use of short urine collection intervals, such as 1-hour, carried out under close

supervision by trained personnel might overcome these difficulties. However, using a shorter collection period, the small errors due to incomplete bladder emptying at the beginning or end of the time would have a greater impact on the estimate of the urine volume and hence the urine flow rate. Indeed, the 1-hour technique has been largely abandoned because the extra effort and personnel required do not significantly improve the accuracy as compared to the 24-hour clearance (112). However, averaging the results of three to four 30-minute collection periods does significantly improve the accuracy, probably due to cancellation of errors from incomplete bladder emptying (113).

Another source for error is the assumption that the patient is in a steady state of creatinine balance. This assumption is not valid for patients with rising or falling serum creatinine, as occurs during the initiation or recovery from acute kidney failure. Under these circumstances, it is necessary to obtain additional blood samples during the urine collection to estimate the average serum creatinine concentration. The most common strategies are to measure serum creatinine at the mid-point of the urine collection, or at the beginning and end of the urine collection, and to average the results.

Clinical conditions that cause errors in the estimation of GFR from measurement of creatinine clearance are shown in Table 11-1. The most serious errors arise from the failure to recognize the increase in the tubular secretion of creatinine in patients with decreased GFR. The overestimation of GFR by creatinine clearance is not clinically significant in individuals with normal GFR. However, in patients with kidney disease, the overestimation is proportionately greater and not predictable. Indeed, within the range of GFR from 40 to 80 mL/minute/1.73 m^2, creatinine clearance can remain normal. Consequently, it can be difficult to detect the onset or early progression of the decline in GFR from measurements of creatinine clearance. As shown in Figure 11-4, creatinine clearance was reduced in only 75% of patients with reduced GFR due to glomerular disease. Once creatinine clearance is reduced, the level of creatinine clearance provides a rough guide to the level of GFR and is generally suitable for decisions regarding drug dosing and timing the onset of dialysis and transplantation.

Serum Creatinine as an Index of Kidney Function

Because of difficulties in obtaining accurately timed and complete urine collections, the serum creatinine concentration itself is generally used to assess GFR. As discussed later, this may result in overestimation of the true level of GFR, which can have important clinical consequences.

The true relationship between serum creatinine and GFR is considerably more complicated than expressed in Eq.12. Based on substitutions and rearrangements of Equations 20 and 24,

$$P_{cr} = (G_{cr} - E_{cr} - TS_{cr})/GFR \qquad [25]$$

Thus, the serum creatinine concentration is indeed inversely proportional to GFR, but is also affected by the generation, extrarenal elimination, and tubular secretion of creatinine. Nonetheless, an elevated serum creatinine usually indicates a reduced level of GFR and a further rise in serum creatinine generally indicates a further decline in GFR. However, as discussed below, a normal serum creatinine does not exclude the possibility of a reduced GFR. Nor does a stable, but elevated, serum creatinine preclude a further decline in GFR if there are concomitant changes in creatinine generation, secretion, or extrarenal elimination.

As discussed earlier, the rate of creatinine generation is lower in women, children, the elderly, malnourished individuals, and those with restricted meat intake. Hence, when creatinine clearance is normal, serum creatinine is lower in these groups. As a corollary, in these groups serum creatinine may remain within the normal range, despite a reduction in creatinine clearance. Therefore, in detecting a reduction in GFR, an elevated serum creatinine is an even less sensitive indicator than a reduced creatinine clearance. In one study (73), only 60% of patients with reduced GFR had an elevated serum creatinine, and in some patients GFR was as low as 20 mL/minute/1.73 m^2, despite normal serum creatinine concentration (Fig. 11-4)

Factors affecting creatinine generation are especially important in patients with chronic kidney disease. Meat intake is often restricted in conjunction with a low protein diet, and malnutrition and muscle wasting are common. Furthermore, due to extrarenal elimination, creatinine excretion may underestimate creatinine generation. These factors tend to blunt the rise in serum creatinine as GFR declines and may cause serious overestimation of the level of GFR from serum creatinine.

Serum Creatinine Assay

Creatinine can be measured easily in serum, plasma, and urine. No systematic differences between serum and plasma have been noted; therefore, throughout this chapter, serum and plasma creatinine have been used interchangeably.

The classic method uses the Jaffe reaction in which creatinine reacts directly with picrate ion under alkaline conditions to form a red-orange complex that is easily detected and quantified (114,115). However, in normal subjects up to 20% of the color reaction in serum or plasma is due to substances other than creatinine (116–120). Two classes of positive interferences have been described: substances such as glucose, ascorbate, and uric acid, which slowly reduce the alkaline picrate, and substances such as acetoacetate, pyruvate, other ketoacids, fluorescein (121), furosemide (122), hemoglobin (123), paraquat and diquat (124), and proteins, including IgG (125) and IgM paraproteins (126), which react with alkaline picrate to form colored complexes. The presence of these noncreatinine chromogens results in an apparent creatinine value that is 20% higher than the true value. The error in measurement can be greater, however, in diabetic ketoacidosis (127), due to the increased concentration of acetoacetate, and in patients taking certain cephalosporins (128,129), which can contribute to the colorimetric reaction. Very high serum bilirubin levels (130–134) can cause falsely lower creatinine levels (135). In patients with kidney disease, noncreatinine chromogens are not retained to the same degree as creatinine. Consequently, the overestimation of serum creatinine, and the corresponding underestimation of creatinine clearance is reduced.

In general, noncreatinine chromogens are not present in sufficient concentration in urine to interfere with creatinine measurement. Hence, measurement of creatinine clearance in normal individuals using the Jaffe reaction results in values that are approximately 20% lower than the true value.

The kinetic alkaline picrate method (136–138) takes advantage of the differential rate of color development for noncreatinine chromogens compared to creatinine. It significantly reduces, but does not eliminate, both types of positive interferences described earlier. This method is included in the Auto-Analyzer method (Technicon Instruments Corporation, Chauncy, NY). A survey by the College of American Pathologists (CAP) in 2004 found it was the most widely used method in clinical laboratories in the United States (139).

To circumvent interferences in the alkaline picrate reaction, a variety of enzymatic methods have been developed (140). The Ektachem (Eastman Kodak Co., Rochester, NY) method, using the enzyme creatinine iminohydrolase, and the creatinine PAP method (Boehringer Mannheim GmbH, Mannheim,

FRG), using the enzymes creatininase, creatinase, and sarcosine oxidase yield creatinine values comparable to those obtained using the kinetic alkaline picrate method. The latter method is more precise than the former. The antifungal agent, flucytosine, interferes with the Ektachem measurement; it may increase the value by as much as 60% (141,142). Bilirburin, dopamine, dobutamine, ascorbic acid, and sarcosine may interfere with the creatinase-creatininease methods (143). Interference from metamizol (144), raffinose cross-linked hemoglobin (145), and monoclonal IgM (146) has also been reported.

High performance liquid chromatography (HPLC) is a fairly sensitive and analytically specific method for measuring serum creatinine. Such methods have become more widely available in recent years. Many of these protocols have included sample pretreatment in order to obviate the effects from interfering compounds with mixed results as to enhanced specificity (147).

All of the commonly used methods, including the kinetic alkaline picrate reaction and the Ektachem method, are imprecise in the lower range of serum creatinine. In one study, the CV for repeated measurement of aliquots from serum samples within the same run were 25.1%, 7.3%, and 1.9%, respectively, for samples with creatinine concentrations of 0.42, 1.32, and 4.38 mg/dL, respectively (148). In another study, the CV for repeated measurements of serum creatinine was 11% in patients with a GFR >30 mL/minute/1.73 m^2 as compared to 6.5% in patients with GFR <30 mL/minute/1.73 m^2 (149). Indeed, analytical variability was estimated to account for 80% to 88% of the day-to-day variability in serum creatinine in an individual (150). The imprecision makes it difficult to interpret changes in serum creatinine within the normal range, as one cannot readily distinguish between differences in serum creatinine levels due to errors in the assay or due to biological variability in GFR.

Calibration of autoanalyzers differs among clinical laboratories, regardless of the method for measurement of serum creatinine. The American College of Pathologists (CAP) 1994 survey documented that the variation across laboratories was higher for serum creatinine than for any of the other ten analytes evaluated (151). In the 2004 CAP survey, the mean bias of the measured serum creatinine in 50 clinical laboratories compared to a true creatinine concentration assigned by isotope dilution mass spectroscopy varied from −0.06 to 0.31 mg/dL (139). Consequently, the range of normal for serum creatinine may differ between laboratories. This variability is greater than the average error in reproducibility of the serum creatinine as discussed earlier. Thus, the inaccuracy of serum creatinine assays appears to be more due to lack of standardization of calibration rather than imprecision of measurement. This has important consequences for clinicians trying to assess the level of kidney function based on serum creatinine reported by different laboratories and also has significant implications in the use of serum creatinine in GFR estimating equations as will be discussed later.

Current national standardization programs are underway by collaboration between the National Kidney Disease Education Program, CAP, and the National Institute for Science and Technology to address the need for a human serum creatinine reference material with acceptable commutability with native clinical specimens in routine field methods. Three isotope dilution-gas chromatography/mass spectrometry methods have been approved by the Joint Committee on Traceability in Laboratory Medicine as reference measurement procedures (152–154). All three of these methods require a separation step to remove creatine, which gives the same derivitization product as creatinine, and are therefore very time consuming with limited ability for sample throughput. This has slowed down the standardization program, but definitive methods are expected.

Estimating Equations: Formulas to Predict GFR and Creatinine Clearance from Serum Creatinine

Because the serum creatinine level is dependent upon physiological factors, such as creatinine generation, extrarenal elimination, and tubular secretion described earlier, as well as GFR, the use of serum creatinine alone to estimate GFR is severely limited. Estimating equations overcome some of these limitations by incorporating known demographic and clinical variables as observed surrogates for the unmeasured physiological factors other than GFR that affect serum creatinine concentration. Estimating equations have been developed to estimate creatinine clearance (18,155–165) and GFR (166–170).

It is relatively straightforward to develop an estimating equation from a study population in which measured GFR, serum creatinine, and relevant predictor variables are available. However, it is likely that in the absence of rigorous development, an equation developed in one population will likely be inaccurate in a second population. Therefore, there are specific criteria by which GFR estimating equations should be evaluated before an equation is accepted for widespread use in clinical practice (Table 11-2).

Due to its relative ease of use, one of the most common estimating equations used to estimate creatinine clearance is the Cockcroft and Gault formula (18).

$$\frac{[140\text{-age} \times \text{body weight (kg)}] \times 0.85 \text{ (if female)}}{[\text{Pcr (mg/dL)} \times 72]} \quad [26]$$

The formula for men was derived from measurements of serum creatinine and urinary creatinine excretion. The formula for women was based on the assumption that creatinine generation is 15% less in women than in men. The Cockcroft and Gault formula was derived in whites; hence, it may underestimate creatinine clearance in African Americans. Because this and other formulas do not explicitly take into account nutritional status, protein, or meat intake, they are likely to overestimate creatinine clearance in vegetarians and in individuals following a low protein diet or with malnutrition. Furthermore, because of differences in creatinine secretion between normal individuals and patients with kidney disease, estimated creatinine clearance is likely to overestimate GFR in patients with kidney disease. One study compared estimated creatinine clearance (factored by 1.73 m^2 body surface area) to measured GFR determinations in 394 subjects (171). As expected, estimated creatinine clearance overestimated GFR at all values.

Recent studies have developed equations to predict GFR rather than creatinine clearance. The most commonly used equation is the Modification of Diet in Renal Disease (MDRD) Study (172,173).

TABLE 11-2

EVALUATION CRITERIA FOR GFR ESTIMATING EQUATIONS PRIOR TO USE IN CLINICAL PRACTICE

- Developed in a large cohort (more than 500 individuals)
- Evaluated in an independent cohort (more than 500 individuals)
- Validated to have adequate precision and low bias against a gold standard measure of GFR (not creatinine clearance)
- Practical to implement taking into consideration cost, required data elements, generalizability, calibration and reliability of the assay

$$\text{GFR} = 170 \times \text{Pcr}^{-0.999} \times \text{Age}^{-0.176} \times \text{BUN}^{-0.170} \times \text{Albumin}^{0.318} \times 0.762 \text{ (if female)} \times 1.180 \text{ (if black)} \quad [27]$$

$$\text{GFR} = 186 \times \text{Pcr}^{-1.154} \times \text{Age}^{-0.203} \times 0.742 \text{ (if female)} \times 1.210 \text{ (if black)} \quad [28]$$

Pcr is expressed in mg/dL, age in years, BUN in mg/dL, and Albumin in g/dL. The MDRD Study equation is more accurate than the Cockcroft-Gault equation as well as measured urinary creatinine clearance with 91% of the estimates being within 30% of the true GFR (Fig. 11-6). Inclusion of the race term significantly improved the prediction, which is likely because of the larger muscle mass in African Americans compared to whites. The MDRD Study Equation was developed in patients with chronic kidney disease (mean GFR 40 mL/minute/1.73 m^2) who were predominantly white and had predominantly nondiabetic kidney disease. The equation has now been validated in African Americans with hypertensive nephrosclerosis, diabetic kidney disease, and kidney transplant recipients (174–176).

There are limitations to the use of estimating equations based on the physiologic, analytical, and statistical principles described earlier (59,177). Armed with this knowledge, clinicians can more properly interpret GFR estimates and determine when to request clearance measurements rather than GFR estimates. Table 11-3 lists clinical situations in which estimating equations for creatinine clearance or GFR may not be accurate and clearance measurements may be indicated.

First, all the estimating equations, including serum creatinine alone depend critically on the assumption of a steady state of creatinine balance. In patients with rising serum creatinine, the GFR estimate is likely to be an overestimate of the true GFR. Conversely, in patients with falling serum creatinine, the GFR estimate is likely to be an underestimate of the true GFR. Accurate estimation of the GFR in the nonsteady state requires a clearance measurement.

Second, measurement of true GFR is complex due to biological variability in GFR over time as well as to errors in measurement of the urine or serum concentrations of the markers during the individual clearance periods. These errors are incorporated into the error term of the estimating equations developed by regression analysis. Because biological variability in GFR and analytical variability in serum creatinine are greater

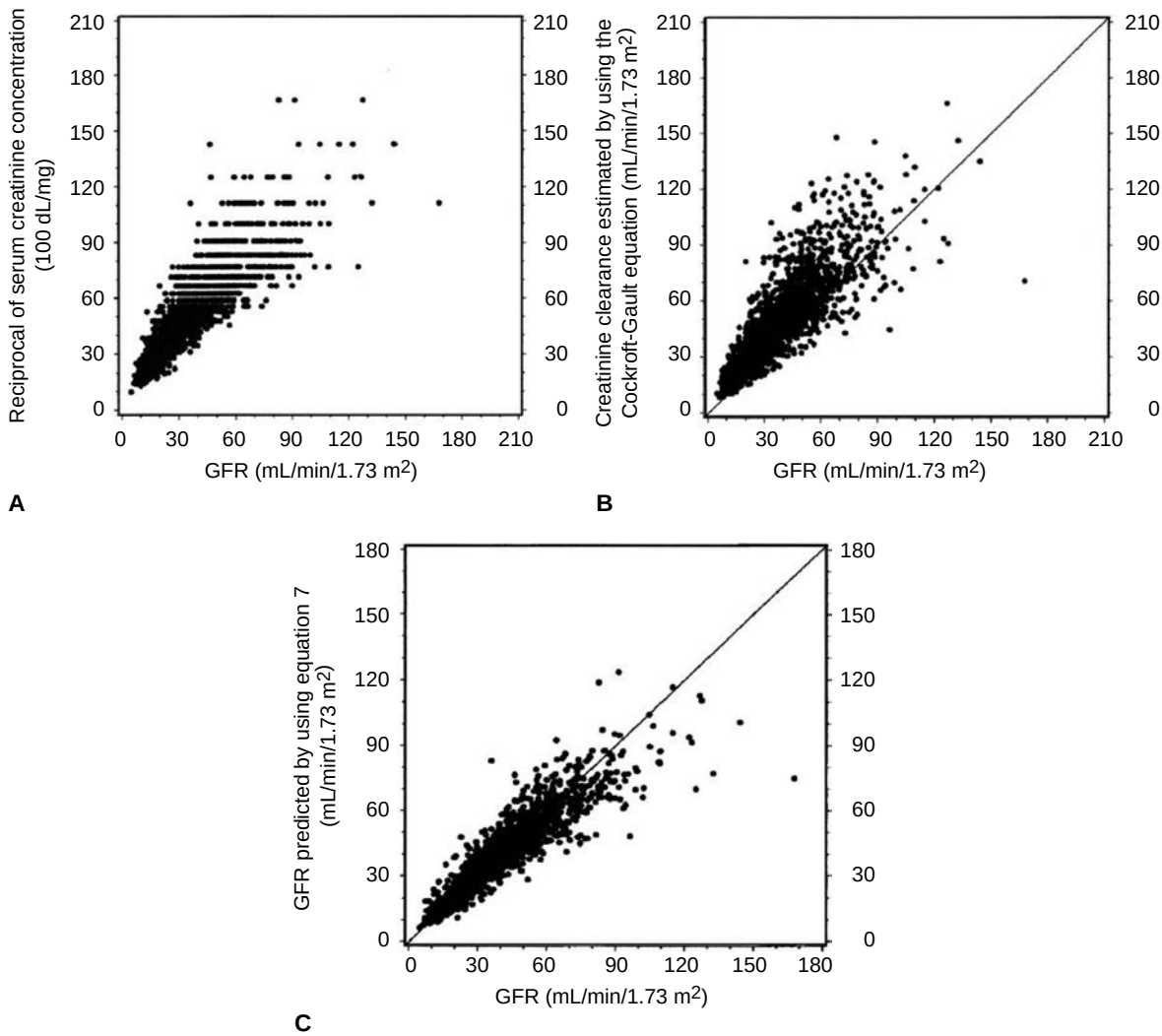

FIGURE 11-6. GFR measured as urinary clearance of ^{125}I-iothalamate and adjusted for body surfae area is 1,628 patients. Estimates include reciprocal serum creatinine ($100/P_{cr}$) ($R^2 = 80.4\%$) (**A**), Cockcroft-Gault equation standardized for body surface area ($R^2 = 84.2\%$) (**B**), and MDRD Study equation 7 (six variable equation including serum creatinine, age, sex, race, BUN, and serum albumin) ($R^2 = 90.3\%$) (**C**).

TABLE 11-3

CLINICAL SITUATIONS IN WHICH ESTIMATING EQUATIONS FOR CREATININE CLEARANCE OR GFR MEASUREMENTS MAY NOT BE ACCURATE AND CLEARANCE MEASUREMENTS MAY BE RECOMMENDED

- Extremes of age and body size
- Severe malnutrition or obesity
- Diseases of skeletal muscle
- Paraplegia or quadriplegia
- Vegetarian diet
- Rapidly changing kidney function
- Pregnancy
- Prior to dosing drugs with significant toxicity that are excreted by the kidneys

in the normal and near-normal range, precision of GFR estimates is lower in these ranges (high GFR range and low serum creatinine range).

Third, use of serum creatinine in estimating equations for either creatinine clearance or GFR requires calibration of the creatinine assay to the laboratory in which the estimating equation was developed. Failure to do so can introduce a systematic error in the estimated GFR, particularly at low levels of serum creatinine (high levels of GFR) (178–181). Calibration is possible to the laboratory where the MDRD Study equation was developed, but is not possible for the Cockcroft and Gault equation or most other equations. For equations for children, inaccuracies may be even greater as the expected serum creatinine level is low, even if GFR is reduced. This issue should be a lesser concern after completion of the national program to standardize creatinine compared to a reference standard, but will likely remain a shortcoming of estimating equations based on serum creatinine compared to other filtration markers.

Fourth, there may be differences among populations or among subgroups in a population in the relationship between serum creatinine and GFR, which may lead to poor performance of the estimating equation. This is most apparent in individuals with substantially high or low muscle mass or protein intake. Possibly, there may be differences in these relationships between normal individuals and those with kidney disease, which may lead to reduced accuracy in screening the normal population for kidney disease compared to estimating the level of GFR in patients with known kidney disease.

Fifth, by virtue of properties of regression analysis and statistics, the use of estimating equations will be most accurate in populations in which the equation was developed. Regression analysis works by developing the best fit to the observed mean and, therefore, the equations are not expected to fit as well in populations with a different range of GFR. This is not specific to any one equation. However, because the MDRD Study equation is the most rigorously and recently developed equation, its validation has been the subject of intense investigation. In general, the MDRD Study equation has been shown to be valid in populations with CKD (i.e., lower levels of GFR) regardless of CKD diagnosis or transplant status. However, the equation does not appear to perform as well in populations with higher levels of GFR (176,182,183).

UREA AS A FILTRATION MARKER

A relationship between plasma urea and kidney function was recognized long before the development of the concept of clearance or of techniques to assess GFR (184). The factors influencing both the production of urea and its renal excretion, however, are considerably more complex and variable than those for creatinine (Table 11-4). As a result, urea clearance is rarely used today as a measure of kidney function, and the serum urea nitrogen concentration (for historical reasons, often referred to as the blood urea nitrogen or BUN) has been replaced largely by the serum creatinine concentration as an index of GFR in routine clinical practice. Nonetheless, measurement of the BUN remains useful both as a diagnostic aid in distinguishing among the various causes of acute decline in GFR and as a rough correlate of uremic symptoms in kidney failure. To understand the utility and shortcomings of BUN measurements, a brief summary of the kidney handling and metabolism of urea is presented subsequently.

Kidney Handling of Urea

Urea (molecular weight 60 daltons) is filtered freely by the glomerulus and reabsorbed in both the proximal and distal nephron. Hence, urea excretion ($U_{UN} \times V$) is determined by both the filtered load and tubular reabsorption (TR_{UN})

$$U_{UN} \times V = GFR \times BUN - TR_{UN} \quad [29]$$

Consequently, clearance of urea (or urea nitrogen, C_{UN}) is less than GFR

$$C_{UN} = GFR - TR_{UN}/BUN \quad [30]$$

In the proximal convoluted tubule, a large fraction of the filtered load of urea is reabsorbed regardless of the state of diuresis. In the medullary collecting duct, urea reabsorption is linked closely to water reabsorption. In the absence of antidiuretic hormone (diuresis), the medullary collecting duct is relatively impermeable to urea; thus, urea reabsorption is minimal. Conversely, in the presence of antidiuretic hormone (antidiuresis), permeability rises and urea reabsorption increases. In normal individuals, the ratio of urea clearance to GFR varies from as high as 0.65 during diuresis to as low as 0.35 during antidiuresis.

In patients with GFR less than 20 mL/minute/1.73 m^2, the ratio of urea clearance to GFR is higher (0.7 to 0.9) and is not influenced greatly by the state of diuresis. Thus, urea clearance is approximately 5 mL/minute less than GFR. By coincidence, at this level of GFR, the difference between the values of GFR and urea clearance is similar to the difference between the values of creatinine clearance and GFR. Hence, the average of the clearances of urea and creatinine approximates the level of GFR (168,185). This coincidence provides a relatively simple method to assess GFR in advanced renal disease. A single blood sample and 24-hour urine collection may be analyzed for creatinine and urea nitrogen and the values for clearance may be averaged. However, the kidney handling of urea and creatinine is influenced by different physiologic and pathologic processes, and may vary independently, causing deviations from this approximation.

Urea Metabolism

The metabolism of urea, its relationship to dietary protein intake, and the effect of renal insufficiency on protein metabolism are discussed in detail in Chapter 101. Briefly, urea is the end product of protein catabolism and is synthesized primarily by the liver. Approximately one-quarter of synthesized urea is metabolized in the intestine to carbon dioxide and ammonia; thus, the ammonia that is generated returns to the liver and is reconverted to urea.

TABLE 11-4

CLINICAL CONDITIONS THAT CAUSE ERRORS IN THE ESTIMATION OF GFR FROM MEASUREMENT OF UREA CLEARANCE OR BUN

Condition	Effect on C_{urea}	Effect on BUN	Comment
Extracellular volume			
Dehydration	Decrease	Increase	Increased urea reabsorption
Reduced renal perfusion (volume depletion, congestive heart failure)	Decrease	Increase	Reduced GFR, increased urea reabsorption, increased urea generation
Overhydration	Increase	Decrease	Reduced urea reabsorption
Increased renal perfusion (volume expansion, pregnancy, syndrome of inappropriate ADH secretion)	Increase	Decrease	Increased GFR, reduced urea reabsorption
Dietary protein or catabolism			
Restriction of dietary protein	Decrease	Decrease	Sustained decrease in GFR and reduced urea generation
Increased dietary protein	Increase	Increase	Sustained increase in GFR and increased urea generation
Accelerated catabolism (fever, trauma, GI bleeding, cell lysis, therapy with tetracycline or corticosteroids)	None	Increase	Increased urea generation
Liver disease	Decrease[a]	Decrease[a]	Decreased GFR, decreased urea reabsorption, decreased urea generation
Kidney disease	None[a]	Decrease[a]	Decreased GFR, no change in urea reabsorption, decreased urea generation (if dietary protein is restricted)

C_{urea}, urea clearance; *BUN*, blood (serum) urea nitrogen; *GFR*, glomerular filtration rate.
[a]Effects on C_{urea} and BUN relative to effects on GFR (i.e., C_{urea} is lower than expected for the reduction in GFR).
(From: Levey AS. Clinical evolution of renal function. Greenberg A, ed. *Primer of kidney diseases*. San Diego: Academic Press, 1998.)

Dietary protein intake is the principal determinant of urea generation and may be estimated as follows:

$$EPI = 6.25 \times G_{UN} \quad [31]$$

where EPI is estimated protein intake, G_{UN} is urea generation, and both are measured in g/day (186). Usual protein intake in the United States is approximately 100 g/day (187–190), corresponding to a usual value for urea nitrogen generation of approximately 15 g/day.

In the steady state, urea generation can be estimated from the measurements of urea excretion, as shown below:

$$G_{UN} = U_{UN} \times V + 0.031 \times \text{weight} \quad [32]$$

where G_{UN} and $U_{UN} \times V$ are measured in g/day, weight is measured in kg, and 0.031 g/kg/day is a predicted value for nitrogen losses other than urine urea nitrogen (191). For a 70 kg individual with a dietary protein intake of 100 g/day, urea excretion and other nitrogen losses would be approximately 13 and 2 g/day, respectively.

Measurement of Urea

The urease method assays the release of ammonia in serum or urine after reaction with the enzyme urease (192). The presence of ammonium in reagents or use of ammonium heparin as an anticoagulant may falsely elevate the BUN, as can the drugs chloral hydrate, chlorbutanol, and guanethidine (193). Urea is also subject to degradation by bacterial urease. Bacterial growth in urine samples can be inhibited by refrigerating the sample until measurement or by adding an acid to the collection container to maintain urine pH <4.0.

BUN as an Index of Kidney Function and Protein Intake

In the steady state, the BUN level reflects the levels of urea clearance and generation.

$$BUN = G_{UN}/C_{UN} = U_{UN} \times V/C_{UN} \quad [33]$$

Consequently, many factors influence the level of BUN (Table 11-4). Nonetheless, the BUN can be a useful tool in some clinical circumstances.

As mentioned earlier, the state of diuresis has a large effect on urea reabsorption and a small effect on GFR, but does not affect creatinine secretion. Hence, the state of diuresis affects urea clearance more than creatinine clearance, and is reflected in the ratio of BUN to serum creatinine. The normal value of BUN is approximately 8 to 12 mg/dL, and the normal ratio of BUN to serum creatinine is approximately 10:1. In principle, a reduction in GFR without a change in the state of diuresis would not alter the ratio. However, conditions causing antidiuresis (dehydration or reduced renal perfusion) would decrease GFR and increase urea reabsorption, thus raising the BUN-to-creatinine ratio. Consequently, the BUN-to-creatinine ratio is a useful aid in the differential diagnosis of acute renal insufficiency. Conversely, overhydration or increased renal perfusion would raise GFR and decrease urea reabsorption, thus lowering the serum creatinine and the BUN-to-creatinine ratio.

Also important is the well-recognized relationship of the level of renal function, the BUN level, and clinical features of uremia. A useful "rule" is that a BUN level greater than 100 mg/dL is associated with a higher risk of complications in both acute and chronic kidney failure and may indicate the

need to initiate dialysis (194,195). In both acute and chronic kidney disease, restriction of dietary protein intake to 40 to 50 g/day would reduce urea nitrogen excretion to approximately 4.5 g/day. Consequently, the BUN level might rise to only 40 to 60 mg/dL, despite severe reduction in GFR. Although protein restriction may temporarily ameliorate some of the uremic symptoms, severe reduction in GFR is associated with development of uremic symptoms despite only moderate elevation in BUN.

Urea generation and the BUN are also influenced by factors other than protein intake (186). An increase is observed after the administration of corticosteroids, diuretics, or tetracyclines; after the absorption of blood from the gut; and in infection, renal failure, trauma, congestive heart failure, and sodium depletion. Decreases in urea generation and BUN may occur in severe malnutrition and liver disease. These conditions may also affect the BUN and the BUN to creatinine ratio.

CYSTATIN C AS A FILTRATION MARKER

Cystatin C has been proposed as an endogenous filtration marker. Studies in human subjects demonstrate a good correlation of serum cystatin C levels with GFR; typically better than that of serum creatinine levels (196–200). A summary of issues related to its kidney handling, metabolism, measurement, and use as an index of GFR is presented subsequently.

Kidney Handling of Cystatin C

Based on its small size (13 kD) and limited direct measurements in the rat, it appears that cystatin C is freely filtered. It is then reabsorbed and catabolized by the renal tubules (201). Cystatin C is found in the urine of patients with tubulointerstitial kidney disease (202), and some glomerular diseases (203), presumably due to impaired catabolism. Direct evaluation of kidney handling in humans has not yet been performed.

Cystatin C Metabolism

Cystatin C is a 13 kD, nonglycosylated basic protein; its mRNA is found in every human tissue (204). Molecular analysis of its promotor suggests that cystatin C is encoded on a "housekeeping" gene (204). There are no direct measurements of its production or degradation in human or whole animal studies nor is there information as to the effect of disease processes, diet, or medications on its synthesis.

Measurement of Cystatin C

Methods for analysis of cystatin C have substantially improved in recent years. Commercially available autoanalyzers using either a particle-enhanced turbidimetric immunoassay (PETIA) (196) or particle-enhanced nephelometric immunoassay (PENIA) (205) provide a high degree of precision and reproducibility. When similarly calibrated, results from these two different methods are highly correlated (206). For the PETIA method, CV of the assay is 1.3% to 3.2% and the analytical range is 0.4 to 14.1 mg/L. The PENIA assay has an intraassay imprecision of 2.0% to 3.0% CV and an interassay imprecision of 3.2% to 4.4% CV. The assay range is 0.23 to 7.25 mg/L. It is the most precise of the automated methods (207). With the PENIA method, no interference is noted with bilirubin, rheumatoid factor, hemoglobin, or triglycerides; bilirubin levels of 150 to 300 (mol/L (8.8 to 17.5 mg/dL) increase cystatin C levels by less than 10% using the PETIA method, but there is no interference by rheumatoid factor, hemoglobin, or triglycerides (196). Cystatin C is very stable in serum, but readily degraded in urine.

Cystatin C as an Index of Kidney Function

Cystatin C levels in surveys of normal adults range from 0.54 to 1.55 mg/L, with slight variability depending on the assay utilized (206). Cystatin C concentrations in subjects with normal GFR (>80 mL/minute/1.73 m^2) were 0.61 to 2.1 mg/L (199,208) with a mean value of 1.08 + 0.14 mg/L (mean ± SD) in another study (200).

Studies have compared serum cystatin and creatinine as filtration markers. When the two analytes are compared alone, cystatin appears to be a better filtration marker. When compared to GFR estimates based on serum creatinine and estimating equations, such as MDRD Study or Cockcroft-Gault equation, there is no clear advantage of cystatin. In acute GFR decline, one study demonstrated that cystatin increases prior to serum creatinine (209). The presence of cystatin C in the urine of patients with kidney disease indicates that low levels of GFR may affect the ability of the kidney to reabsorb and degrade cystatin C. However, it is not clear if this would interfere with the direct relationship between cystatin C and GFR in patients with kidney disease.

It is clear that there are other determinants of cystatin C apart from GFR. Higher levels of cystatin C are observed from birth to 4 months of age (210) and in the elderly (206). It is thought that the elevation after the age of approximately 70 years is related to the age-associated decline in GFR. However, a population based study in Groningen, The Netherlands, showed that even after adjusting for the level of creatinine clearance, age was significantly related to cystatin level, as was male sex, higher body mass index, and higher C-reactive protein (211). Additional studies demonstrated an effect of lipopolysaccharides, interferon α, cigarette smoke, and transforming growth factor β on cystatin C secretion *in vitro* (206). Cystatin level was increased by dexamethasone in a dose-dependent fashion in HeLa cells in vitro (212) and serum cystatin C levels were 25% higher in children with kidney transplants compared to children with chronic kidney disease who had not been transplanted (213), which suggests a possible effect of corticosteroids. Prior to the potential widespread adoption of serum cystatin C levels for the estimation of GFR, more-extensive research is required.

ALTERNATIVE EXOGNEOUS FILTRATION MARKERS AND CLEARANCE METHODS FOR MEASURING GLOMERULAR FILTRATION RATE

In the last 20 years, many variations in the classical method of estimating GFR from urinary clearance of exogenous filtration markers have been introduced. These include:

1. Substitution of constant intravenous infusion by bolus intravenous or subcutaneous injection;
2. Use of plasma clearance techniques to eliminate the need for urine collection;
3. Introduction of radioisotopic markers to facilitate the assay of plasma and urine levels (^{125}I-iothalamate, ^{51}Cr-ethylene diamine tetracetic acid [EDTA], and its analogue, ^{99m}Tc-diethylene triamine pentacetic acid [DTPA]);

4. Introduction of nonradioactive iodinated radiographic contrast agents that can be measured by X-ray fluorescence and HPLC methods to avoid radiation exposure (iothalamate and iohexol); and
5. Increased availability of external counting and imaging techniques to eliminate the need both for urine collection and plasma sampling (radioisotopes).

These new techniques used alone or in combination provide a multiplicity of methods for the safe, accurate, and simple measure of GFR (60,74).

Exogenous Filtration Markers

There is now a wide variety of exogenous isotopic and nonisotopic filtration markers that are more available and simpler to use than inulin (60,149,214,215). Multiple investigations have studied the characteristics and performance of radiolabeled ^{125}I-iothalamate, metal chelates formed with EDTA and DPTA and nonisotopic iodinated contrast agents (iohexol and iopromide), and have demonstrated a high degree of correlation with simultaneous inulin clearances (216–218,219–226).

^{125}I-iothalamate, widely available in a pure, stable form (half life of ^{125}I, 60 days), is bound to protein to a minor degree (227). A number of early studies demonstrated near identity of simultaneous inulin and labeled iothalamate clearances (221,227–229). Subsequent studies have demonstrated tubular secretion of iothalamate in chickens, rats, and humans (230,231). Therefore, ^{125}I-iothalamate clearance provides a reasonable estimate of GFR (60) and satisfies the criteria for safety and ease of measurement. In a study of individuals with chronic kidney disease using a subcutaneous bolus injection of ^{125}I-iothalamate and spontaneous bladder emptying, the median CV for repeated measurements over 3 months was 6.3% (69). These results compare favorably to the reproducibility of inulin clearance measurements in normal adults described earlier.

There is an extensive European experience with ^{51}Cr-EDTA in humans (149). This marker is not commercially available in the United States. The urinary clearance of ^{51}Cr-EDTA consistently underestimates inulin clearance by 5% to 15% in most, although not all, studies (149). DTPA, an analogue of EDTA labeled with ^{99m}Tc, is available in the United States. The advantages of ^{99m}Tc-DTPA include a short half-life (6 hours) that minimizes radiation exposure, its availability on a daily basis in most nuclear medicine departments, and the convenience of using it to measure GFR at the time of renal imaging studies (214,232). The disadvantages include a small but definite (3% to 9%) binding to protein, which leads to an underestimation in GFR. In addition, at least five different chelating kits and three technetium generators are in use in the United States, making standardization among institutions difficult. In patients with chronic kidney disease, studies have yielded correlation coefficients to other GFR standard measurements of greater than 90% (60,214,232,233). In a detailed, simultaneous comparison of ^{125}I-iothalamate, ^{169}Yb-DTPA, ^{99m}Tc-DTPA, and inulin, the investigators concluded that the urinary clearance of any of these radioisotopic agents can be used to accurately measure GFR in patients with graded degrees of chronic kidney disease (60). In Figure 11-7 a comparison between inulin and some of these markers is displayed.

Iohexol is a nonionic radiographic contrast agent that offers significant advantages over other contrast agents in the accuracy and precision of GFR measurement. It may be used for both plasma clearance and urinary clearance measurements. It exhibits neither protein binding nor tubular secretion, extrarenal clearance is minimal, it is stable in biologic fluids, its adverse reactions are rare, and it does not require radioactive tags (224,226,234,235). GFR levels obtained with the single sample technique are highly correlated with those obtained using the multiple sample technique (236). The plasma clearance of iohexol is highly reproducible. The CV of repeated measurements of GFR using the multiple sample plasma clearance of iohexol is 6% (237). For clinical assessment of GFR, extrarenal elimination of ioxol is probably unimportant but may be relevant for assessment of GFR in clinical trials, particularly in patients with GFR below 40 mL/minute/1.73 m^2.

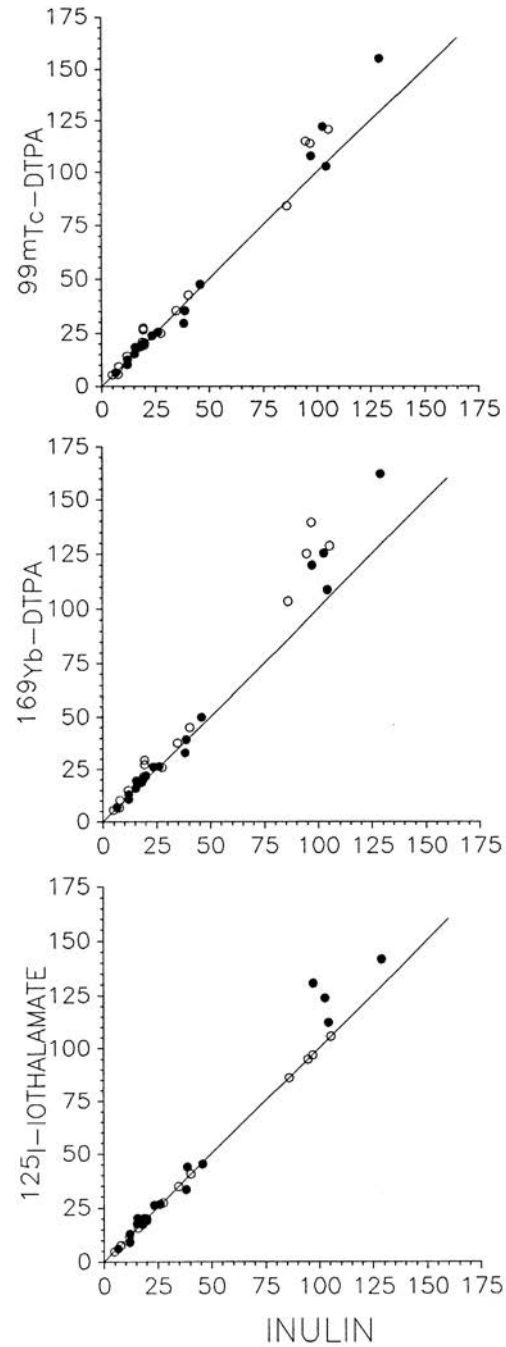

FIGURE 11-7. Relationship between marker clearance and inulin. Values are mean urinary clearance (mL/minute/1.73 m^2) averaged over PRE periods (1 to 3) for day 1 (*open circles*) and day 3 (*solid circles*). The line of identity is indicated. Statistical analysis for subjects with kidney disease were performed on balanced data (n = 13). Results from normal subjects (n = 4) are also shown for comparison.

There are disadvantages of iohexol. High-performance liquid chromotography, requiring a skilled technician and expensive equipment, must be used when low doses of iohexol (e.g., 5 mL of 300 mg/mL iodine) are administered. X-ray fluorescence is simpler to perform but necessitates administration of significantly larger doses of iohexol (10 to 90 mL of 300 mg/mL iodine) (226) and carries an increased risk of adverse reactions. Capillary electrophoresis has been shown to be a promising technique for measurement of radiocontrast media (238). Its advantages include lower cost, increased sensitivity, and more rapid turnaround time. Recently, neutron activation analysis has been validated as a technique to measure plasma iohexol concentrations in the range achieved after administration of 5 mL of 300 mg/mL iodine. The advantages of this technique are a high level of accuracy and reproducibility and the ability to process multiple samples (239).

The magnetic resonance imaging (MRI) contrast agent, gadolinium-DTPA, appears to be comparable to ^{99m}Tc-DTPA for renal clearance determinations (240). Its plasma and urine levels are determined using a nuclear magnetic resonance spectrophotometer.

The decline in serum levels of intravenously administered aminoglycoside antibiotics (gentamycin or tobramycin) or the radiocontrast agents iohexol or iopromide used in a standard plasma clearance calculation yields GFR values very similar to that of simultaneous inulin clearance in critically ill patients (241,242).

Direct Measurement of Kidney Clearance

For direct measurement of urinary clearance, timed urine collections are performed after administration of an exogenous filtration marker. GFR is calculated using the standard formula (Eq. 4). In the modified infusion technique, the marker substance (e.g., inulin, ^{125}I-iothalamate, ^{51}Cr-EDTA) is given subcutaneously, with or without a small dose of aqueous epinephrine, and is released slowly into the circulation, providing fairly constant plasma levels (228,229). An alternative technique using a single IV bolus yields declining plasma levels, which yields reasonably accurate GFR measurements in subjects with reduced GFR, but overestimates GFR in subjects with normal or elevated GFR (60) due to difficulty in determining the exact plasma level corresponding to the time of urine collection. The use of an exponential pattern of decline in serum counts may lead to a better approximation of the relationship between the plasma and urine measurements (69). In some studies, a decline in plasma level is associated with an apparent decline in GFR during the test, which contributes to higher intratest variation (243).

Indirect Measurement of Kidney Clearance

As an alternative to the direct measurement of urinary clearance, GFR can be calculated from plasma clearance (the decline in plasma levels following an intravenous bolus injection of an exogenous filtration marker), using either the entire area under the disappearance curve or one- or two-compartment analysis of the slope of the plasma disappearance plot. Despite the advantage of not requiring urine collections, this method is cumbersome for several reasons. First, if the terminal slope is used (one-compartment analysis), a relatively long time (3 to 5 hours) is required to accurately determine the falling slope of the injected substance; alternatively, multiple samples are required at early time points if the entire plasma disappearance curve is being utilized. Second, filtration markers utilized for this method must meet an additional criterion of rapid equilibration with the extracellular volume. Inulin, the "gold standard" for the urinary clearance technique is not suitable for plasma clearance studies because of markedly delayed equilibration with the extracellular fluid compartment (244). Third, simultaneous assessment of plasma and urinary clearance of a filtration marker typically yields a higher level for plasma clearance, presumably due to extrarenal excretion of the marker (245,246). This underestimation is more apparent at a lower GFR. This systematic bias at low levels of GFR leads to an underestimation of GFR at high levels and to overestimate GFR at low levels (247). Fourth, plasma clearance overestimates GFR in patients with moderate to severe edema probably because of the larger than expected volume of distribution and lower than expected plasma levels of the marker (221).

Measurement of GFR by external counting or imaging over the kidneys and bladder using an exogenous isotopic marker substance is another alternative to urinary clearance (214,248–250). Studies have been done in conjunction with dynamic kidney imaging using ^{99m}Tc-DTPA, comparing the percent kidney (and bladder) uptake at a defined time after injection to simultaneously measured GFR by other techniques. Similar studies may be performed using computed tomographic imaging with nonradioactive radiographic contrast agents or magnetic resonance imaging with gadopentate (251–254). Unfortunately, many of the studies of dynamic renal imaging have not validated it using standard renal clearance measurements and have not assessed their accuracy on external populations. Several studies indicated a poor correlation of ^{99m}Tc-DTPA dynamic renal imaging with simultaneous renal or plasma clearance, especially in the normal and elevated GFR range (249,255). It is premature to recommend external counting or imaging techniques for routine clinical purposes. The main value of dynamic renal imaging would appear to be determination of split kidney function in individuals already undergoing imaging procedures rather than as a primary method of measuring GFR.

CLINICAL ASSESSMENT OF KIDNEY FUNCTION IN CHRONIC KIDNEY DISEASE

Chronic kidney disease (CKD) is a world wide public health problem, with increasing incidence and prevelance, poor outcomes and high cost. Stages in the progression of chronic kidney disease defined by the level of GFR, and therapeutic strategies to improve outcomes are shown in Table 11-5. Chronic kidney disease worsens over time by transitions through these stages, regardless of the specific cause of kidney damage or rate of GFR decline (Chapter 90). In this section, we will review the use of GFR estimates for the detection chronic kidney disease and assessment its progression.

Detection of Chronic Kidney Disease

Clinical practice guidelines of the National Kidney Foundation's Kidney Disease Quality Outcome Initiative (NKF-K/DQOI) define CKD as either GFR less than 60 mL/min per 1.73 m^2 or the presence of kidney damage for 3 or more months, regardless of cause (256). These guidelines, as well as new recommendations from national and international organizations, propose that clinicians use GFR estimated from serum creatinine and estimating equations for routine clinical assessment of kidney function, and that clinical laboratories report estimated GFR in addition to serum creatinine (256–260). In response, intensive educational efforts regarding GFR estimates and chronic kidney disease, as well as active research on improved GFR estimating equations have begun and will continue over the next several years.

TABLE 11-5

DESCRIPTION OF CKD STAGES DEFINED BY GFR LEVEL AND THERAPEUTIC STRATEGIES FOR EACH STAGE

Stage of CKD	Description	GFR ml/min per 1.73 m^2	Therapeutic strategies to improve outcomes
	At ↑increased risk		Detection of CKD. GFR <60 ml/min/1.73 m^2 for ≥3 months defines CKD.
1	Kidney damage with normal or ↑ GFR	>90	Initiate treatment and monitor response to treatment
2	Kidney damage with mild ↓ GFR	60–89	Quantify progression
3	Moderate ↓ GFR	30–59	Selection of appropriate treatment strategies; measure response to treatment; association of comorbid conditions; drug dosing
4	Severe ↓ GFR	15–29	Referral to nephrologist
5	Kidney failure	<15	Interpretation of symptoms, initiate kidney replacement therapy

As discussed earlier, the main limitation of current estimating equations is decreased accuracy at GFR levels >60 mL/minute/1.73 m^2. This limitation was recognized and purposefully incorporated into current guidelines and recommendations. According to the NKF-K/DOQI definition, individuals with a persistent reduction in GFR <60 mL/minute/1.73 m^2 are defined as having CKD. In individuals with GFR ≥60 mL/minute/1.73 m^2, CKD can also be ascertained by assessment of markers of kidney damage, such as proteinuria. Individuals with GFR ≥60 mL/minute/1.73 m^2 without markers of kidney damage do not have chronic kidney disease; precise estimation of GFR is not necessary in these individuals in most clinical circumstances. Consistent with this, the National Kidney Disease Education Plan recommends reporting a specific value only when the estimated GFR is less than 60 mL/minute/1.73 m^2 and otherwise simply report "GFR is ≥60 mL/minute/1.73 m^2." All methods of GFR estimation may lead to misclassification of individuals near the cut-off level for GFR of 60 mL/minute/1.73 m^2. Although we do not know the magnitude of this misclassification with current estimating equations, it is highly likely that use of serum creatinine alone leads to an even larger misclassification and to missed opportunities for early identification and treatment of individuals with CKD.

There may be specific clinical circumstances where accurate measurements of kidney function are required for individuals with higher levels of GFR, such as drug dosing of toxic medications or those with narrow therapeutic windows. In those circumstances, clearance methods should be used to estimate the level of GFR rather than estimating equations (Table 11–3).

Estimating the Rate of Progression of Chronic Kidney Disease

Clinical Trials

Clinical trials are needed to determine therapeutic strategies, such as dietary protein restriction or antihypertensive therapy, to slow the progression of chronic kidney disease. These studies often use serial measurements of serum creatinine to assess the rate of progression, and an active debate has arisen regarding the usefulness of changes in the serum creatinine concentration to assess the rate of decline in GFR. Based on the physiologic considerations discussed above, the rate of decline in estimated GFR (or reciprocal serum creatinine) may differ from the rate of decline in GFR due to changes in creatinine secretion, generation or extrarenal elimination (74,261). Over short intervals of follow-up, the correlation in rates of decline in GFR with rates of decline in reciprocal serum creatinine is relatively weak (44,262,263). In part, the poor correlation may reflect the effects of the therapeutic interventions on determinants of serum creatinine other than GFR, such as the effect of dietary protein restriction on creatinine generation, and may lead to errors in interpretation of the clinical trial (44). However, there are a number of other reasons for the poor correlations. These include the relatively slow rate of progression of kidney disease (3 to 5 mL/minute/year or less in many studies), the short duration of follow-up (<2 year), and deviations from linearity in the decline in GFR and reciprocal serum creatinine (264). Thus, in addition to considering the physiologic relationships among these variables, a number of statistical considerations must be included in the choice of an outcome measure for a clinical trial (265).

Deficiencies of using GFR measurements to assess the rate of progression of kidney disease have also been recognized. First, a decline in GFR may not be a sensitive marker of progressive kidney damage. For example, the earliest stages of glomerular disease appear to be associated with alterations in structure and in permeability to macromolecules. In diabetic kidney disease, increased albumin excretion signals the presence of structural damage before a decline in GFR (266). Similarly, in systemic lupus erythematosus, progression of kidney damage may not be associated with a change in GFR (267). Longer follow-up is required to detect a decline in GFR. Second, a decline in GFR may not be a specific marker of progression of kidney disease. For example, both dietary protein restriction and antihypertensive therapy have short-term effects on GFR which differ from their hypothesized long-term effects on the progression of kidney disease (45,268). Although the magnitude of the change in GFR due to these short-term effects is small, during a short follow-up interval, it may be large in relation to the decline in GFR due to progression of kidney disease.

Thus, it appears that studies of therapies to slow the progression of most chronic kidney diseases may require a long duration of follow-up to assess the effects on either GFR or serum creatinine. In principle, if changes in kidney function during follow-up were large, the rate of change in serum creatinine would indeed reflect the rate of decline in GFR. Several recent clinical trials have shown consistent results using both outcomes measures: a twofold increase "doubling" of baseline serum creatinine and the rate of decline in GFR (269–272) Nonetheless, the cost of such trials is great and only a limited number of them can be performed. For future studies, it would be useful to develop other outcome measures that are more sensitive to the effects of the progression of kidney disease.

Clinical Practice

In clinical practice, measurement of progression is important to identify patients at increased risk for the development of kidney failure (CKD Stage 5, GFR <15 mL/minute/1.73 m^2) and need for kidney replacement therapy, but also for identification of the earlier stages of CKD with their associated with increased risk of complications and consequent need for specific evaluation and management. In particular, identification of CKD Stage 4 (GFR 15 to 29 mL/minute/1.73 m^2) has clinical significance as it is more common than kidney failure, is associated with complications that may result in mortality, morbidity, and decreased quality of life, as well as a significant increase in health care delivery and expenditure. In addition, interventions during this stage, such as creation of vascular access, preparation for dialysis, and evaluation for kidney transplantation, may reduce subsequent morbidity and mortality associated with kidney failure.

Measurement of GFR decline can be imprecise due to variability in GFR or errors in estimation of GFR, especially in the higher range of GFR and for patients with slowly declining kidney function. Use of a longer interval of follow-up can overcome the imprecision. The pattern of GFR decline over time is not well defined. Over short periods of time, GFR decline appears relatively constant. However, the true pattern of GFR decline may not be constant. In one study of 77 patients, 32% to 51% of patients had a spontaneous change in the rate of decline (273). In this study, in 61% of cases, the change in slope was less steep and in 39%, the slope was steeper. Potential explanations for the changes in the rate of decline may be errors in measurement, true nonconstant rate of decline in GFR, effects of treatment, or new or superimposed acute kidney disease.

Progression of kidney disease can also be signified by changes in the markers of kidney damage, such as proteinuria (see the section that follows), size of the kidneys in a polycyststic kidney disease, or presence of complications of kidney disease, such as hyperphosphatemia or anemia. Therefore, progression of chronic kidney disease should be assessed by consideration of all relevant indicators of kidney damage and function for an individual patient.

PROTEINURIA

The plasma contains approximately 11,000 to 14,000 g of protein, yet the final urine is virtually protein-free due to selectivity of glomerular filtration. This conservation of essential proteins is necessary for oncotic regulation, for immune protection, for normal coagulation, and for a host of other vital processes.

An increased protein excretion rate (proteinuria) is usually due to intrinsic kidney disease. Most kidney diseases are associated with some degree of proteinuria. Mild proteinuria does not generally cause clinical signs or symptoms, but when severe (>3 g/day) consequences such as hypoalbuminemia, hypercholesterolemia, hypercoaguability, and hemodynamic instability may occur. The detection and evaluation of proteinuria has gained additional significance in recent years as multiple studies have demonstrated its prognostic importance. In most kidney disease, the degree of proteinuira is a risk factor for kidney disease progression. The presence of even mildly increased amounts of albumin in the urine serves as an independent risk marker for cardiovascular disease, particularly in patients with diabetes, hypertension, or of advancing age. The physiology of normal protein handling and the pathophysiology of proteinuria are extensively covered in Chapter 90.

This section considers (a) mechanisms by which the normal kidney handles protein, (b) patterns of proteinuria, (c) methods to measure urine protein, and (d) clinical interpretation of proteinuria.

NORMAL PROTEIN HANDLING

In normal individuals, the daily urinary protein excretion averages 40 to 80 mg, and the upper limit of normal ranges from 75 to 150 mg. Urine protein is a mixture of plasma proteins that cross the filtration barrier and nonplasma proteins that originate in the tubules and lower urinary tract. Of the total, albumin constitutes 30% to 40%, IgG 5% to 10%, light chains 5%, and IgA 3%. Tamm-Horsfall protein (THP), a glycoprotein not found in plasma (274,275) is the most abundant protein in normal human urine and constitutes the remainder (276). Large molecules, such as IgD and IgM, normally are not detected in the urine (274,277).

The handling of plasma proteins by the kidney is complex, but consists of two major components; the permeability of the glomerular filter to plasma proteins and the tubular metabolism of filtered proteins. For a detailed review of these mechanisms, the reader is referred to Chapter 90.

Urine Proteins of Plasma Origin

Low Molecular Weight Proteins

Low molecular weight proteins (less than 25,000 daltons or less than 2.3 nm) are extensively filtered by the glomeruli, taken up by the tubules, and subsequently handled by proximal tubular degradation (278). Biologically important low molecular weight proteins handled by the kidney include enzymes (lysozyme and ribonuclease), immunoglobulins (light chains and beta-2 microglobulin), fibrin-fibrinogen degradation products, and hormones (insulin, growth hormone and parathyroid hormone). The tubular concentration of these proteins ranges from 50% to 90% of their plasma concentrations (Table 11-6). Low molecular weight proteins are small enough that their charge plays only a minor role in their filtration.

Despite the significant amount of low molecular weight protein that is filtered, only minor amounts appear in the urine. The proteins are taken up in the proximal tubule and hydrolyzed into amino acids by the vacuolar-lysozymal system. Small amounts of these proteins are actually reabsorbed intact (279–281). The tubular capacity for some of these low molecular weight proteins is significantly greater than the filtered load. For example, when purified lysozyme was given in an isolated perfused rat kidney, lysozyme did not appear in the urine until the filtered load was increased nearly threefold (279). Through the process of filtration, tubular absorption and excretion, the kidney accounts for between 30% and 80% of the metabolic clearance of low molecular weight proteins (279).

Light chains are handled in a similar manner. The monomer (molecular weight 22,000 daltons) is filtered freely and then degraded by the tubules with small amounts appearing in the urine. In contrast, the dimer (molecular weight 44,000 daltons, radius 2.8 nm) is restricted with only approximately 10% filtered. Horseradish peroxidase, a neutral tracer molecule of similar weight and size to light chains, is handled in a similar manner (282,283).

Albumin

Albumin, the principal plasma protein with a molecular weight of 69,000 and radius of 3.6 nm, deserves special consideration. Under normal situations, albumin is prevented from entering the urine space by the glomerular permselectivity barrier. However, as discussed earlier, under conditions of reduced glomerular plasma flow, albumin passes into the urine, demonstrating that size selectivity alone is not sufficient to restrict the filtration of albumin. Rather, it appears that the negative charge on the various structures of the glomerular barrier contributes

TABLE 11-6

HANDLING OF PLASMA PROTEINS BY THE KIDNEY

Protein	Molecular weight (daltons)	Approximate Stokes-Einstein radius (nm)	Approximate ratio of glomerular filtrate to plasma concentrations
Inulin (for reference)	5,200	1.4	1.0
Insulin	6,000	1.6	0.9
Lysozyme	14,600	1.9	0.75
Myoglobulin	16,900	1.9	0.75
Parathyroid hormone (cow)	9,000	2.1	0.65
Growth hormone (rat)	20,000	2.1	0.6–0.7
Light chains	44,000	2.8	0.09[a]
Amylase	48,000	2.9	0.02
Albumin	69,000	3.6	0.02
Gamma globulin	160,000	5.5	0.0
Ferritin	480,000	6.1	0.02

[a]Can be as high as 0.45 if light-chain monomers predominate over dimers in plasma.
(From: Kanwar YS. Biology of disease: biophysiology of glomerular filtration and proteinuria. *Lab Invest* 1984;51:7. Maack T, Johnson B, Kau ST, et al. Renal filtration, transport, and metabolism of low-molecular-weight proteins: a review. *Kidney Int* 1979;16:251.)

significantly to restricting the filtration of albumin. Of these structures, the negative charge on the basement membrane has been considered the major obstacle to albumin crossing the glomerular capillary wall (283–289).

Large Plasma Proteins

The large molecular weight plasma proteins are restrained from crossing the glomerular barrier. Proteins such as globulins, (mol. wt. 160,000, radius, 5.5 nm), are undoubtedly restricted by the basement membrane, but the contribution of the endothelial fenestrae is uncertain. Albeit, a tiny fractional clearance of large plasma weight proteins has been established and animal studies suggest that this is due to the presence of very few large pores in the glomerular ultrafiltration barrier (290). Changes in glomerular plasma flow do not alter the restriction of these molecules from the urine space.

Proteins in Urine of Nonplasma Origin

The major protein in normal human urine that has no counterpart in plasma is uromodulin, or THP (276,291,292). This substance, a large glycoprotein with a molecular weight of 7 million, is excreted in amounts of 20 to 100 mg per day (293,294). Immunofluorescent staining techniques in human kidneys have demonstrated that THP is confined to the cells lining the thick ascending limb of Henle's loop and the most proximal part of the distal convoluted tubule, which strongly suggests that these cells are the source of the THP in the urine (295,296). THP is the major protein component of urinary casts (295,297). Excretion of this protein increases only slightly in patients with nephrotic syndrome, and its excretion rate does not appear to be related quantitatively either to the number of casts or to the degree of proteinuria (297). *In vitro* studies indicate that the addition of albumin to THP-containing solutions leads to precipitation of THP (297), which suggests that increased albumin excretion may lead to precipitation of THP in the tubules and thus to cast formation. The structure and function of this unusual glycoprotein has been extensively reviewed (276). In addition to THP, many other discrete proteins unrelated to plasma proteins have been identified in trace amounts (291). These proteins presumably originate in the lower urinary tract and prostate gland. Endothelin, the recently discovered endogenous vasoconstrictor peptide, is produced by renal epithelial cell lines *in vitro*, does appear in human urine, and may serve as a nonspecific marker of renal injury (298).

PATTERNS OF PROTEINURIA

Proteinuria can be classified according to its pathophysiology into three major groups: glomerular proteinuria, tubular proteinuria, and overproduction proteinuria.

Glomerular Protein

Glomerular proteinuria is defined as proteinuria due to increased permselectivity of the glomerular filtration barrier to plasma proteins. Therefore, the hallmark of glomerular proteinuria is albuminuria (Fig. 11-8). Albuminuria may be a transient phenomenon in normal individuals without kidney disease. However, persistent albuminuria indicates the presence of kidney disease. Indeed, albuminuria occurs in the great majority of kidney diseases. Whether the primary site of injury is the glomerulus or the tubulointerstitial compartment, albumin makes up 60% to 90% of the urinary protein (Fig. 11-8). The excretion of low-molecular-weight proteins usually remains minimal (299,300).

Functional Albuminuria

A transient increase in albumin excretion occurs in a variety of physiologic and experimental settings in the absence of kidney disease. Protein excretion is increased twofold to threefold during and immediately following heavy exercise (301–303), and the increase is accounted for largely by plasma protein components (275,304,305). Minor abnormalities can accompany the proteinuria in the urine sediment, but both the proteinuria and the sediment abnormalities usually disappear within hours after the completion of exercise. Similar increases in protein excretion can be induced by fever (306), severe emotional stress (307), infusions of norepinephrine or angiotensin (308,309), and prolonged assumption of the lordotic position (310). In addition, mild to moderate proteinuria often is observed in patients with congestive heart failure (311).

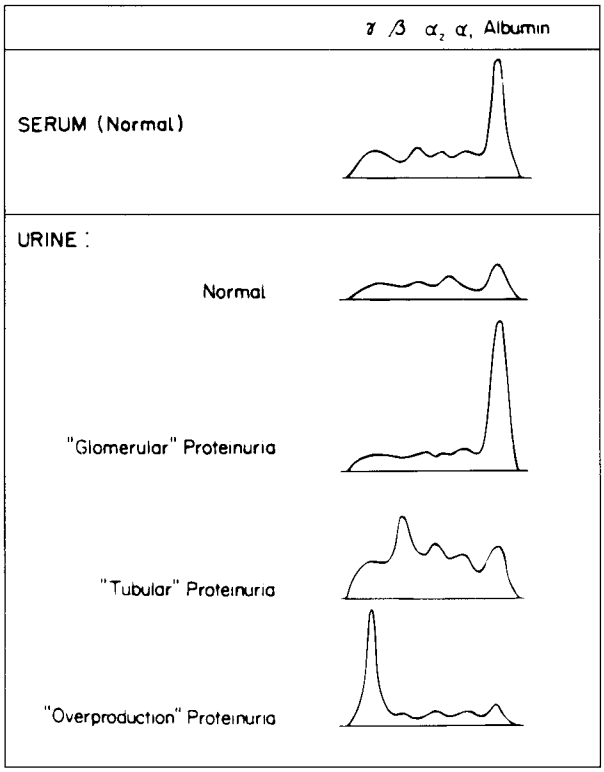

FIGURE 11-8. Electrophoretic patterns of normal serum, normal urine, and urine with three types of abnormal protein excretion. The electrophoresis pattern shown for overproduction proteinuria is taken from a patient with multiple myeloma.

Albuminuria Due to Kidney Disease

Changes in filtered albumin sufficient to account for heavy proteinuria have been documented by micropuncture studies in experimental nephrotic syndrome and by indirect studies in humans. In rats with aminonucleoside nephrosis, the increase in filtered albumin accounts entirely for the increase in protein excretion (312,313). In fact, in animals with either aminonucleoside nephritis or nephrotoxic nephritis, the proximal tubular albumin concentration is increased 8- to 12-fold (313,314). These animals may excrete 100 to 400 times as much albumin as controls (313,314). In humans, clearance studies have provided indirect evidence of increased filtration of protein; in patients with nephrotic syndrome, the minimal protein concentration in the glomerular filtrate (calculated by correcting the urine protein concentration by the fraction of water reabsorbed) far exceeds the concentration of filtered protein observed in the proximal tubular fluid in normal animals (315). Furthermore, urine albumin excretion is linearly related to plasma albumin concentration when the latter is increased by infusion (315,316). Such a relationship is characteristic of substances excreted mainly by glomerular filtration (9).

Although increased filtration of protein appears to be the major factor leading to proteinuria, the specific defects in the capillary wall that are responsible for the protein loss are not completely defined. New information regarding the major role of the podocyte and its slit diaphragm in many proteinuric diseases has become available from multiple studies (317,318). For a detailed discussion regarding the pathophysiology of glomerular proteinuria, the reader is directed to Chapter 2.

Tubular Protein

A pattern of abnormal protein excretion in which low-molecular-weight proteins predominate is found in patients with a diverse group of kidney diseases characterized by primary tubular injury. This includes hereditary tubular disorders, such as Fanconi's syndrome and Wilson's disease (319), chronic potassium depletion, acute renal failure due to acute tubular necrosis, Balkan nephropathy (320), and cadmium poisoning (321,322). The low-molecular-weight proteins excreted by these patients are the plasma constituents described earlier that are present in only minute amounts in the urine of normal individuals (323). As many as 20 of these proteins have been identified. A typical pattern seen in these patients is shown in Figure 11-8. The magnitude of tubular proteinuria exceeds 150 mg per day and rarely is greater than 2 gm per day (324–326).

As described previously, the urinary clearance rates of these low-molecular-weight proteins in normal individuals and in patients with glomerular disease are very low despite the fact that these proteins are filtered readily, suggesting that when the tubules are intact, extensive tubular reabsorption and degradation of these substances occurs (280,319). By contrast, in patients with primary tubular diseases, the clearance rates of these proteins are markedly increased. In fact, in these patients, the clearance rate correlates closely with the predicted filtration rates of these proteins (estimated from molecular size) if the assumption is made that no tubular uptake occurs (319). On the basis of these observations, it appears that tubular proteinuria is due to impaired tubular reabsorption of low-molecular-weight proteins rather than to increased glomerular permeability (300,319).

Among the low-molecular-weight proteins excreted in excess in tubular and interstitial diseases are N-acetyl-beta-D-glucosaminidase (NAG), beta-2 microglobulin (B2M), neutral endopeptidase, and lysozyme (muramidase), an enzyme with a molecular weight of 14,600. The finding of increased amounts of these proteins has received attention as a diagnostic aid in identifying tubular and interstitial disease (327–330). The cause for increased excretion of these low molecular weight proteins is thought to be ineffective reabsortion and catabolism by the injured, dysfunctional tubules (331). Lysozyme excretion is increased in patients with tubular damage secondary to infection, transplant rejection, nephrotoxic agents, and Fanconi's syndrome. Unfortunately, the diagnostic utility of this determination is limited because many patients with interstitial and tubular disease do not have lysozymuria and because increased excretion of lysozyme occurs in some patients with glomerular diseases (328). The largest increase in lysozyme excretion occurs in patients with leukemia, presumably secondary to increased production of this protein (see the Overproduction Proteinuria section within this chapter).

The interpretation of increased excretion of light chains poses a problem similar to that encountered in patients with lysozymuria, namely, to distinguish between increased excretion secondary to tubular disease on the one hand or to overproduction of the protein on the other. In the case of light chains, a slight increase in excretion and a finding of a mixture of both kappa and lambda fragments points to a primary tubular defect, whereas high levels of excretion (greater than 500 mg/day) and the presence of only a single type of light chain points to accelerated synthesis (332,333).

Overproduction of Proteinuria

When the plasma concentration of a filterable protein is increased beyond the capacity of the tubules to reabsorb it, it then appears in the urine. Enhanced excretion of light chains, heavy

chains, and other fragments of immunoglobulins occurs predominantly in the monoclonal gammopathies, including multiple myeloma, macroglobulinemia, heavy-chain disease, and idiopathic light-chain proteinuria (Fig. 11-8). Overproduction with increased filtration rather than a primary tubular defect appears to account for the increased excretion of these substances. Both light-chain and heavy-chain fragments of immunoglobulins are excreted in minute amounts in the urine of normal individuals (307,334). Normally, only 3 mg or so of light chains are excreted daily and the ratio of kappa to lambda light chains is approximately 3 to 1 (335–337). Approximately 25% are present as monomers and the remainder as dimers, even though light chains are normally synthesized as monomers (335,338,339).

Because light chains are small in size and a substantial amount is filtered, it follows that an increase in their delivery into the glomerular filtrate will result in increased excretion unless tubular reabsorption is concomitantly increased. In fact, the clearance of light chains in patients with multiple myeloma is quite high and is inversely related to molecular size, a finding consistent with the view that tubular reabsorption is readily saturated (340). In these patients, light chains have clearances ranging from one-tenth to one-half that of creatinine, depending on the size of the specific protein destined for excretion (Table 11-6). Light-chain proteinuria is most often found in patients with multiple myeloma, and in this disease, some patients have a daily excretion greater than 15 gm. Although a mild increase in albumin excretion is common in patients with monoclonal gammopathies, the excretion of light chains usually predominates, unless renal amyloidosis (or light-chain deposition disease) supervenes. Increased excretion of lysozyme in acute leukemia (341), amylase in pancreatitis, myoglobin in muscle injury, and hemoglobin following hemolysis are other examples of overproduction proteinuria.

Overproduction proteinuria can have important clinical consequences. Patients with light-chain proteinuria can develop acute or chronic kidney failure, and others have the Fanconi syndrome (342,343), distal renal tubular acidosis (333), nephrogenic diabetes insipidus, or various combinations of these disorders (332,333). The association between light-chain proteinuria and tubular nephropathies has led to the speculation that light chains are toxic to renal tubular cells (333,342, 343). Because some patients with increased excretion of light chains have no abnormalities of tubular function, however, the factors producing tubular dysfunction remain to be defined (332). Similarly, the lysozymuria associated with leukemia has been implicated as a cause of renal potassium wasting seen in some patients (344). However, in view of studies demonstrating potassium wasting in some leukemic patients in the absence of lysozymuria, this thesis must be considered unproved (345,346). It should be noted that lysozymuria also occurs in experimental glomerulonephritis and that its excretion is in direct proportion to the magnitude of the albuminuria (347).

MEASUREMENT OF URINE PROTEIN

Urine protein excretion is routinely measured to identify, evaluate, and manage kidney disease. A variety of methods are available to quantify total urine protein, regardless of the type of protein (Table 11-7) (348). The simplest and most widely used methods are semiquantitative tests done on random urine samples. Although these tests are extremely useful in screening for proteinuria, they detect an abnormal concentration of total urine protein, not an abnormal excretion rate. Therefore, they might be positive in patients with low urine volume even if the excretion rate is normal, and they may be negative in patients with high urine volume even if the excretion rate is elevated. For more definitive evaluation and management of patients with proteinuria, quantitative protein analysis must be undertaken. A number of different methods are available (349). Semiautomated, two-dimensional, electrophoretic systems, which employ ultrathin gels, combined with silver staining, allow the detection of a host of specific urinary proteins on a routine basis (350,351). These techniques also improve the characterization of urinary proteins with molecular weights less than 70,000 (352). Specific immunoassays have become available for detection of individual proteins within the urine, as described subsequently for albumin. Additionally, broad descriptions of patterns of urinary protein excretion has become possible by proteomic techniques (353).

Semiquantitative Tests for Total Urine Protein

Semiquantitative tests for urinary protein involve either precipitation of protein or protein-induced color changes of an

TABLE 11-7

DEFINITIONS OF PROTEINURIA AND ALBUMINURIA

	Urine collection method	Normal	Microalbuminuria	Albuminuria or clinical proteinuria
Total Protein	24-Hour Excretion (varies with method)	<300 mg/day	NA	>300 mg/day
	Spot Urine Dipstick	<30 mg/dL	NA	>30 mg/dL
	Spot Urine Protein-to-Creatinine Ratio (varies with method)	<200 mg/g	NA	>200 mg/g
Albumin	24-Hour Excretion	<30 mg/day	30–300 mg/day	>300 mg/day
	Spot Urine Albumin-Specific Dipstick	<3 mg/dL	>3 mg/dL	NA
	Spot Urine Albumin-to-Creatinine Ratio (varies by gender[a])	<30 mg/g <17 mg/g (men) <25 mg/g (women)	30–300 mg/g 17–250 mg/g (men) 25–355 mg/g (women)	>300 mg/g 250 mg/g (men) >355 mg/g (women)

[a] Gender-specific cut-off values are from a single study (392). Use of the same cut-off value for men and women leads to higher values of prevalence for women than men. Current recommendations from the American Diabetes Association define cut-off values for spot urine albumin-to-creatinine ratio for microalbuminuria and albuminuria as 30 and 300 mg/g, respectively, without regard to gender (393).

indicator dye on a dipstick. The precipitation tests may be performed by adding either 5% sulfosalicylic acid or concentrated nitric acid to an aliquot of urine or by heating the urine and adding glacial acetic acid (348). With these methods, the quantity of precipitate is graded from 0 (no precipitate) to 4+ (heavy gelatinous precipitate). If excessive urine turbidity interferes with grading of the precipitate, the urine should be filtered prior to testing. For the heat and acetic acid test, 10 to 15 mL of urine is placed in a test tube and the upper half of the sample is heated to the boiling point. Several drops of acetic acid are then added along the side of the tube, which causes coagulation of the protein in the upper half of the tube. The resultant flocculation is compared with the clear urine in the lower half of the tube (354). Urine samples with a protein concentration as low as 5 to 10 mg/dL will give a positive reaction with the acetic acid precipitation test, but radioopaque contrast materials, tolbutamide, or large amounts of penicillin, nafcillin, or oxacillin may produce a false-positive reaction (348,354,355).

The dipstick test for protein utilizes a paper strip impregnated with a pH indicator dye (tetrabromphenol blue) buffered to maintain the pH in the paper at 3.0. The test is based on the capacity of proteins to change the color of tetrabromphenol (348). The degree of color change is roughly proportional to the amount of protein present, with the color varying from yellow, with low protein concentrations, to blue, with high protein concentrations. The dipstick tests now in common use can detect protein concentrations as low as 6 to 15 mg/dL (356). A color comparison chart is provided with the dipstick that contains a scale of protein concentrations as well as a 0 to 3 or 4+ rating. It should be noted that the correlation between color change and actual protein concentration is only approximate. In one study, for example, comparison with quantitative methods indicated agreement only 60% to 70% of the time (357). The use of the dipstick test is further restricted by the finding of substantial interobserver variation between technicians in interpretation of the results (356,358). Additionally, different brands of dipsticks may have different performance characteristics.

The dipstick method has the advantage that it is not affected by urine turbidity, radio-opaque material, or drugs (348,355). It can give a false-positive value in highly buffered alkaline urine, but such samples are encountered rarely. The major fault of the dipstick test is its insensitivity. The dipstick test detects protein concentrations of 30 mg/dL and above with certainty. Below this level, the test is negative or trace positive in over half the samples tested (357,359). In a patient excreting 300 mg of protein per day in a total volume of 1,500 mL, the protein concentration is only 20 mg/dL, and this concentration may not be detected using the dipstick method. Also, the test is insensitive to light chains and can give a negative reaction even when the excretion of this protein is moderately high (360–362). For these reasons, the physician utilizing the convenient dipstick method must realize that it is less sensitive than the less convenient sulfosalicylic acid or heat and acetic acid methods in detecting low levels of proteinuria. In selected populations, dipstick screening for proteinuria carries a high risk for false positive and negative results with a sensitivity of less than 67% and specificity of 74% (363).

Quantitative Tests for Total Urine Protein

The major limitation to tests for total urine protein is the absence of a criterion standard (gold standard) due to the presence of many different types of protein in the urine in health and disease. Thus, it is not possible to standardize measurements across laboratories or to determine precise cut-off values for the definition of normal or various diseases. Despite this limitation, a large number of tests are available.

Excretion in Timed Collection

Quantitative methods for measuring protein excretion are all based on precipitation of protein, usually accomplished using trichloracetic acid or sulfosalicylic acid. To quantify the amount of protein, sulfosalicylic acid is added to an aliquot of urine, and the turbidity, measured with a photometer or nephelometer, is compared to standards prepared by the addition of known amounts of protein to urine. The sulfosalicylic acid method is only roughly quantitative, however, because it has a CV as large as 20% (348,364). This method is more sensitive to albumin than to globulins (by a factor of 2.4), such that the result is affected by the proportion of albumin in the sample. Light chains (Bence Jones protein), however, are precipitated by this method. The relative insensitivity to globulins can be overcome if trichloroacetic acid is used either in place of the sulfosalicylic acid or in combination with it (364). Other methods of protein precipitation include the use of phosphotungstic acid or Esbach's picric acid-citric acid reagent. Iodinated contrast material can falsely elevate the protein concentration regardless of method, and it is best to wait 24 hours after contrast to determine protein excretion rates (365). With these methods, the volume of precipitate formed is measured in calibrated test tubes. With all of these tests, the protein concentration is multiplied by the total volume of the sample and result reported in milligrams or grams per unit of time (usually 24 hours).

Excretion in an Untimed Collection ("Spot Urine Sample")

Twenty-four-hour protein excretion can be approximated by measurement of both protein and creatinine in a random urine specimen. Because the excretion of both creatinine and protein is fairly stable throughout the day, if the daily creatinine excretion is known, the ratio of the concentrations of protein and creatinine in a random urine specimen provides an estimate of the daily protein excretion (366–368) (Fig. 11–9).

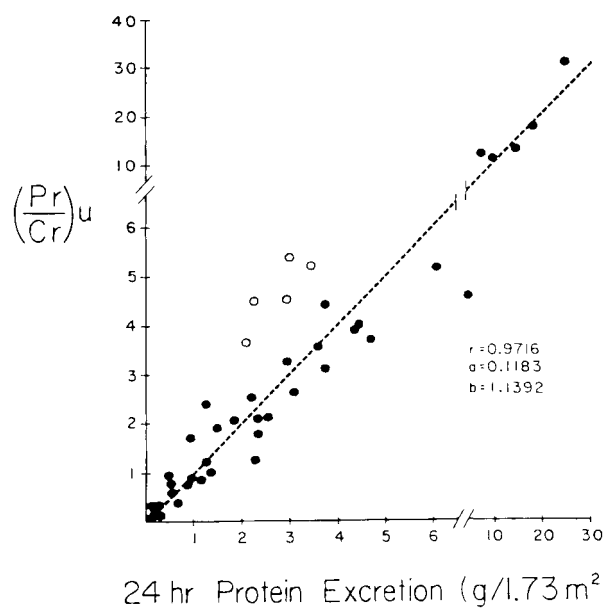

FIGURE 11-9. Ratio of urinary protein to creatinine concentration (Pr/Cr) u of random single voided urine samples expressed as a function of protein excretion per 24 hours per 1.73 m^2. (From: Ginsberg JM, Chang BS, Matarese RA, et al. Use of single voided urine samples to estimate quantitative proteinuria. *N Engl J Med* 1983;309:1543, with permission.)

The correlation between the protein to creatinine ratio and the daily protein excretion is best when the urine sample is taken during the day and with the patient under normal activity, and to be most accurate, the values have to be corrected for age, sex, and body weight. Given the slight, but definite circadian rhythm of proteinuria, it is best to obtain serial samples in an individual patient at the same time of day (369). The validity of the urine protein-to-creatinine method, first proposed in the early 1980s, has now been well supported by several studies (248,370–372), including studies in patients with rheumatic diseases (373), childhood nephrotic syndrome (374,375), routine pregnancy or preeclampsia (376–378), diabetes, and renal transplants (248,370). The results are easy to interpret because the daily creatinine excretion is approximately 1.0 g and the protein-to-creatinine ratio in a random urine specimen is approximately similar to the daily protein excretion in grams (366). It had been demonstrated that the protein-osmolality ratio in a random urine sample may reliably predict protein excretion rates. This ratio was successful in screening for abnormal proteinuria in normal and proteinuric populations with a sensitivity of 96% and a specificity of 93%, superior to routine dipstick performance and equal to the protein-creatinine ratio (379). Validation studies have not been performed. Recently, dipsticks have become available as a method to measure the urine protein-to-creatinine ratio with initial results suggesting excellent correlation with standard measures (380). Tests utilizing the protein-to-creatinine ratio require the patient to be in steady state of creatinine balance and are not valid in acute renal failure. Clinical practice guidelines by NKF-K/DOQI recommend measurement of total urine protein in adults using spot urine protein-to-creatinine ratios and expressing the results as total protein in milligrams per creatinine in gram. The normal value varies with the laboratory, but is approximately <200 mg/g (Table 11-7).

Tests for Specific Proteins

Albumin

The persistent excretion of small amounts of albumin is termed *microalbuminuria*. The term has been defined variably, but usually refers to urinary albumin excretion rates above the normal values of 5 to 20 mg/day, but less than that detected by tests for total urine protein (i.e., 200–300 mg/day). Specific radioimmunoassay is the gold standard for detecting and quantitating albumin concentrations, although turbidometric assays can be used with similar precision (381). HPLC techniques may allow for even more accurate and early detection of abnormal albumin excretion rates (382). Many different screening tests are also available as qualitative screens with varying ability to detect microalbuminuria (383–385). Given the wide variability in urinary albumin excretion in diabetic patients, several urine samples must be tested to reliably estimate the degree of microalbuminuria (386). Due to this variability, timed collections provide a better estimate of albumin excretion than albumin to creatinine ratios. Nonetheless, spot samples for albumin concentration or for albumin-to-creatinine ratio have been advocated in screening and following diabetic patients and other high-risk populations such as patients with hypertension, systemic lupus erythematosis, pregnancy, or malignancy (356,387–391). NKF-K/DOQI and NIDDK NKDEP recommend spot urine albumin-to-creatinine ratio for detection and following chronic kidney disease in adults (256,258,392,393) (Table 11-8). As with the total protein-to-creatinine ratio, dipsticks are also available to measure the albumin-to-creatinine ratio (394) but validation studies are mixed in terms of their sensitivity and specificity (395). Performance of these dipsticks could be improved by adjustment for specific gravity (396). Presently, they may be useful for screening high-risk populations with quantitation better served by laboratory testing.

TABLE 11-8

IMPORTANCE OF PROTEINURIA IN CKD (453)

Interpretation	Explanation
Marker of kidney damage	Spot urine albumin-to-creatinine ratio >30 mg/g or spot urine total protein-to-creatinine ratio >200 mg/g for ≥3 months defines CKD
Clue to the type (diagnosis) of CKD	Spot urine total protein-to-creatinine ratio >500–1000 mg/g suggests diabetic kidney disease, glomerular diseases, or transplant glomerulopathy.
Risk factor for adverse outcomes	Higher proteinuria predicts faster progression of kidney disease and increased risk of CVD.
Effect modifier for interventions	Strict blood pressure control and ACE inhibitors are more effective in slowing kidney disease progression in patients with higher baseline proteinuria.
Hypothesized surrogate outcomes and target for interventions	If validated, then lowering proteinuria would be a goal of therapy.

Light Chains

Identification of immune globulin fragments in the urine is useful in establishing the diagnosis of multiple myeloma and other monoclonal gammopathies. Light chains are often sought by the traditional Bence Jones test, a method that depends on the unusual solubility characteristics of these proteins. When the urine is heated to 45°C to 55°C, light chains precipitate, particularly when the pH is brought to 4.9 by the addition of an acetate buffer (397). When the urine is then brought to a boil, the precipitated light chains redissolve partially or completely. This test is difficult to carry out properly and is also rather insensitive. It is positive only when the concentration of light chains exceeds 800 to 1,600 mg per L (398,399), and even in the presence of such concentrations, it may still be falsely negative (362,398,400). As noted earlier, the semiquantitative dipstick test also may be negative when light chains are present in the urine due to the insensitivity of the indicator dye to globulins (360,361). By far the most sensitive tests for detection of light chains and other immunoglobulin fragments are routine electrophoresis and immunoelectrophoresis. In the presence of light chains, routine electrophoresis discloses a monoclonal peak; and immunoelectrophoresis (of concentrated urine) makes it possible to accurately identify the specific protein.

THE INTERPRETATION OF PROTEINURIA

The pathophysiology of proteinuria, the amount of urine protein, and its pattern of excretion are essential factors in determining the diagnosis and prognosis of patients with kidney disorders. It is most important to first determine whether the urine protein contains albumin. A positive dipstick test is

strongly suggestive of albuminuria, which most likely indicates glomerular proteinuria. A negative dipstick test in the presence of elevated total urine protein excretion suggests nonalbumin protein due to tubular or overload proteinuria. Electrophoresis or other tests should be performed to detect light chains or other low molecular weight proteins. As discussed earlier, these patterns suggest specific diagnoses. Albuminuria, on the other hand, is associated with a variety of kidney diseases. Albuminuria can be divided into the following patterns for purposes of diagnosis and prognosis: (a) intermittent, (b) orthostatic, and (c) persistent.

Intermittant Proteinuria

Although intermittent proteinuria may be the prelude to chronic kidney disease, more often it is the result of hemodynamic alterations in permselectivity associated with heavy exercise, fever, or severe emotional stress in the absence of kidney disease. Even though minor histologic abnormalities may occasionally be seen on a kidney biopsy in such individuals (401), long-term observations indicate that they are at no greater risk than the general population of developing decreased GFR (402). It is likely that this occurrence of mild, intermittent proteinuria in the general population makes routine screening ineffective. It has been suggested that screening of the urine be reserved for populations at higher risk of chronic kidney disease, such as patients with diabetes or hypertension (403).

Orthostatic Proteinuria

In patients with chronic kidney disease, proteinuria typically increases in the upright position to levels above those present in the recumbent position. This orthostatic change in excretion appears to have no special diagnostic or prognostic importance. The finding of proteinuria only in the erect position is known as orthostatic or postural proteinuria (404,405). In this disorder, total daily excretion usually does not exceed 1 g. Postural proteinuria occurs in the healing phase of many glomerular diseases and also in the absence of kidney disease. In the latter group, minor histologic abnormalities are found on kidney biopsy in approximately one-half of the patients (406). Kidney biopsy in patients with postural proteinuria typically discloses few morphologic abnormalities either on light or electron microscopy (406–408), although in two reports both immunoglobulins and complement were identified in a substantial fraction of such patients on immunofluorescence microscopy (409,410). The significance of this finding is uncertain.

To test for postural proteinuria, the patient is instructed to collect a urine sample in the upright position, while carrying out his other usual daily activities. A 16-hour collection can begin in the morning and end just before the patient goes to bed. On retiring, the patient begins an 8-hour recumbent urine collection, including voiding at the time of arising. The amount of protein in both samples is extrapolated to 24 hours. Patients with postural proteinuria have an increased excretion in the specimen collected in the upright position and a normal excretion in the specimen collected when recumbent. If protein excretion is increased in both specimens, the patient has persistent rather than postural proteinuria.

The evidence strongly suggests that postural proteinuria is a benign condition (405,411). After 10 years of follow-up in one study, it was found that over half the patients no longer had proteinuria, somewhat less than half continued to have postural proteinuria, and only a small minority developed persistent proteinuria (412). Decreased GFR was not observed, and hypertension was a rare occurrence. After 20 years of follow-up of many of the same patients, all those examined had normal kidney function, the prevalence of hypertension was no different from that in the general population, and only one-third had proteinuria. In half of the proteinuric group, the pattern of protein excretion was still the postural variety (413). Thus, the prognosis of patients with postural proteinuria appears to be excellent, and patients with this condition should be reassured about the benign nature of the disorder.

Persistent Proteinuria

Persistent proteinuria is almost invariably a sign of kidney damage even when kidney function is normal, when the urine sediment contains no abnormalities, and when the patient has no signs or symptoms of kidney disease (414–416). The amounts of protein excreted by these patients and the pattern of protein selectivity have considerable diagnostic significance.

Magnitude of Proteinuria

Persistent albuminuria (>30 mg/d, microalbuminuria, as discussed previously) is an ominous finding. In Type 1 and Type 2 diabetes, microalbuminuria is the earliest sign of diabetic kidney disease (388). The diabetic patient with microalbuminuria has a 20-fold increased risk of developing progressive kidney disease (417). Furthermore, these patients are likely to express other cardiovascular risk factors, such as obesity, dyslipidemia, and hypertension. Independently, microalbuminuria is a predictor of early cardiovascular morbidity and mortality in diabetes (418). In nondiabetic individuals, microalbuminuria also seems to be a powerful predictor of progression of kidney disease and cardiovascular disease, including coronary disease, peripheral vascular disease, and mortality, especially in elderly populations (419,420). The finding of altered protein excretion is associated with poor blood pressure control, insulin resistance, and lipid disturbances (421). Thus, in addition to serving as a marker of kidney damage, it appears likely that microalbuminuria reflects a diffuse disturbance in endothelial cell function predicting patients at high risk of cardiovascular morbidity and mortality (421,422). Even minimal increases in urinary albumin, still within the normal range >5mcg/min, independently predict cardiovascular events (423,424) and progression of kidney disease (425). Albumin excretion between 30 to 300 mg/day is sometimes referred to as *macroalbuminuria* or *clinical proteinuria*, because it can sometimes be detected by the usual dipstick. Despite their widespread usage, these terms should probably be abandoned, because they are technically incorrect or imprecise. Neither microalbuminuria nor macroalbuminuria refer to the size of urinary albumin fragments, and clinical proteinuria does not convey a specific meaning.

A protein excretion rate between 300 mg and 3 gm per day (i.e., a urine protein-to-creatinine ratio of 300 to 3,000 mg/g) can be seen in many types of chronic kidney disease, whereas an excretion greater than 3 gm per day is almost invariably the result of glomerular disease. Persistent excretion of ≥3 gm per day usually can lead to hypoalbuminemia, edema, and the clinical picture of the nephrotic syndrome. Clinical and laboratory evaluation can identify the cause of chronic kidney disease in many patients with the nephrotic syndrome, but the various histologic subtypes can be definitively identified only by kidney biopsy.

Declining GFR occurs more often with persistent proteinuria than without, but the prognosis is extremely variable (414,426). Prognosis appears to be worse if proteinuria is associated with microscopic hematuria or if heavy proteinuria subsequently develops (414). In one study of 16 patients with asymptomatic proteinuria and with little or no hematuria followed for 8 to 28 years, only 2 patients developed decreased

GFR, and in both instances kidney failure occurred more than 14 years after the onset of proteinuria. By contrast, 7 out of 10 patients who had persistent proteinuria, with the sole additional finding of microscopic hematuria, developed the nephrotic syndrome, hypertension, or decreased GFR within a period of 10 years. Histologic findings also have prognostic implications in persistent proteinuria; notably, the finding of diffuse or membranoproliferative glomerulonephritis or focal glomerular sclerosis implies an unfavorable long-term prognosis.

Protein Selectivity

Persistent proteinuria can be classified into either a selective or a nonselective pattern, based on a comparison of the clearance of albumin with the clearance of larger-molecular weight proteins, such as globulins (349,350,427). Investigators have sought to gauge the severity of the glomerular leak by measuring the relative clearance rates of proteins of various sizes (316,428,429). In patients with proteinuria secondary to a wide variety of chronic kidney diseases, the clearance rates of large molecules, such as IgG, range from less than 10% to greater than 60% of the clearance rate of albumin or transferrin, a protein similar in size to albumin. Patients with a clearance ratio of IgG/albumin (or transferrin) of less than 0.10 are considered to have only a modest increase in glomerular permeability and are defined as having a "highly selective" pattern of protein excretion. Conversely, patients in whom the clearance ratio is 0.5 or greater are considered to have a relatively porous filter and are defined as having a poorly selective pattern.

Studies of the pattern of protein excretion have shown that the majority of patients with proteinuria have a nonselective pattern (428,429). Among patients with the idiopathic nephrotic syndrome, however, two populations emerge. One group has selective proteinuria and, in most cases, has minimal change in the disease. The second group has nonselective proteinuria and usually has one of the more severe histologic varieties, such as membranous nephropathy or membranoproliferative glomerulonephritis. Because of this correlation and the frequency of the minimal change lesion among patients with the idiopathic nephrotic syndrome, selectivity studies have some value in predicting the presence of the minimal change lesion. However, the technical difficulties involved in carrying out the protein analyses, the failure of the test to distinguish among the many subgroups of nephrotic syndrome, and the low risk of kidney biopsy relative to its diagnostic yield have made measurements of selectivity superfluous in the study of the nephrotic patient.

CLINICAL IMPLICATIONS OF PROTEINURIA

Proteinuria is central to the detection, evaluation, and management of chronic kidney disease (Table 11-8). Proteinuria is important to establish a diagnosis of CKD, to identify the specific cause of the disease, to identify patients who are at high risk for adverse outcomes, and to assist in selection of management strategies. In addition, changes in proteinuria appear to be related to the outcome of chronic kidney disease.

Protein Excretion as a Diagnosis for CKD

NKF-K/DOQI guidelines on CKD define persistent proteinuria for 3 months or more is defined as CKD, even in the absence of other markers of kidney damage or decreased GFR. An elevated urinary albumin excretion (30 to 300 mg/day) (microalbuminuria) is widely acknowledged to be the earliest sign of diabetic kidney disease. One recent study showed convincing evidence that elevated urinary albumin in this range or lower was associated with an increased risk of the development of higher rates of urine albumin excretion in both diabetic and nondiabetic patients (425). The threshold level defined by the NKF-K/DOQI guidelines for kidney damage is urinary albumin excretion >30 mg/d, which is roughly equivalent to an albumin-to-creatinine ratio >30 mg/g. Using this definition of proteinuria as a marker of kidney damage has enabled studies of the prevalence of earlier stages of chronic kidney disease, regardless of the cause, in large populations (430).

The National Kidney Foundation has suggested routine testing for urine albumin-to-creatinine ratio in all individuals at increased risk for chronic kidney disease, including those with hypertension, diabetes, a family history of kidney disease, or advancing age (256). The American Diabetes Association also suggests routine testing for albuminuria in all diabetic subjects as part of their evidence-based guidelines (431). Additionally, the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC-7) recommended routine urinalysis in the evaluation of all patients with hypertension with the option of measuring urine albumin excretion as well (432).

Protein Excretion as a Diagnostic Clue to Etiology

Proteinuria is also helpful in diagnosing the cause or type of of kidney disease. Full discussion of what determines the cause of kidney disease is beyond the scope of this chapter. However, as one example, the presence of larger amounts of proteinuria would generally distinguish between glomerular and tubulointerstitial disease.

Protein Excretion as a Prognostic Indicator for Progression of Kidney Disease and Cardiovascular Disease

Initial and repeated measurement of protein excretion is a valuable guide in following the course of patients with kidney disease. Several studies have demonstrated that the magnitude of proteinuria is directly correlated with the risk of progressive decline in kidney function, regardless of the cause or type of kidney disease (425,433,434). Reductions in proteinuria during treatment were associated with slowing progression (270,435,436,437).

There is increasing evidence that proteinuria independently predicts cardiac outcomes and death (438), even at low levels of protein exretion in the microalbumin range (424,439,440).

In patients with specific patterns of proteinuria or types of kidney disease, there are other correlates of proteinuria. The presence of heavy proteinuria predicts a worse kidney disease outcome in many glomerular diseases including diabetes, hypertension, IgA nephropathy, focal glomerulosclerosis, and lupus nephritis (441–444). In patients with the nephrotic syndrome, persistent excretion of massive amounts of protein (>10 gm/day) is a precursor of severe hypoalbuminemia and its complications, such as malnutrition, thrombosis, hypotension, and oliguric renal failure. Day-to-day or week-to-week fluctuations in excretion of as much as 2 or 3 gm in patients with the nephrotic syndrome are probably the result of hemodynamic changes and have little or no clinical significance. However, a progressive decline in excretion to levels less than 2 gm per day (or less) is associated with a favorable prognostic outlook, whether the reduction occurs spontaneously or in response to treatment (445,446). In fact, a decline in protein

excretion to normal in patients with the nephrotic syndrome is the most favorable prognostic sign. A rare exception to this is the proteinuria that can develop during therapy for rapidly progressive glomerulonephritis. In this condition, development of nephrotic syndrome may actually herald recovery (447).

Repeated assessment of protein excretion is also useful in following patients with non-nephrotic proteinuria. In patients with poststreptococcal glomerulonephritis, conversion from persistent to postural proteinuria indicates a good prognosis, and a fall in protein excretion to normal implies healing of the glomerular lesion. By contrast, persistence of proteinuria increases the likelihood of progressive GFR decline (448). In patients with lupus nephritis, the amount of protein excretion correlates well with immunologic activity and histologic changes. However, among patients with severe histologic lesions, the initial protein excretion rate does not predict outcome or response to therapy (449).

Protein Excretion to Assist in Selection of Management Strategies

Secondary analysis of recent clinical trials demonstrates that the beneficial effects of lowering blood pressure and inhibition of angiotensin-converting enzymes are greater in patients with higher levels of proteinuria at the beginning of therapy (435,450–452). Thus, it appears that the magnitude of proteinuria predicts the risk of progression of kidney disease, as well as the response to treatment with antihypertensive agents. Consequently, the magnitude of proteinuria is central to the new guidelines for the management of hypertension in chronic kidney disease (453). For patients with total protein-to-creatinine ratio >200 mg/g, the guidelines recommend initial antihypertensive therapy using an angiotension converting enzyzme inhitor or angiotension receptor blocker.

CONCENTRATION AND DILUTION OF THE URINE

The maintenance of near constancy of body fluid osmolality is of paramount importance. In order to adjust for wide variations in solute and water intake, the kidneys must be able to excrete urine of varied concentrations. This requires tubules to dissociate the handling of salt and water. This goal is accomplished by the integrated functioning of the hypothalamus, pituitary gland, and the kidney (454).

The homeostatic systems regulating normal water balance and the osmolality of body fluids influence both water intake and water excretion by the kidney. The mechanisms controlling water intake through thirst are incompletely understood, although the normal thirst mechanism provides excellent protection against hypertonic dehydration (455). Although adjustments in water intake mediated through variations in thirst play an essential role in the regulation of body fluid tonicity, acquired drinking habits can override these physiologic adjustments in many instances. Water excretion varies widely, depending on intake, and under physiologic conditions, its regulation is independent of solute excretion and GFR (9). As a result, the tonicity of the urine varies from as low as 50 mOsm/kg water or as high as 1,200 mOsm/kg water. When the concentration of solute in the urine is less than that in plasma water (280 to 290 mOsm/kg water), the urine is termed *dilute*, or *hypotonic*. When the concentration of solute in the urine is greater than that in plasma water, the urine is termed *concentrated*, or *hypertonic*. The mechanisms by which the kidney forms concentrated or dilute urine in response to changes in water intake are considered in detail in Chapter 3. The following discussion summarizes the mechanisms of water excretion as they relate to the clinical assessment of this critical aspect of kidney function.

OSMOLALITY

Control of the Osmolality of the Body Fluids

In healthy human beings, the osmolality of the extracellular fluid is maintained within narrow limits (280 to 290 mOsm/kg water) and is performed through alterations in water intake and urinary excretion of water (455–462). The overall effect of the integrated functioning of the hypothalamic-pituitary-kidney mechanism is such that hypertonicity of body fluids stimulates thirst and the production and the release of the antidiuretic hormone (ADH), i.e., arginine vasopressin (AVP). The presence of ADH facilitates distal tubular water resorption, permitting retention of ingested water thus correcting the hyperosmolar state. By contrast, hypotonicity of body fluids suppresses ADH release, and the reduction in hormone concentration, in turn, impedes distal tubular water resorption, which leads to a water diuresis. As extracellular fluid tonicity increases, the water content of hypothalamic osmoreceptors decreases; the converse also is true. Mechanosensitive channels in supraoptic neurons appear to be able to transduce these modifications in intracellular osmolality and influence the production and secretion of ADH by hypothalamic cells, which is then released by the posterior pituitary gland (463). The osmoreceptors have many structural and functional properties in common with those that control the thirst mechanism that are found in approximately the same anatomic location (455). An alteration in osmolality of the body fluids as small as 1.5% to 2% results in marked changes in ADH levels (464). Although factors other than osmotic stimuli (drugs, emotional state, nausea (465,466), and extracellular fluid volume) also have an effect on ADH release, the primary physiologic signal derives from changes in body fluid osmolality (467–469).

An analysis of the functioning of this system is depicted in Figure 11-10. If we start at an initial normal plasma osmolality of 287 mosm/kg (solid arrow in Fig. 11-10A), ingestion of 500 mL of water will lower the plasma osmolality by only 1%, or 3 mosm/kg. This is a sufficient change in plasma osmolality to be recognized by hypothalamic osmoreceptors and results in decreased release of ADH with a fall in the plasma level from 2 to 1 pg/mL. As shown in Figure 11-10B, the reduced ADH level would cause the urine osmolality to fall from 500 to 250 mosm/kg. If we assume constant solute load, this reduced urine concentration will allow the excretion of the ingested water. Conversely, if water is lost such that plasma osmolality increases by 1%, plasma ADH will rise from 2 to 3 pg/mL and urine concentration will rise from 500 to approximately 700 mosm/kg; thus, urine volume will decrease. Through the integrated functioning of the hypothalamic-pituitary-kidney system, small changes in plasma osmolality are translated into large changes in urine osmolality, thereby maintaining water balance.

Relation between Daily Urine Volume and Urine Osmolality

For a given rate of solute excretion, the urine concentration is a direct reflection of the urine volume. For example, an individual in steady state who eats approximately 60 gm/day of protein and 6 gm/day of NaCl excretes approximately 600 mOsm per day. If this solute load is excreted at the minimal urine osmolality of 50 mOsm/kg water, a urine volume of 12 L/day is

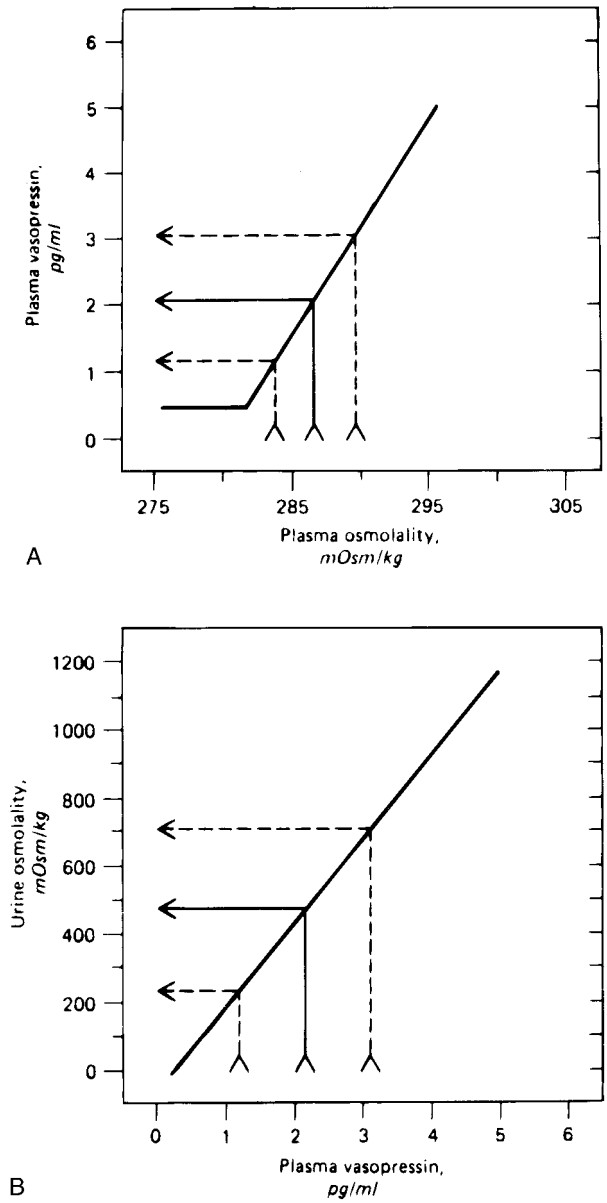

FIGURE 11-10. Schematic representation of the effect of small alterations in basal plasma osmolality on plasma vasopressin (antidiuretic hormone) (**A**) and urine osmolality in healthy adults (**B**). See the text for an explanation. (From: Robertson GL, Athar S, Shelton RL. *Kidney Int* 1976;10:25, with permission.)

required. If the solute load is excreted at the maximal urine osmolality of 1,200 mOsm/kg water, a urine volume of only 0.5 L/day is required. Thus, with normal concentrating and diluting capacity, a wide range of water intake (from 500 to 12,000 mL) can be excreted with no change in water balance. This relationship explains why less than 400 to 500 mL of urine output per day in an adult is termed *oliguria*. Even with maximal urine concentration, the usual solute load cannot be excreted at a urine volume less than 400 mL. If less than 400 mL is excreted, solute is retained leading to azotemia. *Polyuria*, defined arbitrarily as a daily urine volume of greater than 3 L, is generally symptomatic. If the solute load remains constant at 600 mg/day, a daily urine volume of 3 L would be associated with a urine osmolality of 200 mosm/kg water. This relationship can also be used to explore the consequences of defects in concentrating and diluting capacity on the urine volume and on the development of symptoms (Table 11-9).

With a severe concentrating defect, for example, in a patient who can only achieve a maximal urine concentration of 100 mOsm/kg water, on a diet producing 600 mOsm/day, the patient would require a daily urine volume of 6 L to excrete the 600 mOsm. This would be symptomatic. Polyuria would result in hyperosmolality, stimulation of thirst, and polydypsia. If fluid intake is less than 6 L/day (plus an amount sufficient to replace insensible water losses) due to a defective thirst mechanism or inability to obtain water, water depletion and hypernatremia would occur. However, if the patient had a more modest concentrating defect and could achieve a maximal urine osmolality of even 300 mOsm/kg water, the minimum urine volume necessary to excrete the same daily solute load of 600 mOsm would be only 2 L. This volume of urine may not cause symptoms and if water intake is normal, it is not large enough to cause hyperosmolarity, thirst, or polydypsia. However, in patients with increased solute loads, polyuria can occur despite only a modest concentrating defect and, thus, be large enough to be noticed. This is the case in osmotic diuresis where the maximum urine osmolality is about 300 mosm/kg water (470).

Defects in urine diluting ability can be considered in a similar manner. A patient with a severe diluting defect, for example, inability to decrease urine osmolality to no lower than 600 mOsm/kg water, with a daily solute load of 600 mOsm would have a maximum urine volume of 1 L/day. Thus, the patient would retain water if the daily intake of fluid exceeds 1 L (plus an amount sufficient to replace insensible water losses) resulting in hyponatremia. If the defect in diluting ability was mild, (such as a minimum urine concentration of 300 mOsm/kg water), then on the same diet, the maximum urine volume would be 2 L/day. If fluid intake does not exceed 2 L/day, water retention would not occur and the defect in water excretion would have no clinical consequences. Seldom appreciated, however, is the clinically important principle that even a mild defect in water excretion can lead to water retention if water intake is sufficiently large. Referring again to our model, even with the mild defect in diluting ability described previously (maximal urine volume of 2 L/day), if the patient ingests 4 or 5 L of fluid, water retention would occur and body fluids would be diluted. Similarly, a mild defect in water excretion can lead to water retention with low fluid intake if solute intake is low. This phenomenon is observed occasionally in patients with the syndrome of inappropriate ADH secretion; such patients can become hyponatremic even if though their urine tonicity is isotonic.

Methods of Measuring Urine Tonicity

Urinary Osmolality

Osmolality describes the osmotic pressure of a solution. The osmolality is related to the number of particles in solution and not to the size, charge, or density of the particles. Thus, 1 mmol of albumin has the same osmotic effect as 1 mmol of sodium. One mmol of any substance in solution is approximately equivalent to 1 mOsm (for univalent cations and anions, 1 mEq is approximately equivalent to 1 mOsm). If the concentration of all solutes in a solution is known, the osmolality of the solution can be approximated. Urine is a complex mixture of a number of substances. Although it is possible to approximate the osmolality of urine by measuring the electrolyte and urea content, the estimate is only a rough one because of the many other substances contributing to urine tonicity. The osmolality of a solution can be readily determined by taking advantage of the principle that the extent to which the freezing point of a solution is depressed below that of distilled water is linearly related

TABLE 11-9

SYMPTOMS DUE TO CONCENTRATING OR DILUTING DEFECTS BASED ON SOLUTE AND FLUID INTAKE

Solute load (mosm/day)	Urine osmolality (mosm/kg)	Urine volume (L/day)	Concentrating or diluting defect	Symptoms*	Change in sodium concentration
600	50 (minimal physiological limit)	12	Extreme concentrating defect	Polyuria causes water depletion, leading to hyperosmolality, stimulation of thirst, and polydypsia	Hypernatremia if fluid intake <12 L/day occur
600	100 (hypotonic)	6	Severe concentrating defect	Polyuria	Hypernatremia if fluid intake <6 L/day
600	300 (isotonic)	2	Modest concentrating defect	Asymptomatic	Hypernatremia if fluid intake <2 L/day
1200	300 (isotonic)	4	Modest concentrating defect	Polyuria	Hypernatremia if fluid intake <4 L/d
600	1200 (maximal physiologic limit)	0.5	Extreme diluting defect	Water retention leads to hypoosmolality	Hyponatremia if fluid intake >0.5 L/day
600	600 (hypertonic)	1	Severe diluting defect	Depends on magnitiude of hjyponatremia	Hyponatremia if fluid intake >1 L/day
600	300 (isotonic)	2	Modest diluting defect	Depends on magnitiude of hjyponatremia	Hyponatremia if fluid intake >2 L/day
300	300 (isotonic)	2	Modest diluting defect	Depends on magnitiude of hjyponatremia	Hyponatremia if fluid intake >1 L/day

*Estimates of fluid intake associated with symptoms are approximate only. They assume no change in solute intake, insensible water loss, and liberation of water from digestion of solids.

to the osmotic pressure of the solution. The exact relationship is as follows:

$$\text{Osmolality (mOsm/kg)} = \frac{\text{freezing point depression (°C)}}{0.00186} \quad [34]$$

Osmometers using this principle are operated easily and accurate enough for all clinical purposes; the precision of a calibrated osmometer is approximately 0.3% (471,472). Storage of samples for prolonged periods or freeze-thawing can significantly change results (471). This method for assessing urinary tonicity is advantageous not only because of its precision, but also because the results (in contrast to urine specific gravity measurements) are not significantly influenced by such factors as the temperature of the urine or excretion of protein or iodinated contrast agents. Nonetheless, measurements of osmolality require more expensive equipment than do measurements of specific gravity.

Urinary Specific Gravity

The specific gravity of the urine (a measure of the weight of a solution as compared to distilled water) is a function not only of the total number of particles in solution, but also of the relative size and density of these particles. Thus, a solution of glucose (MW = 180 daltons) equal in osmolality to a solution of urea (MW = 60 daltons) has a higher specific gravity (Fig. 11-11). Over a wide range of normal and pathologic variations in urine composition, the specific gravity correlates fairly well with osmolality. Urea, sodium, chloride, sulfate, and phosphate contribute most to the specific gravity of normal urine (348). A urine osmolality of 50 mOsm/kg water is approximately equivalent to a specific gravity of 1.000 to 1.001; a urine osmolality of 300 mOsm/kg water is approximately equivalent to a specific gravity of 1.010; and a urine osmolality of 800 mOsm/kg water or greater is approximately equivalent to a specific gravity of 1.020 (473). Several precautions should be observed in

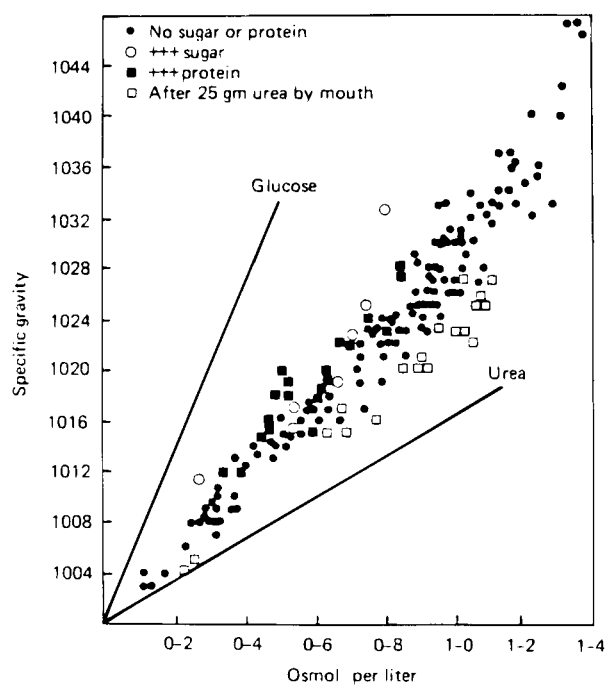

FIGURE 11-11. Relationship between specific gravity and osmolality of the urine. Different urine samples are shown as follows: *small filled circles,* with no sugar or protein; *large open circles,* 3+ sugar; *small filled squares,* 3+ protein; *large open squares,* after 25 g of urea by mouth. The lines show the relation between specific gravity and osmolality for glucose and urea solution. (From: Miles BE, Paton A, de Wardener HE. Maximum urine concentration. *Br Med J* 1954;2:901, with permission.)

interpreting urinary specific gravity measurements. Refrigeration of the urine and excretion of large quantities of protein or glucose both result in moderate increases of specific gravity (473,474). Excretion of radiopaque contrast medium, which is hyperosmolar and contains iodinated compounds (e.g., iothalamate, MW = 614 daltons), has a much more striking effect, which frequently results in urine specific gravity measurements of 1.040 to 1.050, which are values well above the physiologic range.

Urinary specific gravity can be measured readily with a urinometer, but several means of avoiding error should be observed. First, the urine should be corrected for the temperature at which it is measured. For every 3°C difference between the calibration temperature indicated on the urinometer (usually 15.6°C or 60°F) and the temperature at which the specific gravity is measured, the specific gravity will be altered by 0.001 (an increase in temperature decreases the specific gravity). Second, the glassware must be clean and free of detergent; and third, the hydrometer must be calibrated frequently in distilled water (its specific gravity by definition is 1.000) or against a solution of known specific gravity (348). Correction also can be made for the effect of protein or glucose; if these substances are present, one should subtract 0.003 for every 1 gm per 100 mL of either substance (348).

Refractive Index

The refractometer, an instrument that measures the refractive index of solutions, also can be used to measure urine concentration. The refractive index is best correlated with the total solids per unit measure of water, which, of course, is correlated with the specific gravity. In practice, a value for specific gravity is obtained directly from the scale of the refractometer, and this value agrees well with measurements of specific gravity made by the urinometer (475,476). The refractometer method has several advantages over the use of a urinometer. Only one drop of urine is required to obtain a measurement in contrast to the 15 mL required for the urinometer; the instrument automatically compensates for variations in temperature over a reasonable range (60°F to 100° F), and the refractive index is less sensitive to solute density than is the urinometer measurement. For this reason, the specific gravity obtained by the refractometer method is affected less by the presence of radiopaque contrast material than that obtained using the urinometer (476). The refractometer should be checked daily against distilled water.

Dipsticks

The technician time required to measure urine tonicity by osmometer, urinometer, or refractometer has prompted the extension of the reagent strip methodology (dipsticks) to the measurement of urine specific gravity. Commercial strips utilizing this approach are now available. The specific gravity reagent area utilizes the relationship of the concentration of ions to the amount of solids dissolved in the urine. The test assumes that the amount of ions generally increases in the urine as the total solids of urine increase. As the concentration of ions increases, the chemicals impregnated in the specific gravity reagent area (a polyacid) are affected and release hydrogen ions. The resultant change in pH then causes the acid-base indicator (bromthymol blue) impregnated in the specific gravity area of the strip to change color and "read" the specific gravity of the urine. In one study comparing specific gravity by the dipstick method to specific gravity by refractometer, 85% of dipstick results were within ± 0.005 of the refractometer (477,478). Nonionic substances, of course, do not affect this method. Spurious results often occur in alkaline urines and those containing large amounts of protein (477). In infants and children, the specific gravity measurement on dipstick has been found to correlate poorly with measured urine osmolality, and was ineffective as a test for dehydration (479,480). This poor correlation was not due to alkaline pH or proteinuria. A laboratory quality assurance program detected a 13.4% error rate (±1 or 2 color blocks) and a 3.1% gross error rate (±3 color blocks) in the assessment of urine specific gravity by visual inspection in 7,150 spiked urine samples. The error and gross error rates were reduced when an electronic reader was used (481). Thus, the dipstick method is advantageous because of its low cost and convenience, but the relative imprecision suggests it should not be used in any formal test of urine concentration and dilution. This lack of precision seems to be worse in infants and children.

ASSESSMENT OF URINE CONCENTRATING CAPACITY

Assessment of concentrating ability is necessary in patients with polyuria or hyperosmolality. In patients with hyperosmolality, measurement of a random urine osmolality or specific gravity provides important diagnostic information. If the urine osmolality is 900 mOsm/kg water or greater or the specific gravity 1.022 or higher in the absence of protein, glucose, or radiopaque contrast material, it is highly likely that the urine concentrating mechanism is normal. (473,482). The cause of hyperosmolality is inadequate water intake. Lower values of urine osmolality or specific gravity suggest a concentrating defect.

However, in patients with polyuria and normal serum osmolality, formal assessment of concentrating capacity is required. Concentrating ability can be assessed systematically by simple water deprivation (483). This maneuver tests the integrated response of the pituitary and the kidney. Fluid deprivation of 12 to 16 hours leads to insensible water losses of 300 to 400 mL, which is sufficient to slightly raise plasma osmolality which provides a potent stimulus to ADH secretion (469). Fluid deprivation for 18 to 24 hours yields a urine osmolality greater than 900 mOsm/kg water and a specific gravity greater than 1.023 in 90% of normal persons (482,484). In patients with polyuria from primary polydipsia, maximal urine concentration may reach only 500 to 900 mOsm/kg water possibly due to a "washout" of the medullary interstitium and downregulation of collecting duct Aquaporin 2 water channels (485). To reestablish a hypertonic medullary interstitium in such patients, prolonged dehydration (36 to 48 hours) may be necessary for plasma ADH to induce receptors (to ADH) in the medullary thick ascending limb of the loop of Henle, to activate adenylate cyclase, and to increase NaCl transport in this nephron segment (486).

In patients with a urine osmolality of less than 500 mOsm/kg water after water deprivation, the administration of exogenous ADH (5 units of aqueous vasopressin subcutaneously) provides additional important information (483). In patients with central or neurogenic diabetes insipidus, water deprivation does not raise urinary osmolality greater than 100 to 250 mOsm/kg water, despite substantial weight loss and despite rises in plasma osmolality to 300 mOsm/kg water or higher. The response to the administration of exogenous ADH is dramatic and is the definitive test for the diagnosis of central diabetes insipidus (either complete or partial), with urine osmolality rising to at least twice its previous value. In contrast, patients with nephrogenic diabetes insipidus will have virtually no response (of urine osmolality) to exogenous ADH administration. Measurement of plasma ADH by radioimmunoassay at the time of the overnight water-deprivation test enhances the diagnostic usefulness of the test (487). Molecular characterization of mutations in the collecting duct water channel

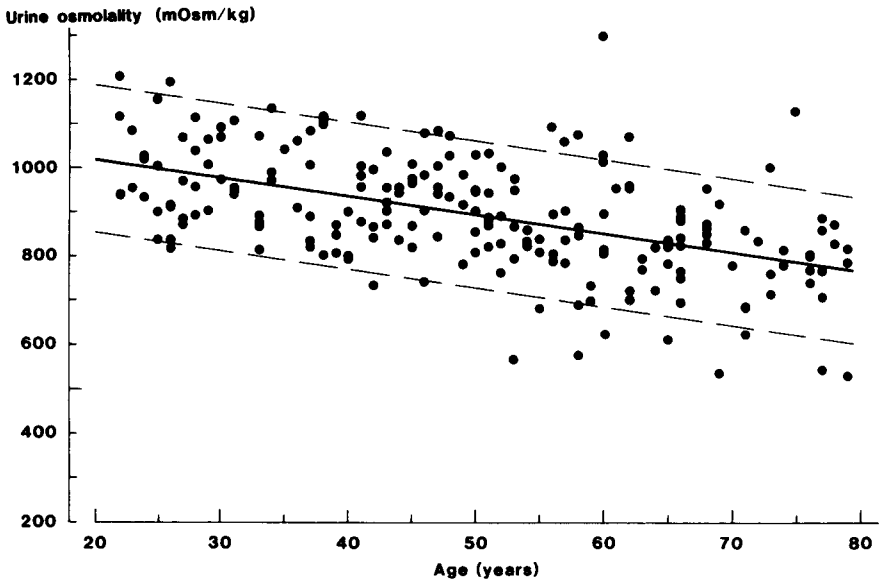

FIGURE 11-12. Peak urine osmolality after l-desamino-8-D-arginine-vasopressin (dDAVP) as a function of age in 211 healthy adults. (From: Tryding N, Berg B, Ekman S, et al. DDAVP test for renal concentrating capacity. *Scand J Urol Nephrol* 1988;22:141, with permission.)

aquaporin 2 or the vasopressin V2 receptor can provide definitive evidence for the basis of nephrogenic diabetes insipidus (488). Administration of exogenous ADH permits assessment of urine concentrating ability (but not pituitary function) without the need for concomitant dehydration. If 5 units of vasopressin tannate in oil is given at bedtime to normal subjects, the maximal urine concentration achieved the following morning approaches that observed with 18 to 24 hours of water deprivation (489). The patient should be cautioned not to consume excessive quantities of fluid because of the danger of ADH-induced water retention and hypoosmolality. ADH administration should be avoided in patients with coronary artery disease.

Alternatively, a shorter period of water deprivation can be followed by administration of exogenous ADH. This method may be more practical than prolonged fluid deprivation, and has fewer side effects than the standard water deprivation test (490). Patients are fluid restricted overnight from 11 PM to 7 AM. Then 4 mcg (micrograms) of an analogue of ADH, l-desamino-8-D-arginine-vasopressin (DDAVP) is administered subcutaneously, and a urine specimen is collected between 3 and 6 hours later for measurement of osmolality. It should be noted that there is an age-dependent reduction in the maximal urine osmolality after DDAVP as seen in Figure 11-12; these reference limits must be kept in mind when evaluating the results of this test (490).

ASSESSMENT OF URINE DILUTING CAPACITY

Assessment of diluting capacity is necessary in patients with hypoosmolality. The finding of urine that is maximally dilute (urine osmolality <80 mOsm/kg water or urine specific gravity <1.003) in the face of a sustained reduction in plasma osmolality indicates massive water ingestion. Greater values indicate an impairment of diluting capacity (491).

Formal assessment of diluting capacity is necessary only in patients with a history of hypoosmolality that has resolved, and the cause has not been defined. Diluting capacity can be assessed with water loading. Normal adults given 1 to 1.5 L of water (20 mL/kg) to drink over a short period excrete more than half this quantity in 3 hours and reduce the specific gravity of at least one voided specimen to 1.003 or less (80 mOsm/kg water or less). However, unlike tests of concentrating ability, the response to this standard test is extremely variable and difficult to standardize (484).

ALTERATION IN CONCENTRATION AND DILUTION AS MARKERS OF KIDNEY DAMAGE

Tests of concentrating capacity are quite sensitive and are occasionally abnormal in patients with kidney disease even before GFR is reduced. This is especially common in individuals with autosomal dominant polycystic kidney disease (ADPKD), in whom the vast majority develops a concentrating defect before GFR decreases. This defect closely parallels the severity of anatomic changes caused by the renal cysts, independent of age and GFR. This observation suggests that the cysts disrupt the medullary architecture and countercurrent mechanisms vital to the creation of a maximally concentrated urine (492). There also appears to be decreased expression of aquaporin-2 protein in the renal epithelial cells of patients with ADPKD (493). Similarly, decreased urinary excretion of aquaporin-2 protein in the urine of patients with diabetic nephropathy correlates with the presence of a concentrating defect (494). In other individuals, the clinical significance of an isolated concentrating defect is often difficult to assess, because a number of factors (e.g., low-protein diet [495], increased rate of solute excretion [496], and prolonged water loading [497] can interfere with the concentrating ability even in the absence of kidney disease.

As described earlier and detailed in Chapter 3, diluting defects occur in a variety of medical conditions. However, many patients with a diluting defect do not have hypoosmolality. Hypoosmolality only occurs if the impairment of water excretion is severe or if they drink a large volume of fluid. In patients without hypoosmolality, the potential risk of a water-loading test to uncover a defect in urinary diluting capacity makes such a study of little practical value. Diluting defects are usually not the result of kidney damage. Usually the cause is failure to suppress ADH, with preserved kidney function. In some patients, the diluting defect is due to decreased delivery of solute to the diluting segment in the distal nephron as a consequence of decreased GFR. However, generally, there is no associated

kidney damage and tubular function is usually preserved, as seen in decreased kidney perfusion.

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CHAPTER 12 ■ INTRAVENOUS UROGRAPHY, ULTRASONOGRAPHY, AND RADIONUCLIDE STUDIES

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Intravenous urography, ultrasonography, and radionuclide studies have for many years been the first-line imaging techniques used most frequently in renal disease. The predominantly structural information provided by urography and ultrasonography complements the largely functional information given by radionuclide studies. An appreciation of the strengths and weaknesses of each technique and its possible hazards is vital to the renal physician. The best single investigation or the optimum combination and sequence of investigations to evaluate a particular clinical problem then can be chosen.

The discussion in this chapter largely concerns the use of these investigations in the adult kidney. The techniques are reviewed and the indications for their use are outlined. The appropriate imaging approaches to a number of common diagnostic problems then are discussed.

INTRAVENOUS UROGRAPHY

Plain Films

Renal calcifications usually are hidden by contrast medium during urography, and problems relating to the presence of renal calcification must be resolved before contrast medium is given. A full-length radiograph on inspiration and a film coned to the renal area on expiration usually resolves whether or not calcification overlying the kidneys is intrarenal. An oblique film of the kidney or renal tomography is necessary if doubt remains. Plain tomography often shows unsuspected calculi (1), especially if they are of low density, and is indicated when feces and bowel gas obscure the renal areas, or when there is suspicion of calculous disease.

Calcifications in the Ureter or Bladder

The line of the ureter must be examined carefully because even large ureteric calculi may be overlooked if they overlie the lumbar transverse processes or the sacrum. Determining whether calculi lie within the ureter or bladder usually necessitates contrast-medium injection to define the relation of the observed calcifications to the urinary tract.

The *renal outlines* usually are seen at least in part on the plain film, especially if abundant perirenal fat is present. Nonetheless, the assessment of renal size and outline is such an important part of the urographic diagnosis that decisions concerning them should be made only after intravenous contrast medium has been given.

Contrast Media and Their Excretion

The contrast media used since the 1960s for urography are triiodinated benzoic acid derivatives. Initially compounds such as iothalamate, diatrizoate, and metrizoate were used, in which iodine-containing anions are complexed to cations (usually sodium or meglumine) to form ionic salts of high osmolality (1,400 to 1,200 mOsm/kg). Dimers (e.g., ioxaglate) and nonionic agents (e.g., iohexol and iopromide) have been formulated over the past 20 years. These have an osmolality approximately one-third of the ionic monomers and a lower incidence of side effects. Although the cost of the nonionic agents is greater than that of the ionic media, the lower osmolality compounds are now the only iodinated contrast media used for intravenous injection in much of the United States and Europe.

Excretion

Both ionic and nonionic contrast media are rapidly distributed throughout the extracellular fluid after intravenous injection (2–4). Mixing is largely complete at 2 hours (3). Binding to plasma protein is minimal (5,6). At the doses used in urography, renal excretion of both ionic and nonionic agents, for practical purposes, can be regarded as being accomplished by glomerular filtration (2,7–10). Renal excretion is rapid; for example, 30% of an injection of sodium diatrizoate is excreted in the first hour and over 90% in the first 24 hours (7).

The *nephrogram* is the opacification of the renal parenchyma, produced by filtered contrast medium in the lumen of the renal tubules, principally the proximal convoluted tubules, because these occupy most of the parenchymal volume (11). Contrast medium in the vascular compartment only contributes to the very early nephrogram if a large intravenous bolus is given rapidly.

The density of the nephrogram depends on the glomerular filtration rate (GFR), the peak plasma contrast medium concentration, and the number and size of the nephrons present. Plasma peak concentration is determined by the patient's size and the dose and rate of injection of contrast medium. Increased doses of contrast medium produce a denser nephrogram (12). The nephrogram is reduced when there is a decreased GFR because of the loss of nephrons; however, this only becomes noticeable in practice when GFR is reduced substantially. The nephrogram is not affected by water reabsorption in the distal nephron and is unchanged by dehydration (13).

The *pyelogram* is produced by contrast medium in the urine in the pelvicaliceal system, which has been subjected to the concentrating process along the length of the nephron. Pyelogram density depends on the concentration of contrast medium in the urine and the degree of distention of the collecting system. The concentration of the contrast medium in the urine depends on the plasma concentration of contrast medium and the effects of the concentrating processes in the nephron on the glomerular filtrate. The concentrating processes are affected by the marked osmotic diuresis induced by the contrast medium (14); dehydration, which increases water reabsorption in the distal nephron; and any underlying renal concentrating defect,

such as in renal failure. The nonionic agents have less osmotic diuretic effect than ionic compounds, and this is reflected in higher urine iodine concentrations (15–17). The resultant pyelograms are only slightly denser than or of similar density to those produced with ionic agents, however (18–22). The distention of the collecting system depends on the urine flow rate, collecting system volume, and effectiveness of ureteric compression applied during urography (23).

Hazards of Urography

The hazards of radiation must be remembered, especially in women of childbearing age. The remainder of this section concerns the hazards associated with the use of contrast media.

General

The incidence of serious side effects is very small in view of the large doses of contrast medium that are routinely given by intravenous injection (e.g., 35 g of sodium iothalamate in a 70-kg patient). The majority of patients, however, experience some discomfort from the contrast-medium injection. A sensation of warmth is common, and a metallic taste, tingling, flushing, nausea, and sneezing are frequent (24). These minor side effects are less common with nonionic agents (25,26). More serious side effects occur in about 5% with ionic agents (27), but only in about 1% with nonionic agents (25). Most of these are of intermediate severity—vomiting, urticaria, bronchospasm, angioneurotic edema, and hypotension. Ionic agents cause less than 0.01% of severe side effects, such as cardiac arrhythmias, cardiac arrest, pulmonary edema, loss of consciousness, and convulsions (28). The incidence of such effects is lower with nonionic agents by a factor of five to ten times (25,29). Death has been reported in 1 in 14,000 (27) and 1 in 75,000 cases with ionic agents (30). The mortality is probably lower with the nonionic agents (29,31). Adverse effects usually occur in the 15 minutes after contrast medium injection but can be delayed for an hour or more in rare cases.

The incidence of side effects increases if the patient has a history of previous contrast medium reaction or of allergy, asthma, or cardiac disease (25,29,32). The risk of reaction is also increased in patients over 60 years of age and with the use of meglumine salts (which are associated with an increased incidence of bronchospasm) (28,32).

The cause of these reactions is poorly understood. There appear to be a variety of complex interactions between the complement, contact, coagulation, and immune systems. The release of a variety of active mediators, such as histamine, bradykinin, leukotrienes, prostaglandins and complement factors has been documented (33,34).

Renal

The early reports of high-dose urography in renal failure suggested that deterioration of renal function did not occur if patients are hydrated adequately (35). A large number of reports appeared in the literature in the late 1970s, however, about acute renal failure after contrast medium injection (36). It is difficult to determine the true incidence of contrast-medium–related renal impairment, but a review of 12 prospective studies suggests that it occurs in about 10% of patients (37). The serum creatinine level rose by 1 mg/dL or more in the 24 hours after injection of contrast medium in these patients, and it subsequently returned to normal in the great majority. Factors that increase the risk of deterioration of renal function after urography include renal impairment, dehydration, diabetes mellitus (38), age over 60 years, the use of high osmolality contrast media and large doses of contrast medium, repeated contrast-medium procedures, and concurrent administration of nephrotoxic drugs (e.g., gentamicin, nonsteroidal antiinflammatory agents) (37,39). The mechanism of this effect is unknown, but one of the favored theories suggests that decreased perfusion of the renal microvasculature (probably caused by release of vasoactive substances such as endothelin) is responsible (39,40).

Multiple myeloma is no longer regarded as a contraindication to urography, provided that patients are well hydrated (41). However, patients with myeloma who are in renal failure are at increased risk for development of acute renal failure after urography and should be evaluated by other means (e.g., ultrasonography) whenever possible.

To protect against contrast-medium–induced nephrotoxicity, patients should be well hydrated, low osmolar contrast media should be used in the lowest dose necessary to achieve the diagnosis, and further administration of contrast medium should be delayed for at least 48 hours (39).

Precautions

Intravenous contrast-medium injection should be slow to reduce the risk of cardiac arrhythmias (42). With modern contrast-medium doses, an injection over 1 minute produces a good nephrogram, and slower injections, over 2 to 3 minutes, are advisable in patients with cardiac disease, elderly people, and neonates. A needle should be left in the vein for the first 15 minutes after contrast-medium injection, and medical personnel should be available throughout this time.

If, for reasons of cost, nonionic contrast media cannot be used in all patients, they should be used selectively in patients at increased risk of reaction or complication. These include patients with a significant previous contrast-medium reaction, asthma, or renal failure, especially patients with diabetes. The use of nonionic media is also recommended in patients with myeloma or cardiac disease, as well as in elderly people, neonates, and infants. *Steroid prophylaxis* (e.g., prednisolone, 30 mg twelve and two hours before urography) should be used in patients with a history of previous significant contrast reaction and in those with a strong history of allergy (43,44). Prophylactic antihistamines are not helpful (24), and pretesting with a preliminary small intravenous injection of contrast medium is no longer considered useful (27,45,46).

Management of Reactions

In all severe reactions, intravenous steroids should be given. Management is otherwise symptomatic, and full resuscitation facilities should be available (47).

Technique of Urography

Preparation

Dehydration is not necessary, but fluids are commonly restricted to 500 mL in the 4 hours before the examination to reduce the hazards of vomiting. It may be helpful to give a laxative for the 2 days before the urogram.

Plain Films

The importance and choice of plain films have been discussed.

Contrast-Medium Dose and Administration

A dose of 300 mg of iodine per kg should be given to patients with normal renal function. Pyelogram quality is unaffected, whether contrast medium is given by bolus or infusion (7); the bolus technique generally is preferred because it gives a higher peak plasma iodine concentration and a denser nephrogram.

Postcontrast-Medium Films

A film is obtained immediately after contrast-medium injection. At this time, the nephrogram is densest and optimal for assessing the parenchyma. A film taken 5 minutes after the end of the injection will show some calyceal filling. If there is no evidence of obstruction or other contraindications (aortic aneurysm, abdominal mass, or recent surgery), ureteric compression then is applied with small inflatable bags placed over the ureters as they cross the pelvic brim. A film coned to the renal areas is obtained after compression has been in place for 5 minutes. This film will show the pelvicalyceal systems and ureters filled with contrast medium and is vital for demonstrating early calyceal and papillary pathology. A full-length abdominal film is obtained immediately after compression is released, and this should show the ureters and bladder. A further full-length film is obtained immediately after the patient has emptied the bladder. This not only allows assessment of bladder emptying, but also permits assessment of the upper tracts when the effects of ureteric compression are no longer present.

Additional films may be helpful in a number of situations. Early tomography (nephrotomography) is used when a renal mass or polycystic kidney is suspected, when the pelvicalyceal system has not been visualized at previous urography, or where bowel shadowing obscures the kidneys. *Oblique renal views,* with ureteric compression applied, are used when calyceal or papillary disease is suspected (especially papillary necrosis and tuberculosis). If ureteric filling is delayed, *prone films* may be helpful. The urographic technique also is modified in a number of special circumstances, suspected pelviureteric junction obstruction, and suspected renal artery stenosis, all of which are described in the following.

Normal Urographic Findings and Common Variants

Friedland has given an excellent account of variants of normal at urography (48).

Renal Size

Renal length measured on the urogram is a good indicator of renal weight (49) despite the problems of radiographic magnification, variable renal axis, and contrast-medium-induced increase in renal size (50). In adults, normal renal length approximates to the first three and a half lumbar vertebrae and their disc spaces (51). Generally, a kidney measuring less than 11 cm is abnormal. The left kidney is usually longer than the right by up to 2 cm. Duplex kidneys are longer than kidneys with single pelvicalyceal systems.

Renal Position

The kidneys lie obliquely in the retroperitoneum, with the upper poles posterior and medial to the lower poles. The left kidney lies higher than the right in the majority of cases.

Renal Parenchyma and Outline

Measuring the distance between the line joining the tips of the papillae and the renal outline should assess parenchymal thickness. Because of renal obliquity, the measurements at the poles are greater than the midkidney measurements. The four polar measurements are usually equal (52).

Splenic and Hepatic Impressions and Humps. Splenic or, less commonly, hepatic compression may flatten the superolateral kidney during development. A "hump" in the renal outline occurs just below this area. The features distinguishing it from a space-occupying lesion are that the nephrogram in the hump is homogeneous and that a calyx passes laterally toward it so that the normal measurement from the renal papilla to the renal outline is maintained (Fig. 12-1).

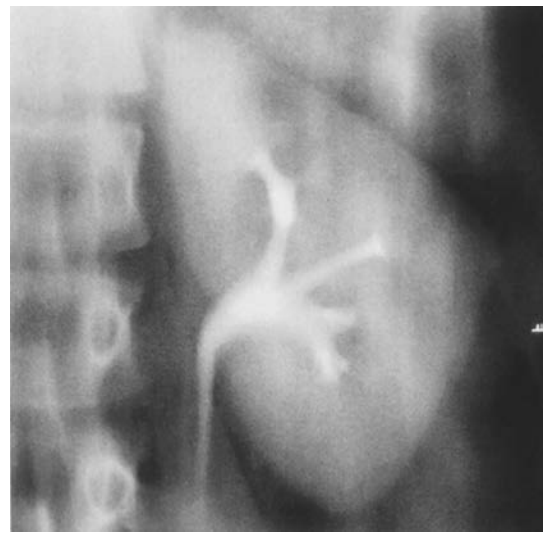


FIGURE 12-1. Splenic hump shown on tomogram.

Fetal lobation may persist into adult life and will be seen as a sharp indentation, which is often in the inferolateral renal outline, commonly symmetrical, and lying between two calyces.

Septum of Bertin. The fused cortices of adjacent lobes may protrude deep into the renal substance and displace the calyces, simulating a mass lesion—one variety of pseudotumor (53). This anomaly is most common in the midkidney and is often associated with a duplex collecting system and with a small truncated calyx that is draining the apparent mass (Fig. 12-2).

FIGURE 12-2. Renal pseudotumor. Enlarged septum of Bertin, causing splaying of the upper and middle calyces.

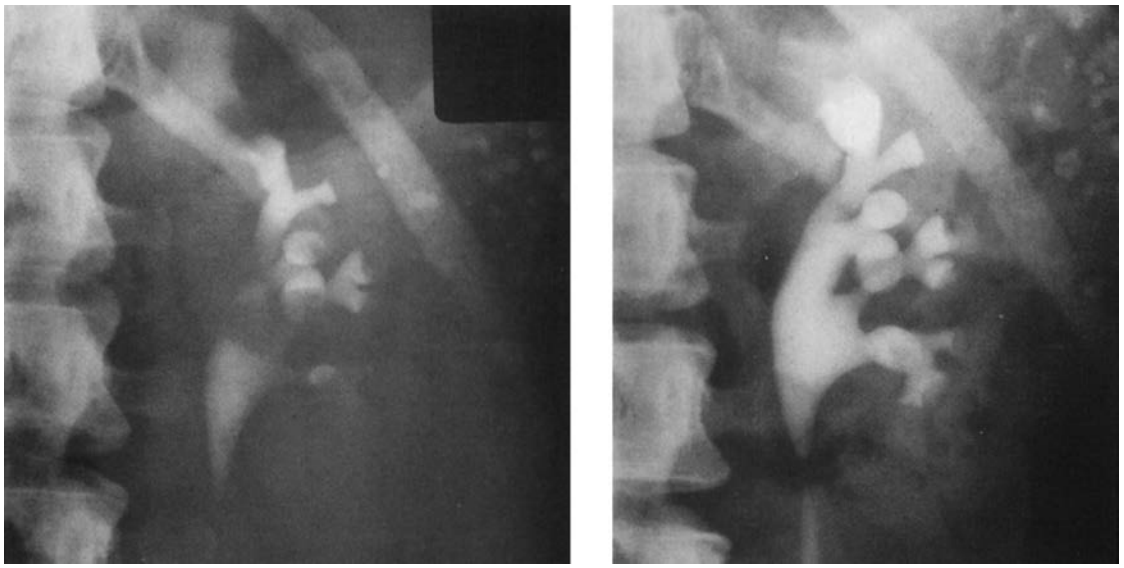


FIGURE 12-3. Vascular impressions on the pelvicaliceal system (*left*) are no longer seen on the film with ureteric compression (*right*).

Pelvicaliceal System and Papillae

Variation in the number and distribution of minor and major calyces is very wide. Calyces may open directly into the pelvis or infundibula of varying length. *Compound calyces* occur throughout the kidney, but especially at the upper and lower poles. *Vessels crossing the pelvicaliceal system* may simulate filling defects, but films with adequate compression usually clarify the diagnosis (Fig. 12-3).

Renal Sinus

The fat in the renal sinus may be apparent during urography, especially if tomography is performed. The amount of sinus fat increases with loss of renal parenchyma, and such an increase may occur during aging. Sometimes this can occur as an isolated anomaly in younger patients, producing uniform stretching of the calyceal infundibula, so-called renal sinus lipomatosis (54).

RENAL ULTRASONOGRAPHY

Ultrasound imaging depends on the reflection of high-frequency sound waves from the interfaces between tissues of different acoustic properties. The information from the reflected sound waves is processed to give a cross-sectional image. In current real-time scanners the transducer head contains multiple transducer elements and the sound beam is automatically moved rapidly through the tissue being examined. The moving gray-scale image so produced is viewed as soon as the transducer head is placed on the body.

The frequencies used in renal scanning are usually 3.5 to 5.0 MHz. Overall image resolution is on the order of 1.5 to 2.0 cm, but depends on the difference in acoustic properties of the tissues being examined. Thus, a 1.0-cm renal cyst has properties markedly different from those of the surrounding parenchyma and will be visualized on a good-quality image, whereas a 1.0-cm solid renal tumor that does not deform the renal contour may well not be shown. Higher frequencies, with improved resolution, can be used for structures closer to the transducer. Thus, 7.5-MHz frequency transducers usually are used for transrectal examination of the prostate.

Ultrasound is reflected by both bone and gas. To image structures deep to these requires that the transducer be angled around the obstacle or that an approach be used such that the sound beam does not encounter bowel gas or bone—thus, the posterior and flank approaches used in renal examination.

Despite recent adverse comments by communication media, no harmful effects of this type of pulse-echo ultrasound technique at the intensities used in diagnostic practice ever have been reported in animals or humans (55). The examination is simple and relatively quick for the patient and involves no x-irradiation or contrast media.

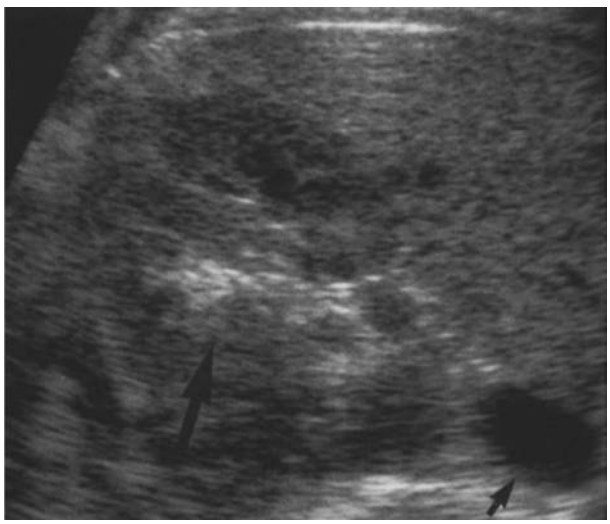
Examination Technique and Normal Appearances

Renal

The patient should empty the bladder before the examination, because a full bladder can cause retention of urine in the upper tracts and give the spurious impression of pelvicaliceal dilatation (56). The long axis of the kidney is identified. Scans along this axis, parallel to it, and transverse to it are carried out so that the whole renal area is covered. Scanning through the flanks, with the patient either in the supine oblique or the lateral decubitus position, is the most favored technique (Figs. 12-4 and 12-5). Supine or supine oblique scanning of the right kidney is often helpful, using an anterior approach with the liver as a window. Prone renal scanning sometimes is helpful, but suffers the disadvantage of loss of resolution as the sound beam traverses the back muscles. Small kidneys with increased parenchymal reflectivity (as in chronic renal failure) may be difficult to identify. Good-quality kidney images are not obtained in obese patients, because fat transmits ultrasound poorly.

The renal outline is ovoid on longitudinal scans, and it is rounded on transverse scans. The renal parenchyma shows a fine, low-level echo pattern of less reflectivity than the normal liver and spleen. In a good-quality image, the lucent medullary pyramids can be differentiated from the surrounding cortex, and the arcuate vessels appear as bright echoes at the bases of the pyramids (Fig. 12-5) (57). A cluster of bright echoes, called the sinus echoes, which are largely produced by the fat in the renal sinus, and, to a lesser extent, by the pelvicaliceal

A



B

C

FIGURE 12-4. Normal ultrasound scans of the right kidney obtained through the flank. **A:** Longitudinal scan. **B:** Transverse scan through hilum. **C:** Transverse scan through lower pole. Long arrow indicates renal sinus; short arrow indicates inferior vena cava.

system and vascular structures, is in the center of the kidney. The normal collecting system usually is not visualized, although some variants (e.g., a large major calyx or renal pelvis) may be appreciated as a central fluid collection in the sinus. The collecting system is more likely to be visualized under conditions of diuresis (e.g., after contrast medium has been given for urography).

FIGURE 12-5. Normal longitudinal ultrasound scan of the right kidney obtained in the anterior oblique position. Note the pyramid (*long arrow*) and arcuate artery (*short arrow*).

Renal length can be measured accurately in the majority of subjects, provided that the measurement is made along the long axis of the kidney (58). In some patients, intervening ribs mean that adequate scans for length measurement will not be obtained. Renal length measurements obtained at ultrasonography are smaller than those obtained at urography because neither the radiographic magnification effect nor the osmotic diuretic effect of contrast medium is present (59). Normal mean renal length should be between 9.4 and 12.2 cm (60).

Ureter

The normal adult ureter is not visualized. A dilated ureter may be seen over a few centimeters as it leaves the renal pelvis and for its last few centimeters before it enters the bladder, but bowel gas usually obscures the rest of its course through the retroperitoneum.

Bladder

Ultrasound measurements of the bladder in three planes before and after bladder emptying provide a simple method of assessing bladder residual volumes (61). This method is acceptable for most urologic assessment of bladder emptying in bladder outflow obstruction and neurogenic bladder, but small residual volumes (less than 50 mL) are not measured accurately. Intrinsic bladder abnormalities such as tumors and calculi can be demonstrated (Fig. 12-6).

FIGURE 12-6. Transverse ultrasound scan of the bladder. Note the polypoid tumor (*arrow*).

Prostate

An enlarged prostate may be shown by suprapubic scanning of the full bladder, but a complete prostatic examination requires the use of a rectal transducer. With high-frequency (e.g., 7.5 MHz) rectal transducers, high-resolution images of the whole prostate gland are obtained. Depending on transducer design, images are obtained either in the longitudinal and transverse planes or multiple planes.

The common prostatic diseases have typical appearances; thus, benign prostatic hypertrophy causes symmetrical enlargement of the transition zone adjacent to the urethra. The abnormal area is usually hypoechoic and well defined, may contain cysts or calculi, and compresses the remaining prostate tissue posteriorly (Fig. 12-7). Prostatic carcinoma classically causes hypoechoic, poorly defined, asymmetrical masses in the peripheral zone of the prostate (Fig. 12-8). However, there is considerable overlap in appearance among the different pathologic processes and the findings often are nonspecific. Prostatitis also affects the peripheral zone and may mimic carcinoma. In one series, only 20% of posterior hypoechoic masses less than 1.5 cm in diameter were caused by carcinoma (62). Carcinoma may develop anteriorly in the periurethral region. Sensitivity, too, is poor, with a detection rate of only 60% for carcinomas greater than 0.5 cm in a multicenter trial (63).

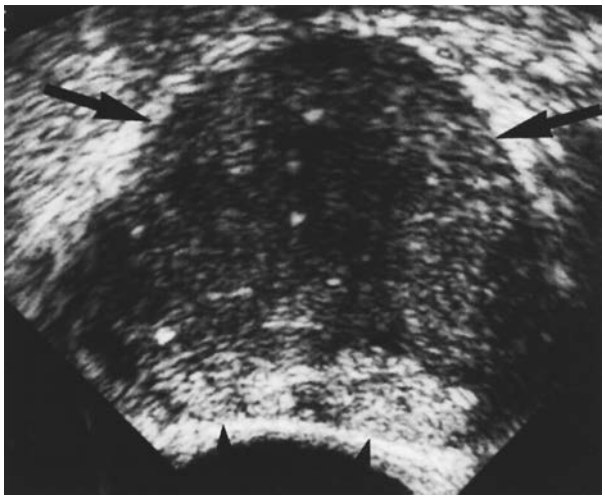


FIGURE 12-7. Benign prostatic hypertrophy. Transverse transrectal ultrasound scan shows enlarged central transition zone (*large arrows*) compressing peripheral zone (*small arrows*). (Posterior gland is in the lower part of the image.)

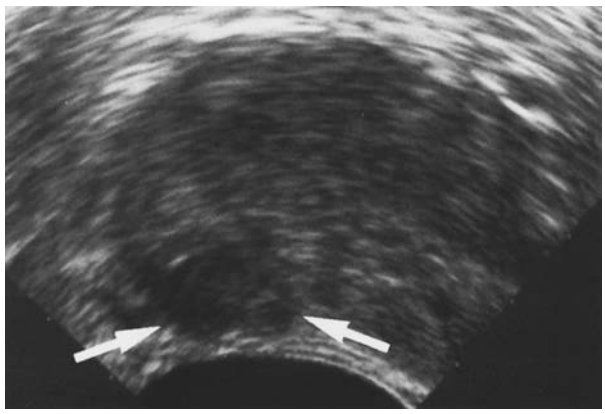


FIGURE 12-8. Carcinoma of prostate. Transverse transrectal ultrasound scan shows the hypoechoic tumor nodule (*arrows*) in the peripheral zone. (Posterior gland is in the lower part of the image.)

For these reasons, screening for prostatic carcinoma with ultrasonography alone is not considered appropriate (64). Nonetheless, transrectal prostate scanning is a very useful diagnostic tool when suspicious findings are obtained on rectal examination, the serum prostate-specific antigen level is elevated, or prostatic abscess is suspected (64). Transrectal ultrasound-guided biopsy of suspicious lesions can be undertaken and systematic sampling of the prostate for malignancy also can be carried out (e.g., eight systematic biopsies) (65).

Doppler Studies

The Doppler principle is used to identify flowing blood, which reflects sound at a frequency other than that transmitted by the transducer. Duplex Doppler equipment combines flow detection with imaging so that the blood flow in specific vessels can be sampled. Doppler color flow mapping samples flow in a block of tissue, providing a perfusion map that is color coded to indicate the direction of flow. This facilitates the identification of small vessels for subsequent duplex waveform analysis and also allows the overall perfusion of organs to be studied.

A major application of Doppler studies in renal diagnosis is in the transplanted kidney. The main renal artery and vein can be examined for evidence of stenosis or occlusion (66), and perfusion of the smaller intrarenal vessels can be evaluated. Examination of the native renal arteries to detect renal artery stenosis has been less successful because of access problems (67). Examination of the flow patterns in the intrarenal vessels has been proposed both for detecting renal artery stenosis (68,69) and for diagnosing renal obstruction (70). Renal perfusion also may be assessed using power Doppler, a method that provides information on Doppler signal energy rather than velocity.

Ultrasound contrast media contain stabilized gas bubbles, which produce enhancement of the Doppler signal. Their possible role in the assessment of the renal vasculature, the detection of renal parenchymal abnormalities, and the characterization of renal masses is being assessed (71,72).

RENAL NUCLEAR MEDICINE

Principles

An understanding of renal physiology and pathophysiology is necessary for the intelligent use of renal radiopharmaceuticals. Conversely, knowledge of the renal handling of radiopharmaceuticals is required for the correct application of nuclear

medicine techniques to disorders of renal function that occur in clinical practice. To understand the transference from anatomic and physiologic reality to a physics test using radionuclides, a physiologic (mathematical) model is applied. The term *isomorphic model* describes the model that is the best compromise between the realistic and the simplistic. The validity of an isomorphic model in a given pathophysiologic situation justifies the use of a particular form of mathematical analysis of the results of a radionuclide test for their application to clinical practice.

The renal function that dominates the clinical assessment of how the kidneys are working is the kidney's ability to remove substances from the blood, that is, their *uptake function*. The different radiopharmaceuticals are taken up with different extraction efficiencies at the different anatomic sites. For chromium ethylene diamine tetraacetic acid (^{51}Cr EDTA) and technetium diethylene triamine pentaacetic acid (^{99}Tc^{m} DTPA), uptake is by glomerular filtration, with 20% extraction efficiency. For radioiodinated orthohippurate (^{131}IOIH and ^{123}IOIH), uptake is by tubular secretion, with 83% extraction efficiency. Mercaptoacetyl triglycine (^{99}Tc^{m} MAG3) is the technetium-labeled substitute for IOIH (73–77). It is now the radiopharmaceutical of choice for dynamic renal studies. A new agent Tc-99m EC, Ethylene Dicysteine (78–80), has the advantages that it is tubularly secreted slightly more than Tc-99m MAG3 with less protein binding and does not require a boiling step in its preparation; however, it has the disadvantages of not being licensed in the United States or the European community. (It is available from Izinta, Hungary.) For technetium dimercaptosuccinate (^{99}Tc^{m} DMSA) and ^{99}Tc^{m} aprotinin, uptake is by tubular fixation. For all these compounds, the kidneys are the only organs that remove them from the blood and their only site of exit from the body. The rate of uptake of the compounds by the kidneys, therefore, equals their rate of loss from the blood—the blood clearance.

A *compartmental model* is used in the analysis of the renal uptake of radiopharmaceuticals. The fundamental assumption of this model is that the rate of mixing within a compartment is more rapid than the rate of exchange between compartments. This applies well to the movement of radiopharmaceutical between the blood and extracellular fluid spaces, so that a compartmental (exponential) analysis is appropriate for studies of blood clearance and renal uptake of compounds. The uptake function measured depends on the radiopharmaceutical used. Thus, IOIH gives effective renal plasma flow (ERPF) and ^{51}Cr EDTA gives GFR. It should be noted that the definition of renal clearance has been altered so that it concerns movement of a compound from blood to kidney rather than from blood to urine. The compartmental model can be used to help in the interpretation of the results obtained in various diseases. For example, in the nephrotic syndrome with edema, the size of the extracellular fluid compartment is increased, and the model indicates the need for both delayed sampling and the appropriate analysis of the data obtained.

For a kidney with a million nephrons, there are a million sites of uptake for IOIH or ^{51}Cr EDTA. Although the nephrons are anatomically separate, they are physiologically interdependent. There is a hierarchy of interacting control systems that interrelate renal plasma flow, GFR, and tubular function through tubuloglomerular balance, nephron autoregulation, and the juxtaglomerular apparatus for cortical nephrons. The same interdependence applies for a kidney with 250,000 functioning nephrons. This means that there is a proportionality between the uptake of ^{51}Cr EDTA or ^{99}Tc^{m} DTPA, MAG3, and IOIH for a pair of kidneys. For example, if the left kidney with a million nephrons takes up 80% of the ^{99}Tc^{m} DTPA and the right kidney with 250,000 nephrons takes up 20% of the ^{99}Tc^{m} DTPA, exactly the same proportions—80% and 20% of IOIH—are taken up by the left and right kidneys under steady-state conditions. Thus, the filtration fraction of one kidney equals that of the other. This is an expression of the intact nephron hypothesis (81) and has been confirmed experimentally (82). It means that any of the compounds can be used to determine the contribution of one kidney to total function. Thus, using a compartmental model, total renal function, whether GFR or ERPF, can be determined by the blood clearance of ^{51}Cr EDTA or ^{99}Tc^{m} DTPA, ^{99}Tc^{m} MAG3 or IOIH, respectively, but relative renal function may be equally well determined by *any* of these compounds.

The compartmental model is not suitable for the study of the renal parenchyma, because the kidney is made up of tubes along which the filtered fluid travels, rather than of spaces in which rapid mixing occurs. The isomorphic model chosen for the movement of fluid along the nephron is a *linear system* that models a series of events in time in which one event follows another. The linear system is based on a number of assumptions. These assumptions include the absence of backflow and stationarity. *Stationarity* means that if a given input gives a particular response, then the same input given later will give the same response. *Linearity* means that the total response to a series of inputs is the sum of the individual responses to the individual inputs. The response of kidney to the blood clearance of a radiopharmaceutical is the *renogram,* the activity–time curve recorded from the kidney. The renogram can be considered as the sum of a series of responses to a succession of individual spike or delta inputs. The term *deconvolution analysis* is applied to the process of obtaining the individual response to a delta input from such complex data as the blood-clearance curve and the renogram. This analysis enables one to obtain the renal response as if to a spike injection into the renal artery; this renal response is called the *impulse retention function*. The mean transit time and the distribution of transit times through the kidney can be obtained from this function.

It can be shown that the majority of nephron transit times are very similar, accounting for the fact that the peak of the renogram from a normal kidney is sharp. This occurs because of nephron autoregulation, a property of the nephrons whose glomeruli lie in the outer cortex and have a juxtaglomerular apparatus, such that each nephron responds in the same way to the perfusion pressure. Because ^{99}Tc^{m} DTPA and IOIH are both nonreabsorbable solutes, their transit time along the nephron depends on two main factors: the urine flow rate in the collecting ducts, which is determined by the functions of the medulla, and the rate of salt and water reabsorption in the tubules, which depends on the relative pressure in the tubular lumen and the peritubular capillary, among other things. The nephron transit times through the renal parenchyma, therefore, can be divided into a minimum transit time common to all nephrons and related to urine flow, and a distribution of the nephron transit times, which is also called the parenchymal transit-time index (PTTI). The transit time through the pelvis depends on its capacity, on the urine flow and turbulence, and on the resistance to outflow. The relationship between the whole-kidney transit time and its subdivisions is shown diagrammatically in Figure 12-9. A summary of their use in clinical practice is given in Table 12-1. The linear system model of the kidney and the determination of ^{99}Tc^{m} DTPA or ^{99}Tc^{m} MAG3 transit times make an important contribution to clinical management of obstructive nephropathy and renovascular hypertension.

Techniques

The determination of the uptake and transit times of radiopharmaceuticals in the kidney requires the external detection of the quantity and distribution of the gamma rays emitted by the radionuclide label. This is performed with a gamma camera

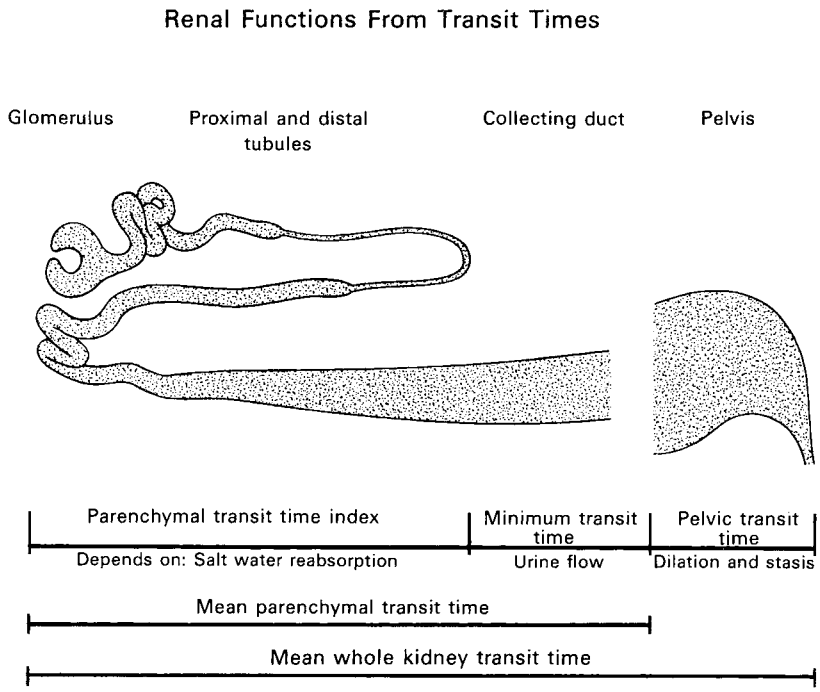


FIGURE 12-9. Diagram of a nephron showing the breakdown of transit times. Minimum transit time is shown as common to all nephrons. It represents collecting duct transit through the medulla and is related to the urine flow rate, which is controlled there. The parenchymal transit-time index varies between nephrons and is related to their salt and water reabsorption.

linked online to a computer. The properties of the main radionuclides used in renal studies are shown in Table 12-2

Radiopharmaceutical Preparation

Unlike contrast media, which are presented as an ampule of sterile fluid, radiopharmaceuticals are prepared on the day of use. Full aseptic precautions in a properly designed radiopharmacy are required. The dispensing of $^{99}Tc^{m}$ from the molybdenum generator and the preparation of $^{99}Tc^{m}$ MAG3 from commercially available kits must be undertaken by technical staff trained both in pharmacy and radiation protection. Quality control is essential to ensure that the product is sterile, pyrogen-free, radiochemically pure, and dispensed in the correct dose for administration at the time of the patient's test. The activity of the injection always must be confirmed in an ionization chamber, and allowance must be made for decay of the radionuclide. $^{99}Tc^{m}$ DTPA, when properly prepared, is a good analog of inulin for glomerular filtration measurement, but some preparations are relatively unstable and will liberate some free $^{99}Tc^{m}$ as pertechnetate if kept for several hours. This is taken up by the stomach after injection and may confuse the interpretation of the images. Similarly, a poor preparation of ^{123}IOIH may contain free ^{123}I that will show stomach uptake and reduce the accuracy of ^{123}IOIH as a measure of ERPF. These effects may be minimized by storage of the injection in a refrigerator at 4°C and by use of a dark glass bottle for IOIH. MAG3 is in the form of a benzoyl precursor to which $^{99}Tc^{m}$ generator eluate is added. The vial is placed in a boiling-water bath for 10 minutes, which splits off the benzoyl group, leaving the $^{99}Tc^{m}$ binding to the three nitrogens and the one sulfur of the MAG3. The kit is then divided into four portions using four sterile syringes. Each is capped and frozen at 4°C. When the patient arrives, the syringe is thawed under a table lamp and used. This freezing step reduces the rate of production of the liver-excreted contaminants by 100-fold (83).

Patient Preparation

The patient should be normally hydrated and, to ensure this, 300 mL of fluid is given orally half an hour before the test. A reassuring explanation of the technique should be given to the patient before he or she enters the gamma camera room. Anxiety and pain cause fluctuations in renal blood flow and produce unsteady-state conditions that affect data analysis. The patient empties the bladder immediately before and after the test, the times and volume of urine being recorded to confirm that the urine flow is suitable; between 1.5 and 4.0 mL/minute is preferred. Emptying the bladder immediately after the test and again an hour later helps to reduce further the radiation to the bladder wall and the small dose to the gonads (which is less than one-fifth of that obtained from intravenous urography).

TABLE 12-1

CLINICAL USE OF RENAL TRANSIT TIMES

	Parenchymal transit-time index	Parenchymal mean transit time	Pelvic transit time
Renovascular disorder	Normal or increased	Increased	Normal
Obstructive nephropathy	Increased	Increased or normal	Increased
Dilated renal pelvis—not obstructive	Normal	Normal	Increased

TABLE 12-2

PROPERTIES OF RADIONUCLIDES

	Gamma-ray energy (meV)	Half-life	Beta emission	Camera detection efficiency
$^{99}Tc^{m}$	0.14	6 hr	None	High
^{123}I	0.159	13 hr	None	High
^{131}I	0.364	8 d	High	Low
^{51}Cr	0.32	27.8	None	Low

Radionuclide Injection and Data Collection

The gamma camera is positioned so that its face is set back about 30 degrees from the vertical plane. The patient sits on a comfortable backless chair with side arms and reclines against the camera face. With the arm abducted, the injection of 10 mCi (400 MBq) of $^{99}Tc^{m}$ DTPA or 3 mCi (120 MBq) of $^{99}Tc^{m}$ MAG3 in less than 1 mL is given rapidly into a deep antecubital vein. Alternatively, a "butterfly" needle, with a sterile extension tube and three-way tap, can be used. The injectate is introduced into the extension tube and flushed in with 10 to 20 mL of saline. This system also allows access for a subsequent injection of 40 mg of furosemide for the diuresis technique. Data are collected at a 10 second frame rate for 20 minutes, or longer if the pelvis is not visualized, and for at least 10 minutes after an injection of furosemide when this is used. Furosemide (40 mg) should not be injected before 18 minutes have elapsed in the adult, and it may be necessary to wait for 30 minutes in a child (0.5 mg/kg body weight).

Hazards

Reactions to radiopharmaceuticals are much less frequent than to intravenous contrast media (84). The rare reactions that occur usually take the form of a feeling of warmth and faintness. The injection of furosemide for diuresis renography may cause postural hypotension.

The absorbed radiation doses for the radiopharmaceuticals used in renal disorders are shown in Table 12-3. If the renal outflow is obstructed and there is good uptake of ^{131}IOIH, then the dose to the kidneys may increase over 100-fold because of its 8-day half-life. Because of its 13-hour half-life, ^{123}IOIH gives no substantial increase in absorbed dose in pathologic conditions and is now used instead of ^{131}IOIH. All the radiopharmaceuticals give a substantially lower gonadal dose than the intravenous urogram, provided the bladder is emptied shortly after the test and again an hour later.

Normal and Abnormal $^{99}Tc^{m}$ DTPA and $^{99}Tc^{m}$ MAG3 Images

Images on transparent film (analog images) usually are combined at 30-second intervals for the first 180 seconds and then at 5-minute intervals for the remainder of the study (Fig. 12-10). The following features should be noted:

1. The length of time the activity in the left ventricular cavity is visible; it is prolonged with renal impairment.
2. The patency and tortuosity of the aorta and possible aneurysm.
3. Whether the times of arrival and tracer distribution are equal in the two kidneys; prolongation on one side may occur with an inflow disorder.
4. The site and position of the kidneys relative to the liver and spleen; possible space-occupying lesions may be between the kidney and these organs.
5. Whether the cortical outline of each kidney is complete and whether there are any defects in the parenchyma—possible tumor, cyst, parenchymal infection, or scar.

A dilated calyx should fill at the pelvic retention stage but not if it contains a stone. A cyst in the parenchyma does not show any tracer uptake, whereas a vascular tumor may show initial tracer activity. This may persist for longer than that in adjacent normal tissue and be followed by a focal defect in the

TABLE 12-3

ABSORBED RADIATION DOSES FROM RENAL RADIOPHARMACEUTICALS

Compound or test	Usual activity given (mCi)	Usual activity given (MBq)	Absorbed dose: Kidney (mrad)	Absorbed dose: Gonad (mrad)	Whole body: effective dose equivalent (mSv)
^{51}Cr EDTA	0.1	4	14	6	0.01
$^{99}Tc^{m}$ DTPA	10.0	400	380	140	4.0
$^{99}Tc^{m}$ MAG3	2.5	100	160	38	1.1
$^{99}Tc^{m}$ DMSA	3.0	120	2250	42	1.5
^{123}IOIH	1.5	60	30–1000[a]	23	0.9
^{131}IOIH	0.5	20	50–10,000[a]	30	1.0
Intravenous urography			400	800	4.5
Selective renal angiography			12,000	80	10.0
Cinecystourethrography				2500	6.0

MBq, megabecquerel; *mSv,* milliSievert.
[a]In a well-functioning obstructed kidney.

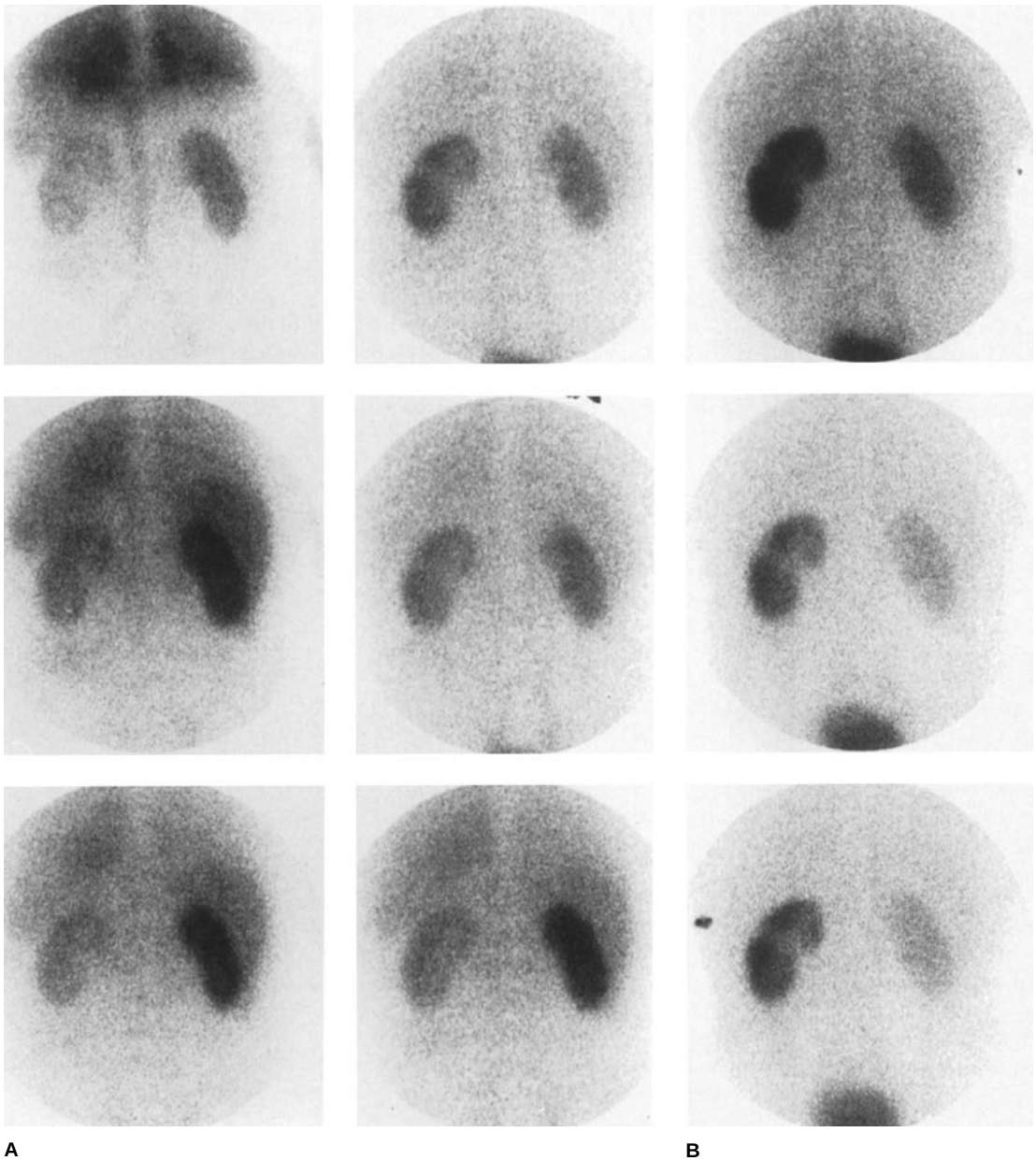
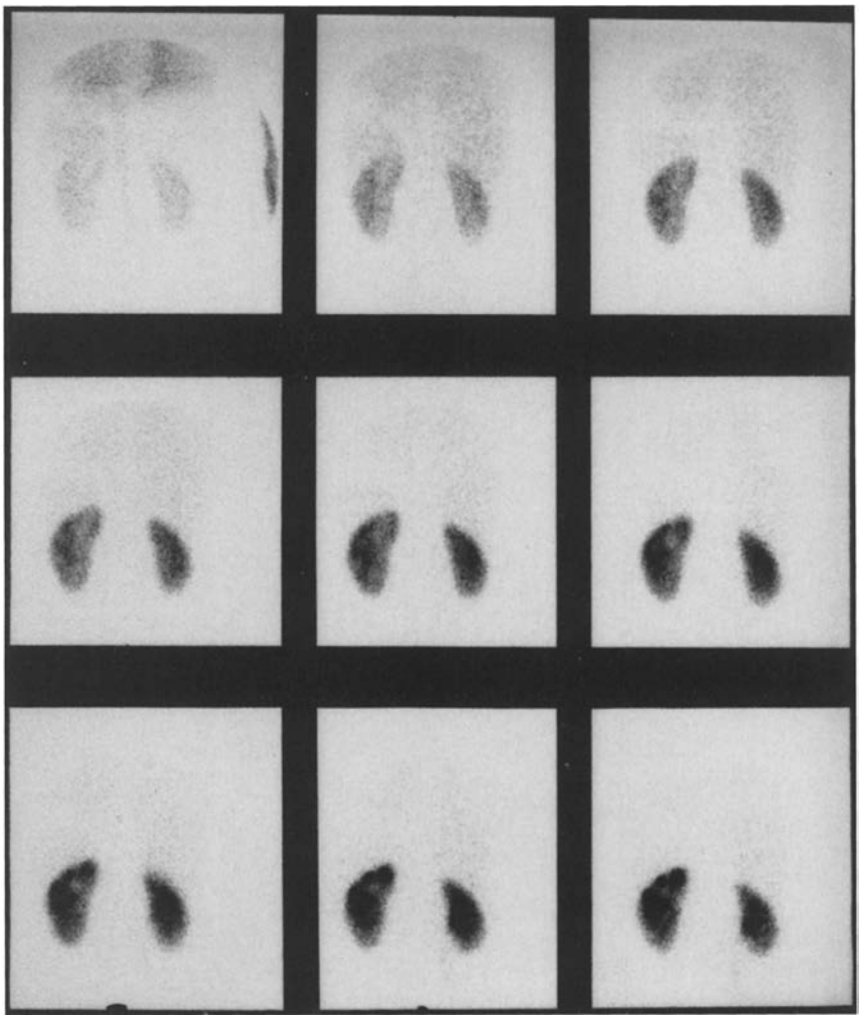


FIGURE 12-10. $^{99}Tc^{m}$ DTPA renal study. Left kidney (*on the left side*) with outflow obstruction due to stone; normal right kidney. **A:** Images taken at 0 to 30, 30 to 60, and 60 to 90 seconds, left side descending, and at 90 to 120 seconds and 5 and 10 minutes, right side ascending. **B:** Same study images at 15, 20, and 25 minutes descending. Note the normal uptake and excretion of tracer by the right kidney. The left kidney shows reduced uptake in the early images as compared with the right and contributes 27% of total uptake function (right, 73%). At 15 minutes, 40 mg of intravenous furosemide is given. Note the normal excretion of tracer by the right kidney and failure to excrete by the left kidney (left obstructing uropathy). The right parenchymal transit-time index (PTTI) was 120 seconds (normal, less than 156 seconds); the left PTTI was prolonged to 260 seconds, confirming obstructive nephropathy. **C:** $^{99}Tc^{m}$ MAG3 renal study in a similar patient. (Posterior views; left kidney is on the left side.) Note the reduced background and better contrast as compared with $^{99}Tc^{m}$ DTPA. Images taken at 30-second intervals (*top left to bottom right*). **D:** Images taken at 5-minute intervals, the first at 5 minutes. *Top left to bottom right:* Furosemide, 40 mg, given at 18 minutes. Note the normal excretion of tracer by the right kidney and the partial response to furosemide by the left kidney. The right PTTI was 39 seconds (normal, less than 156 seconds) and the left 266 seconds, confirming obstructive nephropathy (owing to stone). (*continued*)

distribution of activity resulting from the absence of normal nephrons.

As corticopelvic transfer occurs with the tracer moving from parenchyma to pelvis, the lateral edge of the kidney, noted on the 1- to 2-minute frames, appears to move medially. Pelvic, calyceal, or ureteric retention of tracer, or all of these, occurs when the capacity of these structures has increased through dilatation, but this cannot be considered to indicate that obstruction is present. The ureter should not be considered to have retained tracer unless its whole length or that down to a block persists over several images. Blobs of activity in the ureter are not significant and do not mean ureteric "spasm" or



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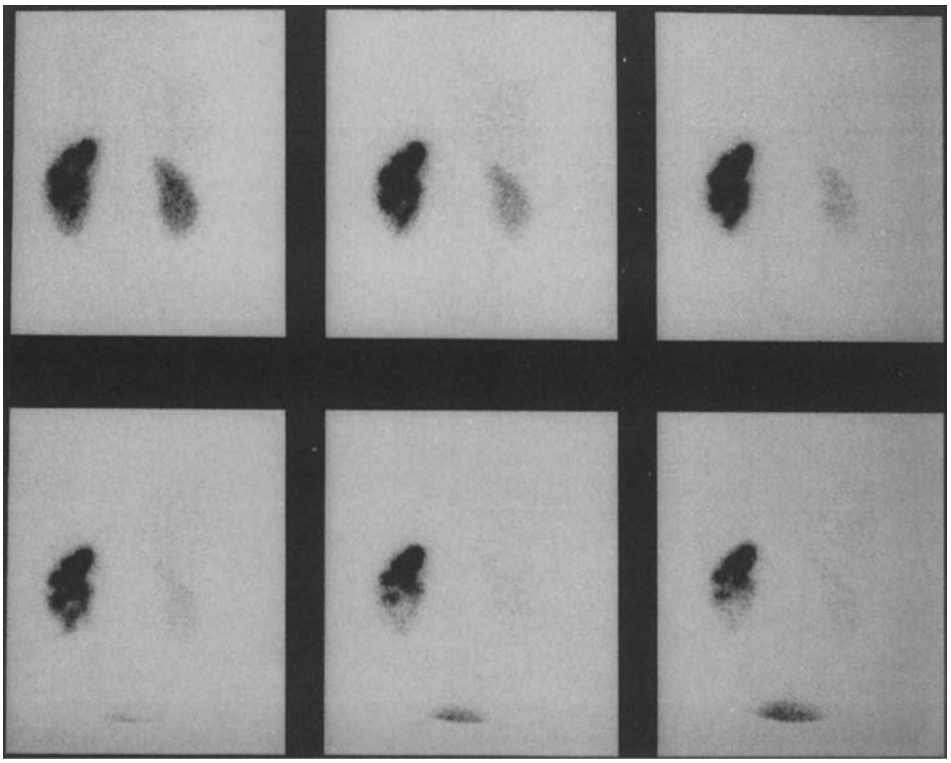

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FIGURE 12-10. (*Continued*)

"hold-up." Activity in the bladder is seen toward the end of the test, and a diverticulum or ureterocele may be observed. If the patient was imaged supine or if there is pelvic retention of activity at the end of the study, a postmicturition picture should be taken.

Normal and Abnormal Measurements

The measurement of overall renal uptake function requires that the rate of uptake of the radiopharmaceutical by the kidneys equals its rate of loss from the blood. For the estimation of the GFR, the compound must have properties that are analogous to inulin. ^{51}Cr EDTA and high-quality preparations of ^{99}Tc^{m} DTPA meet these requirements. The rate of uptake of ^{99}Tc^{m} DTPA by the kidneys per unit of time, K(t), is equal to its rate of supply in plasma flowing to the kidneys, which is given by the product of the renal plasma flow (RPF), the plasma concentration, P(t), and the fraction of plasma that is filtered, the filtration fraction, FF. Thus,

$$K(t) = RPF \times P(t) \times FF = RPF \times P(t) \times (GFR/RPF) = GFR \times P(t) \quad [1]$$

The rate of loss of ^{99}Tc^{m} DTPA from the plasma depends on the dose administered, D, and the clearance rate, λ. This is equal to the rate of uptake of ^{99}Tc^{m} DTPA by the kidneys.

$$K(t) = D \times \lambda \quad [2]$$

Substituting Eq. 1 in Eq. 2, we have

$$GFR \times P(t) = D \times \lambda$$

Thus,

$$GFR = D \times \lambda / P(t) \quad [3]$$

To measure the GFR, it is necessary to measure accurately the dose administered, the rate of clearance of the compound from the blood, and the plasma activity of the compound at particular times. This is not straightforward.

Glomerular Filtration Rate

The GFR can be measured with 100 μCi (4 MBq) of ^{51}Cr EDTA or 2 mCi (80 MBq) of ^{99}Tc^{m} DTPA as part of a full renal dynamic study. Blood samples are taken at 3, 4, 5, and 6 hours and at 24 hours if there is edema or renal failure. The values of the activities of the plasma samples are plotted against time on semilogarithmic paper and a graph is drawn through the points. A tangent is drawn through the apparently straight-line portion of the graph and extrapolated to cut the vertical axis at time zero to give the value of P_o. GFR is then determined from Equation 3.

Alternatively, the counts of the plasma samples taken before and after injection; the sample times; the counts of the standards, background, and syringe washings; and the patient's height, weight, age, sex, and name are entered into the computer programmed to calculate the GFR. The result is printed out together with an error estimate, both uncorrected and corrected for body–surface area. Single or double exponential analysis can be performed on the data, conventionally or by the technique of Nimmon and associates (85).

A number of other approaches have been developed in attempts to simplify the technique of measurement of GFR even further. These simplifications are particularly appropriate when GFR is to be monitored serially over a period in one patient. It is best to undertake a full measurement of GFR in the most accurate way possible first, collecting data for the simpler approach at the same time and then to use the simpler approach for the repeat studies.

The basis of the single-plasma-sample approach depends on the fact that the volume of distribution of the tracer in the steady state, V, is given by the dilution of the activity of the administered dose, D (administered volume × activity/mL) in the plasma of the time of sampling, P(t). Thus,

$$V = D/P(t) \quad [4]$$

There will be a moment when the rates of exchange between the two compartments are equal because the tracer in the plasma is in the process of equilibration with the extracellular fluid. At this time, the rate of loss of tracer is proportional to its volume of distribution, V; thus, GFR α V.

This equilibrium is established at 3 hours in adults. A standard graph is plotted, using the values of GFR obtained with the standard technique and the values of V determined from Eq. 4. One formula for GFR from V (liters) is given by Constable and associates (86).

$$GFR = 24.5(V - 6.2)^{1/2} - 67 \text{ mL/minute} \quad [5]$$

The technique is satisfactory for serial studies in the same patient only when the GFR is over 30 mL/minute. GFR can be measured with ^{99}Tc^{m} DTPA during the routine gamma camera study of renal function without taking any plasma samples. A depth-corrected measurement of the amount taken up by the kidneys and bladder at 22 minutes is made and related to GFR by a previously determined standardization curve (87,88). Multiple blood sampling can be avoided by the use of a solid-state detector strapped to the patient's body over a nonrenal area. Continuous monitoring is undertaken after injection, and only one or two blood samples are needed to calibrate the curve (89,90).

A consensus as to GFR measurement is published (91) and the methodology set out (92). A modern approach is to avoid the errors inherent in measuring the volume of distribution (93) and provide a measure of the rate of clearance alpha3 (94).

Effective Renal Plasma Flow (ERPF)

To measure RPF by a clearance method, a compound that is extracted completely from the blood in a single passage through the kidneys should be used. Because this is not possible, suboptimal compounds, such as p-aminohippurate (PAH), extraction efficiency (E) 87%; IOIH, extraction efficiency 83%; or ^{99}Tc^{m} MAG3, extraction efficiency 55% are used. To acknowledge this discrepancy, the term *effective renal plasma flow* is conventionally used. Thus, ERPF = RPF × E.

IOIH is weakly protein bound and partly taken up by red cells. The 70% protein binding reduces the glomerular filtration of IOIH from 20% to 6% (82). Proximal tubular secretion of IOIH occurs in both cortical and juxtamedullary nephrons and involves two stages of active transport in its passage through the cell. In certain renal disorders, active uptake of IOIH continues, but secretion is prevented, causing accumulation of the compound in the cell. This is called *parenchymal stasis* and is seen in some forms of the nephrotic syndrome, acute glomerulonephritis, and acute tubular necrosis. Competition with tubular uptake of IOIH occurs with PAH, the penicillin group of compounds and probenecid, and certain contaminants of contrast media used for intravenous urography or angiography. Thus, renal IOIH studies should be performed before or at least 8 and preferably 24 hours after these tests. For radionuclide studies, only milligram quantities of IOIH are used, so the tubular maximum for secretion is never approached and the tubular uptake of IOIH is in proportion to the plasma flow. This is the basis of the measurement of ERPF and the intrarenal plasma flow distribution with use of IOIH.

The measurement of ERPF uses the same technique as for GFR. The amount injected and the rate of clearance are determined. Plasma samples are usually obtained at 7, 17, 30, 44, 60, and 120 minutes. Alternatively, external monitoring can be performed with a scintillation probe or a solid-state detector. The activity–time curve is calibrated with one or two plasma samples. The accuracy of the measurement depends on the number of data points and the method of analysis. The simplest index is the 7/17 minute slope, which can be used daily to assess the progress of a renal transplant. The most complex is double or triple exponential analysis of the data. For the single-sample approach, the volume of distribution, V (in liters), is determined at 44 minutes according to either of two formulas (95,96), for example,

$$ERPF = 120(V + 56)^{1/2} - 936 \text{ mL/minute} \quad [6]$$

Preprepared ^{123}I IOIH can be purchased, or OIH can be labeled in-house with the technique of Hawkins and associates (97), which takes about 45 minutes.

^{99}Tc^{m} MAG3 is 90% weakly protein bound and hardly glomerular filtered. It has a volume of distribution about 60% of that of IOIH, but with almost the same plasma clearance rate (73–77). ERPF measurement: IOIH clearance = 1.5 MAG3 clearance + 40 mL/min (SEE ±8%) provided that both volume of distribution and clearance half-life are measured. ^{99}Tc^{m} MAG3 clearance is nearly three times that of ^{99}Tc^{m} DTPA.

A new anionic renal tubular agent with a higher clearance than ^{99}Tc^{m} MAG3 is ^{99}Tc^{m} ethylene dicysteine (98). ^{99}Tc^{m} diaminocyclohexane is a new cationic renal tubular agent that may help to evaluate cyclosporine toxicity (99).

Relative Renal Function

The contribution of one kidney to total function is the most important measurement that is made using radionuclides. It helps the practitioner to decide whether a nephrectomy or restorative operation should be performed. The information cannot be obtained from the urogram, because neither the contrast medium density in the pelvicaliceal system nor the parenchymal thickness is an accurate indicator of function. The relative uptake of the radiopharmaceutical by each kidney is a reliable, routine, and relatively accurate measure to within 6% of their relative function. The normal range is 42.5% to 57.5%. Most of the error is owing to small differences in depth between the two kidneys in a person, causing differences in the degree of gamma-ray attenuation. Correction for depth can be made with an ultrasound probe or by taking true lateral images of the kidneys at the end of the study. There is a consensus report on the quality control of renal function measurements (100).

The uptake of the radiopharmaceutical by a kidney in unit time, K(t), is given by the product of the GFR and the plasma concentration, P(t), of ^{99}Tc^{m} DTPA. Thus,

$$K(t) = GFR \times P(t)$$

For the left kidney,

$$K(t)_L = GFR_L \times P(t)$$

For the right kidney,

$$K(t)_R = GFR_R \times P(t)$$

Dividing gives us

$$K(t)_L/K(t)_R = GFR_L/GFR_R$$

because the plasma concentration is the same for each kidney. Rearranging gives us

$$K(t)_L/K(t)_L + K(t)_R) = GFR_L/total\ GFR \quad [7]$$

The amount in the kidney, K(t), must be determined after adequate mixing in the circulation, usually about 1 minute, and before any has been lost through the kidney, usually about 2 1/2 minutes. Therefore, the measurement of the value of K(t) is taken from each of the activity–time curves obtained for regions of interest set up around each kidney, for example, at 2 minutes. If there is inflow or outflow obstruction, or if renal function is very impaired, the timing of the measurement of K(t) should be delayed until the ratio $K(t)_L$/total K(t) becomes approximately constant. This may be as long as 6 minutes. The assessment of relative renal function may be equally well applied to any radiopharmaceutical taken up by the kidneys, provided that time is allowed for its mixing in the circulation and the measurement of K(t) is made before any is lost from the renal parenchyma.

Relative renal function should be measured whenever nephrectomy is contemplated, because a restorative operation is usually indicated if more than 20% of total renal function is present in a kidney with hydronephrosis, stone, or renovascular disorder. A nephrectomy usually is performed when the kidney has less than 8% of total function, which predicts that recovery would be unlikely. After successful surgery on one kidney, its percentage uptake should rise gradually, assuming unchanging function in the other kidney. The rate of improvement depends on many factors and is difficult to predict. Generally, the lower the level of preoperative function, the longer the recovery, but factors such as the overall level of renal function, the age of the patient, and the chronicity of the disorder influence this time course of recovery.

Static Renal Imaging with ^{99}Tc^{m} DMSA

This is an effective way of demonstrating whether or not a radiologically suspected renal lesion contains normally functioning nephrons. Anatomic variations, lobulation, and "pseudotumors" (101), noted on the intravenous urogram, may be shown to be normal by the parenchymal uptake of ^{99}Tc^{m} DMSA. ^{99}Tc^{m} DMSA imaging is particularly helpful in children because of the delay between the injection and the recording of image data. Information on relative renal function and the amount and distribution of the functioning renal mass can be obtained. It is the recognized technique for detecting renal scarring in children (102–105).

Technique

Using this technique, 2 to 3 mCi (100 MBq) of ^{99}Tc^{m} DMSA is given intravenously in the adult patient. Imaging is performed 3 hours later, with posterior, anterior, oblique, and lateral views being obtained. Anterior and posterior renal regions of interest are corrected for their background levels, and the geometric means are determined for measurement of relative function. An alternative approach to multiple views is to use single-photon emission tomography. This technique images transverse or coronal sections (106) and allows measurement of the volume of the functioning mass of each kidney.

THE USE OF UROGRAPHY, ULTRASONOGRAPHY, AND RADIONUCLIDE STUDIES

For many years, intravenous urography was the principal imaging technique available for renal disease. With the development of ultrasonography and nuclear medicine, urography was used less frequently. Currently, urography is further challenged by the development of CT urography and to a lesser extent by MR urography.

Urography

The high sensitivity of CT for detecting renal masses is well recognized (107). More recently, it has become apparent that spiral unenhanced CT is the most sensitive method for detecting renal and ureteric calculi (108,109). New multislice CT scanners can now generate reformatted images of the pelvicalyceal system and ureter which demonstrate abnormalities previously only detectable with intravenous urography (110,111). These new CT techniques are likely to replace intravenous urography for much urinary tract diagnosis. However, in the current interim phase, traditional intravenous urography is still used in many institutions for at least some of its previous indications.

The particular *strengths* of intravenous urography are:

The demonstration and localization of calcifications and calculi.

The definition of the detailed anatomy of the pelvicaliceal system, together with the demonstration of renal size and contour, a vital combination for the diagnosis of a variety of renal diseases.

The ability to obtain an overview of the kidneys, ureters, and bladder simply and quickly.

These are balanced by a number of *weaknesses:*

To produce a diagnostic result, urography is dependent on a functioning kidney with perfusion, filtration, and tubular concentration. Where renal function is diminished, anatomic information is limited and often delayed.

It provides no assessment of GFR.

It provides little information on parenchymal structure and cannot characterize masses as cystic or solid.

It does not show the whole renal contour and may miss anterior and posterior masses; it does not show the perinephric space.

It necessitates the use of ionizing radiation and contrast medium.

Indications

The main uses of intravenous urography are:

When it is necessary to assess detailed calyceal anatomy and the appearance of the overlying parenchyma, for example, in suspected papillary necrosis, reflux nephropathy, medullary sponge kidney, renal infarct, obstructive nephropathy, and so forth.

When calculous disease is suspected: to localize renal calcification, to localize calcifications that may represent ureteric calculi, and to show detailed pelvicaliceal anatomy, before extracorporeal shock-wave lithotripsy or percutaneous nephrolithotomy. Spiral unenhanced CT, however, is now widely used in suspected ureteric colic.

To diagnose subtle mucosal abnormalities (e.g., transitional cell carcinoma).

After localized blunt loin trauma.

To diagnose congenital anomalies (e.g., duplex kidney and crossed fused ectopia).

For preoperative assessment (e.g., in renal-transplant donors; before endoscopic surgery).

For postoperative assessment (e.g., after ureteric surgery; after resection of transitional cell carcinoma).

Ultrasonography

The major *strengths* of ultrasonography in renal diagnosis are:

It is a sensitive detector of intrarenal fluid collections: pelvicaliceal dilatation and cysts.

It can differentiate cortex and medulla.

It can accurately characterize renal masses as cystic or solid.

It shows the whole renal contour and perinephric space.

It can demonstrate renal blood flow (Doppler technique).

It does not use irradiation or contrast medium.

The *weaknesses* of ultrasonography are:

It does not show fine pelvicaliceal detail.

It does not show the normal ureter.

It shows the retroperitoneum poorly.

It can miss small renal calculi and does not visualize most ureteric calculi.

It gives no functional information.

It is dependent on the skill of the operator.

Indications

Ultrasonography is a good first-line diagnostic method:

To measure renal length.

To diagnose collecting system dilatation, indicating possible obstruction in renal failure, prostatism (112) and pelvic neoplasm, renal transplants, and acute urinary tract infection with suspected pyonephrosis.

To demonstrate renal arterial perfusion and detect renal vein thrombosis in the native kidney

To assess renal transplants—for collecting system dilatation, perinephric fluid collections, or the presence of vascular complications, particularly renal artery stenosis, renal vein thrombosis, or arteriovenous fistulae.

To diagnose adult polycystic kidney disease, both for established disease and to screen involved families (113).

To check for renal masses in patients with hematuria.

To detect perinephric fluid collections such as abscess (114) or hematoma (115).

To guide interventional procedures, such as renal biopsy (116–118), cyst aspiration, and antegrade pyelography. The latter may be preliminary to percutaneous drainage, but the subsequent catheter and guide wire manipulations require fluoroscopic guidance (119).

Ultrasonography is a helpful second-line method:

To characterize masses shown at urography as cystic or solid

When the pelvicaliceal system fails to fill with contrast medium at urography

Ultrasonography and Renal Calculi

Calculi in the kidney are seen as highly reflective foci with posterior acoustic shadowing (Fig. 12-11). However, ultrasonography may miss small renal calculi, and plain films with plain renal tomography are more sensitive than ultrasonography for

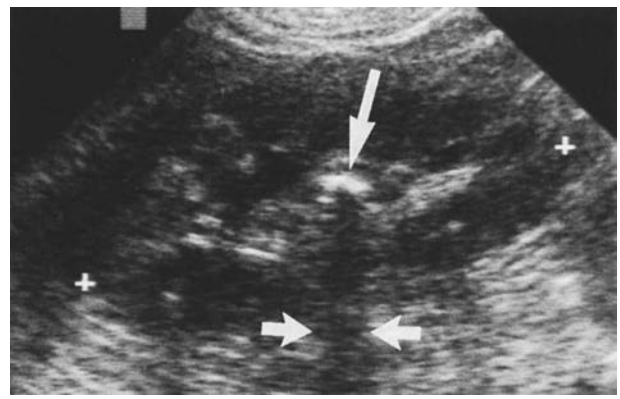


FIGURE 12-11. Renal calculus shown on ultrasound scan. Note the calculus causing an echogenic focus (*long arrow*) with posterior acoustic shadow (*short arrows*). (From: Cattell WR, Webb JAW, Hilson AJW, *Clinical renal imaging.* Chichester: Wiley, 1989, with permission.)

detecting small opaque calculi (120,121). This, combined with the fact that ultrasonography does not detect most ureteric calculi, is the reason that a plain abdominal radiograph always should be obtained if ultrasonography is to be the only renal imaging method used. Unenhanced CT is markedly more sensitive than ultrasonography for detecting renal calculi (122).

Ultrasonography is a helpful investigation when a lucent filling defect is seen in the collecting system at urography. If this represents a calculus, it will show the typical echogenicity with posterior shadowing, thus differentiating it from other lucencies (e.g., tumor or blood clot) (123).

Indications for Radionuclide Studies

The principal indications for radionuclide studies in renal disease are the assessment of (a) total renal function by measurement of GFR or ERPF, (b) relative renal function by measurement of the percentage contribution of one kidney to total renal function, (c) obstructive nephropathy by measurement of the PTTI, (d) obstructing urography by assessment of the response to furosemide visually or using output efficiency (e) renal transplantation by serial assessment of the improvement of acute tubular necrosis and of the onset of rejection, (f) renal parenchymal lesions including renal scarring by the focal loss of $^{99}Tc^{m}$ DMSA uptake, (g) renovascular disorder: case finding in hypertensive patients and captopril renography for the assessment for angioplasty or surgery, and (h) vesicoureteric reflux in children (124) and ureteroureteral reflux in the duplex kidney (125).

RENAL PARENCHYMAL DISEASE

The presence of renal parenchymal disease may be indicated by changes in renal size and symmetry, shown by urography, ultrasonography, and radionuclide imaging or by increased cortical reflectivity at ultrasonography. Such changes, however, usually indicate a broad differential diagnosis rather than a specific disease (57,126,127). Urography, however, can differentiate some types of chronic renal parenchymal disease because of its ability to provide a detailed image of calyceal anatomy together with the associated focal or diffuse parenchymal loss (Table 12-4) (128). Tc-99m DMSA is the method of choice for detecting renal scarring in children (102,103).

Calyceal and Papillary Abnormality without Parenchymal Loss

Papillary Necrosis

The diagnosis of papillary necrosis depends on the demonstration of calyceal and papillary abnormalities without associated parenchymal loss. The calyceal and papillary abnormalities can be considered in two categories (129).

Specific to Papillary Necrosis. Tracks and horns of contrast medium arising from the calyceal fornices (Fig. 12-12) represent necrosis around the papillary margin. Central necrosis produces central pools of contrast medium within the papillae ("egg in a cup" appearance). Papillary sloughing produces a central lucent filling defect with a surrounding ring of contrast medium (ring shadow).

Nonspecific. The end result of papillary sloughing is clubbed or truncated calyces, which, by themselves, are a nonspecific finding.

These changes are bilateral and patchy in distribution in the chronic forms of papillary necrosis associated with analgesic abuse (130), sickle cell trait (131), sickle cell disease (132), and diabetes mellitus. The acute form of papillary necrosis that may occur in severely infected kidneys, especially associated with obstruction or diabetes mellitus, or both, usually is unilateral and causes uniform papillary sloughing. Multiple blunted calyces with ring shadows and lucent filling defects in the collecting system result (Fig. 12-13).

Calcification of affected papillae may be spotty, especially in analgesic abusers (133) or curvilinear around the margins of a sloughed papilla. Early in the disease, the kidneys are normal in size and smooth, but late in the disease, when multiple papillae have sloughed, the kidneys are small with a slightly wavy outline (129).

Tuberculosis

Renal calcification occurs in 30% of cases (134). It is often punctate within the parenchyma (Fig. 12-14). Calcification within pyonephrosis has a cloudy pattern early, but becomes progressively denser as a tuberculous autonephrectomy develops. Papillary irregularity, which gives a ragged appearance to the calyx, is the earliest change observed and can be confused with papillary necrosis. Concentration of contrast medium in

TABLE 12-4

UROGRAPHIC DIAGNOSIS OF CHRONIC RENAL PARENCHYMAL DISEASE

	Calyceal/papillary abnormality	Parenchymal loss	
		Focal	Diffuse
Papillary necrosis	+	−	± (late)
Tuberculosis	+	±	−
Medullary sponge kidney	+	−	−
Megacalyces	+	−	−
Pelvicalyceal cyst	+	−	−
Chronic atrophic focal reflux nephropathy	+	+	−
Stone disease	+	+	−
Back-pressure atrophy			
Obstruction	+	−	+
Diffuse reflux nephropathy	+	−	+
Infarct	−	+	−
Trauma (old)	−	+	−

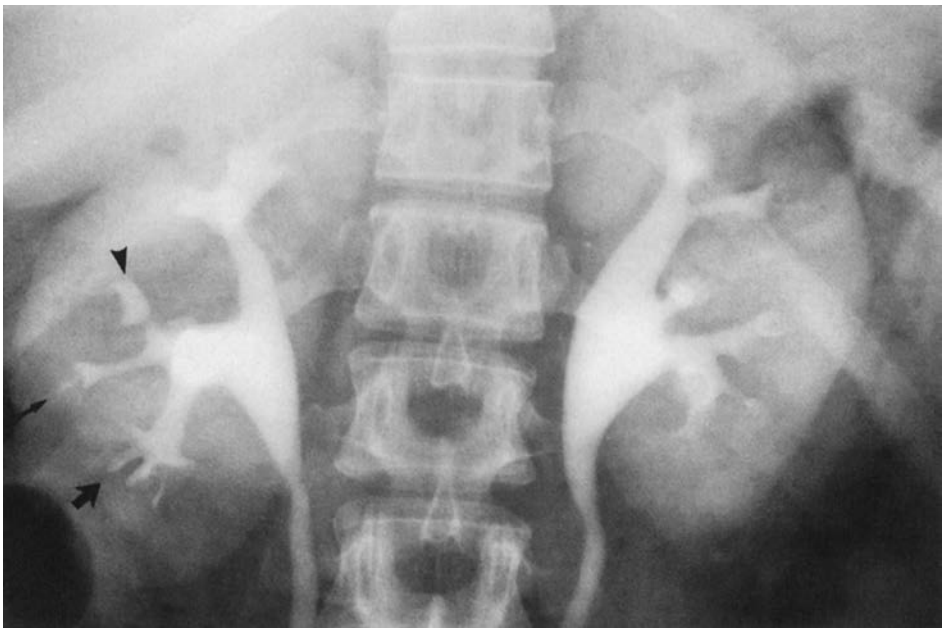


FIGURE 12-12. Renal papillary necrosis in a 48-year-old woman with sickle cell trait. Note the blunted calyx (*arrowhead*), "egg in a cup" (*small arrow*), and contrast track (*large arrow*).

the pelvicaliceal system often is poor. With progression of the disease, cavitation occurs. It is classically irregular, but the cavity walls may be smooth. Fibrosis produces strictures and obstruction of calyceal infundibula (producing hydrocalycosis) and of the ureter (producing hydronephrosis). Focal scarring may be observed overlying affected calyces, and diffuse parenchymal loss accompanies pelviureteric junction obstruction.

Medullary Sponge Kidney

In medullary sponge kidney, papillary involvement may be anything from a single pyramid to the whole of both kidneys and is often patchy. About 30% of patients have nephrocalcinosis (135) with multiple elongated and rounded papillary calcifications. The involved papillae are enlarged and contain streaks, pools, or both, of contrast medium during urography (Fig. 12-15). The linear streaks produced by contrast medium in ectatic tubules can be distinguished from the diffuse papillary blush, observed at urography in normal subjects who concentrate their urine well, by the fact that individual contrast-medium streaks can be detected by the naked eye and persist until late in the urogram. The normal papillary blush is maximal soon after contrast-medium injection and fades during the urogram. It is more prominent with the new low-osmolar contrast media (136).

Megacalyces

This condition, which is believed to be a congenital variant, is characterized by nonobstructive enlargement of the calyces because of decreased thickness of the medullary pyramids, usually unilateral (137). The calyceal infundibula, pelvis, and ureter all appear normal and cortical thickness is not reduced. However, there may be an associated increase in the number of calyces and megaureter.

Pelvicaliceal Cyst

These cysts are usually smooth walled, and their communication with the pelvicaliceal system cannot always be shown (138). Calyceal cysts tend to be located at the corticomedullary junction and polar in distribution (Fig. 12-16), whereas pelvic cysts usually are central. Stasis within the cysts predisposes to stone formation.

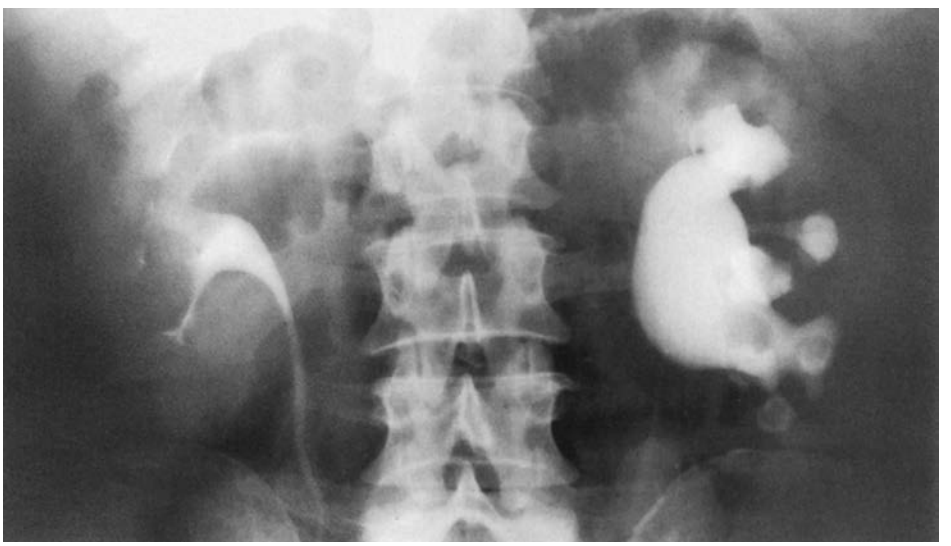


FIGURE 12-13. Unilateral renal papillary necrosis in a diabetic man with left pelviureteric junction obstruction. Note the multiple blunted calyces and sloughed papillae seen as lucent filling defects in the left lower pole collecting system.

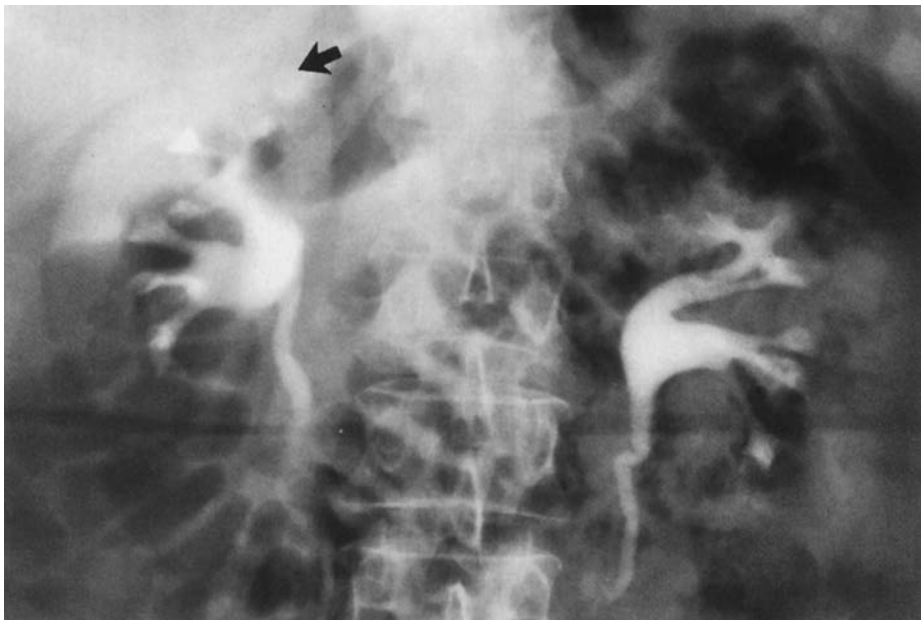


FIGURE 12-14. Tuberculosis of the right kidney in a 64-year-old woman. Note the upper-pole cavity (*arrow*), blunted calyx, and parenchymal loss.

Calyceal or Papillary Abnormality with Focal Parenchymal Loss

Chronic Atrophic Pyelonephritis and Focal Reflux Nephropathy

The diagnosis of focal reflux nephropathy depends on demonstrating clubbed calyces with overlying focal parenchymal scars (52).

Calyceal clubbing is usually smooth and often polar in distribution, because the compound calyces at the renal poles predispose to intrarenal reflux of infected urine (139). The upper pole is more often affected, and the right side more than the left (52,140). The parenchymal scars overlying the clubbed calyces may be noted as irregularities of the outline when seen in profile at urography (Fig. 12-17). When the scarring is polar, however, the renal outline may be smooth and the parenchymal loss indicated by the clubbed calyx lying nearer the renal surface than usual. Urography does not show all scars,

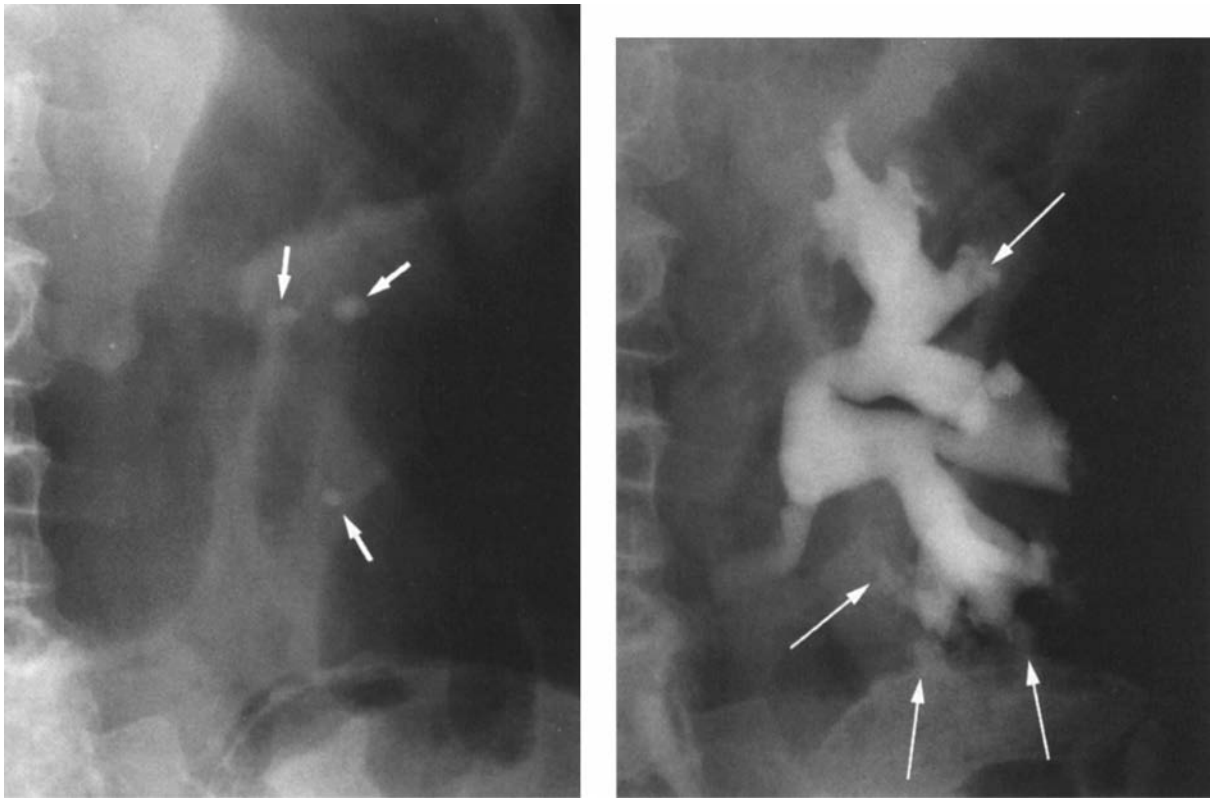


FIGURE 12-15. Medullary sponge kidney. **A:** Plain film: multiple papillary calcifications (*short arrows*). **B:** Urogram: multiple contrast pools in the papillae (*long arrows*). (From: Cattell WR, Webb JAW, Hilson AJW, eds. *Clinical renal imaging.* Chichester: Wiley, 1989, with permission.)

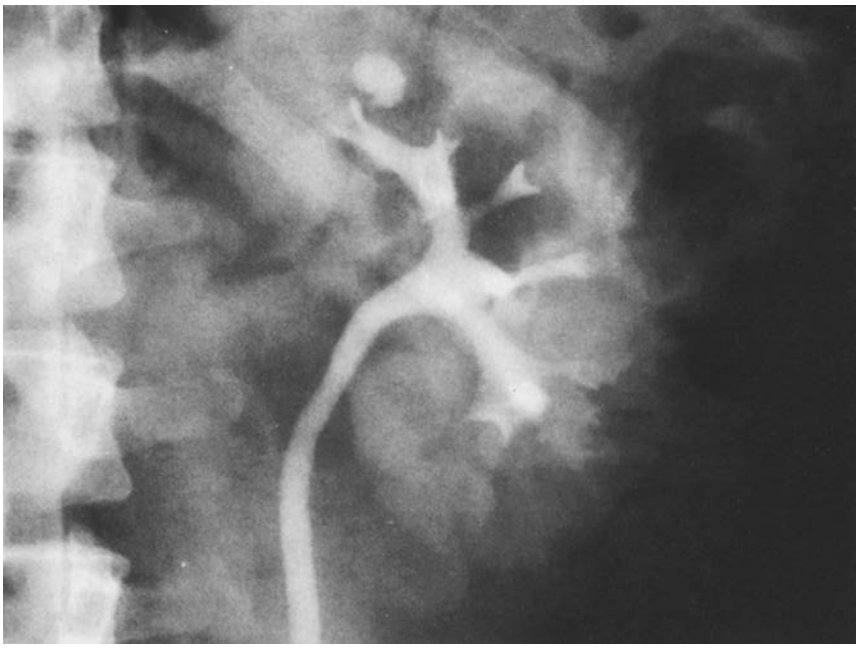


FIGURE 12-16. Calyceal cyst at the left upper pole.

and DMSA nuclear scans are more sensitive in their detection (141).

Involvement of the kidneys is patchy in distribution, and, if both kidneys are affected, asymmetry is common. A small kidney usually, but not invariably, results from involvement. Contrast-medium concentration often is good until late in the disease.

Calculous Disease

Calculous disease with obstruction of a calyceal infundibulum can result in hydrocalycosis with an overlying focal scar and usually only affects one or two calyces.

Calyceal and Papillary Abnormality with Diffuse Parenchymal Loss

Obstructive Atrophy

Obstructive atrophy is characterized by uniform papillary atrophy, which produces blunted calyces with associated diffuse thinning of the parenchyma (Fig. 12-18) (142). A history of obstruction usually, but not invariably, is obtained. In a proportion of cases, the papillary atrophy may be very slight and the predominant change is diffuse parenchymal loss, so that differentiation from an ischemic kidney may be difficult (143).

Diffuse or Generalized Reflux Nephropathy

In some patients with high-pressure reflux of infected urine, a generalized form of reflux nephropathy develops, indistinguishable at urography from obstructive atrophy (144). Such patients, however, are likely to have vesicoureteric reflux at micturating cystography.

Focal Parenchymal Scarring without Calyceal or Papillary Abnormality

Lobar Infarct

A broad-based parenchymal scar characterizes the so-called lobar infarct produced by occlusion of an interlobar artery

FIGURE 12-17. Focal reflux nephropathy in a 19-year-old hypertensive patient. Note the multiple blunted calyces with overlying parenchymal scars.

FIGURE 12-18. Obstructive atrophy of the right kidney secondary to calculous obstruction of the right ureter. Note the generalized calyceal blunting and parenchymal loss. (The round laminate calcification overlying the right upper ureter is a gallstone.)

with no abnormality of the underlying pelvicaliceal system (Fig. 12-19) (145). A very large infarct may be associated with some deformity of the underlying calyx, but this is always slight in relation to the size of the scar.

Renal Trauma

Renal trauma may result in an appearance identical to a renal infarct, but an appropriate history usually establishes the diagnosis.

Renal Mass Lesions

Ultrasonography is a more sensitive detector of small renal masses than is urography (with tomography), but a less sensitive detector than computed tomography (107,146). The more widespread use of ultrasonography in abdominal imaging has led to a great increase in the number of renal cysts diagnosed and to the incidental detection of many small renal adenocarcinomas and angiomyolipomas (147–149).

Ultrasonography is considered to be the most cost effective method to characterize masses detected at urography (150). In cases in which ultrasonography is indeterminate, computed tomography, cyst puncture, or both usually provide a definitive answer (151). Angiography is now hardly ever indicated for renal mass lesions solely to determine their nature. $^{99}Tc^{m}$ DMSA scans are helpful in one particular category of mass lesion: when a normal variant or pseudotumor is suspected. In this situation, the uptake of $^{99}Tc^{m}$ DMSA into the suspicious area indicates a normal variant (101).

Urographic Diagnosis

A number of urographic criteria indicate that a renal mass is a benign cyst. *Simple cysts* are classically rounded, sharply defined from the adjacent renal parenchyma, and lucent. Where they lie peripherally, the free wall of the cyst, which protrudes from the kidney, measures 2 mm or less, and at the margin there is smoothly tapered stretching of the renal parenchyma—the "beak" or "claw" sign (Fig. 12-20). By contrast, tumors are often irregular and poorly defined protrusions from the renal parenchyma, blush after contrast-medium administration, and have irregular thickened walls if they are necrotic (145). Calcification within a renal mass suggests malignancy; although a pencil-thin rim of calcification can occur in the margin of a cyst, 20% of such lesions are malignant (152). The diagnosis of a likely benign cyst is more difficult when the mass is central; in this situation, the wall thickness cannot be measured and the beak sign is absent. Further, the beak sign, classically associated with cysts, can occur with slow-growing tumors. Although the

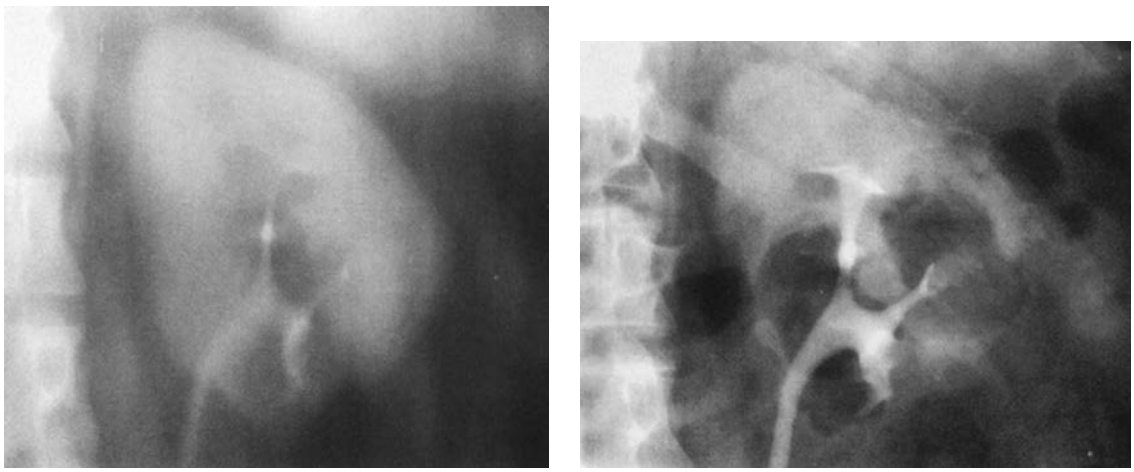


FIGURE 12-19. Infarct at the left lower pole in a 66-year-old hypertensive patient. There is a broad-based irregular scar with a virtually normal underlying calyx.

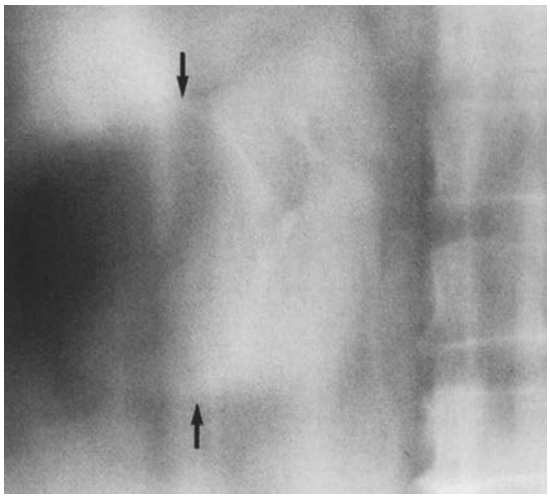


FIGURE 12-20. Simple cyst of the right kidney shown on a tomogram. Note the smooth outline, lucency within the cyst, and peripheral beaks of renal parenchyma (*arrows*).

various urographic signs give some indication of the nature of a mass, their accuracy is probably only about 50% even in the best hands (153). For this reason, all masses found at urography should be evaluated further with ultrasonography, unless there are features that are strongly suggestive of malignancy (e.g., central calcification), when computed tomography is more appropriate.

Ultrasound Diagnosis

The signs of a simple cyst at ultrasonography are that it contains no echoes (apart from anterior reverberation echoes) and that it has a clearly defined posterior wall and shows "increased through transmission" of sound posterior to it. Cysts are usually either spherical or slightly ovoid. The ultrasonographic diagnosis of a simple cyst is accurate in 98% of cases (154) where these criteria are strictly adhered to and no further investigation is required (Fig. 12-21). Patients in whom these criteria are not met require further investigation by computed tomography, cyst puncture, or both. A proportion of these patients have hemorrhagic, infected, or septate cysts (Fig. 12-22); abscesses; or hematomas. Some, however, have necrotic tumors, and, if such tumors are to be detected, further investigation of all atypical fluid collections shown at ultrasonography is essential (151).

FIGURE 12-21. Simple renal cyst on a longitudinal ultrasound scan. Note the smooth-walled cyst (*long arrow*) with "increased through transmission" of sound posteriorly (*short arrows*).

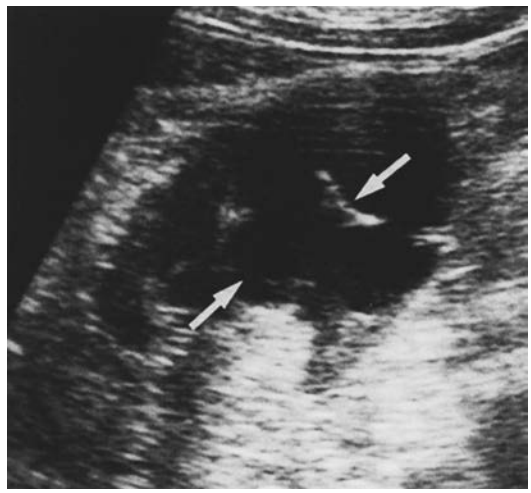


FIGURE 12-22. Septate renal cyst on a transverse ultrasound scan. The two septa are indicated by arrows. Puncture yielded clear yellow fluid with no malignant cells.

Solid masses usually show contained echoes at ultrasonography and no "increased through transmission" of sound posterior to them (Fig. 12-23). The majority of renal carcinomas have a reflectivity similar to or less than the adjacent renal cortex (155). A number of other renal mass lesions are typically hypoechoic: renal lymphoma (Fig. 12-24) (156), infarct, hematoma, focal acute pyelonephritis (157,158), and xanthogranulomatous pyelonephritis (159). The fat content of angiomyolipoma is associated in most cases with markedly increased reflectivity (Fig. 12-25) (160), but this pattern also may occur in renal cell carcinoma, cavernous hemangioma, and oncocytoma (160–162).

Ultrasonography can demonstrate tumor spread into the venous system. This causes enlargement of the renal veins, which are filled with echoes, and a reflective tumor mass may be shown in the inferior vena cava (Fig. 12-26) (163). Assessment

FIGURE 12-23. Renal carcinoma on a longitudinal ultrasound scan. The inhomogeneous solid upper-pole tumor is indicated by arrows. (From: Cattell WR, Webb JAW, Hilson AJW, eds. *Clinical renal imaging*. Chichester: Wiley, 1989, with permission.)

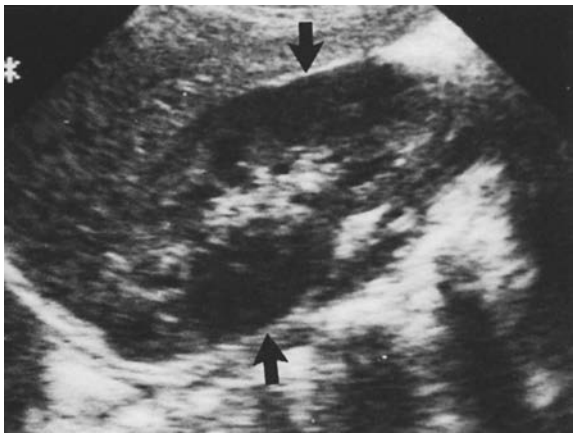


FIGURE 12-24. Renal lymphoma. Longitudinal ultrasound scan of the right kidney shows two hypoechoic solid masses (*arrows*). (From: Meire HB, Cosgrove DO, Dewbury KC, eds. *Clinical ultrasound*. London: Churchill Livingstone, 1993, with permission.)

of tumor extension into the vena cava may be improved by using Doppler (164).

Urinary Tract Obstruction

Obstruction of the upper urinary tract leads to dilatation above the level of obstruction. This effect of the obstructing process on the renal outflow tract is called *obstructing uropathy* and can be shown either by urography or ultrasonography. The effect of the obstructing process on renal function, called *obstructive nephropathy* can be quantitated by nuclear medicine techniques, because obstruction to outflow is associated with increased salt and water reabsorption and slower transit of $^{99}Tc^{m}$ DTPA or $^{99}Tc^{m}$ MAG3 through the nephron.

Urography

For many years urography has been the gold standard for diagnosing *acute obstructions,* which are caused by a ureteric calculus most often. Recently, urography has been challenged by spiral unenhanced CT (108). In the acutely obstructed kidney, usually there is sufficient renal perfusion and filtration for a nephrogram to appear immediately after contrast-medium injection, and the nephrogram increases in density markedly with time because of increased salt and water reabsorption consequent on renal tubular stasis. Pelvicaliceal and ureteric filling is delayed—up to 24 hours after injection of contrast medium—and dilatation is typically mild, even with severe complete obstruction (Fig. 12-27).

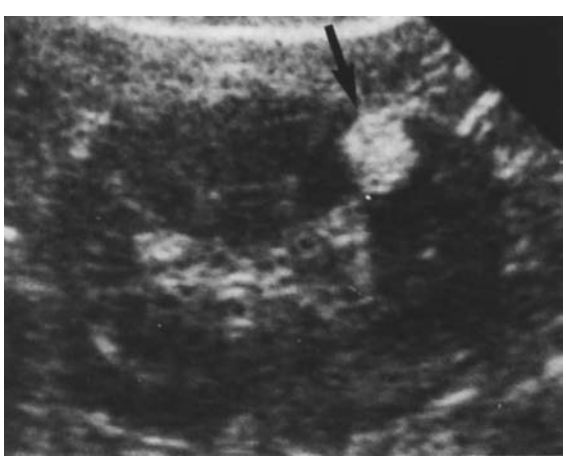


FIGURE 12-25. Renal angiomyolipoma shown at ultrasound. Note the small, well-defined, hyperechoic mass (*arrow*). (From: Meire HB, Cosgrove DO, Dewbury KC, eds. *Clinical ultrasound*. London: Churchill Livingstone, 1993, with permission.)

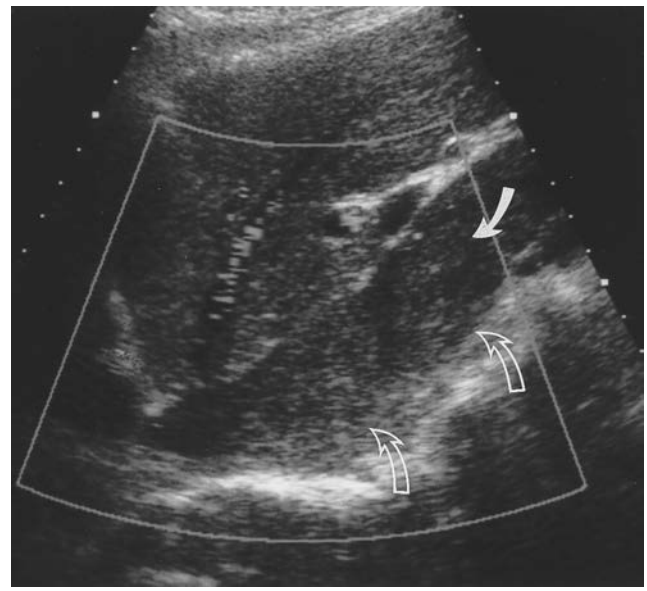


FIGURE 12-26. Extension of renal carcinoma into the inferior vena cava. On this longitudinal ultrasound scan, open arrows indicate the posterior wall of the cava, and the closed arrow points to the tumor mass within it.

In *chronic obstruction* filling of the collecting system with contrast medium also is delayed, and dilatation is usually more marked. Some types of severe chronic obstruction (e.g., those caused by retroperitoneal fibrosis), however, are associated with mild or no pelvicaliceal dilatation (165). Films obtained immediately after injection of contrast medium may show a negative pyelogram, produced by nonopacified urine in the dilated collecting system surrounded by opacified parenchyma. If renal function is sufficient, delayed films show pelvicaliceal opacification (Fig. 12-28). Parenchymal thinning often occurs secondary to obstructive atrophy. Where renal function is markedly impaired, no pelvicaliceal filling occurs, and, in this situation, ultrasonography is indicated (Fig. 12-29).

Not all pelvicaliceal system dilatation indicates obstruction, and careful urography can identify many of the nonobstructive causes. Dilatation may occur because of vesicoureteric reflux, after ileal loop diversion, in some patients with renal transplants (166), and with neurogenic bladder or bladder outflow obstruction. Reflux is suggested by variable dilatation during the course of the urogram, a large postmicturition residue, and striae, which are lucent folds seen in the collapsed collecting system. Congenital anomalies that cause dilatation (e.g., megacalyces or megaureter) often can be recognized by their typical appearance. Common causes of acquired urinary tract dilatation in women are previous pregnancy, infection, or both (167). This may be recognized as being nonobstructive by the prompt upper-tract drainage that occurs after bladder emptying (Fig. 12-30).

The diagnosis of *pelviureteric junction obstruction* sometimes is difficult. The obstruction is often intermittent, and a

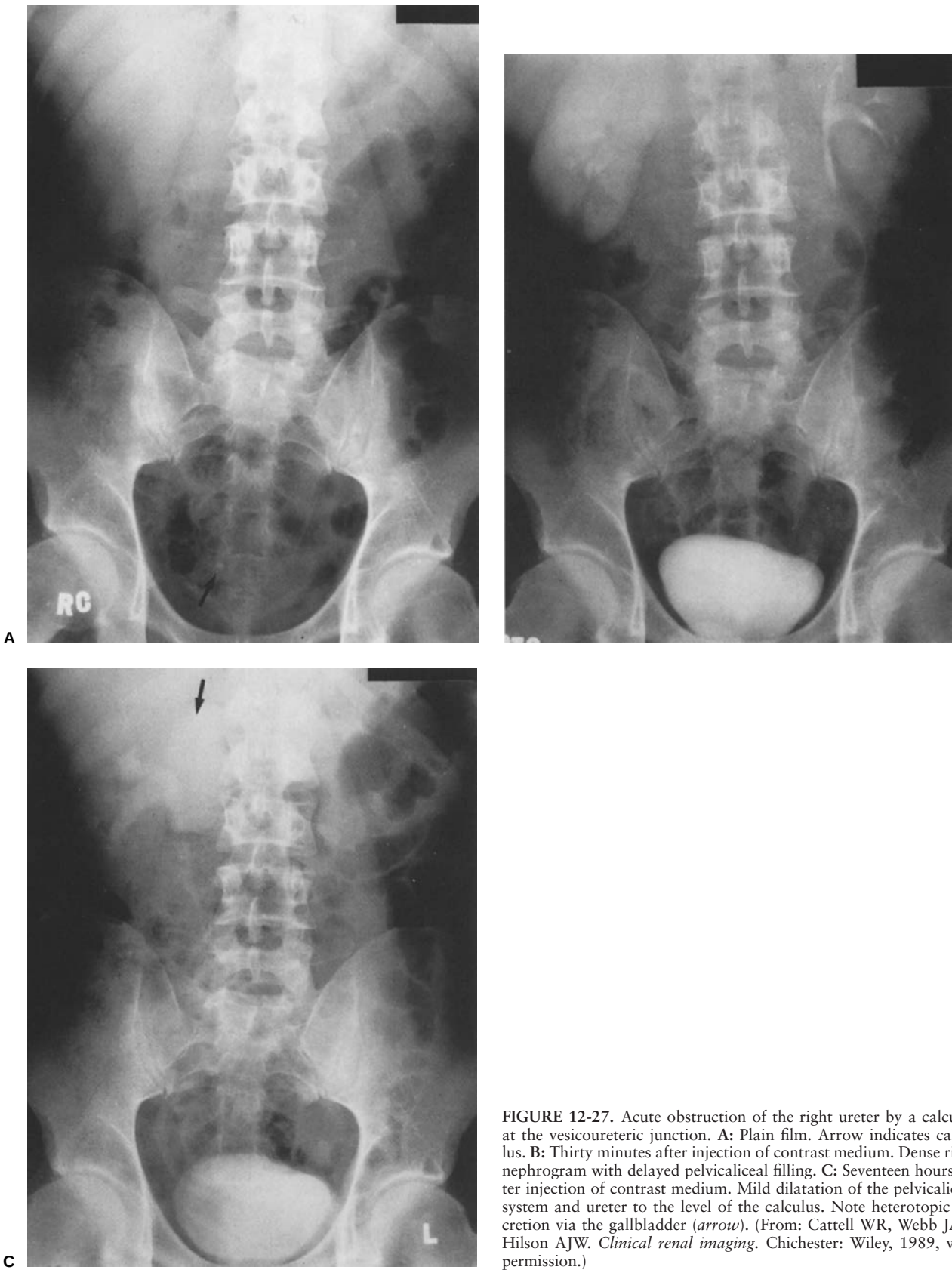


FIGURE 12-27. Acute obstruction of the right ureter by a calculus at the vesicoureteric junction. **A:** Plain film. Arrow indicates calculus. **B:** Thirty minutes after injection of contrast medium. Dense right nephrogram with delayed pelvicaliceal filling. **C:** Seventeen hours after injection of contrast medium. Mild dilatation of the pelvicaliceal system and ureter to the level of the calculus. Note heterotopic excretion via the gallbladder (*arrow*). (From: Cattell WR, Webb JAW, Hilson AJW. *Clinical renal imaging*. Chichester: Wiley, 1989, with permission.)

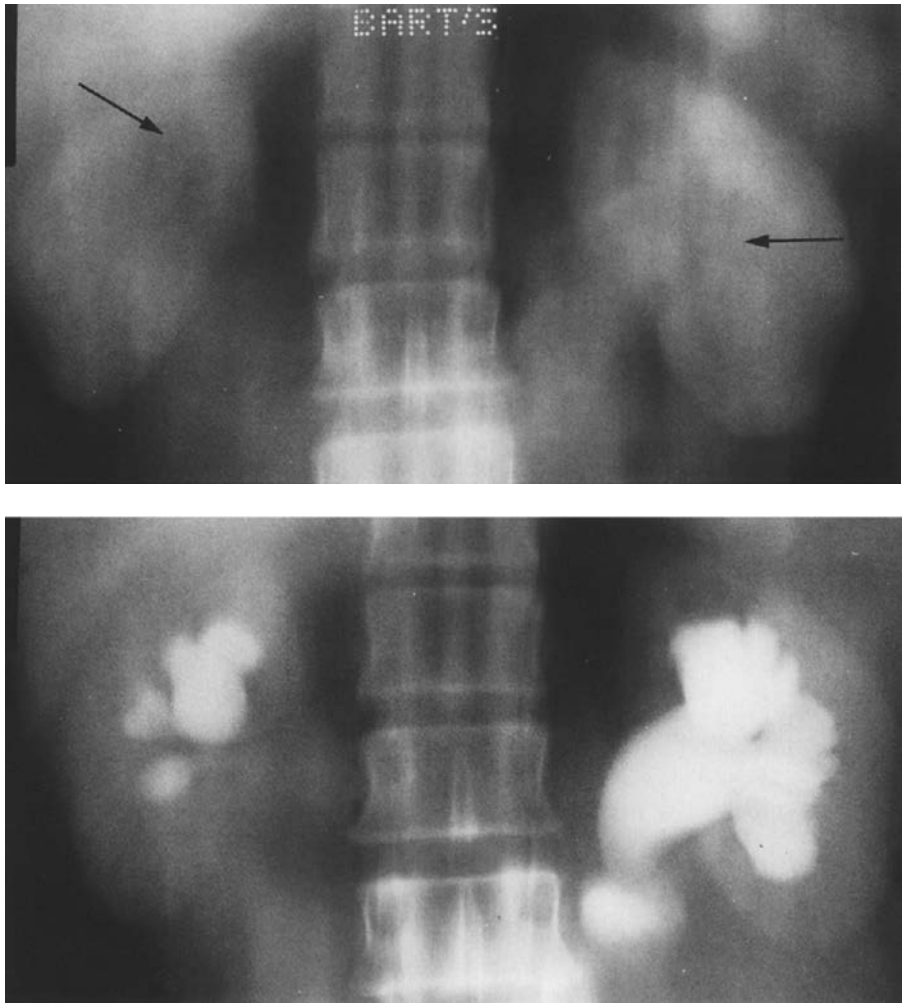


FIGURE 12-28. Chronic bilateral obstruction caused by benign prostatic hypertrophy. **A:** Tomogram obtained immediately after injection of contrast medium. Note the negative pyelogram (*arrows*). **B:** Tomogram 30 minutes after contrast medium: filling of the dilated pelvicaliceal systems is seen. (From: Cattell WR, Webb JAW, Hilson AJW. *Clinical renal imaging*. Chichester: Wiley, 1989, with permission.)

study during pain is diagnostic. The technique of furosemide urography, using a large dose of contrast medium (600 mg of iodine per kg of body weight) with furosemide, 40 mg intravenously, after the 20-minute full-length abdominal film, also is helpful. A further abdominal film obtained 15 minutes after administration of furosemide shows an increase in pelvicaliceal area of 22% or more—readily appreciable to the eye—when pelviureteric junction obstruction is present (Fig. 12-31) (168). This procedure clarifies the diagnosis in 85% of cases (169). It also is helpful to give intravenous furosemide during the course of the urogram when equivocal pelvicaliceal dilatation is seen.

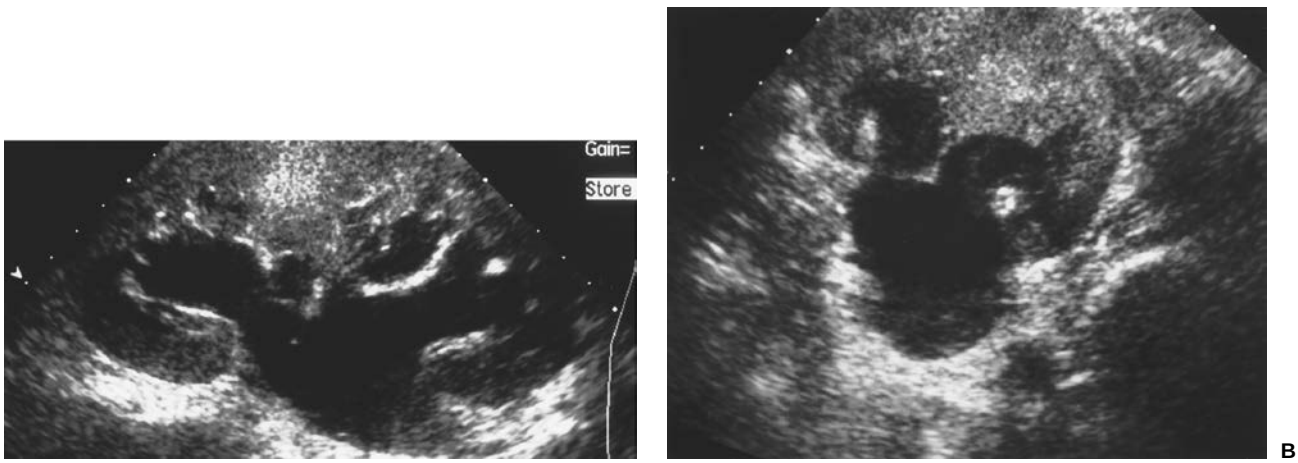
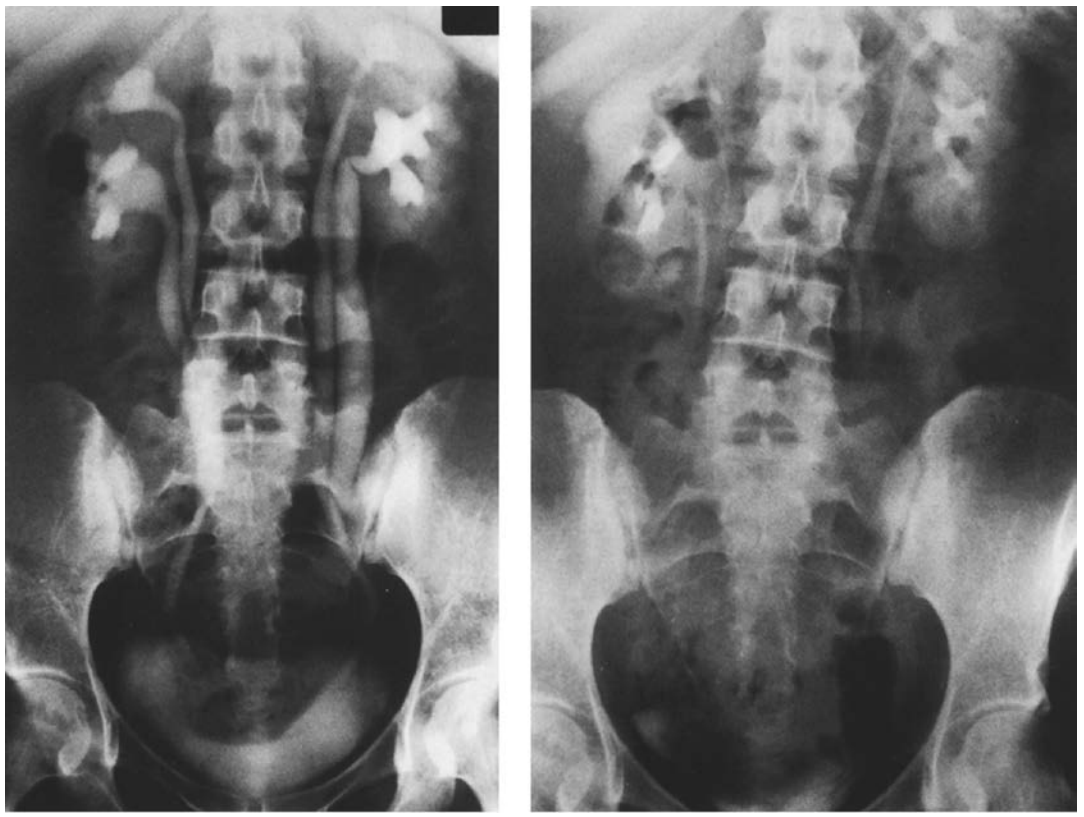


FIGURE 12-29. Renal obstruction: Longitudinal (**A**) and transverse (**B**) ultrasound scans show moderate pelvicaliceal dilatation with the dilated pelvis medially (*arrow*) communicating with the dilated calyces (*arrow*).



A B

FIGURE 12-30. Distensibility of both duplex pelvicaliceal systems and upper ureters in a woman after pregnancy. **A:** Full-length urogram film taken immediately after ureteric compression has been removed shows dilated pelvicaliceal systems and ureters to pelvic brim level. **B:** Postmicturition film shows drainage of the upper tracts.

A B

FIGURE 12-31. Positive furosemide urogram showing left pelviureteric junction obstruction. The film after furosemide (**B**) shows definite increase in the size of the pelvicaliceal system compared to the film before furosemide (**A**) with no filling of the ureter.

Ultrasonography

Ultrasonography is not dependent on renal function and shows a dilated pelvicaliceal system as a communicating multiloculate fluid collection centrally in the renal sinus (Fig. 12-29). It also shows minor pelvicaliceal dilatation and is sufficiently sensitive to be used to screen for collecting system dilatation (170–172). A plain film always should be obtained because collecting system dilatation may be missed in the presence of calculi filling a dilated system (170). Ultrasonography, however, suffers from poor specificity, with false-positive scans occurring in up to 26% of cases (170). Any collecting system visualization with ultrasonography must be considered significant when obstruction is being sought, because mild dilatation can occur with severe obstruction. However, visualization may occur in normal subjects if they have a high fluid intake and a diuresis, or in the presence of some anatomic variants, such as extrarenal pelvis.

Ultrasonography is less good at identifying nonobstructive causes of pelvicaliceal visualization than is urography, largely because it does not show the ureter and cannot assess upper-tract drainage. Normal central vessels, especially veins, may be mistaken for the collecting system. Color or duplex Doppler studies can help by identifying a typical color signal or venous or arterial waveform arising from the central fluid collection (173). Pathologic central fluid collections (e.g., clubbed calyces, cysts) also may be indistinguishable from a dilated collected system.

Renal blood flow decreases from approximately 6 hours after the onset of obstruction in complete renal obstruction. The decreased blood flow can be quantitated by measuring the resistance index (RI) with duplex Doppler ultrasound. An RI of greater than 0.70 has been proposed to distinguish obstructed from nonobstructed pelvicaliceal systems (70). In acute ureteric obstruction the decrease is maximal from 6 to 48 hours (174). An interrenal RI difference greater than 0.08 to 0.10 has been proposed as the most helpful criterion (175). Although initial reports suggested this method was very sensitive (176), subsequent studies have been less successful (177,178) and have drawn attention to the variability of RI in acute obstruction. In chronic obstruction also, RI measurements may be variable and RI measurement following furosemide has been used, with good results obtained when a threshold of 0.75 was used to diagnose obstruction (179). Increases in RI occur with a wide variety of other renal pathology including renal parenchymal disease (127), acute tubular necrosis (180), renal vein thrombosis, acute pyelonephritis, and renal transplant rejection (181,182).

When normal subjects undergoing a diuresis are examined with color Doppler ultrasound, symmetric color streaming (ureteric jets) can be seen in the bladder representing the flow of urine from the ureters into the bladder. No flow or continuous low level flow is seen in patients with high-grade obstruction, whereas the flow may be normal or abnormal in those with lower grade obstruction (183). This method may be helpful when ultrasonography is being used in suspected ureteric obstruction.

Ultrasonography is best used to screen for obstruction when chronic obstruction is suspected, for example, in prostatism (112) or renal failure (58,184,185), or in the presence of a pelvic tumor. It is generally considered less useful than urography in diagnosing acute obstruction, for example, in ureteric colic. If it is used in suspected ureteric colic it should be supplemented both by plain films, and by RI measurement and assessment of ureteric jets. Visualization of the collecting system with ultrasonography often necessitates further imaging by urography, computed tomography, or antegrade pyelography to clarify its significance because of the poor specificity of ultrasonography.

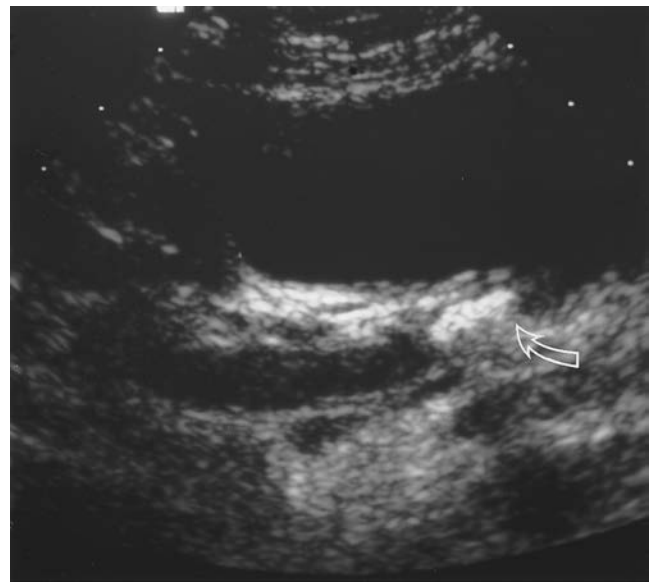


FIGURE 12-32. Calculus (*arrow*) at the vesicoureteric junction with a posterior acoustic shadow, well seen in a dilated ureter through the full bladder on a longitudinal ultrasound scan.

Ultrasonography may show the cause of obstruction if, for example, it is caused by bladder outflow obstruction or by a pelvic tumor. Ultrasonography may also detect ureteric calculi if they lie in the upper ureter or at the vesicoureteric junction (Fig. 12-32). Urography may indicate the cause of obstruction if an intraluminal lesion is present, but often has difficulty in differentiating mural lesions from lesions extrinsic to the ureter. Computed tomography is the method of choice to search the retroperitoneum for obstructing lesions (186). Antegrade pyelography is useful to define the ureteric appearances at the level of obstruction and can be followed by a drainage procedure (percutaneous nephrostomy) (Fig. 12-33). If dilatation is mild, however, retrograde ureterography may be necessary.

Radionuclide Studies

The prime use of radionuclides is in the evaluation of the effect of chronic outflow disorders on renal function. Resistance to outflow is associated with a change in the pressure gradient from the glomerulus to the site of resistance. This causes the nephron intraluminal tubular pressure to exceed slightly the peritubular capillary pressure and associated enhanced reabsorption of salt and water. The parenchymal transit time of nonabsorbed solutes, such as $^{99}Tc^{m}$ DTPA (187–189) or $^{99}Tc^{m}$ MAG3 (71,72), is prolonged.

Pelvic dilatation unassociated with an increased resistance to flow is not obstructive, and the parenchymal transit-time index is normal (Table 12-1; Fig. 12-9). Loin discomfort in such a patient does not benefit from operation on an unobstructed pelvis. Pelvic dilatation associated with significant resistance to outflow causes obstructive nephropathy and prolongs the parenchymal transit-time index above normal. The altered nephron pressure gradient affects tubuloglomerular balance in the cortical nephrons, and GFR and ERPF are reduced. It also reduces the perfusion and concentrating ability of the juxtamedullary nephrons, eventually leading to their atrophy. As obstructive nephropathy progresses, relative uptake function falls. The change is potentially reversible, provided that infection has not supervened and destroyed nephrons, but the more diminished the function, the slower the recovery.

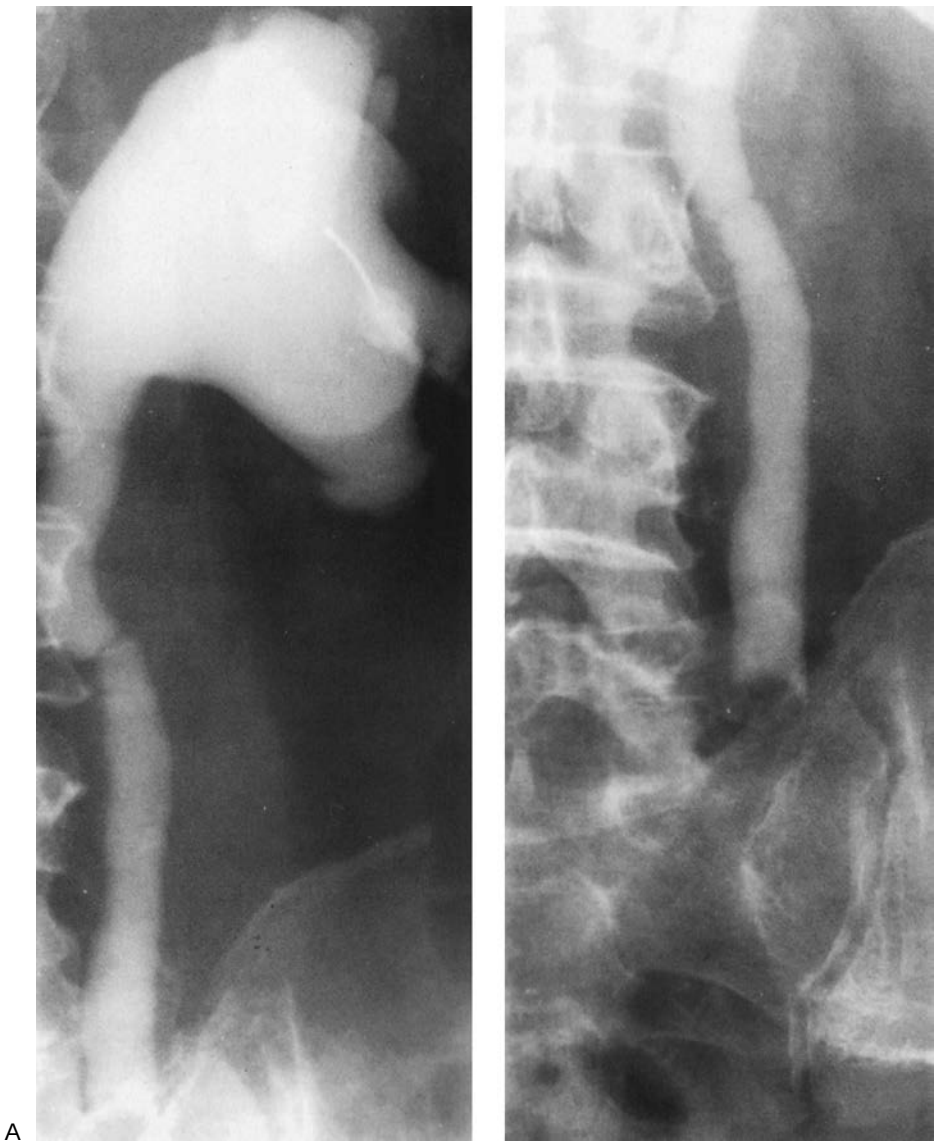


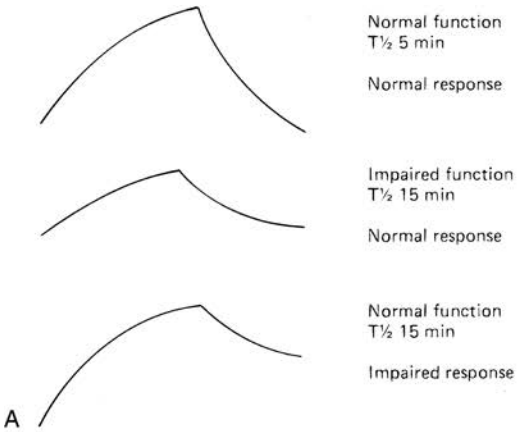
FIGURE 12-33. Left antegrade pyelogram in a 68-year-old man with a transitional cell tumor obstructing the ureter at L5 level. Note the rounded intraluminal lucency of the tumor (**B**).

An alternative approach is to evaluate the effect of a furosemide diuresis (190). Pelvic dilatation with an insignificant resistance to outflow is overcome by the diuresis so that the $^{99}Tc^{m}$ DTPA or $^{99}Tc^{m}$ MAG3 activity–time curve falls and pelvic activity washes out. Failure to show an effect of furosemide indicates obstructing uropathy (191–194). However, the degree of radionuclide response to furosemide depends on the amount of activity previously taken up by the nephrons and thus on the number of nephrons. With renal impairment, there may be too few nephrons to allow sufficient diuresis. An impaired response to furosemide is significant in a normal kidney, whereas the same response may be entirely appropriate in a kidney with poorer function. Thus, the response to furosemide must be judged in relation to the amount of activity taken up by the kidney (Fig. 12-34). Furthermore, a normal kidney may show such a diuresis that a slight but significant resistance may be overcome, leading to the false conclusion that no obstructing uropathy is present (195). Comparison of the furosemide response with antegrade perfusion pressure measurements has not been encouraging (196). A quantitative method for directly relating the response to furosemide to the degree of renal uptake function has been described; it gives an objective measurement of output efficiency (197). Normally, over 78% of the activity that was taken up by the kidney, however little, is put out by 30 minutes. Reduced output efficiency indicates an obstructing uropathy and is more accurate than a visual assessment of the curve or a numerical index based on the postfurosemide response (Fig. 12-34B,C,D) (198–200).

In conclusion, the individual kidney contribution to total renal function, the PTTI, and the response to furosemide given at 20 minutes all can be determined in the same single study. These investigations provide an essential pathophysiologic assessment of obstructive nephropathy and obstructing uropathy before surgery and for postsurgical follow-up study.

RENAL FAILURE

Kidney imaging in renal failure aims to show renal size, because small kidneys indicate irreversible chronic renal failure, and to demonstrate or exclude pelvicaliceal system dilatation, which may indicate obstruction. Ultrasonography and plain films of the renal tract are the imaging methods of choice in patients with renal failure (58,184,185). In established renal failure, nuclear imaging techniques with Tc-99m MAG3 are more helpful than Tc-99m DTPA when the GFR is low. Cr-51 EDTA



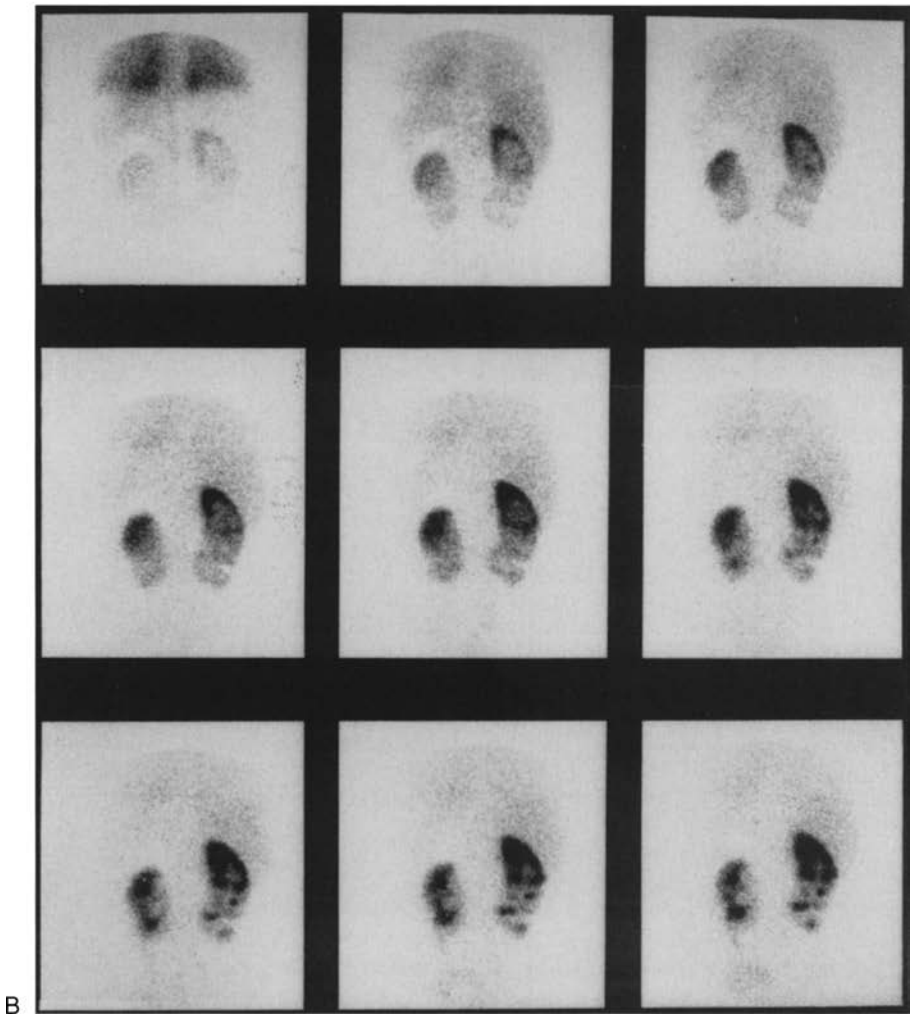


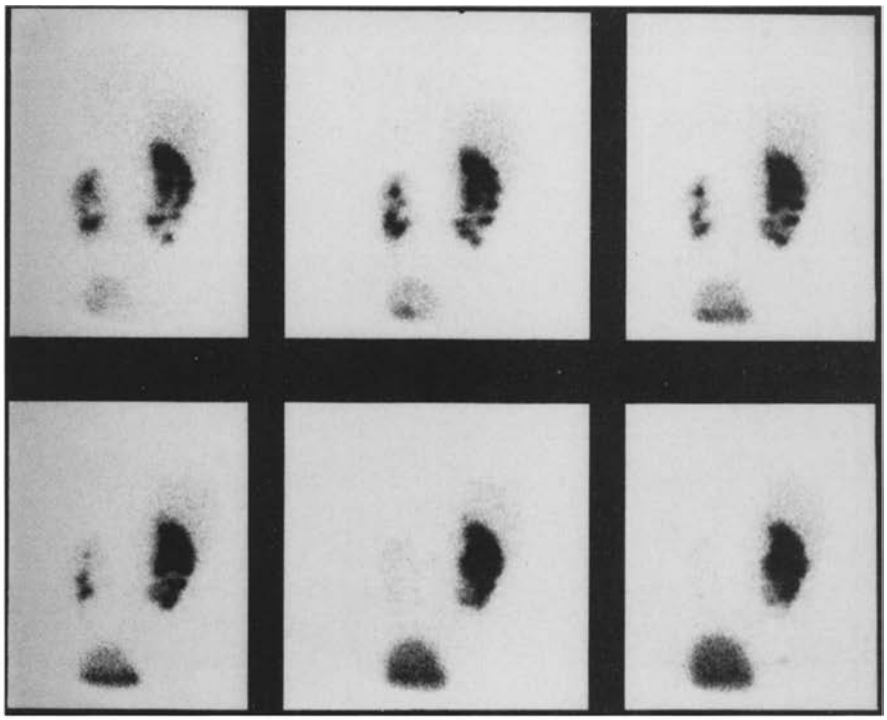
FIGURE 12-34. A: Continuously rising $^{99}Tc^{m}$ DTPA activity time curves and the response to furosemide diagrammatically. The upper curve shows normal uptake and a normal response to furosemide (half-life T1/2 5 minutes). The middle curve shows a poorer uptake and a poorer response to furosemide (T1/2 15 minutes), but it is appropriate to the poorer uptake and thus a normal response for that kidney. The lower curve shows normal uptake and an impaired response to furosemide (T1/2 15 minutes). This response is inappropriate to the normal uptake and indicates an obstructive uropathy. The T1/2 of response or any simple "excretory index" thus cannot be used to determine the presence of obstructive uropathy. **B** and **C:** $^{99}Tc^{m}$ MAG3 renal study in a patient with recurrent obstructing uropathy after Anderson Hynes pyeloplasty to the left kidney. (Posterior views, left kidney is on the left side.) **B:** Images taken at 30-second intervals. Top left to bottom right. (*continued*)

GFR measurements are more accurate than conventional clearances.

Ultrasonography

Renal size can be measured accurately with ultrasonography provided care is taken to obtain the measurements in the true long renal axis. Ultrasonographic measurements are less than those obtained by urography, with 9 cm considered the lower limit of normal for an adult kidney (58). Renal parenchymal thickness should also be assessed and often decreases before renal length (Fig. 12-35).

In renal failure, ultrasonography is usually a sensitive detector of pelvicaliceal dilatation, even when it is minor (Fig. 12-36) (58,184,185). A plain film should be obtained in all cases, and plain tomography to show low-density calculi usually is preferred. Rarely, obstructive renal failure may occur without collecting system dilatation detectable on ultrasonography (201–205). Many of the reported cases did not show pelvicaliceal dilatation on either computed tomography or antegrade pyelography, and the diagnosis was made only when function



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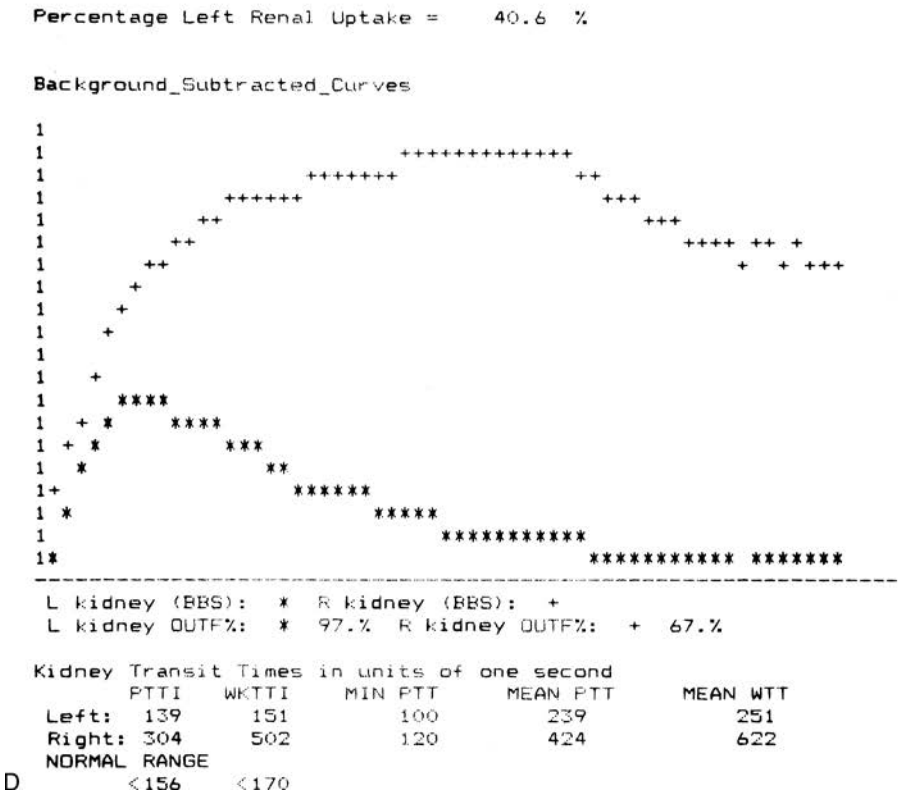


D

FIGURE 12-34. (*Continued*) **C:** Images taken at 5-minute intervals, the first at 5 minutes. *Top left to bottom right:* Furosemide, 40 mg, given at 18 minutes. Note the "negative phase" in the early images of the right kidney, followed by retention of activity in the later images and a poor response to furosemide. The left kidney appears small, with some calyceal retention of activity but with a good response to furosemide. **D:** Renal activity–time curves and quantitation. The left activity–time curve is normal; the right rises to a plateau and shows some response to furosemide; output efficiency (OUT F%) is, however, below normal at 67%. The left kidney contributes 40.6% (right, 59.4%) of total function. The left transit times are normal; the right are prolonged (PTTI 304 seconds, WKTTI 502 seconds; normal less than 156 and less than 170 seconds, respectively), confirming right obstructive nephropathy with the obstructing uropathy.

improved after percutaneous drainage. If no cause for renal failure can be found, therefore, a trial of collecting system drainage by the antegrade or retrograde route occasionally may need to be considered. Because minor dilatation can occur with severe obstructive renal failure, any degree of collecting system visualization warrants further investigation, usually by computed tomography or antegrade pyelography.

Ultrasonography also detects autosomal dominant polycystic kidney disease. The kidneys are enlarged with multiple cysts (too many to count) and loss of the normal parenchymal architecture in the established disease with renal failure (Fig. 12-37).

RENOVASCULAR HYPERTENSION

Renovascular hypertension is the name given to hypertension with a renal cause. It is present in about 5% of the adult

FIGURE 12-35. Chronic renal failure. Longitudinal ultrasound scan shows a small right kidney (8 cm) with thinned parenchyma (1 cm, indicated by +2) that has increased reflectivity compared to the adjacent normal liver parenchyma.

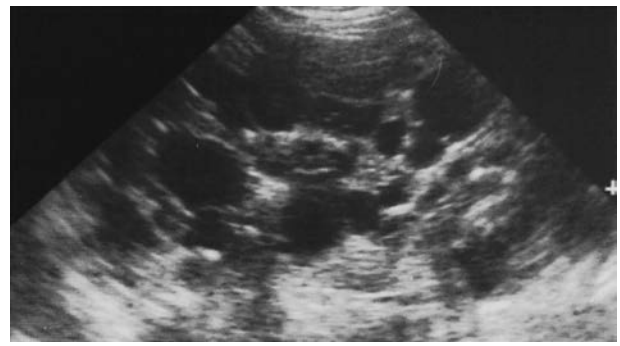


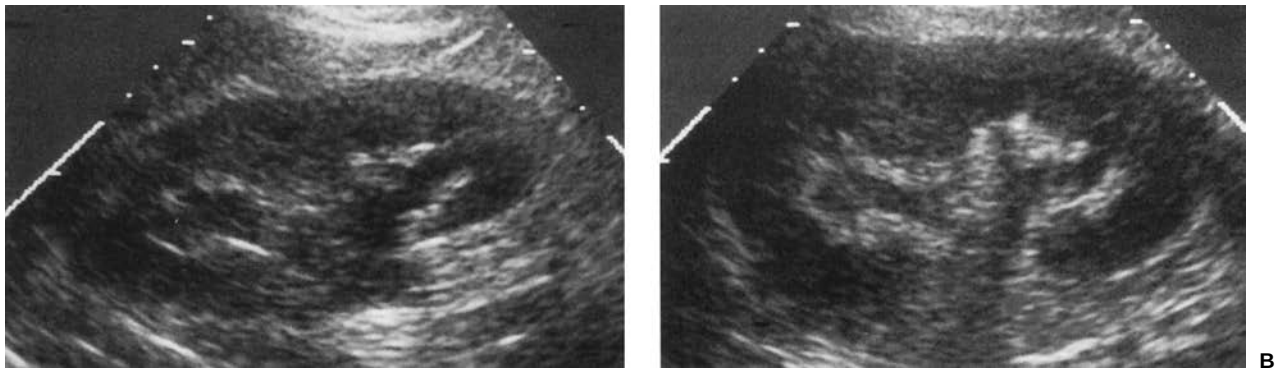
FIGURE 12-37. Adult polycystic kidney disease. Enlarged kidney (18 cm) replaced by multiple cysts of varying sizes. The opposite kidney appeared similar. (From: Meire HB, Cosgrove DO, Dewbury KC, eds. *Clinical ultrasound*. London: Churchill Livingstone, 1993, with permission.)

hypertensive population (206). Renovascular disorder is the name given to the disorder of kidney function that is thought to cause the hypertension. Correction of this disorder of renal function in one kidney leads to the correction of the hypertension, provided that the other kidney is functioning normally. That normality of function may be determined by the renal radionuclide study.

Renovascular disorder may be symmetrical when caused by general pathology such as glomerulonephritis, accelerated hypertension, diabetes, or autoimmune disease. It may be asymmetrical when caused by small vessel disease such as that caused by pyelonephritis, endarteritis (e.g., caused by tuberculosis, amyloid, or renal vein thrombosis). It may be asymmetrical because of large vessel disease, unilateral or bilateral renal artery stenosis or fibromuscular dysplasia, or in association with a resistance to outflow where pelvic dilatation may be seen.

The physiologic consequence of a reduced blood pressure to the glomeruli of cortical nephrons, whether caused by small-vessel disease or main renal artery stenosis, is a reduction in peritubular capillary pressure. Increased salt and water reabsorption prolongs the tubular transit time of nonreabsorbed solutes, such as $^{99}Tc^{m}$ DTPA, $^{99}Tc^{m}$ MAG3, and ^{123}IOIH. This results in a longer than normal mean parenchymal transit time (over 60 seconds), and the peak of the activity–time curve (the renogram) recorded over the affected kidney is delayed more than 60 seconds compared to the uninvolved organ. The reduced renal blood flow on the affected side also causes reduced radionuclide uptake. The combination of reduced uptake and a delayed peak in the affected renogram is the classic finding in functionally significant renovascular disorder, provided that pelvic dilatation is absent (82). Bilateral renovascular disorder prolongs the mean transit time by over 240 seconds. Both captopril and exercise enhance the changes in functionally significant renovascular disorder (207,208).

The early results of screening renography suggested an unacceptably high false-positive rate. This was probably caused by radiologically unrecognized small-vessel disease, because plasma renin was usually elevated (82). More recent quantitative renography with measurement of parenchymal transit times has been shown to be more accurate and suited to screening studies (209). The captopril test has been introduced as an alternative approach to improving the specificity of the changes in renal activity–time curves associated with renovascular disorder (210–212). Captopril, a short-acting angiotensin-converting enzyme (ACE) inhibitor, may act on the afferent arteriolar tone of cortical nephrons, which in renovascular disorder may well be already maximally dilated as an autoregulatory response to the reduction in perfusion pressure. Through inhibition of the local effect of circulating angiotensin



A B

FIGURE 12-36. Mild bilateral collecting system dilatation shown on longitudinal renal ultrasound scans in a patient with severe obstructive renal failure caused by retroperitoneal fibrosis. Right (**A**) and left (**B**) kidneys. (From: Meire HB, Cosgrove DO, Dewbury KC, eds. *Clinical ultrasound*. London: Churchill Livingstone, 1993, with permission.)

II, captopril relaxes the efferent arterioles of the juxtamedullary glomeruli, causing a further fall in glomerular filtrate, in addition to the already compromised flow caused by the reduction in renal perfusion pressure. Oei and associates (208) originally showed that captopril renography increased sensitivity from 75% to 94% and specificity from 84% to 100%, using the response to percutaneous angioplasty as the control for functionally significant renal artery stenosis. Hypotension may occur when renin levels are high or the patient is salt depleted, conditions typically related to diuretic therapy. Therefore, diuretics should be stopped for 2 days before the use of captopril intervention. Blood pressure is monitored before oral captopril administration (25 mg) and at 5-minute intervals afterward. If the diastolic pressure falls by more than 10 mm Hg, then the renal radionuclide study is commenced; otherwise, it is started 1 hour later. It is the effect on angiotensin conversion, not the effect on blood pressure, which selectively increases the sensitivity and specificity of the test.

Because of the extra intervention, it is recommended that a conventional gamma-camera study be performed first and that the captopril intervention then be performed subsequently with the repeat study so that the two can be compared. If results of the first study are normal, the subsequent study is undertaken only if the clinical likelihood of renovascular disorder is high. In the younger age group, once the combination of unilaterally reduced uptake, prolonged mean parenchymal transit time (MPTT) (213), captopril-induced greater abnormality of the activity–time curve, and MPTT typical of renovascular disorder owing to large (or small) vessel disease and preferably a contralateral normal kidney are found, then further investigation with angiography is indicated (210–212). A prolonged MPTT predicts the response to angioplasty (214).

A European multicenter study (215) has confirmed the sensitivity and specificity of captopril renography using $^{99}Tc^{m}$ DTPA. Its sensitivity in detecting unilateral angiographically confirmed stenosis was 73%, and for bilateral stenosis 91%. The specificity in the population overall was 84%, but in subjects with near-normal renal function it was 92%. The sensitivity for prediction of relief of hypertension was 93%, with tests becoming normal in 88%. This study was based on visual criteria and observations of reduction in relative uptake function only, not on transit-time measurements. A consensus report on captopril renography attests to its efficacy (216) and procedure guidelines are given (217). The cost effectiveness of this approach to renovascular disorder has been evaluated (218). The captopril test is also being applied in patients with renal impairment to predict whether ACE inhibitors will be beneficial (as is common in diabetic nephropathy) or detrimental to renal function, thus altering the patient's treatment (219).

Intrarenal blood flow distribution can be measured with ^{123}IOIH in hypertensive patients with near-normal renal function (220,221). It has been shown that cortical nephron flow is reduced in essential hypertension and may be important in its etiology and management, being corrected by certain drugs, such as captopril (195) and ramipril (222). It is postulated that an overautoregulation of cortical nephron flow related to the expression of the angiotensinogen gene may account for some essential hypertension (223).

Urography

Urography should not be used as a screening investigation for either renal or renovascular disease in adult hypertensive patients, because the number of unsuspected abnormalities detected is very low (224). Most cases of renal hypertension can be detected clinically. The rapid-sequence urogram uses films at 2, 3, 5, and 10 minutes after contrast-medium administration to search for the signs of renal ischemia. These signs are a small kidney with delay in pelvicaliceal filling, hyperconcentration of contrast medium in the collecting system, and notching of the collecting system by collateral arteries (Fig. 12-38). Because the rapid-sequence urogram misses approximately one in five patients with unilateral renal artery stenosis and can be normal even in the presence of complete renal artery block (225) it is no longer considered to be an effective screening test.

Ultrasonography

Duplex Doppler ultrasonography may be used to search for renal artery stenosis (226,227). The spectral criteria used to

FIGURE 12-38. Urogram of a hypertensive woman with left renal artery stenosis. Note the increased concentration of contrast medium and the notching of the pelvicaliceal system by collaterals on the affected side. (From: Grainger RG, Allison DJ, eds. *Diagnostic radiology,* 2nd ed. London: Churchill Livingstone, 1992, with permission.)

make the diagnosis are increased peak systolic velocity at the site of stenosis, and an increase in the ratio of the peak systolic velocity in the renal artery to that in the aorta. The combination of a peak systolic velocity greater than 180 cm/second and a renal to aortic ratio of 3.0 or greater appears to give the best sensitivity and specificity (228). The examination, however, is technically difficult because overlying bowel gas obscures much of the course of the renal arteries, especially on the left (67). Multiple renal arteries often cannot be detected (229).

More recently it has been suggested that the altered waveform in the intrarenal arteries can be used as an indirect indicator of renal artery stenosis. Renal artery stenosis is associated with a slowed systolic upstroke (pulsus tardus) and reduced systolic amplitude (pulsus parvus) (68,69). However, one study suggested the method can detect only stenoses greater than 80% (230). Measurement of resistance index (RI) in the intrarenal vessels has also been advocated for detecting renal artery stenosis, with lower RIs occurring with relatively severe stenosis (231). It has been suggested that RI measurement greater than 0.8 identifies patients with renal artery stenosis who are unlikely to benefit from angioplasty or surgery (232).

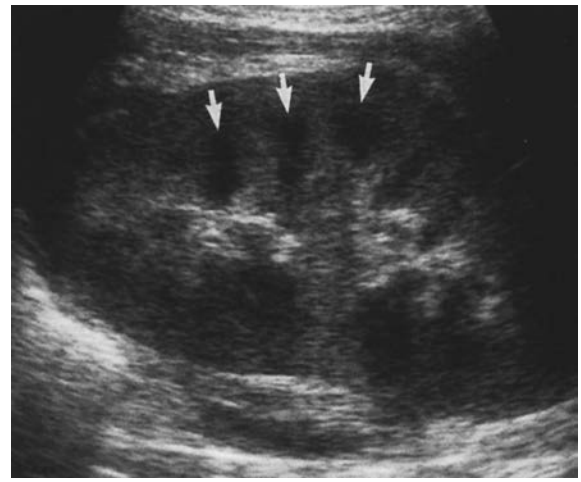


FIGURE 12-39. Longitudinal ultrasound scan of normal transplant kidney. Note the renal pyramids (*arrows*).

RENAL TRANSPLANTS

In the postoperative evaluation of renal transplants, serial radionuclide studies that evaluate both the perfusion and function of the transplanted kidney play an important role in diagnosing rejection and in differentiating it from acute tubular necrosis (ATN). Ultrasound imaging is most helpful in showing pelvicaliceal system dilatation and fluid collections around the transplanted kidney. Duplex Doppler studies are used to assess vascular anatomy and perfusion. Urography is now rarely used, but may be helpful in demonstrating urine leaks.

Radionuclide Studies

A baseline assessment should be made in the first few days after transplantation and followed by serial studies two or three times a week. Resolution of the ATN in the cadaver kidney then can be charted, and failure of improvement or deterioration can be diagnosed early.

The simplest approach is to monitor the blood clearance of ^{131}IOIH using an external detector. More usually, serial gamma-camera studies with ^{99}Tc^{m} DTPA or ^{99}Tc^{m} MAG3 are performed. The vascular blush indicates intact vasculature. ATN is characterized by a good early blush and a prolonged transit time so that no pelvic activity may be seen even after 20 minutes. As ATN resolves, uptake increases and the transit time shortens. If rejection supervenes, there is a reduction both in the early vascular blush and uptake. A perfusion index is derived from comparing the activity–time curve over the iliac vessel distal to the transplant (uptake to peak time) with that over the transplant (uptake to iliac vessel curve peak) corrected for background. This index normally improves with time, whereas rejection causes the index to rise (233). Individual values of this index are much less valuable than serial observation, but inspection of the series of activity–time curves usually is sufficient. Other radionuclide-labeled agents, such as fibrinogen, sulfur colloid, gallium citrate, and ^{111}In (indium)-labeled platelets (234), have their proponents, but none are as early predictors of rejection as repeated serial ^{99}Tc^{m} DTPA or ^{99}Tc^{m} MAG3 studies evaluated in the clinical context of the patient. Other causes of deterioration in an otherwise improving transplant, such as pelvic retention or extravasation of urine, may be identified, but the evaluation of obstructive nephropathy or renovascular disorder is difficult.

Ultrasonography

An examination in the first few days after surgery provides a useful baseline (Fig. 12-39). When transplant function deteriorates, ultrasonography can be used to show or exclude collecting system dilatation as an indicator of possible obstruction. The routine use of ultrasonography has demonstrated that perirenal fluid collections may occur in 50% of cases (235). The majority are small lymph collections that resolve spontaneously. Ultrasonography is unable to differentiate between collections of urine, lymph (Fig. 12-40), pus, or blood, but the presence of internal echoes in the collection suggests that it may be infected or hemorrhagic. Puncture of fluid collections, both for diagnosis and as a preliminary to catheter drainage, can be guided by ultrasonography.

Ultrasound imaging may show a variety of changes in acute rejection: increase in renal size, enlarged pyramids, reduced amplitude of the sinus echoes, loss of corticomedullary differentiation, and local areas of reduced echogenicity (236). However,

FIGURE 12-40. Transplant kidney obstructed by a large lymphocele. Note the dilated collecting system (*short arrow*) and adjacent large lymphocele (*long arrow*). (From: Grainger RG, Allison DJ, eds. *Diagnostic radiology,* 2nd ed. London: Churchill Livingstone, 1992, with permission.)

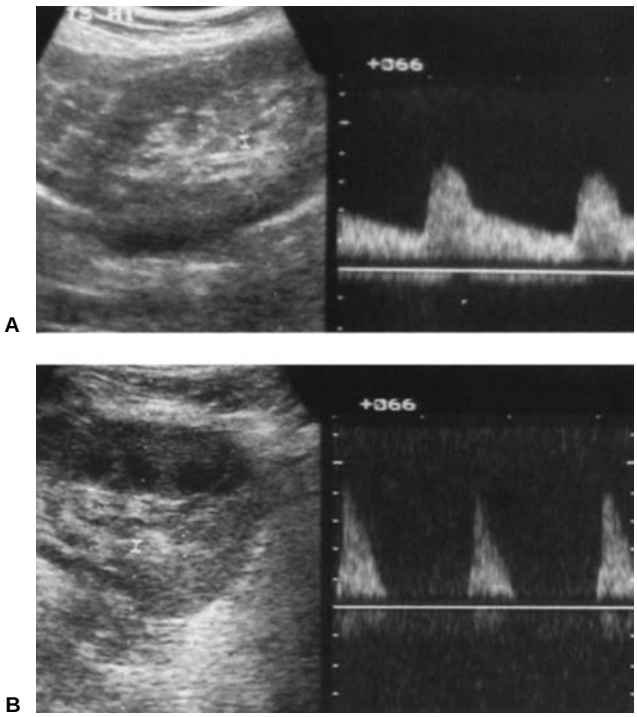


FIGURE 12-41. Duplex Doppler scans of transplant kidney. **A:** Normal intrarenal arterial waveform with flow continuing throughout diastole. **B:** Absence of diastolic flow in a kidney undergoing rejection. (From: Grainger RG, Allison DJ, eds. *Diagnostic radiology,* 2nd ed. London: Churchill Livingstone, 1992, with permission.)

these changes occur late in the development of rejection and are nonspecific, because they may occur in ATN (237–239).

Attempts have been made to diagnose rejection by quantitation of reduced diastolic flow in the segmental, interlobar, and arcuate arteries with duplex Doppler technique (Fig. 12-41). Initial results suggested that an increased resistance index was a good indicator of acute rejection (181,182). More recently, however, the specificity of an increased resistance index as an indicator of acute rejection has been questioned. It has been shown that an increased resistance index may occur in ATN (238,240,241). The definitive diagnosis of rejection requires biopsy, which should be performed with ultrasound guidance.

Duplex and color Doppler studies are most helpful in diagnosing the vascular complications of transplantation. The transplant renal artery is more accessible than the native artery. Renal artery stenosis may be suspected if aliasing is detected on the color image, and duplex diagnosis depends on detecting systolic velocity greater than 250 cm/second and spectral broadening of the waveform (242–244). In renal vein thrombosis, no color flow is seen in the vein, and spectral traces within the kidney show arterial flow reversal in diastole (244,245). Arteriovenous fistulae produce a typical perivascular color mosaic pattern, considered to be caused by turbulent flow and tissue vibration. On spectral examination, the feeding artery is enlarged with high velocity flow and there is pulsatile flow in the draining vein (244,246).

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CHAPTER 13 ■ COMPUTED TOMOGRAPHY AND MAGNETIC RESONANCE IMAGING

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Computed tomography (CT) and magnetic resonance imaging (MRI) have greatly expanded the radiologist's ability to assess renal pathology. Tomographic display of anatomy has allowed accurate, noninvasive evaluation of congenital anomalies, obstructive disease, inflammatory lesions, vascular insufficiency, and benign and malignant tumors. Both CT and MRI offer images of superb anatomic detail. Although tissue-specific diagnoses cannot be reached on the basis of morphologic abnormalities, the use of CT or MRI can narrow a list of diagnostic possibilities and can assist in the direction of percutaneous or operative biopsies. In addition, continued improvements in cross-sectional imaging technology have allowed for better assessment of the vasculature with CT angiography (CTA) and MR angiography (MRA).

COMPUTED TOMOGRAPHY

Technical Considerations

The primary advantages offered by CT lie in its ability to differentiate the x-ray attenuation properties of various tissues and in its generation of cross-sectional images. Superior soft-tissue contrast resolution is provided by the technical factors of scanner design, including a sophisticated x-ray source-detector system, which allows higher signal-to-noise ratios than can be produced with conventional radiographic methods; narrow and precise collimation of the x-ray beam, which decreases scattered radiation; and methods of data analysis and image reconstruction, which eliminate visual effects of structures lying outside the desired imaging plane. Computed tomography scanners presently in clinical use are capable of resolving x-ray attenuation differences of as little as 0.5% and providing spatial resolution of 0.5 to 1.0 mm.

The hardware of a CT system consists of an x-ray gantry that supports a rotating x-ray tube and a set of x-ray detectors, a patient couch, and a computer system interfaced with the gantry, data-storage system, and image-display console. The use of a rotating x-ray beam allows measurement of tissues' x-ray attenuation coefficients from many different projections. These measurements, recorded in digital form, are then converted by a filtered back-projection reconstruction method into analog images, which can be displayed on a cathode-ray-tube screen.

Computed tomography images are displayed over a fixed gray scale. The range of attenuation values to be presented is chosen by the system operator and is called the window width. The midpoint of the chosen range of attenuation values is called the *window level*. Window widths generally vary from 1 to 1,000 Hounsfield units; for renal CT, a width of about 500 Hounsfield units is usually chosen, with a window level of 15 to 30 Hounsfield units.

Multidetector-row CT (MDCT) has replaced helical (spiral) single slice CT as the standard method for performing CT examinations at most institutions. Helical CT previously replaced conventional CT due to faster scanning times (1). Now MDCT allows for even faster scanning time and differs from that of a single slice scanner in that the longitudinal (or slice) direction can be divided into multiple detectors containing multiple elements. The technological design initially used two- or four-detector systems. Currently, four- or eight-detector systems are widely in use and 16-detector systems have been recently introduced into clinical practice. For example a four-detector row CT is capable of sampling the outputs of up to four channels simultaneously and can contain 16 elements. A channel can be the output of a single element or can be the sum of the outputs of neighboring elements. For example, sampling the inner four elements (one element per channel) results in the acquisition of four 1.25-mm channels. The elements can be summed in groups of two, three or four, resulting in four 2.5-mm channels, four 3.75-mm channels, or four 5-mm channels, respectively. This technology allows for faster imaging; improved thin-section imaging (reduced volume averaging artifact), and image reconstruction (2). MDCT also can be employed to obtain angiographic information. This technique, called *CT angiography* (CTA), is able to directly evaluate the renal vasculature, and in most cases, eliminates the need for conventional renal angiography. During CTA, intravenous contrast material is rapidly injected and imaging takes place during peak arterial enhancement. Three-dimensional data reconstruction is performed and presented as a shaded surface display or maximum intensity projection (MIP).

Use of Contrast Media

Computed tomography evaluation of renal anatomy and pathology generally requires intravenous injection of iodinated contrast media. Only when renal or perirenal calcification, intrarenal or perirenal hemorrhage, or urine extravasation is suspected are nonenhanced scans recommended (3). In these situations, scans obtained only after the administration of contrast media may mask abnormalities.

Two different categories of iodinated contrast media are available: the traditional, high-osmolar (ionic) materials and the newer, low-osmolar (nonionic) materials. In high-osmolar contrast agents (HOCAs), iodine atoms are bound to an anion that in solution dissociates from a cation, typically sodium or meglumine. The resultant osmolality in serum is on the order of 1,400 mOsm/kg. In low-osmolar contrast agents (LOCAs), the iodine in solution is in the form of a monomer or dimer, and the associated serum osmolality is approximately 600 mOsm/kg. In low-risk patients, both types of contrast material have low incidences of severe allergic reactions (0.097% for HOCAs, 0.0037% for LOCAs), but the number of minor

reactions is significantly higher for HOCAs (3.19%) than for LOCAs (0.75%). Because LOCAs cost between 10 and 20 times as much as HOCAs, there has been a great deal of debate as to whether LOCAs should be used routinely or selectively in patients with risk factors for contrast reaction (4).

Renal contrast enhancement during CT is divided into four phases, similar to those described in intravenous urography (5,6). The aorta, the renal arteries, and the renal veins are the first structures to be enhanced. Within approximately 1 minute, a vascular nephrogram appears with dramatic enhancement of the renal cortex and visualization of the corticomedullary border. A tubular nephrogram follows during the next minute with the attenuation value of the renal medulla increasing so that it equals or exceeds that of the enhanced cortex. Contrast material appears in the renal collecting system within 2 to 3 minutes of its administration, producing a pyelogram (7,8).

Contrast media can be administered by either rapid intravenous bolus or infusion techniques. Bolus methods provide higher peak plasma concentrations of contrast, whereas infusion methods provide lower but more sustained peak plasma concentrations (7,8). Infusion methods are primarily used in conventional CT because of the long imaging time. The bolus technique is preferable for assessment of vascular and renal anatomy, and it is the standard method for administering intravenous contrast in spiral and multidetector CT. Typically, 150 mL of 60% iodinated contrast is administered by a mechanical injector at a rate of 2 mL per second (up to 4 mL per second during CTA). Imaging should begin no sooner than 45 seconds after injection. Additional delayed images are important in assessing renal pathology to allow opacification of the renal medulla and collecting system.

The rise in attenuation value expressed in Hounsfield units is linearly related to plasma iodine concentration (7,8). Delays in rise to peak attenuation values suggest structural or physiologic abnormalities. For example, the rise in attenuation value of a stenotic renal artery will be slower than that of the aorta, and the enhancement of the renal cortex may be delayed in patients with impaired renal function.

Because much of the diagnostic information available from CT depends on patterns of contrast enhancement, the evaluation of patients with renal failure is difficult. Carefully tailored examinations can allow use of minimal amounts of contrast material, reducing osmotic loads and iodine doses. The need for such technical adjustments should be made clear by the clinical history provided to the radiologist. Only the vascular phase of enhancement is seen reliably in patients with decreased glomerular filtration rates, because of poor contrast excretion.

Normal Anatomy

The retroperitoneum is divided by facial planes into three compartments: the perirenal, anterior pararenal, and posterior pararenal spaces. Abundant fat surrounds the major retroperitoneal organs and provides excellent inherent tissue contrast.

The kidneys, perirenal fat, and adrenal glands lie within the perirenal space, which is bounded anteriorly by Gerota's fascia and posteriorly by Zuckerkandl's fascia. The aorta, the inferior vena cava, the renal and adrenal arteries and veins, and the perivascular lymph nodes are also within this compartment. The kidneys are oval structures, the right lying somewhat caudal to the left. The collecting systems, renal pelvises, and ureters are best seen after administration of contrast agents, but they may be seen on unenhanced scans as water-density structures, particularly if dilated. The adrenal glands lie anterior and medial to the kidneys' upper poles and are bilobed V- or Y-shaped structures. The lateral limb of the right adrenal gland often is closely apposed to the inferior vena cava near the liver. The inferior vena cava and the aorta lie anterior to the spine, slightly to the right and left of midline, respectively. The renal arteries can be seen arising from the aorta and entering the renal hila. The renal veins are visible from their origins in the renal hila to their entrances into the inferior vena cava. The renal veins lie anterior to the renal arteries, and the left renal vein runs between the aorta and the superior mesenteric artery.

The anterior pararenal space contains the descending and transverse duodenum, the pancreas, and the ascending and descending portions of the colon. It is limited anteriorly by the parietal peritoneum and posteriorly by the renal fascia.

The posterior pararenal space contains only fat and is bounded posteriorly by the transversalis fascia. The parietal peritoneum and transversalis fascia fuse laterally to form the lateroconal fascia. The anterior and posterior pararenal spaces communicate to a limited extent above and below the level of the renal vessels. Fascial planes are demonstrated as linear structures of soft-tissue attenuation surrounded by low-density fat.

Congenital Variants of Renal Anatomy

The production of transaxial CT images makes evaluation of variance in renal position and configuration relatively simple. Ectopic kidneys, horseshoe kidneys, duplicated collecting systems, and varying degrees of hypoplasia or dysplasia are routinely detected (Fig. 13-1). Multiplanar and three-dimensional reconstructions may be helpful in the delineation of ectopic or fused kidneys.

Obstructive Disease

Although the CT assessment of renal excretion requires use of contrast agents, the anatomic site, degree, and cause of obstruction can be evaluated on nonenhanced images. The dilated, fluid-filled ureter is visualized as a round structure of water density surrounded by a thin wall. Masses or radiopaque stones obstructing a ureter are readily seen.

Acute lower urinary tract obstruction produces ureteral, pelvic, and calyceal dilatation. The acutely obstructed kidney may be enlarged and edematous. When iodinated contrast is given, a persistent nephrogram may appear and excretion of contrast material into the collecting system will be delayed

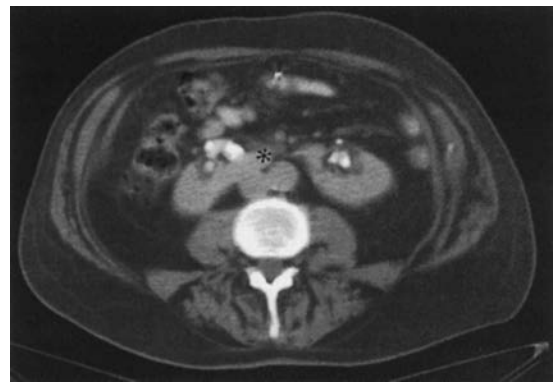


FIGURE 13-1. Contrast-enhanced CT image of a horseshoe kidney. The renal pelvises are directed anteriorly, and the isthmus (*) connecting the two kidneys is visible crossing the midline.

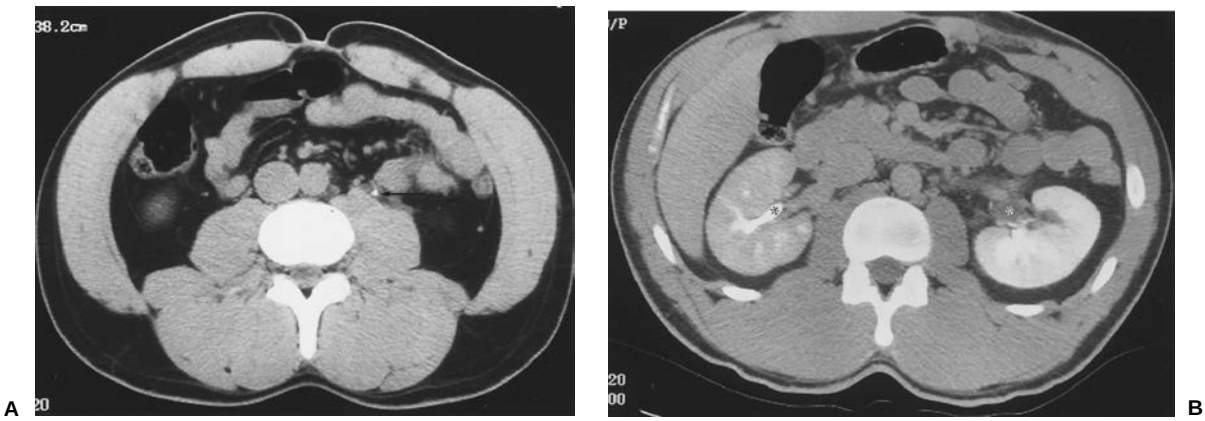


FIGURE 13-2. A: Computed tomography of the kidneys without iodinated contrast demonstrating a left ureteral stone (*arrow*). **B:** Computed tomography of the kidneys following contrast administration shows a left delayed nephrogram caused by the ureteral stone. There is hydronephrosis (*white*) and delayed excretion of contrast into the left renal pelvis; contrast filled normal right collecting system (*black*).

(Fig. 13-2). Chronic obstruction may produce massive distention of the fluid-filled collecting system and atrophy of the renal parenchyma. This loss of renal mass can be detected in noncontrast images (Fig. 13-3).

Nephrolithiasis

Computed tomography has replaced intravenous pyelography (IVP) for the evaluation of suspected renal calculi, in most medical centers, as it is an accurate, safe and rapid technique that does not require contrast. A study comparing CT and IVP in the evaluation of urolithiasis revealed a sensitivity of 100% and specificity of 92% with CT, and a sensitivity of 64% and specificity of 92% with IVP (9). Renal calculi, regardless of chemical composition, are generally radiodense on CT images (10), with the exception of Crixivan induced stones. Computed tomography, therefore, is able to demonstrate cystine and urate calculi that are undetectable on conventional radiographs or tomograms. Calculi of less than 1 mm can be identified.

Crixivan (indinavir sulfate) is a new class of antiviral agent (protease inhibitors) used to treat patients with human immunodeficiency virus (HIV). Crixivan can precipitate in the urinary system forming stones that are radiolucent and isodense on CT (11); therefore, the signs of obstruction, with the appropriate history, suggest their presence on CT.

When nephrolithiasis is suspected, initial images should be obtained before the administration of contrast agents because the very high-attenuation iodine compounds, when excreted into the renal collecting system, obscure underlying stones or calcifications (Fig. 13-4) (3). Oral contrast also should not be given because it can mask or simulate the presence of stones.

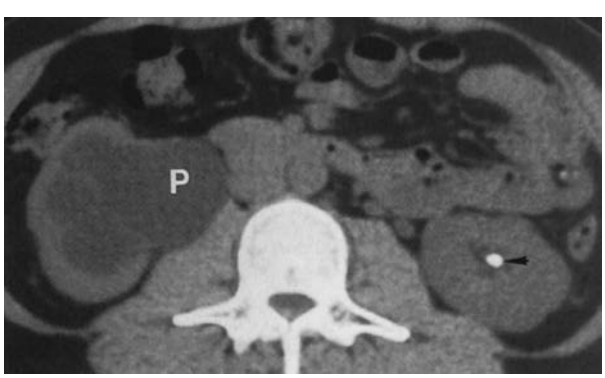


FIGURE 13-3. Computed tomography image obtained without iodinated contrast, demonstrating hydronephrosis of the right kidney caused by ureteral obstruction. The dilated renal pelvis (*P*) and calyces are of water density. The width of the right renal cortex is reduced; parenchymal atrophy has followed chronic obstruction. A calcified stone (*arrowhead*) is present in the left renal collecting system.

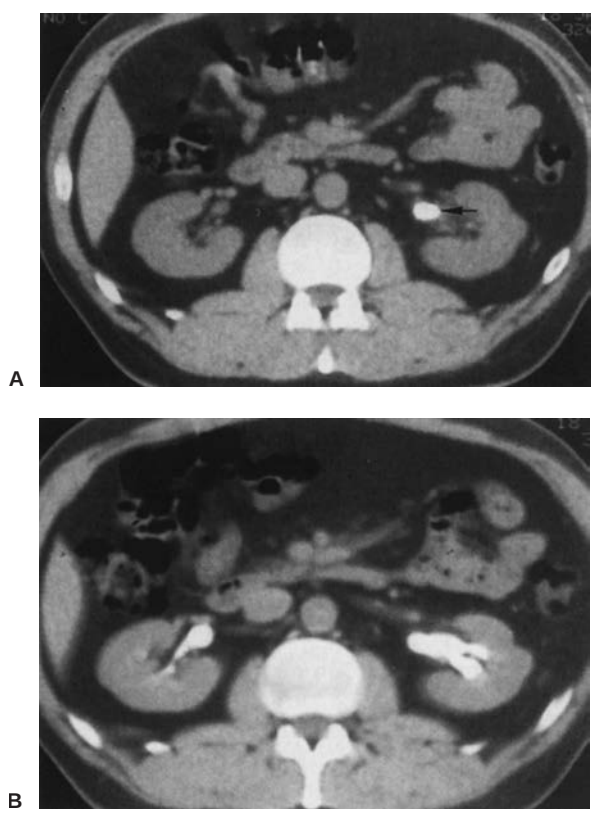


FIGURE 13-4. A: Computed tomography image obtained without iodinated contrast, revealing a calculus (*arrow*) in the left renal pelvis. **B:** After contrast administration, the stone is obscured by opacified urine.

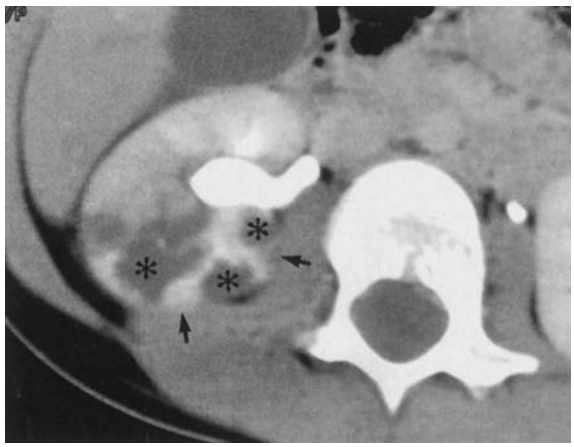


FIGURE 13-5. Contrast-enhanced CT image of a child with focal bacterial nephritis. Irregular areas of low attenuation, representing focal inflammatory masses, are identified posteriorly in the right renal cortex (*). The fat plane between the right kidney and psoas muscle is partially indistinct because of infiltration by the inflammatory process (*arrows*).

Section collimation should be adjusted so that thin slices (no more than 5 mm) are generated.

Inflammatory Disease

Acute Pyelonephritis

A variety of abnormalities are demonstrated on CT images of acutely inflamed kidneys. Precontrast scans may show only renal enlargement caused by parenchymal edema. Functional impairment of the infected kidney leads to heterogeneous patterns of contrast excretion, producing linear areas of low attenuation. Striated collections of renal parenchymal and pelvicaliceal air may be present. Severe inflammation may produce perirenal effusions and thickening of the perirenal fascia (12–14). Computed tomography also is useful in detecting long-term sequelae of renal infections, delineating the extent of renal scarring and atrophy, and explaining morphologic abnormalities of the collecting system. Abnormalities in renal morphology may be detected months after acute infection (15).

It has been well documented that CT is superior to ultrasonography in detecting and delineating renal and perirenal inflammatory masses and abscesses (15,16). In one retrospective study comparing CT and ultrasonography, the latter failed to detect 60% of cases of acute bacterial nephritis and 47% of renal and perirenal abscesses (16).

Focal Bacterial Nephritis

The patchy distribution of renal infection is demonstrated on CT images outlining focal inflammatory masses surrounded by normal parenchyma. Focal bacterial nephritis produces irregularly marginated areas of low attenuation, which enhance heterogeneously. The inflamed regions remain of lower density than surrounding normal tissue in contrast-enhanced images and frequently occupy wedge-shaped, lobar distributions (Fig. 13-5) (17,18).

Renal Abscesses

Abscesses appear as well-defined, rounded collections of low-density fluid in nonlobar distributions. An abscess may have a thick wall that enhances after contrast administration and may contain air (Fig. 13-6). Abscesses may extend into the perirenal fat and may interrupt the perirenal fascia (19). The perirenal fascia often is edematous. Computed tomography has been shown to be superior to sonography in defining the extent of pararenal inflammation and is preferred when surgical intervention is planned (6,18).

Fungal Infections

Fungal infections of the kidney are unusual except in immunocompromised patients. They also may occur in patients with chronic ureteral obstruction. Computed tomography manifestations include focal or global lack of contrast excretion, renal mass, renal enlargement, and filling defects of soft-tissue masses within the renal collecting system (20).

Xanthogranulomatous Pyelonephritis

Xanthogranulomatous pyelonephritis, a rare inflammatory condition that often follows chronic renal obstruction, produces intrapelvic and intracaliceal collections, which are of fluid or fatty density. The renal parenchyma often is atrophic and is replaced by accumulated fat, pus, and cellular debris. Calcification may be present in collecting structures. The pattern of contrast enhancement is variable, depending on the

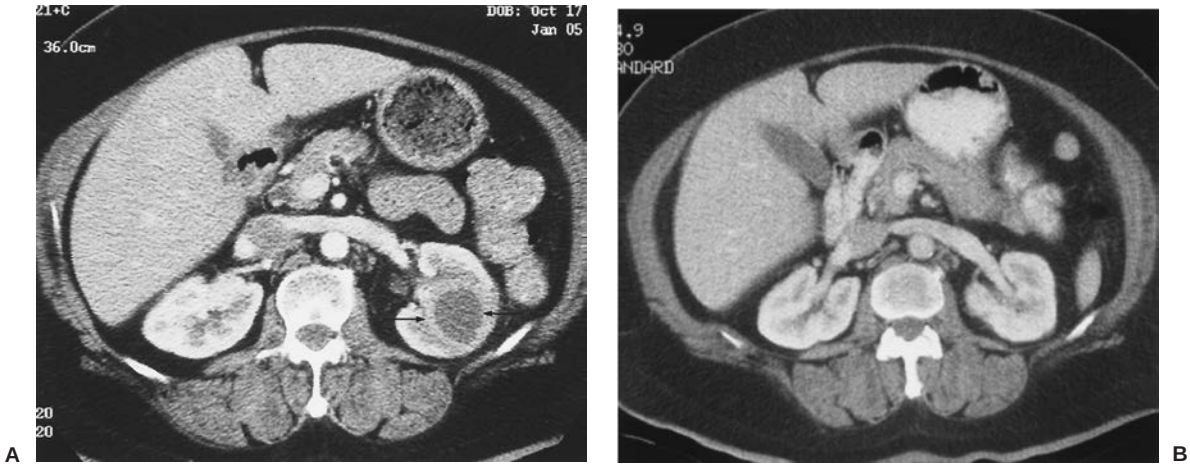


FIGURE 13-6. **A:** Contrast-enhanced CT of a renal abscess (*arrows*) demonstrated as a low-density fluid collection with rim enhancement. **B:** A follow-up CT scan performed 3 weeks later shows resolution of the renal abscess.

FIGURE 13-7. Contrast-enhanced CT image of xanthogranulomatous pyelonephritis. The right kidney is replaced by a low-attenuation inflammatory mass (*M*). A calculus (*arrow*) is present in the renal pelvis.

degree of function in the residual parenchyma. Hyperemic granulation tissue may enhance dramatically (21). Pararenal abscesses frequently are seen (Fig. 13-7).

Cystic Disease

Acquired cystic disease of the kidneys is extremely common, and an uncomplicated renal cyst can be diagnosed reliably by CT. Simple renal cysts are usually located in the renal cortex and are variable in size and number. A renal mass can be confidently called a *simple cyst* when it meets the following criteria on CT (22): (a) the mass has a homogenous low attenuation value near that of water; (b) its wall is so thin that it is nearly indiscernible; (c) it is very sharply delineated from surrounding renal parenchyma; and (4) its fluid contents do not increase in attenuation value after intravenous contrast media is infused (Fig. 13-8).

Complicated renal cysts may have contents of higher density, be irregular in shape, and have thicker or calcified walls. They may simulate solid tumors. Such lesions require further diagnostic evaluation (23). Percutaneous cyst aspiration and biopsy can be performed under CT guidance.

Parapelvic Cysts

Parapelvic cysts may mimic hydronephrosis during sonographic evaluation and on nonenhanced CT images. Computed tomography scans obtained after contrast-medium infusion clearly demonstrate these irregularly shaped thin-walled fluid collections surrounding the contrast-filled renal pelvis. Parapelvic cysts frequently are multiloculated and may be large enough to displace hilar fat and compress renal parenchyma.

Autosomal Dominant Polycystic Kidney Disease

In patients with autosomal dominant polycystic kidney disease, CT scans show replacement of renal parenchyma by innumerable cysts, which vary in size and attenuation value (Fig. 13-9). Kidneys may be of normal size or enlarged, depending on the stage of the disease. Parenchymal replacement often is asymmetrical, as is the amount of residual excretion.

Patients with autosomal dominant polycystic kidney disease are usually referred for CT evaluation because sudden massive renal enlargement causes clinical suspicion of abdominal malignancy or because known cysts may have been complicated by infection or hemorrhage. The detection of intracystic infection or hemorrhage often is extremely difficult because the attenuation values of these cysts vary depending on the presence of blood products, proteinaceous mucoid material, or simple cyst fluid. Sequential studies allow evaluation of fresh hemorrhage and resolution of hematomas. Computed tomography also can be used to demonstrate associated cystic metaplasia in the liver, pancreas, and spleen.

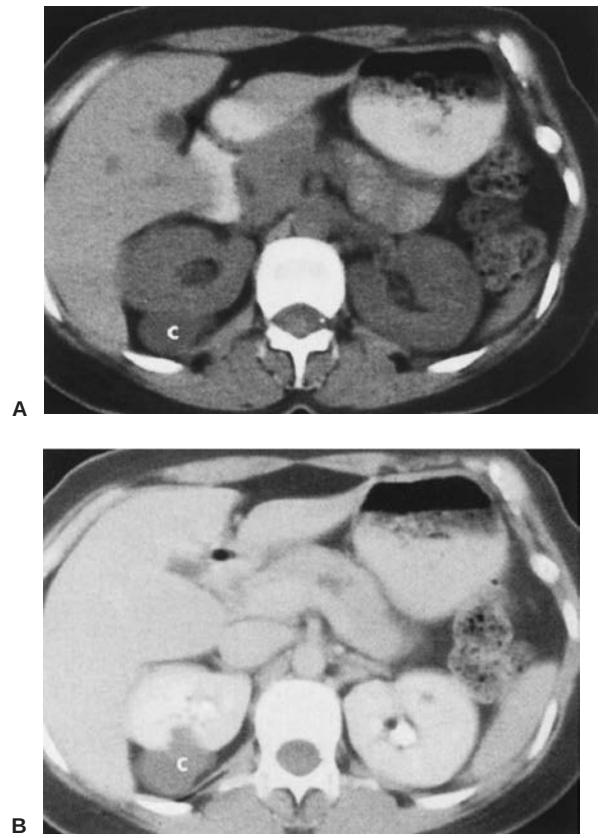


FIGURE 13-8. A: Computed tomography scan obtained without the use of iodinated contrast medium, demonstrating a simple renal cyst. A rounded mass (*c*) projecting from the posterior aspect of the right kidney has a slightly lower attenuation than the adjacent renal parenchyma in this precontrast image. **B:** The cyst (*c*) is clearly defined from the adjacent parenchyma, has no visible wall, and is not enhanced after contrast administration.

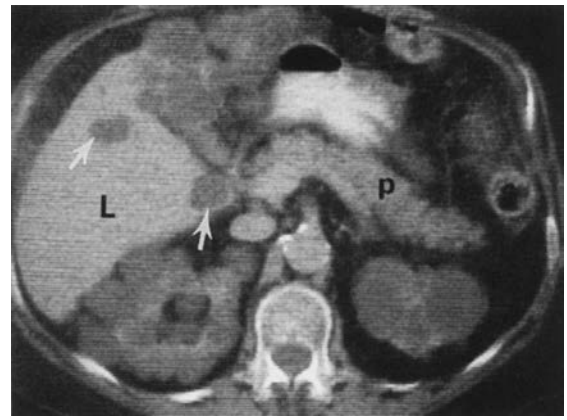


FIGURE 13-9. Contrast-enhanced CT image of a patient with adult polycystic disease. Multiple rounded low-density cysts replace the parenchyma of the kidneys. Cysts (*arrows*) are also present in the liver (*L*). The pancreas (*p*) is normal in appearance.

Acquired Cystic Disease Associated with Chronic Dialysis

Patients with acquired cystic disease associated with hemodialysis are known to have an increased incidence of both benign and malignant renal tumors. Although these patients are usually evaluated by sonography, the superior anatomic detail offered by CT make this technique preferable when signs of pain or hemorrhage suggest metaplastic or neoplastic degeneration (24,25).

Vascular Disease

Computed tomographic images that reveal very small, poorly excreting kidneys are produced in patients with renal failure caused by vascular insufficiency. Such patients include those with atherosclerosis, hypertension, or diabetes (Fig. 13-10).

Segmental renal infarction is most easily detected on contrast-enhanced CT images. Renal infarcts appear as clearly marginated wedge-shaped peripheral areas of low attenuation. They do not enhance after contrast administration (18). Infarcts are occasionally of lower density than normal parenchyma in nonenhanced images.

Computed tomography angiography can be used to assess the number and size of the renal arteries, information that is of particular importance to transplant surgeons before donor nephrectomy (Fig. 13-11). Studies have shown that CTA is

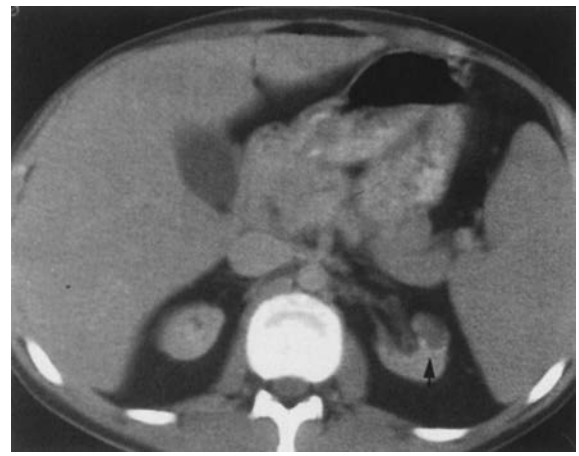


FIGURE 13-10. Computed tomography scan obtained without iodinated contrast. Atrophic kidneys are shown in a patient with chronic renal failure. A cystic lesion with a calcified rim is present in the small left kidney (*arrow*).

FIGURE 13-11. Computed tomography angiogram obtained during contrast enhancement in the arterial phase in a patient being evaluated for donor nephrectomy. The study shows that the patient has two right renal arteries. **A:** Axial image depicting the superior of the two right renal arteries (*arrow*). **B:** Axial image demonstrating the more inferior of the two right renal arteries (*arrow*). **C:** Coronal 3-D reconstructed image demonstrating both right renal arteries (*arrows*). The patient had one single left renal artery, thus the left kidney was selected for donor nephrectomy.

concordant with angiography in 89% to 96% of cases and concordant with surgery in 90% to 98% of cases. The most commonly missed findings are small accessory renal arteries and occasionally renal artery stenosis (26–28). In some institutions, CTA has replaced angiography and IVP in the evaluation of donor nephrectomy; with the benefits of a less expensive and less invasive test. Computed tomography angiography can also demonstrate renal artery aneurysms or stenosis, arteriovenous malformations, and focal or diffuse stenoses caused by atherosclerosis, connective tissue disease, or fibromuscular dysplasia. In one study, the accuracy of CTA in grading renal artery stenosis was 80% when maximum intensity projection images were used (29). Conventional angiography still remains the gold standard for the evaluation of the renal vasculature in most medical centers, but this may rapidly change with the advent of multidetector CTs.

Renal vein thrombosis produces intraluminal filling defects and vein enlargement. Renal enlargement and parenchymal edema are seen in acute venous occlusion and hemorrhagic infarction may occur. A persistent nephrogram may be seen after contrast administration, and contrast excretion is delayed or absent. Chronic renal vein thrombosis produces parenchymal atrophy and may be followed by development of venous collateral channels.

Renal Transplant Evaluation

Computed tomography can be used to evaluate the structural integrity of renal allografts and to detect peritransplant hematomas, urinomas, lymphoceles, and abscesses (Fig. 13-12). Dynamic scanning after bolus injection of contrast material may provide information about contrast excretion. The interval in which the density of the medulla reaches that of the enhanced cortex—the corticomedullary junction time—has been reported to be prolonged in transplanted kidneys undergoing rejection (30). In addition, CTA can be performed to assess the transplant vasculature (31).

FIGURE 13-12. Contrast-enhanced CT image of a renal transplant. A normally functioning renal allograft (*K*) lies in the right iliac fossa. Excreted contrast material is visible in the ureter (*arrow*) and layering dependently in the urinary bladder (*B*).

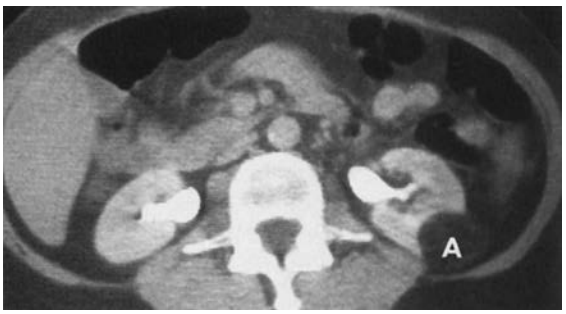


FIGURE 13-13. Contrast-enhanced CT scan of a renal hamartoma. Fatty tissue predominates in this left renal angiomyolipoma (*A*).

Renal Tumors

Benign Tumors

Benign tumors of the kidney are relatively uncommon, and of such masses, only the renal hamartoma or angiomyolipoma has a distinctive appearance on CT (32–34). Angiomyolipoma, which may occur in association with tuberous sclerosis or in the absence of systemic disease, is made up of smooth muscle, fatty, and vascular components in varying proportions. A confident diagnosis can be made with CT if the fatty tissue predominates (Fig. 13-13). This tumor may grow to a large size; masses more that 10 cm in diameter have been reported. Extension into the perinephric space is common, and intralesional hemorrhage is seen often. Angiomyolipomas usually are solitary, but multiple angiomyolipomas frequently are detected in patients with tuberous sclerosis. When vascular or smooth muscle components are present in greater amounts than fat, hamartomas are indistinguishable from other solid tumors and further radiologic workup or biopsy is necessary.

Renal Oncocytomas

Renal oncocytomas are extremely rare tumors that appear on CT images as smoothly marginated solid masses that enhance homogeneously (Fig. 13-14A). These masses are usually of lower attenuation than enhanced normal renal parenchyma, but are isodense in relation to surrounding renal tissue on nonenhanced studies (35,36). They may demonstrate a central linear area of lower attenuation on contrast-enhanced images (Fig. 13-14B). Their appearance on CT is not specific, and diagnosis of these benign masses necessitates an operative biopsy/resection.

Renal Adenomas

Renal adenomas are indistinguishable from other solid renal masses on CT images. They are variable in their pattern of contrast enhancement. Detection of such a mass, which may present as a contour abnormality or that may distort intrarenal anatomy, necessitates further workup and biopsy.

Malignant Tumors

Renal Cell Carcinoma

Renal cell carcinomas are relatively common tumors, accounting for 3% to 5% of all malignancies. Their appearance on CT images varies somewhat with the size and extent of tissue vascularity. The ability of CT to detect and stage renal tumors is good, with accuracy of staging exceeding 90% in many series

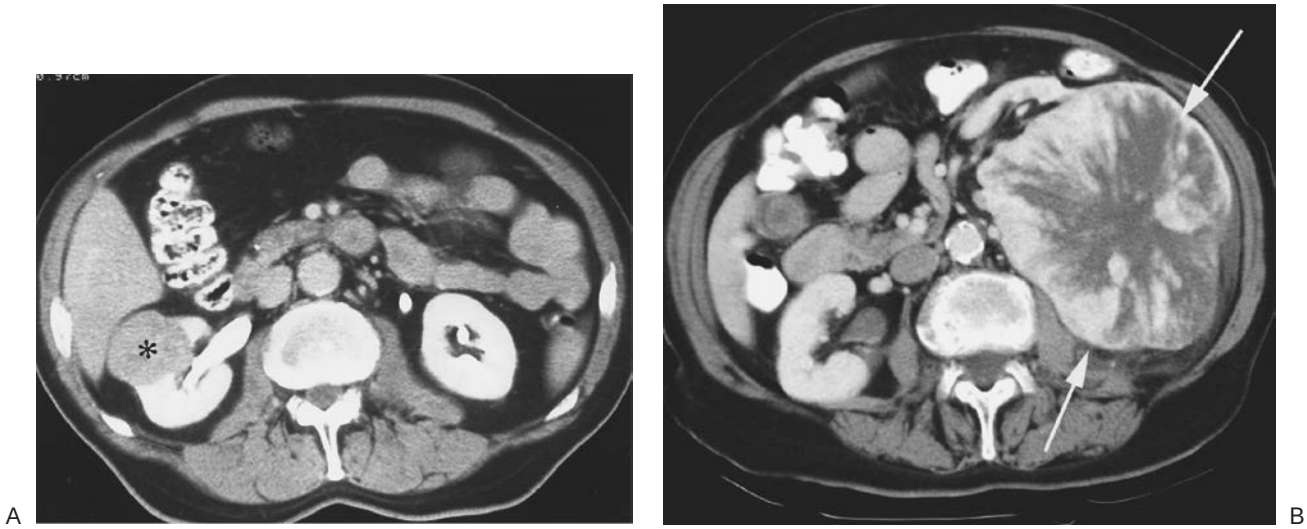


FIGURE 13-14. A: Contrast-enhanced CT scan of a renal oncocytoma (*) demonstrated as a smoothly marginated homogenous enhancing mass that is of lower attenuation than the normal parenchyma in one patient, and **B:** as a large heterogeneous enhancing mass with central low density in another patient (*arrows*).

(32,37,38). Computed tomography has replaced angiography in preoperative evaluation of renal tumors in most medical centers (39–41).

Renal cell carcinomas appear as masses that distort the contour of the kidney or its collecting structures. They are of a density similar to that of surrounding parenchyma in nonenhanced images. The administration of intravenous contrast material leads to heterogeneous enhancement of these tumors, whose attenuation usually remains lower than that of the surrounding cortex (Fig. 13-15) (42). Dynamic scanning after a bolus of contrast agent may reveal dramatic enhancement of relatively vascular tumors and often shows evidence of retroperitoneal feeding or collateral vessels. The interface between tumor and normal renal tissue typically is indistinct. Extension of tumor into perirenal fat or adjacent liver, spleen, or paraspinous musculature; interruption of perirenal fascial planes; and perihilar and perivascular adenopathy can be assessed accurately on good-quality CT images. Evaluation of the renal veins and inferior vena cava for the presence of tumor thrombus requires intravenous contrast media (Fig. 13-16). Dynamic scanning after rapid injection of a bolus of contrast best evaluates the renal veins and inferior vena cava.

Three-dimensional image reconstruction of renal tumors can be obtained with spiral and MDCT. The technique provides an adjunct to axial images in demonstrating the relationship of the tumor to the hilar vessels, collecting system, kidney surface, and adjacent tissues. This information can be important to the surgeon preoperatively, particularly if partial nephrectomy is being considered (Fig. 13-17) (43).

Recently, CT-guided radiofrequency ablation of renal cell carcinomas has been introduced as an alternative treatment option, particularly in patients that are not operative candidates. CT is also used to follow these patients to assess for residual disease or recurrence (Fig. 13-18) (44).

Renal Lymphoma or Leukemia

Leukemic infiltration may produce renal enlargement and heterogeneous density on enhanced and nonenhanced CT images. Renal lymphoma is almost exclusively caused by secondary involvement from systemic disease by direct invasion or hematogenous spread; and is more common in non-Hodgkin's lymphoma than in Hodgkin's lymphoma. The incidence of renal involvement in patients with lymphoma determined by

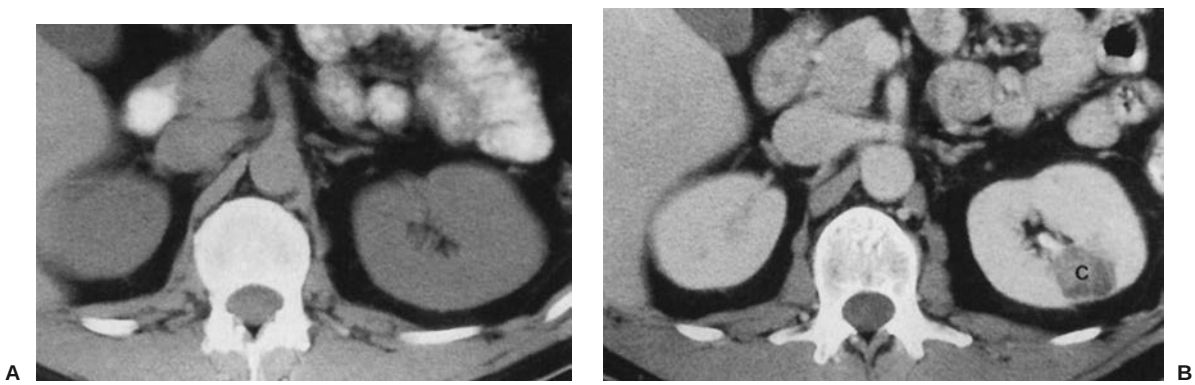


FIGURE 13-15. A: Computed tomography image obtained without iodinated contrast, demonstrating no definite abnormality. **B:** After contrast administration, the renal cell carcinoma (C) in the left kidney enhances to a lesser degree than the normal cortex.

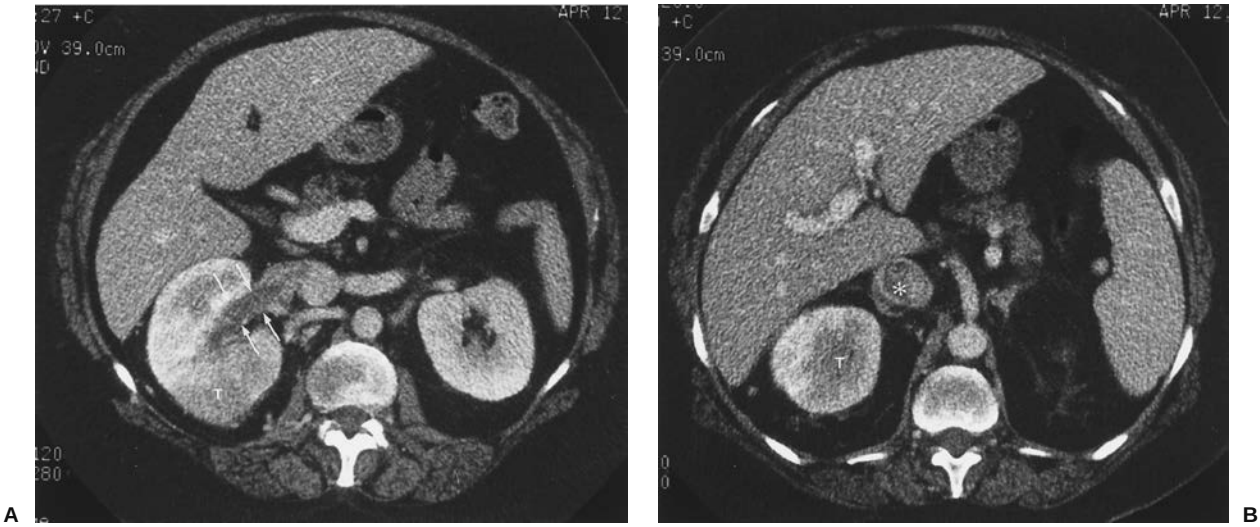


FIGURE 13-16. A: Contrast-enhanced CT scan of Stage IIIA renal cell carcinoma (*T*) with thrombosis of the renal vein (*arrows*). **B:** The thrombosis also extends into the inferior vena cava (∗).

autopsy is 34% to 62%. Computed tomography underestimates the incidence by detecting renal involvement in only 3% to 8% of patients with known lymphoma. Renal lymphoma has a variable radiologic appearance. The most common pattern is multiple masses that are isodense or slightly hyperdense to normal renal parenchyma on noncontrast CT and hypodense on contrast-enhanced CT (Fig. 13-19). Another common appearance is direct invasion from adjacent retroperitoneal lymphadenopathy resulting in distortion of the renal contour. An infiltrative pattern is another form of renal lymphoma, which typically manifests as renal enlargement with preservation of the reniform shape. Less frequently, renal lymphoma can present as a solitary mass (45).

Metastatic Disease

Primary tumors that may metastasize to the kidney include carcinomas of the lung, breast, adrenal gland, and colon, malignant melanoma, and non-Hodgkin's lymphoma. These tumors may produce solitary or multiple focal masses of lower attenuation than enhanced renal parenchyma (46). Direct extension of extrarenal retroperitoneal tumors may lead to renal obstruction and loss of function. Diagnosis generally is based on correlation with clinical history, particularly when multiple lesions are present.

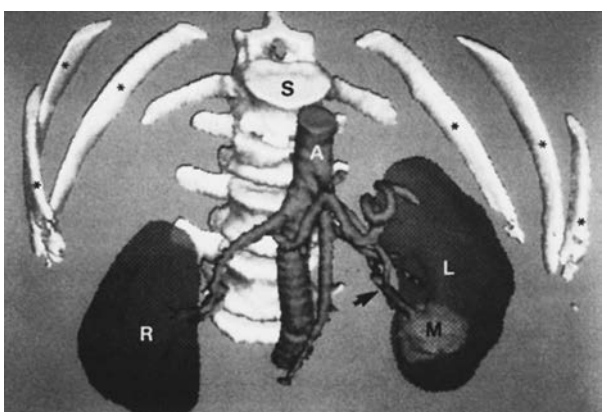


FIGURE 13-17. Three-dimensional reconstruction from a contrast-enhanced spiral CT of a renal cell carcinoma. The relationship of the left lower pole mass (*M*) to the entire left kidney (*L*) and renal arteries (*arrow*) can be assessed. The right kidney (*R*), aorta (*A*), spine (*S*), and ribs (∗) also are demonstrated.

Renal Sarcomas

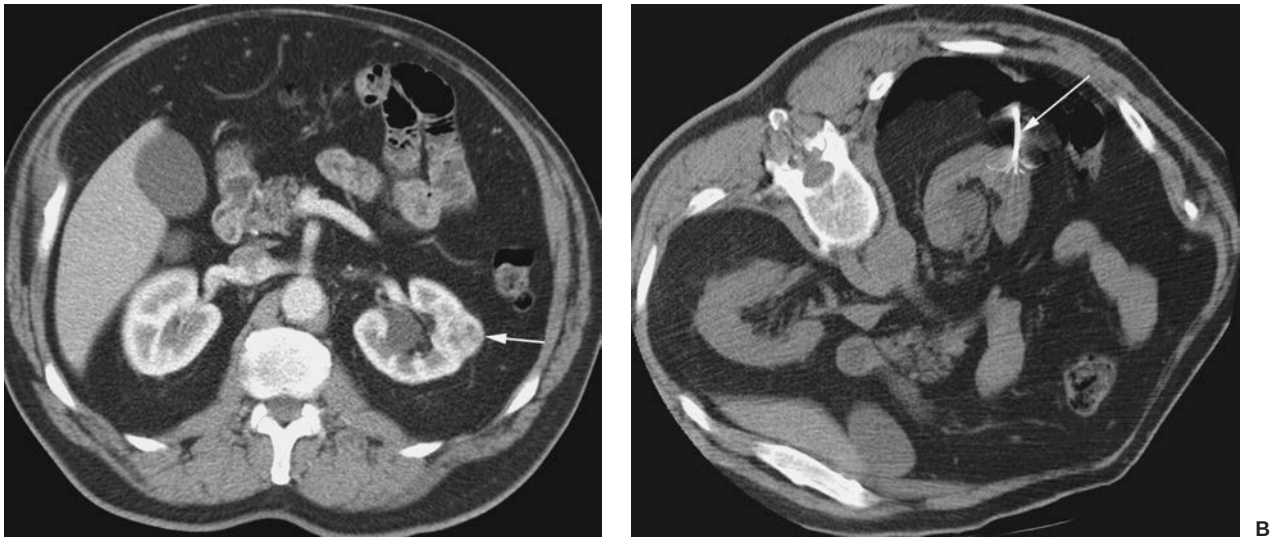
Mesenchymal tumors of the renal parenchyma or renal capsule are rare. They appear as solid masses, which may reach great size and vary in their degree of vascularity. Large tumors often contain central areas of low attenuation caused by necrosis (Fig. 13-20).

Transitional Cell Carcinoma

The appearance of transitional cell tumors of the renal pelvis and ureter on CT is fairly characteristic. Three distinct patterns have been described (47). These masses may present as sessile filling defects of soft-tissue density in the lumen of the opacified renal pelvis or calyx, sites of diffuse symmetrical thickening of the ureteral wall, or infiltrating renal masses that exhibit very little enhancement after contrast-media administration. Tumors may be smooth or papillary in contour and may cause ureteral obstruction and loss of renal function. Computed tomography images are useful for tumor staging, demonstrating invasion of perihilar fat and associated lymph node enlargement (47–49). With the advent of MDCT, many imaging centers have replaced conventional intravenous urography (IVU) with CT urography (with reformatted or 3D images) in the assessment of the renal collecting system (Fig. 13-21). The advantage of CT urography over conventional IVU is that CT in addition to assessing the collecting system can stage the tumor if present, can detect and characterize renal lesions and extra-renal masses not visible on IVU and is more sensitive in detecting renal calculi (50).

Renal Trauma

Most renal injuries (80% to 95%) are secondary to blunt trauma and are evaluated clinically and radiographically. Management is based on severity, which is classified as grade I, minor contusion with or without concomitant subcapsular hematoma; grade II, superficial laceration without involvement of the collecting system; grade III, deep parenchymal laceration



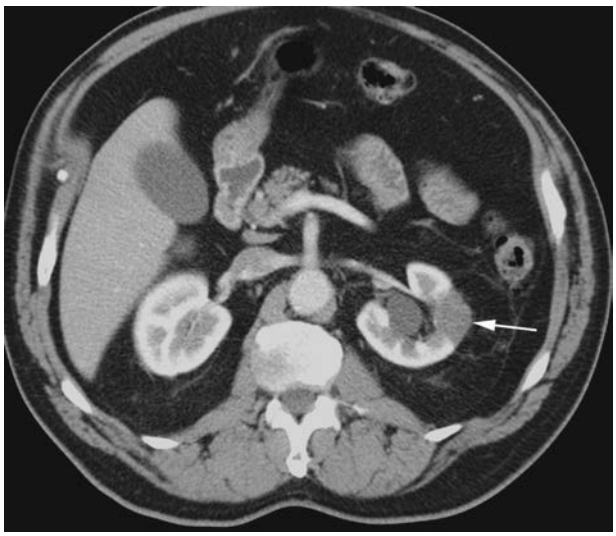


FIGURE 13-18. **A:** Contrast-enhanced CT scan of renal cell carcinoma (*arrow*). **B:** CT-guided radiofrequency ablation of the renal cell carcinoma with the ablation probe (*arrow*) demonstrated within the mass. **C:** Six month postablation contrast-enhanced CT scan shows expected residual low-density changes at the ablation site (*arrow*) with no enhancing components to suggest residual or recurrent tumor.

with or without urinary extravasation (Fig. 13-22); and grade IV, renal pedicle injury (51). The majority (85%–90%) of blunt renal injuries are grade I or II and are managed conservatively. Controversy exists over the management of grade III injury, with the most recent trend favoring conservative therapy (52). Grade IV and penetrating renal injuries are surgically explored. In some cases, injured renal vessels are managed with endovascular techniques, including stent placement.

Although CT does not appropriately evaluate patients with major abdominal injuries who are hemodynamically unstable, those with suspected renal injuries who are clinically stable can benefit greatly from CT assessment (41,53–59). Computed

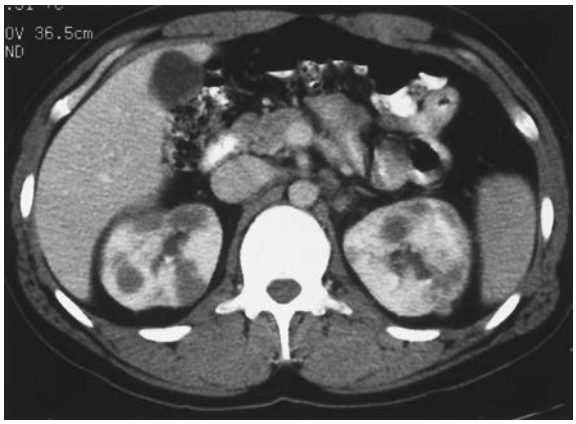
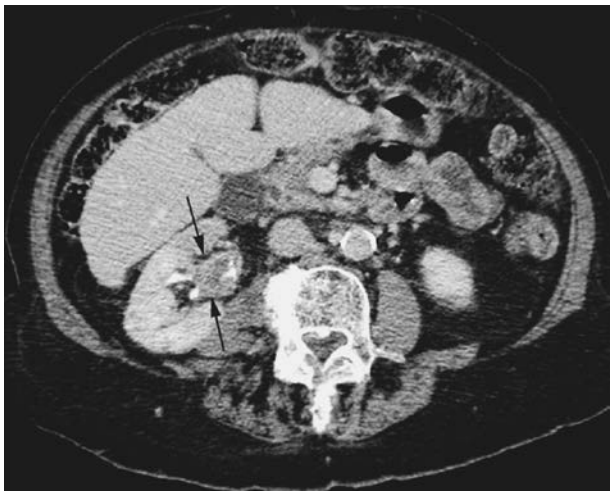


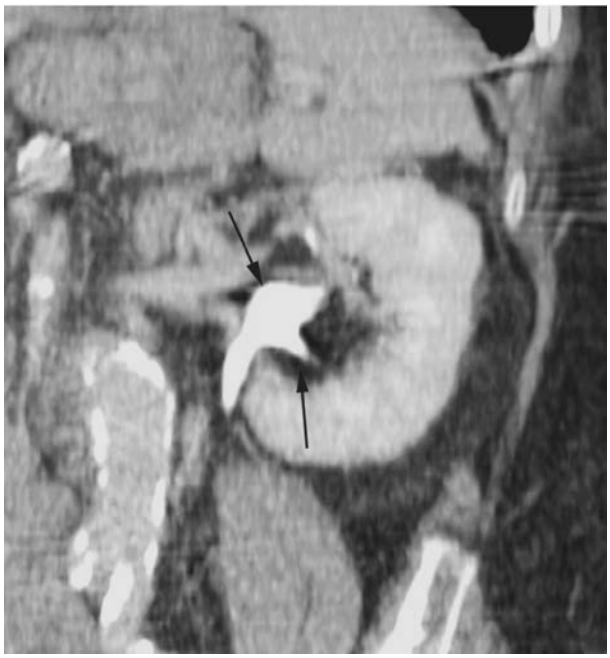
FIGURE 13-19. Contrast-enhanced CT scan of renal lymphoma demonstrated as multiple bilateral low-density masses.

FIGURE 13-20. A fibrosarcoma of the renal capsule on a contrast-enhanced CT image. A large tumor (*T*) of heterogeneous attenuation arises from the left kidney. The kidney is compressed; the mass fills the anterior pararenal space and displaces bowel loops anteriorly.



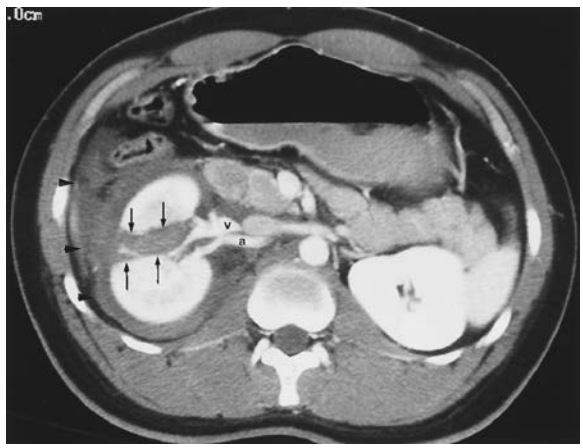
A

B

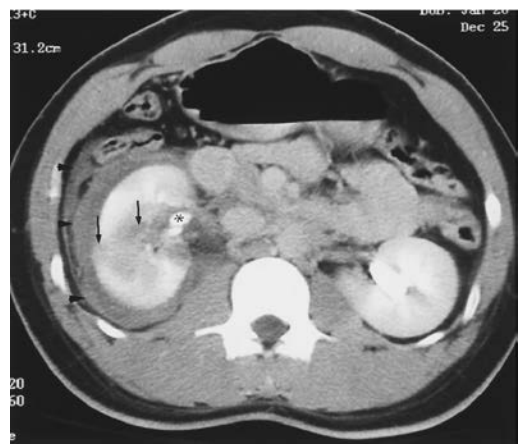


C

FIGURE 13-21. Axial contrast-enhanced CT scan image (**A**) and coronal reformatted image (**B**) of a right renal transitional cell carcinoma demonstrated as a mass/filling defect occupying the renal pelvis and inferior infundibulum (*arrows*). **C:** Coronal reformatted image of contralateral normal renal pelvis (*arrows*).



A



B

FIGURE 13-22. A: Contrast-enhanced CT of grade III renal injury secondary to blunt trauma. A deep laceration is shown by the arrows and surrounding perirenal and pararenal hematoma (*arrowheads*). The renal artery (*a*) and vein (*v*) are intact. **B:** Delayed images show an intact collecting system (∗).

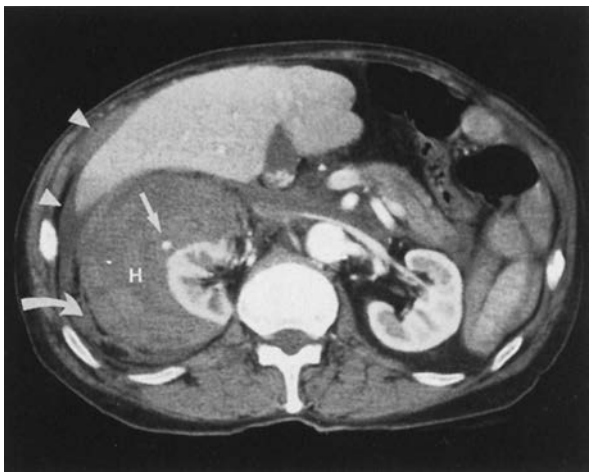


FIGURE 13-23. Contrast-enhanced CT of a patient who sustained blunt abdominal trauma. A large right subcapsular renal hematoma (*H*) contains a focal area of very high attenuation (*arrow*), representing active bleeding of contrast-opacified blood. Hemorrhage also extends into the right perinephric fat (*curved arrow*) and into the peritoneal cavity around the liver (*arrowheads*).

tomography scans obtained after contrast administration can detect renal contusions and are capable of discriminating between incomplete lacerations, which affect the renal cortex but do not communicate with pelvicaliceal structures, and complete lacerations, which interrupt the collecting system. Lacerations produce irregular streaks of low attenuation within the contrast-enhanced renal cortex. Subcapsular hematomas may not be apparent before contrast-media administration but are evident on postcontrast scans, when the enhanced kidney is compressed by a lower-density intracapsular hemorrhage (Fig. 13-23). Extravasated blood varies in its CT attenuation value, depending on the hemoglobin content and the age of the hematoma. Perirenal hemorrhage or urine extravasation (best seen on delayed images) may be diagnosed when fluid collections fill the perirenal or pararenal spaces and displace the kidney. Fresh hemorrhage may appear to have a higher attenuation value than extravasated urine in precontrast scans. Segmental occlusion of polar arteries may cause areas of renal infarction.

Catastrophic injuries, such as shattered kidneys, may be detected. Concomitant injuries to the liver, spleen, pancreas, and bowel may be evaluated during a single examination.

Relatively minor trauma may produce major injuries to kidneys with underlying abnormalities, such as cystic disease, chronic obstruction with hydronephrosis, benign or malignant tumors, or congenital anatomic variations (60).

MAGNETIC RESONANCE IMAGING

Technical Considerations

During MRI, tissue is placed in the central bore of an electromagnet generating a static magnetic field. Nuclei with odd numbers of protons or neutrons, of which hydrogen is the most abundant in biologic tissue, align themselves with their magnetic moments parallel or antiparallel to the external field. A net magnetization vector lies in a direction parallel to the static magnetic field because more nuclei align themselves in the lower-energy parallel orientation than in the higher-energy antiparallel state. If radiofrequency (RF) energy is superimposed on the system, the net magnetization vector will assume a new direction, the amount of its shift varying with the strength and duration of RF pulse. If the RF energy is of an appropriate frequency, resonance of the hydrogen protons between parallel and antiparallel orientations will be induced. The exact value of this resonance frequency depends on the particular nucleus affected and on the strength of the static magnetic field.

On cessation of the RF pulse, the net magnetization vector returns toward its original parallel orientation, and the resonating protons lose their phase coherence. During this process, called *relaxation,* electrical current or signal is induced in receiving coils. To produce magnetic resonance images, RF energy is employed in a pattern of repetitive pulsation and the signal emitted during the intervals between RF pulses is sampled. Two time constants describe the relaxation process: T1, or *spin-lattice* relaxation time, which characterizes the return of the net magnetization vector toward its parallel alignment; and T2, or *spin-spin* relaxation time, which characterizes the loss of the protons' phase coherence. The intensity of the signal produced is related to the magnitude of the net magnetization vector just before the onset of RF irradiation. The decay of the initial signal is caused by the loss of coherent resonance.

Information regarding the anatomic distribution of protons is acquired by establishing magnetic field gradients along the X, Y, and Z axes. Because the protons' resonance frequency depends on magnetic field strength and emitted signal frequency equals the protons' resonance frequency, the pattern of received signal from a selected tissue volume can be interpreted according to computer reconstruction techniques to produce an image in which digital information related to protons' spatial distribution is converted into analog images displayed over a gray scale.

The conventional RF pulse sequences are of spin echo type in biomedical MRI. In spin echo sequences, repetitive 90-degree RF pulses are applied at fixed intervals with 180-degree refocusing pulses interspaced between them to restore proton phase coherence. The interval between 90-degree RF pulses is called the *recovery time,* or TR; the time interval between the 90-degree RF pulses and the sampling of signal is called the echo delay time, or TE. The signal acquired at each interval multiple of TE is often referred to as an echo, hence the use of the terms *first* and *second-echo* images. In spin echo imaging, signal intensity is described by the equation

$$I = n(H)\, f(v) exp^{-TE/T2}(1 - exp^{-TR/T1})$$

where $n(H)$ represents spin density or proton density, and $f(v)$ is related to proton motion (61). The MRI system operator can choose TR and TE from a range of values. T1 and T2 are characteristic of the tissues being examined and vary with the chemical environment surrounding hydrogen protons. T1 also varies with magnetic field strength.

Tissues with short T1 values produce a signal of high intensity at all chosen TR values, because near-complete recovery of magnetization can occur between 90-degree RF pulses. Tissues with long T1 values produce a signal of low intensity when TR values are short, but produce a higher-intensity signal with TR prolongation. Tissues with short T2 values produce a signal that decays rapidly, because coherent resonance is lost quickly; their relative decrease in signal intensity is great when TE is long. Tissues with long T2 values continue to emit a relatively strong signal even when images are obtained with long TE values. For a given TR–TE combination, signal intensity increases with an increase in proton (spin) density, an increase in T2, or a decrease in T1. By varying TR and TE, images can be weighted to reflect differences in tissue T1 (short TR, short TE), T2 (long TR, long TE), or proton density (long TR, short TE).

The variation in signal intensity produced by tissues differing in magnetic relaxation properties is revealed by differences in pixel brightness on the images. Because tissues that vary only slightly in radiographic density may differ dramatically in magnetic relaxation properties, the soft-tissue contrast resolution provided by MRI techniques exceeds that offered by x-ray CT. Interfaces between adjacent structures are more easily detected by MRI. Saturation techniques have been developed that use initial 180-degree pulses, called *inversion recovery,* or selective signal nulling, called *chemical saturation*. These sequences essentially can be used to eliminate the signal from selected tissues, usually fat, which further accentuates tissue interfaces.

A significant limitation of conventional spin echo MRI is the lengthy scanning time required to obtain T2-weighted images, a result of the long TR and TE used on these pulse sequences. Artifacts from patient motion (respiratory, intestinal, and musculoskeletal) can severely degrade image quality, particularly in the upper abdomen. The long scanning period increases patient discomfort and fatigue, and it limits the number of examinations that can be performed in a given time. For these reasons, fast spin echo techniques have been developed. Scanning time is reduced because multiple echoes, called *echo trains,* are acquired per excitation, and the signals are combined to obtain an effective TE. The images are not entirely analogous to conventional spin echo studies for tissue characterization because fast spin echo techniques can only approximate a given TE. However, the faster scanning time and decrease in motion artifacts make the use of fast spin echo a technique superior to conventional spin echo for obtaining T2-weighted renal images. When conventional T2-weighted imaging is performed, proton density images are generally also acquired because no additional scanning time is necessary. Obtaining proton density images prolongs fast spin echo sequences, and they are usually not acquired with this technique. Ultra-fast T2-weighted sequences are now widely available on most scanners. These sequences known as single shot FSE (SSFSE) or HASTE (half-Fourier acquisition single shot turbo spin echo) can acquire images in a single breath hold to further minimize motion artifact.

It is also possible to generate MR images in a rapid fashion by using sequences in which the RF pulses used to disturb the net magnetization vector are of less than the 90 degrees used in spin echo sequences and gradient reversal rather than 180-degree RF pulses is used to restore phase coherence. These sequences, variously referred to as GRASS, FLASH, and gradient reversal (GRE) sequences, can be used to produce images in which contrast is based on differences in the T2 values of biologic tissue but TR values are shorter than 500 msec. Gradient reversal sequences shorten the required imaging time. However, they produce images that have limited signal-to-noise ratios and that are accompanied by specific artifacts that must be recognized by the radiologist interpreting the MRI examination. These sequences usually are added to the spin echo pulse sequences rather than substituted for them. In renal imaging studies, GRE sequences are employed to produce dynamic scans after the administration of intravenous contrast agents.

Gradient MRI can be used to generate angiographic studies, a technique called magnetic resonance angiography (MRA) (61). Using electrocardiographic gating, images also can be obtained at intervals throughout the cardiac cycle and replayed in cine fashion. A recently developed imaging sequence using rapid repetitive GRE called *echo planar* MRI can obtain multiple images in several seconds. Applications of this technique to renal MRI currently are under investigation.

Normal Anatomy

Retroperitoneal anatomic relationships, as depicted in the transverse plane, have been described earlier in this chapter. The appearance of the kidneys on MR images varies with the state of hydration and selection of imaging parameters (62). When T1-weighted spin echo imaging is employed, the renal cortex appears to have a higher signal intensity than the renal medulla, and a very distinct corticomedullary border is visible. The difference in signal intensity between the two regions of the kidney is caused by differences in tissue T1 values, that of the cortex being shorter than that of the medulla. This difference in signal intensity is not present in images obtained with long TR values (proton density and T2-weighted sequences), because more time is then available for recovery of magnetization by the renal medulla (Fig. 13-24).

The calyces, renal pelvises, and ureters are demonstrated as tubular or saccular structures of low signal intensity on T1-weighted images; the long T1 and T2 values of intraluminal

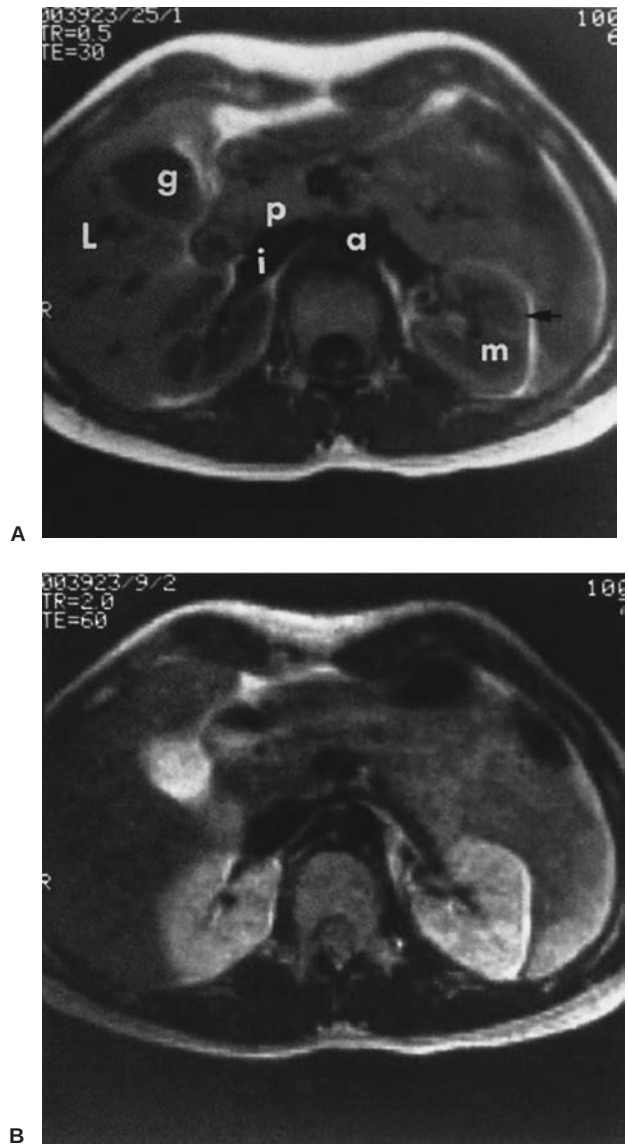


FIGURE 13-24. A: Axial T1-weighted image of normal kidneys demonstrates distinct corticomedullary boundaries. The cortex (*arrow*) and medulla (*m*) are labeled. The lumina of the inferior vena cava (*i*) and aorta (*a*) are devoid of signal. The liver (*L*), gallbladder (*g*), and pancreatic head (*p*) are clearly delineated. **B:** Axial T2-weighted image of the same patient shows that increased signal intensity in the renal medulla has eliminated corticomedullary contrast.

urine, a relatively pure fluid, account for the small amount of signal. These structures demonstrate marked increases in signal intensity on T2-weighted images.

The renal arteries and veins, aorta, and inferior vena cava are depicted as round or tubular structures, depending on the plane of section. They lack an intraluminal signal when blood flow is normal in pattern and velocity (63). The normally rapid-flowing blood within these vessels can be demonstrated as high signal with the use of GRE and angiographic techniques.

The perirenal fat, with short T1 and long T2 values, produces a signal of very high intensity on T1-weighted images. The adrenal glands, surrounded by fat, are visible as bilobed structures, the normal width of their limbs approximating that of the ipsilateral diaphragmatic crura. On T1-weighted images, the adrenal cortex appears to have a higher intensity than the adrenal medulla. The differentiation among adrenal cortex, medulla, and adjacent fat is not as well appreciated on T2-weighted images (64).

The perirenal fascial planes, composed chiefly of fibrous tissue of short T2 value, appear as linear structures of low signal intensity on both T1- and T2-weighted images. The striated paraspinous musculature is of low to medium signal intensity on T1-weighted images, and the signal markedly decreases on T2-weighted images.

The medullary portions of the vertebral bodies appear to have fairly high signal intensity on T1-weighted images because of the prominent fat within the bone marrow. The cortical bone does not produce a MRI signal; this type of bone lacks mobile protons and appears as a region of signal void conforming to expected skeletal contours.

Use of Contrast Media

The only intravenous contrast materials in widespread use for MRI are gadolinium (Gd) chelates. Gd DTPA (gadopentetate dimeglumine), an ionic compound, was the first agent used. Recently, several nonionic Gd compounds also have become available. In distinction to the iodine compounds used in CT, both ionic and nonionic Gd agents are associated with comparably low incidences of allergic reactions. The Gd compounds are filtered at the glomerulus and are neither reabsorbed nor secreted. They parallel iodinated contrast material in their pattern of initial intravascular and subsequent extracellular space distribution and their pattern of excretion. The kidney concentrates them as water is reabsorbed. Gd is paramagnetic, and it shortens both the T1 and T2 values of the tissue in which it is distributed. The relative effect on T1 and T2 depends on concentration; T1 is predominantly affected at low concentrations, and T2 at high concentrations. Gd administration causes renal cortical signal to enhance immediately after intravenous administration. Gd is excreted into the collecting system and renal pelvis within approximately 60 seconds after administration. At first the medullary signal is increased as relatively dilute Gd shortens urine T1 values. As progressive concentration occurs, T2 shortening becomes more important, and medullary and collecting structures emit a weaker signal. In sequential rapid-sequence MRI, a band of bright signal enhancement seems to sweep through the kidney from peripheral cortex to central medulla, followed by a band of signal loss as Gd is concentrated (65). The loss of concentrating ability owing to ureteral obstruction has been demonstrated with Gd in experimental settings and has been observed clinically (25,66–68). Gd contrast agents also can be used to attempt tissue characterization in patients with suspected renal cysts, and they can assist in delineating renal masses by documenting differential mass-normal parenchymal enhancement (69,70).

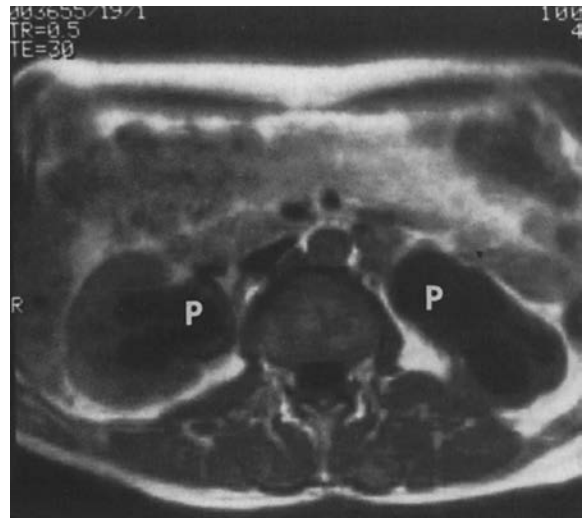


FIGURE 13-25. Axial T1-weighted image in a patient with hydronephrosis. Corticomedullary contrast is lost, and parenchymal atrophy has developed. The dilated renal pelvises (*P*) are labeled.

Congenital Variants of Renal Anatomy

The ability of MRI to generate images in multiple planes facilitates evaluation of anomalies in renal morphology, position, and number. Magnetic resonance imaging is particularly attractive for examining pediatric patients with suspected renal anomalies because no ionizing radiation is used and no exogenous contrast agents are required.

Obstructive Disease

The ureteral, pelvic, and calyceal dilatation caused by distal obstruction is easily appreciated on transaxial, sagittal, or coronal MR images. In addition to the site and possible cause of ureteral obstruction, the duration of ureteral obstruction can be assessed. Acute obstruction does not affect the visibility of the kidney's corticomedullary junction; long-term obstruction leads to loss of this boundary on T1-weighted images (71). Chronic obstruction also may cause detectable parenchymal atrophy (Fig. 13-25). Loss of renal concentrating ability owing to ureteral obstruction can be demonstrated with GRE sequences and intravenous Gd agents (68). Recently, MR urography using heavily T2-weighted images has been shown to be accurate in detecting obstruction and in almost half of cases is able to suggest the underlying pathology (72). Another study found that perirenal fluid was seen in 87% of acutely obstructed kidneys (73).

Nephrolithiasis

Renal stones do not produce a signal on MRI because they lack mobile protons. Calculi may be detected as sites of signal void within the renal parenchyma, collecting system, or ureters.

Inflammatory Disease

On T1-weighted images, acutely inflamed kidneys may show loss of corticomedullary distinction, perhaps caused by

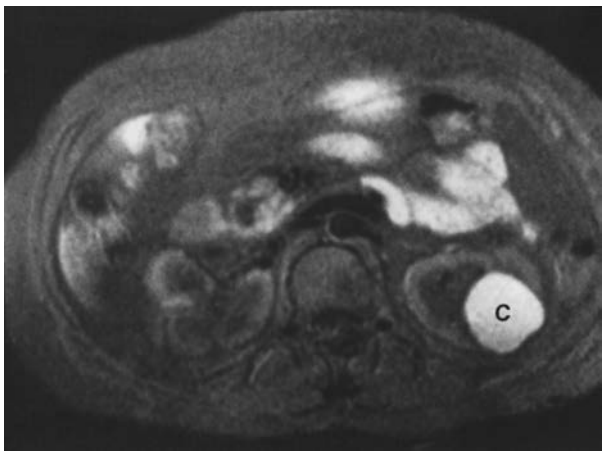


FIGURE 13-26. Axial T1-weighted image with fat suppression of a hemorrhagic cyst. The signal from subcutaneous and intraabdominal fat has been suppressed, but the hemorrhagic left renal cyst (*C*) remains of high signal intensity.

cortical T1 lengthening associated with intrarenal edema. Inflammatory disease may produce diffuse or focal areas of abnormal signal intensity. Perirenal edema may produce a decrease in signal intensity of surrounding fat; thickening of the renal fascia often accompanies inflammation. Renal abscesses appear as well-defined nonlobar fluid collections of variable signal intensity; a high-protein content in abscess fluid causes T1 shortening and produces high signal intensity. Areas of inflammation and abscess cavity walls demonstrate enhancement after Gd administration.

The T2-weighted images of inflammatory disease may demonstrate areas of increased signal within the renal parenchyma, corresponding to edema and inflammatory reaction. Extension into the surrounding fat is best demonstrated by fat-saturation techniques. Abscesses appear as well-defined collections of bright signal. Foci of gas within an abscess appear as signal voids on all sequences.

Cystic Disease

Magnetic resonance imaging demonstrates uncomplicated renal cysts as localized and clearly delineated round structures of low signal intensity on T1-weighted images. Simple cyst fluid has long T1 and T2 values, and it is bright in signal intensity on T2-weighted images. Magnetic resonance imaging can be used in a similar way as CT in cyst diagnosis, because cysts do not enhance after administration of Gd.

Cysts complicated by infection or hemorrhage vary in signal strength on T1-weighted images; high signal intensity is associated with elevated protein content and the presence of blood degradation products. Although signal intensity varies with the stage of the hemorrhage on both T1- and T2-weighted images, blood degradation products are generally bright on T1-weighted images. Differentiation of hemorrhagic cysts from lipomatous masses can be accomplished with fat-saturation techniques (Fig. 13-26).

The walls of complicated cysts may be thick. Calcific foci within cyst walls can be detected as sites of signal void (74). Cysts varying tremendously in size and signal intensity on T1-weighted images replace the kidneys in polycystic disease (Fig. 13-27).

Vascular Disease

Parenchymal atrophy caused by chronic vascular insufficiency may be detected on MR images. In addition, aortic and renal artery aneurysms or stenosis, renal arteriovenous malformations, and renal venous thrombosis can be demonstrated. The renal vessels are readily evaluated by spin echo MRI because their lumina, lacking signal in the presence of normal blood flow patterns, are easily distinguished from the surrounding tissue. GRE techniques and MRA also can be used to assess the aorta and renal vessels, and in many cases are as diagnostic as and have replaced conventional angiographic studies (75).

Various MR imaging techniques are available to assess patients for renal artery stenosis: noncontrast two- or three-dimensional time of flight or phase contrast, with or without gadolinium. All these techniques do come with limitations, but the three-dimensional gadolinium-enhanced is the preferred technique (76–78) (Fig. 13-28). The sensitivity and

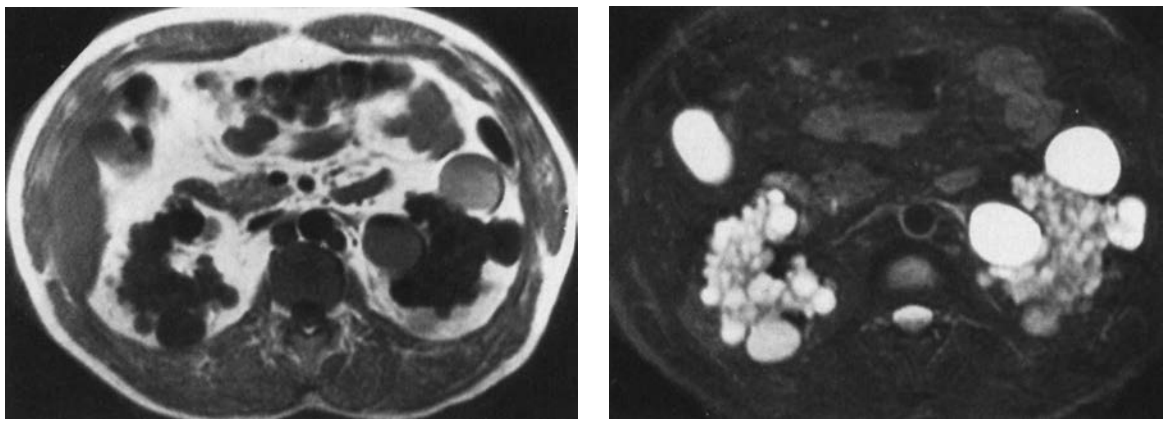


FIGURE 13-27. A: Axial T1-weighted image of adult polycystic disease. Multiple cysts of varying sizes and signal intensities replace the renal parenchyma bilaterally. **B:** Axial T2-weighted image with fat suppression of the same patient. The cysts are all of high signal intensity and are more conspicuous because of greater contrast with adjacent tissues.

FIGURE 13-28. Magnetic resonance angiogram of left renal artery stenosis. **A:** Coronal GRE T1 image obtained during the arterial phase of contrast enhancement demonstrates significant narrowing of the proximal left renal artery just beyond its origin (*arrow*). **B:** The patient underwent subsequent conventional angiography with stent (*arrow*) placement for treatment.

specificity using this technique range from 91% to 100% and 71% to 100%, respectively (76–81). Magnetic resonance angiography allows accurate evaluation without the risks associated with nephrotoxic contrast agents, ionizing radiation, or arterial catheterization.

In the evaluation of donor nephrectomy patients, MRA and MR urography have been shown to be as accurate as the conventional angiographic and urographic studies (82,83). The overall accuracy in detecting renal artery number by MRA is 90%.

Magnetic resonance imaging can demonstrate arterial dissections with intimal flaps appearing as linear structures of medium signal intensity within the dark vascular lumina on T1-weighted images. Slow blood flow often produces signal within the false lumen of an affected vessel, allowing visual distinction of true from false channels. GRE techniques can demonstrate the intimal flap as a low-signal-intensity structure within the bright signal associated with both flowing and stationary blood.

Evaluation of Patients with Renal Failure

Demonstration of detailed renal morphology by MRI does not require the use of exogenous contrast agents. This fact gives MRI a theoretical advantage over CT in evaluation of patients

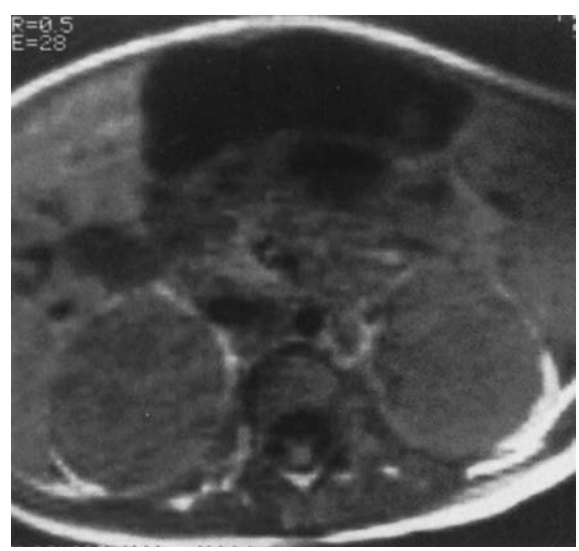


FIGURE 13-29. Axial T1-weighted image of a patient with acute glomerulonephritis. The kidneys are enlarged, and corticomedullary differentiation is lost. These findings reflect intrarenal edema.

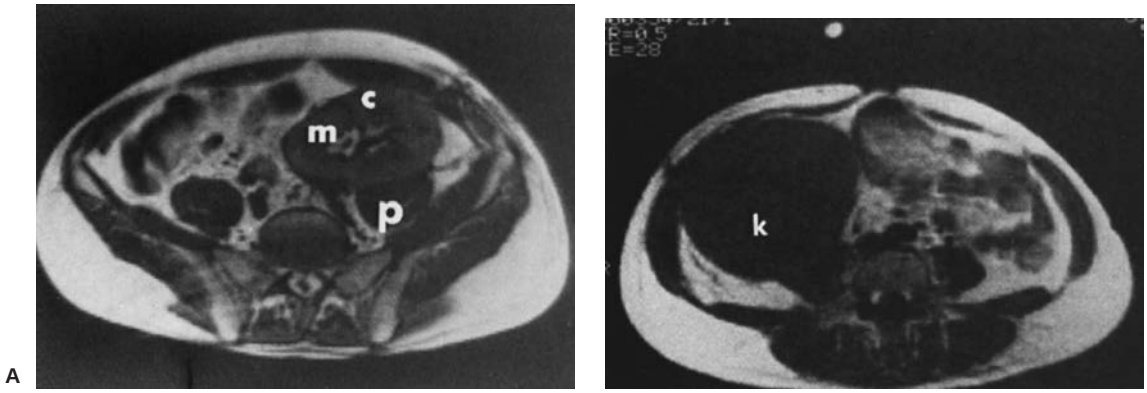


FIGURE 13-30. A: Axial T1-weighted image of a normal renal allograft demonstrates a very distinct interface between the renal medulla with low signal intensity (*m*) and the renal cortex with high signal intensity (*c*). The renal outline is very clearly delineated from the surrounding fat and adjacent psoas muscle (*p*). **B:** Axial T1-weighted image of a renal allograft (*k*) undergoing acute rejection. The kidney is enlarged, and corticomedullary differentiation is lost.

with renal failure. In addition, if contrast agents are desired to assess concentrating ability, Gd chelates have markedly lower incidences of renal toxicity than iodinated contrast media.

In patients with acute glomerulonephritis, MR images show swollen kidneys. Signal intensity is variable, depending on the degree and pattern of tissue edema. The corticomedullary boundary is lost on T1-weighted images (Fig. 13-29). This finding is nonspecific; a variety of pathologic states, including nephrotic syndrome, inflammation, renal vein thrombosis, and intrarenal hemorrhage, can cause loss of corticomedullary differentiation.

Evaluation of Renal Transplants

Magnetic resonance imaging may offer helpful information regarding the differential diagnosis of posttransplantation renal failure. The transplanted kidney undergoing rejection appears swollen in contour and shows no visible corticomedullary junction on T1-weighted images (Fig. 13-30). Associated inflammation may cause peritransplant fluid collections, a decrease in the signal intensity of the surrounding fat, and an increase in the signal intensity of the underlying psoas

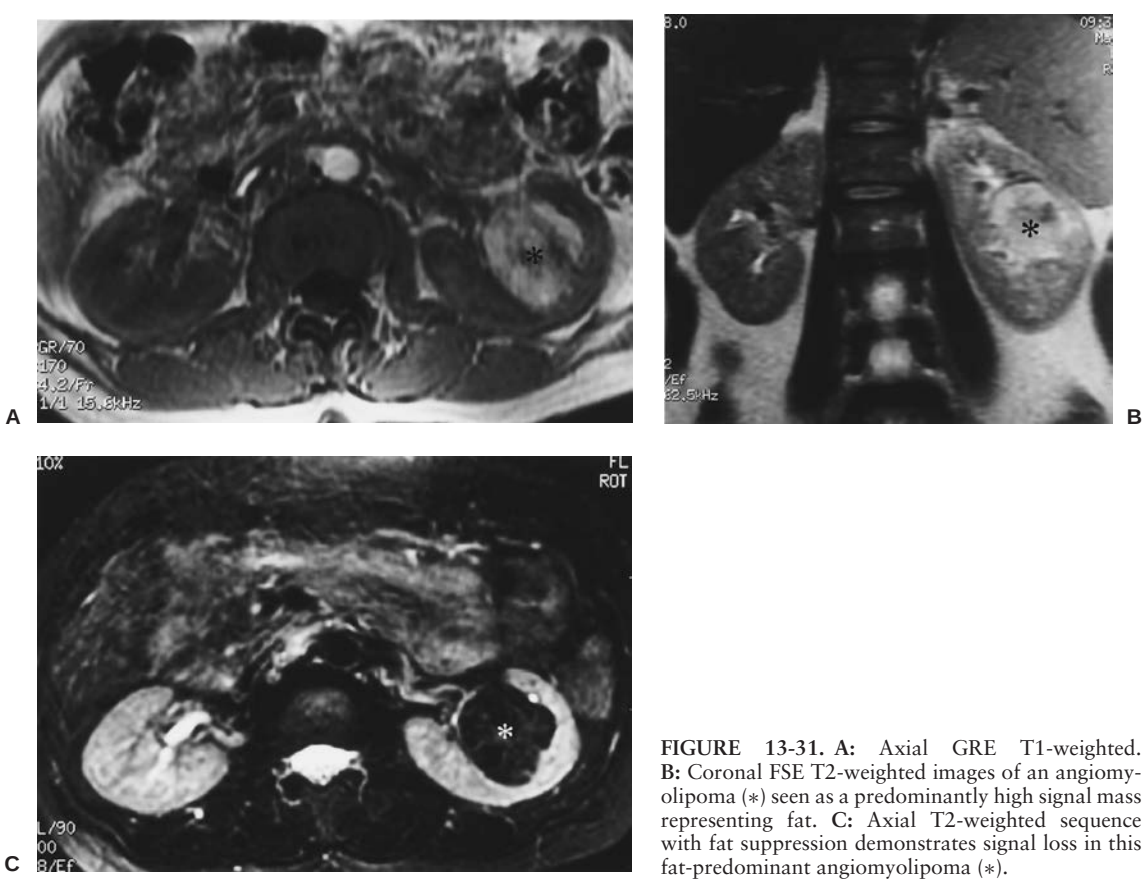


FIGURE 13-31. A: Axial GRE T1-weighted. **B:** Coronal FSE T2-weighted images of an angiomyolipoma (∗) seen as a predominantly high signal mass representing fat. **C:** Axial T2-weighted sequence with fat suppression demonstrates signal loss in this fat-predominant angiomyolipoma (∗).

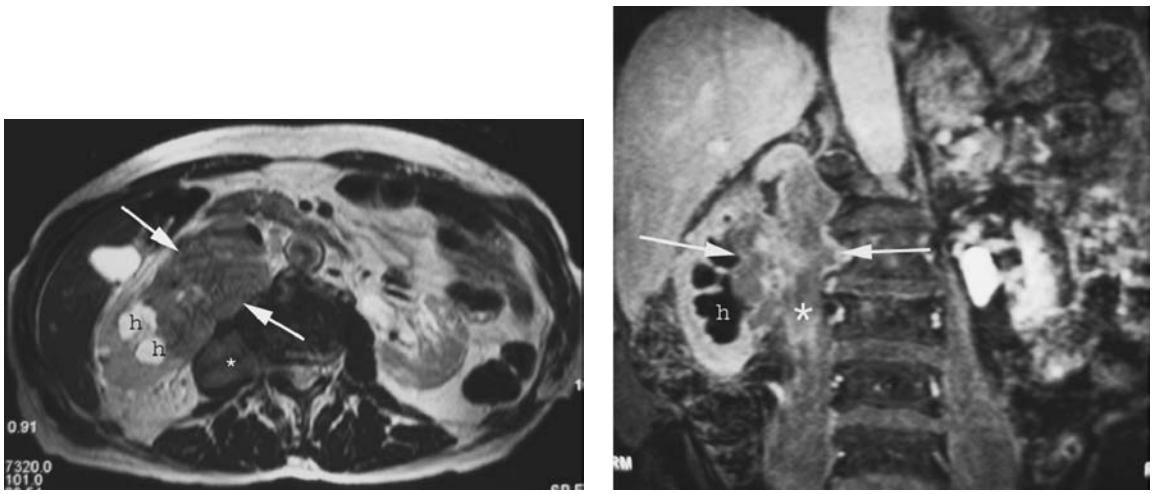


FIGURE 13-32. A: Axial T2-weighted. **B:** Coronal T1-weighted image of lymphoma (*arrow*) involving the right kidney causing hydronephrosis (*h*) and invading the right psoas muscle (∗).

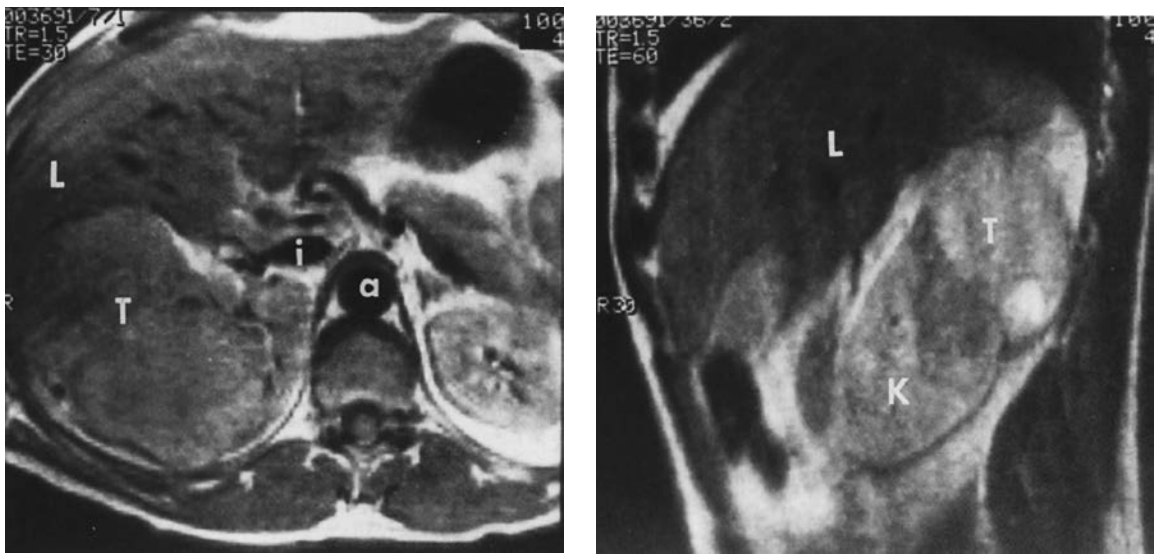


FIGURE 13-33. A: Axial proton density image of a renal cell carcinoma. The large tumor (*T*) arises from the right kidney and abuts the liver (*L*), raising suspicion of direct hepatic invasion. The inferior vena cava (*i*) and aorta (*a*) demonstrate normal signal voids. **B:** Sagittal T2-weighted image of the same patient. The relationship of the tumor (*T*) to the remainder of the right kidney (*K*) is well seen, and a clear interface between tumor and liver (*L*) is present; therefore, this is a stage II lesion, not stage IVA, as was suspected from the axial image.

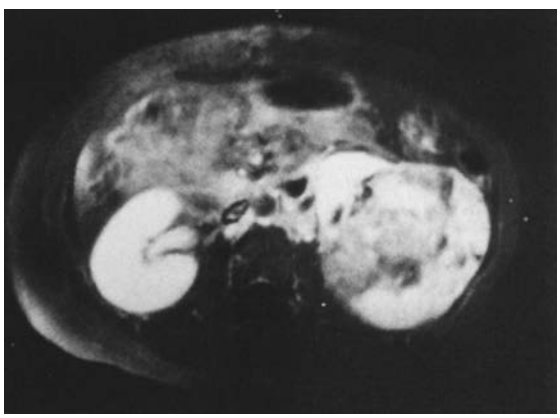


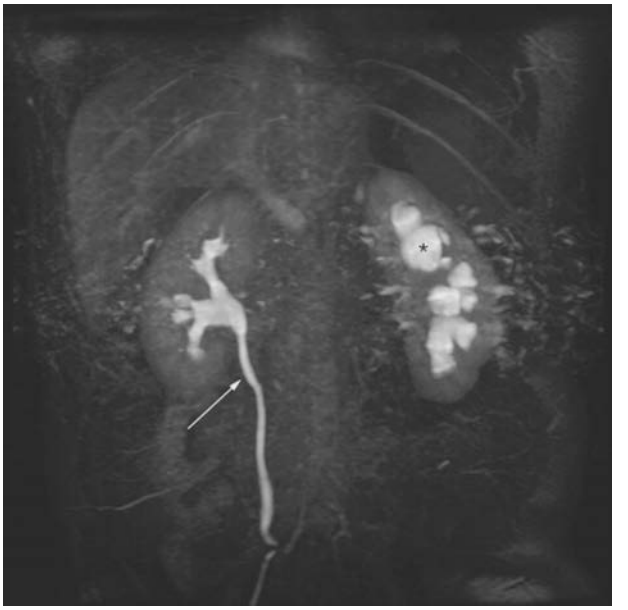
FIGURE 13-34. Axial T1-weighted image of a renal cell carcinoma after fat suppression and Gd DTPA administration. The left renal tumor enhances, but less dramatically than the adjacent normal renal parenchyma.

FIGURE 13-35. Axial GRE image of a renal cell carcinoma. The gradient echo technique displays the rapidly flowing blood in the inferior vena cava (*I*) and aorta (*A*) as bright signal. Tumor thrombus (*arrows*) can be identified clearly extending into the left renal vein and inferior vena cava. The left renal cell carcinoma (*T*) is not visualized optimally by this technique.

A

B

C



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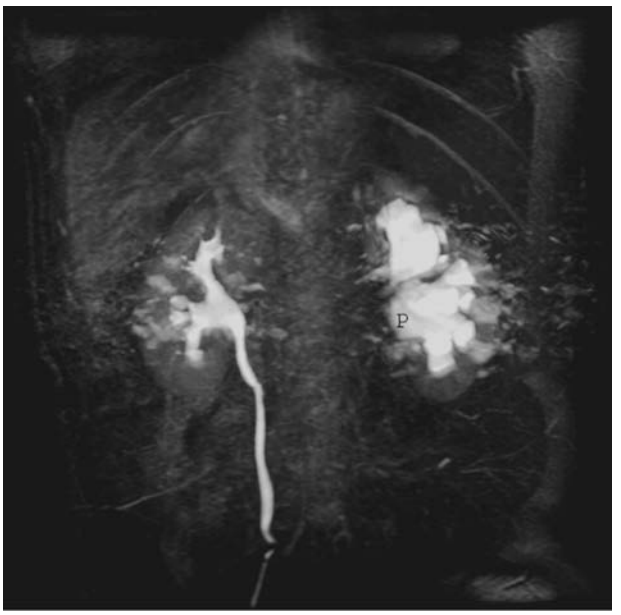

E

FIGURE 13-36. Magnetic resonance angiography and urography of left UPJ obstruction secondary to an accessory left renal artery. **A:** MRA demonstrating main renal arteries (*black arrows*) and bilateral accessory renal arteries (*white arrows*) which are supplying the lower poles of the kidneys. **B:** The accessory left renal artery (*arrow*) crosses anterior to the UPJ causing obstruction with a dilated renal pelvis (*p*). **C–E:** Coronal T1-weighted images 1, 5, and 10 minutes after contrast injection demonstrate normal excretion on the right side (*arrow*) and delayed excretion on the left side with lack of contrast in the ureter up to 10 minutes (*h*, dilated calyx, *P*, dilated renal pelvis).

muscle on these sequences. The renal allograft affected by acute tubular necrosis (ATN) may be normal in appearance or may show signs of edema. Corticomedullary boundaries may be lost and renal contours may be swollen. The T1-weighted images of a renal transplant affected by cyclosporine toxicity will often be normal in appearance with a distinct corticomedullary border (84,85). MRI can assess postoperative mechanical complications, including ureteral obstruction, arterial or venous stenosis, and peritransplant lymphoceles or abscesses. Magnetic resonance angiography can be used to evaluate the allograft vasculature, both arterial and venous, and may be superior to conventional angiography in this regard.

A fairly large degree of overlap, however, is found in the MRI manifestations of ATN, cyclosporine toxicity, and acute rejection, especially if mild. Images can only be interpreted accurately in correlation with detailed clinical data.

Renal Tumors

The MRI appearance of angiomyolipomas depends on the amount of fat, smooth muscle, and vessels within the lesion. Angiomyolipomas composed predominantly of fat have increased signal intensity on T1- and T2-weighted images similar to the surrounding fatty structures and demonstrate loss of signal on the fat suppression sequences (Fig. 13-31).

Renal lymphoma on MRI can be seen as focal parenchymal masses, a diffuse infiltrative pattern, or an invasion by contiguous retroperitoneal disease. Renal lymphoma is typically low to intermediate in signal intensity on T1- and T2-weighted sequences (Fig. 13-32). Lymphomas are hypovascular tumors and demonstrate diminished and heterogeneous enhancement compared to normal renal parenchyma (89).

On MRI renal cell carcinomas appear as masses that distort renal contours or alter intrarenal architecture (Fig. 13-33). These tumors demonstrate variable signal on both T1- and T2-weighted images. The most common appearance is a heterogenous mass of low to intermediate signal on T1-weighted images that increases in signal intensity on T2-weighted images (64). Renal cell carcinomas are vascular lesions and demonstrate enhancement with Gd agents. They often have tortuous tumor vessels; multiplanar MRI clearly demonstrates retroperitoneal feeding arteries and collateral veins (46,86–88).

Magnetic resonance imaging has been shown to be comparable to CT in diagnosing and staging renal cell carcinomas (86). Because of greater spatial resolution, CT is superior to MRI in detecting small lesions, and MRI has not been used as a screening tool in patients with hematuria (86,90). However, MRI has been shown to be more accurate than CT in tumor staging (91,92). Magnetic resonance imaging is particularly beneficial because it offers easy evaluation of the patency of the renal veins and inferior vena cava (Fig. 13-33), has the potential to delineate perivascular lymphadenopathy, and can demonstrate the presence or absence of extension of tumor into adjacent organs. T1-weighted images facilitate differentiation of lymph nodes from perirenal and peripelvic fat. Gd-enhanced images and T2-weighted images, with higher signal-to-noise ratios, can better define tumor boundaries (Fig. 13-34). Sagittal images are of particular use in the evaluation of possible extension of tumor into the liver or spleen and the assessment of the superior extent of tumor thrombus in the inferior vena cava. The fact that iodinated contrast material is not required for evaluation of intravenous or perivascular tumor extension or primary tumor visualization is advantageous, especially in patients with compromised renal function (Fig. 13-35).

Magnetic resonance urography (MRU) can be used to assess the collecting system in patients with a contraindication to iodinated contrast CT (93). Assessment of the collecting system can be performed without the addition of intravenous contrast (T2-weighted sequences) or contrast can be utilized to determine the degree of obstruction and functionality of the affected kidney (Figs. 13-36 and 13-37).

Functional applications of MRI of the kidneys have been investigated, such as early studies on the evaluation of renal perfusion by quantifying flow rate with phase contrast techniques (94). Captopril-sensitized dynamic MR imaging of the

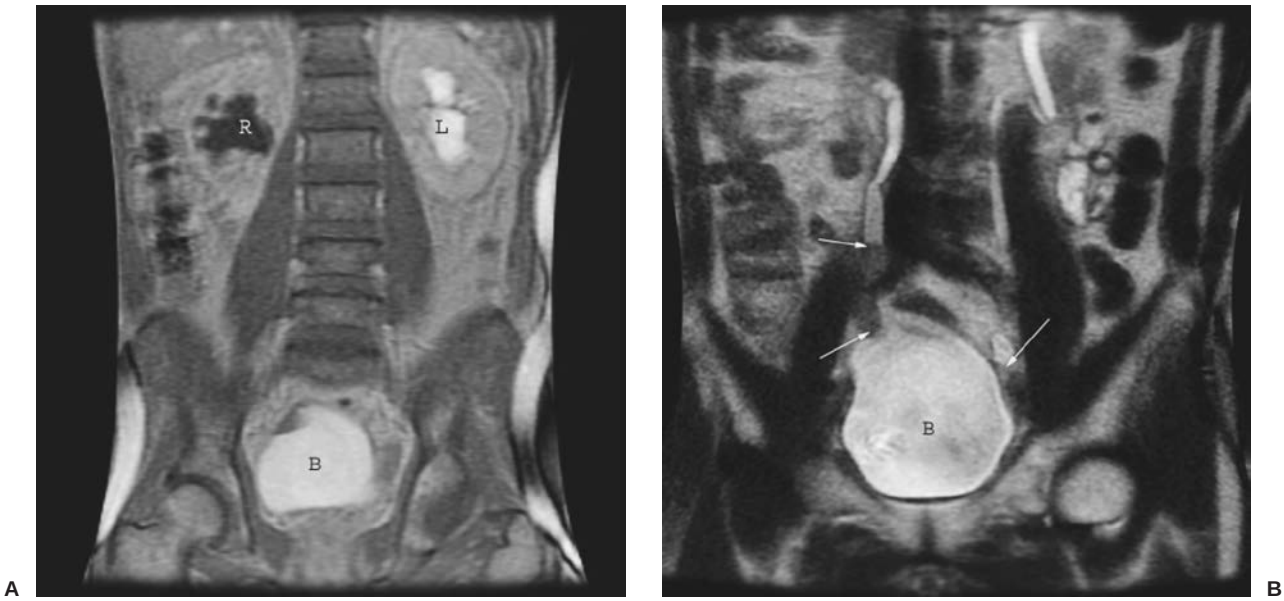

FIGURE 13-37. Magnetic resonance imaging and MRU of bilateral ureteric TCC resulting in bilateral hydronephrosis. **A:** Coronal T1-weighted image post contrast demonstrating chronic obstruction resulting in an atrophic right kidney (*R*) with delay of contrast excretion compared to the left (*L*). B, bladder. **B:** Coronal T2-weighted image inferiorly demonstrating TCC, seen as filling defects (*arrows*) within both distal ureters, causing bilateral ureteral dilatation. *B*, bladder.

kidney also has been investigated in patients with renovascular hypertension (95).

COMPARISON OF COMPUTED TOMOGRAPHY AND MAGNETIC RESONANCE IMAGING

Both CT and MRI provide images of superb anatomic detail. Computed tomography is superior for reliable detection of calcified or fatty structures. Magnetic resonance imaging provides more sensitive tissue-contrast resolution, better assesses the renal vasculature, offers directly obtained multiplanar images, and does not require the use of exogenous contrast agents. Both modalities continue to undergo technical advances that improve their ability to assess renal pathology. Because CT examinations are currently easier and less expensive to perform, they are more commonly used in the evaluation of renal and perirenal disease. Magnetic resonance imaging remains primarily an adjunct to CT in most clinical situations requiring renal imaging.

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CHAPTER 14 ■ DIAGNOSTIC AND THERAPEUTIC ANGIOGRAPHY OF THE RENAL CIRCULATION

CHRISTOPHER S. MORRIS AND JEFFREY M. RIMMER

The introduction of new radiologic techniques has broadened the definition of angiography to include a wide variety of procedures that depict the renal vasculature. During the past two decades, numerous technologic advances have led to changes in the methods of contrast-medium administration, detection of x-rays, evocation of radiofrequency signals and the processing of information from tissue to create images. These approaches have lead to the use of digital angiography, magnetic resonance angiography (MRA) and computed tomography angiography (CTA) as the most common techniques for clinical demonstation of vascular anatomy (Fig. 14-1). Computer manipulation and archiving of these studies also is leading to a revolution in the logistics of analysis, storage, and distribution of imaging procedures. Computed tomography (CT), magnetic resonance imaging (MRI), ultrasonography, and to some extent radionuclide scanning and serologic testing have greatly reduced the role of angiography in the examination of renal parenchymal disease. These procedures almost have eliminated the use of angiography in the investigation of mass lesions, renal venous disease, and vasculitis. At the same time, there has been renewed interest in the role of renovascular disease in hypertension and progressive renal insufficiency (1–3). The majority of renal angiograms now are directed toward the detection of renal vascular abnormalities and the facilitation of their treatment by percutaneous transcatheter techniques. Appropriate utilization of these procedures requires an understanding of their capabilities and the risks associated with them.

ANGIOGRAPHIC ANATOMY

In most cases, the renal arteries originate from the lateral aspect of the abdominal aorta at the level of the L1–L2 interspace, with the left renal artery slightly higher than the right. The ostium of the left renal artery usually is located off the left lateral or posterolateral aspect of the aorta, whereas the right is at the right lateral or anterolateral aspect (4). Because of this, flush abdominal aortography is often performed in the anteroposterior (frontal) as well as the slight left anterior oblique radiographic projections to best profile the ostia. Multiple (duplicated or accessory) renal arteries are common (Fig. 14-2). They are unilateral in 32% of individuals and bilateral in 12% (5). Approximately 10% of multiple arteries are accessory arteries, a term usually applied to small vessels that generally supply a single pole of the kidney, and 20% are aberrant arteries that perfuse larger portions of the kidney but have atypical origins. Accessory renal arteries usually arise from the anterolateral aspect of the infrarenal aorta, whereas aberrant arteries can arise from the thoracic and upper abdominal aorta, as well as the iliac, lumbar, and mesenteric arteries (4,6). As many as seven renal arteries have been observed, but the number seldom exceeds four, and the presence of more than four often is associated with an anomaly, such as a horseshoe kidney or crossed-fused ectopia. The main renal artery separates into anterior and posterior divisions near the renal hilus, with these running anterior and posterior to the renal pelvis (Fig. 14-3). Usually, the posterior division supplies the posterior segment, and the anterior division further divides into the apical, upper, middle, and lower segmental arteries (4). In 20% of kidneys, the posterior division supplies the apical (upper pole) and posterior segments (7). The segmental arteries then subdivide into lobar arteries, which in turn branch to become interlobar arteries. These arteries course between the pyramids and the cortical columns. Each lobar artery subtends one renal pyramid. At the corticomedullary junction, the interlobar arteries divide dichotomously into arcuate arteries, and these terminate in the interlobular arteries that supply the afferent arterioles to the glomeruli. Angiographic resolution usually is limited to the level of the arcuate arteries.

Alternative arterial pathways become apparent with gradual occlusion of the main renal artery, which exploit both potential intrarenal and extrarenal collaterals. Within the kidney, anastomoses may exist among segmental, interlobar, and arcuate arteries (8). Collateral flow to the kidney arises from the lumbar, adrenal, gonadal, or subcostal arteries, which anastomose with the peripelvic, periureteric, and renal capsular arteries (9,10). These anastomotic channels may enlarge and maintain viability of the kidney with chronic obstruction of the renal artery. The reconstituted hilar vessels, as well as the main renal artery distal to an occlusion, often can be visualized by aortography in this setting (Fig. 14-4).

The intrarenal venous drainage system generally parallels the arterial vascular pattern, although multiple intrarenal venous anastomoses are present. In addition, numerous potential retroperitoneal and lumbar venous collaterals are present that may become apparent in renal venous occlusive disease, more frequently on the left than the right. Valves are observed on 15% of renal venograms (11). The right renal vein is 2 to 4 cm long and joins the inferior vena cava at the level of the first lumbar vertebral body. Multiple right renal veins have been reported in 28% of subjects (11). The left renal vein is much longer than the right and courses anterior to the aorta and posterior to the superior mesenteric artery to join the inferior vena cava slightly higher than the right. An anomalous left renal vein runs posterior to the aorta and joins the lower inferior vena cava or iliac vein in 3% of individuals (12). If this vein is single, it is known as a retroaortic left renal vein. It also may be an accessory vein, whereby it is known as a circumaortic left renal vein.

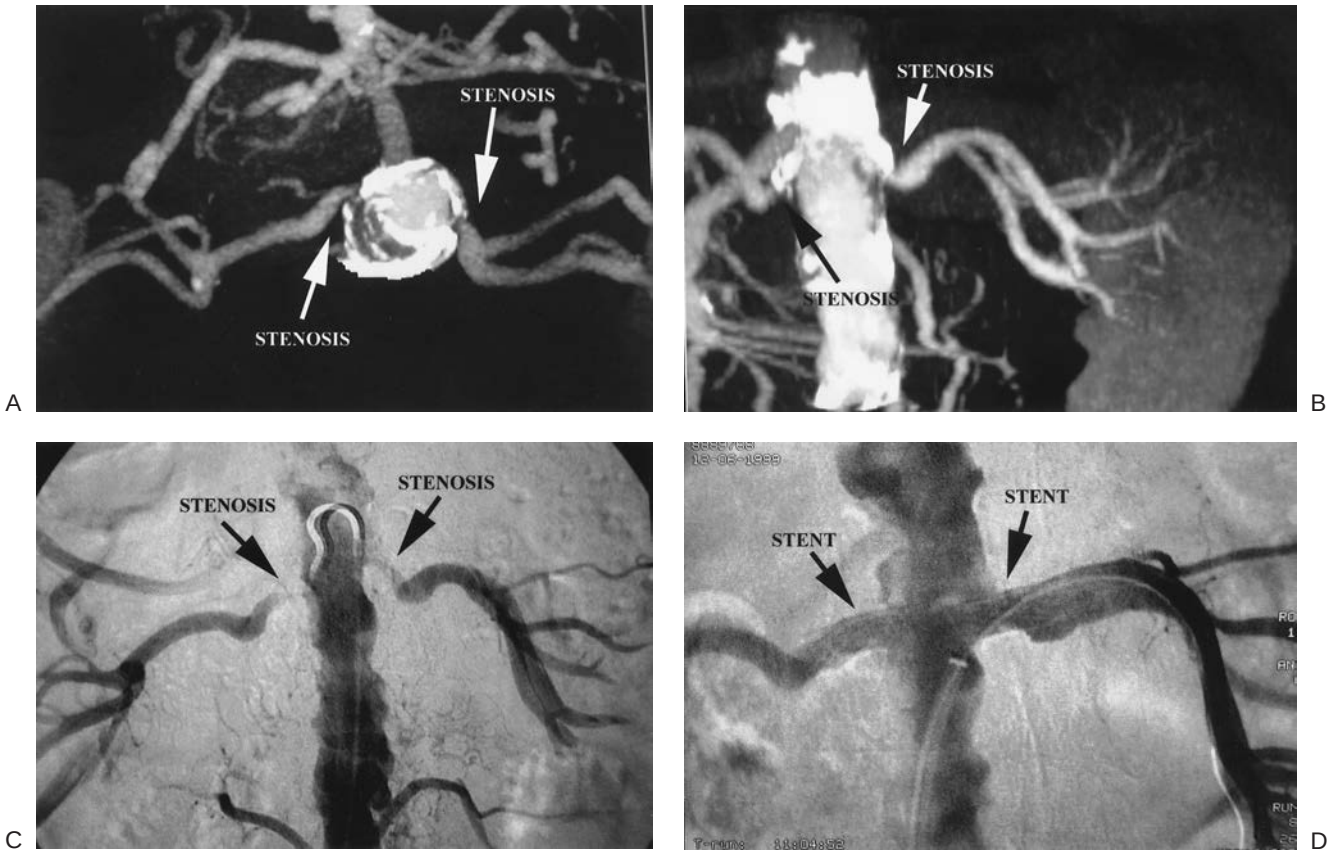


FIGURE 14-1. Digital subtraction aortography and computed tomographic angiography demonstrating bilateral renal artery stenoses in a 79-year-old man with hypertension and renal insufficiency. Axial (**A**) and coronal (**B**) CT angiography reconstructed images demonstrating bilateral renal artery stenoses. Prestent dilatation (**C**) and poststent dilatation (**D**) digital subtraction aortograms on the same patient.

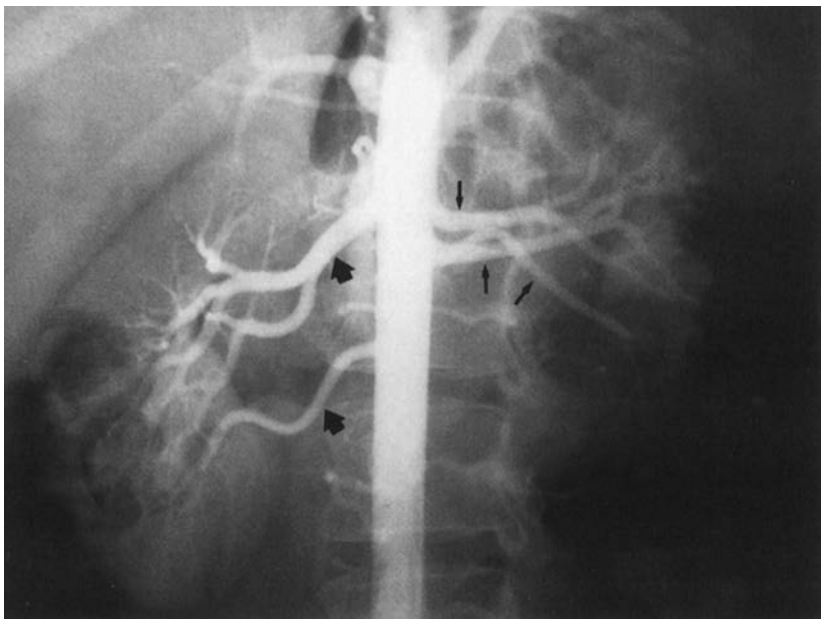


FIGURE 14-2. Multiple renal arteries on a conventional flush aortogram. Two normal renal arteries are seen on the right (*thick arrows*), and three renal arteries, on the left (*thin arrows*).

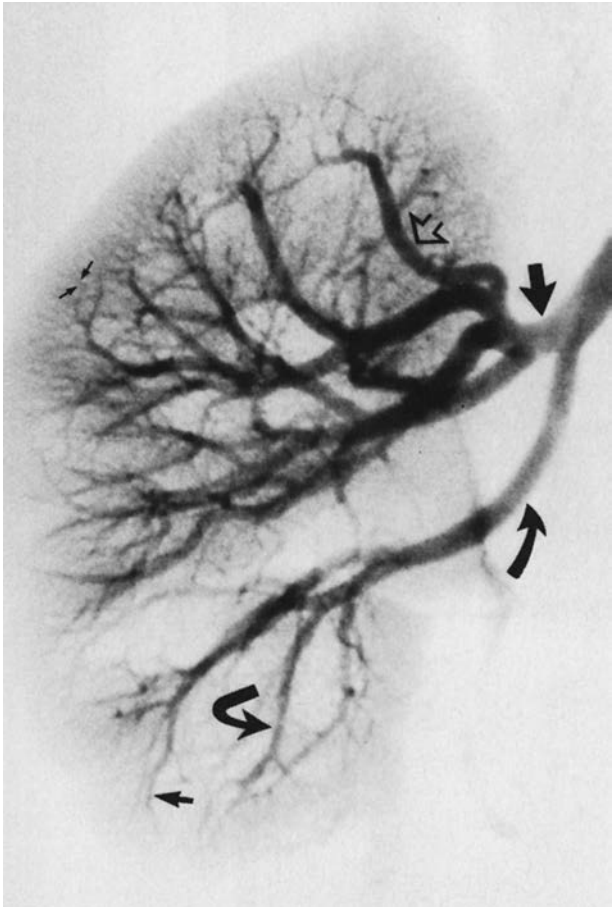


FIGURE 14-3. Manual subtraction conventional arteriogram showing normal renal arterial anatomy. The anterior division (*straight arrow*) and posterior division (*curved arrow*) of the main renal artery are seen. Distal to these are the apical segmental artery (*open arrowhead*), lobar artery (*U-shaped arrow*), interlobar artery (*small arrow*), and arcuate arteries (*smallest arrows*).

ANGIOGRAPHIC CONTRAST MEDIA

Virtually all iodine-containing contrast media in clinical use are triiodinated derivatives of benzoic acid. The prototypic contrast agent, sodium diatrizoate (Hypaque), was developed in 1955 and is the sodium salt of a monomeric, triiodinated substituted benzoic acid (13). This agent and several others that followed are all salts, and have osmolalities in solution from 1,400 to 2,300 mOsm/kg water when infused in concentrations that reliably opacify vessels (14). As salts, these chemicals are highly water soluble, and in high concentrations they provide excellent x-ray attenuation. However, their high osmolality, and possibly their charge, may be related to a number of their toxic properties, now well described (see the subsequent section, Adverse Effects of Iodinated Contrast Media). In 1969, Almén reported the first clinical use of nonionic contrast, metrizamide (Amipaque) (15). He postulated that the substitution of a nonionic hydrophilic moiety for the carboxylic acid on available contrast agents would permit an equal amount of iodine to be infused in a solution of lower osmolality, thus reducing adverse reactions. Metrizamide was predominantly used for myelograms, but a number of other nonionic media appropriate for vascular studies were subsequently synthesized. As predicted, these agents, as used, have osmolalities of about 600 mOsm/kg water and are better tolerated than the earlier ionic agents (16). There now are other low osmolar iodinated contrast agent classes, which include ionic dimers, such as ioxaglate, and true isosmolar agents, such as iodixanol, a nonionic dimer.

Hawkins first described the use of carbon dioxide (CO_2) as an alternative arterial contrast agent in 1971 (17). Its clinical utility was modest until image quality was enhanced by the availability of digital subtraction techniques (Figs. 14-5 and 14-6) (18). In comparison with iodinated contrast agents used for abdominal arteriograms, 91% of the CO_2 studies were judged to be of good or excellent quality (19). Diagnostic agreement was found between 95% of studies with CO_2 and iodinated contrast media, and the findings of CO_2 studies were confirmed at surgery 92% of the time.

CO_2 rapidly dissolves in body fluids and then is excreted by the lungs. It has been found to be safe when used as a contrast agent in humans (17,19), and animal experiments generally have confirmed this safety with little or no direct tissue toxicity. Studies in dogs have shown the potential for ischemic damage owing to "vapor lock" or the transient obstruction of blood flow by gas in vessels superior to the point of injection (20). In humans, this phenomenon has rarely produced mesenteric ischemia (21). For this reason, CO_2 studies should be performed only with injections that do not permit access to the cardiac and cerebral circulations. Overall, there has been little nephrotoxicity and no potential for idiosyncratic reactions when CO_2 is used as the contrast agent.

Gadolinium-based agents (gadopentetate dimeglumine), developed for contrast-enhanced MRI, also have been used as contrast agents for x-ray angiography in the setting of renal insufficiency (22–27) with the hypothesis that this would reduce nephrotoxictiy. When used in concentrations that provide x-ray attenuation equal to a dose of iodinated contrast there is little difference in osmolality (28).

Sequential use of different contrast agents is also reported. Typically carbon dioxide is used as the media for initial survey while small doses of iodine or gadolinium containing contrast are reserved for situations requiring more detail (29,30).

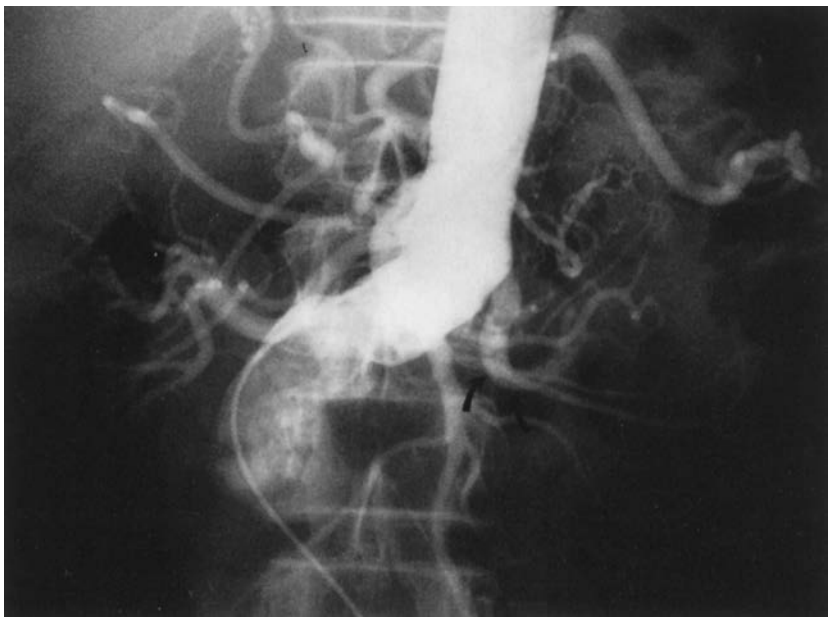
COMPLICATIONS OF ANGIOGRAPHY

Death

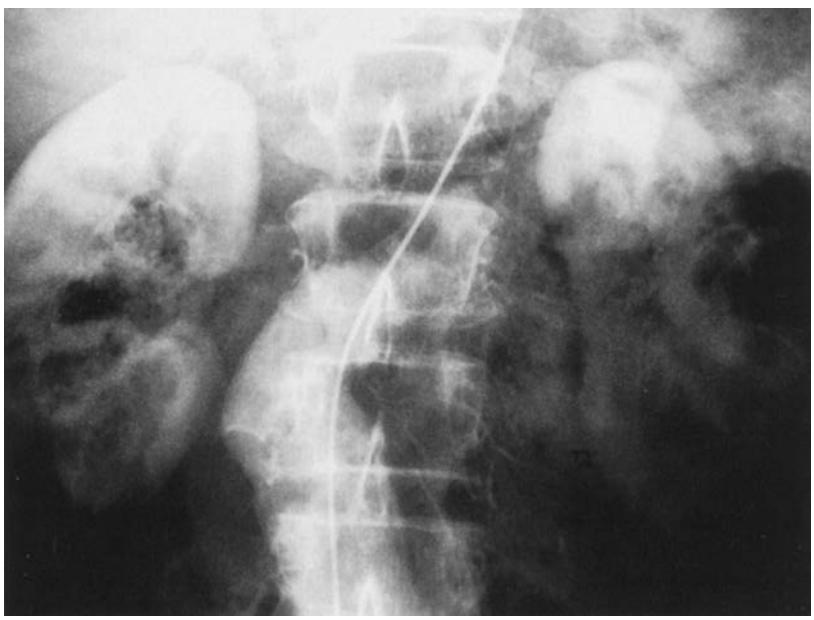
Death directly attributed to arteriography is rare. The overall death rate in a report of conventional arteriography that excluded cardiac and cerebral studies was 30 deaths in 91,776 studies, or 0.03% (31). Death rates in more recent but smaller and more specialized studies have ranged between 0% and 0.7% (32,33).

Adverse Effects of Iodinated Contrast Media

The adverse consequences associated with iodinated contrast media usually are divided into idiosyncratic reactions: (a) those phenomena often termed *allergic* and (b) the toxic effects of the pharmaceuticals. Either of these categories of problems may present with localized or systemic manifestations. Reactions generally are considered mild and require no specific treatment. Regardless of the mechanism, the reported frequency of contrast medium-induced death is very low. A review of seven



A

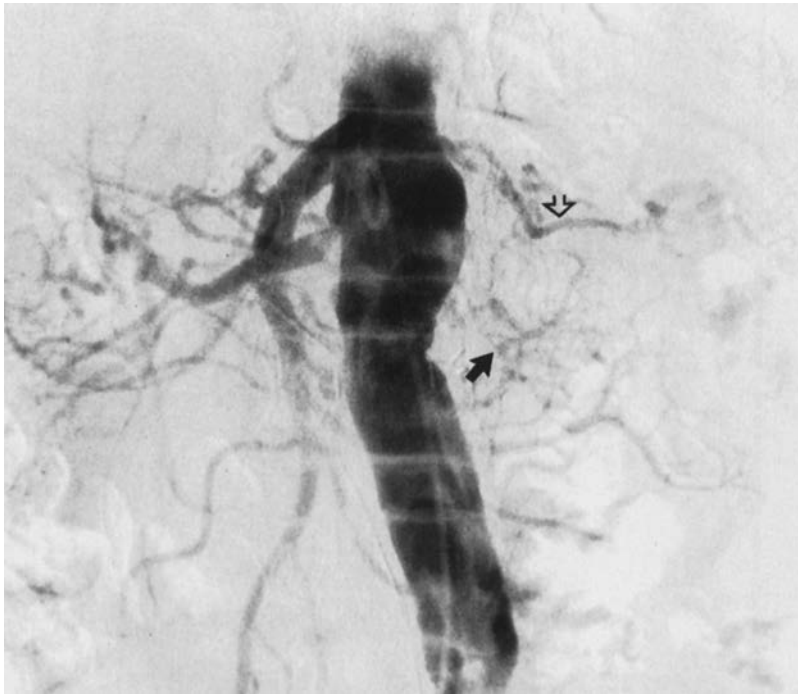


B

FIGURE 14-4. Chronic occlusion of a duplicated left renal artery developing over 2 years. Panels **A** and **B** show aortograms of a 65-year-old man (who was noted to be hypertensive) scheduled for repair of an aortic aneurysm. The initial aortogram shows a patent lower left renal artery (*curved arrows*) and a proximal stenosis. The lower pole nephrogram is normal. (*continued*)

reports of complications associated with urography using ionic contrast agents between 1972 and 1982 showed death rates that ranged from 0.001% to 0.006% (34). More recently, a report of the complications of intravenous ionic and nonionic contrast media described no contrast medium-related deaths after 337,647 administered doses (35).

Idiosyncratic complications include features typically attributed to allergic mechanisms. Despite this, there is little evidence that these events involve antibody–antigen interaction; therefore, they are properly termed anaphylactoid. Nonimmune causes include the possibility of chemically induced mast cell degranulation and toxic activation of complement as well as coagulation and fibrinolytic systems (36). Minor reactions include rash and limited urticaria. A severe reaction may include angioneurotic edema, laryngospasm, bronchospasm, or hypotension and may progress to life-threatening respiratory failure with cardiovascular collapse. The majority of these reactions are unanticipated, although they are more frequent in persons who have had previous reactions to contrast agents, or have a history of allergy, particularly those who have asthma (35). Overall, the incidence of severe reactions is quite low, reported to be between 0.1% and 0.02% during intravenous urography with ionic contrast media (34). In a large study comparing the intravenous use of ionic and nonionic contrast media, severe reactions were reported in 0.22% of examinations with ionic media and 0.04% with nonionic contrast agents. Very severe events were uncommon, with 63 occurring among 169,284 examinations with ionic contrast media and six among 168,363 studies with nonionic contrast media (35). Although adverse responses to contrast media are reduced by the use of nonionic agents (37), their substantially greater cost has created questions about when their use is appropriate (38,39). At present, nonionic contrast media usually are reserved for patients who have an increased risk for



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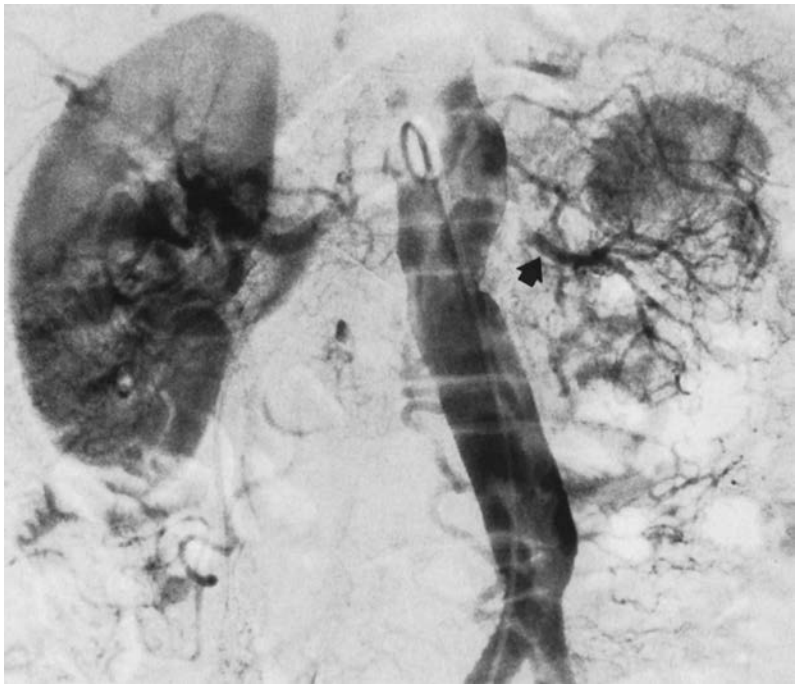


D

FIGURE 14-4. (*Continued*) Panels **C** and **D** show aortograms obtained 2 years after aneurysm repair. They demonstrate that the lower pole artery is now occluded, and the lower pole of the kidney is perfused by collateral arteries (*arrow*). The upper pole artery is still patent (*open arrow*), and late films show opacification of the lower renal artery distal to the occlusion (*thick arrow*).

reactions. The utility of other prophylactic measures is unclear. Premedication of such patients with corticosteroids and H1 histamine blockers in various regimens generally is accepted as useful (37,40). Discontinuation of β-blockers, and prestudy administration of H2 histamine blockers and ephedrine, also has been advocated.

Among toxic reactions, the most common, potentially severe complication is acute renal failure (see Chapter 45 in this text) (41). There is no doubt that iodinated contrast media can transiently reduce renal function. Questions persist about the frequency of this complication, the characteristics of iodinated contrast that contribute to toxicity, and how it may best be avoided. Numerous reports have identified underlying renal insufficiency as a major risk factor for contrast medium-induced acute renal failure (41,42). The importance of other putative risk factors, such as diabetes mellitus with normal renal function, dose of contrast medium, old age, or multiple myeloma, remains controversial (41).

The best approach to minimize contrast-medium toxicity is careful consideration of the utility of the proposed examination and avoiding studies that are not clinically significant. When a study is of critical importance there are essentially no absolute contraindications to contrast-medium administration.

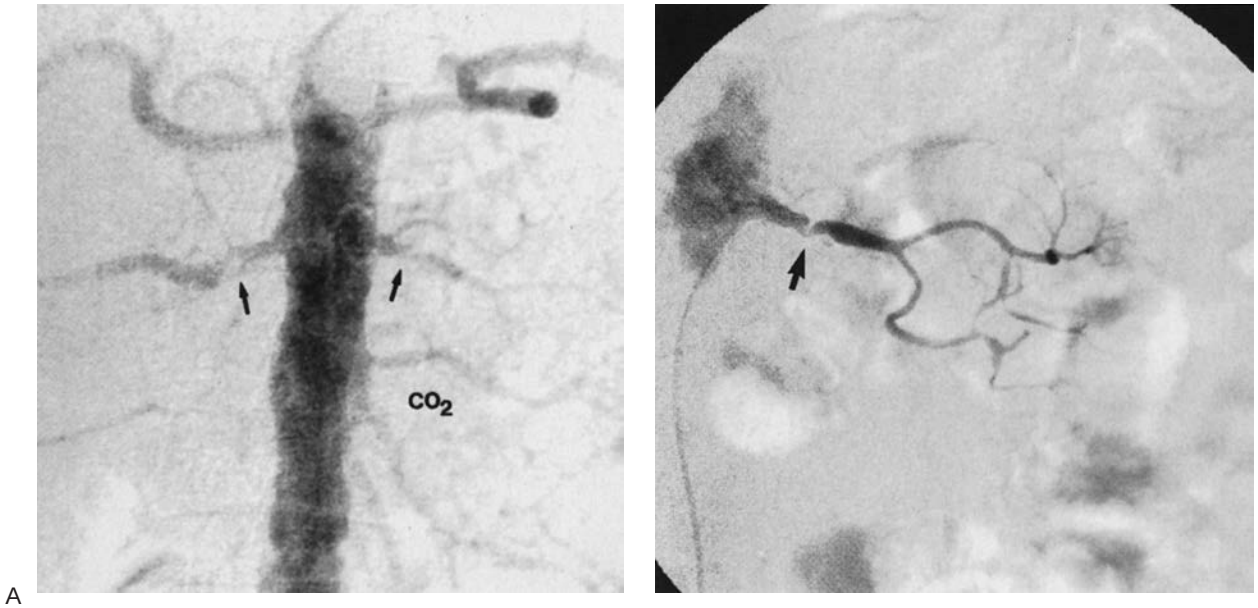


FIGURE 14-5. Intraarterial digital subtraction renal arteriography flush aortogram obtained with CO_2 as contrast medium and a selective renal arteriogram performed with iodinated contrast. **A:** Detection of bilateral renal artery stenosis (*arrow*) using CO_2. **B:** Subsequent selective left renal angiogram with iodinated contrast confirms the initial interpretation.

Recommendations to reduce the incidence of acute renal failure include the use of parenteral volume expansion, prior to the procedure, with hypotonic (43) or isotonic saline (44) and more recently sodium bicarbonate (45). The use of nonionic and low osmolar or isosmolar iodinated contrast in the lowest possible dose (46), or the substitution of gadolinium based contrast or CO2 is also advocated (30). Protocols using the antioxidant n-acetylcysteine have been widely explored (47–54). Administration of numerous other pharmocologic agents including loop diuretics, mannitol (43), dopamine (55), fenoldopam (56), theophylline (57–59), atrial naturetic peptide (ANP) (60), calcium channel blockers (61,62), endothelin receptor blockers (63), and converting enzyme inhibitors have been studied (64,65). Hemodialysis (66,67) and hemofiltration (68) performed around the time of contrast administration have also been investigated. With the exception of volume expansion, the value of most of these procedurers is debateable. The use of n-acetylcesteine has been accepted because of the possibility of benefit, low cost, and little associated risk. The use of the smallest dose of low or isosmolar iodinated contrast also has become routine in the setting of reduced renal function (69).

Many other adverse effects have been attributed to iodinated contrast agents, including cardiotoxicity (70), seizures (71), parotid (72) and pancreatic swelling (73), hyperthyroidism (74), necrotizing vasculitis (75,76), and blistering skin rashes (77). Most serious of these is cardiac toxicity with abnormalities in contractile function (78) and conduction (79,80), which may be confused with a severe idiosyncratic reaction. Common reactions include nausea, flushing, pain at the infusion site, and thrombophlebitis. Most of these problems are decreased by the use of nonionic, low-osmolality contrast media (35).

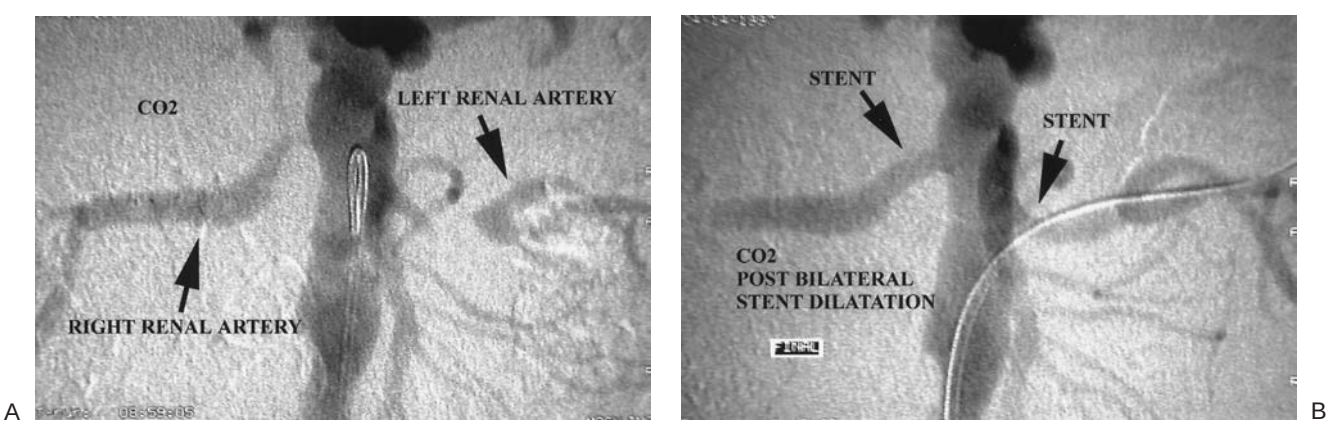


FIGURE 14-6. Digital subtraction angiography obtained with CO_2 as the contrast medium in a patient with hypertension and renal insufficiency. **A:** Detection of bilateral renal artery stenoses. **B:** Poststent dilatation of bilateral renal artery stenoses.

Mechanical Complications

The most common serious systemic complication of arteriography is atheroembolic disease secondary to the dislodgment of material from ulcerated atherosclerotic plaques (cholesterol crystal embolization) (see Chapter 70 in this text). This phenomenon resulting in renal failure can occur during any intraarterial procedure performed proximal to the renal arteries. The true incidence of this process is unknown because there are no sensitive and specific tests for its presence. Its presentation is highly variable, with distal cutaneous and musculoskeletal signs being most common, but diffuse and severe visceral organ damage may occur, including inexorable progression to renal failure (41). Symptoms of atheroembolic disease may not immediately follow arteriography, and the course can be remitting or stuttering. These characteristics are likely to cause underestimation of the incidence of the process, with some authors reporting that it follows less than 0.5% of arteriographic procedures (81,82). Autopsy series probably overstate the true incidence of the disease to be about 25% of patients who have had recent arteriograms, as the small group of patients who died following the procedure is unlikely to be representative of the large population that has undergone arteriographic study (83). The process may be less frequent when an upper extremity is used for arterial access, but beyond this, there are no effective prophylactic or therapeutic measures.

Other serious complications appear to be uncommon (84,85). In a previously cited study of aortic and peripheral vascular studies, there was an overall complication rate of 1.9% (31). Stroke with permanent neurologic deficit was reported in 0.02% of 83,068 transfemoral examinations, myocardial infarction in 0.02%, and cardiac arrest in 0.001% (31). Arterial perforation with extravasation of contrast was noted in 0.44% of cases, an embolus of some form was seen in 0.10%, and breaking of a wire or catheter in 0.10%. Minor puncture-site hematomas are common, occurring in up to 10% of all angiographic procedures. A major hematoma requiring transfusion or surgical evacuation can be expected in 0.5% to 2.0% of procedures (84–87). All other puncture-site complications, including arterial dissection, arteriovenous fistula, or arterial thrombosis, are seen in less than 1% of cases (85). It is likely that more problems accompany puncture of the axillary artery or vascular prostheses, but these approaches have been used with acceptable complication rates (33,87).

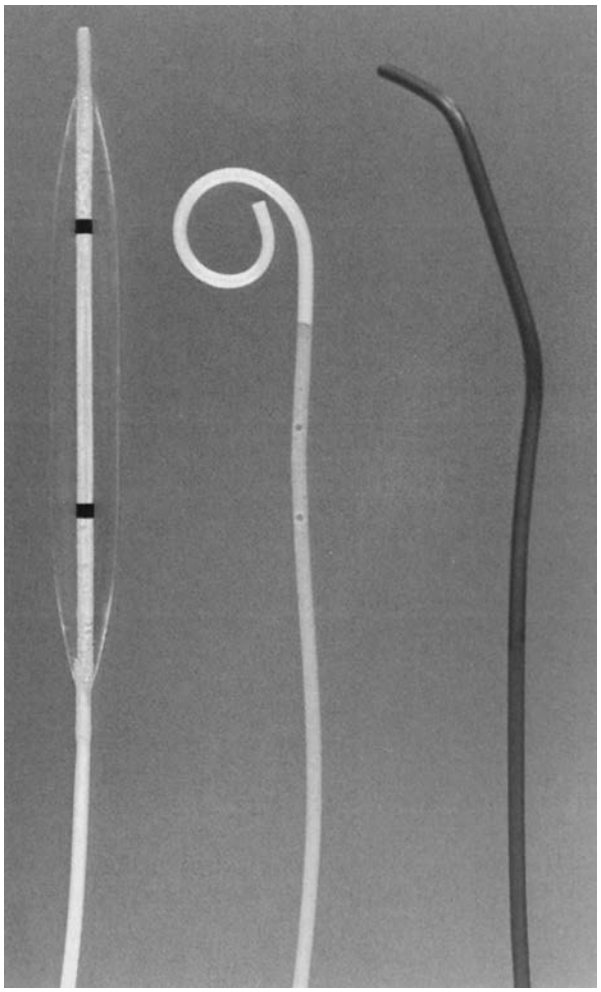


FIGURE 14-7. Catheters commonly used for renal angiography. *On the left* is an inflated balloon angioplasty catheter, *in the center* is a pigtail catheter typically used to perform the aortic flush, and *on the right* is a curved catheter used to selectively cannulate renal arteries.

IMAGE ACQUISITION AND GENERATION

Conventional Arteriogram

Conventional arteriography is the standard for examination of the renal artery and its major branches, and all newer procedures are measured against it. It requires cannulation of the aorta by a catheter inserted through a peripheral artery (88) or, much less commonly, by direct puncture of the aorta. Concentrated iodinated contrast medium is injected into the upper aorta (aortic flush) at a rapid rate through a pigtail or side-hole catheter (Fig. 14-7) that has been positioned with the aid of a guide wire. This opacifies the abdominal aorta and provides a full survey of all the renal arteries. Further injections then are performed in different projections until the renal ostia are optimally visualized. If greater detail is required or an intervention is planned, the pigtail catheter is exchanged for a curved selective catheter that is directed into the renal artery ostium (selective arteriography). Serial exposures are made rapidly on radiographic film 14 inches by 14 inches, which is fed through an automated film changer. Image quality is affected by the patient's characteristics, such as size, bowel preparation, motion, and ability to cooperate, and technical factors, such as positioning of the patient and choice of exposure settings (89).

Because of the use of fine-grain film, conventional renal arteriography is able to provide images with high spatial resolution (the ability to define two points in space) (90). Its drawbacks are predominantly the requirements for arterial puncture, instrumentation of the abdominal aorta, and injection of full-strength contrast medium. In addition, the time taken to develop films is a handicap when the procedure is being used to guide interventions. The films, which are the sole visual record of a study, are bulky, difficult to store, and difficult to distribute among different sites of patient care.

Intravenous Digital Subtraction Arteriogram

Intravenous digital subtraction renal arteriography (DSA) results in a computer-generated visual representation of the renal arteries (91). As in standard arteriography, radiographs are used, but they are detected by electronic receptors (image intensifier), and the response is stored by a computer. Data are

collected from a baseline exposure (mask) before contrast medium is given. These data sets are composed of attenuation values that are determined for each point (pixel) within the potential baseline image. A contrast agent then is administered, usually into the right atrium through a catheter that has been advanced from a puncture site in a peripheral arm vein. Serial exposures are performed as the contrast medium passes through the various phases of the renal circulation. To generate an arterial image, the attenuation values at corresponding locations in the mask and arterial data sets are compared. If the attenuation value at a pixel increases by an increment that satisfies criteria within a computer algorithm, it is considered to be a location opacified by the medium and the computer further increases the density of that pixel. In this way, the faint opacification of the renal arteries that follows venous administration of contrast medium can be enhanced to produce an image that resembles a conventional arteriogram. Contrast resolution (the ability to distinguish a difference in density between two points) with DSA techniques is superior to that provided by conventional arteriography, but as in all electronically generated arteriograms, the much smaller number of points within an image results in a loss of spatial resolution (90).

The same factors that influence the quality of conventional arteriograms affect intravenous DSA. In addition, because contrast medium opacifies all arteries simultaneously after intravenous injection, all intraabdominal arteries are visualized at the same time, which increases the risk that important structures will be obscured by overlying arteries. The method assumes that the anatomic structures represented at corresponding pixels in the data sets are the same in the mask and contrast-medium exposures, but between acquisition of the two exposures there may have been patient motion or physiologic movement of organs. The fidelity of the computer representation is degraded at any pixel where anatomic structures are not in identical positions at the time the compared exposures are made. Delay in the arrival of contrast medium within arteries may result in their inadequate opacification, making the quality of studies poor in patients with reduced cardiac output. These limitations make intravenous DSA most suitable for examinations in which the loss of resolution is not critically important, such as large arteries. Although in one study intravenous DSA was shown to have a sensitivity as high as 100% and a specificity as high as 93% in detecting renal artery stenosis (92), other reports describe it as less accurate. In one large series, studies were nondiagnostic in 37% of patients examined (93). In addition, the results of intravenous DSA in the setting of fibromuscular dysplasia have been disappointing (93,94).

The risks of DSA are the complications associated with the administration of a large intravenous bolus of iodinated contrast medium. The major advantage of intravenous DSA is its potential for examining the renal arterial vasculature without physically cannulating the arterial circulation. Like all computer-generated studies, intravenous DSA is well suited for inclusion in a computerized patient record or for simultaneous viewing at multiple sites for consultation (95).

Intraarterial Digital Subtraction Angiography

Intraarterial digital subtraction angiography, obtained with modern equipment, is now considered the imaging technique of choice for diagnostic as well as therapeutic angiography. The principles of image generation in intraarterial DSA are the same as intravenous DSA. Intraarterial injection of contrast medium improves digital subtraction studies in several ways. Small amounts of medium can be used via the intraarterial route, because minimal opacification of the vessel is required to produce an image. Injection of contrast medium adjacent to the artery of interest reduces the simultaneous filling of other vessels, and delivery is prompt regardless of cardiac output. Motion artifact is reduced substantially, because the interval between mask and arterial opacification is shorter than when intravenous DSA is used. With these changes, the images produced by intraarterial DSA (Fig. 14-8) compare favorably with those produced by conventional arteriography in detecting arterial disease (96,97). Although CTA and MRA have been reported to have sensitivity and specificity approaching that of intraaterial studies in the detection of renal artery stenosis (98), intraarterial digital subtraction angiography is still felt to be superior (99). The cost of the improvement in image quality is the requirement for invasion of the aorta. The increase in risk with aortic cannulation may be offset somewhat by the administration of lower doses of contrast and the use of smaller, potentially less traumatic catheters. Intraarterial DSA is well suited to support percutaneous therapeutic procedures because of the rapid availability of images.

FIGURE 14-8. Intraarterial digital subtraction renal arteriography performed in a potential kidney donor, demonstrating normal arterial anatomy and the results achievable with this technique.

ANGIOGRAPHY OF SPECIFIC CONDITIONS

The Aging Kidney

The arterial supply of the kidney has been shown to undergo predictable changes with age. Arterial changes mimic the pattern of arteriolar nephrosclerosis attributed to systemic hypertension. The renal angiogram demonstrates earliest alterations within the arcuate arteries. Cortical thinning occurs in conjunction with increased tortuosity, rapid tapering, abrupt termination, and intraluminal plaques involving the arcuate and interlobar arteries (Fig. 14-9) (100).

Atherosclerosis

Suspected atherosclerotic renovascular disease is the most common indication for renal arteriography. Signs of this disorder

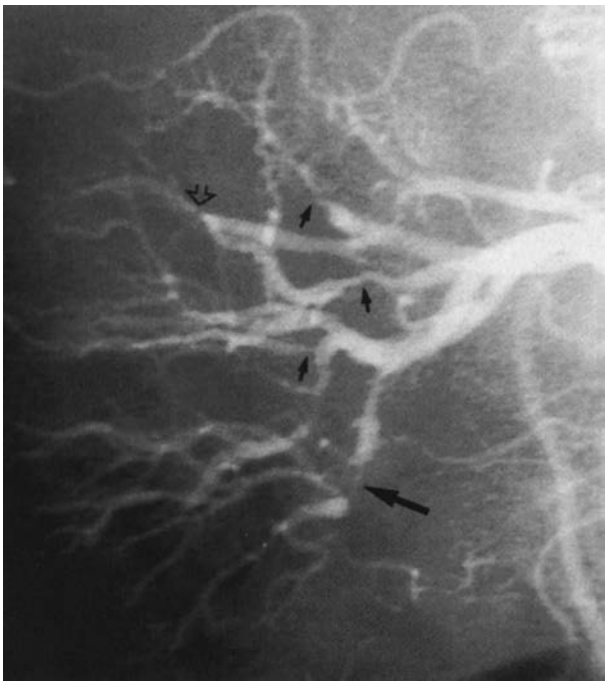


FIGURE 14-9. A nonsubtracted digital renal angiogram showing arterial pruning, segmental occlusion (*large arrow*), atherosclerotic branch stenoses (*small arrows*), and intraluminal plaque causing a filling defect (*open arrowhead*). These findings are typical of those described in the aging kidney and hypertensive nephrosclerosis.

include hypertension and renal insufficiency, either singly or in combination. Most commonly, plaque extends from an aortic site of the disease into the renal artery ostia or arises in the proximal third of the renal artery (101). Typical atherosclerotic narrowing is shown in Figs. 14-10 and 14-11. Bilateral disease has been noted in 46% of patients (3). It is rare to find atherosclerotic disease in a branch artery without narrowing of the main renal artery (102). Lesions may be focal or diffuse and soft or calcified. Stenoses may be concentric, but frequently are eccentric, requiring multiple projections to accurately assess the degree of luminal narrowing. Widely accepted characteristics of a hemodynamically significant stenosis include a 50% or greater reduction in luminal diameter, collateral vessels, poststenotic dilation, diminished kidney size, or a 15% reduction in pressure measured across the lesion (102–104).

Fibromuscular Dysplasia

Fibromuscular dysplasia (FMD) is an uncommon disorder of large arteries of unknown etiology most frequently seen in women (105,106). It is usually found in the renal arteries, but can also affect the carotid and vertebral arteries of the neck, mesenteric arteries, and large arteries of the extremities (107). It is the second most frequent cause of all cases of renovascular hypertension, and the most common cause of this disorder in childhood (108,109).

Early classification resulted in six subtypes of FMD being commonly recognized: intimal fibroplasia, medial fibroplasia, medial hyperplasia, perimedial fibroplasia, medial dissection, and adventitial fibroplasia (110). More recently, a simpler classification has been described that includes intimal, medial, and periarterial subtypes. The medial subtype of FMD is by far the most common, being diagnosed in 70% to 95% of all cases (111). Angiographically, medial FMD appears as a "string of beads" (Figs. 14-12 and 14-13), which consists of alternating areas of stenosis and aneurysmal dilation. The other subtypes may present as a focal stenosis, a long and smooth narrowing, irregular stenoses, medial dissection with a false channel, or a segmental stenosis. Often, fine, flow-limiting intimal webs are present that are difficult to visualize angiographically.

When medial FMD involves the renal arteries, it is bilateral in two-thirds of the cases and it involves branch arteries in 17% of patients (106). Isolated branch artery stenoses are found only 4% of the time (106). Typically, the lesions involve the middle

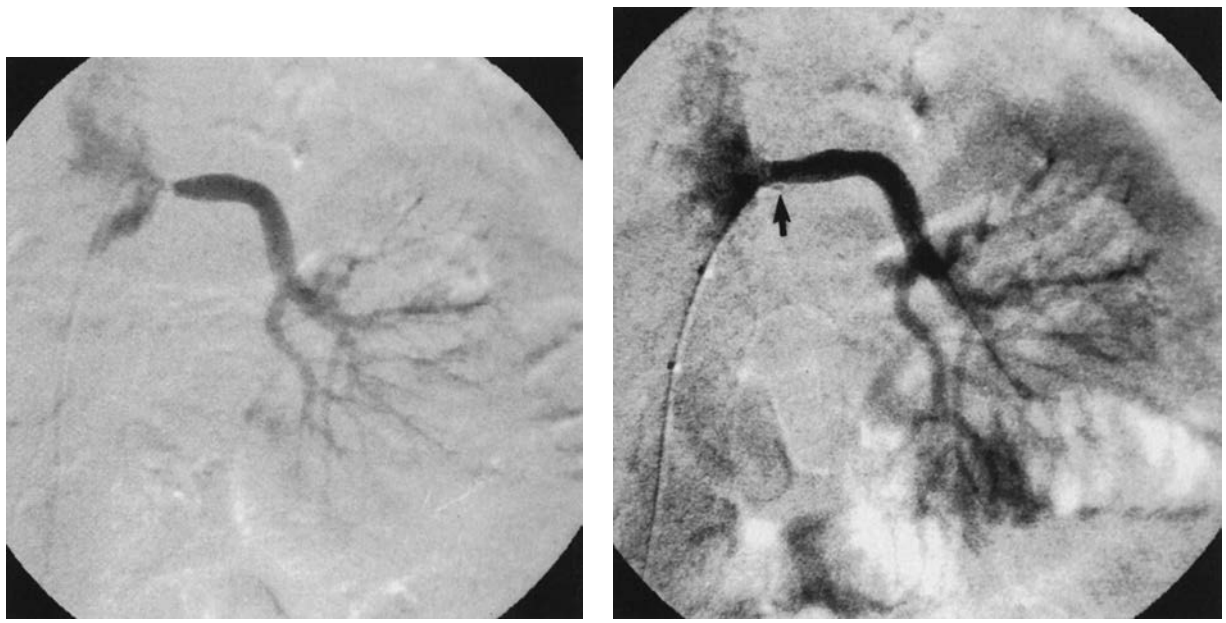
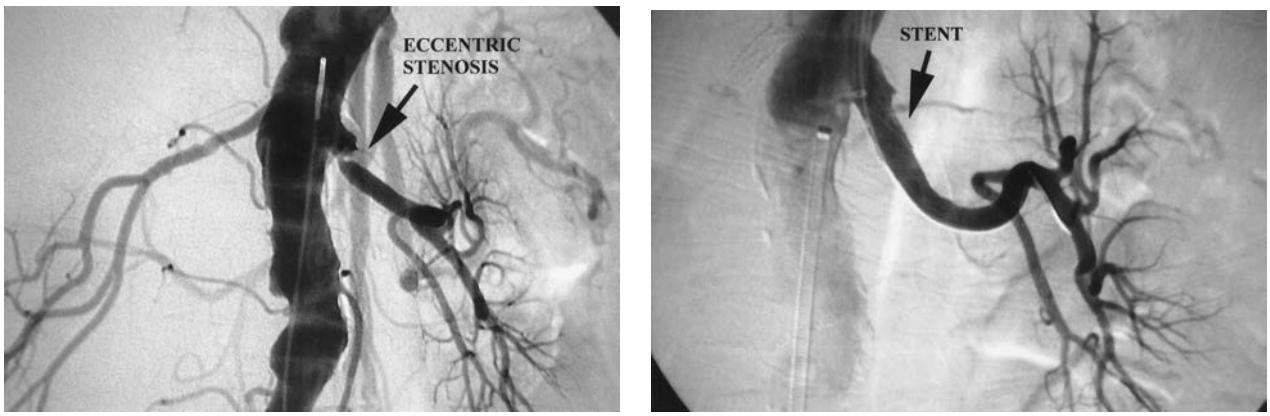


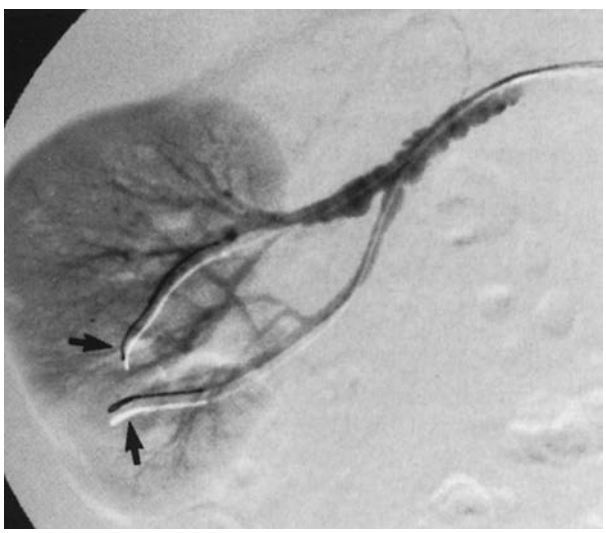
FIGURE 14-10. Atherosclerotic renal artery stenosis before and after percutaneous transluminal renal angioplasty (PTRA). **A:** Semiselective intraarterial digital subtraction renal arteriography (DSA) shows a severe proximal stenosis of the left renal artery. **B:** A "pull-back" intraarterial DSA obtained after PTRA demonstrates improved luminal caliber and a typical intimal "fracture" (*arrow*).



A B

FIGURE 14-11. Digital subtraction angiography demonstrating stent dilatation of an eccentric left renal artery stenosis. These lesions respond less well to percutaneous transluminal renal angioplasty without a stent. **A:** Prestent dilatation aortogram. **B:** Immediate poststent dilatation renal angiogram.

A B



C

FIGURE 14-12. Intraarterial digital subtraction renal arteriography showing the results of percutaneous transluminal renal angioplasty (PTRA) in medial fibromuscular dysplasia (FMD). **A:** The typical "string of beads" appearance of medial FMD involving the distal two-thirds of the main renal artery and the proximal portions of both the anterior and posterior divisions. **B:** A safety wire (*arrow*) in place in the posterior division before dilation of the anterior division. **C:** Safety wires in both the anterior and posterior divisions after PTRA as the results of the angioplasty are assessed.

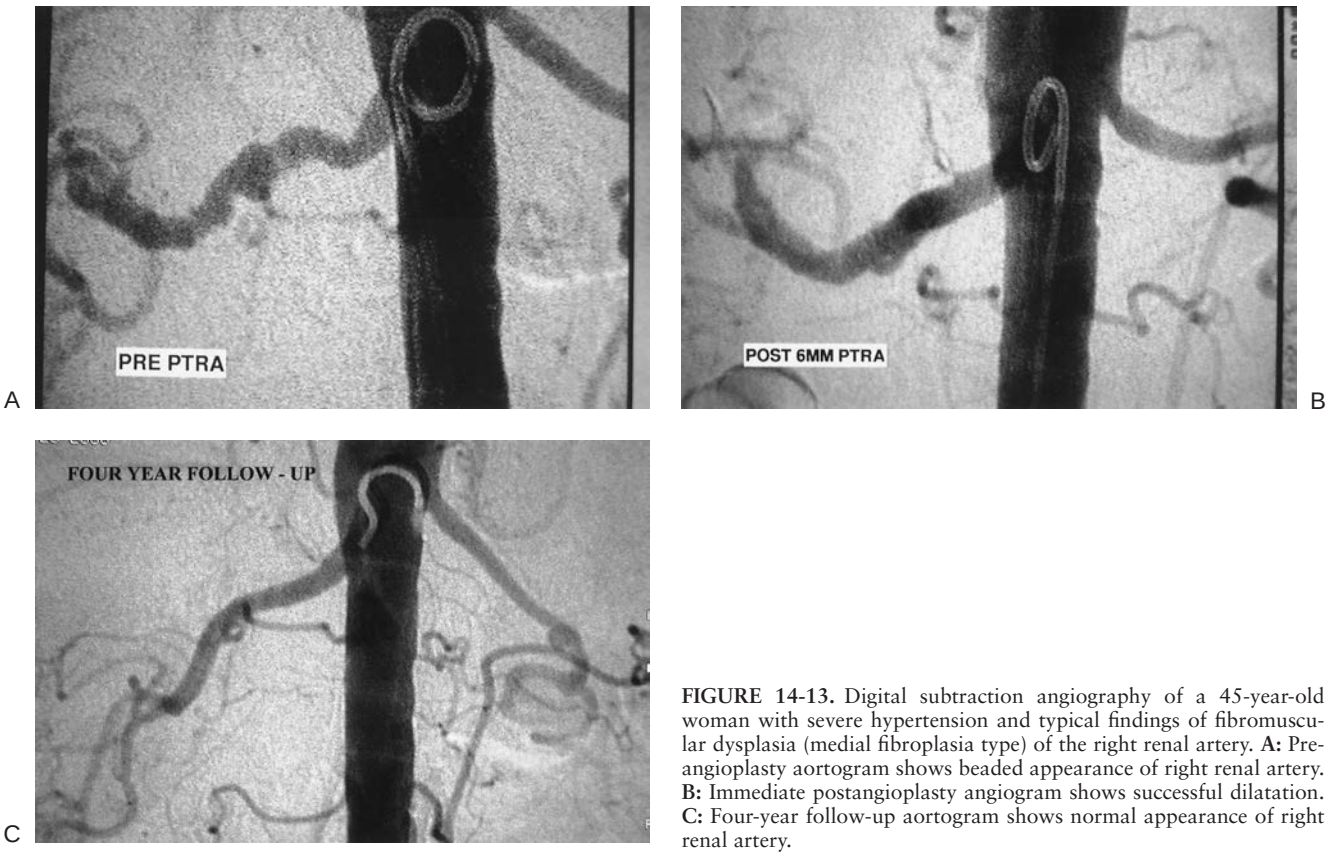


FIGURE 14-13. Digital subtraction angiography of a 45-year-old woman with severe hypertension and typical findings of fibromuscular dysplasia (medial fibroplasia type) of the right renal artery. **A:** Preangioplasty aortogram shows beaded appearance of right renal artery. **B:** Immediate postangioplasty angiogram shows successful dilatation. **C:** Four-year follow-up aortogram shows normal appearance of right renal artery.

or distal segments of the main renal artery and usually spare its proximal or orificial portions, a pattern that differentiates this disease from atherosclerosis (108). Atherosclerosis and FMD may coexist in older patients.

Vasculitis

The vasculitides are classified according to the size and distribution of the arteries involved as well as clinical characteristics, including various serologic markers (112,113). Any systemic vasculitis may include renal involvement, but polyarteritis nodosa, Wegener's granulomatosis, and systemic lupus erythematosus most commonly affect the smaller vessels of the kidneys. The findings in these disorders are indistinguishable angiographically. As shown in Figure 14-14, abnormalities most commonly include microaneurysms and occlusions of the interlobular and arcuate arteries (114,115). Irregularity of the cortical silhouette secondary to small infarctions also may be demonstrated on the angiographic nephrogram. The small aneurysms may rupture, causing large intrarenal, subcapsular, or perinephric hematomas.

Scleroderma, Radiation Nephritis, and Arteriolar Nephrosclerosis

These disorders of diverse pathogenesis appear similar angiographically (115). All three may result in narrowing and occlusion of small arteries and arterioles. Occlusions of the interlobular and arcuate arteries may be documented, but there are no specific findings. The lack of small aneurysms distinguishes these disorders from the vasculitides. Shrunken kidneys with irregular cortical contours may be seen on the nephrogram phase. Stenosis of a main renal artery also may be demonstrated in the presence of malignant hypertension (116,117).

Acute and Chronic Renal Artery Occlusion

Acute renal artery occlusion may occur secondary to an embolus or a traumatic medial dissection (115,118). Vasculitis and thrombosis owing to antiphospholipid antibodies are rare causes (119). Emboli may lodge in the main renal artery or its bifurcation and cause an acute and complete thrombotic occlusion. Acute embolic occlusion is angiographically differentiated from an *in situ* thrombosis within a severe renal artery stenosis by a lack of collateral vessels and normal or near-normal renal size. Smaller emboli can be trapped within the intrarenal arteries, where they may be identified as "filling defects" and abrupt segmental occlusions with small segmental infarcts. Cholesterol emboli have been reported to cause small intrarenal arterial aneurysms that may simulate vasculitis (120).

Spontaneous dissection of the renal artery is rare and usually associated with FMD, atherosclerosis, or blunt trauma (121–123). The left renal artery often is involved in dissections that extend from the abdominal aorta. In this setting, the false channel of the dissected aorta may perfuse the left kidney partly or entirely, or it may become ischemic. Renal artery or branch artery dissection compromising flow is an uncommon complication of balloon angioplasty (124). With this type of dissection, angiography may demonstrate a typical intimal flap that can extend in a spiral fashion into the branch renal arteries. Often, only the false channel is opacified, showing a widened and irregular contour. Both channels may also become occluded, leaving no angiographic evidence of the underlying dissection (102).

Renal artery occlusion is frequently associated with a high-grade renal artery stenosis. Demonstration of the presence of

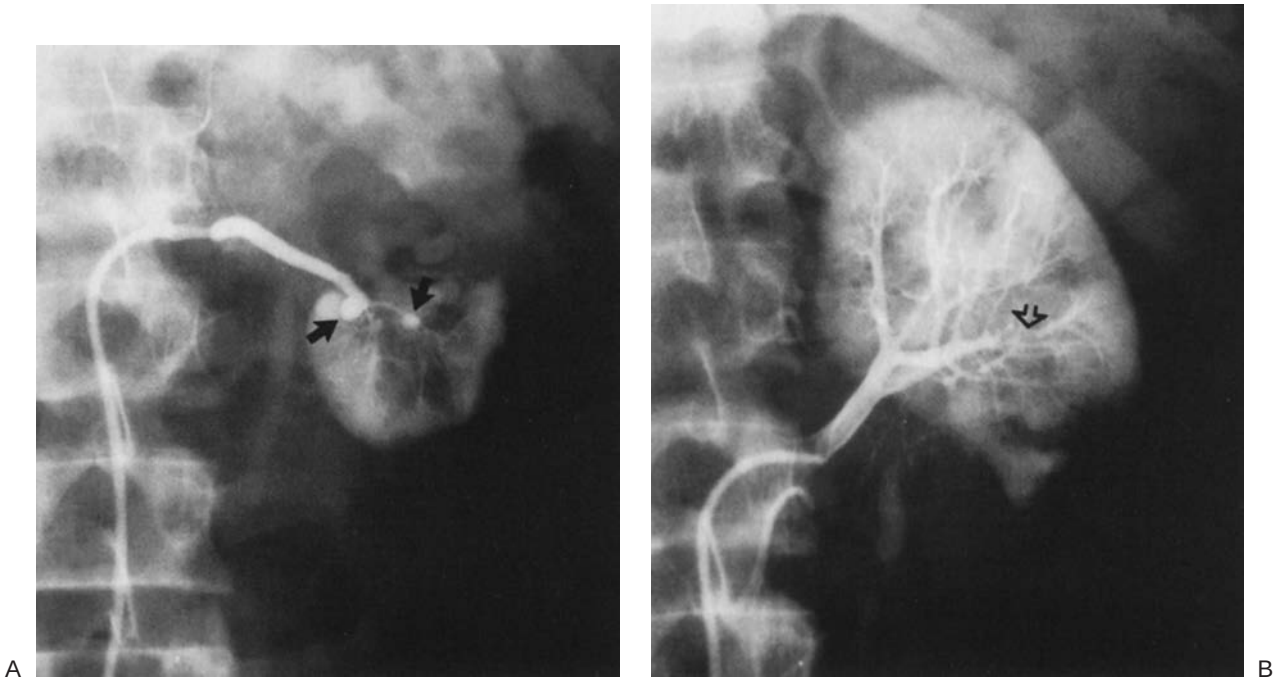


FIGURE 14-14. Typical findings of polyarteritis nodosa in an 18-year-old man presenting with hypertension. **A:** A lower-pole accessory artery injection demonstrates two focal aneurysms involving small arteries (*arrows*). **B:** Injection of the upper pole artery shows a segmental artery occlusion (*open arrow*).

collateral blood flow to the kidney is common (125), and reconstitution of the renal artery distal to the occlusion by aortography (Fig. 14-4) suggests that revascularization is possible and may improve renal function (126).

Aortic (Inflow) Stenosis

Flow to the renal arteries can be reduced by disorders affecting the suprarenal or juxtarenal abdominal aorta. These processes, which may clinically simulate renovascular disease, include abdominal aortic coarctation, middle aortic syndrome, Takayasu's aortitis, neurofibromatosis, radiation aortitis, FMD, and atherosclerosis. Congenital coarctation of the aorta (Fig. 14-15) is the result of failure or incomplete fusion of the primordial dorsal aortae (127), whereas, acquired coarctation (the middle aortic syndrome) usually is triggered by an inflammatory process (128) that may be associated with many diseases, including inflammatory aortitis, atherosclerosis, and cystic medial necrosis. Angiographically, abdominal

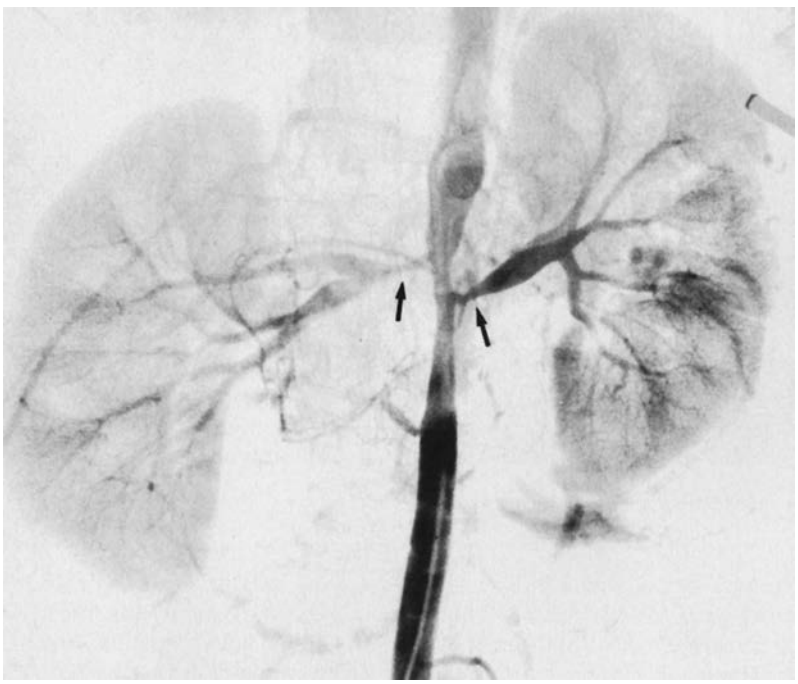


FIGURE 14-15. Conventional aortography demonstrates congenital abdominal coarctation extending into both renal arteries (*arrows*) in a 7-year-old boy with long-standing hypertension.

aortic coarctation appears as a smooth segmental narrowing or diffuse hypoplasia of the abdominal aorta that may involve the renal arteries (129). The associated renal artery disease responds poorly to angioplasty (115).

Takayasu's aortitis (aortoarteritis) is a granulomatous vasculitis that involves the thoracic and abdominal aorta and its major branches and occurs predominantly among younger women (130). It often affects the aortic arch and the arch vessels, but it may be limited to the abdominal aorta (Fig. 14-16). When the disorder is isolated in the abdominal aorta, its angiographic appearance mimics abdominal aortic coarctation. Associated renal artery stenosis responds well to balloon angioplasty (131,132).

Renovascular hypertension caused by neurofibromatosis is usually confined to the pediatric population. At angiography, smooth or nodular-appearing narrowed segments and saccular aneurysms are present. These changes may appear similar to those seen in FMD, but neurofibromatosis is suggested by involvement of the aorta and proximal renal artery, including the orifices (102).

Radiation aortitis is most commonly a complication of radiotherapy for pediatric retroperitoneal tumors, and often it is associated with radiation osteitis of the lumbar spine. It usually results in hypoplasia of the aortic segment and adjacent renal artery that were included in the radiation portal (133).

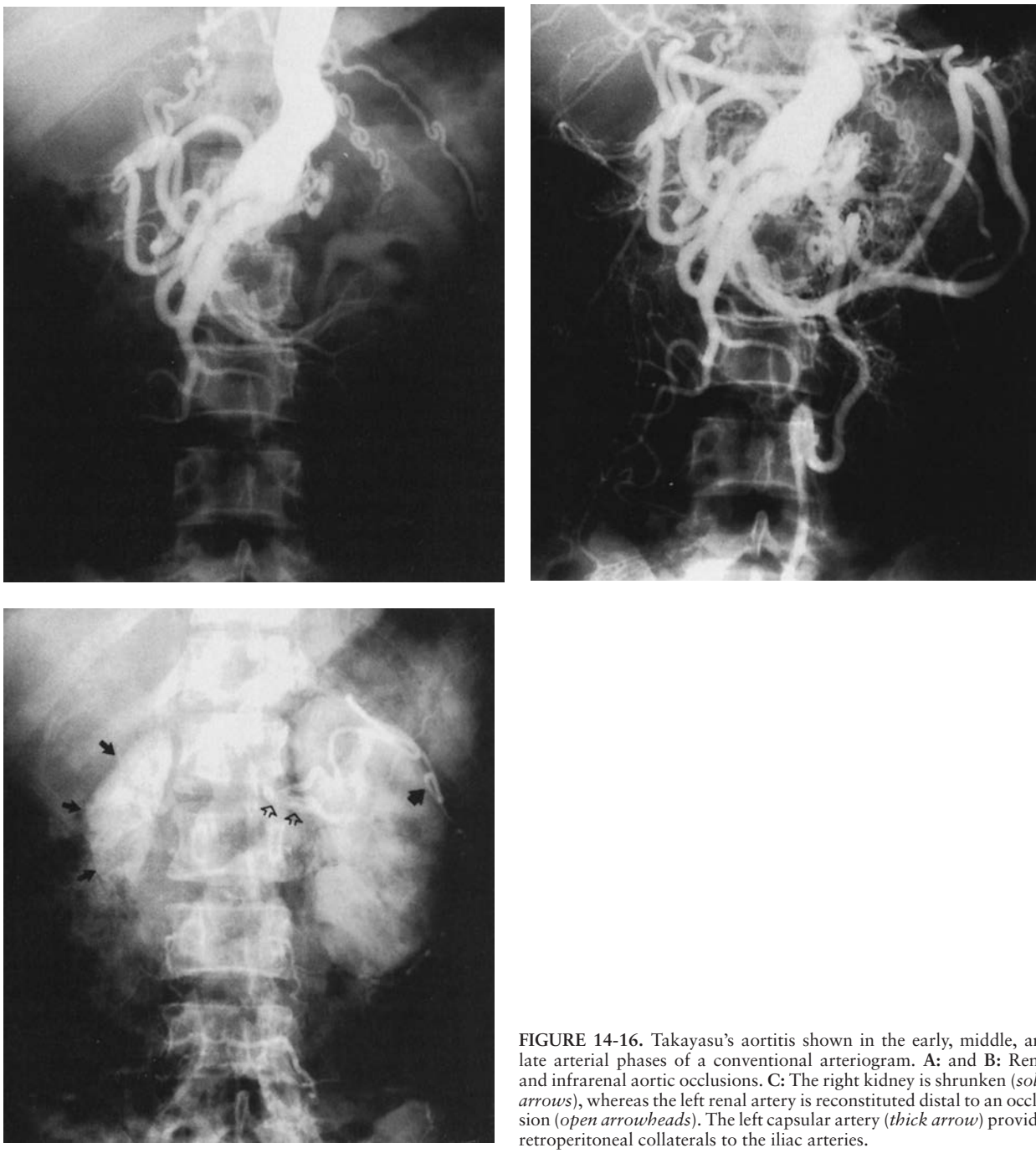
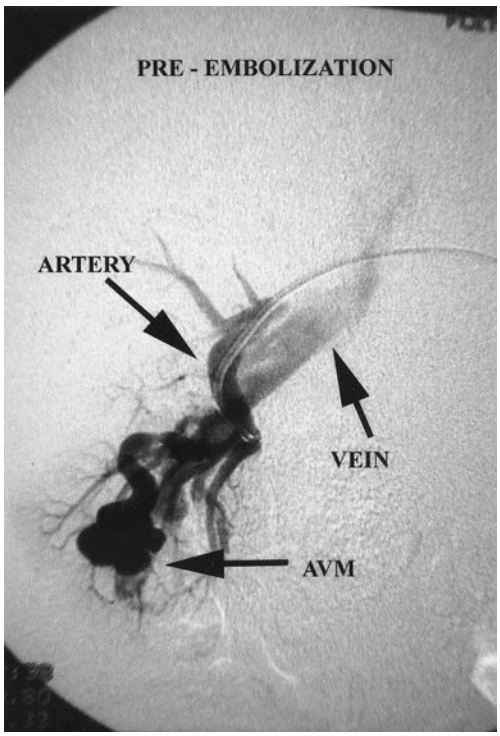


FIGURE 14-16. Takayasu's aortitis shown in the early, middle, and late arterial phases of a conventional arteriogram. **A:** and **B:** Renal and infrarenal aortic occlusions. **C:** The right kidney is shrunken (*solid arrows*), whereas the left renal artery is reconstituted distal to an occlusion (*open arrowheads*). The left capsular artery (*thick arrow*) provides retroperitoneal collaterals to the iliac arteries.



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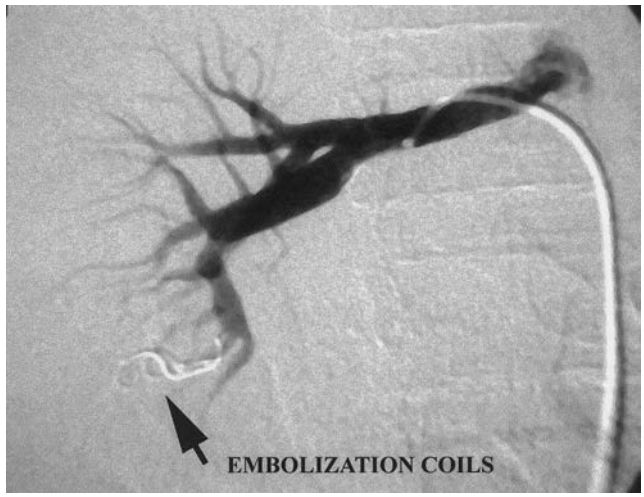


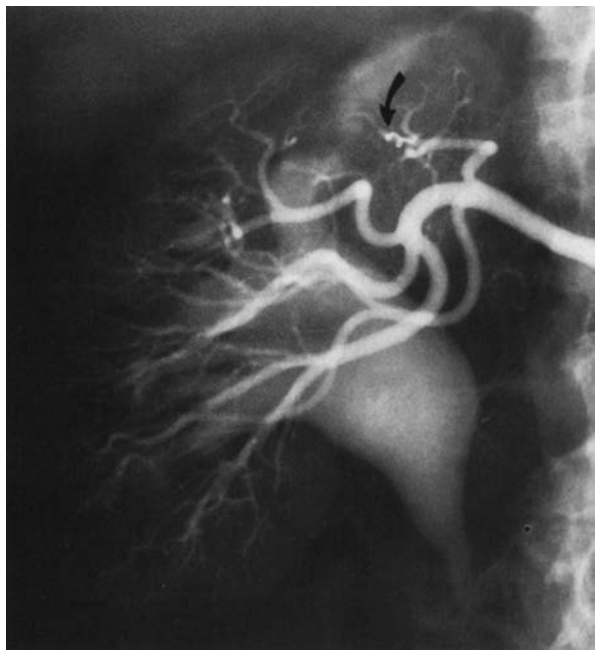
B

FIGURE 14-17. Digital subtraction selective angiography showing an arteriovenous malformation (AVM) of the lower pole of the right kidney, in a patient with hematuria. **A:** Preembolization angiogram depicting the feeding artery and draining vein of the AVM. **B:** Postembolization angiogram demonstrating the stainless steel coils successfully occluding the feeding artery of the AVM.

VASCULAR MALFORMATIONS OF THE KIDNEY

Arteriovenous malformations (AVM) are congenital anomalies that consist of abnormal communications between the arteries and veins that bypass the capillary bed. They are most frequent in women and often present with hematuria. Diversion of blood flow from normal renal structures may result in local ischemia and renin-mediated hypertension. These structures result from focal persistence of primitive vascular elements rather than neoplastic growth (134). Arteriovenous malformations may be small and detectable only by angiography, at which they are characterized by dilated and tortuous vessels with arteriovenous shunting (Fig. 14-17) (135). There are often multiple feeding arteries and communications between arteries and veins. Hemangiomas (Fig. 14-18) are benign neoplasms consisting of a cellular stroma surrounding dilated endothelial channels

A



B

FIGURE 14-18. Renal hemangioma in a patient presenting with hematuria. **A:** The typical cluster of abnormal vascular structures (*arrows*) adjacent to a cortical infarct. **B:** Occlusion of the feeding artery after superselective embolization with a platinum microcoil (*curved arrow*).

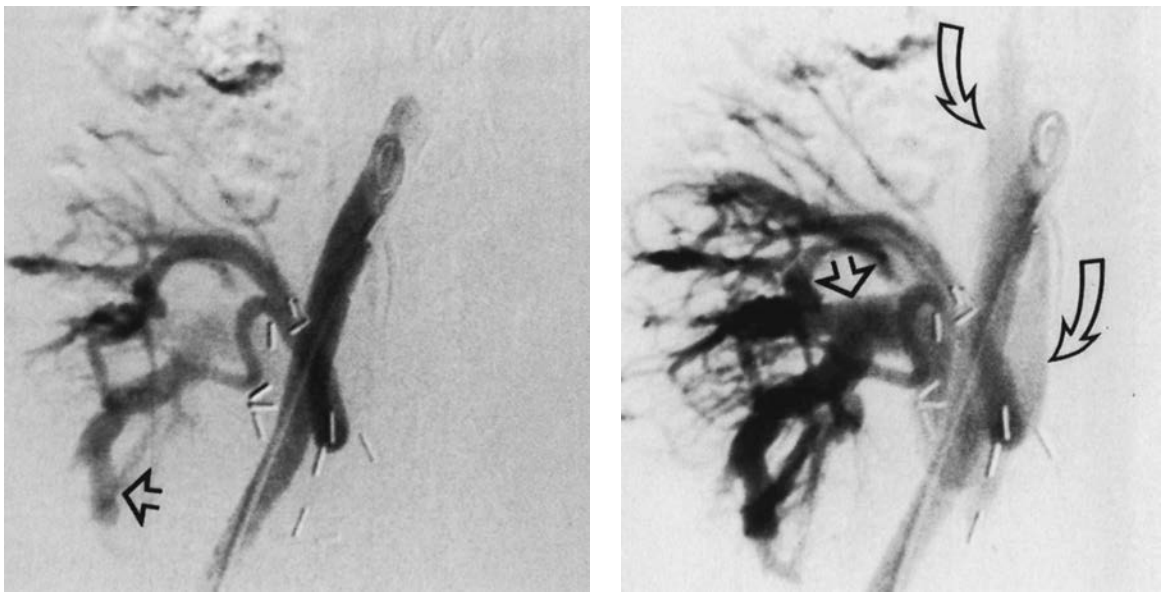


FIGURE 14-19. A large arteriovenous fistula on an intraarterial digital subtraction renal arteriography of a renal allograft after needle biopsy. **A:** The early draining of a segmental vein (*small open arrows*). **B:** Premature filling of the iliac vein (*curved open arrows*).

(134). Like AVMs, they may present with hematuria and colicky flank pain or with renin-mediated hypertension (102). At angiography, a cluster of tortuous vascular spaces with or without vascular shunting may be demonstrated and may be indistinguishable from small AVMs (136). Arteriovenous fistulae are usually acquired communications between a single artery and vein. Fistulae may be created by iatrogenic trauma during renal biopsy (Fig. 14-19), penetrating trauma such as stab wounds, aneurysm rupture, infection, or neoplasms. Arteriovenous malformations, hemangiomas, and arteriovenous fistulae all may be treated with transcatheter embolization (137,138).

RENAL ARTERY ANEURYSM

Renal artery aneurysms (Fig. 14-20) are generally asymptomatic and discovered incidentally during abdominal

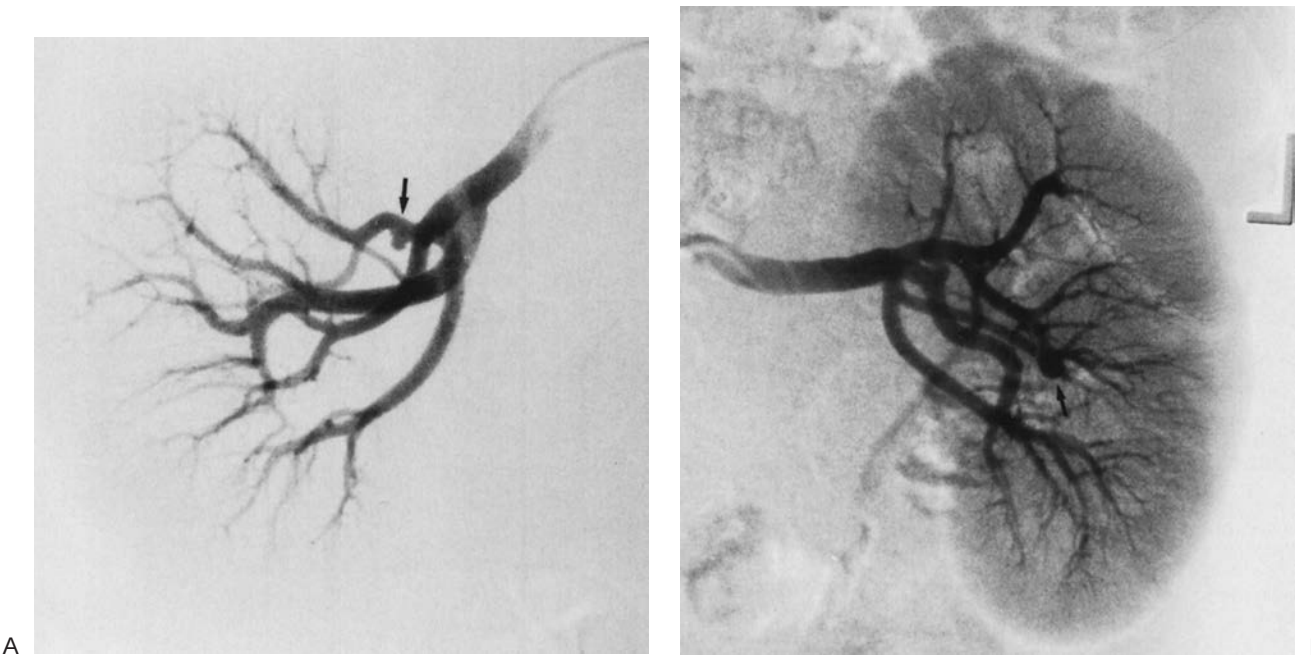


FIGURE 14-20. Bilateral renal artery aneurysms in an asymptomatic patient. Selective conventional arteriograms demonstrate segmental congenital aneurysms (*arrows*) in both kidneys (**A and B**).

FIGURE 14-21. Asymptomatic flow aneurysm (*arrow*), in a patient with a large, hypervascular renal cell carcinoma in a solitary kidney, depicted on a 3-D reconstructed magnetic resonance angiogram.

aortography in about 1% of studies. Aneurysms appear to be secondary to atherosclerosis, FMD, trauma, infection, vasculitis, and congenital anomaly (102,139,140). Renal artery aneurysms rarely rupture, and when they do, it is most commonly in the setting of infection or vasculitis. They may also thrombose and become the source of renal emboli. Hypertension is associated with renal artery aneurysms, but the relationship is unclear. Resolution of hypertension with ipsilateral nephrectomy and the frequent finding of adjacent renal artery stenosis suggest that there may be a causal association (141,142). High flow states, such as an arteriovenous malformation, arteriovenous fistula, and renal cell carcinoma may also be associated with renal artery or branch artery aneurysms (Fig. 14-21).

TRAUMA

Blunt abdominal trauma seldom results in damage to the kidney, yet it is still the most common cause of traumatic renal injury. The kidney is damaged in blunt trauma by either a sudden deceleration or a direct crush injury that compresses the organ against the ribs or spine (143). Hematuria is common and is not by itself an indication for angiography. Most blunt injuries are more effectively evaluated with CT, and angiography is reserved for situations in which there is evidence of life-threatening hemorrhage (144). Angiographic findings include pseudoaneurysms, extravasation of contrast medium, abrupt arterial occlusions owing to either transsections or dissections, and intimal dissections without thrombosis (102). One-third of cases of renal trauma are secondary to penetrating injuries (143). Hematuria in the setting of a penetrating abdominal wound almost always warrants angiography (144). Injury to the kidney or renal vasculature occurs in 12.6% of abdominal stab wounds, and arteriovenous fistulae complicate 16% of renal biopsies (145). Most of these fistulae resolve spontaneously, but some require intervention. Many of the same vascular injuries that cause arteriovenous fistulae also result in arteriocalyceal fistulae (144). The vascular sequelae of blunt and penetrating injuries frequently can be treated effectively with transcatheter embolization (Fig. 14-22).

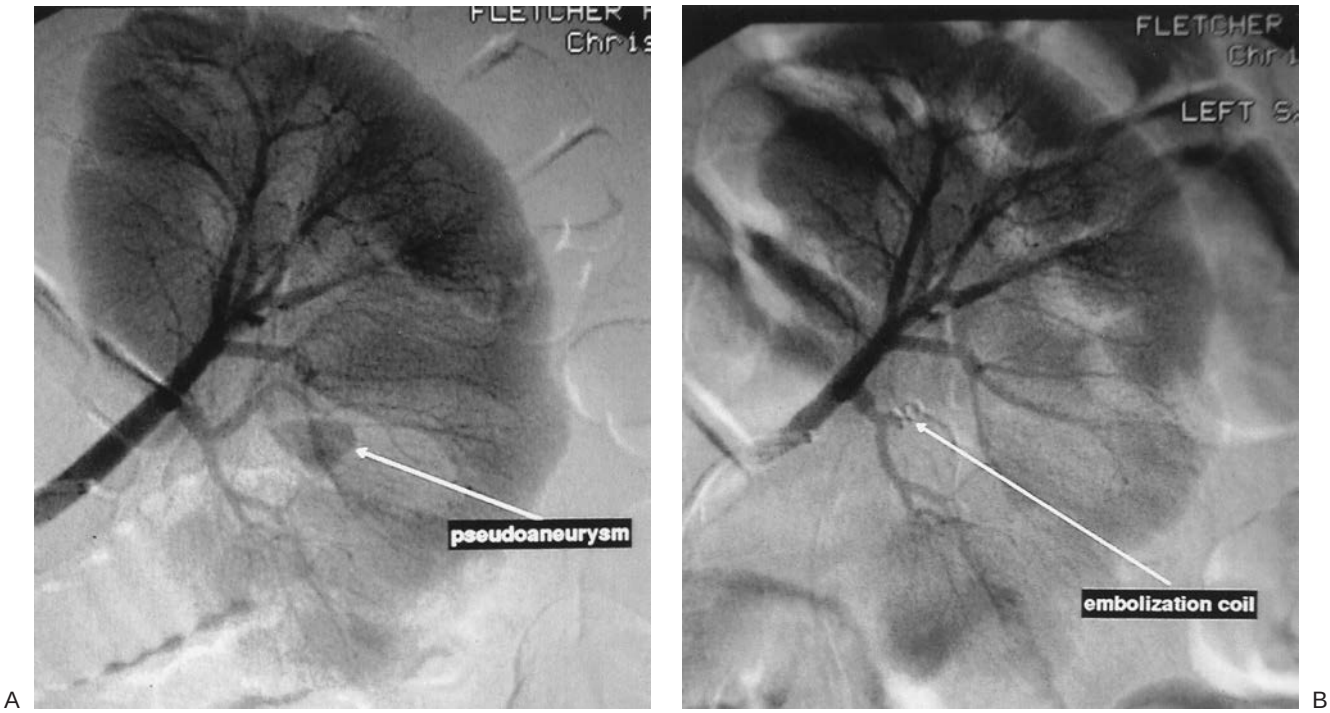


FIGURE 14-22. Digital subtraction angiography of a lacerated left kidney in a 14-year-old boy caused by blunt trauma during a bicycle accident, which resulted in massive hematuria and a large perinephric hematoma. **A:** Preembolization angiogram demonstrates a pseudoaneurysm. **B:** Postembolization angiogram shows the successful placement of a single stainless steel coil, occluding the feeding artery to the pseudoaneurysm.

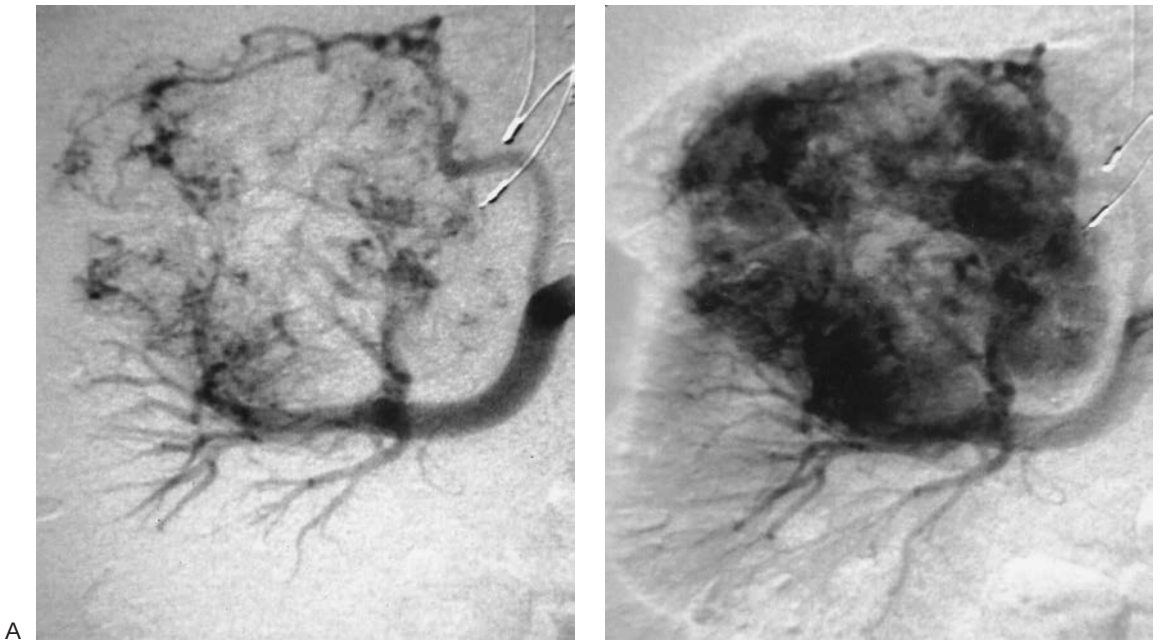


FIGURE 14-23. Digital subtraction angiography of a large renal cell adenocarcinoma involving the upper pole of the right kidney. **A:** Early arterial phase shows vascular puddling within the tumor from contrast material opacifying necrotic areas of the tumor. **B:** Late arterial phase demonstrates an intense tumor blush within the upper pole of the right kidney.

RENAL TUMORS

With advances in noninvasive imaging, the diagnostic utility of angiography for the demonstration of renal masses has markedly diminished. On occasion, renal angiography still plays a role in planning subtotal nephrectomy in the treatment of malignancies. The angiographic appearance of adenocarcinomas is usually hypervascular, with neovascularity and vascular encasement (Fig. 14-23). Few adenocarcinomas are hypovascular, but they still manifest some abnormal vessels. Other findings include dense tumor stain, vascular puddling, arteriovenous shunting, and venous lakes. A benign tumor that is also associated with a dense tumor blush, and often a classic "spoke wheel" pattern of radiating arteries, is the oncocytoma. An angiomyolipoma is a hamartomatous tumor that often exhibits tortuous and aneurysmally dilated vessels (146).

RENAL VEIN STUDIES

Noninvasive techniques have supplanted renal arteriography and venography in the diagnosis of renal vein thrombosis. Rarely, conventional venography may be undertaken to document an isolated thrombosis in a branch renal vein (147). In addition, renal venous catheterization has been used to administer local thrombolytic therapy in renal vein thrombosis (148). Venous catheters also are placed for the measurement of renin activity in the renal vein in order to document the physiologic significance of renal artery stenosis, cystic lesions, obstruction, or a number of intrarenal or extrarenal masses in patients with hypertension (149). It has been recommended that when renal vein renin activity is being determined, samples should be drawn simultaneously from both veins at a time separate from diagnostic arteriography (150). It is not clear whether two catheters must be used or whether contrast-medium administration alters the pattern of renin secretion (151,152). Maneuvers, such as volume expansion or mannitol infusion performed to reduce contrast-medium toxicity, may confound the interpretation of renal vein renin activity measured at the time of arteriography (153). Immediate angioplasty appears to have greater utility than awaiting the results of renal vein renin measurements if a stenosis is discovered during an arteriogram that is part of the workup of a patient with hypertension (154).

PERCUTANEOUS TRANSCATHETER THERAPY

Percutaneous Transluminal Renal Angioplasty and Percutaneous Transluminal Angioplasty with Stent Placement

Percutaneous transluminal renal angioplasty (PTRA) has assumed a major role in the treatment of renovascular disease since its first description by Gruntzig et al. in 1978 (155). Complete explanations of the contemporary techniques of PTRA can be found elsewhere (103,124). The pathophysiologic mechanism of balloon angioplasty is well described (156–165), with the morphologic correlates of successful angioplasty being fracture of the atherosclerotic plaque, localized medial dissection, and overstretching of the arterial wall (166).

In this technique, access is gained to the arterial circulation through puncture of the femoral or axillary artery. Predilation diagnostic arteriography is performed and then heparin and a vasodilating drug such as nifedipine or nitroglycerin is administered to minimize the risk of arterial thrombosis and vasospasm, respectively. A steerable catheter directs a guide wire to the lesion, and both the wire and catheter generally cross the stenosis. When a sufficient length of guide wire is beyond the stenosis, the initial catheter is withdrawn and the balloon catheter (Fig. 14-7) is directed over the indwelling wire so that the balloon portion of the catheter is positioned within the narrowed segment of the vessel. Alternatively, a coaxial guiding

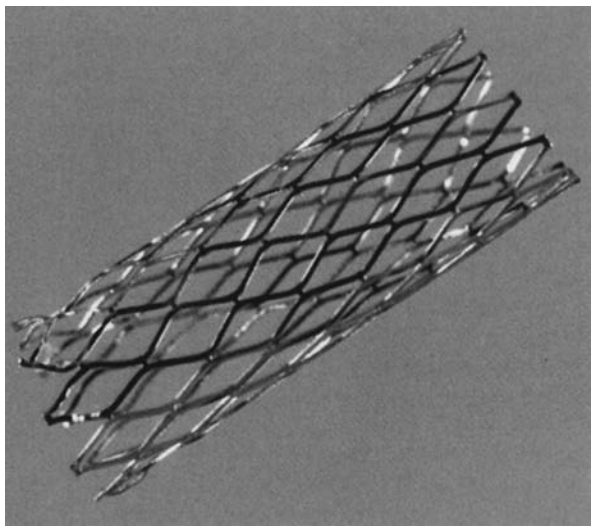
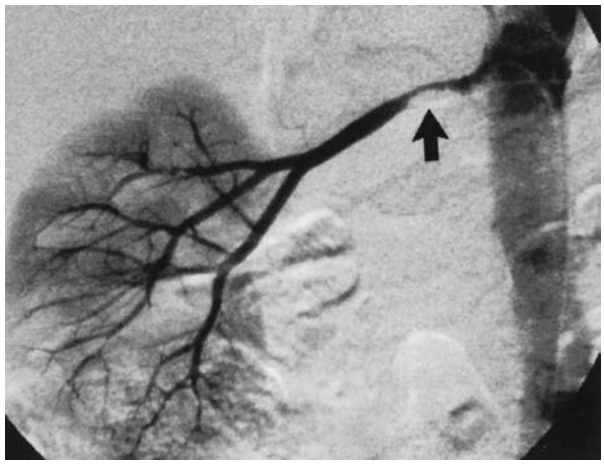


FIGURE 14-24. Photograph of a metallic vascular stent.

catheter can be placed into the origin of the renal artery, with the guide wire and balloon catheter being directed through it to traverse the stenosis. Once the balloon catheter is well positioned across the lesion, it is inflated until either the "waist"—the indentation of the balloon by the arterial narrowing—is eliminated or the maximal pressure ceiling of the balloon is reached. A postdilation angiogram then is obtained, but the guide wire should be left across the treated area until it is certain that no further dilations need to be attempted. Similar techniques are used to dilate lesions of the intrarenal and accessory arteries as well as narrowings at branch points (Fig. 14-10), although special care is taken to avoid occluding adjacent arteries during dilation.

Stents are metallic tubular prostheses that are expanded within the lumen of the artery after being positioned with percutaneous techniques. They are intended to oppose elastic recoil and to stabilize disrupted plaque and arterial wall. Stent placement has become a frequent addition to percutaneous therapy of renal arteries. This ancillary procedure strives to increase the immediate technical success, decrease complications, or increase the long-term patency rates of angioplasty. Renal artery stents (Figs. 14-24, 14-25, and 14-26) are deployed to salvage suboptimal initial angioplasty procedures or to treat



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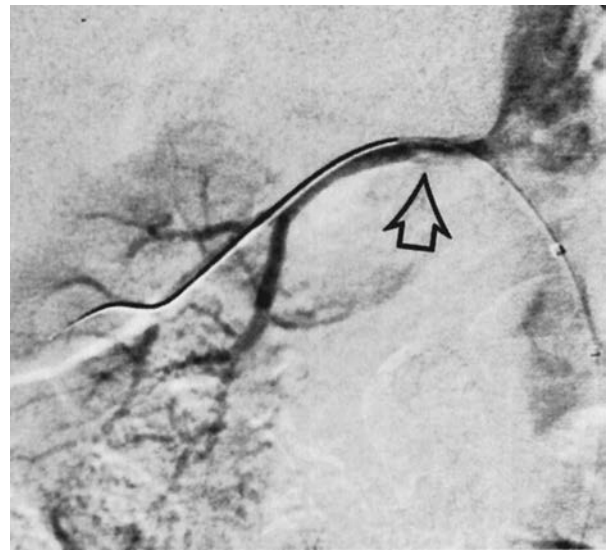

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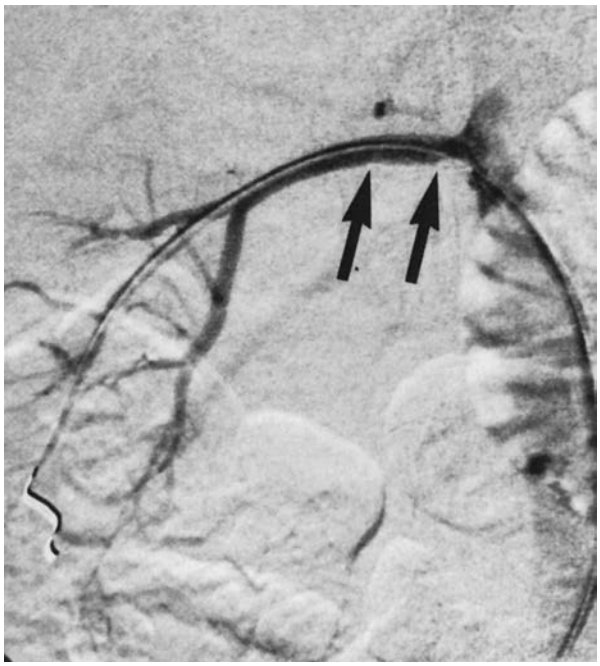

C

FIGURE 14-25. Placement of a renal artery stent. **A:** Intraarterial digital subtraction renal arteriography (DSA) demonstrates a severe eccentric atherosclerotic stenosis. **B:** Intraarterial DSA after percutaneous transluminal renal angioplasty shows an improved lumen, but a residual stenosis secondary to an intimal dissection is present (*open arrowhead*). **C:** Luminal caliber is increased after deployment of a metallic stent within the residual stenosis.

A B

FIGURE 14-26. Digital subtraction angiography of severe ostial (orificial) stenoses involving both adjacent duplicated right renal arteries, which were both successfully dilated with stents. These lesions respond less well to angioplasty alone. **A:** Prestent dilatation aortogram demonstrating atherosclerotic plaque within the aortic wall, extending into both right duplicated renal artery origins. **B:** Poststent dilatation right renal artery angiogram showing successful stent dilatation of both duplicated renal arteries.

recurrent lesions that have previously been treated with balloon angioplasty (168–169). Initial success rates of more than 95% have been reported after a suboptimal or failed angioplasty. Primary stent placement has gained considerable acceptance in the primary treatment of recalcitrant lesions of the renal artery ostium (170).

The reported success rate of PTRA depends on the indication-hypertension or renal insufficiency, and the etiology of the renal artery stenosis. Descriptions of outcomes in patients with fibromuscular dysplasia have generally been so favorable that few doubts have been raised about the use of PTRA for hypertension secondary to this disorder. Analysis of the value of the procedure in patients with atherosclerotic renovascular disease has been limited by a lack of controlled comparisons of adequate size and duration with the options of medical and surgical therapy.

Historically, the results of PTRA were generally reported as a comparison of post procedure measurements with the patients' preprocedure blood pressure or serum creatinine. Pooled data have suggested that with PTRA, atherosclerotic renovascular hypertension is cured following approximately 11% of procedures, improved in about 54%, and unchanged in around 35% of cases. Changes in renal function are characterized in a similar manner with an average of 37% reported as improved, 46% stable, and 16% worsened (171).

Three available randomized studies are different in design and therefore difficult to directly compare. Some of the characteristics are shown in Table 14-1. The Scottish and Newcastle group did not include a target blood pressure as part of their protocol; therefore, there was no early termination and crossover to invasive intervention. Groups were divided by the presence of unilateral or bilateral renal artery stenosis. The only statistically significant difference was in the systolic pressure of those with bilateral renal artery disease. There was no difference in renal function between the medical therapy and PTRA groups during follow-up, which ranged from 3 to 54 months (172). In the Essai Multicentrique Medicaments versus Angioplastie (EMMA) protocol only subjects with unilateral disease were studied and provision was made for early termination in the medical therapy arm if the diastolic blood pressure (BP) remained greater than 104 mm Hg when on maximal medical therapy. The design resulted in 7 (27%) of 26 patients in the medical therapy group undergoing angioplasty prior to the planned 6 months of follow-up. These seven patients were analyzed in the medical therapy arm of the study. Presented in this way, there was no difference in BP control or creatinine clearance between medical therapy and PTRA. The PTRA treated group did require statistically fewer drugs in order to maintain BP control (173). The largest randomized comparison of PTRA and medical therapy for atherosclerotic renovascular hypertension is the Dutch Renal Artery Stenosis Intervention Cooperative (DRASTIC) study. The DRASTIC protocol also allowed for early termination in participation in the medical therapy arm if the diastolic BP remained greater than 95 mm Hg or the serum creatinine rose by 0.2 mg/dL at 3 months. Of the 50 subjects randomized to drug therapy, 28 (56%) were treated with drug therapy alone for the 12 months, whereas 14 (28%)

TABLE 14-1

CHARACTERISTICS OF RANDOMIZED TRIALS OF ANGIOPLASTY VERSUS MEDICAL THERAPY FOR RENOVASCULAR DISEASE

Authors (Ref.)	Offered entry	Randomized	Medical therapy	Angioplasty	Terminated[a] early (HBP)	Stents[b]	Surgery[c]
Webster et al. (143)	135	55	30	25	NA	0	5
Plouin et al. (144)	76	49	26	23	7	2	0
Van Jaarsveld (145)	169	106	50	56	22	2	1

[a]Changed from medical therapy to angioplasty because of failure to control blood pressure.
[b]Number of stents utilized in study.
[c]Number of patients undergoing bypass or nephrectomy.

had early PTRA for persistent hypertension, and 8 (16%) underwent PTRA for progressive renal insufficiency. When presented as an intention to treat analysis, the only significant difference between the groups is that the PTRA group required fewer drugs for blood pressure control. Automated measurements of systolic blood pressure approached being significantly lower (152 ± 20 mm Hg versus 162 ± 27, $P = 0.07$) in the PTRA group at 12 months. When diastolic blood pressures are compared between those in the drug therapy group who did and did not receive PTRA and those who underwent angioplasty, the latter consistently have higher pressures even after the procedure. Despite this, there was a significant drop in both systolic and diastolic blood pressure following PTRA (174). These three studies have now been combined for formal meta-analysis. Pooled data show a significant reduction in systolic (−7 mm Hg) and diastolic (−3 mm Hg) blood pressures as well as a higher probability of having patent renal arteries at 12 months in the intervention group (175). There is no rigorous comparison of medical therapy versus angioplasty with stent placement in renal artery stenosis, although a prospective, randomized study, Cardiovascular Outcomes in Renovascular Lesions (CORAL), has been designed to compare the effectiveness of renal artery stenting with medical therapy to medical therapy alone (176).

Stent placement for ostial lesions has been clearly shown to have a better primary patency rate at 6 months than angioplasty alone (75% versus 29%). If stent placement is used to treat restenosis following angioplasty, a similar 6-month patency rate is achieved (177).

As with PTRA, there are now retrospective and uncontrolled prospective studies that describe outcomes with regard to hypertension or renal function where the most frequent intervention is stent placement.

In the early reports of stenting for atherosclerotic renal artery stenosis, response rates for blood pressure control at 6 to 48 months follow-up have ranged between 39% and 80%, with an average response rate of 64%, in nine recent nonrandomized and uncontrolled renal artery stent studies involving 883 patients and 1,061 treated renal arteries (177,178). In a similar group of patients with azotemia, improvement of renal function ranged between 13% and 36%, with an average improvement being reported in 21% of patients treated. Stabilization of renal function ranged between 38% and 100%, with an average of 73% of patients being reported to have stable renal function (177–180).

A more recent summary of a multicenter registry of 1,058 patients treated with renal artery stenting has reported 4-year outcomes (181). The median length of patient follow-up is not described. At 4 years, the systolic and diastolic blood pressures are reported to have declined significantly from 168 mm Hg to 147 mm Hg and 84 mm Hg to 78 mm Hg, respectively. Also at 4 years, the mean number of antihypertensive medications declined from 2.4 to 2.0 and the mean serum creatinine declined from 1.7 mg/dL to 1.3 mg/dL, both significant changes.

A retrospective review of the experience with PTRA and renal artery stenting in 146 patients at a single institution found divergent outcomes (182). In this series 75% of the interventions included stent placement. Initially, blood pressure was improved in 52% of patients with hypertension, and 68% of 24 patients, who were followed for 5 years, retained a blood pressure benefit. Although an early increase in renal function was seen, this improvement was not long lasting. Creatinine concentration was stabilized or decreased in 87% of patients with renal impairment in the first 3 months following intervention, but only 45% of 26 patients followed for 5 years experienced a benefit.

A prospective study of PTRA and renal artery stenting of 105 patients with severe renal artery stenosis attempted to test the hypothesis that the baseline glomerular filtration rate (GFR) predicts the changes in GFR and blood pressure after treatment (183). At 1-year mean follow-up, these authors found that PTRA and renal artery stenting can reduce blood pressure and increase GFR. In addition, they found discordance between the beneficial effects of treatment on blood pressure and GFR. In patients with normal to mildly impaired renal function, PTRA and stenting usually improved blood pressure. However, in patients with moderate to severely impaired renal function, PTRA and stenting usually improved renal function, but not blood pressure.

A recent small prospective observational study of 28 patients with severe renal failure and atherosclerotic renal artery stenosis attempted to determine whether or not PTRA and renal artery stenting can prevent further deterioration of renal function, and thus potentially delay dialysis (184). Despite deterioration in renal function in 17.9% of patients, a 30-day mortality rate of 10.7%, and a 7% local complication rate, progression of renal failure was significantly slowed following treatment. Of 11 patients expected to be dialysis dependent within 1 year, 8 (72.7%) experienced significant renal function improvement and were dialysis-free at 1-year follow-up.

Because of the concern about adverse outcomes with intervention, the development of techniques that will predict a favorable response is a topic of current interest. The need for such a predictive test has been emphasized by the observation that a considerable survival benefit is conferred on the subset of patients who achieve improved renal function. Doppler ultrasonography has been used to predict the outcome of revascularization for renal artery stenosis (185). Specifically, this study looked at the predictive role of the resistive index, defined as [1 − (end diastolic velocity ÷ maximal systolic velocity)] × 100. In 131 patients that underwent revascularization (angioplasty alone, n = 81; angioplasty and stent, n = 42; surgery, n = 8), the 35 patients with a resistive index value of at least 80 before revascularization had no improvement in renal function, blood pressure, or kidney survival. Other prognostic factors discussed that have strived to determine which patients will respond favorably to renal revascularization have included findings on radionuclide renal scans (186), status of distal renal artery depicted at angiography (187), baseline serum creatinine less than 3.2 mg/dL (188), and the presence of bilateral renal artery stenoses, or renal artery stenosis or occlusion of a single functioning kidney (189) (Figs. 14-27 and 14-28).

Because of the risks associated with PTRA and renal artery stenting, and the lack of a large randomized, controlled trial of intervention with medical therapy, some reports have recommended a cautionary approach to this treatment modality, reserving its use for highly selected patients (190,191). Others have recommended a more liberal use of renal artery stenting in those patients with renal artery stenosis prior to the onset of renal dysfunction (181). A reasonable approach to the role of intervention includes its use when medical therapy fails to achieve adequate blood pressure control. The decision to intervene may be further influenced by physiologic testing, such as the resistive index, and individual patient characteristics. Other indications for endovascular intervention include episodes of congestive heart failure (flash pulmonary edema) in the setting of lesions that affect the entire renal mass or rapidly progressive renal insufficiency (192).

The efficacy of PTRA in the treatment of renovascular hypertension secondary to fibromuscular dysplasia is well documented, with the cumulative primary success rate of seven large series (233 procedures) being 94% (193–199). On the basis of life-table analysis of angiographic and clinical data, a 10-year patency rate of 87% has been reported for this disorder (167). If seven patients who underwent repeat dilations are included, the

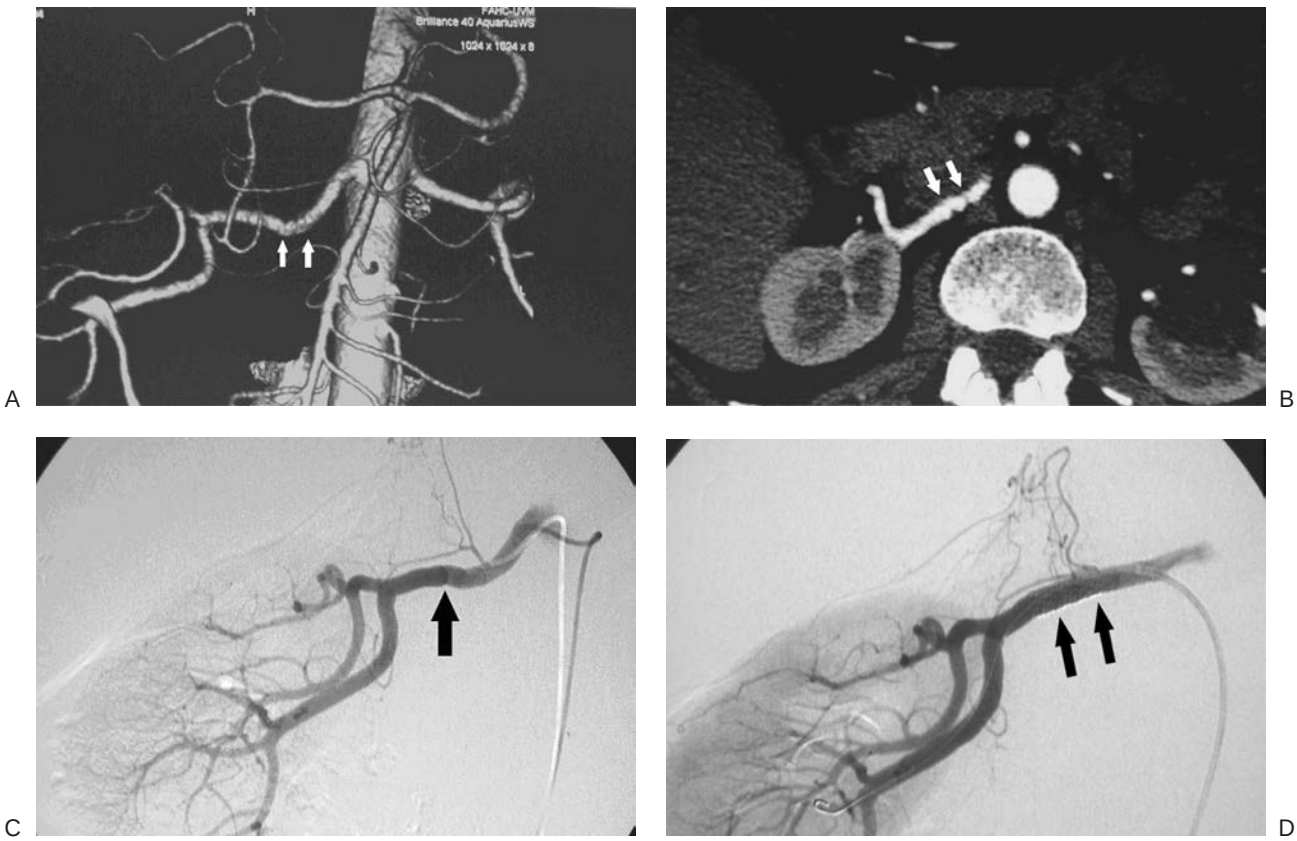

FIGURE 14-27. Residual stenosis of the renal artery in a 66-year-old woman with fibromuscular dysplasia, presenting with recurrent uncontrolled hypertension, despite a "successful" balloon angioplasty 1 month previously. **A**: Three-dimensional reconstructed CT angiogram showing irregularity of the mid right renal artery, at the previous angioplasty site (*arrows*). **B**: Axial CT angiogram confirming multiple web-like stenoses of the right renal artery. **C**: Digital subtraction angiogram confirming the web-like stenoses of the right renal artery. **D**: Post stent-dilatation right renal artery digital subtraction angiogram demonstrates a widely patent right renal artery, with a stent in good position (*arrows*).

secondary patency rate at 10 years is 98%. In general, balloon PTRA, as opposed to renal artery stent dilatation, is the preferred treatment for most stenoses of the renal artery caused by fibromuscular dysplasia. Recurrent stenoses are documented to occur in 11.5% of cases, but as noted these recurrent lesions are usually amenable to repeat PTRA (195). Occasionally, a significant intimal dissection caused by angioplasty of a fibromuscular dysplasia lesion or a suboptimal angioplasty is best treated by placement of a stent (Fig. 14-27).

Renal-transplant arterial stenosis occurs in about 5% of patients with renal allografts (200). Stenoses may be secondary to atherosclerosis of native arteries; perfusion, immunologic, or ischemic injury of the allograft artery; and problems with the surgical anastomosis. PTRA is well tolerated and generally considered the primary treatment for transplant renal artery stenosis. The technical success rate is about 70%, with reduction of hypertension in 67% to 80.5% of cases followed from 12 to 30 months (201–203). Complications are similar in type and frequency to those of angioplasty of native renal arteries. Recurrent stenosis occurs in 20% of patients.

PTRA has also been attempted in renal artery stenosis secondary to inflammatory diseases. The greatest experience is with Takayasu's aortitis or arteritis, in which lesions have been shown to respond well, with an 80% to 85% technical success rate (130,131). However, the most durable and efficacious treatment of renal artery stenosis caused by Takayasu's arteritis has been surgical bypass grafting (204). Most other diseases are uncommon causes of renal artery stenosis, and the response rate to PTRA is not well documented.

Complications of Percutaneous Transluminal Renal Angioplasty

A compilation of numerous PTRA studies encompassing 1,118 patient reports shows an overall complication rate of less than 10% when renal failure is excluded (123). Puncture-site hematomas requiring treatment occurred in 2.3% of procedures and are probably more frequent than in diagnostic studies because of the larger catheter used. Damage to the main renal artery was noted in 2.4% of studies and to branch renal arteries in 2.2%, although surgical intervention was required in less than half of these complications. Some form of embolization was noted in 1.1% of patients. Death is uncommon among patients being treated for hypertension alone, with a 30-day mortality rate of 0.3% reported among 882 patients who had renal angioplasty for diverse indications (123). Mortality rates have been reported to be as high as 5% among patients with atherosclerosis treated for renal insufficiency (3).

In a study of 212 patients (308 renal arteries) treated with either PTRA or stenting of the renal artery, iatrogenic renal artery injuries occurred in 6.1% (13 patients) of patients treated, or 4.2% of renal arteries treated (205). All 13 patients were successfully treated nonoperatively, including the five patients with renal artery rupture, who were treated with balloon tamponade

FIGURE 14-28. A 70-year-old woman with oliguric renal failure and uncontrolled hypertension. Both disorders resolved following bilateral renal artery stent-dilatation. **A**: 3-D reconstructed magnetic resonance angiogram shows bilateral renal artery stenoses (*arrows*). **B**: CO2 renal flush abdominal aortogram confirms the bilateral renal artery stenoses (*arrows*). **C**: CO2 post right renal artery stent-dilatation renal artery angiogram demonstrates a widely patent right renal artery and good position of the stent (*black arrow*). The severe stenosis of the left renal artery is again identified (*white arrow*). **D**: The final CO2 post bilateral renal artery stent-dilatation renal artery angiogram depicts widely patent bilateral renal arteries and stents (*arrows*).

or placement of an additional stent or stent-graft. Six patients suffered acute thrombotic occlusion of the renal artery. Five of these patients were successfully treated with transcatheter thrombolysis, and one was successfully treated with placement of an additional stent.

In another recent series of PTRA and stenting of the renal artery involving 146 patients, the major morbidity rate was 4% and the procedure-related complication rate was 18% (181). These complications included seven stent dislodgements, seven bleeding episodes, five groin hematomas, four acute renal artery dissections, four femoral or axillary artery pseudoaneurysms, three procedure related kidney perforations, two peripheral embolizations to the foot, and one renal artery occlusion. Four of these patients required surgery secondary to the procedure complication, although no complication resulted in the loss of a kidney.

Because of variable reporting techniques, the incidence of renal failure after angioplasty is unknown. In one series of 100 patients, four required dialysis after angioplasty, all of whom had severe renal insufficiency before the procedure (206). Permanent dialysis was required in only one of the four. An additional 26% of this group of patients was noted to have an increase in the serum creatinine level after PTRA. This is consistent with the compiled results of PTRA as a treatment for renal insufficiency, in which 22% to 32% of the patients' renal function was classified as having worsened after angioplasty. It is not possible to determine what proportion of this decline in function is owing to the procedure and what is related to other factors (3).

Investigational Adjuncts to Percutaneous Transluminal Renal Angioplasty

Local administration of antiproliferative agents is now under investigation as a method of decreasing the neointimal hyperplasia that results in recurrent stenosis after PTRA (207–209). Brachytherapy (delivery of localized therapeutic ionizing radiation) has also gained considerable attention recently, as a potential inhibitor of neointimal hyperplasia following angioplasty and stent dilatation procedures (210–215). Although the experience of brachytherapy in the renal arteries is limited, it has been shown to have some promise in increasing long-term patency after angioplasty and stent dilatation of the coronary, and femoral-popliteal arteries. The radiation dose may be administered locally to the treated artery via endovascular techniques, using radioactive balloons, stents, or guide wires.

Atherectomy is a procedure that recanalizes stenoses by resecting plaque with a percutaneously directed cutting catheter. Clinical experience is very limited, but some success has been achieved against nonostial renal artery stenoses with the use of a balloon atherectomy catheter with a blade embedded in its side that is able to shave atheromatous plaque (216).

Inserting a special balloon or umbrellalike device distal to the angioplasty or stent dilatation site can achieve atheroembolic protection of the kidney during renal artery angioplasty and stent dilatation procedures. Following the dilatation procedure, the atheroembolic debris is either collected in the umbrellalike device and removed or aspirated through a catheter located proximal to the balloon. Experience with this technique in the renal arteries is limited, but a study of 16 treated renal arteries demonstrated collection of atheroembolic debris in each case, and no deterioration of renal function (217). A larger, more recent retrospective review of the use of distal protection during renal artery stenting has also shown promising results (218). In this study of 37 patients with renal insufficiency (46 renal arteries), no patients had acute postprocedural deterioration in renal function and 95% of patients had stabilization or improvement in renal function following the procedure. Interestingly, 65% of the protection baskets contained embolic material, including fresh thrombus, chronic thrombus, atheromatous fragments, and cholesterol clefts.

RENAL ARTERY AND RENAL ARTERY BYPASS GRAFT THROMBOLYSIS

Acute thrombosis of the renal artery may be the result of an embolus or *in situ* thrombosis of a preexisting lesion. Renal function may be recovered if a revascularization procedure is performed soon after the acute occlusion. Several authors have found that significant recovery of renal function may still occur despite a delay in the revascularization of up to 2 to 6 weeks (219,220). The presence of a preexisting hemodynamically significant lesion within the renal artery, causing collateral flow to the kidney, has been proposed as a possible protective mechanism. This may be used to predict which patients may experience a return of renal function from a kidney with an acutely thrombosed renal artery, despite a delay in revascularization (221).

Transcatheter thrombolysis is an endovascular technique that entails the direct administration of a thrombolytic agent, such as tissue plasminogen activator, urokinase, or streptokinase, into the clot through a catheter located within the renal artery. It is a nonoperative procedure that is effective in reestablishing patency of the renal artery (222–236).

TRANSCATHETER EMBOLIZATION AND ABLATION

Transcatheter embolotherapy was described as a nonsurgical approach to renal arterial trauma and vascular malformations over 20 years ago (237). It has proved to be an important conservative technique in the treatment of vascular abnormalities that otherwise would require nephrectomy (238). In symptomatic patients, embolization is appropriate if arteriography shows contrast extravasation, pseudoaneurysm formation, and arteriocalyceal or arteriovenous fistula formation 239).

Directing a catheter to the most selective position possible to provide maximal sparing of functioning renal parenchyma achieves embolization. The choice of embolic agent largely depends on the type of lesion to be treated, the size of the feeding artery, the size of the selective catheter employed, and the decision whether or not a temporary or permanent vascular occlusion is desired. A temporary occlusive substance such as a gelatin sponge pledget is used for peripheral traumatic lesions. For vascular malformations and aneurysms, permanent agents such as a stainless steel coil (Figs. 14-17, 14-18, and 14-22) or polyvinyl alcohol particles are used. Success rates should be over 90%. Most patients experience transient flank pain, which is usually related to renal infarct (238). Misdirected emboli that cause ischemia of the bowel, spine, or lower extremity are the most serious complications, but they should be rare when an experienced angiographer performs the procedure.

Transcatheter renal ablation is a procedure that can obviate the need for surgical nephrectomy in selected patients. Indications include uncontrolled renin-mediated hypertension or massive proteinuria in patients with end-stage renal disease and preoperative management of renal cell carcinoma. The usual technique is to infuse absolute alcohol distal to a balloon occlusion of the renal artery (229).

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CHAPTER 15 ■ INDICATIONS FOR AND INTERPRETATION OF THE RENAL BIOPSY: EVALUATION BY LIGHT, ELECTRON, AND IMMUNOHISTOLOGIC MICROSCOPY

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This chapter provides a discussion of the indications for use of renal biopsy, describes the procedure and methods of tissue preparation, and demonstrates the manner in which biopsy specimens are interpreted using a combination of light microscopy, electron microscopy, and immunohistologic microscopy. The technique of percutaneous renal biopsy was introduced clinically in the early 1950s. Iversen and Brun (1), who are generally credited with describing its initial use, believed the technique would be quite useful in obtaining more information about a group of diseases that caused acute renal failure. At that time the diseases were referred to as lower nephron nephrosis. The renal biopsy technique was utilized with increasing frequency during the 1950s, and it has enjoyed wide usage throughout the world since the early 1960s. The technique has provided a wealth of information about the histopathology, pathogenesis, and classification of renal disease that could not have been obtained by any other means.

Proponents of the biopsy procedure employ this technique to diagnose kidney disease, assess prognosis, monitor disease progress, and aid in selection of a rational approach to therapy. It is used extensively in younger patients (2–7) as well as older patients (8–15). The procedure, however, is not without morbidity and occasional mortality. Therefore, the risk:benefit ratio must be considered carefully in each patient who is being evaluated for a biopsy.

As originally described, the biopsy was performed with the patient in the sitting position, and the procedure involved aspiration of the tissue sample. Brun and Raaschou (16) used the Iversen-Rohlm cannula and syringe, which yielded a cylinder of tissue approximately 1.5 mm in diameter and of variable length. However, Kark and Muehrcke (17) chose to place the patient in the prone position and initiated the use of the Franklin modification of the Vim-Silverman cutting needle in place of the aspiration technique. Today, most nephrologists prefer to use one of the spring-loaded, automatic, or semiautomatic biopsy guns. The addition of ultrasonography, computed tomography and image-amplification fluoroscopy to locate the kidneys and aid in positioning of the biopsy needle has greatly simplified the technique.

The percutaneous renal biopsy is a safe and reliable technique in the hands of the experienced operator. The most common complication is bleeding, which occurs in the majority of patients if they are studied carefully after biopsy using ultrasonography (18,19) or computed tomography (20,21). However, the bleeding is self-limited and rarely requires operative intervention or blood transfusion. In a survey (22) of the results of over 5,500 percutaneous renal biopsies, the rate of complications, including the need for blood transfusion or nephrectomy, the puncture of other organs, or the presence of a clinically evident perinephric hematoma, was 2.1%. The overall mortality is approximately 0.1% to 0.2% (22–24), which is comparable to that reported for percutaneous liver biopsy or coronary angiography (22). In a study from a single institution (25) in which 1,000 consecutive percutaneous renal biopsies were analyzed, a total of 94 complications were observed in 81 patients. Gross hematuria, including the passage of blood clots, represented 73% of the complications. Two patients underwent exploration for evacuation of perirenal hematomas, but no kidneys were lost. One patient died of multiple complications after biopsy.

Adequate tissue samples are obtained 90% to 95% of the time. In a retrospective study, Bolton and Vaughn (26) reported that renal tissue was obtained in 97% of their patients with the use of image-amplification fluoroscopy, compared with 81% before the use of fluoroscopy. In contrast, Dias-Buxo and Donadio (25) were unable to demonstrate an increase in their success rate for procuring renal tissue with the use of fluoroscopy. However, these investigators considered image-amplification fluoroscopy to be advantageous in the difficult patient.

TECHNIQUES

It is important to establish that the patient has a normal platelet count, prothrombin time (or INR), and partial thromboplastin time before the procedure is undertaken in all patients in whom a percutaneous renal biopsy is to be performed. In addition, it is advisable to obtain a hematocrit and hemoglobin within 24 hours of the procedure. It has been suggested that the bleeding time is the best predictor of hemorrhagic risk in patients undergoing biopsy for kidney disease (27) when accompanied by a platelet count, hematocrit, and a careful investigation of a family or personal history of bleeding. Currently, most percutaneous biopsies are performed with the guidance of ultrasonography or fluoroscopy to permit more accurate localization of the kidney. These imaging techniques are especially valuable for use by a less experienced operator or in a difficult patient. The use of a premedication, such as intravenous diazepam (Valium), helps alleviate patient anxiety, thus making the procedure less unpleasant. We routinely place an intravenous access in the patient; this is kept open with 5% dextrose in water for the initial 18 to 24 hours after completion of the biopsy.

Most operators prefer to biopsy the lower pole of the left kidney to reduce the risk of inadvertently passing the biopsy needle through a major renal artery or vein. After completion of the biopsy, patients are instructed to remain at bed rest for 18 to 24 hours. In our institution, the blood pressure and pulse are monitored every 15 minutes for 1 hour, every 30 minutes for 1 hour, then hourly for 4 hours, and finally every 4 hours

for the balance of the 24-hour period. The patient is asked to save an aliquot of each voided urine in a separate clear-plastic specimen jar labeled with the date and time, which is kept at the patient's bedside for inspection. This provides a visual check for evidence of bleeding into the intrarenal collecting system. Hematocrits are determined 6 to 8 hours after the biopsy and again at 18 to 24 hours, or earlier, if hypotension or gross hematuria is observed.

Several types of spring-loaded automatic or semiautomatic biopsy guns are being employed to perform percutaneous biopsies of both transplanted (28–38) and native kidneys (36–52). Based on sample of almost 2,000 percutaneous biopsy procedures, the rate of complications, including a clinically evident hematoma, defined without the use of ultrasonography or computed tomography, nephrectomy, blood transfusion, acute urinary tract obstruction, or biopsy of another organ was 1%. Adequate samples of tissue were obtained 94% of the time on the initial attempt at biopsy. These data compare very favorably with the published experience with either the Franklin modification of the Vim-Silverman needle (Popper and Sons, Inc., New Hyde Park, NY) or the Travenol Tru-cut disposable needle (Travenol Laboratories, Deerfield, IL) (22,26). Furthermore, when direct comparisons have been made, the results obtained with the biopsy gun were easily comparable to those achieved with the Travenol disposable needle (31,35,42,43,49,50). In a recent study (36), 1,090 percutaneous kidney biopsies were performed using ultrasound guidance and an automated spring-loaded biopsy device. A total of 114 (10.4%) were performed on renal allografts and 976 (89.6%) on orthotopic kidneys. No serious complications, including loss of kidney, life-threatening hemorrhage, or a persisting hemodynamically relevant arteriovenous (AV) fistula, were encountered. In 98.8% of cases, sufficient tissue was obtained to make a reliable histopathologic diagnosis.

When combined with real-time ultrasound technology, there are some definite advantages to the use of several of the fully automatic biopsy guns. For example, the depth of the biopsy is controlled rather precisely and can be selected for a particular clinical situation. In the case of one of the most commonly used instruments (Biopty, Bard Urological Division, C.R. Bard, Covington, GA), the long-throw device has a depth of 2.3 cm, yielding a specimen with a potential length of up to 1.7 cm. The short-throw device has a depth of 1.15 cm and a potential specimen length of 0.9 cm (53). Fully automatic biopsy guns can be triggered with one hand, thus leaving the operator with a free hand to control the ultrasound probe if necessary. Instruction in the use of the biopsy gun is also easier. Because the procedure does not require the patient to hold their breath during kidney localization, it is both simpler and much faster, thus making it more feasible to perform biopsies on severely ill patients and those less able to cooperate. Many also believe that there is less discomfort with use of the biopsy gun (36,41,42,49).

Currently, there is no universal agreement on the optimum size of the needle that should be used with the various biopsy guns. Many favor the 18-gauge needle, which retrieves almost as many glomeruli per specimen as the larger-gauge needles (28–35,37–42,52). This is due in part to the fact that the individual specimens have cleaner, sharper edges with less crush artifact. Certainly, in pediatric patients, the 18-gauge needle has been found to be quite adequate (37,38,40,52). We favor use of a 15- or 16-gauge needle for biopsy in adult patients.

The fine-needle aspiration biopsy (FNAB) technique applied to the transplanted kidney is used in several major transplantation centers around the world (44,55–57). Pasternack (58) performed the first aspiration biopsies on human renal allografts. Several thousand biopsies have been performed in the renal transplant unit at Helsinki University Central Hospital, where it has been in routine use since 1980. This center has played a major role in the development and subsequent refinement of the technique to monitor allograft rejection (59). Although the FNAB technique cannot substitute entirely for the standard percutaneous biopsy, it does serve as another method of following at frequent intervals the inflammatory events and tubular and endothelial cell alterations that occur in an allograft, including those associated with rejection, acute tubular necrosis, and drug toxicity, and permits close follow-up of the success of maneuvers designed to interrupt or reverse these pathologic events (60). There is now general agreement among investigators utilizing FNAB that the technique can differentiate with a high degree of accuracy among cyclosporine toxicity, acute tubular necrosis, graft necrosis, and acute cellular rejection (59,61). Interestingly, both fungal (62) and bacterial (63) pyelonephritis of the renal allograft have been described through the use of FNAB. A limitation of the technique is inability to thoroughly evaluate vascular lesions. Although invasive in nature, the FNAB technique is relatively innocuous and quite safe and can be used to obtain specimens several times over the course of a day, if desired.

There is growing interest in performing percutaneous kidney biopsies in the outpatient setting. In the United States this movement is driven in part by the need to reduce costs associated with the procedure. Native and transplanted kidneys have been biopsied successfully in the ambulatory setting in both children and adults (64–68). Ultrasonographic evidence suggests that most episodes of major bleeding occur within the initial 6 hours after renal biopsy and that the size of perirenal hematomas actually decreases thereafter (65). These data confirm an earlier report by Carvajal et al. (2) who found only three significant bleeding episodes in 890 consecutive percutaneous biopsies performed in pediatric patients. These data, when linked with the experience in the outpatient setting thus far, suggest that in carefully selected patients in whom the procedure is performed without difficulty, the use of ambulatory percutaneous renal biopsy can be justified. If patients are free of pain at the site of biopsy, have clear urine, and have stable cardiovascular signs for a minimum of 4 to 6 hours after the procedure, they can be safely discharged (66). Activity should be restricted for at least 24 hours, and patients cautioned to seek medical attention immediately if there is macroscopic hematuria or pain over the biopsy site.

INDICATIONS

There is no universal agreement on the precise indications for use of the percutaneous renal biopsy despite 50 years of experience with the technique by the nephrology community. The present section describes several clinical situations in which this technique is either routinely or frequently employed to aid in the evaluation and management of a patient with undiagnosed kidney disease.

Acute Renal Failure

There are many occasions when the etiology of acute renal failure secondary to intrinsic renal disease is not evident despite a carefully performed history and physical examination and the availability of information gained from various laboratory studies. A biopsy can be very useful in establishing the diagnosis, determining the approach to management, and defining the prognosis in this clinical setting. Retrospective studies from several centers have revealed that the diagnosis of acute tubular necrosis (ATN) cannot be established clinically (69–72) in 10% to 25% of patients who present with acute renal failure. The biopsy in this population can be important because other causes of acute renal failure are revealed, such as crescentic proliferative glomerulonephritis, interstitial nephritis, Wegener's

granulomatosis, polyarteritis nodosa, multiple myeloma, amyloidosis, endocapillary proliferative glomerulonephritis, cortical necrosis, hemolytic-uremic syndrome (HUS), systemic lupus erythematosus (SLE), and thrombotic thrombocytopenic purpura, to list just a few. These diseases usually require a different approach to management from that employed normally in uncomplicated ATN.

Occasionally, a biopsy is found necessary in patients who appear to have ATN on clinical grounds at initial presentation, but do not regain renal function after 3 to 4 weeks of supportive therapy, including dialysis (72). The diagnostic possibilities generally are the same as those listed in the preceding paragraph. Careful evaluation of the clinical situation is deemed prudent before a renal biopsy is initiated, because it has been found that this invasive procedure carries a higher risk in the patient with acute uremia (73).

Nephrotic Syndrome

Renal biopsy in the clinical setting of an acute nephrotic syndrome in the absence of a systemic disease is influenced greatly by the age of the patient. It is common practice to treat children initially with high-dose corticosteroids, because most younger children have minimal change nephrotic syndrome (MCNS) on biopsy. The presence of a selective proteinuria and normal renal function and the absence of hypertension strengthen the clinical diagnosis. In children, biopsy is usually reserved for patients with no response to corticosteroid therapy or in whom the clinical and laboratory features of the illness at the time of initial presentation are distinctly atypical for MCNS. These features would include hypertension, azotemia in the absence of volume depletion, nonselective proteinuria, highly active urine sediment including red cell casts, and involvement of other organ systems.

Most nephrologists believe that the adult nephrotic patient without signs of systemic disease should undergo a biopsy before therapy is initiated, because the majority of these patients, including elderly persons (74), have a renal disease other than MCNS (10). The most frequent cause of the nephrotic syndrome in adults is idiopathic membranous glomerulonephritis (10,75,76), a disease in which the efficacy of corticosteroid therapy has yet to be established with certainty. Other leading causes of the nephrotic syndrome in the adult include focal segmental glomerular sclerosis (FSGS), membranoproliferative glomerulonephritis (MPGN), proliferative glomerulonephritis, immunoglobulin A (IgA) nephropathy, and amyloidosis, most of which are unresponsive to corticosteroid administration. Fewer than one-third of adult patients have MCNS. Thus, if the physician elects to administer a short course of high-dose corticosteroid therapy equivalent to that employed in pediatric patients, approximately two-thirds of the patients would not be expected to respond favorably. Despite suggestions to the contrary (22), we believe the risks associated with the use of corticosteroids or other immunosuppressive agents, such as azathioprine, chlorambucil, cyclosporine A, mycophenolate mofetil, and cyclophosphamide, in this population are too great to justify their use in the absence of a specific histologic diagnosis. An exception to this approach would be the patient in whom selective proteinuria was present at initial evaluation.

Isolated Proteinuria

Isolated nonnephrotic proteinuria of 2 g or less/24 hours without hematuria or pyuria in an otherwise asymptomatic patient is a relatively common clinical problem. Often the proteinuria is first detected during a routine physical examination required for participation in school athletics, during a preemployment examination, or at the time of application for life insurance. Unless such a patient requests a kidney biopsy for purposes of reassurance, it is our policy currently to merely monitor the clinical course of such patients at periodic intervals of 6 months to 1 year. There is little evidence to suggest that these patients will progress to renal failure or that they are candidates for any type of specific medical therapy in the absence of impaired renal function (77,78). If there is any evidence during follow-up of functional deterioration or the development of additional clinical signs or symptoms suggesting the presence of a primary renal disease or kidney involvement secondary to systemic disease, the patient is thoroughly reevaluated and is often advised to undergo a kidney biopsy for diagnosis and possible therapeutic intervention.

In asymptomatic patients who remain nonnephrotic but persistently excrete more than 2 g of protein/24 hours, we advise a biopsy at the time of initial presentation. It is this group of patients who are more likely to have an underlying renal abnormality (77). Some of the more common diagnostic possibilities include diabetes mellitus, early idiopathic membranous glomerulonephritis, FSGS, and IgA nephropathy. Patients with urinary abnormalities such as hyaline and granular casts are even more likely to have an underlying glomerular abnormality (79). Patients with true orthostatic proteinuria follow a benign course and do not require biopsy for diagnosis and management (80).

Hematuria with or without Proteinuria

Asymptomatic hematuria is a frequent presenting complaint, especially in children and young adults. It is important that renal neoplasms, infections, and nonrenal causes of hematuria, such as those occurring secondary to neoplasms and infections of the upper and lower collecting system, be excluded before one considers a renal biopsy. In general, the diagnostic value of a renal biopsy in the setting of idiopathic microscopic hematuria relates directly to the extent of associated clinical and laboratory findings. For example, in a series of 76 pediatric patients with isolated hematuria, Trachtman et al. (81) found that almost three-quarters of all biopsy specimens obtained in patients who had either a first-degree relative with hematuria or a history of at least one episode of gross hematuria were abnormal histologically. Renal biopsy was recommended in these two clinical settings when the hematuria had been present for 6 months or longer. IgA nephropathy and Alport's syndrome were the two most common findings. Schröder et al. (82) performed renal biopsies in 65 children with isolated hematuria of at least a 1-year duration. Of the group, 95% had histologic abnormalities that included IgA nephropathy (16 patients), Alport's syndrome (eight patients), thin glomerular basement membrane (33 patients), and nonspecific mesangial abnormalities (five patients). In a later report, Topham et al. (83) evaluated 165 children and adults with isolated hematuria, using cystourethroscopy and renal biopsy. All had a normal intravenous pyelogram, were normotensive with a normal serum creatinine, and were free of both proteinuria and a urinary tract infection. In this group, 47% had significant histologic findings, including IgA nephropathy in 49 patients, whereas only five abnormalities were identified on cystourethroscopy. Renal biopsy abnormalities were most common among patients under 20 years of age (69%), prompting these investigators to conclude that renal biopsy should replace cystoscopy in younger patients as the next step in evaluation if renal imaging yielded normal results. Furthermore, because renal histologic abnormalities are quite frequent in the clinical setting of isolated hematuria, these investigators recommended kidney biopsy in patients over 45 years of age in whom findings at renal imaging and cystoscopy are normal.

The opportunity to obtain significant glomerular pathology is increased considerably when hematuria is accompanied by proteinuria, with or without abnormal urine sediment that includes red blood cell, granular, hyaline, or white blood cell casts. We believe it is important to establish the histologic diagnosis of the renal lesion in this clinical setting, although admittedly a biopsy is not required to identify the source of hematuria. Primary renal diseases that can be seen include IgA nephropathy, acute or resolving postinfectious glomerulonephritis, MPGN, and an occasional example of interstitial nephritis. Heredofamilial and multisystem diseases that may be seen include Fabry's disease, sickle-cell trait and disease, polyarteritis nodosa, Wegener's granulomatosis, diabetes mellitus, SLE, and Henoch-Schönlein disease. Many of these systemic diseases may be evident on clinical grounds if a careful prebiopsy evaluation can be undertaken and are discussed in the next section.

Systemic Disease

There are many systemic diseases in which the extent of kidney involvement varies considerably. Patients often undergo kidney biopsy for diagnosis and management on the basis of either the frequency or severity of the renal lesion. These diseases include SLE, Henoch-Schönlein purpura, polyarteritis nodosa, Goodpasture's syndrome, Wegener's granulomatosis, and various gammopathies.

In approximately 40% to 50% of all patients with type I insulin-requiring diabetes mellitus and comparable percentages with type II, or adult-onset, diabetes mellitus, renal failure develops during the course of their disease (84,85). The natural history of renal disease in both types of diabetes mellitus has been well studied and is reasonably predictable (85,86); thus, in most patients a renal biopsy is seldom indicated for diagnosis or management. However, a biopsy can be helpful in patients whose course may be complicated by the sudden development of renal failure, proteinuria, or the nephrotic syndrome.

Although the nephrotic syndrome is observed in approximately 10% of all patients with diabetes, its sudden appearance, especially in the young diabetic without previous evidence of functional renal impairment, should not be ascribed automatically to the primary underlying disease. This point is well illustrated by the experience of Urizar et al. (87) who described five young diabetic patients with the nephrotic syndrome in whom the renal disease was not distinguishable histologically from MCNS. The nephrotic syndrome appeared either simultaneously or shortly after the recognized onset of diabetes in three of the children. Treatment with corticosteroids in four patients resulted in a prompt response, with loss of edema, cessation of proteinuria, and normalization of all serum abnormalities. No patient had abnormalities suggestive of diabetic nephropathy. Other investigators have reported similar experiences (88,89).

Other types of renal disease also can be seen in association with diabetes mellitus, often in the clinical setting of the nephrotic syndrome. Couser et al. (90) reported the coexistence of dense deposits within the glomerular and tubular basement membranes, resembling those seen in type 2 MPGN and lesions typical of diabetic nephropathy in a 24-year-old nephrotic man with juvenile-onset diabetes mellitus. Other examples of well-recognized renal diseases that have been reported to occur in patients with diabetes mellitus in either the presence or absence of diabetic nephropathy include acute postinfectious proliferative glomerulonephritis (91,92), crescentic proliferative glomerulonephritis (91), and membranous glomerulonephritis (93–96).

Controversy surrounds the role of renal biopsy in the management of SLE (97). At present it is our practice to biopsy all patients who present with clinical evidence of active lupus nephritis unless a medical contraindication exists. Border (98) has suggested that patients with more than six red blood cells (RBCs)/high-power field, a urine protein excretion greater than 200 mg/24 hours, or an abnormal serum creatinine value are candidates for biopsy. There is no other way to establish the type of renal lesion that is present, and the management of lupus nephritis varies considerably, depending on the specific histologic lesion. Although there is little evidence that mesangiopathic or membranous lupus nephritis benefits from therapeutic intervention (99–101), most nephrologists treat patients who have focal proliferative lupus nephritis with corticosteroids and those with diffuse proliferative lupus nephritis with corticosteroids and cytotoxic agents (101).

The value of renal biopsy in predicting prognosis has been debated. The results of earlier studies suggested that the biopsy classification of lupus nephritis was useful in predicting the clinical course (102,103) and this issue was challenged (104–106) from a prognostic standpoint but reaffirmed subsequently (107–111). Correspondingly, we believe it is important to establish as precise a histologic diagnosis as possible because, in general, patients with diffuse proliferative lupus nephritis with signs of disease activity, such as increased cellularity, segmental necrosis, fibrinoid deposits, and crescents, in the glomeruli, have a poorer prognosis than individuals with mesangiopathic, focal proliferative, or membranous lupus nephritis.

Controversy also exists concerning the value of renal biopsy in patients with clinically silent lupus nephritis. In 1977, Mahajan et al. (112) described 12 patients with diffuse lupus nephritis but without clinical or laboratory evidence of renal involvement at the time of renal biopsy. A later report, in which 10 of the original 12 patients were followed from 5 to 11 years, revealed deterioration of renal function in 3 years, with one death as the result of renal failure (113). All patients received prednisone alone or in combination with azathioprine. These investigators concluded that the prognosis for preservation of renal function appeared better in patients with clinically silent diffuse proliferative nephropathy as opposed to clinically active disease, and recommended biopsy in patients with SLE even in the absence of overt clinical renal involvement (113). Woolf et al. (114) described eight patients ranging in age from 6 to 26 years, with clinically silent lupus nephritis, who on biopsy had a variety of histologic lesions indicative of active renal involvement. Although no consensus exists regarding the use of renal biopsy in patients with SLE who are without clinical evidence of renal involvement, it is our policy currently to withhold biopsy in this group of patients.

Renal biopsy often can aid the clinician in selecting appropriate therapy for the treatment of vasculitis when renal involvement is present. Polyarteritis nodosa and Wegener's granulomatosis require aggressive combination therapy with cyclophosphamide and prednisone (115). The prognostic value of crescents in antiglomerular basement membrane (GBM) disease and other conditions is discussed in later paragraphs and Table 15-1. Other systemic diseases that often exhibit renal involvement and, therefore, can be diagnosed with the aid of a renal biopsy when other diagnostic tests have failed or have not been employed include multiple myeloma, kappa light-chain disease (116), amyloidosis (117), fibrillary glomerulonephritis, and mixed cryoglobulinemia with renal failure (71,118).

Transplant Kidney

Renal biopsy is a valuable diagnostic tool in the management of the transplant recipient. Biopsy of an allograft represents the major clinical exception to avoidance of percutaneous biopsy of a single functioning kidney. Numerous studies confirm the value and relative safety of renal biopsy in this setting (28–35,119,120). Biopsy is the most accurate means of determining

TABLE 15-1

RENAL SURVIVAL IN ANTI-GBM DISEASE

	A&W (%)	Renal failure[a] (%)
Percentage crescents		
<85%	48 (81%)	13 (18%)
≥85%	11 (19%)	61 (82%)
Total patients (133)	59	74
Effect of azotemia		
Serum creatinine (mg/dL)		
<6.79	61 (90%)	20 (23%)
≥6.79	7 (10%)	67 (77%)
Total patients (155)	68	87

[a] A&W, alive and well with independent renal function. The renal failure group includes a minority of patients who did not survive. From: Stave GM, Croker BP. Thrombotic microangiopathy in antiglomerular basement membrane glomerulonephritis. *Arch Pathol* 1984;108:747; Johnson JP, Moore J, Austin III HA, et al. Therapy of anti-glomerular basement membrane antibody disease: analysis of prognostic significance of clinical, pathologic and treatment factors. *Medicine* 1985;64:219; Walker RG, Scheinkestel C, Becker GJ, et al. Clinical and morphological aspects of the managment of crescentic anti-glomerular basement membrane antibody (Anti-GBM) Nephritis/Goodpasture's Syndrome. *Quart J Med* 1985;213:75; Briggs WA, Johnson JP, Teichman S, et al. Antiglomerular basement membrane antibody-mediated glomerulonephritis and Goodpasture's syndrome. *Medicine* 1979;58:348; Turner AN, Rees AJ, Pusey CD, et al. *Rapidly progressive glomerulonephritis.* Oxford: Oxford University Press, 1998:5; Herody M, Bobrie G, Couarin C, et al. Anti-GBM disease: predictive value of clinical, histological and serological data. *Clin Nephrol* 1993;40:249; Simpson IJ, Doak PB, Williams LC, et al. Plasma exchange in Goodpasture's syndrome. *Am J Nephrol* 1982;2:301.

the presence of lesions, such as cellular or humoral rejection, ATN, drug-induced or viral (esp BK virus) interstitial nephritis, hemorrhagic infarction, cyclosporine toxicity, and *de novo* or recurrent glomerulonephritis in the allograft. As discussed in an earlier section, the FNAB technique also is useful in identifying the cause of acute renal failure in the renal allograft, except when owing to the presence of extensive vascular lesions (121). There are several clinical settings in which a biopsy of the allograft is often indicated. These include failure of the graft to function within the initial 7 to 10 days (122) after surgery, rapid deterioration in function of unknown etiology after initial good function, absence of a response to adequate antirejection therapy within a reasonable period of time, and unexplained nephrotic syndrome or nephrotic-range proteinuria.

A large number of cadaveric kidneys are engrafted and ischemia-reperfusion injury is a frequent complication. Failure to achieve improved renal function within 7 to 10 days after surgery raises the possibility of a more severe form of renal injury, such as infarction, possible cyclosporine nephrotoxicity, or a superimposed episode of acute rejection. A biopsy is often invaluable in determining the etiology of the renal failure, guiding subsequent therapy, and establishing prognosis. For example, Kiaer et al. (123) reported a 100% graft loss when infarction, capillary thrombosis, and arterial or arteriolar thrombosis were found either singly or in combination on biopsy. Thus, the presence of these lesions in the clinical setting of acute renal failure would obviate the necessity for continued use of antirejection therapy.

The incidence of acute rejection characterized by a sudden decrease in renal function, graft tenderness, and fever is greatest during the first 6 months after transplantation. In most instances the suspicion of acute rejection can be made on clinical grounds. However, acute rejection can occur in the absence of certain of the more obvious clinical features, especially because of the widespread use of cyclosporine and newer therapies, or a patient who was believed to have acute rejection may not respond to a reasonable course of antirejection therapy. It may be desired to tailor the antirejection therapy to vascular versus tubulointerstitial rejection or antibody versus cellular rejection. Biopsy can be extremely helpful at this juncture in the patient's therapy. Other complications, such as ATN; a drug-induced nephrotoxicity, such as cyclosporine; or overt renal infarction may be diagnosed.

The occurrence of the nephrotic syndrome or nephrotic-range proteinuria in a transplant recipient suggests the possibility of recurrent or *de novo* glomerulonephritis (124,125). Those forms of disease that are most likely to recur in the transplant kidney include MPGN, FSGS, diabetic nephropathy, and IgA nephropathy (124,125). To date the most common *de novo* disease reported is membranous glomerulonephritis (124). Although some would take exception, we believe it is worthwhile to establish the lesion responsible for proteinuria, especially if the proteinuria is associated with a decrease in renal function.

CONTRAINDICATIONS

Both the relative and the absolute contraindications for renal biopsy vary among nephrologists. However, most would agree that the potential for increased risk occurs in the presence of severe uncontrolled hypertension, sepsis, known or suspected renal parenchymal infection, a hemorrhagic diathesis, a solitary ectopic or horseshoe kidney (except in the case of a transplanted kidney), or when the patient is unable to cooperate during the procedure.

In 1958, Kark et al. (126) published the results from their initial 500 percutaneous renal biopsies and listed 11 contraindications. These included an uncooperative patient, large cysts, a renal neoplasm, renal artery aneurysm, marked calcific arteriosclerosis, a hemorrhagic diathesis, a single kidney, a perinephric abscess, hydronephrosis or pyonephrosis, a terminal state of illness, and a rising blood nonprotein nitrogen level greater than 100 mg/dL. Hypertension was viewed as a relative contraindication, depending on the importance of the biopsy and the skill of the operator.

Certainly, the presence of a single kidney (except a renal allograft), including a horseshoe kidney, sepsis, or a hemorrhagic diathesis, remains an important contraindication to percutaneous renal biopsy (127). However, in some cases, an open or laproscopic biopsy may well be justified if the clinical situation warrants the risk. This also holds true for the patient with a renal artery aneurysm or calcific arteriosclerosis and an undiagnosed parenchymal renal disease. Many times a coagulation disorder can be corrected, thus allowing the biopsy to be performed. In most clinical situations there is little or no reason to perform a biopsy if the patient has large multiple cysts, a renal neoplasm, or a terminal illness. The same is true in the presence of a perinephric abscess, acute pyelonephritis, hydronephrosis, or pyonephrosis. Today, a rising blood urea nitrogen (BUN) or a BUN greater than 100 mg/dL is not considered a contraindication if the rise is sudden or unexplained and quite likely owing to an acute and potentially reversible process, as discussed earlier in this chapter. The presence of normal-sized or large kidneys increases the likelihood that an acute rather than a chronic form of renal failure is present.

When a patient is hypertensive, we delay the biopsy until the blood pressure is brought under adequate control. Thus, the presence of hypertension should be considered at most a relative contraindication. It is important that blood pressure control is obtained because it is well documented that hypertensive patients are more prone to bleed after a percutaneous renal biopsy (25,26). Diaz-Buxo and Donadio (25) found not

only a significantly greater incidence of complications in hypertensive patients (11.6%) as compared with normotensive subjects (7.1%) undergoing percutaneous renal biopsy, but the higher incidence also correlated positively with both the severity and duration of the hypertension.

GROSS INSPECTION AND TISSUE PROCESSING

Evaluation of a kidney biopsy includes both gross and histologic examination of the specimen. Standard histologic examination includes light microscopy, immunohistology, and transmission electron microscopy. Other less frequently used techniques include scanning electron microscopy, microbiologic cultures, tissue and cell cultures, quantitative or qualitative chemical analyses, enzyme assays, and molecular pathology. Most of the remainder of this chapter is concerned with preparation, histologic examination, and actual evaluation of the biopsy specimen for clinicopathologic interpretation.

Gross Examination

The general purpose of gross examination is to determine adequacy and divide the specimen into appropriate portions for subsequent processing. The overall dimensions, color, and consistency should be noted. In particular, the area of viable cortex should be identified and delineated from the medulla, which is generally paler. Areas of infarction, other necrosis, or pyogenic inflammation that are often pale and highlighted by a hyperemic border, may be evident by gross examination. Ischemia with reflow also may be hyperemic throughout. In general, if the specimen contains both cortex and medulla, the medulla is the deeper tissue as it is removed from the needle, although there are several exceptions. If the needle is thrust deeply into the kidney before the core is taken, the cortex may be missed altogether, or if the direction of the needle is obtuse to the pelvis, the needle may pick up medulla first and then cortex as it passes completely through the medulla. We have observed specimens that contain cortex, medulla, and then more cortex. Chronic disease will make it more difficult to delineate the cortex.

Allografts have additional notable characteristics. First, in older grafts a thick "rind" of fibrous tissue surrounds the graft. This area may be quite pale and should not be confused with cortex. In newer grafts, the surface may be deeply colored from hemorrhage or the presence of granulation tissue. Second, the outermost rim of cortex may be pale from ischemic atrophy or necrosis. The deeper cortex is of interest for the diagnosis of additional disease. Many laboratories employ a dissecting microscope or hand lens to identify glomeruli in the cortex and guide division of the specimen at the time of the biopsy.

Processing for Histologic Examination

Despite considerable effort it has not been possible to develop a single method of tissue fixation and processing that is optimal for light, immunofluorescence, and electron microscopy. Therefore, it is customary to divide the cortical portion of the biopsy into three parts (Fig. 15-1). We prefer to divide the tissue core along its short axis, as illustrated, to minimize tissue damage. For longer cores the largest part is taken for light microscopy, a smaller portion for immunofluorescence microscopy, and the smallest portion for electron microscopy. The exact proportions of this division are variable and depend on the total amount of cortex and the clinical setting (e.g., native vs. allograft biopsy). The basic consideration in division of

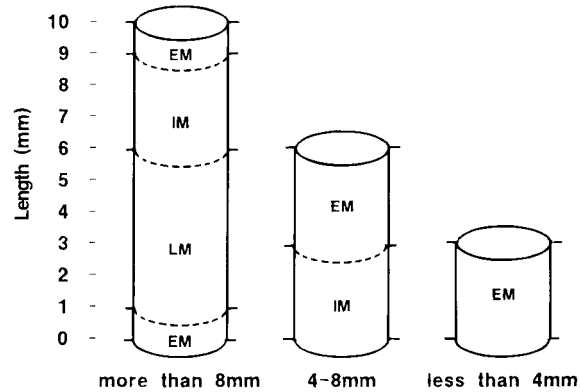

FIGURE 15-1. The methods of dividing cores of native cortical renal tissue obtained by percutaneous biopsy based on sample size. *EM*, electron microscopy; *IM*, immunofluorescence microscopy; *LM*, light microscopy.

the specimen depends on the definition of an adequate sample for each type of histologic examination. Six glomeruli generally are considered an adequate number in a native kidney for light microscopic evaluation. However, in exceptional circumstances the limits are broad. For example, in the evaluation of a patient with the nephrotic syndrome, a disease that is diffuse and generalized such as uncomplicated idiopathic membranous glomerulonephritis may be diagnosed with a single glomerulus. On the other hand, in the early stages of a focal proliferative disease or with the variable pattern of involvement often observed in lupus nephritis, the diagnosis may not be appreciated with six or more glomeruli. We prefer a core of tissue sufficient in length to provide 12 glomeruli for light microscopic examination. The extent or degree of severity of chronic atrophy also is determined best on larger specimens and is especially important for assessment of permanent nephron loss.

The factors that determine the adequacy of a sample for immunohistology are somewhat different. The principal role of immunohistochemistry is to evaluate the type, location, and distribution of serum proteins, particularly those commonly identified in immune complexes or directed against a specific antigen, such as is seen in anti-GBM disease. The biology of these immune diseases is such that the distribution of immune mediators is more diffuse and generalized, even though the light microscopic pattern may be focal and segmental; therefore, sections containing four to six glomeruli usually are adequate.

Electron microscopy is most useful in diffuse and generalized diseases. It is preferable to examine two or three glomeruli as well as tubules and small vessels whenever possible. After the viable cortex has been divided, any remaining tissue such as medulla is also processed for light microscopy.

When only a few millimeters of cortex are obtained, we process the entire specimen for electron microscopy. During the examination for electron microscopy the tissue also is evaluated by light microscopy; therefore, the maximum amount of information can be obtained. In addition, electron microscopic processing is technically most suited for handling small pieces of tissue, and the overall preservation of the tissue is much better. If the core of cortex is 4 to 8 mm in length, we process equal parts for electron microscopy and immunofluorescence microscopy (Fig. 15-1).

Two tissue cores are recommended for allograft biopsies because of the variable nature of rejection (128). We also modify the division of tissue from transplant biopsies. This is done to expedite the diagnosis when rejection is suspected. Approximately half the cortex is submitted for frozen sections. Most of the remainder is processed for routine light microscopy. Small

(1-mm) portions are saved for electron microscopy. Frozen sections are taken immediately for light microscopy stains, hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS), and immunohistology microscopy. These procedures can be completed within 1 to 2 hours. Rejection can be determined from the frozen sections in many patients. The final evaluation has to wait for routine processing in subtle or complex situations, but rapid processing produces permanent sections in 4 hours.

There is diversity of opinion regarding optimal tissue fixation for light microscopy. In part, this is because several fixatives are available that yield generally acceptable results. Fixative for use in general pathology has a formaldehyde base; a 4% solution of formaldehyde in neutral phosphate buffer is acceptable in most situations. Other common fixatives include Zenker's, Van de Grift's, Helly's, and Bouin's solutions (129,130). Probably the most important consideration for someone beginning work in this area is to select one of the generally accepted fixatives and continue with its use. Variations in fixation and other processing steps make less and less difference with time and experience.

There are circumstances when standard fixation and tissue processing should be supplemented with special handling of the tissue. Urate, uric acid, other water-soluble crystals, and glycogen may be dissolved from the tissue during processing in aqueous solutions. Ethanol is the fixative of choice for preservation when the presence of urates and uric acid is suspected. Lipids are extracted from the tissue during the later stages of processing for routine paraffin sections; therefore, frozen sections are preferable for the demonstration of lipids. Some fixatives degrade antibody binding and nucleic acid hybridization to tissue or tissue extracts; therefore, processing must be appropriate for these tests. Because special handling is required in few cases, it is important to have a high index of suspicion when these situations arise and to alert the pathologist and the laboratory before the biopsy is actually performed.

After fixation, the tissue is dehydrated and embedded using one of several techniques. We prefer wax embedding because it is automated and permits the use of the greatest variety of special stains. We normally prepare seven slides with 2-μm–thick sections. The first, fourth, and seventh slides are stained with H&E, the second and fifth with PAS, and the third and sixth with periodic acid-methenamine silver (PAMS) (130). Additional stains, such as Congo red, are used as necessary. Some laboratories use plastic materials for embedding, which produce very clean, thin, crisp sections that also can be stained with the H&E, PAS, and PAMS procedures. Specimens processed in this manner require separate handling and, in our experience, additional special stains often demonstrate poor contrast.

Many types of fixatives are available for electron microscopy, and several are acceptable for the evaluation of a kidney biopsy within certain limits. We recommend either of two initial fixatives for electron microscopy. The first is buffered formaldehyde. In its early use, "formalin" solution was maligned as a fixative for electron microscopy because of poor tissue preservation. This was not because formaldehyde is actually a poor fixative, but because formaldehyde produces a highly acidic solution in water and the increased acidity produces many artifacts. Buffered formaldehyde (pH 7) is a good fixative, inexpensive, and readily available (129). It has a long shelf life, and tissue can remain in the fixative for months if necessary before additional processing.

Glutaraldehyde is employed commonly to preserve kidney biopsy specimens for electron microscopy and is our choice as a primary fixative for ultrastructural preservation, although it may be less readily available in routine histology laboratories. Regardless of the fixative, it is important that tissue to be processed for electron microscopy is divided into pieces less than 1 mm in any dimension to ensure good penetration of the fixative and all other solutions used in subsequent processing. This is not a problem with needle biopsies, but wedge biopsies must be divided accordingly. After primary fixation, the tissue is ready for additional processing, which should be performed in a dedicated electron microscopy laboratory (129).

Tissue for immunohistology can be handled in several ways. For frozen sections, the tissue is placed between gauze sponges, moistened with saline and taken directly to the laboratory. It is important that the tissue not be allowed to float in saline, because tissue specimens left in aqueous solutions absorb water, which distorts the architecture. Ideally, transit time to the laboratory should be less than 30 minutes. If transport is delayed, the tissue should be kept on ice, but not frozen. Alternatively, a second method can be employed. Michel et al. (131) developed a holding solution composed of buffered ammonium sulfate and *N*-ethylmaleimide, which is used at room temperature for preservation of biopsy specimens for immunofluorescence microscopy. The original solution has been modified slightly and is even more broadly applicable than described originally (132). Michel's medium remains valuable for holding tissue at room temperature or shipping kidney specimens without refrigeration provided certain guidelines are followed. First, the tissue pieces should be 2 mm or less in thickness. Second, the tissue should not be kept in the solution for longer than 1 week before it is rinsed and frozen as described for fresh tissue. We block the tissue in gelatin, as described by Burkholder et al. (133), or routine cryomicrotomy solution after which it is "snap" frozen in isopentane or Freon cooled with liquid nitrogen or an electrical refrigeration unit, or in a slurry of dry ice and acetone. Rapid freezing is important to reduce the formation of large ice crystals, because these result in tissue distortion and sectioning artifacts. Tissue stored frozen before and after sectioning should be protected to prevent desiccation and denaturation artifacts.

Frozen sections are cut and stained according to any of several immunohistologic procedures. Direct immunofluorescence staining for serum proteins with fluorescinated heteroantisera or monoclonal antibodies remains the standard procedure. The usual battery of reactants includes antibodies to IgG, IgM, IgA, C1q, C3, and albumin. With the appropriate interpretation, this panel of antisera allows the successful identification of most clinical diseases. Staining for selected amyloid proteins and κ and λ light chains is helpful or necessary in many adult cases. A variety of other antigens have been used in special or experimental situations; however, most are not necessary in everyday clinical practice. After staining, the slides are coverslipped with buffered glycerol at pH 8.2 in preparation for viewing. A fluorescence microscope equipped with epifluorescence is convenient to use and should be outfitted with adequate illumination, a primary interference filter, and an appropriate secondary filter.

A number of methods employing other fixatives or embedding procedures for the immunohistochemical demonstration of serum proteins in kidney biopsies have been described, including the use of wax sections. Some of these alternative procedures are unreliable, but others (134) are suitable for the demonstration of antibodies and some complement proteins in paraffin sections.

Several other tests are required occasionally that can be performed only on frozen sections. These include neutral fat stains and most enzyme histochemistry. The use of immunohistochemistry, combined with a host of specific monoclonal antibodies, has produced a highly specific and sensitive system for the identification of cell and tissue antigens. The impact of this methodology has been most noticeable in our ability to identify lymphohistiocytic cell infiltrates and classify cellular immune responses in the kidney such as: cellular rejection,

interstitial nephritis, and posttransplant lymphoproliferative disease (PTLD). We have found that the "avidin biotin complex" (ABC) procedure yields the best combination of sensitivity, specificity, quality control, and time for completion of the test (135). There are excellent monoclonal antibodies available commercially for the identification of B cells, T-cell subsets, and monocytes. Many of these may be used in tissue following fixation in buffered formalin.

Immunoperoxidase staining of frozen sections can also be used in addition to or instead of immunofluorescence staining. The advantage of immunoperoxidase techniques over immunofluorescence microscopy is the greater sensitivity and permanence of the staining when diaminobenzidine is used as the substrate for color development. The disadvantages of immunoenzyme staining for immunoglobulin localization include the increase in preparation time and the added expense. Endogenous peroxidase and endogenous biotin may produce a bothersome high background and should be blocked (136).

Molecular Biology

In situ hybridization has been a powerful tool for a host of investigational studies (137,138). A variety of molecular probes have been used to study gene expression at the level of messenger ribonucleic acid (mRNA) (139–141) and for the detection of viral sequences (142,143). For example, Epstein-Barr virus (EBV) probes may be useful in the diagnosis of PTLD. Core and FNAB specimens also can be used as a source of DNA or RNA for extraction and nucleic acid blotting or for polymerase chain reaction (PCR)-based techniques (142,144–148). Although great potential for molecular diagnosis of renal disease by microarray analysis and other techniques is near at hand, there are a few standard applications to clinical renal biopsy diagnosis at the present time (149).

HISTOLOGIC EVALUATION

Light Microscopy

The purpose of this section is to present a systematic approach to the histologic interpretation of a kidney biopsy. The discussion also includes the role of several special stains in biopsy diagnosis. The evaluation should begin with a review of all tissue present on the light microscopic sections at a relatively low magnification to assess the adequacy of the specimen and identify any major abnormalities. This is followed by a systematic evaluation of the glomeruli, tubules, interstitium, and vasculature. It is preferable to establish the histopathologic findings before the clinical history is known in order to avoid bias in the final biopsy interpretation. Nevertheless, the biopsy findings must be ultimately reconciled with the clinical presentation: course and prognosis.

It is important that certain terms be carefully defined before continuing. There are four principal definitions that have evolved largely from light microscopic evaluations of kidney biopsies to describe glomerular disease. *Focal* denotes a process in which only some of the glomeruli are altered histologically. The majority of glomeruli are spared. *Generalized* indicates the majority of glomeruli on the biopsy are altered by some process, for instance, proliferation or sclerosis. A local, or *segmental,* lesion is one in which only a portion of a glomerulus exhibits an alteration. A segmental sclerotic process involves only a portion of a glomerulus. The opposite of a segmental process is one that is *global* in nature and generally affects the entire glomerulus. The term *diffuse* has been used ambigiously in the literature. Sometimes it has meant global and other times it has meant generalized; therefore, care must be taken when interpreting this term. Glomerular lesions are generally focal and segmental or generalized and global, but important exceptions exist (e.g., generalized and segmental).

The initial evaluation of the specimen is intended to determine the specific regions of the renal parenchyma that are present on the section, which might include the cortex and the outer and inner medulla. If only cortex is seen, the presence of the renal capsule can aid in orientation of the specimen. The glomeruli should be counted to provide a rough estimate of the sample size. This is generally accomplished best with the PAS-stained sections, in which the glomeruli (including those that are globally sclerotic) can be identified readily. All tissue on each slide should be evaluated because certain features may not be present in all sections. Next, the overall condition of the renal architecture is evaluated to differentiate between chronic or irreversible nephron loss and acute reversible nephron damage. The type, distribution, and intensity of cellular infiltration are accurately established on the H&E and PAS sections. The PAS and PAMS stains are well suited to evaluate the degree of interstitial fibrosis, tubular atrophy, and glomerulosclerosis. In general, the degree of tubular and interstitial injury relates to the reduction in creatinine clearance when the sample size is adequate and the process producing the injury is uniform. The latter feature is important because, with approximately 1 million nephrons in each kidney, a needle biopsy specimen that contains 10 glomeruli only provides a sample of one in 100,000. We employ a simple procedure to estimate the extent of chronic nephron loss in a biopsy. Using a PAS-stained section, the total number of glomeruli is counted and the percentage that is sclerotic is determined. Sclerotic glomeruli generally are shrunken in appearance because of the complete collapse of the capillary bed and the paucity of cells. In a study of chronic glomerulonephritis, we have shown that global glomerular sclerosis of up to 50%, is associated with maintenance of the serum creatinine near the normal range (150). However, an increase in glomerular sclerosis of the global type above 50% to 60% is associated with increases in the serum creatinine. This estimate of chronic nephron loss is based on an interpretation of the intact nephron hypothesis (151). When one portion of the nephron is lost to disease or injury, the remainder of the nephron undergoes atrophy. Thus, the accompanying tubule undergoes atrophy in the presence of global sclerosis. The converse also is true. Tubular atrophy is characterized by a decrease in the outer diameter of the tubule, thickening and wrinkling of the tubular basement membrane, simplification and a decrease in thickness of the tubular epithelium, and an increase in the interstitial connective tissue that surrounds the tubule. These changes all lead to disruption of the normal architecture of the renal parenchyma. As some nephrons are destroyed or atrophy, the remaining nephrons may undergo hypertrophy, showing tubules with larger diameters and cells with increased cytoplasm. This picture of atrophy and hypertrophy is visualized in Figure 15-2. Hyperfiltration in this setting is associated with glomerular changes of increased size and mesangial matrix and segmental sclerosis (152–156). These features are the harbingers of the point of no return or continued deterioration in renal function (157). The normal architecture also can be disrupted by more acute changes such as edema and inflammation. In this setting, there is separation without evidence of atrophy of the renal tubules. Depending on the etiology of the injury, these latter changes may be reversible, as seen in ATN, or occasionally they may progress to loss of the entire nephron.

The next step in interpretation of the biopsy is a detailed evaluation of the glomeruli, which includes a careful assessment of the various structures that comprise the glomerulus or renal corpuscle. The glomerulus is composed of the visceral epithelial cells, endothelial cells, and mesangial cells with the

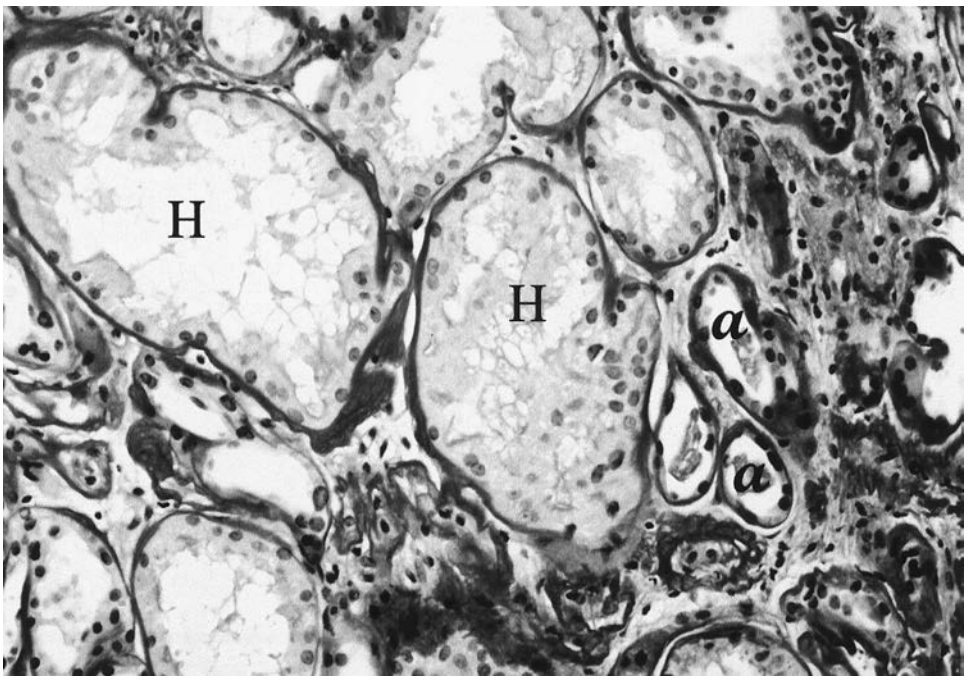

FIGURE 15-2. This figure shows shrunken atrophic tubules (*right side, a*) and hypertrophic tubules (*left and center of field, H*) (PAS, magnification ×210).

mesangial matrix and the basement membranes of the capillary loops, Bowman's capsule and the overlying parietal epithelial cells, the extra glomerular mesangium, and the afferent and efferent arterioles (Fig. 15-3). The first assessment is a determination of the overall glomerular cellularity, which is evaluated using H&E-stained sections (Fig. 15-4A). The cell type should be established if the glomeruli are hypercellular. This includes an estimate of the number of cells normally present in the glomerulus, including the mesangial, endothelial, visceral epithelial, and parietal epithelial cells; and the inflammatory cells that migrate into the glomerulus, including neutrophils, lymphocytes, and monocytes (Fig. 15-5). Most pathologists rely

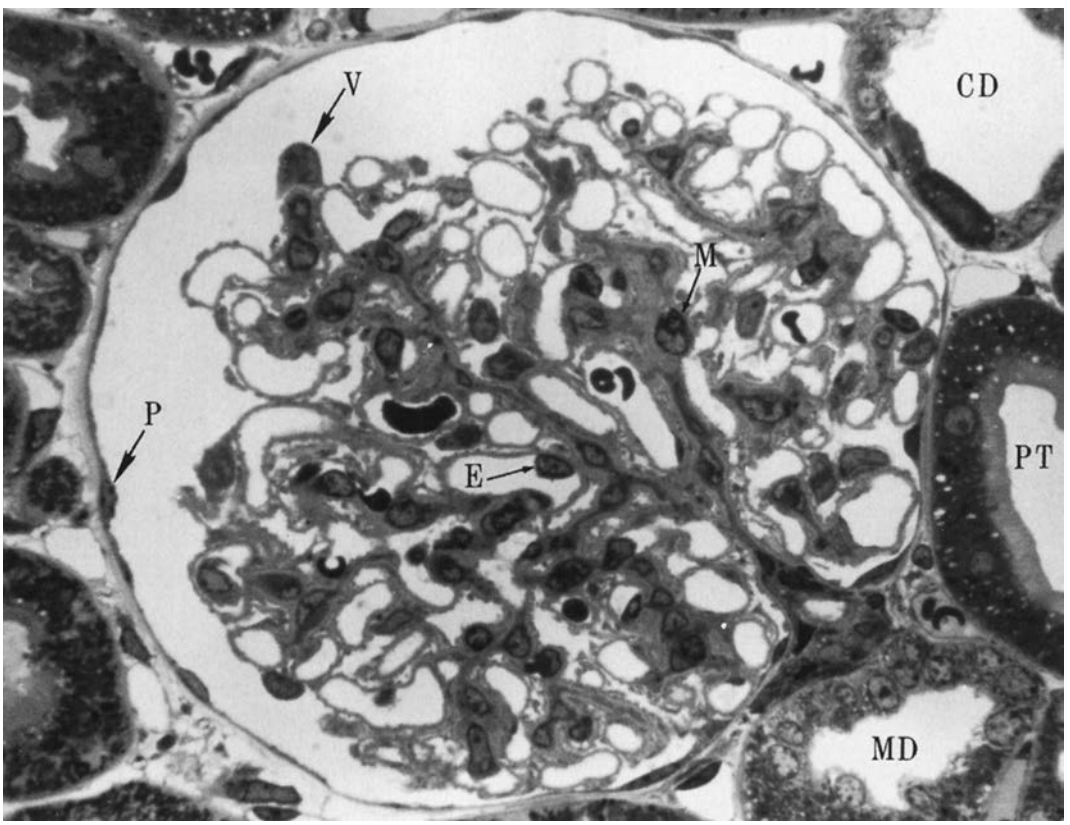

FIGURE 15-3. Photomicrograph of glomerulus (renal corpuscle) depicting major cell types and anatomic regions. *V,* visceral epithelial cell; *M,* mesangial cell; *P,* parietal epithelial cell; *E,* endothelial cell; *MD,* macula densa; *CD,* collecting duct; *PT,* proximal tubule (magnification ×750). (From: Brenner BM, Rector FC Jr, eds. *The kidney,* 3rd ed. Philadelphia: Saunders, 1986, with permission.)

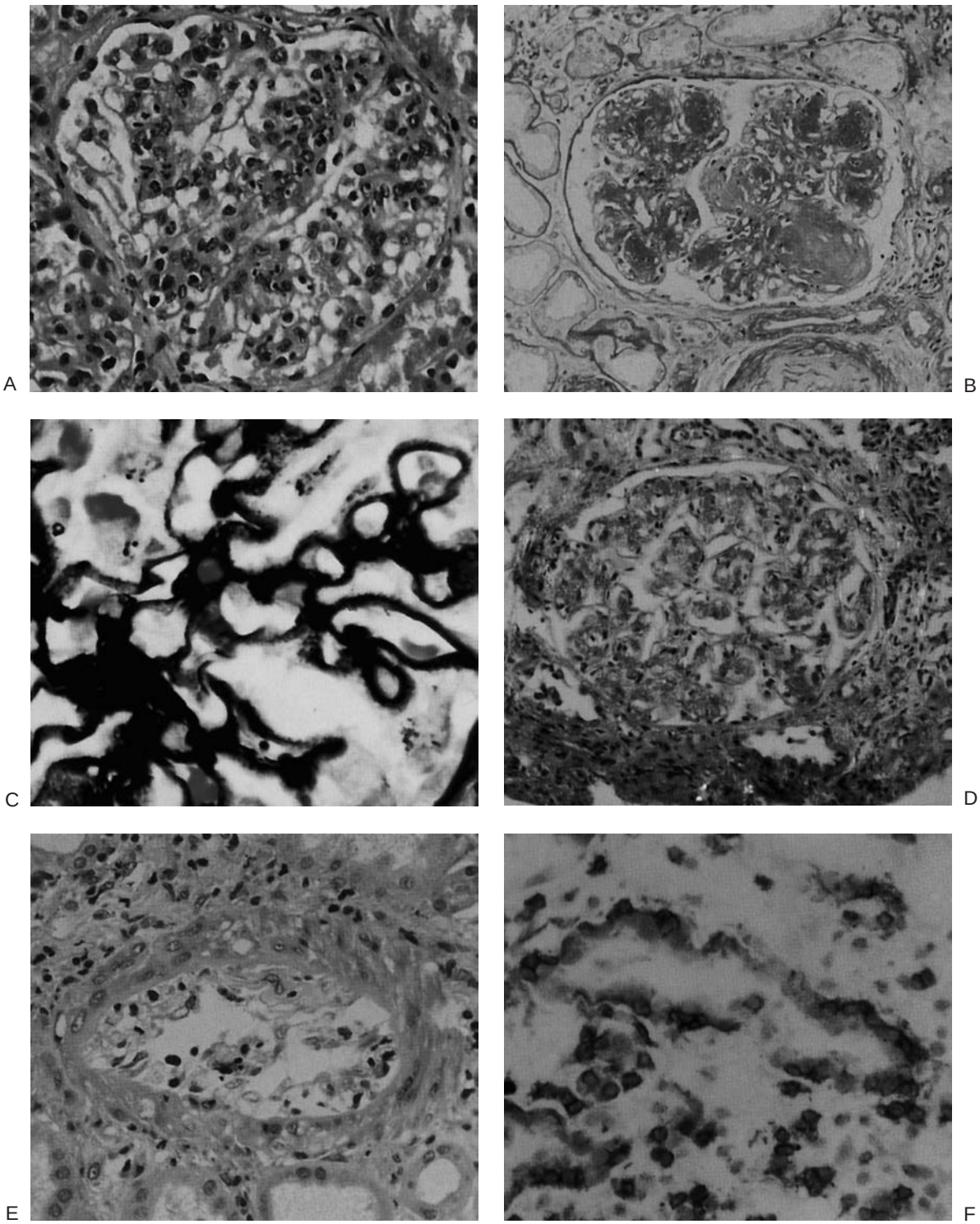

FIGURE 15-4. A: H&E stain of a glomerulus with moderate neutrophilic infiltrate in acute poststreptococcal glomerulonephritis (magnification ×200). **B:** PAS stain of a glomerulus, illustrating early and late nodular mesangial expansion typical of nodular intercapillary glomerulosclerosis of diabetic nephropathy (magnification ×200). **C:** PAMS stain depicting a portion of a capillary tuft with spikelike projections extending outward from the capillary basement membrane. This picture is characteristic of stage II idiopathic membranous glomerulonephritis (magnification ×1,000). **D:** Depiction of the yellow-green birefringence of amyloid when stained with Congo red. Other tissue structures (particularly fibrous tissue) may rotate light and appear white when viewed with the polarizing microscope and should be distinguished from amyloid (Polarization optics; magnification ×200). **E:** This interlobular artery exhibits inflammation primarily in the intima, which is seen commonly in acute vascular rejection. Compare with the immunofluorescence pattern in Figure 15-22F, which demonstrates the transmural nature of the process (H&E; magnification ×400). **F:** Photomicrograph of immunoperoxidase preparation using LEU 2A antibody, an antibody to cytotoxic T cells, and diaminobenzidine. Several cross-sections of tubules and interstitium are shown from a typical example of acute cellular rejection. The T cells infiltrate both the interstitium and the tubular epithelial cells (magnification ×200). (See Color Plate.)

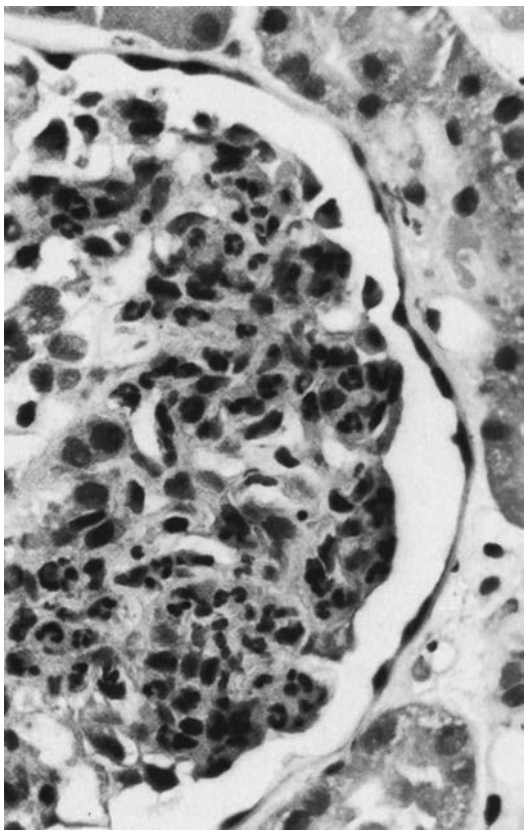

FIGURE 15-5. Photomicrograph of glomerulus illustrating large numbers of neutrophils and monomuclear cells within peripheral capillary loops in biopsy from a patient with poststreptococcal glomerulonephritis (H&E; magnification ×560).

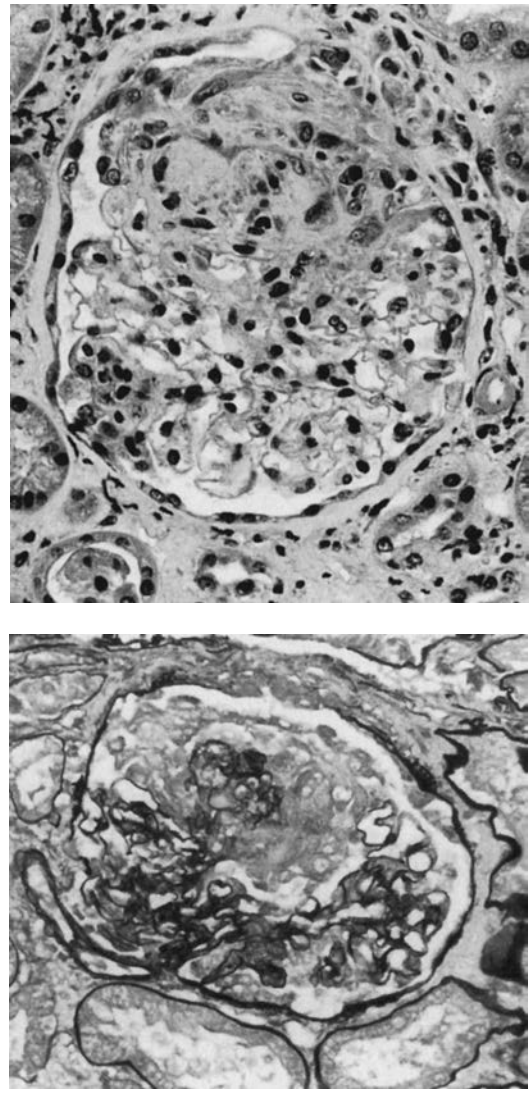

FIGURE 15-6. Photomicrographs demonstrating segmental necrosis of glomerular tuft in a biopsy from a 60-year-old man with a clinical diagnosis of Wegener's granulomatosis (**A:** H&E stain; ×340. **B:** PAMS stain; ×300).

principally on a qualitative assessment of the cellular composition in sections 2- to 3-μm–thick stained with H&E. There are several guidelines for this assessment. Under normal conditions, a typical cross-section through the mesangium contains one to three mesangial cell nuclei (158). An entire glomerulus may contain one to two neutrophils, but more than two is abnormal. There are also a small number (1%) of mononuclear cells (monocytes) in the normal glomerular mesangium (159), but these cells cannot be identified with certainty on routine H&E sections. Similarly, it may not be possible to distinguish monocytes from large lymphocytes or even epithelial cells without immunohistochemistry or electron microscopy. Some cells within the glomerulus may contain inclusions, inspissated material, or numerous vacuoles, such as those that are present in foam cells.

Next, the glomerular capillaries are examined to determine whether they are patent, collapsed, or obstructed with fibrin, platelets, or cells. The PAS and PAMS stains are important in the evaluation of the capillary walls (Figs. 15-4B and C). Both stain normal as well as abnormally thickened basement membrane. If the basement membrane is thickened, it is important to establish the nature of the changes. Both the epithelial and endothelial surfaces are smooth under normal conditions. If one surface is shaggy or irregular or exhibits projections, often referred to as "spikes" when present on the epithelial surface, it is distinctly abnormal (Fig. 15-4C). Occasionally, both surfaces of the basement membrane are irregular in configuration. In necrotizing glomerulonephritis owing to virtually any cause, the capillary wall, including the basement membrane, may be ruptured, discontinuous, or completely lost (Fig. 15-6). This histologic picture usually is associated with fibrin deposition. Fibrin deposition associated with necrosis should be distinguished from fibrin deposits that distend the capillary in the presence of an intact basement membrane. Larger immune complexes can be seen on Masson or PAS stains. These deposits can also be identified on 1-μm thick plastic sections stained with toluidine blue (160) that are cut from tissue that has been prepared for electron microscopy (Fig. 15-7). The capillary (visceral) epithelial cells may exhibit hypertrophy, atrophy, or hyperplasia.

In addition to the mesangial cellularity, there may be an increase in mesangial matrix material with or without an increase in cells. This is evaluated best with the PAS and PAMS stains (Fig. 15-4B). Mesangial expansion may be global and involve the entire glomerular tuft; it may be segmental (Fig. 15-8); or it may involve only the stalk region, which is designated the extraglomerular mesangium and forms part of the juxtaglomerular apparatus. Mesangial matrix expansion may extend into the capillary or be observed in association with subendothelial capillary basement membrane thickening, called mesangial interposition.

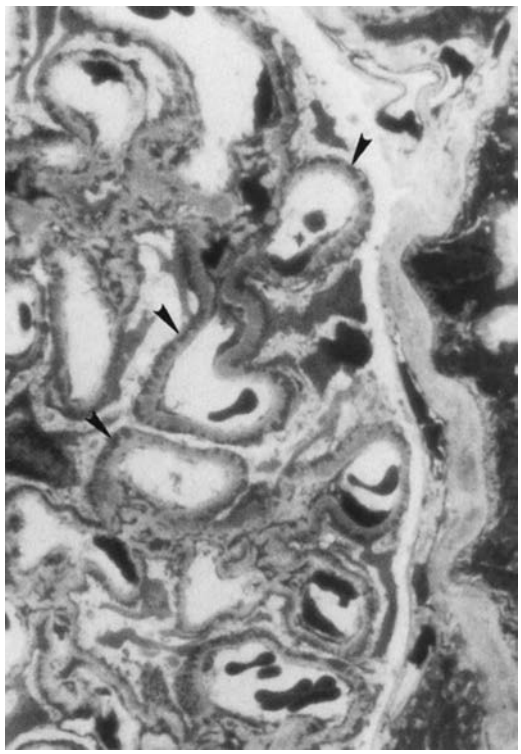

FIGURE 15-7. Micrograph of Epon section stained with toluidine blue, illustrating subepithelial immune complexes (*arrowheads*) along capillary basement membranes in a patient with idiopathic membranous glomerulonephritis (magnification ×1,050).

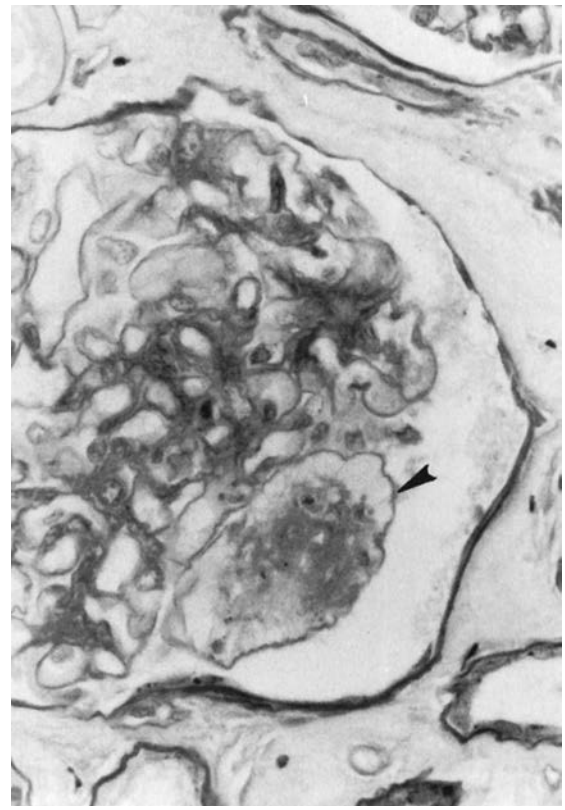

FIGURE 15-9. Light micrograph depicting mesangial lysis (*arrowhead*) in a glomerular tuft (PAS; magnification ×500).

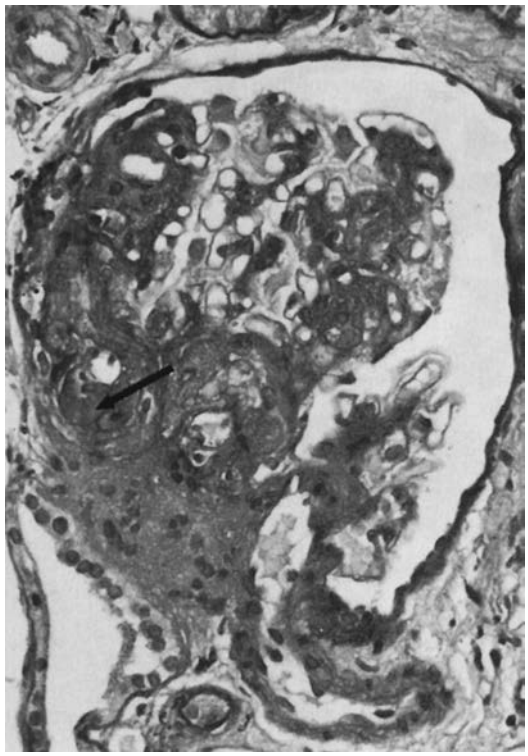

FIGURE 15-8. Glomerulus from a patient with focal segmental glomerular sclerosis, illustrating segmental sclerosis and hyalinosis (*arrow*) (PAS; magnification ×360). (From: Newman WJ, Tisher CC, McCoy RC, et al. Focal glomerular sclerosis: contrasting clinical patterns in children and adults. *Medicine* 1976:55:67, with permission.)

Mesangiolysis is an alteration of the mesangium that is characteristic of thrombotic microangiopathies, but can be observed in diabetes and other conditions (161). The lesion appears as a relaxation or disruption of the attachment of the capillary basement membrane to the mesangium. The basement membrane balloons outward in early lesions. The potential space is filled with disrupted mesangial matrix material, fibrin or platelet thrombi, fragmented erythrocytes, and other material (Fig. 15-9). Eventually, the mesangiolytic lesions sclerose and sclerosis is associated with worsening renal function.

The basement membrane of Bowman's capsule is thicker than the capillary basement membrane in the normal state and, like the capillary basement membrane, may increase in thickness with injury. The parietal epithelium lining Bowman's capsule can respond to injury with hypertrophy or hyperplasia or exhibit infiltration with monocytes, resulting in the formation of crescents (162).

The arterioles of the juxtaglomerular apparatus may be markedly abnormal because of the presence of glassy eosinophilic hyaline material (Fig. 15-10), edema, fibrin, other inflammatory changes, hypertrophy, or sclerosis. They may contain fibrin thrombi (Fig. 15-11) or exhibit segmental fibrinoid necrosis. The pericytes surrounding the arterioles should be examined carefully for evidence of hypercellularity and increased granularity. The PAMS stain is used to screen for the latter feature.

The tubules then are examined in detail. The proximal tubule epithelium should be tall, columnar, and possess a PAS-positive brush border and display a deeply eosinophilic cytoplasm. Acute signs of ischemic or toxic injury include swelling of the cell cytoplasm and disruption of the brush border. Cytoplasmic vacuolization is followed by cell necrosis. Later stages

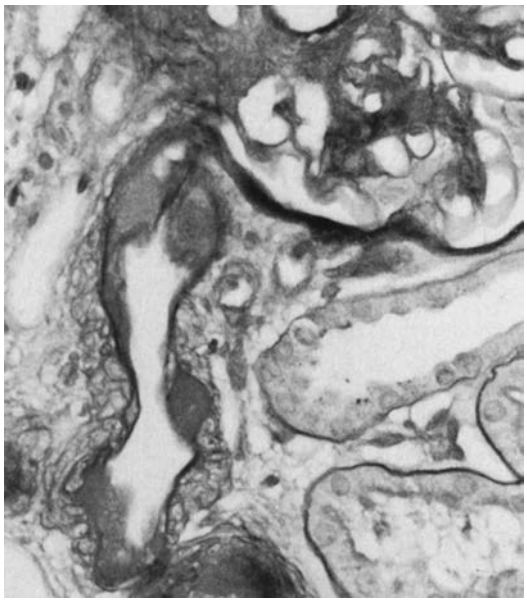

FIGURE 15-10. Arteriole with marked subintimal hyalin arteriolosclerosis from a patient with diabetic nephropathy (PAS; magnification ×460). (From: Suki WN, Eknoyan G, eds. *The kidney in systemic disease,* 2nd ed. New York: Wiley-Liss, 1981, with permission.)

include flattening of the remaining cells with irregular staining of nuclei, and evidence of early regeneration (Fig. 15-12). Apoptosis (163,164) follows a variety of stimuli, including cell-mediated cytotoxicity (165–167) with pyknosis of the nucleus, condensation of the cytoplasm, and extrusion of tubular cells into the lumen. Characteristic PAS-positive droplets that represent lysosomes are present in increased numbers with proteinuria. Additional findings include lipid droplets and cytoplasmic vacuoles. Large irregular vacuoles are seen with severe hypokalemia (Fig. 15-13), whereas fine, diffuse cytoplasmic vacuoles are observed with exposure to osmotic agents, such as mannitol (Fig. 15-14). Vacuoles also are observed in cyclosporine toxicity (Fig. 15-15). Inflammatory cells may infiltrate the tubule and, if present, the type of inflammatory cell should be characterized (Fig. 15-4F). The lumen may contain extracellular material, such as casts or white or red blood cells. The basement membrane may be thickened by atrophy or the presence of immune deposits, or it may exhibit breaks.

Normally the tubules are separated by an inconspicuous interstitium, which contains peritubular capillaries and a few

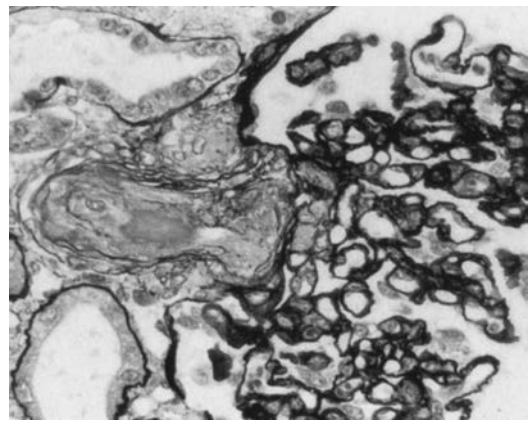

FIGURE 15-11. Fibrin thrombus in afferent arteriole of a kidney biopsy from a 62-year-old woman with acute renal failure and stigmata of a hemolytic-uremic syndrome (PAMS; magnification ×415).

interstitial cells. The interstitium may be abnormally thickened or expanded by a variety of extracellular materials, including collagen, other proteins, crystals, or edema. The type of inflammatory cells should be characterized if inflammation is present. Inflammatory cells may display a specific distribution, such as perivascular or periglomerular, marginated in peritubular capillaries or scattered uniformly throughout the biopsy. Monocytes may aggregate and assume an epithelioid appearance, giving rise to granulomas. Normally, the interstitium is more prominent in the medulla and around the muscular arteries.

Finally, the larger vessels should be examined. The medium-sized renal arteries have a histology typical of arteries elsewhere; however, intrarenal veins have minimal smooth muscle compared to veins of similar caliber in other organs. The most common arterial change is intimal thickening in association with irregular reduplication of the internal elastic lamina, a finding that is seen best with the PAMS stain. The lumen may contain fibrin thrombi, embolized material, or inflammatory cells (Fig. 15-4E). The walls of the arteries also may be thickened as the result of edema, fibrin, other exogenous material, or inflammation. Similar, although less extensive, changes may be observed in the renal veins.

At this point it is useful to compare the relative degree and type of involvement of each of the cortical structures. As discussed in the preceding text, when one portion of the nephron is diseased, the remainder of the nephron eventually is affected. The site of the initial insult usually demonstrates the earliest and most severe changes. Thus, in glomerulonephritis, the

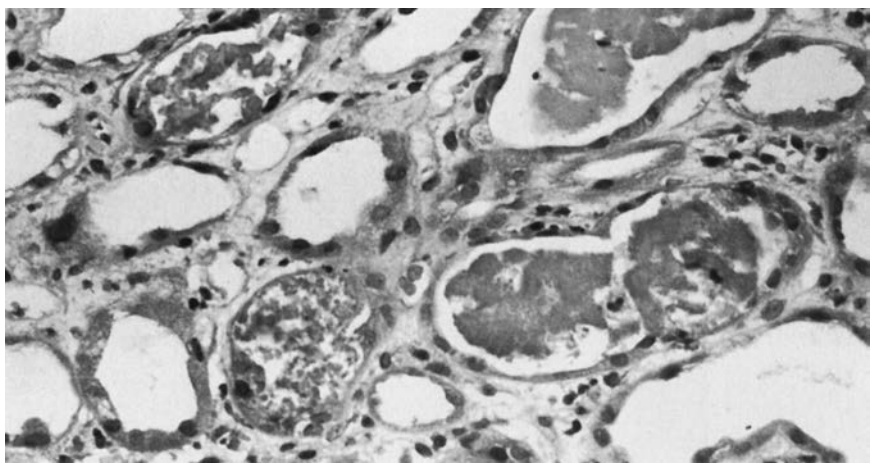

FIGURE 15-12. Photomicrograph depicting acute tubular necrosis (H&E; magnification ×440).

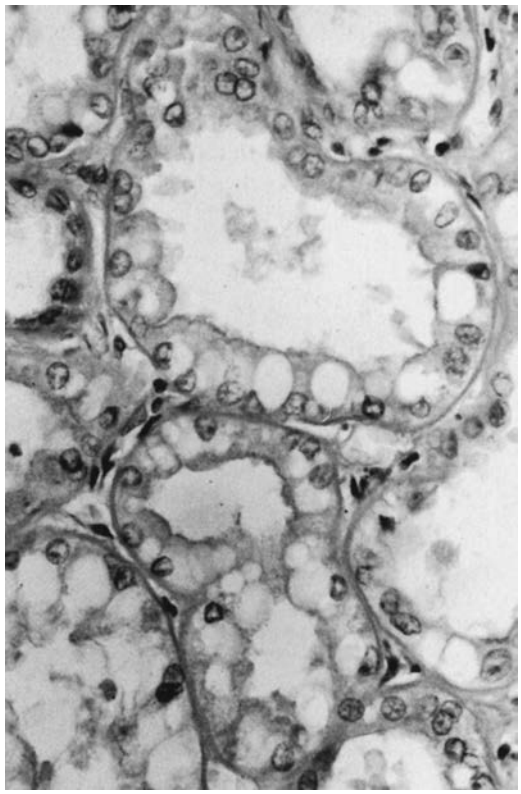

FIGURE 15-13. Example of severe hypokalemic nephropathy. Large irregular vacuoles are evident in proximal tubule epithelium (H&E; magnification ×460).

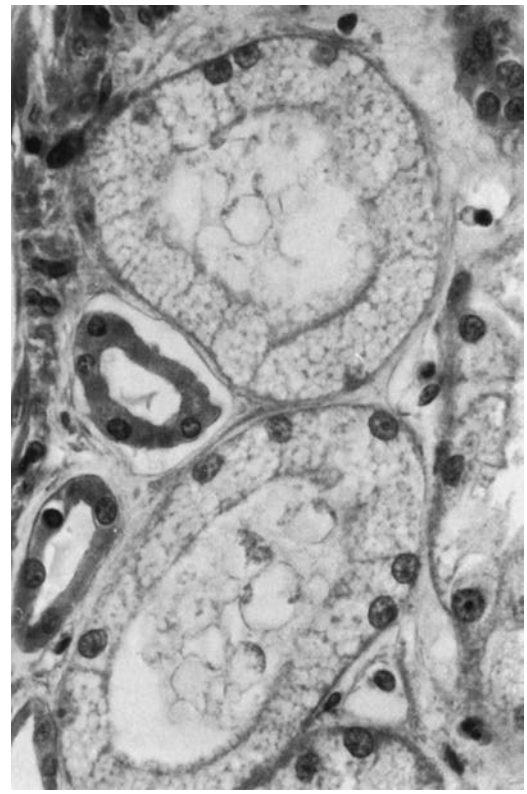

FIGURE 15-14. Photomicrograph illustrating osmotic nephrosis characterized by fine vacuolization of the proximal tubule epithelium (H&E; magnification ×540).

glomeruli usually exhibit the most striking changes, whereas less severe alterations in the form of interstitial inflammation and tubular injury accompany the primary lesion. The interstitium shows the most inflammation in interstitial nephritis, whereas the glomeruli may exhibit secondary involvement. If any process is severe enough and of long enough duration, all structures will atrophy and sometimes leave few clues to the etiology of the original disease. One is then left with a diagnosis of end-stage renal disease.

Electron Microscopy

The major advantage of electron microscopy is its greater resolving power when compared with light microscopy. In kidney biopsy interpretation, electron microscopy is most useful in the examination of glomerular lesions. Immune complexes can be identified by electron microscopy when they are too small to be evident on light microscopy or when the immunofluorescence

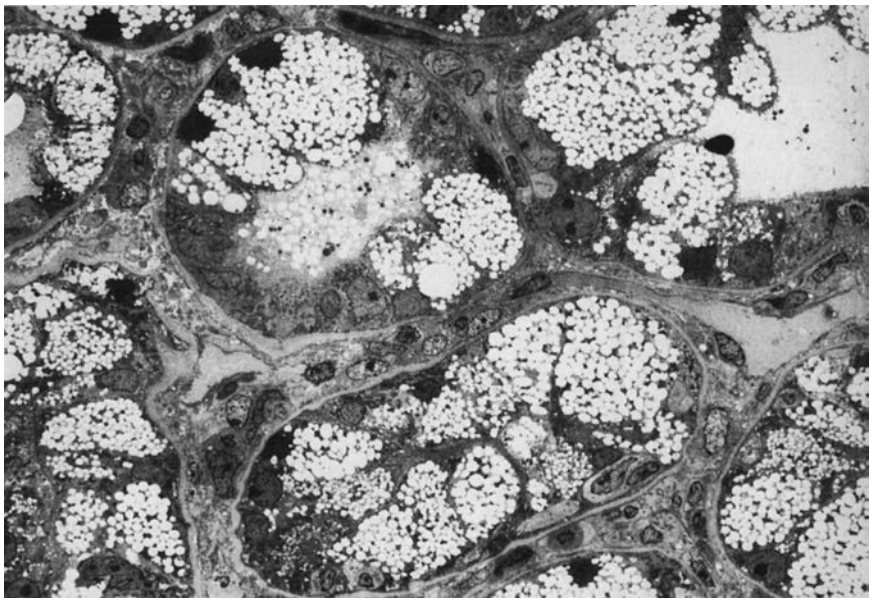

FIGURE 15-15. Electron micrograph illustrating severe tubular vacuolization secondary to cyclosporine toxicity. The nuclei are pyknotic, and the brush border is often disrupted or lost (magnification ×4,900). (From: Tisher CC, Brenner BM, eds. *Renal pathology with clinical and functional correlations*. Philadelphia: Lippincott, 1989, with permission.)

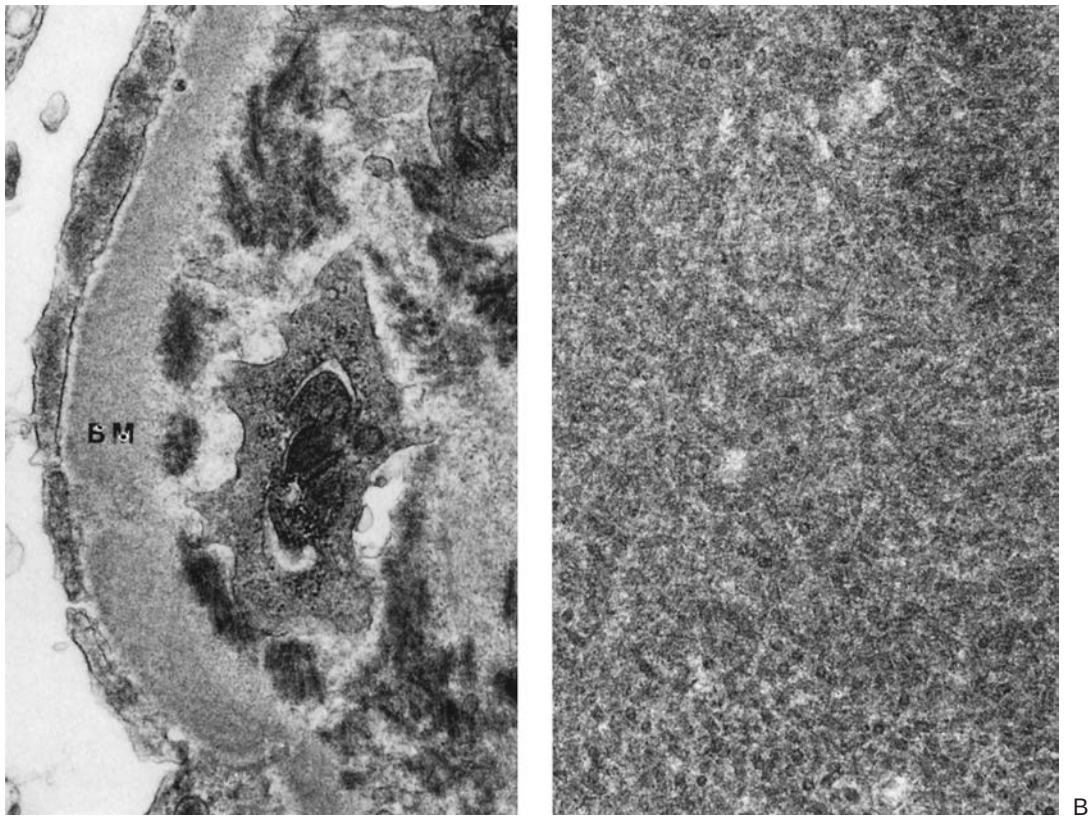

FIGURE 15-16. Electron micrographs illustrating cryoglobulin deposits in a glomerular capillary. **A:** Basement membrane with electron-dense subendothelial deposits (magnification ×15,000). **B:** High-magnification view of the characteristic substructure of cryoglobulin deposits (magnification ×41,000). (Illustrations from: Silva F, Eigenbrodt E, with permission.)

findings lack specificity. Electron microscopy gives the most definitive localization of immune complex deposits, thus making it possible to subcategorize their location as mesangial, subendothelial, subepithelial, or intramembranous. Some deposits have a characteristic substructure, such as those observed in light-chain disease (168), amyloidosis (117), cryoglobulinemic glomerulonephritis (118) (Figs. 15-16A and B), immunotactoid glomerulopathy (169), or fibrillary glomerulonephritis (Figs. 15-17A and B) (170).

The basement membrane of the glomerular capillary loops may be uniformly thickened, as seen in diabetes mellitus (171), or thin or irregular in appearance, as in Alport's hereditary nephritis (172,173) (Fig. 15-18) or thin basement membrane disease (173).

Metabolic abnormalities may be identified by the presence of characteristic accumulations of lipid such as seen in Gaucher's disease (174). Tubuloreticular arrays may be present in the endoplasmic reticulum of endothelial cells and are characteristic of lupus nephritis when seen in large numbers (Fig. 15-19), although they may be observed in other conditions, such as human immunodeficiency virus (HIV)-associated nephropathy (Fig. 15-20). Endothelial cell injury is characteristic of thrombotic microangiopathy and vascular allograft rejection. Immune deposits can also be identified along the tubular basement membrane (TBM) in lupus nephritis (Fig. 15-21) and light chain deposition disease (175,176).

Recently, the importance of electron microscopy in the evaluation of native kidney biopsies was reaffirmed. In a series of 233 biopsies, Haas (177) found that electron microscopy was necessary to arrive at a final diagnosis in 50, representing 21% of the total cases. In another 48 cases, the ultrastructural data were felt to provide important confirmatory information.

One disadvantage of electron microscopy is the limitation in the size of the sample; therefore, ultrastructural findings must be interpreted in the context of other histologic features. In addition, tissue processing for electron microscopy generally takes longer than that for light microscopy. Although rapid processing methods are available, they require special handling and therefore are more costly. Scanning electron microscopy has been used for biopsy investigation, but it is not incorporated into the processing of kidney biopsy specimens for routine clinical evaluation.

Immunohistology

Immunofluorescence and immunoenzyme staining have overlapping but different uses, as discussed previously. Standard immunofluorescence microscopy (as compared to confocal microscopy) is a more rapid but less sensitive technique. It is ideally suited for the detection of immune complex deposits. The major advantages of the immunoperoxidase technique are the greater sensitivity and the ability to examine the tissue with the light microscope, which makes the spatial relationships between tissue structures easier to identify.

There are four major immunoglobulin-staining patterns in glomeruli, which may occur singly or in various combinations (Fig. 15-22). They include linear staining along the basement membrane, granular subepithelial capillary wall staining, granular subendothelial capillary wall staining, and mesangial staining. Paramesangial deposits also may be seen alone, but as a rule they are observed in association with one of the other granular patterns. Intramembranous deposits also are observed, but their exact location is difficult to determine. The

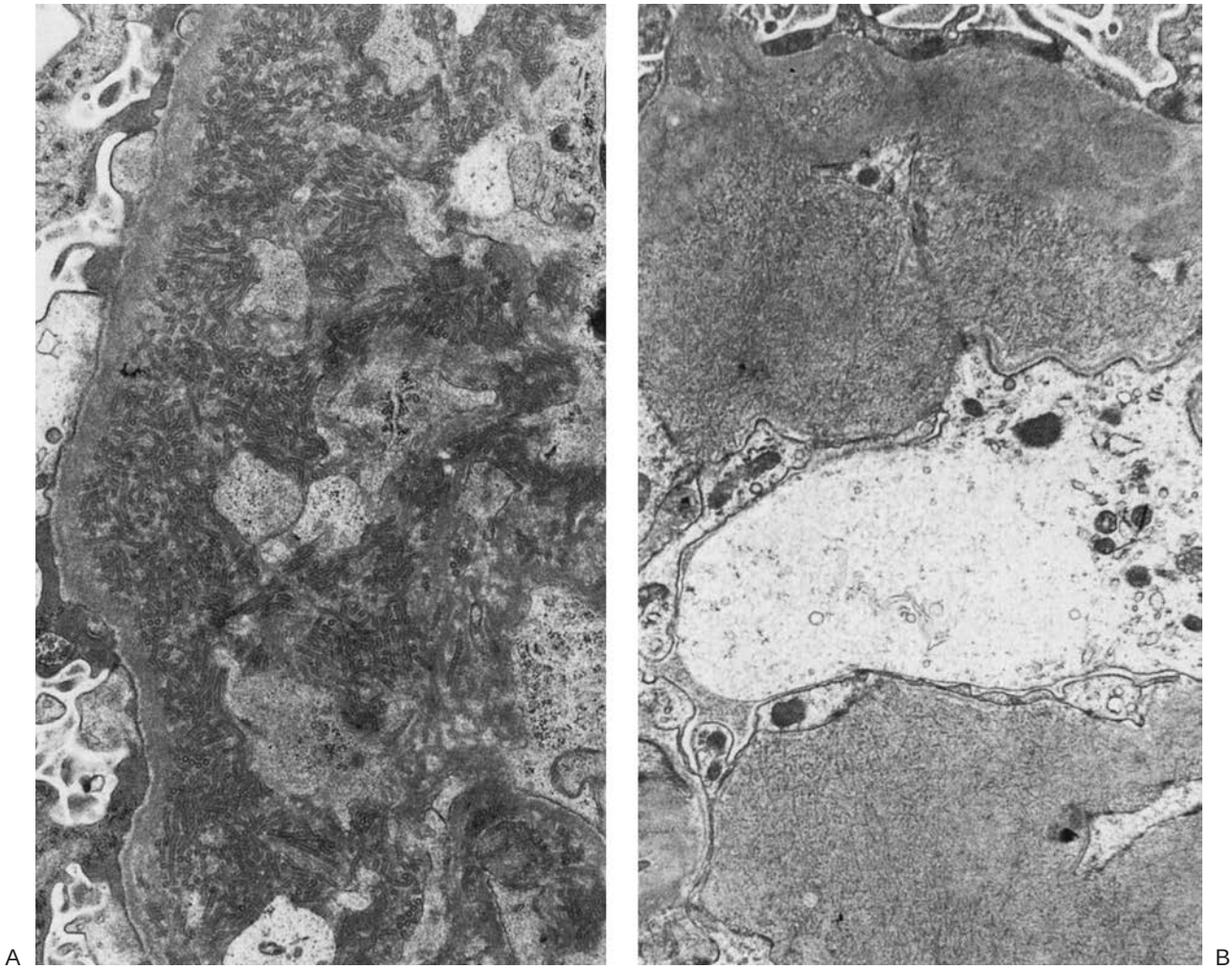

FIGURE 15-17. Electron micrographs depicting the characteristic appearance of immunotactoid glomerulopathy (**A:** magnification ×21,000) and fibrillary glomerulonephritis (**B:** magnification ×18,400). (From: Alpers CE. Immunotactoid (microtubular) glomerulopathy: an entity distinct from fibrillary glomerulonephritis? *Am J Kidney Dis* 1992;19:185, with permission.)

granular deposits may be large and coarse (Fig. 15-22B) or fine (Fig. 15-22C). This difference is readily appreciated when comparing the coarse subepithelial deposits of poststreptococcal glomerulonephritis (Color Fig. 15-22B) with the subepithelial deposits of an early idiopathic membranous glomerulonephritis (Fig. 15-22C). Immune complex deposits may contain IgG, IgM, or IgA in any combination. C1q or C3 staining may be present in any of the same patterns as seen with immunoglobulins, or they may be observed separately. Linear staining requires careful interpretation. First, finely granular capillary wall staining can appear as a confluence of granules yielding a pseudolinear pattern. Electron microscopy resolves any doubt in this situation. Second, diseases such as diabetes mellitus and most other chronic renal diseases, including chronic allograft nephropathy, cause thickening of the basement membrane. This is associated with increased staining of the capillary wall for serum proteins, particularly IgG4 and albumin, owing to electrostatic attraction (178). These situations must be distinguished from the linear capillary basement membrane staining that is specific for IgG as observed in anti-GBM disease (Fig. 15-22A). In anti-GBM disease, the IgG staining clearly exceeds the albumin staining in intensity.

Epithelial cells, especially in the glomerulus and the proximal tubule, commonly have cytoplasmic droplets of protein in proteinuric conditions. Immune complexes also may be seen in the vessels, the interstitium, or along the tubular basement membranes. Vascular or tubular basement membrane staining for C3 in the absence of immunoglobulin is a common and often nonspecific sign of injury.

The use of immunoperoxidase staining with monoclonal antibodies directed against differentiation antigens has made it possible to identify cell populations in tissue sections with great clarity and discrimination (179). Immunofluorescence microscopy also can be used (180), but the spatial discrimination is not as good. Most nonspecific inflammatory infiltrates are an approximately equal mixture of B and T cells, with a predominance of T helper cells (CD4) over T suppressor or cytotoxic (CD8) cells (179–181). These cells form nodular aggregates in interstitial areas. Cellular allograft rejection has predominantly T cells (179–181) (Fig. 15-4F) and monocytes (macrophages).

In some cases, a viral cytopathic effect may be seen by standard histology (e.g., nuclear inclusions) and the viral pathogen inferred from the clinical situation or serologic tests. However, the virus can be specifically identified by immunohistochemistry or nucleic acid hybridization. Common examples in the transplant setting are BK virus, (BKV), cytomegalic virus (CMV), and Epstein-Barr virus (EBV).

FIGURE 15-18. Electron micrograph of a portion of a glomerular capillary loop from a patient with Alport's hereditary nephritis. The typical multilaminated appearance of the thickened basement membrane is evident (magnification ×3,800). (Illustration from: Silva F, with permission.)

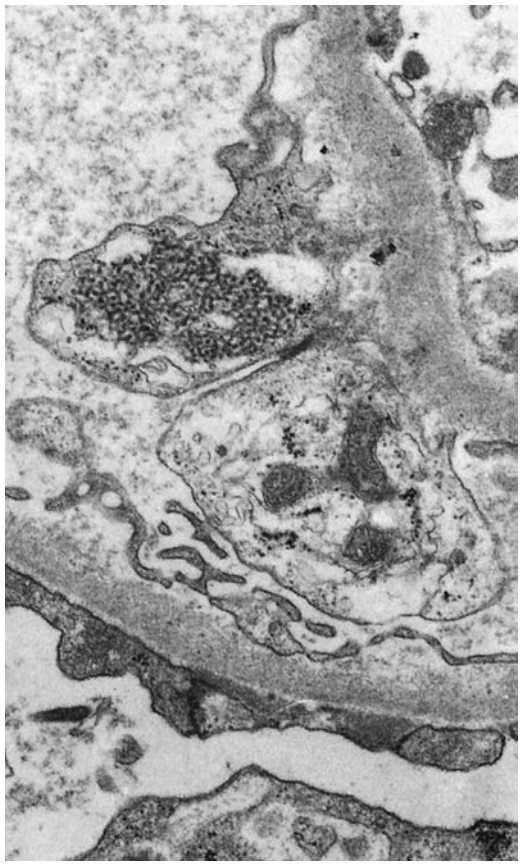

FIGURE 15-19. Electron micrograph depicting tubuloreticular arrays in smooth-surfaced endoplasmic reticulum of glomerular capillary endothelium from a patient with lupus nephritis (magnification ×24,000). (From: Tisher CC, Kelso HB, Robinson RR, et al. Intraendothelial inclusions in kidneys of patients with systemic lupus erythematosus. *Ann Intern Med* 1971;75:537, with permission).

Other enzyme systems can be used to replace peroxidase in immunoenzyme staining. However, immunostaining using horseradish peroxidase and diaminobenzidine as the substrate is the best combination available currently because the staining is crisp and the slides are permanent. Diaminobenzidine is a carcinogen and must be handled with caution. Immunohistochemistry has great diagnostic potential, which is limited primarily by the antibody specificity.

CLINICOPATHOLOGIC CORRELATIONS

Once the light, immunohistology, and electron microscopic findings are completed, they should be integrated to derive a histologic diagnosis that is indicative of the disease process. The histologic diagnosis then is related to the clinical findings to give a clinicopathologic diagnosis that can be used to plan a course of therapy, establish the prognosis, or both. We do not try to describe all of the many histologic patterns of kidney disease in this section, because they are discussed in considerable detail in other chapters of this book. Instead, we briefly discuss selected examples in which the approach we have outlined is utilized.

Several renal diseases fail to reveal significant changes or only nonspecific changes on histologic examination. For instance, in MCNS, the findings are principally the result of proteinuria and include foot-process simplification in the glomerulus and evidence of increased protein resorption by the proximal tubule. The only abnormality in benign recurrent hematuria may be the presence of red blood cells in the tubules. The differential diagnosis should include Alport's disease early in its course and thin basement membrane disease. Differentiation requires thorough electron microscopic examination of the specimen and the appropriate clinical studies.

Mesangial Expansion

In many conditions, mesangial expansion may be the only abnormality observed on light microscopy. The immunofluorescence findings separate a group of immune complex diseases that exhibit mesangial involvement. For instance, if IgA is the predominant immunoglobulin that localizes to the mesangium (Fig. 15-22E), the differential diagnosis should include IgA nephropathy and Henoch-Schönlein disease. If IgG is the principal immunoglobulin, then lupus nephritis should be considered and a careful search should be made by electron microscopy for subendothelial and subepithelial deposits or fibrillary glomerulonephritis. If the predominant immunoglobulin is IgM, then the differential diagnosis should include IgM nephropathy and the mesangiopathic form of lupus nephritis. Mesangial localization of C3 in the absence of immunoglobulins may represent a resolving immune complex disease. Evidence of resolving immune complexes may be seen by electron microscopy. The mesangial expansion may represent early diabetic nephropathy, arterionephrosclerosis, or FSGS if no complexes are noted on electron microscopy. If the material responsible for the mesangial expansion is negative or only weakly positive with the PAMS and PAS stains, it is important to examine additional sections after staining with Congo red in search of evidence of amyloid. On light microscopy the most specific indication of amyloid is a green birefringence that occurs when the Congo red stain is viewed under polarized light (Fig. 15-4D). Occasionally electron microscopy may be necessary to establish the diagnosis, because it is the most sensitive technique to detect amyloid. Other less common immune deposits that are also demonstrated easily with

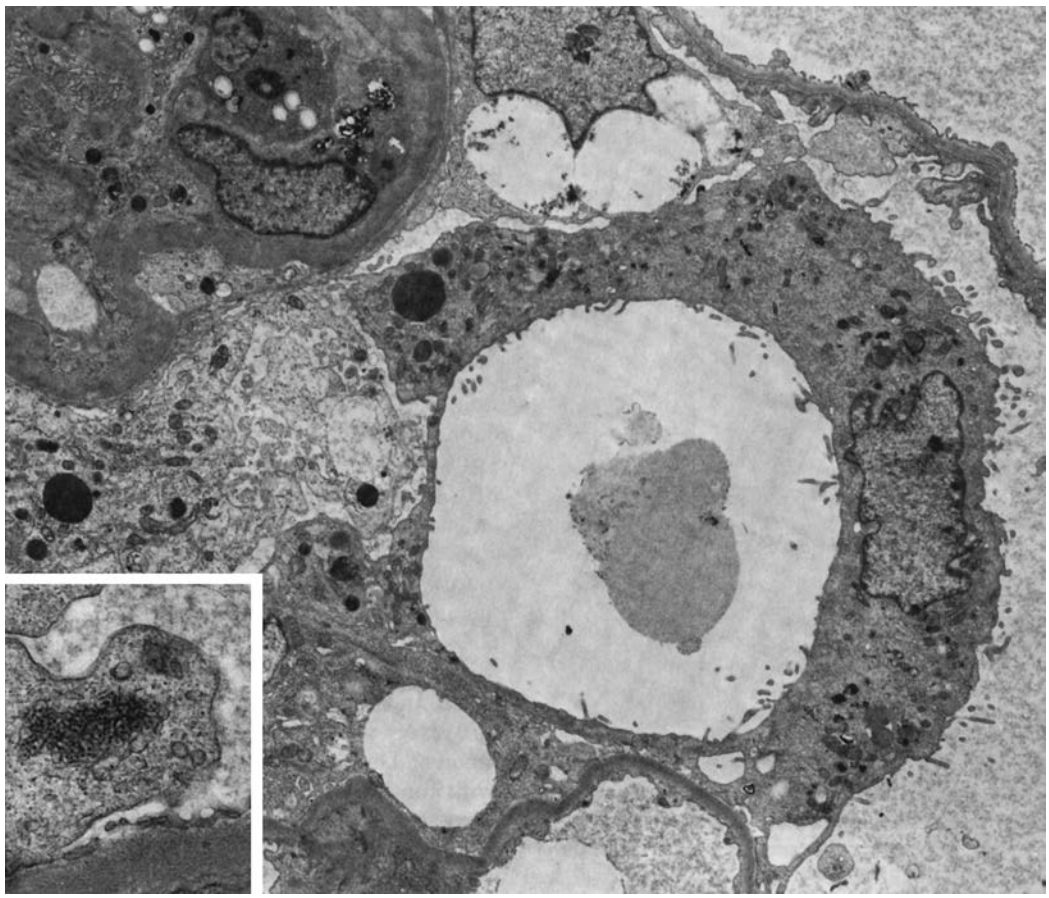

FIGURE 15-20. Electron micrograph illustrating focal and segmental glomerulosclerosis in human immunodeficiency virus-associated nephropathy. The inset depicts the characteristic tubuloreticular arrays located within the endoplasmic reticulum of glomerular endothelial cells that are typically observed in this condition (magnification ×4,000; *inset,* magnification ×16,250). (Illustration from Cohen AH, with permission.)

electron microscopy include κ light-chain disease, cryoglobulinemic glomerulonephritis, fibrillary glomerulonephritis, and immunotactoid glomerulopathy (Figs. 15-16A and B and Figs. 15-17A and B).

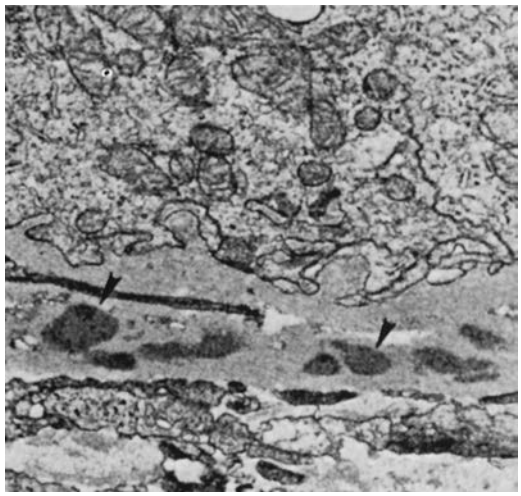

FIGURE 15-21. Electron micrograph illustrating immune complex deposits (*arrowheads*) along a tubular basement membrane in a renal biopsy from a 14-year-old boy with lupus nephritis (magnification ×2,500). (Biopsy specimen from Nash ML, with permission.)

Neutrophilic Exudates

A neutrophilic exudate may be observed in a variety of renal diseases. The most prominent neutrophilic exudate is seen typically in poststreptococcal glomerulonephritis (Fig. 15-5) (182), a disease that does not usually require biopsy for diagnosis. Occasionally, the typical clinical features are obscured, however, and a kidney biopsy is required. The patient may not seek medical attention until later in the disease course, when the light microscopic and immunofluorescence microscopic findings of coarse granular IgG deposits (Fig. 15-22B) may not be present. Often, however, C3 deposits remain. Again, electron microscopy may demonstrate typical large "humplike" subepithelial deposits that are in various stages of resolution. Numerous neutrophils also may be seen in other forms of postinfectious glomerulonephritis, in MPGN (Fig. 15-23), and the proliferative forms of lupus nephritis. Membranoproliferative glomerulonephritis may be identified by its characteristic pattern of mesangial interposition and the electron-dense deposits that are primarily subendothelial in location in type I disease and intramembranous in location in type II disease (Fig. 15-24) when observed by electron microscopy. The proliferative forms

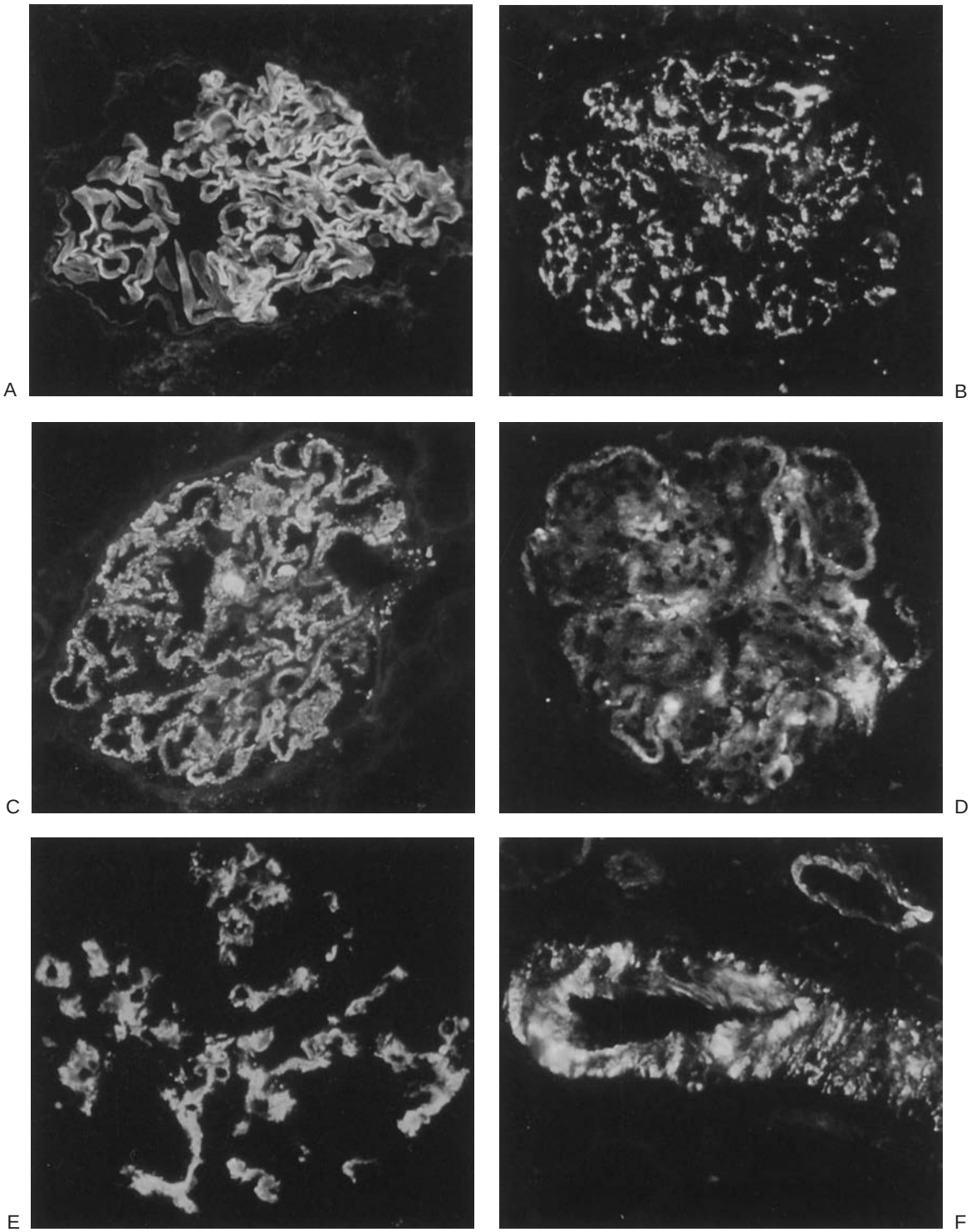

FIGURE 15-22. A: Glomerulus with fine, ribbonlike basement membrane staining with IgG indicative on anti-glomerular basement membrane disease (magnification ×200). **B:** Glomerulus exhibiting coarse granular deposits of IgG distributed irregularly along the glomerular basement membranes. This pattern is typical of poststreptococcal glomerulonephritis. C3 is usually present in the same pattern (magnification ×200). **C:** Glomerulus with fine IgG granules evenly deposited along the glomerular basement membrane. This pattern is typical of the subepithelial deposits of idiopathic membranous glomerulonephritis (magnification ×200). **D:** Glomerulus demonstrating the typical pattern of subendothelial immune complex deposits. The outer edge of the deposits abuts the inner surface of the glomerular basement membrane and is relatively smooth. The inner aspect of the deposits is shaggy and irregular and may merge with the mesangium, which is expanded. This pattern is characteristic of membranoproliferative glomerulonephritis (IgM; magnification ×200). **E:** Glomerulus exhibiting the typical pattern of IgA mesangial immune deposits. Note the absence of basement membrane localization. The dark spaces in the midst of the deposits represent mesangial cells, not capillary lumina. This pattern is typical of IgA nephrology, Henoch-Schönlein purpura, and mesangiopathic lupus nephritis (magnification ×200). **F:** Arteriole with transmural staining for IgM. This pattern is characteristic of relatively mild acute vascular lesions of vascular rejection. In more severe lesions there is greater disruption of the vessel wall, which also can be seen by light microscopy. This pattern should be distinguished from the subintimal glossy deposits of hyaline that are seen in chronic vascular disease (magnification ×400). (See Color Plate.)

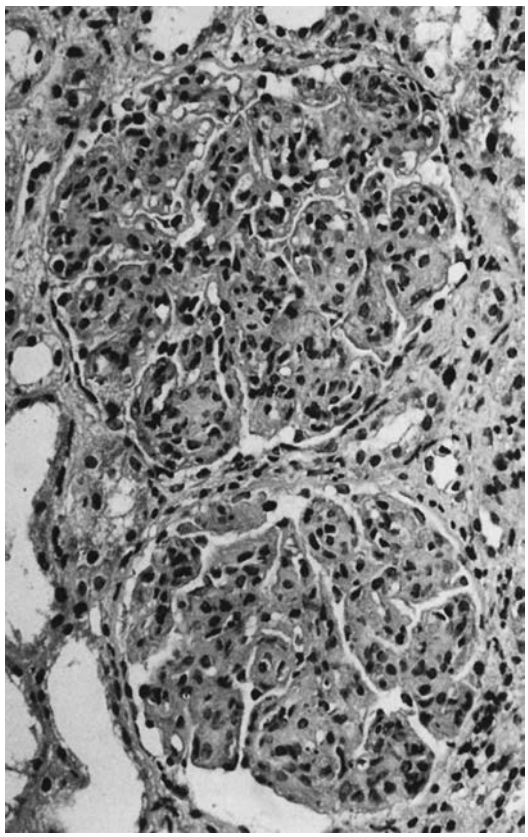

FIGURE 15-23. Photomicrograph demonstrating mesangial hypercellularity and neutrophilic infiltrates in two glomeruli from a patient with type II membranoproliferative glomerulonephritis (H&E; magnification ×300). (From: Lamb V, Tisher CC, McCoy RC, et al. Membranoproliferative glomerulonephritis with dense intramembranous alterations. A clinicopathologic study. *Lab Invest* 1977;36:607, with permission.)

of lupus nephritis are often characterized by the prominent variability in their histologic appearance from one part of a glomerular tuft to another and from one glomerulus to another.

Crescents

Crescents may be present in every type of immune-mediated glomerulonephritis; therefore, they are not diagnostic. Most types of glomerulonephritis associated with crescents can be identified by their characteristic patterns of immunoglobulin localization with immunofluorescence microscopy. These include the more severe forms of IgA nephropathy and Henoch-Schönlein purpura (IgA deposits), lupus nephritis, anti-GBM disease, and MPGN. Serologic studies for antineutrophil cytoplasmic antibodies (ANCA) have helped clarify the diseases with sparse immune deposits (pauci-immune glomerulonephritis) and fibrinoid necrosis or crescents (183). These glomerular changes are indistinguishable from the glomerular involvement observed in the microscopic form of polyarteritis nodosa or Wegener's granulomatosis. In the absence of a vasculitis involving the muscular arteries in the biopsy specimen, these conditions can be separated by the presence or absence of other systemic organ involvement (184).

The association of crescents and clinical outcome is typified by the findings in anti-GBM disease. Several authors have noted the generally benign course of anti-GBM disease in those few patients who do not develop crescents over the course of their disease even with minimal therapy (185–187). Close follow-up is prudent in this group as a rare patient has been shown to progress from noncrescentic to crescentic glomerulonephritis (186). A graded response in renal and patient survival is dependent on the percentage of crescents. Six studies in the literature (185,187–191) had comparable results that could be combined to evaluate the relationship between the percentage of crescents in the biopsy and renal survival in a total of 133 patients. If the percentage of crescents was less than 85%,

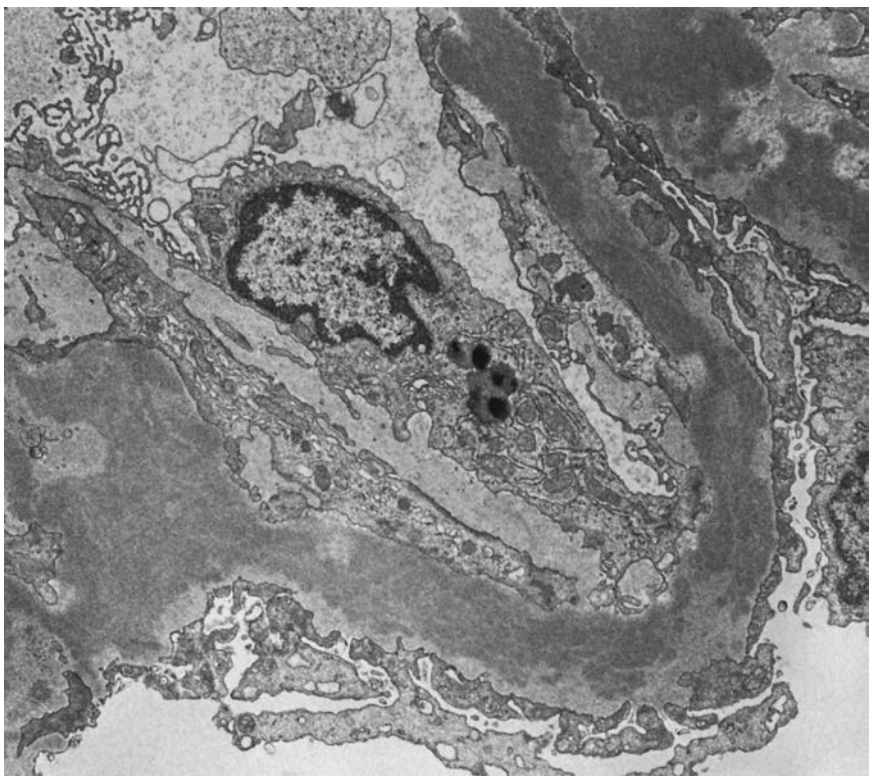

FIGURE 15-24. Electron micrograph of peripheral capillary loop from the glomerulus of a patient with type II membranoproliferative glomerulonephritis. Intramembranous electron-dense deposits are present throughout the widened basement membrane (magnification ×9,650). (From: Lamb V, Tisher CC, McCoy RC, et al. Membranoproliferative glomerulonephritis with dense intramembranous alterations. A clinicopathologic study. *Lab Invest* 1977;36:607, with permission.)

most patients (48 of 61) had independent renal function at follow-up (78%). If the percentage of crescents was 85% or greater, most patients (61 of 72) progressed to renal failure (85%) (Table 15-1). Similar results are found correlating serum creatinine values at presentation with renal survival in anti-GBM disease (Table 15-1) (185,187–192). There also is a cross correlation between serum creatinine and percentage of crescents at presentation (187,190).

In general, the presence of crescents in glomeruli is associated with a more severe prognosis. Exceptions include post-streptococcal glomerulonephritis in children because the crescents may resolve without adverse sequelae (193,194). ANCA associated glomerulonephritis does not have as clear an association of crescents with renal survival (183). Therefore, it is preferable to separate the various causes of crescentic glomerulonephritis for determination of prognosis and treatment.

Glomerulosclerosis

Glomerulosclerosis may be the result of scarring from a prior proliferative or immune complex lesion, or it can be primary in nature (195). Two common examples of the latter are diabetes mellitus and FSGS. FSGS is associated commonly with hyalinosis and foam cells within the glomerular tuft and hyperplasia of the parietal epithelium in the area adjacent to the sclerosis (Fig. 15-8); however, these features may be absent in a given biopsy specimen. In the early stages of FSGS, only a few glomeruli are affected and these are usually located deep in the cortex (196). Not infrequently, a biopsy may miss glomeruli with segmental lesions and the histology will resemble MCNS. With time, the segmental sclerosis progresses to global sclerosis and involves a greater number of glomeruli throughout the cortex. Although diabetes mellitus (diabetic nephropathy) and FSGS are not considered immune complex diseases, the globally or segmentally sclerotic glomeruli usually have staining for IgM and C3. Segmental glomerulosclerosis may also be the end result of any disease that progresses toward chronic renal failure. Therefore, FSGS as a primary disease must be differentiated from the end-stage process of focal glomerulosclerosis. To make a diagnosis of FSGS one must exclude other causes of segmental sclerosis that produce hyperfiltration and secondary changes of glomerular sclerosis. The diagnosis cannot be made with certainty in the presence of advanced nephron destruction or severe vascular disease.

Capillary Wall Thickening

Thickening of the glomerular capillary walls can be seen in a variety of renal diseases. In its early stages, diabetes is characterized by thickening of the *lamina densa,* which may be visualized by electron microscopy. It may occur as a consequence of glomerulosclerosis and take on a wrinkled, ribbonlike appearance in association with other evidence of glomerular tuft ischemia or atrophy. Localized thickening can be seen in postinfectious proliferative glomerulonephritis because of the presence of subepithelial, humplike, immune-complex deposits. In idiopathic membranous glomerulonephritis, discrete granular immune-complex deposits are localized to the subepithelial surface of the capillary wall and are associated with subepithelial extensions of basement membrane material, referred to as "spikes" (Fig. 15-4C). These progress to form bridges to completely enclose the subepithelial deposits, yielding a tram-track configuration. In type I MPGN, subendothelial immune-complex deposits often are seen in association with reduplication of the peripheral glomerular basement membrane, (mesangial interposition, Fig. 15-25). Similar changes can be seen in type II MPGN, in which electron-dense material expands the original basement membrane of the peripheral capillary loop

FIGURE 15-25. Electron micrograph illustrating typical mesangial interposition (*arrowheads*) in type I membranoproliferative glomerulonephritis (magnification ×3,200). (Illustration from Silva F, with permission.)

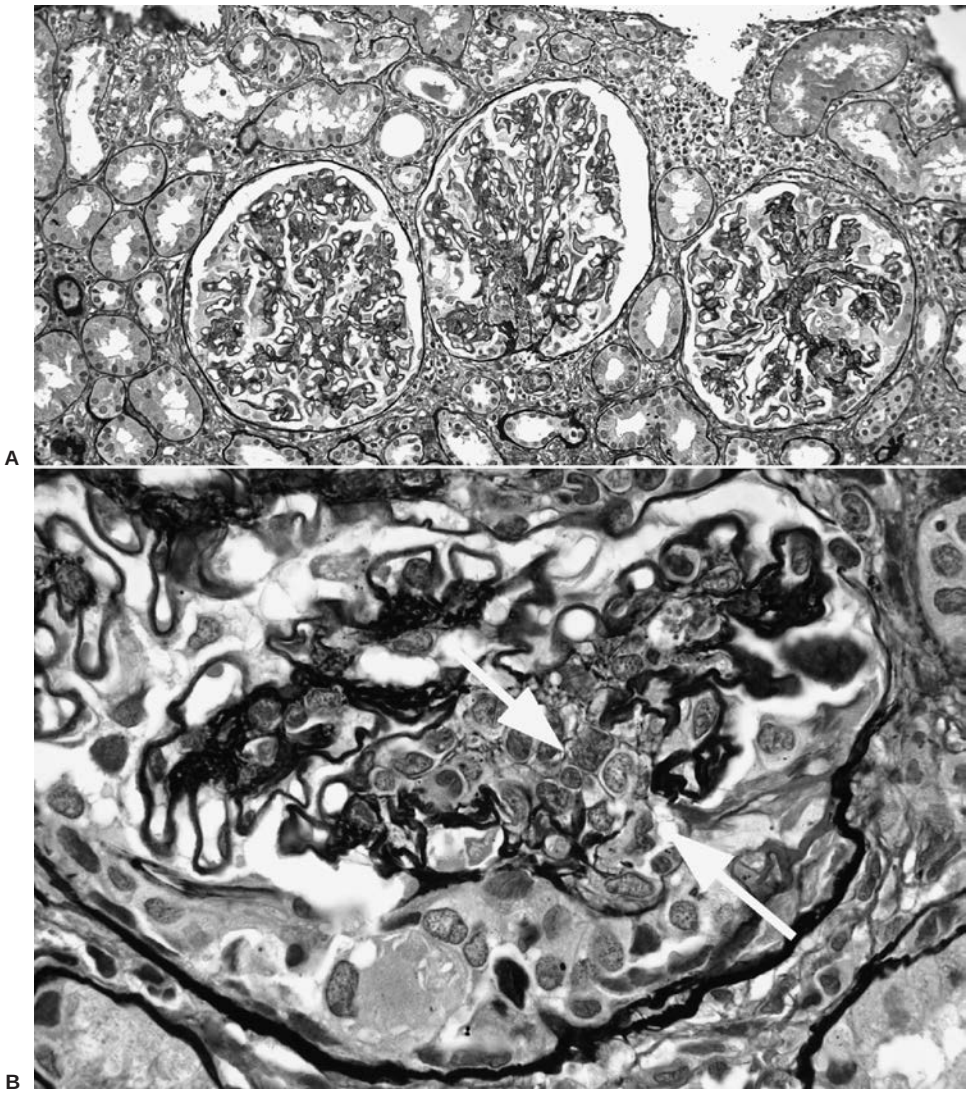

FIGURE 15-26. Segmental Class IV lupus nephritis. **A:** This figure shows three glomeruli with segmental involvement (PAMS magnification ×200). **B:** This is a higher magnification (×500) of a segmental lesion with increased cellularity and fragmentation and disruption of the GBM between the arrows (PAMS).

and also is associated with mesangial interposition (Fig. 15-24). In both type I and II MPGN this combination of histologic changes can give rise to double contouring of the peripheral portion of the glomerular capillary wall.

Regardlless of the particular glomerular alteration it is often important for diagnostic and prognostic evaluation to note whether the lesion is segmental versus global or focal versus generalized. An example of this distinction is emphasized in the most recent classification of lupus nephritis (197) which divided the WHO Class IV (198) into segmental (Class IV-S, Fig. 15-26) and global (Class IV-G) subdivisions. There is a presumption or hypothesis that the segmental subclass may have a different pathogenesis and one published study (199) shows a difference in outcome than the global subclass.

Interstitial Inflammation

The differential diagnosis of interstitial inflammatory infiltrates poses some interesting diagnostic problems for the nephropathologist. Kidneys with nephron atrophy may have fibrosis and associated dense nodular lymphocytic infiltrates composed of mixtures of B cells and T cells. Interstitial infiltrates in the absence of nephron atrophy or acute glomerulonephritis suggest a primary interstitial nephritis. The presence of large numbers of neutrophils in the tubules (neutrophilic tubulitis), Bowman's space, and the interstitium suggests a diagnosis of acute bacterial interstitial nephritis, whereas an occasional tubule with neutrophils may be seen in ATN. Cellular infiltrates composed of lymphocytes and plasma cells, with or without eosinophilia, commonly are the result of a drug allergy or are idiopathic in nature. Urinary outflow obstruction and renal vein thrombosis may cause interstitial edema and mild inflammation, but these changes are relatively nonspecific. Acute tubular necrosis is also associated with mild interstitial infiltrates in the region of the straight portion of the proximal tubule. A more chronic interstitial nephritis can result from an adverse response to nonsteroidal antiinflammatory drugs. A pattern of nephron atrophy that affects the tubules before the glomeruli and vessels is consistent with, but not pathognomonic of, chronic interstitial nephritis. Rarely, a lymphocytic lymphoma or leukemia may be seen on a kidney biopsy.

Vascular Lesions

Arteriosclerosis is the most common vascular lesion observed in kidney biopsies. It may represent a primary vascular

TABLE 15-2

IMMUNOPATHOGENETIC MECHANISMS ASSOCIATED WITH RENAL ALLOGRAFT REJECTION

Type	Onset of clinical manifestation	Morphology	Mechanism
Acute rejection			
Antibody mediated (vascular)			
Hyperacute	0–72 hr	Intracapillary PMN inflammation and thrombosis	Performed antibody with C′ and fibrinogen activation
Accelerated acute	3–7 d	Endovascular inflammation similar to above but histologically less intense	Memory antibody response
Acute rejection	7 d or more	Endovasculitis with PMN but without lymphocytes	De novo antibody response
Cell mediated vascular	7 d to 3 mo	Endovasculitis with varying proporations of CD4 + CD8 lymphocytes and macrophages	May be any of several cell mediated mechanisms
Tubular		Lymphocytic tubulitis with predominately CD8 cells	Cytotoxic lymphocytes
Interstitial		Mononuclear interstitial inflammation with predominately CD4 lymphocytes and macrophages	Delayed type hypersensitivity
Chronic rejection	3 mo or longer		
Vascular		Intimal proliferation	Antibody
Cell mediated		Interstitial nephritis	Continued DTH and cytotoxic responses
Innate response			
Acute		Intrinsic cell activation	Oxidative and other stress injury
Chronic		Chronic interstitial nephritis	Macrophage activation and fibrogenesis

PMN, polymorphonuclear; *DTH*, delayed type hypersensitivity.

disease such as that seen with long-standing hypertension, or it may be seen in association with progressive nephron loss that occurs with end-stage renal disease of any etiology. Extensive hyaline arteriolosclerosis suggests the presence of diabetes mellitus (Fig. 15-10). Milder hyaline lesions are seen in FSGS and sporadically in hypertension and other conditions. Necrotizing vasculitis is rarely seen in kidney biopsies in the absence of the glomerular lesions of polyarteritis, lupus nephritis, or Henoch-Schönlein purpura. Thrombotic microangiopathy is a distinctive lesion of small arteries and glomerular capillaries. It is characterized by insudation of fibrin and RBCs, the latter often in the form of schistocytes, into the walls of the small arteries and glomerular capillaries, which may appear as mesangiolysis. These acute changes often progress to renal ischemia and atrophy. This lesion can be seen in varying degrees of severity in several clinical settings, including the hemolytic–uremic syndrome, malignant hypertension, progressive systemic sclerosis, lupus nephritis, and anti-GBM disease.

Renal Allograft Pathology

Percutaneous biopsies of renal allografts introduce another set of diagnostic problems for the pathologist. Virtually any lesion that occurs in the native kidney also can be found in the allograft in addition to the histologic picture of rejection (125). Absence of function in the immediate postoperative period, especially in a cadaveric kidney, is usually owing to ischemic tubular damage or rejection. A pathogenetic classification of rejection is given in Table 15-2, which incorporates features of immunologic mechanisms, and the clinical setting (200,201). Hyperacute rejection may occur in a small percentage of patients and has a very distinctive morphology characterized by extensive fibrin thrombi and neutrophils in glomerular capillaries. This form of rejection is thought to be secondary to the presence of preformed circulating antibody in the recipient that was not detected with the usual screening procedures.

Acute vascular rejection can occur from the first week of engraftment. It may be antibody- or cell-mediated. It resembles the histologic picture of a low-grade vasculitis, with intimal proliferation or disruption and exudation in the walls of arteries and glomerular capillaries (Color Fig. 15-4E). The vascular lesions usually are patchy and are more frequently missed by light microscopy in biopsies with few arteries. The immunofluorescence findings of IgM, C1q, and C3 in the walls of vessels are more-sensitive indicators of vascular rejection (Fig. 15-22F). Immunoperoxidase staining for the complement component, C4d (Fig. 15-27), is a marker for antibody-mediated rejection and is more widely distributed (202). Electron microscopy reveals swelling of the endothelium and the *lamina rara interna*.

Tubulointerstitial rejection is another type of acute rejection and is a form of acute interstitial nephritis. The infiltrate in most cases is composed of large active cytotoxic T cells (203). The cytotoxic T cells identified by immunohistochemical staining (Fig. 15-4F) infiltrate the tubular cytoplasm, a process termed *emperipolesis,* to produce lymphocytic tubulitis (179). Differentiation of acute cellular rejection from other forms of

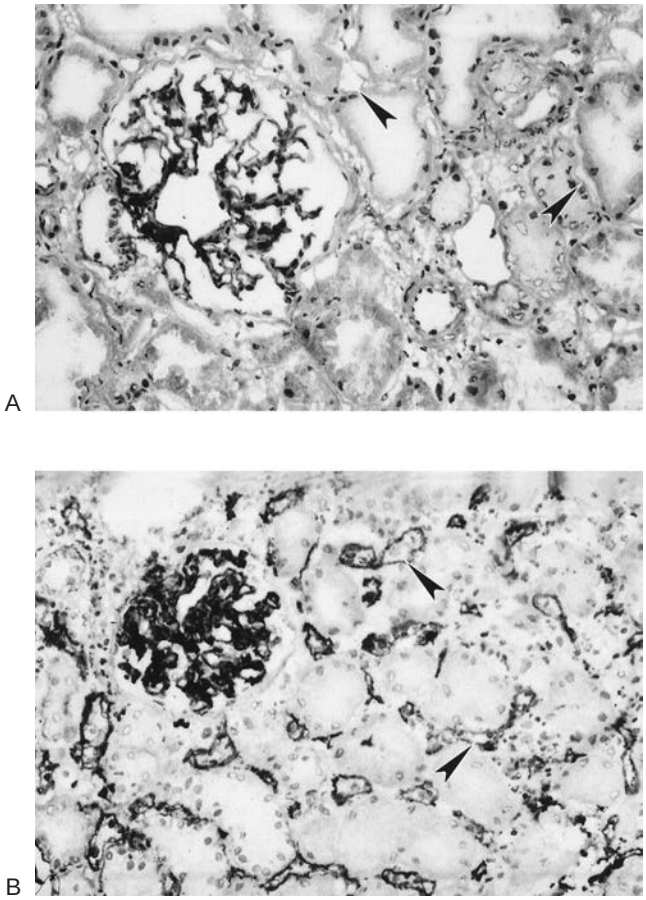

FIGURE 15-27. This figure shows C4d stained. **A:** Allograft with acute tubular necrosis. Peritubular capillaries (*arrows*) are unstained (Immunoperoxidase, C4d, magnification ×210). **B:** Allograft with antibody mediated rejection. Peritubular capillaries (*arrows*) are staining (Immunoperoxidase C4d, magnification ×210).

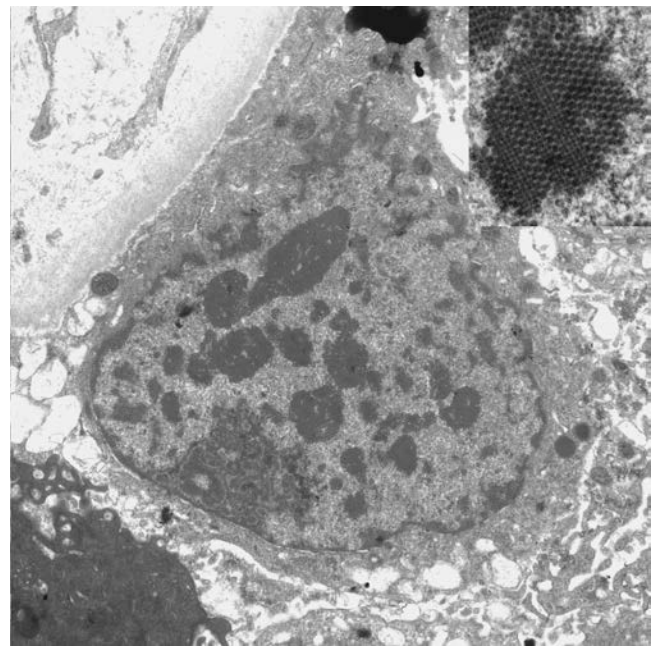

FIGURE 15-28. Electron micrograph of tubular epithelial cell showing coarse clusters of nuclear material. The identification of virus is not obvious at this magnification (magnification ×7,500). **Inset:** Higher magnification of nuclear densities (magnification ×33,000) showing polyoma virus in a crystalloid array.

interstitial nephritis can be difficult in certain settings especially when characterized by a delayed-type hypersensitivity (DTH) response with CD4 T cells and macrophages (179–181).

It is also important to note the distribution of the cellular infiltrate. Both native and allograft kidneys may have nodular infiltrates. These are often located in the adventitia of blood vessels and are composed predominantly of T4 cells and B cells. This type of cellular infiltrate is nonspecific. In cellular rejection, allografts often exhibit a superimposed diffuse interstitial and tubular cytotoxic T-cell infiltrate (179). An increase in cytotoxic T cells during cellular rejection has been confirmed in these infiltrates by fine-needle aspiration (121). B-cell infiltrates suggest PTLD particularly in the presence of EBV and destructive nodules. BKV also produces interstitial nephritis and is indicated by nuclear inclusions and confirmed by EM, immunostatining, or molecular studies (204) (Fig. 15-28).

Cyclosporine and other calcineurin inhibitor toxicity is an important cause of decreased renal function in the allograft. The tubular changes were noted in an earlier section (Fig. 15-14). The most widely accepted demonstrations of cyclosporine-related renal vascular changes have been observed in the setting of transplantation of solid organs other than the kidney (e.g., the heart [205]) or inflammatory diseases of other organs (e.g., type I diabetes [206]).

Although acute changes in renal function are established in many systems (207), the current data suggest that morphologic changes associated with long-term cyclosporine use are characteristic but not pathognomonic (208) of this class of drugs. Features characteristic of chronic cyclosporine toxicity include hyalinosis of arterioles (Fig. 15-29), interstitial fibrosis, and tubular atrophy (205,206,209). When these lesions are noted in the allograft biopsy, cyclosporine toxicity should be considered in the differential diagnosis.

Systemic cytomegalovirus infection continues to be a significant cause of morbidity and mortality among recipients of renal transplants (138). Cytomegalovirus may be detected in the biopsy. Viremia is determined best by peripheral blood studies.

Traditionally, rejection has been defined as an immune response directed against the graft antigens or alloimmune response. More recently years it has become evident that a variety of factors affect graft survival that are unrelated to traditional antigen driven B- and T-cell responses. They include donor factors such as age and gender and events coincident to organ procurement such as cadaveric donation and ischemia time (210,211). The kidney response in these settings recapitulates the responses of innate immunity (Table 15-2).

Finally, late irreversible changes in the graft that are associated with chronic azotemia may represent the sequelae of chronic rejection. This is not necessarily a separate entity, but may be the result of repeated episodes of acute rejection and innate immunity. Therefore, the term *chronic transplant nephropathy* is preferred rather than chronic rejection to identify the nonspecific pathologic changes. The morphologic features are those of severe tubular atrophy, glomerulosclerosis, interstitial fibrosis, and arteriosclerosis with varying degrees of chronic inflammation and tubular necrosis.

In addition to the pathogenetic classification (Table 15-2) noted in the preceding, several schemes for histologic classification of rejection have been developed to facilitate interinstitutional studies and therapeutic trials. The Banff schema (212) was specifically designed toward this end and based on light microscopic features with readily available histologic stains. With

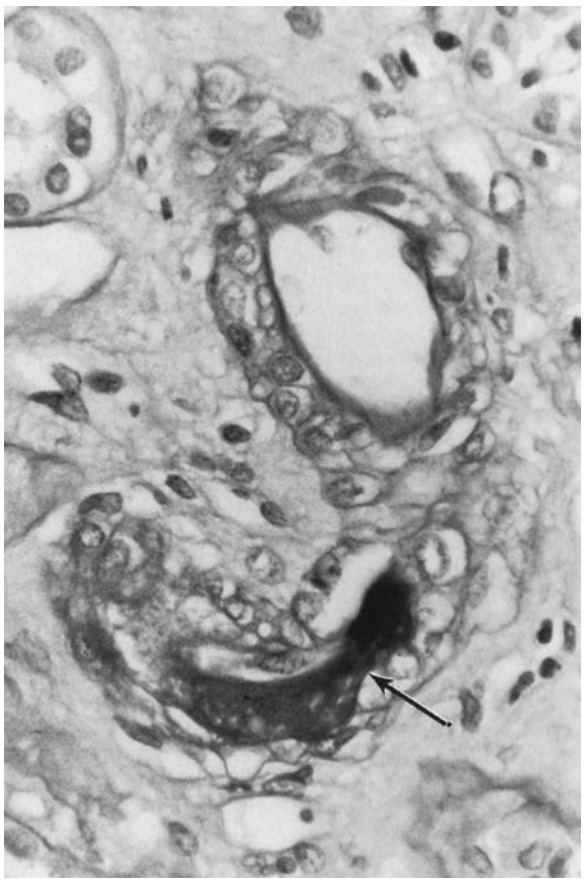

FIGURE 15-29. Photomicrograph illustrating cyclosporine toxicity in a terminal interlobular artery. Degenerated changes are evident in the muscularis in association with a large dense hyaline deposit (*arrow*) (PAS, magnification ×750).

the experience of experimental validation and clinical trials, the original Banff schema was modified in 1995 (213), 1997 (214), and 2003 (215). The NIH-sponsored Combined Clinical Trials in Transplantation (CCTT) classification (216) is simpler but similar in structure for acute rejection. Clinical correlation in the CCTT study indicated mononuclear cell margination to vascular endothelium was an indicator for clinical severity. Although more complex, the strength of the Banff schema is it captures fundamental histologic data. Although acute rejection continues to be a significant problem, a greater problem as noted in the preceding is chronic rejection and chronic allograft nephropathy. Structural relationships are still the cornerstone for understanding these processes and developing the molecular genomic, proteomic, and genetic bases for graft failure or survival. Use of a classification that meets these purposes is essential for understanding and categorizing the many features of renal allograft pathology. Any classification must also be flexible to accommodate morphologic changes that may be associated with new therapeutic measures in this rapidly changing field.

CONCLUSION

We have followed a time-tested step-by-step approach to the use and evaluation of the kidney biopsy. An in-depth discussion of specific diseases that affect the kidney can be found in other chapters of this text. We have selected some common diseases that are amenable to diagnosis on kidney biopsy in an effort to demonstrate the necessity and advantage of utilizing a combination of light, electron, and immunohistologic microscopy to obtain the maximum amount of information from a biopsy specimen to aid in the clinical management of the patient. We expect that molecular genetic, functional genomic, and proteomics studies will provide additional information to the classic structural and functional approach outlined in this chapter.

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SECTION III
HEREDITARY DISEASES

CHAPTER 16 ■ INTRODUCTION TO GENETIC RENAL DISEASE

TERRY WATNICK AND GREGORY G. GERMINO

GENETIC RENAL DISEASE

The success of the human genome project has resulted in dramatic advances in our understanding of inherited renal diseases. There has been an explosion in the number of disease-causing genes that have been identified and in our understanding of the pathogenic mechanisms that underlie these disorders (Tables 16-1–16-8). The spectrum of physiologic and developmental pathways that are disrupted is broad and includes defects in isolated transport mechanisms (e.g., cystininuria, primary hypomagnesia, pseudohypoaldosteronism), defects in complex developmental pathways (e.g., autosomal dominant polycystic kidney disease (ADPKD) and renal coloboma syndrome) and defects in structural proteins (e.g., Alports syndrome and Congenital Nephrotic Syndrome of the Finnish type). The tremendous progress in the field over the past 5 years has made an exhaustive discussion of the topic of genetic renal disease far beyond the scope of this chapter. The interested reader is referred to the Online Mendelian Inheritance of Man (OMIM) for a more complete description. This web-based database provides a complete catalogue of diseases, their clinical features, and their molecular genetics [http://www3.ncbi.nlm.nih.gov/Omim/]. Instead, we will describe the process of gene discovery and how that process has evolved over the past several years. We will then review some of the scientific tools that have been applied in the postcloning stages of gene discovery in order to understand important aspects of renal biology. Finally, we will consider the clinical implications of these insights and how they may ultimately be applied in patient care.

GENE IDENTIFICATION

The basis of any inherited disease is an underlying alteration in genomic DNA that is transmitted from parent to offspring. Theoretically, one could compare the entire genomic sequence of an individual affected with a particular disease to that of unaffected individuals in order to identify the pathogenic difference. As simple as this sounds, the challenges posed by this approach cannot be met easily with current technology. One haploid genome (one copy of each autosome and one sex chromosome) alone consists of 3×10^9 base pairs (bp) while the standard laboratory can only generate about $10^4 - 10^5$ bp of perfect sequence per day. The sequence must be perfect because even a change of one base pair can cause disease depending on its location. In addition, many DNA sequence differences do not produce disease (silent polymorphisms), requiring that the DNA of many normal individuals be sequenced in order to identify changes that are of clinical importance.

Several approaches have been developed to facilitate disease gene discovery. With some disease entities, a broader understanding of underlying pathogenic mechanisms may allow researchers to identify potential "candidate genes." Alport syndrome is an example of the successful application of this approach (1). Biochemical analysis of the Alport glomerular basement membrane (GBM) identified a set of α chains of type IV collagen that were missing (2–5). Molecular techniques were then used to clone the type IV collagen genes, and mutation analyses revealed that sequence variants in a subset of these genes segregated with the disease (see the subsequent text). In a similar manner, the recognition that individuals suffering from the infantile form of Bartter syndrome have a clinical presentation similar to that of patients on loop diuretics prompted investigators to evaluate the drug's target, the Na-K-2Cl cotransporter (SLC12A1), as a probable candidate gene. As predicted for this recessive disease, inactivating mutations were found in both alleles in a subset of families (6).

In certain disorders, methods can be employed to assay functional differences between normal and disease states. One can identify genes responsible for a particular function by transfer of genetic material into cells that lack that function and then screening for activity. Typically, a number of pools of genes are used for the initial screening and then one focuses one's search on only those pools that demonstrate the desired activity. By using a reiterative process of serial dilutions and functional testing, one can ultimately identify the gene or genes responsible for the observed activity. Finally, the cloned candidate genes are scanned for sequence differences (mutations) that segregate with disease. This approach has been termed expression cloning and has been used most successfully to identify various transporters. The genes implicated in cystinuria, *SLC3A1* and *SLC7A9*, were identified by this approach (7–11). Likewise, the three subunits that comprise the epithelial sodium channel, eNaC, were isolated in this manner (12). Inactivating mutations of each of the subunits have been associated with recessive forms of pseudohypoaldosteronism type I (13,14), whereas activating mutations of either the β or γ subunit have been found in Liddle syndrome, an autosomal dominant form of hypertension (15,16).

Unfortunately, gene discovery for many hereditary diseases with complex phenotypes has proven refractory to these methods. This has necessitated the use of a strictly molecular genetic approach, termed positional cloning, which seeks to identify a disease gene solely on the basis of its chromosomal location (17). Occasionally, important clues are provided by cytogenetic analysis of affected individuals who have a chromosomal abnormality that is associated with a disease. Several of the loci involved in the origin of renal tumors were identified by this approach (Table 16-4). The gene responsible for the major form of Wilms tumor, *WT1*, was initially discovered because of its involvement in WAGR syndrome (*W*ilms tumor, *a*niridia, genitourinary anomalies and mental *r*etardation) (18–20). Individuals affected by this disorder were found to have constitutional deletions of 11p13 involving a zinc finger transcription factor, *WT1*, a paired box transcription factor (*Pax 6*), and adjacent DNA sequences. Fine mapping proved that *WT1*

TABLE 16-1

INHERITED DISORDERS OF THE GLOMERULUS

Disease	OMIM #	Mode of inheritance	Chromosomal localization	Gene name(s)	Gene product(s)	Reference
Alport Syndrome						
X-linked	301050	X	Xq22.3	*COL4A5*	α5(IV) Collagen	(1)
X-linked with leiomyomatosis	308940	X	Xq22.3	*COL4A5*& *COL4A6*	α5(IV) and α6(IV) Collagen	(271)
Autosomal recessive	203780	AR	2q35-q36	*COL4A3*	α3(IV) Collagen	(218)
Autosomal recessive	203780	AR	2q35-q36	*COL4A4*	α4(IV) Collagen	(218)
Autosomal dominant	104200	AD	2q35-q36	*COL4A3*	α3(IV) Collagen	(219)
Benign Familial Hematuria*	141200	AD	2q35-q36	*COL4A4*	α4(IV) Collagen	(220)
Congenital Nephrotic Syndrome of the Finnish Type	256300	AR	19q13.1	*NPHS1*	Nephrin	(96)
Diffuse Mesangial Sclerosis	256370	AD	11p13	*WT1*	Wilms Tumor 1, Zinc Finger Protein	(221)
Familial Focal Segmental Sclerosis*						
FSGS1	603278	AD	19q13	*ACTN4*	Alpha-actinin 4	(222)
FSGS2	603965	AR	11q21-q22	*TRPC6*	Transient Receptor Potential Channel C6	(223,224)
Frasier Syndrome (Streak Gonads, Pseudohermaphrodistism, Renal Failure)	136680	AD	11p13	*WT1*	Wilms Tumor 1, Zinc Finger Protein	(225)
Fibronectin Glomerulopathy	601894	AD	1q32	?	?	(226)
Idiopathic Nephrotic Syndrome, Steroid Resistant	600995	AR	1q25-q31	*NPHS2*	Podocin	(39)
IgA Nephropathy*	161950	AD	6q22-23	?	?	(227)
Nail-Patella Syndrome	161200	AD	9q34.1	*LMX1B (NPS1)*	LM1B, LIM-homeodomain protein	(42,43)

*Identifies diseases with probable additional, unmapped loci.

TABLE 16-2

RENAL CYSTIC DISORDERS

Disease	OMIM #	Mode of inheritance	Chromosomal localization	Gene name	Gene product	Reference
Autosomal Dominant Polycystic Kidney Disease	173900					
ADPKD1, most common	601313	AD	16p13.3	*PKD1*	Polycystin-1	(28,228)
ADPKD2	173910	AD	4q21-23	*PKD2*	Polycystin-2	(33)
ADPKD3	600666	AD	?	?	?	(196–199)
Autosomal Recessive Polycystic Kidney Disease (PKHD1)	263200	AR	6p12	*PKHD1*	Polyductin, fibrocystin	(229,230)
Glomerulocystic Kidney Disease (GCD)	137920	AD	?	?	?	(231)
MODY5 (Maturity Onset Diabetes of the Young, Type V)	604284	AD	17cen-q21.3	*HNF1-β* (Tcf2)	Hepatocyte nuclear factor 1-*β*	(232,233)
Overlap syndrome with FJHN and MCKD	603860	AD	16p12	*UMOD*	Uromodulin	(234)
Joubert Syndrome	213300	AR	2q13	*NPHP1*	Nephrocystin-1	(235)
		AR	9q34.3	?	?	(236)
	608629	AR	6q23.3	*AHI1*	Abelson helper integration site-1	(237)
Medullary Cystic Disease*						
MCKD1	174000	AD	1q21	?	?	(238)
MCKD2	603860	AD	16p12	*UMOD*	Uromodulin (Tamm-Horsfall Protein)	(239)
Nephronophthisis*						
NPHP1 (Juvenile), most common	256100	AR	2q13	*NPHP1*	Nephrocystin-1	(35)
NPHP2 (Infantile)	602088	AR	9q22–31	*INVS*	Inversin	(131)
NPHP3 (Adolescent)	604387	AR	3q22	*NPHP3*	Nephrocystin-3	(137)
NPHP4	606966	AR	1p36	*NPHP4*	Nephrocystin-4 (Nephroretinin)	(138,240)
NPHP5	609237	AR	3q21.1	NPH5	Nephrocystin-5	(241)
Polycystic Kidney Disease, Infantile Severe, with Tuberous Sclerosis (PKDTS)	600273	AD, sporadic	16p13.3	*PKD1* & *TSC2* (CGS)	Polycystin-1 and Tuberin	(242,243)
Senior-Loken Syndrome (Renal Dysplasia and Retinal Aplasia Syndrome)	266900	AR	2q13 3q22	*NPHP1* *NPHP3*	Nephrocystin-1 Nephrocystin-3	(244) (131,245)
(See NPHP1, 3,4)			1p36	*NPHP4*	Nephrocystin-4	(138,240,241)

*Identifies diseases with probable additional, unmapped loci.
(*CGS*) indicates contiguous gene syndrome.

TABLE 16-3

GENETIC CAUSES OF HYPERTENSION

Disease	OMIM #	Mode of inheritance	Chromosomal localization	Gene name	Gene product	Reference
Apparent Mineralocorticoid Excess						
AME Type I	218030	AR	16q22	*HSDB2*	11-β-hydroxysteroid dehydrogenase, type 2 isoform	(246)
AME Type 2 (variant of Type I)	207765	AR	16q22	*HSDB2*	same	(247)
Early Onset Hypertension, with Severe Exacerbation by Pregnancy	605115	AD	4q31.1	*NR3C2*	Mineralocorticoid receptor	(95)
Glucocorticoid-Remediable Hypertension, (Glucocorticoid-Remediable Aldosteronism, GRA)	103900	AD	8q21	*CYP11B1-CYP11B2 fusion*	Promoter of CYP11B1 (11-β-hydroxylase) controls expression of CYP11B2 (aldosterone synthase)	(248)
Gordon Syndrome (Pseudoaldosteronism Type II, Gordon Hyperkalemia-Hypertension Syndrome)						
PHA2A	145260	AD	1q31-q42	?	?	(249)
PHA2B	601844	AD	17q21	*WNK4*	Protein kinase, lysine deficient 4	(250)
PHA2C	605232	AD	12q13	*WNK1*	Protein kinase, lysine deficient 1	(250)
Hypertension with Brachydactyly	112410	AD	12p12.2-p11.2	?	?	(251)
Liddle Syndrome (Pseudoaldosteronism)	177200					
Type 1	600760	AD	16p13-p12	*SCNN1B*	β subunit of EnaC, the renal epithelial sodium channel	(15)
Type 2	600761	AD	16p13-p12	*SCNN1G*	γ subunit of EnaC, the renal epithelial sodium channel	(16)
Progressive Nephropathy with Hypertension	161900	AD	1q21	?	?	(252)

TABLE 16-4

RENAL TUMOR LOCI

Disease	OMIM #	Mode of inheritance	Chromosomal localization	Gene name	Gene product(s)	Reference
Wilms Tumor*						
Wilms Tumor 1	194070	AD	11p13	*WT1*	Wilms Tumor 1, Zinc Finger Protein	(18,19)
Denys-Drash Syndrome (Wilms Tumor and Pseudohermaphroditism)	194080	AD	11p13	*WT1*	Wilms Tumor 1, Zinc Finger Protein	(253)
WAGR Syndrome (Wilms Tumor–Aniridia–Genitourinary Anomalies–Mental Retardation Syndrome)	194072	AD	11p13	*WT1*, *PAX6*, nearby DNA sequences (*CGS*)	Wilms Tumor 1, Zinc Finger Protein and Pax 6 Transcription Factor	(18–21)
WT2 (Multiple Tumor Associated Chromosome Region 1, MTACR1)	194071	AD	11p15.5	?	?	(254)
WT3	194090	AD	16q	?	?	(255)
WT4	601363	AD	17q12-q21	?	?	(256)
Von Hippel Lindau (VHL)	193300	AD	3p26-p25	*VHL*	VHL Protein	(83–85)
Renal Cell Carcinoma 1, RCC1	144700	AD	t(3;8)(p14.2;q24.1)	*FHIT/TRC8* gene fusion	TRC8 Protein (similar to patched protein), disruption of Fragile Histidine Triad Gene	(22,23)
Renal Cell Carcinoma (Papillary)	605074					
RCCP1(PRCC)	179755	AD	t(X;1)(p11.2;q21.2)	*PRCC/TFE3* gene fusion	PRCC/TFE3 Helix-Loop-Helix Transcription Factor	(257,258)
RCCP2 (MET)	164860	AD	7q31	*MET*	MET Proto-oncogene	(29)
RCCP3	605075	AD	17q21.32	?	?	(259)
Tuberous Sclerosis (Renal Angiomyolipoma, Renal Cell Carcinoma)	191100					
TSC1	605284	AD	9q34	*TSC1*	Hamartin	(260)
TSC2	191092	AD	16p13.3	*TSC2*	Tuberin (GTPase-Activating Protein for Rheb)	(27)

*Identifies diseases with probable additional, unmapped loci.
(*CGS*) indicates contiguous gene syndrome.

TABLE 16-5

INHERITED DISORDERS OF RENAL SALT WASTING

Disease	OMIM #	Mode of inheritance	Chromosomal localization	Gene name(s)	Gene product(s)	Reference
Bartter Syndrome (Hypokalemic Alkalosis with Hypercalciuria)						
Type I (antenatal)	241200	AR	15q15-q21.1	*SLC12A1*	Na-K-2Cl Co-transporter (Solute Carrier Family 12, Member 1)	(6)
Type 2 (antenatal)	600359	AR	11q24	*ROMK (KCNJ1)*	ROMK, Potassium Channel, Inwardly Rectifying, Subfamily J, Member 1	(261)
Type 3	602023	AR	1p36	*CLCNKB*	Chloride Channel, Kidney B	(262)
Type 4 (with sensorineural deafness) (infantile)	602522	AR	1p31	*BSND*	Barttin	(263)
Hypocalcemia with Bartter Syndrome	601199	AD	3q13.3-q21	*CasR*	Calcium-sensing Receptor	(264)
Digenic Bartter Syndrome	602522	Digenic	1p36	*CLCNKA* and *CLCNKB*	Chloride Channel, Kidney A and Chloride Channel Kidney B	(265)
Gitelman Syndrome (Hypokalemia-Hypomagnesemia with Hypocalciuria)	263800	AR	16q13	*SLC12A3*	Thiazide-Sensitive Na-Cl Co-transporter (Solute Carrier Family 12, Member 3)	(266)
Pseudohypoaldosteronism, Type I (PHAI)	264350	AR	12p13	*SCCN1A*	α Subunit, ENaC	(13)
Recessive Form 1						
Recessive Form 2	264350	AR	16p13.12	*SCNN1B*	β Subunit, ENaC	(13)
Recessive Form 3	264350	AR	16p13.12	*SCNN1G*	γ Subunit, ENaC	(14)
Autosomal Dominant	177735	AD	4q31.1	*NR3C2*	Mineralocorticoid Receptor	(267)

TABLE 16-6

INHERITED DISORDERS OF TUBULAR FUNCTION

Disease	OMIM #	Mode of inheritance	Chromosomal localization	Gene name(s)	Gene product(s)	Reference
Cystinuria						
Type I	220100	AR	2p16.3	*SLC3A1*	rBAT1, Solute Carrier Family 3, Member 1, Amino Acid Transporter	(268)
Type II and Type III (rare Type I)	600918	Incomplete Recessive	19q13.1	*SLC7A9*	bo,+AT, Solute Carrier Family 7, Member 9, Amino Acid Transporter Subunit	(11,269)
Dent Disease (X-linked Nephrolithiasis)	310468	X	Xp11.22	*CLCN5*	Chloride Channel 5	(32)
Nephrogenic Diabetes Insipidus						
X-linked	304800	X	Xq28	*AVPR2*	V2 Receptor	(270,271)
Autosomal Dominant	125800	AD	12q13	*AQP2*	Aquaporin-2	(272)
Autosomal Recessive	222000	AR	12q13	*AQP2*	Aquaporin-2	(273)
Primary Hypomagnesmia	248250	AR	3q	*PCLN1*	Paracellin-1 (Claudin 16)	(40)
Renal Tubular Acidosis						
Renal Tubular Acidosis Type II (Proximal)	604278	AR	4q21	*SLC4A4*	Sodium Bicarbonate Co-Transporter (Solute Carrier Family 4)	(274)
RTA Type I, Classic Distal	179800	AD	17q21-q22	*SLC4A1*	Chloride-Bicarbonate Exchanger AE1	(275,276)
RTA Type I, Classic Distal	179800	AR	17q21-q22	*SLC4A1*	Chloride-Bicarbonate Exchanger AE1	(277)
Distal Renal Tubular Acidosis with Progressive Nerve Deafness	267300	AR	2cen-q13	*ATP6B1*	B1 Subunit of H(+)-ATPase	(278)
Renal Tubular Acidosis, Distal, Autosomal Recessive (RTADR)	602722	AR	7q33-q34	*ATP6N1B*	116-kD subunit of H(+)-ATPase	(279)
Osteopetrosis with Renal Tubular Acidosis	259730	AR	8q22	*CAII*	Carbonic Anhydrase Type II	(280)

TABLE 16-7

MISCELLANEOUS DEVELOPMENTAL DISORDERS

Disease	OMIM #	Mode of inheritance	Chromosomal localization	Gene name(s)	Gene product(s)	References
Bardet-Biedl Syndrome	209900					
Type 1 (BBS1)	209901	AR	11q13	*BBS1*	BBS1	(281)
Type 2 (BBS2)	606151	AR	16q21	*BBS2*	BBS2	(282)
Type 3 (BBS3)	600151	AR	3p13-p12	*ARL6 (BBS3)*	ARL6	(283)
Type 4 (BBS4)	600374	AR	15q22.3-q23	*BBS4*	BBS4	(284)
Type 5 (BBS5)	603650	AR	2q31	*BBS5*	BBS5	(190)
Type 6 (BBS6; MKKS)	604896	AR	20p12	*MKKS*	MKKS Chaperonin Protein	(285,286)
Type 7	607590	AR	4q27	*BBS7*	BBS7	(287)
Type 8	608132	AR	14q32.1	*BBS8 (TTC8)*	BBS8 (TPR Repeat Protein 8)	(288)
Branchio-Oto-Renal Syndrome	601653	AD	8q13.3	*EYA-1*	Eyes Absent Homologue-1 Protein	(289)
CAKUT (Congenital Anomalies of the Kidney and Urinary Tract)*	300034	X	Xq22-q23	*AGTR2*	Angiotensin II Receptor, Type 2	(44)
Kallmann Syndrome I	308700	X	Xp22.3	*KAL (ADMLX)*	Anosmin-1	(290)
Meckel-Gruber Syndrome						
Type 1 (MKS1)	249000	AR	17q22-q23	?	?	(291)
Type 2 (MKS2)	603194	AR	11q13	?	?	(292)
Type 3 (MKS3)	607361	AR	8q24	*TMEM67 (MKS3)*	Meckelin	(293)
Multicystic Renal Dysplasia, Bilateral (MRD)*	143400	AD	6p21	*CDC5L*	Pombe Cell Division Cycle 5-related Protein	(294)
Oro-facial-Digital Syndrome	311200	X	Xp22.3-Xp22.2	*OFD1*	OFD1	(295)
Renal-Coloboma Syndrome	120330	AD	10q24.3-q25.1	*PAX2*	PAX 2 Transcription Factor	(296)
Vesicoureteral Reflux with Reflux Nephropathy*	193000	AD	1p13	?	?	(297)

*Identifies diseases with probable additional, unmapped loci.

TABLE 16-8

MISCELLANEOUS INHERITED DISORDERS WITH RENAL INVOLVEMENT

Disease	OMIM #	Mode of inheritance	Chromosomal localization	Gene name(s)	Gene product(s)	Reference
Cystinosis, Nephropathic (CTNS)	219800	AR	17p13	*CTNS*	Cystinosin	(37)
Cystinosis, Late-Onset Juvenile or Adolescent Nephropathic Type	219900	AR	17p13	*CTNS*	Cystinosin	(298,299)
Cystinosis, Benign or Adult Nonnephropathic Type	219750	AR	17p13	*CTNS*	Cystinosin	(300)
Fabry Disease	301500	X	Xq22	*GLA*	Alpha–galactosidase, A subunit	(301)
Familial Mediterranean Fever (FMF)	249100	AR	16p13	*FMF*	Pyrin	(302,303)
Familial Hemolytic-Uremic Syndrome*						
Autosomal Recessive	235400	AR	1q32	*HF1*	Factor H	(304)
Autosomal Dominant	235400	AD	1q32	*HF1*	Factor H	(305)
Fanconi-Bickel Syndrome	227810	AR	3q26.1-q26.3	*GLUT2*	Facilitative Glucose Transporter 2	(306,307)

*Identifies diseases with probable additional, unmapped loci.

was responsible for the genetic susceptibility to Wilms tumor, whereas *Pax6* was responsible for the aniridia phenotype (21). One of the familial forms of renal cell carcinoma (RCC1) also was identified on the basis of its underlying chromosomal rearrangement. A translocation between chromosomes 3p and 8q t(3;8)(p14.2;q24.1) was found to segregate with renal cell carcinoma (22). Nearly two decades later, investigators identified the genes disrupted by the translocation. They determined that the chromosomal re-arrangement resulted in a novel gene that consisted of 5′ elements of a gene called *FHIT* (Fragile Histidine Triad Gene) fused to the coding sequence of *TRC8* (23). The protein product of *TRC8* has high homology to the basal cell carcinoma/segment polarity gene product, Patched, a signaling receptor, suggesting that it may have a similar function.

Perhaps one of the most striking examples of the power of cytogenetic abnormalities to expedite gene discovery is that provided by the search for *PKD1*, the gene responsible for the most common form of ADPKD. A combination of molecular and genetic techniques had rapidly localized the gene to a 500kb gene-rich segment but the lack of known chromosome re-arrangements or deletions coupled with the large number of potential candidate genes greatly complicated the search (24,25). Several years of mutation screening had failed to determine which one of the many candidates was in fact *PKD1* when an astute clinician identified an unusual family that had individuals with classic ADPKD as well as a child with both tuberous sclerosis and renal cysts. Since it was known that a major form of tuberous sclerosis (TSC2) was located near the *PKD1* gene (26), cytogenetic studies of the family were undertaken. This revealed two individuals in the family with balanced translocations between chromosomes 16 and 22 [t(16;22)(p13.3;q11.21)] (Fig. 16-1) (27). The child with TSC2 had an unbalanced karyotype and was missing a portion of chromosome 22 as well as the telomeric portion of chromosome 16 45XY/-16-22 + der(16)(16qter-16p13.3::22q11.21-22qter)]. It was correctly speculated that the *TSC2* gene was located in the portion of chromosome 16 that was lost while *PKD1* was likely to be the gene bisected by the translocation breakpoint. Further study confirmed this and allowed the identification of both *TSC2* and *PKD1* (27,28).

Although gene hunters may occasionally get lucky and identify a family such as the one described above, most of the time linkage analysis is necessary to pinpoint the location of a gene. Both well-characterized pedigrees and an array of genetic markers are essential for performing linkage analysis. The human genome project has positioned on the chromosomes (mapped) thousands of short stretches of DNA that differ within the normal population in their length or sequence and that can be used to trace inheritance of parental chromosomes within families. Genetic disease develops as a consequence of an inherited DNA sequence variation that disrupts the normal function of a gene product and results in a specific phenotype that can be identified. The inheritance of a disease phenotype can be compared with the inheritance of hundreds of mapped genetic markers and so linkage and a chromosomal address can be established.

The principle underlying this approach is as follows: alleles of loci (a specific chromosomal address of a DNA segment) on nonhomologous chromosomes segregate randomly during meiosis whereas alleles of loci physically close on the same chromosome are inherited together and are said to be linked. Meiotic recombination produces novel haplotypes by exchanging alleles between homologous chromosomes and the frequency with which this occurs depends in part on the distance separating them. In other words, if the alleles of two genes are adjacent to each other on the same chromosome segment, there will be no recombination between them. Statistical programs are used to score the probability that an observed association has not happened by chance. The most common approach determines the ratio of the probability of the observed associations assuming linkage to that of no linkage. The *lod score* is the decimal logarithm of this ratio and is considered significant when greater than 3.

Until recently, it was necessary to clone the chromosomal interval in question and then to screen it for genes, that is, expressed sequences. Each expressed region would be sequenced at least in part in order to develop reagents that could be used to scan for pathogenic mutations. These steps often took many years to complete. The human genome project, as well as all of the resultant databases, has revolutionized this process in a number of important ways. Firstly, virtually the complete sequence of the human genome is presently available in public databases. This has allowed the precise localization of all known genes. Secondly, the genomic sequence has been used to map thousands of genes of unknown function that had been previously identified by the EST mapping project. As part of the genome mapping effort, gene mappers had generated a database of expressed sequence tags (ESTs) for numerous

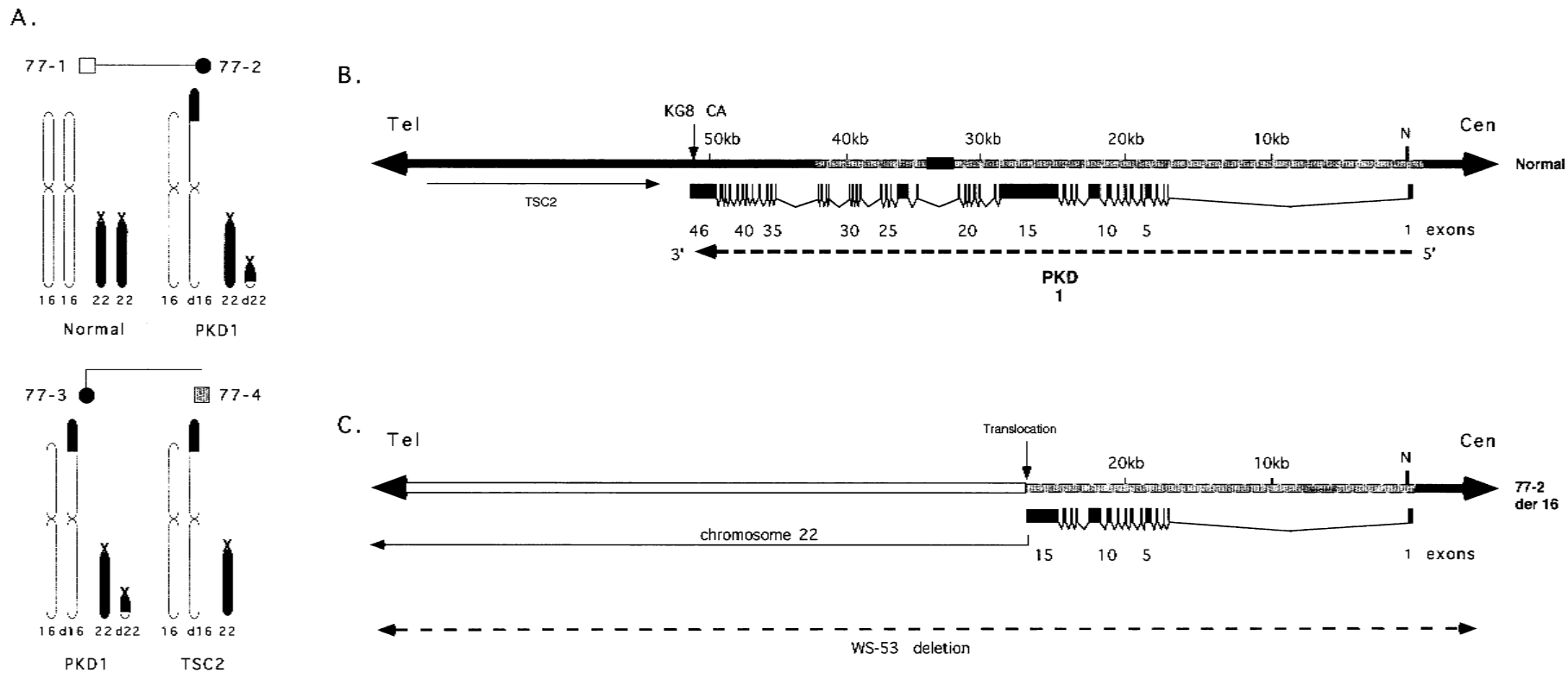


FIGURE 16-1. A: Pedigree 77 with chromosomal abnormalities that segregate with ADPKD and tuberous sclerosis. Unfilled box is the unaffected father. Filled symbols identify individuals affected with ADPKD. The shaded box (77-4) identifies an individual with tuberous sclerosis and cystic disease of the kidney. The homologous pairs of chromosome 16 and 22 for each individual are schematically illustrated beneath each symbol. Individuals 77-2 and 77-3 have ADPKD and a balanced translocation [t(16;22)(p13.3;q11.21)], whereas individual 77-4 has an unbalanced translocation with loss of der22 [−16 − 22 + der(16)(16qter16p13.3:22q11.2122qter)]. *16* and *22* identify normal chromosomes whereas *d16* and *d22* define the derivative chromosomes. (Adapted from European Polycystic Kidney Disease Consortium. The polycystic kidney disease 1 gene encodes a 14 kb transcript and lies within a duplicated region on chromosome 16. *Cell* 1994;77:881, with permission.) **B:** Genomic structure of *PKD1*. The top line represents the chromosome segment with its orientation as listed (tel, telomere, cen, centromere). The segment is marked in kilobases (kb). *N* identifies a *Not* I restriction site that maps to the *5′* end of the gene. The location of the intragenic KG8 CA microsatellite is shown. (From: Germino GG, et al. Positional cloning approach to the dominant polycystic kidney disease gene, PKD1. *Kidney Int Suppl* 1993;39:S20; Snarey A, et al. Linkage disequilbrium in the PKD1 region. *Am J Hum Genet* 1994;55:365.) The shaded portion of the chromosome indicates the segment of the gene that is found replicated elsewhere on chromosome 16. The darkly stippled box within the replicated region indicates the position of the polypyrimidine tract that bisects the gene. The numbered, linked vertical bars and boxes beneath the chromosome represent *PKD1* exons. The direction of transcription for *PKD1* and *TSC2* is indicated by the arrow above each gene name. (Adapted from: European Chromosome 16 Tuberous Sclerosis Consortium. Identification and characterization of the tuberous sclerosis gene on chromosome 16. *Cell* 1993;75:1305; European Polycystic Kidney Disease Consortium. The polycystic kidney disease 1 gene encodes a 14 kb transcript and lies within a duplicated region on chromosome 16. *Cell* 1994;77:881, with permission.) **C:** Molecular map of the PKD1 translocation. The top segment presents the derivative chromosome 16 that is interrupted by the balanced translocation in individuals 77-2 and 77-3. The dashed line at the bottom indicates the approximate extent of a deletion that was discovered in an individual with tuberous sclerosis and severe polycystic kidney disease at any early age. (Adapted from: European Polycystic Kidney Disease Consortium. The polycystic kidney disease 1 gene encodes a 14 kb transcript and lies within a duplicated region on chromosome 16. *Cell* 1994;77:881; Brook-Carter PT, et al. Deletion of the *TSC2* and *PKD1* genes associated with severe infantile polycystic kidney disease—a contiguous gene syndrome. *Nat Genet* 1994;8:328, with permission.)

species. The idea was to generate a catalogue of all genes expressed in a given tissue and organism. Short snippets of sequence, typically several hundreds of base pairs in length from the 5′ and 3′ ends of cDNA clones, had been determined and catalogued for thousands of genes. The availability of the complete human genomic sequence has allowed their accurate localization. Finally, genome annotators have used various gene prediction programs to identify putative genes based on their sequence characteristics. Thus, once linkage analysis defines a chromosomal address for a disease locus, one can identify virtually all of the potential candidate genes without performing a single wet bench experiment. These resources have shaved years off the gene-discovery process.

Despite these advances, the task of identifying a disease-associated gene can still be a time-consuming endeavor. The reason for this is that the resolution of genetic mapping is typically on the order of 500,000 to 1,000,000 bp. With an average gene density of one gene per 30,000 base pairs in gene dense regions, a segment of this length could harbor between 30 to 40 genes! For diseases that are uncommon, the small number of family members available for testing may limit the resolution of genetic mapping to an area millions of base pairs in length. A careful, systematic evaluation of each gene could take years to complete.

Several tricks can be used to minimize the amount of effort. The first is to use the publicly available databases to determine the identity and likely function of the genes within one's target region. This is most successfully accomplished by submitting the putative translated sequence (i.e., protein sequence) of each of the genes to protein databases that report known motifs. In earlier years, one would have to first isolate the complete cDNA for the gene of interest before one could search for hidden homologies. Now, with the availability of gene-prediction software, one can use genomic sequence for these initial analyses and then focus one's effort on the most interesting candidates. A detailed understanding of the pathobiology of a disease can allow one to narrow the field of candidates to those that have functions consistent with the underlying defect. For example, linkage studies revealed that one of the loci responsible for familial papillary renal carcinoma (RCCP2) mapped to an interval on 7q31 that included the MET proto-oncogene. The well-established relationship between MET and other carcinomas prompted the investigators to focus their search on this gene, leading to the rapid discovery of pathogenic mutations (29). In another example, Dent's disease was known to be an X-linked disorder often associated with microdeletions of Xp11.22 (30). Fisher et al. initiated their search for the gene responsible for Dent's disease by screening for expressed sequences that were encoded by the deleted segment. They identified a novel chloride channel family member that was deleted in many patients with Dent's disease, that had a restricted pattern of expression, and whose function was consistent with the pathophysiology of the disease (31). They subsequently showed that mutations of CLCN5 were responsible for this disease (32). Frequently, astute database analysis can be critical for rapid gene identification. In the case of the second gene responsible for ADPKD, *PKD2*, researchers realized that one of the putative proteins in their genetic interval had homology to the gene product of *PKD1*, polycystin-1. This gene was an obvious candidate for PKD2 and mutation analysis quickly confirmed this suspicion (33).

A second approach used to speed the pace of gene discovery is to search for disease-associated microscopic chromosomal abnormalities that are below the level of resolution of standard cytogenetic analyses. The genes responsible for the most common form of nephronophthisis (*NPHP1*) and for cystinosis (*CTNS*) were identified in this manner. In the case of *NPHP1*, large-scale rearrangements were detected in 80% of the patients belonging to inbred or multiplex *NPH1* families and in 65% of the sporadic cases (34). Most of the time, large homozygous deletions of approximately 250 kb involving a 100-kb inverted duplication were discovered to disrupt the gene. In a small number of individuals, oligo-base pair mutations were identified, proving that *NPHP1* was the specific gene responsible for the disorder (35,36) *CTNS* was identified in a similar manner. Investigators found that one of the genetic markers used in their study was homozygously deleted in 23 out of 70 patients (37). They quickly focused their search on the minimal region deleted and identified a ubiquitously expressed transcript that was disrupted in all patients with deletions involving this segment. They subsequently found single or oligo-base pair mutations in many of the remaining patients, thus proving that this gene and not one of its neighbors was in fact responsible for the disease.

A third strategy is to determine the expression pattern of the various candidates and see which, if any, correlates with that predicted by the clinical features of the disorder. Fuchshuber and colleagues had localized a form of steroid-resistant idiopathic nephrotic syndrome (NPHS2) to a 2.5 million-base pair interval on chromosome 1 (38). They had identified multiple putative candidates but focused their search on one whose expression by Northern blot was detectable only in fetal and adult renal tissues. They subsequently discovered recessive, inactivating mutations in *NPHS2*, and further showed that its expression was restricted to glomerular podocytes (39). In a similar manner, the kidney-restricted pattern of expression of *PCLN1* helped to identify it as a probable candidate gene for Primary Hypomagnesemia (40). This approach is not without its limitations, however, since unexpected expression patterns are sometimes observed. In the case of nephronophthisis, for example, the gene found responsible for the disorder, *NPHP1*, is minimally expressed in the kidney (35,41).

There are a number of disorders where none of the aforementioned short cuts have been found to apply. The majority (ADPKD and Bardet Biedl are representative examples) have complex phenotypes involving multiple organ systems, making it difficult to limit one's search to genes with a restricted, compatible pattern of expression. Moreover, the complicated phenotypic presentation makes it more difficult to predict *a priori* what a "reasonable" candidate might be. In these cases, one must resort to the use of sequence-based strategies to identify the disease gene. Fortunately, sequencing technologies have become much more efficient and cost-effective and therefore in difficult situations a systematic comparison of "normal" and "affected" genomes may be justified.

It is important to note one further development in the genomics field that is likely to play an increasing role in the identification and characterization of disease-associated genes. The ability to manipulate the murine genome through gene targeting (described in more detail later) has allowed investigators to generate a lengthy list of murine models of human diseases. In most cases, scientists first identified the disease gene and then created a mutant phenotype in mouse with the intention of modeling the human disease state (see the subsequent text). In some cases, however, the genes targeted for study had not been previously implicated in a genetic disorder; rather, they had been selected for study because the investigators had a fundamental interest in their biological properties. Careful analysis of the murine phenotypes revealed surprisingly similarity to human diseases, leading the investigators to test for mutations in their human homologues. It was in this way that *LMX1B*, which encodes LM1B, a homeodomain protein, was found to be mutated in Nail-Patella Syndrome (NPS). Investigators with an interest in basic developmental processes had targeted this gene for inactivation and discovered limb and kidney defects in *Lmx1b* mutant mice that were remarkably similar to those observed in Nail Patella Syndrome (42). They quickly identified three independent NPS patients with *de novo* heterozygous mutations of *LMX1B* (43). The relationship between mutations of the Angiotensin II Receptor, Type 2 (*AGTR2*) and CAKUT

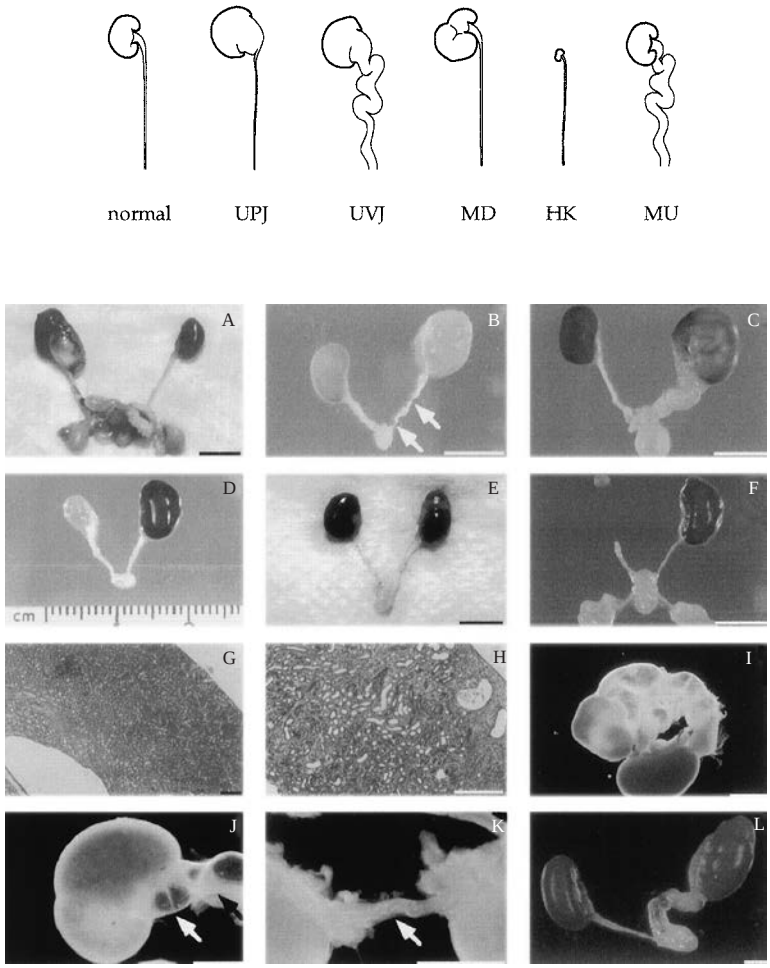


FIGURE 16-2. Gross anatomy and light microscopic patterns of congenital anomalies of GU tract found in *Agtr2* null mutant mice. The schematic drawing on top shows the spectrum of CAKUT found in mutant animals. **A:** Hydronephrotic kidney due to stenosis at the uteropelvic junction (UPJ). **B:** UPJ with atretic ureters (*white arrows*). **C:** Hydronephrotic kidney and hydroureter due to stenosis at the uterovesical junction (UVJ). **D:** Hypoplastic kidney (HK) with microscopically dysplastic kidney. **E:** Multicystic dysplastic kidney (MD). **F:** Aplastic kidney (*on left*). **G, H:** Microscopic analysis of tissue with cortical thinning (**G**) and dysplastic changes (**H**). **I:** Example of massive dilatation and tortuosity of affected ureter (megaureter, MU). **J:** Double ureters in an E17.5 embryo. Upper pole of the kidney is hydronephrotic attached to hydroureter (*white arrow*) while the remaining kidney tissue appears normal with nonhydrotic ureter (*black arrow*). **K:** Tortuous and winding left ureter (*arrow*) encased within undifferentiated mesenchymal tissue of an E17.5 embryo. **L:** Similar pattern observed in a mutant animal at 1 week of age. White or black bars indicate 1 cm, 200 μm, and 1 mm for (**A**) through (**F**), (**G**) and (**H**), and (**I**) through (**L**), respectively. (From: Nishimura H, et al. Role of the angiotensin type 2 receptor gene in congenital anomalies of the kidney and urinary tract, CAKUT, of mice and men. *Mol Cell* 1999;3:1, with permission.)

(Congenital Anomalies of the Kidney and Urinary Tract) was discovered in a similar manner. Mice lacking *AGTR2* had variable penetrance for congenital anomalies similar to those described for the human form of CAKUT (Fig. 16-2). The observation prompted the investigators to analyze human *AGTR2* in patients with CAKUT and they identified a nucleotide transition within the lariat branchpoint motif of intron 1 of human *AGTR2*, which perturbs its mRNA splicing efficiency (44).

Sometimes mouse models created to study a known function of a protein yield surprising results. For example, CD2AP was not known to have a role in glomerular function until mice lacking CD2AP were generated (45,46). Previously, CD2AP was thought to be an adapter protein critical for stabilizing contacts between T cells and antigen presenting cells. Mice that were null for CD2AP had compromised immune function but they were noted to die unexpectedly at 6 to 7 weeks of renal failure. The investigators showed that homozygotes developed proteinuria associated with defects in epithelial cell foot processes and eventual glomerulosclerosis. Based on this data, it could be demonstrated that CD2AP was expressed in podocytes where it associates with nephrin, the primary component of the slit diaphragm. While mutations of CD2AP have not yet been shown to be a cause of familial nephrosis in humans, this gene assuredly will be among the candidates considered for future studies. It is likely that additional fortuitous relationships will be established, as the list of murine genes that are inactivated by gene targeting becomes more complete.

In summary, the combination of multiple avenues of biological data with genetic map position has proved to be a powerful strategy for finding disease genes. Hence we have witnessed a remarkable increase in the number of inherited diseases whose genetic bases has been elucidated.

POSTCLONING PHASE OF GENE DISCOVERY

Using Databases

Identification of a gene is often when the work of defining the biology of a disease begins. This is especially true in diseases such as ADPKD or tuberous sclerosis where a complex phenotype exists and a unifying biochemical defect is not apparent. The first step is usually to perform a series of database analyses, looking for sequence similarities, functional motifs, and structural features that might be used to generate a variety of testable hypotheses regarding a gene's function. In some cases, one finds that a segment of one's query has a high degree of similarity to a family of proteins of known function. In primary hypomagnesemia, the protein encoded by *PCLN1* was found to have sequence and structural similarity to members of the claudin family (40). All other members of this family localize to tight junctions and appear to bridge the intercellular space by homo- or heterotypic interactions, suggesting a similar function for *PLCN1*. This result was particularly intriguing in that renal magnesium ion (Mg2+) resorption occurs predominantly through a paracellular conductance in the thick ascending limb of Henle (TAL).

In other situations, one may identify homologous genes in other organisms, vertebrate or invertebrate, that have already been studied and for which a function may be known. The potential power of this strategy to help expedite the study of disease genes is highlighted by the fact that over 60% of human disease genes have a homologue in the common fruit fly, *Drosophila* melanogaster, and a surprisingly high fraction is even conserved in yeast [47–51). The *TSC1* and *TSC2* genes, mutated in tuberous sclerosis, are representative examples. The sequence of each gene provided little in the way of functional insight. Tuberin, the gene product of *TSC2*, was found to have a region of homology to the GTPase-activing protein GAP3 (27) whereas hamartin, the *TSC1* protein, was homologous to a yeast protein of unknown function (52). Given that other GTP-ase activating proteins had previously been identified as tumor suppressors, it was presumed that tuberin would have a similar function but the mechanism by which it would do so was obscure. Slow progress was made in defining the respective function of each TSC protein until the discovery and functional characterization of their homologues in Drosophila (discussed later). As saturation mutagenesis strategies of simpler organisms help to reveal the function of currently unknown genes, these insights will increasingly help us to better understand the human homologues.

Conservation between species is also helpful because it can be used to identify essential functional components that are hidden in otherwise featureless sequence. In the case of *PKD1*, for example, a comparison of the sequence of human polycystin-1 to that of the puffer fish, *Fugu rubripes*, two species separated by nearly 400 million years of vertebrate evolution, identified several highly conserved regions with no known sequence homologies (53). One of these was subsequently identified as a putative 20-amino-acid heterotrimeric G-protein activation sequence (54). Sequence conservation can also be used to help resolve disputes regarding the putative structure of a protein. In the case of polycystin-1, comparison of the human and *Fugu* sequences led to the identification of a set of 11 conserved probable transmembrane spanning elements (53), subsequently confirmed by biochemical methods (55).

Several databases use specific amino acid patterns culled from an analysis of many proteins to find certain motifs. This can be an especially powerful tool since it can identify patterns otherwise missed by direct sequence comparisons. One example of the successful use of this approach is provided by the identification of a GPS domain (G-protein coupled receptor Proteolytic Site) in polycystin-1 (56). This domain was first identified as the internal cleavage site for the neuronal G-protein coupled receptor, latrophilin, the putative receptor for α-latrotoxin and a member of the LNB-TM7 family of proteins (57–59). The GPS site is a feature common to all members of the family, positioned in the extracellular N-terminus approximately 30 amino acids before the first transmembrane spanning element of each protein. In the case of latrophilin, cleavage at the site yields two fragments: a C-terminal portion that includes a short extracellular stalk followed by the 7 TM-spanning elements and an N-terminal portion that is tethered to the extracellular stalk. It is presumed that cleavage and tethering is essential for creating the active ligand binding site for α-latrotoxin or its endogenous ligands. Recent studies by Qian et al confirm that the GPS site in polycystin-1 similarly undergoes proteolytic cleavage and that cleavage and tethering appear essential for the protein's signaling properties (60). The observation also prompts further consideration of the hypothesis put forward by Parnell et al that polycystin-1 may be the prototype of a novel class of G-protein coupled receptor.

Finally, a variety of algorithms can be used to identify functional protein domains. For example there are a number of programs that can be used to predict hydrophobic areas in a protein that may signify transmembrane regions. This type of information can be extremely important in deciphering the function of a protein. In the case of *PKD2*, this type of analysis suggested that polycystin-2 had six transmembrane domains and resembled a known class of cation channels (61). This hypothesis was testable and it was ultimately confirmed that polycystin-2 could function as a nonselective cation channel in appropriate conditions (61).

MUTATION ANALYSIS AND THE MOLECULAR MECHANISM OF DISEASE

An important step in determining the molecular basis of disease is defining how disease mutations alter the function of the gene product. In recessive disorders, the fact that mutation of both copies of a gene is required to cause the disease suggests that the mutations must impair or result in complete loss of the protein's normal function. In dominant disorders, the mechanism by which mutations cause disease is less straight forward since they can act in one of three ways: (1) by reducing the amount of functional protein (via a hypomorphic allele, haploinsufficiency or by a combination of germ line and somatic mutations); (2) by producing a protein with increased activity or a new function (dominant gain-of-function); (3) or by producing proteins that inhibit the function of the normal allele (dominant negative).

In the case of ADPKD, it was certain clinical features of the disease that provided the clue to unraveling the molecular mechanism of disease. One of the hallmarks of ADPKD is its focal presentation (62). Pathologic examination of cystic kidneys has shown that cysts arise as focal outgrowths of normal tubules. The logical question was that if all cells are genetically identical and carry the same germ line mutation, then why don't all cells form cysts? Considered among the many different potential explanations was the possibility that each cyst might be genetically distinct, forming as the consequence of a second, possibly rate-limiting step. To examine this possibility, a novel method was devised for isolating DNA from the epithelial cells lining individual cysts. Using these techniques, it was demonstrated that renal cysts originate from one cell and thus can be termed monoclonal (63). Furthermore, somatic mutations affecting the normal allele can be detected in a significant fraction of cysts (64–71). This model is depicted in Figure 16-3 and suggests that the first step in cyst formation is functional inactivation of the normal PKD allele. This model implies that PKD is recessive on a molecular level since both copies of PKD are mutated in each cyst. A small minority of cysts from kidneys with a germ line mutation in *PKD2* may instead have as their somatic hit an inactivating *PKD1* mutation and vice versa (68,72). This finding has several clinical implications. First, it means that any type of therapy would have to be aimed at replacing the function of the gene product and also, ideally, it would be targeted to those cells where a "second hit" has actually occurred. Second, these findings explain at least some of the observed clinical variability since the rate at which second hits occur may determine disease severity. Clearly, an understanding of the factors that determine the rate of second hits might be used to slow disease progression. These might include processes that cause DNA instability or alternatively increased cellular proliferation.

Mutation analysis of a large number of pedigrees may also be used to establish genotype-phenotype correlations. One may discover that certain patterns of mutation are associated with specific clinical characteristics. For example, some mutations may lead to an unstable message that is never made into protein. This may have more severe functional consequences than a protein that is made but has only partially reduced activity.

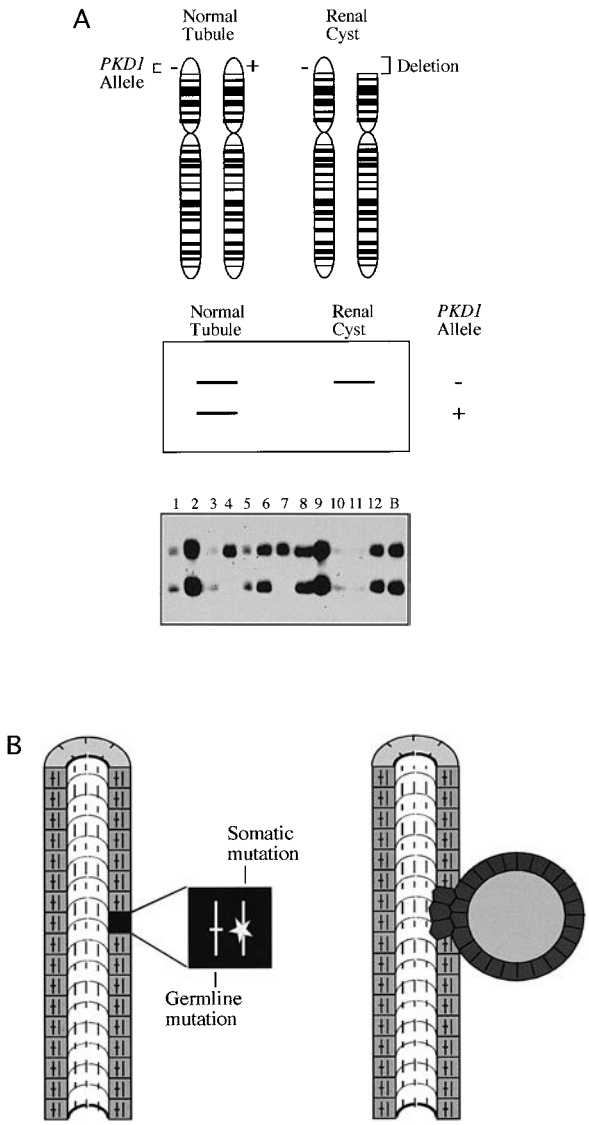
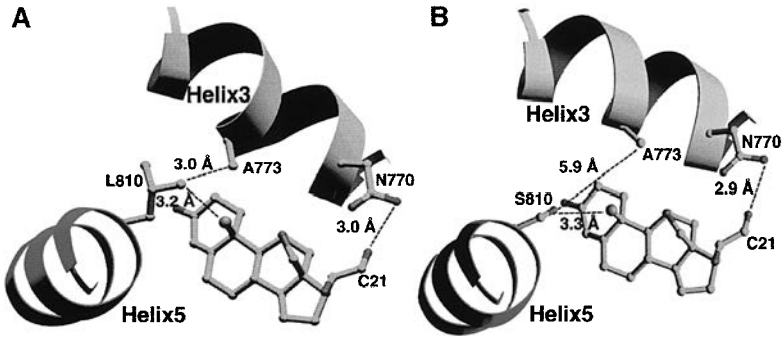


FIGURE 16-3. A: Loss of heterozygosity for the normal *PKD1* allele in renal cystic tissue. *Top panel:* Each renal tubular cell in an individual with ADPKD contains two *PKD1* alleles, one mutant copy (indicated by −) and one normal copy (indicated by +). The alleles can be distinguished using PCR-based assays if the allele-specific PCR products differ in size. Deletions of chromosome 16 that remove the normal *PKD1* locus (*top panel*) will lead to LOH, as evidenced by the disappearance of the PCR product derived from this allele. As a result, only one band remains (*middle panel*). The bottom panel shows the results of this type of analysis on 12 cysts from an individual with ADPKD using the marker KG8, an intragenic *PKD1* marker. Most of the cysts have two *PKD1* alleles, but cysts #4 and #7 have only one band because each has lost the normal *PKD1* allele (*bottom band*). **B:** "Two-hit" model of cyst formation in ADPKD. Each epithelial cell in a renal tubule has the same germline mutation of one *PKD1* allele. The normal allele presumably produces sufficient quantities of polycystin to permit normal differentiation and maintenance of tubular integrity. When a cell acquires a somatic mutation in the normal allele (indicated by a star), the level of polycystin falls below a critical threshold. The cell harboring the somatic mutation undergoes clonal expansion and forms a cyst via pathways that not yet have been identified. (From: Qian F, Watnick TJ. Somatic mutation as mechanism for cyst formation in autosomal dominant polycystic kidney disease. *Mol Genet Metab* 1999;68:237, with permission.)

Mutation studies of affected individuals also may provide a database of allelic variants that can be used to guide the study of important functional domains. Missense mutations that have phenotypic consequences are particularly instructive because they identify essential amino acid residues and critical functional elements. The examples cited below illustrate how the information gained from mutation studies can enhance the understanding of disease pathogenesis.

One of the goals of mutation analysis is to attempt to correlate genotype with phenotype. This information is important because it can allow physicians to counsel patients and potentially to screen presymptomatic individuals for rare disease manifestations. Although this approach has not been universally successful, it has proved to be helpful in some situations. In classic X-linked Alport syndrome, pathogenic mutations involve the 5 chain of type IV collagen. Most of the published mutations have been associated with sensorineural hearing loss and progression to ESRD in the second or third decade of life (73–78). Mutation analysis has identified subgroups of patients, however, that vary from the standard presentation. For example, there is a group of families that have classic Alport's disease coupled with leiomyomatosis of the esophagus and tracheobronchial tree. Affected females also have leiomyomatosis involving the genital tract. These pedigrees appear to have deletions that include the 5′ end of the collagen 4 5 (*COL4A5*) gene and the 5′ end of the collagen 4 6 (*COL4A6*) gene, which abuts it in a head-to-head configuration (79–82). Interestingly, deletions extending beyond the second intron of *COL4A6* are not associated with leiomyomatosis. The reasons for this are not clear. On the other end of the spectrum there are families who exhibit later onset of ESRD and deafness. One group of investigators identified 9 kindreds with a leucine to arginine mutation (L1649R) that results in a mild phenotype (76). The change affects a highly conserved residue in the noncollagenous (NCI) domain of the type IV collagen molecule that is probably involved in key inter- and intramolecular interactions. An analysis of 84 affected males showed that they tended to develop renal failure by the 4th or 5th decade and this preceded the onset of significant hearing loss by approximately 10 years. Haplotype analysis showed that all of these families were likely to have descended from a common ancestor and this mutation is probably a common cause of Alport's in the western United States.

Von Hippel-Lindau disease (VHLD) is another example of the utility of genotype phenotype correlations. VHLD is a dominantly inherited familial cancer syndrome that is characterized by a predisposition to develop renal carcinomas and hemangioblastomas of the retina and central nervous system. The disease is caused by mutations in *VHL* on chromosome 3 (83,84). Consistent with its predicted function as a tumor suppressor gene, somatic mutation of the wild type *VHL* allele has been detected in renal tumors from families with germ line *VHL* mutations (85). The function of the VHL protein is not entirely understood but it has been found to bind to two other proteins, elongin B and C (86,87). Elongin is part of a multiprotein complex that appears to target proteins for proteolytic degradation in proteosomes (88–90). The most widely studied effect of the VHL protein is its effect on the regulation of hypoxia inducible genes (91). Clinically, VHLD falls into two categories: those cases with (VHL2) and those without pheochromocytomas (VHL1) (92–94). It turns out that these clinical phenotypes are closely correlated with the class of mutation that is detected. VHL1 is produced by mutations that completely disrupt VHL protein structure, e.g., frameshift mutations, stop codons and gene deletions. In biochemical assays these proteins have no biological activity. On the other hand, VHL2 appears to be caused by missense mutations in residues that would be predicted to be in contact with elongin C or that map to amino acids where they would



C

Helix 5		Helix 3		Aldosterone	19-NP
S	$-CH_2-OH$	CH_3-	A	100.0 ± 5.8	6.1 ± 1.2
M	$-CH_2-CH-S-CH_3$	CH_3-	A	120.3 ± 9.8	111.0 ± 5.5
L	$-CH_2-CH(CH_3)-CH_3$	CH_3-	A	123.1 ± 15.7	109.2 ± 12.2
V	$-CH(CH_3)-CH_3$	CH_3-	A	116.8 ± 30.4	54.4 ± 4.9
A	$-CH_3$	CH_3-	A	40.0 ± 7.2	3.2 ± 0.2
V	$-CH(CH_3)-CH_3$	H-	G	138.2 ± 38.9	5.7 ± 2.0
L	$-CH_2-CH(CH_3)-CH_3$	H-	G	108.1 ± 8.4	96.8 ± 6.9

FIGURE 16-4. Helix 3-Helix 5 interaction in progesterone-mediated activation of mutant mineralocorticoid receptor, MR_{L810}. **A:** Structural model of a portion of the hormone-binding domain of MR_{L810} bound to aldosterone, based on the crystal structure of progesterone receptor. This model predicts that the side chain of L810 lies sufficiently close to A773 and the C19 methyl group of the steroid to form van der Waals interactions. **B:** Model of $MR_{wild\ type}$. The side chain of S810 does not interact with A773 because the distance between them is too great. **C:** Activity of mineralocorticoid receptors (MRs) with various amino acid substitutions at residues 810 and 773. Mutant receptors containing the indicated substitutions at the two positions were assayed using a luciferase reporter assay in the presence of 1nM aldosterone or 19-NP (19-norprogesterone, a progesterone derivative). MRs with amino acid substitutions that contained larger side chains were activated by 19-NP, as predicted by the model in **A** and **B**. Abbreviations for the amino acid residues are as follows: *A*, Alanine; *G*, Glycine; *L*, Leucine; *M*, Methionine; *S*, Serine; and *V*, Valine. (From: Geller DS, et al. Activating mineralocorticoid receptor mutation in hypertension exacerbated by pregnancy. *Science* 2000;289:119, with permission.)

be likely to cause only local defects. VHL2 alleles tend to retain biologic activity. Mutation analysis in this disease can not only be used to establish a presymptomatic diagnosis but also can provide specific information with respect to the disease phenotype.

As will be discussed in a subsequent section, missense mutations pose a major challenge since it may be difficult to assess their pathogenic significance. Under certain circumstances, however, important information can be gained about the function of a protein by careful analysis of a single amino acid change. Perhaps the best example of this is a recently described mutation in the mineralocorticoid receptor (MR), S810L that causes early onset hypertension in heterozygous carriers (95). This mutation affects an amino acid that lies in the MR hormone binding domain (HBD) and that is conserved in MRs across a wide evolutionary span. In order to test the functional significance of this change, investigators expressed wild type and mutant MRs in Cos-7 cells and were able to show that in the absence of added steroid the mutant MR retained a significant level (27% of maximal) of activity. This suggested that the receptor was constitutively active to some degree. They took their analysis a step further and in an elegant series of experiments showed that certain steroids that normally bind but do not activate wildtype MRs were able to activate the mutant receptor. Steroids with 21-hydroxyl groups such as aldosterone were able to bind and activate both proteins while steroids with 17-keto groups (i.e., estradiol and testosterone) activated neither. Steroids such as progesterone, which lacked both modifications, were found to be potent activators of the mutant MR leading to the conclusion that activation no longer required a steroid 21-hydroxyl group. Using computer manipulation of an established structural model of the steroid HBD the authors were able to understand that the mutation resulted in increased van der Waals interaction between helix 5 and helix 3 of the HBD (Fig. 16-4). This essentially substituted for the interaction of the steroid 21-hydroxyl group with helix 3 in the wild type receptor. There were several insights that were gained from this analysis. First, these findings explained why several affected women in families with the S810L mutation developed severe hypertension during pregnancy, a state that is associated with a drastic increase in progesterone levels. Second, it provided a critical understanding of the general basis for steroid hormone activation since the helix 5-helix 3 interaction is highly conserved among diverse nuclear hormone receptors. Lastly, this type of in-depth structural understanding is often a prerequisite for rational drug therapy.

DEFINING THE EXPRESSION PATTERN OF A GENE AND ITS PROTEIN PRODUCT

The expression pattern of a gene may provide valuable information about its function. Northern blots of tissue are commonly used as a screening tool but are very insensitive and provide little information regarding the specific cell types that express the gene of interest. For example, *NPHS1* and *PCLN1* have a similar pattern on Northern blot—each detects a kidney-specific signal—though they have very different functions (40,96). More-precise localization can be achieved using *in situ* hybridization techniques (Fig. 16-5). Using this method,

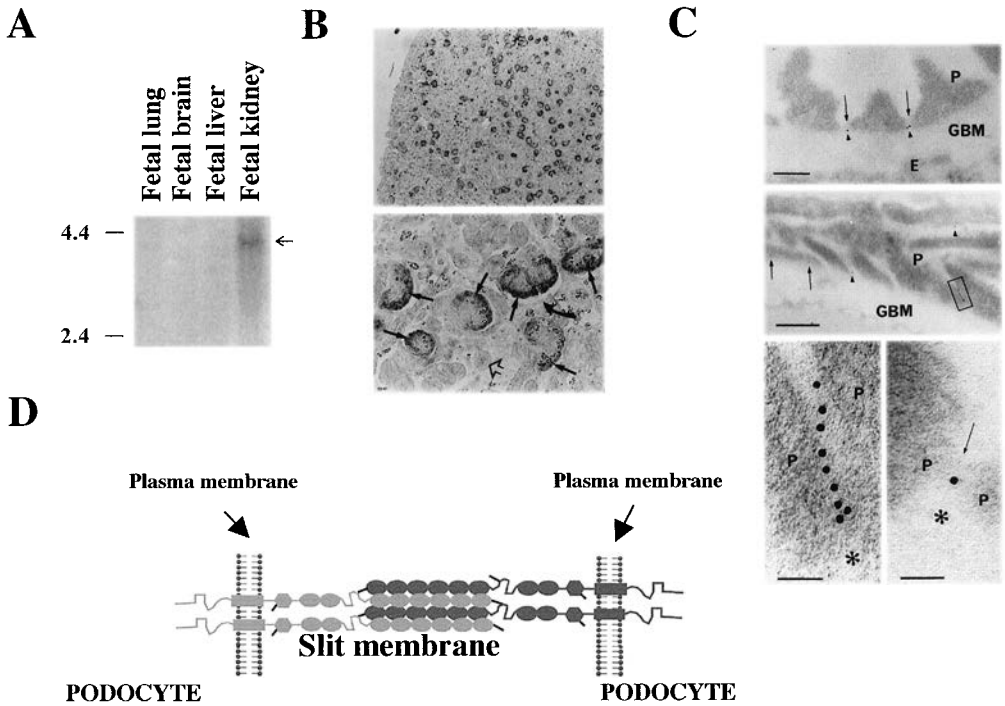


FIGURE 16-5. Expression of *NPHS1* and its protein product, nephrin. **A:** Northern blot of poly(A) RNA from four fetal human tissues, hybridized with a fragment of *NPHS1* cDNA probe. A specific signal can be seen only with fetal kidney RNA (*arrow*). **B:** Expression of *NPHS1* in human fetal kidney by *in situ* hybridization. The top panel shows intense expression in glomeruli throughout the renal cortex with little expression in other structures. A higher magnification is shown in the lower panel, revealing intense expression in the periphery of the glomeruli and little or no expression in Bowman's capsule (*bent arrow*), proximal tubules (*open arrow*), or endothelial cells of vessel walls. (Reprinted with permission from: Kestila M, et al. Positionally cloned gene for a novel glomerular protein–nephrin–is mutated in congenital nephrotic syndrome. *Mol Cell* 1998;1:575.) **C.** Nephrin is localized to slit membranes. Immunoelectron microscopic localization of nephrin in human renal glomeruli. In the top panel, gold label (*arrowheads*) can be seen between foot processes of podocytes (P). The label is located in the central area of the slit, between the glomerular basement membrane (GBM) and the barely visible slit diaphragm (*arrows*). The endothelium is unlabelled. Bar = 200 nm. In the middle panel, several gold particles can be seen lying in a row (*box*) between tangentially sectioned podocytes (P) foot processes. The lower left panel presents a close-up of the boxed section while the lower right shows a gold particle between the slit diaphragm (*arrow*) joining two podocytes (P) and the GBM (*asterisk*) in cross section. Bar = 50 nm D. Hypothetical model of how nephrin molecules assemble to form the slit diaphragm. In this example, four nephrin molecules are shown to associate in the slit between two foot processes. The interdigitation is mediated by Ig repeats 1–6 (*ovals*) and disulfide bridges that cross link the molecules. (Reprinted with permission from: Ruotsalainen V, et al. Nephrin is specifically located at the slit diaphragm of glomerular podocytes. *Proc Natl Acad Sci USA* 1999;96:7962.)

one can readily distinguish the podocyte-specific expression of *NPHS1* from the TAL-specific expression of *PCLN-1*. The temporal and spatial distribution of gene expression is invaluable in defining developmental pathways and identifying co-factors of one gene of interest. RNA-based studies are particularly useful in tracking expression of genes that encode secreted products (i.e., growth factors and cytokines) that are difficult to identify using immunohistochemical methods. *In situ* techniques also have the advantage that they can be used in cases where the specificity of an antibody for one's target has not been adequately demonstrated.

One important limitation of RNA-based studies is that they reveal nothing about the fate of the protein that they encode. Therefore, immunolocalization studies aimed at providing detailed characterization of a protein's cellular and subcellular distribution are key steps in its initial evaluation. In many cases, the protein structure provides clues as to where it is likely to be positioned within the cell. Nuclear localization signals, transmembrane-spanning elements, homologies to other known proteins offer hints, but each of these predictions must be confirmed in cells and tissues. For membrane proteins, one must determine the specific membrane compartment in which they reside since this could have profound implications for understanding their function. Co-staining studies using antisera that recognize known proteins are often helpful.

For novel proteins, one must first develop a set of antibodies that have specific recognition for their target. The strategy used to generate antibodies is straightforward: immunize rabbits, chickens, or other species with either synthetic peptides or recombinant polypeptides derived from the gene of interest; screen for immunoreactivity; and then affinity purify the reactive antibodies using the antigen against which it was raised. Antibodies are generally thought to be specific if they recognize the epitope against which they were raised and if the immunoreactivity was the direct result of the immunization process (i.e., preimmune sera lacked the activity).

In many cases, the results are unambiguous and provide important functional insights. As noted previously, nephrin, the gene product of *NPHS1*, the gene mutated in Congenital Nephrotic Syndrome of the Finnish type, was found to be

exclusively expressed in the glomerular podocyte (96). Immunohistochemical analysis of renal tissue showed that the protein was confined to the slit diaphragm in the pore region of the glomerular basement membrane (Fig. 16-5) (97–101). This invaluable information is unlikely to have been obtained by any other method, and has important implications for understanding the protein's function. This result also helped to explain why loss of this protein could result in the massive nephrotic syndrome observed in those affected with this disease. In a similar manner, immunolocalization studies of *PCLN1*, the protein that is defective in primary hypomagnesemia, revealed that it is located in tight junctions of the thick ascending limb of Henle (40).

Unfortunately, the process of antisera characterization is far less straightforward than it appears, and the commonly accepted standards are insufficiently stringent to insure that one's antisera is truly specific exclusively for one's target. This can, on occasion, result in conflicting data and uncertainty about the true nature of the disease gene product. The problem of antibody specificity is likely responsible for much of the confusion regarding the detailed characterization of polycystin-1 (61,102–125). One ongoing controversy is the apparent overexpression of polycystin-1 in cystic tissue as defined by various polycystin-1 antisera (126). The "two-hit" model of disease initiation predicts that the vast majority of cysts should lack functional polycystin-1. Several arguments have been offered to reconcile the discrepant findings (103,107,110,126). One possibility is that both sets of observations could be true, with the majority of second hits being missense changes that result in functional inactivation without loss of protein expression. The most complete genetic analysis of cystic tissue suggests that this is unlikely, however. Watnick and associates found protein-termination type mutations in nearly all of the cysts examined (64,68,127). A second argument is that somatic inactivation of the wild type allele occurs after the cyst has already formed and is the direct result of the hyperproliferative state of cystic epithelia. This model could account for the large fraction of cysts found to be immunopositive for polycystin-1 and explain why only a subset has detectable somatic mutations. Arguing against this hypothesis are the results of studies performed on a murine model of *PKD2* (128). In this model, the animals have an unstable allele of *Pkd2* that acquires somatic mutations at a very high rate (see the subsequent text). Cystic tissue of these animals lack polycystin-2 while the adjacent normal tissue has intact polycystin-2 expression (Fig. 16-6). Finally, some investigators have suggested that the apparent discordant genetic/immunohistochemical data may be the result of problems with the antisera (104). Nauta et al generated 14 different polyclonal antisera against fragments of polycystin-1 and found that many stained the epithelial cells of fetal, adult, and cystic kidney sections in patterns similar to those reported by others. After further study, however, they concluded that the patterns they observed were not specific for polycystin-1 and they urged cautious interpretation of the published literature. Which of the various explanations is correct remains an open question but the issue highlights the difficulties that sometimes arise in this process.

As invaluable as the immunolocalization studies are in defining essential properties of one's protein, an antibody that has been rigorously confirmed to recognize a protein of interest is an indispensable tool in further biochemical studies. While epitope tags can be included in the design of recombinant molecules that are expressed using cell culture systems, endogenous proteins can only be evaluated using target protein-specific antisera. Many proteins are subject to a number of functionally important posttranslational modifications such as proteolytic cleavage, glycosylation, and phosphorylation. Moreover, most proteins likely act in concert with others via pathways. One way of determining the pathways in which one's protein participates is by identifying its various binding partners. All of these properties are best assessed by immunoblot and immunoprecipitation studies.

FUNCTIONAL ANALYSES

Once the initial description of a new gene is completed, the next goal is to begin its functional characterization. For many of the genes listed in Tables 16-1 through 16-8, their roles have been obvious because they were identified based on their functional properties. For example, each of the transporters listed in Table 16-4 and most in Table 16-5 had been cloned and characterized prior to the discovery of its mutation in the disorder with which it is associated. In these conditions, the focus is on determining how mutations alter the protein's function and how these abnormalities result in the observed pathology. A subset of the genes listed in the tables was discovered to be homologues of known genes that had not been previously associated with the human disease phenotype. For example, mutation of *NPHP2 (invs)*, one of the genes associated with nephronophthisis, had been previously found to result in *situs inversus* and severe polycystic disease in mice (129,130). Its subsequent association with human nephronophthisis provided a critical insight into the functional properties of NPHP-related proteins

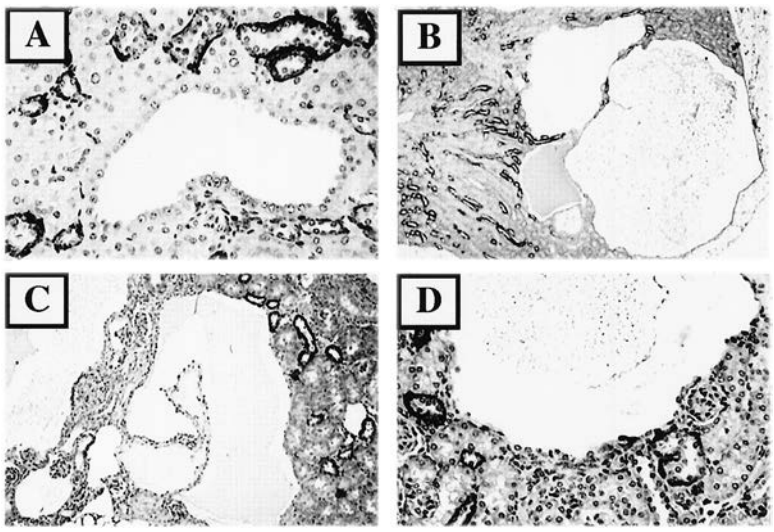


FIGURE 16-6. Renal cysts of mice with an unstable *Pkd2* allele ($Pkd2^{WS25/WS25}$) lack polycystin-2. **A:** An early cyst from a $Pkd2^{WS25/=}$ kidney is negative for immunostains for polycystin-2 multiple positively stained tubules are seen in the adjacent cortex. (×250). **B–D**: Cortical renal cysts consistently are negative for immunostains for polycystin-2 while multiple positive noncystic tubules are clearly positive. (**B** ×80, **C** ×120, **D** ×250). (Reprinted with permission from: Wu G, et al. Somatic inactivation of Pkd2 results in polycystic kidney disease. *Cell* 1998; 93:177.)

(131,132). Member of this group are now thought to comprise essential components of the primary cilia and the basal body. A similar approach is applied to the study of novel genes, like *PKD2*, that have a predicted but unproven function based upon their sequence. A final subset of genes, like *VHL* and *NPHP1*, encode novel proteins of unknown function whose sequence provides few clues.

It is obvious that the particular set of experiments that one uses to characterize a gene depends on the nature of the protein that it encodes. The strategy for studying a putative transporter is very different than the one used to examine a transcription factor. Likewise, one will approach the study of a known gene product like the Na-K-2Cl co-transporter differently than one would a more enigmatic one like nephrocystin-1, the *NPHP1* gene product. In this section, we will briefly review some of the general methodologies used to determine a protein's functional properties and conclude with a discussion of several specific examples.

Identification of Interacting Partners

The specific nature of interactions between proteins is often an important determinant of their functional characteristics. One paradigm of functional protein-protein interactions is that of receptor-based signaling. According to this schema, the receptor, usually situated in a membrane, binds to one or more ligands. This binding usually leads to a cascade of signaling events that may begin, for example, with phosphorylation of the receptor by itself or some other protein. This event may recruit other proteins such as adapter molecules with *src* homology 2 or *src* homology 3 domains to the receptor-ligand complex. These proteins may also be phosphorylated and when activated in this way they move to the nucleus, ultimately leading to the transcription of new genes. Frequently, one receptor may participate in a number of signaling pathways that "talk to one another." In many cases, these factors serve to amplify the signal while parallel or negative feedback systems act to dampen or balance it. In either case, each step in this pathway critically depends on the interaction of proteins capable of transmitting the signal.

Proteins involved in receptor-based signaling are by no means the only types of proteins whose activity depends on protein-protein interactions for their normal function. For example, many channels or transporters are formed by multimeric complexes of a single subunit (in the case of K channels) or multiple independent subunits (e.g., H+ATPase). Moreover, the activity of most channels is regulated by direct binding of other nonchannel proteins. Likewise, transcription factors depend on interactions with specific co-factors, transactivators or inhibitors for their activation or repression. It is fair to say that the function of most proteins critically depend on protein-protein interactions for their activity, and identifying the partners of one's protein of interest can provide important clues into its function. This is especially true if the partners are discovered to be factors that participate in well-defined pathways or regulatory systems. Given the tremendous impact such studies can have on one's understanding of the pathobiology of a new protein, much effort often is directed at identifying the protein partners of a gene product of interest.

Several strategies can be used. The first is guided by an understanding of the disease process. A growing body of literature suggests that inherited disorders that are characterized by genetic heterogeneity often result from mutations of genes whose products either form a multimeric complex (such as a transporter) or function in a common pathway. This property is exploited in Drosophila research as a tool to define the functional pathways of novel genes. In the case of human renal diseases, there are numerous examples that fall into this group: ADPKD, nephronophthisis (NPHP), tuberous sclerosis (TSC), distal RTA (OMIM#267300 and 602722), Liddle Syndrome, PHA-Type I (recessive forms) and cystinuria. In some of these examples, like Liddle Syndrome, the genes associated with the disease had been known to encode interacting subunits prior to their discovery as disease-causing loci (12). In others, like ADPKD, NPHP and TSC, the functional interactions that have been shown for their respective gene products were discovered solely because of a focused search (61,131,133–138).

Sometimes it is possible to make an "informed guess" about potential partners based on the known properties of the protein of interest. For example, a protein that had been shown in immunolocalization studies to be located in focal adhesion plaques might be expected to associate with other components of that complex. One would then pursue a directed search to look for functional associations between the protein and a battery of pertinent interacting factors. Other clues might be provided by homology to known proteins. The identification of a putative G-protein binding site in polycystin-1 resulted from such an analysis (54). In other cases a protein may have domains that are known to be generalized sites of protein-protein interaction. Ankyrin repeats and coiled-coil motifs are examples of such structures and are good places to begin to look for interacting partners.

Finally, in many cases a protein is truly novel and there is no way to predict to what it might bind. This is common when a gene has been identified using a strictly positional approach or when there is no sequence homology to serve as a guide. The Von Hippel Lindau gene product is an example of this situation and necessitates the use of methods that make few prior assumptions about the nature of interacting partners. In such situations, an investigator can purify their protein (either the endogenous protein or a recombinant, epitope-tagged version purified from tissue or cells) and determine the peptide sequence of any factors that copurify. As one might predict, the rate of progress in understanding the function of proteins that fall into this class is usually far slower than it is for others.

MODEL SYSTEMS

Cell Culture Systems

The biochemical approaches described in the preceding section are limited by the nature of *in vitro* studies. They do not generally provide information about the physiological consequences of activation or disruption of a particular pathway. It has become increasingly obvious that cell culture systems can be especially valuable in this regard. Cell culture systems have the advantage that they often can be easily scaled up to perform "bucket biochemistry," they can be used under carefully controlled and well-defined conditions, and they can be scaled down to the single cell level to study molecular processes. They are invaluable tools for studying a molecule's transport properties, its growth-regulatory characteristics, or the signaling pathways in which it participates. If one transfects a cell with a cDNA for a putative ion channel, one can demonstrate and characterize its channel activity using patch clamp methods or calcium photometry for calcium fluxes. Likewise, one can test for the transport of other molecules by simply modifying the approach to look for the movement of the molecules in question. Cell transfection experiments also can be used to quantitate the growth effects of a protein of interest. Once an effect is defined, one can perform cell cycle analysis to determine whether it acts through a cell-cycle dependent process, by altering the rate of programmed cell death or through some other mechanism. Regardless of which mechanism is implicated,

there is a series of well-defined steps that can be followed to define in a precise manner how the protein affects its activities. In the case of transcription factors, cell culture systems may be used to identify potential targets.

A complete review of the many insights that have resulted from the use of cell-culture systems is far beyond the scope of this chapter. Several select examples will serve to illustrate the utility of this approach.

Pseudohypoaldosteronism type II (PHAII, Gordon Hyperkalemia-Hypertension Syndrome) is a rare autosomal dominant disease that is distinct from most other Mendelian genetic forms of hypertension in that affected individuals have both hypertension and hyperkalemia (139). Mutations in *WNK1* and *WNK4*, two related members of the serine threonine kinase super-family, were found to cause the disease. A series of studies performed using recombinant proteins and cell culture systems demonstrated that the proteins act as a molecular switch that allows the kidney to alternate between NaCl-reabsorbing and K+-secreting states through their inhibitory effects on the thiazide-sensitive NaCl co-transporter and the renal outer medullary K+ channel. The observation that the proteins had been localized to the tight junctions in renal epithelia prompted Kahle et al to investigate whether WNKs also function to regulate paracellular ion flux using canine renal epithelial cells (MDCKII cell line) (139). They generated stable cell lines that had inducible expression of either wild type or mutant *WNK4* and showed that the recombinant proteins localized to tight junctions by immunofluorescence. They then showed that *WNK4* decreased transepithelial resistance in MDCK cells grown in monolayers by selectively increasing the paracellular conductance of chloride. They ruled out gross structural changes of the tight-junction complex as a trivial explanation for their findings by transmission and freeze-fracture electron microscopy. The authors concluded that the WNKs likely help coordinate transcellular and paracellular flux to achieve NaCl and K+ homeostasis.

A different kind of cell culture system has recently been developed to study the function of polycystin-1. This protein has been difficult to analyze because of its large size and low abundance in tissues and cells. Boletta et al. used novel methods to establish cell lines with stable over-expression of *PKD1* (140). They had selected the MDCK cell line for this study because it is a renal epithelial cell line commonly used to study the process of tubulogenesis. Under normal conditions, MDCK cells form cysts when cultured in collagen but produce branching tubules if treated with HGF (Hepatocyte Growth Factor) (141). Boletta et al. found that expression of recombinant *PKD1* in MDCK cells resulted in spontaneous tubulogenesis, a reduced rate of growth and induced resistance to apoptotic stimuli. The effects of *PKD1* expression in this model are remarkably similar to what had been predicted based on study of human and murine cystic tissues and suggest that it may serve as a useful tool for understanding pathways regulated by polycystin-1. This unique system offers three phenotypic assays that can be used to test for the functional consequences of altering any of polycystin-1's domains. Given that each of the distinct phenotypes could be affected in a different way, this strategy might allow one to dissect out the various signaling pathways regulated by this multifunctional protein. One might also use such a system to test for functional consequences of missense changes identified solely in affected individuals but of uncertain consequence.

Mouse Models

Although cell culture systems are relatively easy to manipulate, they may not always mimic faithfully what occurs *in vivo*. Over-expression of proteins could conceivably activate pathways that are not important in the whole organism and therefore the significance of any phenotypic readout may be difficult to interpret. Animal models that faithfully reproduce human disease are therefore invaluable tools. They can be used to help define the pathophysiology of a disorder, identify factors that modulate the severity of disease, and to test therapeutic interventions.

For diseases that result from gain-of-function or dominant negative mutations, transgenic mice with over-expression of the mutant gene in a transgenic mouse can be useful (142). In this method, the cDNA of interest is injected into the pronuclei of fertilized eggs and then re-implanted in the uterus of females for carriage to term. Advantages of this approach include its wide availability, relatively low cost, and rapid read-out. Transgenic animals can be produced within less than a month of producing the recombinant construct. Its major limitation is that the spatial and temporal pattern of gene expression of the transgene often fails to recapitulate that of the endogenous gene in a faithful manner. A second problem is that this strategy generally cannot be used for diseases that are recessive in nature. Over-expression of a null or functionally null allele is unlikely to reproduce the human disease since the endogenous murine forms of the genes will be normally expressed. Finally, in most cases the construct used to generate the mice exclusively contains the exonic sequences of one's gene and lacks all but perhaps a single intron (the latter has been shown to improve transgene expression). One consequence is that the transgene does not undergo any of the splicing normally observed for the endogenous gene.

In light of these limitations, a second approach that avoids many of these pitfalls has now become the standard of the community. Its goal is to generate mice with targeted mutations of the endogenous murine gene (143). By altering the gene *in situ*, one preserves most of its intrinsic structural features such as its regulatory elements and introns. In most examples to date, the technique has been used to produce a "knock-out" through the introduction of deletions that abrogate a gene's function. More recent refinements allow one to introduce more subtle changes such as nucleotide substitutions that encode missense changes ("knock-in"). Such strategies allow one to generate models that carry the exact genetic defects observed in humans in a much more faithful manner.

The ability to do gene targeting depends on two principles. First, one must be able to perform an *in vivo* "swap" of the engineered gene segment for the endogenous sequence through the process of homologous recombination. Next, one must have a way of creating an animal from cells with the targeted mutation. Sperm and egg cells would seem like natural choices but unfortunately cannot be used for this purpose. Embryonic stem (ES) cells, removed from the earliest stages of development, do work well because they are easily cultured and totipotent—they can give rise to any organ system or cell type. This allows one to "pass" the mutation through the mouse germ line.

The first step in gene targeting is to design a targeting construct that contains a genomic segment of the target gene into which has been inserted a neomycin resistance gene. The latter step allows for selection of ES cells that have "taken up" one's construct. For "knock-out" constructs, the neomycin gene has replaced a portion of the coding sequence of the target gene so that its integration would interrupt the target's coding region. For "knock-in" constructs, the neomycin gene is typically placed within an intron where it is less likely to disrupt the gene's function. To create the mutation, the targeting construct includes a segment of the normal gene that has been altered to include the desired variant. Properly targeted clones are identified by Southern blot analysis. The appropriate ES cells are then injected into a mouse blastocyst in a process that produces an adult chimeric mouse. Chimeric mice can be readily identified by their mixed black and agouti coat color, because the

ES cells typically come from mice with agouti coat color and the blastocysts are derived from animals whose coat color is black. The chimeric mice are bred and if their germ line is also chimeric, then the F1 generation should have some progeny that are heterozygous for the targeted disruption. In order to produce homozygous mice, the F1 generation can be interbred to one another.

"Knock-out" models are useful in a number of ways. For example, they may confirm the genetic basis of a disease. As noted in a previous section, genetic studies of cystic tissue have indicated that ADPKD is likely to be recessive on a molecular level. This would suggest that mice heterozygous for *Pkd* mutations should survive to adulthood with few if any symptoms. In contrast to their human counterparts, they would be predicted to have relatively few cysts because of their far shorter lifespan and much smaller organ size. A smaller kidney or liver would have far few cells at risk of acquiring mutations over a period of 1 to 2 years of observation. These predictions were confirmed in studies of mice that were heterozygous for inactivating mutations of either *Pkd1* or *Pkd2* (128,144–146). Wu and associates had developed a particularly elegant model (128). They had generated an unstable *Pkd2* allele by inserting a second copy of the first exon of *Pkd2* in tandem to the endogenous exon (Fig. 16-7). This allele was prone to acquiring somatic mutations via intragenic homologous recombination. Mice heterozygous for the null allele and the unstable allele consistently developed an ADPKD phenotype similar to that observed in humans. These mice did not die *in utero* but developed significant renal and hepatic cystic disease by 11 weeks of age. This model has proven to be an invaluable tool for studying the pathophysiology of the disease (147) and testing putative therapies (148). One can easily imagine how it also might be used to identify factors that predispose to the acquisition of somatic mutations or influence the progression of disease.

A common problem limiting the utility of standard gene knockout technology is that complete homozygous inactivation of a gene often results in a nonviable fetus that fails to complete gestation. This situation is usually recognized by a distortion in the Mendelian ratio of observed versus expected genotypes of living offspring. In the case of *Pkd1*, this property severely limited the utility of the many models that had been generated by gene targeting. However, newer technologies offer the hope of circumventing this problem and expanding the capability of manipulating the murine genome at will. Investigators can use gene-targeting techniques to create mice that have latent mutations, which can be activated upon demand (conditional mutations, otherwise known as "floxed alleles") (149–155). In this approach, the targeting construct is designed to include very short DNA sequences that serve as specific recognition sites for non-native DNA recombinase enzymes in introns flanking key functional aspects of a gene of interest. If carefully positioned, the short DNA sequences (either lox P or frt sites) have no effect on the function of the gene until after recombination has been induced. Since the recombinase enzymes (Cre and Flp) are not normally present in mice, one can determine when and where one wishes to inactivate the gene of interest by controlling the spatial and temporal expression of the recombinase. This usually is achieved by breeding mice with either the loxP- or frt-allele to animals transgenic for the corresponding recombinase (Cre and Flp, respectively). By altering the regulatory elements that control Cre or Flp expression, one can define the conditions under which he or she wishes to inactivate his or her gene.

This strategy has been successfully used to generate viable adult mice that otherwise would have died *in utero* of their homozygous mutations. For example, this approach has been recently used to create a viable *Pkd1* model (157). Using a Cre with random expression, the group produced mice with cystic disease of variable severity. Using this new mouse line, investigators will now be able to determine with certainty whether the protein plays a role in the maintenance of tubular integrity by inducing its inactivation once development is completed. This model also will prove invaluable as a resource for testing therapeutic interventions. Finally, a conditional model of gene inactivation allows one to assess the effects of inactivation of a gene in organs other than those that cause its most obvious and life-threatening complications. In the case of ADPKD, viable adult humans or mouse homozygous for germline mutations of either *PKD1* or *PKD2* have not been described, presumably because this genotype results in embryonic lethality (144,146,158,159). Affected humans are likely to have frequent clones of cells with somatic mutations of the wild type allele but these are likely to be widely scattered and comprise only a small fraction of most organs. Thus, we have no way of predicting the phenotype of animals that are engineered to lose polycystin-1 function in a high fraction of cells in an adult animal. The use of a floxed *Pkd1* allele is likely to reveal unexpected complexity and new functional properties for *PKD1*.

The Use of Nonvertebrate Organisms as Models

The numerous genetic tools available for manipulation of the mouse genome along with the wide availability of genetically defined strains has fostered great interest in using mouse models to identify genetic loci that encode modifiers of disease severity or potential interacting partners. Unfortunately, there are currently no efficient techniques for using large-scale genetic screens to identify enhancing or suppressing genes. In some cases, crossing inbred mutant strains to mice of a different background has revealed the influence of genetic modifiers that have been mapped to large chromosomal regions by quantitative trait analysis. Despite great success in localizing many of these factors (160–165), progress in identifying the precise loci has been very slow. For this reason, many groups have turned to use of invertebrate organisms such as *C. elegans* (roundworm) and *Drosophila melanogaster* (fruit fly) to complement the human and mouse studies. As noted in an earlier section, greater than 60% of human disease genes are estimated to have orthologues in Drosophila. In addition, the signaling systems involved in a number of basic processes such as cell-cell signaling (i.e., JAK/STAT/cytokine, Wingless/WNT) appear to have conserved components in vertebrates and flies. We have already alluded to one of the most striking examples of how this approach has enhanced our understanding of a vertebrate gene linked to renal disease. Ito and Rubin identified *Drosophila* homologues of *Tsc2* and showed that its loss resulted in a dramatic change in cell size (166). Several groups linked *Drosophila Tsc1* to the same pathway soon thereafter and, capitalizing on the model system's many advantages, linked both proteins to the insulin signaling pathway (167–169). Subsequent studies soon showed that the *Tsc1/Tsc2* complex regulates the TOR kinase in both Drosophila and vertebrate systems and identified Rheb as the small GTPase that is the likely target of *Tsc2* GAP activity (170–180). The most exciting consequence of these studies is that they had led to a possible therapy (discussed below). The recent development of methodologies that can create Drosophila mutants by homologous recombination may now allow investigators to manipulate its genome at will to make simple models of human disease "on the fly" (181).

Insights are also likely to come from unanticipated sources. In the case of PKD, a strategy of randomly inactivating genes by insertional mutagenesis revealed that the mutation of the *orpkd* gene resulted in a phenotype similar to that of human autosomal recessive polycystic kidney disease (182). The gene

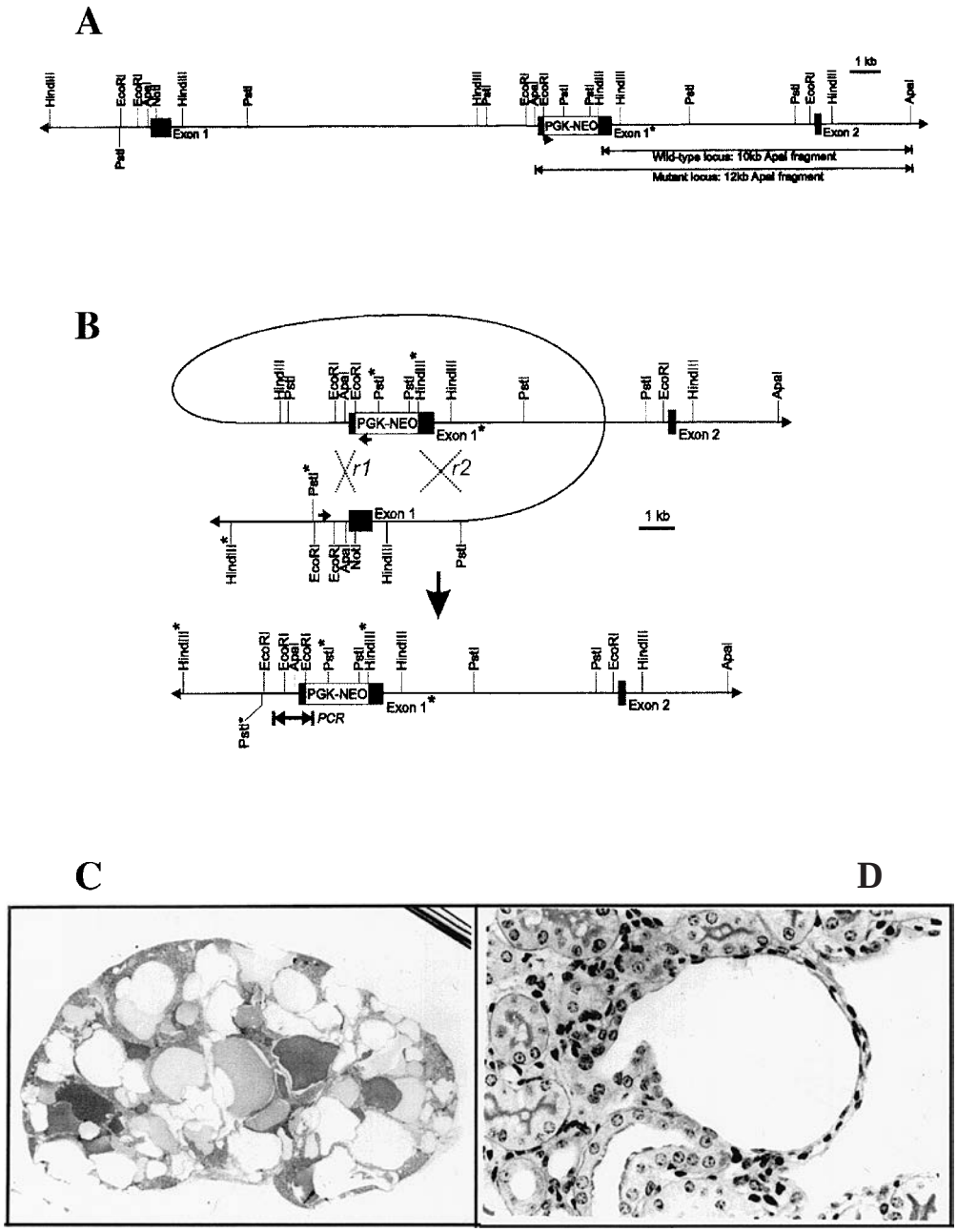


FIGURE 16-7. A: Restriction map of the *Pkd2*^{WS25} allele created by gene targeting. Instead of replacing the normal 5′ end of the *Pkd2* with the mutant sequence, the targeting construct integrated within the 5′ end to create a local duplication. Locally duplicated sequences are known to be prone to genomic rearrangement by unequal sister chromatid exchange. **B:** Schematic representation of intragenic homologous recombination that is observed for the unstable *Pkd2* allele, *Pkd2*^{WS25}. Two different recombination processes can occur. Recombination r1 produces a null allele while recombination r2 results in a wild type allele. Both the wild type and unrecombined *Pkd2*^{WS25} allele have normal functional activity. **C:** A cross section of a murine kidney taken from a homozygous mouse carrying two unstable *Pkd2* alleles (*Pkd2*^{WS25}). Severe cystic disease is observed mimicking the pattern of human ADPKD (×2.5). **D:** Cysts have a focal origin. A magnified view of a murine kidney with the *Pkd2*$^{WS25/WS25}$ genotype. An incipient cyst is shown forming as an outpouching from a normal tubule. Presumably the cyst developed as the result of homozygous inactivation of the *Pkd2* gene in one tubular cell (×320). (Reprinted from: G Wu, et al. Somatic inactivation of *Pkd2* results in polycystic kidney disease. *Cell* 1998;93:177, with permission.)

sequence initially provided few clues as to the protein's function. In an unrelated project focused on identifying genes in algae (*Chlamydomonas*) that regulate formation and function of flagella, investigators determined that one of the genes that caused flagellar defects was a homologue of *orpkd* (183). The gene is thought to encode a protein (IFT 88) that helps to carry "cargo" to the flagella in a process called intraflagellar transport. Subsequent studies showed that IFT88 homologues in other organisms have an essential function in the formation and function of a related structure, the cilia (184). In vertebrates, cilia have been found on almost all cells and are either motile or nonmotile. The latter are thought to possibly

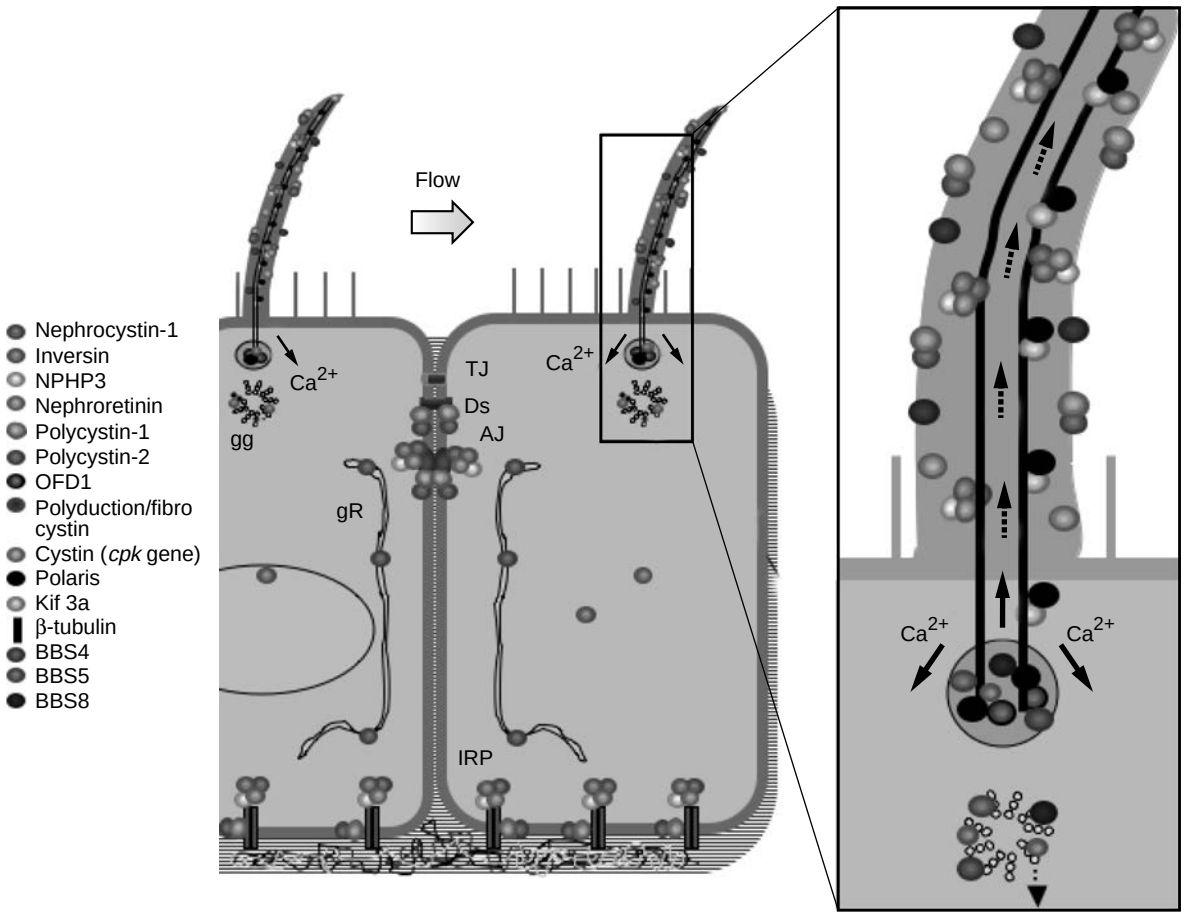


FIGURE 16-8. Subcellular localization of cystoproteins. Many cystoproteins have been localized to more than one intracellular domain. In this illustration, all known members of an interacting complex are shown colocalized to the same locations, even though this has not necessarily been shown for each unit. There are two exceptions: (i) polycystin-2 has been reported to have endoplasmic reticulum–specific functions as a calcium release channel that are independent of polycystin-1, and (ii) at least one form of inversin has been localized to the centriole and to the nucleus. The speckled arrow in the primary cilium indicates the direction of anterograde transport along the microtubule system mediated by kinesin-II, a heterotrimeric protein composed of two motor units (Kif3a, Kif3b) and one nonmotor unit (KAP3). *AJ*, adherens junction; *BB*, basal body; *Cen*, centriole; *ER*, endoplasmic reticulum; *FAP*, focal adhesion plaque; *TJ*, tight junction; *PC-1*, polycystin-1; *PC-2*, polycystin-2. (Adapted from: Watnick T, Germino G. From cilia to cyst. *Nat Genet* 2003;34:355, with permission.) (See Color Plate for protein identification.)

function as sensory organelles for a variety of stimuli (185). In the kidney, they may act as mechanosensors for flow (186,187). There now is a very large body of data that links defects in cilia and related structures such as the basal body/centrosomal complex to renal cystic disease (132,188,189) (Fig. 16-8), and a recent comparative genomic approach capitalized on this observation to identify a novel locus for Bardet-Biedl syndrome (190). In sum, appreciation of the central role that the primary cilium appears to play in normal development and in renal cystic disease was sparked by studies of simple algae.

CLINICAL IMPLICATIONS AND FUTURE DIRECTIONS

The advances described thus far may seem basic in their scope, but they also have reaching implications with respect to diagnosis and ultimately the treatment of inherited renal diseases. The most immediate clinical impact of gene identification is the development of molecular diagnostic tests. There are a number of circumstances in which DNA-based diagnoses may be helpful to clinicians managing the care of affected individuals and their families. In the case of X-linked nephrogenic diabetes insipidus, DNA testing can be used to identify at-risk male fetuses or newborns before they suffer repeated episodes of dehydration (191). DNA testing is particularly useful in the management of families with Von Hippel Lindau disease (192–194). This information can provide reassurance to noncarriers and focus close attention on those individuals most likely to develop complications that will need repeated CT scans or MRIs. An additional benefit of DNA testing in this disorder is that it can identify the subgroup most likely to develop pheochromocytomas.

DNA tests are also used to evaluate presymptomatic individuals who seek genetic counseling regarding their prognosis or for the purpose of family planning. In general, presymptomatic DNA testing will probably be more widely used in the future if protective therapies for diseases such as ADPKD are developed that must be administered early in life before a clinical diagnosis can be made. A final use of DNA testing is in the evaluation of "at risk," presymptomatic individuals as potential living-related transplant donors. This application is particularly important for diseases like ADPKD that have late onset.

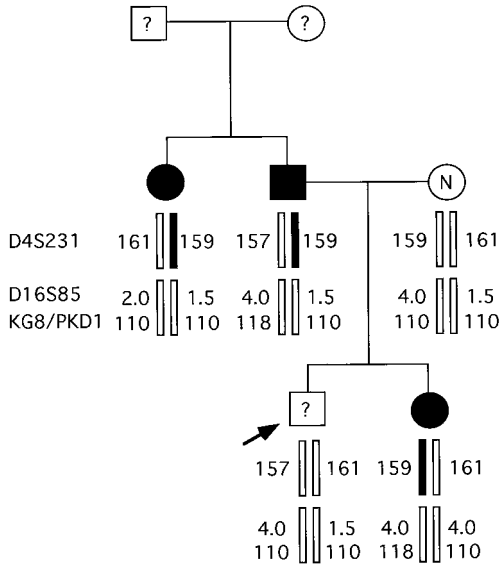


FIGURE 16-9. DNA testing for *PKD1* and *PKD2*. Filled symbols identify individuals affected with ADPKD. Unfilled symbols identify individuals that are either normal (N) or whose status is unknown (?). The vertical bars beneath each symbol represent homologous pairs of chromosome 4 (*top*) and 16 (*bottom*). Genetic markers mapping near each disease locus are listed on the left. D4S231 is tightly linked to *PKD2* on chromosome 4, and D16S85 and KG8/PKD1 map near and within *PKD1*, respectively. The numbers on each side of a chromosome identifies its alleles at these loci. In this family, the disease segregates with the chromosome 4 marker (allele 159) and not with those on chromosome 16. The individual marked by the arrow has less than a 6% chance of being affected with PKD. (Data generously provided by Dr. Allan Bale, Yale University.)

There are two major types of DNA tests that may be offered for a given disease gene, and it is useful for any clinician to appreciate the potential shortcomings of each type of analysis. The first is linkage testing that was discussed in the context of gene discovery previously. In this method, the haplotype that segregates with the disease phenotype is determined and compared to that of the individual seeking testing (Fig. 16-9). The test assesses the probability of an individual being affected based on their haplotype. The accuracy of the estimate depends on the number of family members that participate and the proximity of the disease locus to the DNA markers used to perform the test. Linkage analysis is now available for a number of inherited renal diseases including ADPKD, ARPKD, and Alport's syndrome. These tests can be relatively easily developed for any of the mapped diseases in Tables 16-1–16-8 given the high density of very polymorphic DNA markers.

There are several limitations to linkage testing. The first problem is that since the method is indirect, no information can be obtained from testing the proband alone and the cooperation of multiple family members must be recruited. If the family is small or if many of its members refuse to participate, it may be impossible to determine the disease haplotype with certainty. In a similar vein, the test cannot be used if the proband is suspected to have a *de novo* mutation. It is also important that one be able to establish a definitive clinical diagnosis in the family members who agree to participate so that a correlation can be made. Another factor that limits the potential utility of linkage-based analyses is the problem of genetic recombination. Crossing over between homologous chromosomes occurs during meiosis and may alter the association between DNA markers on an allele. This could result in an erroneous diagnosis on a linkage study. The chance of this happening is related to the distance between the DNA marker and the disease locus. The likelihood can be kept to a minimum by using closely linked markers with less than a 1 percent frequency of recombination.

A final limitation of linkage testing as used in clinical practice is that one rarely achieves a lod score of 3 with one's results. In fact, the calculated genetic risk for a subject as determined by linkage testing is heavily weighted by the prior probability that his/her disease is linked to the locus being examined. For disorders that result from mutations of a single locus (i.e., congenital nephrotic syndrome of the Finnish variety), this poses no difficulty. Many of the diseases listed in Tables 16-1 through 16-8, however, can result from mutations at more than one locus, exhibiting a property called genetic heterogeneity. Tuberous sclerosis, for example, can be caused by mutation of either *TSC1* or *TSC2*. When multiple loci are associated with a disease, then linkage to all must be assayed. When a small number of samples are available for analysis, the probability of the test being noninformative is significantly increased. Finally, it must be remembered that not all of the loci have been identified for each disease, and this factor can sometimes lead to increased uncertainty about the meaning of the test results.

In Figure 16-9 is provided a representative example of linkage-based testing as applied to ADPKD. As noted in Table 16-2, genetic studies have implicated at least three loci in this disorder. Over 85% of the families are affected with a form of the disease that results from mutation of PKD1 on chromosome 16 (195). In virtually all of the remaining families, mutations of PKD2 are responsible for the disease. A small fraction of all ADPKD families, estimated at approximately 1%, has disease that is not linked to either gene (196–199). A chromosomal location has not yet been defined for the locus responsible for the disease in this small subgroup, and there is some question as to whether a third locus even truly exists (200,201). In clinical practice, one tests only for linkage to *PKD1* and *PKD2* and uses these results to estimate the probability of an individual with unknown status to have the disease. In most situations, these data can provide an accurate diagnosis. There may be, however, a small group of families where linkage testing would not be useful.

The second type of DNA testing is direct mutation analysis. Direct testing can determine unequivocally whether the proband has inherited a disease causing mutation without the participation of family members. Most of the techniques that are used for mutation analysis begin with PCR amplification of regions of the gene in question as small overlapping fragments. These PCR products can be screened for changes using a variety of techniques including SSCP (single strand conformational polymorphism), dHPLC (denaturing high-pressure liquid chromatography) or direct sequencing. There are many types of mutations that may be detected including deletions of large or small portions of the gene, small insertions or nucleotide substitutions. Some nucleotide substitutions may lead to the premature truncation of the protein if they result in a stop codon. Others may lead to an amino acid substitution that may affect a critical function of the protein. Alternatively, nucleotide substitutions may either create or destroy splice sites that result in a new message being created. For some diseases, a large proportion of affected individuals will have the same mutation, which means that a directed study can be done. For example in the case of juvenile nephronophthisis type 1 (*NPHP1*), approximately 80% of affected children will have large homozygous deletions (~250 kilobases) around the *NPHP1* interval that can be easily detected (34). A majority of the remaining children will have a heterozygous deletion in combination with a point mutation. A heterozygous deletion can be detected using the technique of fluorescence *in situ* hybridization and the point mutation can be sought by direct sequencing of all 20 *NPHP1* exons. In this way, a renal biopsy diagnosis can be avoided in

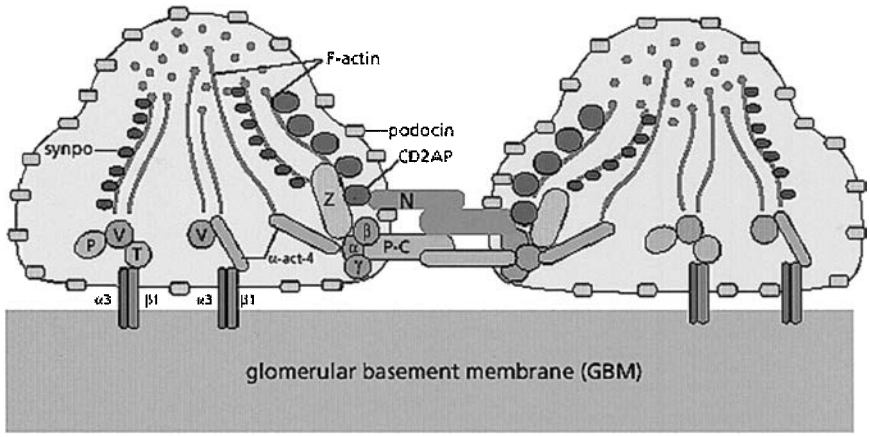


FIGURE 16-10. Schematic model of the molecular structure of the podocyte foot process cytoskeleton. The podocyte foot processes contain a contractile system composed of actin, myosin-II, α-actinin-4 (α-act-4), talin (T), vinculin (V), and synaptopodin (synpo) that is connected to the GBM via $\alpha 3\beta 1$ integrin ($\alpha 3$, $\beta 1$). The linkage of the actin cytoskeleton to the slit diaphragm components, nephrin (N) and P-cadherin (P-C), may be mediated by CD2AP or by a complex of ZO-1 (Z), α-, β- and γ-catenin (α,β,γ). Although this cartoon suggests that podocin is localized to the cell membrane, this point has not yet been established. The actin cytoskeleton is well positioned to integrate different signaling pathways from the matrix-GBM interface, the slit diaphragm, or the cell surface. Disruption of any of these pathways may lead to reorganization of the actin cytoskeleton and foot process effacement as seen with nephrotic syndrome. (Reprinted from: Somlo S, Mundel P. Getting a foothold in nephrotic syndrome [news]. *Nat Genet* 2000;24:333, with permission.)

a fair number of suspected cases. This example illustrates the utility of a molecular approach to diagnosis.

There are certain limitations of direct mutation analysis, however, that need to be considered in interpreting a negative test. For example, one must consider whether genetic heterogeneity exists for the disease in question, and if so, whether all genes had been systematically evaluated. A second problem arises when a disease is not associated with a set of common mutations. In these cases, a laboratory will have to evaluate the gene's entire length for mutations. Depending on the size and complexity of the gene, the laboratory may elect to use screening techniques such as dHPLC (denaturing high performance liquid chromatography) rather than direct sequencing to search for suspected mutations, with DNA sequence analysis reserved only for suspicious changes (202–205). None of the screening methods in common use have 100% sensitivity. A related problem is that the laboratory usually screens for mutations in a set of overlapping PCR products that includes only the coding region and the immediately adjacent splice sites. This strategy will miss pathogenic changes occurring in intronic sequence. Inevitably a proportion of mutations will be missed which may limit the conclusions that can be drawn from the data. Lastly, the implications of all changes may not be absolutely clear. Although some mutations (such as deletions or insertions) are clearly pathogenic, the status of amino acid substitutions may not be obvious. For example, it may be difficult to distinguish between a simple polymorphism and an amino acid change that disrupts a particular protein function.

The list of diseases for which DNA testing is available has grown considerably since the previous edition of this text and includes some forms of nephronophthisis, ADPKD, ARPKD, medullary cystic disease, and hereditary nephrotic syndrome. In some cases, both linkage testing and direct mutation analysis is available. GENETests, an invaluable web-based resource hosted at the University of Washington and funded by the NIH (http://www.genetests.org/) provides a comprehensive list of academic and commercial facilities that offer testing on either a clinical or research basis. The web site also provides a library of contemporaneous reviews on numerous disorders as well as a variety of very informative educational resources.

The impact of molecular studies on the treatment of disease has been, to date, somewhat limited. For diseases that result from inadequate amounts of functional protein (recessive diseases, haploinsuffiency states, tumor suppressor-type genes like *PKD1* or *VHL*), strategies aimed at replacing the lost activity may treat the disorder. The most direct manner is by using gene therapy to express normal amounts of the correct protein in the target tissues. There remain huge obstacles to the widespread implementation of this approach for any disorder. Nonetheless, this strategy remains the "holy-grail" of the field and may, in time, yield rich rewards.

Parallel efforts are underway to develop a detailed understanding of disease-associated proteins and the pathways in which they participate. The hope is that this knowledge will be used to target intermediate steps in disease pathogenesis for therapeutic intervention. This strategy may yield effective treatments before the barriers to gene therapy can be overcome. The cell culture and animal model systems described in earlier sections of this chapter are invaluable reagents for this type of research, and recent studies highlight the important contributions these resources offer. In the case of tuberous sclerosis, the studies first in Drosophila and then in vertebrate cells linked the TSC proteins to regulation of mTOR kinase. Rapamycin, an agent widely used to prevent rejection in transplant patients because of its immunosuppressive properties, is known to inhibit this kinase. Studies in mice and rats have shown that rapamycin or related compounds are effective in reducing the severity of TSC-related disease, and clinical trials in humans are presently underway (206–208).

In the case of ADPKD, insights gleaned from a variety of approaches culminated in an exciting new therapeutic initiative. *In vitro* studies of cultured cyst-lining cells from human specimens suggested that cystic epithelia responded to increased cAMP activity with an increase in cell proliferation (209–211). Reasoning that the V2 receptor was the predominant regulator of cAMP activity in the collecting duct, the most prevalent site of cyst formation, Gattone and colleagues tested a

V2 receptor antagonist previously developed to treat hyponatremia in rodents with a syntenic form of ARPKD (*PKHD1*) and nephronophthisis (*NPHP3*) (212). The investigators found a striking reduction of cyst volume and decreased interstitial fibrosis. In a follow-up report, they tested the same agent in the Pkd2 model generated by gene targeting by the Somlo laboratory and found the same beneficial effects (148). It is expected that a clinical trial examining the efficacy of this class of drugs on the progression of human ADPKD will begin shortly.

Last, much of the focus of this chapter has been on advances in relatively rare monogenic disorders. Knowledge that has been gleaned from these studies, however, may also be applied to understanding the pathophysiology of more common disorders. For example, as a result of the identification of the genes involved in hereditary nephrotic syndromes we have been able to synthesize a more coherent model of normal and abnormal podocyte structure and function (Fig. 16-10) (213). Similarly, mutations of the VHL gene have been implicated in the majority of clear cell renal carcinoma (214,215). The result of studies of Bardet-Biedl syndrome have blurred the boundary between "traditional" Mendelian disorders and complex traits and highlighted the likely continuum that exists between the two sets of conditions (216). Likewise, variants at disease loci may be susceptibility factors for more common diseases such as hypertension and focal segmental glomerulosclerosis. Future studies will most certainly be aimed at deciphering the genetic basis of these and other common polygenic disorders.

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CHAPTER 17 ■
NEPHRONOPHTHISIS–MEDULLARY CYSTIC AND MEDULLARY SPONGE KIDNEY DISEASE

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Both nephronophthisis–medullary cystic kidney disease complex (NPHP-MCKD) and medullary sponge kidney (MSK) involve the medullary region of the kidney. However, as shown later, these two conditions have little in common with respect to clinical presentation, imaging features, and disease progression. In addition, juvenile nephronophthisis, an autosomal recessive disorder, is most likely not restricted to the medulla and is characterized by extensive and progressive interstitial fibrosis. Positional cloning of genes causing nephronophthisis has implicated the function of primary cilia and centrosomes in its pathogenesis.

A group of hereditary renal diseases is summarized under the term *NPHP-MCKD complex* (1,2), because the different disease entities share several features regarding (a) macroscopic pathology, (b) microscopic pathology, and (c) clinical symptoms (Table 17-1A). In this way the complex describes a distinct clinicopathological entity (3). The term *nephronophthisis* (NPHP) is used for the autosomal recessive forms, which lead to end-stage renal disease (ESRD) in the first three decades of life, whereas the term *medullary cystic kidney disease* (MCKD) refers to the autosomal dominant forms, in which ESRD develops in the third to seventh decade of life. Extrarenal manifestations such as ocular motor apraxia, retinitis pigmentosa, hepatic fibrosis, skeletal defects, and cerebellar vermis aplasia have exclusively been described in association with juvenile nephronophthisis. The only extrarenal associations in MCKD are hyperuricemia and gout.

FEATURES SHARED AMONG DISEASES OF THE NPHP-MCKD COMPLEX

Macroscopic Pathology

A major feature shared among the disease entities of the NPHP-MCKD complex (Table 17-1A [i]) is the appearance on macroscopic pathology as described in 27 patients with juvenile nephronophthisis by Waldherr et al. (1). Kidney size is normal or moderately reduced and tends to be small in end-stage kidneys. Cysts appear primarily at the cortico-medullary border of the kidneys (Fig. 17-1). This is quite distinct from autosomal dominant and recessive polycystic kidney disease, where kidneys become grossly enlarged as a result of cystic dilatation throughout the organ. Like in polycystic kidney disease (4), cysts are principally derived from collecting duct epithelia. In NPHP-MCKD there is always bilateral renal involvement. From the external surface, the kidney is indistinguishable from the kidney of glomerulonephritis or pyelonephritis. The surface usually has a finely granular appearance, most likely due to the protrusion of dilated cortical collecting ducts. Calices and pelvis appear completely normal. There are from 5 to over 50 cysts of 1 to 15 mm in diameter, located preferentially at the corticomedullary border (Fig. 17-1). The cysts seem to arise primarily from the distal convoluted and medullary collecting tubules as shown by microdissection (5), but may also appear in the papilla. Cysts are not always present, but occur in about 70% of autopsy cases. They apparently arise late in the course of the disease (6) and do not seem to be important for disease progression to renal failure (7). Therefore, the presence of cysts is not a prerequisite for diagnosis.

Microscopic Pathology

The second shared feature among diseases of the NPHP-MCKD complex pertains to renal histology (Table 17-1A [ii]). The histological changes are characteristic, but not pathognomonic, for the disease group. The characteristic histological triad of NPHP-MCKD consists of (i) tubular basement membrane disintegration with irregular thickening as well as attenuation of the tubular basement membrane, (ii) interstitial round cell infiltration with marked fibrosis, and later on (iii) tubular atrophy with cyst development occurring predominantly at the corticomedullary junction (Fig. 17-2) (1,8). Cysts seem to be the result rather than the cause of the atrophic process, although this time course could not be corroborated by statistical analysis. Sometimes, a communication between a cyst and a tubule can be seen. The tubular basement membrane (TBM) is extremely thickened and multilayered. Fibroblasts are noted between the membrane layers. TBM changes and diverticulum formation are most prominent in the distal tubules, where cysts are lined with a single layer of cuboidal or flattened epithelium. In the advanced stage, the picture merges into a diffuse sclerosing tubulointerstitial nephropathy, the characteristic picture of end-stage NPHP-MCKD. The only significant glomerular change in early stages involves periglomerular fibrosis with splitting and thickening of Bowman's capsule and glomerular obsolescence only in nephrons that have been destroyed by the tubular alterations. An escape of Tamm-Horsfall (uromodulin) protein from damaged collecting tubules into the interstitium has been demonstrated in about 50% of patients with NPHP-MCKD as a periodic acid-Schiff (PAS)-positive material and by specific immunofluorescence staining with an anti-THP antibody (9). Immunofluorescence does not contribute otherwise to the diagnosis of NPHP-MCKD.

Characteristic changes demonstrated by transmission electron microscopy include thickening, splitting, attenuation, and granular disintegration of the TBM (Fig. 17-3). Transition between these alterations is abrupt (8). Fibroblasts are seen in direct contact with the TBM. At the base of the tubular epithelial cells a marked increase of microfilaments is seen. The

TABLE 17-1

SHARED AND DISTINGUISHING FEATURES AMONG DISEASES OF THE NPHP–MCKD COMPLEX

A. *Shared Features*

(i) Symptoms:	polyuria, polydipsia, anemia, growth retardation, ESRD
(ii) Macroscopic pathology:	cortico-medullary cysts
(iii) Microscopic pathology:	tubuli: basement membrane disruption (thickening and attenuation), distal tubular atrophy and cysts interstitium: round cell infiltration, fibrosis glomeruli: periglomerular fibrosis only

B. *Distinguishing Features*

	NPHP	MCKD
(i) Inheritance:	autosomal recessive	autosomal dominant
(ii) Median onset of ESRD:	juvenile NPHP1: 13 yrs infantile NPHP2: 1–3 yrs adolescent NPHP3: 19 yrs NPHP4: 20 yrs NPHP5: 13 yrs	MCKD 1: 62 yrs MCKD 2: 32 yrs
(iii) Extrarenal associations:	(retinal degeneration, cerebellar vermis hypoplasia, hepatic fibrosis, cone-shaped epiphyses)	hyperuricemia, gout

ESRD = end stage renal disease; *NPHP* = nephronophthisis; *MCKD* = autosomal dominant medullary cystic kidney disease.

FIGURE 17-1. Juvenile nephronophthisis (autopsy case, 13-year-old girl). Note numerous cysts of varying size in the medulla and at the corticomedullary junction. (Reproduced with permission from: Hildebrandt F, et al. The nephronophthisis complex: clinical and genetic aspects. *Clin Invest* 1992;70:802.)

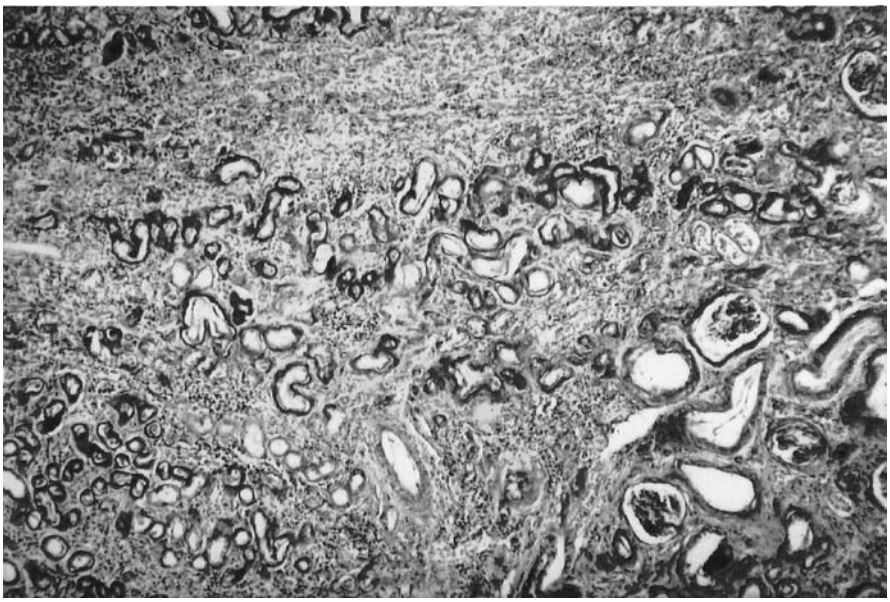

FIGURE 17-2. Renal histology in juvenile nephronophthisis (NPHP1). Note the characteristic triad, which consists of (i) tubular basement membrane disintegration with thickening as well as attenuation of the tubular basement membrane, (ii) interstitial round cell infiltration with marked fibrosis, and later on (iii) tubular atrophy and cyst development. (Courtesy of Prof. R. Waldherr, Heidelberg.)

thickening is either homogeneous or has a lamellated, annular, ring-like appearance. The glomerular basement membrane is normal. Multiple tubular diverticula are seen but the connections between cysts and distal tubular segments are patent.

Clinical Presentation

The third group of features shared among different diseases of the NPHP-MCKD complex involves clinical symptoms (Table 17-1A [iii]). Classical symptoms are polyuria, polydipsia, decreased urinary concentrating ability and, in children, anemia and growth retardation. The insignificance of the symptoms together with frequent lack of edema, hypertension, and urinary tract infections characteristically leads to delayed diagnosis and therapy in NPHP-MCKD. In all variants of NPHP-MCKD, terminal renal failure insidiously ensues at characteristic age ranges, necessitating renal replacement therapy (Fig. 17-4). Disease recurrence has never been reported in kidneys transplanted to NPHP patients (10).

FEATURES DISTINGUISHING DISEASE ENTITIES OF THE NPHP-MCKD COMPLEX

There are three features that clearly distinguish different disease entities of the NPHP-MCKD complex: (i) the mode of inheritance, (ii) the age of onset for ESRD, and (iii) the type of extra-renal organ involvement (Table 17-1B).

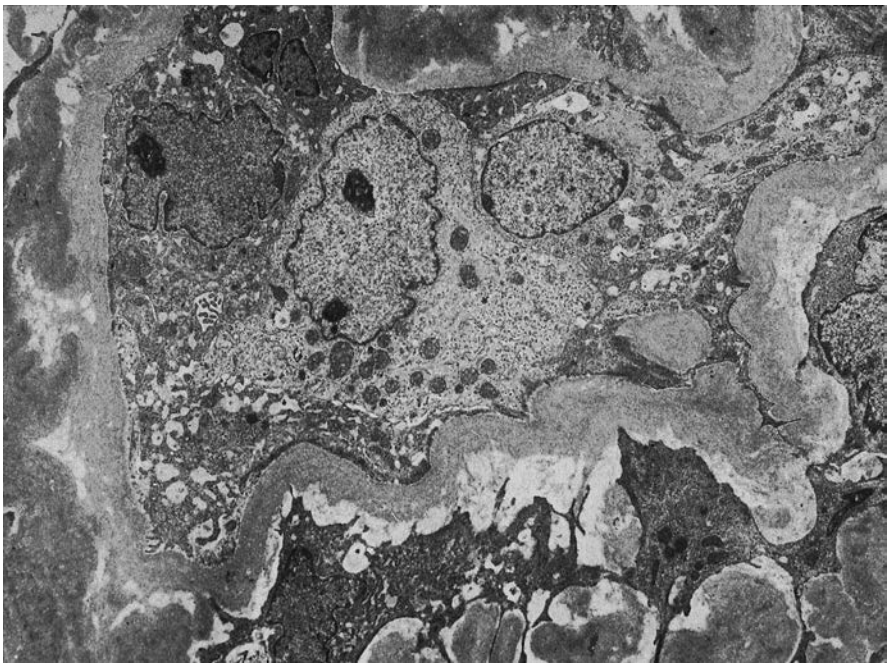

FIGURE 17-3. Thickening, wrinkling, and double-layering of tubular basement membranes with intermembranous fibroblasts and dedifferentiation of tubular epithelial cells. Electron micrograph. (Reproduced with permission from: Hildebrandt F, et al. The nephronophthisis complex: clinical and genetic aspects. *Clin Invest* 1992;70:802.)

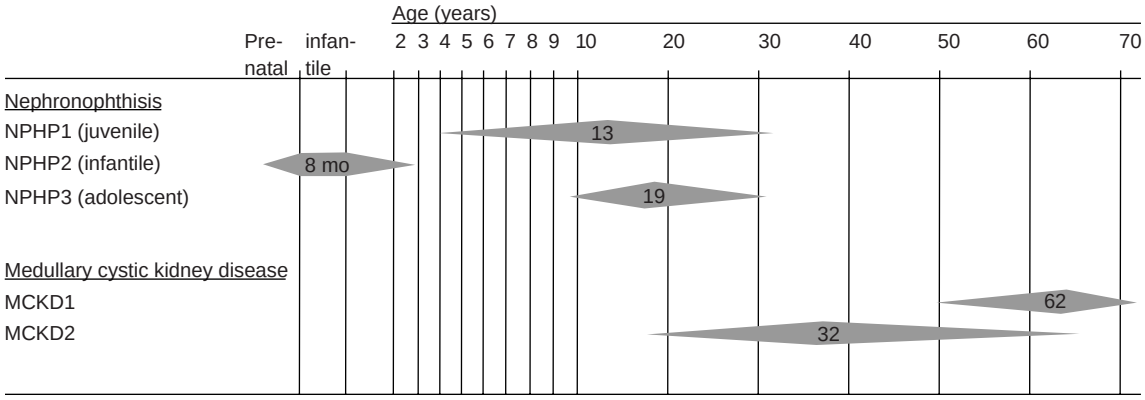

FIGURE 17-4. Time course of renal failure in NPHP-MCKD. Range for age of onset of end-stage renal disease is shown as solid triangles. Numbers indicates median age in years. *NPHP*, nephronophthisis; *MCKD*, medullary cystic kidney disease; *FJHN*, familial juvenile hyperuricemic nephropathy.

Mode of Inheritance

In the NPHP-MCKD complex the mode of inheritance, can be either autosomal recessive or autosomal dominant. For the recessive forms the term "nephronophthisis (NPHP)" is used, while the designation "medullary cystic kidney disease (MCKD)" denotes the dominant variants of the complex (Table 17-1B [i]) (11,12).

Onset of End-Stage Renal Disease

The second distinction pertains to the age of onset for ESRD (Table 17-1B [ii]). In all variants of NPHP-MCKD, ESRD insidiously ensues at characteristic age ranges, necessitating renal replacement therapy (see Fig. 17-4). In NPHP, chronic renal failure develops within the first 3 decades of life (13–15). In a study conducted in 46 children with juvenile nephronophthisis (NPHP1), a serum creatinine of 6 mg/dl was reached at a median age of 13 years of age, (range 4 to 20 years) (2,13). In a study by Waldherr et al. (1), ESRD was reached at a median age of 11.5 years. The rate of deterioration of renal function has been shown by Gretz et al. to be homogeneous in a study of 29 patients with NPHP1 (16). The median time elapsing between a serum creatinine of 2 and 4 mg/dL was 32 months, between 4 and 6 mg/dL 10 months, and between 6 and 8 mg/dL 5 months (16). A high concordance of the development of renal failure was noted in monozygotic twins (17,18). Infantile nephronophthisis (NPHP2) is characterized by an early onset of ESRD, between neonatal age and age 3 years (15). In adolescent nephronophthisis (NPHP3) terminal renal failure develops at a median age of 19 years, which is 6 years later than is known for NPHP1 (14). The median age of ESRD in patients with *NPHP4* and *NPHP5* mutations is 20 years (19) and 13 years (20), respectively. If renal failure has not developed by the age of 25 years, the diagnosis of recessive NPHP should be questioned and pedigree analysis should be intensified to exclude dominant MCKD.

In MCKD, terminal renal failure occurs only in adult life. Two different variants are known, MCKD1 and MCKD2, with a median onset of ESRD at 62 yrs (21) and 32 yrs (22), respectively (Fig. 17-4).

Extrarenal Associations

The third distinguishing feature (iii) among variants of NPHP-MCKD is represented by the degree to which extrarenal associations occur (Table 17-1B [iii]). Extrarenal disease manifestations have only been described in recessive forms. One exception to this rule is the occurrence of hyperuricemia and gout in MCKD1 (23) and MCKD2 (22). MCKD2 patients with UMOD mutations also may exhibit defects in urine concentration (24). NPHP1 can occur in combination with ocular motor apraxia type Cogan (25), with retinitis pigmentosa in Senior-Løken syndrome (SLS), with liver fibrosis and cone-shaped epiphyses in Mainzer-Saldino syndrome (26), and with coloboma of the optic nerve and cerebellar vermis aplasia in Joubert syndrome type B (Table 17-1B (iii) and Table 17-2), as described subsequently. NPHP2 can be associated with *situs inversus* (27) and one case report describes a patient with a nonsense *inversin* mutation with retinitis pigmentosa (28). NPHP4 patients may have retinitis pigmentosa (SLS) and Cogan's syndrome (29). NPHP5 patients display early onset retinitis pigmentosa (SLS) in all known cases (20).

Epidemiology

Nephronophthisis and dominant MCKD seem to be distributed evenly among males and females. Nephronophthisis has been reported from virtually all regions of the world (30). Information on the incidence of the disease is currently solely based on estimates and has been given as 9 patients/8.3 million (31) in the United States or 1 in 50,000 live births in Canada (1,32). The condition constitutes the most frequent genetic cause for ESRD in the first three decades of life, and is a major cause of ESRD in children, accounting for 10% to 25% of these patients (30,33–35). In contrast, in the North American pediatric ESRD population pooled data indicate a prevalence of less than 5% (36,37).

MCKD has initially been reported from the United States (11). Its prevalence in Europe might have been underestimated, since recently kindreds have been reported from Cyprus (23), Italy (22,38), France (39), England, Finland (40,41), Belgium, Czechia (42) and Germany (43,44). Underdiagnosis of MCKD may be common and often requires genetic analysis as clinical symptoms and signs are subtle (45).

MOLECULAR GENETICS OF THE NPHP-MCKD COMPLEX

Classification of disease variants of the NPHP-MCKD complex has recently become more definite through positional cloning of distinct genes and gene loci for the different variants. This

TABLE 17-2

DISEASE VARIANTS, GENE LOCI, AND EXTRARENAL INVOLVEMENT IN THE NPHP–MCKD COMPLEX OF DISEASES

Disease	OMIM#	Inheritance	Locus	Chromosome	Gene (product)	Extrarenal Organ Involvement	Literature
NPHP1 (juvenile)	256100 (607100)	AR	*NPHP1*	2q12-q13	*NPHP1* (nephrocystin-1)	RP, OMA, Coloboma, CVA (JBTS)	(50,51,85,166)
NPHP2 (infantile)	602088	AR	*NPHP2*	9q22-q31	*INVS/NPHP2* (inversin/nephrocystin-2)	RP, *situs inversus*	(15,27)
NPHP3 (adolescent)	604387	AR	*NPHP3*	3q21-q22	*NPHP3* (nephrocystin-3)	RP, LF	(66)
NPHP4	607215	AR	*NPHP4*	1p36	*NPHP4* (nephroretinin/ nephrocystin-4)	RP, OMA	(19,29)
NPHP5	609237	AR	*NPHP5*	3q21.1	IQCB1/NPHP5 (IQ calmodulin-binding motif containing protein-1/nephrocystin-5)	Early onset RP	(20)
Joubert syndrome type 1	213300	AR	*JBTS1/ CORS1*	9q34.3	?	RP, CVA	(87)
Joubert syndrome type 2	608091	AR	*CORS2*	11p12-q13.3	?	RP, Coloboma, CVA	(88)
Joubert syndrome type 3	608894	AR	*AHI1*	6q23.3	*AHI1* (jouberin)	RP, CVA, cortical polymicrogyria	(167–169)
Joubert syndrome type 4	182290	AR	*SMS*	17p11.2	?	CVA	(89)
MCKD1	174000	AD	*MCKD1*	1q21	?	gout, hyperuricemia	(21)
MCKD2	191845	AD	*UMOD*	16p12.3	*UMOD* (uromodulin)	gout, hyperuricemia	(141,142)

AD = autosomal dominant; *MCKD* = autosomal dominant medullary cystic kidney disease; *AR* = autosomal recessive; *CORS* = cerebellooculorenal syndrome; *CVA* = cerebellar vermis aplasia; *NPHP* = nephronophthisis; *FJHN* = familial juvenile hyperuricemic nephropathy; *JBTS1* = Joubert syndrome 1; *OMA* = ocular motor apraxia; *OMIM#* = URL http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM; *RP* = retinitis pigmentosa; *SMS* = Smith-Magenis syndrome; *UMOD* = uromodulin

disease complex is genetically very heterogeneous. Aspects of disease nomenclature, known genes and gene loci and extrarenal involvement within the NPHP-MCKD complex are summarized in Table 17-2.

Recessive Disease Variants: Nephronophthisis

Within recessive variants of the NPHP-MCKD complex, the different forms are distinguished on the basis of the gene mutated. To date, five distinct genes (*NPHP1–5*) have been identified by positional cloning.

Nephronophthisis Type 1 (Juvenile Nephronophthisis): Clinical Features

The first case of juvenile nephronophthisis was described by Smith and Graham in 1945 (46). This report of a sporadic case was followed by publication of two large kindreds with familial disease by Fanconi et al. (47), who introduced the term "familial juvenile nephronophthisis." This disease form today is classified as nephronophthisis type 1 (NPHP1). Since the first description, over 300 cases have been published in the literature (1). NPHP1 is the most common variant within the NPHP-MCKD complex. Penetrance in affected individuals is 100%.

In juvenile NPHP the symptoms of polyuria, polydipsia, decreased urinary concentrating ability, and secondary enuresis are the earliest presenting symptoms in over 80% of cases (30) and occur at around 4 to 6 years of age. Pallor, weakness, and generalized pruritus are also common. Anemia (40) and, in children growth retardation, occur later and are usually pronounced. In juvenile nephronophthisis children usually start to drink regularly at nighttime around age 6 years. This characteristic symptom should actively be sought when taking the patient's history. The insignificance of symptoms together with frequent lack of edema, hypertension, and urinary tract infections characteristically leads to delayed diagnosis and therapy in NPHP-MCKD. Due to late detection of symptoms there is a small but definite risk of sudden death from fluid and electrolyte imbalance. For NPHP1, definite molecular genetic diagnosis is possible (see the subsequent text). Disease recurrence has never been reported in kidneys transplanted to NPHP patients (10).

Nephronophthisis Type 1 (NPHP1): Molecular Genetics

Since little was known about the pathogenesis of NPHP, a positional cloning approach was used to identify a gene for NPHP1.

Through linkage analysis by a total genome search, a gene locus for juvenile NPHP1 was localized to human chromosome 2q12-q13 (48,49). The critical genetic region was subsequently cloned in YAC and PAC contigs (11–15), which lead to the identification of a gene termed *NPHP1*, defects in which are responsible for NPHP1 (50). The identity of this gene as mutated in juvenile nephronophthisis has since been confirmed (51). About 66% of children with juvenile nephronophthisis harbor large (250 kb) homozygous deletions of the *NPHP1* gene, while some carry point mutations in combination with heterozygous deletions (52–54). Through gene identification, molecular genetic diagnosis in NPHP1 has become possible (see the subsequent text) (54,55).

The *NPHP1* gene spans 83 kb, consists of 20 exons, and encodes a mRNA of 4.5 kb. It is flanked by two large (330 kb) inverted duplications. In addition, a second sequence of 45 kb, which is located between the centromeric inverted duplication and the *NPHP1* gene, is repeated directly within the telomeric inverted duplication. In several *NPHP1* families, the deletion breakpoints have been localized to the 45 kb direct repeats using pulsed field gel electrophoresis (53). Chromosomal misalignment followed by unequal crossover or the formation of a loop structure on a single chromosome has been suggested as a potential cause for these deletions. In addition, there is a high degree of further rearrangements known to occur in this region of chromosome 2 (53). In addition, an unusual maternal deletion in a child with *NPHP1* has recently been molecularly characterized. By direct sequencing it was shown that the centromeric breakpoint was localized within a long interspersed nuclear element-1 (LINE1), which belongs to an abundant group of transposable elements within the human genome (56). The *NPHP1* gene is a novel gene, which is not related to any known gene families. Expression studies in man and mouse revealed a broad tissue expression pattern. In addition, *in situ* hybridization studies of whole mount mouse embryos showed ubiquitous but weak *Nphp1* expression at all embryonic stages between days 7.5 and 11.5 p.c. (57). In the adult mouse there was also strong expression in testis.

Nephronophthisis Type 2 (NPHP2)

A second gene locus (*NPHP2*) for recessive NPHP has been localized to chromosome 9q22-q31 in a large Bedouin pedigree, by homozygosity mapping (Table 17-2) (15). This disease form is termed infantile nephronophthisis (NPHP2) due to its prenatal, perinatal, or infantile onset. The clinical course and histology in this disease are quite different form other forms of NPHP (58). The *inv* mouse model, where disruption of the protein inversin led to consistent reversal (rather than randomization) of the left–right axis (59,60) was noted to have cystic kidney disease (61). These observations led to the identification of *inversin* (*INVS*) as the gene mutated in NPHP2 with and without *situs inversus* (27). *INVS* encodes a 1062 amino acid protein containing 16 ankyrin repeats, a nuclear localization signal and an IQ calmodulin domain (62). Yeast two-hybrid and coimmunoprecipitation experiments have confirmed this interaction between inversin and calmodulin. Using a knockdown of inversin expression in zebrafish a PKD-like phenotype in addition to randomization of heart looping was observed (27). Inversin protein has been localized to primary cilia, mitotic spindles and centrosomes (62) and is intimately associated with the microtubule cytoskeleton (63). *INVS/NPHP2* mutations remain a rare cause of NPHP, accounting for <1% of cases.

Nephronophthisis Type 3 (NPHP3)

A third locus (*NPHP3*) for NPHP was localized to chromosome 3q21-q22 in a large Venezuelan kindred by a total genome search for linkage, applying the strategy of homozygosity mapping (14,64,65) (Table 17-2). This disease variant was termed "adolescent nephronophthisis" (NPHP3), since onset of ESRD occurs six years later than in juvenile NPHP1, with a median onset of terminal renal failure at age 19 years (Fig. 17-4).

Identification of the gene causing NPHP3 was carried out using the same Venezuelan kindred (66). This novel protein, named *NPHP3*, encodes a 1,330 amino acid protein which interacts with nephrocystin-1. *NPHP3* mutations were found in patients with isolated nephronophthisis and in families with NPHP and hepatic fibrosis or tapetoretinal degeneration. Murine *Nphp3* was shown to be expressed in the embryonic node, kidney tubules, retina, respiratory epithelium, liver, biliary tract and neural tissues. A homozygous missense mutation in *Nphp3* was identified as the underlying defect in the polycystic kidney disease (*pcy*) mouse phenotype (66).

Nephronophthisis Type 4 (NPHP4)

By total genome search for linkage a fourth gene locus (*NPHP4*) was localized to chromosome 1p36 (67) including a family with SLS. The respective gene (*NPHP4*) was subsequently identified as causing NPHP type 4 and SLS type 4 (19). The gene and its gene product nephroretinin/nephrocystin-4 are novel and highly conserved in evolution. Nephrocystin-4 protein expression has been demonstrated in primary cilia, centrosomes and near to the cortical actin cytoskeleton, with partial colocalization with β-catenin in polarized MDCK cells (68). Co-immunoprecipitation experiments showed that nephrocystin, p130Cas and Pyk2 all complex with nephrocystin-4 (68). This data may reconcile the involvement of nephrocystin complex of proteins in both adherens junction and ciliary hypotheses (Fig. 17-5).

Nephronophthisis Type 5 (NPHP5)

NPHP5 (*ICQB1*) is a novel gene, which has very recently been identified by positional cloning as a cause of NPHP type 5 (20). All of the mutations found in *NPHP5* and its protein product "nephrocystin-5" are the result of truncating mutations. Interestingly, all mutations were associated with the presence of early-onset SLS/retinitis pigmentosa where blindness occurred before the third year of life (20). Nephrocystin-5 protein directly interacts with calmodulin and is in a protein complex with the retinitis pigmentosa GTPase regulator (RPGR), thus explaining the renal-retinal phenotype of the disease.

Nephronophthisis with Extrarenal Associations

With the exception of the occurrence of hyperuricemia and gout in MCKD, extrarenal disease manifestations have only been described in recessive forms of nephronophthisis (Table 17-1B and Table 17-2). Ocular motor apraxia type Cogan is the transient inability of horizontal eye movements in the first few years of life. This has been described in patients with *NPHP1* (25,69) and *NPHP4* mutations (29).

The *Senior-Løken syndrome (SLS)*, represented by concomitant occurrence of NPHP with retinitis pigmentosa, was first described by Contreras (70), Senior (71), and Løken (72). The designation SLS seems more appropriate than the term *retinal-renal dysplasia*, since both, renal and retinal changes are degenerative rather than dysplastic (73). The association of retinitis pigmentosa with NPHP1 was found almost exclusively in families with recessive inheritance (30,74). In NPHP types 1–4 retinitis pigmentosa occurs in about 10% of all affected families. In NPHP type 5 all patients exhibited early-onset retinitis pigmentosa (20). Retinitis pigmentosa is diagnosed by its specific findings on ophthalmoscopy including increased pigment, attenuation of retinal vessels, and pallor of the optic disc, coupled with the results of electroretinography and electrooculography. Retinal degeneration is characterized

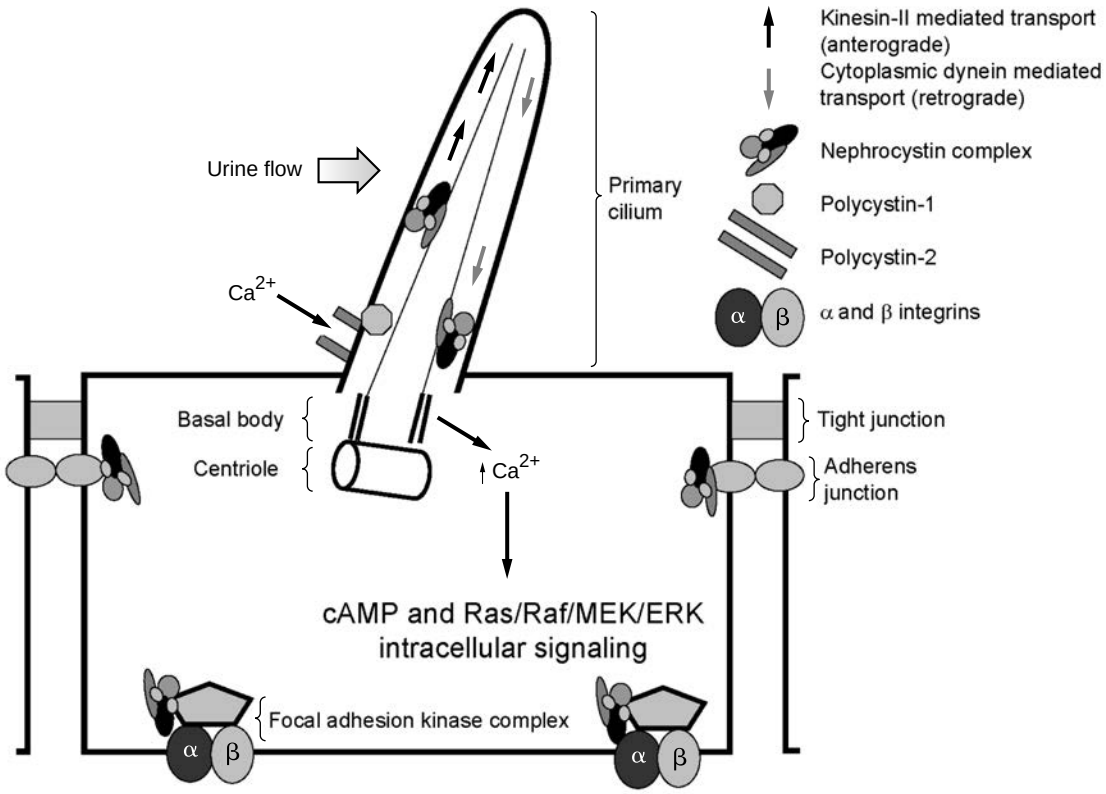

FIGURE 17-5. Localization of the nephrocystin complex to the renal epithelial cell primary cilium, the adherens junction, focal adhesions, and microtubule organizing centers (centrosomes). A primary renal cilium is shown bending as a result of urinary flow. Fluid shear forces lead to an increase in intracellular calcium, mediated by calcium permeant channels, such as polycystin-2, localized to the surface of the cilia. This initial calcium influx may lead to multiple downstream effects including calcium induced calcium release, targeted fusion of cytoplasmic vesicles with the plasma membrane, protein kinase signaling cascades, and gene expression, which may modulate cellular proliferation, differentiation, and apoptosis. (Modified from: Nauli SM, et al. Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nat Genet* 2003;33:129; Praetorius HA, Spring KR. Bending the MDCK cell primary cilium increases intracellular calcium. *J Membr Biol* 2001;184:71; Praetorius HA, et al. Bending the primary cilium opens Ca^{2+}-sensitive intermediate-conductance K^+ channels in MDCK cells. *J Membr Biol* 2003;191:193; Alenghat FJ, et al. Global cytoskeletal control of mechanotransduction in kidney epithelial cells. *Exp Cell Res* 2004;301:23 [refs. 169–172].) Nephrocystin complexes are shown within the cilium, where they are moved up the cilium by intraflagellar transport protein complexes (Kinesin-II) and down via cytoplasmic dynein. The precise function of the nephrocystin complex within the cilium remains to be established. In addition to the cilium, inversin and nephrocystins have been localized to adherens junctions, centrosomes, and the nucleus (not shown). Nephrocystin complexes are also shown localized to the focal adhesion kinase complex, which includes proteins, such as pyk2 and p130Cas, binding partners of Nephrocystin-1 and Nephrocystin-4. (Modified from: Mollet G, et al. Characterization of the nephrocystin/nephrocystin-4 complex and subcellular localization of nephrocystin-4 to primary cilia and centrosomes. *Hum Mol Genet* 2005;14:645; Benzing T, et al. Nephrocystin interacts with Pyk2, p130 (Cas), and tensin and triggers phosphorylation of Pyk2. *Proc Natl Acad Sci USA* 2001;98:9784.) Cell adhesion signaling events and polarity may be regulated by the nephrocystin complex at this location.

by a constant and complete extinction of the electroretinogram, preceding the development of visual and funduscopic signs of retinitis pigmentosa (75). Whether the heterozygous state in the Senior-Løken syndrome can be determined by electroretinography and electrooculography as reported by Polak et al. (76) awaits confirmation. An early-onset and a late-onset type of SLS have been distinguished. The early-onset type seems to represent a form of Leber's congenital amaurosis, since children exhibit coarse nystagmus and/or blindness at birth or within the first 2 years of life (77). Funduscopic alterations are present in all SLS patients by the age of 10 years. The late-onset form is characterized by development of blindness during school age after a preceding night blindness. Funduscopy in these patients usually reveals sector retinitis pigmentosa or retinitis punctata albescens. Other eye symptoms besides tapetoretinal degeneration comprise nystagmus, myopia, coloboma of the chorioidea, strabismus, hyperopia, optical nerve atrophy, and amblyopia (47). Age of onset, symptoms, and histology of renal disease is identical to what is known from patients with juvenile nephronophthisis without ocular involvement.

The association of NPHP with *liver fibrosis* was first noted by Boichis (78) and later also reported by others (79–81). All patients had hepatomegaly and moderate portal fibrosis with mild bile duct proliferation. This pattern differs from that of classical congenital hepatic fibrosis, where biliary dysgenesis is prominent. Cases with skeletal changes, predominantly in

the form of cone shaped epiphyses (type 28 and 28A) are known as *Mainzer-Saldino syndrome*, and were first published by Mainzer (26) in combination with retinal degeneration and cerebellar ataxia. A recessive mutation in the *NPHP3* gene was recently described in a patient with NPHP and liver fibrosis (66).

In *Joubert syndrome type B (JSB)*, a developmental disorder with multiple organ involvement, NPHP occurs in association with coloboma of the eye or retinal degeneration, aplasia of the cerebellar vermis with ataxia and the facultative symptoms of psychomotor retardation, polydactyly and neonatal tachy/dyspnea (82,83). A diagnostic feature of Joubert syndrome on axial magnetic resonance imaging (MRI) of the brain is prominent superior cerebellar peduncles, termed the "molar tooth sign" (MTS) (84). Two genes have now been shown to cause Joubert syndrome. *NPHP1* gene defects are a rare cause of Joubert syndrome in a subset of patients with NPHP (85). A homozygous deletion of *NPHP1* was found in two siblings and a third patient with mild features of Joubert syndrome type B. The second gene defect was found in the *Abelson Helper Integration Site* (*AHI1*) gene, and its protein product has been termed *Jouberin*. The Jouberin protein has three known isoforms and possesses a coiled coil domain, at least six WD40 domains, and an SH3 domain. It is thus likely to be part of the nephrocystin complex of proteins. In the initial reports of *AHI1* mutations, the phenotype included cerebellar abnormalities, but no renal phenotype was reported. We have very recently identified Jouberin mutations in patients with nephronophthisis and Joubert syndrome type B (86).

At least four other gene loci have been reported for Joubert syndrome. These include the *JBTS1* locus on chromosome 9q34 (87), the *CORS2* locus on chromosome 11 (88), the Smith-Magenis locus (89), and a possible locus on chromosome 3q24 (90).

Additional phenotypes have been described in association with NPHP. These include *Jeune syndrome* (asphyxiating thoracic dysplasia) (91–94), *Ellis van Creveld syndrome* (95), ulcerative colitis (96), RHYNS syndrome (97), Alstrom syndrome (98,99), Sensenbrenner syndrome (100,101) and Arima syndrome (102–104).

Bardet-Biedl syndrome (105,106) has been reported to exhibit renal histology findings reminiscent of NPHP. Recently, positional cloning of the responsible genes has revealed that the molecular relation between these syndromes may lie in the expression of the respective gene products in primary cilia, basal bodies, or centrosomes of renal epithelial cells (107).

Animal Models

Genetic animal models resembling nephronophthisis have been fruitful in the identification of underlying gene defects, and more recently in the experimental treatment of cystic kidney disease.

The *Invs* gene when mutated gives rise to renal cysts as well as left–right asymmetry, cardiovascular defects, hepatobiliary defects and premature death in *inv/inv* knockout mice (59,61,108). Collecting ducts of newborn *inv/inv* mice demonstrate diffuse cystic dilatation (109). Mutations in *INVS* give rise to human NPHP type 2, with and without *situs inversus* (27). The *pcy* mouse model (110) demonstrates interstitial fibrosis and cystic kidneys. The underlying defect was shown to be a missense mutation in the *Nphp3* gene (66). This murine model is now being used in a series of trials of inhibitors of cystogenesis (see the subsequent text). The *kd* ("kidney disease") mouse strain has also been reported as a genetic animal model of NPHP (111,112). It shares several clinical and histologic (112) features with human NPHP. The mice are born apparently healthy, then from 8 weeks of age develop severe interstitial nephritis that progresses to ESRD by 4 to 8 months of age. The defect was recently found to be in a novel gene which encodes a mitochondrial protein, namely prenyltransferase-like mitochondrial protein (PLMP) (113). Kd/kd mice were shown to have dysmorphic mitochondria within renal tubular epithelia (113). Additional transgenic mouse models for NPHP, such as the *tensin* knockout mouse (114), the Rho GDI deficient mouse, the *bcl-2* knockout mouse, (115,116) and the *Ace* knockout mouse (117) will hopefully aid our understanding of the pathophysiology of NPHP (118). A canine model of NPHP has also been reported (119–121).

Nephronophthisis: The Renal Cilia, Adherens Junctions, and Focal Adhesion Hypothesis

The identification of mutations in the *inversin* gene, which cause NPHP type 2, established a link between the pathogenesis of NPHP to disease mechanisms of polycystic kidney disease (27). The knockdown of *invs* in the zebrafish embryo caused a renal cystic phenotype. In addition, the positional cloning of the novel gene *NPHP3*, mutated in adolescent NPHP (type 3) and the renal cystic mouse model *pcy* (66) confirmed this paradigm. Nephrocystin interacts with both inversin and with β-tubulin, with colocalization of all three proteins in the primary renal cilia of epithelial cells (27). Inversin was also shown to be localized to mitotic spindles and centrioles (62,122). The IQ calmodulin-binding motif containing protein-1 (IQCB1), also known as *nephrocystin-5,* also reveals a ciliary and basal body colocalization (20). The finding that such nephrocystins colocalize to primary cilia, basal bodies, or centrioles together with other proteins that, if defective, cause renal cystic diseases, suggests a role within a functional module shared with other proteins (Fig. 17-5). Therefore, very recently, the development of a unifying hypothesis of renal cystogenesis has been established (123). This hypothesis states that proteins which, if mutated, cause renal cystic disease in humans, in mice or zebrafish, are part of a functional module, as defined by their subcellular localization to primary cilia, basal bodies or centrioles. This applies to polycystin-1 and –2, fibrocystin/polyductin, nephrocystin-1, -2 (inversin), -3, -4, -5, Bardet-Biedl syndrome (BBS)-associated proteins, cystin, polaris, ALMS1, OFD1 and others.

A model of evolutionary conserved proteins involved in cilia has also recently added weight to the "cystogenes" hypothesis. Following identification the *NPHP1*, *2* and *4* genes, orthologs of these genes in the nematode *C. elegans* have been identified (19,50,57). This strong evolutionary conservation of genes that, if defective, cause nephronophthisis in humans, suggests that their products may be part of a functional module conserved in *C. elegans*. This assumption was also supported the finding of cell-specific GFP expression under the nephrocystin-1 and -4 promoters in the same cell types of head and tail ciliated neurons, in which the *C. elegans* orthologs of other renal cyst-causing genes are expressed such, as *pkd-1* (*lov-1*), *pkd-2*, and *polaris* (*osm-5*) (124).

In addition to the ciliary hypothesis, an adherens junction/focal adhesion hypothesis has also been suggested on the basis that nephrocystin-1 contains an SH3 domain (118,125). This theory is based on the fact that most SH3 domains are found in adapter proteins, which have a function in focal adhesion signaling complexes of cell-matrix contacts (126–128). Several findings support this hypothesis:

(i) Nephrocystin was shown to bind to the protein p130Cas ("crk-associated substrate") (129,130), which is a major mediator of focal adhesion assembly (127) and to compete for binding with Src and Fyn (129).
(ii) In children with NPHP, Rahilly et al. described strong $\alpha 5\beta 1$ integrin expression in proximal tubules, from which $\alpha 5$ integrin is normally absent, which most likely results

from defective $\alpha 6$ integrin expression (131). The $\alpha 5\beta 6$ complex is an important receptor for focal adhesion signaling in renal tubular cells.

(iii) The knockout mouse models for tensin (114) and for the Rho GDIα gene (132) both exhibit an NPHP-like phenotype, thereby implicating proteins of the focal adhesion signal transduction cascade in the pathogenesis of NPHP-like diseases.

Together, these findings may point to a pathogenesis of NPHP, which involves focal adhesion and/or adherens junction signaling processes. Recent data from Mollet et al. demonstrated in addition to ciliary and centrosomal localization, NPHP4 was part of a protein complex which included NPHP1, p130Cas and Pyk2 (68). These data confirm the role of the nephrocystin proteins within both a cilial/centrosomal hypothesis and an adherens junction/focal adhesion hypothesis.

Dominant Disease Variants: Medullary Cystic Kidney Disease

The first large kindreds of autosomal dominant medullary cystic kidney disease (MCKD) were reported by Goldman and by Gardner (11,133,134). Dominant MCKD by renal macroscopic pathology and histology is indistinguishable from recessive nephronophthisis. In MCKD terminal renal failure develops later than in NPHP, within the time frame of the third to the seventh decade of life (Fig. 17-4). The only extrarenal associations known to occur with MCKD are hyperuricemia and gouty arthritis, which have been described in the majority of the kindreds reported.

Medullary Cystic Kidney Disease Type 1 (MCKD1)

A gene locus for MCKD1 has been localized to chromosome 1q21 in large pedigrees from Cyprus (21). Further refinement of this locus has been possible using an observed recombinant from within a Belgian kindred (135), reducing the critical genetic region to 2.1 Mb (Table 17-2). This disease form was associated with hyperuricemia and gout (23). ESRD occurred at a median age of 62 years (Fig. 17-4).

Medullary Cystic Kidney Disease Type 2 (MCKD2)

A second locus (*MCKD2*) for medullary cystic kidney disease was localized to chromosome 16p12 (22,136,137) (Table 17-2). In this variant ESRD develops much earlier, at a median age of 32 years (Fig. 17-4). Very recently, MCKD2 and an autosomal dominant disease formerly known as familial juvenile hyperuricemic nephropathy (FJHN) (138) have been shown to map to the same chromosomal region and suggested that they represent the same disease entity (139,140). This was indeed confirmed with the identification of mutations within the *uromodulin* (*UMOD*) gene in affected patients with phenotypes of FJHN and MCKD2 (141). *UMOD* encodes the Tamm-Horsfall protein, which is a GPI anchored glycoprotein, present abundantly in normal urine. It has been suggested that mutations within *UMOD* may disrupt the tertiary structure of UMOD (141). Additional *UMOD* mutations were recently shown to be causative for MCKD2, with a noted clustering of mutations within the highly conserved exon 4 of the encoded sequence of *UMOD* (142). Investigation of *UMOD* mutations in affected patient's urine and renal biopsies revealed abnormal accumulation of uromodulin within tubular cells and reduced urinary excretion of wild-type uromodulin (143). Glomerulocystic kidney disease (GCKD), characterized by dilatation of Bowman's space and collapse of the glomerular tuft is a distinct renal disorder from MCKD/FJHN although there is sharing of some of the common clinical features. A clinical variant of GCKD demonstrates reduced fractional excretion of uric acid, resulting in hyperuricemia. A mutation in *UMOD* has recently been described in 1 family (3 patients) with this condition, broadening the phenotype associated with *UMOM* mutations further (144). Finally, there is evidence for at least one additional locus for MCKD (43,44).

MOLECULAR GENETIC DIAGNOSIS, IMAGING, AND LABORATORY STUDIES

Molecular Genetic Diagnosis in NPHP

Nephronophthisis types 1 through 5 can now be unequivocally diagnosed, because direct molecular genetic diagnosis has become available through identification of the responsible genes (www.renalgenes.org). Molecular genetic analysis is the only diagnostic procedure, by which the diagnosis of NPHP can be made with certainty. It should be initiated to noninvasively prove or exclude NPHP before the invasive procedure of renal biopsy is performed. However, due to the presence of additional genes for NPHP, the lack of detection of mutations in *NPHP1–5* genes does *not* exclude the diagnosis of NPHP. In a similar manner, *UMOD* mutational analysis will allow a precise genetic diagnosis of MCKD2 but lack of any mutations will not exclude MCKD. Molecular genetic testing should be performed only in the context of genetic counseling and within the guidelines of the National and International Societies for Human Genetics (http://www.ethics.ubc.ca). Prior to genetic counseling a thorough pedigree analysis to distinguish recessive (early-onset) from dominant (late-onset) disease is mandatory, and extrarenal organ involvement should be sought.

Imaging Techniques

Renal ultrasound is a very useful imaging technique in the NPHP-MCKD complex. Kidneys are normal or moderately reduced in size and exhibit, typically, a loss of corticomedullary differentiation and an increased echogenicity. Later in the course of the disease, mostly when patients have reached ESRD, cysts can be detected at the cortico-medullary junction (Fig. 17-6) (145–147). Garel and associates have seen medullary cysts in 13 of 15 children studied at the time of renal failure (mean age 9.7 years) (148).

Magnetic resonance tomography seems to have evolved as a very useful procedure in the diagnosis of NPHP-MCKD (Anlauf, unpublished). Medullary cysts can sometimes also be demonstrated on computed tomography (149–152). The invasive procedure of renal arteriography is not indicated to demonstrate the presence of medullary cysts (153), and caution must be exercised when performing contrast studies in patients with renal failure.

Laboratory Studies and Urinary Concentrating Ability

Besides molecular genetic diagnosis of *NPHP1-5* and *UMOD*, there are no chemical laboratory tests in the NPHP-MCKD complex that specifically establish the diagnosis. Hematuria, proteinuria, and bacteriuria are typically absent in NPHP. In rare cases where proteinuria is present, it is usually mild and of the tubular type. Prolinuria has been reported in two cases, whereas aminoaciduria has been sought but not found in at least 12 other cases (154,155). Laboratory studies are needed

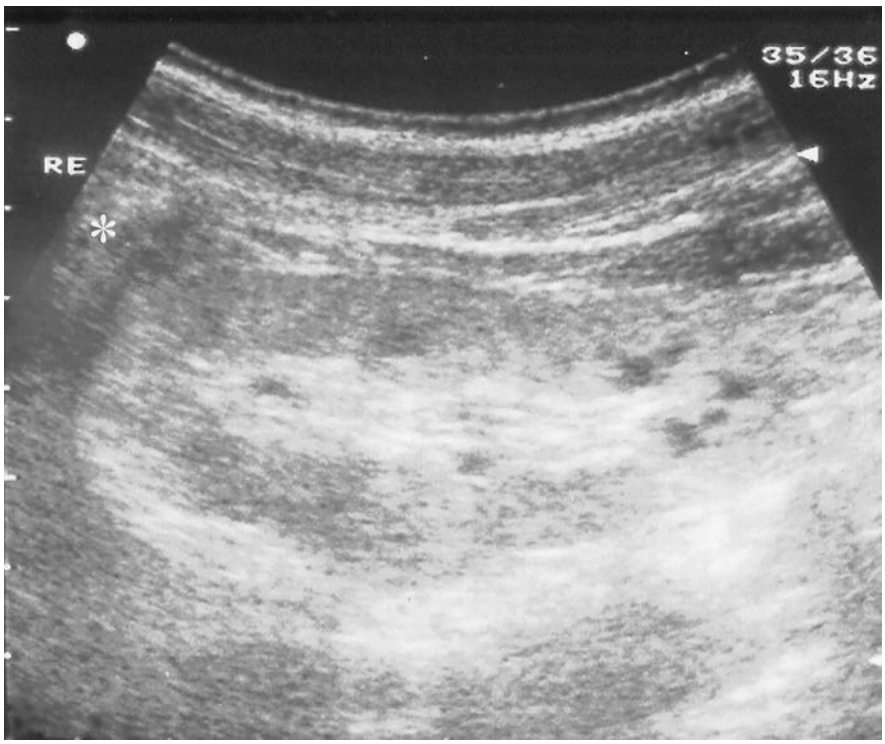

FIGURE 17-6. Characteristic renal ultrasound findings in juvenile nephronophthisis (NPHP1). Note normal kidney size, loss of corticomedullary differentiation, increased echogenicity, which renders the pattern of the kidney similar to the one of the liver (*), together with the presence of cysts at the corticomedullary border of the kidney. (Courtesy of Prof. J. Dippel, Frankfurt, Germany.)

to assess the severity of renal failure and generally demonstrate elevated serum creatinine, BUN, phosphorus, a metabolic acidosis, and anemia at the characteristic ages of onset of ESRD for the different disease entities. Ophthalmoscopy should be performed in any patient to exclude SLS. Liver function and hepatic ultrasonography should also be performed to facilitate detection of patients with hepatic fibrosis.

A characteristic early finding in NPHP is the decreased ability to concentrate the urine following a water deprivation test (30). Functional impairment of tubular function, with the constant finding of a renal concentration defect usually precedes any documentable reduction in glomerular filtration rate (11) and may be present with minimal histologic abnormalities (156).

An intermediate defect of urinary concentration ability has been inconsistently demonstrated in the parents and some siblings of children with NPHP, and has been suggested to reflect the heterozygous state of the disease (157,158). An 8-hour water deprivation test or vasopressin administration can be used to demonstrate a tubular concentration defect. Such tests should be performed with caution, since dehydration may precipitate acute renal failure in patients with the disease or in unrecognized affected family members. In affected individuals urine osmolality after 8 hours of water deprivation or vasopressin administration is <800 mosm/kg/H_2O. The diseases of the complex have also become known as "salt losing nephritis." However, the question of whether sodium loss is a typical finding in the disease complex, has not been clearly answered, upon critical evaluation of the literature (159). Poor renal uptake of 99^m-Technetium-DMSA has been proposed as diagnostic of NPHP (160).

Differential Diagnosis of NPHP-MCKD

On histopathology the NPHP-MCKD complex has to be differentiated from other forms of interstitial nephropathies like chronic pyelonephritis or drug injury. In oligomeganephronic dysplasia (161) kidney size is reduced and histology is distinct from NPHP. The paucity of urinary abnormalities, the frequent lack of hypertension, and the localization of renal cysts (if present) readily differentiate variants of the NPHP-MCKD complex from recessive or dominant polycystic kidney disease. Finally, medullary sponge kidney (162) (see the subsequent text) does usually not lead to chronic renal failure and shows calcifications and calculi on renal ultrasound, and is, therefore, readily distinguishable from the complex.

PROGNOSIS, THERAPY, AND COUNSELING

Therapy of NPHP and MCKD is symptomatic and will pertain to the treatment of hypertension, if present, as well as the correction of disturbances of electrolyte, acid-base, and water balance. Hypokalemia may contribute to the polyuria, so that oral potassium supplementation may alleviate this symptom. Metabolic acidosis should be corrected, and osteodystrophy and secondary hyperparathyroidism treated with adequate calcium supplementation, phosphorus restriction or binders, and vitamin D therapy. Anemia can be treated with erythropoietin and growth retardation may require administration of growth hormone, if the diagnosis is made early enough for an intervention. Adequate nutrition (caloric and amino-acid supplementation) should be maintained with the help of a dietician. Salt wasting seems to be more frequent in the phase just preceding the development of end-stage renal disease. Patients are at risk for sudden water and electrolyte disturbances, due to the high urinary output and salt loss. In some cases an event of severe dehydration with acute renal failure can abruptly precipitate chronic renal failure. Sufficient salt and water supplementation is important at this stage, but may have to be restricted as hypertension develops late in the course of renal failure. Psychological counseling of the patients is an integral part of therapy, because of the poor self-image associated with growth retardation and to alleviate pressures resulting from the need

to comply with complicated medications and dietary prescriptions. All patients will require renal replacement therapy during childhood, adolescence or, in dominant disease, in adult life.

At present, renal transplantation is the treatment of choice ESRF associated with both NPHP and MCDK. Stavrou et al. recently reported the outcomes of renal transplantation for 19 patients with MCKD type 1 (163). Five-year graft survival was 90% with no evidence of recurrence of disease or specific complications.

Prior to genetic counseling a thorough pedigree analysis to distinguish recessive (early onset) from dominant (late-onset) disease is mandatory, and other than renal organ involvement should be excluded. Siblings below 13 years of age should be re-evaluated yearly by maximal urinary concentrating ability, to allow early detection and early prevention of complications. If a transplant recipient's renal histology suggests NPHP or MCKD and a living related donor is considered, an extensive search should be made to exclude or detect renal disease within the family.

Future therapeutic strategies targeted at renal cyst expansion may lead to the successful delay of ESRD. Vasopressin, a major adenyl cyclase agonist, acts via V2 receptors in the collecting duct. Recently, an antagonists of the V2 receptor (OPC31260) has been shown to inhibit renal cystogenesis in the *pcy* mouse, the murine equivalent of human NPHP3 (164). Clinical trials in patients with NPHP are eagerly awaited. Similarly, the antiproliferative agent rapamycin has been used in the Han:SPRD rat model of polycystic kidney disease, where a reduction in cyst volume density and a preservation of renal function was observed (165). Thus the possibility of therapeutic intervention in human patients with cystic kidney disease, including nephronophthisis, looks to be a realistic possibility.

MEDULLARY SPONGE KIDNEY

Definition and History

Medullary sponge kidney is a congenital, benign cystic disorder of the kidney consisting of diffuse ectases or cystic dilatations of the collecting ducts within the medullary pyramids of one or both kidneys (173). The disorder is mainly diagnosed based on radiologic criteria by the presence of radial striations or cystic collections of contrast medium in the affected papillae on excretory urograms (174). The initial description of the urographic aspect of the disease was by Lenarduzzi in 1939 (175), but Cacchi and Ricci were the first comprehensively to describe the radiologic and clinical characteristics of the disease in 1948 (176). Ten years later, Ekstrom et al. reported a series of 44 cases (177), whereas Abeshouse and Abeshouse analyzed 136 cases collected from the literature, including 5 of their own (178). During the past three decades, several hundred cases have been reported, including the large personal series of Yendt et al. (179), as well as series of Kuiper (173), Harrison and Rose (180), and Thomas et al. (181). Our own experience bears on 179 patients with MSK followed from 1982 to 1997, most of whom had been referred for nephrolithiasis (182).

The disease may be studied from two main perspectives: first, as part of the group of cystic diseases of the medulla, especially in view of its multiple pathologic associations, including cystic involvement in other organs and the kidneys themselves, and other developmental defects; and second, as a cause of recurrent nephrolithiasis, indicating that MSK be considered as an important etiology in calcium stone disease.

The term *MSK* is somewhat inadequate because the affected kidney does not resemble a sea sponge, except perhaps in forms with especially numerous and voluminous medullary cyst formations. The terms *tubular ectasia, precaliceal canalicular ectasia,* or *cystic dilatation of renal collecting ducts* would be more accurate, but have never been widely accepted. Continued use has consecrated the terms *MSK* or *Cacchi-Ricci disease*.

Diagnosis

Pathologic Changes

Strictly speaking, the disease is defined on a pathologic basis. Typically only the medulla is affected, especially in the inner papillary portions (183–185). The disease is characterized by spherical, oval, or irregular dilatations of collecting ducts, associated with multiple small cysts measuring 1 to 7 mm in diameter. The dilated ducts connect proximally with collecting tubules of normal diameter (173), and show a relative constriction to an approximately normal diameter at the point of communication with the calix, as shown by microdissection studies (186). Most of the cysts communicate with the dilated collecting ducts, whereas some communicate directly with minor calices (177). The cysts often contain radiopaque spherical concretions usually composed predominantly of carbonate apatite (177). The affected pyramids are often enlarged, and some degree of whole-kidney enlargement may be observed when several papillae are involved (174).

Microscopically, communicating cysts are lined by cuboidal or columnar epithelial cells, but less frequently by a transitional or squamous epithelium, possibly as a response to the presence of intraductal calculi. Closed cysts are lined by atrophic epithelium. The renal cortex, columns of Berlin, calices, and pelvis appear normal unless they are affected by complications secondary to extensive nephrocalcinosis, obstruction, or infection (184).

Radiologic Criteria

The basis for diagnosis of MSK is excretory urography (173,174,185,187–191). Stringent technical conditions are required to demonstrate the characteristic roentgenologic findings of MSK. Typically, dilated collecting tubules, after opacification by the contrast medium, are seen as linear striations described as "streaking" or "brushlike" patterns of affected papillae (Fig. 17-7), whereas ectatic lesions appear as spherical or oval cystic images filled by contrast medium, and are compared with "bouquets of flowers" (Fig. 17-8) or "bunches of grapes" (Fig. 17-9). Thomas et al. observed that the presence of

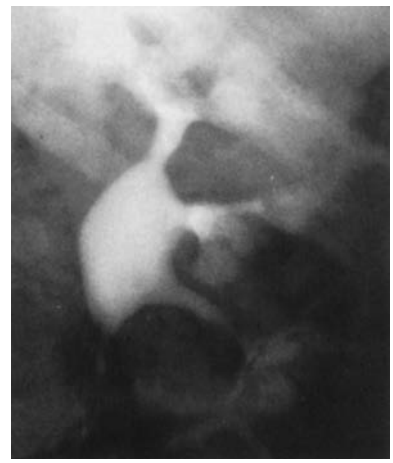

FIGURE 17-7. Excretory urogram in a patient with medullary sponge kidney, showing brushlike linear striations in lower papillae and homogeneous precaliceal opacification (papillary blush) of the upper calices of left kidney.

FIGURE 17-8. Excretory urogram in a patient with medullary sponge kidney, showing multiple spherical or oval dilatations of medullary collecting ducts in all papillary groups of the right kidney, giving a "bouquets of flowers" appearance.

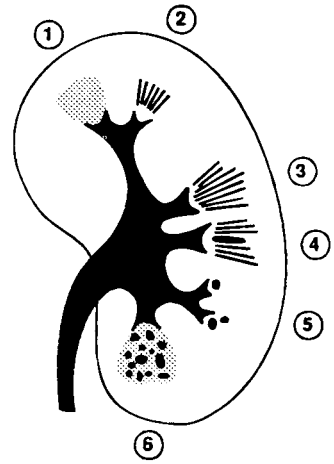

FIGURE 17-10. Schematic representation of excretory urogram findings in a medullary sponge kidney in increasing order of severity: (1) amorphous precaliceal opacification (papillary blush); (2) faint pyramidal striation; (3) heavy pyramidal striations (papillary streaking or brushlike aspect); (4) heavy streaking and pooled contrast medium in a linear tubular dilatation; (5) small, round precaliceal tubular dilatations filled with contrast medium; (6) papillary blush and multiple precaliceal dilatations filled with contrast ("bouquet of flowers" appearance).

a clear zone between abnormally opacified papillae, as well as opacification of the calices, is highly suggestive of MSK (181). Several of these aspects may coexist in diverse papillae in the same patient, as schematically represented in Figure 17-10.

On plain radiographs, spherical radiopaque concretions are often present in one or several papillae, like "stars in a dark sky." They are located at the corticomedullary junction, as later shown on the excretory urogram. In florid cases, multiple coalescent concretions may be present in one or several papillae, looking like calcified blackberries, as schematically represented in Figure 17-11. Such an aspect is virtually pathognomonic of the disease. In addition, stones may be present in calices, pelvis, or ureter. In the most severe forms, extensive medullary nephrocalcinosis may be present in both kidneys.

The true roentgenologic criteria of MSK are still debated. There is no difficulty in florid forms when patent precaliceal striations or multiple cystic dilatations are present, even in only one caliceal group. When only a few lesions are present, Yendt does not accept the diagnosis of MSK unless three or more papillae are involved (154), although he agrees that the diagnosis is justified if several characteristic lesions are identified in only one or two papillae. Ginalski et al. proposed that diagnosis of MSK should be based on the presence of a minimum of three linear or round collections in one or several papillae in the absence of obstruction (192). When only homogeneous precaliceal opacification is present, in the form of isolated papillary "blush," a number of authors do not accept the diagnosis of MSK because such an image is frequently observed in asymptomatic patients. Palubinskas observed such isolated papillary

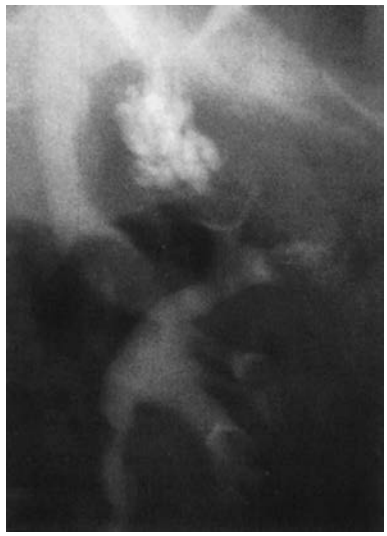

FIGURE 17-9. Excretory urogram in a patient with medullary sponge kidney, showing "bundle of grapes" appearance localized at the upper pole of left kidney.

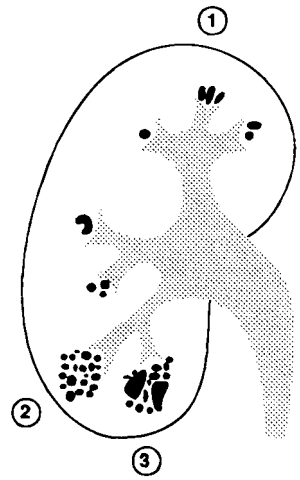

FIGURE 17-11. Schematic representation of the various appearances of medullary nephrocalcinosis on preliminary plain radiographs in medullary sponge kidney: (1) isolated, single or multiple, small precaliceal calcified concretions; (2) clusters of calcifications with appearance of calcified bundle of grapes or blackberries; (3) coalescent aggregates of calcifications.

blushing in almost 1% of subjects who underwent excretory urography (21 cases of 2,465) (185). However, on pathologic examination, ectatic collecting tubules were seen only in those papillae that demonstrated such papillary blush (185) or pyramidal enlargement (177) on excretory urography in some patients. Therefore, many cases of papillary blush probably represent minor degrees of MSK, especially if blushing persists for more than 10 minutes after injection in the absence of obstruction or compression (193).

It was claimed that low-osmolality contrast media should provide better visualization of dilated collecting ducts because of less induction of osmotic diuresis (194). However, Ginalski et al. observed a similar prevalence of clear linear or round precaliceal opacifications regardless of whether high- or low-osmolality contrast media were used (195). With either method, the incidence of such radiologic findings was significantly higher in stone formers (approximately 12%) than in control subjects without urolithiasis (approximately 1%). By contrast, the overall prevalence of isolated papillary blush was markedly higher with the use of low-osmolality media, although the prevalence of this finding was not greater in stone formers than in control subjects. Therefore, these authors suggest that low-osmolality contrast media have no clear advantage for diagnosing MSK; they induce a greater incidence of isolated papillary blush appearance, but this has no demonstrated clinical significance in the absence of concomitant nephrolithiasis or nephrocalcinosis.

To conclude, high-quality intravenous urography, with kidney tomograms before and just after injection of the contrast medium and every 4 minutes during the following 20 minutes before any compression is applied, is a requisite for roentgenologic diagnosis of MSK. Also, careful examination of urograms by a skilled, motivated radiologist is required properly to interpret urographic findings.

Differential Diagnosis

Medullary sponge kidney is more often underdiagnosed than misdiagnosed. When clear linear or round opacifications are seen on urography in one or more papillae, there is usually little difficulty in diagnosis. However, tubular ectases must be differentiated from other pathologic conditions also resulting in abnormal precaliceal opacification, such as renal tuberculosis, caliceal diverticula, or renal papillary necrosis secondary to analgesic nephropathy, diabetes, or sickle cell disease (196–197), as well as from medullary nephrocalcinosis of other causes such as primary hyperparathyroidism, hypercalcemia, distal tubular acidosis, or calcified papillary necrosis (191). Such diseases may usually be differentiated on the basis of associated clinical signs and etiologic conditions. However, MSK may truly coexist with renal tuberculosis (as in 1 of our 102 patients), analgesic nephropathy, or sickle cell trait (198). Caliceal diverticula usually are connected to the fornix of a papilla or appended to a minor calix, but a microcalix can mimic MSK (197). A patient may have both true caliceal diverticula and MSK (199).

Usually, MSK is easily differentiated from other kidney cystic diseases (200–202) (Table 17-3). In juvenile NPH-MCKD complex, imaging and clinical findings are quite different from those of MSK (see earlier). ARPKD is characterized by numerous small fusiform cysts arising from collecting ducts and aligned in a radial distribution. Thus, urographic findings in young children with ARPKD may resemble those of MSK (i.e., striations in the medullary region). However, the clinical and ultrasonographic features differ completely. MSK is very rare in children (203); by ultrasonography, kidneys in ARPKD are enlarged with increased echogenicity and poor differentiation between cortex and medulla; renal macrocysts develop later in older children; and ARPKD is also characterized by the presence of congenital hepatic fibrosis. Differential diagnosis is easy with autosomal-dominant polycystic kidney disease (ADPKD). However, some cases of MSK with voluminous medullary cysts protruding through the cortex may mimic adult-type polycystic renal disease (204). Segmental forms of MSK mimicking a renal tumor on echography and CT scans have been reported (205) and, reciprocally, renal cell carcinoma may mimic MSK (206). In such cases, use of CT scan is helpful to discriminate between MSK and renal tumor.

TABLE 17-3

DIFFERENTIAL DIAGNOSIS OF MEDULLARY SPONGE KIDNEY

Renal tuberculosis
Papillary necrosis (analgesic nephropathy, sickle cell disease, diabetes)
Medullary nephrocalcinosis of other causes (hyperparathyroidism, hypercalcemia, renal distal tubular acidosis, calcified papillary necrosis)
Caliceal diverticula
Microcalices
Parapyelic cysts
Autosomal recessive polycystic kidney disease
Artifactual tubular opacification by ureteral compression during intravenous pyelography
Pyelovenous backflow secondary to disruption of caliceal fornices from excessive intrapelvic pressure during acute ureteric obstruction

Contribution of Other Imaging Techniques

Although diagnosis of MSK mainly relies on urography, there may be specific indications for ultrasonography and CT scan. The small cysts in MSK usually are not seen on sonography. However, sonography may show medullar hyperechogenicity due to nephrocalcinosis (202,207), the presence of cysts in the medulla (when they are large), or images related to a renal abscess or a tumor. Echography is also useful to check for the presence or absence of hepatic cysts. Starinsky et al. observed that antibiotics such as cefazolin, cefotaxime, and gentamicin, when used intravenously, induce attenuation of the collecting system on CT scan, thus possibly leading to confusion with MSK (208).

The CT scan appearance of MSK has not been extensively studied. Ginalski et al. (209) prospectively evaluated the sensitivity of CT scan with thin, 4-mm axial slices compared with excretory urography in 13 patients with typical urographic images of MSK. CT scan showed aspects compatible with possible medullary ectasia in only four patients. Thus, CT scan was much less sensitive than excretory urography in the diagnosis of MSK, but conversely was much more sensitive than plain films and tomograms in the detection of papillary calcifications. On the other hand, CT scan is highly useful for identifying complications such as a renal abscess or renal carcinoma that developed on an MSK kidney (210,211). Therefore, CT scan should be recommended in the case of nephrocalcinosis of unclear origin, MSK with unusually large cysts, presence of a tumoral syndrome, or in the case of associated hepatic or biliary cysts.

Prevalence and Pathogenesis

The prevalence of MSK is not known exactly because its diagnosis depends on the urographic criteria that are adopted. For

ethical reasons, no study involving systematic excretory urography has been performed in asymptomatic subjects. In the earliest reported series, mainly based on presence of evident striations or round collections in the medulla, the incidence of MSK in unselected patients undergoing urography for various urologic symptoms was reported to be approximately 0.5% (174,212). No autopsy series has specifically sought the presence of this condition. From Mayall's study, an estimated prevalence of 1/20,000 in the general population has been proposed, with an equal incidence in both sexes and no obvious racial preponderance (212). More recently, Laube et al. analyzed 444 consecutive urographies in patients with or without urolithiasis. The prevalence of MSK was 1.5% in the control population of non–stone-forming subjects, whereas it was 8.5% in stone formers (213).

Medullary sponge kidney appears to be a developmental anomaly, present from birth (173). Theories suggesting that the dilation of collecting ducts results from their occlusion by uric acid crystals during fetal life (214), or from tubular obstruction by calcium oxalate concretions secondary to infantile idiopathic hypercalciuria (215), are not supported.

A possible hereditary transmission of MSK is supported by the existence of familial forms (216) and by its frequent association with other congenital or hereditary diseases.

Familial Forms

Familial forms of MSK have occasionally been reported even in the absence of any associated developmental abnormality. Families are known in which more than one sibling (217) or generation are affected (200,218). Reviewing the literature up to 1976, Kuiper collected 29 familial cases, including 5 pairs of siblings, 3 examples of 2 successive generations involved, and a family with 6 affected members (173). The same author reported a family in which MSK was well documented in three generations and possibly present in a total of eight subjects over five generations (219). Bennett observed a family with radiographic findings of MSK in five patients over three generations (220). Kliger and Scheer reported the presence of typical MSK in 7 of 12 asymptomatic children of 3 sisters with histologically proven hereditary medullary cystic disease and progressive renal failure (221). We observed a family in which seven subjects over three successive generations were affected by MSK (Fig. 17-12). Taken together, such familial observations with vertical transmission are compatible with a dominant inheritance.

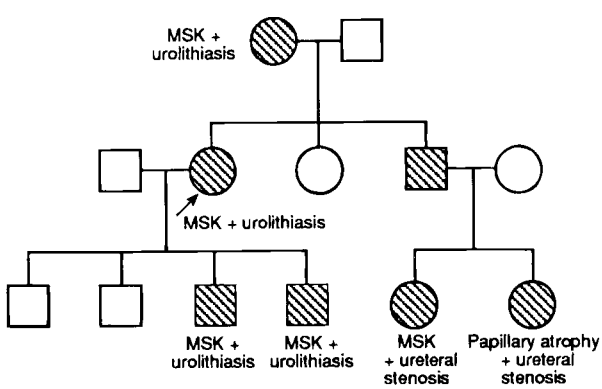

FIGURE 17-12. Pedigree of a family with medullary sponge kidney in seven subjects over three generations. *Circles* denote female, *squares* male family members, and *hatched symbols* affected members.

TABLE 17-4

INHERITED, CONGENITAL, OR ACQUIRED CONDITIONS ASSOCIATED WITH MEDULLARY SPONGE KIDNEY

Congenital hemihypertrophy
Beckwith-Wiedemann syndrome
Congenital hepatic fibrosis
Caroli's disease
Ehlers-Danlos syndrome
Marfan's syndrome
Young's syndrome
Renal arterial fibromuscular dysplasia
Anodontia
Congenital pyloric stenosis
Horseshoe kidney
Autosomal dominant polycystic kidney disease
Medullary cystic disease/nephronophthisis
Wilms' tumor
Adrenal adenoma or carcinoma
Parathyroid adenomatosis
Multiple, complex associations

Pathologic Associations

Of great interest is the frequent association of MSK with other pathologic conditions involving development of cysts in the kidneys or other organs (mainly the liver), or with other developmental anomalies. The most frequent are summarized in Table 17-4 and are schematically depicted in Figure 17-13.

Congenital Hemihypertrophy and Beckwith-Wiedemann Syndrome

Congenital hemihypertrophy (CHH) is one of the most frequent concomitant features found in patients with MSK, as

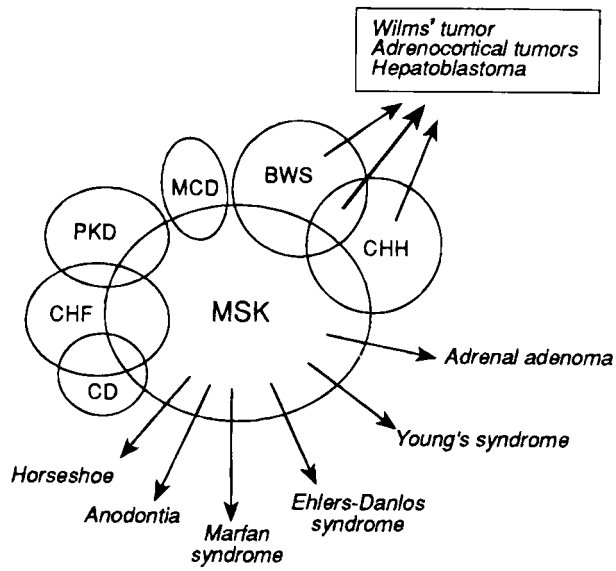

FIGURE 17-13. Schematic representation of the multiple pathologic conditions reported in association with medullary sponge kidney (MSK). *BWS*, Beckwith-Wiedemann syndrome; *CD*, Caroli's disease; *CHF*, congenital hepatic fibrosis; *CHH*, congenital hemihypertrophy; *MCD*; medullary cystic disease; *PKD*, polycystic kidney disease.

first reported in 1964 by Steyn and Logic (222). This association appears to be too frequent to be attributable to chance alone because MSK is found in 5% to 10% of patients with CHH and up to 25% of patients with MSK may have corporeal hemihypertrophy (180,223), whereas the incidence of MSK in the general population is estimated at 5/100,000, and that of CHH at 1/100,000. Harris et al. in 1981 recorded 24 documented cases, including 2 of their own (224). Sporadic observations of MSK associated with CHH have been subsequently reported by Thompson et al. (225) and by Indridason et al. (226). None of the reported patients had evidence of kidney neoplasia despite the high incidence of Wilms' tumor, adrenocortical neoplasms, and hepatoblastoma in isolated CHH (227). Therefore, it was proposed that the propensity to abdominal neoplasms is reduced when MSK coexists with CHH (224). Subsequently, however, Hennessy et al. reported the occurrence of Wilms' tumor ipsilateral to CHH in a child (228), whereas Saypol and Laudone reported the development of adrenocortical carcinoma in a child with MSK and CHH (229), and Tomooka et al. observed an adrenal adenoma contralateral to CHH in a child with MSK (230).

Medullary sponge kidney has been found associated with the Beckwith-Wiedemann syndrome (BWS), especially in cases where congenital somatic asymmetry was part of the syndrome, as occurs in approximately 12.5% of cases (231). This syndrome associates multiple congenital anomalies, including high birth weight, macroglossia, omphalocele, visceromegaly, microcephaly, somatic asymmetry, mental retardation, cysts in the adrenal cortex, nephromegaly with persistent nephrogenesis, medullary dysplasia, and medullary sponge disease (231). Recognizing such a syndrome in an infant or child with MSK is of interest in view of the high oncogenic potential of the association (232). Wiedemann reported the presence of 33 tumors in 29 of 338 children with BWS (a 5% incidence), including Wilms' tumor in 14, adrenocortical carcinoma in 5, and hepatoblastoma in 2 (233). Overall, CHH is present in 12.5% of children with BWS, but in 40% of those with neoplasms, indicating a tumor rate of nearly 25% when CHH is part of the syndrome (231). Because congenital medullary dysplasia is often a component of BWS, Chesney et al. proposed that children with MSK and somatic hemihypertrophy had a minor form of BWS and suggested that such patients be systematically checked for other developmental anomalies and especially for abdominal tumors (234). Beetz et al. reported the coexistence of a Wilms' tumor ipsilateral to CHH in a young girl with BWS and bilateral MSK (235), thus casting doubt on the attenuating effect of MSK on the risk of abdominal neoplasm in patients with CHH, as previously suggested by Harris et al. (224).

As a practical conclusion, in view of the increased risk of abdominal tumors in children with isolated CHH, BWS, or both, every child with either of these conditions should be carefully monitored by frequent abdominal echography to detect early and treat any abdominal neoplasm, especially Wilms' tumor (233,235).

Polycystic Kidney Disease and Congenital Hepatic Fibrosis

There is a spectrum of associations between MSK, polycystic kidney disease or other cystic kidney diseases, and congenital biliary dysgenesis.

There are several reports of coexistent ADPKD and MSK in the same patient or in different members of the same family. Fairley et al. reported on three siblings, all blind from birth, one of whom, aged 22 years at death, was found to have polycystic kidneys at necropsy; another had MSK, and the third had enlarged kidneys with both MSK and polycystic kidney disease (326). The patient reported by Nemoy and Forsberg had recurrent urinary stone passage and evidence of MSK on initial urography, whereas a typical left polycystic kidney was found at surgery some years later (237). Hockley et al., in a young man with subarachnoid hemorrhage, diagnosed MSK on intravenous urography, but subsequently associated polycystic kidney disease was diagnosed on the basis of arteriography and radionuclide scan (238). Abreo and Steele reported two siblings with simultaneous appearance of brushlike pyramidal opacifications characteristic of MSK and of multiple large cysts, seven of the cysts involving the cortex and medulla (and liver in one case) in enlarged kidneys (239). Similarly, Anderson et al. reported the simultaneous presence of diffuse, patent pyramidal striations and of multiple large cysts in both kidneys and liver on echography and CT scan in one patient (240). In most of these observations, there was progressive enlargement of kidneys in patients with concomitant MSK and polycystic kidney disease (238,240). More recently, Torres et al., investigating the prevalence and risk factors of urolithiasis in a series of 751 patients with ADPKD, found that 151 (20%) were affected by kidney stones, and among the latter, nearly 15% had urographic findings of MSK; therefore, approximately 3% of patients had concomitant MSK and polycystic kidney disease (241,242). In our series, 4 of 179 patients with MSK had evidence of concomitant polycystic kidney disease, with a family history of ADPKD in all cases. The course of renal failure was especially rapid in a male patient who reached ESRD at 36 years of age, whereas an affected brother started dialysis at 35 and a sister at 32 years of age. In several of the aforementioned cases the kidneys were enlarged at the time of diagnosis, and a familial history of polycystic disease with progressive renal failure was present. Therefore, it cannot be determined whether MSK lesions are the initial developmental abnormality, followed by further development of large cortical kidney cysts, or if two separate pathologic conditions develop concurrently but at a different pace. However, from a practical point of view, the presence of enlarged kidneys, especially when there is a strong family history of cystic kidney disease, in a patient with urographic evidence of apparently isolated MSK, should alert the clinician to the possibility of underlying polycystic kidney disease and lead to complementary investigation by sonography or CT scan, along with reinforced surveillance. Coexistence of MSK and medullary cystic disease in the same patient or in different members of a same family has been reported, as already mentioned (220,221).

Congenital hepatic fibrosis, an inherited disease characterized by enlargement of portal spaces by bile duct proliferation and fibrosis leading to portal hypertension (243), is associated with a variety of cystic kidney malformations. The most frequent is MSK (73,244), observed in approximately two thirds of cases (245). In some patients, ectatic renal collecting tubules, although not apparent on urograms, were found at histologic examination of the kidneys (246,247). Congenital hepatic fibrosis is also a constant finding in ARPKD, but the clinical manifestations are more or less prominent (see Chapter 18). Surprisingly, only a few PKD1 families with ADPKD and congenital hepatic fibrosis have been reported (248). Other visceral abnormalities may be associated with congenital hepatic fibrosis and MSK, such as cerebral aneurysms (249).

Caroli's disease, another (less frequent) form of fibrocystic hepatobiliary hereditary disease characterized by multifocal segmental cystic dilatation of intrahepatic bile ducts resulting in cholelithiasis and recurrent cholangitis episodes, is also often accompanied by renal tubular ectasia or ARPKD (243,250,251). In most cases, Caroli's disease is associated with congenital hepatic fibrosis (243,251). Complex associations may exist between these abnormalities. Several cases of the association of Caroli's disease, congenital hepatic fibrosis, and MSK have been reported (252–256). Marinone et al. reported the association of congenital hepatic fibrosis, Caroli's disease, and MSK together with cardiac and vertebral malformations

in a 16-year-old boy of consanguineous parents, whereas his sister had asymptomatic congenital hepatic fibrosis and MSK only (257). One of our patients had combined Caroli's disease revealed by recurrent angiocholitis episodes, congenital hepatic fibrosis with portal hypertension and esophageal varices, and MSK complicated by nephrolithiasis. A fatal neonatal case of Caroli's disease associated with severe cardiac and renal lesions was reported by Keane et al. (258). Only two cases of ADPKD associated with Caroli's disease have been reported (259).

Because Caroli's disease and most forms of congenital hepatic fibrosis exhibit autosomal recessive transmission, such observations suggest that concomitant MSK should be a manifestation of a recessive gene (252). Taken together, such observations of associated hepatobiliary and renal cysts emphasize the need for full abdominal echographic study including liver examination in patients with MSK, as already advised for patients with poly cystic kidney disease.

Other Congenital Abnormalities

Other congenital abnormalities found in association with MSK include Ehlers-Danlos syndrome (260,261), congenital pyloric stenosis (260), Marfan's syndrome (262), Young's syndrome or immotile cilia syndrome, clinically manifested by chronic sinobronchial infections and obstructive azoospermia (263), anodontia (264), renal ectopia, horseshoe kidney (265), retinochoroidopathy (266) and unicornuate uterus (267). The main pathologic conditions associated with MSK are summarized in Table 17-4.

Clinical Features

Medullary sponge kidney by itself is asymptomatic, but it may be complicated by, hematuria, urinary tract infection, or nephrolithiasis, which often are the revealing features. The onset of symptoms frequently takes place in the second or third decade of life. Onset during childhood is less common, but late discovery of the condition is far from exceptional.

Hematuria and Urinary Tract Infection

Hematuria is a frequent event. Gross hematuria is present in 10% to 20% of patients, as reported by Ekstrom et al. (177), and may result in clotting and acute renal pelvic obstruction (268). Of note, macroscopic or microscopic hematuria may be observed even in the absence of stones or infection. Urinary tract infection is a frequent complication of MSK in either the presence or absence of nephrocalcinosis or nephrolithiasis, but sterile pyuria is frequent in MSK patients in the absence of any urinary tract infection (269). Parks et al. found the infection rate higher in patients with MSK and nephrolithiasis than in other stone formers, the incidence of infection being markedly higher in women than in men (270).

Nephrolithiasis

Nephrolithiasis, especially calcium nephrolithiasis, appears as the major clinical complication of MSK. Renal colic is the most frequent revealing symptom (177), and most significant complications, including hematuria, urinary tract infection, obstructive episodes, and extensive nephrocalcinosis, ultimately leading to impaired renal function in severe cases, depend on the development of nephrocalcinosis and stones.

The true prevalence of MSK in patients with urolithiasis has long been debated and has been best assessed in more recent studies. In earlier studies, the prevalence of MSK in stone formers had been reported to be as low as 2.6% of 619 patients by Lavan et al. (271), 3.6% of 472 patients by O'Neill et al. (272), and 3.5% of 389 patients by Wikstrom et al. (273). Higher figures were reported by other authors, including Sage et al., who found MSK in 8.5% of 200 randomly selected stone formers (274), Vagelli et al., who reported MSK in 11.6% of 138 consecutive calcium stone formers (275), and Grinalski et al., who identified MSK in 12.5% of 280 consecutive calcium stone formers (192). Over a two-decade period, Yendt et al. observed a prevalence of 9.6% among 1,655 stone formers, with an overall incidence of 21% among the 400 most recent consecutive cases (179). Similar figures were reported in France. Among 500 calcium stone formers followed at Cochin Hospital (Paris), Thomas et al. (181) identified 103 (20.6%) with MSK. This proportion was even higher in our experience, probably because we act as a tertiary referral stone clinic, resulting in an overrepresentation of patients with severe, multirecurrent forms of urolithiasis. Among 702 consecutive calcium stone formers (448 male, 254 female patients) who underwent radiologic and laboratory evaluation at our stone clinic between 1982 and 1997,179 (25.5%) had evidence of MSK on intravenous pyelography. Of these, 92 had obvious precaliceal ectases, whereas the other 87 had minimal precaliceal changes that required meticulous scrutiny not to be overlooked (182).

Parks et al. first pointed out the higher relative incidence of MSK in female than in male stone formers (270). Among 799 calcium stone formers, they observed an overall MSK prevalence of 13.2% with a significantly higher proportion in women (19%) than in men (12%). A similar finding was subsequently reported by Ginalski et al., with a proportion of 10% among 199 male and 17% among 81 female patients (192). In their latest experience, Yendt et al. found that 99 of 1,173 male patients (8.4%) and 60 of 482 female patients (12.4%) had the disorder (179). In the series reported by Thomas et al. (181), prevalence of MSK was significantly higher in female (53 of 190, or 27.9%) than in male stone formers (54 of 310, or 12.6%). The same was true in our recent series (182). The prevalence of MSK was higher in women (73 of 254, or 28.7%) than in men (106 of 448, or 23.6%), although the difference did not reach statistical significance. The median age at first stone episode (29 years in both sexes in our series) is slightly lower in patients with MSK than in other calcium stone formers, especially in men, as was also observed by Yendt et al. (179) and Parks et al. (270).

Clinical manifestations of stone disease in patients with MSK are essentially the same as in other patients with urolithiasis. However, the number of stones passed by patients with MSK is, on the average, higher than in other stone formers, as also observed by Parks et al. (270). In our patients, the average stone activity index was 1.45 stones per year in patients with MSK versus 0.65 stones per year in idiopathic calcium stone formers. The median number of stones passed by patients with MSK was 14 (range, 1 to 55), compared with 5 in idiopathic stone formers (182). Some patients passed a very high number of small calculi, up to more than 10 per year (one of them passed up to 55 stones per year).

Although most patients usually have few precaliceal calcifications on urography at presentation, some have extended nephrocalcinosis with confluent calcified masses in several papillae (Fig. 17-14), whereas others have a number of small calcifications in one or both kidneys along the Hodson line, the virtual line that delineates the bottoms of calices in either kidney. Either of these aspects on plain radiographs or tomograms in a normocalcemic patient is very suggestive of MSK in our experience.

The clinical course of nephrolithiasis in patients with MSK is essentially benign. However, occasional patients exhibit recurrent, incapacitating stone production. In some, renal failure develops because of repeated obstructive episodes or recurrent pyelonephritic episodes, especially if urease-producing microorganisms such as *Proteus* species are involved. In such cases, progression to struvite staghorn calculi has been reported

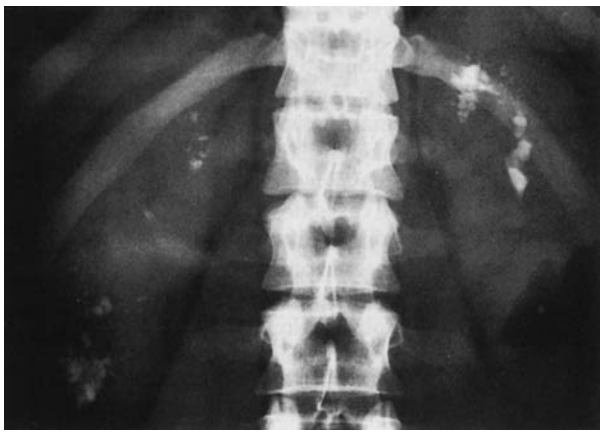

FIGURE 17-14. Extended bilateral nephrocalcinosis on plain radiograph in a patient with medullary sponge kidney. Note the confluent calcified masses in the upper part of left kidney.

(276). Such an unfavorable course is especially to be feared in female patients because of the high urinary infection rate in women with MSK (270,277). Therefore, any urinary tract infection, especially when due to urease-producing microorganisms, should be actively treated in patients with MSK.

Risk Factors for Nephrolithiasis

Formation of nephrocalcinosis and stones in patients with MSK is multifactorial and relates to mechanical as well as metabolic factors that in part are specific to the disease (Table 17-5). The main lithogenic factor in MSK is urine stasis in ectatic tubules, the effect of which is combined with hypercalciuria, hypocitraturia, or tubular acidification defects (277).

Urinary Stasis

Obviously, urinary flux is slowed down in the dilated segments of renal collecting ducts. As an aggravating circumstance, such urinary stasis occurs in the inner part of the medulla, where the concentration of solutes is the highest (278). Impairment of urinary concentrating ability alongside a preserved dilution capacity is often observed in patients with MSK (279,280), but is of no adverse clinical consequence because it may contribute to maintenance of abundant diuresis. Other functional disorders have been reported, such as elevated fractional sodium excretion (279) or impaired kaliuretic response to acute potassium load in patients with MSK who otherwise were able to preserve potassium balance during prolonged potassium loading or depletion 248).

TABLE 17-5

LITHOGENIC FACTORS IN MEDULLARY SPONGE KIDNEY

Local factors
Slowed urinary flux in tubular ectasia
Altered urothelium in tubular ectasia
Intraluminal debris as matrix for stone formation
Locally impaired distal urinary acidification (high pH favoring calcium phosphate precipitation)
General factors
Low diuresis
Hypercalciuria, hyperoxaluria, hyperuricosuria
Hypocitraturia

Hypercalciuria

Whether hypercalciuria is more prevalent in patients with MSK with calcium urolithiasis than in idiopathic calcium stone formers without MSK remains a debated issue. The reported prevalence of hypercalciuria in patients with MSK was 88% by O'Neill et al. (272), 42% by Parks et al. (270), and 30% by Yendt et al. (179). In the study of Parks et al., the relative incidence of hypercalciuria was higher in women (17 of 33 patients, or 52%) than in men (27 of 73 patients, or 37%) (270). Therefore, as pointed out by Yendt et al. (179), the presence of hypercalciuria in a woman with calcium nephrolithiasis may be suggestive of possible underlying MSK. In the series of Thomas et al. (181), hypercalciuria was present in 59% of patients with MSK, hyperoxaluria in 64%, and both disorders in 39%. Among 113 of our patients with MSK investigated at presentation before any treatment, hypercalciuria (calciuria >0.1 mmol/kg/day, or 4 mg/kg/day while on free diet) was present in 44% of patients (48% in men, 40% in women), hyperoxaluria (oxalate excretion >0.5 mmol/day, or 45 mg/day) in 31%, hypocitraturia (citrate excretion <1.5 mmol/day, or 300 mg/day) in 25%, hyperuricosuria (>4.5 mmol/day, or 750 mg/day) in 29%, and low urinary volume (<1.5 L/day) in 27%. Several metabolic abnormalities often coexisted in the same patient. By comparison, initial metabolic evaluation in 196 calcium stone formers without MSK disclosed a similar overall proportion of hypercalciuria (40%) but without a significant sex difference (41% in men versus 39% in women).

The mechanism of hypercalciuria in MSK is debated. On the basis of the location of anatomic lesions, it may be hypothesized that increased calcium excretion results from impaired calcium reabsorption in diseased parts of distal tubules. Hypercalciuria was of the absorptive phenotype in 10 of 15 hypercalciuric patients with MSK evaluated by O'Neill et al. (272), whereas it was of the renal phenotype (or associated with primary hyperparathyroidism) in 8 of 10 patients studied by Maschio et al. (215), and fasting hypercalciuria was found in 16 of 20 patients evaluated by Jaeger et al. (281). In our series, a fasting calcium-to-creatinine ratio above 0.35 mmol/mmol (or 0.12 mg/mg) was found in half of hypercalciuric patients with MSK in both sexes. In any case, hypercalciuria in patients with MSK usually responds to thiazide diuretics regardless of whether hypercalciuria exhibits a renal or absorptive pattern (179).

Another debated issue is whether primary hyperparathyroidism is related to MSK. Several authors reported an unexpectedly high incidence of hyperparathyroidism with confirmed parathyroid adenomas in hypercalciuric patients with MSK (215,282–287). The possible link may be that longstanding renal calcium leak stimulates the secretion of parathyroid hormone, ultimately leading to formation of parathyroid adenomas, as initially suggested by Bordier et al. (288). However, only 2 of 108 patients with MSK evaluated by Parks et al. (270), only 3 of our 179 patients with MSK, and none of the 103 patients with MSK evaluated by Thomas et al. had evidence of hyperparathyroidism (181). In a patient with MSK reported by Siskind and Popovtzer, recurrent postpartum hypercalcemia was attributable not to primary hyperparathyroidism but to the secretion of a parathyroid-like protein during lactation (289). Therefore, no firm conclusion can be drawn based on current knowledge, but a search for possible hyperparathyroidism is advisable in patients with MSK, especially if the serum calcium concentration is elevated or markedly rises after thiazide diuretic administration.

Tubular Acidification Defects

Because the distal tubule is the site of renal ammoniagenesis, the presence of a defective acidification process could be hypothesized in patients with MSK, at least in affected parts of the kidneys.

Impaired distal urine acidification has been reported by several authors in patients with MSK on the basis of the short ammonium chloride test (290–292). Higashihara et al. found 4 of 11 patients with diffuse bilateral MSK unable to lower their urine pH below 5.3 after an acute acid load challenge (293). In this study, the impaired ability to lower urinary pH was associated with reduced excretion of titratable acid, whereas ammonium excretion was normal, and the same authors did not find any acidification defect in patients with partial or unilateral forms of MSK (294). Osther et al. (295) evaluated urinary acidification ability in 10 women with bilateral MSK and 10 healthy women. They found incomplete renal tubular acidosis in four patients, who exhibited significantly higher calcium excretion (7.32 ± 1.45 versus 3.83 ± 1.12 mmol/day, or 293 ± 58 versus 153 ± 45 mg/day) and lower citrate excretion (0.93 ± 0.25 versus 3.58 ± 0.51 mmol/day, or 178 ± 48 versus 687 ± 98 mg/day) compared with patients with MSK and normal urinary acidification. By contrast, in the study of Ginalski and coworkers, only 1 of 35 stone formers with MSK had evidence of distal tubular acidosis (192). We evaluated the response to acute ammonium chloride load in nine patients with diffuse, bilateral forms of MSK complicated by recurrent stone formation and hypercalciuria. We observed a normal response with a decrease in urinary pH below 5.2, and a normal increase in net acid excretion, ammoniagenesis, and titratable acidity in all (296). Among our whole series, a female patient 35 years of age had both MSK and distal tubular acidosis secondary to idiopathic Sjögren's syndrome with polyclonal hypergammaglobulinemia, resulting in diffuse medullary calcinosis and recurrent nephrolithiasis. This observation is reminiscent of that of Popa and Stanescu of a young girl with MSK and renal tubular acidosis associated with hypergammaglobulinemic purpura and suggests the superimposition of acquired tubular acidosis on underlying tubular ectasia (297).

A possible link between MSK and renal tubular acidosis is less clear in cases of congenital distal tubular acidosis concomitant with MSK. Deck reported three patients with MSK and renal tubular acidosis (complete in one case); the father of the first patient had renal tubular acidosis without MSK, whereas the other two patients were a mother and daughter, both affected by incomplete tubular acidosis and MSK (298). Some cases of MSK associated with complete distal tubular acidosis and nephrocalcinosis with decreased plasma bicarbonate level have been reported sporadically (177,261,279). In one patient, hypokalemic paralysis was the revealing symptom (299), whereas another patient was referred for growth failure (300). Renal distal tubular acidosis may contribute to hypercalciuria (301), and alkali therapy has been reported to reduce hypercalciuria in such patients (302).

To summarize, the problem of the relationships between MSK and renal distal tubular acidosis is far from solved. One possibility is that tubular ectasia, through alterations of the tubular epithelium induced by repeated obstruction or infection, results in defective ammoniagenesis, alkaline urine, and intratubular precipitation of calcium phosphate, initiating nephrocalcinosis or lithiasis. Such a hypothesis may be accepted only in bilateral, extensive forms of MSK. In most cases, however, intact nephrons probably compensate for altered tubules so that the final urine has a normal composition, and the patient's response to acute acid load is normal, as observed in our experience (296). Another mechanism, as suggested by Deck, involves renal tubular acidosis as the primary defect, resulting in nephrocalcinosis and nephrolithiasis with secondary dilation of collecting ducts obstructed by calcium deposits (298). A third hypothesis is that MSK and renal tubular acidosis (either congenital or secondary to an acquired disease such as Sjögren's syndrome) coexist as two distinct entities, aggravating each other. The last hypothesis is the most likely in explaining familial forms of renal tubular acidosis associated with MSK.

Hypocitraturia

Renal tubular acidosis, especially in complete forms with systemic acidemia, but also in some patients with incomplete renal tubular acidosis as reported by Higashihara et al. and by Osther et al. (293,295), may result in bone mineral loss and hypocitraturia. However, in our series, marked hypocitraturia (<1.5 mmol/day or 300 mg/day) was observed in only 25% of patients with MSK. The mean urinary citrate to calcium molar ratio was 0.43 in our patients with MSK, not differing from the mean value of 0.43 observed in non-MSK calcium stone formers. Urinary citrate excretion was also usually normal in patients with MSK in the experience of Yendt et al. (179).

Composition of Stones

Hypercalciuria, increased oxalate concentration, subtle tubular acidification defects, or hypocitraturia variably influence the composition of stones in patients with MSK. Dense concretions found in MSK cysts were wholly or predominantly made of apatite by x-ray crystallography in the study of Ekstrom et al. (177). In the experience of Yendt et al. (179), approximately half of passed stones were made of an admixture of calcium oxalate and apatite, and the remaining were composed entirely of calcium oxalate when examined by optical crystallography. We studied stone morphology and composition in 92 patients (57 male, 37 female) with one or more calculus available for analysis, according to our proposed classification (303,304). Infrared spectroscopy disclosed whewellite (calcium oxalate monohydrate) as the main component in 49% (54% in men, 39% in women), and carbonate apatite (carbapatite) in 25% of cases (13% in men, 42% in women), whereas weddellite (calcium oxalate dihydrate) was the main component in 18% of cases (25% in men, 8% in women) and uric acid in only 3%. The core of the calculus was made of carbapatite in 26 cases, and of whewellite in 24 cases. The latter composition is in keeping with the high oxalate concentration in the inner part of the medulla, because whewellite crystals are electively formed in the presence of a high oxalate-to-calcium molar ratio (303); the former composition corresponds to the precipitation of calcium phosphate crystals in urine with increased pH, as can be expected in affected collecting ducts with defective acidification. The two anomalies may coexist, leading to formation of stones of mixed composition, as reported by Yendt et al. (179). In our patients, calculi were of mixed composition in 50 of 92 cases (55%), associating monohydrate or dihydrate calcium oxalate and carbapatite in variable proportions. Several patients passed stones of different composition. Such pleomorphism of calculi in a single patient (Fig. 17-15) is, in our opinion, highly suggestive of MSK. Interestingly, 16 of our patients (4 men, 12 women) who had severe, bilateral forms of MSK passed calcium phosphate stones exhibiting a peculiar morphology (type IVa2 in our classification) similar to that we had observed in stone formers with primary renal tubular acidosis (303). These findings are probably due to local tubular acidification defects in affected parts of the kidneys, leading to intratubular precipitation of calcium phosphate favored by alkaline urine (305).

To summarize, the risk factors for stone formation in patients with MSK are multiple, and several may coexist. Two are

FIGURE 17-15. Heterogeneous morphology of multiple stones passed by a patient with medullary sponge kidney. Calculi range from 0.6 mm to 4 mm in diameter. (Reproduced from: Daudon M, Bader C, Jungers P. Urinary calculi: review of classification methods and correlations with etiology. *Scanning Microsc* 1993;7:1081, with permission.)

directly related to the anatomic disorder, namely urine stasis in dilated tubules and an acidification defect. The others—hypercalciuria, hyperoxaluria, hyperuricosuria, and hypocitraturia—are common to all calcium stone formers. Such considerations have therapeutic implications.

Treatment

Medullary sponge kidney is essentially a benign condition. Therefore, patients with asymptomatic and uncomplicated MSK should be advised about the benign nature of the disorder and require no specific therapy. However, they should be alerted to the possible occurrence of urolithiasis or urinary tract infection, and periodic urinalysis, together with plain radiography or echography, is advisable. In young children affected by MSK, careful examination is required to identify oncologic complications such as Wilms' tumor or another abdominal tumor, and regular surveillance is warranted, especially if other developmental abnormalities coexist.

Microscopic hematuria, often associated with aseptic leukocyturia, is a frequent finding in patients with MSK with nephrocalcinosis or nephrolithiasis and should not require unduly invasive investigation. However, macroscopic hematuria should be fully investigated before accepting MSK as the sole cause. As an example, a bladder tumor was revealed by indolent macroscopic hematuria in one of our patients. This is especially true in children or in patients with associated developmental abnormalities, in view of the frequent occurrence of renal tumors in such a setting.

Any urinary tract infection should be actively treated until eradication is achieved (180), particularly in the presence of nephrocalcinosis or nephrolithiasis, and especially if a urease-producing microorganism is involved, to avoid the possible subsequent development of struvite calculi.

The main problems in patients with MSK arise from nephrolithiasis. Both treatment of symptomatic stones and prophylaxis of recurrent stone formation should be considered. Obstruction of the renal pelvis or ureter should be treated as in non-MSK calcium stone formers by means of extracorporeal shock-wave lithotripsy (ESWL), ureteroscopy, or percutaneous surgery. Several groups have reported satisfying results with ESWL (306–309), whereas others reported disappointing results, at least with precaliceal calcifications, because of impaired drainage of particles (310). Nakatsuka et al. successfully used sequential percutaneous nephrolithotomy, kidney irrigation with ethylenediaminetetraacetic acid by means of nephrostomy, and ESWL (311). In some cases with marked segmental involvement, partial nephrectomy has been used to treat the disorder (312). In other instances, unilateral nephrectomy may be required for severe septic complications, as in one of our patients, but this measure should be undertaken only in case of absolute necessity.

Prophylaxis of stone formation is often difficult. The primary goal is to achieve a high diuresis, at least 2 L/day, to decrease the concentration of stone promoters, especially oxalate, and to reduce urinary stasis in ectatic collecting tubules because no treatment can modify the presence or extent of tubular ectasia. Fluid intake should be taken in divided doses throughout the day. Assessment of urine specific gravity in the first voided morning urine is useful (compared with 24-hour urine) to identify patients with persistently excessive concentration during the night. Such patients should be advised to ingest a large quantity of fluid at bedtime and eventually during the night.

Hypercalciuria, when present, should be treated first by moderation in animal protein and salt intake (313). If such a measure, combined with active diuresis, does not suffice significantly to lower the urine calcium concentration, long-term therapy with a thiazide diuretic should be undertaken, regardless of whether hypercalciuria is of the absorptive or renal phenotype (179). If thiazides induce hypokalemia or hypocitraturia, potassium citrate is a useful adjunct (314). Similarly, potassium citrate should be used in preference to sodium or potassium bicarbonate in patients with impaired acidification and hypocitraturia (314).

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CHAPTER 18 ■ POLYCYSTIC KIDNEY DISEASE

TEVFIK ECDER, GODELA M. FICK-BROSNAHAN, AND ROBERT W. SCHRIER

A cyst is a cavity lined by epithelium and filled with fluid. Renal cysts may develop at any location along the renal tubule, from the Bowman's capsule to the collecting duct, by heritable, developmental, or acquired processes. Single, or simple, renal cysts are acquired, occur commonly in people older than 50 years of age, and are not associated with any disease. In contrast, polycystic kidney diseases can be complicated by renal failure or tumor development.

The heritable polycystic kidney diseases include both autosomal-dominant and autosomal-recessive disorders. The dominant disorders include three systemic diseases: autosomal-dominant polycystic kidney disease (ADPKD), tuberous sclerosis complex (TSC), and von Hippel-Lindau disease (VHL). The major recessive disorder is autosomal-recessive polycystic kidney disease (ARPKD), which is rare. The term *ADPKD* is more accurate than the older nomenclature of *adult polycystic kidney disease,* because ADPKD can manifest in fetuses, newborns, and young children (1–8). On the other hand, ARPKD can remain undiagnosed until young adulthood (9,10). ADPKD is the most common of the heritable polycystic kidney diseases. It is often evident on ultrasound imaging in children, becomes symptomatic in young adulthood, and progresses to end-stage renal failure over a period of decades in 50% to 75% of affected people (11–13). By contrast, ARPKD is most often discovered in the first year of life and terminates in end-stage renal disease (ESRD) in childhood or young adulthood (14–17).

Among the acquired cystic diseases, the most common is acquired cystic kidney disease (ACKD), which occurs in both adults and children with chronic renal insufficiency or who are undergoing dialysis (18–22). Its clinical significance lies in its potential for malignant transformation.

AUTOSOMAL-DOMINANT POLYCYSTIC KIDNEY DISEASE

Definition

Autosomal-dominant polycystic kidney disease is a systemic hereditary disorder transmitted in an autosomal-dominant manner. It is genetically heterogeneous; so far, two genes have been identified (23–26). The systemic manifestations include cystic and noncystic renal and extrarenal abnormalities (27–29). Renal cysts occur in 100% of gene carriers (Fig. 18-1). The array of systemic manifestations is detailed in Table 18-1.

Epidemiology

Autosomal-dominant polycystic kidney disease is the most frequent life-threatening hereditary disease, affecting between 1 in 400 and 1 in 1,000 individuals (30,31). It occurs worldwide and in all races and ethnic groups (32–34). Overall, approximately 600,000 Americans have the disease, making ADPKD one of the most common hereditary disorders in the United States. ADPKD is an important cause of ESRD; it accounts for approximately 4% of ESRD in the United States and 8% to 10% in Europe and Australia (34). In Japan, approximately 5% of patients with ESRD have ADPKD (35).

Genetics

Autosomal-dominant polycystic kidney disease is caused by mutations in at least two different genes, the *PKD1* and *PKD2* genes. The resulting disease phenotypes are very similar, except that ADPKD2 is a milder disease with an older age at diagnosis and later onset of hypertension and renal failure (12,36–40). The first gene, the *PKD1* or *polycystin-1* gene, was localized to the short arm of chromosome 16 in 1985, when linkage to the 3′ hypervariable region of the α-globin locus was discovered (23). The gene was identified in 1994 by the molecular analysis of a family that contained a child with both TSC and ADPKD in addition to members with ADPKD alone (25). A TSC gene, the *TSC2* gene, is also localized on the short arm of chromosome 16, distal to the *PKD1* gene. The child with both TSC and ADPKD had an unbalanced translocation between chromosome 16 and 22, which disrupted the *PKD1* gene and caused the loss of the *TSC2* gene, whereas the family members with ADPKD alone had a balanced translocation that disrupted only the *PKD1* gene but preserved the *TSC2* gene (Fig. 18-2). These studies led to the identification of both *PKD1* and *TSC2* (25,41).

The *PKD1* gene is a very large gene with 46 exons; it stretches over 52 kilobases (kb) of genomic DNA and encodes a 14-kb messenger RNA (mRNA) transcript, which is translated into 4,302 amino acids (25,42,43). The region of the gene extending from exon 1 to exon 33 is duplicated at six other sites on chromosome 16p (44).

Genetic heterogeneity of ADPKD had first been described in 1988, when a large family was reported whose disease was not linked to the *PKD1* locus (24). Further linkage analyses localized the second gene, the *PKD2* or *polycystin-2* gene, to the long arm of chromosome 4 (45,46); positional cloning strategies led to the identification of the gene in 1996 (26). It stretches over approximately 68 kb of genomic DNA, contains 15 exons, and encodes an mRNA transcript of approximately 5.4 kb, which translates into 968 amino acids. In contrast to *PKD1*, the *PKD2* gene has no unusual or complex features, and mutation detection has been more straightforward. The mutation detection rate is 70% to 90% for *PKD2* (47–49), whereas it is less than 60% for *PKD1* (43,50).

For both *ADPKD1* and *ADPKD2*, the mutations described so far are scattered throughout the genes and are unique for most families; only a very few mutations have been found in more than one family. Most mutations in either gene are single base changes or insertions or deletions of a small number of

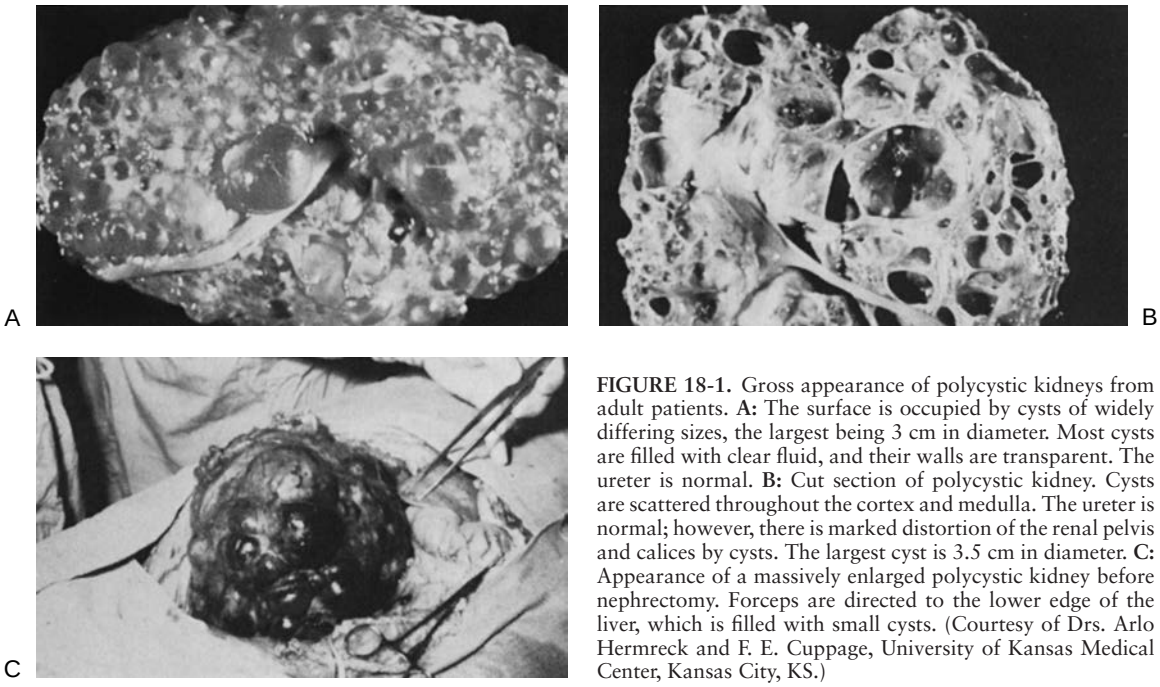


FIGURE 18-1. Gross appearance of polycystic kidneys from adult patients. **A:** The surface is occupied by cysts of widely differing sizes, the largest being 3 cm in diameter. Most cysts are filled with clear fluid, and their walls are transparent. The ureter is normal. **B:** Cut section of polycystic kidney. Cysts are scattered throughout the cortex and medulla. The ureter is normal; however, there is marked distortion of the renal pelvis and calices by cysts. The largest cyst is 3.5 cm in diameter. **C:** Appearance of a massively enlarged polycystic kidney before nephrectomy. Forceps are directed to the lower edge of the liver, which is filled with small cysts. (Courtesy of Drs. Arlo Hermreck and F. E. Cuppage, University of Kansas Medical Center, Kansas City, KS.)

TABLE 18-1

RENAL AND EXTRARENAL MANIFESTATIONS OF AUTOSOMAL-DOMINANT POLYCYSTIC KIDNEY DISEASE

Manifestations	Frequency
Renal	
Cysts	100% after age 30 y
Nephromegaly	95% after age 30 y
Decreased concentrating ability	All adults
Decreased citrate excretion	50% of adults with normal renal function
Hypertension	65% to 80% of adults, 22% to 34% of children
Extrarenal	
Liver cysts	75% by age 60 y
Congenital hepatic fibrosis	Rare
Pancreatic cysts	9% after age 30 y
Seminal vesicle and prostate cysts	60% and 11% at mean age 40 y
Arachnoid cysts	5% to 8%
Mitral valve prolapse	25%
Left ventricular hypertrophy	50% of hypertensive adults without ESRD, up to 90% at autopsy
Intracranial aneurysms	5% to 10%
Intracranial arterial dolichoectasia	2% to 3%
Spontaneous vertebral or coronary artery dissection	Rare
Abdominal wall hernias	45% of patients with ESRD, 10% of children

ESRD, end-stage renal disease.

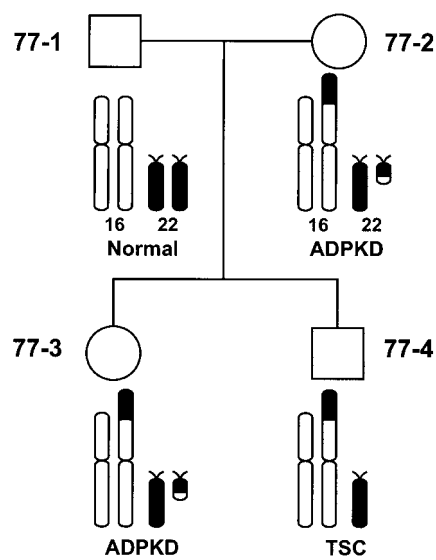


FIGURE 18-2. Pedigree of family 77, which led to the identification of the tuberous sclerosis-2 (TSC-2) gene and the autosomal-dominant polycystic kidney disease type 1 (*ADPKD1*) gene. Person 77-1 has a normal genotype, but persons 77-2 and 77-3 have a balanced translocation of parts of chromosome 22 to chromosome 16; this translocation disrupts the *ADPKD1* gene. The *TSC2* gene is preserved but located on the shortened part of chromosome 22. Phenotypically, these subjects have ADPKD. Person 77-4 has an unbalanced translocation that results in loss of the *TSC2* gene from one allele of chromosome 16 (and loss of part of chromosome 22). Clinically, this person has tuberous sclerosis and a small number of bilateral renal cysts (at age 13 years). (Adapted from: The European Polycystic Kidney Disease Consortium. The polycystic kidney disease 1 gene encodes a 14 kb transcript and lies within a duplicated region on chromosome 16. *Cell* 1994;77:881, with permission.)

base pairs, resulting in truncated proteins and loss of function (44). Patients with mutations in the 5′ region have significantly more severe disease than the 3′ group (51). Similarly, 5′ mutations are more commonly associated with vascular disease, such as ruptured intracranial aneurysm (52).

As mentioned previously, disease caused by *PKD2* mutations is in general milder than disease caused by *PKD1* mutations, with an older age at diagnosis and later onset of hypertension and renal failure. In the reported *ADPKD2* families to date, onset of end stage renal failure occurred at a mean age of 70 to 73 years, whereas in *ADPKD1* families, the mean age at onset of ESRD is 53 to 56 years (12,13,37,39,40). Approximately 85% of white families are linked to the *ADPKD1* gene and 10% to 15% to the *ADPKD2* gene (46,53). A few families have been described whose disease is not linked to either *PKD1* or *PKD2* (54–58). Therefore, a third (or possibly more) gene locus may cause ADPKD. However, the existence of a third locus has been challenged (59). For example, a large Canadian family had been described to show no linkage to either *PKD1* or *PKD2*; however, bilineal transmission was then identified in that family, with one branch of the family linked to the *PKD1* gene and the other to the *PKD2* gene (54,60).

Gene linkage analysis can be used to detect the PKD gene carriers. Gene linkage analysis relies on the fact that DNA markers located around the disease gene are usually inherited together with the disease gene. Certain criteria must be met for this technique to be useful. First, there must be a sufficient number of affected family members to permit identification of the markers that are associated with the ADPKD gene in that particular family. These markers are different in each family. The affected and unaffected parent must carry different marker types; with highly polymorphic probes (many different forms) and different marker probes, this is rarely a limitation. Markers on both sides of the *PKD1* and *PKD2* genes are tested for linkage in a given family to establish whether that family carries the *PKD1* or *PKD2* gene. When this is accomplished, diagnostic testing can be performed for a person who wishes to know his or her status. If that individual carries the same marker haplotype as other affected family members, he or she is affected with 99% probability. The gene linkage technique is detailed in Figure 18-3. In that figure, the two marker types segregating with ADPKD are A and 2. Because the patient in question has these types, he has a greater than 99% probability of carrying the ADPKD gene. This test cannot predict ADPKD with 100% accuracy because a marker very close to the gene is still set apart by some distance and therefore can be separated from the ADPKD gene during meiosis (crossover). This is demonstrated by the proband's brother, who had ADPKD but inherited the B rather than the A type. Gene linkage analysis is performed by commercial DNA diagnostics laboratories. Important applications are for subjects who wish to know their status before donating a kidney, for family planning decisions, and for prenatal diagnosis. Recently, a direct DNA molecular testing using denaturing high-performance liquid chromatography (DHPLC) method has been developed.

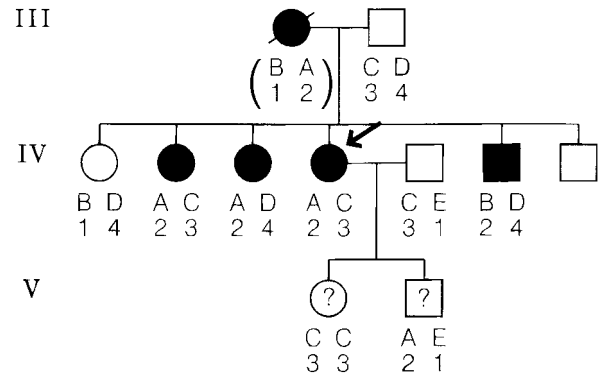


FIGURE 18-3. Linkage data for proband and parents, siblings, and children from a hypothetical family with autosomal-dominant polycystic kidney disease (ADPKD) are demonstrated. Because the unaffected father, who is still living, has one chromosome carrying type C3 and one carrying D4, it can be deduced that the mother's types were B1 and A2. The ADPKD gene appears to be segregating with A2 because three of the affected offspring have that type and the tested unaffected sibling has B1. With this, it can be predicted that the proband's daughter, who has inherited her C3, has a greater than 99% chance of being unaffected, and the proband's son has a 99% chance of carrying the ADPKD gene because he inherited A2 from her. The affected brother of the proband represents a crossover for the ABCD probe. He has ADPKD but has inherited the B, not the A, allele. However, because the 1234 probe is on the other side of the gene, there is no crossover with this marker. □, normal male; ●, affected female; ϕ, deceased ↓, proband.

Pathogenesis

The protein products of the *PKD1* and *PKD2* genes are called *polycystin-1* and *polycystin-2*. Polycystin-1 is a membrane-associated glycoprotein with a large extracellular domain of approximately 3,000 amino acids, with 11 transmembrane domains and a short cytoplasmic tail of approximately 200 amino acids (42,43). The extracellular domain contains several motifs known to be involved in protein–protein or protein–carbohydrate interactions; therefore, polycystin-1 is believed to function as a cell membrane receptor mediating cell–cell or cell–matrix interactions. The intracellular tail interacts with the C-terminal cytoplasmic tail of polycystin-2 (61,62) (Fig. 18-4).

Polycystin-2 is an integral membrane glycoprotein with six transmembrane domains and two intracellular tails. There is evidence that polycystin-2 may be localized in the membranes of the endoplasmic reticulum (63), as well as evidence that it is localized in the cell membrane (26,64). Polycystin-2 has significant homology with the transmembrane regions of polycystin-1, but also with $_{\alpha 1}$ subunits of voltage-gated calcium channels and with transient receptor potential *(trp)* calcium entry channels (26,65,66). These similarities suggest the hypothesis that polycystin-2 functions as an ion channel, possibly regulating calcium flux. Both polycystin-1 and polycystin-2 contain several phosphorylation sites.

The polycystins are expressed in a wide variety of tissues and cell types. Their predominant expression is in epithelial cells of renal tubules and of pancreatic and biliary ducts, but they have also been found in endothelial, neuronal, myocardial, and smooth muscle cells of cardiac valves and large elastic arteries (67–73), which is consistent with the systemic nature of ADPKD. In normal adult kidneys, polycystin-1 is predominantly localized to the lateral aspect of the basolateral cell membrane of collecting ducts, whereas polycystin-2 is localized to the entire basolateral membrane of the thick ascending limb of Henle and the distal convoluted tubule (64). The renal expression of the polycystins is developmentally regulated. Whereas polycystin-1 is expressed at a higher level in the normal human fetal than the adult kidney, polycystin-2 expression is maximal in the mature kidney (64,72–74). In general, cyst lining epithelia from patients with ADPKD, obtained during nephrectomy or at autopsy, display strong staining for polycystin-1 and polycystin-2 by immunohistochemistry, although a few cysts do not stain positively for these proteins (64,67–69,75).

Microdissection studies have shown that only a fraction of nephrons (estimated 1% to 2%) becomes cystic (76). To explain the focal nature of the renal and extrarenal manifestations

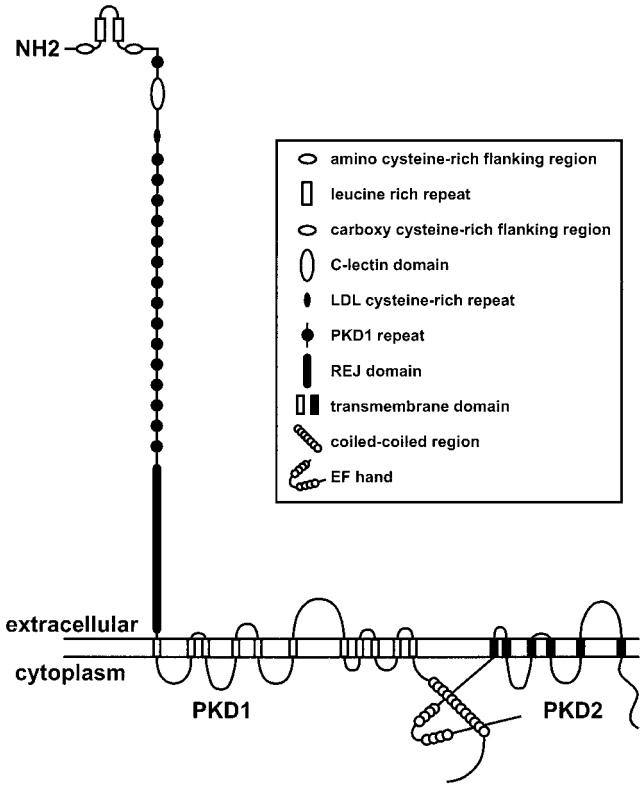


FIGURE 18-4. The predicted structures of polycystin-1 (protein product of the *PKD1* gene) and polycystin-2 (protein product of the *PKD2* gene), and their interaction. Both proteins are transmembrane proteins and interact through their cytoplasmic tails. Polycystin-1 has a long extracellular domain of more than 3,000 amino acids, which is composed of a novel combination of protein domains (shown schematically in the *inset*) that are involved in cell–cell and cell–matrix interactions. Polycystin-1 is predicted to contain 11 transmembrane domains, whereas polycystin-2 has 6 transmembrane domains and 2 intracellular tails. Polycystin-2 has homology to calcium channels, and its carboxy terminus has an EF-hand domain that may bind calcium and serve in a regulatory capacity. The current concept is that polycystin-1 functions as a receptor linking the extracellular matrix to intracellular signaling pathways, some of which may involve ion channel activity mediated by polycystin-2. (Adapted from: Watnick T, Germino GG. Molecular basis of autosomal dominant polycystic kidney disease. *Semin Nephrol* 1999;19:327, with permission.)

of ADPKD, the "two-hit" hypothesis has been advanced, which implies that the germline mutation alone is not sufficient to initiate cyst formation, but that a "second hit" is necessary to inactivate the second PKD allele. This model is supported by the demonstration of loss of heterozygosity and of somatic mutations found in 20% to 30% of renal and liver cysts from patients with *PKD1* or *PKD2* mutations (77–83).

Although the exact function of the polycystins is unknown, polycystin-1 appears to be a cell membrane receptor and polycystin-2 an ion channel. There is evidence that both polycystins are part of a multiprotein complex linking the extracellular matrix with cytoplasmic signaling proteins and the cytoskeleton. Polycystin-1 colocalizes and interacts with the cell adhesion molecules E-cadherin and $\alpha_2\beta_1$-integrin (84,85). E-cadherin is linked to the actin cytoskeleton by cytoplasmic polypeptides, the catenins. The E-cadherin–catenin complexes are part of intercellular adherens junctions, whose proper function is required for regulation of cell proliferation and polarization. Intracellular signaling pathways may involve several protein kinases that regulate nuclear transcription factors. *In vitro* assays have shown stimulation of the transcription factor AP-1 (activation protein-1) by the cytoplasmic tails of either polycystin-1 or polycystin-2, with a dramatically augmented effect when both polycystins were expressed together in a cell culture system (86,87). AP-1 regulates several cellular programs, including proliferation, differentiation, and apoptosis. Disruption of these signaling pathways by mutated polycystin proteins may lead to abnormal polarization and proliferation of ADPKD epithelial cells. Cystic epithelia display an immature phenotype, characterized by hyperproliferation, increased apoptosis, abnormal fluid secretion, and production of an altered extracellular matrix (88–90). The following observations support the pathogenetic roles of these three factors.

Abnormal Cell Proliferation and Apoptosis

The balance between cellular proliferation and apoptosis is disturbed in polycystic kidneys (91,92). Microdissection studies have shown that cysts arise as local outpouchings of renal tubules, which enlarge and, when greater than 2 mm in diameter, separate from the tubule (76). Increased cell numbers are necessary to form the epithelial lining of expanding cysts. Therefore, increased epithelial cell proliferation is a prerequisite for cyst formation and expansion; moreover, hyperplastic polyps and microadenomas in cyst walls are a frequent finding (91% and 24%, respectively) in human nephrectomy or autopsy specimens from patients with early and late stages of ADPKD (93). Epithelial cells from human ADPKD kidneys in culture have demonstrated an exaggerated growth potential (94–96), and the protooncogenes c-*myc,* c-*fos,* c-*jun,* and c-*ras* are overexpressed in these kidneys as well as in animal models of polycystic kidney diseases (97,98).

In human ADPKD and in animal models, the cyst lining cells produce increased amounts of growth factors (99–101). Human renal cyst fluid contains biologically relevant concentrations of epidermal growth factor (EGF) (102–104), hepatocyte growth factor (105), and endothelin (103,106), which also has growth-promoting properties. Moreover, EGF receptor mRNA and protein are overexpressed and mislocalized to the apical membrane in human renal cystic epithelia (ADPKD and ARPKD) (102,107), suggesting autocrine amplification of cell proliferation.

Increased levels of apoptosis are observed in human ADPKD (108) and experimental models of polycystic kidney disease (109–113). Apoptosis was also detected in noncystic tubules in pre-uremic human polycystic kidneys, suggesting that it may lead to the progressive loss of normal nephrons in ADPKD (108).

Abnormal Fluid Secretion

Cellular proliferation alone leads to solid tumors, but renal cysts are composed primarily of fluid. In the early stages of cystogenesis, the tubular outpouchings fill with glomerular filtrate, but after separation of the cyst from the parent tubule, the cyst fluid derives exclusively from transepithelial secretion of solutes and water (76,114). In intact isolated cysts *in vitro* and in monolayer cultures of human kidney cortex cells, fluid secretion can be induced by native cyst fluid or by cyclic adenosine monophosphate (cAMP) agonists (114–116), suggesting that cyst fluid contains autocrine or paracrine substances that promote fluid secretion through cAMP production. A nonpolar lipid has been isolated from human cyst fluid, which potently stimulates fluid secretion by renal epithelial cells *in vitro;* this lipid is called *cyst-activating factor* (117). The cellular transport mechanisms involve active apical chloride secretion through the cystic fibrosis transmembrane conductance regulator (CFTCR)(118,119) and basolateral chloride entry through the Na-K-2Cl-cotransporter, which is inhibited by bumetanide,

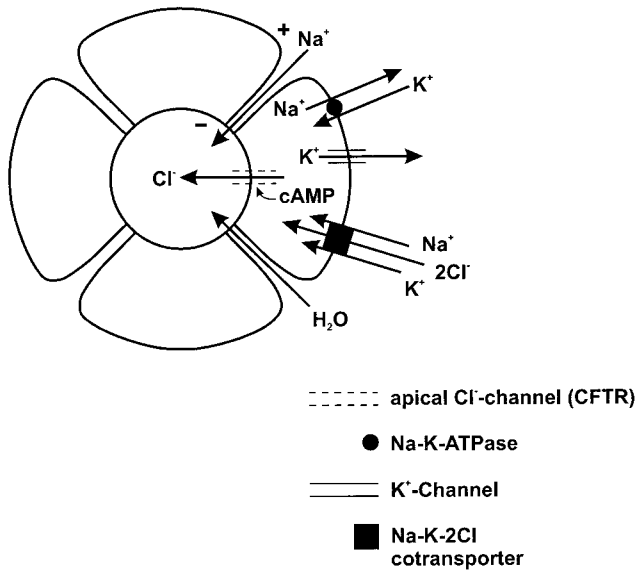


FIGURE 18-5. Model of chloride and fluid secretion by secretory epithelia, including autosomal-dominant polycystic kidney disease (ADPKD) cysts. Required basolateral transporters are the Na-K-ATPase, which establishes the chemical gradient for Na, a potassium channel that allows K to cycle back out of the cell, and the Na-K-2CI-cotransporter, which is driven by the high extracellular-to-intracellular Na gradient. An apical chloride channel provides the pathway for CI efflux into the cyst lumen. In ADPKD cyst epithelia, as well as in some other secretory epithelia, the CI channel is the cystic fibrosis transmembrance conductance regulator (CFTR). Chloride secretion creates cyst lumen negativity with consequent Na flux through paracellular pathways into the lumen; water follows by osmotic forces. The CFTR can be activated by the second messenger cyclic adenosine monophosphate (cAMP). (Adapted from: Sullivan LP, Wallace DP, Grantham JJ. Chloride and fluid secretion in polycystic kidney disease. *J Am Soc Nephrol* 1998;9:903, with permission.)

similar to airway secretory epithelia (120,121) (Fig. 18-5). Other, newly identified chloride channels or additional anion transporters as well as additional basolateral transporters likely also play a role in transepithelial fluid secretion in ADPKD.

Abnormal Extracellular Matrix Metabolism

Electron micrographs reveal a thickened or laminated tubular basement membrane surrounding most cysts (122) (Fig. 18-6). This morphologic finding and the other manifestations of extracellular matrix abnormalities such as mitral valve prolapse, abdominal wall hernias, and cerebral artery aneurysms have suggested an important role of the extracellular matrix in cystogenesis (89,123,124). Abnormalities in the synthesis and metabolism of basement membrane components, particularly increases in laminin, fibronectin, and collagen type IV and decreases in proteoglycans, have been identified in animal models and in cell cultures from human polycystic kidneys (98, 124–126). These alterations disrupt the normal cross talk between epithelia and their underlying basement membrane and thus lead to dysregulation of cell division, cell differentiation, and polarity.

In addition to these basement membrane abnormalities, the renal interstitium is affected as well, particularly as the disease progresses. In animal models and in nephrectomy or autopsy specimens from patients with ADPKD, interstitial inflammatory infiltrates and marked interstitial fibrosis are seen (127,128). Activated fibroblasts and inflammatory cytokines appear to play a role. Fibroblasts from ADPKD kidneys *in vitro* secrete increased amounts of acidic fibroblast growth factor and show a hyperproliferative response to it, suggesting an autocrine stimulatory loop (129). Inflammatory cytokines are present in cyst fluid (130). In addition, a nonpolar lipid fraction from human cyst fluid also possesses chemoattractant activity (131). In animal models, inflammatory and fibrogenic cytokines such as monocyte chemoattractant protein-1, osteopontin, and transforming growth factor-β are upregulated in the kidneys (97,132). The interstitial scarring that follows interstitial inflammation is thought to be a leading cause of renal dysfunction in ADPKD (97,132).

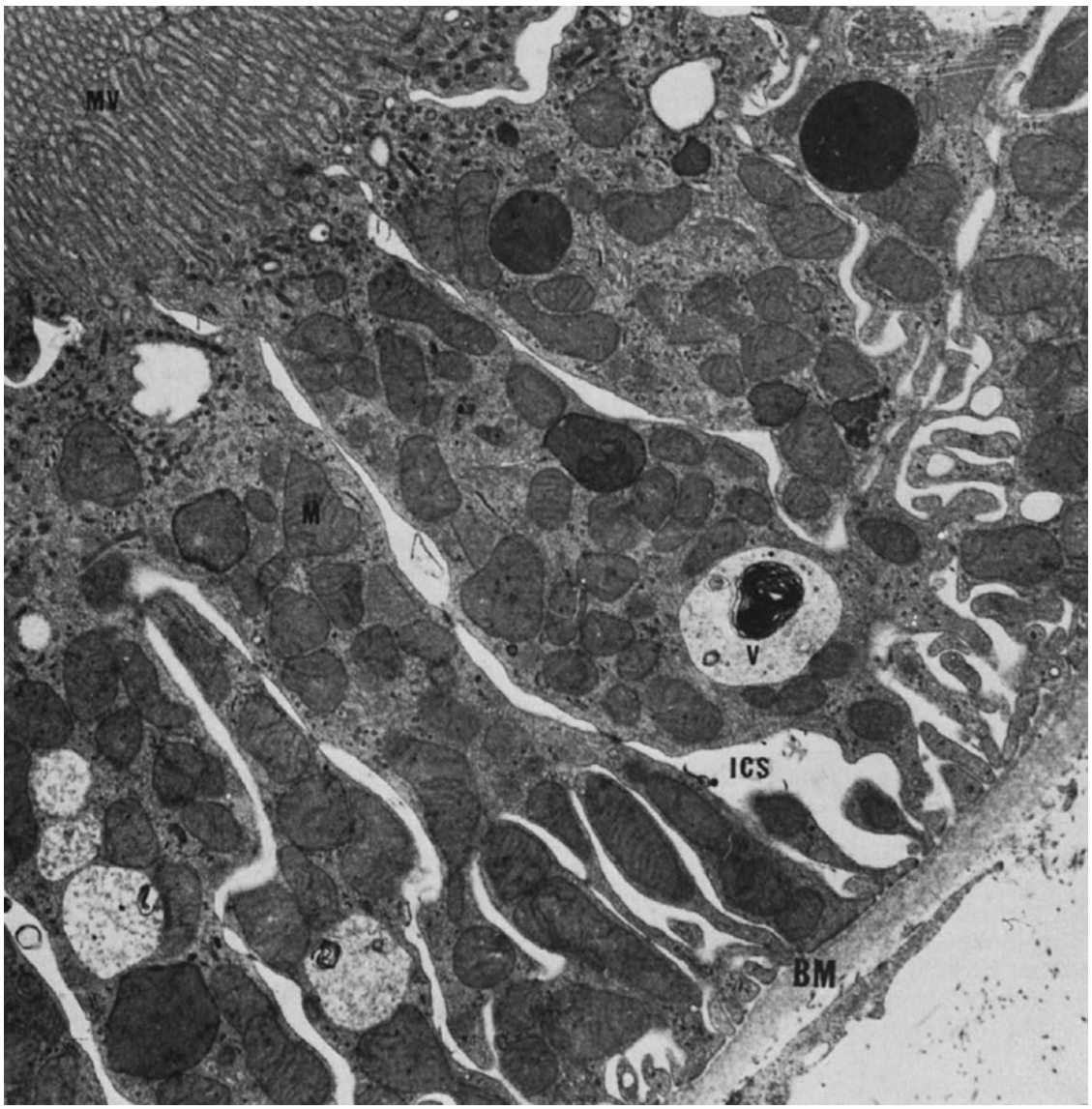
Recently, polycystin-1 and polycystin-2 have also been localized in the primary cilia of renal epithelial cells (133,134). This finding suggests that polycystins might have important functions in primary renal cilia. Primary cilia function as mechanoreceptors where a calcium current is created by bending the cilium by luminal flow (135). The polycystins in the primary cilia can act as mechanosensors receiving signals from the tubule lumen and transducing them into cellular responses that regulate proliferation, apoptosis, adhesion, and differentiation (91,135).

Pathology

Because of the growth of hundreds of cysts, the kidneys in polycystic disease gradually enlarge and can attain an enormous size. Kidneys measuring 40 × 25 × 20 cm and weighing 7 to 8 kg have been reported. Usually these greatly enlarged kidneys are seen in patients with ESRD undergoing nephrectomy, or on autopsy. These end-stage kidneys contain hundreds of fluid-filled cysts of widely differing sizes (Fig. 18-1). The cyst fluid may be clear or cloudy, watery, or have the consistency of chocolate syrup. The color of the fluid can be magenta, due to degraded blood from previous cyst hemorrhage, or various shades of yellow. The cyst walls can be thin and transparent, but calcification of cyst walls is also common (136). The renal capsule may be thickened around infected cysts, and the kidney may be attached to adjacent abdominal organs such as the spleen and the adrenal glands by fibrous tissue.

Cut sections demonstrate cysts throughout the renal parenchyma (Fig. 18-1). Islands of normal-appearing renal parenchyma can usually be found only in kidneys from young, nonazotemic patients (Fig. 18-7). The cysts vary in size from 3 mm to 10 cm or more in diameter. The larger cysts can contain >300 mL of fluid. Cysts in ADPKD arise from all segments of the nephron, and some cysts (~11%) retain the morphologic characteristics of proximal or distal tubules or of collecting ducts (Fig. 18-8A). However, most (84%) are lined by a single layer of poorly differentiated columnar or cuboidal epithelium (76,122) (Fig. 18-6). Approximately 5% of cysts are lined by a markedly hyperplastic epithelium, forming polyps and microadenomas (76,93) (Fig. 18-8B). This hyperproliferative epithelium typically has no signs of dysplasia or premalignant features. The cysts are surrounded by a fibrous stroma, which may contain bundles of smooth muscle-like cells, likely transformed myofibroblasts. Inflammatory interstitial infiltrates are seen, and in advanced cases, the renal interstitium is replaced by fibrosis. Marked arteriosclerosis and arteriolosclerosis are found in nephrectomy specimens, evidence that ischemic injury and damage from hypertension contribute to tubular atrophy and glomerulosclerosis (127).

As noted previously, microdissection studies of human kidneys suggest that only 1% to 2% of nephrons are cystic (76) (Fig. 18-7). These studies also have shown that cysts begin as focal dilatations of tubular segments (137). When these dilatations exceed approximately 2 mm in diameter, they frequently disconnect from the parent tubule; at least 73% of the cysts have no tubular openings when evaluated by scanning electron microscopy (76). The cyst lining epithelial cells are joined together by junctional complexes like those seen in normal proximal tubules or by tight junctions typical of distal renal



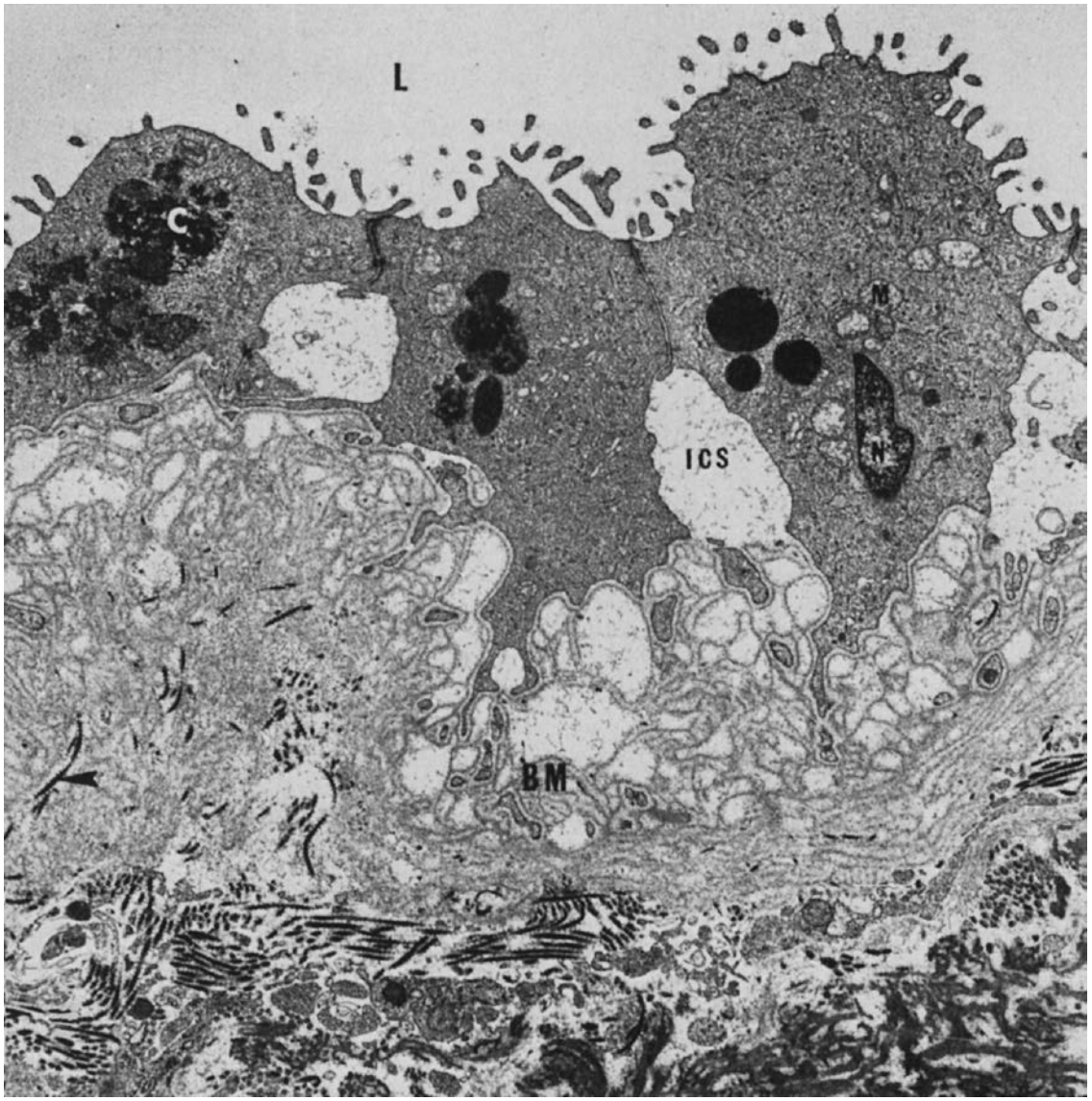
A

FIGURE 18-6. A: Electron micrograph of normal human proximal tubule. The basement membrane (BM) is uniform and thin. The usual numbers of microvilli (MV), mitochondria (M), vesicles (V), and intracellular spaces (ICS) are present (magnification ×8,000). **B, C:** Electron micrographs of cyst walls from adult patients with polycystic renal disease. The basement membrane (BM) is laminated, thickened, and irregular. Collagen fibrils (*arrow*) extend into the membrane. The epithelial lining cells contain few organelles. The intercellular spaces (ICS) are widened. Dense cytosomes (C) are present in some cells. N, nucleus; L, lumen. (Uranyl acetate and lead citrate; magnification ×11,000.) (Courtesy of Dr. F. E. Cuppage, Kansas University Medical Center, Kansas City, KS.)

epithelium (122) (Fig. 18-6). Only a few microvilli are seen on the luminal surface and a few mitochondria in the cytoplasm. Some cells have prominent cilia, and different types of cells are found in some cysts (Fig. 18-8A). Occasional infoldings of the plasma membranes may be found on the basal surface. The basement membrane of most cysts is strikingly abnormal (Fig. 18-6). There is pronounced splitting, duplication, thickening, and lamination of the basal lamina.

The osmolality of cyst fluids is similar to that of plasma, but sodium and nonsodium osmolyte concentrations vary significantly between cysts (90,138,139). Sodium concentrations can vary between 3 and 207 mEq/L, but often are either less than 60 mEq/L or more than 75 mEq/L (138). Therefore, a distinction was made between low-sodium and high-sodium cysts. The high-sodium cysts have sodium concentrations similar to plasma and therefore are also called *nongradient cysts,* whereas the low-sodium cysts are *gradient cysts*, because they are able to maintain steep concentration gradients not only for sodium but for protons, potassium, chloride, phosphates, and other ions (140). Morphologically, the gradient cysts have long tight junctions (zonulae occludens depth >500 μm), making them impermeable to ions, whereas the nongradient cysts have short tight junctions (<500 μm), making them leaky for solutes and water (138). These characteristics suggested that gradient cysts were derived from collecting ducts and nongradient cysts from proximal tubules. However, most nongradient cysts are lined by a poorly differentiated epithelium with few microvilli and few mitochondria, which does not resemble proximal tubular epithelium (138).

More-recent studies have assessed the distribution of aquaporin-1 and -2 in ADPKD cysts. In normal kidneys, aquaporin-1 is expressed in proximal tubules and thin



B

FIGURE 18-6. (*Continued*)

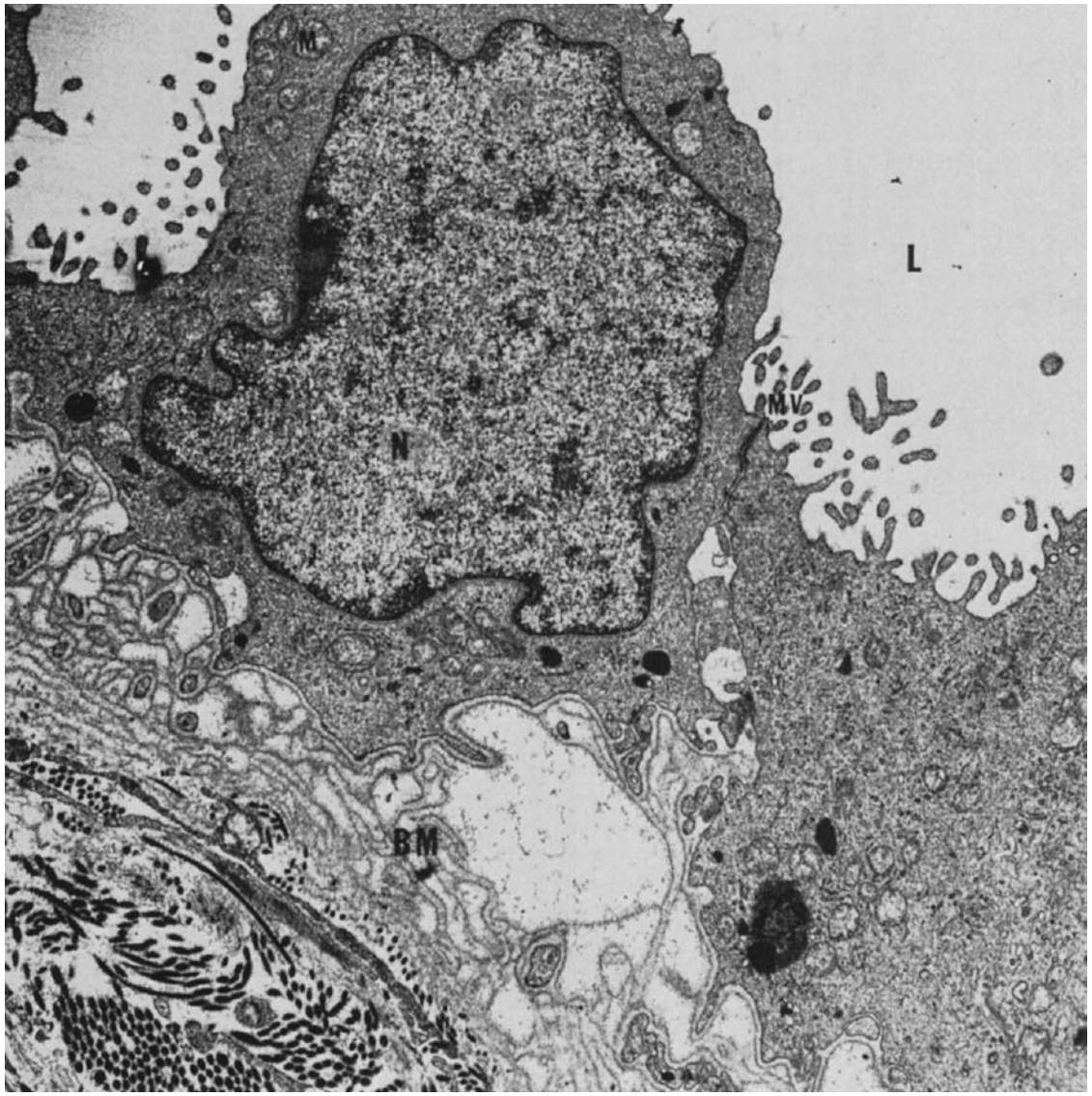
descending limbs of Henle's loop, whereas aquaporin-2 is expressed on the apical surfaces of collecting duct epithelia. In ADPKD kidneys, approximately 30% of cysts stain positive for aquaporin-1, another 30% are positive for aquaporin-2, and the rest are negative for both aquaporin-1 and -2 (141,142). The aquaporin-1–positive cysts presumably are derived from proximal tubules or thin descending limbs, the aquaporin-2–positive cysts from collecting ducts, and the negative cysts from nephron segments that do not express these water channels (i.e., ascending limb of Henle's loop and distal convoluted tubules). These results also imply that expression of water channels is not a prerequisite for cyst expansion. Moreover, the aquaporins appear to retain their segment-specific expression in ADPKD even though the morphologic characteristics of proximal and distal tubules are lost.

In addition to electrolytes and water, cyst fluids also contain amino acids, glucose, urea, idiogenic osmoles such as sorbitol, betaine, and glycerophosphorylcholine, and proteins, such as β_2-microglobulin, erythropoietin, renin, and albumin (139,140). Cytokines, specifically interleukin-1β, interleukin-2, and tumor necrosis factor-α, growth factors (EGF, hepatocyte growth factor, endothelins), and cyst activating factor, a nonpolar lipid, are present in cyst fluids as well and likely play a significant role in the pathophysiology of ADPKD (see earlier) (100,103–105,117,130).

Therefore, the cysts of polycystic kidneys are not simply impermeable cul-de-sacs that collect and store urine from more proximal nephron segments. They are complex structures that proliferate and undergo apoptosis, that synthesize or transport various proteins, hormones, and cytokines, and that actively secrete chloride and water. Under certain conditions, they also may be able to absorb solutes and water (90). Most cysts are permeable to small solutes, but some are highly impermeable. Although most cysts are lined by morphologically undifferentiated epithelium, the differential expression of water channels is maintained.

Diagnosis

History, physical examination, and laboratory findings are neither sensitive nor specific for ADPKD. Therefore, the diagnosis is usually made by renal imaging in combination with the family history, or by gene linkage analysis if at least two affected



C

FIGURE 18-6. (*Continued*)

family members in addition to the proband are available for testing (Fig. 18-3). However, because gene linkage analysis is cumbersome and expensive, it is used only if imaging studies are not informative and there is a need to know the diagnosis.

In clinical practice, ultrasound imaging is the primary tool for diagnosis. Ultrasound is sensitive, noninvasive, does not expose to ionizing radiation or contrast material, and is relatively inexpensive. It can be safely performed in children and pregnant women. Unlike gene linkage analysis, ultrasound imaging also gives information about the structural severity and involvement of other organs. Typical ultrasonographic findings are enlarged kidneys with multiple, often innumerable, bilateral cysts diffusely scattered throughout the parenchyma (143) (Fig. 18-9). If these findings are present in a patient with a family history of ADPKD, the diagnosis is certain.

The diagnosis can be more difficult to establish if only a few cysts are found in normal-sized kidneys or if there is no family history. The first problem can arise when an asymptomatic, young member from an ADPKD family wants to know whether he or she (or his or her child) is affected. Because renal cysts in ADPKD develop and enlarge with age, young family members may have only one or a few cysts in otherwise normal-appearing kidneys. Simple renal cysts are rare in children, but relatively common in the general population, particularly over the age of 50 years. One study examined the prevalence of simple kidney cysts in the general population by performing ultrasound imaging in older subjects with normal renal function and without renal-related symptoms (144). Simple cysts were seen in 11.5% of subjects 50 to 70 years of age and in 22.1% of those older than 70 years. Bilateral cysts are less common in the general population, occurring in approximately 9% of people older than 70 years of age (144). Simple renal cysts are very rare in children; only 0.1% of children younger than 16 years of age have simple renal cysts (145).

Based on the prevalence of simple renal cysts in the general population and on comparison of ultrasound results with gene linkage data (*ADPKD1*), specific age-dependent criteria have been developed to diagnose ADPKD (137). These criteria have been established for subjects with a family history of ADPKD, for ultrasound imaging as opposed to other imaging methods, and for subjects with *ADPKD1* (85% to 90% of ADPKD families). The diagnostic criteria are the following: for someone younger than 30 years of age, at least two renal cysts (unilateral or bilateral) are required to make the diagnosis of ADPKD;

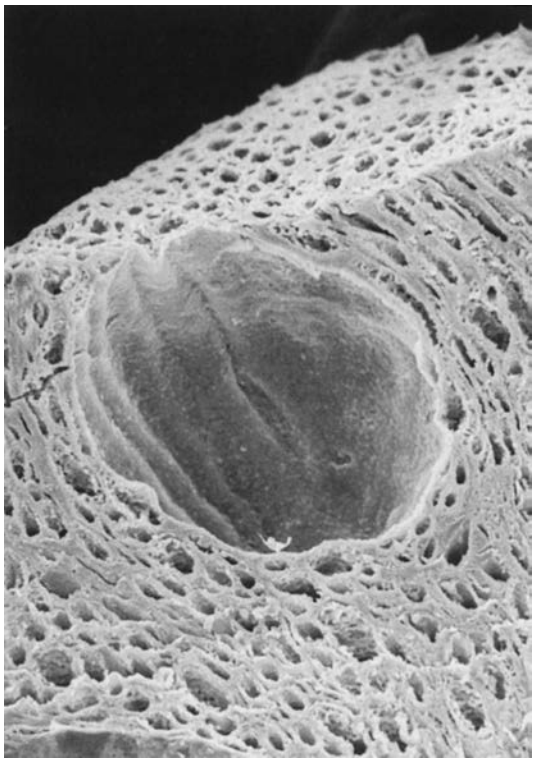


FIGURE 18-7. Scanning electron micrograph of renal cortex from a young, nonazotemic woman with autosomal-dominant polycystic kidney disease. A cyst (500 μm in diameter) is shown surrounded by a large field of normal parenchyma.

for someone 30 to 59 years of age, at least two cysts in each kidney are required; and for someone 60 years of age or older, at least four cysts in each kidney are required to make a diagnosis of ADPKD (146). For a child younger than 18 years of age, any renal cyst is highly suspect for the disease (147,148). Therefore, if a child from an ADPKD family has one renal cyst, we advise the family that the child likely has ADPKD, but that a follow-up ultrasound examination at an older age is required before definitely labeling the child as affected.

For ruling out the diagnosis of ADPKD in a person at risk, the age at which 100% of gene carriers have detectable cysts needs to be known. Several studies have compared ultrasound results with gene linkage data in *ADPKD1* families and have not observed a false-negative ultrasound after age 30 years (12,39,146,149). Therefore, if a subject from an *ADPKD1* family has a negative ultrasound after 30 years of age, he or she can be reassured as not being affected. Similarly, two studies performed in *ADPKD2* families reported that all gene linkage–positive subjects aged 30 years or older had ultrasonographically detectable cysts (150,151). However, the first study included only 19 subjects older than 30 years from 5 families, whereas the second examined 94 subjects older than 30 years of age from 3 *ADPKD2* families. It is likely that some *ADPKD2* families with mild manifestations remain undiagnosed. In these families, the age at first manifestation of cysts could be later. Because these subjects are rare and are expected to have very mild disease, a negative ultrasound after 30 years of age is reassuring and usually indicates absence of ADPKD.

For subjects younger than 30 years of age from ADPKD families, a negative ultrasound does not rule out the disease. This is important if a person with a negative ultrasound who is younger than 30 years of age considers kidney donation for a family member with ESRD. In this situation, a computed tomography (CT) scan with administration of intravenous contrast material, or magnetic resonance imaging (MRI) should be performed. Both methods, particularly MRI, are more sensitive than ultrasound in the detection of cysts (152,153) (Fig. 18-10). If the CT scan or the MRI shows at least two renal cysts, the subject probably has ADPKD.

Another clinical scenario in which a diagnosis of ADPKD is in question is when renal cysts are found incidentally on abdominal imaging in patients without a family history of ADPKD. Then, the most useful diagnostic test is ultrasound imaging of both parents because many subjects with ADPKD are only mildly affected and clinically asymptomatic. If parents are not available, other family members should be imaged. If no family member has the disease and the proband has multiple bilateral cysts scattered throughout normal-sized or enlarged kidneys, the proband likely has a new mutation for ADPKD. If the proband has only a few cysts, follow-up imaging should be performed before confirming the diagnosis. ADPKD is a progressive disease; therefore, follow-up imaging should reveal more or larger cysts.

As stated, ultrasound is the preferred imaging method for the diagnosis of renal cysts. However, in patients with a suspected renal complication of ADPKD, CT scanning with intravenous contrast or MRI are indicated. This includes patients presenting with acute flank or abdominal pain or with persistent infection. In these situations, CT scanning is more sensitive than ultrasound in identifying intracystic or perinephric hemorrhage, stones, obstruction, cyst infection, or perinephric abscess. CT or MRI is also necessary when cysts show atypical features on ultrasound such as a thickened wall or inhomogeneous lumen, to exclude carcinoma (152–154). Renal arteriography occasionally may be helpful when renal carcinoma or a vascular malformation is suspected, but it has no role in the primary diagnosis of ADPKD.

Magnetic resonance imaging has been used to diagnose renal cystic disorders. It is particularly useful for the evaluation of complex cystic lesions and when malignancy is suspected (153,154). Because ultrasound imaging is not very accurate in measuring renal volume, particularly with large kidneys and multiple cysts, MRI is being evaluated as a more reliable tool to determine renal volume (155). In contrast to ultrasound, MRI can detect remaining normal parenchyma between cysts even in advanced cases. Therefore, measuring cyst and parenchyma volume may be useful to assess disease progression before glomerular filtration rate (GFR) declines. This is important if interventions are to be studied at an early stage of ADPKD.

Differentiation of ADPKD from other cystic diseases is occasionally necessary, particularly when there is no family history of ADPKD or when there are unusual features in the patient's presentation (156). For instance, if renal carcinoma occurs in a young patient or in multiple members of a presumed ADPKD family, VHL disease should be suspected because renal cancer is not part of the spectrum of ADPKD manifestations. There are several case reports in the literature describing VHL disease masquerading as ADPKD and being correctly diagnosed only after the occurrence of cancer or hemangioblastoma in the same patient or in the family (157–159). Another autosomal-dominant disorder that can mimic ADPKD in both children and adults is TSC (160,161). VHL disease and TSC are described in more detail later. Usually, the associated extrarenal features point to the correct diagnosis (Table 18-2), but they may be absent in some cases.

In children, it is sometimes difficult to differentiate ADPKD from ARPKD, particularly if there is no family history and the child is severely affected, with enlarged kidneys and hypertension at a young age. This presentation is typical for ARPKD, but can also occur in ADPKD (7,17, 162–164). The most useful test in this situation is ultrasound imaging of both parents. Often one of the parents is found to have asymptomatic ADPKD (6,7,162,165–167). If both parents' ultrasound studies are

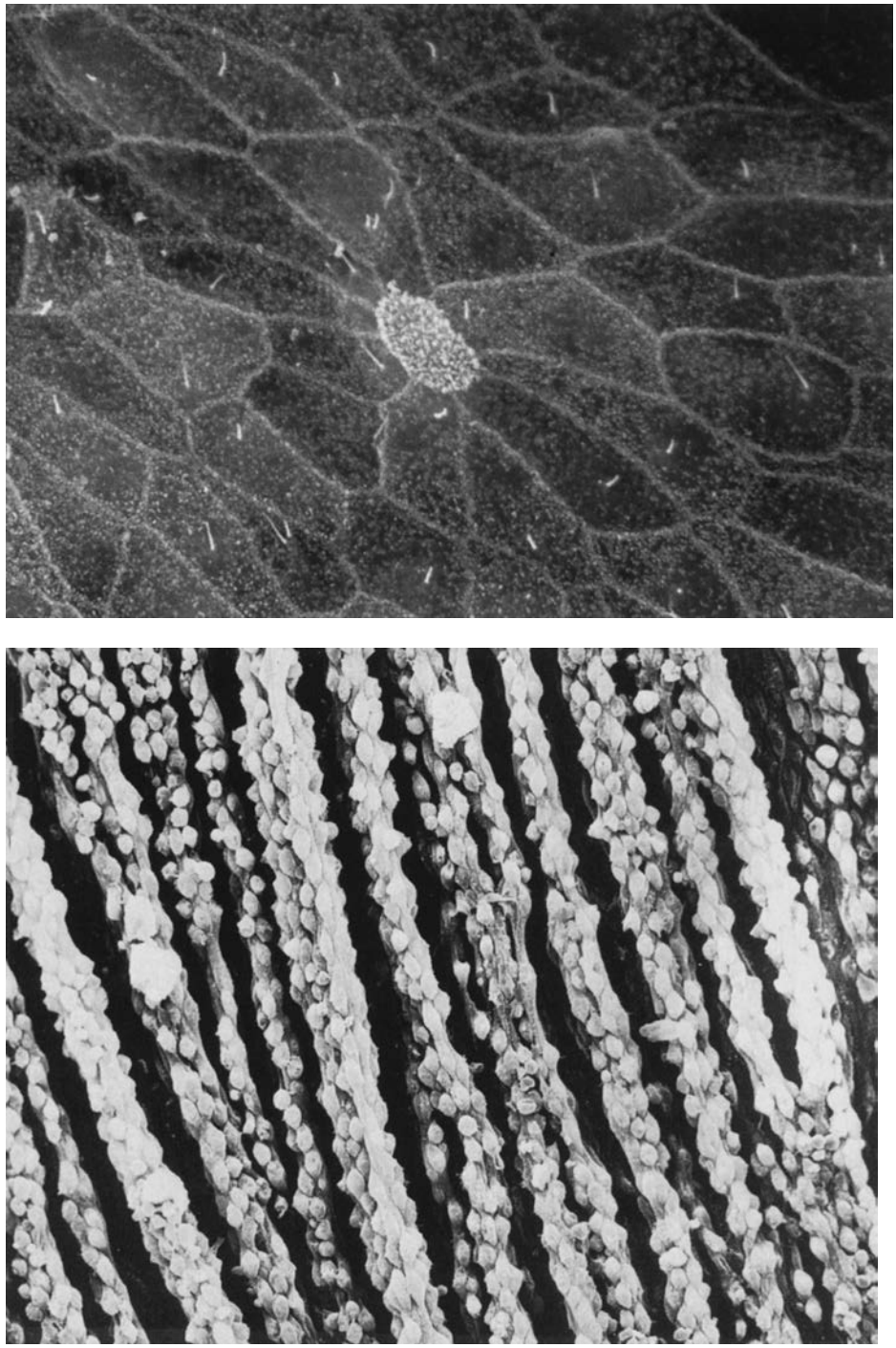


FIGURE 18-8. Scanning electron micrographs of cysts in kidneys of patients with autosomal-dominant polycystic kidney disease. **A:** Apical surface of collecting duct cyst showing two distinct cell types. Note that cell borders are accentuated by microvilli and that most cells have a single cilium (magnification ×1,800). **B:** Apical surface of cyst of unknown tubular origin. Pronounced epithelial hyperplasia gives a cordlike appearance (magnification ×945).

negative, the child may have a new mutation for ADPKD or may have ARPKD. New mutations are estimated to occur in up to 10% of ADPKD cases (28). To differentiate between ARPKD and ADPKD, a liver biopsy may be considered because congenital hepatic fibrosis is invariably associated with ARPKD but only rarely with ADPKD (see later). A renal biopsy is also useful because the histologic features of ARPKD are distinctly different from those of ADPKD (Table 18-2). Sometimes it is not possible to make a diagnosis unequivocally in an infant (163,164,167); in these cases, the course of the disease often allows a differentiation. Progression to ESRD is faster in the more severely affected children with ARPKD, and signs of liver disease often appear as the child with ARPKD becomes older. ESRD and liver disease are very rare in children with ADPKD.

Medullary cystic disease is another cystic disorder that may occur in children and adults. It has an autosomal dominant inheritence. In contrast to ADPKD, the affected individuals have

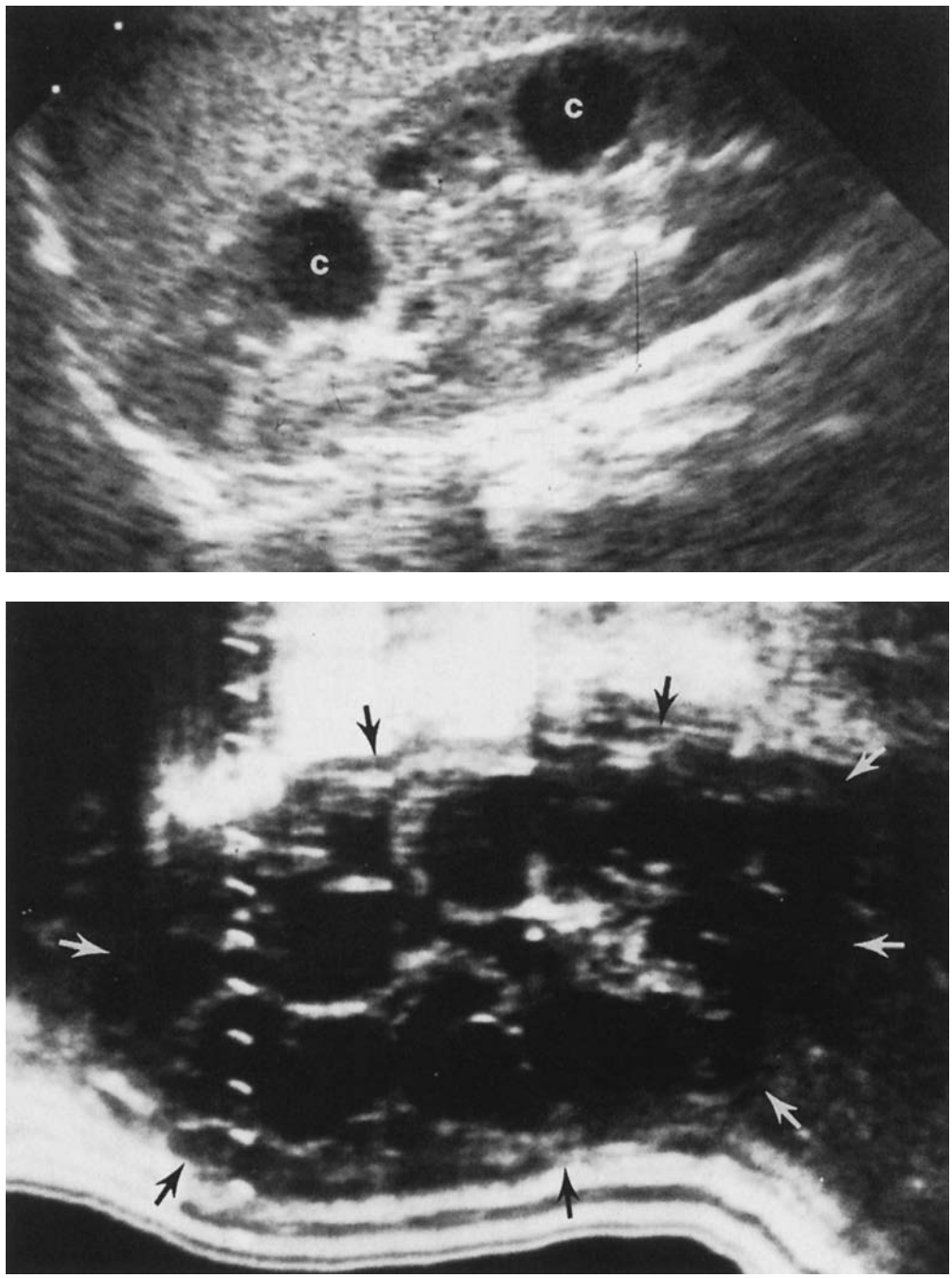


FIGURE 18-9. Renal ultrasonography of two patients with polycystic kidney disease. **A:** Longitudinal ultrasound scan of the left kidney in a 13-year-old boy. The kidney is of normal size, with two cysts (C) measuring approximately 1.5 cm, and several small cysts. **B:** Longitudinal ultrasound scan of the left kidney in a 63-year-old man with advanced autosomal-dominant polycystic kidney disease. The kidney is grossly enlarged, measuring 16 cm, and is essentially replaced by cysts.

normal- to small-sized kidneys. The cysts may be found only at the corticomedullary junction (see Chapter 17). Differentiation from ADPKD is usually straightforward.

Multicystic dysplastic kidney is a developmental disorder of the fetal kidney and is frequently detected by prenatal ultrasonography or as a palpable flank mass in the neonate. It is most often unilateral or may involve only one pole of a kidney, and is frequently associated with ureteropelvic junctional or lower urinary tract obstruction. Contralateral urinary tract anomalies may also be present. Histologically, multicystic renal dysplasia is characterized by the absence of normal nephrons and presence of nonrenal tissue such as cartilage or fat, primitive ducts, and cystic spaces (see Chapter 21). The rare familial types of renal dysplasia are part of malformation syndromes that are associated with abnormalities of the liver, bones, and brain.

Acquired cystic kidney disease occurs in adults and children with chronic renal failure of any etiology (see later). Therefore, the kidneys are usually small, whereas ADPKD kidneys are always enlarged by the time renal insufficiency develops.

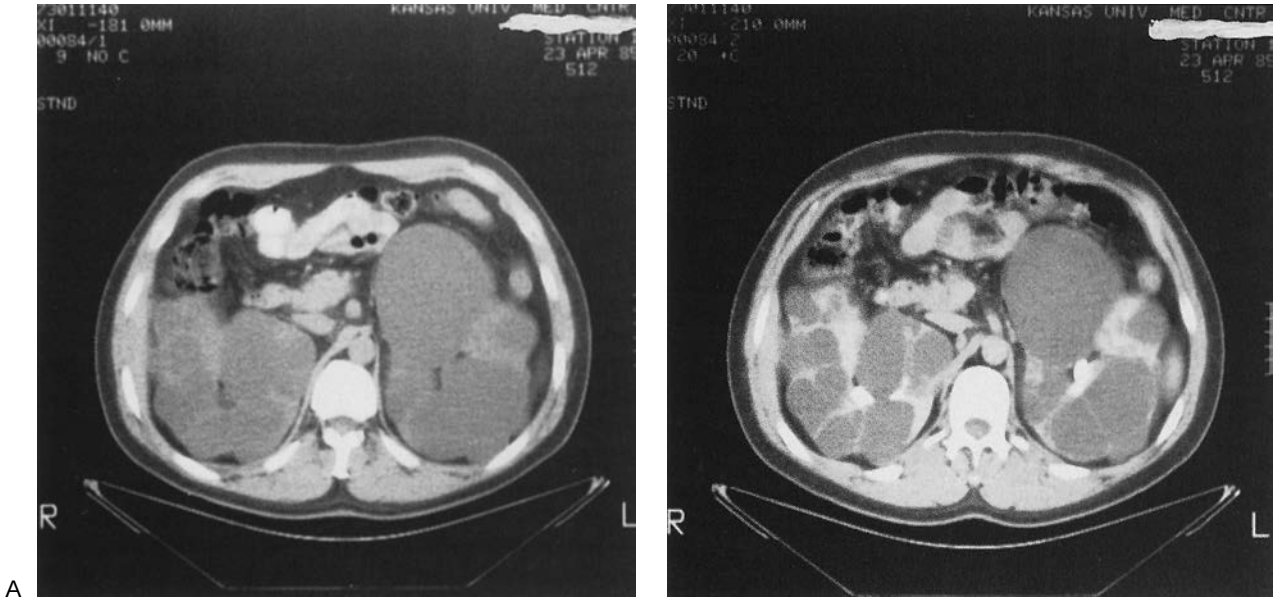


FIGURE 18-10. Computed tomography scans of a nonazotemic patient with autosomal-dominant polycystic kidney disease. **A:** Without contrast. Note the bilateral enlargement of the kidneys and the large cyst in the central portion of the left kidney. **B:** After intravenous infusion of iodinated contrast. Noncystic parenchyma is brightly stained, giving a more distinct view of the individual cysts.

However, a few cases of ACKD have been reported in the literature, in which previously small or normal-sized kidneys enlarged during long-term hemodialysis or peritoneal dialysis therapy so that they resembled ADPKD kidneys (168,169). In these rare cases, the patient's history, the absence of any extrarenal features of ADPKD, and the negative family history serve to differentiate ACKD from ADPKD (Table 18-2).

Clinical Features of Autosomal-Dominant Polycystic Kidney Disease

Multiple renal cysts and progressive renal enlargement are the main clinical features present in 100% of gene carriers after 30 years of age. Extrarenal manifestations occur with variable frequency and in various combinations (Table 18-1) in both *ADPKD1* and *ADPKD2*. As mentioned earlier, the polycystin proteins are expressed in virtually all tissues. Why only the renal phenotype is expressed in 100% of mutation carriers is unknown. The molecular basis for this variability could relate to the frequency with which "second hits" occur in different tissues or cells of an individual. The renal disease course is variable as well. This is in part due to genetic heterogeneity. As stated previously, the mean age at onset of ESRD is 53 to 56 years in *ADPKD1* families, but 70 to 73 years in *ADPKD2* families (12,36–40). However, even in *ADPKD1* families, marked variability of the disease course is observed. Some subjects may remain asymptomatic until old age, others enter ESRD in middle age, and a child of the same family may present with enlarged kidneys and hypertension at birth (7,170).

Renal Cyst Development in Children with Autosomal-Dominant Polycystic Kidney Disease

Although ADPKD is thought of as a disease of adults, renal cysts are present and enlarge in childhood. Research studies on a large number of at-risk children from ADPKD-1 families have shown that renal cysts can be seen on ultrasound in approximately 60% of affected children younger than 5 years of age and in 75% of children between 5 and 18 years of age (148,149). Similarly, a study from Spain revealed an ultrasound sensitivity of 95% for ADPKD-1 subjects younger than age 30 years—that is, 80 of 84 children and young adults diagnosed as affected by gene linkage analysis had at least 2 cysts on ultrasound (150). Although some children have only one or two renal cysts, many have numerous bilateral cysts. For example, of 185 affected children studied at the University of Colorado, 154 had multiple cysts at a mean age of 9.3 ± 0.4 years (171). That study did not select for symptomatic or severely affected children, but all children of participating ADPKD families were examined by ultrasound and gene linkage analysis. Twelve children in that study had a single cyst at their first visit at a mean age of 7.9 ± 1.2 years. Eleven of them progressed to bilateral cysts at a subsequent visit (mean age, 11.2 ± 1.1 years); one girl with a single cyst at age 13 years did not come back for a follow-up visit, but gene linkage analysis confirmed that she carries the *PKD1* gene. This corroborates the notion that even a single cyst in a child from an ADPKD family is highly predictive of the presence of ADPKD in the child.

Cysts detectable by ultrasound are at least 2 to 5 mm in size. However, renal microcysts have been found on histologic examination of aborted ADPKD fetuses as early as the 12th week of gestation; children who had macroscopically normal kidneys and died perinatally of nonrenal causes also had renal microcysts histologically (1,172–175). This suggests that in ADPKD, renal cysts are already present *in utero* and subsequently enlarge over a lifetime. Prenatally detectable ultrasound abnormalities are probably not common. In a small study performed at the University of Colorado, 24 fetuses were studied by ultrasound between 18 and 36 weeks of gestation; renal abnormalities were found in only 5. Abnormal fetal kidneys suggesting ADPKD are enlarged and hyperechogenic, resembling the ultrasound appearance of ARPKD (162–164). Sometimes a few small cysts are found *in utero*.

Renal cystic disease clearly progresses during childhood. With increasing cyst numbers, the kidneys become larger. Renal volume reflects the cyst burden and can be measured

TABLE 18-2

COMPARISON OF VARIOUS CYSTIC RENAL DISORDERS

Feature	ADPKD	TSC	VHL	ARPKD	ACKD
Inheritance	AD, <10% new mutations	AD, ~66% new mutations, somatic mosaicism common	AD, <10% new mutations, somatic mosaicism occurs	AR	None
Chromosome	16, 4 Genes identified	9, 16 Genes identified	3 Gene identified	6 Gene identified	NA
Prevalence	1:400–1:1,000	1:6,000–1:10,000	1:36,000	1:20,000 1:40,000	Up to 90% of patients on long-term (>10 y) dialysis
Age of clinical onset	Usually adults, sometimes children	Children and adults	Usually adults	Newborns, children, rarely young adults	Children and adults
Method of diagnosis	US, CT, MRI, linkage or mutation analysis	US, brain CT or MRI, linkage or mutation analysis	US, brain CT or MRI, linkage or mutation analysis	US, MRI, occasionally liver or renal biopsy, linkage or mutation analysis (prenatally)	CT, US, MRI
Presenting symptom	Pain, hematuria, infection, hypertension, family screening	Seizures, skin lesions, mental retardation, renal bleeding, cardiac arrhythmias	Retinal, brain or spinal cord tumors, renal cysts, pheochromocytoma, renal carcinoma	Abdominal mass, respiratory insufficiency (newborns), hypertension, ESRD, hepatosplenomegaly, portal hypertension	Hematuria, pain, malignancy, screening
Hypertension	65% to 80% of adults	May occur	Patients with pheochromocytoma (7% to 20%)	70% of children who survive the neonatal period	Common
Gross hematuria	50% of adults, 10% of children	May occur	Patients with renal cell carcinoma	May occur	May occur
End-stage renal disease	50% by age 60 y, 75% by age 70 y	Occurs, particularly in patients with the contiguous gene deletion syndrome	Occurs in some patients with polycystic kidneys	Invariable, unless child dies from other cause	Invariable by definition
Renal size	Normal early, later enlarged	Normal or enlarged	Enlarged if tumorous or polycystic	Enlarged early, can regress in size later	Reduced, normal or enlarged
Extrarenal manifestations	Common (e.g., liver cysts, mitral valve prolapse, intracranial aneurysms)	Common, involving skin, brain(tubers), heart, retina, lungs	Common, involving brain, spine (hemangioblastoma), retina, pancreas (cysts); pheochromocytoma	Invariably congenital hepatic fibrosis (may be clinically asymptomatic)	No
Renal malignancy	Rare	Rare (1% to 2.5%)	70% to 80% by age 60 y	Not reported	4–7% after 10 y

AD, autosomal dominant; *ACKD*, acquired cystic kidney disease; *ADPKD*, autosomal-dominant polycystic kidney disease; *AR*, autosomal recessive; *ARPKD*, autosomal-recessive polycystic kidney disease; *CT*, computed tomography; *ESRD*, end-stage renal disease; *MRI*, magnetic resonance imaging; *NA*, not applicable; *TSC*, tuberous sclerosis; *US*, ultrasound; *VHL*, von Hippel-Lindau disease.

FIGURE 18-11. Relationship between mean renal volume (MRV), determined by ultrasound, and age in 185 affected (**A**) and 127 unaffected (**B**) children from the University of Colorado study. Two thirds of the children were examined multiple times (mean, 2.7) between 1985 and 1999. The regression model that gave the best fit for the data was the growth model (general form: $y = e^{b_o + b_1 t}$), which is plotted in *black dots* in the figure, whereas the actual data points are plotted in *gray.* The equation for the affected children is $MRV = e^{3.68 + 103 \times age}$, with $R^2 = 0.61$ for the unaffected children $MRV = e^{3.4 + 0.095 \times age}$, with $R^2 = 0.77$ The β coefficients of the growth curves differ significantly ($P<0.01$) between the affected and unaffected children, with the curve for the affected children being steeper, indicating that renal volume increases faster over time in the affected than the unaffected children. The growth model also implies that renal volume enlarges faster at a higher age. Because R^2 is larger for the unaffected than for the affected children, the model is a better fit for the unaffected children. This is an indication of the greater variability of renal volumes for a given age in the affected children. In unaffected children, renal volumes remain constant after 18 years of age (as shown by the *gray dots;* the *black dots* are fitted to the growth model), whereas renal volumes continue to increase in the affected children. (Adapted from: Fick-Brosnahan GM, Tran ZV, Johnson AM, et al. Progression of autosomal-dominant polycystic kidney disease in children. *Kidney Int* 2001;59:1654, with permission.)

relatively easily using real-time ultrasound (8). The University of Colorado study compared the increase in renal volume over time between affected and unaffected children (Fig. 18-11). Although there was great variability in renal volumes, particularly in the affected children, at all ages mean renal volumes were significantly larger in the affected than in the unaffected children (171). There were no differences in mean renal volumes between boys and girls. Risk factors associated with faster renal enlargement in children were a diagnosis *in utero* or in the first year of life, hypertension, and relatively enlarged kidneys early in life (171).

This observational study from the University of Colorado describes the course of ADPKD in children of whom most were identified by screening (171). However, some children with ADPKD are much more severely affected, and the clinical presentation may mimic that of ARPKD. There are many reports in the literature of children from ADPKD families who presented with abdominal masses shortly after birth or who had abnormally enlarged, hyperechogenic kidneys on prenatal ultrasound imaging (7,162,163,176). These children with very–early-onset disease have an unusually severe form of ADPKD. Some of the reported cases were stillborn, some died shortly after birth of respiratory failure, and others died within the first year of life of renal failure (4,5,177–180). These most severe cases are rare. Many children who are diagnosed *in utero* or in their first year of life have a relatively good prognosis during childhood. Their growth is normal, and usually they maintain normal renal function throughout childhood, although hypertension develops in approximately 50% before 10 years of age, and sometimes in the first year of life (7,147,165). This hypertension can be severe, requiring multiple drug treatment (7,181). In some children with prenatally enlarged kidneys, renal size can normalize after birth (182). However, in many children with early-onset disease, multiple cysts and enlarged kidneys rapidly develop (7). Rarely, these children progress to ESRD during childhood (7,163,166,183), but many of them maintain adequate renal function even as young adults (7,165,167).

As mentioned, ADPKD kidneys enlarge gradually during childhood and adolescence. After 30 years of age, 95% of affected subjects have markedly enlarged kidneys (i.e., renal volumes more than 2 standard deviations above the mean for unaffected adults). Although mean renal volumes are not significantly different between affected boys and girls, men with ADPKD older than 25 years of age have on average significantly larger kidneys than women with ADPKD of similar age (Fig. 18-12; data from the University of Colorado study).

Signs and Symptoms of Autosomal-Dominant Polycystic Kidney Disease

Patients with ADPKD can be asymptomatic, particularly in the early stages of the disease. In a study of 164 young subjects with

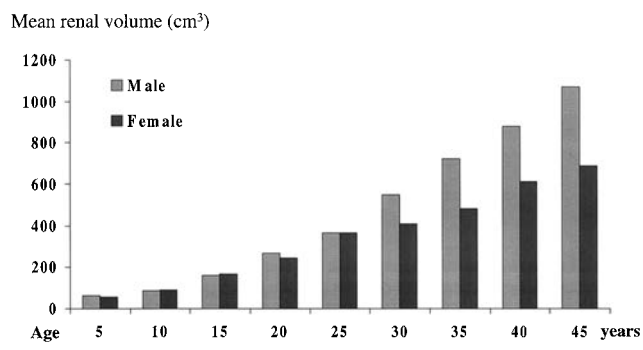


FIGURE 18-12. Mean renal volumes of male and female patients with autosomal-dominant polycystic kidney disease (ADPKD) from birth to 45 years of age. Data from the University of Colorado study. Renal volume determinations (by ultrasound) in 185 children with ADPKD younger than 18 years of age, and 256 men and 403 women with ADPKD 18 to 45 years of age. Up to age 25 years, renal volumes are similar in male and female patients, whereas after 25 years of age, men have significantly larger kidneys than women.

TABLE 18-3

SIGNS AND SYMPTOMS OF AUTOSOMAL-DOMINANT POLYCYSTIC KIDNEY DISEASE

Signs and symptoms	Frequency
Back and flank pain	60% of adults, 20% of children
Hypertension	Up to 80% of adults, 20% to 30% of children
Gross hematuria	~50% of adults, 10% of children
Renal concentrating defect	Potentially all adults
Palpable kidneys	Potentially all adults
Hepatomegaly particularly women	20% to 30% over age 50;
Systolic murmurs or clicks	~20% of young adults
Microscopic hematuria or pyuria	~20% of adults and children
Proteinuria	18% to 68% of adults
Chronic renal insufficiency	Potentially all adults; age dependent

ADPKD (mean age, 35 ± 1 years) diagnosed by ultrasound, 32% had no signs or symptoms (184). The most common complaint is *back and flank pain,* occurring in approximately 60% of adults (185) (Table 18-3). Pain can occur acutely, but can also be a chronic problem. If pain occurs acutely, a renal complication must be considered, such as cyst hemorrhage, infection, or stone colic (see later). Chronic pain is insidious in onset, dull and nagging, and often described as pressure or a pulling sensation. It is believed to be due to stretching of the renal capsule by the enlarging cysts. Severe chronic pain is more common in patients with large cysts or large kidneys (186–188), but it can also occur in patients with many small cysts or a single dominant cyst. For some patients, this chronic pain is disabling, and it can lead to narcotic addiction. A stepwise approach and collaboration with a pain clinic is recommended before referring patients for a surgical procedure. Treatment steps include prescribing acetaminophen, physical measures such as ice massage or heating pads, psychobehavioral measures, transcutaneous electrical nerve stimulation, autonomic plexus blockade, and decompression of renal or liver cysts (185,189). The decompression can be done by percutaneous drainage and alcohol sclerosis in the case of a single or a few large cysts, but more often requires a laparoscopic or open surgical approach to unroof and drain hundreds of cysts (187,190–192). Renal function usually is not affected by these procedures, but hypertension improves sometimes (187,190,191). Immediate pain relief is achieved in approximately 90% of procedures, but chronic pain tends to recur with time. In several studies, the probability of being pain free ranges from 71% to 80% at 1 year to 25% to 60% at 3 years. In patients with ESRD, bilateral nephrectomy, which can now be done laparoscopically, should be considered for relief of severe pain (193).

Hypertension is very common, occurring in 80% of patients with ADPKD. Blood pressures often start to rise in the second and third decade of life, long before renal function starts to decline (194,195). Hypertension can be the presenting symptom leading to the diagnosis of ADPKD. Because hypertension is initially asymptomatic, blood pressure elevations in young patients with ADPKD will be missed unless children from ADPKD families receive regular screening for hypertension. Several studies reported that hypertension occurs in 20% to 30% of children with ADPKD (8,12,147,171,196). A recent study showed that parental hypertension influences the frequency of hypertension with ADPKD, and its presence should alert the clinician for earlier detection and treatment of hypertension in offspring with ADPKD (197). This early blood pressure elevation, although asymptomatic, is biologically significant, as shown by the demonstration of higher left ventricular mass indices in children and young adults with ADPKD compared with unaffected control subjects (198–200).

Hypertension in ADPKD correlates with renal size (186,201). Renal volume is significantly larger in hypertensive subjects with normal renal function than in normotensive subjects matched for age and body surface area (201). This relationship between renal structure and hypertension also exists in children (8,196). Children with more than 10 cysts have significantly larger renal volumes and a higher incidence of hypertension than children with less than 10 cysts (8).

The relationship between renal structural abnormalities and hypertension in ADPKD suggests that the activation of the renin-angiotensin-aldosterone system (RAAS) as a result of cyst expansion and local renal ischemia plays an important role in the development of hypertension in this disease (195,202-207). Immunohistochemical studies of nephrectomy specimens from patients with ADPKD, with normal or impaired renal function have shown hyperplasia of renin-secreting cells of the juxtaglomerular apparatus and in arteriolar walls (127,202,208). Moreover, high levels of active renin were found in cyst fluid obtained from patients with ADPKD, and a large amount of immunoreactive renin was detected in tubular epithelia and cyst lining epithelia of ADPKD kidneys, compared with almost none in normal kidneys (208). Human cystic epithelia in cell culture have the capacity to synthesize renin (208). A recent study showed that in addition to renin, angiotensinogen, angiotensin converting enzyme (ACE), angiotensin II type 1 receptor and angiotensin II peptide are also present in cysts and in dilated tubules in ADPKD kidneys (209).

Activation of the RAAS in human ADPKD has been confirmed by the demonstration of elevated plasma renin activity and aldosterone in hypertensive patients with ADPKD with normal renal function compared with age-matched patients with essential hypertension in the supine and upright position and, even more significantly, after captopril (203). Other studies have provided evidence for an increased intrarenal activity of the RAAS in hypertensive as well as in normotensive patients with ADPKD with normal renal function compared with age-matched, healthy control subjects. Administration of an ACE inhibitor to patients with ADPKD resulted in a significantly greater decrease in renal vascular resistance and increase in renal plasma flow than in the control subjects (203–205). Infusion of angiotensin II led to a smaller decrease in effective renal plasma flow in normotensive subjects with ADPKD than in healthy control subjects, a finding compatible with downregulation of receptors due to tonically increased intrarenal angiotensin II levels (206).

The increased activity of the RAAS observed in patients with ADPKD may also contribute to accelerated cyst growth (195). Angiotensin II has been shown to enhance tubular epithelial proliferation and to stimulate the expression of transforming growth factor-β, which may contribute to interstitial inflammation and fibrosis (210,211). Moreover, increased structural severity of the renal disease promotes increased severity of hypertension and creates a vicious cycle of cyst growth, increased activity of angiotensin II, and further cyst growth (Figure 18-13).

Other factors likely contribute to hypertension in ADPKD. Some studies have found an increase in total body sodium and extracellular fluid volume in subjects with ADPKD with normal renal function (212–214). This observation can be explained by the upregulation of the RAAS, which contributes to sodium retention. There is also evidence for activation of the sympathetic nervous system (207). Muscle sympathetic nerve activity

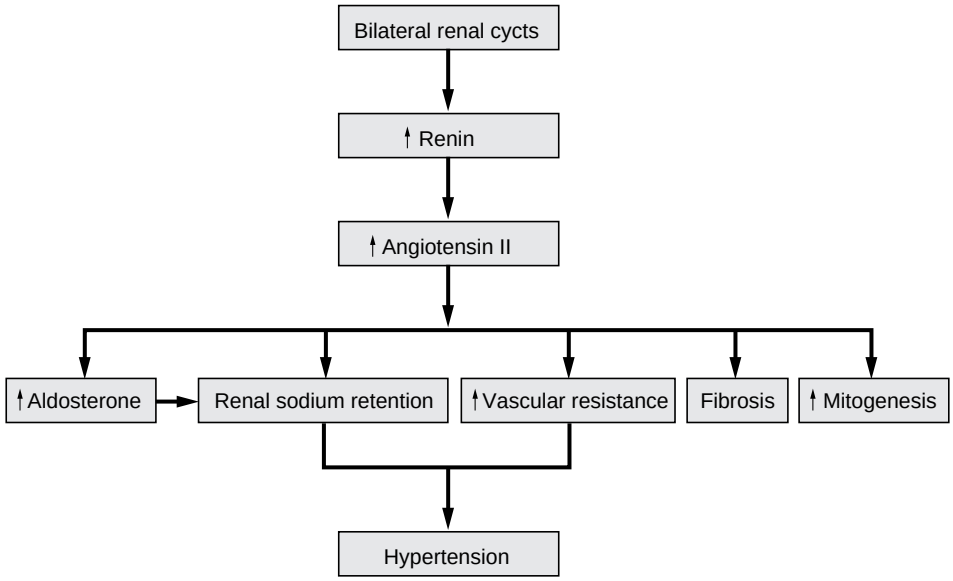


FIGURE 18-13. The effect of the RAAS in the development of hypertension and progression of ADPKD. (Adapted from: Ecder T, Schrier RW. Hypertension in autosomal dominant polycystic kidney disease: early occurrence and unique aspects. *Kidney Int* 2001;12:194, with permission.)

is increased in hypertensive ADPKD patients regardless of renal function, suggesting that sympathetic hyperactivity may contribute to the pathogenesis of hypertension in ADPKD (215).

Tissue sections from patients with ADPKD demonstrate increased expression of endothelin (ET)-1 in the renal cystic epithelium (106). ET-1 can also be detected in the cyst fluid (103). Patients with ADPKD have increased plasma levels of ET-1 compared with healthy controls and patients with essential hypertension (216). Moreover, endothelium-dependent relaxation is impaired and endothelial nitric oxide synthase (eNOS) activity is decreased in patients with ADPKD (217). These findings suggest that endothelial dysfunction secondary to an impaired release of nitric oxide exists in these patients. Therefore, alterations in the natural balance between endothelial-derived constricting and relaxing factors may contribute to the pathogenesis of hypertension in ADPKD (218,219). The recent demonstration of endothelial dysfunction and increased carotid intima-media thickness both in hypertensive and normotensive ADPKD patients with well-preserved renal function shows that the process of atherosclerosis starts very early in the course of this disease (220).

Episodes of *gross hematuria,* which can be painless or associated with dull or colicky pain, are a frequent symptom of ADPKD. Probably they are caused by cyst rupture into the urinary tract. Forty to 50% of patients with ADPKD experience at least one episode, and evaluation for gross hematuria leads to the diagnosis of ADPKD in approximately 20% of adult subjects (221). The first episode occurs at a mean age of 30 years, but 10% of adolescents younger than age 18 years also report having had gross hematuria (221). More than half of subjects report an inciting event for the appearance of gross hematuria, such as infection, sports, or trauma; for other subjects, no precipitating factor is apparent. These episodes usually last 2 to 7 days and cease spontaneously with bed rest. If they are accompanied by severe or colicky pain, renal complications need to be investigated, such as infection or obstruction by a blood clot or stone. Frequent episodes of hematuria have been associated with larger kidneys and worse renal function, but it is unclear whether this is a cause or effect phenomenon (221).

A *renal concentrating defect* occurs early in the course of the disease in patients with normal GFRs (222,223). In both children and adults, subjects with larger kidneys and more extensive cystic disease have lower maximal urinary osmolalities after overnight dehydration than subjects with smaller kidneys or healthy control subjects (8,223). The concentrating defect is thought to be due to disruption of the medullary architecture by the cysts (223), but intrinsic hyposensitivity of ADPKD collecting duct epithelia to arginine vasopressin may also play a role (224). The impairment of renal concentrating ability is mild but may lead to frequency or nocturia in some patients.

Palpable kidneys are found in some children with very–early-onset disease and in older patients in whom renal enlargement is more prominent (184,225). Sometimes the kidneys are enormously enlarged, reaching into the pelvis. Many patients report a dull ache with palpation of their kidneys. If that pain is severe, infection should be ruled out. In general, physical examination is unreliable in estimating kidney size (186,226).

Palpable *hepatomegaly* is found in 20% to 30% of patients with ADPKD older than 50 years of age, particularly in women. Systolic murmurs or *systolic clicks* are found in 14% to 21% of patients with ADPKD compared with 5% to 9% of healthy control subjects of similar age (227). They are likely related to mitral valve prolapse, which occurs more frequently in patients with ADPKD than in the general population (227,228).

Urinary Findings. The urinalysis is often abnormal in patients with ADPKD. Microscopic hematuria is found on routine urinalysis in 23% of adults (184,225) and 20% of children with ADPKD (data from the University of Colorado study). Pyuria is a common finding as well, often in the absence of an infection (30,184). Therefore, a urine culture should be obtained before prescribing antibiotics, particularly if the patient has no symptoms of an infection.

Dipstick-positive proteinuria is found in 34% to 68% of patients with decreased renal function (184,225), but overt proteinuria (>300 mg/24 hours) is uncommon in early stages of the disease. In one study of 270 adults with ADPKD with a wide range of renal function, overt proteinuria was found in only 18% (229). These were the subjects with higher blood pressures, larger renal volumes, lower creatinine clearances, and a greater pack-year smoking history. The amount of proteinuria among all subjects ranged from 7 mg/day to 2 g/day, with

a mean of 260 mg/day (229). Microalbuminuria has been reported in normotensive and hypertensive patients with ADPKD and is associated with higher mean arterial pressures and larger renal volumes (229–231). Proteinuria and microalbuminuria can also be present in children with ADPKD. In a study of 185 children with ADPKD, significant microalbuminuria was found in 30% compared with 10% of control subjects ($P < 0.05$), and overt proteinuria (>4 mg/m^2/hour) was found in 28% compared with 15% of control subjects ($P<0.01$) (232). There was no correlation between microalbuminuria or overt proteinuria and hypertension in the children, and all children had normal renal function. Thus, the significance of proteinuria and microalbuminuria in children with ADPKD is unclear.

Nephrotic-range proteinuria does not occur with ADPKD alone. Several nephrotic patients reported in the literature had a superimposed glomerulonephritis, including immunoglobulin A nephropathy (233), focal sclerosis (234), membranoproliferative glomerulonephritis (235), and membranous glomerulonephritis (236). However, doubly refractile lipid bodies, which may originate in the cysts, can be found in the urine of some patients with ADPKD (237).

Other Laboratory Findings. There are no laboratory data specific for ADPKD. Although some cysts contain high erythropoietin concentrations (238), polycythemia occurs rarely (184). Serum erythropoietin levels are on average twofold higher in patients with ADPKD with renal insufficiency than in subjects with other renal diseases, which may account for the fact that patients with ADPKD with ESRD often have less severe anemia than patients with other types of renal disease (238–240). However, other studies have found no difference in the hematocrits between patients with ADPKD and other subjects with comparable degrees of renal insufficiency (225). Many patients with ADPKD with ESRD require exogenous erythropoietin to maintain their hematocrit.

Serum uric acid levels were once thought to be higher in ADPKD than in the general population. However, this was often due to impaired renal function (184). A study in 163 subjects with ADPKD with normal renal function and 138 unaffected family members demonstrated that serum uric acid levels, uric acid clearance, and fractional uric acid excretion were not different between the two groups (241).

Renal Complications

Acute flank pain is usually a sign of a renal complication such as cyst hemorrhage, infection, or nephrolithiasis with stone colic (Table 18-4). *Cyst hemorrhage* may be associated with gross hematuria if the bleeding cyst has a connection to the ureteral system. In the absence of hematuria, cyst hemorrhage can be diagnosed by CT scan, which shows a high-density cyst without enhancement after administration of contrast material (242). Follow-up CT scans have demonstrated development of cyst calcifications in some patients (242). Renal calcifications are common, being found in 42 of 84 CT scans in one study (136). Symptomatic cyst hemorrhage is treated with bed rest, fluids, and mild analgesics. With these measures, the pain usually resolves within a few days. Rupture of a renal cyst with massive bleeding into the peritoneum has been reported in rare cases (243).

TABLE 18-4

RENAL COMPLICATIONS OF AUTOSOMAL-DOMINANT POLYCYSTIC KIDNEY DISEASE

Complications	Frequency
Cyst hemorrhage	Common
Urinary tract infections (cystitis, pyelonephritis, cyst infection)	78% of women, 33% of men, 21% of children[a]
Nephrolithiasis	20% to 36% of adults
Carcinoma	Rare
End-stage renal disease	50% by age 60 y, 75% by age 70 y[a]

[a]Data from the University of Colorado database.

Symptoms compatible with *urinary tract infections* are reported by 60% to 70% of women and 20% of men with ADPKD (186,225,226). The pathogens found most frequently are *Escherichia coli, Proteus, Klebsiella,* and other Gram-negative enteric organisms (244,245). Most episodes of urinary tract infection present clinically as cystitis and are treated in the same manner as in the general population.

More severe infections are pyelonephritis and cyst infections. The differentiation between pyelonephritis and cyst infection is not always easy. Both are characterized by fever and flank pain; however with cyst infection, the symptoms can be of insidious onset, fever can be low grade, and the patient often complains of severe malaise and localized renal tenderness. With pyelonephritis, urine cultures are positive, whereas they may be negative with cyst infections; however, blood cultures are usually positive with cyst infections (244). Failure to respond to penicillins or aminoglycosides is also indicative of a cyst infection because these antibiotics do not penetrate well into the cyst cavities (244). The penetration of antibiotics into cysts has been studied in patients with ADPKD who received these drugs before cyst aspiration (246–249). Penicillin and cephalothin derivatives, which at physiologic pH are anions, and gentamicin, a cation, did not penetrate well into cysts (246,247). By contrast, lipophilic compounds such as ciprofloxacin, norfloxacin, clindamycin, erythromycin, and trimethoprim accumulated well in renal cysts and have been clinically effective in 80% to 90% of cases (244,247–249). Therefore, patients with presumed cyst infection should be treated with ciprofloxacin or trimetho-prim–sulfamethoxazole. Treatment sometimes needs to be prolonged for 6 weeks or more to cure a smoldering cyst infection. If no response to antibiotics is seen, cyst drainage may be necessary (250). However, it may be difficult to identify the infected cyst by imaging studies. The best imaging methods are contrast-enhanced CT or MRI scanning and scintigraphy with indium-111–labeled leukocytes (250–253). Perinephric abscess may develop in patients with inadequately treated urinary tract infections (254). Emphysematous pyelonephritis has also been reported in patients with ADPKD (255,256). In rare resistant cases, nephrectomy is the best choice for eradicating infection, particularly in patients with ESRD.

Nephrolithiasis occurs in 20% to 36% of patients with ADPKD and is related to metabolic factors and to urinary stasis in a collecting system that is distorted by the cysts (136,225,257,258). Similar to the general population, the stones most commonly contain uric acid or calcium oxalate (257,259). Evaluation and treatment for metabolic factors are the same as in stone formers of the general population. The most common metabolic factor is hypocitruria, which is found in 50% of patients with ADPKD with normal renal function (257,259). Hypocitruria is usually treated with oral potassium citrate in subjects with normal or near normal renal function; it should not be used in the presence of renal failure. Extracorporeal shock-wave lithotripsy and percutaneous nephrolithotomy have been used successfully in patients with ADPKD with symptomatic stones (258,260–262).

As in any patient, particularly the elderly, acute or chronic pain or gross hematuria can be symptoms of *renal cancer.* Other

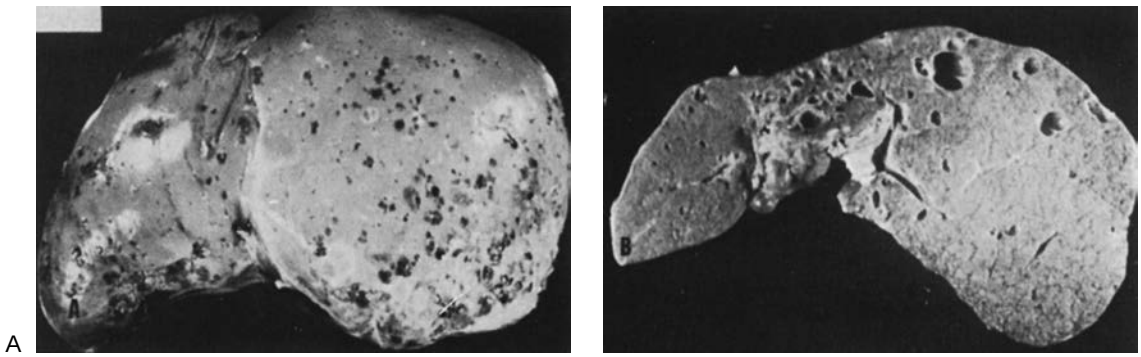


FIGURE 18-14. Polycystic liver from a patient with polycystic kidney disease. **A:** Intact liver with surface cysts. **B:** Liver cut in cross-section.

presenting signs of cancer are unilateral mass, unequal renal size, anemia, fever, night sweats, weight loss, and metastatic disease (263,264). Because the hallmark of cystic epithelium is its proliferative activity, one would expect an increased frequency of renal cancer in ADPKD. Although there are several case reports of renal carcinoma occurring in patients with ADPKD (264–268), comparison with the incidence in the general population does not reveal an increased risk in ADPKD (269–272). The diagnosis can be very difficult to establish in ADPKD because of the underlying distortion of the renal architecture. Delays in diagnosis and treatment are, therefore, common (264). Another specific feature of renal cancer in ADPKD is the increased frequency of bilateral and multicentric involvement and the occurrence of a more sarcomatoid type of tumor (264,267). As mentioned previously, the finding of renal carcinoma in several members of an "ADPKD" family or at a young age should raise the possibility of VHL disease rather than ADPKD.

Extrarenal Manifestations

Liver cysts are the most common extrarenal manifestation (Figs. 18-14 and 18-15; Table 18-1). Arising from bile ducts, they develop later in life than the renal cysts. Liver cysts are very rare in children; their prevalence increases with age and decreasing renal function, so that by 60 years of age at least 75% of patients with ADPKD have liver cysts (184,273–276). Only occasional patients who are at risk for ADPKD have hepatic cysts detected in the absence of renal cysts (184). Liver cysts seem to be as common in patients with *ADPKD2* as in those with *ADPKD1* (39,276), although they may appear later in life in patients with *ADPKD2* compared with *ADPKD1* (277).

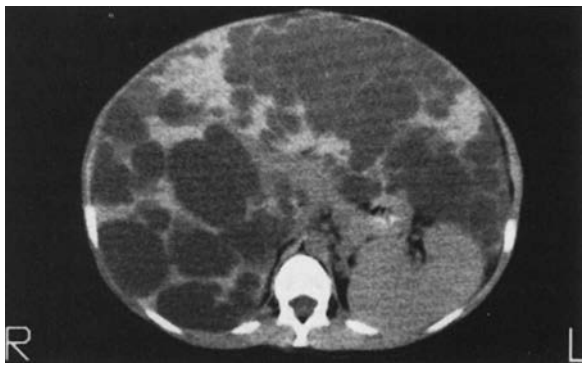


FIGURE 18-15. Computed tomography scan of the liver in a female patient with autosomal-dominant polycystic kidney disease. Results of liver function studies were normal.

Women in general have more severe liver involvement than men of similar age, with greater numbers of cysts, larger cyst sizes, and more frequent hepatomegaly (276). This is particularly true for women who had multiple pregnancies, suggesting a role for estrogens in the pathogenesis of liver cystic disease (276). A small prospective study examined the effect of postmenopausal estrogen replacement on liver cysts and found that estrogen replacement therapy for 1 year was associated with a selective increase in total liver volume but no change in kidney volume (278).

Liver cysts often remain asymptomatic through life; however, patients with more extensive liver involvement may complain of abdominal pain, the feeling of a mass, increased abdominal girth, and early satiety. Although hundreds of liver cysts can develop, routine liver function test results usually remain normal (184,275), and liver failure does not occur, unless a complication supervenes (see later). In contrast to the progressive destruction of the renal parenchyma, the liver parenchyma appears to be preserved. An abdominal CT study demonstrated that even with massive involvement with cysts, hepatic parenchyma volume remains normal, as if the cysts were simply pushing the parenchyma aside rather than destroying it (279). Similarly, sophisticated methods for assessing hepatic function and blood flow demonstrated that function remains normal and shunting does not occur (279).

Complications of liver cystic disease include cyst infection, bleeding, or anatomic obstruction of the intrahepatic bile ducts, the vena cava, or hepatic veins (Budd-Chiari syndrome). *Cyst infection* presents clinically with fever, right upper abdominal pain, leukocytosis, and often elevated liver enzymes (280–282). Associated bacteremia is common. Infecting organisms are Enterobacteriaceae, particularly *Klebsiella pneumoniae, E. coli,* or *Enterobacter cloacae* (282). In contrast to cyst infections of the kidneys, which usually can be managed with antibiotics alone, liver cyst infections require early drainage as well as antibiotics (282). This can often be accomplished by percutaneous puncture under ultrasound or CT guidance, but may require surgical intervention. Useful imaging methods for the localization of infected cysts are contrast-enhanced CT scan or MRI. Cysts with thick walls, heterogeneous contents, and sometimes gas bubbles are seen. ^{111}In-labeled leukocyte scans have been helpful in cases where CT scans were equivocal (282,283).

Cyst bleeding can be associated with right upper abdominal pain without signs of infection. Rarely, rupture of a cyst into the peritoneal cavity can lead to hemoperitoneum and an acute abdomen (284,285).

Jaundice due to compression of the intrahepatic bile ducts by large cysts has been reported; this form of obstructive jaundice resolves with surgical or percutaneous decompression of the cysts (286–288). Compression of the portal vein by cysts with resultant portal hypertension and bleeding esophageal

varices is another complication described in case reports (289,290). Again, treatment is by decompressing the cysts. Rarely, cholangiocarcinoma has occurred in patients with polycystic kidney and liver disease (274,275,291).

Hepatic venous outflow obstruction (Budd-Chiari syndrome) due to compression of the hepatic veins and inferior vena cava by large liver cysts is a serious complication with a high mortality rate, particularly when thrombosis is superimposed (292–294). Affected patients present with acute or insidious onset of abdominal pain, ascites, hepatomegaly, and nonspecific elevations of liver enzymes. Several cases occurred after abdominal surgery, particularly after bilateral nephrectomy. Presumably, a sudden drop in intraabdominal pressure after removal of the kidneys changes the spatial relationship between a large polycystic liver and the inferior vena cava and hepatic veins, resulting in venous hepatic outflow obstruction (293,294). A postoperative hypercoagulable state may also play a role. The diagnosis is best made by MRI (294,295). Aggressive surgical decompression of the liver is necessary to prevent a fatal outcome (294). If the obstruction is caused by a large, dominant cyst, alcohol sclerosis or laparoscopic fenestration may be performed (294), but more often the obstruction is caused by an enlarged liver with multiple cysts. Then, the best surgical option is combined hepatic resection and cyst fenestration (296). With superimposed hepatic vein thrombosis, either a portosystemic shunt procedure or liver transplantation should be performed (294,295).

Percutaneous, laparoscopic, or surgical decompression procedures may also be indicated for patients with massive liver enlargement resulting in severe abdominal pain, malnutrition, or respiratory compromise (284,296,297). Partial liver resection and fenestration leads to greater reduction in liver volume and better long-term results than less invasive procedures, but it carries significant morbidity and should be performed only at experienced centers (296–298). Pain recurrence after variable periods is not uncommon, particularly with the laparoscopic approach. Therefore, if all surgical procedures fail, orthotopic liver transplantation is another option; it may be combined with kidney transplantation in patients with ESRD (299–302).

Congenital hepatic fibrosis, which is invariably associated with ARPKD, has also been observed in some patients with ADPKD (303–307). Histologically, congenital hepatic fibrosis is characterized by irregularly distributed portal fibrosis and bile duct proliferation. Clinically, congenital hepatic fibrosis presents with hepatomegaly and signs of portal hypertension, such as gastrointestinal hemorrhage due to bleeding esophageal varices and splenomegaly, which can cause thrombocytopenia or leukocytopenia. Liver enzymes and function usually remain normal. Congenital hepatic fibrosis in association with ADPKD is rare. It usually manifests in childhood, sometimes in several siblings, but no vertical transmission has been observed (295).

Cysts have been reported in many other organs, including the pancreas, ovaries, testes, seminal vesicles, prostate, spleen, and arachnoid membranes; usually these cysts have no clinical sequelae. *Pancreatic cysts* occur in approximately 9% of patients with ADPKD older than age 30 years (308) and usually are single and asymptomatic. Most patients with pancreatic cysts also have liver cysts (308). Complications due to pancreatic cysts are extremely rare, but one case of chronic obstructive pancreatitis due to a pancreatic cyst in a patient with ADPKD has been reported (309). Extensive involvement of the pancreas with cysts should raise the suspicion of VHL disease (310).

Ovarian cysts are often cited as a manifestation of ADPKD, but one prospective study in 25 premenopausal women with ADPKD and 25 matched control women using transvaginal ultrasound did not find a higher prevalence of ovarian cysts in the women with ADPKD (311). Ovarian cysts were relatively common (12%) in both groups. Another prospective study using transrectal ultrasound in 45 men with ADPKD with a mean age of 40 years and in 46 matched control men revealed seminal vesicle cysts in 60% and prostate cysts in 11% of the men with ADPKD, but in none of the control subjects (312). These cysts do not appear to be of any clinical significance. Intracranial arachnoid cysts occur in 5% to 8% of patients with ADPKD compared with 1% of the general population (313–315). Although they are often asymptomatic, they have recently been associated with the development of chronic subdural hematoma (316).

Noncystic extrarenal manifestations include mitral valve prolapse, left ventricular hypertrophy, intracranial aneurysms, other vascular abnormalities, and abdominal wall hernias. The increased prevalence of these extrarenal manifestations suggests an underlying defect in extracellular matrix production with variable penetrance. *Cardiac valvular abnormalities,* including myxomatous degeneration of the aortic and mitral valves, have been reported in several patients with ADPKD, some of whom required valve replacement surgery (317). Two large, prospective echocardiography studies found mitral valve prolapse in 25% of subjects with ADPKD compared with 2% of a control population (227,228). Mitral valve prolapse can be associated with palpitations and atypical chest pain, but it does not appear to contribute to mortality in ADPKD (269,271,318).

Cardiac disease, particularly *left ventricular hypertrophy,* is very common in patients with ADPKD. Increased left ventricular mass index (LVMI) is associated with a worse renal and patient outcome in patients with ADPKD (13). A significant correlation between hypertension and left ventricular mass index has been reported both in children and in adults with ADPKD (200,319). Left ventricular hypertrophy by echocardiogram has been reported in normotensive young adults with ADPKD (319–321). However, only clinic blood pressures were measured in these patients, and elevations in the 24-hour blood pressure load may have been missed. An attenuated nighttime fall in blood pressure, resulting in an increased 24-hour blood pressure load, was seen in some patients with ADPKD when ambulatory blood pressure monitoring was used (199,322). Valero et al. reported that the nocturnal fall in blood pressure was attenuated even in young normotensive patients with ADPKD and that they had higher LVMI that was closely related to the ambulatory systolic blood pressure (199). Young normotensive patients with ADPKD are also shown to have echocardiographic parameters associated with diastolic dysfunction (320).

Insulin resistance has been found in patients with ADPKD (323,324). A recent study showed that insulin resistance, determined using the homeostasis model assessment (HOMA), was significantly associated with LVMI in healthy relatives and patients with mutations in the PKD1 gene independently of other factors known to increase LVMI, such as age, weight, systolic blood pressure and albuminuria (324).

Rupture of an intracranial aneurysm can be the most devastating of all ADPKD manifestations. The association of ADPKD with intracranial aneurysms had first been observed by Borelius in 1901 (30). Many confirmatory reports have appeared in the literature since (30,325–330). Prospective studies that have attempted to determine the exact prevalence of intracranial aneurysms in the ADPKD population by screening asymptomatic subjects with high-resolution CT, magnetic resonance angiography, and, in selected cases, conventional angiography (Fig. 18-16), have shown that 5% to 10% of asymptomatic adults with ADPKD harbor an intracranial aneurysm (313,314,331,332). However, all aneurysms found by screening were less than 8 mm in diameter, and no change was observed over a mean follow-up period of 2.5 years (333). Small aneurysms that are less than 10 mm in diameter have a very

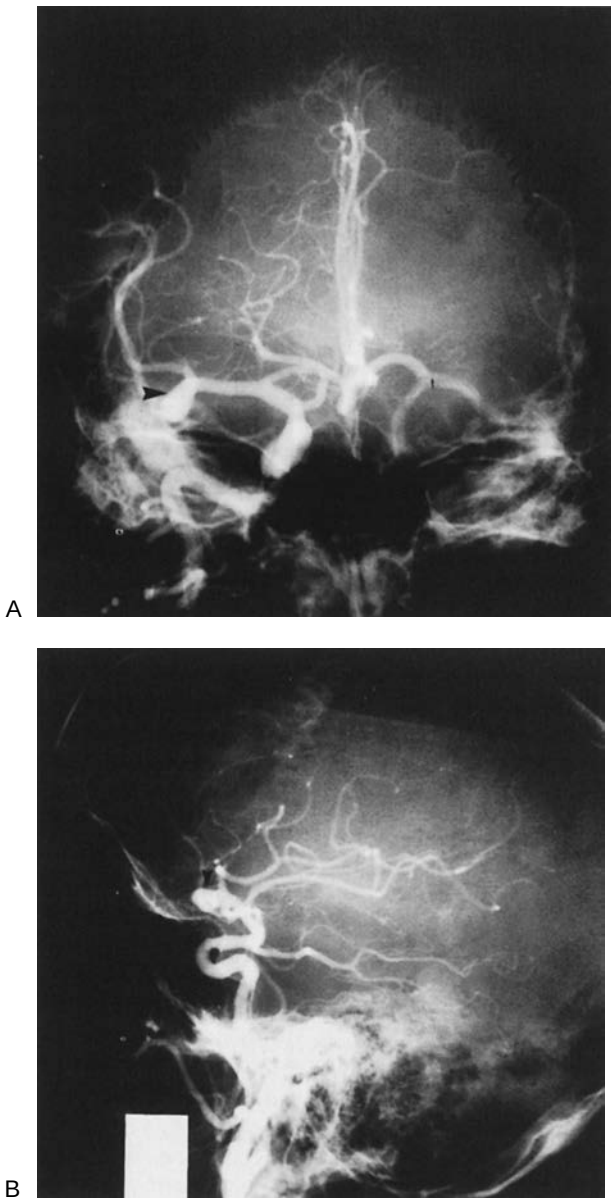


FIGURE 18-16. Cerebral angiogram with aneurysm. Right internal carotid angiogram in a patient with autosomal-dominant polycystic kidney disease and a berry aneurysm. *Arrows* indicate a large saccular middle cerebral artery aneurysm as seen in the anterior (**A**) and lateral (**B**) views.

low risk of rupture (annual risk <0.05%), at least in the general population without ADPKD and in subjects without a previous aneurysm rupture (334). The International Study of Unruptured Intracranial Aneurysms (ISUIA) has shown that the risk of rupture is higher (0.5% per year) in patients with a previously ruptured aneurysm and for aneurysms that are 10 mm or more in diameter (334). The risk of rupture of these larger aneurysms is approximately 1% per year (334). However, the risks of surgery are substantial as well: the ISUIA reported a mortality rate related to elective surgery of 1% to 4% at 1 year and a morbidity rate of approximately 15% at 1 year. Therefore, small aneurysms usually are not considered an indication for elective clipping. Whether these observations from the general population apply to patients with ADPKD as well is not known.

Rupture of an intracranial aneurysm (ICA) seems to cluster in certain ADPKD families (314,329,332,335–337). Familial clustering of aneurysms has been observed in the general population as well. Aneurysms rupture at a younger age in patients with ADPKD (mean age, ~40 years) than in the general population (330,335–337). Multiple aneurysms are found in 20% to 30% of ADPKD patients with an aneurysm (314,331,335). Recurrent rupture of a second aneurysm has also been observed: In one study, recurrent rupture of a second aneurysm occurred in 9% of patients, between 2 days and 14 years after the first rupture (337,338). Other risk factors for intracranial aneurysms are cigarette smoking and uncontrolled hypertension. However, aneurysm ruptures also occur in normotensive subjects with ADPKD (337).

Although it is evident that patients with symptoms suggestive of an aneurysm, such as unusually severe headaches or focal neurologic deficits, need immediate investigation, there is considerable controversy about the need to screen asymptomatic subjects with ADPKD for intracranial aneurysms. A decision analysis performed in 1983 led to the conclusion that cerebral angiography should not be performed routinely because the risks of angiography and elective surgery outweighed the benefits of preventing rare ruptures (339,340). The ISUIA supports these conclusions (334). However, patients with ADPKD with a previous rupture and patients from families with ruptured aneurysms have an increased risk of aneurysm rupture (341). A screening study showed that 5 (25%) of 20 patients with ADPKD with a previous ICA developed a new ICA during the follow-up period of 6 to 33 years (342). On the other hand, in a recent prospective 10-year follow-up study, only 2 (2.6%) of 76 patients with ADPKD with an initially negative ICA imaging study demonstrated an ICA on follow-up, despite the high frequency of risk factors such as hypertension, smoking, and a family history of ruptured ICA (343). Therefore, current recommendations for screening of asymptomatic subjects with ADPKD are to screen all patients with a family history of ruptured aneurysm and those with a previous rupture. Additional recommendations are to screen subjects with high-risk occupations (e.g., pilots) and patients who need screening for peace of mind (336,337,344,345). The best screening method appears to be magnetic resonance angiography, which is noninvasive and does not use contrast material (314,332,346,347). Screening of high-risk subjects should start at 18 years of age and be repeated every 5 to 10 years, unless older age or other conditions limit life expectancy (337,345).

Not all central nervous system events in patients with ADPKD are due to a ruptured aneurysm. Studies on cerebrovascular accidents in ADPKD have shown that ischemic strokes and transient ischemic attacks occur at least as frequently as hemorrhagic events (348,349). In addition, hemorrhagic events are more often due to hypertensive intracerebral hemorrhage than to subarachnoid hemorrhage from a ruptured aneurysm, particularly in patients on renal replacement therapy (270,348–351). The long-standing and sometimes inadequately controlled hypertension of patients with ADPKD contributes importantly to the higher incidence of cerebrovascular death in patients on dialysis with ADPKD than in those without ADPKD (271,351,352).

Other rare vascular abnormalities in ADPKD have been described in case reports. They include aneurysms of the coronary arteries (353–355) and splenic artery (356). Head imaging studies and autopsies have also revealed intracranial arterial dolichoectasia in patients with ADPKD (357,358). Spontaneous cervical and vertebral artery dissections and one spontaneous coronary artery dissection resulting in a myocardial infarction have been reported in several patients with ADPKD (359–361). Annuloaortic ectasia and ascending aortic dissections have occurred in a few patients with ADPKD (317,360,362,363), but it is unclear whether these diagnoses are more frequent in ADPKD than in the general population.

Case reports had suggested an association of ADPKD with abdominal aortic aneurysms (364), although a prospective ultrasound study in 139 patients with ADPKD and 149 control subjects did not find a higher prevalence in ADPKD (365).

Abdominal wall hernias occur with increased frequency in patients with ADPKD, particularly inguinal hernias, but also incisional and umbilical hernias (366). Development of hernias is not simply caused by increased intraabdominal pressure due to enlarged kidneys, because they occur at all ages and without correlation to kidney size (366). In a retrospective study of 85 patients with ADPKD with ESRD, 18 of 41 hernias were detected before the diagnosis of the renal disease, 12 of them in childhood (366). The overall incidence of hernias was 45% in the patients with ADPKD compared with 8% in a sex- and age-matched group of subjects without ADPKD undergoing similar renal replacement therapy (366).

Complications from *colonic diverticula* in patients with ADPKD have been reported in many case series (367–372). Most of these complications occurred in hemodialysis or renal transplant recipients, and patients with ADPKD appear to be overrepresented compared with patients without ADPKD in these mostly retrospective series. One prospective study of 55 non-ESRD subjects with ADPKD older than 40 years of age did not find a higher prevalence of colonic diverticula compared with an age-matched control population. Diverticula were present in 47% of the subjects with ADPKD and in 58% of the control subjects, which was not significantly different (373). This is in agreement with another study in transplant recipients with ADPKD who did not have a higher prevalence of diverticulosis or a higher incidence of complications than the general renal transplant population (374).

Pregnancy in Autosomal-Dominant Polycystic Kidney Disease

The question of fertility and the influence of pregnancy on both the mother and the fetus are important issues in counseling women with ADPKD. Because of reports of ovarian cysts in women with ADPKD (30) and immotile sperm in some men with ADPKD (375), the question of fertility in ADPKD is relevant. A large study of pregnancy outcomes did not find a difference in fertility between men and women with ADPKD and unaffected family members (376).

That study also assessed maternal and fetal complications of pregnancy, comparing 235 women with ADPKD with 108 unaffected female family members (376). Overall, 77% of the 605 pregnancies in the women with ADPKD resulted in a live birth, compared with 82% of 244 pregnancies in unaffected family members, which was not significantly different. The frequency of fetal complications was similar as well. However, maternal complications, mostly new or worsening hypertension, preeclampsia, and edema, were more common in the women with ADPKD than in the unaffected women (35% vs. 19%; $P < 0.001$). Whereas pregnancies in normotensive women were usually uncomplicated, preexisting hypertension was the most important risk factor for a maternal complication. There was no increased risk for infection: uncomplicated urinary tract infections were diagnosed in only 5 of 235 affected compared with 4 of 108 unaffected women. A renal cyst infection or acute symptoms due to nephrolithiasis occurred in three women with ADPKD. Renal function remains normal in most women with ADPKD during their childbearing years. However, having four or more pregnancies seems to exert a negative effect on renal function in women with ADPKD who are hypertensive (376). Rarely, women with ADPKD have decreased renal function before pregnancy. In these women, the risk for a complication and for accelerated progression to ESRD is high (376). This is similar to other renal diseases, in which underlying renal insufficiency carries a relatively high risk of worsening renal function during and after pregnancy.

Autosomal-Dominant Polycystic Kidney Disease in the Elderly

Although ADPKD can be diagnosed by imaging studies in children and often becomes symptomatic in the third and fourth decades of life, some patients remain asymptomatic until their seventh and eighth decades. Asymptomatic older subjects may be evaluated for an elevated serum creatinine or because their children are diagnosed with ADPKD. Some of these elderly subjects have unrecognized complications such as hypertension and renal insufficiency and progress to ESRD, whereas others truly have mild disease without progression to ESRD (226). Mild disease with late presentation is characteristic of *ADPKD2*, but some *ADPKD1* families also have a mild course (377,378). In *ADPKD1* families, late presentation occurs in some parent generations, whereas their children have significantly more severe disease (39,170,379). Thus, an unexpected finding of multiple bilateral renal cysts in a patient aged 70 years or older with normal or only mildly decreased renal function is entirely compatible with a diagnosis of ADPKD. The oligosymptomatic nature of ADPKD in some patients is consistent with the epidemiologic data from Olmstead County, Minnesota, in which one half of all subjects with ADPKD were diagnosed only at autopsy (31).

Risk Factors for End-Stage Renal Disease and Treatment Strategies

Approximately 50% of patients with ADPKD survive without ESRD beyond 58 years of age, and approximately 23% survive without ESRD beyond 70 years of age (11–13). This highly variable rate of progression makes it difficult to predict the prognosis in a given patient. If a patient is from an *ADPKD2* family, the prognosis is much better than for patients from *ADPKD1* families, but usually the genotype of the family is not known. Longitudinal studies of a large number of patients with ADPKD have identified several risk factors associated with worse renal function at a given age. These factors include hypertension, increased left ventricular mass, male sex, a younger age at diagnosis, three or more pregnancies, episodes of gross hematuria, renal size expressed as renal volume, a younger age at incipient renal failure, a history of hypertension in the unaffected parent before age 60 years, and urinary tract infections in men (13,380) (383). A retrospective analysis of 62 patients with ADPKD who had enrolled in a prospective trial of protein restriction showed a strong association between hypertension and progression to ESRD (384). Survival analysis for 1,215 subjects with ADPKD revealed that people who were diagnosed with ADPKD before age 30 years and those with hypertension before age 35 had worse renal survival than those who were diagnosed after age 30 and those who were normotensive until after age 35 years. Median renal survival was 14 years longer for subjects who were normotensive at age 35 years compared with the hypertensive patients (385). A combination of several risk factors predicts a particularly high risk. These analyses allow physicians to identify patients at high risk for progression to ESRD so they can follow them more closely and treat any associated conditions such as infections aggressively. A longitudinal study in 229 patients with ADPKD who underwent renal ultrasound examination showed a significant relationship between between rate of change in GFR and renal volume growth rate (386). This suggests that renal volume and rate of renal volume growth may be useful markers for disease progression in early stages of ADPKD when GFR is preserved. A recent study showed that renal

blood flow measured by MR correlated with anatomic and functional indices of disease severity and this may be the most sensitive measurement that predicts GFR levels in patients with ADPKD (387).

Apart from these clinical risk factors, the genetic background of a person likely influences the severity of the polycystic phenotype. There is evidence for "modifier" genes in several animal models (388,389). A candidate modifier gene for human ADPKD is the insertion/deletion (I/D) polymorphism of the angiotensin-converting enzyme gene. While some studies found that ADPKD patients with ACE DD genotype showed faster progression to ESRD (390,391), others could not find an association (392–396). Another modifying gene present in African Americans could be the sickle-cell gene, because in one study African-American patients with ADPKD with sickle-cell trait (AS hemoglobin) had significantly earlier onset of ESRD than those without (38 vs. 48 years; $P < 0.003$) (32). In the same study, black race *per se* was associated with earlier onset of ESRD, but this was not the case in another larger study: Mean age at onset of ESRD was 65 years in 57 African Americans and 62 years in 180 whites (34).

Despite the importance of hypertension in renal and patient outcome in ADPKD, there have been few clinical trials to examine the role of antihypertensive therapy in preventing or slowing the progression of renal and cardiovascular complications. The Modification of Diet in Renal Disease study, which included 200 patients with ADPKD, evaluated patients with GFRs between 13 and 55 mL/minute/1.73 m^2 at baseline, and found no protective effect of a low-protein diet or of strict blood pressure control compared with standard control over a mean period of 2.2 years (397,398). It is likely that intervention at such an advanced stage of ADPKD is less effective because the structural changes have progressed for four to five decades and are irreversible.

In a randomized prospective 5 year study of 24 patients with well-preserved renal function, blood pressure control less than 140/90 mm Hg treated with either a long-acting calcium channel blocker, amlodipine, or an ACE inhibitor, enalapril, was associated with a mean annual loss of renal function of 3.4 mL/min/1.73 m^2 (399). In this study, ACE inhibition, but not treatment with a calcium channel blocker showed a sustained antialbuminuric effect. In another randomized study in ADPKD patients with well-preserved renal function, a normotensive cohort was randomized to enalapril or placebo and a hypertensive cohort was randomized to enalapril or atenolol and followed for 3 years (400). No difference in loss of renal function was detected in either the hypertensive or normotensive patients with ADPKD. In a 5-year nonrandomized historical prospective study, patients receiving ACE inhibitors had a slowed loss of renal function and less urinary protein excretion compared to patients receiving diuretics (401).

Early and effective treatment of hypertension is also important for the prevention of cardiovascular complications. A 7-year prospective randomized study in 75 hypertensive ADPKD patients with LVH compared the effects of rigorous (<120/80 mm Hg) versus standard (135–140/85–90 mm Hg) blood pressure control on LVH and renal function (402). Both standard and rigorous blood pressure control decreased LVH significantly. However, significantly more subjects in the rigorous blood pressure group (71%) than in the standard blood pressure group (44%) achieved normal LCMI. Moreover, patients receiving an ACE inhibitor, enalapril, had greater benefit from rigorous blood pressure control compared with patients receiving a calcium channel blocker, amlodipine. Therefore, a blood pressure goal of less than 120/80 mm Hg and inhibition of the RAAS should be recommended for hypertensive ADPKD patients with LVH.

A recent epidemiological study compared blood pressure control and renal survival in 513 ADPKD patients studied over two separate periods, 1985–1992 and 1992–2001. The patients seen in the latter period had a significantly lower blood pressure and longer renal survival and increased use of ACE inhibitors compared to the earlier period (403,404). Education programs for patients with ADPKD and their primary care physicians could have contributed to this increased rate of blood pressure control.

Several treatment studies have been performed in experimental animals. The model that closely resembles the human disease is the Han:SPRD rat, which shows autosomal dominant inheritance, a slowly progressive disease course in males, and very mild disease in females, in whom liver and pancreatic cysts also develop (128,405,406). Different interventions have been studied in male Han:SPRD rats, and all treatments were started early (i.e., at 3 to 4 weeks of age). Interventions were dietary modification, blood pressure control, and administration of potassium citrate or other drugs. Dietary modification was aimed at reducing cyst volume and interstitial inflammation. Protein restriction for 4 months in male cystic rats led to a significant decrease in cyst volume and serum creatinine and urea levels compared with cystic rats fed a high-protein diet (407). Substitution of soy protein for casein for 8 or 12 weeks was associated with reduced renal cyst volume, reduced fibrosis, and reduced macrophage infiltration (408–410). Administration of flaxseed showed similar results (411). Lovastatin treatment was used in cystic rats to reduce tubular cell proliferation by interference with the Ras proteins (412). This intervention resulted in a significant reduction of kidney size, cyst volume density, and serum urea levels (413). To test the hypothesis that interstitial inflammation plays an important pathogenetic role, high-dose methylprednisolone was administered to young cystic rats for 6 weeks; this treatment decreased the extent of renal enlargement, reduced interstitial fibrosis, and preserved renal function (414).

Other studies focused on blood pressure control in cystic Han:SPRD rats. The administration of an ACEI or of an angiotensin II receptor blocker showed beneficial effects after 3 to 4 months of treatment, with reduction of cyst volume, histologic severity score, and serum creatinine level compared with untreated cystic rats (415,416). In a long-term study, however, enalapril but not hydralazine limited renal enlargement, but both drugs were associated with improved renal function after 9 months of treatment (417). Conversely, induction of hypertension by sodium chloride loading of cystic rats for 2 months was associated with significantly higher relative kidney weights and higher histologic scores (415).

Because induction of acidosis by ammonium chloride loading accelerated progression of the disease in experimental rats (418), Tanner administered potassium citrate and citric acid to cystic rats by drinking water, starting at 1 month of age and continuing for 5 months. The decline in GFR seen in untreated cystic rats was completely prevented in rats given potassium citrate. This was accompanied by smaller cysts and fewer interstitial abnormalities on histologic examination of the kidneys (419). Unfortunately, no benefit was seen in other animal models of PKD, such as the pcy/pcy mouse (420). No studies of potassium citrate have been performed in humans.

A novel approach targets the neoplastic nature of cystic epithelial cells and aims at inhibiting epithelial hyperproliferation. It is based on the evidence that EGF receptor is overexpressed and mislocalized to the apical membranes of cysts in human ADPKD, human ARPKD, and in animal models of both ADPKD and ARPKD. Activation of this receptor can drive cellular proliferation and cyst expansion (95,100,107,421). Inhibition of EGF receptor activity by a tyrosine kinase inhibitor decreased cyst progression in an organ culture system (422). In other experiments, a tyrosine kinase inhibitor administered to a murine model (BPK mice) resulted in a marked reduction of renal cystic lesions and of biliary duct proliferation, improved

renal function, and increased life span (423). However, a recent study showed showed that EGF receptor tyrosine kinase inhibition was not protective in a rat model (the PCK rat) for ARPKD (424).

A recent study on Han:SPRD rat model of ADPKD showed that rapamycin, which is a potent antiproliferative agent, decreased proliferation in cystic and noncystic tubules, markedly inhibited renal enlargement and cystogenesis and prevented the loss of kidney function (425). This exciting finding needs to be tested in other animal models of PKD and even in future clinical studies.

Since cAMP has a major role in cystogenesis and the vasopressin V2 receptor (VPV2R) is the major cAMP agonist in the collecting duct, a VPV2R antagonist, OPC31260, was administered to animal models of ARPKD. This treatment lowered renal cAMP, inhibited disease development, and either halted progression or caused regression of established disease (426).

Taken together, these studies show that modification of the disease course is possible, and that progression to ESRD will be significantly reduced if antiproliferative or antiinflammatory treatments with low toxicity can be developed. In the meantime, it appears advisable to control blood pressure optimally, treat infections promptly, avoid activities that predictably result in gross hematuria in a given patient, and add soy protein and flaxseed to the diet, given their general health benefits and possible benefits for PKD. The optimal agent to control blood pressure is not known, but current evidence supports the use of ACEI or angiotensin II receptor blockers as the drugs of choice because of their interference with the RAAS, which is activated in ADPKD. Suppression of the RAAS has been shown to decrease the expression of growth-related and inflammatory cytokines in experimental animals and in preliminary studies in humans (427–431). As mentioned in the section on pathogenesis, inflammatory cytokines appear to play a significant role in the renal interstitial scarring process, which ultimately leads to renal failure in ADPKD. The impact of heavy use of nonsteroidal analgesics on the progression of ADPKD has not been examined, but it is generally recommended that patients avoid nephrotoxic insults, including regular use of nonsteroidal analgesic drugs.

Renal Replacement Therapy in Autosomal-Dominant Polycystic Kidney Disease

When patients with ADPKD reach ESRD, treatment options include hemodialysis, peritoneal dialysis, and renal transplantation. The latter is the treatment of choice if no contraindications to surgery or immunosuppressive therapy are present. Patient and graft survival with ADPKD are similar to survival in other primary renal diseases, excluding diabetic patients, who do worse (318,369,432–434). Pretransplantation native nephrectomy usually is not necessary (435). Exceptions are patients with a history of recurrent or recent severe renal infections, with recurrent gross hematuria, or when graft placement is difficult because of the size of the cystic kidneys (434,436). Pretransplantation evaluation should include assessment of cardiovascular status, but routine screening for colonic diverticula or intracranial aneurysms is not necessary because complications from these manifestations after transplantation are uncommon in previously asymptomatic subjects (318,337,344,374,434,435). However, one study recommends that patients with a history of diverticulitis consider colectomy before renal transplantation (369). Similarly, patients with a previously ruptured intracranial aneurysm or a family history of ruptured aneurysm should be screened for intracranial aneurysms (see earlier). During the first year after transplantation, patients with ADPKD may experience one complication more frequently than other patients, namely posttransplantation erythrocytosis. In one report, 14% of patients with ADPKD required phlebotomy compared with 4% of control subjects ($P = 0.02$) (369).

Peritoneal dialysis can be offered to patients who do not have extremely large kidneys, diverticulosis, or abdominal wall hernias. Outcomes are comparable with those in patients with other underlying renal diseases, and no excess complications due to the polycystic kidneys were noted in several reports (35,436,437). Hemodialysis is usually well tolerated by patients with ADPKD, and they appear to have a survival advantage over patients without ADPKD on dialysis, particularly at older ages (433). A study that analyzed data from the United States Renal Data System (USRDS) found a lower mortality rate in ADPKD ESRD patients compared with nondiabetic control ESRD patients (438). This has been attributed to a lower prevalence of severe coronary disease in ADPKD (433), although cardiovascular disease remains the most common cause of death among patients with ADPKD on renal replacement therapy (269,271,318,348,349,439). Specific complications in patients on dialysis related to ADPKD include renal pain, infection, recurrent gross hematuria, and, rarely, liver cyst infection (274,282,318). These complications can usually be managed conservatively (434,440), but in some cases unilateral or bilateral nephrectomy may become necessary. This can now be accomplished laparoscopically, which minimizes operative risk (193). The kidneys decrease in size during dialysis in some (441) but not all patients (442,443), and in some patients, they further increase in size, suggesting superimposed acquired cystic disease (272,444,445). Renal carcinoma was rare in all studies that followed patients with ADPKD on renal replacement therapy or after transplantation.

Counseling and Screening

Genetic counseling is an important aspect of the care of the patient and family with ADPKD. The newly diagnosed patient should be informed about ADPKD in general and the hereditary nature of the disease in particular. He or she should be told that each child of an affected person has a 50% chance of inheriting the disease gene. Each at-risk family member should be informed of the methods of diagnosis and of the availability of prenatal diagnosis. However, before a diagnostic test is performed, every subject also needs to be informed about the consequences of diagnostic screening, particularly regarding insurability (446), to permit informed judgment.

Screening in children requires special consideration. Insurability is a major concern, but there may be an additional psychological burden on the child and the parents. Our current understanding of these issues suggests that children participate in the decision for screening to the degree that their age permits (447). In general, screening outside of research protocols should be performed only if necessary for clinical decisions, such as whether to treat mildly elevated blood pressure, or for evaluation of symptoms, such as gross hematuria or pain. For these clinical questions, ultrasound imaging is the appropriate test. Genetic testing should not be performed in a child, because it does not give any clinical information. Genetic testing is appropriate for reproductive decision making or before kidney donation if ultrasound imaging is negative. Asymptomatic children who are not screened should have their blood pressure monitored at least yearly. If their systolic or diastolic blood pressure consistently exceeds the 95th percentile for age- and sex-matched children, evaluation is appropriate (8).

AUTOSOMAL RECESSIVE POLYCYSTIC KIDNEY DISEASE

Epidemiology and Genetics

Autosomal-recessive polycystic kidney disease is a rare disorder. The exact incidence is not known. Estimates range from 1:6,000 to 1:55,000, with a more recent estimate of 1:20,000 (14,17,448). These figures are from studies in populations of European descent. ARPKD is said to be rare in the African American population, but this is not well established (164). ARPKD is a severe disease, usually manifesting in newborns, infants, or children. Most of the severely affected newborns with massively enlarged kidneys and a history of oligohydramnios *in utero* have pulmonary hypoplasia and die of respiratory insufficiency shortly after birth (449). An estimated 30% to 50% of children with ARPKD are thus severely affected (164). Infants who survive the neonatal period have a mortality rate of 9% to 24% in the first year of life (16,450). Children who survive the first year of life have a relatively good prognosis, with a survival probability of 80% beyond 15 years (16,450).

The genetic defect in ARPKD has been localized to the *PKHD1* gene on chromosome 6p21.1-p12 (451). *PKHD1* is a very large gene of approximately 470 kb, containing 67 exons. The *PKHD1* gene is expressed at high levels in the fetal and adult kidney and at low levels in the liver and pancreas. The *PKHD1* gene encodes a protein called fibrocystin (polyductin), which contains 4074 amino acids (452,453). Fibrocystin is an integral membrane protein with a large extracellular region, a single transmembrane domain and a short cytoplasmic tail. Fibrocystin may act as a receptor with critical roles in collecting-duct and biliary development (454). Fibrocystin is localized to the primary cilia of renal epithelial cells. In tubular epithelial cells, cilia extend into the lumen and may have a mechanosensory role or a function as tubule size sensor (455).

Two recent studies reported mutation detection rates of 51% and 61% in a large cohort of ARPKD patients (456,457). Amino acid substitutions were found to be more commonly associated with a nonlethal presentation, whereas chain truncating mutations were more commonly associated with perinatal or neonatal death (458).

Because inheritance is autosomal recessive, each parent of an affected child carries one mutated allele but is clinically unaffected. The risk of disease for each sibling is one in four, and the risk of being a carrier is one in two.

Pathology

Autosomal-recessive polycystic kidney disease affects both the kidneys and the liver. Macroscopically, the kidneys are often enlarged but retain a normal reniform shape. Cut section reveals radially arranged cystic spaces extending from the cortex to the medulla (Fig. 18-17). Histologic characteristics are fusiform cystic dilatations of the cortical and medullary collecting ducts. During fetal development, there appears to be a transient phase of proximal tubular cyst formation, which disappears after the 26th week of gestation (459). Whereas in neonates and young children the kidneys are almost always grossly enlarged, they tend to regress in size in older children and particularly with progressive renal insufficiency. At the same time, the collecting duct cysts become less uniform and can enlarge to several

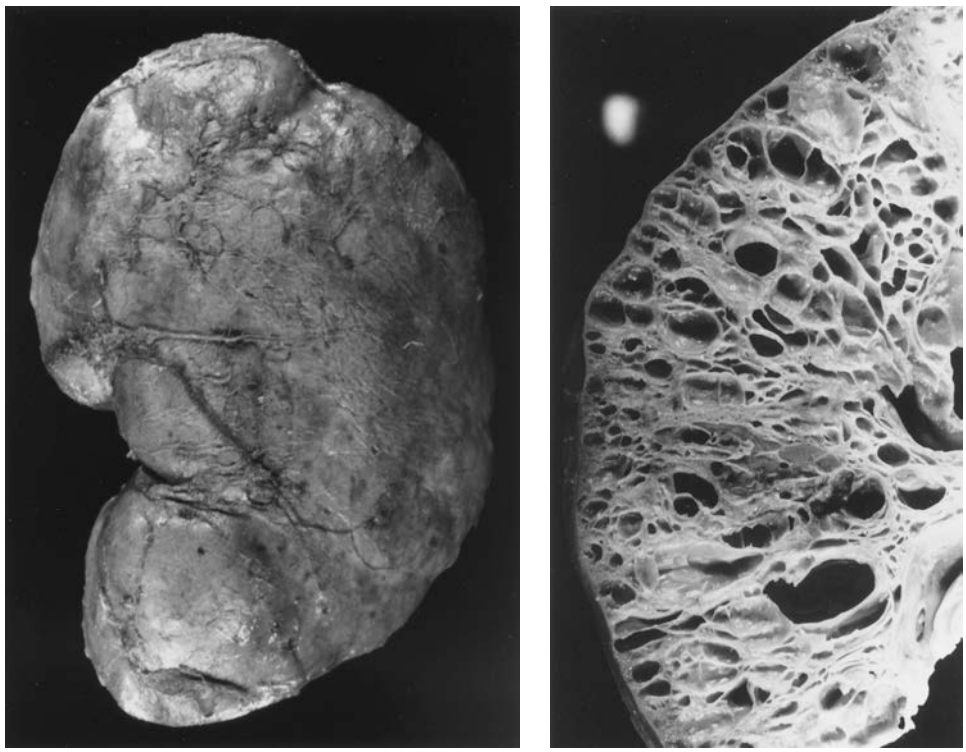


FIGURE 18-17. Gross appearance of external surface (**A**) and cut section (**B**) of a kidney with autosomal recessive polycystic kidney disease. There is little distortion of the external surface, but the cut section shows innumerable fusiform cysts in a radial distribution. (Courtesy of B. Hammond, MD, University of Colorado Health Sciences Center, Denver, CO.)

centimeters in diameter, so that the ultrasound appearance is sometimes indistinguishable from that in children with ADPKD (450). Older children and adults can have mildly enlarged, normal size, or relatively small kidneys with tubular atrophy, interstitial fibrosis, and multiple small cysts (10,450,460).

The liver is always affected on histologic examination, which shows varying degrees of congenital hepatic fibrosis. Congenital hepatic fibrosis is characterized by increased fibrous tissue in the portal tracts and proliferation of small bile ducts. Gross cystic dilatation of the intrahepatic bile ducts, called *Caroli's disease,* can also be seen. Both renal and liver histologic changes have been detected in fetuses as early as 13 weeks' gestation (448). Progressive congenital hepatic fibrosis typically leads to portal hypertension with hepatosplenomegaly and esophageal varices. Caroli's disease is often complicated by episodes of cholangitis.

Diagnosis

A clinical diagnosis is made when the child presents with typical clinical and ultrasonographic findings and both parents have a negative ultrasound after age 30 years, thus excluding ADPKD. Typical ultrasound findings are enlarged, echogenic kidneys with loss of corticomedullary differentiation, giving the appearance of a "pepper and salt" pattern (450,461), combined with signs of hepatic fibrosis and splenomegaly. However, clinical and sonographic features often overlap with those of ADPKD, particularly in older children (462). If the parents are not available for ultrasound imaging or have normal kidneys by ultrasound, the question arises whether the child has ARPKD or a new mutation for ADPKD. If clarification of the diagnosis is needed, histologic examination of a kidney or liver biopsy specimen can usually distinguish between the two diseases.

A newer, noninvasive method of diagnosing ARPKD has been described using MRI. A special MRI protocol, called *rapid acquisition with relaxation enhancement* (RARE-MR), can be used to image the whole urinary tract in a few seconds, which avoids the use of contrast material or radiation (461). In ARPKD, the RARE-MR images show enlarged kidneys with a hyperintense radial signal pattern in cortex and medulla, corresponding to the water-filled, dilated collecting ducts; a few small subcapsular cysts may also be seen (461). Contrast-enhanced CT scanning may also show these characteristics (Fig. 18-18), but is much less desirable to use in infants because of the need for contrast material and significant radiation exposure.

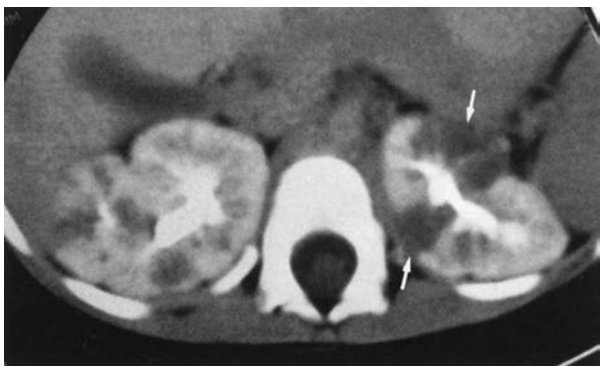


FIGURE 18-18. Autosomal-recessive polycystic kidney disease. Contrast-enhanced computed tomography scan shows both kidneys to be enlarged. The renal medulla shows a "streaky" appearance owing to tubular ectasia. There is less marked involvement of the renal cortex, and a mantle of preserved cortex is seen bilaterally. The left kidney shows some larger cysts (*arrows*). (Courtesy of E. Levine, MD, PhD, Kansas University Medical Center, Kansas City, KS.)

Prenatal diagnosis is possible with linkage analysis if DNA from a previously affected sibling is available to determine the disease-associated haplotype for that family (448). The most critical point regarding the reliability of molecular diagnosis is a correct diagnosis in the previously affected child (448). Prenatal ultrasound is much less reliable than linkage-based diagnosis because detectable abnormalities usually do not occur until after 20 weeks of gestation, and both false-positive and false-negative findings have been reported (463–465). Abnormal prenatal ultrasound findings include enlarged, hyperechogenic kidneys with or without oligohydramnios.

Clinical Course and Treatment

Autosomal-recessive polycystic kidney disease usually manifests in childhood, most commonly in neonates or in the first year of life. Neonates typically present with abdominal masses and respiratory insufficiency due to pulmonary hypoplasia. Many of these most severely affected children die shortly after birth (449). Of 115 children who survived the neonatal period, 10% had been diagnosed prenatally by linkage analysis or abnormal ultrasound, 41% were diagnosed during their first month of life, 23% during their first year, 16% between their second and fifth year, and 10% after 5 years of age (450). Occasionally, ARPKD is diagnosed for the first time in a young adult (10,466). The symptoms that most often lead to the diagnosis in young children are palpable abdominal masses, hypertension, and urinary tract infections. Hypertension is found in approximately 70% of children with ARPKD who have survived the perinatal period. Hypertension is often severe, requiring drug treatment at a median age of 6 months (16,450,462). Urinary tract infections occur in 30% to 40% of affected children (462). Decreased GFR is observed in 72% of children, often starting in the first year of life and slowly progressing to ESRD (450). Growth retardation is observed in 25% of children and correlates with renal insufficiency (462). In older children with less severe renal disease, complications of portal hypertension such as variceal hemorrhage or pancytopenia from hypersplenism may predominate (164,450,466,467). Both the renal and the hepatic manifestations of disease progress with age at variable rates. Whereas the kidney disease terminates in ESRD, which can occur from birth to adulthood, liver function usually remains normal despite the presence of portal hypertension (450). Renal transplantation is the treatment of choice for ESRD due to ARPKD (466). The liver disease may remain clinically asymptomatic, and complications of portal hypertension can often be controlled with sclerotherapy or shunt surgery (466). However, liver transplantation may become necessary in some patients to relieve severe consequences of portal hypertension. Combined liver–kidney transplantation is an option for patients with severe portal hypertension and ESRD.

Associated Disorders

Ehlers-Danlos syndrome type IV was diagnosed at postmortem in an 11-year-old girl with previously known ARPKD who died of a dissecting thoracic and abdominal aortic aneurysm (467). Another report describes the occurrence of multiple (four) intracranial aneurysms in a 31-year-old woman with ARPKD who had received a kidney transplant 2 years earlier (468). Whether these cases represent the simultaneous occurrence of two separate disorders or rare vascular manifestations of ARPKD is not known.

Counseling

As in all autosomal-recessive disorders, the recurrence risk for each sibling of an affected child is 25%. The disease is not transmitted vertically. Because ARPKD is a severe disease with high morbidity and mortality rates in infancy, there is a strong demand for prenatal diagnosis in families who have already had an affected child (448,449). Linkage analysis should be offered to such families in subsequent pregnancies. However, linkage analysis is informative only if adequate DNA from the previously affected child is available. If the fetus, whose DNA can be obtained by chorionic villous sampling at 9 to 11 weeks' gestation, has the same disease-associated haplotype as the previously affected child, the fetus is predicted to be affected, and many parents have chosen termination of the pregnancy (448). The disease severity tends to be similar within families (14,448), which should be taken into account when counseling individual families.

TUBEROUS SCLEROSIS COMPLEX

Definition

Tuberous sclerosis complex is a systemic hereditary disease characterized by hamartomas in multiple organ systems. It has an autosomal-dominant inheritance with high penetrance and an estimated incidence ranging from 1:6,000 to 1:10,000 (469–471). However, 60% to 70% of cases report no family history and are thought to represent new mutations (469,470). The disease shows extreme phenotypic variability, even within families. Some patients are severely affected with epilepsy and mental retardation, whereas others, probably most patients, are of normal intelligence (472).

Genetics

Tuberous sclerosis complex is genetically heterogeneous, with two genes being responsible for the disease. The *TSC1* gene is located on chromosome 9 (9q34), whereas the *TSC2* gene is located on chromosome 16 (16p13), close to the *PKD1* gene (473). Both TSC genes have been identified (41,474). They are tumor suppressor genes, and somatic loss or intragenic mutation of the corresponding wild-type alleles is seen in the hamartomas (475–478). The *TSC1* gene codes for a protein named *hamartin,* which has been shown to interact with the *TSC2* gene product, tuberin (479,480,481). Tuberin has a region of homology to a guanosine triphosphatase-activating protein, which is involved in the control of cell proliferation and differentiation (41,482). Recent discoveries show that the tuberin-hamartin complex inhibits the activity of mammalian target of rapamycin (mTOR), a key protein in a signaling pathway that controls cell growth (478).

The defects in both genes cause similar phenotypic abnormalities; however *TSC2*–associated disease is more common and more severe than that associated with *TSC1* (477,483). In a large mutation analysis in 224 patients with TSC, mutations were found in 186 (83%; 12% *TSC1* and 71% *TSC2*) patients (483). Most mutations are unique for a particular family or patient (477). Somatic mosaicism is common among sporadic cases and in mildly affected parents of more severely affected index patients (484,485). Gonadal mosaicism has been detected in unaffected parents of multiple affected offspring (484).

Clinical Features

Hamartomas, which are the characteristic lesions of TSC, are benign tumors resulting from disordered cell migration and incomplete or abnormal cell differentiation. The skin, brain, retina, heart, kidneys, liver, lungs, and bones are affected by these hamartomatous growths (486,487).

Skin manifestations of TSC include facial angiofibromas (also called *adenoma sebaceum*), fibrous forehead plaques, ungual fibromas, shagreen patches (palpable, indurated lesions mainly of the lumbosacral region, histologically connective tissue nevus), and hypomelanotic macules (487). Examination of the skin with ultraviolet light may reveal early skin lesions such as hypomelanotic macules, which are the earliest and most common skin lesions, occurring in 90% of the cases (488). Facial angiofibromas, fibrous plaques, and ungual fibromas are major features of TSC and confirm the diagnosis when other signs of TSC are present (489).

The hamartomatous lesions affecting the central nervous system, cortical tubers and subependymal glial nodules, are responsible for the most severe clinical manifestations of TSC (474,490). Cortical tubers of the brain develop before birth and consist of differentiated and undifferentiated neurons and glia. These cortical dysplastic lesions are often associated with infantile seizures and mental retardation, but they can also remain asymptomatic. The diagnosis is made by CT or MRI, the latter of which is more sensitive (491,492). Subependymal glial nodules or subependymal giant cell astrocytomas usually arise from the walls of the lateral ventricles; they cause increased intracranial pressure because of obstruction of the foramen of Monro, and focal neurologic deficits.

Astrocytic hamartomas of the retina (mulberry tumor) are observed in approximately one half to two thirds of patients and may be bilateral. They are multinodular structures that may be round or oval. They do not grow and may become calcified in time. Loss of vision is a very rare finding of these tumors, and treatment is rarely needed (493).

Cardiac rhabdomyomas are common in TSC. These benign tumors commonly originate from the ventricle, mostly from the ventricular septum (494,495). They start to grow during intrauterine life. In 90% of cases, cardiac rhabdomyomas are detected before 2 years of age (496). They tend to regress spontaneously during childhood. Clinical findings and death due to these tumors are uncommon. They may rarely cause obstruction of blood flow in the heart, congestive heart failure due to myocardial contractile abnormalities, and arrhythmias.

Renal lesions occur in more than half of the cases. The most common renal lesions associated with TSC are angiomyolipomas, which start to develop in late childhood and continue to progress (497–500). Angiomyolipomas are benign tumors consisting of adipose tissue, smooth muscle cells, and arterial vessels whose thickened walls lack normal elastic tissue. Because of their fat content, these tumors have characteristic features on imaging that usually allow the correct diagnosis (501). On ultrasound they appear echodense, whereas on CT scanning they are characterized by low attenuation. The main complication is bleeding due to the increased vascularity of these lesions (502). Hemorrhage may occur, particularly during pregnancy, and may be life threatening. Lesions larger than 4 cm appear to pose a higher risk of bleeding (500). Renal-sparing surgery has been recommended for large angiomyolipomas, and selective embolization can be performed successfully in cases of severe bleeding (501–503). Another complication of renal angiomyolipomas is destruction of the normal parenchyma, resulting in ESRD. Rarely, these tumors may become malignant; the risk of cancer appears to be approximately 1% to 2.5% of patients with TSC (477), which is increased compared with the general

population (500,504). Angiomyolipomas may occasionally be seen in the liver, adrenal glands, and gonads.

Renal cysts are found in approximately 30% of patients with TSC. They may occur alone or in conjunction with angiomyolipomas. Renal cysts together with angiomyolipomas are highly suggestive of TSC (497–499,505). The cyst epithelium is eosinophilic with nodular hyperplasia (498,506). Glomerular cysts may also be seen. Most patients with TSC have only a small number of cysts, which increase in prevalence with age (500). Renal cysts can develop in subjects with either a *TSC1* or *TSC2* mutation. A small subset of patients with TSC presents in infancy with severe bilateral renal cystic disease resembling ADPKD. These children have a contiguous gene syndrome, with deletion of both the *TSC2* gene and the adjacent *PKD1* gene on chromosome 16 (507,508). Although adults with only a few renal cysts are often asymptomatic, some patients may present with flank and back pain and hematuria (497). If the cysts are multiple and bilateral, hypertension and renal failure may be observed, particularly in patients with the contiguous gene syndrome, in whom ESRD often develops in adolescence or early adulthood. Renal failure has been reported to be the most common cause of death in patients with TSC who live past the age of 10 years (509).

Pulmonary lymphangioleiomyomatosis is estimated to occur in less than 1% of the cases (510). These lesions are characterized by abnormal proliferation of smooth muscle cells around the airways, blood vessels, and lymphatics, resulting in obstructive airway disease, cysts, pneumothorax, chylous effusions, and pulmonary hypertension (494). They are more common in women. Patients may acquire pulmonary insufficiency, necessitating lung transplantation.

The skeletal manifestations of TSC are cysts and sclerosis due to periosteal thickening of bones (486,511). Hamartomatous polyps of the rectum, gingival fibrosis, and enamel pits on the teeth are other manifestations of TSC (486).

Diagnosis

The diagnosis of TSC is usually made by the combination of major and minor features assessed clinically or radiographically (489). No single sign is absolutely specific for TSC or present in all affected patients; therefore, at least two types of lesions need to be found in a person to make the diagnosis of TSC (489). Mutation testing has been developed into another tool with relatively high sensitivity (80%) (477). However, it is complicated by the occurrence of somatic and germline mosaicism (485).

Management

There is no specific therapy for TSC, except for surgical resection of hamartomas that could cause problems and nonspecific symptomatic treatments. A consensus conference recommended that patients should be evaluated every 1 to 3 years with renal ultrasonography, CT or MRI of the brain, and one chest radiograph in adult women (512). The recent discovery of the importance of mTOR in the pathogenesis of TSC lesions suggests that drugs like rapamycin that inhibit mTOR might be useful in the treatment of angiomyolipomas or other lesions associated with TSC. Surgical interventions may be necessary to resect subependymal astrocytomas causing hydrocephalus, or for large renal angiomyolipomas. Surgery is also necessary if radiologic studies cannot exclude a renal malignancy. ESRD is best treated with renal transplantation unless general contraindications are present. Immunosuppressive therapy after transplantation does not appear to affect progression of the angiomyolipomas (501).

VON HIPPEL-LINDAU DISEASE

Definition

Von Hippel-Lindau disease is an autosomal-dominant disorder with high penetrance, characterized by development of benign and malignant tumors in multiple organ systems, particularly the central nervous system and the kidneys. Its incidence ranges from 1:36,000 to 1:53,000 (513,514). VHL disease has been divided into two categories: type 1 is characterized by the absence of pheochromocytomas, and type 2 by the presence of these tumors (7% to 20% of families) (515). Type 2 families may manifest as a low (type 2A) or a high (type 2B) risk of renal cell carcinoma (RCC) (516).

Genetics

Von Hippel-Lindau disease is caused by a single gene, a tumor suppressor gene located on the short arm of chromosome 3 (3p25-26) (517). The VHL gene encodes two proteins, pVHL30 and pVHL19 (516). These are tumor suppressor proteins that bind to elongin B, elongin C and cullin-2 (Cul-2) to form a ubiquitin-ligase complex (516,518). This complex is involved in the suppression of tumors by negatively regulating hypoxia-inducible proteins (516).

Up to one fourth of subjects diagnosed with VHL disease may not have a family history of the disease. As in TSC, somatic mosaicism has been shown to occur in VHL disease (519). Parents of patients without a family history can have a mutation in only some of their cells and tissues with mild or no clinical abnormalities, whereas their offspring may have the mutated gene in all cells because of a germ cell mutation in the parent (520). As with other tumor suppressor genes, the development of tumors depends on a second, somatic mutation in the homologous VHL allele.

Clinical Features

Central nervous system, eyes, kidneys, adrenal glands, pancreas, and epididymis are commonly affected in VHL disease. Central nervous system involvement is an important cause of morbidity and mortality in these patients (521). The typical lesions are hemangioblastomas arising from the cerebellum, the spinal cord, or the brainstem. Supratentorial localization of hemangioblastomas is rare. Although these are benign tumors, they may cause symptoms depending on their size, site, and growth rate. They tend to recur even after radical resection.

Retinal hemangioblastomas develop in more than one half of all the cases. They may be multiple and bilateral (522,523). In the early stage, they appear as red dots, resembling microaneurysms of diabetic retinopathy. They may grow and cause blindness owing to retinal detachment and hemorrhage. Therefore, regular ophthalmologic examination is important for the early detection and treatment of these tumors.

The characteristic renal lesions in VHL disease are cysts and carcinomas (524,525). Renal cysts, which may be multiple and bilateral, are present in 50% to 70% of patients (523). They may be misdiagnosed as ADPKD. RCC is an important cause of morbidity and mortality, being responsible for up to 50% of the deaths due to VHL disease (522). The penetrance of RCC is high; it develops in up to 77% of all patients with VHL disease by 60 years of age (524). In contrast to the general population, it occurs at a younger age (mean age, 44 years) and is often multiple and bilateral (522,525). Local recurrence after nephron-sparing surgery is common, necessitating repeated surgeries.

ESRD eventually develops in approximately 25% of patients because of extensive resections or bilateral nephrectomies and rarely because of polycystic kidney disease (524).

Pheochromocytomas cluster within certain families (VHL disease type 2) (515,522). They occur at a younger age than the sporadic cases and may be multiple, bilateral, and extraadrenal (526). They may cause intermittent or sustained hypertension or remain quiescent. Patients with VHL disease should be screened for pheochromocytomas before renal angiography or any surgical procedure because of the risk of hypertensive crisis.

Pancreatic cysts and serous cyst adenomas are found in approximately 30% of patients with VHL disease (527). Cysts are often multiple and may completely replace the gland. Although cysts are mostly asymptomatic, complications such as biliary obstruction and pain may occur because of their space-occupying effects. Pancreatic islet cell tumors occur rarely, as do adenocarcinomas of the pancreas and Vater's ampulla (310).

Epididymal lesions are found in 10% to 60% of male patients (526). These are cystadenomas that may be unilateral or bilateral. Cysts and angiomas may also be detected in the liver, spleen, and lung. Endolymphatic sac tumors may occur in approximately 10% of cases (528). These tumors are frequently bilateral and may cause hearing loss by local invasion.

Diagnosis

In patients with a family history of hemangioblastomas of the retina or the central nervous system, the finding of a single retinal or cerebellar hemangioblastoma or a visceral lesion (i.e., RCC, pancreatic cyst or tumor, pheochromocytoma, papillary cystadenocarcinoma of the epididymis) is sufficient for the diagnosis of VHL disease. In patients with no family history, two or more hemangioblastomas or one hemangioblastoma and a visceral lesion are necessary for the diagnosis (523). Direct mutation analysis of individual patients and families is now possible as well, with a mutation detection rate of 80% to 100% reported (519,529).

Von Hippel-Lindau disease should be considered in the differential diagnosis of ADPKD. The diagnosis of ADPKD can be made with the findings of bilateral enlarged kidneys and the positive family history. Among extrarenal findings, hepatic cysts are common and pancreatic cysts are rare in ADPKD. On the other hand, in VHL disease, hepatic cysts are rare but pancreatic cysts are common (525,527). VHL disease should be suspected in a patient who has multiple pancreatic cysts without any hepatic cysts.

Management

Patients with VHL disease need to undergo a regular screening program to detect neoplasms early so that they can be resected successfully. This screening program consists of annual physical and ophthalmologic examination starting in infancy; after age 10 years, annual MRI of the central nervous system is added, and after 15 to 18 years of age, annual abdominal imaging by ultrasound, CT, or MRI is done. Periodic screening for pheochromocytoma in affected families is best performed by measurement of plasma normetanephrine and metanephrine levels (530).

Cerebellar or spinal cord hemangioblastomas can be successfully removed in most patients. Retinal hemangioblastomas should be treated with photocoagulation to preserve vision. Surgical treatment is necessary for RCC. Nephron-sparing surgery is recommended for early lesions to preserve renal mass (525). If ESRD occurs as a result of multiple operations or polycystic disease, it is treated by dialysis or transplantation. Successful results of renal transplantation have been reported, despite concerns over increased tumor growth with immunosuppressive therapy (531). A waiting period of 1 to 2 years between surgery for carcinoma and transplantation should be observed to avoid early recurrences in the transplanted patient.

GLOMERULOCYSTIC KIDNEY DISEASE

Glomerulocystic kidney disease (GCKD) is a rare disorder characterized by cystic dilatation of Bowman's space. Most of the reported cases are in infants and children (532–535). GCKD occurs as both a sporadic and familial disease. Autosomal-dominant transmission has been suggested in familial cases (536–538). Familial GCKD can be associated either with hypoplastic or normal sized kidneys. Mutations in hepatocyte nuclear factor-1β gene are reported to be associated with the familial hypoplastic form of GCKD (539).

Glomerular cysts can be observed in other diseases and syndromes apart from GCKD. They can occur in TSC, juvenile nephronophthisis, Meckel-Gruber syndrome, Jeune's syndrome, Zellweger's syndrome, trisomy 13, orofaciodigital syndrome type 1, and early-onset ADPKD (533). The differentiation of GCKD from other cystic kidney diseases is clinically difficult. On ultrasonographic examination, the kidneys appear enlarged with increased echogenicity and loss of corticomedullary junction differentiation (540). The diagnosis can be established only by renal biopsy. Glomerular cysts, which contain primitive glomeruli, are mainly located in the subcapsular area of the renal cortex. The tubules are unaffected. This lack of tubular involvement distinguishes GCKD from other renal cystic diseases. The pathogenesis of GCKD remains unknown. Glomerulotubular junction stenosis and obstruction caused by periglomerular fibrosis have been suggested by some authors (541).

The natural history of GCKD is not clearly known. In infants and children, the disease may present with abdominal mass, hypertension, and renal failure. In adults, hypertension, flank pain, and hematuria may be the presenting findings. Although the course of the disease in adults is not clearly known, it may be associated with slow progression to ESRD in some cases (537). Because biopsy is not performed in adults with multiple renal cysts, there may be undiagnosed cases.

ACQUIRED CYSTIC KIDNEY DISEASE

Acquired cystic kidney disease (ACKD) was first recognized by Simon in 1847 (542). It was described in 1977 by Dunnill et al. in a study of autopsy specimens from patients on hemodialysis (543). ACKD may be defined as the development of multiple, bilateral cysts in kidneys of patients with chronic renal disease due to causes other than cystic kidney diseases. Although the incidence of this disease in patients with chronic renal failure is 10% to 20% before the chronic dialysis treatment, it increases after the start of dialysis (19,543,544). More than half of patients undergoing dialysis treatment for 3 or more years have ACKD (20). The major determinant of cystic transformation is the duration of either chronic renal failure or dialysis. Thus, its prevalence is increasing because of prolonged survival of patients on dialysis. The disease has a similar incidence in patients on hemodialysis and on peritoneal dialysis. Dialysis efficacy is not associated with prevalence of ACKD. It may occur at any age, even in children (22). It is reported to be more common in men and in blacks (19,545). It may develop in any etiology of renal disease. In some studies, it was found to be more common

in patients with nephrosclerosis compared with patients with other causes of renal failure (545).

The cysts in ACKD usually are less than 0.5 cm in diameter. However, they may reach sizes of 2 to 3 cm. They affect both the renal cortex and medulla. The epithelium lining the cysts is typically hyperplastic with multifocal areas of adenomatous changes. Histochemical studies of ACKD cyst epithelium and analysis of cyst fluid suggest that most of the cysts derive from proximal tubules (546–548). The pathogenesis of ACKD is not clear. It has been postulated that during the progressive loss of nephrons in chronic renal failure, various mitogenic and cystogenic substances accumulate, which cause hypertrophy and hyperplasia of the epithelial cells of the remaining nephrons. The elevated levels of parathyroid hormone may contribute to cyst formation by stimulating transepithelial secretion of fluid into the tubular lumen. In addition, the interstitial fibrosis and oxalate crystals may contribute to cyst formation through tubular obstruction (20).

The cysts are observed to regress in some patients after successful renal transplantation (549–551). It is also reported to be more common in transplant recipients on cyclosporine therapy, suggesting that renal ischemia may play a role in cyst formation in failing kidneys (552).

Most patients with ACKD are asymptomatic. However, as in other cystic kidney diseases, flank pain, hematuria, hemorrhage, and infection may occur. Rarely, the affected kidney may be large enough to be felt on physical examination. Flank pain and hematuria are the most common clinical findings. Hemorrhage may be either into the cyst or to the retroperitoneal space. Relative or absolute erythrocytosis may be detected in some patients. This is due to increased levels of erythropoietin in these patients compared with those without cysts. The most serious complication of ACKD is the development of RCC. The incidence of RCC increases with duration of dialysis, and men are four to seven times more likely to be affected than women (19,544,553).

The diagnosis of ACKD can be made by ultrasonography. However, because kidneys and cysts are often small, CT may be more sensitive (19,554) (Fig. 18-19). MRI is preferred if the cysts show atypical features on ultrasound (153,154). No uniform criteria exist for the diagnosis of ACKD. The presence of four or more cysts in each kidney in a patient with chronic renal failure without a history of hereditary renal cystic disease is necessary for the diagnosis. The differentiation from ADPKD is usually easy because the family history is negative, the kidneys are small, and there are no cysts in other organs.

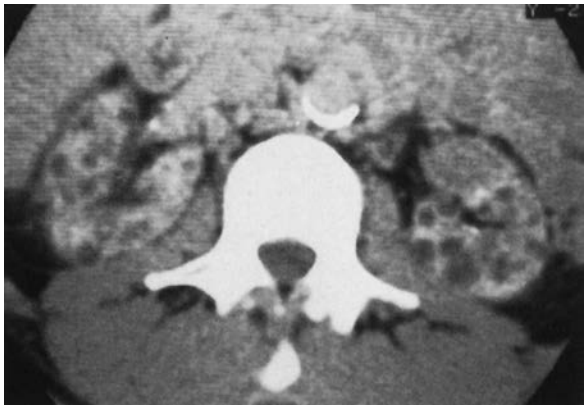


FIGURE 18-19. Acquired cystic kidney disease in a patient on dialysis with diabetes mellitus. Contrast-enhanced computed tomography scan shows both kidneys to be studded with cysts. A solid mass is present on the dorsal surface.

There is controversy over whether periodic renal imaging should be performed for early detection of premalignant or malignant lesions in asymptomatic patients with ESRD (555). Because the incidence of ACKD increases significantly after 3 years of dialysis, some authors suggest annual screening of patients who are on dialysis for more than 3 years (544). Screening can be done with ultrasonography, contrast-enhanced CT, or MRI. If a solid mass is seen on CT or MRI, nephrectomy is indicated (554). On the other hand, because the overall mortality rate of patients with ESRD is high because of cardiovascular disease, survival is often not affected by an incidental RCC. Therefore, screening of all asymptomatic patients with ESRD usually is not recommended (555). Exceptions are young, otherwise healthy patients treated with dialysis for more than 3 years, particularly if awaiting kidney transplantation.

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CHAPTER 19 ■ ALPORT SYNDROME, FABRY DISEASE, AND NAIL-PATELLA SYNDROME

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Hereditary glomerular diseases have diverse and often covert patterns of inheritance and varied clinical presentations. They are more common than is generally appreciated. Vigilance and awareness of these subtleties are important for prompt and accurate diagnoses, which allow optimal management and timely genetic counseling.

ALPORT SYNDROME

Alport syndrome of hereditary nephritis and hearing loss is the best-known hereditary glomerulopathy. Cecil Alport's archetypal kindred (1) had dominantly inherited kidney disease that was characterized in both sexes by hematuria and urinary erythrocyte casts, variable proteinuria, and by hearing loss and renal failure in males. Affected males died in adolescence of uremia.

Since Alport's original description, the phenotype of Alport syndrome has broadened with recognition of other associated clinical features, such as ocular defects and relatively well-preserved hearing. Contiguous gene defects include diffuse leiomyomatosis and facial malformation with mental retardation and elliptocytosis. There are also forms of hereditary nephritis associated with platelet and sometimes granulocyte defects.

PRESENT DEFINITION OF ALPORT SYNDROME

For this chapter, Alport syndrome will be defined as progressive, hereditary, hematuric, nonimmune glomerulonephritis that is characterized ultrastructurally by progressive irregular thickening, thinning, and lamellation of the glomerular basement membrane (GBM) and genetically by a mutation in COL4A3, 4, or 5. In X-linked families (XL-AS), eventual end-stage renal disease (ESRD) of nearly all affected males is a central feature (Figs. 19-1A and 19-1B). Nonrenal features occurring in some kindreds are helpful diagnostic pointers. Conditions relevant to the differential diagnosis and classification of Alport syndrome are collated in Table 19-1.

Diagnosis of Individuals

Chronic hematuria is the cardinal sign of Alport syndrome. Persons with hematuria and a gene for Alport syndrome are affected. One criterion for hematuria is three or more red blood cells/high-power field of the centrifuged fresh urine sediment (2). Carriage of the gene for Alport syndrome may be shown by mutation analysis or inferred in a patient on the line of descent in a family with Alport syndrome. If a mutation has not been shown, typical renal ultrastructure or immunohistochemical findings are strong evidence supporting Alport syndrome. Urinary erythrocyte casts, hearing loss, and ocular lesions support, but are not necessary, for the diagnosis of Alport syndrome. Other urinary findings (pyuria, positive urine cultures, or proteinuria without hematuria) are not signs of Alport syndrome (2).

Asymptomatic Gene Carriers

Clinically normal gene carriers—almost exclusively females in families with XL-AS—should be identified for genetic studies, counseling, and selection of kidney donors. Progressive renal disease will occur in some gene-carrying females. Renal failure is rare in persons of either gender carrying a single gene for autosomal-recessive forms of Alport syndrome.

Ascertainment of Kindreds

Alport syndrome may be ascertained in kindreds either by demonstration of a causative mutation or by demonstration of a family history of chronic glomerulonephritis in many related persons. Unfortunately, many small kindreds contain few or no males with ESRD, even though an individual may meet clinical and histologic criteria. Disease in small kindreds should be considered probable Alport syndrome until further cases occur or a mutation is demonstrated.

Classification of Kindreds by Phenotype and Mode of Inheritance

The severity of symptoms varies from person to person and with age and gender. Large kindreds show modes of inheritance and kindred-specific phenotypes that clearly reflect the genetic heterogeneity of Alport syndrome. Molecular classification now permits rational classification (Table 19-2).

Precise description of each kindred helps in three ways. First, individual prognosis varies widely from one kindred to another, but is generally rather stereotyped in each kindred. Second, genetic counseling can be reliable only if the mode of inheritance has been carefully established. Third, specific features may suggest a specific molecular defect.

Juvenile Versus Adult Types of Alport Syndrome

There is wide variation in the age of ESRD in affected males. Males in some kindreds (with XL-AS) experience ESRD in childhood or adolescence, while in others, males reach ESRD in middle age (3–5). "Juvenile" kindreds are those in which ESRD develops in males at a mean age of 31 years or less; in "adult" kindreds, ESRD occurs at a mean age above 31 years. In Figs. 19-1A and 19-1B, the different kinetics of ESRD in males in Utah kindreds that typify juvenile- or adult-type nephritis are shown. ESRD developed in males at a mean age (± SD) of

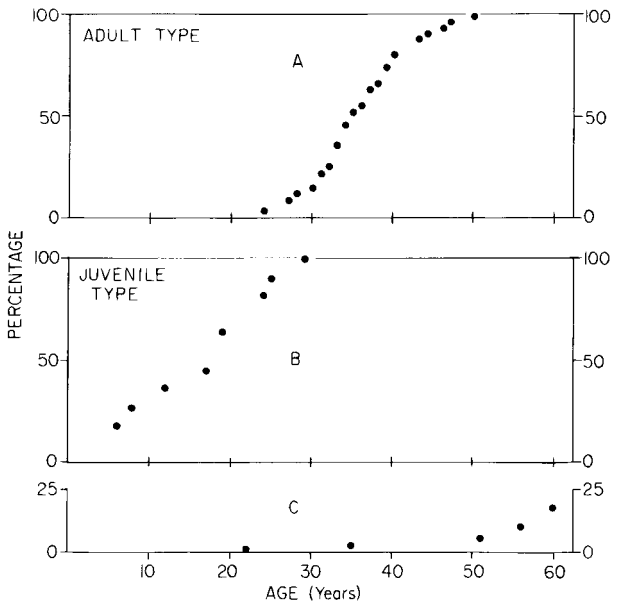

FIGURE 19-1. Prevalence of end-stage renal disease (ESRD) in Alport syndrome. The ordinates show the percent cumulative incidence of ESRD among the same category of gene-carrying persons in representative kindreds, versus age of those persons. At the appropriate ages, instances of other mortality before ESRD were subtracted from number at risk. **A:** All affected males in Utah kindreds C, P, and 3 with adult-type Alport syndrome. **B:** All affected males in Utah kindred PB with juvenile-type Alport syndrome. **C:** All gene-carrier females in all four kindreds.

18 ± 7 years in a kindred with a G635N mutation in COL4A5, and at mean ages of 32 ± 7, and 38 ± 5 years respectively in kindreds, with C1564S and L1649R mutations. Ages at ESRD in males in each of these kindreds were normally distributed. Such data can help in prognosis. Juvenile and adult types of Alport syndrome should not be confused with early or late stages of disease in individuals.

Hearing loss nearly always accompanies juvenile types of XL-AS (6,7), but is variable in adult types (6), and in the largest known adult-type kindred is late or inconspicuous (8). Specific ocular defects such as lenticonus, posterior polymorphous corneal dystrophy, and retinal flecks are more likely in juvenile kindreds.

Genetic Classification of Alport Syndrome

Genetic advances now permit classification of Alport syndrome on a molecular basis (Table 19-2). This classification is still incomplete and will evolve. In practice, many patients will be classified provisionally because genetic testing is not yet widely available. Where possible, correlations with the classification of diseases in the online version of Mendelian Inheritance in Man (OMIM) (9) are given.

X-Linked Alport Syndrome Caused by a Mutation in COL4A5 (OMIM 301050)

This is the most common form of Alport syndrome and is phenotypically very heterogeneous. ESRD is inevitable in males but occurs at widely different ages in different families. The age of ESRD tends to run true within a family, but even within a family there can be quite wide variability. Knowing the mean age of ESRD in males in a family is useful from a prognostic standpoint. Moreover, extrarenal manifestations, such as hearing loss and ocular defects, tend to occur more commonly, more severely, and at an earlier age in kindreds whose males develop ESRD early (7).

X-Linked Alport Syndrome Caused by a Deletion in COL4A5 with Damage to Contiguous Genes

In this form, Alport syndrome is associated with other features caused by extension of the deletion outside COL4A5. Alport syndrome with diffuse leiomyomatosis (OMIM 308940) stems from a deletion embracing the 5′ ends of COL4A5 and COL4A6 (10,11). The AMME syndrome (OMIM 300194/5) consists of Alport syndrome, midface hypoplasia, mental retardation, and elliptocytosis (12–14).

Autosomal-Recessive Alport Syndrome Caused by Homozygous or Mixed Heterozygous Mutations in COL4A3 or COL4A4 (AR-AS, OMIM 203780)

Autosomal-recessive Alport syndrome (AR-AS) is allelic with familial thin GBM disease. It is not yet clear whether all AR-AS or all familial thin GBM disease is caused by mutations of these genes. To date, most examples of AR-AS syndrome have resulted in early ESRD, but this may reflect ascertainment bias (15–22). Males and females are equally severely affected and hearing and ocular defects are usual.

Autosomal-Dominant Alport Syndrome without Hematologic Defects (AD-AS, OMIM 104200)

Autosomal-dominant Alport syndrome (AD-AS) has been described several times, but most descriptions are unconvincing. One well-described family had relatively mild renal impairment and no hearing loss, eye signs, platelet abnormalities, or leiomyomatosis (23). The mutation in this family is a splice site mutation in COL4A3 (24). Another family with autosomal dominant nephropathy and deafness has been linked to 11q24 (25).

Autosomal-Dominant Alport-Like Syndrome Caused by Mutations in MYH9

Epstein syndrome (OMIM 153650) was first described as a variant of Alport syndrome with nephropathy, sensorineural hearing loss, thrombocytopenia, and giant platelets (26). Several kindreds have been described (27–34). When there are inclusions in leukocytes the term Fechtner syndrome (OMIM 153640) is used (35–43). Nonsyndromic hereditary hearing impairment (44), Fechtner syndrome (40,42), and May-Hegglin anomaly all map to 22q11-13. Mutations in MYH9, the gene at this locus coding for nonmuscle myosin heavy chain IIA, were found almost simultaneously in patients with nonsyndromic hereditary hearing impairment (44), May-Hegglin anomaly (45,46), Sebastian symdrome (46), and Fechtner syndrome (46). Epstein syndrome, Fechtner syndrome, nonsyndromic hereditary hearing impairment, May-Hegglin anomaly, Sebastian syndrome, and isolated macrothrobocytopenia have now been shown to result from mutations in MYH9 in nearly all cases (44–55) and are thus described as MYH9 (or NMMHCA [nonmuscle myosin heavy chain A]) diseases.

It is intriguing that the original kindred described by Alport cannot be unequivocally assigned to any of these forms of Alport syndrome because affected males did not reproduce and because the taint has died out, preventing further study. The severity in males suggests strongly that the original kindred was X-linked.

TABLE 19-1

DISEASES RELEVANT TO THE CLINICAL CLASSIFICATION OF ALPORT SYNDROME

Disease or condition	Mode of inheritance	Reference
Alport syndrome		This chapter
X-linked	XD	
With leiomyomatosis	XD	
With mental retardation and midface hypoplasia	XD	
Autosomal dominant	AD	
Autosomal recessive	AR	
Conditions with hearing loss that may be confused with Alport syndrome		
MYH9 syndromes	AD	This chapter
Alström syndrome	AR	(322–326)
Branchiootorenal syndrome	AD	(327)
Charcot-Marie-Tooth disease and nephritis	D	This chapter
Familial immune nephritis		
Familial IgA nephropathy with hearing loss	D	(328)
Familial membranous nephropathy	D	
Hereditary interstitial nephritis with hearing loss	AD	This chapter
Familial spastic paraplegia, deafness, mental retardation, nephropathy		(329)
Muckle-Wells syndrome (urticaria, deafness, amyloidosis)	AD	(330–332)
Renal tubular acidosis with nerve deafness	AR	(333–338)
Various rare syndromes	Various	(339–345)
X-Linked progressive hearing loss	XD	This chapter
Conditions with normal hearing that may be confused with Alport syndrome		
Acquired glomerulonephritis	Not hereditary	
Alagille syndrome	AD	(346–351)
Asphyxiating thoracic dystrophy (Jeune syndrome)	AR	(352,353)
Bardet-Biedl syndrome	AR	(354–357)
Cystinosis	AR	Chapter 79
Diabetic nephropathy	Polygenic	Chapter 73
Fabry disease (angiokeratoma corporis diffusum)	XD	This chapter
Familial interstitial nephritis without hearing loss		This chapter
With hyperuricemia and gout	AD	Chapter 76
Familial Mediterranean fever	AR	Chapter 75
Familial nephropathic amyloidoses	AD	Chapter 75
Finnish nephrotic syndrome	AR	Chapter 79
Hemolytic–uremic syndrome	AR	Chapter 67
Hereditary carpotarsal osteolysis	AD	(358)
Hereditary immune nephritis	D (?)	This chapter
Imerslund–Graesbeck syndrome	AR	(359,360)
Lecithin–cholesterol acyltransferase deficiency	AR	(269,361,362)
Nail–patella syndrome	AD	This chapter
Non-Alport hereditary glomerulonephritis	AD (?)	This chapter
Primary hyperoxaluria	AR	Chapter 26
Thin glomerular basement membrane disease ("benign" familial hematuria)	AD	This chapter

AD, autosomal dominant; *AR*, autosomal recessive; *D*, undifferentiated dominant; *XD*, X-linked dominant.

Familial Thin Basement Membrane Disease or Benign Familial Hematuria (OMIM 141200)

This can be a manifestation of heterozygosity for a mutation in COL4A3 or COL4A4. Mutations in other genes have not yet been found, but have not been excluded.

Canine and Murine Hereditary Nephritis—Models of Alport Syndrome

X-Linked Samoyed Nephritis. X-linked nephritis occurs in Samoyed dogs and leads to renal failure in the males by the age of 9 months (56). Microscopic hematuria may be present at the outset of the disease but does not invariably occur; this is a significant difference from Alport syndrome in humans. Hearing loss and other extrarenal defects have not been described. By light microscopy of sections stained with periodic acid-Schiff (PAS), segmental GBM splitting appears at 4.5 months and progresses to glomerulosclerosis. Segmental sclerosis, synechiae, and parietal cell proliferation appear in some glomeruli. In pups as early as 1 month old, electron microscopy reveals focal subepithelial and subendothelial lucencies and splitting of the lamina densa with granulation. Tubules, interstitial blood vessels, and their basement membranes remain unaffected despite

TABLE 19-2

GENETIC CLASSIFICATION OF ALPORT SYNDROME AND MHY9 SYNDROMES

Description	OMIM	Gene	Gregory/Atkin classification	Synonyms	Comments
Dominant, juvenile-type nephritis with or without hearing loss			I		Because of absence of offspring of affected males, pedigree analysis cannot distinguish X-linked from autosomal-dominant inheritance. Alport's original kindred best fits in this category.
X-linked dominant type nephritis with hearing loss	301050	COL4A5	II, III, IV		Type II is juvenile-type nephritis with hearing loss. Type III is adult-type nephritis with hearing loss. Subtype IIIa displays and subtype IIIb lacks the familial nephritis serum epitope in glomerular basement membrane and epidermal basement membrane. Type IV is adult-type nephritis without overt extrarenal features. Hearing loss typically appears one to two decades later than renal failure in affected men.
X-linked dominant nephritis with diffuse leiomyomyomatosis	308940	COL4A5, COL4A6	VII		Hearing loss and cataracts frequently associated.
X-linked dominant nephritis with midface hypoplasia, mental retardation, and elliptocytosis	300194/5	COL4A5, FACL4, AMMECR1		AMME syndrome	
Autosomal-dominant hereditary nephritis	104200	COL4A3	(VI)		Several uncertain kindreds of autosomal-dominant Alport syndrome with hearing loss. The one kindred linked to COL4A4 did not have overt hearing loss.
Autosomal-recessive hereditary nephritis	203780	COL4A3 or COL4A4	VIII		Allelic with familial thin basement membrane disease.
Autosomal-dominant nephritis with thrombocytopenia, giant platelets, and leukocyte inclusions	153640	MYH9	V	Fechtner syndrome	Phenotypically similar to Epstein syndrome, but leukocytes have inclusions.
Autosomal-dominant nephritis with thrombocytopenia and giant platelets	153650	MYH9	V	Epstein syndrome	
May-Hegglin anomaly	155100	MYH9			Thrombocytopenia, giant platelets, and granulocyte inclusions.
Sebastian syndrome	605249	MYH9			Thrombocytopenia, giant platelets, and granulocyte inclusions of different structure from those in May-Hegglin anomaly and similar to those in Fechtner syndrome.
Nonsyndromic deafness (DFNA 17)	603622	MYH9			

OMIM, Mendelian Inheritance in Man (http://wwwncbinlmnihgov/omim).

progression in extent and severity of the GBM lesions. Modification of the epitope(s) of the noncollagenous (NC) region of type IV collagen in these dogs (57–59) is similar to that in humans (60) with hereditary nephritis. NC is the roughly 30-kd C-terminal noncollagenous domain of basement membrane (type IV) collagen chain that can be isolated as monomers, dimers, and so on from collagenase-treated basement membrane. A mutation found in COL4A5 is precisely analogous to human XL-AS (61).

Autosomal-Dominant Bull Terrier Nephritis. Bull terriers are prone to a hereditary nephritis that is apparently inherited in an autosomal-dominant fashion (62). Proteinuria consistently develops in affected males and females, and slightly fewer than 50% show hematuria. Anterior lenticonus is a constant finding but, because the canine fundus is normally speckled, it is difficult to comment on retinal pigmentary changes (63). Hearing loss occurs in bull terriers but does not segregate with renal disease. Affected dogs show renal ultrastructural changes similar to those in human Alport syndrome although subepithelial "frilling" is more prominent, and they progress to renal failure at varying ages (64). Both the Goodpasture $\alpha 3$(IV) and Alport $\alpha 5$(IV) antigens are present in the GBM of affected bull terriers (63).

Autosomal-Recessive Alport Syndrome in English Cocker Spaniels. Collagen $\alpha 5$(IV) chains are present in GBM, and $\alpha 6$(IV) chains also appear in this location in the hereditary nephritis that occurs spontaneously in this breed. Glomerular ultrastructure is similar to that in human Alport syndrome (65).

Mouse Models of Alport Syndrome by Homozygous Knockout of COL4A3. These closely resemble human AR-AS (66–68).

Epidemiology and Genetics of Alport Syndrome

Incidence of Renal Failure, Gene Frequency

Between 2% and 5% of males with ESRD and probably less than 1% of females have Alport syndrome. Various estimates place the gene frequency from 1:5,000 to 1:10,000 (4). The true prevalence may be higher because affected patients with subtle hearing loss are easily overlooked.

Inheritance

Dominance. Hematuria and ESRD are the main manifestations of Alport syndrome. Defined by hematuria, XL-AS is dominant in most kindreds. If the late manifestation, ESRD, is confused with gene carriage, then many gene carriers will be missed and inheritance misclassified as recessive. As Longo et al. (69) perceptively pointed out, the distinction between AD-AS, AR-AS, and familial thin glomerular basement membrane disease is blurry, with significant intrafamilial heterogeneity and gene-carriers developing no, mild, or late renal disease. Until all pathogenic COL4A3 and COL4A4 mutations, including those in introns, can be identified with certainty, there is a definite possibility that apparent cases of AD-AS may be mixed heterozygotes for AR-AS with the second mutation not yet having been identified.

Penetrance versus Prevalence of Symptoms. Hematuria from birth is a generally reliable indicator of Alport gene carriage. It occurs in 100% of hemizygous males (7,70,71), and 90% to 100% of heterozygous female carriers of XL-AS (70,72,73). In the autosomal forms, data are sparse: all affected patients so far described with AR-AS have had hematuria, but as hematuria was generally a presenting feature or a criterion for the diagnosis there is heavy ascertainment bias: 80% of heterozygous carriers of AR-AS (73) had microhematuria. Most patients with AD-AS have had hematuria (23,24,69,74,75), but the numbers are small and one 90-year-old gene carrier did not have hematuria and was "completely asymptomatic" (74).

Because they are late symptoms, it is unwise to equate chronic renal failure and ESRD with Alport gene penetrance. We define prevalence of ESRD as the fraction of a population at risk in whom ESRD eventually develops. For males in each Alport kindred, ESRD prevalence generally coincides with hematuria penetrance. Figures 19-1A and 19-1B show representations of the kinetics and prevalence of ESRD of males with XL-AS. The Lyon hypothesis of inactivation of X-chromosomes (76) likely explains both incomplete hematuria penetrance among females heterozygous for XL-AS and their low prevalence of ESRD (Fig. 19-1C) (2). Jais et al. (72) found a cumulative prevalence of ESRD of 12% in female carriers of XL-AS by the age of 40 years. The cumulative prevalence of ESRD has not been systematically studied in AR-AS and AD-AS.

Modes of Inheritance, Genes, and Chromosomal Regions. Mutations in several different genes have been implicated in various types of Alport syndrome. The role of mutations in COL4A3 and COL4A4 in autosomal Alport syndrome and of COL4A5 in XL-AS is discussed previously. Autosomal dominant nephropathy associated with MYH9 mutations is best separated as a separate entity (see the previous text).

Sex Ratio. One consequence of X linkage is that twice as many females as males with a nephritis gene will be born (2,76) if there are no prenatal effects and if reproductive fitness is independent of gender of the gene-carrying parent. In kindreds with juvenile XL-AS, most affected children obtain the gene from their mothers, and the sex ratio of gene-carrying newborns approaches 1:1.

Fitness and Mutation

Decreased fertility and increased mortality impair the reproductive fitness (76) of affected males with juvenile types of Alport syndrome. When males have no offspring, pedigree analysis cannot distinguish X-linked from autosomal inheritance. Affected males in 65 kindreds with all types of Alport syndrome had, overall, only 70% fitness as compared to the gene-carrier females (4). In contrast, affected males and carrier females in large Utah kindreds with adult type disease had the same vigorous reproductive fitness as did their noncarrier relatives.

At equilibrium gene frequency (Hardy-Weinberg hypothesis [76]), 30% decreased reproductive fitness will counterbalance new mutations in roughly 15% of newborns with a gene for Alport syndrome. It has been estimated (77), and observed in Japanese schoolchildren (78), that 17% to 18% of cases are new mutations.

Population Genetics

"Juvenile"-type kindreds are more numerous (4) but relatively smaller (i.e., they have fewer affected persons and fewer affected generations). The first affected ancestor in juvenile-type kindreds can often be identified and may be living. Many de novo mutations have been found in juvenile kindreds. The disease tends to die out because uremia hinders reproduction.

"Adult"-type kindreds are fewer but larger. The genealogy can be traced for eight generations in large families (8) and the disease shows no signs of dying out. The first affected ancestor often cannot be identified. Haplotype analysis suggests that

groups of kindreds having identical adult-type mutations are actually branches of one giant kindred (8).

Pathology

Kidney

From being of normal size or slightly enlarged, the kidneys progressively and symmetrically shrink without distortion of the calyces or pelves. In advanced cases, the renal surface is finely granular with an adherent capsule. Yellow flecks may be seen on the subcapsular or cut surface. The essential pathologic features are a characteristic and progressive ultrastructural lesion of the GBM and the absence of immune reactants.

Light Microscopy. Light microscopy is less sensitive than electron microscopy and is never pathognomonic, although patients with early stage disease may show an increased number of immature ("fetal") glomeruli (79–81). Tissue sections stained with PAS or methenamine silver may show focally thickened GBMs early in the course of disease (79,82–84). Red cells in tubular lumina may reveal inconspicuous glomerular damage. Subsequently, stalk enlargement (85), diffuse mesangial proliferation (79,85,86), segmental or global glomerular sclerosis, periglomerular fibrosis, tubular atrophy, and interstitial fibrosis appear, but these changes are not specific. Foam cells in the interstitium are common in Alport syndrome (79,87–91) but also occur in other renal diseases (79,91). Yoshikawa and associates (86) report foam cells mainly in patients over the age of 10 years.

Epithelial crescents are occasionally seen (89–97); they tend to occur in patients with the juvenile type of disease (79,91,96,98). Many older reports neither mentioned the proportion of crescents nor distinguished them from periglomerular fibrosis in advanced stages of disease, but well-defined glomerular epithelial crescents were associated with rapidly progressive renal failure in a few instances. Apart from more rapid progression and the occasional presence of crescents in patients with juvenile-type disease, the histology appears identical in all types of Alport syndrome.

Glomerular Ultrastructure. Although hematuria is detectable from the first few days of life, careful ultrastructural examination of renal biopsy specimens may show no abnormalities until patients are several years older (99). By contrast, pathognomonic ultrastructural changes may be present long before the appearance of any clinical or laboratory signs other than hematuria. Renal ultrastructure of clinically normal gene-carrier females can be normal. Widespread lamellation of the GBM is highly characteristic for Alport syndrome although it is not absolutely specific and may be seen focally and in conjunction with other lesions in a wide variety of nephropathies (100,101). Early in the course, diffuse or focal thinning of the GBM similar to that in thin GBM disease can occur (79,80,84,99,102,103). In more advanced stages of Alport syndrome (Fig. 19-2A), the lamina densa of the GBM splits into two or more independent lamellae, frequently separated

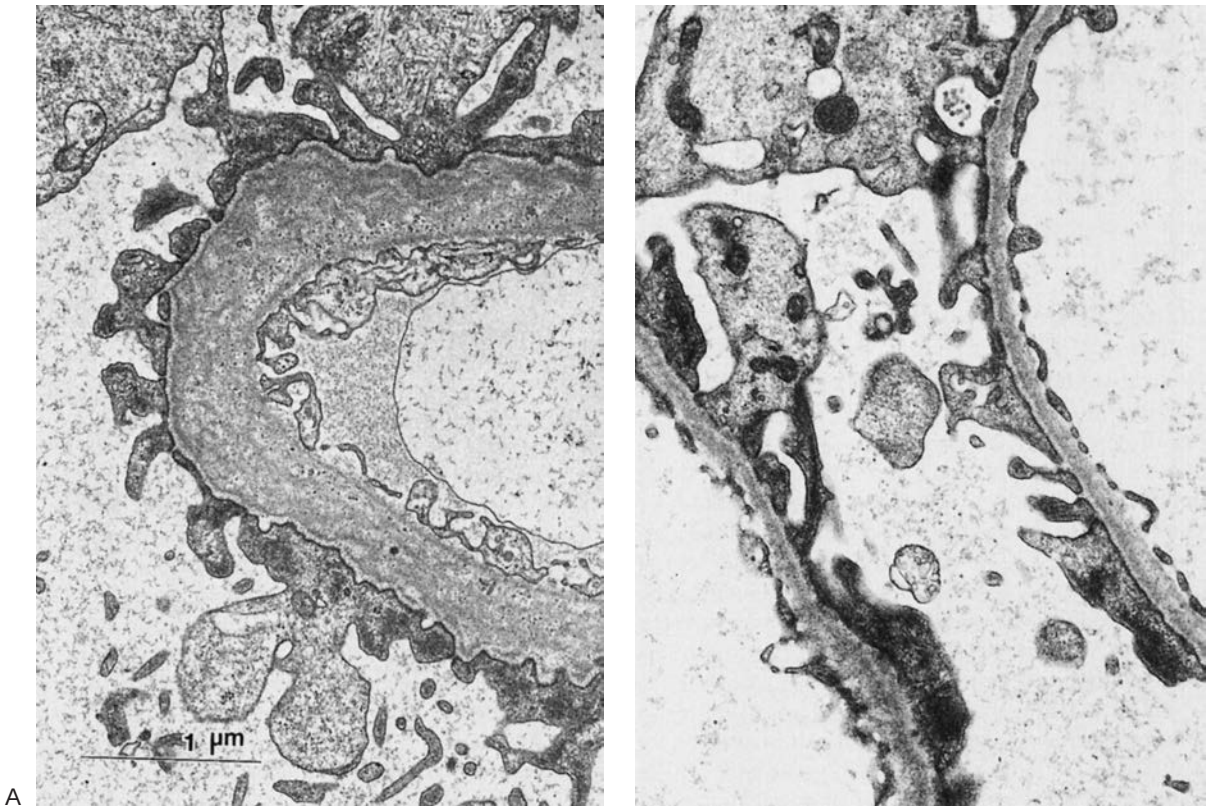

FIGURE 19-2. A: Glomerular ultrastructure in Alport syndrome. Electron micrograph of a renal biopsy specimen from a man in Utah kindred M, illustrating a widened lamina densa of the glomerular basement membrane (GBM). The lamina densa is split into several layers, between which may be seen numerous small electron-dense granules. **B:** Electron micrograph, at same magnification as (**A**), from an affected woman with familial thin GBM disease. The uniform thinning of the GBM can be appreciated by comparison with the width of the epithelial foot processes. (Electron micrographs courtesy of Dr. M.E. Hammond, University of Utah, Salt Lake City.)

by numbers of electron-dense granules with diameters of approximately 50 nm (85,100,104,105). Distortion of the lamina densa may be extreme, at times amounting to a "basket weave" appearance in which the lamellae branch and rejoin in a complex tangle (106). Stretches of GBM thickening may alternate with areas of attenuation.

Splitting of the basement membranes of Bowman's capsule and of tubules has also been described (2,101,104), but these defects were equally common in control subjects with other renal diseases (107). Focal or diffuse foot process fusion is common and seems to bear little relation to the severity of the proteinuria or to the degree of basement membrane damage (102,108). In advanced uremia, sclerosis and scarring rob the morphology of any specificity. Scanning electron micrographs show numerous microvilli and blebs projecting from glomerular epithelial cells, and effacement of foot processes (109); unfortunately, these findings are nonspecific. The ultrastructural lesions of the GBM appear identical in all types of Alport syndrome; in particular there is no discernible difference between kindreds with hearing loss or thrombocytopathy and those without these abnormalities.

Immunopathology. Immunofluorescence is negative (83,85, 100,110) or shows only minor focal staining (79,102,110,111) for various immunoglobulins or complement-3 (C3) except in sclerosed or severely damaged glomeruli, presumably from mechanical trapping.

Absence of a GBM component in Alport syndrome was inferred from nonreactivity of some patients' GBM with sera from patients with Goodpasture's disease (112–114). The Goodpasture epitope was primarily associated with 28-kd NC domains of GBM collagen. In contrast, Habib and coworkers (99) report that the GBM from patients with Alport syndrome consistently reacted with Goodpasture's sera. Others (115–118) have obtained variable results with Goodpasture's and heterologous anti-GBM sera. These discrepancies likely arose from disparate specificities of the antibodies employed.

The occasional occurrence of anti-GBM nephritis in Alport syndrome recipients of renal allografts (116,117,119–121) led to the development of invaluable natural familial nephritis serum (FNS) antibodies that recognize a highly cationic 26-kd NC domain (121–124), as well as monoclonal antibodies that recognize 28-kd domains (125–130). The GBMs of most males with Alport syndrome fail to stain with antibodies against the FNS epitope, and those in females stain in an interrupted fashion, as would be expected from mosaicism and random inactivation of the normal X chromosome (131). A minority of families with otherwise typical Alport syndrome show normal staining of the GBM and epidermal basement membrane (EBM) (131). Some kindreds with undetermined disease type show staining of the GBM and EBM, and some do not (132). Monoclonal antibodies against α2(IV) and α5(IV) are now commercially available for study of GBM and EBM.

The missing or nonreactive NC epitope (Goodpasture antigen) resides in the α3(IV)-collagen chain monomer (133,134) and the Alport antigen is on the α5 chain (135). The explanation of this apparent paradox is that heterotrimers containing α3(IV) cannot assemble in the absence of functional α5(IV) (136,137). In humans, unlike dogs, α3(IV) and α4(IV) are transcribed independently of α5(IV). The defect of heterotrimer formation thus occurs downstream of transcription (138).

Cochlea

The inner ear is much less amenable to histopathologic study than is the kidney. Specific lesions in Alport syndrome include a zone of separation between the basilar membrane and the overlying basement membrane of the organ of Corti and the presence of cells filling the tunnel of Corti and the extracellular spaces of Nuel (139). Cellular infilling of the tunnel of Corti and spaces of Nuel likely represents a persistence of the fetal cochlear structure (139).

Although no studies have been made on Alport syndrome patients or gene carriers, the FNS epitope is normally present in basement membranes of the spiral limbus, inner sulcus, basilar membrane, and spiral prominence of the cochlea (124). A broadly similar distribution of α3,4,5 chains has been shown in mice (140) and dogs (141) although these chains extend into the spiral ligament and stria vascularis in the animal models.

Eye

In anterior lenticonus the basement membrane of the anterior lens capsule is thinned (142,143) and more fibrillar than normal (143), allowing anterior bulging of the cortex of the lens, most prominently in the pupillary region (142,144).

The FNS epitope is widely distributed in the normal eye. It has been shown in conjunctival, corneal, and iridal basement membranes; Descemet's membrane; and anterior and posterior lens capsules as well as all other basement membranes studied in the eye (124,145). The α3, α4, and α5 chains of type IV collagen may be present or lacking in the anterior lens capsule of Alport syndrome patients (146).

Skin

No light microscopic or consistent ultrastructural abnormalities have been described, although systematic ultrastructural studies of the skin are needed. The FNS epitope is normally present in the EBM at the dermal–epidermal junction (131) (Fig. 19-3A). While a few Alport syndrome families possess normal reactivity for the epitope at this site, EBMs from most males with Alport syndrome lack it (123,131) (Fig. 19-3C). Female heterozygotes display interrupted staining (131) (Fig. 19-3B).

Platelets and Granulocytes

In families with COL4 mutations, no abnormalities have been recognized in the formed elements of the blood. See the subsequent text for the specific abnormalities in the MYH9 syndromes.

Smooth Muscle

In Alport-leiomyomatosis syndrome, orderly hyperplasia of smooth muscle may involve the trachea and bronchi, all muscle layers of the esophagus, the clitoris, and uterus. True leiomyomas, characterized by disorderly smooth muscle proliferation, have been found in the trachea and lungs, esophagus, upper part of the stomach, clitoris, vagina, vulva, and perineum (147). Malignant transformation has not been observed. Ultrastructurally, basement membranes are normal in esophageal tissue (147).

Pathogenesis

Speculations that defects of basement membrane (type IV) collagen, or specifically of its cross-linking cysteine residues, are implicated in the pathogenesis of Alport syndrome date back more than 20 years (85,148).

The Oulu group cloned the COL4A5 collagen gene, physically mapped it to the Xq22 chromosomal region, showed that it coded for the heretofore unknown basement collagen monomer chain 5(IV), and speculated that mutations of it might cause Alport syndrome (149). The findings were soon confirmed (150,151). Probing Southern blots of restricted leukocyte DNAs with complementary DNA (cDNA) probes from COL4A5, showed different mutations of COL4A5 in three Utah kindreds (150). Defective basement membranes in

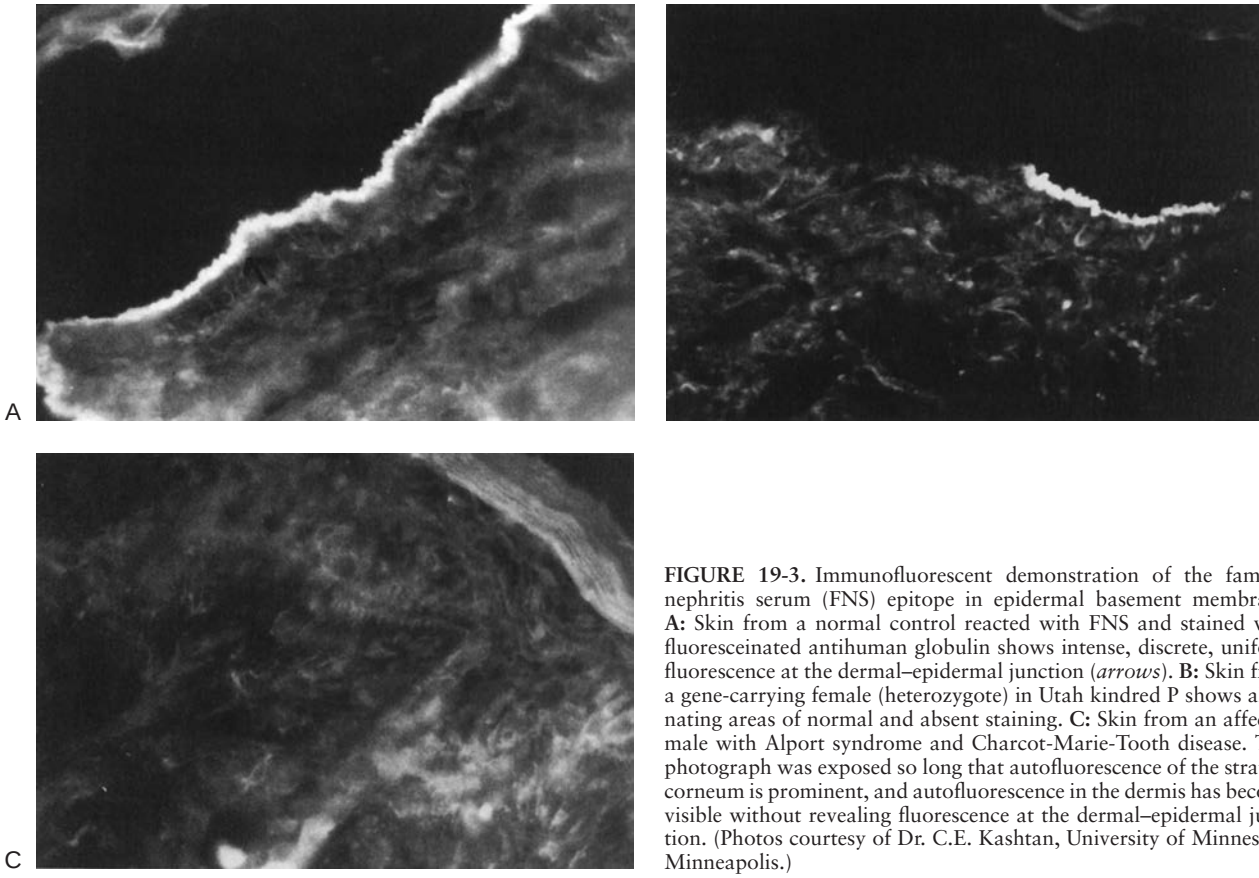

FIGURE 19-3. Immunofluorescent demonstration of the familial nephritis serum (FNS) epitope in epidermal basement membrane. **A:** Skin from a normal control reacted with FNS and stained with fluoresceinated antihuman globulin shows intense, discrete, uniform fluorescence at the dermal–epidermal junction (*arrows*). **B:** Skin from a gene-carrying female (heterozygote) in Utah kindred P shows alternating areas of normal and absent staining. **C:** Skin from an affected male with Alport syndrome and Charcot-Marie-Tooth disease. This photograph was exposed so long that autofluorescence of the stratum corneum is prominent, and autofluorescence in the dermis has become visible without revealing fluorescence at the dermal–epidermal junction. (Photos courtesy of Dr. C.E. Kashtan, University of Minnesota, Minneapolis.)

some are explicable in terms of loss of essential cross-linking cysteine residues in the C-terminal NC domains of $\alpha 5$(IV) (152). In others there is interruption of repetitive Gly-X-Y sequences in the collagenous domain, or truncation of this triple helical region. Zhou and associates (153) discuss this in more detail and provide explanatory figures. An extensive correlation of genotypes and phenotypes has been published (7,72) and extended (154).

Clinical Features

Renal Symptoms and Signs

Hematuria is the cardinal feature, persistent and present from birth in males (7) and in a fraction of females (72) who have a nephritis gene. Hematuria is a sine qua non for the diagnosis of Alport syndrome in males. A child's mother may note occasionally or persistently red diapers, but hematuria is usually inconspicuous in adult-type disease. Episodes of gross hematuria may follow sore throats or other infections in children and may be the presenting symptom (79,80,155). The red cell excretion rate is increased by acute infections and by pregnancy.

Microscopy reveals dysmorphic red cells, renal tubular cells, and red cell casts. Early reports of pyuria have been abundantly refuted (2). Urinary tract infection is no more frequent than in the general population. Proteinuria is variable, ranging from barely detectable in early stages to nephrotic range (7) in some patients in advanced stages. Very heavy or increasing proteinuria implies a worse prognosis (79,156). The nephrotic syndrome may be more likely to occur in juvenile types of disease. In Utah kindreds with adult types of Alport syndrome, heavy proteinuria is usually well compensated by increased plasma protein synthesis; while increased plasma $\alpha 2$-globulin levels and hypercholesterolemia do occur, we have rarely observed edema. Serum complement concentration is normal (80,83,93,157).

In adult types of Alport syndrome, renal function is typically normal for years and then wanes inexorably to renal failure. The reciprocal of serum creatinine falls linearly with time during this phase (roughly 6 years from early to end-stage renal failure in adult-type Alport syndrome); hypertension appears and worsens as renal function deteriorates. Crescentic glomerulonephritis may occur, especially in juvenile types of Alport syndrome, and be accompanied by rapidly progressive renal failure (98).

With either juvenile- or adult-type XL-AS, renal failure is inevitable for affected males, and 30% to 40% females become uremic, generally in late adulthood (72), (Fig. 19-1C). In kindreds with AD-AS, ESRD in females may occur as frequently as in males.

Sensorineural Hearing Loss

Some families with adult type XL-AS Alport syndrome have socially normal hearing (8,158), whereas progressive and ultimately profound, bilateral, sensorineural hearing loss distinguishes kindreds with all other types (6,7,159). Patients can be unaware of a high-frequency loss that is readily shown by audiometry (159). Hearing loss generally occurs later, less severely, and less frequently in females (6,72) (Fig. 19-4B), although some women and girls may have a profound loss. In some families with Alport syndrome and hearing loss, affected members may have apparently normal hearing even after ESRD (8), but in other kindreds, family members without hearing loss appear to have less severe renal disease than their counterparts with hearing loss (79,160). In a large Utah kindred with a C1549S mutation in the NC domain of 5(IV),

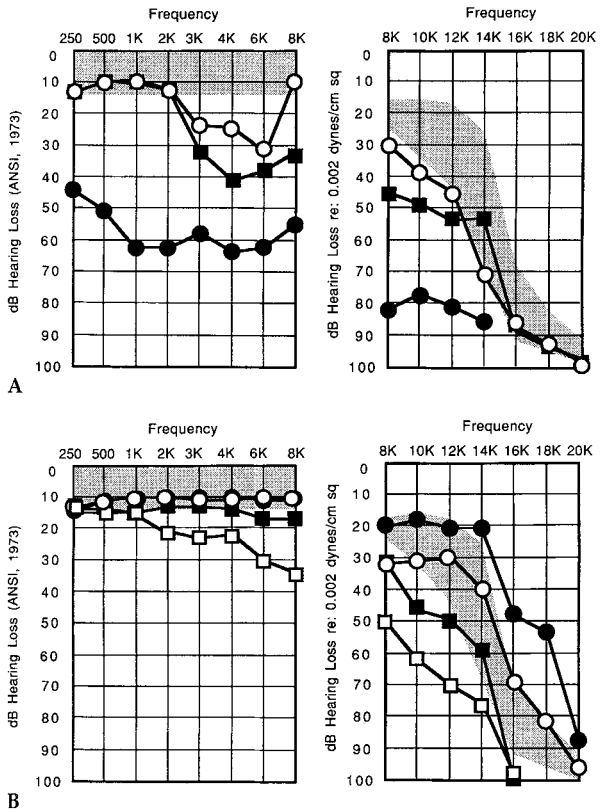

FIGURE 19-4. A: Audiograms of affected males with adult types of Alport syndrome. Standard test frequencies (*left panel*) and ultra–high-frequency (*right panel*) audiograms in men aged between 31 and 40 years of age. *Solid circles* represent men with C1549S mutation (n = 3); *open circles*, men with L1564A mutation (n = 5); and *solid squares*, unaffected men with significant noise exposure (n = 4). The *shaded area* represents one standard deviation either side of the mean for a reference normal population (n = 24). **B:** Audiograms of females heterozygous for C1549S mutation. Standard test frequencies (*left panel*) and ultra-high frequency (*right panel*). *Solid circles* represent ages 8 to 20 years (n = 5); *open circles*, ages 21 to 30 years (n = 3). Note that solid and open circles overlap entirely in the left-hand panel. *Solid squares*, ages 31 to 40 years (n = 6); and *open squares*, ages 41 to 50 years (n = 8). The *shaded area* represents one standard deviation either side of the mean for a reference normal population (n = 25) (all ages combined). (From: Dr. Derin Wester, University of California, San Diego, with permission.)

noticeable hearing loss generally coincided with the onset of renal failure (8).

Most audiologic studies (79,159,160) were carried out on an unspecified mixture of patients with mainly juvenile phenotypes. The only systematic audiologic study in which different Alport phenotypes were distinguished was carried out in patients with adult-type disease by Wester and colleagues (6). Males with the C1549S mutation had severe hearing loss both at standard testing frequencies and at ultra-high frequencies (Fig. 19-4A). Their hearing loss was clearly discriminated from the mild loss in females heterozygous for the same mutation (Fig. 19-4B) and the equivocal impairment in males with the L1564A mutation (Fig. 19-4A). No impairment was demonstrable in C1564S males before age 20, but the loss increased steadily thereafter to at least age 40 (6).

There is no anatomic abnormality of the tympanic membrane or ossicular chain; middle ear pressures are normal and air conduction is normal. A suspicion that purulent otitis media was common in Alport syndrome (160,161) has not held up under systematic scrutiny (79,159). Hearing loss is usually worse above 1,000 Hz (Fig. 19-4), with an abnormal short increment sensitivity index, negligible tone decay, and normal brainstem auditory evoked responses (159), proving cochlear rather than neural dysfunction. Caloric test results are normal (162), but more subtle testing reveals impaired vestibular function (159); flat intensity function curves locate the lesion in the end organ itself.

Renal disease with hearing loss, even if familial, should not be equated with Alport syndrome. In a series of 24 patients with inborn chronic renal disease and hearing loss, Bergstrom and colleagues (163) report only a minority with Alport syndrome. Hearing loss from the time of birth is unlikely to be due to Alport syndrome (6–8).

Ocular Features

Eye defects appear limited to kindreds with juvenile-type nephritis with hearing loss. In stark contrast to hearing loss, which is common in hereditary nephritis, but not specific for it, anterior lenticonus (79,142,164–169) (Fig. 19-5), although uncommon, is nearly pathognomonic. All cases of anterior lenticonus reported between 1964 and 1982 were associated with hearing loss with or without nephritis (166,167). Lenticonus is more common in males and is usually, but not invariably, bilateral. It is easy to recognize anterior lenticonus when it is fully developed. The red reflex is present but it is impossible to

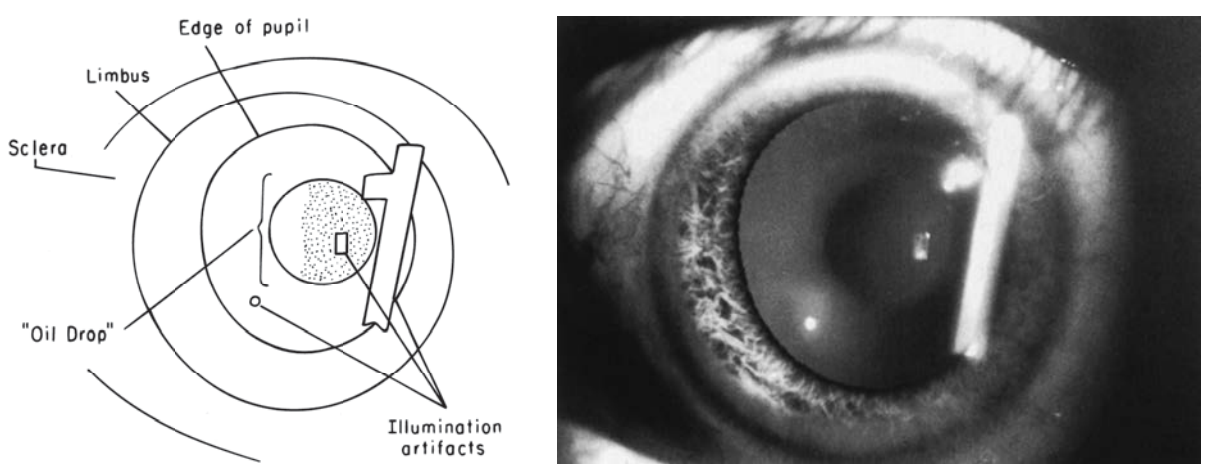

FIGURE 19-5. Anterior lenticonus. "Oil drop" refractive anomaly in the central pupillary area of the right eye of a man with Alport syndrome, retroillumination view. The appearance is similar by direct ophthalmoscopy. (Photograph courtesy of Dr. J. Kivlin, University of Wisconsin, Madison.)

see the fundus clearly because of the severe refractive error. Examination through a dilated pupil with a strong convex lens in the ophthalmoscope reveals an "oil drop" bulging the anterior surface of the lens. Lesser degrees of lenticonus may be difficult to diagnose even by slit-lamp examination (165). The lens appears normal at birth (157), and lenticonus develops later, frequently during the teens. Severe degrees of lenticonus cause grave visual impairment, not correctable by glasses or contact lenses.

Macular and perimacular lesions are more common than lenticonus, occurring in a highly variable proportion of affected family members (79,98,165–167,170–172). These changes occur more frequently in males (79,170), and in the context of a personal or family history of hematuric nephritis, are almost specific to Alport syndrome. Yellowish or whitish spots around the macula or loss of the foveal reflex with alterations in macular pigmentation are common appearances, although more peripheral pigmentary disturbances, either white or dark, can also occur (165). The lesions may be described as fundus albipunctatus. Visual acuity is unaffected by the presence of these retinal lesions. They may be present when the lens is normal, but lenticonus implies that retinal lesions are almost certainly present (166). Electroretinograms and electro-oculograms are normal in patients with and without visible retinal lesions (165,172). Posterior polymorphous corneal dystrophy is closely associated with Alport syndrome in some families (168,173).

Cataracts are common but of little diagnostic value (165–168). They are usually subcapsular, but may be cortical or lamellar. Association of Alport syndrome with refractive errors other than lenticonus, particularly myopia (144,162,166, 174,175) possibly reflects common eye problems rather than genetic linkage. Spherophakia, the first ocular problem described in hereditary nephritis (162), proved to be a misdiagnosis of anterior lenticonus (144). Many ocular abnormalities listed subsequently have been noted in patients with Alport syndrome (165). Some are variants of the lesions described previously; others are likely chance associations.

Established associations are:

Anterior lenticonus
Retinal flecks
Posterior polymorphous corneal dystrophy
Cataracts
Macular holes
Possible associations
Posterior lenticonus
Various corneal dystrophies and opacities
Keratoconus
Megalocornea
Flat lenses
Arcus cornealis
Probably coincidental
Antimongoloid slant
Strabismus
Coloboma
Partial atrophy of the iris
Refractive errors
Pseudopapilledema
Retinal drusen
Retinal telangiectasia
Retinal detachment
Macular degeneration
Macula
Nystagmus
Color blindness

Esophageal and Genital Leiomyomatosis

In several kindreds (10,11,176–179) and in isolated patients (180–186), hematuric nephropathy was associated with striking muscular hypertrophy or leiomyomas of the esophagus. In females there was also hypertrophy of the clitoris, vulva, and adjacent structures (176,187). Hearing loss and cataracts were common (176–178,187), and anterior lenticonus was occasionally present (187). Alport-leiomyomatosis syndrome has been comprehensively reviewed (147,187).

Inheritance is X-linked dominant with deletions having been shown in the adjacent 5′ ends of COL4A5 and COL4A6 (179,188–190). In cases examined by electron microscopy, lamellation and granulation were seen in renal (147,176,178) but not in esophageal (147) basement membranes.

Rare or Chance Associations

Many strange associations with Alport syndrome have been described. Often the diagnosis of Alport syndrome was insecure, and in others a coincidence noted on a few cases was not confirmed in larger studies. Further examples are needed to confirm these associations, which are collated in Table 19-3.

Familial nephritis with iminoaciduria has been described in eight families (97,191–197), but the association of hyperprolinemia and glomerulonephritis is likely fortuitous. Phang and Scriver (198) emphasize that inheritance of renal disease was dominant in the archetypal family (192,196), whereas that of hyperprolinemia was recessive. Other reports are old or describe small kindreds. No diagnostic renal electron microscopy studies are available.

Diagnosis

The path to the correct diagnosis lies through a carefully collected, extended family history and personal examination of the urinary sediment, specifically for hematuria. The proband will often be a child with unexplained hematuria or an adolescent to middle-aged male with ESRD, with a vague history of kidney disease in brothers or relatives on the maternal side. Systematic urinalyses may reveal several relatives with hematuria.

There are several points to remember:

Microscopy of urine sediment—do it yourself!
Family history—extend to as many generations and collaterals as possible.
Age of ESRD in males helps to clarify the phenotype.
Female gene carriers—look for hematuria, although not all carriers have it.

When a member on the line of descent in a well-studied kindred is found to have hematuria, a renal biopsy is generally superfluous. The poorer the family history and the more remote the nearest affected relative, the stronger the case for a biopsy. Typical extrarenal features, specifically anterior lenticonus, retinal pigmentary changes, and to a lesser extent hearing loss, in the patient or family strengthen the presumption of Alport syndrome and diminish the need for a biopsy. Unless male-to-male transmission has been well documented in the kindred concerned, there are strong grounds to perform a biopsy on the apparently affected son of an affected man. In a family with a defined mutation, demonstration of this mutation in an individual defines him or her as a gene carrier. Linkage studies can identify gene carriers with near certainty in large families, but this is frequently not practical for routine diagnosis.

From time to time, a renal biopsy in a patient with hematuria will unexpectedly reveal ultrastructural features of Alport syndrome. Naturally this finding will prompt a search for affected relatives. Diagnoses in persons without positive family histories often result from new mutations. Without a family history or proof of a mutation, a secure diagnosis is impossible

TABLE 19-3

RARE ANOMALIES IN ALPORT SYNDROME THAT ARE LIKELY TO BE MERE CHANCE ASSOCIATIONS

Anomaly	Reference
Anorectal anomaly	(341)
Asthma or allergy	(89)
Cancer	
Wilms' tumor	(195)
Chromosomal abnormality	
Supernumerary	(95,363)
Truncation of a short arm	(30,157)
Turner's syndrome	(364)
Circulatory system	
Aortic medionecrosis, deformed aortic valve	(208)
α-Thalassemia minor	(365)
Capillary fragility	(366)
Congenital heart disease	(195)
Rheumatic heart disease	(27,367)
Ruptured intracranial aneurysm	(368)
Cryptorchidism	(157)
Dentition, absent or delayed	(160)
External ear	
Deformed pinnae	(369)
Preauricular tag	(341)
Fibromuscular dysplasia	(370)
Growth retardation	(160)
Hyperprolinemia	See text
Immunoglobulin A deficiency	(371)
Kidney	
Membranoproliferative glomerulonephritis, type I	(372)
Proximal tubular defects	(373,374)
Tubular acidosis	(375)
Marfan-like features	(162)
Nervous systems	
Central nervous system disease/epilepsy/electroencephalographic abnormality	(195,376)
Mental retardation and macrocephaly	(79,377)
Peripheral neuropathy	(378)
Psychosis	(379,380)
Retrobulbar neuritis	(381)
Situs inversus	(374)
Skin	
Epidermolysis bullosa	(365)
Ichthyosis	(194)
Spinal anomalies	(197)
Thyroid	
Antithyroid antibodies	(371,382,383)
With thyroiditis, hypothyroidism, goiter	(383,384)

because the biopsy findings are never absolutely specific. The family history may eventually appear, although males with juvenile Alport syndrome are unlikely to sire children.

Juvenile and adult types of Alport syndrome cannot be distinguished with certainty in kindreds with fewer than two examples of ESRD in males, but there are suggestive nonrenal indicators. Normal hearing suggests an adult type of Alport syndrome or thin GBM disease. The high prevalence of hearing abnormalities in the general population and the uncertain kinetics of progressive hearing loss in Alport syndrome limit the value of audiometry except in large kindreds. Eye defects, especially anterior lenticonus, appear limited to the juvenile types of Alport syndrome. Thrombocytopenia and male-to-male transmission are features of AD-AS.

Of great interest are the forthcoming genetic methods of diagnosis. Mutations in COL4A3-5 are currently demonstrable in a sizable minority of kindreds and MYH9 mutations exclude the diagnosis of Alport syndrome by establishing an alternative etiology. Emerging techniques with microsatellite markers within collagen genes, exon scanning, single-strand DNA fragment conformational analyses, and direct sequencing of COL4A3-5 should soon provide specific genetic tests for gene-carrier status in most families.

Treatment

Kidney Disease

No specific treatment is known to affect the underlying pathologic process or to alter the clinical course of kidney disease. Antibiotics, anticoagulants, steroids, and immunosuppressives have in general wrought no benefit, although in one uncontrolled series cyclosporine appeared to be surprisingly effective (199). Control of hypertension is mandated on general grounds and protein restriction may prove to be of value once nephron loss gives rise to hyperfiltration. Management of advancing renal failure is along conventional lines. When terminal uremia occurs, dialysis and transplantation pose no particular problems, although the lack of certain GBM antigens invites a slender risk of de novo anti-GBM nephritis after transplantation (116–121,200). Except for one unconvincing example (201), the glomerular defect of Alport syndrome has not recurred after transplantation (117). Particular care must be taken in the selection of living donors; meticulous and repeated urinalysis for hematuria is the most important step. Specific genetic tests will be increasingly helpful.

Hearing and Vision

Great care should be taken to avoid adding insults from drug ototoxicity to the advancing aural injury. Improvement or stabilization of hearing loss in Alport syndrome patients has occasionally been noted after transplantation (79,202); others noted no benefit (203), nor have we. Interpretation of these findings is difficult because dialysis or the uremic state (204) has been blamed for reversible hearing loss. When hearing loss worsens, the patient will become more dependent on lip reading and other visual cues. We have observed fair success with hearing aids. Visual acuity should be monitored at intervals in those with or at risk of lenticonus and consideration given to early lens extraction and intraocular lens implantation (177). Steroid doses should be kept low after transplantation, and patients monitored regularly for cataracts: poor vision is a disproportionate handicap to the deaf.

Genetic Counseling

Inheritance will be dominant in most families. As a group, men with Alport syndrome have about 30% fewer children than do men without Alport syndrome; many men with juvenile-type disease will sire no offspring.

Incomplete penetrance of Alport syndrome in females must always be kept in mind (205). In kindreds with X linkage, daughters of affected males will all be gene carriers regardless of their urinalysis results. Each clinically normal daughter of the three other sorts of dominant gene carriers (mothers in kindreds with X linkage, and parents of either sex in kindreds with autosomal dominance) has a 50% chance of having an undetected nephritis gene. Information from genetic tests or

from urinalyses of the next generation may help decide whether these females have inherited a gene.

CONDITIONS THAT CAN BE CONFUSED WITH ALPORT SYNDROME

Conditions with Hearing Loss

Many conditions affect both the ear and the kidney (Table 19-1), perhaps because of simultaneous embryogenesis or because of structural and physiologic homology (206).

Autosomal-Dominant Alport-Like Syndrome Caused by Mutations in MYH9

Epstein and colleagues (26) described a syndrome of hereditary macrothyrombocytopenia and deafness that had clear parallels with Alport syndrome, but in neither the original kindred nor in subsequent descriptions were ultrastructural or immunological renal features of Alport syndrome shown (26,27, 29,31,32,34,35,37–39,51,207,208). The phenotype was expanded to include patients with granulocyte inclusions (Fechtner syndrome) (35). Clarity was brought to the classification by the demonstration that Epstein syndrome, May Heggelin anomaly, Fechtner syndrome, Sebastian syndrome, isolated macrothrobocytopenia, and nonsyndromic hereditary hearing loss (DFNA17 phenotype) were all caused by mutations in the MYH9 gene at 22q11.2 (44–47,54,55,209).

Clinical and Laboratory Features of MYH9 Disorders

Although some genotype-phenotype correlations have been described (46,54,55), there is considerable overlap between these syndromes. Most patients with overt renal disease had impaired hearing, and cataracts were common in patients with Fechtner syndrome (35,39).

In patients with macrothrombocytopathic thrombocytopenia, bleeding complications are customarily mild, although severe bleeding after renal biopsy has occurred (34), as have intracranial hemorrhages in patients with terminal uremia (26, 27,32,34). The platelet count is usually between 25,000 and 100,000 per μl and responds to neither steroids nor splenectomy. Bleeding times may be normal or substantially prolonged, even in nonuremics. Childbirth is possible without excessive blood loss and major operations have been performed without incident, usually after platelet transfusion.

Specific renal ultrastructural features include focal and segmental effacement of podocytes and loss of the interpodocyte slit diaphragm (51). Immunohistochemistry showed apical localization of NMMHC-IIA in tubular epithelia and reduced podocyte staining, in contrast to the diffuse localization in normal epithelia (51).

In families with giant platelets, the mean platelet diameter has varied from 3.6 to 11.0 mm (26,27,30,32,34,35,38,208). Platelet aggregation may be normal or impaired and platelet factor 3 availability either increased or decreased. Clot retraction is normal or only slightly impaired. Thrombocyte content of purine nucleotides is increased, but probably only in proportion to the volume of the platelets.

Platelet ultrastructure being variably reported as normal (27,35) or disorganized (26,30,32,34,38,208). By light microscopy, "Fechtner" granulocytic inclusion bodies resemble toxic Döhle bodies and May-Hegglin inclusions (35,39, 46,207), but they are smaller and stain less well (210). Ultrastructurally, the inclusions contain clusters of ribosomes and small segments of rough endoplasmic reticulum (35) and are distinct from May-Hegglin inclusions which contain parallel longitudinal filaments (48).

In affected families granulocytes may (41,129,131,132,165) or may not (128,130) show Fechtner inclusion bodies. Cataracts have been frequent findings in patients with the Fechtner syndrome (41,132). The Sebastian syndrome (168) shows hematologic anomalies identical to those of the Fechtner syndrome but lacks renal, ocular, or auditory disease.

Charcot-Marie-Tooth Disease and Nephritis

Peroneal muscular atrophy and renal disease have been found together in three small kindreds (211,212) and in ten sporadic cases (213–217). About half the patients had hearing loss. Renal biopsy specimens generally showed focal segmental glomerulosclerosis; GBM splitting and granulation typical of Alport syndrome were not seen.

Hereditary Interstitial Nephritis with Hearing Loss

One quite large kindred with autosomal-dominant inheritance of high-tone hearing loss, proteinuria, casts, and pyuria, but without hematuria or infection has been described (218). Biopsy findings in four siblings were interpreted as interstitial nephritis. Renal impairment was generally mild although renal failure supervened late in life in four men and one woman. Familial reflux nephropathy (see Chapter 24) was not excluded as a possible cause of this disorder.

X-Linked Progressive Hearing Loss

These are rare disorders that can mimic the kinetics and audiologic features of adult-type Alport syndrome. At least two forms segregate to loci at Xq21 and Xq22, close to the site of COL4A5 (219,220).

Conditions with Normal Hearing

Familial Thin GBM Nephropathy (Benign Familial Hematuria, OMIM 141200)

Familial hematuria does not always have an ominous prognosis and large families in which renal impairment never occurred have been described (221,222). Early descriptions of "benign recurrent," "primary," or "essential" hematuria were imprecise and included a variety of conditions. Rogers and colleagues (222) delineated an entity characterized by uniform thinning of the GBM and prolonged survival without deterioration of renal function. Familial thin GBM nephropathy appears the best name for this condition once biopsy has shown the ultrastructure in a family. Alternatives, such as "benign familial hematuria" or "benign recurrent hematuria" imply knowledge of prognosis, which is not available at diagnosis.

Familial thin GBM disease displays autosomal-dominant inheritance (223,224), although in some areas sporadic cases are extremely frequent (225). Most cases have been detected in childhood although hematuria with erythrocyte casts persists throughout life. Hematuria is usually microscopic and continuous, but may be punctuated by episodes of visible hematuria, particularly during or after an upper respiratory tract infection. As a rule, hearing is normal even by audiometry, there is no proteinuria, blood pressure is normal, and renal function is unaltered (222,226,227). Flank pain occurs in some patients (226–228), and the distinction of these cases from the so-called loin pain–hematuria syndrome is vexatious. Serum complement is normal (222), and imaging of the urinary tract shows normal anatomy.

Renal biopsy specimens, apart from the presence of erythrocytes in Bowman's space and in tubules, appear normal by

light microscopy (222,229) or show minimal mesangial proliferation and an increase in mesangial matrix. Immunofluorescence examination reveals normal findings (222,229) or occasionally shows focal and segmental deposits of immunoglobulins and complement in glomeruli (230). An Australian series (228) noted prominent deposits of the third component of C3 in arterioles. In thin GBM disease, the GBM stains continuously, albeit perhaps less intensely than normal, with anti-NC monoclonal antibodies. This property has been used to distinguish between Alport syndrome and thin GBM disease (125,231,232) and may become a useful diagnostic aid. The characteristic ultrastructural finding is uniform thinning of the lamina densa of the GBM. The overall width of the GBM is reduced by about one-third to approximately 200 nm (222,229,230,233–235) (Fig. 19-2B). Breaks may be seen in the GBM (222) through which red cells can cross (236). There are no alterations in cellularity, no deposits, and foot process fusion is not observed.

Even with adequate material for high-resolution electron microscopy, the distinction from Alport syndrome is not always clear. Biopsy tissue removed early in Alport syndrome can show areas of uniform attenuation, but abnormally thick areas will usually be found if sufficient search is made (79,84,230). Piel and co-workers (237) report some degree of lamellation of the GBM in 14 of 20 patients with thin GBM disease; details of family histories were not given and follow-up was limited to a mean of 6.7 years, during which renal failure developed in one patient. Because renal failure in males with Alport syndrome may occasionally be delayed until after the age of 50 years (8,89,158,175,238–240), many or all of these patients could have had Alport syndrome. Nevertheless, the paradigm of Alport syndrome shows that different mutations even within the same gene can cause renal and auditory effects differing both in type and in degree. Occasional cases of thin GBM disease with hearing loss (237,241) or renal insufficiency (126,237,241) may be examples of pleotropism of thin GBM disease rather than misdiagnoses of Alport syndrome.

In practice, until specific immunologic or genetic markers become available, features that help distinguish thin GBM disease from Alport syndrome include male-to-male transmission, normal hearing and longevity of several affected family members, and wholly characteristic biopsy findings in at least one member of the family. Abnormal EBM or GBM reactivity for the a5(IV) collagen chain can help distinguish cases of Alport syndrome.

Sporadic, thin GBM disease has been described as frequently or more frequently than the familial disease in some series (126,242,243). In Melbourne, Australia, thin GBM disease is the most common cause of isolated microscopic hematuria (227). Using systematic morphometry in renal transplant donors, Dische and colleagues (225) report thin GBMs in 5.2% to 9.2% of the population. If confirmed, this finding will cast considerable doubt on the significance of a thin GBM as the cause of hematuria in a patient with sporadic disease unless casts or altered erythrocytes are also seen in tubular lumina. If thin membranes are generally as common as these reports suggest, then the condition might reasonably be regarded as a variant of normal. If other features such as flank pain are associated, they might reasonably be regarded as coincidental and an alternative physical or behavioral cause for pain should be considered.

Familial Immune Nephritis

A familial incidence is occasionally noted in many forms of glomerulonephritis, including immunoglobulin A (IgA) nephropathy (244,245), systemic lupus erythematosus, membranous nephropathy (246–252), IgM mesangial proliferative glomerulonephritis (253), focal segmental glomerulosclerosis (254–263), membranoproliferative glomerulonephritis (264–271), and partial lipodystrophy (272,273). Congenital complement deficiencies, most commonly C3 (274), predispose to membranoproliferative nephritis.

Kourilsky and colleagues (275) described glomerulonephritis in a mother, son, and daughter. All had proteinuria. Hemolytic–uremic syndrome and malignant hypertension developed in the son at age 24 and a bilateral nephrectomy was performed. Examination of biopsy samples showed large mesangial deposits containing complement in all three patients, although circulating complement components were repeatedly normal. Similar deposits recurred in a cadaver allograft.

Familial Nephropathy and Tumor Syndromes

The Wilms' tumor suppressor gene (*WT1*) at 11p13 is a transcriptional regulator of many growth factor genes (276), and inactivation of both *WT1* alleles is important in tumorogenesis (277). The gene consists of 10 exons with a complex pattern of alternative splicing (276). Mutations of *WT1* produce three distinct but related syndromes (276–278). Deletions of the whole gene or insertions or mutations in exon 1 that result in a truncated protein are frequently associated with the WAGR syndrome of Wilms' tumor, aniridia, genitourinary malformation, and mental retardation (277). Point mutations within exon 9 in the region of the gene encoding the zinc finger domains result in the Denys-Drash syndrome of diffuse mesangial sclerosis, early progression to ESRD, XY pseudohermaphroditism, and a high risk of Wilms' tumor (277,279,280). Diffuse mesangial sclerosis in Denys-Drash syndrome frequently occurs before the age of one year (281). Mutations in intron 9 affecting the KTS exon 9 donor splice site can result in Frasier syndrome of focal segmental glomerulosclerosis, XY pseudohermaphroditism, streak gonads and propensity to gonadoblastoma (279,281–283). Denys-Drash and Frasier syndromes appear to be closely related and mutations that might be expected to cause one at times produce the other. In Denys-Drash and WAGR syndromes, Wilms' tumor commonly occurs at a younger age than sporadic Wilms' tumor (279) but Wilms' tumor is very uncommon in Frasier syndrome (281). The cumulative incidence of renal failure by 20 years after Wilms' tumor diagnosis was 62% in Denys-Drash syndrome and 38% in WAGR syndrome (280). Formes frustes of the *WT1* syndromes may present as apparently normal females with infantile nephrotic syndrome but without other syndromic features (284,285). These are important to recognize because of the potential for development of genitourinary and other tumors in the patient or offspring. Five percent of males and 9% of females with idiopathic steroid-resistant nephrotic syndrome had a *WT1* mutation in a recent series, whereas none of 110 cases of steroid-sensitive nephrotic syndrome carried a mutation (286). In another series, idiopathic nephrotic syndrome segregated with the *WT1* locus, although patients with HIV-1 associated nephrotic syndrome segregated negatively at this locus (287).

A second Wilms' tumor gene, WT2, at 11p15.5 is linked to the Beckwith-Wiedemann syndrome of exomphalos, macroglossia, gigantism, hemi-hypertrophy, and a tendency to develop Wilms' tumor, rhabdomyosarcoma, hepatoblastoma, and adrenal adenoma or carcinoma (276,288,289). Renal abnormalities in this syndrome include nephromegaly, calyceal abnormalities and renal cysts (290,291).

Hereditary Interstitial Nephritides

Familial Juvenile Hyperuricemic Nephropathy. In this entity, well reviewed by Cameron and Simmonds (292), hyperuricemia and sometimes gout appear in childhood or

adolescence with progression to uremia by the third to the fifth decade. Isosthenuria and hypertension develop, but proteinuria is absent or slight and the urine sediment is normal or contains only a few urate crystals or epithelial cells. The chronic interstitial nephritis is histologically nonspecific. Although intratubular crystal deposits may initiate the interstitial nephritis (293), uric acid crystals are rarely seen in biopsies (293,294). Inheritance is usually autosomal dominant and in up to 50% of cases is associated with mutations in the UMOD gene at 16p11-12, the same gene that is mutated in some cases of medullary cystic disease (292). Early in the disease, plasma urate concentration may appear normal although diagnoses have been delayed by failure to use reference values appropriate for the patient's age and sex. Purine pathway enzyme levels in erythrocyte lysates are normal (295–297), distinguishing this condition from the Lesch-Nyhan syndrome. Striking purine underexcretion appears to be a constant feature (292). Reducing serum uric acid levels early in the progression of the disease is effective in halting progression (292), but is ineffective later (297–299). The pathogenesis of the nephropathy is debated: a defect in renal blood flow may lie at the root of both hyperuricemia and progressive renal damage; hyperuricemia may incite renal fibrosis by a mechanism unrelated to crystal deposition (300,301); or abnormal urate handling may result from abnormal regulation of nephrogenesis (302). Because of prominent apoptosis of proximal tubule cells, it has been suggested that a gain of function mutation of the proximal tubule luminal anion exchanger could explain reduced uric acid excretion (303).

Familial Interstitial Nephritis without Hyperuricemia

The family described by Stabellini et al. (304) is the best characterized of this rather imprecise group. Renal biopsy specimens from three of six affected family members showed tubular atrophy, interstitial fibrosis, mononuclear cell infiltrates, and severe vascular lesions.

Familial Interstitial Nephritis with Hypokalemia

The association of hypokalemia, sodium wasting with high plasma renin levels, and interstitial nephritis is an autosomal recessive, possibly human leukocyte antigen-linked trait (305,306). Glomerular function is usually unaffected although uremia developed in the three siblings of one family (305).

NEWLY DESCRIBED INHERITED DISEASES OF STRUCTURAL GLOMERULAR PROTEINS

Collagen III Glomerulopathy

Also known as collagenofibrotic glomerulopathy, this new type of hereditary glomerulopathy was initially seen in 10 children with progressive glomerular disease (307). Hypertension was common and several children and their siblings had hemolytic anemia. Some also had pulmonary disease. Light microscopy showed a diffuse increase in the mesangial matrix and generalized widening of the capillary walls. Ultrastructurally, fibrillar collagen was found within the mesangial matrix and the subendothelial aspect of the GBM, adjacent to normal lamina densa. Immunohistochemical studies identified the fibrillar collagen as type III collagen. Neither affected children nor their relatives showed clinical features of nail-patella syndrome (NPS). The tendency to early renal failure, the possible extrarenal features, and the transmission as an autosomal-recessive trait strongly suggest that collagen type III glomerulopathy (307–313) is a distinct hereditary disease.

Fibronectin Glomerulopathy

Families from Europe, the United States, and Asia have now been described with a lobular glomerulonephritis and deposition of PAS-positive and Congo red-negative material in the mesangium and subendothelial space (309,314–321). The deposits stain bright red with acid fuchsin-orange G. Electron microscopy confirmed the deposits shown by light microscopy. The mesangial and subendothelial osmiophilic deposits were generally granular and homogeneous; rarely, fibrils 12 to 16 nm in diameter were seen, but the appearances were not suggestive of amyloidosis. In most patients studied, the deposits stained only weakly for immunoglobulins and complement, but strongly and consistently for fibronectin (319). Nearly all patients had proteinuria, often in the nephrotic range, and about half had microhematuria. Serum creatinine level was usually near normal at diagnosis, and tended to rise slowly or not at all. Inheritance is autosomal dominance (317,318) and linkage to 1q32 has been reported (316).

FABRY DISEASE (OMIM 301500)

Fabry or Anderson–Fabry disease was identified over a century ago, in 1898.This inborn error (also called angiokeratoma corporis diffusum, ceramide trihexosidosis) is a rare metabolic disorder that particularly affects vascular endothelium, leads to renal, cardiac, and cerebrovascular manifestations and early death. Fabry disease is the only known X-linked sphingolipid storage disease. Lack of α-galactosidase (also termed *ceramide trihexosidase*) (E.C. 3.2.1.22; GalA), a lysosomal hydrolase crucial in glycosphingolipid metabolism, causes accumulation of neutral glycosphingolipids in many tissues.

Epidemiology

The disease is panethnic, and estimates of incidence range from about 1 in 40,000 to 60,000 males (385,386) but may be higher (387). Fabry disease predominantly affects males, although carrier (heterozygous) females also can be affected to a mild or severe degree because of random X-chromosomal inactivation (388). That most reported patients have been white may reflect reporting bias rather than a true racial difference.

Genetics

Inheritance is X-linked and the gene coding for α-galactosidase A (GLA) is situated at Xq22.1 on the long arm of the X chromosome, just centromeric to the Alport syndrome locus. The GLA gene has been cloned and its DNA sequence determined. It is a relatively small gene of ~12-kb containing seven exons. To date, over 300 mutations that cause Fabry disease have been identified, including missense, nonsense, small deletions and insertions, large gene rearrangements, and splice mutations. The mutations are spread throughout the entire GLA gene, and most are "private," occurring in one or a few affected families (389–395). Most mutations result in the typical phenotype, but five missense mutations produced signs and symptoms confined to the heart, or no symptoms at all (392).

Fewer than 5% of families with Fabry disease have had large mutations demonstrable by Southern blots, implying that most mutations are single-base changes or small insertions,

deletions, or rearrangements. For further details, Eng and colleagues provide an excellent review (392).

Normally, α-galactosidase is synthesized as a precursor protein that is then glycosylated in rough endoplasmic reticulum. A signal peptide splits from the glycosylated precursor protein, which then undergoes further glycosylation in the Golgi apparatus. This mature, heavily glycosylated protein then moves to its site of action in lysosomes. Errors can occur at many of the steps in this complex process. The results may be total lack of a precursor, formation of an unstable precursor, abnormal processing of a normally sized but subtly abnormal precursor, or normal processing of a mutant enzyme. Several of these specific errors have been linked to particular phenotypes. For example, whereas most mutations produce the full classic phenotype, hemizygotes with some missense mutations may be asymptomatic or have disease limited to the heart (392,396).

A structure-based human a-Gal A model was generated, and used to locate various a-Gal A mutations that caused Fabry disease (397). Genotype–phenotype correlations are complex in Fabry disease, as the same mutation can lead to both classic and atypical disease, even within the same family (389,398). Patients with atypical variants of Fabry have been found to exhibit missense mutations that lead to a reduction, but not absence of α-galactosidase A activity (399).

Pathogenesis

Alpha-galactosidase hydrolyzes neutral glycosphingolipids with terminal α-galactosyl residues. If the enzyme is defective, several glycosphingolipids, particularly globotriaosylceramide (ceramide trihexoside), will accumulate.

Pathology

Kidney

Glycolipids accumulate in blood vessels, glomeruli, and vascular smooth muscle, and to a lesser extent in tubular epithelium. Lipids deposited in vascular endothelium and glomerular visceral epithelial cells are dissolved out with routine processing. The cells that contained them appear as foam cells. If the lipids are preserved in frozen sections, they can be stained with fat stains such as Sudan III or oil red O. Likewise, the glutaraldehyde fixation and epoxy embedding for electron microscopy preserve fat, which is readily visible in toluidine blue-stained sections (Fig. 19-6). Electron microscopy shows striking stacks or whorls of dense, flat, osmiophilic inclusions in the lysosomes of blood vessels, and glomerular (Fig. 19-7) and tubular epithelial cells with a periodicity of 35-50 Å. These myeline bodies are 1 to 3 μm in diameter, showing a characteristic "zebra" or "onion-skin" appearance (400,401).

Nonrenal Tissues

The most striking changes are in blood vessels and are similar to those just described for the kidney. Thromboses can occur in many organs and seem to occur as a result of platelet aggregation on areas of sphingolipid accumulation in the endothelium. Tissue infarction, the consequence of vascular occlusion, has no features specific to Fabry disease. Aside from ischemic sequelae, the heart may show glycosphingolipid deposition in myocytes and valvular fibrocytes (396). All four chambers of the heart may enlarge, the mitral and tricuspid valves thicken, and the mitral valve may prolapse. Cerebral vessels are strikingly involved; glycosphingolipid accumulation in neural tissue is confined to the perineurium of peripheral nerves, autonomic neurons, and some primary somatic afferent neurons.

Clinical Features and Course

Extrarenal Manifestations

In childhood and early adolescence, affected males experience agonizing pains in the limbs, more marked distally (385). Pain can be chronic and/or acute with episodic crises often precipitated by changes in temperature, exercise, or stress. They occur in 80% to 90% of male patients in the first decade and about 10% to 70% of female patients, often in the later course of the disease (402,403). A typical pattern of pain is acroparesthesia or burning sensations in the palms of the hands or soles of the feet. They become less intense or may even disappear in later life. In severe attacks, pain radiates proximally and may even simulate an acute abdomen. The pain is a result of damage of small nerve fibers caused by diffusion into and accumulation of Gb3 in the nerve axons and dorsal root ganglia. The diffusion is related to the surface area of the axons, although the myelin sheath may provide some protection. As the longest axons are those with the largest surface area this can explain why the pain occurs initially in the periphers (404). Hypohydrosis is a nearly constant feature. Angiokeratomas, apparent as slightly raised, cherry red to black, nonblanching macules or maculopapules 1 to 3 mm in diameter, are small hyperkratotic areas of dilated blood vessels, appear in adolescence and progressively increase on the lower trunk and back, being particularly marked on the scrotum and bathing suit area (Fig. 19-8). Usually they appear in groups or in generalized forms but they also can be isolated. At least minimal angiokeratomas can also be observed in about 30% of carrier females (403). Angiokeratomas are not specific of Fabry disease, as they occur also in other lipid storage diseases, and their absence does not rule out a diagnosis of Fabry disease (405).

Cardiac and cerebral (406) ischemic episodes are remarkable only for the early age at which they occur; strokes and cardiac infarction during the third and fourth decades are fairly common. Congestive heart failure and severe mitral regurgitation are major complications and can be fatal (407,408). There are no differences in valvular changes between hemi- and heterozygotes, and so both genders are affected with more or less the same incidence (405). Cardiac rhythm and conduction disturbances may also occur (396). Fabry disease apparently confined to the heart may account for 10% of patients with left ventricular hypertrophy without obvious cause (396).

Cornea verticillata is a distinctive whorled corneal opacity (409,410) that is closely similar to the opacities that can occur with prolonged chloroquine or amiodarone therapy. Opacities appear within the first few years of life and are generally quite asymptomatic. Even the posterior radial cataracts that eventually occur in 50% of patients scarcely interfere with vision. Retinal vascular occlusion and ischemic optic atrophy may cause visual loss (411,412).

Female heterozygotes have variable but generally mild manifestations; rarely can they be as severely affected as males. Surprisingly, about four of five females show corneal opacities (413).

Renal Manifestations

Female Heterozygotes. In heterozygous female patients, manifestations are highly variable. They take the same form as in male patients, but are generally milder (414). Nevertheless, end stage renal disease (ESRD) can occur (415).

Male Hemizygotes. Urinary concentration defects may be the earliest functional manifestation of Fabry renal disease, leading

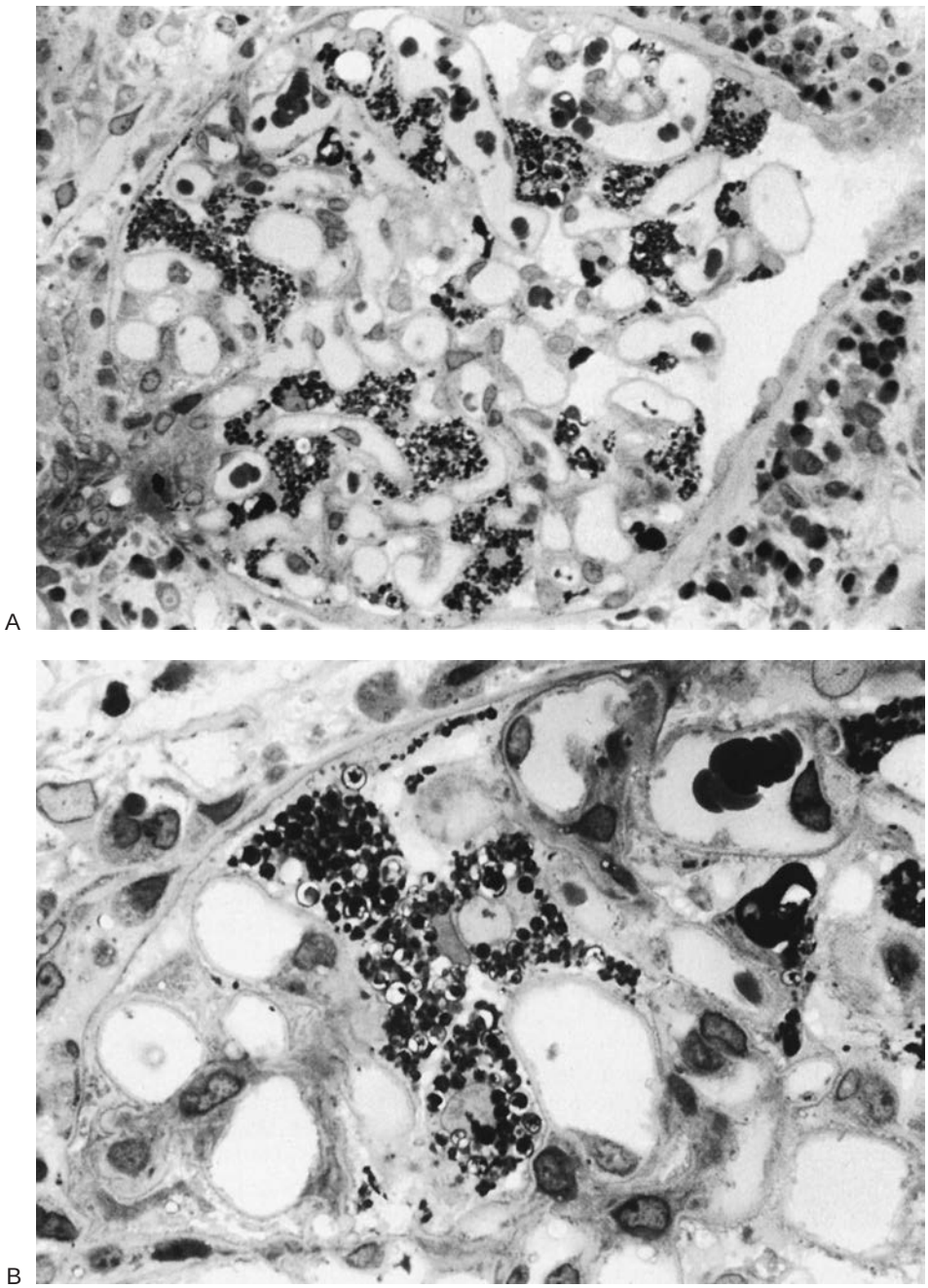

FIGURE 19-6. A: Foam cells in renal glomerular epithelium in Fabry disease. (Magnification ×400.) **B:** Lipid deposits in renal glomerular epithelium. (Magnification ×1,000.) (Glomerulus embedded in epoxy resin and stained with toluidine blue.) (Photographs courtesy of Dr. Melvin M. Schwartz, Rush-Presbyterian-St. Luke's Medical Center, Chicago.)

to polyuria and nocturia. Nephrology referral is more typically initiated because of the development of proteinuria. Proteinuria may begin in the teenage years and becomes more frequent when patients reach their 20s and 30s (rarely in nephrotic range) (416). The progression to ESRAD is common in 3rd to 5th decade of life (417). Hypertension is a late manifestation and usually after onset of renal failure (418). Usually Fabry patients show glomerular hyperfiltration rate similar to diabetic nephropathy (419). Additionally, nephrogenic diabetic insipidus (420), distal renal tubular acidosis (421), and impaired ammoniagenesis (422) have been reported. Microscopy of the urine sediment may show red blood cells, renal tubular cells, casts, and lipid. Lipid droplets with their characteristic "Maltese cross" appearance under polarization microscopy may be found even when proteinuria is slight. Electron microscopy of the urine sediment may show "myelin bodies" morphologically identical to those seen in the lysosomes of renal tubular cells on renal biopsy specimens (Fig. 19-9).

Diagnosis

The most important step is to consider the diagnosis of Fabry disease. The index patient in most families goes undiagnosed for years or decades. In affected males with fully expressed disease, the constellation of symptoms and signs is highly suggestive. Slit-lamp microscopy will confirm the diagnosis in nearly all patients. A certain diagnosis can be made by the determination of the α-galactosidase A activity in blood leukocytes and Gb3 (globotriaosylceramide) measurement in urine and blood (416). Biopsy, for example of kidney or skin, typically shows lipid deposition and, in electron micrographs, multilamellar

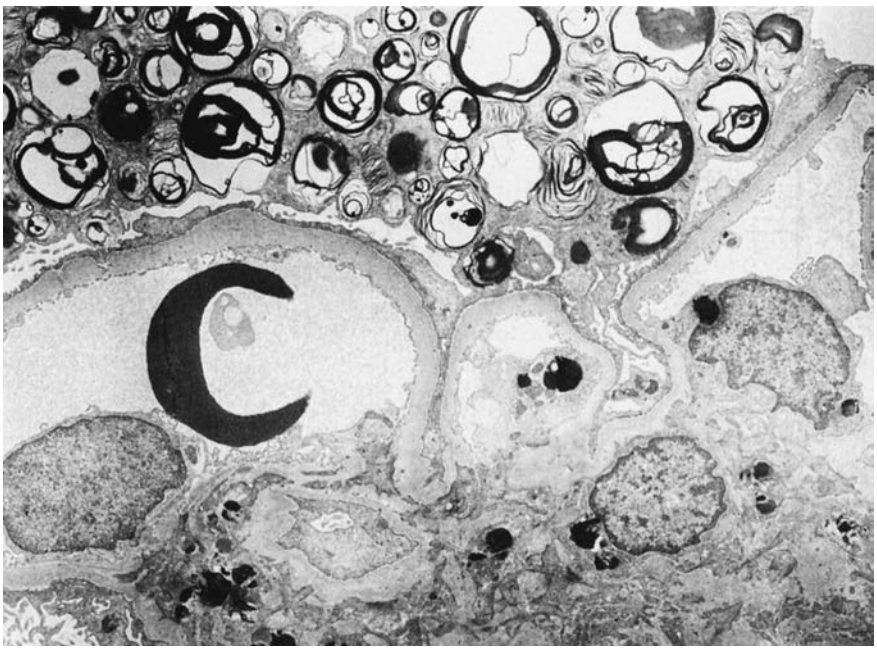

FIGURE 19-7. Myelin figures in Fabry disease. Electron micrograph of glomerular capillary loops with ultrastructural changes characteristic of Fabry disease. (Magnification ×5,500.) Many whorled "myelin figures" or "zebra bodies" are visible in glomerular visceral epithelial cells. (Photograph courtesy of Dr. Daniel Terreros, Salt Lake City VA Medical Center, Salt Lake City, Utah.)

myelin bodies (423). Genetic testing detects the mutation in *GLA*, the gene coding for α-galactosidase A (424). Family screening is helpful in order to identify additional cases in general and patients who have an atypical course of the disease or are of young age with just developing symptoms in particular.

Prenatal Diagnosis

The severity of Fabry disease in males is the principal motivation for prenatal diagnosis. Because of the relatively mild involvement in females, prenatal diagnosis is relevant chiefly for the offspring of an affected woman and may start with determination of gender. Enzymatic analysis of chorionic villous tissue or of cultured amniotic cells can confirm or exclude the disease in a male fetus; confirmation of carrier status in a female is tricky, but possible (425). Prenatal diagnosis can also be done by DNA mutation analysis, if the mutation has been identified in the family. They can be performed on chorionic villi or cultured amniocytes (426).

Implication of New Knowledge for Genetic Counseling

Counseling should be based on information that is as precise as possible. The status of relevant family members should be determined. For example, is a woman in the line of descent who seeks advice truly heterozygous or just at risk, or perhaps through nonpaternity or another explanation, not a carrier at all? The severity of the phenotype in the family should be defined as accurately as possible, by obtaining information about many affected family members. If possible, the mutation should

FIGURE 19-8. Angiokeratomas of the anterior abdomen of a man hemizygous for Fabry disease.

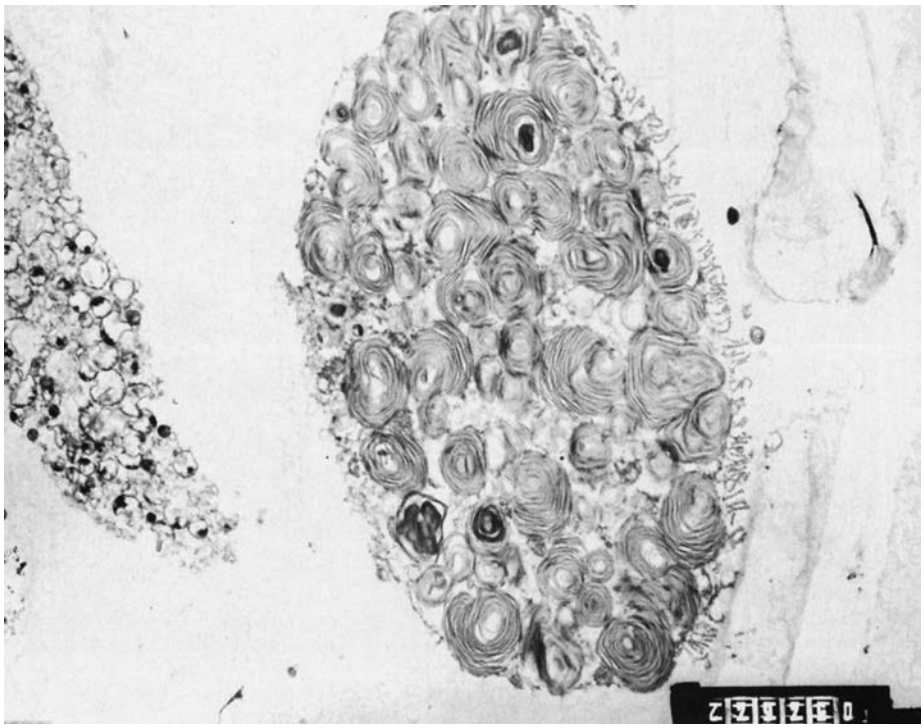

FIGURE 19-9. Urinary myelin bodies. Electron micrograph of a cell in the urine of a female heterozygous for Fabry disease. It is filled with myelin figures. (Uranyl acetate and lead citrate stain, ×20,000.) (Photograph courtesy of Dr. Melvin M. Schwartz, Rush-Presbyterian-St. Luke's Medical Center, Chicago.)

be defined, because this facilitates both precise diagnosis in uncertain cases or in a fetus at risk, and because it may help predict the clinical severity of the disease.

When one is informing couples considering termination of pregnancy of the chances of various outcomes, it is well to bear in mind that therapy has changed in the last few years and is likely to advance rapidly. Although gene therapy is not yet practical, enzyme replacement is feasible now; however, the cost of the therapy should be considered as well.

Treatment

Until recently, Fabry disease management was limited to symptomatic and palliative treatment, but this has changed with the availability of the recombinant human a-Gal A enzyme, agalsidase. Two different agalsidase formulations have been obtained: one from human fibroblast (agalsidase a), and one from Chinese hamster ovary (CHO) cells (agalsidase b). Both preparations underwent clinical trials that documented the feasibility, efficacy, and safety of the treatment. Based on observations made during these trials, both agalsidase a (Replagal) (0.2 mg/kg every other week) and agalsidase b (Fabrazyme) (1.0 mg/kg every other week) have been approved in Europe, and agalsidase b was approved recently in the United States.

A double-blind placebo-controlled trial showed that glomerular mesangial widening decreased by a mean of 12.5% for patients receiving α-gal vs a 16.5% increase for placebo. Mean creatinine clearance increased by 2.1 mL/min (0.4 mL/s) for patients receiving α-gal A versus a decrease of 16.1 mL/min (0.3 mL/s) for placebo. In patients treated with α-gal A, there was an approximately 50% reduction in plasma glycosphingolipid levels, a significant improvement in cardiac conduction, a significant increase in body weight, and also remarkable improvement in their pain control (427). In another study, 27 men and two women received Fabrazyme, and 29 men received placebo. Inulin clearance did not change from baseline in either group after 20 weeks of double-blind treatment or after 6 months of open-label treatment. However, enzyme treatment cleared microvascular endothelial deposits of globotriaosylceramide from the kidneys, heart, and skin (428).

In a long-term trial, enzyme replacement therapy (ERT) for 30 to 36 mo with agalsidase beta resulted in continuously decreased plasma GL-3 levels, sustained endothelial GL-3 clearance, stable kidney function, and a favorable safety profile (429). However, larger trials to show additional dose finding and comparative studies are needed to find the most cost-effective dose and drug. A point to keep in mind is that ERT is extremely expensive.

The debilitating pain accompanying Fabry disease may be considerably eased by phenytoin or carbamazepine. Those with valvular heart disease should receive standard antibiotic prophylaxis for dental extractions and other interventions (430). Excess glycosphingolipid can be removed by plasmapheresis (431) with temporary symptomatic benefit. Although plasmapheresis could be carried out repeatedly, this is cumbersome and expensive, and there have been no systematic trials. Chemical chaperone treatment with 1-deoxygalactonojirimycin can retard degradation of mutant enzyme and thus prolong its activity (432). This approach has been promising *in vitro*, but has not been assessed clinically, and it might be limited by side effects such as weight loss and lymphoid depletion. Inhibitors of glucosylceramide synthase can lower globotriaosylceramide levels in α-GLA knockout mice (433,434).

When renal failure supervenes, both hemodialysis and peritoneal dialysis can be carried out with no specific problems (435,436). Fabry patients are more likely to be started on hemodialysis than on peritoneal dialysis (64% vs. 34%) (437). In both the United States and Europe, Fabry patients who initiate dialysis have a worse survival compared with non-Fabry starting dialysis, even when one accounts for age (437,438). Renal transplantation successfully reverses uremia. Although survival was poor after transplantation in an early series (439), One-, five-, and ten-year graft survival in patients with Fabry disease are now equivalent to that in case-matched controls (440,441). Thus, at this time, renal transplantation should be recommended for eligible Fabry patients either

before or within a few years following initiation of dialysis (437). There are, however, particular problems in choosing living related transplant donors. Asymptomatic hemizygous males can be reliably detected by their predictably low serum α-galactosidase levels. The variability of manifestations in female heterozygotes raises greater problems. Most assays for carrier detection are about 80% to 90% sensitive, so several assays should be employed to reduce the risk of inadvertently choosing a female carrier (442). A reasonable plan is careful ophthalmologic examination together with a test for α-galactosidase activity in serum, plasma, urine, leukocytes, fibroblasts, or hair roots (443). Mutation analysis would be decisive in a family with a known mutation.

Recurrent myelin figures develop in the endothelium of normal kidneys transplanted into recipients with Fabry disease (439,443–446), nevertheless it has not been shown that disease recurrence in the endothelial cells of the allograft results in graft dysfunction or premature graft loss (441). Some clinical experiences demonstrate that ERT is safe and effective in kidney transplant recipients suffering from Fabry disease. ERT preserves renal allograft function, it improves extrarenal Fabry disease involvement, and ERT could prevent severe cardiomyopathy progression, which can be present in patients on renal replacement therapy (447). However, studies in larger groups of patients and long-term follow-up will be required to confirm these preliminary clinical observations.

Variants and Diseases Related to Fabry Disease

Recently, a variant form of Fabry disease was identified with manifestations primarily limited to the heart (396,448); these "cardiac variants" lack the classical disease symptoms, and present in the sixth or seventh decade of life with left ventricular hypertrophy (LVH) and/or cardiomyopathy. They may have proteinuria, but their renal function is typically normal for age. Of note, "cardiac variants" have residual α-Gal A activity due to missense mutations and lack the systemic vascular endothelial glycosphingolipid deposition characteristic of classically affected patients (399). Screening of 230 consecutive Japanese male patients with LVH and 153 British male patients with hypertrophic cardiomyopathy by plasma α-Gal A assays revealed that 3% and 3.9%, respectively, were previously unrecognized "cardiac variants"(396,449).

There is mounting evidence that renal disease with end-stage renal failure can also occur in absence of other typical organ manifestation as has been described in patients with solely cardiac manifestation. Such patients are predisposed to remain undiagnosed or misdiagnosed. Nakao et al. recently carrying out a large screening programme on male Japanese haemodialysis patients and identifying patients with exclusively renal manifestation suggest that such types should be defined as renal variant of the disease. Interestingly, these patients had residual α-galactosidase A activity being about 7% to 28.6% of the mean activity of healthy males and reached end-stage renal failure at an age similar to patients with full-blown disease (450).

Angiokeratoma corporis diffusum with glycopeptiduria is an autosomal-recessive disorder (451,452) resulting from deficiency of α-*N*-acetylgalactosaminidase (E.C. 3.2.1.49; α-Ga1NAc). Features are similar to Fabry disease, with angiokeratomas and peripheral neuropathy. Both infantile-onset and adult-onset forms of the disease have been described. The former is a severe disease with neuroaxonal dystrophy but without angiokeratomas or visceral lysosomal inclusions, whereas the latter manifests itself much later with angiokeratomas, mild intellectual impairment, and peripheral neuropathy (451,453).

Animal Models and Directions for Future Research

Alpha–GLA-deficient knockout mice provide a useful experimental model for Fabry disease (453).

NAIL-PATELLA SYNDROME (NPS)(OMIM 161200)

Nail-Patella syndrome (NPS) is a rare autosomal dominant disorder involving organs of ectodermal and mesodermal origin and characterized by the tetrad of dysplastic nails, hypoplastic or absent patellae, iliac horns and deformities of the elbow. It has a truly bewildering range of pseudonyms including hereditary onychoosteodysplasia (HOOD), Fong's syndrome, Turner-Keiser syndrome, and Osterreicher-Turner syndrome.

Epidemiology and Genetics

The prevalence of NPS has been estimated at 4.5 per million in United States and 2.2 per million in United Kingdom. The incidence approaches 1 in 50,000 live births (454,455). The condition maps to 9q34.1 (9,456,457) which is the site of the LMX1B gene (456). LMX1B codes for Lmx1b, a member of the LIM homeodomain protein family which is essential for wide range of developmental processes including dorsoventral patterning of the limb, differentiation of dopaminergic and serotinergic neurons, patterning of the skull and normal development of the kidney and eye (458–461). These developmental processes need two normal genes. A mutation that impairs the function of one LMX1B results in NPS; that is to say haploinsufficiency of LMX1B produces the clinical syndrome (462–464).

To date over 100 mutations have been reported in patients with NPS, but no genotype-phenotype correlation has been identified (454,456,465).

Pathology

Light microscopy of renal biopsy specimen is nonspecific and usually demonstrates normal findings. When present the only consistent but nonspecific glomerular feature is focal glomerular basement membrane (GBM) thickening (466–467). If there is clinical evidence of renal diseases light microscopy findings are related to degree of renal failure and include focal segmental glomerulosclerosis, proliferative glomerulonephritis with crescent sclerosis, and hyalinization of the glomeruli (466–469).

Immunofluorescence microscopy is negative or shows presumably nonspecific trapping of IgM, and complement (466). Staining of the GBM with MCA-P1, a monoclonal antibody directed against the α3 chain of type IV collagen, was absent in two of three patients with NPS (470). Absent reactivity with MCA-P1 is otherwise confined to patients with Alport syndrome indicating an abnormality of the GBM that interferes with incorporation of α3 chains.

Ultrastructural changes are characteristic and specific and have been found in all NPS patients. The GBM is irregularly thickened. Overlying pedicles are effaced. Patches of dense fibrillar material with periodicity of collagen are scattered throughout the entire thickness of the GBM (466,467, 469,471–473) (Fig. 19-10). Lucent areas and areas of fluffy low-density material give rise to the sobriquet "moth-eaten" basement membrane. Bowman's capsule and tubular basement membranes are not specifically affected, but the mesangium

FIGURE 19-10. Glomerular basement membrane (GBM) in nail-patella syndrome (NPS). High-resolution (magnification ×55,000) electron micrograph of the GBM showing fluffy lucencies expanding the GBM and blurring its endothelial margin. Several collagen fibrils are visibly embedded in the GBM. (Photograph courtesy of Dr. Daniel Terreros, Salt Lake City VA Medical Center, Salt Lake City, Utah.)

may show fibrillar collagen similar to that in the GBM and usually accompanied by mesangial cell proliferation. Although somehow similar mesangial lesions have been seen in other conditions such as diabetes, membranoproliferative glomerulonephritis, amyloidosis, and glomerulosclerosis, in these conditions the material is not collagen and can be distinguished by its relatively weak staining with phosphotungstic acid.

For such a rare condition, NPS has been associated surprisingly often with other glomerular lesions. These include membranous nephropathy (474), systemic vasculitis (475), IgA nephropathy (476), Goodpasture's syndrome (477), hemolytic-uremic syndrome (478), and bilateral renal stones (479). Perhaps abnormal structure or immunogenicity renders the GBM more vulnerable to other insults.

Clinical Features and Course

Exterarenal Manifestations

Patients with NPS have a lean body mass, which is more apparent in adolescents and young adults. This decrease in muscle mass in more prominent in the upper arms and upper legs. Legs often appeared short compared to the torso and head appear larger compared to the body. Other abnormalities include lumbar lordosis, less developed biceps and triceps with full developed deltoids and forearm muscles, and high forehead and male pattern hairlines in female patients (454).

The classic tetrad of NPS consists of dystrophic nails (Fig. 19-11), patellar and lateral femoral condylar hpoplasia (Fig. 19-12), hypoplasia of the radial head and capitellum of the elbow (Fig. 19-13), and bilateral iliac horns (Fig. 19-14).

Nail dystrophy is present at birth in more than 90% of those affected and is the most constant feature of NPS. The presence of triangular nail is a pathognomonic sign for NPS. The lunula appears stretched toward the free border of the nail, and the central portion of the nail is deformed. Commonly, affected nails are smaller than normal and may even be absent. It affects the fingernails more than the toenail and the thumb and

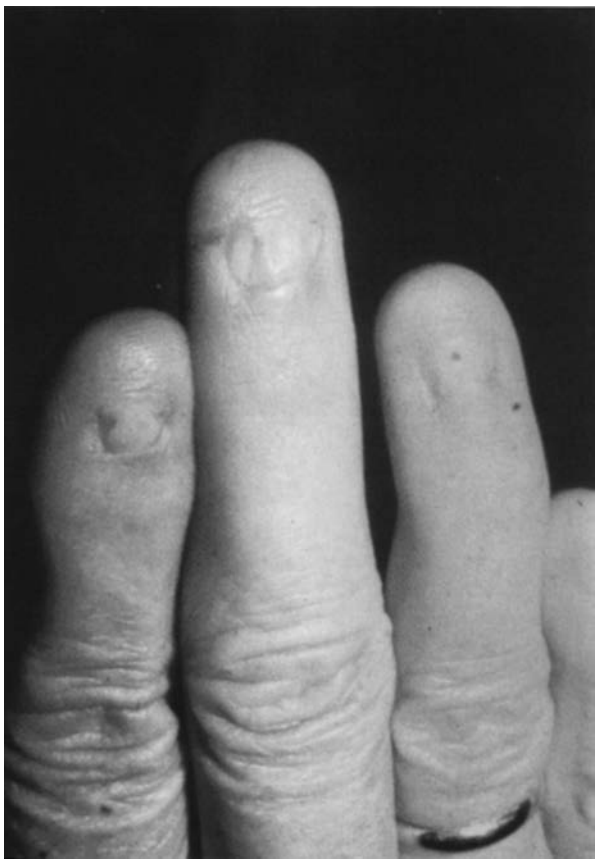

FIGURE 19-11. Characteristic nail changes of nail-patella syndrome (NPS). All the nails are small. The long finger shows the typical central triangular dystrophy with its base at the lunula. Usually, the changes are more marked toward the radial side of the hand.

FIGURE 19-12. Absence of patella in nail-patella syndrome (NPS). Radiograph of the left knee of a 19-year-old girl with NPS. The patella is congenitally absent and the lateral femoral condyle is hypoplastic.

FIGURE 19-13. Elbow contracture in nail-patella syndrome (NPS). The left elbow of a 44-year-old woman with NPS and end-stage renal disease (same patient as in Figure 19-11). She is holding her elbow as straight as possible. A web of soft tissue fills the antecubital fossa.

FIGURE 19-14. Iliac horns. Pelvic x-ray film of an 8-year-old girl with nail-patella syndrome (NPS) (same patient as in Figure 19-12, but 11 years earlier). The *arrow* indicates the characteristic iliac horn, visible medial to the outer margin of the blade of the ileum. The radiograph incidentally shows a fusion defect and lumbarization of S1.

radial-side digits more than the ulnar-side digits. Furthermore, anonychia, hemianoncia, nail hypoplasia, longitudinal ridging, splitting and spoon-shaped and flaky nails have been associated with NPS.

The patella is small, misshapen or absent in more than 90% of patients (Fig. 19-12). Concurrent hypoplasia of the lateral femoral condyle and poor development of the vastus medialis muscle may lead to recurrent subluxation or dislocation of the patella, knee instability and knee pain complicated by patellofemoral arthrosis. Involvement of the elbow is almost as common, with hypoplasia of the radial head and capitellum being characteristic. This predisposes to posterior subluxation of the radial head. Synechiae may lock the elbows in permanent and disabling flexion (Fig. 19-13).

Conical bony horns projecting from the back of the blade of the ileum is seen in about 80% of the patients and considered pathognomonic of NPS. Often palpable in thin subjects, they are readily seen radiographically (Fig. 19-14).

Although not part of the classic tetrad, the most common presentations to orthopedists are foot or ankle deformities and the most common being talipes equinovarus and equinovalgus (480–481). Usually these deformities require surgical correction.

In addition to the common deformities found in NPS, many other skeletal changes may be discovered (482). These include Madelung's deformity (483), scoliosis, spondylolisthesis (484), spina bifida occulta, straight clavicles, hypoplasia of the first ribs, sternal deformities, cranial vault defect, absence of the fibulae, and shoulder dysplasia (485). Urinary tract malformations also occur (466,471,486).

Ocular effects, such as heterochromia iridis (Lester's line) (487), glaucoma, cataracts, strabismus, and microcornea attest to the widespread nature of the structural defects. Sensorineural hearing loss has been described (488). Chronic open angle glaucoma is strongly associated with NPS, although ocular hypertension may also be seen (489). In addition, patients often describe redundant and wrinkled skin on the back of the hands.

Renal Manifestations. It is the renal manifestation of NPS that influence mortality and occurs in 30% to 50% of the patients. Previous studies have estimated renal involvement to occur in between 12% to 55% and renal failure in between 5% and 14% of NPS patients (454). The most typical signs of renal

disease are moderate proteinuria with or without hematuria. Nephrotic syndrome sometimes occurs (466).

The relationship between the somatic features of NPS, clinical signs of nephropathy, and the GBM lesion is perplexing and has not been studies systematically. There appear to be families with the somatic features in whom nephropathy does not develop (491–492). It is not clear whether this is variable expression or genetic heterogeneity. Even families that clearly express the full spectrum of somatic and renal features may have affected individuals without clinical renal signs. Other individual patients have shown musculoskeletal features and typical renal ultrastructure without clinical renal abnormalities (471,469). As described subsequently, several families have clinical renal disease and typical renal ultrastructure without the somatic manifestations.

Variants and Diseases Related to Nail-Patella Syndrome

More than 20 patients showing the ultrastructural features of NPS without the somatic findings have been described (493–495). In seven families, inheritance appeared to be autosomal recessive. It is possible that these cases were examples of collagen III glomerulopathy as previously described (307). There are also some evidences for an autosomal dominant NPS-like syndrome without extrarenal symptoms. These GBM diseases might be due to partial expression of NPS or another type of hereditary glomerulopathy (455).

A girl with a heterozygous complete deletion of the COL5A1 gene at 9q34 and underexpression of α-1(V) chains had dysplastic nails, normal patellae, but other mesenchymal and ectodermal features more suggestive of Goltz's syndrome than of NPS (496).

Animal Models

Renal changes similar to those seen in human with NPS have been observed in LMX1B mutant mice. Expression of α3(IV) α4(IV) chains is decreased in the GBM of LMX1B mutant mice, whereas α1(IV), α2(IV), and α5(IV) chain expression is unaffected (461,497). LMX1B mutations are suggested to contribute to renal pathology by preventing a transcriptional switch from α1/α2 to an α3/α4/α5 network of type IV collagen during GBM development and by disrupting maintenance of COL4A3 and COL4A4 expression, which is important for the long-term structure and function of the GBM.

LMX1B may regulate COL4A4 and possibly COL4A3 expression by a direct action on enhancer sequences (461,455). It is postulated that persistence of the fetal α1/α2 network predisposes the GBM to proteolytic degradation. Furthermore, Lxm1b may contribute to nephropathy by decreasing the expression of genes that have a likely role in podocyte function and maintenance (455). Despite these findings in animal models, immunohistochemical studies in patients with NPS showed normal expression of podocin and α3(IV) and α4(IV) (455).

Diagnosis

Typical NPS is readily recognized clinically. If doubt remains, radiographs of the knees and pelvis usually show absent, rudimentary, or deformed patellae and bilateral iliac horns.

Renal biopsy is rarely required to make the diagnosis of NPS. It may be justified in patients with atypical disease and in patients with evidence of nephropathy because of the apparently high incidence of other and potentially treatable renal disorders in NPS patients.

Prenatal Diagnosis

In families with a defined mutation, chorionic villous sampling can potentially be used for mutation analysis (504); in families without a known mutation, but sufficiently large enough to be informative, linkage could be sought with restriction fragment length polymorphic markers near 9q34 (499). The typical renal ultrastructural features of NPS were found in an 18-week abortus (505), and the diagnosis of NPS has been made by intrauterine kidney biopsy (506). Skeletal anomalies may be recognized by fetal sonography (507–508).

Treatment

As long as we cannot correct the molecular defect of NPS, treatment for all aspects is supportive. Orthopedic surgery to relieve contractures and to fuse or realign joints confers major benefits. Knee, ankle and foot surgery is frequently helpful, whereas elbow surgery is rarely needed (480). Treatment of advanced renal insufficiency is along general line and hemodialysis, peritoneal dialysis, and transplantation have been carried out successfully. In three patients who have had biopsy of the allograft (78,509), there was no evidence of recurrence or NPS lesions or of anti-GBM nephritis. Most intriguing, in a single patient (509) nail lesions appeared to improve after transplantation.

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CHAPTER 20 ■ ISOLATED RENAL TUBULAR DISORDERS: MOLECULAR MECHANISMS AND CLINICAL EXPRESSION OF DISEASE

MAYBIN SIMFUKWE, SANDRA SABATINI, AND EUGENE DAPHNIS

The proximal and distal renal tubules are primarily responsible for solute fluid and reabsorption; thus, it is likely that abnormalities in the mechanisms involved will have clinical expression and that most of the defects will have a genetic basis. Because the transporters (or enzymes) for carbohydrates, amino acids, and ions are polarized to either the apical or basolateral membrane, and because their localization (or function) may differ (integral or constitutive), the possibility of biologic error is virtually infinite. Renal tubular transport disorders are a fascinating study as some of these errors shed light on both evolution and genetic control. That mutant gene products can be expressed in one tissue but not in others is evidence that these carriers are controlled by a variety of gene loci. The advantage conferred by this diversity of control is an evolutionary success. Although many of the tubular disorders remain descriptive, knowledge about the cellular mechanisms is advancing rapidly. In this chapter, we review the major tubular transport defects of carbohydrates, amino acids, and some of the ions (H^+, K^+, Na^+). Abnormalities of calcium, phosphate, and water are discussed elsewhere. We also describe the clinical features of these disorders in humans and summarize some of the insights learned from their animal models.

DISORDERS OF CARBOHYDRATE TRANSPORT

Mechanisms of Carbohydrate Reabsorption

The proximal tubule is responsible for reclamation of approximately two thirds of filtered salt, water, and bicarbonate and for the complete reabsorption of glucose and amino acids (1–6) (Fig. 20-1). Most of the filtered glucose is reclaimed in the first phase of proximal reabsorption (i.e., in the first 25% of the proximal tubule). Reabsorption occurs through sodium–glucose symporters localized in both the S_1 and S_3 segments of the apical brush-border membrane. The lumen-to-cell sodium concentration gradient, maintained by the basolateral Na-K-adenosine triphosphatase (Na,K-ATPase), provides the energy for optimum activity of these transporters (7,8). The lumen-negative potential difference in the early proximal tubule (−2 mV) is thought to be an active transport potential difference driven by sodium-coupled glucose and amino acid reabsorption. Proximal tubule intracellular glucose concentration approximates 5 mmol/L; the portion not used for cellular metabolism returns to the peritubular capillary across the basolateral membrane by facilitative glucose transporters.

Wright and colleagues (9) were the first to identify a Na^+ cotransporter. The gene for this cotransporter localizes to the long arm of chromosome 22 encoding a 73-kDa integral membrane protein of the gastrointestinal apical membrane. It turns out that this ancient gene family (with 11 members in the human genome) has remarkably diverse and versatile functions even separate from glucose (hexose) uptake.

The SGLT1 cotransporter has been cloned in the rabbit kidney (S_3 segment) and is identical to one found in the intestine (10,12). The gene encoding the human sodium–glucose renal symporter is also found on chromosome 22. Less is known about SLGT2 (also identified in the human kidney, early proximal tubule), but complementary DNA sequences of the two transporters show 80% homology. In the S_1 segment, basolateral glucose exits the cell by the glucose transporter type 2 (GLUT2) isoform (and in the S_3 segment by the GLUT1 isoform), a protein carrier that is independent of ion transport. As stated, eleven isoforms have been identified in a number of tissues, including adipose tissue, brain, heart, kidney, and liver (9). Renal handling of other sugars is incompletely understood, but there are data suggesting separate transport systems for fructose and mannose (3,4).

The normal kidney reabsorbs all of the filtered monosaccharides if their plasma concentrations are normal or low. If plasma concentration increases because of disease or a systemic infusion, however, the sugar begins to appear in the urine. A plot of the plasma concentration versus the urinary excretion rate allows the maximum rate of tubular reabsorption (T_{max}) to be obtained (14). As seen in Figure 20-2, above a certain filtered load, no more glucose is reabsorbed by the renal epithelium. Intravenous titration studies in humans document that a splay is always noted; this has been interpreted to indicate that individual nephrons have different thresholds or that the protein transporters have different solute affinities (9,13–15). The higher the dissociation constant (K_D) a specific monosaccharide has for its receptor, the greater its documented splay. In the following sections, a review of specific hexosurias and pentosurias is presented.

The Hexosurias

Primary Renal Glucosuria

Primary renal glucosuria is a disorder characterized by a normal glomerular filtration rate (GFR) and increased urinary glucose excretion in the presence of a normal plasma glucose concentration. Normal intestinal absorption of carbohydrates is also a feature of the disease. The incidence of this disorder varies substantially, although when the diagnostic criteria proposed by Marble in 1947 (15) are strictly applied, the condition is not common. These criteria are:

1. Glucosuria without hyperglycemia, which may increase during pregnancy;
2. Glucosuria independent of dietary intake, present after an overnight fast;

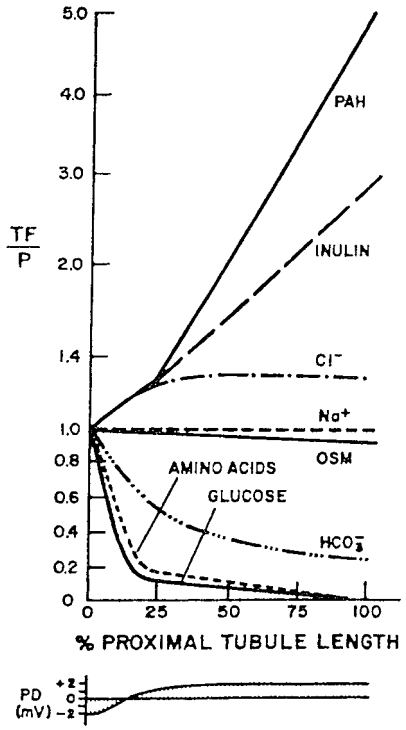

FIGURE 20-1. Transport of glucose, amino acids, sodium, water, chloride, and bicarbonate along the proximal tubule. *OSM*, osmolality; *PAH*, para-aminohippurate; *TF/P*, ratio of tubule fluid to plasma concentration.

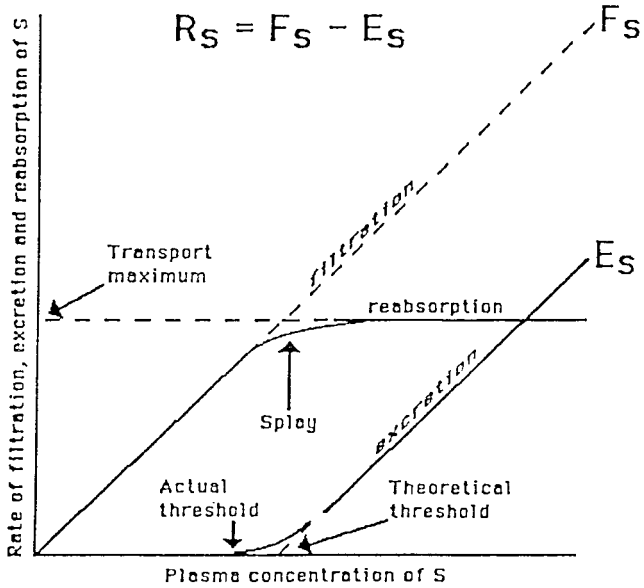

FIGURE 20-2. Proximal tubule reabsorption of a solute, S, by a saturable active transport. E_s, solute excretion (direct measurement); F_s, solute filtration (plasma concentration of S × glomerular filtration rate); R_s, solute reabsorption; TmS, transport maximum, filtered load at which no more solute can be reabsorbed. The theoretical threshold is TmS/TF, or plasma concentration of S at which it begins to appear in the urine. (Adapted from: Haycock GB. Isolated defects of tubular function. In: Cameron A, Davison A, Grünfeld JP, et al., eds. *Oxford Textbook of Clinical Nephrology.* Oxford: Oxford University Press, 1992:1039.)

3. A normal (or flat) glucose tolerance curve in the presence of normal plasma insulin, free fatty acids, and glycosylated hemoglobin;
4. Normal renal transport of other sugars; and
5. Normal carbohydrate utilization in affected subjects.

Glucose excretion rates above 2.7 mmol/day/1.73 m^2 are definitely abnormal even if all these criteria are not met.

Classically, three variants of primary renal glucosuria (Fig. 20-3), as assessed by glucose titration studies (types A, B, and O), have been described (3,14). In type A glucosuria, there is a decrease in the renal threshold as well as a decrease in T_{max}, resulting in massive glucosuria even when the plasma glucose level is normal; very little splay in the titration curve is observed. Some patients with Fanconi syndrome fall into this category. In type B glucosuria, there is a decrease in the renal threshold but T_{max} is normal; titration studies always document increased splay. In affected patients, there is always some degree of glucosuria even when plasma glucose is low. Type B glucosuria is usually an isolated disorder, but may be found in gluco-glycinuria and renal phosphaturia (16). The third variant, type O glucosuria, was first reported by Oemar et al. (17) in a teenager unable to reabsorb glucose under any circumstances. The pattern of inheritance was not documented, because titration studies were not performed in family members. Bagga et al. (18) described a child with type O glucosuria whose mother and two siblings had type A glucosuria.

The pathophysiologic process of primary renal glucosuria is complex and not completely understood. The descriptive terms provided previously will no doubt be reassessed as we now know of many pedigrees with defects in SGLT2 gene. Some of these are associated with aminoaciduria or hypercalciuria. That many of these nonsense mutations are found in highly consanguinous families are suggestive of autosomal recessive inheritance. In 23 families, mutations for the SGLT2 gene were noted in 21. Some of these patients were homozygous and some were heterozygous (19). Because the degree of glucosuria was greater in the homozygotes, the authors suggest the disorder to be codominant trait with variable penetrance.

Types A and B primary renal glucosuria are usually considered to be benign conditions diagnosed during routine examination of the urine. Usually, no clinical manifestations are evident. Type O glucosuria, however, may result in severe dehydration and ketoacidosis during pregnancy or after periods of prolonged fasting.

An acquired form of renal glucosuria has been described by Rezaian et al. (20) in subjects with tetanus. In 63 patients requiring intensive care treatment for tetanus, more than 50% had glucosuria that normalized approximately 4 weeks postdischarge. There was no difference in disease severity, sex, or age in those with or without the renal leak. These patients are probably most similar to those described earlier for type B. It would be of interest to follow these patients to see if the renal leak or diabetes occurs later in life.

Glucose–Galactose Malabsorption

This rare autosomal-recessive disorder (~200 patients identified worldwide) is characterized by impaired gastrointestinal sugar transport in the jejunum resulting in severe diarrhea that is fatal within a few weeks after birth unless lactose (which consists of glucose and galactose) is removed from the diet. There is minimal renal involvement, which is usually a decreased tubular threshold for glucose with a normal T_{max} (i.e., type B glucosuria). No galactosemia occurs because carbohydrate metabolism *per se* is not impaired. This disease is seen in consanguineous marriages and is due to mutations in the SGLT1 gene resulting in a truncated SLGT1 protein (or mistrafficking) in the gut. The disorder is most reliably diagnosed with the hydrogen breath test after an oral glucose (but not

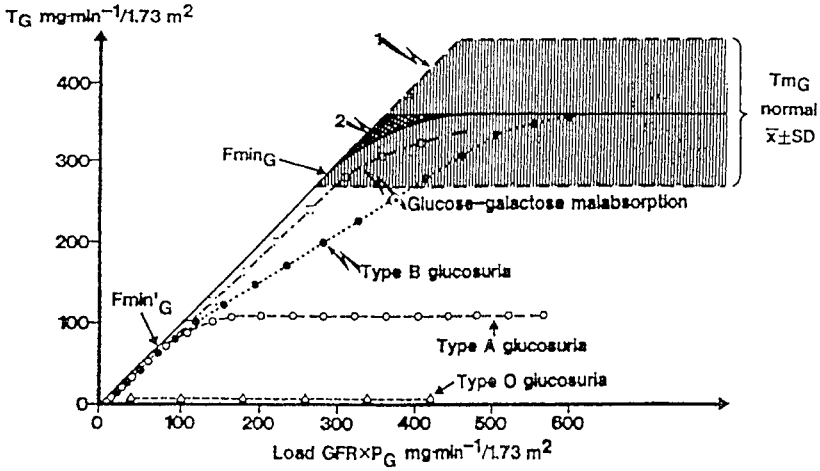

FIGURE 20-3. Schematic drawing of normal and pathologic renal tubular glucose reabsorption (T_G) as a function of the filtered load. The curve marked *1* is the theoretical curve, and the curve marked *2* is the actual curve in normal subjects. $Fmin_G$, minimal renal glucose threshold in normal subjects; $Fmin'_G$, minimal renal glucose threshold in patients with renal glucosuria. (From: Oemar BS, Byrd DJ, Brodehl J. Complete absence of tubular glucose reabsorption: a new type of renal glucosuria. *Clin Nephrol* 1987;27:156, with permission.)

fructose) challenge. Children with the disorder thrive normally on a fructose formula, but even as adults, a relapse occurs after the ingestion of even a few grams of glucose or lactose (21–24).

Glucosuria of Pregnancy

It is well known that glucosuria may occur during pregnancy. Chen et al. (25) found that 1.7% of pregnant patients had glucosuria during routine antenatal testing. The glucose T_{max} falls during pregnancy for two reasons: a marked increase in GFR without a parallel increase in maximum glucose reabsorptive rate and a significant inhibition of proximal tubule reabsorption due to volume expansion. In affected women, renal glucose transport tends to normalize approximately 2 months after delivery. Some have suggested that these women have a mild case of one of primary renal glucosurias discussed earlier, but further studies are needed to document this possibility (26). There is no clear evidence that women with glucosuria of pregnancy are at higher risk for the subsequent development of diabetes mellitus.

Glucosuria in Infancy

Urinary glucose excretion is higher in preterm infants than in term infants; normal adults have virtually no glucose in the urine. When adjusted for body surface area, both the glucose T_{max} and the GFR tend to be lower in preterm infants than those born at term; both parameters are lower than in adults. This phenomenon is not seen in laboratory animals and, consequently, the mechanisms have yet to be elucidated. Perhaps, during the maturation process the capacity to reabsorb glucose rises because of an increase in the amount of the apical symporter proteins or because of a decrease in the turnover of these proteins. Also, as development proceeds, a more favorable electrochemical gradient may be established in the proximal tubule or there may be a decrease in proximal tubule membrane fluidity. Any or all of these mechanisms could occur, but they remain to be documented. Despite the substantial caloric and calcium losses in infants having this disorder, there seems to be no significant long-term clinical consequence (27). A slow learning ability has been shown in long-term follow-up of very small preterm infants, a number of whom are now in their early teenage years. Whether they are a subset of those who previously had significant glucosuria remains to be determined.

Essential Fructosuria

Essential fructosuria is an autosomal-recessive disorder of metabolism occurring with an incidence of approximately 1:130,000 in the general population. Phosphofructokinase activity is decreased; therefore, fructose-1-phosphate is not formed. Significant fructosemia and fructosuria occur despite a large portion of the hexose being metabolized in the muscle and adipose tissue by alternative mechanisms. Surprisingly, the condition is benign and is usually discovered on routine urinalysis (28). This disorder should be distinguished from the more common and potentially fatal disorder, hereditary fructose intolerance (discussed later).

The Pentosurias

Essential Pentosuria

Essential pentosuria, an autosomal-recessive disorder occurring mainly among Jews, is clinically benign. Its incidence among Askenazi Jews is between 1:2,000 and 1:5,000. In humans, the pentoses are derived predominantly from renal and hepatic glucuronic acid metabolism. Subjects who have eaten fruit (i.e., saccharose) excrete L-arabinose and L-xylose. Because the enzyme nicotinamide adenine dinucleotide phosphate–xylitol dehydrogenase is decreased, xylose cannot be further metabolized (28). If $CuSO_4$ is used to measure glucose colorimetrically, these patients may be erroneously diagnosed with diabetes mellitus; the glucose oxidase method clarifies the issue.

Other Pentosurias

Muscular dystrophy results in the release of ribose from muscle, and therefore its excretion in the urine is increased (29). The other "overflow" pentosurias related to abnormal fructose metabolism include D-glyceric aciduria and erythrocyte aldolase A deficiency.

For additional information on renal glucose and the clinical abnormalities in carbohydrate metabolism, the reader is referred to Brosius (30) and Brodhel (31) for reviews.

DISORDERS OF AMINO ACID TRANSPORT

Mechanisms of Amino Acid Transport

The turnover rate of endogenous protein averages 80 to 100 g/day; it is highest in the gut mucosa and virtually absent in collagen and myelin. Together with ingested protein,

TABLE 20-1

THE AMINOACIDURIAS

Amino acids	Transport system involved	Aminoaciduria	Species
Acidic	Aspartate, glutamate	Acidic aminoaciduria or hyperdicarboxylic aminoaciduria	Humans
Basic	Arginine, lysine, ornithine	Hyperdibasic aminoaciduria I and II "Classic" cystinuria	Humans, dogs
Neutral	Cystine/cysteine	Isolated cystinuria	Humans, dogs
	Proline, hydroxyproline, glycine	Iminoaciduria	Humans
	Glycine	Isolated glycinuria	Humans
	Alanine, histidine, leucine, isoleucine, methionine, serine, threonine, tryptophan, valine, glycine, glutamine, citrulline	Hartnup disease	Humans
	β-Alanine, γ-aminobutyric acid, taurine	Taurinuria	Mice

See text for discussion of each disorder.

the amino acid pool supplies the daily needs of the body. Each day the kidney filters approximately 70 g of protein, mostly as small peptides and amino acids, but only 2% to 3% of the amino acids are excreted in the urine. The percentage of amino acids metabolized by the kidney is 0.4% of that found in blood, although this value doubles during starvation and increases up to 52% during metabolic acidosis (32). Under normal circumstances, virtually all the amino acids filtered are returned to the circulation by reabsorption in the first quarter of the proximal tubule (Fig. 20-1). Depending on the specific amino acid, plasma levels in humans vary from 30 to 300 μmol/L. Because the intracellular amino acid concentration may be 10- to 50-fold greater than that found in the glomerular filtrate or plasma, specific mechanisms must be present to accomplish the process of transcellular tubular transport.

Sodium-dependent cotransport systems for many of the acidic and neutral amino acids have been identified (5,6) (Table 20-1). Activity for these electrogenic transporters is energized, once again, by the electrochemical gradient generated by basolateral Na,K-ATPase (8,33,34). Substantial heterogeneity exists for the rate of amino acid transport in the nephron, with the highest rates noted in the S_1 segment and the lowest in the S_3 segment. Amino acid exit from the cell into the peritubular capillary occurs by facilitated diffusion. Although paracellular transport of amino acids does occur, it is of no physiologic significance. The nutritionally essential amino acids in adults are valine, leucine, isoleucine, threonine, methionine, phenylalanine, lysine, arginine, and histidine (taurine appears to be an essential amino acid for infants).

Evidence suggests that there are at least six separate transport systems for the different types of amino acids (for a recent review of the structure-function relationship of some of these transporters see the article by Broer and Wagner [35]):

1. Dicarboxylic amino acids–glutamate, aspartate (32,36);
2. Dibasic amino acids–lysine, arginine, ornithine, cystine (36,37);
3. Neutral amino acids–the group found in Hartnup disease–glycine, valine, leucine, isoleucine (and 13 other amino acids) (5,37,38);
4. Imino amino acids–proline, hydroxyproline, sarcosine (and other *N*-methyl amino acids) (39);
5. β-Amino acids–β-alanine, β-aminoisobutyric acid, β-aminobutyric acid, taurine (40,41); and
6. Cystine–cysteine (5,37).

Substantial data have been presented on the molecular biology of two groups of amino acid and oligopeptide transporters in mammalian tissues, including kidney (40,42–44). For the dicarboxylic amino acids (i.e., acidic), mRNA for the high-affinity glutamate transporter EAAC (system XAG-) is highest in proximal tubule (S_2 and S_3 segments), but is also present in the medulla (45). This system, which has four isoforms, is important in activating brain glutamine. The dibasic amino acids, some neutral amino acids, and cystine are associated with the heavy-chain protein, rBAT (system $b^{O,+}$); the light-chain unit is $b^{O,+}$AT. This system then affects cellular nitric oxide, polyamine, and carnitine biosynthesis. The other heavy-chain unit of the amino acid transporters in this class is 4F2hc, which, if associated with the Y^+LAT-1 light chain, results in the Y^+L transport system. Under the Human Genome Organization (HUGO) nomenclature, the abbreviation for these two systems is SLC. Oligopeptides are reabsorbed in the proximal tubule by two transporters, PEPT1 and PEPT2 (43,46). PEPT1, a low-affinity but high-capacity transporter, is confined mainly to the pars convoluta, whereas PEPT2 is found mostly in the S3 segment, with a small amount present in the outer stripe of the outer medulla. There are some interesting differences between the mammalian and insect amino acid transporters that may be parlayed into selective therapies in the future (40,47).

Abnormal Amino Acid Transport

A number of mechanisms have been postulated to explain the aminoacidurias in humans (40,48). A brief review of the six most commonly suggested mechanisms follows:

1. The simplest explanation is that the apical proximal tubule transporter for an amino acid is defective. This mechanism should be the most specific, and perhaps the one best explained by a single genetic defect.
2. Another proposed mechanism relates to a defective carrier located on the basolateral membrane. Such a defect would then lead to "backflux" of a specific amino acid (or to a class of amino acids) into the tubular lumen. This is hypothesized to occur in classic cystinuria and lysinuric protein intolerance (both discussed later).
3. This mechanism postulates that the primary defect resides in either the apical or basolateral membrane *per se*. The theory states that although the amino acid transporters (or carriers)

are intact and normal, other parts of the membrane are not. In such a setting, amino acid uptake and exit are normal but back-diffusion, nonspecific in nature, occurs because other components of the membrane are aberrant. This has been the suggested explanation for some aspects of Fanconi syndrome (discussed later).

4. This intriguing mechanism proposes that the proximal tubular cell accumulates some metabolic end product(s), which then decreases amino acid reabsorption. The aminoaciduria of galactosemia or hereditary fructose intolerance may be the result of a massive intracellular accumulation of galactose-1-phosphate or fructose-1-phosphate, respectively.
5. This mechanism states that a single amino acid is reabsorbed by the appropriate transport system, but the other amino acids, using the same carrier, have no access to it. These others are then excreted in excessive amounts. Were an amino acid to accumulate because of an inherited defect in metabolism, excessive amounts of it would be filtered; if it overwhelmed the carrier, reabsorption of other amino acids would be depressed.
6. The final postulate, and the one receiving the most attention, is that related to "overload": when the filtered load of an amino acid exceeds the transport capacity of the tubule, aminoaciduria results. This mechanism probably prevails in renal insufficiency where the single-nephron GFR is high and the transporters are saturated continuously. Depending on the kinetics of the carrier, aminoaciduria varies with the absolute excretion rate and fractional excretion. For example, a transporter with a high capacity for reabsorption (but low affinity) would lead to an increase in absolute excretion of the amino acid (i.e., glycine, histidine), but fractional excretion would be normal. By contrast, a transporter with a low capacity (but high affinity) would result in an increase in both absolute excretion and fractional excretion (i.e., aspartate, glutamate, and β-amino acids).

We recognize that much of the information presented here is descriptive; however, there are data supporting each of these mechanisms. With the power of molecular biology, and the recent mapping of the human genome, the additional studies generated should allow eventually an understanding of the mechanisms behind these diseases more precisely. For the most recent information concerning aspects of amino acid transport and selected aminoacidurias, the reader is referred to the excellent reviews of Palacin et al. (48) and Avila-Chavez et al. (49).

Specific Clinical Diseases Associated with Aminoaciduria

Hyperdicarboxylic Aminoaciduria

This disorder is associated with increased excretion of glutamic and aspartic acids while their plasma levels remain normal (50,51). It is thought to be inherited as an autosomal-recessive trait. It appears to be clinically benign, but in cultured skin fibroblasts of affected patients, amino acid uptake is impaired.

Hyperdibasic Aminoaciduria

Two types of hyperdibasic aminoaciduria have been identified, types I and II. Type II is perhaps the best known (also called *lysinuric protein intolerance*) (48). It is inherited in an autosomal-recessive pattern and is seen mainly in Finland, Italy, and Spain. An estimated 1 in 60,000 live births in Finland involves this disorder. Homozygotes are intolerant of a protein-rich diet. The defect has been localized to the basolateral membrane in the kidney; thus, the step for the exit of the amino acids is abnormal. In 31 Finnish patients, Torrents et al. (52) found a missense mutation leading to a premature stop codon. The mutation is presumably on the long arm of chromosome 14 in the Y^+L amino acid transport system. Prenatal diagnosis is now possible (53).

Patients have failure to thrive, vomiting, hypotonia, and episodes of stupor or coma after a high-protein meal. They also have hepatomegaly, hyperammonemia, low blood urea nitrogen levels, and orotic aciduria. Osteoporosis is commonly seen and a severe interstitial lung disease may occur at any time during life. Intestinal absorption of three amino acids (lysine, ornithine, and arginine) is also abnormal, as is their transport in the brain.

Therapy consists of L-citrulline (0.5 mg per g) with dietary protein restriction (1.5 g per kg per day) (54). L-Citrulline supplements improve the patient's tolerance for protein intake, further suggesting that hepatocyte arginine and ornithine transport is defective, subsequently compromising activity of the urea cycle. Citrulline is a precursor of both arginine and ornithine; it enters the hepatocyte through the neutral amino acid transporter. Renal excretion of lysine, ornithine, and arginine is enhanced, but urinary cystine excretion is normal. Some have reported that immunosuppressive agents may also be required for optimum therapy (55).

Type I hyperdibasic aminoaciduria is also an autosomal-recessive disorder. Those affected appear to have an incomplete phenotype and modest aminoaciduria (lysine, ornithine, and arginine). It has a different ethnic distribution from that of the type II disorder in that cases are not clustered in one area. Although homozygotes have no hyperammonemia, mental retardation occasionally is observed (56,57).

Iminoglycinuria

This benign autosomal-recessive defect is characterized by increased excretion of proline, hydroxyproline, and glycine (16,39). The defect is thought to reside in the apical membrane amino acid transporter. Genetically, the disorder is heterogeneous and has an estimated incidence of 1 in 15,000 live births. Four alleles result in different phenotypes (types I, II, III, and IV iminoglycinuria). Some heterozygotes may have only enhanced excretion of glycine (types III and IV), whereas others are silent (types I and II). Homozygotes have both aminoaciduria and a defect in intestinal transport (type I). Careful study of both gut and renal biopsy specimens using molecular probes should yield important information regarding future nomenclature, mechanisms, and possible therapeutic strategies.

Neutral Aminoaciduria (Hartnup Disease)

One of the best described disorders of amino acid transport is that of enhanced excretion of the neutral amino acids (*excluding* proline, hydroxyproline, and glycine), or Hartnup disease (38). The neutral monocarboxylic amino acids have no net charge at physiologic pH and include tryptophan, phenylalanine, valine, leucine, isoleucine, alanine, serine, histidine, glutamine, asparagine, and threonine. Hartnup disease involves massive urinary and fecal excretion of all of these amino acids.

The disorder occurs with an estimated incidence of 1 in 20,000 live births, but the disease itself is multifactorial and depends on both diet and environment. Three forms of the disease have been described: the "classic" form has defects in both the kidney and intestine; the second form has a defect only in the kidney; and the third form affects only the intestine. The heterozygous state is silent. Some variants have been described

in which there is an abnormality in only one amino acid (e.g., tryptophan or histidine malabsorption).

The mechanism proposed for Hartnup disease is that a widespread defect exists in the transporter(s) for the α-amino acids and the neutral amino acids in both the brush border of the renal tubule and the jejunum. The primary evidence for this suggestion is the finding that dipeptides of these amino acids are transported normally but the free amino acids are not. The transport abnormality appears to occur only in kidney and gut; it has been shown that tryptophan transport in leukocytes, placenta, and cultured skin fibroblasts from affected patients is identical to that in control subjects (58–61). Unusual findings in some homozygotes include cerebellar ataxia, psychiatric manifestations, and a pellagra-like photosensitive skin rash (38). These features occur in approximately 10% of patients and are thought due to the impaired conversion of tryptophan to nicotinamide and kynurenine.

Symula et al. (62) described a mouse model that, in the homozygous state, excretes large amounts of neutral amino acids, especially phenylalanine. The mutation maps to chromosome 7 (in humans, on chromosome 5) (63). These animals also acquire niacin deficiency, similar to that noted in patients.

Treatment in affected subjects includes increasing protein intake and nicotinic acid supplementation (40 to 100 mg per day) to prevent the pellagra-like symptoms. Exposure to sunlight should also be limited. Tryptophan ethylester, a lipid-soluble form of the amino acid, is said completely to reverse the clinical symptoms (64).

Cystinuria

In 1810, Woolaston determined the chemistry of two novel bladder stones, which he named *cystic oxide stones*; in 1833 Berzelius called them *cystine stones*. Alexander Garrod, in his 1908 Croonian lecture, discussed Woolaston's original findings in the context of disordered amino acid metabolism, but it was not until 50 years ago that cystinuria was proposed to be an inborn error of dibasic amino acid transport. The disorder is an autosomal-recessive trait and has been characterized clinically into three types (65):

> *Type I cystinuria* is characterized by increased excretion of cystine, lysine, arginine, and ornithine in association with urinary stone disease in homozygotes. Heterozygotes do not have aminoaciduria but may have urinary stone disease because of the low solubility of cystine. Intestinal amino acid absorption is impaired. Heterozygotes are silent, and 60% of cases worldwide are of this type.
>
> *Type II cystinuria* is inherited in an incomplete recessive pattern because heterozygotes have moderately increased excretion of cystine and lysine. Intestinal lysine absorption is impaired.
>
> *Type III cystinuria (Hartnup disease)* is associated with normal intestinal transport of the amino acid in heterozygotes, but excess cystine is excreted in the urine. (A phenotype of cystinuria is found in nonhuman vertebrates [65].)

Now, we classify Types II and III cystinuria as *non-type I cystinuria*; it is characterized as an incomplete recessive form of the disease with heterozygotes having variable amounts of urinary cystine excretion. A pedigree with *non-type I cystinuria* has been localized to the long arm of chromosome 19.

The incidence of cystinuria varies from 1 in 2,000 to 1 in 15,000 live births, and is highest in Scandinavia (48,66). The sexes are equally affected, as would be predicted; however, male patients appear to have a more severe form of the disease.

With respect to pathogenesis, brush-border vesicles and nephron microperfusion studies show that transport of cystine, lysine, ornithine, and arginine is defective. The abnormality cannot be demonstrated in cortical slices, and addition of sulfur-containing amino acids is without effect. The basolateral membrane is thought to have multiple carriers, but none for the four shared amino acids listed previously. By contrast the apical membrane is thought to have one transporter shared by the four amino acids. Studies of intestinal epithelium show that brush-border uptake of these amino acids is defective. According to some reports, urinary excretion of glycine, methionine, cystathionine, and homocysteine–cysteine disulfide also occurs (65).

Studies suggest that kidney rBAT (a single transmembrane glycoprotein related to the system $b^{O,+}$ amino acid transporters) is implicated in cystinuria (64). This protein, whose gene (i.e., SLC3A1) has been localized to the short arm of chromosome 2, may regulate, or be a part of, some amino acid cotransporters (for review, see Avila-Chavez et al. [49]).

Many mutations in the SLC3A1 gene have been noted in type 1 cystinuria (67,68). Stoller et al. (69) reported that *non-type I cystinuria* maps to the long arm of chromosome 19. The incidence of *non-type I cystinuria* is high in Jews of Libyan origin (70). The literature is rapidly being clarified, and the reader is specifically referred to the review by Palacin et al. (48) and Shekarriz and Stoller (66).

Clinically, the disease presents during the second or third decade of life, with renal colic and stone disease the usual symptoms. Urinary tract obstruction, recurrent infection, and hypertension occur; an occasional patient may require transplantation. Commonly associated diseases include hyperuricemia, hemophilia, Down syndrome, hypotonia, retinitis pigmentosa, and pancreatitis. The intestinal absorption defects in cystinuria, however, do not appear to impair growth.

The diagnosis may be made with the urinary cyanide-nitroprusside test, in which the urine turns magenta red; characteristic cystine crystals may also be seen on microscopic urinalysis. Ion exchange chromatography reveals excretion of excess cystine and cationic amino acids with normal plasma levels.

Dietary control with low methionine intake is of variable effectiveness; however, salt intake should be restricted (71). The water solubility of cystine at pH 7.4 is 0.8 mmol/L (25°C). As a consequence, stone formation is predictable when urinary pH is low. Alkalinizing the urine to pH 7.5 is of no practical benefit because urinary cystine concentration is always greater than 0.8 mmol/L in affected subjects. Penicillamine (1 to 2 g/24 hours), by contrast, is efficacious in dissolving stones and preventing their further formation. This compound, a well-known chelator of copper and lead, acts to convert cystine to a relatively soluble compound (50-fold more soluble) by complexing with sulfhydryl groups (72,73). The drug, however, has numerous and common (as high as 85%) side effects, including nephrotic syndrome, membranous nephropathy, serum sickness, fever, rash, and pancytopenia. As a result, its long-term use is quite limited. The compound α-mercaptoproprionylglycine has also been used. This drug has a higher redox potential than penicillamine; it is said to be less toxic. Even so, proteinuria, membranous glomerulonephritis, fever, rash, and positive antinuclear antibodies have been reported (74). Angiotensin-converting enzyme inhibitors have been used in a few cases with beneficial results (75). The mechanism of action appears to be related to the increased solubility of a cysteine–captopril complex excreted in the urine. Bladder irrigation using *N*-acetylpenicillamine, penicillamine, mercaptoproprionylglycine, or trimethamine has been attempted with some success. Cases in which the urinary tract is obstructed have been treated with lithotomy. Lithotripsy is of no benefit in this disorder because cystine stones are particularly refractory to extracorporeal shock waves. Use of this approach may result in substantial problems, including hematuria, infection,

and renal parenchymal scarring. If transplantation is required, the disease does not appear to recur in the transplanted kidney (76–78).

Hypercystinuria

This is an autosomal-recessive disorder associated with enhanced urinary cystine excretion but normal excretion of the dibasic amino acids lysine, ornithine, and arginine. Only two siblings have been described, and neither had stone disease. It is not known whether other tissues are affected.

Miscellaneous

"Physiologic" hyperaminoaciduria may occur in premature and normal infants and in children. The mechanism postulated for this is that the renal clearance of certain amino acids (e.g., proline, glycine, cystine, and the cationic amino acids) is higher than the clearance of other amino acids (6). The syndrome tends to resolve as the child grows. An inbred mouse strain, C57B1/6J, is reported to have β-aminoaciduria with enhanced excretion of β-alanine, taurine, and β-aminoisobutyric acid. No inborn errors of the β-amino acids (and β-aminobutyric acid) have been documented in humans. This is of some interest because taurine is an "essential" amino acid in infants, and therefore such a defect would be expected to have clinical consequences.

Additional works are available for the reader interested in a further review of the amino acid transporters in mammalian cells, techniques available to study them, and controversies in the mechanisms and therapies of genetic diseases (78–82).

HETEROGENEOUS TUBULAR DEFECTS (FANCONI SYNDROME)

Generalized dysfunction of the proximal tubule leads to excess urinary excretion of glucose, amino acids, phosphate, bicarbonate (mechanisms of bicarbonate reabsorption and proton secretion are discussed in the next section), and other solutes handled by this nephron segment (83,85). Endogenous compounds lost include low–molecular-weight proteins (i.e., <50 kD) comprising oligopeptides, peptide hormones, enzymes, and immunologic light chains. Loss of sodium, potassium, calcium, magnesium, and carnitine also occurs. The disorder may be congenital or acquired, primary or secondary, complete or incomplete. The Lignac-deToni-Debre-Fanconi syndrome (Fanconi syndrome) is a generalized tubular defect that leads to hypokalemic metabolic acidosis (proximal renal tubular acidosis [RTA], type II RTA) associated with vitamin D–resistant metabolic bone disease. The abnormalities in calcium, phosphate, and cholecalciferol metabolism lead to rickets in children and osteomalacia in adults.

In humans, no major morphologic lesions are seen unless renal failure is present. Ultrastructural changes seen in the proximal tubule in some of the experimental models include cytoplasmic vacuolization, mitochondrial matrix edema, engorgement of lysosomes, distention of granular endoplasmic reticulum, loss of basolateral membrane infoldings, and cellular necrosis. Because none of the experimental models is completely pure, most experts consider these abnormalities nonspecific.

The expression of Fanconi syndrome in humans and animals is highly variable. The onset of symptoms in a patient with hereditary fructose intolerance occurs within minutes of an intravenous administration of fructose (85,86) (for a review, see Ali et al. [87]). It has been noted after a few days in patients with galactosemia given galactose (88,89), and more than 25 years after exposure to cadmium (90). In some instances, Fanconi syndrome can be reversed.

Pathophysiologic Mechanisms

Studies of the complex pathophysiologic mechanisms behind Fanconi syndrome center around four areas: defects in the plasma membrane, disturbances in energy generation, paracellular (or cellular) backflux, and abnormalities in specific subcellular organelles.

With respect to the plasma membrane, some have proposed that an intrinsic defect exists. If true, the most likely postulate is an abnormality in the sodium-binding domain of the multiple heterogeneous carriers in the brush-border membrane. There may, however, be an abnormality in the way the different carriers are moved to (inserted into or retrieved from) the brush-border membrane. If this postulate is true, the major defect would involve the cytoskeleton. With animal models, doubtless both occur; in humans, both are possible.

Dysfunction of the proximal tubule Na,K-ATPase (basolateral) would result in abnormal energy generation. This important enzyme provides the energy for transport in the proximal nephron; it is composed of two α and two β subunits, with the catalytic site located in the α subunit (8). If either subunit were abnormal in the proximal tubule, transepithelial gradients would be altered. Hereditary fructose intolerance, galactosemia, cytochrome C oxidase deficiency, and heavy metal poisoning may act by inhibiting Na,K-ATPase activity. Decreased ATP levels have been found in some, but not all, of the animal models of Fanconi syndrome. Depending on the dose or protocol, maleic acid infusion, succinylacetone, and cystine dimethylester administration are all reported to decrease tissue ATP or alter Na,K-ATPase activity in animals (31,91,92). Na,K-ATPase is a P-type ATPase and in the kidney both it and the H-ATPase (a V-type ATPase) are present (83). The extent to which the latter is associated with these four possibilities is incompletely understood as most proximal tubule function depends on "normal" Na,K-ATPase activity.

With respect to membrane permeability, enhanced efflux from either the cell or peritubular capillary would cause increased urinary electrolyte and solute excretion. Both mechanisms have been demonstrated in some studies using the maleic acid model of proximal renal tubule acidosis (93,94). Glucosuria in maleate-treated dogs may be related both to the enhanced efflux of glucose across the brush-border membrane and to partial inhibition of the sugar as it exits the cell across the basolateral membrane (95,96).

Specific abnormalities in proximal tubule subcellular organelles and amino acid transport have also been reported. High-dose maleic acid induces lysozymuria after accumulation of vacuoles in the apical cytoplasm (97,98). Mitochondria also concentrate certain compounds (e.g., maleate, cadmium, and mercury), resulting in abnormal function. The decreased activity of 25-OH-D_3-1-hydroxylase is probably due to accumulation of these compounds in mitochondrial membranes. In this regard, rats injected with cadmium (total dose ~2.25 mg) show mitochondrial enlargement after 3 weeks of administration (99,100). Cadmium toxicity is also associated with loss of the basolateral membrane surface area; associated with this loss is a decrease in cellular ATP by approximately 40%, a decrease in Na,K-ATPase by approximately 60%, and altered glucose and amino acid flux (99). It is not clear, however, which of these events is primary.

A single mechanism for Fanconi syndrome can best be hypothesized by assuming that the primary abnormality is related to a defect in energy generation. The single most important abnormality would be one affecting Na,K-ATPase activity

at the basolateral membrane of the proximal tubule. Such an inhibition would alter the sodium gradient across the luminal membrane, decreasing sodium-coupled reabsorption. This same defect would also interfere with the normal functioning of intracellular organelles (e.g., endosomes, mitochondria) by limiting substrate availability for maximum energy utilization. Most of the solutes lost in the Fanconi syndrome are those coupled to apical sodium reabsorption (e.g., Na-H antiporter, sodium–glucose symporter, sodium–amino acid symporter, or sodium–phosphate symporter). Alternatively, direct damage to endosomes or mitochondria would cause aspects of cellular metabolism to fall. This would secondarily decrease the basolateral exit of sodium, which, in turn, would markedly alter gradient-dependent sodium entry and sodium-coupled transport. Consequently, an increase in the urinary excretion of those ions and molecules coupled to sodium would occur. The protective effect of acetoacetate in minimizing maleic acid-induced bicarbonaturia and phosphaturia seems related to its ability to provide acetate for transfer to coenzyme A (CoA) in mitochondria (101,102). Phosphate loading also prevents maleic acid-induced bicarbonaturia, but the precise mechanism for this remains to be elucidated because it is protective at a maleic acid dose that does *not* change cortical ATP levels (91,103).

Fanconi syndrome is also associated with distal nephron dysfunction in some patients. Micropuncture studies also suggest that maleic acid has an additional site of action in the thick ascending limb (95). The mechanisms involved are not known, but the evidence suggests that there may be a defect in Na,K-ATPase throughout the nephron.

Experimental Models of Fanconi Syndrome

As with many human diseases, there is no pure animal model of Fanconi syndrome. Thus, information gleaned from several models, some of them discussed previously, must be synthesized and interpreted in light of known findings in humans. One of the best studied of the experimental models is that of *maleic acid injection;* maleic acid is the *cis* isomer of fumaric acid. Depending on the species studied, a single intravenous infusion of the compound results in proximal RTA, glucosuria, aminoaciduria, phosphaturia, and hypokalemia, as well as affecting the GFR (91,98). Some, but not all, researchers have demonstrated a 40% decrease in renal cortical ATP (91). That this fall appears to be dose related is of significance because glucosuria, bicarbonaturia, phosphaturia, and decreased proximal tubule Na,K-ATPase have all been documented after administration of a dose of maleic acid that does *not* affect cortical ATP levels (101,102). Maleic acid has several cellular effects, although these have been studied primarily at high doses only (103). The agent appears to bind to acetyl-CoA and impair activity of the trichloroacetic acid cycle. As stated, acetoacetate partially prevents development of Fanconi syndrome, suggesting that the effect could be secondary to the trapping of acetyl-CoA as malonyl-CoA (102). Maleic acid decreases tissue levels of acetyl-CoA, free CoA, and acid-soluble CoA, all of which are reversed by administration of acetoacetate (102). Maleic acid also blocks the conversion of 25-hydroxyvitamin D_3 to 1,25-dihydroxyvitamin D_3, the hydroxylation step known to occur in the mitochondria of cortical tissue (104,105). Although maleic acid decreases oxidation of α-ketoglutarate, there is no direct evidence that the dehydrogenase enzyme is altered.

Futhermore, maleic acid impairs sugar and amino acid transport in cortical slices and isolated rat proximal tubules (106,107). Although the uptake of these compounds is not altered, efflux from vesicles is accelerated (108). Maleic acid does not appear to affect the physical characteristics of the luminal or basolateral membranes, implying that membrane fluidity and, consequently, the lipid profile of these membranes is intact (109). We examined this issue and found that lipid incorporation into the brush-border membranes was altered (110). Maleic acid also decreases sulfhydryl groups by reacting with tissue thiols.

Low-dose maleic acid (i.e., a dose which does *not* affect tissue ATP levels) decreases Na,K-ATPase and H-ATPase in microdissected segments of rat proximal tubule. The latter effect is selective for the proximal tubule because the collecting duct H-ATPase is not changed (91). If endocytotic recycling is a major cause for proximal tubule acidosis, as has been suggested, these findings are now understandable. While phosphate infusion only partially ameliorates the effects in the proximal enzymes, the mechanism for this finding is not known.

In summary, although maleic acid injection is a well-studied experimental model of Fanconi syndrome, it is one in which multiple other biochemical interactions occur. The model does offer some insights into the pathophysiologic process of the heritable forms of the heterogeneous tubular defects in humans, but many questions remain to be answered. Some other animal models are discussed subsequently.

Cadmium given to rats for approximately 3 weeks (200 μg/g of body weight) results in Fanconi syndrome (and proteinuria) that reverses 4 to 5 days after stopping the compound (90,99). Radiolabeling studies show that cadmium localizes to the proximal tubular cells bound to metallothionein. At peak effect, the cortical ATP level decreases by approximately 40% and Na,K-ATPase activity falls by 60% (90). It is not clear, however, whether the decrease in cortical ATP is the primary event or a secondary event. Tubular dysfunction similar to that produced by cadmium has been noted after the administration of *lead*, an element that also causes proximal RTA (111). Lead partially uncouples oxidative phosphorylation, but it is not known whether this is its sole mechanism of action (112). *Uranium and mercury* also cause proximal tubular lesions. Both typically result in acute tubular necrosis and a fall in GFR, thus it is difficult to separate out the pathogenesis of human Fanconi syndrome where the GFR is normal.

Outdated tetracyclines cause reversible Fanconi syndrome in some animals (113,114). With time, heat, and moisture, the tetracyclines are converted to anhydro-4-epitetracycline, a compound that decreases metabolism in the cell cytosol. The effect is magnified at low pH. In rats, the injection of *cystine* results in tubular dysfunction, a fall in cortical ATP levels, a decrease in mitochondrial respiration, and low intracellular potassium. Na,K-ATPase levels, however, remain normal (115,116). The defect in proximal tubular transport capacity is partly restored when ATP is added to the incubation medium. Renal tubular cells incubated *in vitro* with cystine dimethyl ester show increased intracellular cystine levels and altered proximal solute reabsorption (117). Administration of *succinylacetone*, a compound structurally related to maleic acid, causes a Fanconi syndrome in rats (118). When renal tubules are incubated *in vitro* in the presence of this compound, amino acid, glucose, and phosphate reabsorption falls (118–120). The short-chain fatty acid, *4-pentenoate*, causes a Fanconi-like syndrome in dogs. This compound, which inhibits mitochondrial metabolism and β-oxidation of fatty acids only in the proximal tubule, results in the accumulation of the toxic metabolic compound, 3-keto-4-pentanoic-CoA. Finally, Fanconi syndrome spontaneously develops in Basenji dogs; the proximal tubular lesions appear to be minimal, but these animals are subject to debilitating bone disease (121). Very few studies have been performed in this naturally occurring "experiment of nature," but such an avenue of research may prove promising, particularly given ongoing advances in molecular biology (for a recent review of drug-induced Fanconi syndrome, see the article by

Izzedine et al. [122]. New reports now include the effects of valproic acid [123–125]).

Signs and Symptoms of Fanconi Syndrome

A number of important features characterize this syndrome. Glucosuria occurs in the presence of normal plasma glucose concentration. Urinary excretion of glucose is in general less than 10 g/day except in patients with glycogenosis (i.e., Fanconi-Bickel disease, discussed subsequently), in whom excretion may exceed 200 g/day.

A generalized aminoaciduria also occurs, and ion exchange chromatography is the most accurate method for quantitating the degree of tubular dysfunction. The pattern of amino acid excretion in Fanconi syndrome appears to be similar to that seen with "physiologic" aminoaciduria, except in a single pediatric case report in which renal biopsy revealed complete absence of proximal tubule brush-border membranes (126). In this subject, the clearance rate for all amino acids was identical to the GFR. Surprisingly, amino acid losses do not appear to result in deficiency states, thus, supplementation is not part of the therapy for Fanconi syndrome.

Phosphaturia results only when the plasma phosphate level is greater than steady-state levels. 1,25-Dihydroxyvitamin D_3 is low in some, but not all, patients (127,128). Some have impaired conversion of 25-hydroxyvitamin D_3 to 1,25-dihydroxyvitamin D_3. This impaired conversion is also noted in the maleic acid model (104). A low pH is reported to decrease the conversion of 25-hydroxyvitamin D_3 to 1,25-dihydroxyvitamin D_3. All enzymes have fairly narrow pH optima, and that reported for the 1α-hydroxylase is pH 7.4; any change in blood pH, as is seen with maleic acid, would decrease the normal rate of formation to the active metabolite. The role of parathyroid hormone in Fanconi syndrome is not clear because both low and high levels have been reported. The combination of hypophosphatemia and low 1,25-dihydroxyvitamin D_3 universally seen in patients with Fanconi syndrome leads to osteomalacia in adults and rickets in children. In both age groups, the bone disease is quite marked and therapy is less than optimum.

Hyperchloremic metabolic acidosis, a state which also adversely affects bone growth, is caused by decreased proximal reabsorption of bicarbonate when plasma bicarbonate is near normal. At the T_M for bicarbonate, an increase in splay and bicarbonaturia is noted (acidification mechanisms are discussed later). Its fractional excretion may be greater than 30%, leading to massive fluid losses and a plasma bicarbonate which ranges from 12 to 18 mEq/L. As the filtered load of bicarbonate decreases, the percentage of bicarbonate reabsorbed by the proximal tubule increases (due to volume contraction) and no bicarbonate appears in the urine. Compounding the proximal effect on proton transport are results suggesting that a distal acidification defect may also be seen. If present, it is likely due to the prolonged hypokalemia and nephrocalcinosis resulting in a continuous bicarbonate "leak" from the distal nephron.

Sodium and potassium losses occur; these losses may be massive, resulting in severe secondary hyperaldosteronism and even metabolic alkalosis. Given the detailed studies on the interaction of high aldosterone and low potassium to stimulate collecting duct H-ATPase and H,K-ATPase activities, respectively, levels of both enzymes would be expected to be markedly elevated (129). Using the low-dose maleic acid model, however, our laboratory reported levels of both enzymes to be normal, not increased, in the distal nephron segments (cortical and medullary collecting ducts) (91). We did not, however, perform complete enzyme kinetics to elucidate a mechanism.

Volume contraction is seen and is due to a combination of the osmotic diuresis induced by glucosuria and the concentrating defect secondary to hypokalemia. Hypouricemia and uricosuria occur, but the cause is unknown. Urolithiasis is rare, although if noted is probably due to low urine flow rate and high urine pH. Proteinuria is minimal, and in children excretion is less than 40 mg/m^2/hour. The proteinuria consists of low–molecular-weight proteins of 1.9 to 3.0 kD. Growth retardation is universally seen in children and is multifactorial, as noted previously.

Specific Diseases Associated with Fanconi Syndrome

Several excellent reviews concerning aspects of the abnormalities associated with Fanconi syndrome have been written (83,122,180). Studies of megalin and other renal tubular and interstitial proteins related to the pathophysiologic process in Fanconi syndrome are newly published (131). Leheste et al. (132) reported some of the characteristics of a knockout mouse for the megalin receptor. These knockouts exhibit low–molecular-weight proteinuria, including losses of vitamin D-binding protein, α-microglobulin, and retinol-binding protein, as is seen in Fanconi syndrome. Megalin is believed to be responsible for normal proximal tubule endocytosis and tubular uptake of selected filtered molecules.

Inherited Forms

Cystinosis. In cystinosis, massive amounts of cystine accumulate in the kidney and other organs (133,134). The disorder is inherited as an autosomal-recessive trait and occurs in 1:200,000 live births. It is not the same as cystinuria (a disorder in which *no* intracellular cystine accumulation occurs). Three types of cystinosis have been identified. The *infantile* (or nephropathic) type is the most common. It is associated with severe renal injury and end-stage renal disease occurring by late childhood. Patients have very high levels of cystine in leukocytes and cultured fibroblasts. The second type has been termed the *benign* (or adult) type. It is marked by lower levels of intracellular cystine, with crystals found in bone marrow and cornea. There is no renal involvement. In the third type, termed the *adolescent* (or intermediate) type, renal involvement usually occurs during the teenage years. Bone disease is rare in this type; however, progression to end-stage renal disease is common (135). Glucosuria and aminoaciduria are present, but other manifestations of proximal tubule abnormality are absent.

Cystine accumulates in lysosomes of different tissues probably because of a defect in its efflux from cells. No abnormalities in cystine metabolism or intracellular degradation have been documented. The origin of the intracellular cystine is thought to be protein degradation (in lysosomes), but some studies have shown the involvement of plasma cystine. The definitive diagnosis is made by measuring an elevated intracellular cystine in leukocytes or in a rectal biopsy specimen; occasionally, needle-shaped, tinsel-like refractile opacities are seen in the cornea. Early in the disease, crystals are found in renal tubular epithelial cells and interstitial cells, but rarely in glomerular epithelial cells. The cystine-loaded tubules have decreased oxygen consumption, reduced ATP levels, and abnormalities in mitochondrial oxidation. Later in the disease, abundant crystal deposition occurs in the kidney, with tubular atrophy, interstitial fibrosis, and glomerular segmental sclerosis. In the infantile form, end-stage renal disease occurs by the age of 7 to 10 years, and in the adolescent form, by the second or third decade.

In the nephropathic type, the first clinical symptoms usually appear in the latter half of the first year of life. Polyuria, polydipsia, dehydration, and metabolic acidosis occur along with anorexia, failure to thrive, and rickets. Renal glucose losses

of 1 to 5 g/day are seen. Occasionally, metabolic alkalosis is noted and is thought due to the massive amounts of bicarbonate and citrate given to increase urinary pH. The disorder is more common among blond, fair-skinned whites than in other ethnic groups. Initial reports stating a high incidence of mental illness are incorrect.

Shotelersuk et al. (136) have identified abnormalities in the cystinosis gene (deletions of varying size and mutations). In general, specific mutations in the CTNS gene appear to result in the three different forms of the disease seen clinically.

Medical treatment for cystinosis includes fluid and electrolyte therapy along with the administration of alkali, phosphate, and vitamin D. Penicillamine, ascorbic acid, and dithiothreitol have been used to lower intracellular cystine concentration, but they are without proven benefit. Cysteamine, by contrast, appears to decrease leukocyte cystine content, slow the progression of renal insufficiency, and increase linear growth. This drug has significant side effects, including nausea, vomiting, and serum sickness (137). Dialysis or transplantation is eventually required, and the disease recurs in a transplanted kidney.

Galactosemia. Galactosemia is an autosomal-recessive disorder of galactose metabolism, most commonly due to a deficiency of galactose-1-phosphate uridyltransferase that is caused by mutations in the GALT gene (88). The deficiency leads to the intracellular accumulation of galactose-1-phosphate, a metabolite that damages the liver, brain, lens, and proximal renal tubule. The disorder has substantial allelic heterogeneity with worldwide variability. In Europeans, Q188R mutations account for approximately two thirds of affected subjects, whereas in South African blacks, more than 90% of patients have S135L mutations (88,138). In 16 unrelated Turkish patients, 57% had the Q188R mutation (139). Other mutations also have been noted in galactosemia (140–142). Galactose kinase deficiency has occasionally been noted in patients who have only cataracts.

The disorder is usually diagnosed in infants when vomiting and diarrhea develop after milk ingestion. Later, jaundice, hepatomegaly, cataracts, mental retardation, and Fanconi syndrome result. Galactosuria, but *not* glucosuria, occurs. Galactose is a urinary reducing substance that does not react in the glucose oxidase strip test. The diagnosis is made by demonstrating the enzyme deficiency in erythrocytes, fibroblasts, leukocytes, or hepatocytes. The disorder is treated by eliminating galactose from the diet. Topical aspirin appears to prevent galactosemic cataracts (143).

Hereditary Fructose Intolerance. Hereditary fructose intolerance is an autosomal-recessive trait caused by a deficiency of the catalytic site of fructose-1-phosphatase aldolase B (87). Mutations in some exons of the aldolase B gene, along with a partial deletion on exon 6, have been reported in patients (144). The latter has been shown to perturb the proper orientation of adjacent catalytic residues of the aldolase enzyme. The diagnosis may be suspected when nausea, vomiting, and hypoglycemia occur after the ingestion of fructose. Continued exposure to the sugar results in seizures, coma, and death. Infants in whom exposure is chronic, but low, have failure to thrive, hepatomegaly, jaundice, cirrhosis, and nephrocalcinosis. Although Fanconi syndrome appears virtually immediately after the ingestion of fructose, complete resolution is seen only days to weeks after the compound is withdrawn.

Fructose intolerance is diagnosed by measuring aldolase levels in a liver biopsy specimen. New and faster screening methods are also available (145). The pathogenesis of this disorder is incompletely understood, but acute fructose loading in animals results in intracellular accumulation of fructose-1-phosphate with subsequent marked depletion of ATP, adenosine diphosphate, adenosine monophosphate, and intracellular phosphate. Therapy mandates the strict avoidance of fructose and sucrose-containing foods.

Glycogenosis. At least ten specific defects of glycogen metabolism have now been identified. The pattern of inheritance varies, as does the expression of the disorder (146–150). In the X-linked form of glycogenosis, mutations occur in the α subunit of liver phosphorylase kinase and, until recently, no reports of proximal RTA had been noted. Burwinkal et al. (146) described two patients with a defect in the PHKA2 liver isoform who also had renal involvement. Typically, the autosomal-recessive form of the disease is found with proximal tubule dysfunction (i.e., the Fanconi-Bickel syndrome) (151, see also 152, and 153 for recent reviews). The disorder is more common in Europe than elsewhere. Glucosuria may be massive and may approaching 200 g/1.73 m^2 daily. Other features of Fanconi syndrome appear quickly in the first few months of life and precede the development of hepatomegaly consequent to the excess glycogen storage. Rickets, osteoporosis, and growth retardation are common. Despite the severity of renal tubular dysfunction and renal glycogen accumulation, the GFR does not decline (154). Santer and colleagues (147,151) reported follow-up on the original Swiss patient described in 1949 and reviewed 82 cases of patients with Fanconi-Bickel syndrome. Mutations in the GLUT2 gene were the most common abnormality noted.

Tyrosinemia. Tyrosinemia is an autosomal-recessive trait (also called *tyrosyluria*) with severe hypermethioninemia or inborn hepatorenal dysfunction (155–157). The disorder may present in an acute form in which acute hepatic dysfunction develops early in life or in a chronic form presenting with Fanconi syndrome, cirrhosis, hepatic carcinoma and vitamin D–resistant rickets. The disorder is thought to be due to deficiency of two enzymes, fumaryl-acetoacetate hydrolase (Type 1 tyrosinemia) and malelyl-acetoacetate hydrolase, resulting in the accumulation of fumaryl- and malelyl-acetate compounds, both of which are subsequently converted to succinylacetone (in lymphocytes) in humans (158). As stated earlier, succinylacetone is a compound structurally similar to maleic acid and is well known to cause Fanconi syndrome in animals.

Kubo et al. (159) reported a new mouse model defective in the fumaryl-acetoacetate hydrolase gene (and another enzyme). Proximal tubule cell dysfunction, rapid apoptosis, increased succinylacetone excretion, and a Fanconi-like syndrome occurred when one of the precursors was administered. Some of these abnormalities were partially reversed with caspase inhibitors, particularly the proximal tubule apoptosis. Studies of these doubly mutant mice should aid in our understanding of events leading to proximal renal cell death (160).

In affected humans, the renal abnormality improves with a diet low in phenylalanine and tyrosine, often coupled with drug therapy using the fluorinated derivative of 1,3-cyclohexanedione. Despite dietary intervention, however, cirrhosis eventually ensues. Orthotopic liver transplantation in eight children with 6.7 years' follow-up showed good outcome even though succinylacetone levels were not normal; thus, it should be considered in patients at risk of malignancy or who are nonresponders to drug therapy (161).

Wilson Disease. Wilson disease is an autosomal-recessive trait associated with marked intracellular accumulation of copper in the liver, brain (resulting in severe extrapyramidal symptoms), cornea, and renal proximal tubule (162–164). Procreation is also impaired with abnormal endocrine function noted both in men and women. Twenty-six percent of treated or untreated women had pathology (most had spontaneous abortion) and 10% of women had difficulty getting pregnant and about 4% of

men were impotent (165). In humans, the defective gene is localized to chromosome 13 in humans, coding for a copper-transporting ATPase–ATP7B (162,163). About 200 mutations have been identified in patients with the most common being the His1069Gln point mutation. The phenotypic expression varies significantly (166,167). In those homozygous for the aforementioned point mutation, less severe neurologic disease is noted when ApoE allele ε 3/3 is also found. The Long-Evans cinnamon rat appears to be a good animal model of the disease, and many interesting results using it have been published (168–170); for recent reviews see references (171–174).

Varying degrees of renal tubular dysfunction occur in most patients; aminoaciduria is common, followed by moderate glucosuria and bicarbonate wasting. Evidence of distal renal tubular dysfunction may also be observed, although the precise mechanisms for this are incompletely understood. Even with a progressive decrease in the GFR, frank renal failure typically does not occur. Despite the bicarbonate wasting observed in Wilson disease, there is no bone disease. No renal abnormality is evident on light microscopy; however, electron microscopy may show loss of brush-border membrane, cavitation of mitochondria, and accumulation of electron-dense bodies in the subapical area of the proximal tubule. Fanconi syndrome appears before the onset of hepatic failure.

The disease has a worldwide prevalence of approximately 1 in 30,000 births, and the deficiency of plasma ceruloplasmin is a characteristic feature. Once copper storage capacity of the hepatocyte is exceeded, the heavy metal appears in the circulation, where it is taken up by other tissues. Copper probably damages the kidney, because it accumulates in the cortex in its free form. Carrier states and prenatal diagnosis can now be identified (175). Treatment with trientine hydrochloride or penicillamine (1 g per day, usually in divided doses) reverses renal dysfunction, but the latter is associated with a variety of toxic effects, including the nephrotic syndrome and systemic lupus erythematosus in 25% of patients (176). Both are copper chelators. In 1997, the U.S. Food and Drug Administration approved zinc acetate as therapy for Wilson disease. This drug prevents gut absorption of copper, and follow-up of the initially treated subjects (up to 10 years) documents its substantial efficacy and low toxicity, even in pregnancy (172–174,177,178). Eghtesad et al. (179) reported that orthotopic liver transplantation is very successful in patients with hepatic failure and may prevent irreversible neurologic deterioration as well as most of the other abnormalities; more successful cases continue to be reported (174,180,181).

Lowe Syndrome. Lowe syndrome, also known as *oculocerebrorenal syndrome,* is transmitted as an X-linked recessive disorder that has been mapped to the Xq24-q26 region of the sex chromosome (182). This region of the gene appears to encode a protein that has 70% homology with human inositol polyphosphate-5-phosphatase, suggesting that this may be an inborn error of inositol phosphate metabolism and endosomal acidification (83,183). Many mutations and increased excretion of twenty-one urinary proteins have been reported (183,184). Because a few female patients are affected (<5%), there may be variable expression of the disorder, which depends on X chromosome inactivation (185,186). A spontaneous mutation could also explain the incidence of the disorder in women.

The disease is associated with severe mental retardation, congenital cataracts with glaucoma, and Fanconi syndrome. Late in the course of the disease, the GFR falls. Renal biopsy reveals only fusion of foot processes and swelling of endothelial cells early in the disease (187). Later, thickening of the basement membrane, tubular atrophy, glomerular sclerosis, and renal failure occur. The disorder is said to have three distinct stages: early, when central nervous system and renal abnormalities develop; later, when bone disease becomes evident; and finally, the onset of death. Treatment is symptomatic only and is directed at the organ systems involved. Results of long-term follow-up of liver transplant recipients are variable.

Cytochrome C Oxidase Deficiency. At least two cytochrome deficiencies (complex III and complex IV deficiencies) are now reported to have an associated proximal RTA. Originally described in 1977, cytochrome C oxidase deficiency (complex IV) is characterized by a generalized myopathy, encephalopathy, and Fanconi syndrome (188,189). It is the most common of the mitochondrial disorders of the respiratory chain. Symptoms are seen early in life and are associated with progressive muscle weakness, respiratory failure, and death (190,191). Skeletal muscle fibers contain lipid-laden vacuoles with swollen mitochondria. Muscle biopsy reveals both histochemical and biochemical deficiencies of cytochrome C oxidase. Antibodies to the various subunits of the enzyme show selective loss of the subunit 7a,b, representing a defect in the nuclear-encoded polypeptide of the respiratory chain. Immunocytochemistry also reveals the loss of subunit 2 of mitochondrial DNA. One or both of these abnormalities are associated with impaired activity of the electron transport chain and abnormalities in oxidative phosphorylation.

Mourmans et al. (192) have described six children with an isolated complex III deficiency in muscle. One half of the patients presented in the neonatal period, and two of these infants had a severe form of Fanconi syndrome. Although presentation varied substantially, clinical outcome ranged from neonatal death to failure to thrive. Extraction of mitochondrial DNA revealed no common deletions or point mutations.

The genetics of these disorders are different from those of autosomal disorders. Mitochondrial DNA is directly inherited from the cytoplasm of germ cells, primarily the oocyte. Because the spermatocyte contributes few of its mitochondria at fertilization, maternal inheritance is apparent in all mitochondrial disorders.

Idiopathic Fanconi Syndrome. The idiopathic form of Fanconi syndrome has been described in both adults and children. The pattern of inheritance is variable, being autosomal recessive, autosomal dominant recently localized to chromosome 15 (193), or X-linked. In most cases, however, no clear pattern can be described, and these cases, as such, are considered sporadic. Although the patients have virtually all aspects of the renal tubular abnormalities, others have been described with glucose intolerance, insulinopenia, and ketonuria. Patients progress to renal failure, and the syndrome recurs in an allograft after transplantation. Marshansky et al. (83) is an excellent review as to cellular and molecular mechanisms of proximal tubule abnormalities.

Acquired Forms

Exogenous Agents. In humans, *lead* intoxication results in Fanconi syndrome (194,195). In general, the severity of the syndrome correlates with the degree of intoxication. The changes are similar to those noted in laboratory animals in which partial uncoupling of oxidative phosphorylation has been described (101,102). Chronic exposure to *cadmium,* an element used in electroplating and other metal-processing activities, also may cause Fanconi syndrome and bone disease (90,196). Although cadmium is present in small amounts in food, drinking water, and dust, intake by the oral route is not thought significant. One cigarette contains 1 to 2 μg of cadmium, and even if pulmonary cadmium absorption is as low as 10%, smoking one pack per day results in an annual dose of approximately 1 mg. The half-life of cadmium in biologic tissues is approximately 20 years; thus, with continuous exposure, the total body burden

is quite high. Cadmium is now a well-known cause of renal cell adenocarcinoma, being seen as late as 25 years after exposure. *Outdated tetracyclines* cause a reversible Fanconi syndrome associated with muscular weakness and occasionally neurologic symptoms. Anhydro-4-epitetracycline is considered the offending agent; it is formed by heat, moisture, and an acidic pH (113). Substances such as *toluene, Lysol, gentamicin, 6-mercaptopurine, diachrome,* and *streptozotocin* may also cause a reversible form of the syndrome (31,185). *Cisplatin,* a compound frequently used in cancer chemotherapy, results in a Fanconi syndrome that is irreversible and dose dependent (197). An unusual feature of this syndrome is massive magnesium wasting, indicative of damage to the thick ascending limb of Henle. *Ifosfamide* commonly causes Fanconi syndrome in humans and animals (198). Badarly (199,200) has shown that both taurine and L-histidinol, an analog of histidine given orally, markedly attenuate this chemotherapeutic agent's toxicity. Taurine administration does not appear to affect the antitumor activity of ifosfamide. Children with ifosfamide-induced Fanconi syndrome have specific deletions in mitochondrial DNA that are *not* maternally inherited. The clinical syndrome was noted to occur for as long as 2 years after the drug had been discontinued (201).

Other Causes. Dysproteinemia with a "Fanconi-like" syndrome occurs in adults who have amyloidosis, multiple myeloma, light-chain disease, or Sjögren syndrome (202–204). The patients with Sjögren's had distal tubular dysfunction with hypokalemic, a few with osteomalacia (205). A mouse model for myeloma with Fanconi syndrome has been reported, and doubtless new data regarding mechanisms should be forthcoming (206). Using x-ray crystallography, Deret et al. (207) examined the molecular structure of the κ light chains in several patients with the proximal tubular disorder. Most were found to have a unique chemical moiety in one part of the molecule and an absence of specific side chains in another part, observations associated with dimer formation and toxicity. Also, a single patient has been described with a malignant mesenchymal bone tumor and Fanconi syndrome, which reversed after tumor excision (208). Rarely, Fanconi syndrome is associated with nephrotic-range proteinuria (209). These patients, however, typically have focal segmental glomerulosclerosis on renal biopsy.

DISORDERS OF ACIDIFICATION

Overview of Urinary Acidification Mechanisms in the Proximal and Distal Nephron

The kidney preserves acid–base balance by two mechanisms: *bicarbonate reclamation* (mainly a function of the proximal tubule) and *bicarbonate regeneration* (largely a function of the distal nephron). Both mechanisms must be intact to excrete the 1 mEq/kg acid ash protein ingested daily and to maintain the arterial pH at 7.4 and the plasma HCO_3 concentration at 24 mEq/L.

Proximal Tubule Acidification

Most evidence suggests that the major part of bicarbonate reabsorption in the proximal tubule is mediated by hydrogen ion secretion by the Na-H antiporter (i.e., NHE-3 isoform) localized in the brush-border membrane (bicarbonate reclamation) (Fig. 20-1). The source of energy for this transporter appears to be basolateral Na,K-ATPase. The enzyme generates the electrochemical gradient for luminal sodium entry by maintaining a low intracellular sodium concentration (~10 mEq/L). There is also an electrogenic H-ATPase present in the proximal tubule, although its precise role in proximal acidification is incompletely understood (210–213). Large quantities of bicarbonate are normally absorbed in the proximal tubule (~80%), and a number of factors come into play to alter this process such that virtually all of the bicarbonate may be absorbed here (Table 20-2).

TABLE 20-2

FACTORS INFLUENCING PROXIMAL BICARBONATE REABSORPTION

Increased HCO_3 reabsorption	Decreased HCO_3 reabsorption
Volume contraction	Volume expansion
Chloride deficiency[a]	—
Potassium depletion	Potassium excess
Hypercapnia (↑ arterial P_{CO_2})	Hypocapnia (↓ arterial P_{CO_2})
↓ Arterial pH (by intracellular acidosis?)	↑ Arterial pH (by intracellular alkalosis?)
—	Carbonic anhydrase inhibitors (e.g., acetazolamide)
Hypoparathyroidism	Hyperparathyroidism
Hypercalcemia	Hypocalcemia
Vitamin D excess	Vitamin D deficiency
—	Phosphate depletion
Glucose	—

[a]In the clinical setting, this is synonymous with volume contraction. (From: Alpern RJ, Rector FC Jr. Renal acidification: cellular mechanisms of tubular transport and regulation. In: Windhager E, ed. *Handbook of Physiology.* New York: Oxford University Press, 1992:767; Sabatini S. Distribution and function of the renal ATPase (H-ATPase). *Semin Nephrol* 1991;11:37; and Sabatini S, Kurtzman NA. Pathophysiology of the renal tubular acidosis. *Semin Nephrol* 1991;11:202.)

Factors affecting proximal bicarbonate reabsorption may be summarized as follows (214). The state of extracellular volume (either absolute or "effective" arterial volume) is the most potent regulator of proximal bicarbonate reabsorption. When volume is contracted, proximal sodium reabsorption increases markedly as a consequence of increased activity of the Na-H antiporter; thus, bicarbonate reabsorption increases secondarily. Carbonic anhydrase plays an essential role in proximal bicarbonate reabsorption because it is present on the brush-border membrane as well as inside the cell. Enzyme inhibition results in substantial bicarbonaturia and metabolic acidosis. Hyperkalemia, independent of volume, inhibits proximal bicarbonate reabsorption, as does a fall in arterial pH, low P_{CO_2}, parathyroid hormone excess, and hypocalcemia (for reviews, see Sabatini and Kurtzman [214] and Eiam-Ong and Sabatini [215]).

Of the many possible mechanisms for proximal RTA, three seem most likely: inhibition (or absence) of proximal tubule carbonic anhydrase, direct inhibition of Na-H exchange activity, and inhibition of basolateral Na,K-ATPase (210,216).

Inhibition of proximal carbonic anhydrase would lead to bicarbonate wasting and hypokalemia and should result in massive bicarbonaturia. Acidemia would result to the extent that the distal nephron is incapable of reabsorbing the bicarbonate remaining in the tubular lumen. Because the enzyme is present in both the proximal and distal nephron, one would expect features of both acidification processes to be present. Indeed, the defect in urinary acidification noted in some family members

with carbonic anhydrase B deficiency is compatible with this notion (discussed later).

A direct inhibitory effect of the apical Na-H exchanger (NHE-3) would also lead to proximal RTA. The magnitude of this effect is difficult to estimate. Some think that two thirds of proximal acidification occurs through the Na-H exchanger, with the rest occurring through the proton pump (i.e., H-ATPase) (33,216–218). If this is so, then a selective defect in the antiporter would result in a "partial"-type proximal RTA. The precise relationship between these two mechanisms of acidification is not yet understood because activity of one may affect the other. For example, in chronic metabolic acidosis (induced by NH_4Cl loading), Na-H exchange activity is increased, and some report proximal H-ATPase activity to be decreased (213,219). In chronic metabolic alkalosis, however, the exchanger is inhibited but the H-ATPase is unaffected. Furthermore, effects of the various parameters given in Table 20-2 and activity of proximal tubule H-ATPase are not yet defined. Recent defects in distal H-ATPase have been noted and are associated with impaired proton secretion (discussed later).

Inhibition of basolateral Na,K-ATPase activity should also lead to proximal RTA. It would also lead to loss of all ions and solutes transported by sodium-coupled mechanisms. Unless there are major differences in the proximal tubule enzyme, its various subunits, and their interactions, compared with other tissues or with the distal nephron, this lesion may be incompatible with life.

A decrease in proximal tubule H-ATPase due to gene mutations or postgenomic signaling defects could result in a fall in acidification, but thus far we have no evidence for such suggestions. Although our own study with NH_4Cl loading to intact rats does not support such a hypothesis (i.e., we should have seen an increase in biochemical activity), there may be factors that we did not take into account (different isoforms, different enzyme kinetics, different cellular localization, etc.). See also the recent review by Marchansky et al. (83).

Distal Nephron Acidification

Until the 1970s, distal RTA was considered a single disorder. The prevailing view was that patients were unable either to generate or maintain a hydrogen ion gradient across the distal nephron because they were unable to secrete protons or because the secreted protons then back-diffused to the cell (i.e., backleak). The observation that amphotericin B administration resulted in altered proton permeability (and backleak) of the membrane substantiated the latter view (217,218,220). Because of this observation, this form of RTA was referred to as *gradient* RTA. A different mechanism was proposed in the mid-1970s based on the observation that the urinary P_{CO_2}, after alkalinization (an index of collecting tubule proton secretion), was low in patients with distal RTA. It was argued that a low urine P_{CO_2} was incompatible with a gradient mechanism because, during alkalinization of the urine, the tubular pH was raised to a value higher than that of the peritubular blood so that the chemical gradient favored secretion; it did not favor backleak. Thus, the low urinary P_{CO_2} detected in patients with distal RTA argued for a secretory defect rather than a backleak defect. We now have a better understanding of the biochemical mechanism of these two defects, as well as other abnormalities of proton transport.

In the distal nephron, proton secretion occurs by three major mechanisms (33,221–223): an electrogenic proton H-ATPase located in the luminal membrane of α-intercalated cell (and also found in the basolateral membrane of the β-intercalated cell), an electroneutral H,K-ATPase also located in the intercalated cell, and the transepithelial voltage gradient (up to –40 mV) generated by sodium reabsorption and activity of the basolateral Na,K-ATPase. Because protons must be secreted into a urine containing buffer, the amount (and type) of buffer present also affects overall proton secretion (217). In humans, most of the urinary buffer is HPO_4^{2-} as a consequence of dietary protein intake. The other significant buffer for proton secretion is that coupled to ammonium (NH_4^+).

There are three major factors that affect activity of the two proton pumps either directly or indirectly. The adrenal hormone, aldosterone, directly stimulates the electrogenic H-ATPase both *in vivo* and *in vitro* (91,211,225). This is true in mammalian cortical and medullary collecting tubules and in certain nonmammalian analogs of the distal nephron. Aldosterone deficiency inhibits or markedly decreases activity of this enzyme (91). Distal sodium delivery, a maneuver that generates a large transepithelial gradient (lumen negative), also stimulates this enzyme in that it creates a favorable electrical gradient for the secretion of a positively charged ion (i.e., hydrogen). This effect seen in the cortical collecting tubule, however, is a secondary one because enhanced distal sodium delivery directly increases Na,K-ATPase activity (the medullary collecting tubule does not reabsorb sodium) (217,226). Hypokalemia or low urinary potassium markedly stimulates the H,K-ATPase in the cortical collecting duct, and hyperkalemia inhibits the enzyme. These effects are independent of any action on aldosterone (91). Hypokalemia also stimulates medullary collecting duct H,K-ATPase (where the transtubular potential is lumen +10 mV and is due to proton secretion by the H-ATPase).

Thus, defects in distal proton secretion in the *cortical* collecting duct appear due to a direct action on the electrogenic H-ATPase, a direct action on the electroneutral H,K-ATPase (the second proton secretory enzyme in the kidney), a direct action on Na,K-ATPase, or any combination of the three. In the *medullary* segment of the collecting duct, the primary mechanism for deranged acidification seems to be defective H-ATPase activity. Significant quantities, although lesser amounts, of the electroneutral H,K-ATPase are present in the medullary collecting tubule, and although the enzyme does aid in urinary acidification at this site, it probably does not contribute to electrical gradients (218,222).

Proximal (Type II) Renal Tubular Acidosis*

See the section on Heterogeneous Tubular Defects (Fanconi syndrome) (Table 20-3) (227,228).

Carbonic Anhydrase Deficiency

Carbonic anhydrase II deficiency is an extraordinarily rare defect (229,230). RTA may be proximal, distal, or both, as would be expected if the type II carbonic anhydrase isoform were present inside the renal tubular cell throughout the nephron. It is associated with osteopetrosis, severe mental retardation, short stature, and frequent fractures. Bilateral renal stones, nephrocalcinosis, and hypercalciuria are predictable. Hypokalemia is commonly noted, as may be found with proximal or distal RTA. Diagnosis is made by showing fractional HCO_3 excretion greater than 15% after HCO_3 infusion. Large amounts of bicarbonate are required (and potassium if hypokalemia is found), often without achieving therapeutic success. A mouse transgenic for carbonic anhydrase II deficiency has been studied that, when given the enzyme, partially responds by decreasing urine pH. Mutations continue to be reported (231,232) as well as the complete absence of band 3 protein and protein 4.2 in a birth (233). Three publications have particular relevance to the kidney (234–236).

*There is no type III renal tubular acidosis.

TABLE 20-3

SUMMARY OF SELECTED ISOLATED TUBULAR TRANSPORT DISEASES OF THE RENAL ATPases

Site	Disorder	Mechanism
Proximal tubule	Fanconi syndrome (type II RTA)	↓ PCT Na, K-ATPase
	Isolated proximal RTA	↓ H-ATPase; ↓ Na/H antiporter
MTAL	Bartter syndrome	↓ MTAL Na, K-ATPase
		↓ Na/K/2Cl cotransporter
Collecting duct	Classic distal RTA (type I RTA) (with hypokalemia)	↓ CCT H, K-ATPase
	Distal RTA (with hyperkalemia) (type IV RTA)[a]	
	Urinary tract obstruction	↓ CD Na, K-ATPase
		↓ H-ATPase
	Amiloride	↓ CD Na, K-ATPase
	Lithium	↓ CT Na, K-ATPase
		↓ CCT H-ATPase
		↓ H, K-ATPase
	Sickle hemoglobinopathy	?

CCT, cortical collecting duct; CD, collecting duct; *MTAL*, medullary thick ascending limb; *PCT*, proximal convoluted tubule; *RTA*, renal tubular acidosis.
[a] Also called *voltage-dependent* distal RTA. (There is no type III RTA.)
Summarized from references: Roth KS, Goldmann DR, Segal S. Developmental aspects of maleic acid-induced inhibition of sugar and amino acid transport in the rat renal tubule. *Pediatr Res* 1987;12:1121; Burwinkel B, Amat L, Gray RG, et al. Variability of biochemical and clinical phenotype in X-linked liver glycogenosis with mutations in the phosphorylase kinase PHKA2 gene. *Hum Genet* 1998;102:423; Ribeiro ML, Alloisio N, Almeida H, et al. Severe hereditary spherocytosis and distal renal tubular acidosis associated with the total absence of band 3. *Blood* 2000;96:1602; Dafnis E, Kurtzman NA, Sabatini S. Effect of lithium and amiloride on collecting tubule transport enzymes. *J Pharmacol Exp Ther* 1992;261:701; Ogita K, Takada N, Taguchi T, et al. Renal tubular acidosis secondary to FK506 in living donor liver transplantation: a case report. *Asian J Surgery* 2003;26:218; Cho BS, Kim HS, Jung JY, et al. Severe renal tubular acidosis in a renal transplant recipient with repeated acute rejections and chronic allograft nephropathy. *Am J Kidney Dis* 2003;41:E6; Seldin DW, Coleman AJ, Carter NW, et al. The effect of Na_2SO_4 on urinary acidification in chronic renal disease. *J Lab Clin Med* 1967;69:893.

Classic (Type I) Distal Renal Tubular Acidosis

The syndrome of classic distal RTA consists of hypokalemia, hyperchloremic metabolic acidosis, and inability to lower the final urinary pH to less than 5.5. Nephrocalcinosis, nephrolithiasis, and osteomalacia (or renal rickets) also occur. Urinary P_{CO_2} does not rise normally after bicarbonate infusion (217,220) (Table 20-3).

Hypokalemic RTA is reported to be endemic in northeastern Thailand (237). The condition may affect as many as 450,000 people. These patients also have gastric hypoacidity; therefore, it was suggested that they might have an abnormality in H,K-ATPase activity (251). The soil and well water in this part of Thailand are rich in vanadium, an inhibitor of H,K-ATPase. In addition, the local population has higher than normal concentrations of vanadium in urine and tissue. This syndrome affects not only humans but water buffalo, indicating that it may be caused by an environmental inhibitor of the H,K-ATPase pump (239,240).

Long-term administration of sodium orthovanadate to normal rats results in hypokalemic distal RTA, with the urinary pH higher than expected for the level of acidemia and a vanishingly low fractional bicarbonate excretion (241). In microdissected nephron fragments from these animals, activity of H,K-ATPase and Na,K-ATPase was markedly depressed in the collecting duct. H-ATPase activity, by contrast, was normal. It is likely that the hypokalemia observed in these animals depressed aldosterone secretion, preventing the rise in H-ATPase activity despite metabolic acidosis. To the extent that this model represents human type I distal RTA, these enzyme defects are predictive. In all likelihood, a singular defect in collecting duct H,K-ATPase is responsible for the lesion.

Using monoclonal antibodies to the 31-kD subunit of the H-ATPase demonstrated an absence of the enzyme in intercalated cells in a renal biopsy from a patient with Sjögren syndrome and hypokalemic type I distal RTA (242). No H-ATPase staining was found in the proximal tubule brush-border microvilli, but it was noted in the subvillous invaginations. No data concerning H,K-ATPase immunoreactivity were presented. More cases of Sjögren syndrome are being identified with defects in acidification (243,244).

Hereditary Classic Distal Renal Tubular Acidosis (Dominant/Recessive)

The dominant form of classic distal RTA is thought to be due to point mutations in the Cl/HCO_3 exchanger located on the basolateral membrane of the α-intercalated cell (245,246). These patients have a mild form of distal RTA, with nephrocalcinosis and stones noted in one half of the patients. Serum potassium is low and therapy consists of 2 mEq HCO_3 per kg body weight and potassium. In children, more bicarbonate is required to affect linear growth even partially. Recently, Wrong's group (235) reported a dominant form of RTA in the AE1 enzyme in erythrocytes (i.e., 11-COOH deletion called human band

3 Walton). This band 3 Walton inhibits the movement of normal band 3 to the cell surface, retaining it inside the cell. Apparently, chloride transport is normal (247).

The recessive form of the disease is apparent in the first year of life and has been noted to be associated with mutations in the β_1 subunit of the H-ATPase in some families. Abnormalities in the enzyme in the cochlea have also been noted in those with sensorineural hearing loss (248,249). Now abnormalities have been noted in other isoforms of the kidney resulting in recessive distal RTA (250,251). Plasma HCO_3 and potassium are lower in these subjects than in those with the dominant form of distal RTA. Bone disease is quite marked (osteopetrosis) and multiple stones and nephrocalcinosis invariably occur. Some of these patients are CAII defective and have severe mental retardation. None of these groups of patients can lower the urine pH below 5.5. Therapy, which consists of HCO_3 and potassium, is less than optimum.

Voltage-Dependent Distal (Type IV) Renal Tubular Acidosis

The primary defect in "voltage-dependent" distal RTA is considered to be the loss of the lumen-negative potential difference in the cortical collecting duct (Table 20-3). The absence of the lumen-negative potential difference would lead to a reduction in both hydrogen and potassium secretion (218,252). Therefore, the hallmark features of this syndrome are a hyperkalemic, hyperchloremic metabolic acidosis associated with salt wastage (detectable only during salt restriction). In humans, plasma aldosterone is normal or increased and urinary pH is greater than 5.5. Urinary P_{CO_2} during bicarbonate loading is low, and the response to furosemide administration is abnormal (i.e., P_{CO_2} does not rise).

Urinary tract obstruction (UTO), amiloride, and lithium (Li^+) induce this syndrome clinically (218,253–255). Careful assessment of these animal models, however, reveals significant differences. Amiloride administration and UTO are associated with hyperkalemia and the inability to lower urinary pH below 5.5. Giving Li^+ to humans or animals, however, does not induce hyperkalemia and does not affect the ability to lower urinary pH after sulfate administration. Li^+ has a greater propensity to cause systemic acidosis than does amiloride.

Our studies on the effects of short-term unilateral ureteral obstruction and long-term administration of amiloride and Li^+ (in rats) on acid–base status, electrolyte composition, and collecting tubule ATPases provide further insight into the pathogenesis of this type of distal RTA. After 24 hours, rats with unilateral obstruction have normal systemic base and potassium levels, as expected with a normal contralateral kidney. Potassium and proton excretion in the postobstructed kidney is markedly reduced. Rats given amiloride have normal acid–base status, but become hyperkalemic. Metabolic acidosis developed in Li^+ treated rats, but potassium levels were normal to low. In the postobstructed kidney, Na,K-ATPase activity was depressed throughout the entire nephron (from the proximal tubule to the collecting duct). H-ATPase, however, was markedly decreased in medullary collecting duct. By contrast, the H,K-ATPase enzyme was increased significantly in the cortical collecting duct, whereas it was low in the medullary collecting tubule. Long-term amiloride administration in rats resulted in low Na,K-ATPase activity in cortical and medullary collecting ducts (254). H-ATPase activity was low, but only in the cortical collecting duct. H,K-ATPase activity was unaffected by amiloride. After long-term Li^+ therapy, Na,K-ATPase activity was low in the cortical collecting duct; activities of H-ATPase and H,K-ATPase were also decreased, but only in the cortical collecting tubule.

In the 24-hour model of unilateral ureteral obstruction, the enhanced cortical collecting duct H,K-ATPase activity probably mitigates the reduced H-ATPase activity at this site. Therefore, the overall acidification defect observed in the model is probably due primarily to impaired medullary collecting duct proton transport. The potassium retention observed in acute UTO is probably due to a fall in potassium secretion secondary to reduced Na,K-ATPase activity and enhanced H,K-ATPase activity of the cortical collecting duct. Studies in isolated perfused tubules show acidification to be intact (or even increased) in cortical collecting ducts after unilateral ureteral obstruction, but decreased in medullary collecting ducts (see also references 256–257).

The alterations seen during UTO appear to be specific because certain of the enzymes remain normal (253). It seems likely that there is "cross-talk" between the two proton ATPases in intercalated cells of the cortical collecting duct, such that the inhibition of one activates the other. The underlying mechanisms that trigger and control this phenomenon remain unknown. Rats subjected to 24-hour UTO have moderately high aldosterone levels, but aldosterone plays no part in regulating H,K-ATPase (129). In addition, the increased H,K-ATPase activity detected only in the cortical segment suggests that the proximate cause is at this level. This indicates there are additional forces beyond systemic potassium balance that control the H,K-ATPase enzyme.

A report examining the renal cellular distribution of H-ATPase (30-kD subunit) in unilateral ureteral obstruction showed that changes in its cellular distribution were seen only in the intercalated cells (258). The morphologic change that correlated most closely with the onset and resolution of the acidification defect was the appearance of gaps in apical H-ATPase staining, suggesting a cytoskeletal defect. Because staining of H,K-ATPase was not performed, full correlation between the immunologic and enzymatic data has not yet been established.

Despite the acidification defect demonstrable in kidney after 24-hour unilateral obstruction, acid–base status is normal. The contralateral kidney, therefore, adapts quickly to the increased workload. In additional studies, we have shown that the contralateral kidney has a 40% increase in collecting tubule H-ATPase, H,K-ATPase, and Na,K-ATPase activities (259). Therefore, the contralateral kidney responds to events in the obstructed kidney even in the absence of measurable changes in blood chemistry. The signals that initiate these changes remain to be elucidated.

Thus, it appears that these experimental models of voltage-dependent distal RTA exhibit renal pump defects in addition to any electrical changes secondary to diminished Na,K-ATPase activity, lending some insight into human type IV RTA.

Normokalemic Distal Renal Tubular Acidosis

Metabolic acidosis develops when functional renal mass is reduced to a level at which the GFR is 20% to 30% of normal. When the GFR falls below 20 mL/minute, normochloremic acidosis with a high anion gap develops. In patients with tubular interstitial disease, acidosis develops earlier and is more profound than in patients with glomerular disease. This pattern may be the first indication of chronic transplant rejection (260–262).

Patients with renal insufficiency have a relatively high urinary pH and low net acid excretion even when plasma bicarbonate is in the normal range. Distal proton secretion, however, is qualitatively normal, as indicated by the ability maximally to lower the urinary pH and mount a normal response to sodium sulfate or phosphate loading (217,263). The P_{CO_2} gradient (urine value minus blood value) is relatively normal in these

patients after correction for the associated concentrating defect. A reduction in ammonia production secondary to decreased renal mass or impaired ammonia trapping in the collecting duct is considered to be the basic defect in these patients. Urinary phosphate excretion and formation of titratable acids are relatively normal.

A defect in one of the two proton pumps may be the cause of this disorder. Because these patients are normokalemic, an acidification defect could be caused by abnormal H-ATPase activity in the collecting duct. If this enzyme fails but the Na,K-ATPase and H,K-ATPase pumps remain intact, normokalemic distal RTA should ensue. There are no human or animal studies examining this issue directly, as has been done for the other forms of distal RTA.

Aldosterone Deficiency

The syndrome of isolated aldosterone deficiency was originally described in 1957 by Hudson et al. (264). Only since the early 1980s has the disorder been recognized with any regularity. Theoretically, aldosterone deficiency should cause a voltage-dependent acidification defect and proton secretory failure. In aldosterone-deficient humans and laboratory animals with acidosis, the ability to generate urinary pH gradients in the collecting duct is thought to be relatively normal, but the rate of acid secretion is reduced (217,218). Patients with aldosterone deficiency can lower urinary pH below 5.5, have a normal urine P_{CO_2} in an alkaline urine, and respond appropriately to phosphate and sulfate infusion. The rate of titratable acid excretion is normal, but urinary ammonium and potassium excretion after sodium sulfate infusion is reduced. The decreased ammonia production and excretion may be due mainly to the hyperkalemia. When hyperkalemia is corrected by cation exchange resin or by dietary potassium restriction, both ammonia production and excretion increase, but not to normal levels. Net acid excretion also increases, but the finding that it never reaches control levels suggests an intrinsic defect in hydrogen ion secretion.

Aldosterone deficiency results in an absolute decrease in H-ATPase activity in both cortical and medullary collecting duct segments (129). A series of experiments examined the interaction of aldosterone and potassium on the H-ATPase and H,K-ATPase enzymes in the collecting duct. These studies were designed to control potassium and aldosterone levels precisely because each exerts powerful effects on the other (129). Adrenalectomized animals were given normal or pharmacologic amounts of aldosterone by a chronically implanted osmotic minipump (or given no aldosterone) and then placed on one of three potassium diets (normal, low, or high). The most severe metabolic acidosis resulted in the group placed on the high-potassium diet that received no aldosterone. In microdissected segments (cortical and medullary collecting tubules) from the kidneys of these animals, H-ATPase activity was approximately three-fourths lower than that in normal animals. Regardless of the level of dietary potassium (normal, low, or high), animals not given aldosterone had very low H-ATPase activity. By contrast, even when the aldosterone level was varied markedly (zero, normal, or high), H,K-ATPase activity changed only in response to potassium. H,K-ATPase activity in the collecting duct was stimulated when plasma and muscle potassium levels were low, and it was inhibited when hyperkalemia was present (129). The most profound metabolic alkalosis was seen in the group of rats with pharmacologic aldosterone and plasma potassium levels of approximately 2 mEq/L. In these animals, levels of both enzymes were very high and the H-ATPase varied only with aldosterone, whereas the H,K-ATPase varied only with potassium.

Pseudohypoaldosteronism Type 1 and Liddle Syndrome

Also called *Cheek-Perry syndrome,* pseudohypoaldosteronism type 1 may appear as either an autosomal-dominant or autosomal-recessive feature (in families who have intermarried) (265). It is characterized by salt wasting, dehydration, metabolic acidosis, and hyperkalemia (Table 20-4). Extremely elevated aldosterone secretion rates have been reported, along with high plasma renin activity. It was postulated that the collecting tubules were refractory to endogenous aldosterone; however, spironolactone worsened the salt loss, suggesting that an absolute lack of responsiveness to aldosterone was not present. Mineralocorticoid receptors are reported absent (or markedly decreased) in mononuclear leukocytes of patients with the disease, and some investigators, therefore, have postulated that the defect is in the expression of the mineralocorticoid receptor gene (266,267). In one study in a single patient, however, there was no evidence of rearrangement or deletion in genomic DNA, and normal "qualities" of mineralocorticoid receptor RNA were present (268). In 11 families, the gene loci subsequently were found to be on the short arm of chromosome

TABLE 20-4

METABOLIC ABNORMALITIES OF SOME TRANSPORT DISORDERS

Disorder	Acid–base status	Serum				Plasma renin activity	Plasma aldosterone	Pattern inheritance	Molecular mechanism
		Na^+	K^+	Cl^-	HCO_3^-				
Pseudohypoaldosteronism (Cheek-Perry syndrome)	Metabolic alkalosis	↓	↑	↓	↑	↑	↑	AR/AD	Defective ENaC channel
Pseudohyperaldosteronism (Liddle syndrome)	Metabolic alkalosis	↑ or nl	↓	nl	↑	↓↓	↓↓	AD	Stimulating ENaC channel
Bartter syndrome	Metabolic alkalosis	↓	↓	↓	↑	↑↑	↑↑	AR	Thick limb Na/K/2Cl defect
Renal tubular K^+ secretion defect (Spitzer-Weinstein syndrome)	Metabolic acidosis	nl	↑	nl	↓	↑ or nl	nl	unknown	?

AD, autosomal dominant; *AR*, autosomal recessive; *ENaC*, amiloride-sensitive epithelial sodium channel; *nl*, normal.
See the text for relevant information regarding clinical expression of the disorders.

12 or chromosome 16 in an area where the subunits of the amiloride-sensitive epithelial sodium channel (ENaC) resides, not in the aldosterone receptor *per se* (269–272).

The differential diagnosis includes Fanconi syndrome, adrenal hyperplasia with virilization, RTA, and salt-losing nephritis. Treatment consists of a high-sodium diet (3 to 6 g of NaCl per day), an intervention that does not decrease the elevated plasma renin activity.

Liddle syndrome is an autosomal-dominant disorder characterized by hypertension, hypokalemia, and metabolic alkalosis; aldosterone and plasma renin activity are extremely low (Table 20-4) (271,273). The GFR is usually normal. Patients with this disorder respond to triamterene but not to spironolactone. Red blood cell sodium efflux is increased, whereas the Na:K ratio in salivary and sweat glands is normal. The primary mechanism appears to be due to overactivity of the collecting duct ENaC channel (in the α, β, or γ subunits), resulting in enhanced sodium reabsorption in the collecting duct. This disorder is therefore the exact opposite of that of pseudohypoaldosteronism type 1 (274,275). The differential diagnosis includes Bartter syndrome, primary aldosteronism, and 11β-hydroxysteroid dehydrogenase deficiency. In addition to triamterene, patients usually require sodium restriction and potassium supplementation.

Bartter Syndrome and Gitelman Syndrome

These syndromes are characterized by severe hypokalemia, salt wastage, and metabolic alkalosis with hyperaldosteronism, hyperreninemia, as well as hypertrophy and hyperplasia of the juxtaglomerular apparatus (Table 20-4). Patients with Bartter syndrome are also refractory to the rise in blood pressure in response to angiotensin II infusion; urinary prostaglandin E_2 levels are high (276–279). Some cases are sporadic, but most are inherited as an autosomal-recessive trait. The three types of Bartter syndrome must be distinguished from Gitelman syndrome, which is also a primary renal tubular disorder with hypokalemic metabolic alkalosis and salt wastage, but one associated with marked magnesium deficiency and hypocalciuria. Urine prostaglandin levels are normal. The molecular basis for Gitelman syndrome is due to mutation(s) in the thiazide-sensitive NaCl cotransporter in the distal convoluted tubule on chromosome 16 (long arm) or the chloride channel gene (278–280). Mutant alleles occur in approximately 1% of the population, making the disorder quite common.

It appears that the abnormality in Bartter syndrome is multifactorial. In type I, mutations are found in the Na/K/2Cl cotransporter in the thick ascending limb, thus creating a lesion that resembles the result of chronic furosemide use (281,282). In type II, mutations are found in the luminal K channel (283,284), and in "classic" Bartter's (type III), mutations are found in the basolateral Cl channel. All of these lesions result in salt wastage, hypokalemia, and most of the features described previously. See also recent reviews (278,285–288) for molecular genetics and cell signaling abnormalities.

Bartter syndrome typically manifests early in life with polyhydramnios, failure to thrive, growth retardation, polydipsia, dehydration, salt craving, and marked muscle weakness. Blood pressure is characteristically low or normal. The GFR is normal, but there is inadequate urinary acid excretion after NH_4Cl challenge. Nephrogenic diabetes insipidus also may be seen. Sodium transport in erythrocytes and salivary glands is impaired, which led some to suggest that the primary problem was due to altered responsiveness to aldosterone. As early as 1975 Kurtzman and Gutierrez (281) postulated that Bartter syndrome resembled one of inhibited function of the thick ascending limb, and the most recent genetic studies seem to confirm this proposal. Renal biopsy demonstrates hyperplasia and hypertrophy of the juxtaglomerular cells as well as of the medullary interstitial cells, the site of prostaglandin E_2 synthesis.

In a model of Bartter syndrome (long-term furosemide administration in rats), we demonstrated that the three renal ATPases were increased in cortical and medullary collecting tubules (289). The high aldosterone level stimulated the H-ATPase, and the hypokalemia stimulated the H,K-ATPase (Table 20-3). Na,K-ATPase was also increased in cortical and medullary collecting tubules by aldosterone and hypokalemia, respectively. With respect to the human syndrome, because we had postulated in 1975 that the primary defect was in the thick ascending limb (impaired sodium chloride transport) (281), we think the changes we observed in the cortical and medullary collecting tubule enzymes after furosemide are secondary, not primary. Aldosterone is not causative in kaliuresis because humans with Bartter syndrome are hypokalemic after adrenalectomy (290).

The differential diagnosis includes Fanconi syndrome, laxative and diuretic abuse, chronic vomiting, licorice ingestion, primary and secondary aldosteronism, and familial chloride diarrhea. Some patients with cystic fibrosis also present similarly. Treatment includes large quantities of potassium (150 to 300 mEq per day) with spironolactone, triamterene, or amiloride daily. Indomethacin (and aspirin) has also been used to alter renal prostaglandin E_2 levels, and although rarely correcting all the metabolic abnormalities, the nonsteroidal agents do promote sodium retention and thus increase the blood pressure. Theoretically, the selective cyclooxygenase inhibitors should be more effective, but no large studies have been performed. A preliminary report in one patient with type II Bartter syndrome treated with a cyclooxygenase-2 inhibitor showed remarkable results, including height and weight gain. Angiotensin-converting enzyme inhibitors have been used experimentally, but thus far they have not reversed the metabolic defects; drug-induced hypotension is a major problem with their use (291).

OTHER DISORDERS

Renal Tubular Potassium Secretion Defect (Spitzer-Weinstein Syndrome)

This disorder is characterized by metabolic acidosis and hyperkalemia associated with low aldosterone and plasma renin activity. The GFR is normal. The mode of inheritance is not clear. The biochemical basis of this disorder is unknown; a defect in collecting duct H,K-ATPase is a possible mechanism, although tubular K-APTase could also be abnormal. Treatment includes thiazide diuretic administration, sodium restriction, and occasionally alkali administration.

Renal Tubular Magnesium Wasting Defect

This autosomal-recessive disorder involves magnesium excretion three to five times greater than normal that is associated with potassium wasting and a mild metabolic alkalosis. It may also be seen after aminoglycoside and cisplatin administration, although both nephrotoxins usually result in acute tubular necrosis. The differential diagnosis includes hyperaldosteronism, hyperparathyroidism, magnesium malabsorption, Fanconi syndrome, Bartter syndrome, and RTA. If this is really Gitelman syndrome, where the defect is in the distal convoluted thiazide-sensitive NaCl cotransporter, it is not yet clear as to the mechanism by which magnesium wastage occurs. Several reviews have been written recently that discuss magnesium wasting (286–288,292). Patients with the disorder usually

respond well to oral magnesium supplementation, and the disease is thought to be of little clinical consequence.

SUMMARY

Although the isolated renal tubular disorders are complex, some of their features can be predicted from information known about the transport of carbohydrates, amino acids, and ions throughout the nephron. Doubtless, most of these disorders have a genetic basis, and as our technology improves, more new and exciting information about their underlying mechanisms will be revealed. Despite the marked increase in knowledge expected, it remains to be seen whether these insights will further aid our approach to therapy or in fact cure any of these abnormalities totally.

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SECTION IV: UROLOGICAL DISEASES OF THE GENITOURINARY TRACT

CHAPTER 21 ■ CONGENITAL UROLOGIC ANOMALIES

ANTHONY ATALA

The most common congenital anomalies involve the genitourinary system. The ability to visualize the genitourinary tract *in utero* with ultrasonography has led to the early diagnosis of many abnormalities, often before they are symptomatic. Congenital urologic anomalies that are of interest to the nephrologist are discussed in an anatomically ascending order: testis, urethra, bladder, ureter, and renal disorders. The topics of vesicoureteral reflux and urinary obstruction are covered in other chapters.

CRYPTORCHIDISM

The incidence of cryptorchidism is approximately 6% at birth and 1.6% at 3 months of age (1). An increased rate is reported in premature and lower–birth-weight neonates. The incidence of cryptorchidism also increases in neural tube defect disorders, myelomeningocele, omphalocele, anencephalia, abnormalities of testosterone biosynthetic defects, 5α-reductase deficiency, and peripheral androgen receptor abnormalities, and in disorders of gonadotropin deficiency such as Kallmann syndrome, Prader-Labhart-Willi syndrome, and Laurence-Moon–Biedl syndromes (2). There are several other dysmorphic syndromes that may also be associated with cryptorchidism. Genetic predisposition seems to play a role, which has been noted in 1% to 4% of siblings of cryptorchid children and 6% of fathers with cryptorchid children (3).

Descent of the testes takes place in two separate stages. In the first stage, called the *transabdominal descent,* the testes move from the urogenital ridge to the level of the internal inguinal ring. The testes remain at the internal ring until the 28th week of gestation, when they begin their descent into the scrotum. This second stage of descent is called the *inguinoscrotal stage* and is normally complete at 40 weeks' gestation. The second stage is believed to be secondary to gubernacular regression. Hormonal factors may also be involved. A low–molecular-weight fraction of fetal testicular extract called *descendin,* which is stimulatory to gubernacular cells, might be involved (4). There is also some indication that testosterone plays a role in the initiation of gubernacular regression (4). A hormonal mechanism for testicular descent has long been suspected. Many studies cite an abnormality in the pituitary–gonadal axis under both static and stimulated conditions (5). Other studies have suggested the role of the Müllerian inhibitor factor as a promoter of testicular descent, which initiates descent by stimulating growth and shortening of the gubernaculum (6). The genitofemoral nerve has also been implicated as having a role in testicular descent. Androgen receptors are located in the central nervous system, and the genitofemoral nerve may act as a mediator of testosterone on the gubernaculum, enabling testicular descent (6). Other proposed theories for testicular descent involve changes in intraabdominal pressure with development and the increased weight of the epididymis (7).

There is no standard classification system for categorizing the cryptorchid testis. Nonetheless, the testis may be defined as being cryptorchid, retractile, sliding, associated with a later ascent, or absent. The location of the testis on physical examination should be documented. The position of the testis should be described as either palpable or nonpalpable. If palpable, its location on examination should be described: internal inguinal ring, external inguinal ring, scrotal inlet, or ectopic. Ectopic locations are outside the normal course of descent. Ectopic positions may be the superficial pouch of Denis Browne or a perineal, femoral, or penile location. The child should be lying on an examination table in a warm room with the abdomen and genitalia exposed. Visual inspection of the abdomen and scrotum is performed first. The cryptorchid patient often has underdevelopment of the ipsilateral hemiscrotum. An abdominal examination is performed and gentle pressure is placed just medial to the iliac crest (level of the internal inguinal ring). The examining hand is gently advanced down the inguinal crease as the other hand palpates the ipsilateral hemiscrotum. The ipsilateral hemiscrotum is gently inverted to meet the advancing upper hand. The testis is often corralled in this fashion. An attempt is made to bring the testis down into the most dependent portion of the hemiscrotum. Care should be taken not to mistake a low cryptorchid testis for a fully descended one by bringing the scrotal tissue up around the testis. If the testis is not located, the child should be placed in the tailor's position (seated with legs crossed) and the scrotum reexamined. This maneuver usually abolishes the cremasteric reflex. A hot compress applied to the inguinal region may also relax the cremasteric muscle. The cremasteric reflex, which displaces the testis proximally, is not usually present until 3 months of age; therefore, the neonatal period is the ideal time to examine the genitalia. If this opportunity is not available, then records of the neonatal examination should be reviewed. There is a clinical entity called *testicular ascent;* however, controversy surrounds its etiology. This may represent ascent of a previously descended testicle, usually due to an associated hernia. Intervention is indicated. The patient may have a retractile testicle. A retractile testis often is visualized in the hemiscrotum when the patient is lying undisturbed. Upon manipulation, the testis may become "cryptorchid" due to a hyperactive cremasteric reflux. One can usually confirm the diagnosis of a retractile testis by manipulating the testis down into the scrotum, and noticing that it will stay down in the scrotum once released. These patients can be observed, as the retractile testis status usually normalizes once the cremasteric reflex dampens with age. The patient may have a sliding testis, in which case the testicle may be brought down into the scrotum, but once released, it immediately reverts to its cryptorchid position. These testicles are by definition, cryptorchid, and intervention is indicated. The testicle may be nonpalpable.

In patients with bilateral impalpable testes, a human chorionic gonadotropin (hCG) stimulation test should be performed to determine the presence of testicular tissue. If there is no corresponding rise of testosterone to hCG challenge, and the

basal level of follicle-stimulating hormone is high, testicular absence can usually be diagnosed and surgical exploration may not be necessary. An alternative strategy is to detect for the presence of Müllerian inhibiting factor and, if absent, testicular absence can be confirmed. However, only few labs perform this test routinely.

In patients with either unilateral or bilateral nonpalpable testes, ultrasound and magnetic resonance imaging (MRI) may demonstrate the position of the testis (intraabdominal, ectopic, etc.); however, nonvisualization by these techniques does not exclude the presence of testicular tissue, and these tests are not usually recommended.

Surgical treatment should be performed after the age of 6 months, but before 18 months. Surgical therapy is successful in 90% of patients with an impalpable testis, and in over 95% in patients with a palpable testis (8). Surgery affords the best chance for preservation of germinal epithelium. If the preoperative examination demonstrates a unilateral impalpable testis, then the possibility of gonadal absence should be discussed with the parents. The use of a testicular prosthesis should also be discussed. Laparoscopy continues to play a greater role in the diagnosis and treatment of impalpable testes. In the case of a unilateral impalpable testis, laparoscopy demonstrates its position and may help in planning therapy. If the testis is located at the level of, or just proximal, to the internal inguinal ring, a standard orchidopexy may be performed. If the testicle is located higher in the abdomen, a two-stage orchidopexy or microvascular reanastomosis may be planned. Low intraabdominal testes can be brought down into the scrotum in one stage by dissecting the vas deferens and vessels retroperitoneally. This can be performed either with standard open surgery techniques or laparoscopically. An atrophied intraabdominal testis may be removed at the time of initial laparoscopy.

Hormonal treatment in cryptorchid children should be limited to older children (>7 years) with retractile testes (9). Several double-blinded hormonal treatment studies show little or no benefit to the treatment of the truly cryptorchid testis. Hormonal treatment is primarily performed with hCG. Dosage schedules of hCG injections vary, and there is no consensus. Position of the testicle at the time of treatment appears to be important: the lower the testicle, the higher the reported success rate. Perhaps the main role of hormonal treatment is to distinguish the retractile testis from a truly cryptorchid testis (2).

The naturally acquired complications of cryptorchidism are infertility and testicular cancer. Infertility has a more pronounced effect because it is so much more prevalent among cryptorchid patients compared with testicular cancer. Some evidence is beginning to accumulate that early orchidopexy can preserve fertility (2). Of all patients diagnosed with testicular cancer, 7.3% have a history of cryptorchidism. In addition, a man with cryptorchidism has a 9.7 times greater chance of dying from testicular cancer. In unilateral cryptorchidism, the testicular cancer arises in the contralateral, normally descended, testis in 20% of cases (2). Orchidopexy does not appear to affect the incidence of subsequent testicular cancer. The risk of cancer correlates with the degree of cryptorchidism; therefore, patients with intraabdominal testes carry the highest risk for development of testicular malignancy. Testicular self-examinations should be performed routinely by patients with a history of cryptorchidism.

DISORDERS OF THE URETHRA

Meatal Stenosis

Meatal stenosis in boys may occasionally be associated with another urologic anomaly. However, it is most often an acquired lesion after circumcision, usually secondary to meatitis or trauma. Although many theories exist regarding the etiology of meatitis, it is widely believed that it is due to a continued inflammatory response of the meatus to ammonia retained in diapers. The meatitis usually starts to develop in the first months of life and may be prevented by frequent diaper changes. A diagnosis can be established only after a careful physical examination. This may include the observation of voiding. If the meatus has a pinpoint opening, with a narrow urine stream, and deflection of the urinary stream is evident, then a diagnosis of meatal stenosis can be ascertained. The child may also strain to empty his bladder. A meatotomy, which involves a ventral incision in the thin fused area of the glans alleviates the stenosis. This can be performed in the outpatient setting with either local or mask anesthesia. Alternatively, if mild, steroid creams may be applied daily, however, the success rates are low (10).

Hypospadias

Hypospadias is a congenital defect of the penis in which there is an incomplete development of the anterior urethra. The urethral opening is proximal to its usual location at the center of the glans. The abnormal urethral opening may be anywhere along the ventral aspect of the penis, the scrotum, or perineum. Approximately 60% of hypospadias are distal, involving either the proximal glanular, coronal, or distal penile shaft regions. Approximately 20% of hypospadias occur in the mid-penile region along the midshaft. Another 20% occur proximally, either in the penile, penoscrotal, scrotal, or perineal region (11).

The incidence of hypospadias has been reported to be increasing, which is believed to be because of the more common use of fertility drugs during pregnancy. The incidence of hypospadias is currently 1 of every 150 male births (11). The diagnosis is made during physical examination. The physical examination determines the severity of the hypospadias and is also aimed at identifying any associated anomalies. These include meatal stenosis, penile torsion, urethrocutaneous fistula, chordee (a ventral curvature of the penis), and penoscrotal transposition, wherein the superior portion of the scrotum is located above the base of the penis. A neonatal circumcision should not be performed in a patient with hypospadias. The prepuce is routinely used during the repair, and the cosmetic results may be compromised if there is a shortage of preputial tissue. In the rare event that the hypospadias is noted only after the initial preputial cut is made during the circumcision, the surgery should be stopped, and a compressive dressing should be placed around the head of the penis and the cut skin surface, leaving the skin intact.

Approximately one-fourth of patients with hypospadias and undescended testes (unilateral or bilateral) have an intersex anomaly. These patients should be evaluated for intersex with a workup that includes a determination of karyotype, serum analysis for follicle-stimulating hormone, luteinizing hormone, testosterone, and urinary 17-ketosteroids and 17- hydroxycorticosteroids. If a phallus is small, its size may be enlarged before surgery by the use of topical testosterone or dihydrotestosterone cream or parenteral testosterone.

Early referral for surgery is preferable. Surgical treatment is performed preferably at 4 to 8 months of age. The earlier age for surgery is recommended so the repair can be completed before the patient starts crawling or is ambulatory. This is particularly important for the more complex defects that may require multiple procedures. Although most hypospadias are amenable to a single-stage surgical repair (12), a few more complex defects require two or even three separate surgical procedures, each separated by about 6 months in order to maximize vascular recovery to the surgical region (13). Regardless of the

severity of hypospadias, the goals of surgery are the same. These include creating a straight penis, positioning the meatus on the penile tip, normalizing the direction of voiding, creating a urethra of adequate and uniform caliber, and achieving symmetry in the appearance of the glans and shaft. The glanular hypospadias can be repaired with a meatal advancement procedure. The more proximal subglanular and penile shaft defect usually can be corrected by advancing the urethra (14). Grafts from other donor sites, such as nonpenile skin, bladder, or buccal mucosa, can also be used for the more complex repairs.

Even in the hands of the most experienced surgeons, hypospadias repair may be associated with a complication rate as high as 30% for the more complex repairs. Complications include urethrocutaneous fistula, stricture, diverticula, and persistent chordee (15). A careful preoperative evaluation, precise surgical technique, and appropriate postoperative care are required to minimize the complication rate and achieve the desired objectives of hypospadias surgery.

Posterior Urethral Valves

Posterior urethral valves represent the most common cause of bladder outlet obstruction in the male newborn. Although initially described as three types of valves in 1919 (16), with a fourth type described in 1983 (17), only type 1 and type 3 valves are clinically significant. Type 1 valves arise from either side of the verumontanum as leaflets that extend distally to the anterior urethral wall at the level of the urogenital diaphragm. Type 2 valves represent membranous valves that run outward from the verumontanum to the bladder neck. Type 3 valves consist of a diaphragm usually located at the verumontanum with a small central perforation. Type 4 valves are seen in patients with prune-belly syndrome, when the prostate folds on itself and causes a partial obstruction. Type 1 makes up more than 95% of the valves seen; type 3 makes up over 4%. Even though different embryologic defects are present, there is no difference in the clinical presentation, pathophysiologic process, or management of children with either type 1 or type 3 valves.

A large number of infants with posterior urethral valves are now being diagnosed prenatally with ultrasonography. The prenatal ultrasound may show a large, thickened bladder with a dilated prostatic urethra and varying degrees of unilateral or bilateral hydroureteronephrosis. Valves cause varying degrees of urinary tract obstructive changes, ranging from severe hydronephrosis with renal dysplasia to a relatively normal upper urinary tract. Severe obstructive uropathy owing to posterior urethral valves may lead to the development of oligohydramnios with subsequent pulmonary hypoplasia. Survival in these instances is markedly decreased. Prenatal intervention for the severe forms of this condition was performed in the early 1980s. The frequency of this type of intervention, however, has slowed considerably because of poor results in terms of survival and associated infant morbidity. Although prenatal intervention is performed at several centers, it is still considered experimental.

The prenatal finding of posterior urethral valves should be confirmed at birth with a voiding cystourethrogram. The classic appearance is that of a dilated and elongated posterior urethra, a cut-off of the urethral caliber just proximal to the membranous urethra, bladder neck hypertrophy, and occasional filling defects caused by the valve leaflets. A baseline renal and bladder ultrasound should be obtained (18).

Newborns with posterior urethral valves, who are not diagnosed prenatally, usually present with an abdominal mass, failure to thrive, urinary ascites, azotemia, or urosepsis. When the newborn or infant is acutely ill, prompt management of acidosis, sepsis, and fluid and electrolyte abnormalities is essential. The serum creatinine is monitored, bearing in mind that it reflects the mother's levels for the first week of life. An increase in the serum creatinine levels with serial measurements during the first week in life, however, is indicative of worsening renal function in the neonate.

Once the diagnosis of posterior urethral valves is made, the bladder is drained transurethrally with a catheter or small-caliber feeding tube. A Foley balloon catheter is not recommended, because of its tendency to occlude the ureteral orifices. Broad-spectrum antibiotics are administered. When the patient is stable, valve ablation is performed cystoscopically. Valve ablation by either a perineal urethrostomy or transvesically may be necessary in the small infant whose penile urethra cannot accommodate the cystoscope. In rare situations, a vesicostomy may be performed. If the valves are ablated and there is continued obstruction or an increase in serum creatinine, a higher diversion may be indicated, using either a cutaneous pyelostomy or loop ureterostomy. Even if valve ablation results in adequate urinary emptying, close observation is still imperative.

Although the radiographic appearance of the urethra after valve ablation returns to normal almost immediately, the trabeculation and hypertrophy of the bladder take much longer to resolve. Vesicoureteral reflux, if present, may improve or cease entirely. In a subset of patients there is continued impairment in bladder compliance, which may be associated with a decreased bladder capacity or high voiding pressures. These patients may require periodic urodynamic assessment and long-term management with intermittent catheterization alone or in conjunction with anticholinergic or α-sympathomimetic agents. Failure of these methods of treatment may lead to bladder augmentation with urinary reconstruction.

Older boys with valves may present at any age, depending, in part, on the severity of obstruction. Voiding dysfunction, hematuria, and urinary tract infections are frequent reasons for older boys with posterior urethral valves to seek medical attention. A full urodynamic and radiographic evaluation is necessary before proceeding with valve ablation. Careful follow-up is essential, because of the common sequela of impaired bladder function.

Anterior Urethral Valves/Diverticula

An anterior urethral diverticulum may arise because of a segmental defect of the corpus spongiosum or as a result of a ruptured Cowper's duct cyst (19). The diverticulum may enlarge, extending beyond the anterior margin, producing an obstructing valvular mechanism. Children usually present with some symptoms of urinary obstruction or infection. On physical examination, there may be a swelling present just proximal to the penoscrotal junction that produces urinary drainage, if pressure is applied. A voiding cystourethrogram is diagnostic. Small diverticula may be managed with endoscopic resection, whereas the larger ones may require open excision with urethroplasty.

Urethral Polyps

Urethral polyps are congenital lesions that are always benign (20). They arise in the area of the verumontanum and usually have a long stalk. Children present with urinary infection, hematuria, or intermittent obstruction. A voiding cystourethrogram usually shows a filling defect that may vary in location. Although less reliable, an ultrasound may also show a polyp. Removal of the polyp usually can be performed cystoscopically. Incomplete removal may lead to recurrence.

Urethral Prolapse

Urethral prolapse occurs most commonly in young black girls. The presenting symptoms are bloody spotting and dysuria. The diagnosis is made on physical examination when an inflamed, everted mucosa is seen surrounding the urethral meatus. The condition usually can be managed with estrogen cream applications two to three times daily for 2 weeks. If a prolapsed urethra persists for several months, formal excision may be necessary (21).

Urethral Duplication

Duplication of the urethra is a rare anomaly. Four major varieties may occur (22). In type 1, there is incomplete duplication of the urethra. In type 2, there is a bifid phallic urethra, which may or may not have two external openings. Type 3 indicates that the ventral meatus is in a perineal location. In type 4, urethral duplication is a component of partial or complete caudal duplication. In all of these categories, the ventral urethra is the most normal. Presenting symptoms include voiding with two urinary streams, infection, or purulent discharge. A voiding cystourethrogram is helpful in delineating the anatomy and confirming the diagnosis. Treatment depends on the anatomy of the duplication and its clinical manifestations. Surgery should be considered for disturbing symptoms, such as a divergent double stream, incontinence, or a cosmetic deformity, such as an epispadiac meatus. Surgical treatment may involve complete excision of the accessory urethra, transurethral excision of the intraurethral system, or formal urethroplasty.

Megalourethra

Megalourethra is a rare entity that may be associated with developmental abnormalities of two types: the fusiform (complete type) and scaphoid (incomplete type). A fusiform megalourethra has a deficiency of both the corpora cavernosa and the corpus spongiosum. The penis is flaccid and markedly distended. This type is usually associated with severe forms of the prune-belly syndrome (23). If the patients survive, treatment of this defect is usually unsatisfactory and sex conversion may be necessary. The scaphoid variety has an abnormal corpus spongiosum. Treatment usually involves trimming of the urethra.

Urethral Gland Abnormalities

There are two groups of periurethral glands: the diaphragmatic glands and the bulbar glands. These glands empty their secretions through their appropriate ducts into the urethra. Any obstruction in the ducts may cause retention cysts, abscess formation, or a dilated duct. In addition, urine may reflux into a dilated duct. Any of these conditions may lead to symptoms of obstruction or infection. If symptomatic, transurethral fulguration of the retention cysts may be performed. Most children are asymptomatic and do not require any treatment (24).

CONGENITAL BLADDER ANOMALIES

Bladder Exstrophy

Bladder exstrophy is one of the most severe abnormalities in urologic practice. It occurs once in every 30,000 births (25). Embryologically, there is a failure of mesodermal ingrowth that extends from the umbilicus to the urogenital area. The embryologic defect leads to the absence of the anterior portion of the urinary bladder and the lower abdominal wall. Boys with exstrophy may have an associated epispadiac penis. The testes usually are descended, but may be retracted to the pubic or inguinal areas. Bilateral inguinal hernias are common with this condition. In girls, the clitoris is bifid and separated by an open urethral groove. The vagina may be stenotic and anteriorly placed.

It was believed until the 1920s that simply closing the bladder was sufficient treatment. Unfortunately, hydronephrosis and eventual kidney failure developed in most patients. Most were also incontinent. Later, diversion of the ureters to the sigmoid became the standard surgical approach. However, ureterosigmoidostomy failed to prevent reflux in many patients and infection, pyelonephritis, and calculi ensued. Newer techniques to prevent reflux were introduced in the 1950s that produced more-satisfactory results. However, it was later recognized that these patients were at a high risk for development of adenocarcinoma of the sigmoid colon. It is estimated that these patients have at least a 400 times greater than expected risk for development of sigmoid cancer (26). Many adults who had ureterosigmoidostomies in the past have been converted to other forms of diversion, not only because of the high risk of malignancy, but because of the development of renal obstruction in some. Ureterosigmoidostomies no longer play a major role in the management of bladder exstrophy.

Even when bladder closure was attempted, the results were poor. A literature review of 329 cases managed between 1906 and 1966 showed that only 5% achieved continence with preservation of renal function (27). Considerable advances have been made over the last three decades in the management of bladder exstrophy that have substantially improved the results. Two approaches are widely used for the surgical management of bladder exstrophy. One approach involves the staging of the reconstructive procedure. This includes three separate procedures: bladder closure, bladder neck reconstruction with an antireflux procedure, and epispadias repair. The other approach involves a single-stage primary closure, wherein the bladder closure and the epispadias repair are performed initially (28).

With the current approaches to the treatment of bladder exstrophy, it is possible to achieve five objectives: an adequate abdominal wall closure, urinary continence, preservation of renal function, sexual functionality, and an acceptable physical appearance. The bladder closure should be done as early as possible and, preferably, within the first 72 hours. The diastasis of the symphysis pubis does not incur any orthopedic disability; however, its correction is necessary for facilitation of the abdominal wall closure, decreasing the dehiscence rate, and improving eventual continence (29,30). The symphysis pubis may be approximated without iliac osteotomies in neonates younger than 72 hours of age, because of the pliability of the pelvis (31). Neonates who present after 72 hours of age require bilateral osteotomies before or, preferably, at the time of bladder closure. The most common procedure used is posterior iliac osteotomies, as originally described in 1958 (32). Attempts should be made to achieve symphyseal approximation of 2 cm or less to improve the chances of long-term urinary continence (33). Anterior bilateral osteotomies have also been advocated as an alternative to the standard posterior approach. This involves a bilateral transverse incision of the innominate bones anteriorly (34). Immobilization of the patient is essential to prevent separation of the pelvis after surgery.

If a staged reconstructive approach is followed, the primary bladder closure converts the patient into one with complete epispadias and incontinence. Growth of the bladder occurs during the incontinent interval. By 3 to 5 years of age, the capacity

often reaches 50 to 60 mL, and bladder neck reconstruction is then possible. The continence procedure may also be delayed and epispadias repair performed first in anticipation of further bladder growth. Gearhart and Jeffs (35) have advocated doing the epispadias repair before the bladder neck reconstruction. In their experience of 28 patients who were believed to have a bladder capacity inadequate for bladder neck reconstruction (<60 mL), epispadias repair was performed. After a median interval of 22 months, bladder capacity increased by a median of 55 mL. Twenty-five of 28 patients had bladder neck reconstruction, and 88% of these were continent with a median follow-up of 4.5 years (35).

If a single-stage primary closure is performed, both the epispadiac defect and the bladder exstrophy are initially repaired. Bladder neck repair and ureteral reimplantation are not initially performed (36). Overall, the rate of urinary continence in this group approaches 80% after toilet training.

Patients who remain incontinent need further evaluation with urodynamic and urethral pressure profile studies. The direction of treatment depends on the underlying pathologic process. If the urethral pressure is low, a repeat (staged approach) or first (single-stage primary closure) bladder neck reconstruction may be necessary. If the vesical pressure is high and the bladder capacity is limited, augmentation cystoplasty should be considered. If the bladder is small and additional urethral length is needed, tubularization of the bladder into a neourethra and formation of a neobladder with bowel may be considered (37). The use of the artificial urinary sphincter for continence, either alone or in combination with augmentation cystoplasty, may be beneficial in some children. Patients who have had multiple failed procedures and have a small, fibrotic bladder with multiple scarring may benefit from construction of a continent urinary reservoir. However, the clinician must be aware of the associated morbidity of each type of continent reservoir and choose accordingly (38).

Despite the numerous advances in the management of the patient with bladder exstrophy, there is still a percentage of patients who do not achieve continence. This is true in some patients even after repeated surgical procedures, including repeat bladder neck reconstruction, suspension, and bladder augmentation. Medical management with α-adrenergic agonists and anticholinergics is of little help in these patients. Several centers inject collagen in the periurethral region to increase urethral resistance and treat incontinence in these patients. The injected collagen is mostly reabsorbed within 1 year and, therefore, the treatment is only temporary. However, other injectable materials are being used that may be permanent, such as the endoscopic placement of a detachable balloon system (39) or the injection of autologous substances, such as chondrocytes (40–42).

Cloacal Exstrophy

Cloacal exstrophy is the most severe urologic congenital anomaly. It occurs in approximately 1 in 200,000 to 400,000 live births. Early during development, before 6 weeks of age, the genital, urinary, and gastrointestinal tracts empty into a single opening called the *cloaca*. During normal development, the urorectal septum divides the cloaca into an anterior urogenital sinus and a posterior anorectal canal. During this process, primitive mesoderm migrates distally, forming the infraumbilical abdominal wall. The abnormal migration of the cloacal membrane may create a mechanical barrier to mesodermal migration (43). Clinically, the infant presents with an absent lower abdominal wall, two hemibladders divided medially by a foreshortened cecum, the surface of the inverted cecum representing a prolapsed terminal ileum, an inferior orifice representing a blind-ending tailgut, and a laterally single or paired appendix. There is a wide separation of the pubic symphysis, and the genitalia are separated into halves bilaterally with adjacent scrotum or labium.

Associated anomalies can occur in up to 85% of patients (43). An omphalocele is present superiorly. Surgical management after birth depends on the overall condition of the child. Sexual assignment is usually female because of the severe phallic deformity, although this is being reconsidered from a psychological endpoint (44). Gonadectomy is performed if it is decided to raise a male infant as female. Surgical reconstruction entails omphalocele repair and either a terminal colostomy or ileostomy at birth. The bladder is closed and the bifid phallus is reapproximated. Staged reconstruction is then indicated to achieve an adequate bladder. Associated renal anomalies include pelvic kidneys, unilateral renal agenesis, and multicystic kidneys.

Urachal Anomalies

The allantois appears around day 16 of gestation as an outpouching from the caudal wall of the yolk sac. As the bladder enlarges, the allantois involutes to form a thick tube called the *urachus* (45). The urachus allows free communication between the urinary bladder and the umbilicus in the abdominal wall. After birth, the urachus becomes a fibrous cord called the *median umbilical ligament*. The lumen in the inferior portion of the urachus remains patent approximately one-third of the time. The abnormalities associated with the umbilicus are usually because of embryologic abnormalities of the urachus. Urachal anomalies include a patent urachus and a urachal cyst or sinus (46).

Failure of the urachus to regress completely results in a patent urachus, a communication between the bladder and umbilicus. The usual presenting sign is a discharge of fluid from the umbilicus that increases with voiding. Urethral obstruction may be present. Umbilical swelling because of an associated umbilical hernia or urachal vascular engorgement and skin discoloration may be present.

A bladder ultrasound or voiding cystourethrogram may show the patent urachus and may also rule out the presence of a lower urinary tract obstruction. Fluid analysis for blood urea nitrogen and creatinine may be helpful. Injection of methylene blue transurethrally may confirm the communication. Treatment consists of identification and extraperitoneal surgical excision of the urachal tract with a bladder cuff.

Persistence of the distal urachus with an opening at the umbilicus results in an external urachal sinus. This may become symptomatic at any age, but usually occurs during childhood. Drainage, fever, paraumbilical tenderness, and inflammation may be present. A voiding cystourethrogram should be done to rule out a patent urachus and the presence of lower urinary tract obstruction. Sonography or sinography may aid in the diagnosis. Treatment consists of excision of the sinus. Concomitant removal of any attached intraperitoneal structures is necessary if omphalomesenteric duct remnants are present.

A urachal cyst occurs when both ends of the urachus are closed and only the central portion of the canal remains patent. The central lumen may enlarge from epithelial desquamation and degeneration. These cysts are usually asymptomatic until they attain a considerable size or become infected. Lower abdominal pain is the most frequent presenting complaint and may be associated with fever, voiding symptoms, gastrointestinal upset, and a palpable mass. Peritoneal irritation may give rise to symptoms that mimic an acute abdomen. Rupture of the cyst into the bladder, umbilicus, or peritoneal cavity may occur. Diagnostic studies may include a voiding cystourethrogram in the lateral position to demonstrate the compressive effect on the bladder and an ultrasound. Treatment consists

of removal of the cyst, if it is uninfected, and drainage and marsupialization if an abscess is present. Another alternative is to first drain the cyst percutaneously with ultrasound guidance and then perform a surgical excision at a later date.

Bladder Diverticula

Bladder diverticula are herniations of bladder mucosa between the detrusor muscle. Most diverticula are congenital, although a few are acquired because of outlet obstruction or infection. The most common location is lateral and cephalad to the ureteral orifice (47). Congenital bladder diverticula may cause vesicoureteral reflux, because of the paucity of smooth muscle in the periureteral region. Bladder diverticula can also cause obstruction either at the bladder neck or urethral region. Usually, however, they are entirely asymptomatic and are found incidentally. Bladder diverticula are best demonstrated by voiding cystourethrography when the postvoid film is obtained. Surgical excision is indicated if the diverticulum is obstructive, is a locus for infection, or is associated with high degrees of vesicoureteral reflux.

CONGENITAL URETERAL ANOMALIES

Embryologically, the ureteral bud develops from the mesonephric duct. The segment of mesonephric duct between the ureteral bud and future urogenital sinus is termed the *common excretory duct*. This region is absorbed progressively into the developing sinus and contributes mesenchyme to the trigone and underlying detrusor. The ureteral bud and mesonephric duct acquire separate openings, with the ureteral bud initially located medial and distal to the mesonephric duct. There is migration of the two orifices in opposing directions, rotating as they do so. The mesonephric duct crosses over the ureter as it moves medially and caudally, and the ureteral orifice migrates cephalad and laterally with the developing vesicourethral canal. Variations in the site of origin of the ureteral bud on the mesonephric duct and supernumerary budding explain a variety of ureteral and renal anomalies.

Ureteral Duplication

Ureteral duplication is the most common ureteral anomaly. In most situations, it is an incidental finding. However, there is a higher incidence of duplication in cases of urinary infection, and it may be associated with upper urinary tract stasis, obstruction, or reflux. A duplicated ureter usually drains a duplex kidney or, rarely, a normal supernumerary kidney. Ureteral duplications may be either partial or complete. In complete ureteral duplication, a second orifice enters the bladder, the urethra, or other structure. The Weigert–Meyer law (48,49) defines the position of the orifices in double ureters. The two orifices are characteristically inverted in relation to the collecting system they drain. The orifice to the lower pole ureter occupies the more cranial and lateral position than the upper pole ureter, which has a caudal and more medial position. Only rare exceptions to this configuration have been observed.

Rarely, a ureteral duplication does not drain a renal segment and is, therefore, blind ending. Embryologically, the affected ureteral bud is abortive and fails to make contact with the metanephros. Most of the blind segments are asymptomatic, but some may present with vague abdominal pain, because of either infection or calculi. Retrograde pyelography may be required for diagnosis, because a blind segment may not fill on excretory urography.

Rarely, ureteral triplication occurs, which is due to the presence of three buds from the mesonephric duct. Triplication can be asymptomatic or may present with signs of reflux, obstruction, ureterocele, or ectopia.

Ectopic Ureters or Ureteroceles

The proper location of the ureteral orifice is the trigone of the bladder. Any other location is ectopic, which occurs more commonly in female patients and is usually associated with an upper pole duplication (50).

Embryologically, if the ureteral bud arises proximal to its normal position on the mesonephric duct, the ureteral orifice accompanies the mesonephric duct to a final location caudal to the trigone. The mesonephric duct proximal to the ureteric bud in the male embryo becomes a wolffian duct structure, which includes the seminal vesicles, vas deferens, and epididymis. In the female, this part of the mesonephric duct becomes the epoophoron, oophoron, and Gartner's duct (51). The ectopic ureter can drain into any of the mesonephric structures.

In boys, ectopic ureters are more commonly associated with a single unit, and they usually drain into the prostatic urethra or bladder neck. Urinary tract infections, urgency, and frequency may be the presenting symptoms. If the ectopic ureter ends in a wolffian duct remnant, epididymitis may develop. In boys, the ectopic ureter always drains proximal to the external sphincter, and, therefore, urinary incontinence does not occur.

An ectopic ureter in girls is associated with incontinence because of its terminal location below the external sphincter. The classic history of a girl with an ectopic ureter is a normal voiding pattern associated with continuous wetting (52). A history of continuous dribbling or never having a dry diaper should arouse strong suspicion of this diagnosis. Vaginal discharge may be common when the ureter inserts into the fallopian tube, uterus, upper vagina, urethra, or vestibule.

The ectopic ureter is almost always obstructed, to some extent, and it may contribute to renal dysplasia or atrophy. As a rule, the more ectopic the orifice, the more dysplastic is the renal moiety that it drains. Sonography is usually the first radiographic study obtained. A duplex system with upper pole hydroureteronephrosis usually can be fairly easily demonstrated. The upper pole cortex may be thinned to varying degrees. The dilated ureter may be seen posterior to the bladder. There may be enough dysplasia or atrophy of the upper renal pole that it may be difficult to visualize it on sonography. Excretory urography usually demonstrates absent or delayed function of the upper pole. However, associated signs point to the diagnosis. These include a lower kidney pole that is pushed inferiorly by the upper pole (drooping lilly sign), a laterally displaced ureter due to the mass effect of the hydroureteronephrosis, and an increased distance between the superior calix and the upper limits of the renal contour, suggesting the presence of an upper pole segment (53).

Reflux can be demonstrated into the lower pole moiety by voiding cystourethrography in one-half of the cases. Occasionally, the ectopic ureter also refluxes. If reflux is present before voiding, the orifice may be at the bladder neck; if it refluxes during voiding exclusively, a urethral orifice may be suspected. Methylene blue can be instilled intravesically while careful cystoscopic and vaginoscopic examinations are performed to determine the exact location of the ectopic orifice.

Treatment for ectopic ureters associated with a duplex system depends on the overall function of the upper pole renal moiety. If the renal segment has sufficient function to be worth salvaging, many surgical options are available. These include ureteropyelostomy, ureteroureterostomy, or common sheath

reimplantation. In some cases, the ectopic ureter drains an upper pole kidney that has minimal function, and an upper pole nephrectomy is recommended (54,55). If the ectopic ureter does not reflux, it can be resected as distally as possible through a flank incision and left open. If there is concomitant reflux, a second incision is necessary to resect the ureter in its entirety. When the ectopic ureter drains a single system, the ureter can be reimplanted if function has been preserved. Otherwise, a nephrectomy is performed.

With a unilateral single-system ectopic ureter, the ipsilateral hemitrigone fails to develop, and one side of the bladder neck is incompletely developed. Nevertheless, the hemitrigone and bladder neck usually are adequate for urinary control, provided that, in the girl, the ectopic ureter is above the external urethral sphincter. With bilateral single ectopic ureters into the urethra, the bladder fails to develop properly. The bladder usually is small, and the trigone is absent. Continuous incontinence is usual. Treatment consists of reimplanting the ectopic ureters and performing a bladder neck reconstruction. If a gradual increase in bladder capacity does not occur, augmentation cystoplasty may be necessary.

Ureteroceles

A ureterocele is a cystic dilatation of the intravesical submucosal ureter. The appearance is that of a thin-walled, often translucent, mass that varies in size from a small bulge of 1 cm to a lesion that almost fills the entire bladder. A ureterocele may involve either a single collecting system or the ureter to the upper pole of a duplicated system. The opening may be either intravesical or ectopic. Ureteroceles are divided into either simple (intravesical) or ectopic. An intravesical ureterocele is entirely contained within the urinary bladder. Ectopic ureteroceles extend into the bladder neck or urethra. Other terms may also be used in describing ureteroceles. A stenotic ureterocele has a narrowed orifice located on the dome of a rounded intravesical mass and is usually located in a normal or near-normal intravesical location. In a sphincteric ureterocele, part of the orifice is contained in the internal sphincter, either in the bladder neck or proximal urethra. The orifice is obstructed by the sphincter-like action of the vesical outlet and empties only during voiding, when the bladder neck is open. Reflux may also be present during voiding. A sphincterostenotic ureterocele is located within the internal sphincter and has a narrowed orifice. This type of ureterocele often achieves a large size and may extend into the bladder outlet, obstructing it to a varying degree. Prolapse of the ureterocele through the urethra on to the perineum may occur occasionally. A cecoureterocele has an intravesical orifice and submucosal projection of the ureterocele into the urethra.

Embryologically, the ureterocele occurs because of a delayed rupture of Chwalla's membrane (56), which is a two-layered epithelial structure that is normally present in the embryo and is located between the developing ureter and the urogenital sinus. Delayed opening of the membrane results in dilatation of the terminal ureteral segment and proximal stenosis of the orifice.

Of all ureteroceles, 80% arise from the upper poles of duplicated systems. Single-system ureteroceles (simple ureteroceles) are usually found in adults. Simple ureteroceles are more prone to severe obstruction and dysplasia than those associated with duplicated systems (54).

In the past, the most common presentation for a ureterocele was that of an infant with a urinary tract infection (55). The diagnosis now is made more frequently with prenatal sonography. Ureteroceles are predisposed to urinary stasis and subsequent infection. Therefore, children should be placed on prophylactic antibiotics. If a ureterocele is ectopic, it may prolapse out of the urethra and present as a vaginal mass. This mass can be distinguished from other interlabial masses, such as rhabdomyosarcoma, urethral prolapse, periurethral cyst, and hydrometrocolpos, by its smooth, round wall and location (21). A prolapsed ureterocele is located anterior to the vagina, and the urethra can usually be demonstrated to be anterior. The prolapsed ureterocele may cause vesicular obstruction and urinary retention, requiring decompression with a small-gauge needle until more definitive treatment can be instituted.

An ultrasound should be the first study obtained for evaluation of the ureterocele. Usually, the ureterocele is associated with a duplicated collecting system, specifically draining the upper pole. Sonographically, two separate renal pelves surrounded by their echogenic hila can be seen. A dilated upper pole ureter is usually visualized. If the lower pole ureter refluxes urine, hydronephrosis may appear in the lower pole as well. Similarly, the ureterocele may impinge on the contralateral ureteral orifice or obstruct the bladder neck and cause hydronephrosis in the opposite kidney. The bladder frequently displays a thin-walled cyst, which is the ureterocele.

Sonographic imaging, however, may not show the ureterocele because it may be effaced by bladder overdistention, or it may be difficult to visualize if the bladder is empty, and there is little distinction between the bladder wall and the wall of the ureterocele. The diagnosis of ureterocele may be overlooked if the upper pole parenchyma is atrophied and is not well visualized. This entity has been termed *nonobstructive ureterocele* (57) as well as *ureterocele disproportion* (58).

Excretory urographic findings in a patient with a ureterocele include a poorly functioning upper pole, which may be laterally deviated from the spine because of hydronephrosis. The upper pole hydronephrosis pushes the lower pole laterally and inferiorly. The presence of the upper pole ureter may be inferred from its effect on the lower pole ureter. The lower pole ureter may be laterally deviated and notched. A voiding cystourethrogram demonstrates the presence or absence of vesicoureteral reflux, which may occur 50% of the time in the ipsilateral lower pole (59). The cystogram also demonstrates the size and location of the ureterocele, as well as the presence or absence of contralateral reflux. Nuclear scans with agents, such as dimercaptosuccinic acid and MAG-3, can provide valuable estimates of the upper pole contribution to overall renal function, as well as degrees of obstruction. This information is often helpful in determining whether the upper pole moiety is worth saving.

Treatment depends on the degree of renal function present in the upper pole moiety, as well as on the presence or absence of reflux in the lower pole. If there is adequate renal function in the upper pole moiety, a ureteral pyelostomy, ureteroureterostomy, ureterocele excision, and common sheath reimplantation, or transurethral incision may be performed. If the upper pole renal function is minimal, an upper pole nephrectomy and partial ureterectomy is performed. Alternatively, transurethral incision of the ureterocele can be performed, leaving the poorly functioning upper pole intact.

Transurethral incision of the ureterocele is the most common initial treatment for all patients. A follow-up cystogram and intravenous pyelogram are performed 2 to 6 months after surgery. If reflux is present, it can be managed either with further observation or reimplantation, depending on the degree.

Megaureters

The term *megaureter* simply means "large ureter." Gradually, the term has acquired such wide usage that it now includes a variety of secondary, as well as primary, lesions. A useful

classification for megaureters is one that uses three major categories: refluxing, obstructed, and nonrefluxing, nonobstructed megaureter (59). A wide ureter, due to reflux, is termed a *primary refluxing megaureter.* If the reflux is because of urethral obstruction, neuropathic bladder, or other etiologies, it is termed a *secondary refluxing megaureter.* Obstructed megaureters are primary if the obstruction is intrinsic, such as that owning to stenosis or an adynamic segment. If the obstruction is extrinsic, such as that owing to a retroperitoneal tumor, or is secondary to other etiologies outside the ureter, like a neuropathic bladder or urethral obstruction, a secondary obstructed megaureter is recognized. A nonrefluxing, nonobstructed megaureter is primary if it is identified as idiopathic. The secondary etiology indicates whether it is because of other conditions, such as polyuria, infection, or a ureter that remains wide after relief of distal obstruction.

Treatment, if any is required, depends on the particular etiology. Refluxing megaureters are reimplanted and tapered if the degree of reflux justifies its correction. Obstructed megaureters, most commonly because of a distal adynamic segment, are assessed radiographically to determine both the degree of obstruction and the overall renal function. Nuclear scans, such as MAG-3 with furosemide, are used to assess the overall degree of obstruction. If the degree of obstruction is mild, serial radiographic follow-up is maintained. If the degree of obstruction is severe, corrective surgery often achieves favorable results. Nonrefluxing, nonobstructed megaureters require serial radiographic follow-up, often with ultrasound. Surgical treatment usually is not necessary.

Ureteral Diverticula

Diverticula of the ureter can be due to abortive ureteral duplications (blind-ending bifid ureters), true congenital diverticula containing all tissue layers of the normal ureter, or acquired diverticula representing mucosal herniations. Congenital diverticula are very uncommon and have been reported to arise from the distal ureter above the ureterovesical junction, mid-ureter, and ureteropelvic junction (60). These can become very large, and secondary hydronephrosis can ensue. A patient may present with abdominal pain, renal colic, or a palpable cystic mass. Acquired diverticula may be associated with strictures or calculi, may follow trauma, or may be because of chronic infection. Large, symptomatic diverticula usually can be removed surgically.

Ureteral diverticulosis refers to multiple ureteral diverticula. This condition is extremely rare, more so than single ureteral diverticula. Fewer than 30 cases of ureteral diverticulosis have been reported in the literature. The diverticula are usually small (<5 mm).

This condition is usually diagnosed with excretory urography, particularly with the use of high-dose, high-concentration contrast agents, although a few cases in the literature were diagnosed with retrograde pyelography and not high-dose urography, even with compression. Such lesions, shown only by high nonphysiologic pressures, may be congenital variants with weakness of the ureteral wall rather than acquired conditions. Others believe that ureteral diverticulosis is a result of chronic infection alone. There is no evidence to support either hypothesis.

Ureteral diverticulosis is usually asymptomatic. If symptoms do occur, they are usually the same as those associated with a single ureteral diverticulum (ureteral obstruction, hematuria, infection, and stone formation). The surgical approach depends on the location of the diverticula and the state of the associated kidney. Simple diverticulectomy with a watertight reanastomoses of the ureteral wall is usually all that is necessary in the rare instances where surgical therapy is indicated (61).

Ureteral Valves

Ureteral valves are an uncommon cause of ureteral obstruction, consisting of transverse folds of redundant mucosa that contain smooth muscle. The lesions may be single or multiple, annular or diaphragmatic, with a pinpoint opening. A ureter is dilated above the obstruction and normal below it. This entity should not be confused with ureteral dilatation secondary to folds or kinks, which are normally occurring in the newborn period (62). With true valves, the dilated ureter at its junction with the undilated segment assumes a pink coloration and eccentric position from elongation and pull from the surrounding adventitia. If obstruction is present, surgical resection of the ureteral valve is necessary.

Ureteral Stenosis

Ureteral stenosis can occur anywhere along the length of the ureter, but is more common in three areas, in order of decreasing frequency: the distal ureter just above the ureterovesical junction, the ureteropelvic junction, and the mid-ureter at the pelvic brim. More than one area of stenosis may be present in the same ureter. If the area of stenosis is obstructed, as judged by radiographic studies, such as an excretory urogram or a nuclear renal scan, such as a MAG-3 with furosemide infusion, then surgical correction is required. A more extensive discussion of this condition is provided in the chapter on ureteropelvic function obstruction.

Circumcaval Ureter

Embryologically, the inferior vena cava develops on the right side from a plexus of fetal veins, which include the posterior cardinal and supracardinal veins dorsally and the subcardinals ventrally. Normally, the left supracardinal veins and the lumbar portion of the right posterior cardinal veins atrophy. The subcardinals become the internal spermatic veins. The right supracardinal veins become the right-sided inferior vena cava. If the subcardinal vein in the lumbar portion fails to atrophy, the ureter is trapped dorsal to it. The vena cava traps the right ureter, which deviates medially behind the inferior vena cava, winding about and crossing in front of it from a medial to a lateral direction, to assume a normal course distal to the bladder.

Retrograde ureteral pyelography or excretory urography usually demonstrates an "S" curve to the point of obstruction, with a retrocaval segment lying at the level of L3 or L4. Ultrasound, computed tomography, and MR imaging have also been useful in defining the vascular malformation (63). If the obstruction is significant, surgical correction involving ureteral division with relocation and ureteroureteral or ureteropelvic reanastomosis is performed.

CONGENITAL ANOMALIES OF THE KIDNEY

Bilateral Renal Agenesis

Renal agenesis results from either the absence of a nephrogenic ridge on the dorsolateral aspect of the celomic cavity or failure

of a ureteral bud to develop from the wolffian duct. The absence of both kidneys requires a common factor causing renal or ureteral maldevelopment on both sides of the midline. Besides the absence of functioning kidneys, each ureter may be either wholly or partially absent. Complete ureteral atresia is observed in slightly more than 50% of affected individuals (64). The bladder is present only in approximately 50% of cases and is usually hypoplastic from the lack of stimulation by fetal urine production.

Bilateral renal agenesis is an infrequent abnormality that occurs in approximately 1 in 10,000 births (65). Complete differentiation of the metanephric blastema into adult renal parenchyma requires the presence and orderly branching of a ureteral bud. This normally occurs between the fifth and seventh week of gestation, after the ureteral bud arises from the mesonephric wolffian duct.

Newborns with bilateral renal agenesis usually have low birth weights and oligohydramnios. There is usually a characteristic "Potter's" facial appearance, which consists of a prominent fold and skin crease beneath each eye, with a blunted nose and depression between the lower lip and chin (66). The ears give an impression of being low set, because the lobes are broad and drawn forward, but actually, the ear canals are normally located. The legs are often bowed and the feet clubbed, with excessive flexion at the hip and knee joints. These characteristics are because of compression of the fetus against the internal uterine walls without the cushioning effect of the amniotic fluid. Normally, urine produced by the developing kidneys is a major source of amniotic fluid, constituting greater than 90% by the third trimester. Thus, the absence of kidneys reduces severely the amount of amniotic fluid produced during pregnancy.

Pulmonary hypoplasia is common with this condition and is associated with other situations where an oligohydramniotic state occurs. The diagnosis usually is suspected when the infant is born with the characteristic Potter's appearance and the presence of oligohydramnios. Close to 40% of affected newborns are stillborn. Children who are born alive usually do not survive beyond the first 48 hours of life, because of the respiratory distress associated with pulmonary hypoplasia. Failure to urinate within the first 24 hours of life is not uncommon in most normal newborns and, therefore, should not arouse suspicion. Sonography may be helpful in the diagnosis. If the sonogram is inconclusive, a renal scan may be performed.

Unilateral Renal Agenesis

Unilateral renal agenesis occurs in approximately 1 in 1,100 births (67). Usually, there are no signs to suggest a unilateral absent kidney. The diagnosis often is not suspected and the condition remains undetected unless prenatal or postnatal sonography or other radiographic studies are performed for unrelated reasons. The embryologic defect for this abnormality does not differ significantly from that described for the bilateral type. Structures derived from the Müllerian or wolffian duct may not develop in a normal fashion. Structures that develop from the wolffian duct in the male embryo include the vas deferens, seminal vesical, ampulla, and ejaculatory duct. These structures may be absent in up to 50% of patients with renal agenesis (68). Conversely, almost 80% of patients with an absent vas deferens have an absent kidney on the ipsilateral side (69).

Approximately one-third of females with renal agenesis have an abnormality of the internal genitalia. Most commonly, there is a true unicornous uterus with complete absence of the ipsilateral horn and fallopian tube or a bicornuate uterus with rudimentary development of the horn on the affected side. An absent, septated, or hypoplastic vagina is usually associated with a unicornous or bicornuate uterus.

Because of the high frequency of unilateral renal agenesis in female patients with internal genital anomalies, assessment of the entire genitourinary system should be performed in this patient population.

Other organ system anomalies are frequently found in patients with unilateral renal agenesis. The more common sites include the cardiovascular, gastrointestinal, and musculoskeletal systems.

Usually, unilateral renal agenesis is entirely asymptomatic. The diagnosis is usually made incidentally when radiographic studies are performed for other reasons, or when a physical examination shows either an absent vas deferens or an absent, septated, or hypoplastic vagina. Except for ectopia or malrotation, anomalies of the contralateral kidney are infrequent. Unilateral renal agenesis with an otherwise normal contralateral kidney does not predispose the patient to greater than normal risks. Patients should be advised, however, to avoid participation in contact sports.

Renal Ectopia

Renal ectopia results when the mature kidney fails to reach its normal location; it occurs in approximately 1 in 900 births (70). An ectopic kidney is found in a number of locations, including pelvic, iliac, abdominal, thoracic, and contralateral or crossed. Embryologically, the ureteral bud first arises from the wolffian duct at the end of the fourth week of gestation. It then grows craniad toward the urogenital ridge and acquires a cap of metanephric blastema by the end of the fifth week. As elongation and straightening of the caudal end of the embryo start, the developing reniform mass migrates on its own and is forcibly extruded from the true pelvis. During renal ascent, the upper ureteral bud matures into a normal collecting system, and medial rotation of the renal pelvis takes place. The process of migration and rotation is completed by the end of the eighth week of gestation. Several factors may prevent the orderly movement of kidneys cranially. These include ureteral bud maldevelopment, genetic abnormalities, defective metanephric tissue that by itself fails to induce ascent, maternal illness, thoracogenic causes, or a vascular barrier that may prevent upward migration secondary to persistence of the fetal blood supply.

The kidney usually is smaller and may not conform to the usual reniform shape. There usually is incomplete rotation, and the renal pelvis is usually anterior instead of medial to the parenchyma. The renal axis is usually slightly medial or vertical. The ureter is of an appropriate length for the position of the kidney, but may be slightly tortuous. The ureter usually enters the bladder on its normal location. The vascular pattern is anomalous and depends on the ultimate resting place of the kidney.

Most ectopic kidneys are clinically asymptomatic. A vague abdominal symptom of frank ureteral colic secondary to an obstructing stone is still the most frequent symptom leading to discovery of an ectopic kidney. The abnormal position of the kidney results in a pattern of direct and referred pain that usually is atypical for colic.

Malposition of the colon may be a clue to the ectopic position of a lumbar or pelvic kidney (as with renal agenesis). Radiographic studies usually confirm the diagnosis. Except for the development of hydronephrosis or urinary calculus formation, the ectopic kidney is no more susceptible to disease than the normally positioned kidney. The anteriorly placed pelvis and malrotated kidney may lead to impaired urinary drainage from a high ureteropelvic junction or an anomalous vasculature that partially blocks one of the major calices or the upper

ureter. Because the kidney is low lying and unprotected by the rib cage, there may also be an increased exposure to injury from blunt trauma. This increased risk is not present when the kidney is an intrathoracic ectopia. With any type of renal ectopia, the adrenal gland is usually in its normal location (70).

When a kidney is located on the side opposite from which its ureter inserts into the bladder, the condition is known as *crossed ectopia*. More than 90% of these are fused to their ipsilateral mate. Crossed renal ectopia may occur with or without fusion; bilaterally crossed renal ectopia also may be found. In any of these anomalies, the ureter usually is not ectopic. Most individuals are entirely asymptomatic. The defects are usually discovered incidentally, as with other renal anomalies thus far described.

The horseshoe kidney is probably the most common of all renal fusion anomalies. This anomaly consists of two distinct renal masses lying vertically on either side of the midline connected at the respective lower poles by a fibrous isthmus that crosses the midpoint of the body. Horseshoe kidney occurs in approximately 1 in 400 births (71). Embryologically, it has been postulated that at 4.5 weeks of gestation, the developing metanephric masses lie close to one another; any disturbance in this relationship may result in joining at their inferior poles. A slight alteration in the position of the umbilical or common iliac artery could change the orientation of the migrating kidneys, thus leading to contact and fusion. It is presumed that the inferior mesenteric artery prevents full ascent of the horseshoe kidney by obstructing the movement of the isthmus. In over 95% of patients, the kidneys join at the lower poles; in a small number, however, the isthmus connects both upper poles instead (72). In general, the isthmus lies anterior to the aorta and vena cava, and it is not unusual for it to pass between the inferior vena cava and the aorta or even behind the great vessels (73). The blood supply to the horseshoe kidney can be quite variable. The calices are atypical in orientation, pointing posteriorly. Probably as a result of incomplete renal rotation, the ureter may insert high on the renal pelvis and lie laterally. Patients with horseshoe kidneys may have associated anomalies. Horseshoe kidney is found in 3% of children with neural tube defects. Horseshoe kidneys may also be seen in 20% of patients with trisomy-18 and in as many as 60% of girls with Turner syndrome (74).

Most patients remain asymptomatic throughout their lifetime. If symptoms do occur, they are usually related to hydronephrosis, infection, or calculus formation. Ureteropelvic junction obstruction may occur in as many as one-third of patients. Usually, horseshoe kidneys are detected when radiographic studies are performed for other unrelated reasons or because of a palpable midline abdominal mass. Because of the horseshoe kidney's location above the pelvic inlet, pregnancy or delivery should not be adversely affected.

Caliceal Diverticulum

A caliceal diverticulum is a cystic cavity lined by transitional epithelium, encased in the renal substance, and situated peripheral to a minor calix to which it is connected by a narrow channel. The upper calix is most frequently affected. Some of the ureteral branches of the third and fourth generation in the fetus, which ordinarily degenerate, may persist as isolated branches, resulting in the formation of a caliceal diverticulum. The caliceal diverticulum could also be acquired because of an abscess, obstruction secondary to stone, infection in the calix, renal injury, or dysfunction at the base of a minor calix.

Although this entity is usually asymptomatic, complications of stasis or obstruction can produce symptoms. Infection, milk of calcium (crystallization of calcium salts without actual stone formation), and true stone formation are complications of stasis or obstruction that can produce symptoms. Hematuria, pain, and urinary tract infection may be seen. Excretory urography with delayed films is helpful in showing the diverticulum. Retrograde pyelography, MRI, and ultrasound may also be useful. Asymptomatic patients do not require treatment. Symptomatic patients may require surgery, which may be through an open surgical approach, percutaneously, or laparoscopically (75).

Congenital Megacalices

Congenital megacalices, or *megacalicosis,* was first described by Puigvert in 1963 (76). The renal calices, although usually ectatic, may be normal in number, but are often increased to as many as 36 (instead of the normal 8 to 12). The renal pelvis is not dilated and has a normal wall thickness. The ureteropelvic junction is normal without any evidence of obstruction. Although the cortical thickness is normal, the medullary pyramids are underdeveloped. There usually is a malformation of the renal papillae and a defect in tubular-concentrating ability. The collecting tubules are not dilated, but are shorter than normal, with a transverse, rather than a vertical, orientation from the cortical medullary junction. Glomerular filtration, tubular isotope uptake, and plasma flow are normal.

The embryologic cause of congenital megacalices is unknown. Several theories have been proposed, including faulty metanephric ureteral bud embryogenesis. It is believed that there may be a transient delay in the recanalization of the upper ureter after the branches of the ureteral bud connect with the metanephric blastema. This delay may produce a limited period of obstruction when the embryonic glomeruli start producing urine. The calices may dilate and then retain their obstructed appearance despite the lack of postnatal obstruction. The increased number of calices may be due to an arrest of the coalescence of terminal ureteral buddings and may occur in response to a transient period of obstruction at a critical time in development. Other theories proposed for the etiology of congenital megacalices include hypoplasia of the juxtamedullary glomeruli, which may account for a lack of renal-concentrating ability, as well as maldevelopment of the pelvicaliceal muscle.

Megacalicosis occurs predominantly in boys, in a ratio of 6:1. Segmental unilateral involvement is seen exclusively in girls, whereas bilateral disease has been seen only in boys. This distribution suggests that an X-linked partially recessive gene with reduced penetrance in girls is a possible causative factor (77).

Megacalicosis may lead to urinary stasis, which predispose children to urinary tract infection and adults to stone formation and hematuria. The diagnosis is usually made with an excretory urogram. Although the ureteropelvic junction does not appear obstructed, there may be segmental dilatation of the distal third of the ureter. In addition, megacalicosis has been associated with an ipsilateral megaureter. This combination of anatomic abnormalities may be mistaken for congenital ureteropelvic or ureterovesical junction obstruction, but no obstruction at these locations has been demonstrated. It is important, therefore, to be aware of this condition in order to avoid unnecessary surgery. Once this condition has been diagnosed, an increased fluid intake is recommended to reduce the chances for stone formation and the other complications that might arise.

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CHAPTER 22 ■ DISORDERS OF MICTURITION

DIRK-HENRIK ZERMANN AND JÖRG SCHŪBERT

Voiding disorders are defined as problems with emptying the bladder in a timely and complete manner. However, in most patients, there is a combination of disorders of micturition and proper urine storage. Therefore disorders of micturition and urine storage are summarized as *lower urinary tract dysfunction*. Symptoms occurring because of lower urinary tract dysfunction are, in general, termed as *lower urinary tract symptoms* (LUTS).

Diagnosis, treatment, and rehabilitation of patients with LUTS are major fields of urology, especially neurologic and gynecologic urology. The primary goal of all medical efforts in micturition disorders is the prevention of upper urinary tract damage, especially the preservation of renal function. Achieving urinary continence, proper micturition, and social rehabilitation is the second most important aim of urologic diagnostics and treatment. These are the basic theses, and this important message should be kept in mind whenever an individual patient is seen for urologic disease affecting micturition.

The lower urinary tract is located in the pelvic area, bordering on the male/female genital tract, the rectum, and the pelvic floor. There is a broad relationship and interconnectivity between the different pelvic organ systems regarding anatomy, physiology, and pathology. This includes, for instance, bladder and bowel emptying, urinary and anal continence and incontinence, erectile/sexual function and dysfunction, chronic pelvic pain syndromes, and inflammatory diseases.

BASIC SCIENCE

Local Anatomy of the Lower Genitourinary Tract

The empty bladder lies behind the pubic symphysis. When full, the urinary bladder rises above the symphysis, where it then can be palpated and percussed. The normal bladder is described as having an apex, a superior surface, two anterolateral surfaces, a bladder base, and a bladder neck. The bladder apex reaches a short distance above the pubic bone and ends up as a fibrous cord, an embryologic derivate of the urachus. This fibrous cord extends from the bladder apex to the umbilicus between the peritoneum and the transversalis fascia, then called the median umbilical ligament. The superior surface is the only bladder area covered by peritoneum. In the female, the bladder is in relationship with the uterus, the vagina, the pelvic floor, and the pubic bone; in the male, it is in relationship to the prostate, the seminal vesicles, the rectum, and the pelvic floor. The interior of the bladder is completely covered by transitional epithelium. There is a loose underlying connective tissue that permits considerable stretching of the mucosa. The bladder musculature is arranged as a network of coarse muscle bundles, widely separated without any clear sheet formation. The description of the bladder wall as having three muscular coats is only true by definition in the bladder outlet area. In the remaining part, the layering is not as strict as often reported in textbooks (Fig. 22-1).

In males the prostate gland lies directly below the bladder base, above the pelvic floor. This accessory genital gland is shaped like a chestnut. The prostate has a base, an apex, and anterior, inferolateral, and posterior surfaces. The base is the upper surface adjacent to the bladder neck; the apex is the lowest part bordering the pelvic floor muscles. The ejaculatory ducts pierce the posterior surface just below the bladder base and pass obliquely through the prostate gland to open separately into the prostatic urethra on the verumontanum. The fibromuscular stroma gains size and weight with age, potentially obstructing the prostatic urethra and causing voiding problems.

The male urethra can be considered in different regional parts: membranous, prostatic, and spongiose. The prostatic urethra is approximately 3 to 4 cm in length and tunnels through the prostate gland. The membranous part is the shortest and narrowest section of the urethra. The wall of the membranous urethra consists of a muscle coat that is separated from the epithelium by a small layer of fibroelastic connective tissue. This muscle coat consists of a thin layer of smooth muscle bundles continuous with a layer of circularly orientated striated muscle fibers forming the external urinary sphincter—the rhabdosphincter. The slow-twitch fibers of the rhabdosphincter are capable of sustained contraction over relatively long periods of time and actively contribute to the tone that closes the urethra and maintains urinary continence. There is still scientific dispute about the exact anatomy of the urinary sphincter (1). The spongiose part is about 12 to 16 cm long and is contained in the corpus spongiosum of the penis. Functional problems usually result from pathology in the prostatic and membranous part of the urethra. The female urethra is approximately 3 to 4 cm long. The wall of the female urethra comprises an outer muscle coat and an inner mucous membrane that lines the lumen and is continuous with that of the bladder. The muscle coat consists of an outer sleeve of striated muscle and an inner coat of smooth muscle fibers. The female rhabdosphincter is anatomically separate from adjacent periurethral striated muscle of the pelvic floor. Dysfunction results from sphincter insufficiency, overactivity, or dislocation of the urethra due to prolapse.

The anatomy of the pelvic floor striated muscle system is complex and basically encompasses two functional layers. The deeper (upper) layer is composed of the levator and its various components. This is a hammocklike structure attached to the pelvic brim (2–4). It slings across the pelvic outlet supporting the internal pelvic organs. Contraction of the levator occurs with stimulation of the third and fourth sacral nerves. The pelvic organs are then lifted upward and anteriorly toward the pubis. With this movement, there is actual physical compression of the bladder neck. The second functional layer to the pelvis is composed of all of the muscles below the levator—the transversus perineal, ileal, and bulbocavernosus muscles,

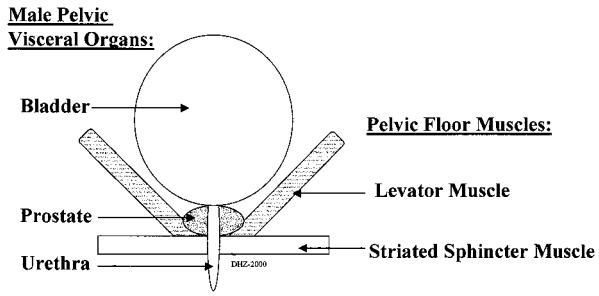


FIGURE 22-1. Schematic drawing of the male pelvic organs/lower genitourinary tract.

the urethral sphincter, and the superficial anal sphincter. These muscles are innervated by the pudendal nerve, which is primarily derived from S2.

Neuroanatomy of the Lower Urinary Tract

The pelvic viscera receives a rich nerve supply from both divisions of the autonomic nervous system. The sympathetic nerve supply originates from the lower thoracic (T11 and T12) and upper lumbar (L1 and L2) segments. These sympathetic nerve fibers descend into the sympathetic trunk and then to the lumbar splanchnic nerves, which reach the superior hypogastric plexus. The latter separates into a right and a left plexus, the hypogastric nerves. The hypogastric nerves extend inferiorly to join the pelvic plexus coming from the pelvic parasympathetic and adjoin it to proceed toward the pelvic viscera. The parasympathetic nerve supply, however, arises from the sacral segments S2 to S4, which proceed to form the rich pelvic parasympathetic plexus, joined by the hypogastric plexus.

The vesical plexus, the major extension of the pelvic plexus, reaches the lateral side of the urinary bladder, innervating both bladder and urethra. In males a separate part will reach the prostate gland, forming the prostate plexus. From the prostate plexus, the cavernosus nerves emerge to supply penile erectile tissue. In females, these nerves supply the tissue of the clitoris.

The pudendal nerves (S2, S3, S4, and bilateral) supply sensation throughout the pelvic area. These are complex nerves that have a variety of responsibilities on both sensory and motor levels (5). The S2 nerve is much larger than the S3 and provides a much greater contribution to the pudendal as well as to the sciatic nerve. Diagnostic electrical stimulation of the S2 nerve produces muscle contractions ("clamplike movements") in the perineum and the leg. The S3 nerve supplies some branches to the pudendal nerve as well, but there is wide variation among patients because of pre- and postfixation of the spinal nuclei. The S3 nerves predictably will supply the more anterior levator muscles via pelvic branches that leave the nerve trunk before it contributes to the sciatic and the pudendal nerves. Direct stimulation of the S3 nerves produces a visible contraction of the levator muscles ("bellowslike movements") and a contraction of the large toe or the distal foot. Referred sensation to the rectum and scrotum are common. The S4 nerves do not contribute to the pudendal innervation of the pelvis or sciatic nerves. Stimulation of these nerves produces a contraction of the posterior portion of the levator, accompanied by referred sensation to the rectum and/or to the tip of the coccyx.

Animal tracer studies have shown that the somatic innervation of the pelvic floor muscles is mainly derived from Onuf's motor nucleus in S1 and S2. The innervation of the striated urethral sphincter also originates in this nucleus, but there is no difference when it is compared to the innervation of other pelvic floor muscles (6).

Bladder and pelvic muscle control is directly linked to the brainstem, and proper function is dependent on regulation from a number of these brainstem nuclei. Pathology in these regions can have tremendous impact on the functional integrity of the lower urinary tract, usually in the form of hyperreflexic behavior stemming from a loss of control in central nervous system (CNS) inhibition. Neuroanatomic studies, using the retrograde transneuronal tracer pseudorabies virus (PRV) (7,8), showed a significant overlap of areas in the spinal cord and the brainstem, which are involved in the innervation of perineal muscles of the pelvic floor (m. ischiocavernosus, m. bulbospongiosus) (9), the external urethral sphincter (10), the urethra (11), the bladder (12,13), and the prostate (14,14a) (Fig. 22-2 and Table 22-1).

Many of the spinal cord- and brain-labeled neurons belong to areas representing the sympathetic autonomic system (15). It is noteworthy to emphasize that the premotor autonomic area in the hypothalamus and ventrolateral medulla contain topographically segregated populations of neurons that innervate individual sympathetic preganglionic functional units and control specific patterns of their activity (16,17).

The paraventricular nucleus of the hypothalamus (PVN) is a well-known way station of vagal and sympathetic activity. It seems to serve as a "master controller" for the autonomic nervous system because it provides highly specialized innervation to all autonomic relay centers. It contains many putative peptides and there are important projections from this area to the hypophysis, the spinal control centers for the pelvic floor (nucleus Onuf), the parasympathetic motor nucleus, and the intermediolateral cell column (IML, sympathetic motor nucleus), as well as the dorsal horns (lamina I, lamina X) that can tie pelvic physiology to hormonal regulation of the hypophysis, allowing for the potential linkage in patients between dysmenorrhea symptoms (e.g., oxytocin-mediated cramps) and bladder dysfunction.

Electrical stimulation of the dorsolateral pontine reticular formation (DPRF) (i.e., locus coeruleus) produces bladder contractions, while lesioning leads to a depressed bladder reflex (18–20). There are two areas of concern: (a) a medial area that results in sphincter relaxation and detrusor contraction when stimulated and (b) a lateral area that leads to sphincter contraction and detrusor inhibition when stimulated. The DPRF gives projections to the nucleus retroambiguus, which, in turn, projects heavily to both the abdominal muscles and the pelvic floor muscles. Discomfort in the upper abdomen can therefore be associated with or stem from spastic dysfunction of the pelvic muscles. From these areas of the brainstem there are noradrenaline projections throughout the length of the cord, including the parasympathetic motor nucleus, the intermediolateral nucleus, and Onuf nucleus (21). They have a general excitatory influence. α-Blockers, because they are lipophilic and can cross the blood–brain barrier, could produce significant clinical benefit in downregulating the excitability of these brainstem areas.

Similarly, the nucleus pallidus and ventromedullary reticular formation have strong projections to the somatic and autonomic motor neurons throughout the cord. Many of these projections are serotonergic, but contain substance P and leuenkephalin. They have a facilitatory role. New serotonergic blockers would thus have a potential role in modulating micturition dysfunction.

Given the neurologic linkage between the brainstem and lower urinary tract, the integrity of these peptide pools could be reflected in the activity or behavior of the bladder/pelvic floor. It is difficult to imagine the lower urinary tract as being normal in the face of brainstem neuropathology. It is difficult to consider these same brainstem nuclei, with regulatory influence over micturition, as being normal in the face of a significantly dysfunctional lower urinary tract. Functional

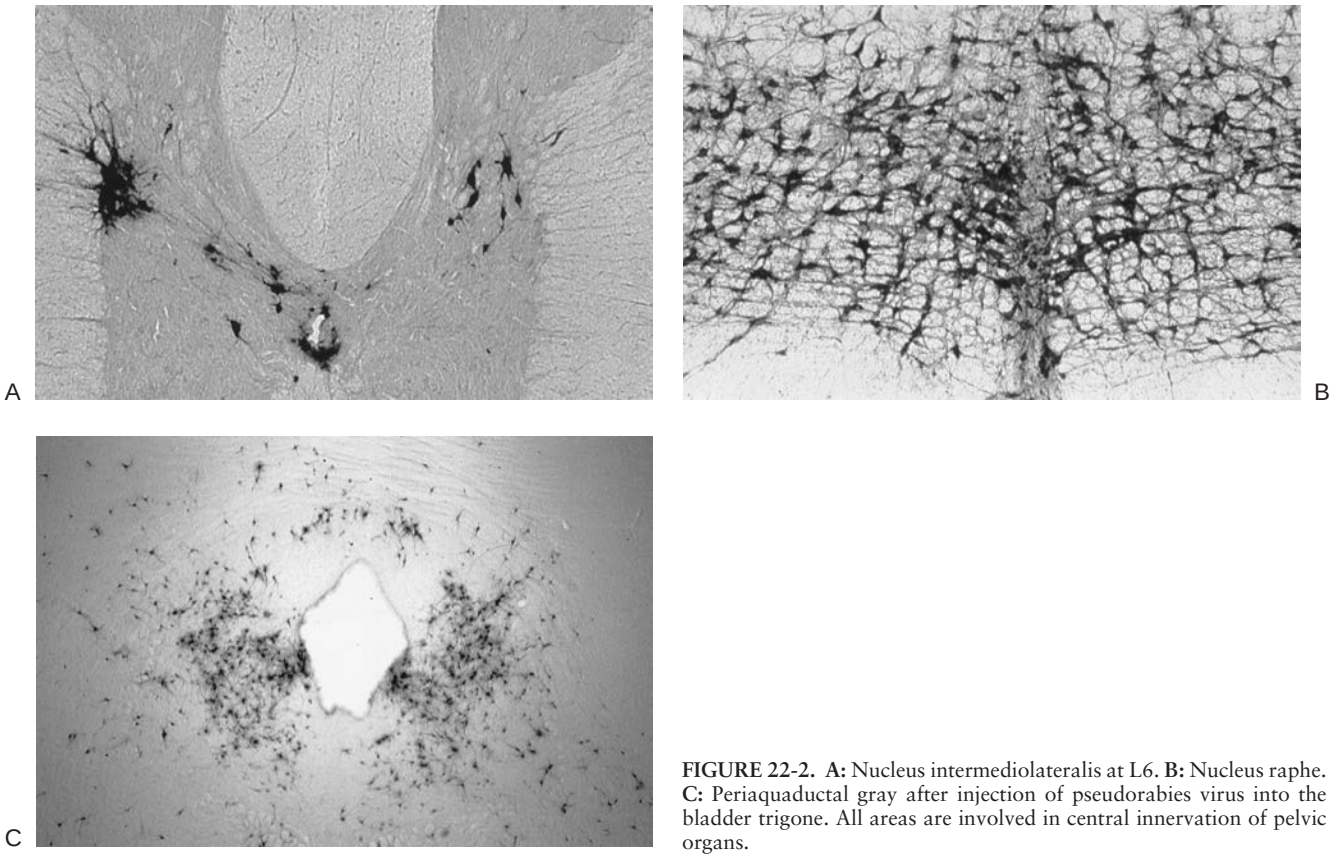


FIGURE 22-2. A: Nucleus intermediolateralis at L6. **B:** Nucleus raphe. **C:** Periaquaductal gray after injection of pseudorabies virus into the bladder trigone. All areas are involved in central innervation of pelvic organs.

imaging of the brain will provide the insight into the integrity of these various nuclei and how they are affected in patients with "idiopathic" lower urinary tract/pelvic organ dysfunction.

Normally, bladder distention produces reflex excitation of parasympathetic nuclei, depression of sympathetics, and depression of somatic sphincter control (22). Detrusor afferent activity associated with filling is relayed via pelvic nerves to the sacral cord (23,24). No discharge activity occurs unless the detrusor is distended. The reflex discharge of spinal cells has a long latency, afferent limb 30 to 40 milliseconds and efferent limb 45 to 60 milliseconds, which is not found after sectioning of spinal cord, but is preserved with decerebration. Short latency reflex contractions are not normally found. Hence, reflex bladder contraction is a supraspinal reflex in patients with intact spinal cords. Stimulation of regions above (i.e., rostral to the locus caeruleus) produces inhibition. Areas rostral to the pons are, therefore, thought to have modulating influences on detrusor excitability.

In contrast to the intact nervous system, spinal cord section leads to the appearance of short latency reflexes (3 to 5 and 7 to 25 milliseconds, respectively). There is an emergence of somatovesical reflex excitation normally suppressed by higher centers.

TABLE 22-1

PSEUDORABIES VIRUS STUDIES ON THE CENTRAL NEUROANATOMIC PATHWAYS INVOLVED IN INNERVATION OF THE PELVIC AREA (ANIMAL EXPERIMENTS)

Authors	Mapped structure	Spinal representation	Brainstem/brain representation (major areas)
Nadelhaft et al., 1992 (12)	Bladder	L6–S1	PMC, RO, NGC, LC, A5, PVN
Zermann et al., 1998 (13)	Bladder trigone	T12–L2 L6–S2	C, PMC, NGC, LC, A5. PVN, RM, RN
Marson and McKenna, 1996 (9)	Perineal muscles (IC, BS)	T13–L2 L6–S1	PMC, RP, VML, A5, LC, PVN
Nadelhaft and Vera, 1996 (10)	External urethral sphincter	L6–S1	PMC, RM, A5, A7, LC, PAG
Vizzard et al., 1995 (11)	Urethra	L6–S1	PMC, RN, LC, PVN
Zermann et al., 2000 (13)	Prostate	T13–L2 S1–S2	PMC, NGC, LC, A5. PVN, RM, RN

[a] *A5*, A5 noradrenergic cell group; *A7*, A7 noradrenergic cell group; *BS*, m. bulbospongiosus; *C*, cortex; *IC*, m. ischiocavernosus; *LC*, locus ceruleus; *NGC*, nucleus gigantocellularis; *PAG*, periaquaductal gray; *PMC*, pontine micturition center; *PVN*, paraventricular nucleus of the hypothalamus; *RM*, raphe magnus; *RN*, red nucleus; *RO*, raphe obscurus; *RP*, raphe pallidus; *VML*, ventrolateral medulla.

Under certain conditions there can be a reemergence of short latency reflex activity. This would appear to occur in patients without neuropathology who develop urge urinary incontinence. It could also be argued that adult onset incontinence could be the long-term consequence of chronic dysfunctional voiding habits, which evolved *de novo* in early adulthood, or were chronically present from childhood.

Behavioral Aspects of the Development of Proper Micturition and Continence

The evolution of bladder control is a gradual process. On the surface it would seem to be rather automatic as most children achieve night time continence by 3 years of age. It is, however, a process that is not completed until well beyond the age of reason, when children are capable of responding to messages from their bladders in an appropriate manner. Children are fine tuning the regulatory pathways for the micturition control process well beyond the time when they have gained nighttime control. Micturition is, in part, an automated process linked to the growth of the brain. More important, it is a learned process dependent on information that is delivered to the brain (25). To appreciate this principle it is helpful to understand the way in which the brain develops. There is an overall basic organization to the brain and CNS. However, within this gross organization, dendritic interconnectivity is influenced by the afferent information delivered into the brain circuitry. Experiences, even the simplest of behaviors, generate change in our nervous systems (26–34). They shape language, coordinate movements, and develop cognition. Adjustments and change of the way in which information is handled and processed goes on throughout life, but much more slowly as the nervous system ages. Changes in the amount, source, and competitive nature of the sensory input can have a profound influence on neural interconnectivity. Even a few minutes a day for several days a week can produce major changes in control of somatosensory regulatory areas of the brain. This is especially true during childhood when the brain is much more plastic and moldable.

Inappropriate learning strategies adopted by our self-organizing nervous system early in life can create problems. For example, if the pelvic floor muscles are tightened repetitively to delay or postpone voiding, or voiding is accomplished through straining, the integrity of the entire CNS regulatory apparatus involved in micturition could be affected. Once an incorrect behavior is in place, it can be experientially reinforced by repetition. Eventually the circuitry for incorrect behavior becomes etched or burned in. Children have a strong tendency for retentive behavior. They do not recognize urges to empty their bladders until the last minute. They can get "caught" in a car ride, in school, at a playground, or in a department store, as they can not judge time until access to a bathroom. Often, they must be reminded to void as they do not break away from play easily. There are all kinds of reasons for "abnormal" learning of void behavior—tension in the home, overbearing parents pushing their children to be toilet trained, a day care center that demands that the child be toilet trained prior to entry, unsympathetic teachers at a school, and excessive disciplining for a bedwetting accident can all lead to disturbances in bladder and/or bowel behavior.

Plasticity slows considerably by age 10. A child with retentive tendencies from early childhood can evolve a permanent circuitry that can guarantee this type of behavior. Such behavior is readily reinforced with time, because of tensions and pressures that result from our deadline-orientated society, such as exams in school, grant and contract applications, economic strains, or work-related bathroom inaccessibility. Tensions naturally affect automated muscle systems in the body. Inappropriate muscular dynamics, excessive pelvic floor tension, poor relaxation, and straining to void can too easily become the norm for micturition behavior, leading to a breakdown in the functional and anatomical integrity of the system over time and with age.

It is especially important to appreciate that incorrect conditioning of these reflexes during the time the nervous system is maturing, can result in voiding dysfunctions ranging from incontinence to retention. Symptoms may not appear for years, even decades. Many of the problems in adult life may stem from toileting habits evolved during childhood. Prevention of adult disorders of micturition and urinary incontinence by addressing toileting habits of children is, therefore, not only logical but critically important to the long-term management of these lower urinary tract disorders.

The Basics of Lower Urinary Tract Function—The "Four-Phase Concept"

The normal bladder/lower urinary tract function involves apparently two discrete processes: (a) bladder filling and urine storage at a low pressure, and (b) complete and coordinated bladder emptying. This includes urinary continence at all times.

In more physiologic detail, it is obvious that the "two-phase concept" (35) does not characterize bladder function as much as necessary in order to understand all aspects of bladder dysfunction. Based on analyses of thousands of urodynamic charts a "four-phase concept" that involves (a) bladder-filling phase; (b) transition phase (switching from "hold" to "release"); (c) micturition phase; and (d) recovery phase (switching from "release" to "hold") seems to be more appropriate. This concept is not based only on anatomic–physiologic aspects of peripheral organ function. Moreover, this general approach includes central and peripheral neurophysiologic processes involved in proper lower urinary tract function.

During the first phase, the urinary bladder is able to hold increasing amounts of urine without a parallel rise in intravesical pressure. This is due to the elastic and viscoelastic properties of the detrusor muscle. Elasticity allows all constituents of the bladder wall to stretch up to a certain degree without any increase in tension. Viscoelasticity differs in that stretch induces an initial rise in tension, which then decays with bladder filling, causing the stretch to slow or stop (i.e., stress relaxation). The ratio of bladder volume increase to intravesical pressure rise is called bladder compliance. Compliance is of major interest in diagnostics and follow-up of bladder-storage problems, especially because of the risk of upper urinary tract deterioration and renal failure. Bladder compliance can be altered by any process within the bladder wall (e.g., fibrosis, inflammation), the peripheral (e.g., peripheral neuropathy), or CNS (e.g., spinal cord injury, multiple sclerosis). Initially, there is no sensation during bladder filling. As filling progresses, a slight sensation develops in the bladder region, which can easily be ignored. With further bladder filling, this sensation becomes more distinct and the time for voluntary voiding is established. The sensation is brought to consciousness via afferent nerves originating in the bladder wall, traveling in the pelvic nerves and the spinal cord up to the brainstem and cortex (Fig. 22-3).

During the second transition phase, important changes emerge at the level of the CNS allowing a switch from urine storage to bladder emptying. This short process requires a proper functioning of the CNS system. Furthermore, the ability to switch from phase 1 to 3 depends on a sufficient organ function and continence training in childhood and adolescence. Many patients who are unable to initiate micturition are suffering from a failure of this particular second phase. The basic

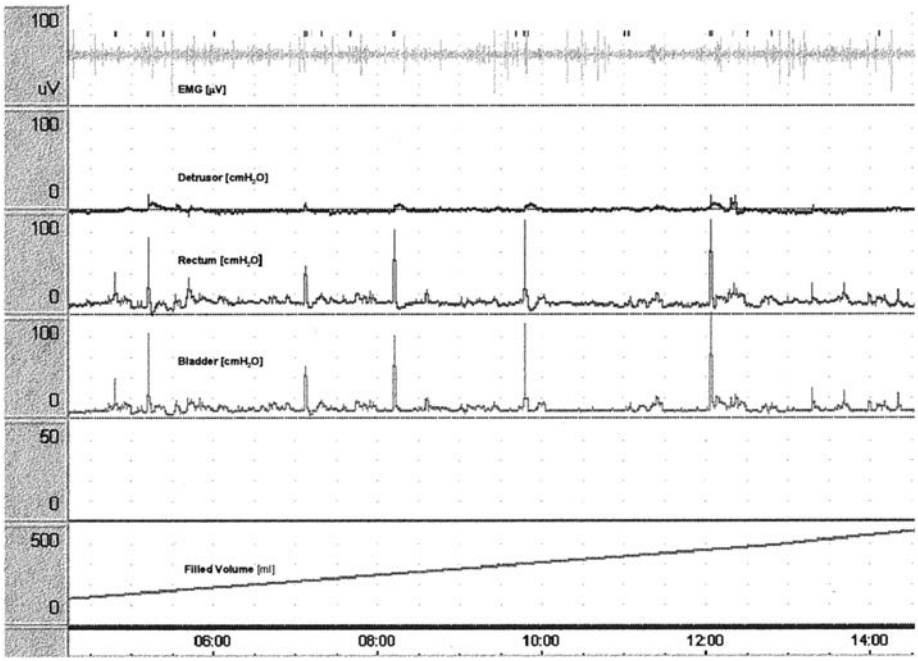


FIGURE 22-3. Normal bladder filling (phase 1) without functional abnormalities.

neuronal processes initiating and coordinating the switch are not yet known (Fig. 22-4).

Bladder emptying, the third phase, is achieved by a rise in intravesical pressure, which is normally sustained until the bladder is empty. Synchronously, the urethral pressure falls during voiding and returns to normal when the intravesical pressure has fallen to resting levels. The initial fall in urethral pressure, which precedes the rise in intravesical pressure by seconds, belongs to the transition phase. In the third phase, voiding starts when the intravesical pressure is above the closure pressure level of the bladder neck and urinary sphincter, allowing a urinary flow (Fig. 22-5).

When the bladder is empty, one basic cycle of bladder function is complete. The switch from urine release to urine hold requires a total "turnaround" in autonomic innervation (i.e., facilitation, inhibition, and neuromodulation), as well as voluntary pelvic floor behavior. These changes are characterized by the fourth phase, the recovery phase (Fig. 22-6).

DIAGNOSTICS OF MICTURITION DISORDERS AND LOWER URINARY TRACT DYSFUNCTION

History

On an initial visit to a urology clinic, patients usually complain about a variety of symptoms, including frequency,

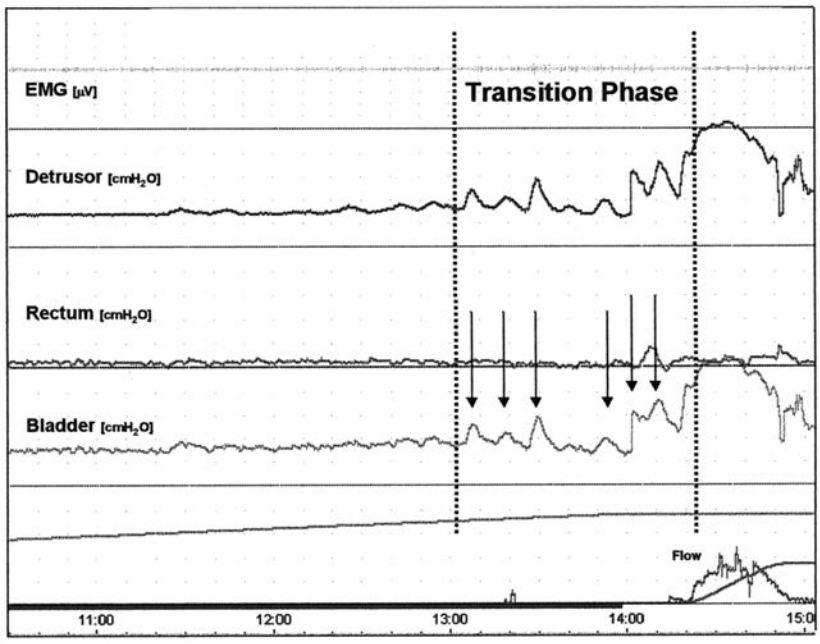


FIGURE 22-4. Failure of proper switching from "urine hold" to "release." Note repeated inefficient detrusor contractions during the transition phase without micturition, before micturition finally happens.

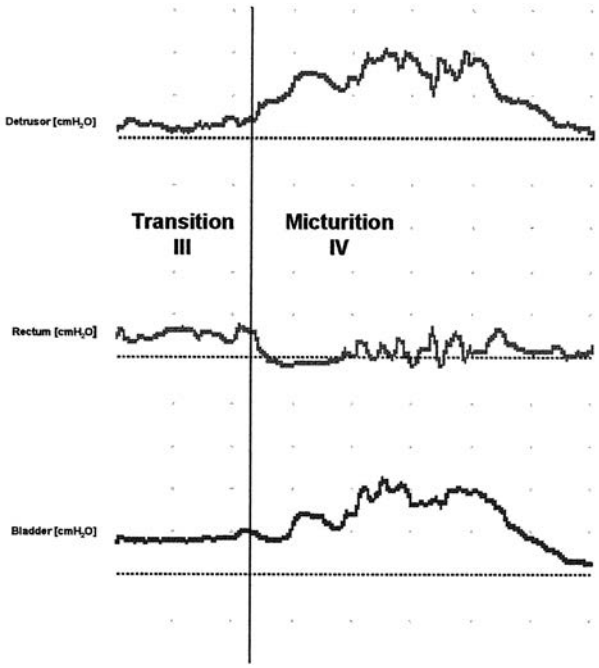


FIGURE 22-5. Normal micturition after sufficient transition from "hold" to "release."

urgency, slow urinary stream, the necessity of double voiding, and recurrent urinary tract infections or pain. A carefully obtained history (Table 22-2) provides a detailed description of the patient's symptoms and their onset and duration. This includes a micturition diary of at least 7 days, or, better, 14 days. The patient is asked to note emptying parameters (time, volume, leakage, pad change) as well as fluid intake data (time, volume). Comments on pain, autonomic signs (headache, sweating, tachycardia, palpitation), and defecation are requested.

The history recorded should include at least those data shown in Table 22-2.

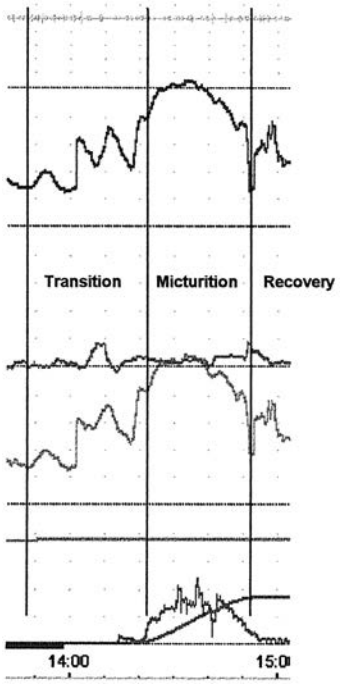


FIGURE 22-6. Normal recovery of bladder function after micturition is completed.

TABLE 22-2

RECORDED DATA FOR BASIC HISTORY

CURRENT SYMPTOMS (Since when? Development or change over time?)

Micturition
Any problems? Double voiding? Infections? Burning? Inability to void?

Defecation
Frequency? Consistency?

Pain
Where? Character? Intensity (using visual analog scale, 0–10)? Change over time?

Sexual life
Erectile/sexual dysfunction? Emotional problems? Vaginism (female)?

CHILDHOOD
Prolonged bedwetting? Excessive excercises to achieve early urinary continence? Punishment for bedwetting? Retentive voiding habits (i.e., low micturition frequency)? Sexual abuse (female)?

ADOLESCENCE
Female: Painful menses? Frequent urinary tract infections?
Male: Urinary tract infections?

ADULTHOOD
Female: Childbirths? Vaginal delivery? Pelvic surgery? Infections? Voiding habits over time? Professional, personal satisfaction?
Male: Voiding habits? Professional, social life?

PREVIOUS TREATMENT
Surgery (pelvis)? Radiation therapy, chemotherapy?

CURRENT MEDICATION

Physical Examination

A general clinical examination is performed in all patients seen for voiding dysfunction. Special attention should be paid to the abdomen, hernias, scars, palpable masses, and signs of neurologic impairments.

A main issue is the pelvic floor and digital rectal examination. Initially, the clinician should observe voluntary attempts to tighten and relax the perineum. This is done with the patient in lithotomy position, or stooped over, with arms on an examination table. A visual assessment is then carried out. With a "hold" effort, contraction and relaxation of the pelvic floor muscles should be clearly evident. There should be an instantaneous, unhesitating identity with the rectal muscles. The movement should be effortless and free of extraneous muscular activity (i.e., abdominal tightening, Valsalva, and pelvic tilt). The "bellowslike" movement of the levator should be repeatable with consistency and the contraction should be sustainable for 2 to 3 seconds before relaxation is permitted. Often the effort is flawed dynamically, with contraction or relaxation

inconsistencies, or frank tightness/weakness is evident. Subsequently, the buttock should be slightly separated and the anal skin observed for "clamplike" wrinkling on the hold effort. Occasionally one may see fibrillations in the superficial anal muscle fibers. Alternately, an easier way to check on voluntary recruitment of the pudendal innervated perineal muscles is to observe contraction of the base of the penis in males (or the clitoris in females) during hold and relaxation. Shortening of the ischiocavernosus muscles should pull the penis (or clitoris) toward the pubic bone. Poor identity with the pelvic muscles, or inconsistencies in recruitment, reflect inefficiencies in neural regulation of the sphincter.

The digital rectal examination is then performed, both for anatomical aberrations, and functional behavior. The following should be assessed and mentally quantified:

1. Tonus of the deep and superficial anal sphincter—is the tone of the sphincter normal, weak, or high?
2. Evidence of hypersensitivity—is there tenderness of the levator posterolaterally? Is there tenderness of the prostate apex, base, or the external sphincter?
3. Motor identity—can the patient contract on the examining finger? Is the movement repetitively consistent? Is there a distinct contraction as well as relaxation above and below the basal tone? Is the levator edge distinct or mushy? Is there movement in both the levator and pudendal sphincter components?

In female patients a gynecologic investigation is also necessary.

The clinician should learn to recognize degrees of inefficiency that affect the behavior of the pelvic floor between voids, as well as during the void. Patients with symptomatic lower urinary tract dysfunction (e.g., "shy bladders" " recurrent urinary tract infections," or a "frequency issue") often have high pressure, tender, and inefficient pelvic floor muscles, and muscular dynamics.

This examination should follow a careful clinical examination of the patient. For this undertaking, the patient can be examined standing, sitting, or in the lateral decubitus position. A systematic assessment of pelvic floor sensation, anal tone, strength of the lower limbs and feet, and the lability of reflexes involving the bulbocavernosus, anal, knee, and ankle muscles should be carried out.

When completed, this examination should provide useful insight into the functional integrity of the sacral nerve roots controlling bladder and sphincter behavior. Reflex excitability should be an important focus of the examination. Brisk lower extremity reflexes are often a good indication of a hyperexcitable pelvic floor (Table 22-3).

TABLE 22-3

SUGGESTED SCHEME FOR THE CLINICAL EVALUATION OF THE PELVIC AREA

CLINICAL ASSESSMENT OF PELVIC FLOOR AND SPHINCTER		
Asymmetry of gluteal–thigh creases	None (0), some (1+), major (2+)	2
Symmetry of greater trochanters (pelvic tilt)	None (0), some (1+), major (2+)	2
Total		**4**
VISUAL ASSESSMENT		
Bellows (levator)		
Gluteal cleft	Deep (2+), flat (2+), moderate (0)	2
Hold	Definitive (0), some (1+), none (2+)	2
Relaxation	Complete (0), some (1+), none (2+)	2
Repetitious consistency on three tries: identical movement (0), some (1+), none (2+)		2
Penile retraction (sphincter)		
Hold	Definitive (0), some (1+), none (2+)	2
Relaxation	Complete (0), some (1+), none (2+)	2
Repetitious consistency on three tries: identical movement (0), some (1+), none (2+)		2
Visual total		**14**
VOLUNTARY CONTRACTION ASSESSMENT (DIGITAL RECTAL EXAMINATION)		
Levator (bellows)		
Resting Tone	None (0), moderate/normal (1+), increased (2+)	2
Hold	Definitive (0), some (1+), none (2+)	2
Relaxation	Complete (0), some (1+), none (2+)	2
Repetitious consistency on three tries identical movement (0), close (1+), none (2+)		2
Rectal (levator) sensation		2
No pain (0), some discomfort (1+), very painful (2+)		
Anal sphincter (clamp)		
Resting tone	Moderate, normal (0), increased, weakened (1+), tight, none (2+)	2
Hold	Definitive (0), some, flutter, or premature release (1+), none (2+)	2
Relaxation	Complete (0), some (1+), none (2+)	2
Repetitious consistency on three tries: identical movement (0), some (1+), none (2+)		2
Perianal skin sensation	Normal (0), increased, decreased (1+), hyperesthetic, numb (2+)	2
TOTAL EXAM POINTS		**20**
TOTAL POSSIBLE SCORE		**38**

Laboratory Testing

Laboratory testing including urinanalysis and serum creatinine measurement are basic tests in a neurourology clinic.

Urinanalysis and bacteriologic studies are needed to diagnose or exclude a urinary tract infection. In the case of bladder dysfunction, acute, recurrent, and chronic urinary tract infections are often found. These are often due to bladder emptying problems. The most common bacteriologic species found in patients with severe bladder dysfunction are *Escherichia coli, Enterococcus, Pseudomonas aeruginosa,* and *Proteus mirabilis* (36). During follow-up, depending on the type of lower urinary tract dysfunction, the urine also needs to be periodically checked (e.g., hyperreflexic bladder owing to spinal cord injury every 3 months).

An elevated serum creatinine concentration may be a first sign of renal deterioration. However, in patients with lower urinary tract dysfunction it is a late sign indicating upper urinary tract damage secondary to insufficient bladder management. In a bladder dysfunction clinic, the serum creatinine level is an important follow-up marker, controlled on an annual basis in case of stable bladder function under treatment. However, radionuclide studies are also needed in patients with impairment of bladder function.

Imaging Techniques

The introduction of ultrasonography in clinical medicine was a revolutionary step. Modern neuro–urology benefits from this imaging technique. In the past, a catheterization was necessary in order to evaluate residual volume. Today, an ultrasound scan provides this information in a fast and reliable manner. Another indication in neuro–urology is renal ultrasound. Significant information can be obtained about both the renal parenchyma and the collecting system. Hydronephrosis (e.g., because of bladder dysfunction), will appear as a dilated collecting system separating the normally echogenic renal sinus, creating an anechogenic central area surrounded by parenchyma. Echoes within the collecting system may indicate an upper urinary tract infection. The thickness of the renal parenchyma is a good indicator for the duration of upper urinary tract obstruction because of bladder-storage problems.

Transrectal ultrasonography allows an evaluation of the prostate gland and the seminal vesicles. Prostate size and changes of the echo pattern can be easily evaluated. This information is of interest in the diagnostics of benign prostate hyperplasia, prostate inflammation, pain, and prostate cancer.

Transvaginal and introitus sonography are other fields of modern ultrasound. These investigations provide additional information in the diagnosis of stress urinary incontinence in women.

Radionuclide imaging is used to estimate total and split renal function at the time of primary diagnosis and during follow-up. Renal scans are particularly useful to follow patients with known obstruction of the upper urinary tract because of long-time inadequate bladder management, as well as patients with spinal cord injury who are at a high risk for impaired renal function.

Intravenous pyelography remains a standard modality to evaluate hematuria. Hematuria is commonly observed in functional disorders because of the problem of chronic inflammation. In order to exclude tumors of the collecting system, urography is used.

In the evaluation of stress urinary incontinence and obstructive voiding patterns in women, another radiographic technique in use is colpocystourethrography. Two different contrast media are given intravesically and intravaginally. A chain marks the urethra (Fig. 22-7). The position and contour of the bladder, vagina, and urethra are evaluated during relaxation, straining, and squeezing to determine dislocation of pelvic organs causing functional disorders.

FIGURE 22-7. Colpocystourethrography in a grade-3 stress incontinent woman: The urethra is marked by a metal chain and bladder and vagina by different contrast media. Note the extreme descensus vesicae et urethrae.

Urethrocystoscopy/cystoscopy are indicated to assess bladder mucosa morphology to exclude chronic inflammatory changes and malignant disease. In males, the bladder outlet and prostatic urethra are evaluated for obstructive disease.

Bladder Biopsies

In order to exclude malignant, chronic inflammatory disease or interstitial cystitis, a bladder biopsy may need to be obtained. A histologic evaluation will provide additional information if imaging techniques are not adequate. In most cases, light microscopy will be sufficient; in some cases, however, electron microscopy will provide additional information regarding ultrastructural changes (37–40) (Fig. 22-8).

Urodynamics

Urodynamic studies are meant to include all techniques used to provide objective documentation of lower urinary tract functional integrity (25,41,42). Traditionally, urodynamic testing includes a cystometrogram (CMG), a urethral pressure profile, a urinary flow rate, and a documentation of residual urine. Additional tests include the leak point pressure and various neurologic tests, electromyographic (EMG) monitoring of the

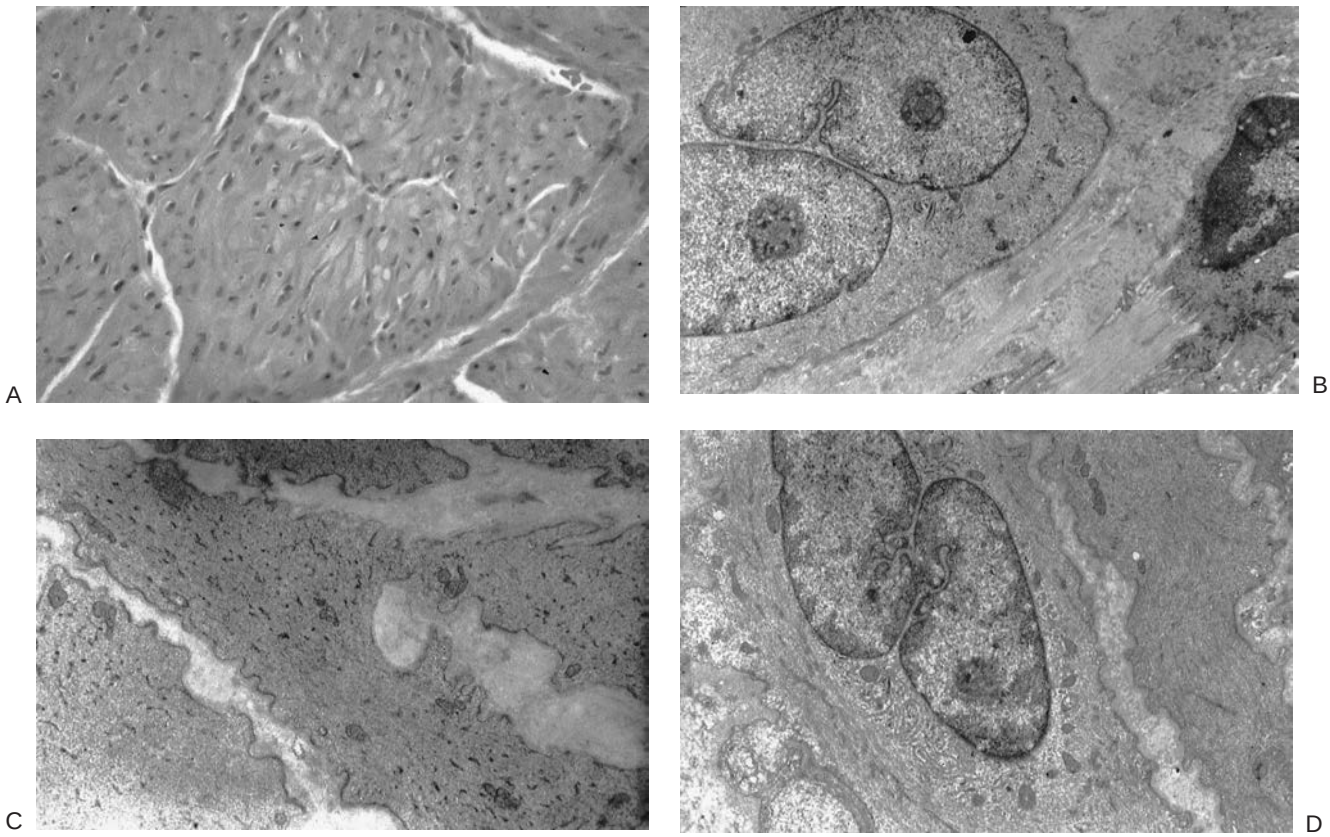


FIGURE 22-8. Bladder specimen from a patient with obstruction secondary to pelvic floor hyperactivity. **A:** Light microscopy. Note the decrease of cell/nuclei numbers due to muscle overdistention. **B:** Electron microscopy. Note increased collagen between cells. **C, D:** Electron microscopy. Note the increased distance between detrusor muscle cells.

pelvic floor, pudendal motor term latencies, and evoked potential studies. Radiologic tests include video monitoring of the bladder during filling and emptying (videourodynamics). The most meaningful assessment is achievable via recordings of lower urinary tract behavior.

Uroflowmetry is a noninvasive functional investigation evaluating bladder emptying. The urinary flow rate and pattern reflect the net effect of detrusor pressure and urethral resistance. The maximum and medium uroflow rates are the most widely used parameters of flowmetry. If the urinary flow rate and flow pattern are normal and the voided volume is over 200 mL, it is unlikely that a micturition disorder is present. In daily practice, uroflowmetry is an ideal screening tool for disorders of micturition. However, it is insufficient to diagnose a bladder outlet obstruction or to differentiate those from decreased bladder contractility or a combination of both.

Uroflowmetry–Electromyography

The combination of uroflowmetry and recording electric pelvic floor activity during voiding provides additional information about lower urinary tract function (i.e., detrusor–sphincter–externus dyssynergia).

Cystometry

Just what is normal bladder function? There is good urodynamic evidence that the adult bladder should be able to hold 400 to 500 mL volume without much more than a sense of fullness. As with low urinary volumes, storage above 500 mL is considered abnormal and quite possibly a sign of a distinct lower urinary tract dysfunction. There is a range and the greater the departure from the ideal normal, the greater the neuromuscular abnormality of the bladder, the greater the risk of complications, and the greater the difficulty to be expected in correcting the problem.

Void diaries are quite helpful, but can be misleading. A void frequency of four to six times per day may be normal with a 24-hour output of 2 L (500 mL/void), but abnormal with a 24-hour output of 800 mL (200 mL/void). Smaller volume voids reflect such abnormalities of the bladder wall as instability, hyperreflexia, hypersensitivity, or poor compliance.

A CMG is obtained with the patient lying down and a filling rate of 20 mL/minute. The cool temperature of the filling fluid, or performing this procedure with the patient, is provocative to the void reflex. Contraction and voiding should occur as a voluntary event with filling near the ideal range. Otherwise, the test should be considered a departure from an idealized norm.

The value of urethral pressure profile (UPP) recording lies not so much in the absolute pressure reading, but in documentation of the degree of facilitation or inhibition that may exist within CNS regulatory circuits. The UPP is recorded with the bladder filled to roughly 25% and 75% of suspected capacity, as there are different micturition gates operational at the different fill volumes. Sensitivity to the presence of the catheter is noted. Hypersensitive urethras are most often associated with labile activity and with irritative voiding symptoms. Each time the profile is repeated, changes in urethral tone, reflex excitability of the pelvic floor striated muscles, and any triggering of an unstable bladder contraction are noted. The real value of the UPP is achieved only when the data are collected and viewed with a dynamic perspective. Static pressure readings have

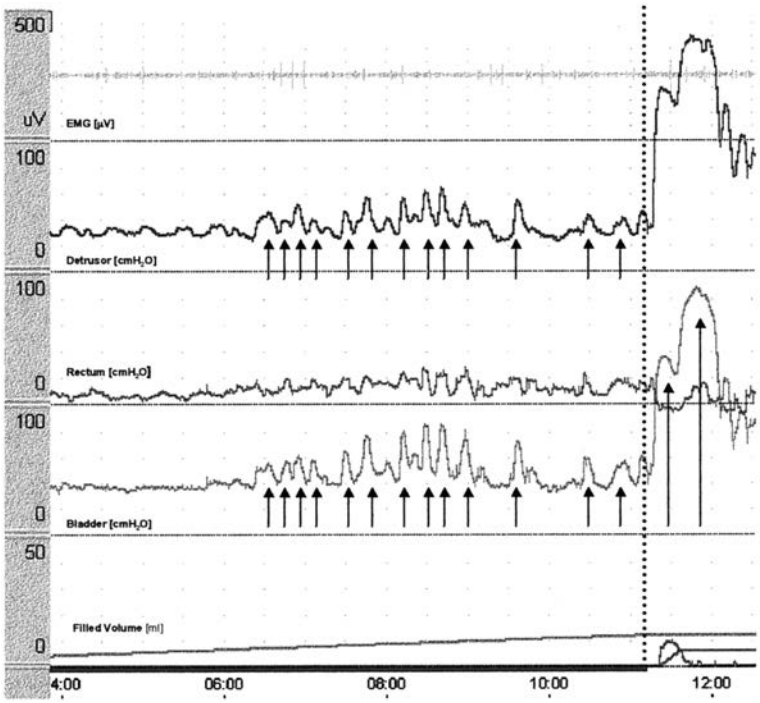


FIGURE 22-9. Detrusor instability in a 38-year-old woman with spinal cord injury.

limited value as may be the case with pure intrinsic sphincter weakness. Dynamic recording is able to show sphincter overactivity and spasticity (Figs. 22-9 and 22-10).

Pressure-Flow Studies

These studies are used to differentiate between patients with a low maximum uroflow secondary to obstruction (e.g., benign prostatic hyperplasia [BPH]), and those whose weak uroflow is caused by a decompensated detrusor function (e.g., neurogenic bladder). Pressure-flow studies may also identify high-pressure obstruction in patients with BPH with normal flow rates. The test–retest reliability of this technique appears to be reasonable (43,44).

DISORDERS OF MICTURITION AND LOWER URINARY TRACT FUNCTION

Benign Prostatic Hyperplasia

BPH is one of the most commonly diagnosed urologic disorders in men. No single definition has gained general acceptance in urology. BPH has been variably defined as prostatic enlargement, bladder outlet obstruction (BOO), histologic hyperplasia, LUTS, as well as various other conditions. The prostate undergoes significant growth during life, starting during fetal development, proceeding throughout puberty, and, in most men, continuing into late middle age. Fifty percent of men between the ages of 51 to 60 and 90% of men over 80 have histologic evidence of BPH (45). At the age of 55, approximately 25% of men note a decrease in the force of their urinary stream (46). The occurrence of this symptom increases linearly to 50% by age 75 and reaches almost 90% by age 85 (45). Patients often present with a combination of irritative and obstructive symptoms including urinary frequency, urgency, nocturia, urge, and urge incontinence, as well as slow stream, intermittent urinary flow, difficulty in initiating the stream, prolonged and double voiding, incomplete bladder emptying, and straining with urination. A typical emergency is the acute urinary retention with the absolute inability to void and overflow incontinence (discussed later in this chapter). These patients need a catheter in order to empty the bladder. On initial presentation for symptomatic BPH, as many as 10% of men have renal insufficiency. Correcting long-standing postrenal obstruction may lead to a profuse diuresis, marked natriuresis, and volume depletion in the absence of fluid replacement. Therefore hospitalization, intensive monitoring, and treatment are mandatory. The condition is usually reversible, with normal renal function returning within 3 to 8 days.

The diagnostic workup of BPH includes a history, clinical examination, urine analysis, a repeated uroflow, and ultrasound scanning of kidneys, prostate, and bladder, including measurement of residual volume. Pressure-flow studies are necessary if the history and clinical examination are suggestive of a primary bladder disorder.

Patients with an enlarged prostate without symptoms do not require any treatment. For patients suffering from symptomatic BPH, different treatment options are available, ranging from watchful waiting and prescribing medications to a variety of different surgical interventions. Watchful waiting is recommended in patients with minimal symptoms and no significant impact on the quality of life. Periodic reevaluations are necessary to ensure that no BPH-related complications will occur. The risks are chronic renal failure owing to obstructive uropathy, urinary tract infection, and bladder stones because of residual volume and detrusor decompensation owing to the high bladder outlet resistance.

Different plant extracts (*Urtica dioica* [stinging nettle], *Sabal serrulatum,* semen curcurbitae, *Serenoa repens,* etc.) have been used to treat the irritative components of BPH with more or less effect (47–56). The idea that there may be some possible placebo effect associated with these treatments has been considered. "Uroselective" α-adrenergic blockers relax the bladder outlet and allow an improvement of the uroflow (57–63,63a). α-Blockers currently in use include tamsulosin, alfuzosin,

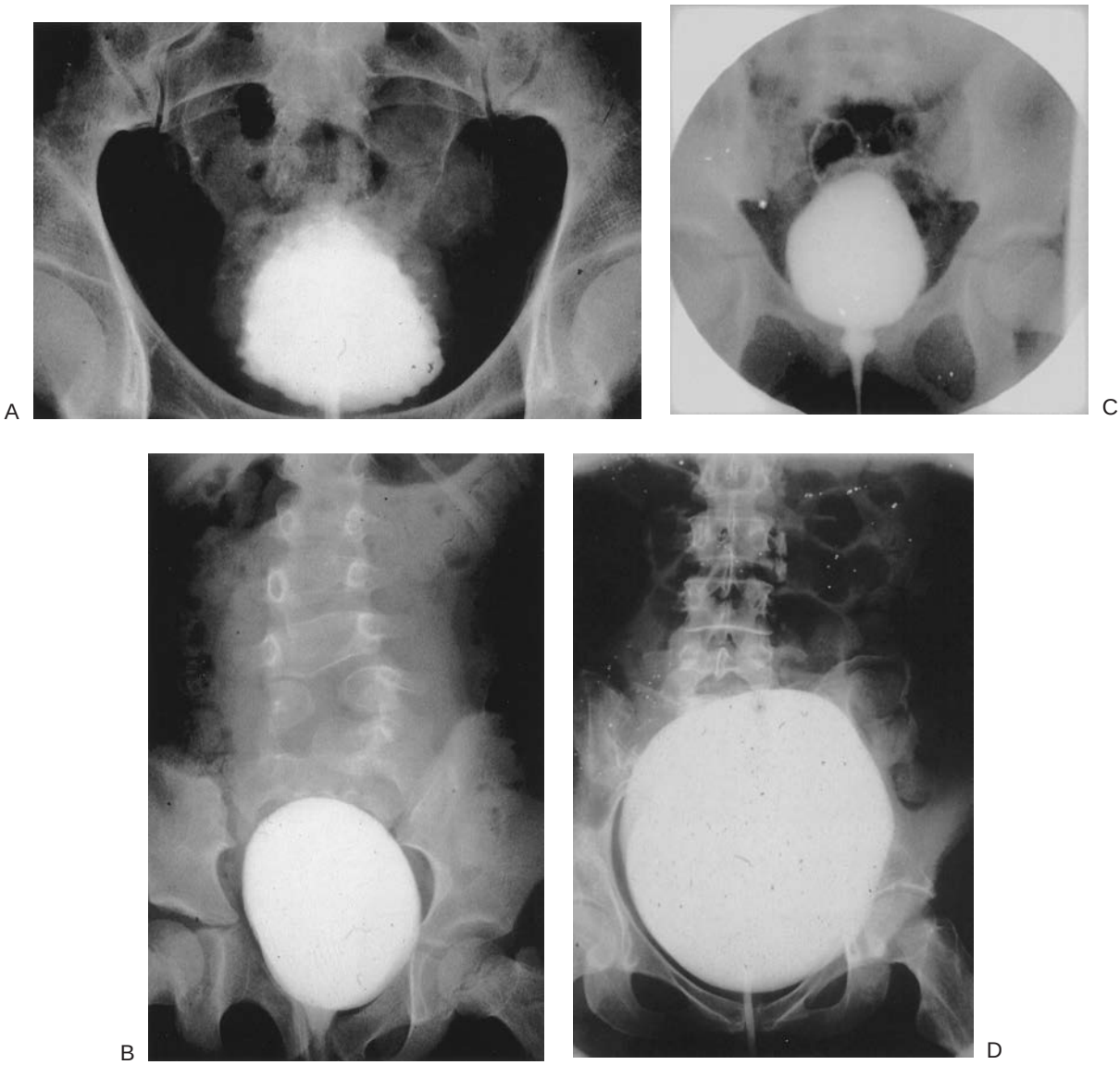


FIGURE 22-10. Videourodynamics. **A:** Spastic bladder in a spinal cord injury patient without adequate management, beginning "Christmas tree" shape, trabeculation, and many small diverticula. **B:** Detrusor–sphincter dyssynergia in myelodysplasia patient. Note the abnormalities of the spinal column. **C:** Detrusor–sphincter dyssynergia in a child with persistent enuresis due to pelvic floor dysbehavior. **D:** Bladder acontractility because of overdistention in a psychiatric patient. Note the small white dots in the lower abdomen. This was an accidental finding during urodynamics caused by mercury—the patient destroyed a thermometer and took in the mercury fluid.

doxazosin, prazosin, and terazosin. Terazosin, in particular, has had a significant impact on blood pressure decrease, with a number of patients suffering from tiredness, dizziness, and headache. 5α-Reductase inhibitors (64–70) (e.g., finasteride), prevent the conversion from testosterone to dihydrotestosterone, thus resulting in shrinkage of the prostate gland. It takes up to 6 months to obtain a maximal clinical response. Usually the subvesical obstruction remains unchanged. In the urology clinic, a conservative approach should be discussed with the patient if there is no absolute indication for surgical therapy (Table 22-4).

The transurethral resection (TUR) of the prostate is still the "gold standard" of surgical BPH therapy (71,72). This method offers the best chances of reducing symptoms and improving flow parameters. If the prostate is too large, open surgery is indicated (adenomas exceeding 60 to 80 g). In these cases, we

TABLE 22-4

ABSOLUTE INDICATIONS FOR SURGICAL THERAPY OF BENIGN PROSTATIC HYPERPLASIA

Recurrent urinary retention
Bladder stones
Impairment of renal function
Recurrent urinary tract infection
Recurrent gross hematuria

prefer the transvesical enucleation of the hyperplastic prostatic adenoma.

Alternative treatment modalities, such as transurethral incision (73), laser ablation (74,75), hyperthermia (heat energy used to destroy tissue at <45°C) (76,77), thermal therapy (heat energy at >45°C) (78), balloon dilation (79,80), focused ultrasound (81,82), and cryotherapy (83) have been discussed. None of these options have gained general acceptance however, and they are used only in a few centers.

Prostate Cancer

Locally advanced prostate cancer can cause severe obstruction. In the case of newly diagnosed advanced prostate cancer, immediate onset of hormone therapy (orchiectomy, LH–RH-analogs, estrogens) accompanied by temporary urine drainage via a suprapubic or transurethral catheter is indicated. Within 8 to 12 weeks the prostate gland will shrink and micturition should be possible again. If not, a palliative transurethral resection of the prostate may be performed (84,85).

Urethral Strictures

Injury of the urethral epithelium or the underlying corpus spongiosum with healing resulting in a scar can cause a urethral stricture. Today most strictures are of traumatic (i.e., straddle trauma), inflammatory (i.e., gonorrhea), or iatrogenic genesis (i.e., coarse catheterization or instrumentation). Patients with urethral strictures usually present with obstructive voiding symptoms and/or genitourinary tract infections (e.g., acute epididymitis, prostatitis). Only a few patients present with urinary retention. Uroflow is performed in order to evaluate the impairment of voiding. The length and location of the stricture can be determined using retrograde urethrography and urethroscopy. The depth and density of the scar in the corpus spongiosum can be deduced from physical examination and evidenced by ultrasound examination. Treatment modalities include urethral dilation, 12 o'clock internal urethrotomy, laser urethrotomy, and open reconstruction (86,87) (Fig. 22-11).

A special form of urethral stricture is the meatal stenosis, seen in hypospadias and after inflammation. A meatoplasty should be performed.

Posterior Urethral Valves

Posterior urethral valves are an inborn error and represent incomplete regression of urethral folds that are remnants of the mesonephric wolffian duct. This disorder is estimated to occur in one of every 5,000 to 8,000 male births (88). Obstruction of the urethra by valves affects the entire urinary system. Consequences are hydronephrosis with impairment of renal function, vesicoureteral reflux, and vesical dysfunction. Ultrasound and voiding cystourethrography confirm the diagnosis. Treatment involves surgical ablation of the valves. Most newborns with posterior urethral valves need temporary suprapubic drainage of urine to allow 4 to 6 weeks of growth in order to improve conditions for transurethral surgery. After surgical therapy all patients require close follow-up. A significant number of patients will continue to have voiding dysfunction because of secondary bladder wall changes (e.g., poor compliance—"valve bladder"). Prevention of renal dysfunction is the primary goal. In fact, 35% of patients will progress to chronic renal failure (89,90); therefore, bladder and renal function need to be checked on a regular basis. In case of persistent severe bladder dysfunction refractory to medication, augmentation cystoplasty may be necessary.

Phimosis

A high-grade phimosis may cause obstruction (91). A typical clinical sign of this is a ballooning of the foreskin. It is rarely seen, but may appear in newborns, infants, and patients with chronic balanoposthitis and severe diabetes mellitus. Treatment of choice is a reconstruction of the foreskin in infants and a circumcision in adults.

Bladder Neck Obstruction in Females

Subvesical obstruction in females is rare (92). In contrast to males, females more often present with irritative symptoms of urinary urgency, frequency, and urge incontinence. These symptoms arise from bladder instability because of obstruction or urinary tract infections. Complete obstructions are rare and may be caused by diverticula, urethral valves, Skene cysts, and benign or malignant tumors of the urethra. Partial obstruction may be due to a prolapse of pelvic organs. Iatrogenically induced bladder outlet obstruction after bladder suspension

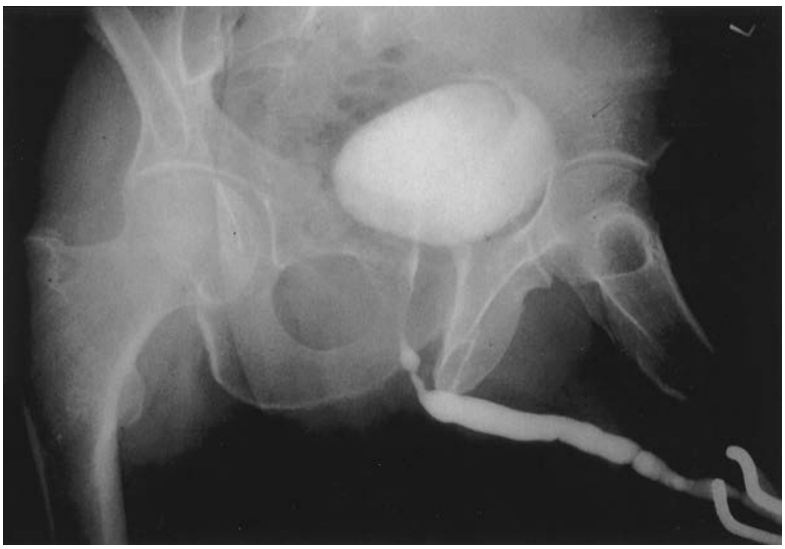


FIGURE 22-11. Retrograde urethrocystogram shows strictures in the spongious urethra and directly below the membranous area.

procedures for stress urinary incontinence is the most common cause (93–96). This problem is often only temporary and only a few patients require a permanent intermittent catheterization of the urinary bladder.

Hypertonic/Spastic Urinary Sphincter

Overactivity of the external urinary sphincter is an underestimated problem in functional urology. Chronic urinary retention, chronic pelvic pain, recurrent urinary tract infections, and bladder stones may result from permanent sphincter overactivity. The absence of an anatomical obstruction suggests a functional problem. A continuous recording of the urethral sphincter pressure during cystometry helps to diagnose this disorder. Usually sphincter pressures over 90 cm H_2O will be found in these disorders. During digital rectal examination, poor sphincter identity and a high-resting sphincter tone will be found. Over time, a decompensation of the detrusor muscle will be the consequence (Figs. 22-12 and 22-13).

In spinal cord injury a spastic urethral sphincter behavior will be found. Owing to missing central inhibition of sacral reflex activity, the striated pelvic floor muscle will be spastic with maximum sphincter pressures of more than 300 cm H_2O.

Detrusor–Sphincter Dyssynergia

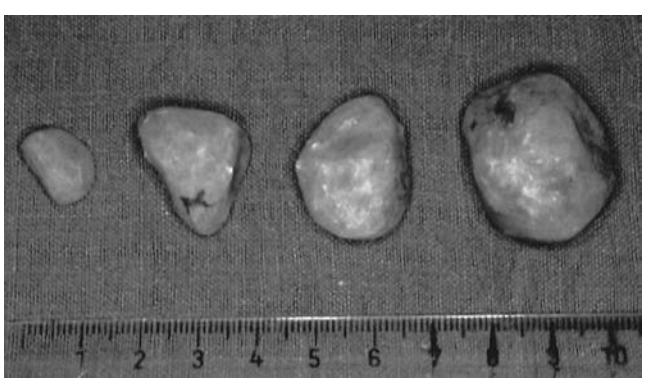
Dyssynergic behavior of the detrusor muscle and the urinary sphincter can occur in both women and men and may be secondary to neurologic and nonneurologic disease. There is only a severity difference to the "spastic urinary sphincter" (see above). In men, this syndrome tends to present at an earlier age than BPH and is manifested by long-standing obstructive and irritative symptoms. Uroflow, uroflow–EMG studies, and pressure-flow studies confirm an obstructive pattern. Residual volume can be evaluated by ultrasound. Endoscopy usually demonstrates bladder trabeculation and sometimes diverticula.

FIGURE 22-12. Megalocystis, owing to pelvic floor/urinary sphincter overactivity, chronic urinary retention as a consequence of detrusor decompensation, and intermittent catheterization are imperative in this female patient.

Videourodynamics is necessary in order to localize the level of obstruction (Fig. 22-14).

Women present with this syndrome generally between 30 and 60 years of age and suffer from incomplete emptying or absolute urinary retention. A detailed history of childhood and adolescence often reveal distinct psychologic problems, which may be attributed to the undesirable development of lower

A



B

FIGURE 22-13. One complication of inefficient bladder emptying is bladder stone formation. **A:** Four large bladder stones on a plain radiograph in a 32-year-old man. **B:** Stones after surgical intervention.

FIGURE 22-14. Ballooning of the proximal urethra during micturition due to detrusor–sphincter dyssynergia.

urinary tract function. Therapeutic attempts are often frustrating. Perisphincteric injection of botulinum toxin subtype A or sacral neuromodulation may be an option in these patients. However, children suffering from enuresis secondary to functional problems, such as detrusor–sphincter dyssynergia may benefit from acoustic or visual biofeedback treatment (97).

Urinary Incontinence

Urinary incontinence is defined as the involuntary loss of urine (25). There are four categories of urinary incontinence, which can occur in various combinations: (i) stress incontinence, (ii) urge incontinence, (iii) overflow incontinence, and (iv) neurogenic incontinence.

Stress incontinence is defined as leakage with an increase in intraabdominal pressure. The urethral sphincter fails to protect against loss of urine either because of intrinsic weakness or a failure to protectively contract. Overflow incontinence occurs when the bladder is unable to empty and filling occurs to the stretch limit of the bladder. Pressure inside the bladder builds until urethral resistance is exceeded and leakage takes place. Most forms of incontinence have components of two of the three elements. Pure stress incontinence is, in reality, relatively uncommon. Void diaries from patients with "stress" incontinence will most always evidence inappropriate voiding, not explainable as simply prophylactic voiding. Urge incontinence is a voiding dysfunction associated with an involuntary loss of urine. There is an inability to inhibit the bladder long enough to reach a toilet. Detrusor instability or overactivity is a common finding in patients with urge incontinence. Discovering the underlying cause of urge incontinence or detrusor instability, apart from obvious neuropathology, is difficult given the complexity of the neural regulation system. Given that prophylaxis is, ultimately, the most cost-effective therapy, understanding the possible causes of incontinence should be a paramount issue in management. Overflow incontinence is a complication of severe bladder neck obstruction (e.g., BPH). A key symptom is a continuous dribbling of urine—every milliliter of urine produced by the kidneys and transferred to the bladder will "overflow."

Neurogenic incontinence is associated with detrusor hyperreflexia, because of a loss of central inhibition of bladder activity in patients with neurologic disease (e.g., spinal cord injury, multiple sclerosis, or Parkinson's disease). Detrusor–sphincter dyssynergia (as discussed previously) can cause incomplete bladder emptying.

Chronic Pelvic Pain

From a clinical perspective, nonmalignant chronic pelvic pain conditions could be caused by bacterial (e.g., chronic bacterial prostatitis) (98,99) and neurogenic inflammation (e.g., prostatodynia) (100–103), nerve injury (e.g., after surgery), (104–107), and chronic organ dysfunction (e.g., tension floor myalgia and perineal pain) (103,108,109). However, in a given case, it is not easy to determine the initiating mechanism generating a chronic pain situation. Patients complain about pain deep in the pelvis or in the perineum region. Furthermore, they suffer most often from symptoms of lower urinary tract dysfunction: frequency, urgency, and weak urinary stream. Micturition, in particular, is reported to be very painful.

In recent years, the daily practice in the diagnosis and treatment of chronic pelvic pain has shown that success rates in diagnostics and treatment are mainly unsatisfactory. Scientifically based multicenter studies on different diagnostic and treatment approaches are still unavailable. In the literature, different names are used for the same clinical features. For example, male chronic pelvic pain syndrome (mCPPS) has been referred to as chronic abacterial prostatitis (110,111), prostatodynia (101), prostatosis (108,112), interstitial cystitis (113), orchalgia (114), penile pain (102), trigonitis (115), chronic urethritis (116), urethral syndrome (117), rectal pain (118–120), tension floor myalgia (108,109), perineal pain (121,122), and so on. Etiology and pathophysiology of chronic pelvic pain are mainly hypothetical or unknown. The diagnostic approach and treatment strategies are often empirical and polypragmatic. Therefore, chronic pelvic pain provides a challenge for further basic and clinical research.

Bladder Dysfunction Resulting from Metabolic Disorders

Diabetes mellitus leads to autonomic neuropathy causing cardiovascular, bladder, sexual, and gastrointestinal dysfunction. Involvement of the lower urinary tract by peripheral diabetic autonomic neuropathy is referred as "diabetic bladder" or "diabetic cystopathy" and is thought to occur in 27% to 85% of patients (123). The real incidence of bladder dysfunction in diabetic patients is unknown, because many patients do not complain of voiding symptoms. Insidious onset of reduced bladder sensation is often an early symptom preceding a gradual increase in voiding intervals. Other symptoms include urge, urge incontinence, recurrent urinary tract infection, weak force of stream prompting abdominal straining to void, and incomplete bladder emptying (124). The urodynamic evaluation will typically reveal an impaired bladder sensation, increased bladder capacity, impaired detrusor contractility, a decreased urine flow, and postvoid residual urine. Bladder instability, accounting for symptoms of urgency and urge incontinence, can arise in as many patients as 25% of patients with advanced diabetes (125).

Bladder Dysfunction Resulting from Specific Inflammation

The incidence of tuberculosis continues to rise. Genitourinary tuberculosis is diagnosed after several occasions of vague urinary symptoms: sterile pyuria, hematuria, hematospermia, recurrent infections, and fistulae. Bladder tuberculosis secondary to renal tuberculosis leads to irritative symptoms, such as frequency and nocturia. The tuberculosis-related inflammation

causes a decreased bladder compliance and bladder wall fibrosis (126,127).

Bilharziasis is caused by an infection with *Schistosoma haematobia*. A calcified bladder on a plain x-ray film is pathognomonic of chronic bladder schistosomiasis. Symptoms are urgency, frequency, weak urine stream, and bladder pain. The disease is most commonly seen in Africa and Arabia, but rarely in Central and South America or Southeast Asia. Obstruction of the lower urinary tract, bladder fibrosis with bladder shrinkage, and bladder cancer are important late complications of bladder bilharziasis (128–130).

Bladder Dysfunction and Acquired Immunodeficiency Syndrome

The acquired immunodeficiency syndrome (AIDS) involves nearly every organ system, including the genitourinary tract. Patients with AIDS are predisposed to recurrent urinary tract infections with both common and uncommon pathogens. Of all cases of urinary retention in patients with AIDS, 90% are of neurogenic origin. Urodynamic studies demonstrate detrusor hyporeflexia or areflexia in 83% and detrusor sphincter dyssynergia in the remaining 7%. The acute and subacute myelopathies related to AIDS can involve the spinal cord, and the resulting type of bladder dysfunction would depend on the level of the spinal cord lesion. Urinary incontinence is usually observed in late stages of the disease, because of dementia related to AIDS–encephalitis (131–133).

Bladder Dysfunction in Neurologic Diseases

Stroke

Brain damage caused by a cerebrovascular accident (CVA) has a great potential for producing a wide variety of types of bladder dysfunction, depending on the distribution and the extent of the cerebral damage and the time since the insult. Immediately after a CVA, urinary retention is common. Later on, urinary incontinence affects the majority (57% to 83%) of early stage CVA patients. Incontinence can be transient, with 41% of patients returning to continence within the first 2 weeks following the onset of the stroke. However, incontinence can be a continuing problem in 15% of patients 1 year after the stroke. It seems that the severity of urinary incontinence and bladder dysfunction is related to the severity of the brain damage. Factors associated with incontinence are the degree of motor deficit, impaired mobility, and mental impairment. Continuing incontinence in CVA patients is associated with poor outcome (134–137).

Parkinson's Disease

The management of lower urinary tract dysfunction in Parkinson's disease is complicated by the physical features of rigidity, hypokinesia, bradykinesia, and gait disturbances that make the toileting process difficult. Urinary symptoms are common in patients with Parkinson's disease. Some 37% to 71% of patients complain of frequency, urgency, and/or incontinence. Urodynamic studies have shown that detrusor instability and sphincter disturbances are commonly found in Parkinson's disease.

Prostate problems are common in older adult men with Parkinson's disease. The risk for urinary incontinence increases in these patients after transurethral resection of the prostate due to an abnormal sphincter control (138–140).

Multiple Sclerosis

Multiple sclerosis (MS) can produce urinary symptoms from a very wide range of neurologic sites. The presence of symptoms correlates well with the severity of pyramidal and sensory lesions and the total disability score. The pattern of urodynamic findings depends on the site of the lesion and, therefore, it should not be surprising that there are a number of different types. In a study of 86 patients with MS, symptomatic lower urinary tract dysfunction was found in 97% (141). Hyperreflexic bladder has been found in 67% to 83% of MS patients (142,143) compared with hyporeflexic or areflexic bladders in 16% to 20% of patients and normal bladders in 1% to 12% of patients. Somatic dyssynergia was found in about 50% of the patients in most studies (Fig. 22-15) (142–148).

Spinal Cord Injury

Impaired bladder, bowel, and sexual function are three of the most serious complications of spinal cord injury. Renal failure has been described as "the main killer of sufferers from spinal cord injury or disease" (149). Although the principles of management, introduced by Guttmann, have now reduced the number of deaths from this cause, complications in these systems still account for much morbidity and social restriction. Depending on the level of the spinal cord lesions, there are different clinical features. Lesions of the conus medullaris may destroy the cell bodies of the sacral parasympathetic preganglionic neurons and interneurons in the lateral horn and the cells within the anterior horn supplying the pelvic floor and external sphincter. Spinal injury at this level or at the level of the cauda equina may damage the nerve roots intradurally or extradurally. The consequence is an areflexic bladder and sphincter. Although patients with such a lesion may be able to void via abdominal straining, incontinence is likely. Lesions of the spinal cord above the sacral segments may leave the sacral reflex arc intact, but interrupt ascending and descending connections to the brainstem and higher centers within the diencephalon and cortex. These central centers play an important role in modulation, facilitation, and inhibition of the reflex activity within the sacral arc. Bladder storage and micturition are affected. Such patients may have hyperreflexic contractions of the detrusor muscle in response to stretch the bladder wall or other external stimuli resulting in neurogenic incontinence. Hypertonicity of the detrusor may also reduce its capacity and compliance, producing raised pressure during the storage phase and micturition. This pressure hinders ureteric drainage and may produce ureteric reflux, and lead to hydronephrosis, pyelonephritis, and, over time, to renal failure. The external urinary sphincter is likely to be capable of reflex contraction and may be spastic. Often the sphincter fails to relax during bladder contraction. This dyssynergia between detrusor and sphincter contributes to high pressures and incomplete voiding, with resultant recurrent urinary tract infection, stone formation, bladder trabeculation, formation of diverticula, and changes of the gross morphology of the bladder (e.g., "tower bladder," "Christmas tree bladder") (150–157) (Figs. 22-16 and 22-17).

Myelodysplasia/Tethered Cord

Myelodysplasia is the most common cause of neurogenic bladder dysfunction in children. Because of advances in medicine, care of these children has become a managerial disease. The term myelodysplasia refers to a spectrum of congenital anomalies involving primarily the vertebral column of the lumbosacral segments of the cord and their spinal roots. Urodynamic testing identifies children and adults at risk for upper urinary tract deterioration. Detrusor hyperreflexia occurs in 54% of myelodysplastic patients, with the remainder having abnormal bladder compliance. Over one-half of these patients have associated

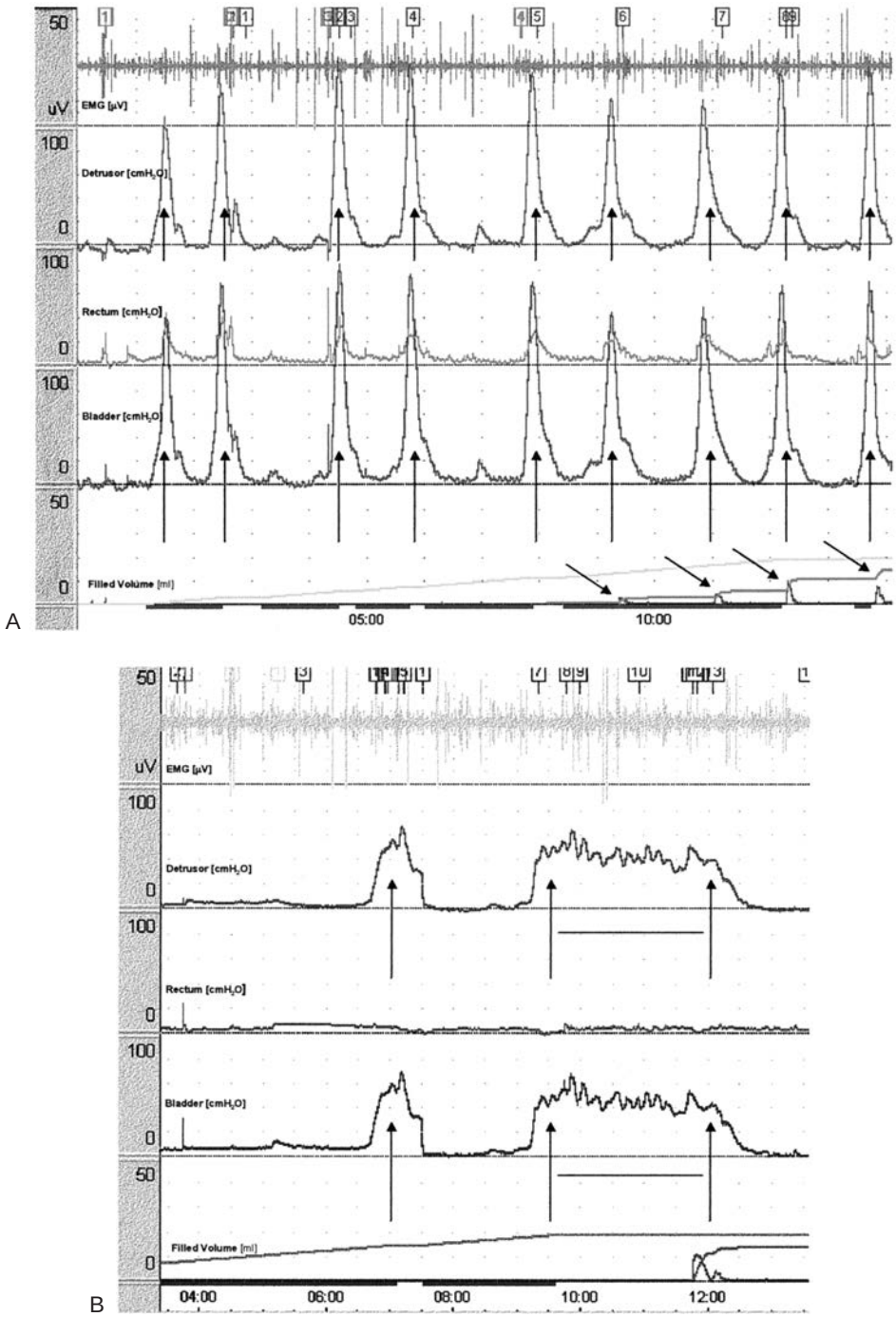


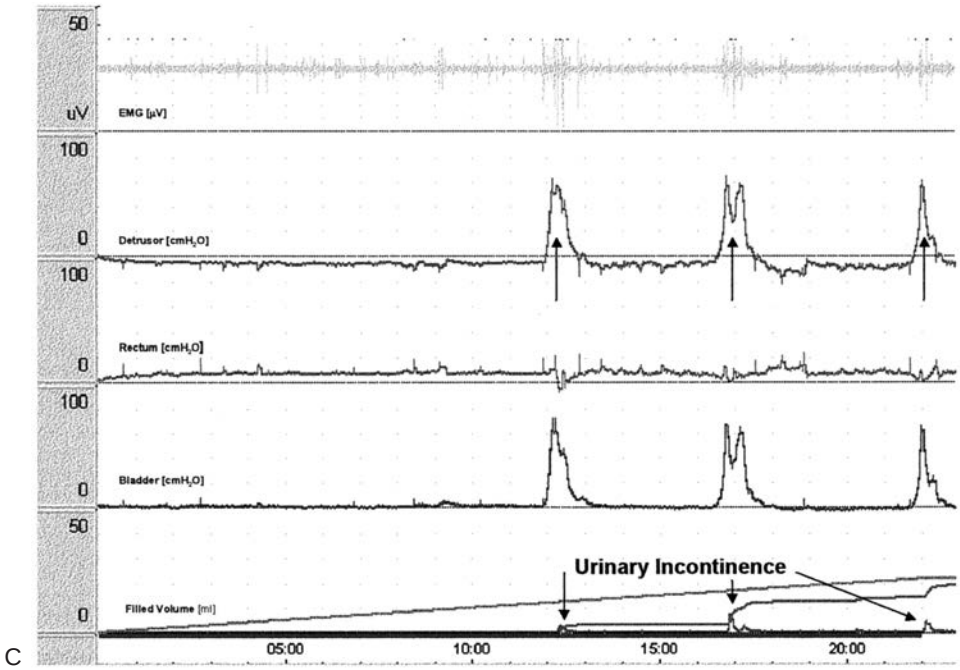
FIGURE 22-15. Different urodynamic features of detrusor instability/detrusor hyperreflexia in multiple sclerosis patients. **A:** Continuous uninhibited detrusor contractions during bladder filling; incontinence is starting by a bladder volume of approximately 200 mL. **B:** Detrusor instability, near the end of bladder, filling with a maximum bladder volume of approximately 250 mL; normal micturition is still possible. (*Continued*)

detrusor–sphincter dyssynergia. This uncoordinated activity of the external sphincter, especially in the presence of high bladder filling pressures, is an indicator for the risk of bladder decompensation and upper urinary tract deterioration.

The tethered cord syndrome is a form of spinal dysraphism arising as a primary disorder during development or as a secondary complication from adhesions after neurosurgical correction of myelodysplasia. Abnormal stretching of the conus medullaris will be the consequence of traction by the filum terminale. This prevents the ascent of the conus to its normal level. Neurologic symptoms develop when the child is in the upright position and during growth spurts. A variety of symptoms and clinical signs occur, including gait disturbances, skin lesions of the lower back, pain or numbness in the lower extremities, and bladder and/or bowel dysfunction. There can be a wide spectrum of bladder and external sphincter dysfunction. Early neurosurgical intervention is warranted before significant denervation develops (158–162) (Figs. 22-18 and 22-19).

Bladder Dysfunction and End-Stage Renal Disease/Renal Transplantation

Abnormal lower urinary tract dysfunction is no longer a contraindication to renal transplantation. However, successful renal transplantation requires diagnosis and treatment of correctable abnormalities of the lower urinary tract. Ideally, it is preferred to transplant the renal graft into the native bladder, whenever possible. If impossible, urinary diversion is necessary.



C

FIGURE 22-15. (*Continued*) **C:** Detrusor instability with urinary incontinence; no normal micturition owing to hyperreflexia and detrusor sphincter dyssynergia.

Urodynamic testing is also indicated in patients with long-term anuria prior to transplantation. Over time changes in the morphology and function of the lower urinary tract may occur and can cause severe problems after transplantation. Normally these "disuse" bladders can respond to repetitive hydrodistention and regain normal capacity and compliance. However, close follow-up is necessary to recognize problems of lower urinary tract function, urine storage, and micturition after transplantation. Diabetic patients with acontractile bladders and high residual volumes have been successfully treated with intermittent self-catheterization. Noncompliant, fibrotic bladders may require bladder augmentation or urinary diversion (Fig. 22-20) (163–166).

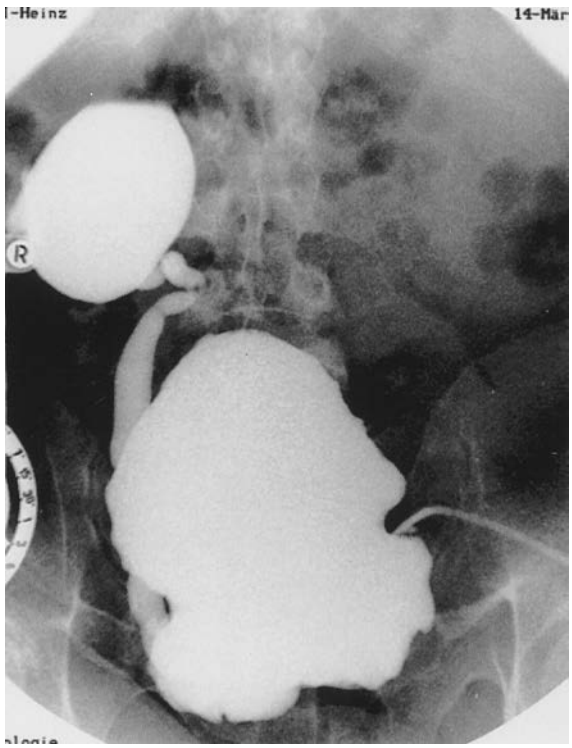


FIGURE 22-16. Detrusor hyperreflexia and high-grade vesicoureteral reflux with hydronephroses on the right side. A nephrectomy was necessary for recurrent urinary tract infection; the split function was 0%.

TREATMENT OF LOWER URINARY TRACT DYSFUNCTION

Credé/Valsalva Maneuver

In patients with low bladder outlet resistance and concomitant impaired detrusor contractility external compression of the bladder (Credé maneuver) or abdominal straining (Valsalva maneuver) may result in bladder emptying. However, because of the possible generation of high intravesical pressures, it is dangerous for the morphologic integrity of the urinary tract and the renal function if this procedure is recommended and done without close urodynamic control and follow-up. It is a "last resort" treatment option and other treatment options (e.g., intermittent catheterization) should be discussed in these patients (Fig. 22-21).

Biofeedback

The essential goal of biofeedback is to reestablish the proper dynamic behavior to the pelvic musculature. Often patients simply have no concept of how to relax these muscles. They may not even be able to identify these muscles or to exert any voluntary influence on their behavior. The goal of biofeedback is to restore appropriate concepts of pelvic muscle relaxation. This can be as simple as a suggestion to take one's time with voiding and never to push, strain, or rush the event.

It is important for patients with an overactive urinary sphincter to be made aware of adverse voiding habits or of uncontrolled anxiety states. Patients need to be consciously aware of the goals of biofeedback and to be actively involved

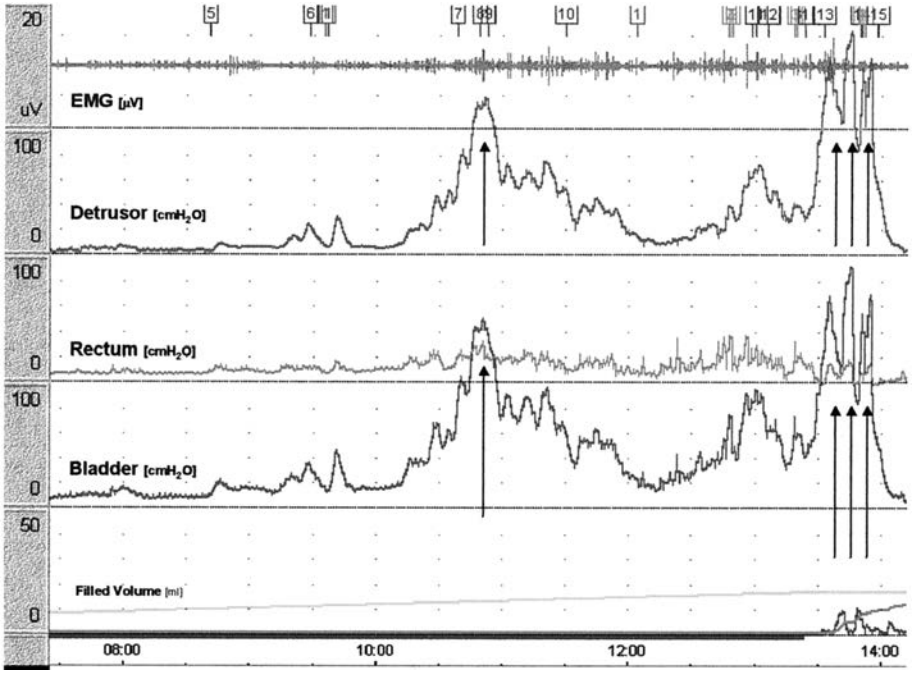


FIGURE 22-17. Detrusor hyperreflexia with maximum intravesical pressures during normal bladder filling of up to 135 cm H_2O.

on a daily basis with retraining of the void technique if long-term success is to be achieved. If awkward voiding methods continue, biofeedback approaches will ultimately fail to affect symptoms. If necessary, the patient should be counseled on how to deal with stress and lifestyle patterns that contribute to anxiety, as emotions can impact sphincter tension via the brainstem.

At times, patients will need more specific instruction. Biofeedback equipment and software programs are one option. A simple rectal/vaginal examination is less expensive and can be very useful in helping patients to learn to control their pelvic muscles. Patients are asked to tighten and relax on a rectal/vaginal balloon, or the gloved finger, and the physician can assess the efficiency and specificity of these movements.

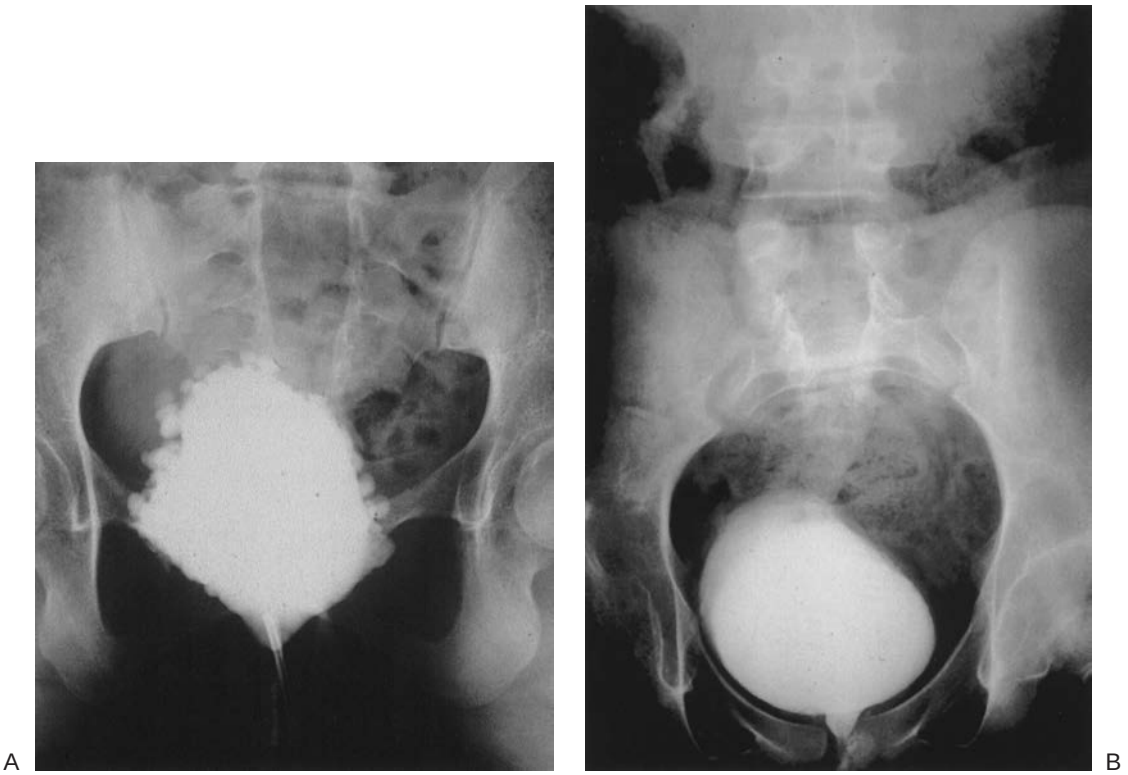


FIGURE 22-18. A: Bladder changes in myelodysplasia owing to detrusor hyperreflexia. **B:** Dilation of the proximal urethra due to detrusor–sphincter dyssynergia in myelodysplasia. Note bone abnormalities in both cases.

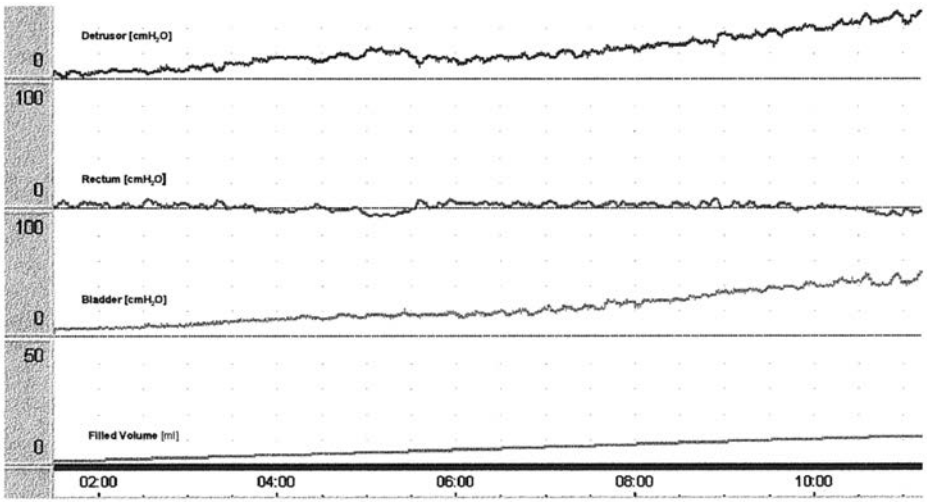


FIGURE 22-19. Low compliance bladder owing to bladder wall fibrosis. Note continuously increasing bladder pressure during bladder filling.

Patients should practice this dynamic, emphasizing relaxation, at regular intervals daily. Progress can be monitored with void diaries, repeat rectal examinations, or flow rates. If treatment is successful, there should be a precipitous decline in symptoms, (e.g., intermittency, slowness of the urinary stream, and pain). Biofeedback approaches should be combined with medications as well as other treatments for maximum results (Table 22-5) (167– 173).

Pharmacologic Treatment

α-Blockers

It is known that the urethral sphincteric overactivity, spasticity or irritability, and BPH can result in poor bladder emptying. α-Blockers, via their peripheral and central actions, can help to lower bladder outlet resistance in neurourologic disorders and BPH (see previous discussion under "Benign Prostatic Hyperplasia"). Phenoxybenzamine, a nonselective α-blocker, was shown to be highly effective in the management of high bladder outlet resistance. The disadvantages were the high incidence and severity of adverse clinical events. Two subtypes of α-adrenoceptors are known: α_1- and α_2-adrenoceptors. Prazosin was one of the first α_1-adrenoceptor antagonists. Prazosin and other α-blockers require at least twice-daily dosing because of their relatively short serum elimination half-lives. Further research led to the development of drugs with serum elimination half-lives that allowed for once-daily dosing: terazosin and doxazosin. Molecular studies have now identified three subtypes of α_1-adrenoceptors. Tamsulosin, used in BPH and neurourologic patients, exhibits some degree of selectivity for the α_{1a}-adrenoceptor. Prostate smooth muscle tension is mediated by the α_{1a}-adrenoceptor (Table 22-6) (57–63,63a).

Striated Muscle Relaxants

Baclofen and dantamacrin are useful in the treatment of high bladder outlet resistance due to spastic striated pelvic floor and urinary sphincter in spinal cord injury patients with bladder hyperreflexia. Because of severe side effects, intrathecal administration of baclofen has been used in an attempt to increase efficacy and reduce side effects of systemic administration (174–176).

Cholinergics

Carbachol, distigmine bromide, and bethanechol chloride are drugs used for acute bladder- and bowel-emptying problems. However, in chronic disorders of micturition, a clinical success is also possible. Before invasive therapies are scheduled, an attempt should be undertaken in patients with bladder-emptying problems and without bladder neck obstruction. Sometimes an improvement of symptoms can be achieved, especially in patients with psychosomatic problems (177).

Anticholinergics

Anticholinergic drugs block the parasympathetic neurotransmitter acetylcholine at postganglionic neuromuscular junction muscarinergic receptors. Anticholinergics are mainly used in the treatment of bladder-storage disorders (e.g., urge incontinence and hyperreflexive bladder dysfunction). Anticholinergics increase bladder capacity, improve bladder compliance, and decrease the amplitude of or avoid involuntary detrusor contractions. One important side effect of anticholinergics and other detrusor relaxants (e.g., flavoxate) is the impairment of bladder emptying because of the "reversible paralyses" of the detrusor. These drugs are contraindicated in patients with BPH, narrow-angle glaucoma, and bowel obstruction (Table 22-7) (178–182).

Tricyclic Antidepressants

Tricyclic antidepressants (e.g., amitriptyline, imipramine) have been used in bladder-storage disorders with moderate success. The weak antimuscarinergic effect in decreasing bladder contractility and the mild α-adrenergic agonist action that increases bladder outlet resistance explain the efficacy of these drugs in stress and urge incontinence (183). Duloxetine, a new serotonin and noradrenalin reuptake inhibitor, has proved to be efficacious in stress-incontinent women (183a,b,c).

Acupuncture

Acupuncture needles placed above the medial malleolus in the inside of the leg, similar to surface pelvic floor and direct S3 nerve root stimulation, have been proposed to have a suppressive effect on urge-based urinary incontinence and may also be promising in the treatment of other lower urinary tract dysfunctions. The mechanism is probably direct activation of larger muscle afferents, which effectively modulate synaptic transmission in the spinal cord. The technique is simple to administer and complements biofeedback and drug therapy. For reasons that are unclear, the technique is only effective if the needles are placed through the skin at a point of lowest skin impedance,

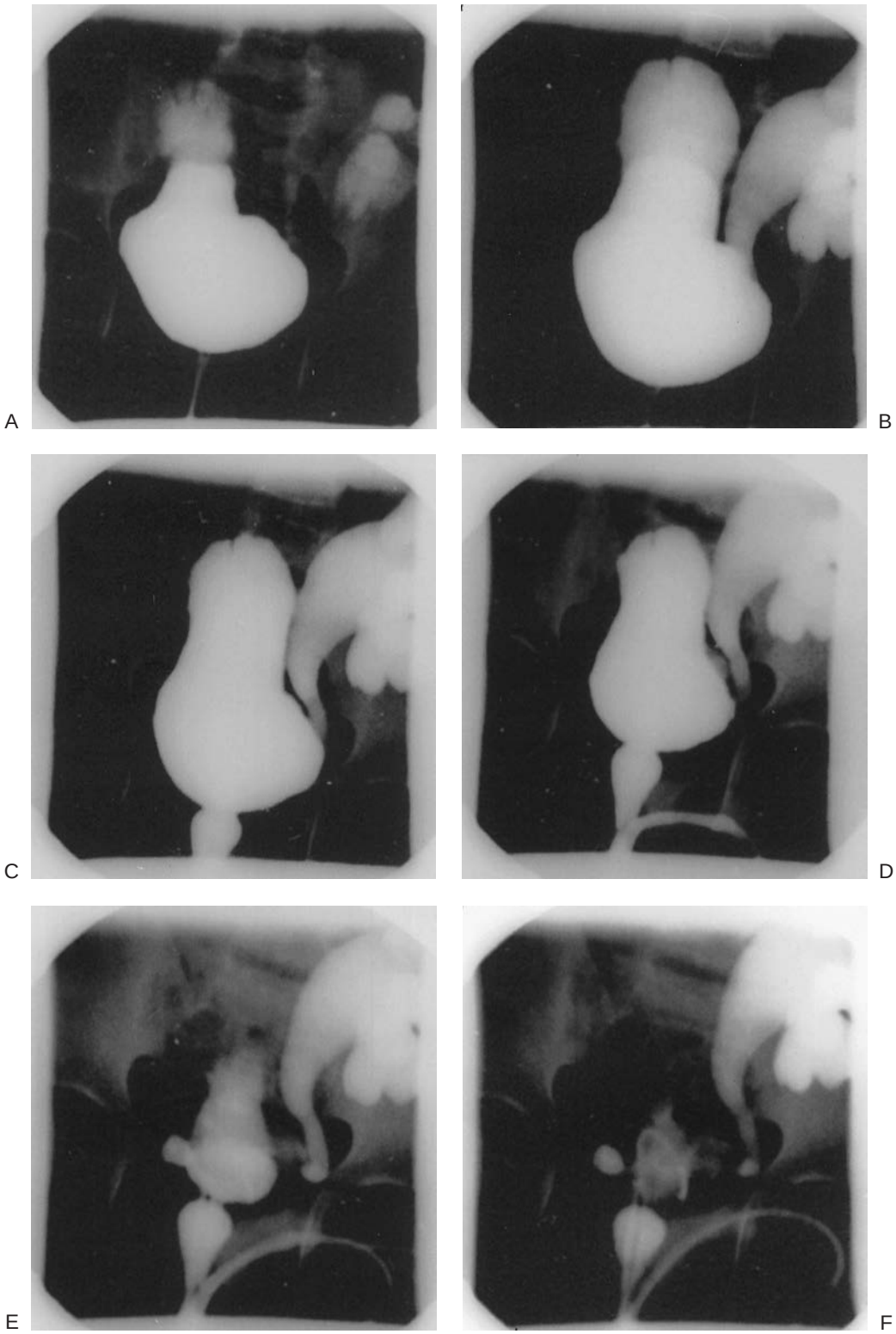


FIGURE 22-20. Videourodynamics after renal transplantation in a 7-year-old boy. **A:** Initial bladder filling shows a "self-augmented" bladder secondary to high pressures, beginning urine reflux into the renal graft. **B:** Bladder and collecting system of the renal graft are filled with contrast medium (reflux 4°). **C, D:** Micturition shows a ballooning of the proximal urethra and detrusor sphincter dyssynergia at the internal and external sphincter level. **E, F:** The bladder is nearly empty, but the collecting system remains filled with urine. These findings are because of inadequate diagnostics before renal transplantation.

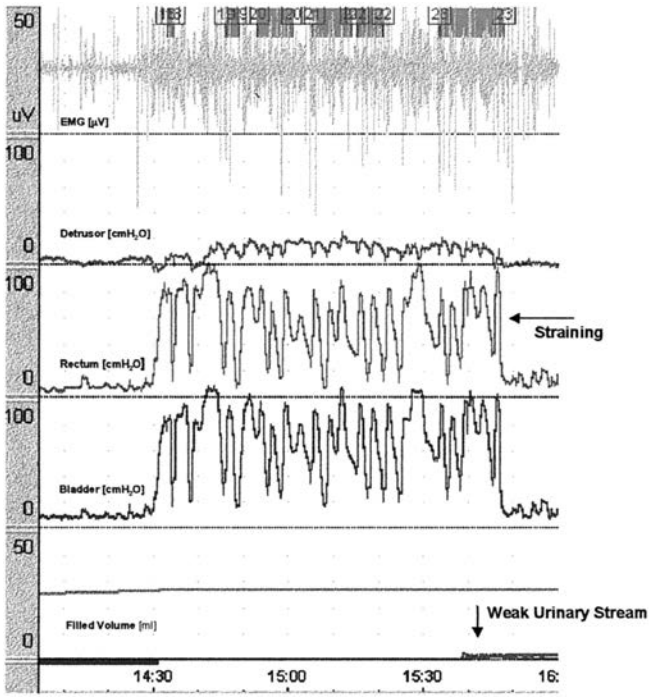


FIGURE 22-21. Straining does not allow proper micturition in this patient, high pressure can damage the upper urinary tract.

4 cm above the medial malleolus, and just behind the tibia (posttibial nerve) (184–186).

Intermittent Self-Catheterization/ Long-Term Catheters

Occasionally catheter management is the only option. It is a pure symptomatic treatment option. The method of intermittent self-catheterization is generally preferred over insertion of a long-term catheter. Learning to carry out self-catheterization allows patients to empty the bladder alone and with low risk of side effects. This procedure must be done on a strictly adhered to time schedule because of missing bladder sensitivity in most patients who are in chronic urinary retention. Patients with a hypertonic/spastic urethra or severe detrusor–sphincter dyssynergia must be aware of possible problems passing the sphincter with the catheter. Skillful catheterization will prevent hematuria, sphincter lesions, and via falsa.

Insertion of a suprapubic or transurethral long-term catheter is a last resort in management of voiding disorders. Generally, these patients have severe motor disabilities related to CNS pathology. Long-term catheters are associated with an increased risk of problems. These include recurring infections, sepsis, stones, pain, and cancer. Catheter-related complications are greatly increased by hyperactive, hypertensive, or hypertonic bladders. Patients can do very well with catheters if their bladder is kept in a low-pressure, low-activity state, they are well hydrated, and exercise good perineal hygiene with regular catheter changes. New catheter designs are in development that will both facilitate emptying and allow a patient more mobility and, hence, a better quality of life (187–191).

TABLE 22-5

GENERAL RECOMMENDATIONS FOR CHANGING WRONG VOIDING BEHAVIOR

Take time for micturition, do not rush
Sit for voiding every time (also men)
Double voiding at home (after 5–10 minutes)
Do not strain
Practice timed voiding

TABLE 22-6

α-ADRENERGIC BLOCKERS USED IN UROLOGY TO LOWER BLADDER OUTLET RESISTANCE

Class	Drug name
Nonselective α_1	Phenoxybenzamine Prazosin Alfuzosin Indoramin
Long-acting α_1	Doxazosin Terazosin
Selective α_{1a}	Tamsulosin

Capsaicin/Resiniferatoxin

Small unmyelinated C-fibers and the slightly larger A-delta fibers are felt to be involved in unstable detrusor activity. These same C-fibers are felt to reflexively drive urethral dyssynergic behavior. High doses of capsaicin cause depletion of the sensitive nerve cell supply of substance P and neurokinin A in these fibers.

The application of 1 to 2 mmol/L of capsaicin in 30% ethanol in saline was found to be effective in increasing bladder capacity and decreasing detrusor hyperreflexia. Capsaicin is, therefore, a useful approach for patients unable to tolerate the side effects of anticholinergic medications or are simply refractory to these medications. The downside of capsaicin is that it must be given under general anesthesia. There is a 10- to 14-day period of induced inflammation during which time incontinence can be aggravated. Finally, capsaicin will not improve bladder compliance.

Because of severe side effects (e.g., pain, burning, and autonomic sensations) capsaicin is problematic in nonneurogenic patients. A new substance, resiniferatoxin, promises nearly equal efficacy with fewer side effects. Randomized multicenter studies are now beginning. Experiences in our own patients with dosages between 10^{-6} and 10^{-8} M diluted in 50 mL of sterile NaCl solution have been positive (192–200).

TABLE 22-7

DETRUSOR RELAXANTS

Tolterodin
Solifenacin
Propantheline
Hyoscyamine
Oxybutynin
Propiverin
Flavoxate

Transcutaneous Nerve Stimulation Therapy

The principle of transcutaneous nerve stimulation (TENS) has been established as a valuable tool in the treatment of a variety of urinary incontinence and pain problems over the last 15 years. The technique is simple and virtually risk-free. Electrical stimulation is applied to critical areas within the sacral dermatomes. Sites reported to be associated with beneficial results have included the sacral, suprapubic, common perineal, and posterior tibial nerves. Large skin afferents, when stimulated, suppress spontaneous reflex activity within the same dermatome. Vaginal and anal plugs have also been repeatedly reported to be useful.

While it is encouraging to know that relatively simple approaches can be effective, there are drawbacks. As a general rule, even when it is effective, the effects of neurostimulation do not last and patients become dependent on frequent, ongoing treatment. In addition, the results of superficial stimulation are rather unpredictable from patient to patient. Vaginal and anal plug devices, or both, have not proved popular with patients in Europe and North America if more than a few sessions of stimulation are necessary. The technique is, however, simple to apply, and is one that may be considered in a patient before resorting to destructive surgical procedures (201–206).

Botulinus Toxin Type A

Botulinus toxin type A (BTX) is a potent neurotoxin with a high affinity toward acetylcholine (ACh) receptors. It is produced by the Gram-negative, rod-shaped, anaerobic bacterium *Clostridium botulinum*. The neurotoxin, synthesized in seven different serotypes, acts on peripheral cholinergic nerve endings to inhibit ACh release (207). Although the neurotoxin is most potent at somatic neuromuscular junctions, it does have the ability to inhibit autonomic cholinergic transmission (208). BTX is, therefore, capable of inhibiting transmitter release from pre- and postganglionic cholinergic nerve endings of the autonomic nervous system, as well as interfering with smooth muscle visceral function. Injected BTX will be quickly attached to somatic ACh receptors when injected into the target muscle. There is very little risk that the BTX will adversely affect neighboring striated or visceral smooth muscle or even ganglionic transmission. BTX does not penetrate the blood–brain barrier in doses used therapeutically. There is, therefore, no significant risk that transmitter function in the CNS will be affected. BTX, in doses used clinically, is a highly selective and safe neurotoxin.

The treatment of striated muscle with BTX results in an accelerated loss of junctional ACh receptors (209). Paralysis and a nearly complete decline of miniature end-plate potentials occur within a few hours after injection of BTX (210,211). The muscle becomes functionally denervated, atrophies, and develops extrajunctional ACh receptors (212). The clinical effect is to weaken the injected muscle.

In the early 1980s, BTX was successfully introduced for the treatment of strabismus (213), and its use subsequently expanded to other conditions manifested by involuntary muscle spasm, spasticity, and tremors (214).

BTX-selective urethral sphincter injection can reduce the hypertonic and hyperreflexic sphincter activity, with the potential to provide pain relief and improvement of LUTS.

Many patients suffering from chronic urinary retention and chronic pelvic pain show pelvic floor muscle tenderness, myofascial trigger points, and/or sphincter hypersensitivity, hypertonicity, and hyperactivity (215,216). The clinical examination typically reveals restricted pelvic floor dynamic control (i.e., contraction and relaxation) (see "Diagnostics of Micturition Disorders and Lower Urinary Tract Dysfunction," earlier in this chapter).

Whether pelvic muscular dysfunction is a root cause of pain, or an integral part of the CNS failure that underlies pain, addressing the muscular dysfunction directly makes sense. It is a logical step toward reducing the neurogenic cascade of events that can feed inflammation and hypersensitivity in tissue. It has been shown that BTX can not only weaken injected muscle, but also affect neighboring muscle within the same dermatome (217). This may occur via local diffusion of the BTX, or by its impact on the spinal reflex regulation of muscle within the dermatome. The latter, indirect, effect of BTX on spinal cord circuits could occur via BTX action at the intrafusal (218) and/or extrafusal neuromuscular junction (219,220). An observed change in muscle activity pattern after BTX injection suggested that a central reorganization is induced by the therapy. This position is supported by results from a positron emission tomography (PET) study, which showed an increased activation within the parietal cortex and the caudal supplementary motor area after BTX therapy for writer's cramp. The changes that occurred reflected changes in muscle movement strategy and in the associated CNS activation patterns that controlled these muscle activities. These changes could also be explained by a cortical reorganization secondary to loss of motor neurons and decreased sensory afferent input (221). Over the last years, many studies have been performed in spinal cord injury, multiple sclerosis and myelomeningocele patients (221a,b). These studies support the high value of this treatment modality in patients with detrusor hyperreflexia and the need for intermittent self-catheterization.

Sacral Nerve Stimulation—Neuromodulation

Stimulation of the sacral nerves will evoke striated and smooth muscle contractions in the pelvis within a pain-free window of applied current. Painful amplitudes of current applied to either the S3 or the S4 nerve will produce a contraction of the bladder at thresholds four to five times greater than that for the striated pelvic floor muscle. There is, therefore, a tolerable pain-free window for stimulation of the pelvic striated muscle.

The S3 nerves supply some branches to the pudendal nerves. However, the significance of this contribution can vary widely among patients because of pre- and postfixation of the spinal nuclei. The S3 nerves predictably will supply the more anterior levator muscle systems via pelvic branches that leave the nerve trunk before they contribute to the sciatic and pudendal nerves (i.e., the ischial spine). Direct stimulation of S3 will produce a visible contraction of the levator muscles ("bellowslike" movement) and contraction of the large toe or the distal foot (i.e., all the toes). Referred sensation to the rectum and scrotum is common. Contraction of the large toe, and less often, the forefoot, may be evident along with referred sensation to the scrotum.

The S4 nerves do not contribute to the pudendal innervation of the pelvis or to the sciatic nerves. Direct stimulation of this nerve will produce a contraction of the posterior portion of the levator. This is a referred sensation to the rectum or to the tip of the coccyx, or both.

The bladder neck is pulled open by the contracting detrusor body. The longitudinal muscle of the bladder is known to extend down and around the bladder neck, forming the detrusor loops. When the bladder contracts, the loops are pulled back into the body of the bladder, and the neck of the bladder is mechanically opened. This observation does not negate the theory that there is active relaxation of the bladder neck via sympathetics, but it does support a concept wherein the actual

funneling of the bladder neck is dependent on cholinergically mediated active smooth muscle contraction.

Implanted devices have specific advantages and are able to circumvent most of the disadvantages of TENS therapy. Those patients who benefit from neurostimulation trials can be helped regardless of the time, place, need, or dependence on the neurostimulation. Inefficiencies associated with the delivery of TENS current are minimized with implants because of direct application of current to a specific sacral nerve. Finally, nerve stimulation is the most efficient way to elicit a continuous influence over pelvic muscle failure. This, in turn, provides the best hope of regulating striated pelvic floor muscle behavior. It is therapeutically attractive to specifically neutralize hyperreflexic striated sphincter activity, without compromising the reflex contractility of the bladder. Usually, with stimulation of a sacral nerve, a pleasant pulling or tugging in the perineum is felt by the patient.

A period of testing is required to determine if the proper set of nerve fibers can be found for chronic neurostimulation, then if the stimulation can be applied on a continuing basis. In this procedure, an insulated needle is positioned in one or more of the sacral foramen. Stimulation is applied by gradually increasing (ramping) the current to a comfortable intensity. Usually the stimulation intensities will be much higher and more comfortable when good muscle contraction is obtained. If it is not, the stimulation will be unpleasant. When it is effective, tenderness will be markedly reduced in tissues during and after application of the stimulation. A temporary electrode is then inserted into the foramen through the test needle and left in place for 3 to 7 days. If the patient does well during this period of trial stimulation, then there is the option of placing the electrode permanently or waiting and repeating the trial.

Few patients who benefit from the stimulation will remain in remission from their symptoms once the stimulation therapy is withdrawn. Trial therapies over several days have been performed on over 600 nerves for a variety of reasons. Permanent implants were performed specifically for the treatment of pain in 40 patients. None of these patients has shown evidence of nerve injury. Thus, the techniques of evaluation and chronic stimulation, when successful, do not appear to present undue risks to the patient. Within this experience those patients who did best were those who had identifiable dysfunction in the pelvic muscles (Figs. 22-22 and 22-23) (222–235).

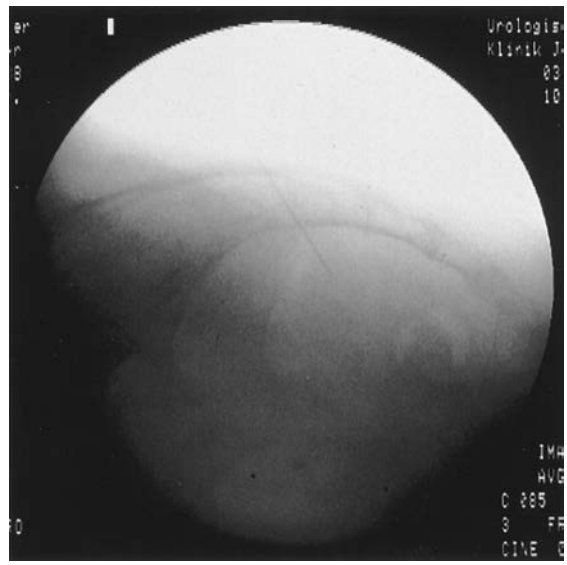


FIGURE 22-22. Intraoperative monitoring during acute sacral nerve stimulation. The test needle is located at the third sacral foramen (lateral view).

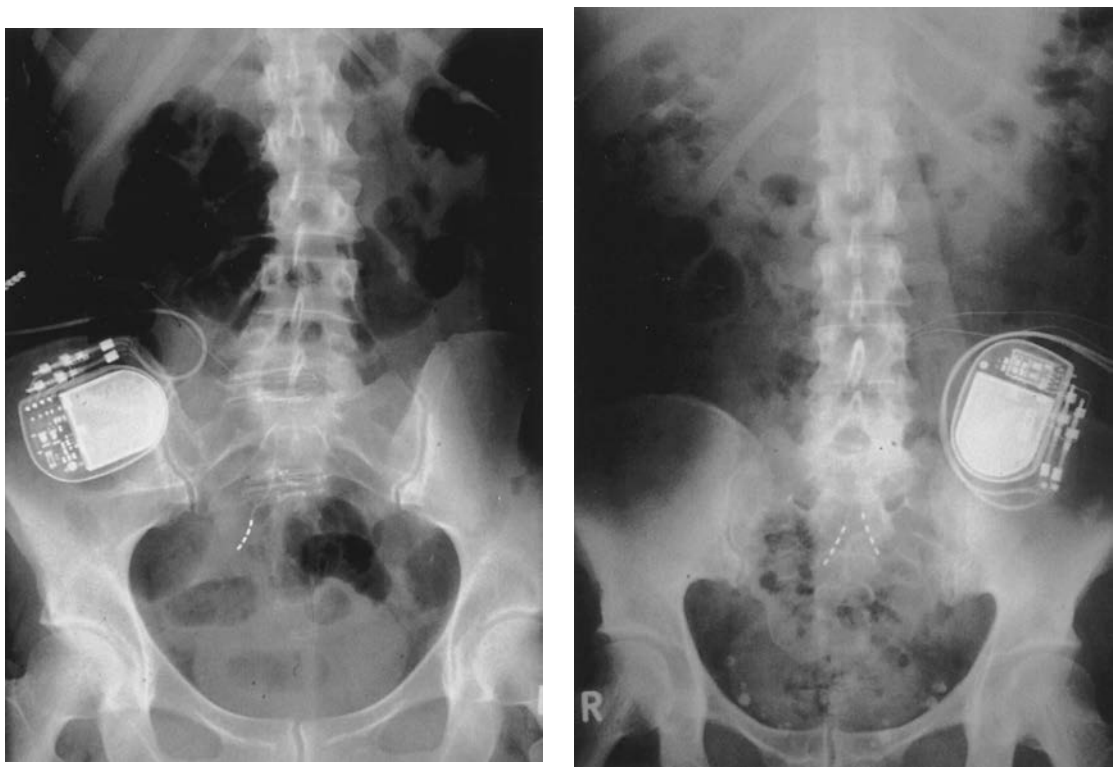


FIGURE 22-23. Permanent sacral nerve stimulation by an implanted neuroprosthesis. **A:** Unilateral (*right*) sacral nerve stimulation (S3). **B:** Bilateral sacral nerve stimulation (S3).

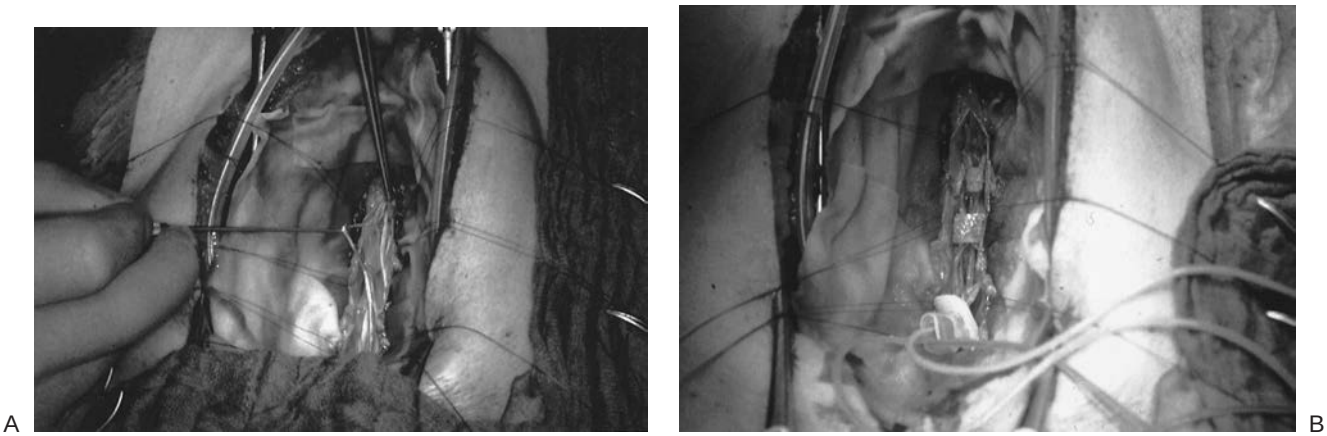


FIGURE 22-24. Intraoperative sites. **A:** Intradural dissection of the sacral nerves and resecting the afferent fibers. **B:** Implantation of electrodes at the anterior roots.

Special Surgical Techniques in Neurogenic Bladder/Spinal Cord Injury Patients

External Sphincterotomy

The external sphincterotomy (12 o'clock sphincterotomy) is a suitable method to reduce the bladder outlet resistance in patients with spinal cord injury with bladder–sphincter hyperreflexia and detrusor–sphincter dyssynergia. The procedure protects the upper urinary tract from hydronephrosis and the detrusor from secondary morphologic changes and allows a low-pressure emptying of the bladder by "triggering" Incontinence may worsen in a few patients; however, this is acceptable in tetraplegics who are unable to perform intermittent self-catheterization and who have decided to wear an external collecting system to reduce the need for transfers for toileting (236–239).

Sacral Deafferentation and Electrical Stimulation of the Sacral Anterior Root

Sacral deafferentation produces an areflexic bladder by interrupting the uninhibited sacral reflex arc. This procedure itself allows storing of large urine volumes at low pressure, thereby protecting the upper tract and contributing to continence. It also serves to abolish the active component of detrusor–sphincter dyssynergia and to abolish autonomic dysreflexia triggered by sacral afferents. Electrical stimulation of the anterior roots produces detrusor contraction and allows "electromicturition." The problem of simultaneous stimulation of the sphincter and the pelvic floor can be improved by using different types of current impulses (Figs. 22-24 and 22-25) (240–247).

Bladder Augmentation

The use of a detubularized ileum, ileocecum, or sigmoid colon to enlarge the bladder can reduce intravesical pressure to levels that are less damaging to the kidneys and that allow patients to be continent on intermittent self-catheterization. This method is indicated in low-compliance bladders secondary to bladder wall fibrosis and in cases of "malignant" hyperreflexia, refractory to conservative management (248–253).

Urinary Diversion

Diversion of urine, usually in an ileal conduit opening to the right anterior abdominal wall was a widely used procedure in children with myelodysplasia and also a treatment option in patients with spinal cord injury. However, the long-term results often showed significant renal deterioration. Today this procedure is rarely used. It remains a "last possibility" if all other neurourologic options have failed to manage lower urinary tract dysfunction (254–256).

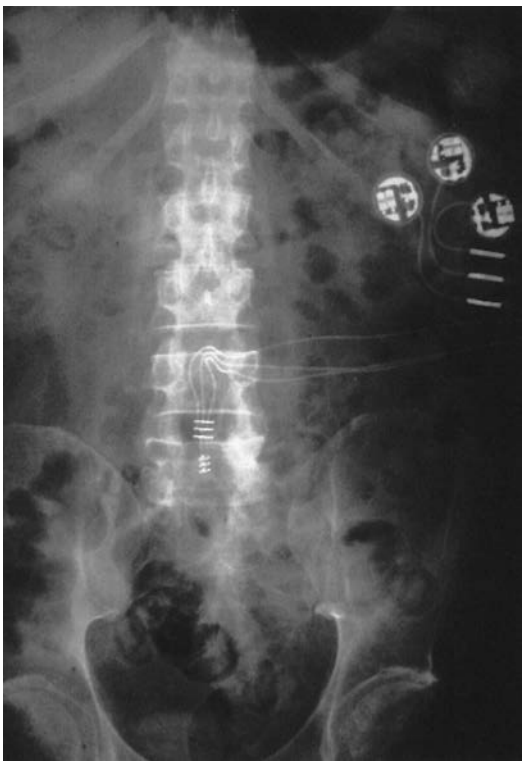


FIGURE 22-25. Sacral anterior root stimulator for electromicturition in spinal cord injury patients (x-ray, anterior-posterior).

Artificial Urinary Sphincter

The artificial urinary sphincter consists of a hollow cuff implanted around the bladder neck or proximal urethra and a fluid reservoir connected to the cuff. Fluid can be transferred between the two components by manual operation of a pump located in the scrotum or major labia. The main indication for this procedure is stress urinary incontinence (i.e., postprostatectomy incontinence) and neurogenic incontinence due to a weak sphincter, secondary to a lower motor neuron lesion (257–260).

CONCLUSION

Advances in the neurosciences allow a growing understanding of central mechanism influencing and modulating lower urinary tract function. Recent progress in the clinical sciences provides the basis for comprehensive care of the neurourologic patient in daily practice. However, basic and clinical scientific efforts are imperative to further improve our knowledge in these fields, since we often are unable to help the patient properly and alleviate chronic pain states, chronic neurologic impairment, and psychosomatic/behavioral disturbances.

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CHAPTER 23 ■ PROSTATITIS, ORCHITIS, AND EPIDIDYMITIS

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PROSTATITIS

Prostatitis is an important male medical condition that may clinically afflict as many men as the other two important prostate diseases—benign prostatic hyperplasia (BPH) and prostate cancer. Approximately 8% (1) of visits to U.S. urologists are reported to be for inflammatory diseases of the prostate gland. It is even more prevalent in urological practice in other countries (2). It is the most common urologic diagnosis in men under 50 years of age and the third most common urologic diagnosis in men over 50 years of age (1). It is estimated that 4% to 5% of young men (age less than 50 years) have a history of prostatitis (3,4). In older men, the overall prevalence of a prostatitis diagnosis is 11% to 16% (5, 2). An international study determined that 35% of men had at least one symptom of prostatitis over the previous year and for 8% of men, this represented at least a minor problem (6). A North American population-based study (7) employing a validated prostatitis symptom index (8) determined that 9.7% of male respondents, aged 20 to 74 years, reported pain/discomfort in the perineum and/or with ejaculation. They reported a total pain score of greater than or equal to 4 (based on a possible range of 0 to 21). The location and severity of pain would be sufficient to lead most physicians to make a diagnosis of chronic prostatitis. Similar symptoms with a score of greater than or equal to 8 were reported by 6.6% of the men in this group. This score would place them in the moderate or severe category. While it has traditionally been reported that prostatitis is a disease of young men, epidemiologic studies (5,7,9) show that there is not an age-specific prevalence of either a physician-assigned diagnosis of prostatitis or the prevalence of prostatitis-like symptoms.

Classification

The traditional classification system (10) was based on a diagnostic plan described by Meares and Stamey in 1968 (11). The classification system was based on chronicity of symptoms, white ("pus") blood cells, and the presence of "uropathogenic" bacteria in prostate-specific specimens. These specimens included expressed prostatic secretion (EPS) obtained during prostatic massage and/or a voided bladder 3 specimen (VB3 or postprostatic massage initial stream urine specimen). These specimens were to be quantitatively cultured and the specimen examined microscopically for the presence of inflammatory cells and the results subsequently compared to an initial specimen or voided bladder 1 specimen (VB1) and a midstream or voided bladder 2 specimen (VB2). Purulent, prostate-specific specimens combined with positive cultures for uropathogenic bacteria in a patient, who is acutely and systemically ill defined a patient with acute bacterial prostatitis. Chronic bacterial prostatitis occurred in a patient who was less ill, characteristically with documented recurrent urinary tract infections (UTIs) who also had purulent and culture-positive prostate-specific specimens. Nonbacterial prostatitis included symptomatic patients with significant numbers of leukocytes in prostate-specific specimens, but no significant number of uropathogenic bacteria. Prostatodynia patients had symptoms similar to those with nonbacterial prostatitis, but the prostate-specific specimens did not show either leukocytosis or uropathogenic bacteria.

The National Institutes of Health (NIH) consensus meeting on prostatitis (Bethesda, Maryland, December 1995) led to the development of a specific NIH classification system for research purposes (12). Categories I and II are similar to the traditional categorization system for acute and chronic bacterial prostatitis. It was recognized that the primary diagnostic criterion for chronic nonbacterial prostatitis and prostatodynia was genitourinary pain. It was also accepted that leukocytosis (either the degree of leukocytosis or the absence of white blood cells) and the presence of presumed "nonpathogenic" microorganisms in prostate-specific specimens has never been adequately validated as a reliable method to differentiate patients with genitourinary pain. These conclusions formed the background for the development of the new NIH definition of Category III chronic pelvic pain syndrome (CPPS), which is defined as "the presence of genitourinary pain in the absence of uropathogenic bacteria detected by standard microbiological methodology." This syndrome is further categorized as Category IIIA or inflammatory CPPS and Category IIIB or noninflammatory CPPS, based on the presence or absence of excessive leukocytes in prostate-specific specimens, including semen.

Patients with infertility, BPH, and prostate cancer, as well as asymptomatic men in autopsy series, have prostatic inflammation noted on histologic examination or prostate-specific specimens (including semen). The traditional classification system omitted this potentially very important group of patients with prostatic inflammation. This problem is addressed in the new NIH classification system as Category IV or asymptomatic inflammatory prostatitis (AIP).

The traditional and NIH classification systems are described in Table 23-1. In 1998, the NIH International Prostatitis Collaborative Network confirmed the value of this classification system, not only in clinic research studies, but also in clinical practice (13).

Etiology

Pathologically, prostatitis is defined as the presence of inflammatory infiltrates, usually glandular, periglandular, or stromal (Fig. 23-1) within the prostate gland. Clinically, prostatitis is defined by an acute or chronic bacterial infection of the prostate gland, a nonspecific inflammation of the prostate, or by symptoms, usually pain, localized to the prostate, perineum, or pelvis. Except for acute bacterial prostatitis and recurrent urinary tract infection in males, the significance of bacteria,

TABLE 23-1

CLASSIFICATION SYSTEMS FOR THE PROSTATITIS SYNDROMES

Traditional classification system	National Institutes of health classification system
Acute bacterial prostatitis	Category I
Chronic bacterial prostatitis	Category II
	Category III, chronic pelvic pain syndrome (CPPS)
Nonbacterial prostatitis	Category IIIA, inflammatory CPPS
Prostatodynia	Category IIIB, noninflammatory CPPS
N/A	Category IV, asymptomatic inflammatory prostatitis (AIP)

white blood cells, or even histologic documentation of inflammation within the prostate gland remains essentially unknown. However, these factors do appear to be interrelated in some pathogenic way in the development of not only bacterial infection of the lower urinary tract, but also in the development of the symptom complex defined by CPPS (chronic nonbacterial prostatitis and/or prostatodynia).

An Infectious Etiology?

Acute bacterial prostatitis and chronic bacterial prostatitis are associated with Gram-negative uropathogenic bacteria (originally residing in the gastrointestinal tract). Enterobacteriaceae, such as *Escherichia coli,* are cultured in 65% to 80% of cases (14,15); *Pseudomonas aeruginosa, Serratia* sp., *Klebsiella* sp., and *Enterobacter* sp. are cultured in an additional 10% to 15% of cases (15,16). *Enterococcus* sp. may account for as many as 5% to 10% of culture-proven prostate infections (16–18). In acute bacterial prostatitis, the bacteria cause a generalized infection of the entire prostate gland and there is usually an associated lower urinary tract infection (i.e., cystitis) that is often accompanied by generalized sepsis (bacteremia). Chronic bacterial prostatitis is associated with focal, localized microcolonies of bacteria deep within the prostate gland, which act as a nidus for recurrent lower urinary tract infections. Between episodes of cystitis, standard midstream urine cultures are usually sterile.

Other Gram-positive bacteria such as *Staphylococcus saprophyticus* and hemolytic *Streptococcus* sp. can be localized to prostate-specific specimens (17–24). However, the significance of these organisms in regard to both prostatic inflammation and the resulting symptom complex is really unknown (25). In other words, these organisms may be simply colonizing the prostate rather than causing an actual infection. Anaerobic bacteria can be localized to prostate-specific specimens (26–31), but the significance of this localization in regard to symptoms is unknown. *Corynebacterium* sp., which has usually been acknowledged as prostate nonpathogens, have also been suggested as potential etiologic agents in this disease (29,30). Tanner and colleagues (31), using polymerase chain reaction (PCR) techniques, were able to identify a bacterial signal in 65% of 17 patients with chronic prostatitis—most

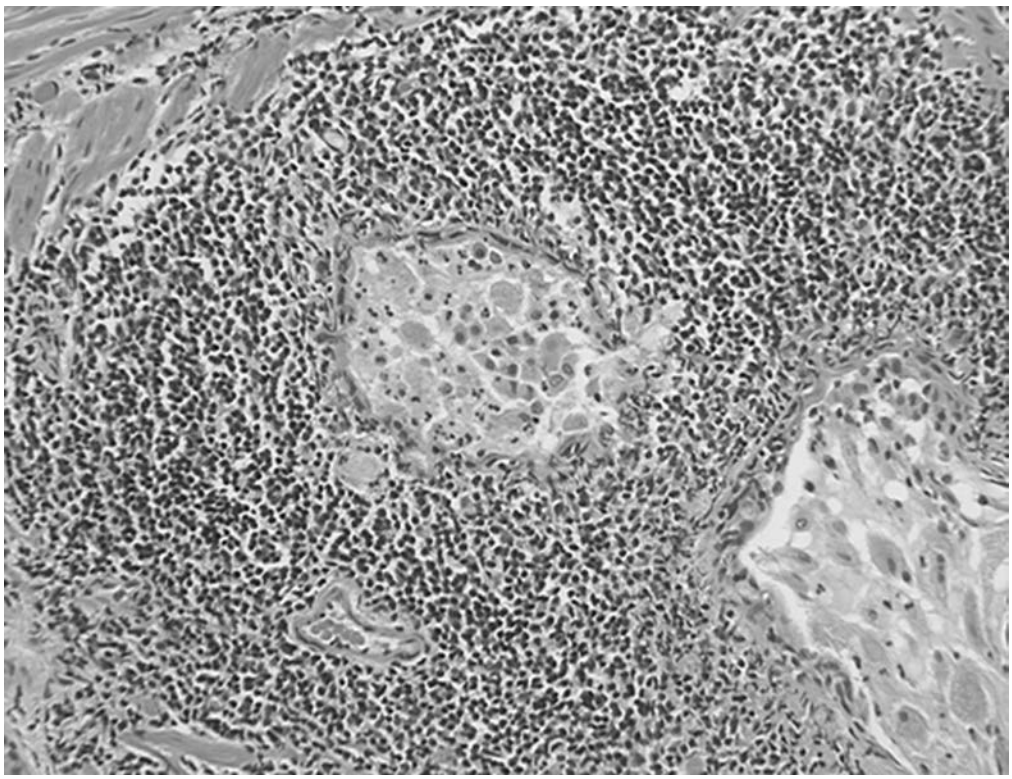

FIGURE 23-1. Prostatitis, by definition, is inflammation of the prostate gland. This histopathologic micrograph demonstrates the glandular, periglandular, and stromal inflammation seen in patients with chronic inflammatory conditions of the prostate. Magnification ×400. (Photo courtesy of Dr. Alexander Boag.)

appeared to be Gram-positive organisms with a predominance of *Corynebacterium* sp. *Chlamydia trachomatis* and *Ureaplasma urealyticum,* which are etiologic agents for nonspecific urethritis, have also been proposed as agents for the development of chronic prostatitis. The vast and conflicting literature on the potential role of *C. trachomatis* (32–48) presents a confusing picture of the importance of this organism in the prostate gland. Similarly, *U. urealyticum,* which can be commonly isolated from the urethra of men without a history of nonspecific urethritis, can also be found in prostate-specific specimens (27,49–52). A critical review of all these studies, however, does not lead to a definite conclusion that *U. urealyticum* is implicated in prostatic inflammation and/or symptoms associated with chronic prostatitis. The problems encountered in studies with *Chlamydia* and *Ureaplasma* include the absence of controls and the fact that it is very difficult to account for possible urethral contamination in collecting specific prostate specimens.

Candida (53,54), viruses (55,56), and *Trichomonas* (57–60) have also been implicated in prostatic inflammation, but, in most cases, it is usually an isolated finding, many times in immunosuppressed patients.

Studies employing specialized culture techniques (30,61, 62), cultures of percutaneous prostate biopsies (63,64), and molecular biologic techniques, such as PCR (31,47,65) have provided evidence that fastidious or nonculturable microorganisms might be present in the prostate gland, and that such organisms may be involved in the inflammatory process and subsequent development of symptoms.

To further complicate the controversy, an analysis of a large cohort studied by the NIH–Chronic Prostatitis Collaborative Research Network (CPCRN) did not show an association between bacterial cultures, inflammation, and symptoms (66). A further case-control study published by the NIH–CPCRN observed similar positive bacterial culture localization of both uropathogens and traditional nonpathogens in both prostatitis cases and age-matched asymptomatic control men (25).

A critical review (67) of the evidence for and against the role of microorganisms—culturable, fastidious, or nonculturable—is available if the reader would like to examine the role of microorganisms in the etiology of chronic prostatitis in more depth.

A Noninfectious Etiology?

A very popular hypothesis for the development of symptoms (whether inflammatory or not) is that anatomic or neurophysiologic obstruction results in high-pressure dysfunctional voiding (68–73). This dysfunctional or dyssynergic voiding flow pattern may lead to autonomic overstimulation of the local neural system with subsequent development of a chronic neuropathic state (74). Reflux of urine (and possibly bacteria) in the prostatic ducts has also been postulated as one of the most important etiologic mechanisms involved in the pathogenesis of both chronic bacterial and nonbacterial prostatic inflammation. High-pressure, dysfunctional voiding combined with intraprostatic ductal reflux may occur in susceptible individuals (68,70,75,76). It has been demonstrated (76) that urine and its metabolites (i.e., urate) are present in prostatic secretion in patients with chronic prostatitis. It has been hypothesized that the prostatic inflammation may simply be because of a chemically induced inflammation secondary to noxious substances in the urine that have refluxed into the prostatic duct.

Bacterial-specific immunologic alterations of the local prostatic immune system are observable in acute and chronic bacterial prostatitis. In acute bacterial prostatitis, bacterial- specific immunoglobulin G (IgG) and immunoglobulin A (IgA) can be detected immediately after onset of infection in both serum and prostatic fluid; these decline over the next 6 to 12 months following successful antibiotic therapy (77–80). Prostate-specific antigen (PSA) can also be markedly elevated during an acute episode of bacterial prostatitis (81,82) that slowly resolves to normal over the course of about 6 weeks, provided there is no recurrence of infection. In chronic bacterial prostatitis (Category II), the prostatic fluid IgA and IgG are both increased, but no serum immunoglobulin elevation is detected (44,80). It takes several months for IgG levels to return to normal, but IgA (particularly secretory IgA) can remain elevated for up to 2 years (44,47). Specific antibody-coated bacteria can be detected in urine, EPS, and semen in patients with chronic bacterial prostatitis (83,84). In CPPS (Category III), nonspecific IgA and IgM antibody elevation is detected (33,47,87) and similar antibodies, as well as fibrinogen and complement C3 (86,87), have been identified in prostatic biopsies. It has been suggested that this nonspecific inflammation may be because of an autoimmune process (88,89), with cytokine production playing a subsequent role in the process of inflammation (90–92). In all cases of prostatic inflammation (bacterial or nonspecific), the immunologic cascade does appear to play an important role in the development of prostatic inflammation (92–94).

Other investigators have proposed that sensory and/or motor disturbances consistent with neural dysregulation of the lower urinary tract may be a consequence of acquired abnormalities of the central nervous system (CNS) (95,96). Alternatively, the source of pain may be due to specific problems of the pelvic musculature attachment area at the sacrum, coccyx, ischial tuberosity, pubic rami, and endopelvic fascia (97).

The close association between interstitial cystitis, an ill-defined condition marked by a nonspecific inflammation of the bladder that occurs primarily in women, and the signs and symptoms of chronic prostatitis that occur in men have led a number of investigators to hypothesize that these two lower urinary tract inflammatory syndromes have a similar etiology (98,99). The pain and voiding symptoms of interstitial cystitis and chronic prostatitis are similar (99–101), and men diagnosed with chronic prostatitis have cystoscopic (102) and urodynamic findings (103) very similar to patients with interstitial cystitis. The problem, however, is that the etiology of interstitial cystitis is as obscure as that for chronic prostatic inflammation.

It is becoming clear to many investigators that chronic prostatitis/CPPS may represent a spectrum of interrelated lower urinary tract abnormalities. Alternatively, the syndrome may result from a progression of related immunologic and/or neurogenic mechanisms that occur after some initiating event (i.e., infections, dysfunctional voiding, and intraprostatic ductal reflux). The end result is pain and voiding symptoms with or without demonstrable prostatic inflammation.

Diagnosis

History and Physical Examination

The presentation and diagnostic workup of Category I, acute bacterial prostatitis, is very different from that of chronic prostatitis/CPPS (categories II and III). These patients present with an acute, usually febrile, illness with severe obstructive and irritative voiding symptoms. Patients complain of urinary frequency, urgency, dysuria, hesitancy, poor interrupted stream, and stranguria, as well as quite severe discomfort and/or pain localized to the perineum, suprapubic area, or external genitalia. Physical examination may reveal a febrile, nauseated patient who might be tachycardic and/or hypotensive. Suprapubic palpation and/or percussion may disclose a distended painful bladder. Digital rectal examination will detect anal sphincter spasm and a swollen, soft, and inflamed prostate gland. A prostatic massage to collect prostatic fluid is not required (and

may even be dangerous), but urine and blood cultures should be obtained before initiating therapy.

The prevalence of bacterial prostatitis ranges from 5% to 15% (15,104,105) of all chronic cases of prostatitis (chronic prostatitis/CPPS), but, in most large, carefully evaluated series, the incidence of bacteria localization to prostate-specific specimens is less than 5% of patients with symptoms of chronic prostatitis (106). Patients with Category II chronic bacterial prostatitis may present with episodes of recurrent cystitis (106,107) and may be asymptomatic between these acute episodes. However, in many cases, presentation of patients with chronic bacterial prostatitis (Category II) and those with CPPS (Category III) is indistinguishable. It is also impossible on clinical grounds to differentiate patients with inflammatory CPPS (Category IIIA) from patients with noninflammatory CPPS (Category IIIB). The predominant symptom in patients with chronic prostatitis/CPPS is pain, which is most commonly localized to the perineum, suprapubic area, and penis, but can also occur in the testes, groin, or lower back (108–112). Pain during or after ejaculation is another prominent feature in many patients. Irritative and obstructive voiding symptoms, including urgency, frequency, hesitancy, and poor, interrupted flow are also common. Erectile dysfunction is reported in patients with CPPS, but is not pathognomonic of this syndrome. By definition, the syndrome becomes chronic after 3 months duration. While the symptoms wax and wane over time, the natural history of CPPS over the years is unknown. However, the impact of this condition on quality of life is known and is significant. The quality of life of many patients diagnosed with chronic prostatitis/CPPS is dismal and generic health status measures show that men with chronic prostatitis have scores similar to those for patients with a history of myocardial infarction, angina, or Crohn's disease (113–115).

Category IV asymptomatic inflammatory prostatitis, by definition, does not include symptoms as a diagnostic criterion. These patients are characteristically diagnosed by observing leukospermia in infertile patients, by identifying inflammatory infiltrates in patients who have been surgically treated for benign and malignant prostate diseases (benign prostatic hyperplasia or prostate cancer), or in biopsies in patients being investigated for elevated PSA.

The evaluation of a man presenting with a chronic prostatitis syndrome has been the subject of a number of international consensus conferences in North America (116) and internationally (117,118). The North American recommendations for the evaluation of a chronic prostatitis patient are presented in Table 23-2. A number of attempts have been made to define the symptom complex associated with chronic prostatitis/CPPS into a formal standardized symptom index. It has been acknowledged by most researchers in the field that in a syndrome characterized and defined by a constellation of symptoms, a system to formally assess symptoms would be useful for baseline evaluation and follow-up of patients, both in research studies and in clinical practice. A number of such symptom indices have been proposed (109–112,119) and, while none of these symptom indices were properly validated according to the rigorous standards that now must be met for an accepted disease-specific index (120), they provided the basis for the recently validated and published National Institutes of Health Chronic Prostatitis Symptom Index (NIH/CPSI) (8). The NIH/CPSI consists of nine questions that address the three most important domains of the chronic prostatitis experience: pain was captured in four questions that focused on location, severity, and frequency; urinary function was captured in two questions focusing on irritative and obstructive symptoms; and quality-of-life impact was captured in three questions that explored the effect of symptoms on daily activities. The NIH/CPSI (Fig. 23-2) has now been approved by the International Prostatitis Research Community as an accepted outcome measure (13) and has proved useful in evaluation and follow-up of patients in general clinical practice (121).

TABLE 23-2

RECOMMENDED EVALUATION FOR A MAN PRESENTING WITH CHRONIC PROSTATITIS-LIKE SYMPTOMS

Mandatory
History
Physical examination, including DRE
Urinalysis and urine culture
Recommended
Lower urinary tract localization test
Symptom inventory or index (NIH–CPSI)
Flow rate
Residual urine determination
Urine cytology
Optional
Semen analysis and culture
Urethral swab for culture
Pressure flow studies
Videourodynamics (including flow-EMG)
Cystoscopy
Transrectal ultrasound (TRUS)
Pelvic imaging (ultrasound, CT scan, MRI)
Prostate-specific antigen (PSA)

(From: Nickel JC. Clinical evaluation of the man with chronic prostatitis/chronic pelvic pain syndrome. *Urology* 2003;60[Suppl 6A]:20.)

Physical examination of patients with Category II/III chronic prostatitis/CPPS is usually unremarkable, but careful examination and palpation of the external genitalia, groin, perineum, coccyx, external anal sphincter, and internal pelvic side walls may pinpoint prominent areas of spasm/pain/discomfort. The digital rectal examination must be performed after initial urine specimens (discussed later in this chapter) are produced. The prostate gland in the chronic prostatitis syndromes has been described as "boggy and tender," but, in reality, the prostate may be normal, firm, soft, and may not be tender at all until prostatic massage is employed to obtain prostate-specific specimens.

Lower Urinary Tract Evaluation

The "gold standard" Meares-Stamey four-glass test, first formally described over three decades ago (11), outlines a rigorous method to localize bacteria and inflammation to a urethral, bladder, and/or prostate site. The first 10 ml of initial voided urine (VB1) represents the urethral specimen; a midstream urine specimen (VB2) represents the bladder specimen. During and/or following a vigorous prostatic massage, a drop or two of EPS represents the optimal prostatic specimen while a postprostatic massage (initial 10 mL) urine specimen (VB3) represents an alternative prostate-specific specimen. All specimens are sent for quantitative culture, while a wet-mount specimen of EPS and a sediment specimen of the urine samples are examined microscopically for the presence of leukocytes. Category II, chronic bacterial prostatitis, is defined by significantly more uropathogenic bacteria detected in the prostate-specific specimens compared to the urine specimens before prostatic massage. Category III, CPPS, is defined by the absence of uropathogenic bacteria in the prostate-specific specimens. Category IIIA (inflammatory CPPS) is defined by significant

NIH-Chronic Prostatitis Symptom Index (NIH-CPSI)

Pain or Discomfort

1. In the last week, have you experienced any pain or discomfort in the following areas?

	Yes	No
a. Area between rectum and testicles (perineum)	❑ 1	❑ 0
b. Testicles	❑ 1	❑ 0
c. Tip of the penis (not related to urination)	❑ 1	❑ 0
d. Below your waist, in your pubic or bladder area	❑ 1	❑ 0

2. In the last week, have you experienced:

	Yes	No
a. Pain or burning during urination?	❑ 1	❑ 0
b. Pain or discomfort during or after sexual climax (ejaculation)?	❑ 1	❑ 0

3. How often have you had pain or discomfort in any of these areas over the last week?

❑ 0 Never
❑ 1 Rarely
❑ 2 Sometimes
❑ 3 Often
❑ 4 Usually
❑ 5 Always

4. Which number best describes your AVERAGE pain or discomfort on the days that you had it, over the last week?

❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
0	1	2	3	4	5	6	7	8	9	10
NO PAIN									PAIN AS BAD AS YOU CAN IMAGINE	

Urination

5. How often have you had a sensation of not emptying your bladder completely after you finished urinating, over the last week?

❑ 0 Not at all
❑ 1 Less than 1 time in 5
❑ 2 Less than half the time
❑ 3 About half the time
❑ 4 More than half the time
❑ 5 Almost always

6. How often have you had to urinate again less than two hours after you finished urinating, over the last week?

❑ 0 Not at all
❑ 1 Less than 1 time in 5
❑ 2 Less than half the time
❑ 3 About half the time
❑ 4 More than half the time
❑ 5 Almost always

Impact of Symptoms

7. How much have your symptoms kept you from doing the kinds of things you would usually do, over the last week?

❑ 0 None
❑ 1 Only a little
❑ 2 Some
❑ 3 A lot

8. How much did you think about your symptoms, over the last week?

❑ 0 None
❑ 1 Only a little
❑ 2 Some
❑ 3 A lot

Quality of Life

9. If you were to spend the rest of your life with your symptoms just the way they have been during the last week, how would you feel about that?

❑ 0 Delighted
❑ 1 Pleased
❑ 2 Mostly satisfied
❑ 3 Mixed (about equally satisfied and dissatisfied)
❑ 4 Mostly dissatisfied
❑ 5 Unhappy
❑ 6 Terrible

Scoring the NIH-Chronic Prostatitis Symptom Index Domains

Pain: Total of items 1a, 1b, 1c, 1d, 2a, 2b, 3, and 4 = ____

Urinary Symptoms: Total of items 5 and 6 = ____

Quality of Life Impact: Total of items 7, 8, and 9 = ____

FIGURE 23-2. The National Institutes of Health Chronic Prostatitis Symptom Index (NIH–CPSI) has been validated for use in determining the severity and following the symptom complex of patients with chronic prostatitis/chronic pelvic pain syndrome in clinical trials and scientific studies. This simple-to-administer questionnaire which explores the important domains of pain, voiding disturbances, and quality of life/impact has proved useful in clinical practice to follow patients diagnosed with chronic prostatitis. (From: Litwin MS, McNaughton-Collins M, Fowler FJJ, et al. The National Institutes of Health Chronic Prostatitis Symptom Index: development and validation of a new outcome measure. *J Urol* 1999;162:369.)

leukocytosis in the prostate-specific specimens, while Category IIIB (noninflammatory CPPS) is defined by an absence of significant leukocytosis in these particular specimens. The critical value of leukocytes has never been validated, but suggestions of significant leukocytosis range from 2 to 20 WBC/hpf.

The Meares-Stamey four-glass test is rarely performed by urologists and almost never by primary care physicians (122–125). Physicians, including urologists, found the procedure time consuming, cumbersome, expensive, and rarely leading to a significant change in management, since the bacterial forms of prostatitis are extremely rare and acute bacterial prostatitis is very easily diagnosed on clinical grounds. The pre- and postmassage (two-glass) test, originally suggested by Weidner and Ebner (126) and subsequently popularized by Nickel (127,128), is a simple, cost-effective screen for patients presenting with chronic prostatitis syndrome. It is easily accomplished by physicians who do not have access to their own laboratory facilities and its interpretation is almost as accurate as the more cumbersome four-glass test (128,129). Examination and culture of EPS, however, remains the optimal test to differentiate chronic prostatitis patients.

The four- and two-glass tests are shown in Figure 23-3. The presentation and interpretation of lower urinary tract evaluation in patients presenting with symptoms of chronic prostatitis is described in Table 23-3.

Other Tests

Cystoscopy is indicated in patients whose history (i.e., hematuria, lower urinary tract trauma), lower urinary tract evaluation (i.e., VB1 urinalysis), or ancillary studies (i.e., urodynamics) indicate the possibility of a diagnosis other than chronic

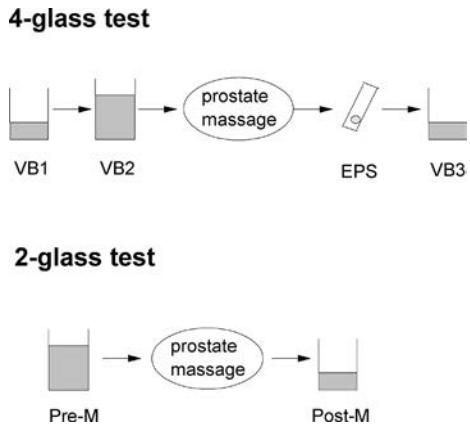

FIGURE 23-3. The lower urinary tract should be evaluated in chronic prostatitis syndrome with examination for white blood cells and microbiologic culture of prostate-specific specimens. While the "gold standard" method compares the examination and culture of the expressed prostatic fluid and voided bladder 3 to the urethral (voided bladder 1) and bladder urine (voided bladder 2) employing the Meares-Stamey four-glass test, the simpler two-glass method of evaluating the pre- and postprostatic massage urine (the pre- and postmassage tests) provides for a clinically adequate screening. See text for interpretation of the results of this testing.

prostatitis/CPPS. Occasionally, lower urinary tract stones or malignancies, urethral strictures, bladder neck problems, and so forth are discovered that can be surgically corrected. Urodynamics has been suggested by a number of investigators in patients with chronic prostatitis (103,130–133). It appears particularly useful in patients who present with lower urinary tract voiding symptoms as their primary complaint. In these patients, many conditions have been found, including detrusor–vesical neck or external sphincter dyssynergia, proximal or distal urethral obstruction, fibrosis or hypertrophy of the vesical neck, bladder areflexia with nonrelaxing perineal floor, and bladder hyperreflexia (68–73,103,130,134–137). A urinary flow rate study, combined with a bladder ultrasound, can be employed to screen patients for obstructive voiding patterns and/or poorly emptying bladders who would be candidates for further, more sophisticated, urodynamic evaluation.

While transrectal ultrasound has become the best radiologic method to evaluate prostate disease (especially to determine prostate volume and assist guidance of biopsy needles), the diagnostic value of ultrasound in prostate disease remains controversial. While a number of investigators (138–144) describe possible ultrasound characteristics of chronic prostatic inflammations, the results are not conclusive enough to indicate that this is a clinically useful tool. However, transrectal ultrasonography can be valuable in diagnosing prostatic cysts (145), diagnosing and draining prostatic abscesses (146), or diagnosing and draining obstructed seminal vesicles (147).

Research studies have employed transperineal biopsies to localize bacteria and other histologic abnormalities within the prostate gland (46,47,63,64,87). At this time, however, histologic, culture, and molecular biologic evaluation of prostate biopsies in patients with chronic prostatitis/CPPS remains a research tool only. A number of investigators (33,44,85, 148,149) differentiate patients by determining whether or not their prostate-specific specimens contain specific antibodies to uropathogens. While very interesting, this has not proved to be a useful clinical tool. Other investigators (90,91,150,151) suggest that various proinflammatory cytokines (interleukin-1β [IL-1β], tumor necrosis factor-α [TNF-α], and interferon 6 and 8 [IL-6 and IL-8]) in EPS or semen in men with chronic prostatitis may provide an objective measure of disease. This is a very exciting avenue of research, but, at this time, the sensitivity or specificity of these immunologic tests and their prognostic value are really unknown and are not yet indicated in clinical practice.

Screening for prostate cancer by determining serum PSA levels should be considered in patients in whom a physician would

TABLE 23-3

DESCRIPTION AND PRESENTATION OF THE PROSTATITIS SYNDROMES

Classification	Description	Presentation
Category I	Acute infection of the prostate gland	Acute febrile illness associated with perineal and suprapubic pain, dysuria, and obstructive voiding symptoms
Category II	Chronic infection of the prostate gland	Recurrent urinary tract infections with variable pain and voiding disturbances
Category III	Chronic genitourinary pain in the absence of uropathogenic bacteria localized to the prostate gland employing standard methodology	Chronic perineal, suprapubic, groin, testicular, penile, and ejaculatory pain associated with of uropathogenic bacteria localized voiding symptoms variable dysuria and obstructive and irritative to the prostate gland employing standard
Category IIIA	Significant number of white blood cells in expressed prostatic secretions, postprostatic massage urine sediment (VB3), or semen	See above (Category III)
Category IIIB	Insignificant number of white blood cells in expressed prostatic secretions, postprostatic massage urine sediment (VB3), or semen	See above (Category III)
Category IV	White blood cells (and /or bacteria) in expressed prostatic secretions, postprostatic massage urine sediment (VB3), semen, or histologic specimens of prostate gland	Asymptomatic

normally screen in his/her routine practice (except for bacterial prostatitis [Categories I and II], until the infection is cleared) (152). PSA is not helpful in the diagnosis of prostatitis.

Treatment

Category I: Acute Bacterial Prostatitis

Antibiotic therapy should be initiated in patients presenting with acute bacterial prostatitis as soon as the physical examination and necessary cultures have been obtained. These patients are usually very ill and may need ancillary support, such as intravenous fluids, antipyretics, and antiemetics. If the patient has severe voiding symptoms and feels he is not emptying his bladder or if physical examination reveals a distended bladder, bladder drainage is required. Although most experts have advocated suprapubic cystotomy, most practicing urologists in standard clinical practice actually insert a small-bore urethral Foley catheter. If this is too painful to insert or very uncomfortable once it is in, a suprapubic tube then can be inserted. In patients who are ill, the parenteral route of antibiotic administration is initially indicated (even if only a single dose is given) and wide-spectrum coverage should be employed to cover the potential Gram-negative and Gram-positive pathogens. A combination of aminoglycoside and ampicillin (and/or cephalosporin) provides adequate wide-spectrum coverage against most Gram-negative uropathogens, as well as *Enterococcus faecalis*. Second- or third-generation cephalosporins or one of the intravenous fluoroquinolones (ciprofloxacin or levofloxacin) are also optimal agents to consider. Once the acute symptoms have abated, therapy should be continued with one of the oral antibiotics appropriate for the treatment of chronic bacterial prostatitis (i.e., trimethoprim or fluoroquinolones). Although the duration of optimal therapy is unknown, 2 to 4 weeks have been suggested (121). If patients do not respond to appropriate antibiotic therapy within 36 to 48 hours, the possibility of a prostatic abscess needs to be clinically explored. Transrectal ultrasound, computed tomography (CT) scan, and magnetic resonance imaging (MRI) are useful in detecting such an abscess. Most abscesses are best drained by a transurethral surgical route, but transperineal and/or transrectal drainage may also be appropriate in special circumstances.

Category II: Chronic Bacterial Prostatitis

Antibiotics do not concentrate well within the prostate, particularly within the prostatic ducts. There is a huge body of pharmacokinetic data available, particularly in animal models (14,153–158), and these studies explain the poor antibiotic penetration by the presence of a pH gradient across the prostatic epithelium. Drug penetration was felt to be a passive transport mechanism, based on diffusion and concentration, which made drug penetration dependent on lipid solubility, degree of ionization and protein binding, size and shape of the antimicrobial molecule, pK_a of the antibiotic, and the pH of the plasma and ductal fluids. From these models, it was anticipated that trimethoprim and, subsequently, the fluoroquinolones (which are neither pure acids nor bases but have characteristics of both; known as zwitterion drugs) should result in higher drug concentrations within the prostatic ducts and acini. However, it is difficult to extrapolate from the animal model for pharmacokinetic studies to those of humans (159), and it has been subsequently confirmed that the pH of prostatic secretion in men with prostatic infection was markedly different from that of dogs and other animal models (160–163). A review of human pharmacokinetic studies (164) suggested that fluoroquinolones resulted in the best concentrations of drug in prostatic secretions compared to plasma.

Surveys have confirmed that trimethoprim/sulfamethoxazole (or, to a lesser extent, trimethoprim alone) has been, historically, the most commonly used antimicrobial agent in chronic prostatitis (122,123). However, most clinical studies demonstrated poor results. Eradication of pathogens in patients with chronic bacterial prostatitis with trimethoprim/sulfamethoxazole or trimethoprim alone ranged from a low of 0% (165) to a high of 67% (166), with most studies demonstrating an efficacy rate between 30% to 50% (167–171). It appears that for trimethoprim/sulfamethoxazole, therapy duration of 90 days provides the best clinical results. Most other antibiotics (except for the fluoroquinolones), including minocycline, cephalexin, and carbenicillin, do not demonstrate significant clinical efficacy in clinical studies in which patients were followed for sufficient time (166,172,173), except for one notable exception evaluating a combination of erythromycin and sodium bicarbonate (174). The fluoroquinolones provide the best eradication rate and improved therapeutic results compared to other antibiotic therapeutic regimens. Eight comparable studies evaluating fluoroquinolones and chronic bacterial prostatitis in which the diagnosis was obtained by localization studies and the patients were followed for sufficient time after completion of therapy (for at least 6 months) are available (175–182). These studies, which have been reviewed by Nabor (183), evaluated norfloxacin, ciprofloxacin, ofloxacin, and lomefloxacin and demonstrated a bacterial eradication rate between 57% to 86% (averaging about a 65% bacteriologic cure rate). The treatment duration of 1 month for the fluoroquinolones (for chronic prostatitis caused by *E. coli*) seems to be superior to the usual 3-month treatment with trimethoprim/sulfamethoxazole. A recently published trial (184) comparing four weeks of ciprofloxacin 500 mg BID with 4 weeks of levofloxacin 500 mg OD in men with chronic bacterial prostatitis showed an equivalent bacterial eradication rate (76.8% versus 75%, respectively) and clinical success rate (includes cured and improved) (72.8% versus 75%, respectively).

A direct injection of antibiotics into the prostate gland has been advocated by a number of investigators (185–187), but this method has never been rigorously evaluated or become standard practice among urologists. In a patient in whom the symptoms and offending bacteria are refractory to antibiotic therapy, some have advocated a program of repetitive prostatic massage combined with antibiotics (188–190), and some patients do appear to have significant clinical benefit. This finding has yet to be substantiated in clinical trials (191).

As a last resort, radical transurethral resection of the prostate (192) and open radical prostatectomy (194,195) has been advocated for patients with refractory chronic bacterial prostatitis. No prospective clinical series or long-term follow-up has ever been presented for this type of surgical therapy and it should not be encouraged or recommended at this time.

Category III: Chronic Pelvic Pain Syndrome (Chronic Nonbacterial Prostatitis and Prostatodynia)

A differential treatment algorithm has been recommended (121,196) based on the NIH categorization of inflammatory (Category IIIA) and noninflammatory (Category IIIB) CPPS. Although theoretically attractive and clinically helpful, such an approach has never been vigorously evaluated in prospective clinical trials. Until recently, there were few well-designed, randomized, placebo-controlled clinical trials evaluating various treatment options in CPPS. Those that did exist were small, had variable inclusion and exclusion criteria, undefined outcome parameters, and, for the most part, very short follow-up. However, since 2000, a number of randomized placebo controlled trials, based on the NIH definition for CPPS (12), standardized

inclusion and exclusion criteria (118,197), and validated outcome index, the NIH–CPSI (8) have changed treatment algorithms from traditional and empirical to evidence-based.

It appears that most patients with chronic prostatitis, irrespective of culture results, are treated with antimicrobial therapy (2,122,123,125). Studies have generally indicated that approximately 40% of patients with chronic prostatitis (or CPPS) have some symptomatic improvement with antimicrobial therapy (18,31,45,106,121,149,198–200). This perceived beneficial antibiotic effect in CPPS may be secondary to a very strong placebo effect, eradication or suppression of nonculturable microorganisms (67), or related to the recently discovered independent antiinflammatory effect of some antibiotics (201,202). A European consensus group, evaluating the role of antibiotics in the treatment of chronic prostatitis (200), suggests that antibiotics should be considered as empirical treatment for Category IIIA CPPS, but the benefit should be appraised after a minimum of 2 to 4 weeks of therapy. However, two recently published studies evaluating levofloxacin (203) and ciprofloxacin (204) have clearly shown that in chronic heavily pretreated (including previous antibiotic therapy), antibiotics produce no better results in amelioration of symptoms than placebo. Perhaps a trial of antibiotics is indicated (especially in Category IIIA) in recently diagnosed, antibiotic-naïve CPPS patients, but if they do not respond within 4 weeks, no further antibiotics are indicated.

The bladder neck and prostate are rich in α-receptors and lower urinary tract symptomatology associated with CPPS may be related to poor relaxation of the bladder neck during voiding (71,73,133,136). Nonspecific α-blockers (diphenoxybenzamine and phenoxybenzamine) demonstrated significant symptomatic improvement compared to placebo in two studies (205,206); however, there was an unacceptable high rate of adverse effects. More selective α1-blockers (alfuzosin, terazosin, and doxazosin) have demonstrated a reduction in symptoms in a number of trials (109,207,208). Only one of these early studies (207) compared one of these more selective α-blockers to placebo, and the improvement in symptom scores in the treatment arm compared to placebo was not startling. In the last several years, four well-designed randomized placebo controlled trials (204, 209–211) employing standardized patients and outcomes have been reported for terazosin, alfuzosin, and tamsulosin. These trials have taught us that α-blockers are modestly effective in more recently diagnosed, α-blocker naïve patients when given for over 6 weeks and that symptoms can return if the medication is discontinued, even after 6 months (212). α-Blockers do not appear to be superior to placebo in very chronic, intensely treated (including previous treatment with α-blockers) CPPS (204).

Prostatic inflammation is associated with Category IIIA CPPS and elevated cytokine levels are noted in the semen (90,150) and EPS (91,151) (semen and EPS). Antiinflammatory drugs should theoretically improve the inflammatory parameters within the prostate and possibly result in the reduction of symptoms. A nonsteroidal antiinflammatory agent, nimesulide, did reduce inflammatory type symptoms, such as dysuria, stranguria, and painful ejaculation (213,214) and the results of a North American randomized trial (215) comparing the cyclooxygenase-2 inhibitor, rofecoxib (now off the market), showed very modest amelioration of symptoms compared to placebo at 6 weeks, but only at higher doses (50 mg/day). The efficacy of pentosan polysulfate, an antiinflammatory glycosaminoglycan that has been used for the treatment of interstitial cystitis, was compared to placebo in patients with chronic prostatitis (216). In this small study, the treated group had a statistically significant improvement in symptoms, but the major improvement was in nonspecific myalgia and arthralgia. A recent study (217) evaluated oral pentosan polysulfate and demonstrated symptom and quality-of-life improvement in 40% of patients after 6 months. A larger multicenter randomized trial (218) reported statistically significant and marginal clinically significant improvement after 16 weeks of therapy compared to placebo.

Many patients with CPPS, particularly Category IIIB, appear to have some form of neuromuscular dysregulatory phenomena of the perineum/pelvic floor musculature. Skeletal muscle relaxants combined with adjuvant medical and physical therapies have been advocated and promoted (96,97) and, in one small study (206), baclofen (a striated muscle relaxant) showed significant improvement compared to placebo in patients with prostatodynia (Category IIIB). Another small study comparing diazepam to an antibiotic found no difference in symptom improvement between the two groups (219).

Prostate growth and function, and perhaps inflammation, are influenced by the local hormonal milieu, especially androgens (220). Finasteride, a 5α-reductase inhibitor, that is used to reduce prostatic size in BPH, has been compared to placebo in men with chronic prostatitis (221,222). In both studies, finasteride reduced symptoms better than placebo, but the difference was disappointing. Perhaps these agents, which are used for the treatment of BPH, will work better in older men who are diagnosed with both BPH and prostatitis (223).

Phytotherapeutic agents have become very popular in the treatment of other prostate diseases such as BPH and prostate cancer. Three specific phytotherapeutic agents, Cernilton (224,225), quercetin (226), and saw palmetto (227) have been tested in clinical trials and have demonstrated favorable improvement in pain and irritative voiding symptoms.

Allopurinol therapy has been suggested as a therapeutic agent based on the theory that intraprostatic ductal reflux of urine increases concentrations and metabolites containing purine and pyridinium bases in the prostatic ducts causing inflammation (76). Investigators comparing allopurinol to placebo were able to show a small difference between the mean patient reported discomfort score between the study and control groups at certain times in this long prospective trial (228); however, upon closer analysis, the data are not convincing (229).

For most of the 20th century, repetitive prostate massage was the main therapy for the chronic prostatitis syndrome. Although it fell into disfavor among urologists and physicians in the late 1960s, it may be making a comeback (188,189). Independent, but uncontrolled, studies (190,230) showed that about one- to two-thirds of patients do obtain some benefit in subjective symptoms with repetitive prostate massage for 4 to 6 weeks. However, prostatitis "experts" (191) could not come to a consensus on the potential benefits of repetitive prostate massage. Many physicians recommend frequent ejaculation, and there is some evidence that it might be beneficial (231). It has also been suggested that treatment of pelvic/perineal myofascial trigger points with heat therapy, physiotherapeutic massage, ischemic compression, stretching, anesthetic injections, and electroneural modulation may benefit some patients (97). Biofeedback has been shown in small, controlled studies to ameliorate some specific prostatitis-like symptoms in some men (73,209). A recent report described quite satisfactory results in 12 men with refractory CPPS treated with acupuncture (223), but these results should be replicated by other groups, preferably in a sham-controlled trial.

A number of minimally invasive therapies, including balloon dilation of the prostate (234), balloon dilation with prostatic hyperthermia (235,236), transurethral needle ablation of the prostate (119), and neodymium:yttrium–aluminum–garnet (Nd:YAG) laser therapy (237), have all been advocated and tested for men with chronic prostatitis/CPPS. Sham-control trials will be required before any of these therapies can be recommended. Heat therapy applied directly to the prostate either transrectally or transurethrally has been shown in a number of

TABLE 23-4A

MEDICAL THERAPIES ADVOCATED FOR THE PROSTATITIS SYNDROMES

Category I	Category II	Category IIIA	Category IIIB
Antibiotics	Antibiotics α-Blockers	Trial of antibiotics α-Blockers Antiinflammatories Phytotherapy Finasteride Pentosanpolysulfate	α-Blockers Antiinflammatories Muscle relaxants Antidepressants

studies to provide symptomatic relief to patients with nonbacterial prostatitis and prostatodynia (238). Heat therapy may accelerate the natural resolution of the inflammatory process (accelerating fibrosis or scar formation), alter or destroy afferent pain nerve fibers (239), or even kill nonculturable or cryptic bacteria within the prostate gland (240). Three published studies have used sham controls, two with transrectal microwave hyperthermia (241,242) and one with transurethral microwave thermal therapy (111). These studies demonstrate improved symptom amelioration compared to sham treatment. Larger-scale studies need to be performed in patients with refractory or end-stage symptoms.

Surgery does not have an important role in the treatment of most patients with CPPS unless a specific indication is discovered during evaluation of the patient. Patients with urethral strictures or urodynamic evidence of bladder neck obstruction may benefit from endoscopic incision (72). Open removal of the prostate gland has been advocated (194,195) and while the potential complications of radical prostatectomy are well known, the long-term potential benefits have never been adequately demonstrated.

Tables 23-4A and B illustrate the various potential treatment modalities suggested in the literature, based on the NIH categorization of the prostatitis syndromes.

Summary

Prostatitis is a common clinical entity that will be encountered by all physicians dealing with the urinary tract, not only urologists, but also primary care physicians, internists, and nephrologists. An appreciation of the epidemiology of this syndrome, an understanding of the possible etiologic mechanisms, a rational diagnostic plan, and a working knowledge of the evidence available for development of a sound treatment plan are required for any physician who deals with patients potentially suffering from prostatitis.

ORCHITIS

Definitions

By definition, orchitis is inflammation of the testicle, but the term has been used to describe testicular pain localized to the testicle without objective evidence of inflammation. Acute orchitis represents sudden occurrence of pain and swelling of the testicle associated with acute inflammation of that testicle. Chronic orchitis involves inflammation and pain in the testicle, usually without swelling, and persisting for more than 6 weeks.

Classification

Although no formal universally accepted classification for orchitis exists, we use the classification presented in Table 23-5. Bacterial orchitis is usually caused by organisms associated with urinary tract infections (UTIs) or sexually transmitted diseases (STDs). Nonbacterial, but infectious, orchitis can be subdivided into viral, fungal, parasitic, and rickettsial types. Truly noninfectious orchitis is often idiopathic or related to trauma, although autoimmune disease has rarely been implicated (243). Chronic orchitis can be attributed to any infectious or noninfectious cause, while chronic orchalgia has no identifiable cause and may not even be associated with inflammation. It may be impossible to clinically distinguish chronic orchitis from chronic orchalgia.

TABLE 23-4B

PHYSICAL/SURGICAL THERAPIES ADVOCATED FOR THE PROSTATITIS SYNDROMES

Category I	Category II	Category IIIA	Category IIIB
Catheterization—suprapubic or urethral	Repetitive prostatic massage Bladder neck incision Radical TURP[a] Radical open prostatectomy	Repetitive prostatic massage Microwave thermotherapy Bladder neck incision TUNA[a] Laser therapy Radical open prostatectomy	Biofeedback therapy Heat therapy Acupuncture Massage therapy Bladder neck incision

[a] *TURP*, transurethral resection of prostate; *TUNA*, transurethral needle ablation of the prostate.

TABLE 23-5

CLASSIFICATION OF ORCHITIS

Acute bacterial orchitis
Secondary to UTI[a]
Secondary to STD[a]
Nonbacterial infectious orchitis
Viral
Fungal
Parasitic
Rickettsial
Noninfectious orchitis
Idiopathic
Traumatic
Autoimmune
Chronic orchitis
Chronic orchalgia

[a] *UTI*, urinary tract infection; *STD*, sexually transmitted disease.

Pathogenesis

Isolated orchitis is a relatively rare condition and is usually viral in origin. It spreads to the testicle by a hematogenous route. Most cases of orchitis occur secondary to local spread of an ipsilateral epididymitis and are termed "epididymoorchitis." UTIs are usually the underlying source in boys and elderly men (244). In young sexually active men, STDs are often responsible (244). The etiology of chronic orchitis/orchalgia may be implied by history, but is sometimes unclear.

Microbiology

Bacterial orchitis is usually associated with epididymitis and is, therefore, often caused by urinary pathogens, including *E. coli* and *Pseudomonas*. Less commonly, *Staphylococcus* sp. or *Streptococcus* sp. are responsible. The most common sexually transmitted microorganisms responsible are *Neisseria gonorrhoeae, Chlamydia trachomatis,* and *Treponema pallidum*.

Mycobacterial infections can also cause orchitis, with tuberculosis (245) being much more common than leprosy. The most common cause of viral orchitis is mumps (246), but infectious mononucleosis has also been implicated (247). Fungal infections occasionally involve the testicle, with candidiasis, aspergillosis, histoplasmosis, coccidiomycosis, blastomycosis, and actinomycosis all having been reported as causes of orchitis (248). Parasitic infections rarely cause orchitis in the Western Hemisphere, but filariasis has been described in some endemic areas of Africa, Asia, and South America, and can be associated with elephantiasis (249).

Diagnosis

History and physical examination become important in the diagnosis of orchitis, given that definitive testicular cultures are almost impossible to obtain and urine/urethral cultures are often negative or contaminated. In patients presenting with acute infectious orchitis, history discloses a recent onset of testicular pain, often associated with abdominal discomfort, nausea, and vomiting. These symptoms may be preceded by symptoms of parotitis in boys or young men, UTIs in boys or elderly men, or alternatively by symptoms of an STD in sexually active men. Although the process is usually unilateral, it is sometimes bilateral, especially if viral (250). Physical examination reveals a toxic and febrile patient. The skin of the involved hemiscrotum is erythematous and edematous and the testicle is quite tender to palpation. Transillumination of the scrotum often reveals a hydrocele on the ipsilateral side. A large prostate gland is palpated on digital rectal examination in elderly men, while acute prostatitis (a tender, boggy prostate) may be noted in younger men. For acute noninfectious orchitis, the clinical picture resembles the above description, except that these patients lack the toxic appearance and fever. A history of trauma may be elicited from the patient.

For chronic orchitis and orchalgia, there may have been a history of previous episodes of testicular pain, usually secondary to acute bacterial orchitis, trauma, or other causes. The patient has chronic testicular (and possibly epididymal) pain to a degree that could seriously affect his day-to-day functioning and quality of life. Patients with this diagnosis usually become very frustrated with this problem. On examination, the patient does not appear toxic and does not have a fever. The scrotum is not usually erythematous, but the testicle may be somewhat indurated and is almost always tender to palpation.

Laboratory tests employed to assist in the diagnosis include urinalysis, urine microscopy, and urine culture. For a patient in whom an STD is suspected, a urethral swab should also be taken for culture. If the diagnosis is not evident from the history, physical examination, and these simple tests, scrotal ultrasonography should be performed (to rule out malignancy in patients with chronic orchitis/orchalgia). An important differential diagnosis in young men and boys is testicular torsion, which is often difficult to differentiate from an acute inflammatory condition. Scrotal ultrasounds (with use of Doppler to determine testicular blood flow) are especially helpful in differential diagnosis, but occasionally it will miss the diagnosis (particularly with intermittent or partial torsion) and the clinician should err in favor of the surgically correctable diagnosis of torsion (251).

Treatment

General principles of therapy include bed rest, scrotal support, hydration, antipyretics, antiinflammatories, and analgesics. Antibiotic therapy should be employed for infectious orchitis and is ideally based on culture and sensitivity testing, but may be based on microscopic or Gram's stain results. There are no specific antiviral agents available to treat orchitis caused by mumps, and the previously mentioned supportive measures are important. For orchitis associated with cystitis or prostatitis (usually epidymoorchitis), oral trimethoprim/sulfamethoxazole (TMP/SMX), one double-strength tablet (160 mg TMP, 800 mg SMX) twice daily for 3 to 4 weeks is administered. Alternatively, an oral fluoroquinolone can be used, namely, ciprofloxacin 500 mg twice daily or levofloxacin 500 mg once a day. For orchitis associated with gonococcal urethritis, a single intramuscular dose of ceftriaxone, 250 mg, is adequate. Since *Chlamydia* is associated with gonococcal urethritis 30% to 50% of the time (244), a 2- to 3-week course of either oral tetracycline 500 mg four times daily or oral doxycycline 100 mg twice daily is also administered. Alternatively, the fluoroquinolones are effective against both *N. gonorrhoeae* and *C. trachomatis,* and either oral ciprofloxacin 500 mg twice daily or oral ofloxacin 300 or 400 mg twice daily can be given for 2 to 3 weeks. If early testing is negative or results are unavailable, empirical treatment should be initiated, directed at the most likely pathogens, based on the available clinical information; a fluoroquinolone would be the best agent in this scenario. Most patients can be readily managed on an outpatient basis. Surgical intervention

is rarely indicated, unless testicular torsion is suspected (as discussed previously). Spermatic cord blocks with injection of a local anesthetic may sometimes be needed to relieve the patient of severe pain. Abscess formation is rare, but if it does occur, percutaneous or open drainage is necessary.

Treatment for chronic orchitis/orchalgia is supportive. Anti-inflammatories, analgesics, support, heat therapies, and nerve blocks all have a role in ameliorating symptoms. It is generally believed that the condition is self-limited, but could take years (and sometimes decades) to resolve. Orchidectomy is indicated only in cases where pain control is refractory to all other measures (and even this might not be successful in alleviating the chronic pain).

EPIDIDYMITIS

Definitions

Epididymitis by definition is inflammation of the epididymis. Acute epididymitis represents sudden occurrence of pain and swelling of the epididymis associated with acute inflammation of the epididymis (244).

Chronic epididymitis refers to inflammation and pain in the epididymis, usually without swelling (but with induration in long-standing cases), persisting for over 6 weeks (244). Inflammation is not always clinically evident in many cases of localized epididymal pain.

Classification

As for orchitis, there is no universally accepted classification for epididymitis, but the classification we use parallels the one used for orchitis (Table 23-6) (252).

Pathogenesis

Epididymitis usually results from the spread of infection from the bladder, urethra, or prostate via the ejaculatory ducts and vas deferens into the epididymis. The process starts in the tail of the epididymis and then spreads through the body to the head of the epididymis. In infants and boys, epididymitis is often related to a UTI and/or an underlying genitourinary congenital anomaly (253). In young boys, there is evidence that a relationship exists between epididymitis and the presence of a foreskin (254). When an infant or young boy is diagnosed with epididymitis, he should be further evaluated with abdominopelvic ultrasound, voiding cystourethrography, and possibly cystoscopy (244,255). In elderly men, BPH leads to urinary obstruction (and urethral catheterization), stasis, and urine infection. This is usually the cause of epididymitis in this age group. Bacterial prostatitis is associated with epididymal infection in postpubertal males of all ages. In sexually active men under 35 years of age, epididymitis is commonly the result of an STD (244). In most cases of acute epididymitis, the testicle is also involved in the process and referred to as epididymoorchitis.

Chronic epididymitis may result from inadequately treated acute epididymitis, recurrent epididymitis, or some other cause, including associations with other disease processes such as Behcet's Disease (256). The cause of chronic epididymalgia is usually unclear.

TABLE 23-6

CLASSIFICATION OF EPIDIDYMITIS

Acute bacterial epididymitis
Secondary to UTI[a]
Secondary to STD[a]
Nonbacterial infectious epididymitis
Viral
Fungal
Parasitic
Noninfectious epididymitis
Idiopathic
Traumatic
Autoimmune
Amiodarone-induced
Associated with a known syndrome (e.g., Behcet's Disease)
Chronic epididymitis
Chronic epididymalgia

[a] *UTI*, urinary tract infection; *STD*, sexually transmitted disease.

Microbiology

In all age groups, the most common microorganisms responsible for epididymitis are those microorganisms that are the most common cause of genitourinary infection in that group. For example, the most common causative microorganisms in the pediatric and elderly age groups are the coliform organisms that cause bacteruria (257). Likewise, in men under the age of 35 who are sexually active with women, the most common offending organisms causing epididymitis are the usual bacteria that cause urethritis, namely *N. gonorrhoeae* and *C. trachomatis*. In homosexual men practicing anal intercourse, *E. coli* and *Haemophilus influenzae* are most commonly responsible. As with orchitis, viral, fungal, and parasitic microorganisms have all been implicated in epididymitis (244,248,249).

Diagnosis

As for orchitis, the history and physical examination are very important in establishing a diagnosis of epididymitis. Both acute infectious and acute noninfectious epididymitis present in much the same way as do acute infectious and acute noninfectious orchitis, respectively. Physical examination localizes the tenderness to the epididymis (although in many cases the testicle is also involved in the inflammatory process and subsequent pain—referred to as epididymoorchitis). The spermatic cord is usually tender and swollen. Early on in the process, only the tail of the epididymis is tender, but the inflammation quickly spreads to the rest of the epididymis and, if it continues to the testicle, the swollen epididymis becomes indistinguishable from the testicle.

Chronic epididymitis is a frustrating problem for the patient. There may be no clinical or etiologic differentiation between these two chronic conditions of chronic epididymitis and epididymalgia, and they should be considered a similar entity when initiating a management plan. The patient usually presents with a long-standing history of pain (waxing and waning or constant) localized to the epididymis and, like chronic orchitis/orchalgia, these symptoms may have a significant impact on the patient's quality of life (252).

Laboratory tests should include Gram's stains of a urethral smear and a midstream urine specimen. Gram-negative bacilli can usually be identified in patients with underlying cystitis. If the urethral smear reveals the presence of intracellular Gram-negative diplococci, a diagnosis of *N. gonorrhoeae* is

established. If only white cells are seen on the urethral smear, a diagnosis of *C. trachomatis* will be established two-thirds of the time. A urethral swab and midstream urine specimen should be sent for culture and sensitivity testing. If the diagnosis is uncertain, duplex Doppler scrotal ultrasonography to look for increased blood flow to the affected epididymis may be performed (also to rule out torsion as described under orchitis)

Treatment

The general principles of management employed for orchitis are applicable to epididymitis. Specific antibiotic therapy is the same for epididymitis as it is for orchitis and depends upon the clinical scenario (i.e., UTI versus STD). Antibiotics should be continued for 4 weeks.

A 4- to 6-week trial of antibiotics that would potentially be effective against possible bacterial pathogens and, particularly, *C. trachomatis,* is appropriate for chronic epididymitis. Antiinflammatories, analgesics, scrotal support, and nerve blocks have all been recommended as empirical treatment. It is generally believed that chronic epididymitis is a self-limited condition that will eventually "burn out," but this could take years (or even decades). Surgical removal of the epididymis (epididymectomy) should only be considered when all conservative measures have been exhausted and the patient accepts that the operation will have at best a 50% chance of curing his pain (258).

Complications

The two major early complications from acute epididymitis are abscess formation and testicular infarction (244,259,260). Long-term complications are usually related to the development of chronic epididymitis and include testicular atrophy, infertility, and chronic pain (222,226).

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CHAPTER 24 ■ VESICOURETERIC REFLUX AND REFLUX NEPHROPATHY

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The small, irregularly scarred, contracted kidney has been one of the enigmas of medicine. For many years the kidney illustrated in Figure 24-1 was described as "chronic nonobstructive atrophic pyelonephritis." Later, this description was replaced by the term *reflux nephropathy* (1), because the latter emphasizes the central role that vesicoureteric has in its pathogenesis. The precise role of complicating bacterial infections in the etiology of the renal parenchymal scarring has been widely debated. The precise nature of the relationship between primary vesicoureteric reflux (VUR), urinary tract infection (UTI), and renal scarring has stimulated considerable controversy. Elucidation of this relationship has been impeded by having to deal with the end result of the disorder before the pathogenesis has been fully understood. Experimental studies have filled the gap and contributed significantly to its understanding.

This chapter attempts to provide a comprehensive review of current knowledge on this common and important nephrourologic condition.

HISTORY

A description of what is now recognized as reflux nephropathy was given over a century ago (2). "Schrumpfniere" was what clinicians feared if UTIs were untreated or treated inadequately. An early clue to the development of Schrumpfniere came from Leonardo da Vinci in A.D. 1500, who considered that there was free backflow of urine from the bladder to the kidneys. This was illustrated in his anatomic drawings, now housed in the Royal Library at Windsor Castle. In a pen and brown ink drawing (Fig. 24-2) da Vinci drew scars on two small kidneys and showed the ureters opening directly into the bladder. It seems likely that Leonardo had dissected a patient with reflux nephropathy. There is no evidence that he recognized the association, but neither did anyone else for another four centuries.

Galen (3), in A.D. 150, demonstrated that reflux of urine from the bladder to the ureters did not normally occur, thus suggesting a valvular mechanism at the vesicoureteric junction. The anatomy of the intramural ureter was described by Bell in 1812 (4). In 1893, Pozzi (5) reported that during a hysterectomy he accidentally cut the ureter and observed that urine spurted from both cut ends. In 1903, Sampson (6) suggested a relationship between VUR and renal scarring and theorized that the length and obliquity of the intramural ureter was an important factor. Although cystography was first performed in 1905, it was left to Bumpus (7) to point out, in 1924, a direct relationship between VUR and renal scarring. Five years later, Gruber (8) noted that the presence of reflux was related to the development of the trigone and the length of the intravesical ureter.

Löhlein (9), in 1917, was probably the first to offer a clinical account of reflux nephropathy when he described the findings in three young women dying from uremia. Staemmler and Dopheide (10), in 1930, provided a classic description of the pathologic process, while Longcope and Winkenwerder (11) reported nine fatal cases in young women and commented that "it is remarkable to what extent the insufficiency of renal function may advance in these patients without noticeable impairment in health to the individual." In 1939, Weiss and Parker (12) described in detail the pathology of chronic atrophic pyelonephritis and drew attention to the development of this entity in childhood.

The criteria for the radiologic diagnosis of reflux nephropathy were proposed by Hodson (13) in 1959, while Hodson and Edwards (14) made the important observation that the renal damage resulting from VUR was radiographically indistinguishable from chronic pyelonephritis. Interest was then rekindled in the anatomy of the intramural ureter by Hutch (15). Smellie (16) observed that "reflux was seen in all our children with radiologic chronic pyelonephritis in whom a micturating cystogram was performed before puberty." In 1969, Hodson (17) concluded that in his experience the coarse scars of atrophic pyelonephritis were associated with VUR in 100% of cases, while Williams (18) stated that "urinary infections without reflux in childhood are not associated with atrophic pyelonephritis."

At the same time that Hodson and Edwards were performing their studies at the University College Hospital, London, in the late 1950s, Rolleston, Shannon, and Utley (19) became interested in VUR and its relation to renal damage in Christchurch. In 1970, this New Zealand group published their observations on the natural history of VUR in infants and children (20). They stressed the importance of grading the degree of reflux and observed that gross reflux in infants was accompanied by a high incidence of initial and progressive anatomical renal damage (19). The latter could occur even if the urine was sterile. Lesser degrees of reflux were not associated with renal damage. The natural history of reflux was for a spontaneous lessening of its severity and the majority of children with slight or moderate reflux and some of those with gross reflux, who had suffered renal damage, lost the reflux by puberty (21). They also noted that infancy and early childhood were the critical periods for the development of renal damage associated with VUR. Bailey (1) utilized these key observations to propose in 1973 that the term *reflux nephropathy* was more appropriate than "chronic pyelonephritis" to describe the renal damage resulting from severe VUR. The following year, Rolleston, Maling, and Hodson (22) reported the significance of intrarenal reflux (pyelotubular backflow) in the development of focal renal scars.

The observation of intrarenal reflux stimulated the most relevant experimental work yet undertaken on this subject. Hodson and associates (23) showed that in the growing miniature pig VUR in the presence of high voiding pressure caused by placing a metal ring around the proximal urethra was damaging to the kidney, that intrarenal reflux occurred above a critical pressure, and that scarring developed at the sites of intrarenal reflux. Later, Ransley and Risdon (24,25) made the important

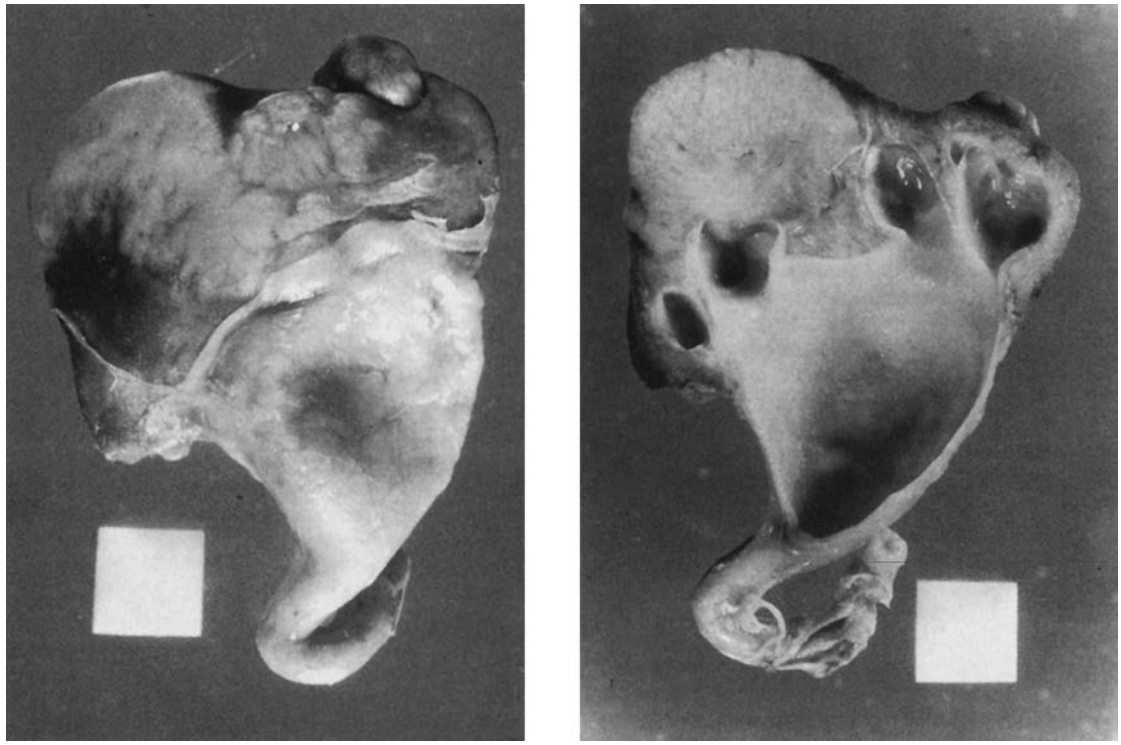


FIGURE 24-1. An example of a kidney showing gross reflux nephropathy. Note the arrest of renal growth with areas of focal cortical scarring and adjacent calyceal clubbing.

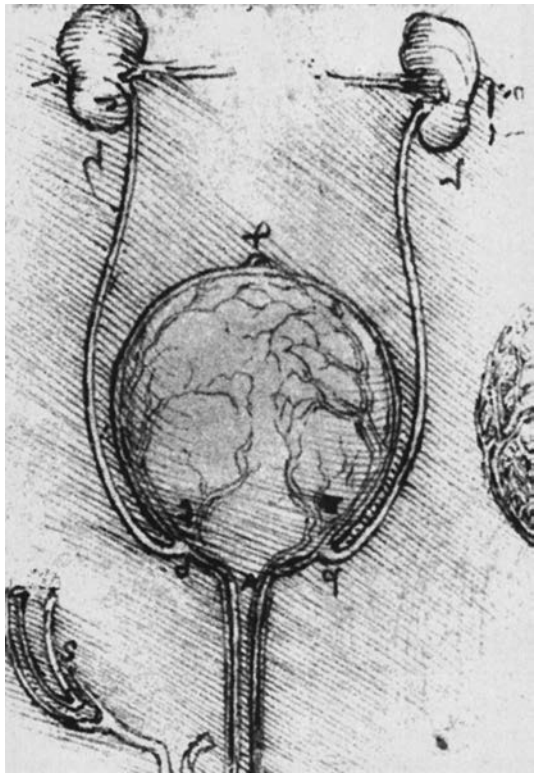


FIGURE 24-2. Drawing by Leonardo da Vinci showing scars on two small kidneys and the ureters opening directly into the bladder. From a collection of anatomic drawings now housed in the Royal Library at Windsor Castle. (From: Asscher AW. *The challenge of urinary tract infections*. London: Academic Press, 1980.)

observation that intrarenal reflux depended on the architecture of the renal papillae. They found that the scars always occurred directly over a renal papilla, were invariably associated with a "refluxing-type" of papilla situated mainly in the renal poles, and developed following reflux of infected urine or high pressure reflux of sterile urine. Such "refluxing" papillae, unlike "nonrefluxing" papillae, had a concave valleylike or deeply indented area cribosa.

Over the past 20 years, research has concentrated on the mechanisms of the complicating hypertension, the pathogenesis of the renal injury including the associated glomerulopathy, improving the organ imaging methods of demonstrating reflux and renal scarring, the indications for antireflux surgery, the familial and genetic aspects, and a search for reliable, noninvasive, and cost-effective methods of diagnosing potentially damaging degrees of reflux at birth or *in utero* (26–31).

PRIMARY VESICOURETERIC REFLUX

Primary VUR is the regurgitation of urine through a vesicoureteric junction rendered incompetent by a congenital defect of the length, diameter, muscle, or innervation of the submucosal segment of ureter. The vesicoureteric junction works normally as a flap-valve, which permits the flow of urine from the ureter into the bladder, but not in the opposite direction. The submucosal segment of ureter holds the key to ureteric continence and is dependent on factors, such as the length of the intramural portion of ureter, the nature of the ureteric orifice, and the integrity of the bladder wall musculature.

The most common explanation for the defective valve mechanism of the vesicoureteric junction is the shortness of the

submucosal segment, because of congenital lateral ectopia of the ureteric orifice (32). When the bladder fills, further shortening of the submucosal segment of ureter may take place, accounting for the observation that reflux may only occur if the bladder is either full or in the act of voiding. The complex anatomy of the vesicoureteric junction and its relationship to the bladder trigone and urethra is best approached from a developmental point of view (33). In its lower 2 to 3 cm, the outer circular layer of muscle of the ureter is replaced by Waldeyer's sheath, a rigid fibrous band that traverses the detrusor muscle obliquely and creates a tunnel for the intramural ureter. There are two mechanisms that prevent urine refluxing into the ureters. The first of these is an increase in bladder pressure, which results in approximation of the walls of the collapsible submucosal ureter that is not encased by Waldeyer's sheath and, second, the contraction of the muscles of the superficial trigone lengthens both the submucosal and intramural ureter and so narrows it even further. Recently, Oswald et al. (34) have paid attention to changes occurring in the intravesical part of the ureteric wall (reduced smooth muscle α-actin, myosin, and desmin expression and increased extracellular matrix and macrophages) and proposed that a congenital muscular structural insufficiency of the distal ureter is important in the pathogenesis of vesicoureteral reflux.

Tanagho and Hutch (35) stressed that the characteristic endoscopic sign of primary reflux was the gaping or golf-hole ureteric orifice. These workers pointed out that in the normal adult the ureteric orifice was 10 to 15 mm below the ureteric hiatus and to give a golf-hole appearance these two apertures would need to be superimposed on each other.

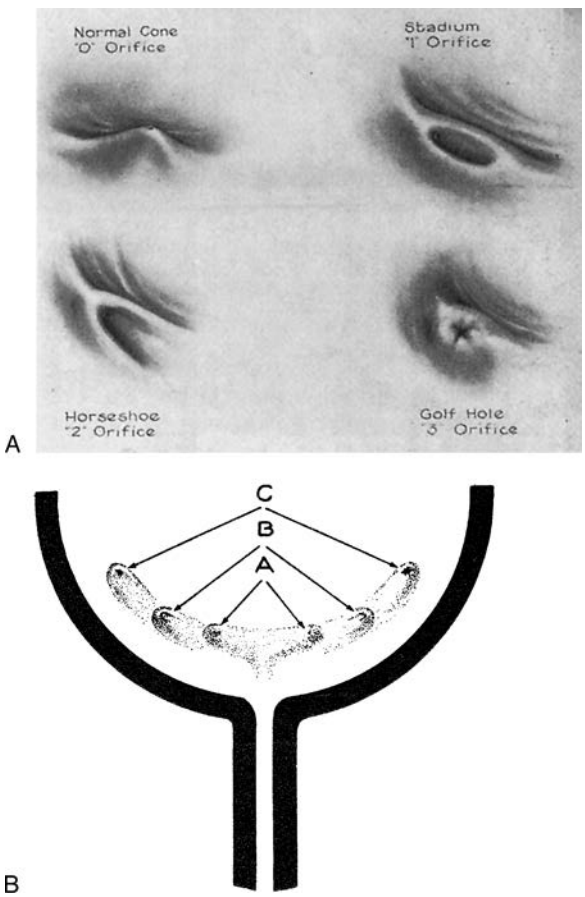


FIGURE 24-3. Appearances of the ureteric orifice configurations in the distended bladder showing various grades of competency (**A**), and positions of the ureteric orifices (**B**). *A,* normal; *B*, moderate degree of lateral displacement; *C,* extreme lateral displacement. (From: Lyon RP, Marshall S, Tanagho EA. The ureteral orifice: its configuration and competency. *J Urol* 1969;102:504, with permission.)

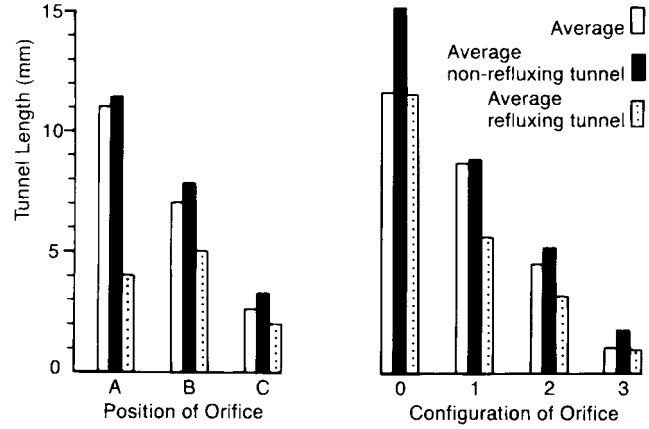


FIGURE 24-4. Average tunnel length related to grade and position of the ureteric orifices. (From: Vermillion CD, Heale WF. Position and configuration of the ureteral orifice and its relationship to renal scarring in adults. *J Urol* 1973;109:579, with permission.)

Hutch (15) proposed the maturation theory, such that as a child grows, the intravesical ureter lengthens, and the tendency for reflux to diminish or disappear with age. Lyon et al. (36) using endoscopic evaluation, both visually and with cinefilm, studied the configuration and position of 171 ureteric orifices in 330 girls and correlated these observations with the presence of VUR (Fig. 24-3). Vermillion and Heale (37) performed cystoscopy on 67 patients over the age of 12 years with known reflux nephropathy. Of the 134 ureteric orifices, 97 were associated with renal scarring and 45 still had demonstrable VUR. All orifices draining ureters with continuing reflux were abnormal. Of the ureteric orifices associated with renal scarring, but no longer refluxing, 41 of 52 (79%) were found to have abnormalities of such a degree that reflux seemed likely to have occurred in the past. As the orifice became more abnormal in position and configuration, the average tunnel length decreased (Fig. 24-4). The average length of the ureteric tunnels associated with reflux was shorter than the length of those in which reflux was no longer demonstrable. Cystoscopic assessment of the position and configuration of the ureteric orifices is of value, particularly when assessing a patient with a scarred kidney in whom VUR is no longer present.

Yucel and Baskin (38) have described in detail the neuroanatomy of the distal ureter and vesicoureteric junction. At the vesicoureteric junction, the nerves encircle the entire ureter and travel just outside Waldeyer's sheath, leaving a safe area for surgical dissection under the sheath. As the ureter tunnels into the bladder, the nerves localize to the lateral wall of the detrusor muscle. Careful dissection within Waldeyer's sheath during ureteral reconstructive surgery is critical for preserving bladder function. If this neural network is injured during dissection, it can lead to decreased contractility of the affected detrussor side (39).

INTRARENAL REFLUX

Another radiologic phenomenon has been recognized as important in patients with VUR. This is the entity of intrarenal reflux in which contrast medium can be shown to flow into the renal parenchyma during voiding cystourethrography and

opacifying segments of kidney by means of pyelotubular backflow (22,40,41).

The earliest type of pyelorenal backflow described was pyelovenous. In 1856, Gigon (42) demonstrated with the starch test the intrusion of potassium iodide from the pelvic cavity into the renal veins in fresh cadaver kidneys and that this depended on the intrapelvic pressure. This observation was supported by Ludwig and Zawarykin (43), but attracted little attention until retrograde pyelography was introduced 43 years later (44). Blum (45) was the first to report in the English literature pyelorenal reflux occurring *in situ* in humans. Köhler (46) studied 628 retrograde pyelograms and found that the pressure necessary for the development of backflow in clinically normal kidneys was 80 to 100 mm Hg. Pyelorenal reflux occurred at lower pressures in damaged kidneys.

Rolleston et al. (21) directed attention to the importance of intrarenal reflux by reviewing 386 voiding cystourethrograms in children known to have VUR. Intrarenal reflux was observed in 20 kidneys from 16 children. Ten of these 16 children were under 1 year of age and intrarenal reflux was not observed in any patient over the age of 4 years. Intrarenal reflux was present in 6.7% of the voiding cystourethrograms in children below the age of 5 years. Renal damage characteristic of reflux nephropathy was shown in 13 of the 20 kidneys with intrarenal reflux and, in 12 of the 13, the damage corresponded exactly to those segments of the kidney in which intrarenal reflux had been demonstrated. Maling and Rolleston (47) described the radiologic appearances of intrarenal reflux (Fig. 24-5) and the techniques necessary for demonstrating it. Subsequently, Bourne et al. (48) found intrarenal reflux in 8 (13.5%) of 119 children with known VUR. All eight patients were under 6 years of age. There was a high correlation between the areas of intrarenal reflux and the associated cortical atrophy and calyceal clubbing. Uldall et al. (49) found intrarenal reflux in 7 (9.2%) of 76 children, with a total of 123 ureters showing VUR, and observed that focal renal damage was confined to the site of intrarenal reflux. Reifferscheid et al. (50) studied 74 children under the age of 6 years and found intrarenal reflux in 21 (28%).

Intrarenal reflux almost certainly occurs more commonly than is diagnosed on standard cystography. The critical intrapelvic pressure required to produce intrarenal reflux in susceptible papillae is about 45 cm of water, which is close to the filtration pressure (23,41).

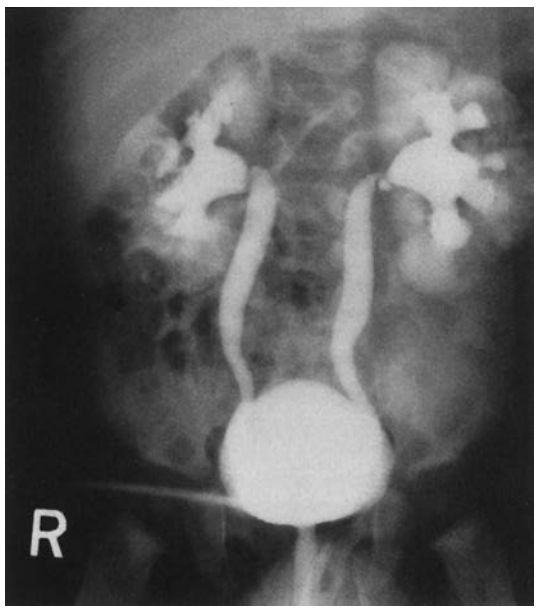


FIGURE 24-5. Voiding cystourethrogram in a 10-month-old girl showing bilateral VUR (Christchurch grade IV) and widespread intrarenal reflux.

RENAL PAPILLAE

Ransley and Risdon (51) studied the kidneys of 18 children aged between 2 days and 16 months who died from nonrenal causes and were examined at autopsy. As in the piglet, extensively fused papillae of the type associated with intrarenal reflux occurred almost exclusively at the upper and lower poles of their kidneys. However, the incidence of this kind of papilla was lower in human kidneys and, in over 25% of the specimens examined, only cone-shaped papillae were found.

Funston and Cremin (52) studied kidneys obtained at autopsy from 100 infants and children dying from nonrenal causes and showed that the ratio of compound to simple papillae was 1:4 and that the majority of compound papillae occurred in the upper poles. Injection of barium at measured pressures showed that the compound papillae were the first to exhibit intrarenal reflux and that intrarenal reflux occurred at much lower pressures (22 mm Hg) in infants under the age of 1 year.

Tamminen and Kaprio (53) investigated the morphology of the collecting duct openings of different types of papillae in infant and child cadaver material. They assessed whether there were morphologic differences on the papillary surface in the younger age groups that may suggest why intrarenal reflux was more commonly detected in the first few years of life. Two-thirds of the kidneys studied had nonconvex papillae with vestibular openings, and it was the only type of papilla that allowed intrarenal reflux. It was suggested that the relatively large size of the duct openings was a reason why neonates and younger infants were predisposed to detectable intrarenal reflux and the formation of renal scarring.

BLADDER DYSFUNCTION

Increasing evidence suggests that bladder dysfunction or dysfunctional elimination syndrome is common in patients with VUR and that may contribute to the severity and persistence of the reflux and to the development of reflux nephropathy (54,55). Two patterns of bladder dysfunction have been recognized, detrusor instability and detrusor–sphincter dyssynergia (56,57). Detrusor instability is characterized by urinary obstruction during bladder filling, because of voluntary sphincter contractions in an appropriate attempt to maintain continence during uninhibited bladder contractions. Symptoms associated with detrusor instability are frequency, urgency, incontinence, and holding maneuvers, such as squatting. Detrusor–sphincter dyssynergia is characterized by obstruction during voiding, because of voluntary contractions of the urinary sphincter. The symptoms associated with detrusor–sphincter dyssynergia are staccato voiding, urgency, diurnal and nocturnal incontinence, and recurrent UTIs. It often results in increased residual urines and high bladder capacity. It is a more severe form of bladder dysfunction than detrusor instability, and it is more often associated with increased severity of the VUR and renal damage. Detrusor instability occurs in 8% to 75% and detrusor–sphincter dyssynergia in 8% to 68% of children with VUR (58–60). They can occur in infants, as well as in older children, and are viewed by some as an inherited congenital phenomenon (61).

Chen et al. (62) have recently quantified the relationship between dysfunctional elimination syndrome and gender, UTI, and VUR in 2,759 pediatric patients who underwent renal sonography and voiding cystourethrography at a referral

practice. They found that girls had a significantly higher rate of dysfunctional voiding than boys (36% versus 21%) and that UTI significantly impacts the occurrence of dysfunctional voiding in patients with VUR.

PREVALENCE OF VESICOURETERIC REFLUX IN HEALTHY INFANTS AND CHILDREN

The prevalence of VUR in healthy infants and children is uncertain. In 1916, Kretschmer (63) reported unilateral reflux in 3 of 10 healthy children aged from 3 to 10 years and concluded that reflux may occur in normal children. The cystourethrograms, however, were done under ether anesthesia and irritative contrast media were used. Prather (64) reviewed the early literature and found a wide divergence of opinion. Most stated, however, that reflux never occurred normally.

Gibson (65) undertook voiding cystourethrograms on 43 children, aged up to 12 years,who had no symptoms or signs of urinary tract disease. He injected contrast medium using a 50-mL syringe until the bladder pressure was "sufficient to cause the plunger of the syringe to move backward." A boy, aged 13 months, and a girl, aged 16 months, showed unilateral reflux. Gibson concluded that reflux may occur in children who have an apparently normal urinary tract. Campbell (66) expressed doubt as to whether reflux was ever present in the absence of demonstrable pathologic changes. Of 722 unanesthetized infants and children, reflux was not found in the absence of a urologic abnormality. Iannaccone and Panzironi (67) examined 50 healthy infants aged 10 days to 6 months. Unilateral reflux was found in one infant at 14 weeks of age. This infant had no reflux when earlier studied at 10 days and again at 4 months of age. Kjellberg et al. (68) undertook cystograms on 101 children, aged 2 days to 13 years, who had no urinary tract signs or symptoms. They were unable to demonstrate reflux. Jones and Headstream (69) studied 70 boys and 30 girls (aged 14 days to 14 years; mean age 3.5 years) with a nonurologic hospital admission. Only one showed reflux. Subsequent examination of this 4-month-old boy revealed a bladder neck contracture.

McGovern et al. (32) reviewed the literature, including 330 children, under the age of 14 years, who were free of urinary tract symptoms. Six (1.8%) were found to have primary reflux, but several did not withstand a critical appraisal. These authors concluded that reflux was rare in normal children. Lich et al. (70) found no reflux in 26 newborn infants.

The observations of Köllermann and Ludwig (71) are at variance with the above studies. These workers examined 102 children under the age of 5 years hospitalized for a nonurologic reason. Thirty percent showed reflux, all under the age of 3 years. There is criticism of this study, however, as 60% urografin was injected rapidly from a syringe.

Peters et al. (72) studied 66 healthy premature infants (48 boys) using suprapubically injected contrast medium. None showed reflux. Importantly, 56 of these infants were blacks, in whom reflux is known to be uncommon. These authors reviewed the literature and found 6 cases (0.6%) of reflux among 1,015 normal infants and children. The authors misread one paper (70); the total should have been 4 (0.4%).

Many of the above reports can be criticized because children of all ages were examined, there was inadequate information on the radiologic technique used, and, in some, it was unclear as to why the studies were done. Most importantly, there was insufficient information given concerning the degree of reflux.

Bailey (73) reviewed the published literature until 1979 and concluded that reflux may be present in 0.4 to 1.8% of healthy children. Abbott et al (74), however, suggested that it may be higher. These authors studied 50 infants under the age of 1 year who were hospitalized for a variety of reasons but not thought to have a UTI. All infants had a voided urine culture of greater than 10^8 organisms/L ($>10^5$/mL) and then had an uncontaminated specimen taken by suprapubic bladder aspiration. After urine was aspirated for culture, 30% urovision was instilled through the same needle into the bladder by gravity and a single radiograph taken as the infant voided. Of the 50 infants, five had asymptomatic bacteriuria and two of these five had VUR. Of the remaining 45 infants with sterile bladder urine, 4 (9%) had reflux (3 moderate and 1 gross). The infant with gross reflux had a repeat cystogram 6 weeks later and the reflux had disappeared. Bourchier et al. (75) demonstrated that reflux may be present in some premature infants, but that it had ceased by the time of their expected maturity. In a recent review, Arant (76) did not consider some of the important studies mentioned above when he concluded that "VUR in humans should be considered rare."

Recent studies using ultrasonography have provided a more precise approximation of the prevalence of VUR in the neonate. A large population-based prospective study at Christchurch Hospital using antenatal ultrasound screening showed that VUR occurs at a rate higher than 1.2% births (77). Gunn et al. (78) performed ultrasonography on 3,856 fetuses after 26 weeks of gestation. When anomalies were identified, the newborns and babies were evaluated at 6 days and 6 weeks of age. Dilatation of the pelvis was found in 298 of the fetuses, but it was not verified postnatally in 216. VUR was identified in 14 children of the remaining 82. The incidence of VUR was estimated to be 0.36:100 births. Tsai et al. (79) performed ultrasonography on 2,384 asymptomatic neonates. Voiding cystourethrography was performed in cases with moderate to severe hydronephrosis or persistent mild hydronephrosis. VUR was diagnosed in 30 infants with a prevalence of 1.26%. Hiraoka (80) studied 2,000 newborns using voiding ultrasonography. Those who showed transient renal pelvic dilatation during voiding underwent voiding cystourethrography. VUR was detected in 0.8% of the neonates. Because of the low sensitivity of ultrasonography to detect VUR, these figures underestimate its true prevalence at birth.

PREVALENCE OF VESICOURETERIC REFLUX IN RELATION TO AGE, GENDER, AND RACE

Chand et al. (81) reviewed the records of 15,504 children who underwent a voiding cystourethrogram or radionuclide cystogram between 1993 and 2001 to determine if the incidence and grade of vesiocoureteral reflux (VUR) differs in children based on age, race, and gender, and if the incidence and severity of VUR are related to race in girls younger than 7 years presenting for evaluation after UTI. Overall, black children were one-third as likely as white children ($p < 0.0001$) and females were twice as likely as males ($p < 0.0001$) to have VUR. Compared to children 0 to 2 years old, the occurrence of reflux was 0.5 times as likely in those 3 to 6 years old ($p < 0.0001$), 0.3 times as likely in those 7 to 11 years old ($p < 0.0001$), and 0.15 times as likely in those 12 to 21 years old ($p < 0.0001$). When analyzing children with UTI, results were similar. Of the patients with VUR, 65% were younger than 7 years. The incidence of VUR in black girls young than 7 years with a diagnosis of UTI was less than 10% compared to white girls, and

no black girl had high-grade reflux. In young children referred for UTI, the incidence and severity of VUR in black patients were significantly lower than those of white girls (81).

SECONDARY VESICOURETERIC REFLUX

Secondary VUR may be a complication of inflammatory disorders involving the vesicoureteric junction, such as tuberculosis and bilharziasis. It may also be a sequel to bladder neck obstruction, interstitial irradiation of the bladder, and surgery to or near the ureteric orifice, and associated with other congenital anomalies or with neurogenic bladder defects.

Although the position and configuration of the ureteric orifice and the length of the intramural ureter are major factors in determining the competence of the vesicoureteric junction, there are other factors that may influence whether a junction of marginal competence could permit reflux. Such factors include lower urinary tract dysfunction with unstable bladder contractions and high-pressure voiding and the development of a paraureteric saccule or diverticulum in the neuropathic bladder as described by Hutch (33).

UTI with associated bladder inflammation does not appear to be a primary cause of reflux when the submucosal segment of the ureter is normal in all other respects. If this segment is defective in some way, bacterial infection may temporarily induce reflux, but this is not usually of a severe degree.

Neurogenic Bladder and Vesicoureteric Reflux

Meningomyelocele and Myelodysplasia

In children with meningomyelocele, the incidence of reflux appears to increase with age. Woods and Atwell (82) found that at 1 month of age, 18 of 64 children had reflux, but by 5 years of age this had increased to 29. Although urodynamic studies were not included in this report, the increase with age may reflect developing lower urinary tract dysfunction.

McGuire et al. (32) studied 42 children with myelodysplasia and found 35 with detrusor areflexia. Of the latter, 5 had flat detrusor pressures during bladder filling and the remaining 30 had steeply rising low-compliance patterns. These workers did not find reflux in the eight children in whom the end-filling pressure at which urethral leakage occurred was less than 40 cm of water. However, of the 22 children in whom the pressure to produce leakage was higher than 40 cm of water, 15 showed reflux.

Cohen and co-workers (83) reviewed the records of 180 patients (98 males; mean age 9.3 years) with myelodysplasia and an intact urinary tract who had been followed in a multidisciplinary spina bifida clinic for 3 to 18 years. Seventy-two (40%) patients had a total of 103 renal tracts with VUR. Of the latter, 45 were classified as grades III through V on international classification. Seventeen of these 72 patients underwent ureteric reimplantation, because of persistent high-grade reflux, or progressive renal parenchymal damage. Renal scarring was found in 19 girls and 9 boys, was bilateral in 7, and affected the left kidney in two-thirds. Three-fourths of the patients who had developed renal scarring had documented VUR. As anticipated, renal scarring correlated directly with the grade of reflux, affecting 33% of those kidneys with grade III, 56% with grade IV and 100% with grade V reflux.

Spinal Cord Injuries

The incidence of VUR in spinal cord injury patients is not well documented, but over the last few decades better urologic management of the bladder has probably reduced the occurrence (84). The data are incomplete, however, because most early reports were retrospective and only patients with a clinical problem were studied.

It is possible that some patients sustaining a spinal cord injury will have had reflux in childhood and will have a preexisting marginally competent vesicoureteric junction (85). The superimposed neurologically disordered lower urinary tract function in these patients may then be sufficient to compromise its function and allow reflux. Once VUR has commenced, the intrarenal reflux of urine (often infected) seems to be associated with the development of reflux nephropathy (Fig. 24-6), just as in the infant with primary reflux.

It is important to note that reflux is not confined to high-pressure uninhibited bladders. Damanski (84) found 43 of 154 such bladders to have reflux, but this was also present in 13 of 68 flaccid areflexic bladders. During vigorous Credé or Valsalva maneuvers to express and empty the bladder of patients with flaccid areflexic bladders, transmitted bladder pressures of up to 100 cm of water or more can be achieved for short intervals. While this abdominal pressure is transmitted to the renal pelvis as well as the bladder, if this is not equal, then in a refluxing system intrapelvic pressures could be raised significantly.

In a retrospective analysis of 280 patients with spinal cord injuries seen in Christchurch between 1965 and 1976, with a minimum follow-up of 8 years, 28 (9.3%) had radiologic appearances of reflux nephropathy (Arnold, personal communication). The incidence of VUR, however, would have been much higher. The incidence of renal scarring in those who do have reflux is, therefore, not yet established.

In a prospective study, Arnold et al. (86) found VUR in 6 of 58 spinal injury patients followed by repeated urodynamic studies for 3 years. The reflux was discovered between 4 and 24 months (mean 12.4 months) after injury. Two of the six developed reflux nephropathy with maximum detrusor pressures of 35 and 70 cm of water. It would appear that VUR and reflux nephropathy usually occur early after the injury, at a time when bladder dysfunction is changing and unbalanced.

There has been considerable interest in the time necessary for the full development of a parenchymal scar. The reason for this is that most infants and children with severe grades of primary VUR already have renal scarring at presentation and the documented development of new renal scarring is uncommon (87,88). Rolleston (89) has used the spinal injury patient as a model for the development of the scarring process. About 10% of such patients develop VUR soon after their injury, thus enabling the start of the scarring process to be fairly accurately assessed. In a meticulously studied small group of patients, the development time of the scars varied from 14 to 25 months (mean 21 months), although contraction was maximal during the first year.

The need to recognize lower urinary tract dysfunction and to manage it early is being more widely appreciated in spinal injury units, where urodynamic studies are an integral part of patient monitoring and management. It is to be hoped that this will reduce the incidence of secondary VUR and resulting reflux nephropathy.

Vesicoureteric Reflux into the Transplanted Kidney

The clinical significance of VUR in the transplanted kidney has been controversial. In 1977, Mathew et al. (90) reported that VUR may be an important cause of late failure of renal allografts. Graft failure occurred in 14 of 29 refluxing transplants compared with 14 of 90 without reflux. In the refluxing group, failure of the graft was slowly progressive and associated with

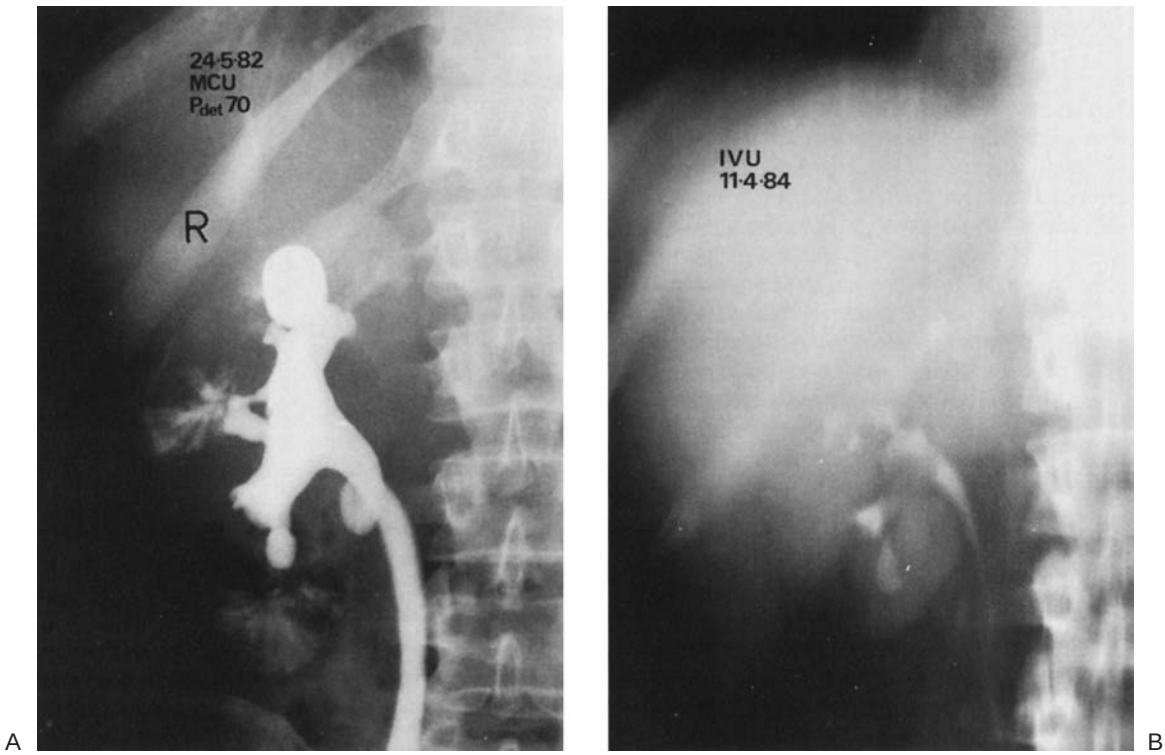


FIGURE 24-6. A: A voiding cystourethrogram in a 33-year-old man, 3 months after becoming a partial quadriplegic and suffering several episodes of acute pyelonephritis. Grade III VUR (Christchurch classification) and areas of intrarenal reflux are demonstrated at a detrusor pressure of 70 cm of water. A calyceal diverticulum is present in the upper pole. **B:** An intravenous urogram, almost 2 years later, shows areas of focal scarring best seen in the lower pole. The scars are at the sites of the previously demonstrated intrarenal reflux.

increasing proteinuria, microscopic hematuria, hypertension, and a mesangiocapillary glomerulopathy. Is this a specific lesion or chronic rejection producing an allograft nephropathy? Grunberger et al. (91) also reported a 10% difference in graft survival between the patients without and with VUR into the transplant. McMorrow et al. (91a), however, were unable to confirm these observations. More recent studies have not found any significant effect of VUR on the medium and long-term outcome of renal transplantation (92–94).

Thomsen (95) undertook retrograde pyelography on 239 grafts transplanted between 1971 and 1980. Pyelotubular backflow was present in 34 grafts and was seen in one-third of those patients whose transplants were removed within 60 days, because of rejection, but in only 4% of those with surviving grafts. It has been hypothesized that VUR may increase graft immunogenicity through increased MHC molecule expression. This hypothesis has not been supported by Coosemans et al. (96), who found no difference in the number of rejection episodes and early graft survival between the patients without or with VUR.

While VUR into the transplanted kidney seems to be of limited significance in adults, the same may not be true in the pediatric population. A number of small studies have shown that VUR into the graft system is a significant risk factor for acute perinephritis and renal damage in children (96–98). A study by Fontana et al. (99) did not find significant difference in renal function of graft survival between children without and with VUR. This study, however, is less than reassuring, because all children with grades I to III VUR were kept on chronic antimicrobial prophylaxis, and those with grade IV reflux were surgically corrected. Therefore, it seems reasonable to recommend that ureteroneocystostomy in young transplant recipients be performed using a careful antireflux technique.

There is no indication for pretransplantation surgical correction or nephroureterectomy for those patients with end-stage reflux nephropathy who still have VUR to reduce the risk of UTI following transplantation, except possibly for those with recurrent urinary sepsis, bacteremia, or urinary calculi, while maintained on dialysis (100,101).

PATHOLOGY OF REFLUX NEPHROPATHY

The recognition of the entity of reflux nephropathy has been a major step in elucidating the pathology of chronic pyelonephritis. Heptinstall (102) reviewed the phases in the pathologic diagnosis of chronic pyelonephritis. The role of urinary tract obstruction in establishing and maintaining bacterial infection in the kidney has long been appreciated by pathologists and the entity of "chronic nonobstructive pyelonephritis" has been a puzzling exception. It is now clear that the pattern of renal injury resulting from VUR corresponds well with descriptions of both chronic nonobstructive pyelonephritis and the Ask-Upmark kidney (103,104).

An understanding of the pathology of human reflux nephropathy has largely been built by the observations made on the pig experimental model (23–25,105,106). Although it is now clear from these studies that intrarenal reflux of sterile urine at high pressure can lead to focal scarring, there is still controversy as to whether such scarring can be produced in humans without infection. The reason is that it is difficult to establish that a newly diagnosed patient with reflux nephropathy has never suffered from a urinary infection. Consequently, the pathology of reflux nephropathy in humans has

largely been inferred from descriptions of chronic pyelonephritis. There are few explicit accounts of the pathology of human reflux nephropathy.

The pathologic findings in human reflux nephropathy are based mainly on the examination of kidneys removed because of hypertension or uncontrollable UTIs, or prior to renal transplantation. The pattern of evolving reflux nephropathy can also be inferred from appearances in the kidneys of paraplegic patients. Pathologic appearances depend on the severity of VUR and the presence of intrarenal reflux and UTI.

Where gross reflux is continuing, the ureter is tortuous, dilated, and hypertrophied, and there may be fairly generalized dilatation of the pelvicalycine system with shrinkage of the pyramids and hypertrophy of pelvicalycine smooth muscle so that the appearances are similar to a pure obstructive process. However, there is a tendency for the degree of atrophy to vary from one lobe to another. Macroscopic evidence of infection may be minimal. Microscopically there is tubular atrophy in the cortex with regressive changes in the medulla similar to those seen in less severe forms.

Nephrectomy specimens show the changes that are associated specifically with reflux nephropathy. In these cases, reflux may be less than gross or even have ceased completely, leaving a scarred kidney. Many of these kidneys may weigh only 15 to 30 g. Areas of gross cortical atrophy alternate with areas of normal cortex. There is a marked tendency for atrophy to be concentrated at the poles of the kidney, especially the upper pole, but the pattern is extremely variable. Careful examination shows the large flat areas of atrophy to correspond with the renal lobes. Because the papillae at the poles are mainly of a compound reflux type, polar scars are usually extensive, representing atrophy of several lobes. Smaller irregular areas of scarring are also seen in other lobes, typically in lobe centers and in the form of bands and wedges. Where infection has been a prominent clinical feature, the capsule may be thickened and adherent. The degree of lobar atrophy is variable and, in extreme cases, the combined width of cortex and medulla may not exceed 1 mm. The corresponding calyx is greatly expanded by atrophy of the papilla and outer medulla. However, other calyces appear normal, and the ureter may be of normal caliber, although thick-walled. Inflammatory changes are frequently inconspicuous. Risdon et al. (107) emphasized that congenital maldevelopment of the kidney is also a crucial factor in the development of reflux nephropathy in young children, particularly males.

Histologic progression of atrophy can be traced. Tubular atrophy advances with diminution of tubular diameter and thinning of epithelial cells. Atrophic tubules commonly contain colloid casts and some tubules may undergo cystic dilatation (Fig. 24-7). Glomeruli are initially approximated by tubular atrophy and then undergo hyalinization. In long-standing cases, they may disappear entirely. The interstitium is fibrotic with a sparse lymphocytic infiltrate. Arterioles and interlobular arteries undergo obliterative changes with contraction and intimal thickening. In the outer medulla, atrophic tubules with flattened epithelium are often prominent with the appearance of thyroidization, classically associated (albeit erroneously) with chronic pyelonephritis. In some cases, focal "blow-out" lesions may be seen in the outer medulla and cortex. These consist of rounded masses of pale-staining material, which include Tamm-Horsfall protein, apparently extravasating from ruptured tubules into the interstitium. Such foci are frequently surrounded by aggregates of lymphocytes and plasma cells. In the inner medulla, loops of Henle have disappeared, and only a few markedly dilated ducts of Bellini remain. They are surrounded by an interstitium containing large numbers of spindle cells. Sometimes these are arranged concentrically around collecting ducts, a pattern that is associated with marked hypertrophy of pelvicalycine smooth muscle. Frequently, focal calcification is present both within dilated ducts and in the interstitium. Chronic inflammatory changes are often striking in the inner medulla and calyces, even when cortical inflammation is minimal and when urinary infection is absent. Heavy lymphocytic infiltration of medullary interstitium and of calycine lamina propria may include follicle formation.

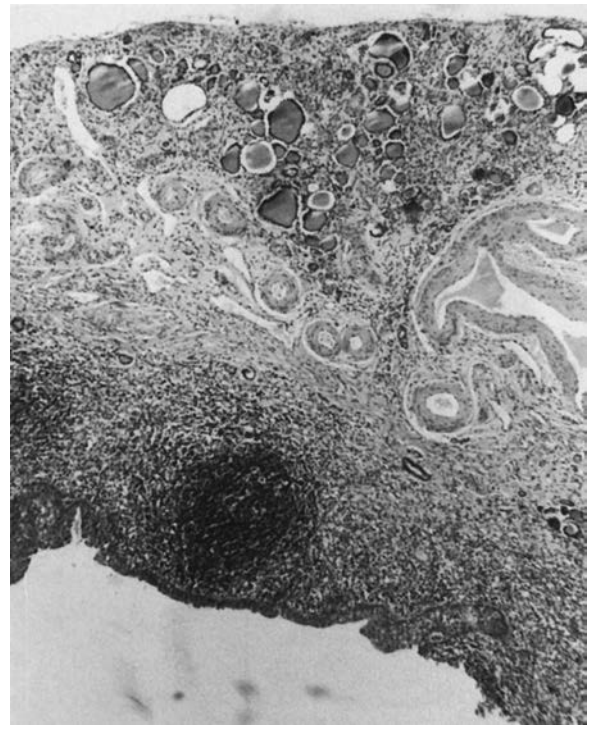


FIGURE 24-7. Section from a scarred area in a kidney from a woman aged 22 years with sterile reflux nephropathy. In the cortex no glomeruli can be identified. Most tubules are atrophic, but some are dilated and contain "colloid." Arteries are thick walled. There is a heavy chronic inflammatory infiltrate in the thinned atrophic medulla with a lymphoid follicle. (Hematoxylin and eosin. Magnification ×70.)

While the classic pattern of reflux nephropathy is expressed in total lobar atrophy, it is evident that intrarenal reflux may involve some ducts of Bellini in some pyramids, while sparing others. This results in macroscopic cortical scarring in a smaller and more irregularly disposed form. Corresponding radial atrophic and inflammatory changes involving part of the lobe are histologically evident.

In a proportion of cases where continuing UTI has been noted, cortical atrophic changes are accompanied by a heavy inflammatory reaction in a radial distribution extending from the medulla and involving all or parts of a lobe. There is corresponding radial inflammation in the medulla. Periglomerular fibrosis is usually obvious and frequent. Scanty neutrophils may be seen in occasional cortical tubules, as well as in the medulla.

The wide range of changes seen macroscopically and microscopically extend from gross atrophy, with minimal inflammatory change to heavily inflamed and less atrophic kidneys falling within the ambit of chronic pyelonephritis. It is the integration of cortical atrophy with segmental or duct reflux that validates the pathological entity of reflux nephropathy.

Glomerular Lesion

While some patients with severe bilateral reflux nephropathy may develop renal failure because of severe scarring, a further

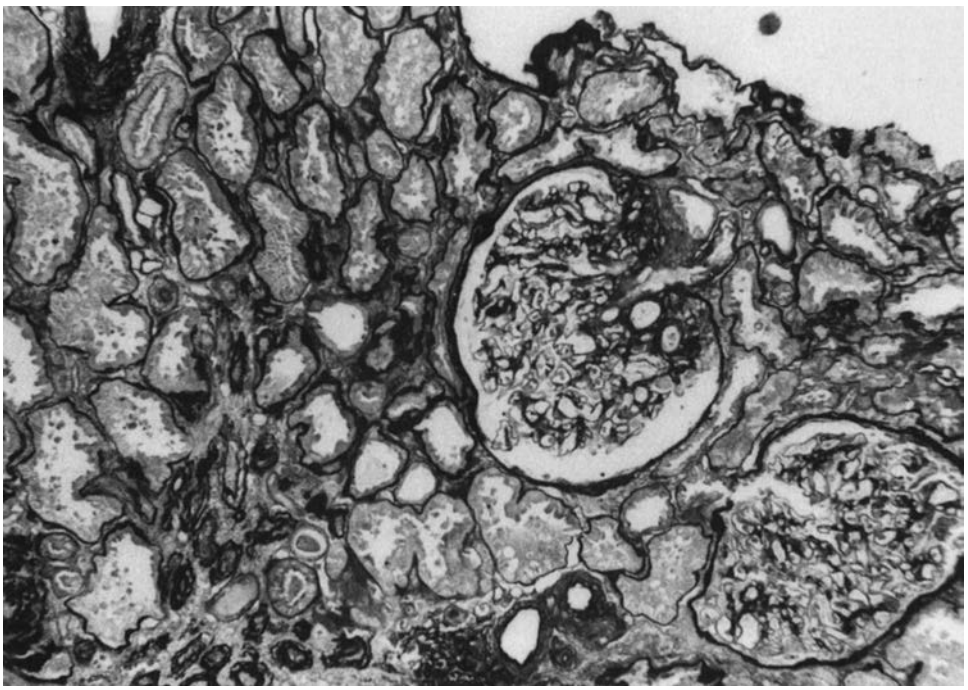


FIGURE 24-8. Biopsy specimen from the hypertrophied left kidney of a 49-year-old man with severe reflux nephropathy in the right kidney. One hypertrophied glomerulus shows sclerosis of segments adjacent to the stalk. (Silver methenamine. Magnification ×175.)

group has been defined whose renal function deteriorates despite reflux having spontaneously ceased or having been corrected surgically, and in the absence of complicating UTI. The latter group is characterized by persistent proteinuria and less consistently hypertension. This histologic hallmark is the hypertrophy of surviving nephrons, with focal and segmental glomerulosclerosis and hyalinosis involving unscarred segments of kidney or the structurally normal contralateral kidney in patients with unilateral reflux nephropathy (Fig. 24-8). Morita et al. (108) reported a strong positive correlation between the extent of glomerular involvement and both the amount of proteinuria and the reduction of glomerular filtration rate (GFR).

In the early chronic pyelonephritis literature, proteinuria was a constant feature in patients developing uremia. A variety of glomerular lesions were described in scarred tissue, including segmental and global sclerosis. The relationship of these lesions to the vascular obliterative lesions of chronic pyelonephritis has not been clearly defined.

Several authors have reported finding focal and segmental glomerulosclerosis in both children (108–111) and adults (112–115) with reflux nephropathy, who developed chronic renal failure. Most of these patients had proteinuria exceeding 2 g/day. Zimmerman et al. (110) examined nephrectomy specimens from eight young patients with bilateral reflux nephropathy and renal failure. In six of these, the daily protein excretion was over 2.3 g. Apart from changes of chronic pyelonephritis, they also noted focal and segmental glomerulosclerosis with hyalinosis. Sclerosed glomeruli contained IgM, complement, and subendothelial electron-dense deposits. Kincaid-Smith (112) reported focal and segmental glomerulosclerosis in 88 of 105 patients with reflux nephropathy, including all 52 with end-stage renal failure. In four cases, this glomerular lesion was demonstrated in the normal contralateral kidney of patients with unilateral reflux nephropathy. Torres et al. (114) described 54 patients with reflux nephropathy. Although the majority presented with renal failure, in some the renal biopsy was done because of proteinuria. The most common lesion was focal and segmental glomerulosclerosis with widespread glomerulomegaly. Deposits of IgM, C3, and properdin were present in sclerosed segments. Antireflux surgery did not influence the development of these glomerular lesions. Not all reports agree. Marek and Dvoracek (104) presented a series of nephrectomy specimens from children and adults with reflux nephropathy that did not show focal and segmental glomerulosclerosis, but only severe scarring and interstitial damage. Acute poststreptococcal glomerulonephritis may cause an acute deterioration of renal function in patients with reflux nephropathy; recovery has been reported to be poor (116).

Cotran (117,118) discussed some of the mechanisms that may lead to focal and segmental glomerulosclerosis in patients with reflux nephropathy. Based on studies in a rat model, Hostetter et al. (119) constructed an elegant hypothesis that may explain the development of this glomerular lesion. They proposed that hemodynamic adaptation by surviving glomeruli to the loss of renal mass leads to glomerulosclerosis. The hypothesis is that each surviving glomerulus is subjected to a supranormal single-nephron blood flow causing raised intraglomerular pressure and enlargement of glomerular capillaries (glomerulomegaly). This causes stretching and widening of the slit pores of the epithelial cells, resulting in proteinuria, sclerosis, and filtration failure. Clinical observations suggest that a maladaptive response to a loss of renal mass may be a cause of glomerulosclerosis and progressive renal damage in reflux nephropathy. Proteinuria may not appear for many years after severe scarring has occurred, and the proteinuria increases as renal function declines. Hypertension is a late complication. The change in renal hemodynamics may explain why this glomerular lesion may affect the contralateral unscarred kidney in patients with unilateral reflux nephropathy (120).

Focal and segmental glomerulosclerosis, therefore, appears to be the most common glomerular lesion found in end-stage reflux nephropathy. The relative contributions to chronic renal failure from chronic obliterative vascular disease/scarring and the development of a secondary glomerular disease are still uncertain. Matsuoka et al. (121) undertook open renal biopsies on 49 patients (43 children) with coarse renal scarring and/or with more than grade III reflux. Most cases of glomerulosclerosis were global, while focal segmental glomerulosclerosis was

uncommon. Attention paid to the tubulointerstitium and the relationships between the cellular infiltrates (mainly T4 cells) and glomerular, tubular, and vascular damage (122).

POSSIBLE MECHANISMS OF DAMAGE

The majority of young patients shown to have VUR come to attention because of a complicating UTI. Over 90% of patients with renal damage associated with reflux will already have demonstrable damage at the initial presentation. Of those patients who present with end-stage reflux nephropathy, however, only about 60% have a history of UTI. What happened to the rest? Did they have asymptomatic infections? Or an illness that could not be recalled? Or was the reflux silently causing damage by an effect, such as the colorful and often quoted analogy to the destructive "water–hammer" action of the Severn Bore, a periodic tidal wave in the River Severn in England? Or was the damage because of renal dysplasia, a related, but separate, expression of a malformation of the urinary tract during renal development? It is clear from experimental work in the pig and monkey that high-pressure VUR can cause renal damage in the absence of infection. It is clear that complicating UTIs make VUR more harmful and are important in the pathogenesis of some renal scars. A debatable issue is whether UTI without VUR or obstruction can result in renal scarring.

Urodynamic Factors

There is strong support for the suggestion that high-pressure VUR and intrarenal reflux in the sensitive, growing newborn kidney can be potentially damaging by way of urodynamic factors. This is the age when bladder and sphincteric control is developing and during which dysfunction is not uncommon. It is characterized by uninhibited detrusor contractions during filling and higher bladder pressures during voiding, because of the obstructive effect of uncoordinated contractions in the urethral sphincter. With the more severe degrees of reflux, these pressures are transmitted to the renal pelvis and to any refluxing collecting ducts. In fact, fetal ultrasonography studies are providing increasing evidence that renal damage is occurring *in utero*.

In reflux, each time the bladder contracts, a "water-hammer" pulse of pressure of up to 100 cm of water or higher reaches the kidney and could produce severe disruption if intrarenal reflux should occur. The fact that the bladder pressures usually rapidly fall again suggests that ischemia is unlikely to be the major factor in the pathogenesis of reflux nephropathy. In contrast, in ureteric obstruction, a pelvic pressure of 30 to 60 cm of water sustained for several hours may well exceed filtration pressure and efferent arteriolar pressure and cause ischemia and papillary atrophy. Elevation of the intrapelvic pressure causes a decrease in renal blood flow in the presence of intrarenal backflow (41).

In clinical practice, increasing attention is now being given to the detection of lower urinary tract dysfunction that might raise the intrapelvic pressure associated with reflux. This is not to deny that reflux in the presence of normal lower urinary tract function and voiding pressures may carry a significant risk of reflux nephropathy.

In infants and young children, the risk of developing reflux nephropathy is related to the grade of reflux, which, in turn, can be altered by detrusor dysfunction and high pressures. It is thus logical to include synchronous urodynamic studies whenever voiding cystourethrography is indicated in search of reflux. If lower urinary tract dysfunction is demonstrated in addition to reflux, then anticholinergic drugs could be used to lower the pressures during filling and voiding and possibly hasten the cessation of reflux. There is, however, as yet, no evidence that anticholinergic agents can reduce the risk of renal damage.

Role of Tamm-Horsfall Glycoprotein

It has been suggested that mechanisms other than urodynamic factors or bacterial infection may contribute to the renal damage of reflux nephropathy and, in particular, to the deterioration in renal function that may occur in the absence of continuing reflux, UTI, or hypertension (117,118). One of these mechanisms involves the escape of urine into the renal parenchyma. The urinary constituent that has received the most attention is Tamm-Horsfall glycoprotein, which is secreted by the epithelial cells of the ascending limb of the loop of Henle and the distal convoluted tubule (DCT).

Hodson et al. (23) postulated that following intrarenal reflux, Tamm-Horsfall protein may excite an immunologic response and that the detection of autoantibodies to it might prove a useful marker of reflux. Anti–Tamm-Horsfall glycoprotein autoantibodies have been detected in the sera of normal infants, children, and adults and in increased titers in patients with upper urinary infection (123,124). Nevertheless, these autoantibodies were not found in increased titers in girls with asymptomatic bacteriuria and reflux.

The mechanism of formation of autoantibodies to this glycoprotein is unclear, and it is difficult to reconcile the presence of true autoantibodies with the presence of free Tamm-Horsfall protein in normal serum. Moreover, it has been suggested that the binding between Tamm-Horsfall glycoprotein and immunoglobulin exhibited by these immunoassays is nonspecific (125). Sensitive immunoassays can detect Tamm-Horsfall glycoprotein in serum (126,127).

Tamm-Horsfall glycoprotein is found in the glomerular space, renal interstitial tissue, perihilar lymph nodes, and veins of diseased kidneys. Interstitial deposits of Tamm-Horsfall protein are observed in 25% to 30% of patients with tubulointerstitial diseases, including reflux nephropathy (128,129). Extratubular Tamm-Horsfall glycoprotein serves as a marker for parenchymal urinary extravasation, but any etiologic role in renal injury remains to be elucidated. In many patients, the deposits are surrounded by a pleomorphic immune cell infiltrate that comprises monocytes/macrophages, lymphocytes, neutrophils, and occasional plasma cells. In the pig model of reflux nephropathy, high-pressure reflux is associated with extratubular localization of Tamm-Horsfall protein and circulating autoantibodies (23,130). Thomas et al. (131) demonstrated *in vitro* release of proinflammatory compounds from human neutrophils and mononuclear phagocytosis following stimulation with aggregated Tamm-Horsfall glycoprotein.

From studies in both humans and pigs, it is clear that the histologic damage cannot be accounted for by the demonstrable deposits of Tamm-Horsfall glycoprotein, as these deposits are restricted to a small portion of the tissue exhibiting histologic change and are not found in all human cases or animal models. Any immunologic mechanism proposed for the continuing renal damage in reflux nephropathy must account for the localized lesions that occur and the fact that the condition may be unilateral. Although there is some evidence in animals that an immune response to Tamm-Horsfall glycoprotein may be operative in disease models of tubulointerstitial nephritis, the case for this glycoprotein having a role in the pathogenesis of the tubulointerstitial nephritis of reflux nephropathy in humans remains unproved.

Role of Infection

Most UTIs complicating VUR or resulting in acute pyelonephritis are caused by *Escherichia coli*. The initiating step in the onset of the UTI is adhesion of the *E. coli* to the urinary tract epithelial cells (132). This adhesion is mediated by nonspecific, as well as by specific receptor–ligand interactions. Among the factors involved, P-fimbriae (so named because they act as ligands for P blood group antigens on the urothelial cells) have received special attention. Additional virulence factors, such as hemolysin, aerobactin, particular O and K serotypes, and serum resistance may also be important in the pathogenesis of acute pyelonephritis (133–136). The role of P-fimbriated *E. coli* in the development of reflux nephropathy is unclear. While some reports indicate that most episodes of nonobstructive acute pyelonephritis in children and in adults are caused by P-fimbriated *E. coli*, most pyelonephritic episodes associated with renal scarring are caused by non-P-fimbriated strains (137,138). Bacterial adhesion to the urothelium results in the release of endotoxin, activation of the alternative complement pathway and formation of membrane attack complex and chemotactic cleavage products (139,140). The release of endotoxin has been proposed to inhibit ureteral peristalsis causing functional obstruction and facilitating intrarenal reflux. Macrophages in the renal interstitium respond to the bacterial invasion by releasing cytokines and chemokines, as well as by digesting and presenting bacterial antigens to CD 4 + T lymphocytes. The activation of lymphocytes results in further release of cytokines and stimulation of lymphocyte proliferation. Invading neutrophils engulf bacteria that have been coated with serum-derived proteins. The release of reactive oxygen species (ROS) and lysosomal enzymes by these cells results not only in bacterial killing, but also in renal damage. Some studies suggest that urine and serum cytokine levels may be useful as noninvasive markers of parenchymal damage in reflux nephropathy (141,142). Early treatment of the infection with antibiotics and inhibition of the inflammatory response by the administration of glucocorticoids have been proved experimentally to reduce renal damage (143).

EXPERIMENTAL REFLUX NEPHROPATHY

Observations on the pig experimental model have contributed enormously to the understanding of the natural history and pathology of VUR and reflux nephropathy (23–25,106,107). The reason why the pig has been used as a model to study pyelorenal reflux is that, like humans (and the gorilla, cow, sheep, and otter), it possesses a multipapillary kidney.

Hodson et al. (23) observed the development of focal scarring in the multipapillary kidneys of young female miniature pigs with intrarenal reflux while their urine remained sterile. The scars only developed in areas affected by intrarenal reflux. A characteristic feature of intrarenal reflux was that it occurred mainly at the poles of the kidneys, while the mid-zone tended to be spared. The reason for this was unexplained until the important observations on the morphology of renal papillae were made, as discussed earlier (24,25).

The initial studies by Ransley and Risdon (24,25) indicated that UTI was of prime importance in the scarring process and that intrarenal reflux of infected urine could produce focal scarring very rapidly ("big bang" theory). These workers later confirmed (107) the work of Hodson et al. (23) that high-pressure sterile reflux resulted in renal scarring and defined the urodynamic conditions under which damage developed.

There has been debate as to the effect of an acute increase in ureteric pressure on renal blood flow. However, Thomsen et al. (41) studied piglets using electromagnetic flow probes and contrast material infused through a ureteric catheter and showed that renal blood flow decreased with increasing intrapelvic pressure, but only if intrarenal reflux was present. This was thought to be because of the mechanical effects of interstitial edema and the resulting increase in interstitial pressure. Focal ischemia produced by intrarenal reflux may be an important factor in the development of renal scarring in reflux nephropathy.

The pathology of the pig experimental renal lesion following sterile reflux has been described in detail (106,107). The sterile focal scarring was in the same distribution as the scarring resulting from intrarenal reflux of infected urine. Macroscopically the most striking changes are the development of extensive flat scars corresponding to lobes draining through compound papillae. Less extensive "slit" scars, traversing the center of the lobe in the plane of its axis, occurred most commonly in the mid-zone of the kidney, and small "pit" scars were scattered throughout the kidneys. Changes varied in severity with the voiding pressure. In some animals with a high voiding pressure, there was generalized dilatation of the pelvicalycine system with scarring in most lobes. Where there was lobar scarring, the corresponding papillae were flattened, while with slit scars only the centers of papillae were scarred. The histologic changes have been described elsewhere (106,107).

Over the past 20 years, Roberts (139,140,144) has undertaken a series of well-organized experiments using adult macaque monkeys to study the interrelationships between VUR of varying degrees, intrarenal reflux, urodynamic factors, and untreated UTI. These studies have shown that a loss of renal function will occur in the presence of sterile urine only if the bladder neck obstruction is severe. VUR by itself did not cause renal parenchymal damage in the absence of obstruction or infection. On the other hand, the studies by Roberts in the monkey have shown that inoculation of bacteria into the bladder can cause acute pyelonephritis and scarring in the absence of VUR (139,140). The cause of this different susceptibility to UTI in the pig and the monkey and the relevance of these observations to reflux nephropathy in humans continue to be a subject of debate.

TECHNIQUES FOR DEMONSTRATING VESICOURETERIC REFLUX

Voiding Cystourethrography

The voiding cystogram is the most precise method available for defining VUR and will almost certainly remain the reference against which new techniques will be evaluated. However, the procedure is invasive and unpleasant, especially in the 2- to 5-year-old child. If repeated studies are required, the cumulative radiation dose should be considered. The radiation dose with tailored, low-dose voiding cystourethrography techniques, however, is much lower, comparable to those of radionuclide techniques. Voiding cystourethrography is also important for assessing bladder function and is best combined with measurements of bladder pressure and urine flow rate. The bladder is filled with water-soluble contrast medium run into the bladder through either a urethral or suprapubic catheter (145). A helpful outline with recommendations on how to optimize the performance of voiding cystography has been recently published (146). The authors provide recommendations for patient and parent preparation, catheterization technique, radiological contrast, cyclical voiding, imaging, and reporting of results, and discuss contraindications and possible

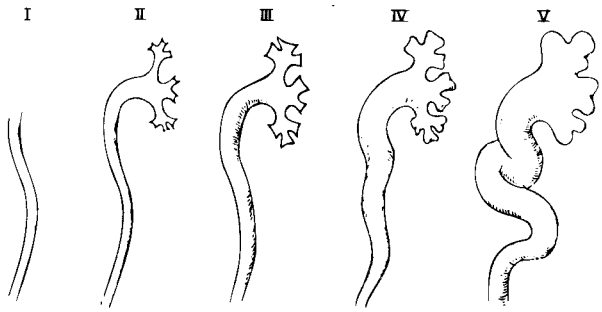


FIGURE 24-9. Classification of grades of VUR used by the International Reflux Study Committee. (From: Report of the International Reflux Study Committee. Medical versus surgical treatment of primary vesicoureteral reflux. *Pediatrics* 1981;67:392, with permission.)

complications. If VUR is demonstrated, it must be classified according to the extent and degree of ureteric filling and the degree of dilatation of the collecting system and, in particular, the minor calyces. The grading classification of VUR agreed upon by the 26 units participating in the International Reflux Study in Children (144,147,148) has become widely used (Fig. 24-9):

- Grade I Ureter only.
- Grade II Ureter, pelvis, and calyces with no dilatation and normal calyceal fornices.
- Grade III Mild or moderate dilatation and/or tortuosity of the ureter and mild or moderate dilatation of the pelvis. No or only slight blunting of the fornices.
- Grade IV Moderate dilatation and/or tortuosity of the ureter, and moderate dilatation of the pelvis and calyces. Complete obliteration of the sharp angles of the fornices, but maintenance of papillary impressions in the majority of calyces.
- Grade V Gross dilatation and tortuosity of the ureter, pelvis, and calyces. The papillary impressions are no longer visible in the majority of the calyces.

The grading of reflux is determined by the most severe reflux, which usually coincides with the peak of voiding. The bladder volume at which reflux is first seen, as well as any intrarenal reflux, should be noted, although these are not relevant to the current grading systems. Intrarenal reflux may occur in association with grades III, IV, or V on the International Classification. This classification is now widely used and its utilization by all radiologists encouraged.

Alternative Methods of Demonstrating Vesicoureteric Reflux

Alternative methods for demonstrating VUR could be considered clinically useful if they were less invasive, better tolerated, cheaper, or associated with a lower radiation dose to the gonads than voiding cystourethrography. However, such methods must not produce false-negative results, especially for the more severe degrees of reflux, and should have a low incidence of false-positive results. It is essential that any new technique should enable the degree of reflux to be classified (145).

Indirect Methods

Unfortunately there is no clinical test available for diagnosing VUR. Careful cystoscopic evaluation by an experienced urologist will give valuable information on the position and configuration of the ureteric orifices when assessing the etiology of a scarred kidney where reflux is no longer demonstrable by radiologic techniques.

Silber and McAlister (149) assessed the frequency of longitudinal folds in the calyceal system, renal pelvis, or ureter, demonstrated during intravenous urography, as an indirect sign of VUR. Longitudinal folds were observed in 30% of 354 renal units examined. These folds were most frequent in those individuals with the most severe degree of reflux. The authors concluded that such longitudinal folds were a valuable indirect indicator of reflux. However, these folds are not specific for reflux.

While the wide ureter on an intravenous urogram in a neuropathic patient should arouse suspicion that reflux or lower urinary tract dysfunction might be present, these conditions are not necessarily excluded by the findings of a ureter of normal size (150).

Orr et al. (151) demonstrated experimentally that VUR was associated with a sharp drop in renal blood flow coinciding with a peak in bladder pressure at the commencement of micturition. Whether this technique would prove valuable as a clinical test seems doubtful. A reduction in either the renal length or mass correlates well with the continuing presence of gross VUR. Individual renal function tests may assist in identifying which kidneys are being subjected to potentially damaging reflux.

Radionuclide Micturating Cystography

In 1959, Winter (152) was the first to suggest the use of radionuclides in the investigation of VUR. Dodge (153) drew attention to the potential value of the indirect radionuclide micturating cystogram when he observed that if a patient with reflux voided at the end of a renogram there was often a brisk rise in renal activity.

Other workers used this indirect method, which relied on the rapid and complete renal clearance of an intravenously injected radionuclide, such as ^{99m}Tc DTPA or ^{99m}Tc MAG-3, which are often used for split renal function measurements. When the radionuclide had accumulated in the bladder, the residual activity in the kidneys was low enough to permit visualization of any reflux activity when the patient was placed under the gamma camera. As the patient voided, any significant increase in radioactivity over the upper urinary tract indicated reflux. The major drawback of this indirect method was the necessity for considerable patient cooperation, which makes it unsuitable for children under 5 years of age. Furthermore, reflux can be detected only during the voiding and postvoiding phases, and the morphology of the bladder and urethra cannot be evaluated. There are advantages, however, including the fact that the bladder is not catheterized and that renal function can be measured.

Blaufox et al. (154) reported the use of direct radionuclide micturating cystography, which is performed by instillation into the bladder through a urethral catheter of a radionuclide, such as ^{99m}Tc-pertechnetate or ^{99m}Tc-DTPA in saline. This procedure required about 50 minutes to perform and resulted in an estimated gonadal radiation dosage of 4 or 5 millirads. The authors showed good correlation with conventional voiding cystourethrography and concluded that the technique was a useful adjunct to conventional radiologic studies for the diagnosis of VUR in children. This technique has been used extensively by Conway et al (155,156), but, again, the radionuclide was instilled through a urethral catheter, a technique that is unpleasant and potentially hazardous. Conway et al. (155) used 1 millicurie of ^{99m}Tc-pertechnetate and estimated the radiation exposure to the bladder wall to be 30 millirads. Conway et al. (157) measured the residual urine volume and other urodynamic measurements during the examination. Conway et al. (157) later reported their clinical experience of more than 3,500

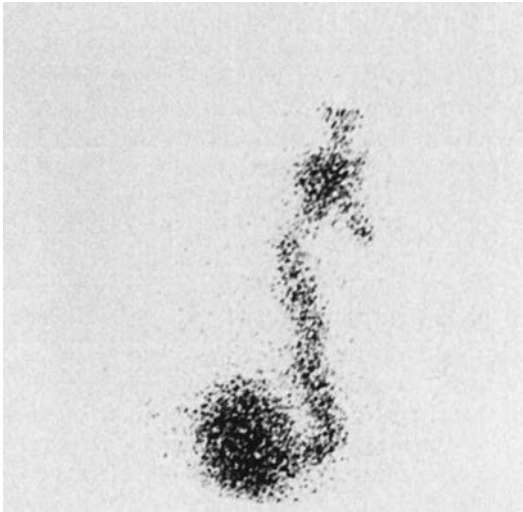


FIGURE 24-10. Radionuclide micturating cystogram showing VUR into the left kidney.

radionuclide micturating cystograms over a 12-year period and concluded that the method was sensitive and, possibly more so, than conventional radiologic methods. Cyclic (filling the bladder and having the infant void around the catheter several times) direct radionuclide cystography may increase the sensitivity of the procedure (158).

Bailey et al. (159) modified the direct method by instilling the radionuclide suprapubically into the distended bladder (Fig. 24-10). This method was simple, highly acceptable to the patients, and preferred to urethral catheterization. These authors concluded that this technique was ideal for the follow-up of patients with VUR treated either surgically or conservatively. More recently, indirect radionuclide cystography has been shown to have some advantages for following children with VUR (83,160).

Godley et al. (161) have reported a method for the quantitation of VUR by synchronous combined direct radionuclide cystography and urodynamics. Intravesical pressures and volumes are coordinated with volumes of reflux. This method requires further study to assess its potential for providing a functional classification of VUR.

Recently, the Pediatric Committee of the European Association of Nuclear Medicine has published guidelines on the indications, acquisition, processing, and interpretation of direct radionuclide cystography in children (162). Advantages of radionuclide cystography are the higher sensitivity and lower radiation burden. Disadvantages, compared to conventional voiding cystourethrography, are that the morphology of the bladder and urethra and the dimensions of the collecting system cannot be evaluated.

Ultrasonography

For some time there has been hope that ultrasound would be able to detect VUR. Despite the report of Tremewan et al. (163), there has been disappointment in this respect. One of the methods used has been conventional ultrasonography of kidneys and ureter in order to detect dilatation of the collecting system or other indirect signs of VUR. Jureidini et al. (164) assessed the urinary tract of children using real-time ultrasonography. The bladder and lower ureters were observed closely for at least 5 minutes for dilatation. Ultrasonography was shown by these investigators to be reliable for diagnosing reflux with dilatation. Others (161–163,165–167), however, believe that sonography is not sufficiently sensitive or specific for detecting VUR. Blane et al. (165) found that 74% of kidneys with reflux at voiding cystourethrography were ultrasonographically normal and that 28% of the refluxing kidneys that were missed had reflux of grade III or more. Stokland et al. (166) and DiPietro et al. (167) have reported similar results.

More recently, Avni et al. (168) have again confirmed that dilatation of the renal pelvis alone detected by static ultrasonography is a poor predictor of the presence of VUR. Nevertheless, when these investigators included other abnormalities, in addition to pelvic dilatation, such as calyceal or ureteral dilatation, pelvic or ureteral wall thickening, absence of corticomedullary differentiation, and signs of renal dysplasia (small kidney, thinned or hypoechoic cortex and cortical cysts), they found that only 12.3% of renal refluxing units were normal. This study has been criticized, because it only included infants with VUR, therefore allowing an estimate of specificity of the variables studied, but not of their sensitivity.

Hiraoka et al. (80) have used ultrasound scanning of the kidneys during voiding (voiding ultrasonography) to diagnose VUR. They examined the kidneys before, during, and after voiding provocation using a B-mode ultrasound scanner equipped with a videocassette recorder. Voiding provocation was attempted for up to 5 minutes and was successful in 60% of the cases. The presence of transient pelvic dilatation, defined as an increase in diameter of 3 mm or more by comparison to before voiding and returning to its basal size within 2 minutes, was considered indicative of VUR. By comparison to voiding cystourethrography, voiding ultrasonography detected transient renal pelvic dilatation in 71% of 24 kidneys with VUR and 94% of 17 kidneys with high-grade (grade III or more) reflux.

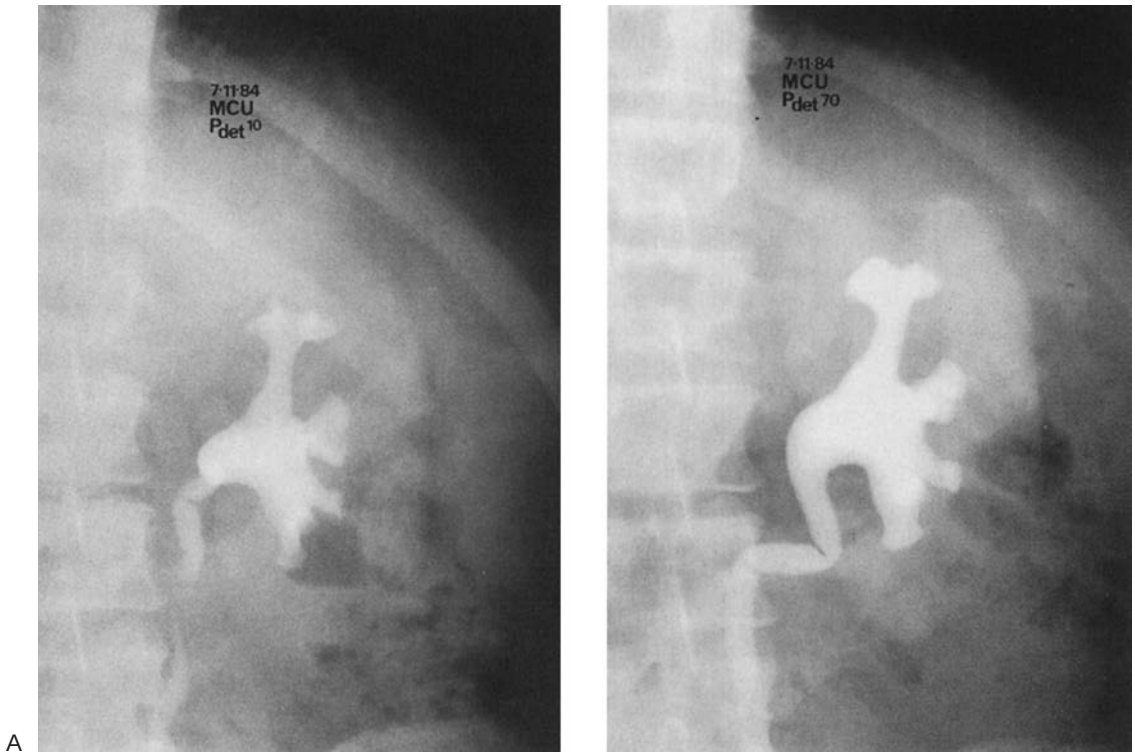
Another ultrasonography approach has been the introduction of fluids with echogenic characteristics into the bladder followed by ultrasonography of the kidneys and ureters to detect the echogenic refluxing material (169,170). Obviously, the major disadvantage of this technique is that it still requires bladder catheterization, and its clinical usefulness has not been proved.

Marshall et al. (171) used color Doppler to detect the presence of VUR. This method is noninvasive, avoids ionizing radiation, does not require bladder catheterization, and enables the position of the ureteric orifice to be determined in relation to the midline of the bladder. These authors concluded that the more laterally positioned the ureteric orifice was, the more likely it was to reflux and suggested that color Doppler ultrasound measurement of laterality of the ureteric orifice may be useful for predicting which children with a UTI would benefit from voiding cystourethrography. Oak et al. (172) reported 87% accuracy for grade I, 92% for grade II, and 100% for grades III, IV, and V reflux using a color Doppler real-time machine as a diagnostic method. Kosar et al. (173) found color-flow Doppler ultrasonography to be 90% sensitive with a specificity of 93%. Color-Doppler ultrasound has some limitations. It is influenced by hydration status and is unsuitable for patients aged <2 years because it requires significant patient cooperation. Another important limitation is the suboptimal delineation of the posterior veins, which imposes restrictions on the evaluation of posterior urethral valves and of the ureters, which imposes restrictions on the grading of reflux.

Resistive index determined by color-Doppler ultrasound has also been reported to correlate with the severity of the VUR, but the clinical usefulness of this method is doubtful (174).

BLADDER PRESSURE AND BLADDER DYSFUNCTION

The potential importance of bladder pressure in patients with VUR has become recognized since the availability of



A B

FIGURE 24-11. A voiding cystourethrogram of a patient showing grade III (Christchurch classification) VUR at a detrusor pressure of 10 cm of water (**A**), and both grade IV and areas of intrarenal reflux at 70 cm of water (**B**).

urodynamic studies. The pressure within the renal pelvis and any refluxing tubules is determined by the filtration pressure and the urinary production rate and by the contractility of the smooth muscle, the degree of distention, and the efficiency of peristalsis in the pelvis and ureter, all modified by the ureterovesical resistance. In the more severe degrees of reflux, intrapelvic pressure is dominated by the transmitted bladder pressure where a continuous column of fluid joins the bladder and pelvis. The grade of reflux in any one patient can change with the bladder pressure (Fig. 24-11) and, hence, if the grade of reflux is to be documented or used as a basis for any management decision, it is important to record instantaneous bladder pressures on any still x-ray film showing reflux.

Assessment of Lower Urinary Tract Dysfunction

The two main features of lower urinary tract dysfunction that can lead to increased bladder pressures are detrusor instability and detrusor–sphincter dyssynergia (56, 57).

In children, after the toilet-training age, lower urinary tract dysfunction can be grossly assessed from the history (54,55). The diagnosis can be facilitated by documentation of the voiding pattern using a urinary diary. Noninvasive measurement of urine flow and residual urine can be easily obtained using a flow meter and a sonographic device. If these are abnormal, further urodynamic studies are recommended. These studies may be difficult to interpret, as findings depend on the observer being confident that the child is cooperating and remaining relaxed throughout. This difficulty has been overcome in some centers by repeating the cystometry three times in a relaxed environment. Another technique is the dysfunctional voiding symptom score, which is an aggregate score of various clinical features of the dysfunctional voiding symptom complex. It provides a quantitative means of monitoring the response to bladder behavioral modification (62).

The assessment of low urinary tract dysfunction in infants and non–toilet-trained children is more difficult. To provide a basic assessment of bladder function in these patients, Holmdahl et al. (175) have developed a 4-hour voiding observation test, including the number of voidings, voiding volume (from diaper weights), bladder capacity, and residual urine (using a bladder ultrasound). Using this test, refluxing infant boys with very high pressure levels and low capacity on urodynamic investigation were shown to have a different voiding pattern from healthy infants of comparable age.

TECHNIQUES FOR DEMONSTRATING REFLUX NEPHROPATHY

Intravenous Urography

The intravenous urogram has been the traditional imaging technique for diagnosing reflux nephropathy. For many years this was the only technique available, but real-time ultrasound and dimercaptosuccinic acid (DMSA) radionuclide scanning are now available. These other methods do not provide as much anatomic detail, but can be used to determine renal size and focal loss of parenchyma. The role of ultrasound and, in particular, DMSA scanning has changed the status of the intravenous urogram with regard to the examination of patients with UTI. Whereas the intravenous urogram was formerly widely used as the primary examination in patients with UTI, it now plays a secondary role to either ultrasonography or DMSA scanning.

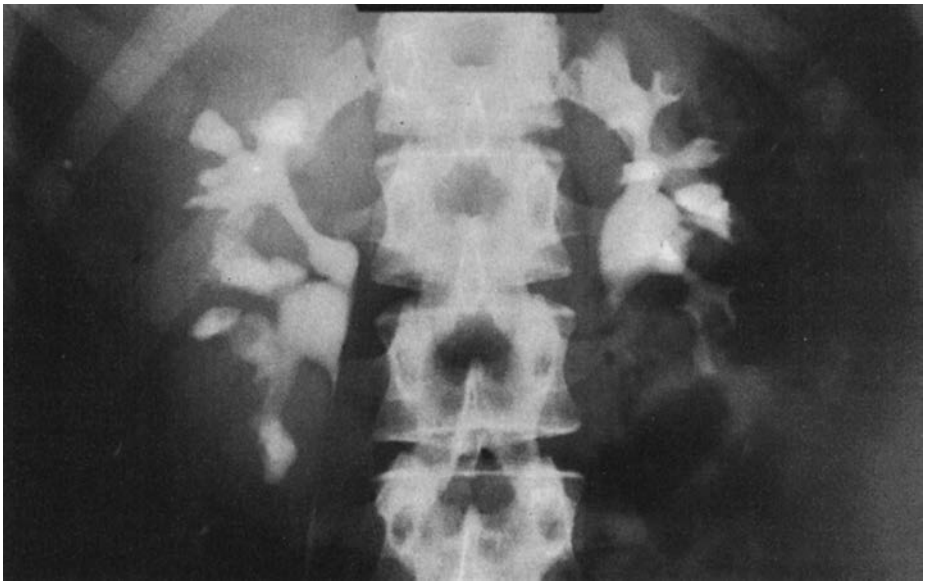


FIGURE 24-12. Intravenous urogram of a 35-year-old woman showing bilateral reflux nephropathy, which is more severe on the right side. Note the focal nature of the scars involving the full thickness of renal parenchyma, mainly in the polar region (*arrow*). There are other areas where the renal lobes are normal.

The place of the intravenous urogram is to confirm the diagnosis of reflux nephropathy, where this is suspected, from one of the other examinations.

A good-quality intravenous urogram will demonstrate the irregularly scarred surface of the kidney along with clubbing of the underlying calyx. The latter indicates damage to the papilla. This is evidence of full-thickness scarring on a lobar basis, which is the hallmark of reflux nephropathy. Depending on the extent and severity of this scarring process, two quite distinct types of radiographic damage emerge:

1. Full-thickness focal scars with calyceal clubbing and atrophy and retraction of the overlying cortex, involving one or more renal lobes, and found most frequently in the polar region. There is always preservation of at least one normal renal lobe (Fig. 24-12). These appearances are pathognomonic of reflux nephropathy. This is the most common form of reflux nephropathy, and the number of renal lobes involved determines the severity of the scarring process.
2. Occasionally, and associated with the most severe degrees of VUR, the renal damage is diffuse with involvement of all lobes of the kidney, resulting in a generalized reduction in parenchymal thickening with uniform papillary changes (Fig. 24-13). The use of nomograms for renal length, renal parenchymal area, and parenchymal thickness are helpful in its identification. This type of reflux nephropathy resembles the changes seen in obstructive atrophy.

Classifications of the type and degree of renal scarring have been proposed by Smellie et al. (176) (Fig. 24-14) and by Monsour et al. (177).

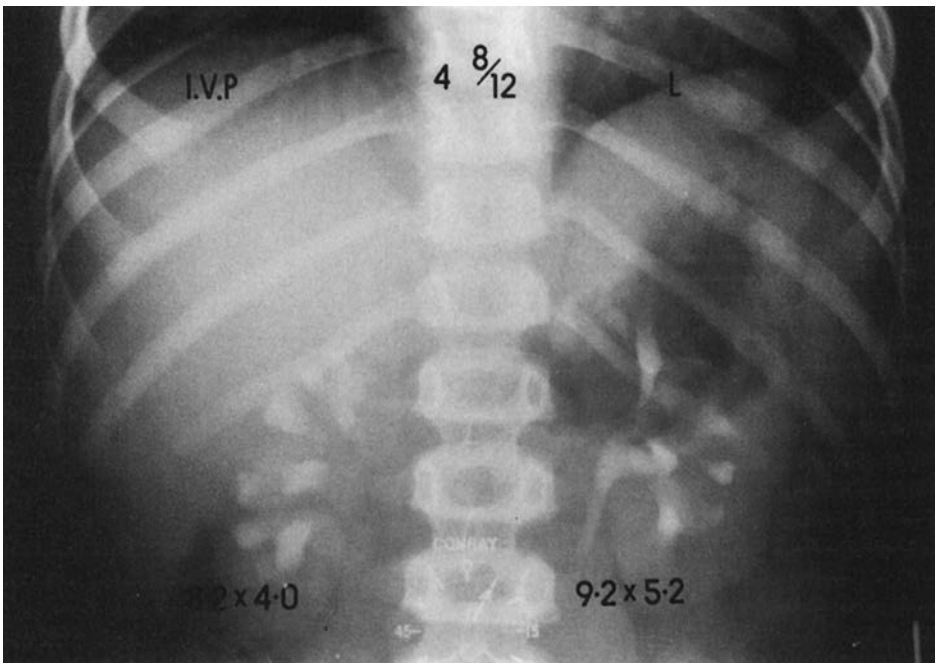


FIGURE 24-13. Intravenous urogram of a 4-year, 8-month-old boy showing generalized scarring in the right kidney and a normal left kidney.

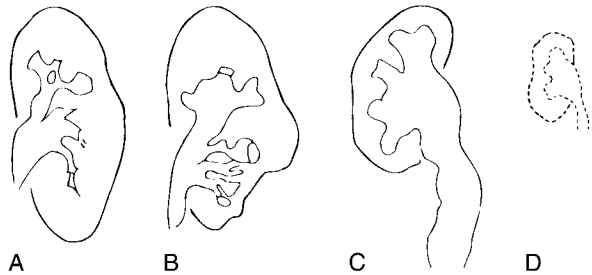


FIGURE 24-14. Grading of parenchymal scarring in reflux nephropathy. **A:** Mild scarring with not more than two scarred areas. **B:** More than two scarred areas but some areas of normal tissue persist. **C:** Diffuse thinning of parenchyma with generalized calyceal clubbing—"backpressure type." **D:** Shrunken kidney with insignificant parenchymal thickness. (From: Smellie JM, Edwards D, Hunter N, et al. VUR and renal scarring. *Kidney Int* 1975;8:S-65, with permission.)

Radionuclide Techniques

Not long ago, it was necessary to undertake bilateral ureteric catheterization to measure individual renal function. This required general anesthesia, was time-consuming, and was potentially hazardous. In a dilated urinary collecting system, such as one subjected to reflux, it was often difficult to place a tapered ureteric catheter that did not leak. Recently, the use of noninvasive radionuclide techniques for measuring individual renal function have become popular and have been shown to correlate well with measurements made during ureteric catheterization (178) (Fig. 24-15).

Although the intravenous urogram has been the key imaging modality for renal parenchymal injury, at times it fails to demonstrate focal scarring, especially at the upper poles, and is of no value in determining renal function. There has been recent interest in the use of radionuclides for the renal imaging of patients with reflux nephropathy. The most widely used radionuclides are those labeled with ^{99m}Tc because of their excellent physical properties. Compounds, such as diethylenetriamine pentaacetic acid (DTPA), DMSA, glucoheptonate, and mercaptoacetyltriglycine (MAG-3) are available and have their own specific advantages and disadvantages. DTPA is predominantly filtered with little cortical retention, but the other agents are retained to varying degrees (DMSA > glucoheptonate > MAG-3) by chemical combination in the cells of the proximal tubules, allowing good cortical pictures to be obtained (179).

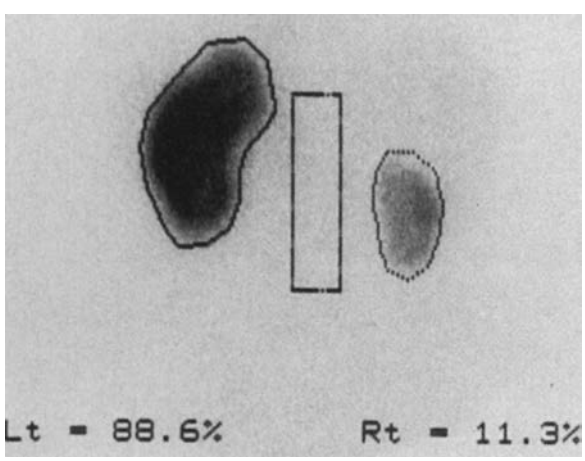


FIGURE 24-15. Posterior view of a DMSA scan showing a hypertrophied normal left kidney and a small scarred atrophic right kidney contributing only 11.3% to overall renal uptake.

Radionuclides have also been used to evaluate possible renal sepsis. [^{67}Ga]citrate localizes in polymorphonuclear leukocytes and an increased uptake of Ga can be expected in bacterial infections within the renal parenchyma. ^{111}In- and ^{99m}Tc-labeled autologous leukocytes have also been reported to be useful in acute pyelonephritis, but clinical experience is limited.

Dillon et al. (180) reported in 1983 an excellent correlation between the findings on intravenous urography and the DMSA scan in 12 children with reflux nephropathy. The latter detected two additional scars that had been missed on conventional radiology. Focal defects on DMSA scanning reflect underperfusion, either because the proximal tubules in the damaged area extract a smaller amount of the radionuclide than the normal areas or because they are damaged by ischemia and are incapable of taking up the DMSA. Many other workers subsequently confirmed the value of DMSA scanning for identifying renal scars (181,182), in addition to providing valuable functional information based on the relative uptake of the tracer in the two kidneys in the posterior view, corrected for background activity. In 1984, Goldraich et al. (182) proposed a four-grade classification of renal parenchymal damage based on the DMSA scan (Fig. 24-16). These Brazilian workers (183) later reported an 88% concordance between the findings on intravenous urography and the DMSA scan in a large series of children with primary VUR. In 34 kidneys, the intravenous urogram was considered normal but the DMSA scan showed scars. The authors concluded that abnormalities detected by the DMSA scan may precede the radiologic findings. On the other hand, acute perfusion defects on a DMSA scan may occur as a result of an acute renal parenchymal infection and resolve within 3 months of the clinical episode.

Most clinicians now consider the DMSA scan and the intravenous urogram to be complementary investigations in the diagnosis of renal scarring (184), but some believe that the DMSA scan is the new "gold standard" investigation for a child with a UTI (185). Posterior and left and right posterior oblique planar images are usually obtained, but some authors prefer the single-photon emission-computed tomography (SPECT) technique. Although DMSA scans have been used for many years, the interpretation of DMSA images is not always straightforward. There is significant interobserver variability in the classification of DMSA scans as normal or abnormal (186,187).

Ultrasonography

Ultrasonography has evolved considerably in recent years with the development of real-time and Doppler imaging. The information obtainable from ultrasound examination will increase in the future with continuing technologic advances. Ultrasound now provides an excellent screening examination of the urinary tract, providing information on kidney size, the presence of obstruction, severe scarring in adults, and examination of the bladder with assessment of bladder emptying. The assessment of renal size in children is facilitated by the use of nomograms (188,189). It can be used to screen out children in whom intravenous urography is not necessary (190,191) and to evaluate adults presenting with UTI (192). It has the advantage of being less expensive and noninvasive when compared to intravenous urography. On the other hand, ultrasonography is very operator dependent and less accurate than intravenous urography or DMSA scan to detect renal scarring.

Other Imaging Methods

Computed tomography (CT) and magnetic resonance imaging (MRI) have not been extensively used to diagnose reflux

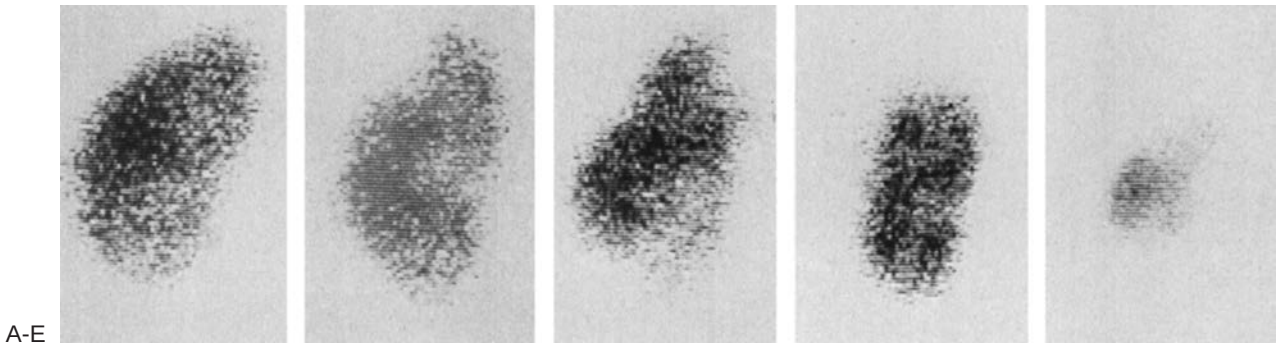


FIGURE 24-16. Classification of kidneys with reflux nephropathy (RN) on ^{99m}Tc-DMSA scanning. **A**: Normal. **B**: Type 1 (no more than two scarred areas). **C**: Type 2 (more than two scarred areas, with some areas of normal parenchyma). **D**: Type 3 (generalized damage to whole kidney, similar to obstructive nephropathy, i.e., contraction of entire kidney with or without focal scars). **E**: Type 4 ("end-stage" kidney with little or no uptake of radionuclide, i.e., less than 10% of overall function). (Courtesy of Dr. Noemia P. Goldraich, Porto Alegre, Brazil.)

nephropathy. Fast CT techniques, such as spiral and electron-beam CT, may be more sensitive and specific than excretory urography and DMSA scan to detect small scars, particularly in the anterior and posterior part of the kidneys, which are more difficult to visualize by urography or DMSA scanning. The disadvantage of these techniques, however, is that they require breathhold imaging, which may not be feasible in young children. The potential advantages of MRI techniques to detect renal scarring are, at present, outweighed by their cost and need for sedation or anesthesia in young children. Normative renal growth curves can be constructed from CT and MRI derived renal volumes. In reflux nephropathy, the cortical fraction is reduced and differential function on nuclear scan correlates with MRI-derived differential volume (193).

NATURAL HISTORY OF VESICOURETERIC REFLUX

Some kidneys subjected to VUR become progressively damaged, while others remain apparently unaffected. The observation, however, that the severity of reflux was the single most important determining factor as to whether renal damage would occur was a major breakthrough in the understanding of this entity (1,19,21,194). Reflux becomes less marked with increasing age and frequently completely disappears. Even the severest degrees of reflux may cease, especially when renal scarring has become severe. An abnormal ureteric orifice in the bladder may remain as the only marker of previous reflux. What may have been a grossly refluxing ureter in infancy may be a moderately or slightly refluxing ureter in childhood. This phenomenon is best explained by the maturation theory of Hutch (15).

Rolleston et al. (19) investigated 91 males and 84 females under the age of 12 months. Ninety-eight were under 8 weeks of age. Those with obstructive uropathy were excluded. VUR was found in 86 (49%) of the infants and was bilateral in 44. The 130 refluxing ureters were classified into three grades of severity. At the initial presentation, 26 of the 32 kidneys subjected to gross reflux already had renal damage. In comparison only 3 of 74 kidneys subjected to moderate reflux were possibly slightly damaged. None of the 24 kidneys with slight reflux was damaged. When these infants were followed for 6 months to 6 years, progressive renal parenchymal damage only occurred in those kidneys with continuing gross reflux. No progressive damage was detected in kidneys associated with moderate or slight reflux. What was of great significance was that progressive lesions were demonstrated in some of these children in the continued absence of UTIs.

Rolleston et al. (21) extended their study to 350 infants (age range 3 days–12 months; mean age, 3 months) and demonstrated reflux in 147 (42%). Of the 226 refluxing ureters, 49 were classified as gross. Only 79% of these infants were investigated because of a complicating UTI. At the initial investigation, 29 of 49 kidneys with gross reflux, or 13% of all kidneys with reflux, were already damaged. No damage was associated with the lesser degrees of reflux. Of the 16 kidneys associated with gross reflux but no evidence of renal damage at the initial examination, 7 showed subsequent damage. In the majority of these kidneys the renal damage was generalized. These key studies by Rolleston et al. (19,21) clearly indicated that infancy or early childhood was the critical period in the natural history of the renal damage associated with reflux.

Acton and Drew (195) reported 160 neonates undergoing urinary tract investigations primarily because of a urinary infection. Of this group, there were 54 between the ages of 7 days and 8 weeks in whom reflux was demonstrated. Reflux nephropathy was found only in those with severe reflux. Five of the six with grade IV reflux had a generalized reduction of renal parenchyma at a mean age of 3 weeks of life. Of those infants with less severe reflux, 77% ceased to reflux spontaneously, the majority in the first year of life. None of these infants developed any radiologic evidence of renal damage.

Support for anatomic renal damage occurring in association with continuing gross reflux was provided by Aperia et al. (196), who demonstrated that kidneys subjected to gross reflux underwent a steady functional deterioration. Kidneys with lesser degrees of reflux, or no reflux, had no change in individual renal function. Klare (197) assessed individual renal function using radionuclide techniques and demonstrated that only those kidneys subjected to gross reflux had reduced function.

There have been few long-term studies of infants with gross VUR. Bailey et al. (198,199) reported the long-term follow-up of 31 infants with gross reflux diagnosed between 1952 and 1970. Prior to 1970, all infants with gross reflux diagnosed in that institution were treated with prophylactic antimicrobial therapy and did not undergo ureteric reimplantation unless there was radiologic evidence of renal damage. In this series, there were 16 boys and 15 girls with a total of 44 grossly reflux ureters. Twenty-four of the 31 infants presented with a UTI. The mean age at presentation was 15.3 weeks (boys

11.3 weeks; girls 19.5 weeks). In 12 of the 26 in whom reflux nephropathy was eventually documented, renal scarring was already present when first investigated at 4 to 48 weeks of life (mean 20 weeks). Of the 14 additional patients, who developed reflux nephropathy during follow-up, all did so within 3 years of birth. Clearly the damage resulting from gross reflux developed extremely early in life and in some was probably present at birth. Of the original 31 infants, 21 had remained under regular supervision for a mean of 23.9 years (range 16 to 37 years) (199). Four of the 21 had normal kidneys on intravenous urography and normal renal function. Four patients had developed bilateral reflux nephropathy; two of these had proteinuria and a glomerular filtration rate of less than 70 mL/min with one progressing toward end-stage renal failure. One of the two patients with renal insufficiency also had mild hypertension. The remaining 13 patients had unilateral reflux nephropathy. Two of these had hypertension and one (born with a single kidney) developed end-stage renal failure at 23 years of age. While this study was in progress, 24 patients born between 1952 and 1970 entered the same department's renal replacement program, because of end-stage reflux nephropathy. These 24 patients did not present in infancy or early childhood with UTIs but came to attention in later life with hypertension, proteinuria, or renal failure.

Jacobson et al. (200) reported a 25- to 35-year retrospective follow-up on 30 patients who presented with acute pyelonephritis in childhood (aged 1 to 13 years; mean age 6 years) and were shown to have reflux nephropathy. Three of the patients developed end-stage renal failure and seven, hypertension. These studies demonstrated that the more severe the renal parenchymal damage, the greater the risk of long-term complications. Such patients should be kept under supervision for the development of proteinuria, hypertension, or renal insufficiency.

Smellie et al. (201) reported a long-term (10 to 35 years) follow-up of 226 patients with VUR and UTI, who had a mean age of 5 years at presentation. Of the 226 patients (20% of those with grade I, 26% of grade II, and 69% of grade III through V reflux), 37% had cortical scars at presentation. One hundred ninety-three patients were treated with uninterrupted low-dose antibacterial prophylaxis, until two consecutive negative cystograms were obtained, and a regimen designed to achieve complete bladder emptying by regular drinks and voiding, double voiding at bedtime, and correction of constipation. The remaining 33 patients were treated surgically. New scars developed in two boys, one with and one without previous scars, at the age of 7 and 9 years. Both had VUR into the corresponding ureter and each scar followed a symptomatic infection.

In 1984, the Southwest Pediatric Nephrology Study Group (202) initiated a prospective study to follow the natural history of children with grades I, II, or III VUR on the International Classification. A total of 113 normotensive children (92 girls), aged between 1 month and 5 years, with reflux in at least one ureter and without radiographic evidence of renal scarring were enrolled. VUR was unilateral in 65 and bilateral in 48 of the children. The protocol included antimicrobial prophylaxis and surveillance for UTI, an annual voiding cystourethrogram until the reflux had resolved, intravenous urography at 1, 3, and 5 years to assess renal growth (planimetric surface area) and scarring, and an annual screening for evidence of renal dysfunction and hypertension.

At the initial report, 83 patients had completed 2 years of follow-up (202). Breakthrough UTI had occurred in 18 children. Of 45 ureters with no reflux at the initial diagnosis, 5 had grade I or II reflux, while of 121 ureters with reflux at diagnosis, 28% had resolved, 22% had no reflux, 19% had a lower grade of reflux, 21% had the same degree, and 10% a more severe degree of reflux. VUR resolved more often when the reflux was initially grade I than grade II or III, while the reflux worsened in only one ureter with grade III. Twelve percent of those with grade II and 21% with grade I reflux had a higher grade of reflux at the 2-year follow-up than at the initial diagnosis. Blood pressure, GFR, and plasma renin activity, relative to age, were normal in all children. New unilateral renal scars, unrelated to recurrent UTI or the grade of reflux, was observed in three children.

Arant (203) reported that 74 of the 113 children had completed 3 years of follow-up. At this time, reflux had resolved in 67% of those with grade I, 57% with grade II, and only 19% with grade III reflux. A total of 6% had a higher grade of reflux between diagnosis and the 3-year follow-up and 23% of nonrefluxing ureters at diagnosis exhibited reflux during a follow-up study. A breakthrough UTI had occurred in 24% of children, but was unrelated to resolution of the reflux or to renal damage. No child had a significant abnormality of the urinary sediment, while one had mild proteinuria. Five patients had a blood pressure measured at or above the 95th percentile for age, and 70 had a plasma renin activity more than two standard deviations above the age-related mean value. The GFR had remained normal in every child. Arant indicated that the most significant finding to date in this prospective study was that definite evidence of renal scarring (cortical thinning over a deformed calyx) had developed in eight kidneys (eight children), including some children without documented UTI during the period of observation. In this group, renal scarring occurred nearly three times more commonly in grade III VUR than it did in grades I or II.

Several large prospective studies have confirmed the relationship between the presence and severity of VUR and the occurrence and extent of the cortical scars. Many children already had evidence of renal parenchymal scarring at the time that UTI and VUR were detected. The International Reflux Study in Children (IRSC) studied 306 children under 11 years of age with grades III or IV VUR (204–211). The frequency of parenchymal scarring or thinning increased from 10% in children with nonrefluxing renal units, who had contralateral VUR to 60% in those with severely refluxing grade IV kidneys. The Birmingham Reflux Study Group (BRSG), reported renal scarring in 54% of 151 children under 14 years of age with severe VUR, resulting in ureteral dilatation (greater than grade III using the classification system adopted by the IRSC) at the time reflux was detected (212,213). Participants in these studies were children previously diagnosed as having UTI. Parenchymal scarring also occurred after the diagnosis and initiation of therapy. The frequency of new scar formation in the IRSC was inversely related to age, occurring in 24% under 2 years of age, 10% from 2 to 4 years of age, and 5% over 4 years of age. This is consistent with the initial observations by Rolleston in 1975. In another prospective study by Jodal of 1,177 children with first time symptomatic UTI, 66% of those with grade III or more VUR developed renal scarring, compared to only 5% of those without VUR (214).

Despite the evidence reviewed above, other studies using DMSA scanning as a tool to identify renal parenchyma scars, have challenged the concepts that VUR plays the primary role in the development of cortical scarring (215–218) and that younger children are at a higher risk of renal sequelae after pyelonephritis (219). Some methodological issues, such as the difficulty in distinguishing between acute and old lesions using DMSA scans, the lack of generally accepted criteria for interpretation of DMSA images, the utilization in some studies of radionuclide cystography to grade the severity of the VUR, and the high variability of VUR, which may be detected on one examination and not on another the following day, may account for these differences (186,187,220–222).

CLINICAL PRESENTATIONS OF VESICOURETERIC REFLUX AND REFLUX NEPHROPATHY

VUR may present in many ways. The most frequent clinical marker, particularly in infants and children, is a complicating UTI. The other clinical presentations are included in Table 24-1.

Like VUR, reflux nephropathy may also present in several ways (Table 24-2), some of which obviously overlap.

The various clinical presentations of VUR and reflux nephropathy are discussed individually.

Urinary Tract Infections

As discussed earlier, complicating UTIs are the most common manifestation of VUR or reflux nephropathy in infants and children. The reason why patients with reflux are predisposed to UTI is not clear, but is certainly multifactorial and beyond the scope of this discussion.

Depending on the age at which the initial investigations are performed, 15% to 60% of infants and children with UTI will be found to have some degree of reflux (1), and 8% to 13% of the total will have radiologic evidence of reflux nephropathy (Fig. 24-17). Arant (76) stated that "the highest incidence of primary VUR therefore, may be expected from young, nonblack patients with UTI and their siblings, male and female, studied by radionuclide cystography immediately after the urinary tract becomes sterile. These are the patients who should be identified early and observed closely to minimize any further renal injury as a consequence of recurrent UTI or bladder dysfunction."

In Christchurch Hospital, Abbott (223) examined 1,460 consecutive infants (757 males), age 3 to 6 days of life, and found 14 (1%) to have bacteriuria. Nine were asymptomatic. Of these 11 boys and 3 girls with bacteriuria, 8 had reflux (slight in 2, moderate in 6). Maherzi et al. (224) studied 1,950 infants in a neonatal special care unit and found bacteriuria on suprapubic bladder aspiration in 43 (2.2%). Fourteen of these 43 neonates (36 boys) had reflux. Drew and Acton (225) undertook suprapubic aspiration on infants presenting with jaundice of unknown etiology, failure to thrive, excessive weight loss, diarrhea, vomiting, or possible sepsis. There were 905 such infants among 12,942 consecutive neonates, and 64 of them had bacteriuria (0.5% of all live births). Of the infected infants, 84% were males, and VUR was detected in 27.

Bourchier et al. (75) investigated 100 infants with a UTI. Twenty-seven of the 68 boys (40%) and 20 of the 32 girls (63%) had a urinary tract abnormality, which, in 29%, was considered clinically significant. Thirty-six of the infants had VUR and, in 23, it was grade III or IV.

What these and other studies clearly demonstrate is that in neonates and young infants UTIs are more frequent in boys than in girls and, in both sexes, there is a high incidence of urinary tract abnormalities, the most common of which is VUR. It could be suggested that the infant or young child with severe reflux, who has a clinical urinary infection or detected asymptomatic bacteriuria and thereby comes to clinical attention is more fortunate than the child whose reflux is not complicated by a UTI and, therefore, goes undetected, resulting in renal damage.

After the first year of life, the prevalence of asymptomatic bacteriuria in boys is extremely low, but for girls it remains at about 1%. McKerrow et al. (226) investigated 572 consecutive children with a UTI and found 51% to have a urologic abnormality. More specifically, reflux was seen in 272 ureters of 178 children (31%).

In prospective studies of girls over the age of 4 years, reflux nephropathy rarely developed in kidneys that were normal at the outset, even when reflux was present and asymptomatic UTIs were left untreated (227–229). These observations suggest that antimicrobial therapy may not be necessary in girls over 4 years of age in whom the kidneys are radiologically normal. In Cardiff schoolgirls over 4 years of age, prolonged asymptomatic bacteriuria was not associated with a reduction in renal function, and there was no benefit from intermittent antimicrobial treatment (230). In those with reflux nephropathy, who might have been expected to show progressive renal damage, there was no difference in renal function between kidneys exposed to gross reflux or continuing infections, or both, and those not so exposed. Hansson et al. (231) concluded likewise and provided evidence that girls with asymptomatic bacteriuria may actually benefit from not receiving antimicrobial treatment. These results differ from the earlier observations of Smellie et al. (176,232), who showed, in uncontrolled studies, that long-term antimicrobial prophylaxis benefited renal growth.

With increasing age, other clinical presentations of VUR or reflux nephropathy become relatively more common, although young women with an onset of UTIs after the commencement of sexual activity can be shown to have underlying reflux nephropathy that did not present in infancy or early childhood. About 4% to 5% of sexually active women with symptomatic UTIs have been shown to have reflux nephropathy (233). These patients may present with either bacterial cystitis or acute pyelonephritis. As discussed later, the disorder may also come to attention because of the detection of asymptomatic bacteriuria in pregnancy.

TABLE 24-1

CLINICAL PRESENTATIONS OF VESICOURETERIC REFLUX

1. Complicating urinary tract infections
2. Loin pain
3. Family studies
4. Nocturnal enuresis or other urologic complications
5. Associated with other congenital abnormalities
6. Fetal ultrasonography
7. Coincidental

TABLE 24-2

CLINICAL PRESENTATIONS OF REFLUX NEPHROPATHY

1. Complicating urinary tract infections
2. Hypertension—benign or accelerated
3. During pregnancy—syndrome mimicking preeclampsia
4. Proteinuria
5. Renal failure
6. Renal calculi
7. Family studies
8. Nocturnal enuresis or other urologic complications
9. Associated with other congenital abnormalities
10. Coincidental

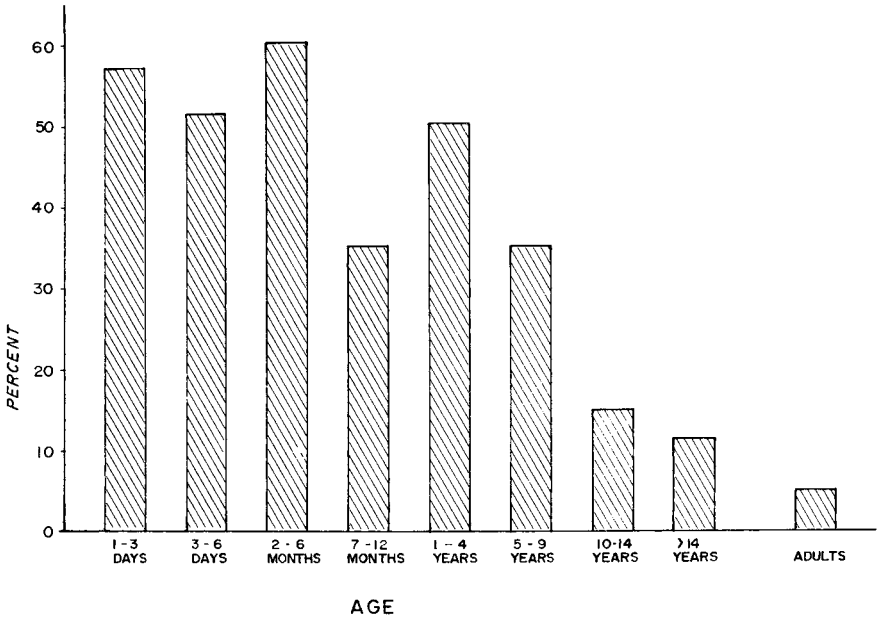


FIGURE 24-17. Histogram depicting the percentage of patients of various age groups with UTI shown to have VUR (based on a variety of published data). Both sexes are included in those under 1 year of age, and only females in the remainder. (From: Bailey RR. The relationship of vesico-ureteric reflux to urinary tract infection and chronic pyelonephritis–reflux nephropathy. *Clin Nephrol* 1973;1: 132, with permission.)

The bacteriologic diagnosis of UTI in the pediatric age group is difficult. If in doubt, uncontaminated urine must be obtained for culture by suprapubic bladder aspiration. Similarly, young children may not develop the classic clinical features of acute pyelonephritis, but present with fever, jaundice, or failure to thrive. A UTI should always be excluded in any ill child.

It is clear that any infant or child, whether asymptomatic or not, should have urinary tract investigations following the first bacteriologically proven UTI (234). The objective should be to detect as many children as possible with significant VUR as early in life as possible so that adequate therapeutic measures can be undertaken before significant damage occurs. The most accurate and precise method of detecting significant VUR is still the voiding cystourethrogram. In infants and children up to 2 years of age, this is a relatively simple examination. However, beyond this age it is usually difficult and traumatic for the child. As a result, any protocol for radiologic investigation of UTI must be a compromise. Voiding cystourethrography does not provide any information about the upper urinary tracts other than the degree of VUR, and, for assessment of renal parenchymal scarring and obstructive uropathy, either DMSA scanning, ultrasound, or intravenous urography should be considered.

As ultrasonographers become more experienced and clinicians become more confident in ultrasonic interpretation, it may be possible to undertake ultrasonography in preference to intravenous urography (235,236). Gordon (237) has already advocated ultrasound examination as the primary investigation in all infants and children. This is accompanied by DMSA scanning in all children under 5 years and, for those under 1 year, a voiding cystourethrogram is performed, in addition. In children over the age of 5 years in whom the ultrasound examination is normal, Gordon advocates no further investigation. If ultrasonography is abnormal, then further studies will be required and may include DMSA scanning, intravenous urography, and voiding cystourethrography. This protocol appeals as one that will detect significant VUR at an early age and will not subject a large number of otherwise normal children with UTI to the trauma of a normal voiding cystourethrogram.

Whyte et al. (238) reported on a protocol that restricts the number of voiding cystourethrograms in children over the age of 2 years by reliance on a good-quality intravenous urogram. This has appeal in centers where DMSA scanning is not readily available. In this protocol, children under 2 years with UTI are investigated with both ultrasound and voiding cystourethrography. If these are normal, then no further imaging is required. If abnormal, then DMSA scanning and intravenous urography will be necessary. In children between the ages of 2 and 5 years, the primary examination is an intravenous urogram where the objective is to determine the presence of underlying significant VUR by assessment of ureteric caliber. If the examination is of good quality and normal, then no further investigations are performed unless UTI continues to be a clinical problem.

Recent recommendations for initial work-up at the first upper UTI from a Swedish state-of-the-art conference include a clinical history, examination of abdomen, genitals and back, determinations of blood pressure and serum creatinine, and imaging studies, which are determined by the age of the patient. For children below 2 years of age, ultrasonography within 2 to 4 weeks and a voiding cystouretherogram within 1 to 2 months are recommended. Children 2 years of age and older should have an ultrasonography within 1 to 2 months and a DMSA scan after 6 to 12 months. A voiding cystoureterogram is added in these older children if DMSA scanning is not available or if the DMSA scan reveals uptake defects or uptake asymmetry, with one kidney having below 45% of total function (239).

Hansson et al. (240) reviewed the records of 303 children younger than 2 years with initial UTI investigated with DMSA scintigraphy; voiding cystourethrography was performed within 3 months after UTI. In 156 of the 303 children (51%), DMSA scintigraphy showed renal lesions. VUR was found in 80 of the 156 patients with renal lesions (51%), and VUR grade significantly correlated with their presence. Seven infants had a normal DMSA scintigraphy and dilating VUR (grade III) on the voiding cystourethrography. At follow-up after 1 to 2 years, six of these seven patients had normal DMSA scans and one had a scarred duplex kidney. VUR resolved spontaneously in five and improved spontaneously to grade 1 in two patients. None of the seven children had recurrent

UTI. Based on these observations, the authors proposed a strategy that replaces voiding cystourethrography by DMSA scintigraphy as a first-line investigation in infants with UTI and utilizes voiding cystourethrography only in patients with renal lesions.

Hypertension

Hypertension is a frequent late complication of reflux nephropathy. The hypertension is usually benign but occasionally may follow an accelerated course with deteriorating renal function. Reflux nephropathy is the most common cause of severe hypertension in childhood, but is uncommon in the first five years of life. Not infrequently, accelerated hypertension in children or teenagers is diagnosed by an ophthalmologist.

Rance et al. (241) found 29 of 96 children with persistent hypertension to have "chronic pyelonephritis," making it the most common cause. Smellie and Normand (242) reported 83 children with reflux nephropathy, of whom 11 were hypertensive at presentation. In six of these eleven children, the hypertension was malignant, and in seven with bilateral reflux nephropathy, there was renal insufficiency. During the 4 to 20 years of follow-up, an additional 14 children developed hypertension.

In a long-term follow-up study of 294 patients (235 females) over 15 years of age with reflux nephropathy (176 unilateral), 25 (8.5%) had hypertension at their initial presentation at a mean age of 17.3 years. At their last follow-up, at a mean age of 34.2 years, 113 patients (38%) had hypertension or were on antihypertensive therapy. Hypertension occurred significantly more frequently in those with severe bilateral renal parenchymal scarring (243).

Kincaid-Smith and Becker (244) reported 48 women and 7 men 17 years or older with reflux nephropathy. At presentation, 21 of these patients had a diastolic blood pressure above 99 mm Hg. Hypertension was more frequent in those with bilateral scarring or renal insufficiency. Kincaid-Smith et al. (245) later reported that hypertension was the presenting manifestation in 26 of 123 women and 5 of 22 men with reflux nephropathy. However, almost one-half of the total group had a blood pressure exceeding 140/90 mm Hg at presentation.

Torres et al. (246) studied 7 men and 60 women with primary bilateral VUR and noted that 23 (34%) had a diastolic blood pressure exceeding 90 mm Hg at presentation. Of those with hypertension, 3 had unilateral renal scarring, 19 had bilateral scars, and 1 had no scarring. The presence of hypertension in the 19 patients with bilateral scars correlated positively with both the cumulative grade of renal scarring and the degree of renal insufficiency. Similar results have been reported by Köhler et al. (247).

More recently, ambulatory blood pressure monitoring (ABPM) has been used to detect early blood pressure (BP) changes in patients with reflux nephropathy. Patzer et al. (248) compared ambulatory blood pressure values in 61 reflux nephropathy patients and 904 healthy controls. They found a significant positive correlation between the extent of renal scarring and systolic and diastolic BP. Mean systolic daytime BP was significantly elevated in girls with renal scars compared with healthy controls. Mean systolic and diastolic night-time BP were significantly elevated in these patients. Diastolic dipping was significantly lower in patients compared with controls. Elevated night-time BP may be the most sensitive indicator of BP elevation in these children. Lama et al. (249) evaluated the relationship between ambulatory BP and the severity of renal damage in 100 (70 females, 30 males, mean age of 13.5 ± 5) normotensive children with reflux nephropathy. There was no significant difference in casual BP between the groups. The mean 24–hour systolic and diastolic BP and load were significantly higher in patients with grades III/IV than in those with grades I/II reflux nephropathy, while casual BP measurements were not different. In the patients with grades III/IV reflux nephropathy, who had significantly higher serum creatinine concentrations, microalbuminuria correlated with ambulatory BP, and plasma renin activity failed to decrease with chronological age. In 3 of 12 patients with grades III/IV reflux nephropathy and increased load BP, left ventricular geometry, by integrated backscatter, was abnormal.

UNILATERAL REFLUX NEPHROPATHY AND HYPERTENSION

From the time Ask-Upmark (103) suggested a possible relationship between hypertension and unilateral renal disease and the report by Butler (250), 8 years later, that the removal of a small scarred kidney cured the hypertension in a 10-year-old girl with a UTI and a blood pressure of 190/120 mm Hg, there has been interest in the question of whether hypertensive patients with unilateral reflux nephropathy may benefit from nephrectomy. The evidence still remains unclear despite an enormous amount of published literature, none of it controlled and most of it anecdotal.

Smith (251) was one of the first to attempt to assess the chaos of conflicting opinion when reviewing the literature published between 1937 and 1957. There were 575 documented patients who underwent unilateral nephrectomy for established hypertension. Of these, 149 (26%) had a fall in blood pressure to 140/90 mm Hg or less for at least 1 year. Of the 149 patients, 62 had unilateral "chronic pyelonephritis." Smith (251) considered that many more failures than successes remained unreported and concluded that "even in the presence of obvious unilateral renal disease, nephrectomy remains an experimental procedure and subject to experimental error."

In 1957, Thompson (252) reviewed 3,000 patients who had undergone unilateral nephrectomy at the Mayo Clinic. Of these, 344 had hypertension, of whom 337 were assessable. Of the latter, 100 had unilateral chronic pyelonephritis, and 63 of these had a normal blood pressure after 1 month. The majority of these patients were followed for 1 year; 55% remained normotensive.

Kincaid-Smith (253) reviewed the literature from 1956 to 1961 and collated data on 326 patients with unilateral renal disease and hypertension. Fifty-seven of these had reflux nephropathy, and 23 of them were normotensive after 1 year; 33 showed a substantial benefit after nephrectomy. Luke et al. (254) reported 28 patients with unilateral reflux nephropathy who underwent nephrectomy because of hypertension. Eleven were cured, 3 improved, and 14 were not helped. It was concluded that the best results were in young patients with a short history of hypertension and that nephrectomy should be considered if the scarred kidney contributed less than 25% of total renal function, and the other kidney was completely normal.

Siamopoulos et al. (255) reported on a retrospective assessment of 31 patients with hypertension and unilateral scarred kidneys treated over a 9-year period. Fifteen of the patients were subjected to nephrectomy and 16 managed conservatively (not randomized). Nephrectomy was of value in the management of some of the patients. Wanner et al. (256) studied 43 patients with unilateral parenchymatous renal disease (predominantly reflux nephropathy) and hypertension, 20 of whom were nephrectomized and 23 treated with antihypertensive drugs. Both therapeutic approaches showed an excellent blood pressure-lowering effect, which was sustained for up to

4 years. These authors also reviewed the more recent published literature and commented on the lack of prospective, randomized studies.

Haycock (257) reported two hypertensive girls with unilateral reflux nephropathy who were cured by nephrectomy and reviewed the literature on the results of nephrectomy for unilateral renal disease in hypertensive patients under the age of 15 years. Of a total of 45 children (23 with unilateral reflux nephropathy), 34 were cured, 1 improved, 9 failed, and there was 1 postoperative death. The results of surgery in childhood appeared better than in adults.

There has been only one long-term prospective, randomized trial to study the benefit of nephrectomy on the hypertension of patients with unilateral reflux nephropathy. This study (258) was designed to assess whether prophylactic nephrectomy of the damaged kidney could prevent hypertension developing in those patients who were still normotensive and whether nephrectomy would be beneficial to those already hypertensive. This study only included patients with well-documented unilateral reflux nephropathy in whom the affected kidney had a glomerular filtration rate of 10 mL/min or less, and the contralateral kidney was completely normal radiologically, showed compensatory hypertrophy, and had an overall glomerular filtration rate exceeding 72 mL/min. This study was terminated, because of increasing difficulties in obtaining informed consent from patients eligible for enrollment. The results confirmed that normotensive patients are at risk of developing hypertension, but there was no convincing evidence to demonstrate that this could be prevented by prophylactic nephrectomy. There was statistically significant evidence, however, that hypertensive patients benefited from nephrectomy (259).

To complicate the situation, de Jong et al. (260) reported eight patients in whom unilateral reflux nephropathy was thought to be responsible for the hypertension, but, in fact, the contralateral renal artery was stenosed because of fibromuscular dysplasia. Clearly, this previously unrecognized scenario should be considered in any hypertensive patient with a unilateral small kidney.

ROLE OF THE RENIN–ANGIOTENSIN SYSTEM

Peripheral Plasma Renin Activity

Savage et al. (261) showed that the peripheral plasma renin activity was raised in 9 of 15 hypertensive children and 8 of 100 normotensive children with either unilateral or bilateral reflux nephropathy. It was suggested that an elevated plasma renin activity may indicate those children at risk of developing hypertension. Dillon and Smellie (262) then reported on 26 normotensive children with reflux nephropathy, and, of these, 5 had an elevated peripheral plasma renin activity. Of 51 children with reflux nephropathy and nonmalignant hypertension, 36 had a plasma renin activity markedly above the normal range, while in 15 the values were high in the normal range. These findings suggested that the renin–angiotensin system may be implicated in the hypertension of children with reflux nephropathy. The same authors reported their 5-year (263), 10-year (264), and 15-year (265) findings on the original 100 normotensive children. At 5 years, 85 patients were available for assessment, and 11 (13%) were found to have an elevated plasma renin activity, but not 5 of the 8 original normotensive children with an increased level in the first study. Of eight children identified as hypertensive at the 5-year follow-up, only three had an increased plasma renin activity 5 years previously. Although the plasma renin activity tended to rise in children with reflux nephropathy as they grew older, there was no direct relationship between blood pressure and plasma renin activity, plasma creatinine concentration, and the degree of renal scarring. The 10-year assessment (264) of 45 of the 95 traceable patients has shown 10 (22%) to have an elevated plasma renin activity. Once again there was no correlation between blood pressure and plasma renin activity or plasma creatinine. The 15-year follow-up (265) traced 78 of the original 100 normotensive children, of whom 55 (median age 27.3 years; 40 females) were available for assessment. Systolic blood pressure was above 140 mm Hg in 5 and diastolic BP was above 90 mm Hg in 2 patients. Whereas previously in this long-term study, the BP and peripheral plasma renin activity had increased progressively with age, the latest findings suggested that in early adult life there was a leveling out of the BP and a reduction in the plasma renin activity, even though the latter's regression with age remained abnormal.

Bailey et al. (266,267) measured the peripheral plasma renin activity of 17 normotensive and 12 hypertensive adults with unilateral reflux nephropathy. Eight of the normotensive and 6 of the hypertensive patients had a low plasma renin activity, while 4 of the 17 normotensive and none of the 12 hypertensive patients had an elevated peripheral plasma renin activity. In a carefully controlled study, Jacobson et al. (268) investigated 22 adult women with reflux nephropathy and concluded that the renin–angiotensin–aldosterone system was abnormally activated and was probably more important than hypervolemia in the development of hypertension in this group compared with 9 healthy control women.

Renal Vein Renin Studies

Hypertension in a patient with a unilateral renal parenchymal abnormality raises the possibility of surgical cure. The ratio of the plasma renin activity in the renal veins of the normal and abnormal kidneys has been widely used as an index of significant asymmetric renin release and thus a predictor of which patients with renal artery stenosis may have their hypertension cured by revascularization or nephrectomy. The same concept has been followed to assess the possible role of the renin–angiotensin system in the hypertension associated with unilateral reflux nephropathy.

Several early studies concluded that patients with unilateral nonvascular renal disease showed few, if any, indications of participation of the renin–angiotensin system in the pathogenesis of their hypertension. Bailey et al. (266,267) studied 29 patients with unilateral reflux nephropathy, 17 of whom were normotensive and 12 had untreated hypertension. The ratio of the plasma renin activity between the renal veins exceeded 1.5 in 3 normotensive and only 2 hypertensive patients, all of whom had a low peripheral plasma renin activity. All patients in this study also had differential renal function tests undertaken and 4 of the normotensive and 1 of the hypertensive patients had criteria suggesting that the damaged kidney was acting ischemically. One of these 4 normotensive patients had a renal vein renin concentration exceeding 1.5, while the hypertensive patient had a ratio of 1.1. From these data, it was concluded that there was neither consistent evidence to support the concept that the renin–angiotensin system played a primary role in the nonmalignant hypertension associated with unilateral reflux nephropathy, nor could any guide be obtained from renal vein renin estimations to support the idea that prophylactic nephrectomy should be considered in a normotensive patient.

Dillon and Smellie (262), however, studied 21 hypertensive children with unilateral reflux nephropathy and found the renal vein renin concentrations of some predictive value as to whether surgery would be beneficial or not. There is abundant evidence that the renin–angiotensin system is activated

when accelerated hypertension complicates reflux nephropathy (269).

Presentation During Pregnancy

If a female with reflux nephropathy does not present early in life with a complicating UTI or as a teenager with hypertension, she is likely to do so when she becomes pregnant. Women with reflux nephropathy are also prone to become hypertensive with any type of hormonal contraception.

It is well established that approximately 5% to 6% of pregnant women have asymptomatic bacteriuria in the first trimester. In some racial groups, such as the New Zealand Maori, the prevalence rate may be as high as 18% (270,271). Of women found to have asymptomatic bacteriuria during pregnancy, 5% to 33% have been reported to have a urinary tract abnormality, the most common of which is reflux nephropathy.

Women with a history of previous UTIs have been shown to have a much higher incidence of bacteriuria during pregnancy, while those with renal scarring and persistent reflux are more prone to develop acute pyelonephritis in pregnancy (272). These authors noted few serious complications in pregnancy in those women with reflux nephropathy and attributed this to their continuous clinical supervision.

Apart from a UTI, symptomatic or asymptomatic, the most common presentation of reflux nephropathy in pregnancy is with a syndrome mimicking preeclampsia, often in the first or second trimester. Bailey (266) reported 226 women who presented with severe or complicated preeclampsia or preeclampsia in a second or subsequent pregnancy. Nine (4%) of these women were found to have reflux nephropathy. This was comparable to the incidence of reflux nephropathy found in women investigated because of asymptomatic bacteriuria in pregnancy.

Kincaid-Smith and Becker (244) reported 48 women 16 years or older with reflux nephropathy. One was diagnosed because of hypertension in pregnancy and two because of postpartum edema and proteinuria. Thirty-seven of these women had a total of 85 pregnancies. Complications such as hypertension, fluid retention, and UTIs developed in 59 of the pregnancies. Kincaid-Smith et al. (245) expanded their study and reported that 23 (19%) of 123 women presented with a pregnancy-related complication. Recently, this group (273) reported on 345 pregnancies in 137 women with reflux nephropathy. Overall, the number of fetuses lost was 48 (14%), while maternal complications occurred in 39% of the pregnancies. Bilateral renal scarring was associated with increased maternal complications during pregnancy. McGladdery et al. (274) reached similar conclusions when they reviewed the outcome of 50 pregnancies in 28 women with reflux nephropathy.

Smellie et al. (201) have reported on 91 pregnancies in 52 women with a history of VUR during childhood. Twenty-four of these women had scarred kidneys and 10 still had VUR on their last cystogram. Of these 52 women, 26 had 43 completely normal pregnancies, while the other 26 women had 11 uncomplicated and 37 complicated pregnancies. The complications include hypertension, UTI, and low infant birth weight. Women with scarred kidneys were more likely to have abnormal pregnancies than those without, including an increased frequency of UTIs. The frequency of UTIs was not different in women who had undergone ureteric reimplantation from those who had not been treated surgically.

Women with reflux nephropathy, normal renal function, and hypertension should be encouraged to become pregnant, but require careful antenatal supervision. These patients usually have an improvement in their BP during the second trimester, but in the third trimester may require increased antihypertensive therapy. The pregnant women with reflux nephropathy who get into major problems are those who have renal insufficiency and proteinuria prior to becoming pregnant. They should be monitored carefully and their BP aggressively controlled. Becker et al. (275) studied 20 women with reflux nephropathy and renal insufficiency (plasma creatinine 0.2 to 0.4 mmol/L). Six of these 20 women experienced pregnancies exceeding 12 weeks' gestation. Pregnancy was associated with a rapid deterioration in renal function in all six, resulting in end-stage renal failure in four women within 2 years of delivery despite adequate BP control. These authors concluded that pregnancy in women with reflux nephropathy and moderately severe renal failure had a deleterious effect on renal function and that they should be informed of this risk. The majority of pregnant women with reflux nephropathy, however, will provide few management problems.

If any woman develops hypertension during early pregnancy, or severe or atypical preeclampsia in a second or subsequent pregnancy, then reflux nephropathy should be excluded. Although some of these women will be detected by routine urine culture, many will not have covert bacteriuria.

Proteinuria

Persistent proteinuria is a bad prognostic feature. It indicates a complicating glomerulopathy, the histologic hallmark of which is focal and segmental glomerulosclerosis with hyalinosis. This lesion may involve the unscarred segments of kidney or the structurally normal contralateral kidney in patients with unilateral reflux nephropathy (110,112,114,276).

Proteinuria may not appear for many years after severe scarring has occurred, and the proteinuria tends to increase as renal function declines. Hypertension then becomes an associated complication. As the glomerulopathy is the most sinister long-term complication of reflux nephropathy, there clearly needs to be a great deal more investigational work done in this area.

End-Stage Reflux Nephropathy

Chronic renal failure may be the end result of VUR (277–283). Early descriptions (11,12) of what is now recognized as reflux nephropathy were of young individuals dying from uremia. Yet, in 1975, neither VUR nor reflux nephropathy were listed as etiologies of renal failure in the United States Human Renal Transplant Registry. Undoubtedly these patients were categorized under diagnoses such as "pyelonephritis" or "congenital anomalies."

In one study, 32 (3.3%) of 965 patients referred for renal transplantation had bilateral primary, low-pressure VUR (284). The mean age at referral was 21.1 years. Ten of the 32 patients had sterile reflux. The severity of the reflux, rather than UTI, was considered the significant factor in the development of renal failure. In another study (285), 15 of 50 patients with end-stage renal failure had VUR. The 7 males and 8 females had a mean age of 30 years. Nine of the 15 patients had a history of UTI.

Bailey (277) and Bailey and Lynn (280) initially reported that about 12% of patients entering their dialysis–transplant program had reflux nephropathy. The majority presented with renal failure, hypertension, and significant proteinuria. Bailey et al. (283) extended these observations and reported that 42 patients (18 males) with reflux nephropathy, representing 11.3% of 371 patients, had entered their renal replacement program. Documented UTIs only occurred in 18 patients. Eight of the 24 women presented during a pregnancy, usually with features resembling preeclampsia. The mean age at commencement of dialysis treatment was 27.9 years for the males and

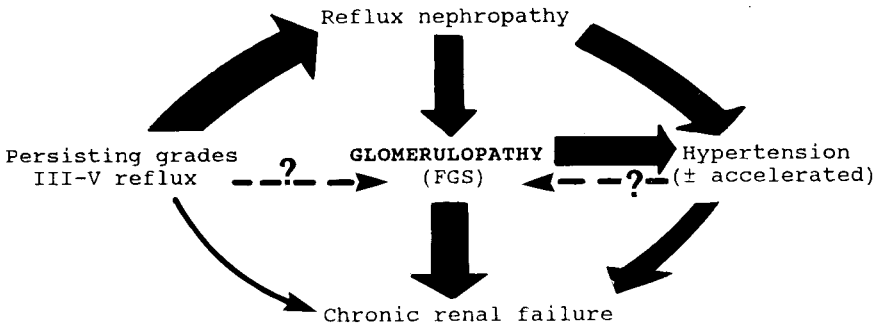


FIGURE 24-18. Diagram suggesting the possible mechanisms by which reflux nephropathy may progress to end-stage renal failure. *FGS*, focal and segmental glomerulosclerosis and hyalinosis. (From: Bailey RR, Lynn KL, Robson RA. End-stage reflux nephropathy. *Renal Failure* 1994;16:27, with permission.)

28.5 years for the females. Six of these 42 patients started dialysis before the age of 16 years and represented one-half of the children entering this program.

Kincaid-Smith et al. (245) reported that, between 1963 and 1982, 533 patients presented with end-stage renal failure and, in 82 (15.3%), it was because of reflux nephropathy. There were 30 men and 52 women; the mean age at which the men developed renal failure was 22 years and for the women 33 years. These authors also published details of 145 patients (123 women) with reflux nephropathy, aged 14 to 65 years, who presented over a decade (245). Seven of these patients reached end-stage renal failure during follow-up.

All investigators have stressed that proteinuria is the major prognostic feature of those patients whose renal function deteriorates. The proteinuria reflects the complicating glomerulopathy that may develop and progress in the absence of complicating UTIs or hypertension and after the reflux has either been corrected surgically or resolved spontaneously. El-Kharib et al. (286) reported that the risk factors for an increase in plasma creatinine in patients with reflux nephropathy were, in descending order, the presence of proteinuria, an elevated plasma creatinine, bilateral renal scarring, male sex, and the presence of hypertension.

A diagram suggesting the mechanisms by which reflux nephropathy may progress to renal failure is shown in Figure 24-18.

The Australia and New Zealand Dialysis and Transplant Registry has complete data for all patients entering renal replacement programs in both countries. The figures for 1971 to 1992 inclusive are included in Table 24-3. Of patients under 16 years of age, 23% had reflux nephropathy.

Using the European Dialysis and Transplant Association Registry Report of 1977, it has been assessed that 0.3 to 0.4 children per million of the population per year under the age of 15 years will present with renal failure due to reflux nephropathy (287). This may have been an underestimate, as it is known that some of the most severely affected patients, particularly boys, die from uremia in early life.

During the years 1979 to 1983 inclusive, 81,108 patients were enrolled in the European Dialysis and Transplant Association Registry (S. Challah, personal communication). Unfortunately as many as seven different diagnostic categories probably included patients with end-stage reflux nephropathy. It has been assessed from these data (288) that somewhere between 1.8% and 15.3% of all these patients with end-stage renal failure, and 8.1% to 24.7% of those under the age of 15 years, had reflux nephropathy (Table 24-4).

The North American Pediatric Renal Transplant Core Comparative Study reviewed more than 3,000 patients who received renal transplants between 1987 and 1995. Reflux nephropathy was found to be the cause of end-stage renal failure in 5.7% of these patients (289).

Marra et al. (290) used data from the ItalKid Project, a prospective population-based registry of Italian children, which collects information of all patients with CRF born over the last 30 years, to compare the prevalence of chronic renal failure secondary to VUR in males and females. They found a striking prevalence of males, a finding that conflicts with the data of the Australia and New Zealand Dialysis and Transplant Registry where the distribution of chronic renal failure secondary to reflux is similar in the two sexes. It is possible that this difference in the most recent study is the result of the more widespread and aggressive management of UTIs over the last 30 years. It is also possible that reflux nephropathy in females leads to chronic renal failure at a later age.

Although many editorials and reviews (115,291–297) have drawn attention to reflux nephropathy being an important cause of end-stage renal failure, it continues to be regarded by many as a minor or insignificant problem. Any patient presenting with renal insufficiency and proteinuria, with or without hypertension or UTIs, should have reflux nephropathy excluded.

TABLE 24-3

PATIENTS ENTERING RENAL REPLACEMENT PROGRAMS IN AUSTRALIA AND NEW ZEALAND AND THE NUMBER WITH END-STAGE REFLUX NEPHROPATHY, 1971–1992

	Australia		New Zealand	
	Male	Female	Male	Female
Total no. patients entering renal replacement programs	7368	6033	1432	1039
No. with reflux nephropathy	434 (5.9%)	523 (8.7%)	92 (6.4%)	119 (11.5%)
Total no. patients <16 yr	261	198	44	52
No. <16 yr with reflux nephropathy	57 (21.8%)	49 (24.7%)	7 (15.9%)	12 (23.1%)

(From: Disney APS, Australia and New Zealand Dialysis and Transplant Registry, personal communication.)

TABLE 24-4

DATA FROM EUROPEAN DIALYSIS AND TRANSPLANT ASSOCIATION REGISTRY, 1979–1983 INCLUSIVE

		Registry category[a]					Registry category[a]			
Sex	Total no. patients	24	20	29	60–63	Total no. <15 yr	24	20	29	60–63
Male	47,646	663	3,769	406	575	1,334	88	47	7	164
Female	33,462	761	5,275	395	467	1,006	101	67	11	91
Total	81,108	1,424	9,044	801	1,042	2,340	189	114	18	255
		(1.8%)	(11.2%)	(1.0%)	(1.3%)		(8.1%)	(4.9%)	(0.8%)	(10.9%)

[a] Category 24, Pyelonephritis/interstitial nephritis, because of VUR without obstruction; category 20, pyelonephritis/interstitial nephritis, cause not specified; category 29, pyelonephritis/interstitial nephritis, because of other causes; category 60–3, congenital renal hypoplasia, oligomeganephronic hypoplasia, segmental renal hypoplasia (Ask-Upmark kidney), congenital renal dysplasia ± urinary tract malformation.
(From: Challah S, EDTA Registry, personal communication.)

Urinary Calculi

It is not widely appreciated that there is an increased frequency of urinary calculi in patients with reflux nephropathy. The emphasis has been on the association with staghorn calculi in patients with uncontrolled UTIs, particularly with *Proteus mirabilis* or other urea-splitting organisms.

Torres et al. (246) reported 16 radiopaque calculi in 12 of 67 adults with primary bilateral VUR. Eight of the 12 patients were asymptomatic. In 11 patients, the calculi were in the scarred kidneys and one-half of the stones were contained in medullary cavities, while others were in clubbed calyces or associated with papillary tips. None of these patients had an accompanying renal tubular acidosis. Six of the 12 underwent metabolic investigations and the only abnormality detected was a patient with hyperuricosuria. When stone analysis was possible, the composition consisted of calcium carbonate, calcium phosphate, or struvite. The location of some calculi suggested that urinary stasis may promote stone formation. Very similar observations have been made by Köhler et al. (247).

Roberts and Atwell (298) reported five children (four boys) with VUR, a *Proteus* species UTI and urinary calculi; they estimated the incidence of calculi in patients with VUR to be 0.5%.

Recently, García-Nieto et al. (299) studied the prevalence of nephrolithiasis and hypercalciuria in 46 children with VUR and in their parents. The prevalence of renal colic and calyceal microlithiasis in these children was 6.5% and 19.6%, respectively. Twenty-seven out of 46 (58.6%) children had hypercalciuria, a prevalence much higher than that found in their control pediatric population (3.8%). The presence of hypercalciuria was not correlated with the severity or status of the VUR or with the presence of reflux nephropathy. The children with hypercalciuria were shorter and had lower maximal urine osmolalities compared to those without hypercalciuria. Sixty-three percent of the children with hypercalciuria and 52.6% of the children without hypercalciuria had one or both parents with hypercalciuria. The authors concluded that hypercalciuria is an important metabolic factor that contributes to the pathogenesis of nephrolithiasis in patients with VUR and suggested a common link between these two inheritable conditions. Other authors have not found a correlation between VUR and hypercalciuria (300).

Familial Association

After Stephens et al. (301) reported VUR in identical twins in 1972, there were several similar reports, including a set of triplets (302). Additional evidence indicated familial aggregation, but the mode of inheritance was unclear. Several authors suggested either a multifactorial trait (303–305) or an autosomal dominant gene with reduced penetrance (306–308). Two single-family studies suggested X-linked inheritance (309,310).

Three New Zealand studies have more clearly elucidated the inheritance of VUR. First, Heale (311) reported 15 families who had come to attention on casual enquiry of 213 patients with reflux nephropathy. Radiologic abnormalities of the urinary tract were found in 67 (38%) of 175 relatives of the 15 propositi. In 12 of these 15 families there was a hereditary pattern, suggesting autosomal dominant inheritance with variable penetrance. Bailey (194) then reported 56 further families from the same unit and noted that in 17 (30%) of these there were one or more affected members. Finally, data from a total of 88 affected families were subjected to segregation analysis (312,313), using a mixed model (314) and a computer program (POINTER). Eighteen families were ascertained through a proband with end-stage reflux nephropathy, 2 through a proband with severe renal failure, and 68 through a proband with reflux and normal renal function. In 30 of the 88 families, additional affected members were found. Some of these are depicted in Figure 24-19. Of the 242 relatives investigated, 48 were affected. Three generations were affected in 6 families, and 2 generations in a further 15 families. In a few cases, apparently nonaffected relatives were likely asymptomatic carriers of the gene, as they had both an affected offspring and an affected parent (Fig. 24-19, No. 31). In one family (Fig. 24-19, No. 41), both parents were affected, but the only child was unaffected. These results are consistent with a dominant, but not recessive, mode of inheritance.

Segregation analysis indicated that the hypothesis of no genetic effect could be rejected, as could the recessive model. Polygenic inheritance fitted better, but the best model was that of a single dominant gene acting together with random environmental effect. Detailed testing of the subjects with VUR, reflux nephropathy, or end-stage renal failure showed no heterogeneity in the data, supporting the hypothesis that the same major locus was responsible. The model indicated that the gene frequency was 1 in 600 and that mutation was uncommon. According to the model, and using information on the prevalence of end-stage reflux nephropathy, it was calculated that 45% of gene carriers will have VUR or reflux nephropathy as adults and 15% will develop end-stage renal failure. Of noncarriers of the gene, 0.05% will have VUR or reflux nephropathy and 0.001% will go on to renal failure from this cause. Thus, the trait for reflux is one of the most common mendelian dominant traits known in humans.

Hollowell et al. (315) performed a meta-analysis of 11 recent studies on the value of screening siblings of affected

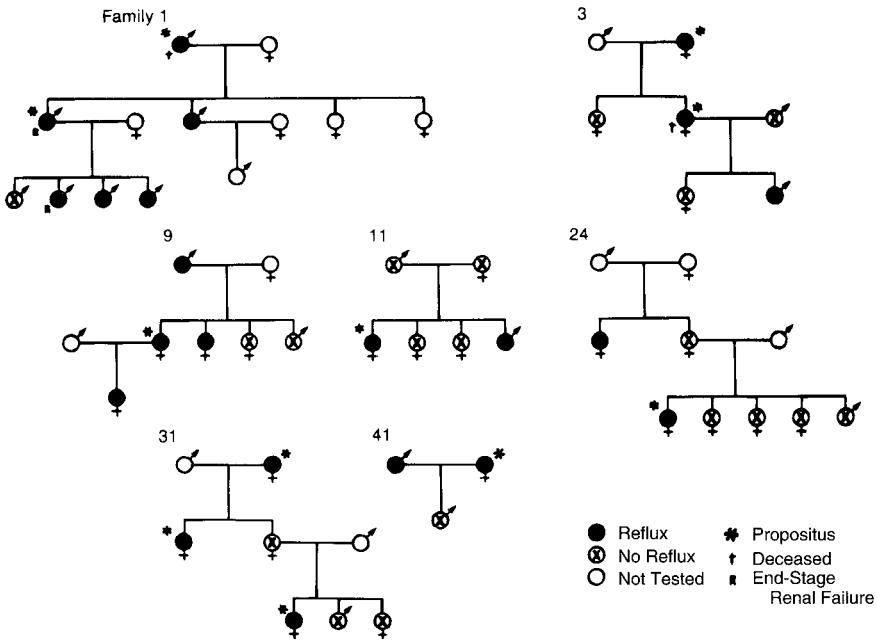


FIGURE 24-19. Pedigrees of some of the families in which more than one member had VUR or reflux nephropathy. The data are consistent with an autosomal dominant mode of inheritance with incomplete penetrance. (From Bailey RR, Janus E, McLoughlin K, et al. Familial and Genetic Data in Reflux Nephropathy. In *Reflux nephropathy update: 1983*, Hodson CJ, Heptinstall, RH, Winberg, J, eds. Basel: Karger, 1984, with permission.)

individuals for VUR (315). Of 1768 siblings, 32% had reflux rate substantially higher than that seen in the general pediatric population. Only 2%, however, had reflux greater than grade III. Some of these children go on to develop progressive renal scarring with renal dysfunction. The incidence of reflux and renal abnormality in the sibling population was 3%. In more than one-half of these, there was no history of UTI. Hollowell et al. (315) noted that the four largest studies found a 5% to 23% incidence of renal insufficiency in siblings with reflux, compared to a 3% risk of renal disease in the entire sibling population. Kaefer et al. (316) analyzed a database of voiding cystourethrogram (VCUG) for multiple gestation births. Overall, 50% of siblings of index cases had demonstrable VUR (316). Eighty percent of identical twins were concordant for VUR, whereas only 35% of nonidentical twins were concordant for VUR. When only the youngest individuals in each group were considered, 100% of the monozygotics and 50% of the dizygotics exhibited VUR. The investigators concluded that the frequency strongly supported autosomal dominant inheritance.

In view of the familial aggregation of VUR and reflux nephropathy and the evidence for an inherited basis, a search has been made for possible genetic markers. An early study (194,317) showed that HLA-B12 was significantly more frequent in patients reaching end-stage reflux nephropathy compared with those entering two dialysis–transplant programs from all other causes. Sengar et al. (318) studied the histocompatibility antigens of 36 unrelated French Canadian children (34 girls) with a history of at least two UTIs and found HLA-AW32 to be present in 6 of 16 with VUR, compared with none of 20 without reflux. These authors suggested that a gene or genes other than those governing the HLA were responsible for the genesis of reflux.

The frequency of 40 HLA antigen specificities was determined in 44 Americans with end-stage reflux nephropathy and compared with 526 blood donors (319). Higher-than-normal frequencies were observed for HLA-B12 in female patients and HLA-B15 in both sexes. In addition, there was an increase in the frequency of the combinations A9/B8, A9/BW15, A1/A9, and B8/BW15 in the male patients. It was concluded that the major histocompatibility antigens may be in linkage disequilibrium with the gene(s) determining the susceptibility to renal damage by reflux and that this linkage, or its clinical expression, may be influenced by variations in relation to the sex of patients.

Bailey et al. (317) extended their earlier study to 33 patients with end-stage reflux nephropathy and 108 patients with end-stage renal failure from all other causes and compared them with 179 blood donors. Comparisons of HLA antigen and HLA haplotype (the unit of inheritance of the HLA system) frequencies in patients and controls were made. An analysis of the HLA antigen frequency in the control subjects versus patients with end-stage reflux nephropathy showed that only for HLA-A9 was there a significantly higher incidence in the patients. When the patients were subdivided according to sex, the trend persisted, but statistical significance was lost. The haplotypes that showed a difference between the patients with end-stage reflux nephropathy and the controls were HLA-A9/B12 and HLA-A2/B8. However, neither combination was statistically significant. When patients with end-stage reflux nephropathy were compared with those with renal failure from all other causes, the relative incidence of these two haplotypes was significantly more frequent in the former.

Clearly the search must continue for a genetic marker or markers for VUR. It seems likely that VUR will be found to be genetically heterogeneous. An autosomal dominant inheritance with variable expression and reduced prevalence has also been documented for other types of congenital urological malformations, such as renal adysplasia, ureteropelvic junction obstruction, ureterovesical junction obstruction, ureteral duplication, and multicystic renal dysplasia (320). Interestingly, contralateral urological malformations of different types often coexist in the same patients. Numerous genes are involved in the regulation of the complex reciprocal inductive interactions between the ureteric bud and the metanephric blastema, which is responsible for the development of a normal urinary tract (321). It seems likely that mutations in many of these genes will be found to be responsible for congenital urological malformations. Mutations in the *PAX2* gene have been found to be responsible for the renal–coloboma syndrome, which is characterized by optic nerve colobomas, VUR, and other renal malformations (322). *PAX2*, however, has been excluded as a major locus for primary familiar VUR (323). Uroplakin III appeared to be an excellent candidate gene for familial VUR,

since its knock-out in the mouse causes VUR without other congenital abnormalities of the urogenital tract or other organ systems. However, no pathogenic mutations were detected in 25 patients with familial VUR (324).

The angiotensinogen, angiotensin-converting enzyme (ACE), angiotensin II type 1 (AT_1) and type 2 (AT_2) receptor genes are necessary for the normal development of the kidney and/or urinary tract (325–327). While disruptions of the angiotensinogen, *ACE*, and AT_1 genes in mice result in atrophic renal papillae and enlargement of the renal pelvis, disruption of the AT_2 gene results in congenital hydronephrosis (328). A particular polymorphism of the AT_2 gene has been found with increased frequency in patients with primary obstructive megaureter (329), but not in patients with primary VUR (330). While some of the genes may not be directly responsible for VUR, they may increase the susceptibility to development of renal damage. A study has suggested that patients with VUR and the *ACE* gene *DD* polymorphism are more likely to have cortical scars than those with the *DI* or *II* genotype (331). Rigoli et al. (332) recently investigated the genotype distribution of the *ACE* insertion/deletion polymorphism and AT_2R single nucleotide polymorphisms in Italian children with congenital urinary tract abnormalities, as well as the association between these polymorphisms and renal disease progression. For these studies, 92 healthy control subjects and 102 Italian children with congenital urinary tract abnormalities were recruited; 27 with vesicoureteral reflux, 12 with hypoplastic kidneys, 20 with multicystic dysplastic kidneys, 13 with ureteropelvic junctions stenosis/atresia, 18 with nonobstructed, nonrefluxing primary megaureters, and 12 with posterior urethral valves. No difference was observed in the *ACE* genotype distribution, but the *D/D* genotype was overrepresented in the subgroup of patients with radiographically demonstrated renal parenchymal abnormalities. Moreover, a significant correlation was observed between the *G* allele of AT_2R and congenital urinary tract abnormalities. These data suggest that the *ACE*-related *DD* polymorphism is a risk factor for progressive renal parenchymal damage in these patients, while allelic variants in AT_2R may play a role in the development of some congenital urinary tract abnormalities (332). Haszon et al. (333) also found a significant association between the *DD* phenotype and renal scarring. Other studies do not confirm these observations (334,335). Yoneda et al. (334) performed a study to identify significant independent risk factors, including *ACE* gene polymorphism, associated with renal parenchymal damage in patients with primary familial vesicoureteral reflux (334). They identified 88 families in which 2 or more members had primary vesicoureteral reflux, comprising 162 patients with a mean age of 2.3 years (range 3 weeks to 12 years). Renal scarring was detected in 45 of the 162 patients (28%). There were no significant differences in *ACE* genotype distribution in patients with and without renal damage. The same authors also failed to find an association between polymorphisms and the presence of VUR.

Recent studies in mice have demonstrated that genes encoding bone morphogenetic protein 4 (BMP4) and forkhead transcription factor 1 (FOXC1) are involved in the organogenesis of the kidney and urinary tract and that loss of function of either gene causes congenital urinary tract abnormalities. To explore the possible role of *BMP4* or *FOXC1*, Nakano et al. (336) screened both genes for mutations in seven patients with congenital urinary tract abnormalities. Initial analysis revealed a triplet (GCG) insertion in *FOXC1* in three of the seven patients. No mutations were detected in the coding sequence of *BMP4*. The TGF-β1 at position −509 T-C polymorphism, which is associated with higher circulating levels of TGF-β1, the tissue kalikrein (*KLK1*) at position –130 12 *G* allele, which is associated with low kallikrein expression, and the TNF-α gene polymorphism at position −308 involving a guanidine to adenosine transition (−308G and −308A) have been found more frequently in patients with progressive reflux nephropathy than in controls (337,383).

Nocturnal Enuresis or Other Urologic Complications

Infants and children with VUR have a high incidence of nocturnal enuresis (339) or evidence of lower urinary tract dysfunction such as detrusor instability or detrusor–sphincter dyssynergia. Hypospadias, undescended testicles, bifid pelvicalyceal collecting systems, ureteric duplication, pelviureteric junction obstruction, multicystic renal dysplasia, and other urologic conditions may be associated with primary VUR.

Association with Other Congenital Abnormalities

VUR has been reported in association with a range of other congenital disorders such as Hirschsprung's disease, anorectal abnormalities (e.g., short-colon syndrome), renal–coloboma syndrome, and the prune-belly syndrome.

Loin Pain

Loin pain is probably the only urinary tract symptom that can be attributed specifically to VUR. Although infants and young children rarely complain of loin pain, older patients may give a clear history of loin pain when their bladder is full, with worsening at the start of micturition and then rapid relief after voiding. This is arguably the only indication for antireflux surgery in an older child or adult.

Coincidental Finding

As more patients are having upper abdominal ultrasonography to evaluate abdominal symptoms, so is reflux nephropathy being detected coincidentally with greater frequency. The most common situation is to find in a previously healthy individual an irregularly scarred kidney on one side with a hypertrophied contralateral kidney. Rarely, however, does the chance finding of a small kidney explain the abdominal symptoms, unless the patient has a UTI or urinary calculi.

Fetal Ultrasonography

Since the evidence became available that renal damage occurs as a result of gross VUR in the first few years of life, and probably even *in utero*, there has been a search for a simple, rapid, reliable, safe, and noninvasive method of screening infants for the presence of gross reflux. To date prenatal ultrasonography appears the most promising.

Improved sophistication of high-resolution ultrasonographic equipment has permitted visualization of fetal anatomy. The potential now exists for the detection of certain congenital anomalies as early as 12 to 15 weeks of gestation. Kramer (340) pointed out that at 10 weeks of gestation the intercortical nephrons produce urine, the ureter becomes patent, and urine flows into the urogenital sinus. Shortly after

this the cloacal membrane ruptures and allows communication between the urinary tract and the amniotic cavity. Ultrasonography may identify up to 90% of fetal kidneys by 17 to 20 weeks of intrauterine life. After 20 weeks' gestation, the rate of urine production can be measured by serial estimation of bladder volume.

This knowledge has stimulated great interest in the examination of fetal kidneys by ultrasonography. A number of investigators have reported a range of urinary tract abnormalities observed *in utero* (341–348). The most common prenatal ultrasound findings are those of hydronephrosis or hydroureter. In approximately 10% to 30% of cases this is because of VUR (349,350). Most neonates with VUR diagnosed prenatally are boys owing to the higher voiding pressures in males as compared to females. Prenatal hydronephrosis in blacks, however, is very rarely because of VUR (351). VUR detected prenatally is usually bilateral and high grade. Of kidneys exposed to prenatal VUR detected by fetal ultrasonography, 23% to 60% are renographically abnormal. Many of these kidneys show generalized damage, but others exhibit focal lesions. These observations clearly point to the fact that a significant proportion of reflux nephropathy is congenital. Whether these lesions are primary, occurring pari passu with the VUR, or whether they are secondary, as a result of the VUR, is uncertain (352,353). Nevertheless, the predominance of boys suggests that the renal damage in congenital reflux nephropathy is, indeed, secondary to VUR *in utero*, since the "ureteric bud theory" cannot satisfactorily account for the marked male predominance.

It is currently recommended to offer maternal ultrasonography to all pregnant patients to detect potentially significant congenital urological malformation in asymptomatic fetuses (349,350). Patients with pelvic dilatation measuring more than 4 mm in the anterior–posterior dimension should undergo repeat ultrasonography in the early postnatal period between 2 and 7 days of life. Those with initial negative studies should have a repeat ultrasonography within 1 month of life, since low urinary output within the first days of life may cause false-negative studies. Patients in whom the hydronephrosis is confirmed at birth should be screened for VUR by voiding cystourethrography. Some authors recommend screening with voiding cystourethrography (VCUG), even when hydronephrosis has resolved at birth. Ismaili et al. (354) prospectively followed 264 infants with antenatal renal pelvis dilation. Two successive neonatal renal ultrasound examinations were performed at day 5 and 1 month after birth. VCUG was performed in all infants. Neonatal ultrasound findings were abnormal in 190 infants (72% of total). Among them, 63 (33%) had an abnormal VCUG. When both neonatal ultrasound findings were normal (74 infants), the VCUG showed abnormalities in only 5 (6.7%) patients (4 low-grade primary VUR and 1 posterior urethral valve). Therefore, a normal-appearing urinary tract on two successive neonatal ultrasound scans rarely coexists with abnormal findings at VCUG. In such patients, VCUG does not seem justified. Moorthy et al. (355) also concluded in a retrospective study that children with antenatal hydronephrosis and a normal postnatal ultrasound are highly unlikely to have an undetected urinary tract abnormality.

It has been suggested that male infants with VUR may benefit from circumcision, because uncircumcised boys have a fourfold higher likelihood of UTI compared to the circumcised population despite antibiotic prophylaxis (349). However, a recent study reporting on 27 children circumcised at the time of antireflux surgery and 50 not circumcised showed no significant difference between the groups in the number of UTI episodes or morphologic changes on renal scans after antireflux surgery (356). These findings indicate that circumcision during antireflux surgery has no effect on the incidence of postoperative UTI.

MANAGEMENT OF VESICOURETERIC REFLUX

Surgery to Correct Vesicoureteric Reflux

There is still considerable disagreement concerning the indications for antireflux surgery (357–362). At one extreme, are those clinicians who do not correct VUR of any degree in any individual, regardless of age, but choose to use low-dose antimicrobial prophylaxis for varying periods of time. At the other extreme, are those who operate on any patient, at any age, and with any degree of reflux. The truth, as always, probably lies somewhere in between.

In the past, many urologists have recommended antireflux surgery in an attempt to control complicating UTIs. The balance of evidence suggests, however, that surgical correction has no effect on the overall incidence of these infections. The latter are invariably easy to manage if attention is paid to careful bacteriologic assessment, appropriate antimicrobial treatment, and regular follow-up.

Recently, there has been a more scientific approach to antireflux surgery. This has resulted directly from studies of the natural history of the different degrees of reflux in infants and young children. The intentions of surgery must be either to prevent renal parenchymal damage developing in normal kidneys subjected to continuing reflux or to reduce the risk of further damage to those kidneys that are already scarred. The important questions that have been addressed concern which kidneys are at risk of scarring, at what age should surgery be done, and does surgery prevent scarring, lessen further damage, or even allow the kidney to regain normal growth?

There have been many uncontrolled studies reporting surgery having little or no benefit, but the procedure was invariably done long after renal damage developed or was undertaken on patients with a degree of reflux that is now known not to be associated with parenchymal scarring.

In Christchurch Hospital, an uncontrolled study (363) showed that some kidneys subjected to gross reflux may recommence normal renal growth if the reflux was successfully corrected early in life. In other patients, renal growth did not occur after surgery, but further renal damage was prevented. Other investigators made similar observations (364–368).

Most clinicians now agree that surgery is of no benefit if the patient has developed proteinuria, renal insufficiency, or hypertension. The latter persists, while renal function continues to deteriorate. All of the information available would suggest that if surgery is beneficial, it must be undertaken very early in life, as renal damage associated with reflux invariably develops within the first 2 or 3 years of life and usually within the first few months.

Probably the only specific indication for antireflux surgery in an adult is the very occasional patient who is troubled by loin pain when the bladder is full or during micturition.

Controlled Trials

There have been several prospective, controlled trials to assess the benefits of antireflux surgery compared with medical management of the more severe grades of reflux. These trials have studied the natural history of sterile VUR of a severe degree. As far as the long-term outcome of patients is concerned, it is probably more important to assess the effect of surgery on the natural history of reflux nephropathy and its complications.

The Birmingham Reflux Study Group (212) started in 1975 and compared operative versus nonoperative management of

children with gross reflux (Rolleston classification), whether or not scarring was present, and with moderate reflux (Rolleston classification—equivalent to International Classification grade II) when scarring was already present. Three age groups were included (<1 year, 1 to 5 years, >6 years). Children in both arms of the study received antimicrobial prophylaxis. Unfortunately, most of the children already had scarred kidneys at enrollment and only 15 were under the age of 1 year. After 2 years of follow-up, no short-term advantage was shown for either form of treatment, with neither antimicrobial treatment nor antireflux surgery diminishing the scarring process once it had developed (212). The initial follow-up report was extended to 161 children observed for 2 years, of whom 104 were followed for 5 years (213,369). Between the treatment groups no significant differences emerged in the incidence of breakthrough UTIs, GFR, renal concentrating ability, renal growth, the progression of existing scars, or new scar formation. The authors concluded that neither treatment could claim superiority, nor could they fully protect the kidneys from further damage, and stressed that efforts must be directed toward identifying infants at risk before scarring develops. This study was concluded after the 5-year report.

The largest prospective trial was undertaken under the auspices of the International Reflux Study Group as a collaborative effort enrolling children under the age of 11 years (204,211). The 15 collaborating units in the United States enrolled 138 children with grade IV reflux (International Classification), while the 8 units in the European arm of the study enrolled 402 children with both grade III and grade IV reflux. Unfortunately, children with grade V reflux were not included because the clinicians all agreed that the most severe form of reflux in an infant or a young child warranted antireflux surgery. Enrollment was completed in early 1985 and all children were randomized to receive either antireflux surgery or no surgery, which included complete bladder emptying, regular bowel movement, and careful perineal toilet. Most of the children already had renal scarring at entry. All were given antimicrobial prophylaxis, which was continued in the medically treated group until the reflux had stopped and in the surgical group for 6 months after surgery.

In the European arm of the study, the children had a repeat voiding cystourethrogram 3 months after enrollment and, if they still had dilating reflux (i.e., grade III or more), were then considered to have stable reflux, entered in the main study, and randomized to either treatment with surgery or no surgery. This preallocation period resulted in 81 children in the European arm being excluded because of "unstable reflux." These 81 children are being followed as a separate group and appear to differ biologically from those remaining in the main study. Thus, 321 children in the European arm of the study were followed in the main study.

There was no preallocation phase in the United States arm of the study, which definitely had problems with recruitment. In addition, it is difficult to explain why in this group there were only 14 boys (11% of the total) compared with 97 (24% of the total) in the European group. This difference was statistically significant and was not explained by an older age group in the American children. If the latter were true, fewer boys would be expected, as boys tend to present within the first year of life. It has been shown that the risk of UTIs in boys is higher in those who are uncircumcised (370), and it has been postulated that the practice of widespread circumcision in the United States may be responsible for the small number of boys enrolled in that arm of the trial. In addition, 67% of the children in the United States were already known to have reflux before the index presentation, compared with only 19% of the European group.

Of the 321 children in the European arm, 306 were treated according to the random allocation, 155 medically and 151 surgically. During the first 5 years of medical observation, the VUR disappeared in 16%, diminished in 33%, and did not change in 51% of the cases. The disappearance rate was higher for unilateral (54%) than for bilateral (12%) reflux. Postoperative obstruction occurred in 10 ureters (10 patients) of the 151 surgically treated patients. Reoperation was needed in seven (4.6%). Recurrence of UTI was observed in 39% of the medical and 40% of the surgical patients. Acute pyelonephritis, however, was more frequent in the medical than in the surgical group (22% versus 10%, $p < 0.01$). Recurrent infections in boys occurred almost exclusively during the first year of age. New scars developed in 12% of medical and 13% of surgical patients. Following surgery, new scars developed earlier than in the medical group. The younger the child at entry, the higher the risk of new scars. More boys than girls develop new scars. Children with grade IV reflux were more likely to develop new lesions than those with grade III reflux. Progression of the scars was noted in 42% of children with renal scars at entry, with no difference between the study groups.

After completing the 5-year study, five of the eight original European participating centers agreed to extend the follow-up period with clinical observation and an additional intravenous urogram at 10 years (371). Among 223 patients (113 medical and 110 surgical) who continued follow up with urography at 10 years, new scars developed in only 2 patients. Overall, 47 new scars were acquired in 42 patients (20 medical and 22 surgical), 25 of them in children with unscarred kidneys at entry (13 medical and 12 surgical). New scars occurred mostly in children under 5 years of age and were observed more frequently in children with grade IV than grade II reflux. The authors concluded that with careful management, only a small proportion of children with severe reflux developed new scars and rarely after the first 5-year follow-up period and that there was no difference between children treated medically or surgically.

A 10-year follow-up of this study focusing on the evolution of the height and weight of these children has also been published (372). No differences in these parameters were detected between the surgically treated children and those on long-term antibiotic prophylaxis (3%).

In the United States arm of the study, 68 children were allocated to medical and 64 to surgical treatment. Treatment changes, however, occurred during the study, resulting in surgical correction of VUR in 87 patients. There were no episodes of postsurgical obstruction and persistent reflux was noted in less than 1% of the patients. The overall rate of UTIs was the same in both groups, but pyelonephritic infections occurred more frequently in the medical than in the surgical group (22% versus 8%, $p < 0.05$). The VUR disappeared in 24% of the medically treated patients. New scars developed in 22% of the children treated medically and in 31% of those treated surgically.

A group from Rotterdam (373–375) published three reports of two prospective studies conducted between 1982 and 1986. Ninety-four children (63 girls), aged between 3 months and 14 years, with a total of 137 refluxing ureters, have been followed for at least 5 years. All children with reflux grade III (International Classification) or less had antimicrobial prophylaxis only, those with grade IV were randomized to antimicrobial treatment alone or surgery plus antimicrobial treatment, and those with grade V reflux were all treated surgically. The protocol and the findings of these studies are difficult to follow, but the authors concluded that if detrusor instability has been excluded, surgery is the preferred treatment for children with grade IV and V reflux.

A prospective, randomized study was undertaken in Auckland, New Zealand to assess the effect of sterile grade III or IV reflux (International Classification) on individual kidney function and renal growth in children in three age groups (6 to 23 months, 2 to 5 years, and 6 to 10 years). A total of 138 children (112 girls) were randomized to receive either conservative treatment (continuous low-dose antimicrobial prophylaxis) or

TABLE 24-5

TREATMENT RECOMMENDATIONS: BOYS AND GIRLS WITH PRIMARY VESICOURETERAL REFLUX AND NO RENAL SCARRING

Clinical presentation		Treatment					
		Initial (antibiotic prophylaxis or open surgical repair)			Follow-up (continued antibiotic prophylaxis, cystography, or open surgical repair)[a]		
Reflux grade/ laterality	Pt. age (yr)	Guideline	Preferred option	Reasonable alternative	Guideline[b]	Preferred option[b]	No consensus[c]
I–II/Unilat. or bilat.	Younger than 1	Antibiotic prophylaxis					Boys and girls
	1–5	Antibiotic prophylaxis					Boys and girls
	6–10	Antibiotic prophylaxis					Boys and girls
III–IV/Unilat. or bilat.	Younger than 1	Antibiotic prophylaxis			Bilat: Surgery if persistent	Unilat: Surgery if persistent	
	1–5	Unilat: Antibiotic prophylaxis	Bilat: Antibiotic prophylaxis			Surgery if persistent	
	6–10		Unilat: Antibiotic prophylaxis Bilat: Surgery	Bilat: Antibiotic prophylaxis		Surgery if persistent	
V/Unilat. or bilat.	Younger than 1		Antibiotic prophylaxis		Surgery if persistent		
	1–5		Bilat: Surgery Unilat: Antibiotic prophylaxis	Bilat: Antibiotic prophylaxis	Surgery if persistent		
	6–10	Surgery		Unilat: Surgery			

[a]For patients with persistent uncomplicated reflux after extended treatment with continuous antibiotic therapy.
[b]See duration of reflux regarding the time that clinicians should wait before recommending surgery.
[c]No consensus was reached regarding the role of continued antibiotic prophylaxsis, cystography or surgery.
(From: Elder JS, Peters CA, Avant BS Jr, et al. Pediatric vesicoureteral reflux guidelines panel summary report on the management of primary vesicoureteral reflux in children. *J Urol* 1997;157:1846.)

antireflux surgery. There were 45 infants under 1 year of age, but only 7 of these were boys, while in this age group there were only 6 with grade IV reflux. Complete 2-year data are available for 115 children (376). The significant observation was that in those children aged 2 to 10 years at entry, those treated surgically had a significant increase in GFR, but those in the medical group did not. A further report on this study is unlikely.

Recently, Wheeler et al. (377) conducted a meta-analyses of randomized controlled trials using a random effects model to evaluate the benefits and adverse effects of treatments for VUR in children. Main outcome measures were incidence of UTI, new or progressive renal damage, renal growth, hypertension, and GFR. Eight trials involving 859 evaluable children comparing long-term antibiotics with surgical correction of reflux (VUR) and antibiotics (seven trials) and antibiotics compared with no treatment (one trial) were identified. Risk of UTI by 1 to 2 and 5 years was not significantly different between surgical and medical groups (relative risk [RR] by 2 years 1.07; 95% confidence interval (CI) 0.55 to 2.09, RR by 5 years 0.99; 95% CI 0.79 to 1.26). Combined treatment resulted in a 60% reduction in febrile UTI by 5 years (RR 0.43; 95% CI 0.27 to 0.70), but no concomitant significant reduction in risk of new or progressive renal damage at 5 years (RR 1.05; 95% CI 0.85 to 1.29). In one small study, no significant differences in risk for UTI or renal damage were found between antibiotic prophylaxis and no treatment. It is uncertain whether the identification and treatment of children with VUR confers clinically important benefit. The additional benefit of surgery over antibiotics alone is small, at best. Assuming a UTI rate of 20% for children with VUR on antibiotics for 5 years, nine reimplantations would be required to prevent one febrile UTI, with no reduction in the number of children developing any UTI or renal damage.

The place of surgical management of VUR will continue to be debated. As the results of the control trials do not clearly favor a surgical versus a medical approach, strong opinions for and against surgical management of VUR will continue to be promoted. Because of the lack of consensus regarding management of this common condition, panels of experts have been convened by the American Urologic Association and the Swedish Medical Research Counsel, and their recommendations have been published (239,378). The treatment recommendations of the American Urologic Association panel of experts are presented in tabular form (Tables 24-5 and 24-6) and are based on scientific evidence and expert opinion. Although cost reduction cannot be the basis for decision making, cost analysis of the treatment of VUR have shown that medical management is less costly than up-front surgery (379,380).

Surgical Techniques

In experienced hands, antireflux surgery is a successful procedure and associated with a low morbidity rate. A number of

TABLE 24-6

TREATMENT RECOMMENDATIONS: BOYS AND GIRLS WITH PRIMARY VESICOURETERAL REFLUX AND WITH RENAL SCARRING

Clinical presentation		Treatment					
		Initial (antibiotic prophylaxis or open surgical repair)			Follow-up (continued antibiotic prophylaxis, cystography, or open surgical repair)[a]		
Reflux grade/ laterality	Pt. age (yr)	Guideline	Preferred option	Reasonable alternative	Guideline[b]	Preferred option[b]	No consensus[c]
I–II/Unilat. or bilat.	Younger than 1	Antibiotic prophylaxis					Boys and girls
	1–5	Antibiotic prophylaxis					Boys and girls
	6–10	Antibiotic prophylaxis					Boys and girls
III–IV/Unilat.	Younger than 1	Antibiotic prophylaxis			Girls: surgery if persistent	Boys: surgery if persistent	
	1–5	Antibiotic prophylaxis			Girls: surgery if persistent	Boys: surgery if persistent	
	6–10		Antibiotic prophylaxis		Surgery if persistent		
III–IV/Bilat.	Younger than 1	Antibiotic prophylaxas			Surgery if persistent		
	1–5		Antibiotic prophylaxis	Surgery	Surgery if persistent		
	6–10	Surgery					
V/Unilat. or bilat.	Younger than 1		Antibiotic prophylaxis	Surgery	Surgery if persistent		
	1–5	Bilat: surgery	Unilat: surgery			Surgery if persistent	
	6–10	Surgery					

[a]For patients with persistent uncomplicated reflux after extended treatment with continuous antibiotic therapy.
[b]See duration of reflux regarding the time that clinicians should wait before recommending surgery.
[c]No consensus was reached regarding the role of continued antibiotic prophylaxis, cystography or surgery.
(From: Elder JS, Peters CA, Avant BS Jr, et al. Pediatric vesicoureteral reflux guidelines panel summary report on the management of primary vesicoureteral reflux in children. *J Urol* 1997;157:1846.)

techniques are available, with Cohen's transtrigonal advancement technique being the most popular (381). The basic principle with this procedure is to lengthen the submucosal ureteric tunnel. Austin and Cooper (382) have recently reviewed the different surgical techniques, postoperative management, and complications, as well as new endoscopic techniques used to correct VUR and their outcomes (Tables 24-7 and 24-8).

The first endoscopic technique to correct VUR was based on the endoscopic injection of biologically inert polytetrafluoroethylene (Teflon) paste suspended in glycine into the lamina propria behind the submucosal ureter (383). This is a simple and reliable procedure that can be undertaken on an outpatient basis (384,385). Because of concerns with migration of the injected Teflon, granulomatous formation, and possible carcinogenic properties, this product has been withdrawn from use in some countries (386,387). Silicone, glutaraldehyde cross-linked bovine collagen (GAX-collagen), polyvinyl alcohol foam, bioglass, dextranomer microspheres suspended in a sodium hyaluronan solution, autologous fat or blood, chondrocyte-alginate or human bladder muscle cell–alginate gel suspensions, and detachable self-sealing membrane balloon systems are alternative substances under investigation (388,389). The use of particulate silicone microimplants has been limited, because of reports of silicone particle migration. The main drawback of GAX-collagen is its inability to conserve volume over time. Polyvinyl alcohol has been shown to have a potential tumorigenic effect in laboratory animals. Recently, the U.S. Food and Drug Administration (FDA) has approved an injectable implant of dextranomer/hyaluronic acid copolymer for use in children, making the endoscopic treatment of reflux a potential alternative to open surgical correction in native kidneys. Continuous observation will be necessary to establish the long-term success of this intervention. Uncontrolled voiding dysfunction may contribute to endoscopic treatment failure and treatment with anticholinergic agents and voiding rehabilitation for at least 6 months prior to the procedure has been recommended for these patients (390). Endoscopic correction of VUR in transplanted kidneys is often unsuccessful (391).

Management of Neurogenic Bladder and Vesicoureteric Reflux

Intermittent bladder catheterization, together with prophylactic antimicrobial therapy and anticholinergic agents, when indicated, have been used with some success to circumvent the high detrusor pressures associated with neuropathic lower urinary tract dysfunction.

In spinal cord injury patients, lowering bladder outlet resistance, either pharmacologically using α-adrenergic blocking drugs and striated muscle antispasmodics, or surgically by endoscopic division of the external urethral sphincter or the insertion of ureteral stents or springs, can reduce the height of

TABLE 24-7

COMMONLY USED TECHNIQUES OF SURGICAL URETERAL REIMPLANTATION

Author	Technique	Advantages	Disadvantages
Politano–Leadbetter	Intravesical ureteroneocystotomy	Potential for a long submucosal tunnel; anatomic alignment of the ureters for retrograde catheterization	Potential complications as a result of neocystotomy (obstruction, transperitoneal placement, bowel injury)
Glenn–Anderson	Intravesical trigonal advancement	No neocystotomy and anatomic alignment of ureters; simple to perform	Not suitable for dilated ureters as a result of limitation of submucosal tunnel length
Cohen	Intravesical cross-trigonal advancement	No neocystotomy and potential for long tunnel length; simple to perform	Ureters not in anatomic alignment could prevent retrograde catheterization
Gil–Vernet	Intravesical medial advancement	No neocystotomy; simple and rapid procedure because of limited dissection of ureters	Less successful than other intravesical techniques and only suitable if ureters are highly mobile within the bladder
Lich–Gregior and Zaontz and colleagues	Extravesical neocystotomy with or without advancement	Ureters in anatomic alignment; less postoperative bladder symptoms and hematuria	Difficult dissection; potential temporary urinary retention when performed bilaterally

(From: Austin JC, Cooper CS. Vesicoureteral reflux: surgical approaches. *Urol Clin North Am* 2004;31:543, with permission.)

reflex bladder contractions and stop reflux, or at least reduce its severity. Intermittent self-catheterization of the bladder can reduce the incidence of complicating UTIs, but whether this lowers detrusor pressure is not yet fully established.

Management of Complicating Urinary Tract Infections

The management of symptomatic UTIs in either children or adults with VUR or reflux nephropathy should be the same as that for any complicated UTI. Such patients invariably respond to a curative course of any appropriate antimicrobial agent. A 5-day course of treatment is generally sufficient. There is no indication for combinations of antimicrobial agents to be used. Similarly, there is no place for single-dose therapy in the treatment of UTIs in infants or young children, patients with acute pyelonephritis, or those patients known to have VUR or reflux nephropathy.

Infants or children with VUR, particularly grade III and above, should be placed on long-term, low-dose antimicrobial prophylaxis with a drug, such as nitrofurantoin or trimethoprim, until puberty or after the reflux has stopped, either spontaneously or following successful surgical correction. If recurrent symptomatic infections are a clinical problem, careful attention should be given to increasing the fluid intake and instructing the patient to practice complete or double micturition. Cognitive bladder training may also be appropriate (392). Recently, it has been suggested that suspension of antibiotic

TABLE 24-8

DIFFERENT IMPLANT MATERIALS STUDIED FOR ENDOSCOPIC TREATMENT OF REFLUX

Implant	% Reflux cured with one injection[a]	Biodegradable	Comments
Autologous chondrocytes	55	Autologous	Requires two procedures: cartilage harvest and the subsequent subureteric injection
Bovine cross-linked collagen	72	Yes	Skin allergy testing prior to treatment; high relapse rate after 1 year
Dextranomer/ hyaluronic acid copolymer	72	Yes	Nonimmunogenic; easy to inject with syringe and needle; only implant FDA approved for the treatment of reflux in children in the United States
Polydimethylsiloxane	82[b]	No	Solid silicone elastomere soft tissue-bulking agent suspended in bioexcreteable carrier gel
Polytetrafluoroethylene	73	No	Teflon particles suspended in glycerine; small particle size makes migration a concern; most studied of all implants with largest series

[a] Overall for all reflux grades.
[b] Grades I–III only.
(From: Austin JC, Cooper CS. Vesicoureteral reflux: surgical approaches. *Urol Clin North Am* 2004;31:543, with permission.)

prophylaxis may be a reasonable option for a highly select group of children with VUR old enough to verbalize symptoms of UTI, with normal voiding patterns, and a minor history of infections and minimal or no renal scarring (393). For now, however, this practice should be used only with extreme caution.

For sexually active women, a vaginal source of sepsis should be excluded. Many of these women will benefit from postcoital micturition. Other patients have been assisted by applying an antiseptic cream to the periurethral area prior to intercourse. Failing that, long-term, low-dose antimicrobial prophylaxis may be necessary. The drugs that are most appropriate include nitrofurantoin, 50 mg, trimethoprim, 100 mg, cotrimoxazole, 0.24 g, or norfloxacin, 200 mg. If the patient has renal insufficiency, cephalexin, 125 mg, can be used successfully. Initially prophylactic treatment is best started on a nightly basis. The duration of treatment is an individual clinical decision. The drug could then be reduced to alternate nights, 3 nights weekly, or even just following intercourse, if that has been the clinical pattern of the infections. Some patients are enthusiastic to remain on long-term medication if their symptomatic infections are troublesome, while others may wish to abandon treatment after a 3- to 6-month period.

As long as there is no evidence of associated urinary tract obstruction or complicating urinary calculi, there is no risk of any further renal damage occurring in adult patients who have symptomatic or asymptomatic infections.

Management of Hypertension

All patients with reflux nephropathy should have their BP monitored and treatment started as soon as it becomes persistently elevated. Nonpharmacologic approaches should be instituted initially. Hypertension due to reflux nephropathy is generally easy to manage with conventional antihypertensive therapy, while accelerated hypertension invariably responds well to treatment. Meticulous control of hypertension is probably the most important aspect of the long-term management of any patient with reflux nephropathy. The angiotensin-converting enzyme inhibitors are proving particularly valuable antihypertensive agents in these patients. In addition, there is a possibility that this group of drugs may affect the rate of deterioration of renal function in the small proportion of patients with reflux nephropathy who have proteinuria and renal insufficiency (394).

As mentioned earlier, hypertensive patients with unilateral reflux nephropathy may benefit from nephrectomy of the affected kidney. However, apart from one prospective study (201), much of the evidence for such a benefit remains anecdotal.

Management of Pregnant Women with Reflux Nephropathy

Women with known reflux nephropathy, like all pregnant women, should have their urine screened for asymptomatic bacteriuria at the first antenatal clinic visit and thereafter in each trimester. If bacteriuria is present, it should be eradicated so as to reduce the risk of acute pyelonephritis developing later in the pregnancy or early puerperium. If bacteriuria recurs in the pregnancy, then low-dose antimicrobial prophylaxis (e.g., nitrofurantoin, 50 mg) should be introduced for the remainder of the pregnancy.

Those women with reflux nephropathy who are hypertensive, but have normal renal function and no proteinuria, should not be dissuaded from becoming pregnant, but encouraged to have careful antenatal supervision. Such women often have an improvement in their BP control during the second trimester, but require increased antihypertensive treatment in the third trimester and puerperium. ACE inhibitors and angiotensin II antagonists are contraindicated during pregnancy.

The women with reflux nephropathy who get into major problems during or following a pregnancy are those who have proteinuria and renal insufficiency prior to conception (217). These women should be monitored carefully and their BP well controlled. Fortunately, the majority of pregnant women with reflux nephropathy provide few management problems.

Investigation of Family Members

The familial nature of VUR raises the question of family studies. VUR is found in approximately one-third of first-degree relatives of individuals with this condition (395–400). The frequency of VUR is much higher in children younger than 1 year than in those older, declining from 67% in infants, 52% in 1 to 2 years old to 29% after 2 years of age. In the absence of a simple screening test for gross VUR, it has been suggested that all children born to parents with known VUR or reflux nephropathy, or who have affected siblings, should have their urinary tract investigated as soon after birth as possible. A limited voiding cystourethrogram in the neonatal period is a simple procedure and will reliably exclude a potentially damaging degree of VUR. Such investigations are invariably acceptable to well-informed parents and enable the diagnosis of VUR to be made before a UTI or reflux nephropathy develops. This approach is recommended irrespective of the finding of fetal renal pelvic dilatation on prenatal ultrasonography.

Several recent studies have addressed the value of familial screening for VUR (reviewed by Chertin and Puri [401]). Cascio et al. (402) compared the incidence of renal damage in index patients ($n = 107$, median age 1.5 years) with VUR and UTI, symptomatic siblings with reflux and a documented history of UTI ($n = 64$, median age 1.2 years), and asymptomatic siblings with reflux diagnosed during a screening program for hereditary reflux ($n = 55$, median age 6 months) DMSA scanning revealed reflux nephropathy in 36 of 166 (22%) refluxing units in index patients, 25 of 97 (26%) in siblings who presented with UTI, and 6 of 89 (7%) in asymptomatic siblings who underwent screening voiding cystourethrography. However, the prevalence of moderate and severe renal scarring in asymptomatic siblings was comparable to that in siblings with reflux and UTI. Sweeney et al. (402) compared the incidence of renal scarring in infants with high-grade vesicoureteral reflux presenting with UTI ($n = 97$) and those diagnosed by familial screening with no history of UTI ($n = 30$). DMSA renal scan demonstrated renal scarring in 38% and 67% of grade IV and V refluxing units in UTI cases, but only 29% and 36% of those in asymptomatic siblings. These observations suggest that early intervention in infants with high-grade reflux may change its natural history and protect against renal scarring. Other studies raise doubts about the potential value of screening programs. Bonnin et al. (403) performed DMSA scanning in 46 symptomatic index patients, 17 siblings with reflux, and 48 siblings without reflux. Four of the 17 siblings had a previous febrile UTI and the remaining 13 were asymptomatic. On DMSA renal scan, only six of all screened siblings (10%) had abnormalities, including asymmetrical differential function in five and a parenchymal defect in one. Only one of these six patients had reflux. Based on their finding, Bonnin et al. (403) concluded that a systematic screening in this group is not beneficial, despite significant 26% incidence of reflux in asymptomatic siblings of patients with primary reflux. A limitation of this study is that most of the 65 siblings described were older than 18 months.

Management of Patients with Urinary Calculi

Patients with reflux nephropathy who develop loin pain, ureteric colic, or UTIs that are difficult to eradicate should be investigated for urinary calculi. Most of the stones will be radiopaque and, therefore, a good-quality plain abdominal radiograph with nephrotomography should detect them. If ureteric colic is present, an intravenous urogram should be undertaken.

Metabolic evaluation of stone disease associated with VUR is indicated, although the role of metabolic factors is controversial. If a UTI is present, every attempt should be made to eradicate it. This may require a course of parenteral antimicrobial therapy. If the infection cannot be eradicated, and the stone is growing, consideration should be given to its removal. Most of these stones can be extracted using the percutaneous technique, although this may provide technical problems in small, irregularly scarred kidneys. There is little information available on the use of lithotripsy, but in experienced hands it would undoubtedly be beneficial.

CONCLUSION

Primary VUR is an important and common congenital abnormality, which may be inherited, and is a central component in the pathogenesis of reflux nephropathy. There is compelling evidence that infancy and early childhood comprise the critical period in the natural history of the renal scarring associated with reflux. In some infants renal damage occurs *in utero*. The pattern and extent of the renal damage are related to the severity of the reflux and the presence of intrarenal reflux. The precise role of complicating bacterial infections in the etiology and pathogenesis of reflux nephropathy remains uncertain, but renal damage may occur in the continued absence of UTIs. However, the most frequent and most important clinical presentation of VUR is with a complicating UTI early in childhood.

More investigation on the pathogenesis of this important cause of serious renal disease is necessary. What is required is a reliable way of detecting VUR at birth, or even *in utero*, so as to prevent or at least reduce the risk of renal damage and to enable UTIs to be avoided. The search must continue for a genetic marker as well as a simple and efficient way of screening newborn infants for the presence of the more serious degrees of VUR. At the present time, there is strong evidence to investigate those infants at birth who were shown to have fetal renal pelvic dilatation on prenatal ultrasonography or who have parents or siblings with either VUR or reflux nephropathy.

It is still uncertain as to the mechanisms for the development of both hypertension and the glomerulopathy. The latter is manifested as proteinuria and is the indicator of those patients who may progress to end-stage reflux nephropathy.

The role of antireflux surgery is still unclear despite several prospective studies. If surgery has a place, it is for the correction of the most severe degrees (i.e., dilating forms) of reflux, as early in life as possible, and ideally before any renal parenchymal damage has occurred.

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CHAPTER 25 ■ URINARY TRACT OBSTRUCTION

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Obstructive nephropathy is a renal disorder that begins with hydrodynamic and hemodynamic responses, leading to cellular changes in all renal compartments, and, finally, to interstitial fibrosis and tubular atrophy.

Urinary tract obstruction is a common cause of loss of renal function, which may be reversible with treatment. Urinary tract obstruction occurs in a variety of clinical circumstances, not only in diseases intrinsic to the urinary tract, but also in diseases originating in other organ systems. Symptoms and signs of urinary tract obstruction may be minimal or absent, and diagnosis, therefore, requires a high index of suspicion. Early recognition of the presence of obstruction is important, since the degree to which nephron function is irreversibly lost is related to the duration of obstruction.

Several terms and definitions are used in this chapter: *Obstructive uropathy* is used as a general term indicating complete or partial obstruction of the flow of urine at any level from the renal calyx to the external urethral meatus. *Hydronephrosis* refers to abnormal dilation of the renal pelvis and calyces with varying degrees of renal parenchymal atrophy and is usually, but not always, the result of obstructive uropathy. *Obstructive nephropathy* refers to the functional and pathologic changes in the kidney that result from obstruction to the flow of urine.

INCIDENCE AND CAUSES

Obstructive uropathy is a common clinical problem. The incidence of hydronephrosis reported by Bell (1) in a series of 32,360 autopsies was 3.8% (3.9% in males, 3.6% in females). The incidence of clinical manifestations of obstructive uropathy prior to death was not reported, and it is likely that hydronephrosis was an incidental autopsy finding in many of these patients. The incidence of hydronephrosis at autopsy is somewhat lower in children than in adults, being 2% in one series of 16,000 autopsies (2). Over 80% of children with hydronephrosis at autopsy were less than 1 year old, with the balance of childhood cases being distributed uniformly through the childhood years. About 166 patients per 100,000 population had a presumptive diagnosis of obstructive uropathy on admission to hospitals in the United States in 1985 (3). Among male patients with kidney and urologic disorders, obstructive uropathy ranked fourth at discharge (242 patients/100,000 discharges). In females with kidney and urologic problems, obstructive uropathy ranked sixth as a diagnosis at discharge (94 patients/100,000 discharges). In the United States in 1985, about 387 visits per 100,000 population were related to obstructive uropathy (3). New ultrasound techniques have made possible the diagnosis of obstructive uropathy in the fetus during pregnancy (4,5). In the adult, the incidence and causes of urinary tract obstruction vary with the age and sex of the patient. In young and middle-aged males, acute obstruction from renal calculi is common, but temporary, and such cases would not be included in autopsy surveys. In females of this age group, on the other hand, pelvic cancer is an important cause. In the older age group, urinary tract obstruction is more common in men, resulting from prostatic hypertrophy or malignancy. In the United States, 7,913 patients with urologic problems were on dialysis in 2001.

Anatomic factors are also important in establishing the site of the obstruction. The degree of obstruction in the urinary tract and whether it is intraluminal, intramural (intrinsic), or extramural (extrinsic) are helpful. The usual sites of obstruction in the urinary tract are the urethra and the bladder neck, the bladder and ureterovesical junction, the ureter, and the renal pelvis and ureteropelvic junction. Table 25-1 shows a clinical–anatomic classification of the causes of urinary tract obstruction based on age, sex, and anatomic level of obstruction.

Intraluminal obstruction may be because of renal calculi, blood clots, or sloughed renal papillae and most likely occurs at three locations—the ureteropelvic junction, the ureterovesical junction, or the bladder neck, although it may occur at any level. Intramural (intrinsic) obstruction may be because of functional or structural lesions in the wall of the urinary tract. Functional obstruction is because of impaired peristaltic activity, such as neurogenic bladder, while structural lesions include inflammatory ureteric strictures or malignant disease of the uroepithelium. Common extramural obstructive lesions include prostatic disease and gynecologic, colorectal, or retroperitoneal malignancy.

CLINICAL SYNDROMES AND COMPLICATIONS

Patients with obstructive uropathy may present with an acute and life-threatening illness, with chronic and slowly progressive symptoms, or with virtually no symptoms or signs. The clinical presentation will depend not only on the underlying cause of obstruction but also on the duration of the obstruction (acute or chronic), on its severity (unilateral or bilateral, partial or complete), and on the presence or absence of complications, such as urinary tract infection (UTI).

There are five common clinical syndromes that may indicate the presence of obstructive uropathy and several additional clinical presentations because of complications of this disorder (Table 25-2). Clinical syndromes owing to urinary tract obstruction itself include lower urinary tract symptoms, such as difficulty in voiding, increasing frequency and nocturia, flank pain and renal colic with or without an abdominal mass, or the finding of an asymptomatic abdominal mass, often in a child. Patients with obstructive uropathy may present with acute renal failure, particularly with anuria or widely varying urinary output, or with symptoms and signs of chronic renal failure, often with few symptoms and signs to suggest obstruction. Of interest because of their pathophysiology, are the renal tubular defects associated with obstructive nephropathy, including the

TABLE 25-1

COMMON CAUSES OF OBSTRUCTIVE UROPATHY, BY AGE AND LEVEL OF OBSTRUCTION

Infants and children	Adults
Urethra or bladder neck	
Meatal stenosis	Urethral stricture (male)
Phimosis	Benign prostatic hypertrophy
Urethral valves (male)	
Bladder	
Neurogenic bladder	Neurogenic bladder
Calculus (Southeast Asia)	Blood clot
Blood clot	Carcinoma of bladder
	Foreign body
	Calculus
Ureter	
Stricture (congenital)	Calculus (male predominance)
Ureterocele	Blood clot
Megaureter	Renal papilla
Blood clot	Stricture—tuberculosis, radiation
Retroperitoneal tumor	Vesicoureteral reflux (female preponderance)
Vesicoureteral reflux (female preponderance)	Carcinoma of prostate
	Retroperitoneal tumor
	Retroperitoneal fibrosis
	Pelvic neoplasm—carcinoma of cervix
	Pregnancy, uterine prolapse
	Inflammatory bowel disease
	Abdominal aortic aneurysm
	Surgical ligation
	Carcinoma of ureter
Ureteropelvic junction and renal pelvis	
Congenital stricture	Calculus
Aberrant renal artery	Blood clot
Blood clot	Renal papillary tissue
	Aberrant renal artery
	Fibrous band

clinical syndromes of polyuria (nephrogenic diabetes insipidus, postobstructive diuresis), salt and water depletion, and hyperchloremic hyperkalemic metabolic acidosis.

Unilateral obstruction of the ureter or ureteropelvic junction may be asymptomatic, may be accompanied by dull aching flank pain, or, in the case of a renal stone obstructing the ureter, there may be severe renal colic presenting as an acute abdominal emergency with associated paralytic ileus. Renal colic in milder cases may subside spontaneously, leaving no symptoms or only intermittent mild flank pain.

Bilateral obstruction of acute onset may be accompanied by lower abdominal or back pain and progressive symptoms of renal failure. Since chronic lower urinary tract obstruction often precedes acute obstruction, as in the older male with benign prostatic hypertrophy, there may be a history of increasing "bladder" symptoms. Gross hematuria in a patient with acute or chronic renal failure should alert one to the possibility of obstructive uropathy from tumor, blood clots, or stones. Patients with slowly progressive bilateral urinary tract obstruction may present with symptoms of chronic renal failure, such as fatigue, anorexia, nausea, vomiting, dyspnea, peripheral edema, or drowsiness.

Renal tubular defects resulting in water and salt depletion are particularly characteristic of obstructive nephropathy.

TABLE 25-2

CLINICAL SYNDROMES OF URINARY TRACT OBSTRUCTION

Clinical syndrome	Type of obstruction
1. Bladder symptoms	Partial bilateral obstruction
2. Acute renal failure (often with anuria)	Complete bilateral obstruction (or complete obstruction of a solitary kidney)
3. Chronic renal failure	Severe partial bilateral obstruction
4. Renal tubular disorder with:	Chronic partial bilateral obstruction, or
Polyuria, polydipsia	Relief of complete or severe partial bilateral obstruction (postobstructive diuresis)
Sodium and water depletion	Complete or partial unilateral obstruction
Renal tubular acidosis	Any obstructive lesion
5. Flank pain or enlarged tender kidney	
6. Other complications of obstruction:	
Resistant or recurrent urinary tract infections	
Infective (struvite) calculi	
Hypertension	
Polycythemia	
Neonatal ascites	

Polyuria, nocturia, thirst, and polydipsia may be prominent symptoms. Patients with chronic partial urinary tract obstruction may present with hyperosmolar dehydration and hypernatremia because of severe impairment of urine-concentrating ability resulting in nephrogenic diabetes insipidus. This is particularly true in older adults, who frequently have hypodipsia. If renal tubular damage results in urinary salt wasting, patients with chronic obstructive uropathy may present with postural hypotension and progressive manifestations of uremia owing to intravascular volume contraction. Hyperchloremic acidosis, often with hyperkalemia, may also result from distal nephron or collecting duct damage (type 4 distal renal tubular acidosis). After relief of severe bilateral obstruction, a disorder referred to as postobstructive diuresis may occur with a marked increase in sodium and water excretion.

In addition to these clinical syndromes, patients with obstructive uropathy may present with the clinicopathologic consequences of urinary tract obstruction, specifically hydronephrosis, renal parenchymal atrophy, and chronic renal failure, or with other complications of obstruction, such as urinary tract infection, renal calculi, hypertension, polycythemia, and neonatal ascites.

Hydronephrosis, Renal Parenchymal Atrophy, and Renal Failure

The volume of urine normally contained in the calyces and pelvis of each kidney is small (5 to 10 mL), but with persistent obstruction the urinary tract proximal to the site of obstruction becomes dilated and, with long-standing obstruction, there can be massive enlargement of the kidney. In such situations, the calyces and pelvis are greatly dilated, the medulla is

almost completely destroyed, and the cortex may be reduced to a thin, extensively sclerotic rim. With severe acute obstruction, in childhood, atrophy of the parenchyma may be very rapid, and a shrunken kidney with minimal dilation of the pelvis and calyces may result. Microscopic examination of obstructed kidneys shows tubular dilation and atrophy with chronic interstitial inflammatory changes and fibrosis, which are more severe than glomerular changes. However, glomeruli become hyalinized in a pattern similar to that seen in nephrosclerosis. When both kidneys are affected by lower urinary obstruction, the changes are usually asymmetric, but chronic renal failure eventually results.

Although hydronephrosis is a striking sign of obstructive uropathy, it is the associated atrophy of the renal parenchyma that leads to loss of renal function. Three mechanisms have been proposed to explain the loss of kidney function.

1. *Pressure atrophy.* Increased pressure in the renal pelvis and calyces may be responsible for the atrophy observed in the renal medulla of obstructed kidneys. The morphologic finding that renal papillae are often flattened and atrophied out of proportion to the cortex is in agreement with this concept. This structural finding is compatible with the distal tubular and collecting duct defects in sodium, potassium, and water handling and urinary acidification that often accompany urinary tract obstruction.
2. *Intrarenal reflux.* Intrarenal reflux refers to pyelotubular backflow of urine or of radiographic contrast material instilled in the bladder. It usually occurs during micturition and results in segmental radiographic opacification of the kidney parenchyma. Rolleston and colleagues (6) found that intrarenal reflux, sometimes extending outward as far as the renal capsule, was common in children with severe vesicoureteral reflux, and, in addition, that the scarred and atrophic segments of the kidney corresponded exactly with the segments in which intrarenal reflux was observed. Hodson and colleagues (7,8) made the same observations in piglets with experimental urethral obstruction and vesicoureteral reflux. Intrarenal reflux probably results from increased pyelocalyceal pressure, which changes the shape of the pliant renal papilla from conical to flat, causing the slitlike openings of the ducts of Bellini (terminal collecting ducts) to gape open, thus allowing pyelotubular backflow of urine (9). Further evidence of retrograde intratubular movement of urine is obtained from the finding of Tamm-Horsfall protein, which is secreted in the distal nephron, in the Bowman's space of glomeruli, and in the interstitium of obstructed rat kidneys (10). The finding of intrarenal reflux in the cortex helps explain the presence of cortical as well as medullary atrophy in the obstructed kidney.
3. *Ischemia.* Renal blood flow is reduced in chronic obstruction not only because of the vasomotor changes but also because enlargement of the renal pelvis may cause arterial or venous obstruction owing to stretching or kinking of vessels. Decreased renal blood flow predisposes the obstructed kidney to ischemic atrophy. Collapse of the inner medullary blood vessels and corresponding medullary tubular injury occur in rats with unilateral obstruction (11). If hypertension develops owing to obstructive nephropathy, there may be sclerosis and narrowing of the renal blood vessels. The resultant ischemia could play an important role in the progression of parenchymal atrophy.

Urinary Tract Infection Associated with Urinary Tract Obstruction

UTI is a common and serious complication of obstruction. Acute pyelonephritis with fever, costovertebral angle pain, and tenderness, or bacteremia may be the presenting clinical picture. Recurrent bacteriuria and/or UTI may be present. In males, the first attack of UTI is sufficient justification for performing an intravenous urogram to exclude a structural (often congenital) abnormality causing partial obstruction and predisposing to infection. Chronic bacteriuria is frequent and difficult to eradicate in patients with obstructive uropathy.

If the obstruction is below the level of the bladder, spontaneous development of bacteriuria is very common. Two factors are important in the development of such infections. First, in the presence of an obstruction to bladder outflow, there is a larger than normal volume of residual urine after voiding. This urine provides an excellent culture medium in which bacteria may persist and multiply. Second, defenses against bacterial growth may be impaired in the walls of the distended ureter and bladder.

Obstructive uropathy above the level of the bladder is not necessarily associated with infection. Biopsy evidence shows that an obstructed kidney is more easily infected than an unobstructed kidney (12,13). With time, however, the susceptibility to infection induced by urinary tract obstruction may decrease function only modestly and not progressively. If urine from such a hydronephrotic kidney is sterile, it is important not to undertake procedures such as retrograde pyelography. This procedure can introduce infection that is often refractory to treatment and that may then cause progressive renal damage.

Renal Calculi

Renal calculi are both a cause and a serious potential complication of obstruction. Stones complicating obstruction are invariably of the struvite (magnesium ammonium phosphate–calcium carbonate) type resulting from the association of urinary infection with urea-splitting bacteria, which produce urease and break down urea in the urine to ammonia, which, in turn, buffers hydrogen ions and raises urine pH, precipitating struvite. The usual sequence of events is the presence of partial urinary tract obstruction followed by infection, with formation of a renal pelvic staghorn stone. Such stones tend to grow rapidly and recur after removal unless (a) all stone fragments are removed, (b) infection is eradicated on a long-term basis, and (c) obstruction to urine flow is corrected. The combination of ureteropelvic junction obstruction with recurrent staghorn calculi may result in loss of renal function.

Hypertension Associated with Urinary Tract Obstruction

In obstructive nephropathy, hypertension may develop because of fluid retention with extracellular fluid (ECF) volume expansion, increased renin secretion, or possibly from decreased synthesis of vasodepressor substances, such as prostaglandins (PGs). After relief of bilateral obstruction, volume-dependent hypertension may improve as fluid retention is corrected.

Hypertension can occur as a consequence of unilateral urinary tract obstruction. Hypertension was associated with elevated renin concentration in the renal venous blood from the obstructed kidney (14–16) and relief of the obstruction has, at times, resulted in disappearance of the hypertension and restoration of the renal venous renin levels to normal (14). This sequence of events resembles the hypertension associated with unilateral renal artery stenosis. Indeed, ureteral occlusion does cause an acute increase in renin release (17,18).

Complicating an evaluation of the role of renin, however, are patients with hypertension and unilateral hydronephrosis,

but normal renin secretion (19–21). Weidmann et al. (21), however, studying six patients before and after surgery for unilateral ureteropelvic and ureteral obstruction, observed a correlation between the postoperative magnitude of the fall in blood pressure and the decrement in plasma renin levels. These authors emphasized that surgery is most likely to cure hypertension in patients who have not only elevated renin secretion in the ipsilateral kidney but also suppressed renin secretion in the contralateral kidney.

Several clinical studies suggest that hypertension associated with unilateral hydronephrosis may be renin-dependent. However, other aspects of the pathophysiology of unilateral ureteral obstruction may also be contributing to the hypertension (20).

Polycythemia

Occasionally, patients with obstructive nephropathy have an abnormally high erythrocyte mass. This polycythemia is because of increased synthesis and release of erythropoietin (22).

Neonatal Ascites

Urinary tract obstruction (bilateral), usually owing to urethral valves in male infants, may present with ascites in the neonatal period.

PATHOPHYSIOLOGY OF URINARY TRACT OBSTRUCTION

The clinical syndromes outlined previously also influence the pathophysiology of obstructive uropathy. Specifically, the age of the patient and level of obstruction are important, as well as the severity and duration of obstruction and the presence or absence of complications.

The effects of obstruction on renal function and structure, that is, obstructive nephropathy, may be discussed by considering the functions of the kidney, including renal blood flow, glomerular filtration rate (GFR), and tubular function, as indicated by urinary-concentrating ability and salt, other solutes, and acid excretion. In addition, the endocrine–metabolic aspects of renal function must be considered, particularly the renin–angiotensin and prostaglandin systems. Some aspects of obstructive nephropathy have been studied in humans, usually with chronic obstruction, but our current understanding of this disorder is based largely on the use of animal models that resemble the clinical syndromes seen in humans.

Hydrodynamics in the Urinary Tract

The movement of urine from the kidney to the bladder under normal conditions is the result of three factors: (a) hydrostatic pressure, (b) ureteral and pelvic peristalsis, and (c) the rate of urine flow (23,24). The urinary collecting system is lined by a transitional epithelium and surrounded by circular and longitudinal layers of smooth muscle. Action potentials that originate in smooth muscle cells of the renal pelvis are conducted along the pelvis and ureter and are followed by a wave of contraction. Urine leaves the renal pelvis and enters the ureter passively during the resting phase after contraction. The normal ureteropelvic junction does not demonstrate an anatomic sphincter. This junction, however, appears to act as a functional sphincter, since distortion of this area is a common cause of hydronephrosis.

In the ureter, urine is propelled in boluses by the peristaltic waves of contraction. Initially, the circular smooth muscle components of the ureteral wall contract proximally and completely occlude the lumen, a process referred to as *coaptation*, and, subsequently, as circular muscle relaxes, the longitudinal smooth muscle components contract and the bolus of urine is propelled down the ureter. An essential feature of normal peristalsis is bolus formation, which is achieved only by occlusion of the lumen. Dilation of the ureter clearly interferes with this process. The nature of normal ureteral peristalsis also prevents retrograde transmission of the pressures generated during coaptation (10 to 25 mm Hg) to the renal pelvis and renal pelvic pressures seldom rise above 4 mm Hg (23).

The effects of obstructive uropathy on hydrodynamics will depend on the rate of urine flow at the time of obstruction, with very high pressures being generated during diuretic states. It will also depend on the level of obstruction, with higher pressures being generated by obstruction at higher levels in the urinary tract, particularly above the level of the bladder. Whether obstruction is unilateral or bilateral and partial or complete is also important. Immediately after acute ureteral obstruction, both the baseline and peak intraluminal pressures are increased, and 1 hour after obstruction, baseline and peak pressures are similar and three to five times greater than pressures prior to obstruction (25). At this point, coaptation of the ureteral walls does not occur, and pressures generated may be transmitted directly to the renal pelvis and papillae. The effects of ureteral obstruction on ureteral diameter are best understood by examining the relationship between pressure and tension, which is expressed in Laplace's law:

$$p = k\,(t/r),$$

where p is the transmural pressure gradient p_i (inside) p_o (outside), k is a constant, t is the wall tension, and r is the radius of the ureter. Assuming constant extraluminal pressure, an increase in ureteral pressure will be observed after obstruction as long as the increase in wall tension (increased t) is greater than the degree of ureteral dilation (increased r) of the urinary tract. After acute obstruction, smooth muscle fibers in the urinary tract respond to the increase in pressure by contracting and increasing the tension. With persistent obstruction, the smooth muscle of the urinary tract contracts less forcefully, the tension of the walls of the obstructed tract increases no further, and, after prolonged obstruction with dilation, the tension may decrease. With superimposed urinary infection, as often occurs in chronic obstruction, the loss of muscle tone is even more dramatic and progressive dilation occurs with no further increase in wall tension, or with a decrease (26). From the Laplace formula it can be seen that with dilation (increased r and constant or decreased t), intraluminal pressure will decrease, and renal pelvic pressures will return to only mildly elevated levels as has been demonstrated with chronic obstruction in both animals and humans (27). These findings have several clinical implications, most notably (a) the major renal damage resulting directly from increased pressure will occur early after the development of obstruction, and (b) patients with chronic partial obstruction may not experience deleterious effects owing to elevated ureteral pressures, since these are often in the near-normal range, unless conditions of high urine output or worsening obstruction ensue, when intrarenal pressure may increase.

Decompression of the chronically obstructed urinary tract cannot, however, be entirely accounted for by dilation and decreased smooth muscle tone in the walls of the urinary tract. The driving force for intrarenal pressure and urine propulsion, namely, the glomerular filtration pressure, is also reduced. After 12 to 24 hours of complete unilateral ureteral ligation or after

relief of acute obstruction, intraluminal hydrostatic pressure is normal, but glomerular filtration pressure and renal blood flow are reduced (28–32). These findings indicate that the GFR is depressed by factors other than high intraluminal hydrostatic pressure opposing filtration pressure. This fall in GFR involves renal vasoconstriction associated with prolonged obstruction.

The escape of urine across walls of the collecting system is a third mechanism by which the urinary tract may be decompressed with prolonged obstruction. There is evidence that complete obstruction of the urinary tract does not completely abolish glomerular filtration. Direct micropuncture of glomeruli and proximal tubules indicates the presence of significant filtration (29,33,34). If there is persistence of filtration, then reabsorption of filtrate must also occur.

In addition to renal tubular reabsorption, urine may be reabsorbed directly across the walls of the renal pelvis through the lymphatics (pyelolymphatic reflux) or renal venous system (pyelovenous reflux). Markers of GFR experimentally introduced into the renal pelvis may appear in renal venous blood or renal lymph. The lymphatic flow from the kidneys is increased markedly during acute and chronic obstruction. Most of this increase in lymph flow, however, appears to be because of increased lymph production from the renal vascular bed, perhaps due to increased renal venous pressure rather than to urine reabsorbed from the renal pelvis (35). Thus, the quantitative significance of pyelolymphatic or pyelovenous backflow is uncertain, but probably small.

Changes in Intrarenal Pressure, Glomerular Filtration, and Renal Hemodynamics

The factors determining the fall in GFR during obstructive uropathy have been clarified by micropuncture studies of glomerular dynamics in experimental animals. Changes in intratubular pressure, including stop-flow pressure, which represents glomerular filtration pressure, have provided an important insight on the pathophysiology of obstructive nephropathy after unilateral (UUO) and bilateral ureteral obstruction (BUO). Glomerular filtration may be expressed by the formula:

$$GFR = K_f\ (P_{GC} - [P_T + \pi_{GS}])$$

where K_f is the glomerular ultrafiltration coefficient, P_{GC} is the glomerular capillary pressure, P_T is the intratubular pressure, and π_{GC} is the mean oncotic pressure along the glomerular capillary. δP is the difference between P_{GC} and P_T and represents the pressure gradient across the glomerular capillary wall. An increase in P_T without a concomitant increase in P_{GC} will result in a decrease in δP, the driving force for filtration. Glomerular filtration also depends on the rate of blood flow entering the glomerular capillary; a decrease in glomerular blood flow during obstructive uropathy will decrease GFR, because the rate at which capillary oncotic pressure rises is accelerated when a given volume of filtrate is removed from a smaller volume of blood. Both glomerular blood flow and hydrostatic pressure depend on renal vascular resistance, which is largely divided between two resistance segments—the preglomerular segment (afferent arteriole) and the postglomerular segment (efferent glomerular arteriole). Peritubular capillaries may also provide a postglomerular vascular resistance in urinary tract obstruction.

During Obstruction

After either unilateral or bilateral ureteral obstruction, renal blood flow increases significantly (15% to 25%) in the first 1 to 2 hours. This decrease in renal vascular resistance immediately after complete ureteral obstruction is probably secondary to the synthesis and release of vasodilator PGs. With persisting unilateral or bilateral ureteral obstruction, renal blood flow progressively decreases to 40% to 50% of normal by 24 hours (32,36) (Fig. 25-1). GFR is more markedly reduced than renal blood flow–that is, filtration fraction is low, and GFR is 20% to 30% of normal in both UUO and BUO obstruction after 24 hours (29,33). However, the site of changes in intrarenal vascular resistance and, therefore, the mechanisms responsible for the decrease in GFR differ in these two models of obstructive nephropathy.

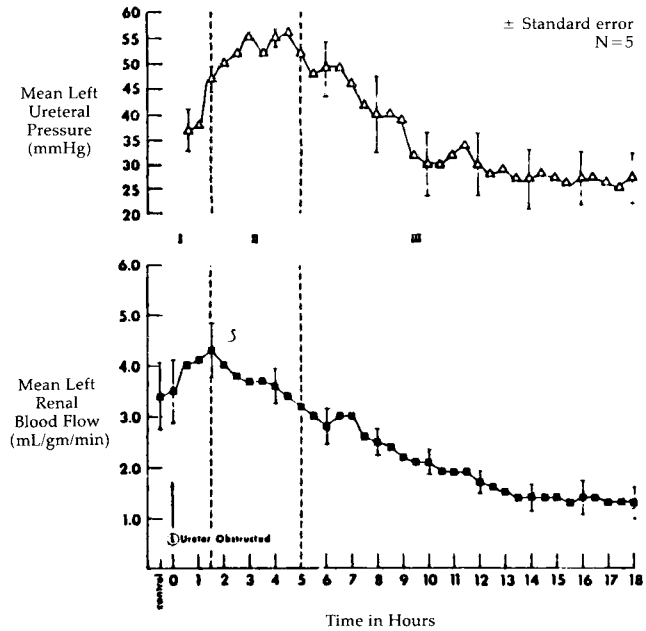

FIGURE 25-1. The triphasic relationships between ipsilateral renal blood flow and left ureteral pressure during 18 hours of left ureteral occlusion. The three phases are designated by roman numerals and divided by vertical dashed lines. In phase I, the left renal blood flow and ureteral pressure rise together. In phase II, the left renal blood flow begins to decline while the ureteral pressure remains elevated and, in fact, continues to rise. Phase III shows the left renal blood flow and ureteral pressure declining together. (From: Moody TE, Vaughan ED, Gillenwater JY. Relationship between renal blood flow and ureteral pressure during 18 hours of total unilateral ureteral occlusion. *Invest Urol* 1975;13:246, with permission.)

After acute UUO, there is an immediate increase in intrapelvic and proximal tubular hydrostatic pressure, the severity of which depends on the diuretic state of the animal (37). Despite this increase in intratubular pressure, the GFR in surface nephrons is about 80% of normal, because of an increase in glomerular capillary hydrostatic pressure and glomerular plasma flow, secondary to afferent arteriolar dilation and decreased renal vascular resistance (34). As unilateral obstruction persists, progressive vasoconstriction and a decrease in nephron filtration rate develop within about 4 hours and, by 24 hours, surface nephron GFR is 30% of normal because of a decrease in glomerular capillary pressure and plasma flow associated with an increase in renal vascular resistance, presumably at the level of the afferent arteriole (29). Proximal intratubular pressure is now normal rather than increased, as during the first few hours of obstruction (28–32).

During acute BUO, proximal tubular hydrostatic pressure increases to a higher level than after unilateral obstruction and, in sharp contrast to unilateral obstruction, intrarenal pressure remains twice normal after 24 hours. Renal blood flow increases for the first few hours after obstruction and then decreases to 40% to 50% of normal, similar to unilateral obstruction (36,38,39). Surface nephron GFR after 24 hours is

TABLE 25-3

COMPARISON OF CHANGES IN HEMODYNAMICS AND FILTRATION DYNAMICS IN COMPLETE UNILATERAL AND BILATERAL URETERAL OBSTRUCTION[a]

	Unilateral ureteral obstruction			Bilateral ureteral obstruction		
	During			During		
	1–2 hr	18–24 hr	After	1–2 hr	18–24 hr	After
RBF	↑	↓	↓	↑	↓	↓
P_T	↑	N	N	↑↑	↑	N
GFR	↓	↓↓	↓↓	↓	↓↓	↓↓
GPF	↑	↓	↓	↑	N	↓
P_{GC}	↑	↓	↓	↑	N	↓

[a]*RBF*, renal blood flow; P_T, proximal tubular pressure; *GFR*, glomerular filtration rate; *GPF*, glomerular plasma flow; P_{GC}, glomerular capillary pressure; ↑, ↓, *N*, increase, decrease, normal.

reduced to about 30% of normal in BUO, as it is in UUO, but the decrease in GFR in BUO is because of a persistent increase in proximal tubular hydrostatic pressure while glomerular capillary pressure and plasma flow are normal (33). Comparison of UUO and BUO is shown in Table 25-3. The predominant site of increased vascular resistance thus appears to be postglomerular during bilateral obstruction compared to preglomerular with unilateral obstruction.

The increase in renal vascular resistance that develops after several hours of UUO also occurs at the level of the single nephron when it is blocked with viscous oil for 12 to 24 hours (28,40–42). Prolonged tubular obstruction results in decreased glomerular capillary hydrostatic pressure through slowly developing vasoconstrictor mechanisms at the level of the single nephron. This single-nephron vasoconstrictor response does not occur if both ureters have been ligated prior to individual nephron obstruction (40), again indicating the differing effects of bilateral, compared to unilateral obstruction, on the intrarenal sites determining renal vascular resistance.

Heterogeneity of nephron function is also prominent in models of acute unilateral or bilateral obstruction after 24 hours, but does not appear to contribute significantly to the distinctive changes in total renal function (30,31,43–45). Similarly, changes in the intracortical distribution of blood flow, as measured by microspheres, do not explain the differences between bilateral and unilateral obstruction. Inner medullary plasma flow, in contrast to the initial increase in total renal blood flow, decreases progressively during complete ureteral obstruction (46). After 24 hours, inner medullary plasma flow is more markedly decreased than total renal blood flow, being 10% to 20% of normal, and is more severely affected in bilateral than in unilateral ureteral obstruction. Reversible inner medullary vascular obstruction has also been demonstrated histologically (11). These changes in medullary blood flow may be important in the impaired sodium and water reabsorption in deep nephrons and collecting ducts of the postobstructive kidney.

Acute ureteral obstruction has hemodynamic effects in addition to increasing renal blood flow for several hours and increasing pressures in the ureter and renal tubules (47). Vasoconstrictor responses to several stimuli are blunted, decreasing responses to electrical stimulation of the renal nerves or brain or norepinephrine (48). In addition, autoregulation of renal blood flow is virtually abolished by high ureteral pressure of 75 mm Hg and renal blood flow, while increased, becomes directly related to arterial blood pressure (49,50). A third result of acute ureteral obstruction, which may also be associated with its vasodilator effect, is a marked increase in renin release into the renal vein (17,18).

During chronic complete ureteral obstruction, renal blood flow progressively decreases. After 24 hours, renal blood flow is 40% to 50% of normal in both unilateral and bilateral obstruction. Prolonged UUO is associated with a further decrease in blood flow to 30% at 6 days, 20% at 2 weeks, and 12% at 8 weeks (51).

During chronic partial ureteral obstruction, GFR may remain unchanged or decrease, depending on the severity and duration of the obstruction and the extracellular fluid (ECF) volume status. Maintenance of normal nephron filtration rates appears to depend on an increase in glomerular capillary hydrostatic pressure, which results from a more marked increase in efferent compared to afferent arteriolar vascular resistance and which may also be associated with a decrease in glomerular capillary ultrafiltration coefficient (52–54). Decreased nephron filtration rates in chronic partial obstruction have been associated with a mild persistent increase in proximal pressure (55). Nephron loss, which may be more marked in deep, compared to superficial nephrons (44,55,56), also contributes to decreased GFR with chronic partial obstruction.

After Relief of Obstruction

Following relief of unilateral obstruction of 24 hours' duration, intratubular pressure is normal, but GFR remains reduced and renal vascular resistance increased, with a gradual return to normal after approximately 1 week (29–31,57). Following relief of 24 hours of bilateral obstruction, intratubular pressure decreases from elevated levels to normal, but glomerular capillary pressure and plasma flow also decrease because of afferent arteriolar vasoconstriction, resulting in a persistent decrease in GFR (33,39,45,57,58). Thus, the glomerular filtration dynamics and renal blood flow following relief of 24 hours of UUO or BUO appear to be similar and dominated by afferent arteriolar vasoconstriction. The observation that marked postobstructive diuresis occurs after relief of BUO, but not of UUO, indicates that factors other than changes in filtration and blood flow dynamics must be important in determining this state (see below).

The recovery of glomerular filtration after relief of obstruction is a problem of major clinical importance. The potential for recovery of GFR will seriously affect decisions regarding treatment of obstructive uropathy.

The factors that determine the extent to which the decrease in GFR is reversible have been studied in dogs by measuring the rate and degree of return of renal function after varying periods of complete UUO (59,60). The maximum GFR attained after the release of obstruction of 7 days' duration was about two-thirds of the GFR before obstruction. If the duration of ureteral obstruction was 28 days, the GFR returned to only

one-fifth of the original rate. In general, the maximal degree of recovery was observed within 2 to 4 weeks after release of the obstruction. These studies indicate that the longer the duration of obstruction, the less the degree of recovery that can be expected.

Recovery of nephron function after obstruction is not uniform throughout the kidney. There is a greater decrease in filtering juxtamedullary nephrons as compared to superficial cortical nephrons immediately after release of 24 hours of UUO in the rat (30). Whole kidney GFR recovered by 2 weeks to normal values (after release of 24 hours of obstruction), but only 85% of nephrons in the postobstructive kidney were filtering compared to 100% in the contralateral kidney (61). Single-nephron GFR of functioning superficial and juxtamedullary nephrons was higher in the postobstructive than in the contralateral normal kidney. The normal whole kidney GFR in the postobstructive kidney was, therefore, at the expense of hyperfiltration in the remaining nephrons and there was a permanent decrement in functioning nephrons. The mechanism responsible for this permanent loss of nephrons remains to be defined, as does its long-term significance for the development of chronic renal failure after obstructive uropathy.

The conclusion that recovery of function is related to the duration of obstruction also appears to apply to humans, but the number of supporting clinical studies is limited. In children with severe congenital lesions, the earlier the obstruction is relieved by reconstructive surgery, the greater is the GFR after long-term follow-up (62,63). Most reports of functional recovery following relief of urinary tract obstruction in humans have been based on radiographic or isotopic methods of assessing renal function rather than on renal clearance techniques. By radiographic techniques, recovery of function is often observed after obstruction of less than 3 weeks' duration, and some recovery of function was demonstrated in one case when the duration of obstruction was almost 5 months. The obstruction in the latter case, however, may have been only partial (64). One patient was carefully studied after the relief of complete unilateral obstruction of 3 months' duration (65). In that case, the GFR in the postobstructed kidney returned to 20% of normal for one kidney (10.2 mL/min). It is likely that the potential for recovery of function is better in partial than in complete obstruction and when the obstruction is not complicated by urinary tract infection (63,66).

Mechanisms of Changes in Glomerular Filtration Rate and Renal Hemodynamics

The factors responsible for the changes in renal hemodynamics and GFR in obstructive nephropathy are of interest, because of their potential clinical significance. Nishikawa et al. (67) demonstrated increased PG synthesis in the hydronephrotic isolated perfused kidney. Vasodilator and vasoconstrictor PGs have been studied in relation to the functional changes of urinary tract obstruction. The relevance of such studies to human obstructive uropathy is suggested by reports of increased thromboxane (TX) synthesis by human hydronephrotic kidneys (68) and increased prostaglandin E_2 (PGE_2) excretion during postobstructive diuresis in humans (69).

Vasodilator Prostaglandins. The early hemodynamic consequences of acute ureteral obstruction are blunted or prevented by inhibition of PG synthesis. The increase in ureteral pressure following UUO is reduced by prior administration of indomethacin (70) and the increases in proximal intratubular pressure and glomerular capillary pressure are similarly reduced (70–73). The increase in renal blood flow that begins immediately after UUO is prevented by indomethacin or meclofenamate and a similar effect is seen on the vasodilation that follows BUO (50,71,72,74–76). The impairment of autoregulation that is seen in the kidney with ureteral obstruction is prevented by indomethacin and normal autoregulation of renal blood flow with changes in arterial pressure is restored in the obstructed kidney (74). In addition, the renin release that accompanies ureteral obstruction is nearly abolished by inhibitors of PG synthesis (74,75). The stimulus for the release of vasodilatory PGE_2 or PGI_2 after acute ureteral obstruction is unclear, but appears to depend on elevated pressure in the renal tubule or lumen of Bowman's space (47).

Vasodilator PGs may also be important in maintaining GFR during chronic partial obstruction, as suggested by studies in an animal model (54). After PG inhibition, nephron GFR and plasma flow fell from previously normal levels, suggesting that intrarenal PGs were acting as a vasodilator and antagonizing a local vasoconstrictor substance. Thromboxanes, the group of vasoconstrictor PGs, were not thought to be involved because their release would also be inhibited by indomethacin and meclofenamate. Angiotensin II (Ang II) is known to increase efferent arteriolar constriction and reduce the ultrafiltration coefficient (K_f), changes that were observed with chronic partial ureteral obstruction. It was suggested that the vasoconstrictor Ang II may be interacting with vasodilator PGs to determine glomerular filtration dynamics in this experimental model (54).

Vasoconstrictor Prostaglandins. Two vasoconstrictors, TXA_2 and Ang II, play a major role in the decrease in renal plasma flow per nephron and the decline in single-nephron GFR seen following ureteral obstruction (77). Both TXA_2 (78) and Ang II (79) are able to contract mesangial cells in culture and, therefore, can potentially reduce the glomerular capillary area available for filtration. Figure 25-2 summarizes the effects of these two hormones on renal function. The central role of these two vasoconstrictors in modulating postobstructive renal hemodynamics is illustrated by the fact that if rats are pretreated with ACE inhibitors and thromboxane synthase inhibitors, the decline in renal function seen after ureteral obstruction is virtually prevented (80).

After the onset of ureteral obstruction, the hydronephrotic kidney exhibits an enhanced ability to generate TXA_2 (81,82). This prostanoid is a potent vasoconstrictor in the hydronephrotic kidney (83). Inhibitors of TX synthesis partially reverse the decline in renal function seen after obstruction (84–86), with prior administration giving more benefit (80). The generation of TX after ureteral obstruction is conditioned by the diet of the animals prior to the onset of ureteral obstruction (87–89). Animals fed a low-protein diet do not generate as much TX and exhibit less vasoconstriction in response to ureteral obstruction than animals fed a high-protein diet (84). Furthermore, thromboxane synthase inhibitors were not effective in reversing the postobstructive vasoconstriction in animals fed a low-protein diet. Although several studies support the suggestion that TX generation is important in the alteration in renal hemodynamics following ureteral obstruction, this has not universally been found (90,91); the reason for this discrepancy is unclear.

Chronic renal obstruction in rabbits is associated with a proliferation of interstitial fibroblasts and infiltration of mononuclear cells (92,93). This mononuclear cell infiltrate has been linked to the increase in TX and PGE_2 production and release by the chronically hydronephrotic rabbit kidney (73,94,95). It is now apparent that there is an acute (within 4 hours) infiltration of leukocytes, predominantly macrophages, into the kidney following the onset of ureteral obstruction (82) (Fig. 25-3), which correlates with the increase in TX generation and the decline in GFR. Furthermore, when the macrophage infiltration is eliminated by prior irradiation of the animals, the enhanced TX generation is blunted and postobstructive renal function is improved (96), suggesting that the infiltrate is, indeed,

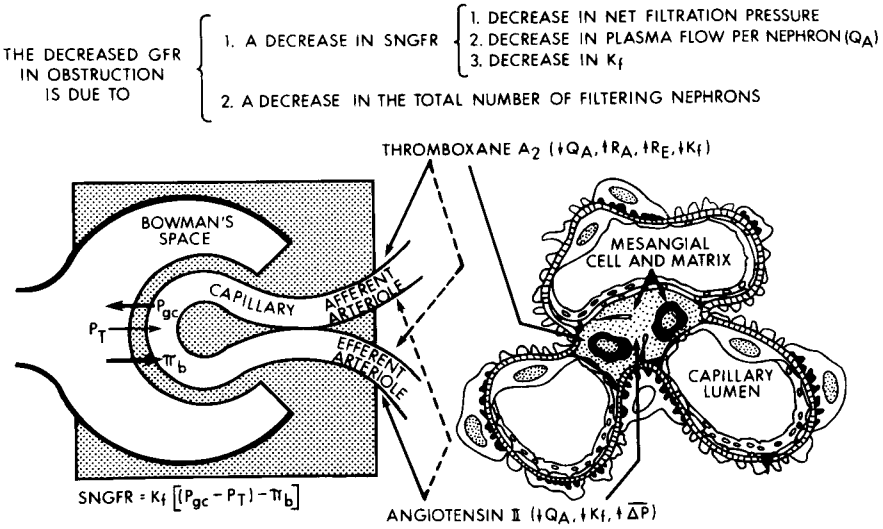

FIGURE 25-2. Glomerular filtration rate (*GFR*) is reduced in obstructive nephropathy because of a decrease in both single nephron GFR (*SNGFR*) and in the total number of filtering nephrons. The potential mechanisms responsible for the decrease in SNGFR in obstruction are shown. Initially (3 to 6 hours after the onset of obstruction) SNGFR falls because of reduced net filtration pressure as a consequence of a marked elevation in hydrostatic pressure (P_T) in Bowman's space without a comparable increase in intraglomerular pressure (P_{GC}) After 24 hours of obstruction, the decrease in SNGFR is due mainly to a fall in intraglomerular hydrostatic pressure (P_{GC}) This decrease in P_{GC} may be related to decreased plasma flow per nephron (Q_A) as a consequence of increased intrarenal levels of TXA_2 and Ang II. Inhibition of Tx synthesis in rats with ureteral obstruction increases Q_A, decreases afferent (R_A) and efferent (R_E) arteriolar resistances, and increases the ultrafiltration coefficient (K_f), suggesting that this vasoconstrictor decreases Q_A and K_f and increases R_A and R_E. Angiotensin administration in normal rats has been shown to decrease Q_A and K_f and increase net filtration pressure ($\overline{\Delta P}$), presumably due to preferential constriction of the efferent arteriole. Both TXA_2 and Ang II may decrease K_f by contraction of mesangial cells and, hence, decrease the total glomerular surface area available for filtration. (From: Klahr S, Harris K, Purkerson ML. Effects of obstruction on renal function. *Pediatr Nephrol* 1988;2:342, with permission.)

functionally significant. Despite irradiation of the animals prior to the onset of ureteral obstruction, TX generation remains significantly elevated over baseline values. Thus, in addition to the infiltrate of metabolically active cells acting as a source of TX in the hydronephrotic kidney, there is also an intrinsic renal source of enhanced TX production. Indeed, glomeruli isolated from rats following a short period of ureteral obstruction exhibit an enhanced production of prostanoids, including TX (97,98). Mesangial cells are most likely responsible for the increased production of eicosanoids in isolated glomeruli from rats with obstruction. The mechanism underlying this increased synthesis may relate to exposure of these cells to increased Ang II secretion *in vivo*, as treatment of the animals with the ACE-I inhibitor enalapril, prior to ureteral obstruction, effectively prevented the enhanced glomerular prostanoid production (98). Enhanced activity of enzymes of both the cyclooxygenase and lipoxygenase pathways has been demonstrated in kidneys of rats with ureteral obstruction (99).

Renin–Angiotensin System

Ureteral obstruction increases renin secretion into the renal vein (17,18). The maximum renin release during ureteral occlusion coincides with complete arteriolar dilation (100), suggesting that arteriolar dilation is the predominant stimulus to renin secretion. Because the increased renin is almost completely abolished by pretreatment with the cyclooxygenase inhibitors indomethacin and meclofenamate (74,101), it is likely that the formation of vasodilatory prostaglandins such as prostacyclin or PGE_2 is a necessary step for the release of renin from juxtaglomerular cells (102). In addition, the generation of renal cortical prostaglandins may act as a direct stimulus to the release of renin, as prostaglandins are able to release renin from renal cortical slices (103).

There is evidence that Ang II plays a central role in the modulation of hemodynamic changes following ureteral obstruction. The angiotensin II antagonist saralasin ameliorates the vasoconstriction that is seen in response to ureteral obstruction in rats (104), although other authors found it ineffective in dogs (105). Similarly, the ACE inhibitors captopril (84,86) and enalapril (80) appear to be highly effective in ameliorating the decline in GFR and renal plasma flow in response to ureteral obstruction, and this effect is particularly marked if the inhibitor is administered prior to the onset of obstruction (80). Because ACE inhibitors also activate kinins, their effect could be secondary to enhancing the levels of vasodilator kinins, rather than inhibiting the production of the vasoconstrictor angiotensin. However, neither aprotinin, a kallikrein inhibitor that blocks kinin production, nor carboxypeptidase, an enzyme that increases kinin destruction, block the beneficial effect of captopril on postobstructive renal function (86). Thus the beneficial effect of ACE inhibitors appears to be because of inhibition of Ang II generation.

Antidiuretic hormone (ADH) contributes to renal vasoconstriction and the decrease in GFR observed in rats with BUO (106). Rats with BUO of 24 hours' duration had significantly higher plasma values of ADH than did sham-operated rats. Pretreatment with a specific antagonist of the V_1-type receptor for ADH significantly increased GFR and effective renal plasma flow and decreased mean arterial blood pressure in rats with BUO. Leukotrienes (LKs), potent mediators of inflammation, are synthesized by cells through the 5-lipoxygenase

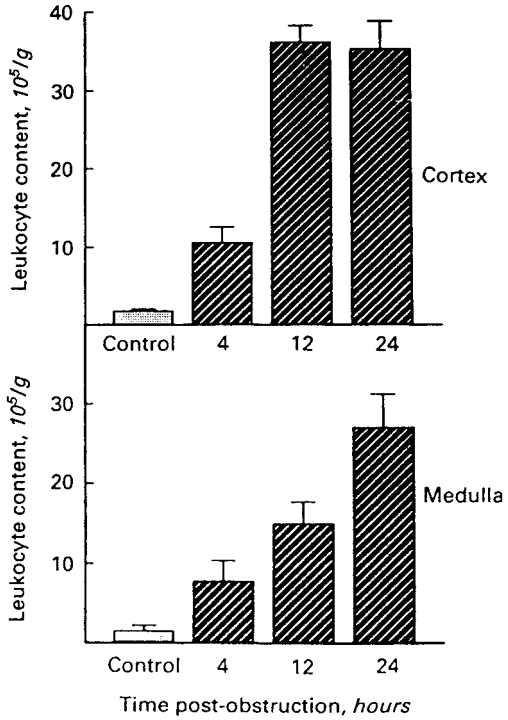

FIGURE 25-3. The effect of bilateral ureteral obstruction on the number of cells expressing the leukocyte common antigen in kidneys at timed intervals after obstruction. The results represent the mean ± SE of single kidneys of three rats. Representative portions of cortex and medulla were dissected out, weighed, and subjected to enzymatic digestion and labeled. The control kidneys were taken from littermates that did not undergo any procedure. *SE*, standard error. (From: Schreiner G, Harris KPG, Purkerson ML, et al. The immunological aspects of acute ureteral obstruction: immune cell infiltrate in the kidney. *Kidney Int* 1988;34:487, with permission.)

pathway. We found increased synthesis of leukotriene B_4 (LKB_4) in isolated glomeruli from rats with BUO. Inhibition of the 5-lipoxygenase pathway *in vivo* ameliorated the decrease in GFR and effective renal plasma flow seen after unilateral release of BUO (107).

Atrial Natriuretic Peptide

A contributing factor to the differences in hemodynamics after UUO and BUO may be the differing plasma levels of atrial natriuretic peptide (ANP). ANP levels are higher in animals with BUO than in those with UUO (108). Atrial peptide causes preglomerular vasodilation and postglomerular vasoconstriction (109) and increases K_f in the isolated perfused glomerulus preparation (110). In addition, the administration of exogenous ANP is able to increase GFR following both UUO and BUO (80,108). A scheme for the interactions of the various vasoactive hormones following ureteral obstruction is shown in Figure 25-4.

Nitric oxide (endothelium-derived relaxing factor), a vasodilator, appears to have a role in the hemodynamic changes that occur after ureteral ligation. In the model of BUO with unilateral release of obstruction after 24 hours, infusion of L-arginine, the substrate for nitric oxide synthase (NOS), increased GFR and effective renal plasma flow in postobstructed kidneys compared to control kidneys (111). Infusion of the NO synthase inhibitor N^6-nitro-L-arginine methyl ester (L-NAME) markedly impaired renal function after unilateral release of BUO of 24 hours' duration (111). In another study (112), L-NAME produced greater vasoconstriction in kidneys with ipsilateral UUO than in those of sham-operated rats.

Other Factors

Renal nerve activity is increased by elevated ureteral pressure (113), and the compensatory vasoconstriction that develops acutely in the contralateral normal kidney is prevented by ipsilateral or contralateral renal denervation (113,114). Endogenous opioids may also be involved in mediating excretory responses in the contralateral kidney (115). Acute renal denervation improves blood flow and GFR after release of UUO, but prior renal denervation was ineffective (116). In contrast to the effect of renal denervation on unilateral obstruction, denervation had no effect on the kidney with postobstructive diuresis after release of bilateral obstruction, and such kidneys had decreased norepinephrine content compared to unilaterally obstructed kidneys, suggesting partial denervation (117,118). Partial renal denervation could, therefore, be a contributing factor to postobstructive diuresis.

The possible role of the tubuloglomerular feedback (TGF) mechanism in determining changes in glomerular filtration dynamics in experimental obstructive nephropathy has also received attention (119,120). After 2 hours of UUO, the sensitivity of the TGF mechanism to increased flow through the loop of Henle is decreased when intrarenal pressure and renal blood flow are increased (119). After 24 hours of UUO, however, TGF sensitivity is enhanced and could contribute to the afferent arteriolar vasoconstriction noted at this time, which persists after relief of obstruction (120). With BUO, in contrast, TGF sensitivity is blunted at 24 hours before and after relief of obstruction. Decreased TGF sensitivity after relief of BUO could contribute to the phenomenon of postobstructive diuresis (120).

Sodium Excretion and Postobstructive Diuresis

During and After Relief of Partial Obstruction. During acute partial UUO, there is a significant decrease in sodium, potassium, and solute excretion, with a decrease in urine sodium concentration and an increase in urine osmolality (16,104,121). The clinical importance of acute partial obstruction causing decreased urine sodium concentration and increased osmolality, thus mimicking prerenal oliguria owing to volume depletion, has been emphasized (121). Decreased sodium excretion was initially attributed to decreased GFR (122,123), but studies undertaken during saline diuresis indicate that with mild or moderate increases in ureteral pressure there is a significant increase in tubular reabsorption of sodium and water (121). The factors responsible for this increased reabsorption are not clear. Increased reabsorption is the reverse of that expected with an increase in blood flow to the juxtamedullary cortex that is known to occur during partial UUO (124) and increased renal nerve activity does not appear to be involved (125). Decreased flow rate in the distal nephron has been suggested (16). In one study during elevation of ureteral pressure to 30 mm Hg, fractional reabsorption of fluid in the proximal tubule was not significantly increased (126). With an increase in proximal tubular pressure to 30 mm Hg during mannitol diuresis, however, a rapid and reversible increase in proximal and distal renal tubular permeability to creatinine, mannitol, sucrose, and iothalamate, but not inulin, was seen (127).

During chronic partial obstruction, the gradual decrease in GFR is accompanied by an increase in the fractional excretion of filtered sodium, indicating decreased tubular reabsorption. Micropuncture of surface nephrons in chronic partial UUO or of a solitary kidney with partial obstruction in the rat indicates that increased fractional excretion of sodium is not dependent on increased filtration or decreased proximal tubular reabsorption in surface nephrons, but results from decreased

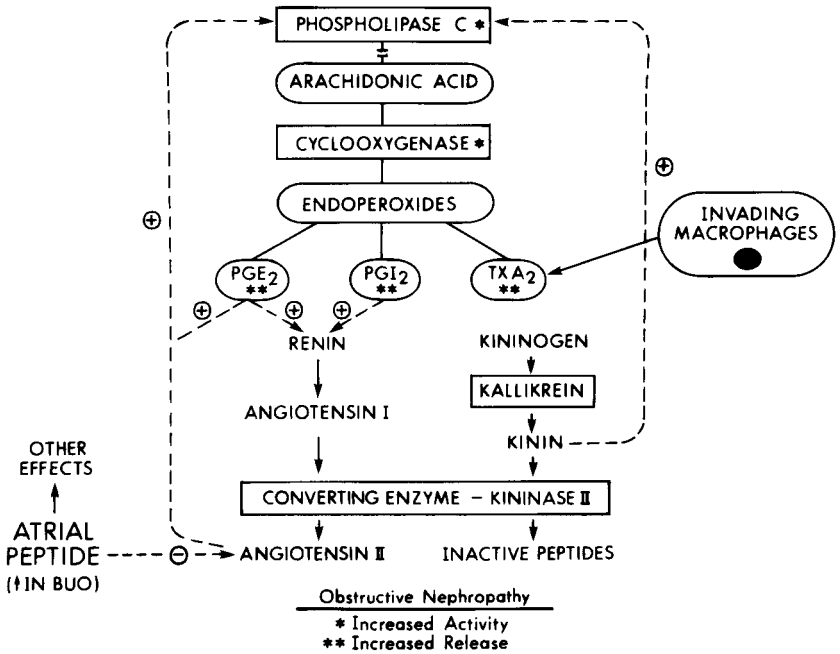

FIGURE 25-4. In obstructive nephropathy, the activities of the enzymes phospholipase C and cyclooxygenase are increased. There is also increased production of vasodilatory prostaglandins (*PGE_2,PGI_2–prostacyclin*); PGE_2 and PGI_2, in turn, may increase the production of the vasoconstrictor Ang II by activating renin. The production of another vasoconstrictor, thromboxane A_2 (*TXA_2*), is also increased in the obstructed kidney. This increased production of TXA_2 is, in part, because of its synthesis by macrophages that invade the renal parenchyma. Kinin excretion, per unit of glomerular filtration rate (*GFR*), is also increased in obstruction. Plasma levels of atrial peptide (a vasodilator) are increased in rats with bilateral ureteral obstruction (*BUO*), but not in rats with unilateral ureteral obstruction. Atrial peptide, in turn, may antagonize the effects of Ang II. Ang II may activate phospholipase C and increase the release of PGE_2. Kinins are also known to activate phospholipase C. It is suggested that these four interrelated systems encompassing both vasoconstrictor (*renin–angiotensin, TXA_2*) and vasodilatory substances (*PGE_2, PGI_2, kinins, atrial peptide*) condition the changes in renal plasma flow and GFR seen in obstructive nephropathy. (From: Klahr S, Harris K, Purkerson ML. Effects of obstruction on renal function. *Pediatr Nephrol* 1988;2:342, with permission.)

reabsorption in the distal tubule, in juxtamedullary nephrons, or in the collecting duct (55,128).

After relief of chronic partial UUO in humans, there is no increase in absolute sodium and water excretion from the hydronephrotic kidney, although decreased concentrating ability and increased fractional excretion of sodium are observed (66), presumably because of altered function in the deep nephrons and collecting ducts. The results indicate that other factors such as volume expansion or further reduction in functioning nephron mass with uremia are necessary to bring about an increase in salt and water excretion (postobstructive diuresis) following relief of obstruction. With hypotonic saline loading, there was a disproportionate diuresis from the hydronephrotic kidney (56), which was associated with reduced reabsorption in the distal nephron, indicating that volume expansion may be a significant factor in postobstructive diuresis.

A mild diuresis developed after relief of chronic partial ureteral obstruction of a solitary hydronephrotic kidney in the rat (128). Partial obstruction of a solitary kidney in humans and animals is comparable to BUO in its association with azotemia and postobstructive diuresis. In contrast to the marked postobstructive diuresis seen after relief of acute complete BUO, the modest increase in urine flow and sodium excretion that occurred after relief of chronic partial obstruction was due entirely to an increase in GFR after release of obstruction and there was no increase in fractional sodium excretion. Surface-nephron GFR increased by only 20%, and tubular reabsorption did not change significantly after relief of obstruction, whereas whole kidney GFR doubled, suggesting an improvement in function of deep nephrons. Animals with a high urea concentration in plasma had a greater diuresis and decreased water reabsorption in the distal tubule. Thus, changes in GFR and blood urea concentration may influence the degree of postobstructive diuresis after relief of chronic partial BUO.

After Relief of Complete Obstruction. A marked and sometimes prolonged diuresis may occur after the relief of obstruction of both kidneys or of a solitary kidney. This diuresis is characterized by massive losses of water, sodium, and other solutes. In one case report, after relief of acute obstruction, the rate of urine flow approached one-half the GFR (129). If not replaced, such losses could lead to severe hypovolemia and life-threatening electrolyte imbalance. However, a brisk diuresis following relief of urinary tract obstruction may also be physiologically appropriate or even iatrogenic rather than an indicator of tubular malfunction. Many factors influence the urinary flow rate following relief of obstruction, and the appropriateness of fluid replacement in a given case will depend on the mechanisms involved. Table 25-4 lists some of the mechanisms contributing to postobstructive polyuria.

1. *Saline diuresis owing to expansion of ECF volume.* When obstruction of the urinary tract is bilateral and prolonged, renal insufficiency occurs, so that continued intake of fluid

TABLE 25-4

MECHANISMS RESPONSIBLE FOR POSTOBSTRUCTIVE DIURESIS

Physiologic mechanisms
Excretion of excess salt and water retained during the period of obstruction
Excretion of excess salt and water administered intravenously after relief of obstruction
Osmotic diuresis because of retained urea or administered glucose
Pathologic mechanisms
Inappropriate losses of sodium and water because of intrinsic defect in tubular reabsorption of sodium or circulating natriuretic factors, such as atrial natriuretic peptide (ANP)
Inappropriate losses of water because of an impaired renalconcentrating capacity
Rate and degree of recovery of glomerular filtration

and electrolytes results in expansion of the ECF volume. ECF volume expansion activates natriuretic forces, such as ANP, which will become manifest when obstruction is relieved and GFR increases. With hypotonic expansion of ECF volume, the secretion of vasopressin may be suppressed, and a water diuresis may be superimposed on the natriuresis after release of the obstruction. Excretion of retained water and electrolytes allows normal restoration of the ECF volume and its composition. This effect accounts, at least in part, for the diuresis in most patients who have polyuria after relief of BUO (130,131).

2. *Osmotic diuresis owing to retained urea.* During urinary tract obstruction there is progressive azotemia, with accumulation of urea and other poorly reabsorbable solutes. After relief of obstruction, the high concentration of urea and other solutes in the glomerular filtrate will create an osmotic diuresis similar to that produced by intravenous infusion of urea or mannitol (132,133). However, some patients with high osmotic loads do not display a significant natriuresis or diuresis after relief of obstruction and, in those who do have a natriuresis, there may be no urea retention (134). This finding implicates factors other than, or in addition to, an osmotic load to account for the phenomenon of postobstructive diuresis.
3. *Osmotic diuresis owing to excessive infusion of intravenous solutions.* The brisk diuresis observed after relief of urinary tract obstruction is often so striking that the replacement of urinary losses with intravenous fluids is begun before ECF volume has been restored to normal. When this is done, however, the patient will continue to have iatrogenic polyuria and natriuresis until the rate of fluid administration is reduced and ECF volume is allowed to decrease to normal. Thus many cases of postobstructive polyuria attributed to abnormal tubular function are actually caused by infusion of intravenous solutions.
4. *Recovery of glomerular filtration.* The rate and degree of recovery of glomerular filtration after relief of obstruction is important in determining the occurrence of a marked postobstructive diuresis (135). In spite of the presence of many factors favoring rapid diuresis, including urea retention and impaired tubular function, the majority of patients with chronic obstructive uropathy do not demonstrate a clinically important natriuresis after correction of the obstructing lesion because the GFR is low. In the reported cases of striking postobstructive diuresis, GFR was relatively high, 30% to 70% of normal, thus delivering a large load of solute and water to tubules with impaired reabsorptive capacity.
5. *Diuresis owing to defect in tubular reabsorption of sodium.* A massive and prolonged postobstructive diuresis associated with a defect in tubular sodium reabsorption and thus with "inappropriate" sodium losses in the urine has been described in (129,132,134,136,137). Osmolar clearances of up to 62 mL/min and fractional sodium excretion in the range of 20% to 30% have been observed. In the cases described, the obstruction was relatively acute and bilateral (or involved a solitary kidney), and, prior to relief of the obstruction, the patients were in severe renal failure.

   Animal experiments indicate that a dramatic increase in sodium and water excretion (about fivefold) occurs after relief of bilateral obstruction for 1 day, despite the low GFR. Volume expansion is not the major factor involved, since food and water are withheld during the 24 hours of obstruction and animals lose weight. Micropuncture studies demonstrated decreased proximal (57) and distal (39,43,57) tubular sodium reabsorption, but the most dramatic abnormalities occur in the medullary collecting duct. Bilateral obstruction caused a marked decline in sodium reabsorption in this segment of the nephron, and there was a net addition of sodium to the tubular fluid as it passes down the collecting duct (138). Impairment of reabsorption in deep nephrons may also be important in postobstructive diuresis and papillary (terminal) collecting duct reabsorption was normal in this study (139). After relief of unilateral obstruction in experimental animals, fractional reabsorption of sodium is decreased, but net sodium excretion is usually similar or less than that of the unobstructed kidney, because GFR is low (30,31). In contrast, after relief of bilateral obstruction, proximal tubular reabsorption of sodium is normal or increased in the surface nephrons of the unilateral postobstructive kidney, and thus delivery of sodium to the collecting ducts is reduced (30,140). Therefore, in spite of impaired deep nephron (141) and collecting duct reabsorption (138), the excretion of sodium remains normal. These results are in agreement with the clinical findings that significant postobstructive natriuresis occurs only after relief of bilateral obstruction or obstruction of a solitary kidney (134,142).

   A period of functional anuria from bilateral obstruction or obstruction of a solitary kidney may impair tubular sodium reabsorption directly (an intrinsic defect) or may be associated with the accumulation of natriuretic factors in the plasma. Experimental data indicate that both occur but that circulating natriuretic factors are critical for the occurrence of postobstructive diuresis. Impaired function of deep juxtamedullary nephrons and medullary collecting ducts has been described after relief of both unilateral and bilateral obstruction (138–140,143). Cross-circulation studies, however, demonstrated the presence of plasma natriuretic factors only in rats with bilateral obstruction (144) (Fig. 25-5). Total intravenous reinfusion of urine also resulted in marked natriuresis and diuresis (58,144,145) without impairment of medullary collecting duct function (138). Urea acting as an osmotic agent in plasma is undoubtedly important, but did not account for the degree of natriuresis and diuresis observed (58,144). ANP was recently shown to increase markedly in BUO compared to UUO and is probably an important critical circulating natriuretic factor in postobstructive diuresis (108). Clinical studies have confirmed elevated ANP levels in patients with bilateral obstruction and azotemia (146).
6. *Diuresis owing to impaired water reabsorption.* The development of vasopressin-insensitive nephrogenic diabetes insipidus during partial urinary tract obstruction is discussed subsequently in this chapter. This type of abnormality can also continue or become manifest after relief of obstruction. Impaired water reabsorption in the collecting duct has been

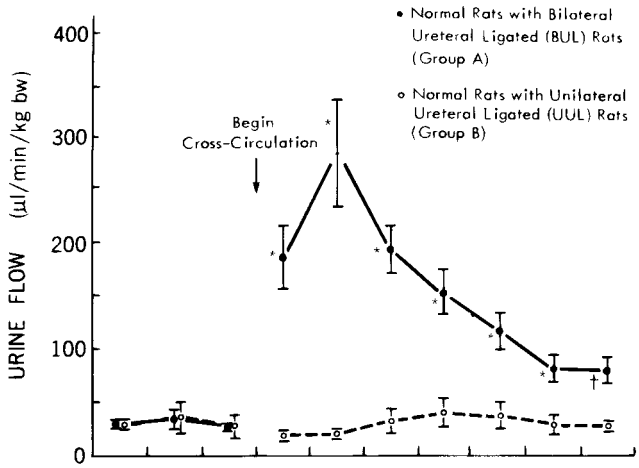

FIGURE 25-5. Changes in sodium excretion in normal rats undergoing cross-circulation with donor rats having bilateral (•) or unilateral (o) ureteral ligation of 24-hour duration. Standard error of mean value is shown and significance of the difference from the mean control value. *, P <0.01 or less; P <0.05. (From: *J Clin Invest* 1976;57:380, reprinted with permission from the © American Society of Clinical Investigation.)

observed following relief of bilateral urinary tract obstruction (138). Micropuncture experiments in rats and chronic unilateral hydronephrosis indicate that volume expansion may magnify this defect in distal tubular salt and water reabsorption (56).

Impaired Urinary-Concentrating Ability

Impaired ability to concentrate the urine is a characteristic feature of obstructive nephropathy whether urinary tract obstruction has been acute or chronic, unilateral or bilateral, or complete or partial. The only exception is seen during acute partial obstruction when urine osmolality is increased and sodium concentration decreased because of increased tubular reabsorption (16,121). In patients with chronic partial obstructive uropathy or with recently relieved partial or complete obstruction, a decrease in renal-concentrating ability can usually be demonstrated (66,147,148). Patients with marked impairment may present with nephrogenic diabetes insipidus and demonstrate polyuria and persistently hypotonic urine (149–152). If fluid intake is inadequate in these patients, severe dehydration and hypernatremia can develop (153). After relief of partial or complete urinary tract obstruction, impairment of concentrating ability, which persists initially, may gradually disappear over a period of months (150). The loop of Henle, distal tubule, and collecting duct, particularly in the juxtamedullary nephrons, are the major sites affected (143,154). Loss of the normal medullary solute concentration gradient and lack of responsiveness to the administration of vasopressin (VP) are the two characteristic pathophysiologic features and, as expected, several mechanisms participate in producing these abnormalities, which lead to impaired urinary-concentrating ability (Fig. 25-6).

Inability to Establish Hypertonicity in the Medullary Interstitium

A defect in the transtubular transport of sodium chloride in the ascending limb of the loop of Henle may contribute to the concentrating defect of urinary tract obstruction. Such a defect would lower the tonicity of the medullary interstitium and thus the osmotic driving force for water movement from the collecting duct into the interstitium. In support of this possibility is the finding that in dogs (16,155) and rabbits (156), the tissue concentration gradient of sodium and urea between the cortex and the papillary tip was abolished in the obstructed kidney. Furthermore, Hanley and Davidson (143) showed that chloride transport was markedly decreased in the thick ascending limb of the loop of Henle, microdissected from obstructed kidneys and perfused *in vitro*. Decreased Na^+– K^+–ATPase activity in the outer medulla of obstructed kidneys contribute to this defect (157,158) and enhanced PGE_2 production may play a role (159).

Loss of the medullary solute gradient has also been attributed to renal hemodynamic changes that "wash out" the medullary tonicity. However, inner medullary plasma flow is

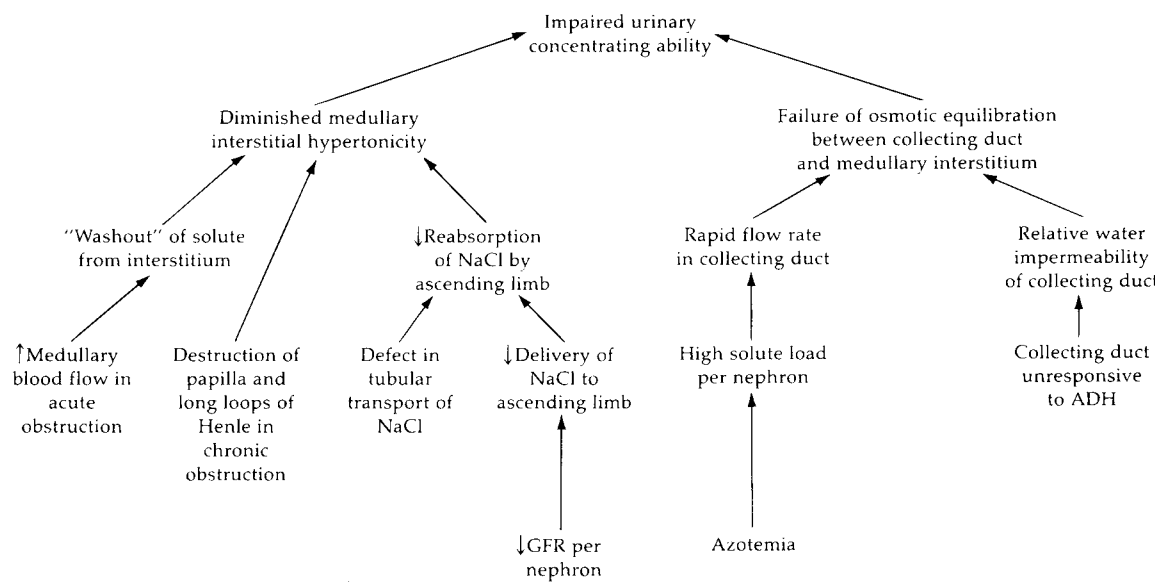

FIGURE 25-6. Mechanisms by which urinary tract obstruction may impair urinary concentrating capacity of the kidney. (From: Schrier RW. *Renal and electrolyte disorders,* 3rd ed. Boston: Little, Brown, 1985, with permission.)

markedly decreased, not increased, during ureteral obstruction (11,46), and prior clamping of the renal artery did not prevent loss of the medullary solute concentration gradient (156). Medullary plasma flow does, however, increase rapidly toward normal after relief of obstruction (46,141) and could delay reestablishment of the medullary solute gradient. Flattening of the renal papillae and destruction of the long loops of Henle in chronic obstruction contribute to a decrease in the maximal tonicity of the medullary interstitium.

Insensitivity of the Tubule to Vasopressin

Administration of VP to patients with water-losing states, secondary to partial urinary tract obstruction, may not decrease the urine flow rate nor increase the tonicity of the urine (149,152). Furthermore, VP did not increase urine cyclic adenosine monophosphate (cAMP) excretion or α VP-sensitive adenyl cyclase in experimental animals (160,161). A VP-resistant isotonic urine can be attributed to factors that impair the generation of a hypertonic medullary interstitium. However, since the medullary interstitium should never be less than isotonic, the presence of VP-resistant hypotonic urine indicates a failure of complete osmotic equilibration between the collecting duct and the interstitium owing either to failure of VP to increase water permeability of the collecting duct maximally or to rapid tubular flow through the collecting duct. VP-dependent water reabsorption was reduced in the isolated perfused cortical collecting duct from obstructed kidneys and the VP resistance was not overcome by cAMP (143,154). Increased synthesis of PGE_2 in the medulla of the obstructed kidney may play a role in the decreased collecting duct response to VP (159,162, 163).

Role of Aquaporins

The aquaporins are a family of membrane water channels. Aquaporin-2 (AQP2) is predominantly found in the apical domain of the collecting duct principal cells (164). Frokiaer and colleagues report (165,166) that AQP2 expression is decreased in the setting of bilateral or unilateral obstruction. The reduction in aquaporin expression may explain the postobstructive polyuria and the impairment in urinary-concentrating capacity found in bilateral ureteral obstruction. Recently Shi et al. (167) found that major sodium transporters and aquaporins in the obstructed kidney are downregulated in response to neonatally induced partial UUO, which indicates that these transporters may play a crucial role for the persistent reduction in renal handling of sodium and water in response to partial UUO.

Increased Osmotic Load

Increased solute excretion and thus increased solute delivery to the concentrating and diluting segment of the nephron, is associated with diminished urine concentration in spite of maximal doses of VP (133). In chronic obstruction and in other forms of chronic renal disease, total solute excretion is normal, but, because of the fall in GFR, solute excretion per milliliter of glomerular filtrate or per nephron may be high. Thus the rate of solute delivery to the concentrating and diluting segment of each nephron may be high. Such an osmotic diuresis per nephron may provide a partial explanation for the impairment in renal-concentrating capacity associated with urinary tract obstruction. Impairment of urinary-concentrating ability is also observed, however, in acute and unilateral obstruction (131,150,168,169), situations in which azotemia is not present, and the solute load per unit of GFR may not be excessive.

Defects in Urinary Acidification and Potassium Excretion

Patients with obstructive uropathy may be unable to acidify the urine. Impaired urinary acidification is seen in patients after relief of unilateral obstruction (65,68,169) and after relief of bilateral obstruction (147,149) or obstruction of a solitary kidney (142). Distal renal tubular acidosis, with inability to lower the urine pH to a normal minimum value in response to acidemia, has been reported. Proximal, bicarbonate-wasting, renal tubular acidosis has also been reported during postobstructive diuresis (142).

Berlyne (147) reports distal renal tubular acidosis in six of seven patients with chronic obstruction. In six patients studied by Gillenwater et al. (66), 1 week after release of unilateral obstruction, H^+ excretion was significantly less in the previously obstructed kidney than in the contralateral kidney. After relief of obstruction, the acidification defect may disappear over a period of weeks, or it may persist. In one study, the acidification defect correlated with recovery of function (170); urine pH below 6.0 in patients with chronic obstruction was associated with recovery of renal function after relief of obstruction, while with higher urine pH, recovery of function was only partial.

Animal studies found that the decreased H^+ excretion is because of either decreased bicarbonate reabsorption in the deep juxtamedullary nephrons or impaired H^+ secretion in the late distal tubule and collecting ducts (171,172). Defective acidification is associated with decreased excretion of both ammonium and titratable acid. Walls and colleagues (172) found no significant change in fractional bicarbonate reabsorption in the proximal and distal tubules of surface nephrons of the rat, but a marked decrease in urine P_{CO_2} during bicarbonate loading, suggesting impaired ability of the deep nephrons or collecting ducts to secrete hydrogen. Thirakomen et al. (171) found a low urine P_{CO_2} during bicarbonate loading in the dog. Sodium sulfate infusion failed to reduce urine pH to the same extent in the postobstructive kidney, thus suggesting impaired H^+ secretion.

Studies of isolated perfused nephron segments suggest that H^+ secretion in the outer medullary and cortical collecting ducts is reduced by obstruction (173), a change associated with a decrease in H^+-ATPase in the apical portion of intercalated cells (173–175). Batlle and associates (176) describe hyperkalemic distal renal tubular acidosis in patients with chronic obstructive uropathy. Hyperkalemia owing to decreased urinary K^+ excretion was common in these patients. Hyperkalemic hyperchloremic acidosis was the presenting feature of obstructive uropathy in several patients and resulted from three mechanisms:

1. A selective deficiency of aldosterone secretion, probably secondary to decreased renal renin production (hyporeninemic hypoaldosteronism). Such patients lower urine pH appropriately in response to an ammonium chloride load, but have reduced ammonia excretion.
2. A defect in H^+ secretion, with inability to maximally lower urine pH in the presence of systemic acidosis (distal renal tubular acidosis).
3. A combination of the above two defects (type 4 distal renal tubular acidosis). A defect in distal nephron (collecting duct) sodium reabsorption with decreased intraluminal negative potential difference could be the primary factor in both diminished H^+ secretion and K^+ secretion (a so-called voltage-dependent defect).

In animal models, K^+ excretion, both absolute and fractional, is markedly decreased from the UUO kidney after relief of 24 hours of obstruction. This abnormality has been attributed to decreased sodium delivery to the distal nephron (135). Decreased potassium excretion persists, however, when

distal sodium delivery is increased with volume expansion or sodium sulfate administration (171), suggesting that there may be an intrinsic defect in the distal K^+-secreting mechanism that could be associated with the defect in sodium reabsorption. During postobstructive diuresis, K^+ excretion is increased, both in absolute and in fractional terms. This increased potassium excretion is due to enhanced secretion in the distal tubule and collecting duct (138), perhaps because of increased sodium delivery, increased tubular flow rate, and particularly increased plasma K^+ levels.

In vitro perfusion of the cortical collecting duct of the rabbit revealed that UUO led to decreases in the lumen-negative transepithelial voltage (143,154) and in Na^+–K^+ pump *in situ* turnover (177). Muto and co-workers (178) found that in the cortical collecting duct obtained from obstructed kidneys of rabbits with 24 hours of UUO, the conductances of Na^+ and K^+ in the apical membrane were decreased. The Na^+–K^+– ATPase pump activity and the relative K^+ conductance in the basolateral membrane of this nephron segment were also inhibited, whereas the relative chloride conductance in the basolateral membrane was stimulated. Muto and Asano (179) also showed that renal decapsulation in rabbits with UUO partially corrects the decreased conductances of Na^+ and K^+ in the apical membrane and the basolateral Na^+– K^+ pump activity. Thus, increased renal pressure may initiate the defects in Na^+ and K^+ transport observed in the cortical collecting duct of the obstructed kidney. Others (180,181) also report transport defects in the thick ascending limb and the inner medullary conducting ducts of obstructed kidneys in the rabbit.

Magnesium and Calcium Excretion

Hypomagnesemia is seen in humans following relief of BUO (182). Magnesium excretion increases inappropriately after release of both bilateral and unilateral obstruction (65,142,183) The increase in Mg^{2+} excretion correlates with the increase in sodium excretion during postobstructive diuresis; a similar increase in calcium excretion is seen. Magnesium, but not Ca^{2+}, excretion is high following release of UUO in both humans (65) and animals (183). Decreased reabsorptive capacity of the ascending limb of the loop of Henle during UUO or BUO has been suggested as the explanation for increased fractional excretion of Mg^{2+}.

Phosphate Excretion

Hypophosphatemia because of high urinary excretion of inorganic prosphate (P_i) may develop during postobstructive diuresis in humans (142) and animals (183–186). The striking increase in absolute and fractional excretion of P_i appears to parallel the increase in sodium and water excretion and may, in part, be because of extrarenal factors. Phosphaturia in experimental animals can be prevented by dietary restriction of P_i, suggesting that changes in filtered phosphate load are important, while the fact that P_i is largely reabsorbed in the proximal tubules suggests that this nephron segment must be a major site of decreased reabsorption.

After release of UUO, in contrast to bilateral obstruction, P_i excretion is markedly decreased in humans and experimental animals (65,183,185,186). P_i excretion and urinary cAMP excretion from the obstructed kidney are relatively insensitive to parathyroid hormone (PTH) (65). Animal studies indicate that decreased P_i excretion is because of a decrease in nephron GFR and an increase in reabsorption of phosphate in the proximal tubule, similar to the changes that occur in sodium handling after release of UUO (185). No intrinsic abnormality in phosphate transport or in the response to PTH has been found in the proximal tubule of the UUO kidney using brush-border vesicles from luminal membranes (186). These results suggest that hemodynamic factors may enhance proximal tubular reabsorption of phosphate.

Recovery of Tubular Function After Obstruction

While the effects of ureteral obstruction on tubular function in the immediate period that follows relief of obstruction have been extensively studied, the long-term effects of ureteral obstruction on tubular function are less well known. Studies after release of UUO of 24 hours in rats revealed that abnormalities of tubular function persist beyond a time (14 days) when whole kidney GFR had returned to normal (61). Urine osmolality was lower in the postobstructed kidney up to 60 days following release of the obstruction. Net acid secretion was decreased and ammonium excretion was less in the postobstructive kidney, the latter owing to a defect in ammonia reentrapment in the collecting duct. Urine pH remained higher in the postobstructive kidney up to 14 days after the release of the obstruction and K^+ excretion remained lower. These observations on tubular function, following release of ureteral obstruction, are consistent with persistent alterations in distal tubule-collecting duct function, a loss of functioning juxtaglomerular nephrons following the release of the obstruction, or both. The temporal recovery of tubular function following the release of ureteral obstruction is illustrated in Figure 25-7.

Effect of Age on Obstructive Uropathy

Urinary tract obstruction is common in infants and children (62,63,187). It is almost always partial and congenital. Long-term obstructive nephropathy is the most important cause of renal failure in infants and children (188). Josephson et al. (189) induced partial UUO in newborn rats and examined the effects at 9 weeks in the adult animal. Marked hydronephrosis occurred, the obstructed pelvis being enlarged sevenfold, but whole kidney blood flow and GFR were decreased by only 10%. There was a compensatory increase in blood flow and GFR in the contralateral nonobstructed kidney. Kidney weights were similar to normal and, despite distortion, the degree of atrophy appeared to be small. Thus, the effects of partial ureteric obstruction on renal function were not proportionate to the degree of hydronephrosis and were fully compensated. Taki et al. (190) examined the relationship between the age at which obstruction occurs and the resulting changes in renal function in guinea pigs. Partial ureteral obstruction at 1, 2, 3, 4, or 5 weeks of age resulted in a progressively decreasing amount of compensatory hypertrophy in the contralateral kidney. GFR was markedly reduced in the obstructed kidney at all ages, but younger animals were more severely affected. Urinary-concentrating ability, however, was similarly affected at all ages.

Renal Metabolism in Obstructive Nephropathy

Changes in enzyme activity and in energy and substrate metabolism in the obstructed kidney have been described (191). Altered responsiveness to hormones and altered hormone synthesis have also been described. The significance of changes in enzyme activity, such as alkaline phosphatase and glucose 6-phosphate dehydrogenase, which increase and decrease, respectively, with obstruction, has not been determined (92,192,193). The decrease in Na–P-ATPase observed 1 to

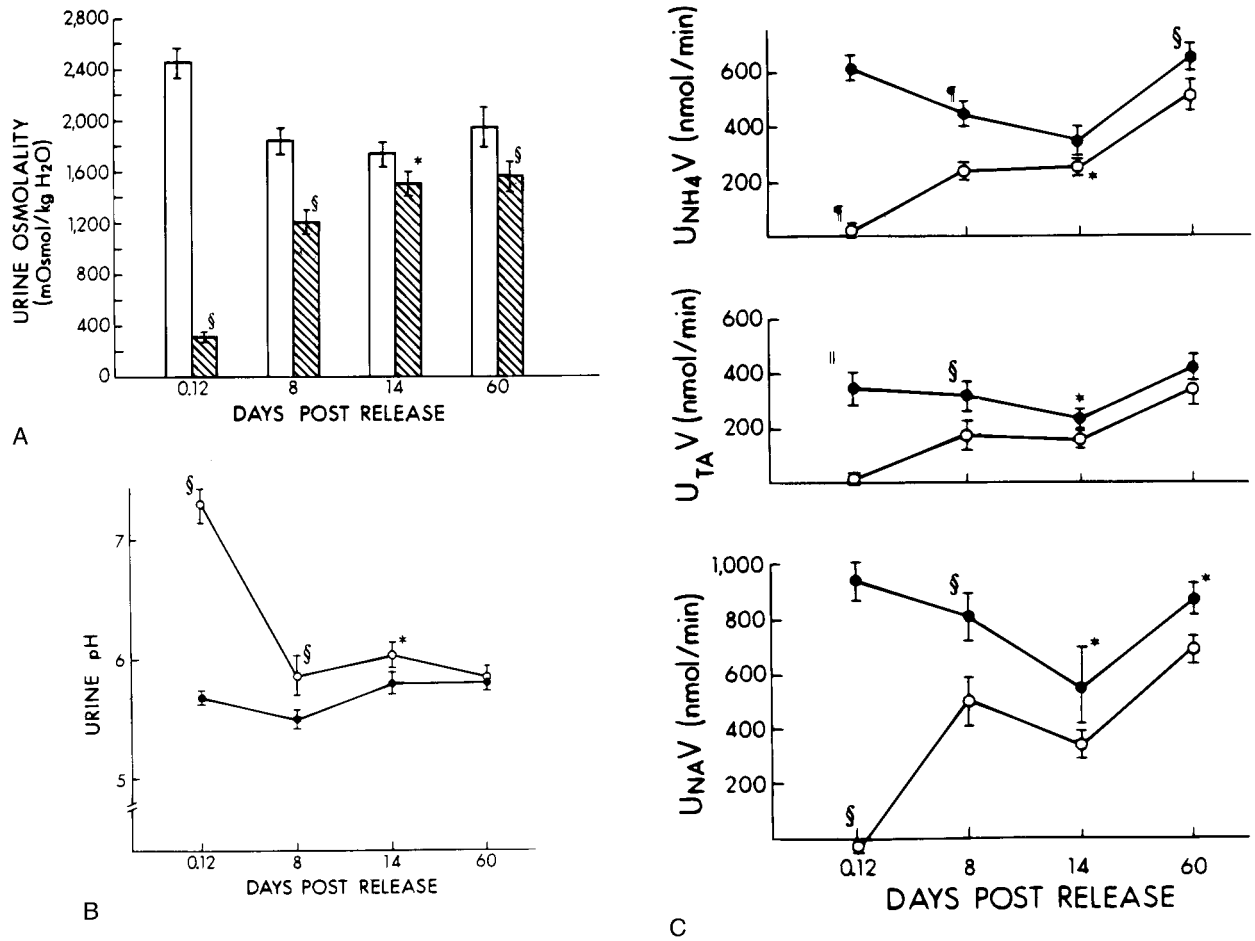

FIGURE 25-7. A: Effect of unilateral ureteral obstruction on the urine osmolality (mean ± SE) for the contralateral kidney (□) and the postobstructed kidney (▨) is depicted 3 hours (0.12 day) and 8, 14, and 60 days postrelease. **B:** Relation between urine pH and the number of days after ureteral release. (•) Represents mean values obtained for the contralateral kidney; (o) values obtained from the postobstructed kidney. **C:** Long-term effects of unilateral ureteral obstruction on acid excretion. (o) Represents mean values for the postobstructed kidney; (•) values for the contralateral kidney. *Upper panel* depicts absolute ammonium excretion ($U_{NH_4}+V$). *Middle panel* depicts absolute titratable acid excretion ($U_{TA}V$). *Lower panel* depicts the absolute excretion of net acid ($U_{Na}V$). Values in **A**, **B**, and **C** are significantly different from those for the contralateral control kidney at *P <0.05; §P <0.01; ‖P <0.005; and ¶P <0.001. (From: Bander SJ, Buerkert JE. Long-term effects of 24 hour unilateral obstruction on renal function in the rat. *Kidney Int* 1985;28:614, with permission.)

7 days following release of obstruction, particularly in the outer medullary portion of the kidney, could be important in the pathogenesis of increased fractional sodium and water excretion and impaired urinary-concentrating ability (157,158).

The GFR decrease in the obstructed kidney is associated with decreased delivery and reabsorption of sodium and lower energy requirements. Oxygen consumption and substrate uptake decrease in proportion to the fall in GFR and, with a decrease in oxygen consumption, there is a corresponding increase in anaerobic glycolysis (194). Adenine nucleotide (ATP) levels fall in the obstructed kidney after 24 hours of ureteral obstruction, probably because of mitochondrial damage and decreased oxidative metabolism (93,195). Renal gluconeogenesis is markedly reduced in the obstructed kidney within 24 hours, perhaps because of inhibition of phosphoenolpyruvate carboxykinase (PEPCK) activity, and this may play a role in modulating the decrease in renal phosphate reabsorption (195). Renal ammoniagenesis is reduced and probably plays a role in the defective urinary acidification (194).

Hormonal abnormalities in obstructive nephropathy include (a) altered hormonal responsiveness and/or sensitivity of the obstructed kidney and (b) altered production of hormones within the kidney, specifically renin–angiotensin and the PGs. Decreased renal excretion and/or altered metabolism of hormones also occur in obstructive nephropathy, but are not known to be affected in a way that is different from other renal parenchymal diseases. VP insensitivity of the collecting duct may be important in the decrease in urine-concentrating ability (143,154,160,161). Decreased sensitivity to aldosterone (171,176) may be important in the syndrome of hyperkalemic distal renal tubular acidosis described in patients with obstructive uropathy. Administration of mineralocorticoid fails to increase urinary K^+ excretion in some patients, but it is uncertain whether blunting of the tubular response to aldosterone or a primary defect in K^+ secretion, or both, is responsible.

The postobstructive kidney has an increased ability to release prostaglandins in response to Ang II, and dose-response curves suggest that there is an increase in the number and/or affinity of the receptors for the hormone (67). In addition, the contractile response of strips of renal cortex to angiotensin was enhanced in rabbit kidneys that had been obstructed for 8 to 32 days compared to normal kidneys, which showed minimal response (196), suggesting that the sensitivity of the postobstructive kidney to angiotensin is increased.

PTH responsiveness is blunted in the postobstructive kidney, as indicated by response of urinary cAMP and phosphate excretion (65), but the abnormalities in phosphate excretion do not appear to be explained by this finding (185). This decrease in response to PTH is accompanied by decreased generation of cAMP, decreased activation of adenylate cyclase by the hormone in basolateral membranes obtained from the proximal tubule of postobstructive kidneys, and the apparent loss of PTH receptors in the same membrane (197). After relief of BUO, absolute and fractional phosphate excretions are increased and do not increase further with exogenous PTH administration (65,183). However, reducing the filtered load of phosphate (which is high following BUO because of hyperphosphatemia) restores phosphate excretion toward normal and the response to PTH is normalized. Ureteral obstruction blunts the calcemic response of the skeleton to PTH, probably as a result of the decreased production of 1,25-dihydroxyvitamin D by the obstructed kidney (198) or increased levels of circulating PTH.

Lipid metabolism is altered in the obstructed kidney. There is an increase in triglyceride content and a decrease in total phospholipid content (199). The net synthetic rate of triglyceride by the obstructed kidney is increased because of a decrease in fatty acid oxidation and an increase in the release of fatty acids from phospholipids, presumably due to increased phospholipase activity. Following obstruction, the phospholipid content of the basolateral membranes of tubular cells is decreased (200). Because the lipid composition or physical state of the membrane (fluidity) affects the activity of membrane-bound enzymes and water permeability, it is possible that selective changes in the lipid composition of basolateral membranes following obstruction could account for both the altered transport characteristics and the altered response to hormones seen following ureteral obstruction.

FACTORS LEADING TO EXTRACELLULAR MATRIX ACCUMULATION IN OBSTRUCTIVE NEPHROPATHY

Growth Factors

Interstitial fibrosis is a common outcome of long-term ureteral obstruction. The process of fibrosis, in part, represents an imbalance between extracellular matrix (ECM) protein synthesis and degradation. An initial event in the interstitial fibrosis process is macrophage infiltration of the tubulointerstitial compartment. Because the macrophage is a potent source of numerous peptide growth factors, it is not surprising that these moieties play a critical role in initiating and propagating the fibrogenic response to urinary tract obstruction. The renal cortical tubular cell can also produce a myriad of peptide growth factors in response to ureteral obstruction. Renal proximal tubular cells and macrophages are a potent source of an array of growth factors, such as transforming growth factor (TGF)-β, interleukin-1 (IL-1), interleukin-6 (IL-6), fibroblast growth factor (FGF), tumor necrosis factor (TNF), and platelet-derived growth factor (PDGF). These peptide growth factors are important regulators of cell growth and differentiation.

Considerable attention has focused on the pluripotent peptide growth factor, TGF-β in the renal fibrotic response to injury (201). This polypeptide elicits a variety of cellular responses that promote fibrosis, including stimulation of ECM genes, downregulation of degradative matrix metalloproteinases, and upregulation of tissue inhibitor of metalloproteinase-1 (TIMP-1) (202,203). TGF-β1 is a 25 kDa protein secreted in an inactive (latent) form that requires activation before it can exert its biological effect. Latent TGF-β1 is stored at the cell surface and in the ECM and is converted to active TGF-β1 by an unknown mechanism(s). TGF-β1 induces the production of collagen types I, III, and V by cultured renal fibroblasts and stimulates production of proteoglycans and type IV collagen. The role of upregulated TGF-β1 expression in tubulointerstitial fibrosis has been described in a number of experimental models of progressive renal disease. In these models a major profibrogenic effect of TGF-β is to produce a state of net ECM accumulation, which is achieved by the disordered balance of oversynthesis of ECM components and a combination of inhibition of the degradation of matrix protein by increased generation of proteinase inhibitors and decreased expression of degradative proteins.

In the model of UUO, increased TGF-β1 expression occurs (204–206). Walton et al (206) noted a gradual increase in TGF-β messenger RNA (mRNA) expression with time from the onset of ureteral ligation. Kaneto (205) found that 3 days after ureteral ligation, the obstructed kidney exhibits significantly increased TGF-β1 mRNA expression, as compared with control (unoperated rats) kidneys. In addition, TGF-β1 mRNA levels in glomeruli from the obstructed kidney are essentially the same as those obtained in glomeruli from the contralateral kidney (205). However, TGF-β1 mRNA in isolated tubules is increased in the obstructed, but not in the contralateral unobstructed kidney, supporting the contention that the tubular epithelium is the source for this growth factor in the obstructed kidney (205).

Diamond et al. (204) found highly significant 2.6-, 5.8-, and 7.0-fold increments in renal cortical TGF-β1 mRNA levels at 12, 48, and 96 hours, respectively, in the obstructed kidney versus the contralateral unobstructed specimens. Intracellular TGF-β1, on immunolabeling, was detected only in the obstructed kidneys of rats with unilateral ureteral obstruction at all three time points and was confined to peritubular cells of the renal interstitium. A significant correlation ($p < 0.005$) correlation ($r = 0.95$) between interstitial macrophage number and cortical TGF-β1 mRNA levels was noted (204), suggesting the infiltrating renal interstitial macrophage as a cellular source for this growth factor's upregulated expression. In subsequent studies (207), these investigators measured levels of active TGF-β1 in tissue culture medium conditioned by obstructed and unobstructed cortical tissue, harvested at 48 and 96 hours after unilateral ureteral obstruction. The renal cortex of obstructed kidneys had 1.9- and 3.6-fold increments in active TGF-β1 concentrations (pg/mL/mg cortical protein) at 48 and 96 hours after unilateral ureteral ligation intervals, respectively, versus contralateral unobstructed kidney specimens from the same animals with unilateral ureteral obstruction or cortical specimens from normal, sham-operated rats. Pimental et al. (208) noted a strong TGF-β immunoreactivity in Bowman's capsule and in peritubular interstitial tissue in obstructed kidneys of rats at both 1 and 7 days after UUO. These investigators consistently found TGF-β1 immunoreactive mononuclear cells within the interstitium at 24 hours after UUO (208). Collectively, these data show that after ureteral ligation, macrophages increase within the obstructed renal cortex in association with increased renal cortical TGF-β1 mRNA and protein expression, as well as TGF-β1 activity, implicating this inflammatory cell as a source for this pluripotent cytokine.

Modulation of Tissue Inhibitors of Metalloproteinases and Matrix Metalloproteinases

The accumulation of ECM following tubulointerstitial injury represents an imbalance between ECM deposition and

dysregulation of proteases, which degrade matrix components. Proteases are a family of enzymes collectively termed metalloproteinases, capable of degrading both the collagenous and noncollagenous components of the extracellular matrix. Matrix metalloproteinases (MMPs), such as meprin, stromelysin, collagenase, and gelatinase are present in renal tissue and play an important role in the regulation of normal tissue remodeling (209). The activity of MMP is controlled, in part, by inhibitory proteins or TIMPs, which are a family of at least three proteins that can bind to metalloproteinases to inhibit ECM degradation (210). Macrophage-derived cytokines, PDGF, EGF, and FGF, can increase collagenase, transin, and stromelysin mRNA expression and activity. IL-1 is a potent activator of TIMP expression. TGF-β is a potent repressor of collagenase and stromelysin and can activate increased TIMP-1 expression, particularly in combination with IL-1. The ability of TGF-β to inhibit collagenase expression has been shown in human fibroblast cultures. *In situ* hybridization studies have revealed that tissue localization of TIMP-1 transcripts is present at sites of active tissue remodeling, where its expression significantly overlaps with that of TGF-β.

Alterations in the regulation of TIMP expression by TGF-β may contribute to the development of interstitial fibrosis after unrelieved UUO. A marked elevation of TIMP-1 mRNA expression was shown as early as 12 hours after unilateral ureteral obstruction (211). After 96 hours of unilateral ureteral obstruction, there was a 30-fold increment in TIMP-1 mRNA in the obstructed kidneys compared with the contralateral unobstructed kidneys (211). Sharma and co-workers (203) found that expression of TIMP mRNA is significantly increased at all time points after ureteral ligation, although it is maximal at day 3. Immunohistochemically, increased TIMP reactivity localized to the interstitial space and TIMP mRNA expression is observed to parallel the interstitial macrophage infiltration that accompanies ureteral obstruction (203). In regard to MMP-2, which has degrading activity against both collagen IV and denatured collagen, there is an early increase in mRNA expression at day 3 in the hydronephrotic rabbit kidney; however, it returns to normal by day 7 after ureteral ligation (203).

The membrane-bound metalloproteinase, meprin, is also implicated in the development of renal injury. Meprin is a metalloendopeptidase located in the brush-border membranes of the rat and mouse kidney. A decreased meprin α and β mRNA expression in kidneys has been observed in rats as early as 12 hours after unilateral obstruction (212). Meprin protein, on Western blotting, is also decreased in a proximal tubule membrane preparation from obstructed kidneys as early as 48 hours after unilateral ureteral ligation and persists throughout 96 hours of obstruction (212). These studies suggest that dysregulation of the renal proximal tubule cells after ureteral obstruction may lead to diminished meprin activity and hence alterations in ECM accumulation.

It has been shown (213,214) that macrophage-derived TGF-β1 may be pivotal in the process of myofibroblast transformation. Johnson et al. (215) note that infusion of Ang II to rats leads to focal tubulointerstitial injury. Alpers et al. (216) have identified accumulation of the renal cortical interstitial cells expressing α-smooth muscle actin (αSMA) at sites of chronic tubulointerstitial injury. Diamond and associates (217) show that αSMA mRNA and protein are only evident in the renal cortex of the obstructed kidney.

Decorin is a 92.5-kDa protein that possesses a 40-kDa core protein and a single chondroitin sulfate side chain. TGF-β binds only to the core protein of decorin and becomes inactive. When released from decorin, TGF-β regains activity, suggesting a biological interaction of this growth factor with decorin that transiently inactivates it in the ECM. In the rat unilateral ureteral obstruction model of hydronephrosis, active TGF-β1 may induce a physiological upregulation of decorin mRNA and protein with immunolocalization of this proteoglycan to the periglomerular and peritubular interstitium (207). One possible explanation to account for the progressive interstitial fibrosis that develops in experimental rat hydronephrosis, despite an increased decorin mRNA and protein expression (207), is that a physiological upregulation of renal cortical decorin is inadequate to neutralize the available TGF-β1, which, consequently, initiates a profibrogenic cascade by a variety of mechanisms.

DIAGNOSIS OF URINARY TRACT OBSTRUCTION

Symptoms and Signs

The diagnosis of obstructive uropathy is not always obvious. Until excluded by specific diagnostic tests, urinary tract obstruction should be suspected in patients presenting with acute or chronic renal failure. Obstructive uropathy may present at any age—in the infant, child, and all stages of adulthood. Early recognition and prompt treatment are essential, since irreversible renal damage resulting from obstruction is related to its duration as well as its severity.

The clinical syndromes and complications of urinary tract obstruction have been described previously. Difficulties with urinary stream, such as hesitancy, frequency, dribbling, nocturia, and the need to double-void are all obvious indications of a history of possible urinary tract obstruction. Patients may present with acute abdominal pain, because of sudden ureteral obstruction, with mild persistent flank aching, with lower abdominal pain, or with gross hematuria. A past history of renal calculi, gynecologic disease or surgery, or bowel disease or surgery may also be clues.

On physical examination, signs of intravascular volume contraction may be found in patients with salt and water depletion, secondary to chronic urinary tract obstruction. With severe renal failure, on the other hand, peripheral edema, pulmonary congestion, and hypertension may be observed. Features of chronic renal failure, such as pallor, drowsiness, increase in neuromuscular irritability, or pericarditis may be prominent. A palpable kidney or a distended bladder provides more direct evidence of urinary tract obstruction. Paralytic ileus may be prominent with acute renal colic. A careful rectal examination is essential and in the female a complete pelvic examination is required.

Urinalysis and Laboratory Findings

Urinalysis may be normal in acute or chronic urinary tract obstruction despite the presence of severe impairment of renal function. Usually, however, there are both red cells and white cells in the urine depending on the cause of obstruction. Proteinuria is absent or mild (less than 1.5 g/24 h), in most cases. Casts in the urine are also not distinctive and consist of granular, hyaline, or waxy casts, or occasionally white cell casts in the presence of renal infection. Urine culture should be obtained in all patients with obstructive uropathy even in the absence of pyuria. The urine sediment should be examined carefully for the presence of crystals. Sulfonamides, cystine, or uric acid crystals may be the first indication as to the type of stone causing the ureteral obstruction or the intrarenal obstruction resulting in acute renal failure.

When acute renal failure is the presenting syndrome of obstructive nephropathy, urinary sodium, creatinine concentrations, and urine osmolality may be difficult to interpret. Usually the urinary diagnostic indices are similar to those seen with acute tubular necrosis with decreased osmolality, high urine

sodium concentration, and decreased urine-to-plasma creatinine ratio (218) owing to previous chronic obstruction resulting in tubular damage. With recent obstruction and without severe renal failure, however, the indices may be similar to those for prerenal azotemia with low urine sodium concentration and increased osmolality (121).

Serum electrolytes may indicate a hyperchloremic metabolic acidosis, because of acquired distal renal tubular acidosis and this may be combined with an anion gap type of metabolic acidosis when renal failure is present. Hyperkalemia with hyperchloremic metabolic acidosis (type 4 distal renal tubular acidosis) may be a clue to the presence of obstructive nephropathy (176), although it also may be seen in chronic interstitial renal disease and diabetic nephropathy. The blood urea nitrogen may be elevated out of proportion to the serum creatinine, the ratio normally being approximately 10:1, because in obstructive disease, with slowing of intratubular fluid flow, urea reabsorption is increased in the distal nephron or in the bladder. The earliest functional abnormality in obstructive nephropathy is impairment of maximum urinary-concentrating ability, which can be assessed by an overnight concentration test. Assessment of urine acidification may also be important; this is undertaken by using the short ammonium chloride load test or the bicarbonate loading urine P_{CO_2} test or both.

Diagnostic Imaging in Urinary Tract Obstruction

Several diagnostic imaging techniques are available to detect urinary tract obstruction, to assess its severity, and to determine its cause. In all patients with acute or chronic renal failure when no obvious cause is found, obstructive uropathy must be excluded by appropriate investigation. A wide range of procedures is available and may be appropriate, depending on the circumstances, including abdominal plain films and tomograms, intravenous urography, ultrasonography, radionuclide scanning, computed tomography, and the invasive techniques of retrograde or antegrade pyelography (reviewed in reference 219).

The selection of diagnostic imaging techniques depends on the clinical setting and presenting symptoms. In patients with acute unilateral obstruction presenting with flank pain, abdominal plain films, and intravenous urography are the initial and possibly the only tests required. With chronic unilateral hydronephrosis, however, an assessment of the severity and degree of parenchymal atrophy will require ultrasonography and diuresis urography, with possibly antegrade pyelography and pressure measurement, as well. Determination of the cause of obstruction may require computed tomography (CT) and retrograde pyelography. With chronic bilateral obstruction, a similar range of tests may be needed, with invasive procedures usually applied first to the more severely affected kidney.

In patients presenting with acute or chronic renal failure, abdominal plain films or renal tomograms and ultrasonography are the procedures of choice since urographic contrast agents may provide little visualization and are associated with a risk of worsening renal function. If hydronephrosis is detected, other procedures are then required to determine the severity and cause of the obstruction.

Plain Films and Tomograms

A plain abdominal radiograph (KUB—kidneys, uereters, bladder) may provide clues to the presence of obstructive uropathy. It should be obtained routinely in the early investigation of patients with renal failure and is a standard initial step prior to intravenous urography. A difference in size of more than 2 cm between the two kidneys or the finding of suggestive intraabdominal calcifications is an immediate clue to possible obstructive uropathy. The presence of normal-size kidneys in a patient with chronic renal failure is also suggestive. Renal tomograms provide better definition of renal size and shape and also detect smaller calcifications (as small as 2 mm in diameter).

Intravenous Urography

The intravenous urogram or pyelogram (IVP) is the examination of choice in most cases of urinary tract obstruction. The IVP combines the features of accurate anatomic demonstration of the kidneys, calyces, pelvis, and ureters with gross estimate of renal function (220). Other diagnostic imaging procedures provide more precise information concerning one or another aspect of urinary tract structure and function, but the IVP remains a helpful screening procedure.

Urographic contrast agents are hypertonic water-soluble salts of iodinated benzoic acid derivatives (e.g., diatrizoate) that are rapidly excreted by the kidney following intravenous administration. These agents are excreted almost entirely by glomerular filtration, without significant tubular reabsorption, and are concentrated in the tubules by sodium and water reabsorption. With normal renal function, a uniform, dense nephrogram, because of contrast material in the proximal tubules and peritubular capillaries is seen within 30 seconds of injection, and approximately 2 minutes later, contrast agent begins to appear in the renal calyces. The IVP may not be helpful in patients with severely compromised renal function. The urinary tract is usually visualized poorly in patients with serum creatinine levels greater than 5 mg/dL. In early acute obstruction there may be minimal dilation of the collecting system, whereas, with chronic or repeated episodes of obstruction, hydronephrosis may be marked and is eventually accompanied by renal parenchymal atrophy. The IVP may also indicate the level of obstruction at the renal pelvis, ureter, ureterovesical junction, or bladder outlet. Close attention should be focused on the bladder for evidence of thickening (trabeculation) of the wall, an enlarged prostate, and, particularly, residual urine after voiding. Lower urinary tract (bladder neck) obstruction may be further evaluated by a voiding cystourethrogram in which dye is inserted by catheter into the bladder and timed films are taken during voiding. This technique is also useful for the assessment of vesicoureteral reflux.

It is important to emphasize that dilation of the urinary tract cannot always be attributed to obstruction nor does the absence of hydronephrosis exclude obstruction. Functional abnormalities of the ureter or bladder, vesicoureteral reflux, or even a chronic massive diuresis, such as that accompanying primary diabetes insipidus may produce urinary tract dilation. On the other hand, intermittent or partial obstruction may not be accompanied by urinary tract dilation unless urine flow rates are high. Intravenous urography during a diuresis induced by hypertonic saline or furosemide may be helpful in showing a dilated renal pelvis (221) (Figs. 25-8 and 25-9).

Ultrasonography

Diagnostic ultrasound is the procedure of choice to determine the presence or absence of dilated calyces or renal pelvis and, thus, to suggest the presence of obstructive uropathy (222). Because it is not invasive and not dependent on renal function, ultrasonography is particularly useful to exclude hydronephrosis in patients with acute or chronic renal failure (Fig. 25-10). It is very useful in detecting hydronephrosis in the fetus during pregnancy (4,5,223,224). Ultrasound is an extremely sensitive test for hydronephrosis, with a reported accuracy of greater than 90% (225–227). It can also be used to assess the degree of renal parenchymal atrophy accompanying hydronephrosis. Like all diagnostic procedures, it is not foolproof. Examples of conditions causing obstruction without urinary tract dilation

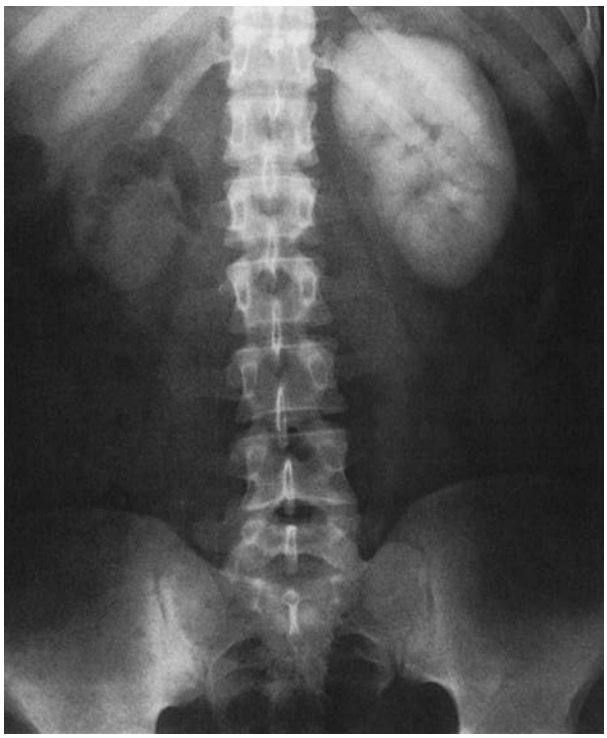

FIGURE 25-8. Intravenous pyelogram from a patient with severe left flank pain that lasted for 3 hours. Radiograph taken 6 hours after injection of contrast media shows a very dense nephrogram on the left. A faint right pyelogram is seen, but a clear left pyelogram is not seen. The findings suggest nearly complete left ureteral obstruction; a stone was subsequently passed. (From: Flamenbaum W, Hamburger RJ. *Nephrology* Philadelphia: Lippincott, 1982, with permission.)

include staghorn calculi filling the renal pelvis, retroperitoneal fibrosis with constriction of the ureters and pelvis, and acute obstruction in a patient with ECV contraction and low urine flow rate.

A limitation of ultrasonography is, paradoxically, its extreme sensitivity to detect small increases in volume of the renal pelvis. False-positive diagnosis has been reported in 8% to 26% of urographically nonobstructed kidneys (225,228). A second drawback is its relative inability to provide information on the localization and cause of urinary tract obstruction.

Isotopic Renography

This procedure requires the intravenous injection of a radionuclide and imaging with a gamma scintillation camera. Intravenous injection of tracer amounts of technetium-labeled diethylenetriamine pentaacetic acid (^{99m}Tc-DPTA) or ^{131}iodine-labeled orthoiodohippurate and sequential imaging provide a dynamic pattern of urinary excretion. Radionuclide examinations are 90% sensitive in detecting obstruction, with false-negative diagnoses, because of poor excretory function and, therefore, poor visualization (229). It is also highly specific, although dilation of the renal pelvis or ureters from nonobstructive causes may result in a false-positive diagnosis. Diuresis renography carried out by administering a diuretic (furosemide 0.3 to 0.5 mg/kg intravenously) during the study may help to determine the presence or absence of mechanical obstruction, since, with functional obstruction, the diuretic promptly and markedly increases tracer drainage from the urinary tract. Alternatively, diuretic administration may increase dilation during diuresis; an increase of greater than 20% suggests significant ureteropelvic junction obstruction (230,231).

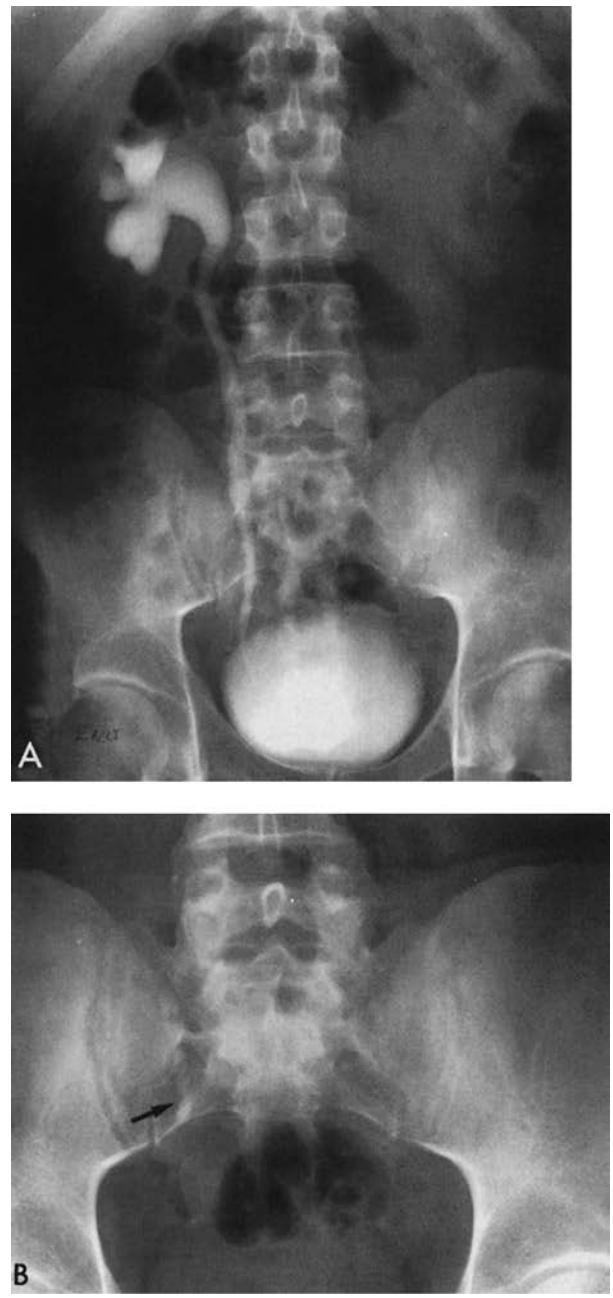

FIGURE 25-9. A: Intravenous pyelogram from a patient with right flank pain and hematuria intermittently for several weeks. Radiograph taken 1 hour after injection of contrast media shows a markedly dilated right renal pelvis and a dilated, completely filled right ureter. **B:** Examination of the plain radiograph before dye injection shows several radiopaque stones overlying the sacrum. (From: Flamenbaum W, Hamburger RJ. *Nephrology* Philadelphia: Lippincott, 1982.)

The main limitation of isotopic renography compared to intravenous urography is the inferior anatomic definition, whereas its advantages are the small radiation dose and lack of risk of systemic reaction or renal injury.

Newer developments with radionuclide imaging, including computer analysis of excretion of radioactivity (deconvolution analysis), suggest that while the uptake function may be normal despite significant outflow obstruction, the renal parenchymal transit time of radionuclide is a more sensitive indicator of obstruction (232,233). Similarly, analysis of activity in the renal parenchyma compared to the renal pelvis or whole kidney

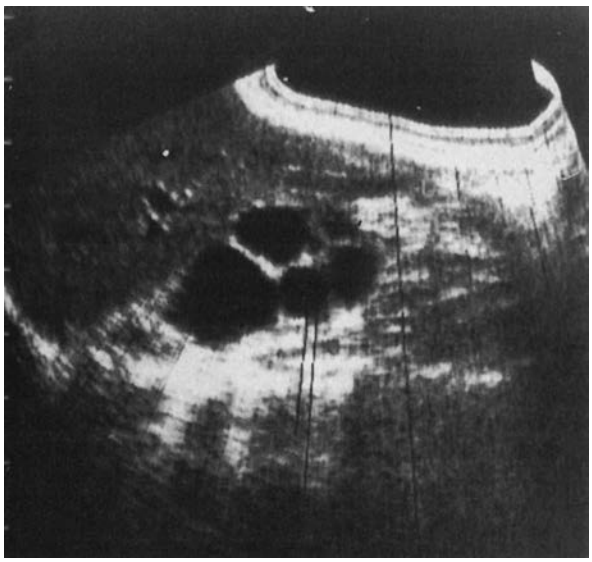

FIGURE 25-10. Supine right sagittal sonogram of the abdomen in a patient presenting with anuric acute renal failure and a history of cervical carcinoma. The film shows markedly dilated calyces in the right kidney lying inferior to the liver, with the anterior abdominal wall at the top of the photograph. Similar findings were present in the left kidney. (From: Flamenbaum W, Hamburger RJ. *Nephrology* Philadelphia: Lippincott, 1982.)

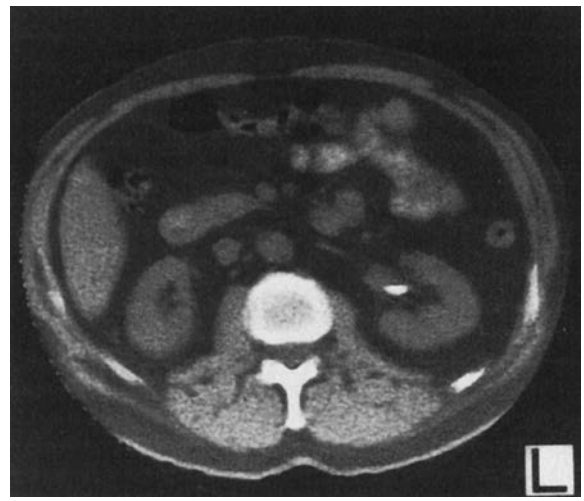

FIGURE 25-11. Transverse view of abdominal computerized axial tomogram (CT) in a patient with intermittent left flank aching in whom a radiolucent filling was visualized in the renal pelvis by intravenous pyelography. The CT scan shows that this filling defect at the ureteropelvic junction is dense (*white*), indicating that it is a uric acid stone rather than a tumor, blood clot, or necrotic papilla. (From: Flamenbaum W, Hamburger RJ. *Nephrology* Philadelphia: Lippincott, 1982.)

may provide a more accurate estimate of the potential recovery of renal function following surgical relief of obstruction (233,234). It is important to remember that even nonvisualization on renal scan does not preclude recovery of significant renal function after relief of obstruction (235).

Computed Tomography and Magnetic Resonance Imaging

Computed tomography (CT) may be useful as a secondary study to determine the etiology of urinary tract obstruction (236). It can provide detailed anatomic information, but is not used as an initial procedure because of its high cost, lack of immediate availability, and relatively large radiation dose. CT is particularly helpful to determine potential causes of obstructive uropathy in the retroperitoneal area, since it is able to determine the density of different types of tissues. Nonradiopaque calculi composed of uric acid are also readily detectable by CT. Thus CT scanning has replaced the invasive diagnostic procedures of retrograde and antegrade pyelography in evaluating patients with urinary tract obstruction (Fig. 25-11).

Nuclear magnetic resonance (NMR) or magnetic resonance imaging (MRI) provides computer-generated displays showing the distribution of hydrogen nuclei-emitting NMR when subjected to applied magnetic fields and radiofrequency energy. NMR is particularly useful in determining tissue densities and, hence, like CT, may prove to be useful in determining the etiology of obstructive uropathy (237,238).

Ureteropyelography

Pyelography provides detailed information about the location and cause of urinary tract obstruction previously detected by IVP, ultrasonography, or isotopic renography. When further anatomic information is required or when renal contrast excretion is poor or nonexistent, pyelography becomes an essential part of the investigation. Urologic consultation is an integral part of the diagnosis and management of such patients. Cystoscopy provides definitive information about possible causes of obstruction located in the posterior urethra and bladder, which are difficult to demonstrate by noninvasive methods. Retrograde ureteric catheterization permits samples of urine to be obtained for diagnosis from one or both ureters, allows the injection of contrast material for exact localization of the level of ureteric obstruction, and may be therapeutic in revealing ureteric or ureteropelvic obstruction. In the hands of a skilled urologist, the incidence of infection or postcatheterization ureteral edema is minimal.

Antegrade ureteropyelography is also used as a diagnostic and, at times, therapeutic procedure in obstructive uropathy. The technique involves the percutaneous puncture of the dilated renal pelvis that has been previously localized by ultrasonography or contrast injection. Measurement of resting pressures and samples of urine for culture or cytology are obtained from the renal pelvis, and, following injection of contrast media, excellent visualization of the ureter is obtained. If desired, temporary or permanent relief of obstruction may be accomplished by percutaneous nephrostomy.

The advantages of antegrade pyelography include the use of local anesthesia, sterile technique, physiologic route of contrast injection, and the ability to make pressure measurements and obtain samples of fluid or brushings of intraluminal lesions. The risks include bleeding or the extravasation of urine.

In patients with chronic partial unilateral obstruction and hydronephrosis, it is difficult to determine whether a surgical procedure should be done for an abnormality at the ureteropelvic junction. The gold standard in this situation is considered to be renal perfusion–pressure studies carried out by antegrade pyelography (239,240). For these studies, fluid is infused through a needle in the dilated renal pelvis at a standard rate and intrapelvic pressure is recorded while bladder pressure is monitored simultaneously through an indwelling catheter (Whitaker test). At a flow rate of 10 mL/min, the pressure fall to the bladder should not exceed 15 cm of water. With significant obstruction, the pressure decrement will, in almost all cases, be more than 22 cm of water (238). It should be recognized, however, that such procedures have technical pitfalls and that factors other than outlet obstruction may affect the pressure drop.

TREATMENT OF URINARY TRACT OBSTRUCTION

The overriding principles in the treatment of urinary tract obstruction should be as follows:

1. Eliminate any life-threatening aspect of this disorder.
2. Preserve renal function by relief of obstruction and treatment of complications, particularly, urinary tract infection (UTI).
3. Determine the cause of obstruction and its specific treatment.

The first principle in treating obstructive uropathy is to eliminate any life-threatening aspect of this disorder.

1. Gram-negative septicemia may occur when pyelonephritis develops in the presence of severe partial or complete obstruction. Thus, fever in a patient with urinary tract obstruction must be managed aggressively with diagnostic blood and urine cultures and appropriate antibiotics administered parenterally while awaiting the results of cultures.
2. Acute papillary necrosis secondary to pyelonephritis and obstruction may cause rapid destruction of renal tissue and this possibility is another reason for using immediate corrective measures. Emergency surgical relief of obstruction by the most simple appropriate procedure, such as bladder catheterization or percutaneous nephrostomy, is usually required in such patients.
3. Acute or chronic renal failure with hyperkalemia, acidosis, convulsions, coma, or pericarditis is another life-threatening clinical presentation of obstructive uropathy that may require immediate treatment by dialysis until definitive measures can be taken to correct the obstruction.
4. Occasionally, intravascular volume contraction and hypotension may present as a complication of postobstructive diuresis or of chronic partial urinary tract obstruction with inadequate oral intake.

The second principle of treatment is to prevent further deterioration of renal function and to allow recovery of function. Surgical therapy that decreases elevated intrarenal pressure or corrects progressive anatomic abnormality is essential. Meticulous management of urinary tract infection is also important. Treatment of other complications, such as renal calculi or hypertension, and detection of recurrent obstruction by careful follow-up, may preserve renal function.

The need to accurately determine the cause and rate of progression (natural history) of urinary tract obstruction is an important third principle of treatment. Before a decision is made regarding the need for specific surgical therapy, it may be necessary to perform serial assessments of renal function over several months or longer. Elective surgical repair is required for progressive lower urinary tract symptoms, recurrent serious urinary tract infections, urinary retention, or evidence of progressive deterioration in renal function. Surgical treatment, however, is usually not indicated simply because of the finding of residual urine in the bladder, mild to moderate vesicoureteral reflux, or a dilated renal pelvis. Once a decision has been made that surgical intervention is required, optimal therapy consists of removal of the obstructing lesion and reestablishment of continuity of the urinary tract. At times, however, the obstructing lesion cannot be removed, and urinary diversion may be required for weeks, months, or years. Placement of a bladder catheter (intermittent or continuous), nephrostomy, pyelostomy, cutaneous ureterostomy, suprapubic cystostomy, or ureteroileal cutaneous anastomosis (ileal conduit), as well as other procedures, may be used in appropriate cases, but each is accompanied by risks of recurrent obstruction, ascending urinary tract infection, electrolyte abnormalities, volume depletion, and formation of renal calculi. At times, nephrectomy is the procedure of choice when return of useful renal function is unlikely.

It is clear that management of obstructive nephropathy should involve consultation with a skilled urologist. Definitive therapy to preserve renal function and correct the cause of obstruction is frequently surgical. Nevertheless, it is appropriate for the internist or nephrologist to remain directly involved in the care of many of these patients, particularly with respect to evaluation of the underlying causes, such as renal calculi, treatment of complications, such as urinary tract infection, and careful follow-up of renal function.

Surgical Treatment

To determine the likely benefit from definitive surgical therapy, it is important to consider the potential for recovery of GFR after relief of obstruction. Assessment of the severity, duration, and cause of obstruction by analysis of symptoms, signs, and appropriate specialized tests is essential. In general, if the obstruction is of recent onset (weeks), a gradual increase in GFR over a period of 1 to 4 weeks may be expected. However, if obstruction is long-standing (months or years), renal parenchymal function may be irreversibly lost and little or no improvement in function should be expected. A discussion of the surgical treatment of obstructive uropathy is available elsewhere (241).

Renal Pelvic Obstruction

Congenital ureteropelvic obstruction is usually corrected surgically in patients who are symptomatic (flank pain or mass), patients who experience recurrent infections, or patients in whom progressive renal damage can be demonstrated. In addition to removal of any extrinsic obstructing lesion, such as an aberrant renal artery, surgical treatment (240) involves some form of plastic repair (pyeloplasty) of the ureteropelvic junction, such that redundant tissue is removed and the junction remains funnel-shaped and dependent. Radiographic improvement in the appearance of the renal pelvis following surgery should not be expected, but deterioration of renal function and recurrent infections can be largely eliminated. In patients in whom a stable level of renal function has been achieved with a dilated renal pelvis and mild ureteropelvic obstruction, surgery is not required.

Renal calculi obstructing the ureteropelvic junction for several days usually require surgical removal, since they are too large to be passed spontaneously, and may cause progressive renal damage. Smaller stones in the renal pelvis or calyces are usually not removed surgically unless they are causing intermittent obstruction, as they may be difficult to locate and surgery is associated with significant risk of renal damage. Surgical intervention for large, nonobstructing renal pelvic stones, which are usually of the staghorn infective (or struvite) type, is controversial, because surgery is difficult and rapid stone recurrence is frequent unless all infected stone fragments can be removed and recurrent infection prevented.

Ureteral Obstruction

Renal calculi are a common cause of ureteric obstruction in the adult. Most stones pass spontaneously if they are less than 5 to 7 mm in diameter and, on occasion, larger stones may be passed. Thus, in the absence of infection, such stones should be treated conservatively at first with control of renal colic by analgesics and increased hydration. If the stone reaches the lower end of the ureter, basket extraction through cystoscopy may be possible, whereas stones impacted higher in the ureter

require surgical removal by ureterolithotomy. Extracorporeal shock-wave lithotripsy (242) involves the focusing of electrohydraulically or ultrasonically generated shock waves to disintegrate the stone. The procedure is effective for calculi of 7 to 15 mm; in 90% of patients, the stone will be fragmented and all particulate matter will pass within a 3-month period. Morbidity is low. In selected individuals, the procedure can be done on an outpatient basis. Most patients are back at work 2 to 3 days after shock-wave therapy. Ureteric obstruction from other causes, such as tuberculous stricture or extrinsic compression from tumor or retroperitoneal fibrosis, often requires more extensive surgical therapy, which may include urinary diversion. The management of vesicoureteral reflux and reflux nephropathy is discussed in Chapter 24 (Vesicoureteric Reflux and Reflux Nephropathy).

Lower Urinary Tract Obstruction

Surgical treatment for bladder neck or urethral obstruction is indicated for patients with severe difficulties in voiding, decreased renal function, or recurrent urinary tract infection. In patients with mild or moderate symptoms, a decision should be made on an individual basis with periodic assessment of renal function. Severe bladder dysfunction, such as a neurogenic bladder or obstruction from pelvic malignant disease, may require surgical urinary diversion, usually in the form of an ileal conduit.

Nephrectomy as a Treatment for Unilateral Obstruction

There are few indications for removing an obstructed kidney. In general, refractory pyelonephritis in a unilaterally obstructed, but functioning, kidney is not sufficient reason for a nephrectomy, because it is likely that pyelonephritis has developed in the unobstructed contralateral kidney by ascending spread from infected bladder urine. In this situation, removal of functioning tissue in the obstructed kidney could hasten development of chronic renal failure. Nephrectomy is warranted only if the obstructed kidney is severely and irreversibly damaged and pyelonephritis within it is recurrent, severe, and refractory to treatment. Rarely, removal of a functioning, but obstructed, kidney may be indicated if surgical repair has been unsuccessful and acute infection in that kidney appears to have been the source of life-threatening episodes of Gram-negative septicemia.

Medical Treatment

Fluid and Electrolyte Management

Patients with chronic partial obstruction and renal tubular dysfunction may have excessive losses of water (including nephrogenic diabetes insipidus), sodium chloride, or sodium bicarbonate in the urine. Accordingly, they may require oral sodium chloride and/or sodium bicarbonate supplements, as well as a high fluid intake. They should be warned to replace excessive fluid losses occurring with intercurrent illness causing diarrhea, vomiting, or excessive sweating.

Postobstructive diuresis after relief of severe partial or complete obstruction is an uncommon event, but requires careful assessment and management of fluid and electrolyte balance including replacement of deficits of sodium chloride, bicarbonate, potassium, and water, and replacement of insensible losses and of ongoing losses in the urine. Usually 0.45% NaCl is a suitable replacement solution for urinary losses, but urinary electrolytes should be checked periodically, and potassium supplements are usually required. Postobstructive diuresis must be distinguished from the physiologic diuresis of retained fluid and from iatrogenic diuresis resulting from overenthusiastic administration of intravenous fluids. Prolongation of the natriuresis by volume expansion may be assessed by decreasing the intravenous replacement of urinary losses for 8 to 12 hours, combined with clinical assessment of intravascular volume and of changes in urine volume and body weight.

Urinary Tract Infection

Bacteriuria is difficult to eradicate in the presence of urinary tract obstruction and occurs with increased frequency in such patients. Acute UTI, with or without clinical pyelonephritis, should be treated with appropriate antibiotics based on *in vitro* bacterial sensitivity testing, although therapy must often be initiated before such results are available and then altered as required. Antibiotics that reach high concentrations in the kidney and urine should generally be used.

Prophylactic antibiotics given parenterally 1 hour before and a few hours after instrumentation of the urinary tract in patients with obstructive nephropathy may reduce the incidence of infection. Prolonged prophylactic antibiotic therapy, for months or years, may be useful to reduce episodes of recurrent acute symptomatic UTI with pyelonephritis in patients with chronic obstruction and/or struvite stones.

Other Complications: Hypertension and Renal Failure

Hypertension associated with obstructive uropathy is usually treated medically with antihypertensive drugs, although occasionally surgical correction of acute unilateral obstruction may result in control of blood pressure. Renal failure associated with obstruction is treated by dialysis as required for management of acute renal failure or end-stage renal disease resulting from obstruction. Patients with end-stage renal disease owing to obstruction are suitable for renal transplantation, but usually require bilateral nephrectomy prior to such a procedure in order to eliminate foci of infection. Even patients with urinary diversions, such as an ileal conduit, may receive a kidney transplant, although the high risk of infective complications often makes long-term dialysis a safer alternative.

Long-Term Follow-Up

Patients who have had surgical treatment for obstruction or who have chronic obstruction require careful long-term follow-up by a physician. Such follow-up involves careful clinical assessment, urinalysis and urine culture, periodic radiologic evaluation, and, most important, an assessment of renal function, usually by endogenous creatinine clearance.

Obstructive Nephropathy and Renal Fibrosis

A model of renal fibrosis that encompasses many aspects of other models of kidney disease is unilateral ureteral obstruction (UUO) (243–246)

There are features of the UUO model that occur within 1 week of the onset of ureteral ligation. There are many readily quantifiable cellular and molecular events during the initiation and progression of renal fibrosis that make UUO an increasingly good experimental model for study.

Table 25-5 describes factors, the expression of which is increased in kidneys with ureteral obstruction; Table 25-6 depicts factors, the expression of which is decreased in kidneys with ureteral obstruction.

In a rat model of UUO, Sweeney et al. (247) visualized changes in renal blood flow (RBF) using a radiolabeled compound and radioautography. They measured an initial vasodilation in the cortex within 10 min of obstruction. This was

TABLE 25-5

FACTORS THAT ARE INCREASED IN THE SETTING OF URETERAL OBSTRUCTION

Vasoactive compounds
Angiotensinogen
Angiotensin II
Endothelin
Thromboxane A_2
Prostaglandins
Transforming growth factor-β
Protein 53
Protein 21 (WAF1)
Tissue inhibitor of metalloproteinase-1
Decorin
Nuclear factor κB
Tumor necrosis factor-β
Protooncogenes
c-fos
c-jun
jun B
c-myc
cH-Ras
Growth factors
Interleukin-6
Platelet-activating factor
Basic fibroblast growth factor
Proteins involved in apoptosis
Clusterin (SGP-2)
Osteopontin
Chemoattractants
Monocyte chemoattractant peptide-1
Osteopontin
Adhesion proteins
Intercellular adhesion molecule-1
Vascular cell adhesion molecule-1
Fibronectin (alternate splice forms)
Matrix/basement membrane proteins
Collagen types I, III, IV, XV, and XVIII

followed by a significant vasoconstriction in both the cortical and medullary beds (247). The changes in blood flow were mediated by prostanoid, Ang II, and NO. We have identified a significant pathophysiological role of several vasoconstrictive substances during ureteral obstruction. These vasoconstrictors include Ang II, TXA_2, and ADH (248).

A recent study by Moridaira et al. (249) confirms that enalapril inhibits the interstitial fibrosis in UUO kidneys. It also suggests a beneficial and unforeseen effect of enalapril on the obstructed kidney by potentially stimulating the production of NO through an increased expression of the ET_B receptor.

TABLE 25-6

FACTORS THAT ARE DECREASED IN THE SETTING OF URETERAL OBSTRUCTION

Growth or homeostatic factors
Bone morphogenetic protein-7 (BMP-7)
Hepatocyte growth factor
Epidermal growth factor
Redox state
Copper, zinc superoxide dismutase
Catalase
Reduced glutathione
Others
Meparin
Inducible nitric oxide synthase (iNOS)

TABLE 25-7

EFFECTS OF VASOCONSTRICTORS AND VASODILATORS ON RENAL HEMODYNAMICS IN RATS AFTER UNILATERAL RELEASE OF BILATERAL URETERAL OBSTRUCTION[a]

	GFR (%)	
Maneuver	**Before maneuver**	**After maneuver**
Inhibition of synthesis or activity of vasoconstrictors		
RAS inhibition	20.7	75.8
RAS and TXA_2 inhibition	23.5	90.5
Prevention of macrophage infiltration	21.5	52.3
TXA_2 synthesis inhibition	17.0	29.7
LT synthesis inhibition	21.5	49.5
Inhibition of ADH V_1 receptors	22.3	35.8
Endothelin antibody	21.0	33.8
Deendothelialization of main renal arteries	21.0	10.2
Administration or activation of vasodilators		
L-Arginine administration (for NO synthesis)	24.3	49.2
TXA_2 inhibition and PAF administration	17.0	57.6
ANP administration	24.8	39.5

[a] GFR, glomerular filtration rate was measured in rats after unilateral release of bilateral obstruction versus that in one kidney of sham-operated rats; *RAS*, renin–angiotensin system; *TXA_2*, thromboxane A_2; *LT*, leukotrienes; *ADH*, antidiuretic hormone; *NO*, nitric oxide; *PAF*, platelet-activating factor; *ANP*, atrial natriuretic peptide. (From: Gaudio KM, et al. Renal perfusion and intratubular pressure during ureteral occlusion in the rat. *Am J Physiol* 1980;238:F205; Mene P, Dunn MJ. Contractile effects of TXA_2 and endoperoxidase analogues on cultured rat glomerular mesangial cells. *Am J Physiol* 1986;251:F1029, with permission.)

We also found a vasoconstrictive effect of leukotrienes in the setting of bilateral ureteral obstruction (248). On the other hand, it is likely that vasodilatory substances are decreased in the setting of ureteral obstruction or are not increased to a sufficient degree to balance the increased activity of the vasoconstrictor compounds. Administration of vasodilators (PGE_2 and PGI_2) and platelet-activating factor (PAF) after inhibition of the synthesis of TXA_2 increased GFR and effective renal plasma flow (ERPF) significantly but not to normal levels. We also found that endothelin has an important role in the renal functional alterations observed in rats after unilateral release of 24-hour duration bilateral ureteral ligation (250). Rats given a specific anti-endothelin antibody had significantly higher GFR and ERPF values than untreated rats subjected to bilateral ureteral obstruction. Table 25-7 summarizes the overall role of vasoconstrictors and vasodilators in the renal hemodynamics observed after unilateral release of bilateral ureteral obstruction. The overall role of endothelin in the altered hemodynamics of bilateral ureteral obstruction seems to be modest compared with that of other vasoconstrictors or the decreased activity of other vasodilators known to have a role in this setting. L-Arginine, PAF, and atrial natriuretic peptide (ANP) are among the vasodilators that have a role in the hemodynamics of bilateral ureteral ligation. The administration of L-arginine,

the substrate for NO synthesis, increased GFR to ~50% of the values obtained for one kidney in sham-operated rats.

Inducible nitric oxide synthase (iNOS) has been identified by immunohistochemistry in the renal tubules after UUO (251) Vasodilation of the afferent arteriole during acute UUO (<5 hours) may account for the increase in RBF and ureteral pressure. Several studies have explored the role of NO during ureteral obstruction. Intrarenal infusion of N^G-monomethyl-L-arginine, a nonspecific NOS inhibitor, attenuated the increase in RBF after acute ureteral obstruction (251). These effects of N^G-monomethyl-L-arginine were reversed by the intrarenal infusion of L-arginine.

The protective effect of NO has been demonstrated in several renal disease models. We augmented renal NO production by transfer of the inducible nitric oxide synthase (iNOS) gene into rat kidney in controls and in unilateral ureteral obstruction (UUO).

This study demonstrates the feasibility of liposome-mediated iNOS gene transfer into the kidney. Furthermore, the improvement of renal function in UUO demonstrates that the transfected iNOS gene is active and suggests that decreased iNOS activity contributes to the decreased renal function in UUO. This iNOS construct may have therapeutic utility in the pathophysiologic sequelae of UUO and other renal diseases (252).

Another aid in the recovery from acute ischemic renal injury is treatment with hepatocyte growth factor (HGF) (253). Indeed, a decrease in HGF levels induced by either an increase in Ang II or TGF-β was hypothesized to be a factor in the development of glomerulonephritis (254). HGF has been described as a substance produced by mesenchymal cells that maintains epithelial homeostasis (255). Mizuno et al. (256) used a mouse model of UUO and treated the animals with vehicle, a neutralizing antibody to HGF, or recombinant human HGF. Treatment with the HGF-neutralizing antibody was found to increase TGF-β expression, decrease tubular cell proliferation, and accelerate apoptosis. Treatment with exogenous HGF attenuated apoptosis and TGF-β expression (256). The authors suggested that the reduction in endogenous HGF can account for the progression of renal fibrosis in tubulointerstitial-based disease (256). This was underscored by a study in which HFG was administered by tail-vein injection every 12 hours for the 7 days of UUO (257). The injection of HGF blunted the expected increase in TGF-β1 mRNA and various histological indices of fibrosis (257).

Recently, another protein has emerged as a potential renotrophic factor: bone morphogenetic protein-7 (BMP-7). In a preventative protocol, BMP-7 treatment was found to significantly decrease renal injury in a rat model of UUO when treatment was initiated at the time of injury (258). Subsequent studies suggested that BMP-7 treatment will also attenuate renal fibrosis when administered after renal fibrosis has begun (259). This treatment protocol was also found to significantly increase renal function from the levels measured in the vehicle-treated group (259). These studies underscore the value of histological parameters as an indicator of renal function and the potential of renal homeostatic factors in the treatment of kidney disease.

Recently, arginine infusions in dogs with 18 hours of UUO were shown to increase renal blood flow, leading to the conclusion that a lack of substrate may contribute to the profound vasoconstriction resulting from chronic UUO (260,261). Chronic UUO in mice leads to a progressive reduction in the production of iNOS by the obstructed kidney and tubular apoptosis is even more severe following UUO in mice lacking the gene for iNOS (262). Mechanical stretch of tubular epithelial cells, because of tubular dilatation, appears to be a major factor contributing to apoptosis following UUO, and axial strain on cultured tubular cells increases iNOS expression (263). Moreover, inhibition of endogenous iNOS increases stretch-induced apoptosis in rat renal tubular cells (262).

In this very well-controlled study using cationic liposomes as the vehicle, iNOS was localized to collecting ducts, distal tubules, and glomeruli. Importantly, renal expression of iNOS and urinary NO_2/NO_3 remained elevated more than 30 days after transfection (previous reports of kidney-targeted viral-mediated delivery of genes have not shown long-term expression) (252). While iNOS transduction was performed 7 days prior to ureteral obstruction, and functional studies were performed after only 24 hours of UUO, the authors indicate that longer-term studies of morphologic effects of iNOS transduction are to follow.

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CHAPTER 26 ■ NEPHROLITHIASIS

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The crystals that form into renal stones consist of calcium salts, uric acid, cystine, or struvite (magnesium ammonium phosphate). Calcium stones are the predominant variety (Table 26-1) and are composed of calcium oxalate (CaOx), CaOx and calcium phosphate as apatite, or apatite alone (1–7). Two forms of CaOx crystals—monohydrate and dihydrate—differ in their lattice structure and microscopic appearance (8) and this may be relevant to pathophysiology. The calcium phosphate crystals are most commonly apatite or hydroxyapatite. Calcium carbonate is a crystal form usually found mixed in struvite stones or a high pH environment. Occasionally, brushite (calcium hydrogen phosphate), whitlockite (calcium orthophosphate), and octacalcium phosphate are found (6). Calcium phosphate crystals are as common in stones as are CaOx crystals (Table 26-1), but the amount of CaOx in mixed stones generally exceeds that of calcium phosphate. In addition, pure CaOx stones are much more frequent than are pure calcium phosphate stones. The incidence of renal stones appears to be gradually increasing, but remains poorly defined. A survey reported from a well-defined, upper-Midwest population in 1979 found the incidence in women to be 36 per 100,000 population. For men, the incidence had risen from 79 per 100,000 in 1950 to 124 per 100,000 in 1974 (9).

Nephrolithiasis is an extremely costly malady to society in terms of lost productivity and treatment cost. Estimates document the loss of several billion dollars annually from the United States economy each year to nephrolithiasis and missed workdays.

Calcium, uric acid, cystine, and struvite stones differ from one another in terms of pathogenesis and treatment. Therefore, each stone type is described separately. The clinical manifestations by which the stones present are not related to the composition of the stone and this should be kept in mind.

CALCIUM STONES

Natural History of Nephrolithiasis

Kidney stones account for about one of every 1,000 hospital admissions, and up to 10% of Americans will develop a stone in their lifetime; the vast majority of these are calcium stones. The incidence of nephrolithiasis in the United States is highest in the southeast (10,11), with peak incidence occurring in the late summer months. In addition, sedentary white-collar workers are more likely to form stones than are active blue-collar laborers (12,13). Stones are distinctly less common in African Americans, American Indians, and people of Asian descent. The age and sex distribution of patients referred for evaluation of nephrolithiasis at our institution shows a 1.6:1 ratio of male to female patients and a maximum incidence in the 30- to 50-year-old group (excluding patients with cystinuria and infection stones).

The natural history of stone disease is characterized by recurrence. Although many studies of the natural history and incidence of stones have been biased by referral practices and differences in definition of recurrence, there is clear evidence in the literature of the recurrent nature of stone disease.

A common dilemma faced by the clinician is whether or not to evaluate and treat the first-time stone former. The risk of recurrence after an initial episode has been estimated to be about 50% within 5 years, with almost two-thirds of patients having a recurrence by 9 years (13,14). Even when nephrolithiasis is evaluated and treated appropriately, the incidence of recurrence in first-time stone formers is not significantly different from that in treated patients with a history of multiple stone episodes (15). In fact, these patients most likely represent recurrent stone formers in the initial stage of their disease. These and other data demonstrating Randall's plaque (1,16) suggest that such patients should be evaluated and treated in the same manner as patients with recurrent nephrolithiasis.

Patients who have undergone shock-wave lithotripsy represent another group at risk for stone recurrence. Stone fragmentation often leads to regrowth of stone material, as residual fragments may act as a nidus for ongoing crystal deposition and stone formation. Medical therapy aimed at correcting underlying urinary abnormalities in these patients may prevent or limit stone growth and recurrence (17).

Despite the almost inevitable risk of nephrolithiasis recurring if it is left untreated, diagnostic evaluation and selective treatment of metabolic abnormalities clearly decrease the incidence of new stone formation and can induce complete remission (18–21).

Pathogenesis

Formation of Renal Stones

The formation of renal stones composed of calcium salts is a complex process that remains poorly understood despite considerable efforts over many centuries. The process consists of a calcium salt precipitating from solution (nucleation) forming a crystal. Subsequent crystal growth and aggregation lead to a stone nidus. When the aggregate adheres to the tubulopelvic uroepithelium, continued epitaxial growth of the crystal aggregate eventually leads to a detectable size, making it a renal stone.

Nucleation

Nucleation describes the process that occurs when the activity of calcium salts reaches the level at which the solid phase begins to appear. If one compares urine to an aqueous solution, it quickly becomes apparent that urine is able to hold much higher levels of calcium salt in solution than is water. If one considers an aqueous solution containing crystals of a calcium salt when the crystal neither grows nor shrinks, the solution is in equilibrium. The product of the free ion concentrations (activity product) at this equilibrium determines the equilibrium solubility product (SP) of the salt (Fig. 26-1). Solutions with concentrations of salt less than the equilibrium SP are undersaturated. A higher free-ion activity product will cause the

TABLE 26-1

TYPES OF RENAL STONES FORMED AND FREQUENCY OF OCCURRENCE[a]

	CaOx and cap	CaOx	Cap	Uric acid	Cystine	Struvite	Number of stones
Nordin and Hodgkinson (2)	46.0	14.7	8.0	2.9	3.3	25.1	243
Lagergen (3)	44.2	15.1	7.6	3.6	1.1	28.1	460
Melick and Henneman (4)	30.3	27.1	20.6	12.9	2.6	14.8	155
Prien (5)	34.3	32.7	5.3	5.8	2.9	19.0	1,000
Sutor, et al. (6)	35.9	28.5	7.4	2.47	1.61	24.1	810
All series	37.2	26.3	7.4	4.5	2.2	22.3	2,668

[a] *CaOX*, calcium oxalate; *Cap*, calcium phosphate. Numbers represent the percentage of each stone type in the series. Total numbers of stones surveyed are shown in the last column.
(From: Coe FL, Favus MJ. Nephrolithiasis. In: Brenner BM, Rector FC Jr., eds., *The kidney*, 2nd ed. Philadelphia: Saunders, 1991, with permission.)

solid phase (the crystals) to grow (epitaxy). However, if the crystals are removed from a solution at the level of the equilibrium SP and then the ion activity product is elevated, the activity product that would have caused growth of preformed crystals now results in no appearance of a new solid phase. This solution is called *metastably supersaturated* (Fig. 26-2). The activity products of calcium salts in urine are almost constantly in the range of metastable supersaturation. In the range of metastable supersaturation, if the activity products are sufficiently raised, new crystals will appear. The activity product at which new crystals form is called the *formation product* (FP), or the upper limit of metastability (Fig. 26-2). Above the level of the FP, a solution is unstable, creating new crystal nuclei. Urine may be undersaturated, metastably supersaturated, or unstable with respect to CaOx or the stone-forming calcium phosphate crystals (brushite, octacalcium phosphate, hydroxyapatite, and apatite). However, most of the time, it is metastably supersaturated and, particularly for brushite, close to the FP.

Factors Influencing Urinary Supersaturation

The multiple factors influencing urinary supersaturation in a clinical setting are shown in Table 26-2. The renal excretion of calcium salts that precipitate and take part in stone formation is a primary determinant of urinary supersaturation. Thus, urinary volume, calcium, oxalate, and phosphate ions all participate in the risk of calcium stone formation. In addition, binding of calcium and oxalate to cells, or the solid phase, and urine pH (which influences relative amounts of monohydrogen phosphate and dihydrogen phosphate), drastically alter free-ion concentrations and have great importance in regulating saturation—at least equal to the role of the total concentrations of the respective substances. This is the reason why hypercalciuria, oxaluria, unduly alkaline urine, and low urinary volumes are not sufficient to ensure that stones will form in and of themselves. Binding of the components of calcium salts also complicates the measurement of urine saturation and simple concentration measurements give only small clues to actual free-ion activity products.

Alternative substances to calcium salts may be considered as inhibitors of urinary saturation and contribute to the ability of urine to hold salts in solution to a much greater extent than does a simple aqueous solution. The known inhibitors of urinary saturation include the divalent cation magnesium, which forms oxalate, and phosphate salts, which are more soluble compared to those of calcium. In addition, citrate and sulfate are anions with which calcium forms soluble complexes as alternatives to phosphate or oxalate. Urine also contains substances to which calcium binds, thereby reducing the free-ion activity. Pyrophosphate, nephrocalcin, and osteopontin are other inorganic and organic crystal inhibitory calcium-binding sites and are discussed in greater detail later in this chapter. In addition, certain substances to which calcium salts may complex actually promote precipitation. In this category, uric acid and sodium urate are found. These substances are also discussed later in this chapter.

Measurements of Urinary Supersaturation

Because simple concentration measurements provide few clues to the activity of specific ions in urine, several strategies have been designed to estimate urinary supersaturation (22–24). These approaches are computer-based calculations of urinary free-ion activity for calcium, oxalate, and phosphate derived from their concentrations and their known tendencies to form

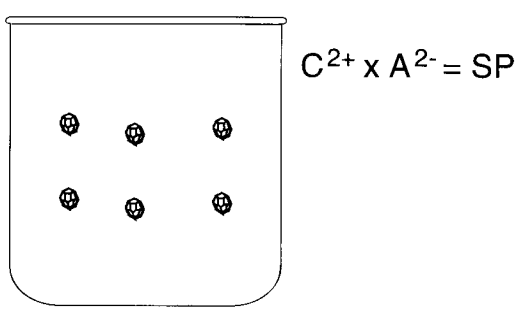

FIGURE 26-1. A solution containing calcium salt (a calcium salt consists of cations, C^{2+}, and anions, A^{2-}) crystals is in equilibrium when the crystals neither grow nor shrink. At this point, the product of the free-ion concentration (activity product) is the equilibrium solubility product (SP) of the salt.

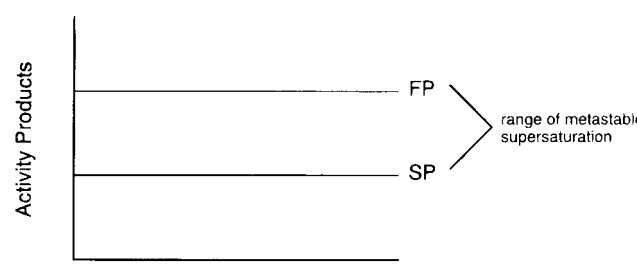

FIGURE 26-2. When calcium salts are added to a solution, precipitation (nucleation) does not occur until free-ion activity products well above the SP are reached. The activity product at which solid phase begins to form is the formation product (FP).

TABLE 26-2

FACTORS AFFECTING URINARY SUPERSATURATION IN THE CLINIC

Renal excretion rates
Calcium
Oxalate
Phosphate
Protons
Water
Inhibitors
Magnesium
Citrate
Nephrocalcin
Osteopontin
Tamm-Horsfall protein
Others (pyrophosphate, glycosaminoglycans)
Promoters
Uric acid, urate
Altered epithelial calcium oxalate binding

soluble complexes with each other and with other ligands, such as citrate and sulfate. A calculated free-ion activity product, such as the CaOx ion product, when divided by the corresponding equilibrium SP, yields an activity product ratio (APR), which estimates the degree of saturation (Fig. 26-3). A ratio above 1 indicates urinary supersaturation. Ratios below 1 represent undersaturation. The upper limit of metastable supersaturation can be determined by raising the APRs to the point at which precipitation or solid-phase formation begins to appear. The APR at this point is called the *formation product ratio* (FPR) (22). Pak and Holt (25) modified the approach to measuring urinary supersaturation by adding seed crystals to urine and incubating for 2 days at 37°C, with stirring at constant pH. The ratio of concentration products at the start and end of the incubation must equal the APR, even though the concentration products themselves do not equal the activity products. Pak and Holt (23,25) show that the assumption of stable activity coefficients is valid, so that that the empirical concentration product ratio is a valid estimate of the APR, within limits.

Observations of Urinary Supersaturation

Several investigators (26–30) with varying approaches have accumulated evidence indicating that urine from stone formers is more supersaturated than normal (Table 26-3). Because of differences in methodology, the absolute values of activity products differ among investigators. However, stone formers, whether hypercalciuric or normocalciuric, had higher average values of urine saturation than did those who did not form stones. This held whether saturation was measured with respect to CaOx, brushite, octacalcium phosphate, or hydroxyapatite.

An important observation common to approaches both with and without the use of seed crystals is that activity products of normal urine, on average, are above the equilibrium SP (Fig. 26-2) or oversaturated, except with respect to brushite. In the data from Pak and Holt (25,29) and Weber (30), this is a visible fact: Added crystals grew in urine from most normal persons. The use of urine measurements to assess supersaturation may be insufficient to reveal the full crystallization potential that exists in the renal tubule. Hautmann and colleagues (31) studied the calcium and oxalate concentrations in tissue from cortex, medulla, and papillae of human kidneys. The CaOx concentration product in the papillae exceeded that of urine and the concentrations in the medulla and cortex. If the high chemical concentration product in the papillae reflects a high free-ion product in tubular fluid or interstitium, CaOx crystallization in this region may occur more rapidly than would be predicted from the ion product of the final urine.

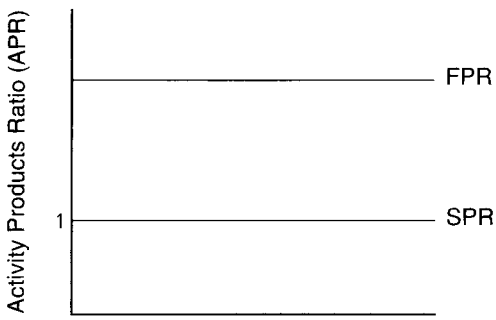

FIGURE 26-3. The calculated activity product of a calcium salt factored by its solubility product (SP) yields a ratio (R) describing undersaturation (<1), saturation (1), metastable supersaturation (>1), and the formation product ratio (FPR) at the point when the solid phase begins to form. The FPR determined for calcium oxalate (CaOx) by Pak and Holt (25) was 11 ± 3.

Formation Products

The urinary APR at which the urine produces new crystals has been measured for CaOx and brushite for those with no signs of stone formation and hypercalciuric, normocalciuric, and hyperparathyroid stone formers. Surprising variability was reported by Pak (29) (Fig. 26-4). However, the APR at the limit of metastability, the FPR, is higher in normal urine than in urine from stone formers. Furthermore, the FPR in urine from patients with hyperparathyroidism may be below the value observed for simple aqueous salt solution. This low value of FPR suggests facilitation of crystal formation. This type of data from several investigators yielded several conclusions. The first is that urine is abnormally supersaturated in stone formers. The values of APRs lie close enough to the FPR, at least for CaOx, so that new crystal formation would be expected. Most urine, even from those without stone formations, is metastably supersaturated with respect to CaOx, so that growth of crystal nuclei into a significant mass is predictable.

Homogeneous Versus Heterogeneous Nucleation

Nucleation, the initial precipitory event in stone formation, may be homogeneous or heterogeneous. In an unstable solution, crystals form spontaneously by homogeneous nucleation. Much higher levels of supersaturation are required to produce homogeneous nucleation than heterogeneous nucleation (32). The latter occurs in metastably supersaturated urine as certain macromolecules, or other crystals that can act as nuclei, stimulate precipitation. Because urine contains a number of macromolecular and cellular degradation products, crystallization is most often heterogeneous (32,33).

The efficiency of heterogeneous nucleation depends on the similarity between the spacing of charged sites on the preformed surface and the spacing in the lattice of the crystal that is to grow on that surface. This matching is referred to as *epitaxis*, and its extent is usually referred to as a *good* or *poor epitaxial relationship* (34). A number of urine crystals have good epitaxial matching and behave toward one another as heterogeneous nuclei. Monosodium urate and uric acid are excellent heterogeneous nuclei for CaOx (35,36). Thus, uric acid or urate could, by crystallization, lower the FPR for CaOx. Heterogeneous nucleation is thought to play a role in linking hyperuricosuria to CaOx stones (37–41), a matter discussed

TABLE 26-3

URINE CALCIUM OXALATE AND CALCIUM PHOSPHATE ACTIVITY PRODUCT RATIOS (APRs) IN NORMAL SUBJECTS AND IN STONEFORMERS[a]

	Normal subjects	Hypercalciuric stone formers	TCH normocalciuric TCH stone formers
Calcium oxalate monohydrate			
Robertson[b]	3 ± 1.2 to 10.7 ± 1.3	5.5 ± 1.3 to 18.2 ± 1.3	—
Pak[c]	1.45 ± 0.70	2.8 ± 1.4	2.2 ± 6.1
Weber[d]	1.97 ± 0.90	3.3 ± 2.2	2.2 ± 1.0
Brushite			
Pak[e]	0.35 to 0.26	1.74 ± 0.79	0.9 ± 0.5
Marshall[f]	1.15 ± 0.60	1.35 ± 0.70	4 ± 1.4
Octacalcium phosphate	63	79	200
Hydroxyapatite	4.6×10^5	9.1×10^5	2.9×10^8

[a]All values are means ± standard deviations.
[b]From: Lonsdale K. Human stones. *Science* 1968;159:1199; Robertson WG, Peacock M, Nordin BEC. Activity products in stone-forming and non-stone-forming urine. *Clin Sci* 1968;34:579; Robertson WG. Saturation inhibition index as a measure of the risk of calcium oxalate stone formation in the urinary tract. *New Engl J Med* 1976;294:249; and Marshall RW. The relationship between the concentration of calcium salts in the urine and renal stone composition in patients with calcium-containing renal stones. *Clin Sci* 1972;43:433; values of APR were calculated from activity products; the equilibrium solubility product (K_{sp}) was taken as 1.7×10^{-9} m^2.
[c]From: Pak CYC. Effects of orthophosphate therapy on the crystallization of calcium salts in urine. *Miner Electrolyte Metab* 1978;1:147; values of APR were measured by experiments.
[d]From: Weber DV. Urinary saturation measurements in calcium nephrolithiasis. *Ann Intern Med* 1979;90:180; values of concentration product ratio (CPR) (see text) were measured by seeding experiments.
[e]From: Pak CYC, Holt K. Nucleation and growth of brushite and calcium oxalate in urine of stone-formers. *Metabolism* 1976;25:665; K_{sp} of brushite was taken as 9.32×10^{-7} m^2; values of APR were calculated.
[f]From Marshall RW. The relationship between the concentration of calcium salts in the urine and renal stone composition in patients with calcium-containing renal stones. *Clin Sci* 1972;43:433; K_{sp} of octacalcium was taken as 2.3×10^{-18} m^2 and of hydroxyapatite as 1.1×10^{-56} m^2; values of APR were calculated.
(Modified from: Coe FL, Favus MJ. Nephrolithiasis. In: Brenner BM, Rector FC Jr., eds. *The kidney,* 2nd ed. Philadelphia: Saunders, 1991, with permission.)

later in this chapter. Epitaxial overgrowth of CaOx on a surface of uric acid has been experimentally documented (42). At a pH above 6.9, brushite may transform to hydroxyapatite, which can serve as a nucleus for CaOx (43). Based on observations that calcium phosphate is the most common crystal in human urine (44), is ubiquitous in human urinary stones, and is often seen at the center of mixed CaOx/calcium phosphate urinary stones (45), nucleation of CaOx crystals is proposed to be induced by calcium phosphate (1,16). In addition, both apatite and brushite crystals induce crystallization of CaOx *in vitro* from metastable solutions of CaOx (46,47). The other possible epitaxial relationships have not yet been linked explicitly to particular varieties of stone disease (48). However, the low FPRs in primary hyperparathyroidism suggest that heterogeneous nucleation may be occurring.

Crystal Growth

Once present, crystal nuclei grow if suspended in urine with an APR above 1 (Fig. 26-3). Crystal growth is critical to stone disease, as microscopic nuclei are too small to cause obstruction. Crystals are regular lattices, composed of repeating subunits, and they grow by incorporation of calcium and oxalate, or phosphate, into new subunits on their surfaces. In metastable solutions at 37°C, growth rates of CaOx and the stone-forming calcium phosphate crystals are rapid. Appreciable changes in macroscopic dimensions occur over hours to days. Growth rate increases with the extent of oversaturation and tends to be most rapid in urine having the highest APR.

Factors Influencing Crystal Growth

In urine, the upper limit of metastability is higher and crystal growth rates lower than in a salt solution with the same APR. The nature of the materials that confer crystal growth rate inhibition on urine is not completely known. Crystal growth inhibitors for calcium phosphate crystals may not be the same as the substances that affect CaOx crystal growth.

Inorganic pyrophosphate increases the FPs of calcium phosphate and CaOx in salt solutions and by absorbing their surfaces, retards the growth of hydroxyapatite (41) and CaOx crystals (43). Urinary pyrophosphate concentrations range from 20 to 40 μM in adults. This concentration is sufficient to inhibit crystal growth (49). Fleisch and Bisaz (40) suggest that urine raises the FP for calcium phosphate above the level expected from the pyrophosphate it contains. They suggest that other inhibitors accounted for approximately 50% of the total inhibition of calcium phosphate crystal growth. Smith and colleagues (50) have produced similar estimates. Bisaz (51) suggests that pyrophosphate, citrate, and magnesium ions contribute about 77% of the total calcium phosphate—pyrophosphate contributes insignificantly to CaOx crystal growth inhibition.

Inhibitors of Calcium Oxalate Crystal Growth

Some progress has been made describing the urinary inhibitors of CaOx crystal growth. The studies of Robertson and associates (53,54) suggesting that a urinary proteoglycan may significantly contribute to CaOx crystal growth inhibition have

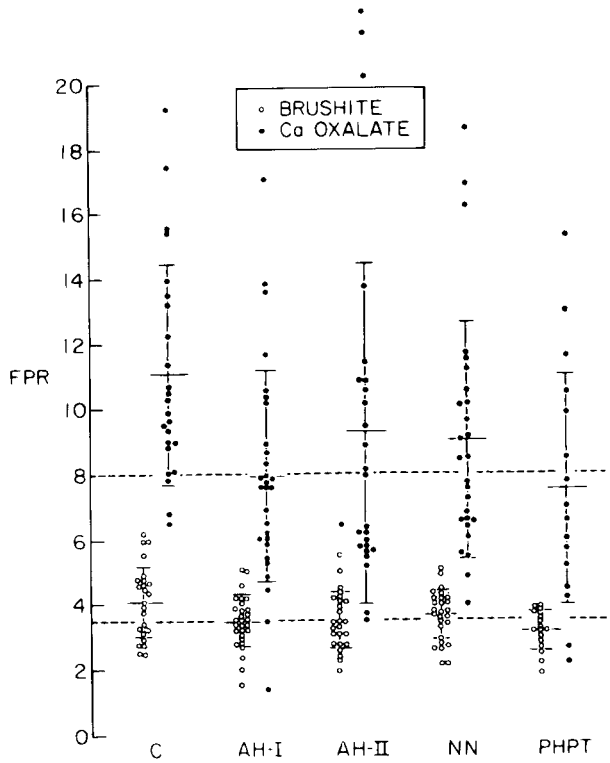

FIGURE 26-4. Formation product ratios (FPRs) for calcium oxalate (CaOx) and brushite of urine from normal subjects and stone formers. Each point shows the value for a single urine sample. For an aqueous solution, the activity product ratios at which spontaneous crystallization of CaOx and brushite occurs, the so-called FPRs, are shown by dotted lines at 8 and 3.6, respectively. *C*, control subject; *AH-1* and *AH-2*, severe and mild absorptive hypercalciuria; *NN*, normal calciuric stone formers; *PHPT*, primary hyperparathyroidism. Mean values in standard deviations (*SD*) are shown by horizontal lines. (From: Pak CYC, Holt K. Nucleation and growth of brushite and CaOx in urinary stone-formers. *Metabolism* 1976;25:665, with permission.)

not been further supported. Strongly acidic peptides, such as nephrocalcin and osteopontin have been described as important inhibitors of CaOx crystal growth (49,55–59). Strongly acidic peptides, such as poly-L-aspartate and poly-L-glutamic acids inhibit CaOx crystal growth and urine appears to contain several glycopeptides unusually rich in these two amino acids. Two of the best known of these are nephrocalcin and osteopontin (59). Treatment of urine with nonselective proteases diminishes the inhibition of CaOx crystal growth (49), which may be related to urinary glycoproteins. Nephrocalcin contains γ-carboxyglutamic acid (Gla) and is an amphiphilic molecule with a molecular mass of about 15 kDa. It tends to self-aggregate into a series of higher molecular-mass polymers (55,56).

Nephrocalcin from stone formers lacks the Gla residues, which is associated with a loss of ability to form stable film at an air–water interface, perhaps reflecting decreased amphiphilicity. The nature of the molecular abnormality leading to decreased inhibition of CaOx crystal growth is unknown. A separate abnormality in nephrocalcin has been identified from the urine of patients with X-linked recessive nephrolithiasis (60). Nephrocalcin from affected males and carrier females with X-linked recessive nephrolithiasis is poorly phosphorylated, with decreased ability to inhibit crystal growth.

Osteopontin (OPN) is a more recently isolated CaOx crystal growth inhibitor (57). Expression of OPN under basal conditions is limited to bone matrix, kidney, inner ear, decidual glands, and smooth muscle (61). OPN is found in breast milk and serum (62,63). It is expressed in response to various mitogens and growth factors, including phorbol esters and transforming growth factor-beta (TGF-β) (64,65) and is also expressed by cells in response to injury. Kleinman (66) finds that renal tubular OPN expression increases markedly after ischemic injury. However, OPN excretion is not decreased in renal stone formers. It appears that the level of phosphorylation is the critical issue in regards to the function of OPN as a urinary crystal inhibitor (59). The full OPN protein is not required for functional activity. Small domains of the OPN primary sequence were able to independently inhibit calcium oxalate monohydrate (COM) crystal growth. The phosphorylated OPN peptides are potent inhibitiors of COM crystal growth, but not COM crystal aggregation (59). The extent of phosphorylation of OPN is responsive to hormonal influences, such as those of vitamin D (67). The role of OPN in the kidney is not fully understood, although it is protective against CaOx monohydrate crystal aggregation and retention in the kidney as demonstrated by studies in OPN null mice, genetic hypercalciuric stone-forming rats, and humans (68,69).

The role of OPN in biology is complex (70). It is an osteoclast autocrine-regulating motility and bone resorption (71). OPN is a ligand for the cell surface receptors of $\alpha_v\beta_3$ and CD44, and stimulates osteoclast signal transduction upon binding (71,72). In the kidney $\alpha_v\beta_3$ is found largely on the basolateral surface of the distal nephron (73), and CD44 localization is unknown, whereas OPN is secreted mainly by the thick ascending limb into the tubular fluid at the luminal surface of the epithelial cell. OPN is structurally related to proteins found in other mineralized tissues, notably mollusk shells, which have contrasting effects on crystallization, depending on whether they are in solution or immobilized on a surface. When in solution, these proteins inhibit calcite, CaOx, and apatite crystal growth (74). However, if these proteins are immobilized on a support before incubation with a supersaturated solution of the mineral phase, they are able to initiate crystal nucleation in specific orientations (75). It is believed that OPN serves as a modulator of crystallization and is important for ordered crystal structure of the bone (76). In its phosphorylated state, it inhibits and, thereby, regulates apatite formation (77). OPN is also found in association with the pathologic calcification of stone matrix (78) and atherosclerotic plaques (64,64,79). Phosphorylation of OPN renders it inhibitory to vascular calcification (80) and skeletal mineralization (76)

Tamm–Horsfall protein (THP) is the major urinary glycoprotein of normal urine. THP from stone formers is abnormal, having a higher tendency to aggregate under conditions of increased ionic strength and low pH (81). Normal THP is an inhibitor of crystal aggregation, but THP from stone formers is less active in preventing aggregation and, under some conditions, THP from stone formers may promote the formation of crystal aggregates, especially in the presence of high concentrations of calcium. The structural abnormalities responsible for impaired inhibitory activity are not completely understood (82). One study demonstrated that Tamm-Horsfall glycoprotein and citrate concentrations are linearly related to CaOx monohydrate agglomeration inhibition (83). The effects of the two substances are synergistic. Tamm-Horsfall glycoprotein removal from the urine dramatically reduced CaOx agglomeration (83).

Hallson and Rose (84) suggested that certain materials in urine, which they refer to as *uromucoids,* might promote calcium phosphate crystallization and aggregation. The significance of these findings is unclear. Of greater interest, urate anions in urine appear to bind and adsorb inhibitor substances, suggesting that hyperuricosuria could promote CaOx stones by reducing levels of urine crystal growth inhibitors (53). When incubated *in vitro*, monosodium urate is able to bind heparin, a potent proteoglycan inhibitor of CaOx crystallization (85).

TABLE 26-4

PATHOGENETIC MECHANISMS IN SOME ESTABLISHED FORMS OF CALCIUM NEPHROLITHIASIS

		Mechanisms	
	Overexcretion	Heterogeneous nucleation	Reduced inhibition of stone formation
Idiopathic hypercalciuria	+	−	Unknown
Primary hyperparathyroidism	+	+	−
Hyperuricosuria	−	+	+
Renal tubular acidosis	+	Unknown	+
Hyperoxaluria	+	−	+
No detectable metabolic abnormality	−	−	+

This demonstrates that at least one specific polyanion inhibitor could be adsorbed by a solid phase of uric acid.

Relationship of Oversaturation and Crystal Growth Inhibition to Clinical Nephrolithiasis

The force that drives calcium salts out of solution, into the solid phase, is oversaturation. Compared to homogeneous nucleation, heterogeneous nucleation facilitates stone formation by decreasing the degree of oversaturation required for nucleation. Inhibitors, such as magnesium, pyrophosphate, osteopontin, and nephrocalcin suppress nucleation, increase the supersaturation needed to produce the solid phase, and retard the growth of nuclei already formed. In the most important stone-forming conditions, oversaturation, heterogeneous nucleation, and reduced inhibitors have documented, or at least postulated, roles that vary from one disease to another (Table 26-4). Treatment is often successful in reversing stone formation by eliminating the disturbances that enhance the risk of stones or, in some cases, by introducing secondary biochemical changes that compensate for the underlying defect.

Oversaturation occurs in idiopathic hypercalciuria, primary hyperparathyroidism, and hyperoxaluria because of overexcretion (Table 26-4). Both hypercalciuria and phosphaturia occur in renal tubular acidosis (RTA). Oversaturation with respect to calcium phosphate salts, which make up most of the stones in RTA, is also increased by an alkaline urine and by low levels of urinary citrate, an important calcium-binding agent.

The finding of urine formation products below those of simple salt solutions provides evidence for heterogeneous nucleation in hyperparathyroidism. The basis for this finding is unknown. Hyperuricosuria is thought to engender urine crystals of uric acid or sodium hydrogen urate, which are efficient heterogeneous nuclei for CaOx (35,36,38). It is uncertain whether these crystals are in a gel state (53).

Low levels of urine inhibitors have been demonstrated in some hypercalciuric and normocalciuric stone formers (27). Robertson (27) reports lower inhibitor levels in hyperuricosuric stone formers. In general, the levels of crystallization inhibitors in the urine of stone formers differ from those in non–stone formers and, consequently, their urine samples can be distinguished from samples of normal people more reliably when inhibitor content is measured than by the use of supersaturation measurements alone (Fig. 26-5). This fact highlights the presence of low inhibitor levels in stone-forming patients and suggests that inhibitors are very important in preventing stones.

More recent measurements of urinary inhibitors of CaOx monohydrate crystal growth show that the lowest inhibitor levels occur in patients with hypercalciuria, but not hyperuricosuria (85), and that samples from normal subjects can be distinguished from those of stone formers no more reliably by a combination of inhibitor and supersaturation measurements than by measurements of inhibition alone. Nephrocalcin from urine of stone-forming patients (86) or CaOx stones (87) seems abnormal; it lacks Gla and forms weak air–water films. The difference between these results and those of Robertson (27) is probably related to differences in methodology. Hallson and Rose (84) present additional evidence that inhibitors of crystallization are functionally important and differ in stone formers from those in normal subjects.

Classification of Calcium Nephrolithiasis by Urinary Chemistries

In Table 26-5 are shown the classifications of biochemical and physical disturbances that contribute to the formation of

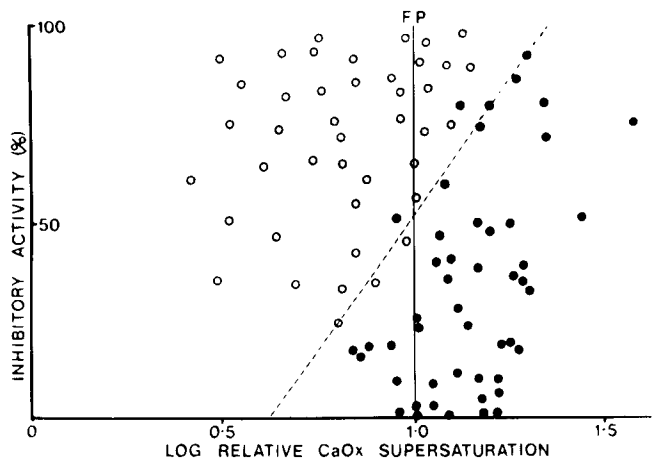

FIGURE 26-5. Saturation-inhibition index of urine from stone formers in normal subjects. The ability of dilute urine to prevent crystal aggregation in an *in vitro* system (inhibitory activity) is shown as a function of relative supersaturation with respect to calcium oxalate (CaOx) (*x* axis). A value of 1.0 represents the saturation at the level of the formation product (FP); a value of 0.1 would be near the solubility product. Stone formers (*solid circles*) and normal subjects (*open circles*) fall into separate zones; the dotted line, obtained by statistical analysis, is the best plane of separation. (From: Robertson WG, Peacock M, Marshall RW, et al. Saturation inhibition index as a measure of the risk of calcium oxalate stone formation in the urinary tract. *N Engl J Med* 1976;294:249, with permission.)

TABLE 26-5

URINARY CHEMISTRIES IN EVALUATION OF NEPHROLITHIASIS[a]

Category	Seltzer and Hruska (89) Jewish Hospital ($N = 587$)		Levy et al. (88) University of Texas Southwestern ($N = 1270$)	
	Sole occurrence (%)	Combined occurrence (%)	Sole occurrence (%)	Combined occurrence (%)
Hypercalciuria	14	51	—	—
Male (>250 mg/24 hr)	8	34	—	—
Female (>225 mg/24 hr)	6	17	—	—
Absorptive hypercalciuria	—	37	6.1	23.1
Fasting hypercalciuria	—	14	4.3	13.9
Renal hypercalciuria	—	1	0.3	1.3
Renal phosphaturia	—	2	2.1	7.6
Primary hyperparathyroidism	—	1	0.8	1.3
Hyperuricosuria	8	42	—	—
Male (>0.75 g/24 hr)	6	30	—	—
Female (>0.70 g/24 hr)	2	12	—	—
Hyperuricosuric calcium nephrolithiasis	—	—	8.3	27.6
Gouty diathesis	—	—	3.1	6.9
Hypocitraturia	9	34	—	—
Male (<250 mg/24 hr)	5	18	—	—
Female (<300 mg/24 hr)	4	16	—	—
Complete distal RTA	—	—	0.08	0.16
Incomplete distal RTA	—	—	0.0	1.1
Chronic diarrheal syndrome	—	—	0.2	1.8
Idiopathic	—	—	3.5	24.4
Hyperoxaluria (>40 mg/24 hr)	8	34	—	—
Enteric hyperoxaluria	—	—	0.2	1.4
Primary hyperoxaluria	—	—	0.0	0.4
Dietary hyperoxaluria	—	—	0.4	5.7
Hypomagnesuria (<5 mEq/24 hr)	5	26	0.3	6.5 (<50 mg/24 hr)
Low urinary volumes (<1,500 ml/24 hr)	26	61	1.7	13.5 (<1,000 ml/24 hr)
No diagnosis/difficult to classify	—	2	—	4.0

[a] *RTA*, renal tubular acidosis. The category definitions in the table refer to Jewish Hospital and Washington University, St. Louis. Criteria for the University of Texas Southwestern data are provided in the text.

calcium stones, based on two surveys from the mid-1990s (88,89). A number of specific disturbances have the potential to create an environment conducive to renal stone formation. Several investigators utilize the presence of such disturbances as the basis for diagnostic categorization of nephrolithiasis (18,88,89). In 1980, based on ambulatory evaluations of patients with nephrolithiasis, Pak (90) reported ten metabolic etiologies composing four types of hypercalciuria hyperuricosuria, hyperoxaluria, RTA, uric acid stones, and infection stones. Pak reported an 11% incidence of finding no metabolic abnormalities (90). Since 1980, the understanding of the pathophysiology of renal stone disease has progressed. As shown in Table 26-5, in large series, more than 15 etiologic categories of nephrolithiasis have been described. A single diagnosis is found in the minority of patients, while approximately 60% have more than one diagnosis. The finding of no metabolic abnormality has been reduced to the range of 2% to 4% of patients with nephrolithiasis. Hypercalciuric nephrolithiasis accounts for about 60% of the patients. Hyperuricosuria associated with calcium nephrolithiasis can be subdivided into hyperuricosuric calcium nephrolithiasis and patients with gouty diathesis. Hyperoxaluric calcium nephrolithiasis, which occurs in about 8% of patients with recurrent stones, has been subdivided into enteric, primary, and dietary variants. Hypocitraturic calcium nephrolithiasis, which affects about 30% of patients in its idiopathic variant, is also associated with incomplete RTA and chronic diarrheal syndrome. Hypomagnesiuric calcium nephrolithiasis, infection stones, and cystinuria are uncommon, accounting for 7%, 6%, and 1% of patients, respectively. The acquired problem of low urinary volume, less than 1 L/day, according to Levy and colleagues (88), and less than 1.5 L/day, according to Seltzer and Hruska (89), is the single most common abnormality.

The description of clinical subtypes that follow represents the minimal diagnostic criteria utilized to establish the presence of the entities listed in Table 26-5 according to Hruska and Seltzer.

Absorptive Hypercalciuria Type I

Diagnostic criteria include: calcium nephrolithiasis, normocalcemia, normophosphatemia, hypercalciuria (>200 mg/day) on a calcium-restricted diet, normal fasting urinary calcium (<0.11 mg/dL glomerular filtrate [dL GF]), exaggerated calciuric response to an oral calcium load (>0.20 mg urinary calcium/mg urinary creatinine), and normal to suppressed serum parathyroid hormone (PTH) function (Fig. 26-6) (91–98).

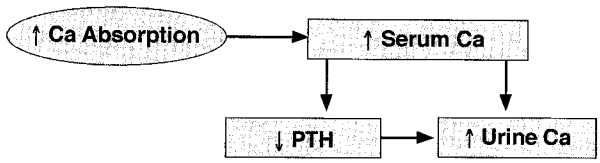

FIGURE 26-6. Pathogenesis of absorptive hypercalciuria. Intestinal hyperabsorption of calcium leads to an increase in serum calcium and a reduction of parathyroid hormone (*PTH*). The increase in the filtered load of calcium and the reduced calcium reabsorption produced by loss of PTH activity lead to hypercalciuria.

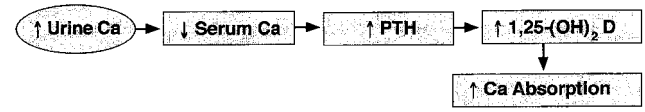

FIGURE 26-8. The pathogenesis of renal hypercalciuria. Defective renal calcium reabsorption leads to hypocalcemia and a stimulation of parathyroid hormone (*PTH*) secretion. The latter increases the production of $1,25(OH)_2D_3$, stimulating calcium absorption and leading to hypercalciuria. The hypercalciuria, in turn, compensates for the reduction in serum calcium, but compensation never completely restores normal calcium, and elevated PTH values are required in this syndrome.

Absorptive Hypercalciuria Type II

Criteria are same as for type I, except for normal urinary calcium (<200 mg/day) on the restricted diet (91–97,99).

Absorptive Hypercalciuria Type III (Renal Phosphaturia)

Diagnostic criteria are characterized as similar to type I, except for persistent severe hypophosphatemia (2.0 mg/dL or less) (Fig. 26-7) (100).

Sodium-Linked Phosphate Transporter

Low serum phosphate concentrations owing to a decrease in renal phosphate reabsorption occur in some patients with renal calcium stones and/or bone demineralization. Two different heterozygous mutations in the sodium-linked phosphate transport protein encoded by the *NPT2a* gene have been associated with this disorder (101).

Renal Hypercalciuria

Diagnostic criteria include calcium nephrolithiasis, normocalcemia, normophosphatemia, hypercalciuria on the restricted diet, elevated fasting urinary calcium (>0.11 mg/dL GF), and elevated serum PTH (Fig. 26-8) (102).

Primary Hyperparathyroidism (Resorptive Hypercalciuria)

Criteria for diagnosis include: nephrolithiasis, hypercalcemia, hypercalciuria, and high serum PTH with surgical confirmation of abnormal parathyroid tissue (Fig. 26-9) (91–94,96,103,104).

Fasting Hypercalciuria and Elevated Fasting Urinary Calcium

Calcium nephrolithiasis and hypercalciuria on the restricted diet that can be categorized into a resorptive form because of fasting hypercalciuria. Fasting hypercalciuria is further characterized by normal to suppressed parathyroid function, eliminating renal calciuria, normocalcemia, and normophosphatemia (>2.0 mg/dL).

Hyperuricosuric Calcium Nephrolithiasis (HUCN)

The diagnostic criteria for hyperuricosuric calcium nephrolithiasis (HUCN) include: calcium nephrolithiasis, hyperuricosuria (>700 mg/day for females; >750 mg/day for males), and, frequently, a low urinary pH of <5.5 (38,105,106).

Gouty Diathesis

Criteria include uric acid or calcium nephrolithiasis and low urinary pH (<5.5) in the absence of excessive gastrointestinal alkali losses (107–109) or animal protein excess. Hyperuricemia, hypertriglyceridemia, and a history of gouty arthritis may be present.

Hyperoxaluric Calcium Nephrolithiasis

Criteria include calcium nephrolithiasis and hyperoxaluria (>44 mg/day). The three forms of hyperoxaluric calcium nephrolithiasis are:

1. Enteric hyperoxaluria, defined as the presence of ileal disease (Crohn's disease, ulcerative colitis, jejunoileal bypass, or intestinal resection), or fat malabsorption with hyperoxaluria on the random and restricted diets (110–113).
2. Primary hyperoxaluria, consisting of marked hyperoxaluria (>80 mg/day) without evidence of bowel disease, high-oxalate diet, low-calcium diet, treatment with calcium-binding agents, enhanced oxalate absorption, or high doses of vitamin C (114).
3. Dietary hyperoxaluria, marked by high-oxalate diet, hyperoxaluria on a random diet, and normal urinary oxalate excretion on the restricted diet (113,115,116).

Enteric hyperoxaluria is typically associated with hypocitraturia because of intestinal loss of HCO_3, low urinary volume, and low normal urinary calcium excretion.

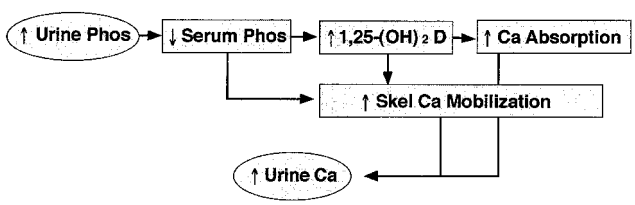

FIGURE 26-7. Renal phosphaturia. Hypophosphatemia leads to increased production of $1,25(OH)_2D_3$, intestinal hyperabsorption of calcium, and increased skeletal calcium mobilization. As a result, hypercalciuria develops.

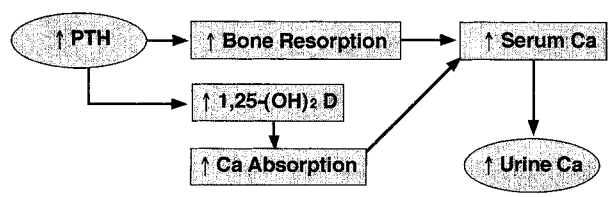

FIGURE 26-9. Pathogenesis of hypercalciuria and primary hyperparathyroidism. Excess secretion of parathyroid hormone (*PTH*) stimulates bone remodeling and elevations of $1,25(OH)_2D_3$ and calcium absorption. The resultant increase in serum calcium leads to an increase in the filtered load of calcium. The latter overwhelms the stimulation of renal tubular calcium transport by PTH, and hypercalciuria results.

Hypocitraturic Calcium Nephrolithiasis

Diagnostic criteria include calcium nephrolithiasis and hypocitraturia (<320 mg/day) [108] that compose:

1. Distal RTA, characterized by systemic metabolic acidosis or defective urinary acidification following an ammonium chloride load and urinary pH above 6.5. The acidosis is a hypokalemic, hyperchloremic nonunion-gap metabolic acidosis (116). In the complete form, metabolic acidosis is present before an ammonium chloride load, whereas in the incomplete form, urinary acidification following an ammonium chloride load is impaired despite normal serum electrolytes before the load.
2. Chronic diarrheal syndrome, defined as chronic diarrhea with excessive alkali loss from various gastrointestinal disorders (e.g., gastric resection, ileal disease, Crohn's disease, and ulcerative colitis) (117).
3. Idiopathic hypocitraturia of unknown etiology.

Hypomagnesiuric Calcium Nephrolithiasis

Criteria for diagnosis include: nephrolithiasis, hypomagnesuria (<50 mg/day) on the random diet, and absence of a diarrheal disorder (118).

Infection Stones

Criteria for diagnosis include struvite or carbonate-apatite nephrolithiasis (119).

Cystinuria

Diagnosis is based on cystine nephrolithiasis and urinary cystine level higher than 200 mg/day (120).

Low Urine Volume

Diagnosis is based on calcium or uric acid nephrolithiasis and urine volume less than 1 L/day (121).

Lack of Metabolic Abnormality

This is attributed to calcium nephrolithiasis and a normal biochemical evaluation (122).

Difficult Classification

Those that fall into this category are generally nephrolithiasis with recognized stone risk factors (123). A definitive diagnosis cannot be made because of borderline or inconsistent laboratory values or to the absence of critical data (e.g., stone analysis or roentgenographic visualization).

HYPERCALCIURIC NEPHROLITHIASIS

The majority of patients with calcium nephrolithiasis exhibit hypercalciuria (Table 26-5) and have idiopathic hypercalciuria, which is a term used to describe recurrent nephrolithiasis associated with hypercalciuria, and is probably a distinct entity. However, most of the data suggest that multiple metabolic abnormalities besides excess calcium excretion contribute to nephrolithiasis associated with hypercalciuria. Furthermore, there is a much higher incidence of idiopathic hypercalciuria than nephrolithiasis in the general population (124). Several estimates place the incidence of idiopathic hypercalciuria at 2% to 4% (56), while the incidence of nephrolithiasis is no more than 0.5% to 1.0%. Between 40% and 50% of calcium stone formers excrete excess calcium in their urine, defined as more than 300 mg/24 hour (men), 250 mg/24 hour (women) on ≥1000 mg calcium intake, or 4 mg/kg body weight/24 hour (either sex). The term *idiopathic hypercalciuria* applies if the serum calcium level is normal and sarcoidosis, RTA, hyperthyroidism, malignant tumors, rapidly progressive bone disease, immobilization, Paget's disease, Cushing's disease (or syndrome), and furosemide administration have been excluded. Virtually all normocalcemic hypercalciuria encountered in patients with nephrolithiasis falls under the umbrella of "idiopathic hypercalciuria" (88).

Idiopathic Hypercalciuria

Idiopathic hypercalciuria is an inherited syndrome. Studies of families of patients with hypercalciuric nephrolithiasis reveal a high incidence of hypercalciuria in first-degree relatives (125). The pattern of inheritance in consecutive generations with high frequency is compatible with an inherited trait with the broad characteristics of autosomal dominant transmission. This pattern of inheritance was demonstrated in large kindred (126). Hypercalciuria also occurs in children at the same rate as in adults (127). Spontaneous hypercalciuria similar to that observed in hypercalciuric nephrolithiasis of humans has been demonstrated in the laboratory rat (69,128), which has become an animal model of the human disorder.

The pathogenesis of idiopathic hypercalciuria involves excessive intestinal calcium absorption and depressed renal tubular calcium reabsorption (Fig. 26-6). The latter is largely due to suppression of PTH (129,130) and can be considered as a major factor in preventing hypercalcemia associated with increased intestinal absorption. When placed on low-calcium diets, patients with hypercalciuric nephrolithiasis often demonstrate a negative calcium balance (131). This could be because of defective renal tubular calcium reabsorption, but renal hypercalciuria should produce secondary hyperparathyroidism, which is rarely observed (88,89). In addition, considerable evidence indicates that PTH levels are suppressed and that the negative calcium balance stems from excessive skeletal remodeling and bone resorption (129,130). The question of whether depressed renal tubular calcium reabsorption greater than that expected with PTH suppression contributes to the hypercalciuria of nephrolithiasis remains unanswered. It is supported only by data from a few patients in whom secondary hyperparathyroidism has been documented (see "Renal hypercalciuria" in Table 26-5).

In hypercalciuric calcium oxalate stone formers, the initial site of calcium/phosphate crystal deposition is the basement membrane of the thin limbs of Henle's loop. There is subsequent extension to the vasa section, then the interstitium and, in the most severe cases, to the papillae. Alternatively, in patients with hyperoxaluria secondary to intestinal bypass, the initial crystals were again calcium/phosphate complex, but these arose within the tubule lumens of terminal collecting ducts. Non–stone formers, subjected to nephrectomy had neither plaque nor crystals. Thus, there are different sites of crystallization depending upon the metabolic abnormalities leading to stone formation.

In addition, in patients with idiopathic hypercalciuria, there was evidence for crystal-induced cell injury in areas of dense crystal deposition, while in the bypass patients there was not only cell injury, but also cell death (16).

The genetically hypercalciuric stone forming rats spontaneously form calcium/phosphate stones unless their diet is augmented with an oxalate precursor (132).

In the genetic hypercalciuric stone forming rats, calcium oxalate, but not calcium/phosphate, stones induce marked proliferation of the urothelium resulting in sequestration of stones (69).

Thus, rats and humans appear protected against calcium oxalate stone formation unless a nucleation site, such as the calcium/phosphate crystal is present.

Absorptive Hypercalciuria

Increased intestinal absorption of calcium is a uniform finding in patients with hypercalciuric nephrolithiasis (89) (Fig. 26-6). At issue is whether increased absorption is the primary defect or caused secondarily in the idiopathic hypercalciuric syndrome (Figs. 26-6 through 26-9). All forms of hypercalciuric nephrolithiasis are associated with increased intestinal calcium absorption. Those associated with intestinal calcium hyperabsorption on a secondary basis—renal hypercalciuria, primary hyperparathyroidism, and renal phosphaturia—are relatively uncommon forms of hypercalciuric nephrolithiasis. Furthermore, fasting hypercalciuria appears to be the expression of increased skeletal remodeling and intestinal calcium hyperabsorption together. All of this indirectly suggests that a specific problem producing intestinal calcium hyperabsorption is a major, if not the basic, underlying defect in idiopathic nephrolithiasis.

Intestinal Calcium Absorption

Net calcium absorption is the difference between the mucosal absorptive rate and the secretion of calcium into biliary, duodenal, and pancreatic fluids. While calcium absorption rates may be measured using oral radiolabeled calcium, only overall balance studies in which fecal losses are measured can quantitate net calcium absorption. The mucosal to serosal absorptive rate is higher in patients with hypercalciuric nephrolithiasis than in healthy individuals (94) (103,133–140) (Table 26-6), but overlap is extensive. In six studies, individuals with no signs of hypercalciuric nephrolithiasis absorbed an average of 27% to 52% of an oral dose of radioactive calcium, whereas those with hypercalciuric nephrolithiasis absorbed 22% to 80%. If one chooses only the six studies incorporating normal control subjects, the more efficient calcium absorption by hypercalciuric nephrolithiasis subjects is particularly evident. Increased mucosal-to-blood transport of calcium, but not magnesium, has also been demonstrated directly by *in vivo* jejunal perfusion in hypercalciuric nephrolithiasis (97). The molecular and electrophysiology of intestinal calcium transport is discussed in Chapter 89. At normal calcium intakes, <1,500 mg/day, calcium absorption in the duodenum and proximal jejunum is an active process mediated by a mucosal membrane calcium pump (ECaC) (141,142) and efficient cytosolic calcium-binding proteins (calbindin) (143), both transcriptionally regulated by calcitriol. At high calcium intakes, passive transport mechanisms in the more distal small bowel and colon may account for most of calcium absorption as the proximal active calcitriol-regulated mechanisms are suppressed.

In normal individuals, urine calcium excretion rises slowly with net absorption (144) (Fig. 26-10), and calcium balance is usually positive when the absorption rate exceeds 200 mg/24 hour. At all levels of net absorption, urinary calcium excretion was higher in hypercalciuric than in normal subjects, so much so that none of the patient data fell within the 95% confidence band derived from studies of normal individuals (Fig. 26-11). For example, in the range of 200 to 300 mg of net calcium absorption, not one of 38 normal subjects excreted as much as 300 mg of calcium in the urine, whereas 16 hypercalciuric patients did (compare Figs. 26-10 and 26-11). In other words, hypercalciuric nephrolithiasis subjects excreted in the urine an abnormally high percentage of the calcium they absorbed from the intestine. This is compatible with suppression

TABLE 26-6

INTESTINAL CALCIUM ABSORPTION IN NORMAL SUBJECTS AND PATIENTS WITH IDIOPATHIC HYPERCALCIURIA

			Dietary calcium absorbed (%)[a]	
Reference (no.)	Method	Calcium intake (mg/24 hr)	Normal subjects	Idiopathic hypercalciuria
Caniggia et al. (134)	Fecal ^{45}Ca	Free diet[b]	None studied	22.0 (1)
Birge et al. (133)	^{47}Ca, PO/IV	800	52.2 ± 13.2 (6)	58.5 ± 8.6 (4)
Wills (135)	^{47}Ca, PO/IV	400	49.0 ± 10.0 (4)	76.0 ± 17.0 (5)
Pak (136)	Fecal ^{47}Ca	400	45.6 ± 9.0 (29)	69.7 ± 7.0 (9)
				58.1 ± 13.0 (11)[c]
Pak et al. (94)	Fecal ^{47}Ca	400	50.0 ± 7.0 (20)	71.0 ± 7.0 (22)[d]
				50.0 ± 17.0 (2)[e]
Ehrig et al. (137)	^{47}Ca/^{45}Ca, PO/IV	462–952	None studied	47.8 ± 11.0 (22)[f]
				37.6 ± 11.0 (22)[g]
Kaplan (103)	Fecal ^{47}Ca	400	48.0 ± 8.0 (11)	80.0 ± 9.0 (211)[d]
				73.0 ± 7.0 (3)[e]
Shen (138)	^{47}Ca/^{45}Ca, PO/IV	Free diet[b]	27.0 ± 9.0 (14)	40.0 ± 9.0 (15)
Barilla (139)	Fecal ^{47}Ca	400	None studied	69.5 ± 6.4 (10)[d]
				70.1 ± 10.4 (8)[e]
Zerwekh and Pak (140)	Fecal ^{47}Ca	400	None studied	69.0 ± 7.0 (11)[d]
				68.0 ± 9.0 (10)[d]

[a] *Ca*, calcium; *PO*, orally; *IV*, intravenously. Values are means ± standard deviations; numbers in parentheses represent numbers of patients studied.
[b] Usual diet, but not measured.
[c] Eleven patients listed as having normocalcemic primary hyperparathyroidism may be considered hypercalciuric.
[d] "Absorptive" idiopathic hypercalciuria.
[e] "Renal" idiopathic hypercalciuria.
[f] Prior to therapy.
[g] Three to 16 months after administration of hydrochlorothiazide.

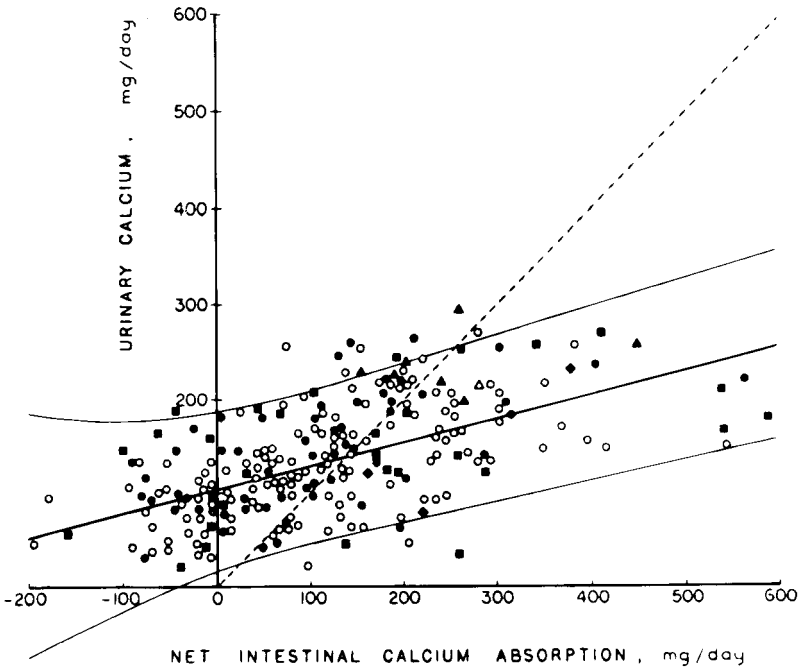

FIGURE 26-10. Urinary calcium excretion as a function of net intestinal calcium absorption. Data are derived from 6-day balance studies on 195 normal adults. Each symbol represents individual subjects from different sources: *circles*, Knapp (274); *squares*, Lafferty and Pearson (275); *diamonds*, Liberman et al. (276); and *triangles*, Edwards and Hodgkinson (277). Open figures represent women; solid figures represent men. Solid lines represent mean and 2 SD. The dotted line is the line of identity; points above the line reflect negative calcium balance. (From: Coe FL, Favus MJ. Nephrolithiasis. In: Brenner BM, Rector FC, eds. *The kidney*, 2nd ed. Philadelphia: Saunders, 1991, with permission.)

of renal tubular calcium transport rates by low levels of PTH. Net absorption rates exceeded 200 mg/24 hour in 55 normal subjects (Fig. 26-10). Urine calcium excretion was less than net absorption; that is, calcium balance was positive in 48 subjects. If a generous margin for error (50 mg/24 hr) is allowed in the balance data, none of the 55 normal individuals were in negative calcium balance. Among 37 hypercalciuric patients with a calcium absorption rate above 200 mg/24 hour, however, calcium excretion exceeded net absorption in 23 patients by more than 50 mg/24 hour (Fig. 26-11). In other words, negative calcium balance was frequent in idiopathic hypercalciuria subjects, but not in normal individuals. This is compatible with either reduced tubular reabsorption, which should produce elevated levels of PTH, or with excessive bone resorption. The latter appears most likely.

Renal Tubular Calcium Reabsorption

Two systematic studies (145,146) have evaluated overall renal fractional calcium reabsorption (Table 26-7). In both, the filtered load of calcium was calculated from inulin clearance or creatinine clearance and ultrafilterable serum calcium concentration. The fraction of the filtered calcium load excreted was calculated for several clearance periods in normal and hypercalciuric nephrolithiasis subjects. Fractional calcium excretion was clearly high in the hypercalciuric nephrolithiasis subjects. The effects of hydrochlorothiazide and acetazolamide on the renal tubular handling of sodium, magnesium, and calcium suggested to the authors a generalized defect in proximal tubular reabsorption (145–147). These studies did not examine the role of suppressed or elevated PTH levels in the subjects with hypercalciuric nephrolithiasis. The general finding of a

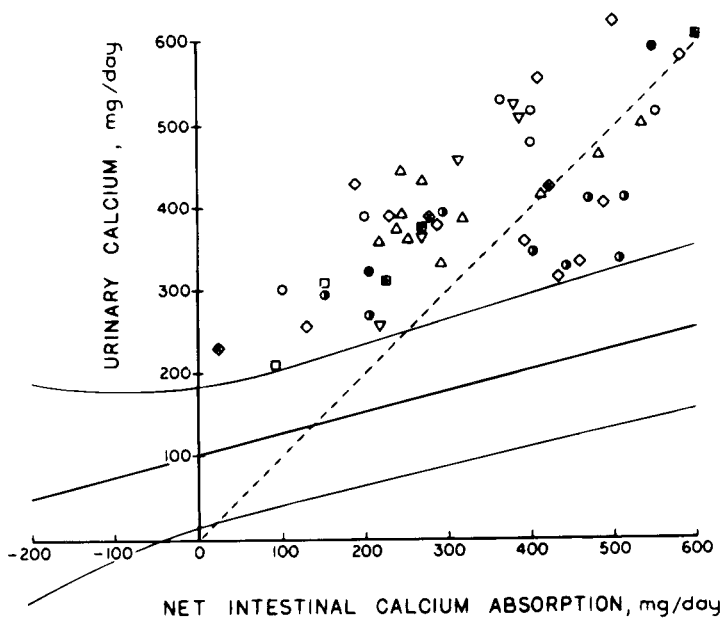

FIGURE 26-11. Urinary calcium excretion as a function of net intestinal calcium absorption from 6-day balance studies performed on 51 patients with idiopathic hypercalciuria reported as follows: *open circle*, Nassim and Higgins (278); *open square*, Henneman et al. (279); *open squares with dot in center*, Jackson and Lancaster (280); *open triangles*, Harrison (281); *open circles with dot in center*, Dent et al. (282); *open inverted triangles*, Parfitt et al. (283); *closed diamonds*, Edwards and Hodgkinson (277); *open diamonds*, Liberman et al. (276); *half-darkened circles*, Lemann (284). Solid lines represent mean and 2 SD derived from balance studies from 195 normal adults, shown in Figure 26-10. The dotted line is the line of identity, with positive calcium balance below the line. (From: Coe FL, Favus MJ. Nephrolithiasis. In: Brenner BM, Rector FC, eds. *The kidney*, 2nd ed. Philadelphia: Saunders, 1991, with permission.)

TABLE 26-7

FRACTION OF FILTERED CALCIUM EXCRETED IN THE URINE BY NORMAL AND HYPERCALCIURIC SUBJECTS[a]

	Normal subjects	Hypercalciuric subjects[b]
Edwards and Hodgkinson (145)	0.94% (7)	2.94% (14), $p < 0.001$
Peacock and Nordin (146)	1.27% (5)	4.25% (9), $p < 0.01$

[a]Number of subjects studied are shown in parentheses next to fractional excretion values.
[b]Urine calcium >300 mg/24 hr (men) or 250 mg/24 hr (women).

tendency for PTH levels to be suppressed and the rarity of secondary hyperparathyroidism call for a reexamination of the issue regarding renal tubular calcium fluxes.

A rare syndrome, X-linked hypercalciuric nephrolithiasis (XLHN) or Dent's Disease is characterized by recurrent calcium nephrolithiasis and has been found to be because of mutations in a proximal tubular intracellular vesicle chloride transport protein, CLCN5 (148–152). Two other types of hypercalciuric nephrolithiasis, which map to the same defective gene on the X chromosome (Xp11.22) as Dent's disease, X-linked recessive nephrolithiasis and recessive hypophosphatemic rickets, are associated with inactivating mutations in CLCN-5 (148).

The *CLC-5* gene is a member of a family of genes that encode voltage-gated chloride channels (149). CLC-5 is found in the kidney tubules and in bone cells. All mutations in the *CLCN-5* gene found to date have been functional, with loss of function manifested as a lowered conductance of the mutated channel. CLC-5 is distributed in the human kidney in the proximal tubule, in the thick ascending limb of the loop of Henle, and in the α-type intercalated cells of the collecting duct (149). These sites are where calcium is resorbed from the filtrate. CLC-5 knockout animals are hypercalciuric and proteinuric (150).

CLC-5 colocalizes with the vacuolar H+-ATPase in proximal tubular cells and α-type intercalated cells. CLC-5 mutations are associated with modifications in the polarity and expression of H+-ATPase, but not ultrastructural alterations in proximal tubular cells (152). The variability in diseases with the CLCN-5 mutations involves impaired solute reabsorption by the proximal tubule and range from kaliuresis, glycosuria, phosphaturia, and/or hypouricemia. Constant findings of Dent's disease include hematuria and low-molecular weight proteinuria. Dent's disease progresses to renal failure because of nephrocalcinosis and tubulointerstitial nephritis.

Thiazide diuretics reduce hypercalciuria in patients with CLC-5 mutations, but thiazides can make these patients become hypokalemic. The beneficial effect must be weighed against the potential side effect profile (151). Langman finds little reduction in stone formation even when urinary calcium is lowered to normal in such individuals (153).

Another Voltage-Gated Chloride Channel

Bartter's syndrome is a disease arising from one of three possible genes in the thick ascending limb that bear mutations in the Na^+-K^+-2Cl-gene *NKCC2*, in the K^+ channel *ROMK* or in the chloride channel *CLCNKB*. Each of these mutations produces a phenotype that includes hypercalciuria and kidney stone formation with or without nephrocalcinosis. A misense mutation in the *CLCNKB* gene leads to disease of intrafamilial heterogeneity of urinary calcium levels. Some family members have Bartter's syndrome with frank hypercalciuria, while others have hypocalciuria and a clinical phenotype of Gitelman's syndrome (154).

Hypomagnesemia/Hypercalciuria

Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) is an autosomal recessive tubular disorder that is frequently associated with progressive renal failure. The primary defect is related to impaired tubular reabsorption of magnesium and calcium in the thick ascending loop of Henle. Mutations in *PCLN-1*, which encodes the renal tight junction protein paracillin-1, were identified as the underlying genetic defects. Affected patients usually present in childhood or adolescence with symptomatic hypocalcemia (155–157). Recurrent nephrolithiasis and nephrocalcinosis are also seen and progression to renal insufficiency and an acidification defect are common. The problem with acidification has been attributed to defective ammonia transfer to the deep nephrons and impaired medullary hydrogen ion secretion because of nephrocalcinosis (158). Treatment with magnesium salts and thiazides seems to have no effect on the progression of the disease.

A second form of primary hypomagnesemia, Gitelman's syndrome, is associated with hypocalciuria. It is because of mutations in the gene encoding the thiazide-sensitive sodium-chloride-cotransporter. Since thiazides are used to treat hypercalciuric nephrolithiasis, it is important to know that they can mimic the syndrome of hypomagnesemia including hypokalemia induced by magnesuria (159).

Skeletal Remodeling

The high frequency of negative calcium balance in patients with idiopathic hypercalciuria on low calcium diets was the first indication that exaggerated bone resorption characterized the syndrome. Additional evidence for elevated skeletal remodeling in hypercalciuric nephrolithiasis has accrued. Several investigators (130,160) have documented reduced vertebral bone density in hypercalciuric nephrolithiasis by both computed tomography and dual-energy x-ray absorptiometry. Patients who exhibit fasting hypercalciuria tend to have a greater reduction in trabecular bone density than do other hypercalciuric patients, but there is significant overlap, and patients with absorptive hypercalciuria and normal fasting calcium excretion exhibit a high prevalence of reduced bone mineral density. Increased rates of skeletal remodeling with resorption favored over formation are supported by the findings of increased osteocalcin secretion and increased urinary hydroxyproline levels in patients with fasting hypercalciuria (130). The pathogenesis of exaggerated bone remodeling rates may be due to elevations in 1α,25-dihydroxycholecalciferol ($1\alpha,25[OH]_2D_3$) levels, or because of elevations in bone cytokine activity, such as prostaglandin (PG) activity (161) and interleukin-1 (IL-1) activity (130). The result of this exaggerated skeletal remodeling is an increase in calcium release to the systemic circulation and suppression of PTH secretion (162). One possibility is that exaggerated skeletal remodeling is a component of the syndrome of idiopathic hypercalciuria. Activation of skeletal remodeling

in the hypercalciuric patient results in increased skeletal remodeling, leading to the loss of a quantum of the skeleton before counterregulatory influences decrease remodeling rates, removing the component of fasting hypercalciuria from the hypercalciuric syndrome. Such a scenario is sufficient to explain the clinical picture, as we currently understand it. Greater clarification of the roles of fasting hypercalciuria, and of bone remodeling, and their pathogenesis is required in patients with hypercalciuric nephrolithiasis. The role of skeletal remodeling in nephrolithiasis was further clarified by the recent discovery of a mutation in the Type 2a, sodium-dependent phosphate cotransport gene found in the proximal tubule and osteoclasts (101).

Fasting Hypercalciuria

Except for negative calcium balance, either primary intestinal calcium absorption or a primary renal calcium leak could produce the findings summarized in Tables 26-6 and 26-7 and in Figs. 26-10 and 26-11. Primary intestinal overabsorption increases postprandial serum calcium levels above normal and increases the filtered load of calcium (Fig. 26-6). PTH secretion is reduced by the hypercalcemia and suppression of PTH secretion would reduce calcium reabsorption because PTH stimulates renal tubular calcium reabsorption (see Chapter 88, Disorders of Potassium and Acid-Base Metabolism in Association with Renal Disease). In contrast, a renal tubular transport defect (Fig. 26-7) leading to hypercalciuria would produce secondary hyperparathyroidism. PTH, in turn, would stimulate the production of $1\alpha,25(OH)_2D_3$ and produce intestinal calcium hyperabsorption. Hyperabsorption would elevate postprandial serum calcium levels, raising the filtered calcium load and decreasing the magnitude of secondary hyperparathyroidism. The only way of distinguishing one mechanism from the other is by testing specific predictions that differ in the two forms of hypercalciuria. Clinically, PTH levels are the most clear-cut basis of distinction. Fasting hypercalciuria is not a means of detecting a renal calcium leak because it can be and is caused by exaggerated bone remodeling.

Absorptive hypercalciuria is associated with low or normal fasting immunoreactive PTH (iPTH) levels. The absorptive hypercalciuria hypothesis predicts a spectrum of fasting PTH values, but it forbids the combination of elevated fasting urinary calcium–creatinine ratio and normal-to-suppressed iPTH levels. Normal PTH levels are typically observed in patients with fasting hypercalciuria and hypercalciuric nephrolithiasis. The renal model requires elevated fasting urinary calcium–creatinine ratios and a high serum iPTH level. This is seen uncommonly (Table 26-5).

On the other hand, evidence exists for suppressed PTH levels in patients with hypercalciuric nephrolithiasis who exhibit fasting hypercalciuria (162) (Fig. 26-12). When fasting hypercalciuric subjects are treated with sulindac, a nonsteroidal antiinflammatory agent, their urinary calcium excretion is decreased, but, more important, their PTH levels are increased. This suggests that a bone resorptive process is releasing calcium to the circulation and suppressing PTH. Inhibition of bone resorption by sulindac results in an increase in PTH levels, suggesting that the levels are suppressed in patients with fasting hypercalciuria (162).

Past studies attempting to detect low or normal PTH levels (94,102,138,163,164) suffer from difficulties with the radioimmunoassay for PTH. More recent double-antibody techniques that enable the measurement of intact hormone and the detection of low circulating PTH levels circumvent these problems and support the finding of low or normal PTH levels in patients with hypercalciuric nephrolithiasis.

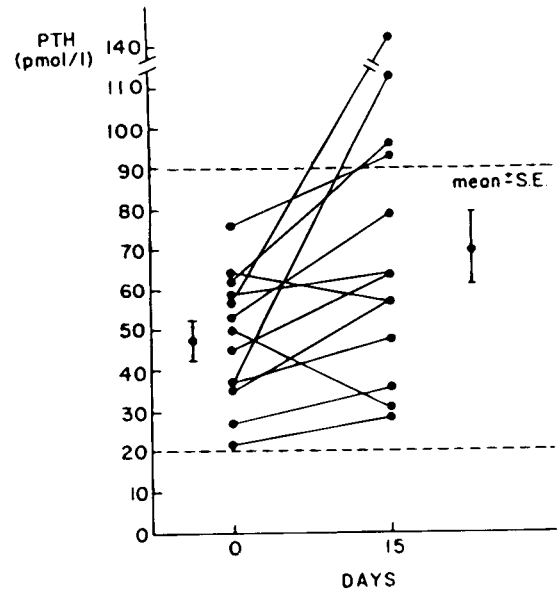

FIGURE 26-12. Changes in serum immunoreactive parathyroid hormone (*PTH*) in patients with fasting hypercalciuria after 15 days of diclofenac treatment. Dotted lines indicate the normal range of PTH. (From: Filipponi P, Mannarelli C, Pacifici R, et al. Evidence for a prostaglandin-mediated bone resorption mechanism in subjects with fasting hypercalciuria. *Calcif Tissue Int* 1988;43:61, with permission.)

Pathogenesis of Absorptive Hypercalciuria

A potential explanation for the pathogenetic process identified in absorptive hypercalciuria is abnormally elevated $1,25(OH)_2D_3$ levels. Patients with idiopathic hypercalciuria tend to exhibit elevations in $1,25(OH)_2D_3$ levels (100,103,131,138,165). The frequency of high $1,25(OH)_2D_3$ levels in idiopathic hypercalciuria is controversial, but it appears to range from 30% to 40%. Kaplan (103) demonstrates that fractional calcium absorption correlates with the serum concentration of $1,25(OH)_2D_3$. Two-thirds of the patients in this study did not have elevated $1,25(OH)_2D_3$ levels.

On the other hand, evidence exists showing that increased intestinal absorption of calcium may be primary and independent of vitamin D. Several studies indicated that the hypophosphatemia observed in idiopathic hypercalciuria is not sufficient to stimulate $1,25(OH)_2D_3$ levels (166,167). Breslau (168) used ketoconazole, an imidazole antimycotic agent (169) capable of reducing serum $1,25(OH)_2D_3$ levels by 40% in normal subjects and in patients with primary hyperparathyroidism after 1 week of therapy (170). Ketoconazole was used as a probe to investigate the pathogenetic importance of $1,25(OH)_2D_3$ in patients with absorptive hypercalciuria. Twelve of 19 patients responded to ketoconazole with a reduction in serum $1,25(OH)_2D_3$ levels, intestinal calcium absorption, and 24-hour urinary calcium excretion. In the responding patients, intestinal calcium absorption was directly correlated with serum $1,25(OH)_2D_3$ levels and 24-hour urinary calcium excretion. In seven nonresponders, a reduction in $1,25(OH)_2D_3$ produced no change in intestinal calcium absorption or 24-hour urinary calcium excretion. The authors conclude that absorptive hypercalciuria is a heterogeneous disorder composed of both vitamin D-dependent and vitamin D-independent subsets (168). The vitamin D-dependent subsets incorporate patients with elevated $1,25(OH)_2D_3$ levels, patients with abnormally responsive vitamin D receptors, and patients with allelic variations in the vitamin D receptor that have been incriminated in causing osteoporosis (171). Animal studies in the

genetically hypercalciuric rat support the possibility that an abnormal vitamin D receptor could contribute to the pathogenesis of absorptive hypercalciuria (172).

Medical Management of Hypercalciuric Nephrolithiasis

The conservative recommendations made for all patients, regardless of the underlying etiology of stone disease, are affected somewhat by the diagnosis of hypercalciuria. Measures undertaken to increase urinary output to more than 2 L/day, to avoid high oxalate and sodium intake, and to restrict animal proteins with extremely high purine levels, are generally recommended in any patient with recurrent nephrolithiasis. With these conservative measures alone, a significant number of patients are able to normalize urinary risk factors for stone formation and reduce the incidence of recurrence. After 3 to 4 months of conservative therapy, the patient needs reevaluation. If the patient's metabolic or environmental abnormalities have been corrected, the conservative approach to therapy should be considered and the patient followed every 6 months with repeat 24-hour urine testing. Follow-up is essential, not only to monitor the efficiency of treatment but also to encourage patient compliance. However, if a metabolic defect persists despite conservative treatment, medical therapy may be instituted. For example, if significant sodium-dependent hypercalciuria persists despite dietary recommendations of restricting salt intake, medical therapy with thiazides or citrate may be instituted.

The issue of dietary calcium in patients with hypercalciuric nephrolithiasis has become an important one. It is clear that adequate calcium intake is needed to maintain skeletal homeostasis during adulthood and old age. However, high calcium intakes are not tolerable in patients with hypercalciuric nephrolithiasis and absorptive hypercalciuria. Nevertheless, a low-calcium diet promotes oxalate absorption by decreasing CaOx salt formation in the intestine. In the absence of calcium, increased free oxalate anion is available for absorption. The impact of low-calcium diets favoring hyperoxaluria has been tested in a large population study from Framingham, Massachusetts (173). Curhan and co-workers (173) show that dietary calcium intake is inversely associated with the risk of kidney stones in patients who did not have a prior history of kidney stones. Thus, low-calcium diets increased the risk of the development of a kidney stone. It is important to realize that this study specifically excluded most patients with idiopathic hypercalciuria who had already had a renal stone. It is a mistake to recommend high-calcium diets in patients with hypercalciuric nephrolithiasis, especially those who have absorptive hypercalciuria.

Despite improved elucidation of pathophysiology and formulation of diagnostic criteria for nephrolithiasis of different causes, selective medical treatment programs for nephrolithiasis have not been widely implemented. Confusion and controversy regarding the pathogenesis of hypercalciuria, and the absence of an ideal therapeutic approach to absorptive hypercalciuria, have contributed to this situation. Thiazide diuretic therapy remains the mainstay of the medical approach to hypercalciuria. Treatment with thiazide is ideal, pathophysiologically, for renal hypercalciuria. Thiazides directly stimulate renal tubular calcium transport and suppress the secondary hyperparathyroidism associated with the condition. However, renal hypercalciuria, as previously discussed, is an uncommon form of hypercalciuria associated with nephrolithiasis. As idiopathic hypercalciuria is largely an intestinal overabsorption problem, an ideal treatment would be one that directly and specifically inhibits calcium absorption. Such a pharmacologic agent is not available.

Sodium Cellulose Phosphate

Sodium cellulose phosphate reduces urinary calcium excretion, especially in patients with intestinal hyperabsorption. However, sodium cellulose phosphate induces a reciprocal increase in oxalate excretion because of the binding of intestinal calcium. The resultant hyperoxaluria offsets the beneficial effects of the induced hypocalciuria. In one study, the oxalate excretion rate rose from 30 to 60 mg/24 hour (174) and the mean APR for CaOx fell only 20%, from 2.75 to 2.19. Other investigators (175) described the clinical response of recurrent CaOx stone disease to cellulose phosphate. The therapy reduced stone recurrence in patients who received cellulose phosphate to the same degree accomplished by just reducing dietary calcium intake. The latter is known to be ineffective as a single therapeutic approach. Thus, cellulose phosphate is not a treatment for the typical calcium stone former. Whereas, the rationale for its use might seem reasonable, it is flawed by the reciprocal hyperoxaluria. In addition, the high frequency of negative calcium balance in patients with idiopathic hypercalciuria must also be of concern with use of this type of agent.

Thiazides

Thiazides have been extensively used for the treatment of hypercalciuric nephrolithiasis (18,176,177). Hydrochlorothiazide is most effective in patients with renal hypercalciuria, because by reducing the high PTH levels, hydrochlorothiazide inhibits $1\alpha,25(OH)_2D_3$ production and reduces intestinal calcium absorption. Thiazides exert significant hypocalciuric action in patients with intestinal hyperabsorption without reducing intestinal calcium absorption (98). Nonetheless, thiazides may show long-term effectiveness in absorptive hypercalciuria. Despite the hypocalciuric action of thiazides and the resultant increase in serum calcium, intestinal calcium absorption remains persistently elevated. These studies suggest that retained calcium is added to the skeleton at least during the first few years of therapy. Bone density, determined in the distal third of the radius by photon absorptiometry, increases significantly during thiazide treatment, as does absorptive hypercalciuria, with an annual increment of 1.34% (178). With continued therapy, however, the increase in bone density stabilizes and the hypocalciuric effect of thiazide becomes attenuated. These results suggest that thiazide treatment causes low turnover of bone, which interferes with the continued calcium accretion in the skeleton. The resulting hypocalciuric effect of thiazide is blunted by an increase in the serum calcium level and the resultant increase in filtered calcium load (178).

In addition to the aforementioned studies, several controlled and uncontrolled studies documented the effectiveness of thiazide therapy in hypercalciuric nephrolithiasis; see reference (7) for review. Thiazides directly inhibit the sodium chloride cotransport protein for apical sodium entry in the diluting segment of the cortical thick ascending limb and the early distal nephron. This transport protein is also expressed in the osteoblast, which may further contribute to the positive actions of thiazides in patients with absorptive hypercalciuria leading to increased skeletal calcium accretion. Additional studies will be required to delineate the role of this potential mechanism in the therapeutic benefits resulting from thiazides.

Orthophosphate

Orthophosphate (neutral or alkaline salt of sodium/potassium phosphate given in doses of 0.5 g of phosphorus 3 to 4 times/day) reduces $1,25(OH)_2D_3$ synthesis. However, no convincing evidence exists that this treatment restores normal intestinal calcium absorption. Orthophosphate reduces urinary calcium by directly impairing the renal tubular reabsorption of calcium and by binding calcium in the intestinal tract. Urinary

phosphorus is markedly increased during therapy, a finding reflecting the absorbability of soluble phosphate. Physicochemically, orthophosphate reduces the urinary saturation of CaOx, but increases that of brushite. Moreover, the urinary inhibitor activity is increased, probably because of the stimulated renal excretion of pyrophosphate and citrate. The results of studies on the efficacy of orthophosphate are mixed (178–180). Although reports to the contrary have appeared, orthophosphate therapy can cause soft tissue calcification and PTH stimulation (181). Furthermore, orthophosphate therapy is contraindicated in patients with nephrolithiasis complicated by urinary tract infection and in patients with renal insufficiency.

PRIMARY HYPERPARATHYROIDISM

In the past, primary hyperparathyroidism was a major cause of hypercalciuric nephrolithiasis. The advent of routine screening of serum chemistries in hypercalcemic patients led to early detection of primary hyperparathyroidism. Consequently, most patients with primary hyperparathyroidism are detected in an asymptomatic phase before nephrolithiasis becomes a problem. Currently, primary hyperparathyroidism accounts for less than 1% of hypercalciuric nephrolithiasis (88,89,182) (Table 26-5).

The pathogenesis of nephrolithiasis in primary hyperparathyroidism is a direct response to the presence of excessive PTH levels in the circulation. PTH is the primary regulator of renal tubular calcium transport and one of the primary mechanisms regulating bone remodeling. PTH regulates intestinal calcium absorption secondarily through $1,25(OH)_2D_3$. PTH acts on the proximal nephron by decreasing phosphate reabsorption and increasing the production of $1,25(OH)_2D_3$ (see Chapter 89). In the thick ascending limb and the distal nephron, PTH directly stimulates tubular calcium reabsorption by regulating both transcriptional and posttranslational modifications of transport proteins both at the entry step in the luminal membrane and the exit step, the calcium ATPase, on the basolateral membrane. Measurement of tubular calcium reabsorption in hypercalcemic patients is technically difficult, but perhaps of a diagnostic value (183). Enhanced tubule reabsorption of calcium is responsible for normal urinary calcium excretion rates in some hyperparathyroid patients (184,185), and for the observation that for any given level of serum calcium, urinary calcium excretion is lower in hyperparathyroidism than in other nonparathyroid types of hypercalcemia (i.e., sarcoidosis and multiple myeloma).

Although PTH stimulates tubule calcium reabsorption, urinary calcium excretion is greatly elevated in primary hyperparathyroidism because of the increase in filtered calcium load (186). The elevation of circulating $1,25(OH)_2D_3$ contributes to the hypercalcemia and as a result, adds to the hypercalciuria seen in patients with primary hyperparathyroidism.

A major component of hypercalcemia stems from PTH stimulation of bone remodeling. PTH affects both bone formation and bone resorption through its direct actions on marrow cells at varying stages of osteoblast differentiation. Stimulation of osteoprotegerin ligand in the osteoblast and marrow stromal cells stimulates osteoclast differentiation.

Renal stones observed in patients with primary hyperparathyroidism are usually composed of hydroxyapatite and CaOx. Stones often recur and become bilateral if the diagnosis is not made early in the course of the disease. Nephrocalcinosis may be the only renal manifestation of hyperparathyroidism. PTH increases the APRs for CaOx and brushite mainly due to hypercalciuria. Phosphate overexcretion may be observed, but it also may be absent because chronic phosphaturia tends to cause a negative phosphorus balance. The urine pH is not abnormally alkaline in primary hyperparathyroidism, and the magnitude of the acidosis is negligible when glomerular function is normal. This suggests that altered acid-base metabolism in hyperparathyroidism does not contribute significantly to stone formation (8). Pak and Holt (25) demonstrate an unexplained reduction of the FPR for CaOx and brushite in primary hyperparathyroidism (Fig. 26-4). The diagnosis of primary hyperparathyroidism rests with repeated determination of the serum calcium and iPTH levels. The assays for circulating PTH levels using double-antibody techniques enable the detection and measurement of the intact hormone (187,188). More recently, the "intact" hormone assays have been recognized as also detecting PTH inhibitory peptides lacking the first few amino acids from the NH_2-terminus (189). Assays that specifically recognize PTH and its first amino acid have been developed and are referred to as "bioactive" PTH assays (189,190). In the diagnosis of primary hyperparathyroidism, detection of familial hypocalciuric hypercalcemia is critical. This inherited disease is produced by a mutation in the PTH gland calcium sensor such that PTH secretion is not adequately sensitive to the serum calcium levels. Affected patients may exhibit mild hypercalcemia and mild hyperparathyroidism, but do not have complications related to the hyperparathyroid state. Thus, detection of patients with the familial disease is crucial so that they are not exposed to surgical therapy for primary hyperparathyroidism. The very low urinary clearance of calcium factored by creatinine excretion is currently the most sensitive means of separating primary hyperparathyroidism from familial hypocalciuric hypercalcemia (191).

Surgical removal of adenomas or excess parathyroid tissue is indicated in patients with primary hyperparathyroidism who have sustained complications of the disease. Nephrolithiasis is one of the main complications of primary hyperparathyroidism, and any patient who has had a renal stone should undergo surgical correction of the disease state.

HYPERURICOSURIA

Hyperuricosuria can be a significant factor in the formation of calcium stones. It can also result in the formation of uric acid stones (as discussed later in this chapter in the section entitled "Uric Acid Stones") or hematuria without stones. Hyperuricosuria is defined as a urinary excretion rate of uric acid that exceeds 700 mg/24 hour in females or 750 mg/24 hour in males. In about 8% of patients with recurrent calcium stones, hyperuricosuria is the sole urinary abnormality (88,89) (Table 26-5). Furthermore, patients with gout may form calcium stones (192), which formation also suggests that uric acid plays a role in calcium nephrolithiasis. Although the serum uric acid level is higher in hyperuricosuric calcium stone-forming patients, compared to other calcium stone formers, levels may still be within the normal range (18). As 12% of those without stones may have hyperuricosuria, other unknown differences between the nonstone-forming and the stone-forming populations (105) may also exist. Nevertheless, the dramatic reduction in calcium stone formation in hyperuricosuric patients treated with allopurinol firmly establishes hyperuricosuria as a contributory agent (18,37,193).

Either of two theories may explain the mechanism by which uric acid promotes calcium stone formation. The prevailing hypothesis states that through epitaxy (194), the growth of one crystal on the substance of another, uric acid can serve as a seed for precipitation of a calcium salt. Studies have demonstrated that sodium urate accelerates precipitation of CaOx or calcium phosphate *in vitro* (35,36). This has been attributed to physiologically relevant urinary concentrations of monosodium urate (0.1 mg/mL) (35). An alternative hypothesis states that urate can complex with and thus neutralize endogenous urinary acid

mucopolysaccharides, which normally retard the crystallization of CaOx (195).

Urinary uric acid depends on both the renal filtered load of uric acid and its subsequent tubular transport. Hyperuricosuria is usually due to a high filtered load. The source of this uric acid are purines, which come from the dietary intake of purine-rich foods, endogenous synthesis of purines from nonpurine precursors, and salvage of purine bases from tissue catabolism (196). Coe and colleagues. (197) have studied the contribution of dietary purine overconsumption to hyperuricosuria in CaOx stone formers. They find that hyperuricosuric patients consume more purine than do control subjects eating isocaloric diets. Overall, a very close correlation exists between urine uric acid and dietary purine intake. Coe and colleagues (197) also find that excessive purine intake does not fully account for the hyperuricosuria, as some patients excrete more urate than normal subjects consuming equivalent amounts of purine. A purine-rich diet is also rich in protein. Gutman (198) hypothesizes that in some instances, a high-protein diet causes overproduction of uric acid, as the increase in urinary uric acid during a high-protein diet is only partially accounted for by the purine content. It is possible that abnormal tubular handling of urate resulting in hyperuricosuria and normal or low serum urate levels also occurs. Patients with such a defect and stones have been described (196). Similarly, the Dalmatian dog, which has been well studied, can have hyperuricosuria because of enhanced tubular secretion of urate (199). Although purine load is the most common mechanism for hyperuricosuria, other factors are contributory.

The treatment of hyperuricosuric calcium stone formers focuses on decreasing the hyperuricosuria. All of these patients should be administered a low-purine diet, which will decrease urinary uric acid excretion (105,197,200), although, in some patients, the effectiveness in stone prevention is unproved. A low-purine diet involves avoiding meat, fish, and poultry. Hyperuricosuric calcium stone formers may also be treated with allopurinol, which blocks xanthine oxidase, the last enzymatic step in the purine degradative pathway before uric acid is produced. Very good evidence shows that allopurinol decreases stone formation in this group of patients (18,37), including the results of a prospective controlled study by Ettinger and colleagues (193). The usual starting dose of allopurinol is 300 mg/day. A follow-up measurement of 24-hour urinary uric acid excretion after institution of allopurinol will determine the adequacy of the dose. Potassium citrate may be an alternative to allopurinol in patients with mild hyperuricosuria (600 to 1,000 mg/day) in whom hypocitraturia is present, as citrate will inhibit CaOx precipitation (201).

RENAL TUBULAR ACIDOSIS

Renal tubular acidosis (RTA) is a known risk factor for both nephrocalcinosis and calcium stones. The patients may have severe disease, as in one study by Caruana and Buckalew (202) in which the patients had a mean of 51 ± 14 stone episodes. Only the hypokalemic form of distal RTA (type I RTA) is associated with kidney stones, but the cause of the RTA may be idiopathic or secondary to systemic diseases (202). Most patients present with nephrolithiasis, a normal serum bicarbonate level but abnormal urinary acidification in response to an ammonium chloride challenge. These patients are believed to have incomplete RTA (203). The complete form of RTA is often accompanied by nephrocalcinosis. An early description by Albright (204) was of a 13-year-old girl severely affected by RTA with renal calculi, but also with rickets combined with dwarfism. The calculi may be CaOx, calcium phosphate, or a mixture of both, but the presence of calcium phosphate makes RTA a likely etiology (202,205,206). When both nephrocalcinosis and RTA appear in concert, it may be difficult to define the primary event because nephrocalcinosis can both cause and RTA or occur as a result of it. For example, when medullary sponge kidney is associated with kidney stones, nephrocalcinosis, and RTA, it is unclear which is the primary problem (207).

RTA results in hypercalciuria and hypocitraturia, both of which predispose to nephrolithiasis. Induction of metabolic acidosis with ammonium chloride results in hypercalciuria (208), which is associated with a net systemic positive acid balance with a stable serum bicarbonate level, suggesting that the acid load is buffered. Because gastrointestinal absorption of calcium does not increase with metabolic acidosis, bone is the likely source of both the urinary calcium and the buffering capacity. This is most likely the link between distal RTA and rickets (204). Furthermore, as expected, alkali therapy corrects the acidosis and the osteomalacia (209). Citrate excretion, a known inhibitor of calcium precipitation, is low in patients with RTA because of increased reabsorption (202). Simpson (210) demonstrated *in vitro* inhibition of citrate oxidation by renal cortical slices incubated in medium with a high pH. Furthermore, hypokalemia, a frequent accompaniment of distal RTA, also results in hypocitraturia due to increased reabsorption.

Distal renal tubular acidosis often presents as a familial disease. Primary distal RTA is inherited as either an autosomal-dominant or autosomal-recessive trait (OMIM179800, 276300, and 602722). Autosomal-recessive distal RTA often presents in infancy, whereas autosomal-dominant distal RTA may not present until adolescence or young adulthood (211). Patients with autosomal-dominant and -recessive distal RTA have been shown to harbor mutations in the gene encoding the chloride–bicarbonate exchanger AE1 (SLC4A1) (211–217). Mutations in ATP6N1B, encoding a kidney vacuolar proton pump protein 116-kDa subunit, cause recessive distal RTA with preserved hearing (211,218). On the other hand, mutations in the gene encoding B1 subunit of the proton pump, (ATP6V1B1) and (ATP6V0A4), cause renal tubular acidosis with sensory neurodeafness (211,219,220). Mutations in the genes encoding carbonic anhydrase (CA) II (221–223), kidney anion exchanger 1 (K AE1) (213,216–218,224), and subunits of the H+-ATPase (211,220,225) have also been identified in patients with distal RTA (211). In the familial forms of RTA, renal deposition of calcium salts (nephrocalcinosis) and renal stone formation commonly occur. Replacement of alkali corrects the systemic metabolic defects and improves the nephrocalcinosis and nephrolithiasis. Such treatment does not affect the hearing loss of the patients with recessive distal RTA.

Pseudohypoaldosteronism type II is a genetic disorder that produces a clinical phenotype that includes hypertension, hyperkalemia, and metabolic acidosis. It has been linked to mutations in the gene encoding WNK-1 or -4 and is localized to the distal nephron (226). A recent study demonstrated that in an affected family with a mutation in the *WNK4* gene, there was marked hypercalciuria and osteopenia. Thiazide diuretics completely reversed both the urine and bone abnormalities (226). Distal RTA is possible only if fasting urine pH is above 5.3. If so, an ammonium chloride challenge is necessary to prove the diagnosis. A urine pH above 5.3 after ammonium chloride-induced (100 mg/kg) acidification indicates the presence of RTA.

The treatment of RTA-associated nephrolithiasis consists of alkali therapy. Coe and Parks (227) and Wilansky and Schneiderman (228) document dramatic decreases in stone disease when patients with RTA are treated with alkali. This treatment may also result in an associated decrease in nephrocalcinosis. An oral dose of alkali is 1 mEq/kg (approximately 80 mEq/day) in four divided doses, but the dose should be

adjusted to normalize 24-hour urinary citrate excretion. The alkali is usually given in the form of citrate, which both normalizes urinary citrate (229) and decreases urinary calcium (209,228,229). Potassium citrate is preferable over sodium citrate, because potassium citrate causes a greater decrease in calcium excretion (229). By replacing buffer capacity with oral alkali, potassium citrate causes calcium excretion to fall as less bone buffers are mobilized. It is likely that sodium citrate does not decrease hypercalciuria as much as the potassium salt, because the extra sodium may have a calciuric effect. Potassium citrate is contraindicated in severe renal insufficiency, and care should be taken to avoid hyperkalemia in the presence of mild or moderate renal insufficiency.

HYPEROXALURIA

Excessive urinary oxalate excretion (>40 to 45 mg/day) contributes to stone formation by increasing the saturation of urine with respect to CaOx. Signifying the importance of hyperoxaluria, increased urinary concentration of oxalate has a greater impact than does urinary calcium on the saturation of CaOx (230). Hyperoxaluria is a relatively frequent finding in patients with kidney stones and was detected in 34% of 587 consecutive patients evaluated for recurrent nephrolithiasis at the Jewish Hospital of St. Louis Kidney Stone Center from 1987 to 1993. In 8% of these patients, hyperoxaluria was the only identifiable defect (Table 26-5) (231).

Oxalate is an end product of metabolism excreted primarily by the kidneys. Under normal conditions, oxalate is poorly absorbed from the gastrointestinal tract and only about 10% of urinary oxalate can be accounted for by dietary intake (232). Urinary oxalate excretion varies among patients and is determined by intrinsic oxalate production and metabolism, and gastrointestinal oxalate absorption.

Oxalate Production and Metabolism

Oxalate production occurs through a number of metabolic pathways, some of which remain incompletely characterized (Fig. 26-13). The oxidative metabolism of glyoxylate is a major contributor to oxalate production; in addition, ascorbic acid and tryptophan are converted directly to oxalate. Pyridoxine (vitamin B_6) is required as a cofactor for the transamination of glyoxylate to glycine. Moreover, deficiency of vitamin B_6 may result in accumulation of glyoxylate, increased production of oxalate, and hyperoxaluria (233). Disordered red cell oxalate exchange and defective oxalate transport may occur as an inherited trait and has been proposed to be a factor in hyperoxaluria within certain families (234).

Increased availability of substrate for oxalate production can occur clinically in patients taking large doses of ascorbic acid (vitamin C) and in those who ingest ethylene glycol. Metabolism of ethylene glycol results in increased production of glycolate, increased glyoxylate, oxalate formation, and hyperoxaluria. Ascorbic acid, when taken in large doses (4 to 8 g/day), may lead to marked increases in urinary oxalate (235); however, in some patients hyperoxaluria may develop with doses as small as 500 mg/day.

Primary hyperoxaluria (PHO) is a rare metabolic disorder with autosomal recessive inheritance. PHO is induced by one of two enzymatic defects, both of which result in markedly enhanced conversion of glyoxalate to poorly soluble oxalate, which is then excreted in the urine. Glyoxalate is normally metabolized in three ways. Glyoxalate is converted to glycine by the hepatic peroxisomal enzyme alanine:glyoxalate aminotransferase (AGT). AGT is abnormal in type I PHO (236). Pyridoxine (vitamin B_6) is a coenzyme of AGT. Glyoxalate

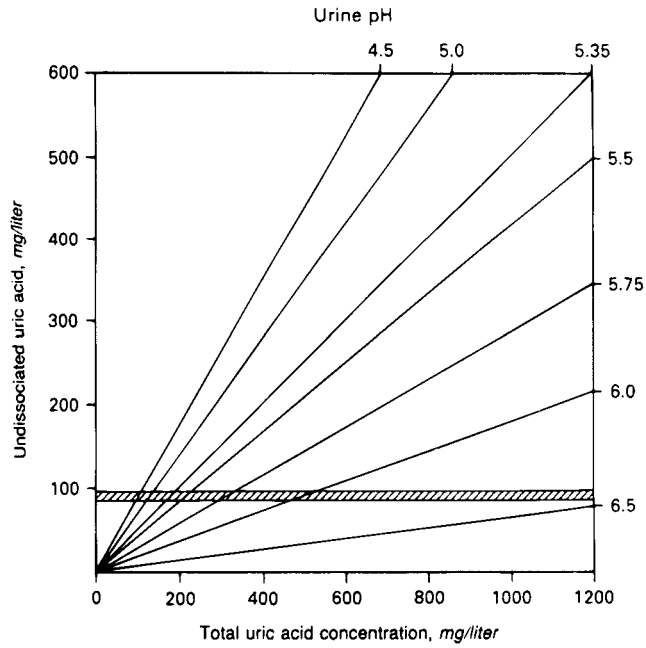

FIGURE 26-13. Metabolic pathways of oxalate production. Oxidative metabolism of glyoxylate is the major endogenous source of oxalate. Ascorbic acid (vitamin C) is also directly converted to oxalate.

is converted to glycolate by the cytosolic enzyme glyoxalate reductase/D-glycerate dehydrogenase (GRHPR). GRHPR is deficient in type 2 PHO (237). Patients with this disorder excrete increased amounts of L-glyceric acid as well as oxalate. Glyoxalate is also converted to oxalate by lactate dehydrogenase.

Type I PHO is much more common than type 2. The defect in AGT, which normally converts glyoxalate to glycine, results in an increase in the glyoxalate pool available for conversion to oxalate. The AGT gene maps to chromosome 2q36-37 and encodes for a 43-kDa protein. A number of different mutations have been identified in the coding region of the AGT gene in type 1 PHO. These defects lead to absent AGT protein and/or absent AGT catalytic activity (236). The *GRHPR* gene has been mapped to chromosome 9 and contains nine exons, spanning 9 kb (238).

In most patients with type 1 PHO, glycolate excretion is increased. Practically all patients with type 2 disease have increased excretion of L-glyceric acid. In general, hyperoxaluria plus increased urinary excretion of glycolate or L-glyceric acid is strongly suggestive, but not absolutely diagnostic, of either type 1 or 2 PHO. In addition, hyperoxaluria alone, without increased glycolate or L-glyceric acid excretion, does not exclude the diagnosis of PHO (114).

The presence of AGT deficiency can be confirmed by liver biopsy. Evaluation of the hepatic tissue includes quantification of enzymatic activity, an immunoblot to analyze the protein, and an immunoelectronic examination to demonstrate the virtual absence of AGT in the peroxisomes. AGT activity is less than 2% of normal in one-third of cases and ranges from 2 to 48% of normal in the remaining patients (237).

Phenotypically, PH1 is heterogeneous, ranging from severe infantile oxalosis and death to milder forms with renal stone disease in later life. PH2 has a less severe clinical course, but there may be a decline in renal function associated with the presence of nephrocalcinosis. Children with nephrolithiasis secondary to hyperoxaluria should have urinary glycerate measured to exclude PH2 (15).

Gastrointestinal Oxalate Absorption

Increased absorption of oxalate occurs with excess dietary oxalate intake, diminished binding of oxalate by dietary calcium and magnesium, or enhanced permeability of the colon to oxalate. Foods with a relatively high oxalate content include spinach, beets, rhubarb, asparagus, cranberries, wheat germ, colas, teas, chocolates, nuts, beans, and various green leafy vegetables. Once ingested, oxalate forms insoluble salts with available calcium (and magnesium) in the intestinal lumen and is poorly absorbed; any free unbound oxalate is available for absorption distally in the colon.

Disorders characterized by absent or dysfunctional small bowel, as well as any causes of fat or bile acid malabsorption can lead to hyperoxaluria (239). Fat malabsorption allows luminal calcium and magnesium to saponify, leaving inadequate free calcium (and magnesium) to bind oxalate. Bile acid malabsorption causes increased permeability of the colon to oxalate (240). Thus, patients with inflammatory bowel disease, those who have had ileal bypass surgery or resection, and those with disorders associated with malabsorption develop hyperoxaluria because of an increase in unbound oxalate and enhanced colonic absorption.

Patients following a low-calcium diet and those taking sodium cellulose phosphate for the treatment of absorptive hypercalciuria may also manifest hyperoxaluria as a result of such therapy. Diminished oral calcium intake (with a low-calcium diet) and binding of calcium and magnesium in the gut by sodium cellulose phosphate allow increased amounts of unbound oxalate to be presented distally in the colon where it is readily absorbed (241,242).

Therapy

Treatment of primary hyperoxaluria is difficult. Reducing sodium intake to 2 to 3 gm/day and limiting or avoiding high-oxalate foods is recommended. Supplemental citrate, magnesium, and phosphorus may help decrease urinary oxalate crystallization. Calcium intake should not be restricted because this can increase intestinal calcium absorption. Approximately 10% to 40% of patients respond to pyridoxine supplementation, but vitamin C and D supplements should be avoided. Dialysis does not remove oxalate adequately. Hepatic transplantation remains the only therapy capable of correcting the underlying abnormalities in these patients (243).

Treatment of patients with enteric hyperoxaluria should include a low-fat diet with restriction of oxalate-rich foods, appropriate therapy of any underlying gastrointestinal disorders, and avoidance of a low calcium intake. Some patients may benefit from the addition of oral calcium and magnesium supplements taken with meals, which act to bind dietary oxalate in the intestinal lumen, making it unavailable for absorption. Cholestyramine may also be of some benefit in those patients with significant fat and bile acid malabsorption as it acts as a nonabsorbable resin to bind fats and bile acids. Pyridoxine supplements may be effective in patients with moderate to severe hyperoxaluria (244).

Patients with chronic diarrhea frequently have hypomagnesemia, hypokalemia, metabolic acidosis, hypocitraturia, and low urinary volumes. For these reasons, they also are prone to the development of uric acid stones. Therapy involves increased fluid intake, correction of hypokalemia and hypomagnesemia, and oral citrate supplements. Attention must also be paid to treatment of the underlying intestinal disorder and diarrhea.

URIC ACID STONES

Uric acid stones are radiolucent stones responsible for approximately 5% of kidney stones in the United States. Other populations may have a higher relative incidence of uric acid stones as a cause of urolithiasis. Because of the difficulty in visualizing these stones on an abdominal radiograph, an intravenous pyelogram is often necessary to make the diagnosis. Stones containing some calcium may be visualized on the radiograph, which may have important therapeutic implications.

Pathogenesis

Uric acid is the normal breakdown product of purine metabolism and is a natural urinary constituent. Precipitation of uric acid to form a stone can be demonstrated best by the relationships demonstrated in Figure 26-1 (108). The solubility limit of undissociated uric acid is 96 ± 2 mg/L at 37°C. In a given sample of urine, undissociated uric acid is dependent on the total uric acid concentration and urinary pH. The clinical laboratory routinely measures total uric acid excretion, but the undissociated uric acid can be inferred from both the total uric acid concentration and the urinary pH (Fig. 26-14).

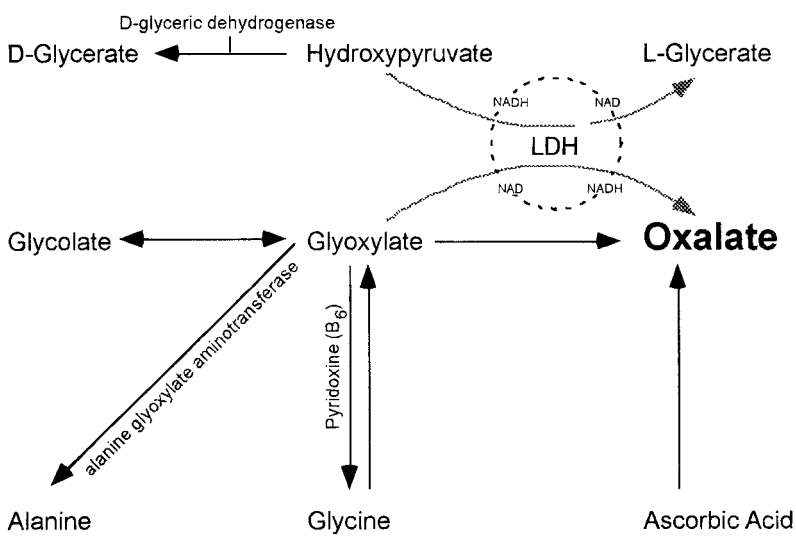

FIGURE 26-14. Nomograms showing undissociated uric acid concentration at values of urine pH and total uric acid concentration. The solubility limit for uric acid is shown by crossed hatched bars (96 ± 2 mg/L). (From: Coe FL. Uric acid and calcium oxalate nephrolithiasis. *Kidney Int* 1983;24:392, with permission.)

The total urinary uric acid concentration is a function of both uric acid excretion and urinary volume. Hyperuricosuria is defined as a urinary excretion rate of uric acid that exceeds 700 mg/24 hour in females or 750 mg/24 hour in males. When patients with gout were assessed for risk factors for stone disease, it was found that the incidence of stones increases with increasing degrees of hyperuricosuria (192). A urinary excretion rate of uric acid of more than 1 g/24 hour is associated with a 50% incidence of stones (192). Urinary uric acid excretion depends on both the renal filtered load of uric acid and its subsequent tubular transport. Hyperuricosuria is usually because of a high filtered load. As stated earlier, the source of this uric acid is mostly purines (196), usually from meat, fish, and poultry. A purine-rich diet is also rich in protein and, as Gutman (198) hypothesizes,, in some instances, a high-protein diet also causes overproduction of uric acid as the increase in urinary uric acid during a high-protein diet is only partially accounted for by the purine content. Urinary uric acid is probably not the only factor predisposing patients with gout to stone formation. This is supported by the finding that patients with gout have a threefold greater risk of stones compared to hyperuricemic control subjects who were not stone formers (245). Furthermore, most patients with gout and uric acid lithiasis are not hyperuricosuric (246).

Uric acid nephrolithiasis in patients with gout may be due to an abnormal urine pH. The precipitation of uric acid is highly dependent on urine pH within the usual physiologic range. Urate is capable of binding two protons. It is the dibasic (containing two protons) form, which is also referred to as *undissociated uric acid* and which has a low solubility in urine. The dissociation constant (pK_a) of the first proton is 5.35 at 37°C. Thus, at pH 5.0, there may be tenfold more undissociated uric acid than at pH 6.5 (Fig. 26-14). Interestingly, patients with uric acid stones (246,247) and gout (192) have lower urine pH than do control subjects. This has been attributed to an abnormality in glutamine metabolism and decreased ammoniagenesis (246), resulting in secretion of the remaining endogenous acid produced as titratable acidity. Both diet and underlying diseases can influence urinary pH and contribute to uric acid stones in the gout-free population. A high-protein diet generates an acid load that has to be excreted by the kidneys, whereas diarrhea results in bicarbonate losses that have to be regenerated by the kidney, resulting in an acidic urine.

Diseases Associated with Uric Acid Lithiasis

Among patients with uric acid lithiasis, a family history of gout or kidney stones often exists, predominantly in males. Most cases of uric acid lithiasis are idiopathic, but some disease associations should be considered when treating a patient with uric acid stones.

Primary Gout

Of patients with primary gout, 22% have uric acid stones (192). Of these stones, 83% are pure uric acid, while 4% are mixed stones, while the rest are calcium stones. Often, the uric acid stone disease antedates the diagnosis of gout. Conversely, the stones may only appear after administration of uricosuric drugs for gout. As discussed previously, uricosuric agents may treat the gout, but cause uric acid stones.

Secondary Gout

Underlying diseases that cause gout confer a higher risk for kidney stones than does primary gout (42% versus 22%). These diseases are associated with excess generation of uric acid because of nucleotide turnover (e.g., myeloproliferative disease, polycythemia because of congenital heart disease, and chronic granulocytic leukemia).

Chronic Diarrhea

Because intestinal fluid losses may result in urinary concentration of excreted urate, chronic diarrhea can result in uric acid stones. Likewise, fecal bicarbonate loss causes renal regeneration of bicarbonate with subsequent urinary acidification and more undissociated urate.

Familial Disease

Hereditary disorders associated with overproduction of uric acid include inborn errors of metabolism, such as Lesch–Nyhan syndrome and type I glycogen storage disease. Lesch–Nyhan is caused by hypoxanthine-guanine phosphoribosyltransferase (HGPRT) deficiency and Kelley Seegmiller syndrome is a partial enzymatic defect (248). Type I glycogen storage disease is secondary to glucose-6-phosphatase deficiency (249). A single gene that is located distally on the long-arm of the X chromosomes codes for HGPRT. Purification of mutant HGPRT genes in patients with partial or complete deficiency of HGPRT has lead to the identification of single amino acid substitutions in five known variants of HGPRT. Several families with inherited predispositions to uric acid stones have been described (247), though the underlying cause is unknown. A candidate gene for uric acid nephrolithiasis has been identified on 10g21-g22 from a local Sardinian population with known susceptibility to uric acid kidney stone disease (9).

Treatment

The fact that urinary uric acid concentration and urine pH can influence urate precipitation underlies the following treatment modalities.

Existing stones can be dissolved with alkali (to keep the urine pH at 6.5 or above) and a large amount of fluids to keep the urine output at more than 2 L/d. Allopurinol should also be given to reduce hyperuricosuria. Uric acid stones that contain calcium may be refractory to dissolution. Furthermore, the presence of obstruction or refractory pain may necessitate more rapid therapies such as lithotripsy or invasive urologic techniques.

Stone prevention necessitates dietary counseling and alkali therapy. Dietary modification entails keeping urine output at more than 2 L/day and reducing dietary purine intake (i.e., meat, fish, and poultry). Furthermore, patients should also reduce their protein intake to decrease both urine uric acid and the acid load. Likewise, alcohol intake should be limited because it may increase uric acid production. Alkali treatment should be given, aiming for a urine pH of 6.5. Approximately 1 mEq/kg of potassium citrate daily given in three divided doses is effective (198). Sodium bicarbonate may be a less desirable form of alkali because the sodium load may aggravate hypercalciuria, which may aggravate nephrolithiasis.

Allopurinol can decrease urinary uric acid excretion by blocking the conversion of xanthine to uric acid by xanthine oxidase. Allopurinol serves as a second-line agent used when patients either refuse treatment or diet and when alkali therapy fails. Furthermore, it may be of benefit when uric acid loads are large, such as prior to chemotherapy for large-volume rapidly growing tumors, or if the urinary uric acid excretion rate is more than 1 g/24 hour. In the treatment of hyperuricosuria, because of chemotherapy, a brisk diuresis should still be maintained as xanthine, the precursor of urate, may accumulate and cause acute renal failure, as can other products of tumor lysis.

STRUVITE STONES

Struvite stones are composed of magnesium ammonium phosphate with variable amounts of carbonate apatite. This compound forms only in the presence of chronic urinary tract infection with bacteria capable of producing urease. The action of bacterial urease on urine urea yields ammonia and carbon dioxide. These are further hydrolyzed to ammonium and carbonate, resulting in a urine pH above 7.2—ideal conditions for struvite formation. Most species of *Proteus* and *Providencia* produce urease. *Klebsiella, Pseudomonas, Serratia, Haemophilus, Staphylococcus,* and *Corynebacterium* species are all capable of urease production. *Escherichia coli* do not possess urease activity.

Struvite now accounts for less than 10% of all stones and occurs most often in women and patients with spinal cord injury, neurogenic bladder, urinary diversion, or chronic indwelling bladder catheters, because of their increased frequency of chronic urinary tract infection. Clinical findings may include evidence of urinary tract infection, hematuria, flank pain, or obstructive uropathy. Rarely, infection stones may cause xanthogranulomatous pyelonephritis. Struvite, when calcified, presents radiographically with a characteristic multilobulated shape and laminated appearance and may extend to involve all calyces forming so-called staghorn calculi.

Because struvite formation occurs in the region surrounding bacterial colonies, all struvite stone material is infected. In addition, antimicrobial agents are unable to adequately penetrate struvite and achieve bactericidal levels. Therefore, the only curative treatment is eradication of infection with antimicrobials and removal of all stone material. Combined percutaneous nephrostolithotomy and extracorporeal shock wave lithotripsy is recommended as the first-line treatment choice (250). In those patients who are not candidates for surgery, a conservative approach may be indicated. Chronic antibiotic therapy may limit stone growth and result in partial dissolution (251) in nonsurgical patients. Another potentially useful agent is acetohydroxamic acid (AHA), which is a potent inhibitor of bacterial urease and can limit stone growth (252). Despite the potential usefulness of AHA, it has been associated with frequent side effects, including potentially carcinogenic effects (253), particularly in patients with renal insufficiency. For these reasons, use of AHA should be limited to patients with normal renal function who are unable to undergo surgical intervention.

CYSTINE STONES

Cystinuria is an inherited abnormality in amino acid transport affecting the intestinal and renal epithelia. As a result of abnormal renal tubular transport of cystine, abnormally large amounts of the amino acid are excreted in the urine. The solubility of cystine in urine is approximately 300 mg/L When overexcretion leads to higher concentrations than the solubility limit, cystine stones tend to form. Biochemically, it has been known for decades that there exist different phenotypes in cystinuric populations. The disease has been differentiated by amino acid excretions in obligate heterozygotes as type I (normal urinary amino acids), type II (high excretion of dibasic amino acids), or type III (modest elevation in dibasic amino acids). However, mutations of only two genes, SLC3A1 and SLC7A9, identified by the International Cystinuria Consortium, have been found to be responsible for all three types of the disease making a case for a new classification (254). The first known gene of the cystine transporter, *SLC3A1*, was found to encode a subunit glycoprotein rBAT and thought to be responsible for type I disease. *SCL3A1* is located on chromosome 2. This gene encodes a protein that mediates sodium-independent transport of cystine and dibasic acids in the proximal tubule and small intestine. The severity of the defect in protein function and, therefore, in cystine handling varies in part with the type of mutation that is present (255). A second component of the transporter, b(o)+AT, encoded by the gene, *SCL7A9*, is thought to be responsible for type II and III (or nontype I) cystinuria (255–257). *SCL7A9* encodes an amino acid transporter that is located on chromosome 19q13.1. However, some carriers of *SCL7A9* mutations display a type I clinical phenotype (254,258). Urinary cystine levels, initial and recurrent stone formation rates, and gender differences do not segregate with the mutation in either part of the transporter protein (259).

Cystinuria frequently causes staghorn calculi or multiple large and bilateral stones. Urinary tract obstruction, infection, and renal failure are common, especially in men. Cystine stones are visible on roentgenograms, because of the density of the sulfur in the cystine molecule. Recurrence of stones is the rule following surgical treatment, including lithotripsy, of nephrolithiasis. Cystinuria should not be confused with cystinosis. The latter is a genetic disorder in which intracellular cystine accumulation leads to widespread tissue damage, including renal failure. In cystinuria, the amino acid accumulates only in the lumen of the renal tubules.

Despite molecular unraveling of cystine transport and cystinuria, it has not reached a degree of clinical significance that mandates genetic testing in affected patients. The response to tiopronin therapy by reductions in urinary cystine does not seem dependent on genotype (153).

Pathogenesis

Cystine overexcretion raises urinary cystine concentration above the limits of solubility for this relatively insoluble amino acid. Characteristic hexagonal crystals are identified in cystinuric patients, particularly in the first voided morning urine, which is concentrated and usually acidic. Normal adults excrete less than 30 mg of cystine in 24 hours (19 mg/g of creatinine) (260), whereas homozygous cystine stone formers usually excrete more than 350 mg of cystine per day (250 mg/g of creatinine). Intermediate excretion rates are observed in heterozygote patients (261). Urinary excretion of the dibasic amino acids arginine, ornithine, and lysine is also increased in patients with cystinuria. Of interest, patients with heterozygous cystinuria may have an increased risk of forming CaOx stones (262).

Amino acids are filtered and normally completely reabsorbed by the proximal tubule. Excessive urinary excretion of cystine and dibasic amino acids in cystinuria occurs with normal or subnormal blood levels, indicating the tubular reabsorption defect in the common transport mechanism for the dibasic amino acids (263–266). A similar transport defect exists in intestinal epithelial cells. After oral administration of cystine, urinary excretion of cystine does not rise, as it does in normal subjects. Urinary excretion of orally ingested arginine, lysine, or ornithine is variable and often low in cystinuric subjects. Jejunal perfusion studies showed defects in arginine, as well as cystine, absorption (267).

Treatment

Therapy is designed to reduce the excretion and increase the solubility of cystine. Methionine is the precursor of cystine and dietary restriction of methionine reduces urinary excretion of cystine (268). However, methionine is an essential amino acid and dietary restriction is, therefore, not a practical mode of

treatment. Lowering urinary cystine concentration by increasing urinary volume reduces the likelihood of precipitation and thus provides the basis for clinical treatment. An intake of more than 4 L/day may be required. Patients may excrete up to 1 g of cystine daily and the solubility limit for cystine is about 300 mg/L unless the urine pH is above 7.5. However, alkaline therapy to raise urinary pH is of limited benefit because the maximal pH is usually around 7.9 and is difficult to maintain. Use of sulfhydryl agents to produce soluble mixed disulfides instead of cystine provides the cornerstone of medical therapy. Classically, D-penicillamine has been the drug of choice until recently (269). This drug forms a mixed disulfide with cystine and reduces cystine excretion. A newer compound, mercaptopropionylglycine (Thiola), is also capable of reducing the free cystine concentration of the urine and is more efficacious than D-penicillamine, because side effects are less common (270). The principal side effects of mercaptopropionylglycine therapy are similar to those of D-penicillamine and include anosmia, loss of taste, skin rashes, and the nephrotic syndrome. More severe side effects require discontinuation of the medication. Recent interest in the use of captopril, a sulfhydryl-containing angiotensin II-converting enzyme inhibitor used to treat congestive heart failure and hypertension, has produced several reports. Results have been mixed, with some studies showing a reduction in cystine excretion (271,272). However, other investigators found captopril ineffective in reducing cystine excretion in children and adults (273). The mechanism of captopril action is unknown as its dose of approximately 100 mg/day cannot suffice to account for a 1:1 exchange for cystine and the formation of a mixed disulfide.

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SECTION V: NEOPLASMS OF THE GENITOURINARY TRACT

CHAPTER 27 ■ MOLECULAR PATHOGENESIS OF RENAL CANCER AND RELATED TREATMENT ASPECTS

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The age of molecular medicine is rapidly uncovering key events in the pathogenesis of human cancer and other diseases. This chapter is focused on critical discoveries and pathways that are presently known to affect the pathogenesis of renal cell cancer (RCC), specifically clear-cell carcinoma, papillary carcinoma, and Wilms' tumor. We also review certain key findings in kidney development that pertain to renal carcinogenesis. As is the nature of science, new findings will likely confirm, extend, or even refute current concepts. For this edition, we have incorporated a number of recent observations that reveal new mechanisms and reinforce the importance of established mechanisms. We have also included a section on treatment of metastatic clear-cell RCC, particularly those aspects that pertain to the pathogenetic process.

KIDNEY DEVELOPMENTAL PATHWAYS AND CANCER

Development of the vertebrate kidney has been studied extensively, first by biologists interested in inductive events between tissues and, more recently, by molecular biologists interested in the biochemical details of these events. It is striking that many molecular signaling pathways implicated in normal kidney development have also been implicated in cancer, either uniquely in the kidney or in other organs. Thus, normal and neoplastic development can be viewed as different aspects of fundamentally similar processes and a great deal of insight can be obtained by contrasting the two. Our understanding of molecular signaling pathways, which play critical roles during kidney development, derives primarily from gene knock-outs in the mouse, developmental expression patterns, and experimental manipulations in tissue or organ culture.

There are now hundreds of genes whose expression patterns have suggested involvement in kidney development. We make no attempt to discuss these comprehensively, but provide a synopsis of selected relevant pathways.

Morphological Events

The metanephric kidney is initiated as an evagination of the Wolffian duct into surrounding metanephrogenic mesenchyme at the duct's caudal end. The resulting ureteric bud induces mesenchymal cells, which condense into aggregates around the growing tip, proliferate, and undergo mesenchymal to epithelial transition. Peripheral mesenchyme, which remains undifferentiated, induces the ureteric bud to grow and arborize, generating more growing tips. Each tip is capable of condensing more mesenchymal cells into aggregates. Aggregated epithelial cells form the renal vesicle, a spherical cyst that undergoes subsequent shape changes to generate comma-shaped and then S-shaped bodies as it connects to the developing ureter. The S-shaped bodies ultimately form the glomerulus and tubule system of the mature nephron, while the branched ureteric bud forms the collecting ducts and ureter. This process occurs in a radial manner such that mature nephrons are formed in the central portion of the growing kidney with newer nephrons and cells in earlier stages of development at the periphery. The process of nephron development is complete in humans by 34 to 36 weeks gestation, but continues for up to 10 days postpartum in the mouse.

Signaling Pathways in Kidney Development

It has long been clear from tissue transplantation experiments that reciprocal tissue inductions take place between the ureteric bud and the surrounding metanephrogenic mesenchyme. Glial cell–derived neurotrophic factor (GDNF) is produced by uninduced metanephric mesenchyme while its receptor complex, GFR-α1, together with the receptor tyrosine kinase RET, is expressed at the growing tips of the ureteric bud. Mouse knock-outs for RET or GDNF lack kidneys (1,2) The ureteric bud produces signaling molecules that induce the metanephric mesenchyme. Two of these are WNT-11 and FGF2. Another WNT ligand, WNT-4, as well as BMP-7, are expressed by the aggregating mesenchymal cells and are required for the mesenchymal–epithelial conversion. Condensing mesenchymal cells also express WT1 and c-MET, both of which are directly involved in kidney tumorigenesis.

WT1 is the Wilms' tumor suppressor gene that encodes a zinc-finger transcription factor. Kreidberg et al. (3) found that mice homozygous for a WT1 knockout died as embryos between stages E13 and E15 with failed kidney development. The ureteric bud was absent and a high proportion of blastemal cells underwent apoptosis. Tissue culture experiments revealed that mutant metanephric mesenchyme was incapable of being induced. The receptor tyrosine kinase MET, which is the receptor for hepatocyte growth factor/scatter factor (HGF/SF), plays an important role in kidney development (4). MET stimulation induces tubulelike structures in cultured epithelial cells and in organ culture, while neutralizing antibodies against HGF/SF inhibit differentiation of metanephric mesenchymal cells (5). Importantly, activating mutations in MET lead to papillary renal carcinoma. The most frequently mutated gene in clear-cell renal cell carcinoma (RCC), the von Hippel–Lindau (VHL) gene, has no known role in kidney development. However, the murine VHL knock-out dies *in utero* at E10.5 to 12.5 because of placental vascular dysgenesis, making evaluation of kidney development difficult (6). A link between VHL and WT1 is exemplified by the fact that hypoxia induces WT1 in a HIF-1 dependent manner (7).

HEREDITARY KIDNEY CANCER

Tumor suppressor genes (TSGs) encode proteins that prevent cells from acquiring neoplastic properties, such as uncontrolled cell-cycle progression and proliferation, growth, immortalization and escape from programmed cell death (apoptosis). As first shown for the retinoblastoma gene (8), loss of TSG function is a rate-limiting event. Since most genes occur in two copies, one on each chromosomal homolog, inactivation of both alleles requires two separate events. Familial cases result, in part, from an inherited mutation in one allele with a subsequent somatic mutation eliminating the second. While still a rare event, the frequency of the second "hit" is sufficient (~1 in 10^6 cells) that individuals who inherit a mutant allele have a high risk for tumor development. On the other hand, sporadic cases result from equally rare somatic mutations in both alleles. As a consequence, bilateral disease is rare in sporadic cases, but common in familial ones. The development of both VHL disease and Wilms' tumor involves loss of TSGs, whereas papillary renal cancer involves an activation event.

CLEAR-CELL RENAL CANCER

von Hippel-Lindau Disease (VHL)

In 1904, von Hippel described progressive retinal changes in two patients and, with knowledge of other reports, indicated that the primary retinal lesion was a hemangioblastoma. In 1921, with the autopsy of one of von Hippel's patients, multiple tumors in the central nervous system (CNS), hypernephromas (RCCs), bladder papillomas, along with numerous pancreatic, renal, and epididymal cysts were observed. In 1926, Lindau, following his work on cerebellar cysts often containing small angioblastic tumors, was able to assemble enough patient data to substantiate the syndromic nature of these various lesions.

More recently, Maher and colleagues (9) reported the spectrum of disease in a series of 152 English VHL patients. Retinal angiomas and cerebellar hemangioblastomas were the most frequent initial manifestations, with a mean age of onset of 25 and 29 years, respectively. In contrast, the mean age of onset for renal cancer was 44 years. Given enough time, however, nearly all patients were predicted to develop both renal cancer and CNS vascular neoplasms. The development of pheochromocytoma, on the other hand, is related to the nature of the VHL mutation and clinically has been used to subclassify VHL patients into those with (type 2), and those without (type 1) this manifestation.

VHL disease has previously been considered as one of the multiple endocrine neoplasias (MEN) (10). Interestingly, the presence of pheochromocytoma in VHL provides phenotypic overlap with both MEN2A (medullary thyroid cancer, pheochromocytoma, and hyperparathyroidism) and MEN2B (medullary thyroid cancer, pheochromocytoma, marfanoid habitus, and neuromas of the lips, tongue, and gastrointestinal [GI] tract) (10,11). In a study of 82 patients with pheochromocytoma from Freiburg, Germany, 19% were found to have VHL syndrome while 4% had MEN2 (12). While these percentages are not representative of more heterogeneous populations, it is important to consider both syndromes in the evaluation of patients with pheochromocytoma. The causative gene in MEN2 is the transmembrane tyrosine kinase, RET, which functions as the signal-transduction component for four coreceptors, GFRα-1, 2, 3, and 4. These co-receptors bind four related ligands: glial-derived neurotrophic factor (GDNF), neurturin (NTN), artemin (ART), and persephin (PSP) in a partially overlapping manner (13,14). While activating mutations of RET are responsible for the MEN2 syndrome, loss of either RET or GDNF results in renal agenesis (1,2), and persephin is known to promote branching of the ureteric bud (15).

Using a positional cloning strategy, Latif et al. (16) were able to isolate the VHL gene in 1993. Following this breakthrough, Iliopoulos et al. (17) produced anti-VHL antibodies and found the protein had an approximate size of ~30 kDa and was localized predominantly in the cytoplasm. The hypothesis that VHL suppressed tumor formation was verified by inserting a wild-type gene in a VHL mutant renal carcinoma cell line (17). The initial clue as to VHL function came from coimmunoprecipitating proteins, which were identified as ElonginC and B (18). These proteins were known to be part of a complex with ElonginA, which suppressed the pausing of RNA polymerase II during transcription. However, VHL–ElonginC/B complexes were mutually exclusive with those consisting of ElonginA–ElonginC/B. This suggested that VHL might compete with ElonginA to inhibit gene transcription. Experiments by Kibel et al. (19) reached parallel conclusions and, in addition, demonstrated that many VHL mutations clustered in the region that interacted with the ElonginC and B complex. However, the story was just beginning.

Gnarra et al. (20) also observed that wild-type VHL inhibited *in vivo* tumor growth while having no effect *in vitro*. VHL also had no effect on the tumorigenicity of papillary renal carcinoma. These investigators hypothesized that this discrepancy might be due to the expression of angiogenic factors and demonstrated that the production of vascular endothelial growth factor (VEGF) was upregulated in VHL mutant cell lines. However, while VEGF mRNA was dramatically upregulated, this did not occur at the level of transcriptional elongation, suggesting there must be an alternative VHL function. The answer came again from coimmunoprecipitation experiments, which identified a new component, cullin-2 (21,22). At the protein level, cullin-2 was similar to yeast CDC53, which was part of a ubiquitin ligase complex. Since ubiquitination targets proteins for destruction, these results suggested that VHL might function in the control of protein stability. We now know that a major function of VHL involves targeting of specific proteins for polyubiquitination, especially HIF-α subunits, and subsequent degradation by the 26S proteosome (Fig. 27- 1).

STRUCTURE OF THE VHL, ElonginC, ElonginB (VCB) COMPLEX

Stebbins et al. (23) described the x-ray crystal structure of VHL complexed with ElonginC and ElonginB. VHL provides the targeting subunit to this E3–ubiquitin ligase complex. The prototypic pathway was discovered in yeast mutants that affected the G1/S cell cycle transition by failing to degrade the cell cycle inhibitor, Sic1. The yeast complex is known as SCF (*S*kp1, *C*dc53/Cullin, *F*-box receptor). ElonginC is highly related to Skp1 and interacts with VHL through a motif (present in VHL) known as the F-box. Cullin-2 functions as a scaffold positioning the VCB proteins on one side of the substrate and RBX1, a small protein containing a RING-H2 domain with a high-affinity binding site for the Ub-conjugated E2 (24,25), on the other side. Interestingly, the TRC8 (26) hereditary renal/thyroid cancer gene also contains a RING-H2 domain (see below) and has been implicated in ubiquitination (27).

Approximately one-half of the mutations in VHL affect its F-box-like α-helical region and disrupt interaction with ElonginC (23). The β-domain comprises the other major portion of VHL and is formed by extensive β-pleated sheets. The β-domain recruits substrates independent of ElonginC/B

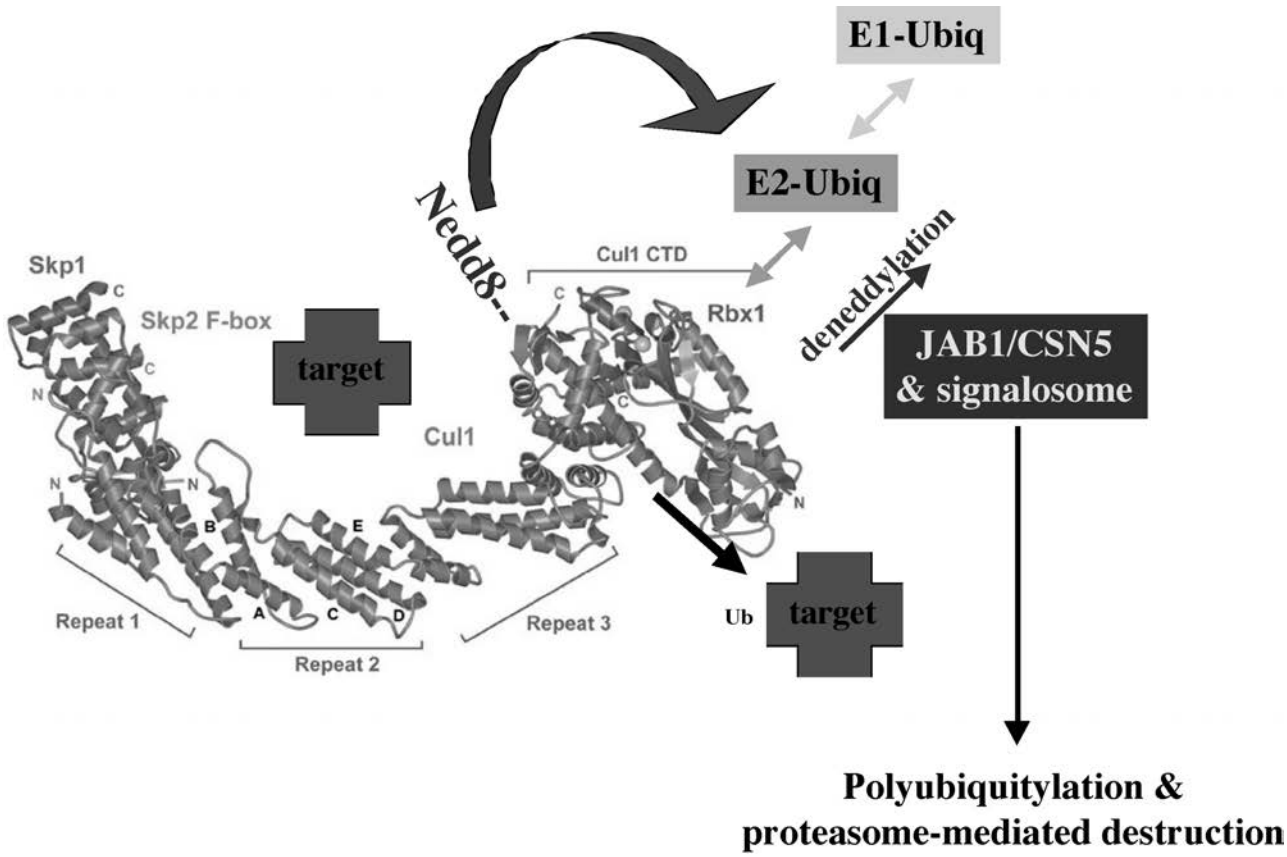

FIGURE 27-1. Anatomy of an SCF complex.

binding and is also frequently disrupted by mutations. The correct folding of newly synthesized proteins is facilitated by chaperones, multisubunit complexes containing a central cavity that function in an ATP-dependent manner. A 55-amino acid segment of VHL has been shown to bind to the chaperonin, TRiC, along with the heat-shock protein, HSP70 (28). VHL mutations involving this region interfere with TRiC binding and result in incorrect folding, as evidenced by increased protease sensitivity. Thus, VHL mutations diminish the polyubiquitination of substrates through either decreased binding of the substrate to VHL or by disrupting the binding of VHL to the ElonginC/B/Cul-2/Rbx1 complex.

E3–ubiquitin ligases are combinatorially complexed, with six cullins and multiple Skp1-related proteins. Database analyses have found a much larger number of F-box proteins. In support of this complexity, based on quantitative Western blots, there is about 1μg of VHL protein per 150 μg of ElonginB (29). The ElonginBC complex has been shown to bind a group of molecules containing a common ~50 amino acid domain in their C-terminus referred to as the SOCS box. SOCS-box proteins, so named for their ability to *s*uppress *c*ytokine *s*ignaling, are induced by various cytokines, including interleukin-6 (IL-6), erythropoietin (EPO), GH, leptin, leukemia-inhibitory factor, and others, in an apparent negative feedback loop. The SOCS-box motif is structurally similar to the F-box (23) and mediates binding to ElonginBC (reviewed in Zhang et al.[30]). Various F-box proteins are themselves unstable and undergo ubiquitin-dependent degradation (31). It has been suggested that the ubiquitin ligase core components, such as ElonginBC/Cul-2/E2 ligase constantly associate with different F-box proteins in a dynamic manner (31).

Cullins are modified by the ubiquitin-like peptide, Nedd8, on a specific lysine residue close to the RBX1 interaction site (32–38) . This modification is necessary for maximal activity of E3-ubiquitin ligases. While normal cells contain a small proportion of neddylated cullins, mutations in the COP9 signalosome (CSN) complex lead to high modification levels. The signalosome contains a deneddylase activity that appears to reside in the CSN5 subunit (39). While increased neddylation levels might be expected to constitutively activate E3 ligases, in fact, the converse is true, as this interferes with function (40). Modification by Nedd8 is thought to increase the binding affinity of cullin/RBX1 for ubiquitin-conjugated E2s. These observations have suggested a model in which neddylation and deneddylation must occur cyclically for maximum E3 ligase activity.

Consequences of VHL Mutations

The VHL protein has a substantial effect on the transcriptional regulation of many genes that function physiologically in response to hypoxia, including VEGF and erythropoietin. Renal carcinomas are particularly vascular tumors often associated with polycythemia. The link between these phenomena involves the transcription factor, hypoxia-inducible factor (HIF), which binds hypoxia response elements (HREs) present in the promoter regions of these genes. The presence of HREs, or regulation by HIF, has been shown for a variety of genes including VEGF, the VEGF receptor FLT-1, erythropoietin, glucose transporters 1 and 3, insulinlike growth factor 2, and its binding proteins, etc. (see Semenza, 1999 and 2004 for detailed reviews [41,42]). HIF is a heterodimeric transcription factor composed of an α and β subunit. HIFα protein levels are carefully regulated at the transcriptional, translational, and posttranslational

levels while HIF β is constitutive. Importantly, VHL has been shown to bind HIFα subunits and influence its stability (43).

While most E3-Ub ligases recognize polyubiquitinated target proteins after they are marked by phosphorylation, HIFα subunits are modified by proline hydroxylation to create a high-affinity binding site for VHL. Three proline hydroxylases are known, PHD1, 2, and 3, and the reaction also requires Fe(II), 2-oxoglutarate, molecular oxygen, and the cofactor ascorbic acid. Hydroxylation of the two proline residues (Pro-564 and Pro 402) on the 4 position also generates succinate as a byproduct. An asparagine hydroxylase termed FIH-1 (*F*actor *I*nhibiting *H*IF-1) modifies an interaction site for transcriptional coactivator, CBP/p300, and blocks binding. Thus, normoxia leads to inhibition of HIF transcriptional activity, as well as its degradation. Hypoxia robs the PHDs of the critical oxygen cofactor, leading to reduction or loss of activity and a corresponding increase in HIF transactivation (44). Interestingly, the iron chelator desferrioxamine also stabilizes HIFα, as does cobalt, which can replace the Fe(II) atom and block PHD activity. Both ascorbic acid and 2-oxoglutarate are intermediates in the Krebs cycle, as is the hydroxylation by-product, succinate. Thus conditions that alter levels of these metabolic intermediates may influence activities of the proline and asparagine hydroxylases and potentially upregulate HIF. This is discussed in more detail later.

While increased levels of HIF1α, HIF2α, and HIF target genes appear necessary for renal tumor development (45), recent data suggests these changes are not sufficient. Nevertheless, deregulated HIF contributes to the high vascularity characteristic of kidney tumors and likely provides an important boost to tumor growth. More insight into the possible role of VHL in tumorigenesis came from studies in model organisms. In mammalian cells, the ElonginC/B complex binds Cul-2. Disruption of the worm gene, *C. elegans cul-2*, leads to growth arrest of cells at the G1/S boundary (e.g, a checkpoint during the cell cycle, which regulates the beginning of DNA replication) (46). Cell-cycle control is mediated partly by the activities of cell-cycle dependent kinases, CDKs, and CDK inhibitors. The loss of *cul-2* leads to elevated levels of a particular CDK inhibitor and reduction of this inhibitor was shown to correct the cell cycle arrest. Loss of *cul-2* also resulted in cell division with uncondensed chromosomes and unequal DNA segregation. Thus, in the worm, defects in ubiquitin targeting can lead to alterations in the cell cycle and aneuploidy. Experimentally, overexpression of VHL in human cells leads to elevated levels of the CDK inhibitor $p27^{kip1}$ (47), which is known to be regulated by ubiquitination and degradation. Carrano and coworkers (48) showed that p27 ubiquitination is dependent on the presence of an F-box-containing protein, SKP2, the kinase activity of CDK2/cyclinE, which phosphorylates p27, and Cul-1. Moreover, the F-box protein SKP2 is limiting in the cell for this reaction to occur. Studies in *Drosophila* identified the VHL homologous gene, DVhl (49). Development of the trachea in *Drosophila* involves the migration and elongation of a set of primordial cells, which fuse and branch in a manner that is reminiscent of vascular development. Loss of VHL results in excessive branching, similar to what is observed during hypoxia in *Drosophila*. In contrast, overexpression of VHL results in blunted trachea formation, consistent with the absence of cell movement. In human RCC, VHL inhibits branching morphogenesis induced by HGF/SF (50) and inhibits matrix metalloproteinases, which degrade the extracellular matrix during migration and invasion.

VHL has also been shown to bind and regulate secretion of fibronectin, a prime constituent of the extracellular matrix (51). The importance of this interaction has been unclear, yet all tumor-associated VHL mutations tested have also dysregulated fibronectin deposition (52). Integrins, which are transmembrane proteins, bind fibronectin and other matrix components, and several of these have important roles in kidney development. The interaction with fibronectin occurs with a fraction of VHL that is localized within, or at, the endoplasmic reticulum (ER) (52). Renal carcinomas with mutant VHL are deficient in fibronectin secretion and this interaction appears to be independent of ElonginC/B. In at least one instance, a renal carcinoma cell line (ACHN) lacking a VHL mutation was shown to have a fibronectin mutation (reviewed in Ohh et al [52]). Ohh and colleagues (52) have recently discovered a VHL mutation (K159R) that fails to deposit fibronectin properly, yet retains the ability to regulate HIFα. Of interest is the fact that expression of this mutant VHL cannot block tumor formation, despite its ability to downregulate HIF. This suggests that deregulation of fibronectin may be at least as important for tumorigenicity as HIF. Moreover, specific lysine mutated is the target for modification by Nedd8.

Hereditary Renal Carcinoma and TRC8

A 3;8 chromosomal translocation, t(3;8)(p14.2;q24.1), was described in a family with classic features of hereditary RCC, that is, autosomal dominant inheritance, early onset and bilateral disease (53). The translocation and RCC segregated concordantly and a follow-up analysis reported the occurrence of thyroid cancer in two translocation carriers (54). Frequent 3p loss of heterozygosity (LOH) in sporadic RCC led to the initial assumption that a critical tumor suppressor gene would be located at 3p14. However, identification of the VHL gene at 3p25 (see above), provided an alternative explanation for at least some observed 3p LOH. Within 3p14, Ohta et al. (54a) identified the FHIT gene, which was interrupted in its 5′ untranslated region by the 3;8 translocation. The human gene, like its yeast homolog, encodes diadenosine (5′5″-P1,P3-triphosphate) hydrolase activity. Although several reports described FHIT alterations in diverse carcinomas using nested RT-PCR, other results were contradictory (55–57). In fact, most FHIT abnormalities occurred in the presence of wild-type transcripts and resulted from low-abundance splicing alterations. Furthermore, Boldog et al. (58) demonstrated that the 3p14 deletions coincided with FRA3B, the most inducible fragile region in the genome and suggested FHIT alterations might be primarily related to genomic instability.

Based on an observation from one case of a parotid adenoma in which FHIT underwent a chromosomal translocation with HMGIC, the known causative gene in a variety of benign tumors, a novel gene fused to FHIT from the chromosome 8 breakpoint, was found (26). This gene, TRC8, was found to be partially related to the *Drosophila* segment polarity gene, *patched*. *Patched* is responsible for both hereditary and sporadic basal cell carcinomas, as well as medulloblastomas (Fig. 27-2).

TRC8 encodes a predicted 664 amino acid, multitransmembrane protein. The *patched* similarity includes the second extracellular domain of *patched*, which is involved in binding sonic hedgehog, as well as its putative sterol-sensing domain. Sterol-sensing domains are regulatory motifs best characterized in SCAP, the sterol regulatory element-binding protein (SREBP) cleavage-activating protein (59). Under conditions of low cholesterol, SCAP escorts SREBP to the Golgi, where proteases cleave and activate three SREBP proteins (60). When cells are replete with cholesterol, the SREBP/SCAP complex is retained in the ER, bound to the anchoring protein, INSIG. When cholesterol levels are low, interaction between SCAP and INSIG is lost and the SCAP/SREBP complex is escorted to the Golgi for proteolytic activation. While the function of the sterol-sensing domain in *patched* is unknown, hedgehog is actually tethered to the plasma membrane by cholesterol. A

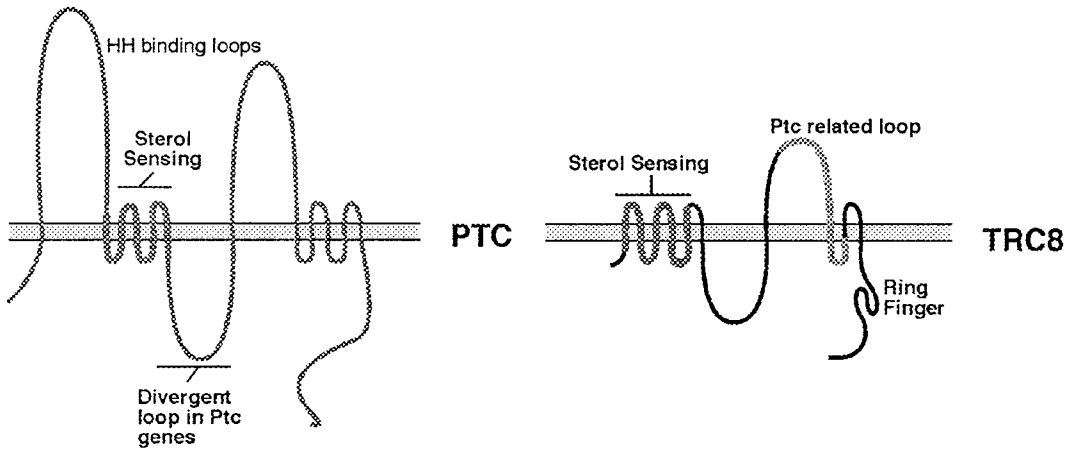

FIGURE 27-2. A schematic of the predicated TRC8 structure and patched similarities.

schematic of the predicted TRC8 stucture and patched similarities is shown in Figure 27-2.

TRC8 also contains a RING-H2 domain ($CX_2CX_{9-27}CXHX_2HX_2CX_{6-17}CX_2C$), which is known to catalyze ubiquitination reactions (61). As discussed above, the VHL–Elongin complex contains a smaller RING-H2 protein, RBX1. The *Drosophila* TRC8 homolog has been shown to function as a growth inhibitor, consistent with tumor suppressor function (62). Given the probable role of TRC8 in ubiquitination, it is perhaps not surprising that the COP9 signalosome subunit CSN5 was identified as an interacting protein (62). Moreover, selected mutations in CSN5 could restore growth to fly structures inhibited by TRC8 (63). *In vitro* growth inhibition has now been observed in mammalian cells expressing wild-type TRC8, but not when mutations in the RING-H2 domain were present (A. Brauweiler, H. Drabkin and R. Gemmill, unpublished).

Tuberous Sclerosis (TSC)

Tuberous sclerosis (TSC) is a dominantly inherited disease marked by the formation of hamartomas in different organs, including the brain, kidney, and skin. The incidence is 1 in 5 to 10,000 live births (64) with about one-third of cases being familial (65). The disease is caused by mutations in either TSC1 (hamartin) or TSC2 (tuberin) (66,67). Loss or reduction in the function of the hamartin–tuberin complex in patients with TSC leads to unregulated mTOR signaling (68,69). In the kidney, angiomyolipomas are the most common manifestations of TSC, although benign cysts and lymphangiomas occur. In addition to benign lesions, clear-cell RCC may develop in 1% to 2% of adult patients with TSC.

Angiomyolipomas are composed of varying amounts of mature smooth muscle, adipose tissue, and blood vessels (70). The natural history of the renal manifestations of TSC was evaluated in a series of 59 patients (mean age 11.4 years), who were prospectively followed by different imaging studies (71). Angiomyolipomas were present in approximately 80% of patients, commonly being numerous and bilateral. In patients with normal baseline imaging studies, renal lesions (angiomyolipomas or renal cysts) presented at an average age of 9 years. It has been suggested that lesions <4 cm in diameter can be monitored with periodic ultrasonography. Angiomyolipomas >4 cm can become symptomatic and require intervention (72,73). Routinely, renal-sparing approaches are preferred, while total nephrectomies are reserved for patients with nonfunctioning kidneys causing uncontrolled hypertension or other complications, as well as for tumors suspected of being malignant (72).

PAPILLARY RENAL CANCER

Papillary renal tumors account for between 5% to 20% of renal carcinomas (74,75). In addition to spontaneous tumors, papillary renal carcinomas can be hereditary. In contrast to the frequent loss of chromosome 3p seen in clear-cell carcinomas, papillary tumors are characterized by a gain of chromosomes 7, 17, 16, and loss of the Y chromosome in males. From genetic linkage studies in papillary renal carcinoma families, a large 27 cM region of chromosome 7 was identified as containing the responsible gene. Because of the increased copy number of chromosome 7 in papillary tumors, Schmidt et al. (76) considered that the causative gene might be an oncogene instead of a tumor suppressor gene. Using candidate genes localized with the 27 cM interval, the MET oncogene, a receptor tyrosine kinase, was shown to contain activating mutations in affected family members. Mutations were also seen in nonhereditary papillary tumors, although their frequency was low (3/60) (76). Moreover, the extra copies of chromosome 7 that occur in papillary renal carcinomas were shown to contain the active mutant MET gene (77).

Morphologically, papillary renal cancer can be classified as type 1 and type 2. Type 1 tumors are composed of small cells organized in single layers, while type 2 tumors have larger cells and pseudostratification (78). These pathologic subtypes correlate with differences in molecular pathogenesis and outcome (79). Germ-line activating mutations in MET are found in patients with hereditary papillary renal cancer type 1. Interestingly, MET is also a HIF target gene (80) and clear-cell RCCs with VHL mutations are hyperresponsive to hepatocyte growth factor (50). However, MET mutations have not been described in clear-cell RCC, although tumors with mixed clear-cell/papillary features occur (78).

A second type of hereditary papillary renal cancer occurs in the leiomyoma renal cell carcinoma syndrome (HLRCC), characterized by cutaneous and uterine leiomyomas, together with type 2 papillary RCC. In this disorder, papillary renal cancer can be aggressive with early metastases. Germ-line mutations of the Krebs cycle component, fumarate hydratase, have been identified in affected individuals (81). Interestingly, mutations in another Krebs cycle enzyme, succinate dehydrogenase (SDH), are responsible for some forms of hereditary

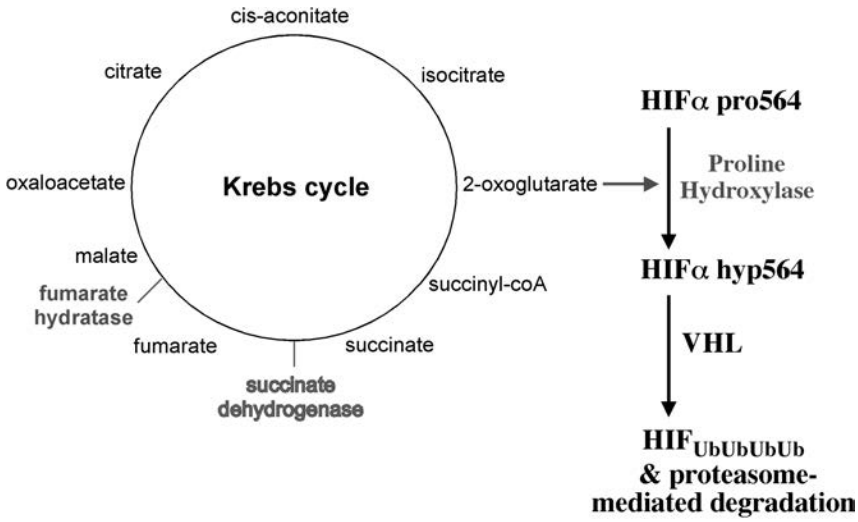

FIGURE 27-3. Krebs Cycle.

paragangliomas and pheochromocytomas, tumor types seen in the VHL syndrome (82). Even in apparently nonhereditary cases of paraganglioma/pheochromocytoma, mutations in SDHB or SDHD subunits were identified in ~14% with the frequency about equal between the two genes. However, while mutations in the SDH-D subunit are associated with benign tumors, about one-third of tumors arising in individuals with SDH-B mutations are malignant (83). Moreover, early-onset clear-cell RCC has been reported in some of these individuals (84).

SDH converts succinate to fumarate and fumarate hydratase converts fumarate to malate (85). Thus, two consecutive enzymes in the Krebs cycle are linked to renal cancer, paragangliomas, and pheochromocytoma. As shown in Figure 27-3, 2-oxoglutarate is an intermediate in the Krebs cycle and is used by prolyl hydroxylases to modify HIF, making it a higher affinity substrate for VHL (86–88). Succinate dehydrogenase (SDH) converts succinate to fumarate and mutations in the D-subunit, SDHD, lead to elevated levels of succinate, which inhibit prolyl hydroxylase activity (89). In addition, DNA damage by free radicals has been reported in a SDH-C mutation model (90). Although the cellular phenotype of fumarate hydratase mutations in cases of HLRCC has not yet been described, it is possible that there are similar alterations involving either HIF or DNA damage (Fig. 27-3).

Overall, when confined to the kidney papillary, RCCs have a better prognosis than the clear-cell subtype. In a review of 1985 patients with sporadic clear-cell versus 270 patients with papillary carcinoma without metastatic disease, the 5-year survival was 87.4% for papillary, but only 68.9% for clear-cell carcinoma (91). In the metastatic setting, however, papillary RCCs may have a worse prognosis (92). Currently, there is no standard treatment for metastatic papillary RCC and objective responses to immunotherapy are uncommon. However, *in vitro* data suggest that therapy directed against MET may be a promising strategy for type 1 cancers (93). Taken together, these data suggest that papillary and clear-cell cancers share at least some features, including MET and HIF deregulation, while survival with metastatic disease and responses to immunotherapy differ.

Other molecular alterations have also been identified in hereditary papillary renal cancers. From cytogenetic studies, a recurrent translocation between chromosomes X and 1 was identified (75), and a basic helix-loop-helix type of transcription factor, TFE3, was shown to be involved. This rearrangement fuses an intact TFE3 gene downstream of the PRCC gene. The normal TFE3 gene is lost in tumors (94). The normal PRCC gene encodes a nuclear protein and compared to normal TFE3, the PRCC-TFE3 fusion has enhanced transcriptional activity (95). The N-terminal region of PRCC has been shown to interact with a mitotic spindle checkpoint protein, Mad2B, stimulating translocation of Mad2B into the nucleus (96). The PRCC/TFE3 fusion appears to block this effect in a dominant negative fashion.

WILMS' TUMOR

Wilms' tumor or nephroblastoma is an embryonal neoplasm originating from undifferentiated metanephric mesenchyme. Affecting about 1 in 8 to 10,000 children, Wilms' tumor is frequently triphasic, containing blastemal, stromal, and epithelial cell components (97). However, as all three of these cell types originate from the condensing metanephric mesenchyme of the developing kidney, finding them together in a single tumor is not surprising. Development of Wilms' tumor requires loss of activity for the tumor suppressor locus, WT1, which encodes a transcriptional regulator. However, while loss of WT1 activity is necessary, it is not by itself sufficient. Clusters of preneoplastic cells called *nephrogenic rests* are frequent in the kidneys of Wilms' tumor patients (98). These poorly differentiated cells are believed to represent precursors to Wilms' tumor. Nephrogenic rests were shown by mutation analysis to contain the identical somatic mutation, as the accompanying Wilms' tumor in two cases (99), demonstrating their clonal relatedness and suggesting that mutations of WT1 constituted an early lesion. Wilms' tumors occur both sporadically and in a heritable fashion (100). Familial cases constitute about 1% of all Wilms' tumors; tumor risk is inherited in an autosomal dominant fashion with variable penetrance and expressivity. Like most tumor suppressor genes associated with familial cancers, the risk for tumor development is dominant, but the mechanism for generating neoplasia is recessive at the cellular level, requiring loss of both alleles.

FUNCTION OF WT1

The protein encoded by WT1 is shown schematically in Figure 27-4. WT1 contains a DNA binding domain made up of four

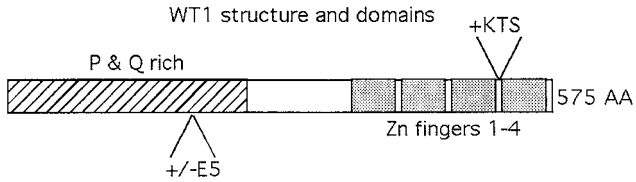

FIGURE 27-4. Wilms' tumor 1 (WT1) structure and domains.

zinc fingers of the Cys2– His2 class together with a praline- and glutamine-rich domain that can regulate transcription. Mutations found in Wilms' tumors either eliminate the protein entirely or disrupt the zinc-finger domain and, thus, interfere with DNA binding. The zinc fingers consist of polypeptide regions, which can coordinate Zn^{2+} ions, stabilizing their structure, and holding key amino acid side chains into specific configurations. Zinc fingers have been found in many transcription factors where they function to hold the DNA interaction domain in a specific configuration necessary for binding. A developmental abnormality of the genitourinary tract called Denys-Drash syndrome results from dominant negative mutations in WT1 (101,102). Denys-Drash patients have severe urogenital aberrations, renal failure, pseudohermaphroditism, and Wilms' tumor. These mutations predominantly affect the third zinc finger (occasionally the second) of WT1, preventing the protein from binding DNA. Kidney abnormalities resulting from dominant negative mutations support the critical role of WT1 in kidney development identified by murine knock-out studies previously mentioned.

There are two alternatively spliced sites in the WT1 pre-mRNA that give rise to four distinct WT1 transcripts and four distinct protein products. Transfection of a Wilms' tumor cell line with constructs expressing these four variants demonstrated that all could suppress growth (103). Most experimental attention has been focused on the two WT isoforms, which either contain or lack a three amino acid motif consisting of lysine, threonine, and serine (KTS). These three amino acids are inserted into a linker region between zinc fingers 3 and 4 and, as a consequence, alter the interactions between WT1 and its DNA binding site (104). The −KTS form generates a more stable interaction with its DNA binding site, while insertion of KTS disrupts important interactions between the linker region and the adjacent zinc fingers, lowering the stability of the complex. These differences translate into altered nuclear localization for the two variants. The −KTS form is distributed diffusely throughout the nucleus (105), while the +KTS variant resides in nuclear speckles, as do WT1 mutant proteins with a disrupted zinc finger domain. While it has been suggested that the +KTS form participates in pre-mRNA splicing, its speckled location was distinct from that of the essential splicing factor, SC35, questioning this role. Coexpression of the −KTS form with truncated mutant WT1 protein led to their physical association and an altered subnuclear compartmentalization. This suggests that dominant negative WT1 proteins may alter WT1 (−KTS) function by changing its subnuclear localization.

WT1(−KTS) binds to both GC-rich and TC-repeat elements in a variety of promoters and represses transcription, at least in transient transfection assays (106). Genes whose expression is known or suspected of being repressed by WT1, include Pax-2 (107), insulinlike growth factor 2 (IGF-2), the IGF receptor, platelet-derived growth factor A, EGF receptor, TGFβ, BCL2, c-MYC, and WT1 itself (108), among others (109). Several of these genes are known to be critical factors in kidney development, and at least Pax2 is upregulated in WT1 mutant Wilms' tumors. The repression of these genes has led to the broad hypothesis that WT1 functions as a transcriptional repressor to block growth-promoting genes and, thereby, allow differentiation. In addition, it appears necessary for cell survival by blocking apoptosis. However, not all target genes are repressed by WT1. The cell surface proteoglycan syndecan-1 was shown to be transcriptionally activated by WT1 (109). This observation takes on added significance as syndecan-1 expression is induced at the time of epithelial differentiation of metanephric mesenchyme, and its loss is correlated with malignant transformation and loss of differentiation. The view of WT1 as a general transcriptional repressor was challenged further by Lee et al. (110). They pointed out that many genes, which possess WT1 responsive elements in their promoters and are transcriptionally repressed in transient transfection assays, are not regulated by WT1 *in vivo* (110). Moreover, WT1 can either repress or activate GC-rich promoters depending upon experimental conditions. Using expression profiling coupled with tetracycline-regulated expression of WT1 (−KTS) they found the gene encoding amphiregulin to be strongly induced (110). A ligand for the epidermal growth factor receptor, amphiregulin is known to induce proliferation of some epithelial cells and yet inhibit growth of many cancer cell lines (111). Amphiregulin expression was coincident with WT1 in differentiating structures within the nephrogenic zone of the kidney. Condensed mesenchyme or blastemal cells surrounding the ureteric bud showed low-level expression for both genes, while developing glomeruli had high levels, particularly in the podocyte cells. Mature glomeruli had little to no expression of either gene. Importantly, recombinant amphiregulin was capable of inducing branching morphogenesis in cultured murine kidney rudiments. The results of Lee et al. (110) confirm that WT1 can function as a transcriptional activator on a physiologically relevant target and suggest that repression of gene expression is only a part of the picture. Additional genes activated by WT1 include Müllerian-inhibiting substance (MIS), which is upregulated by WT1 (−KTS) in conjunction with steroidogenic factor-1 (SF1), during sexual development (112). Sex reversal (SRY) is also a target (113,114). Transfection of WT1 can increase expression of E-cadherin, an important cell-adhesion molecule that is induced in condensing mesenchyme at about the same time as WT1 expression (115). Anti-apoptotic effects of WT1 may result from its upregulation of Bcl2 (116).

The absence of kidneys in WT1 mutant mice has been described above. Besides kidney agenesis, $WT1^{-/-}$ embryos had abnormal gonadal development with total loss occurring by day E14. This feature is consistent with the genitourinary defects observed in WAGR and Denys-Drash syndromes. Recent findings have demonstrated the upregulation of WT1 mRNA and protein in hypoxic heart (and kidney) tissues in a HIF-1 dependent manner (7). Thus, remarkably, WT1 is a target gene of the HIF system forming a potential connection between the deregulated events in clear-cell renal carcinoma and Wilms' tumor.

TREATMENT OF RENAL CELL CARCINOMA

Metastatic renal cell carcinoma is notoriously resistant to conventional chemotherapy and is currently an incurable disease. However, new biologic agents are beginning to break the resistance barrier. Following a brief discussion of conventional chemotherapeutic agents, we review the use of immunotherapy in RCC, then move to anti-VEGF, anti-EGFR, as well as proteasome and mTOR inhibitors, before considering the multikinase inhibitors, which are generating considerable enthusiasm in the treatment of RCC.

CONVENTIONAL CHEMOTHERAPY

Many conventional cytotoxic agents have been tried in metastatic RCC with uniformly poor results (average response rate of 6%). Vinblastine and 5-FU appear to be the most active (117). Newer agents, including Gemcitabine, Irinotecan, Oxaliplatin, Paclitaxel, Carboplatin, and Ixabepilone, are being tested alone or in combination in Phase II trials. Recently, the combination of Capecitabine and Gemcitabine produced a 15.8% partial-response rate in 21 patients with metastatic RCC, although no complete responses were observed (118).

IMMUNOTHERAPY

RCC has been one of the malignant diseases where presumed stimulation of the immune system, either by removing the primary tumor or through treatment with IL-2, interferon or stem-cell transplantation (to induce a graft versus tumor effect) has repeatedly produced beneficial results.

Interleukin-2 (IL-2)

Reports of rare, but recurrently demonstrated spontaneous remissions, in RCC suggested that the immune system, in some circumstances, was able to recognize cancer cells as non-self and organize an effective response (119). In the early 1980s, such observations triggered a search for therapeutic agents with potential to improve the immunologic response against tumor cells. Basic research in T-cell stimulating molecules led to the discovery of interleukin-2 (IL-2) (120). IL-2 is naturally produced by TH-1 lymphocytes and normal CD4 and CD8 T-lymphocytes respond to IL-2 by activation and proliferation, enhancing the ability of these cells to recognize antigens and amplifying the population of cells capable of causing direct cytotoxicity. Initial attempts to use IL-2 as an anticancer agent were generally unsuccessful, except for responses in melanoma and renal cell carcinoma (121). Early trials were based on *in vitro* stimulation of T-cells with IL-2 to induce the formation of lymphokine-activated killer cells (LAK cells), which were administered with high-dose IL-2.

Patients exposed to high doses of IL-2 develop fever, rash, flulike symptoms and, most concerning, a capillary-leak syndrome, characterized by lung edema and hypotension requiring volume support and vasopressors (122). Once established, such syndromes can be complicated by infection, renal failure, and acute coronary events. Although toxic, the effect of high-dose IL-2 in metastatic RCC can sometimes be spectacular. However, in a different series, only about 15% of the patients achieved an objective response, and about 5% of patients obtained a complete response, which could be long-lasting (123).

With more experience in IL-2 therapy, the exclusion of high-risk patients has resulted in a marked reduction in lethality (124). The addition of LAK cells to IL-2 also increases the complexity and cost of treatment, without a clear benefit. One major randomized trial compared high-dose IL-2 with or without LAK cells. In patients with RCC, there was no significant difference in activity between arms (125). Subsequent trials using low-dose IL-2, with or without INF-α, have produced fewer overall complete responses, which were usually short-lasting (126).

The importance of dose intensity of IL-2 for patients with metastatic RCC was clarified in a NIH trial that randomized patients to receive high-dose IL-2 (156 patients) or a dose that was 10 times lower (150 patients). The trial design was subsequently amended (after 117 patients) to include a third arm of low-dose subcutaneous IL-2. All patients had clear-cell histology and a favorable performance status. Although there were no treatment-associated deaths, morbidity was higher with high-dose IL-2. There was also a significantly higher response rate with high-dose IL-2 compared to low-dose IV IL-2 (21% versus 13%, $p = .048$), but no overall survival difference. High-dose IL-2 was also superior to subcutaneous IL-2 with response rates of 21% versus 10%, $p = .033$ (127).

Recently, the results of a multi-institution Phase III trial involving 192 patients with metastatic RCC and good performance status was reported (128). Patients were randomized to receive intravenous high-dose IL-2 or low dose, subcutaneous IL-2 plus INF-α. The response rate was significantly higher in patients treated with high-dose IL-2 (23.2% versus 9.9%, $p = 0.018$). While there was no significant difference in overall survival (17 versus 13 months, $p = 0.211$), patients with prior nephrectomy ($p = .04$) or bone or liver metastasis ($p = 0.001$) appeared to benefit. As expected, there were more severe grade 3 and 4 toxicities in the high-dose IL-2 arm, although treatment-related mortality was rare and not different. Based on these reports, we conclude that high-dose IL-2 may be the best option for younger patients with good performance status, who do not have access to a clinical trial, particularly if they have bone or liver involvement.

Interferon

Interferons provide primary defenses against infectious organisms, particularly viruses. Interferon-alpha (INF-α) is naturally produced by macrophages and lymphocytes and induces several biological effects including immunomodulation, antiproliferation, and enhanced of expression of cell-surface antigens. In phase II studies, recombinant INF-α was reported to induce responses in RCC in up to 29% of cases (129). However, in contrast to IL-2, INF-α has no curative potential and complete responses are rare and of short duration. In a randomized trial comparing INF-α with medroxyprogesterone acetate, INF treatment was associated with an improved survival, although the benefit was minimal (median survival of 8.5 versus 6 months), and patients treated with INF-α had more symptoms and worse quality of life (129a). INF-α can be self-administered subcutaneously. Most patients experience a flulike reaction with low-grade fever, myalgia, arthralgia, and headache. Other side effects including alopecia, depression, rash, autoimmune phenomenon, and cytopenias are much less frequent (129a).

Stem-Cell Transplantation

In leukemia, an important aspect of stem-cell transplantation involves graft-versus-leukemia effects. Based on this observation, and responses of RCCs to immunologic manipulations, attempts have been made to achieve graft-versus-tumor effects using nonmyeloablative, allogeneic stem-cell transplantation. In an initial report of 19 patients, 3 obtained a complete response, and 7 obtained a partial response. Two patients, however, died of transplant-associated events (130). However, a recent report failed to show similar benefit (131). Considering the complexity and treatment-associated toxicity, allogeneic stem-cell transplantation remains an experimental approach for RCC and should be performed in the context of a clinical trial. Other approaches, such as vaccines prepared from the individual's tumor, also remain experimental.

ANTI-VEGF THERAPY

Bevacizumab

At least one-half of clear-cell RCCs have upregulation of HIF-1/2α and HIF target genes including VEGF (131). Bevacizumab (rhuMAB VEGF [Avastin, Genentech, San Francisco, CA]) is a humanized recombinant anti-VEGF antibody that binds all forms of VEGF-A isoforms (with an affinity of ~1.8 nM) and neutralizes their activities (132). Despite *in vitro* absence of antiproliferative activity against primary cancer cell lines, *in vivo* tumor growth of MDA-MB435 breast cancer or A673 rhabdomyosarcoma cells in nude mice was inhibited by 95% following treatment with bevacizumab.

Bevacizumab was subsequently tested in Phase I trials in patients with advanced malignancies. Among eight advanced RCCs, minor tumor shrinkage was noted in two cases and stable disease for at least 2 months was observed in five others (133,134). In a Phase II trial of 116 patients with metastatic clear-cell RCC randomized to placebo, low-dose (3 mg/kg) or high-dose (10 mg/kg) bevacizumab every 2 weeks, there was a 10% objective partial-response rate, which was confined to the high-dose arm (135). Compared to placebo, there was also a significant prolongation in the time to disease progression (4.8 versus 2.5 months; $p<.001$ by log-rank test) and even by 8 months, 30% of these patients had not progressed compared to 14% and 5% treated with low-dose bevacizumab or placebo, respectively. At the time of the report, four patients were still undergoing treatment in the absence of disease progression for 3 to 5 years (6). Two patients, who discontinued therapy after 2 years, had subsequent disease progression. Both responded after the reinstitution of bevacizumab and have remained stable on treatment for an additional 3 to 3.5 years. Two other patients remained stable while undergoing protocol therapy, but then subsequently received 10 mg/kg under compassionate use circumstances. Both remained stable and continued to take bevacizumab for a total of >4 years (136). Some of these patients have experienced substantial proteinuria (>3.5 g/day), although other aspects of renal function remained normal.

Thalidomide

Thalidomide, a previously banned agent because of fetal limb developmental abnormalities (phocomelia) when taken during pregnancy, has found new uses in oncology, particularly multiple myeloma and other solid tumors (137). While there is an antiangiogenic effect, its mechanism of action is unclear. Downregulation of COX-2, decreased VEGF-induced endothelial cell activation, reduced expression of TNF-α and inhibition of IL-6 have been reported (138; 138a; 139). Hansen et al., as well as others (140), have shown that thalidomide induces oxidative stress in susceptible species with impairment of the transcription factor, NF-κB, and its downstream target genes including fibroblast growth factors. These results appear to fit well with the role of NF-κB and FGFs in multiple myeloma, and the ability of FGFs to induce VEGF production (141). In several Phase II studies, partial responses to thalidomide were observed in up to 10% of cases (138). Increased doses of thalidomide were toxic, with somnolence, constipation, peripheral neuropathy, and increased incidence of thrombosis (138,142). Thalidomide combined with INF-α produced partial responses in 3/14 patients (21%) (142a). Among 37 patients treated with thalidomide and subcutaneous IL-2, there was 1 complete and 14 partial responses (response rate of 41%) with tolerable toxicity.

EGFR INHIBITORS

The EGF receptor (EGFR) belongs to the ErbB family of tyrosine kinase receptors, which is comprised of EGFR itself (ErbB1/EGFR/HER1), ErbB2 (HER2/*neu*), ErbB3 (HER3), and ErbB4 (HER4) (143–145). Overexpression of the EGF receptor (EGFR) in RCC has been recognized for some time and EGFR signaling is mitogenic for malignant and normal renal tubular cells (146–149). TGFα, an EGFR ligand under transcriptional control by HIF, is constitutively expressed in VHL mutant cells (150). Downstream consequences of ErbB signaling include activation of the Ras/Raf/MAPK and phosphatidylinositol 3-OH kinase (PI_3K)/AKT pathways, as well as JAK/STAT signaling, which mediate a host of cell responses including cell growth and proliferation.

Monoclonal Antibody Therapy

There are currently two different monoclonal antibodies in clinical trials in patients with metastatic renal cancer. Cetuximab (cetuximab, IMC-C225, Erbitux ImClone Systems Inc, New York) is a chimeric human–murine antibody directed against the EGFR, while ABX-EGF (panitumumab, Abgenix, Fremont, CA) is a fully humanized antibody. Both bind to the extracellular domain of the receptor, compete with ligand binding and block activation by EGF or TGFα (151–153). They also induce receptor dimerization and downregulation, which may be important for growth inhibition (154).

In a Phase II trial of patients with RCC, treated at varying doses of ABX-EGF, there were two of 31 patients with objective responses, while 58% had minor responses or stable disease (155). However, despite similar *in vitro* results (156), treatment with cetuximab did not produce any responses among 55 patients. Whether these different results reflect real variation in the antibodies, the doses used, or are due to other factors is unknown. In any case, the investigators concluded that further study of cetuximab was not recommended (157), while trials with ABX-EGF are ongoing in RCC and in colorectal cancer (158).

Small Molecule Inhibitors of EGFR

Small molecule inhibitors of EGFR have been developed that compete with ATP binding and block the kinase activity of the receptor. Two of these inhibitors have been tested in patients with metastatic RCC, gefitinib (IressaTM; AstraZeneca, Wilmington, DE) and erlotinib (Tarceva, OSI-774, OSI Pharmaceuticals, Melville, NY). These small molecules are also able to block the catalytic activity of EGFR mutants lacking the extracellular binding domain (159). In terms of downstream signaling pathways, the monoclonal antibodies (MABs) and tyrosine kinase inhibitors (TKIs) seem to have similar effects and toxicities.

Phase I studies with both gefitinib and erlotinib demonstrated that rash, diarrhea, nausea, vomiting, and fatigue were the most common side effects with maximum tolerated doses of 500 and 150 mg/day, respectively (160). As single agents in RCC, the antitumor effects of these compounds (in terms of tumor regression) are modest, at best. In a Phase II study of 21 patients treated with 500 mg gefitinib daily as a 28-day cycle, stable disease was reported in eight (38%), all of whom were previously progressing. Median progression-free survival was 2.7 months and median overall survival was 8.3 months: the difference in overall survival between patients with stable disease and progressive disease was 16 + versus 6.1 months,

respectively ($p = 0.0007$). To our knowledge, no studies with single-agent erlotinib in RCC have been performed.

COMBINED ANTI-VEGF AND ANTI EGFR-THERAPY

Since RCCs frequently have upregulation of hypoxia-inducible genes (including VEGF and TGFα), the combination of an anti-VEGF agent (bevacizumab) plus an antagonist of EGFR (erlotinib) was a logical strategy. In xenograft models, the combination of Avastin and Tarceva results in greater efficacy than either agent alone (161). There also appears to be little to no overlap in the toxicity profile between the two agents. Based on these factors, a Phase II trial of the bevacizumab/erlotinib combination in patients with metastatic RCC was undertaken. Bevacizumab was given 10 mg/kg IV every 2 weeks, and erlotinib was used at 150 mg PO daily. A total of 57 patients, with 0 or 1 prior treatment, were enrolled. Out of 40 evaluatable patients, overall responses were noted in 16 patients (40%) (partial response in 10 [25%]). Stable disease was noted in 19 patients (47%), and the actuarial progression-free survival at 6 months was 71%. The treatment was generally well tolerated with the most common grade 3/4 toxicities being hypertension, diarrhea, rash, and nausea/vomiting. Thus, the combination appears to be more active than either agent alone. A randomized Phase II trial of bevacizumab versus bevacizumab plus erlotinib in patients with metastatic RCC is in progress and has completed accrual.

PROTEASOME INHIBITION

The normal degradation of proteins is crucial for maintenance of cellular homeostasis. The 26 S proteasome is a barrel-shaped structure with internally located proteases. However, entry of proteins into the 20 S proteasome core is sterically inhibited in the absence of a 19 S regulatory particle consisting of a multisubunit proteasome lid and base, which, together with the core particle, form the 26 S proteasome (162). The usual scenario is that proteins are first targeted by specific phosphorylations and subsequently recognized by E3-ubiquitin ligase complexes by which polyubiquitin chains are added. These polyubiquitinated proteins are bound by the proteasome lid, unfolded by the activity of ATPases that form the adjacent base, and finally threaded into the 20 S core where they are hydrolyzed (162). Bortezomib (PS-341) (Velcade TM; Millennium Pharmaceuticals, Inc., Cambridge, MA) is a potent, reversible inhibitor of this proteolytic activity.

While proteasome inhibition might be expected to be nonspecific, in fact, certain systems appear particularly vulnerable, such as the transcription factor NF-κB, which is sequestered in the cytoplasm by IκB (163). When IκB is phosphorylated (164) by IKK (165), IκB is polyubiquitinated and degraded allowing NF-κB to enter the nucleus and activate target genes. B-cell malignant diseases, especially multiple myeloma, are particularly dependent on NF-κB and sensitive to proteasome inhibition (166). Substantial constitutive NF-κB activation has been observed in RCC cell lines (167,168). Importantly, the frequency of constitutive NF-κB activation is reported to be greater in locally advanced and metastatic RCCs compared to localized cases (169). Bortezomib is able to induce apoptosis in RCC cell lines with high constitutive NF-κB activity in a dose-dependent fashion (170). Furthermore, the presence a wild-type VHL gene appears to sensitize RCCs to the antitumor effects of bortezomib (170).

Two independent Phase II trials of PS-341 (Bortezomib) have been published (171,172). In the first (171), PS-341 was administered IV twice weekly and patients received the drug 2 out of every 3 weeks (the maximally tolerated dose was 1.3 mg/m^2). Of 37 assessable patients, there were 4 partial responses (11%) and stable disease in 14 patients (38%). These responses lasted between 8 and 20+ months with sensory neuropathy being the most frequent significant toxicity (53%). The second study (172) used 1.5 mg/m^2 administered IV twice weekly for 2 out of every 3 weeks with escalation to 1.7 mg/m^2 in the absence of significant toxicity. Twenty-three patients were enrolled and eighteen patients completed at least three cycles of therapy. The most common severe side effects were arthralgia, diarrhea, and vomiting. However, only one objective response was seen. In summary, proteasome inhibitors likely will have a role in the treatment of RCC, although not as single agents and not in all patients.

mTOR INHIBITORS

All mRNAs are not created equal. Messenger RNAs with long, structured 5′-untranslated regions often associated with growth and cell-cycle regulatory genes (e.g., c-MYC, cyclin D1) are poorly translated into protein unless stimulated by growth factor signaling (173). AKT, MAPK, and the *m*ammalian *t*arget *o*f *r*apamycin, mTOR, are critical components in this regulation. In at least one form of hereditary RCC, because of mutations in the tuberous sclerosis (TSC1/2) complex, the mTOR pathway is constitutively activated (174). Mutations in the tumor suppressor gene, PTEN, also activate mTOR. PTEN mutations occur in ~5% of RCCs and are associated with advanced-stage, aggressive disease (175). Thus, there is rationale for mTOR inhibitors in RCC.

Rapamycin (sirolimus), an immunosuppressant macrolide produced by *Streptomyces hygroscopicus*, binds FKBP-12, creating a molecular complex that specifically inhibits mTOR functions (176). Inhibition of mTOR by rapamycin leads to downregulation of cyclin/cdk complexes and p27 accumulation, which blocks progression in late G1/S phase of cell cycle (177,178). In addition to immunosuppression, sirolimus is thought to retard proliferation of endothelial and vascular smooth muscle cells required for tumor angiogenesis (179). Recently, studies showed that sirolimus also inhibited the oncogenic transformation of human cells induced by either PI3K or AKT in mice with loss of the normal *PTEN* allele (180–182). CCI-779 is a water-soluble ester of sirolimus. At several nontoxic doses, CCI-779 demonstrated antitumor activity in a variety of human cancer models (183,184). As with sirolimus, *PTEN*-deficient human tumors are more sensitive to CCI-779-mediated growth inhibition than *PTEN*-expressing cells (180–182). Preclinical studies suggest that intermittent administration of CCI-779 reduces its immunosuppressive properties, while retaining antitumor activity (185).

In a Phase II study (185), 111 patients were treated with varying doses of CCI-779. Overall, there was one durable complete response and an overall response rate of ~35%. The median time to tumor progression was 5.8 months, and median survival was 15.0 months. The most common adverse events were rash, mucositis, and nausea, although severe toxicities were rare and reversible upon discontinuation of CCI-779. Since mTOR is one of the downstream targets of growth factor signaling, mTOR inhibitors will likely find their greatest use when combined with other agents. For example, we have shown that rapamycin plus an EGFR inhibitor synergistically inhibits growth in RCC cell lines with wild-type VHL (186). Other reports have shown that rapamycin augments the actions of other tyrosine kinase inhibitors, as well as standard chemotherapeutic agents (142).

MULTIKINASE INHIBITORS

Small-molecule kinase inhibitors that have more than one target (multikinase inhibitors) are generating considerable excitement in the treatment of metastatic RCC. Although these agents have potent activities against specific kinases, the true biologic targets responsible for tumor regressions are not precisely known. Sequencing of the human genome has identified 518 putative kinases, of which 90 are tyrosine kinases, and 58 are receptor tyrosine kinases (187). Moreover, the *in vivo* targets are likely to include kinases present in both the tumor cells, as well as stroma (e.g., endothelial cells). Because deregulation of HIF is an important aspect of RCC development, agents that affect HIF target genes, especially VEGF and its receptors, may be particularly useful.

BAY 43-9006

The BAY 43-9006 compound (Sorafenib) was initially found to block the RAF proteins, CRAF and BRAF *in vitro* (IC50 concentrations of 2 to 22 nM), but further examination demonstrated that this compound also blocked other kinases, including the VEGF receptors, VEGFR2 and VEGFR3 (IC50, 10-90 nM), platelet-derived growth factor receptor (PDGF-β, IC50 28nM), as well as the receptors for FLT-3 ligand (FLT-3, IC50, 45 nM) and steel factor/stem-cell factor (c-KIT, IC50, 50 nM) (188). At higher concentrations (IC50, 0.58 μM), the fibroblast growth factor receptor, FGFR1, is inhibited. Enthusiasm for the compound was initially because of, in part, to the high frequency of BRAF mutations in melanoma (~70%) and papillary thyroid cancer (33%). Signaling through many receptors involves activation of RAS, recruitment of RAF to the membrane, and subsequent activation of the MAP kinase (ERK) pathway, which affects a large number of downstream cellular responses. *In vitro* studies have demonstrated that BAY 43-9006 is a potent inhibitor of ERK phosphorylation in many, but not all, cancer cell lines (189). In particular, cell lines with activating mutations of KRAS appear nonresponsive. However, *in vivo* growth of these resistant cell lines could still be inhibited by BAY 43-9006, suggesting an anti-angiogenic effect.

BAY 43-9006 is taken orally. Phase I studies in patients with solid tumors indicated that diarrhea, hand–foot syndrome, and fatigue were the common toxicities. A Phase II trial of 63 metastatic RCC patients treated with BAY 43-9006 for at least 12 weeks resulted in 25 partial responses, 18 patients with stable disease and 15 with progressive disease (190). There is currently a large randomized trial BAY 43-9006 versus placebo in patients with metastatic RCC who failed one previous treatment (immunotherapy or chemotherapy), although patients with previous anti-angiogenic treatments were excluded.

SU011248

Like BAY 43-9006, SU011248 is an oral multikinase inhibitor that blocks the activity of the VEGF receptor (VEGFR2, IC_{50}, 9 nM) and PDGFR-β (IC_{50}, 8 nM), as well as SRC, ABL, IGFR-1, and FGFR-1 tyrosine kinases at IC_{50} doses ranging from 0.6 to 0.8 μM. Phase I studies identified fatigue as the most common dose-limiting toxicity and showed encouraging responses in 3 out of 4 patients with metastatic RCC (191) A Phase II trial of 63 previously treated RCC patients showed a response rate of 40% with an additional 27% of patients with stable disease lasting at least three months (192). A Phase III randomized trial comparing SU011248 versus INF-α in previously untreated patients is underway.

PTK787/ZK 222584

This agent was developed as an oral selective inhibitor of VEGFR-1, VEGFR-2, and VEGFR-3. VEGFR-3 is major regulator of lymphatic proliferation, which correlates with the amount of lung metastases in experimental systems (193). Other kinases, such as PDGFR and c-KIT are inhibited, whereas there appears to be no activity against c-ABL (194). In a Phase I study involving 37 evaluatable patients, 7 (19%) had minimal or partial responses, while 17 others had stable disease, suggesting the compound had activity in RCC (195) Nausea, fatigue, vomiting, dizziness and headaches were observed, but overall PTK787 appears to be relatively safe.

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CHAPTER 28 ■ PRIMARY NEOPLASMS OF THE KIDNEY AND RENAL PELVIS

BRIAN I. RINI, JOHN F. WARD, AND NICHOLAS J. VOGELZANG

Kidney tumors account for approximately 3% of malignancies in the United States with 36,160 new cases and 12,660 deaths expected in 2005 (1). Renal cell carcinoma (RCC) constitutes 90% of all tumors of the renal cortex, but is not comprised of a single biologic or histologic cell type. Instead, RCC is a heterogenous group of malignant cell types each having a particular biologic behavior ultimately impacting the response to treatment and cancer-specific mortality (1). Conventional clear-cell renal cortical neoplasms (CCRCC) represent more than 65% of all RCC and are the most virulent of the subtypes. The other 35% of RCC is composed of generally more indolent subtypes (chromophobe and papillary) or are not known to systemically disseminate (oncocytoma). Tumors arising from the lining of the upper urinary collecting system and ureter may sometimes be mistaken for primary renal cortex tumors, but are a distinct entity of urothelial origin. These urothelial carinomas (transitional cell carcinomas) require treatment aligned with the management of bladder cancers. Cancers arising in other organs will occasionally metastasize to the kidney (gastrointestinal, pulmonary) but, the most common nonrenal neoplasm of the kidey are of hematologic origin (lymphoma). Wilms' tumor (nephroblastoma), which represents the most common solid neoplasm of children, accounts for less than 1% of primary renal cancers in adults.

RENAL CELL CARCINOMA

Historical Perspective

Renal carcinoma was first described in 1826 by Konig. In 1855, Robin hypothesized that these tumors arose from cells of the renal tubular epithelium, a notion that was later verified. In 1883, Grawitz noted that the microscopic appearance of renal tumors was similar to that of adrenal cells. He concluded, incorrectly, that renal cancers that arose from the adrenal, rests within the parenchyma of the kidney. The term *hypernephroid tumor* derives from this incorrect hypothesis; however, the term *hypernephroma,* as applied to renal tumors in general, survives today.

Epidemiology

Renal cell carcinoma accounts for 2% to 3% of all cancers. It affects men almost twice as often as women (2) and is more common in North American and European countries than other areas (3). The incidence of RCC increases with age with a median age at diagnosis of approximately 65 years. Although RCC is uncommon in children, such cases are occasionally encountered.

The incidence of RCC is rising in the United States among all races and both sexes. From 1975 to 1995, the incidence among white men, white women, black men, and black women increased by 2.3%, 3.1%, 3.9%, and 4.3%, respectively (4) (Fig. 28-1). This increase in incidence occurred for all tumor stages, but was greatest for localized tumors. The increasing use of ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI) has increased the proportion of incidentally detected renal tumors (5,6). Most studies conclude that incidentally detected tumors are of lower stage and have a better outcome than symptomatic lesions (see below). In spite of increased incidental detection of the disease, the overall mortality rate from kidney cancer continues to rise. Furthermore, there is an increasing disparity of RCC incidence and mortality between whites and blacks, the latter group having disproportionately higher rates of both disease and death from disease (7).

Risk Factors

Cigarette smoking is generally regarded as a risk factor for RCC. The largest published case-control study reported a relative risk of 2.0 among patients with over 42 pack-years of exposure (8). Attributable risks based on these data suggest that about one-fourth to one-third of all RCC in men may be caused by cigarette smoking. The attributable risk appears less pronounced in women. After smoking cessation, the risk of RCC seems to decline (8). Other tobacco products have not consistently been shown to increase the risk of RCC (7,9). The component of cigarette smoke responsible for tumorigenesis is not known (9).

Both case-control and cohort studies have reported an association between obesity and the risk of RCC. This association is consistently demonstrated in women; the relationship is less apparent in men (10). The mechanism by which obesity predisposes to RCC is not clear.

Hypertension has been reported to increase the risk of RCC; however, when epidemiologic data are adjusted for antihypertensive medications, it is unclear whether the hypertension or the medicines cause the increased risk (7). Diuretics have been implicated in particular (8). When hypertension is combined with cigarette smoking and obesity, the three factors are estimated to account for 50% of the RCC cases in the United States (11). Trends in incidence of these three risk factors may partially explain the rising incidence and racial disparity of RCC.

Many individual studies report an increased risk of RCC among members of various occupational groups, but no group has demonstrated consistent and conclusive evidence of increased risk. One large case-control study reported a significant association (relative risk 1.4 to 2.0) among blast-furnace and coke-oven workers, as well as iron and steel workers. In addition, workers exposed to asbestos, cadmium, dry-cleaning solvents, gasoline, and other petroleum products were at increased risk (12). None of these associations have been consistently seen in other studies so that the true risk of RCC from

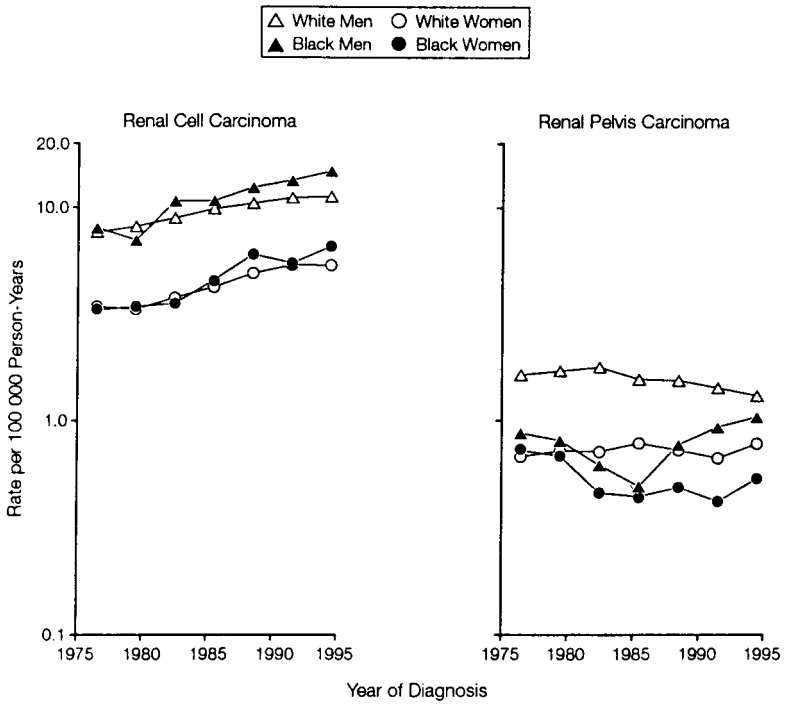

FIGURE 28-1. Age-adjusted (1970 U.S. standard) incidence rate for renal cell carcinoma and renal pelvis carcinoma per 100,000 persons/year by sex and race (SEER), 1975–1977 to 1993–1995. SEER indicates surveillance, epidemiology, and end results program. (From: Chow WH, Devesa SS, Warren JL, et al. Rising incidence of renal cell cancer in the United States. *JAMA* 1999; 281: 1629, with permission.)

various occupations remains unknown. Cases of RCC following therapeutic radiation exposure are rare, but have been reported (13).

Trichloroethylene (TCE), an industrial solvent, is classified by IARC (International Association of Renal Carcinoma) as a carcinogen in animals. In humans, mutations in the von Hippel-Lindau (*VHL*) gene (implicated in RCC tumorigenesis; see "Genetics/Molecular Biology," later in this chapter) have been noted in tumors from patients with high cumulative exposure to trichloroethylene, more so than tumors from nonexposed patients (14). This study demonstrated an association between the number of mutations and the severity of the trichloroethylene exposure. Nucleotide 454 in the *VHL* gene appears to be a hot spot for mutational changes in patients exposed to trichloroethylene. Epidemiologic studies attempting to define the risk of RCC in TCE-exposed patients have had conflicting results (15,16). The cytochrome enzymes responsible for TCE metabolism have genetic differences, which may produce carcinogenic metabolites in rare individuals. In order to more precisely quantify risk of RCC in occupational groups, a large epidemiological study is underway in formerly communist Eastern Europe where occupational safety laws were lax or nonexistent for many years.

Patients receiving hemodialysis are at increased risk for RCC, particularly patients with acquired cystic kidney disease (ACKD), who have a 50-fold increased risk compared to the general population (17). The tumors arise from cells lining the cysts and are generally of papillary histology. Some authors have recommended regular CT or ultrasound imaging to screen for renal tumors (18).

Clinical Presentation

Renal cell carcinoma has been referred to as the "internist's tumor," a tribute to the protean symptoms, signs, and laboratory abnormalities that can be seen at presentation and throughout the clinical course. A "classic triad" of hematuria, flank pain, and a palpable mass has been long associated with RCC, but this constellation of findings is seen in less than 1% of patients with localized disease (2,19). In Table 28-1 are listed the frequency of the more common symptoms and signs encountered at diagnosis in a series from UCLA of over 1,500 newly diagnosed patients (20).

In a case series of over 2,000 patients diagnosed with RCC in Illinois from 1975 to 1985, 42% had stage I tumors at diagnosis, 19% had stage II, 21% had stage III tumors, and 18% had stage IV (metastatic) disease (20). More recent series have reported 10% to 50% of patients as presenting with metastatic disease (21,22). The most common sites of spread are the lungs (61%), lymph nodes (37%), bones (27%), adrenal gland (16%), and liver (16%), About 5% of RCC patients present with brain metastases (21). RCC has been known to metastasize to virtually any site in the body (Fig. 28-2); metastases to the thyroid gland, heart, pancreas, digits, and skeletal muscle are all uncommon, but well documented. RCC is unique in its predilection to spread locoregionally via the lumen of the renal vein and inferior vena cava (19). Contiguous spread to the right atrium is not uncommon.

Paraneoplastic Syndromes

The list of signs and symptoms (paraneoplastic syndromes) associated with RCC is long and varied. In the series from UCLA,

TABLE 28-1

PRESENTING SYMPTOMS OF RENAL CELL CARCINOMA

	% of cases
Hematuria	40–60
Pain	25–50
Palpable mass	30–50
Fever	5–20
Weight loss	20–30
Varicocele	1–2
Incidental finding	4–10

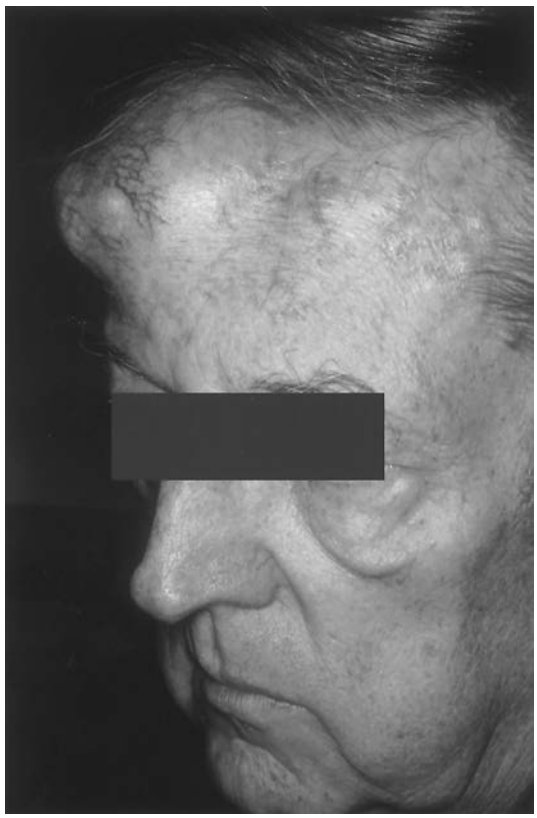

FIGURE 28-2. Renal cell carcinoma with metastatic spread to the forehead. (See Color Plate.)

the most common findings at presentation were anemia, hepatic dysfunction, unintended weight loss, malaise, hypoalbuminemia, and hypercalcemia (Table 28-2). Confirmation that a sign or symptom is paraneoplastic requires documentation of resolution of the sign or symptom following complete resection. Kim et al. (23) showed that the majority of the findings (Table 28-2) occurred more frequently in patients with localized RCC, strongly implicating systemically acting proteins secreted by the tumor or the host in response to the tumor as the cause for the syndrome. Thus, the presence or absence of a paraneoplastic syndrome does not predict metastatic disease. As noted by the UCLA group, the presence of virtually any sign or symptom conveyed a significantly worse prognosis and that "the highest hazard scores were associated with anorexia, unintended weight loss, malaise, thrombocytosis and night sweats" (23). The UCLA investigators reported that the symptom complex of hypoalbuminemia, weight loss, anorexia, and malaise (so called "cachexia-related finding") were predictors of poor survival that were independent of stage, grade, and performance status. The difference in survival between patients with one versus all four cachexia-related findings was not statistically different. In patients with localized RCC, the 2-year survival rate was 95% in patients without cachexia compared to 79% in patients with at least one cachexia-related finding ($p < 0.0001$). In patients with metastatic RCC, the median survival was 12 months in patients with cachexia, compared to 31 months in patients without cachexia ($p < 0.001$).

Kim et al. (23) also investigated whether cachexia was an independent predictor for both tumor recurrence and survival following nephrectomy in patients with localized, T1 RCC. At least one cachexia-related finding was found in approximately 15% of the T1 patients. The effect of cachexia on survival rates was the most pronounced for patients with high-grade T1 RCC. where the presence and absence of cachexia were associated with 5-year survival rates of 55% and 75%, respectively.

Previous reports suggesting that there was no difference in prognosis for patients with RCC presenting with or without

TABLE 28-2

PRESENTATION OF RENAL CELL CARCINOMA (LOCALIZED AND METASTATIC) AND EFFECT ON DISEASE-SPECIFIC SURVIVAL

	Percentage (*n*)	Localized/ metastatic	Hazard ratio (HR)	*p* value (univariate)
Paraneoplastic				
Anemia	52	0.7	2.0	<0.0001
Hepatic dysfunction	32	0.6	2.1	<0.0001
Weight loss	23	0.3	3.0	<0.0001
Malaise	19	0.6	2.9	<0.0001
Hypoalbuminemia	20	0.6	2.3	<0.0001
Hypercalcemia	13	0.6	1.8	0.0223
Anorexia	11	0.4	3.1	<0.0001
Thrombocytosis	9	0.6	2.6	<0.0001
Night sweats	8	0.6	2.5	<0.0001
Fever	8	0.5	2.0	<0.0001
Hypertension	3	0.6	1.4	0.3945
Erythrocytosis	4	0.47	1.4	0.1810
Chills	3	0.6	1.7	0.0457
Classic triad				
Gross hematuria[a]	24	0.7	1.2	0.0744
Flank pain[a]	20	0.6	1.3	0.0631
Flank/abd mass[a]	4	0.4	1.8	0.0064

[a] Disease-specific survival.

symptoms and were likely flawed due to small sample size, leaving the investigators with inadequate power to investigate the specific types of signs and symptoms (i.e., cachexia-related versus noncachexia-related findings). The more common paraneoplastic syndromes are discussed in the following sections.

Anemia

Anemia is much more common than erythrocytosis. The low hematocrit is rarely explained by hematuria or bone marrow replacement by tumor. Laboratory analysis often reveals normochromic, normocytic morphology with depressed serum iron and iron-binding capacity, consistent with the anemia of chronic disease.

Fever

RCC commonly causes tumor fever. Interleukin-6 (IL-6), a known pyrogen, may be secreted by tumor cells. One series of RCC patients reported elevated IL-6 levels in 18 out of 71 (25%) patients, with fever documented in 78% of those with elevated levels (24). Other cytokines have been implicated but the data are less convincing. Anti-IL-6 antibodies are being developed clinically.

Hypercalcemia

Hypercalcemia was seen in 13% of the newly diagnosed patients in the UCLA series. Secretion of parathyroid hormone-related peptide (PTHrP) by the tumor is the predominant cause of hypercalcemia associated with RCC. One series of 42 RCC cases used PTHrP-specific monoclonal antibodies to demonstrate PTHrP in 40 of the tumors (25). Other humoral polypeptides that may contribute include; PTH, osteoclast-activating factor (OAF), transforming growth factor-α (TGF-α), interkeukin-1, and tumor necrosis factor. Hypercalcemia is virtually the only paraneoplastic syndrome associated with RCC that will respond to conventional medical therapy (intravascular fluid replacement, loop diuretics, biphosphonates, etc.) without treatment directed at the underlying tumor (26,27). In metastatic RCC, stimulation of osteoclasts by metastatic bone deposits appears to contribute relatively little to the hypercalcemia. Nonetheless a search for bone metastases is warranted in all patients with hypercalcemia.

Hepatic Dysfunction (Stauffer's Syndrome)

Abnormal serum liver function studies have been associated with RCC independent of liver metastases. This syndrome was first reported by Stauffer in 1961 (28). The etiology of this syndrome is poorly understood, but tumor resection typically improves the abnormalities. Hepatic biopsies demonstrate a nonspecific hepatitis with no biliary obstruction to explain an elevated alkaline phosphatase (29). It is speculated that cytokines and acute-phase reactants produced either by, or in response to, the tumor may cause the hepatic dysfunction, but this relationship remains ill defined. Treatment of the malignancy has been shown to improve Stauffer's syndrome in animal models (30).

Erythrocytosis

Erythropoietin levels were once thought to be elevated in the majority of RCC cases (31). The paradoxically low incidence of erythrocytosis was believed to be due to the concomitant presence of anemia in these patients. A more recent series demonstrated elevated erythropoietin levels in only 1 out of 49 RCC patients (32). Despite this study, it is believed that the erythrocytosis associated with RCC is due to aberrant production of erythropoietin, or a closely related substance, by the tumor cells (33). This follows in that erythropoietin production is regulated by hypoxia-inducible factors that are dysregulated in RCC (*vide infra*). Small series have suggested that erythropoietin-producing tumors may be more responsive to immunotherapy (34).

Hypertension

Elevated blood pressure that responds poorly to antihypertensive therapy is well documented in RCC. Ectopic renin production by the tumor has been implicated (35). In the absence of renal artery stenosis, nephrectomy will generally lower the blood pressure. Erythrocytosis, if present, can exacerbate preexisting or paraneoplastic hypertension.

Amyloidosis

Secondary amyloidosis is an unusual manifestation of RCC; however, RCC is the most common solid tumor associated with amyloidosis. This phenomenon has been the subject of several small series and case reports (36–41). Still, the mechanism by which amyloid is formed has not been determined. Nephrotic syndrome and hepatosplenomegaly have both been described.

Other Syndromes

RCC has been reported to express gonadotropic hormones (42), prolactin (43), and adrenocorticotrophic hormone (ACTH) (44).

Pathology/Genetics/Molecular Biology

With the increasingly widespread availability of cytogenetic and molecular studies (i.e., microarray) efforts have been made to correlate the genetic with the morphologic features of renal epithelial neoplasms (45–47). More recent studies have demonstrated that distinct pathologic subtypes have distinct gene expression profiles (48). Further research has also identified gene expression patterns associated with survival (49). Additional work, however, is required in this area on larger data sets to incorporate this technology into clinical practice. However even before microarray analysis was widely available, by the late 1990s pathologists had incorporated cytogenetics into the morphologic classification of renal neoplasms (50,51). While that classification system represented an advance in our understanding of these diseases, it also rendered older medical literature difficult to interpret. For example, the "sarcomatoid" variant of RCC does not exist as a distinct clinicopathologic entity in the updated classification system. Rather, it is considered a dedifferentiated form of any of the subtypes.

The recent classification system does not yet include microarray but does define the three most common malignant epithelial neoplasms in adults; conventional (clear-cell) RCC, papillary RCC, and chromophobe RCC. The genetic and morphologic features of each are briefly discussed below and, where appropriate, discussion of inherited predisposition syndromes is included. These syndromes are often divided into two types: those giving rise to the clear-cell variety of RCC and those associated with papillary histology.

Conventional (Clear-Cell) Renal Cell Carcinoma

This pathologic entity arises from the proximal convoluted tubule and comprises more than one-half of renal epithelial tumors. Most cases are sporadic, but these tumors are also associated with von Hippel-Lindau (VHL) disease. Among patients with VHL disease who develop clear-cell RCC, deletion (partial or complete) of the 3p chromosome arm is nearly universal. Of note, among patients without clinical VHL disease who develop clear-cell RCC, somatic inactivation (via mutation

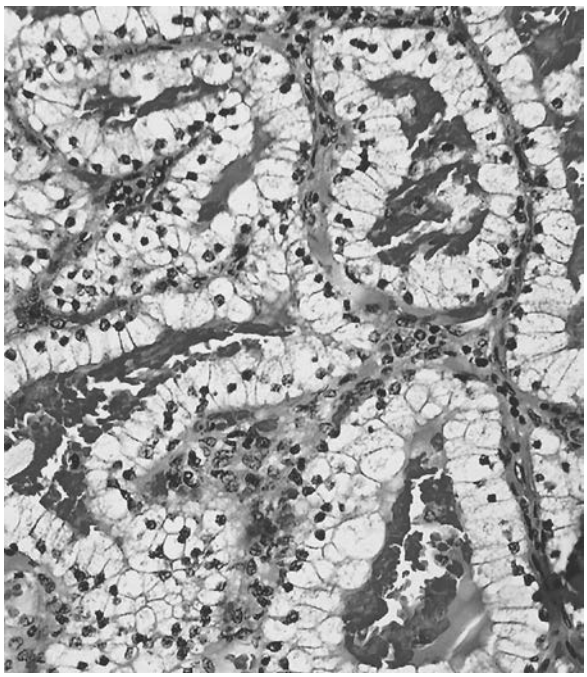

FIGURE 28-3. Renal cell carcinoma, clear-cell type: The cells are in an acinar arrangement and feature optically clear cytoplasm. (From: Murphy WM, Beckwith JB, Farrow GM. *Tumors of the kidney, bladder, and related urinary structures,* 3rd series. Washington, DC: The American Registry of Pathology, 1994, with permission.) (See Color Plate.)

or gene methylation) of both alleles of the *VHL* gene are found in a significant number of patients (52,53).

Grossly, these tumors appear golden yellow, but the color varies with tumor grade. By light microscopy, the tissue can demonstrate a variety of growth patterns including solid, acinar, cystic, papillary, pseudopapillary, tubular, and sarcomatoid. An individual tumor can exhibit multiple growth patterns. The cytoplasm is typically clear or granular–eosinophilic (Fig. 28-3).

The tumors are graded according to nuclear characteristics and given a score of 1 to 4, based on the system of Fuhrman and associates (54). This score has been shown to correlate with prognosis in clear-cell RCC. Most high-grade tumors are solid, pseudopapillary, or sarcomatoid. Most low-grade lesions have acinar features.

von Hippel-Lindau Disease. VHL disease is an autosomal-dominant familial cancer syndrome involving multiple organs. It is due to mutation of the *VHL* gene on 3p25. The disorder involves abnormalities of vascular proliferation and an increased risk of some neoplasms. Characteristic lesions other than RCC include hemangioblastomas of the cerebellum and retina, pheochromocytomas, and renal, pancreatic, and epididymal cysts. Of patients with VHL disease, 60% to 80% will develop central nervous system hemangioblastomas and retinal angiomas, the most characteristic lesions of VHL disease (55,56). These lesions are nonmalignant, but can cause significant morbidity. VHL disease is also the most common cause of hereditary-type pheochromocytoma (57). Diagnostic criteria derived in 1963 by Melmon and Rosen (58) are still in use today. Screening for symptoms of VHL should begin early in families at risk and include: yearly eye examinations and blood pressure monitoring starting by age 5, yearly abdominal ultrasounds, magnetic resonance imaging (MRI), and computerized tomography (CT or CAT) scans starting in late childhood, and yearly 24-hour urine collection test for elevated catacholamines.

Of patients with VHL disease, 40% to 60% will develop RCC, which is characteristically multicentric, arising from renal cysts. The kidneys of patients with VHL disease harbor thousands of small cysts that appear to derive from proximal renal tubule epithelial cells. Although the majority of these cysts will be clinically silent, each is potentially a site of RCC development (Fig. 28-4). The propensity for multifocality makes management difficult. Serial imaging with CT scans is always indicated, beginning at around age 20. Likewise, nephron-sparing surgery, when mass lesions reach approximately 3 cm in size, is indicated whenever possible (59,60). Such an approach may delay the need for renal dialysis.

The *VHL* gene was cloned in 1993 (61) and characterized as a tumor suppressor gene shortly afterward. The function

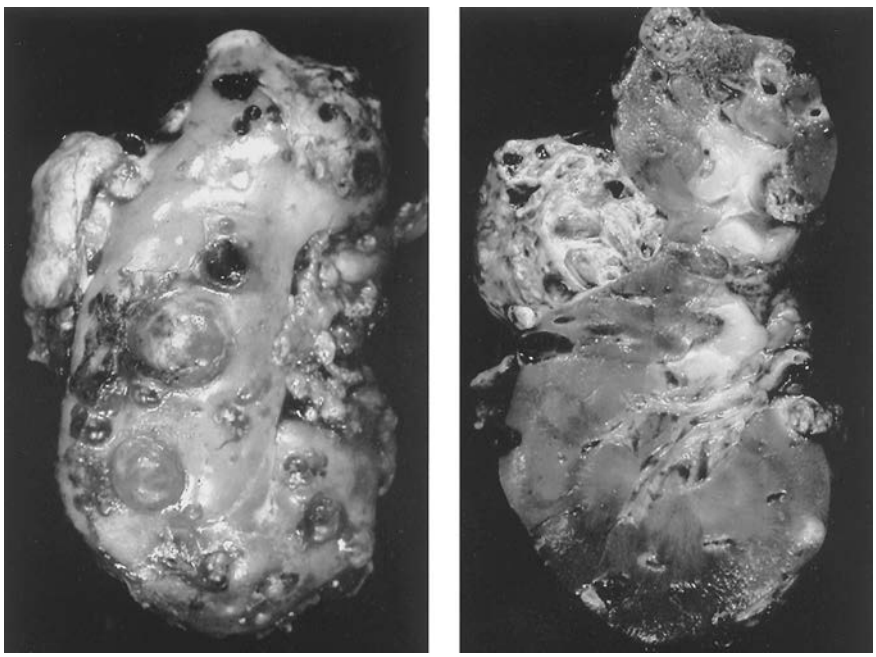

FIGURE 28-4. von Hippel-Lindau syndrome. *Left*: Multiple renal cysts and tumors are evident. *Right*: Cut surface of the same kidney. (From: Murphy WM, Beckwith JB, Farrow GM. *Tumors of the kidney, bladder, and related urinary structures,* 3rd series. Washington, DC: The American Registry of Pathology, 1994, with permission.)

of the *VHL* gene protein product (pVHL) has recently been elucidated. In normal cells, pVHL controls the degradation of hypoxia-inducible factor-α (HIF-α) proteins. These proteins have a variety of cellular functions, among them to upregulate proangiogenic proteins (like vascular endothelial growth factor [VEGF]) in the presence of hypoxia (62). It is now clear that reduced cellular levels of pVHL result in an overexpression of VEGF (63). These data support the belief that the dysregulation of angiogenesis is an important step in the development of RCC and cancer, in general. With the development of new pharmacologic agents that target aberrant angiogenesis (see below), VHL syndrome may provide a unique opportunity to study the long-term effects of such therapy and its impact in preventing or slowing tumorigenesis (64).

According to the Knudson "two hit" genetic model of tumorigenesis (65), the sporadic form of a known hereditary tumor can arise if both copies of a tumor suppressor gene are inactivated. This phenomenon has been described in 60% of sporadic clear-cell RCC in which allelic loss of *VHL* and somatic mutation or promotor methylation of the remaining *VHL* allele occurs (66,67). Inactivation of *VHL* appears to predispose only for clear-cell histology in patients afflicted both with VHL disease and sporadic RCC. Furthermore, *VHL* inactivation is only seen in tumors associated with VHL disease and their sporadic counterparts.

Familial Clear-Cell Renal Cell Carcinoma. Dominantly inherited clear-cell RCC, independent of VHL syndrome, has also been described. One extended family with a germline t(3:8)(p21;q24) translocation has been studied extensively. Ten members of the family have developed clear-cell RCC, six with bilateral disease. The age at diagnosis ranged from 37 to 59 years (68). The relationship between this translocation and the *VHL* gene is the subject of ongoing investigation, but it is clear that the *VHL* gene is not mutated in this syndrome. Yearly screening for kidney cancer with ultrasound, MRI, and CT scan beginning at age 20 has been suggested for families with this rare condition.

Tuberous Sclerosis. Also called *tuberous sclerosis complex,* this disorder is characterized by multiple benign hamartomas associated with epilepsy and learning disabilities. It is dominantly inherited with two major susceptibility loci at 9q34 and 16p13.3. Like *VHL,* these genes appear to encode for proteins involved in tumor suppression (69). Although tuberous sclerosis is associated with RCC, benign hamartomas (angiomyolipomas) of the kidney are much more common in afflicted patients.

Papillary Renal Cell Carcinoma

This entity accounts for approximately 10% of RCC tumors (70). A number of cytogenetic abnormalities characterize these tumors including trisomy of chromosome 7, 16, and 17 and the loss of chromosome Y (47,53–55). The molecular basis for this malignancy is unknown. Although hereditary papillary RCC is caused by an activating mutation of the MET oncogene (see below), only about 5% of sporadic papillary RCC have MET mutations. The application of the Fuhrman grading system to papillary tumors is controversial, in contrast to clear-cell lesions where the prognostic significance of tumor grade has been clearly demonstrated.

Multifocal disease, either microscopically or grossly, is a prominent pathologic feature of papillary RCC. By light microscopy, necrosis is often seen and and racemase can be used as an immunohistochemical marker.

Foamy macrophages and psammoma bodies are frequently encountered, as is hemosiderin pigment in the cytoplasm of the tumor cells. Most papillary tumors exhibit elements of papillary, papillary-trabecular, and papillary-solid tissue (70,71) (Fig. 28-5). Tumors that demonstrate all three types of growth are the rule. In addition, many papillary tumors have areas with solid, tubular, and glomeruloid characteristics; however, sarcomatoid growth is unusual.

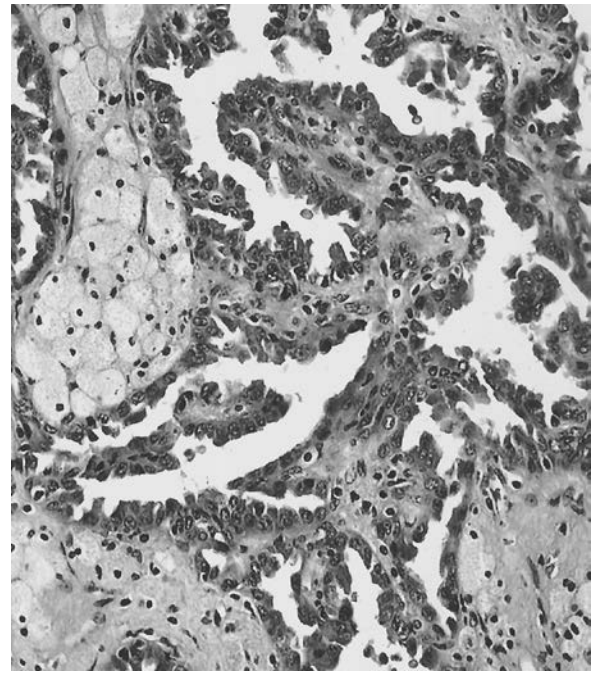

FIGURE 28-5. Renal cell carcinoma, papillary type: neoplastic cells over papillary structures with numerous stromal xanthoma cells. (From: Murphy WM, Beckwith JB, Farrow GM. *Tumors of the kidney, bladder, and related urinary structures,* 3rd series. Washington, DC: The American Registry of Pathology, 1994, with permission.) (See Color Plate.)

Hereditary Papillary Renal Cell Carcinoma. Hereditary papillary RCC (HPRCC) is an inherited form of RCC in which patients develop bilateral, multifocal type 1 papillary RCC. The susceptibility gene for hereditary papillary RCC has been identified as the *MET* proto-oncogene residing on 7q31-q34 (72). *MET* encodes a precursor protein of the C-MET receptor, whose ligand is hepatocyte growth factor (HGF). The HGF/C-MET signaling cascade has been implicated in normal liver and placenta organogenesis. In cancer cells, this signaling pathway appears to enhance the proliferative and metastatic capabilities of the cell. Management of multifocal tumors is similar to VHL syndrome with surgical extirpation when tumors reach a size of 3 cm. Individuals in families where HPRCC is suspected should have yearly screening with ultrasound, MRI, or CT scan beginning around age 30.

At least one family segregating both papillary RCC and papillary thyroid cancer has been described. This familial syndrome was not linked to markers for the *MET* proto-oncogene. Whether this syndrome represents a distinct clinical entity or a variant of organ-specific hereditary papillary RCC is not clear.

Hereditary Leiomyomatous Renal Cell Carcinoma. Hereditary leiomyomatous RCC (HLRCC) is a disease characterized by papillary type II RCC, multiple cutaneous leiomyomas, uterine leiomyomas, and leiomyosarcomas. The renal tumors tend to be solitary, characterized by aggressive clinical behavior with early metastases (73). Screening for kidney cancer should be

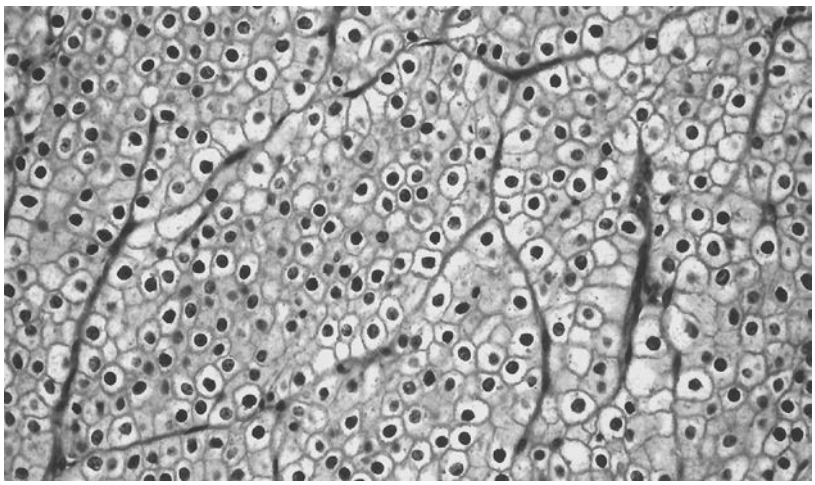

FIGURE 28-6. Renal cell carcinoma, chromophobe cell type: The tumor features a mixture of acidophilic granular cells and pale transparent cells. Prominent cell borders impart a plantlike or "cobblestone" appearance. (From: Murphy WM, Beckwith JB, Farrow GM. *Tumors of the kidney, bladder, and related urinary structures,* 3rd series. Washington, DC: The American Registry of Pathology, 1994, with permission.)

considered in families with HLRCC. Women in these families should also be screened for uterine leiomyomata and treatment with hysterectomy may be necessary. The gene associated with HLRCC is called FH (fumarate hydratase) and genetic testing is available.

Chromophobe Renal Cell Carcinoma. This variant comprises an estimated 5% of RCC (74) and carries a significantly better prognosis than clear-cell RCC, likely because more chromophobe tumors are localized at diagnosis. It was this description of chromophobe RCC that eventually led to a reassessment of the renal tumor classification system. While the morphology of these tumors may overlap with other subtypes, the genetic abnormalities help define this entity. Many chromophobe RCC's feature hypodiploid tumor cells with frequent loss of chromosomes 1, 6, 10, 13, 17, or 21 (75,76). Like oncocytomas, chromophobe RCCs are felt to arise from intercalated cells of the renal cortex; some authors have postulated that "hybrid tumors" (oncocytoma and chromophobe RCC) may exist (77).

While metastatic disease occurs in cases of chromophobe RCC, these tumors are less aggressive than either clear-cell or papillary RCC. They tend to be larger at diagnosis, with a greater proportion of organ-confined disease (78–80).

Grossly, these tumors are characteristically beige or light tan. Microscopically, the growth pattern is mostly solid, but can be admixed with areas of tubular, trabecular, or cystic growth. As in papillary RCC, sarcomatoid growth can be seen and is associated with more aggressive behavior. The cells demonstrate perinuclear halos, a visual product of the conglomeration of cytoplasmic organelles at the periphery of the cell. This phenomenon accentuates the cell membrane granting the tissue a "cobblestone" appearance at low microscopic power (Fig. 28-6).

Hereditary Chromophobe Renal Cell Carcinoma and Oncocytoma (Birt-Hogg-Dube Syndrome). Birt-Hogg-Dube (BHD) syndrome is an inherited disorder characterized by hair follicle tumors (fibrofolliculomas and trichodiscomas) on the face, neck and trunk, pulmonary cysts, and renal tumors (73). Most of the kidney cancers associated with BHD are classified as the chromophobe type or oncocytoma, but clear-cell and papillary kidney cancers have also been reported in families with BHD. Due to the increased risk of kidney cancer, yearly screening with ultrasound, MRI, or CT scan should be considered beginning at age 25. The BHD gene has been localized to the short arm of chromosome 17 and genetic testing is available.

Other Genetic Syndromes

Autosomal-Dominant Polycystic Kidney Disease. Patients with autosomal-dominant polycystic kidney disease (ADPKD) do not have a higher prevalence of RCC (81); however, the disease may behave differently in the presence of ADPKD. A systematic review of the published RCC cases in the setting of ADPKD revealed an earlier age of onset, more frequent incidence of bilateral tumors, and a higher incidence of sarcomatoid histologic features compared to sporadic RCC cases (82). Because of the baseline radiologic abnormalities in patients with ADPKD, the diagnosis of renal tumors can be challenging and potentially delayed (Fig. 28-7).

FIGURE 28-7. Polycystic kidney disease. This renal cell carcinoma of the mid-kidney arose in a patient with multiple renal cysts. (From: Murphy WM, Beckwith JB, Farrow GM. *Tumors of the kidney, bladder, and related urinary structures,* 3rd series. Washington, DC: The American Registry of Pathology, 1994, with permission.)

Imaging

When a patient is suspected of having a renal mass, based either on clinical signs and symptoms, retroperitoneal imaging is generally indicated. These tests help identify renal masses and differentiate cystic from solid lesions, as well as assess for nodal involvement, renal vein or inferior vena caval tumor thrombus, and metastasis to other sites. There is little role for biopsy of renal masses. The indications, limitations, and interpretation of the most commonly used tests are discussed in the following sections.

Intravenous Urography (IVU)

Although this test is not as sensitive as axial imaging in the detection of RCCs, particularly smaller lesions, it is very often the first study obtained. Irregularities in the renal contour, abnormal distortion of a renal calyces, and parenchymal calcifications or loss of the psoas shadow are all suggest the presence of a renal mass. In general, any lesion, with or without calcifications, that is not sharply defined should be further investigated with ultrasound or CT scanning. Usually, solid renal masses are iso- to hyperintense when compared to normal contrast enhanced renal parenchyma, while renal cysts are usually hypointense.

IVU is not a sensitive means to detect renal lesions smaller than 2 to 3 cm, detecting only 52% of confirmed masses in one study (83). IVU, a functional anatomic study, is still a valuable tool for assessing the anatomic relationships of the kidney and ureters and may still be superior to CT urography for assessing the urothelium of the renal pelvis and ureters.

Ultrasonography

When a mass is detected by IVU, ultrasonography is useful to differentiate solid from cystic masses. Despite the fact that a proportion of lesions will be solid or indeterminate and require CT imaging, the approach of using ultrasound before CT may still be cost-effective in some settings (84). Solid renal masses can appear hyperechoic, isoechoic, or hypoechoic, when compared to surrounding parencyma. All solid masses noted on ultrasound merit CT scanning. RCC can occasionally contain cystic areas or even be predominately cystic. Unlike benign cysts that have sharply defined, smooth walls and posterior enhancement of the ultrasonic signal, the cystic component of a RCC will usually have irregular, nodular walls. Cystic lesions of the kidney can be categorized I–IV for the likelihood of malignancies. Category I to II lesions are usually followed with sequential imaging, while category III to IV cysts have a much higher (50% to 95%) association with malignancy and should generally be treated as such. Malignant lesions can escape detection by ultrasound if they are isoechoic and do not deform the borders of the kidney (85,86). As in IVU, if there is a high clinical suspicion of RCC, a CT should be obtained, even in the face of a normal ultrasound. Retroperitoneal ultrasound can also be useful in assessing the renal vein and inferior vena cava for the presence of tumor thrombus.

Computed Tomography (CT)

CT of the abdomen before and after the adminstration of intravenous contrast and using 5-mm reconstruction is the imaging study of choice for diagnosis and staging of renal neoplasms. The sensitivity to detect a lesion less than 3 cm in diameter has been estimated at 94% (87).

The vast majority of malignant renal lesions display hyperattenuation with IV contrast when compared to surrounding normal parenchyma, a function of the prominent vascularity of these tumors. Any solid renal mass should be considered malignant until proved otherwise. There are no reliable CT characteristics to differentiate between RCC cell types or oncocytomas. Some <1-cm renal masses have been longitudinally followed in patients at high risk for morbidity from any intervention and can demonstrate very slow (<0.5 cm/year) growth. Malignant cystic lesions (class III and IV cysts) contain nodularity or irregularity to the walls or septae within the mass (88).

Magnetic Resonance Imaging (MRI)

Despite the widespread availability of MRI scanning, CT is still the mainstay for radiologic evaluation of renal tumors. MRI with IV gadopentetate dimeglumine (Gd-DTPA) enhancement offers an excellent option, in patients with renal failure or an allergy to iodinated contrast material. Using intravenous contrast, the ability of MRI to characterize renal masses is at least as good as CT (89). MRI offers better resolution in the detection of very small lesions and may better demonstrate hemorrhage into a cyst (89,90). On the other hand, MRI studies are more expensive than CT, have more frequent imaging artifacts, and do not reliably demonstrate calcifications.

MRI is not required in the staging of RCC, in most circumstances, although it can more reliably detect tumor thrombus into the renal vein and the cephalic extent in the inferior vena cava compared to CT (90–93) (Fig. 28-8).

Angiography

MRI has largely replaced angiography in the evaluation of the inferior vena cava in cases of known or suspected tumor thrombus. Angiography remains an option for patients who cannot undergo MRI. In cases where nephron-sparing surgery is being planned, angiography is sometimes used to accurately define the renal vasculature. In general, however, this modality is rarely used in the initial imaging or staging of renal neoplasms.

Staging/Grading

The American Joint Committee on Cancer (94) tumor, node, and metastasis (TNM) system and the Robson (95) staging

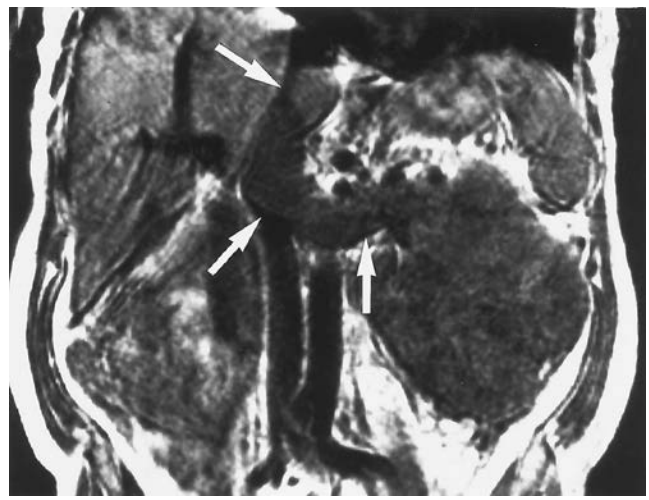

FIGURE 28-8. Renal cell carcinoma as seen with MRI. There is massive extension of tumor into the inferior vena cava (*arrows*). (From: Murphy WM, Beckwith JB, Farrow GM. *Tumors of the kidney, bladder, and related urinary structures,* 3rd series. Washington, DC: The American Registry of Pathology, 1994, with permission.)

TABLE 28-3

RENAL CELL CARCINOMA STAGING[a]

Primary tumor (T)		Robson stage
TX	Primary tumor cannot be assessed	
T0	No evidence of primary tumor	I
T1	Tumor 7 cm or less in greatest dimension limited to the kidney	I
T1a	Tumor 4 cm or less in greatest dimension, limited to the kidney	I
T1b	Tumor more than 4 cm but not more than 7 cm in greatest dimension, limited to the kidney	I
T2	Tumor more than 7 cm in greatest dimension limited to the kidney	II
T3	Tumor extends into major veins or invades the adrenal gland or perinephric tissues, but not beyond Gerota's fascia	III
T3a	Tumor invades the adrenal gland or perinephric tissues but not beyond Gerota's fascia	II
T3b	Tumor grossly extends into the renal vein(s) or vena cava below the diaphragm	IIIA
T3c	Tumor grossly extends into the renal vein(s) or vena cava above the diaphragm	IIIA
T4	Tumor invades beyond Gerota's fascia	IV
Regional lymph nodes (N)		
NX	Regional lymph nodes cannot be assessed	
N0	No regional lymph node metastases	
N1	Metastasis in a single regional lymph node	IIIB
N2	Metastasis in more than one regional lymph node	IIIB

[a] American Joint Committee on Cancer, 6th ed. Robson Staging System.

system are both used in the United States (Table 28-3). Although CT scanning is useful for clinical staging, prognosis is based upon pathologic staging of the resected tumor. The most recent version of the TNM system has divided T1 lesions into T1a (≤4 cm) and T1b (4 to 7 cm). This revised system has been strongly correlated with patient survival (96).

The most widely used system of grading renal tumors was proposed by Fuhrman and associates in 1982 (54). This system takes into account nuclear size, nuclear membrane irregularity, and nucleolar prominence and assigns the tissue a score of 1 to 4. This score is based on subjective observations by the pathologist; hence, there may be significant variability from one observer to another (97). Nonetheless, tumor grade is an independent predictor of survival. In one series of 643 patients, the 5-year survival was 88.7% among patients with grade 1 disease, but 46.1% with grade 3 and 4 (98). Lower grade lesions have demonstrated a statistically significant improvement in survival at all tumor stages (98).

Prognosis

Wide variability in the clinical behavior of RCC reflects a complex molecular mechanism of tumor growth, spread, and the role of the immune system. Although progress has been made in identifying the genetic changes and biomolecular markers of RCC, they are not currently validated or widely available. The most important determinant of survival remains stage (Table 28-3), while histologic subtype has a well-described impact on prognosis (98). Other multiple variables have an interplay with stage in determining the liklihood of a single event (disease recurrence and survival). Exponential formulas, which integrate these variables, have been gathered from large databases of patients having long-term outcome information to provide prognostic information for a single patient. The most widely used of these systems is the University of California Los Angeles Integrated Staging System (UISS), which has been validated across many institutions (99). It is commonly applied in patients being considered for protocol enrollment to adjunct treatment trials.

The UISS defines subgroups with different risks of death following nephrectomy by assimilating TNM stage, Eastern Cooperative Oncology Group (ECOG) performance status, and Fuhrman grade into prognostic algorithms and nomograms. This results in three risk groups (low- [LR], intermediate- [IR], and high-risk [HR]) for localized and metastatic disease (99). For localized disease, 5-year survival rates following partial or radical nephrectomy are approximately 92%, 67%, and 44% for LR, IR, and HR disease, respectively. For patients with metastatic disease, 3-year survival rate treated with nephrectomy followed by interferon (IFN) or interleukin-2 (IL-2)-based immunotherapy are approximately 37%, 23%, and 12%, respectively. The fact that this figure is greater than zero underscores the curious and variable natural history of RCC.

Treatment

Localized Tumors

Surgery. Despite advancing understanding of the molecular pathophysiology and cytogenetic abnormalities of RCC, surgical extirpation of the tumor remains the only curative treatment for early-stage disease. Surgical nephrectomy, removal of the kidney from within Gerota's fascia, was first successfully performed in 1869 and became routine by the end of the 19th century. In 1963, Robson (100) claimed superior survival over simple nephrectomy when the perirenal fat and

ipsilateral adrenal gland were also included in the surgical resection As a greater understanding of the pathophysiology of RCC was achieved and fewer patients presented with large, symptomatic or locally advanced tumors, nephron-sparing surgery (NSS) (i.e., partial nephrectomy) evolved into a standard surgical approach for most tumors ≤4 cm, even when a normal contralateral kidney is present (101). Early favorable results with NSS at the beginning of the 1990s were solidified with 10-year reports showing rare local recurrence and almost 100% survival with NSS by the end of the decade (102–104). Maximal nephron preservation is associated with decreased long-term (>10-year) hyperfiltration injury, proteinuria, and hypertension, as well as renal insufficiency, when compared to radical nephrectomy, especially in patients with or at risk for hypertension and diabetes mellitus (105).

Concurrent to the establishment of open partial nephrectomy, laparoscopic radical nephrectomy was being developed and applied to patients with RCC. Since 1992, the number of patients undergoing laparoscopic radical nephrectomy has increased explosively worldwide and at select centers is now extended to patients with advanced disease. Laparoscopic nephrectomy has advantages in many indexes of perioperative morbidity, including estimated blood loss, postoperative narcotic requirements, length of hospitalization and duration of convalescence (102). The complication rate during laparoscopic nephrectomy varies in the published literature from 7.5% to 34%, but is generally higher than following open radical nephrectomy.

Laparoscopic radical nephrectomy is generally reported to confer long-term oncologic effectiveness equivalent to traditional open radical nephrectomy, although local recurrences and port site recurrences are well documented (102).

In 4% to 9% of newly diagnosed patients RCC has invaded the venous system (renal vein, inferior vena cava, right atrium). This cancerous thrombus can embolize and cause sudden death or invade the wall of the IVC, complicating resection. Several studies have examined the prognostic significance of the level of venous extension (106,107). Although the risk of recurrence after nephrectomy is significantly increased in patients with venous thrombus, compared to patients without venous thrombus, this difference is eliminated in multivariate analysis that includes T stage, Fuhrman grade, and ECOG performance status. Clinical variables, such as stage, grade, and ECOG should primarily guide treatment decisions.

The role of regional or extended lymph node dissection at the time of nephrectomy is controversial and is best individualized based on nuclear grade, the presence of sarcomatoid component, tumor size and stage, and the presence of tumor necrosis obtained by frozen section analysis at the time of nephrectomy. Some, but not all, authors have reported extended lymph node dissection (diaphragmatic crus to bifurcation of the aorta) to confer improved cancer-specific survival, even when no lymph node involvement is identified (108,109). The only Phase III randomized trial of the role of lymphadenectomy in 772 patients has revealed no increase in morbidity, but equivalent survival, suggesting it should only be used in patients likely to receive the most benefit through cytoreduction (110).

Adjuvant Therapy. RCC carries a very poor prognosis following relapse; the minority of patients respond to disease-specific therapy. Following nephrectomy with curative intent, 20% to 30% of patients will relapse (111,112). It is possible to identify patients at a particularly high risk for recurrence. For example, renal vein and lymph node involvement are associated with higher relapse rates. Hence, the concept of adjuvant systemic therapy to lower the risk of relapse after "curative" resection is of considerable interest. However, there is currently no evidence to support routine adjuvant therapy outside of participation in a clinical trial. Postnephrectomy radiation failed to reduce the risk of relapse in randomized trials (113,114). Three randomized trials of adjuvant interferon-α (IFN-α) versus observation failed to demonstrate improved time to relapse or overall survival in the treated group (115–117). Studies of adjuvant interleukin-2 (IL-2) have, likewise, not demonstrated any clinical benefit.

Relapsed or Metastatic Disease

The prognosis for patients with metastatic disease is poor. Approximately one-third of patients will either present or return with metastatic disease, 90% of these from conventional clear-cell RCC. The median overall survival time of patients with advanced RCC is approximately 10 months, with a 3-year survival rate of about 10% (118). Factors associated with shorter survival include poor performance status, high lactate dehydrogenase, low hemoglobin, high calcium, and no nephrectomy (118).

Immunotherapy. The host's immune response to cancer has been studied extensively in recent years, leading to the development of immunomodulating agents in the treatment of RCC (and cancer, in general). Today, with the success of IFN-α and IL-2 in the treatment of advanced RCC, the importance of the immune system in cancer treatment is well established.

Interferon-α. Interferons occur naturally in all persons. *In vitro,* these glycoproteins display a number of immunomodulating effects. IFN-α stimulates mononuclear cells and promotes expression of the major histocompatibility complex. Beyond these immune effects, there is evidence that interferons inhibit angiogenesis (119). When this agent is used alone to treat RCC, the overall response is 10% (120,121), although rates as high as 30% have been reported in more selected patients (122). The patients most likely to respond to IFN-α include those with high-performance status, prior nephrectomy, and lung-predominant metastases (120,123). The typical duration of response is 4 to 6 months. Prolonged responses are rare but do occur (123).

The time from the start of treatment to objective response can be prolonged; the average time is 3 to 4 months (121), with lengths as long as 1 year reported. The most effective duration of treatment in those who respond has not been established. Several randomized trials examining the dose of IFN-α suggest that 5 to 10 million units 2 to 3 times per week has maximal efficacy, with less toxicity than that seen at higher doses (124). The toxicity of IFN-α can be significant including flulike symptoms, fatigue, and anorexia. An estimated 10% to 20% of patients cannot tolerate treatment (120).

IFN-α has been compared to hormonal agents and cytotoxic chemotherapy in four randomized trials (Table 28-4). Two of the trials were underpowered with less than 100 total patients (125,126). The two larger trials demonstrated a statistically significant survival advantage in the interferon arm (127,128). The largest, with 335 patients randomized to treatment with interferon alone versus medroxyprogesterone (127), showed a median survival of 8.5 months in the interferon arm versus 6 months in the steroid arm. The second largest study compared vinblastine to vinblastine plus interferon. In this trial, survival improved by 7 months in the interferon arm (16 months versus 9 months) (128). Despite these significant results, long-term survival with interferon treatment is rare and the toxicities of this agent are not insignificant. Nevertheless, IFN-α treatment is a standard of care for patients with metastatic RCC who cannot be treated on clinical trials.

Interleukin-2. The Food and Drug Administration (FDA) approved IL-2 for the treatment of RCC in 1992 and it is the

TABLE 28-4

RANDOMIZED TRIALS OF INTERFERON-α IN PATIENTS WITH METASTATIC RENAL CELL CARCINOMA

	N	Response rate (%)	Median survival (months)
Steineck et al., 1990 (125)			
Interferon-α2a	30	6	7
Medroxyprogesterone acetate	30	3	7 (*NS*)
Kriegmair et al., 1995 (126)			
Interferon-α + vinblastine	41	20.5	16
Medroxyprogesterone acetate	35	0	10 ($p = 0.19$)
Medical Research Council Renal Cancer Collaborators 1999 (127)			
Interferon-α	167	16	8.5
Medroxyprogesterone acetate	168	2	6 ($p = 0.017$)
Pyrhonen et al., 1999 (128)			
Interferon-α2a + vinblastine	79	16.5	16
Vinblastine	81	2.5	9 ($p = 0.0049$)

only agent so indicated. This decision was based on a study of 225 patients treated with high-dose IL-2. The overall response rate was 14% with a median response duration of 23 months (129). Longer durations have been reported, but in more selected patient populations. Randomized trials of high-dose IL-2 versus lower dose IL-2 or IFN-α regimens have demonstrated a slightly higher overall response rate, complete response rate, and durable complete response rate with high-dose IL-2, but no overall survival advantage has yet been demonstrated for high-dose IL-2. Administration of high-dose bolus IL-2 is complicated by significant toxicity, mainly the result of capillary leak. This phenomenon can lead to hypotension and pulmonary edema that often requires monitoring in the intensive care unit. As a result, the small but real benefit of high-dose IL-2 realized in the durable complete response rate must be balanced against these side effects.

IL-2 does not inhibit tumor cells directly, rather, it activates lymphocytes, both *in vitro* and *in vivo*. This observation prompted the administration of IL-2 in conjunction with lymphocytes derived from the host, so-called "lymphokine-activated killer (LAK) cells." When IL-2/LAK cell treatment was compared to IL-2 treatment alone in three randomized trials (130,131), the efficacy of the therapy was shown to be entirely derived from IL-2. Similarly, infusion of tumor-infiltrating lymphocytes (TILs) with high-dose IL-2 did not augment clinical benefit when studied in a randomized trial.

Combined Immunotherapy. In 1993, Vogelzang and colleagues (132) reviewed the literature on IL-2 combined with interferon. A total of 607 patients treated in 23 clinical trials had an overall response rate of 19%, which is not markedly different than the response rate with IL-2 alone. A randomized phase II trial of high-dose IL-2 and IFN-α versus IL-2 alone showed no difference in response (133). A second randomized trial showed a higher response rate with IL-2 and IFN-α compared to either agent alone, but survival with the combination was not better than with IL-2 alone (134). Although IL-2 and IFN-α are frequently given in combination for the treatment of RCC, the advantages and benefits of this approach have not been firmly established.

Other Immune Modulators. GM-CSF likewise proved ineffective (135,136). Interleukin-12 (IL-12) has shown variable efficacy in Phase II trials (137); however, it appeared to demonstrate synergy with IL-2 in animal models (138). Although no cytokines other than IL-2 and interferon have proved successful, ongoing efforts using different methods of immunomodulation are encouraging. For example, one trial of host-derived tumor cells fused to allogeneic dendritic cells demonstrated a complete response in 4 of 17 patients (139), although further follow-up has failed to confirm the initial findings. Efforts to induce the "graft versus tumor" effect with the administration of allogeneic lymphocytes (nonmyeloablative allogeneic peripheral blood stem cell transplantation or "mini bone marrow transplant") are ongoing in patients with RCC. The technique appears to have preliminary efficacy and substantial toxicity in a recently published series (140).

VEGF-Targeted Therapy. As described above, inactivation of the VHL tumor suppressor gene is observed in the majority of clear-cell RCC. The resulting VHL gene silencing leads to induction of hypoxia-regulated genes, including VEGF, a potent proangiogenic protein, and platelet-derived growth factor (PDGF). Therapy to inhibit VEGF has thus been pursued in RCC.

A recombinant human monoclonal antibody against VEGF (rhuMAb VEGF, bevacizumab, Avastin [Genentech, San Francisco, CA]) binds and neutralizes all biologically active isoforms of VEGF (141). The clinical utility of bevacizumab in metastatic RCC has been investigated in a randomized Phase II trial in which 116 patients with treatment-refractory, metastatic clear-cell RCC were randomized to receive placebo, low-dose (3 mg/kg) bevacizumab or high-dose (10 mg/kg) bevacizumab given intravenously every 2 weeks (142). An intent-to-treat analysis demonstrated a significant prolongation of TTP in the high-dose bevacizumab arm compared to placebo (4.8 versus 2.5 months; $p < 0.001$ by log rank test). Given the promising data demonstrating an effect of bevacizumab on TTP in RCC, an intergroup Phase III trial, investigating the addition of bevacizumab to initial IFN-α therapy in RCC, is underway (143).

Additional small molecule inhibitors of the VEGF and related receptors have also undergone initial clinical testing in metastatic RCC. Sorafenib is an orally bioavailable biaryl urea originally identified as an inhibitor of raf kinase, an enzyme involved in cellular proliferation and, subsequently, found to inhibit the VEGF and platelet-derived growth factor (PDGF) receptor families (144). Sorafenib was investigated in a Phase II randomized discontinuation trial in which 202 patients with metastatic RCC were evaluated. The overall percentage

of patients experiencing any tumor regression was 71%, with a progression-free survival (PFS) advantage in the subset of patients who were randomized to remain on sorafenib versus patients crossed over to placebo (24 versus 6 weeks; $p = 0.0087$) (145). A subsequent placebo-controlled, randomized Phase III trial of sorafenib in 905 cytokine-refractory RCC patients was conducted. This trial observed a PFS advantage in the sorafenib arm of 24 versus 12 weeks ($p < 0.000001$) (146). Based on these data, sorafenib was FDA approved for advanced RRC in December, 2005.

Sunitinib is an orally bioavailable oxindole small-molecule tyrosine kinase inhibitor of VEGF and PDGF receptor families (147,148). Two sequential Phase II trials of sunitinib in cytokine-refractory, metastatic RCC have been conducted (149,150). Objective response rates of 40% were observed, with additional patients experiencing tumor shrinkage. Based on these data, sunitinib was FDA-approved for advanced RCC in January, 2006.

Chemotherapy. RCC is particularly resistant to cytotoxic agents. The cells exhibit nearly uniform expression of P-glycoprotein, the protein product of the multidrug resistance (MDR) gene (151). Perhaps, for this reason, most standard chemotherapeutic drugs (such as doxorubicin and paclitaxel), which are transported from the cell by the MDR protein, have no demonstrable activity against RCC. Despite our growing understanding of the molecular and genetic basis of chemotherapy resistance, efforts to circumvent the P-glycoprotein resistance mechanism have been unsuccessful (152,153).

Motzer and Russo reviewed the results of Phase II trials conducted with 33 agents from 1990 to 1998. These studies tended to be small; few included more than 50 patients. The most extensively studied agents were floxuridine, fluorouracil, and vinblastine, with response rates ranging from 0% to 20%, depending largely on patient selection. The only single agents with consistently demonstrable, albeit modest, efficacy are the fluorinated pyrimidines (fluorouracil, capecitabine, floxuridine). Given the low response rates with these agents, patients with unresectable disease are appropriate candidates for investigational approaches. The more recently developed chemotherapeutic agents are being evaluated. For example, the combination of gemcitabine and fluorouracil initially demonstrated an objective response rate of 17% (154). Further experience with this regimen substantiates a modest 10% to 15% response rate, thus making it a viable alternative for treatment-refractory metastatic RCC.

Surgery. The role of surgical intervention in patients with metastatic RCC can be twofold: First, to render a patient clinically free of all sites of metastases ("metastasectomy"); and second, to resect the primary tumor when metastases are unresectable prior to initiation of systemic therapy ("cytoreductive nephrectomy"). Debulking nephrectomy has become a standard of care in selected metastatic RCC patients on the basis of two identically designed, prospective randomized trials (155). Eligibility for both trials included biopsy-proved T_{any}, $N_{any,}$ M1 RCC with a primary tumor amenable to resection as determined by the operating surgeon. Additional eligibility included ECOG performance status 0 or 1, no prior radiotherapy or systemic treatment of any kind, and adequate end-organ function. Eligible patients were randomized to radical nephrectomy (with or without lymphadenectomy), followed within 1 month after surgery by interferon-α (5 million units subcutaneously 3×/week) or to immediate interferon without preceding nephrectomy. Both trials were powered to detect an overall survival improvement, with analysis based on intent-to-treat criteria using Kaplan–Meier technique for survival duration. A combined analysis of 341 total randomized patients demonstrated an overall survival advantage for the nephrectomized group of 13.6 months versus 7.8 months ($p = 0.002$). Not surprisingly, the benefit was most pronounced in performance status 0 patients, but was not dependent on site of metastasis or disease measurability. The combined response rate in 253 patients with measurable disease revealed a 6.9% response rate in the nephrectomy arm versus a 5.7% response rate in the interferon-only arm ($p = 0.60$). Surgical morbidity and mortality were acceptable and did not prevent subsequent administration of interferon in 95% of nephrectomized patients at a median of 19 days after surgery. These trials provide convincing evidence of an overall survival benefit in appropriately selected patients. These data have been translated into the clinical practice of initial debulking nephrectomy followed by systemic therapy. Importantly, proper patient selection can maximize the benefit of this approach. Good performance-status patients with a resectable primary tumor representing the majority of tumor burden and without rapidly progressing extra renal disease or medical comorbidities should be considered for initial nephrectomy.

More recent metastasectomy studies have identified favorable clinical and surgical characteristics, but have never been assessed in a randomized clinical trial. Favorable subgroups include those patients with a solitary site of metastases (pulmonary most favorable), disease-free interval of >1 year, and <60 years of age (156). Surgical resection after a partial response to systemic treatment may be an option in selected patients.

TRANSITIONAL CELL CARCINOMA OF THE RENAL PELVIS AND URETER

The epithelial cells lining the urethra, urinary bladder, ureter, and renal pelvis, referred to collectively as the urothelium, can give rise to carcinoma at any point. When they arise in the renal pelvis, these transitional cell carcinomas (TCC) account for 5% of all urothelial tumors and about 5% to 10% of all renal tumors. In 1989, there were approximately 1,600 new cases of renal pelvic TCC, accounting for 700 deaths. The peak age at diagnosis is around 60 to 65 years (157). TCC is uncommon in persons less than 40 years of age. Published series consistently report a male predominance.

Clinical Presentation

The presenting symptoms are not unlike those of RCC. Hematuria is the most commonly reported symptom, occurring in 60% to 100% of patients depending on the series. Flank pain is reported in about one-third of patients. A palpable mass is seen less frequently and, like RCC, the triad of hematuria, pain, and mass is not commonly encountered. Multicentric disease, especially in the bladder, occurs in about 20% to 50% of patients (161–164). Therefore, diagnosis of one urothelial tumor mandates evaluation of the entire urinary tract.

Diagnosis

Intravenous pyelogram has long been the mainstay of renal pelvic tumor detection, but ultrasound, CT, and MRI are becoming more important. Urine cytology is helpful, but the number of false-negative results is high (164). The most definitive method of diagnosis is achieved via ureteroendoscopic techniques (165).

Staging

The staging of renal pelvic TCC has gone through numerous permutations and revisions over the past 60 years. The most commonly employed system was originally proposed by Grabstald and colleagues in 1971 (161). It was based on the staging of bladder TCC proposed by Jewett and Strong. Other staging systems have been based on those for RCC. In an effort to standardize renal pelvic TCC staging, the TNM system of the American Joint Committee on Cancer is recommended.

Treatment

Early stage disease has traditionally been treated with total nephroureterectomy with removal of a cuff of bladder. Studies demonstrating a high incidence of recurrence in the residual ureteral stump (166) illustrate the need for this bladder cuff resection. More conservative approaches via endourologic procedures have recently proved feasible. These procedures may be appropriate for patients with bilateral disease, significant comorbidities, or poor performance status. Laparoscopic nephroureterectomy (168,169), percutaneous management techniques (170), and retrograde ureteroscopic management (171,172) have all been used with success.

Metastatic or unresectable disease can be treated with the same chemotherapy utilized in metastatic bladder cancer. MVAC (methotrexate, vinblastine, doxorubicin, and cisplatin) has been the most commonly used chemotherapeutic regimen. A recent study suggested that treatment with gemcitabine and cisplatin was as efficacious as and less toxic than MVAC (173). High-dose MVAC with G-CSF may be associated with a greater 2-year survival rate than standard dose MVAC (174).

Prognosis

Stage is the most important predictor of survival, regardless of which staging system is employed. Early stage disease can be treated surgically with curative intent. In surgically treated patients, 5-year disease-specific survival rates have been reported as follows: 100% for Ta/*cis,* 91.7% for T1, 72.6% for T2, and 40.5% for T3. The outlook for patients with T4 tumors is poor, with a median survival of 6 months (175).

WILMS' TUMOR

Wilms' tumor, sometimes called nephroblastoma, is a primary renal cancer. Predominantly a pediatric disease, Wilms' tumor can occur in young adults. About 400 new cases are reported each year in the United States. Unlike RCC, Wilms' tumor responds well to treatment and curative therapy is available, even for metastatic disease.

Epidemiology

The majority of Wilms' tumors occur in children under 5 years of age (167). Although rare, adult cases are well documented (176). The prevalence among boys and girls is essentially the same. A number of risk factors for Wilms' tumor have been proposed, including paternal exposure to hydrocarbons and lead (178) and maternal exposure to cigarette smoke, coffee, oral contraceptives, and a number of other environmental factors (179). There have been several studies that fail to show these associations, however, leading most investigators to conclude that the bulk of available data does not definitively support the existence of environmental risk factors for Wilms' tumor (180).

Genetics

A number of congenital abnormalities are associated with Wilms' tumor including aniridia, WAGR syndrome (*W*ilms' tumor, *a*niridia, *g*enitourinary abnormalities, and mental *r*etardation), Denys-Drash syndrome, Beckwith-Wiedemann syndrome, hemihypertrophy, and trisomy 18 (181,182). Wilms' tumor has been associated with abnormalities in three genes: *WT1, WT2,* and an unnamed gene on the 16q chromosomal arm. Of Wilms' tumors, 20% show loss of heterozygosity at *WT1* located on the 11p13 chromosomal arm. Abnormalities at this gene are associated with WAGR syndrome and Denys-Drash syndrome (183). *WT2* is located at 11p15 and is linked to Beckwith-Wiedemann syndrome. Familial Wilms' tumor without any associated congenital abnormalities or mutations at 11p13, 11p15, or 16q has been described, suggesting that other gene loci are involved.

Presentation and Evaluation

The mean age at diagnosis is about 4 years (184). The most common finding at diagnosis is an abdominal mass, associated with pain in about one-half of cases. Hypertension, fever, and anemia (from hematuria or intratumoral hemorrhage) are not uncommonly seen. Ultrasound is the initial imaging procedure of choice, mainly for its ability to demonstrate the presence of tumor thrombi in the inferior vena cava. CT of the abdomen helps distinguish Wilms' tumor from neuroblastoma, which often arises suprarenally. Whether CT imaging of the chest is indicated for staging is controversial. Tissue specimens are classified into favorable and unfavorable histology, a distinction that requires an experienced pathologist. In general, unfavorable histology demonstrates anaplastic or sarcomatous features, while favorable findings include rhabdomyomatous or teratoid histology (185).

Treatment

The study of pediatric tumors has contributed a great deal to the general understanding of multimodality therapy. This approach has proved successful in Wilms' tumor, where treatment can involve surgery, radiation, and chemotherapy. Curative treatment generally requires two out of the three modalities.

Surgery

Surgical resection of unilateral disease remains the mainstay of treatment. After a full preoperative evaluation to determine the extent of the tumor as accurately as possible, the resection is generally performed via a transverse supraumbilical incision. The adrenal gland is generally removed only for lesions of the upper renal pole. Regional lymph nodes are sampled, but a formal lymph node dissection has not been proved beneficial.

Radiotherapy

Despite impressive cure rates with multimodality treatment, therapy-related toxicity is a significant problem. Radiation can contribute to poor bone growth, scoliosis, pulmonary fibrosis, fertility problems, and second malignancies. For this reason,

much effort has been directed toward defining more precisely the risks and benefits of radiation. Recent large randomized trials have helped clarify the indications for radiation therapy. For example, radiation is no longer recommended for patients with early-stage tumors and favorable histology. Radiation is given to all patients with stage III and IV disease, and stage II patients with unfavorable histology. The dose and field of radiation is based on histology, stage at diagnosis, and sites of gross residual disease after surgery.

Chemotherapy

Wilms' tumor was the first tumor in which adjuvant chemotherapy was successfully used. Current recommendations call for chemotherapeutic treatment at every stage of disease. The primary agents used are vincristine, dactinomycin, and doxorubicin, often administered together. Other agents with activity against Wilms' tumor include etoposide, cisplatin, carboplatin, cyclophosphamide, and ifosfamide. Reducing the toxicity of chemotherapeutic treatment, particularly doxorubicin, has been the goal of recent studies.

Relapsed Disease

Survival after recurrence is poor. One study found that the 3-year postrelapse survival of 367 children was only 30% (185). Salvage treatment with single agents or combination chemotherapy does have efficacy, but durable complete responses are rare. High-dose chemotherapy with stem cell rescue has been used for relapsed Wilms' tumor with some short-term success (186). Experience with this technique is too limited to characterize the impact on long-term survival.

Prognosis

Overall survival is excellent with current therapy. Early-stage, favorable histology patients have a 4-year survival of greater than 90%. Even patients with bilateral, stage IV, or unfavorable histology have an overall survival of about 70% (185).

SARCOMA OF RENAL ORIGIN

Primary renal sarcomas are rare in adults. One retrospective analysis identified 21 patients with the diagnosis out of 4,018 cases of primary renal malignancy (187). The most common histology is leiomyosarcoma, accounting for approximately 50% of these tumors. Complete surgical resection is the therapeutic goal. Resectability is related to vascular involvement, distant metastases, and the extent of local disease. As with soft-tissue sarcoma, in general, the resection of widespread disease may provide a survival benefit in treated patients (188); thus "resectable" is a relative term.

Extrapolation of data regarding soft-tissue sarcomas suggests that there is no role for adjuvant chemotherapy (189). The use of adjuvant radiotherapy has never been studied in a randomized prospective trial and available data have been inconclusive (190,191). Ifosfamide and doxorubicin have been used in advanced or unresectable soft-tissue sarcomas with some success (192,193).

RENAL ONCOCYTOMA

Renal oncocytoma is a rare primary renal neoplasm with unique microscopic features. The tumor consists of eosinophilic epithelial cells (oncocytes) with dense, mitochondria-rich cytoplasm (Fig. 28-9). The cell of origin is uncertain, but believed to be in the distal tubule or collecting duct (194). The diagnosis of oncocytoma can be suggested by CT findings of a sharply marginated, homogeneous mass with occasional stellate scar formation (195) (Fig. 28-10); however, imaging studies are not sufficiently accurate to forgo tissue sampling if RCC is suspected.

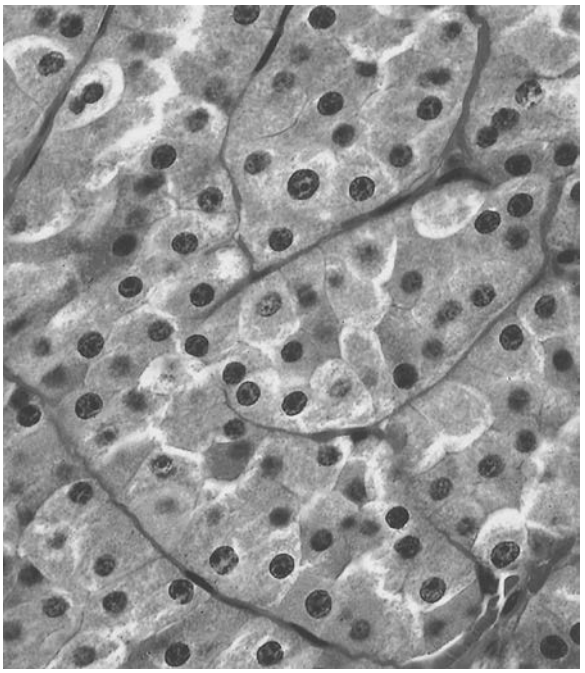

FIGURE 28-9. Renal oncocytoma. The cells are in tight alveolar arrangements and feature acidophilic cytoplasm. The nuclei are round and regular and exhibit no or single small nucleoli. (From: Murphy WM, Beckwith JB, Farrow GM. *Tumors of the kidney, bladder, and related urinary structures,* 3rd series. Washington, DC: The American Registry of Pathology, 1994, with permission.) (See Color Plate.)

The issue of whether these tumors metastasize is complicated by the limitations of past classification schemes for renal neoplasia. Early published series of oncocytoma cases almost certainly contain what we would today classify as RCC with eosinophilic cytoplasm. More recent, large series with better follow-up (196,197) have led some authors to state definitively that these are benign tumors.

The pathologic differentiation between an oncocytoma and a RCC with oncocytic features can be difficult. Furthermore, there are reports of both oncocytoma and RCC elements existing within the same tumor (198), or within the same kidney (199). Given these complicating factors, most authors agree with the surgical resection of large solid tumors of the kidney. In the setting of significant comorbidities and clinical data suggestive of oncocytoma, careful watchful waiting may be appropriate.

COLLECTING DUCT CARCINOMA

Also known as Bellini duct carcinoma (BDC), this rare tumor comprises much less than 1% of primary renal neoplasms. It is believed to arise from the collecting duct in the renal medulla. These tumors tend to be aggressive; most patients present with metastatic disease. Cytogenetic abnormalities have been reported, but all such studies include few patients. These investigators have noted monosomy of chromosome 1, 6, 14, 15, and 22 (200) and loss of heterozygosity of chromosomal arm 1q (201). The classic histology is described as neoplastic ducts and tubules associated with stromal desmoplasia (202). Few prospective data regarding systemic treatment strategies were

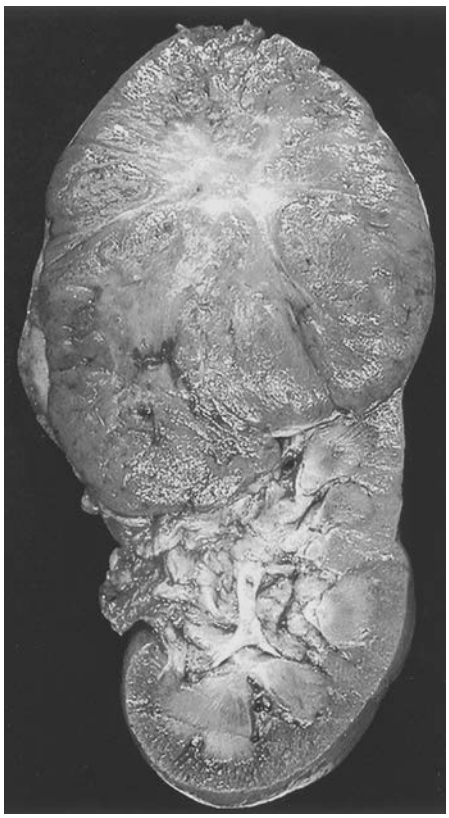

FIGURE 28-10. Renal oncocytoma. This mahogany-brown tumor exhibits a stellate central scar. Such scars can be visible on computed tomography (*CT*) images and suggest the diagnosis of oncocytoma. (From: Murphy WM, Beckwith JB, Farrow GM. *Tumors of the kidney, bladder, and related urinary structures,* 3rd series. Washington, DC: The American Registry of Pathology, 1994, with permission.) (See Color Plate.)

available, but, recently, a French group reported on a prospective trial using gemcitabine and either cisplatin (or carboplatin in patients with renal insufficiency). They treated 22 BDC patients; (median age 62; M:F ratio of 1:1) and reported 1 complete response, 6 partial responses, and 7 patients with stable disease. The median time to progression was 9.5 months and the median survival was 12.9 months; toxicity was modest. This is the first prospective clinical trial to be conducted in BDC and is the current standard of care (203).

MEDULLARY CARCINOMA

In 1995, Davis and colleagues published a case-series of 33 patients with sickle cell trait (one patient had sickle cell disease) who presented with an aggressive neoplasm originating in the renal medulla (204). The description of this entity has now been confirmed in other published reports (205–207). Like collecting duct carcinomas, these tumors generally behave aggressively and are often metastatic at diagnosis. In the series presented by Davis and colleagues (204), the mean survival after surgery was 15 weeks. Patients tended to be young; the mean age was 22 years with a male predominance. Microscopically, the tumor cells appear high grade and are intermixed with inflammatory cells. The pathology is suggestive of collecting duct carcinoma (CDC) and some authors have suggested that medullary carcinoma is an aggressive variant of CDC (205). Molecular or cytogenetic features that define this entity rarely been described. One series reported ABL gene amplification in 3 patients (208). As with CDC, these tumors do occasionally respond to conventional chemotherapy agents.

PHASE III TRIAL OF SUNITINIB VERSUS IFN-α

Motzer et al. (209) recently reported on a phase III trial of sunitinib versus IFN-α as first line therapy in 750 patients with good and intermediate risk RCC using Memorial Sloan Kettering Cancer Center risk group categorization. The primary end point was progression-free survival (PFS) with secondary end points being patient related outcomes, overall survival, response rate, and adverse events and safety. The trial had a 90% power to detect a 35% improvement in median PFS from 20 weeks to 27 weeks. Patients were randomized 1:1 to receive sunitinib 50 mg/day, by mouth, for 4 weeks followed by a 2 week off period (6 week cycles) or IFN-α 9MU subcutaneous TIW. There was no crossover, and the arms were well balanced. The median age was 60, and 90% had a prior nephrectomy. Approximately 35% had good risk disease, 58% had intermediate risk disease, and 7% had poor risk disease. The median PFS was 11 months for the sunitinib arm versus 5 months for the IFN-α arm (Hazard ratio 0.415, $P < 0.000001$). The objective response rate assessed by the investigators was 37% for sunitinib versus 9% for IFN-α ($P < 0.000001$) while the independent third party review recorded an objective response rate of 31% versus 6% ($P < 0.000001$). There was only one complete response (in the sunitinib arm). Of the sunitinib patients, 66% remain on study, while only 34% of the IFN-α patients remain on study. All subgroups benefited from sunitinib, including the poor risk patients (HR = 0.53). All adverse events were more common in the IFN-α arm except diarrhea, hand foot syndrome, and hypertension, which were more common in the sunitunib arm. Adverse events leading to withdrawal from the study occurred in 8% of patients on sunitinib and 13% on IFN-α. Only 1% of patients withdrew consent from the sunitinib arm, while 8% withdrew consent from the IFN-α arm. Although survival end points were still premature, the HR was 0.65 ($P < .02$) in favor of sunitinib. The investigators concluded that sunitinib was the new standard of care for first line treatment of metastatic RCC.

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CHAPTER 29 ■ BLADDER CANCER

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Cancer of the urinary bladder ranks eleventh in incidence among cancers worldwide. Internationally, approximately 310,000 new cases are diagnosed each year. The age-standardized incidence of bladder cancer is highest in industrialized countries (1). In the United States, cancer of the urinary bladder is the fourth most common cancer in men and the tenth most common cancer in women; an estimated 61,420 cases will be diagnosed (44,690 in men, 16,730 women) and 13,060 (8,990 men, 4,070 women) will die of their disease in 2006. The majority of patients (approximately 75%) present with localized disease (2).

EPIDEMIOLOGY

The incidence of cancer of the urinary bladder increases with age. In the United States, the probability of developing bladder cancer is low below age 40 (approximately 1 in 4,600) but increases markedly after age 60. Bladder cancer is more common in men than women with a ratio of 2:1 to 4:1. The disease is almost twice as frequent in white men as black men (2).

RISK FACTORS

Several chemical and environmental exposures have been linked to the development of bladder cancer. However, in most cases, the long latency period between the exposure and development of clinical disease has limited the ability to establish causality.

Industry-Related Carcinogens

A link between chemical carcinogens and the development of bladder cancer was first made in the early 1800s. Since then, multiple compounds have been implicated. The majority of these carcinogens are aromatic amines, including 2-naphthamine, 4-aminobiphenyl, and benzidine, or derivatives of aromatic amines. The time from exposure to death from bladder cancer has been estimated at over 20 years (3).

Occupations linked to the development of bladder cancer include workers in the aluminum, dye, paint, petroleum, rubber, and textile industries. Occupations involving exposure to "combustion gases" including chimney sweeps, truck drivers, and garage or gas station workers have also been associated with an increased risk (4–7). Case-control studies have suggested an increased risk in hairdressers and barbers, thought to be related to exposure to hair dyes (8). No significant association has been shown with personal hair dye use (9).

Tobacco Smoking

Cigarette smoking is the most important risk factor for bladder cancer and is thought to contribute to 1 to 2 of every three cases of bladder cancer diagnosed (10–12). The risk of developing bladder cancer increases with the extent of exposure to cigarette smoking; heavy smokers have a six- to tenfold increase in risk compared with nonsmokers. Smoking cessation leads to a decreased risk. In a meta-analysis of case-control studies, the risk decreased by 60% after 25 years (13). The precise carcinogens in tobacco linked to the development of bladder cancer are unclear. However, the aromatic amines 2-naphthylamine and 4-aminobiphenyl and the heterocyclic amine Trp-p-2 are likely candidates. Presumably as a result of less inhalation of smoke, the risk is lower in pipe and cigar smokers compared with cigarette smokers (14).

Diet

Several dietary factors have been linked to the development of bladder cancer. A case-control study suggested an increased risk with consumption of fried or fatty foods and a decreased risk with vitamin A intake (15). The quality of drinking water, through the by-products of water chlorination and concentration of arsenic, has also been associated with increased risk (16,17). Consumption of coffee and dietary sweeteners has not convincingly been associated with the development of bladder cancer.

Drugs and Diseases

Heavy use of the analgesic phenacetin has been associated with chronic renal disease and development of cancer of the bladder, ureter, and renal pelvis (18). There does not appear to be an association with acetaminophen, the major metabolite of phenacetin. The cytotoxic/immunosuppressive agent cyclophosphamide increases the risk of bladder cancer up to ninefold with a latency period of generally less than 10 years (19). Pelvic irradiation for prostate, cervical, or ovarian cancer, increases the risk of a secondary bladder cancer by a factor of 1.5 to 4 (19).

Schistosomiasis, caused by the trematode *Schistosoma haematobium*, is endemic to the Middle East, Southeast Asia, and Africa. Bilharzial bladder disease, secondary to infection with *S. haematobium*, has been linked to the development of bladder cancer (20). In endemic regions, bladder cancer is the most common solid tumor. The majority of cases are squamous cell carcinomas. Paraplegic patients (often with bladder stones or chronic indwelling Foley catheters) are also felt to be at increased risk for squamous cell carcinoma of the bladder.

PATHOLOGY

The lining of the urinary tract, the urothelium, extends from the renal pelvis to the urethra. The majority (90% to 95% in the United States) of cancers of the urothelial tract are transitional cell carcinomas (TCC) (21). Approximately 90% of transitional cell carcinomas develop within the bladder, whereas 8% develop in the renal pelvis and 2% develop in the ureter

and urethra. In North America, less common histologies include squamous cell carcinomas (3%), adenocarcinomas (2%), and small-cell carcinomas (<1%) (22). The distribution of histologic subtypes differs in other parts of the world. In *Schistosoma haematobium* endemic regions, squamous cell carcinomas predominate.

Adenocarcinomas of the bladder can arise in the urachal remnant at the dome of the bladder or in the periurethral tissues. Mixed histologies against a background of transitional cell carcinoma are not uncommon, and generally do not portend a worse prognosis. Lymphomas or melanomas of the bladder are rare.

Cancers of the urothelium demonstrate distinct growth patterns. The majority are papillary, arising from hyperplastic epithelium (21). While these lesions have a propensity to recur, they rarely progress to more invasive cancers (22). Conversely, *carcinoma in situ* (CIS) is a high-grade lesion and is thought to be a precursor in up to two-thirds of invasive cancers (23).

PATHOGENESIS

Cancers of the urothelium are characterized by a propensity to recur, both at the same site and at distant sites in the urothelial tract. This has led to the hypothesis that a field defect develops in the urothelium, leading to the tendency to form new tumors. Whether tumors that originate at separate sites in the urothelial tract are derived from the same clone is an area of controversy, although several studies support this concept (24,25).

A series of genetic events likely leads to the development (primary events) and progression (secondary events) of bladder cancer. These events are believed to result in the activation of protooncogenes and/or inactivation of tumor suppressor genes. Multiple studies support a key role for alterations in the p53 and retinoblastoma-susceptibility (RB) gene pathways. Altered expression of the RB gene product in primary bladder tumors obtained from cystectomy or transurethral resection specimens has been correlated with poorer survival (26,27). Nuclear accumulation of p53 protein, as determined by immunohistochemical analysis, has been associated with a higher probability of disease progression in superficial tumors (28) and disease recurrence/death in invasive tumors (29). The cooperative effects of alterations of these genes and others, including p21 and p16, has been highlighted by recent work (30–32).

CLINICAL PRESENTATION, DIAGNOSIS, AND STAGING

The majority of patients with bladder cancer present with superficial disease (approximately 75%), while 15% to 20% have invasive disease and 5% present with metastases (2). Hematuria is the most common presenting sign of bladder cancer. Most often, the hematuria is gross and painless and may be intermittent. Other disorders may present in a similar fashion. In a series of 1,000 patients presenting with gross hematuria, 15% were diagnosed with bladder cancer (33). While microscopic hematuria was detected in 13% of the population in one study, it was attributed to urothelial cancer in only 0.4% (34).

Pain related to bladder cancer typically results from locally advanced or metastatic disease. Flank pain may indicate ureteral obstruction. Suprapubic pain may be related to invasion of the perivesical soft tissue or bladder outlet obstruction and urinary retention. Bone pain may be a sign of bone metastases.

Irritative voiding symptoms including urinary frequency, urgency, dysuria, or urge incontinence most commonly occurs with CIS or tumors decreasing the bladder capacity or causing detrusor overactivity. Obstructive voiding symptoms, such as an intermittent stream or the sensation of incomplete voiding, are less common.

TABLE 29-1

CLINICAL STAGING OF PRIMARY BLADDER TUMOR

Noninvasive papillary tumor	Ta
Carcinoma *in situ*	Tis
Invasion of subepithelial connective tissue	T1
Invasion of superficial muscle	T2a
Invasion of deep muscle	T2b
Invasion of perivesical fat (microscopic)	T3a
Invasion of perivesicular fat (macroscopic)	T3b
Invasion of adjacent structures (prostate, uterus, vagina, bowel) but still mobile	T4a
Fixed to pelvic wall, abdominal wall	T4b

The diagnosis of bladder cancer is typically made by urine cytology and/or cystoscopic examination and a transurethral resection specimen. Clinical staging involves both information obtained from a pathologic review of the transurethral resection specimen and an examination under anesthesia. The transurethral resection specimen will determine if the muscle has been invaded (at least clinical stage T2); therefore, muscle must be present in the specimen for an adequate assessment. The exam under anesthesia affords an opportunity to determine whether there is a palpable mass (clinical stage T3b) and whether or not the mass is fixed (clinical stage T4b).

The cystoscopic examination begins with a bladder barbotage to examine urine cytology. The bladder is then systematically inspected and the size, number, location, and growth pattern of tumors are noted. Biopsies are obtained from visible tumors or tumors are resected transurethrally. Muscle must be present in the specimen for adequate staging. Random biopsies should also be taken from grossly normal urothelium and from the prostatic urethra.

Notably, there is marked discordance between clinical staging and the information derived from pathological review of the cystectomy specimen (pathologic staging). In a trial of patients receiving neoadjuvant chemotherapy in which 57% had a complete clinical response (no evidence of tumor by cystoscopic examination or transurethral resection), only 30% had no evidence of disease on pathologic review of the subsequent cystectomy specimen (35).

Computed tomography (CT) and magnetic resonance imaging (MRI) can be helpful in determining the presence of extension to the perivesical fat (T3) or involvement of the regional lymph nodes (nodes confined within the true pelvis). However, these imaging modalities do not accurately predict the depth of invasion in the bladder wall. Radionuclide bone scans are generally recommended in patients with locally advanced tumors and/or symptoms of skeletal involvement or in patients with unexplained elevations in alkaline phosphatase.

Clinical stage is most commonly assigned using the tumor, node, and metastasis (TNM) system (Table 29-1) (36). Postcystectomy, the TNM system is also used to assign the pathologic stage, which further refines prognosis. Nodal stage is determined by the size and number of involved regional lymph nodes.

TREATMENT

The selection of treatment for patients with bladder cancer involves consideration of the extent of disease and the patient's comorbidities, renal function, and preferences. Treatment

options range from endoscopic resection to multimodality approaches involving surgery, radiation therapy, and chemotherapy.

Superficial Disease

Superficial bladder cancer refers to patients with Tis (carcinoma *in situ*), Ta (papillary tumors confined to the urothelium above the basement membrane), or T1 (extension into the lamina propria) disease. Three main goals define the treatment of patients with superficial disease: removal of existing disease, prophylaxis against recurrence, and prevention of the development of more invasive disease. Generally, these lesions are first managed by complete endoscopic resection. In patients with tumors involving the lamina propria, a repeat cystoscopy is usually performed to document the absence of residual disease as a significant number of patients have more advanced disease than initially appreciated (37).

Approximately 40% to 80% of patients with complete resection of superficial disease will develop a recurrence within 6 to 12 months and 10% to 25% will have progression to more advanced stages. Therefore, vigilant follow-up with cystoscopic examination and urine cytology every 3 to 6 months is indicated. Higher-grade tumors are more likely to recur and progress to more advanced stages (38). Papillary noninvasive lesions (Ta) are typically low grade and recur multiple times before progression to invasive disease. Alternatively, CIS is generally high grade and frequently associated with progression to invasive disease. The majority of T1 lesions are high grade and 90% of patients will have a recurrence by 5 years (39).

Intravesical therapies have been developed in an attempt to minimize this high risk of recurrence with superficial bladder cancer. The most common indication for intravesical therapy is in the adjuvant setting after complete endoscopic resection of disease. Less frequently, intravesical approaches are utilized for patients with residual disease after transurethral resection.

Various guidelines have been proposed for selecting patients for intravesical therapy. Patients at highest risk for recurrence appear to benefit most from such therapy, which includes patients with multiple superficial recurrences within a short time period, multiple tumors, diffuse CIS, or T1 disease. In a study of intravesical bacillus Calmette-Guerin (BCG) in patients with these characteristics, treatment-delayed progression, decreased the need for cystectomy, and improved survival (40).

Several agents have been administered as intravesical therapy including BCG, mitomycin, the anthracyclines, interferon, and gemcitabine. The mechanism of action of these agents is not clear, although a nonspecific inflammatory reaction is felt to play a role. Randomized trials have demonstrated the superiority of BCG compared with doxorubicin or mitomycin (41,42). Side effects from these agents are similar including cystitis, fever, and hematuria.

BCG is instilled weekly for 6 weeks. Close surveillance is then performed with urine cytology and cystoscopy every 3 to 6 months. Some recommend a repeat 6-week course of BCG if a recurrence is detected. The criteria for proceeding with cystectomy in patients in whom intravesical therapy has failed are not well defined. Patients with impaired bladder function or recurrent T1 tumors within 6 to 12 months after transurethral resection and intravesical therapy are generally offered cystectomy.

Muscle-Infiltrating Tumors

Surgery

For patients with tumors infiltrating the muscularis propria (clinical stage T2), the standard approach in the United States is a radical cystectomy with pelvic lymph node dissection. Organ-sparing procedures may be possible in a select subgroup of patients. A radical cystectomy involves a wide resection of the bladder with all of the perivesical fat and tissue in an attempt to achieve negative margins. In men, a prostatectomy is also performed. In women, the urethra, uterus, fallopian tubes, ovaries, anterior vaginal wall, and surrounding fascia are removed. Urinary flow can either be directed through a conduit diversion or a continent reservoir. With a conduit diversion, urine is drained directly from the ureters to the skin surface with no internal reservoir. A segment of bowel is used to bridge the gap between the ureters and the skin. Continent reservoirs include continent stomas, in which patients self-catheterize at regular intervals, and internal, orthotopic neobladders, in which case patients may void in the natural position.

The prognosis after surgery for muscle-invasive disease is defined by the pathologic stage (Table 29-2). In a series of 1,054 patients with TCC treated with radical cystectomy and pelvic lymph node dissection, the 5-year disease-free survival for patients with node-negative organ confined disease exceeded 80%, but dropped to 62% to 78% for patients with extravesical disease (pT3) and 50% for patients with invasion of local organs (pT4) (43). While, pathologic involvement of the regional lymph node portends an even worse prognosis, some patients with lymph node involvement are cured with surgery alone. Five-year survival rates of up to 57% have been reported for patients with a single lymph node involved, compared with 0% to 27% for those with more extensive involvement (44–46).

The importance of an adequate pelvic lymph node dissection and pathologic assessment has recently been highlighted. With a standard pelvic lymph node dissection, the distal common iliac, external iliac, obturator, and hypogastric nodes are removed, generally yielding 10 to 14 lymph nodes (47). Retrospective studies have correlated the extent of lymphadenectomy with improved survival. In one such analysis, the association between surgical/pathologic variables and survival

TABLE 29-2

FIVE-YEAR SURVIVAL OF PATIENTS WITH BLADDER CANCER TREATED WITH CYSTECTOMY BY PATHOLOGIC STAGE[a]

Number patients	Year	T2	T3a	T3b	PT4	N−	N+	Reference
261	1991	63%	50%	15%	21%	60%	4%	(88)
269	2001	59%	22%	28%	27%	NA	NA	(89)
1054	2001	72%	58%	38%	33%	69%	31%	(43)

[a]T stages include patients with node- positive disease.

was explored in a series of 637 patients (48). Improved survival and decreased local recurrence was associated with an increased number of lymph nodes removed (and examined by the pathologist). This association was observed in both patients with node-negative and node-positive disease. The 5-year survival rate for patients with 0 to 6, 6 to 10, 11 to 14, and >14 lymph nodes examined was 33%, 44%, 73%, and 79%, respectively. Lymph nodes are dissected from the pathologic specimen by the pathologist and then counted. Similar results were reported from a second study in which surveillance, epidemiology, and end results (SEER) data on 1923 patients who underwent radical cystectomy was analyzed (49). Notably, the influence of surgical factors on outcome has been shown to be independent of the impact of perioperative chemotherapy. In an analysis of a randomized cooperative group study exploring neoadjuvant chemotherapy, a multivariate model adjusted for perioperative chemotherapy demonstrated that negative margins and ≥10 nodes removed were significantly associated with longer postcystectomy survival (50).

External-Beam Radiation Therapy

In many countries, external-beam radiation therapy is standard initial therapy for patients with invasive bladder cancer. A recent analysis reviewed the results of three randomized trials conducted before 1980 comparing preoperative radiation therapy followed by cystectomy with radiation therapy alone (51). While these trials were small and did not specifically address the issue of cystectomy versus radiation therapy, the authors did conclude that cystectomy provided a survival benefit. Randomized studies of radiation therapy versus surgery, using modern radiotherapy techniques, have not been performed. In a contemporary series, the cause-specific survival at 5-years among 163 patients with bladder cancer treated with external-beam radiation therapy (and salvage cystectomy) was 87% for T1, 48% for T2, and 26% for T3 disease (52). Patients who retain their bladder are at continued risk of developing new tumors and require close surveillance.

Side effects related to external-beam radiation therapy to the bladder include irritative symptoms of the bowel and bladder, inflammation of the skin, and fatigue. Sexual function may be impaired. Rarely, a reduction in bladder capacity due to fibrosis may necessitate cystectomy or urinary diversion.

Perioperative Chemotherapy

A significant proportion of patients undergoing cystectomy with curative intent will have micrometastases and ultimately succumb to their disease. Given the chemosensitivity of TCC, multiple clinical trials have sought to administer chemotherapy in the perioperative setting in an attempt to eradicate subclinical disease. These trials have utilized a variety of regimens either administered before (neoadjuvant) or after (adjuvant) surgery.

There are several potential advantages to a neoadjuvant approach. Before surgery, patients may be better able to tolerate chemotherapy. Systemic treatment is initiated sooner, theoretically against a smaller volume of microscopic disease. Importantly, this approach allows the response to treatment to be assessed by monitoring the primary tumor; this has been shown to be of prognostic significance. In a study of patients treated with neoadjuvant cisplatin-based chemotherapy, at a median follow-up of 25 months, 91% of patients who responded to chemotherapy (defined as pathologic stage ≤T1) were alive compared to 37% of nonresponders (53).

A disadvantage of the neoadjuvant approach is the discordance between clinical response (based on repeat cystoscopy/TURBT) and pathologic response (based on pathologic review of the cystectomy specimen). In one study of neoadjuvant chemotherapy, 57% of patients achieved a clinical complete response, but only 30% had a complete pathologic response (54). While neoadjuvant therapy may allow the consideration of organ preservation in a select subgroup of patients, chemotherapy is not a substitute for surgery and the majority of patients will still require cystectomy. In patients with a complete clinical response to chemotherapy, refusal of cystectomy becomes problematic. A theoretical disadvantage of neoadjuvant chemotherapy is the risk of delaying potentially curative surgery during administration of chemotherapy which may be ineffective.

Several randomized trials have explored the role of neoadjuvant chemotherapy in TCC (Table 29-3). While the majority failed to demonstrate a survival benefit with the use of preoperative chemotherapy, many of these studies were underpowered (55), utilized suboptimal chemotherapy (56,57), closed early (58), or were reported with inadequate follow-up time (59). Recently, large well-designed trials and a meta-analysis have been reported, which have shifted the standard of care in muscle-invasive TCC toward the routine use of neoadjuvant chemotherapy (60–62).

The largest neoadjuvant chemotherapy trial in TCC (63) randomized 976 patients with T2 to T4aNx disease to neoadjuvant CMV (cisplatin, methotrexate, and vinblastine) versus no chemotherapy. Management of the primary tumor was left to the discretion of the treating physician and could include cystectomy, radiation therapy, or both. When initially reported, a

TABLE 29-3

RANDOMIZED TRIALS OF NEOADJUVANT CHEMOTHERAPY[a]

Number patients	Neoadjuvant chemotherapy	Local therapy	Survival benefit	Reference
975	CMV	RT/Cyst/Both	Yes	(60)
317	MVAC	Cyst	Yes	(61)
325	C+A	RT/Cyst	Yes[b]	(62)
317	M+C	Cyst	No	(90)
206	MVAC	Cyst	No	(55)
104	C+5FU	Cyst	No	(91)
122	C	Cyst	No	(57)
123	MCV	Cyst/RT	No	(58)
171	MVEC	Cyst	No	(59)
194	Carboplatin MV	Cyst	No	(92)

[a] *M*, methotrexate; *C*, cisplatin; *V*, vinblastine; *A*, doxorubicin; *5-FU*, 5-fluorouracil; *E*, epirubicin; *Cyst*, cystectomy; *RT*, radiation therapy.
[b] Benefit for subset with T3 to T4.

TABLE 29-4

RANDOMIZED TRIALS OF ADJUVANT CHEMOTHERAPY[a]

Number patients	Adjuvant chemotherapy	Local therapy	Survival benefit	Reference
91	CAP	Cyst (CAP Cyst	Yes	(67)
49	MVA(E)C	Cyst	Yes	(66)
77	C	Cyst (C Cyst	No	(68)
83	CM	Cyst (CMCystCyst	No	(65)
50	CMV	Cyst	No	(69)

[a] *M*, methotrexate; *C*, cisplatin; *V*, vinblastine; *A*, doxorubicin; *5-FU*, 5-flurouracil; *E*, epirubicin; *Cyst*, cystectomy.

5.5% difference in the absolute 3-year survival (HR = 0.85; 95% CI 0.71 to 1.02) favoring the chemotherapy arm was observed, which did not achieve statistical significance. Updated results, with a median follow-up of 7 years, demonstrated a significant improvement in survival favoring neoadjuvant chemotherapy ($p = 0.048$; HR = 0.85; 95% CI 0.72 to 1.0) (60).

A United States intergroup trial has also shown a survival benefit with neoadjuvant chemotherapy (61). This study randomized 317 patients with T2 to T4a TCC of the bladder to three cycles of neoadjuvant MVAC (methotrexate, vinblastine, doxorubicin, and cisplatin) followed by radical cystectomy versus radical cystectomy alone. At 8.7 years median follow-up, both median survival (77 versus 46 months, $p = 0.06$) and 5-year survival (57% versus 43%, $p = 0.06$) favored the combined modality arm. Neoadjuvant chemotherapy did not impair the ability to proceed with surgery or increase the rate of postoperative complications.

A meta-analysis including data from 2688 patients (but not including the U.S. intergroup trial) further supports the use of neoadjuvant chemotherapy (64). Compared to local therapy alone, the use of neoadjuvant cisplatin-based combination chemotherapy was associated with a significant improvement in overall survival (HR = 0.87 [95% CI, 0.78 to 0.98, $p = 0.016$]) with a 5% absolute survival benefit at 5 years. Notably, the survival benefit did not reach statistical significance when trials utilizing single-agent cisplatin were included (HR = 0.91 [95% CI 0.83 to 1.01, $p = 0.084$]).

Delivery of chemotherapy in the adjuvant setting also has potential advantages and disadvantages. With postoperative chemotherapy, a treatment decision can be based on information derived from the pathologic rather than the clinical stage. This may avoid "over-treating" patients who are estimated to have a reasonable outcome from surgery alone (e.g., patients with organ-confined disease). Disadvantages include potential difficulties tolerating chemotherapy in the postoperative setting and the lack of objective means of assessing treatment response after the primary tumor is removed.

The role of adjuvant chemotherapy following cystectomy has been evaluated in several clinical trials (Table 29-4) (65–69). All of these trials were small, with the largest enrolling only 91 patients. Two of these trials did report a survival benefit with adjuvant chemotherapy. Skinner et al. (67) randomized patients with pT3-4 or node-positive TCC to receive CISCA (cisplatin, doxorubicin, and cyclophosphamide) for four cycles versus no further treatment after cystectomy. The median survival was 4.3 years in the adjuvant chemotherapy group versus 2.4 years in the observation group ($p = 0.006$). However, only 91 of 498 eligible patients were enrolled, raising concerns of selection bias. In a trial conducted by Studer et al. (68), patients with pT3b-T4a or node positive TCC were randomized to receive either three cycles of MVA(E)C (methotrexate, vinblastine, doxorubicin/epirubicin, cisplatin) or no further treatment after cystectomy. This study, designed to detect a 35% improvement in disease-free survival, enrolled 100 patients and was terminated early after 49 patients were enrolled after an interim analysis showed a significant improvement in 3-year disease-free survival (63% versus 13%, $p = 0.0015$). Additional randomized trials, limited by inadequate sample size and suboptimal chemotherapy, have not show a survival advantage with the use of adjuvant chemotherapy (65,68).

Neoadjuvant chemotherapy is the current standard for patients with (muscle-invasive) TCC of the bladder. Based on the limitations of the reported trials, the data supporting adjuvant chemotherapy is less compelling. The optimal management for patients with locally advanced TCC of the bladder, who did not receive neoadjuvant chemotherapy, is controversial and divergent opinions exist regarding the use of adjuvant therapy. This controversy is reflected in the design of two large ongoing international trials, one randomizing patients between adjuvant chemotherapy and cystectomy alone and the other randomizing patients between two different adjuvant chemotherapy regimens.

BLADDER-SPARING APPROACHES

In carefully selected patients, muscle-invasive disease may be treated with curative intent while attempting to spare the bladder. Attempts to spare the bladder have included transurethral resection of tumors alone, radiation therapy alone, chemotherapy plus conservative surgery, and combinations of all three modalities. In general, multimodality approaches seem optimal and such therapy requires the close collaboration of an experienced team. Given the risk of recurrent disease and new tumors in the retained bladder, ongoing surveillance is mandatory after completion of therapy.

In a small subgroup of patients, a partial cystectomy alone may be performed in an attempt to spare the bladder. Suitable patients for consideration of partial cystectomy include the following: a solitary tumor at the dome, posterior, anterior, or lateral bladder wall; a 2-cm margin of normal bladder around the base of the tumor; no prior history of TCC or CIS; and normal bladder capacity. However, few patients meet these criteria and local recurrence rates from 38% to 78% have been reported (70).

The combination of transurethral resection, chemotherapy, and radiation therapy may optimize bladder preservation. The best candidates are patients with a solitary early-stage lesion and no evidence of hydronephrosis. Critical to the success of this approach is the ability to initially perform a complete transurethral resection of tumor. Subsequently, patients receive

TABLE 29-5

SELECTED TRIALS OF COMBINED MODALITY BLADDER-SPARING THERAPY

Number patients	Adjuvant/Neoadjuvant chemotherapy	Radiation therapy	Radiation sensitizer	Survival	Intact bladder	Reference
42	None	Daily	C	52% 5-yr	NA	(93)
91	Neoadjuvant CMV[a]	Daily	C	62% 4-yr	44% 4-yr	(94)
34	None	Hypofractionated	C + FU	83% 3-yr	66% 3-yr	(95)
52	Adjuvant CMV	Twice daily	C	61% 3-yr	48% 3-yr	(96)

[a] *M*, methotrexate; *C*, cisplatin; *V*, vinblastine; *5-FU*, 5-fluorouracil.

concurrent chemoradiation with or without adjuvant or neoadjuvant chemotherapy. During (and after) the treatment period, cystoscopic surveillance of the bladder is maintained and cystectomy is performed if there is evidence of persistent invasive disease.

The optimal combined modality bladder-sparing regimen is not well defined. A series of trials exploring various regimens have been performed, many by the Radiation Therapy Oncology Group (RTOG) (Table 29-5). The necessity of neoadjuvant chemotherapy was explored in RTOG 89-03 in which patients were randomized to neoadjuvant cisplatin, methotrexate, plus vinblastine (CMV) followed by concurrent radiation plus cisplatin versus concurrent chemoradiation alone. There was no difference between the arms in survival with an intact bladder at 5 years median follow-up (58).

There have been no randomized trials comparing the results of bladder-sparing therapy with radical cystectomy. Compared with historical controls, the use of a combined modality approach in appropriately selected patients has yielded similar outcomes. Five-year survival rates of 50% to 60% have been reported with approximately 70% of those patients maintaining an intact bladder (71). In a recent analysis of quality of life in patients treated with bladder preservation, the majority of patients with an intact bladder preserved normal bladder function and low rates of bowel symptoms and urinary incontinence were reported (72).

METASTATIC DISEASE

Cisplatin is the most active single agent in urothelial TCC. During the late 1970s, trials evaluating single-agent cisplatin in patients with advanced TCC reported response rates ranging from 26% to 65%. Multiagent regimens followed shortly. In 1985, a landmark study explored the MVAC regimen. In this trial, 24 patients with advanced/metastatic disease were treated and responses were observed in 71% (95% CI, 53% to 89%) of patients (73). Complete responses were achieved in 50% (95% CI, 30% to 70%). Subsequently larger studies confirmed the activity of MVAC and randomized trials showed improved survival with MVAC compared to single-agent cisplatin (74) and CISCA (cyclophosphamide, cisplatin, and doxorubicin) (Table 29-6) (75).

Despite the benefits of MVAC, the limitations of this regimen in patients with advanced disease became readily apparent. While a substantial proportion of patients that responded to this regimen, median survivals were consistently less than 13 months. The durability of complete responses was poor, with

TABLE 29-6

RANDOMIZED TRIALS OF CISPLATIN-BASED CHEMOTHERAPY IN METASTATIC TRANSITIONAL CELL CARCINOMA

Treatment arms	Number patients	OR	CR	Survival (months)	*P*	Reference
MVAC[a]	120	36%	13%	12.5	<0.0002	(74)
Cisplatin	126	11%	3%	8.2		
MVAC	55	65%	35%	12.6	<0.05	(75)
CISCA	55	46%	25%	10		
MVAC	86	59%	24%	12.5	0.17	(97)
FAP	83	42%	10%	12.5		
MVAC	129	58%	9%	14.1	0.122	(98)
HD-MVAC	134	72%	21%	15.5		
MVAC	205	46%	12%	14.8	0.746	(77)
Gemcitabine + Cisplatin	203	50%	12%	13.8		
MVAC	109	54%	23%	14.2	0.025	(78)
Docetaxel + Cisplatin	111	37%	13%	9.3		
MVAC[b]	44	40%	13%	14.2	0.41	(83)
Paclitaxel + Carboplatin	41	28%	3%	13.8		

[a] *MVAC*, methotrexate, vinblastine, doxorubicin, cisplatin; *CISCA*, cyclophosphamide, cisplatin, doxorubicin; *CMV*, cisplatin, methotrexate, vinblastine; *MV*, methotrexate, vinblastine; *FAP*, 5-fluorouracil, interferon-α2b, cisplatin; *HD-MVAC*, high-dose MVAC; *OR*, overall response; *CR*, complete response.
[b] Trial terminated early with only 85 patients.

less than 4% of patients alive and disease-free at 6 years or more (76). Perhaps most limiting was the associated toxicity; febrile neutropenia and mucositis were common and treatment-related deaths occurred in 2% to 4% of patients. Other side effects included decreased renal function, hearing loss, and peripheral neuropathy.

Over the past decade, efforts have focused on not only improving the efficacy of treatment in patients with advanced TCC, but also on improving the tolerability of treatment. Several newer agents with activity in TCC have been identified and incorporated into multiagent regimens.

A landmark multicenter Phase III trial randomized 405 patients to gemcitabine plus cisplatin (GC) versus MVAC (Table 29-6) (77). While this was not designed as a noninferiority trial, the response rates and median survivals were similar on both arms. Importantly, MVAC was associated with a greater incidence of neutropenic fever (14% compared to 2%), neutropenic sepsis (12% compared to 1%), grade ≥3 mucositis (22% compared to 1%), and treatment-related deaths (3% compared to 1%). Gemcitabine plus cisplatin has since become a widely used standard treatment regimen for patients with metastatic TCC.

Other cisplatin-based doublets and triplets have been recently been explored. In a randomized Phase III trial, docetaxel plus cisplatin (DC) was compared with MVAC (78). Although DC was better tolerated, response rates and survival favored the MVAC arm. Phase II trials exploring triplet combinations of gemcitabine, cisplatin, plus paclitaxel (GCT) and ifosfamide, cisplatin, plus paclitaxel have reported promising activity (ITP, ifosfamide, paclitaxel, and cisplatin) (79,80). A randomized Phase III study comparing gemcitabine plus cisplatin versus gemcitabine, cisplatin, plus paclitaxel is ongoing.

Up to 40% of patients with TCC may be ineligible for cisplatin-based therapy due to impaired renal function. As a result, carboplatin-based therapy has been extensively explored. No Phase III trials have directly compared carboplatin-based therapy with cisplatin-based therapy and controversy exists regarding the equivalence of such regimens. In randomized Phase II trials, consistently higher response rates have been reported with cisplatin- compared with carboplatin-based therapy (81,82). The Eastern Cooperative Oncology Group (ECOG) initiated a Phase III trial comparing MVAC with paclitaxel plus carboplatin; however, this study was terminated after 2 years of slow accrual (83). In patients with advanced TCC without contraindications (e.g., poor creatinine clearance, solitary kidney, poor performance status), cisplatin-based therapy should be considered the treatment of choice.

The majority of patients with advanced TCC still succumb to their disease, necessitating novel approaches. One new approach is the administration of sequential dose-dense chemotherapy based on the Norton–Simon hypothesis, a mathematical prediction model of chemotherapy sensitivity derived from the Gompertzian growth rates of tumors (84). Other approaches involve the integration of novel targeted therapies.

Based on the promising results with the ITP regimen, a pilot study of sequenced therapy with doxorubicin and gemcitabine (AG) followed by ITP was performed (85). A preliminary analysis of 21 patients demonstrated an major overall response rate of 87% (95% CI, 71% to 100%) and a complete response of 43% (95% CI, 22% to 64%) (86). This regimen is being further explored in the setting of advanced disease and a modified version (AG followed by paclitaxel plus cisplatin) is the experimental arm of a national adjuvant therapy trial.

Therapies targeting the epidermal growth-factor receptor (EGFR) pathway given in combination with cytotoxic agents are also being explored. Cooperative group trials are evaluating trastuzumab given in combination with paclitaxel, carboplatin, and gemcitabine or gefitinib in combination with either gemcitabine/cisplatin or gemcitabine/carboplatin.

PROGNOSIS IN ADVANCED DISEASE

Pretreatment patient characteristics can be used to assess prognosis in patients with advanced TCC treated with cisplatin-based combination chemotherapy. In a multivariate analysis of predictors of survival in 203 patients with TCC, two factors had independent prognostic significance: Karnofsky performance status ≤80% and visceral (lung, liver or bone) metastases (87). The median survival for patients with 0, 1, or 2 risk factors was 33, 13.4, and 9.3 months, respectively ($p = 0.0001$).

SUMMARY

Bladder cancer is a common disease. Over the past decade, much progress has been made in understanding the pathogenesis of this disorder and elucidating the key genetic pathways involved. The clinical course and management of bladder cancer is heterogenous. The management of superficial lesions typically involves endoscopic resection with or without intravesical therapy. Muscle invasive disease is best managed by radical cystectomy with neoadjuvant chemotherapy. In a select subgroup of patients, a combined modality approach involving surgery, radiation therapy, and chemotherapy may successfully spare the bladder. For patients with advanced disease, strides have been made in the activity and tolerability of systemic chemotherapy yet few patients are cured. Novel approaches, integrating agents targeting pathways involved in the pathogenesis of bladder cancer, are being explored in an attempt to further improve outcomes.

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CHAPTER 30 ■ PROSTATIC CARCINOMA

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EPIDEMIOLOGY AND ETIOLOGY

Prostatic carcinoma is the most commonly diagnosed noncutaneous malignancy in American males, with approximately 170,000 new cases diagnosed each year (1). Prostate cancer is the second most common cause of male deaths caused by cancer and, although the government initiative *Healthy People 2010* aims for the mortality of prostate cancer to be less than 28 per 100,000, few states have yet to achieve this in 2004. Death rates range from 25 to 37/100,000 in Caucasians across the United States and are generally more than twice this for African American men (1). In the year 2000, there were more than 400 million people worldwide aged 65 or above. This number is projected to increase to almost 1.5 billion by the year 2050, 25% of whom will be aged 80 or above. This close to fourfold increase is only partially accounted for by the projected 50% increase for the global population, as a whole, and is due largely in part to improvements in longevity (2). Specifically in America, in 2020, 5.9% of the population—or 17,000,000 individuals—will be age 75 or older. Prostate cancer may appear in epidemic proportions as approximately one-third of these seventeen million citizens are male and autopsy studies suggest that up to 60% of men harbor tumors (3).

The fact that prostate cancer has the lowest *average* number of life-years lost to all major cancers in men or women prompts some to suggest that early detection and treatment of this neoplasm are a low priority, despite the fact that it causes more deaths among men less than 65 years of age than diseases that receive greater research support, such as nephritis, lymphoma, and leukemia (4). Yet, when placed in the context of the enormous numbers of men who die of prostate cancer, the total number of potential life-years lost to this cancer ranks it *third* among 17 malignancies studied in men (5). Thus, the overall impact of prostate cancer is significant.

One of the risk factors for prostate cancer is age. Most autopsy series show a positive correlation of prostate cancer incidence and age. However, prostate cancer prevalence in younger men may be higher than expected from clinical practice. One autopsy study of men less than 50 years old who died of trauma demonstrated microscopic foci of prostate cancer in 29% of those 30 to 39 years old and in 32% of those in their forties (6). Exposure to environmental agents has been evaluated and cadmium appears to be a risk factor for the development of prostate cancer (7). History of benign prostatic hypertrophy (8) or previous vasectomy (9) do not appear to have any bearing on the risk of developing prostate cancer.

Race, as alluded to earlier, is also a risk factor for prostate cancer. Northern European and black populations, particularly in the United States, tend to have high mortality rates for prostate cancer, whereas persons of Asian origin have low clinical incidence rates (10,11). Although serum testosterone quantitation has been used to explain this variation (long-term testosterone injections in rats results fairly predictably in prostate cancer), the true etiology and mechanism of this discrepancy is still being investigated. A recent study out of Finland found *decreased* androgen levels to be a significant risk factor for prostate cancer (12). Leptin, insulin, and insulin-like growth factor (IGF-1), and their interactions with testosterone may be responsible for these findings. A recent analysis suggests that IGF-1 may be directly related to the rate of developing prostate cancer. Men in the upper quartile for IGF-1 serum levels have a twofold increased risk compared to the general population (13). Recent data on incidence of latent carcinoma in Japan show a significant upward trend, whereas clinical prevalence remains low (14). This suggests similarity of initiating factors in malignant transformation, with important genetic or geographic differences in promoting factors. A landmark study (prostate cancer prevention trial) recently evaluated the effect of 5-α reductase inhibition and prostate cancer risk. The prospective study randomized 18,882 men (55 years of age or older) with a normal digital rectal examination (DRE) and a prostate-specific antigen (PSA) of $\leq$3.0 to daily finasteride or placebo. DREs were performed and PSAs were drawn at routine clinical intervals; transrectal ultrasound guided-needle sextant biopsies were performed when there was a corrected PSA of >4 or an abnormal DRE. At the end of 7 years, all the men who had not previously had a biopsy for PSA elevation or change in DRE underwent a biopsy. Of the men who had data for the final analysis, prostate cancer was detected in 18.4% of the 4368 men treated with finasteride and 24.4% of the 4,692 men who received placebo, resulting in a 24.8% reduction in the prevalence of prostate cancer over 7 years of therapy. Tumors of Gleason grade 7–10 were significantly more common in the finasteride group (37.0%) than in the placebo group (22.2%) (p <0.001). This represented 6.4% of the men included in the final analysis of the finasteride group and 5.1% of the men in the placebo group ($p = 0.005$) (15).

Family history also plays an important role in prostate cancer risk. A man has a threefold increased risk of prostate cancer if his father or brother has been diagnosed with the disease and up to a ninefold increased risk if he has more than two first-degree relatives have been diagnosed with the disease (16). Studies comparing concordance rates for monozygotic and dizygotic twins indicate that as much as 42% of prostate cancer can be attributable to heritable factors (17). The first locus found to be implicated in prostate cancer is HPC1 on chromosome 1. Now others on various chromosomes (17q and 8p) have been identified. However, despite extensive efforts using linkage analysis, a gene that accounts for more than a small percentage of familial prostate cancer cases has not been found (18).

Dietary or other cultural influences are implicated by studies showing that persons of Japanese ancestry living in the United States have a prostate cancer mortality rate that is between that of Japanese men in Japan and Caucasian men in the United States (19). Several other studies suggest a relationship between diet and carcinoma of the prostate. Dietary risk factors identified to date include a high intake of proteins, total fat, saturated fat, and possibly high intakes of vitamins A and C (20). Studies performed for skin cancer revealed that selenium and vitamin E may provide some protection from prostate cancer development. A trial coordinated by the Southwest Oncology Group (SWOG 9917) is a prospective randomized trial designed with

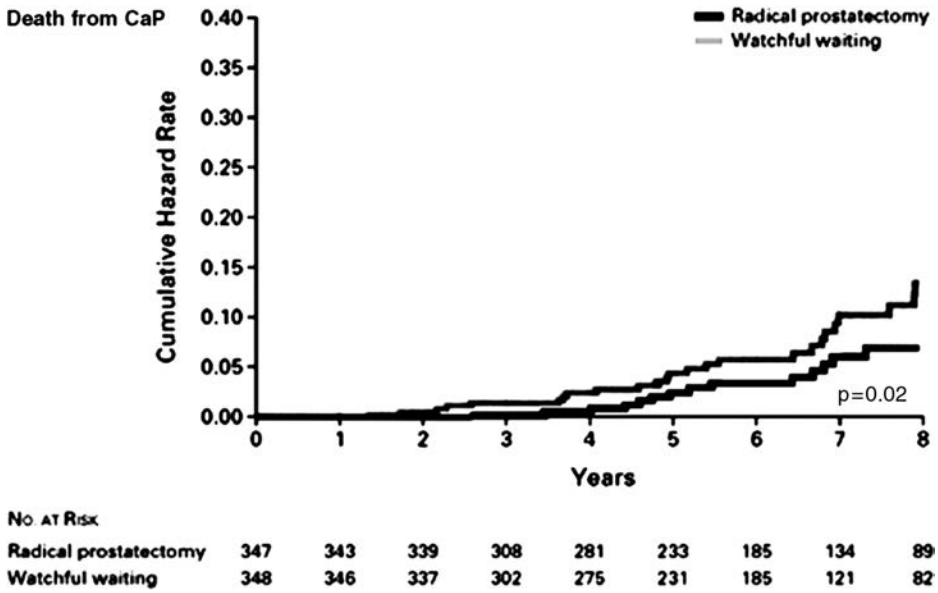

FIGURE 30-1. Eight-year analysis of 695 men randomized to radical prostatectomy or watchful waiting with cancers detected by PSA screening. Death in surveillance group 13.6%, prostatectomy group 7.1% with surveillance patients allowed to be placed on androgen deprivation when disease was progressing. (From: Holmberg L, Bill-Axelson A, Helgessen F, et al. A randomized trial comparing radical prostatectomy with watchful waiting in early prostate cancer. *N Engl J Med* 2002;347:781.)

enough power to draw conclusions about the effect of selenium on patients with prostatic intraepithelial neoplasia. Similarly the SELECT trial is a prospective, randomized four-arm trial evaluating the benefit of selenium alone, selenium with vitamin E, vitamin E alone or placebo in patients with PSA ≤4 ng/mL. Lycopene is the third focus in prostate cancer prevention. Lycopene has been shown to decrease adverse pathology on radical prostatectomy and will likely continue to be evaluated (21). Phase I and II studies are also ongoing to evaluate the efficacy of vitamin D analogs in the prevention of prostate cancer (22). Other investigations into prevention include soy, green tea, and cycloogenase inhibitors.

PROSTATE CANCER SCREENING

The value of early detection is probably one of the more controversial areas topics in prostate cancer. Early detection in asymptomatic men is of major interest because it is generally believed that men presenting with symptomatic prostate cancer usually are incurable at the time of diagnosis. This simple observation has prompted recent efforts to detect more cases of prostate cancer at an asymptomatic stage, when cure is possible. The discovery of prostate-specific antigen (PSA) has enhanced these efforts. Salient arguments against routine screening center around a number of issues, including lead-time and lag-time bias, financial as well as quality-of-life treatment costs, imperfect screening tests, and lack of consistent evidence that current prostate cancer treatments decrease mortality. Proponents, on the other hand, point out that properly conducted screening utilizes one of the best tumor markers available, detects significant cancers, is cost effective, and has lead to significant stage migration.

The debate regarding the efficacy of treatment of early prostatic carcinoma includes lead- and length-time bias arguments. Opponents of screening state that because of lead-time bias, a diagnosis of prostate cancer may be made earlier, but the patient dies at the same point in time. Opponents also argue that because of length-time bias, generally insignificant nonthreatening tumors are detected and that when aggressive tumors are detected, they present at such clinically advanced stage that current treatments are ineffective.

The lead time provided by PSA, appears to be between 5.4 and 12.3 years (23,24). This is significantly longer than the 1.7-year time frame provided by mammography screening (25). With the results of the Scandinavian trial showing a huge trend toward improvement in disease-specific survival with prostate cancer treatment versus observation at 7-year follow-up (13.6% death due to prostate cancer with surveillance versus 7.1% with extripation; Fig. 30-1) and decreased painful bony metastases with treatment (27.3% rate of metastatic disease with surveillance versus 13.4% with definitive therapy; Fig. 30-2), it appears that a pure lead-time bias is not occurring (26). It is expected with further follow-up of this study a benefit in overall survival with treatment will also become evident. The fact that definitive prostate cancer treatment appears to decrease metastases and increase disease-specific survival helps explain why the overall mortality in the United States is declining, although the population as a whole is getting older and the incidence has increased (Fig. 30-3). These data obviously argue strongly in support of the effectiveness of prostate cancer screening (27).

Opponents are also concerned that prostate cancer screening detects nonthreatening latent tumors. However, autopsy series show the incidence of latent prostate cancer approaches 50% in men less than 50 years old and 70% in men 70 to 80 years of age (28). Prostate cancer incidences in the United States in areas where screening is prevalent are reported to be 4% to 6.4% in men 50 to 70 years of age and 10% in men 70 to 80 years of age (29). Clearly, all latent tumors are not being detected. Artificial neural networks evaluating autopsy and prostatectomy data predict that screening detects only 29% to 44% of latent tumors (30). Jonathan Epstein estimates the percentage of the low-volume low-grade potentially clinically "insignificant" tumors to comprise only 14% to 16% of the cancers removed by radical prostatectomy at Johns Hopkins (31). Gerald Andriole has evaluated the difference of cancers detected by screening at Washington University and incidental cancers found in cystoprostatectomy and autopsy specimens. The volume of tumor, Gleason's scoring, and percentage of tumors that are extracapsular are all significantly higher in tumors detected by PSA screening (Table 30-1).

Opponents of PSA screening have concerns about the cost of screening as well as the costs associated with treatment. Annual costs of prostate cancer screening appear to be in line with other life-saving programs. Annual costs of PSA screening are estimated to range between $8,700 to $145,000 per patient (depending on PSA cut points and inclusion or exclusion of biopsy

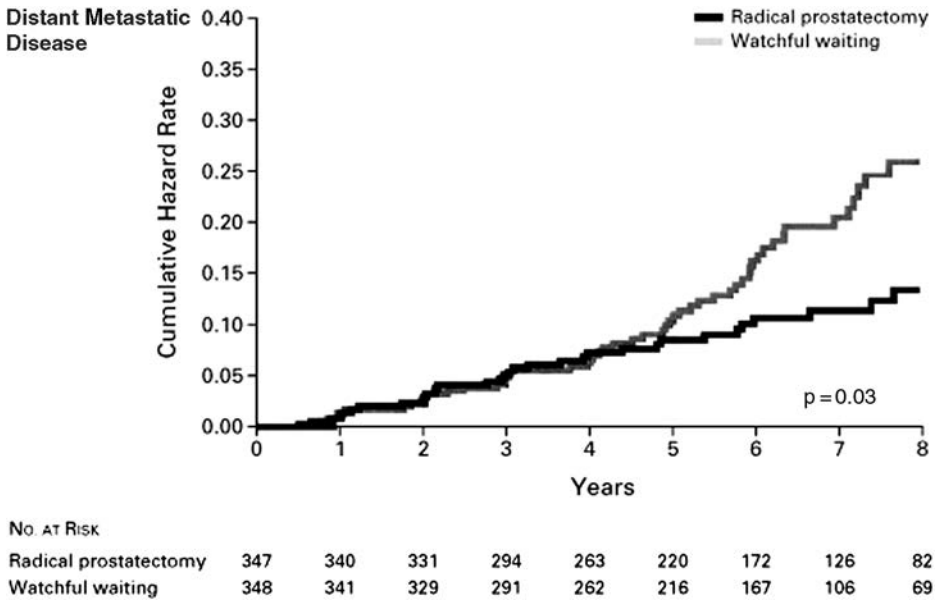

FIGURE 30-2. Eight-year analysis of 695 men randomized to radical prostatectomy or watchful waiting with cancers detected by PSA screening. Metastatic rate in surveillance group 27.3%, prostatectomy group 13.4% (From: Holmberg L, Bill-Axelson A, Helgessen F et al. A randomized trial comparing radical prostatectomy with watchful waiting in early prostate cancer. *New Engl J Med* 2002;347:781.)

and treatment costs). Even in the worst-case scenario, this is considerably less than mammography ($232,000 per patient per year), which has similar screening efficacy concerns, but is widely accepted (32). In addition, the costs of treatment to a patient's quality of life have also significantly declined. Although older studies find incontinence and erectile dysfunction rates to be 63% and 90%, respectively, modern series reporting on patients who undergo nerve-sparing procedures indicate these rates are closer to 5% and 40% (33,34). Furthermore, studies show 3.5 years of quality-adjusted life expectancy in patients who undergo definitive treatment, even in patients with moderately to poorly differentiated prostate cancers (35).

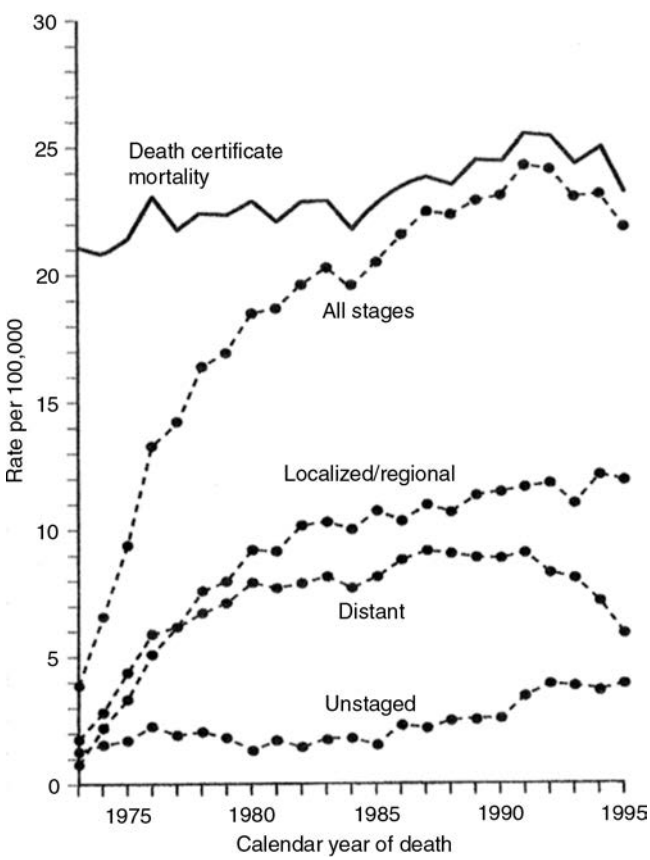

FIGURE 30-3. Incidence and mortality rates of prostate cancer in the United States. (From: *Campbell's Urology*, 8th ed. Philadelphia: Walsh, Retik, Vaughan, and Wein, 2002.)

Unfortunately, PSA is an imperfect marker. With use of these databases, sensitivity, specificity, and positive predictive values have been developed for the test (Table 30-2) (36–39). Digital rectal exam is also not sensitive in screening for early disease when used alone. Historically, the detection rate by DRE and digitally guided biopsy was reported to be 1.3% to 1.7% (40,41). However, when DRE, PSA measurement, and transrectal ultrasound (TRUS) were used together in this large screening study of over 1,000 men, the detection rate increased to 3.6%. In one prostate-cancer screening program, 38% (12 of 32) of carcinomas detected would have been missed by DRE alone, whereas another study reported that 32% to 43% would have remained undetected by DRE or TRUS alone (42,43). Age-specific reference ranges have been developed that attempt to increase the sensitivity of detection in a younger age group, while increasing the specificity in older men. In fact, these age-specific reference ranges are now widely utilized by many reporting laboratories (Table 30-3). PSA specificity is also improved by considering the use of PSA velocity (rate of rise over time) (with a change greater than 0.75 being considered a significant risk of cancer). PSA density (amount of PSA in serum divided by prostate volume on ultrasound), although intuitively reasonable (with values greater than 0.15 implying greater risk) does not improve specificity as much as expected and can miss significant cancers (44). Consideration of percentage of free PSA, does, however, increase specificity (the more PSA protein that is unbound to serum proteins the higher the risk of cancer with 15% considered a general cut point) (45). Fortunately, intense work is being performed on other PSA polymorphisms and proteomics, which may add more sensitivity and specificity to the diagnosis of cancer. Proteomics uses mass spectroscropy to measure the weights of various proteins using a surfaced-enhanced laser. Each mass spectroscropy creates a "signature," which correlates well with the presence or absence of cancer. Early reports using this technique show both a sensitivity and specificity of over 85% (46).

The controversy of the true efficacy of prostate cancer screening will not be resolved until formal, controlled

TABLE 30-1

COMPARISON OF SCREENED VERSUS INCIDENTAL PROSTATE CANCERS

	Screening cancer	Cancer at autopsy	Cancer-cystectomy
Extracapsular (%)	20	0	2
Volume cancer (mL)	0.5–2.5	0.5–0.15	0.02–0.7
Gleason score ≤6 (%)	4–26	94	78

(Modified from: Lecture by Dr. G. Andriole National Urologic Oncology, Update 2003, Long Beach, CA.)

large-scale trials have been completed. These trials are underway and involve long-term follow-up of thousands of men in America and Europe (PLCO and ERSCP trials, respectively). Until the data are available, however, it is important to know that prostate cancer mortality continues to decline in America where screening is heavily practiced, prostate cancer screening appears to have similar or lower costs of other screening programs, and sensitivities and specificities of screening can be improved by using age-adjusted PSA, PSA velocity, and percentage of free PSA. It is also important to know that because of screening in the United States, currently only 2% to 4% of patients with prostate cancer are diagnosed with metastatic disease and only one-third of patients are diagnosed with extracapsular extension or large-volume disease (47–49). Prior to widespread screening efforts two thirds of patients diagnosed with prostate cancer were found to have either locally advanced or metastatic disease (50,51).

DIAGNOSIS

There has been an effort to reestablish PSA threshold that is considered normal. Although a value less than 4 ng/mL is usually considered within normal limits, men with a PSA between 2 to 3 ng/mL, have a relative risk of having or developing prostate cancer five times greater than men with a PSA <1 ng/mL. According to Catalona, 82% of the cancers in younger men and 65% of the cancers in older men would have been missed using the traditional PSA threshold of 4.0 (52,53). An analysis of blood samples collected prospectively in men who developed prostate cancer, mathematically determined the best PSA threshold to be 3.3 ng/mL in the Physician's Health Study (54). In men undergoing biopsy in the previously discussed Prostate Cancer Prevention Trial (PCPT), 15.2% of the control patients diagnosed with prostate cancer had a PSA ≤3.0 ng/mL and 14.9% of these were Gleason's grade 7 or higher (55). The results of this study and of those already discussed suggest that the biopsy incidence of prostate cancer in men with PSA levels of 1 to 10 ng/mL is relatively similar and suggest that PSA values in this range are more a reflection of benign prostatic hyperplasia than cancer stage. However, no study has evaluated the outcome of men who have undergone therapy with lower PSA threshold-detected cancer and compared them to outcomes of therapy in men with prostate cancer detected by traditional PSA thresholds. Most urologists make the decision on when to proceed with prostate biopsy by considering PSA value in the context of several other factors such as patient age, family history, race, PSA polymorphism and velocity determinations, as well as patient comorbidities.

When a biopsy is performed, cancer detection rates can be improved with an increased number of cores and a laterally directed technique. Ten to twelve core samples is considered the current standard of care and decreases the false-negative biopsy rate (56). Laterally directed prostate biopsies, as shown in Figure 30-4, contain greater volumes of peripheral zone and, therefore, are more sensitive in detecting prostate cancer (57). Color doppler has been combined with conventional ultrasound technology resulting in a small improvement in the sensitivity of ultrasound in detecting prostate cancer (58).

STAGING

Although there is no true "premalignant lesion," which has been definitively shown to progress to invasive prostate cancer, prostatic intraepithelial neoplasia (PIN) is thought to be associated with a high risk of concurrent or subsequent development of adenocarcinoma. In a case-controlled study of 100 patients showing high-grade PIN matched with 112 biopsied patients without PIN, 36% of those with PIN were diagnosed with prostate cancer within 24 months. In contrast, only 13% of the PIN-negative group with otherwise similar risk factors developed invasive disease in the same time frame (59).

TABLE 30-2

CALCULATED PERCENTAGES OF SENSITIVITY, SPECIFICITY, POSITIVE/NEGATIVE PREDICTIVE VALUE, AND OVERALL ACCURACY OF PSA DETERMINATION AS SCREENING TEST FOR PROSTATE CANCER

				Predictive value		
Study	Patients	Sensitivity (%)	Specificity (%)	Positive (%)	Negative (%)	Overall accuracy (%)
Catalona (24)[a]	235	79.0	59.0	40	89.0	64.0
Cooner (25)	1807	80.2	74.6	35	95.7	75.5
Labrie (26)	1002	72.0	91.2	33	98.1	90.1
Powell (27)	211	89.5	90.0	47	NA[b]	NA[b]

[a]Comparison group.
[b]NA, not available.

TABLE 30-3

AGE-SPECIFIC PSA RECOMMENDATIONS

Age (years)	PSA (ng/mL)
50–60	<2.5
60–70	<3.5
70–80	<4.5

Accurate staging of prostatic carcinoma remains a difficult problem. When surgical staging is carefully performed, it is highly predictive of survival. In Table 30-4 are shown the current tumor/mode/metastases (TNM) pathological staging system for prostatic carcinoma (60). Pathologic stage correlates with recurrence risk, as well as overall survival. Gleason score, which has been shown to be remarkably reproducible among pathologists, is a critical element in this evaluation (61). The primary Gleason score refers to the dominant histologic pattern, whereas the secondary score represents the bulk of the nondominant pattern. The addition of these two scores, the Gleason sum, then can be used to predict (along with the PSA and clinical stage) the likelihood of lymph nodes at the time of radical retropubic prostatectomy (Tables 30-5a–d).

Preoperative staging using pelvic computed tomography (CT) scans remains unsatisfactory for the majority of patients. Only 50% sensitivity to extracapsular extension was found among 32 patients in whom CT was compared with histologic examination after radical prostatectomy (63). CT scanning, however, can be used for directing fine-needle aspiration of an enlarged lymph nodes in high-risk patients (64,65). Magnetic resonance imaging (MRI) using endorectal coils offers an improvement in the sensitivity of preoperative staging, particularly in high-risk patients. One study showed 100% sensitivity and 94% specificity in predicting seminal vesicle invasion at surgery (66). However, this technology remains costly and is somewhat invasive. In the past, bone scans were considered mandatory before a decision to pursue aggressive local management. However, this practice has fallen under increasing scrutiny in the era of cost containment. The incidence of positive bone scans in patients with clinically locally confined prostate cancers and PSA values of less than 8.0 ng/mL was 0 in a study of 467 men studied at the Mayo Clinic. In addition, only three of 561 patients with PSA values of 10 ng/mL or less had a positive scan (67). It is generally recommended that these imaging procedures be performed in patients with a substantial risk of seminal vesical or lymph node involvement (Gleason sum ≥7 or PSA >10–20 ng/mL) (68).

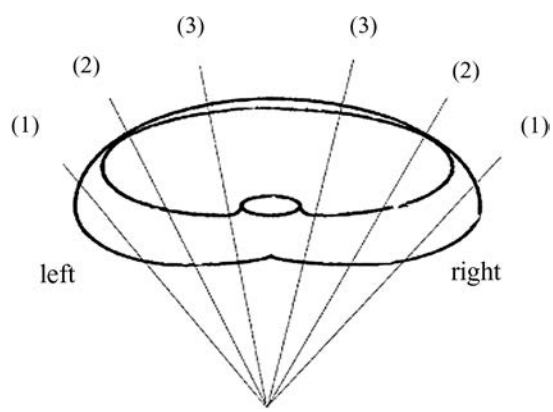

FIGURE 30-4. Laterally directed prostate biopsies (#1's) are more likely to contain greater volumes of peripheral zone and, therefore, prostate cancer. (From: Keetch DW, Catalona WJ, Smith DS. Serial prostatic biopsies in men with persistently elevated serum prostate-specific antigen values. *J Urol* 1994;151:1571.)

TABLE 30-4

TUMOR–NODE METASTASES STAGING OF PROSTATE CANCER FROM AMERICAN JOINT COUNCIL OF CANCER

TNM	Definition
T_{1-2}	Tumor confined to the prostate
T_{1a}	Cancer detected in <5% of specimen removed for obstruction
T_{1b}	Cancer detected in >5% of specimen removed for obstruction
T_{1c}	Cancer detected by elevated PSA alone
T_{2a}	Cancer confined to one lobe of prostate, palpable clinically
T_{2b}	Cancer found bilaterally in prostate, palpable clinically
T_{3-4}	Tumor extension beyond the prostate
T_{3a}	Cancer with extension extracapsularly; fatty tissue only
T_{3b}	Cancer invasion into seminal vesicle
T_{4a}	Cancer extension to bladder or rectum
T_{4b}	Cancer extension to pelvic side wall
$N_{1-4}M_1$	Metastases
N_{1-4}	Regional nodal metastases
M_1	Distant metastases

Current CAP staging.

^{111}Indium capromab pendetide (ProstaScint) is monoclonal antibody directed to prostate- specific membrane antigen (PSMA). PSMA is a type 2 transmembrane glycoprotein with three domains. Some studies suggest it may be more frequently expressed in aggressive cancers. In one study, the sensitivity of immunoscintigraphy for lymph node detection was only 62% and the specificity was 72% when used preoperatively in 152 patients before pelvic lymph node dissection (69). Positron-emission tomography (PET) scans are generally felt to be of little value in prostate cancer due to the generally slow rate of growth of this cancer. Despite advances in imaging technology, consideration of PSA and Gleason score remains one of the best predictors of lymph node status (70). For completely accurate staging, patients at high risk may undergo laproscopic pelvic lymph node sampling with minimal morbidity.

Interestingly, recent studies evaluating reverse transcriptase polymerase chain reaction (RT-PCR) for messenger RNA contained in archival specimens, suggest that many patients formerly thought to have negative nodes by routing histologic examination, have, in fact, evidence of prostate cancer cells. Edelstein evaluated archival specimens from 57 patients who were felt to have negative nodes at the time of radical prostatectomy. Of evaluable specimens, 47% contained mRNA for PSA using RT-PCR. Of these, 14 of 16 had PSA biochemical relapse within 5 years, emphasizing the inability of localized surgical or radiotherapy to cure patients with pathologic N1 disease (71). Ferrari (72), who studied 58 nodes from 33 patients, only four of whom were found to be positive by routine evaluation, published similar findings. In contrast, RT-PCR found message for PSA or PSMA in 23 of 29 "negative" patients. Although the prognostic significance of these findings is not available,

TABLE 30-5

RISK OF LYMPH NODE INVOLVEMENT GIVEN PSA AND GLEASON SUM

Gleason score	Stage T1a	Stage T1b	Stage T1c	Stage T2a	Stage T2b	Stage T2c	Stage T3a
a. Probability of lymph node involvement—PSA = 0.0–4.0 ng/mL							
2–4	0	0	0	0	0	0	—
5	0	1	0	0	1	1	2
6	1	2	0	1	2	2	5
7	—	6	1	2	5	5	9
8–10	—	14	4	5	10	10	—
b. Prediction of probability of lymph node involvement—PSA = 4.1–10.0 ng/mL							
2–4	0	1	0	0	1	1	1
5	1	2	0	1	2	2	3
6	3	5	1	2	4	4	9
7	8	12	3	4	9	9	15
8–10	18	23	8	9	16	17	24
c. Prediction of probability of lymph node involvement—PSA = 10.1–20.0 ng/mL							
2–4	0	2	0	1	1	1	—
5	3	5	1	2	4	4	7
6	—	13	3	4	10	10	18
7	18	24	8	9	17	18	26
8–10	—	40	16	17	29	29	37
d. Prediction of probability of lymph node involvement—PSA >20.0 ng/mL							
2–4	—	4	1	1	3	—	—
5	—	10	3	3	7	7	11
6	—	23	7	8	16	17	26
7	—	—	14	14	25	25	32
8–10	—	51	24	24	36	35	42

(From: Gleason DF. Histologic grading of clinical staging of prostatic carcinoma. In: Tannenbaum M, ed. *Urologic Pathology: The prostate.* Philadelphia: Lea & Febiger, 1977:171.)

studies such as these, performed with standardized reagents and techniques, are ongoing, and may provide useful information in the future regarding therapy decisions. There has been one adjuvant randomized clinical trial evaluating the value of early hormonal blockade in men with positive lymph nodes diagnosed at the time of radical prostatectomy. This study, although small, clearly revealed a significant survival benefit for early androgen deprivation in patients with node-positive disease following radical prostatectomy (73).

THERAPY OF LOCALIZED DISEASE

For patients with stage T1–2 (i.e., tumor is confined to the prostate), the condition and age of the patients dictate therapy. Stage T1a and T1b tumors may be able to be observed with minimal impact to a patient's overall survival, although this strategy may need to be modified in younger men.

For stage T1c and T2 tumors, several localized treatment options are available, depending on patient preference, comorbidities, and Gleason grade. One option for such patients is expectant management, or "watchful waiting." This technique involves digital exam and PSA monitoring quarterly or semiannually, with the option of deferred definitive or palliative treatment. Although this technique subscribes to the "do no harm" dictum, the progression of tumors detected by screening is substantially more than one would hope. In Sweden, Johansson (74) studied the natural history of untreated low-grade, generally well-differentiated (only 4% of tumors were high-grade), prostate cancer in men with a median age of 73 years and has recently updated his observations (74). This study involves 223 consecutive patients with T0–2, NX, M0 disease who have now been followed for an average of 21 years treated only with androgen deprivation upon tumor progression. During observation, it appears that the rate of death due to prostate cancer increases threefold between 15 and 20 years of observation (from 15 per 1,000 patient-years to 44; $p = 0.01$). Of patients, 64% progressed, and 48.8% developed metastases at 20 years. These values are substantially increased from the 55% rate of progression and 23.1% rate of metastases seen at 15-year follow-up. Kaplan-Meyer curves demonstrating this natural history of prostate cancer stratified by clinical stage and pathologic grade are seen in Figure 30-5. To avoid the development of metastases and death from prostate cancer, these data suggest that men with a life expectancy longer than 15 to 20 years should be treated definitively—even when the disease is well differentiated and diagnosed at an early stage. In Holberg's study (26), the metastatic rate was 27.3% at 8 years in men undergoing watchful waiting compared to 13.4% in men treated definitively ($p = 0.03$). Watchful waiting may be a consideration

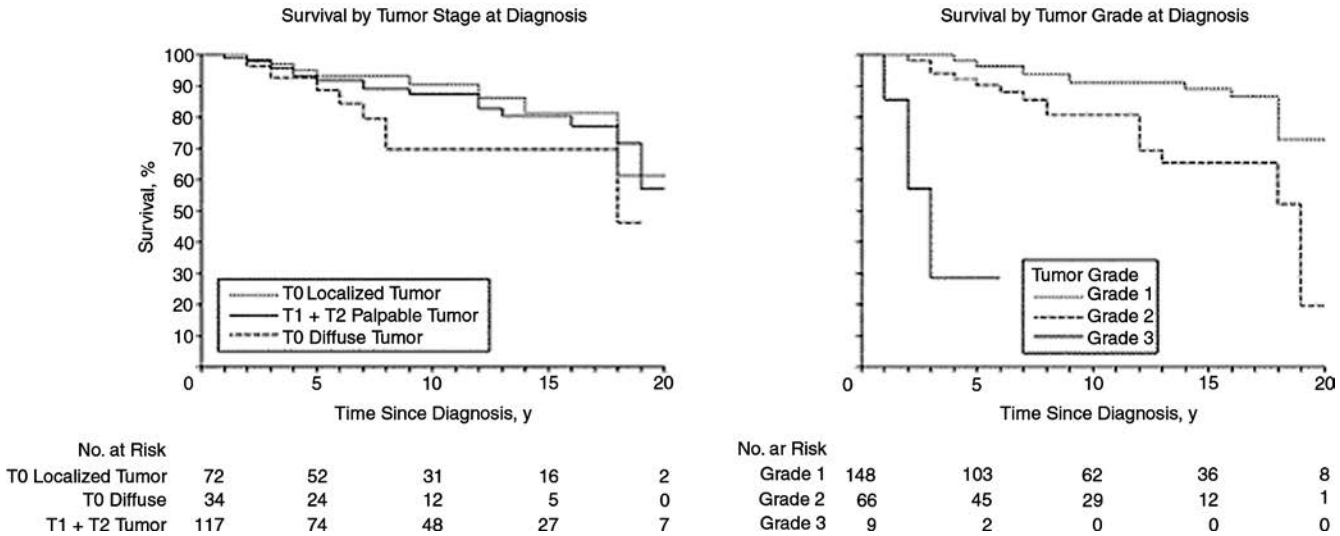

FIGURE 30-5. Survival of patients with prostate cancer treated only with androgen deprivation upon progression stratified by clinical stage and pathologic grade (From: Messing EM, Manola J, Sarosdy M, et al. Immediate hormonal therapy compared with observation after radical prostatectomy and pelvic lymphadenectomy in men with node-positive prostate cancer (see comments). *N Engl J Med* 1999;341:1781). (Reprinted with permission from Johansson JE, Adren O, Andersson SO, et al. Natural history of early, localized prostate cancer *JAMA* 2004;291:2713.)

for patients who refuse therapy or who have protracted life expectancies, however, the likely rate of metastatic development should be kept in mind. Novel observation protocols that include definitive therapy for failures that incorporate PSA doubling time, clinical stage, and pathologic grade may reach a balance between overtreatment and prevention of metastatic disease, but have yet to be fully evaluated (75).

Data from Harvard Medical School (76) suggest that 57% of patients at 5 years and 74% of patients at 7 years of patients on observation were eventually converted to active therapy (76% treated definitively upon treatment).

Surgical Prostatectomy

The lack of consensus on definitive treatment of early prostate cancer continues to present confusion for patients and practitioners. In addition to the lack of supporting data found in the published literature, marked bias exists among treating subspecialists. Moore and colleagues (77) surveyed prostate cancer "experts" with a case summary that included an early-stage prostate cancer scenario and asked these individuals how they would wish to be treated if the case was their personal medical history. Radiation oncologists overwhelmingly chose radiation therapy, whereas urologists chose surgery, and medical oncologists were divided in their choice. Interestingly, British respondents generally were less likely to choose any therapy, perhaps indicating a more conservative approach to the disease at the time of the survey (1988) than may exist now. Although more than a decade has passed since Moore's survey, recent polls suggest that the biases of treating physicians to recommend their own modality persist (78).

In spite of these uncertainties, the number of patients undergoing radical retropubic prostatectomy has increased approximately sixfold over the past decade. This may reflect the fact that urologists are generally the physicians who diagnose prostate cancer. It may also reflect improvements in surgical technique, which allows nerve-sparing approaches in properly selected patients with concomitant preservation of erectile function and is also a result of the increased number of patients being diagnosed with early-stage disease by current screening efforts (79).

A number of papers have been published on the outcome of radical retropubic prostatectomy in terms of disease control rate, cancer specific, and overall survival (80–83). The appropriate patient for such therapy has clinical stage T1 or T2 prostate cancer and expected longevity exceeding 10 years. The results of such treatment are shown in Table 30-6.

TABLE 30-6

RESULTS OF RADICAL PROSTATECTOMY

10-Year actuarial survival rates	
Overall	44–88%
Biochemical progression-free	82%
Disease-specific	88–93%
10-Year local failure	12%
Dead from competing causes	10–30%
Complications	
Impotence[a]	25–100%
Incontinence[a]	10–40%
Rectal injury	0–5%
Cardiovascular	0.5–4%
Death	0–2%

[a]Incontinence and impotence rates depend on definitions and methodology of assessment.
(From: Smart CR. The results of prostate carcinoma screening in the U.S. as reflected in the surveillance, epidemiology, and end results program. *Cancer* 1997;80:1835; Lepor H, Walsh PC. Long-term results of radical prostatectomy in clinically localized prostate cancer: experience at the Johns Hopkins Hospital. *NCI Monogr* 1988;7:117; Walsh PC. Follow-up prostate cancer treatments after radical prostatectomy: a population-based study. *J Urol* 1996;156:301; Oefelein MG, Grayhack JT, Mcvary KT. Survival after radical retropubic prostatectomy of men with clinically localized high grade carcinoma of the prostate [see comments]. *Cancer* 1995;76:2535.)

Surgical Prostatectomy with Neoadjuvant Hormonal Ablation

A number of centers have studied the potential of using androgen ablation prior to radical prostatectomy in an effort to improve on the foregoing results. In these studies, patients are treated with a gonadotropin-releasing hormone (GnRH) analog plus or minus an antiandrogen or other means of androgen ablation for varying periods of time prior to prostatectomy. The results of four such studies are shown in Table 30-7 (84–87).

In each study, the incidence of a positive surgical margin was significantly reduced by the neoadjuvant treatment. However, this may because at least in part, to difficulty of pathological assessment following androgen deprivation as no prospective randomized trial to date has shown an improvement in overall outcome with neoadjuvant androgen deprivation. Currently, patients who are candidates for prostatectomy should undergo surgery immediately unless they are enrolled in a clinical trial (on-going studies continue evaluating various durations of neoadjuvant androgen deprivation and novel suppression techniques are ongoing). These recommendations differ substantially from the recommendation for definite use of neoadjuvant hormonal ablation in patients who chose radiotherapy as the primary treatment modality, as is discussed below.

Radiation Therapy

Radiation therapy for early prostate cancer has been used to treat prostate cancer for over 25 years. The complications of proctitis, fissures, and lymphedema, seen at the initiation of this treatment modality have, in essence, been eliminated through improvements in technique. Diarrhea, urinary obstruction, hemorrhagic cystitis, remain complications of radiation therapy, but are generally self-limited (88). Using data from the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) data base, the cumulative proportion of patients who had undergone radiation for prostate cancer, who developed bladder cancer, was 5.5% over a follow-up period of 20 years (HR = 1.5, CI 1.07 to 2.02; 95% CI, 4.1% to 6.9%) (89). Impotence following radiation therapy has been less well studied, but approaches that of nerve-sparing prostatectomy at 2 years (90,91). Three-dimensional conformal radiotherapy (3D-CRT) allows the treatment of the prostate with higher doses with sparing of the rectal and bladder wall resulting in less fecal urgency/frequency and urinary incontinence (92). Intensity Modulated Radiation Therapy (IMRT) refines dosing further using special software to allow for even more concentrated dosimetry to the prostate while sparing surrounding organs, although a difference in survival with this technique has yet to be proved. The use of a proton beam appears to be feasible and to have similar outcomes to traditional photon beam treatment as well (93). The radiation oncology group at Fox Chase Cancer Center in Philadelphia evaluated external-beam radiation dose and outcome. The group found that lower dosages of radiation was one of the most predictive factors of biochemical failure after treatment (76% failure for dosage <72 Gy, 35% failure for 72.1 to 75.9 Gy, and 21% failure for dosage >76 Gy, when pretreatment PSA = 10 to 19.9 (94). Three-dimensional conformal radiation allows for consistent delivery of 78 Gy to the prostate. A PSA nadir ≥1.0 ng/mL appears to be an important predictor of biochemical failure after radiation treatment (95).

Several large prospective randomized trials have shown the benefit of androgen deprivation with radiation. The Radiation Oncology Group (RTOG) showed that androgen deprivation with radiation provided a survival advantage over radiation alone, particularly in patients with high-grade tumors in the 85-31 trial (96). A European trial (EORTC 22863) confirmed a survival advantage with adjuvant androgen deprivation in patients with high-grade, as well as large low-grade tumors, with only 3 years of deprivation (97). RTOG 86-10 showed this same benefit with only 4 months of neoadjuvant/adjuvant therapy in men with bulky tumors and Gleason score ≤6 (98). RTOG 92-02 showed a possible survival advantage for long-term over short-term androgen suppression in high-grade tumors (99). As a result of these studies, virtually all patients who elect radiotherapy for the initial treatment of their prostate cancer now receive neoadjuvant hormonal ablation. Studies continue to investigate the ideal length of therapy required both prior to and following radiation.

Brachytherapy, or "seed implants," have been used since the turn of the century, when Barringer inserted radium seeds into the gland. Recent advances, in which the urologist and radiation oncologist participate in the three-dimensional planning and placement of seeds, have improved the dosimetry of this technique. Retrospective studies of patients who have undergone treatment with this modality indicate comparable results with potentially fewer side effects in patients who have low-grade, low-stage tumors (100). The use of transperineal implantation with a template to control the "x,y" coordinates in conjunction with transrectal ultrasonography to control the "z" coordinate has allowed brachytherapists to improve the accuracy of the interstitial implant. Improved implant imagery, combined with better patient selection, computer-assisted dosimetry, and the availability of additional radioactive sources, such as ^{125}Iodine, ^{103}Palladium, and ^{192}Iridium, with different photon energy and half-life, all contribute to the

TABLE 30-7

SURGICAL PROSTATECTOMY WITH NEOADJUVANT HORMONAL ABLATION STUDIES

Study	*N* Initial androgen ablation	*N* Immediate surgery	Positive surgical margin	Reference
Canadian Urologic Oncology Group	101	112	28/101 59/91	Goldenberg et al. (84)
Belgian Uro-oncology Group	65	62	18/65 23/62	Van Poppel et al. (85)
Lupron Depot Neoadjuvant Group	149	138	24/149 65/138	Soloway et al. (86)
Sloan-Kettering Trial	69	72	13/69 31/72	Fair et al. (87)

superior results compared with older implant studies. Localized therapy with permanent or temporary radiation seed placement can also be used in combination with external-beam therapy in patients with high- or intermediate-risk disease (101). Results of such treatments are currently under investigation. Most radiation oncologists agree that pretreatment androgen deprivation is not required for patients with low-risk disease (PSA <10, clinical stage T1c or T2a, Gleason score <7).

Comparative Studies of Early Treatment Outcomes

Unfortunately, there are simply no randomized prospective studies comparing the outcome of patients who choose radiotherapy or surgery for treatment. However, retrospective data exist. One such study compares patients treated with radiation at Harvard and the surgically managed patients at the University of Pennsylvania Hospital. Surgical treatment consisted of a radical retropubic prostatectomy and bilateral pelvic lymph node sampling, whereas radiotherapy was given with the 3D-CRT technique without neoadjuvant hormonal manipulation. Patients with Gleason scores of 5 to 6 who represent about 50% of the total patients reported in the entire series. Perhaps the most remarkable aspect of the data is the absolute consistency of worsening prognosis with higher Gleason grade or clinical stage, regardless of the treatment modality. As far as comparative results, the confidence intervals between radiation and surgery overlap considerably, indicating probable equivalence in outcome—at least at the time of publication with 2-years follow-up. The authors of the study also concluded that the very high relapse rate at 2 years for patients with Gleason scores of 8 to 10 or pretreatment PSA more than 20 ng/mL make these patients ideal candidates for involvement in a clinical trial (102).

THERAPY OF ADVANCED DISEASE

Rising PSA after local treatment of prostate cancer is a hotly debated issue in urologic oncology. Biochemical failure may be the only manifestation of disease recurrence. Ultrasensitive PSA value, Gleason score, timing, velocity of detectable PSA, and PSA doubling time are the most helpful predictors of eventual imaging-detectable recurrence (103–105). TRUS–guided biopsies of prostatic fossa are more sensitive than digital rectal examination (DRE) when local recurrence is suspected, although a false negative is not infrequent with this modality. The yield of bone scan is limited until serum PSA rises above 20 ng/mL, immunoscintigraphy and CT scan have only moderate sensitivity in early disease, and PET scanning has limited value in prostate cancer evaluation because of the generally slow-growing nature of the tumor. There has been some investigation into a fusion of these two studies to help provide physiologic and anatomic detail.

Local salvage therapies include:

- Radiation for patients who underwent radical prostatectomy. The patients most likely to benefit from this therapy are those with good preradiation continence and PSA levels <1.0 ng/mL (104).
- Salvage surgery for those with organ-confined disease who failed radiation. Salvage prostatectomy includes a high risk of incontinence and rectal injury in most series and should be reserved for carefully selected patients.
- Salvage cryotherapy is an alternative to prostatectomy following radiation. This surgery carries a minimal risk of rectovesical fistula and almost no risk of bowel injury or total incontinence. Early reports suggest a cure rate similar to radical surgery (106). The rates of erectile dysfunction with cryotherapy, however, approach 100%.

It is important to remember that localized salvage therapies should be reserved only for patients who were considered candidates for cure prior to their initial treatment.

Systemic therapy includes a combined, intermittent, or monotherapeutic androgen deprivation. Controversy continues as to the best time of initiation of systemic therapy. Studies have shown that patients with rapid PSA doubling times are more likely to die of prostate cancer than of other causes (107,108). "Vaccines," which stimulate a patient's immune system in this scenario, are currently being evaluated in clinical trials.

Hormonal Manipulations

The standard of care for patients with metastatic prostate cancer remains androgen deprivation. In 1794 John Hunter, who recognized that removal of the testes caused prostatic atrophy in animals, made the original insight into a potential role for testosterone in the trophic maintenance of prostatic carcinoma (109). Wide knowledge of the hormonal sensitivity of prostate epithelial cells showing favorable results obtained with castration date back to the 1890s. The role of surgical castration for advanced prostatic carcinoma was firmly established by Huggins and colleagues in the early 1940s wherein a survival advantage was seen in androgen-deprived patients (110,111).

The role of diethylstilbestrol (DES) therapy and orchiectomy did not emerge until completion of the Veterans Administration Cooperative Urology Research Group trials, which were organized in the 1960s (112–116). The first of these trials was designed to determine whether DES therapy, either alone or in combination with orchiectomy, truly was superior to placebo administration in patients with locally extensive or metastatic disease. Investigators participating in these trials were allowed to change therapy when disease progression occurred, thus making it difficult to compare the various assigned treatments. In fact, the investigators really were comparing delayed therapy in the placebo group with early therapy in the DES groups. Of subjects assigned to placebo, 70% of stage C and 100% of stage D patients were eventually given another form of therapy after developing symptomatic disease (117). The main conclusion of the first trial was that there was no survival advantage to hormonal manipulation used at the diagnosis of disease, as compared with waiting until symptomatology appeared.

The central change in therapy during the past two decades is the widespread availability of GnRH analogs as an alternative to surgical castration and DES. Patients prefer this treatment modality, since it provides the opportunity for intermittent androgen deprivation and also provides the opportunity to avoid the potential cardiovascular complications of high-dose DES (118). In two studies in which patients were given a choice between a GnRH analog and orchiectomy, more than three-fourths elected to avoid surgery (119,120). GnRH analog therapy has become even more convenient, since these studies, with the availability of 3-, 4-, and 6-month depot injections.

The underlying biochemical mechanisms of androgen activity are summarized as follows. Target tissues including the prostate gland and hair follicles in males, which contain membrane-bound 5-α reductase. This enzyme converts circulating testosterone to a more potent molecule, dihydrotestosterone (DHT). Active DHT is bound by a specific cytoplasmic receptor. The receptor–steroid complex is then translocated to the nucleus, where it is bound to acceptor sites on DNA. This interaction with DNA results in the transcription of new mRNA and protein synthesis. The net result in prostatic tissue appears to be inhibition of cell death (121). Although some

relationship exists between responsiveness to androgen depletion and the quantitative measurement of androgen receptor, the degree of heterogeneity of tumor tissue, combined with a high subjective response rate to androgen depletion, restricts the clinical usefulness of routine androgen-receptor measurements in managing prostate cancer (122,123). Hormone-refractory prostate cancer seems to result from amplification and overexpression of the wild-type receptor, as opposed to mutations in the gene (124).

In general, the subjective and objective cancer response rates to all methods of reducing testicular androgen production are the same. Orchiectomy, DES, GnRH analog, as well as the newly formulated GnRH antagonist (which does not require administration of an anti-androgen with the initiation of treatment) all reduce circulating testosterone levels to less than 50 μg/dL, although occasional patients may have an incomplete response (125). The disappointing fact is, however, that none of these approaches used alone have resulted in increased survival (126–131). Regardless of how androgen deprivation is achieved, most studies show the majority of patients with metastatic disease have disease progression 12 to 36 months after the initiation of therapy. Performance status, extent of disease, and other variables are more important prognostic factors of survival than the means of androgen deprivation (132–134).

Progression to androgen independence has been evaluated by several scholars. One theory explains the failure of androgen-deprivation therapy to increase survival by postulating the existence of several clones of cells in prostate cancer. The majority of the clones are likely androgen dependent whereby androgen deprivation results in apoptosis and death (reflected clinically by partial or complete remission). However, a small number of cell clones would be androgen independent and continue to proliferate, resulting in the clinical reappearance of cancer. A Southwest Oncology Group study, based on this hypothesis, compared androgen ablation plus chemotherapy with androgen ablation alone, eventually followed by chemotherapy, but was unable to show a difference in overall survival (135,136).

Another hypothesis assumes that different prostate cancer cell lines have different levels of androgen dependence (137). This hypothesis is largely based on the work of Geller, who showed that DES administration or bilateral orchiectomy does not result in complete elimination of dihydrotestosterone in prostatic tissue (138). Low levels of intraprostatic dihydrotestosterone synthesized by conversion of adrenal androgens still can be measured after any surgical or medical castration. Reducing testosterone levels with castration would then cause the death of the most androgen-dependent cells, with clinical remission as a result, but the less androgen-dependent cells would survive and continue to proliferate, eventually resulting in clinical progression. The latter hypothesis led to the concept of total or maximal androgen blockade as initial approach to metastatic prostate cancer. Labrie (139) first promoted this in 1985, when he demonstrated, for the first time, that overall survival was increased by combining bilateral orchiectomy or a luteinizing hormone-releasing hormone (LHRH) analog with the antiandrogen flutamide. His results were received with considerable skepticism because the trial treated a relatively small number of patients and lacked an adequate control group. Subsequently, large meta-analyses have confirmed a small advantage to combined androgen blockade. The first meta-analysis, published in 1995 by the Prostate Cancer Trialists Collaborative Group, examined 22 published and unpublished trials totaling 5,710 patients (140). Original patient-based data were obtained from the primary investigators and analyzed by the log-rank statistic. It compared the number of deaths observed in the combined androgen blockade arm to the number expected on the basis of the average pattern of survival in a combination of the two treatment arms. Median follow-up was 40 months; 57% of the patient cohort had died at the time of analysis. Types of castration included orchiectomy, goserelin, leuprolide, or buserelin; antiandrogens included cyproterone acetate, nilutamide, or flutamide. No statistically significant differences were observed among the different forms of castration (alone or combined with antiandrogens), or among individual antiandrogens. The overall mortality among patients treated by castration alone was 58.4% versus 56.3% in those treated by combined androgen blockade, corresponding to a nonsignificant 6.4% reduction in the annual odds of death. A plot of survival estimate demonstrated no difference between arms at 2 years, but a 3.4-month (3.5%, 95% CI, 0% to 7%) improvement (26.2% versus 22.8%) in estimated survival in favor of combined androgen blockade at 5 years. No differences in the study findings were observed when patients were stratified by age or stage of disease. No data were analyzed with respect to cost or quality of life in this study. A second meta-analysis also was published in 1999 by the Agency for Health Care Policy and Research (141). This study included published randomized, placebo-controlled trials for survival estimates, Phase II trials for adverse events, and all trials that included quality of life assessments. The castration arms included leuprolide, goserelin, buserelin, diethylstilbestrol, and orchiectomy; antiandrogens included flutamide, nilutamide, bicalutamide, and cyproterone acetate. Survival data were combined from the randomized studies using a random effects model. A detailed cost-effectiveness analysis also was performed. The results were reviewed by a broad range of professionals with expertise in urology, medical oncology, technology assessment, cost-effectiveness analysis, meta-analysis, and relevant lay issues. These included representatives from the American Urological Association, the American Society of Clinical Oncology, the American Society for Therapeutic Radiation and Oncology, and the pharmaceutical industry. Finally, the most recent analysis included 21 trials comparing outcomes between castration and combined androgen blockade in 6,871 patients. No statistically significant differences in outcome were observed among different forms of castration or individual antiandrogens. Eighteen trials, including the largest trial of 1,386 patients, analyzed 80% of the patients and showed no statistically significant difference in overall survival among treatment arms (142). Three trials found a statistically significant difference in overall survival in favor of combined androgen blockade, with an improvement of median survival at 5 years of 3.7 to 7 months, or 3% to 9%. The analysis of all 21 trials found no difference in overall survival at 2 years (HR 0.97, 95% CI 0.87 to 1.09), but an improved survival for combined androgen blockade at 5 years (HR 0.92, 95% CI 0.85 to 0.99). Six trials reported outcomes stratified by prognostic group, but only one included prospective stratification and had sufficient statistical power to detect a meaningful difference in survival (142). Both the meta-analysis and this single trial found no differences in survival among prognostic groups or therapeutic arms. Although limited data are available on toxicity, a higher proportion of patients randomized to combined androgen blockade (10%) withdrew from therapy because of adverse effects versus the castration arm (4%). Only a single study (which compared orchiectomy to orchiectomy plus flutamide) included quality of life endpoints, with a clear advantage in emotional functioning and less diarrhea in the orchiectomy arm (14). The cost-effectiveness analysis suggested that combined androgen blockade costs more than $100,000 per quality-adjusted life year compared to orchiectomy, and that its efficacy must increase to 20% to fall below this widely used benchmark.

Alternative approaches to the use of combined androgen blockade also are receiving attention. The use of antiandrogens alone remains an interesting possibility, producing responses in about 75% of patients treated. A possible advantage of this

category of drugs is better maintenance of sexual potency, albeit at the expense of estrogenic side effects (143). Progestins, such as megestrol and medroxyprogesterone acetate, have been studied in some trials and appear to avoid some of the estrogenic side effects of DES (144). Agents that inhibit steroid synthesis (aminoglutethimide, ketoconazole, and spironolactone) have undergone limited clinical trials in patients with prostatic carcinoma, but appear to introduce additional side effects that make them unsatisfactory as first-line agents (145–147).

One of the more intriguing new approaches to hormone refractory disease is intermittent androgen blockade. The theory for this is that some cells remain sensitive to androgens and that by allowing these cells periodic reexposure to androgen by withdrawal of the GnRH analog or antiandrogen, such cells will remain the majority population. Completed preliminary clinical studies suggest that quality of life may be improved, but it remains uncertain as to whether such an approach is equivalent (or superior) to continuous androgen ablation (148).

Androgen-Independent Disease

Androgen deprivation is the only effective form of systemic therapy for men with advanced disease, producing symptomatic and/or objective response in 80% of patients. Unfortunately, androgen independence (AI) frequently predicts death within a few years (149). Hormone refractory or androgen-independent prostate cancer (HRPC/AIPC) is highly chemoresistant with objective response rates of 10% and most studies have shown no survival benefit with treatment (150). In 1985, Eisenberger and colleagues (151) reviewed 17 randomized clinical trials of 1,464 evaluable patients and found an objective response rate of 4.5% (151). In 1992, Yagoda and Petrylak (152) reviewed 26 trials of chemotherapy conducted between 1987 and 1991; the reported overall response rate was 8.7%. Lack of effective treatment of HRPC remains the main obstacle to improving the survival and quality of life for patients with advanced disease; therefore, novel therapeutic strategies that target the molecular basis of androgen resistance and chemoresistance are required.

Definition of Hormone Refractory Prostate Cancer

Hormone refractory prostate cancer is commonly defined as demonstration of two to three serial rises in serum PSA obtained at least 2 weeks apart with castrate serum testosterone levels. Using these criteria alone, a very heterogeneous population of patients is included. It is helpful to subcategorize patients in terms of extent of disease and presence or absence of symptoms such as bone pain.

Patients with localized prostate cancer and rising PSA levels after definitive surgery or radiotherapy and no demonstrable metastatic disease may be treated with androgen deprivation. When serum PSA begins to rise while on androgen deprivation therapy, restaging studies are often negative for metastases. This situation, sometimes referred to as biochemical HRPC, represents a growing population of otherwise asymptomatic patients. There is no standard approach to these patients, although many clinical trials now are targeting this population.

Most often, HRPC is thought of in the context of widely metastatic bone disease. In this setting, it is helpful to distinguish between those who are asymptomatic and those who have bone pain or other symptoms of metastatic disease. The median time to symptomatic progression after a rise in PSA over 4 ng/mL is approximately 6 to 8 months, whereas the median time to death from PSA progression is 12 to 18 months (150). Once patients become symptomatic, however, the median survival ranges from 8 to 12 months. Symptomatic patients need immediate attention; palliation of pain and treatment options is varied and is reviewed in the following. A recent analysis of patients involved in Cancer and Leukemia Group B protocols suggested that overall survival in men with androgen-independent prostate cancer ranges from 7.5 to 27 months. This study suggested that risk factors such as PSA, alkaline phosphatase, lactate dehydrogenase, hemoglobin, Gleason Score, ECOG performance status, and presence or absence of visceral metastases could help predict an individual's risk (153).

Finally, it is important to know that the term androgen independence or hormone refractory is somewhat of a misnomer in terms of contemplating treatment options. In some cases, the prostate cancer may still exhibit some dependence on the hormonal milieu, whereas in other cases the disease may function entirely independently. Basic science investigation using androgen independent prostate cancer cells *in vitro* suggests that apoptosis rates decline when testosterone is provided (Hari Koul, personal communication November, 2004). Unfortunately, at present there is no way to predict which patients may benefit from a second or subsequent hormonal manipulation and androgen dependence or independence can only be determined retrospectively, based on response to further hormonal manipulation.

Second-Line Hormonal Manipulations

Antiandrogen Withdrawal. Patients with a rising serum PSA while on combined androgen blockade should undergo a trial with antiandrogen discontinuation to evaluate for a positive antiandrogen withdrawal response. This phenomenon was first observed after flutamide was discontinued and the PSA subsequently declined without other interventions (154). It has now been described with other hormones such as megestrol acetate and diethylstilbestrol, as well as the other nonsteroidal antiandrogens, bicalutamide and nilutamide (155–158). The antiandrogen withdrawal response occurs in approximately 15% to 30% of patients. Although the median duration of PSA response is only 3.5 months, the range of duration of response varies from 4 weeks to well over 12 months. Both subjective and objective responses have been reported, along with a decline in PSA (159). Although the precise mechanism of the antiandrogen withdrawal response remains undefined, it likely results from altered structure of the steroid-binding domain of the androgen receptor (i.e., mutations of the promoter with hypermethylation or cleavage) or presence of coactivators (such as interleukin-6). One or both of these factors permits an antiandrogen to act as an agonist rather than an antagonist with subsequent transcriptional activation of cell survival and mitogenesis signaling pathways (160,161). Discontinuation of the antiandrogen thus disrupts this stimulus.

Antiandrogen Addition. An antiandrogen trial may result in a PSA response (>50% decline) in 15% to 80% of cases, if the patient has not been on antiandrogens previously; however, the median duration of response is only 4 months (162–164). Subjective responses have been reported as well. The effects of second-line antiandrogen therapy in HRPC on survival remain unknown.

Ketoconazole. The combination of ketoconazole (KC) and hydrocortisone (HC) generally is well tolerated and often effective, although patients on this regimen require careful monitoring. PSA responses (>50% decline) have been reported in 62% of patients and subjective responses have been observed in 20% to 50% (166–168). At a dose of 400 mg TID, KC blocks production of adrenal hormones including hydrocortisone; therefore, replacement doses of 20 to 25 mg in the morning and 10 to 15 mg at 4:00 PM should be prescribed. Mineralocorticoid replacement is also frequently necessary. KC must

be taken on an empty stomach and requires an acid stomach environment for adequate absorption. If patients are on acid-lowering drugs, such as H2 blockers or ion-pump inhibitors, 1,000 mg vitamin C can be added to each KC dose to improve acidity. Adverse events secondary to drug–drug interactions are significant. Coadministration of KC with terfenadine, astemizole, cisapride, fluconazole, and erythromycin can cause arrhythmias. Patients on drugs such as warfarin, which are metabolized by cytochrome P-450, need to be monitored more closely, because KC interacts with this pathway. Side effects of KC include nausea, gastrointestinal bloating, hepatotoxicity, sticky-skin syndrome, and asthenia.

Corticosteroids. Corticosteroids alone have definite activity against prostate cancer and provide significant palliation in terms of anorexia, pain, and depression. The optimal drug and dosage have not been determined, but even prednisone at a dose of 5 mg BID resulted in subjective and PSA responses in the Canadian randomized trial discussed below (168). Higher doses of corticosteroids often are reserved for patients with very end-stage disease or those with spinal cord compression, because long-term administration results in manifestations of Cushingoid syndrome, including fluid retention, hypertension, bruisability, glucose intolerance, truncal obesity, facial erythema, and osteoporosis.

Radiation Therapy

EBRT traditionally has provided successful palliation of bone pain for patients with symptomatic HRPC or patients with pending spinal cord compression (169). Radiopharmaceuticals may be indicated in a patient with multiple painful bone metastases who is otherwise not eligible to receive chemotherapy. ^{89}Strontium and ^{153}Samarium, are preferentially taken up and deliver beta irradiation in sites where there is new bone formation (170,171). The exact mechanism of action of pain relief is not known, although PSA declines reported after treatment in some studies suggest there is some direct cell kill. A randomized trial of EBRT versus EBRT with ^{153}Samarium showed a delay in the appearance of painful new bone metastases, decreased analgesic intake, greater freedom from metastases at 3 months, and improved quality of life compared with EBRT alone (172). A randomized multicenter clinical trial initiated by M.D. Anderson is currently evaluating the benefit of ^{89}Strontium with doxorubicin as compared to doxorubicin alone (MDA-3410). A difficulty with strontium is the incidence of neutropenia. A small study evaluating the benefit of ^{188}Rhenium, given in one or two doses, showed an increase in objective response rate, time to progression, and overall survival when two doses of radiopharmaceutical were given (173).

Chemotherapeutic Options

Historically, prostate cancer has not been considered a chemosensitive disease because of the poor survival outcomes reported in older clinical trials. Many of these trials were performed in the pre-PSA era when patients were treated at later and more symptomatic stages and survival or objective response rates were primary (and often underpowered) endpoints. Single-agent chemotherapy has been associated with relevant palliative effects, but no single agent has been associated with an objective response rate greater than 30%. The use of palliation as an endpoint, along with the availability of PSA as an indicator of disease response, have renewed interest in the use of chemotherapy in multiple Phase II and several Phase III trials. PSA, as a measure of disease outcome in metastatic HRPC, has emerged as an acceptable surrogate endpoint to evaluate new agents in this clinical setting (174,175). There are also is some evidence of correlation between PSA response and survival, based on retrospective data, but a cause and effect relationship remains to be confirmed through prospective investigations.

Anthracyclines. Palliative effects have been observed in HRPC patients following the administration of corticosteroids, as well as mitoxantrone. A Phase III Canadian trial randomized 161 symptomatic patients with HRPC to receive either the best palliative care with prednisone 5 mg BID or prednisone plus mitoxantrone 12 mg/m^2(176). The primary endpoints were palliation and duration of palliation with secondary endpoints of improved time to progression and survival. A reduction of two or more points in a six-point pain scale lasting for at least two evaluations, 3 weeks apart, without any increase in analgesic use was achieved in 29% of patients in the mitoxantrone arm versus 12% of those receiving prednisone alone. Importantly, the median duration of palliation was significantly longer in the mitoxantrone arm, 43 weeks versus 18 weeks in the prednisone alone arm (p <0.0001). Although PSA was not an endpoint of the trial, PSA responses (>50% decline) were higher in the mitoxantrone arm as well (44% compared to 21% in the prednisone alone arm). Of 50 patients who were crossed over to the mitoxantrone arm at progression, 22% achieved a palliative response that lasted a median of 18 weeks. Median time to progression also was better in the mitoxantrone arm, 24 versus 10 weeks ($p = 0.0001$); however, there was no difference in survival between the treatment arms.

The Cancer and Leukemia Group B (CALGB) recently published results of a similar trial conducted in the United States (177). Unlike the Canadian trial, survival was the primary endpoint of this study with palliation secondary and no crossover to the mitoxantrone containing arm was allowed. Furthermore, patients did not need to be symptomatic. Over one-third of the 242 patients in the CALGB trial were asymptomatic at randomization. As in the Canadian trial, there was a significant difference between arms in time to disease progression (median 16.1 versus 9.9 weeks, $p = 0.0218$). Forty-two of 112 patients (38%) receiving mitoxantrone had a 50% reduction in PSA as opposed to 25 of 116 patients (22%) receiving hydrocortisone alone (p <0.008). Evaluation of survival by PSA reduction in both groups ($N = 228$) demonstrated a survival advantage for those patients with a PSA response. Despite the improvement in time to disease progression and PSA response in the mitoxantrone-containing arm, overall survival was not significantly improved (median 12.3 versus 12.6 months, respectively). It should be noted that similar results were previously reported with corticosteroids and doxorubicin and there may be little difference between the anthracyclines (178).

Estramustine Combinations. When used alone, estramustine is a relatively ineffective drug with significant nausea and vomiting, edema, thromboembolic events, and gynecomastia. In combination with other antimicrotubule drugs, including vinblastine, etoposide, paclitaxel, and docetaxel, however, estramustine appears to be synergistic. Multiple Phase II trials have evaluated estramustine-based combinations and have demonstrated objective, PSA, and palliative responses in the 40% to 50% range (Table 30-8) (179–182). A Phase III trial of vinblastine alone versus vinblastine plus estramustine showed statistically significant improvement in progression-free survival (3.7 versus 2.1 months), but whether this difference is significant clinically is debatable (183).

As reported in Phase I to II trials, the combination of estramustine and docetaxel appears to be active, as well as reasonably well tolerated (182). Estramustine at a dose of 280 mg TID on an empty stomach is administered for 5 days. Toxicities include granulocytopenia, thrombocytopenia, nausea, edema, transaminitis, and esophagitis. A new Phase III trial will compare this combination with mitoxantrone and corticosteroids.

TABLE 30-8

ESTRAMUSTINE-BASED PHASE II TRIALS

Estramustine combined with (reference)	Number of patients	Percentage with <50 decline in PSA	Percentage with palliative response	Median survival (months)
Vinblastine (179)	36	61	43	11.5
Etoposide (180)	42	52	—	10.5
Paclitaxel (181)	34	53	—	17
Docetaxel (182)	32	63	53	22.8

Taxanes. Paclitaxel (Taxol) and docetaxel (Taxotere) are taxanes that inhibit microtubule formation and phosphorylate the cell survival oncoprotein, Bcl-2 (183). Paclitaxel is indicated in the treatment of metastatic breast and ovarian cancer and has been evaluated alone and in combination in patients with prostate cancer. A Phase II trial by the Eastern Cooperative Oncology Group reported minimal activity with single-agent paclitaxel in 23 men with HRPC (184). Increased activity is reported, however, when paclitaxel is combined with other inhibitors of microtubular function. A Phase II trial combining paclitaxel with estramustine reported PSA responses (>50% decline) in 53% of 32 patients and objective responses in four of nine men with measurable disease (181). A Phase II study of estramustine, oral etoposide, and paclitaxel in 40 men with HRPC reported and overall response rate of 45% and a >50% decrease in PSA in 65% of patients (185). Median duration of response was 3.2 months and median overall survival was 12.8 months. The regimen was well tolerated with no significant change in quality of life as a result of therapy.

Docetaxel monotherapy is indicated in the treatment of patients with locally advanced or metastatic breast cancer after failure of cytotoxic therapy, and in some countries for the treatment of non–small cell lung and ovarian cancer. In Phase I and II trials in HRPC, weekly or every third week, docetaxel exhibits significant activity (186,187). Docetaxel monotherapy of 75 mg/m^2 every third week resulted in PSA declines of >50% and >80% in 45% and 20%, of patients, respectively. An objective response was seen in 28% of patients with measurable disease (188). Docetaxel has also been combined with zoledronic acid, thalidomide, calcotrol with some response in Phase II studies (189–191). The CALGB study found a 19-month overall survival when docetaxel was used in combination with estramustine and carboplatin (192).

Although the every third week conventional schedule is the currently approved schedule with an extensive clinical experience in solid tumors, such as breast, lung, and ovarian cancer, there is a growing clinical experience with the docetaxel weekly schedule, including HRPC. Early studies in HRPC suggested that weekly docetaxel could provide a suitable alternative resulting in higher dose intensity without increased toxicity. The weekly schedule appeared to have similar activity as the conventional 3-weekly schedule, but a better safety profile. Unfortunately, the results of TAX327, a three-armed randomized Phase III trial evaluating docetaxel and prednisone on a 3-weekly schedule against docetatel and prednisone weekly versus mitoxantrone and prednisone found that there was a statistically significant median survival advantage on patients given docetaxel every third week over mitoxantrone and prednisone (18.9 versus 16.5 months, respectively, $p = 0.009$). This finding was not statistically significant when weekly administration of docetaxel was evaluated (17.4 months for weekly docetaxol versus 16.5. months for mitoxantrone and prednisone) (193). An important Phase III randomized study recently showed a survival advantage when chemotherapy was given for androgen-independent prostate cancer. This study of 770 men showed a 17.5-month versus 16.5- month survival in men treated with estramustine and docetaxel verses mitoxantrone and prednisone ($p = 0.002$). Although neutropenic fevers, nausea, and vomiting were higher in the estramustine/docetaxel arm, this may now be considered standard of care for patients with HRPC (194).

Bisphosphonates

Bisphosphonates are stable analogs of calcium pyrophosphate that inhibit osteoclast activity in bone. They have been FDA-approved for palliation of pain owing to osteolytic bone metastases of breast cancer and myeloma and have been reported to be palliative in small Phase II studies in prostate cancer (195,196). Clodronate, a relatively low-potency bisphosphonate, available in Europe, has resulted in a reduction in the incidence and number of new bone and visceral metastases in breast cancer (197). A study presented in the *Journal of the National Cancer Institute* in October, 2002 evaluated zoledronic acid, a potent new IV bisphosphonate. Six hundred forty-three patients with documented metastatic hormone refractory prostate cancer were randomly assigned to three treatment groups, including 8 mg of zoledronic acid every 3 weeks for 15 months, 4 mg of zoledronic acid at a similar dosing interval or a placebo group. All patients were given vitamin D and calcium supplementation. Unfortunately, secondary to renal toxicity the patients on the 8-mg dose were later relegated to 4-mg dose. Skeletal-related events (SREs), time to skeletal related events, pain levels, and quality of life scores were recorded. The number of patients who suffered skeletal-related events was significantly less in the 4-mg group than the placebo group 44% versus 33% ($p = 0.02$) and the median time to the first skeletal related event was significantly different for these two groups as well (50% incidence not reached by 420 days in patients on 4 mg zoledronic acid and 50% incidence achieved at 363 days in patients on placebo). There was no difference seen in overall survival, disease progression, performance status, or quality of life in this study, and only 32% of the patients enrolled completed the study. Fatigue, anemia, myalgia, fever, and lower-limb edema occurred in at least 5% more patients in both zoledronic acid groups than in placebo groups (198). Skeletal-related events, as suggested above, are potentially more common in patients with metastatic disease or osteoporosis. An interesting study looking at the prevention of osteoporosis in prostate cancer was presented recently in the *Journal of Urology*. This multicenter, double-blind, randomized, placebo controlled study evaluated 106 men with nonmetastatic prostate cancer who were started on androgen blockade for the first time during the study period. All patients were given calcium and vitamin D supplementation and underwent bone density evaluation at enrollment and at the termination of the trial at 1 year. Study candidates were randomized to receive 4 mg of IV zoledronic acid or placebo upon enrollment and every 3 months during the study. Data showed that the mean bone mineral density increased significantly in

the lower spine, femoral neck, trochanter, and total hip in patients on the IV bisphosphonate, but decreased in patients on placebo. This difference in density was statistically significant ($p < 0.001$) (199).

Investigational Trials of Novel Agents

Ongoing clinical trials using investigational agents continue either alone or in combination with more conventional approaches discussed previously. Some of these approaches include gene or vaccine therapy, matrix metalloproteinase inhibitors, antiangiogenesis drugs, telomerase inhibitors, endothelian A receptor blockers, nonsteroidal antiinflammatory medications, gene therapy using antisense oligodeoxynucleotides (AS-ODNs) (chemically modified stretches of single-strand DNA complementary to mRNA that inhibit translation by forming RNA/DNA duplexes thereby reducing transcription of a target gene), and signal transduction inhibitors. A comprehensive discussion of these agents is beyond the scope of this chapter. Improved understanding of the pathways regulating apoptosis after androgen deprivation or cytotoxic chemotherapy has helped identify cell survival genes that may serve as new therapeutic targets in the future.

CONCLUSION

Treating patients with HRPC is challenging and requires consideration of multiple disease and patient-related factors. It is important to individualize the approach to therapy based on the patient's performance status, other comorbidities, symptoms, logistical issues, patient desires, and expectations. It is imperative to consider maintaining or improving quality of life, in addition to increasing survival when treating patients with hormone refractory prostate cancer.

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CHAPTER 31 ■ TESTICULAR CARCINOMA

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Germ cell tumors (GCTs) of the testis, arising from the malignant transformation of primordial germ cells, constitute 95% of all testicular neoplasms. GCTs infrequently arise from an extragonadal site, including the mediastinum, retroperitoneum, and, rarely, the pineal gland. This disease is notable for the young age of the afflicted patients and serves as a model for curable cancer. Ninety-five percent of all newly diagnosed patients are cured, including 70% to 80% of patients with metastatic disease treated with cisplatin-containing chemotherapy. Knowledge of management and experience are critical and studies show that patient outcome is superior at centers with expertise in the management of GCTs (1).

EPIDEMIOLOGY AND RISK FACTORS

Germ cell tumors are the most common solid tumors in men between the ages of 15 and 35 years. There are three modal peaks: infancy, ages 25 to 40, and approximately age 60. Approximately 8,900 new cases of testicular GCTs and 400 deaths due to testicular cancer were diagnosed in the United States in 2004, and the incidence is increasing (2).The worldwide incidence has more than doubled in the past 40 years. It is highest in Scandinavia, Switzerland, Germany, and New Zealand, intermediate in the United States and Great Britain, and lowest in Africa and Asia. GCTs are seen principally in whites; the white to black incidence is approximately 5:1.

The cause of GCTs is unknown. Familial clustering has been observed, particularly among siblings (3).Cryptorchidism and Klinefelter syndrome predispose a person to the development of GCTs arising from the testis and mediastinum, respectively (4).Orchiopexy performed prior to puberty reduces the risk of GCTs and improves the ability to observe the testis. Few data support a higher incidence of GCTs in persons with the human immunodeficiency virus infection and the results of treatment are similar (5,6).Reports suggesting that having a vasectomy may be a risk factor for GCTs have not been confirmed in cohort analysis (7).

PATHOLOGY

GCTs are classified into two major subgroups: seminoma and nonseminoma (Table 31-1). The Mostofi adaptation of the Dixon/Moore classification (8), adopted by the World Health Organization (WHO), is the classification commonly used in North America and Europe (9).

Carcinoma in situ (CIS), present in every case of testicular GCTs, is the precursor of invasive GCTs. CIS is frequently present in retroperitoneal presentations, but rarely in mediastinal presentations (10). The incidence of CIS is low in the general population; in men with impaired fertility, the incidence is about 0.5%. The incidence of CIS is between 2% and 5% in both cryptorchid testes and the contralateral testis in patients with a prior documented testicular GCTs (11,12).

Seminoma accounts for approximately one-half of all testicular GCTs and most frequently occurs in the fourth decade of life. The typical or classic form consists of sheets of large cells with abundant cytoplasm and round, hyperchromatic nuclei with prominent nucleoli. A lymphocytic infiltrate (and/or granulomatous reaction with giant cells) is frequently present. A high mitotic rate (anaplastic), syncytiotrophoblastic giant cells, and increased serum concentration of human chorionic gonadotropin may be present, but do not change management. An "atypical" form of seminoma has been described with unusual immunohistochemical features. The cells cytologically resemble classical seminoma, but lymphocytic infiltrate and granulomatous reaction are absent, necrosis is more common, and the nuclear-cytoplasmic ratio is higher than typical seminoma. "Atypical" seminoma frequently shows cytoplasmic expression of low-molecular-weight keratin or the type-1 precursor to the blood group antigens, while typical seminoma stains negative (13). Electron microscopic studies have shown that the individual tumor cells of "atypical seminoma" acquire cytoplasmic cytokeratin intermediate filaments, suggesting epithelial differentiation. There has been no specific association of "atypical" seminoma with an adverse prognosis, and its management is currently the same as any other seminoma.

The incidence of nonseminoma (which comprises 50% of GCTs) peaks in the third decade of life. Most tumors are mixed, consisting of two or more cell types, and consist of embryonal carcinoma, yolk sac carcinoma (endodermal sinus tumor), choriocarcinoma, and teratoma histologies. Seminoma may be a component, but the definition of a pure seminoma excludes the presence of any nonseminoma cell type. The presence of any nonseminomatous element (other than syncytiotrophoblasts) imparts the prognosis and directs management.

Embryonal carcinoma is the most undifferentiated cell type, with a capacity to differentiate to other nonseminoma cell types. Embryonal carcinoma may produce elevated serum concentrations of human chorionic gonadotropin (HCG), alpha-fetoprotein (AFP), or both. Pure choriocarcinoma, which is rare, consists of both cytotrophoblasts and syncytiotrophoblasts and is usually associated with widely metastatic disease and elevated serum concentrations of HCG. Hemorrhage into the primary tumor may occur and is occasionally a severe complication when it occurs spontaneously at a metastatic site. Yolk sac tumors histologically mimic the embryonic yolk sac and nearly always produce AFP. Pure yolk sac histology is frequently present in GCTs arising in the mediastinum.

Teratoma, derived from a totipotential, malignant precursor (embryonal carcinoma or yolk sac tumor), is composed of somatic cell types from two or more germ cell layers (ectoderm, mesoderm, and endoderm). Mature teratomas consist of adult-type differentiated cell types, such as cartilage or mucin-producing glandular epithelium. Immature teratoma refers to a tumor with partial somatic differentiation, similar to that seen in a fetus. Both mature and immature teratomas are generally histologically benign. Rarely, however, any teratoma may develop an aggressive growth pattern and histologically resemble a somatic malignancy such as rhabdomyosarcoma, adenocarcinoma, or primitive neuroectodermal tumor (14). This entity is

TABLE 31-1

HISTOLOGIC CLASSIFICATION OF GERM CELL TUMORS

1. Seminoma
   a. Classic (typical) seminoma
   b. Anaplastic seminoma
   c. Spermatocytic seminoma
2. Embryonal carcinoma
3. Teratoma
   a. Mature
   b. Immature
   c. Mature or immature teratoma with malignant transformation
4. Choriocarcinoma
5. Yolk sac tumor (endodermal sinus tumor)

referred to as "teratoma with malignant transformation," and may occur in the setting of GCTs arising from any primary site (14). Acute leukemia has been associated with GCTs arising from the mediastinum, but not from other primary sites (15). The clinical behavior of teratoma with malignant transformation follows the natural history of the transformed cell type, not GCTs (16).

BIOLOGY

The genetic analysis of male GCTs has revealed important data relevant to the mechanism of germ cell transformation. An isochromosome of the short arm of chromosome 12 [i(12p)] is a specific genetic marker of a GCT, and has been identified in all histologic subtypes (17). In tumors not displaying an i(12p), excess 12p genetic material is found on marker chromosomes with aberrant banding consisting of repetitive 12p segments (18). Therefore, excess 12p genetic material is present in all GCTs and represents one of the earliest genetic events in malignant transformation. i(12p) has been identified in the acute leukemia associated with mediastinal GCTs and in teratoma with malignant transformation, reflecting clonal GCT origin (19,20).

A candidate gene, *CCND2,* mapped to 12p13, is a possible oncogene on 12p whose deregulated expression may lead to GCT development (21). It is abundantly expressed in CIS as well as many lineages of GCTs (21). Cyclin D2 is one of the D-type cyclins that, along with the cyclin-dependent kinases cdk4 and/or cdk6, regulate the phosphorylation of pRB and controls the G1/S cell cycle checkpoint. Disruption of this checkpoint through the amplification/overexpression of D-type cyclins has been shown to be one of the most important pathways in human tumor development. Extensive molecular analysis has identified the loss of genomic or functional expression, or both, of several known tumor suppressor genes, such as *RB1*, *DCC*, and *NME*, and genomic loss at several previously recognized as well as at novel chromosomal sites (18,22). More recently, microarray technology has been used to identify candidate amplified and overexpressed genes on 12p that may ultimately provide a mechanistic basis for this genetic abnormality and its role in GCT development (23,24).

Male GCTs display patterns of differentiation that mimic stages normally undergone by the developing zygote. The pluripotentiality of the tumor cells manifests as histological differentiation into germ-cell-like undifferentiated (seminoma), primitive zygotic (embryonal carcinoma), embryonal-like somatically differentiated (teratoma), and extra-embryonally differentiated (choriocarcinoma and yolk sac tumor) phenotypes. Seminoma can be viewed as transformed germ cells that have retained the inhibitory mechanism for zygotic-like differentiation, a feature of germ cells prior to fertilization. The kit receptor, normally expressed by spermatogonia and primary spermatocytes (25), is expressed mainly by CIS and seminoma (26). Nonseminoma appears to down-regulate kit and up-regulate stem cell factor, which is consistent with loss of germ cell phenotype and acquisition of somatic fates (26).

Molecular genetic studies have identified a subset of clinically resistant GCTs that harbor *TP53* gene mutations (27). Evaluation of cellular response to cisplatin in one GCT-derived cell line with a *TP53* gene mutation showed resistance to cisplatin (27). This was in contrast to the extreme sensitivity of another GCT-derived cell line with wild-type *TP53*(27). The cisplatin resistance in a subset of GCT may be linked to an inability to mount apoptotic response after drug exposure due to inactivating *TP53* gene mutation. The rapid apoptotic response of GCTs upon exposure to chemotherapeutic agents may be due to a high ratio of the pro-apoptotic bax protein to the anti-apoptotic bcl-2 protein, favoring apoptosis (28).

A cohort of cisplatin-resistant GCTs was analyzed for the presence of amplified DNA sequences (29). In this study, comparative genomic hybridization was performed on a panel of GCTs comprising of 17 resistant and 17 sensitive tumors. High level amplification of eight chromosomal regions (other than 12p) was detected in five of the resistant tumors, but none in the sensitive group (29). Once the identity and function of the amplified genes are determined, they may become relevant to understanding GCT chemotherapy resistance.

INITIAL PRESENTATION

Clinical Presentation

The pathognomonic presentation of a primary testicular tumor is a painless testicular mass; however the painless testicular mass occurs in a minority of patients with testicular tumor. A majority present with diffuse testicular pain, swelling, hardness, or a combination of these findings. Since infectious epididymitis or orchitis is more common, a trial of antibiotic therapy is often undertaken first. If testicular discomfort does not abate or findings do not revert to normal within 2 to 4 weeks, a testicular ultrasound is indicated. On ultrasound, the typical testicular tumor is intratesticular and may produce one or more discrete hypoechoic masses or diffuse abnormalities with microcalcifications. The association between testicular microlithiasis and GCTs is not clearly defined (30,31). Bilateral tumors are rare at diagnosis, but 2% of patients with one testicular GCT will develop a metachronous new primary tumor in the remaining testis. Therefore, self-examination is taught to all patients.

A radical inguinal orchiectomy with clamping and ligation of the spermatic cord at the internal ring is required for all suspected testicular tumors. Since the testis embryologically originates in the genital ridge and descends through the abdomen and inguinal canal into the scrotum during fetal life, the primary lymphatic and vascular drainage of the testis is to the retroperitoneal lymph nodes and the renal or great vessels, respectively. Trans-scrotal orchiectomy is contraindicated because this leaves the inguinal portion of the spermatic cord intact and predisposes to scrotal skin recurrence and inguinal and pelvic nodal metastases.

GCTs also arise from extragonadal primary sites. The mediastinum and retroperitoneum are the most common primary extragonadal sites and comprise fewer than 10% of all GCT presentations. Retroperitoneal primary tumors present with back pain (psoas muscle invasion) or an abdominal mass.

Mediastinal primary tumors usually present with shortness of breath, chest pain, or superior vena cava syndrome. In all extragonadal presentations, testicular ultrasound is performed to exclude the testis as the primary site. The management of extragonadal and testicular GCTs is the same, and the primary site is an independent factor in staging and risk classification.

Staging

A comprehensive evaluation is necessary to define the extent of disease and determine the appropriate treatment. It includes pathology; physical examination; determination of serum concentrations of AFP, HCG, and LDH; and radiographic studies.

Pattern of Metastases

The regional draining lymph nodes for the testis are in the retroperitoneum and are the initial route of metastasis. The vascular supply originates from the great vessels with drainage to the inferior vena cava (for the right testis) or the left renal vein (for the left testis). As a result, the lymph nodes involved first by a right testicular tumor are the interaortocaval lymph nodes just below the renal vessels, between the aorta and inferior vena cava. For a left testicular tumor, the first involved lymph nodes are lateral to the aorta (para-aortic) and below the left renal vessels. In both cases, further nodal spread is inferior and contralateral and, less commonly, above the renal hilum.

Lymphatic involvement can extend cephalad to the retrocrural, posterior mediastinal, and supraclavicular lymph nodes. Left supraclavicular adenopathy and pulmonary nodules may occur with or without retroperitoneal disease. Pulmonary metastases are the most common site of visceral organ metastases. Liver, bone, and brain metastases are less common.

Radiographic Evaluation

A computed tomography (CT) scan of the abdomen and pelvis and a chest radiograph are required. Nodes in the retroperitoneum at landing zones measuring between 10 and 20 mm are involved by GCTs in about 70% of patients. In evaluation of the postchemotherapy mass, the CT scan detects residual masses which may contain residual malignant tumor, teratoma, or necrosis/fibrosis. If mediastinal, hilar or lung parenchymal disease is suspected, CT of the chest is required. CT or magnetic resonance imaging of the brain is performed for patients with neurologic signs or symptoms, but is usually not part of routine staging.

MRI occasionally provides valuable preoperative information regarding vascular anatomy and patency of the great vessels for patients with bulky retroperitoneal disease following chemotherapy. However, MRI adds little to the management of most patients with GCT. The role of lymphangiography is limited to assessing precise ports in patients with Stage I seminoma.

Studies have compared positron emission tomography (PET) to CT for evaluation of patients with newly diagnosed disease or residual disease after completion of chemotherapy. Although early studies suggest that PET may be more sensitive than CT, disease <0.5 cm was not detected by PET (32). In patients with NSGCT, PET has not been consistently able to identify residual viable malignant GCTs and does not detect teratoma (33,34). A study has shown that PET is useful in detection of residual viable seminoma in patients with masses larger than 3 cm in diameter after chemotherapy (35).

Serum Tumor Markers

AFP production is restricted to nonseminoma, specifically embryonal carcinoma and yolk sac tumor. The serum half-life of clearance is 5 to 7 days. Increased AFP concentrations are seen at all stages; between 40% and 60% of the patients with metastasis have increased serum levels. Conditions other than GCTs in which elevated AFP may be detected in serum include liver injury (infectious, drug/alcohol induced), hepatocellular carcinoma, and other malignancies arising from the gastrointestinal tract. Elevated serum concentrations of AFP for patients with pure seminoma reflect an otherwise undetected nonseminoma presence.

Increased HCG serum concentrations (produced by embryonal carcinoma, seminoma, or syncytiotrophoblasts) are observed in 40% to 60% of metastatic nonseminomatous GCTs, and 15% and 20% of metastatic seminomas (36). The serum half-life of clearance is 18 to 36 hours. The α-subunit is identical to that of luteinizing, follicle-stimulating, and thyroid-stimulating hormones. Since the a-subunit is common to several pituitary hormones, radioimmunoassays for HCG are directed at the β-subunit. False-positive estimations of HCG concentrations include cross-reactivity of the antibody with luteinizing hormone and treatment-induced hypogonadism, or pituitary production of HCG.

Lactate dehydrogenase (LDH) is less specific than AFP and HCG. Serum levels are increased in approximately 60% of advanced nonseminoma and 80% of advanced seminoma (36,37). The serum level of LDH has independent prognostic significance for patients with advanced GCTs.

Serum tumor marker levels are assessed before, during, and following treatment, and throughout follow-up. Increased levels of AFP and/or HCG without radiographic or clinical findings imply active disease and are sufficient reason to initiate treatment if false-positive causes are excluded. After surgery and chemotherapy, marker concentrations should decrease according to known half-lives. A plateau or slow half-life clearance suggests residual active disease (38).

STAGING

A comprehensive evaluation is necessary to define the extent of disease and to determine appropriate treatment. This includes pathologic examination of the primary tumor; physical examination; determination of serum concentrations of AFP, HCG, and LDH; and radiographic studies. The GCT's extent is assigned as follows: Stage I—disease limited to testis, epididymis, or spermatic cord; Stage II—disease limited to retroperitoneal lymph nodes; and Stage III—metastatic disease to supradiaphragmatic nodal or visceral sites, or markedly elevated serum tumor markers. Revised TNM and stage groupings of the American Joint Committee on Cancer (AJCC) and the Union Internationale Centre le Cancre (UICC) were adopted in 1997 (Table 31-2) (39). Serum concentrations of AFP, HCG, and LDH were incorporated because of their independent prognostic significance. The classification of retroperitoneal lymph node involvement is based either on pathological evaluation after retroperitoneal lymph node dissection (RPLND) or clinical evidence of retroperitoneal lymph node involvement.

PROGNOSTIC FACTORS

T-stage of the primary lesion (not size), histology, and serum tumor marker concentrations predict the likelihood of retroperitoneal disease for clinical Stage I tumors. For nonseminoma, the presence of lymphatic and/or vascular invasion is associated

TABLE 31-2

TNM STAGE

Primary tumor (T)

pT_X	Primary tumor cannot be assessed.
pT_O	No evidence of primary tumor (eg., histologic scar in testis).
pT_{is}	Intratubular germ cell neoplasia (carcinoma in situ).
pT_1	Tumor limited to the testis and epididymis and no vascular/lymphatic invasion. Tumor may invade into the tunica albuginea but not the tunica vaginalis.
pT_2	Tumor limited to the testis and epididymis with vascular/lymphatic invasion or tumor extending through the tunica albuginea with involvement of tunica vaginalis.
pT_3	Tumor invades the spermatic cord with or without vascular/lymphatic invasion.
pT_4	Tumor invades the scrotum with or without vascular/lymphatic invasion.

Regional lymph nodes (N)

Clinical

N_X	Regional lymph nodes cannot be assessed.
N_O	No regional lymph node metastasis.
N_1	Lymph node mass 2 cm or less in greatest dimension; or multiple lymph nodes, none more than 2 cm in greatest dimension.
N_2	Lymph node mass more than 2 cm, but not more than 5 cm in greatest dimension; or multiple lymph nodes, any one mass >2 cm, but not more than 5 cm in greatest dimension.
N_3	Lymph node mass more than 5 cm in greatest dimension.

Pathological

pN_0	No evidence of tumor in lymph nodes.
pN_1	Lymph node mass 2 cm or less in greatest dimension and ≤5 nodes positive, none >2 cm in greatest dimension.
pN_2	Lymph node mass more than 2 cm but not more than 5 cm in greatest dimension; more than 5 nodes positive, none >5 cm; evidence of extranodal extension of tumor.
pN_3	Lymph node mass more than 5 cm in greatest dimension.

Distant metastases (M)

MO	No evidence of distant metastases.
M1	Nonregional nodal or pulmonary metastases.
M2	Nonpulmonary visceral metastases.

Serum tumor markers (S)

	LDH	HCG (mIu/mL)	AFP (ng/mL)
S1	<1.5 × N	<5,000	<1,000
S2	1.5–10 × N	5,000–50,000	1,000–10,000
S3	>10 × N	>50,000	>10,000

N indicates the upper limit of normal for the LDH assay.

Stage GROUPING

	T	N	M	S
Stage I				
IA	T_1	N_0	M_0	S_0
IB	T_2	N_0	M_0	S_0
	T_3	N_0	M_0	S_0
	T_4	N_0	M_0	S_0
IS	T_{ANY}	N_0	M_0	S_{ANY}
Stage II				
IIA	T_{ANY}	N_1	M_0	S_0
	T_{ANY}	N_1	M_0	S_1
IIB	T_{ANY}	N_2	M_0	S_0
	T_{ANY}	N_2	M_0	S_1
IIC	T_{ANY}	N_3	M_0	S_0
	T_{ANY}	N_3	M_0	S_1
Stage III				
IIIA	T_{ANY}	N_{ANY}	M_1	S_0
	T_{ANY}	N_{ANY}	M_1	S_1
IIIB	T_{ANY}	N_{ANY}	M_0	S_2
	T_{ANY}	N_{ANY}	M_1	S_2
IIIC	T_{ANY}	N_{ANY}	M_1	S_3
	T_{ANY}	N_{ANY}	M_2	S_{ANY}

(From: *AJCC staging handbook*. Philadelphia-New York: Lippincott-Raven, 1998, with permission.)

TABLE 31-3

GERM CELL TUMOR RISK CLASSIFICATION

	Seminoma	Nonseminoma
Good Risk	Any HCG Any LDH Nonpulmonary visceral metastases absent Any primary site	AFP <1000 ng/mL HCG <5000 mIu/mL LDH <1.5 × upper limit of normal Nonpulmonary visceral metastases absent Gonadal or retroperitoneal primary tumor
Intermediate Risk	Nonpulmonary visceral metastases present Any HCG Any LDH Any primary site	AFP 1,000–10,000 ng/mL HCG 5,000–50,000 mIu/mL LDH 1.5–10.0 times upper Limit of normal Nonpulmonary visceral metastases absent Gonadal or retroperitoneal primary site
Poor Risk	–	Mediastinal primary site Nonpulmonary visceral metastases present (eg., bone, liver, brain) AFP ≥10,000 ng/mL HCG ≥ 50,000 mIu/mL LDH ≥10 × upper limit of normal

(From: International Germ Cell Cancer Collaborative Group. International germ cell concensus classification: a prognostic factor-based staging system for metastatic germ cell cancers. *J Clin Oncol* 1997;15:594, with permission.)

with a higher likelihood of retroperitoneal metastases (about 50%) and is included in the definition of T_2. Invasion through the tunica albuginea into tunica vaginalis (also T_2), spermatic cord (T_3), or scrotum (T_4) are additional adverse features. Reproducible prognostic factors predicting retroperitoneal disease in seminoma have not been identified. Persistent elevation of serum tumor markers after orchiectomy implies metastatic disease.

The number and size of retroperitoneal lymph nodes found at RPLND for nonseminoma have prognostic importance (pathologic stage). Most retrospective studies report a ≤30% incidence of relapse when fewer than six nodes are involved with tumor, *and* the largest node is <2 cm, *and* no extranodal tumor extension is evident. More extensive tumor involvement at RPLND is generally associated with a recurrence rate of ≥50%.

The transverse diameter of the largest lymph node on CT scan is used to subcategorize Stage II disease (clinical stage). For seminoma, the size of retroperitoneal adenopathy on CT scan dictates treatment modality, and relapse proportion after definitive radiation therapy increases from 15% or less for nodes <5 cm to between 40% and 60% for nodes >5 cm (40). For patients with nonseminoma, treatment is based on retroperitoneal lymph node size, location of adenopathy, and presence of increased serum tumor marker concentrations.

Between 70% and 80% of the patients with advanced GCTs are cured with cisplatin- combination chemotherapy programs. Treatment is stratified according to the probability of cure. Patients more likely to be cured are classified as "good-risk." They constitute the majority with advanced disease and are treated with regimens showing maximum efficacy with minimal toxicity. In contrast, the patients who are unlikely to be cured are categorized as "poor-risk." For poor-risk patients, effective therapy is the priority.

Between 1980 and 1997, several classification algorithms were used to assign good- and poor-risk status based on extent of disease, specific sites of disease, and pretreatment serum tumor marker concentrations. The Memorial Sloan-Kettering Cancer Center (MSKCC) (41) and Indiana University (42) allocation criteria were used in the United States. A comparison of these and other risk criteria for patients with advanced nonseminoma demonstrated marked differences (43). The International Germ Cell Cancer Collaborative Group (IGCCCG) developed a common classification system based on data from over 5,000 patients treated with chemotherapy. Independent prognostic factors for progression-free survival with nonseminoma were pretreatment levels of LDH, HCG, AFP; site of primary tumor; and the presence of nonpulmonary visceral metastases (bone, brain, or liver) (44). Nonpulmonary visceral metastasis was the only significant prognostic factor in seminoma. Investigators agreed on three strata of good-, intermediate-, and poor-prognosis patients (Table 31-3). These strata were incorporated into the revised TNM classification (39). The IGCCCG grouping should be used in future clinical trials and in treatment decisions.

MANAGEMENT OF LOW-STAGE GCT

Seminoma

Patients with clinical Stage I seminoma are treated with radiation therapy. A randomized trial showed that a simple paraaortic port excluding the ipsilateral iliac and pelvic nodes is as effective as the "dog-leg" portal. Although toxicity appears less, more pelvic relapses may be seen (45). Stage I seminoma is treated with 150 to 180 cGy/day five sessions per week using high-energy linear accelerator beams to a total dose of 2,500 to 3,000 cGy. Approximately 4% will relapse elsewhere, and the death rate is less than 2% (45–49).

Long-term sequelae of radiation therapy include the potential for gastrointestinal neoplasms (50). Therefore, surveillance alone has been studied as management after orchiectomy, with chemotherapy reserved for patients who relapse. The relapse rate to surveillance is approximately 15%, and relapses occur at intervals more than 5 years from diagnosis. Therefore, in the United States, observation for clinical Stage I seminoma is not recommended.

Low-tumor-burden Stage II seminoma includes all patients with retroperitoneal metastases measuring <5 cm in maximum transverse diameter (clinical Stages IIA and IIB); radiation therapy is the treatment of choice. A dog-leg radiation portal is used. Fractionation is the same as clinical Stage I disease, with a boost of approximately 500 to 750 rads to involved lymph nodes. The relapse proportion is 5% to 15%, with death from seminoma rare (51,52). Prophylactic mediastinal radiation therapy is not given, since relapses solely in the anterior or posterior mediastinum seldom occur.

A "horseshoe kidney" is a contraindication to retroperitoneal radiation therapy due to the high likelihood of radiation-induced renal failure. In this circumstance, observation is preferred in clinical Stage I, and primary chemotherapy is preferred for clinical Stage II. Patients with second metachronous testicular GCTs following RPLND or radiation are observed for clinical Stage I disease. Inflammatory bowel disease may also be a contraindication to radiation therapy, and the same management policies are followed as for patients with a horseshoe kidney.

Nonseminoma

The choice of management options for clinical Stage I nonseminoma depends upon specific histologic features and serum tumor marker concentrations. The conventional approach has been RPLND. Retroperitoneal metastases will be found in about 30% of patients judged preoperatively to be clinical Stage I (53). In the past, most patients undergoing bilateral RPLND experienced retrograde ejaculation and subsequent infertility. An improved understanding of the neuroanatomy of seminal emission and ejaculation, the pattern of retroperitoneal metastasis for right- and left-sided tumors, and surgical mapping studies have led to modification of infrahilar surgical boundaries and techniques.

RPLND may be of two types: nerve-dissecting or nerve-avoiding. Modified, nerve-avoiding RPLND templates were designed to avoid the hypogastric plexus and contralateral sympathetic fibers responsible for ejaculation in clinical Stage I or IIA disease. These template dissections do not identify specific nerve fibers. Rather, their design minimizes trauma to the hypogastric plexus by limiting contralateral dissection above the takeoff of inferior mesenteric artery. This method avoids transsection of contralateral nerves and results in preservation of ejaculation in approximately 50% to 80% of patients. Because the para-aortic nodes may be involved with right sided primary tumors, a dissection of these nodes is important in right RPLND template to minimize the need for reoperation (54). Preservation of ejaculation appears to be more successful when nerves are prospectively identified and spared, compared to modified template dissection, although the operation usually takes longer. With nerve dissection, about 95% of patients have normal ejaculatory status postoperatively. Regardless of technique, retrograde ejaculation remains a risk with any RPLND, and preoperative sperm banking is recommended.

The impetus for surveillance in clinical Stage I patients includes the infertility resulting from RPLND, lack of tumor detected at RPLND, and high cure rate associated with cisplatin-based chemotherapy for advanced disease. Approximately 25% of patients with $T_1N_0M_0$ disease and normal serum tumor markers relapse during surveillance (55). A higher likelihood of retroperitoneal and/or systemic relapse is associated with T_{2-4} tumors.

Clinical Stage I nonseminoma patients with a T_1 tumor and serum tumor markers that are normal or declining at half-life are offered both surgical and observation options. When RPLND is chosen, the procedure should be a nerve-sparing type. If surveillance is selected, patient compliance is essential, as follow-up is rigorous and includes physical examination, chest x-ray, determinations of AFP and HCG levels, and regular CT scans. Patients with clinical Stage I and persistently elevated or rising serum tumor markers following orchiectomy are treated with chemotherapy, since the disease is often not limited to the retroperitoneum (56). The experience is limited regarding chemotherapy as initial treatment of clinical Stage I disease in the setting of normal tumor markers when the risk of retroperitoneal disease is high (T_2 or greater). In three reports of patients receiving two cycles of cisplatin-based chemotherapy, fewer than 5% relapsed and about 1% died of GCT (57,58,59). This approach avoids RPLND, but patients are exposed to the transient (e.g., myelosuppression), permanent (e.g., neuropathy), and delayed (e.g., Raynaud's phenomenon, acute leukemia, cardiovascular) toxicities of chemotherapy.

Low-tumor-burden clinical Stage II nonseminoma includes disease ipsilateral to the primary tumor, at or below the renal hilum, not associated with tumor-related back pain, and limited to the primary landing zone. Ipsilateral solitary lymph nodes <3 cm are managed by RPLND. However, elevated serum tumor markers usually reflect systemic disease, and these patients are considered for primary cisplatin-based chemotherapy. Lymph nodes between 3 and 5 cm, even if solitary, are associated with more extensive disease than can be detected on abdominal CT scan, and are generally treated with chemotherapy followed by RPLND.

Surveillance is a treatment choice for compliant patients with fewer than six involved nodes and none larger than 2 cm (pN1) (60). Approximately 20% of such patients relapse. Surveillance requires close monitoring and a compliant patient. Lack of patient compliance, psychologic factors, occupation, geography or other issues may make adjuvant chemotherapy the preferred choice in rare patients. Those who relapse during surveillance require three or four cycles of cisplatin-based therapy according to disease status at that time.

Adjuvant chemotherapy is an important consideration when any involved node resected at RPLND is >2 cm in diameter, or six or greater nodes are involved, or extranodal extension is present (pN2-pN3) (60). A majority of patients relapse in this setting when adjuvant chemotherapy is not given. A randomized trial showed that observation with standard treatment at relapse and two cycles of adjuvant chemotherapy had equivalent survival (61). Treatment with two cycles of adjuvant etoposide plus cisplatin with or without bleomycin administered at three-week intervals results in a relapse-free survival of nearly 100% (61–66). Although survival is the same when chemotherapy is withheld until relapse, the number of cycles of adjuvant chemotherapy is less than that given at relapse and additional surgery is avoided.

ADVANCED METASTATIC DISEASE

The treatment for patients with clinical Stage IIC and primary retroperitoneal and mediastinal seminoma is chemotherapy, since relapse frequent occurs when these patients are managed with radiation therapy only. In nonseminoma, Stage III patients and Stage II patients with multifocal retroperitoneal lymph node involvement, lymphadenopathy >3 cm in diameter, increased AFP or HCG, and/or tumor-related back pain receive initial chemotherapy.

The first combination chemotherapy regimens containing cisplatin, vinblastine, and bleomycin (with or without other drugs) resulted in a 70% to 80% complete response proportion in patients with metastatic GCT (37,67). Subsequent studies showed that prolonged maintenance chemotherapy was unnecessary, and etoposide, less toxic and more efficacious, replaced vinblastine (68). Significant toxicities included neuromuscular toxicity (68), mortality due to myelosuppression or bleomycin-induced pulmonary fibrosis (68), and Raynaud's phenomenon (68,69).

TABLE 31-4

CHEMOTHERAPY REGIMENS

A. Previously untreated—good risk
1. Etoposide 100 mg/m^2 IV daily × 5 days
   Cisplatin 20 mg/m^2 IV daily × 5 days
   Four cycles administered at 21-day intervals
2. Etoposide 100 mg/m^2 IV daily × 5 days
   Cisplatin 20 mg/m^2 IV daily × 5 days
   Bleomycin 30 units IV weekly on days 2, 9, 16
   Three cycles administered at 21-day intervals

B. Previously untreated—intermediate and poor risk
   Etoposide 100 mg/m^2 IV daily × 5 days
   Cisplatin 20 mg/m^2 IV daily × 5 days
   Bleomycin 30 units IV weekly on days 2, 9, 16
   Four cycles administered at 21-day intervals

The high cure rate with significant toxicity resulted in an effort to identify patients more likely ("good-risk") and less likely ("poor-risk") to be cured with standard chemotherapy. Extent of disease and serum tumor marker elevations were identified as independent predictors of prognosis (44), and previously untreated patients with metastatic disease were stratified into good-risk and poor-risk groups. Good- and poor-risk allocation algorithms were developed, and clinical trials addressed issues represented by risks of treatment failure. Standard treatment regimens are summarized in Table 31-4. Response proportions range from 88% to 95% among good-risk patients with favorable survival distributions (43). Since the advent of good-risk stratification, trials focused on eliminating bleomycin from treatment, reducing the number of cycles of chemotherapy, and substituting carboplatin for cisplatin (Table 31-5) (70–76).

Three randomized clinical trials have evaluated the elimination of bleomycin from regimens containing etoposide and cisplatin. Etoposide and cisplatin (EP) for four cycles was compared to a five-drug, bleomycin-containing regimen (VAB-6) and was found to be therapeutically equivalent and less toxic (71). A randomized trial of cisplatin and etoposide with (BEP) and without bleomycin (EP) was performed by the EORTC. The dose of etoposide in this study was 360 mg/m^2 per cycle, with dose modifications for thrombocytopenia in contrast to 500 mg/m^2 per cycle without dose attenuations in American trials (72). The bleomycin arm was more toxic; the complete response proportion was lower in EP patients (72). No differences were observed in relapses, time to progression, or survival in over 7 years of follow-up (72). This study concluded that bleomycin cannot be deleted from good-risk therapy when European doses of etoposide are used.

A French trial compared three cycles of BEP to four cycles of EP. The criteria for good risk were those developed by the Institute Gustave Roussy but most patients were good risk by the IGCCCG criteria. At the primary endpoint of "favorable" response, BEP (92%) and EP (91%) were equivalent. With a median follow-up of 4 years, neither event-free survival nor overall survival was statistically different (76). The trial has been criticized for a number of reasons including the failure to establish noninferiority, and the retrospective assignment to IGCCCG criteria. A retrospective analysis evaluated late relapse in patients receiving EP, and none were observed with a median follow-up of 7 years (77).

Because carboplatin has less neurologic and gastrointestinal toxicity than cisplatin, two randomized trials compared their efficacy in combination chemotherapy. Both trials showed carboplatin was inferior to cisplatin (74,75). Carboplatin-containing programs have no role in the initial treatment of advanced GCTs.

These trials collectively establish that four cycles of etoposide (500 mg/m^2/cycle) plus cisplatin (≥100 mg/m^2/cycle) or three cycles of bleomycin, etoposide plus cisplatin are equally effective and well tolerated, and in conjunction with adjunctive surgery, will cure approximately 90% of good-risk patients. Either regimen may be considered standard treatment.

Between 20% and 25% of patients with advanced GCT present with hepatic, osseous, and/or brain metastases; high serum tumor marker levels; or mediastinal nonseminomatous primary tumors. These features are associated with a low likelihood of cure, and clinical trials directed at improving treatment efficacy remain the priority. New agents and dose-intense therapy have been evaluated to improve the treatment outcome for poor-risk GCT patients. A randomized trial comparing cisplatin 100 and 200 mg/m^2 per cycle given in combination

TABLE 31-5

RANDOMIZED TRIALS IN GOOD-RISK GERM CELL TUMORS

Reference	Good-risk criteria	Regimen (cycles)	Complete response (%)	Durable response (%)	Recommended regimen
70	Indiana	BEP(4)	97	92	BEP (3)
		BEP(3)	98	92	
71	MSKCC	VAB-6(3)	96	85	EP(4)
		EP(4)	93	82	
72	EORTC	BEP(4)	95	91	BEP (4)
		EP(4)	87	83	
73	Indiana	BEP(3)	94	86	BEP(3)
		EP(3)	88	69	
74	MSKCC	EC(4)	90	87	EP (4)
		EP(4)	88	76	
75	MRC/EORTC	CEB(4)	87	77	BEP (4)
		BEP(4)	94	91	
76	GETUG	BEP(3)	92	—	Regimens equivalent
		EP(4)	91	—	

MSKCC, Memorial Sloan-Kettering Cancer Center; *EORTC*, European Organization for the Research and Treatment of Cancer; *MRC*, Medical Research Council; *GETUG*, Genito-Urinary Group of the French Federation of Cancer Centers; *B*, bleomycin; *E*, etoposide; *P*, cisplatin; *C*, carboplatin; *VAB-6*, cisplatin + vinblastine + dactinomycin + bleomycin + cyclophosphamide; *NS*, not stated.

with etoposide plus bleomycin showed that the higher cisplatin dose resulted in equal efficacy but greater toxicity (78). In another randomized trial, ifosfamide replaced bleomycin, but no therapeutic benefit was observed and toxicity was worse (79). Therefore, in poor-risk patients, four cycles of BEP remain the standard regimen to which investigational regimens should be compared. Likewise, in intermediate-risk patients by IGCCCG criteria, four cycles of BEP is considered standard therapy. Durable complete responses occur in 75% and 40% of intermediate- and poor-risk patients, respectively.

Reports that high-dose therapy with stem cell rescue cured a minority of patients with refractory GCT (80,81) led to the study of dose-intensive regimens as part of first-line therapy. Two studies incorporated high-dose carboplatin plus etoposide into first-line therapy for poor-risk patients. In the first study, poor-risk patients who were at high risk of failure based on presenting factors were selected to receive high-dose therapy. Patient selection was based on a prolonged clearance of serum AFP or HCG, or both, during standard induction chemotherapy (82,83). Hematopoietic reconstitution was rapid, and a trend to improved survival was observed in patients who received high-dose chemotherapy compared to an historical poor-risk experience with conventional-dose, cisplatin-based chemotherapy (83). In a separate report, a single-arm study of high-dose chemotherapy with autologous stem cell support as first-line therapy in advanced GCT patients with poor prognostic features, also suggested a favorable outcome in patients receiving high-dose chemotherapy compared to historical controls treated on conventional-dose therapy (84). A national, randomized trial comparing four cycles of BEP to two cycles of BEP followed by two cycles of three-drug, high-dose therapy in poor- and intermediate-risk patients has completed accrual and data are maturing.

POSTCHEMOTHERAPY SURGERY

Surgical resection of residual disease is necessary in nonseminomatous GCT patients who respond to cisplatin-containing chemotherapy by achieving normal serum tumor marker concentration. Necrotic debris/fibrosis, teratoma, and/or viable carcinoma may be found at resection. The pathologic presence of necrotic debris or mature teratoma requires no further chemotherapy; between 5% and 10% of patients will relapse. If a viable residual GCT is completely resected, two additional cycles of chemotherapy are given, but 30% to 50% of patients relapse (85).

There is general agreement over the need to resect all measurable residual disease. In the retroperitoneum, necrosis/fibrotic debris comprises 45% to 50% of pathologic findings, teratoma another 35%, and viable GCT the remaining 15% to 20% (86–88). No single criterion predicts a negative pathology with sufficient accuracy to eliminate the risk of residual teratoma or viable GCTs and obviate postchemotherapy RPLND. The need for postchemotherapy RPLND is controversial when the postchemotherapy CT scan, usually of the retroperitoneum, is interpreted as "normal." But, larger retrospective series show that 10% to 20% of such patients will have either mature teratoma or viable carcinoma in previously involved areas (86,87,88).

The approach to patients with pure seminoma and a postchemotherapy residual mass is controversial. Two important features distinguish seminoma from nonseminoma. First, teratoma in the residual mass is rare. Second, a complete RPLND following chemotherapy is often not technically feasible, secondary to severe desmoplastic reaction and obliteration of tissue planes. Consequently, perioperative morbidity is reported as higher for seminoma than for nonseminoma (89,90).

The majority of patients with persistent radiographic abnormalities after chemotherapy do not have viable seminoma. Studies conflict with regard to size of residual mass as a predictor. In a series of 104 patients, 8 of 30 (27%) patients with a residual mass >3 cm relapsed or had residual seminoma (91). Conversely, among 74 with tumors measuring <3 cm, no viable tumor was identified, and only two patients (3%) relapsed at the site of residual "disease." Their conclusion was that resection or biopsy of a residual mass ≥3 cm is preferable to observation, recognizing that full RPLND was usually not feasible. If viable seminoma was documented, additional therapy was required (91). Conversely, other series report that the size of the residual mass is not predictive of residual malignancy. A fluorodeoxyglucose (FDG)-PET scan study in 52 patients with residual mass after chemotherapy for bulky seminoma reported that all 8 positive scans and 42 of 44 negative scans were accurate (35).

In seminoma, residual masses <3 cm are observed. Controversy exists regarding the minority of patients with a residual mass measuring ≥3 cm. An FDG-PET scan should be done in patients with a residual mass measuring 3 cm or larger. A negative PET scan in this setting is associated with a high likelihood of freedom from disease. A positive PET scan implies presence of viable disease and intervention should be considered. Careful observation is one alternative, but relapse with subsequent ifosfamide-based salvage chemotherapy has a low cure rate. Surgical resection, which can identify residual disease and direct immediate therapy, has low morbidity in experienced hands given modern techniques and perioperative care (91). There is no role for radiation to the area of residual disease (92).

MANAGEMENT OF RELAPSE AFTER CHEMOTHERAPY

Twenty to thirty percent of patients with advanced GCT will relapse or fail to achieve a complete response to conventional-dose cisplatin-based chemotherapy. Patients who relapse after complete response are treated with second-line ifosfamide plus cisplatin-containing salvage chemotherapy, and approximately 25% are cured (93). High-dose chemotherapy with stem cell rescue is the treatment of choice for patients not cured by ifosfamide plus cisplatin-containing chemotherapy or who fail to achieve complete response to first-line chemotherapy (i.e., incomplete response). Two cycles of high-dose carboplatin plus etoposide with or without high-dose cyclophosphamide (or ifosfamide) with stem cell rescue given as third-line therapy cures approximately 20% of patients who fail prior cisplatin plus ifosfamide-containing therapies (81,94).

The major toxicities of dose-intensive therapy are hematologic and infectious, with most studies reporting 10% treatment-related mortality (81). Hematopoietic growth factor support decreases the duration of neutropenia and hospitalization (95). Peripheral blood-derived stem cells have replaced the use of autologous bone marrow, and a randomized trial showed that peripheral blood-derived stem cells result not only in rapid neutrophil reconstitution but also faster platelet engraftment (96).

Prognostic factors may be used to select patients for either standard ifosfamide based chemotherapy or high-dose chemotherapy with stem cell rescue. Patients with testicular GCT who fail to achieve an initial complete remission have a cure rate of <10% with ifosfamide-based therapy (97) and are treated with high-dose etoposide plus carboplatin-based regimens. In contrast, patients with GCTs who progress within 1 month of cisplatin, have high HCG levels, or nonseminoma histology arising from a mediastinal primary site generally

do not achieve long-term benefit from high-dose chemotherapy and should be considered for Phase II studies with novel agents.

Complete and partial responses to paclitaxel have been reported in approximately 25% of patients who fail prior therapy (98). The combination of ifosfamide, cisplatin, and vinblastine (VeIP) is a standard regimen in patients who relapse from complete remission with a durable complete remission rate of 25%. The substitution of paclitaxel for vinblastine shows promise in both high-dose and conventional-dose salvage programs (99,100). Of 46 patients treated with paclitaxel, ifosfamide plus cisplatin (TIP) as second-line therapy, 36 (78%) were progression-free more than 18 months from the start of therapy (101). All had favorable prognostic features for response: testis primary site and prior complete response to first-line chemotherapy program. Four cycles of TIP was associated with a high proportion of patients who achieved a complete response, a low likelihood of relapse, and tolerable toxicity.

In contrast, an incomplete response to first-line therapy or extragonadal (mediastinal) primary site results in a durable complete remission in fewer than <10% of patients treated with cisplatin plus ifosfamide salvage chemotherapy. Three series of patients with refractory progressive GCTs, who received high dose carboplatin and etoposide with stem cell rescue (with or without cyclophosphamide or ifosfamide), reported that 15% to 21% of patients survived and remained disease-free with long-term follow-up (102–104). The major toxicities are hematologic and infection, with these series reporting a treatment-related mortality rate of 10% to 12%. The treatment of these patients with repetitive cycles of dose-intensified paclitaxel, ifosfamide, carboplatin, and etoposide with autologous stem cell rescue resulted in a complete remission in 21of 37 (57%) treated with this program and 13 (35%) remain in durable complete response with follow-up >18 months (100). Hematopoietic growth factor and peripheral blood-derived stem cells decreased the duration of neutropenia and hospitalization. Although these series demonstrate that refractory disease remains potentially curable, the majority of cisplatin-refractory GCTs patients die of their disease, and new drugs and strategies are still an important are of research.

Histologic findings of resected masses following second-line chemotherapy shows viable tumor occurs in approximately 50% of specimens, teratoma in 40%, and necrosis in only 10% (105). Additional standard-dose chemotherapy adds no benefit to patients with viable NSGCT in the resected specimen after salvage chemotherapy.

In general, surgery should be avoided in patients with elevated serum tumor markers. While this general rule holds true, surgery has curative potential in a highly select group of patients with increased marker levels, even after salvage chemotherapy. Patients with a solitary retroperitoneal mass and increased AFP seem to be the best candidates. Technically difficult, this surgery should be performed at a tertiary center.

TREATMENT SEQUELAE

Acute nausea and vomiting occurs but is controllable with 5-HT_3 antagonist plus dexamethasone. Cisplatin can be associated with delayed emesis, and administration of oral metoclopramide and a benzodiazepine plus dexamethasone for 2 to 4 days after therapy is sometimes necessary.

Due to the effect of cisplatin on proximal tubules, progressive reduction in glomerular filtration and cumulative hypomagnesemia may result (106), particularly after ifosfamide-combination or high-dose salvage chemotherapy. An increased frequency of diastolic hypertension and cardiac events, increased mean cholesterol levels, and increased low-density and decreased high-density lipoprotein levels have been reported years after chemotherapy for GCTs (107–109). All patients treated with cisplatin-based chemotherapy for GCTs should be monitored for blood pressure, lipids, and cholesterol, and symptoms suggestive of cardiovascular disease.

Neutropenic fever occurs in about 10% to 15% of patients receiving EP, more frequently with the addition of bleomycin, and in more than 50% of patients receiving salvage chemotherapy. Hematopoietic growth factor support should be used prophylactically from the beginning of ifosfamide salvage therapy. Replacing vinblastine with etoposide decreased the frequency of neuropathy, although cisplatin still causes symptomatic neuropathy in a minority of patients. Auditory toxicity from cisplatin is associated with reduced high-tone hearing and, less frequently, tinnitus, although patients rarely require hearing aids.

Pulmonary toxicity from bleomycin is rare but can be fatal. In good-risk patients, a reduction in the number of bleomycin doses from 12 to 9 resulted in no bleomycin-related, deaths (70). Vascular toxicity, most prominently as Raynaud's phenomenon, occurs in a minority (<10%) of patients receiving bleomycin by weekly bolus (69).

Infertility is an important consideration, since patients are young and the cure rate is high. A standard, modified bilateral RPLND results in retrograde ejaculation in nearly all patients. Nerve-dissecting and nerve-avoiding RPLND reduce, but do not eliminate, that risk. Chemotherapy may affect the germinal epithelium directly, and Leydig cell insufficiency is frequent. After chemotherapy, persistent oligospermia and abnormal forms and motility have been reported (110), but conception may occur despite oligospermia.

Etoposide causes secondary leukemia characterized by translocations involving chromosome 11q in less than 0.5% of patients receiving a total dose <2,000 mg/m^2 (111,112) and as many as 6% of patients receiving total etoposide doses of >3,000 mg/m^2(113). However, recent reports showed acute leukemia in 0.8% to 1.3% of patients receiving median cumulative etoposide doses >2,400 mg/m^2(114).

The incidence of gastrointestinal malignancies increased after radiation therapy or radiation plus chemotherapy (50,115). The relative risk increases with time and is greatest after 10 years (50). An excess of soft tissue sarcoma has also been observed, with radiation therapy implicated as the major cause (50). These second malignancies do not outweigh the benefits of treatment intervention.

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SECTION VI: INFECTIONS OF THE URINARY TRACT AND THE KIDNEY

CHAPTER 32 ■ HOST–PATHOGEN INTERACTIONS AND HOST DEFENSE MECHANISMS

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The urinary tract is a complicated epithelium-lined tube with an opening to the body surface, making it susceptible to infection by exogenous organisms. Indeed, urinary tract infections (UTIs), which are almost always caused by colonic organisms in the normal host, may be the most common bacterial infection of humans (1). UTIs represent the most common kidney and urologic disease in the United States, whether judged by visits to physicians or hospital discharge summaries (2), and entail direct and indirect medical costs approaching $2 billion annually (3).

In the course of producing, storing, and voiding urine, the urinary tract is continually flushed, and infecting organisms are usually recovered by the clinician or investigator in a voided urine specimen. This fortunate situation allows for relatively easy assessment of bacteriuria, which almost always accompanies UTIs. Bacteriuria generally manifests in one of three clinical presentations. The first category is *asymptomatic bacteriuria*, in which bacteria may be limited to the bladder or may be found in both the bladder and kidney. The second is *cystitis*, a syndrome characterized by dysuria, frequency, and urgency of urination, sometimes with lower abdominal pain and hematuria. The third is *acute pyelonephritis*, which is clinically indicated by flank pain and fever and complicated by bacteremia in about 30% of the cases. Although most clinicians ascribe cystitis to an infection of the bladder and pyelonephritis to one including the kidney, this may be too facile a distinction. For instance, as judged by bladder washout tests or ureteral catheterization, 15% to 25% of "cystitis" cases actually may have bacteria above the bladder, and, conversely, 30% to 50% of patients with flank pain and/or fever do not have bacteria in the kidney (4,5). However, while most of the work described here has been performed without localization studies, investigations of bacterial virulence factors and host response mechanisms over the past two decades have shown excellent correlation with the clinical syndromes of acute cystitis and pyelonephritis.

This chapter is devoted to understanding the means by which the most common uropathogen, *Escherichia coli,* causes acute cystitis or pyelonephritis in the normal urinary tract (i.e., one without structural or functional abnormalities, or prior instrumentation) after entering the bladder through the urethra. Thus, other organisms, other means of colonization and entry, recurrent and chronic infections, and complicated UTIs are discussed in other chapters. Neither do we discuss hematogenous infection, a sequence beginning at another site in the body and resulting in a minority of UTI cases, and usually caused by pathogens such as *Staphylococcus aureus,* salmonella, and *Candida*.

This chapter is divided into two parts. The first concerns the pathogen and the features that allow it to occupy and produce disease within the urinary tract. The second part concerns the host and is divided into six areas: urine, bladder epithelium, ureter, kidney, inflammatory/immune response, and genetic factors. To the extent possible, studies of organisms stress comparisons of isogenic mutants; that is, strains genetically identical but for the factor being investigated.

UROPATHOGENIC *ESCHERICHIA COLI*

Strains of *E. coli* colonize the colon of human newborns soon after birth; these organisms and a succession of different *E. coli*—some transient, some more persistent—inhabit this site for the rest of the individual's life. Some of these strains may colonize the periurethral area and enter the bladder. Those that do and persist without symptoms are similar in their characteristics to the spectrum of *E. coli* colonizing the gut (6). However, those that enter and elicit symptoms are not a random sample of fecal *E. coli*, but rather a subset of strains—the uropathogenic *E. coli* (UPEC)—that differ from the majority of fecal strains in that they are more likely to possess a variety of putative virulence factors, including polysaccharide capsules, adhesins, siderophores, and cytotoxins (7,8).

A large proportion of UTIs are caused by a limited number of UPEC "clones" (clusters of closely related strains) that typically derive from two of the four main phylogenetic groups of the *E. coli* species, which are groups B2 and D, moreso than groups A and B1 (9,10). Although virulence factor carriage remains a better predictor of experimental virulence than does phylogenetic background (11), the concentration of virulence factors in strains from groups B2 and D (12) suggests that the evolution of certain *E. coli* lineages has equipped these strains well for production of extraintestinal disease. For example, serotype O18:K1:H7 (phylogenetic group B2), long known as the leading Gram-negative cause of newborn meningitis (13), is also the most frequently recovered serotype in acute cystitis the United States (9,14). Such pathogenic versatility highlights the notion of "extraintestinal fitness" that has informed much recent work on the UPEC strains (10) and is consistent with evidence that colonization of the lower genitourinary tract serves an important role in the ecology of these strains (15,16).

Uropathogenic *E. coli* are, thus, defined not only by their source (they are cultured from the urine or blood of individuals with symptomatic UTI), but also by the presence of putative virulence factors; however, mere association does not establish whether these factors are truly involved in infection. The concept of molecular Koch's postulates has been very useful for determining whether a putative virulence factor is indeed important for infection. According to these postulates, a factor is considered a virulence determinant if the following three conditions are met: (a) the factor is associated with virulent strains, (b) inactivation of the gene encoding the factor results

in attenuation of virulence in a relevant animal model, and (c) virulence is restored when the gene encoding the factor is reintroduced to the mutant (17). In the sections that follow, we examine putative UPEC virulence factors and review what is known regarding their role in infection, with emphasis on fulfillment of these postulates.

Pathogenicity Associated Islands

Studying model UPEC strain 536, Hacker and colleagues noted the spontaneous deletion of chromosomal regions leading to simultaneous loss of multiple, distinct virulence-associated phenotypes (18,19). Further characterization of the deleted segments led to the discovery of horizontally mobile, virulence factor-rich clusters of DNA that they termed "pathogenicity associated islands" (PAIs). Subsequently, these elements have been discovered in a wide variety of bacterial pathogens, Gram-positive and Gram-negative alike, with multiple PAIs described in each of model UPEC strains 536, J96, and CFT073 (19–21). In general, PAIs are 10 to 200 kb in size, carry virulence genes, are inserted near or within tRNA genes, contain insertion sequences or other mobile genetic elements, and have a G+C content different from the rest of the chromosome (22).

The contribution of mobile genetic elements to the emergence of pathogenic strains from commensal populations has been highlighted by the recent publication of several bacterial chromosome sequences, including nonpathogenic K-12 lab strain MG1655 (23), enterohemorrhagic O157:H7 strain EDL933 (24), and UPEC strain CFT073 (25). The chromosomes of these model strains differ not only in size—ranging from 4.6 Mb (MG1655) to 5.5 Mb (EDL933)—but in content, such that only 39.2% of their combined protein set are common to all 3 strains (25). *E. coli* chromosomes, then, appear to be highly diverse mosaic structures, featuring a core genome with a mostly homogeneous G+C content, along with a large, flexible gene pool formed of mobile genetic elements such as PAIs, conjugative plasmids, bacteriophages, transposons, insertion elements, as well as by the recombination of foreign DNA into host DNA (22). The dynamic properties of bacterial genomes may provide for continued emergence of novel clones. Indeed, the recent detection of a globally disseminated *E. coli* clone ("clonal group A"), accounting for up to 50% of trimethoprim-sulfamethoxazole-resistant cystitis and pyelonephritis isolates (26,27), indicates the need for ongoing surveillance.

Adhesins

The host-pathogen interactions that lead to urinary tract disease are initiated upon attachment of bacteria to epithelial cells. This type of adherence is a highly specific phenomenon: tropism for and within the urinary tract is mediated by molecular interactions between *adhesins* on the bacterial surface and complementary *receptors* on the epithelial cell membrane. Bacterial adhesins often are integrated into fimbriae (also known as *pili*), hair-like appendages composed of repeating subunits in a helical array that protrude from the surface of the bacteria. Alternatively, adhesins may take a variety of other forms.

Type 1 Fimbriae

Genes for the production of type 1 fimbriae are ubiquitous in *E. coli* and related Gram-negative bacteria, and the fimbriae are produced by more than 90% of all *E. coli*. Following initial visualization of these fimbrial organelles in 1950 (28), Brinton (29) and Duguid (30) and colleagues independently made the important observation that the ability of type 1 fimbriae to agglutinate erythrocytes was inhibited by mannose; thus, they are known as "mannose-sensitive" or "mannose-specific" adhesive organelles. The nine-gene *fim* cluster encodes FimA (the major structural subunit) and FimH (the tip adhesin), as well as machinery for biosynthesis of the type 1 fimbriae (31–34), via the chaperone/usher pathway (described in detail for the structurally more complex P fimbria, subsequently).

By virtue of their ability to bind mannose-containing glycoproteins via the FimH adhesin, type 1-fimbriated bacteria and/or isolated type 1 fimbriae adhere to a wide range of human target cells, including buccal cells, colonic epithelial cells and polymorphonuclear leukocytes (35–38). Comparative analysis of UPEC and fecal strains, however, reveals that type 1 fimbriae expressed by UPEC mediate up to 20-fold higher ability to bind uroepithelial cells than the type 1 fimbriae expressed by intestinal isolates (39,40). This distinct tissue tropism is due to increased affinity of UPEC FimH variants for terminal mono-mannose (M1) residues, resulting from acquisition of single amino acid replacements that occur at various locations throughout the adhesin (41). The structural basis for this functional heterogeneity has been clarified by the FimH crystal structure, which was recently resolved (42,43).

Consisting of a *lectin* (receptor-binding) domain and a *pilin* (fimbria-adapting) domain joined by a inter-domain linker chain (42), the FimH adhesin binds mannose residues at the distal end of the lectin domain within a deep cavity composed of residues highly conserved among UPEC strains (43). Curiously, however, the mutations conferring strong M1 adherence map away from this binding cavity, primarily to the interface between the lectin and pilin domains (44). The functional basis for this unexpected distribution was clarified by study of conformational change between the two FimH domains during mechanical stress. Thomas and colleagues observed that fecal-type, low-M1-binding FimH variants mediate distinctly stronger bacterial binding to target cells when shear force is applied (*shear-dependent binding*), whereas UPEC-type, high-M1-binding FimH variants mediate strong bacterial binding regardless of shear force (*shear-independent binding*) (45). Computational simulations based on the FimH crystal structure predicted that when shear force was applied to receptor-bound FimH, the inter-domain linker chain would be extended, allowing the two domains to move apart. Then, a specific linker chain mutation predicted to ease linker extension (by reducing hydrogen bonding to adjacent lectin domain loops) was introduced into FimH by site-directed mutagenesis and its binding properties evaluated. Like the naturally occurring, UPEC variants of FimH, the mutant exhibited shear-independent binding with dramatically increased M1 binding strength, indicating the functional relevance of linker chain extension in altering adhesin-receptor interactions (45). The "force sensor" function of FimH provides a molecular mechanism for bacterial discrimination between soluble and surface-anchored receptor molecules. This capacity would allow bacteria to avoid inhibition of binding during colonization of niches rich in soluble mannosylated glycoproteins (such as the oropharynx). In contrast, shear-independent, strong M1 binding may mediate rapid colonization of certain niches; however, the increased sensitivity to soluble inhibitors, as well as the limited ability to spread across target surfaces during low shear, may represent an important functional trade-off with deleterious implications for long-term fitness (45,46).

Elegant electron microscopy images by Mulvey and colleagues have demonstrated *E. coli* adhering via type 1 fimbriae to umbrella cells of the murine bladder with the tips of the fimbriae seen embedded in the central cavity of a hexagonal array of uroplakin molecules (47). Uroplakins are glycoproteins that constitute the "asymmetric membrane unit" in uroepithelium; they express terminal M1 residues and have been

identified as the specific receptors for type 1 fimbriae in murine bladder (48). Following type 1 fimbrial binding to superficial epithelium, bacteria are rapidly engulfed by these cells, using a zipper-like mechanism (49). Internalization is not observed with an isogenic FimH-null mutant (50), but is observed using polystyrene latex beads coated with FimH (49). Epithelial cell engulfment of *E. coli* may then lead to the formation of "intracellular bacterial communities" (discussed later in the chapter) by which UPEC may avert the robust host inflammatory response (51).

Additional efforts to demonstrate a UTI-related virulence phenotype for type 1-fimbriated *E. coli* have focused on type 1 fimbrial expression, as *fim* cluster carriage is common to pathogenic and commensal strains alike. Expression of these pili is governed by an invertible genetic element containing the promoter for the operon. When the invertible element is in one position, transcription proceeds into the operon and pili are made, whereas the opposite orientation precludes fimbrial expression (52). Using mixed inocula of wild-type and phase-locked mutants of CFT073, Gunther and colleagues studied the role of phase variation during urinary tract colonization in the CBA mouse model, in which bacteria are inoculated by transurethral colonization into the bladders of strain CBA mice (53). Phase-locked-on mutants were recovered from the bladder in greater numbers than wild-type bacteria at 24 hours, but not 48 or 72 hours, indicating that phase variation is essential to bladder colonization, particularly in the early stages of infection. In addition, transcriptome analysis from microarray studies of the CBA mouse model indicates high levels of type 1 fimbrial expression during bladder colonization (54). More importantly, evidence from mouse studies strongly supports the essential role of type 1 fimbriae in UTIs. A mutant unable to make FimH is severely deficient in colonization of the urinary tract in the murine model and complementation of the mutant restores virulence (55); thus, molecular Koch's postulates have been fulfilled for the type 1 pili.

P Fimbriae

In 1976, Svanborg and colleagues demonstrated that *E. coli* causing acute pyelonephritis adhered in greater numbers *in vitro* to uroepithelial cells obtained from the urine of volunteers than did strains causing asymptomatic bacteriuria (56). Such adherence was not inhibited by mannose, an observation that excluded the well-studied type 1 fimbria as the responsible binding organelle. This work rapidly led to the identification of a new adhesin, the P fimbria. Other adhesins have been identified since, but none have supplanted P fimbriae in their predominance in acute pyelonephritis. Cloning of the fimbrial genes by Hull and associates (31) and subsequently others (57, 58) led to the elucidation of the genetics and structure of P fimbriae, much of which has been done by Normark and Hultgren and their colleagues (59–64). Typically encoded within chromosomal PAIs (22), the *pap* (pyelonephritis-associated pilus) operon comprises 11 genes (Fig. 32-1) and is distributed selectively within the *E. coli* species (12), unlike the ubiquitous *fim* operon.

P fimbrial biogenesis has served as the paradigm for the assembly of numerous other pili (65). Biogenesis of the fimbria is dependent on an outer membrane channel, PapC, which has a pore of sufficient diameter to allow pilin subunits, but not intact pili, to pass (66). The energy driving pilus export across the outer membrane is thought to arise in the favorable thermodynamics resulting from pilin subunit interactions in the tightly packed pili. PapC functions as an usher with an active role in selecting the order in which fimbrial subunits are assembled (67). The PapD protein serves as a molecular chaperone, capping interactive surfaces on nascent pilin subunits as they emerge through the cytoplasmic membrane and preventing premature subunit–subunit interactions (68). The fimbria is composed of a proximal shaft joined end-to-end with a flexible fibrillar tip, at the end of which is the receptor-specific PapG adhesin. The fimbrial shaft is composed of about 1,000 PapA proteins arranged in a helical pattern and is anchored to the PapC outer membrane usher by PapH. At the distal end of the shaft, PapK links the shaft to the fibrillum, which is composed of repeating subunits of PapE. PapF links the tip adhesin, PapG, to the distal end of the fiber. The structure of the P fimbria allows the tip adhesin to project beyond the lipopolysaccharide and capsular material covering the organism. The fiber on the distal end of the shaft appears to be flexible, a property that is likely required by the "side-on" binding pocket orientation of the PapG adhesin, the crystal structure of which (PapG II variant) has been resolved (69).

Kallenius and associates found that pyelonephritogenic *E. coli* strains agglutinated erythrocytes expressing the P blood group antigens, which are glycosphingolipids consisting of a lipid moiety anchored in the cell membrane and a carbohydrate chain on the erythrocyte surface. A review of these known carbohydrate portions suggested that the common receptor moiety for P fimbriae may be a digalactoside, α-D-Gal-(1α4)-ß-D-Gal (termed hereafter *gal-gal disaccharide*) (70). Demonstrating that pyelonephritogenic *E. coli* bound red blood cells obtained from persons of P antigen phenotype much more avidly than from those lacking this phenotype, and that binding was inhibited by the synthetic gal-gal disaccharide, they named the fimbriae "P fimbriae" for P blood group antigen (71). These data are consistent with knowledge that globosides containing the gal-gal disaccharide are the predominant glycolipid found in human kidney tissue, particularly epithelial cells (72). Korhonen and colleagues subsequently used frozen sections of human tissue to show binding of purified P fimbriae to proximal tubular, distal tubular, collecting duct, and bladder epithelial cells (Table 32-1) (73–75).

The epidemiologic association of P fimbriae with acute pyelonephritis (and to a lesser extent, with cystitis) identified by Svanborg and colleagues was subsequently confirmed by numerous studies (7,76–86). In an analysis of published, controlled studies, Donnenberg and Welch reported that *E. coli* from otherwise healthy patients with pyelonephritis are six times more likely to possess P fimbriae than are strains from the feces of controls (87). Approximately 80% of such strains have P fimbriae. Furthermore, there is a strong relationship between the severity of infection and the prevalence of P fimbriae. It is interesting to note that unlike type 1 fimbriae, only *E. coli,* not other Gram-negative rods, carry the genes for P fimbriae (88).

Mouse and monkey models of ascending UTIs have been particularly useful in confirming the importance of P fimbriae, as these species express renal epithelial receptors similar to those in humans (89–93). Studies using genetic mutants of P fimbriae (90,91,94), soluble gal-gal receptors as inhibitors (95), and purified P fimbriae as vaccines (91,96–99) have supported the concept that P fimbriae are important in acute pyelonephritis. In one such study, a mutant identical to the virulent clinical isolate except for lack of the PapG adhesin caused significantly less renal damage than its adhesin-positive relative, although both strains were able to colonize the kidney. There was no difference between the strains in colonization of the bladder (94). The result of complementing the mutant to restore production of P fimbriae was not studied, and thus molecular Koch's postulates were not fulfilled. Furthermore, in a mouse model of ascending UTIs, deletion of both copies of the tip adhesins from a highly virulent pyelonephritis strain that has two copies of the *pap* operon had no effect on colonization of bladder or kidneys (100).

P fimbriae comprise a family in which the P fimbrial shaft is homologous; the distinction among family members is in

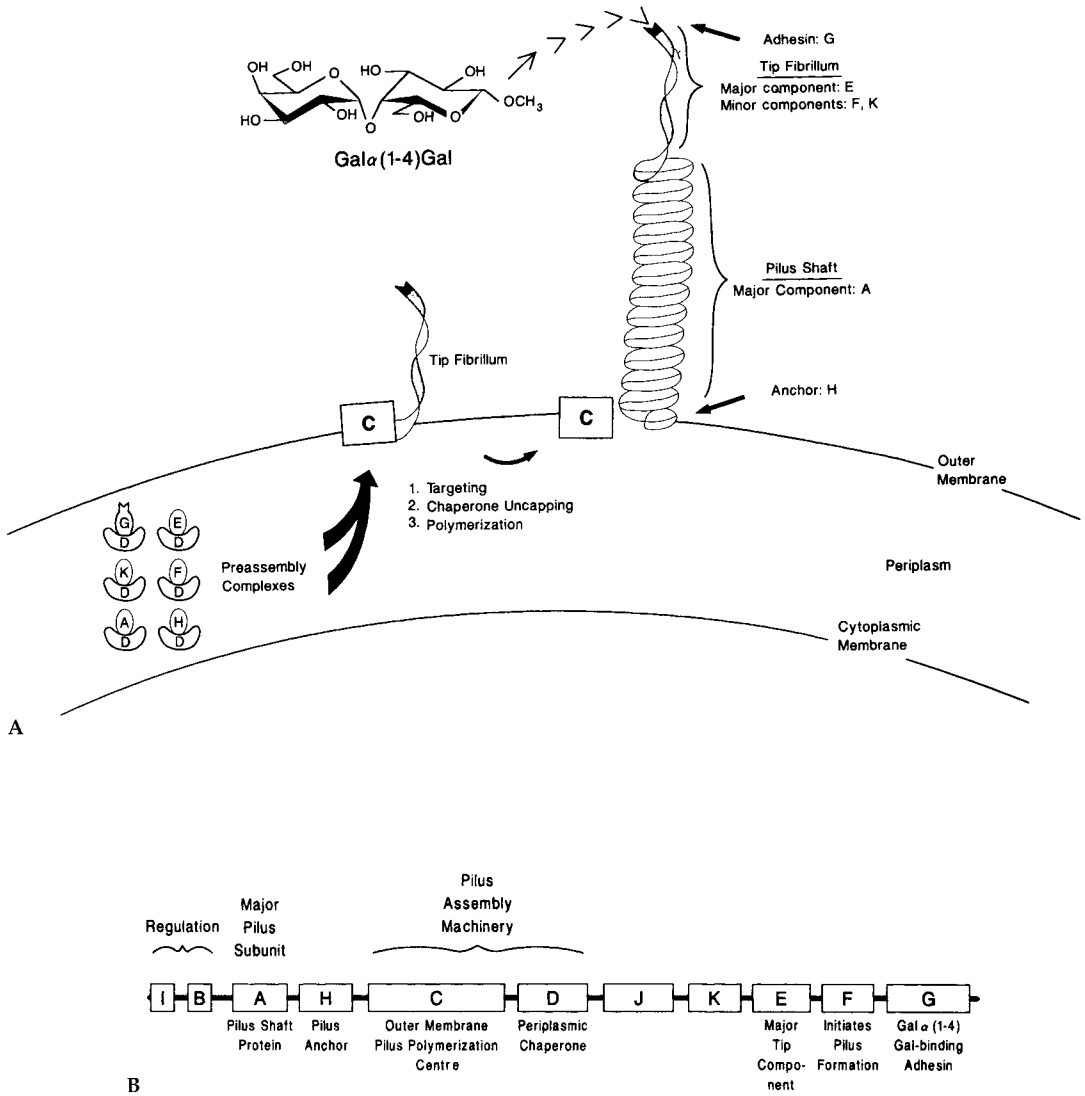

FIGURE 32-1. A: Model of P-fimbria construction. **B:** *Pap* gene operon for P fimbria. (Reprinted from: Kuehn MJ, et al. *P. pili* in uropathogenic *E. coli* are composite fibers with distinct fibrillar adhesive tips. *Nature* 1992;356:252, with permission.)

TABLE 32-1

BINDING OF *E. COLI* ADHESINS TO HUMAN KIDNEY AND BLADDER

	Adhesin binding[a]				
	P fimbria	FIC fimbria	S fimbria	Type 1 fimbria	Dr adhesin
Kidney					
Bowman's capsule	+++	—	+++	—	+++[b]
Glomerulus	+++	—	+++	—	—
Proximal tubule	++	—	++	+++	+++[b]
Distal tubule	++	++	++	(+)	+++[b]
Collecting duct	+	++	++	(+)	+++[b]
Vessel walls	+++[c]	+++[c]	+++[c]	+++	—
Urinary bladder					
Epithelium	+	—	++	—	+
Vessel walls	+++[c]	+++[c]	+++[c]	++	—
Muscular layer	+	+	+	+++	+
Connective tissue	—	—	++	—	+++

[a]Binding to tissues is graded from +++ (intense) to – (not detectable); (+) denotes very weak binding.
[b]To capsular or tubular basement membranes.
[c]Mainly to endothelial cells.
(Reprinted from: Korhonen TK, et al. Tissue tropism of *Escherichia coli* adhesins in human extraintestinal infections. *Curr Top Microb Immunol* 1990;151:115, with permission.)

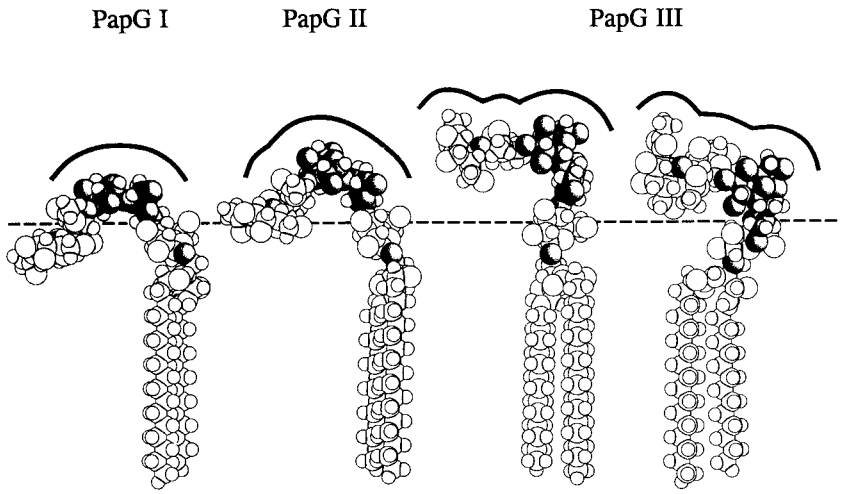

FIGURE 32-2. Preferred conformations and epitope presentations of gal-gal disaccharide-containing glycosphingolipids as receptors for the three PapG adhesins of P fimbriae. (Reprinted from: Stromberg N, et al. Saccharide orientation at the cell surface affects glycolipid receptor function. *Proc Natl Acad Sci USA* 1991;88:9340, with permission.)

the tip adhesin, PapG (101,102). The distinct adhesin types were first identified by differences in binding characteristics; it now appears that each adhesin type attaches to a distinct functional portion of the gal-gal disaccharide-containing glycosphingolipids, based on the length of the carbohydrate chain exposed on the surface of the eukaryotic cell, and thus on the stereometric positioning of the gal-gal moiety (Fig. 32-2) (69,102). PapG I, the adhesin variant coded by the first *pap* cluster cloned by Hull and associates (31), has subsequently been found in limited numbers of other UPEC strains (103). P fimbriae bearing adhesin PapG II are much more common and are in fact the predominant P fimbrial class in pyelonephritis. PapG III fimbriae, bearing the so-called PrsG adhesin, is more common in cystitis strains (104–106).

Adhesins with Less Established Roles in Urinary Tract Infections

Dr Adhesin

The Dr adhesin family is composed of fimbrial and afimbrial structures on the surface of *E. coli* that bind to the Dr blood group antigen (107), a portion of the membrane protein decay-accelerating factor (DAF) that prevents host cell lysis by complement (108, 109). Dr adhesin-bearing *E. coli* bind to bladder epithelium and have been associated epidemiologically with recurrent UTI in young adults and pyelonephritis in pregnant women (110). While all Dr family adhesins characteristically bind DAF, only DraE binds type IV collagen of basement membranes as well (Table 32-1) (111). This dual receptor specificity was investigated by creation of a mutant DraE in which type IV collagen binding was abolished but DAF binding retained. In a mouse model of chronic UTI, the mutant-bearing strain was able to colonize the kidney, but was eliminated in 6 to 8 weeks, while the native protein-bearing, isogenic strain caused persistent infection lasting at least 14 weeks (112). These provocative findings indicate that in addition to DAF binding, the extracellular matrix binding mediated by Dr fimbriae may contribute to establishment of chronic UTI by *E. coli*.

F1C Fimbriae and the S Fimbria Family

A family of *E. coli* fimbriae that includes S, F1C, and Sfa pili has highly related biogenesis genes but different adhesin alleles. These pili have been linked to UTIs and other extraintestinal infections, particularly neonatal meningitis. Although the epidemiology of these pili remains poorly studied, pooled data from several reports indicate that F1C fimbriae in particular are more common among pyelonephritis and cystitis strains than among fecal strains of *E. coli* (87). Although they do not agglutinate erythrocytes, F1C fimbriae are expressed *in vivo* and adhere to human distal tubular and collecting tubular epithelium and vascular endothelium on kidney cross sections (Table 32-1) (75). Recently, the F1C fimbriae were reported to interact with two minor glycosphingolipids isolated from rat, canine, and human urinary tracts: galactosylceramide, found throughout the urinary tract (except in urethra), and globotriaosylceramide, expressed specifically in the kidney (113). Further, F1C-fimbriated bacteria induced IL-8 production in renal epithelial cells to levels produced by adhesion of type 1- and P-fimbriated bacteria, supporting a role for F1C in pyelonephritis. However, isogenic mutant experiments to determine the role of these pili in UTIs have not been reported.

Other Putative Virulence Factors

One might imagine that mere attachment of bacteria to an epithelial cell surface is insufficient to cause symptomatic disease. Clues to additional virulence-related bacterial features have been derived from epidemiologic studies (7). In many cases, possible roles for these putative virulence factors have been explored in animal and *in vitro* systems.

Hemolysin

Since the early 1900s, certain *E. coli* strains have been known to lyse erythrocytes, detected as a zone of clearing around the bacterial colony on a blood agar plate. In 1921, Dudgeon and colleagues found that about 50% of *E. coli* that cause UTIs were hemolytic (114), a finding substantiated by numerous recent studies. The responsible factor, hemolysin, is a 107-kDa toxin belonging to the RTX ("repeats in toxins") family, a group of proteins sharing a glycine- and aspartate-rich nonapeptide motif repeated in tandem near the C terminus (115). The four-gene hemolysin operon is encoded on the chromosome of UPEC strains, often adjacent to genes encoding P fimbriae on the same PAI (116,117). Thus, it is not surprising that UPEC are twofold to threefold more likely to have the *hly* genes than are fecal strains (87).

The gene cluster for hemolysin encodes four proteins: HlyC, HlyA, HlyB, and HlyD (118,119). The *hlyA* gene encodes prohemolysin, which is acylated to the active, mature lipoprotein by HlyC (120). HlyB (an ATP-binding cytoplasmic membrane protein) and HlyD (a cytoplasmic membrane protein with a large periplasmic domain), together with TolC (an outer

membrane protein not encoded within the hemolysin operon), form an export complex by which hemolysin is secreted into the surrounding media (121,122). Once in the external environment, hemolysin binds calcium using its RTX consensus repeats to generate a functional tertiary structure and bind to cell membranes (123–125). Target cell surface receptors identified for HlyA include a β2-integrin in leukocytes (126) and glycophorin, an integral membrane protein, in red blood cells (127). Amino acid residues 914–936 appear to constitute the major glycophorin-binding region, as demonstrated by the ability of a peptide containing these residues to protect red blood cell suspensions against HlyA challenge (128). Rather than forming a pore through the membrane, as originally proposed, it appears that hemolysin produces cytolysis by inserting only into the outer leaflet of the host cell membrane (125), via its N-terminus domain.

In addition to its ability to lyse erythrocytes, *E. coli* hemolysin is cytotoxic to other human blood cells, such as polymorphonuclear leukocytes, monocytes, mast cells, basophils, and lymphocytes (129–133). In cystitis and acute pyelonephritis, however, *E. coli* hemolysin may also directly contribute to infection through its effects on uroepithelial cells. The lethality of the toxin has been demonstrated in renal tubular epithelial cells from rat (134) and human (135,136), as well as in human bladder epithelial cells (137). More recently, sublytic concentrations of hemolysin were reported to induce oscillations of intracellular calcium concentrations in renal tubular epithelial cells, and these oscillations were associated with induction of IL-6 and IL-8 expression (138). Abolition of the oscillations by use of a glutamate receptor antagonist indicate that, unlike the lethal effects, the sublethal effects of hemolysin may be due to an inhibitable, receptor mediated interaction with the host cell (139). These results suggest a role for hemolysin in stimulating the host response to the bacteria. In spite of evidence for its contribution to virulence in several infection models, hemolysin's role in UTI has not been clearly demonstrated by appropriate studies using deletion mutants.

Cytotoxic Necrotizing Factor (CNF1)

CNF1 is a fascinating, 115-kDa toxin that induces the formation of stress fibers in cells by deamidating and constitutively activating members of the Rho family of small, actin-regulatory GTPase proteins (140,141). Paradoxically, once thus activated by CNF1, Rho proteins are rapidly degraded in the ubiquitin-mediated proteasomal pathway of the host cell (142). Nonetheless, CNF1 intoxication effectively confers epithelium-invasive properties upon UPEC (142,143), and triggers production of proinflammatory cytokines in both epithelial (144) and endothelial cells (145). The physical linkage of CNF1 and hemolysin on PAIs (146,147) led to studies indicating that these two important toxins may in fact be coregulated in UPEC, by novel means (148) involving the virulence-related, regulatory protein RfaH (149). Although epidemiologic data and *in vitro* studies indicate an important role for CNF1 in urinary tract disease, direct comparisons of the virulence of wild-type UPEC strains with their isogenic *cnf1* mutants in different murine models of UTIs have so far yielded conflicting results (150,151).

Secreted Autotransporter Toxin (Sat)

Enterobacterial autotransporter systems enable translocation of proteins across the inner membrane, presumably via the Sec system, and across the outer membrane through a ß-barrel porin structure formed by the C-terminus autotransporter domain. A 107-kDa protein termed Sat (secreted autotransporter toxin) is secreted significantly more often by *E. coli* strains causing acute pyelonephritis than by fecal strains (152). Originally isolated from model pyelonephritis strain CFT073, Sat showed highest similarity to two known SPATE (serine protease autotransporters of *Enterobacteriaceae*) (153) proteins, Pet and EspC, but has homology with virulence-related proteins (including adhesins, invasins, and toxins) from a range of *E. coli* pathotypes. In addition to serine protease activity, Sat displays cytopathic (specifically, cytoplasmic vacuolating) activity on human bladder and kidney cell lines (154). In an animal model of ascending UTIs, Sat produced cytopathic effects in the CBA mouse kidney (though not in the bladder), but was not required for urinary tract colonization (154). In addition, SPATE proteins Pic and Tsh are expressed by CFT073 in the urine of transurethrally infected mice and are more prevalent among UPEC than among fecal isolates, but do not yet have a demonstrated role in urinary tract virulence (155).

Siderophores

Like all living cells, bacteria require iron for important functions such as electron transport and oxygen transport and storage. Infecting bacteria are confronted with iron limitation because most mammalian hosts sequester iron using proteins such as transferrin and lactoferrin. However, bacteria have evolved low-molecular-weight chelators called siderophores (Greek *sideros,* iron; and *phoros,* bearing) in order to acquire host iron for their own use. Aerobactin, the most effective siderophore produced by *E. coli,* is secreted into the medium, binds iron, and is then reclaimed by the bacterium via a receptor protein. To date, several additional siderophores and receptors, including yersiniabactin (*fyu*) (156), *ireA* (157) and *iroN* (158) have been identified among UPEC strains. The siderophore receptor *iroN* contributed to *E. coli* virulence in a mouse model of UTI (158) and has been proposed as a UTI vaccine candidate (159). Individual UPEC isolates frequently encode multiple iron acquisition systems (25), and many strains can also utilize heme as an iron source by expressing the *chuA* outer membrane receptor (160). It is not yet understood to what extent these systems serve distinctive roles (161) or may be redundant in these bacteria. However, the importance of iron utilization in UTI has been emphasized by fulfillment of molecular Koch's postulates for TonB (162), a cytoplasmic membrane protein that provides energy to the outer membrane receptors involved in transport of iron compounds (163). A TonB-negative derivative of CFT073, unable to utilize heme or the siderophores enterobactin and aerobactin, exhibited attenuated virulence in a murine UTI model and restoration of virulence upon introduction of a TonB-containing plasmid. In addition, CFT073 mutants deficient in either aerobactin or heme transport (*chuA*) were able to colonize murine bladder and kidney when inoculated in pure culture, but were outcompeted by the wild-type strain when introduced in mixed culture (162).

O Antigens

E. coli strains are serotyped on the basis of a system developed by Kauffmann in the 1940s (164). The three determinants of serotype still in use are the O, or somatic antigen; the K, or capsular antigen; and the H, or flagellar antigen (165). Precise serotype determination is a laborious process that involves the use of hundreds of cross-absorbed antisera to determine to which of the 173 O, 80 K, and 56 H types a strain belongs. As many as 50,000 different *E. coli* serotypes exist (in addition to nontypable strains, commonly found in fecal flora); yet, as pointed out decades ago by Kauffmann, a limited number account for a large proportion of the strains that cause human infection. Although certain of the O-antigen serogroups are overrepresented in symptomatic UTIs, with O1, O2, O4, O6, O7, O8, O16, O18, O25, and O75 accounting for the majority of isolates (7), the association of particular serogroups with disease is confounded by other potential virulence factors that are found significantly more frequently in serogroups

associated with infection (81,166,167). It should also be noted that carriage of shared serotype antigens does not guarantee the phylogenetic relatedness of *E. coli* strains (168), due to the horizontal mobility of antigen determinant clusters. Although isogenic mutant studies have suggested that ability to synthesize an O antigen in general is required for UTI pathogenesis (169), there is no experimental evidence that having a particular O antigen type *per se* increases the pathogenic potential for an *E. coli* strain.

Capsular Polysaccharides and Serum Resistance

The ability to grow in the presence of human serum is a complex phenotype in *E. coli* that depends on multigenic properties, including lipopolysaccharide, outer membrane proteins, and the ability to make a carbohydrate capsule (170,171). Capsules also play a role in resistance to phagocytosis in the absence of anticapsular opsonizing antibody (172). The epidemiologic data concerning the occurrence of capsule in strains isolated from patients with UTIs are contradictory and quite confusing. A review of the published data did not confirm the common belief that certain capsules are more common in UPEC than other *E. coli,* nor did it confirm that particular encapsulated strains were more likely to be resistant to human serum (87). Likewise, experimental studies of different capsule types in which wild-type isolates were compared with isogenic, unencapsulated mutants in mouse models of ascending UTIs have yielded conflicting results in support of a role for capsule production in urovirulence (22,169). While insertion of a signature-tagged transposon into the (K2) *kps* cluster of strain CFT073 attenuated virulence in a murine model of UTIs, the defect was not complemented (173).

Vaccine

Langermann and colleagues (174) developed a vaccine using the type 1 fimbrial adhesin protein (FimH), which binds to mannosylated receptors in the urinary tract, for protection against the development of UTIs. This vaccine, administered intraperitoneally, successfully protected mice from transurethral challenge with a uropathogenic *E. coli* strain; bladder colonization was reduced by 99%. Similar success was achieved in cynomolgus monkeys (175), but clinical experience in humans has yet to be published. A similar strategy utilizing the purified P fimbrial adhesin (in an adhesin/chaperone complex) as a vaccine antigen protected cynomolgus monkeys against pyelonephritis on challenge by a P-fimbriated *E. coli* strain (176). Other vaccine candidates recently proposed and studied in animal models include the Dr adhesin (177) and siderophore receptor *iroN*(159).

Summary

The concept that certain traits are more common among *E. coli* isolated from patients with UTIs than among fecal *E. coli* is now well established; however, it is important to note that none of the recognized putative virulence factors is present in all strains from cases of pyelonephritis or cystitis, even when only normal hosts are considered. Exploring the genotypic diversity of UPEC strains, comparative genomic approaches have revealed the mosaic structure and highly dynamic nature of bacterial chromosomes, while the detailed study of PAIs has begun to elaborate how multiple virulence factors may be coordinately regulated in the course of complex programs of pathogenesis. Given these considerations, what generalizations are possible regarding UPEC? First, UPEC are a heterogeneous collection of strains that differ greatly in phenotypic properties within the group, as well as in comparison to other *E. coli* strains. Second, UPEC often express specific adhesins—most notably type 1 fimbriae and P fimbriae, which aid in colonization of the urinary tract. Third, many UPEC possess toxins such as hemolysin and CNF that may contribute to tissue damage, but the actual roles of these factors in disease remain only partially understood. Likewise, the contributions to UTI pathogenesis of other attributes common among UPEC strains (e.g., O antigen, capsule and serum resistance) have not been fully elucidated to date. Thus, despite years of study, much remains to be learned about the disease-causing abilities of uropathogenic *E. coli*.

THE HOST

The ability of an organism to cause symptomatic disease is the net effect of the bacterium's virulence properties and the host's responses to prevent colonization, inhibit bacterial growth, clear the organism from the body, contain the invader or its effects, or kill the organism.

Urine

Voiding

The most effective mechanism of the host to protect itself from UTIs is one of the simplest. The process of voiding is a highly efficient means of clearing bacteria from the normally functioning urinary tract (178–181); urination flushes out more than 99.9% of organisms that have recently entered the bladder (180). A shear-dependent mode of fimbrial binding (described for type 1 fimbriae, above) may provide means to counter this defense, by firmly anchoring the bacteria to urinary tract epithelium during periods of urine flow (45).

Composition

Even after effective voiding, a film of urine remains adherent to the bladder mucosa and can then inoculate subsequently formed urine. A number of investigators have demonstrated that human urine often allows growth of known uropathogens (178,180), especially *E. coli,* which can outcompete other organisms (182). Although urine composition varies among individuals, and varies with diet, hydration, and related factors, it has been established that high osmolality and urea concentrations inhibit bacterial growth (183,184), and that measurable oxygen tension in urine can kill anaerobic bacteria that prevail among colonic strains (182).

Tamm-Horsfall Protein

Urinary mucus is primarily composed of aggregates of Tamm-Horsfall protein (THP, also known as *uromodulin*), a glycoprotein produced by epithelial cells of the ascending limb of the loop of Henle. Orskov and colleagues noted that type 1-fimbriated *E. coli* bound to urinary mucus (185, 186) via what was later determined to be mannose-sensitive binding to THP (187). This prompted speculation that THP suspended in urine might bind type 1-fimbriated *E. coli* and prevent adherence to bladder epithelium. However, subsequent studies indicated not only that THP levels typically found in urine were unlikely to block target cell adhesion (188–191), but also that very low THP concentrations may even enhance binding of type 1-fimbriated bacteria to uroepithelium (190). Recently, however, comparison of wild-type and THP-knockout mice demonstrated that type 1-fimbriated, but not P-fimbriated, bacteria produce longer duration of bacteriuria and more intense colonization of the bladder in the absence of THP (192).

Bladder Epithelium

Glycosaminoglycan

A layer of glycosaminoglycan (GAG) overlies epithelial cells in the normal bladder. A series of studies by Parsons and associates demonstrated that this lining inhibits bacterial adherence to the underlying epithelium (193–197), while removal of this layer enhances adherence. Regeneration occurs within 24 hours, and bacterial adherence to epithelium is once again inhibited. GAG makes the bladder surface more hydrophilic, forming a low-energy interface with urine that tends to inhibit bacterial adhesion (198). In experimental cystitis, this layer can be disrupted by infection, with subsequent binding of organisms to the bare epithelium (199,200). Based on these observations, intravesical instillation of hyaluronic acid to restore integrity of the GAG layer has shown promise in an uncontrolled study of human volunteers with recurrent UTIs (201).

Attachment, Internalization, and Exfoliation

Upon reaching the bladder, *E. coli* attach via type 1 fimbriae to mannosylated uroplakin proteins present on the superficial umbrella cells of the urinary mucosa (50), triggering a complex series of events that has been carefully studied by Hultgren, Mulvey and colleagues. Activation of host cell GTPases of the Rho family (including Cdc42 and Rac1) leads to induction of focal adhesin kinase/phosphoinositide-3 kinase and vinculin/α-actinin complexes, producing localized actin rearrangements by which type 1-fimbriated *E. coli*, but not isogenic *fimH*-null mutants, are promptly engulfed (49). This invasion of the superficial epithelium thus provides a protected intracellular niche that allows some proportion of the infecting UPEC to avert the robust response of the host innate immune system.

At six hours following inoculation, massive exfoliation of apoptotic bladder epithelial cells takes place (47), carrying many bound organisms into the urine for subsequent voiding. This phenomenon has been described by several investigators in previous animal models (200,202–204), and indicated by the morphologic changes observed by Elliott and colleagues on scanning electron microscopy of human bladder biopsy tissue (205,206). This innate host defense mechanism leaves exposed the underlying, less differentiated intermediate cells of the bladder mucosa, which rapidly differentiate to superficial epithelial cells over the next 12 to 48 hours (207). Using a functional genomics approach, Mysorekar and colleagues characterized the dramatic response of murine bladder epithelium to the binding of type 1-fimbriated *E. coli*. Quite distinct from the extremely low rate of localized turnover that normally marks sloughing during bladder epithelial renewal (208), UPEC-infected bladder activates an intricate network of regulatory and signaling pathways that mediate urothelial regeneration and induce expression of proinflammatory mediators, including inducible nitric oxide synthase, IL-6 and Mip-2, the mouse ortholog of IL-8 (207).

Persistent Intracellular Reservoirs

A number of observations, both clinical and experimental, have led to interest in the possibility of long-term intracellular colonization of the bladder by UPEC. Following acute infection of the bladder, approximately one in four women experience recurrence within 6 months despite antibiotic therapy, with one-third of recurrent *E. coli* UTIs being caused by the index-episode strain (209). Although bacteria have been observed adhering to the bladder tissue of previously infected individuals whose urine had been sterilized by antibiotics (205,206), biopsy specimens from human cystitis cases have not, to our knowledge, been examined by transmission electron microscopy to evaluate internalization of organisms by bladder epithelial cells. Animal models of cystitis (199,202,203), however, as well as *in vitro* studies of cultured human bladder cells (47), have demonstrated this phenomenon. Finally, over a 6-week period following acute bladder infection with UPEC, Schilling and colleagues detected at least one bacteriuric recurrence in 5 of 14 mice, and more than one recurrence in 3 of 14, and observed persistence of bacteria in the murine bladder despite 10 days of treatment with trimethoprim-sulfamethoxazole (210). These provocative findings raise the question as to whether recurrent UTIs are due primarily to *de novo* infections with the same strains by reintroduction from a persistent vaginal, fecal or environmental (household member or pet) reservoir, or rather to reemergence of bacteria from a persistent reservoir within the bladder.

Recently, Justice and colleagues used time-lapse video microscopy to visualize the postinternalization behavior of GFP-labeled UPEC inside murine bladder epithelial cells, and described distinctive stages in the development of what they have termed *intracellular bacterial communities* (IBCs) (51). *Early IBC formation* occurs in the first 6 to 8 hours after inoculation, and consists of rapid bacterial division that produces loosely associated, amorphous clumps of organisms in the host cell cytoplasm. Between 6 to 8 hours after inoculation, a dramatic phenotype switch occurs, featuring a shortening of bacterial morphology and a twofold increase in doubling time. This *IBC maturation* produces a tight, highly organized collection of bacteria that is appreciated as a pod-like protrusion on the bladder surface (211). The resulting *middle IBC* exhibits many properties typical of biofilms—collections of surface-attached bacteria demonstrating community behavior (212)—including expression of type 1 fimbriae (213), antigen 43 (214) and polysaccharide matrix (212). Once established in the intracellular niche, UPEC are protected from engulfment by arriving neutrophils, while extracellular bacteria are readily cleared (211). Thus, intracellular biofilm formation promotes bacterial persistence in the bladder by protecting from immune effectors and presumably, by virtue of barriers to permeability and a reduced rate of growth, from antibiotics (210).

Starting 12 hours postinoculation, the *late IBC* forms, as bacteria along the edge of the biofilm begin to differentiate into typical rod morphology and detach from the group. These bacteria are highly motile (likely due to flagellar expression) and begin "fluxing" out of infected cells, individually or *en masse* (215), completing the first round of the developmental process. Once escaped, the bacteria may filament up to 70 μm or more in length (51, 215), in which form PMNs appear unable to ingest or kill them. By attaching to and invading another epithelial cell, the bacteria initiate a second round of IBC formation, which proceeds more slowly than the first, and subsequent rounds may follow, by which time the reservoir is established. After several days, a quiescent reservoir of bacteria remains inside an intact layer of superficial epithelium. Without further apparent action by host or bacteria, the UPEC may persist in such state for months (210, 215). Events that lead to recurrence are unclear, but may involve reentry into the characterized cycle at the point of early IBC formation (51). While this elegant and important study represents the first description of intracellular biofilms in living tissue, the formation of such communities in human bladder remains to be demonstrated.

Ureter

Vesicoureteral reflux is uncommon in the normal adult urinary tract, and the means of bacterial ascent from bladder to

kidneys in these individuals is unclear; however, it is known that *E. coli* adhere to human ureteral mucosa (37,216,217). This is mediated by type 1 fimbriae, which can bind to superficial as well as intermediate cells, and by P fimbriae, which can bind to intermediate cells. Furthermore, there are *in vivo* and *in vitro* findings indicating that disordered ureteral peristalsis during infection may also contribute to bacterial ascent (218,219).

Kidney

Once organisms reach the pelvis of the kidney, the subsequent steps that lead to acute pyelonephritis are unknown. Whether pyelonephritogenic organisms bind to pelvic epithelium or move into the collecting system and bind to collecting duct or distal or proximal tubular epithelium is unclear. The medullary kidney may be more susceptible to infection than the cortex possibly owing to lower blood flow, higher osmolality, and/or lower pH, which may adversely affect leukocyte activity (220).

While organisms adherent to epithelium have not been sought in the few biopsy or autopsy specimens of human cases of early acute pyelonephritis, such biopsies have demonstrated bacteria internalized in renal tubular epithelial cells (221). In animals, bacteria adherent to pelvic and distal and proximal tubular epithelium have been identified, often tethered to the epithelium by fimbria-like structures (222–228). Furthermore, a number of investigators have observed bacteria inside pelvic, proximal tubular and/or distal tubular epithelial cells, usually contained within membrane-bound vacuoles, suggesting that the epithelial cell is capable of a process similar to phagocytosis (223–228). Internalization of bacteria into cultured human renal epithelial cells has been demonstrated (229) in a process involving tyrosine phosphorylation (230,231), but the fate of the epithelial cell and internalized bacteria in such a situation is unclear (225,227,228). The human renal epithelial cell, then, appears capable of active internalization of bacteria during upper tract infection, but the significance of this activity in tissue damage and/or bacterial clearance remains to be studied.

Inflammatory and Immune Responses

Symptomatic UTI—whether cystitis or pyelonephritis—represents an acute inflammatory response, and the severity of infection reflects the magnitude of inflammation. Specifically, the outcome of bacterial intrusion into the urinary tract appears to be determined primarily at the mucosal surface by the innate immune response generated by the host (232). This response begins with specific interactions between bacterial surface structures and epithelial cell receptors, leads to activation of host cell signaling pathways and release of immunomodulators (including cytokines and chemokines), and results in the recruitment of leukocytes. On the other hand, the adaptive response, with humoral and cell-mediated components, develops more slowly, and thus may contribute more to chronic/repeat UTIs than to acute infections.

Innate Immunity

By recognizing conserved molecular elements of commonly encountered microorganisms, the toll-like receptors (TLRs) promote the mammalian host's rapid response to bacterial infection. While sharing structural features and host cell signaling pathways, the TLRs each recognize a distinct, specific ligand, with the best studied of the receptors to date, TLR4, recognizing bacterial lipopolysaccharide (LPS), in concert with host molecules CD14, MD-2, and LPS binding protein (233). Discovery that an inactivating point mutation in the cytoplasmic tail of TLR4 accounts for the well-known, LPS-unresponsive phenotype of mouse strain C3H/HeJ (234) has led to extensive research into the host innate immune response to *E. coli* UTI.

After UPEC infection of the urinary tract, TLR4 signaling leads to a rapid influx of polymorphonuclear leukocytes (PMNs). Haraoka and colleagues demonstrated that this TLR4-dependent recruitment is essential to successful resolution of UTI, with the finding that TLR4-competent C3H/HeN mice recruit PMNs and are able to clear virulent UPEC (235), while TLR4-deficient C3H/HeJ mice demonstrate minimal PMN response and experience UPEC persistence in tissue (235,236). With TLR4 expression by hematopoietic cells (such as macrophages and dendritic cells) already well established, Schilling and colleagues undertook a reciprocal bone marrow transplantation approach to evaluate the role of TLR4 expression by epithelial cells in the murine response to UTI (237). The investigators compared the responses to UPEC challenge of wild-type, TLR4-competent mice with two chimeric strains: TLR4-competent mice transplanted with marrow from TLR4-deficient mice (thus, TLR4-competent epithelium and TLR4-deficient neutrophils) and TLR4-deficient mice transplanted with marrow from TLR4-competent mice. The finding that neither chimeric strain was effective in clearing the infection supported a conclusion that the hematopoietic compartment alone is insufficient in mounting a protective inflammatory response to *E. coli* UTI in the mouse, and thus TLR4-dependent, epithelial activation and signaling play an essential role (237). Expression of TLR4 and responsiveness to LPS have been demonstrated in human bladder epithelium using several distinct tumor cell lines (238), but there have been conflicting findings (due to differences in tumor cell line selection) as to the involvement of other host factors—particularly membrane-bound versus soluble CD14—in the TLR4/LPS interaction (238,239).

Type 1 and P fimbrial adhesion also contribute to epithelial cell activation, although by distinct (and not fully understood) mechanisms. While CD14-negative epithelial cells (e.g., human cell line A498) are poorly reponsive to free LPS, type 1 fimbriae are able to deliver an LPS-dependent signal to CD14-negative epithelial cells via the TLR4 pathway (239). P-fimbriated bacteria appear to stimulate uroepithelial cells in two ways. First, by binding to glycosphingolipid (GSL) receptors on human kidney epithelial cells *in vivo*, P-fimbriated bacteria trigger release of ceramide, the GSL receptor's membrane-anchoring domain, and activate a cellular signaling pathway leading to IL-6 secretion (240). Second, P-fimbriated bacteria recruit TLR4 as a co-receptor and activate an LPS-independent signal in CD14-negative, human kidney epithelial cells, leading to an increase in TLR4 mRNA levels (241). While it is unclear whether P fimbriae might also deliver an LPS-dependent signal in CD14-positive cells, it is clear that specific fimbrial interactions are important in host cell activation.

Polymorphonuclear leukocytes have been noted within the urine of patients with UTI since the microscope was invented, and while most of these PMNs are viable, only 2% to 3% contain ingested bacteria (242). PMNs may ingest pathogens with or without prior opsonization. Opsonins are host factors that enhance phagocytosis (Greek *opson,* seasoning; and *phagein,* to eat), and the process by which they enhance ingestion by leukocytes is known as *opsonophagocytosis*. The most important serum opsonins are complement components and immunoglobulins, but evidence indicates that in urine, IgG and IgA may serve as the main opsonins (243).

The ability of phagocytes to ingest *E. coli* even in the absence of complement or immunoglobulin has been partially explained by observations that certain bacterial surface components can mediate phagocytosis. Type 1 fimbriae, and the FimH adhesin in particular, are important in this process (244–247); thus, this process has become known as *lectinophagocytosis* (*lectin,* in this sense is a synonym for *adhesin*) (244). It

is important to note, however, that type 1-mediated phagocytosis is associated with relatively low bactericidal activity by both PMNs and macrophages (248–250). In fact, a wide variety of host and bacterial factors have been shown to attenuate the lethality of the PMN/bacteria interaction, including Tamm-Horsfall protein (251,252), Dr family adhesins (253), P fimbriae (254–256) and surface carbohydrates such as O-antigen and capsular polysaccharide (257). It is interesting to speculate, then, that for clearance of bacteria from the urinary tract, the bactericidal capacities of PMNs may not be crucial. Merely the ability to attach and perhaps ingest bacteria may be sufficient as PMNs work their way through the epithelium to the mucosal surface, where they are released into the urine to be voided with their captured prey. Additionally, increased expression by PMNs (as well as bladder epithelial cells) of inducible nitric oxide synthase, leading to production of nitric oxide and its breakdown products, may contribute to clearance of UPEC from the bladder (258).

The presence of PMNs in the kidney during pyelonephritis is well established, and likely contributes substantially to both bacterial clearance and tissue damage. Using a monkey model of acute pyelonephritis, Fussell and Roberts demonstrated binding of P-fimbriated *E. coli* to distal and proximal tubular epithelial cells at 6 hours. Neutrophils were not seen until 24 hours but grew to large numbers within the interstitium, between epithelial cells, and within the lumen, often containing phagocytized bacteria (222). The innate immune properties of the kidney itself, however, are less well understood. While epithelial cells in the mouse kidney appear to lack TLR4 expression (237,259), a recent study of urinary tract biopsy tissue has demonstrated TLR4 expression in human renal tubular epithelial cells (260), in contrast to previous findings in a human kidney cell line (259). The possibility of renal epithelial signaling grows more likely in light of the recent discovery of TLR11 (261). Expressed preferentially in the urinary tract, TLR11 appears to recognize UPEC specifically, although a specific ligand remains to be identified. UPEC infection in TLR11-deficient mice results in dramatically lowered inflammation and a severely increased bacterial load in kidneys (261). Finally, the kidney may mount innate immune responses through renal tubule epithelial cell production of the antimicrobial peptides beta-defensins, demonstrated in response to both IL-1α and *E. coli* (262).

Interleukin (IL)-6 is an endogenous pyrogen that elicits synthesis of acute-phase reactants and induces IgA secretion. IL-8 is the main human chemokine promoting transepithelial PMN migration, and macrophage inflammatory protein (MIP)-2 was identified as the mouse homolog (263–265). Within an hour or so of challenge with UPEC, cultured human renal and bladder epithelial cells produce IL-6 and -8 (266–268), a process now known to be mediated by LPS stimulation of eptihelial cell TLR4, and possibly enhanced by type 1 and/or P fimbrial adhesion (259). Concentrations of both cytokines are commonly elevated in the urine and serum patients with various manifestations of UTI, particularly acute pyelonephritis (269–272). Antibody against MIP-2 blocks and traps PMNs.

The high-affinity, human IL-8 receptors (IL8Rs) CXCR1 and CXCR2 are serpentine molecules with seven transmembrane domains that couple to heterotrimeric G proteins for signal transduction. Godaly and colleagues have shown that both receptors are expressed in human urinary tract biopsy tissue, and both are upregulated during *in vitro* infection of human uroepithelial cell lines (263). However, in this same model, antibodies to CXCR1, but not to CXCR2, inhibited neutrophil migration across infected epithelium, indicating a more essential role for CXCR1 (263). Furthermore, knockout of the murine IL-8R homolog (mIL-8Rh KO) produces a dramatic *in vivo* phenotype of infection, featuring an aberrant PMN response with delayed influx, subepithelial trapping, and abscess formation (265). In mice that do not die of sepsis, the natural history of UPEC infection in mIL-8Rh KO strains eventually involves renal scarring, in a pattern resembling human disease (273).

The ability of uropathogenic *E. coli* to elicit an inflammatory response from bladder, as well as renal, epithelium may help to explain the previously noted finding that localization studies do not always confirm that the cystitis syndrome is confined to the bladder or that the acute pyelonephritis syndrome includes the kidney (4,5). Two reports have examined characteristics of infecting bacteria and have localized UTI through the use of either antibody-coated bacteria or renal scanning with dimercaptosuccinic acid (79,274). Both showed that a substantial number of pyelonephritis syndromes caused by P-fimbriated organisms appear to be caused by infections restricted to the lower urinary tract. It is tempting to speculate that certain bladder infections may elicit sufficient IL-6, IL-8, and other cytokines to manifest as fever with circulating acute-phase reactants and leukocytosis—that is, to mimic acute pyelonephritis.

Humoral Immunity

There is a local and systemic antibody response to UTIs. Patients with acute pyelonephritis have higher circulating concentrations of antibodies against the infecting organism than those with cystitis; indeed, the latter often have circulating antibodies at concentrations no higher than control patients (275–277). Antibodies to infecting organisms have also been identified in the urine of patients with UTIs (278–280), with IgA and IgG predominating, and IgM uncommonly found (281). Substantially greater numbers of patients with pyelonephritis can be shown to have antibody-coated organisms in their urine than patients with cystitis; indeed, for some time this was used to distinguish kidney from bladder infection (282,283). Animal models have confirmed local production of antibodies, demonstrating IgA-producing lymphocytes in the submucosa of the bladder (284) and the appearance of IgA and IgG, but usually not IgM, in the urine (285–287).

Antibody in urine provides host protection in several ways. First, antibody acts as an opsonin for phagocytosis by PMNs migrating to the urinary tract; that is, within a few days lectinophagocytosis is joined by opsonophagocytosis. Second, antibodies to adhesins can inhibit bacterial attachment to urinary epithelial cells (287). Third, antibodies can agglutinate organisms by immune mechanisms, presumably allowing more effective eradication by voiding. Fourth, antibodies to hemolysin have been demonstrated in patients (288,289) and may be important in its neutralization. The use of various *E. coli* components as vaccines in animals suggests that antibodies play a role in diminishing the incidence of subsequent infections (96,97,175,290). The roles of complement activation and antibody-dependent cytotoxicity in the urinary tract have not yet been well explored.

Cell-Mediated Immunity

Although human T cells increase in 2 to 3 days in infected organs during pyelonephritis and cystitis, the importance of cell-mediated immunity in acute infection is unclear. Marked T-cell scarcity owing to thymectomy, radiation, or genetic absence of the thymus makes little difference to outcome of experimental bacterial infection of the kidney (291–293). Because CD4 cells are often seen with plasma cells (284,294), a reasonable speculation is that within a day or so of the acute infection, CD4 cells are acting as helper cells for antibody response to the infecting organism (294), and thus may contribute to host response upon rechallenge with the same strain (295).

Experimental studies of knockout mouse strains have generally confirmed the inefficiency of the specific immune response

in UTI. Nude (T-cell deficient) mice, for example, are resistant to experimental UTI, as are T-cell receptor (TCR) $\alpha\beta$ subunit-complex knockout and RAG knockout mice (232) and mice lacking B cells due to chromosomal deletion of a portion of the antibody-receptor gene complex (296). However, SCID (B- and T-cell deficient) mice do appear to be more susceptible to UTI (297), as do mice deficient in the TCR $\gamma\delta$ subunit complex (296). Notably, $\gamma\delta$ T lymphocytes are absent from SCID mice but present in nude mice, and may account for the differing UTI susceptibility of these two model mouse strains. The $\gamma\delta$ T lymphocytes are considered important for mucosal immunity, and may contribute to epithelial repair (such as that following bladder exfoliation) by growth factor production (298). Nonetheless, these data suggest that the role of adaptive immunity may be in the response to chronic or repeat infections of the kidneys and bladder, rather than in the defense against acute infection.

Acute Renal Damage

There are at least three possible modes of injury to renal parenchyma during UTI. The first is due to direct effects of the infecting bacteria. Among known *E. coli* virulence factors, only hemolysin is known to damage bladder and renal epithelial cells directly (134–137). Many *E. coli* organisms causing acute pyelonephritis and cystitis do not carry hemolysin, thus any direct damage to host tissue must be by other means.

In contrast, there is strong evidence that in acute pyelonephritis, much of the renal damage is caused by the inflammatory response, particularly that of PMNs. *In vitro,* PMNs isolated from experimental acute pyelonephritis can kill syngeneic renal epithelial cells in culture within 24 to 48 hours (299). In animals, suppression of acute suppuration, even though causing a greater bacterial burden, diminishes tubular epithelial cell damage and renal scarring (227,228,300–302). In IL-8 receptor knockout mice, PMNs fail to exit properly and accumulate in the subepithelial space, forming abscesses and producing renal scarring in surviving mice (273). The importance of PMN-mediated oxidative injury of renal cells has been confirmed in additional *in vivo* studies (303,304). Thus, the inflammatory response by PMNs must be considered a double-edged sword.

The third possibility is renal ischemia and reperfusion injury, which may be induced during acute pyelonephritis, possibly by granulocyte aggregation in capillaries (305).

Genetic Factors

In addition to the many behavioral and environmental risk factors that predispose to UTI, which have been reviewed elsewhere (306), several interesting findings point to genetic factors that may contribute to UTI susceptibility. Of these, ABH blood group secretor status and its relationship to risk of recurrent UTI (307) and pyelonephritis (308) have been studied most extensively. Reduced expression of the human IL-8 receptor CXCR1 has been found in children prone to acute pyelonephritis (264), and PMN bactericidal function may be defective in some patients with recurrent UTIs (309). Also, although a contribution to UTI susceptibility of genetic polymorphism in the human TLR4 locus (310) has yet to be determined, TLR polymorphism has been linked with susceptibility to disease from other pathogens (311). Statistical analysis of experimental UTI outcomes in inbred and backcross mouse strains also indicates the polygenic nature of host susceptibility to disease, and suggests that C3H/HeJ strain susceptibility may, in fact, be due to more than a single (TLR4) locus (312). The interface of epidemiology, immunology, and microbiology promises to further our understanding of host genetic factors in disease susceptibility.

CONCLUSION

At least three general principles can be derived from a synthesis of the preceding data. First, adherence of bacteria to urinary tract epithelial cells is a critical step in the pathogenesis of symptomatic UTIs caused by *E. coli*. Type 1 and P fimbriae are important mediators of *E. coli* adherence to specific receptors on uroepithelium.

Second, adherence is not sufficient. The epithelium must be perturbed for symptomatic infection to become manifest, and such perturbations may be of benefit or detriment to the host. For example, epithelial cell lysis induced by hemolysin is probably detrimental to the host; on the other hand, the production of cytokines by the epithelial cell is probably beneficial to the host. However, the effects of some epithelial perturbations are unclear. Examples include cytoskeletal changes induced by cytotoxic necrotizing factor, ingestion of bacteria by the epithelial cell and sloughing of the epithelial cell. Are these steps in the progression of the bacterial infection, or in the response of the host to protect itself?

Third, each of the symptomatic UTI syndromes must represent the common manifestation of several different pathogenic pathways. That is, no single *E. coli* strain causes all cases of pyelonephritis, or all cases of cystitis. For instance, simply on the basis of genetic make-up, no more than 50% of pyelonephritogenic *E. coli* organisms employ hemolysin as a virulence factor. Nonhemolytic strains causing acute pyelonephritis must use other virulence factors, known and as yet undiscovered, to produce this symptomatic UTI. Likewise, hosts differ genetically (as well as behaviorally), and this variation is certain to interact with pathogen diversity to determine the outcome of bacterial intrusions into the urinary tract.

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CHAPTER 33 ■ CYSTITIS AND URETHRITIS

WALTER E. STAMM

DEFINITIONS

Bacteriuria

Bacteriuria, or bacteria in the urine, does not necessarily imply infection of the urinary tract. One must interpret urine culture results within the context of the clinical situation, the method of specimen collection, and the results of urine microscopy. Contamination of voided urine specimens with bacteria resident in the anterior urethra or periurethral skin inevitably occurs in some patients. For this reason, the number and type of bacteria in the urine are quantified and differentiated bacteriologically.

In voided urine samples obtained from patients with urinary tract symptoms, the finding of more than 10^5 organisms of a single bacterial species is highly predictive of infection (1). In addition, the finding of a combination of several organisms present in quantities greater than 10^5/mL of urine, together with pyuria, dysuria, urgency, and frequency, is considered to be diagnostic of infection (2). The specificity of this traditional threshold (i.e., $\geq 10^5$ bacteria/mL) for the diagnosis of acute coliform urinary tract infections (UTIs) in women is very high (0.99), but the sensitivity in populations of women with acute dysuria is often low (0.51) (3). About one-third of women with symptoms characteristic of cystitis and associated pyuria have midstream coliform counts between 10^2 and 10^5/mL. They have a satisfactory response to appropriate antimicrobials (4). These patients, formerly considered to have the acute urethral syndrome, should now be regarded as a subset of women with cystitis. A reduction in the threshold for significant bacteriuria in this setting to 10^2 coliforms/mL raises the sensitivity of the midstream urine culture to 0.95, whereas specificity only drops to 0.85 (3). Although not yet tested, this threshold is probably also suitable for uropathogenic Gram-positive bacteria in women presenting with acute dysuria. For example, *Staphyloccus saprophyticus* has a longer generation time in urine than the Enterobacteriaceae (5) and is usually found in lower counts (10^2 to 10^4) in urine obtained by suprapubic aspiration from dysuric women (6). The type of organism recovered from the urine also helps to distinguish true bacteriuria from contamination; lactobacilli, the other staphylococci, diphtheroids, and *Neisseria* are all rare causes of UTI. However, the presence of one or more of these organisms along with a typical uropathogen, such as *Escherichia coli* or *S. saprophyticus,* is not uncommon in voided specimens. Mixed cultures, particularly with low colony counts, are very likely to represent contamination. True mixed infections occur in 3% to 5% of cases (7).

In voided specimens from women with no symptoms of UTI, $\geq 10^5$ bacteria represents true bacteriuria in four out of five cases (8,9). If the same organism is isolated in $\geq 10^5$ organisms/mL in a repeat sample, then the possibility of contamination falls to 5% (10). Urine obtained by catheterization is less likely to be contaminated; a single catheter-derived specimen containing $\geq 10^5$ organisms/mL indicates infection in 95% of cases, and lower counts are more likely to be significant than low counts obtained from clean-catch samples. Urine obtained by suprapubic aspiration is least likely to be contaminated, although small numbers of bacteria are sometimes found in uninfected patients (11). Contamination is less likely in men, and a diagnostic threshold of 10^4 bacteria/mL in clean-catch samples is more appropriate (12). Bacterial counts in clean-catch samples of less than 10^5 in asymptomatic women and less than 10^4 in asymptomatic men are most unlikely to represent true bacteriuria (5%).

Cystitis and Urethritis

There is a broad spectrum of symptoms in UTIs, ranging from patients who are completely asymptomatic to those with symptoms referable to the urethra, bladder, or both, and to those with the full-blown syndrome of acute pyelonephritis with fever and loin pain.

Acute bacterial infection of the bladder is referred to as acute cystitis. Histopathologically, acute cystitis is characterized by inflammation limited to the superficial mucosal layer of the bladder. Patients with cystitis generally complain of dysuria, urgency, and frequency. Hematuria, low back pain, and lower abdominal pain also may be present. Fever and costovertebral-angle tenderness are both absent in most cases. Clinical signs and symptoms are notoriously inaccurate in localizing the site of infection, however, and up to 50% of women with symptoms and signs of cystitis on clinical examination are found to have silent renal infection when evaluated with localization procedures, such as ureteral catheterization, bladder washout, or the antibody-coated bacteria test.

The term *acute urethral syndrome* has been used to describe women who experience symptoms of cystitis but who have urine cultures with less than 10^5 colony-forming units (CFU)/mL. This term should be discarded in that it implies both unknown causation and a urethral origin of symptoms. Recent work, as outlined in the following section, has demonstrated that most women who formerly were classified as having the urethral syndrome can now be classified as having either acute cystitis or acute urethritis, and specific causative pathogens usually can be identified.

Recurrent Infections

Recurrent urinary tract infections are either relapses with the original infecting organism or reinfections with a different organism. In general, relapses develop within 3 weeks after cessation of therapy for the previous infection. Occasionally, reinfections occur with the same organism that has persisted in the vagina or feces and may be mistaken for relapse. Reinfections account for 80% of recurrent UTIs (13) and result from reintroduction of organisms from the perineal flora. Relapses arise after inadequate or inappropriate treatment, when there is a sequestered focus of infection in the kidney or prostate, or because of a structural abnormality of the urinary tract.

Continual relapses (i.e., persistence of a single infecting organism despite appropriate treatment) or true chronic urinary tract infection are unusual and usually suggest a structural abnormality or stone.

PATHOGENESIS

Route of Infection

Bacterial cystitis almost always results from the entry of bacteria colonizing the anterior urethra and periurethral skin into the bladder (13). Hematogenous or lymphatic spread from sites of infection elsewhere is very unusual. Retrograde infection from foci in the prostrate or kidney may occur and recent data in animals suggest that foci of infection may be established in the bladder mucosa (14). Rarely, bacteria spread from the bowel to the bladder by means of a fistulous communication (i.e., in Crohn's disease or malignancy). Characteristically, polymicrobial infection and pneumaturia result in such cases.

The short female urethra is an insufficient anatomic barrier to the entry of urethral bacteria, which may be massaged easily into the bladder. This may explain the association of urinary tract infections and bacteriuria with sexual activity (15). The annual incidence of bacteriuria is significantly lower (0.4%) among nuns in the age range of 13 to 54 years than among working women of the same age (1.6%) (16). Presumably, bacteria are massaged into the bladder during sexual intercourse.

Bacteria are also introduced into the bladder during catheterization of the urethra. Single catheterization of the bladder in ambulatory patients results in a 1% incidence of subsequent UTI (17). Further support for the theory that UTIs arise from ascending spread of bacteria from the perineum comes from the consistent observation that the organisms that eventually cause UTIs usually colonize the vagina and the periurethral areas beforehand (13). In women with normal urinary tracts, the development of infection following colonization probably depends on mechanical factors, such as sexual intercourse and pregnancy, and the balance between contributions of bacterial virulence factors and host defenses.

Pathogens

In 95% of infections, a single bacterial species is responsible, and in about 80% of acute uncomplicated cases, the organism is *E. coli* (Fig. 33-1) (18). Interestingly, relatively few serogroups, defined on the basis of O, H, and K antigens, cause the majority of infections namely, O antigen groups 2, 4, 8, 18, and 75 (19). These pathogens are known as *uropathogenic E. coli*. In patients who have repeated urinary tract infections, the likelihood of isolating more than one organism or an antibiotic-resistant organism increases. Among hospital inpatients, there is a similar increase in the frequency with which organisms other than *E. coli* are isolated. Instrumentation, catheterization, cross infection, and underlying illnesses all contribute. Antibiotic-resistant isolates are common, particularly if the patient has received multiple or protracted courses of antimicrobial treatment.

In the past, coagulase-negative staphylococci often have been regarded as urinary contaminants, but studies have now clearly demonstrated a pathogenic role for one species, *S. saprophyticus* (7,20). In urine, this organism can be identified reliably as coagulase-negative staphylococci that are resistant to novobiocin (21). *S. saprophyticus* infections characteristically occur in young women between the ages of 16 and 25 years but may occur in men or women of any age. They occur most commonly during the spring and summer. In studies from Europe (20,22), *S. saprophyticus* has accounted for up to one-third of acute UTIs. Wallmark (22) reported that 22% of a series of 787 Swedish women 16 to 25 years old were infected with the organism. In the United States, the proportion of UTIs owing to *S. saprophyticus* infection has generally been reported to be 5% to 15%.

Anaerobic bacteria, lactobacilli, corynebacteria, streptococci (other than enterococci), and other staphylococci are the predominant organisms isolated from the perineum and distal urethra, but they seldom cause UTI. These organisms are among the most common urinary contaminants. Bacteria may be found in the urine during certain systemic infections (e.g., leptospirosis and *Salmonella* bacteremia), and in metastatic infections of the kidney (usually *Staphylococcus aureus*), but cystitis caused by these species is very unusual. Adenoviruses (type 11) may cause epidemic hemorrhagic cystitis in children,

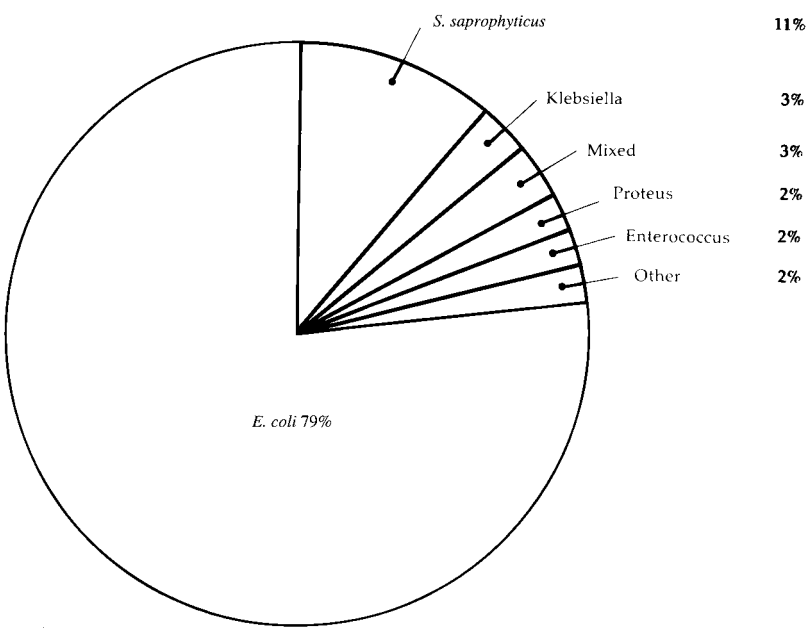

FIGURE 33-1. Bacterial causes of acute cystitis.

particularly in boys (23), and may be underestimated as an endemic cause of cystitis. Other viruses rarely cause acute cystitis.

Virulence Factors

In comparison with *nonuropathogenic E. coli* derived from the normal fecal flora, *uropathogenic E. coli* exhibit specific virulence factors, which include increased adherence to vaginal (24) and uroepithelial cells mediated by fimbrial adhesins (25,26), resistance to the bactericidal activity of human serum (27), production of hemolysin and other cytotoxins (19), siderophores to bind iron and increased amounts of K capsular antigen (28). The adhesive properties of these organisms are important determinants, not only of infectivity, but also of a propensity to develop upper tract infections. Adhesion is mediated by specific surface ligands, which attach as lectins to host cell-wall carbohydrate residues (receptors). These ligands are usually pili or fimbria, which can be seen on electron micrographs as narrow hairlike projections extending from the bacterial capsule (29,30). Most of the Enterobacteriaceae possess type 1 fimbriae, which bind mannoside residues in mannosylated glycoproteins on vaginal epithelial and uroepithelial cells (31). Binding can be competitively inhibited by *a*-methyl mannoside and thus is termed *mannose-sensitive* (MS) (32). Type 1 fimbriae mediated binding appears particularly important in bladder infections and in internalization of E coli within epithelial cells (14). *Escherichia coli* strains that cause upper urinary tract infections usually express additional surface adhesins that cannot be inhibited by an *a*-methyl mannoside and are thus termed *mannose-resistant adhesins* (MR); 80% to 90% of uropathogenic *E. coli* that express MR adhesins recognize a glycolipid receptor found on human erythrocytes and uroepithelial cells (33,34). The minimal recognition structure on this globoseries glycosphingolipid is a disaccharide moiety, α-D-galactopyranosyl-(1-4)-ß-galactopyranoside [α-gal(1-4)-ß-gal] (35). This structure also constitutes a major part of the P blood group erythrocyte surface antigens (36), and these pili have, therefore, been named P fimbriae. The MR adhesin or adhesins that do not recognize this disaccharide moiety are termed x-specific (34); they are also fimbrial but recognize receptor structures other than α-gal-(1-4)-ß-gal. Further molecular details of the role of specific adhesins in UTI are provided in Chapter 32, Host–Pathogen Interactions and Host Defense Mechanisms.

Several lines of evidence emphasize the importance of bacterial attachment in uropathogenicity (37) *Escherichia coli* colonization of the mouse bladder can be prevented by mannose (i.e., competitive blockade of the MS adhesin) (38). Subjects who lack the P blood group phenotype and, therefore, do not express the P-specific recognition site on uroepithelium appear to be less susceptible to UTI (39). Antibodies directed against the fimbriae that mediate uroepithelial attachment block adherence *in vitro* (39a) adhesion probably also play a central role in the uropathogenicity of other bacterial species; *S. saprophyticus* adherence to uroepithelial cells is significantly greater than that of *S. aureus* or the other coagulase-negative staphylococci (40). Fimbrial adhesion and uropathogenicity have also been correlated in *Proteus mirabilis* (41) and *Klebsiella* UTIs (42). Once attachment to uroepithelial cells occurs, hemolysin production and bacterial endotoxins may be important in initiating tissue invasion and cell damage. The presence of K-antigen protects bacteria from phagocytosis by leukocytes. Most uropathogenic *E. coli* also produce aerobactin, which facilitates acquisition of iron. These virulence factors are undoubtedly important in predisposing otherwise normal subjects to UTIs, but they are less important in patients with structural abnormalities of the urinary tract. Organisms infecting the upper tracts of children with vesicoureteric reflux, for example, do not exhibit urovirulence factors (43).

In summary, in women and children with normal urinary tracts, bacteria enter the bladder after periurethral colonization. Their ability to survive in the bladder depends on adherence to vesical uroepithelium mediated by fimbriae and resistance to host factors (see the following). Other bacterial virulence factors then determine whether ascending infection will develop. Of these, P fimbriae have been identified as being important because they presumably mediate adhesion to the uroepithelium of the renal pelvis and collecting system (9,30).

Host Factors

Entry of bacteria to the female bladder is probably a frequent occurrence, but established infection does not necessarily ensue. Urethral catheterization introduces a shower of bacteria into the bladder, but natural clearance occurs in all but 1% of ambulatory patients. Several factors act in concert to defend against infection. The flushing and diluting effects of urine accumulation and voiding are important (44), but at normal urine flow rates these factors alone are insufficient to clear infection. The acidity, high urea concentration, and extremes of osmolality of urine make it a poor culture medium (45) for many anaerobic and fastidious bacteria and inhibit growth of many other organisms. The increased frequency of UTIs in pregnancy may, in part, be related to a more favorable urine pH and osmolality. Bacteria grow less well in urine collected from men than women, probably because of inhibitory activity of prostatic fluid (46). Urinary mucus containing Tamm-Horsfall protein may act as a physical barrier to infection with the Enterobacteriaceae, because it contains large numbers of mannose residues that bind to the MS adhesins on these bacteria (47). The bladder mucosa itself resists adhesins, a property that appears to be related to surface mucopolysaccharides and can be reversed by application of acid (48). Although these factors may defend against small inocula of bacteria, they may be overcome by larger inocula or more adhesive bacteria (49). Foreign bodies such as stones and structural abnormalities provide a refuge for bacteria that may be extremely difficult or impossible to eradicate with antimicrobials (50). Recent studies have defined the role of innate host defense mechanisms in mediating the inflammatory response in cystitis (Chapter 32). In particular, attachment of *E. coli* to mucosal epithelial cells initiates IL-6 and IL-8 secretion, which produces an inward migration of polymorphonuclear (PMN) leukocytes and macrophages, which remove and destroy bacteria (49). Urine inhibits the phagocytic functions of PMN leukocytes, including migration, aggregation, and killing. The inflammatory response is in part responsible for the symptoms of cystitis. In contrast to pyelonephritis (51), cystitis is seldom associated with a marked systemic or local antibody response to the infecting organism (51). Recent studies have more clearly defined the role of specific cytokines in mediating the inflammatory response in cystitis (Chapter 32).

Two main theories have been advanced to explain the propensity of certain women to develop repeated UTIs. The first, propounded by Stamey and colleagues, holds that these women have a defect in their local defense mechanisms that renders them more vulnerable to periurethral colonization (14,52,53). The host, therefore, has a biologic predisposition to infection. An alternative theory proposed by Kunin and colleagues (54) holds that the virulent properties of uropathogenic bacteria are the clinical determinants of infection. According to this theory, there is no intrinsic defect in local defense mechanisms, provided that the urinary tract is structurally normal. All women, therefore, are equally prone to infection, but once they have developed a UTI, the infection itself and the antimicrobial treatment used to treat the infection may predispose them to persistent or recurrent periurethral colonization with

uropathogens. The corollary of this theory holds that the longer the interval between infections, the less likely is a recurrent infection. Periurethral colonization predisposing to UTI is central to both theories, but, according to the latter theory, it is not the primary abnormality. Although longitudinal studies generally have confirmed that periurethral colonization precedes infection, the evidence that some women are intrinsically more likely to be colonized than others is conflicting. Some studies have reported an increased incidence of prolonged colonization with Enterobacteriaceae in women with recurrent UTIs (14,53,55–58) but others have not (54,59–61). The factors predisposing to colonization have been investigated; low vaginal pH was the major important factor preventing colonization (28), and serotypes of *E. coli* associated with UTI were significantly more resistant to an acid environment than those serotypes not associated with UTI (57). Vaginal fluid is inhibitory to the growth of *Pseudomonas aeruginosa* and *P. mirabilis* but less so to *E. coli* (62). Biochemical changes in vaginal fluid might reasonably be attributed to alterations in the nonpathogenic resident vaginal bacterial flora. In earlier studies, no detectable quantitative or qualitative differences in vaginal concentrations of lactobacilli or anaerobic bacteria were demonstrated between women with and without recurrent UTI (63). More recent studies, however, have demonstrated an inverse relationship between *E. coli* vaginal colonization and the presence of H_2O_2 producing lactobacilli in women with recurrent UTI (64). These studies indicate that diaphragm or spermicide use markedly alters the vaginal microflora, promoting colonization with *E. coli* and other uropathogenic species (9), and reducing lactobacilli populations.

Studies of bacterial adherence in women prone to recurrent UTIs generally have demonstrated an increased attachment of *E. coli* to periurethral (65), uroepithelial (66), buccal, and vaginal epithelial cells from such women (14,67). More recently, studies have demonstrated that nonsecretors of blood group antigens have an increased risk of recurrent UTIs (9) and that the epithelial cells of nonsecretors support bacterial attachment to a greater extent than do cells from secretors (9), suggesting a genetic predisposition to UTIs in some women. No defects in local immunoglobulin production in women with previous UTIs have been demonstrated (68). Thus, at present, there is increasing evidence in support of host factors as the primary explanation for recurrent UTIs in women. Both inherited (i.e., P blood group and nonsecretor status) and acquired factors (i.e., spermicide and diaphragm use, as well as antibiotic exposure) are likely of importance. Polymorphisms in host response genes, such as IL-6, IL-8, TNF and others, are now being studied as possible factors predisposing a person to recurrent UTIs.

EPIDEMIOLOGY

Cystitis or inflammation of the bladder is among the most common of all bacterial infections. In the first year of life, the incidence of UTI is higher in males than females (69). The overall prevalence of bacteriuria in the neonatal period is approximately 1% (70,71). Established UTIs during this period are often associated with bacteremia. Between the ages of 1 and 5 years, the prevalence of bacteriuria in girls rises to 4.5% per year, whereas the incidence in boys falls to 0.5% per year. Infections in boys are often associated with congenital abnormalities of the urinary tract or with lack of circumcision. Between one-third and one-half of UTIs during the first 5 years are associated with vesicoureteric reflux, and this appears to be the critical period, determining whether progression to renal scarring will occur or not (72,73). Bacteriuria in this age group is usually associated with symptoms, whereas asymptomatic bacteriuria is more usual during the school years. The prevalence of bacteriuria among schoolgirls in the United States is approximately 1.2%, with a total of 5% of girls having bacteriuria at some time. Most of these cases are not associated with renal abnormalities (74,75). Bacteriuria is rare in school-age boys. During the reproductive period, UTIs are some 50-fold more common in women. Approximately 20% of women between 24 and 64 years old have at least one episode of dysuria each year, most of these being caused by bacterial infections (76). In later years, the incidence of UTIs increases sharply in both sexes, with a progressive reduction in the female:male ratio. In the United States, UTIs result in approximately 6 million outpatient visits each year (77). This figure probably underestimates the true incidence, because at least one-half of all UTIs resolve without medical attention.

CLINICAL MANIFESTATIONS

The symptoms of a UTI in young children are notoriously nonspecific, with fever, poor feeding, and vomiting often being the major manifestations (70). Abdominal discomfort is inconstant. Urinary tract infections must always be excluded in children with unexplained fever. After infancy, the classic symptoms of dysuria, with urgency and frequency, are more usual. Adults with cystitis have frequent urgent voiding of small volumes of urine. Nocturia is common. There is often a sensation of lower abdominal heaviness or discomfort. The urine may be turbid and is frankly bloody in one-third of cases. The onset of dysuria may be abrupt. Most cases resolve promptly with antimicrobial treatment, and many resolve spontaneously without therapy. However, some progress after 1 or 2 days to develop signs and symptoms of upper tract involvement, including fever, rigors, vomiting, flank pain, and hematuria. However, studies comparing the localization of bacteria by laboratory techniques with clinical signs and symptoms have demonstrated a poor correlation between clinical manifestations and localization results (71,72). Urethritis may be more common than cystitis in men who experience dysuria (73). Urinary tract infections often are asymptomatic in the elderly (74); furthermore, frequency, urgency, nocturia, and incontinence may have multiple causes in this age group. There should be a low threshold for microbiologic examination of the urine in elderly patients with unexplained fever, increased urinary frequency, incontinence, or lower abdominal discomfort. Patients with neurogenic bladders or an indwelling catheter usually have no symptoms referable to the bladder when a UTI develops; presenting signs and symptoms of pyelonephritis and unexplained fever or septicemia are more usual.

Investigations

In a series of classic studies in the 1950s, Kass (1,8,75) demonstrated the value of quantitative cultures of the urine in the diagnosis of asymptomatic bacteriuria and pyelonephritis. A threshold of $\geq 10^5$ bacteria/mL of urine reliably distinguished contaminated specimens from true bacteriuria in asymptomatic women and accurately diagnosed women with acute pyelonephritis. Many clinicians subsequently adopted this single criterion to diagnose cystitis, although Kass had not, in fact, studied women with lower tract symptoms. Approximately 40% of women who experience symptoms of cystitis have midstream urine cultures containing less than 10^5 bacteria/mL (61,76,77). These women closely resemble those with cystitis (characterized by "significant" bacteriuria, that is, $\geq 10^5$/mL) in age, marital status, presenting symptoms, prevalence of periurethral colonization, bacterial species, serotypes, bacterial virulence factors, and response to antimicrobials (76–78). It has become apparent that cystitis with significant bacteriuria and cystitis with lower bacterial counts have a

similar pathogenesis and represent a clinical continuum (3,79,80). Both illnesses occur in the same women; approximately 20% of symptomatic episodes in women with recurrent UTIs are associated with less than 10^5 organisms/mL. In a prospective study of women with the acute urethral syndrome, over 50% subsequently developed significant bacteriuria (61).

In a study of 59 women presenting with the acute urethral syndrome (80), cultures of bladder urine obtained by suprapubic aspiration (the majority of cases) or sterile urethral catheterization grew uropathogens in 27 (46%). In 26 of these cases (96%), there were $\geq$8 leukocytes/mL of unspun midstream urine. This study confirmed that approximately half of the women who present with acute dysuria have midstream urine containing 10^2 to 10^4 coliforms/mL and indeed have bacterial cystitis.

Urethritis and vaginitis account for urinary symptoms in most women with urine specimens that are truly culture-negative for bacteria. The sexually transmitted pathogens *Chlamydia trachomatis, Neisseria gonorrhoeae,* and herpes simplex virus are also important causes of dysuria (4); approximately 20% of women with proved gonococcal or chlamydial genital infections who were attending a clinic for sexually transmitted diseases complained of these symptoms. In one study, the incidence of gonorrhea in women presenting with symptoms of acute UTI to an urban emergency room was approximately 30% (81). In a population of college students presenting with the acute urethral syndrome, 10 of 16 patients with sterile bladder urine and pyuria were found to have *C. trachomatis* infection, and no cases of gonococcal infections were detected (80). Clearly, the relative frequencies of these two infections depend on their prevalence in the community. Genital herpes simplex infection produces dysuria in 10% of women with primary infections (73). Lesions usually are evident on the vulva or cervix, but occasionally dysuria precedes the external lesions and may cause diagnostic confusion.

Vulvovaginitis associated with *Candida albicans* or *Trichomonas vaginalis* also may cause dysuria. The discomfort is characteristically external, because it is related to the passage of urine across the inflamed labia (73). The patient's history helps to distinguish vaginitis from cystitis, because complaints of urgency and suprapubic pain are unusual in vaginitis, and dysuria is characteristically external. Associated symptoms of vaginitis (discharge, odor, or soreness) are usually present as well.

Several other organisms have been implicated in the acute urethral syndrome, but their role is controversial. Those include *Mycoplasma hominis, Ureaplasma urealyticum,* Mycoplasma genitalium and a variety of anaerobic, microaerophilic, and fastidious bacteria (80,82–84). Approximately 15% of women have no identifiable infectious cause of dysuria. In those women, the urine is sterile and there is usually no pyuria. Some of these women experience repeated episodes of dysuria for which no cause is identified; cold weather (85), allergies (86), stress (86), anxiety (86); obstruction (87,88), trauma, and senile atrophy of the periurethral glands (89) all have been suggested as possible causes, and treatment strategies, such as surgery to improve urine flow (90) and estrogen suppositories (89), have been recommended, but no approach is consistently beneficial (4).

DIAGNOSIS

Microscopic examination and culture of clean midstream urine specimens are the primary laboratory investigations in suspected UTIs (2,91). Uninfected people excrete less than 400,000 leukocytes/hour, whereas more than 95% of patients with symptomatic UTIs excrete considerably more than this number (92). Unfortunately, measurement of pyuria by examination of centrifuged urine sediment lacks precision (2). A simple and reproducible method of screening for pyuria is to examine a fresh, uncentrifuged specimen of urine in a hemocytometer chamber. A count of 10 or more leukocytes/mL is considered abnormal. Most women with cystitis and midstream bacterial counts of uropathogens $\geq 10^2$/mL have more than 60 leukocytes/mL (91). The finding of pyuria is not specific for cystitis, but it serves as a useful indication of infection because most patients with infections have white cells in their urine. In the absence of positive midstream urine cultures ($<10^2$ uropathogens/mL), pyuria suggests chlamydial infection or gonorrhea (3). Microscopic or occasional gross hematuria may be observed in cystitis, but it is rare in gonorrhea or chlamydial infection; white cell casts strongly suggest pyelonephritis in a patient with symptoms of a UTI.

The presence of one or more bacteria per oil immersion field in Gram-stained uncentrifuged urine correlates with $\geq 10^5$ organisms/mL on culture with a sensitivity and specificity over 90% (9); therefore, this method represents a simple and rapid screening test for high-grade bacteriuria, but does not detect low-count infections (10^2 to 10^4 bacteria/mL). The gram stain is also useful because organism morphology is helpful in selecting empiric therapy.

Rapid Diagnostic Methods

Several screening methods can be used for the rapid detection of bacteriuria and pyuria. They may be used in several settings: The clinician may use them to facilitate immediate diagnosis; the laboratory may use them as a rapid screen to select which specimens require further microbiologic investigation; and they may be used for the mass screening of various populations (e.g., school-age girls or pregnant women) to detect bacteriuria. Four biochemical tests have been devised: the glucose oxidase, catalase, nitrite, and leukocyte esterase tests.

Glucose Oxidase Test

The urine normally contains glucose, but if bacteria are present, glucose is metabolized. This test detects the absence of glucose in a single specimen. It has two drawbacks: First, the urine must remain in the bladder for approximately 4 hours for the bacteria to consume sufficient glucose; and second, it is not appropriate when abnormal glycosuria is present (i.e., in pregnancy or diabetes).

Catalase Test

Most uropathogens generate catalase. If hydrogen peroxide is added to infected urine, bubbles of oxygen are then released. Unfortunately, this test does not reliably identify bacteriuria, because blood elements also contain catalase; therefore, the test has a high false-positive rate.

Nitrite Test

Nitrite is formed when bacteria reduce the nitrates that are present in normal urine (93). Colorimetric detection of nitrite, therefore, provides a simple and rapid method of detecting bacteriuria. The Griess test has been adapted to a dipstick, which turns pink when positive. The major disadvantage of this test is lack of sensitivity; the urine must incubate in the bladder for at least 4 hours (94) to allow adequate nitrate reduction. In addition, some organisms, such as yeasts and enterococci, do not reduce nitrates. Finally, urobilinogen, ascorbic acid, and low urinary pH may all produce false-negative results. Detection of bacterial dehydrogenase activity by triphenyltetrazolium chloride reduction (also a colorimetric method) also lacks sensitivity and may give false-negative results.

Leukocyte Esterase Test

The presence of leukocytes in urine may be detected by a colorimetric test of esterase activity. This method has been reported to detect pyuria down to levels of 25 to 50 white cells/mL (72). The sensitivity of a combined dipstick test, which utilizes both the leukocyte esterase and the nitrite test (LN strip), has ranged from 70% to 95% with a specificity of 65% to 85% (93).

Automated Methods for the Detection of Bacteriuria

Several automated labor-saving devices have been introduced for the rapid detection of bacteriuria (95).

Photometric Methods

Photometric methods detect changes in light scatter, resulting from bacterial growth, and measure the consequent drop in voltage in the photometer. Obviously, the sensitivity of these methods is directly related to growth rates and incubation times. Currently available systems require incubation times between 1 and 13 hours. False-negative rates have tended to be high for slower growing organisms (e.g., *Pseudomonas*). Using a threshold colony count of more than 10^5 CFU/mL, overall false-negative rates have ranged from 3% to 15% for the three available systems, and detection times have been between 2 and 5 hours (96). These methods also have been adapted for speciation of organisms using selective liquid media and have been extended to perform antimicrobial sensitivity testing.

A more rapid turbidometric method using the Limulus amoebocyte (LAL) test has been introduced, which can detect Gram-negative bacteriuria in 20 minutes, with a sensitivity of approximately 95% for more than 10^5 CFU/mL (97). It cannot detect Gram-positive bacteriuria.

Bioluminescence Methods

Bioluminescence methods of rapid detection do not require bacterial growth and are therefore more rapid than the photometric assays (current methods take between 2 and 25 minutes for sample processing). They are based on the firefly luciferin/luciferase system, which converts the energy of cellular adenosine triphosphate (ATP) into light (98). These methods have had a reported sensitivity that is comparable to the photometric methods, but they are generally less specific with false-positive rates over 15%.

Filtration Methods

A very rapid method of diagnosis (Bac-T-Screen) has been introduced in which urine is filtered, stained, and quantitated colorimetrically (99). This test takes 2 minutes. Studies report a sensitivity of 85% to 98% and a specificity of 40% to 60% with this method, using cultures of probable pathogens at more than 10^5 CFU/mL as the standard (100). Approximately 5% to 10% of samples are uninterpretable because of clogging or deposition of residual pigment on the filter. This method has the advantage of also detecting pyuria, which may be of value in identifying patients who have infections characterized by pyuria and 10^2 to 10^4 bacteria/mL of urine.

None of these currently available methods has emerged as clearly superior to the others, and the choice of a given method depends in part on its intended use. There are continuous technologic improvements, and it is likely that automated techniques will progressively replace conventional diagnostic methods for the detection of pyuria and bacteriuria, particularly for large-scale screening. A major limitation of these methods is their lack of sensitivity when applied to the diagnosis of acute dysuria. The sensitivities of all of these methods for detecting bacteriuria $\geq 10^5$ CFU/mL exceeds 90%, but all are considerably less able to detect infections characterized by 10^2 to 10^4 CFU/mL. Because most of these low-count infections are also characterized by pyuria, however, methods such as the L-N strip or the Bac-T-Screen, which are capable of detecting either bacteriuria or pyuria, appear to be useful diagnostically in this setting. In addition, the rapidity of these two tests makes them appealing in the setting of acute symptoms.

Urine Culture

Conventional microbiologic quantitation of bacteriuria is performed by streaking a known volume of urine from a calibrated platinum loop (either 0.01 or 0.001 mL) onto agar plates, incubating at 37°C for 24 hours, and then counting the number of bacterial colonies (101). Other methods include the accurate but time-consuming pour-plate method and the dip inoculum method, in which an agar surface is simply immersed in urine and then incubated. The dip inoculum method is a simple and practical alternative for outpatient diagnosis when access to a microbiology laboratory is limited.

MANAGEMENT

General Principles

Asymptomatic Bacteriuria

Children with asymptomatic bacteriuria generally should be treated. This, however, begs the question as to who should be screened, and there is no consensus on this issue (47). By the time a child goes to school, it is probably too late to significantly affect the natural history of vesicoureteric reflux and progressive renal scarring (70,102,103). The cost:benefit ratio, efficacy, and practicalities of screening younger children for bacteriuria have yet to be determined (74,104–106).

There is little convincing evidence that treatment of asymptomatic bacteriuria is beneficial in adults who are not pregnant (72). Bacteriuria in pregnancy is associated with a high risk of developing acute pyelonephritis and jeopardizing the pregnancy (107,108). Women should be screened for bacteriuria during pregnancy and promptly treated. At present, there is no clear reason to routinely treat asymptomatic bacteriuria in elderly patients (10,73). Treatment may be advisable in selected high-risk cases. Although asymptomatic bacteriuria in hospitalized patients has been associated with a higher mortality than in patients without bacteriuria (110), this probably relates to the frequency with which seriously ill patients are catheterized and the mortality associated with bacteremic catheter-associated UTIs. Asymptmatic bacteriuria in patients about to undergo urologic surgery should also be treated in order to reduce the risk of postoperative infection. The Infectious Disease Society of America has recently released guidelines for the treatment of aymptomatic bacteriuria (109).

Symptomatic Bacteriuria

All symptomatic infections should be treated with antimicrobial drugs. The principles of antimicrobial therapy are the same as those applying to treatment of any infection. Ideally, the least toxic, least expensive drug should be prescribed for long enough to eradicate the infection (111,112). The antibacterial spectrum of the agent should cover the likely infecting organisms but preferably not disrupt the normal gut and perineal flora. Successful treatment of uncomplicated lower tract infections closely correlates with the inhibitory concentration of

TABLE 33-1

TREATMENT OF UNCOMPLICATED LOWER URINARY TRACT INFECTION

Drug	Dose (adult; mg)	Dose (child; mg/kg)	Dose interval (hours)	Recommended duration (days)
Trimethoprim-sulfamethoxazole	160/800	2.5/12.5	12	3
Trimethoprim	200	3	12	3
Norfloxacin	400	N/A	12	3
Ciprofloxacin	250	N/A	12	3
Ofloxacin	200	N/A	12	3
Amoxicillin	250–500	5	8	7
Amoxicillin-clavulanate	250–500/125	4/2	8	7
Nitrofurantoin	100	3	12	7

NA, not applicable.

antimicrobials achieved in the urine rather than plasma or tissue concentration (12). Some antimicrobials that are successfully used to treat cystitis (e.g., nitrofurantoin and nalidixic acid) do not achieve microbicidal blood or tissue levels but are excreted in high concentrations in the urine. Urinary concentrations of many antibiotics are considerably higher than corresponding levels in other body fluids and may even exceed the minimal inhibitory concentration and minimum bactericidal concentration of some "resistant" organisms. This finding probably accounts for the clinical observation that antibiotics to which their infecting organism was apparently resistant cure some patients with cystitis.

Urinary pH is an important determinant of *in vitro* antibacterial activity of some antimicrobials, such as erythromycin and the aminoglycosides, but is rarely clinically important. The urinary antiseptic methenamine exerts its antibacterial activity by the liberation of formaldehyde and un-ionized organic acid at pH 5.5 or lower. Two organic acid salts are available, methenamine mandelate, and methenamine hippurate. Acidification of the urine by restriction of alkalizing agents, such as milk and fruit juice, and by ingestion of methionine may enhance formation of uric acid stones, and taking ascorbic acid may accelerate formation of oxalate stones. Furthermore, it may be impossible to acidify the urine, particularly if it is infected with urea-splitting organisms, such as *Proteus*. Methenamine sometimes is used for long-term suppressive treatment if renal function is normal, although it remains questionable whether adequate urinary acidification is possible. The aminoglycoside antibiotics must be given parenterally; therefore, they are used only for the treatment of cystitis with organisms that are resistant to all other antimicrobials. Urinary pH may be an important determinant of their activity in renal failure when urinary concentrations are lower (because less drug is filtered) (113). In this situation, low urinary pH together with high urinary concentrations of calcium and magnesium may reduce their antibacterial activity sufficiently to impair therapeutic efficacy.

A variety of nonspecific adjunctive measures are often recommended to supplement antimicrobial treatment. Patients with a UTI are commonly advised to drink as much as they can. The diluting and flushing out of nonadherent bacteria from the bladder may rapidly reduce bacterial counts in the urine and provide temporary symptomatic relief. In some cases, a very large fluid load (7,000 mL/24 hours) may be sufficient to clear the infection but, more usually, as urine flow falls, bacterial counts rise again and symptoms recur. Other effects of forced fluids may not be beneficial; urinary acidification is reduced, antibacterial substances in the urine are diluted, and obstruction or reflux may be exacerbated. Nonspecific treatment to reduce bladder discomfort and dysuria, such as potassium citrate and phenazopyridine, has little use in the management of bacterial cystitis. Phenazopyridine occasionally is helpful in women with recurrent dysuria who have no documented infections.

Antimicrobial Therapy for Uncomplicated Cystitis

Increasingly, women with uncomplicated cystitis are being treated empirically without obtaining a urine culture or antimicrobial sensitivity data. Empiric antimicrobial regimens are chosen for their lack of toxicity, patient acceptability, activity against potential uropathogens, and cost (114,115). The Infectious Diseases Society of America has published evidence-based treatment guidelines (116). Most experience has been gained with sulfonamides, ampicillin or amoxicillin, nitrofurantoin, trimethoprim-sulfamethoxazole, and the newer fluoroquinolones (Table 33-1), although the oral cephalosporins, nalidixic acid, and the tetracyclines have all been used as well.

Sulfonamides

Traditionally, sulfonamides with rapid renal clearance have been used for outpatient treatment of cystitis. In the past, drugs were effective *in vitro* against 70% to 85% of outpatient strains of *E. coli, S. saprophyticus,* and *P. mirabilis; Klebsiella* strains are variably sensitive, but enterococci are uniformly resistant. They also have useful activity against *C. trachomatis* (117). Adverse reactions are rare but potentially serious, including rashes, erythema nodosum, erythema multiforme (occasionally progressing to the Stevens-Johnson syndrome), hemolysis, blood dyscrasias, and a variety of other hypersensitivity phenomena (118,119). Sulfonamide crystalluria is rare with the more soluble sulfonamides. These drugs should not be given to premature infants or women before delivery, because they displace bilirubin from plasma albumin binding sites and therefore may precipitate kernicterus. Although they remain a generally effective, well-tolerated, and inexpensive treatment for outpatient UTIs, they have been superseded in recent years because of increasing *in vitro* resistance of *E. coli* and the availability of newer antimicrobials with a broader antibacterial spectrum. Up to 30% to 40% of strains causing acute cystitis are sulfonamide resistant.

Ampicillin and Amoxicillin

Although potentially effective against all the major uropathogens, the proportion of community-acquired *E. coli* strains demonstrating *in vitro* resistance to ampicillin has been rising in recent years and now exceeds 30% in many communities. In addition, at least half of *S. saprophyticus* (7) and almost all *Klebsiella* strains are resistant *in vitro*. The antibacterial spectra of other ampicillin-related compounds, such as amoxicillin and cyclacillin, and the prodrugs pivampicillin and talampicillin are essentially similar (120), although they are both well absorbed from the gut and, therefore, have less associated diarrhea (3% to 5% versus 15% to 20%). Adverse effects from ampicillin treatment are seldom serious. Rashes are relatively common (10%) (121), but serious allergic reactions are rare.

Clavulanate-Potentiated Amoxicillin

Clavulanic acid is the first of a new generation of ß-lactamase inhibitors (122). These drugs are structurally related to penicillin, have little intrinsic antibacterial activity, but inactivate staphylococcal and Gram-negative bacterial enzymes of Richmond classes II to V (123). Potassium clavulanate combined with amoxicillin (Augmentin) has excellent *in vitro* and *in vivo* activity against a variety of ß-lactamase-producing amoxicillin-resistant organisms. The pharmacokinetics of the two drugs are similar, and approximately 40% of the oral dose of clavulate is eliminated unchanged in the urine (124). Several studies have demonstrated that clavulate-potentiated amoxicillin is effective in the treatment of UTIs caused by ampicillin-resistant organisms (mainly ß-lactamase-producing *E. coli*) (114,115,125). However, gastrointestinal side effects (usually loose stools or diarrhea) have been frequent (about one-quarter of patients) and sometimes necessitate cessation of therapy.

Nitrofurantoin

Nitrofurantoin is effective *in vitro* against most of the common uropathogens seen in acute cystitis (126). Antimicrobial activity is confined to the urinary tract because tissue concentrations are subinhibitory, whereas urine concentrations are bactericidal. Mild nausea is observed in many patients, but vomiting is less common (4%). Gastrointestinal intolerance occurs less frequently with the macrocrystalline preparations (119). A variety of allergic manifestations have been reported, including fever, pulmonary infiltrates, pulmonary fibrosis, pseudotumor cerebri, megaloblastic anemia, and hepatitis, but all are rare (127–130). Peripheral neuropathy is observed mainly in patients with renal failure (in whom the drug is relatively ineffective) (132). As with the sulfonamide drugs, hemolysis may be precipitated in patients who are deficient in glucose-6-phosphate dehydrogenase (G_6PD). Overall side effects are more common than with the short-acting sulfonamides (approximately 9% versus 3%). The prevalence of these adverse effects increases with age. Interestingly, despite many years of use, most uropathogens seen in acute cystitis remain sensitive *in vitro* to nitrofurantoin (131).

Trimethoprim and Trimethoprim-Sulfamethoxazole

Trimethoprim usually is combined with sulfamethoxazole in a fixed 1:5 ratio. Although this combination demonstrates synergy against a wide variety of bacteria, it is not clear whether the sulfonamide component is necessary for the treatment or prevention of UTIs (133). Trimethoprim alone appears to be equally effective. However, the sulfonamide component does provide useful activity against *C. trachomatis,* which may have some value in the empiric outpatient treatment of dysuric women. Trimethoprim-sulfamethoxazole is highly effective against all the major uropathogens except the enterococci and pseudomonads. Most of the adverse effects associated with trimethoprim-sulfamethoxazole can be attributed to the sulfonamide component (134,135). Sensitivity reactions and gastrointestinal symptoms constitute nearly three-quarters of the reported adverse effects (135). Skin rashes occur in approximately 3.5% of patients (136), but serious reactions, including Lyell's syndrome and Stevens-Johnson syndrome, are rare. Nausea and, occasionally, vomiting occur in 3.5% of patients (137), but monilial overgrowth and diarrhea are rare. Hematologic toxicity, including leukopenia, thrombocytopenia, and hemolytic anemia, has been reported in about 0.5% of patients (135,137). Rare cases of agranulocytosis and aplastic anemia also have occurred (135). Surprisingly, megaloblastic anemia secondary to folate antagonism is unusual but may occur in patients who are already folate-deficient (138). Deterioration of renal function has been reported after trimethoprim-sulfamethoxazole administration (139), but this may be partly accounted for by the observation that trimethoprim specifically inhibits tubular secretion of creatinine and may cause a rise in serum creatinine without affecting the glomerular filtration rate (140). In addition, trimethoprim may interfere with the assay of creatinine (141). Occasional headaches, dysphoria, and other central nervous system side effects also have been reported, particularly after high doses (142). Trimethoprim should be avoided during early pregnancy because folate antagonism, theoretically, may be teratogenic, although there is no evidence of this in humans. Overall, despite an apparently long list of potential adverse effects, trimethoprim-sulfamethoxazole has been used extensively for the past 15 years with an excellent record of safety and effectiveness. Increasing antimicrobial resistance in *E. coli* strains causing UTIs (now 15% to 20%) is limiting its usefulness in many geographic areas (131).

Fluoroquinolones

Most of the recently developed fluoroquinolones are excellent agents for the treatment of UTIs. Ciprofloxacin and levofloxacin are the two drugs for which there is the most clinical experience and data treating UTIs, and there is little evidence that any of the more recently released drugs in this class offer advantages over these two (116,143). They are highly active against most of the major uropathogens, reach high concentrations in the urine, have little activity against the resident perineal and vaginal anaerobic flora, and are well absorbed and well tolerated when taken by mouth. Except for ciprofloxacin, *in vitro* activity against *P. aeruginosa* is limited. The fluoroquinolones have limited *in vitro* activity against enterococci. Clinical experience with these drugs indicates that they are highly effective for treatment of cystitis (116,118,146,147).

Duration of Therapy

Although older treatment regimens of 7 and 14 days for uncomplicated UTIs generally were very effective, they were also expensive and relatively toxic. Several lines of evidence suggest that shorter courses of treatment may be preferable. First, pathologic observations indicate that cystitis is a superficial mucosal infection in most cases (113). Second, patients often discontinue treatment themselves, when symptomatically improved after taking only a few doses of antibiotic, and are cured. Third, the bladder washout procedure, which involves a single instillation of antimicrobials into the bladder, has a cure rate of about 90% in patients with infection limited to the bladder (146). In addition, there is the analogy with the treatment of bacterial sexually transmitted diseases, in which single-dose therapy is commonly employed. Finally, after a single dose of

antimicrobials, therapeutic bactericidal concentrations may be present in the urine for 24 to 72 hours with some drugs.

Early clinical studies with single-dose treatment were encouraging with most cure rates being 75% to 100% (146–151). However, most of these studies were confined to infecting organisms that were sensitive to the antimicrobials and were not antibody-coated bacteria test positive (i.e., confined to the lower urinary tract). Many studies now have documented a higher failure rate of antibody-coated bacteria (ACB) positive infection, even with longer courses of treatment. In addition, follow-up in some of these single-dose treatment studies was 1 week or less (i.e., insufficient to exclude relapsing or recurring infection), and many of the early studies had small sample sizes and inadequate power.

Single-dose therapy has the advantages of simplicity, high compliance, reduced cost and side effects, and less adverse selective pressure on the perineal flora (152). It has the disadvantages of low efficacy against upper tract infection (pyelonephritis has been documented to follow single-dose therapy in one case) (114) and, commonly, failure to clear vaginal and periurethral coliform colonization, resulting in rapid reinfection. Trimethoprim-sulfamethoxazole is superior to the ampicillin-related drugs in the single-dose treatment of uncomplicated cystitis (111,112,114). Cure rates with trimethoprim-sulfamethoxazole have varied between 76% and 95% (114,148,149), whereas amoxicillin results have been generally lower, varying between 50% and 85%. Bacampicillin (28%) (153) and cyclacillin (30%) (114) were even less effective in similar studies. The most likely explanation for this discrepancy lies with the different pharmacokinetic properties of these drugs. Ampicillin is rapidly eliminated ($t^{1/2}ß = 30$ minutes), and although clearance is predominantly renal, urinary concentrations 6 hours after oral administration are almost undetectable (154). Fifty to 60% of trimethoprim and 25% to 50% of sulfamethoxazole are eliminated unchanged in the urine, and the resulting ratio in the urine is approximately 2:1 (155). The elimination rates of both drugs are considerably slower than that of ampicillin ($t^{1/2}ß$ for both is approximately 10 hours) (156). Consequently, urinary concentrations remain high for up to 24 hours. Bacteria in the urine, therefore, are exposed to lethal concentrations of antimicrobials for considerably longer than in the case of ampicillin or other rapidly eliminated ß-lactam antibiotics. The same is true for many fluoroquinolones, which have long half-lives and are effective as single-dose therapy (114,116).

The biologic effects of the drugs are also quite different. Ampicillin eliminates much of the microaerophilic and anaerobic vaginal flora. This encourages colonization by enteric Gram-negative organisms, whereas trimethoprim-sulfamethoxazole and fluoroquinolones have less effect on the normal vaginal flora, even when used for long periods. The high rates of recurrence with ampicillin may be related to persistent perineal colonization with coliforms and to the high frequency with which this drug selects resistant organisms in fecal flora.

Although trimethoprim-sulfamethoxazole has emerged as superior to ampicillin, it is not clear whether the sulfonamide moiety is at all necessary. Trimethoprim alone is highly effective for treatment of UTIs (157–159). Sulfonamide-sensitive, trimethoprim-resistant uropathogens are rare, and most of the serious toxicity associated with the combined preparations can be ascribed to the sulfonamide component. It has been argued that trimethoprim alone should replace the combination, but this is not universally accepted. Most large series reported have used the combined preparation.

Current evidence thus suggests that single-dose treatment of uncomplicated lower urinary tract infection in women is a satisfactory alternative to longer courses of antimicrobials. Specific regimens include trimethoprim-sulfamethoxazole (320 to 1,600 mg), trimethoprim (400 mg), ofloxacin (400 mg), ciprofloxacin (500 mg), amoxicillin (3 g), and nitrofurantoin (200 mg). However, more recent studies and meta-analyses suggest that 3 days of therapy with trimethoprim-sulfamethoxazole or a fluoroquinolone reduces the failure rate but preserves the advantages of single-dose therapy (i.e., low cost, low toxicity, good patient acceptability). Available data indicate that 3-day regimens of trimethoprim-sulfamethoxazole, norfloxacin, ofloxacin, and ciprofloxacin are highly effective in the treatment of acute cystitis (114,116). These regimens appear to be as effective as 7- to 10-day regimens but have fewer side effects (114–116), and they are more effective than single-dose therapy (111,112). Thus, 3-day regimens have become the treatment of choice for acute uncomplicated cystitis (116). Regimens of 7 to 10 days should be used in patients with complicating factors or in patients being treated with beta-lactams or nitrofurantoin.

Asymptomatic Bacteriuria

Children and pregnant women with asymptomatic bacteriuria ($>10^5$ organisms/mL) should be treated. One approach is outlined in Figure 33-2. Three days of therapy may be effective, but follow-up must be guaranteed to ensure clearance of bacteriuria. The evidence that treatment benefits other groups of patients is less convincing. In particular, asymptomatic bacteriuria in elderly patients is a common and apparently benign condition that probably does not require treatment. Patients found to have asymptomatic bacteriuria prior to urologic surgery should be given 3 to 7 days of antimicrobial prior to surgery (109).

Acute Dysuria

In the treatment of women presenting with acute dysuria and frequency, a careful history and microscopic examination of the urine occupy the central part of the decision-making tree and usually allow definitive treatment to be started immediately.

Figure 33-3 shows one practical approach to diagnosis and treatment. It may, however, be more cost effective to empirically treat women who have symptoms of a UTI, or who have symptoms and pyuria on urinalysis, with 3 days of an antimicrobial agent, using response to therapy rather than positive urine culture as the means of diagnosis (160). This approach costs the patient less and results in fewer symptom days. Any features in the history or examination (i.e., fevers, flank pain, costovertebral tenderness) that suggest upper urinary tract infection warrant an extended course of treatment (Chapter 37). In addition, short-course therapy is only appropriate for uncomplicated infections in women and children. It is not recommended for patients with abnormalities of the urinary tract or for the treatment of males.

Acute Pyelopnephritis

Acute pyelonephritis can be divided into cases that are considered uncomplicated and cases considered complicated. The former occur in young women with no evidence of anatomic or structural abnormalities. The latter are all other cases. Recent data suggest that most uncomplicated cases can be successfully treated with oral antimicrobials in the outpatient setting as long as they have no vomiting and have normal blood pressure, pulse and only mild to moderate pain. A regimen of 7 to 14 days of a flouroquinolone can be used as empiric therapy. One initial dose of parenteral medication followed by oral therapy is an alternative approach. Patients with moderate to severe uncomplicated pyelonephritis or complicated disease should be hospitalized for initial parenteral therapy. They can usually

FIGURE 33-2. Algorithm for management of asymptomatic bacteriuria.

be switched to oral therapy in 2 to 3 days when fever resolves (Table 33-1).

Relapsing Infection

Relapses usually are caused by unsuspected persistent upper tract infections (females), prostatitis (males), or structural abnormalities, such as stones or diverticula. If a patient relapses after 1 to 7 days of treatment, then a 14-day course of treatment should be prescribed. Before therapy, sensitivity to the prescribed antimicrobial agent should be confirmed. Trimethoprim-sulfamethoxazole or a fluoroquinolone probably is the most effective therapy. If there is another relapse, further investigations are indicated to identify potentially remediable structural lesions. Meanwhile, a 6-week course of treatment should be initiated, because this may be effective when 2-week courses have failed (155).

Recurrent Infection

Many women have periodic episodes of cystitis, but a small minority has attacks with such disabling frequency that antimicrobial prophylaxis is justified. This group of patients may be divided into those with structural or functional abnormalities of the urinary tract (and an associated tendency to develop pyelonephritis) and those with a normal intravenous pyelogram and cystoscopy and infections confined to the lower urinary tract. The vast majority of women fall into the latter category. Recurrent infections can be related to sexual intercourse (15) or diaphragm use (161) in a proportion of women, but no predisposing factors are apparent in the majority. Recurrent infections tend to cluster in time (162); that is, the likelihood of a subsequent infection is greatest at the end of the treatment course and then diminishes progressively the longer the woman is infection free.

Simple measures, such as voiding immediately after sexual intercourse and changing from the diaphragm to an alternative form of contraception, may be effective in women in whom these factors are related to recurrent infection. Three antimicrobial strategies can be employed: (a) continuous low-dose prophylaxis (163,164), (b) self-administered short-course treatment (165), and (c) postcoital single-dose prophylaxis (Table 33-2) (166).

Trimethoprim, with or without sulfamethoxazole, and nitrofurantoin have been evaluated extensively and are highly effective (164). Most experience has been gained with trimethoprim-sulfamethoxazole; it is well tolerated, with an excellent therapeutic index when used for a long term (163–165,167). Side effects are rare and usually develop in the first few weeks of therapy. Emergence of resistant strains is unusual with these drugs, and their efficacy does not appear to diminish with extended use. Excellent results have also been obtained with norfloxacin, ciprofloxacin, and levofloxin. Nitrofurantoin and oral cephalosporins have been used for long-term prophylaxis, but experience is more limited and, in general, results have not been as good.

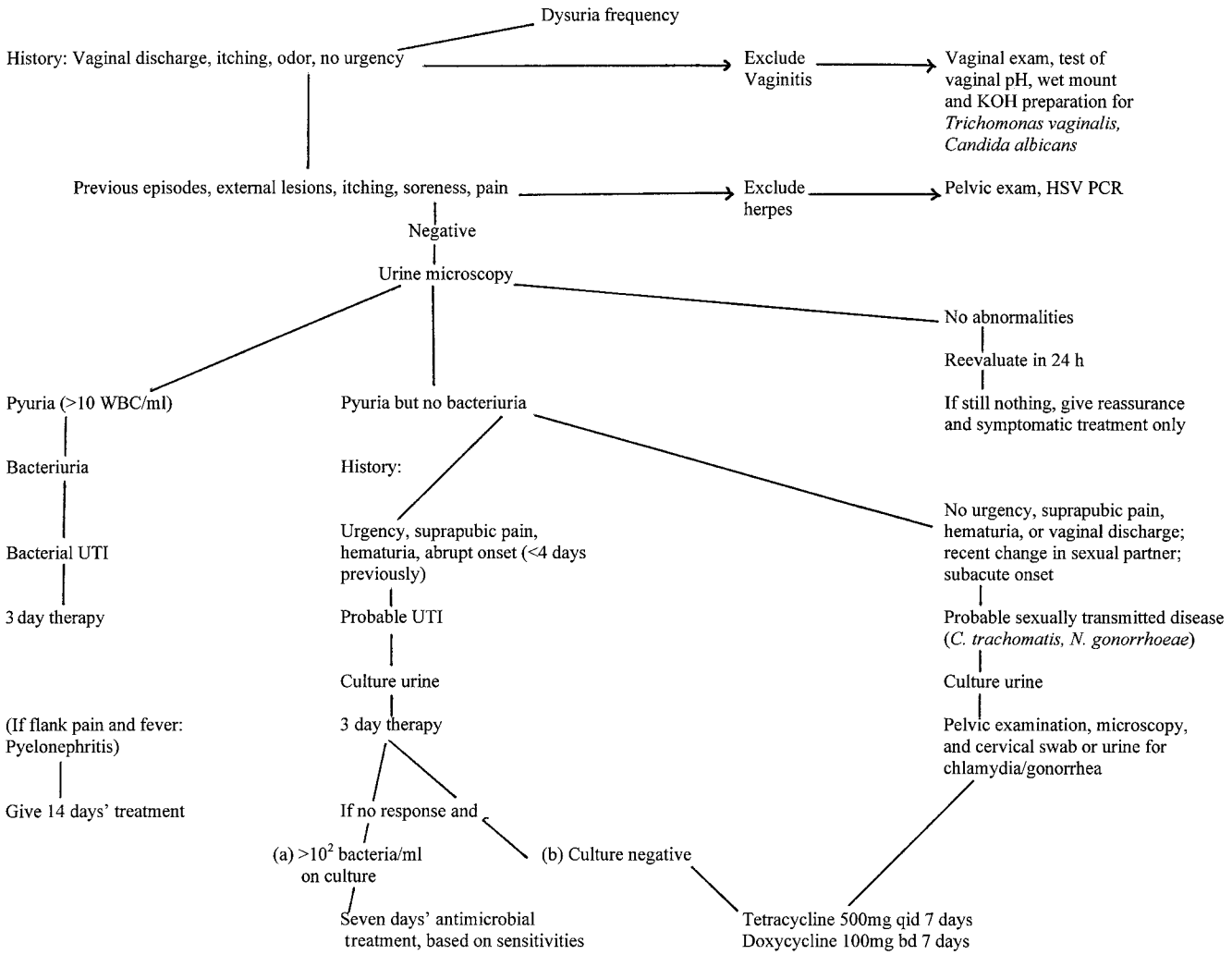

FIGURE 33-3. Management of dysuria and frequency in women.

The choice of management strategy depends on the factors predisposing to recurrent infection (if any), the numbers of infections per year, and the preference of the patient. In general, continuous prophylaxis is preferred in women who experience three or more infections per year. Patient-administered single-dose therapy should be reserved for women with two to three infections per year, and postintercourse prophylaxis should be used for women who temporally relate infection to sexual activity. The costs of prophylaxis and patient-administered single-dose therapy with trimethoprim-sulfamethoxazole are approximately the same (165). Obviously, the infection rate is higher with self-treatment, but patient acceptability also may be higher. There are no clear guidelines concerning when to stop prophylaxis; often, 6 months of drug treatment is given. Most women return to their preprophylaxis rate of infection when the drug is withdrawn. During prophylaxis, patients should initially be seen twice yearly to evaluate efficacy and toxicity and to check urine cultures. If significant bacteriuria (10^5/mL) or symptomatic infection develops during prophylaxis, an antibiotic other than the prophylactic agent should be selected initially. Therapy should then be modified, dependent on antimicrobial susceptibility.

TABLE 33-2

REGIMENS FOR MANAGEMENT OF RECURRENT CYSTITIS

Daily: Trimethoprim 40 mg-sulfamethoxazole 200 mg or nitrofurantoin 100 mg or trimethoprim 100 mg
Self-treatment: Trimethoprim 320 mg-sulfamethoxazole 1600 mg at first symptoms of infection
Postcoital: Trimethoprim 40 mg-sulfamethoxazole 200 mg or nitrofurantoin 100 mg or trimethoprim 100 mg

Pregnancy

Asymptomatic bacteriuria occurs in 4% to 7% of pregnant patients (108,168). The prevalence of bacteriuria is directly proportional to age and parity (169) and inversely proportional to socioeconomic status (170). Asymptomatic bacteriuria in early pregnancy is a major risk factor for the development of acute pyelonephritis in the second and third trimesters (110,170). Thus, patients presenting with bacteriuria at their first antenatal clinical visit should be treated. Approximately one-third of untreated patients with bacteriuria develop upper tract infections at a later stage (usually in the third trimester). This contrasts with a rate of less than 1% in patients without bacteriuria in early pregnancy (108). The incidence of pyelonephritis

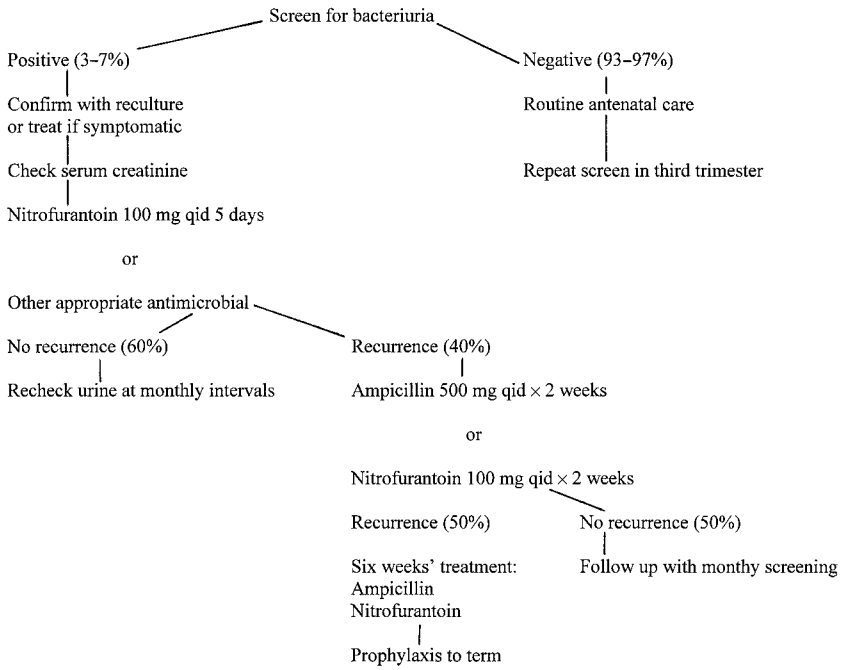

FIGURE 33-4. Management of bacteriuria in pregnancy.

in women with bacteriuria can be reduced to less than 5% by appropriate therapy. Thus, over 75% of acute pyelonephritis in pregnant patients can be prevented by screening for and eliminating asymptomatic bacteriuria in early pregnancy. Prevention of pyelonephritis in pregnancy also prevents associated fetal morbidity (primarily prematurity).

Several physiologic factors probably account for this predisposition to infection in pregnancy. Estrogens and progesterone induce dilatation of the ureter and renal pelvis, which increases progressively toward term (171). Bladder capacity almost doubles in late pregnancy with distortion of shape by the gravid uterus. These changes revert to normal by the second month postpartum. Bacteriuria is a frequent finding in postpartum women with asymptomatic bacteriuria in early pregnancy, even if this has been treated appropriately (172,173). Treatment of bacteriuria in pregnancy does not appear to influence the long-term course in these women.

Women should be screened for bacteriuria at their first antenatal clinic visit and treatment should be given if significant bacteriuria is found. Sulfonamides, nitrofurantoin, ampicillin, cephalexin, and nalidixic acid all are considered relatively safe in early pregnancy. Sulfonamides should not be used near term, because there is the theoretic risk that kernicterus may be induced in the newborn. Trimethoprim, a dihydrofolate reductase inhibitor, is not generally recommended in pregnancy, because there is some evidence of fetal toxicity with very high doses in animals. However, there is no evidence of teratogenicity or other adverse effects in human pregnancy. Tetracyclines are also contraindicated in pregnancy; fetal toxicity secondary to calcium chelation may occur after the fifth month, and acute fatty liver may develop in the mother at any stage. Nitrofurantoin is well tolerated, and many studies attest to its safety in pregnancy (174–176). It may induce anemia in both mother and fetus with G_6PD deficiency (177) and has also been shown to produce coagulation abnormalities, but these have not been associated with adverse effects (178). The fluoroquinolones should not be used during pregnancy.

Single-dose therapy has not been evaluated widely in pregnant patients; single-dose ampicillin (2 g) plus probenecid, nitrofurantoin (200 mg), or sulfisoxazole (2 g) were effective in approximately 75% of patients in one study (179). Given the greater effectiveness of 7-day therapy in nonpregnant women, a 7-day course of nitrofurantoin would seem to be a reasonable treatment of choice for asymptomatic bacteriuria or uncomplicated lower UTIs if careful follow-up can be achieved (Fig. 33-4). These patients should be followed up 2 weeks after completing therapy and then at monthly intervals. The aim of management is to detect and treat asymptomatic bacteriuria as promptly as possible and therefore to prevent the development of acute pyelonephritis.

Children

Urinary tract infections in newborn infants may be nosocomially acquired; babies nursed in neonatal units are at a threefold increased risk as compared with babies nursed at home (180). After the neonatal period, UTIs continue to be an important cause of fever, and they are a potential cause of progressive renal damage in children with vesicoureteric reflux (73,103,181). Early detection and treatment of recurrent infections have been shown to reduce renal scarring (182). Symptomatic infections in young children may occur with fever, abdominal pain, convulsions, or other nonspecific signs and symptoms. In older preschool children, symptoms are usually referable to the urinary tract (i.e., frequency, dysuria, hematuria, urgency, fever, and often a return of enuresis). Asymptomatic infections are common in young girls and also represent a threat to the developing kidneys, because many girls with asymptomatic bacteriuria have vesicoureteric reflux and renal cortical scars that are indicative of reflux nephropathy. Overall, renal scarring is found in 12% to 20% of children evaluated for infections of the urinary tract (71). Because this is a major cause of hypertension and end-stage renal failure (183), all infections in childhood should be treated promptly. Although much of the renal damage occurs within the first 5 years of life, recent evidence suggests that children over 5 years old are still vulnerable (182).

As soon as a UTI is suspected, urine should be obtained for culture and empiric treatment started before waiting for bacteriologic confirmation of the diagnosis. As in adults, pyuria is a valuable indicator of a UTI and the most likely infecting organism is *E. coli*. The initial antimicrobial agent of choice is probably trimethoprim-sulfamethoxazole or trimethoprim

alone. The initial duration of treatment should be 7 to 10 days. Single-dose or short-course treatment is effective in uncomplicated lower tract infections, but upper tract involvement should be assumed in febrile children in order to err on the side of safety. The traditional teaching that girls are "allowed" one urinary tract infection but boys are not allowed any before initiating investigations is still probably valid, although an ultrasound examination of the bladder and kidneys is a reasonable first step in any child admitted to a hospital with a documented UTI. Girls, at their second infection, all boys, and children with large bladders, abnormal kidneys, hypertension, or azotemia should be evaluated. This should include routine blood biochemistry, ultrasound of the urinary tract, and a plain abdominal radiograph before proceeding to intravenous urography, micturating cystourethrography, and, in some cases, cystoscopy in consultation with a pediatric urologist.

Recurrent infections in children with vesicoureteric reflux can be prevented by antimicrobial prophylaxis (183,184). This treatment appears to prevent fresh scarring, whereas 20% of children with reflux who receive intermittent short courses of treatment for recurrent infections develop new scars (185). This finding supports the initiation of prophylaxis immediately following the course of treatment until the underlying cause of the infection has been determined.

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CHAPTER 34 ■ INFECTIONS OF THE UPPER URINARY TRACT

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In the normal urinary tract, ascending upper tract infection is prevented or delayed by the dynamics of urine flow and the interference of the vesicoureteral junction. The microbial etiology of urinary tract infections is similar throughout the urinary system, but the infection site determines the clinical features, response to therapy, complications, and ultimate prognosis; therefore, it is appropriate to identify upper tract infection in this chapter as a unique and significant illness. Parasitic, fungal, and mycobacterial infections of the upper tract are discussed in other chapters. Viruses are commonly excreted in the urine, but with the exception of the syndrome of hemorrhagic fever with renal insufficiency, their role in renal disease is ill defined; therefore, this chapter focuses primarily on bacterial infections. Infection of the upper urinary tract is not readily diagnosed in the absence of the classic clinical features of acute pyelonephritis. As a result, much of the information available and compiled for this review is derived from studies in which the diagnosis of renal infection was imprecise. Complicated urinary infections are reviewed in Chapter 36. Uncomplicated upper tract infection in adults is the principle focus of this review.

DEFINITIONS

Acute Pyelonephritis

Acute pyelonephritis is a clinical syndrome of flank pain, renal tenderness, fever, and chills, accompanied by bacteriuria. It may be associated with bacteremia, which can progress to the systemic inflammatory response syndrome, "septic shock," and death. Acute nonobstructive pyelonephritis, also called acute uncomplicated pyelonephritis, occurs in women with a normal genitourinary tract (1). Pyelonephritis also occurs as one of the more severe symptomatic presentations of complicated urinary infection in persons with structural or functional abnormalities of the genitourinary tract.

Chronic Pyelonephritis

Chronic pyelonephritis is a radiologic diagnosis characterized by renal scarring and destructive changes in the calyceal system, presumed to be caused by bacterial infection, vesicoureteral reflux, or both. Although the classic histologic features of chronic pyelonephritis have included interstitial inflammation and fibrosis, this description is now recognized to lack specificity for bacterial infection and is common to a variety of pathologic processes (2).

Bacteriuria

Bacteriuria is the presence of bacteria in the urine. Kass introduced the term significant bacteriuria to differentiate contaminated from infected urine by using quantitative urinary bacterial counts (3). Significant bacteriuria is the presence of at least 10^5 colony-forming units (CFU)/mL or at least 10^8 CFU/L (International System of Units). Patients with symptomatic urinary infection may demonstrate lower bacterial counts in voided urine.

Asymptomatic Bacteriuria

Asymptomatic bacteriuria is bacteriuria in the absence of any subjective symptoms of urinary infection. Presumably, if pyuria or a serologic response to the infecting organism occurs, the patient is infected and not simply colonized. In females, two voided urine samples with at least 10^5 CFU/mL of the same organism are required to diagnose asymptomatic bacteriuria (3). In men, only one urine culture is necessary (4). A single specimen obtained by catheterization or suprapubic aspiration in men or women is also sufficient.

Pyuria

Pyuria is the presence of more than 5 polymorphonuclear leukocytes/high-power field (HPF) on microscopic examination of spun urine sediment or the presence of more than 10 polymorphonuclear leukocytes/μL of unspun urine (5).

HISTORICAL PERSPECTIVES

Asscher credited the ninth-century Arabic physician Rhazes for the initial diagnosis of acute pyelonephritis in a patient in Baghdad (6). The patient had an acute febrile illness complicated by necrotizing papillitis. In 1841, Rayer, in his textbook of kidney disease, noted the frequent occurrence of pyelonephritis during pregnancy (7); however, it was not until 1881 that Roberts noted bacteria in the urine of patients with urinary symptoms (8). A year later, Wagner associated the focal histopathologic changes of chronic pyelonephritis with a history of recurrent urinary infection (9). He also cited the "Schrumpfniere" or "the shrunken kidney" as the end product of urinary infection. In 1894, Escherich identified *"Bacillus coli"* in the urine of children with urinary infections (10). Lohlein, in 1917, described the occurrence of urinary infection in three girls who had progressive renal impairment and bilateral contracted kidneys (11); all three died with uremia.

Several investigators followed groups of patients with recurrent infection prior to the antimicrobial era. Longcope and Winkenwerder, in 1933, identified hypertension and atrophic pyelonephritis as important complications of longstanding recurrent renal infection (12). Weiss and Parker, in their 1939 classic monograph, described the histologic lesions in patients with chronic pyelonephritis and noted in particular the

vascular changes in the renal arterioles (13). They postulated that chronic pyelonephritis frequently led to hypertension and end-stage renal failure. In 1937, Crabtree and colleagues followed 45 patients with acute pyelonephritis of pregnancy (14,15). During the subsequent 5 to 10 years, hypertension developed in six women and three progressed to end-stage renal disease. Marple, in 1941, recognized that bacteria were commonly present in the urinary tract in large numbers without associated symptoms (16). Kass, in 1956 showed the significance of quantitative assessment of bacteriuria and identified the importance of asymptomatic bacteriuria during pregnancy (3). The subsequent availability of antimicrobial therapy has substantially modified the natural history and complications of renal infection.

PATHOGENESIS

Bacteriology

Escherichia coli is responsible for 75% or more of community-acquired renal infections (Table 34-1). Organism characteristics associated with renal invasion belong to a restricted number of O:K:H serotypes, and are characterized by the production of hemolysin, production of aerobactin, resistance to the bactericidal activity of normal human serum, and the presence of specific adhesion proteins that bind to receptors on epithelial cells (22). Strains of *E. coli* isolated from patients with nonobstructive pyelonephritis consistently contain genes for hemolysin production and one particular adhesion protein, P fimbriae (23). These and other virulence genes are often clustered in the genome in pathogenicity islands (24). The P fimbrae family is one of the mannose resistant adhesins with a binding specificity for the gal (α1–4) gal-β disaccharide galabiose. The pap G class II allele is identified in approximately 95% of *E. coli* identified in individuals with acute nonobstructive pyelonephritis but only 70% to 75% of those with complicated urinary infection (25). *E. coli* strains isolated from acute nonobstructive pyelonephritis are from a restricted group of virulent *E. coli,* whereas those isolated from complicated urinary infection or asymptomatic bacteriuria are highly heterogeneous with a significantly lower frequency of virulence characteristics (25,26). One clonal group of *E. coli*, which was also multidrug resistant, has been described in outbreaks occurring in both Europe and the United States (27). Prats and colleagues described a uropathogenic clone (*E. coli* 015:K52:H1) during a 1-year period in Barcelona, Spain, and showed that this strain is overrepresented in patients with acute pyelonephritis (28).

Other Gram-negative rods, including *Klebsiella* spp., *Proteus mirabilis,* and *Enterobacter* species, are isolated in 10% to 15% of patients with community-acquired renal infections. *Proteus mirabilis* accounts for more than 40% of infections in infant boys (29). *Proteus mirabilis* is particularly significant as a renal pathogen because of its propensity to promote struvite calculi. Coagulase-negative staphylococci and *Enterococcus faecalis* each cause 2% to 3% of invasive renal infections. The latter is a more important pathogen in elderly men. Group B streptococci are isolated in less than 1% of urinary infections, but appear to have a propensity for patients with diabetes and pregnant women (30). Group A streptococci, *Streptococcus pneumoniae,* and *Neisseria* spp. are rare upper tract pathogens. *Haemophilus influenzae* has been implicated occasionally in childhood urinary infections, including acute bacteremic pyelonephritis. *Staphylococcus aureus* renal infection and bacteriuria commonly occur in patients with bacteremic staphylococcal infection (31). Although *Staphylococcus saprophyticus* is an important cause of acute cystitis in females, its role in invasive upper tract infection is uncertain (32). *Gardnerella vaginalis* may be isolated from women with bacteriuria, particularly during pregnancy (33). Both *Mycoplasma hominis* and *Ureaplasma urealyticum* have, infrequently, been isolated as the sole pathogen in patients with classic acute pyelonephritis, and increases in specific antibody titers to these agents support a role for infection (34).

The etiology of pyelonephritis in patients with complicated urinary infection, including catheter associated hospital-acquired urinary infection is substantially different (Table 34-1). *Escherichia coli,* usually arising from the patient's own gastrointestinal tract, remains the most common urinary pathogen, but other species are more often isolated, and resistant bacteria are more common. Gram-negative rod infections, including *Pseudomonas aeruginosa* and *Serratia marcescens,* account for 10% to 15% of hospital-acquired invasive upper

TABLE 34-1

DISTRIBUTIONS OF BACTERIAL SPECIES ISOLATED IN PYELONEPHRITIS AND BACTEREMIC URINARY INFECTION IN SELECTED REPORTS

	Pyelonephritis		Bacteremic infection		
Organism	Safrin et al. (17)	Pinson et al. (18)	Jerkeman and Braconier (19)[a]	Bryan and Reynolds (20)[a]	Bryan and Reynolds (21)[b]
Escherichia coli	140	63	52	201	100
Klebsiella spp.	5	5	7	—	—
Citrobacter spp.	—	—	2	29	41
Enterobacter spp.	—	3	—	—	—
Proteus mirabilis	2	2	9	37	28
Pseudomonas spp.	—	4	5	8	21
Staphylococcus aureus	—	1	1	—	—
Staphylococcus saprophyticus	8	4	—	17[c]	19[c]
Enterococcus faecalis	1	—	8	—	—
Other	—	6	—	4	12

[a]Community acquired, including both complicated and uncomplicated infection.
[b]Hospital acquired.
[c]All Gram-positive organisms.

tract infections in some reports, and may occur in outbreaks (35,36). *Staphylococcus epidermidis,* often resistant to the antibacterial agents usually prescribed for urinary infection, occurs frequently as an acquired cause of bacteriuria in patients with indwelling catheters but is seldom a cause of symptomatic infection (37). In one study, of 175 pathogens cultured from the initial urinary infections in 110 patients following catheterization, coagulase-negative staphylococci were isolated from 31% of infections; *Candida* spp. from 32%; *P. aeruginosa* and *Klebsiella* spp. each from 17%; and *E. coli* from 16% (38). The spectrum of infecting organisms will be influenced by antimicrobial exposure of the patient. *Corynebacterium* group D2 has been identified as a unique etiologic agent of nosocomial urinary infection, particularly in catheterized patients (39). These organisms are urease producers and may produce persistent infection, bladder and renal calculi, pyelonephritis, and bacteremia. They are slow growing and may be missed if routine cultures are discarded after 24 hours.

Some organisms that are relatively uncommon in North America can also cause urinary infection. Leptospiral infection usually involves the kidneys, with pathologic changes of interstitial inflammation, hemorrhage, and tubuloepithelial damage (40). The pathogenesis of these lesions and the specific role of the leptospiral spirochete are uncertain. Although renal insufficiency is common, localized renal symptoms are unusual. Leptospirosis has not been reported to cause chronic renal impairment. Culture of urine on special leptospiral media is usually positive during the acute illness (40). *Brucella* spp. have been associated with bacterial pyelonephritis (41). In 1905, Kennedy reported that 54% of patients with *Brucella melitensis* infection had positive urine cultures (42). The clinical features vary from those of acute pyelonephritis to a chronic debilitating illness. Chronic infection results in a granulomatous response, which may progress to a destructive cavitating process indistinguishable from renal tuberculosis (42,43). *Salmonella* spp. are common renal pathogens in patients with urinary schistosomiasis and may cause acute pyelonephritis in patients with *Salmonella* gastroenteritis (44).

Organisms that are unable to use urine as a nutrient source only rarely cause pyelonephritis. These organisms include most species of obligate anaerobes. The relatively high oxygen tensions in normal urine also likely inhibit anaerobic organisms. In a prospective study of 5,781 specimens, Segura et al. identified only 10 patients with anaerobic bacteriuria among those with positive urine Gram stains and negative aerobic cultures, an overall prevalence of 1.2% (45). All but one of these 10 patients had complicated urologic problems.

Protoplasts, or bacterial L forms with cell walls, have been postulated to have a significant role in pyelonephritis, enabling organisms to persist and cause disease in the hypertonic environment of the renal medulla (46). Studies are inconclusive with respect to the significance of these in urinary infection, although occasionally they are isolated in urine.

Host Factors and Host Response

Host factors and response are described in detail in another chapter (Chapter 32). Genetic factors have a role in development of acute, unobstructed pyelonephritis. The prevalence of urinary infection is increased twofold to sixfold in the female siblings and mothers of girls with urinary infections (47). The P1 blood group is associated with recurrent pyelonephritis in girls without vesicoureteral reflux (48). Epithelial cell receptors necessary for *E. coli* binding are glycolipids of the globoseries, the antigens of the P blood group system. This observation may provide a genetic basis for the increased susceptibility of some females for urinary infection. Women who are nonsecretors of blood group substance also have an increased risk of recurrent urinary infection (49).

Individuals with structural or functional abnormalities of the urinary tract have a greatly increased risk of pyelonephritis, apparently independent of genetic factors or bacterial virulence factors. Obstruction at the level of the kidney or ureter may directly inhibit urine flow from the upper tract and bacteria will establish infection behind the obstruction. Voiding abnormalities, as with neurogenic bladders, are frequently associated with reflux and upper tract infection.

Acute pyelonephritis is characterized by an intense and extensive inflammatory and immune response. Pyuria is present, and urinary and serum cytokines and chemokines are elevated (50–52). The extent of the inflammatory response appears to correlate with the severity of the clinical presentation. The specific virulence factor, the P fimbria, appears to be important in stimulating an epithelial cytokine response (52). The inflammatory response resolves promptly with the institution of effective antimicrobial therapy (53). Upper tract infection is associated with a greater inflammatory response than lower tract infection, and cytokines are increased in symptomatic infection as compared to asymptomatic infection. Upper tract infection is associated with both a local and a systemic humoral immune response. Local urine antibody includes both an IgA and an IgG response to the infecting bacteria (54). This specific antibody response develops about 7 to 10 days from infection onset. The protective effect of these antibodies remains controversial (54).

PATHOLOGY

Acute Pyelonephritis

The histologic hallmarks of acute pyelonephritis include abscess formation and edema in the renal parenchyma and accumulation of polymorphonuclear leukocytes in and around tubules. Bacteria are often demonstrable in foci of acute renal suppuration. In general, glomeruli are spared, although small abscesses may surround them. Areas of infection are characteristically wedge-shaped with the apex in the medulla resembling an infarct. Tissue destruction is greater in the cortex than in the medulla as a result of this configuration, but the relative smaller size of the medulla means the inflammatory response appears to have a greater effect on medullary anatomy and function. The distribution of wedge-shaped areas of suppuration is characteristically focal, usually corresponding to renal lobes, and sharply demarcated from areas of uninvolved renal parenchyma. In adults, the kidney is unlikely to be uniformly affected, unless concomitant obstruction is present. Histologic features of acute and chronic pyelonephritis can overlap. The relative degrees of edema and fibrosis rather than the interstitial cellular response are the most useful criteria to delineate these entities.

Circulatory compromise, perhaps because of toxins or increased intrarenal pressure, is present in the early stages of acute pyelonephritis. In animal models, short periods of vascular occlusion result in changes that closely resemble those of pyelonephritis (2). Vascular spasm, therefore, may be a significant factor in the pathogenesis of the pyelonephritic scar. Many systemic infections cause an acute interstitial nephritis with edema and a predominant infiltration of mononuclear cells. *Salmonella typhosa, Corynebacterium* diphtheriae, *Mycoplasma* pneumoniae, *Leptospira* spp., and other organisms have been associated with an acute diffuse interstitial renal response without direct bacterial invasion (2). The pathogenesis of this response is not well understood.

Chronic Pyelonephritis

Chronic pyelonephritis is a focal parenchymal disease with associated changes in the renal collecting system owing to inflammation and deformation (55). Fibrosis with atrophy of overlying renal tissue leads to surface depression or scars. Neighboring unaffected renal tissue often undergoes hypertrophy and may appear to be a mass lesion. A sharply defined border between normal and diseased tissue is characteristic of chronic pyelonephritis. The capsule is adherent and the cortical surface irregular. Calyceal clubbing results from papillary retraction into the scar. Dilatation, muscular hypertrophy, and fibrotic inflammation causing thickening of the calyceal system, all occur to a variable extent. The two kidneys are usually markedly asymmetrically involved, whereas other diseases that cause interstitial inflammation usually affect both kidneys equally.

The histology of chronic pyelonephritis is the pathology of interstitial or tubulointerstitial nephritis and is characterized by a pleomorphic infiltrate of lymphocytes, plasma cells, and macrophages in the interstitium of the kidney (2). Polymorphonuclear leukocytes and eosinophils may also be present. Recent and longstanding interstitial renal inflammation can appear similar. However, with longstanding disease, tubular atrophy and fibrosis as well as foci of tubular dilatation and colloid cast formation become evident. These blind, obstructed tubules can superficially resemble thyroid acini. Zones of normal parenchyma usually separate scars. Arteries and arterioles within the scars show medial hyaline change and intimal proliferation. The vessels in zones of normal kidney may be normal or may demonstrate hyaline intimal changes (55).

The status of the glomerulus differentiates primary tubular interstitial disorders from glomerulopathies. Although glomeruli in tubular interstitial disorders may be partially or completely hyalinized, glomeruli in severely scarred zones may be relatively normal, or may show compensatory glomerular hypertrophy.

Previously, many end-stage kidneys were referred to pathologically by the term *chronic pyelonephritis*. Freedman reviewed the body of clinical and experimental data that documents the difficulty of attributing chronic interstitial renal inflammation, in the end-stage kidney, to infection (55). Four points were noted. First, histologic findings of chronic interstitial renal inflammation occur in many clinical entities that are not associated with bacterial infection. Second, interstitial changes in the kidney can be produced experimentally by methods that do not employ bacterial infection. Third, the equal sex distribution of histopathologic pyelonephritis does not correspond to the known predominance of urinary tract infections in females (56). Fourth, bacteriuria is a very common nosocomial complication of hospitalization and occurs with increased incidence in patients with a variety of underlying renal and urologic diseases. Thus, the contribution of urinary infection to the pathologic features found at necropsy has been overemphasized. In most instances, bacteriuria has played no role in the development of the underlying renal disease.

PREVALENCE AND INCIDENCE

Symptomatic Pyelonephritis

Bacteriuria occurs in 0.7% of full-term infants, and clinically evident urinary infection occurs in 0.3% (57,58). Males predominate in both groups, and account for almost 80% of neonatal urinary infections. Most of these infections appear to involve the upper tracts. Tullus and colleagues retrospectively reviewed the incidence of pyelonephritis among 11,655 children born at a Stockholm hospital between January 1, 1979, and June 30, 1982 (59). The annual incidence of pyelonephritis during the first 2 years of life in these children was 34 per 10,000, but 89/10,000 in infants cared for in one neonatal unit. An epidemic of a virulent, multiple resistant *E. coli* strain, serotype 06:K5, occurred in this unit (59). Acute pyelonephritis occurred after a mean of 5.6 months following discharge, with a range of 1 week to 17 months; only one infant had an underlying malformation of the urinary tract.

It is estimated at least 250,000 episodes of acute pyelonephritis with over 160,000 hospitalizations occur each year in adult women in the United States (60). The highest rates of acute nonobstructive pyelonephritis are in young women (Table 34-2). Ikaheimo and associates reported an incidence of 2.7 episodes of pyelonephritis/100 patient years during 1-year follow-up of women in a family practice in Finland who had originally presented with acute cystitis (61). The ratio of episodes of cystitis to pyelonephritis was 29:1. Stamm and associates, in the United States, followed 51 women with recurrent uncomplicated urinary infection for a median of 9 years (62).

TABLE 34-2

REPORTED INCIDENCE OF PYELONEPHRITIS IN WELL CHARACTERIZED POPULATIONS

Study design (reference)	Population	Rate
Retrospective (59), Sweden	children <2 years	34/10,000 population
Prospective, 12 months, Finland (61)	179 women initially presenting to GP with acute cystitis	2.7/100 patient years
Retrospective (62), United States 1969–1985	51 women with recurrent cystitis	0.1 ± 0.3/patient year
Administrative data (63), Canada, hospitalized	women—all	10.9/10,000 population
	women >60 years	175/10,000 population
	men	3.3/10,000 population
Administrative data (60) United States, hospitalized	women <60 years	7.8–15.0/10,00 population
	women >60 years	13.5–23.3/10,000 population
	men <60 years	1.1–2.4/10,000 population
	men >60 years	6.3–12.9/10,000 population
Prospective (64), Canada	diabetic women with bacteriuria	7.2/100 patient years

The mean infection rate was 2.6 per patient year with the ratio of cystitis to pyelonephritis episodes being 18:1 in women not receiving prophylactic antibiotics.

Hospitalization for treatment of acute pyelonephritis reported from one province in Canada was 11/10,000 for all women (63). Pyelonephritis accounted for 0.4% of all hospital admissions. The frequency of hospitalization underestimates the incidence of pyelonephritis because many patients with pyelonephritis, especially young healthy women, are not admitted for treatment. Peak rates occurred in women 20 to 29 years old, and men and women older than 50 years. From 0.3% to 0.7% of all pregnancies required hospitalization for pyelonephritis. Women with diabetes were six to 24 times more likely to be admitted with pyelonephritis than nondiabetic women, stratified by age. An American study reported similar hospitalization rates of 11.7/10,000 for women (60) (Table 34-2). Rates were not increased in patients with diabetes. They were highest for younger women at 15/10,000 (age 20 to 39 years) and in women over 80 years (23.3/10,000).

Pyelonephritis in men is usually in the context of complicated urinary infection, but acute nonobstructive pyelonephritis does occur. Krieger and colleagues reported an incidence of symptomatic urinary infection including both lower and upper tract infection, of 4.9/10,000 men per year in a 6-year study of male university students (65). Rates of hospitalization for acute pyelonephritis in men were reported to be 3.3/10,000 per year in Manitoba (63) and 2.4/10,000 in the United States (60). In other groups with complicated urinary infection, Waites and associates reported 1.8 episodes/person year of urinary infection associated with fever and chills, presumably upper tract infections, in 64 spinal cord-injured patients managed with intermittent catheterization or condom drainage (66). Fever occurred with 11% of all episodes of urinary infection identified by monthly screening. In a prospective study of episodes of febrile urinary infection in residents of long-term care facilities, a rate of febrile urinary infection of 1.1/10,000 resident days was reported (67). The rate was 0.8/10,000 resident days for individuals without chronic indwelling catheters, and 4.6/10,000 for those with chronic indwelling catheters. The high rates in elderly populations reflect underlying functional and structural abnormalities, consistent with complicated urinary infection.

Asymptomatic Upper Tract Infection

Asymptomatic bacteriuria is common in many populations (68). Although no epidemiologic studies have prospectively localized the site of infection, some individuals with asymptomatic bacteriuria do have upper tract involvement. Presumably, factors such as duration of bacteriuria, infecting organism, associated medical illnesses, the presence of vesicoureteral reflux, and urinary obstruction influence the prevalence of upper tract infection in patients with asymptomatic bacteriuria.

Lincoln and Winberg found that eight of 298 male neonates had asymptomatic bacteriuria, whereas none of 286 female neonates were infected at birth (69). The reasons for male predominance are unknown. In cross-sectional and longitudinal studies, Kunin and associates determined the prevalence and incidence of urinary infection in both schoolgirls and adult women (70–72). The prevalence was 1.2% among 16,000 girls and the annual incidence of infections was 0.4% in previously nonbacteriuric girls (70). About 5% of girls had one or more episodes of bacteriuria between the ages of 6 and 18 years. Two-thirds of these infections were asymptomatic. Girls found to be bacteriuric at the initial examination and those who acquired bacteriuria during the study did not differ from their nonbacteriuric cohorts with regard to personal hygiene, socioeconomic status, or race.

Large populations of women have been studied in different settings. The prevalence of bacteriuria is strikingly similar in Wales, Jamaica, Japan, and Virginia (United States) (72,73). Between 3% and 7% of women surveyed from the ages of 16 to 65 years have asymptomatic bacteriuria, compared with less than 0.1% of men. In 1,758 Dutch women, the prevalence of bacteriuria increased from 2.7% of women aged 15 to 24 years to 9.3% of women aged 65 years or older (74). Kunin and McCormack studied the prevalence of bacteriuria in 3,304 nuns and 2,698 control subjects matched for age and race (72). The prevalence of bacteriuria in nuns below the age of 34 was one-twelfth that of control subjects, and similar for white and black nuns. The frequency of bacteriuria in the nuns gradually increased so that by the age of 65 there was no significant difference between nuns and control subjects. In the control subjects there was no evidence that menstruation, personal hygiene, or oral contraceptive practice altered the prevalence of bacteriuria.

The prevalence of asymptomatic bacteriuria during pregnancy has varied from 3% to 10%. The incidence of acquiring bacteriuria during pregnancy is about 1%, similar to the incidence of new infections in these patients in the nonpregnant state (75). Whether patients with diabetes have an increased prevalence or incidence of urinary infection has been controversial. The prevalence has been reported to be increased about threefold in women with diabetes, but is not increased in men with diabetes (76). There are large differences in the prevalence of bacteriuria reported in different studies, reflecting variations in study populations.

The prevalence of bacteriuria increases consistently with age, with a rapid increase over the age of 65 (77). Boscia and associates found an initial prevalence of bacteriuria of 18% for women and 6% for men in one primarily ambulant elderly population (78). By 1 year of follow-up, 30% of women and 10% of men had at least one positive urine culture. This high prevalence is likely related to associated chronic illness, particularly neurologic diseases. The prevalence of bacteriuria in residents of nursing homes is exceptionally high, reaching 20% to 50% in both men and women (79–81). Residents who are more functionally impaired and have neurologic diseases with voiding impairment are more likely to have bacteriuria. For institutionalized women, at least 50% with bacteriuria have upper tract localization (82,83). Bacteriuria in these populations often persists and remains asymptomatic for months to years (79). Episodes of febrile infection are infrequent given the high prevalence of bacteriuria in this population (67). Warren and associates, however, showed that at autopsy 38% of elderly bacteriuric women resident in nursing homes with a long-term indwelling catheter, and 5% without a catheter had renal parenchymal inflammation (84).

NATURAL HISTORY AND CONSEQUENCES

Infection during Infancy and Childhood

In the anatomically normal urinary tract, recurrent infections rarely lead to end-stage renal disease unless infections begin during infancy or childhood. Established risk factors for the development of renal parenchymal scarring include the presence of vesico-ureteric reflux, recurrent infection, and delayed treatment in young age at the time of infection (85). Why the acute inflammation accompanying infection results in renal scarring in some children but does not lead to scarring in others is not well understood. Recently, studies of the inflammatory response and genetic polymorphisms have suggested

that cytokine gene polymorphisms may partly explain this observation (85).

Renal scarring is found almost exclusively in patients with vesicoureteral reflux. Several investigators have argued that vesicoureteral reflux is essential for the development of renal scars and renal impairment. However, consensus seems to have emerged that, in infants and children with vesicoureteral reflux, infection is the primary cause of renal scars (86). Few patients have progressive or new scarring when infections are adequately treated and recurrences prevented (87). Specific diagnosis and adequate treatment in infancy may prevent renal scars and chronic atrophic pyelonephritis (88).

The ability to diagnose vesico-ureteral reflux in utero has provided further information about the association of vesico-ureteral reflux, urinary infection, and renal failure in infants. Severe prenatal reflux combined with kidney damage is seen virtually exclusively in males (89). Kidney damage in female infants is focal, as it is with older children and male infants with less severe vesico-ureteral reflux. Asale et al. (90) followed 108 children from birth in whom vesico-ureteral reflux was diagnosed before any infection had occurred. Mild to moderate kidney damage was present at birth in only 9 of the neonates, and 7 of these were male. Urinary infection was avoided through continuing antimicrobial prophylaxis while vesico-ureteral reflux persisted. There was no evidence of renal function deterioration in the first 30 months of life. Thus, renal failure associated with vesico-ureteral reflux in infants is often due to abnormalities present in the prenatal period, but further renal deterioration and renal function can be avoided by preventing urinary infections.

Scarring after pyelonephritis in infancy is associated with renal growth arrest in the involved kidney. Careful follow-up with immediate diagnosis and adequate treatment of all recurrent episodes of infection, particularly during infancy and early childhood, is the major factor in the prevention of progressive renal impairment (91). Smellie and colleagues confirmed that renal growth proceeded normally in children with nonrefluxing ureters and children with reflux who were kept free of infection (87). Although ureteral surgery for reflux can prevent some symptomatic episodes of pyelonephritis, it does not prevent the progression of renal scarring which may occur if infections are not prevented (92).

Asymptomatic bacteriuria occurring in girls 5 years or older with normal kidneys on study entry is benign (93). It is not associated with renal scar development regardless of antimicrobial treatment. A follow-up of young girls with asymptomatic bacteriuria detected in screening programs subsequently followed into adult life and pregnancy, however, reported that subjects treated for covert bacteriuria as schoolgirls, usually 10 to 15 years earlier, had a greater capacity to increase glomerular filtration rate during pregnancy, compared to untreated control subjects (94). Programs to screen for bacteriuria and treat infections in school-age girls have not proven to be worthwhile.

Adult-Onset Infection and Renal Impairment

Kunin critically reviewed the relationship between kidney infection and renal failure (95). Following observations in the preantimicrobial era implicating urinary infection as a major factor in the etiology of renal failure, the relationship has been clarified by: (a) a more precise definition of bacterial pyelonephritis and the recognition of its similarity to the lesions produced by vascular disease, interstitial nephritis of varying etiology, and papillary necrosis; (b) substantial evidence that infection and vesicoureteral reflux are synergistically associated with focal renal cortical scars and distortion of the renal calyces and that most renal damage occurs during the first 3 years of life; (c) the description of analgesic nephropathy and its role in producing interstitial nephritis and papillary necrosis, which must be distinguished from bacterial pyelonephritis; (d) improved bacteriologic procedures for the diagnosis of urinary tract infection; and (e) results of long-term prospective epidemiologic studies that evaluated the outcome of infection.

Careful autopsy studies performed since 1960 have evaluated the contribution of renal infection to renal disease (55,96). Weiss and Parker, in reviewing autopsy data of end-stage kidneys, noted that healed pyelonephritis was indistinguishable from primary vascular disease (13). In the longest and most definitive study, Freedman correlated autopsy with clinical data (55). He excluded patients with genitourinary abnormalities and serious nonurinary infections. Pathologic criteria for pyelonephritis were found in 1.4% of 4,686 autopsied subjects. Anatomic findings were equally distributed bilaterally, and the kidneys lacked the broad scars characteristic of pyelonephritis. He concluded that these abnormalities could be explained on the basis of vascular disease, papillary necrosis, or medullary cysts. A detailed analysis of the clinical course suggested that the role of infection was negligible.

Huland and Busch reviewed 15,921 cases in the twelfth report of the Human Transplant Registry (96). Thirteen percent were considered to have pyelonephritis; however, all had vesicoureteral reflux, analgesic abuse, nephrolithiasis, or obstruction as an underlying contributory factor in the etiology of pyelonephritis. They concluded that renal damage from adult-onset pyelonephritis sufficient to cause end-stage renal disease rarely, if ever, occurs in the absence of underlying structural abnormality.

Stamm and colleagues followed 51 women with recurrent urinary infection for an average follow-up time of 9 years (62). They experienced 696 episodes of infection; approximately 5% were clinically diagnosed as acute pyelonephritis. No significant impairment in renal function occurred during almost a decade of follow-up. The Bristol Pyelonephritis Registry followed 375 individuals after a clinical diagnosis of recurrent pyelonephritis from 1 to 13 years. Only one patient had radiographic progression of renal scars (97).

Where progressive renal failure occurs, diagnoses other than infection are usually present. Parker and Kunin retrospectively reviewed 74 of 163 women hospitalized for acute pyelonephritis 10 to 20 years earlier (98). Continuing clinical illness following the index hospitalization had occurred in more than 40% of patients; 28% had had an operative urologic procedure, and 23% had renal stones. Seventeen percent were bacteriuric at follow-up examination. One patient died of complications of pyelonephritis, one required transplantation for end-stage renal disease, and two others had significant renal impairment. Seven patients had undergone unilateral nephrectomy for pyelonephritis. Gower followed 62 adult women with a radiologic diagnosis of chronic pyelonephritis, for a mean follow-up period of almost 5 years (99). Infection was treated, so bacteriuria was present during the observation period in only 3% and 20% of women with unilateral and bilateral pyelonephritis, respectively. Serial studies demonstrated radiographic progression in 11 women. Persisting bacteriuria and analgesic ingestion were both important factors in progressive radiologic damage, but not hypertension or vesicoureteral reflux. Two of the 36 patients with bilateral pyelonephritis had renal failure or died during the follow-up. Alwall described a selected series of 29 women followed for 1 to 15 years (100). After an initial normal-appearing IUD, contract needs to be developed in all patients and several went on to end-stage renal disease. It is likely, however, that analgesic abuse was a concomitant factor that accounted for disease progression.

A recent study by Raz (101) and colleagues describes long-term outcomes for women admitted with acute pyelonephritis

to a hospital in Israel between 1982 and 1992. Only 31% of the patients were available for 10-year follow-up, likely a group representing those with more serious disease. With technetium ^{99}Tcm–labelled dimercaptosuccinic acid (^{99}Tcm-DMSA) scanning, 46% of these women had evidence of renal scarring. Pregnancy and hypoalbuminemia at hospitalization for pyelonephritis were independent risk factors correlated with the finding of renal scars at 10 years follow-up. While four women with scars had a GFR of less than 75 mL per minute, none had developed renal impairment. Despite the high proportion of renal scarring in this selected group of women, there were no clinically relevant adverse outcomes.

A small number of well-documented anecdotal reports describe acute renal failure, which is usually reversible, in acute nonobstructive pyelonephritis with *E. coli* (102). In some of these cases, concomitant nonsteroidal antiinflammatory drugs may have contributed to the pathogenesis of renal failure.

These long-term prospective studies in adult women support the hypothesis that recurrent urinary tract infection usually has a benign natural history. Current evidence suggests that a very small proportion of adults with recurrent urinary tract infection and no other complicating illness are at risk of renal damage directly attributable to infection. Most persons with renal impairment and infection have significant underlying urologic abnormalities or associated renal diseases.

Hypertension

Miall and associates initially reported a significantly higher mean blood pressure among women with bacteriuria compared to noninfected women (103). Kincaid-Smith and colleagues found blood pressure to be higher in patients with urinary tract infection and radiologic evidence of renal damage, compared to those without renal damage (104). However, long-term follow-up of the large female cohort of Kunin and associates demonstrated no significant differences in blood pressure between patients with bacteriuria and those without bacteriuria (72). In the 1930s, Longcope described young women with severe progressive renal disease caused by bilateral renal infection (105). Hypertension usually was not seen during the course of acute pyelonephritis, but only after renal insufficiency developed, usually an interval of 10 to 15 years. Patients entered into the Bristol Pyelonephritis Registry and followed for up to 13 years developed hypertension at the same rate as the general population (106). Raz (101) found a similar frequency of hypertension in women with and without renal scarring 10 to 20 years after hospitalization for acute pyelonephritis. Thus, urinary infection is not directly associated with hypertension.

Nephrolithiasis

The pathophysiology of nephrolithiasis is described in Chapter 26. Stone formation within the urinary tract increases the susceptibility of the kidney to infection by obstructing the flow of urine (107). The reported rate of infection in the presence of renal calculi has varied from 2% to 47% (107). The importance of infection with urease-producing organisms in the development of triple phosphate (calcium, magnesium, and ammonium phosphate) stones composed of struvite ($MgNH_4[PO_4] \times 6H_2O$) and calcium apatite ($Ca_{10}\ [PO_4]_6 \times CO_3$) is well established (107). Successful treatment of struvite stones requires complete eradication of all stone material. Antimicrobial therapy is an adjunct to maintain urine sterile while complete stone dissolution is achieved (108).

Mortality

A comprehensive population-based study of the incidence and prevalence of bacteremic acute pyelonephritis from 1977 to 1981 in an urban population of 400,000 was reported by Bryan and Reynolds (20). They identified all episodes of bacteremia prospectively over 5 years. Of community-acquired bacteremias, 22% were attributed to invasive urinary infection, an annual incidence of 15.7 per 100,000. The mortality directly attributable to bacteremic urinary infection was 4.8%. All 15 deaths occurred in patients with significant underlying illness. During the same period, these investigators observed 1,520 episodes of hospital-acquired bacteremia, of which 221 episodes originated from the urinary tract, yielding a rate of 7.3 per 10,000 hospitalized persons (21). The mortality rate attributed directly to infection in these patients with nosocomial bacteremic pyelonephritis was 12.7% and 71% occurred in catheterized patients. The mortality of Gram-negative bacteremia originating from sites other than the urinary tract was more than three times the mortality of bacteremic acute pyelonephritis.

Neurogenic Bladder

Following traumatic spinal cord injury, patients managed with permanent indwelling catheters have substantial subsequent morbidity and mortality attributable to renal infection. The mortality rate from renal failure was 20% within three decades from injury in survivors of World War II spinal injury. This was a consequence of obstruction, nephrolithiasis, suppurative renal disease, and progressive nonobstructive chronic pyelonephritis (109). More than one-half of the deaths during the second decade after cord injury were caused by renal failure (110). The introduction and widespread practice of bladder management to maintain a low-pressure system within the genitourinary tract, particularly intermittent catheterization, have dramatically altered the occurrence of and mortality from complications of urinary infection in this population (111). Spinal cord injured patients, however, remain at risk of urosepsis and complications including urethritis, periurethral abscess, bladder and renal calculi, vesicoureteral reflux, and renal or perinephric abscesses.

Pregnancy

Acute pyelonephritis occurs in 1% to 2% of all obstetric patients in the absence of bacteriuria screening and treatment programs (75). It is the most common medical complication requiring hospitalization during pregnancy (75) and is associated with preterm labor (112). Acute pyelonephritis in pregnancy occurs primarily in the second and third trimesters. Duff reported that 52% of episodes occur in the second trimester, 46% in the third, and only 2% in the first (113).

The attributable risk of asymptomatic bacteriuria or acute pyelonephritis to maternal toxemia, prematurity, and perinatal mortality remains controversial (75). Sever and associates, in data collected from more than 55,000 pregnant women, noted a higher incidence of low-birth-weight infants and stillbirths in the 3.5% of women with documented urinary tract infection (114). Naeye reported a combined perinatal mortality rate of 42 per 10,000 births in bacteriuric women as opposed to 21 per 10,000 births in nonbacteriuric women (115). McGrady and colleagues, using birth certificate data from Washington State, showed that the fetal mortality rate was 2.4 times higher for urinary tract infection-associated pregnancies (116). Romero and colleagues, in a meta-analysis, documented an increased

occurrence of low birth weight and preterm delivery with asymptomatic bacteriuria (117). Smaill (in a Cochrane review) also showed that antibiotic treatment significantly reduced the risk of low birth weight (118). Thus, acute pyelonephritis, as with any febrile bacterial illness during pregnancy, is associated with premature labor in late pregnancy (117). Asymptomatic bacteriuria is associated with prematurity and low birth weight, but it is not clear if it is causative.

Pregnant patients have a reduced glomerular filtration rate following acute pyelonephritis, which reverts to normal within 8 weeks of effective treatment (119). Long-term follow-up studies of women known to have previously experienced bacteriuria during pregnancy showed a benign course in the majority of patients (120,121). In a follow-up period of 10 to 14 years, almost 40% of 134 women with bacteriuria during pregnancy also had bacteriuria on follow-up culture (121). Although pyelography showed renal scarring in 28% of these patients, creatinine clearance was normal for both the bacteriuric and the nonbacteriuric women (121). In addition, no significant elevation of blood pressure was found in the persistently bacteriuric group. Raz (101) reported that pregnancy at hospitalization for acute pyelonephritis was an independent risk factor for presence of renal scars 10 to 20 years later. Subtle differences in renal function were found in long-term follow-up between women with and without urinary infection during pregnancy, but these did not appear to be clinically significant.

LABORATORY DIAGNOSIS

Urine Culture

Quantitative bacterial counts from the urine of patients with acute pyelonephritis generally exceed 10^5 CFU/mL (3,112). In patients with renal infection, ureteral urine bacterial counts vary between 10^1 and 10^6 CFU/mL (123). The maximum stationary phase growth is reached in the bladder after a period without emptying; thus, whenever possible, urine cultures should be obtained following overnight "incubation" of urine within the urinary bladder. A number of factors, including diuresis, frequent voiding, partially effective antibacterial chemotherapy, infection owing to fastidious organisms, and extraluminal infection, may reduce bacterial counts to lower levels. No study to date has shown a correlation between bacterial counts in urine and the severity of urinary tract symptoms.

The diagnosis of asymptomatic bacteriuria in women can only be made with 95% assurance if two consecutive urine cultures are positive for the same organisms at numbers equal to or greater than 10^5 CFU/mL (3). In men, a single positive urine culture establishes the diagnosis of bacteriuria (4).

Urine cultures from which more than one organism is isolated may be difficult to interpret. Such "mixed cultures" may reflect contamination, but true multiple organism infection of the urinary tract is not uncommon. Mixed cultures are particularly common in patients with foreign bodies or renal stones. In a study of catheter-associated infections, 10% of low-level and 6% of high-level bacterial count infections had two or more organisms isolated (38). Different organisms may be present in each kidney. Differential growth rates in the bladder or suppression of one organism by the other may result in the report of a single organism in voided urine despite different infecting organisms in ureteral urine cultures or even the bloodstream.

Pyuria

Demonstrating pyuria is the most readily available means of establishing the presence of a host response, presumably differentiating colonization from infection. In some clinical presentations it is also a sensitive but nonspecific screening test for bacteriuria. Renal infection is characterized by a higher urinary leukocyte count than bladder infection (124,83). Leukocytes disintegrate at alkaline pH, potentially leading to false-negative findings for pyuria in patients with infections with urease-producing organisms (125). In addition, neutropenic patients with symptomatic urinary infection may fail to demonstrate pyuria. The sensitivity, specificity, and predictive value of pyuria as a diagnostic test for acute pyelonephritis have not been determined.

The routine clinical practice of measuring pyuria by the number of cells in centrifuged urine per high power field is imprecise with many sources of error (5). The enumeration of the absolute number of leukocytes in a measured volume of urine excreted over a fixed period is highly reproducible and separates levels of pyuria in symptomatic infected patients from those in normal volunteers without bacteriuria (126). Leukocyte excretion rates, however, are not applicable to routine patient care. Excretion rates do correlate well with absolute leukocyte counts from random, unspun urine counted in a hemocytometer. Neutrophil counts in excess of $10/mm^3$ represent an abnormal host response (5). Less than 1% of healthy males and females without bacteriuria excrete 10 leukocytes/mm^3 or more (61).

Bacteremia

Between 15% and 30% of hospitalized patients with acute pyelonephritis have bacteremia (17–19,127). Elderly women, patients with diabetes, and individuals with obstruction are more likely to be bacteremic (128). Bacteremic patients are presumably at greater risk of metastatic infection to other sites.

Recent studies have reviewed the utility of routine blood cultures for all patients presenting to the emergency department with acute pyelonephritis. McMurray reported that 56 (18%) of 307 patients hospitalized with acute pyelonephritis from 1990 to 1992 had positive blood cultures; 32% of positive blood cultures were coagulase negative staphylococci and presumed to be contaminants (129). Only one blood culture grew a pathogenic organism not also isolated from urine culture, and clinical management was not altered by positive blood cultures. Velasco (130) obtained routine blood cultures from outpatients presenting with a diagnosis of acute pyelonephritis at a Spanish hospital. Positive blood cultures were found in 147 (25%) of 583 patients, with coagulase negative staphylococci isolated in only 17. *Escherichia coli* grew from 91% of positive blood cultures, and 23% of women with *E. coli* pyelonephritis had positive blood cultures. Blood culture results were consistent with the urine culture result in 98% of positive cases. Both these studies concluded that routine blood cultures were not clinically useful and should not be recommended. Wing (131) pooled data from three randomized control trials in pregnant women with acute pyelonephritis where routine blood cultures obtained at study entry. Among 391 women, 24% had positive blood cultures and 5 (1.3%) grew *S. epidermidis*. They also observed that blood cultures were consistent with urine cultures, and did not change management. Smith (132), however, retrospectively reviewed results from nonpregnant women admitted with acute pyelonephritis through the emergency department of an urban county teaching hospital where blood cultures were obtained selectively rather than routinely. Bacteremia occurred in 36% of 64 women, and was more frequent in black women, women with genitourinary abnormalities, with higher pulse rates on admission, higher levels of pyuria, and more prolonged fever. Blood cultures were positive in two patients with negative urine cultures.

TABLE 34-3

LOCALIZATION OF INFECTION IN BACTERIURIC POPULATIONS

Reference	Method	Population investigated	Symptoms	Site of infection		
				Renal	Bladder	Equivocal
123	Ureteral catheterization	95 Women 26 Males	Variable	67	54	—
134	Bladder washout	125 Women 30 Upper 43 Lower	52 Asymptomatic	63	52	10
135	Bladder washout	133 Women 15 Upper 28 Lower	90 Asymptomatic	56	54	23
136	Bladder washout	50 Women 25 Lower	25 Upper	21	22	7
137	Bladder washout	105 Women 24 Lower 20 Upper	60 Asymptomatic	65	39	—

These studies suggest women who present with acute pyelonephritis and who are clinically stable with moderate symptoms are unlikely to benefit from the practice of obtaining routine blood cultures. However, women who are more severely ill, where the diagnosis is uncertain, or where an underlying abnormality is present or suspected, should have blood cultures. This is particularly the case for pregnant women, where failure to initiate appropriate antimicrobial therapy may have adverse fetal outcomes.

Leukocyte Casts

Leukocyte casts indicate intrarenal inflammation. They are present in about two-thirds of patients with invasive renal infection, but are nonspecific, also being present in many interstitial and glomerular renal diseases.

Hematuria

Microscopic hematuria is common in patients with renal infection and has no documented significance. It usually disappears with treatment. Red blood cell casts are unusual. Persistent hematuria after antimicrobial treatment may require urologic investigation to exclude other causes.

Proteinuria

Quantitative proteinuria with a urinary protein excretion rate exceeding 100 mg/24 hours is unusual in either acute or chronic renal infection unless a second renal disease associated with proteinuria is present. A syndrome of vesicoureteral reflux, chronic pyelonephritis, and massive proteinuria associated with focal glomerulosclerosis has been reported (133). The cause of this syndrome is not known.

INFECTION LOCALIZATION

Bacteriuria was at one time assumed to be synonymous with renal infection. Localization studies by Stamey and colleagues in 1965, using the ureteral catheterization technique, demonstrated that many asymptomatic patients and most patients with symptoms confined to the bladder had only lower tract infection (123). In Table 34-3 are summarized several studies performed to localize infections (123,134–137). From these studies, it is apparent that as many as one-half of women with asymptomatic infection and a substantial minority of women who experience only bladder symptoms, have renal infection. No epidemiologic studies and few therapeutic studies of bacteriuria and its complications have attempted to prospectively localize renal bacteriuria. A partial list of tests and procedures proposed for localization of the site of infection within the urinary tract are listed in Table 34-4.

Cystoscopy with ureteral catheterization is a direct approach to localization and continues to be the only certain way to diagnose renal infection. It also permits localization of infection to one kidney and can identify different infecting organisms in each collecting system (138). In practice, however, this procedure has limited usefulness. Expert urologic manipulation with meticulous attention to avoid contamination is required. Cystoscopy and ureteral catheter insertion must be performed in an infected urinary tract because antibacterial therapy is withheld until the urine collections are complete. Infected bladder urine must be removed by repeated washing with sterile irrigating fluid before ureteral catheters are introduced through the bladder (123). Otherwise, positive ureteral urine

TABLE 34-4

METHODS PROPOSED TO IDENTIFY URINARY INFECTION LOCALIZED TO THE KIDNEY

Ureteral catheterization with differential urine cultures
Intravenous pyelography/CT scanning
Nuclear medicine imaging
Bladder washout
Renal biopsy with culture
Fluorescent examination of renal tissue for bacterial antigen
Serum antibodies to lipopolysaccharide antigen
Urinary enzymes
C-reactive protein
Serum procalcitonin
Tamm-Horsfall antibodies
Maximal renal concentrating ability
Antibody-coated bacteria (ACB)
Relapse following short course therapy

cultures may result from carriage of infected bladder urine into the ureters during catheterization.

Histopathologic examination of renal tissue obtained by biopsy or at necropsy is a direct approach to the diagnosis of renal infection. Pyelonephritis, however, is a focal disease, and a random kidney biopsy specimen may not produce either a pathologic or bacteriologic diagnosis (139). The correlation between the histologic diagnosis of chronic pyelonephritis and bacteriologic findings at postmortem is tenuous, and bacterial cultures of urine or renal tissue obtained at necropsy have not proved useful. Percutaneous renal biopsies are seldom used to diagnose chronic pyelonephritis. Bacteriologic studies of renal tissue rarely are helpful. The nonspecificity of the histologic findings and their focal nature further limit the diagnostic utility of biopsy. Obstruction or concomitant diseases such as diabetes mellitus and hypertension frequently distort the gross and histologic features and make it impossible to attribute changes specifically to bacterial inflammation. At necropsy the normal renal architecture may be totally destroyed.

An intravenous pyelogram has a low sensitivity for diagnosing renal infection (140). Imaging of the kidneys with ^{67}Ga citrate has been proposed to localize infection. Hurwitz and associates compared imaging with ^{67}Ga citrate to cultures obtained by ureteral catheterization or bladder washout in 47 patients and found a false-positive rate of 15% and a false-negative rate of 13% for the imaging procedure (136). Radioisotopic localization, particularly with DMSA may be a more sensitive procedure for diagnosing renal infection and assessing renal scarring. These tests are often positive in patients with invasive upper tract infection, but are less reliable in those with asymptomatic infection (141–143).

Fairley and colleagues described a technique for diagnosing renal infection that involves Foley catheterization followed by bladder irrigation with a mixture of an antibiotic and a debriding enzyme (135). The antimicrobial agent is then rinsed from the bladder, and timed specimens are obtained. Organisms found in post-rinse specimens are considered to have originated from the kidneys. Although this technique seldom has been compared to the more definitive ureteral catheterization technique, it has been used widely to validate other procedures (Table 34-2). However, the procedure is uncomfortable and there are problems of interpretation in at least 20% of women (144). In patients with significant reflux, antimicrobial agents can ascend into the upper tract and suppress positive cultures. Antimicrobial agents persist in the bladder in some patients following irrigation and can render renal urine cultures falsely negative.

Indirect techniques to localize upper tract infection have, to date, consistently not been validated over time. Antibody directed against the lipopolysaccharide antigen of the infecting microorganism is frequently present in patients with symptomatic upper tract infection and usually absent in patients with cystitis or asymptomatic infection. Essentially all studies of antibody response to date have limited their investigation to *E. coli.* Although several investigators suggested that this technique has a sensitivity of 80% or higher in patients with renal infection and a specificity approaching 80% in patients with bladder infection, other investigators found that the sensitivity and specificity are inadequate for the clinical evaluation of an individual patient (145). The test is not useful for routine patient care.

In 1974, Thomas and colleagues described an *in vitro* procedure for the localization of the site of infection in patients with bacteriuria (146). The test depends on the presence of fluorescing immunoglobulins directed at the O somatic antigen on the bacterial surface (the ACB test). Bacteria in urinary sediment are incubated with fluorescein-tagged antihuman immunoglobulins. Even within the same laboratory, difficulties with interpretation can be considerable. Thus, as with other localizing procedures, the ACB test lacks the sensitivity and specificity to be a reliable guide for clinical decision making for individual patients.

Maximal urinary concentrating ability is reduced in many patients with renal bacteriuria. Fluid deprivation for 24 hours with administration of antidiuretic hormone achieves maximum urinary concentration of more than 800 mOsm/kg of water in more than 80% of patients with bladder bacteriuria, but at least 70% of patients with renal infection are unable to concentrate urine to this level following maximal stimulation (147,148); however, urinary concentration is altered in a number of pathologic states, and the test is not sufficiently sensitive or specific to be of value for an individual patient.

Measurements of urinary enzymes, including urinary dehydrogenase, leucine aminopeptidase, ß-glucuronidase, catalase, lactic dehydrogenase, lysozyme, and urinary $\alpha 1$ microglobulin, have been proposed as localization methods. Considerable overlap occurs in the urinary enzyme range for renal and bladder infections. Although at least one study has found that each of these can differentiate renal infection from bladder infection, further evaluation has not proved them to be clinically useful for localizing infection. Excretion of Tamm-Horsfall protein is increased in some patients with renal infection, particularly those with vesicoureteral reflux. IgG autoantibodies to this protein have also been proposed to diagnose renal infection.

Measurement of C-reactive protein has been used as a test to localize renal infection in symptomatic children (149). Serum procalcitonin is also being studied to diagnose pyelonephritis and upper tract infection in febrile infants and children, a presentation which is often a diagnostic challenge. A sensitivity of 70% to 94.1% and, specificity of 83% to 94%, of procalcintonin levels using DMSA scintography as the reference test has been reported (150–152). This is superior to C-reactive protein (150,152). However, variable levels of procalcitonin are used in these studies, and the optimal level above which a test is considered abnormal has yet to be defined. Serum procalcitinon levels have also been used to predict outcomes. Pratt (153) showed that elevated proclacitonin (using a cut-off of 1 ng/mL) correlated with the presence of renal scars and was more specific than C-reactive protein or leukocyte count.

In practice, clinical presentations are used to assess site of infection. Smeets and Gower screened 43 symptomatic women with the bladder washout technique (154). Among these women, only fever higher than 38°C correlated with renal involvement. Two-thirds of the patients with upper tract infections were febrile at the time of localization compared to one-third of patients with lower tract infections. These studies and others suggest that no clinical criteria are uniformly reliable to localize the site of the infection (155).

A short course of effective antimicrobial therapy will cure most uncomplicated bladder infections in women. It has been proposed that differential outcomes with short-course therapy may localize the site of infection. Comparisons of single-dose therapy outcome with other localization methods have documented an association between failure of single-dose therapy and renal infection (156–158). However, single-dose therapy will fail in 10% to 20% of individuals with lower tract infection, and a proportion of individuals with upper tract infection will be cured. Single-dose treatment regimens can predict upper tract abnormalities on radiographic studies. In one study of 37 women (159), eight had abnormal-appearing IVPs. Treatment with a single injection of kanamycin failed in seven of these eight patients. Bailey found that 76 of 80 patients treated with a single dose of amoxicillin with a radiologically normal urinary tract were cured, compared with only 12 of 21 with radiologic abnormalities (160). Thus, the use of single-dose

therapy as a localization method has some specificity, but limited sensitivity.

ORGAN IMAGING

Acute Pyelonephritis

An abdominal film of the kidneys as initial screening may provide some information in patients with presumed acute pyelonephritis, as renal calculi are visualized in about 5% of patients. One or both kidneys may be enlarged or gross changes, such as hydronephrosis or renal atrophy, may be apparent from the renal outline. Occasionally, perinephric gas or other changes in the retroperitoneal space may provide diagnostic clues (161). Other findings recognized on the abdominal films may lead to diagnoses other than acute pyelonephritis, such as a perforated viscus. There is currently little role for intravenous pyelography in patients during an episode of acute pyelonephritis. An early study (162) reported that three-fourths of 40 patients had normal IVPs. Nonobstructive hydronephrosis may be diagnosed in patients presenting acutely with invasive upper tract infection and must be differentiated from stones, congenital anomalies, sloughed papillae, or other lesions obstructing urine flow.

Renal ultrasonography and computerized tomographic (CT) scanning have largely replaced IVP for initial screening for genitourinary obstruction in patients with acute pyelonephritis (163,164). In addition to identifying anatomic abnormalities and focal complications such as intrarenal or perinephric abscess, ultrasonography usually shows swollen kidneys, although this may be appreciated only by repeat scanning following antimicrobial therapy. The degree of renal enlargement on ultrasonography has been correlated with prolonged pretherapy symptoms, higher leukocyte counts, and prolonged hospitalization (163).

An unenhanced CT is usually sufficient and will detect most calculi, gas forming infections, hemorrhage, parenchymal calcifications, obstruction and inflammatory masses. However, a contrast enhanced study is necessary to fully define changes in renal excretion that occur as a result of inflammation and can be identified through use of contrast material. The helical (or spiral) fast scanning technology using a CT scan can provide information addressing specific phases of contrast medium excretion (165). To date there has been little experience in using magnetic resonance imaging in the evaluation of acute renal infection (165).

A contrast-enhanced CT scan may be indicated in acute pyelonephritis if the differential diagnosis includes other intraabdominal or retroperitoneal pathology, or if there is a delayed response to therapy and the ultrasound is normal or equivocal. With an unenhanced CT, there is global swelling of the infected kidney (164). With contrast-enhanced CT either an enlarged kidney with a uniformly homogeneous nephrogram or a striated parenchymal nephrogram or wedge-shaped segmental defects are seen (164). The striated pattern is owing to localization of inflammatory cells and fluid within the collecting ducts. The wedge-shaped low-attenuation areas reflect renal parenchyma with impaired function caused by vascular spasm, tubular obstruction, or interstitial edema. Renal enlargement, delayed visualization, and poor definition of calyceal architecture in the involved kidney are also common findings. Renal parenchymal volume increases by 25% to 50% during an episode of acute pyelonephritis and can take 4 to 6 weeks to regress (166). Little and colleagues described four patients with unilateral pyelonephritis in whom the involved kidney was 1.5 cm or longer than the noninvolved kidney (167). Mucosal edema of the ureter or renal pelvis may appear as delicate parallel lucent striations in the collecting system and the infundibulum and calyces may be narrowed by edema (164).

In children, DMSA scintigraphy is more sensitive then intravenous pyelography or ultrasound for detecting acute pyelonephritis and renal scarring (165,168). Scintigraphy has also been evaluated as a predictor of outcomes at longer-term follow-up. Wallin (169) reported that scintigraphy defects present at acute infection persist in 34% of kidneys at 6 months. Hitsal (170) evaluated quantitative analysis of DMSA scintigraphy to predict long-term renal scarring following acute pyelonephritis in children. The intensity of abnormality with DMSA scintigraphy was predictive of subsequent scarring. In adult populations, Attari (171) found contrast enhanced CT scanning more accurate for identifying acute pyelonephritis then DMSA scintigraphy. Small lesions and those localized to the inner layer of the renal cortex were present on the CT scan but not appreciated with the lower resolution of scintigraphic images.

Rosenfield and colleagues described 12 patients with an edematous renal mass resulting from localized renal inflammation, called a nephronia (172). The term *nephronia* refers to a renal mass from localized inflammation but without suppuration, analogous to lobar pneumonia (173). Gallium scanning demonstrates increased uptake in the area of the mass, usually accompanied by generalized increased activity elsewhere in the same or opposite kidney. Ultrasonography shows a sonolucent ovoid mass that disrupts the normal corticomedullary definition and produces low-level echoes. These findings permit differentiation of nephronia from renal abscess or tumor.

Radiologic changes induced by acute inflammation are usually reversible with antimicrobial treatment (136,163). However, in isolated instances, a progressive reduction in renal size or the development of a scar has been described following an episode of acute pyelonephritis (167). In one study, 10 of 44 men and women with acute nonobstructive pyelonephritis and hypodense images on CT had persistent abnormalities at follow-up CT (174). Early scar formation was present in two patients, and two patients with atrophy had renal biopsy which showed chronic interstitial nephritis.

Chronic Pyelonephritis

Hodson described the classic large, deep, segmental, coarse cortical scar usually related to one or more of the renal calyces, which is the hallmark of atrophic or chronic pyelonephritis (175). The involved kidney is irregular in outline and below average size unless hypertrophy has occurred owing to compensatory enlargement. Scars may involve an entire pole of the kidney or, in patients with atrophic pyelonephritis, the entire kidney. The upper pole is the most common site for scars. In serial studies, the initial abnormality was loss of renal cortex, with the renal parenchyma becoming thinned. Calyceal clubbing then developed as the renal papilla is retracted into the scar. The cup of the calyx is no longer a "cup," because the papilla does not project into it.

Renal atrophy with coarse scars that histologically contain tubules but no glomeruli had been considered to be a congenital malformation and designated as the Ask-Upmark kidney; however, vesicoureteral reflux and recurrent urinary infection are associated with this abnormality, and these features may be related causally (176). Uninvolved renal tissue may hypertrophy locally with segmental enlargement in patients with chronic pyelonephritis, resulting in the formation of a pseudotumor that must be differentiated from other causes of renal masses. Brucellosis may cause chronic renal infection that mimics renal

cell carcinoma with calyceal clubbing, deposition of calcium in renal tissue, and mass formation (41).

Indications for Radiologic Investigation

There are several indications for imaging in individuals with suspected acute pyelonephritis: (a) to assist with diagnosis of acute pyelonephritis, (b) to assess whether there are underlying abnormalities present that may require intervention, (c) to assess the severity of infection or, (d) in follow-up, to determine the extent of persistent damage such as renal scarring.

Imaging of the genitourinary tract for patients presenting with acute pyelonephritis should be selective (165). In patients with acute pyelonephritis evaluated by IVP, among 170 patients (163 women, seven men), 85 had normal pyelograms, 75 had structural or functional abnormalities that were attributable to the acute infection or represented a risk for relapse, and only 10 (5.9%) had specific disorders that resulted in a change of management (177). Thus, routine imaging studies for all patients are not recommended. Men and women with atypical presentations, with a delayed response to therapy, or in septic shock where obstruction must be excluded, should have imaging studies. They may also be considered in patients who relapse shortly after therapy is discontinued.

Renal ultrasonography or contrast enhanced CT scanning is the preferred initial screening test for upper tract infection in adults because they are rapid and noninvasive. In children, initial screening with ultrasonography or DMSA scintigraphy detects scarring and other renal abnormalities (141,143). Voiding cystourethrogram is added for young children or if there is evidence of upper tract disease. Investigations should be performed in male infants and boys with proven bacterial urinary infection, and in girls with recurrent or complicated infection. Intravascular injection of radiologic contrast media has risks of hypersensitivity or contrast media-induced renal failure. The concomitant presence of diabetes mellitus, particularly with renal impairment, is a relative contraindication.

Repeated radiologic evaluation following an initial normal examination should be discouraged. Unless specific new indications emerge, serial periodic studies are redundant in adults with recurring infection. Even among patients with prior radiologic evidence of chronic pyelonephritis, the development of new findings is unusual (99). Upper tract pathology rarely develops after the age of 1 year in adequately treated children, and repeated examinations are seldom indicated (91,93,178).

CLINICAL PRESENTATION

Adults

The classic clinical presentation of acute upper urinary tract infection in adults includes fever, usually over 38.5°C, chills, unilateral or bilateral pain in the lumbar flank region, and generalized constitutional symptoms, including malaise, anorexia, nausea, emesis, diarrhea, myalgia, and headache. The illness may progress rapidly, and many patients seek care within 24 hours of onset of symptoms. Between 15% and 30% of patients experience concomitant symptoms suggestive of lower urinary tract infection, including dysuria, frequency, urgency, and suprapubic discomfort. More than one-half of patients presenting with acute pyelonephritis have a history of lower tract symptoms during the previous 6 months. Renal pain may radiate to the epigastrium or the lower abdominal quadrants. Severe flank pain with radiation to the groin is unusual and suggests ureteral obstruction. Gastrointestinal symptoms, primarily nausea, vomiting, and diarrhea, occur frequently and predominate in about 10% of patients. Although patients presenting with acute pyelonephritis may be severely ill, the spectrum of disease also includes individuals with only low-grade fever or mild flank discomfort. Flank pain or discomfort on palpation or fist percussion is usually present and may be accentuated by superimposed obstruction. The physical findings are variable, however, ranging from none to the clinical features of septic shock.

Other diseases, both above and below the diaphragm, can mimic the pain of acute pyelonephritis. The differential diagnosis includes acute bacterial pneumonia, appendicitis, cholecystitis, a perforated viscus, diverticulitis, splenic infarction, acute pancreatitis, and aortic dissection. Acute pelvic inflammatory disease may be misdiagnosed as acute pyelonephritis and should be excluded by a pelvic examination in women at risk of sexually transmitted infections. Acute varicella-zoster viral infection in an appropriate dermatome can also mimic renal pain. Renal infarction, acute renal vein thrombosis, obstructive uropathy, and acute glomerulonephritis can each occur with a clinical picture that can be confused with acute pyelonephritis.

Pinsent and colleagues (179) assessed the utility of fever in differentiating acute pyelonephritis from other potential diagnoses. In a retrospective chart review, 93% of women who presented to the emergency department with pyuria, leukocytosis, costovertebral angle tenderness, and two or more of abdominal pain or tenderness, back pain, and history of nausea and vomiting were ultimately diagnosed as acute pyelonephritis when fever was also present. When fever was not present, 35% of women with these symptoms ultimately had an alternate diagnosis. All nonhospitalized patients with fever were ultimately diagnosed as pyelonephritis, and 13% without fever had an alternate diagnosis. They concluded that when patients present with findings compatible with acute pyelonephritis but without feverthere should be a high index of suspicion for an alternate diagnosis (179).

The presenting clinical features in pregnant women are similar to those in nonpregnant women. In one study, septic shock developed in 3% of pregnant patients with acute pyelonephritis (180). Patients with diabetes mellitus may experience worsening of hyperglycemia. Renal infection in patients with diabetes mellitus and hyperglycemia may, rarely, present as emphysematous pyelonephritis owing to carbon dioxide production from fermentation of glucose by Gram-negative rods (161,181). Pneumaturia also may be present. Acute pyelonephritis in patients with diabetes may be accompanied by papillary necrosis. Fragments of the renal papillae can block the ureter, producing colic and hydronephrosis, often accompanied by gross hematuria.

In elderly patients, symptoms of urinary infection may be more difficult to ascertain and chronic symptoms of dysuria, frequency, urgency, and incontinence often occur unrelated to infection. Flank tenderness is less common in elderly patients, and confusion, sometimes with other neurologic symptoms or signs, is more common compared to younger age groups. However, classic features of upper tract infection do occur in elderly patients. Gleckman and colleagues reported that septic shock occurred in five and bacteremia in 19 of 35 elderly patients who presented to a hospital with acute pyelonephritis, although only one patient died during the acute illness (182).

The clinical manifestations of chronic pyelonephritis are usually nonspecific. Some patients have recurrent acute symptomatic exacerbations of renal infection. Others have no clear-cut symptoms of infection despite persistent bacteriuria. Others may complain of vague flank discomfort, abdominal pain, or intermittent low-grade pyrexia. Only improvement after a trial of therapy may clarify an association between symptoms and persistent renal infection.

Genitourinary sepsis can lead to metastatic infection at other sites. Siroky and colleagues, in a comprehensive review, identified 175 patients in whom metastatic infections developed from a primary source in the genitourinary tract (183). One hundred six patients (59%) had infection of the skeletal system; 51 patients (28%), infection of the endocardium; and the remainder, infection of miscellaneous sites, including the eye and the central nervous system. Men predominated (86%) and the mean age was 57 years. Many of the men were considered to have a primary prostatic focus from which dissemination occurred, but in 46 patients the upper urinary tract was considered to be the source of the bacteremia. About one-third of these patients had undergone manipulation of the upper urinary tract prior to development of metastatic infection. Underlying host factors that would impair resistance to infection were unusual. The mean latent period separating an episode of acute urinary infection from the onset of symptoms of vertebral osteomyelitis was 54 days in a Danish review (184). Almost 70% of the skeletal infections were caused by Gram-negative rods and the spine was the metastatic site in 83 of these patients. In patients with endocarditis, Gram-positive organisms were responsible for two-thirds of the infections and usually occurred in patients with preexisting heart disease.

Infants and Children

In the neonatal period, urinary tract infection usually presents as sepsis. The clinical picture, however, can vary from life-threatening septicemia in association with pyelonephritis to asymptomatic bacteriuria (57,185). Nonspecific symptoms associated with urinary infections in infants include fever; inadequate weight gain; gastrointestinal symptoms such as anorexia, emesis, diarrhea, and paralytic ileus; meningitis; seizures; lethargy; irritability; hypotonicity; respiratory irregularity; pallor; cyanosis; abdominal distention; gray skin color; and jaundice. Other studies have identified jaundice as the hallmark of ongoing infection in the neonate (186). The frequent occurrence of generalized sepsis, premonitory symptoms prior to the onset of bacteriuria, and necropsy findings demonstrating cortical infection in the presence of a normal renal pelvis support a hematogenous route of renal infection in neonates. The predominance of males is unexplained. The intestinal tract is the presumptive source.

In older children, the clinical features of urinary infection more closely approximate those in adults. Burbige and colleagues reviewed 83 bacteriuric boys and found only nine of the 83 to be asymptomatic (187). The most common clinical presentation included fever, irritative bladder symptoms, abdominal or flank mass, hematuria, and enuresis. Almost 75% of these boys had an anatomic abnormality, most commonly vesicoureteral reflux. One-fourth, however, had obstructive lesions, usually in the lower urinary tract. As girls mature, abdominal tenderness, vaginal discharge, vomiting, and anorexia become less common features. Fever and flank pain become the predominant symptoms. The epidemiologic studies of Kunin and colleagues confirmed that asymptomatic urinary tract infection in childhood occurs almost exclusively in females (70,71).

TREATMENT OF UPPER TRACT INFECTION

Therapeutic Principles

The following should be considered whenever upper urinary infection is diagnosed and treated:

1. Antimicrobial selection is based on known presumed organism susceptibility and patient tolerance. The efficacy of most regimens for upper urinary tract infections has not been assessed optimally in clinical trials (188). Many reported studies combine patients presenting with acute nonobstructive pyelonephritis and those with upper or lower tract complicated urinary infection. Thus, the evidence base to support a specific regimen is limited.
2. The clinical findings of invasive upper tract infection can be mimicked by many illnesses. Laboratory confirmation is necessary.
3. Bacteriuria by itself is a nonspecific finding. Patients with asymptomatic bacteriuria, particularly elderly patients, frequently have illnesses that do not arise from renal infection.
4. An agent that provides adequate blood and tissue levels as well as a high urinary level is preferred for the treatment of acute invasive renal disease. Agents that provide high sustained medullary levels of antimicrobial activity are more effective for upper tract infection in an animal model (189), although human studies are lacking.
5. Urine pH alters antibacterial activity and concentration. For example, a higher pH simultaneously increases the concentration of sulfonamides and decreases the concentration of trimethoprim. Aminoglycosides are more active in alkaline urine. Urine pH has not been shown, however, to be an important determinant of therapeutic outcome in acutely ill patients treated with parenteral agents.
6. Suppurative and obstructive complications commonly complicate invasive pyelonephritis and occur in 5% to 15% of patients. Patients with life-threatening infection, patients who do not respond within 72 hours of antimicrobial therapy, patients who deteriorate after the onset of therapy, and patients who rapidly recur after therapy must be investigated urgently to exclude obstruction or abscesses that require surgical responses.
7. Clinical improvement may not equate with pathogen eradication. Urinary infection is usually a recurrent disease and patients should be aware of this possibility. Follow-up urine cultures are not necessary to document bacteriologic cure, but should be obtained if symptoms recur.

Acute Pyelonephritis

A suspected clinical diagnosis of acute pyelonephritis requires urgent assessment and institution of therapy. Appropriate diagnostic tests, including urine culture prior to antimicrobial therapy in all cases, and CBC and blood cultures for subjects with more severe presentations, should be obtained. Initial therapeutic decisions include whether hospitalization is required and whether diagnostic imaging studies should be obtained to exclude obstruction or other complicating factors, as well as selection of an empiric antimicrobial agent.

Hospitalization

Patients with severe clinical presentations, including moderate to severe costovertebral angle pain, rigors, high fever (>38.5°C), or severe nausea or vomiting, should be hospitalized for initial investigation and parenteral antibacterial treatment. Intravenous fluids, analgesics, and bed rest are usually prescribed during the initial 24 to 48 hours. Hypotension and diminished urine output should be identified early and appropriate interventions undertaken. Patients with clinical features of septic shock should be cared for in a critical care unit. Where it is unclear whether a patient needs to be admitted, observation for a 12-hour period with initial parenteral therapy is an appropriate and frequently used option. If the patient is stable without substantial nausea or vomiting after initial

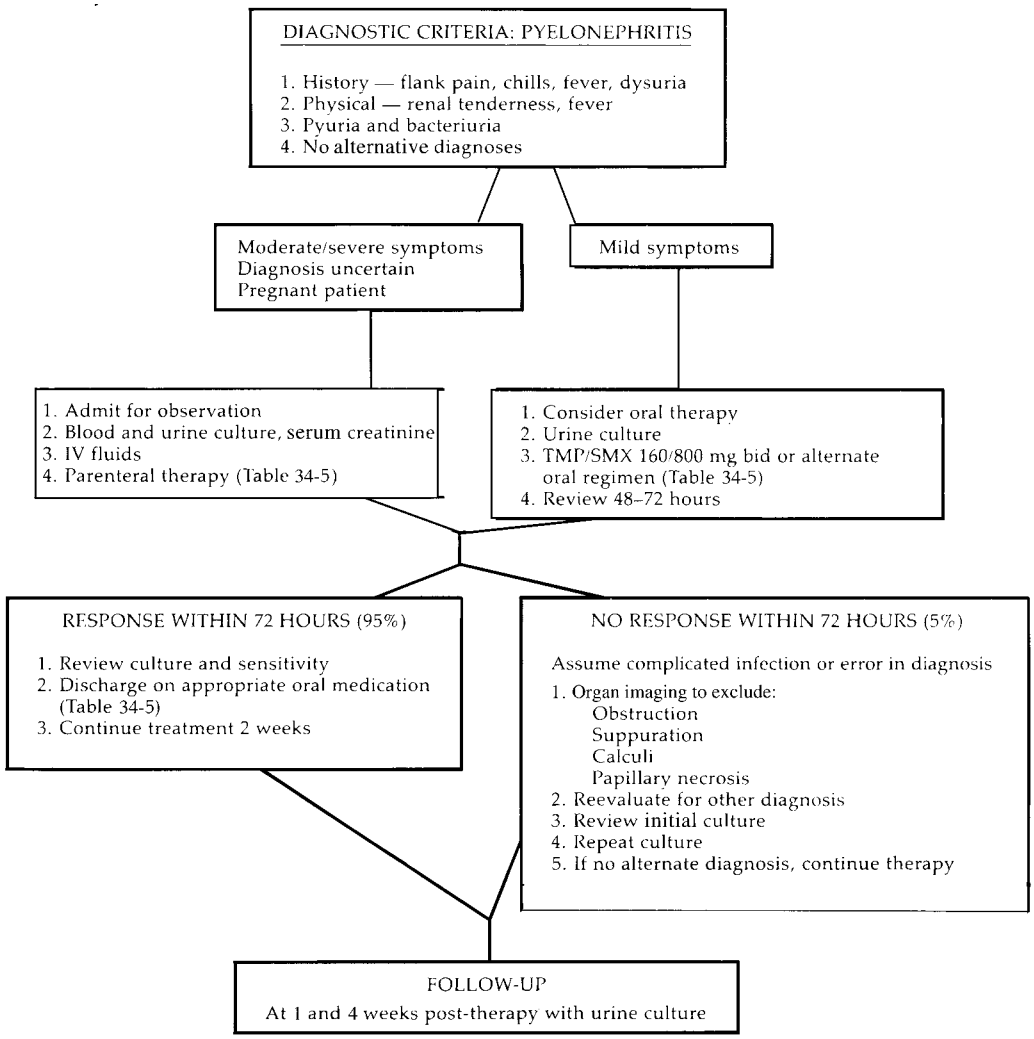

FIGURE 34-1. Management of pyelonephritis. *TMP/SMX,* trimethoprim/sulfamethoxazole.

parenteral therapy, discharge home to complete oral therapy is appropriate.

Patients with acute pyelonephritis may be only mildly or moderately symptomatic with low-grade fever, mild flank pain, and few constitutional symptoms. These patients may be managed as outpatients with an oral antimicrobial regimen (Fig. 34-1). Specific criteria to identify patients appropriate for outpatient therapy are not well defined; however, patient compliance; lack of significant nausea, vomiting, or other constitutional signs; and absent or low-grade fever (38°C or less) seem appropriate. In addition, women who are pregnant or where there is diagnostic uncertainty require more careful follow-up, and hospital admission is recommended in these cases.

Safrin and associates (17) reported a retrospective review of therapy and showed similar outcomes for parenteral and oral therapy with unsatisfactory outcomes in 10% of either group. Ambulatory patients were clearly less ill, as evidenced by lower temperatures and leukocytosis, as compared to the inpatient group, but specific criteria used to identify patients for outpatient therapy were not stated. Pinson and associates (18) also reviewed management and outcomes of 111 febrile women presenting to an emergency room with acute pyelonephritis. Eighty-three (75%) were not hospitalized. Women hospitalized for management were older, more frequently had diabetes or known genitourinary abnormalities, and were more ill as evidenced by vomiting and a higher temperature. Management of nonhospitalized patients (80%) usually included a single parenteral antimicrobial injection followed by oral therapy (usually trimethoprim-sulfamethoxazole). Nine (12%) of 75 outpatients with follow-up returned with continuing symptoms of acute pyelonephritis, usually within 24 hours, and seven were subsequently admitted and cured. The authors concluded that most febrile women with acute pyelonephritis could be treated out of hospital, but recommended early follow-up after initiation of therapy.

Elkharrat (190) used an algorithmic approach for women presenting to the outpatient department with acute pyelonephritis. All received a single intravenous dose of a flouroquinolone after cultures were obtained, and had an ultrasound of the abdomen and pelvis. Patients with normal ultrasonography could be discharged. Of 68 patients, 10 were discharged directly from the emergency ward, 48 after a 12 to 24 hour stay in the observation unit, and 10 were hospitalized. Favorable outcomes occurred in all but one woman.

Parenteral Antimicrobial Therapy

Patients usually require initial parenteral therapy if their symptoms are severe. A full course of parenteral therapy is indicated only for patients who cannot tolerate oral therapy or are infected with a resistant organism for which no oral therapy is available. The Infectious Disease Society of America (IDSA)

guidelines note that there are limited data available to support recommendations for specific parenteral agents (188). However, based on substantial clinical experience, an aminoglycoside with or without ampicillin, a parenteral fluoroquinolone, or an extended spectrum cephalosporin with or without an aminoglycoside is an appropriate empiric option (188). Johnson et al.(191) reported parenteral ampicillin or TMP/SMX, both with initial gentamicin, were equally effective.

Treatment regimens have been investigated in several animal models of acute pyelonephritis. Ampicillin and gentamicin in combination were more effective than either drug alone in sterilizing renal tissue, preventing relapse, and reducing the severity of renal lesions in acute pyelonephritis (192). Miller and Phillips, in a similar model, showed that antimicrobial therapy markedly reduced histopathologic changes when it was given up to 4 days after bacterial challenge, but had little effect on the development of renal scars when it was initiated later in the course of the infection (193). Thus, at least in the animal model, early effective treatment can prevent a destructive pyogenic and cellular immune response owing to bacterial invasion.

Any empiric regimen for initial treatment of patients with acute pyelonephritis should include in its antibacterial spectrum at least 95% of the potential organisms in the population treated. Standard initial therapy, pending culture and susceptibility results, is an aminoglycoside, such as gentamicin, netilmicin, or tobramycin, in a dose of 1.5 mg/kg initially, followed by 1.0 mg/kg every 8 hours combined with ampicillin 1 g every 4 hours (Table 34-4). Once daily aminoglycoside dosing is an alternative. The ampicillin therapy provides coverage for *E. faecalis,* an uncommon pathogen in younger populations. For the penicillin-allergic patient, an aminoglycoside alone may be adequate empiric parenteral therapy. This approach is depicted in an algorithm in Figure 34-1. The selection of a "standard" antimicrobial regimen for empiric therapy requires knowledge of antimicrobial susceptibilities in the community or hospital in which the infection has developed. If widespread resistance to gentamicin or tobramycin is present, amikacin may be the aminoglycoside of choice for empiric treatment, especially for nosocomial pyelonephritis where more resistant isolates may be anticipated.

Aminoglycoside-related nephrotoxicity and ototoxicity are unusual if high sustained trough levels are avoided and the duration of therapy is 4 days or less. Aminoglycoside levels should be monitored if the patient has renal impairment or will be continued on therapy longer than 5 days. Trough levels should not exceed 2 μg/mL for gentamicin, netilmicin, and tobramycin or 5 μg/mL for amikacin. Bailey (194) enrolled 53 patients, the majority of whom were women with acute pyelonephritis, into a comparative study of a single large dose of 10 mg/kg of gentamicin followed by oral ciprofloxacin or 2.5 mg/kg q8h followed by oral ciprofloxacin if patients were clinically stable. The two regimens appeared to be equally efficacious, but there was a trend to increased autotoxicity in the standard gentamicin therapy group and lower costs associated with the single large dose to initiate therapy. Sandberg (195) reported that the addition of a two-day course of tobramycin to initial cefotaxime therapy, followed by oral cefadroxil did not improve outcomes in women with acute pyelonephritis.

Cefazolin should not be used alone for empiric treatment of invasive renal infection owing to the prevalence of resistance to these agents among nosocomial and community-acquired pathogens. Wide-spectrum penicillin derivatives and third-generation cephalosporins have been compared prospectively to the aminoglycoside with ampicillin regimen (Table 34-4) (188,196–199). In clinical studies reported to date, all regimens appear equivalent. Piperacillin, mezlocillin, and azlocillin are active *in vitro* against most strains of *P. aeruginosa* and *E. faecalis*. They are preferred over the third-generation cephalosporins for empiric treatment if either of these pathogens is a probable etiologic agent. Piperacillin should generally be used in combination with an aminoglycoside. Aztreonam is a monobactam ß-lactam antibiotic, with an antibacterial spectrum limited to aerobic Gram-negative rods, including *P. aeruginosa* (196). It has been used successfully to treat patients with acute pyelonephritis. The third-generation cephalosporins, including cefotaxime, ceftizoxime, ceftazidime, and ceftriaxone, have each been studied as single-agent alternatives to the combination of ampicillin and an aminoglycoside, with cure rates for acute pyelonephritis of about 90% in patients with normal renal function and a normal urinary tract (188,197–199). The ß-lactam inhibitors in combination with a ß-lactam antibiotic are also effective and equivalent to other regimens and may be drugs of choice for hospital-acquired infections. Piperacillin-tazobactam, ampicillin-clavulanic acid, ticarcillin-clavulanic acid, and cefoperazone with sulbactam are all effective regimens. Imipenem-cilastatin or meropenem have been used successfully to treat complicated urinary infection with multiply resistant organisms. In a compilation of several studies, however, nine of 41 adequately evaluated patients experienced relapse following a course of imipenem therapy (199). Parenteral fluoroquinolones such as ciprofloxacin, ofloxacin, and levofloxacin are also useful agents for the treatment of acute pyelonephritis (200,201). Vancomycin may be required to treat enterococci or staphylococci resistant to the ß-lactam antibiotics. Newer parenteral antimicrobial agents are more expensive than the standard therapy of ampicillin plus aminoglycoside and potential therapeutic advantages must be weighed against the increased expense.

Oral Antimicrobial Therapy

The usual approach is to switch patients to oral therapy once they have improved. In addition, patients presenting with mild to moderate symptoms may be treated entirely with oral therapy. Pinson and colleagues (202) and Warren and colleagues (188) reviewed studies that document efficacy of oral regimens for treatment of acute pyelonephritis. At the time of review, they suggested trimethoprim-sulfamethoxazole, amoxicillin-clavulanic acid, and norfloxacin have been studied well enough to document efficacy, but that cephalosporins and amoxicillin cannot be recommended (Table 34-5). Amoxicillin by itself should not be used for nonenterococcal or nonstreptococcal infection because of high community rates of resistance to Gram-negative organisms and a tendency to promote colonization with resistant organisms that may cause subsequent infections (188,203). However, amoxicillin in combination with the ß-lactamase inhibitor clavulanic acid can be prescribed as an oral regimen for multiply resistant organisms.

Stamm and colleagues (204) compared 2- and 6-week regimens of trimethoprim-sulfamethoxazole or ampicillin in the outpatient management of women with pyelonephritis. They found trimethoprim-sulfamethoxazole to be superior to ampicillin for both treatment durations; the 6-week regimen did not improve cure rates. For trimethoprim-sulfamethoxazole, 83% to 90% of women remained cured at 6-week follow-up following discontinuation of antibiotics. Thus, for populations where resistance to trimethoprim-sulfamethoxazole is less than 5% in community isolates, trimethoprim-sulfamethoxazole remains a therapy of choice for oral outpatient management of pyelonephritis (188).

A comparative study randomized 378 women with acute pyelonephritis to oral trimethoprim/sulfamethoxazole for 14 days compared to oral ciprofloxacin for 7 days (205). In either regimen, an initial parenteral dose of ceftriaxone or ciprofloxacin, respectively, could be given. In this study, therapy was not modified once antimicrobial susceptibilities were obtained (i.e., therapy with TMP/SMX was continued even if

TABLE 34-5

SELECTED THERAPEUTIC REGIMENS APPROPRIATE FOR TREATMENT OF PYELONEPHRITIS IN PATIENTS WITH NORMAL RENAL FUNCTION

Parenteral therapy
Standard
Ampicillin 1 g q4–6h *plus* aminoglycoside[a] (gentamicin, tobramycin, or netilmicin at 1.5 mg/kg q8h or 3–5 mg/kg q24h, or amikacin at 5 mg/kg q8h or 15 mg/kg q24h)
Alternatives
Cefotaxime, 1 g q8h
Ceftriaxone, 1 g q12–24h
Ceftazidime, 1 g q8–12h
Ceftizoxime, 1 g q8–12h
Aztreonam, 1 g q8h
Piperacillin-tazobactam, IV, 3.375 g q8h
Ticarcillin-clavulanate, 3 g/100 mg q4h
Ampicillin-sulbactam, 2 g/0.5–1.0 g q6h
Piperacillin, 2 g q6h *plus* aminoglycoside
Ciprofloxacin, 400 mg IV q12h
Ofloxacin, 200–400 mg IV q12h
Levofloxacin, 500 mg 1 g OD
Gatifloxacin, 400 mg OD
Meropenem 500 mg–1g q8h
Ertapenem 1g OD
Oral Therapy
Standard
Amoxicillin 500 mg TID
Trimethoprim-sulfamethoxazole, 160/800 mg BID
Trimethoprim, 100 mg BID
Cephalexin, 500 mg QID
Norfloxacin, 400 mg BID
Ciprofloxacin, 500 mg BID
Alternatives
Amoxicillin-clavulanate, 500 TID or 875 mg BID
Cefuroxime axetil 500 mg BID
Ofloxacin, 400 mg BID
Lomefloxacin, 400 mg OD
Levofloxacin, 500 mg OD
Gatifloxacin, 400 mg OD

[a] Aminoglycosides may be prescribed in a once-daily dose.

the infecting isolate was found to be resistant). The regimens were equally effective when the infecting organism was susceptible to the antimicrobial given. Because of a higher rate of resistance of *E. coli* to trimethoprim/sulfamethoxazole, the 1-week ciprofloxacin arm was superior overall with a 96% cure rate at 4 to 11 days and 85% at 22 to 44 days posttherapy. TMP/SMX therapy was not effective when given by itself to patients with TMP/SMX resistant organisms.

Cronberg (206) enrolled 171 patients, about 60% of whom were women with acute pyelonephritis, into a comparative study of initial cefuroxime for 2 to 3 days and, once fever had subsided and culture results were available, randomized to ceftibuten 200 mg bid or norfloxacin 400 mg bid to complete 10 days. The norfloxacin treatment arm was superior for eradication of bacteriuria. Sanchez (207) enrolled 105 women into a study comparing ceftriaxone one gram daily for 10 days compared with a single initial dose of ceftriaxone followed by oral cefixime for 9 days. The cure rate was 91% for both groups. These studies and substantial clinical experience all support oral therapy for infection, with limited initial parenteral therapy for selected patients. In a modelling exercise, Yen (208) compared trimethoprim/sulfamethoxazole to norfloxacin for 10 day outpatient oral therapy for acute pyelonephritis in young women. Using the assumptions in their model they concluded that norfloxacin would be more effective and less costly then trimethoprim/sulfamethoxazole. Their base assumptions were a lower cure rate with TMP/SMX and a higher adverse event rate, which explains the benefit of the norfloxacin regimen. Several studies have also examined the use of quinolones in oral therapy (201). Ciprofloxacin, norfloxacin, ofloxacin, and levofloxacin are all efficacious for the treatment of acute pyelonephritis. It is likely that all fluoroquinolones with primarily renal excretion are equally effective (201). Quinolone antimicrobials, which are extensively metabolized and have low urinary levels, such as sparfloxacin or grepafloxacin, may not be as effective. The newer quinolones with an extended spectrum of greater Gram-positive and anaerobic coverage do not provide additional benefit in treatment of pyelonephritis compared to earlier agents.

Pregnant Women

It is generally recommended that pregnant women with acute pyelonephritis be admitted to hospital, at least the first several days, to ensure that they respond to antimicrobial therapy and ascertain the infecting organism and susceptibilities. Parenteral outpatient therapy with close monitoring is an option for some patients in the first and early second trimesters only (209). A prospective, randomized trial of intravenous ampicillin and gentamicin, intravenous cefazolin, or intramuscular ceftriaxone found all three to be equally effective for parenteral treatment of acute pyelonephritis in pregnancy (210). Despite this study, empiric cefazolin therapy should be avoided given the relatively high prevalence of resistance in community *E. coli.*

Children

There are limited clinical trials which address treatment of acute pyelonephritis in children. Infants of one month or less require hospitalization for treatment and investigation because of the very high prevalence of concomitant bacteremia and uropathology. For parenteral therapy aminoglycosides or a third generation cephalosporin are preferred, and trimethoprim/sulfamethoxazole or amoxicillin/clavulanic acid are first line agents for oral therapy (211). Fluoroquinolones are avoided because of concerns about adverse effects on cartilage. Outpatient parenteral therapy is an option (212). Clinical trials that have compared oral beta lactam therapy with intravenous ceftriaxone found no differences in outcome for older children treated for 10 to 14 days (211). Studies which have examined short courses of parenteral antibiotics followed by oral therapy compared with longer courses of intravenous treatment have shown that at long-term follow-up at 3 or 6 months there was no significant difference between the two treatment regimens with regard to renal scarring (213,214). Thus, initiating therapy with a short initial course of intravenous therapy and completing with oral therapy for children who are clinically stable, with or without initial parenteral therapy, is recommended.

Duration of Therapy and Follow-Up

Most patients become afebrile within 72 hours of the onset of therapy; other clinical findings, including renal-angle tenderness, rapidly improve. Behr et al. (215) reported in a series of patients admitted with both complicated and uncomplicated acute pyelonephritis, that 26% remained febrile at 48 hours and 13% at 72 hours. The median duration of fever was 34 hours. Johnson et al. reported that 30% of women with acute uncomplicated pyelonephritis remained febrile at two days, but virtually all were afebrile by 72 hours (191).

TABLE 34-6

ABNORMALITIES ASSOCIATED WITH COMPLICATED RENAL INFECTION

Associated systemic diseases
Diabetes mellitus
Neutropenia
Immunosuppression
Postrenal transplantation
Renal insufficiency
Underlying structural abnormalities
Congenital abnormalities (obstruction and/or stasis)
Acquired obstruction (pelvocalyceal, ureteral, urethral)
Neurogenic bladder
Catheter associated (continuous or intermittent)
Cystocele
Bladder diverticula
Renal cyst
Renal calculus
Atrophic or malfunctioning kidney
Less susceptible pathogens
Pseudomonas aeruginosa
Proteus mirabilis
Candida albicans

The duration of therapy required for optimal cure rates in patients with acute pyelonephritis is uncertain. A minimum of 2 weeks of therapy has been routinely recommended. However, 10 days are clearly sufficient in many studies (192,207) and 7 days (205) and 5 days (194) have also been reported effective in carefully controlled clinical trials. Currently, 10 to 14 days are recommended.

About 10% of women with acute pyelonephritis have a relapse with the initial infecting organism within 4 weeks of completing therapy (216). For men with an initial episode of urinary infection, as many as 30% will relapse, presumably because of prostatic localization of infection. About 50% of patients with urinary infection in the setting of structural or functional genitourinary abnormalities (Table 34-6) recur by 6 weeks after therapy (217,218). Generally, urine cultures posttherapy are obtained only if symptoms recur. Patients with symptomatic relapse should be reassessed clinically and, if appropriate, investigated radiologically to exclude calculi, obstruction, or other abnormalities.

Recurrent Upper Tract Infection

Urinary infection recurrence, either upper or lower tract, can be anticipated in about one-half of patients within 1 year of treatment (219). The pattern of recurrence is a predictor of infection site, with relapse predictive of renal infection in women and renal or prostatic infection in men. For women who have a symptomatic relapse, retreatment with a 4- or 6-week course of antimicrobial therapy results in cure for most of those with a normal genitourinary tract (204,217). Longer courses of antimicrobial therapy (4 weeks or more) may be considered in patients with relapsing symptomatic infection or where progressive renal damage is a concern. Men should receive therapy for 6 or 12 weeks because of presumed prostatic infection (220).

Several principles should be considered in all patients with recurrent infections:

1. A negative urine culture on therapy provides *in vivo* evidence of antimicrobial effectiveness against the pathogen. The urine culture becomes negative within 24 hours of institution of effective chemotherapy. Isolation of any quantitative count of the initial infecting organism is failure of therapy. Pyuria usually disappears within 4 days. Bacterial persistence with positive urine cultures occurs owing to inadequate levels of the antimicrobial agent in the urine, the presence of resistant organisms, or patient noncompliance. Continuing the same antimicrobial regimen, even with a clinical response, may be inappropriate if urine cultures remain positive.
2. Relapse is frequently associated with urologic abnormalities, an atrophic or poorly functioning obstructed kidney, or prostatic infection. Infected renal cysts (221), calculi, and prostatic infection allow organisms to remain in sites where effective antimicrobial levels are not achieved. Patients with relapse require careful reevaluation including radiologic and urologic investigation. In the absence of urologic or radiologic abnormalities, most women who have a relapse can be cured with a sufficiently prolonged course of therapy. On the other hand, infection recurs in patients with calculi in the kidneys or prostate infection following even prolonged treatment courses of several months.
3. Urinary infection treatment trials in men have not generally localized infection to the prostate or kidneys. Owing to the frequency of complicating urologic abnormalities in men with urinary infection, attempts to define a homogeneous group with renal infection and determine treatment response have generally been unsuccessful. About one-half of men with relapsing bacteriuria appear to have renal infection, and an equal number have an infected prostate (222). Some may have infection at both sites. Treatment should be undertaken only if recurring symptoms or complicating disease indicate that these men are at risk of morbidity from infection. A prolonged course of therapy with TMP/SMX or a quinolone for at least 6 to 12 weeks should be prescribed to eradicate a persisting but curable renal or prostatic focus. Even with prolonged treatment, however, a cure is obtained in less than one-half of men.
4. Antimicrobial concentration in both renal tissue and urine may be markedly diminished with impaired renal function caused by acute or chronic parenchymal renal disease or obstruction. This phenomenon has not been well studied in acute renal infection. In patients with unilateral renal impairment, the antimicrobial concentration of some agents may be inadequate to inhibit bacterial growth or sterilize urine originating from the diseased kidney (223). The contralateral normal or hypertrophied kidney may be excreting the antibacterial agent effectively, so drug does not accumulate in serum. Excretion of the antimicrobial agent in the urine from the healthy kidney may be adequate to sterilize bladder urine and make it appear that the bacteriologic outcome is satisfactory, despite persistence or continuing bacterial multiplication in the diseased kidney.
5. Patients with frequent reinfections have altered bacterial flora, reflecting the impact of repeated courses of antibacterial agents on the fecal reservoir (224). Sulfonamides, penicillins, cephalosporins, and flouroquinolones eradicate susceptible Gram-negative organisms within the intestinal tract, which may be replaced by resistant Enterobacteriaceae or *Pseudomonas* spp. Broad-spectrum antimicrobial agents excreted preferentially in bile have a greater impact on the fecal flora than do agents preferentially excreted in the urinary tract. The "next infection," if it occurs within 2 to 4 weeks, often will be caused by an organism that is resistant to the therapeutic regimen previously prescribed. With frequent reinfections, particularly in patients with devices such as ureteric stents or an indwelling catheter, the pathogens become progressively resistant to antimicrobial therapy.

6. Treatment of asymptomatic bacteriuria should be avoided except for bacteriuria in pregnancy or prior to an invasive urologic procedure with potential trauma to the genitourinary mucosa (68).
7. Patients with ileal conduits and long-term indwelling suprapubic or urethral catheters are always infected. Although transient suppression may occasionally yield a negative urine culture, bacteriological failure is predictable. In the absence of controlled studies demonstrating sustained benefits, patients should only be treated for symptomatic episodes. Continuous suppressive antibacterial regimens select multiply resistant organisms and are not recommended.

Treatment in the Presence of Impaired Renal Function

There are additional considerations in treating patients with impaired renal function. Agents which might further compromise function should be avoided, if possible, and agents which are effective in the face of decreased renal perfusion should be selected. In addition, antimicrobial therapy may ameliorate symptoms but frequently will not cure infection in the presence of moderate to severe renal impairment. Long-term suppressive therapy with an oral antimicrobial may be required in selected cases for management of recurrent symptomatic episodes.

Aminoglycosides should be avoided. In the presence of unstable renal function, a rise in blood urea or creatinine level may be incorrectly attributed to an aminoglycoside. Nitrofurantoin is contraindicated. It does not achieve adequate urinary levels to inhibit bacterial growth in renal infection and may cause irreversible peripheral neuropathy if prescribed to patients with renal impairment (225). Doxycycline is relatively safe in renal impairment but usually fails to achieve adequate urine levels. Other tetracyclines are contraindicated. Sulfonamides have been associated with further loss of renal function and should not be used. Methenamine mandelate or other organic salts also are contraindicated in patients with renal impairment.

The ß-lactam antibacterials, both penicillins and cephalosporins, have little dose-related toxicity and are relatively safe in patients with renal failure. Patients with impaired renal function are at increased risk of seizures with imipenem, but not meropenem therapy. Interstitial nephritis occurs rarely in patients receiving ß-lactam antibacterial agents, but the risk of this complication is not increased by coexisting renal impairment. Moderate dose reduction of most ß-lactam agents is required if glomerular filtration falls below 25% of normal. No untoward consequences of trimethoprim prescribed without sulfonamide have been reported in patients with moderately advanced renal impairment. The fluoroquinolone antimicrobials, ciprofloxacin, ofloxacin, levofloxacin, and gatifloxacin also are effective in renal failure. Dosage adjustments are required for patients with creatinine clearance rates less than 30 mL/minute. There have been reports of renal failure with concomitant administration of quinolones with cyclosporine and these agents should be used with caution in transplant recipients (226).

PREVENTION

New infections can be prevented by both continuous and intermittent chemoprophylactic regimens. Persistent infections in renal or prostatic tissue can be suppressed indefinitely without "cure" by long-term treatment regimens, preventing symptomatic recurrence.

Prophylaxis

Prophylaxis of symptomatic lower tract infections is highly effective (Chapter 33). These prophylactic regimens also are effective for women with recurrent acute nonobstructive pyelonephritis and should be considered in any woman who experiences two or more episodes of symptomatic infection, either cystitis or pyelonephritis, in 1 year. For males, only Freeman et al. (227) prospectively studied the effectiveness of preventing asymptomatic infection in reducing the occurrence of acute pyelonephritis. This study demonstrated that both sulfonamides alone and nitrofurantoin could suppress endogenous infection and prevent reacquisition in males. Studies with current antimicrobials including trimethoprim-sulfamethoxazole or the fluoroquinolones to prevent new infections have not been reported in men.

Following renal transplantation, continuous prophylaxis with trimethoprim-sulfamethoxazole reduced the incidence of bacteriuria from 38% to 8% during the initial year after transplantation (228). Both symptomatic and asymptomatic urinary infections are prevented with current strategies of TMP/SMX prophylaxis in renal transplant recipients (229).

In children with vesicoureteral reflux, upper tract infection can occur with each reinfection. Long-term prophylaxis prevents most reinfections. In prospective longitudinal studies, Smellie and colleagues (230) and Winberg and colleagues (91) reported that prolonged prophylaxis prevents infection and can prevent progressive renal impairment during infancy and childhood, with resumption of normal renal growth.

Immunization has been suggested as an alternative to chemoprophylaxis. Both capsular antigen and pili have been used to prevent ascending infection in animal models (231–233). Antigenic variations in lipopolysaccharides and *E. coli* pili have made this difficult to achieve. Vaccination of monkeys with PapG prevented ascending pyelonephritis, but human studies are not yet available (234).

Prevention in Pregnancy

Prevention of invasive upper tract infection in pregnant women should be a part of the antenatal care of all patients (75). Pregnant women should be screened for bacteriuria once at 12 to 16 weeks' gestation and those with positive cultures should be treated. Subsequent screening cultures later in pregnancy are not recommended for asymptomatic women whose initial culture is negative. In the absence of a screening and treatment program, acute pyelonephritis will occur in 20 to 30 per 1,000 pregnant women. With an intervention program for treatment of asymptomatic bacteriuria, acute pyelonephritis can be reduced to three to five per 1,000 (75). Pregnant women who have had bacteriuria should be followed with urine cultures throughout pregnancy. Although continuous low-dose prophylaxis has been recommended for patients who have recurrent infections, careful follow-up and treatment may be as effective.

The choice of a regimen for treatment of asymptomatic bacteriuria or for prophylaxis of symptomatic or asymptomatic infection in pregnancy must address potential adverse effects on the fetus. The flouroquinolones are contraindicated, because of potential harmful effects on fetal cartilage development. Although trimethoprim/sulfamethoxazole is generally considered safe during pregnancy, residual concerns about a potential teratogenic effect from the trimethoprim component limits use, especially during the first trimester. The penicillins and cephalosporins are safe in pregnancy, and nitrofurantoin is safe but is avoided at term because of potential hemolysis of fetal hemoglobin. Although ampicillin or a cephalosporin is not

usually recommended, where the organism susceptibility is known, as is the case for asymptomatic bacteriuria, they are appropriate for treatment. Cephalexin may also be used for prophylaxis, at 250 mg/day, and nitrofurantoin may be used for either treatment or prophylaxis.

Prevention of Catheter-Associated Upper Tract Infection

Catheter-associated infections are the most common hospital-acquired infection and a frequent cause of hospital-acquired bacteremia (21). Infections can be prevented or delayed during short-term catheterization by limiting the duration of catheterization, maintaining a closed drainage system, and insertion under sterile technique. Infection control programs must ensure that prevention practices are current and maintained to minimize acquisition of nosocomial bacteriuria.

Prevention of Infection in Patients with Neurogenic Bladders

Bacteriuria is an anticipated complication following neurologic injury or disease complicated by a neurogenic bladder. Increased residual urine and reflux are major reasons for the development of bacteriuria. Maintenance of a low-pressure voiding system is important to prevent complications of urinary infection (111). Prophylactic antimicrobials are not recommended in spinal cord-injured patients managed with intermittent catheterization (235). Although trimethoprim-sulfamethoxazole, trimethoprim, or nitrofurantoin prophylaxis may prevent infection in the acute or early injury phase, they are not effective in the long term. Problems associated with induction of resistant bacteria outweigh benefits.

Continuous Suppression

Some patients experience repeated relapse despite prolonged antimicrobial treatment. Continuous long-term suppression should be considered if these patients have symptomatic recurrences or underlying complicating urologic abnormalities. For instance, patients with infected renal calculi or obstructive lesions leading to recurrent symptomatic infection that cannot be remedied by surgery should receive continuous suppressive antimicrobial treatment.

In a large study of bacteriuric males, Freeman and colleagues compared continuous suppressive therapy with one of three regimens (sulfamethoxazole, nitrofurantoin, and methenamine mandelate) to placebo in 229 men with bacteriuria (237). During a 10-year follow-up, continuous therapy reduced the number of symptomatic recurrences and maintained the urinary tract free of bacteriuria more effectively than did placebo. Whereas only 44% of the patients on treatment regimens required additional therapy for acute exacerbations, 93% of the patients on placebo required treatment. Antimicrobial agents currently available may be even more effective. Suppression could not be achieved in all patients with the treatment regimens used. Prostatic and renal calculi, previous renal scars, and *E. faecalis* infections all predicted failure of suppressive therapy.

Sheehan and colleagues (236) compared 12 to 24 weeks of norfloxacin therapy for complicated recurrent urinary infection in a prospective, randomized, blinded study. The longer antimicrobial course led to fewer failures or reinfections compared to placebo, serving as prophylaxis, suppressive, and curative therapy.

Chinn and associates also demonstrated that antibacterial suppression is effective in patients with renal calculi (237). No patients had further loss of renal function during a cumulative 77 years of continuous observation despite the presence of stones and partial obstruction. Renal calculi increased in size in only four of the 22 patients during the period of antibacterial suppression, and four of six patients with impaired renal function had a fall in serum creatinine during suppression. Long-term antibacterial treatment should be combined with operative removal to prevent stone recurrence. Advances in the endourologic treatment of infected renal calculi make it possible for most patients with renal stones to be treated with complete removal of all calculous material (108). Continuous antibacterial treatment for as long as 3 to 6 months is recommended with careful follow-up for recurrence of infection with urea-splitting pathogens, particularly *Proteus* spp. Permanent suppression may be necessary if any calculous material remains in the upper tract.

The antimicrobial dosage required to maintain patients free of bacteriuria with suppressive therapy is not well documented in clinical studies, but generally one-half the treatment dose may be used. In stable patients, the dose may sometimes be reduced further. Patients on suppressive regimens should be reviewed periodically to ensure compliance, obtain urine cultures, and repeat renal function assessment. "Breakthrough" bacteriuria occurs occasionally. If the initial infecting organism has reappeared, then susceptibility testing will determine whether it has become resistant or has recurred because of an inadequate suppressive dose of medication. New infections may occur because of entry of a new infecting pathogen and are usually resistant to the existing treatment regimen. Without stopping suppressive therapy, the patient should receive a 2-week course of a second agent to eradicate the new pathogen. The suppressive regimen should be continued throughout if the underlying "permanent" pathogen is being suppressed effectively. No guidelines have been developed for the optimal length of continuous suppressive therapy. If the indications for continuous suppressive therapy persist, therapy may need to be continued indefinitely.

VIRAL INFECTIONS OF THE KIDNEY

Viruria commonly occurs during viremic infections as a manifestation of acute generalized disease with limited, if any, involvement of the kidneys. Many viruses multiply in tubular epithelium and are excreted in the urine as exfoliated infected cells. The presence of characteristic inclusions in urine sediment is a useful diagnostic parameter for several viral illnesses. Viral infections of the kidney, however, rarely produce acute inflammatory changes in renal tissue or precipitate symptoms that lead to recognized clinical renal illness. Chronic renal impairment is not a known consequence of viral infections.

Viruses have been isolated from the urine in at least 16 viral diseases (238). Rubella, varicella-zoster, measles, and cytomegalovirus are commonly isolated during clinical illness, but no disease with renal manifestations owing to these agents has been recognized (238). Mumps frequently produces transient renal impairment with a reduction in glomerular filtration and significant proteinuria and hematuria. These abnormalities disappear within 1 month of the illness (239). Histologic studies suggest that viral multiplication in renal tubular cells induces these changes. Coxsackie B virus has also been associated with mild renal impairment.

Infectious mononucleosis may have renal manifestations. Hematuria and proteinuria occur in 11% and 18% of patients,

respectively (240). Acute renal failure owing to interstitial nephritis with few glomerular changes has been described (241). The duration of the renal disease appears to be shortened with high-dose corticosteroid treatment.

Papovaviruses (the BK virus strain) are frequently isolated from the urine of patients during treatment with immunosuppressive drugs following transplantation and the immunocompromised states (242). Viruses can be seen by electron microscopy in uroepithelial cells from the renal pelvis, virus-specific antibody is present in urine, and decoy cells can be visualized with Papanicolaou's stain. BK virus nephropathy with graft dysfunction and tubular necrosis occurs in some immunocompromised patients with positive PCR for BK virus DNA in plasma and urine (242). The only treatment is reduction of immunosuppression.

The hemorrhagic fever viruses frequently cause renal impairment and are important health problems in many parts of the world (243). Viral hemorrhagic fever with renal syndrome occurring in Korea, Scandinavia, the Soviet Far East, and the Balkans is caused by members of the Bunyaviridae genera (243,244). The prototype agent is the Hantaan virus, originally isolated in Korea in 1978 (245). Rodents are the reservoirs for these agents, and transmission to humans occurs by respiratory aerosols with no intermediary vector (232). Secondary cases have not been described in humans. The virus has been isolated from rats trapped in East Coast seaports of the United States (244).

High fever, myalgias, severe headache, and a petechial rash characterize the illness. Thrombocytopenia is common. Hypotension and oliguria develop on about the fifth day of fever. Pathologic examination discloses widespread capillary damage with leakage of intravascular plasma and red cells. Acute oliguric renal failure with massive proteinuria is a frequent complication. Renal biopsy specimens show extensive necrosis of tubular epithelium with anatomically normal glomeruli except for the presence of extravasated red cells and protein-rich fluid. The overall mortality can be markedly reduced by supportive therapy, including dialysis, and in recent series, it has been 5% or less.

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CHAPTER 35 ■ RENAL AND PERIRENAL ABSCESSES

LOUISE-MARIE DEMBRY AND VINCENT T. ANDRIOLE

Bacterial infections of the kidney and perinephric space include a spectrum of pathologic conditions that can be divided into intrarenal and perirenal abscesses. Both conditions are suppurative infections localized either within the parenchyma of the kidney (intrarenal abscess, i.e., renal cortical abscess and corticomedullary abscess) or within the perirenal fascia external to the kidney capsule (perinephric abscess), and each can be identified by specific diagnostic techniques. The incidence of intrarenal and perirenal abscesses ranges from 1 to 10 cases per 10,000 hospital admissions. In the preantibiotic era, most cases were caused by hematogenous seeding from distant foci of infection and were predominantly in young males without an antecedent history of renal disease. Currently, most cases occur as a complication of urinary tract infection and affect males and females with equal frequency. The incidence increases with age and an abnormality of the genitourinary tract often exists. This chapter covers only the more common types of these renal and perirenal infections.

INTRARENAL ABSCESS

Renal Cortical Abscess (Renal Carbuncle)

Etiology

A renal carbuncle (from the Latin, *carbunculus,* or "little coal") is a circumscribed, multilocular abscess of the renal parenchyma, which forms from a coalescence of multiple cortical microabscesses. It is most commonly caused by staphylococci *(Staphylococcus aureus)* and is the result of metastatic spread from a primary focus of infection elsewhere in the body most commonly the skin. Renal carbuncles were first described by Israel in 1905, in a presentation before the Free Society of Berlin Surgeons (1). Although numerous reports and reviews (2–12) have been published since Israel's initial description, the total number of reported cases of renal carbuncle remains relatively small.

Pathogenesis

A renal cortical abscess results from a primary focus of infection elsewhere in the body (Fig. 35-1). Common primary foci are cutaneous carbuncle, furunculosis, cellulitis, paronychia, osteomyelitis, endovascular infection, and infection of the respiratory tract. Important predisposing conditions that increase the risk of bacteremia and hematogenous abscess are injection drug use, hemodialysis, and diabetes mellitus. *Staphylococcus aureus* is the most common causative agent (90%) and infects the cortex of the kidney by hematogenous dissemination from the primary focus, resulting in several interconnecting furuncles or microabscesses. Coalescence may occur with progression of the infection so that the lesion consists of a fluid-filled mass with a relatively thick wall. Rarely, the process may extend to the periphery of the renal cortex and rupture through the capsule, leading to formation of a perinephric abscess. The majority of renal cortical abscesses are unilateral (97%), single lesions (77%), in the right kidney (63%), and are not associated with perinephric abscesses (90%). The reason for unilateral localization is not clear, although diminished resistance of the kidney resulting from previous disease or injury, including trauma, has been cited as a predisposing factor (13). Infrequently, ascending infection causes a renal cortical abscess (14,15). Because the interval between the original staphylococcal infection and the onset of clinical symptoms of a renal cortical abscess may vary from a few days to many months (average time of approximately 7 weeks) (13), the primary focus of infection may have healed and is not apparent in one-third of affected patients (6,8,11).

Clinical Features

Renal cortical abscesses are three times more common in males than females. The disease occurs at all ages but is most common between the second and the fourth decades of life (6,7,13). The clinical picture of a renal cortical abscess is nonspecific. Most patients have chills, fever, and abdominal or back pain (8,11,13). Some may have a palpable flank mass. Others present with a clinical picture of fever of undetermined origin, with few or no localizing signs (16). Most patients have no urinary symptoms (13), because the abscess occupies a circumscribed area within the parenchyma of the kidney, which may not communicate with the excretory passages.

Physical examination often reveals tenderness in or near the region of the kidney. Pain on fist percussion of the costovertebral angle is the most constant physical finding, often accompanied by moderate muscle rigidity in the upper abdominal and lumbar muscles. A flank mass or a bulge in the lumbar region, with loss of the natural concave lumbar outline, may be present. Examination of the chest on the affected side may be abnormal, with decreased respiratory excursion, tenderness over the lower ribs, dullness, diminished breath sounds, increased fremitus, or rales.

Basic laboratory data are variable. Peripheral white blood cell counts are moderately elevated in 95% of the patients (6,13). The urinalysis usually presents no pathognomonic findings. Proteinuria, pyuria, or microscopic hematuria usually are present and a Gram stain of the urine will demonstrate the pathogen if the abscess communicates with the collecting system of the kidney. However, negative urinalyses are seen in most patients and blood cultures are usually negative (13).

Diagnosis

Renal cortical abscesses must be differentiated from other space-occupying lesions in the kidney. Renal tumors, cysts, intrarenal abscesses caused by aerobic Gram-negative bacilli, and perinephric abscesses can mimic renal cortical abscesses. In the past, surgical exploration was performed to differentiate the renal mass from a carcinoma (5). The clinical presentation of a

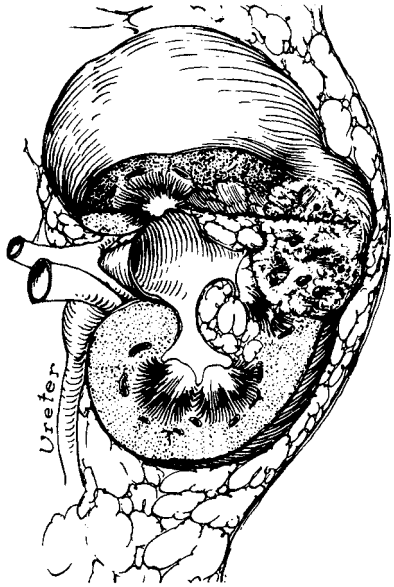

FIGURE 35-1. Diagram of the pathogenesis of a staphylococcal renal carbuncle. (From: Andriole VT. Renal carbuncle. *Medical Grand Rounds* 1983;2:259, with permission.)

renal cortical abscess is nonspecific and not helpful in differentiating this disease from a renal tumor or perinephric abscess. Chills, fever, malaise, and back pain may be seen in each. A renal cortical abscess on the anterior surface of the kidney may produce abdominal symptoms and lead to an erroneous diagnosis of an intraabdominal process. Renal cortical abscesses may also be confused with abscesses of the renal medulla, particularly in children (11,13–15,17–19). Radiologic techniques can define the character of the renal mass and establish the correct diagnosis (20–29).

In intravenous pyelograms, a renal cortical abscess appears as a mass of diminished density, frequently associated with distortion of the calyces, infundibulum, and renal outline. An abscess that extends to the periphery of the renal cortex may produce sufficient edema of the renal capsule to obliterate a segment of the perirenal fat shadow. However, there is no displacement of the kidney, as is frequently seen with a perinephric abscess. Thus, an abnormal intravenous pyelogram that demonstrates an intrinsic mass with caliceal distortion, but without displacement of the kidney in a patient with sterile urine, suggests a diagnosis of renal cortical abscess or tumor.

Ultrasonography has been extremely helpful in establishing the diagnosis of renal cortical abscess (13). Renal ultrasonography can provide morphologic detail of the kidneys; it is capable of identifying cystic lesions, tumorlike masses, or abscess cavities; and can show the size and location of the lesion. Early in the development of a renal cortical abscess, however, internal echoes may be present, giving the appearance of a solid or semisolid mass. Because these findings are compatible with either a renal cortical abscess or tumor, renal angiography may be performed to define the lesion further and to establish the correct diagnosis (4,8,11,13,20,22,26,27,29). After coalescence, an abscess can be identified by ultrasound as a fluid-filled mass with a relatively thick wall (Fig. 35-2). Ultrasonography also can be used to guide aspiration of the lesion and to follow its resolution with antibiotic treatment (11,28,30) (Fig. 35-3).

Selective renal arteriography may be required to differentiate a renal cortical abscess from a tumor. A renal cortical abscess can be identified angiographically as a mass that produces arcing, stretching, and attenuation of adjacent arteries, with the vessels located around the circumference rather than within the mass (Fig. 35-4). Early in the course, the rim around the abscess is poorly visualized, but arterial circulation to the periphery gradually increases with time so that a late study may identify a dense rim in the parenchymal phase. An untreated abscess may progress to a stage in which the rim is thick and poorly vascularized.

Renal and perirenal abscesses can be arteriographically distinguished from tumors, because the major portion of an abscess is avascular while the wall of the abscess is hypervascular. Renal carcinoma may be hypervascular or hypovascular

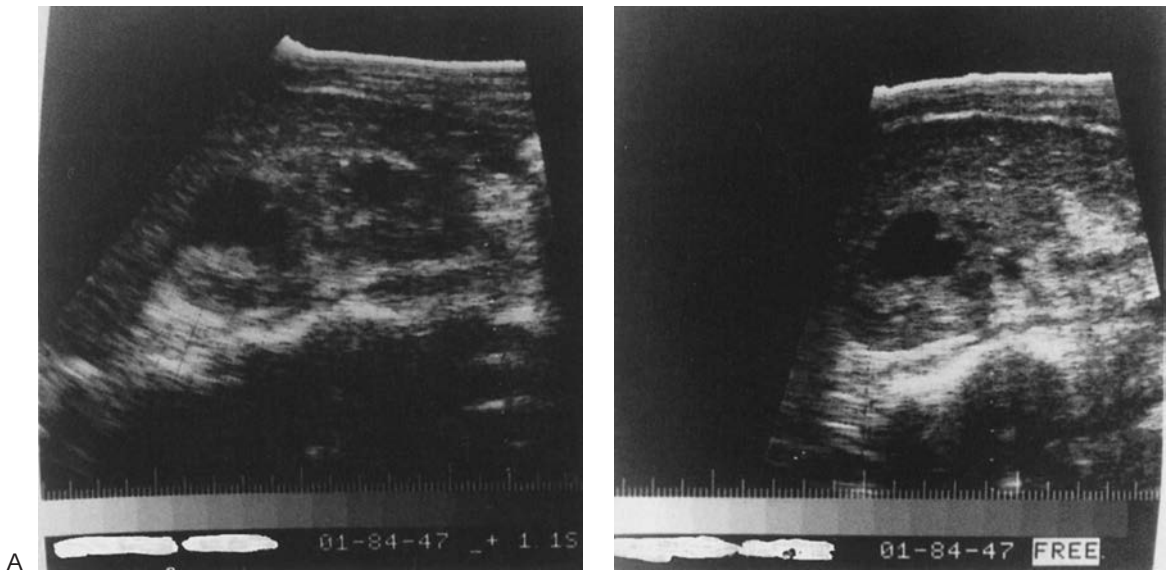

FIGURE 35-2. Ultrasonogram of the right kidney on admission to the hospital. **A:** Longitudinal view, demonstrating two echolucent fluid-filled lesions. **B:** Transverse view, demonstrating fluid-filled masses with thickened margins. (From: Andriole VT. Renal carbuncle. *Medical Grand Rounds* 1983;2:259, with permission.)

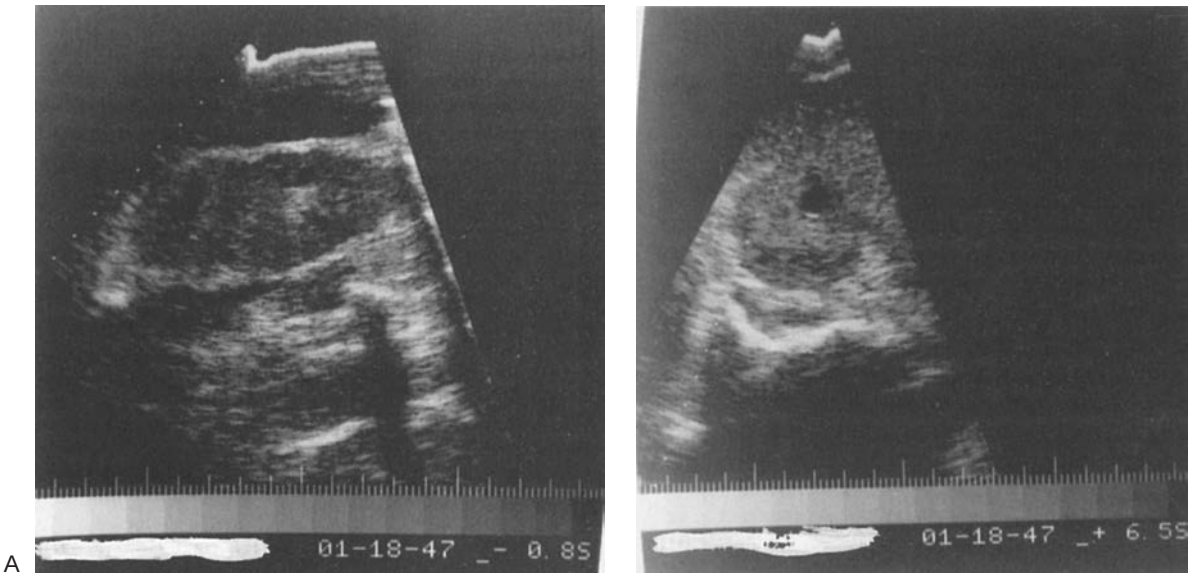

FIGURE 35-3. Ultrasonogram of the right kidney after 4 weeks of antibiotic therapy (from the same patient as in Figure 35-2). Longitudinal view (**A**) and transverse view (**B**) showing a decrease in the size of the fluid-filled echolucent lesions. (From: Andriole VT. Renal carbuncle. *Medical Grand Rounds* 1983;2:259, with permission.)

(necrotic), but rarely both. In an abscess, the arteries retain their normal organization and branching pattern. Tumor neovascularity, in contrast, consists of abnormal vessels. Tumor vessels have no recognizable organization, may enlarge instead of taper as they course peripherally, and have an abnormal branching pattern.

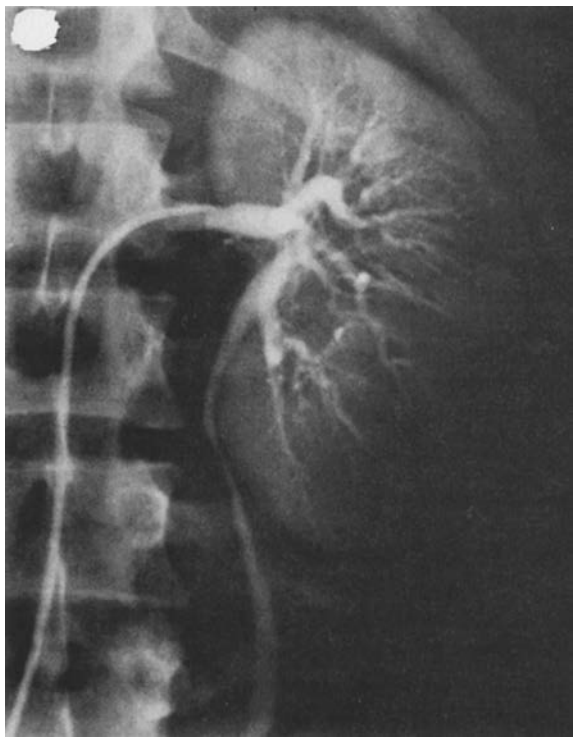

FIGURE 35-4. Arterial phase of left renal arteriogram. Peripheral vessels of the lower pole are attenuated and separated in comparison to normal vessels in the upper pole. No tumor vessels are present. (From: Andriole VT. Renal carbuncle. *Medical Grand Rounds* 1983;2:259, with permission.)

Occasional chronic inflammatory lesions may mimic tumors angiographically, especially if the mass is solid and contains central vascularity. However, in the presence of intrinsically normal peripheral arteries and absent central vascularity, a diagnosis of abscess can be established accurately. Oblique projections may be necessary to verify the absence of central vascularity (11,25,26).

Renal scanning with ^{67}Ga citrate (Fig. 35-5) also has been useful in localizing a renal abscess in adults (21,30,31). A subtraction technique using ^{67}Ga citrate and ^{99}Tc glucoheptonate can define the extent of perinephric involvement and eliminate any false-positive scans seen with gallium alone (31).

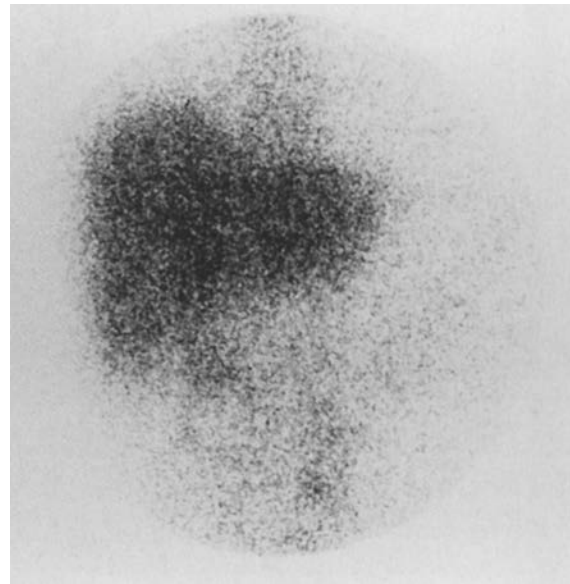

FIGURE 35-5. Radionuclide scan with ^{67}Ga citrate at 48 hours, showing abnormal uptake in the right upper quadrant, inferior to the liver and right in the area of the kidney. (From: Andriole VT. Renal carbuncle. *Medical Grand Rounds* 1983;2:259, with permission.)

The latter may occur in patients with renal carcinoma, severe pyelonephritis without abscess formation, or ureteral obstruction. ^{111}In-labeled white cell scanning identifies a renal abscess, but does not demonstrate a renal carcinoma.

Computed tomography (CT) is the most accurate noninvasive technique currently in widespread use and permits detection of abscesses smaller than 2 cm (32–34). Computed tomography is useful if ultrasonography is negative or equivocal and allows for the detection of unsuspected occult abscesses. Computed tomography also is useful as a guide to percutaneous aspiration of an abscess and to follow a known lesion. An abscess appears as a sharply demarcated low-density lesion on CT. The abscess does not enhance with contrast because of its avascular nature; however, the wall of the abscess enhances because of the presence of dilated and inflamed vessels (30,33,34). The finding of gas in a low density mass is pathognomonic for an abscess (33). Such noninvasive techniques (ultrasound and CT) have reduced the need for angiography to further define intrarenal masses.

Radiologic techniques can correctly establish a diagnosis of a renal cortical abscess only when this diagnosis is considered. Unfortunately, clinicians generally do not think of the diagnosis of renal cortical abscess early in its course. An average delay of 62 days before the correct diagnosis was established and proper treatment instituted has been reported (13).

Treatment

Historically, the treatment of a renal cortical abscess has been surgical and has varied with the condition of the patient (6,7,9,12). However, since a renal cortical abscess is usually hematogenous in origin and caused by *S. aureus,* it often responds to antistaphylococcal antimicrobial therapy alone, thus obviating the need for surgical intervention (13).

If the diagnosis of renal cortical abscess is suspected from the history, physical findings, and renal ultrasonography (abscess localized to the renal parenchyma) or CT, and large, Gram-positive cocci or no bacteria are seen on microscopic examination of the urine, antimicrobial therapy should be directed against *S. aureus*. Oxacillin or nafcillin 1 to 2 g IV every 4 to 6 hours is appropriate initial therapy. If a history of penicillin allergy (rash) is present, cefazolin (2 g every 8 hours) or vancomycin (1 g every 12 hours) IV is a recommended alternative in patients with normal renal function. Patients with severe immediate penicillin allergy may manifest a cross-reacting allergy when a cephalosporin is administered and should receive vancomycin instead. Renal cortical abscesses can be cured with parenteral antibiotic therapy administered for a minimum of 10 days to 2 weeks, followed by oral antistaphylococcal therapy for at least an additional 2 to 4 weeks. Fever gradually subsides over a 5- to 6-day period without recurrence. Flank or back pain abates rather quickly, and patients display significant clinical improvement within 24 hours of initiating antibiotic therapy. A prompt response to treatment justifies continuing antibiotic therapy without surgical intervention, and serial ultrasound or CT examinations can be used to show progressive reduction and ultimate disappearance of the renal mass. A contrary clinical course should suggest misdiagnosis or uncontrolled infection, with the development of perinephritis, perinephric abscess, or infection with an organism resistant to the antibiotics being administered. In such cases, modification of therapy may be required, based on the results of cultures of blood, urine, or purulent material percutaneously aspirated from the abscess, or surgical drainage may be necessary. Nevertheless, a trial of intensive antibiotic treatment is warranted in lesions localized to the renal parenchyma. If the patient does not respond within 48 hours, percutaneous, ultrasonically, or CT-guided needle aspiration of the intrarenal fluid-filled lesion can be attempted as a substitute for surgical drainage (35–38). If this treatment is unsuccessful, operative intervention should be undertaken.

Renal Corticomedullary Abscess

Etiology

Enteric aerobic Gram-negative bacilli, predominantly *Escherichia coli, Proteus* spp., and less commonly, *Klebsiella* spp., *Enterobacter* spp., and *Pseudomonas* spp. are usually responsible for intrarenal corticomedullary infections in association with vesicoureteral reflux or other urinary tract abnormalities.

Pathogenesis

Renal corticomedullary bacterial infections include a variety of acute and chronic parenchymal inflammatory processes. The more severe forms of these infections include acute focal bacterial nephritis, acute multifocal bacterial nephritis, and xanthogranulomatous pyelonephritis, which almost always involve only one kidney. *Acute focal bacterial nephritis* is an uncommon, severe form of acute infectious interstitial nephritis presenting with a renal mass caused by acute focal infection without liquefaction (39). This entity is also referred to as focal pyelonephritis or acute lobar nephronia, because the pathology consists of a heavy leukocytic infiltrate confined to a single renal lobe with focal areas of tissue necrosis. *Acute multifocal bacterial nephritis* is also a severe form of acute renal infection in which a heavy leukocytic infiltrate occurs throughout the kidney with frank intrarenal abscess formation. Acute focal bacterial nephritis may represent an early phase of acute multifocal bacterial nephritis (40). *Xanthogranulomatous pyelonephritis* is a very rare and atypical form of severe chronic renal infection. Schlagenhaufer initially described the pathologic features of xanthogranulomatous pyelonephritis (41) in 1916. Grossly, the entire kidney or its involved segment is enlarged and may be fixed by perirenal fibrosis or retroperitoneal extension of the granulomatous process, which often resembles an inoperable tumor. Xanthogranulomatous pyelonephritis is classified into three stages based on the extent of involvement of renal and adjacent tissue by the xanthogranulomatous process (42). In stage I (nephric), the xanthogranulomatous inflammatory process is confined to the kidney. Stage II lesions (perinephric) involve the renal parenchyma and Gerota's fat, whereas stage III lesions (paranephric) involve the renal parenchyma and its surrounding fat with widespread retroperitoneal involvement. Each stage is further divided into focal or diffuse, depending on the amount of parenchymal involvement. Microscopically, the disease is characterized by massive tissue necrosis and phagocytosis of liberated cholesterol and other lipids by xanthoma cells (macrophages). These foamy xanthomatous histiocytes appear to simulate clear-cell renal carcinoma (43,44).

Acute focal bacterial nephritis, acute multifocal bacterial nephritis, and xanthogranulomatous pyelonephritis most commonly occur as a complication of bacteriuria and ascending infection, associated with tubular obstruction or scarring from prior infections, renal calculi, vesicoureteral reflux, urinary tract obstruction, or other abnormalities of the genitourinary system or in association with the endocrinopathies of diabetes mellitus or primary hyperparathyroidism (13,15, 18,19,39,40,44–49). These predisposing factors, particularly vesicoureteral reflux in children and renal calculi or other forms of urinary obstruction in adults, lead to intrarenal reflux and provide an avenue for bacteria to inoculate the renal parenchyma. Parenchymal infection develops with abscess

formation because the kidney is unable to clear the infection in the presence of reflux, urinary obstruction, medullary scarring, or other causes of tubular obstruction. In adults, two-thirds of intrarenal abscesses caused by aerobic Gram-negative bacilli are associated with renal calculi or damaged kidneys, whereas in children this process is often associated only with vesicoureteral reflux. The incidence of renal abscesses in patients with diabetes mellitus is twice that in nondiabetic persons. In contrast to the staphylococcal renal cortical abscess of hematogenous origin, the Gram-negative bacillary corticomedullary abscess of the kidney frequently produces severe renal infection. Although renal corticomedullary infections are confined within the substance of the kidney, they may perforate the renal capsule and form a perinephric abscess, extend toward the renal pelvis and drain into the collecting system, or develop into a chronic abscess (47). The etiology of xanthogranulomatous pyelonephritis is undefined; however, it appears to be related to a combination of chronic urinary tract infection and renal obstruction. Seventy-five percent of patients have renal calculi, 50% of which are staghorn calculi. Additional predisposing factors include chronic segmental or diffuse renal ischemia resulting in alterations in renal or lipid metabolism or both, lymphatic obstruction, abnormal immune response, diabetes mellitus, and primary hyperparathyroidism (44,50,51).

Clinical Features

Renal corticomedullary abscesses affect males and females with equal frequency except for xanthogranulomatous pyelonephritis in adults, where females are more frequently affected than males (50,51). Although these infections occur in all age groups, the incidence increases with advancing age. Peak incidence for xanthogranulomatous pyelonephritis occurs in the fifth to seventh decade and has been reported to occur in transplanted kidneys as well as native kidneys (52). Most patients with acute focal bacterial nephritis, multifocal bacterial nephritis, or xanthogranulomatous pyelonephritis experience fever, chills, and flank or abdominal pain. Two-thirds of patients have nausea and vomiting but dysuria is not necessarily present thus mimicking an abdominal process. Some patients may have a palpable flank or abdominal mass. Clinical signs of severe urinary tract infection with urosepsis may be seen in patients with acute multifocal bacterial nephritis, half of whom have diabetes mellitus. Nonspecific constitutional complaints of malaise, fatigue, and lethargy are particularly common (74%) in patients with xanthogranulomatous pyelonephritis, who may also complain of weight loss (24%). Significant physical findings include a renal mass (60%), hepatomegaly (30%), and rarely, a draining flank sinus in patients with a past medical history of recurrent urinary tract infection (65%), renal stones (30%), or prior urinary tract instrumentation (26%). Peripheral white blood cell counts are elevated in most patients. The urinalysis is often abnormal, with pyuria, proteinuria, bacteriuria, and occasionally hematuria. However, the urinalysis may be normal in as many as 30% of patients. *Escherichia coli, P. mirabilis,* and *Klebsiella* spp. are the most common organisms recovered from urine cultures. Blood cultures are also frequently positive in patients with acute focal bacterial nephritis or acute multifocal bacterial nephritis. Anemia is present in 75%, abnormal liver function tests (bilirubin, AST, alkaline phosphatase, and prothrombin time) in 38% to 63%, hypoalbuminemia in 60%, hypergammaglobulinemia (α_1- and α_2-globulin) in 79%, and hyperuricemia in 50% of patients with xanthogranulomatous pyelonephritis (44,53). In general, the clinical and laboratory findings may or may not point to the urinary tract as the focus of infection and may not distinguish renal abscess from other abnormalities of the urinary tract.

Diagnosis

Renal corticomedullary abscesses must be differentiated from other space-occupying lesions in the kidney. Renal tumors, intrarenal cysts, renal cortical abscesses, and perinephric abscesses may mimic renal corticomedullary abscesses, because the clinical presentation of each of these conditions is similar. Fever, chills, malaise, and back pain may be seen in each. Clinical signs of urosepsis may be present in patients with renal corticomedullary abscesses and to a lesser extent, in patients with perinephric abscesses. In contrast, these signs are usually absent in patients with renal tumors, cysts, and renal cortical abscesses. Patients with renal corticomedullary abscesses often have an abnormal urinalysis (70%) with pyuria, proteinuria, and bacteriuria, and blood cultures frequently are positive (40).

Radiographic techniques are essential to identify renal corticomedullary abscesses. The urographic findings in patients with acute focal bacterial nephritis are: (a) a poorly marginated and relatively sonolucent ovoid mass disrupting the corticomedullary definition and producing some low-level echoes on ultrasound examination; (b) a solid-appearing mass on excretory urography, CT, or angiography; and (c) abnormal uptake of gallium at the location of the mass, which may be associated with increased activity elsewhere in the same or opposite kidney (39). A fluid-filled mass or a fluid debris level typical of a frank renal abscess is not found in acute focal bacterial nephritis on ultrasonography (39,40). On a non–contrast-enhanced CT, the lesion of acute focal bacterial nephritis is typically imperceptible. There are three characteristics seen with contrast enhanced CT: lobar distribution of inflammatory areas, poorly defined wedge-shaped areas of diminished contrast enhancement without frank liquefaction, and masslike hypodense lesions in severe cases (40,54). Renal abscesses, however, are usually round, have liquid centers, and are visible with and without contrast enhancement. The lack of a defined wall by ultrasound or CT in acute focal bacterial nephritis is an important factor distinguishing this entity from an abscess (30). On angiography, narrowing and obstruction of veins within the mass, along with only minor arteriographic abnormalities, are characteristic of acute focal bacterial nephritis (39). Focal abnormalities in the kidney on gallium images may be seen in some neoplasms and renal abscesses as well as acute focal bacterial nephritis. However, the diagnosis of acute focal bacterial nephritis is strongly suggested whenever the abnormalities are larger on the gallium image than on the urogram or sonogram or whenever bilateral abnormalities are seen on gallium images that correlate with a focal mass on the urogram or sonogram (39). The distinction between acute focal bacterial nephritis, renal abscess, and tumor can be made also by needle aspiration (55). However, in most patients, a combination of imaging techniques is sufficient to diagnose acute focal bacterial nephritis and permit conservative medical therapy without confirmation by needle biopsy or surgery (39,56). In this context, serial uroradiologic studies should be performed to follow the process to resolution (57,58).

The urographic findings in patients with acute multifocal bacterial nephritis typically show severe impairment of excretion of contrast material on the affected side, with renal enlargement, a diminished nephrogram, and a delayed pyelogram (40,59,60). Ultrasonography may demonstrate areas of decreased echogenicity throughout the affected kidney. Poorly defined wedge-shaped areas of decreased contrast enhancement similar to those described in patients with acute focal bacterial nephritis can be seen on contrast-enhanced CT, except that multiple renal lobes are involved (40,61) (Fig. 35-6). If angiography is performed, the number and caliber of interlobar arterial branches are diminished, and fine linear stripes of alternating density and lucency in the angiographic nephrogram

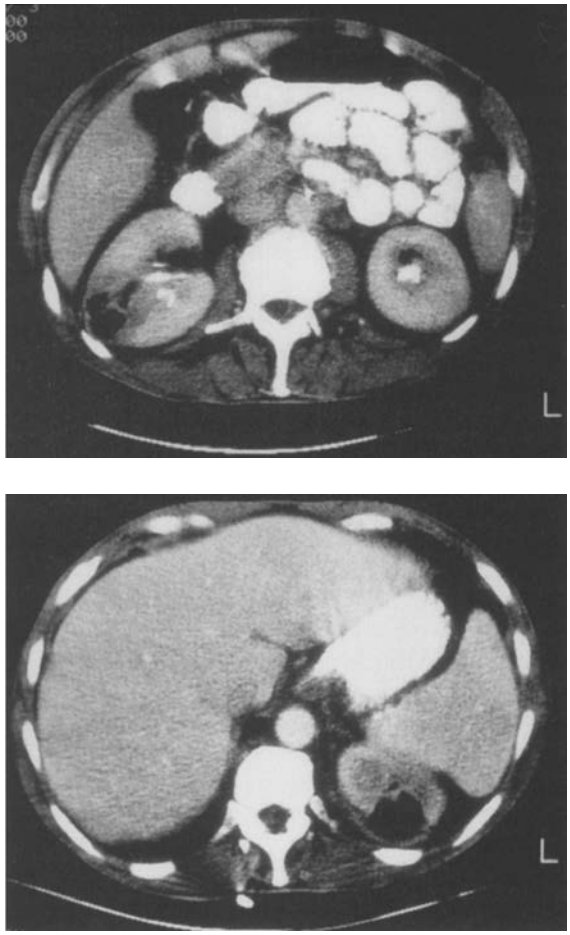

FIGURE 35-6. Computed tomography (CT) scans of a patient with bilateral intrarenal abscesses.

are present throughout the kidney (40). Computed tomography is more sensitive than ultrasonography for the detection of intrarenal bacterial infections and defining the extent of disease (30,58,62).

The radiographic findings in patients with xanthogranulomatous pyelonephritis are varied and uncharacteristic. The xanthogranulomatous process may occur in a localized (unifocal) or diffuse (multifocal) form in either a previously normal kidney or one that is obstructed, contains a stone, or has an anomalous collecting system or caliceal diverticulum. Perinephric extension of the xanthogranulomatous process produces obscure renal margins and ablation of the perinephric and paranephric fat (44). Radiographically, xanthogranulomatous pyelonephritis appears as either localized or diffuse enlargement of one kidney with an indistinct renal outline. Urographically, the most frequent finding has been a stone-bearing (70%) and functionless (80%) kidney (63). Caliceal deformity and irregularity (46%) may be present (44,53). Neovascularity may be present on angiography, but most xanthogranulomatous renal masses are hypovascular or avascular, and the majority cannot be definitively distinguished from renal cell carcinoma (44,64,65). There are no specific ultrasonographic findings that reliably distinguish renal tumors and abscesses from xanthogranulomatous pyelonephritis (66). The sonographic features of xanthogranulomatous pyelonephritis include a diffusely enlarged kidney with multiple areas of increased anechoicity and a central echogenic focus with acoustic shadowing (67). Although these findings are also seen in a hydronephrotic kidney, the presence of a central strongly echogenic focus suggests xanthogranulomatous pyelonephritis (67,68). On CT, *focal* xanthogranulomatous pyelonephritis appears as low-density mass lesions, with wall enhancement surrounding dilated calices, which may contain stones, or as a focal mass in one pole of a duplicated kidney (69). In *diffuse* xanthogranulomatous pyelonephritis, CT demonstrates an enlarged kidney, often with central calcification in the renal pelvis without dilatation, and with multiple, rounded, low-density areas representing dilated calices and abscess cavities. On enhanced scans, the walls of these cavities demonstrate a prominent blush because of increased vascularity within the granulation tissue and the compressed normal parenchyma (63,69). Extension of the xanthogranulomatous process through the renal capsule, with involvement of the perirenal and pararenal spaces and the psoas muscle, when present, also can be observed on CT (63,69). The diagnosis is suggested by CT findings in 44% of cases. This imaging study plays an important role in determining the extent of extrarenal disease and planning of operative treatment. Ultrasound examination is less specific than CT, and magnetic resonance imaging (MRI) generally offers no additional information over CT scans but may be useful in patients with renal insufficiency or allergy to iodinated contrast material (30,70).

Treatment

In the past, surgical drainage, débridement, or nephrectomy was the accepted treatment for renal corticomedullary abscesses. Recent experience indicates that successful therapy of acute focal and multifocal bacterial nephritis with antimicrobial agents alone will produce a symptomatic response within 1 week in most patients and result in no sequelae (31,32,38,40,46,47,71). Radiologic techniques should be used to document resolution of the infection (58); nevertheless, the time at which the intrarenal infection is discovered and its degree of suppuration should guide its management. A well-established large abscess cavity may be more difficult to eradicate with antibiotics alone than an earlier lesion in the preabscess state; however, a trial of intensive antibiotic treatment is appropriate for lesions localized to the renal parenchyma before determining the need for operative drainage, particularly in a promptly diagnosed and otherwise healthy person. Intravenous fluids and parenteral antibiotics should be started as soon as the diagnosis is entertained. In patients with acute focal or multifocal bacterial nephritis, initial antibiotic selection (empiric therapy), before the results of urine cultures and sensitivities are available, should be aimed at the most common uropathic, enteric Gram-negative aerobic bacilli (e.g., *E. coli*, *Klebsiella,* and *Proteus* spp.).

Monotherapy with a third-generation cephalosporin (e.g., cefotaxime, ceftriaxone, or ceftazidime), an extended-spectrum penicillin (e.g., piperacillin), or ciprofloxacin is acceptable empiric therapy. Combined therapy with a ß-lactam, such as ampicillin or cefazolin, with an aminoglycoside is no more successful than single-agent therapy in the treatment of acute focal or multifocal bacterial nephritis (38). Empiric therapy should be modified to the most effective single agent based on the results of the antibiotic sensitivities of the organisms recovered from cultures of urine, blood, or both. Although the duration of treatment has not been defined, current recommendations are to continue parenteral antibiotics for at least 24 to 48 hours after the resolution of fever and clinical improvement are attained. Oral antibiotic therapy, based on the results of antimicrobial susceptibility tests, can then be continued for an additional 2 weeks. Patients with acute focal bacterial nephritis typically respond to medical therapy alone (at least 14 days of an appropriate antimicrobial agent), and follow-up studies have shown resolution of the intrarenal lesion without the need for surgical

drainage (40,49,56). Although many patients with acute multifocal bacterial nephritis slowly improve with antibiotic therapy alone, some may require surgical intervention. Patients who are likely to fail appropriate antibiotic therapy alone are those who have radiologic evidence of a large intrarenal abscess, significant obstructive uropathy, severe vesicoureteral reflux (primarily in children with Gram-negative bacillary multifocal bacterial nephritis) with extensive parenchymal involvement, patients with diabetes mellitus with gas-forming infections, and patients of advanced age or with urosepsis (18,40,45–47,72). In general, surgical intervention is indicated in the patient who has radiologic evidence of a large intrarenal abscess and persistent fever with an absence of a clinical response after 5 to 7 days of adequate antibiotic therapy. In patients requiring drainage, percutaneous aspiration of the abscess combined with systemic antibiotic therapy has been successful (47,73,74). If a significant obstructive uropathy is present, prompt drainage, usually by percutaneous nephrostomy, is necessary with correction of the lesion, if possible, when the patient is afebrile and stable (40). If surgical intervention is necessary, the abscess should be incised and drained, and nephrectomy should be reserved for diffusely damaged kidneys or for patients of advanced age who are septic and require urgent surgical intervention for survival (45). Also, all children with renal parenchymal infection caused by Gram-negative bacilli should undergo voiding cystourethrography to look for lower urinary tract abnormalities (18). Thus, current clinical experience indicates that many patients with acute multifocal bacterial nephritis may not require surgery as they did in the past but may be treated successfully with antibiotics alone. The decision to drain the abscess mechanically should be based on the radiologic findings and response of the patient to initial drug therapy.

In contrast, patients with xanthogranulomatous pyelonephritis generally are not cured by antibiotic therapy alone. These patients often require surgical removal of the xanthogranulomatous process to cure this disease; however, there have been several case reports of successful treatment with antibiotics without surgical intervention (50). The diagnosis of xanthogranulomatous pyelonephritis is not commonly made preoperatively; however, once the involved tissue is resected, the xanthogranulomatous process ceases and does not seem to recur. The prognosis in patients who have an otherwise normal urinary tract is excellent. Total nephrectomy is the usual procedure, but Malek and Elder (44) suggested that partial nephrectomy for selected localized disease, such as cases confined to the kidney (stage I) or involving the perinephric fat (stage II), may be sufficient. Partial nephrectomy is especially suitable in children, who usually present with localized disease (44). In adults, the disease is frequently diffuse throughout most, or all, of the kidney and advanced stages extend to the perinephric fat (stage II) and beyond (stage III). Although removal of the kidney and perirenal fat is preferred, it may be technically difficult and complicated by fistulization of adjacent bowel. Open surgical nephrectomy is preferred over laparoscopic nephrectomy as it is quicker, leads to less complications and results in a similar post-operative course (75). Even though xanthogranulomatous pyelonephritis does not recur following successful surgery, bacteriuria may continue in some patients and will require appropriate treatment (44,53).

Infected Renal Cyst

Spontaneous infection of preexisting solitary renal cysts has been described (40,76,77). In contrast, patients with autosomal dominant polycystic renal disease may have one or more cysts that become infected (78). The most common etiologic agents are Gram-negative uropathogens that are thought to infect the cysts as a consequence of bacteriuria and ascending infection (79). Infection may also occur as a result of iatrogenic cyst instrumentation (40). The clinical features of infected cysts are similar to those of an acute renal abscess and include nausea, chills, fever, flank or back pain, and dysuria. The diagnosis is made radiographically. Ultrasonography or CT may demonstrate a solitary renal mass that is compatible with an uncomplicated simple renal cyst or multiple lesions characteristic of polycystic renal disease. Gallium or indium imaging or gadolinium-enhanced magnetic resonance may help to identify the infected cyst (or cysts) in patients with polycystic kidneys (80). A definitive diagnosis can be made by ultrasound or CT-guided percutaneous cyst puncture with culture. Effective treatment for infected solitary cysts includes percutaneous drainage combined with 2 weeks of appropriate antimicrobial therapy (38). Surgical drainage is rarely required. In contrast, the therapy of infected cysts in patients with polycystic renal disease is more difficult and not well defined. A conservative approach with long-term (6 to 8 weeks) oral antibiotic treatment directed against the most likely pathogens or those isolated from urine or blood cultures is successful in some cases (79). In general, surgical drainage is generally avoided because of the difficulty in identifying which cyst is infected.

PERINEPHRIC ABSCESS

Etiology

Staphylococcus aureus, E. coli, and *Proteus* spp. are the most common causes of perinephric abscesses. Other, less common causes include *Klebsiella* spp., *Enterobacter* spp., *Pseudomonas* spp., *Serratia* spp., and *Citrobacter* spp. Occasionally enterococci and streptococci are implicated, including two cases of *S. pneumoniae* (81,82) and one case of Group B streptococcus in a diabetic patient (83). Perinephric abscesses also may be caused by various anaerobic bacteria, including Gram-negative bacilli and anaerobic cocci, *Clostridium* spp., and *Actinomyces* spp. (84). These anaerobic bacteria may be the pathogens in patients with abscess cultures reported to be sterile (32,47). *Mycobacterium tuberculosis* is also an important cause of perinephric abscess, as are certain fungi, particularly *Candida* spp. Nocardia has been reported as a cause of perinephric abscess in immunocompromised patients (85). More than one microorganism has been simultaneously recovered from perinephric abscesses in as many as 25% of patients (45,47,48,86,87). Although bacteria isolated from the urine frequently correlate with those isolated from the abscess, in some patients, urine cultures are positive for microorganisms different from those subsequently isolated from the abscess material (47,86,88,89). Blood cultures may be positive (20% to 40%) in some patients (86,88).

Pathogenesis

A perinephric abscess is a collection of purulent material in the space between the capsule of the kidney and Gerota's fascia (Fig. 35-7). The abscess usually is confined to this space, but may extend beyond Gerota's fascia into the pararenal space, or even into the flank muscles or psoas muscle (90). It may present as a draining flank abscess through Petit's triangle or as an abscess in the groin or paravesical area by extending caudally between the diverging layers of Gerota's fascia (48). It rarely perforates into the peritoneal cavity or ruptures into the colon. Cephalad extension may result in a subphrenic abscess, penetration of the diaphragm and empyema, lung abscess, or formation of a nephrobronchial fistula (2,47,73,87,91). The most frequent initiating event is the direct extension or rupture of an abscess within the renal parenchyma into the perinephric space (5,14,17). This is the most common mechanism responsible for

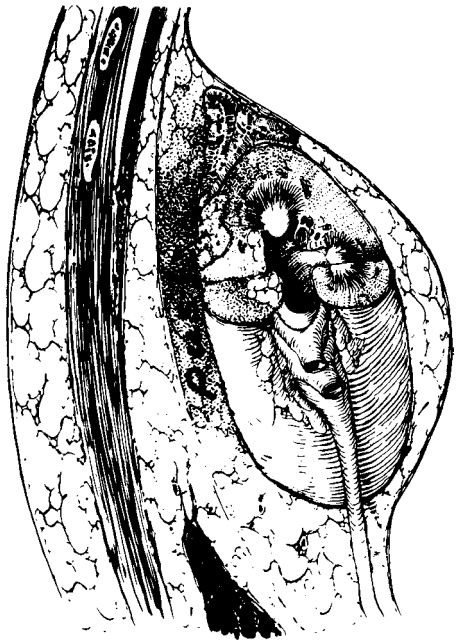

FIGURE 35-7. Diagram of the pathogenesis of a perinephric abscess.

staphylococcal perinephric abscesses that occur when a renal cortical abscess ruptures into the perinephric space (13). Other causes include hematogenous or regional lymphatic seeding of the perinephric space, usually from sites of skin infection (47,86,92). Rarely, spread of infection from inflammatory lesions of the liver, gallbladder, pancreas, pleura, prostate, or female reproductive organs as well as diverticulitis, appendicitis, perforated carcinoma of the colon, and osteomyelitis of adjacent ribs or vertebrae has been implicated in the pathogenesis of perinephric abscess (32,47,93–95). The majority of patients with perinephric abscesses have some form of obstruction to urinary outflow. Specific predisposing factors include renal or ureteral calculi, ureteral stricture, neurogenic bladder, vesicoureteral reflux, mechanical bladder outlet obstruction, neoplasm, renal papillary necrosis, polycystic kidney disease, genitourinary tuberculosis, immunosuppresion including renal transplantation, trauma (including urinary tract instrumentation, renal biopsy, or aspiration), and the associated conditions of diabetes mellitus (a major contributing factor), glucocorticoid therapy, and injection drug use (40,47,78,81,96).

Clinical Features

The onset of perinephric abscess is characteristically insidious. Patients are often ill for 1 to 3 weeks before they seek medical care, and early recognition of this condition is difficult. The most common symptoms are fever, which occurs in almost all patients; unilateral flank pain, in 70% to 80%; and chills and dysuria, in 40% of patients (47,86,97,98). Weight loss, nausea, and vomiting are less common. Diarrhea is very rarely a symptom associated with perinephric abscess (99). On physical examination, flank and costovertebral angle tenderness are the most common findings, but abdominal tenderness may be present in about 60% of cases. In some patients, the pain may be referred to the corresponding hip, thigh, or knee. Scoliosis, with splinting on the affected side, pain on bending toward the contralateral side and during either active flexion of the ipsilateral thigh against pressure, or extension of the thigh while walking may be present in some patients. A flank or abdominal mass is present in less than half the patients (32,40,47,86). Routine laboratory studies are nonspecific. The peripheral white blood cell count is usually modestly elevated with associated neutrophilia. Anemia and azotemia may be present in 40% and 25% of patients, respectively (97). Pyuria and proteinuria are common; however, hematuria is present in only 10% of patients and the urinalysis may be entirely normal in 25% to 30% of the cases. Two-thirds of patients have positive urine cultures, with more than 10^5 bacteria/mL of urine. Approximately 40% of patients are bacteremic (32,40,47,86).

Diagnosis

A perinephric abscess must be differentiated from other infections of the urinary tract and from other occult abscesses. Patients with this disease may present with fever of undetermined origin or with unexplained peritonitis, empyema, or pelvic abscess resulting from extension of the perinephric abscess. The diagnosis should also be considered in patients with urinary tract infection who do not respond promptly to antibiotics and have an abnormality of the urinary tract or diabetes. Prompt diagnosis of this disease is made in less than one-third of patients at the time of admission (47,48,86,88). Up to 25% to 30% of patients are diagnosed only at autopsy (47,86). This disease should be considered in the differential diagnosis of patients who present with the signs and symptoms described previously. Radiologic examinations with ultrasonography, CT, arteriography, and ^{67}Ga or ^{111}In imaging are essential diagnostic aids in most cases. Roentgenogram of the chest may be normal or may show a pleural effusion, elevated hemidiaphragm either with or without decreased diaphragmatic excursion, or a lower lobe infiltrate (47,86). Supine abdominal roentgenogram may demonstrate an upper quadrant mass, obliteration of the renal outline, vertebral scoliosis, or absent psoas shadows. However, obliteration of the psoas margin is not a reliable diagnostic sign for perinephric abscess (13,17). Although uncommon, the presence of extraluminal, retroperitoneal gas bubbles in the area of the kidney suggests a perinephric abscess produced by gas-forming organisms. This condition, termed emphysematous pyelonephritis, occurs primarily in patients with diabetes mellitus, with or without urinary obstruction, and more rarely in nondiabetic patients who have urinary obstruction (72,100,101). On excretory urography, important radiographic findings of perirenal abscesses include decreased renal mobility with respiration or position, absent or diminished renal function, caliectasis and other caliceal abnormalities, and displacement (usually medially and upward) of the kidney or ureter. Extrarenal extravasation of contrast material, though uncommon, is virtually diagnostic of perirenal abscess (47,90). Also, fistula formation occurs occasionally between the perirenal space and other structures, such as the colon. Retrograde pyelography is usually not necessary, but it is occasionally helpful in identifying obstructive lesions distal to the renal pelvis. Ultrasonography may demonstrate an intrinsic mass in addition to the more characteristic findings of a perinephric fluid collection, along with displacement of the kidney, loss of a distinct renal outline, and renal fixation. These findings indicate extension of the inflammatory process to the perinephric space (13,17). The sonographic appearance may vary from a nearly anechoic mass, displacing the kidney, to an echogenic collection that merges with normally echogenic fat within Gerota's fascia (40). Ultrasound also may be useful to determine the extent of the abscess (Fig. 35-8) and detect an associated obstruction of the collecting system. However, ultrasound may be falsely negative in 36% of cases as compared to computed tomography (97). Findings on CT include thickening of the renal fascia and perirenal fluid collection. In most cases, pus can be differentiated from other causes of perirenal fluids collections, such as urine, blood, lymph, exudates, and transudates (102). Computed tomography also provides the most precise anatomic information and can demonstrate the extent and route of the

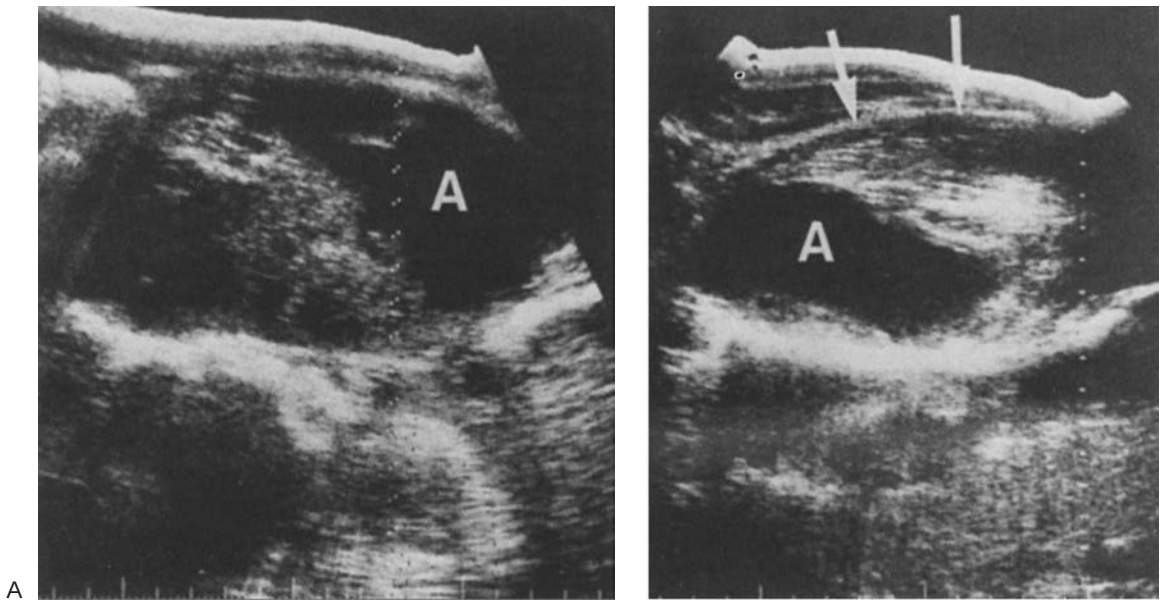

FIGURE 35-8. A: Longitudinal ultrasonogram down the right paravertebral region, demonstrating a huge complex mass with a large irregular fluid component, labeled A. **B:** Marked enlargement of the psoas major muscle (*arrows*) with a large contained fluid collection, labeled A. (From: Andriole VT. The clinician's viewpoint. *Clin Diagn Ultrasound* 1982;11:1, with permission.)

abscess beyond the renal capsule (e.g., extension to the flank or the psoas muscle) (Fig. 35-9). This detail is important in planning surgical drainage of the abscess. Radionuclide imaging with ^{67}Ga or ^{111}In may be used occasionally to confirm the presence of renal or perirenal inflammation (Fig. 35-10) or evaluate renal function. Gallium or indium imaging may provide the first evidence of a perirenal abscess in patients with suspected infections but without localizing signs or symptoms. However, ^{67}Ga is not sufficiently definitive to exclude renal carcinoma, pyelonephritis, intrarenal abscess, or ureteral obstruction (13,31,33). Thus, a subtraction technique using ^{67}Ga citrate and ^{99m}Tc glucoheptonate has been used to define the extent of perinephric involvement as well as to eliminate any false-positive scans seen with gallium alone (13,31). On angiography, characteristic findings of perinephric abscesses include an increase in number and size of the perforating arteries extending from the kidney into the abscess, stretching, and prominent tortuous capsular arteries around the abscess and a contrast blush (11,13,25,26). Perirenal and renal abscesses can be arteriographically distinguished from tumors, as described previously; however, angiography is not necessary in most patients with perinephric abscess because of the availability of the newer, noninvasive imaging modalities.

Treatment

Early surgical or percutaneous (under imaging guidance) drainage of the perinephric abscess is imperative (40,47, 86,102). Antibiotic therapy alone is inadequate and should be used as adjunctive treatment. In some patients, the perinephric abscess has been drained by percutaneous tube placement, aspiration of pus, and antibiotic irrigation prior to definitive surgery (nephrectomy), which is frequently necessary (40,47). In others, acute nephrectomy is performed at the time of initial surgical drainage. There are rare case reports of immunocompetent patients with small ($\leq$3.5 cm) abscesses who were successfully treated with prolonged courses of intravenous antibiotics without drainage (74,103); however, immunocompromised patients or those with abscesses larger than 2 cm did poorly and had high mortality rates when treated with

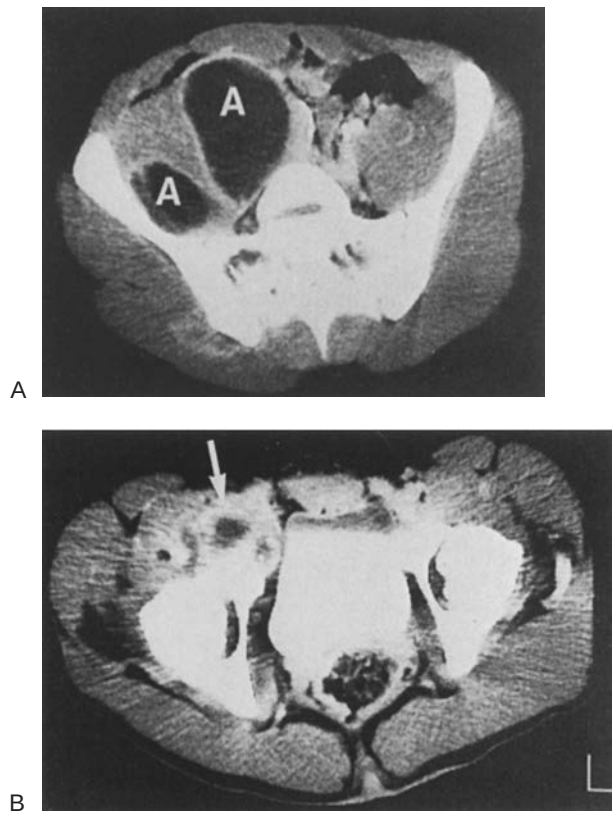

FIGURE 35-9. A: Computed tomography (CT) scan through midabdomen, demonstrating marked enlargement of the right psoas major muscle with a bilobed fluid-filled cavity, labeled A (from the same patient as in Figure 35-8). **B:** Transverse CT scan through the level of the femoral head, showing the inferior extent of the abscess (*arrow*) pointing below the inguinal ligament. (From: Andriole VT. The clinician's viewpoint. *Clin Diagn Ultrasound* 1982;11:1, with permission.)

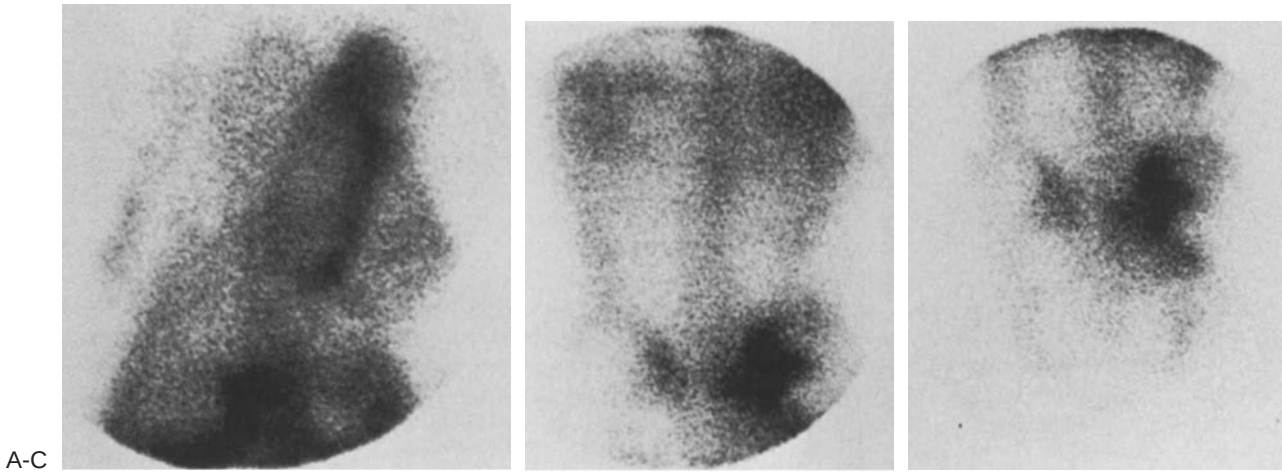

FIGURE 35-10. A: Oblique ^{67}Ga scan, demonstrating activity of the entire right paravertebral region (from the same patient as in Figure 35-8). **B:** Posterior ^{67}Ga scan, demonstrating activity in the region of the femoral head, indicating the inferior extent of this abscess cavity. (From: Andriole VT. The clinician's viewpoint. *Clin Diagn Ultrasound* 1982;11:1, with permission.)

antibiotics alone. Computed tomography should be used to follow the response to treatment.

Initial antimicrobial therapy should be aimed at the most common uropathic Gram-negative bacilli as well as against staphylococci, since some perinephric abscesses are a consequence of staphylococcal renal carbuncles. An aminoglycoside (gentamicin or tobramycin) in a dose of 1 to 1.5 mg/kg of body weight every 8 to 12 hours in patients with normal renal function should be combined with an antistaphylococcal ß-lactam, oxacillin, nafcillin, or cefazolin, intravenously. If bacterial resistance to gentamicin or tobramycin is suspected because the patient has had previous antimicrobial therapy or hospitalization, amikacin in a dose of 7.5 mg/kg of body weight every 12 hours in patients with normal renal function should be used. The dose of gentamicin, tobramycin, or amikacin must be adjusted for those patients with compromised renal function. Therapy should be modified based on the results of the antibiotic sensitivities of the organisms recovered from cultures of the abscess material. An antipseudomonal ß-lactam (e.g., ticarcillin, piperacillin, cefoperazone, or ceftazidime) should be added to the aminoglycoside if *P. aeruginosa* is the cause of the infection, and clindamycin, chloramphenicol, or metronidazole should be added if anaerobic bacteria are involved. A combination of penicillin or ampicillin plus gentamicin is the treatment of choice in enterococcal infections. Isoniazid plus rifampin and ethambutol or streptomycin is necessary for abscesses caused by *M. tuberculosis* and amphotericin B for those caused by fungi.

The prognosis in patients with perinephric abscess is poor. Even though there have been major improvements in diagnostic technology, surgical therapy, and antimicrobial treatment, the mortality associated with perinephric abscess remains high and is in a range from 20% to more than 50% (32,45,47,74). Prompt diagnosis of perinephric abscess, immediate surgical or percutaneous drainage, and appropriate antimicrobial therapy, followed by definitive surgical therapy for cases with a poor response, may be effective in reducing this high mortality rate.

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CHAPTER 36 ■ COMPLICATED URINARY TRACT INFECTIONS

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Complicated infections of the genitourinary (GU) tract refer to those infections that occur in the presence of anatomic or functional abnormalities in the kidney, bladder, or collecting systems (including vesicoureteral reflux and neurogenic bladders in patients with spinal cord injury); obstruction to normal urine flow (including renal, ureteric and bladder calculi, and prostatic hypertrophy); urinary tract catheterization or instrumentation; cystic renal disease; specific diseases such as diabetes mellitus; and abnormalities in host defense mechanisms and those infections that follow surgery or renal transplantation (Table 36-1). In addition, infections that are caused by organisms that resist antimicrobial therapy (such as multidrug-resistant enterococci) or might otherwise be difficult to eradicate may be considered in this category. Aerobic or anaerobic bacteria, mycobacteria, fungi, parasites, and even viruses may cause complicated infections.

Other conditions associated with complicated urinary infections include prostatic, kidney, or perinephric abscesses; pyonephrosis; emphysematous pyelonephritis and cystitis; malakoplakia and xanthogranulomatous pyelonephritis; intramural vesical abscesses; pyelonephritis with bacteremia and sepsis; and tuberculosis. Some of these conditions are discussed in other chapters (Chapters 35, 37, and 38). Complicated urinary infections may occur either in the upper or lower urinary tract and may be acute or chronic. Not included in this category are asymptomatic bacteriuria, urethritis, acute cystitis, acute pyelonephritis, or recurrent upper or lower urinary tract infection occurring in the presence of a normal urinary system.

ANATOMIC OR STRUCTURAL RISK FACTORS

Obstructive Uropathy

Obstructive uropathy may include calculi at any level of the urinary tract; prostatic hypertrophy; cancer of the prostate, bladder, or uterus; external compression by uterine or other tumors; neurogenic bladder; and congenital abnormalities. Any of these conditions may be associated with a complicated urinary tract infection. Obstruction above the bladder may lead to renal pelvic dilatation and hydronephrosis with subsequent pressure atrophy of renal cortical tissue. When infection does occur in the setting of partial or complete obstruction, clearance of infection is more difficult because drainage may be limited, antibiotic penetration might decrease, and host responses may be impaired.

Obstruction of the urinary tract may be acute or chronic, unilateral or bilateral, and complete or incomplete. Acute obstruction of the upper urinary tract may be associated with retroperitoneal or flank pain, especially if calculi are present. Obstruction at the bladder level increases risk of infection by decreasing the effect of micturition on reducing bacterial inocula as well as allowing for multiplication of bacteria to the degree that mucosal antibacterial and other host factors are overwhelmed or inactivated. Obstruction at higher levels of the urinary tract may predispose to infection, as dilatation and pressure necrosis may decrease defense mechanisms in the kidney and allow disseminating hematogenous bacteria to alight and form a nidus of infection in the renal cortex or medulla. Also, once the normal architecture of the urinary tract has been damaged, whether as a result of reflux or obstruction, bacteria that lack the virulence factors necessary to cause renal infection in the absence of structural lesions may be responsible for serious upper tract infection once introduced into the kidney.

Treatment of urinary infections in the face of obstructive uropathy or stone disease usually requires antibiotic therapy for a longer duration than that for uncomplicated infections; treatment may be required for up to 6 weeks. Obviously, correction of the obstruction and removal of calculi are important adjunctive measures. In general, a 6-week course of a bactericidal antibiotic that achieves adequate concentration in renal tissue and bladder urine is recommended. Depending on the organism and susceptibility testing results, intravenous or oral therapy can be used, for example, a fluoroquinolone or a ß-lactam antibiotic. Special consideration is needed in the face of staghorn calculi, which often form in the presence of urease-producing organisms such as *Proteus* sp. Once the organism has been eradicated with a long course of antibiotics, "prophylactic" or "suppressive" therapy can be given with low-dose trimethoprim-sulfamethoxazole, daily or every other day, or with a combination of trimethoprim and a methenamine compound. Nephrolithiasis is considered in more detail in Chapter 26.

Catheterization and Instrumentation

The distal third of the urethra is normally colonized with perineal and skin flora. Instrumentation for any reason may introduce these organisms into the bladder. In the presence of an indwelling catheter, bacteria can ascend from the periurethral area along the mucous sheath that develops between the urethral mucosa and the latex rubber catheter (1). Although several techniques have been introduced to prevent urinary infection in chronically catheterized patients, such as closed sterile drainage, continuous irrigation through a three-way catheter with antibiotic or acetic acid, and systemic antibiotic prophylaxis, all patients with prolonged urinary catheter drainage ultimately become colonized with high counts of bacteria (from 2 to about 21 days).

Urinary tract infections (UTIs) are the most common infection acquired in hospitals; elderly patients are at greatest risk. A review of nosocomial infections from 1986 to 1990 analyzed a total of 101,479 infections in 75,398 patients (2).

TABLE 36-1

COMPLICATED INFECTIONS OF THE URINARY TRACT

Anatomic or structural risk factors
Obstructive uropathy—stones, strictures, tumors
Prostate associated (see Chapter 34)
Instrumentation—catheter associated and nosocomial
Renal cystic disease
Ureteral stents and surgical urinary diversions
Ileal loop constructions
Other anatomic risk factors
Vesicoureteral reflux
Urachal remnants
Functional risk factors
Diabetes mellitus
Renal transplantation
Spinal cord injury and neurologic dysfunction
Neutropenia
Human immunodeficiency virus
Miscellaneous complicated infections
Pyonephrosis
Emphysematous pyelonephritis and cystitis
Malakoplakia and xanthogranulomatous pyelonephritis
Intramural vesical abscess
Urosepsis
Tuberculosis (see Chapter 38)
Infections caused by atypical or resistant organisms (e.g., vancomycin-resistant enterococci, anaerobes)

Of these, 44% originated in the urinary tract and *Escherichia coli* was responsible for 25% of these infections, followed by *Enterococcus* sp. in 15%, *Pseudomonas aeruginosa* in 13%, and *Candida* sp. in 7%. Indwelling Foley catheters were present in 84% of the elderly patients reviewed in this series and 78% of younger patients (ages 15 to 64 years). Bacteremia, an important sequela of complicated UTIs, developed in 4% of patients with indwelling bladder catheter-associated urinary infections and case-fatality rates of 13% to 30% have been reported for these bacteremic nosocomial urinary infections (3–6).

Catheter-associated urinary infections may account for 40% of nosocomial infections in American hospitals (7,8). Patients with catheter-associated urinary infections have a case-fatality rate ten times higher than that for nonbacteriuric catheterized patients (9). This is most likely attributable to associated bacteremia and sepsis (5,7).

Catheter-associated nosocomial UTIs (CAUTI) have been recently described in a study of 1,497 patients. These infections were more frequent in women (23.2%) than men (8.9%). They were unimicrobial in 94% and polymicrobial (primarily with enterococci and Gram-negative bacilli) in 6%. The distribution of single isolates in these patients included Gram-negative bacilli in 34%, enterococci or staphylococci in 27% and *Candida* sp. in 27%. Patients with CAUTI only rarely have symptoms (<10%) in the face of infections and pyuria, and they may not have peripheral leukocytosis (10). Catheters left in place without bona fide medical necessity often contribute to nosocomial infections and are accompanied by an increase in associated antibiotic costs (11).

Environmental factors may relate to the nosocomial acquisition of bacteriuria in catheterized patients. Prevalent bacteria in the hospital colonize patients, or the patient's endogenous flora may enter the urinary collecting system or drainage bag. Within 24 to 48 hours they may be found in the bladder (12,13), and they increase to high colony counts over the subsequent 48 hours (14). Bacteria may attach to the luminal surface of the catheter in association with the production of a mucoid biofilm, and this may predispose to urinary infection or catheter blockage and obstruction (7,12–17).

Urea splitting bacteria may lead to mucosal encrustations and encrusted cystitis and pyelitis, most commonly owing to *Corynebacterium* group D_2 (18). There is often a history of prior urologic procedure or chronic illness, including immune compromise or renal transplantation. Patients may describe symptoms of cystitis, dysuria, gross hematuria, passage of encrusted debris, often with complaints of an ammonia odor to the urine. Failure to diagnose this condition can lead to renal impairment or ureteric obstruction and loss of renal graft as a result of infection, renal abscess, or obstructive uropathy.

Treatment consists of antibiotics. The glycopeptides vancomycin and teicoplanin have *in vitro* activity against *Corynebacterium* group D_2. These organisms are frequently resistant to fluoroquinolone antibiotics (>50%). Additional treatment includes acidification of urine as well as chemolysis and removal of infected calcified plaques that contain the organisms (18).

Some caution is warranted in interpreting the results of cultures of material obtained from urinary collection devices. Several populations of bacteria may grow within the catheter and include planktonic bacteria in the urine and surface bacteria associated with the bacterial biofilm (19). Bacteria may be cultured from catheter lumen encrustations when bladder urine might be sterile (20). Because bladder urine is normally sterile, Garibaldi and associates (21) suggested that the presence of 100 or more organisms per mL should be considered as evidence of a positive urine culture in a catheterized patient. These authors demonstrated that breaks in the catheter-collecting system junction were associated with early acquisition of bacteriuria.

Condom catheters are the usual alternatives to indwelling bladder catheters in incontinent male patients without obstructive uropathy. Although associated with fewer infectious complications, at least one outbreak implicated these devices in 64 geriatric patients, 40 (63%) of whom had asymptomatic infections, frequently with mechanical obstruction of urine flow including kinking of the outlet, or blockage of flow by adhesive devices with associated penile cyanosis and ulceration. These problems may lead to urinary stasis, bacteriuria, and bladder wall distention, all of which may predispose to complicated urinary infections (22,23).

Treatment of catheter-associated urinary infections depends on the clinical setting. In general, asymptomatic bacteriuria in catheterized patients is not treated. In patients with catheters in place for the long term, there is some risk of dissemination of bladder bacteriuria to the blood during manipulation of the urinary tract as during catheter changes (generally done to minimize concretions and obstruction). Antimicrobial agents have not been shown to prevent catheter associated UTIs in persons with long term indwelling urethral catheters (24). Preventive strategies that avoid antibiotics are needed for these patients. We occasionally advocate treating the colonizing bacteria 8 to 24 hours prior to the catheter change, with a single dose of a bactericidal antibiotic based on susceptibility testing of the organisms (e.g., a quinolone or an aminoglycoside).

Bacteriuria

Interventional attempts to decrease the incidence of nosocomial UTIs using a silver-alloy, hydrogel-coated latex urinary catheter have been compared with standard silicone-coated latex catheters (25). In older studies, catheters significantly reduced the rates of bacteriuria in male surgical patients not

receiving antibiotics (26,27). In the more recent study (25), silver-coated catheters were associated with a 32% decrease in infection risk in male patients. Infection rates in females were similar in both catheter groups. In addition to a decrease in nosocomial infection rates, significant savings on hospitalization and other infection related costs were described. The economic consequences of nosocomial symptomatic UTIs can cost over $650 (USD) and catheter-related bacteriuria over $2,800 (USD) (28). A recent study by Rupp et al. demonstrated additional cost savings with the silver-alloy hydrogen-coated urinary catheters and also showed a decline in nosocomial UTIs (29). No evidence of silver-resistant urinary pathogens was found.

Symptomatic urinary infection or urosepsis in the presence of an indwelling catheter is best treated with rapidly bactericidal antibiotics such as an aminoglycoside, a quinolone, or a ß-lactam-aminoglycoside combination. Bacteremia is usually easily cleared, but eradication of the urinary infecting organism may be difficult in the continued presence of the catheter. Management of catheter-related urinary infections has been reviewed (30).

Renal Cysts (Including Polycystic Renal Disease)

Complicated infections within or associated with isolated renal cysts, autosomal dominant polycystic renal disease, or acquired renal cystic disease (three or more renal cysts or cystic involvement of >25% of renal mass in the absence of autosomal dominant polycystic kidney disease) (31,32) remain important diagnostic and therapeutic challenges.

Patients with polycystic kidney disease may develop typical infections of the urinary bladder and ascending pyelonephritis with renal parenchymal involvement as well as infection within the renal cysts themselves (33–35). The presence of polycystic kidney disease is associated with a 50% to 70% lifetime risk of UTI (36). In an autopsy study of 23 patients with polycystic kidney disease, 13 (56%) had findings consistent with pyelonephritis (37). It may be difficult to implicate infection as a cause of hematuria or flank pain in patients with cystic abnormalities of the kidney because these symptoms may be present in the absence of infection (38). Also, pyuria (≥10 leukocytes/high-power field) may be present in more than 40% of patients with polycystic kidney disease, with or without other symptoms suggestive of urinary infection (39); however, infection is documented in only about 10% of these patients (40). Findings suggestive of UTI in the presence of cystic renal disease include positive blood cultures, leukocytosis, fever, and lower GU tract symptoms such as dysuria. Negative urine cultures do *not* exclude infection of a renal cyst (35).

In a review of renal infections in patients with polycystic kidney disease, Sklar and associates (38) described four types of infections according to anatomic involvement: (a) localized infected cyst (pyocyst), (b) pyonephrosis (intrarenal abscess associated with ureteral obstruction), (c) acute bacterial interstitial nephritis, and (d) perinephric abscess. Clinical findings may vary with the anatomic location of bacterial infections in these patients.

The diagnosis of complicated UTIs in patients with renal cystic disease is usually based on the results of clinical examination, laboratory testing, and diagnostic imaging. Radiologic evaluation with plain radiography, ultrasound, computed tomography, and gallium imaging has been utilized to determine the presence and location of infection (38). Gallium imaging may show uptake within the kidney, but it does not provide specific information to determine whether an abscess or infected cyst is present. Computed tomography may be necessary to define pyocysts (38). Plain radiography may be useful if calculi are contributing to the clinical presentation. Renal ultrasonography also may identify calculi and can differentiate hydronephrosis from pyonephrosis and perinephric abscess. Percutaneous drainage of infected cysts in adult polycystic kidney disease has been described as has laparoscopic cyst decortication using transperitoneal or retroperitoneal access (41,42).

When cysts are infected, the Enterobacteriaceae (especially *E. coli, Klebsiella* sp., and *Proteus* sp.) and *P. aeruginosa* are most frequently implicated, with *Staphylococcus aureus, Salmonella* sp., *Streptococcus* sp., *Corynebacterium* sp., and others isolated less frequently (43,44). A gas producing *Clostridium perfringens* infection in a renal cyst has been reported in a patient with polycystic kidney disease (45).

Attempts should be made to isolate the infecting organism from the blood, urine, or cyst drainage. Appropriate treatment of infections in patients with polycystic kidney disease depends on the use of antibiotics that are able to concentrate within the infected cysts in addition to providing bactericidal activity against the infecting organism. Aminoglycosides, penicillins, and cephalosporins have relatively poor penetration into renal cysts, although pH, cyst physiology, and histology may affect diffusion (46–48). These antibiotics are relatively lipophobic and do not diffuse across cyst epithelial layers (46–50). Lipophilic agents such as clindamycin, chloramphenicol, macrolides, metronidazole, and trimethoprim are able to penetrate and accumulate within cysts, but they may or may not be active against the infecting organisms (46–50). Fluoroquinolone antibiotics such as ciprofloxacin and norfloxacin accumulate in cystic fluid, and they have been used successfully to treat infected renal cysts (51–53). In one study, ciprofloxacin achieved therapeutic concentrations in cystic fluid in seven patients with polycystic kidney disease and proximal and distal cysts. Because most of the causative organisms in infected cysts are facultative Gram-negative bacilli with quinolone susceptibility, these agents are quite useful clinically. Oral therapy is acceptable unless patients are septic. Infection in multiple cysts has been associated with sepsis and may require surgical intervention (nephrectomy) in rare cases (54). Intravenous quinolone therapy should be used in bacteremic patients and in patients with decreased gastrointestinal (GI) quinolone absorption (e.g., antacid use).

Urinary Diversion

Ureteral Stents

Ureteral stents are placed in the treatment of hydronephrosis and obstruction caused by nephrolithiasis or malignancies, and as adjuncts to lithotripsy and open surgical procedures on the urinary tract. These stents are made of synthetic polymers and are either indwelling (self-retained in place between the renal pelvis and the bladder) or external.

Complications of indwelling ureteral stents include fever, infection, gross or microscopic hematuria, biofilm development and stent rupture, catheter migration, encrustation and bladder stone formation, and vesicoureteral reflux (55–57). Infection in the presence of stent obstruction is problematic and difficult to eradicate. Also, it is often difficult to differentiate symptoms caused by infection from those associated with the presence of a stent, which include hematuria, dysuria, increased urinary frequency, nocturia, and loin pain (58). Bacterial colonization of stents has been reported but there are no formal guidelines to distinguish colonization (which may be associated with encrustation, stone formation, and obstruction) from active infection (55).

Positive urine cultures with supporting clinical evidence for urinary infection should stimulate prompt antibiotic treatment to eradicate the infecting pathogens. Ureteral stents that develop biofilm formation and encrustations treated *in vivo* with oral ciprofloxacin or ofloxacin absorb these antibiotics (59). Attempts to determine the presence of bacterial stent colonization are not reliable. Despite negative urine cultures, stents may be colonized with bacteria or fungi. Double-J ureteral stents can become colonized with Gram-negative bacteria within 2 weeks of placement. *E. coli*, the most common organism in one study, was cultured on stents prior to urinary colonization (60). Silicone ureteral stents compared with low surface energy stents and hydrogel-coated stents demonstrated less encrustation in the presence of urease-producing bacteria (*Proteus mirabilis*). These results may indicate a reduced risk of encrustation and *Proteus mirabilis* infection with this stent (61). Some investigators have recommended prophylactic antibiotic treatment directed at common Gram-negative uropathogens and enterococci prior to endourologic procedures (62). The presence of bacteremia and other signs of systemic infection suggest infection in the face of possible occlusion or obstruction of the stent, and stent removal is essential (55).

Surgical Urinary Diversion

Urinary diversions are performed to reroute urine in patients with obstructive uropathy from many causes—urinary bladder carcinoma or prostatic or gynecologic malignancies—and in patients with congenital abnormalities, neurologic disorders, and pelvic trauma. Although intermittent catheterization may be preferable in some patients with neurogenic bladder dysfunction (e.g., multiple sclerosis, paraplegia), the creation of a ureteroileal conduit is a popular alternative to achieve control of urine excretion. This procedure does not carry the associated metabolic and electrolyte complications seen with jejunal bypass procedures (63). The surgical construction of an ileal loop conduit is associated with few serious complications and a low mortality rate.

Infectious complications have been well described in pediatric and adult populations, however (64–67). An increased incidence of infectious complications has been noted when the ureteric component becomes obstructed; pyelonephritis may result. In long-term follow-up studies of patients with ureteroileal conduit diversions, Pitts and Muecke (68) and Schmidt and associates (69) observed pyelonephritis in 10% to 20% of patients. A large number of these patients had renal calculi and renal deterioration. Renal calculi are encountered frequently after urinary diversion and are often caused by urea-splitting organisms such as *Proteus mirabilis* and *Proteus morganii,* as reported in 31 of 34 patients studied by Dretler (66). The urease produced by these organisms splits urea to form an alkaline pH, and the solubility product constant for calcium and phosphate is exceeded with the resultant precipitation of crystals, which form the nidus for renal stones. In a study of renal calculi that developed following ileal conduit diversion, Dretler (66) found that 14 of 15 were composed of calcium and struvite (magnesium ammonium phosphate).

Recommendations for management of these patients include aggressive control of infection using bactericidal drugs active against urea-splitting organisms and acidification of the urine or avoidance of alkaline urinary pH, which encourages stone formation. The detection of urinary infection in these patients is difficult, because the ileal loops are almost always colonized. Asymptomatic bacteriuria in the presence of a ureteroileal conduit should not be treated and prophylactic antibiotics are not recommended. However, positive urine cultures associated with physical findings of fever, chills, and flank pain should prompt initiation of appropriate bactericidal antibiotics directed against Gram-negative enteric rods including *Proteus* sp. Aminoglycosides, quinolones, cephalosporins, and penicillin-ß-lactamase inhibitor combinations (e.g., ampicillin-sulbactam, piperacillin-tazobactam, ticarcillin-clavulanate) are useful in this setting. When susceptibilities are known, specific therapy can be instituted.

Other Anatomic Risk Factors

Vesicoureteral Reflux

Conditions that allow reflux of urine from the bladder to the ureters and subsequently the kidneys are associated with increased frequencies of acute and chronic pyelonephritis (70). Various degrees of vesicoureteral reflux may occur congenitally, and once bladder urine is infected, upper tract infection is facilitated by the retrograde flow. In this setting, organisms that cause infection may be relatively free of virulence factors such as hemolysins, pili, and other adhesions (Chapter 24). Vesicoureteral reflux is associated with progressive renal scarring in children, even in the absence of infected urine, and these patients have an increased risk of renal damage and even renal failure when infection does occur (71,72).

In a study of the etiologies of renal scarring, Huland and Busch (73) evaluated 213 patients with recurrent UTIs. Forty-two patients (26%) were found to have pyelonephritic scarring and renal insufficiency. Twenty-eight (67%) of these patients had urinary infections in the presence of vesicoureteral reflux. Young children under 4 years of age with intrarenal reflux have a well-demonstrated risk of renal scarring (71,74).

The approach to treatment of patients with documented vesicoureteral reflux and recurrent UTIs includes antibiotic management plus surgical reconstruction (antireflux operation). Hendren reported that more than 70% of very young children no longer had urinary infections after the surgical procedure (75). Some success has been achieved with continual antibiotic prophylaxis in these patients. Long-term chemoprophylaxis to prevent recurring UTIs has been studied in addition to surgical reconstruction. Regimens that include trimethoprim with and without sulfamethoxazole, quinolones, nitrofurantoin, and cephalexin can be effective at low doses over the long term (76,77).

Infected Urachal Remnants/Cysts

Acute and recurrent UTIs in the presence of abnormalities of urachal development with patent urachal remnants may be difficult to diagnose. Four primary developmental defects exist and present with varying signs and symptoms: patent urachus, umbilical urachal sinus, vesicourachal diverticulum, and urachal cyst (78–82). Umbilical urachal sinuses and urachal cysts are only rarely infected and many urachal cysts are found incidentally during surgery or radiographic studies. Although most patent urachal remnants in adults are undetected and asymptomatic, umbilical urachal sinuses occasionally may be infected and present with purulent umbilical drainage or periumbilical erythema. These infections may present as infraumbilical abdominal wall abscesses or with contiguous involvement of the peritoneal cavity with an enteric fistula (83).

Vesicourachal diverticula may present with recurrent urinary infections, and urethral discharge, pneumaturia, and a suprapubic mass also may be found (78,83). Imaging studies may help make the diagnosis, but often cystoscopy may be necessary, especially if the diverticulum is associated with malignant transformation of the remnant. The organisms that infect vesicourachal diverticula include *E. coli* and other Enterobacteriaceae, *S. aureus, Enterococcus* sp., and actinomycetes (78,84,85).

FUNCTIONAL RISK FACTORS

Diabetes Mellitus

Many factors that predispose the diabetic patient to infections in the urinary tract have been described. Diabetes mellitus is associated with increased risk of these infections as a result of poorly controlled plasma glucose concentrations, which in turn may impair granulocyte function and cell-mediated immunity. Also, the neurologic dysfunction associated with diabetic neuropathy may result in a neurogenic bladder with incomplete bladder emptying, urinary stasis, and retention. The increased likelihood of urethral instrumentation may predispose these patients to infection, as may diabetic microangiopathy, which can contribute to local ischemia and impaired host defenses (86,87).

In Table 36-2 are listed the manifestations of UTIs in patients with diabetes mellitus. Asymptomatic bacteriuria has been described as occurring two to four times more frequently in diabetic women (87,88) and as being more prevalent in diabetic women than men (89). Bacteriuria in diabetic patients may be associated with a disproportionate risk of infection in the upper urinary tract and kidneys and one study reported that upper tract infection could be documented in 79% of diabetic women with asymptomatic bacteriuria (88). Other renal parenchymal complications of UTI in diabetic patients include pyelonephritis, emphysematous pyelonephritis, papillary necrosis, and perinephric abscesses; these conditions should be considered in the evaluation of nonresponse to appropriate antibiotic therapy for urinary infection in diabetic patients.

Diabetic patients with urinary infections are more likely to be bacteremic or uroseptic than are nondiabetic patients (90,91). These patients are also more likely to develop acute pyelonephritis at a fivefold greater risk than nondiabetic patients (92). Diabetic patients with systemic signs of urinary infection should be studied with abdominal radiography to detect renal emphysematous pyelonephritis. Ultrasound or computerized tomography should be performed if obstruction of an abscess is suspected. In a large review of bacteremia in hospitalized patients, MacFarlane and associates reported that 49 (29%) of 168 patients with bacteremia were diabetic patients and the urinary tract was the focus of infection in 21 (43%) of them (90). The urinary tract is implicated as the source of bacteremia more frequently in diabetic than nondiabetic patients (91). Postmenopausal women with diabetes are at higher risk of UTI and this is related to duration of diabetes and insulin dependence (93). Type 2 diabetic women with histories of UTI (especially upper UTI) are at increased risk for renal scarring and damage, as demonstrated by renal cortex scans (94). Most of the bacteria responsible for urosepsis in diabetics are Gram-negative rods, with *E. coli* and *Klebsiella* sp. accounting for about 70%. Notably, *Klebsiella* sp. are isolated twice as frequently in diabetic patients with bacteremic urinary infections, and a large proportion of these patients had indwelling urinary bladder catheters.

TABLE 36-2

MANIFESTATIONS OF URINARY INFECTIONS IN PATIENTS WITH DIABETES MELLITUS

Clinical manifestation	Reference
Asymptomatic bacteriuria	(88,90,224,225)
Acute papillary necrosis	(87,97)
Emphysematous cystitis	(98,226)
Emphysematous pyelonephritis	(99,100)
Fungal infections	(227)
Perinephric abscess	(228)
Xanthogranulomatous pyelonephritis	(229,230)

In diabetic patients taking oral hypoglycemic agents, trimethoprim-sulfamethoxazole could lead to further hypoglycemia. No such potentiation is seen with most fluoroquinolone antibiotics but hypoglycemia is reported with gatifloxacin (95). Invasive staphylococci also can cause complicated infections and they may or may not result in abscess formation. Treatment for staphylococcal urinary infections requires oxacillin, nafcillin, or vancomycin, based on susceptibility testing. Intravenous antibiotics should be administered for the first 2 to 4 days in complicated infections, monitoring for decreasing symptoms and fever. Oral step-down treatment should be continued for approximately 14 days thereafter.

Opal and associates described 29 adult patients with *Streptococcus agalactiae* (group B streptococcus) bacteremia reviewed over 10 years at Walter Reed Medical Center. Nine (31%) of these bacteremic patients had diabetes, and six of these had involvement of the urinary tract (96).

Renal papillary necrosis is a well-known complication of UTIs in diabetic patients. In fact, more than half of patients with renal papillary necrosis are diabetic, possibly reflecting microvascular insufficiency leading to ischemia and necrosis of the renal papilla (97). These patients may present with flank pain, chills, and fever, and 15% may have renal insufficiency (87).

Emphysematous infectious processes may involve the bladder *(emphysematous cystitis)* or the kidney *(emphysematous pyelonephritis)* and are more frequent in diabetic patients. Emphysematous cystitis is usually caused by common facultative bacteria such as *E. coli,* although a few cases caused by *Clostridium perfringens* have been reported (98). This condition is identified on plain films, urographic roentgenograms, or computerized tomography by finding gas in the bladder wall. Tissue ischemia or trauma is usually involved in the pathogenesis.

Emphysematous pyelonephritis usually results from invasion of the renal parenchyma by gas-producing organisms. In patients with diabetes mellitus, high levels of blood glucose offer an enhanced environment for bacteria and gas formation may result from the accompanying mixed acid fermentation of glucose by enteric organisms. In patients with diabetes, emphysematous pyelonephritis presents with fever, flank pain, and often the finding of a flank mass or a renal mass on pyelography (99). *Escherichia coli* is the most frequently isolated bacteria, followed by *Proteus, Pseudomonas,* and *Klebsiella* sp., but *Clostridium* sp. are not typically involved (100). The diagnosis is established by the radiologic finding of gas in the renal parenchyma and bacteremia is usually present. Cultures of the urine are positive and blood cultures also may be positive.

Treatment includes intravenous fluid support, appropriate antibiotics, and percutaneous catheter drainage. Hyperbaric oxygen may be an additional treatment option along with antibiotics (101). Surgical intervention may be required if the response to medical therapy is delayed. Obstructing lesions must be removed and collections drained; nephrectomy may be required to effect a cure (102), especially in patients with extensive renal involvement and/or multiorgan system dysfunction (103).

Renal Transplantation

Complicated UTIs may occur in patients following renal transplantation, with an incidence varying from 35% to 79% (104).

Most of these infections occur within the first 4 months following transplantation (105) and are usually caused by common Enterobacteriaceae, including *E. coli, Klebsiella pneumoniae,* and *P. aeruginosa,* or by fungi, especially *Candida* sp. and invasive aspergillosis (106). Infections that occur 6 months or later after transplantation are less common and are more likely to involve the lower urinary tract, including the bladder. Some unusual organisms may cause urinary infections in transplant recipients; these include *Ureaplasma urealyticum* and *Gardnerella vaginalis* (107), the latter of which was implicated as a cause of perinephric abscess (108).

In the early posttransplantation period, symptoms of urinary infection may be mild or absent and fever may be absent; however, pyelonephritis and associated bacteremia are not uncommon (104). Uremia and corticosteroid or other immunosuppressive therapy may be responsible for this picture. Significant risk factors for post transplant UTIs include advanced age, female gender, cadaveric donor, reflux kidney disease and use of azathioprine (109). Posttransplantation renal infection (especially with *Enterococcus faecalis*) may lead to elevated serum creatinine levels and may result in a cascade of immunologic responses that ultimately precipitate allograft dysfunction or rejection (110). In addition to common cytomegalovirus infections in transplant patients, infections with hepatitis C virus and BK polyomavirus have been described after renal transplantation, and viral-associated interstitial nephritis may occur. In one center BK polyomavirus with associated transplant dysfunction and graft loss was reported in 2.5% of transplanted patients. Clinical features include ureteral obstruction, lymphocele, bacterial urinary infection, and hematuria. The diagnosis may be established with renal biopsy and electron microscopy of urine. Interruption of progression may be achieved with immunosuppressive treatment (111,112). Relapse rates may be high if posttransplantation urinary infections are not treated aggressively with antimicrobial agents (104,113,114).

Chronic urinary infection may occur in these patients and can be particularly problematic if associated with anatomic or structural defects in the ureter, bladder, or urethra as a result of the surgical procedure itself or secondary to fistula formation (113). Vesicoureteral reflux may develop at the ureteral anastomotic site in up to 25% of patients and may lead to hydronephrosis and infection. Graft failure may also occur as a result of mesangiocapillary glomerulopathy (115,116).

In a review of infectious complications in 450 renal transplant patients who received cyclosporine, Takahashi and colleagues found 41 patients (9.1%) with UTIs (117). Diabetes mellitus, high serum cyclosporine levels, and prolonged urinary catheterization were identified as factors that increased the risk of urinary infection.

Prophylactic use of trimethoprim-sulfamethoxazole for 6 months following renal transplantation may prevent complicated UTIs, including Gram-negative rod bacteremia and sepsis (104,118–121) and may decrease the incidence of posttransplantation urinary infection to less than 10%. This drug also might reduce the development of opportunistic infections caused by *Listeria monocytogenes, Nocardia asteroides,* and *Pneumocystis jiroveci* (104,120). However, reports of trimethoprim-sulfamethoxazole-induced alteration of renal function or synergistic exacerbation of cyclosporine nephrotoxicity have stimulated the search for other prophylactic compounds.

Treatment of pyelonephritis in the posttransplantation patient should include 6 weeks of an appropriate antibiotic based on susceptibility testing plus chronic "suppressive" antibiotic therapy thereafter (104). A recent report suggested that ciprofloxacin is associated with high cure rates and few serious side effects in the treatment of acute pyelonephritis and other urinary infections in these patients (122). Late UTI after renal transplantation may be a risk factor for serious complications including graft loss and death as described in a recent retrospective review of 728,000 renal transplant patients (123). Pharmacokinetic or pharmacodynamic interactions between ciprofloxacin and cyclosporine have not been reported, which suggests that they can be used together without additional monitoring (124).

Patients are at increased risk of catheter-associated urinary infection because they often receive Foley catheters in the posttransplantation period. To screen for these infections, Burleson recommended culturing the catheter tip on removal (125). In a study of 15 patients with negative catheter tip cultures, none developed a subsequent urinary infection; however, in 24 patients with positive catheter tip cultures and negative urine cultures, 16 developed a urinary infection. Tolkoff-Rubin and colleagues (118,126) used prophylactic trimethoprim-sulfamethoxazole (160 mg of trimethoprim, 800 mg of sulfamethoxazole) for 4 months after urinary catheter removal and reported a decrease in the catheter-associated infection rate from 38% to 8% with the use of this regimen.

Spinal Cord Injury and Neurologic Dysfunction

Frequent bladder catheterization is necessary as a result of bladder neuropathy in paraplegic or quadriplegic patients following traumatic or surgical injury to the spinal cord. This leads to colonization of the lower urinary tract with pathogenic bacteria and results in bacteriuria in about 80% or more of these patients (127). Bacteremia may follow and urinary infection may be associated with high mortality rates in patients with spinal cord injury and neuropathic bladders.

Urinary infections and the related sepsis as well as the high frequency of renal disease in these patients are probably associated with vesicoureteral reflux, hydronephrosis, accompanying renal calculi, and pyelonephritis, which were responsible for death in 43% of patients with spinal cord injury followed prospectively for 25 years in a veterans' hospital (128).

These patients may not present with typical symptoms of fever, chills, dysuria, or flank pain, and the presence or absence of symptoms usually is not helpful in predicting the results of urine cultures. Perkash and Giroux were able to document the presence of typical symptoms in only 3% of 110 patients with spinal cord injury and bacteriuria ($>10^5$ bacterial colonies/mL) (129).

The prevention of infection in patients with spinal cord injury is a major priority. Unfortunately, intermittent catheterization or self-catheterization as an alternative to indwelling bladder catheters may be associated with complications, including urethral fistulization, periurethral abscess formation, and epididymitis. Banovac and associates compared methenamine hippurate to no prophylactic antimicrobial agent in 56 patients with spinal cord injury who utilized intermittent catheterization or self-catheterization (130). Although there was some imbalance in the patient groups, positive urine cultures occurred in eight (23%) of 24 patients who received methenamine compounds and in 13 (58%) of 22 patients who received no prophylactic agents (130). Methenamine compounds (methenamine mandelate and methenamine hippurate) are urinary antiseptics that decompose in acid urine to formaldehyde. Urine pH must be kept in the range of 5 to 6 for this reaction to occur (131). Methenamine mandelate may be combined with trimethoprim to minimize or prevent infection with *Proteus* sp., which contribute to alkalization of the urine by means of urease production (132,133).

The high rates of bacteriuria (80% to 90%) that develops within 2 to 3 weeks in spinal cord-injured patients who require self-catheterization have prompted the study of many other prophylactic antimicrobial regimens. Some of these

studies were well-designed double-blind or placebo-controlled trials and some demonstrated a decrease in bacteriuria (134); however, little effect on symptomatic infection and bacteriuria was demonstrated. A double-blind, placebo-controlled, prospective, randomized study of low-dose trimethoprim-sulfamethoxazole (40 mg of trimethoprim, 200 mg of sulfamethoxazole, every day) was performed in 117 evaluable patients with recent spinal cord injury who required intermittent urinary bladder catheterization and who were followed for the first 4 months following injury. A reduction in the frequency of bacteriuria was demonstrated in men in the treatment group and the time to onset of bacteriuria was prolonged. Symptomatic urinary infection was more common in the placebo-treated men (18 of 52, 35%) than in the trimethoprim-sulfamethoxazole-treated men (four of 57, 7%; $P < 0.0003$). However, resistant Gram-negative bacilli and breakthrough bacteriuria were found in both populations, side effects were common, and the authors concluded that these events limit the overall utility of this approach (134,135).

Recent attention to the development of adherent biofilms in the urinary tract may offer explanations for recurring and difficult to treat infections in this population. Uropathogens can develop dense urethral biofilms with glycocalyx material on the bladder wall or catheters. Recently, biofilms have been described with intracellular bacteria that cause bulges appearing like pods on the bladder surface of infected mice. The pods have been shown to have polysaccharide matrices with a protective shell of uroplakin. It is becoming increasingly more evident how bacteria may evade antimicrobial killing within an environment that protects microorganisms in a uroplakin shell. These mechanisms support the ability of biofilms to allow bacterial microcolonies to survive and cause chronic or recurrent infections (136,137). This may contribute to the pathogenesis of recurrent urinary infections. Bacterial biofilms also can be detected on bladder epithelial cells and may respond better to fluoroquinolones than to trimethoprim-sulfamethoxazole. In addition to clinical cure of urinary infection, ofloxacin eradicated bladder biofilms in patients with spinal cord injury (138). In spinal cord-injured patients with neuropathic bladders and urinary infection, *E. coli* isolates are more virulent and more often hemolytic and demonstrate D-mannose–resistant hemagglutination of human erythrocytes more often than isolates from patients with asymptomatic bacteriuria (139). Increasing antibiotic resistant Gram-negative bacteria and multidrug resistant staphylococci are being reported in outpatients with spinal cord injury and UTI (140).

An experimental approach to prophylaxis has been reported that utilizes a nonpathogenic *E. coli* strain 83972 to colonize urinary bladders in spinal cord injury patients. Intravesical inoculation and colonization with this bacteria was not associated with urinary infection symptoms but was associated with improved quality of life (141). Other approaches, including cranberry juice intake, may lead to reduction of uroepithelial cell biofilms (142).

As mentioned already, frequent monitoring and treatment of symptomatic infection with appropriate bactericidal antibiotics and single doses of these agents prior to catheter changes may reduce the morbidity of urinary infections in spinal cord-injured patients with long-term indwelling catheters.

Neutropenia

Patients whose neutrophil count has fallen below 1,000 granulocytes/μL are at increased risk of bacterial infections. Most of these patients have received anticancer chemotherapy for leukemia, lymphoma, or solid tumors. As the gastrointestinal (GI) flora is the usual source of bacteremia in these patients, unless instrumentation in the urinary tract has been performed, urinary infections are not particularly frequent. In most series, fewer than 5% of the infections in neutropenic patients arise from the urinary tract (143). In patients with profound and prolonged granulocytopenia (<100 cells/μL), bacteremia is not uncommon; however, the urinary tract is infrequently the source of these infections. In fact, bacteremia in this population is more frequently caused by Gram-positive cocci than Gram-negative rods, probably in part because of the presence of chemotherapy-induced oral mucositis and the dissemination of oral Gram-positive cocci to the bloodstream.

The usual symptoms and signs of bacterial urinary infection may not be manifest because granulocytopenic patients may not respond locally to the presence of infection. Dysuria and burning may or may not be present and pyuria is often minimal because of the absence of granulocytes; therefore, it is important to culture the urine in febrile granulocytopenic patients even though the yield may be low. Fungal urinary infections may occur, especially following long courses of antibiotics. Patients with hematological malignancies and neutropenia have been reported to have *Achromobacter* and *Alcaligenes* sp. bacteremic infections, with urinary tract involvement or origin. Resistance patterns for these species show susceptibility to antipseudomonal penicillins, carbapenems, and trimethoprim-sulfamethoxazole, but resistance to quinolones and aminoglycosides (144).

Empirical use of antimicrobial agents early in the course of fever after appropriate cultures of blood, urine, and material from other presumed infected sites are obtained has reduced the mortality owing to infections in neutropenic patients. Gram stain should be performed on urine specimens from these patients and antibiotics directed against the common bacterial pathogens in this population: *E. coli, S. aureus, P. aeruginosa,* and streptococci (especially viridans Streptococcus). Amphotericin B or fluconazole should be considered for candidal UTIs.

Acquired Immunodeficiency Syndrome and Human Immunodeficiency Virus Infection

Acquired immunodeficiency syndrome (AIDS) and infection with the human immunodeficiency virus (HIV) have been associated with infectious and noninfectious complications in the GU tract (145–150). Noninfectious complications include HIV-associated nephropathy, which has become the third leading cause of end stage renal disease in Blacks between 20 and 64 years old (151). Other noninfectious complications are associated with protease inhibitor therapy, usually used in combination with two other highly active agents to prevent HIV replication. Indinavir sulfate has been implicated with crystallization and stone formation in the urinary tract, not usually visible on plain radiographs. Patients complain of ipsilateral flank pain with nausea, vomiting, dysuria, and hematuria. Computerized tomography is not diagnostic, but calcifications may be seen ultrasonically in approximately 35%. Up to 20% of patients treated with indinavir may have urologic side effects. Conservative treatment with hydration, analgesia, and brief discontinuation of therapy is effective, but permanent withdrawal may be necessary in 5% of patients (152,153). Infections caused by commonly encountered bacteria may present as cystitis, pyelonephritis, or renal abscesses. Also, *Mycobacterium tuberculosis* as well as atypical or nontuberculous mycobacteria may be found in upper and lower UTIs in these patients. Of the usual infecting bacteria, *E. coli* accounts for 25% and *Pseudomonas, Proteus, Klebsiella,* and *Enterobacter* sp. are found frequently. *Acinetobacter, Salmonella,* and *Candida* sp. also may be encountered (150). A recent review of HIV patients showed that UTIs were more frequent than in HIV-negative patients.

Enterococcus sp. were among the most frequently isolated organisms (154).

Gardenswartz and associates described a cryptococcal renal abscess in a patient with AIDS, and there are reports of prostatic abscesses caused by *S. aureus, Enterococcus* sp., *M. tuberculosis,* and *Mycobacterium avium-intracellulare* (155,156). *Histoplasma capsulatum* can cause prostatic infection, which presents clinically as prostatitis with fever and tenderness on examination, but urine cultures are only rarely positive (157,158). Granulomatous interstitial nephritis resulting from disseminated histoplasmosis has been reported (159). Microsporidial infection of the urinary tract owing to *Vittaforma corneae* has been described (160). Urinary infection with aspergillus infecting the kidney has been reported in an AIDS patient and was treated with Amphotericin B locally via a nephrostomy tube, as well as with systemic antifungals (161). Nosocomial infection with *Stenotrophomonas maltophilia* of presumed urinary tract origin has been reported in HIV-infected patients by Calza et al., with high-level resistance to beta-lactam antibiotics, monobactams, carbapenems, and aminoglycosides (162).

Cytomegalovirus can cause cystitis with hematuria in HIV-infected persons (126) and toxoplasmic cystitis also has been described (163). *Trichomonas vaginalis* urethritis, documented both by wet-mount and culture and polymerase chain reaction, has been reported in men with and without symptoms (164). Latent urinary BK virus, a human polyomavirus, can be activated in patients with AIDS and nephritis may result (165). Adenovirus hemorrhagic cystitis with gross hematuria and dysuria has been described in an HIV-infected patient with Burkitt's lymphoma (166). Noninfectious vesical involvement with Kaposi's sarcoma (145) and urethral lymphoma (described by Lopez and colleagues) in HIV-infected patients may have unusual presentations that initially could be confused with urinary infection (149).

Patients with neurogenic bladder that complicates HIV infection or AIDS may present with urinary retention, urinary frequency, and altered urinary flow as a result of bladder hyperreflexia or hyporeflexia (167). Most of the patients with bladder hyperreflexia had concomitant cerebral toxoplasmosis. Other patients with AIDS may have urinary retention secondary to central nervous system lymphoma, myelopathy, or prostatic hypertrophy.

MISCELLANEOUS CAUSES

Pyonephrosis is an acute suppurative infectious process with gross pus within the renal parenchyma that usually results from ureteral obstruction (168,169). The clinical differentiation of pyonephrosis from infected hydronephrosis is difficult even with the use of ultrasound evaluation (169). Infection may be associated with obstruction owing to congenital anomalies, calculi, malignancy, ureteral strictures, nephrolithiasis, diabetes, and functional disorders of the renal collecting system.

In a review of 23 patients with pyonephrosis, St. Lezin and associates reported 15 patients with virgin stone formation that produced obstruction at various sites including the calyces, renal pelvis, and middle and distal aspects of the ureter (168). The clinical presentation may vary from asymptomatic bacteriuria to resembling that of pyelonephritis with fever, flank or abdominal pain, leukocytosis, pyuria, and septic shock. The responsible organisms include the Enterobacteriaceae, anaerobic bacteria such as *Bacteroides* sp., and *Candida* sp. (168).

Radiographic determination of pyonephrosis may be limited even with the use of ultrasonography and computerized tomography; the correct diagnosis requires a high degree of suspicion. Ultrasound or computerized tomography-guided renal urine aspiration or retrograde ureteral catheter placement may be required. Nephrectomy may be necessary if drainage and appropriate antibiotic therapy directed at organisms isolated from aspirated and drained infected material does not prove successful (170–172).

Xanthogranulomatous Pyelonephritis

Xanthogranulomatous pyelonephritis is a unique pathologic presentation of chronic bacterial pyelonephritis. Schlagenhaufer initially described it in 1916, and almost 400 cases have now been reported in the literature (173). During the past 20 years, several reviews have been published (173–175). Xanthogranulomatous pyelonephritis is relatively uncommon and accounts for less than 1% of surgically or pathologically proved cases of chronic pyelonephritis. Only 18 patients with this diagnosis were identified at the Mayo Clinic in a five year period, during which 3,000 consecutive nephrectomies were investigated (175). Most cases are diagnosed in elderly patients, with almost 70% occurring in women.

The pathogenesis of xanthogranulomatous pyelonephritis is uncertain. Although *P. mirabilis* infection is present in most patients, it is not essential for the pathologic process to occur. Urinary obstruction usually has a role. Macrophages filled with periodic acid-Schiff (PAS)-positive granules have been produced in a rat infection model with *P. mirabilis*, *E. coli*, and *S. aureus*. On electron microscopy, macrophages appear to have ingested bacteria and developed phagolysosomes filled with amorphous material. It is hypothesized that xanthogranulomatous pyelonephritis may be caused by a lysosomal defect of macrophages that prevents complete digestion of ingested bacteria. Familial disease has not been described.

Presenting symptoms are recurrent flank pain, fever, and constitutional fatigue. Persistent anemia and leukocytosis occur in about 75% of patients. The urinalysis shows pyuria and often hematuria. Urine cultures are positive for *P. mirabilis* in about two-thirds of patients, and *E. coli*, *Klebsiella* sp., and *S. aureus* are each reported from a small proportion of patients. Multiple pathogens occur in about 25% of patients (174). In a small proportion of patients, urine cultures are negative despite ongoing disease activity and positive cultures may be obtained from resected renal tissue (175). *Staphylococcus aureus* may be more likely to produce localized disease and usually is not associated with nephrolithiasis (176). Occasionally pathogens isolated from resected renal tissue are different than those from voided urine (177).

Most patients have a history of recurrent urinary infection, often complicated by renal calculi, obstructive uropathy, and previous urologic procedures. Often patients have had a chronic undiagnosed illness for several months before the diagnosis of xanthogranulomatous pyelonephritis has been considered. On physical examination, a renal mass is palpable in more than 50% and hypertension is present in about one-fourth of patients (174). Hepatic dysfunction with elevated enzymes and indirect bilirubin levels is present in one-quarter of patients and these revert to normal after appropriate treatment (174).

Intravenous pyelography (IVP) discloses a nonfunctioning kidney in 85% of patients. Struvite renal calculi occur in 80% and are virtually universal with *P. mirabilis* infection. Radiologic investigation may also demonstrate cavitary masses and calyceal deformities. Angiography usually discloses hypovascular renal masses with no neovascularization. Computerized tomography is the diagnostic procedure of choice. It demonstrates the extent of involvement of perirenal structures and may permit a specific diagnosis by recognition of abnormal fatty tissue in the renal mass (178).

The disease is almost always unilateral and appears to be caused by chronic renal infection with an unusual inflammatory response (175). On gross examination, the kidney is

enlarged with either local or generalized involvement of renal tissue. Calyces are usually dilated and the renal parenchyma is replaced by yellow-orange soft tissue, which is usually surrounded by abscesses. The localization of this peculiar tissue in the renal pelvis is characteristic. Perirenal fat is usually inflamed, edematous, and adherent to the kidney. The inflammation may spread beyond Gerota's fascia and involve the perirenal fat of the retroperitoneal space. On microscopic examination, the lipidlike tissue is composed of a mixture of large foamy lipid-laden macrophages (xanthoma cells) together with neutrophils, plasma cells, fibroblasts, and necrotic debris. The cytoplasm of the xanthoma cells stains strongly with PAS. Although these cells form the basis of the microscopic identification of the lesion, they are not specific and may only reflect phagocytosis of tissue within the lipid. Foreign-body giant cells and microscopic calcification are also frequently present.

Xanthogranulomatous pyelonephritis is frequently mistaken for renal carcinoma or renal tuberculosis. Prior to the availability of CT, the diagnosis was seldom considered preoperatively. Kidneys were often removed surgically because of an incorrect preoperative diagnosis. Current organ imaging technologies enable a preoperative diagnosis (178). If the disease is localized in the kidney, total nephrectomy may be avoided and local resection with removal of renal calculi and intensive treatment of urinary infection may salvage residual functioning renal tissue (177). The disease rarely involves both kidneys and does not recur after treatment. The disease has not been observed to progress serially from one kidney to the other, so radical removal of involved tissue is not necessary. Further study of conservative management is warranted (177,179).

Malakoplakia

Renal malakoplakia is a rare granulomatous disease of uncertain etiology that occurs in similar clinical settings to xanthogranulomatous pyelonephritis. The term *malakoplakia* is derived from the Greek word for soft plaque. Over 200 cases have been reported to date, predominantly in women and the elderly. Many of the clinical and laboratory features of this disease resemble those of xanthogranulomatous pyelonephritis, but most patients have *E. coli* rather than *P. mirabilis* urinary infection (180).

The gross lesion is a soft yellow-brown plaque of variable size. Renal tissue is involved in one-fifth of patients and is bilateral in about 50%. The renal pelvis and ureters are involved in an additional one-fifth of patients and ureteral stricture may develop. Renal involvement appears frequently to be an ascending progression of bladder malakoplakia. Histologically, the plaques show large histiocytes with foamy eosinophilic cytoplasm, called von Hansemann macrophages. The cytoplasm contains PAS-positive granules and large renal concentric crystals, named Michaelis-Gutmann bodies. These bodies show a typical crystalline structure on electron microscopy and are primarily calcium and iron on chemical analysis. These lesions may be confined to the urinary tract but occasionally are seen in skin, prostate, testes, and the gastrointestinal tract.

The disease is caused by a defect in macrophage function, with impairment of bactericidal activity of monocytes for *E. coli* (181). Movement of lysosomes to phagocytic vacuoles is delayed owing to low levels of cyclic guanosine monophosphate. A cholinergic agonist can correct this defect (182). The Michaelis-Gutmann bodies are presumed to result from abnormal deposition of calcium phosphate and iron in the overloaded phagosomes (183).

Renal parenchymal malakoplakia usually occurs as an upper tract infection with fever and flank pain (184). A palpable flank mass occasionally may be present. IVP reveals enlarged kidneys with multiple filling defects (185). Renal malakoplakia may progress to renal impairment and failure (186,187). The disease occurs more frequently in immunosuppressed patients and has been observed in several patients following renal transplantation (188).

Several patients have been treated successfully with the cholinergic agonist bethanechol chloride and long courses of trimethoprim-sulfamethoxazole (181,184,189). Immunosuppression may have to be modified when the disease occurs in renal transplant recipients.

Urosepsis

The urinary tract is the most common site of origin for Gram-negative rod bacteremia, and the development of bacteremia from a urinary focus is termed *urosepsis*. Urosepsis is one of the most common presentations for bacteremic illness in nursing homes and hospitals. As discussed already, *E. coli* accounts for almost half of these uroseptic infections, with other enteric Gram-negative bacilli and enterococci following in frequency. Interleukin 8 (IL-8), a small chemotactic protein, plays an important role in neutrophil migration during UTI (193–195). Increased urinary IL-8 levels occur in urinary tract infection and urosepsis. Patients with acute pyelonephritis have higher IL-8 levels in urine than plasma (194). During urosepsis, this phenomenon stimulates the delivery of neutrophils to the GU system and results in pyuria. Although endotoxin (the bacterial cell wall lipopolysaccharide) may enter the circulation from a well-localized focus of Gram-negative bacterial infection, the presence of sepsis originating from the urinary tract usually implies bacteremia, which in turn usually results from infection in the kidney or renal pelvis.

Clinically, most uroseptic patients present with fever, shaking chills, flank pain, hypotension, cloudy urine, and leukocytosis. However, obtunded patients may not have urinary complaints or clinical signs referable to the urinary tract, and patients with obstructive uropathy might not present with the usual laboratory clues of pyuria and bacteriuria (196). Bacteremia in a patient with urinary infection usually implies that the infection originates from the kidney, but in the presence of an indwelling bladder catheter, erosive urethritis or cystitis might be the responsible focus. Also, sepsis may follow instrumentation or manipulation of the lower urinary tract such as occurs during percutaneous nephrolithotomy or ureteroscopy (197). Staghorn calculi (struvite or apatite) can become embedded with Gram-negative bacteria. A recent study has identified endotoxin associated with sepsis in stone fragments in a child who died from sepsis syndrome after percutaneous staghorn stone manipulation (198).

The diagnosis of urosepsis is usually confirmed by blood cultures positive for the same organism cultured from the urine. Despite initial hopes that rapid diagnostic tests for the presence of circulating endotoxin might speed the diagnosis of bacterial sepsis, no such test is available currently. Careful examination for the presence of bacteria on a drop of unspun urine (under a coverglass, or a Gram-stained smear) plus the findings of pyuria in a septic patient point to the urinary tract as the source of the infection in most cases. However, strong clinical suspicion is necessary for less typical patients and at least two and preferably three blood cultures should be obtained to identify the responsible organism.

Treatment requires intravenous fluid support, maintenance of blood pressure with pressors if necessary, and antibiotics. If the organism is known (as in the case of recently cultured urine in a septic patient), then a single active antibiotic can suffice. If no clues exist as to the identity of the organism, intravenous therapy with extended-spectrum penicillin (e.g.,

piperacillin) or a penicillin-penicillinase inhibitor combination (e.g., ticarcillin-clavulanic acid, ampicillin-sulbactam; piperacillin-tazobactam) can be used. Third-generation cephalosporins also can be used, but these agents are not effective against enterococci. Aminoglycosides (gentamicin, tobramycin, or amikacin) may be used in combination with a penicillin if enterococci or *P. aeruginosa* are suspected. Combination therapy with cephalosporin or extended-spectrum penicillin plus aminoglycoside is suggested if Gram-negative rod bacteremia occurs in a patient with granulocytopenia. Intravenous fluoroquinolones (e.g., ciprofloxacin, levofloxacin) also are useful, but resistance is increasing in some centers.

Adjunctive therapy with corticosteroids is not recommended. Considerable research activity has been directed to blocking cytokine activity in sepsis (not limited to urosepsis), but recent trials of antiendotoxin monoclonal antibodies, antitumor necrosis factor antibodies, and IL-1 receptor antagonist have been disappointing.

Others

Tuberculosis (Chapter 38) and renal and perinephric abscesses (Chapter 35) also are discussed elsewhere. Abscesses may rarely develop in the bladder wall. These intramural vesical abscesses are only rarely reported (190–192) and are usually caused by coliform bacteria, often in the presence of inflammatory bowel disease, diverticular disease, or a foreign body.

Infections Caused by Unusual or Resistant Organisms

Enterococci are not uncommon causes of UTIs and GU sites account for the majority of clinical bacteriology laboratory isolates. These organisms are more commonly isolated from nosocomial infections than from the community. Enterococci may be the causative agents in lower UTIs (e.g., cystitis), in catheter-related infections, in infections following urinary tract instrumentation, and in patients with GU anatomic abnormalities (199–201).

The incidence of enterococcal infection in general is increasing at an alarming rate according to the National Nosocomial Infection Surveillance (NNIS) Study; for example, from 1980 to 1989, the incidence of bloodstream infections with enterococci has increased 120% (201), and the urinary tract is frequently identified as the source. These infections may originate from the patient's own GU or GI flora or may be nosocomially spread from other patients (202).

Unfortunately, antimicrobial resistance in enterococci is also increasing dramatically, not unlike the rise in methicillin-resistant *S. aureus* seen over the past 30 years (203). A large number of enterococcal strains including up to 70% of *Enterococcus faecium* isolates show vancomycin resistance. This has been attributed to the almost 160-fold increase in vancomycin usage per 1,000 patient-days in hospitals between 1978 and 1992 (204). Vancomycin resistance is often classified by phenotypic expression of the presence of certain resistance genes such as *VanA*, which mediates resistance to vancomycin and teicoplanin; *VanB* with strains susceptible to teicoplanin but resistant to vancomycin; and *VanC,* which imparts low-level resistance to vancomycin in strains of *Enterococcus gallinarum* and *Enterococcus casseliflavus* (205,206).

Also, enterococcal strains that were previously susceptible to penicillin and gentamicin have been identified. Even penicillin plus gentamicin therapy may fail to eradicate some of these infecting organisms (207). Although ampicillin and vancomycin have been used as mainstays in the therapy of complicated nosocomial enterococcal urinary infections, strains resistant to both are threatening to overcome the efficacy of these drugs. Alternative treatment with drugs such as teicoplanin and quinupristin-dalfopristin (Synercid) or with chloramphenicol may be necessary. Moellering and associates described the efficacy of quinupristin/dalfopristin for vancomycin-resistant enterococcal (VRE) infections including those in the urinary tract. These patients were most often hospitalized, chronically ill, with multiple comorbidities. Clinical response to treatment occurred in 74% (208). Newer agents include linezolid, an oxazolidinone compound, effective in the treatment of serious Gram-positive infections including VRE (209). Daptomycin has been introduced recently with activity against resistant Gram-positive bacteria (VRE/MRSA) (210). Increasing multidrug resistant strains of *E. coli* causing community acquired pyelonephritis and other complicated UTIs are being reported, with identified clonal groups responsible for the antimicrobial resistance (211).

Hemolytic-uremic syndrome (HUS) associated with enterohemorrhagic *E. coli* urinary infection has been reported. Hemolytic-uremic syndrome includes a triad of microangiopathic hemolytic anemia, thrombocytopenia, and acute renal impairment. Thirteen cases of HUS associated with *E. coli* UTI have been described. Most cases are not associated with diarrhea or a prodrome, which usually occur when HUS occurs as a result of consumption of fecally contaminated products from cattle or sheep (212). Extended spectrum beta-lactamase producing bacteria (ESBLs) are other resistant Gram-negative enterobacteria that cause nosocomial and community acquired UTIs. Susceptibility patterns usually show resistance to quinolones, aminoglycosides, cephalosporins, and monobactams. Identified antibiotics to which these isolates (usually *Klebsiella* sp., *E. coli*, *Acinetobacter* and other Gram-negative bacteria) are susceptible include ertapenem, imipenem, and amikacin (213–216).

Atraumatic rupture of the urinary bladder associated with methicillin-resistant *Staphylococcus aureus* (MRSA) has been reported recently (217). Methicillin-resistant *Staphylococcus aureus* UTIs usually require therapy with intravenous vancomycin. Involvement of the upper urinary tract, pyelonephritis, renal abscess, and bacterial endocarditis should be excluded in these patients.

Anaerobic bacteria are unusual causes of urinary infection, but they are implicated in some patients (Table 36-3). Although they are major components of the normal urethral, periurethral, and vaginal flora, their presence in these areas may prevent other more invasive aerobic or facultative organisms from colonizing these sites and subsequently causing infection (207). Anaerobic bacteria may be identified in the urine in chronically catheterized patients but this is often short-lived and without clinical significance (218). Anaerobic bacteriuria is more common in patients who have undergone renal transplantation (219).

The mechanism by which these organisms cause disease in the bladder, kidney, or prostate depends on their ability to ascend from the periurethral area to the bladder or kidney. This process is accelerated in the presence of obstruction, urinary stasis, calculi, trauma, and catheters or other foreign bodies (219–222). Anaerobic bacteria have been documented to cause prostatitis and prostatic abscesses as well as bacteriuria, pyelonephritis, and urosepsis. Some of these organisms (e.g., *Bacteroides* or *Sphaerophorus* sp.) have been implicated in urinary or prostate infections following colonic surgery (223). Clindamycin and metronidazole are usually active against anaerobic organisms and cefoxitin or cefotetan and penicillins with ß-lactamase inhibitors also may be effective.

TABLE 36-3

ANAEROBIC CAUSES OF COMPLICATED URINARY TRACT INFECTIONS

Clinical diagnosis	Organism	Reference
Renal transplantation	*Bacteroides fragilis*	(219)
	Bacteroides melaninogenicus	
Prostatitis	*Peptostreptococcus*	(231)
	Sphaerophorus funduliformis	(220)
Prostate abscess	*S. funduliformis*	(233)
	Sphaerophorus gonidiaformans	(232)
	B. fragilis	(233)
Renal abscess or Pyelonephritis	*B. fragilis*	(234)
	Peptococcus asaccharolyticus	(235)
	Fusobacterium nucleatum	(236)
Perinephric abscess	*B. fragilis*	(237)
Urosepsis with bacteremia	*Peptococcus*	(238)
	Clostridium perfringens	(239)
Recurrent cystitis	*Actinobaculum massiiae*	(240)
Pyeloureteral junction obstruction/ pyelonephritis	*Actinobaculum schaalii*	(241)

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CHAPTER 37 ■ FUNGAL URINARY TRACT INFECTIONS

SCOTT F. DAVIES AND GEORGE A. SAROSI

Although thousands of different fungi exist in nature, only a few can cause illness in humans. Most of these are organisms that cause only superficial skin infections. Only a very small number of fungi are true pathogens for humans and other mammals, able to invade normal hosts and infect deep tissues. In addition, there are a number of fungal species that can produce human illness under special circumstances, especially when the patient's immune status has been altered, either by native disease or by therapy directed at some other illness.

Although customarily listed with bacteria as microorganisms capable of causing human illness, fungi are distinctly different from bacteria. Bacteria do not have a true nucleus (prokaryotes); fungi are the first group that does have a true nucleus, surrounded by a nuclear membrane similar to those of mammalian cells (eukaryotes).

The vast majority of human fungal illnesses are caused by only a handful of organisms. Among these organisms are the causative agents of the endemic mycoses—histoplasmosis, blastomycosis, coccidioidomycosis, and paracoccidioidomycosis. In addition, in rare instances, invasive fungal infection may be caused by another fungus, *Sporothrix schenckii.* These five fungi share a common characteristic—they are dimorphic. These fungi exist in the environment as hyphae (molds) but, following invasion of human tissues, they convert to their tissue-invasive form. The causative agents of histoplasmosis, blastomycosis, and sporotrichosis convert to unicellular yeasts that reproduce by simple budding. Paracoccidioidomycosis is similar, but reproduction of the tissue-invasive form occurs by multiple budding of daughter cells peripherally around the parent cell. Tissue replication of the fungus *Coccidioides immitis* is unique. The tissue-invasive form of the organism is a spherule. As the spherule grows, cleavage lines develop and the spherule is divided into a large number of endospores. Following maturation of the giant spherule, the outer membrane ruptures, and the multiple endospores are released. Each of these endospores can in turn mature into another giant spherule. A sixth fungal organism, *Cryptococcus neoformans,* can also produce invasive infections in normal hosts. This organism, both in nature and infected human tissues, is a single budding yeast with a carbohydrate capsule. It is the only encapsulated yeast that can infect mammals. The infecting particles are likely small, desiccated yeast that are aerosolized easily and are the correct size to reach the alveoli if they are inhaled. Once in the lung, the yeast grows in size and the carbohydrate capsule becomes more prominent.

These six fungal organisms are often referred to as "pathogenic fungi." They are capable of invading any nonimmune host and producing an infection. All these fungi share one important feature. Their tissue-invasive form cannot be killed by nonimmune phagocytes, including polymorphonuclear leukocytes (PMN) and macrophages (1).

Following inhalation and conversion to their tissue-invasive form, these organisms may remain in the lungs, or they may disseminate widely throughout the body. Their unchecked propagation will be contained only by the eventual development of effective cell-mediated immunity. The vast majority of people are able to mount effective cell-mediated immunity and, thus, are able to eliminate these slowly propagating fungi before severe illness ensues; therefore, recovery from the infections is the rule rather than the exception. However, the ordinarily benign clinical course can be profoundly altered if the infecting dose is massive, if there are overwhelming immune defenses, or if cell-mediated immunity does not develop because of an underlying immunodeficiency (whether naturally occurring or induced by immunosuppressive therapy with glucocorticoids and/or cytotoxic agents).

The second group of fungi that cause human illness is often called "opportunistic" fungi. A number of filamentous fungi, including aspergillus species and members of the family Mucorales, can cause invasive disease when normal immunity is altered. Aspergillus infections are perhaps 10 times as common as mucor infections, with other rare filamentous organisms such as *Pseudallescheria boydii* and *Fusarium* infections much less common. When the spores of the filamentous fungi are inhaled, normal neutrophils are able to dispose of these invading particles (1). These phagocytes form an efficient nonimmune barrier to infection that is breached easily when cytotoxic chemotherapy greatly reduces the number of neutrophils. Similarly, human infection with these organisms can occur in patients whose native neutrophils have abnormal fungicidal mechanisms, which are either inherited or acquired. High-dose glucocorticoid therapy depresses T-cell function, but is also a common cause of acquired phagocyte dysfunction.

Candidiasis, the most common opportunistic fungal infection, is caused by *Candida* species, most commonly *Candida albicans*. The epidemiology of candidiasis differs greatly from the epidemiology of the other normally pathogenic fungi. *Candida albicans* is a common commensal and is part of the endogenous flora of humans, colonizing skin, mucous membranes, the gastrointestinal (GI) tract, and the female genital tract (2,3). Unlike other fungal infections, which are usually acquired by inhaling the infecting particles, candidiasis is almost always the result of endogenous infection.

Candidiasis is an overlap disease. The organism can flourish on mucosal surfaces when T-cell function is depressed by a primary immunosuppressive illness, especially acquired immunodeficiency syndrome (AIDS). Glucocorticoid therapy (systemic or topical), antibacterial antibiotics, and diabetes also enhance overgrowth of Candida organisms on mucosal surfaces; however, phagocytes can kill the yeast and there is little tendency to deep invasion except with severe neutropenia. Because most cases of invasive candidiasis complicate neutropenia, it is best classified (with aspergillus species) as an opportunist.

Another important difference between Candida infections and aspergillus infections is that barrier function is much more important. Invasive Candida infections can occur when barrier function (especially skin and bowel mucosa) is breached in

the setting of corticosteroid therapy, severe illness, or even diabetes. The organisms can transmigrate across bowel and can invade through the skin (either along vascular access catheters or directly through diseased or injured areas of skin); however, deep tissue invasion leading to multiple abscesses throughout the body is uncommon except in neutropenic patients and corticosteroid-treated patients.

Candida organisms can also readily ascend from the urethra to the bladder, especially when there is an indwelling catheter. This colonization of the bladder is analogous to mucous membrane infection in most instances and does not usually set the stage for deep invasion.

It is helpful to divide fungal pathogens into two groups—one group requiring intact T-cell function for elimination, and the other group requiring intact PMN and macrophage function. Rather than considering these fungi as "pathogens" versus "opportunists," it is perhaps better to think in terms of T-cell opportunists (the endemic mycoses plus cryptococcus) and phagocyte opportunists (the filamentous fungi and Candida) (4). Although both limbs of immune defenses are abnormal in many immunocompromised patients, the more pronounced form of immune defect determines which type of fungal infection is likely to occur.

Phagocyte opportunists are discussed first, followed by T-cell opportunists, because candidiasis is by far the most important fungal infection of the genitourinary (GU) tract.

INFECTIONS CAUSED BY PHAGOCYTE OPPORTUNISTS

Candidiasis

Candida albicans, the most common organism associated with the syndromes of candidiasis, is a normal inhabitant of the human GI tract, the female genital tract and frequently the oropharynx (2,3). The organism does not usually colonize normal skin, but moist intertriginous skin, damaged skin, or skin of elderly individuals is frequently colonized (5). In addition, members of the genus Candida have been isolated from hospital environments (6), food, and many animals. Although the organism has also been isolated from soil, it is not believed that human infections result from exposure to soil.

The vast majority of human infections are indigenous in origin. Although human-to-human transmission has been seen, especially in neonates who pass through a contaminated birth canal, this is a rare and unusual mechanism of infection. Although rare episodes of Candidiasis have been traced back to putative sources in a hospital (7), the lack of a convenient biotyping system interferes with careful epidemiologic investigation; it is also entirely possible that the reported infections were indigenous in origin.

Pathogenesis

Intact skin and GI mucosa provide a powerful barrier to infection by Candida. Any abnormality in normal integument, such as maceration in intertriginous areas, alters this barrier and allows Candida colonization—setting the stage for possible invasion. Similarly, alteration of the normal mucosal barrier in the GI tract (e.g., by cytotoxic chemotherapy) facilitates transinfection across the gut. The first line of defense is the neutrophils once the organism crosses the skin or gut and tissue invasion occurs. Normal neutrophils can phagocytose and kill Candida blastospores and damage pseudohyphae (8,9). Monocytes and perhaps eosinophils also are capable of attacking and destroying Candida. In fact, some evidence suggests that monocytes are more efficient in destroying ingested Candida than are neutrophils (10). Myeloperoxidase, hydrogen peroxide, and the superoxide anion system are the primary mechanisms of intercellular killing by neutrophils. Individuals whose neutrophils lack these killing mechanisms are highly susceptible to invasion by Candida species (10,11).

The role of lymphocytes in normal defense against Candida species is less certain (12). Although it is apparent that patients with dysfunctional lymphocyte function (e.g., chronic mucocutaneous candidiasis) are superficially infected with Candida species, deep tissue invasion apparently does not take place. This situation is analogous to human immunodeficiency virus (HIV)-infected individuals with marked decrease in helper T cells. Mucocutaneous candidiasis is ubiquitous in these patients, but deep tissue invasion of Candida is rare (13).

The role of humoral immunity in candidiasis is complex. Serum and plasma, even though they contain antibodies directed against Candida, are incapable of killing the fungi (14). Complement is necessary for opsonization of the organism (15), and the clinical observation that complement-deficient animals are more susceptible to candidiasis indicates a possible role for the complement system in host defense against Candida species (16). Both the classical and the alternate pathways of complement activation are involved; the alternate pathway may be more important. The ability of Candida species to adhere to epithelial cells, fibrin clots, and synthetic material is important; however, the precise role of adherence in the pathogenesis of candidiasis has not been fully elucidated yet (17).

It is apparent from the complex interaction of phagocytotic cells, lymphocytes, and humoral immunity that any immune defect will likely increase the risk of Candida invasion. Perhaps the most important factor predisposing to invasion by Candida has been the wide and increasing usage of broad-spectrum antibiotics. Both oral and parenteral antibiotics reach significant concentrations in the gut lumen and markedly alter the bacterial flora of the normal human GI tract. This alteration allows organisms normally at a competitive disadvantage to grow unimpeded, creating a microenvironment where Candida may overgrow.

The GI tract is one site for the entrance of Candida into the bloodstream. When an overwhelming amount of Candida is ingested (as was done deliberately in the often-cited article by Krause) the organisms transmigrate even normal GI mucosa to invade the bloodstream (18). Any interruption of the normal GI tract mucosal barrier will increase the likelihood of bloodstream invasion. When surgical manipulation of the gut (especially procedures that transect bowel) is combined with antimicrobial therapy, the likelihood of this occurrence increases many times (19). Similarly, when chemotherapy causes destruction of the rapidly dividing cells of the gut lining, bloodstream invasion by Candida can occur.

The development of intravenous devices to administer parenteral fluid, therapeutics, and nutrients has provided another route for Candida to enter the bloodstream. In fact, intravascular catheter-related candidemia is likely the most common cause of positive Candida blood cultures. Patients receiving hyperalimentation are more susceptible to hematogenous candidiasis (20). It is uncertain whether the hyperalimentation itself is the major predisposition or whether much of the increased risk is owing to the catheter and factors related to the underlying condition that necessitated the hyperalimentation. Colonization with Candida species is common when chemical or thermal burns damage the normal skin (21). It should not be surprising that hematogenous candidiasis is frequent in these patients because burn patients frequently receive multiple antibiotics, hyperalimentation, intravenous fluids and multiple antibiotics.

Once the fungus enters the bloodstream and disseminates widely throughout the body, microabscesses are formed in many deep tissues (22). In these microabscesses, both yeasts and pseudohyphae are present. The initial tissue response is

a neutrophil-rich exudate; monocytes and histiocytes appear later. In some patients, a granulomatous response may be seen. If patients have neutropenia, the reaction frequently lacks the neutrophil component, and the microabscesses may contain only tissue debris, endothelial cells, and occasional chronic inflammatory cells. The clinical picture includes fever, chills, and in some cases renal failure. Macronodular skin lesions and endophthalmitis (cotton wool exudates visible with an ophthalmoscope) may be clues to the diagnosis.

Diagnosis

Candida organisms are small, 4- to 5-μm cells that are usually ovoid in shape and reproduce by single budding, or (for albicans species) by serial budding or germ tube formation. These latter forms of reproduction lead to pseudohyphae and sprout mycelia. All species of Candida grow readily in most laboratory media, whether in vented blood culture bottles or on agar plates. Special fungal media are not required for recovery. The use of the lysis centrifugation blood culture system increases the recovery of Candida from blood cultures (23).

Candida albicans is easily separated from other species of Candida by placing freshly cultured organisms in serum. The organisms immediately begin to produce pseudohyphae. The process begins by formation of germ tubes that project from the cell surface, which can usually be observed within 2 hours. Among the remaining species of Candida, speciation can be accomplished relatively quickly by chemical tests (24). These are usually important, because the different species have different antifungal sensitivity patterns.

The organisms are readily seen in histopathologic sections, even in hematoxylin and eosin (H&E)-stained sections. The organisms also are easily identified after staining with one of the various modifications of the silver stain. The gold standard for the diagnosis of disseminated candidiasis is recovery of the organism from blood culture. Even with the increased sensitivity of the lysis centrifugation system, perhaps as many as half of the patients with disseminated candidiasis never have a positive blood culture (25).

Accurate serodiagnosis of candidiasis would be a great help, but unfortunately there are no widely accepted serologic tests available (26). Generally speaking, tests that measure antibody to Candida are not reliable, because most individuals have antibodies to Candida. Although a rising titer may carry some weight, it is not uncommon to see equally high or even rising titers from patients who are only colonized. Serodiagnostic tests vary a great deal from investigator to investigator; no single test has gained widespread acceptance or proved itself in the clinical arena. Tests that measure circulating Candida antigens have more theoretical appeal because of higher specificity, but sensitivity has been variable and there are no promising candidates for a highly useful clinical test.

Candidemia. Perhaps the single most common error made in the diagnosis of candidiasis is to disregard so-called "isolated" candidemia (27). Isolation of Candida from any biological specimen should be taken seriously, but this is especially true for isolation from blood. Although occasional patients may have only transient episodes of Candida dissemination via the bloodstream, even a single positive blood culture for Candida should lead to further investigation to rule out possible sites of deep-tissue involvement. All patients with Candidemia need a full course of treatment.

Invasive candidiasis may occur following insertion of intravenous devices or after alteration of the gut mucosal barrier. Whatever the mechanism, once the organism is in the bloodstream, widespread dissemination can ensue, especially in the setting of profound and prolonged neutropenia. In the vast majority of instances, Candida infection of the renal parenchyma is the result of hematogenous dissemination from a skin or gut source; however, renal candidiasis is the cause of only a tiny fraction of positive Candida urine cultures.

Upper and Lower Urinary Tract Candidiasis

Candidiasis is by far the most important fungal infection of the urinary tract. About half of all Candida isolates from the urine are *Candida albicans*. About 20% to 30% are *Candida glabrata* and about 10% each are *Candida parapsilosis* and *Candida tropicalis*. A few percent of isolates are owing to other species, including *C. kreusei, C. Guilliermondi,* and *Candida lusitaneae.*

The problem is that there are several different syndromes associated with positive Candida cultures in the urine that vary in severity from trivial to life threatening or even fatal. Sometimes it is very hard to tell these syndromes apart.

Positive urine cultures for Candida may be: (a) contamination, (b) colonization of the lower urinary tract (usually harmless), (c) infection that has already ascended to the upper urinary tract with possible serious consequences and even a small chance of hematogenous spread from the kidney, or (d) a marker of hematogenous Candida infection from another source (usually intravenous catheter or bowel) that has seeded the kidney and is being excreted into the urine. In this last case, blood cultures sometimes are positive; however, positive urine cultures may be the only marker of disseminated candidiasis.

Simple contamination should be excluded first, especially in female diabetic patients without a urinary catheter (who have increased numbers of Candida organisms in the vagina and at the urethral orifice). Two consecutive positive urine cultures should be required before a diagnosis is made and therapy considered.

Most positive urine cultures for Candida are not contaminants. They reflect simple colonization of the lower urinary tract. Candida species, usually *C. albicans* or *C. glabrata,* occasionally are recovered from the urine of normal females without catheters (28,29). Although less frequent than in females, the normal male urinary tract may also occasionally harbor candida organisms. Whether this actually represents colonization of the male bladder or whether it just reflects colonization of the male urethra secondary to infection in the female sexual partner is unclear. Unlike in normals, colonization of the lower urinary tract is very common in hospitalized patients. The major risk factors for colonization of the bladder are broad-spectrum antibiotic administration, diabetes mellitus, age, and most important, an indwelling urinary catheter (30). Diabetes is a risk factor because of increased perineal colonization and autonomic impairment of bladder function, which often necessitates catheter drainage of the bladder. Even without the use of antibiotics, the longer a urinary catheter stays in place, the greater the risk of Candida infection of the bladder (31). Candida isolates make up 10% or more of all positive urine cultures in hospitalized patients and 25% or more of all positive urine cultures in hospitalized patients with a urinary catheter (32). Candida isolates are even more common (sometimes more than 50% of all urinary isolates) in patients from surgical intensive care units.

Ascending infection from the bladder to the kidneys is uncommon and usually occurs when the upper urinary tract is obstructed and/or instrumented, especially in diabetic patients. Diagnosis is difficult. Symptoms of pyelonephritis (fever with flank pain) are rare. Hyphae within renal casts are diagnostic but rare. Unfortunately, pyuria and positive KOH smear from yeast and/or pseudohyphae in the urine are not helpful in distinguishing upper tract disease for colonization of the bladder. Ascending infection should be suspected when Candida urine cultures are persistently positive in appropriate patients (with stones, strictures, stents, or other urinary tract abnormalities) who have fever and sepsis syndrome or decreasing renal function (reflecting bilateral obstruction).

Renal ultrasound examination and computed tomographic scans can demonstrate abscesses in the kidneys or filling defects in the proximal collecting system. As discussed previously, positive blood cultures for Candida species are usually associated with central venous catheters (often in patients with other risk factors) and bowel transmigration in patients with neutropenia, and with bowel trauma and surgery. Candidemia rarely results from ascending infection of the upper urinary tract that began as colonization of the bladder.

The final and least likely explanation for candiduria is that the positive urine culture is a marker of hematogenous Candida infection that has seeded the kidney and is being shed in the urine. In this case, there is a systemic fungal infection that needs aggressive systemic therapy. In one large prospective study of 861 hospitalized patients (mostly asymptomatic) with positive urine cultures for Candida, only 7 patients had positive blood cultures and only 2 died of systemic Candida infections (33); however, there are other situations where a positive urine culture may be more significant. A positive urine culture in a neutropenic patient who has never had a urinary drainage catheter is more likely a marker of occult systemic infection. Multiple urine cultures with *C. parapsilosis* sometimes are a marker for a catheter-related systemic Candida infection.

High fever in a patient with candiduria is usually caused by another infection; however, if no other infection is found then the combination of persistent candiduria and fever warrants investigation to exclude clinically silent hematogenous renal candidiasis that is seeding the urine. In that setting, it is also important (but difficult) to exclude disseminated candidiasis, especially in high-risk patients. Some intensive care unit (ICU) studies suggest that 10% or more (rather than 1%) of positive urine cultures are a marker of disseminated candidiasis (34). Blood cultures can be negative and the infection can be fatal if left untreated. Deciding which patients to treat for possible disseminated candidiasis is extremely difficult. Many centers choose to treat empirically based on some combination of the following: fever without other cause and unresponsive to antibacterial antibiotics; colonization of multiple sites (usually urine and sputum or other respiratory specimens); prolonged therapy with antibacterial antibiotics; central vascular lines; hyperalimentation; abdominal trauma requiring bowel resection; and abdominal surgical procedures that transect bowel wall.

Unfortunately to date there are no good tests to exclude disseminated candidiasis. Many patients have negative blood cultures. Negative urine cultures are evidence against disseminated candidiasis in neutropenic patients with fever (35), but probably have less value in non-neutropenic patients. Overtreatment of patients with positive urine cultures results from the extreme difficulty of absolutely proving that a positive urine culture is not a marker of invasive candidiasis in a high-risk patient. It is unlikely that this situation can be improved unless better tests are developed to diagnose and exclude disseminated candidiasis. The positive urine culture has exceedingly poor specificity, because over 90% of the time there is only colonization of no particular clinical import.

Treatment

Treatment depends on the clinical syndrome, species of Candida isolated, and susceptibility testing. The most important agents are intravenous amphotericin B, intravenous and oral azoles, and caspofungin, the first of a new class of antifungals, the echinocandins. Amphotericin B has more toxicity, especially nephrotoxicity. Fluconazole is nontoxic and is excreted unchanged into the urine in high concentrations. Itraconazole is highly protein bound and is poorly excreted into the urine and has a lesser role. It does have a somewhat broader spectrum against non-*Candida albicans*. Voriconazole has the same broad spectrum of itraconazole, but is not excreted in active form into the urine. Caspofungin is an echinocandin that is potent across the range of candida species. Although caspofungin has fairly low urine levels, it has been reported to successfully clear candiduria in a small number of cases. Other agents that are coming include additional azoles and echinocandins. Clinical data about these newer agents are incomplete.

Most strains of *C. albicans* are sensitive to fluconazole and to Amphotericin B (AMB) and fluconazole. Many strains of *C. glabrata* and especially *C. krusei* may have significant resistance to both these drugs. Lipid-based formulations of AMB have less renal toxicity, but are not generally utilized as first line therapy for candida infections.

There is finally reliable susceptibility testing for yeast isolates, the NCCLS M28-A system (36). It tests various yeast isolates against fluconazole, itraconazole and other azoles. Isolates are divided into S (susceptible), S-DD (susceptible but dose-and delivery-dependent), and R (resistant). Testing for susceptibility to AMB is not as well developed. The exact species isolated and the results of susceptibility testing influence the treatment plan.

The Infectious Diseases Society of America (IDSA) published extensive practice guidelines for treatment of candidiasis in 2004 (37). There were many changes from the previous guidelines of 2000 (38), because the release of caspofungin and voriconazole (and large clinical studies with these agents) has led to major changes in clinical practice. The reader can refer to the original documents for more complete information. The recommendations from 2004 will also change over time as new studies and even newer agents emerge.

For candidemia in particular there have been major shifts. Several large trials have shown equivalency for fluconazole with AMB (39,40,41). Caspofungin in one excellent study showed equivalency with AMB and possible superiority for non-albicans species (42). An open label trial showed that voriconazole was equal to combined therapy with AMB and fluconazole (43).

The new agents and the new information support the following approach. For first line therapy, consider fluconazole 400 mg/day continued 2 weeks beyond negative cultures and resolution of symptoms. Caspofungin (70 mg loading dose followed by 50 mg/day) is alternate therapy. Other options include fluconazole 800 mg/day (especially for S-DD isolates), AMB 0.6 –1.0 mg/kg/day, fluconazole 800 mg/day plus AMB 0.6 to 1.0 mg/day for the first 5 to 6 days, or voriconazole 6 mg/kg q 12 hours times two and then 3 mg/kg q 12 hours. As stated in the ISDA guidelines (37), the choice among these regimens is complex and multifactorial. "The choice ... depends on the clinical status of the patients, ... knowledge of the species and/or antifungal susceptibility ... , relative drug toxicity, presence of organ dysfunction, knowledge for use of the drug in the given patient population, and the patients prior exposure to antifungal drugs."

For *C. glabrata* and S-DD isolates of other fungal species, caspofungin and fluconazole 800 mg/day are good options. Fluconazole 400 mg/day is generally preferred for *C.albicans*, *C. tropicalis*, and *C. parapsilosi*, barring other factors. Fluconazole 600 mg/day is preferred for *C. lusitaneae*. Caspofungin and AMB are preferred agents for *C. krusei*. Other factors, as stated earlier, may tilt treatment toward ABM, combination therapy, or voriconazole. A cytokine that accelerates recovery from neutropenia (G-CSF or GM-CSF) should be used for patients who develop suspected or proven disseminated candidiasis while neutropenic.

The following are considerations for treatment of urinary candidiasis:

1. For all noncatheterized patients with asymptomatic candiduria, repeat urine cultures are indicated to exclude simple contamination.

2. For patients with asymptomatic candiduria with little reason to suspect ascending infection or clinically silent invasive candidiasis, no treatment is indicated. There is no evidence that these patients benefit from therapy. In a prospective double-blind trial, Candida was cleared from urine at 14 days in 50% of patients treated with fluconazole at 200 mg/day as compared to 29% treated with placebo. Two weeks later, however, candiduria rates were the same in treated and untreated patients (68% versus 65% clear of Candida) (44). Remove the urinary drainage catheter if possible. Repeat urine cultures 2 weeks later.
3. Amphotericin bladder washes can be used as a test. If candiduria clears, that is evidence that the positive cultures were only colonization. If candiduria cannot be cleared, it is somewhat suggestive (but not diagnostic) of seeding of the urine from silent disseminated candidiasis. AMB bladder washes are rarely indicated.
4. For rare symptomatic Candida cystitis (frequency, dysuria, urgency, hematuria), treat with fluconazole 200 mg/day for 7 to 14 days or AMB 0.3 to 1.0 mg/kg for 1 to 7 days.
5. Treat candiduria patients who have a higher risk of progression from the lower to the upper tract (ascending infection that might possibly lead to hematogenous spread from the kidney) the same as patients with symptomatic candiduria. This includes patients with renal allografts and patients scheduled for urologic procedures.
6. Perform ultrasound and/or renal computerized tomography (CT) to diagnose ascending disease involving the upper tract in appropriate persistent candiduria patients (those with ureteral or renal pelvic catheters, obstructed upper urinary tract, diabetes, or renal allograft recipients). Treat patients with proven upper tract infection the same as those with invasive disease. Occasionally, patients with fungus balls may require surgery.
7. For selected patients (defined in the preceding text) with candiduria but lacking evidence of infection at any other site treat as possible occult invasive candidiasis with the same regimens used for candidemia and proven disseminated candidiasis (see the previous text). Continue treatment for 2 weeks after all cultures are negative and signs and symptoms of infection are resolved. (The usual course of therapy is 4 to 6 weeks.) As mentioned, selection of this treatment group is difficult and uses criteria that include: fever without other cause that is unresponsive to antibacterial antibiotics; colonization of multiple sites (usually urine and sputum or other respiratory specimens); prolonged antibacterial antibiotics; central vascular lines; hyperalimentation; abdominal trauma requiring bowel resection; and surgery that transects bowel wall.
8. Fluconazole is excreted unchanged in the urine and does not reach high urine concentrations in patients with renal failure. Treatment of candiduria with fluconazole is one of the few situations where the drug dosage must be increased in renal failure.

In Table 37-1 treatment strategies are outlined for various forms of candidiasis in non-neutropenic and neutropenic hosts, and in patients with AIDS.

INFECTIONS CAUSED BY FILAMENTOUS FUNGI

The filamentous fungi are true phagocyte opportunists. These organisms are extremely common in nature and are found in decaying organic material. Members of the genus Aspergillus are clinically most important. More than 300 different species of aspergillus have been identified, over 100 of which have been associated with human illness. However, 90% or more of human infections are caused by *Aspergillus fumigatus,* followed in decreasing frequency by *Aspergillus niger, Aspergillus terreus, Aspergillus nidulans,* and *Aspergillus clavatus*. Infection by any other species is uncommon. Members of the class Zygomycetes (mucormycoses) are also becoming more important as pathogens in immunosuppressed patients, although the total number of human infections from these agents is less than one-tenth that from aspergillus species. Recently, other soil fungi also have been associated with clinical illness. For the sake of simplicity, these have been categorized as hyalohyphomycosis (pigmented hyphae) and phaeohyphomycosis (nonpigmented hyphae).

These fungal species are primarily respiratory pathogens, although members of the class Zygomycetes frequently invade the upper respiratory tract, diseased skin, and even diseased bowel, in addition to the lung. Following inhalation (or implantation into the skin or sinuses) all of these organisms may invade blood vessels, which leads to widespread dissemination to the skin, brain, and visceral organs, often including the kidneys. A major pathogenic mechanism shared by all filamentous fungi is vascular invasion leading to distal tissue necrosis (45). Subsequent to tissue necrosis, the fungi may continue to grow in the necrotic tissue producing a fungus ball or fungal bezoar as a cavity forms (45,46,47). Although the vast majority of all renal aspergillosis is pyemic, there has been one report of inadvertent transplantation of two kidneys already infected with aspergillus; the donor was a failed liver transplant recipient (48).

Aspergillus renal disease takes two main forms: parenchymal and pelvic aspergillosis. The first form is more common and it is assumed that both types of infection are usually blood-borne, although occasional reports have shown apparently isolated fungus balls in the bladder without upper tract disease (49). Parenchymal renal disease is most common in patients with impaired phagocyte function (50), whereas pelvic aspergillosis is more frequently encountered in diabetic patients (51). Flank pain is the most common manifestation of renal aspergillosis, analogous to the left upper quadrant pain that occurs with splenic infarction. Urinalysis often shows white blood cells without bacteria. KOH preparations of the urine may show hyphae. Positive aspergillus cultures from urine usually are the result of blood-borne seeding of the renal parenchyma and only rarely reflect isolated disease of the bladder. For Candida, over 90% of positive urine cultures are the result of bladder colonization; for aspergillus, over 90% of positive urine cultures are the result of hematogenous spread of infection to the kidneys from a lung source and shedding of the organisms into the urine. Autopsy evaluation of patients with invasive aspergillosis shows a much higher frequency of renal disease than was clinically apparent during life (50). A test to detect aspergillus antigens in blood has recently been standardized and has become widely commercially available (52). That test may be helpful to diagnosis aspergillus infection as one of the possible causes of multiple renal abscesses.

Renal disease from Zygomycetes includes renal parenchymal disease as well as invasion of renal veins (53). Disease almost always is owing to blood-borne seeding of the kidneys. Isolated renal mucormycosis has been described in AIDS patients who abuse drugs intravenously or have long-term intravascular access catheters for various therapies.

Therapy for filamentous fungi infections is less successful and less well defined than therapy for other fungal diseases. High-dose AMB (even 1 to 1.5 mg/kg/day) has the mainstay of therapy for invasive aspergillosis, but at this dose AMB is poorly tolerated because of nephrotoxicity. Newer lipid based preparations of AMB permit higher AMB dosing with less renal toxicity. Therapy for invasive aspergillosis is rapidly evolving with the advent of newer agents and new clinical studies. Voriconazole 6 mg/kg q 12 hours times two doses followed by 300 mg q 12 hours is a first line agent (54). Liposomal AMB 5 mg/kg/day (or higher doses) is an alternate therapy.

TABLE 37-1

TREATMENT STRATEGIES FOR CANDIDIASIS

Form of illness	Nonneutropenic host	Neutropenic host	HIV-infected host
Cutaneous candidiasis	Topical clotrimazole, econazole, miconazole, nystatin. Keeping the area dry is also important.	Same as nonneutropenic	Same as nonneutropenic
Oral pharyngeal	Topical clotrimazole or nystatin, FLU 100 mg/day PO for 7 to 14 days. ITRA solution 200 mg/day PO is equally effective and treats some patients who fail FLU therapy.	Same as nonneutropenic	FLU 200 mg PO × 1 day, then 100 mg PO × 1 week. Continue long-term suppression with 100 mg PO q week. ITRA solution 200 mg/day PO for 7 to 14 days treats some patients who fail FLU therapy. Consider AMB 0.3 mg/kg/day for refractory cases.
Esophageal candidiasis	FLU 200 mg PO × 1 day, then 100 mg PO q day 1 to 2 weeks. RA solution 200 mg/day PO is equally effective and treats some patients who fail therapy.	Same as nonneutropenic	FLU 200 mg PO × 1 day, then 100 mg q day × 1 week. Continue long-term suppression with 100 mg PO q week. ITRA solution 200 mg/day PO for 7 to 14 days treats some patients who fail FLU therapy. Consider AMB 0.3 mg/kg/day for refractory cases.
Vaginal candidiasis	Intravaginal miconazole, butaconazole, tioconazole, or terconazole. FLU 150 mg PO single dose or ITRA 200 mg BID for 1 day. Recurrent infections may require maintenance therapy with FLU 150 mg q week.	Same as nonneutropenic	FLU 200 mg PO q day until symptomatic improvement; then 200 mg q week. Some patients need continuous daily suppression.
Invasive candidiasis (includes all patients with positive blood cultures)[a]	Remove central venous lines. FLU 6 mg/kg (or 400 mg in usual adult) IV q day × 7 days, then 400 mg PO until 14 days after last positive culture. Caspofungin, AMB, and voriconazole are alternate treatments (see text).	Same as nonneutropenic. The evidence for removing central venous lines is less clear, because many cases are of bowel origin.	Rarely seen. Treat as non-neutropenic, except continue long-term suppression with FLU 100 to 200 mg PO q day.
Hepatosplenic candidiasis	Not seen	Frequent AMB failures. FLU 400 mg/day × 6 to 12 months may be effective.	Not seen
Chronic mucocutaneous candidiasis	FLU 200 to 400 mg/day PO	Not seen	Not seen
Urinary candidiasis (bladder, not renal)[b]	Remove urinary catheter if possible. No evidence of benefit from treatment of asymptomatic patients with low risk of invasive candidiasis. Do serial cultures after catheter removed or changed. For treatment of bladder colonization, FLU 200 mg/day × 7 to 14 days. If invasive candidiasis is highly suspected, treat for that indication.	Treat all cases of candiduria. FLU 200 mg PO for 7 to 14 days if isolated bladder colonization is suspected. However, there is more concern that the patient may have invasive candidiasis. Treat as invasive disease if suspicion is high.	Same as nonneutropenic, but may need long-term suppression with FLU 100 to 200 mg PO q day.
Peritoneal (on peritoneal dialysis)	AMB intraperitoneal or combination therapy with flucytosine and FLU intraperitoneal.	Not seen	Not seen

AMB, amphotericin B; *FLU*, fluconazole; *ITRA*, itraconazole, *VORI*, voriconazole.
[a]These recommendations are for sensitive strains of *C. albicans, C. tropicalis,* and *C. parapsilosis*. Infections with azole resistant strains including *C. glabrata* require different strategies and higher dosing. For *C. glabrata* caspofungin, FLU 12 mg/kg (or 800 mg/day), VORI, and AMB at least 0.7 mg/kg are possible choices. For *C. krusei*, Caspofungin and AMB at 1.0 mg/kg/day are preferred.
[b]Renal indicates invasive candidiasis.

Caspofungin 70 mg on day one and then 50 mg/day is another option (55). For the sickest patients, combination therapy with two or even all three of these agents is increasing in clinical practice, without as yet firm evidence from clinical trials. Itraconazole in doses of 200 to 400 mg twice daily has also been shown to be effective in many forms of aspergillosis (56).

Renal excretion of these agents is somewhat limited. There is poor urinary excretion of itraconazole, which is highly protein bound, drug levels in urine for caspofungin are low, and there is no excretion in the urine of the active form for voriconazole. In some cases, surgical removal of an aspergillus infected kidney may yield a higher cure rate than pharmacologic therapy alone and should be considered if appropriate in the context of the underlying illness and the extent of disease in other organs. Therapy of *Pseudallescheria boydii,* an aspergillus-like fungus, has to be mentioned separately. This organism is rare but important because it is resistant to AMB. Some isolates are susceptible to itraconazole and/or voriconazole. It can be distinguished from aspergillus only by culture.

Therapy of Zygomycosis is unsatisfactory. Most authorities recommend the use of AMB (57). Neither the exact dose nor the duration of therapy is known. Surgical resection of devitalized infected tissue is extremely important. Anecdotal reports suggest that hyperbaric oxygen may have a minor adjunctive role (57). Isolated renal mucormycosis in AIDS patients is best treated by antifungal therapy in combination with surgical resection if possible and appropriate in the overall clinical situation.

INFECTIONS CAUSED BY T-CELL OPPORTUNISTS: THE ENDEMIC MYCOSES

The endemic mycoses share many features, but there are also significant differences among them. One important difference among the endemic mycoses of North America is their geographic location. Although histoplasmosis (58) and blastomycosis are coendemic in the Midwest and south central United States, blastomycosis extends much further north than histoplasmosis and extends into adjacent areas of central Canada (59). For unexplained reasons, New England seems to be free of both fungi. *Coccidioides immitis* is endemic in the southwest United States, especially in the more arid parts of this area as well as the contiguous areas of northwest Mexico (60). *Cryptococcus neoformans* is cosmopolitan, and cases have been reported worldwide.

These organisms all enter the body by way of the lung, but the histopathologic appearance of the initial infection is quite different. Histoplasmosis is predominantly a granulomatous infection with very few neutrophils present among well-developed granulomas (58). The tissue response to blastomycosis is predominantly neutrophilic and scattered granulomas appear only with the development of cell-mediated immunity (61). Neutrophils do not disappear in virtually all instances of blastomycotic infection; thus, the characteristic tissue response is the presence of a mixed pyogenic and granulomatous exudate (59,61). The tissue response in *Paracoccidioides immitis* is similar to blastomycosis (62). *Coccidioides immitis* is unique—its form of reproduction (endospores evolving into giant spherules) is different from the other endemic mycoses. In this infection, the initial tissue response is rich in neutrophils, and granulomas make their appearance later. Although occasionally the final tissue response is predominantly granulomatous, usually the tissue response retains a heavy component of neutrophils, and the pathology remains both pyogenic and granulomatous (60).

The tissue response to *C. neoformans* is different than that of the endemic mycoses. Sometimes inflammatory cells are virtually absent. The thick polysaccharide capsule of the yeast is antiphagocytic (63), and it is not uncommon to see cryptococcal infection without any inflammatory tissue response, especially in the lung, where huge masses of rapidly dividing cryptococci can form a gelatinous mass.

Histoplasmosis

Epidemiology

The thermal dimorphic fungus *Histoplasma capsulatum* is endemic throughout the world, with its highest concentration along river valleys. By far the most endemic area in the entire world, however, is in the Midwest and south central United States around the Ohio and Mississippi River valleys (58). Although the exact growth requirements for the fungus are not known, soil enriched with organic nitrogen, commonly in the form of bird or bat droppings, is most suitable for fungal propagation. Infection in humans follows inhalation of wind-borne particles (69).

Pathogenesis

The lung is the portal of entry. Following inhalation, a few spores reach the alveolar spaces, where multiplication by binary fission begins. There may be a brief initial influx of neutrophils, but macrophages actively phagocytose the organisms and quickly become the predominant inflammatory cell. These macrophages are unable to kill the fungus during the preimmune phase of the illness (65). The organism grows unchecked within the macrophages and disseminates throughout the body through the bloodstream and lymphatic system. With the development of specific T-cell mediated immunity, the now "armed" macrophages can either kill the fungus or wall it off within granulomas. There is often central necrosis in the granulomas. The clinical illness ends as the infection is controlled. The granuloma is surrounded by peripheral fibrosis and may eventually calcify. It is important to realize, however, that prior to the development of specific T-cell–mediated immunity, the organism has disseminated widely throughout the body, and multiple organs (especially organs rich in reticuloendothelial [RE] cells) may be heavily seeded, including the liver, spleen, adrenal glands, and lungs. Calcified granulomas often are found in these organs in residents of endemic areas (66) and do not imply progressive dissemination but only uneventful healing of the fungemia and benign dissemination that accompany many primary infections.

Clinical Manifestations

The Primary Infection. The vast majority of human infections caused by *H. capsulatum* are asymptomatic and discovered only during the course of an epidemic investigation by identifying individuals with a positive histoplasmin skin test. Usually there is no history of any significant prior respiratory illness (67,68). When the primary infection is symptomatic, it produces a lower respiratory syndrome indistinguishable from other influenza-like illnesses with nonproductive cough, fever, headache, and myalgias (69). Recovery from the clinical illness takes anywhere from several days to several weeks, but virtually all primary infections resolve spontaneously. In the rare individual with abnormal T-cells, the initial, usually self-limited dissemination progresses, and a rapidly fatal, progressive disseminated illness ensues (70) called progressive disseminated histoplasmosis (PDH), which distinguishes it from the benign early dissemination of primary histoplasmosis.

Radiographically, the primary infection appears as single or multiple areas of nodular infiltrate, frequently with involvement of ipsilateral hilar lymph nodes. A diffuse micronodular infiltrate may be seen when the initial inoculum is extremely heavy (69). In rare patients with heavy exposure, rapidly progressive gas exchange problems can develop, leading to the manifestations of the adult respiratory distress syndrome (ARDS).

In patients with preexisting pulmonary abnormalities, especially smokers with centrilobular emphysema, the primary infection often involves the upper zones where the lung is most abnormal (71,72). Most of these infections resolve spontaneously, although slowly. As many as 25% of these patients develop a progressive, chronically destructive infection. The chest radiograph mimics reinfection tuberculosis, and the fungus grows in abnormal air spaces and advances to adjacent areas of the lung, producing a fibrocavitary infiltrate (73).

Progressive Disseminated Histoplasmosis. Histoplasma organisms disseminate widely throughout the body, involving cells of the RE system, during the preimmune phase of the primary infection. With the development of T-cell–mediated immunity, these foci of infectious particles are either killed or walled off, and the infection is localized (70). Patients without an effective T-cell–mediated immune system, however, often develop a rapidly progressive infection. The tissue response in T-cell–deficient individuals is poor, without any attempt at granuloma formation. Although macrophages are seen throughout the infiltrate, organisms multiply freely within these macrophages as well as in the necrotic material within the infected tissue. High fever, hepatosplenomegaly, and evidence of suppressed bone marrow function are seen. The pace of the illness is rapid and may lead to death within days or weeks.

Progressive disseminated histoplasmosis follows a more chronic course in patients with at least partially preserved T-cell function—attempt at granuloma formation is seen, and the number of organisms is considerably smaller (70). Ulcerations of the buccal and pharyngeal mucosa and the rectum as well as the glans penis may be seen, and occasionally cutaneous ulcers are present. Fever is usually low grade, and the time course of the illness is months to years (70).

The chest radiograph in PDH is variable. In the more rapidly progressive form of PDH, the chest radiograph frequently shows a diffuse micronodular infiltrate (70). Small nodules may coalesce as the infection progresses. In patients with partially intact T-cell mediated immunity, chest radiograph may mimic reinfection tuberculosis with fibrocavitary infiltrates in the upper zones.

The vast majority of patients with the rapidly progressive form of PDH are immunocompromised, and it is especially common in patients with AIDS who have a CD4 count under 100/dL (74). In this clinical setting, the chest radiograph may appear normal initially and then rapidly progress to a diffuse reticulonodular infiltrate, mimicking *Pneumocystis carinii* pneumonia (74).

The mechanism of infection in HIV-infected patients (75) (as well as in other immunocompromised patients) (76) may be endogenous reactivation or may result from rapid progression of the primary infection in a patient with deficient T-cell–mediated immunity who inhales the organisms while immunosuppressed (77). In rare instances, PDH has been acquired by renal transplantation of an infected allograft (78).

Diagnosis

The diagnostic gold standard is recovery of the fungus in culture. Although it is usually not difficult, it is time consuming because the laboratory has to grow the organism in its mycelial phase at 23°C, convert it to the yeast phase at 37°C, and finally convert it back to the mycelial phase at 23°C. This entire process may take up to 30 days. It is important to notify the laboratory when histoplasmosis is suspected clinically. Exoantigen testing on early mycelial isolates can prove that the isolate is *H. capsulatum* and shorten the diagnostic sequence.

Primary pulmonary histoplasmosis is seldom diagnosed by culture. Most cases are either never diagnosed or are diagnosed retrospectively by serologic tests because sputum production is minimal and the illness is usually self-limited. With rapidly progressive pulmonary disease leading to gas exchange problems, lung biopsy is usually required, and the organism often can be seen directly in histopathologic sections and cultured from the tissue.

The most frequently utilized diagnostic tests for histoplasmosis are serologic. The immunodiffusion test has fair specificity but is not sensitive, and it cannot be highly recommended. The complement fixation (CF) serologic test is more sensitive, with more than three-quarters of the patients with primary pulmonary histoplasmosis showing a diagnostic titer rise in 6 weeks. Unfortunately, both acute and convalescent sera are required to show this titer rise. It often takes 4 to 6 weeks to collect both sera and get the results back from a reference laboratory (79). A single titer of 1:32 or higher to the yeast-phase antigen is highly suggestive of acute histoplasma infection in the appropriate clinical setting. Another immunodiagnostic test is the measurement of the histoplasma polysaccharide antigen (HPA) (80), which is now available in a kit form. The test measures fungal antigen in urine and serum and has high specificity. It is also very sensitive in patients with a heavy burden of organisms, mainly AIDS patients with PDH (81). Sensitivity of the test is low in more benign, self-limited forms of the disease, including primary pulmonary histoplasmosis in normal hosts.

Except in AIDS serodiagnostic tests cannot be relied on for diagnosis in patients with ARDS or rapidly progressive PDH because they are too slow and insensitive. In AIDS patients, the urine can be checked for HPA; a positive test is diagnostic. For non-AIDS patients the organism must be documented histopathologically in tissue or cultured from the blood or other specimens. Cultural recovery from blood using the lysis–centrifugation system is faster than cultural methods, especially if there is a large burden of organisms, as occurs in AIDS patients (82). Within 3 to 5 days, adequate fungal growth can be visualized. When the pace of the illness is even more rapid, attempts at direct histopathologic diagnosis should be made. The most simple and direct way to establish a direct diagnosis is the examination of bone marrow using an aspirate and bilateral trephine biopsies (83). Special stains, either methenamine silver or the periodic acid-Schiff (PAS), are extremely helpful. The diagnosis can be established within hours if the characteristic yeast is seen within bone marrow macrophages. In occasional patients (again usually AIDS patients with large burden of organisms), the characteristic yeast can be identified directly on peripheral blood smears, where they are seen within circulating phagocytes (84).

Involvement of the Genitourinary Tract. Although the renal parenchyma may be involved at autopsy examination in more than 40% of patients who die of PDH (70,85,86), clinically apparent renal disease during life is uncommon. In occasional patients, renal papillary necrosis has been reported (87). In one reported instance, a calcified histoplasma granuloma eroded from the kidney to the skin producing a pyelocutaneous fistula (88). The organism may be recovered in culture from urine in patients with PDH. The incidence of cultural recovery depends on the severity of infection and intensity of effort made to culture the urine. Some authors have reported positive urine cultures in up to 40% of patients in selected series of PDH (86,89). AIDS patients with PDH have a very high

density of organisms and are most likely to have positive urine cultures.

Prostatitis and cystitis have been reported at autopsy, but clinically these are exceedingly rare (70,85,90). The glans penis may be involved by an ulcer in PDH, especially in uncircumcised males.

Histoplasma polysaccharide antigen can be detected in the urine of patients with PDH with a heavy fungal burden. This test is essential in AIDS patients with PDH, both for initial diagnosis (81) and for monitoring relapse (91). Histoplasma polysaccharide antigen has a significant but lesser role in diagnosis and management of PDH in non-AIDS patients where the burden of fungal organisms is much smaller.

Treatment

Treatment is seldom indicated for primary infections because the vast majority of them resolve spontaneously. For patients with severe acute pulmonary histoplasmosis with ARDS, the treatment is amphotericin-B (AMB) to a dose of 500 mg or until improvement has occurred (92). Patients with PDH usually require AMB to clinical stabilization (up to 500 to 1,000 mg) followed by itraconazole 200 mg twice daily (93). Itraconazole should be continued for life in patients whose immunosuppressed state cannot be reversed, including all patients with AIDS. Patients with chronic pulmonary histoplasmosis and milder forms of PDH can be treated with itraconazole (same dose) for 6 months.

Blastomycosis

Epidemiology

The thermal dimorphic fungus *Blastomyces dermatitides* is endemic primarily in North America. The disease is common only in the United States and Canada. The endemic area largely overlaps the endemic area of histoplasmosis except that it extends further north across northern Wisconsin and Minnesota and into the central provinces of Canada. The organism resides in soil, and it appears to require high humidity and organic nitrogen for optimum growth (59). Infection in humans and susceptible mammals, such as the dog (94), follows inhalation of aerosolized particles.

Pathogenesis

The lung is the portal of entry for most infections. Following inhalation of the infecting particles, an area of pneumonitis occurs in the alveoli. The large yeasts multiply once the organism converts to its yeast phase. There is single budding with a characteristic broad neck of attachment between the bud and parent cell. The initial tissue response is predominantly pyogenic (61). Only later do macrophages participate in large number. After establishment of cell-mediated immunity, the macrophages ingesting the organisms are capable of killing them, and the infection is usually controlled. Usually the infection is confined to the lung and hilar nodes. In some patients, the organism disseminates by way of the bloodstream throughout the body. Spontaneous resolution is unlikely once extrapulmonary dissemination occurs. Unlike in histoplasmosis where the final histopathology is primarily granulomatous, in blastomycosis the histopathology usually retains a heavy PMN infiltrate, producing characteristic microabscess formation and pyogenic-granulomatous tissue response. Extrapulmonary dissemination usually involves the skin and bones, and less commonly the prostate and meninges (59,61). Other involved organs have been reported on occasion.

Clinical Manifestations

Some primary infections are asymptomatic. When symptomatic, the disease may manifest itself as an acute lower respiratory tract infection with fever, myalgia, and arthralgia, and a cough productive of mucopurulent sputum (95). Many individuals slowly recover following the clinically symptomatic illness, but a sizable number do not. They develop a subacute to chronic, slowly progressive pulmonary infection (59).

Chest radiographic findings are highly variable. The infiltrates are frequently nodular, but may involve entire lobes as well (96). Hilar lymph nodes may be involved, and pleural disease is not uncommon.

Once extrapulmonary dissemination occurs, the resultant cutaneous ulcers and bony lesions become symptomatic. Prostatic involvement may be silent or symptomatic (59,61), whereas meningeal disease usually leads to the development of subacute or chronic meningitis. Patients may have headache and altered mental status, but frank meningeal signs including stiff neck are usually absent (97).

Involvement of the Genitourinary Tract

Renal involvement, although reported, is extremely rare (98). When the kidney is involved, there are usually no clinical findings. Autopsy series show a higher incidence of renal involvement, but these include mainly patients with well-advanced cases of blastomycosis who died of widely disseminated disease. Even in these patients, the renal involvement was clinically silent during life (61).

The prostate as well as the testes and epididymis may be involved with extrapulmonary dissemination (59,61,99). Findings frequently are that of a nonspecific prostatitis with signs and symptoms of a urinary tract infection. Occasional patients present with lower urinary tract obstruction, suggesting benign prostatic hypertrophy. The combination of prostatic disease in combination with a chronic lung infection, with or without skin and bony lesions, should raise suspicion of blastomycosis (59).

Diagnosis

Diagnosis usually requires either isolation in culture or direct visualization of the organism from biologic material. Cultural recovery is not difficult, but may take as long as 30 days to complete. When the laboratory is properly alerted, earlier, tentative identification can be made as soon as 3 to 5 days after plating the material by using exoantigen testing to positively identify early mycelial isolates.

The simplest and most direct diagnostic test is direct examination of sputum and other biological material after digestion with 10% KOH. The potassium hydroxide dissolves extraneous material, leaving the infecting organisms readily visible. When large, single budding yeast with a wide neck of attachment between parent and daughter cell is visualized, diagnosis is certain. KOH preparations may be performed on respiratory secretions, skin scrapings, aspirates from cutaneous microabscesses, and prostatic fluid obtained after transrectal prostatic massage (59).

Histopathology of biopsied material frequently shows the organism. In addition to the more frequently performed silver stain, the PAS stain is highly effective for identifying the organism in tissue sections (61).

Although serologic tests exist, they are neither as specific nor as sensitive as for histoplasmosis (100). A positive serologic test should raise suspicion and prompt more intensive diagnostic investigation, including direct smears, cultures, and tissue biopsies as appropriate, although one should not make a definite diagnosis of blastomycosis on the basis of serologic tests. The immunodiffusion test has fairly good specificity

(it should never be ignored when positive) but has poor sensitivity so that a negative result can never exclude the diagnosis.

Treatment

Itraconazole is highly effective in nonmeningeal blastomycosis. Over 90% of patients with mild to moderate pulmonary and nonmeningeal extrapulmonary blastomycosis can be successfully treated with itraconazole at a dose of 200 mg twice daily for 6 months (101).

Amphotericin-B should be used when the disease is rapidly progressive or involves the meninges. In the majority of instances, 1.5 to 2.0 g of AMB is curative (102). Amphotericin-B is more effective and results in more rapid improvement than oral agents. Amphotericin-B should be used for all life-threatening disease. Once such patients stabilize (usually after 0.5 to 1.0 g of AMB) treatment can be switched to itraconazole, 200 mg twice daily, which is continued for 6 to 12 months.

Coccidioidomycosis

Epidemiology

The tissue dimorphic fungus *Coccidioides immitis* is endemic only in North and South America. The most heavily endemic area exists in the desert southwest United States and contiguous areas of Mexico. The organism survives the desert heat in rodent burrows. Mycelial growth occurs following the rainy season, when rapidly rising temperatures are highly favorable for growth (60). The barrel-shaped Arthroconidia become airborne following any disturbance of the soil and may travel considerable distances to cause human illness (103).

Pathogenesis

The lung is the portal of entry. Upon arrival at the alveoli, the arthrospores convert to the tissue-invasive form, leading to the formation of giant spherules. Septation occurs within the giant spherules, leading to the formation of a large number of endospores. Following maturation of the giant spherule, the cell wall ruptures, releasing the endospores. Each endospore can mature to another giant spherule. Following initial PMN influx, macrophages increase in numbers and ingest the fungus. Following initial PMN exudate, macrophages move in to ingest the fungus. Following establishment of effective cell-mediated immunity, infected areas are walled off, creating focal nodules that have granulomatous and pyogenic elements. These nodules are essentially microabscesses. In patients at risk for the development of disseminated disease, the organism can spread beyond the lung. Involvement of the skin and bones is common. In approximately one-third of the patients extrapulmonary dissemination involves the meninges, either as one of many organs or as the sole extrapulmonary site of infection (60).

Clinical Manifestations

The Primary Infection. Most primary infections are asymptomatic and heal spontaneously. Symptomatic patients have fever, arthralgias, and myalgias, frequently followed by the development of severe pleuritic chest pain and cough productive of mucopurulent sputum. Recovery may take anywhere from a few days to 6 or more weeks. One to 3 weeks are typical. In some patients, the disease slowly progresses causing substantial morbidity (57).

Extrapulmonary dissemination, when present, usually follows in the wake of the acute illness. With meningeal disease, overt meningeal signs are usually absent, and the patient presents primarily with either headache or confusion. Bone and joint infection is usually symptomatic. If the patient lives long with disseminated disease, involvement of the skin, which is usually quite obvious to the patient and the examiner, is nearly universal (57,60).

Chest radiographic findings during the acute infection range from small areas of pneumonitis to nodular infiltrates. These nodules frequently shell out, leading initially to a thick-walled cavity. The development of a thin-walled cavity is considered a hallmark of the disease. Radiographic manifestations of bone disease often include destruction of bone, but are not specific (104).

Genitourinary Tract Involvement

Symptomatic involvement of the kidneys is extremely rare, although renal involvement in an autopsy series is considerably more common, reaching 60% in an older series that included many patients with widely disseminated disease (105,106). The organism often can be cultured from the urine in cases of disseminated coccidioidomycosis. However, the presence of funguria during a primary infection does not always indicate progressive extrapulmonary dissemination. Many patients with funguria recover spontaneously (107). The prostate and epididymis may also be involved. Symptomatic prostatitis is quite rare (104,106,108).

Diagnosis

Although the organism is easy to culture, it is extremely hazardous to laboratory personnel. Most laboratories that attempt to culture it use a closed culture system with exoantigen testing as the identification procedure, to reduce risk to laboratory personnel (109). Papanicolaou stain of biologic material (including sputum and pus from sinus tracts or aspirate from microabscesses) frequently shows the fungus (110). Although 10% KOH may be used, the Papanicolaou smear is more sensitive.

Serodiagnosis in coccidioidomycosis is highly developed. The complement fixation (CF) test in the right laboratory is both diagnostic and provides useful prognostic information. A high or rising titer (to 1:32 or higher) should increase suspicion of extrapulmonary dissemination (111). Many laboratories now use the much simpler immunodiffusion test for both IgM and IgG antibodies. Once the diagnosis is established by immunodiffusion testing, a CF test also should be done because it has greater prognostic value. Complement fixation test is not necessary if the immunodiffusion tests are negative.

The organism is readily seen in histopathologic sections even without special stains. Giant spherules are large (30 to 100 μm) and are so characteristic in appearance that they are seldom misinterpreted. Special stains also are helpful, and both the silver and PAS stains may be used. Individual endospores are best seen with these special stains and can resemble small yeast; however, if spherules are also present, there is no confusion.

Treatment

Although AMB has been used for almost 40 years in coccidioidomycosis, it is not as effective as it is for histoplasmosis and blastomycosis and its exact role is still poorly defined. Most authorities believe that severely ill patients should receive AMB initially, followed by fluconazole after clinical stabilization.

Since fluconazole became available, the majority of opinion has swung toward the use of fluconazole early in daily doses of 400 to 800 mg (112). Even meningitis seems to respond well (113). Although it is perhaps too early to speak of cures of coccidioidal meningitis, long-term functional improvement may be obtained.

INFECTIONS CAUSED BY T-CELL OPPORTUNISTS: CRYPTOCOCCOSIS

Epidemiology

Cryptococcosis is different from the endemic mycoses because it is neither geographically restricted nor dimorphic. The organism exists in a yeast form in nature and infected tissue. The organism is found in soil, usually in association with pigeon and other bird excreta (114). It occurs in both urban and rural areas. Although the exact nature of the infecting particle is unclear, it is widely accepted that desiccated small yeast is the likely infectious particle.

Pathogenesis

The lungs are the portals of entry. Following inhalation of the infecting particle (the small, nonencapsulated yeast), the yeast forms reach the alveoli, and begin multiplication and capsule formation. Because the large carbohydrate capsule is antiphagocytic, the recruitment of PMN and other inflammatory cells is markedly decreased (63). It is not uncommon to see large masses of cryptococci growing in tissue without any visible inflammatory response. Elimination of the organism usually proceeds only after development of adequate cell-mediated immunity (115). Presumably during the preimmune phase of the infection, the organism gains access to the circulation and disseminates throughout the body. The yeast is neurotropic, and the most frequent extrapulmonary site of lodgment is the meninges and brain tissue itself.

Clinical Manifestations

Primary Infection. The vast majority of patients have no symptoms during the initial pulmonary infection. Most patients come to medical attention only if they later develop meningeal disease. At the time cryptococcal meningitis is recognized, occasional patients still have evidence of healing pulmonary cryptococcosis, usually manifest as a small infiltrate or round nodule on the chest radiograph. Occasional patients develop symptoms during the primary pulmonary infection, which are usually mild. Mild fever and cough may be present, but the temperature elevation is usually short-lived, and the cough is seldom severe. The chest radiograph in symptomatic patients with pulmonary cryptococcosis is variable. The majority of individuals show single, or occasionally multiple, nodules that attain surprisingly large size, even up to 10 cm in diameter. Healing usually takes place without scar formation and complete disappearance of the lesions from the pulmonary parenchyma is the rule.

Cryptococcal meningitis is the most common clinical syndrome recognized. The onset of meningitis is usually subacute, and seldom there are florid signs of meningitis. Most frequent complaints include headache and occasionally confusion. Focal neurologic findings are uncommon. When present, they usually result from entrapment of various cranial nerves. The sixth cranial nerve is most frequently involved, causing lateral rectus palsy. The tempo of disease is highly variable. Although in many patients the disease follows a subacute or even chronic course, the organism is capable of producing an explosive and rapidly progressive infection leading to death in days to weeks. Extrapulmonary cryptococcosis, including meningitis, is a disease seen almost exclusively in patients whose T-cell function is impaired. Prior to the onset of the HIV epidemic, many patients with lymphoreticular malignancies (most commonly Hodgkin's disease) developed the illness (116). Because of the advent of the HIV epidemic, the vast majority of cryptococcal infections are seen in patients with AIDS (117). Development of cryptococcal disease is usually a late finding during the course of HIV infection. Early in the AIDS epidemic in North America, approximately 7% to 10% of all HIV-infected patients developed cryptococcal disease. This incidence has

TABLE 37-2

CLINICAL MANIFESTATIONS OF T-CELL OPPORTUNISTS

	Histoplasmosis	Blastomycosis	Coccidioidomycosis	Cryptococcosis
Habitat	Soil enriched by organic nitrogen in the form of bird or animal feces.	Soil enriched by organic nitrogen in the form of bird or animal feces.	Soil enriched by organic nitrogen in the form of bird or animal feces.	Soil enriched by organic nitrogen in the form of bird or animal feces.
Portal of entry of primary infections	Lung Mild Self-limited. Occasional adult respiratory distress syndrome.	Lung Moderately severe. May be self-limited, usually subacute and progressive. Can mimic bacterial pneumonia occasional ARDS.	Lung Mild to severe pleuric pain common, usually self-limited. Progressive in significant minority of patients.	Lung Symptomatic to mild.
Tissue response	Granulomatous	Pyogranulomatous	Pyogranulomatous	Usually no response to occasional granuloma
Extrapulmonary spread	Always (benign Fungemia) Progressive in T-cell immunocompromised host.	Frequent Frequently no evidence of T-cell abnormality.	Occasional Males, blacks, T-cell immunocompromised hosts at risk.	Rare in normals Extemely common in T-cell immuno-compromised host.
	Regiculoendothelial organs	Skin, bone, prostate, meninges	Skin, bones, meninges	Meninges, bones, prostate, kidney, skin
Complicating human immuno-deficiency virus infection	Common	Rare	Common	Very common

TABLE 37-3

DIAGNOSIS OF INFECTIONS WITH T-CELL OPPORTUNISTS

	Histoplasmosis	Blastomycosis	Coccidioidomycosis	Cryptococcosis
Histopathology	2 to 5-μ single budding yeast; silver or PAS stain.	−20-μ single budding yeast, with broad neck of attachment; multiple nuclei; thick, doubly refractive wall; silver or PAS stain.	Huge, up to 100-μ giant spherule, with multiple endospores H&E; silver stain.	Encapsulated single budding yeast; capsule size variable; silver or mucicarmine stain. India ink preparation of CSF and other fluids.
Culture	Time consuming, up to 30 days. Tuberculate macroconidia may be seen by 5 to 7 days. Exoantigen testing quicker.	Time consuming, up to 30 days. Lollipop-shared conidia are seen by 5 to 7 days.	Extreme biohazard Barrel-shaped arthroconidia are seen by 7 days. Closed-system exoantigen testing avoids aerosol formation.	Rapid, 3 to 5 days. Capsule formation is variable.
Rapid diagnosis by examination of fresh specimen	Silver stain of sputum may show small yeasts, but is unreliable and has low sensitivity. Intracellular organisms may be seen on peripheral blood smear, especially in AIDS patients.	KOH digest is less highly reliable. Papanicolaou stain frequently is positive.	Papanicolaou stain is positive in 50%. KOH digest is less reliable.	India ink preparation of CSF or prostatic secretions is highly specific and 70% sensitive.
Serology	Immunodiffusion is not sensitive CF test is 75% sensitive; fourfold rise usually takes 3 to 6 weeks. HPA is sensitive in disseminated disease, especially in AIDS patients. HPA has high specificity.	Tests are not highly reliable; however, positive results should lead to renewed efforts to investigate further with cultures and tissue biopsies for histopathology.	Immunodiffusion test is useful as initial screening test. CF test is sensitive and provides prognostic information.	Antigen determination is highly sensitive in CSF; serum antigen titer usually positive in AIDS patients, but usually negative in others.

HPA, Histoplasma polysaccharide antigen; *PAS*, periodic acid-Schiff; *H&E*, hematoxylin and eosin; *AIDS*, acquired immune deficiency syndrome; *KOH*, potassium hydroxide; *CSF*, cerebrospinal fluid; *CF*, complement fixation.

decreased because of the widespread use of fluconazole (for oral and esophageal candidiasis and primary prevention of cryptococcus) and because of the advent of effective antiretroviral therapy that preserves and even restores immune function. It is estimated that up to one-third of AIDS patients in Africa develop cryptococcal infection during the course of their HIV infection.

Diagnosis

Recovery of the organism in culture is usually rapid and easy. The organism grows in unenriched media and is frequently cultured in 3 to 4 days.

India ink preparations of spinal fluid (and prostatic fluid) are extremely helpful in experienced hands. Fine particles of carbon are held away from the yeast cell wall by the capsule, leaving an obvious halo around the organism. Although the India ink test is highly specific, sensitivity, even in the best hands, is only 50% to 70% (118).

Diagnosis has been simplified since cryptococcal antigen determination became available. Cryptococcal antigen may be measured in serum or in cerebrospinal fluid (CSF). In HIV-infected patients, serum cryptococcal antigen titers are almost always as high if not higher than concurrent cerebrospinal fluid levels (117). In non-AIDS immunocompromised patients, serum cryptococcal titers are usually negative, and the CSF must always be tested for the antigen.

Examination of CSF usually reveals a lymphocytic pleocytosis of varying degree. The protein is usually elevated, and the glucose is depressed. In non-AIDS patients, a white count below 20/mm^2 implies poor prognosis (119). It is important to recognize that, especially in AIDS patients, the cerebrospinal fluid frequently is entirely normal without any lymphocytic pleocytosis and normal protein and glucose. In these instances, the diagnosis depends on a positive cryptococcal antigen test on CSF or a positive culture of the CSF (120).

Histopathologic examination of biopsy material shows large masses of budding yeasts with a large carbohydrate

capsule. Although the organism is readily visible on H&E stained sections, the mucicarmine stain stains the capsule a brilliant red, aiding diagnosis.

Involvement of the Genitourinary Tract

During the preimmune phase of dissemination, the organisms frequently spread to the kidney and prostate. Renal involvement is usually asymptomatic, and only occasionally will patients develop any urinary tract findings such as dysuria and urinary frequency (121). Progressive involvement of the kidneys has been reported but is rare. Pyelonephritis and renal papillary necrosis are also quite uncommon (121,122). In contrast to the infrequent clinical findings of renal involvement, autopsy studies of AIDS patients with cryptococcal infection show a surprisingly high frequency of renal involvement with up to one-half of patients showing fungi within renal parenchyma (123,124).

Symptomatic prostatitis is uncommon, but does occur (123,125,126). The prostate appears to be a "sanctuary" site of involvement (127). Following apparently successful treatment of cryptococcal meningitis, recurrence of the cryptococcal infection is sometimes recognized first in the prostate, especially in AIDS patients (128).

Treatment

Pulmonary cryptococcal disease in immunologically intact patients is usually a self-limited illness. Prior to the availability of fluconazole, most experts suggested a period of careful observation prior to starting AMB treatment, after CSF sampling to exclude meningitis (129). Since fluconazole became available, it is probably reasonable to initiate treatment with this agent at 400 mg/day to hasten resolution and perhaps lessen the chance of meningeal seeding. Whether one elects to observe or treat such a patient, it is mandatory to perform a lumbar puncture to rule out the presence of meningeal involvement, which may be totally asymptomatic.

Pulmonary cryptococcal disease in T-cell immunocompromised patients usually progresses to extrapulmonary dissemination. After a lumbar puncture is performed, all such patients should be treated. In stable patients, fluconazole 400 mg daily usually is adequate. When the pace of the infection is rapid, AMB 0.7 mg/kg daily with 5-fluorocytosine (5FC) 100 mg/kg daily in four divided doses (with monitoring of serum levels and dosage adjustments as needed to maintain serum levels of 100 to 150 μg/dL) is recommended (93).

Amphotericin-B 0.7 mg/kg daily plus 5-FC 100 mg/kg in four divided doses also is the recommended initial treatment for patients with disseminated cryptococcal disease (130). 5-FC is bone marrow toxic and is particularly difficult to use in AIDS patients, whose bone marrow is already suppressed from HIV infection, other opportunistic infections and from retroviral treatment. Drug levels must be monitored and 5-FC dosing adjusted downward as needed. The goal in AIDS patients is to keep the serum level less than 100 μg per dL. Even with reduced dosing, many AIDS patients simply will not tolerate 5-FC without major bone marrow suppression. In fact in some centers 5-FC is rarely used. AMB alone is an alternative for initial therapy of cryptococcal meningitis (doses at least 0.7 mg/kg/day) for AIDS patients unlikely to tolerate 5-FC. Fluconazole (400 to 800 mg/day) is also effective and may be safely tried as initial therapy in patients who are stable and do not have focal neurologic findings or altered mental state (131). Care must be taken to watch for the development of increased intracranial pressure, which usually becomes manifest by deterioration of cognitive function as well as the development of papilledema. When increased intracranial pressure becomes clinically evident, then removal of spinal fluid by repeated lumbar puncture (done under pressure monitoring with removal of sufficient volume to reduce the opening pressure by half) or decompression of the lateral ventricles by use of an intraventricular shunt should be performed.

Following successful treatment of the meningeal disease, fluconazole 200 mg daily should be given to all AIDS patients and other selected severely immunocompromised patients for the remainder of their lives. This form of treatment is more effective than continuous suppressive therapy with weekly doses of AMB (132). It is thought that persistence of the organisms in the prostate may give rise to many clinically significant recurrences. The continuous use of fluconazole appears to successfully suppress the organism in extrapulmonary sites and prevent relapses (118). In Table 37-2 are highlighted the clinical manifestations of the important T-cell opportunists and in Table 37-3 are highlighted the diagnostic tests that are useful in these infections.

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CHAPTER 38 ■ URINARY TRACT TUBERCULOSIS

MARK S. PASTERNACK AND ROBERT H. RUBIN

Nearly a century ago, Osler pointed out that the clinical entity tuberculosis is the net result of two pathologic processes: "In all tubercles two processes go on: the one—caseation—destructive and dangerous; and the other—sclerosis—conservative and healing. The ultimate result in a given case depends upon the capabilities of the body to restrict and limit the growth of the bacilli" (1).

Perhaps in no other form of tuberculosis are both these processes so important in determining the clinical impact of the tubercle bacillus on an organ system as in genitourinary tuberculosis. On the one hand, particularly in the preantimicrobial era, progressive destruction and caseating necrosis of the kidney leading to "autonephrectomy" have long been recognized as possible results of tuberculous seeding of the kidney. On the other hand, ureteral and calyceal scarring as part of the healing process can result in obstructive uropathy and comparable loss of renal function. Therefore, medical and surgical management of genitourinary tuberculosis must focus on both aspects of the tuberculous process, with emphasis on the early diagnosis and prevention of both tissue damage and excessive scarring. Because genitourinary tract disease can be a particularly occult form of tuberculosis, clinicians are challenged greatly in their efforts to achieve these objectives. The purpose of this chapter is to outline a practical approach to this problem, based on established epidemiologic, pathogenetic, and clinical principles.

ETIOLOGY

Robert Koch first identified the tubercle bacillus in 1882 over 100 years ago. In his classic report, he defined staining procedures for the direct observation of bacilli in clinical specimens (including the use of aniline dyes, which are now used to define "acid-fastness"), culture techniques on solid medium for the *in vitro* passage of bacilli isolated from clinical or experimental lesions, and subsequent inoculation of guinea pigs with cultured material to confirm its etiologic role in tuberculosis (2). This demonstration of an etiologic role for the tubercle bacillus in tuberculosis became the basis of "Koch's postulates," the standard criteria for etiologic research in infectious disease.

Mycobacterium tuberculosis, the human tubercle bacillus, is now classified as one of approximately 90 species of higher bacteria with unusual shared structural and tinctorial properties (3,4). All mycobacteria, members of the genus *Mycobacterium,* have the ability to take up aniline dyes, such as those contained in carbolfuchsin, and to resist decolorization by washing in alcohol acidified with inorganic acid (e.g., 95% ethanol, 3% HCl). This unique property correlates with the extremely high lipid content of mycobacterial cell walls. Although all mycobacteria are obligate aerobes, they are found in nature in disparate settings: some species exist primarily as soil and water saprophytes, while others are true pathogens of amphibians, reptiles, birds, and various mammals. *M. bovis,* the bovine tubercle bacillus, has virtually disappeared as a human pathogen in modern societies through tuberculin testing of cattle and pasteurization of dairy products. A variety of mycobacterial species can be pathogenic in humans (e.g., *M. avium-intracellulare*), whereas still others have been identified as human saprophytes (*M. gastri, M. smegmatis*). *Mycobacterium tuberculosis* is distinguished from the many other "atypical" mycobacteria by its metabolic properties, rate of growth, pigment production, and virulence in experimental infection in guinea pigs. *Mycobacterium tuberculosis* characteristically appears as a small, slender, slightly curved rod 2 to 4 μm in length with a diameter of 0.3 to 0.6 μm. Bacilli can appear singly or in small clusters on clinical specimens. Unlike infected pulmonary secretions where the density of organisms commonly is high, the low density of bacilli in urine samples, as well as their possible confusion with saprophytic mycobacteria, makes urine acid-fast stains impractical for rapid diagnosis. Although *M. tuberculosis* can grow on simple synthetic media, typically in intertwining aggregates known as *serpentine cords,* its slow growth rate (15 to 20 hours doubling time) necessitates culture periods of up to 6 to 8 weeks for the appearance of visible colonies. Optimal growth requires high partial pressures of oxygen, as in air, although bacilli can remain viable but metabolically dormant under greatly reduced PO_2. This is particularly relevant for the progression of renal tuberculosis (see the following discussion).

The unique features of the mycobacterial cell wall are responsible not only for the characteristic acid-fast staining properties but for some of the important host–parasite interactions as well. In addition to a peptidoglycan cell wall layer common to conventional bacteria, a second glycan layer encases the organism (5,6). This arabino-galactan layer is covalently linked to the peptidoglycan layer and also contains esters of mycolic acids, which are very large fatty acids that are unique to mycobacteria. A number of complex glycolipids reside in the outermost layer—"cord factor," phosphatides, and sulfatides. These glycolipids can be extracted from viable mycobacteria by organic solvents and thus are not covalently linked to the glycan layers. Cord factor, trehalose dimycolate, is responsible for growth in serpentine cords *in vitro* and is a virulence factor *in vivo* (7). The mycobacterial sulfatides are lysosomotropic within macrophages and inhibit the fusion of sulfatide-containing lysosomes with phagosomes containing engulfed viable bacilli (8). These agents may thus be protective against the intracellular killing mechanism of macrophages. In contrast, the most striking immunologic feature of mycobacteria, their immunoadjuvant capacity, appears to be due to muramyl dipeptide, which is an essentially ubiquitous component of bacterial peptidoglycan cell walls (9,10). Presumably, the association of the peptidoglycan layer with the lipid-rich outer layers potentiates its adjuvant activity (7).

EPIDEMIOLOGY

Tuberculosis remains the single greatest cause of death due to microbial invasion in the world. The World Health Organization (WHO) estimates that approximately one-third of the world's population is latently infected with *Mycobacterium tuberculosis;* and approximately 8 million new cases and 3 million deaths due to tuberculosis occur each year. Although more than 90% of cases occur in the developing world (where there is significant overlap with the acquired immune deficiency syndrome (AIDS) epidemic), approximately 15 million individuals in the United States are infected with *M. tuberculosis* (11–15).

Prior to 1980, significant progress, particularly in the developed countries, was being made in the control of tuberculosis. However, in the 1980s the epidemiology of tuberculosis, both in developed countries like the United States and in the emerging countries of Asia, Africa, and Latin America, underwent a catastrophic change. The advent of the AIDS epidemic, further exacerbated by war, famine, and poverty, and the large-scale immigration of populations caused by these conditions, brought to a halt the progress that was being made in the worldwide control of tuberculosis. For example, in the United States, the number of recognized cases of tuberculosis decreased between 1953 and 1984 by 73.6% (84,304 to 22,255); however, from 1985 to 1992, there was an increase of 12.6% in the annual incidence of reported cases of clinical tuberculosis (26,673 in 1992). Factors other than the AIDS epidemic postulated to account for this increase in cases included the following: (a) a decrease in the availability of resources for tuberculosis control programs; (b) an increasing homeless population; and (c) an increase in the number of individuals incarcerated within the prison system—these last two, like the AIDS epidemic, particularly affecting those populations in which tuberculosis had traditionally been a significant problem—immigrants, the poor, non-Caucasians, and those without supporting social structures. Included in this resurgence in tuberculosis cases was a high rate of transmission to vulnerable populations in hospitals, correctional facilities, residential care facilities, and shelters for the homeless. Indeed, in a particularly alarming development, outbreaks of *M. tuberculosis* resistant to isoniazid and rifampin were now being reported (15–19).

Recognition of this growing problem resulted in an intensification of tuberculosis control measures, including the use of directly observed therapy. By 1999, the number of new U.S. cases of tuberculosis had fallen to an all-time low, 18,361 (6.4 per 100,000), a 34% reduction since 1992. This decline reflected improvement in preventing both the transmission of the infection (clustered cases) and reactivation disease (15,20,21). Tuberculosis in the United States is primarily an urban disease with 75% of the new cases occurring in the 99 metropolitan areas that have populations of more than 500,000. Increasingly, in the United States tuberculosis is found among immigrants. Thus, although from 1992 to 1999 the number of cases decreased 46% among those born in the United States, the number of cases increased 3% among those born outside the United States. Approximately half these cases were found in immigrants from Latin America, the Philippines, and Southeast Asia (15). The overall incidence of clinical tuberculosis among foreign-born residents of the United States is approximately 30/100,000, more than four times that of native-born residents. In Europe similar patterns of increased tuberculosis have been seen among immigrants from high-tuberculosis–incidence countries (15,16,22–24). In addition, relatively high rates of extrapulmonary tuberculosis and drug-resistant tuberculosis have been observed among these immigrant populations (25).

Of these extrapulmonary forms of tuberculosis, genitourinary tract disease and infection of regional lymph nodes are the ones most frequently encountered by the clinician with genitourinary tract disease accounting for approximately 20% of extrapulmonary infection (26–29). As outlined in the following discussion and in Figure 38-1, extrapulmonary tuberculosis is the result of hematogenous spread from a pulmonary site of primary infection. Accordingly, genitourinary tuberculosis is observed in two clinical settings: commonly, as a late manifestation of earlier clinical or subclinical pulmonary infection and rarely, as part of the multiorgan infection that is characteristic of disseminated (miliary) tuberculosis.

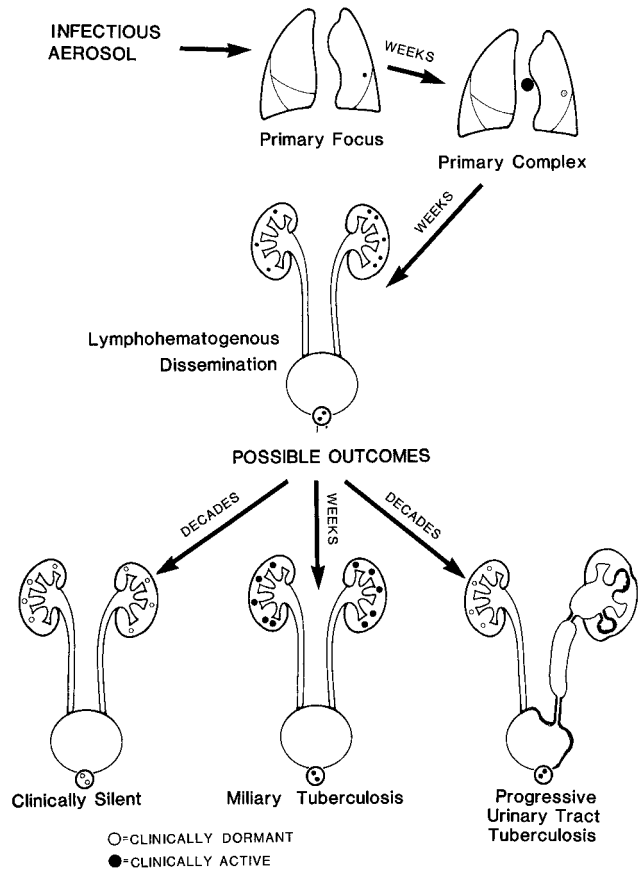

FIGURE 38-1. Schematic representation of the pathogenesis of urinary tract tuberculosis.

Statistics generated in the prechemotherapy era indicated that approximately 3% of unselected autopsy patients and 26% of those dying of tuberculosis had evidence of genitourinary tract tuberculosis at postmortem examination (30). In the early chemotherapeutic era (1952–1960), approximately 11% of patients dying of tuberculosis had genitourinary involvement at autopsy with most of this decline attributable to a decrease in early genitourinary lesions in patients with miliary tuberculosis (31). At the present time, it is estimated that significant genitourinary disease will develop in approximately 4% to 8% of non-HIV–infected individuals with pulmonary tuberculosis if adequate therapy is not instituted (29).

Traditionally, genitourinary tuberculosis has been a disease of young to middle-aged adults with a male to female ratio of approximately 5:3 (29–31,33–35). Although genitourinary tuberculosis has been reported in children (32,36,37), it is uncommon, and seen today almost exclusively in the rare child with miliary disease. Approximately one-quarter of the patients with genitourinary tuberculosis have a history of diagnosed tuberculosis (usually of the lung). In an additional 25% to 50% of patients, changes compatible with old pulmonary tuberculosis

can be found on chest x-ray films made at the time of diagnosis of genitourinary tract disease (29,33,35).

Thus, in the non-HIV–infected individual there is a considerable lapse of time between the onset of pulmonary infection and the diagnosis of active genitourinary tuberculosis. If one considers patients with early manifestations of primary tuberculous infection (i.e., erythema nodosum, pleurisy, or hilar adenopathy), the time lapse is most commonly 16 to 25 years, and intervals of longer than 40 years have been well documented. If one looks at patients with reactivation pulmonary tuberculosis, then the time lapse is usually about 4 to 8 years, but it may be as long as several decades (29,33,38).

There are several epidemiologic implications of these observations. First, the best way to decrease the incidence of genitourinary tuberculosis is by identifying and treating persons with pulmonary infection prior to the development of extrapulmonary disease. Second, long after the incidence of pulmonary tuberculosis falls, the incidence of genitourinary disease will remain relatively high, because a reservoir of patients with silent genitourinary tract infection will persist for decades after the incidence of new pulmonary infection falls. Thus, the rise in tuberculosis cases that occurred in the late 1980s and early 1990s virtually guarantees an increase in extrapulmonary infections such as genitourinary disease in the next several decades, unless extensive case finding and effective treatment of these individuals are accomplished in the interim. This task will be rendered particularly difficult because the burden of disease has fallen particularly heavily upon those with poorer access to skilled medical care—foreign born, the homeless, the incarcerated, migrant farm workers, and inner city ethnic minority groups (15,23,28,39–42). Finally, it is clear that the age of the population with genitourinary tract tuberculosis, and other forms of extrapulmonary tuberculosis, reflects the average age at presentation of active pulmonary tuberculosis—significantly younger among immigrants, urban ethnic minority populations, and the disadvantaged, and over the age of 50 among other groups (25,29,43,44).

In assessing epidemiologic factors that might predispose to tuberculosis, it is important to emphasize that only 5% to 15% of persons infected with the tubercle bacillus (as measured by a positive response to the tuberculin test) ever become ill from this infection (45). Of this group, approximately 3% to 5% have manifestations of genitourinary tract disease (26). In addition to exposure to tuberculosis in countries where the disease is rampant, such public health factors as crowding, homelessness, poverty, and drug addiction, all amplified by the AIDS epidemic, play a significant role in the spread of tuberculosis (11–17,40,43,46-52).

A minimum estimate of clinical tuberculosis in patients with AIDS is approximately 15% in the United States, with an even higher incidence (27%) in adults 25 to 44 years of age. The annual rate of tuberculosis among tuberculin skin test positive HIV-infected individuals in the United States has been reported to be 1.7 to 7.9 cases per 100 person years. This variation reflects the differences in the prevalence of tuberculosis in different population groups. To put this statistic in perspective, the rate of tuberculous disease among HIV-infected, tuberculin positive individuals is 4 to 26 times higher than that among comparable HIV-infected, tuberculin negative individuals, and is approximately 200 to 800 times that of the general American population (53–55). In addition, HIV-infected individuals presenting with clinical tuberculosis have a significantly increased probability of infection with *M. tuberculosis* resistant to first line antituberculous agents, particularly rifampin (55). The consequences of dual infection are far greater than those of either agent by itself. Thus, the mortality rate for HIV-infected individuals with tuberculosis is approximately four times higher than that of tuberculosis itself, with at least part of this excess mortality being caused by acceleration in the progression of the HIV process (15,55–61). There is a bi-directional intraction between AIDS and tuberculosis: tuberculosis is best treated in the setting of highly active AIDS retrovirus therapy (HAART) for the HIV infection, and there is some evidence that dual infection results in an escalation in the progression of the HIV infection. In addition, drug interactions become particularly important in patients with dual infection; for example, rifabutin is used instead of rifampin if a protease inhibitor is used. Fluconazole metabolism is increased in the presence of rifampin and other agents, thus complicating care in these patients. Despite the need for care in constructing the therapeutic regimen, early diagnosis and appropriate therapy of both infections are critical to the patient's survival (56–66).

Two epidemiologic patterns of mycobacterial infection are observed in HIV-infected individuals. Individuals from population groups with a low rate of endemic tuberculosis, such as gay men and those with posttransfusion HIV disease, primarily have difficulties with disseminated *M. avium-intracellulare* infection. In contrast, HIV-infected individuals who either belong to or come into contact with population groups with a high rate of endemic tuberculosis (those in developing countries, immigrants from these countries, the homeless, intravenous drug users, prisoners, and, in the United States and other developed countries, the inner city poor) are primarily afflicted with disseminated *M. tuberculosis* (15,46–52,55,67–72). Careful molecular epidemiologic studies have demonstrated that in HIV-infected individuals, outbreaks of tuberculosis resulted in either progressive primary disease or reinfection of individuals whose immunity had been attenuated by the effects of progressive HIV disease (73–75).

In HIV-infected individuals tuberculosis is often the AIDS-defining illness, not infrequently occurring early in the course of HIV infection. Extrapulmonary disease, often in conjunction with pulmonary disease, is the rule, with the time course for the development of disseminated disease being greatly abbreviated in these individuals. In this setting, genitourinary disease is rarely seen as an isolated phenomenon, but rather is part of disseminated infection. The incidence of HIV infection among patients with extrapulmonary tuberculosis is approximately twice that of those with tuberculosis restricted to the lungs (55,74,75).

The coexistence of tuberculosis with HIV infection has already had major public health consequences on the control of tuberculosis. Because HIV infection both increases the burden of infectious tubercle bacilli and obscures the symptoms (owing to the impaired inflammatory response of these immunosuppressed individuals), these people are highly efficient transmitters of tuberculosis. The expected consequence of this is the potential for a marked increase in the occurrence of secondary cases, and even epidemics, in contacts of these individuals, particularly in medical settings, crowded living conditions, prisons, and shelters for the homeless. Those populations most at risk for both HIV infection and tuberculosis, and the coexistence of these two infections, are the same populations with the highest incidence of drug-resistant tuberculosis. The result of this concordance of events is that the tuberculosis that can be amplified by the HIV epidemic includes a high potential for drug-resistant disease, thus complicating greatly both the management of individual patients and the public health strategies that must be taken to protect the community (15,48–52,55,74,76,77).

One additional epidemiologic consideration is the possibility that urine from patients with urinary tract tuberculosis could transmit tuberculosis to household members. Vasquez and Lattimer (78) reported a doubling of the incidence of tuberculin positivity among children of parents with active urinary tract tuberculosis without active pulmonary disease. Other observers have been unable to confirm this finding. Our policy has been not to isolate persons with urinary tract tuberculosis but

to consider their urine potentially infectious and to maintain contact precautions when handling it.

PATHOGENESIS OF TUBERCULOSIS

Systemic Aspects

The host–parasite interaction in tuberculosis (i.e., the interaction of a slowly proliferating pathogen that resists the usual microbicidal mechanisms of the host) stands in stark contrast to conventional bacterial disease, where despite the pathogen's rapid proliferation, the host's resources (complement fixation, opsonization, phagocytosis, and ready lysis within phagocytic cells) are formidable. The pathogenesis of tuberculosis reflects the balance of intrinsic mycobacterial virulence and the host immunologic response. The classic response in tuberculosis, the formation of granulomas, is ordinarily protective for the host by limiting the proliferation and spread of *M. tuberculosis,* but may be pathogenic as well, because it may lead directly to tissue injury in the form of caseation (79). Thus, the clinical manifestations of tuberculosis represent not only the consequences of mycobacterial proliferation but also host reparative and destructive responses.It is well known that in the absence of immunosuppression, the lifetime risk of symptomatic M. tuberculosis infection among latently infected individuals is only ~10%. Similarly, the risk of developing extrapulmonary disease, such as genitourinary tuberculosis, is rather low. An increased understanding of the molecular mechanisms responsible for host defense against mycobacterial infections has led to a growing appreciation that human susceptibility to mycobacterial infection may be attributable in significant measure to host genetic factors, as well as to the intrinsic virulence of M. tuberculosis isolates (80,81).

Tubercle bacilli are inhaled as small-particle aerosols and gain direct access to the alveoli (79). Presently, ingestion of *M. tuberculosis* with primary localization of disease in the intestinal tract or oropharynx is rare. The small aerosol inoculum multiplies slowly and is phagocytosed by polymorphonuclear leukocytes and pulmonary macrophages. Mycobacteria interact with respiratory epithelium (82), alveolar surfactant proteins (83), and the alternate complement system (84), but interactions with macrophage Toll-like receptors (TLRs 2 and 4, (85,86)) play a critical role in initiating the host immune response. TLRs are pattern-recognition proteins, expressed on macrophages and dendritic cells, which serve as innate immune receptors (87). Each TLR binds one or more of a variety of microbial products [endotoxin (lipopolysaccharide), bacterial DNA, flagellin, mycobacterial lipoarabinomannan, etc.] and transduce inflammatory signals culminating in the activation of NF-κB and transcription of tumor necrosis factor α (TNFα) and interferon γ (88). Following opsonization by C3, *Mycobacterium tuberculosis* binds to phagocytic cell surface complement receptors and is phagocytosed (84). Mycobacteria suppress the intracellular calcium flux that normally accompanies phagocytosis and inhibit macrophage activation and phagolysosome maturation (89). *M. tuberculosis* can also directly adhere to, infect, and lyse type II alveolar epithelial cells (90,91), facilitating access to lung interstitium and enhancing early dissemination to extrapulmonary foci.

The host response to mycobacterial infection has been called the IL-12—interferon γ axis (80). Macrophage activation via TLR signaling and other early events is associated with secretion of TNFα and IL-12 and the related cytokines IL-18 and IL-23 (92). The latter cytokines program resting T lymphocytes toward an inflammatory Th1 response. Activated Th1 lymphoctyes secrete as their dominant cytokines interferon γ as well as TNFα, and this in turn activates macrophages and enhances their mycobactericidal activity. The use of TNFα antagonists (etanarcept, infliximab, and adalimumab) as disease-modifying agents in the treatment of rheumatoid arthritis and other inflammatory diseases confirms a central role for TNFα in the host response against mycobacterial infection. These therapies have been associated with rapidly progressive tuberculosis, impaired granulomatous reactions in tissue biopsies, and a high rate of extrapulmonary disease (93). In the initial stages of primary infection, resting macrophages have a limited ability to lyse mycobacteria, and the bacillary titer rises despite entrapment within macrophage and granulocyte phagosomes and lysophagosomes. Some bacilli can even escape from these organelles and replicate freely within the cytoplasmic compartment (94). Macrophage-mediated killing of intracellular *M. tuberculosis* requires the L-arginine-dependent generation of reactive nitrogen intermediates, such as nitric oxide, and this capability is greatly enhanced following macrophage activation by interferon-γ and tumor necrosis factor (95). Thus, infected macrophages program T cells toward a Th1 response which in turn augments macrophage-mediated mycobacterial killing.

T cell-infected macrophage interactions are more complex, however, since several cytolytic T cell effector populations are generated which can lyse infected macrophages (96). In addition to the expansion of conventional peptide-specific CD4+ and CD8+ $\alpha\beta$T-cell receptor-expressing populations (97), T cells with a double negative phenotype (CD4-, CD8-) expressing $\gamma\delta$ T-cell receptors recognize mycobacterial phospholipid antigens presented by MHC-like molecules (e.g., CD-1) and lyse infected macrophages via Fas–FasL interaction. These phospholipid antigens are also recognized by CD8+ $\alpha\beta$T-cell receptor-expressing cytotoxic T lymphocytes, triggering perforin-mediated cytotoxicity, and by natural killer T (NKT) cells, which possess NK markers as well as $\alpha\beta$T-cell receptors (98). In at least some instances, immune lysis of infected macrophages appears beneficial to the host. Perforin-mediated lysis of infected macrophages reduces *M. tuberculosis* viability by 50% *in vitro* (97), and may be important *in vivo* in reducing the number of infecting tubercle bacilli. The cytotoxic granules that contain perforin also contain granulysin, a lipid-binding protein that has potent mycobactericidal activity in the presence of perforin (99). In contrast, Fas-mediated lysis of macrophages does not affect mycobacterial viability, but may be important in reducing antigen presentation and dampening the immune response. The immune lysis of heavily infected macrophages may facilitate the phagocytosis of released mycobacteria by additional activated macrophages. These newly recruited cells have a lower bacillary burden and may be more effective at killing their intracellular bacilli, or serve as target cells for cytotoxic T lymphocytes.

In addition to their direct cytotoxic activities, the activated lymphocytes secrete a variety of cytokines, including interferon-γ, migration inhibitory factor (MIF), granulocyte-macrophage colony-stimulating factor, TNFα, IL-12, and inhibitory cytokines such as interleukin-4 (IL-4) (100) and IL-10 (101). IL-4 production undermines the host Th1 response, and triggers tissue fibrosis (102), a characteristic finding in chronic tuberculosis. In HIV-infected individuals coinfected with *M. tuberculosis,* immunosuppressive cytokines (e.g., IL-10) produced by macrophages/monocytes diminish the T-lymphocyte response *in vitro,* suggesting that Th2-like activity contributes to uncontrolled, systemic spread in these patients (103). Macrophages are recruited to infiltrate the area of mycobacterial growth to form granulomas and mature into epithelioid cells by the macrophages' elaboration of tumor necrosis factor (104); this process requires NKT cells (98). In spite of macrophage activation, the killing of intracellular mycobacteria by human macrophages is often incomplete (105,106).

The outcome of early tuberculous disease covers a spectrum from granuloma formation with efficient containment and healing to slowly progressive disease at the site of the primary pulmonary infection, or to clinically significant systemic spread of disease. Mycobacterial dissemination is actually the rule rather than the exception (Fig. 38-1). Although most bacilli are contained within macrophages initially, their continued proliferation disrupts the macrophages and the bacilli return to the extracellular environment. Most are engulfed again, but some are carried in the lymphatic drainage and produce regional lymphadenitis. Alternatively, some viable mycobacteria may reach regional lymph nodes while entrapped within migratory macrophages. Progressive infection within the lymph node contaminates efferent lymph, and when sequential lymph node barriers fail, thoracic duct lymph delivers mycobacteria to the venous blood, seeding the pulmonary bed as well as extrapulmonary sites, such as the skeletal system, lymph nodes, and most frequently, the kidneys.

Thus, limited hematogenous dissemination due to low grade bacillemia can occur early in the process of granuloma formation when the number of mycobacteria is small, and most organisms are found intracellularly within the macrophages comprising the granuloma. Small granulomas rapidly form at the metastatic foci because mycobacterial immunity is evolving or is already established at the time of dissemination. Although the bacilli may remain viable, the granulomas may remain clinically silent for decades.

Granuloma formation may itself contribute to the pathogenesis of severe tuberculosis (79). Granulomas are active lesions with continued ingress of immune T lymphocytes and monocytes (107). Shortly after microscopic granulomas become well established, polymorphonuclear leukocytes and monocytes enter the lesion (108). The resultant phagocytosis is accompanied by exocytosis of lysosomal contents with local tissue destruction. This leads to a characteristic local necrotic process, known as *caseation*. Macrophage disruption returns the mycobacteria to the extracellular environment, where their proliferation accelerates (79). Communication of the caseating granuloma with the bronchial tree restores favorable metabolic conditions, and mycobacterial titers can increase by several logarithms. Such highly infected material can spread endobronchially to produce additional foci of pulmonary tuberculosis or excavate into a pulmonary vessel, leading to intense bacillemia. Such severe hematogenous dissemination commonly is responsible for miliary tuberculosis rather than limited extrapulmonary disease. In miliary tuberculosis, the systemic features of illness overshadow the asymptomatic renal involvement.

Pathogenesis of Renal Tuberculosis

Local factors play a significant role in the evolution of clinically significant renal tuberculosis. The small silent renal granulomas resulting from silent hematogenous dissemination are typically found bilaterally in the renal cortex and arise from capillaries within and adjacent to glomeruli (109) (Fig. 38-2). A glomerular location is not surprising in view of their high rate of perfusion (increasing the likelihood of delivering a bacillus in the setting of sparse bacillemia) and their favorable oxygen tension. Such cortical granulomas usually remain dormant for decades. In some patients, however, bacillary proliferation within the glomerular capillary leads to capillary rupture and delivery of organisms into the proximal tubule. Clinically important renal tuberculosis, therefore, is usually initially localized to the medulla. This is likely caused by entrapment of mycobacteria and infected macrophage debris within the loop of Henle (109) and the known impairment of phagocyte function associated with the hypertonic environment found in the medulla (110,111).

FIGURE 38-2. Early renal tuberculosis. Three small granulomas are visible in the cortex. The adjacent papillary tip is involved as well. (From: Kollins SA, Hartman GW, Carr DT, et al. Roentgenographic findings in urinary tract tuberculosis. *Am J Roengentol Radium Ther Nucl Med* 1971;121:487, with permission.)

Analogous to progressive pulmonary disease, granulomas may enlarge in the medulla, leading to caseation and papillary necrosis. Such intraparenchymal granulomas may persist as mass lesions but commonly cavitate into the calyceal system. Despite bilateral hematogenous seeding of the kidneys, clinically significant disease is usually unilateral (109). Communication of the caseating granuloma with the collecting system usually is responsible for the spread of bacilli to the renal pelvis, ureters, bladder, and accessory genital organs and is analogous to the endobronchial spread of infection seen in cavitary lung disease (30). Lymphatic spread to contiguous structures also occurs in addition to epithelial infection by a luminal mechanism (112), and direct hematogenous seeding of pelvic genital organs with clinical sparing of the kidney can occur occasionally (30). In addition to the direct parenchymal destruction associated with advanced renal lesions, the fibrosis that accompanies the granulomatous process within the collecting system, such as infundibular strictures and renal pelvic kinking, adds an obstructive mechanism that may contribute significantly to progressive renal dysfunction.

PATHOLOGY

The caseating granuloma is the classic microscopic finding in essentially all forms of tuberculosis (Figs. 38-3 and 38-4). Medlar (109) characterized the early pathologic findings in renal tuberculosis by meticulously examining microscopic sections of kidneys from patients who died of pulmonary tuberculosis. Bilateral microscopic renal involvement is the rule, although the extent of involvement usually is asymmetric. Granulomas vary greatly in size, from lesions contained within a single

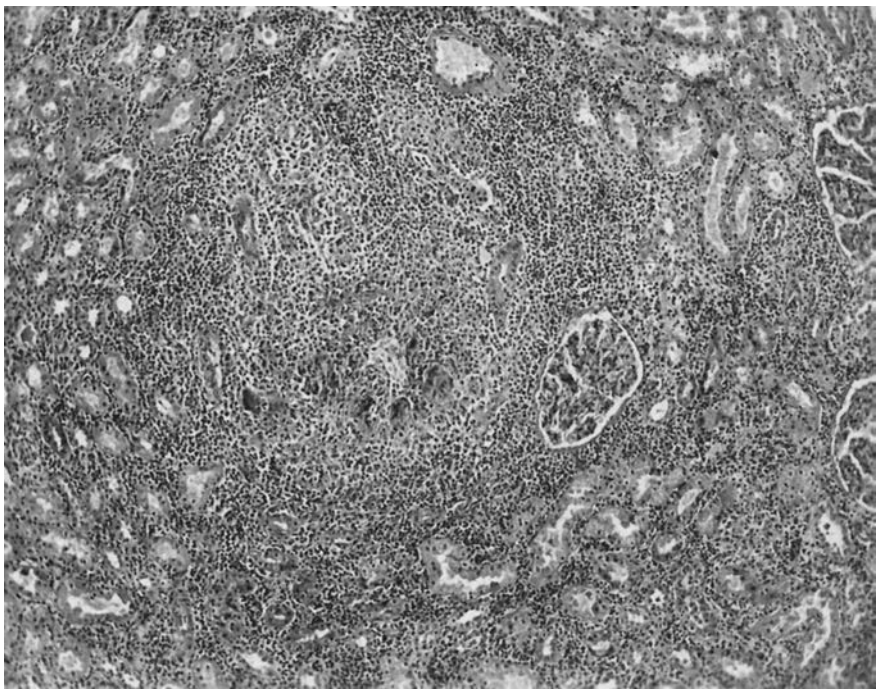

FIGURE 38-3. A caseating granuloma in the renal cortex. In addition to the necrotizing granuloma in the center of the field, a diffuse interstitial infiltrate of lymphocytes is seen. Some tubules are preserved (H&E, magnification × 79).

glomerulus to large caseous abscesses, and in the apparent density of acid-fast bacilli.

Most renal granulomas originate as vascular lesions in the cortex. Although glomerular lesions predominate, with foci within the capillary tuft, granulomas may develop within capillaries in relation to the convoluted or collecting tubules. Lesions within the collecting system per se are usually at the nadir of the loop of Henle or in the pyramidal collecting tubule, always draining a vascular granuloma (Fig. 38-2), and presumably developing in response to ulceration and discharge of these lesions into the collecting system. Focal sparing of tubules, glomeruli, or both within the granulomas is characteristic of renal tuberculosis (Figs. 38-3 and 38-4).

Clinically significant caseation progresses from the medullary collecting system lesions (113). The enlarging medullary abscess extends to the papilla and commonly produces papillary necrosis. It may replace the medullary pyramid and persist as a parenchymal cavity, or tuberculoma, or discharge into the draining calyx. Several pyramids may be involved individually with a variable extent of destruction or may coalesce to destroy the bulk of the renal parenchyma (Fig. 38-5). Infection of the calyces, pelvis, and ureter is followed by stricture formation, so that caliectasis (114) and tuberculous pyonephrosis ("caseocavernous renal tuberculosis") are common in advanced disease. The end-stage kidney is nonfunctional ("autonephrectomy") and destroyed by the combined

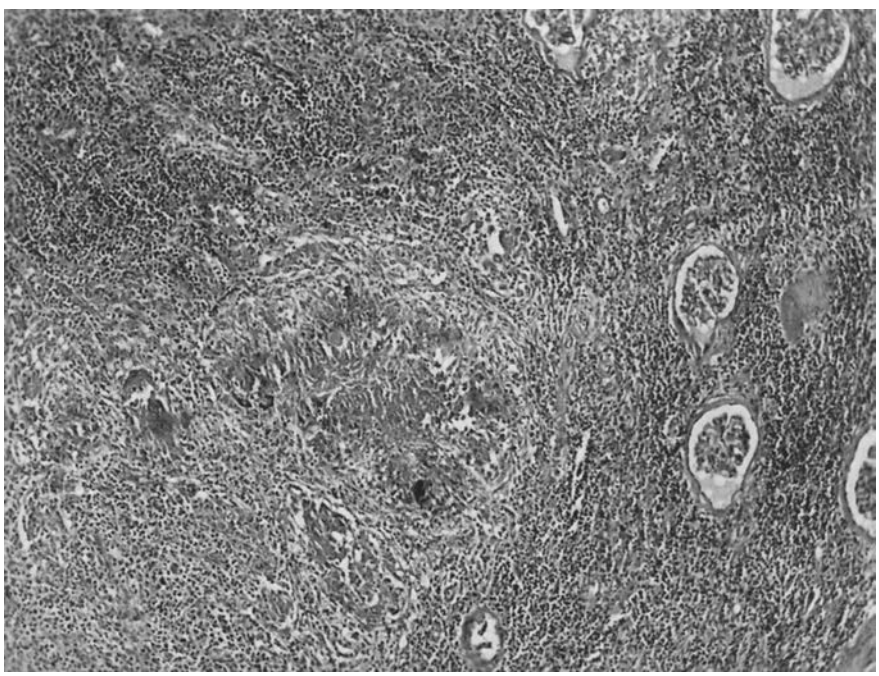

FIGURE 38-4. Renal cortical tissue showing destruction of tubules and a diffuse lymphocytic infiltrate with focal caseating granulomas. The glomeruli are spared (H&E, magnification ×79).

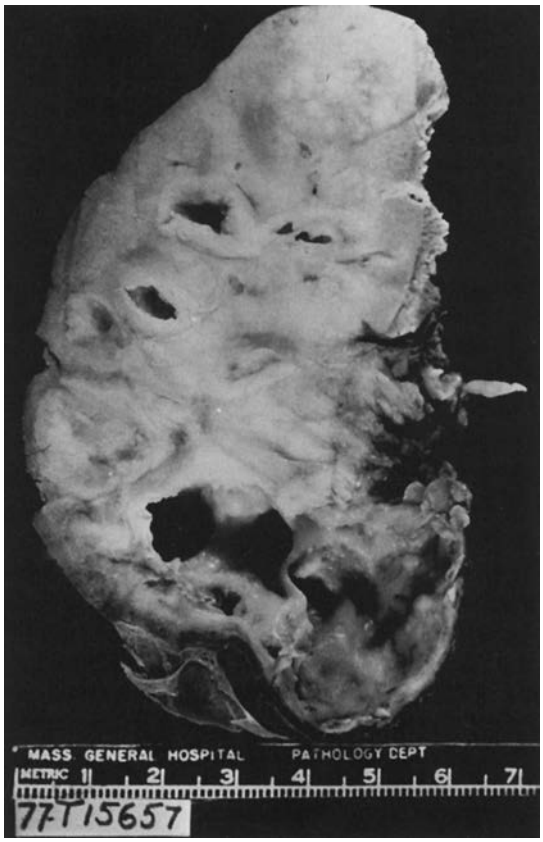

FIGURE 38-5. Renal tuberculosis with replacement of most of the parenchyma by caseous necrosis. There is cavitation of pyramids.

necrotizing and obstructive processes. Calcification in advanced lesions is common and may be focal or generalized, which produces a "putty" or "cement" kidney.

CLINICAL FEATURES

Urinary tract tuberculosis is notorious for its insidious mode of presentation, with approximately 20% of cases diagnosed unexpectedly at operation or autopsy. As many as 20% to 56% of patients with active urinary tract tuberculosis detected on the basis of an abnormal urinalysis or culture deny either constitutional symptoms of tuberculosis or symptoms referable to the urinary tract (29,33,35,36,114,115). One measure of the frequently occult nature of urinary tract tuberculosis comes from Lattimer's report (115) of 18 of 25 physicians with renal tuberculosis being diagnosed only after far-advanced cavitary disease had developed. If physicians with ready access to medical care have their renal tuberculosis overlooked, then the problem is only compounded in the populations who today have the greatest risk of tuberculosis—inner-city minority groups, immigrants from the developing countries of the world (particularly those undergoing social disruption), and the indigent elderly—all of whom have relatively poor access to medical care.

Approximately 75% of patients with urinary tract tuberculosis present with symptoms suggesting urinary tract inflammation. Such symptoms resemble those of conventional bacterial urinary tract infection—dysuria, mild or moderately severe back or flank pain, hematuria, nocturia, and pyuria. Renal colic, owing to the passage of clots or stones, may be observed in as many as 10% of patients (29,33–35,44,114,115). Severe pain localized to the kidneys is uncommon but has been reported (116). Noteworthy for their infrequency are the constitutional symptoms usually associated with tuberculosis—fever, weight loss, night sweats, and anorexia. Fewer than 20% of patients with tuberculosis restricted to the urinary tract have constitutional symptoms. Indeed, the presence of such constitutional symptoms should suggest the presence of active tuberculosis in other organs as well (29,33–35,44,114,115).

As noted previously, the extent of renal dysfunction secondary to tuberculous infection can be quite variable, from small focal areas of infection and scarring unassociated with any functional impairment to gross parenchymatous destruction and complete loss of function. As in any other form of tubulointerstitial nephritis (Chapter 48, Acute Tubulointerstitial Nephritis), patients with renal tuberculosis may be subject to dehydration because of a concentrating defect, a tendency to lose salt, or both (117). However, in any patient in whom this is documented, particularly when associated with constitutional symptoms, concomitant tuberculous adrenal disease (Addison's disease) must also be sought (118,119).

The only way that renal function loss owing to parenchymatous infection and destruction can be limited is by early diagnosis and therapy. It is also important to identify patients subject to the second major cause of renal functional loss in this setting—those with hydronephrosis secondary to obstruction induced by the tuberculous process. Here the second element of the pathologic process induced by tuberculosis, sclerosis, exerts its effects. Strictures, usually either at the ureteropelvic junction or at the lower end of the ureter, can result in hydronephrosis and loss of renal function. Obstruction and hydronephrosis can develop during therapy because such sclerotic strictures are frequently part of the healing process. The clinician must be alert to this possibility, because correction of such obstruction is the best way to preserve renal function in patients with tuberculosis (29,33–35,114,120–123).

There are three other major complications of renal tuberculosis: hypertension, superinfection with conventional bacteria, and nephrolithiasis. In 1940, Nesbit and Ratliff (124) reported that hypertension could be cured by the removal of a tuberculous kidney, an observation subsequently confirmed by other authors (125–127). More recent data, however, suggest that this is an uncommon event. First, hypertension may not be more common in patients with renal tuberculosis (<5% of those with tuberculous kidney infection are hypertensive) than in the general population. Second, surgical cure of hypertension in these patients appears to be the exception rather than the rule (29,33–35,44,120,128,129). Renal vein renin sampling may be useful in predicting the outcome of surgery for patients with hypertension and renal tuberculosis (130).

Both nephrolithiasis and bacterial superinfection of a urinary tract rendered anatomically abnormal by the tuberculous process are not uncommon. Nephrolithiasis has been reported in 7% to 18% of patients with renal tuberculosis, and superinfection has been reported in 12% to 50% of patients with urinary tract tuberculosis (29,33,34,44,120).

The delivery of large numbers of *M. tuberculosis* into the urine of patients with renal tuberculosis is the major cause of tuberculous infection of the ureters and bladder. In the bladder, as in the ureters, scarring and contracture is the major result of tuberculous infection, again the not-so-benign effects of "healing." The result is a small, contracted bladder with greatly thickened walls. There are three functional consequences of this process: a small bladder capacity, incomplete emptying and thus a predisposition to secondary bacterial infection, and most serious of all, vesicoureteral reflux (120,131,132).

The incidence of genital infection in association with urinary tract tuberculosis is very different in the two sexes. In men, such

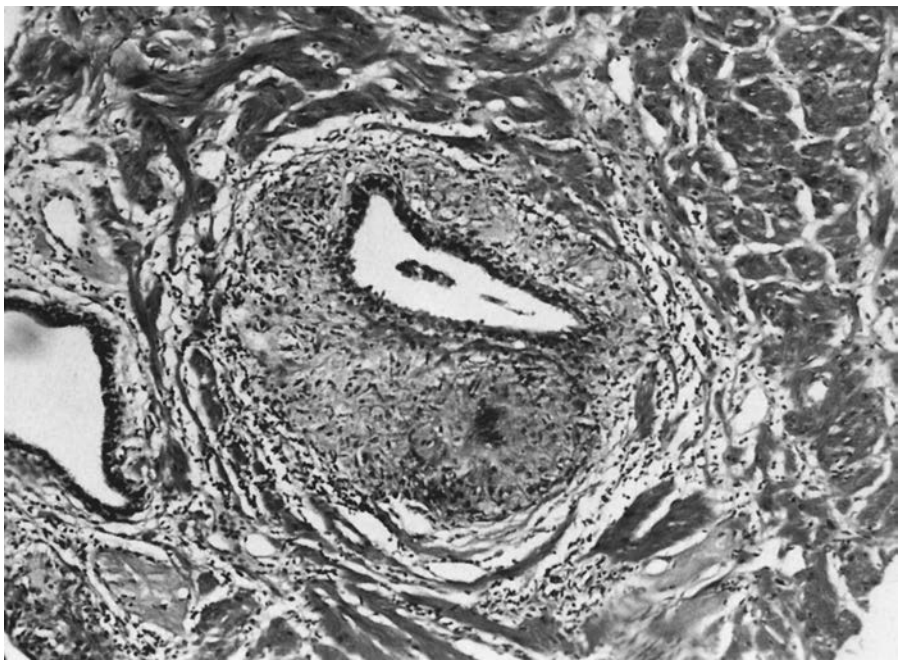

FIGURE 38-6. Prostatic tissue with epithelioid granulomatous reaction surrounding a duct (H&E, magnification ×125).

dual involvement is relatively common. Indeed, genital disease may lead to the recognition of extensive urinary tract infection. Epididymitis, with or without orchitis, presenting as a scrotal mass or discomfort, is the most common manifestation of male genital tuberculosis. The majority of such patients are free of constitutional complaints (35,115,120,133,134). The importance of this form of tuberculosis is underlined by the report of Ferrie and Rundle (133) that 75% of their patients with tuberculous epididymoorchitis already had evidence on pyelogram of tuberculosis involving the bladder, ureters, kidneys, or all of these organs at the time they presented with their epididymal disease. Epididymitis can present decades after apparently adequate therapy for renal tuberculosis (135).

Tuberculous prostatitis, also heralded by a mass lesion, pain, or both, rather than systemic symptoms in most patients, is likewise frequently associated with urinary tract disease (Fig. 38-6). The prostate, like the epididymis and testes, can be infected either by means of the hematogenous route or more directly from infected urine. Two important points for the clinician regarding tuberculous prostatitis are as follows: Tuberculosis can be transmitted by means of infected semen in such patients (115), and viable organisms frequently persist in the prostate long after other parts of the genitourinary system have been sterilized (29,120,136,137). Persistent prostatic infection is presumably related to the same difficulty in delivering effective antimicrobial therapy to the prostate that is responsible for persistent conventional bacterial infection of the male genitourinary system.

Urethral and penile tuberculosis are both uncommon but may present with strictures, fistulous tracts, and ulcerating or papillonecrotic skin lesions. Both these forms of tuberculous infection are usually secondary to seeding from infected urine, but primary penile tuberculosis has been reported to occur owing to the direct inoculation of *M. tuberculosis* into a wound following ritual circumcision, after the use of contaminated surgical instruments, or from contaminated clothing (138–140).

In contrast, tuberculosis of the female reproductive tract is quite distinct from urinary tract tuberculosis. The incidence of renal disease in women with genital tuberculosis is less than 5%, which is little different from that found in patients with pulmonary or skeletal tuberculosis (141). The explanation for this difference between men and women is clear: Whereas involvement of the male genital tract usually occurs from direct extension or from infected urine, the female genital tract is almost always infected hematogenously, with seeding of the fallopian tubes and then secondary extension from this site. Thus, the major manifestations of female genital tuberculosis—infertility, secondary amenorrhea, vaginal bleeding, and pelvic pain—are quite separate from the manifestations of urinary tract tuberculosis (29,33,115,141–143).

Given the insidious nature of urinary tract tuberculosis, routine laboratory studies can be quite important in raising the possibility of tuberculous infection. By far the most useful screening test is urinalysis. As emphasized by Simon and associates (29), virtually the only time when a urinalysis is normal and a urine culture positive for *M. tuberculosis* is in the patient with miliary dissemination, in whom the urinary tract seeding is a recent and perhaps insignificant event. In contrast, essentially every patient with established urinary tract tuberculosis has an abnormal urinalysis with pyuria, hematuria, or both. The old clinical teaching that the asymptomatic patient with pyuria, particularly with an acid urine and a urine culture that fails to reveal conventional bacterial pathogens, must be considered as having tuberculosis until proved otherwise remains true today (29,33-35,44,115,120,144). Although there are other causes of "sterile pyuria," such as *Chlamydia trachomatis* or invasive fungal infection, tuberculosis must be excluded in patients with these findings. Perhaps most useful as a next step in the evaluation of such a patient is the placement of an intermediate-strength tuberculin test (5 TU). Virtually every patient with urinary tract tuberculosis who is not receiving systemic steroids or who has not been rendered anergic by such debilitating conditions as advanced malignancy or HIV infection has a positive tuberculin test. In contrast, fewer than 20% of patients with urinary tract tuberculosis have abnormalities on tests that measure systemic illness (i.e., anemia, changes in white blood cell count, low serum albumin, etc.) (29,33–35,44,115,120,144). Similarly, azotemia at presentation is quite uncommon. Approximately two-thirds of patients with urinary tract tuberculosis have evidence of old or current tuberculosis on chest roentgenography (29,33,44,115,120,144).

ATYPICAL MYCOBACTERIAL INFECTION

Genitourinary infection caused by atypical mycobacterial organisms is quite rare, although a few cases of prostatic or epididymal infection, or both, owing to *M. kansasii* have been reported (145,146). In addition, disseminated disease due to this organism has been reported in immunocompromised patients, with such disseminated infections seeding the kidney and leading to a granulomatous reaction in the kidney and the isolation of *M. kansasii* in the urine (147,148). As noted, this form of genitourinary infection is particularly prominent in patients with AIDS with disseminated infection caused by *M. avium-intracellulare* and other atypical mycobacterial species (66,69–71,149).

Diagnosis

The isolation of *M. tuberculosis* by urine culture is the definitive diagnostic test in renal tuberculosis. Early-morning urine specimens are preferred over 24-hour urine samples (144, 150), because mycobacterial viability falls with prolonged exposure to acid urine (151). In order to detect the low rate of bacilluria, three to five specimens should be submitted. Samples are routinely decontaminated by limited exposure to acid or alkaline solutions and then concentrated by centrifugation. Neither direct nor amplified nucleic acid hybridization probes are licensed for rapid diagnosis of urinary M. tuberculosis given their borderline sensitivity (~70%) compared to traditional culture (152). Cultures are established on standard solid mycobacterial media, either egg-based (Löwenstein-Jensen) or agar-based (Middlebrook 7H10). These media also contain aniline dyes, such as malachite green, to inhibit the growth of bacterial contaminants. The transparent agar medium facilitates early visualization of microcolonies by approximately 1 week (3).

Microbiologic identification is based on colonial morphology, growth rate and optima (37°C, carbon dioxide-enriched atmosphere), absence of pigment production, accumulation of niacin, reduction of nitrate, and absence of significant catalase activity (3,144,150). Recently, radiometric techniques have been employed to recover mycobacteria with greater sensitivity and increased speed. Commercial reagents are available for the rapid speciation of primary isolates by nucleic acid hybridization techniques (e.g., Accuprobe® [Gen-Probe, San Diego, CA]).

The development of commercial broth systems for mycobacterial growth detection has permitted the automation of the diagnostic process. Such systems as the BACTEC 460 (Becton Dickinson Microbiology Systems, Sparks, MD), mycobacterial growth indicator tube (MGIT) systems, ESP (Extra Sensing Power) Myco-ESP culture System II (Trek Diagnostic Systems, Inc., Westlake, OH), and BacT/ALERT MB Susceptibility Kit (Organon Teknika, Durham, NC) use Middlebrook 7H12 media with added material for detection of mycobacteria (radiometric or colorimetric systems). With these systems, mycobacterial growth can be detected in 1 to 3 weeks, as compared to solid media, which takes 3 to 8 weeks (151).

Other rapid diagnostic techniques have been developed and are in the process of being deployed in major diagnostic laboratories. These include high performance liquid chromatography to identify the spectrum of mycolic acids in the bacterial cell (which is useful after 7 to 10 days of culture), ELISA techniques to detect mycobacterial protein antigens, and the detection of tuberculostearic acid by gas chromatography and mass spectroscopy. A high priority is being given to making more rapid diagnostic approaches widely available (15,153–157).

In retrospective analyses of patients with renal tuberculosis, urine cultures were reported to be positive in 80% to 90% of cases (29,33). In patients with negative cultures despite optimal processing of multiple samples, the diagnosis is often reached by the recovery of *M. tuberculosis* from other sites (e.g., sputum or surgical specimens) in the setting of abnormal urinalyses and intravenous pyelography, together with a positive tuberculin reaction. When tissue specimens are submitted for mycobacterial culture, they are best macerated using sterile sand with a mortar and pestle, because automated homogenization methods may heat samples excessively and kill any mycobacteria present (151).

Primary mycobacterial isolation provides an opportunity to perform drug susceptibility testing in addition to confirming the diagnosis of genitourinary tuberculosis. Drug susceptibility testing is recommended on initial isolates from all patients, but is particularly important in evaluating patients who previously received chemotherapy and in patients epidemiologically linked to known cases of drug-resistant tuberculosis (144).

Radiology

Radiologic evaluation plays a central role in the diagnosis and long-term management of patients with renal tuberculosis (34,158–160). There is excellent correlation between the pathology of renal tuberculosis and the corresponding abnormalities seen by excretory urography (161). Even plain films of the abdomen are valuable, as genitourinary calcifications (present in up to 50%) (34,162) as well as other extrapulmonary foci of mycobacterial disease (vertebral, mesenteric lymph node, adrenal glands) may be present (approximately 10%) (34) (Figs. 38-7 and 38-8). Chest radiographs show evidence of tuberculosis in 50% to 75% of patients with active renal disease (29,33,34). In the remainder, the primary pulmonary granuloma, responsible for hematogenous spread, heals and may no longer be detectable by radiograph, but the metastatic renal granulomas progress to cause local destruction.

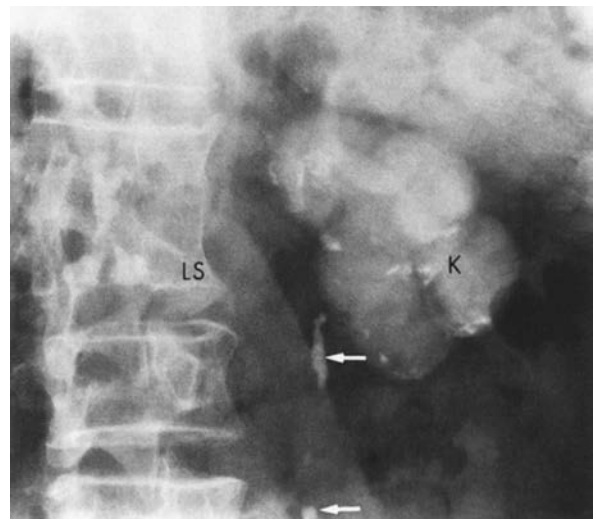

FIGURE 38-7. Plain film of the abdomen shows a left autonephrectomy with calcification of the kidney parenchyma and the left ureter (*arrows*). There is tuberculous involvement of the lumbar spine, resulting in fusion of the intervertebral disc space. *K,* kidney parenchyma; *LS,* lumbar spine.

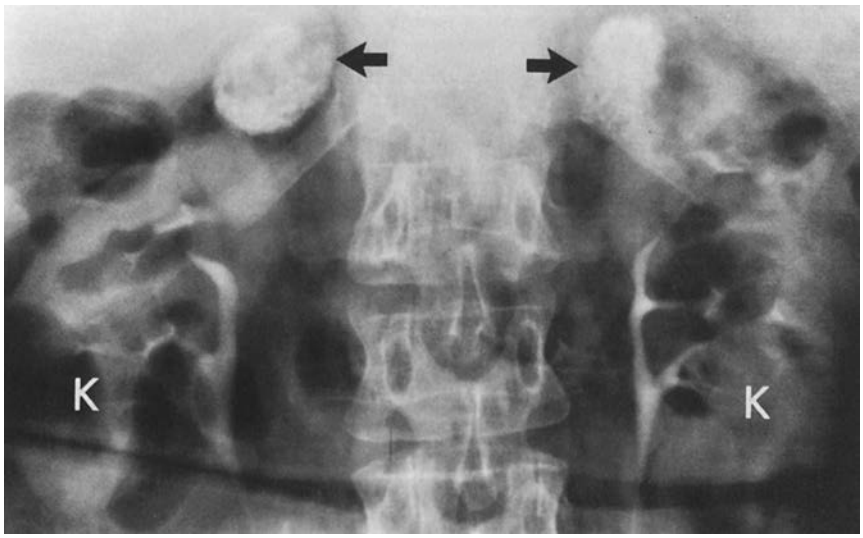

FIGURE 38-8. Tuberculous calcification of both adrenal glands (*arrows*). Intravenous urogram demonstrates the normal kidneys. *K,* normal kidneys.

Excretory urography traditionally has been the standard diagnostic imaging technique. Nephrotomograms are particularly valuable in assessing moderate or advanced disease, especially with decreasing clearance of dye. In recent years, computerized tomography (CT) with contrast, particularly helical CT, has provided increased delineation of renal parenchymal abnormalities (163–165), although contrast administration (with risks of allergy and nephrotoxicity) is still required, and the total radiation dose exceeds that of traditional excretory urography. The earliest stage of renal involvement, the cortical granuloma (Fig. 38-2), is associated with positive urine mycobacterial cultures but negative excretory urograms. The spread of infection to the medulla and the evolution of cavitary disease in the papillae represent the earliest changes detectable radiologically (Fig. 38-9). Papillary granulomas caseate and rupture into the collecting system. The resulting small communicating cavities may be single or multiple but are usually unilateral and may mimic papillary necrosis of other etiologies. With time, they enlarge in an irregular fashion, with shaggy margins, and may progress to involve the entire medullary pyramid. A consequence of papillary cavitation is spread of infection to the uroepithelium and submucosa of the draining calyx. The resulting fibrotic reaction leads to stenosis and even complete stricture of the calyceal infundibulum. Thus a medullary cavity may be excluded from the collecting system and an abscess, or localized tuberculous pyonephrosis, ensues. These lesions may be difficult to distinguish from a truly noncommunicating caseous parenchymal cavity. Advanced medullary disease may lead to renal cortical scarring as well. Amorphous calcification is frequently seen (Fig. 38-7) and may progress to outline the entire granuloma. Both the parenchymal tuberculous granuloma and the noncommunicating pyonephrosis may be confused with primary mass lesions, but CT (163–165), sonography (166), and, in selected cases, angiography (167) may be necessary to resolve this issue by confirming the cystic and avascular nature of the mass.

The discharge of caseous material infects the renal pelvis, ureter, and bladder as well. Renal pelvic involvement is manifested by obstructive changes involving portions of the kidney due to stenosis or kinking of the pelvis or the entire organ due to ureteropelvic junction stenosis (Figs. 38-10 and 38-11). Thus poor visualization by excretory urography may be segmental or involve the entire kidney. Ureteral disease initially presents as mucosal irregularity, together with diffuse dilatation or narrowing due to inflammation or edema (168) (Figs. 38-9 and 38-10). There may be a single focus or multiple areas of ureteral involvement. In addition to an irregular border, the combination of multiple strictures and accompanying segmental dilation leads to a beaded or corkscrew configuration (Fig. 38-10). Unlike nonspecific strictures, tuberculous lesions may extend for several centimeters and are usually present together with ipsilateral renal disease. In some instances, intramural fibrosis leads to a rigid "pipestem" ureter. Calcification and even

FIGURE 38-9. Intravenous urogram in a patient with active urinary tuberculosis, demonstrating papillary necrosis diffusely involving all the calyces in both kidneys. There is a small irregular bladder and narrowing of both distal ureters (*arrows*). *B,* bladder; *C,* calcyes.

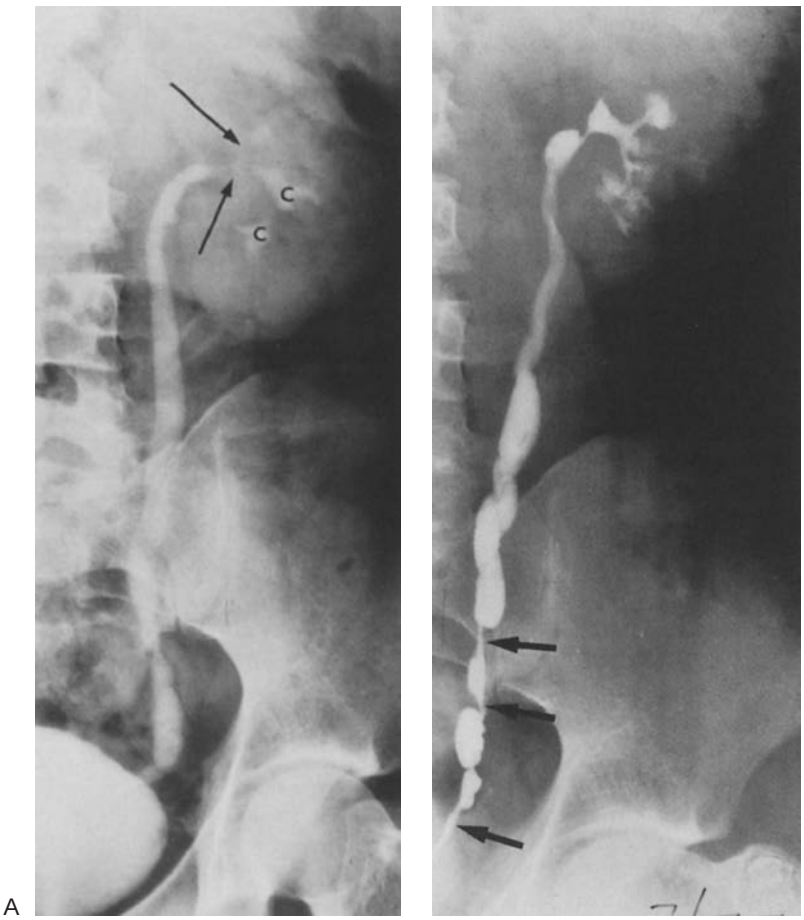

FIGURE 38-10. A: Intravenous urogram shows tuberculous narrowing of the left renal pelvis and upper pole infundibulum (*arrows*) and papillary necrosis of the lower pole calyces. The left ureter is moderately dilated and irregular in contour. **B:** Left retrograde pyelogram 6 months later, documenting fibrotic narrowing of the distal ureter (*arrows*) during antituberculous therapy. Tuberculous ureteritis commonly heals by cicatrization and may lead to severe obstruction of the collecting system. *C,* calcyes.

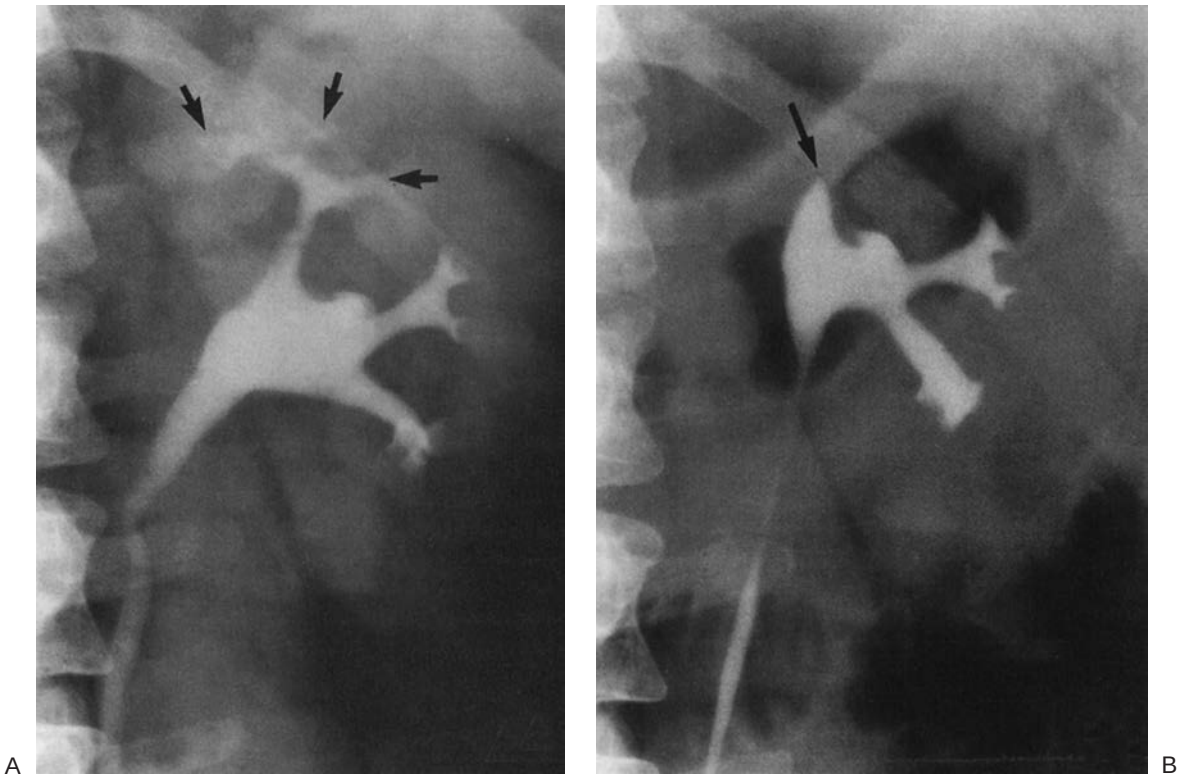

FIGURE 38-11. Intravenous urograms 14 years apart. **A:** Early change of renal tuberculosis with papillary necrosis of the upper pole calyces (*arrows*). **B:** Fourteen years later, there has been complete stenosis of the left upper pole infundibulum (*arrow*) as the tuberculous process heals by scarring.

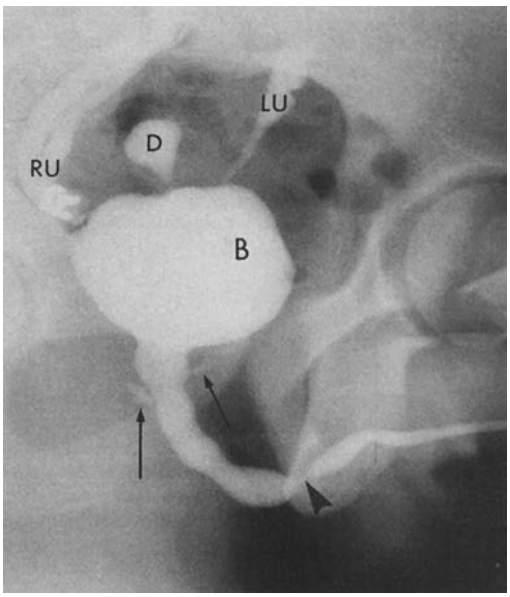

FIGURE 38-12. Voiding cystourethrogram in a young man with left renal tuberculosis, showing reflux into duplicated ureters of a normal right kidney and into an abnormal single left ureter, with a concomitant distal stenosis. The bladder is markedly reduced in volume and there is a diverticulum at the dome. There is reflux of contrast into the prostatic gland (*arrows*) secondary to tuberculous prostatitis. There is a stricture of the midbulbous urethra (*arrowhead*), which is an unusual site for tuberculous involvement. *B,* bladder; *D,* diverticulum; *LU,* left ureter; *RU,* duplicated ureters.

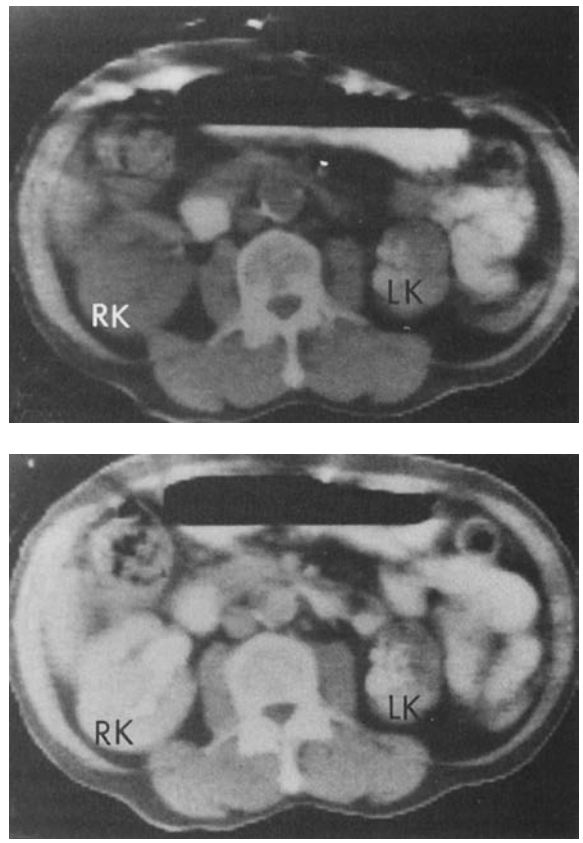

FIGURE 38-13. Computed tomography scans *(A)* before and *(B)* after the intravenous administration of contrast. There is a calcified nonfunctioning left kidney characteristic of a tuberculous autonephrectomy in a patient with old pulmonary tuberculosis. The right kidney is normal. *LK,* left kidney; *RK,* right kidney.

calculi may be present as well. Ureterovesical junction involvement may produce a stricture responsible for ureteral obstruction or a patulous, rigidly dilated orifice associated with vesicoureteral reflux.

Bladder involvement similarly begins with focal mucosal irregularity and progresses to produce a small, contracted trabeculated bladder with intramural thickening (Figs. 38-9 and 38-12). Occasionally, calcification of the bladder wall may occur.

The excretory urogram has long been the radiologic standard for evaluating patients with renal tuberculosis because of its capacity to record focal calyceal abnormalities, including cavitation, scarring, and obstruction, and to survey the collecting system in equal detail simultaneously. Sonography cannot record erosive calyceal abnormalities or distinguish advanced caseous disease from focal pyonephrosis. Sonography may, however, play a role in monitoring the possible development of hydronephrosis (166). Computerized tomography studies, particularly when intravenous contrast reagents are administered, have become the procedure of choice for diagnosing and assessing renal tuberculosis (Fig. 38-13). Thus, in early or focal disease, obstruction of a single major calyx or a group of minor calyces may be observed; tuberculous involvement of the renal pelvis may be visualized as either dilatation owing to ureteropelvic junction obstruction or diffuse pelvic contraction. With advanced disease, small, atrophic kidneys, often with one or more low-density areas, are observed (163–166). Calcifications of the genitourinary system and extrarenal intra-abdominal disease can likewise be observed at the same time (169,170). Sonographic and CT studies are particularly useful in patients with advanced disease when there is nonvisualization of the affected kidney by excretory urography. Angiography has been used in the occasional patient when isolated focal disease caused by obstruction or cavitation has mimicked a primary renal mass. The obliterative arteritis that accompanies progressive caseation is responsible for the avascular appearance of the granulomatous mass with pruning and obliteration of the interlobar arteries (167). Radionuclide imaging may demonstrate an inflammatory lesion but is entirely nonspecific and does not replace urine culture or intravenous pyelography (or CT) (171).

Clinical Management

The advent of effective chemotherapy has revolutionized the clinical management of urinary tract tuberculosis, although the recent increase in drug-resistant infection, amplified by the AIDS epidemic, threatens this success. Whereas in the prechemotherapeutic era extirpative surgery was the only hope of controlling infection, today medical cure is the rule. There is a continuing need for surgical intervention, but now it is for the correction of anatomic abnormalities caused by scarring rather than for the removal of infected tissues. The two goals, then, in the management of urinary tract tuberculosis are the conservation of tissue and function (both with medical treatment and surgical relief of obstruction resulting from tuberculous scarring) and antimycobacterial cure.

CHEMOTHERAPY OF URINARY TRACT TUBERCULOSIS

The chemotherapeutic approach to tuberculosis is based on the following general principles (15,172–176).

1. *Mycobacterium tuberculosis* may persist in a viable form while multiplying slowly or even intermittently. It is believed that there are three populations of organisms that must be considered when treating patients with active tuberculous infection (177). The largest number, and fortunately the most easily treated, are those that are extracellular, as within a cavity, where the pH is either neutral or alkaline. Because this group of organisms is actively multiplying, this is the population most easily treated with two or more bactericidal drugs. Also, because it is quantitatively the largest population, drug resistance is most apt to emerge within this population if an inappropriate therapeutic program is employed. A much smaller population of slowly or intermittently multiplying organisms is found at an acid pH within macrophages. Finally, there are a variable number of organisms exhibiting slow or intermittent multiplication at a neutral pH within closed caseous lesions.

   Curative therapy requires eradication of all three populations of organisms. Because of pH constraints, differing abilities to penetrate at different sites, and inherent effects on the tubercle bacillus, each of the available antituberculous drugs is more or less effective for these different populations of *M. tuberculosis*. Rifampin and rifabutin are the only drugs that are bactericidal for all three populations of *M. tuberculosis*. Isoniazid (INH) is bactericidal both for the actively growing organisms in cavities and for those slowly multiplying within macrophages. Streptomycin and the other injectable aminoglycosides are bactericidal only for the actively replicating extracellular organisms. Pyrazinamide is bactericidal only for intracellular organisms. All other drugs currently licensed for the treatment of tuberculosis are bacteriostatic. The fluoroquinolones—ciprofloxacin, ofloxacin, and levofloxacin—are bactericidal for *M. tuberculosis in vitro* (minimum bactericidal concentration of 2 μg/mL, in the setting of peak blood levels >4 μg/mL) and achieve excellent concentrations intracellularly. Not surprisingly, although they are not yet formally licensed for the treatment of tuberculosis, initial studies of these drugs appear promising when one or the other is administered together with at least one first-line bactericidal agent (INH, rifampin, or pyrazinamide) to which the particular isolate is susceptible (173,178–182). *The primary role of bacteriostatic antituberculous drugs is to inhibit the development of mutants resistant to simultaneously administered bactericidal agents.*
2. The spontaneous development of drug-resistant mutants of *M. tuberculosis* occurs at a rate of approximately 1×10^{-6} (183). The probability that a single organism would be resistant to two drugs simultaneously is thought to be equal to the product of the probabilities of resistance to each drug alone ($1 \times 10^{-6} \times 1 \times 10^{-6} = 1 \times 10^{-12}$). Therefore, a major determinant of the number of drugs necessary to treat tuberculous infection is the number of organisms harbored by the individual. In the case of urinary tract tuberculosis without active infection at other sites, an organism burden of approximately 1×10^{7} is likely. Therefore, a minimum of two drugs to which the patient's particular isolate is susceptible is necessary to treat these infections. Use of only one effective drug, given the rate of mutation to drug resistance and the number of organisms present, would lead to not only clinical failure but also the selection of resistant organisms.
3. Different patient groups have different rates of primary drug resistance. In the United States as a whole, a survey of *M. tuberculosis* isolates from 13,511 patients newly diagnosed (and previously untreated) in the time period 1994 to 1997 revealed that 8.2% were primarily resistant to one of the major drugs (isoniazid, rifampin, streptomycin, ethambutol), and an additional 4.1% were resistant to more than one drug. Of an additional 833 isolates taken from previously treated individuals in the United States, 12.5% were resistant to one drug (8.1% resistant to isoniazid), 11.2% were resistant to more than one drug, and 2.0% were resistant to all four drugs (184). Hispanics, Asians, blacks, and new immigrants from Asia and Latin America have rates of primary resistance two to four times higher than those cited for the rest of the country. As previously noted, these are the patient groups in whom the coexistence of HIV infection and tuberculosis is most likely to occur, thus greatly complicating the therapy of such individuals. Other patient groups with a particularly high rate of drug resistance are those who have undergone previous therapeutic courses that failed and those who remain persistently culture positive on a therapeutic regimen consisting of two or more first-line drugs (12,15,23,173,184) (Table 38-1).
4. Because of the increase in incidence of drug-resistant tuberculosis in the United States in recent years, it is now recommended that drug susceptibility testing be carried out on isolates from all patients, rather than just on isolates from the high-risk patient groups. Because the most common cause of the development of *de novo* drug resistance is failure of compliance by the patient, increasing attention is now directed to the use of directly observed therapy in which public health workers directly administer the therapy to the patient. Particularly in urban areas, this strategy of enforced antituberculous therapy compliance has proved to be quite successful in blocking the spread of tuberculosis, particularly drug-resistant tuberculosis (15,173,184–186).

The standard of care for urinary tract tuberculosis due to drug-sensitive organisms in a compliant patient now consists of a 6-month regimen of INH, rifampin, and pyrazinamide for 2 months, followed by INH and rifampin for 4 months (15,173,184–189). Unless the possibility of drug resistance is vanishingly small (a <4% incidence of drug resistance in the patient's community, no previous treatment with antituberculous drugs, no known exposure to a drug resistant case, etc.), a fourth drug—ethambutol, streptomycin, or one of the fluoroquinolones—should be included in the initial regimen until the results of sensitivity testing are available. Patients not tolerating pyrazinamide should be treated for 9 months with INH and rifampin (plus one or more of the other drugs listed until susceptibility testing results are known). More prolonged courses of therapy are indicated for any patient slow to respond to one of the standard regimens, those with miliary or central nervous system disease, those with significant immunosuppression (e.g., AIDS, organ transplant recipients, etc.), and children with multiple sites of involvement (including the skeleton) (173,190).

HIV-associated tuberculosis is managed similarly to that observed in the HIV-negative population (although our preference is to prolong therapy for an additional 3 to 6 months in those with AIDS), with the following modifications to increase the probability of success: directly observed therapy for all patients with HIV-related tuberculosis; the substitution of rifabutin for rifampin in individuals receiving anti-HIV protease inhibitors because of the risk of drug interactions that affect the efficacy of treatment of both the HIV and the tuberculosis; monitoring the responses to antituberculosis treatment to individualize the appropriate duration of antituberculosis therapy (191,192).

The management of drug-resistant disease is determined by the results of *in vitro* susceptibility testing. At a minimum, at least two drugs, preferably including at least one of the front-line bactericidal agents (INH, rifampin, and pyrazinamide) to which the isolate is susceptible, are prescribed. If the isolate is susceptible to two of these agents, and the patient tolerates them, then a 6- to 9-month regimen is usually sufficient. If the isolate is susceptible to only one of these, and the additional agent is ethambutol or a fluoroquinolone, then therapy should

TABLE 38-1

TREATMENT OF MYCOBACTERIAL DISEASE IN ADULTS AND CHILDREN

Commonly used agents	Daily dose	Twice-weekly dose	Most common side effects	Remarks
Isoniazid	5 to 10 mg/kg up to 300 mg PO or IM	15 mg/kg PO or IM	Peripheral neuritis, hepatitis, hypersensitivity	Bactericidal to both extracellular and intracellular organisms. Pyridoxine as prophylaxis for neuritis.
Rifampin	10 mg/kg up 600 mg PO	10 mg/kg up to 600 mg PO	Hepatitis, febrile reaction, purpura (rare)	Bactericidal to all populations of organisms.
Streptomycin	15 to 20 mg/kg up to 1 g IM	25 to 30 mg/kg IM	Eighth nerve damage, nephrotoxicity	Bactericidal to extracellular organisms.
Pyrazinamide	15 to 30 mg/kg up to 2 g PO	50 to 70 mg/kg PO	Hepatotoxicity, hyperuricemia	Bactericidal to intracellular organisms. Combination with an aminoglycoside is bactericidal.
Ethambutol	15 to 25 mg/kg PO	50 mg/kg PO	Optic neuritis (reversible with discontinuation of drug; very rare at 15 mg/kg), skin rash	Bacteriostatic to both intracellular and extracellular organisms, primarily used to inhibit development of resistant mutants. Use with caution with renal disease or when eye testing is not feasible.
Capreomycin	15 to 30 mg/kg up to 1 g IM		Eighth nerve damage, nephrotoxicity	Bactericidal to extracellular organisms in cavities. Use with caution in older patients. Rarely used with renal disease.
Kanamycin	15 to 30 mg/kg up to 1 g IM		Eighth nerve damage, nephrotoxicity	Bactericidal to extracellular organisms. Use with caution in older patients. Rarely used with renal disease.
Ethionamide	15 to 30 mg/kg up to 1 g PO		GI disturbance, hepatotoxicity, hypersensitivity	Bacteriostatic to both intracellular and extracellular organisms. Divided dose may help GI side effects; has a metallic taste.
Para-aminosalicyclic acid	150 mg/kg up to 12 g PO		GI disturbance, hypersensitivity, sodium load	Bacteriostatic to extracellular organisms only. GI side effects are very frequent, making cooperation difficult.
Cycloserine	10 to 20 mg/kg up to 1 g PO		Psychosis, personality changes, convulsions, rash	Bacteriostatic to both intracellular and extracellular organisms. Alcohol may aggravate psychotic problems. Side effects may be blocked by pyridoxine, ataractic agents, or anticonvulsant drugs.
Ciprofloxacin	500 to 1,000 mg		GI upset, dizziness, hypersensitivity, drug interactions, headaches, restlessness	Bactericidal. Not approved by FDA for tuberculosis treatment. Should not be used in children.
Ofloxacin	400 to 500 mg		GI upset, dizziness, hypersensitivity, drug interactions, headaches, restlessness	Bactericidal. Not approved by FDA for tuberculosis treatment. Should not be used in children.

GI, gastrointestinal; *FDA*, U.S. Food and Drug Administration.
(Adapted from: American Thoracic Society. Treatment of tuberculosis and other mycobacterial diseases [Official Statement]. *Am Rev Respir Dis* 1983;129:790.)

be prolonged for at least 12 months. In patients with infection caused by multiresistant organisms, a multidrug regimen of the second-line agents (Table 38-1) is administered for 24 months (172).

Two types of genitourinary tract tuberculosis are particularly difficult to treat—"the autonephrectomized" kidney destroyed by tuberculosis and now presenting as a nonfunctioning, avascular, calcified, caseous mass, and prostatic tuberculosis. In both these circumstances, delivery of antituberculous therapy to the site of infection is fraught with difficulty. Indeed, as observed in the following, some authorities believe that all end-stage tuberculous kidneys should be removed surgically, and Dutt and Stead (190) noted that tuberculous abscesses must be surgically drained in any patient undergoing short-course therapy. As far as prostatic tuberculosis is concerned, we have observed patients who had been rendered culture negative with 2 years of therapy for urinary tract tuberculosis who, a few years later at the time of an incidental prostatectomy, were shown to harbor living *M. tuberculosis* at this sequestered site.

SURGICAL MANAGEMENT OF URINARY TRACT TUBERCULOSIS

In the prechemotherapy era, surgical ablation of infected foci was the only therapy available for renal tuberculosis. Without surgery, the 5-year survival rate for patients with renal tuberculosis was 15% to 42%, but with surgery, 10-year survival rates approached 50% (193). With modern chemotherapy, no one should die of urinary tract tuberculosis; however, there still remains a role for surgery today for this disease. The primary form of surgical intervention is in the relief of strictures, particularly those of the ureters, which can result from the scarring process. Thus, ureteral dilatations, ureteral reimplantations, and in some cases, relief of intrarenal obstruction to urine flow are important aspects of the modern function-conserving approach to urinary tract tuberculosis (131,132,194–199). Because such strictures can develop on therapy, the possible need for surgical intervention continues long after cultures for *M. tuberculosis* turn negative, and close follow-up with frequent radiologic assessment is mandatory (see the previous discussion). Less commonly, patients whose bladders have been badly scarred by the tuberculosis process have such poor bladder function that bladder augmentation or even urinary diversion may be necessary to deal with unbearable urinary frequency, inadequate emptying, or both (197,199–201).

In the case of ureteral strictures, it has been suggested that the administration of steroids in conjunction with antituberculous therapy can prevent scarring and lessen the need for reparative surgery (202,203). A controlled trial demonstrating this benefit has not been done, and the use of steroids remains controversial (29). We do not use steroids in this setting. Newer techniques for balloon dilatation and stent placement to deal with internal strictures have proven to be very useful (204).

Perhaps the most controversial area of surgical management is whether or not nephrectomy is necessary for the nonfunctioning end-stage tuberculous kidney. Lattimer and his colleagues (115,128,205,206) pioneered the concept that a 2-year course of chemotherapy can sterilize these caseous, calcified masses and leave the patient with no further risk to his or her health. Other investigators have felt that such sequestered, caseous tissue should be removed during the course of chemotherapy in order to prevent late tuberculous reactivation, the development of hypertension, and other untoward sequelae (207–211). It is our opinion that with modern regimens that include INH and rifampin for periods longer than 18 months, routine removal of the tuberculous "cement" kidney is not necessary. We reserve such nephrectomies for patients with unilateral, end-stage tuberculous kidneys whose course is complicated by conventional bacterial sepsis, hemorrhage, intractable pain, newly developed severe hypertension, inability to sterilize the urine because of patient unreliability, drug resistance, or some other factor (29). For the great majority of patients, however, ablative surgery is not indicated.

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SECTION VII
ACUTE RENAL FAILURE

CHAPTER 39 ■ PATHOPHYSIOLOGY OF ISCHEMIC ACUTE RENAL INJURY

CHARLES L. EDELSTEIN AND ROBERT W. SCHRIER

Acute renal injury may lead to the clinical syndrome, which has been termed *acute tubular necrosis* (ATN). This term emerged from the early observation on renal biopsy that necrosis of some renal tubular epithelial cells may occur in humans with acute renal injury (1). Tubular epithelial casts (muddy brown casts) are excreted in the urine of these patients. It is now known, however, that the tubular necrosis is quite patchy and alone could not account for glomerular filtration rates (GFR) less than 10 mL/minute/1.73m^2, the functional hallmark of clinically significant ATN (1). Moreover, a percentage, ranging from 30% to 70%, of the urinary tubular epithelial cells has been shown to be viable by culture and exclusion of vital dyes (2,3). This observation is somewhat surprising since generally cells, which are separated from their extracellular matrix, undergo apoptosis (4–7). Emerging results, however, suggest that adhesion molecules (e.g., cadherins and integrins) may allow cell to cell or cell to matrix adhesion, which not only avoids apoptosis but may contribute to intratubular obstruction (8–11). Intraluminal tubular casts on renal biopsy are a hallmark of clinical ATN and earlier nephron dissection studies by Jean Oliver demonstrated a preferential location of these casts in the medullary collecting duct (12). This location is of particular relevance to the overall low GFR in ATN, since thousands of nephrons drain into a single medullary collecting duct.

In the past, a debate emerged about whether the pathophysiology of clinical ATN was primarily tubular or vascular. In fact, initial rat micropuncture studies were unable to consistently detect an elevation in tubular pressures in experimental acute renal failure (ARF) and thus suggested that the term *vasomotor nephropathy* replace the ATN term for the clinical syndrome (13–15). However, against a purely vascular pathogenesis was the observation that the intrarenal infusion of a vasodilator (e.g., dopamine), which restored renal blood flow to normal, failed to reverse the ARF failure clinical syndrome in either human or experimental animals (16,17). Experimental results have emerged that both vascular and tubular factors are involved in the pathogenesis of clinical ATN. Most recently, the role of endothelial injury and dysfunction (18) in promoting an inflammatory response in ARF (19) has received prominence. Thus, this chapter will discuss the potential tubular and vascular factors, as well as inflammatory processes, involved in the pathogenesis of ischemic ARF (Fig. 39-1). Toxic ARF will be discussed in another chapter. However, it must be emphasized that ATN in humans is frequently multifactorial. Both ischemic and toxic insults combine to cause clinical ATN. In the present chapter, ATN and ischemic ARF will be used interchangeably with the assumption that causes of prerenal and postrenal (i.e., urinary tract obstruction) azotemia have been excluded (see Chapter 25).

Regarding the definition of ARF, criteria based on the serum creatinine and urine output, have been recently proposed for classifying ARF (19a). These RIFLE criteria (Risk, Injury, Failure, Loss of function, and End stage kidney disease) may facilitate early diagnosis and interpretation of future clinical trials.

The understanding of the pathogenesis of ischemic ARF is of considerable importance for several reasons. First of all, this clinical syndrome is quite frequent, occurring in 5% to 10% of hospitalized patients and 30% to 40% of intensive care unit patients (20–22). The incidence is likely to increase in the future because of the use of newer nephrotoxic drugs and the performance of more complex procedures in older patients (23). Secondly, ischemic ARF has a very high mortality particularly when requiring dialysis to treat the resultant uremic syndrome. Overall mortality averages 40% to 50%; however, patients in an intensive care unit with ischemic ARF may have mortality in excess of 80%, particularly if they have multiorgan failure (24–29). It is widely quoted that the mortality of ARF has only improved slightly in the last 40 years (30). However, a study suggests that there has been improvement in ARF mortality between the late 1970s and the early 1990s (31). Thirdly, there is considerable evidence that a functional component of the renal failure exists. Specifically, histologic examination of the kidney from patients with clinical ATN exhibits normal glomeruli, occasional tubular necrosis, some intraluminal casts, and modest interstitial edema (1). There is virtually no evidence for irreversible tissue damage and the morphological changes alone fail to support the presence of a GFR less than 10 mL/minute/1.73m^2. Lastly, the vast majority of patients who recover from ischemic ARF demonstrate grossly normal renal function (27). Thus, on recovery, patients with this clinical syndrome, which have a dramatically high mortality, are not left with residual dead tissue, which occurs after a myocardial infarction or stroke.

EXPERIMENTAL MODELS OF ACUTE RENAL FAILURE

Available models to study the pathophysiology of renal cell ischemia are listed in Table 39-1 (32). An understanding of these models will allow better interpretation of the multiple studies discussed in this chapter.

Proximal and distal tubular cells in culture have been widely used to study tubular injury. These cells change from their normal dependence on oxidative, mitochondrial metabolism to glycolysis under culture conditions (33). As a result, these cultured tubules become less susceptible to oxygen deprivation. Thus, exposure to drugs like antimycin-A, ionomycin, or a combination to induce "chemical" ATP depletion and subsequent necrosis or apoptosis is used. Cultured cells also undergo considerable structural change that includes simplification of both their apical and basolateral compartments (34). The presence of necrosis rather than apoptosis in these cells may be related to the level of ATP depletion. In cultured mouse proximal tubules subjected to ATP depletion below 15% of control values, the cells died of necrosis, whereas in ATP depletion from 25% to 70% of control values all the cells died of apoptosis

Pathogenesis of ischemic acute renal failure (ARF)

Tubular factors:
Back-leak of glomerular filtrate
Decreased proximal tubular sodium reabsorption
Increased tubuloglomerular feedback
Tubular cast formation and obstruction

Vascular factors:
Renal vasoconstriction

Inflammatory response:
Endothelial injury
Leukocyte adhesion/infiltration
Inflammatory mediators

FIGURE 39-1. Vascular and tubular factors and inflammatory processes are involved in the pathophysiology of ischemic acute renal failure.

(35). While cultured tubules are the least complex model and allow understanding of mechanisms involved, the therapeutic implications for *in vivo* ARF is limited.

Freshly isolated rat or rabbit proximal tubules in suspension are also widely used to study proximal tubular injury (36–44). The method of isolation of tubules is by collagenase digestion and Percoll centrifugation. The isolation of proximal tubules from mice has recently been described (41,45). Hypoxia is achieved by gassing the suspension with 95%N_2/5%CO_2 for up to 15 minutes thereby reducing the pO_2 to approximately 30 mm Hg. Percent lactic dehydrogenase (LDH) release into the suspension medium is measured as an index of lethal membrane injury (46,47). The tubules are preincubated with cytoprotective agents and enzyme inhibitors before induction of hypoxia and the effect of these agents on cell membrane injury can be determined. The presence of necrosis rather than apoptosis during short periods of hypoxia (15 to 30 minutes) in this model was demonstrated using DNA-specific dyes, such as Hoechst 33342 and propidium iodide (48). Another study has also demonstrated that during hypoxia there is endonuclease activation without morphologic features of apoptosis in the same model of rat proximal tubules (49). Freshly isolated tubules are valuable for both structural and metabolic investigations as they retain the biochemical properties of the *in vivo* state, a high degree of structural integrity, and are highly polarized and fully differentiated (32). However, the tubules are highly sensitive to ATP depletion and severe hypoxia or anoxia results in necrosis of more than 50% of the cells after 30 minutes.

TABLE 39-1

AVAILABLE MODELS TO STUDY THE PATHOPHYSIOLOGY OF RENAL CELL ISCHEMIA (INCREASING ORDER OF COMPLEXITY)

Model	Origin
Cultured tubular cells	Primary culture of human, rat and mouse; Madin-Darby canine kidney (MDCK) cells (distal); porcine renal epithelial (LLC-PK1) cells (proximal); opossum kidney (OK) cells; human kidney (HK) cells.
Freshly isolated proximal tubules in suspension	Rabbits, rats, mice
Isolated perfused kidney	Rat
Whole animals	Rabbits, rats, mice, dogs (not much used anymore)
Human patients	Renal biopsy studies. Urine and serum biomarkers of ARF.

(Adapted from: Lieberthal W, Nigam SK. Acute renal failure: II. Experimental models of acute renal failure; imperfect but indispensable. *Am J Physiol* 2000;278:F1.)

In whole animal studies, usually rats, rabbits, or mice, a clamp model of ischemic ARF is used (50–52). Ischemic ARF is generally induced by (i) clamping of both the right and left renal pedicles or renal arteries or (ii) unilateral renal pedicle or artery clamp preceded by contralateral nephrectomy. The renal vessels are clamped for varying periods of time, generally from 45 to 60 min, followed by varying periods of reperfusion. This results in a reversible model of ARF in which the BUN and serum creatinine reach a peak at 24 to 48 hours reperfusion and then gradually normalize over the next 7 days (50–53). However, renal vessel clamping in rats results in extensive necrosis of proximal tubules. This necrosis is much more extensive than is seen in humans with ischemic ARF. Nevertheless, while animal models of ischemic ARF are complex with many experimental limitations, they provide important leads for future therapeutic clinical interventions.

In the isolated perfused kidney model, the kidney is removed from the animal. The perfusate usually consists of Krebs-Henseleit buffer with albumin. Urine is collected by cannulation of the ureter. This model allows study of factors independent of changes in systemic hemodynamics and neural activity. The further advantages of this model are that it allows the study of specific circulatory factors or pharmacological agents that are added to the perfusate. These agents are thus delivered directly to the kidney. The disadvantages of the model are (i) the absence of red blood cells in the perfusate impairs oxygen delivery to the medullary thick ascending limb (MTAL) and (ii) perfusate flow greatly exceeds normal *in vivo* values. The isolated perfused kidney is regarded as a model of selective hypoxia to the medullary thick ascending limb (54).

Study of human patients with ARF, while having important experimental limitations, of course, has the most direct therapeutic value. Analysis of urine cytology represents a noninvasive method for potentially defining the cause of ARF (1). Myers and coworkers have examined patients with ischemic ARF postrenal transplantation by obtaining biopsies of these allografts at the time of transplantation (55–57). Biomarkers of kidney injury would greatly facilitate the early detection and precise diagnosis of ATN.

BIOMARKERS OF ARF

Acute renal failure is usually diagnosed by recording increases in serum creatinine and decreased urine output over several days. However, serum creatinine is not a good marker of renal function in ARF because its concentration can be affected by factors not related to renal function such as volume of distribution, muscle mass, and creatinine secretion (27). When the kidney is injured and the true GFR suddenly drops, there is a slow increase in serum creatinine over days. A new steady state, that may take up to 7 days, is reached when creatinine generation equals creatinine excretion. In contrast to serum troponin in myocardial infarction, an increase in serum creatinine is not directly related to tubular injury in ARF. Recent studies have examined urine and serum biomarkers of kidney injury that would facilitate the diagnosis of ATN.

Although the initial studies on some molecules like tubular enzymes (e.g., L-alanine aminopeptidase, N-acetyl-[beta]-D- glucosaminadase, and adenosine deaminase binding

protein) were promising, the larger and more detailed studies have shown inadequate sensitivity or specificity to advocate clinical use (58–60). Recently described molecules such as the cytokine IL-18 (61), kidney injury molecule-1 (KIM-1) (62), cystein-rich protein 61 (Cry61) (63), neutrophil gelatinase-associated lipocalin (NGAL) (64), and sodium/hydrogen exchanger isoform 3 (NHE3) (65) have demonstrated compelling results as markers of ARF at the preclinical level. Studies are being initiated to explore these molecules in human ARF.

Urinary IL-18 is an excellent test for diagnosis of ATN and delayed graft function in humans (66). The mature form of IL-18, as detected by ELISA assay, is massively increased in the urine of patients with ischemic ATN compared to normal controls and other acute and chronic renal diseases. Urinary IL-18 increases in the first 24 hours after kidney transplantation in patients with delayed graft function (DGF). Lower levels of urinary IL-18 in the first 24 hours after kidney transplantation are associated with a better functioning kidney. On receiver operating characteristic (ROC) curve analysis, the discriminatory power of the urine IL-18 test to detect ATN is 97%. The accuracy of this test compares favorably with the recently described markers of ARF, actin, IL-6, and IL-8 (67).

Because of the crucial importance of earlier therapies and management of ARF, markers are being explored for early diagnosis of ARF. NGAL were investigated as an early biomarker for ARF following cardiopulmonary bypass in 45 patients (68). Urine and serum was collected at baseline and at frequent intervals for 5 days following cardiopulmonary bypass. All patients who developed ARF (defined as a 50% increase in serum creatinine) displayed a significant increase in serum and urine NGAL very early after cardiopulmonary bypass compared to patients without ARF. These results show that NGAL may be a sensitive, early urinary, and serum biomarker for ARF.

In a nested case-control study within the Adult Respiratory Distress Syndrome (ARDS) network trial, urinary IL-18 was investigated as an early marker of ARF (69). Median urine IL-18 levels were significantly higher in ARF cases (defined as a 50% increase in serum creatinine) as compared to controls. On multivariable analysis, urine IL-18 values predicted development of ARF 24 and 48 hours later after adjusting for demographics, sepsis, APACHE III score, serum creatinine, and urine output. After controlling for other parameters, a rise in urine IL-18 by 25 pg/mL is associated with increased odds of ARF by 19% in the next 24 hours. Urine IL-18 performs well as a diagnostic test with an area under the receiver operator characteristic curve of 73%. The conclusion of this study is that urinary IL-18 levels can be used for the early diagnosis of ARF.

A recent study demonstrated that serum cystatin C appears to increase 24 to 48 hours before creatinine in patients with ARF (70). However, cystatin C is a marker of clearance and not a marker of renal tubular injury.

An early biomarker of ischemic ATN may lead to earlier treatments (e.g., appropriate fluid balance, avoidance of nephrotoxic drugs, appropriate drug dosage, earlier nephrology consultation, and earlier initiation of dialysis) (71). Also, an early marker with a high sensitivity and specificity will help in designing interventional trials for the treatment of ARF by allowing for the timely initiation of the investigational drug. Future clinical trials and prospective studies in ARF should incorporate biomarker measurements to hasten the clinical development of these tests.

BACKLEAK OF GLOMERULAR FILTRATE POSTRENAL ISCHEMIA

It has been proposed that GFR in ischemic ARF is really not as low as measured since glomerular filtrate is leaking across damaged epithelium or tubular basement membranes. In some toxic experimental models of ischemic ARF, diffuse tubular necrosis and basement membrane damage have been associated with evidence for backleak of glomerular filtrate. The term *backleak* of glomerular filtrate refers to the unregulated passage of salt and water from the tubular lumen into the interstitium and later back into the renal venous capillaries and renal veins (72). However, the level of epithelial and basement membrane damage with these experimental toxic models (e.g., cisplatin and mercuric chloride) is virtually never observed in human ATN. Myers et al. have performed studies using solute sieving curves in search of tubular backleak of glomerular filtrate (73). They found that dextran sieving curves could sometimes exceed inulin sieving curves, thus providing evidence in support of backleak of solutes (i.e., inulin) which are normally unable to cross intact tubular epithelial basement membranes. Even when accepting the validity of this method for documenting tubular backleak of filtrate, however, the calculated amount would only account for a decrease in renal function of 8% to 10%. Thus, while tubular backleak of glomerular filtrate might occasionally occur in patients with severe ischemic ARF, it is unlikely to be a dominant pathogenic factor.

The tight junction of polarized tubular epithelial cells is the most apical component of the junctional complex and serves as an important permeability barrier (74). Tight junctions also control cell polarity (75). However, the tight junction in the proximal tubule is relatively "leaky" with as much as one-third of proximal sodium reabsorption occurring via the paracellular route. The tight junctional complex is a dynamic and regulated structure. Some of its protein components have been identified and include the transmembrane protein occludin. Nontransmembrane proteins on the cytosolic leaflet include zona occludens (ZO)-1, ZO-2, cingulin, 7H6, and several unidentified phosphoproteins. Interactions of some of these proteins with the actin cytoskeleton are major determinants of tight junction structure and may also play a role in the regulation of tight junction assembly (74). The integrity of the tight junction is disrupted during ischemic injury and must be reestablished for recovery (76). There is *in vitro* experimental evidence in cell culture studies for an impaired tight junction between tubular epithelial cells undergoing chemical anoxia (77). Ruthenium red, which normally is impermeable to tight junctions, has been shown to enter the zona occludans after chemical hypoxia and renal ischemia (77,78). Energy depletion abolishes the gate function of the tight junction, as determined by the dramatic decrease in transepithelial resistance, but it leaves the fence function intact, as determined by the maintenance of lipid polarity (79). In an ATP depletion-repletion model in Madin-Darby canine kidney cells, tight junction proteins, such as ZO-1, reversibly form large complexes and associate with cytoskeletal proteins (80). A model has been proposed in which a key, potentially regulated, step in the generation of the ischemic epithelial cell phenotype is the interaction between tight junction proteins and fodrin and/or other cytoskeletal proteins (80). Intracellullar calcium plays a role in tight junction reassembly after ATP depletion (76). In this study, the role of intracellular calcium in tight junction reassembly after ATP depletion-repletion was studied using the cell-permeable calcium chelator 1, 2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid-AM (BAPTA-AM). Lowering intracellular calcium during ATP depletion was associated with significant inhibition of the reestablishment of the permeability barrier following ATP repletion as measured by transepithelial electrical resistance and mannitol flux, marked alterations in the subcellular localization of occludin by immunofluorescent analysis and decreased solubility of ZO-1 and other tight junction proteins by Triton X-100 extraction assay. This suggested that lowering intracellular calcium potentiates the interaction of tight junction proteins with the cytoskeleton.

Recent studies have also shown the importance of small GTPases (Rho, Rac, and dc-42) in the integrity of the zona occludans (81). ATP depletion with chemical hypoxia has been demonstrated to inactivate these GTPases and thereby contribute to increased paracellular backleak of filtrate (75,82). Expression of constitutively active RhoA GTPase in ATP-depleted MDCK cells prevents tight junction disassembly (75). Cyanide-induced chemical hypoxia increases the kinase activity of c-Src and causes its translocation to cell-cell junctions where it binds to and phosphorylates beta-catenin and p120, suggesting that this may contribute to the loss of epithelial barrier function (83). It is likey that the effects on tight junction integrity seen in ATP depletion are due, at least in part, to the inhibition of Na-K-ATPase (84).The relevance of these cell culture observations to clinical ischemic ARF in patients remains to be proven.

DECREASED TUBULAR SODIUM REABSORPTION POSTRENAL ISCHEMIA

Proximal tubular injury, whether it be sublethal reversible dysfunction, necrosis, or apoptosis has been extensively studied. Mechanisms of proximal tubular injury that will be discussed in this section are outlined in Table 39-2. The study of proximal tubular injury is of special relevance in order to explain the decreased tubular sodium reabsorption that occurs in postrenal ischemia.

In the normal kidney, Na^+ is vectorially transported from the proximal tubule lumen across the apical membrane microvilli into the tubular epithelial cells and then across the basolateral membrane into the interstitium and the peritubular circulation (85). Na^+-influx into the polarized proximal tubular epithelial cells across the apical membrane is passive down the Na^+-gradient via the H^+/Na^+ exchanger and various Na^+-cotransporters. The Na^+- gradient is maintained by an active transport via the Na^+/K^+-ATPase at the basolateral membrane of the proximal tubular cells (Fig. 39-2).

The earliest signs of clinical ATN are urinary muddy brown casts and an increased fractional excretion of sodium (FE_{Na}) (86,87). The proximal tubule is the most frequent morphological site of injury in ischemic ARF in both humans and animals (88). The S_3 segment of the proximal tubule is particularly prone to ischemic injury, perhaps because of its location in the outer medulla, which is relatively hypoxic compared to the renal cortex (88). The proximal tubule nephron site is also associated with impaired vectorial sodium transport. The earliest morphological changes with ischemic injury are invagination and sloughing of the brush border membrane into the lumen, an abnormality compatible with impairment of apical sodium antiporters and cotransporters responsible for sodium entry into proximal tubular epithelium (89–91). The tubules lose their polarity (87). *In vitro* studies have shown that ATP depletion leads to dephosphorylation and inactivation of the actin binding protein, ezrin, and activation of actin depolarizing protein in the proximal tubule membrane (92,93). This leads to disruption of the microvillar actin and loss of the brush border membrane (94). Loss of polarity of proximal tubule cells during chemical anoxia and ischemia has also been shown with the translocation of the Na-K-ATPase to the apical membrane (95–97) (Fig. 39-3). The translocated Na-K-ATPase remains functional (98). In MDCK cells exposed to ATP depletion, there is loss of polarity of Na-K-ATPase and dissociation of the membrane-cytoskeleton complex at the spectrin-ankyrin interface (99). Thus, sodium transport into the proximal lumen and decreased proximal tubular sodium reabsorption have been proposed in response to hypoxia and ischemia.

TABLE 39-2

MECHANISMS OF HYPOXIC/ISCHEMIC PROXIMAL TUBULAR INJURY

SUBLETHAL REVERSIBLE INJURY
Cytoskeletal disruption and loss of polarity
Loss of tight junction function
Loss of cell-matrix adhesion
Abnormal gene expression
NECROSIS
Severe ATP depletion (15% of normal)
Calcium influx
Calcium-dependent phospholipase A_2($cPLA_2$)
Calcium-dependent cysteine proteases e.g., calpain
Calcium-independent PLA_2
Caspase-1
Interleukin-18 (IL-18)
Metalloproteases
Oxygen radicals
Lipid peroxidation
Deficiency of glycine
Nitric oxide (generated by iNOS)
Endonuclease activation
Deficient heat stress response
Potassium efflux
APOPTOSIS
Mild ATP depletion (25% to 50% of normal)
Caspase-3
Caspase-1
Caspase-6
Endonuclease activation
Serine proteases
Insulin-like growth factor I receptor deficiency
Deficient heat stress response
Erythropoietin
Bcl-2 proteins
Mitochondrial injury

Studies in cadaveric transplanted kidneys with prompt and delayed graft function have been compared relative to the cellular location of the actin binding proteins, ankyrin and spectrin, and Na-K-ATPase using selective antibodies. In those kidneys with delayed graft function approximately 50% of the ankyrin, spectrin, and Na-K-ATPase was translocated from the basolateral membrane to the cytoplasm, whereas those kidneys with prompt graft function had only minimal translocation of these

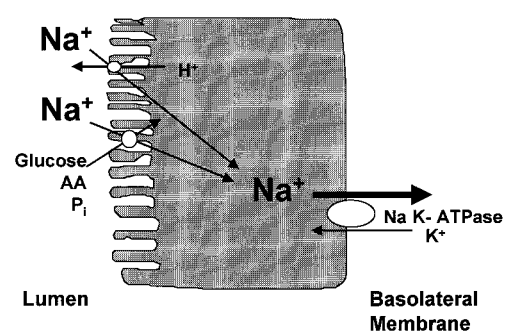

FIGURE 39-2. Normal reabsorption of sodium in the proximal tubule. The NaK-ATPase pump is located on the basolateral surface of the proximal tubule.

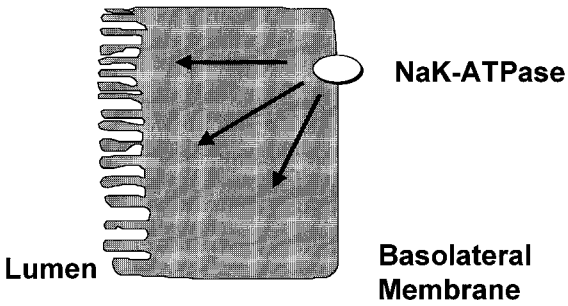

FIGURE 39-3. Tranlocation of the Na-K-ATPase pump away from the basolateral proximal tubule membrane during hypoxia/ischemia. Loss of polarity of proximal tubule cells during chemical anoxia and ischemia results in a translocation of the Na-K-ATPase. During ATP depletion in cultured cells the Na-K-ATPase translocates to the apical membrane. In human kidney allografts with delayed function, the Na-K-ATPase translocates to the cytoplasm. The translocated Na-K-ATPase remains functional.

proteins from the basolateral membrane (55) (Fig. 39-4). These observations, therefore, have contributed to our understanding of reversible and sublethal tubular dysfunction in ischemic kidneys *in vivo*.

Since proximal tubular cells in culture convert from primarily oxidative to glycolytic metabolism and alter their phenotype (33,34), confirmation of experimental results in other systems is advisable. The use of freshly isolated proximal tubules to study the response to hypoxia has also been enlightening. These tubules in general maintain their phenotype and oxidative metabolism but are more sensitive to hypoxia, as assessed by LDH release, than *in vivo* tubules (100–103). Hypoxia for 15 to 30 minutes causes a reproducible release of LDH and the cells die by necrosis (48,49). The central role of intracellular calcium and various protective maneuvers against hypoxic injury have been demonstrated in these isolated proximal tubules. However, before discussing the role of intracellular calcium in proximal tubular injury, we shall briefly consider adenine nucleotides. It is unquestioned that the first effect of ischemia, hypoxia, or mitochondrial inhibition in most *in vitro* and *in vivo* models is to compromise adenine nucleotide metabolism.

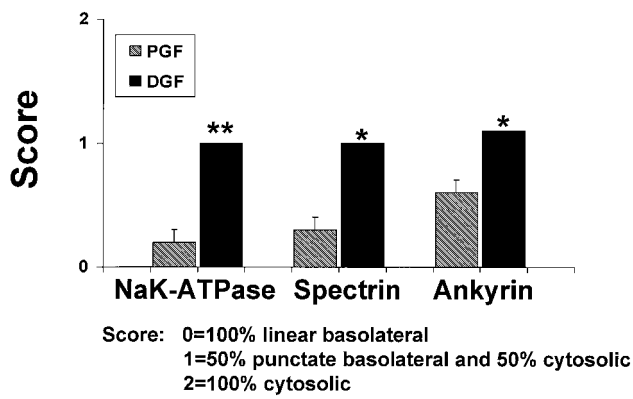

FIGURE 39-4. The cellular location on immunohistochemistry of the actin binding proteins, ankyrin and spectrin, and Na-K-ATPase in cadaveric transplanted kidneys with prompt graft function (PGF) and delayed graft function (DGF) was compared. In those kidneys with delayed graft function approximately 50% of the ankyrin, spectrin, and Na-K-ATPase was translocated from the basolateral membrane to the cytoplasm. Whereas those kidneys with prompt graft function had only minimal translocation of these proteins from the basolateral membrane. $^{**}p < 0.01$ vs PGF, $^{**}p < 0.05$ vs PGF. (Adapted from: Alejandro VS, Nelson WJ, Huie P, et al. Postischemic injury, delayed function and NaK-ATPase distribution in the transplanted kidney. *Kidney Int* 1995;48:1308.)

Decreased production of ATP precedes the increase in intracellular calcium.

Adenine Nucleotides

Removal of oxygen from renal cells or whole kidneys results in prompt decreases in the cellular ATP pool. Initially, adenosine diphosphate (ADP) and adenosine monophosphate (AMP) concentrations increase (52), and further catabolism of AMP to adenosine and then to hypoxanthine and, in some species, to xanthine occurs as the ischemic period is prolonged (104,105). Provision of exogenous ATP-$MgCl_2$ to ischemic rat kidneys protects against ischemic injury (106). Mechanisms whereby loss of ATP results in cellular injury include the loss of purine nucleosides themselves, in some species the generation of oxygen free radicals during reperfusion, and the loss of many metabolic functions (e.g., phosphorylation) of important enzymes and ion channels and the functions of ion transporters that are dependent on adequate ATP levels.

Ischemic preconditioning protects the heart and in some studies, the kidneys, from subsequent ischemia/reperfusion injury. Ischemic preconditioning appears to be mediated via activation of adenosine receptors, specifically A_1 adenosine receptors. In support of this are studies that the exogenous administration of adenosine, or A_1 adenosine agonists mimic ischemic preconditioning in cardiac muscle (107). It was recently demonstrated that rat kidneys can be preconditioned to attenuate ischemic-reperfusion injury. In this study, adenosine infusion before the ischemic insult protects renal function via A_1 adenosine receptor activation and adenosine A_1 antagonism blocks adenosine-induced protection. In addition, adenosine A_3 receptor activation before ischemia worsens renal ischemia-reperfusion injury and adenosine A_3 receptor antagonism protects renal function (107). A_{2A} adenosine receptors mediate inhibition of ischemic ARF in rats due to an inhibitory effect on neutrophil adhesion (108,109). Combined infusion of an A_{2A} adenosine receptor agonist and a type IV phosphodiesterase (PDE 4) inhibitor leads to enhanced protection against ischemia-reperfusion injury in mice (110). Protection against renal ischemia-reperfusion injury by adenosine A_2A receptor agonists or endogenous adenosine requires activation of receptors expressed on bone marrow–derived cells (111). The adenosine A_2 receptor may be a novel therapeutic target in renal ischemia-reperfusion injury (112).

Intracellular Calcium

The normal regulation of epithelial cell calcium is demonstrated in Figure 39-5. Calcium exists in the cell as cytosolic free calcium, which is the smallest amount, but the most critical for regulation of intracellular events. Calcium is also bound to proteins and anions in the cytosol and to membrane phospholipids and glycoproteins. The largest pool of intracellular calcium is in the mitochondria and the endoplasmic reticulum (113,114). The concentration of calcium in the cytosol is about 100 nM, which is 1/10,000 of the extracellular calcium that is in a mM concentration (113). The large electrochemical gradient between intracellular and extracellular calcium is maintained by binding of calcium to intracellular components and by apical and basolateral transport systems. Transport systems may be voltage dependent or ATP dependent. Calcium efflux is mediated in basolateral membranes by both calcium ATPase, which is ATP dependent, and by a Na^+/Ca^{2+} exchanger on the basolateral membrane, which is ATP independent (115). Normally, the cell membrane is impermeable to calcium and maintains the steep calcium gradient between cytosolic free calcium and the extracellular space (114). However, when cytosolic calcium increases in response to either increased cellular

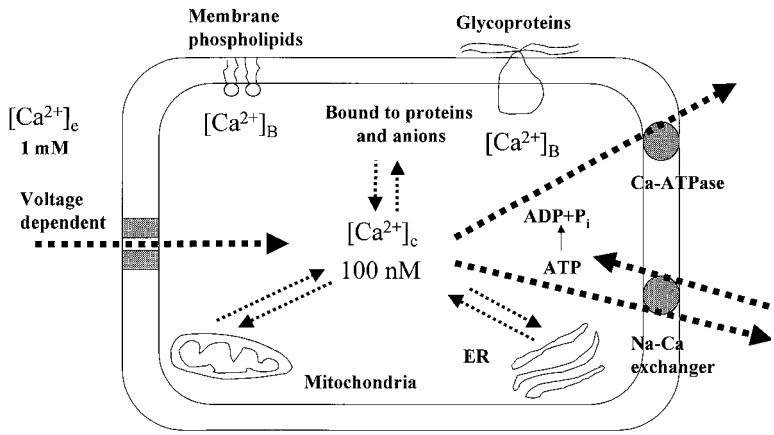

FIGURE 39-5. The normal regulation of epithelial cell calcium. The cytosolic free calcium $[Ca^{2+}]_c$ is the smallest amount, but the most critical for regulation of intracellular events. The concentration of calcium in the cytosol is about 100 nM, which is 1/10000 of the extracellular calcium, which is in a mM concentration $[Ca^{2+}]_e$ Calcium is also bound to proteins and anions in the cytosol and to membrane phospholipids and glycoproteins $[Ca^{2+}]_b$. The largest pool of intracellular calcium is in the mitochondria and the endoplasmic reticulum. The large electrochemical gradient is maintained by binding of calcium to intracellular components and by apical and basolateral transport systems. Transport systems, which maintain the large electrochemical gradient between intracellular and extracellular Ca^{2+} may be voltage dependent or ATP dependent like the Ca^{2+} ATPase pump and the Na^+/Ca^{2+} exchanger. During cell injury active mitochondrial sequestration appears to be quantitatively the most important process for buffering elevations in cytosolic calcium.

membrane permeability or decreased calcium efflux or both, the mitochondria and endoplasmic reticulum actively increase their calcium uptake. Mitochondrial uptake and retention of calcium becomes substantial only when cytosolic levels exceed 400 to 500 nM as occurs with cell injury (113). Mitochondrial uptake is regulated by a calcium uniporter in the mitochondrial inner membrane. Thus, during cell injury active mitochondrial sequestration appears to be quantitatively the most important process for buffering elevations in cytosolic calcium.

During epithelial cell injury, several factors favor increases in cytosolic free calcium (Fig. 39-6). There is (i) decreased mitochondrial electron transport leading to decreased ATP levels; (ii) increased membrane permeability; and (iii) depolarization or opening of voltage dependent channels. The decreased ATP leads to decreased calcium uptake by mitochondria and endoplasmic reticula and decreased ability to pump calcium out of the cell.

With this background on the normal regulation of cell calcium, we shall now consider the role of intracellular calcium in tubular injury. In 1981, it was proposed that calcium ions were important participants in the functional, biochemical and morphologic disturbances that characterize ARF (116,117). Numerous studies over the past 15 years in different injury models and cell types have demonstrated an increase in cytosolic calcium in renal epithelial cell injury. These studies are summarized in Table 39-3.

Initial interest in this area began with the demonstration that chemically dissimilar Ca2+ channel blockers (CCB) are effective in preventing or attenuating the courses of experimental ARF (50,115). On the background of these experimental results, the efficacy of CCB has been shown in preventing ARF associated with cadaveric transplantation (118,119) and radiocontrast media (120). These results led to a series of experiments examining the role of cellular Ca^{2+} in the pathogenesis of ischemic ARF. Early studies demonstrated that renal mitochondrial Ca^{2+} accumulation characterizes ischemic ARF and that this Ca^{2+} uptake impairs renal mitochondrial oxidative phosphorylation and ATP synthesis (51,52,121,122). Moreover, protection against ischemic ARF with CCB was associated with an attenuation of this mitochondrial Ca^{2+} accumulation and improved mitochondrial respiratory function (50).

In vitro experiments using cultured renal tubules suggested a pathogenic role for cellular Ca^{2+} independent of any vascular effect (123,124). In other experiments, either removal of

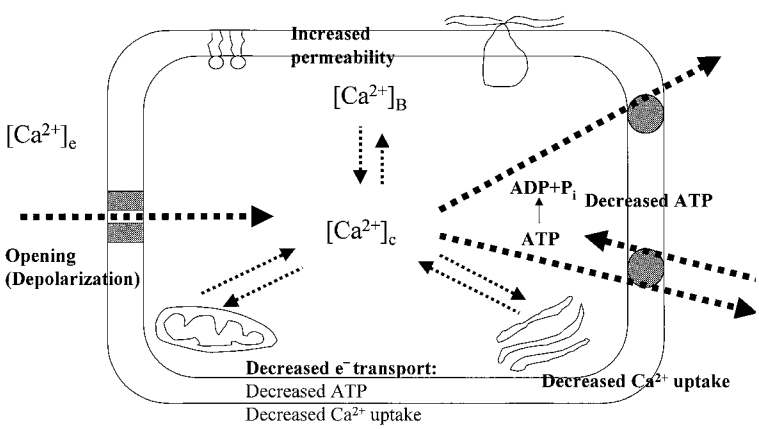

FIGURE 39-6. During epithelial cell injury, several factors favor increases in cytosolic free calcium. There is (1) depolarization or opening of voltage dependent channels; (2) increased membrane permeability and (3) decreased mitochondrial electron transport leading to decreased ATP levels. The decreased ATP leads to decreased calcium uptake by mitochondria and ER and decreased ability to pump calcium out of the cell.

TABLE 39-3

INCREASES IN CYTOSOLIC CALCIUM IN RENAL EPITHELIAL CELL INJURY

Injury model	Cell type	Reference
Calcium ionophore	Rabbit proximal tubules	(454)
Anoxia	LLCMK2 cells	(455)
Chemical ATP depletion	MDCK cells	(456)
Calcium ionophore	Cultured rabbit proximal tubules	(457)
Chemical ATP depletion		
Chemical anoxia	Rabbit proximal tubules	(178)
Hypoxia	Rabbit proximal tubules	(458)
Hydrogen peroxide	LLCPK1 cells	(459)
Anoxia and hypoxia	Rat proximal tubules	(127)
Chemical anoxia	Opossum kidney cells	(460)
Hypoxia-reoxygenation	Primary culture rat proximal tubules	(461)
Hypoxia	Rat proximal tubules	(103)
Anoxia	Rabbit proximal tubules	(462)

(Reproduced from: Edelstein CL. Editorial Comment: calcium-mediated proximal tubular injury—what is the role of cysteine proteases? *Nephrol Dial Transplant* 2000;15:141, with permission.)

Ca^{2+} from the medium during early reoxygenation (125) or the administration of CCB (126) was found to improve cell viability after anoxia. Further experiments were designed to examine the role of cellular Ca^{2+} and the direct effects of CCB on renal tubular epithelium without vascular influences; these experiments utilized isolated proximal tubules, a consistent tubular site of ischemic injury. The use of this preparation allowed the study of direct tubular effects of protective agents, which could not have been demonstrated to be independent of vascular effects in whole animal studies. Models of injury were developed in the isolated tubule preparation in response to hypoxia, anoxia, and phosphate depletion (127–129). Studies demonstrated that ^{45}Ca uptake is increased with hypoxia and that CCB prevent this increase (127). Moreover, the protective effect of a decreased pH was also shown to be associated with a decrease in hypoxia related enhanced ^{45}Ca influx (130). In addition, Wetzels et al. showed that hypoxia-induced proximal tubule membrane damage can be significantly reduced by lowering the extracellular Ca^{2+} concentration or by the addition of verapamil (46). In this latter study, the protective effect of verapamil was associated with improved cellular ATP and K^+ levels, an effect that is not observed with other protective agents, such as glycine or alanine. This finding suggested that verapamil could act directly on the mitochondria and affect the plasma membrane. Subsequent studies using isolated renal cortical mitochondria did, in fact, demonstrate that verapamil exerts a protective effect against Ca^{2+}-induced mitochondrial damage and that this effect may be mediated by the inhibition of mitochondrial Ca^{2+} uptake and mitochondrial phospholipase activity (131). Furthermore, studies in isolated tubules confirmed hypoxia-induced free fatty acid and lysophospholipid accumulation (132) and suggested a relationship between these events and the rate of cellular Ca^{2+} uptake (133).

One question is to what level the free cytosolic calcium rises during ATP depletion. Previously it was difficult to determine peak cytosolic calcium levels using the high affinity calcium fluorophore Fura-2. Weinberg's group showed that the cytosolic free calcium increases to greater than 100 μM in ATP depleted proximal tubules using the low-affinity calcium fluorophore Mag-Fura-2 (101). Experiments were done in the presence of 2mM glycine which approximates the physiological concentration *in vivo*. In the tubules studied, 91% had a free cytosolic calcium that exceeded 10 μM. Thirty-five percent had levels greater than 500 μM with no cell membrane damage. In this study, proximal tubules seemed to have a remarkable resistance to the deleterious effects of increased calcium during ATP depletion in the presence of glycine. In the isolated perfused rat kidney, intracellular calcium increases have also been measured using 19^F NMR and 5^F BAPTA and demonstrated a partially reversible increase from 256 nM to 660 nM (134,135).

Another question is what level of oxygen deprivation is required to increase cytosolic calcium. Peters et al. demonstrated the rise in cytosolic calcium in anoxic but not hypoxic tubules (136). In hypoxic perfusion oxygen tension measured with a very sensitive electrode was 5 to 6 mm Hg. Complete anoxia was achieved with oxyrase in a nonperfused system. Calcium did not increase during hypoxia, but there was an increase in calcium during anoxia. This increase paralleled the collapse in mitochondrial membrane potential as measured by rhodamine fluorescence. Because cell membrane damage occurred during both anoxia and hypoxia, it was concluded that an increase in cell calcium is not always necessary for cell injury.

The crucial questions to implicate calcium as a primary factor in cell injury are (i) whether the increase in cytosolic calcium precedes the injury and (ii) whether preventing the rise in cytosolic calcium attenuates the injury (137,138). To investigate whether hypoxia is associated with an increase in free cytosolic calcium in proximal tubular cells, which precedes any evidence of membrane damage, a video imaging technique was developed in which free intracellular calcium could be measured simultaneously with the staining of nuclei with the membrane impermeable indicator, propidium iodide, as an index of hypoxia-induced membrane damage (139). Propidium iodide enters the cell through the damaged plasma membrane and stains the cell nucleus. The percent of nuclei that stain with propidium iodide is quantitated and is an index of plasma membrane damage. Hypoxia in rat proximal tubules is associated with a significant rise in cytosolic calcium that antecedes evidence of membrane damage as assessed by propidium iodide staining (103). Cytosolic calcium increased from 170 to 390 nM during 5 minutes of hypoxia. The increase in cytosolic calcium preceded propidium iodide detectable cell injury (Fig. 39-7). The increase in cytosolic calcium that preceded the hypoxic membrane damage was promptly reversible with reoxygenation after 8 minutes hypoxia. This is important because if cytosolic calcium is increased only after lethal cell membrane damage, reoxygenation should not have normalized cytosolic calcium. The 10-minute cytosolic calcium rise correlated significantly with subsequent cell damage observed at 20 minutes. The pivotal role of the rise in cytosolic calcium during hypoxia was further demonstrated by using the intracellular

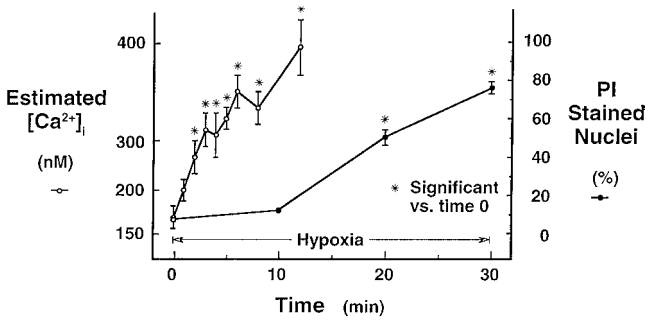

FIGURE 39-7. In isolated proximal tubules, the increase in free cytosolic calcium as measured by Fura-2 precedes the cell membrane damage as assessed by Propidium Iodide staining. (Adapted from: Kribben A, Wieder ED, Wetzels JF, et al. Evidence for role of cytosolic free calcium in hypoxia-induced proximal tubule injury. *J Clin Invest* 1994;93:1922.)

Ca^{2+} chelator 1,22-Bis (2-aminophenoxy) ethane-N,N,N′,N′-tetraacetic acid (BAPTA) to prevent the rise in cytosolic calcium; this approach resulted in marked cytoprotection against hypoxic tubular injury.

Thus, low extracellular calcium also prevented the hypoxia induced increase in free intracellular calcium, indicating that hypoxic injury may be primarily due to net calcium entry (i.e., calcium influx > efflux) from the extracellular compartment into the cells (103). While this result suggests an increase in calcium influx during hypoxia, it does not exclude a decrease in calcium efflux. Direct measurement of the calcium efflux is not possible with the current technology. The estimation of the calcium influx with different techniques during hypoxia has provided contradictory results. Using $^{45}Ca^{2+}$ an increase in calcium influx was observed, while with manganese-quenching of fura-2, a decrease of the calcium influx was proposed (127,140). The latter study hypothesized that reducing calcium influx during hypoxia provides the cell with a means to prevent cellular calcium overload during ATP depletion when calcium extrusion is limited. In this regard, it was demonstrated that endoplasmic reticulum calcium stores play a role in determining the cytosolic calcium during hypoxia (141).

In summary, there are various factors that may explain the differences between the cytosolic calcium found in proximal tubules during hypoxic injury. These factors include (i) species differences between rat and rabbit tubules, (ii) differences in injury models caused by oxygen deprivation as compared to metabolic inhibitors (chemical hypoxia), (iii) the severity of the oxygen deprivation and the energy state of the mitochondria, and (iv) different methods in measuring cytosolic calcium.

What are the mechanisms whereby increases in cytosolic free calcium could lead to cell membrane injury? Potential calcium-dependent mechanisms include changes in the actin cytoskeleton of proximal tubule microvilli (142), activation of calcium-dependent PLA_2(39), and activation of the cysteine protease, calpain (137,138,143,144).

Calcium-Dependent Changes in the Actin Cytoskeleton

The role of calcium in pathophysiological alterations of the proximal tubule microvillus actin cytoskeleton was studied in freshly isolated tubules (142). Precisely defined medium calcium levels were defined using a combination of the metabolic inhibitor, antimycin, and the ionophore, ionomycin, in the presence of glycine, to prevent lethal membrane damage. Increases of intracellular calcium to 10 μM were sufficient to initiate concurrent actin depolymerization, fragmentation of F-actin into forms requiring high-speed centrifugation for recovery, redistribution of villin to sedimentable fractions, and structural microvillar damage consisting of severe swelling and fragmentation of actin cores. However, during ATP depletion induced by antimycin alone or hypoxia alone, initial microvillar damage was calcium-independent. This study suggests that both ATP depletion-dependent but Ca^{2+}-independent, as well as Ca^{2+}-mediated processes can disrupt the actin cytoskeleton during acute proximal tubule cell injury. It also suggests that both types of change occur, despite protection afforded by glycine and reduced pH against lethal membrane damage; and that Ca^{2+}-independent processes primarily account for prelethal actin cytoskeletal alterations during simple ATP depletion of proximal tubule cells.

In normal proximal tubule cells, actin is concentrated in apical brush border microvilli, along with the actin-binding protein villin. Villin plays an important role in actin bundling and in microvillar assembly, but can also act as an actin-fragmenting protein at higher calcium concentrations. The effects of ischemic injury and reperfusion on the distribution of villin and actin in proximal tubule cells of rat kidney were examined (145). This study demonstrated that villin may be involved in the initial disruption of the actin cytoskeleton during reperfusion injury and that its migration back to the apical domain of these cells accompanies the reestablishment of a normal actin distribution in the brush border.

ATP depletion results in the conversion of monomeric G-actin to polymeric F-actin during tissue ischemia (146). This conversion results from altering the ratio of ATP-G-actin and ADP-G actin, causing a net decrease in the concentration of thymosinactin complexes as a cosequence of the differential affinity of thymosin beta 4 for ATP and ADP-G actin (147). Recent studies suggest that the actin binding protein tropomysin binds to and stabilizes the apical actin microvilli under physiological conditions in proximal tubules (148).

Activation of Phospholipase A_2 (PLA_2)

PLA_2 enzymes are important regulators of prostaglandin and leukotriene synthesis and can directly modify the composition of cellular membranes (149). PLA_2enzymes are also potent regulators of inflammation. The cytosolic form, $cPLA_2$, preferentially releases arachidonic acid from phospholipids and is regulated by changes in intracellular calcium concentration (150).

PLA_2 enzymatic activity was measured in cell-free extracts prepared from rat renal proximal tubules (39). Both soluble and membrane-associated PLA_2 activity were detected. All PLA_2 activity detected during normoxia was calcium dependent. Fractionation of cytosolic extracts by gel filtration revealed three peaks of PLA_2 activity. Exposure of tubules to hypoxia resulted in stable activation of soluble PLA_2 activity, which correlated with disappearance of the highest molecular mass form (>100 kDa) and appearance of a low-molecular-mass form (approximately 15 kDa) of PLA_2. Hypoxia also resulted in release of a low-molecular-mass form of PLA_2 into the extracellular medium. Pretreatment of tubules with glycine before hypoxia blocked this release of PLA_2 but not activation of soluble PLA_2 activity. This study provides direct evidence for calcium-dependent PLA_2 activation during hypoxia. However, calcium-independent forms of PLA_2have also been found to play a role in hypoxic proximal tubular injury (151).

The mechanism of PLA_2-induced cell membrane damage is interesting. Membrane phospholipid breakdown has been observed to occur in a number of tissues during ischemia (152). In proximal tubules hypoxia has been shown to cause an increase in free fatty acids, which was initially believed to contribute to cell injury (153). However, a study from our laboratory has shown that unsaturated free fatty acids protect against

hypoxic injury in proximal tubules and that this protection may be mediated by negative feedback inhibition of PLA_2activity (40). This protective effect of unsaturated free fatty acids has been confirmed by Zager et al. (154). The injurious effect of PLA_2 could be related to a direct disruption of cell membrane integrity by attacking the phospholipid component of cell membranes or through accumulation of lysophospholipids, which have been shown to disrupt cell membranes and cause cytotoxicity (155).

$cPLA_2$knockout mice have been developed (156). After transient middle cerebral artery ischemia, the cPLA2 knockout mice have smaller infarcts and developed less brain edema and fewer neurological deficits (156). The effect of ischemic ARF in cPLA2 knockout mice has not been reported.

Activation of Calpain

The cysteine proteases are a group of intracellular proteases that have a cysteine residue at their active site. The cysteine proteases consist of three major groups: cathepsins, calpains, and the newly discovered caspases. The major groups of cysteine proteases are shown in Table 39-4. The cathepsins are noncalcium-dependent lysosomal proteases that do not appear to play a role in lethal cell injury (157–159). Calpain is a calcium activated neutral protease (CANP) (160). It has absolute dependence on calcium. It is a heterodimer and has 2 subunits, an 80-kDa catalytic subunit and a 30-kDa regulatory subunit. There are two major ubiquitous or conventional isoforms of calpain, the low calcium sensitive μ-calpain and the high calcium sensitive *m*-calpain (161,162). The isoenzymes have the same substrate specificity, but differ in affinity for Ca^{2+}. μ-calpain is activated by micromolar concentrations of Ca^{2+} and *m*-calpain is activated by millimolar concentrations of Ca^{2+}. The millimolar concentrations of intracellular calcium needed for activation of m-calpain are not seen in normal cells and phosphophatidylinositol is thought to lower the calcium concentration required for half maximal autolysis of m-calpain (163).

Suzuki's membrane activation theory is thought to explain the regulation of calpain activity (164,165). Specifically, procalpain exists in the cytoplasm as an inactive proenzyme and becomes active proteolytically only after it has become autolysed at the cell membrane. Activity of the autolyzed calpain is subject to a final regulation by calpastatin (164,165). Calpastatin is a specific endogenous inhibitor of calpain. It is as widely distributed in nature as the enzyme itself. Calcium is required for calpastatin to bind to calpain and thus for the inhibitory effect of calpastatin on calpain.

Calpain substrates include cytoskeletal proteins (e.g., spectrin), receptor proteins (e.g., glutamate), and enzyme proteins (e.g., kinases and phosphatases). Postulated functions of calpain include platelet activation and aggregation, cytoskeleton and cell-membrane organization (166), and regulation of cell growth (167–170).

The calcium-dependent calpains have been shown to be mediators of hypoxic/ischemic injury to the brain, liver, and heart (171–174). The role of the calcium dependent cytosolic protease, calpain, in hypoxia-induced renal proximal tubular injury has also been investigated (42). Tubular calpain activity increased significantly by 7.5 minutes of hypoxia, before there was significant LDH release, and further increased during 20 minutes of hypoxia. Chemically dissimilar cysteine protease inhibitors markedly decreased LDH release after 20 minutes of hypoxia and completely prevented the rise in calpain activity during hypoxia. This role of calpain in proximal tubule injury has subsequently been confirmed by other groups (175,176). This increased calpain activity has subsequently been shown to be associated with breakdown of the cytoskeletal protein, spectrin, both *in vitro* (44) and *in vivo* (177) as well as increasing Na-K-ATPase into the cytoplasmic fraction of the cell.

Acidosis has been shown to protect the isolated proximal tubule from membrane damage (178). The effects of low intracellular pH (pH_i) or low free cytosolic calcium $[Ca^{2+}]_i$ on this hypoxia-induced calpain activity were also determined. Both low pH_i and low $[Ca^{2+}]_i$ attenuated the hypoxia-induced increase in calpain activity. This attenuation of calpain activity was observed early before hypoxia-induced membrane damage and was associated with marked reduction in the typical pattern of hypoxia-induced cell membrane damage observed in this model (43).

Recent studies have demonstrated that calpain mediates progressive plasma membrane permeability and proteolysis of cytoskeleton-associated paxillin, talin, and vinculin during antimycin A or hypoxia-induced proximal tubular cell death (179). Novel nonpeptide calpain inhibitors are protective against antimycin A-induced calcium influx and hypoxia/reoxygenation-induced proximal tubular cell death (180).

Caspases

Caspases participate in two distinct signaling pathways: (i) activation of proinflammatory cytokines and (ii) promotion of apoptotic cell death (6,181–186). The term *caspase* embodies two properties of these cysteine proteases in which "c" refers to "cysteine" and "aspase" refers to their specific ability to cleave substrates after an aspartate residue. There are now

TABLE 39-4

THE MAJOR GROUPS OF CYSTEINE PROTEASES

	Cathepsins	Calpains	Caspases
Family	B,H,L,S (lysosomal)	μ and *m*-calpain Tissue specific isoforms	1 to 14
Location	Lysosome	Cytoplasm	Cytoplasm
Activation	Calcium-independent	Calcium-dependent	Caspase activated
Optimal pH	5 to 6	7.4	7.4
Functions	Intracellular protein degradation	Intracellular signaling Cytoskeletal stability Necrosis and apoptosis	Apoptosis/necrosis Cytokine activation

(Adapted from: Edelstein CL. Editorial Comment: Calcium-mediated proximal tubular injury—what is the role of cysteine proteases? *Nephrol Dial Transplant* 2000;15:141, with permission.)

14 members of the caspase family, caspases 1–14. Caspase-14 has recently been characterized and found to be present in embryonic tissues but absent from adult tissues (187). Caspases share a predilection for cleavage of their substrates after an aspartate residue at P1 (183,188). The members of the caspase family can be divided into three subfamilies based on substrate specificity and function (189). The peptide preferences and function within each group are remarkably similar (189). Members of Group 1 (of which caspase-1 is the most important) prefer the tetrapeptide sequences WEHD and YVAD. This specificity is similar to its activation sequence suggesting that caspase-1 may employ an autocatalytic mechanism of activation. Caspase-1 (previously known as interleukin-1 converting enzyme or ICE) plays a major role in the activation of proinflammatory cytokines. Caspase-1 is remarkably specific for the precursors of interleukin-1 (IL-1) and IL-18 (interferon-gamma-inducing factor), making a single initial cut in each procytokine that activates them and allows exit from the cytosol (190,191). Group III "initiator" caspases-8 and 9 prefer the sequence (L/V)EXD. This recognition motif resembles activation sites within the "executioner" caspase proenzymes, implicating this group as upstream components in the proteolytic cascade that serve to amplify the death signal. These "initiator" caspases pronounce the death sentence. They are activated in response to signals indicating that the cell has been stressed or damaged or has received an order to die. They clip and activate another family of caspases, the "executioners." The optimal peptide sequence motif for Group II or "executioner caspases" (of which caspase-3 is the most important) is DEXD (182,189,192). This optimal recognition motif is identical to proteins that are cleaved during cell death.

Activation of caspases-1, 8, 9, and 3 have been described in hypoxic renal epithelial cells (193–195) and cerebral ischemia (196). Caspase-1 may also cause cell injury by activation of the proinflammatory cytokines IL-1 and IL-18 (183,191). However, IL-1 was not found to play a role in ischemic ARF in mice (29). Caspase-3 knockout mice have decreased apoptosis in the brain and most have premature lethality dying at 1 to 3 weeks of age (197). However, caspase-3 deficient mice that have been backcrossed into C57BL/6 and reaching 6 to 12 weeks of age are protected against Fas-mediated fulminant hepatitis (198). These mice provide an ideal opportunity to evaluate the role of the "executioner" and the most abundant caspase, caspase-3, in ischemic and hypoxic cell injury. While cells contain many caspases, targeted disruption of specific caspase genes in mice has provided much insight into the functions of individual caspases during cell death (199).

While caspases play a crucial and extensively studied role in apoptosis, there is now considerable evidence that the caspase pathway may also be involved in necrotic cell death (200). Caspase inhibition has been demonstrated to reduce ischemic and excitotoxic neuronal damage (201–203). Moreover, mice deficient in caspase-1 demonstrate reduced ischemic brain injury produced by occlusion of the middle cerebral artery (203,204). Inhibition of caspases also protects against necrotic cell death induced by the mitochondrial inhibitor, antimycin A, in PC12 cells, Hep G2 cells, and renal tubules in culture (205,206). Caspases are also involved in hypoxic and reperfusion injury in cultured endothelial cells (207). Rat kidneys subjected to ischemia demonstrate an increase in both caspase-1 and caspase-3 mRNA and protein expression (208). Caspases play a role in hypoxia-induced injury of isolated rat renal proximal tubules (48). In this study, caspase activity was increased in association with cell membrane damage as assessed by lactate dehydrogenase (LDH) release. A specific caspase inhibitor attenuated the increase in caspase activity and markedly protected against cell membrane damage.

Like caspase-3, caspase-6 is also an "executioner" caspase (209). Caspase-6 was cloned and characterized in rat kidneys (210). In rat kidney ischemia-reperfusion injury, there is increased expression of caspase-6 and translocation from the cytoplasm to the nucleus (210).

A recent study investigated the role of caspase inhibition and apoptosis in ischemic ARF in mice *in vivo* (211). A relationship between apoptosis and subsequent inflammation was found. At the time of reperfusion, administration of the antiapoptotic agents IGF-1 and ZVAD-fmk (a caspase inactivator) prevented the early onset of not only renal apoptosis, but also inflammation and tissue injury. Conversely, when the antiapoptotic agents were administered after onset of apoptosis, these protective effects were completely abrogated.

There appears to be an interaction between caspases and calpain during hypoxia-induced injury in the proximal tubule, since caspase inhibition was shown to decrease calpain activity during hypoxia (48,144). Recent *in vivo* studies suggest that caspase mediated degradation of the endogenous inhibitor of calpain, calpastatin, is a mechanism whereby the calcium-mediated activity of calpain is increased (177).

Caspases in Cold Ischemia

Preservation injury, also known as cold ischemia, is an important clinical problem in kidney transplantation. Significant damage to the kidney may occur during harvest, cold storage and transport. An ongoing area of interest is identifying methods to reduce organ injury during this process. The primary consequence of cold ischemic injury is delayed graft function (DGF) in kidney transplants (212–214). These consequences have both short-term and long-term effects. In kidney transplant, for example, DGF increases patient morbidity in the short term as a hospital stay is longer and dialysis may be required. In the long term, DGF independently predicts reduced 1- and 5-year graft survival (215).

Both human and animal studies suggest that the adverse impact of cold ischemia may be associated with apoptosis. In human kidney transplant biopsies performed after 1 hour of reperfusion, apoptosis of tubular cells correlated significantly with cold ischemic time (216). Biopsies of human donor kidneys that subsequently developed postoperative ATN demonstrated increased renal tubular epithelial cell apoptosis (217). Prolonged cold ischemia has also been shown to increase apoptotic cell death in rat kidney allografts at 24 weeks posttransplant (218). Mitochondria undergo significant changes during ischemia and may contribute to preservation injury (219).

Caspases have been studied in cold ischemic kidneys (220). Kidneys of mice were perfused with cold University of Wisconsin (UW) solution containing a pancaspase inhibitor or vehicle via the left ventricle. The contralateral right kidney was used as a control. The left kidney was stored for 48 hours at 4°C to produce cold ischemia. Caspase-3 activity was massively (100-fold) increased in cold ischemic kidneys compared to controls. On immunoblot analysis, the processed form of caspase-3 was increased in cold ischemic kidneys compared to controls. The increase in caspase-3 was associated with significantly more renal tubular apoptosis and brush border injury. In addition, caspase-2, 8, and 9 activities were increased in cold ischemic kidneys. The pancaspase inhibitor prevented the formation of the processed form of caspase-3 and the increase in caspase activity, and reduced apoptosis and brush border injury. The results of this study suggest that caspase inhibition may prove useful in kidney preservation.

The relevance of these studies to organ preservation and the subsequent risk of graft dysfunction is substantial. In the models of liver organ harvest and storage, addition of a caspase inhibitor to the UW storage solution was effective at reducing caspase activation and cell death in both liver and kidney models (221,222). Phase I clinical trials regarding IDN-6556

developed by Idun Pharmaceuticals have been completed. IDN-6556 decreased the elevation of liver enzymes in patients with hepatic dysfunction (223). A placebo controlled phase II trial is being conduced to evaluate whether addition of IDN-6556 to the flush and cold storage solutions as well as administration to the recipient prior to transplant can improve markers of liver injury following liver transplantation. Caspase inhibitors are a particularly attractive approach to reducing the incidence of DGF in kidney transplantation.

Caspase-1 and IL-18

Caspase-1 is a proinflammatory caspase that cleaves precursor interleukin-1β (IL-1β) and precursor interleukin-18 (IL-18). Caspase-1 –/– mice develop less ischemic ARF as judged by renal function and renal histology (224) (Fig. 39-8A). IL-1β receptor knockout mice or mice treated with IL-1β receptor antagonist (IL-1Ra) are not protected against ischemic ARF (29). Since caspase-1 also activates IL-18, lack of the mature form of IL-18 in these caspase-1 –/– mice was investigated as a possible mechanism of this protection against ARF. Kidney IL-18 was more than 100% increased in wild-type ARF as compared to sham-operated controls. On immunoblot analysis, there was a conversion of the precursor to the mature form of IL-18 in ARF wild-type mice, but not in the caspase-1 –/– ARF mice and sham-operated controls. To further analyze the role of IL-18, wild-type mice were injected with rabbit anti-murine IL-18 neutralizing antiserum prior to the ischemic insult. These mice were protected against ARF to a similar degree as caspase-1 –/– mice (Fig. 39-8B).

Caspase deficient mice have provided extensive information on the role of individual caspases in disease processes. The study of caspase inhibitors is an important step toward the

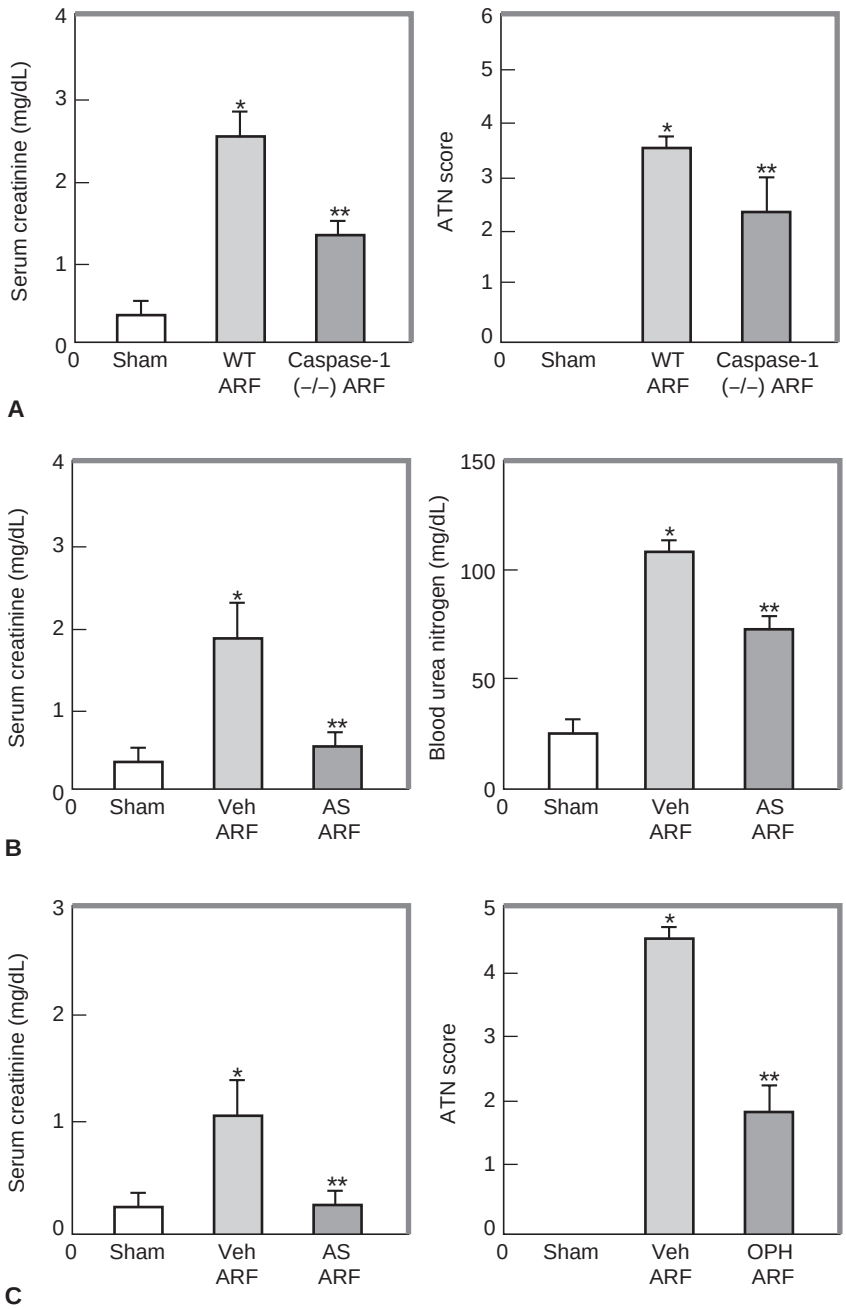

FIGURE 39-8. Caspases and IL-18 in ischemic ARF. A. Caspase-1 –/– mice are protected against ischemic ARF. Caspase-1 –/– mice developed less severe ARF, as determined by serum creatinine and ATN score compared with wild-type (WT) mice with ARF. *p <0.001 vs sham, **p <0.01 vs wild type ARF. B. Caspase-1 converts the pro to mature IL-18. Mice treated with IL-18 antiserum (AS) are functionally protected against ischemic ARF. In vehicle-treated mice with ischemic ARF (Veh ARF), serum creatinine and BUN was significantly increased at 24 hours compared with sham-operated controls. In mice treated with neutralizing IL-18 antiserum (AS), the serum creatinine and BUN were significantly reduced. *p <0.01 vs sham, **p <0.01 vs vehicle-treated mice with ARF (Veh ARF). C. Mice treated with the pancaspase inhibitor OPH-001 are protected against ischemic ARF. In vehicle-treated mice with ischemic ARF, serum creatinine and ATN score was significantly increased at 24 hours of post-ischemic reperfusion compared with sham-operated controls. In mice treated with OPH-001 (OPH) before induction of ischemic ARF, the serum creatinine and ATN score were significantly decreased compared with sham-operated controls. *p <0.001 vs sham, **p <0.01 vs vehicle-treated mice with ARF (Veh ARF), not significant vs sham. (Reproduced from: Melnikov VY, Faubel SG, Siegmund B, et al. Neutrophil-independent mechanisms of caspase-1- and IL-18-mediated ischemic acute tubular necrosis in mice. *J Clin Invest* 2002;110:1083, with permission.)

possible therapeutic effect of caspase inhibition in ischemic ARF. Mice with ischemic ARF treated with newly developed caspase inhibitor, Q-VD-(Ome)-OPH (OPH-001) had a marked (100%) reduction in blood urea nitrogen (BUN) and serum creatinine and a highly significant reduction in the morphological ATN score than did vehicle-treated mice (225) (Fig. 39-8C). OPH-001 significantly reduced the increase in caspase-1 activity and IL-18 and prevented neutrophil infiltration in the kidney during ischemic ARF. To further investigate whether this lack of neutrophil infiltration was contributing to the protection against ischemic ARF, a model of neutrophil depletion was developed. Neutrophil-depleted mice had a small (18%) reduction in serum creatinine during ischemic ARF but no reduction in the ATN score despite a lack of neutrophil infiltration in the kidney. Remarkably, caspase-1 activity and IL-18 were still significantly increased in the kidney in neutrophil-depleted mice with ARF. Thus, to investigate the role of IL-18 in ischemic ARF in the absence of neutrophils, neutrophil-depleted mice were treated with IL-18-neutralizing antiserum. IL-18-antiserum-treated neutrophil-depleted mice with ischemic ARF had a significant (75%) reduction in serum creatinine and a significant reduction in the ATN score compared to vehicle-treated neutrophil-depleted mice. These results suggest a novel neutrophil-independent mechanism of IL-18-mediated ischemic ARF.

The effects of different caspase inhibitors on ischemic ARF in the rat kidney has been studied (226). A caspase-1 inhibitor significantly reduced functional and histological evidence of ischemic ARF compared to a caspase-3 inhibitor.

Another group of investigators found that caspase-1-deficient mice were not protected against renal ischemia (227). In this study, the model of renal ischemia was 45 minutes of unilateral renal pedicle clamping with contralateral nephrectomy. This model produces a milder form of functional injury than bilateral clamping. At 24 hours, BUN and creatinine were lower in the caspase-1−/− mice than in wild type, but the decrease was not statistically significance. Studies of kidney tissue revealed that renal myelperoxidase (MPO) was reduced and DNA laddering was the same. On histological examination, immunohistochemistry for neutrophil infiltration and TUNEL positive cells were the same between both groups; necrosis, however, was not examined. In addition, in the same study (227), treatment with IL-1 receptor antagonist, anti-IL-1 receptor antibody, or anti-IL-18 antibody minimally reduced renal functional deterioration, inflammation, and apoptosis.

Caspase-1 also contributes to cisplatin-induced ARF and ATN (228). In this study, both caspase-1 and 3 activities were increased in the kidney. Caspase-1-deficient mice were protected against cisplatin-induced apoptosis and ARF. Surpirisingly the caspase-1 deficient mice had less caspase-3 activation, less tubular apoptosis on day 2 after cisplatin injection and less ATN on day 3 after cisplatin injection.

Matrix Metalloproteinases

Matrix metalloproteinases are a large family of zinc-dependent matrix-degrading enzymes, which include interstitial collagenases, stromelysins, gelatinases, elastases, and secreted as well as membrane-type matrix metalloproteinases. They play a crucial role in remodeling of the extracellular matrix, which is an important physiological feature of normal growth and development. In the kidney, interstitial sclerosis and glomerulosclerosis have been associated with an imbalance of extracellular matrix synthesis and degradation (229). Alterations in renal tubular basement membrane matrix proteins, laminin and fibronectin, occur after renal ischemia-reperfusion injury (230). The role of matrix metalloproteinases in this process has been studied.

Meprin A is a zinc-dependent metallo-endopeptidase that is present in the brush border membrane of renal proximal tubular epithelial cells. The redistribution of this metalloendopeptidase to the basolateral membrane domain during ARF results in degradation of the extracellular matrix and damage to adjacent peritubular structures. The effect of meprin A, the major matrix degrading metalloproteinase in rat kidney, on the laminin-nidogen complex was examined. Nidogen-1 (entactin) acts as a bridge between the extracellular matrix molecules, laminin-1 and type IV collagen, and thus participates in the assembly of basement membranes. Following ischemic injury, meprin A undergoes redistribution and/or adherence to the tubular basement membrane. Nidogen breakdown products are produced as the result of partial degradation of tubular basement membrane by meprin A following renal tubular ischemia-reperfusion injury (231).

The susceptibility of inbred strains of mice to ischemic and nephrotoxic ARF was studied in mice with normal and low meprin A activity (232). The strains of mice with normal meprin A developed more severe renal functional and structural injury following renal ischemia or the injection of hypertonic glycerol compared to the two low meprin A strains. These findings suggest that meprin A plays a role in the pathophysiology of ARF following ischemic and nephrotoxic ARF insults to the kidney (232). A recent study demonstrated that meprin inhibition protects against ischemic ARF *in vivo* in rats (233).The characteristics of tubular metalloproteinases have been studied and indicate that it is distinct from classic matrix-degrading metalloproteinases (234).

Nitric Oxide (NO)

NO is a lipophylic, highly reactive free radical gas with diverse biomessenger functions (235). NO mediates diverse functions including vasodilatation, platelet aggregation inhibition, neurotransmission, inflammation, antimicrobial, antitumor, and apoptosis (235). Whether the net effects of NO are beneficial or deleterious is determined by the cell type, concentration of NO, duration of production, and the composition of surrounding microenvironment (235). There are three major nitric oxide synthase (NOS) isoforms in the kidney: neuronal NOS or nNOS (also known as NOS1), inducible NOS or iNOS (also known as NOS2), and endothelial NOS or eNOS (also known as NOS3) (236) (Table 39-5). The macula densa is the principal site of nNOS expression in the kidney (237,238). In situ hybridization studies in normal rat kidney demonstrate iNOS mRNA in the S3 segment of the proximal tubule, the cortical and medullary thick ascending limb, the distal convoluted tubule, and the cortical collecting duct and inner medullary collecting duct (239). eNOS mRNA has been detected in glomeruli and preglomerular vasculature, as well as proximal and distal tubules (240). eNOS protein is mainly present in the endothelium of intrarenal, afferent, efferent, glomerular arterioles, and medullary vas recta (237). Expression of eNOS protein in tubules has not yet been reported (236). nNOS and eNOS are continuously present, activated by calcium, and are also termed *constitutive NOS* (cNOS) (241,242). In contrast, iNOS is induced when the cells have been stimulated by certain cytokines, microbes, and microbial products, and thus is called *inducible NOS* (iNOS) (243,244). The time course of both calcium-dependent and independent NOS activity in rat renal cortex and medulla has been studied (245). Calcium-dependent NOS activity in cortex and medulla decreased in the early phase of ARF and then increased in the recovery phase in the cortex. iNOS activity increased in the early phase of ARF in both cortex and medulla and was maintained at higher levels in the medulla. In another study, L-arginine improved the deficiency

TABLE 39-5

NITRIC OXIDE SYNTHASE (NOS) ISOFORMS

Isoform	Tissue distribution: Body	Tissue distribution: Renal	Phenotype of knockout mouse
nNOS (Type 1)	Neurons, skeletal muscle, penis	Macula densa	Protection against cerebral ischemia (463,464)
iNOS (Type 2)	Constitutive: ileum, uterus, skeletal muscle Induced: macrophage, VSMC	Constitutive: mTAL, proximal tubule	Less hypotensive response to LPS (465) Increased mortality in polymicrobial sepsis (466) No protection against LPS-induced ARF (398) Protection against ischemic ARF (247)
eNOS (Type 3)	Endothelium	Glomerular vessels, intrarenal arteries	Hypertension (467) Increased susceptibility to stroke (468) and myocardial ischemia (469)

of constitutive NOS activity and improved the recovery phase of ischemic ARF in rats (246).

Studies in freshly isolated proximal tubules from knockout mice have also been revealing about the role of nitric oxide in hypoxic/ischemic tubular injury. Hypoxia-induced proximal tubule damage, as assessed by LDH release, was no different between wild type and mice in which eNOS and nNOS has been "knocked out." However, proximal tubules from the inducible NOS (iNOS) knockout mice demonstrated resistance to the same degree of hypoxia (41). The iNOS knockout mice also had less renal failure and better survival than the wild type mice after renal artery clamping (247). An induction of heat shock protein was also observed in the iNOS knockout mice as a potential contributor to the protection. Star et al. produced further results in a renal artery clamp model in mice in which alpha melanocyte stimulating hormone (ά MSH) was shown to block the induction of iNOS, decrease neutrophil infiltration and afford functional protection (248). A subsequent study examined the relative importance of ά MSH on the neutrophil pathway by examining the effects of ά MSH in ICAM-1 knockout mice and neutrophil poor isolated perfused kidneys where neutrophil effects are minimal or absent (249,250). In this study, it was found that ά MSH decreases renal injury when neutrophil effects are minimal or absent indicating that ά MSH inhibits neutrophil-independent pathways of renal injury.

Hypoxia was found to increase nitric oxide (NO) release from freshly isolated proximal tubules and this effect was blocked by L-NAME, a nonspecific NOS inhibitor, but not by the inactive D-NAME compound (38,251). The NO release during hypoxia was accompanied by LDH release and was reversed by L-NAME administration. Interestingly, however, L-NAME administration to the rat kidney clamp model actually worsened the renal failure (252). This result was interpreted as an overriding blocking effect of eNOS activity with the nonspecific effects of L-NAME (72). This would worsen the renal vasoconstriction and resultant injury, thus obscuring any salutary effect at the level of the proximal tubule (253). Thus, opposing abnormalities in NO production within the endothelial and tubular compartments of the kidney may contribute to renal injury (72). Reduced eNOS derived NO production causes vasoconstriction and worsens ischemia; increased iNOS derived NO production by tubular cells adds to the injurious effects of ischemia on these cells. Therapeutic interventions to modulate NO production in ischemic ARF may require selective modulation of different NOS isoforms in the tubular and vascular compartments of the kidney (254). On this background the group of Goligorsky performed studies using a specific antisense oligonucleotide to iNOS (252). The ischemia-induced upregulation of iNOS and nitrite production were both blocked by the antisense oligonucleotide. Most importantly the BUN and serum creatinine did not rise after the renal ischemic insult in the animals treated with the antisense oligonucleotide against iNOS.

The Goligorsky group also studied the relationship between nitric oxide and osteopontin during ischemic ARF. Osteopontin is a negatively charged glycosylated phosphoprotein that is expressed in many tissues including renal epithelial cells. Osteopontin serves both a cell attachment function and a cell signaling function via the alpha v beta 3 integrin. Effects on gene expression include suppression of the induction of nitric oxide synthase by inflammatory mediators. Osteopontin may play an important role in the pathophysiology of ARF. Osteopontin knockout mice subjected to renal ischemia developed worse renal failure and more structural damage than wild type controls (255). This was associated with the augmented expression of inducible nitric oxide synthase and the prevalence of nitrotyrosine residues in kidneys from osteopontin knockout mice versus wild-type counterparts. This study provides strong evidence of renoprotective action of osteopontin in acute renal ischemia.

The microvillar actin and cellular integrins are potential substrates of NO action, which could contribute to the ischemia-mediated sloughing of the brush border membrane and detachment of proximal tubule epithelial cells from their extracellular matrix (8,256–258). Such an effect would not only result in impaired tubular sodium reabsorption, but also provide intraluminal cellular debris as a component of tubular cast formation.

Glycine

Glycine is a well-known cytoprotective agent against proximal tubular injury (259,260). The mechanism of this cytoprotective effect remains unknown. However, many of the pathophysiological events that occur in *in vitro* models of proximal tubule injury, for example, ATP depletion (261,262), cytoskeletal changes (92,102), prelethal influx of Ca^{2+} (103), activation of phospholipase A2 (PLA2)(132), activation of iNOS (38), calpain activation (43) are not affected by glycine. These events therefore are more likely to be potential mediators of *in vivo* proximal tubule injury where glycine is present at physiological concentrations (259,263). It is also well known that the availability of glycine in a cell is a major determinant of lethal cell membrane damage to anoxic, hypoxic, ischemic and toxin-induced insults in hepatocytes, proximal tubules, and

endothelial cells (259,260,264–266). Since glycine is present at physiological concentrations *in vivo* (259,263), the mechanism of structural and functional changes during cell injury is better understood and needs to be assessed in the presence of glycine.

Heat Shock Proteins

The stress response is a highly conserved homeostatic mechanism that allows cells to survive a variety of different stresses (267). Stresses that trigger the heat shock response include hyperthermia, hypothermia, generation of oxygen radicals, hypoxia/ischemia, and toxins (268). On a molecular level, their function is to protect cells from environmental stress damage by binding to partially denatured proteins, dissociating protein aggregates, to regulate the correct folding, and to cooperate in transporting newly synthesized polypeptides to the target organelles.

The proteins induced by these stresses belong to a family of proteins called heat shock proteins (HSP). The proteins are identified by their molecular weight. The most important families include proteins of 90, 70, 60, and 27 kDa (268). HSP 90 is essential for cell viability. It is associated with the steroid hormone receptor and is a general chaperone with ATPase like activity. In stressed cells it associates with the cytoskeletal protein, actin. The HSP 70 family includes proteins that are both constitutively expressed and induced by stress. They are the most highly induced proteins by stress and function as chaperones binding to unfolded or misfolded proteins. The HSP 60 family is restricted to the mitochondrial matrix where it functions as an unfoldase. The HSP 27 family has functions similar to HSP 70. Ubiquitin is a stress protein that binds denatured proteins and targets them for proteolysis by the proteasome.

Renal ischemia results in both a profound fall in cellular ATP and a rapid induction of the 70 kD heat-shock protein family, HSP-70 (269,270). The relationship between cellular ATP and induction of the stress response in renal cortex during renal ischemia has been studied. Van Why et al. demonstrated that a 50% reduction in cellular ATP in the renal cortex must occur before the stress response is detectable, that reduction of ATP below 25% control levels produces a more vigorous response, and that reperfusion is not required for initiation of a heat-shock response in the kidney (271).

Ischemic ARF also induces differential expression of small HSPs. In sham-operated kidneys, HSP 25 localized to glomeruli, vessels, and collecting ducts, while another stress protein, alphaB-crystallin, localized primarily in medullary thin limbs of Henle's loop and collecting ducts. After ischemia, HSP 25 accumulated in proximal tubules in cortex and outer medulla, while alphaB-crystallin labeling became nonhomogeneous in outer medulla, and increased in Bowman's capsule. This study demonstrates that there is striking differential expression of HSP 25 and alphaB-crystallin in various renal compartments (272).

In vitro studies have demonstrated that HSP induction protects cultured renal epithelial cells from injury. It has been determined that prior heat stress protects opossum kidney (OK) cells, a cultured renal epithelial cell line, from injury mediated by ATP depletion (273). Also HSP 70 overexpression is sufficient to protect LLC- PK1 proximal tubular cells from hyperthermia but is not sufficient for protection from hypoxia (274).

During ischemic ARF, the question of whether prior HSP induction by hyperthermia is protective is controversial. One study found that prior heat shock protected kidneys against warm ischemia (275). Another study investigated the protective effect of heat-shock proteins on ischemic injury to renal cells in two different experimental models: ischemia-reflow in intact rats and medullary hypoxic injury as seen in the isolated perfused rat kidney. Prior induction of HSP by hyperthermia was not protective against the functional and morphological parameters of ischemic ARF in either of these models (276). These variable results may be explained by the complexity of the intact animal compared to cultured cells, the degree, duration, and timing of the hyperthermic stimulus and the differential response of mature and immature kidneys (23,277).

Pharmacological agents have been used to increase stress protein expression. Recently, inhibitors of the proteasome have been identified that can block the rapid degradation of abnormal cytosolic and ER-associated proteins. The hypothesis that proteasome inhibitors, by causing the accumulation of abnormal proteins, might stimulate the expression of cytosolic heat-shock proteins and/or ER molecular chaperones and thereby induce thermotolerance was tested in Madin-Darby canine kidney cell culture (278). Inhibition of proteasome function induced heat-shock proteins and ER chaperones and conferred thermotolerance in these cells. Thus, these agents may have applications in protecting against cell injury (278). Another study determined that proteasome inhibition protects against the morphological and functional abnormalities in ischemic ARF in rats (279). However, the effect of proteasome inhibition on HSP induction during ischemic ARF was not determined in this study.

The mechanism of HSP protection against ischemic ARF is interesting. It has been suggested that HSPs participate in the postischemic restructuring of the cytoskeleton of proximal tubules (280). It was found that HSP 72 complexes with aggregated cellular proteins in an ATP-dependent manner suggesting that enhancing HSP 72 function after ischemic renal injury assists refolding and stabilization of Na(+)-K(+)-ATPase or aggregated elements of the cytoskeleton, allowing reassembly into a more organized state (281). Another study examined the temporal and spatial patterns of HSP-25 induction in relation to the actin cytoskeleton (282). This study suggested that there are specific interactions between HSP 25 and actin during the early postischemic reorganization of the cytoskeleton. In another study, the Brown Norway rat was resistant to renal failure and ATN compared to the Sprague-Dawley rat (283). The Brown Norway rat had no distribution of Na-K-ATPase into detergent soluble cortical extracts and immunohistochemistry showed that baseline HSP-72 and 25 expression was increased in proximal tubules of the Brown Norway rats compared to the Sprague-Dawley rats.

Another potential mechanism of HSP protection against proximal tubular injury is the inhibition of apoptosis. Opossum kidney proximal tubule (OK) cells exposed to ATP depletion develop apoptosis by morphological and biochemical criteria. Prior heat stress reduced the number of apoptotic-appearing cells, significantly decreased DNA fragmentation, and improved cell survival compared with controls (284). This study demonstrated that novel interactions between HSP 72 and the antiapoptotic protein, Bcl2, may be responsible, at least in part, for the protection afforded by prior heat stress against ATP depletion injury.

Altered Gene Expression

During renal ischemia *in vivo*, the reaction of the renal epithelial cells is heterogeneous (285). Some cells, especially those of the proximal tubule, undergo necrosis. Other cells undergo apoptosis, and still others survive the ischemic injury intact. In addition, injured tubules are relined with new cells actively engaged in DNA synthesis. Thus, surviving tubular cells re-enter the cell cycle and replicate. These cells may undergo partial dedifferentiation that allows them to undergo mitosis (286). The complex events that mediate this heterogeneous response

of tubular cells are being studied. This response of tubular cells may involve the early immediate gene response.

Immediate early genes and protooncogenes are induced during the early reperfusion period after renal ischemia (287). There is c-fos and c-jun activation as well as an increase in DNA synthesis (288). There is accumulation of early growth response factor-1 (Egr-1) and c-fos mRNAs in the mouse kidney after occlusion of the renal artery and reperfusion (289,290). Transient expression of the genes cfos and Egr-1 may code for DNA binding transcription factors and initiate the transcription of other genes necessary for cell division (291). JE and KC, growth-factor-responsive genes with cytokine-like properties that play a role in inflammation, are also expressed during early renal ischemia (292). These genes may code for proteins with chemotactic effects that can attract monocytes and neutrophils into areas of injury (290). Studies demonstrate that c-fos and c-jun are expressed following renal ischemia as a typical immediate early gene response, but they are expressed in cells that do not enter the cell cycle (286,293). The failure of the cells to enter the cell cycle may depend on the coexpression of other genes.

DNA synthesis occurs in the proximal tubule, whereas the induction of the early gene response is restricted to cells of the thick ascending limb and collecting duct (290). Thus, the immediate early gene response does not always occur in cells that undergo DNA synthesis, suggesting that the role of the early gene response is not necessarily proliferative in this setting. The role of the stress response during renal ischemia and the fate of the cells undergoing it are unknown. This immediate early gene response may play a role in the protection of tubular cells against injury. Alternatively, it may be important in mounting a response that will later help the regeneration of other tubular cells as the products of some of these genes are localized to cells that are not undergoing cell death from apoptosis or necrosis (294). The immediate early gene response may be the response to sublethal injury allowing the cell to dedifferentiate (291).

The pathways that lead to the early gene response are interesting. At least two quite different pathways lead to the activation of c-jun (295–297). Growth factors activate c-jun via the mitogen activated protein kinases (MAPKs), which include extracellular regulated kinases, (ERKs) –1 and 2. This pathway is proliferative in nature. In contrast, the stress-activated protein kinase (SAPK) pathway is separate from the MAPK pathway. These kinases include c-Jun N-terminal kinase (JNK)- 1 and 2. Activation and the effect on cell fate of the SAPK pathway is very different from the MAPK pathway. The SAPK pathway is essentially antiproliferative and can lead to either cell survival or cell death. During renal ischemia, SAPKs are activated and inhibition of SAPK after ischemia protects against renal failure (298,299). Thus, it is possible that manipulation of this pathway could lead to therapies that may ameliorate ARF.

Numerous recent studies have analyzed gene expression during ischemic ARF. Cell communication, apoptosis and inflammation genes distinguish primary allograft function in human kidney transplantation (300). In renal ischemia-reperfusion in the mouse, there was an increase in genes involved in cell structure, extracellular matrix, intracellular calcium binding, and cell division/differentiation (301). In another study in mice, there were consistent patterns of altered gene expression in the first 24 hours of postischemic reperfusion (302). These genes included transcription factors, growth factors, signal transduction molecules and apoptotic factors. In ischemia-reperfusion in the rat, alterations in the expression of 18 genes were identified by microarray analysis (303). Nine genes were up-regulated: ADAM2, HO-1, UCP-2, and thymosin beta4 in the early phase and clusterin, vanin1, fibronectin, heat-responsive protein 12, and FK506 binding protein in the established phase. Nine genes were down-regulated: glutamine synthetase, cytochrome p450 IId6, and cyp 2d9 in the early phase and cyp 4a14, Xist gene, PPARgamma, alpha-albumin, uromodulin, and ADH B2 in the established phase. Changes in gene expression of ADAM2, cyp2d6, fibronectin, HO-1, and PPARgamma were confirmed by quantitative real-time polymerase chain reaction (PCR). One of the problems with microarray analysis in the whole kidney during ischemic ARF *in vivo* is identifying which of the numerous cell types in the kidney is the source of the gene alteration. Laser capture microdissection of immunofluorescently defined cells (IF-LCM) can isolate pure populations of targeted cells from a sea of surrounding cells with excellent preservation of mRNA (304). This technique has been used to label and isolate thick ascending limb cells in the kidney for mRNA analysis (304).

Exploration of the early gene response in renal ischemia using DNA microarrays and other genome-scale technologies should narrow the gap in our knowledge of gene function and molecular biology (305). Gene expression in early ischemic ARF may provide clues toward pathogenesis, biomarker discovery and novel therapeutics (306).

Apoptosis

Apoptosis was first described by Kerr et al. (307). The term comes from the ancient Greek that means "the dropping off as of leaves from a tree." The term stresses the facts that apoptosis is a physiological form of cell death, occurs in the individual cell (or leaf) in a programmed pattern, and can be triggered according to a program regulated by external stimuli (autumn) (4). Thus, apoptosis is the name given to a process of physiologic or programmed cell death. Apoptotic cells undergo a series of morphologically identifiable changes in their pathway to cell death (308). The morphological, biochemical and molecular characteristics of apoptosis versus necrosis are very different (Table 39-6).

The triggers of apoptosis include (i) cell injury e.g. ischemia, hypoxia, oxidant injury, nitric oxide and cisplatinum; (ii) loss of survival factors e.g. deficiency of renal growth factors, impaired cell to cell or cell to matrix adhesion and (iii) receptor-mediated apoptosis (e.g., Fas [CD 295] and TGFβ) (309).

TABLE 39-6

MORPHOLOGICAL, BIOCHEMICAL, AND MOLECULAR DIFFERENCES BETWEEN APOPTOSIS AND NECROSIS

Apoptosis	Necrosis
Individual cells shrink and detach from other cells	Multiple cells swell but remain attached
Plasma membranes remain intact	Plasma membrane disruption
Cell excludes DNA specific dye, propidium iodide	Propidium iodide enters cell and stains nucleus
Nuclear condensation, fragmentation, and pyknosis	Nuclear swelling and autolysis
Apoptotic bodies	No apoptotic bodies
Nuclear DNA fragmentation	Nuclear DNA fragmentation
Programmed by gene activation	No gene activation
Phagocytosis of cellular fragments	Cellular lysis
No inflammation	Inflammation

The two major pathways of apoptosis involve Fas and p53 (6,7,310). In the TNF receptor superfamily, Fas antigen (CD 295) is the most important factor. Engagement of Fas by its ligand (FasL) results in apoptosis. The tumor suppressor gene, p53, mediates apoptosis in cells whose DNA has been damaged. The cascades involving Fas and p53, which are centrally important in cell death, are shown in Figure 39-9.

In the p53 pathway, cytochrome c release from mitochondria is an apoptosis trigger (311). Cytochrome c binds to a protein called apoptotic protease activating factor-1 (Apaf-1). This binding allows Apaf-1 to activate caspase-9, an "initiator" caspase which then activates caspase-3. During this form of apoptosis, the mitochondrial inner transmembrane potential collapses indicating the opening of a large conductance channel known as the mitochondrial permeability transition (PT) pore. The presence of an excess of the antiapoptotic protein Bcl-2 on mitochondria inhibits cytochrome c release. Bcl-2 can also prevent the mitochondrial PT pore opening. P53 levels are increased during renal ischemia reperfusion in rat kidneys (312). Mitochondrial dysfunction has been demonstrated during ischemic ARF (51). Thus, cytochrome c release from mitochondria and subsequent caspase-9 activation may be a common pathway in both apoptosis and necrosis in the kidney.

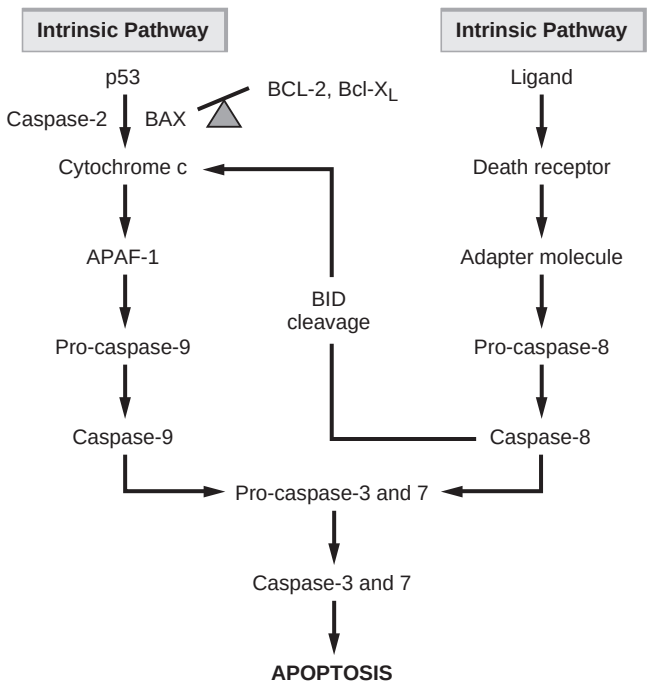

FIGURE 39-9. Major pathways of apoptosis. There are two major pathways of caspase-mediated apoptosis [451]. In the mitochondrial or "intrinsic" pathway, stress-induced signals (for example p53) act via Bcl-2 proteins to cause cytochrome c release from mitochondria. In the "extrinsic" pathway, there is the binding of a ligand (for example, Fas ligand) to its death receptor (for example, Fas) that recruits an adaptor protein. In the "intrinsic" pathway cytochrome c release from mitochondria is an apoptosis trigger [311]. Cytochrome c binds to a protein called apoptotic protease activating factor-1 (Apaf-1). This binding allows Apaf-1 to activate caspase-9, an "initiator" caspase, which then activates caspase-3 and 7. The presence of an excess of the anti-apoptotic protein Bcl-2 on mitochondria inhibits cytochrome c release. Caspase-2 is a recently discovered caspase that is a critical initiator of the mitochondrial apoptosis pathway [452]. Activation and increased activity of caspase-2 is required for the permeabilization of mitochondria and release of cytochrome c [452]. In the "extrinsic" pathway, the death receptors (CD95/Fas/APO-1, TNFR1, DR3/WSL-1/TRAMP. DR4/TRAIL-R1, DR5/TRAIL-R2, DR6) are a subset of the TNF/NGF receptor family of cell surface molecules that possess a common motif within their cytoplasmic tails, called the death domain. The death domains of these receptors recruit adapter molecules that, in turn, recruit caspases to the receptor complex. For example, Fas antigen (CD 95) is engaged by its ligand (FasL) resulting in apoptosis. Activation of procaspase-8 requires association with its cofactor Fas-associated death domain (FADD). The pathways may be linked as caspase-8 may cleave a member of the Bcl-2 family, BID, which can release cytochrome c. Apoptosis mediated by both pathways has been described during renal ischemia/reperfusion in rats [312;313]. Both caspase-3 and 7 play a crucial and extensively studied role in the promotion of all forms of apoptotic cell death [184]. Caspase-7, like caspase-3, is an "executioner" caspase and is downstream of the "initiators" caspase-8 and 9.

In the Fas pathway, Fas antigen (CD 295) is engaged by its ligand (FasL) resulting in apoptosis. Activation of procaspase-8 requires association with its cofactor Fas-associated death domain (FADD). The p53 and Fas pathways may be linked as caspase-8 may cleave a member of the Bcl-2 family, BID, which can release cytochrome c. Fas mediated apoptosis during renal ischemia/reperfusion has been described in rats (313).

Caspases are the major mediators of the cell death in apoptosis and also play a role in necrotic cell death. The central role of caspases in cell death is supported by caspase-8, 9, and 3 knockout mice that have strong phenotypes based on apoptotic cell death defects, developmental defects and usually fetal/perinatal mortality (186). Caspase-7, like caspase-3, is an "executioner" caspase and is downstream of the "initiators" caspase-8 and 9. Both the intrinsic and extrinsic pathways activate caspase-7. Caspase-3 and 7 exhibit very similar substrate specificities in peptide hydrolysis assays *in vitro* (314). However, the role of caspase-7 during the execution phase of apoptosis is obscure (315). Caspase-7 is unable to cleave the well-known caspase-3 substrates including fodrin, gelsolin, DNA fragmentation factor 45 (DFF45), inhibitor of apoptosis proteins (IAP) and signal transducer and activator of transcription-1 (STAT-1) (315). Also, caspase-7 is unable to activate caspases that would normally be activated by caspase-3 (316). It is known that caspase-3 and 7 can act independently as "executioners" of apoptosis (317). Both caspase-3 and caspase-7 deficient mice are perinatally lethal due to a lack of apoptosis (318). Caspase-7 may play a more specialized role in apoptosis than caspase-3 (315). Caspases have been described in detail earlier in this chapter.

Caspase-dependent or independent endogenous endonuclease activation, resulting in DNA fragmentation, is considered a characteristic biochemical marker for apoptosis (49). However, DNA fragmentation also occurs in cellular necrosis (49,319,320). The differentiation of apoptosis from necrosis in tubular cells therefore is still difficult (321) and requires both demonstration of DNA fragmentation, usually using a histochemical technique based on terminal deoxynucleotidyl transferase (TdT) reactivity with DNA breaks, as well as morphological evidence of apoptosis by light and electron microscopy. Pathways usually associated with apoptosis (e.g., endonuclease activation and increased mitochondrial permeability) may also be associated with necrosis, suggesting that apoptotic and necrotic cell death may share the same pathways (322). Both apoptosis and necrosis can occur in tissues exposed to ischemia/reperfusion or cultured cells exposed to hypoxia (323).

The number of *in vitro* and *in vivo* studies where apoptosis is described in renal tubules, is increasing. These studies are summarized in Tables 39-7 and 39-8. A feature of *in vitro* studies in tubules in culture (Table 39-7) is that severe or prolonged ATP depletion leads to necrosis while milder and shorter ATP depletion leads to apoptotic cell death. A similar pattern has emerged from the *in vivo* studies (Table 39-8) (i.e., the same insult in a mild form can lead to apoptosis and when severe can lead to necrosis).

TABLE 39-7

APOPTOSIS IN HYPOXIC/ANOXIC TUBULAR INJURY IN VITRO (CULTURED CELLS)

Cell type	Type of injury	Signals	Comment	Ref
MDCK and primary culture rat proximal tubules	Hypoxia		Necrosis also observed.	(470)
Primary culture mouse proximal tubules	Partial ATP depletion (Antimycin A)	Renal growth factors did not ameliorate apoptosis.	Severe ATP depletion caused necrosis.	(35)
LLC-PK1 (proximal) and MDCK cells	ATP depletion		Same pattern in LLC-PK1 and MDCK.	(471)
MDCK (distal)	Partial ATP depletion (Antimycin A)	Fas, FADD, Caspases, PARP	Severe ATP depletion caused necrosis.	(472)
Opossum kidney (proximal)	ATP depletion (cyanide, 2-deoxy-D-glucose)	Bcl-2/Bax	Prior heat shock attenuated apoptosis.	(284)
Rat proximal tubules	Hypoxia	Caspases Bcl-2	Serine protease inhibitors suppressed caspase-9 activation and apoptosis.	(473)
Rat proximal tubules	Antimycin A	Mitochondrial Ca^{2+} and permeability	L-type calcium channel blocker decreased apoptosis.	(474)
Human tubular cells	Fas transfection	Fas	High, but not basal Fas expression caused apoptosis.	(475)
MDCK	Overexpression of ankyrin death domain	Fas	Inhibition of ankyrin Fas interaction decreased apoptosis.	(476)
Rat proximal tubules	Hypoxia Cisplatin Staurosporine	Mitochondrial cytochrome c release	Minocycline upregulates Bcl-2 and decreases apoptosis.	(477)
Rat proximal tubules	ATP depletion	Bid cleavage	Bid cleavage and apoptosis blocked by anti apoptotic Bcl-2 overexpression and caspase-9 inhibition.	(478)

Numerous recent studies have demonstrated that erythropoietin (EPO) protects against ischemic ARF by affecting apoptotic cell death (324–326). A single dose of EPO either preischemia or just before reperfusion, improves renal function and tubular injury, prevents the activation of caspase-3, -8, and -9 and reduces apoptotic tubular cell death (324). EPO also protects against hypoxia-induced apoptosis in human proximal tubule cells (325). In the same study, EPO protected functionally against ischemic ARF in rats *in vivo* and reduced outer medullary thick ascending limb apoptosis while potentiating tubular mitosis and proliferation (325). In another study in rats with ischemic ARF, EPO decreased serum creatinine, decreased tubular apoptosis and necrosis, decreased tubular cell proliferation, increased antiapoptotic Bcl-2 protein expression, decreased caspase-3 activity and increased heat shock protein 70 (HSP70) expression (326). Thus, EPO may be a potential new therapy for ARF in humans.

Complement

The complement system is a mediator of ischemia-reperfusion injury in heart, lung, brain, intestine and muscle (327). A predominant role for C5b-9 in renal ischemia/reperfusion injury has been demonstrated (328). In this study, the primary damaging effect of complement was on parencymal cells rather than vascular endothelial cells. In another study, lack of a functional alternative complement pathway ameliorated ischemic ARF in mice (329). In this study, mice deficient in factor B, an essential protein in the alternative complement pathway, were functionally and histologically protected against ischemic ARF.

Relative Importance of Proximal Versus Distal Tubular Injury

There is an ongoing debate regarding which nephron segments are most severely injured in ischemic ARF (88). The target zone for hypoxic injury has also been extensively studied in the isolated rat perfused kidney (IPRK). This target zone predominantly involves the S3 segments of the proximal tubule and also distal tubules located within the outer stripe of the outer medulla and their cortical equivalent, the medullary rays, those straight sections of the proximal and distal tubules draining the superficial cortical glomeruli. While the sensitivity of the proximal tubules to injury is well recognized in all models of ARF, the debate over whether the proximal or distal nephron segments is the primary target for hypoxic/ischemic injury has been well reviewed recently (88). Of interest in the IPRK model of injury is the presence of a consistent artifact as a result of the absence of an oxygen carrier during erythrocyte-free perfusion. This artifact is the necrosis of medullary thick ascending limb (MTAL) cells first described by Alcorn (330). It had been observed by Leichtweiss et al. that tissue oxygen tension fell sharply in the region of the corticomedullary

TABLE 39-8

APOPTOSIS IN RENAL ISCHEMIA/REPERFUSION INJURY IN VIVO

Model	Type of injury	Location of apoptosis	Signals	Comment	Ref
Sprague Dawley rats	5 to 45 minutes I, 12 to 24 hours R	Distal	Sulfated glycoprotein 2	Longer periods of ischemia induced necrosis.	(479)
Wistar rats	60 minutes I, 7 to 14 days R	NT	NT	Necrosis at day 1 reperfusion.	(480)
Sprague Dawley rats	60 minutes I, 3 to 12 hours R	NT	Proliferating cell nuclear antigen (PCNA)	Uninephrectomy enhanced proliferation and decreased apoptosis.	(481)
Sprague Dawley rats	45 minutes I, 24 hours R	NT	P53 and c-myc	Verapamil attenuated apoptosis.	(312)
ICR mice	30 to 120 minutes I, 24 hours R	Distal	Fas	Proximal tubules developed necrosis. Less apoptosis in Lpr mice lacking Fas.	(313)
Human kidney allografts	Reperfusion after cold preservation	NT	NT	Less apoptosis in kidneys from living related donors.	(482)
Wistar rats	60 minutes I, 24 hours R	Proximal and distal	EGF, LGF1, and TGFβ	Osteogenic protein-1 reduced proximal but not distal apoptosis.	(483)
Sprague Dawley rats	60 minutes I, 1 and 5 days R	Proximal	Proapoptotic protein, SIVA	*Peaks of apoptosis at 1 and 7 days reperfusion.	(484)
Human kidney allografts	Cold ischemia	Distal	No change in Bcl-2	Apoptosis predicts early graft function.	(217)
Sprague Dawley rats	30 minutes I, 2 hours R	NT	NT	CCBs attenuated apoptosis.	(485)
Sprague Dawley rats	60 minutes I, 1 and 7 days R	Proximal		Fibroblast growth factor (FGF) attenuated proximal apoptosis and distal necrosis.	(486)
Swiss mice	45 minutes I, 2 hours R	Distal	Caspase-1 + 3.	Caspase inhibition attenuated renal failure and inflammation.	(211)
Sprague Dawley rats	60 minutes I, 7 days R			*Endothelin receptor antagonist attenuated apoptosis.	(487)
Sprague Dawley rats	30 minutes I, 4 to 14 days R	Proximal and distal	Bcl-2, Bcl-XL, and Bax	Distal tubules enhanced Bcl-2 expression.	(344)
NMRI mice	15 or 35 minutes I, 1 day R	NT	Fas	Small interfering RNA (siRNA) increased survival.	(488)
C57BL6 mice	22 minutes I, 1 day R	OSOM	Caspase-1 IL-18	Pancaspase inhibitor decreased tubular necrosis and apoptosis.	(225)
Sprague Dawley rats	45 minutes I, 6 hours R	NT	Nitrotyrosine, MDAde	Caspase-1 inhibitor decreased necrosis.	(226)
Death-associated protein kinase (DAPK) mutant mice	30 minutes I, 16 to 120 hours R	NT	DAPK	Mutant mice had less apoptosis and renal failure despite same degree of necrosis as wild type.	(489)

I, ischemia; *R*, reperfusion; *NT*, not tested; *NMRI*, Naval Medical Research Institute; *OSOM*, outer stripe of outer medulla; *MDA*, malondialdehyde; *, apoptosis not confirmed morphologically.

junction (331). Studies by Brezis et al. demonstrated that the MTAL lesion resulted from hypoxia and provided support for the hypothesis that countercurrent diffusion of oxygen from descending to ascending limbs of the vasa recta is responsible for the prevailing low oxygen tension of the renal medulla (332). Subsequently this group was able to induce a similar lesion in a number of models of renal injury. They have championed the notion that the MTAL segment lies on the brink of hypoxia as a result of the unique architecture of the kidney, which facilitates the countercurrent multiplier required for the formation of concentrated urine (333). Other groups have also suggested that arteriovenous diffusion of oxygen (between adjacent parallel arterial and veins) is responsible for lowering tissue PO_2 in the corticomedullary region and for maintaining the very low medullary PO_2 (334,335). However, Endre et al. demonstrated in the IPRK in the presence of low concentrations of erythrocytes that MTAL injury was prevented both under control conditions with high perfusate oxygen tension and in the

presence of hypoxia (336). The proximal tubule continued to be injured by hypoxia in the presence of erythrocytes, confirming that MTAL necrosis is an artifact of cell-free perfusion in this model. Nevertheless, coupled with the evidence for preglomerular arteriovenous diffusion of oxygen, which reduces average cortical PO_2 to sub-venous levels (337), the IPRK studies suggest that an even greater amount of the kidney is under threat from hypoxia when renal perfusion is reduced. Such widespread borderline hypoxia may be adaptively useful in priming the oxygen sensor in renal erythropoietin producing cells. However, when there is reduced renal perfusion, critically low levels of oxygen can be reached in tubular regions, particularly where there is high energy demand from transepithelial transport. Clearly, both proximal straight tubules (S3) and MTAL exist in such a region under constant threat of hypoxia. Magnetic resonance (MR) microscopy studies of the IPRK have demonstrated swelling of the cells in these interbundle regions in the outer medulla and their cortical equivalent, the medullary rays, restricting flow through the vascular bundles (338). These MR observations complement the earlier observations by Mason et al. and others that there is erythrocyte aggregation and stasis in the outer stripe of the model after reperfusion following ischemia (339–341).

Of greater interest, however, is the observation that while frank necrosis of MTAL cells is rarely seen *in vivo*, several studies in the IPRK have observed DNA fragmentation in MTAL cells after brief hypoxia (54) and after 15 or 60 minutes reperfusion after ischemia (342). DNA fragmentation has been observed after 24 hours reperfusion following ischemia *in vivo* in rats (343,344) although little or no morphological evidence of apoptosis has been observed in any of these studies. Similar DNA fragmentation was observed in human autopsy specimens after renal hypoperfusion (345). Recent studies of the Bcl-2 multigene family and growth factors by Gobé et al. (343,345) in a 30-minute bilateral arterial clamp model of ischemia–reperfusion have suggested a way of reconciling the observations of proximal cell necrosis and DNA fragmentation without apoptosis in nearby MTAL. After 24 hours of reperfusion, distal tubules showed a marked increase in expression of antiapoptotic Bcl-2 and a moderate increase in antiapoptotic Bcl-X_L and proapoptotic Bax. Proximal tubules showed a marked increase in Bax expression and a moderate increase in Bcl-X_L. Twenty-four hours after expression of the Bcl-2 proteins was increased, IGF-1 and EGF protein levels were increased in the distal tubule, similar to the Bcl-2 antiapoptotic proteins, and were also detected in the adjacent proximal tubules suggestive of paracrine action in these tubules. TGF-beta expression was moderately increased in regenerating proximal tubules, but no relationship was seen with the pattern of expression of the Bcl-2 genes. An explanation of these results is that the distal tubule is adaptively resistant to ischemic injury via promotion of survival by antiapoptotic Bcl-2 genes, and its survival allows expression of growth factors critical not only to the maintenance and regeneration of its own cell population (autocrine action), but also to the adjacent ischemia-sensitive proximal tubular cells (paracrine action).

The hypothesis has therefore been proposed that both the S3 proximal tubule and MTAL cells reside in regions where oxygen availability is borderline. Hypoxia induces both necrosis and apoptosis in proximal tubular cells. Hypoxia triggers apoptosis in MTAL cells, but the presence of antiapoptotic Bcl-2 genes prevents completion of programmed cell death and the DNA fragmentation is repaired. The induction of the growth factors EGF and IGF in these MTAL and distal tubule cells then provides both autocrine and paracrine mechanisms respectively for the recovery of the MTAL and proximal tubules. Because proximal cells are necrotic or have sloughed due to loss of cell adhesion, proximal tubule recovery is delayed compared to the MTAL. This hypothesis also provides a mechanism for tubular obstruction by casts since viable MTAL cells are the source of Tamm-Horsfall protein.

TUBULOGLOMERULAR FEEDBACK

Tubuloglomerular feedback (TGF) ("tubular communication with the glomerulus") operates within the juxtaglomerular apparatus (JGA) of each nephron where changes are sensed in the salt content of fluid at the luminal macula densa and that information is transmitted to the afferent arteriole to cause compensatory changes in single nephron GFR (346). nNOS (NOS 1) is expressed in the macula densa and may influence TGF. However, micropuncture experiments using NOS antagonists have shown that nitric oxide (NO) may modulate TGF (346). Instead, local NOS blockade causes the curve that represents TGF to shift leftward and become more steep. Changes in macula densa NO production may underlie the resetting of TGF, which is required in order to keep the TGF curve aligned with ambient tubular flow as tubular flow changes to accommodate physiologic circumstances. Also, macula densa NO production may be substrate limited and dissociated from NOS protein content. The importance of NO to TGF resetting and the substrate dependence of NO production have both been found during changes in dietary salt (347,348). In addition, nNOS inhibition sensitizes the tubuloglomerular feedback mechanism after volume expansion (349). Macula densa cells detect changes in distal sodium chloride concentration, at least in part, through an apical Na:2Cl:K cotransporter (350). Macula densa NO directly inhibits Na:2Cl:K cotransport and NO and angiotensin II independently alter cotransporter activity (350). To determine the role of the local renin-angiotensin system on TGF, mice with absent renal tissue expression of ACE were studied (351). TGF was absent in mice without ACE in the kidney suggesting that renal tissue ACE is an important contributor to TGF (351). Mice deficient in adenosine A_1 receptors lack TGF (352). Mice deficient in ecto-5′nucleotidase/CD73, the enzyme responsible for adenosine formation from AMP have an impairment of TGF regulation of GFR (353).

Taken together, the proximal tubular injury and resultant dysfunction could contribute to the drastic fall in GFR, the hallmark of ischemic ARF. One potential mechanism is increased TGF. Specifically in ARF, the decreased proximal tubule reabsorption would increase solute delivery to the macular densa with the resultant constriction of the afferent arteriole and fall in GFR (354). In normal nephrons, the maximal fall in GFR with increased solute delivery to the macular densa is approximately 50%. Thus, increased TGF could be a major factor in mediating the pathway whereby proximal tubule damage could lower GFR. However, because clinical ATN or ischemic ARF is associated with a 90% fall in GFR, either additional factors or increased sensitivity of TGF postischemic injury to the kidney must occur. In that regard, dissected afferent arterioles from ischemic kidneys have been shown to have increased cytosolic calcium concentrations and enhanced vasoconstriction responses to angiotensin II and endothelin (355,356). It is thus theoretically tenable that the sensitivity of the TGF is indeed enhanced postischemia. However, the role of TGF in ischemic ARF remains controversial (357).

In support of a pathogenic role of TGF in ischemic ARF are studies by Brian Meyer's group that demonstrated the following (55,56): (i) Translocation of NaK-ATPase to the cytoplasm results in depolarization confined to the proximal tubule; (ii) Fractional excretion of lithium, a surrogate measure for the fraction of filtered sodium that is delivered to the macula densa, the site of tubuloglomerular feedback, is massively increased, and (iii) These abnormalities persist for the duration of the

maintenance phase of postischemic ARF. This study provides evidence for decreased proximal reabsorption of sodium, resultant increased sodium delivery to macula densa, tubuloglomerular feedback and resultant filtration failure that accompanies ischemic ARF.

Another pathway whereby tubular injury can contribute to a fall in GFR is by causing intraluminal cast formation and tubule obstruction. This will be the next topic discussed.

TUBULAR CAST FORMATION AND OBSTRUCTION POSTRENAL ISCHEMIA

The classic radiological findings in early ARF, prior to the realization that contrast is nephrotoxic, was an early dense nephrogram not followed by a pyelogram. Because the nephrogram phase represents contrast entering the tubules by filtration, a persistent nephrogram suggests tubular obstruction with ongoing glomerular filtration.

Kidneys with ischemic ARF or ATN are swollen and, therefore, it was suggested that interstitial edema may lead to tubular collapse secondary to extraluminal-mediated compression. It is clear however that recovery from ATN can occur when the kidneys are still enlarged and swollen. Increased excretion of tubular epithelial casts are however a hallmark of recovery from ATN (23). The presence of tubular casts on renal biopsy as well as urinary casts has provided morphological support for a role of tubular obstruction due to intraluminal cast formation in the pathogenesis of ischemic ARF (358). As noted earlier, while earlier micropuncture studies failed to consistently demonstrate increased tubular pressures postischemia, several subsequent studies provided convincing evidence for the presence of tubular obstruction in experimental ischemic ARF. Finn and Gottschalk using micropuncture techniques during saline loading demonstrated clear evidence of increased tubular pressures in postischemic, as compared to normal kidneys (359). Renal vasodilation to restore renal blood flow also demonstrated increased tubular pressures in ischemic ARF in the rat. Perhaps the most compelling studies, however, were those micropuncture experiments performed by Tanner et al. (360). They found that perfusing the proximal tubule with artificial tubular fluid at a rate, which did not increase tubule pressure in normal animals, increased tubule pressures in animals after a renal ischemic insult. Moreover, venting those obstructed tubules led to improved nephron filtration rates. Burke et al. also demonstrated that prevention of ischemic ARF in dogs with mannitol led to a decrease in intratubular pressures, suggesting that the induced-solute diuresis led to relief of cast-mediated tubular obstruction (361).

While it is clear that brush border membranes, necrotic cells, viable cells and perhaps apoptotic tubular epithelial cells enter tubular fluid after an acute renal ischemic insult, the actual process and predominant location of the cast formation is however less clear. It is known, however, that the casts uniformly stain for Tamm Horsfall protein (THP) (358).

Integrins

Integrins are heterodimeric glycoproteins consisting of different combinations of alpha and beta subunits; they recognize the most common universal tripeptide sequence, arginine-glycine-aspartic acid (RGD), which is present in a variety of matrix proteins (9). These integrins can mediate cell-cell adhesion via an RGD inhibitable mechanism (8).

In normal kidneys, proximal tubular cells are stained by the RGD peptide, RhoG-RGD, basolaterally in a punctuate pattern and with Bt-RGD only minimally. On the other hand, ischemic kidneys labeling with RhoG-RGD and Bt-RGD occurred at the basolateral and apical aspect of tubular cells as well as on desquamating or desquamated cells within the tubular lumen and also on the vasa rectae (362). In ischemic kidneys, antibodies to beta1 and αV subunits of integrins stained glomeruli and the apical aspect of the proximal and distal tubules. Desquamated cells and cellular conglomerates obstructing the tubular lumina were intensely stained with RGD peptides (363). Dual labeling experiments with Bt-RGD and antibodies against integrin receptors demonstrated $\alpha V\beta 3$ binding sites for RGD peptides in the vasculature and some desquamated cells, whereas the majority of the desquamated cells bind Bt-RGD via $\beta 1$ integrins (362).

Experimental results support a role for adhesion molecules in the formation of casts. It has been shown that a translocation of integrins to the apical membrane of tubular epithelial cells may occur with ischemia (8,257,258). Possible mechanisms for the loss of the polarized distribution of integrins include cytoskeletal disruption, state of phosphorylation, activation of proteases, and production of NO (364,365). These integrins are known to recognize arginine, glycine, aspartate (RGD) tripeptide sequences (10,363). Thus, viable intra-luminal cells could adhere to other luminal or paraluminal cells. The Goligorsky group provided experimental evidence for this cell-cell adhesion process as a contributor to tubule obstruction in ischemic ARF. Synthetic cyclical RGD peptides were infused prior to the renal ischemic insult in order to block cell to cell adhesion as a component of tubule obstruction (11,366–369). Using micropuncture techniques the cyclic RGD tripeptides blocked the rise in tubular pressure postischemic insult (10). *In vivo* study of RGD peptides (cyclic RGDDFLG and RGDDFV) in ischemic ARF in rats demonstrated attenuation of renal injury and accelerated recovery of renal function (11). Systemic administration of fluorescent derivatives of two different cyclic RGD peptides, a cyclic Bt-RGD peptide and a linear RhoG-RGD peptide, infused after the release of renal artery clamp ameliorated ischemic ARF in rats (11,368). The staining of these peptides suggests that cyclic RGD peptides inhibited tubular obstruction by predominantly preventing cell-to-cell adhesion, rather than cell-to-matrix adhesion (363).

In addition to cell-cell adhesion, it is worthy to note that Bonventre et al. have demonstrated increased fibronectin in the tubular lumen after an ischemic insult and fibronectin is known to possess RGD sequences, which are recognized by cellular integrins (370). Moreover, THP is known to possess an RGD sequence, which may or may not be in a position to be recognized by integrins. This possibility however led to *in vitro* cellular adhesion studies in which $LLCPK_1$ cell adhesion to several different matrices, (i.e., collagen I and collagen IV), was examined (364). Interestingly, THP diminished cell adhesion in artificial fluid mimicking distal tubular fluid but not tubular fluid similar to ATN or collecting duct fluid have significantly higher ionic concentrations (371). In this regard, it has been suggested that THP becomes a polymeric gel in the presence of high ionic strength fluid, but is a nongel monomeric substance in low ionic strength fluid. Recent studies documented that the gel formation by THP is an active process that can be abolished by boiling. A role of the oligosaccharide component of THP in the gel formation was demonstrated, since n-glycanase treatment to remove the oligosaccharide abolished the gel formation (371).

Thus, the intraluminal presence of brush border membranes, viable and nonviable cells in association with extracellular matrix, (e.g., fibronectin), THP and adhesion molecules support their involvement in cast formation in ischemic ARF. The actual tubular obstruction by the casts however may only occur in the presence of the impaired vascular responses to renal ischemia. More specifically, if net glomerular filtration pressure were normal, the majority of the tubular casts may be

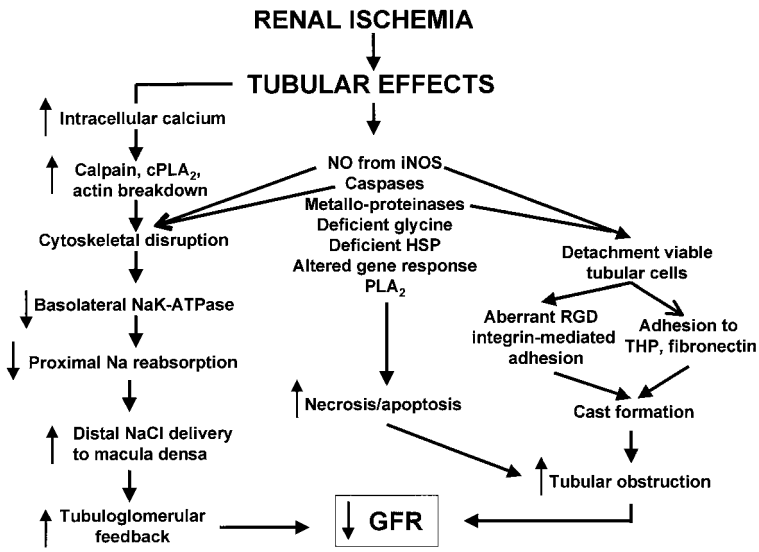

FIGURE 39-10. Tubular factors in the pathogenesis of ischemic ARF. *THP* = Tamm-Horsfall protein, PLA_2 = phospholipase A_2. (Reproduced from: Kribben A, Edelstein CL, Schrier RW. Pathophysiology of acute renal failure. *J Nephrol* 1999;12[Suppl 2]:S142, with permission.)

excreted in the urine rather than lodging in the collecting duct and other nephron sites. Tubular factors in the pathogenesis of ischemic ARF are shown in Figure 39-10. The various perturbations in the renal vasculature, which occur in association with a renal ischemic insult, will now be discussed.

VASCULAR PERTURBATIONS POSTRENAL ISCHEMIA

Ischemic ARF is associated with renal vasoconstriction with a resultant decrease in glomerular hydrostatic pressure and renal plasma flow (16,17,372). Not only are circulatory vasoconstrictors, such as catecholamines, angiotensin II and endothelin, as well as renal sympathetic tone, frequently increased in the setting of ischemic ARF (72), but the renal vascular response has been shown to be enhanced. This increased response to vasoconstrictors is due in part to the earlier mentioned increase in cytosolic calcium concentration in the afferent arterioles of the glomerulus. The endothelial damage is also associated with a diminution of the renal vasodilators which oppose the action of vasoconstrictors. In experimental sepsis, the NO secondary to iNOS has been suggested to downregulate renal eNOS (373). Moreover, the renal clamp model of ARF in the rat has been shown to be associated with downregulation of endothelial-derived nitric oxide (eDNO) (355,374). Recent studies have also shown that endothelin receptor antagonists ameliorate the diminution in renal hemodynamics associated with renal ischemia in the isolated perfused rat kidney (375,376). Further support for endothelin as an important mediator of ischemia-reperfusion induced renal injury is the protective effect of an endothelin-A receptor antagonist in rats after clamping of the renal arteries (377). Impairment of prostaglandin synthesis by damaged endothelium can also profoundly enhance renal vascular resistance associated with renal ischemia. In this regard, infusion of prostaglandin E1 may protect against ischemic ARF (378). Also, inhibition of thromboxane A2 improves renal function in rats exposed to warm ischemia-reperfusion (379).

Oxygen Free Radicals

A large number of studies have been performed in the isolated perfused rat kidney (IPRK), which cast light on the role of oxygen radicals in the biochemical and morphological changes which follow hypoxia and reperfusion. Studies with ^{31}P magnetic resonance spectroscopy (MRS) confirmed the rapid onset of ATP depletion with induction of hypoxia (380). These same studies also demonstrated that the extent of morphological injury during different degrees of hypoxic perfusion was proportional to the extent of ATP depletion. Further studies with ^{23}Na, ^{31}P and ^{87}Rb (a congener of potassium) MRS demonstrated that increases in intracellular sodium and decreases in potassium accompanied the decrease in ATP induced by hypoxia (381). Subsequent investigation revealed that the rate and extent of increase in sodium was reduced by pretreatment with dimethylthiourea (DMTU) and dimethylsulfoxide (DMSO), both scavengers of oxygen-derived free radicals (OFR) (382). These studies supported similar indirect evidence for injury induced by OFR during reperfusion (383,384). Whereas some studies have demonstrated that activated neutrophils produce OFR injury after ischemia (385,386), other studies in isolated proximal tubules (387) and the studies in the cell-free isolated perfused rat kidney, for example, (382) indicated that OFR were generated and contributed to the injury process even in the absence of neutrophils. The identification of the actual species of OFR involved in reperfusion injury required direct methods of detection rather than a reliance on scavengers.

Direct detection of hydoxyl radicals was initially achieved in isolated proximal tubules by using biochemical traps (387). Similar studies were subsequently performed in the intact kidney using 0.5 mM salicylate to react with hydroxyl radicals during reperfusion for 15 minutes after ischemia of 15 minutes (388). An increase in 2,5 dihydroxybenzoic acid was observed using high performance liquid chromotography (HPLC) with electrochemical detection. Subsequent studies by Endre et al. (389) utilized electron paramagnetic resonance (EPR) and 5,5-dimethyl-l-pyrroline N-oxide (DMPO) as a spin trap confirmed that hydroxyl radicals were generated during a briefer 3-minute reperfusion period following 20 minutes of ischemia. Interestingly, both studies demonstrated a significant generation of hydroxyl radicals in control kidneys, which was abolished by the addition of the scavenger, DMTU. An increase in an unidentified carbon-centered radical was also identified during reperfusion in the EPR study and could represent an early lipid peroxidation product (389).

With 60 minutes of reperfusion after 20 minutes of ischemia in the IPRK, tubular damage was prominent in both the cortical and medullary proximal tubule and in the MTAL (342). The morphological changes included cellular blebbing, brush

border damage, tubular casts, and occasional apoptosis. The MTAL always demonstrates signs of injury during cell-free perfusion (see the subsequent text), so caution is required in interpreting damage in this region. DNA fragmentation was detected predominantly in the MTAL and distal tubule using the technique of in-situ end labeling (342). Pretreatment with either allopurinol, which acts both to inhibit xanthine oxidase and as an OFR scavenger, or DMTU, reduced both the morphological features of injury the extent of DNA fragmentation in the MTAL. Taken together, these results suggest that hydroxyl radicals formed during reperfusion after ischemia play a significant role in both necrotic and apoptotic cell injury.

On the background of these postischemic vascular perturbations is the observation that a decrease in renal perfusion pressure is not associated with autoregulation of either GFR or renal blood flow (17,355,372,390–392). In fact, rather than renal vasodilation, renal vasoconstriction occurs with a fall in renal perfusion pressure in the postischemic kidney. Thus, a degree of hypotension, which is of no clinical significance in the normal kidney, may cause renal damage in the kidney during the recovery phase of ATN. The same increased sensitivity in the postischemic kidney has also been shown to occur with nephrotoxic agents such as aminoglycosides.

The increased vulnerability to recurrent ischemic injury is a possible reason for the clinical finding of fresh tubular necrosis even 4 weeks after the initiation of ARF. Specifically, in patients with prolonged ARF after combat injury, a prominent finding in biopsy or autopsy specimens obtained 3 to 4 weeks after the initial insult was fresh tubular renal ischemic lesions that could not be related to the remote initial ischemic insult (393). A possible explanation for the fresh ischemic lesions was altered reactivity of the renal vasculature. Transient reduction of blood pressure within the normal autoregulatory range, which frequently occurs during intermittent hemodialysis treatment, can actually result in recurrent ischemic injury and prolongation of ARF due to the altered vascular reactivity of the injured kidneys (394). In humans, a deterioration of renal function parameters during hemodialysis are in accordance with this proposal of altered autoregulation (365).

Studies to examine the role of Ca^{2+} and CCB in the vascular perturbations in experimental ischemic ARF have been performed. These studies demonstrate that intrarenal CCB can reverse the increased sensitivity to renal nerve stimulation as well as the loss of renal autoregulation, both of which characterize experimental ARF (395). In addition, other studies in the rat showed that atrial natriuretic peptide (ANP), which attenuates vasoconstrictor-induced increases in $[Ca^{2+}]_i$ in cultured vascular smooth muscle cells (396), is also protective against ischemic ARF (397), despite the fact that its systemic administration causes a fall in arterial pressure.

Endotoxemia-Induced ARF

Recent experimental studies in septic mice have incriminated still another mediator of renal vasoconstriction. The intraperitoneal administration of lipopolysaccharide (LPS) as an endotoxin was associated with a profound decrease in GFR and renal blood flow both in wild type and iNOS knockout mice. A soluble receptor of tumor necrosis factor (TNF), however, was associated with profound improvement in renal hemodynamics in both wild type and iNOS knockout mice (398). Since septic patients with renal failure have a high mortality, this observation has potential therapeutic importance.

Sepsis in mice has also been shown to be associated with an impaired response of NO-mediated cyclic GMP in the renal cortex, the agent's secondary messenger for vasodilation (399). The renal nerves and activation of the renin-angiotensin system contribute to renal vasoconstriction during sepsis. In this study, renal denervation decreased the high plasma renin levels during endotoxemia and was protective against the decreased GFR and renal blood flow in a normotensive model of endotoxemia-induced sepsis (400).

Oxygen radicals may contribute to the vasoconstriction in endotoxemia-induced ARF (401). ARF during sepsis is associated with increased nitric oxide and oxygen radicals including superoxide. Renal extracellular superoxide dismutase (EC-SOD) is decreased in endoteoxemia (402). Antioxidant therapy with chemically dissimilar antioxidants, metalloporphyrin and tempol, preserved GFR and renal blood flow during endotoxemia (402). This protective effect was reversed by inhibition of iNOS suggesting the importance of the bioavailability of NO for preservation of renal function during endotoxemia (402).

The demonstration of global renal vasoconstriction in sepsis may depend on the model used. In a nonlethal hyperdynamic model of sepsis in sheep injected with *Escherichia coli*, renal failure developed despite markedly increased renal blood flow (403).

In another study in endotoxemia-induced ARF, the role of renal inflammation and apoptosis was determined (404). In this study, LPS acted on extrarenal Toll-like receptor 4 leading to systemic TNF release and subsequent ARF. Mice with a mutation in Toll-like receptor 4 were resistant to LPS-induced ARF and had less neutrophil infiltration and renal cell apoptosis (404).

INFLAMMATION IN POSTRENAL ISCHEMIA

Ischemic ARF has been described as an inflammatory disease (19). This is evidenced by numerous studies demonstrating endothelial injury, leukocyte infiltration in the kidney and the generation of inflammatory mediators by tubular cells (19).

Endothelial Cell injury

Silver nitrate staining of blood vessels was studied in rats at 4 hr after release of a renal artery clamp (405). Ischemic ARF resulted in disorganization of endothelial integrity with areas of denudation, partial disappearance of cell-cell borders and distortion of cell–cell contacts most prominent in the renal microvasculature. Intravital microscopy of blood flow in peritubular capillaries provided direct evidence for the existence of a "no flow" phenomenon caused by endothelial injury (405,406). Transplantation of endothelial cells or surrogate cells expressing endothelial NOS, either intravenously or intraarterially, resulted in functional protection against ischemic ARF (405). These studies suggested that endothelial cell injury is the primary cause of the "no-flow" phenomenon and that when ameliorated there is attenuation of renal function.

It has been demonstrated in mice that injury of renal microvascular endothelium alters barrier function after ischemia (407). In this study, circulating von Willebrand factor (vWF), a marker of endothelial injury, was increased in the circulation 24 hours after ischemia. In FVB-TIE2/GFP mice, in which the microvasculature can be visualized, there were alterations in the cytoskeleton and alterations in the integrity of adherens junctions that correlated with a permeability defect identified using fluorescent dextrans and two-photon intravital imaging (407).

The extension phase of ARF is marked by continued hypoxia and an inflammatory response, which are more marked in the corticomedullary junction (18). Severely reduced blood flow, stasis and accumulation of red blood cells has been documented in the corticomedullary region (18). Endothelial cell

injury is thought to play an important role in the initiation and extension phase of ischemic ARF (18).

Neutrophil Activation

Renal ischemia-reperfusion injury is associated with an increase in infiltrating neutrophils (408). The adherence of neutrophils to the vascular endothelium is an essential step in the extravasation of these cells into ischemic tissue (28). Therefore, leukocyte adhesion molecules have been studied in renal injury (409). After adherence and chemotaxis, infiltrating leukocytes release reactive oxygen species and enzymes that damage the cells (28). Infusion of normal neutrophils accentuates severe ischemia/reperfusion injury and decreases GFR during ischemia. Activated neutrophils have been shown to enhance the decrease in GFR in response to renal ischemia at least in part due to release of oxygen radicals (386,410–412). In contrast, infusion of oxygen radical deficient neutrophils from patients with chronic granulomatous disease patients did not worsen the course of ischemic injury (411). The mechanism by which adherent leukocytes cause ischemic injury is unclear, but likely involves both the release of potent vasoconstrictors including the prostaglandins, leukotrienes, and thromboxanes (413), as well as direct endothelial injury via release of endothelin and a decrease in NO (72,414).

Intracellular adhesion molecule I (ICAM I) has been suggested to play an important role in the pathophysiology of ischemic ARF (408,415). Increased systemic levels of the cytokines, tumor necrosis factor (TNF-alpha) and interleukin-1 (IL-1) may upregulate ICAM-1 after ischemia and reperfusion in the kidney (415). ICAM-1 on endothelial cells promotes the adhesion of neutrophils to these cells and causes tissue damage. The administration of a monoclonal antibody against ICAM-1 protected against ischemic ARF in rats (408,411). Pretreatment with an ICAM-1 antisense oligodeoxyribonucleotide ameliorated the ischemia-induced infiltration of granulocytes and macrophages and resulted in less cortical renal damage as assessed by a quantitative pathologic grading scale (416). In parallel, ICAM-1 deficient mice are protected against renal ischemia (415). Thus, ICAM-1 is a mediator of ischemic ARF probably by potentiating neutrophil-endothelial interactions.

Red blood cell swelling has been suggested to cause the medullary blood flow congestion which occurs postrenal ischemia and worsens the relative hypoxia in that region of the kidney. The restoration of renal blood flow in experimental renal ischemia in the dog, however, occurs with either an isotonic or hypertonic mannitol induced-diuresis. There is now evidence that upregulation of adhesion molecules may contribute to this impaired medullary blood flow postischemic injury (409,417,418).

P-selectin, an important molecule involved in adherence of circulating leukocytes to tissue in inflammatory states, also seems to be involved in the infiltration of the leukocytes during ischemic injury. In fact, renal ischemia has also been shown to be associated with upregulation of endothelial P-selectin with enhanced adhesion of neutrophils (419). A soluble P-selectin glycoprotein ligand prevented infiltration of leukocytes and ameliorated ischemia induced renal dysfunction (365). In contrast to P-selectin, the L-selectin does not appear to mediate tubular damage in postischemic kidney (409). After adherence and chemotaxis, neutrophils release reactive oxygen species or oxygen free radicals.

There is evidence that neutrophils, mediate tubular injury in ARF (420). This evidence is derived from studies that show an accumulation of neutrophils in ischemic ARF and studies demonstrating a beneficial role of anti-ICAM-1 therapy in ARF (415). Also, mice depleted of peripheral neutrophils by antineutrophil serum were protected against ischemic ARF (415). However, in another study, rats depleted of peripheral neutrophils by anti-neutrophil serum were not protected against ischemic ARF (421). In another study, mice were injected with 0.1 mg of the rat IgG2b monoclonal antibody RB6-8C5 (BD Pharmingen) intraperitoneally 24 hours before renal pedicle clamp (225). This results in depletion of neutrophils in the peripheral blood and in the kidney during ischemic ARF. In this study, there was the slight functional protection and no histological protection against ischemic ARF in neutrophil-depleted mice (225).

Lymphocytes

The role of lymphocytes in ischemic ARF is an ongoing area of study (422,423). Lymphocytes has been examined in genetically altered, immune deficient mice. In one study, mice with a combined deficiency of both CD4 and CD8 cells were protected against ischemic ARF at 48 hours, but not 24 hours, postischemic reperfusion (424). In a follow-up report by the same investigators, *nu/nu* mice that are athymic and deficient in both CD4 and CD8 T cells, were protected against ischemic ARF 24 and 48 hours postischemic reperfusion (425). To determine the pathogenic T cell type, mice with targeted genetic deficiencies of either CD4 or CD8 T cells were also studied. CD4 deficient mice, but not CD8 deficient, are protected against ischemic ARF (425). Therefore, it appears that the pathogenic T cell subtype in ischemic ARF is the CD4 T cell. However, RAG-1 −/− mice, which lack mature T and B lymphocytes, are not protected against ischemic ARF despite lacking both CD4 and CD8 T cells (426). In a recent study, mice deficient in B lymphocytes alone *were* protected against ischemic ARF (427). In summary, CD4 T cell deficient, *nu/nu* (lacking mature T cells), and B cell deficient mice are protected against ischemic ARF while CD8 T cell and RAG-1 −/− (lacking mature B and T cells) mice are not protected.

The effect of complete depletion of CD4 T cells with a monoclonal antibody in ischemic ARF is not known. In one report, the use of GK1.5 antibody alone to deplete CD4 T cells did not protect against ischemic ARF; however, complete depletion of CD4 T cells as judged by FACS analysis did not occur (428). Protection did occur when GK1.5 was used with two other antibodies, which resulted in the depletion of both CD4 and CD8 T cells (428). In another study, complete depletion of CD4 T cells using the GK1.5 antibody was not protective against ischemic ARF in mice (429). In summary, it is believed that CD4 T cells are important in the pathogenesis of ischemic ARF and that very few T cells in the kidney are enough to contribute to injury (425) (423,428).

Monocyte/macrophages

Another inflammatory cell that is a potential mediator of injury in ischemic ARF is the monocyte/macrophage (423). Macrophages infiltrate the postischemic rat kidney (430). Macrophage chemoattractants, for example, monocyte chemoattractant protein-1 (MCP-1) are increased in the postischemic rat kidney (423). In a model of macrophage depletion using liposomal clodronate, it was demonstrated that macrophages contribute to tissue damage during acute rejection (431). An anti-B7-1 antibody blocks mononuclear cell adherence in vasa recta in rats and attenuates ischemic ARF both functionally and histologically (432). Gene therapy in rats expressing an amino-terminal truncated MCP-1 reduced macrophage infiltration and ATN (433).Two recent studies have demonstrated that macrophage depletion using liposomal clodronate is protective against ischemic ARF in mice (434,435).

Inflammatory Mediators

In renal ischemia/reperfusion, tubular epithelial cells produce TNF-alpha, IL-1, IL-6, IL-8, IL-18 TGF-beta, MCP-1, RANTES and fractalkines (19,224,225). Leukocytes produce TNF-alpha, IL-1, IL-8, MCP-1, reactive oxygen species and eicosanoids (19). The antiinflammatory cytokine, IL-10, inhibits TNF-alpha, ICAM-1 and iNOS and protects against ischemic and cisplatin-induced renal failure and ATN (436).

Statins are potent antiinflammatory drugs (437). Both *in vitro* and *in vivo* studies suggest lipid lowering–independent antiinflammatory functions of statins (437). After adhesion to the vascular endothelium, inflammatory cells migrate to the site of inflammation (437). Statins act independently of lipid lowering to selectively inhibit leukocyte adhesion by direct interactions with the leukocyte-function antigen-1 (LFA-1) (437). Statins reduce macrophage influx and chemokine expression in rat kidneys (438). Statins are known to decrease the expression of pro-inflammatory cytokines by inflammatory cells (437). Simvastatin reduces expression of IL-6 and monocyte chemoattractant protein (MCP-1) in monocytes from hypercholestrolemic patients and in cultured endothelial cells (437). Pravastatin downregulates TNF-alpha and MCP-1 (439] in human monocytes *in vitro*. Some of the antiinflammatory effects of statins are mediated by nitric oxide.

Rats were treated with cerivastatin or vehicle for 3 days before induction of ischemic ARF (440). Statin treatment reduced the increase in serum creatinine by 40% and protected against tubular necrosis. In addition, monocyte and macrophage infiltration was almost completely prevented, ICAM-1 upregulation was decreased and iNOS expression was reduced (440). In another study, atorvastatin improved the course of ischemic ARF in aging rats by enhancing nitric oxide availability and improving renal hemodynamics (441).

RECOVERY FROM ISCHEMIC ARF

It has been demonstrated in rats that severe ischemic ARF results in permanent alteration in renal capillary density that contributes to a urinary concentrating defect and renal fibrosis (442,443). However, in contrast to other organs, the kidney can recover from tubular necrosis, at least in the short term. There are 2 potential mechanisms of recovery: (i) dedifferentiation and proliferation of the surviving non necrotic tubular cells (444) and (ii) mobilization and delivery of bone marrow stem cells to the injured kidney (445).

After ischemic ARF there is proliferation of tubular cells that mimics events in the developing kidney (446). Epithelial cells are also dedifferentiated during the recovery period (444). In the postiscehmic kidney, there is also expression of genes that encode growth factors (301,303,306). Dedifferentiation and proliferation of tubular epithelial cells may result in the spreading of cells over the damaged basement membrane. Cell adhesion molecules like neural cell adhesion molecule (NCAM), cytokines and kidney injury molecule-1 (Kim-1) (62,447) may play a role in these processes. Targeted delivery of hepatocyte growth factor (HGF) to the proximal tubule in transgenic mice results in marked protection against ischemic ARF (448). Understanding the physiology of repair and recovery of surviving tubular cells may lead to therapies to hasten the recovery process in humans.

Bone marrow stem cells have the capacity to migrate to other organs (445). Bone marrow derived cells can populate and contribute to turnover of both the normal and injured renal tubular epithelium (449). Cells from the adult mouse bone marrow are mobilized into the circulation by transient renal ischemia and home specifically to the injured kidney where they differentiate into tubular epithelial cells (445). It was investigated whether an increase in circulating stem cells, using pharmacological mobilization from the bone marrow, would improve renal function in mice with ischemic ARF (450). The pharmacological increase in stem cells was associated with marked granulocytosis and worsening of renal failure. In the future, therapies aimed at stimulating the proliferation, mobilization and targeting of stem cells may enhance recovery from ischemic ARF (445).

SUMMARY

Tubular and vascular perturbations and inflammation combine to cause ischemic ARF. The tubular and vascular events in ischemic ARF are summarized in Figures 39-10 and 39-11. The inflammatory events are summarized in Figure 39-12. Recent laboratory studies using *in vivo*, cellular and molecular approaches have provided substantial insight into the pathogenesis of the syndrome. These studies have identified several potential therapeutic interventions, which need to be tested with prospective clinical trials. Interventions which have

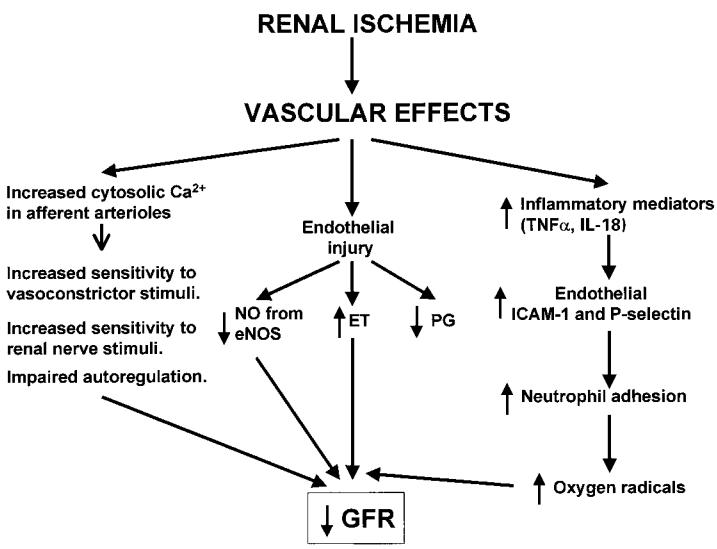

FIGURE 39-11. Vascular factors in the pathogenesis of ischemic ARF. *ET*, endothelin; *PG*, prostaglandins; *TNF α*, tumor necrosis factor. (Reproduced from: Kribben A, Edelstein CL, Schrier RW. Pathophysiology of acute renal failure. *J Nephrol* 1999;12[Suppl 2]:S142, with permission.)

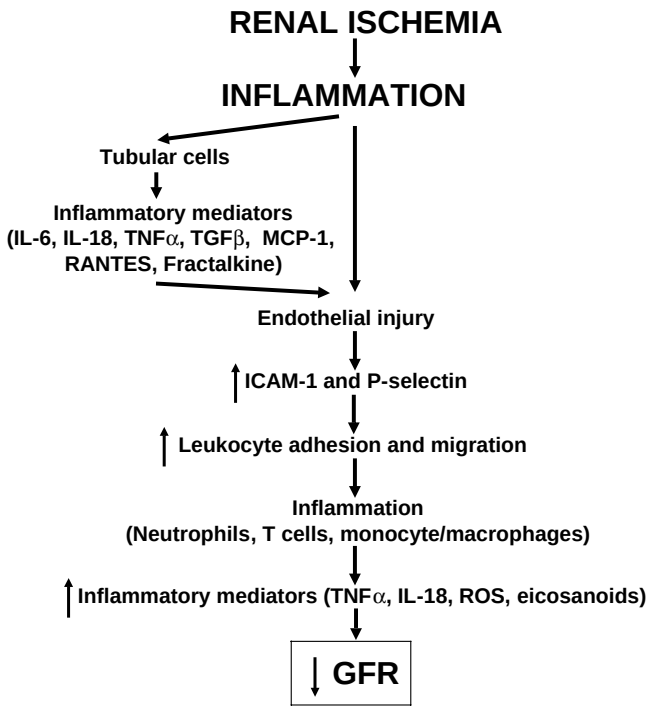

FIGURE 39-12. Inflammatory response in ischemic ARF. *IL-1*, interleukin-1; *IL-18*, interleukin-18; *TNFα*, tumor necrosis factor alpha; *TGFβ*, transforming growth factor beta; *RANTES*, regulated upon activation, normal T cell expressed and secreted, *ROS*, reactive oxygen species; *ICAM-1*, intracellular adhesion molecule-1; *MCP-1*, monocyte chemoattractant protein-1.

attenuated experimental ischemic/hypoxic proximal tubule damage include cysteine protease inhibitors, ά MSH, specific iNOS inhibition, synthetic cyclical RGD sequences, mannitol, oxygen radical scavengers, TNF soluble receptors, inducers of HSP and anti-ICAM antibodies, endothelin antagonists, IL-18 antiserum, erythropoietin, CTLA4 immunoglobulin to mention a few (Table 39-9). The intrarenal administration of these compounds may be necessary to avoid systemic complications. In that regard, the hypotensive effect of atrial natriuretic peptide in recent trials no doubt obscured any intrarenal beneficial effect of the compound.

TABLE 39-9

SOME EMERGING THERAPIES FOR ISCHEMIC ACUTE RENAL FAILURE

Cysteine protease inhibitors
Caspase inhibitors
IL-18 inhibition
ά MSH
Specific iNOS inhibition
Synthetic cyclical RGD sequences
Oxygen radical scavengers
TNF soluble receptors
Inducers of HSP
Anti-ICAM antibodies
Endothelin antagonists
Endothelial cell infusion
Mannitol with natriuretic peptides or calcium channel blockers
Erythropoeitin
CTLA4 immunoglobulin

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CHAPTER 40 ■ PATHOPHYSIOLOGY OF NEPHROTOXIC CELL INJURY

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INTRODUCTION

Nephrotoxic epithelial cell injury induced by chemicals can result in acute renal failure (ARF) and can be mediated via a variety of mechanisms. After direct or indirect chemical-induced injury, renal epithelial cells either repair and regenerate or die. If the degree of cell death is sufficient, then overall renal function declines and ARF results. Indirect chemical insults may decrease renal blood flow, thereby causing renal ischemia and reperfusion-induced cell injury and death. Furthermore, recent studies demonstrate that the T and B cells and, to a lesser extent, macrophages and neutrophils inhibit renal function in ARF (1–3). The role of inflammatory cells in ARF is likely secondary to the initial insult and occurs during the maintenance phase of ARF (4,5). These examples highlight the premise that multiple mechanisms are involved in the pathophysiology of nephrotoxic epithelial cell injury and ARF.

A secondary effect of nephrotoxicant-induced cell death in ARF can be the generation of "backleak." After an insult, injured and dead epithelial cells release from the basement membrane and adhere via integrins to other released and attached epithelial cells (4–11). The cellular aggregates can form tubular casts that block the flow of filtrate and increase intraluminal pressure, decreasing the single nephron glomerular filtration rate (4). In addition, the loss of epithelial cells can leave gaps in the basement membrane allowing tubular filtrate to "backleak" into the circulation, further decreasing the apparent single nephron glomerular filtration rate as currently measured. Thus, "backleak" and the loss of epithelial cells contribute to the decreased renal function.

As a consequence of either direct or indirect renal epithelial cell injury, cells die or repair and regenerate. Renal epithelial cells are known to repair and regenerate, and significant advances have been made to enhance our understanding of this process (12–16). Regeneration begins when cells adjacent to the injured area de-differentiate, proliferate, and migrate into the denuded areas. Ultimately, the cells differentiate and tubular structure and function are restored. Chemicals also may protract the maintenance phase of renal dysfunction through their ability to inhibit cellular repair and regeneration. If a nephrotoxicant inhibits this process, then recovery of normal renal function may be delayed or inhibited. For example, the recovery of renal function after treatment with the aminoglycoside antibiotic tobramycin and cisplatin were compared (10,11). A 4-day treatment with tobramycin resulted in renal dysfunction with proximal tubular necrosis that was followed by a proliferative response. Renal dysfunction was fully reversible and serum creatinine levels and proximal tubular morphology returned to the control values by day 14. Although a 4-day regimen of cisplatin also resulted in renal dysfunction with proximal tubular necrosis, the renal dysfunction persisted. These studies provide evidence that renal proximal tubules damaged by cisplatin may not undergo normal regeneration, which results in protracted ARF.

In vitro studies support the hypothesis that nephrotoxicants can inhibit the proliferative and/or migratory processes during regeneration. For example, Counts and colleagues (17) used an *in vitro* model of renal proximal tubular cell (RPTC) regeneration to determine whether nephrotoxicants can inhibit the proliferative and/or migratory processes during regeneration. Subsequent to mechanically induced injury to a confluent monolayer, $HgCl_2$, the mycotoxin fumonisin B_1, and the haloalkene cysteine conjugate, S-(1,2)-dichlorovinyl-L-cysteine, inhibited the normal proliferative and migratory response that resulted in the closure of the denuded areas in the absence overt cytotoxicity to the monolayer. Therefore, it is important to determine if the mechanisms involved in the pathophysiology of nephrotoxic-induced ARF are directly inducing cell death or inhibiting regeneration after cell death.

The goal of this chapter is to review some of the mechanisms by which chemicals produce renal epithelial cell injury and death. Other chapters in this volume, and several excellent reviews, discuss renal cell death and ARF produced by specific chemicals; the reader is referred to these for additional insights and perspectives (18–20).

SUSCEPTIBILITY OF THE KIDNEY TO INJURY

The susceptibility of the kidney to various agents can be attributed to several functional properties of this organ. These properties include: (a) receiving 20% to 25% of the cardiac output, ensuring high levels of toxicant delivery over a period of time; (b) extensive reabsorptive capacity with specialized transporters promoting cellular uptake of the toxicant; (c) urinary concentrating capacity that results in high concentrations of toxicants in the medullary lumen and interstitium; (d) biotransformation enzymes resulting in the formation of toxic metabolites and reactive intermediates; (e) high metabolic rates and workload of renal cells resulting in increased sensitivity to toxicants; and (f) sensitivity of the kidney to vasoactive agents.

Nephrotoxicants generally damage specific segments of the nephron, with the proximal tubule epithelial cell being the primary target. Furthermore, different segments of the proximal tubule (S_1, S_2, and S_3) are targets for different nephrotoxicants. For example, aminoglycoside antibiotics, chromate, cadmium chloride, and the mycotoxin citrinin primarily affect the S_1 and S_2 segments, whereas cyclosporine, $HgCl_2$, uranyl nitrate, cisplatin, bromobenzene, and cysteine conjugates of halogenated hydrocarbons affect the S_3 segment (21). In addition, interferon-α, gold, and penicillamine may target cells in the glomeruli, whereas nonsteroidal antiinflammatory drugs (NSAIDs) and angiotensin convertising enzyme (ACE) inhibitors may target cells in renal vessels (22). The reasons for these segmental differences may include: (a) differences in toxicant delivery to a given segment, (b) differences in transport

and uptake among segments, and (c) differences in biotransformation enzymes among segments.

XENOBIOTIC TRANSPORT

For most xenobiotics, transport into the epithelial cell is required for the expression of toxicity and xenobiotics, in general, enter cells by passive diffusion or by active or facilitated transport. With xenobiotic accumulation, injury is initiated with altered cellular functions. Likewise, inhibition of the transporter(s) responsible for xenobiotic import blocks injury. As stated earlier, differences in transporter expression among different tubular segments are one indicator of whether a given tubular segment is the target of xenobiotic toxicity.

Several transporters that participate in basal physiological functions of renal cells, such as reabsorption and secretion, participate in the transport of nephrotoxicants (23–25). These transporters include, but are not limited to, the organic cation transporters (OCT [26–28]), organic anion transporters (OATs [29–31]), amino acid transporters (32,33), and transporters involved in multi-drug resistance such as P-glycoprotein (34).

OATs and OCTs transport a variety of physiological compounds into renal cells (35). In addition, they transport drugs, natural products, and industrial chemical and pollutants (23,29,31,35–37). For example, the mycotoxin ochratoxin A is transported into renal tubular cells by OAT1, OAT 3, and OAT 5 (35,36,38) and induces renal cell death. Ochratoxin A transport into renal cells is inhibited by probenecid (39), an inhibitor of most OAT proteins, and probenecid blocks ochratoxin A-induced cell death (40). The uptake and cell death induced by the trichloroethylene metabolite S-(1,2)-dichlorovinyl-L-cysteine also is inhibited by probenecid (41) and para-aminohippurate (PAH, an OAT substrate, [37]) in isolated RPTC. The ability of Hg^{+2} and its cysteine-conjugates to induce cell death *in vivo* and in canine kidney cells is altered by inhibitors or substrates of OAT proteins, suggesting that the nephrotoxicity of this environmental contaminant is regulated by these transporters (29,31).

OCT proteins also play a significant role in xenobiotic transport and injury in renal cells. For example, OCT 1 and OCT 2 are required for the nephrotoxicity of platinum compounds including cisplatin, oxaliplatin, and carboplatin in Madin-Darby canine kidney cells (27). OCT 1 and OCT 2 also mediate the transport of 1-methyl-4-phenyl-pyridinium, disopyramide, and chlorpheniramine into renal cells (42).

The role of P-glycoprotein in chemical-induced renal cell death is still being investigated. However, the nephrotoxicity of chemotherapeutics, such as cisplatin, is altered by over expression of P-glcyoprotein (43). Further, P-glycoprotein may mediate the nephrotoxicity of diallyl disulfide and S-allyl-cysteine, two investigational chemotherapeutics (44).

CELL DEATH

In many cases, transport of toxicants into renal cells results in cell death. Cell death is generally thought to occur through one of two mechanisms: oncosis or apoptosis (45–47). Apoptosis is a tightly controlled, organized process that was initially defined morphologically, and morphologic changes remain the hallmark of apoptosis. *Necrosis* or *necrotic cell death* affects masses of contiguous cells and is characterized by the swelling of organelles and increases in cell volume, after which the cell membrane becomes more permeable and ruptures with the release of cellular contents, followed by inflammation (Fig. 40-1). Majno and Joris (45) pointed out that necrotic cell death is a poor term to describe cell death characterized by cellular volume increases and cell rupture. Historically, necrosis has been used to describe drastic tissue changes occurring after cell death. These changes include karyorrhexis, karyolysis, pyknosis, condensation of the cytoplasm, and intense eosinophilia, and alterations representing the cadaver of a cell. Temporally speaking, cells die long before necrotic changes are observed.

Manjo and Joris (45) suggested that the term *oncosis* (from *ónkos,* meaning "swelling"), a word coined by von Recklinghausen in 1910 to describe cell death with swelling, be used generically to describe cell death characterized by cellular swelling, organelle swelling, blebbing, and rupture (48). Levin and associates (49) have supported the use of the term oncosis and further suggested that the term necrosis be used to designate groups of dead cells, defined on histological sections, regardless of the type of cell death (apoptosis or oncosis).

Despite the fact that both apoptosis and oncosis induce necrosis, the morphologic and biochemical characteristics of oncosis and apoptosis are very different (45–47) (Fig. 40-1). The morphology associated with oncosis has been explained previously. Apoptosis usually affects scattered individual cells, and morphologically, the cell shrinks while organelle integrity is retained. The chromatin becomes pyknotic and marginates against the nuclear membrane. Ultimately, the cell shrinks to a dense round mass (apoptotic body) or forms pseudopodia (i.e., buds) containing nuclear fragments and/or organelles that break off into small fragments (apoptotic bodies). In either case, adjacent cells, or macrophages phagocytize the apoptotic bodies, and inflammation does not occur. Numerous authors incorrectly use the terms *programmed cell death* and *apoptosis* synonymously. The phrase "programmed cell death" refers to situations in which cells are programmed to die at a fixed time (e.g., during development) (46). Although the process and morphology of programmed cell death and apoptosis may be similar, the term apoptosis is more appropriate for chemical- and ischemia-induced cell death.

Apoptosis

One key difference between oncosis and apoptosis is the activation of caspases in the latter. Caspases belong to a 14-member family of cysteine proteases and are key mediators of apoptosis (50–52). Caspases can be divided into three groups based on structural differences and substrate preferences: initiator caspases (caspase -2, -8, -9, -10, and possibly -12), executioner or effector caspases (caspases -3, -6, and -7), and cytokine processors (caspases -1, -4, -5, and -13). Initiator caspases are activated by numerous processes including receptor-directed mechanisms and chemical exposures, and mediate chemical-induced apoptosis in numerous cell types, including proximal tubular cells (53–55), glomerular cells (56), medullary cells (57,58), and cells present in the collecting ducts (57–61). Activation of initiator caspases results in the activation of executioner caspases that leads to several of the biochemical characteristics of apoptosis. However, recent studies in nonrenal cells suggest that caspase-2 can induce apoptosis independent of its catalytic activity via a mechanism involving permeabilization of the mitochondrial membrane and subsequent release of pro-apoptotic proteins inducing cytochrome c, Smac/Diablo (62,63) (see the subsequent text).

Caspase-8, an initiator caspase, plays an integral role in receptor-mediated apoptosis (64–67). Caspase-8 is activated by membrane receptors such as Fas-ligand and TNFα receptors (66;68–70) and, in turn, cleaves the Bcl-2 family protein Bid to form tBid (64-67). tBid acts on mitochondria to cause the release of pro-apoptotic proteins and result in the

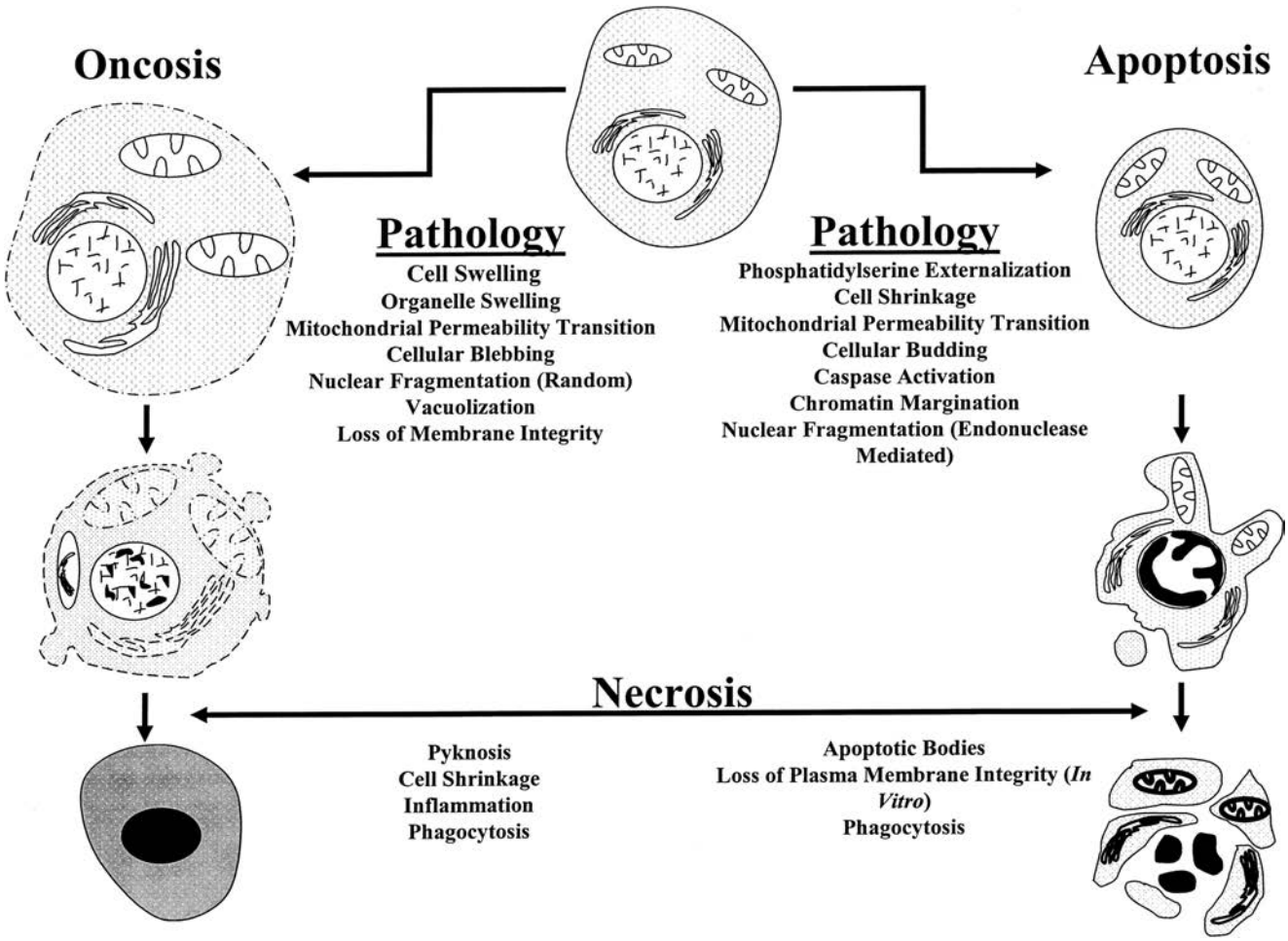

FIGURE 40-1. Comparison of the morphologic features of oncosis and apoptosis. At the top middle, a normal cell. **Left:** Cell and organelle swelling, followed by vacuolization, blebbing, and increased membrane permeability (lysis) and finally necrotic changes (i.e., coagulation, shrinkage, and karyolysis). **Right:** Cell shrinkage followed by budding and karyorrhexis and finally necrotic changes (i.e., breakup into cluster of apoptotic bodies). The pathology of each event is listed next to each pathway. (Adapted from: Cummings BS, Schnellmann RG. Measurement of cell death in mammalian cells. In: *Protocols in pharmacology*. New York: John Wiley & Sons, 2004;12:8, with permission.)

activation of caspase-9 and caspase-3. In contrast, caspase-8 may directly activate caspase-9 or caspase-3 independently of the mitochondrion (Figure 40-2). Chemicals may also activate caspase-8 independently of receptor-mediated mechanims. For example, cisplatin and etoposide activate caspase-8, caspase-9 and caspase-3 in LLC-PK1 cells (71) and U937 cells in the absence of receptor-stimulation (66). In contrast, cisplatin and cyclosporine activated caspase-3 in the absence of caspase-8 in mouse and rabbit RPTC (49–51). Thus, the role of caspase-8 in chemical-induced apoptosis is variable.

Executioner, or effector, caspases are responsible for the cleavage of a number of substrates that typically result in the morphologic features of apoptosis. For example, cleavage of the *I*nhibitor of *C*aspase-*A*ctivated *D*eoxyribonuclease (ICAD/DFF45) by caspases results in its deactivation and allows caspase-activated deoxyribonuclease to cleave DNA, resulting in the "ladder-like" pattern after agarose gel electrophoresis (72,73). Further, cleavage of poly(ADP-ribose) polymerase (PARP) by caspases-3 and -7 results in its activation and the addition of ADP-ribose monomers to a variety of proteins (74). Other substrates for caspases include DNA repair enzymes that prevent cells from making a futile attempt at repair, and scaffolding proteins that allow for the reorganization of the cytoskeleton and the packaging of the cell constituents into apoptotic bodies (51).

Role of Mitochondria in Apoptosis

Mitochondria are integral in the apoptotic process through the production of ATP and the release of pro-apoptotic factors. For example, the release of cytochrome c from mitochondria results in the binding of cytochrome c to apoptotic protease activating factor 1 (APAF-1), which promotes the binding and proteolytic cleavage of pro-caspase-9 to caspase-9 (51; 75). Activated caspase-9 cleaves and activates executioner caspases (i.e., caspases-3, -6, and -7) (Fig. 40-2). A number of toxicants, including cisplatin and S-(1,2)-dichlorovinyl-L cysteine, cause cytochrome c release and apoptosis (51,66,76,77). Cytochrome c release from the mitochondria is associated with a decrease in the mitochondrial inner membrane potential and the accumulation of several pro-apoptotic proteins such as Bad, Bak, and Bax at the mitochondria (Fig. 40-2). Other pro-apoptotic proteins released from the mitochondria include apoptosis-inducing factor (AIF), Smac/Diablo, Omi and Endo G (64–66,78–85) (Fig. 40-2).

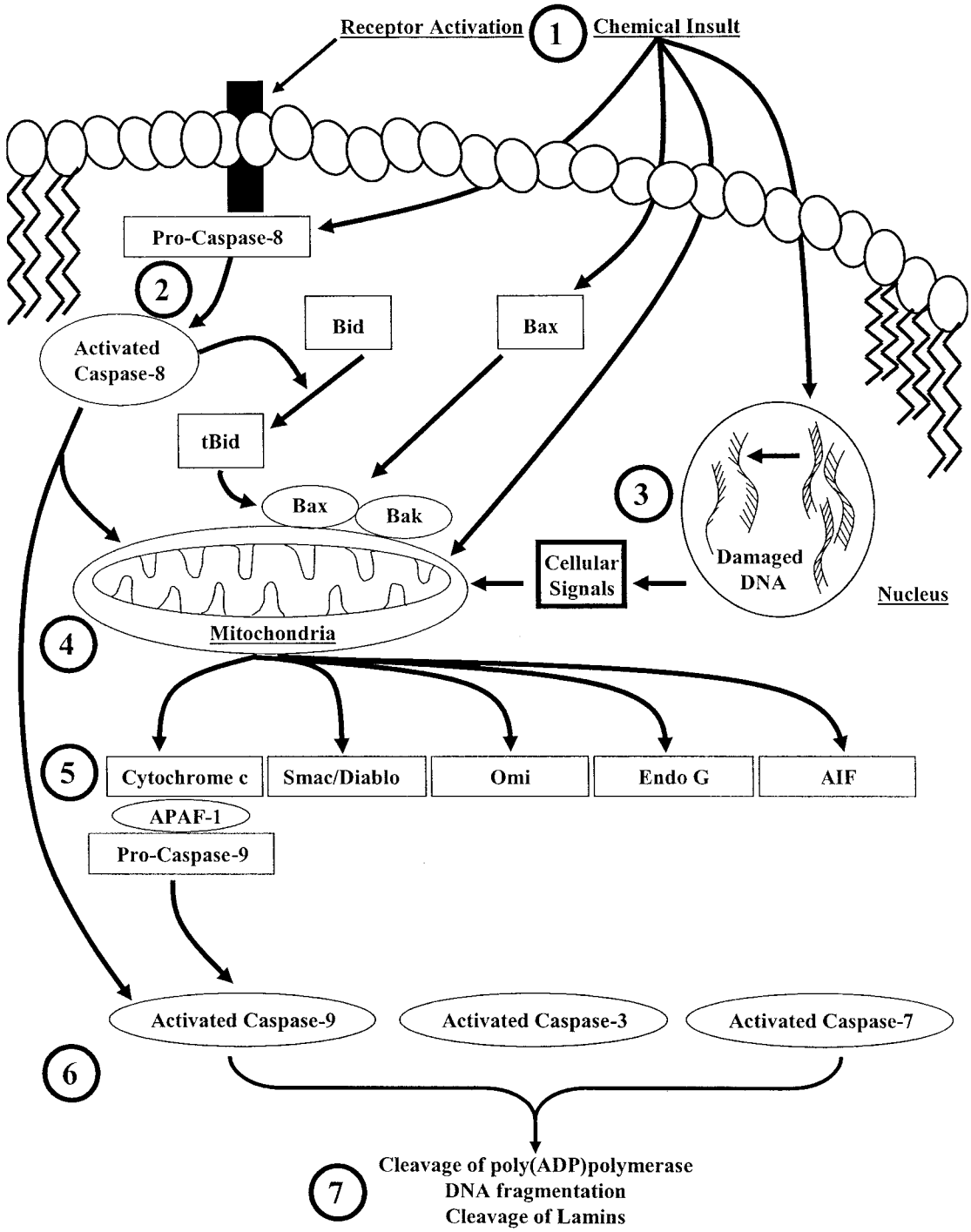

FIGURE 40-2. Cell signaling cascades involved in the activation of caspases and apoptosis. **1:** Receptor-mediated death signals or chemicals can initiate apoptosis through multiple mechanisms. **2:** Pro-caspase-8 is activated by receptor-mediated signals at the cellular membrane or directly by chemicals. Once activated, caspase-8 cleaves Bid to t-Bid, which interacts with Bax/Bak to induce mitochondrial-mediated apoptosis or directly activates caspase-9 and other caspases. **3:** Some chemicals cause DNA damage that signals the release of pro-apoptotic proteins from the mitochondria. **4:** Receptor-mediated signals, direct chemical injury, or signals resulting from DNA damage can all cause cytochrome c, Smac/Diablo, Endo G, and AIF release from the mitochondria. **5:** Released cytochrome c forms a complex with APAF-1 and pro-caspase-9, resulting in caspase-9 activation. **6:** Activated caspase-9 cleaves and activates pro-caspase-3 and -7, which can also be activated by caspase-8 independently of cytochrome c. **7:** Activated caspases (e.g., 3 and 7), AIF, and Endo G cause the classical markers of apoptosis, such as cleavage and activation of poly(ADP)polymerase, inactivation of inhibitors of DNases leading to DNA fragmentation, cleaved lamins, and the activation of other caspases.

Bad, Bak, Bax, and Bid belong to the Bcl-2 family of pro-apoptotic proteins, which are characterized by specific regions of homology termed Bcl-2 homology domains (86). Under non-stressed conditions these proteins exist bound to proteins in the mitochondria and cytosol (64). After toxicant exposure Bax, Bid, or Bak can dissociate and translocate to the mitochondria. Translocation to the mitochondria typically initiates alterations in the mitochondrial membrane potential and release of cytochrome c, Endo G, Smac/Diablo, Omi, and AIF (61,64,87) (Fig. 40-2). The exact mechanisms involved in the regulation of these proteins, or whether caspases or cytochrome c can act independently of Bax, Bid, or Bak in renal cells is presently unclear. However, current studies suggest that these proteins are partially regulated by trans-activation factors such as p53 (88–91). Furthermore, studies show that Bid mediates apoptosis induced by hypoxia and ATP depletion in cultures of rat RPTC (92); Bax mediates proximal tubular apoptosis in mice treated with cisplatin *in vivo* (58); and Bak is elevated during apoptosis in primary bovine glomerular endothelial cells induced by TNFα or lipopolysaccharide (93) or during ischemia-reperfusion-induced renal cell apoptosis in mice (94).

In contrast to Bax, Bid, and Bak, Bcl-2 is an antiapoptotic protein (60). Increased levels of Bcl-2 prior to toxicant exposure protects numerous cells, including renal cells (94), from toxicant-induced apoptosis (78,86,94,95). The protective effect of Bcl-2 may be the result of its ability to bind Bax, Bid, and Bak, preventing them from altering mitochondrial membrane permeability, initiating the release of mitochondrial pro-apoptotic proteins, and activating caspases (95). In support of this hypothesis, over expression of Bcl-2 protects against ATP-depletion-induced apoptosis in cultures of rat RPTC (92), and up regulation of Bcl-2 protected kidney epithelial cells both *in vitro* and *in vivo* against apoptosis induced by hypoxia, azide, cisplatin, and staurosporine (67).

AIF is released from the mitochondria in response to decreases in the mitochondrial membrane potential induced by ATP depletion (78,96); ischemia-reperfusion; anti-Fas antibodies (97); or exposure to high concentrations of Ca^{2+} (98), *t*-butyl hydroperoxide (98), or atractyloside (98). Cellular pathologies associated with AIF release are similar to those seen with caspases (chromatin condensation and oligonucleosomal DNA fragmentation) (97). AIF is a distinct protease with properties similar to those of caspases. In opposum kidney cells AIF is released after ATP depletion-induced by sodium cyanide and 2-deoxy-D-glucose (79,96). However, the ability of AIF to participate in toxicant-induced renal cell death *in vivo* or in other renal cell models *in vitro* has not been fully explored.

Smac/Diablo is a pro-apoptotic protein released from the mitochondria to the cytosol during apoptosis where it blocks the antiapoptotic activity of inhibitors of apoptosis proteins (IAP) (81). However, the ability of Smac/Diablo to promote apoptosis may not exclusively be the result of its ability to bind IAP. This was demonstrated in human embryonic kidney cells (HEK293) in which a mutant Smac/Diablo was unable to bind IAP. In this model both receptor- and chemical-induced apoptosis was increased in the presence of mutant Smac/Diablo, supporting the hypothesis that this protein's ability to induce apoptosis is partially independent of its ability to bind IAP (82). Despite these data, released Smac/Diablo functions at the level of executioner caspases and downstream of the Bcl-2 family of proteins (83). Smac/Diablo is expressed in the mouse kidney and in several renal cell models (99) and mediates apoptosis *in vivo* in mice after treatment with high concentrations of folic acid or after exposure of cultures of renal epithelial cells to TNFα (81). Increased expression of Smac/Diablo potentiates TNFα- and etoposide-induced apoptosis in HEK293 cells (82). However, similar to several other pro-apoptotic proteins, expression of Smac/Diablo is not essential for apoptosis in kidney cells. For example, acetaminophen-induced renal cell apoptosis proceeds in a caspase-dependent manner in the absence of Smac/Diablo activity (100).

Omi is a mammalian serine protease that has homology to bacterial HtrA endoprotease (101). Omi localizes to the endoplasmic reticulum and mitochondria and is expressed ubiquitously in a number of cell types including RPTC (84,85,101,102). After exposure to apoptotic stimuli Omi is released from the mitochondria and binds to, and cleaves, inhibitors of IAP (84). Omi-directed degradation of IAP facilitates caspase activation and the subsequent biochemical and morphological features of apoptosis. Inhibition of Omi using the synthetic inhibitor ucf-101, or siRNA against Omi, decreased cisplatin-induced apoptosis in primary cultures of mouse RPTC (84). Furthermore, treatment of mice with ucf-101 prior to cisplatin treatment decreased nephrotoxicity and renal cell death *in vivo* (84). These recent studies support the hypothesis that Omi may mediate other types of toxicant-induced renal cell apoptosis.

INITIATORS OF CELLULAR INJURY

Xenobiotics initiate cell injury by a variety of mechanisms. Some chemicals initiate toxicity directly because of their reactivity with selected cellular macromolecules. For example, the antifungal drug amphotericin B increases the permeability of the plasma membrane to cations (103), the mycotoxin fumonisin B_1 inhibits sphinganine (sphingosine) *N*-acyltransferase (104), and aminoglycosides bind initially to cellular anionic phospholipids (105,106). Other xenobiotics initiate toxicity following biotransformation to a reactive intermediate or a stable metabolite. Xenobiotics can also initiate toxicity indirectly through the production of reactive oxygen species.

Role of Biotransformation

Renal xenobiotic metabolism can contribute significantly to whole-body metabolism and/or renal toxicity of a chemical because a primary route of xenobiotic excretion is the kidney. Some chemicals need to be metabolized or biotransformed to a toxic reactive intermediate for cellular injury to occur (Fig. 40-3). The reactive intermediate binds covalently to critical cellular macromolecules, which are thought to interfere with the normal functioning of the macromolecules and thereby initiate cellular injury. In many cases, these reactive intermediates or "alkylating" agents are electrophiles that bind to cellular nucleophiles. The renal xenobiotic-metabolizing enzymes found in experimental animals and humans have been reviewed by Lock (107) and Lohr (108) and are summarized in Table 40-1.

Although the kidney contains many of the xenobiotic-metabolizing enzymes found in the liver, in general their concentration within the kidney is lower. For example, renal cytochrome P-450 levels are between 0.1 and 0.2 nmol/mg microsomal protein across a variety of species, which represents approximately 20% of cytochrome P-450 levels found in the liver (107,109). The distribution of cytochrome P-450 also varies in different renal cells. For example, cytochrome P-450 levels are highest in the S_2 segment, followed by the S_3 and S_1 segments, respectively with the other tubular segments having less than 10% of that of the S_1 segment (107).

The renal cytochrome P-450 system is very active against a variety of endogenous and exogenous compounds, and

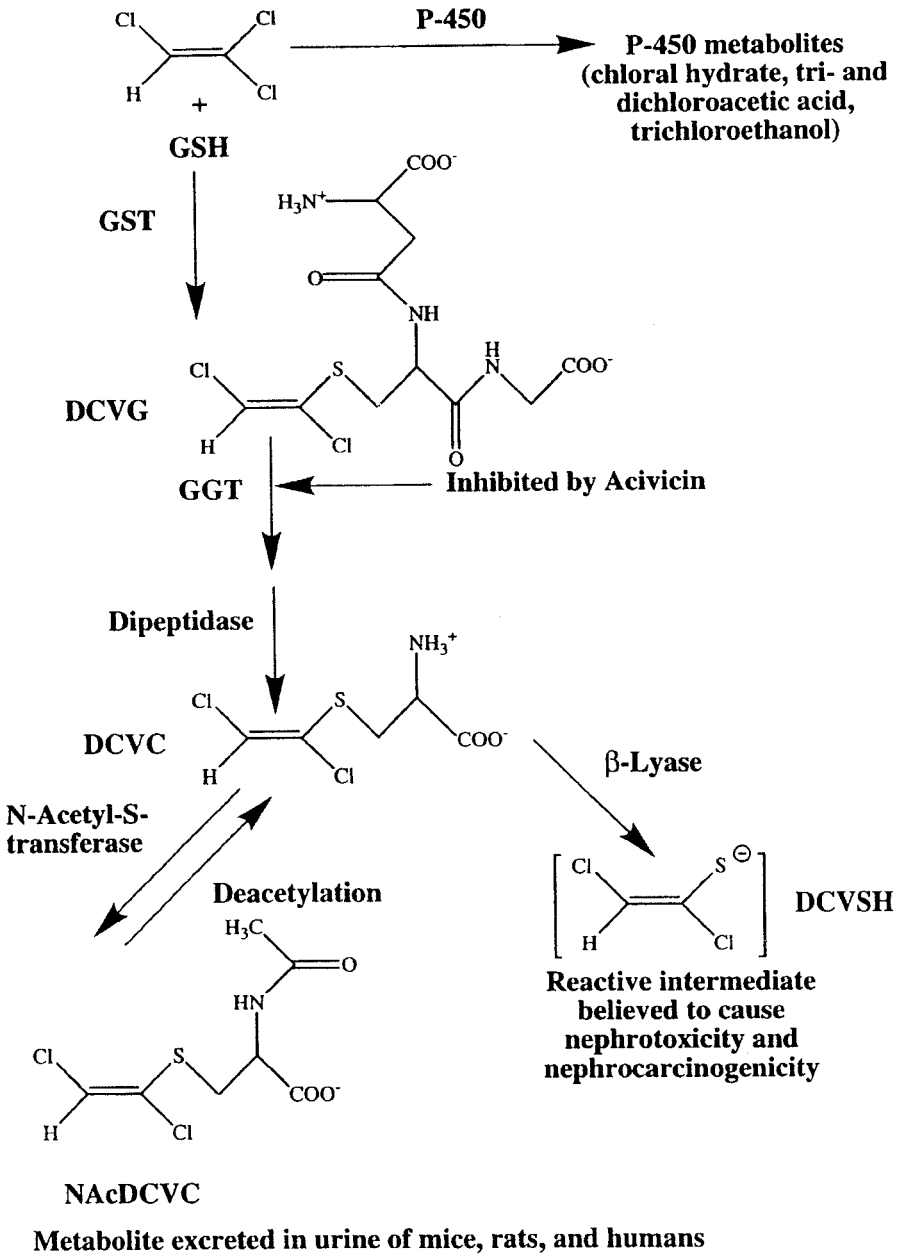

FIGURE 40-3. The bioactivation of trichloroethylene by the glutathione-(GSH-) conjugation pathway. Trichloroethylene (top left) can be metabolized by either cytochrome P-450 to the compound listed (top right) or be conjugated to GSH by the glutathione S-transferase (GST) to form S-(1,2)-dichlorovinyl-glutathione (DCVG). These reactions can occur either in the liver or in the kidney. DCVG formed in the liver is delivered to the kidney via the bile or the blood where the high concentrations of γ-glutamyltransferase (GGT) and dipeptidase in the kidney results in the cleavage of the GSH moiety and the formation of S-(1,2)-dichlorovinyl-L cysteine (DCVC). Metabolism of DCVC by N-acetyl-S-transferase produces N-acetyl-S-(1,2)-dichlorovinyl-L-cysteine (NAcDCVC), which is excreted in the urine of mice, rats, and humans exposed to trichloroethylene. NAcDCVC also can be deacetylated back to DCVC. Metabolism of DCVC by cysteine-conjugate ß-lyase results in the formation of a reactive thiol that can rearrange to form a protein acylating species. (From: Cummings BS, et al. Role of cytochrome P450 and glutathione S-transferase alpha in the metabolism and cytotoxicity of trichloroethylene in rat kidney. *Biochem Pharmacol* 2000;59:531, with permission.)

numerous cytochrome P-450 isoforms have been identified in renal tissue. For example, cytochromes P-450 IA1, IA2, IIB2, IIC2, IIC11, IID, IIE1, IIJ2, IIJ3, IIJ5, IIJ9, IIIA, IVA1, IVA2, IVA3, and IVF have been identified in RPT of the mouse, rat, and rabbit kidney (107,108,110 –114). The distal tubular cells also express several cytochrome P-450 isoforms, including IIB1, IIC11, IIE1, IVA2, IVA3, and IVF (108,110,113,115). There are species- and sex-dependent differences in the expression of cytochrome P-450 isoforms. For example, cytochrome P-450 IIA, IIC, and IIE are present in male mouse kidneys but are absent, or present at very low levels, in female mouse kidneys (116). Several studies also report that there are differences in the expression of cytochrome P-450 isoforms between the human and rodent kidney. One important example is the expression of cytochrome P-450 IIE1, which has been detected in renal proximal and distal tubular cells of mice and rats but not human kidneys (107,108,110,111,117 – 119). In contrast, both human and rodent kidneys express high amounts of cytochrome P-450 IVA isoforms. However, rat kidneys express IVA1, IVA2, and IVA3, whereas human kidneys appear to express IVA11 (110,119). Such differences in xenobiotic expression must be taken into account when the role of biotransformation in chemical-induced nephrotoxicity is assessed.

In contrast to the numerous compounds known to be biotransformed to reactive intermediates by P-450 isoforms in the liver, few compounds have been documented to produce nephrotoxicity through renal cytochrome P-450 bioactivation. Renal cytochrome P-450 plays a role in the nephrotoxicity of chloroform (120–127). Renal cytochrome P-450 metabolizes chloroform to trichloromethanol, which is unstable, and releases HCl to form phosgene. Phosgene can react with: (a) two molecules of glutathione to produce diglutathionyl dithiocarbonate, (b) water to produce two molecules of HCl and CO_2, (c) cysteine to produce oxothizolidine-4-carboxylic acid, or (d) cellular macromolecules to initiate toxicity (120,121,127).

TABLE 40-1

EXPRESSION OF SELECTED XENOBIOTIC BIOTRANSFORMATION ENZYMES IN THE KIDNEY

Enzyme	Cell type	Species	References
CYTOCHROME P450 MONOOXYGENASES			
IA	Proximal tubules	Rat, mouse, human	(107,108)
IA2	Proximal tubules		(112)
IIB	Proximal tubules	Rat and mouse	(110)
	Distal tubules	Rat and mouse	(110)
IIC2	Proximal tubules		
IIC9	Unknown[a]	Human but not rat	(107)
IIC11	Distal tubules	Male rat	(110)
IID	Proximal tubules		(114)
IIE1	Proximal tubules	Rat, mouse, not human	(110,119,375)
	Distal tubules		(110)
IIJ	Proximal tubules	Human, rat, mouse	(112,376–378)
IIIA1	Glomerulus	Rat, mouse, not human	(107,108,110)
IIIA4	Proximal tubules	Human, not rat or mouse	(107)
IVA2	Proximal tubules	Rat, mouse, not human	(107,110)
	Distal tubules		(107,110)
IVA3	Proximal tubules	Rat, mouse, not human	(107,110)
	Distal tubules		(107,110)
IVA11	Proximal tubules	Human, not rat or mouse	(117,119)
IVF	Proximal tubule	Human and mouse	(113,119)
	Distal tubules	Mouse	(113)
FLAVIN-CONTAINING MONOOXYGENASES			
FMO1	Unknown[a]	Rat, mouse, and human	(107,137)
FMO3	Unknown[a]	Rat, mouse, and human	(107,137)
FMO5	Unknown[a]	Human	(137)
GLUTATHIONE S-TRANSFERASES			
GSTα	Proximal tubules	Rat, mouse, and human	(115,117,136)
	Distal tubules		
GSTμ	Proximal tubules	Rat, mouse, not human[b]	(115,117,136,140)
GST π	Proximal tubules	Rat, mouse, and human	(115,117)
GST θ	Proximal tubules	Human	(117)

[a]Activity and expression have been measured in kidney microsomes only.
[b]GSTμ is expressed in some human kidney malignancies.

Chloroform bioactivation by renal cytochrome P-450 is dependent on the sex of the species. The marked sex difference in the nephrotoxicity of chloroform is reversed by castration of males or the treatment of females with testosterone, suggesting that the renal cytochrome P-450 responsible for chloroform bioactivation is under androgenic control (120,128). Because cytochrome P-450 isozymes IIB1 and IIE1 are present in male mice and are expressed in female mice treated with testosterone, these isozymes may be responsible for renal chloroform bioactivation (107,127).

Acetaminophen is metabolized in the mouse kidney by cytochrome P-450 IIE1 to the reactive intermediate *N*-acetyl-*p*-benzoquinonimine, which binds to cellular proteins (122,124,128). Numerous hepatic proteins to which *N*-acetyl-*p*-benzoquinonimine binds covalently have been identified. These include a cytosolic protein that has sequence homology to a selenium binding protein (58 kDa) (129–131), microsomal glutamine synthetase (44 kDa) (126), cytosolic *N*-10-formyl tetrahydrofolate dehydrogenase (100 kDa) (130,132), and mitochondrial glutamate dehydrogenase (50 kDa) (132). Recent studies suggest that acetaminophen can mediate renal cell death in mouse RPTC by inducing endoplasmic reticulum (ER) stress (100). In this model, acetaminophen treatment increased the expression of GADD153, an ER stress protein, and induced caspase-12 cleavage and apoptosis, independently of caspase-3, -9, or the release of the mitochondrial pro-apoptotic protein Smac/Diablo. It is not known if *N*-acetyl-*p*-benzoquinonimine mediates ER stress via cytochrome P-450 bioactivation or if novel mechanisms are involved.

Flavin-containing monooxygenases (FMOs) are a family of enzymes that oxidize the nucleophilic nitrogen, sulfur, and phosphorus moieties of a number of chemicals, including S-(1,2)-dichlorovinyl-L-cysteine, tamoxifen, and cimetidine (108,133 –135). The role of FMOs in nephrotoxicity has received little attention, but FMO isozyme FMO3 activity has been detected in the kidneys of rats, dogs, mice, rabbits, and humans (136). Like cytochrome P-450, species differences do exist in FMO activity in the kidney with the rat exhibiting a two-fold to six-fold greater activity (as determined by methionine S-oxidase activity) than other species, including humans. Studies in human kidney microsomes demonstrate that FMO1, FMO3, and FMO5 are all expressed but at different levels (137). Further, samples from African American patients exhibited significantly higher levels of FMO1 activity compared to their Caucasian counterparts, suggesting that the expression of renal FMO isoforms may differ depending on ethnic

background (137). However, the expression of FMO1, FMO3, or FMO5 does not appear to be sex-dependent as no difference in the activity or expression of these enzymes was detected between human male and female kidney microsomes (137).

In vitro, FMO1, FMO3, FMO4, and FM05 metabolize cysteine S-conjugated S-allyl cysteine, while FMO3 metabolizes S-(1,2)-dichlorovinyl-L-cysteine (137). However, little S-(1,2)-dichlorovinyl-L-cysteine was metabolized in human kidney microsomes, even though FMO3 was expressed in these tissues, suggesting that FMO may not contribute to the nephrotoxicity of this compound in human renal cells. In contrast, in studies using human proximal tubular cells, treatment with the FMO inhibitor methimazole decreased S-(1,2)-dichlorovinyl-L-cysteine-induced apoptosis (138). Recent studies also suggest that FMO catalyzed sulfoxidation of the sevoflurane (a commonly used anesthetic) degradation product fluoromethyl-2,2-difluoro-1-(trifluoromethyl)vinyl ether may be key to its renal toxicity (139). However the exact isoforms involved in the process, or the mechanisms involved, are not known.

The conjugation enzymes glucuronosyltransferase, sulfotransferase, and glutathione S-transferase are located in the kidney where they conjugate both endogenous and exogenous compounds. The action of these enzymes on nephrotoxicants increases their water solubility and promotes their excretion and elimination (108). Glutathione-S-transferases are a diverse family of enzymes with at least five different subfamilies. Glutathione-S-transferase α, μ, and π are expressed in RPTC of the rat, and α and μ are expressed in rat distal tubular cells (115,136). The expression of glutathione-S-transferases in human RPTC appear to be similar to that observed in rat RPTC (117). The μ class of glutathione-S-transferase is expressed in some patients exhibiting kidney neoplasias and/or tumor growth (140).

Although glutathione conjugation is normally recognized as a detoxification pathway in which electrophiles are neutralized, numerous extrarenally formed glutathione conjugates have proved to be nephrotoxic. In fact, glutathione conjugates and mercapturic acids may be considered targeting moieties for toxicant delivery to the kidney. For example, the extrarenal conjugation of glutathione is important for the nephrotoxicity of $HgCl_2$ (141), halogenated alkenes, and aromatics, and possibly acetaminophen (127,142–144). The nephrotoxicity of the halogenated alkene trichloroethylene in rats and humans is believed to be a direct result of its conjugation with glutathione to form S-(1,2)-dichlorovinyl-glutathione, and the subsequent processing of the glutathione-conjugate to S-(1,2)-dichlorovinyl-L-cysteine in RPTC (115,145) (Fig. 40-3). *In vivo,* trichloroethylene is conjugated with glutathione in the liver and delivered via the bile or blood to the kidney. The expression of enzymes, such as γ-glutamyl transferase and dipeptidase in the RPTC and biliary and intestinal tract results in the cleavage of the γ-glutamyl and glycyl moieties, respectively, and the formation of S-(1,2)-dichlorovinyl-L-cysteine. Metabolism of S-(1,2)-dichlorovinyl-L-cysteine by *N*-acetyl-S-transferase produces *N*-acetyl-S-(1,2)-dichlorovinyl-L-cysteine, which is excreted in the urine of mice, rats, and humans exposed to trichloroethylene (146,147). *N*-acetyl-S-(1,2)-dichlorovinyl-L-cysteine also can be deacetylated back to S-(1,2)-dichlorovinyl-L-cysteine. Metabolism of S-(1,2)-dichlorovinyl-L-cysteine by cysteine-conjugate ß-lyase results in the formation of a reactive thiol that can rearrange to form a protein acylating species. A strong correlation exists between increases in markers of renal injury (proteinuria, creatinine clearance, glucosuria) and the levels of glutathione metabolites of trichloroethylene in the blood and urine of humans exposed to high amounts of trichloroethylene (148). Key determinants in the nephrotoxicity of trichloroethylene and similar chemicals, such as sevoflurane, isoflurane, and desflurane, which utilize this common pathway of biotransformation (139,149), appear to be the high levels of γ-glutamyl transferase, dipeptidase, and cysteine-conjugate ß-lyase activities found in the kidney.

Role of Reactive Oxygen Species

Reactive oxygen species (ROS) are mediators of cellular injury during inflammatory responses, ischemia-reperfusion, and after nephrotoxicant exposure. Cellular ROS are generated during the normal function of the mitochondrial and microsomal electron transport chains as a result of the incomplete reduction of O_2 to water (150–152) (Fig. 40-4). Superoxide anion free radical is produced by a one-electron reduction of O_2, and H_2O_2 is produced by a two-electron reduction of O_2. Superoxide anion can dismutate to form H_2O_2, or H_2O_2 can be formed directly. The hydroxyl radical is formed from H_2O_2, and the superoxide anion free radical via the metal-catalyzed Haber-Weiss reaction or the superoxide-driven Fenton reaction. Ferrous iron (Fe^{2+}) appears to be the major intracellular initiator of the reaction, but cuprous ions may participate as well. The precise source and form (e.g., ferritin) of the ferrous iron is still unclear.

One source of Fe^{2+} may be the heme-moiety that resides in the active site of cytochrome P-450 isoforms (153). Evidence for this source includes the observation that rats treated intraperitoneally with cisplatin for 4 days had significantly lower levels of renal cytochrome P-450 content compared to control rats, and the decrease in P-450 content correlated with increases in bleomycin-detectable iron content in the kidney. Piperonyl butoxide (a cytochrome P-450 inhibitor) decreased the cisplatin-induced release of iron in the kidney and the functional and morphologic markers of kidney toxicity (154). These same effects were observed in LLC-PK_1 cells, a porcine kidney cell culture line. Thus, P-450 may serve as one source of Fe^{2+} to initiate the formation of ROS.

Superoxide anion acts as a reductant for Fe^{3+}, and the Fe^{2+} generated reduces $H_2O_2$to the hydroxyl radical. The hydroxyl radical is a highly reactive species and reacts rapidly with adjacent molecules. Superoxide anion and H_2O_2 are less reactive, and H_2O_2 may diffuse away from the initial site of formation to produce injury at a distant site within the cell. Although H_2O_2 readily crosses cell membranes, superoxide anion and hydroxyl radical do not. Because ROS production is a natural byproduct of metabolically active cells, such as the kidney, significant defenses against the normal production of ROS or those produced under pathologic conditions exist (Fig. 40-4).

The term oxidative stress is commonly used to describe those conditions in which there is an increase in ROS formation. Chemicals may initiate oxidative stress indirectly by augmenting the production of ROS. For example, Walker and Shah (155) showed that gentamicin enhances H_2O_2 generation in isolated rat renal cortical mitochondria, and Lund and associates (156) showed that mitochondria isolated from rats treated with $HgCl_2$ exhibit elevated levels of H_2O_2 production. Another mechanism by which chemicals may produce oxidative stress is through *redox cycling*. Certain compounds, especially quinones, can undergo a one-electron reduction to a semiquinone radical and a second one-electron reduction to the hydroquinone. The hydroquinone is oxidized to the quinone, and the cycle begins again, hence the term *redox cycling*. During the reduction process, superoxide anion is formed from O_2, and oxidative stress ensues. For example, Brown and colleagues (157) have provided evidence that menadione (2-methyl-1,4-naphthoquinone) produces toxicity in isolated rat renal epithelial cells through its ability to undergo redox cycling and cause oxidative stress. However, it should be recognized that the ability of quinones to undergo redox cycling varies with the

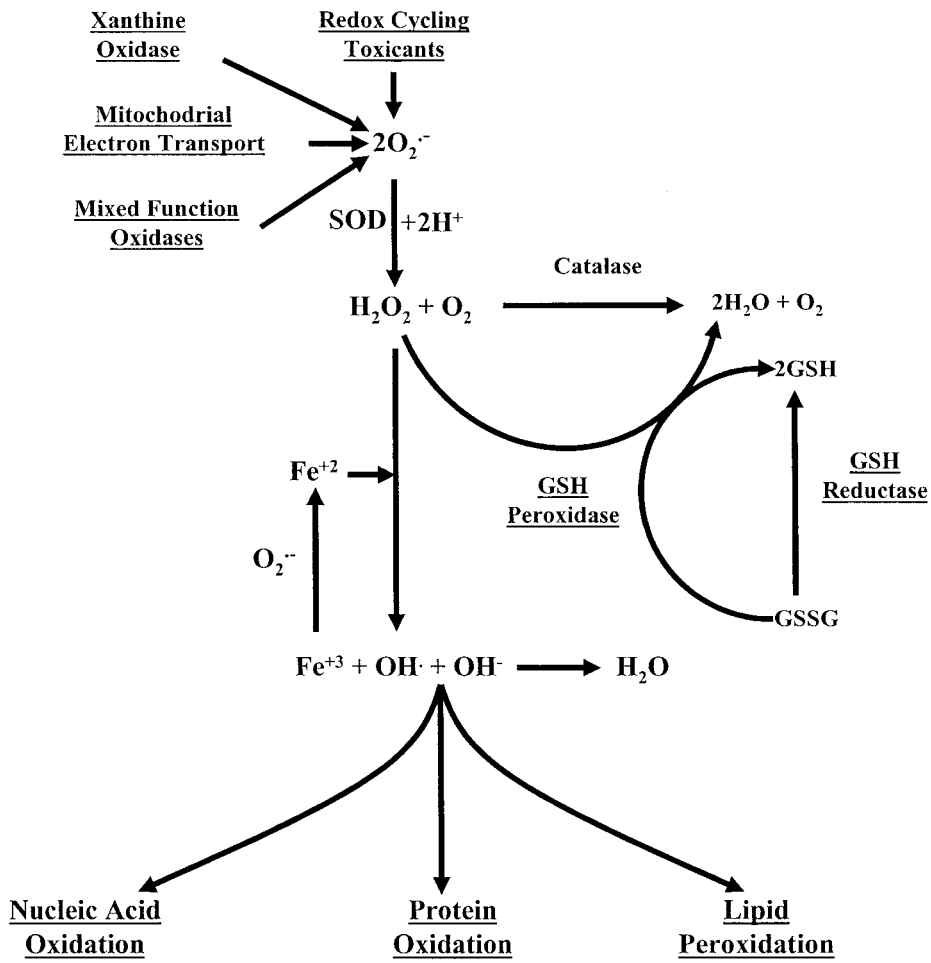

FIGURE 40-4. A schematic representation of the major pathways and possible intracellular targets and oxidants. Detoxification pathways and protective agents are also shown. See text for details. *GSH*, glutathione; *GSSG*, glutathione disulfide; *SOD*, superoxide dismutase.

quinone, and some quinones produce toxicity through their ability to arylate cellular macromolecules, particularly protein sulfhydryls (157–159).

ROS can induce lipid peroxidation, inactivate enzymes by directly oxidizing protein sulfhydryl or amino groups, depolymerize polysaccharides, and induce DNA strand breaks. Lipid peroxidation results from the interaction of free radicals with polyunsaturated fatty acid side chains of membrane phospholipids to form free radicals and relatively stable lipid hydroperoxides (160). Transition metals can catalyze the decomposition of lipid hydroperoxides, which results in the formation of alkoxyl and peroxyl free radicals that propagate the reaction. Lipid breakdown products such as hydroxylated fatty acids, 2-alkenyls, and 4-hydroxyalkenyls also are produced, are toxic, and may contribute to organelle and cellular dysfunction. Thus, ROS-induced degradation of membrane lipids can result in decreased cellular membrane integrity, altered enzymatic activity, and transport properties, and anisotropy (161,162). The oxidation of protein sulfhydryl and amino groups by ROS can produce dramatic alterations in enzymatic activity and membrane structure and function that also lead to cell death. Finally, ROS can produce DNA strand breaks that may lead to cell death. Although H_2O_2 does not directly damage DNA, because of its stability and ability to diffuse throughout the cell, it is generally thought that DNA damage results from the metal-catalyzed activation of H_2O_2 to the hydroxyl radical near the DNA.

A wide variety of structurally diverse nephrotoxicants appear to produce renal cell injury at least in part by oxidative stress, including $HgCl_2$ (141,156,160), haloalkene cysteine conjugates (163–166), cyclosporine A (166), and cisplatin (167–172). The diversity of these nephrotoxicants highlights the critical and common roles that ROS play in the mechanism of cell death. Further, the generation of ROS is typically an initiator signal for renal cell death.

MEDIATORS OF CELL INJURY

A number of common cellular pathways that mediate cell death have been identified. It is generally thought that after an initial cellular interaction, a sequence of events occurs that leads to cell injury and death. In the case of oncosis and apoptosis, there is a point along the sequence, yet to be identified, that is the point of no return; the point at which the cell will die irrespective of any intervention. Investigators have tried to identify the sequence of deleterious events, the point of no return, and the relative importance of each observed change in a variety of tissues for a number of years with some success. This has led to the identification of numerous intracellular mediators that are keys in the generation of renal cell injury and death.

p53 and p21

The tumor suppressor protein p53 and the cell cycle inhibitor protein p21 play important roles in renal cell death and acute renal failure (171,173–179). p53 and p21 appear to have opposite effects in that activation of p53 increases renal cell death (171) while activation of p21 appears to be protective (174,176). However, recent reports suggest that p53 can activate p21 during renal cell death induced by selected toxicants,

such as cisplatin (175). Thus the exact mechanisms controlling p21 and p53 cross talk, and the effect of this cross talk on renal cell death and cellular proteins have yet to be determined.

p53 is activated in renal cells after exposure to agents that induce DNA damage, such as cisplatin (172,175). The mechanism of p53 activation involves its phosphorylation at numerous serine-residues and its subsequent release from a regulator protein and translocation to the nucleus. Once in the nucleus, p53 can induce the transcription of a number of genes, including p21 (175), and activate several apoptotic pathways such as those involving caspases. Inhibition of p53 activation with a pharmacological agent decreased cisplatin-induced apoptosis in rabbit RPTC through a mechanism that includes the inhibition of caspase-3 (171). Caspase-3 activity in this model preceded both phosphatidylserine externalization and chromatin condensation, but not p53 nuclear translocation. In support of this hypothesis others have demonstrated that inhibition of p53 translocation inhibits ARF and renal cell death induced by cisplatin in rat RPTC (180), and after renal ischemia in rats (181,182).

Induction of p21 occurs in response to DNA damage and p53-induced cell cycle arrest (174). Once activated, p21 inhibits one or more of the cyclin-dependent kinase activities (173,174) and it is hypothesized that inhibition of these processes prevents renal cell cycle activity and apoptosis, and protects against nephrotoxicity. In support of this hypothesis, knock-out mice lacking p21 exhibit increased renal cell cycle activity, apoptosis, and are more susceptible to cisplatin and ischemia-induced ARF compared to wild type controls (174). The downstream mediators of apoptosis include caspases, as treatment of RPTC from p21 knock-out mice with cisplatin resulted in significantly higher levels of caspase-3 activity and apoptosis compared to control cells expressing p21 (176). Although these studies illustrate that caspase activity is one mechanism by which p53 induces and p21 protects against renal cell death, the exact pathways needed to activate caspases have not fully determined.

Signaling Kinases

Signaling kinases refer to enzymes that alter the activity, expression, or localization of another protein by altering its phosphorylation, including other signaling kinases. Signaling kinases differ in terms of the amino acids targeted for phosphorylation (serine/threonine/tyrosine), the location within a cell (membrane-bound or cytosolic), and the protein targeted for phosphorylation. Table 40-2 lists several signaling kinases identified in the kidney, the site within the kidney or cell involved, the nephrotoxicant involved, and several references to studies that suggest critical roles for signaling kinases both in the development of renal cell death and in the recovery of renal cells after toxicant-induced injury (12,183–189).

Protein kinase C (PKC) refers to a family of serine/threonine kinases comprised of 11 different isoforms divided into conventional PKC (cPKC; α, $\beta_{1/2}$, and γ), novel PKC (nPKC; ε, δ, η, and θ), and atypical PKC (aPKC; τ, λ and ζ) groups (190). These isoforms differ in terms of preferred substrates and mechanisms of action (190–192). Activation of cPKC is Ca^{2+}- and diacylglycerol-dependent while activation of nPKC is Ca^{2+}-independent (190,193). In contrast, activation of aPKC is independent of both Ca^{2+} and diacylglycerol. RPTC have been reported to express α, β_1, β_2, ζ, δ, λ, and ϵ (194–197), and several other isoforms are expressed in the kidney of rats, mice, and humans (183,185,198,199).

TABLE 40-2

SELECTED SIGNALING KINASES INVOLVED IN RENAL CELL INJURY, SURVIVAL, OR REPAIR

Kinase	Location	Nephrotoxicant	Reference
PROTEIN KINASE C (PKC)			
CONVENTIONAL PKC			
PKCα	Proximal tubules	Cisplatin DCVC[a]	(185,379)
ATYPICAL PKC			
PKCζ	Proximal tubules	*t*-butylhydroperoxide	(184)
MITOGEN ACTIVATED PROTIEN KIANSE (MAPK)			
ERK1/2	Proximal tubules	Cisplatin H_20_2 TGHQ[c]	(185,186,202, 205,380)
JNK/SAPK[a]	Proximal tubules	Cisplatin	(186)
P38	Proximal tubules	Cisplatin H_20_2 TGHG[c]	(186,202,205)
OTHER KINASES			
Protein kinase B[b]	LLC-PK1 Proximal tubules	Cisplatin Mechanical Injury H_20_2	(12,380)
Phosphoinositide-3-kinase	LLC-PK1 Proximal tubules	Cisplatin Mechanical Injury	(12)

[a] S-(1,2)-dichlorovinyl-L-cysteine
[b] Also known as AKT
[c] 2,3,5-tris-(glutathion-S-yl)hydroquinone

The toxicity of both fumonison-B1 and TNF are mediated by PKC in human renal cells and LLC-PK1 cells (185,188,189). The exact role of PKC in renal cell death depends on the toxicant and the specific isoform(s) involved (183–185). For example, activation of PKCα in rabbit RPTC during cisplatin treatment results in mitochondrial dysfunction and cell death (185). In contrast, activation of PKC-ζ after exposure to t-butylhdyroperoxide mediates cellular repair (183,184).

One possible mechanism by which PKC mediates renal cell death following toxicant exposure is by activating a group of kinases called mitogen-activated protein kinases (MAPK). MAPK is the general name for a family of tyrosine/threonine kinases that are activated by oxidants, environmental chemicals, and anticancer agents (200–209). Family members of MAPK include ERK 1 and 2 (ERK1/2), the c-jun N-terminal kinase/stress activated protein kinases (JNK/SAPK), and p38 (206). Studies in RPTC support the hypothesis that activation of ERK1/2 is a participant in cisplatin-induced mitochondrial dysfunction (185). In particular cisplatin-induced PKCα activation was followed by ERK1/2 activation, which caused a decrease in mitochondrial membrane potential, caspase 3 activation, and apoptosis. Other studies supporting the hypothesis that MAPK mediates cisplatin-induced renal cell death include those performed *in vivo* in mouse kidneys (58,186,210). The targets of MAPK include mitochondrial-associated proteins (58,185), forkhead transcription protein (211), alpha-adducin (a prominent protein in the regulation of cytoskeleton filament, (210)), and proteins that are part of the mitochondrial electron transport chain (185).

Similar to PKC, the role of MAPK in toxicant-induced renal cell death is toxicant-specific. Arany et al. (186) demonstrated that ERK activation mediated cisplatin-induced renal cell death *in vivo* and *in vitro* in mouse models. However, the same investigators observed that ERK activation protected against oxidant-induced (H_2O_2) cell death (186). Interestingly, cisplatin-induced ERK activation and renal cell death was dependent on the epidermal growth factor-receptor (EGF-R) and c-Src. Although studies by Zhuang and colleagues (12) (personal communication) demonstrated that H_2O_2 exposure of RPTC results in Src, EGF-R, ERK1/2, protein kinase B (Akt) and phosphoinositide-3-kinase (PI3K) activation, ERK1/2 inhibition was associated with cell death and Src inhibition potentiated cell death. Finally, EGF-R-induced activation of ERK is believed to mediate 2,3,5-tris-(glutathion-S-yl)hydroquinone (TGHQ)-induced death in LLC-PK1 cells (202,205). The detailed mechanisms of Src, EGF-R, PI3K, ERK1/2 and p38 activation, the targets of these kinases and their role in cell death produced by different toxicants remain to be determined.

Altered Calcium Homeostasis

Intracellular Ca^{2+} homeostasis is important to cell viability because Ca^{2+} is a second messenger and plays a critical role in a variety of cellular functions (212–216). Cytosolic free Ca^{2+} concentration is approximately 100 nM and is tightly regulated in the face of a large extracellular-intracellular gradient (10,000:1) by a series of pumps and channels located on the plasma membrane and ER. Mitochondria are not thought to be involved significantly in the normal regulation of Ca^{2+}; however, they accumulate Ca^{2+} after toxicant exposure or hypoxia (212).

Considering the preceding, toxicants must increase cytosolic free Ca^{2+} levels supraphysiologically or produce sustained increases to initiate or mediate cell death. In turn, these increases in cytosolic free Ca^{2+} can activate a number of degradative Ca^{2+}-dependent enzymes, such as phospholipases, endonucleases, and proteinases, and alter the cytoskeleton. The precise role of Ca^{2+} influx remains unclear. However, decreasing the extracellular Ca^{2+} concentration or blocking extracellular Ca^{2+} influx decreases cell death (217–220). Increases in cytosolic free Ca^{2+} levels were observed in a hypoxia model using rat RPTC and in a mitochondrial inhibitor model using rabbit RPTC, and experimental manipulations that chelate intracellular Ca^{2+}, or decrease the influx of extracellular Ca^{2+}, decreased cell death (219–224). These results reveal that intracellular and extracellular Ca^{2+} mediate the late phase of cell injury. Studies demonstrating that inhibitors of Ca^{2+}-activated neutral proteases (calpains) decreased cell death produced by a variety of diverse toxicants, and anoxia, in rabbit RPTC, further supports the role of Ca^{2+} in cell death (219,220,224,225). Nephrotoxicants that have been shown to increase cytosolic free Ca^{2+} include $HgCl_2$ (226,227), pentachlorobutadienyl-glutathione (167), pentachlorobutadienyl-L-cysteine (228), tetrafluoroethyl-L-cysteine (229), S-(1,2)-dichlorovinyl-L-cysteine (230–233), oxidants (234,235), sevoflurane, miconazole (236,237), cyclosporine A (87,238), and gentamicin (155,239,240).

Proteinases

Nonphysiologic activation of proteinases in the cytosol or those associated with organelles or membranes could disrupt normal membrane, cytoskeleton, or organelle function and lead to cell death. Lysosomes are one source of proteinases because they normally degrade proteins with acid hydrolases. The lysosomal membrane could rupture under conditions of cell injury, releasing the proteinases into the cytosol to degrade susceptible critical proteins. Experimental studies have found little evidence of lysosomal rupture during injurious conditions (212). There was neither evidence of lysosomal rupture prior to cell death nor beneficial effects of lysosomal enzyme depletion in primary cultures of individually microdissected human and rabbit RPTC treated with cyclosporine A (241). The cysteine proteinase inhibitor *t*-trans-epoxysuccinyl-leucylamido(4-guandino)butane (E64), however, was cytoprotective.

A variety of cysteine and serine proteinase inhibitors were shown to be ineffective in protecting rabbit RPTC segments from antimycin A, tetrafluoroethyl-L-cysteine, bromohydroquinone, and *t*-butylhydroperoxide (242). However, E64 and the aspartic acid proteinase inhibitor pepstatin produced a small degree of cytoprotection in RPTC exposed to antimycin A or tetrafluoroethyl-L-cysteine. Although loss of lysosomal membrane potential was observed after antimycin A exposure, and extensive inhibition of lysosomal cathepsins B and L by E64 was correlated with cytoprotection, E64 cytoprotection was only observed after some cell death had occurred. These results reveal that lysosomal cysteine and aspartic acid proteinases do not play a significant role in RPTC death produced by nephrotoxicants.

Ca^{2+}-activated neutral cysteine proteinases (calpains) are a 14-member family of Ca^{2+}-activated proteinases that have cytoskeletal proteins, membrane proteins, and enzymes as substrates (243). The kidney expresses calpain-1 and -2, the two most-studied calpains and Schnellmann and colleagues (221,224,244,245) showed that calpain inhibitor II and the calpain inhibitor PD150606 decreased calpain activity and decreased cell death produced by a variety of toxicants including bromohydroquinone, antimycin A, tetrafluoroethyl-L-cysteine, and *t*-butylhydroperoxide. Inhibition of calpains may serve to protect the cell by preventing calpain-mediated extracellular Ca^{2+} influx and/or cytoskeletal alterations (221,244). Edelstein and associates (246) reported that calpain activity was increased early (within 10 minutes) in the genesis of hypoxic injury to rat RPTC and that inhibition of the initial calpain-mediated influx of extracellular Ca^{2+} protected these

cells. Furthermore, Takaoka and associates (247) reported that treatment with calpeptin increased renal function in rats subjected to ischemia-reperfusion. These results suggest that calpains play a critical role in cell death produced by a wide range of nephrotoxicants and renal dysfunction produced by ischemia-reperfusion.

Recent evidence has revealed that mitochondrial Ca^{2+} accumulation results in the activation of a mitochondrial calpain, which causes mitochondrial dysfunction (248–250). This atypical mitochondrial calpain has been identified as calpain-10, and inhibition of it prevented mitochondrial dysfunction. Further, mitochondrial calpain-10 targets complex I of the electron transport chain. While cytosolic calpains-1 and -2 are mediators of cytoskeleton and membrane disruption in cell injury, it is now clear that mitochondrial calpain plays a role in cell injury.

In several reports, the activation of calpains resulted in the deactivation of caspases and increased oncosis (251,252). Likewise, in a neuronal apoptosis system, addition of calpain I inhibited the release of cytochrome c and the subsequent cleavage and activation of pro-caspase -3 and -9 (251). In contrast, inhibition of calpains in this same model increased apoptosis. Furthermore, calpains inactivate caspases -3, -7, -8, and -9 by cleaving them at sites distinct from their activation site (253). Thus, calpains may mediate key processes that determine whether a cell will die by either apoptosis or oncosis. The mechanisms and targets of calpains in renal cell injury are still to be determined. Caspases, a family of cysteine proteases, are discussed under the previous apoptosis section. Caspase activity, per se, is not always needed for apoptosis to occur. For example, caspase-2-directed permeabilization of the mitochondrial membrane results in the release of the pro-apoptotic proteins cytochrome c and Omi is independent of its catalytic activity (62,63,254). Furthermore, a recent study demonstrated that apoptosis induced by four diverse toxicants (cisplatin, vincristine, staurosporine, and A23187) proceeded in rabbit RPTC in the presence of caspase inhibitors and the absence of caspase-3, -8, and -9 activity (255). Further, cisplatin-induced apoptosis in RPTC was shown to be both dependent and independent of caspase-3 and p53 activity (171). Although these studies support the hypothesis that renal cell apoptosis can proceed in the absence of caspase activity, the exact mechanisms involved are not known. Recent data in renal cells show that cisplatin-induced apoptosis is mediated via endonuclease G as opposed to caspases (256,257).

Phospholipase A_2

Phospholipase A_2s (PLA_2) are a family of enzymes that hydrolyze the acyl bond at the *sn*-2 position of phospholipids, resulting in the release of arachidonic acid and a lysophospholipid (258). The enzymes in this group have different substrate preferences, Ca^{2+} dependencies, and biochemical characteristics. Several Ca^{2+}-dependent forms have been identified in the rat and rabbit kidney cytosolic fraction with molecular weights of 14, 85, and 100 kDa (258,259). The most thoroughly characterized renal cytosolic PLA_2 is the Ca^{2+}-dependent 85 kDa PLA_2 ($cPLA_2$). A Ca^{2+}-independent plasmalogen-selective PLA_2 has been described in the rabbit kidney (260), which is located in the cytosol and has a molecular weight of approximately 28 kDa. Recently an ER Ca^{2+}- independent 80 kDa PLA_2, distinct from the 85-kDa cytosolic $cPLA_2$, has been found in the rabbit kidney (261). Furthermore, Mancuso and associates (262) have reported that human kidneys express mRNA corresponding to an 85-kDa membrane bound Ca^{2+}-independent PLA_2. This PLA_2 was subsequently demonstrated to be localized to at least the ER of rabbit RPTC and in rat kidney (261,263). Thus, both Ca^{2+}-dependent and Ca^{2+}-independent PLA_2 are present in the kidney. There are likely additional forms of PLA_2 found in the kidney, and additional characterization of all renal forms is needed.

It is generally thought that a toxicant-induced nonphysiological increase in PLA_2 activity could result in the loss of membrane phospholipids and consequently impair membrane function. Because many PLA_2 are Ca^{2+}-dependent, the increase in PLA_2 activity may be secondary to an increase in cytosolic free Ca^{2+} (258). For example, an increase in PLA_2 activity was observed in rabbit RPTC subjected to anoxia (264), and the phospholipase inhibitors mepacrine and dibucaine decreased hypoxia-induced rat RPTC death (265). The generated lysophospholipids and free fatty acids also may contribute to the injury by altering membrane permeability or uncoupling mitochondrial respiration (266–269). If ATP levels are limited during the injury process, reacylation of the lysophospholipids (270,271), *de novo* phospholipid synthesis (272), and esterification of free fatty acids (273,274) may all be inhibited.

The role of PLA_2 activation in various forms of renal cell injury and apoptosis is controversial (69,172,222,258–261,264,275–279). Nevertheless, the role PLA_2 in cell injury and death appears to be toxicant-specific. For example, inhibition of membrane-bound Ca^{2+}-independent PLA_2 with bromoenol lactone increased rabbit RPTC oncosis induced by the oxidants *t*-butylhydroperoxide, menadione, duraquinone, cumene hydroperoxide, and cisplatin (at high concentrations) but had no affect on oncosis induced by the nonoxidant antimycin A (261). The increase in cell death induced by these oxidants correlated to increased lipid peroxidation at early time points during injury, prior to decreases in membrane integrity. Thus, Ca^{2+}-independent PLA_2 in renal cells appears to protect against oxidant-induced renal cell oncosis. In contrast, inhibition of membrane-bound Ca^{2+}-independent PLA_2 in the same cell type decreased cisplatin-induced apoptosis and inhibited caspase-3 activation, phosphatidylserine externalization and chromatin condensation (172). Thus, membrane-bound $iPLA_2$ appears to induce apoptosis and protect against oxidant-induced oncosis in rabbit RPTC. The exact mechanisms controlling these opposing roles have yet to be fully determined. However, oxidants inactivate $iPLA_2$ activity in rabbit RPTC, whereas cisplatin, at an apoptotic inducing concentration, does not (275).

In contrast to the protective role of membrane-bound Ca^{2+}-independent PLA_2 following oxidative stress, increasing $cPLA_2$ activity increased the susceptibility of renal epithelial cells to oxidative stress (280). Increased H_2O_2 toxicity was not due to decreases in the activity of the antioxidant defense enzymes superoxide dismutase, catalase, or glutathione peroxidase. In contrast, chelation of cytosolic free Ca^{2+} decreased H_2O_2 toxicity, suggesting a key role for Ca^{2+} in the mediation of $cPLA_2$-mediated oncosis in renal cells. Kohjimoto and colleagues (281) demonstrated that preincubation of MDCK cells with an inhibitor of $cPLA_2$ (arachiondyl trifluoromethyl ketone) significantly reduced the toxicity of oxalate. Thus, $cPLA_2$ can mediate some forms of oncotic renal cell death.

Another possible role for PLA_2 in renal cell death is their ability to metabolize and release fatty acids from glycerophospholipids. Cell membranes are rich in polyunsaturated fatty acids and as such are susceptible to lipid peroxidation under normal and pathologic conditions. Peroxidized lipids are predisposed to degradation by PLA_2, resulting in increased PLA_2 activity and the formation of arachidonic acid metabolites and lysophospholipids. Ultimately, the lipids are reacylated and repaired. As discussed earlier, selective inhibition of an ER-Ca^{2+}-independent, plasmalogen-selective PLA_2-potentiated *t*-butylhydroperoxide induced rabbit RPTC death. Furthermore, *t*-butylhydroperoxide itself decreased $iPLA_2$ activity prior to the onset of cell death (275). These results reveal that the microsomal Ca^{2+}-independent, plasmalogen-selective PLA_2 may

function to hydrolyze oxidized or damaged phospholipids and is therefore a *phospholipid repair enzyme*. The observation that *t*-butylhydroperoxide decreased the microsomal Ca^{2+}-independent, plasmalogen-selective PLA_2 activity suggests that one pathway by which *t*-butylhydroperoxide is toxic is through its ability to produce oxidized phospholipids and inhibit their removal. The importance of not only Ca^{2+}-independent PLA_2, but also all PLA_2 in renal cell death is still under investigation.

MITOCHONDRIA, ENDOPLASMIC RETICULUM, LYSOSOMES, AND THE CELL MEMBRANE

Mitochondria, ER, lysosomes, and the cell membrane all play a role in nephrotoxic cell injury that leads to oncosis and apoptosis. Apoptosis and oncosis do not proceed through mutually exclusive pathways consisting of single sequences of events. Cell death by apoptosis or oncosis is often determined by the organelles affected and the pathway(s) activated in these organelles. Typically, one pathway predominates over others, depending on the time of exposure, the concentration used, and the toxicant itself.

Because of the presence of multiple cell death pathways and multiple targets, inhibition of one pathway may not block nephrotoxicant-induced cell death. For example, if one blocks the oxidative stress associated with S-(1,2)-dichlorovinyl-L-cysteine, pentachlorobutadienyl-L-cysteine, or tetrafluoroethyl-L-cysteine exposure to RPTC, the rate of cell death is diminished, but the cells eventually die because of the mitochondrial dysfunction produced by these compounds (282) (Fig. 40-5). Thus, a given chemical can cause cell death by interacting at numerous organelles, and blocking one interaction at one organelle may not decrease cell death, rather it may alter the organelle targeted, and thus the mechanism of cell death.

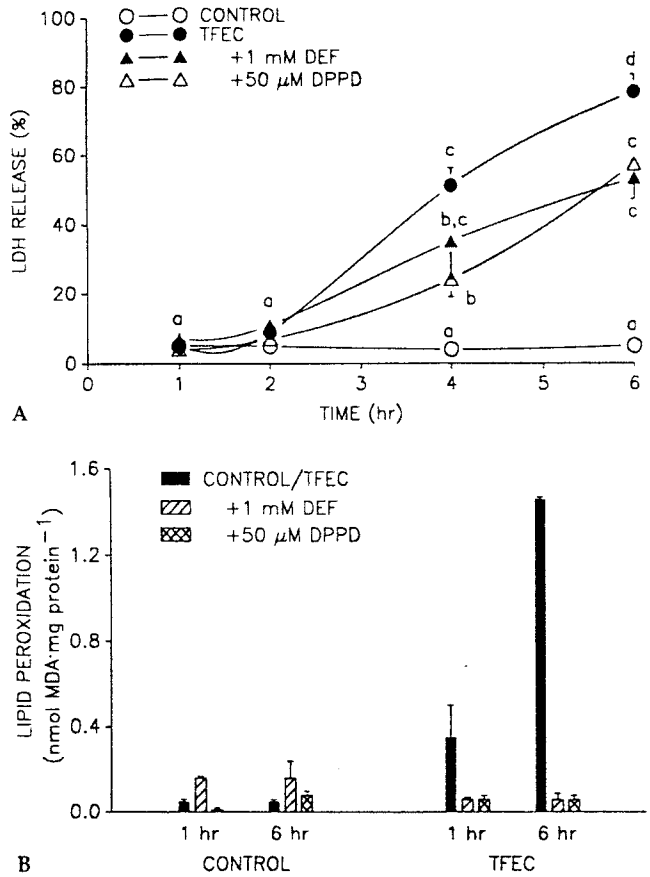

FIGURE 40-5. A: The time-dependent effects of deferoxamine (DEF) and N, N′-diphenyl-1,4-phenylenediamine (DPPD) on S-(1,1,2,2-tetrafluoroethyl)-L-cysteine (TFEC)-induced cell death (lactate dehydrogenase [LDH] release) from rabbit renal proximal tubules. DEF and DPPD were added at the same time as TFEC (25 μM). Values are means ± SEM. Values at a given time point or within a given treatment with different superscripts are significantly different from one another ($P \leq 0.05$). **B:** The time-dependent effects of DEF and DPPD on TFEC-induced lipid peroxidation (malondialdehyde [MDA] formation) in rabbit renal proximal tubule suspensions. Values are means ± SEM. Only the TFEC alone is significantly different from controls ($P \leq 0.05$). (Adapted from: Groves CE, Lock EA, Schnellmann RG. Role of lipid peroxidation in renal proximal tubule cell death induced by haloalkene cysteine conjugates. *Toxicol Appl Pharmacol* 1991;107:54, with permission.)

Mitochondria

The renal tubular reabsorption of solutes and water requires a large expenditure of energy. Although ATP is generated by both oxidative phosphorylation and glycolysis, approximately 95% of renal ATP is formed by oxidative phosphorylation (283). The amount of oxidative phosphorylation that occurs within a given cell varies along the nephron. Thus, toxicants that interfere with mitochondrial function and anoxia will produce cell injury and death, particularly in tubular cells that have limited glycolytic capabilities, such as the S_1 and S_2 segments of the proximal tubules.

Mitochondria can act as primary or secondary mediators in apoptosis and oncosis (284,285). When mitochondria are the primary target of nephrotoxicants, release of cytochrome c and other apoptotic inducing proteins can occur early in the apoptotic process. If mitochondria are not a direct target of the nephrotoxicant, these proteins may still be released but later in the apoptotic process. As stated, central to the role of the mitochondrion in apoptosis is its ability to release cytochrome c and other apoptotic inducing proteins, leading to the activation of caspases-9 and -3 and other downstream caspases (75) (Fig. 40-2). Other regulators of apoptosis that interact with the mitochondria have been discussed previously.

A key difference in mitochondrial function during apoptosis and oncosis is the maintenance of ATP during apoptosis. ATP production must be maintained long enough for apoptosis to ensue. Cellular ATP levels act with the mitochondrial membrane potential as one switch that dictates whether a cell dies by apoptosis or oncosis (286). If the mitochondrial membrane potential is lost quickly and cellular ATP levels are drastically decreased (below 10% to 20% of normal), then oncosis occurs. Events that result in the rapid loss of mitochondrial membrane potential include the rapid influx of Ca^{2+} into the mitochondria and the rupture of the inner and/or outer mitochondrial membranes (286). In contrast, if the loss of membrane potential is slower and ATP levels are maintained through oxidative phosphorylation or glycolysis, then the cell dies through apoptosis. It should be noted that the majority of cells in culture derive their energy from glycolysis and can maintain ATP levels in the presence of mitochondrial dysfunction. Consequently, cultured cells are generally more susceptible to apoptosis than cells *in vivo*.

Many nephrotoxicants cause mitochondrial dysfunction (285). The mechanisms of cell death for many of these toxicants are still under investigation, whereas others are fairly well known. For example, $HgCl_2$ altered isolated renal cortical mitochondrial function and mitochondrial morphology prior to proximal tubule necrosis after an *in vivo* exposure

(287,288). When added to isolated rat renal cortical mitochondria, $HgCl_2$ produced similar changes in various respiratory parameters (287; 288). Rabbit RPTC exposed to $HgCl_2$ also exhibit decreased mitochondrial function prior to the onset of cell death (289). Pentachlorobutadienyl-L-cysteine initially uncouples oxidative phosphorylation in RPTC cells by dissipating the proton gradient (290–293). In contrast, tetrafluoroethyl-L-cysteine does not uncouple oxidative phosphorylation but rather inhibits state-3 respiration by inhibiting sites I and II of the electron transport chain (293). Other nephrotoxicants that have been shown to affect mitochondrial function include cisplatin (294–296), citrinin (297–301), ochratoxin A (302–304), cephaloridine (305,306), N-(3,5)-dichlorophenylsuccinimide (307), S-1,2-(dichlorovinyl)-L-cysteine (308,309), and 2-bromohydroquinone (159).

Because of the vast number of nephrotoxicants that induce mitochondrial dysfunction, studies are starting to focus on the exact protein targets within the mitochondria. For example, studies determining the effect of hypoxia on mitochondrial electron transport chain constituents in rabbit RPTC reveal that complex I may be particularly sensitive (310,311). Studies also illustrate that cisplatin-induced changes in oxidative phosphorylation, membrane potential, and ATP levels in rabbit RPTC are all preceded by inhibition of F(0)F(1)-ATPase (complex IV) (185).

Recent studies in renal cortical mitochondria suggest that an increase in Ca^{2+} influx activates a mitochondrial calpain that targets complex I of the electron transport chain and induces mitochondrial dysfunction (see the previous text) (248–250). These recent studies are of critical importance to the understanding of the pathology of mitochondrial-mediated renal cell death and to identifying novel therapeutic targets for inhibition of renal cell death and possibly ARF.

Endoplasmic Reticulum

The ER is the site of protein synthesis and processing, and bioactivation and detoxification pathways, including those involving cytochrome P-450 and FMO. The ER is also a key regulator of cellular Ca^{2+} homeostasis. Under physiologic conditions, ER Ca^{2+} is typically released following receptor activation through the binding of inositol triphosphate (IP_3) to IP_3 receptors on the ER. Cytosolic free Ca^{2+} increases as a consequence of the ER Ca^{2+} release and is subsequently decreased by ER uptake via the smooth ER Ca^{2+}-ATPases (SERCA) or extrusion via the plasma membrane Ca^{2+}-ATPase.

Schnellmann and colleagues demonstrated that the release of ER Ca^{2+} is an important signaling pathway in oncosis (219,312). In this case, depletion of ER Ca^{2+} stores with the SERCA inhibitors thapsigargin or cyclopiazonic acid prior to antimycin A or hypoxia exposure resulted in the inhibition of oncosis (219,312). It was demonstrated that Ca^{2+} release from the ER activates calpains (Ca^{2+}-dependent cysteine proteases), which subsequently leads to further disruption of ion homeostasis, cleavage of cytoskeleton proteins, and cell swelling, which ultimately results in oncosis (221,224,244,312). Recent studies demonstrate that the cytoprotective effects of some stress proteins may be through their ability to regulate ER Ca^{2+}. For example, iodacetamide and S-(1,2)-dichlorovinyl-L-cysteine, both alkylating toxicants, can activate heat shock proteins (HSPs), calreticulin, and glucose related protein 78 (GRP78) in the renal epithelial cell line, LLC-PK1 (313,314). HSPs are typically ER localized proteins that are critical mediators of protein folding. Glucose related protein 78 and calreticulin are Ca^{2+} binding proteins that appear to aid in the sequestering of Ca^{2+} during toxic stress. Sequestering of Ca^{2+} by these proteins may also protect renal cells by preventing cellular oxidative stress that is induced by Ca^{2+}-mediated mitochondrial injury (315,316). The increased expression of Ca^{2+}-sequestering proteins and HSPs at the ER is a response to a previous injury and is meant to condition the cell to withstand further injury.

The ER is also a site within the kidney for genesis of apoptosis induced by a number of compounds. Caspase-12 is a murine caspase, is similar to caspase-1 and -11, and is localized to the ER. Further, caspases-12 is found in mice kidneys and is expressed in RPTC (317). Mice that do not express caspases-12 were resistant to renal cell apoptosis induced by the ER stress agents tunicaymycin, brefeldin A, and thapsigargin compared to wild type animals. In contrast, kidneys from mice null for caspases-12 underwent similar levels of apoptosis caused by the fas antibody, TNF-α plus cycloheximide, or staurosporine, agents that cause apoptosis by mechanisms other than ER stress. The key to the activation of caspases-12 as opposed to other caspases may be perturbations in the ER membrane and/or Ca^{2+} levels. Recently, acetaminophen has also been suggested to activate caspase-12 in mouse RPTC (100). Thus, ER can mediate both renal cell oncosis, via the release of Ca^{2+} and calpain activation, or renal cell apoptosis via the activation of caspases-12, and it appears that both mechanisms involve changes in the ability of the ER to regulate Ca^{2+}. The exact mechanistic differences between ER-mediated apoptosis and oncosis are under investigation.

Plasma and Organelle Membranes

Some compounds can interact with the plasma membrane directly, increase ion permeability, and disrupt ion homeostasis. For example, amphotericin B is an antifungal polyene that binds to cholesterol in the plasma membrane and forms a pore that increases potassium and proton permeabilities (103,318,319). Several heavy metals such as silver, gold, mercury, and copper also appear to react with the plasma membrane and increase potassium permeability (320,321). It remains to be determined how these changes in potassium and proton permeabilities ultimately lead to cell death; however, Reeves and Shah (322) reported that inhibition of potassium channels decreases hypoxic injury in rat RPTC.

Toxicants generally disrupt cell volume and ion homeostasis by inhibiting energy production either directly or indirectly. The loss of ATP results in the inhibition of membrane transporters that maintain the differential ion gradients across the plasma membrane. The Na^+-K^+-ATPase is responsible for maintaining the normal Na^+ and K^+ gradients and the secondary ion transport processes. As ATP levels decrease, Na^+-K^+-ATPase activity decreases, resulting in K^+ efflux and Na^+ influx and a decrease in the normally negative membrane potential (323,324). The decrease in the negative membrane potential allows Cl^-, as well as additional Na^+, to enter down a concentration gradient resulting in water influx and cellular swelling. For example, treatment of rabbit RPTC suspensions with the mitochondrial inhibitor antimycin A inhibits respiration within 1 minute, followed by ATP depletion, and the loss of the sodium and potassium gradients and transport over the next 5 to 10 minutes (224,325) (Fig. 40-6). Miller and Schnellmann (326) demonstrated that increased Cl^- influx does not occur during the initial 15 minutes but between 15 and 30 minutes, during the late stages of cellular injury, followed by cellular rupture. Decreasing extracellular NaCl concentrations by 50% with isoosmotic substitution of mannitol decreased Cl^- influx, cellular swelling, and cellular rupture (327). Furthermore, hyperosmotic incubation buffer decreased the cellular swelling and cellular lysis but not the increased Cl^- influx (327). Thus, the delayed increase in Cl^- influx may be the trigger for the water influx and additional Na^+ influx that provides the osmotic force for cellular swelling and rupture.

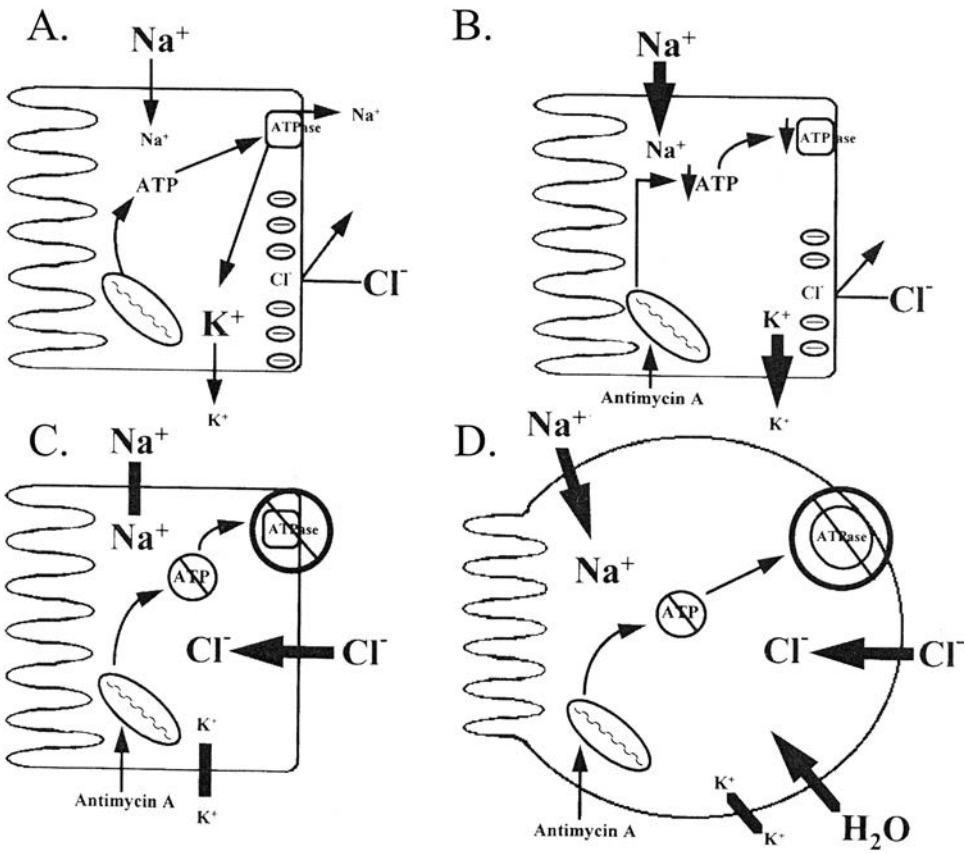

FIGURE 40-6. A: A schematic representation of a normally functioning renal cell. Note that the inside of the cell is negative with respect to the outside, which decreases the ability of Cl^- to enter the cell. **B:** The addition of a mitochondrial inhibitor such as antimycin A blocks cellular respiration, decreases ATP levels and Na^+-K^+-ATPase activity, increases Na^+ influx and K^+ efflux, and decreases the membrane potential. **C:** Subsequently, there is an increase in Cl^- influx (down the concentration gradient) by an unidentified pathway. **D:** The increase in Cl^- influx results in water influx, increased Na^+ influx, and cellular swelling. These processes provide the osmotic force that ultimately leads to cellular lysis.

Increased Cl^- influx occurs during the late stages of cell injury in RPTC and LLC-PK1 cells exposed to a variety of injury stimuli and toxicants, including $HgCl_2$, *t*-butylhydroperoxide, bromohydroquinone, tetrafluoroethyl-L-cysteine, and hypoxia (327,328). The mechanism by which Cl^- influx occurs under these conditions is not known, but it is inhibited by blockers of Ca^{2+}-activated Cl^- channels (e.g., niflumic acid, indanyloxyacetic acid (IAA-94), 5-nitro-2-(3)-phenylpropylamino-benzoate (NPPB), and diphenylamine-2-carboxylate (DPC)) (150–153). The Cl^- influx is insensitive to the Cl^- channel blockers 4-acetamide-4′-isothiocyanostilbene-2,2′-disulfonic acid (SITS) and diisothiocyanostilbene-2,2′-disulfonic acid (DIDS) or to the Cl^- transport inhibitors bumetanide and hydrochlorothiazide (327). Therefore, the Cl^- influx that occurs during the late phase of cell death may be through a Ca^{2+}-activated Cl^- channel.

Lysosomes

Lysosomes are membrane-bound vesicles that pinch off from the Golgi-apparatus and contain a variety of hydrolytic enzymes (329). Under normal conditions lysosomes contain hydrolytic enzymes that function as intracellular digestive enzymes. However, lysosomes can play an important role in the toxicity of several compounds including aminoglycoside antibiotics, and in α_{2u}-nephropathy. α_{2u}-nephropathy occurs in male rats when compounds such as unleaded gasoline, *d*-limonene, 1,4-dichlorobenzene, tetrachloroethylene, decalin, 2,2,4-trimethylpentane, and lindane bind to α_{2u}-globulin and prevent its normal renal proximal tubular lysosomal degradation (330–333). α_{2u}-Globulin is synthesized in the liver of male rats under androgen control. Serum α_{2u}-globulin (18.7 kDa) is freely filtered by the glomerulus with approximately half being reabsorbed via endocytosis in the S_2 segment of the proximal tubule. The binding of these agents to α_{2u}-globulin inhibits its normal degradation and results in the accumulation of α_{2u}-globulin in the proximal tubule. Over time, the size and number of lysosomes increase, and characteristic protein-droplet morphology is observed. Ultimately, this leads to single-cell oncosis, the formation of granular casts at the junction of the proximal tubule and the thin loop of Henle, and cellular regeneration. Recent data show that an HS protein cognate of 73 kDa mediates the binding of α_{2u}-globulin to a 96-kDa membrane glycoprotein in male rat kidney lysosomes (334). This HSP also is involved in the degradation of other cellular proteins. Treatment of rats with 2,2,4-trimethylpentane increases the rate of transport of not only α_{2u}-globulin into the lysosome, but also increases the rate of lysosomal transport of many proteins. The increase in transport is a result of α_{2u}-globulin-mediated increases in the level of the receptor proteins in the lysosomal membrane. Thus, α_{2u}-globulin may induce lysosomal overload by increasing the rate of transport of cellular proteins to the lysosome. In this manner, chronic exposure to the preceding compounds may lead to a chronic nephropathy and in some cases results in an increased incidence of renal adenomas/carcinomas by nongenotoxic mechanisms.

α_{2u}-nephropathy is sex- and species-specific, occurring in particular strains of male rats but not female rats, male or female mice, rabbits, or guinea pigs. It does not occur in female rats, other species, or male Black Reiter rats because they do not produce α_{2u}-globulin. Because humans are exposed to these diverse compounds regularly, they may be at risk for α_{2u}-globulin-induced nephropathy and renal tumors. Current evidence suggests that humans are not at risk because: (a) humans do not synthesize α_{2u}-globulin; (b) humans secrete fewer proteins in general and, in particular, fewer

low-molecular-weight proteins in the urine than the rat; (c) the low-molecular-weight proteins in human urine are either not related structurally to α_{2u}-globulin, do not bind to compounds that bind to α_{2u}-globulin, or are similar to proteins in female rats, male Black Reiter rats, rabbits, or guinea pigs that do not exhibit α_{2u}-globulin nephropathy; and (d) mice excrete a low-molecular-weight urinary protein that is 90% homologous to α_{2u}-globulin, but do not exhibit α_{2u}-globulin nephropathy and renal tumors after exposure to α_{2u}-globulin nephropathy-inducing agents (335).

The aminoglycoside antibiotics also induce lysosomal dysfunction and cause ARF failure (105,106,336) (Chapter 42, Antibiotic- and Immunosuppression-Related Renal Failure). In this case, the aminoglycosides are filtered, bound to anionic phospholipids in the brush border, reabsorbed by endocytosis in the S_1 and S_2 segments of the proximal tubule, and accumulated in the lysosomes. Over time, the size and number of lysosomes increase and electron-dense lamellar structures called myeloid bodies appear. The myeloid bodies contain undegraded phospholipids and are thought to occur through aminoglycoside-induced inhibition of lysosomal hydrolases such as sphingomyelinase and phospholipases. However, the steps between lysosomal phospholipid overload and tubule cell death are less clear.

CELLULAR DEFENSES

The RPT cell has numerous defenses against both reactive intermediates and ROS (Fig. 40-4). Glutathione, a primary cellular protectant and the most abundant cellular nonprotein thiol, is found at high concentrations in at least three subcellular compartments (cytosol, mitochondrion, nucleus) (337). Normally, glutathione detoxifies electrophiles by forming a glutathione conjugate either directly or with the aid of glutathione S-transferases. For example, RPT cells detoxify compounds containing a quinone nucleus, such as bromohydroquinone, by conjugating it to glutathione, forming mono- and di-substituted glutathione conjugates (159). Glutathione also acts in conjunction with glutathione peroxidase and glutathione reductase to neutralize ROS. In this case, an organic peroxide is reduced to water and an alcohol by glutathione peroxidase, forming glutathione disulfide (Fig. 40-4). Glutathione disulfide is reduced to glutathione by glutathione reductase in an NADPH-dependent reaction. Catalase and superoxide dismutase are two other enzymes that detoxify ROS. Superoxide dismutase converts the superoxide anion to H_2O_2, and catalase converts the hydrogen peroxide to water.

Several studies have investigated the differences in the activity of glutathione-dependent enzymes among different cells of the kidney. This area is of interest as differences in the activity of these enzymes may account for differences in the susceptibility of different kidney regions to oxidative stress. Cummings and associates (145) reported that the levels of glutathione peroxidase and γ-glutamylcysteinyl synthetase are higher in rat RPT cells than distal tubule cells. The activity of glutathione reductase and glutathione S-transferase appears to be equal between the two cell populations; however, the proximal tubular cells have a much higher concentration of glutathione than distal tubular cells (27 nmol/mg for proximal tubular cells versus 13 nmol/mg for distal tubular cells) (338).

Lash and colleagues have demonstrated that overexpression of the dicarboxylate carrier, a protein responsible for the transport of gluthathione into mitochondria, protected normal rat kidney-52E cell lines from both oxidant (*t*-butylhydroperoxide) and S-(1,2)-dichlorovinyl-L-cysteine-induced apoptosis (339). The mechanism of protection correlated to increased transport of glutathione into the mitochondria. The increased level of glutathione decreased oxidant-induced mitochondrial dysfunction and the subsequent release of cytochrome c and caspase activation.

Vitamin C (ascorbic acid) under normal circumstances is a very effective reducing agent and free radical scavenger and functions in the recycling of the Vitamin E radical back to Vitamin E (340,341). Like glutathione, Vitamin C can detoxify compounds containing a quinone nucleus such as bromohydroquinone, but in this case Vitamin C reduces the bromoquinone and the bromoquinone radical back to bromohydroquinone (159); however, Vitamin C can act as a prooxidant in the presence of iron under some circumstances.

Reports have suggested that Vitamin C may function in rabbit RPTC to promote repair and regeneration after exposure to *t*-butylhydroperoxide and S-(1,2)-dichlorovinyl-L-cysteine (14–16). Vitamin C added in pharmacological concentrations to primary cultures of rabbit RPTC prior to, during, and after exposure to toxicants improved recovery in these cultures as measured by increases in cell number and mitochondrial function (16). The effect of Vitamin C was not the result of its antioxidant function, because both *t*-butylhydroperoxide and S-(1,2)-dichlorovinyl-L-cysteine caused the same amount of damage in treated and untreated cultures. Rather, the increase in cellular function observed in cultures treated with Vitamin C was linked to its ability to aid cells in recovery after damage, potentially through its ability to promote collagen deposition in the extracellular matrix (16).

Vitamin E (α-tocopherol) is a lipid-soluble antioxidant found in cell membranes (342). Vitamin E is known as a chain-breaking antioxidant because it contributes an electron to a peroxyl radical formed during lipid peroxidation and thereby prevents further lipid peroxidation. The Vitamin E radical produced is unreactive and is recycled back to Vitamin E. Vitamin E can suppress cyclosporin A-mediated toxicity *in vivo* in rat renal kidneys by inhibiting lipid peroxidation (343). Vitamin E also can protect freshly isolated rat proximal tubule cells from the toxicity of cephaloridine, a beta-lactam antibiotic that causes cell specific acute tubular necrosis *in vivo* (344). The protective effect of Vitamin E on proximal tubule cells in this study also correlated with the decreases in the level of lipid peroxidation.

Glycine

During studies designed to examine the cytoprotective effects of glutathione, it was observed that glycine was cytoprotective in a number of models (345). In addition to glycine, a few other small amino acids of similar structure, including D- and L-alanine, ß-alanine, and 1-aminocylopropane-1-carboxylic acid, were protective, indicating a stringent structural requirement for cytoprotection. Studies have demonstrated that glycine is cytoprotective against a diverse group of chemical insults, such as anoxia, metabolic inhibitors, bromohydroquinone, halogenated alkene, and alkane cysteine conjugates, and to a lesser extent *t*-butylhydroperoxide and $HgCl_2$ (217,346). The mechanism of glycine cytoprotection has remained elusive, but recent studies demonstrate that glycine acts during the terminal phase of cell injury (346). Furthermore, Aleo and Schnellmann (347) and Miller and associates (326,327,348,349) observed that the neuronal glycine receptor antagonist strychnine was cytoprotective and acted in a similar manner to that of glycine under a variety of conditions. Thus, strychnine and glycine may be cytoprotective through a ligand–acceptor interaction. Strychnine binds to a low-affinity binding site on the basolateral membrane of the rabbit RPT cell in a saturable and reversible manner at concentrations equivalent to that necessary for cytoprotection (245). Proteins corresponding to two of the three subunits of the neuronal strychnine-sensitive glycine receptor have been identified on the basolateral

membrane of the RPT and a recent report has provided evidence that one of these is the glycine receptor ß-subunit (348–350). The signal transduction pathway for the neuronal glycine receptor is Cl^-. Because Cl^- influx plays a major role in providing the osmotic force for the swelling during cell injury and glycine and strychnine block Cl^- influx, glycine and strychnine are cytoprotective by directly or indirectly altering Cl^- influx.

Alternatively, Nichols and associates (351) proposed that glycine is cytoprotective in hepatocytes through its ability to inhibit calpains. However, Edelstein and coworkers (246) reported that glycine did not inhibit calpain activity in rat RPT exposed to hypoxia. Studies in rabbit RPT demonstrated that glycine and strychnine did not inhibit basal calpain activity but did inhibit the increase in calpain activity observed during the late phase of cellular injury (352). Later studies confirmed that glycine does not directly affect calpain activity, but rather inhibited toxicant-mediated extracellular Ca^{2+} influx, calpain translocation, and Cl^- influx (352). Further studies are needed to conclusively identify the mechanism of glycine cytoprotection.

Acidosis

Although acidosis is not a normal cellular defense mechanism per se, decreasing intracellular pH has been shown to be cytoprotective in a variety of *in vitro* models, and as such has contributed to our understanding of cell death (353,354). Using freshly isolated RPT, extracellular pH reduced to 6.8 to pH 7.0 results in cytoprotection (354–356). Interestingly, Weinberg (355) demonstrated that the protective effect of extracellular acidosis not only was limited to the addition of acids to the extracellular medium, but also was observed in high-density suspensions of RPT subjected to anoxia. Under these conditions, the RPT spontaneously lowered the pH of surrounding medium to pH 7.0, presumably due to the hydrolysis of ATP and the accumulation of protons. Thus, under conditions of ischemia *in vivo,* it is possible that localized acidosis may afford a degree of cytoprotection.

A number of findings have contributed to our understanding of the cytoprotective effect of extracellular acidosis. Rodeheaver and Schnellmann (353) demonstrated that extracellular acidosis (pH 6.4) ameliorated renal proximal tubular cell death produced by a series of mitochondrial inhibitors (antimycin A, rotenone, carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone, oligomycin) and ion exchangers (nigericin, monensin, valinomycin) but potentiated cell death produced by the oxidants *t*-butylhydroperoxide, hydrogen peroxide, and ochratoxin A. Associated with the extracellular acidosis-induced increases in cell death was an increase in glutathione disulfide formation, lipid peroxidation, and mitochondrial dysfunction and a decrease in glutathione peroxidase and reductase activities. Thus, the mechanism for this extracellular acidosis-induced potentiation of oxidant toxicity is most likely the result of a decrease in free radical detoxification.

Previous studies indicated that extracellular acidosis was not cytoprotective through its ability to preserve mitochondrial function or ATP levels (353–355). Studies have demonstrated that extracellular acidosis initiated at various times after toxicant exposure was still cytoprotective (212,354). For example, extracellular acidosis initiated 15 minutes after antimycin A or carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone addition, a time point after the cessation of respiration, depletion of ATP, and increases in intracellular sodium and decreases in intracellular potassium, was completely cytoprotective at 45 and 105 minutes later, respectively. However, the cytoprotection did not prevent the increase in Cl^- influx that occurs in the late stages of cell injury. Extracellular acidosis initiated 2 hours after tetrafluoroethyl-L-cysteine or *t*-butylhydroperoxide addition also was cytoprotective 2 hours later. These results demonstrate that the cytoprotective effect of extracellular acidosis occurs very late in the cell injury process distal to Cl^- influx.

Peroxisomes and Peroxisomal Proliferating Activated Receptors (PPAR)

Peroxisomes are membrane-bound vesicles that contain degradative enzymes for fatty acids and amino acids (329). In addition peroxisomes contain catalase, which converts H_2O_2 to oxygen and water. Although the ability of peroxisomes to metabolize H_2O_2 is crucial in the protection of cells against oxidative stress, peroxisomal proliferation has been linked to the preservation of mitochondrial function (357) and the reduction in renal cell death following injury induced by gallic acid (358), cisplatin (256,359), and ischemia/reperfusion-induced injury.

Peroxisomes also may protect against renal cell death via mechanisms linked to the activation of peroxisomal proliferator receptors (PPAR). PPAR are members of a nuclear hormone-activated receptor and transactivation protein family (360). At this time, three different PPAR have been identified and cloned (PPAR-α, PPAR-β/δ, and PPAR-γ) (360). PPAR-β/δ appears to be expressed in almost all tissues including the kidney cortex (360–362). PPAR-γ is present in distal medullary collecting ducts, glomeruli, and the renal microvasculature (362,363). PPAR-α is expressed in the proximal tubule, medullary thick ascending limbs, and the glomerular mesangial cells. It is hypothesized that differences in the distribution PPAR isoforms may result in different mechanisms of protection between different cells.

Activators of PPAR are structurally diverse and include plasticizers (di[2-ethyhexl]phthalate) (364), herbicides (365), hypolipidemic drugs (fenofibrate, clofibrate, and clofibric acid) (360) and antidiabetic drugs (360,365,366) (e.g., troglitazone and rosiglitazone) (366–369). Activators of PPAR increase the number of peroxisomes within the cell and increase the expression of enzymes for fatty acid β-oxidation including fatty acyl-CoA oxidase, enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase bifunctional enzyme, and 3-ketoacyl-CoA thiolase (364,365,370,371). Activators of PPAR isoforms also increase mitochondrial enzymes including carnitine palmitoyl transferase, medium chain acyl-CoA dehydrogenase, and pyruvate dehydrogenase complex (359,362). The increase in these proteins is believed to be key in the protection against nephrotoxicants (360,362,365,372).

The overall affect of the activation of PPAR, at least activation of PPAR-α, on renal cell death appears to be beneficial. The PPAR-α agonist clofibrate and WY14643 reduce renal cell dysfunction and injury induced by ischemia/reperfusion in male rat kidneys (362,373,374). *In vivo* studies demonstrate that induction of PPAR-α also correlated to increases in mitochondrial medium-chain acyl-CoA dehydrogenase and pyruvate dehydrogenase complex activity and decreases in cisplatin-induced proximal tubular necrosis (359). The exact mechanism involved in the PPAR-α mediated protection against nephrotoxicants has yet to be determined.

SPECIFIC TOXICANTS

It is critical to identify the ultimate toxic species and the cell type targeted in order to understand the mechanism by which a chemical produces nephrotoxicity. For example, is the glomerulus, proximal convoluted tubule, proximal straight tubule, the thick ascending limb of Henle, or the distal convoluted tubule the target of the parent compound, a primary

or secondary metabolite? Thus, excellent biotransformation, toxicokinetic, and morphologic studies are paramount in determining the sites of biotransformation, which metabolites reach the kidney, the quantity of metabolites in the kidney, the target cell type in the kidney, and ultimately the mechanism of nephrotoxicity. Other chapters in this book focus on specific toxicants such as analgesics (Chapter 44, Nephrotoxicity of Nonsteroidal Antiinflammatory Agents, Analgesics, and Angiotensin-Converting Enzyme Inhibitors), antibiotics (Chapter 42, Antibiotic- and Immunosuppression-Related Renal Failure), antineoplastics (Chapter 43, Renal Diseases Induced by Antineoplastic Agents), heavy metals (Chapter 47, Nephrotoxicity Secondary to Environmental Agents and Heavy Metals), immunosuppressives (Chapter 42, Antibiotic- and Immunosuppression-Related Renal Failure), and radiocontrast media (Chapter 45, Radiocontrast Media-Induced Acute Renal Failure).

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CHAPTER 41 ■ ACUTE RENAL FAILURE

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Acute renal failure (ARF) is defined as an abrupt decrease in renal function sufficient to result in retention of nitrogenous waste (e.g., blood urea nitrogen [BUN] and creatinine) in the body. Although there is unanimity of opinion regarding this general definition, there is no consensus regarding the magnitude of elevation of serum creatinine and BUN sufficient to ascribe a diagnosis of ARF (1–3). Moreover, there is a nonlinear relationship between decreasing glomerular filtration rate (GFR) and rising serum creatinine concentration in individuals with a normal basal serum creatinine. Thus, in individuals with a normal basal serum creatinine, significant decreases in GFR are often associated with either slight or modest increases in serum creatinine concentration (4). Also, not only renal elimination, but also rate of production and volume of distribution are significant determinants of serum creatinine concentration (5). Taken together, although practical and currently our most useful tool for diagnosis, increases in serum creatinine concentration can be a somewhat insensitive marker of ARF.

Not only differences in diagnostic criteria, but also differences in frequency of surveillance and populations studied render definitive conclusions about the incidence of ARF difficult. Nonetheless, the data depicted in Table 41-1 demonstrate that ARF occurs with relatively high frequency, especially in seriously ill hospitalized patients (6–34). Figure 41-1 depicts potential high incidence settings of ARF.

Acute renal failure not only occurs with high frequency, but is also associated with significant morbidity and mortality. A matched-pairs cohort study of mild-to-moderate radiocontrast-associated ARF found a mortality of 7% in controls that was increased about sixfold in patients with ARF (35). A prospective case control study of 17,126 intensive care unit (ICU) patients found that patients with ARF treated with dialysis had an over four times higher hospital mortality than control patients (62.8% vs. 15.6%, $p < .001$), which remained significantly higher even when dialysis patients were matched with control subjects for age, severity of illness, and treatment center (36). Contemporary mortality of patients with oliguric and nonoliguric ARF remains in the 40% to 80% and 15% to 20% ranges, respectively (Fig. 41-2) (37–44). It is noteworthy that a direct relationship exists between the magnitude of rise in serum creatinine concentration and mortality of ARF (Fig. 41-3) (37). This emphasizes the need for early assessment and intervention in all cases of ARF. Together, it is apparent that ARF is associated with substantial morbidity, mortality, and cost.

The financial costs of ARF are high. In a Canadian ICU, the cost of dialysis was $3,486 to $5,117 (Canadian) per week for continuous renal replacement therapy (CRRT), and $1,342 a week for ischemic heart disease (IHD). The total direct cost of care for the subsequent year for patients who remained on dialysis was substantially higher ($73,273) than for those patients with renal recovery ($11,192) (45). A prospective cohort study of 490 ARF patients requiring dialysis from five geographically distant teaching hospitals estimated costs per quality-adjusted life year saved to be from $62,000 to $274,000 (46). In a Finnish study carried out in 1992, the mean cost of the treatment for ARF was $36,000 per patient and $80,000 for ARF survivors (47). These latter data are now more than 12 years old, suggesting even greater contemporary cost.

Abrupt and progressive renal failure is the final common pathway for several disease processes (Tables 41-2 to 41-4; Fig. 41-4). Thus, multiple disease entities and diverse pathologic conditions can produce a similar clinical entity of ARF. The high frequency of occurrence, multiple causes, and potential for high morbidity and mortality demand a logical approach to the patient with ARF. In this chapter, we use the term ARF in its most generic sense to describe acute impairment of the kidney function independent of cause and mechanism. We discuss the causes, clinical settings, diagnostic approaches, consequences, and therapy of such clinically encountered ARF.

This chapter will discuss the general clinical aspects of acute renal failure. The pathophysiology of ARF (Chapters 39 and 40), antibiotic- and immunosuppression-related aspects of ARF (Chapter 42), renal diseases induced by antineoplastic agents (Chapter 43), nephrotoxicity of nonsteroidal anti-inflammatory agents, analgesics and angiotensin-converting enzyme inhibitors (Chapter 44), radiocontrast media–induced ARF (Chapter 45), nephrotoxicity secondary to drug abuse and lithium use (Chapter 46), nephrotoxicity secondary to environmental agents and heavy metals (Chapter 47), acute tubulointerstitial nephritis (Chapter 48), acute renal failure associated with pigmenturia or crystal deposits (Chapter 49) are discussed elsewhere.

DIAGNOSIS OF ACUTE RENAL FAILURE

Acute renal failure is most often diagnosed by finding increasing concentrations of serum creatinine and/or blood urea nitrogen (BUN). The usual BUN:serum creatinine ratio is about 15:1 and the BUN and serum creatinine increase by 10 to 15 and 1.0 to 1.5 mg/dL/day, respectively, in the absence of GFR. Several clinical situations can disproportionately affect either the BUN or serum creatinine thereby altering the BUN:serum creatinine ratio (48). Moreover, as is apparent from Figure 41-5, factors other than a reduction in GFR can be associated with increased concentrations of BUN (e.g., catabolic state with enhanced urea nitrogen formation) and occasionally serum creatinine (e.g., medication effects to impair renal tubular secretion of creatinine and chemically interfere with creatinine measurements).

The serum creatinine concentration is usually a better marker of glomerular filtration rate (GFR) than the BUN. In a steady state setting, a reasonable approximation is that each time the GFR halves the serum creatinine concentration doubles. Thus, steady state GFRs of 100, 50, 25, 12.5, and 6.25 mL/minute are associated with serum creatinine concentrations of about 1.0, 2.0, 4.0, 8.0, and 16.0 mg/dL, respectively. Acute renal failure often occurs in a non–steady state in which the three determinants of serum creatinine concentration (production, volume of distribution, and renal elimination) fluctuate (5). Computerized models derived from ARF patients

TABLE 41-1

INCIDENCE OF ACUTE RENAL FAILURE

Population with ARF	Any ARF (%)	Severe ARF (%)
General public (18,19)		
<50 yr of age	—	0.0017*
15–64 yr of age	—	0.0006*
>65 yr of age	—	0.0047*
>80 yr of age	—	0.095*
Pregnancy (20,21)		
Presumably healthy	—	0.00006
Underprivileged, inner city	—	0.0002
Heart disease (22–26)		
Admission to a coronary care unit	—	4
After coronary intervention	14	1
Cardiopulmonary resuscitation survivors	—	29
Infections (27–31)		
Hospitalized for pneumonia	8	—
Community-acquired bacteremia	24	4
Rocky Mountain spotted fever	20	—
Human immunodeficiency virus	20–40	10–30
Extensive trauma (32,33)	20–40	0.1–5.0
Significant burns (34,80)	15–30	1–5
Liver disease (96,224)		
Spontaneous bacterial peritonitis	30–40	0–1
End-stage liver disease	20–40	10–20
Hospital admission (37,54)		
General medical—surgical ward	1–4	0.1–1.0
Intensive care units	10–30	1–5
Nonrenal transplantation (96,185,192,195)		
Cardiac	25–50	1–15
Liver	30–50	20–40
Bone marrow autologous	5–15	2–7
Bone marrow allogeneic	30–60	15–30
Postoperative (24,25,161,167)		
Elective abdominal aortic aneurysm	10–30	2–10
Obstructive jaundice	5–10	2–3
General surgery	20–30	0.1–2.0

ARF, acute renal failure.
Severe ARF, ARF requiring dialysis except in those data marked by * where severe ARF is defined by a rise in serum creatinine to over 500 μmol/L with subsequent fall below 500 μmol/L.
— = not stated or assessed.

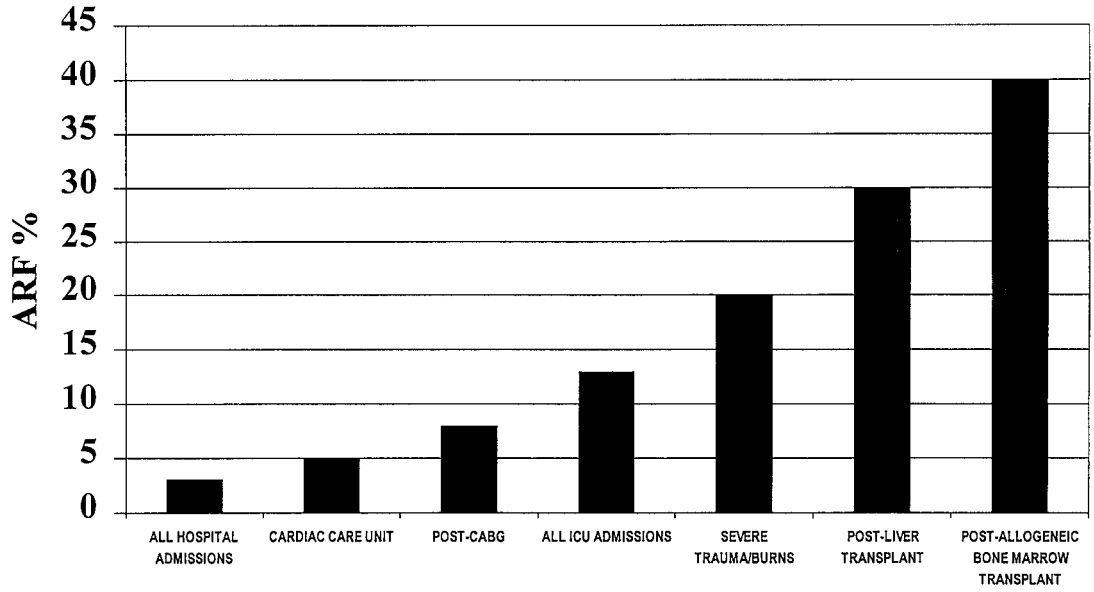

FIGURE 41-1. Frequency of acute renal failure (ARF) in selected settings.

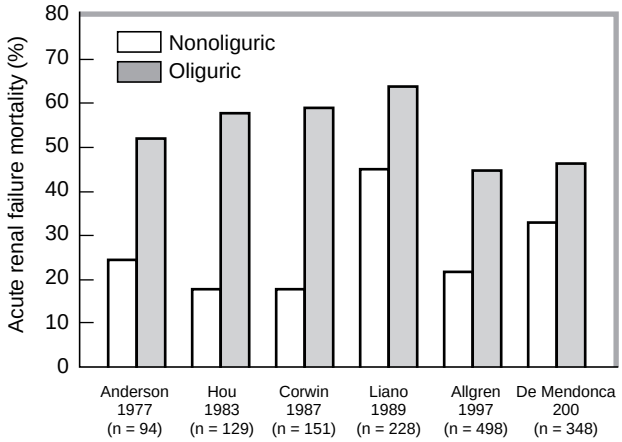

FIGURE 41-2. Mortality of oliguric and nonoliguric acute renal failure in six studies published in the past quarter of a century.

demonstrate that several patterns of change in GFR occur during development and recovery from ARF. These GFR changes are poorly reflected by daily changes in serum creatinine concentration (5). Moreover, the rise in serum creatinine that occurs in ARF is a *post facto* finding. Unfortunately, although real-time, noninvasive monitoring of GFR can be done in seriously ill patients, these techniques are currently expensive and not widely available (49).

In selected circumstances it is not clear if an elevated BUN:serum creatinine ratio is due to an acute or chronic process (Table 41-5). In this setting, review of previous records is helpful. In the absence of previous values, measurement of carbamylated hemoglobin can be helpful. Nonenzymatic carbamylation of the terminal valine of hemoglobin occurs in direct relationship to the magnitude and duration of increase in BUN (50,51). A carbamylated hemoglobin level greater than 80 to 100 μg carbamyl valine per gram hemoglobin suggests the diagnosis of acute rather than chronic renal failure (52,53). The presence of small kidney size on an imaging study strongly supports a diagnosis of chronic renal disease and the other factors noted in Table 41-5 may also help to differentiate acute from chronic renal failure. Because reversible factors often are operative in both acute and chronic renal failure, the clinician should assume the presence of potentially treatable conditions in all cases of renal failure.

Other biochemical markers of ARF that are more sensitive than serum creatinine have been sought (Table 41-6). Urinary markers of early renal injury have been studied recently. Kidney injury molecule (KIM-1) is a transmembrane protein found in proximal tubule cells that was shown to be upregulated in a postischemic rat model (7). Han and colleagues found (8) that urinary KIM-1 protein concentration was significantly higher in urine samples from patients with ischemic acute tubular necrosis (ATN) compared to urine samples from patients with other forms of acute and chronic renal failure. Muramatsu and associates (9) found that the secreted, cysteine-rich, heparin binding protein Cyr61 was rapidly induced in proximal straight tubules following renal ischemia, and excreted in the urine where it might serve as an early biomarker of renal injury. No clinical studies have so far been undertaken. In a prospective study of critically ill patients, high values of urinary gamma glutamyl transpeptidase, alkaline phosphatase,

TABLE 41-2

CAUSES OF PRERENAL ACUTE RENAL FAILURE

Decreased intravascular fluid volume
Extracellular fluid loss—burns, diarrhea, vomiting, diuretics, salt-wasting renal disease, primary adrenal insufficiency, gastrointestinal hemorrhage
Extracellular fluid sequestration—pancreatitis, burns, crush injury, nephrotic syndrome, malnutrition, advanced liver disease

Decreased cardiac output
Myocardial dysfunction—myocardial infarction, arrhythmias, ischemic heart disease, cardiomyopathies, valvular disease, hypertensive disease, severe cor pulmonale

Peripheral vasodilation
Drugs—antihypertensive agents
Sepsis
Miscellaneous—adrenal cortical insufficiency, hypermagnesemia, hypercapnia, hypoxia

Severe renal vasoconstriction
Sepsis
Drugs—nonsteroidal antiinflammatory agents, β-adrenergic agonists
Hepatorenal syndrome

Mechanical occlusion of renal arteries
Thrombotic occlusion
Miscellaneous (emboli, trauma [e.g. angioplasty])

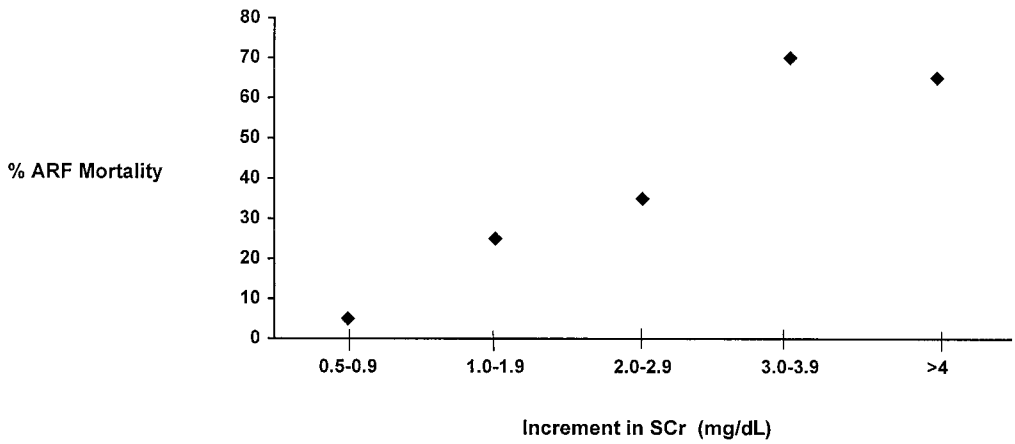

FIGURE 41-3. Relationship of acute renal failure mortality to magnitude of resultant rise in serum creatinine concentration. (From: Hou S, Bushinsky D, Wish JB, et al. Hospital-acquired renal insufficiency: a prospective study. *Am J Med* 1983;74:243, with permission.)

TABLE 41-3

CAUSES OF POSTRENAL ACUTE RENAL FAILURE

Intrarenal (intratubular)
Crystal deposition—uric acid, oxalic acid, methotrexate, acyclovir, triamterene, sulfonamides, indinavir, tenofovir
Protein deposition—light chains, myoglobin, hemoglobin
Extrarenal
Ureteral/pelvic
Intrinsic obstruction—tumor, stone, clot, pus, fungal ball, papilla
Extrinsic obstruction—retroperitoneal and pelvic malignancy, fibrosis, ligation, abdominal aortic aneurysm
Bladder
Prostate hypertrophy/malignancy
Stones
Clots
Tumor
Neurogenic
Medication
Urethral
Stricture
Phimosis

N-acetyl-glucosaminidase, and alpha- and pi-glutathione *S*-transferase, indexed to urinary creatinine, were predictive of ARF. Urinary interleukin-18 was found to be elevated in patients with ATN and delayed graft function compared with other renal diseases (12). In patients with acute renal allograft dysfunction, selected chemokines that bind to the CXCR3 receptor appear to be useful (14). In patients with ARF, the apical sodium transporter Na^+/H^+ exchanger isoform 3 (NHE3) protein appears to be a marker of renal tubule damage, helping to differentiate prerenal azotemia, ATN, and intrinsic ARF other than ATN (13). Serum cystatin C appears to be more sensitive than serum creatinine for detecting early decrements in GFR (11), but is not validated in ARF. Further studies are required to assess the usefulness of these tests in clinical practice.

Kidney regulation of the normal volume and composition of body fluids and the process of urine formation begins with ultrafiltration of the blood delivered to the kidney, proceeds through intrarenal processing of the ultrafiltrate by tubular reabsorption and secretion, and ends by elimination of the formed urine through the ureters, bladder, and urethra. It follows that ARF can result from a decrease in renal blood flow (prerenal azotemia; Table 41-2), intrinsic renal parenchymal diseases (renal azotemia; Table 41-4), or obstruction to urine flow (postrenal azotemia; Table 41-3). Because appropriate therapy of ARF depends on delineating the underlying cause, the initial step in determining the cause of ARF is to attempt to classify the site of origin of ARF as prerenal, renal, or postrenal. From a practical perspective, patients with hospital-acquired ARF tend to have more than one cause, whereas those with community-acquired ARF often have a single cause of ARF (2,18,37,41,54–58). Individuals with community-acquired ARF have fewer numbers of comorbidities and greater prevalence of ARF of postrenal cause than those with hospital-acquired ARF (3,59). A number of clinical and laboratory clues may assist in determining the site of ARF.

Prerenal Acute Renal Failure

The process of urine formation begins with delivery of blood to the glomerulus. The highly selective permeability of the glomerular capillary combined with the glomerular capillary hydrostatic pressure (which exceeds glomerular capillary oncotic pressure and intratubular hydrostatic pressure) results in formation of glomerular filtrate. Under unusual circumstances such as mannitol intoxication, high-dose dextran infusion, or marked hyperproteinemia, a "hyperoncotic state" occurs in which glomerular capillary oncotic pressure exceeds hydrostatic pressure (60–62). This results in cessation of glomerular filtration and ARF, often accompanied by an anuric state. Rapid reversal of this form of ARF occurs with removal (plasmapheresis) of the osmotically active substance from plasma.

The central role of delivery of blood to the glomerulus as the starting point of formation of glomerular filtrate dictates that clinical disorders that can decrease renal perfusion are potential causes of ARF. In clinical practice, ARF owing to hypoperfusion with a resultant fall in glomerular capillary filtration pressure is one of the most common forms of ARF

TABLE 41-4

RENAL CAUSES OF ACUTE RENAL FAILURE

Renal vascular disorders
Vasculitis
Malignant hypertension
Scleroderma
Thrombotic thrombocytopenic purpura
Hemolytic–uremic syndrome
Disseminated intravascular coagulation
Mechanical renal artery occlusion (surgery, emboli, thrombotic occlusion)
Renal vein thrombosis
Glomerulonephritis
Postinfectious
Membranoproliferative
Rapidly progressive glomerulonephritis (idiopathic, polyarteritis nodosa, systemic lupus erythematosus, Wegener's syndrome, microscopic polyarteritis, Goodpasture's syndrome, Henoch-Schonlein purpura)
Drugs
Interstitial nephritis
Drugs (penicillin, sulfonamide, rifampin, ciprofloxacin, phenindiones, cimetidine, proton pump inhibitors [omeprazole, lansoprazole], azathioprine, phenytoin, captopril, thiazides, furosemide, bumetanide, allopurinol, NSAIDs including selective cyclooxygenase-2 inhibitors, 5-aminosalicylates)
Hypercalcemia
Infections
Nonspecific due to frank septicemia or systemic antiinflammatory response syndrome
Specific organisms (Legionella, Leptospira, Rickettsia, Hantavirus, Candida, malaria)
Specific organ involvement (bacterial endocarditis, visceral abscess, pyelonephritis)
Infiltration
Sarcoid
Lymphoma
Leukemia
Connective tissue disease
Tubular necrosis
Renal ischemia (prolonged prerenal)
Nephrotoxins (aminoglycosides, radiocontrast agents, heavy metals, organic solvents, other antimicrobials)
Pigmenturia (myoglobinuria, hemoglobinuria)
Miscellaneous

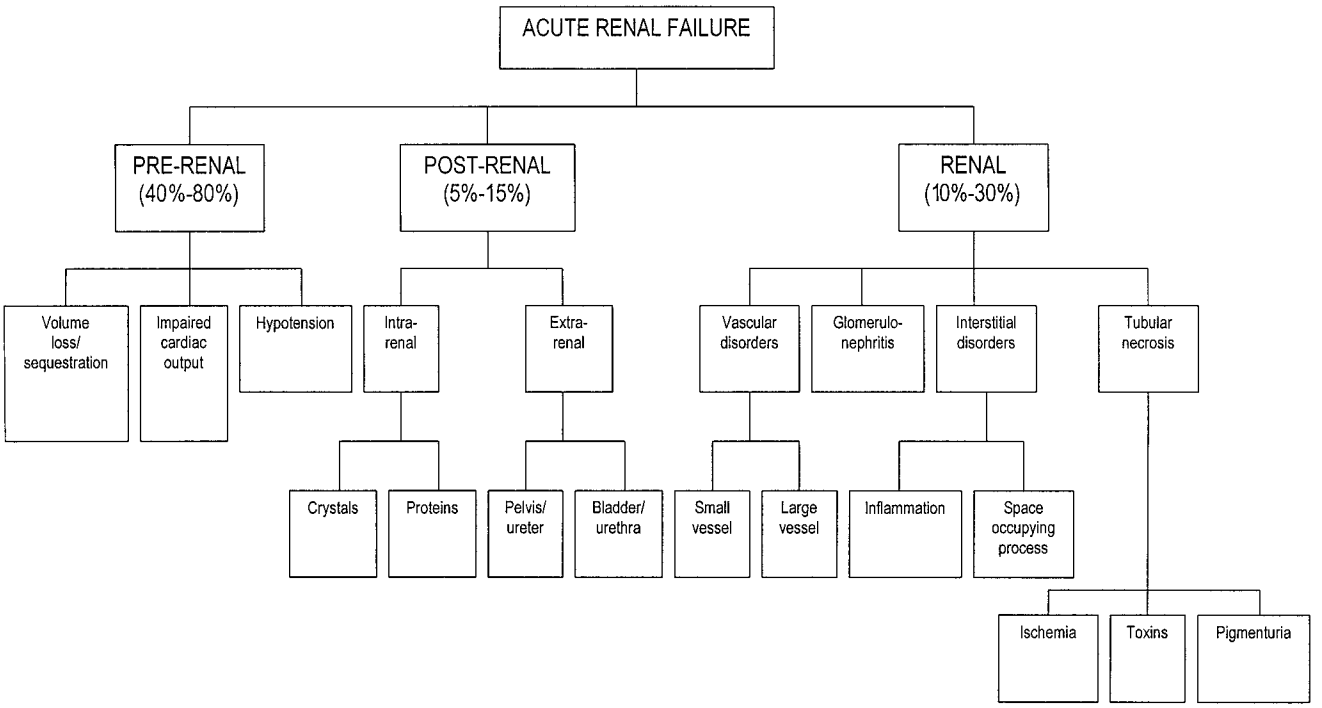

FIGURE 41-4. Causes of acute renal failure.

(1,2,18,35,38–40,44,55). The frequency of prerenal azotemia as a cause of ARF varies with the clinical setting. A prospective study by Hou et al. (37) found prerenal azotemia to be the single most common cause of ARF in a general medical-surgical hospital. Liano found that prerenal causes of ARF among the elderly accounted for 48% of community-acquired ARF and 58% of hospital-acquired ARF (63). In blacks, Obialo reported that 19% of community-acquired ARF and 35% of hospital-acquired ARF were prerenal in origin (3). Brivet observed that in critically ill patients, prerenal causes accounted for 17% of cases of ARF (44). Shusterman and associates found that the odds ratios for development of hospital-associated ARF were increased by 9.4- and 9.2-fold in the presence of the prerenal insults of volume depletion and congestive heart failure, respectively (55). Prerenal forms of ARF also appear to be common causes of community-acquired ARF and constituted 70% of all such cases in the experience of Kaufman and associates (57). Not only is prerenal azotemia common but also it is often potentially reversible. Moreover, prolonged prerenal azotemia can lead to ischemic acute tubular necrosis (ATN) with significant morbidity. Thus, recognition and prompt therapy of prerenal causes of ARF are important.

Under normal circumstances, renal blood flow and glomerular filtration rate (GFR) are relatively constant over a wide range of renal perfusion pressures, a phenomenon termed *autoregulation* (64). Renal autoregulation not only allows constancy of GFR and filtered load of solutes but also maintains constancy of oxygen delivery in spite of variable renal perfusion pressures. This autoregulatory response normally renders an individual relatively resistant to prerenal forms of ARF; however, a marked decrease in renal perfusion pressure below the autoregulatory range can lead to an abrupt decrease

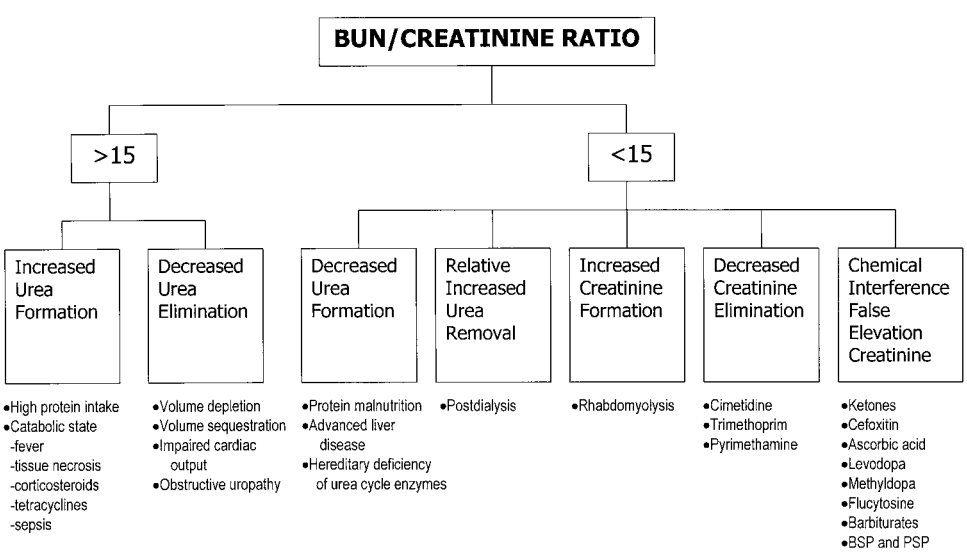

FIGURE 41-5. Causes of abnormalities in blood urea nitrogen (BUN) to serum creatinine ratio.

TABLE 41-5

DIFFERENTIATION OF ACUTE VERSUS CHRONIC RENAL FAILURE

	Favors chronic	Favors acute
History of kidney disease, hypertension, abnormal urinalysis	+	0
Small kidney size	+	0
Urinalysis with broad casts (i.e., more than two to three white blood cells in diameter)	+	0
Return of renal function to normal with time	0	+
Low carbamylated hemoglobin	0	+
Hyperkalemia, acidemia, hyperphosphatemia,[a] anemia[a]	+	+

[a] More common in chronic renal failure.

in GFR and ARF. Within the autoregulatory range, a reduction in renal perfusion, as occurs with either diminished cardiac output or depletion of extracellular fluid volume, normally results in dilation of the glomerular afferent arteriole and constriction of the glomerular efferent arteriole so that glomerular capillary-hydrostatic pressure and GFR usually remain constant (64). The afferent dilation is mediated in part by vasodilatory eicosanoids, whereas efferent constriction can be attributed in part to angiotensin II. It follows that in the setting of compromised cardiac output or intravascular volume depletion, prevention of afferent arteriolar dilation (as occurs following nonsteroidal antiinflammatory agent therapy, which impairs synthesis of selective eicosanoids) and attenuation of efferent arteriolar constriction (as occurs following angiotensin converting enzyme inhibition, angiotensin receptor inhibition, and perhaps calcium channel blocking agents) can potentially decrease glomerular capillary filtration pressure and potentially cause an abrupt decline in GFR.

From a clinical perspective, a potentially reversible "prerenal" form of ARF can be seen when nonsteroidal antiinflammatory drugs (NSAIDs) are given to patients with volume depletion, hypoalbuminemia, an edematous disorder, advancing age, underlying chronic renal failure, or recent diuretic (especially triamterene) use (65) (see Chapter 44). These clinical conditions are often associated with enhanced renal vasoconstriction owing to renal adrenergic neural tone, norepinephrine, and angiotensin II. If the nonsteroidal antiinflammatory drugs (NSAIDs) are stopped early, the renal failure readily reverses. With continued administration of the agent, a more severe form of ARF owing to ischemic ATN may occur. Selective cyclooxygenase-2 inhibitor therapy has also been associated with acute renal failure, hypertension, edema, hyponatremia, hyperkalemia, type 4 renal tubular acidosis, and acute tubulointerstitial nephritis (66).

A similar prerenal form of ARF can complicate angiotensin converting enzyme therapy (67,68). In the presence of a decrease in renal blood flow from severe bilateral renal artery stenosis, renal artery stenosis in a solitary kidney, and other high-renin, high-angiotensin II states (i.e., edematous states and volume depletion disorders), angiotensin II converting enzyme inhibition with a resultant decrease in both renal perfusion pressure and efferent arteriolar constriction can precipitously decrease GFR. For example, about one-third of patients with severe congestive heart failure experience an abrupt rise in serum creatinine concentration following angiotensin converting enzyme inhibitor therapy (68). In the setting of heart failure, this increase in serum creatinine following angiotensin converting enzyme inhibition tends to be mild and readily reversible on discontinuation of the drug.

Several clinical conditions can result in enhanced renal afferent arteriolar vasoconstriction, which potentially impairs renal autoregulation, thereby decreasing glomerular capillary hydrostatic pressure and inducing ARF. For example, in the setting of hemorrhagic hypotension or Gram-negative sepsis, the combined effect of intense renal adrenergic neural traffic, norepinephrine, angiotensin II, endothelin, a variety of lipid-derived mediators (thromboxanes, leukotrienes, and prostaglandin F_{2a}-like compounds), and endotoxin can all exert potent vasoconstrictor influences on the afferent arteriole of the kidney. Thus, the decrease in systemic and, therefore, renal perfusion pressure that accompanies septic or hemorrhagic shock, combined with intense afferent arteriolar constriction, can result in a precipitous fall in GFR and ARF.

It is noteworthy that experimental studies demonstrate that one effect of ischemic ATN may be to impair renal autoregulation (69). Some of this impaired autoregulation appears to be due to the effect of enhanced renal adrenergic neural tone and increased action of renal vasoconstrictors such as thromboxane to prevent afferent arteriolar dilation as renal perfusion pressure is reduced (69). Impaired generation of endothelial-derived relaxing factors such as nitric oxide may be important determinants of abnormal autoregulatory responses under normal and pathologic conditions. Sublethal endothelial injury and endothelial dysfunction, resulting in an impaired release of nitric oxide produced by endothelial-derived nitric oxide synthase, have been described in ischemic kidneys (70,71). From a clinical perspective in the setting of ATN, it is possible that modest decreases in renal perfusion pressure can serve to maintain a decrease in GFR in the maintenance phase of ARF, thus preventing or prolonging recovery from ATN.

TABLE 41-6

EXPERIMENTAL URINARY MARKERS IN ARF (ADAPTED FROM [6])

Enzymes e.g., gamma glutamyl transpeptidase, alkaline phosphatase, *N*-acetyl-glucosaminidase, alpha- and pi-glutathione *S*-transferase
Tubular proteins, e.g., adenosine-deaminase binding protein, kidney injury molecule, cyr61 (cysteine-rich heparin binding protein),
Growth factors, e.g., human epidermal growth factor, hepatocyte growth factor
Low-molecular-weight proteins
Proteases, e.g., L-alanine aminopeptidase, *N*-acetyl-[beta]-D-glucosaminidase
Cytokines, e.g., IL-2, IL-6, IL-18
Chemokines, e.g., CXCR3 binding chemokines, monocyte chemotactic peptide-1
Adhesion molecules, e.g., intercellular adhesion molecule-1, CD8, CD25
Amino acids
Cytodiagnostics, e.g., proximal tubule cells
Coagulation factors
Ion and water transporters, e.g., NHE3

IL, interleukin.

It is also of interest that fixed renal artery stenosis is an important determinant of the renal autoregulatory response and thus predisposition to ARF. Studies by Textor and associates suggest a "critical renal perfusion pressure" in patients with fixed renal artery stenosis (72). In these studies, eight patients with unilateral renal artery stenosis tolerated sodium nitroprusside–induced arterial pressure reduction without a change in either GFR or estimated renal plasma flow. By contrast, a similar pressure reduction in eight patients with bilateral renal artery stenosis resulted in marked reductions in GFR and renal plasma flow. Sensitivity of GFR and renal plasma flow to blood pressure reduction was eliminated following revascularization in four patients with bilateral renal vascular stenosis (72). In addition to impairing renal autoregulation, fixed renal artery stenosis may also impair renal blood flow sufficient to cause a reversible form of prerenal azotemia (73). Correction of renal artery stenosis may not improve blood pressure or renal function if there is evidence of structural renal damage such as high resistance to renal blood flow (74). Although chronic renal hypoperfusion can lead to ischemic glomerulopathy, improvement in renal function often occurs in kidneys >9 cm in size once chronic vascular occlusion is relieved (73).

A number of disorders that result in extracellular fluid volume depletion can override normal autoregulatory responses and induce a prerenal form of ARF (64). For example, gastrointestinal losses of fluid can precipitate prerenal ARF. Prolonged nasogastric and biliary drainage (sodium concentration 150 mEq/L) of large amounts of fluid can cause ARF. Diarrhea of any cause can induce volume depletion and ARF. In this regard, aggressive restoration of extracellular fluid volume dramatically decreased the frequency of ARF owing to prerenal causes that complicate cholera (75). In a collected series of 22 patients with villous adenoma (stool sodium 70 to 150 mEq/L), all had azotemia with BUN as high as 200 mg/mL (76). Sequestration of extracellular fluid, as occurs with acute pancreatitis, can cause ARF. In some studies, 1% to 7% of cases of ARF have occurred in the setting of acute pancreatitis (77,78). In a case series of 363 individuals with severe acute pancreatitis, ARF occurred in 35.8% of patients and was an independent predictor of mortality (79). Extensive dermal losses of salt, albumin and water occur in the setting of burns (34,80,81). Early in the course of patients with burns, ARF is often due to volume depletion with prerenal azotemia and rarely ischemic ATN (34,80). Later, sepsis and aminoglycoside antimicrobial agents produce a nonoliguric form of ARF in the setting of burns. Burn size, inhalation injury, and albumin level at presentation are risk factors for ARF in burn patients (34,81). Delay in fluid resuscitation is associated with higher mortality (80). Excessive dermal losses with extracellular volume depletion and ARF also occur in heat stroke; however, rhabdomyolysis also is present in many cases of heat stroke (82–84).

Acute renal failure resulting from excessive renal loss of extracellular fluid also is common (55,62,85,86). Often this is due to overzealous use of diuretic agents. Mild degrees of azotemia often are seen in conditions of renal loss of fluid as occurs with diabetic ketoacidosis; fortunately, ischemic ATN is rare in this setting (86). Of note, ketoacidosis can interfere with automated serum creatinine assays, producing artifactually high values. Renal loss of salt and water also can lead to ARF in hyperosmolar, nonketotic states (87).

Interestingly, hemorrhagic shock, except in either occasional circumstances or in the obstetric setting, rarely leads to severe ARF. Of 590 patients with serious upper gastrointestinal hemorrhage, only 8 died with ARF (88). Of 175 episodes of gastrointestinal bleeding in 161 patients with cirrhosis, ARF occurred in 20 of these episodes, and 11 patients died (89). ARF occurring in the setting of acute myocardial infarction also is rare. Causes of ARF in the setting of myocardial infarction include prolonged hypotension with cardiogenic shock, contrast nephropathy, cholesterol embolism (from angiography or thrombolysis [90]), drugs, renal tract obstruction due to hemorrhage (91) and rarely, rhabdomyolysis from cardiopulmonary resuscitation or cardioversion (92). In two series of patients with acute myocardial infarction who underwent percutaneous coronary angioplasty, ARF occurred in 3% (93) in one series and 0.6% of patients required dialysis in another series (94). The risk of ARF increases with the degree of hemodynamic compromise. Of 118 patients with cardiogenic shock following acute myocardial infarction, 39 developed ARF and 35 died (95).

The hepatorenal syndrome (see Chapter 75) is a life-threatening complication of severe liver disease that shares many features with prerenal azotemia (96,97). In 234 patients with cirrhotic ascites, the probability of development of this syndrome was about 40% over a 5-year period (97). It is generally agreed that severe renal vasoconstriction occurs with the hepatorenal syndrome and is responsible in large part for the ARF. The renal vasoconstriction may be caused by a combination of enhanced renal vasoconstrictors (norepinephrine, angiotensin II, vasopressin, endotoxin, endothelin, selected thromboxanes, leukotrienes, and increased renal adrenergic traffic) as well as decreased renal vasodilators (eicosanoids, kinins, and nitric oxide). Other key hemodynamic features of hepatorenal syndrome are systemic arterial hypotension, portal hypertension, and splanchnic vasodilatation (98). Despite some understanding of the pathophysiology of this disorder, no therapeutic modality has been shown to be consistently beneficial. Liver transplantation has been lifesaving in selected cases. As a bridge to liver transplantation, intravenous albumin and vasoconstrictors of the splanchnic circulatory bed such as terlipressin are helpful short-term agents which may improve renal function in 50% to 75% of patients (98,99).

Postrenal Acute Renal Failure

Obstruction of urine flow is generally considered a less common cause of ARF. In several series, obstructive uropathy is encountered in 2% to 10% of all cases in ARF (18,22,37,38,44,54,56,57) (Table 41-3). However, obstructive uropathy is more common in selected patient populations such as the very young or older men with prostatic disease and patients with a single kidney or intraabdominal cancer, particularly pelvic cancer (18,100,101). Obstructive uropathy is most frequently encountered in community- and hospital ward-associated ARF and is less common in ICU-related ARF (56,63). For example, obstructive uropathy constitutes 20% to 40% of all community-acquired ARF. Finally, the cause of obstructive uropathy is often amenable to therapy. Thus, obstructive uropathy should be considered in each case of ARF.

The cause of obstruction of urine flow can be classified as intrarenal or extrarenal (Table 41-3; Fig. 41-4). Intratubular deposition of either crystalline or proteinaceous material can increase intratubular pressure, thereby decreasing effective glomerular filtration pressure. For example, intratubular precipitation of uric acid can cause tubule obstruction and ARF. Acute uric acid nephropathy is most often seen following chemotherapy for leukemias and lymphomas (102,103). In this setting, the liver converts the purine load generated by cytolysis into uric acid. The high filtered load of uric acid and tubular reabsorption combine to produce high tubular concentrations of soluble urate and uric acid. Acidification of tubular fluid converts urate to uric acid, which can occlude tubular lumens.

Abrupt exposure of the kidneys to high filtered loads of other insoluble crystalline substances can also cause an

intrarenal form of obstructive uropathy (104). For example, ARF associated with calcium oxalate crystalluria can accompany ethylene glycol ingestion, administration of the anesthetic agent methoxyflurane, and small-bowel bypass operations (105,106). Administration of high doses of methotrexate can be associated with ARF, possibly owing to intratubular precipitation of the insoluble 7-hydroxy metabolite of methotrexate (104). Other crystalline substances that can potentially precipitate within renal tubules and lead to ARF include acyclovir, triamterene, sulfonamides, and protease inhibitors such as indinavir (104,107–109). Intratubular precipitation of myeloma proteins and perhaps other proteins also can lead to ARF (110). Dehydration with resultant high tubular water reabsorption and radiographic contrast material can facilitate intratubular myeloma protein deposition.

Recognition of intratubular obstruction as a potential cause of ARF has important therapeutic implications. For example, prophylactic therapy with the xanthine oxidase inhibitor allopurinol can prevent accumulation of uric acid in tumor lysis syndrome. Moreover, forced diuresis decreases tubular salt and water reabsorption, thereby diluting tubular fluid with decreases in crystal and protein concentrations. Finally, manipulations that increase urinary pH can increase solubility of crystalline substances such as methotrexate, uric acid, and sulfonamides (104).

Micropuncture and morphologic studies of an animal model of ARF owing to uric acid nephropathy illustrate these points (111,112). In this model, the underlying pathologic lesion is deposition of uric acid crystals within collecting ducts and deep cortical and medullary vessels. In these studies, high rates of urine flow, either induced by high-dose furosemide (which produced a solute diuresis) or in animals with central diabetes insipidus (which resulted in a water diuresis), protected against the development of uric acid–induced ARF. By contrast, either urinary alkalinization or induction of a mild diuresis with a furosemide provided only minimal protection. These observations suggest that maintenance of high urinary flow rates should be a major objective in cases of high uric acid loads. It is also noteworthy that clinical experience suggests that maintenance of high urine flow may protect against development of ARF in the setting of high doses of methotrexate infusion and myeloma cases. Finally, in patients with ARF owing to uric acid nephropathy, hemodialysis can remove large amounts of uric acid and restore renal function.

Extrarenal lesions are the most common cause of postrenal ARF and are listed in Table 41-3. Several factors determine renal response to extrarenal obstruction. The site, degree, and rapidity of onset of obstruction are all important. Without complicating infection, substantial improvement in renal function can follow decompression of the urinary tract after several days of complete obstruction. Prostatic obstruction is by far the most common cause of postrenal ARF encountered in men because of its critical location at the bladder outlet. Obstruction of the upper urinary tract is a less common cause of ARF because it requires simultaneous obstruction of both ureters or unilateral ureteric obstruction with either absence of or severe disease in the contralateral kidney. Intraureteric obstruction can be due to stone, necrotic papillae, tumor, pus, blood clots, and fungal balls. Papillary necrosis usually occurs in the setting of sickle cell disorders, diabetes mellitus, chronic urinary tract infections, analgesic abuse, obstructive uropathy, and possibly chronic alcoholism. Extraureteric lesions producing obstruction include retroperitoneal fibrosis, adenopathy, and tumors; pelvic tumors; and surgical ligation. Retroperitoneal fibrosis is often idiopathic but may be encountered in response to retroperitoneal neoplasia as well as in the setting of some pharmacologic agents (methysergide, methyldopa, β-blockers) and connective tissue diseases. A high frequency of ARF due to prostatic carcinoma in males and pelvic carcinoma (predominantly cancer of the cervix) in females causing ureteric occlusion has been reported (18,101). Less commonly encountered causes of extrinsic ureteric obstruction include inflammatory bowel disease (predominantly right-sided obstruction), an inflammatory reaction resulting from a leaking abdominal aortic aneurysm, and the late stages of pregnancy.

Norman and colleagues examined their experience with 50 cases of renal failure caused by ureteric obstruction experienced over a 5-year interval (113). The cause of obstruction was malignant disease (cervix, prostate, bladder, bowel, or ovary) in 76% of cases. Nonmalignant causes of obstructive uropathy included retroperitoneal fibrosis (16%), calculi (4%), and ligated ureters (4%). Substantial survival time was observed following relief of obstruction (often with percutaneous nephrostomy or ureteral stents) in many of the patients with extensive malignant disease.

Kaufman and associates (57) performed a prospective study looking at ARF that develops while living in the community. Of 100 patients with ARF, 20 cases were due to obstruction. Causes of obstruction included benign prostatic hypertrophy (12 patients); prostatic carcinoma (2 patients); and individual cases of lymphoma, ureteral stones, and bladder stones (57).

Recent reports emphasize that stone-induced obstruction may be a relatively frequent cause of ARF in selected populations (114,115). Grundy and collaborators described urosepsis as the setting of ARF in five quadriplegic patients. In three of these five patients, stone-induced obstructive uropathy played a role in the sepsis (114). Ansari et al. (115) have emphasized that uric acid obstruction of the ureters with anuric ARF can be seen in young male residents of the Near East. From 1978 to 1981, they encountered eight male patients with ARF, loin pain, and anuria owing to ureteral obstruction with uric acid calculi. Retrograde pyelography and either local or systemic alkalinization dissolved the stones, relieved the obstruction, and returned renal function to normal.

Pharmacologic agents with potential anticholinergic effects (e.g., tricyclic antidepressants, phenothiazines, antihistamines) and cold remedies containing α-adrenergic agents (e.g., phenylpropanolamine) often precipitate acute urinary retention by impairing detrusor function and enhancing bladder sphincter tone, respectively.

Acute Renal Failure

A variety of renal disorders can cause ARF (Table 41-4). These diagnoses should be considered when prerenal and postrenal disorders have been excluded. The frequency with which renal causes are encountered in patients with ARF varies between 25% and 80%. In a series of pediatric patients, as many as 50% of all cases of ARF can be attributed to such renal parenchymal disorders as acute glomerulonephritis and hemolytic-uremic syndrome. In hospitalized adults in whom prerenal and postrenal azotemia have been excluded, ARF is often caused by ATN. By contrast, in an outpatient setting in which prerenal and postrenal causes have been excluded, other renal parenchymal diseases more often cause ARF. The renal causes of ARF can be categorized most systematically by their anatomic site of origin. Thus, vascular, glomerular, interstitial, and tubular disorders occur. Acute renal failure due to renovascular (Chapters 52 and 70), glomerular (Chapters 58–69), and interstitial disorders (Chapter 48) are discussed in detail in other chapters.

The underlying disorders that usually predispose to ATN are listed in Table 41-4 and Figure 41-4. Basically, three major categories of insults predispose to ATN. These categories include renal ischemia (prolonged prerenal failure), nephrotoxins, and pigmenturia (myoglobinuria and hemoglobinuria). Studies of patients with ATN emphasize that multiple insults to

renal function are usually present. For example, in more than 600 well-characterized patients with ATN, about half had more than a single insult to renal function (35,38–42,55). Several experimental studies in animal models of ARF demonstrate that multiple renal insults such as fever, bacteremia, endotoxemia, relative hypotension, and aminoglycoside agents individually produce relatively minor decrease in renal function. Collectively, however, these insults can produce marked decrements in renal function with resulting ARF (116,117).

The most common predisposing factor in the development of ATN appears to be renal ischemia resulting from prolonged prerenal azotemia (37,38,41–44,55). Sepsis, and particularly septic shock, has assumed an ever-increasing role as a major predisposing factor in the occurrence of ATN (37,38,42,55,56). Nephrotoxins (Chapters 42–47) account for about 25% of all cases of ATN (85,118). Contemporary nephrotoxins commonly encountered include the aminoglycoside antimicrobial agents, radiographic contrast materials, nonsteroidal antiinflammatory drugs (NSAIDs), organic solvents, and heavy metals such as cisplatin and carboplatin. A prospective case control study found that the relative risk for development of ARF was increased five- to sixfold by exposure to either aminoglycoside antimicrobials or radiocontrast agents (55). The increasing number of infections in patients with AIDS has served as a strong reminder of the significant nephrotoxicity and ARF that can accompany therapy with pentamidine, sulfamethoxazole–trimethoprim, amphotericin B, foscarnet, acyclovir, cidofovir, aminoglycosides, and β-lactam antibiotics (30,31,119). The use of recombinant cytokine therapy for advanced solid tumors has been associated with nephrotoxicity following gamma interferon and interleukin-2 treatment (120,121). Intravenous immunoglobulin therapy has been used to treat a variety of immunologic disorders. Such therapy can occasionally be associated with ARF, although the mechanism appears unclear (122). In addition to ischemia and nephrotoxins, a third common predisposing factor in ATN cases is pigmenturia caused by either hemoglobin or myoglobin. Finally, no specific identifiable cause is found in a few cases of ATN.

Rasmussen and Ibels examined risk factors for the development of ATN (123). In this study, the records of 143 patients who developed an acute increase in serum creatinine of more than 2.2 mg/dL and who did not have prerenal or postrenal azotemia, glomerulonephritis, and interstitial nephritis were examined by retrospective multivariate analyses. Approximately 60% of these patients were seen in a surgical setting. The following were considered possible acute insults: hypotension (74%), sepsis (31%), contrast media and aminoglycosides (25%), pigmenturia (hemoglobin, myoglobin, 22%), and dehydration (35%). Nearly two-thirds (64%) of the 143 patients had more than one acute insult before ATN. In three more recent series of ICU-associated ARF, 30% to 48% of patients experienced sepsis, 17% to 30% either impaired cardiac output or volume depletion, and 20% to 30% were exposed to one or more nephrotoxins (43,44,56).

CLINICAL SETTINGS OF ACUTE RENAL FAILURE

Multiple Organ Failure

Acute renal failure is increasingly recognized to occur commonly in the context of multiple organ failure. This is particularly true when ARF is encountered in critically ill patients. In the extensive experience of Liano and associates, only 11% of intensive care unit (ICU)–associated ARF occurred without failure of at least one additional organ system (56,124). By contrast, in Liano's series, 69% of hospital ward-associated ARF occurred without failure of another organ system. Of more than 200 cases of intensive care unit-associated ARF, 11% had none, 24% had one, 40% had two, and 26% had concomitant failure of three or more concomitant organs (56). Allgren and colleagues reported that of ARF patients, 48% to 55% were on assisted ventilation, 26% to 35% had congestive cardiac failure, 27% to 42% were septic, and 13% to 33% had acute hepatic dysfunction at the time of presentation of ARF (42). Groeneveld and co-workers found that 90% of ICU patients who developed ARF had failure of other organ systems (125). In their experience, other organ systems failed 90% of the time before the ARF became apparent. Together these and other observations emphasize that intensive care unit-associated ARF usually occurs in the context of dysfunction of two or more additional organ systems. Moreover, this ARF usually appears after the onset of dysfunction of other organ systems.

A case-controlled study provides a unique perspective on the association of ARF and multiple organ dysfunction (35). This analysis of moderate radiocontrast-associated ARF found that preexisting sepsis (22%), respiratory failure (36%), mental status changes (41%), and clinically significant bleeding (15%) were commonly present before the onset of ARF (35). However, after the onset of ARF the percentage of patients that previously did not have the condition that experienced sepsis was 45%, respiratory failure 78%, mental status changes 68%, and bleeding 27%. These observations emphasize that although ARF often complicates the course of patients with multiple organ dysfunction, the occurrence of ARF often heralds either the onset or the further development of multiple organ failure.

The cause of ARF in the setting of multiple organ dysfunction can rarely be attributed to a single insult (37,38,41–44,55,125). Renal ischemia owing to hemodynamic instability and impaired cardiac output, intravascular volume depletion, sepsis, and exposure to potential nephrotoxins are nearly uniform accompaniments of ARF when it occurs in the setting of multiple organ dysfunction.

The basic principles of treatment for ARF occurring in the setting of multiple organ failure do not differ substantially from isolated ARF. However, with multiple organ failure, the associated hemodynamic instability and requirement for continuous adjustment of volume status often demands early continuous modalities of renal replacement therapy (125).

Older Age

The frequency of development of severe ARF is undoubtedly increased with advancing age. In a prospective, 2-year study of a 450,000 person population, Feest and co-workers found that individuals over 70 years of age comprised more than 70% of all cases of ARF (18). The frequency of severe ARF was 17 per million population in those under 50 years of age and was increased by 56-fold (949 per million population) in those aged 80 to 89 years (18). The mean age of several recent series of patients with severe ARF has ranged from 54 through 69 years. Pascual et al. (126) found that 14% of all ARF encountered in Madrid, Spain referral centers occurred in patients aged 80 years or older.

Elderly individuals have multiple other comorbidities that put them at higher risk of ARF. Fortescue et al. (127) found that in a prospective cohort of 8,797 patients undergoing coronary artery bypass grafting, 1.8 to 1.9% of patients over 70 years of age required dialysis versus 0.5 to 1.0% under the age of 70. However, in a stepwise logistic regression analysis, cardiogenic shock, history of renal disease, elevated left-ventricular end diastolic pressure in addition to age were also found to be independent predictors of development of ARF.

The causes and underlying predisposing factors of ARF in the elderly generally mirror those seen in a younger population. However, prerenal and postrenal causes of ARF may be especially common in the aging population. For example, Pascual et al. (126) found prerenal causes increased from 21% to 30% when individuals less than 65 years of age were compared with those over 80 years of age. Moreover, postrenal causes increased from 7% to 21% when those less than 65 were compared with those over 80 years of age. Together, prerenal and postrenal causes of ARF comprised 52% of all causes of ARF in the over 80 years of age population (128). Renal causes including those of vascular, glomerular, interstitial, and tubular origins are also encountered in the elderly population. Preston and collaborators found that 55 of 363 renal biopsies done on an over 65 years of age group were performed because of ARF (128). Of those 55, 42% revealed acute glomerulonephritis, 27% ATN, 16% renal vascular diseases, and 15% acute interstitial nephritis (128).

One specific concern with regard to ARF in an elderly population is use of NSAIDs and selective cyclooxygenase-2 inhibitors. About 10% to 15% of older adults in the United States consume these medications on a regular basis (19,129). Population-based case-control studies demonstrated that the incidence of ARF is rare (two per 100,000 person-years) but is increased fourfold by NSAID usage (19). In a population of individuals with an average age of 79 years, NSAID usage was associated with twofold-increased likelihood of having a blood urea nitrogen (BUN) >23 mg/dL and a serum creatinine >1.4 mg/dL (129). Selective cyclooxygenase-2 inhibitors appear to have a potential to reduce GFR similar to conventional NSAIDs in the elderly (66). There are several case reports in the elderly of ARF due to selective cyclooxygenase-2 inhibitors (130–132). A randomized trial of 8059 patients (39% aged over 65) compared a selective cyclooxygenase-2 inhibitor to a NSAID (133). Increased creatinine levels occurred in 0.7% of the selective cyclooxygenase-2 inhibitor versus 1.2% of the NSAID group (133).

The influence of increasing age on ARF outcome is debated. Some but not all studies demonstrate a direct relationship between advancing age and ARF mortality (126). There are, however, numerous outcome variables in the setting of ARF and a contribution of age, independent of comorbidity, on ARF outcome is difficult to ascertain. When stratified by severity of illness, recent studies do not suggest that age is an important ARF outcome determinant.

Sepsis or Infection

The occurrence of either frank septicemia or systemic inflammatory response syndrome is commonly noted in conjunction with development of ARF. Shusterman and collaborators found that septic shock was the clinical condition associated with the highest likelihood of development of hospital-acquired ARF with an adjusted odds ratio near infinity (55). Three studies of ICU-associated ARF found that sepsis was a predisposing factor in 30% to 48% of cases (40,43,44). In a multicenter study of 81 ICU's across Australia, septic shock accounted for 45% of cases of ARF requiring dialysis (134). In 234 patients with community-acquired bacteremia, 24% developed some degree of ARF (28). In about half of these patients, the ARF was mild to moderate in severity and readily reversible, whereas in the remainder it was severe and persisted (28). In a hospital-based study (135), ARF developed in 19% with sepsis, 23% with severe sepsis, and 51% with septic shock in culture-positive individuals.

Several types of infections with prominent systemic manifestations such as leptospirosis are occasionally complicated by ARF (see Table 41-4). A national pneumonia surveillance study found that 8% of patients hospitalized with pneumonia have some element of ARF (27). Some degree of ARF can be encountered in the context of specific causes of pneumonia such as that due to *Legionella* species and other organisms (136,137). Several systemic bacterial infections such as those due to bacterial endocarditis and visceral abscesses are associated with development of ARF (138). Severe bacterial pyelonephritis can be associated with bacteremia and ARF (139). Systemic infections of nonbacterial origin are also associated with ARF. Examples include Rocky Mountain spotted fever where ARF occurs in up to 20% of cases (29). Other examples of systemic, nonbacterial infections associated with ARF include Hantavirus and candidiasis (140,141). Malaria-associated ARF is common in many geographic areas (142).

The causes of ARF in patients with sepsis and infection are diverse (143–148)(see Table 41-7). Prospective studies have identified several risk factors for acute renal failure in patients with sepsis. Older age, elevated serum creatinine level despite elevated central venous pressure (CVP), and presence of hepatic failure were predictive for ARF in 2442 patients with sepsis admitted to an intensive care unit (149).

Therapeutic efforts for sepsis-related ARF, other than identification and treatment of the offending infectious agents, generally are supportive in nature. Some enthusiasm for continuous hemofiltration for treating sepsis-associated ARF has been generated (150,151). One rationale supporting such therapy is potential removal of proinflammatory cytokines; however, the brief half-life of many of these substances and direct measurements have demonstrated very limited removal of circulating mediators by continuous hemofiltration (151). In addition, large controlled trials of monoclonal antibodies and receptor antagonists directed against either tumor necrosis factor or interleukin-1 in patients with the sepsis syndrome have been disappointing (151).

TABLE 41-7

PATHOPHYSIOLOGIC FACTORS IN SEPTIC ARF

Prerenal
Hypoperfusion
Renal
Intrarenal vasoconstriction with redistribution of blood flow away from outer medulla (143)
Activation of coagulation and fibrinolysis (intraglomerular thrombosis and disseminated intravascular coagulation)
Norepinephrine
Angiotensin II
Endothelin
Interleukins (IL-6, Il-8, IL-10) (144)
Extrarenal toll-like receptor 4 (146)
Platelet activating factor
Eicosanoids
Tumor necrosis factor (145)
Apoptosis (144)
Acute tubular necrosis (312)
Interstitial nephritis (140,312)
Disseminated intrarenal microabscesses (147)
Rhabdomyolysis, hemoglobinuria, and myoglobinuria (142,148)
Glomerulonephritis (138)
Postrenal
Drug-induced intratubular crystallization (acyclovir, sulfonamides, protease inhibitors, foscarnet)
Fungus balls
Sloughed papilla
Renal calculi

Human Immunodeficiency Virus Infection

Acute renal failure is common in the setting of human immunodeficiency virus (HIV) infection (30,31,119,152–155) (see Table 41-8). The frequency of occurrence of ARF complicating the course of HIV infection is dependent on the stage of the disease, treatment regimen, comorbid complications, and sociodemographic features of the population studied. An early study that antedated current treatment protocols found 55% of 246 patients with advanced HIV infection experienced one or more episodes of a >0.3 mg/dL increase of serum creatinine over a 42-month interval (152). In this study, 20% of patients experienced a >2.0 mg/dL rise in serum creatinine and 7% had a peak serum creatinine that exceeded 6 mg/dL (152). Several other studies demonstrated a high frequency of occurrence of ARF in HIV populations in the context of the treatment regimens in use in the 1980s (30,31,154). Many clinicians experienced in the care of HIV-infected patients feel that advances in overall management have decreased the frequency and improved the prognosis of HIV-associated ARF (30). In a single center series of 92 HIV positive patients with ARF admitted between 1988 and 1997, most patients recovered with symptomatic treatment, but 18% of patients died within 2 months of their diagnosis of ARF (155).

Multiple potential causes are usually present in patients with HIV infection that develop ARF (30,31,152–155). The relative frequency of the cause depends on the characteristics of the HIV population being reported, the frequency with which renal biopsies are performed, and the academic interests of the reporting center. Often comorbid and/or associated conditions such as hypertension, diabetes mellitus, malignancy, intravenous drug abuse, and multiple infections are present that can contribute to renal dysfunction. Potential prerenal causes include gastrointestinal volume loss/sequestration, poor fluid intake, severe hypoalbuminemia, and early sepsis (30,31,152–155). Postrenal possibilities include intrarenal (tubular precipitation of sulfadiazine, acyclovir and protease inhibitors, and tubular precipitation of myeloma protein) (105), extrarenal (extraureteric blockage from fibrosis, tumor and nodes, and intraureteric obstruction from stones, pus, fungus balls, and papillae), and bladder/urethra (obstructing prostate) origin (30,31,152–155).

A renal source appears to be an especially common cause of ARF in the HIV population. Such renal causes include those of vascular (shiga–toxin independent hemolytic-uremic syndrome) and glomerular (HIV-associated glomerulosclerosis; cytomegalovirus [CMV], hepatitis B and C and other infection-associated glomerulopathies; tumor-associated glomerulopathy; and lupus-like glomerulopathies) origin (155). Although earlier studies suggested ATN as a common renal cause of ARF in HIV populations, more recent reports suggest a declining frequency of ATN (30). Potential causes of ATN include ischemia (prerenal disorders, sepsis), drugs (NSAIDs, radiocontrast, aminoglycoside, pentamidine, foscarnet, amphotericin), and pigmenturia from rhabdomyolysis. Other renal causes of ARF in HIV-infected individuals include allergic interstitial nephritis (trimethoprim–sulfamethoxazole, phenytoin, rifampin, and multiple other agents) and a rare but treatable disorder characterized by plasmacytic interstitial nephritis (154). The nucleotide analog tenofovir causes a reversible acute renal impairment and Fanconi syndrome (156,157). The use of highly active antiretroviral therapy (HAART) is associated with reconstitution of CD4 positive T cells. Reactivation of latent infection such as tuberculosis and cryptococcosis is a recognized consequence of HAART (158). Immune reconstitution may cause ARF due to reactivation of urinary tuberculosis (159).

A recent report of 92 HIV-infected individuals with ARF illustrates the spectrum of causes and potential utility of renal biopsy (155). The patients in this series were generally late in the course of HIV infection and had a mean CD4+ count of 76/mm^3. A renal biopsy was performed in 60 patients. The most common cause of ARF was hemolytic-uremic syndrome (36%). Other causes included ATN (27%), intrarenal or extrarenal obstruction (17%), HIV-associated glomerulosclerosis (16%), other glomerulopathies (4%), interstitial nephritis (2%), and myeloma kidney (1%).

The prognosis of ARF complicating HIV infection is dependent on the cause. A report of severe ARF (serum creatinine of 6 mg/dL or higher) found roughly comparable renal recovery in HIV-infected and noninfected ARF patients (56% and 47%, respectively) and comparable overall ARF mortality of 55% to 60% (30). A significantly higher percentage of the HIV ARF population than the non-HIV ARF group was considered to be terminal and was not dialyzed (36% vs. 18%, respectively). Many of the non-ATN causes of HIV-associated ARF appear to be responsive to therapeutic modalities (160).

TABLE 41-8

CAUSES OF ARF IN HIV-POSITIVE PATIENTS

Prerenal
Hypovolemia—gastrointestinal losses, poor fluid intake, severe hypoalbuminemia, renal salt wasting, diabetes insipidus
Hypotension and reduced renal perfusion—sepsis, cardiomyopathy, cirrhosis/hepatic insufficiency, pancreatitis, nephrotic syndrome
Intrarenal
Vascular—microangiopathy (hemolytic uremic syndrome and thrombotic thrombocytopenic purpura), drug-induced, infection, plasmacytic interstitial nephritis
Glomerular—HIV associated glomerulosclerosis, IgA nephropathy, immune complex glomerulonephritis
Tubulointerstitial—acute tubular necrosis (nephrotoxic, ischemic, pigment-related)
Postrenal
Intrarenal—tubular precipitation of uric acid, sulfadiazine, acyclovir, protease inhibitors, foscarnet
Extrarenal—extraureteric (fibrosis, tumor, lymph nodes), intraureteric (stones, pus, fungus balls, sloughed papilla), bladder (structural, e.g., stones and blood clots and functional, e.g., neuropathic), urethral obstruction (urethritis, stricture, blood clots)

IgA, immunoglobulin A.
(Adapted from: Perazella MA. Acute renal failure in HIV-infected patients: a brief review of common causes. *Am J Med Sci* 2000;319:385.)

Postoperative State

The postoperative period is currently one of the most prevalent settings of ARF. For example, 27% of the 748 cases of ARF reported by Liano and Pascual were encountered in the postoperative setting (40). Postoperative ARF accounted for 25% of all intensive care unit-associated ARF and 8% of all hospital ward-associated ARF in this study (40). Older studies by Charlson and associates indicated that 25% of elective, noncardiac surgical procedures were complicated by an acute rise in serum creatinine of 20% or greater (161). A 50% decline in endogenous creatinine clearance occurred in 11% of these patients (161). More recent studies indicate that mild ARF occurs in 7% to 8% of patients undergoing cardiac surgery

and that about 1% of such patients will require renal replacement (24,25).

In studies by Chertow and associates (25), the development of postcardiac surgery ARF sufficient to require renal replacement therapy increased 30-day mortality by 15-fold (4% to 64%). Recursive partitioning could allocate patients into distinct risk groups for development of ARF (25). For example, use of intraaortic balloon pump, cardiomegaly plus New York Heart Association Class IV functional status or the presence of peripheral vascular disease plus a cardiac valve replacement operation were the three clinical settings associated with relatively high risk (>5%) of developing severe postoperative ARF (25). Leacche and colleagues (162) followed 13,847 consecutive patients undergoing cardiac surgery, 40 (0.3%) of whom developed postoperative ARF requiring dialysis. Of these 40 patients, 29 died and 7 of the remaining 11 survivors required permanent dialysis (162).

Several risk factors have been identified for ARF after cardiac surgery. The use of cardiopulmonary bypass was an independent predictor of ARF in a series of 2199 patients undergoing coronary artery bypass surgery (163). Mangano and colleagues (164) performed a multicenter prospective study of 2,222 patients undergoing cardiac surgery. Use of cardiopulmonary bypass for greater than 2 hours was associated with a 3.7 times relative risk of acute renal impairment (serum creatinine >2.0 mg/dL or increase of 0.7 mg/dL) or dialysis. Other risk factors identified included older age, congestive heart failure, previous coronary artery bypass surgery, preexisting renal impairment, diabetes, hyperglycemia, and poor ventricular function (164).

The use of hypothermia during cardiopulmonary bypass has been associated with worse post-operative renal function (165) (compare to effects of therapeutic hypothermia in section on cardiopulmonary resuscitation below). In a series of 1,442 patients undergoing coronary artery bypass surgery, the use of hypothermia was associated with worse renal function at 24 hours postoperatively, but similar renal function 1 week postoperatively (166). Duration on cardiopulmonary bypass was longer in the hypothermia group (because of the need for cooling down and rewarming), which may be the explanation for why the patients undergoing hypothermia had transiently worse postoperative renal function.

Another clinical situation in which a relationship between renal ischemia and ATN is well established involves surgical procedures on the aorta. In 25 series of more than 7,000 patients with an operation for an abdominal aortic aneurysm, 17% developed mild and 6% severe ARF. Mortality rate for ARF was 61% (167). Gornick and Kjellstrand reported their experience with 47 patients with ATN who required dialysis following repair of an abdominal aortic aneurysm (168). These 47 patients constituted approximately 15% of the patient population that required dialysis for ATN over an 11-year interval. Survival in patients with ATN after surgery for abdominal aortic aneurysms was low (21%).

Individuals undergoing aortic or thoracoabdominal aortic surgery commonly have renal arterial disease. A retrospective nonrandomized study (169) suggested that ARF requiring dialysis was less common in patients who had previously undergone renal artery endarterectomy for occlusive disease.

A third operative setting felt to be associated with a high frequency of ARF is that of the jaundiced patient. A review of 16 series made up of 2,300 jaundiced patients found an overall frequency of development of ARF of 8% (167). Recently, a very small ($N = 23$) prospective study of patients undergoing surgery for relief of obstructive jaundice studied the frequency of development of ARF (170). All patients were aggressively hydrated preoperatively and then assigned to receive either dopamine or no dopamine. No cases of ARF were seen in either group (170).

Abdominal operations may be associated with ARF postoperatively. Sugrue and colleagues (171) identified increased intraabdominal pressure as an independent risk factor contributing to postoperative ARF in patients undergoing abdominal surgery, a phenomenon known as the abdominal compartment syndrome. Operative exploration and decompression of 279 patients whose intraabdominal pressure exceeded 25 mm Hg resulted in diuresis and resolution of ARF in 7 patients (172). Proposed mechanisms include increased renal vascular resistance and increased levels of antidiuretic hormone, renin, aldosterone, and catecholamine concentrations (171,173–176). Pressure on the ureter is not thought to be an important factor (171).

What underlies the relatively high frequency of ARF that occurs in relation to elective surgical procedures? In many cases, underlying comorbidity (diabetes mellitus, chronic hypertension, vascular disease, congestive heart failure, myeloma, cirrhosis) leads to diminished baseline GFR and reduced renal reserve (161,177). With this background, the "surgical experience" may induce afferent arteriolar renal vasoconstriction and diminished GFR (178,179). Clinical ARF may then occur if an additional renal insult is encountered. These additional renal insults often are referred to as "second hits," and include reoperation, sepsis, nephrotoxin exposure, circulatory/volume deficits, and heart failure (161,177).

Trauma and Burns

The frequency with which ARF accompanies major trauma is dependent on the definition of ARF and the trauma population studied. A retrospective review of nearly 73,000 admissions to nine referral trauma centers over 5 years revealed only a 0.1% incidence of ARF sufficient to require renal replacement therapy (32). By contrast, 31% of prospectively studied trauma patients admitted to a single ICU developed either a rise in serum creatinine of >2 mg/dL or a 20% increase if baseline values were abnormal (32,33,180). The frequency of ARF complicating earthquake trauma is unknown but may be substantial in patients with significant degrees of rhabdomyolysis (181). In the earthquake in the city of Marmara, Turkey, 639 victims developed ARF, 477 required dialysis, and 97 died (182,183).

The causes of posttraumatic ARF depend on the timing of onset of ARF (32,33,180,181). When present early in the posttraumatic course, ischemia caused by hypotension and pigment-associated ARF, either alone or in combination, are the most common predisposing factors. When ARF occurs later in the course of the traumatized patient, it usually arises in the context of multiple organ failure and sepsis. Risk factors for development of posttraumatic ARF include high injury severity scores, hypotension at admission, high creatine phosphokinase (CPK) values (over 100,000 to 250,000), and requirement for mechanical ventilation.

About half of posttraumatic ARF is modest in degree with peak serum creatinine concentration less than 4 mg/dL (33). Generally speaking, any degree of posttraumatic ARF is associated with significant mortality. Thus, in the study of Vivino and associates, the mortality of mild ARF (peak serum creatinine <4.0 mg/dL) was 71%, whereas with greater degrees of severity of ARF it was 93% (33).

Acute renal failure complicating burn injury occurs in 5% to 15% of patients with third-degree burns exceeding 10% of body surface area (34,80). As with posttraumatic ARF, the occurrence of postburn ARF appears to be biphasic. Volume depletion, hypotension, and myoglobinuria usually are contributing factors when ARF occurs early postburn (34,80). Acute renal failure occurring later in the course of burn injury usually occurs in the context of sepsis, nephrotoxin exposure, and multiple organ failure (34,80). The degree of burn injury

and the occurrence of infection are closely related to development of ARF (34,80). Mortality of burn injury complicated by ARF is exceptionally high and exceeds 85% in nearly all studies (34,80). Survivors tend to be younger, with less extensive burns and with clinical courses not complicated by sepsis (34,80).

Acute Renal Failure Complicating Nonrenal Solid Organ Transplantation

The widespread success and availability of transplantation of hearts, pancreases, lungs, bone marrow, and livers has been associated with substantial occurrence of renal failure that is both acute and chronic in nature. Much of the chronic renal failure seen after nonrenal solid organ transplantation appears attributable to cyclosporine and tacrolimus (184).

The frequency with which ARF complicates cardiac transplantation varies depending on the immunosuppressive regimen used and the underlying health of the recipient. Patients at highest risk are those with abnormal baseline renal function, recipients of high-dose cyclosporine, significant comorbidity, and associated posttransplant cardiac dysfunction (185). The frequency of occurrence of severe ARF after cardiac transplantation ranges from 5% to 15% in most series. In their series of 56 cardiac transplant patients, Stevens and colleagues (186) reported that 6 (11%) required renal replacement therapy. However, mild degrees of ARF are much more common and are seen in 25% to 50% of all cardiac transplant recipients (185,187,188). Typically, ARF after heart transplantation occurs within the first week posttransplantation and is characterized by an oliguric state, unremarkable urine sediment, and urinary diagnostic indices typical of prerenal azotemia (185). Often, a high BUN to creatinine ratio and disproportionate hyperkalemia are present. Late ARF can be seen in the setting of rhabdomyolysis in patients taking an HMG-COA (3-hydroxy-3-methyl-glutaryl-coenzyme A) reductase inhibitor and cyclosporine (189). General supportive treatment and reduction or elimination of cyclosporine are the mainstays of therapy (185–188). Even with severe ARF, survival may be good, perhaps because of the younger age of recipients and the presence of single organ failure (185). A small randomized trial did not demonstrate benefit of pretreatment with the renal vasodilator urodilatin (188).

Acute renal failure occurs commonly in patients awaiting and following liver transplantation. Over 18 months at a single large liver transplantation center, ARF occurred in 36% of end-stage liver disease patients who were not transplanted and in 30% of transplanted patients during their postoperative course (96). In this series, ARF was defined as either doubling of the serum creatinine within 24 hours or development of oliguria with need for dialysis. Patients with reversible prerenal azotemia were excluded. In another study, ARF occurred after liver transplantation in 51% of cases (190). Most (66%) of this ARF was modest in degree, although 34% of patients required dialysis (190). Patients at highest risk for the development of ARF in the setting of liver transplantation are those with higher comorbidity as reflected by higher Acute Physiology and Chronic Health Evaluation (APACHE) scores as well as those with abnormal basal renal function and impaired graft function.

The causes of ARF pretransplantation are usually attributed to either the hepatorenal syndrome or ischemic ATN (96,190). Acute renal failure after liver transplantation usually is due to ATN (about 60% of cases), which is most commonly ischemic and occasionally nephrotoxic in origin. Other common contributors to postliver transplantation ARF include cyclosporine (30% to 40%), hepatorenal syndrome (5% to 10%), sepsis (1% to 10%), and rhabdomyolysis (1% to 5%).

Liver transplant recipients who develop ARF exhibit postoperative mortality that exceeds that seen in those without ARF fourfold to sevenfold, an average of 30% to 50% (187,190). Interestingly, transplanted patients who develop ARF prior to liver transplant appear to have comparable mortality (29%) to those who develop ARF posttransplant (41%) (96).

Patients who develop ARF after liver transplantation not uncommonly progress to end-stage renal failure. Paramesh and colleagues studied 1,602 orthotopic liver transplant recipients, of whom 350 (22%) required dialysis postoperatively. Forty-three (2.7%) developed end-stage renal failure. Risk factors included cyclosporine and the presence of diabetes pretransplantation (191).

Acute renal failure is a common accompaniment of bone marrow transplantation, where it is estimated to complicate the course of 30% to 60% of allogeneic, 5% to 15% of autologous, and 5% of nonmyeloablative transplants (192–198) (see Table 41-9). About half of all patients with ARF have a sufficient degree of renal failure to require renal replacement

TABLE 41-9

HEMOPOIETIC CELL TRANSPLANTATION AND ARF

	Autologous	Allogeneic	Nonmyeloablative
Study	(198)	(194)	(193)
Number of patients	160	88	253
Population studied	Breast cancer (regional lymph node spread or metastases)	Lymphomas and leukemias ($N = 79$), breast cancer ($N = 7$), multiple myeloma ($N = 1$), myelodysplastic syndrome ($N = 1$)	Lymphomas and leukemias ($N = 183$), multiple myeloma ($N = 70$)
Grade 0 renal dysfunction	44%	8%	10%
Grade 1 renal dysfunction	35%	23%	50%
Grade 2 renal dysfunction	14%	36%	36%
Grade 3 renal dysfunction	7%	33%	4%
Mortality	7%	58%	34%

Grade 0 = decrement in GFR (as calculated by Cockcroft and Gault formula) of less than 25% from baseline.
Grade 1 = fall in GFR >25% from baseline but rise in serum creatinine less than twofold.
Grade 2 = fall in GFR >25% from baseline and rise in serum creatinine greater than twofold but without requiring dialysis.
Grade 3 = same as grade 2 but with dialysis.

therapy. Traditionally, the causes of ARF after bone marrow transplantation have been managed by time of onset (192,195–197). Very early ARF (within 1 week) is rare and usually attributable to either tumor lysis syndrome from cytoreductive therapy or bone marrow infusion toxicity. Bone marrow infusion toxicity often is caused by the presence of free hemoglobin from the red cell destructive action of freeze–thaw cycles and dimethylsulfoxide (DMSO) exposure. Acute renal failure is a relatively common occurrence 20 to 30 days after transplantation. This ARF is categorized as resulting from a form of hepatorenal syndrome owing to graft versus host disease or nephrotoxins or is multifactorial (prerenal factors, sepsis) in nature. Adenovirus associated nephritis is an unusual cause of ARF that occurs in allogeneic stem cell transplantation (199). These patients developed ARF in association with symptoms such as hematuria, flank pain, fever, and cystitis. Kidney biopsy revealed nuclear inclusions within renal tubular epithelial cells which were positive for adenovirus immunocytochemical stains (199). An even later form of ARF occurs usually in the context of thrombotic microangiopathy or cyclosporine nephrotoxicity. Mild forms of ARF (doubling of serum creatinine) after bone marrow transplant are associated with 20% to 45% mortality. Post–bone marrow transplant ARF sufficient to require hemodialysis is associated with greater than 80% mortality.

Cardiovascular Disease

Heart disease is the leading cause of mortality and hospitalization in patients over 65 years of age in the United States (200). Patients with cardiovascular disorders may be uniquely susceptible to the development of ARF. Thus, such patients often have substantial comorbidity (hypertension, atherosclerotic disease, and diabetes mellitus), which can decrease renal reserve. The presence of diminished cardiac output, therapy with multiple pharmacologic agents (angiotensin converting enzyme [ACE] inhibitors, receptor blockers, and diuretics), selected cardiac events (arrhythmias and arrest), exposure to nephrotoxins (radiocontrast media), and invasive procedures such as surgery and catheterizations all can result in development of ARF.

Several recent studies highlight many of these points. Behrend and Miller published a retrospective review of ARF as it occurred within a cardiac care unit (22). The frequency of ARF was 4%. More than 50% of these patients had hypertension and diabetes mellitus. Congestive heart failure accounted for 35% of ARF cases and contributed to an additional 25% of cases that had more than a single cause. Other etiologies included arrest or arrhythmia in 13%, radiocontrast in 11%, volume depletion in 6%, sepsis in 6%, and urinary tract obstruction in 3%. The overall mortality rate of these cardiac care unit patients with ARF was 50%.

McCullough and collaborators (23) reported on their experience with over 3,600 patients undergoing coronary intervention (angioplasty, atherectomy, or stenting). The incidence of ARF (defined as a 25% increase in serum creatinine concentration) was 14%, and 0.8% of patients required hemodialysis. Multivariable analysis found that decreased creatinine clearance, the presence of diabetes mellitus, and increasing dose of radiocontrast were independently associated with the development of ARF. In-hospital mortality was 1.1% for those without ARF, 7.1% for those with ARF who did not require dialysis, and 35.7% for those with ARF who needed dialysis (23).

Mattana and Singhal have reported on ARF accompanying cardiopulmonary arrest. These investigators found that 16 of 56 patients who survived cardiopulmonary arrest developed ARF (26). Those with ARF had longer duration of arrest (12 versus 7 minutes), received more epinephrine (1.8 vs. 0.9 mg), and had more heart failure (44% vs. 13%) than those without ARF. Survival to discharge was observed in 6% of those with and 48% of those without ARF. In cardiopulmonary resuscitation, the use of mild therapeutic hypothermia (compared to normothermia) is associated with reversible acute renal dysfunction (201) (also see earlier section, "Postoperative State," for ARF associated with cardiopulmonary bypass surgery). The mechanism of this effect is unknown. In animal and human studies, hypothermia causes renal afferent arteriolar vasoconstriction, reducing renal plasma flow (201–203). In a rat model of hypothermia, mean blood pressure and heart rate fell, leading to reduced cardiac output and renal perfusion (204).

Other causes of ARF encountered in patients with cardiovascular diseases include prerenal forms of ARF caused by diuretics and ACE inhibitors, atheroembolic disease, and other vascular disorders (67,205–207).

Pregnancy

Acute renal failure occurring in the setting of pregnancy has dramatically declined in incidence in western societies but remains a major issue in selected less well-developed countries (20,21,208–212). Current estimates of the frequency with which ARF complicates pregnancy range from one in 5,000 pregnancies in an inner-city U.S. urban population to one in 18,000 pregnancies in an Italian population (20,21). The incidence in the Italian population decreased from one in 3,000 to one in 18,000 pregnancies when the 1956 to 1967 and 1988 to 1994 time periods, respectively, were examined (21). A strikingly high frequency of pregnancy-related ARF continues to be reported in some developing countries (208,209).

The causes and predisposing factors for pregnancy-associated ARF are multiple. Occasionally, prerenal factors from either associated illnesses or hyperemesis gravidarum are encountered. Rarely, the gravid uterus leads to obstructive uropathy, especially with twin pregnancies, polyhydramnios, or other intrapelvic processes (25). Pregnancy-associated ARF has occurred often in the context of septic abortion in the past and in some underdeveloped countries. Where septic abortions are rare, most contemporary pregnancy-related ARF occurs in the setting of the HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets), eclampsia or preeclampsia, postpartum hemorrhage, or placental abruption. Cortical necrosis or idiopathic postpartum ARF occurs rarely. These disorders may lead to nonreversible renal failure (211,212). Pregnancy-associated pyelonephritis with sepsis can be associated with ARF infrequently.

Sibai and Ramadan provided information on ARF complicating the HELLP syndrome (211). Of 32 pregnant patients with HELLP, 6 had preexisting hypertension, and 26 were previously normotensive. Maternal and perinatal mortality rates were 13% and 35%, respectively, in these HELLP cases, whereas 72% of births were preterm. Acute dialysis was required in 10 of 32 patients with ARF complicating the HELLP syndrome. Eleven subsequent pregnancies occurred in eight normotensive women, and only one was complicated by preeclampsia. By contrast, preeclampsia occurred in three of six subsequent pregnancies in four hypertensive women. No residual renal dysfunction or hypertension was seen after 5 years of follow-up in 23 surviving normotensive women, whereas 2 of 5 surviving hypertensive women required chronic dialysis.

Overall, most contemporary series of ARF complicating pregnancy report relatively low mortality rates (approximately 25%). Nowadays, cortical necrosis with lack of recovery of renal function occurs much less commonly during pregnancy at present but continues to be seen when ARF accompanies pregnancies in developing countries (212).

See Chapter 74 for a detailed discussion of pregnancy-related ARF and Chapter 53 for discussion on syndromes of preeclampsia.

Malignant Disease

One setting in which ARF is encountered with high frequency is in patients with malignant disease (104,213–218) (see Chapters 43 and 49). For example, Lanore and collaborators found that 43% of 349 patients with hematologic malignancies encountered over 6 years developed some degree of ARF (213). Recent studies of patients with multiple myeloma find that about one-fourth develop ARF (214).

The causes of ARF in patients with neoplastic disease are diverse (213–217). Prerenal factors from lack of fluid intake, vomiting, and diarrhea are common. Less commonly encountered prerenal failures seen in patients with cancer include chemotherapeutic agent–induced heart failure owing to cardiomyopathy and cancer-related pericardial tamponade and interleukin-2–induced capillary leak syndrome (215,218). Postrenal factors potentially operative as causes of ARF in patients with neoplastic disorders include intrarenal obstruction from crystals (uric acid, methotrexate, myeloma protein) and extrarenal obstruction owing either to ureteric blockage (nodes, tumor mass, fibrosis) or bladder obstruction (prostatic or bladder cancer, neurogenic bladder caused by neoplastic spinal cord compression) (104,113,214–218). Acute tubular necrosis appears to be the most common renal cause of ARF in patients with neoplasm. This ATN is often attributable to sepsis or drugs (e.g., NSAIDs, radiocontrast, aminoglycosides, mithramycin, gallium, nitrosoureas, and amphotericin B). Other pharmacologic agents such as mitomycin, tacrolimus, and cyclosporine can induce renal vascular injury and ARF. Tumor-lysis syndrome, hyperuricemia, and hypercalcemia also are relatively common causes of neoplasia-associated ARF. Tumor-lysis syndrome may occur after cytotoxic therapy or may occur spontaneously (219–221). Other disorders (e.g., tumor infiltration, tumor-related glomerulopathy, and either tumor-related or chemotherapeutic agent-related thrombotic microangiopathy) are occasional causes of ARF in cancer patients (213–218,222,223). In patients with plasma cell dyscrasias, ARF can be attributable to light-chain tubular toxicity, amyloid renal involvement, and renal vein thrombosis (214).

Acute renal failure outcome occurring in the cancer setting is dependent on the cause and extent of the cancer, and is usually associated with a guarded prognosis; however, survival of up to 30% can be seen, even in patients requiring hemodialysis for the ARF (213). Recovery of renal function and at least 1-year survival can be seen in 40% to 50% of patients with multiple myeloma and ARF (214).

Liver Disease

Renal dysfunction is often encountered in patients with advanced liver disease (96,97,224–227). Acute renal failure occurred in 19% in 493 patients with end-stage liver disease who were being evaluated for possible liver transplantation (96). In this study, all cases of reversible prerenal forms of ARF were excluded. The likelihood of development of ARF owing to the hepatorenal syndrome was about 40% over a 5-year period in a prospective study of 234 patients with cirrhotic ascites (97,110).

There are several diagnostic considerations in patients with liver disease and ARF. With acute liver disease, prerenal factors (e.g., vomiting or diarrhea) always can play a role in ARF. Also toxins (e.g., acetaminophen, carbon tetrachloride and other hydrocarbons, and amanita phalloides), infections (e.g., sepsis; hepatitis A, B, and C; leptospirosis; and yellow fever), and circulatory disturbances (e.g., shock or hypotensive states and severe congestive heart failure) can result in concomitant acute renal and hepatic dysfunction. Prerenal and postrenal factors must be considered in the setting of chronic liver disease. Renal causes of ARF in patients with advanced liver disease usually fall into one of four categories: hepatorenal syndrome, ischemic ATN, nephrotoxin-induced ATN (especially NSAIDs and radiocontrast), and sepsis-associated ARF.

One common setting of ARF in patients with advanced liver disease is spontaneous bacterial peritonitis (224). With spontaneous bacterial peritonitis, about one-third of patients develop some degree of ARF. A recent prospective randomized trial found that intravenous albumin (1.5 g/kg initially and 1.0 g/kg on day 3) plus cefotaxime resulted in significantly less ARF (10% vs. 33%) and hospital death (10% vs. 29%) than cefotaxime alone in patients with cirrhosis and spontaneous bacterial peritonitis (36,224).

The prognosis of ARF complicating liver disease is dependent on the clinical setting and cause of the liver and renal dysfunction but generally is not good. Acute renal failure was associated with more than 80% mortality in patients with end-stage liver disease evaluated for transplantation (37,96).

Rhabdomyolysis

Dissolution of skeletal muscle with resultant extravasation of intracellular contents into the circulation constitutes the clinical syndrome of rhabdomyolysis. Rhabdomyolysis can be traumatic (muscle crush, and/or pressure injury, extreme physical exertion) or nontraumatic in nature (41,228–236). Nontraumatic causes of rhabdomyolysis are legion (Table 41-10 or see table in Chapter 49) and include ischemic, infectious, toxic, metabolic, and thermic insults (237–239). The frequency with which rhabdomyolysis is associated with ARF is variable, depending on associated medical conditions, the degree of muscle injury, the presence or absence of volume depletion and hemodynamic instability, and the duration of the rhabdomyolysis. In general, about 10% to 40% of patients undergoing significant rhabdomyolysis develop some degree of ARF (228–236). About half of the cases are mild in degree and responsive to volume repletion, whereas the remainder are more severe, with a clinical course typical of ATN. The logistic regression model for prediction of ARF in patients with rhabdomyolysis developed by Ward found that the magnitude of elevation of creatine kinase, potassium, and phosphorus; and the presence of volume depletion, hypoalbuminemia, and sepsis as causes of rhabdomyolysis were predictive of prolonged ARF (235).

Recent reviews highlight several key aspects of ARF that can be seen in the context of traumatic rhabdomyolysis (240). The immediate cause of morbidity is leak of the sarcolemmal membrane to potentially toxic factors (potassium and phosphorus) and metabolites (myoglobin and uric acid) as well as uptake by damaged muscles of extracellular fluid and calcium, which can lead to hypovolemic and hypocalcemic shock as well as neurovascular entrapment. Persons surviving the cardiotoxicity of hyperkalemia and the hypovolemic shock are susceptible to ATN, which is a consequence of ischemia (hypovolemia, hypotension), direct tubular toxicity of myoglobin, and myoglobin tubular cast formation. Management includes prehospital intravenous volume replacement and maintenance of high urine flow (>100 cc/hour) until myoglobinuria subsides. There is anecdotal evidence that a mannitol–alkaline diuresis can help prevent the associated ARF (229,232,236,240).

TABLE 41-10

CAUSES OF RHABDOMYOLYSIS

Physical causes	Nonphysical causes
Trauma and compression Traffic or working accidents Disasters Torture Abuse Long-term confinement to the same Position Occlusion or hypoperfusion of muscle Vessels thrombosis Embolism Vessel clamping Shock Excessive or prolonged contraction of muscles Exercise Epilepsy Psychiatric agitation Delirium tremens Tetanus Amphetamine overdose Ecstasy Status asthmaticus Electrical current High-voltage electrical injury Lightning Cardioversion Hyperthermia Exercise High ambient temperatures Sepsis Neuroleptic malignant syndrome Malignant hyperthermia	Metabolic myopathies McArdle's disease Mitochondrial respiratory chain Enzyme deficiencies Carnitine palmitoyl transferase Deficiency Myoadenylate deaminase deficiency Phosphofructokinase deficiency Drugs and toxins Regular and illegal drugs (see Chapter 46) Toxins Snake and insect venoms Buffalo fish (United States), burbot (Northern Europe)—Haff disease Infections Local infection with muscle invasion (pyomyositis) Metastatic infection (sepsis) Systemic effects Toxic shock syndrome *Legionella* Tularemia *Salmonella* Falciparum malaria Influenza HIV Herpes viruses Coxsackievirus Dengue fever Electrolyte abnormalities Hypokalemia Hypocalcemia Hypophosphatemia Hyponatremia Hypernatremia Hyperosmotic conditions Endocrine disorders Hypothyroidism Diabetic coma, related to electrolyte disturbances Polymyositis/dermatomyositis

(Adapted from: Vanholder R, Sever MS, Erek E, et al. Rhabdomyolysis *J Am Soc Nephrol* 2000;11:1553; Birewar S, Oppenheimer M, Zawada ET Jr. Hypothyroid acute renal failure *S D J Med* 2004;57:109; Davis JS, Bourke P. Rhabdomyolysis associated with dengue virus infection *Clin Infect Dis* 2004;38:e109.)

The International Society of Nephrology established the Renal Disaster Relief Task Force in 1995 and gave it responsibility for preparing stocks of goods (intravenous fluids, dialysis equipment) and lists of medical, nursing staff and volunteers who could intervene immediately in the event of a large-scale disaster (241). Victims of crush injury associated with earthquake damage were shown to benefit from early administration of mannitol and intravenous fluids (241,242).

Gabow and collaborators reported experience with 87 episodes of rhabdomyolysis in 77 patients seen over a 4-year interval (243). Rhabdomyolysis was defined as an at least sixfold increase in serum creatine kinase in the absence of myocardial infarction or cerebrovascular accident and an increase in MM isoenzymes of creatine kinase. Most patients (66 of 77) were men, with mean age of 48 years. Causes of rhabdomyolysis included alcoholism (67%), muscle compression (39%), seizures (24%), trauma (17%), drugs (5%), and metabolic (8%) factors. Muscle pain was noted in half the patients, but muscle swelling rarely was present. In the 87 episodes, 52 (60%) were not associated with ARF. Prerenal azotemia was seen in six (7%), whereas 29 (33%) developed ATN. Of the 29 patients who developed ATN, half had advanced ARF on hospital admission. Nearly one-half of the 29 patients with ARF were oliguric and 13 required dialysis. Rhabdomyolysis following major trauma appeared particularly to predispose to ARF. Six of the 29 ARF patients (21%) died. Only two patients required fasciotomy for neurovascular compression.

DIAGNOSTIC APPROACH

Presenting Features

There are numerous causes of ARF, as noted in the previous section, some of which are amenable to specific therapeutic interventions. For these interventions to be effective, they must usually be applied early in the course of the disease process. It follows that key considerations in the diagnosis of ARF are early detection and timely evaluation to determine the cause of the disorder.

With regard to early detection, hospital-acquired ARF usually comes to the attention of the clinician by finding a rising serum creatinine and/or BUN concentration. Less commonly, hospital-acquired ARF is detected by the development of oligoanuria. Rarely, hospital-associated ARF is detected when evaluation for one of the biochemical or clinical consequences of loss of renal function (i.e., hyperkalemia, metabolic acidosis, hyperphosphatemia, hypocalcemia, hyperuricemia, bleeding, or encephalopathy) is undertaken. Community-acquired ARF is usually detected by finding an elevated serum creatinine and/or BUN or abnormal urinalysis on multiphasic screening of patients with nonspecific complaints. Difficulties with the urinary stream also are a common presenting manifestation of ARF owing to postrenal causes in men in the outpatient setting.

It is important to acknowledge that early detection of the presence of ARF may be difficult. Currently, there is no reliable, practical "real time" method that provides accurate information on renal function. An abnormality in renal function certainly is present in the oliguric patient (244); however, most contemporary ARF is nonoliguric in nature. Also an increase in the serum creatinine concentration may not be a sensitive indicator of ARF, particularly in patients with normal baseline renal function and serum creatinine concentration. It follows that relatively large decreases in GFR in patients with a normal baseline GFR, as occurs early in the course of ARF, initially may be associated with only small increases in serum creatinine concentration; moreover, the serum creatinine concentration is also influenced by such variables as production rate and volume of distribution (5). Heightened awareness of the clinical settings of ARF and careful attention to small increases in serum creatinine concentration and abnormalities of urinalysis in these settings are necessary for early detection of ARF.

Tests of tubular function have been developed to aid early diagnosis of ARF (see Table 41-6). Early reports are promising, but at present, these tests have no specific role in clinical practice. Similarly, radionuclide methods of real-time noninvasive monitoring of renal function are expensive and impractical for day-to-day use.

The importance of early detection and elucidation of the specific cause of ARF is illustrated by data in Figure 41-3. These data, from patients with hospital-acquired ARF, demonstrate that the mortality of ARF is directly proportional to the magnitude of subsequent rise in serum creatinine concentration (37). In addition to this observation, several disease entities that can produce ARF (e.g., acute glomerulonephritis, acute interstitial nephritis, and renal vascular disorders) may respond to specific therapeutic interventions. With regard to rapidly progressive glomerulonephritis, there is convincing evidence that therapy is beneficial primarily when given early in the course of the disorder. A recent study documents the relatively long delays that are often encountered before hospital or specialist referral for patients with potentially treatable disorders such as renal failure caused by Wegener's granulomatosis, Goodpasture's syndrome, and other forms of rapidly progressive glomerulonephritis (245) (see Chapters 60 and 68). Maximal effort should be directed to early detection in view of the high morbidity, mortality, and numerous causes of ARF. Once detected, timely determination of the cause of ARF depends on a systematic approach similar to that depicted in Figure 41-6. Other approaches that provide generalizations about the course of ARF include the "site" of onset (Table 41-11) and the associated clinical conditions.

Chart Review, History, and Physical Examination

Chart analysis for determination of the underlying disease states and recent clinical events is needed. A meticulous history with regard to prescription drugs, over-the-counter agents, and herbal preparations as well as possible environmental exposure is critical in view of the frequency with which nephrotoxins contribute to development of ARF (1,37,38,54,85,246,247)

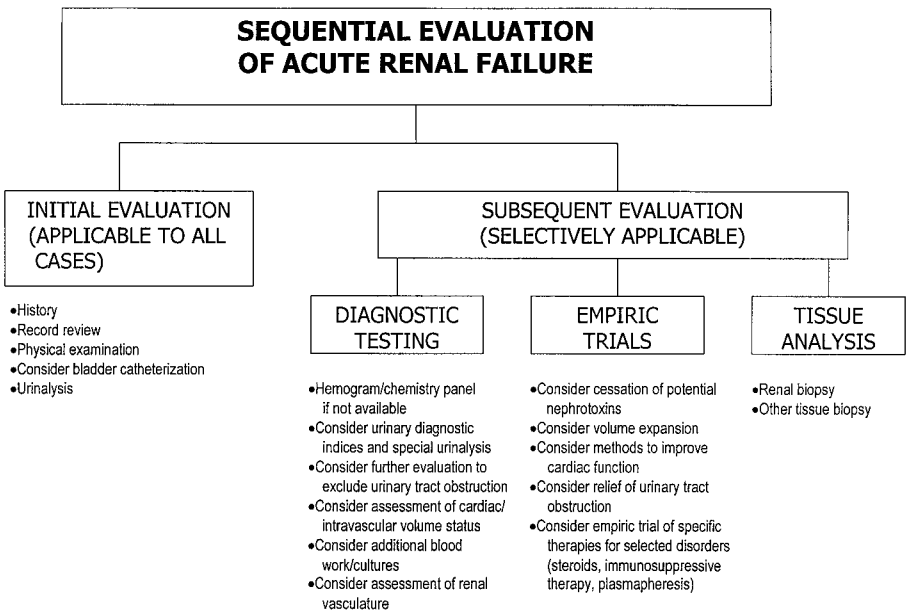

FIGURE 41-6. Suggested sequential diagnostic evaluation to determine the cause of acute renal failure.

TABLE 41-11

COMPARISONS OF ACUTE RENAL FAILURE BY SITE OF ONSET

Factor	Community	Hospital ward	Intensive care unit
Age	Older	Older	Younger
Cause (%)			
Prerenal	30–50	30	20
Postrenal	20–40	10–20	1
Renal	15–30	30–50	70–80
Isolated (%)	80	70	10
Renal replacement therapy need (%)	10	20	70
Mortality (%)	20	30	70

(see Chapters 42–45, 47, 48). The relationship between medication exposure and ARF may not be readily apparent in some cases (248). Careful attention to a history of loss or sequestration of extracellular fluid volume, signs and symptoms of sepsis or heart failure, and symptoms related to the genitourinary tract (urine output, pyuria, dysuria, hematuria, and flank or abdominal pain) can provide helpful diagnostic information. Intense thirst, salt craving, orthostatic syncope, and muscle cramps often are symptoms of extracellular fluid volume depletion. Examination and careful recording, in flow sheet fashion, of available data on the clinical course of each patient with ARF are necessary. Examination of serial vital signs, hemodynamic data, intake and output, and daily weight can provide important data regarding the cause of ARF. A weight change of greater than 0.25 to 0.50 kg/day indicates gain or loss of salt and/or water. Recording of serial renal functional data and correlation of any deterioration in renal function with clinical events such as those altering systemic hemodynamics (Table 41-2) and use of potential nephrotoxins often are of great diagnostic value. As is discussed subsequently, analysis of the hemogram, routine biochemical data, and special serologic studies also can be of great diagnostic assistance.

Physical examination can be of value in determining the presence or absence of prerenal and postrenal causes of ARF as well as the presence of a systemic disorder that could result in a renal cause of ARF. The effect of either loss or sequestration of extracellular fluid volume on systemic hemodynamic responses depends on several variables, including the composition and rate of fluid loss and the underlying health state of the patient. For example, a 20% to 40% decrease in extracellular fluid volume by sodium depletion over 4 to 5 hours decreases mean arterial pressure by about 35% (249). This decrease in mean arterial pressure is associated with a decline in cardiac output and an increase in total peripheral resistance (249). By contrast, a 30% decrease in extracellular fluid volume by sodium depletion over 11 days causes no decrease in mean arterial pressure (249). The presence of a significant increase in pulse and a decrease in arterial pressure 2 to 3 minutes following change from a supine to either a sitting or standing position is compatible with the presence of extracellular fluid volume depletion. Dry mucous membranes, low jugular venous pressure, absence of axillary moisture, decreased turgor of skin over the forehead and sternum, and absence of skin sheet marks over the back are all findings compatible with either loss or sequestration of extracellular fluid volume.

A recent metaanalysis examined the sensitivity, specificity, and predictive value of physical examination in the setting of volume depletion (250). A large (>30 beats/minute) postural pulse rise and postural dizziness was highly associated with blood loss hypovolemia, whereas dry mucous membranes and longitudinal tongue furrows were more than 80% sensitive for detecting non-blood loss hypovolemia (250). Physical examination also can provide evidence of cardiac dysfunction. Elevated jugular venous pressure, pulsus paradoxus, the presence of moist pulmonary rales, S_3 sounds, and murmurs are all compatible with prerenal azotemia owing to impaired cardiac function. The presence of significant edema is compatible with a number of disorders characterized by a decrease in effective arterial blood volume and prerenal azotemia. These include cardiac failure, hepatic cirrhosis, nephrotic syndrome, and severe hypoalbuminemia. Also, retroperitoneal fibrosis and intraabdominal lymphomas and other extensive cancers as well as acute inferior vena caval or renal vein thrombosis can present as ARF associated with pedal edema.

Physical examination must also include palpation for determining the state of peripheral circulation, renal size, and the possibility of abdominal aortic aneurysms. Palpation or percussion of the suprapubic area is necessary to detect bladder distention, and rectal and pelvic examinations are needed to detect prostatic and pelvic disorders. Examination of the skin may reveal palpable purpura suggestive of vasculitis; lower-extremity evaluation may reveal livedo reticularis and evidence of emboli, suggesting atheroembolic disease. It is beyond the scope of this chapter to detail all the physical findings that can be associated with causes of ARF other than ATN; however, the presence of neurologic or pulmonary disease, fever, skin lesions, joint abnormalities, or diffuse lymphadenopathy suggests the presence of a systemic disorder associated with ARF.

Urinalysis and Urinary Flow Rate

Chemical and microscopic examination of the urine is critical in assessment of the cause of ARF (6,37,251,252). Urinalyses in 103 patients with ATN yielded diagnostically useful information in approximately 75% of cases (37). Routine microscopic urinalysis may also provide prognostic information. Hou and associates found that about half of 97 patients with ARF had an abnormal microscopic urinalysis (37). An abnormal urinalysis (probable "renal" cause of ARF) was associated with 35% mortality, whereas a normal urinalysis (probable "prerenal" cause of ARF) was associated with 15% mortality (37). Such a relationship between routine urinalysis and prognosis was not found in another study of ARF (252). Marcussen et al. (251) recently used quantitative Papanicolaou smears of urine sediment in patients with ARF. Patients with ATN had higher numbers of collecting duct cells and casts than those without tubular necrosis; however, there was substantial overlap between the two groups. Moreover, significant numbers of patients with rapidly reversible ARF demonstrated tubular epithelial cells and casts on this type of urinary sediment examination (251). An entirely normal microscopic urinalysis in the setting of ARF suggests the presence of either prerenal azotemia or obstructive uropathy.

The "dipstick" can provide helpful information in ARF. A positive reaction for "blood" by an orthotoluidine test indicates the presence of red blood cells (>3/high-power field). If no red blood cells are present, this reaction will be positive in the presence of either myoglobin or hemoglobin. Because the myoglobin molecule is smaller (17,000 MW) and is not bound to plasma proteins, it is readily filtered and cleared from plasma. By contrast, the larger hemoglobin molecule (65,000 MW), which is bound to haptoglobin, is less readily cleared from the plasma and thus gives plasma a pink-red color. Definitive differentiation of hemoglobin from myoglobin in the urine is best done by electrophoretic or immunochemical techniques that are not widely available.

The dipstick protein reading reflects the presence of albumin. Urinary protein determination performed by the acid precipitation method (e.g., Exton's reagent) detects the presence of all types of protein in the urine. Thus, a quantitative estimate of proteinuria that is lower with the dipstick method than with Exton's reagent suggests the presence of light chains (globulins) in the urine. Immunoelectrophoretic techniques remain the definitive method for identifying urinary light chains. If heavy proteinuria (2 to 3 g/day) is present in ARF, the presence of vasculitis or glomerular or other renal parenchymal cause of ARF should be sought. It is important to correlate the dipstick proteinuria assessment with the urinary specific gravity. For example, a 1+ reading in a concentrated specimen may not indicate significant proteinuria. Conversely, a 1+ reading in a dilute specimen may indicate significant proteinuria. Recent studies indicate that the ratio of a urinary measurement of albumin-to-creatinine concentration in a spot urinary sample provides a reasonable estimate of the grams per day of urinary protein.

Examination of the urinary sediment is of great value in the differential diagnosis of acute impairment of renal function. Sediment containing few formed elements or only hyaline casts strongly suggests prerenal azotemia or obstructive uropathy. With ATN, brownish pigmented cellular casts and many renal tubular epithelial cells are observed in more than 75% of patients. Sufficient red blood cells to cause microscopic hematuria are traditionally thought to be incompatible with a diagnosis of ATN and usually result from glomerulonephritis or structural renal disorders (stones, tumor, infection, or trauma). However, a case report suggested that macroscopic hematuria might be a rare presenting manifestation of ATN (253). Red blood cell casts suggest the presence of glomerular or vascular inflammatory diseases of the kidney and rarely if ever occur with ATN. Red blood cell casts, however, can be seen rarely in acute interstitial nephritis. The presence of large numbers of polymorphonuclear leukocytes, singly or in clumps, suggests acute diffuse pyelonephritis or papillary necrosis. Eosinophilic casts on Hansel's stain of urine sediment may be diagnostically helpful (254). The stains that best detect the bilobed eosinophil include Hansel's stain and the May-Grunwald-Giemsa stain. These stains are less pH dependent than Wright's stain and often detect eosinophiluria in allergic interstitial nephritis. However, eosinophiluria also is seen in some forms of glomerulonephritis and in atheroembolic renal disease but is rarely encountered in ATN (205,254). The combination of brownish-pigmented granular casts and positive occult blood tests on urine in the absence of hematuria indicates either hemoglobinuria or myoglobinuria. In ARF, the finding in fresh, warm urine of large numbers of football-shaped uric acid crystals may suggest a diagnosis of acute uric acid nephropathy, whereas the finding of large numbers of "back-of-envelope–shaped" oxalic acid suggests ethylene glycol toxicity. Other agents (e.g., indinavir, sulfadiazine, acyclovir, and methotrexate) also can induce ARF with characteristic crystal appearance on urinalysis (104). The presence of broad casts (defined as more than three white blood cells in diameter) suggests chronic renal disease.

An older study used the technique of transmission electron microscopy to study urinary sediment in the setting of ARF (255,256). In this study, renal tubular epithelial cells were present in urine sediment only in the presence of ATN (255). There was a correlation between the severity of cellular damage to urinary renal tubular epithelial cells and clinical course. These observations suggest that transmission electron microscopy may be helpful in determining the cause and severity of ARF. Unfortunately, the test is not practical.

The urinary flow rate also may provide helpful information about the cause of ARF. Anuria should be defined as absence of urine by bladder catheterization. Sustained periods of anuria suggest urinary tract obstruction as the cause of ARF. Other rare causes of anuria include rapidly progressive glomerulonephritis, mechanical occlusion of renal blood flow, and diffuse renal cortical necrosis. Brief intervals of severe oliguria (<100 mL/day) may be encountered early in the course of some patients with ATN and may be especially common in the setting of ATN and heat stroke (82).

Oliguria (<400 mL/day) traditionally has been considered the cardinal feature of renal failure (257); however, the first clinical descriptions of progressive azotemia occurring with little or no oliguria were reported in the early 1940s (258). In the mid-1950s, additional reports suggested that nonoliguric varieties of ARF may be especially common following head injury, military combat casualties, and burns (259,260). Small series of selected patients in the 1960s and 1970s reemphasized the occurrence of nonoliguric ARF in burned and traumatized patients (261,262). Since then, it has become apparent that nonoliguric ARF is more common than generally appreciated. In a 1977 prospective study, we found that the majority (59%) of patients with ARF encountered in a general medical-surgical hospital were nonoliguric despite progressive azotemia (38). The nonoliguric state was seen with all types of ARF, including those following surgery, trauma, hypotension, nephrotoxins, and rhabdomyolysis. Additional studies report similar results and demonstrate a frequency of nonoliguric ARF ranging from 25% to 80% of all cases of ARF (Fig. 41-2). More recently in a nationwide study, Allgren et al. (42) enrolled ARF patients in a clinical trial of a renal vasodilator. Of enrolled patients, 24% were oliguric and 76% nonoliguric (42).

Why was the high frequency of nonoliguric ARF recognized only relatively recently? Daily biochemical monitoring of renal function in seriously ill patients, regardless of urine output, has contributed to greater recognition of nonoliguric ARF. Aminoglycoside nephrotoxicity, a frequent contemporary cause of hospital-acquired ARF, often is nonoliguric. Prophylactic use of volume expansion, high-dose potent diuretic agents, and renal vasodilators may also contribute to a high frequency of nonoliguric ARF, especially after aortic and open-heart surgery. It is possible also that aggressive resuscitation and improved supportive management of the seriously ill, traumatized patient have altered the natural history of ARF so that nonoliguric varieties are more common. For example, the Maryland Institute of Emergency Medical Services demonstrated an increasing incidence of nonoliguric and a decreasing incidence of oliguric ARF concomitant with more aggressive fluid resuscitation of traumatized patients (263). Another influence on the frequency of occurrence of nonoliguric ARF is the means of patient selection. When ARF patients are selected by need for dialytic therapy as the only criterion, few if any nonoliguric patients are encountered. When all patients with an increasing serum creatinine level are studied (2), the frequency of occurrence of nonoliguric ARF is very high (Fig. 41-2).

Oliguria with avid renal salt and water retention is the hallmark of reversible prerenal azotemia. Decreasing effective circulating arterial volume results in increased renal vascular resistance (occurring predominantly at the afferent arteriole) and diminished renal blood flow. A decrease in glomerular capillary hydrostatic pressures decreases glomerular filtration. The resultant decline in peritubular hydrostatic pressure and increase in filtration fraction (with an increased peritubular colloid oncotic pressure) may act to increase proximal tubular fluid reabsorption. Increases in plasma concentrations of aldosterone and antidiuretic hormone, decreases in glomerular capillary pressure and altered peritubular factors combine to result in avid tubular reabsorption of salt and water and decreased urine flow. Thus, urine flow in patients with prerenal azotemia usually is diminished, and the urine is concentrated with a low fractional excretion of sodium.

In 1980, Miller and associates reported nine patients with apparent polyuric prerenal failure (264). These patients ranged

from 28 to 76 years of age. No evidence of diabetes insipidus, exposure to nephrotoxins or diuretic drugs, or glycosuria was present. Two patients had mild chronic interstitial renal disease, whereas the other seven had normal basal serum creatinine concentrations. Azotemia developed in each patient (mean serum creatinine, 2.5 mg/dL), and high urine outputs (ranging from 980 to 2140 mL/day at the time of peak serum creatinine) were present. A negative fluid balance, signs of intravascular volume depletion, low urinary sodium concentration (<22 mEq/L), and resolution of azotemia with intravenous fluid administration suggested prerenal azotemia. The authors postulated that a depletion of medullary interstitial solute prevented maximal urinary concentrating ability in these seriously ill patients, allowing the "polyuric" prerenal state to occur. However, because slow recovery of renal function following correction of prerenal factors was seen, some of these patients may have had nonoliguric ATN.

It is important to emphasize that all disease processes including prerenal, postrenal, and diseases of the kidney (vascular, glomerular, interstitial, and tubular) that can produce ARF are often nonoliguric (265). In nearly all of the reported cases, a nonoliguric clinical picture is associated with lower morbidity and mortality and better long-term outcome than an oliguric state (38,42,265).

Recent clinical studies and a review of experimental studies conducted in laboratory models of ARF have clarified several aspects of the pathophysiology underlying the variations of urine flow rate in ARF (266,267). In a study of 25 patients with predominantly renal ischemia-associated ARF, Rahman and Conger found that the urine flow rate strongly correlated with residual GFR (266). By contrast, urine flow rate did not correlate with selected aspects of renal tubular function such as urine:serum ratio of creatinine or fractional excretion of sodium. These clinical observations plus a large body of experimental data suggest that the residual level of GFR is the primary determinant of urine flow in patients with ARF (266,267). The higher level of residual GFR in nonoliguric patients is compatible with improved survival and lower morbidity in these patients.

Urinary Chemical Indices

Urinary concentrations of electrolytes and nitrogenous wastes were first measured in patients with ATN in the late 1940s (268,269). These studies found low urinary-plasma (U/P) creatinine and urea nitrogen ratios and high urinary concentrations of sodium (U_{Na}) and chloride (U_{Cl}) in the established phase of ATN. As renal function improved, U/P creatinine and urea–nitrogen ratios increased. These early observations were subsequently confirmed in nearly all studies (270–280). In the 1950s, Welt (281) and Waugh (282) first suggested that spot urinalysis, specific gravity, and U_{Na} would be helpful in distinguishing between reversible prerenal azotemia and ATN. In 1967 and 1976, Handa (273) and Espinel (276), respectively, suggested that the diagnostic accuracy of U_{Na} alone in determining the cause of ARF was limited. However, either the renal failure index ($U_{Na} \div$ U/P creatinine) or the fractional excretion of sodium (FE_{Na} or $U/P_{Na} \div$ U/P creatinine $\times$ 100) was found to have a high degree of accuracy in differentiating between reversible prerenal azotemia and ATN. Prospective studies have confirmed the utility of selected urinary indices as diagnostic aids in assessing the patient with ARF (270) (Table 41-12; Fig. 41-7). More recently, the fractional excretion of uric acid and trace lithium has been proposed to be even more sensitive and specific aids in determining the cause of ARF (278,279).

The primary use of spot urine chemistries in ARF is to assist in differentiation between potentially reversible prerenal states and ATN. For prerenal states, the combination of reduced renal perfusion and intact tubular function results in enhanced renal tubular reabsorption of filtered salts and water in an effort to expand extracellular fluid volume, thereby restoring renal perfusion. This increase in tubular reabsorption of salts and other organic substances results in relatively low urine concentrations and fractional excretion of sodium, chloride, lithium,

TABLE 41-12

FRACTIONAL EXCRETION OF SODIUM (FE_{Na}) IN ACUTE RENAL FAILURE

FE_{Na} <1%	FE_{Na} >1%
Prerenal azotemia	Diuretic use
ECF volume loss or sequestration	Nonreabsorbable solute
Impaired cardiac output	Bicarbonate
Severe renal vasoconstriction	Glucose
Hepatorenal syndrome	Mannitol
Nonsteroidal antiinflammatory agents	Mineralocorticoid deficiency
Disease of afferent arteriole (e.g., TTP, scleroderma)	Late obstructive uropathy
Sepsis	Chronic renal failure
Nephropathy	Acute tubular necrosis
Early phase of myoglobinuric renal failure	Severe ischemic
Acute glomerulonephritis	
Early obstructive uropathy	
10%–15% of nonoliguric acute tubular necrosis	

TTP, thrombotic thrombocytopenic purpura.

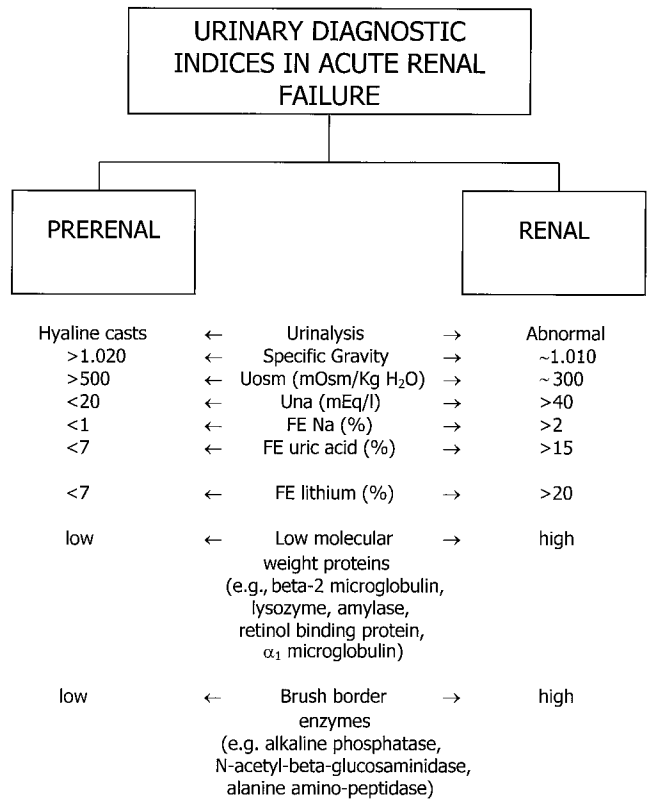

FIGURE 41-7. Urinary diagnostic indices to differentiate between prerenal and renal causes of acute renal failure.

and uric acid (Fig. 41-7). The enhanced tubular water reabsorption produces concentrated urine with relatively high U/P ratios of osmolality, urea nitrogen, and creatinine. By contrast, ATN is associated with impaired tubular reabsorption of salts and water with consequent higher urinary concentrations of sodium, chloride, lithium, and uric acid and lower U/P ratios of osmolality, urea nitrogen, and creatinine (Fig. 41-7). Some of the impaired tubular reabsorption of filtered salts in ATN can be attributed to loss of renal tubular epithelial cell polarity with translocation of the sodium pump (Na-K-ATPase) from a normal basolateral to an apical location (283). Also, a major Na exchanger in the proximal tubule (NHE-3), is transcriptionally downregulated with ischemic injury, as are other more distal transport systems that regulate tubular sodium reabsorption, such as the bumetanide sensitive Na-K-2Cl transporter and the thiazide sensitive Na-Cl-cotransporter (284,285).

Several caveats are in order in using spot urine chemistries as diagnostic aids in evaluating the cause of ARF. Despite widespread, routine use, no study has demonstrated that knowledge of these indices either changes management or improves outcome of ARF. There is currently no "gold standard" for the diagnosis of ATN and most data obtained on urine chemistries in ARF rely on variable and arbitrary criteria for determining the cause of the renal failure. Nearly all studies of spot chemistries have been performed at a single time point relatively late in the course of ARF. The lack of serial data is important because ARF state is often dynamic in nature (277). For example, early in the course of ARF, renal tubular function is intact. Later, ischemia may result in loss of tubular cell polarity. The resulting urine chemistries therefore are dependent on the phase of the course in which they were obtained. This may limit the sensitivity and specificity of urine chemical indices. For example, the early course of ARF occurring in the setting of sepsis, radiocontrast exposure, rhabdomyolysis, and NSAID use often is associated with renal vasoconstriction and low FE_{Na} (286–290). Later in the course, the FE_{Na} often increases, especially if tubular necrosis occurs.

Two other points deserve emphasis with regard to use of urine chemistries as ARF diagnostic aids. Early in the course of urinary tract obstruction, in some patients with nonoliguric ATN and in some vascular/glomerular disorders (acute glomerulonephritis, vasculitis, thrombotic thrombocytopenic purpura), urinary chemical indices can be indistinguishable from those seen with prerenal ARF (274,280,290). Conversely, several acute renal parenchymal disorders (e.g., interstitial nephritis, severe ischemic nephropathy, and exacerbations of chronic renal failure) can be associated with urine chemical parameters indistinguishable from ATN, suggesting a lack of specificity (291,292). Finally, it is important to acknowledge that potentially reversible prerenal ARF with a $FE_{Na}>1\%$ occurs in selected settings such as recent diuretic use, bicarbonaturia, salt-wasting nephropathy, glycosuria, and mineralocorticoid deficiency (Table 41-12). In the setting of prerenal ARF associated with bicarbonaturia, the urinary chloride concentration is low, confirming a prerenal state (293). In the setting of prerenal ARF associated with diuretic use, the fractional excretion of trace lithium and uric acid continue to be low (279). Unfortunately, measurement of fractional excretion of trace lithium requires atomic absorption spectrophotometry and thus is not widely available for timely clinical use at present.

Although urine chemical indices are most often used as diagnostic adjuncts in patients with ARF, it is of interest that they may also provide prognostic information. For example, several studies suggest that, in oliguric patients with ARF, lower values for FE_{Na} and higher values for U/P osmolality predict a high likelihood of a diuretic response to loop diuretics, mannitol, and/or dopamine (38,294,295). Moreover, the more urine chemistries resemble those seen in prerenal states, the higher the likelihood of survival from ARF (38,279).

One specific urine index deserves special comment. The ratio between urinary uric acid and urinary creatinine has been used as an aid to determine the nature of ARF (296). In an older study by Kelton et al. (296), ratios of higher than 1 in patients with renal failure were observed in patients with acute uric acid nephropathy compared with ratios below 1 in those who had ARF from other causes (296). For example, the ratio was found to be less than 1 in prerenal azotemia, chronic renal failure, and obstructive uropathy. It was concluded that a ratio of more than 1 in ARF signified a diagnosis of acute uric acid nephropathy. In a more recent study, the urinary uric acid–urinary creatinine ratio was studied in 23 patients with ARF (297). This ratio was >1 in 12 patients. These 12 patients had high fever, catabolism, hyperbilirubinemia, and nonoliguric ARF. The 11 patients with ratios of <1 tended to be less catabolic and oliguric. These results suggest limited diagnostic utility of the urinary uric acid–urinary creatinine ratio in determining the cause of ARF (297).

Consider Urinary Tract Obstruction

A postrenal cause is encountered in a significant percentage of all cases of ARF, especially in cases of community-acquired ARF (18,57) (see Chapter 25). The potential for therapeutic intervention suggests that the possibility of urinary tract obstruction should be considered in all cases of ARF. A cost-effectiveness analysis of a global strategy of excluding urinary tract obstruction in all cases of ARF has not been done. The presence of prostatic, pelvic, or intraabdominal cancer, a single kidney, anuria, widely fluctuating urinary volumes, recent surgery on the genitourinary tract or within the retroperitoneal space, or a normal urine sediment demand evaluation for potential obstruction.

The best means of excluding obstructive uropathy as a cause of ARF remains debatable. Physical examination (suprapubic palpation, and pelvic and rectal examinations) and postvoiding bladder catheterization continue to be the best methods for bladder neck obstruction. Ultrasonography in experienced hands is an excellent screening tool for the presence of extrarenal obstruction; however, it is recognized that significant obstructive uropathy may occur in the presence of minimal ureteral and renal pelvic dilation. For example, in studies by Curry and associates (298), 27 patients with obstructive uropathy were examined by ultrasound. Three of these 27 (11%) were found to have minimal dilation and high-grade obstruction that improved with percutaneous drainage. Other series of nearly 250 patients report a somewhat lower frequency (4%) of nondilated obstructive uropathy (299,300). Because the majority of patients with ARF and nondilated renal collecting systems do not have either retrograde pyelography or percutaneous nephrostomy, it is likely that the reported frequency of nondilated obstructive uropathy represents a minimal estimate. Abdominal and pelvic conventional and spiral computerized tomography scanning technologies appear to be exceptionally helpful in the diagnosis of acute obstructive uropathy (301). Occasionally, retrograde pyelography, stent replacement, or empiric percutaneous nephrostomy drainage may be necessary to exclude obstructive uropathy.

Miscellaneous Tests

In some circumstances, the cause of ARF is not apparent after chart review, history, physical examination, and urinalysis. Review of the hemogram may be helpful in this setting. A peripheral blood smear that reveals rouleaux formation

may suggest the presence of a plasma cell dyscrasia. Serum and/or urinary protein electrophoresis are needed to substantiate this diagnosis. Eosinophilia is compatible with allergic interstitial nephritis, atheroembolic disease, and polyarteritis nodosa. A microangiopathic picture with thrombocytopenia suggests vasculitis, malignant hypertension, the HELLP syndrome, hemolytic-uremic syndrome, and thrombotic thrombocytopenic purpura. The presence of coagulopathy can suggest either disseminated intravascular coagulation or an antiphospholipid antibody syndrome as the cause of ARF. If glomerulonephritis is a diagnostic possibility, then the presence of antineutrophilic cytoplasmic antibodies may suggest a diagnosis of either Wegener's granulomatosis (primarily a cytoplasmic pattern) or pauci-immune glomerulonephritis (primarily a perinuclear pattern). Antibodies to glomerular basement membrane are strongly suggestive of Goodpasture's syndrome, whereas antinuclear antibodies and antibodies against DNA suggest the presence of systemic lupus erythematosus. The presence of cryoglobulins may point to the presence of circulating immune complexes, a plasma cell disorder, or primary cryoglobulinemia. A review of blood chemistries may be helpful. Hypercalcemia of diverse causes can be associated with the development of ARF, and very high uric acid concentrations may suggest the presence of diffuse tissue injury such as occasionally occurs in tumor lysis syndrome and rhabdomyolysis. If mechanical obstruction of the renal vasculature is suspected, then isotope studies, Doppler procedures, magnetic resonance angiography, or conventional angiography might be considered.

Therapeutic Trials

Response to specific interventions may provide diagnostically helpful information in the ARF setting. Restoration of renal function with either extracellular fluid volume replacement or improvement in cardiac output (dopamine/dobutamine) supports a diagnosis of prerenal azotemia. Improved renal function after relief of obstructive uropathy suggests postrenal azotemia. Improvement or amelioration of ARF after cessation of pharmacologic agents such as NSAIDs or ACE inhibitors suggests a pathogenetic role for those substances in ARF. Mechanical (angioplasty or surgical manipulations) or pharmacologic (thrombolytic) improvement in renal blood flow in the appropriate clinical setting supports an ischemic basis for worsening of renal function (72,73,302,303).

Renal Biopsy

Occasionally, despite a logical sequential approach, the cause of ARF remains unclear. Renal biopsy may be considered under such circumstances. A renal biopsy often is considered in the setting of ARF when some of the following are present: (a) no obvious cause of ARF, (b) either extrarenal clinical evidence or a history of systemic disease, (c) heavy proteinuria and persistent hematuria, (d) marked hypertension in the absence of volume expansion, (e) prolonged (>2 to 3 weeks) oliguria, and (f) anuria in the absence of obstructive uropathy. It is worth reemphasizing the necessity for aggressive, timely evaluation if glomerular disease is a diagnostic possibility as a cause of ARF (245). Once the serum creatinine exceeds 2 to 5 mg/dL in cases of rapidly progressive glomerulonephritis, and perhaps acute interstitial nephritis, the possibility of nonreversible scarring and lack of recovery of renal function increases significantly (245).

Several studies have examined the clinical utility of renal biopsy in the setting of ARF (304–309). Mustonen and associates (305) performed biopsies in 91 of 99 patients and compared biopsy results with the prebiopsy clinical diagnoses. A clinical impression of acute tubular and/or interstitial disease was confirmed by biopsy in 44 of 51 (86%) of cases. A clinical impression of acute glomerular disease was confirmed by biopsy in 16 of 23 (70%) of cases. In 30% of cases, either no clinical diagnosis was obvious or the clinical diagnosis differed from the biopsy diagnosis. Wilson et al. (307) performed a renal biopsy on 84 patients with ARF. All of these patients had undefined "atypical features" for ATN. On biopsy, 52% had glomerular disease, 30% had acute tubulointerstitial disease, and 18% had renal vascular disorders (307). In this study, a clinical diagnosis of an acute tubulointerstitial process was 77% sensitive and 86% specific. By contrast, a clinical diagnosis of acute glomerulonephritis was less sensitive (56%) and less specific (66%). In another prospective study, Cohen and associates (304) found that only one-third of the clinical diagnoses of the cause of ARF were substantiated by renal biopsy. Moreover, in this small series, the renal biopsy led to a significant change in therapy in more than half of the patients with ARF who were undergoing a biopsy (304).

Solez and colleagues (306) reported on their experience with 976 patients with ARF encountered over a 10-year interval. Twenty-two percent of these patients underwent biopsy because the cause of ARF was not apparent. Most of the patients had features atypical of ATN such as gradual onset of ARF, significant hypertension, marked proteinuria, significant hematuria, prolonged (>3 weeks) oliguria, and underlying systemic disease. Half of the patients, in whom the ARF diagnosis was not apparent, had glomerular disease on biopsy, whereas the remainder had tubulointerstitial disease.

In addition to indications for performing a renal biopsy in ARF, two other issues are of concern. The first is safety. Recent developments in treating the coagulopathy associated with renal failure plus improvements in biopsy techniques (e.g., biopsy guided by real time ultrasonography or CT imaging, use of smaller needles and biopsy guns) have improved safety. Indeed, reasonable safety has been demonstrated in the setting of uncooperative ICU patients requiring mechanical ventilation (310). In this small study, percutaneous renal biopsy performed on critically ill, ICU patients undergoing mechanical ventilation was compared with open biopsy. Sufficient renal tissue for diagnosis was obtained on all seven patients undergoing percutaneous biopsy and the rate of complications was roughly comparable to patients undergoing open biopsy (310). Renal biopsy utilizing a transjugular approach has been used in high-risk patients. Thompson and colleagues (311) reported on 25 patients who underwent transjugular renal biopsy—these procedures were performed by an interventional radiologist experienced in transjugular liver biopsies, and diagnostic tissue was obtained in 21 (84%) of cases. Seventeen out of 25 patients developed perforation of the renal capsule, 6 of whom required coil embolization. One patient developed renal vein thrombosis 6 days after a failed transjugular biopsy attempt (311).

The timing of biopsy in ARF remains a key issue (245). In the past, lack of recovery of renal function and persisting anuria after several days were considered as indicators for ARF biopsy. Presently, concerns about the irreversible nature of many forms of severe glomerulopathy and acute interstitial disorders, if left untreated, have led to a much more timely approach to biopsy when the cause of ARF is not clear.

COMPLICATIONS OF ACUTE RENAL FAILURE

Determination of the rate of occurrence of complications in patients with ARF sometimes is difficult. Patients with the

highest rate of development of ARF often have significant comorbidity and preexisting multiple organ dysfunction prior to development of ARF. For example, in a case cohort study of mild-to-moderate radiocontrast-induced ARF, preexisting sepsis (22%), respiratory failure (36%), mental status changes (41%), and bleeding (15%) were relatively common (35). However, the rate of new occurrence of these complications following development of ARF in patients that did not have the complication before ARF was strikingly high (sepsis 45%, respiratory failure 78%, mental status changes 68%, and bleeding 27%) (35).

Cardiovascular System Complication

Hemodynamic instability is often encountered before and after development of ARF. Liano found that 60% of ICU-associated and 19% of hospital ward-associated ARF cases experienced hypotension (56). Myocardial ischemia (13% to 19%) and cardiac arrhythmias that required treatment (22% to 29%) were observed commonly in a large multicenter study of patients with ARF (42). Volume overload and congestive cardiac failure are also common complications occurring in ARF patients (38,40,77,312,313). Of course, congestive heart failure and its treatment (angiotensin converting enzyme [ACE] inhibitors and diuretics) can be associated with ARF. Pericardial tamponade also can be associated with ARF. The causes of cardiac tamponade in ARF are pericardial effusion (usually of serosanguinous type), hemorrhage into the pericardial sac, and collagenization of pericardial exudate (314,315). The concomitant presence of significant cardiac dysfunction and ARF may be a manifestation of either systemic disease (i.e., systemic lupus erythematosus, scleroderma) or a complication of cardiac disease (i.e., subacute bacterial endocarditis, atrial fibrillation with emboli). Spontaneous and catheter-induced atheroemboli can cause abdominal and peripheral vascular manifestations as well as ARF. Indwelling arterial and venous catheters can lead to vascular occlusion, inflammation, and infection in the setting of ARF. Cardiac arrest in the setting of ARF always should arouse immediate suspicion of hyperkalemia, a potentially treatable cause of cardiac arrest.

Pulmonary System Complications

Pulmonary infiltrates caused by edema from volume overload and/or infection are encountered frequently in ARF. In eight series comprising 1,900 patients with severe ARF, more than 50% had concomitant respiratory failure sufficient to warrant mechanical ventilation (316).

Respiratory failure occurred commonly before (36%) and especially after (78%) the development of ARF in the experience of Levy and associates (35). Liano et al. (40) found that 82% of ICU-associated cases of ARF were on mechanical ventilation, whereas Allgren reported that 48% to 55% of all ARF patients were intubated for respiratory support (42). Pulmonary infiltrates appeared in 26 of 92 (28%) patients with ATN in our experience (38). Pulmonary complications including aspiration pneumonia, and adult respiratory distress syndrome occurred in 54% of 462 patients in another recent study (312). McMurray and associates found 81 episodes of pneumonia in 276 patients with ATN (77).

There are several disease processes that can cause simultaneous pulmonary involvement and impairment of renal function (316). These processes include Goodpasture's syndrome, systemic lupus erythematosus, Wegener's granulomatosis, allergic granulomatous angiitis (Churg-Strauss syndrome), polyarteritis nodosa, cryoglobulinemia, sarcoidosis, renal vein thrombosis with pulmonary emboli, and bronchogenic carcinoma with immune complex glomerulonephritis. The development of pulmonary complications is an adverse prognostic factor in ARF (312,313,316,317). In a study of prognostic risk factors in 462 patients with ATN, Bullock et al. (312) found the development of pulmonary complications to be the single most significant risk factor for death in ATN; and ARF occurring in the respiratory ICU was associated with an 80% mortality. In five series of patients with severe ARF, mortality increased from 49% in nonventilated patients to over 80% in patients who required mechanical ventilation (316).

Gastrointestinal System Complications

The primary gastrointestinal complications of ARF include symptoms of anorexia and nausea, vomiting, and upper gastrointestinal bleeding. Stress ulcers and gastritis are common. In a prospective study of 514 subjects with ARF (318), acute gastrointestinal bleeding occurred in 69 (13.4%) patients. Forty (7.8%) patients had bleeding that was severe, that is, resulted in hemodynamic compromise or required transfusion or surgery. The most common causes of bleeding were gastric erosions/ulcers, duodenal ulcers and esophageal varices. Independent risk factors for bleeding included thrombocytopenia, liver cirrhosis, *de novo* ARF, chronic liver disease, increased severity of ARF, and APACHE II score (318). Gastrointestinal bleeding complicated the course of 8% to 13% of patients with ARF recently reported by Allgren and associates (42).

Mild hyperamylasemia (two or three times normal) can be seen in ARF (319). Also noteworthy is the fact that several disease processes (e.g., atheroemboli, vasculitis, and common bile duct stones with bacteremia) can present with both acute pancreatitis and ARF. Acute and chronic renal failure can elevate the ratio of the renal clearances of amylase to creatinine (319). Thus, lipase determinations and clinical assessment often are necessary to assist in the diagnosis of pancreatitis in the setting of ARF. Acute pancreatitis can result in ARF. In two recent series of more than 1,000 patients with acute pancreatitis, 4% to 14% developed some degree of ARF (320,321). Allgren found that 2% to 10% of all ARF patients had a clinical diagnosis of acute pancreatitis (42).

Jaundice often occurs during the course of ARF. Jaundice occurred in 43% of 462 patients with ATN in the experience of Bullock et al. (312). Jaundice occurred in 28% of ICU-associated and 13% of hospital-ward associated ARF in the experience of Liano and associates (40). Jaundice is often multifactorial, with contributing factors including passive hepatic congestion, blood transfusions, hypotension, medications and toxins, and sepsis. A number of infections (Gram-negative sepsis, especially in patients with bile duct obstruction, leptospirosis, or hepatitis A, B, and C) and toxins (hydrocarbons, acetaminophen, *Amanita phalloides* toxin) can cause simultaneous hepatitis and ARF. The development of jaundice in the setting of ARF may be an ominous prognostic finding (312).

Neurologic System Complications

Central nervous system disorders have long been recognized as frequent accompaniments of ARF. Liano and associates emphasized that the presence of coma associated with ARF signals an exceptionally poor prognosis (40). Mental status changes were noted by Levy et al. (35) in 41% of patients before and 68% of patients after development of ARF. In another study, Liano found that about 25% of ARF patients were sedated, making assessment of mental status difficult, and that 5% were comatose (56). Levy et al. found that the course cases of 12% of ARF patients were complicated by an acute stroke, whereas a stroke occurred in only 2% of case controls (35).

There are a number of causes of neurologic dysfunction in the setting of ARF such as primary neurologic disease, other metabolic disturbances, and the presence of systemic disease involving both the kidneys and nervous system (vasculitis, systemic lupus erythematosus, subacute bacterial endocarditis, thrombotic thrombocytopenic purpura, hemolytic-uremic syndrome, malignant hypertension). Pharmacologic agent–induced (e.g., sedative-hypnotic drugs) encephalopathy is a common cause of central nervous system dysfunction in patients with renal failure (322). Neurologic symptoms appearing after dialytic therapy should arouse suspicion of a dialysis disequilibrium syndrome. Development of peripheral neuropathy in the setting of ARF should raise consideration of neurovascular entrapment (rhabdomyolysis), ischemic neuropathy (polyarteritis, emboli), and heavy metal intoxication.

Infectious Complications

Acute renal failure and infections are commonly associated. In the experience of Rasmussen and Ibels, septicemia appeared to be an important factor contributing to the development of ARF in 26% of 143 ARF patients (123). In a case-control study, Shusterman and colleagues (55) found that septic shock was the single clinical condition associated with the highest likelihood of development of hospital-acquired ARF.

Allgren found that sepsis occurred in 27% to 42% of patients with ARF and that the overall rate of associated infection ranged from 44% to 57% (42). In a recent prospective study of patients with septic shock, 21% of patients were oliguric at the time of study entry (323).

Not only is septicemia frequently associated with the onset of ARF, but also infections frequency complicates the course of patients with ARF. Levy and colleagues found a 22% frequency of preexisting infection before and an additional 45% frequency of infections after ARF (35). McMurray et al. (77) found infections in 74% of 276 patients with ATN. In their study, the most common sites of infection were pulmonary (29%), urinary tract (32%), and peritonitis (22%). A total of 56 patients had abscesses, and 97 had documented bacteremia. Infections from indwelling venous and arterial lines and indwelling bladder catheters also occur in ARF; however, urinary tract infections can occur even in patients without indwelling catheters. Patients with ARF on dialysis in ICU are especially susceptible to infection. Hoste (324) found that in 704 ICU patients treated for ARF with dialysis, 62 patients developed nosocomial bacteremia. The lungs were the most important source of bloodstream infection (26%), followed by the abdomen (23%), catheters for vascular access (16%), urogenital tract (10%), wounds (6%), and unknown (19%) (324). Pulmonary infections appear to occur later in the course of ARF. In a prospective analysis, we found that 11% of 92 patients with ARF developed septicemia. In the experience of Bullock et al. (312) with 462 ARF patients, 91% received an average of three antibiotics for 16 days. Keane et al. (325) recently published a small ($N = 35$) prospective, controlled study examining the efficacy of intravenous immunoglobulin G as adjunctive therapy in patients with ARF. A striking 71% of 35 ARF patients developed culture-proven infections (325). Interestingly, mortality was significantly lower in immunoglobulin-treated patients (12%) than controls (44%). Also of interest, there was no difference in the rate of infectious complications when the immunoglobulin-treated patients were compared with controls. Confirmation of these results as well as documentation of the mechanisms of improved survival is needed.

Infectious complications are a leading source of morbidity and mortality in ARF. McMurray et al. (77) found that infectious complications were the cause of death in 54% of 102 ATN deaths. In the extensive experience of Liano and associates in which progression of underlying disease was excluded, infection was the single leading case of death (40).

Endocrine System Complications

Acute tubular necrosis is often associated with disturbances in divalent ion metabolism (hypocalcemia, hyperphosphatemia, and hypermagnesemia) (326,327). Altered parathyroid hormone (PTH) action and vitamin D metabolism may play a pathogenetic role in the hypocalcemia and hyperphosphatemia. Several studies demonstrate a high plasma PTH in ATN (233,328–335). This probably occurs in response to hypocalcemia. The simultaneous presence of hypocalcemia with high PTH levels suggests impaired bone response to PTH, which has been demonstrated in humans with ATN (330). In some ARF patients, decreased 1,25-dihydroxy vitamin D is present and many ARF patients have hyperphosphatemia. These latter two factors may underlie the skeletal resistance to PTH that occurs with ATN (326–331,333-335). Monomeric calcitonin levels have been found to be increased with ARF (335). The role of calcitonin in the deranged calcium/phosphorus homeostasis of ARF remains to be determined.

Calcium homeostasis in ARF related to rhabdomyolysis has been investigated (233,329,335). In general, hypocalcemia, hyperphosphatemia and low levels of 1,25-dihydroxy vitamin D are present. Hypercalcemia can rarely occur early in the polyuric phase of ARF and is associated with increases in 1,25-dihydroxy vitamin D and PTH (both amino-terminal and carboxy-terminal) (233). Later in the polyuric phase, serum calcium vitamin D, and PTH levels returned to normal. A close correlation between serum calcium and vitamin D levels can be present (233). These observations suggest that the hypocalcemia seen during the early phase of ARF is due to hyperphosphatemia and decreased 1,25-dihydroxy vitamin D synthesis. In summary, high PTH, skeletal resistance to PTH, and low 1,25-dihydroxy vitamin D levels occur in ARF. Rarely, the recovery of rhabdomyolysis-associated ARF can be complicated by hypercalcemia. The cause of this late syndrome may be mobilization of calcium sequestered by necrotic muscle tissue rather than a disturbance of the PTH-vitamin D axis (336,337). Calciphylaxis has been reported as a rare complication of ARF (338).

Data on male gonadal function in ARF are available. Two groups of investigators found high blood prolactin levels and low testosterone in males with ARF (331,332). The abnormalities in prolactin and testosterone resolved as renal function normalized. In the study of Kokot and Kuska (331), increased blood concentrations of luteinizing hormone and estradiol also were present. These workers found a negative correlation between plasma prolactin and testosterone but no correlation between PTH and either testosterone or prolactin (331). By contrast, Levitan et al. (332) found a direct relationship between prolactin and PTH. Together, these observations demonstrate that male gonadal failure frequently occurs in the setting of ARF. Unfortunately, data on comparably ill control subjects are not available.

Thyroid function tests have been examined in the setting of ARF (339,340). Total thyroxine (T_4) and triiodothyronine (T_3) are decreased in ARF and return to normal with recovery of renal function. In one but not another study, free T_3 and free T_4 also were decreased in ARF (339,340); however, all patients appeared clinically to be euthyroid and thyroid-stimulating hormone was normal. Thus, patients with ARF resemble other critically ill patients, and thyroid function is normal.

Kokot and Kuska (331) studied glycogenic insulin release in patients with ARF. Insulin release from beta cells was studied by intravenous glucose infusions. Fasting insulin concentrations were normal. In response to glucose infusion, insulin

concentrations were higher than in controls, and plasma disappearance was prolonged. These findings suggest the presence of insulin resistance such as is commonly seen in chronic renal failure. This conclusion was further documented by finding that insulin (0.1 μm/kg) decreased fasting plasma glucose from 96 to 58 mg/dL in patients with ARF and from 90 to 38 mg/dL in normal subjects. In acutely uremic rats, insulin resistance appears to be caused by skeletal muscle resistance to insulin-mediated glucose uptake (341). Also, there appears to be impaired hepatic glucose response to glucagon but not to cyclic adenosine monophosphate (cAMP). Together, these clinical and experimental studies suggest that ARF produces insulin resistance and the resistance occurs at the skeletal muscle level. Also, acute uremia appears to include a "pre-cAMP" defect in hepatic glucose response to glucagon (341). High plasma renin activity (PRA) and angiotensin II often occur in the setting of ARF. In a clinical study, Mitch and Walker (342) found elevated plasma levels of angiotensin II in 13 patients with shock and ARF. Of these 13 patients, 10 had increases in PRA; however, only slight increases in angiotensin II and PRA levels were frequently found. Furthermore, comparable increases in PRA and angiotensin II were observed in six patients undergoing elective drug-induced hypotension who did not develop ARF (342). The observations confirm that high levels of angiotensin II and PRA are often observed in the clinical setting of ARF. However, similar levels also occur in settings in which renal function is clinically well maintained. Whether the high angiotensin II levels contribute to hypertension that occasionally complicates ARF remains to be determined. Transient hyperreninemic hypoaldosteronism is common in patients with septic shock. These abnormal aldosterone levels are associated with greater sodium and fluid depletion and are followed by enhanced incidence of acute renal failure requiring renal replacement therapy and prolonged length of stay in the ICU (343).

Kokot and Kuska (331) examined growth hormone response in insulin in patients with ARF. Patients with ARF had a threefold greater increase in growth hormone compared with controls. Following hemodialysis, the exaggerated increase in growth hormone response was significantly less but still present.

BIOCHEMICAL ABNORMALITIES IN ACUTE RENAL FAILURE

Nitrogen Balance

Plasma urea nitrogen and serum creatinine increase because decreased urinary excretion of nitrogenous waste occurs in ARF. The magnitude of increase is dependent on the nitrogen intake, the degree of renal impairment, and the degree of protein catabolism (see Table 41-13). Urea nitrogen appearance rates ranging from 5 to 50 mg/day or greater can occur, depending on the catabolic state of the patient (344). In the noncatabolic patient with mild renal impairment, daily BUN usually increases <10 to 15 mg/dL per day and serum creatinine <1.5 mg/dL per day. Conversely, in the catabolic patient, daily increments of BUN can exceed 50 mg/dL. In 462 patients with ATN, Bullock et al. (312) found that 35 were "catabolic" as defined by an increase of BUN of >30 mg/dL on 2 consecutive days. Biesenbach et al. (317) found that 10% to 20% of 710 cases of ARF were hypercatabolic. The degree of renal impairment is also an important determinant of BUN and plasma creatinine in ARF. We found that the duration of BUN of >50 mg/dL in ARF was 18 ± 2.0 days in oliguric patients and 8 ± 0.8 days in nonoliguric patients who had a higher GFR (38).

TABLE 41-13

SELECTED BIOCHEMICAL COMPLICATIONS IN ACUTE RENAL FAILURE

	Change/day	
	Noncatabolic	Catabolic
Blood urea nitrogen (mg/dL)	10–20	>30
Creatinine (mg/dL)	<1.5	>1.5
K^+ (mEq/L)	<0.5	>0.5
$HCO3^-$ (mEq/L)	<2	>2
Phosphorus (mg/dL)	0.5	>1

The precise cause of the catabolic state in ARF cannot be stated with certainty. Many patients have necrotic tissue, muscle damage, fever, and sepsis and may be receiving corticosteroids. Several hormonal abnormalities are present in ARF (e.g., elevated levels of glucagon, catecholamines, growth hormone, cortisol, and insulin resistance) that could alter muscle protein metabolism (331,344). A major cause of protein catabolism in ARF is insulin resistance (345). Insulin resistance leads to reduced protein synthesis, enhanced degradation of muscle protein and excessive release of amino acids into the circulation (345). Gluconeogenesis and ureagenesis are increased due to hepatic uptake of these amino acids from the circulation (345). Circulating proteases released from leukocytes have been found in the blood of catabolic patients with ARF (346). ARF is associated with a reduction in protease inhibitors such as α-2-macroglobulin (347). The use of dialysis may remove amino acids and other nutritional elements (348). Finally, diminished nutritional intake can potentially contribute to the depressed level of muscle protein synthesis in ARF.

Enhanced muscle breakdown with release of creatine can lead to a disproportionate increase in serum creatinine relative to BUN in the setting of rhabdomyolysis-induced ARF. For example, Koffler and associates (349) found that about 25% of their patients with rhabdomyolysis-induced ARF had disproportionate increases in creatinine relative to BUN. Grossman and associates (234) found daily increments in plasma creatinine varying from 1.6 to 6.6 mg/dL. In 15 patients with rhabdomyolysis-related ARF, nine had daily increments of plasma creatinine of >3.0 mg/dL (234). However, Gabow et al. (243) did not find any difference in daily increment in plasma creatinine when ARF patients with (1.3 ± 0.7 mg/dL) and without (1.4 ± 0.8 mg/dL) rhabdomyolysis were compared.

Clinicians usually follow daily serum creatinine concentrations to assess whether GFR is increasing, decreasing, or constant in patients with ARF. The serum creatinine concentration, however, is dependent on creatinine production, volume of distribution, and renal elimination, and all of these variables are subject to fluctuations in patients with ARF. Moran and Myers (5) developed a simple, computerized model of creatinine kinetics in patients with postischemic ARF. This model allows calculation of GFR based on serum creatinine concentration corrected for changes in creatinine volume of distribution and was validated by direct measurements of GFR. Using this model, two clinically noteworthy observations were made. First, in patients with ARF, changes in GFR often correlated poorly with changes in serum creatinine concentration. Second, several patterns (abrupt and large, slow and progressive, and stepwise) of change in glomerular filtration occurred during development of and recovery from postischemic ARF and were poorly reflected by daily changes in serum creatinine concentration (5). These observations suggest that definite conclusions regarding changes in GFR are difficult to make using serum creatinine concentration alone in the setting of ARF.

In this regard, isotopic methods (utilizing radioisotope or radiocontrast) can provide noninvasive, real-time monitoring of renal function in critically ill patients (49,350,351). Unfortunately, this methodology is not widely available at present.

Disorders of Electrolyte and Uric Acid Metabolism

Hyperkalemia, hyponatremia, metabolic acidosis, and hyperuricemia often occur in ARF. In our experience, a rise in plasma potassium concentrations to >5.5 mEq/L was seen in 50% of patients with ARF (38). Minuth and colleagues (252) found hyperkalemia in 75% of 94 patients. Wheeler and associates (352) found that 30% of patients with ATN develop an increase in plasma potassium concentration to >6.0 mEq/L; however, an increase in serum potassium may not occur in cases of mild ARF (326). The hyperkalemia is due to continued potassium release from cells in the face of impaired renal potassium elimination. For example, the potassium concentration of intracellular water is about 155 mEq/L in skeletal muscle. Thus, tumor lysis syndrome and rhabdomyolysis can induce dangerous levels of hyperkalemia quickly. A study of seven patients with rhabdomyolysis induced by extensive traumatic muscle crush injury revealed plasma potassium concentrations on admission of 4.5 to 8.3 mEq/L despite rapid evacuation from the site of injury (236). Three of the seven patients had potassium concentrations of >6.9 mEq/L. Gabow and colleagues (243) found significantly higher peak potassium concentrations in ARF associated with rhabdomyolysis than in other forms of ARF (5.6 ± 0.9 vs. 4.7 + 0.6 mEq/L, respectively; $p < 0.05$). Other factors including a cellular shift of potassium owing to acidemia and hyperosmolality and potassium loads from exogenous sources such as blood, dietary intake, potassium salts (e.g., salt substitutes), or large doses of penicillin G can also contribute to hyperkalemia. Acute renal failure induced by NSAIDs can also be associated with marked hyperkalemia (353). An effect of these agents in suppressing renin and aldosterone secretion may be responsible in part.

Metabolic acidosis occurs often in ARF. In 92 patients with ARF, we found metabolic acidosis (plasma bicarbonate of <15 mEq/L, pH <7.40) in 19% (38). In a group of patients with mild ARF (rise in serum creatinine of 2 mg/dL), a 2 to 4 mEq/L decrease in serum bicarbonate occurred (339). Stable patients without kidney function on chronic hemodialysis usually have a decline of plasma bicarbonate of 2 mEq/L/day (344). The metabolic acidosis is often associated with an increased anion gap. Thus, Gabow and associates (243) found an anion gap of 17 ± 6 mEq/L in ARF; however, when rhabdomyolysis was present, the anion gap increased to 28 ± 14 mEq/L. By contrast, Dolson found an anion gap of 14 ± 0.6 mEq/L in series of patients with mild ARF (326). The metabolic acidosis results from continued production of nonvolatile acid and decreased renal ability to excrete acid. In severely catabolic states, the usual daily production of 1 mEq/L of nonvolatile acid can be increased markedly. It is noteworthy that other causes of anion gap metabolic acidosis such as ingestion of ethylene glycol and clinical settings associated with lactic acidosis are often associated with ARF.

Hyponatremia is a common complication of ARF and is caused by an absolute or relative increase in solute-free water intake. In a prospective analysis of hyponatremia, we found that 19% of all cases of hyponatremia occur in the setting of excess solute-free water intake in the presence of renal failure (354). Rare associations with hyponatremia and ARF include toxin ingestion (355,356), rhabdomyolysis, infection (357) and hypothyroidism (358). Hyperuricemia usually occurs in ARF. Peak uric acid was 9.2 ± 3.7 mg/dL in 38 patients with ATN (243) and 8.6 mg/dL in 96 patients with mild ARF (326). Much higher blood uric acid concentrations occur in cell injury-associated ARF, and peak uric acid concentrations of 14.1 ± 4.4 mg/dL were found in patients with ARF and rhabdomyolysis (243). Uric acid concentrations of greater than 20.0 mg/dL were seen in five of 21 patients with rhabdomyolysis and ARF in other studies (234,349). Striking increases in uric acid concentrations also occur with ARF in the setting of heat stroke, tumor lysis, and catabolism. Despite high blood uric acid concentrations, there is little evidence of irreversible end-organ disease resulting from hyperuricemia (82,349).

Disorders of Divalent and Trivalent Ion Metabolism

Hyperphosphatemia in the range of 5 to 8 mg/dL often occurs in ARF (233,327,329,335). With mild ARF, the rise in serum phosphorus may be very modest, to the 4 to 5 mg/dL range (326). Decreased urinary excretion of phosphorus contributes to hyperphosphatemia. In addition, phosphorus is released from injured tissue, in which intracellular phosphate concentrations average 100 mmol/L. In the presence of tissue destruction, as in the tumor lysis syndrome and in rhabdomyolysis, extremely high serum phosphorus concentrations are seen. For example, 14 of 34 patients with ARF due to nontraumatic rhabdomyolysis had hyperphosphatemia (>9 mg/dL) in two studies (234,349). In another study, peak serum phosphorus in patients with ARF with and without rhabdomyolysis was 7.0 ± 2.7 and 4.5 ± 1.8 mg/dL, respectively (243). Acute acidosis can also contribute to hyperphosphatemia, by decreasing the glycolytic rate and increasing the rate of hydrolysis of sugar phosphates intracellularly.

Hypocalcemia is also an expected finding in ARF. In the absence of rhabdomyolysis, calcium concentrations usually exceed 6.5 mg/dL (327,329,335). In mild ARF, the serum calcium concentration usually is 8.5 mg/dL or higher (326). Profound hypocalcemia can be observed in rhabdomyolysis-associated ARF. Thus, eight of 35 patients with rhabdomyolysis and ARF had serum calcium concentrations of <6.5 mg/dL (233). The cause of the hypocalcemia of ARF is debated. Phosphate retention with calcium-phosphate deposition in soft tissues can cause some degree of hypocalcemia. However, in some studies, no relationship between hyperphosphatemia and hypocalcemia can be demonstrated, suggesting that additional mechanisms are operative (330). As noted previously, skeletal resistance to the calcemic effect of PTH is present in ARF.

Rarely, hypercalcemia complicates the course of ARF (243,359-361). Although this hypercalcemia usually occurs during the diuretic phase of rhabdomyolysis-induced ARF, it has been reported during the oliguric phase as well (359). The mechanism of this hypercalcemia is unclear. Both increased and decreased PTH levels are reported (243,359). Utilizing electron microscopy and technetium pyrophosphate scanning in four patients with rhabdomyolysis, Akmal et al. (359) demonstrated muscle tissue calcification that disappeared during recovery from ARF. Sperling (336) described one case of rhabdomyolysis in which plasma parathyroid hormone and 1,25-dihydroxyvitamin D levels were suppressed during the period of maximal hypercalcemia while in the recovery phase of ARF. A technetium pyrophosphate scan demonstrated extensive deposition of calcium throughout the pelvic and lower extremity muscles. Meroney et al. (362) administered radiolabeled calcium to dogs with experimental muscle trauma. They found that the isotope was deposited in injured skeletal muscle at a rate nine times that observed in control animals. As noted earlier, hypercalcemia occurring in the early polyuric phase of rhabdomyolysis-associated ARF is associated with increases in

1,25-dihydroxy vitamin D and PTH (233). Two studies demonstrated widespread tissue calcium deposition with calcium-related organ dysfunction in patients becoming hypercalcemic after rhabdomyolysis-associated ARF (243,349). These observations emphasize the importance of recognition and control of this complication of ARF.

Hypermagnesemia in the range of 2.5 to 4.0 mg/dL occurs frequently in ARF (326,327). This range of hypermagnesemia is almost always asymptomatic; however, striking hypermagnesemia can occur if magnesium-containing antacids are administered to patients with ARF.

High plasma aluminum concentrations are traditionally thought to occur exclusively in the setting of chronic renal failure. However, more recent reports demonstrate high plasma aluminum concentrations occurring in the setting of ATN (363,364). In the majority of these patients with ARF, the source of the aluminum appeared to be untreated water used for dialysis (363). Other potential sources include intravesicular alum therapy for hemorrhagic cystitis (364,365), oral feeding, and intravenous albumin (366).

Hematologic Status

Anemia is common with ARF. A recent study by Hales and associates (367) found anemia (hematocrit <35) in 91% of 56 patients with ARF. In this study, the degree of anemia correlated directly with the magnitude of elevation of BUN and was worse in oliguric than in nonoliguric patients (367). The causes of anemia in the ARF setting are multiple and include blood loss from surgical procedures/trauma, the gastrointestinal tract, and phlebotomy for laboratory testing. Contemporary reports establish that ARF may be associated with suppressed serum erythropoietin concentrations (334,368). For example, a report of 10 patients with ATN includes data on serial measurements (radioimmunoassay) of serum erythropoietin (368). Serum erythropoietin levels were very low in patients with ATN. Moreover, several weeks to months were required before serum erythropoietin levels returned to normal, and the normalization of serum erythropoietin lagged far behind the normalization of the GFR. In one patient with ATN, exogenous recombinant erythropoietin resulted in a brisk reticulocytosis and a progressive increase in hemoglobin, suggesting the lack of any endogenous inhibitor of erythropoiesis.

A number of disease states resulting in ARF are associated with significant abnormalities of the hemogram. Thus, ARF associated with a microangiopathic hemolytic process suggests a "vascular" form of ARF (Table 41-4). Eosinophilia suggests the possibility of allergic interstitial nephritis, polyarteritis nodosa, and atheroembolic disease (205,369–371). Anemia and rouleaux formation suggest a plasma cell dyscrasia. Leukopenia and thrombocytopenia often occur in systemic lupus erythematosus.

Coagulation disturbances occur frequently in ARF. Disseminated intravascular coagulation commonly accompanies ARF. Allgren found that of a large number of ARF patients (32% to 42%) were thrombocytopenic and 13% to 27% had coagulopathy (42). Acute renal failure associated with traumatic and atraumatic rhabdomyolysis is often accompanied by thrombocytopenia and disseminated intravascular coagulation (240). Cocaine-associated rhabdomyolysis appears to be associated with an especially high frequency of thrombocytopenia and coagulopathy (372). Preeclampsia and the HELLP syndrome may be associated in severe cases with ARF, thrombocytopenia and disseminated intravascular coagulation (373,374). The use of bioincompatible components in dialysis (intermittent or continuous therapies) may cause activation of platelets, plasmatic coagulation and fibrinolysis (375). Sepsis may cause activation of plasma coagulation, reduction of natural inhibitors of coagulation, defective fibrinolysis, and thrombocytopenia. Consequently septic patients with ARF may develop a "consumptive thrombohemorrhagic disorder" where the initial presentation is hemorrhagic, whilst the initial pathologic event is thrombotic (376). In one study, 19 of 47 patients with intravascular coagulation developed ARF concurrently with or shortly after onset of the coagulopathy (377). However, the absence of either cortical necrosis or microthrombi in the 11 kidneys examined histologically argues against a role for coagulopathy in directly affecting renal function in these patients. Studies in three patients with ARF owing to cardiogenic shock also suggest that intravascular coagulation can contribute to ARF (378). A falling platelet count and a rise in fibrinogen degradation products occurred simultaneously with onset of ARF. Increasing urine output and improved renal function occurred after heparin therapy.

A bleeding diathesis complicating the course of ARF occurred in 16% of the patients reported by Liano and associates (40). A functional defect in platelets leading to a prolongation in the bleeding time is seen in ARF. Thus, hemorrhagic complications contributing to hemostatic abnormalities are seen in 5% to 20% of patients with ARF (77). Bleeding was encountered in 15% of patients before and in an additional 27% of patients after development of ARF in one study (35). Cardiac bypass surgery affects hemostasis by decreasing coagulation factors, due to hemodilution and consumption (provoked by endothelial activation from trauma and artificial surface exposure) (376). Massive transfusion may cause hemostatic failure due to dilution of clotting factors, while storage of blood leads to platelet dysfunction and reduced clotting factors (376). Associated liver disease is often present in patients with ARF.

TREATMENT OF ACUTE RENAL FAILURE

Prevention

In view of the high morbidity and mortality of ARF, it is imperative that maximal effort be directed to prevention of this disorder. General preventative strategies are outlined in Tables 41-14 and 41-15. With regard to nephrotoxins, about 20% to 30% of all cases of ARF can be attributed to one or more nephrotoxic agents (1,37,38,41,55,85) (see Chapters 42–45, 47, 48). The clinical characteristics that place a patient in a high-risk category for nephrotoxin-induced ARF are well known and include the presence of underlying renal ischemia (e.g., volume depletion, congestive heart failure, or an edematous disorder), concomitant exposure to more than a single nephrotoxin, and exposure to high doses of the nephrotoxin. Other characteristics such as advanced age and underlying renal insufficiency may also be important. When good indications for use of a potential nephrotoxin are present in high-risk patients, reasonable guidelines for usage include implementation of the smallest possible dose, clear end points for stopping the drug, measurement and maintenance of normal blood levels (if available), avoidance of concomitant nephrotoxins, maintenance of euvolemia, and serial monitoring of serum creatinine concentration. In some cases, such as with once daily dosage of aminoglycosides (379) or lipid encapsulated amphotericin B (380), dosing and formulation modification may modestly reduce nephrotoxicity (381).

A contemporary study has nicely illustrated the potential value of a computerized surveillance system with electronic notification of clinicians to attenuate nephrotoxin-induced ARF (382). In this study, e-mail messages were sent to clinicians to notify them whenever mild increases in serum creatinine occurred in their patients who were receiving a potential nephrotoxin (382). This notification led to earlier discontinuation of

TABLE 41-14

PREVENTION OF ACUTE RENAL FAILURE

Avoidance of nephrotoxicity
Recognition of agents with nephrotoxic potential
Recognition of high-risk populations
Avoidance of concomitant use of more than one nephrotoxin
Consideration of alternative therapies
Use of smallest dose and briefest duration
Formulation/dosing modification
Monitoring of blood levels if available
Frequent measurement of renal function
Surveillance systems to alert clinicians to changes in renal function
Hydration
Minimization of nosocomial infection
Meticulous handwashing
Conservative use and rapid removal of intravascular and intravesicular catheters
Cautious use of antibiotics based on culture data with automatic stop orders to ensure periodic reassessment
Aspiration pneumonia precautions (elevate head of bed, attention to gastric residual volume, conservative use of sedatives/hypnotics)
Selected application of pharmacologic intervention
Extracellular fluid expansion
Maintenance of high urine flow
Maintenance of cardiac index and mean arterial pressure
Renal vasodilators
Intravenous albumin
Growth factors
Calcium channel blockers
Miscellaneous agents
Selected application of nonpharmacologic interventions
Preoperative optimization
Maintenance of high oxygen delivery
Minimization of artificial ventilation
Supranormal optimization of cardiovascular hemodynamics
Prophylactic hemofiltration

the offending agent than when clinicians were not notified. Earlier notification and cessation of the offending agent decreased the frequency of development of severe ARF from 7.5% to 3.4%. It is likely that a more powerful intervention (e.g., automatic stop order, clinician telephone notification) could have even earlier, more meaningful impact.

Recommendations to prevent ARF are not uniformly followed. Weisbord and associates (383) reviewed the medical records of "at risk" patients who underwent radiologic investigations using radiocontrast. They found that of 144 patients eligible for intravenous volume expansion, 16% failed to receive any intravenous fluids. NSAIDs and COX-2 inhibitors were prescribed for 8% of patients (383).

Volume Expansion

A large body of experimental data and clinical studies suggest numerous clinical settings in which volume expansion and maintenance of high urine flow rates can protect the kidney from development of ARF (Tables 41-14 and 41-15). Based on a comparison with historic controls, Eisenberg and associates (384) suggested that volume expansion is 100% effective in preventing contrast agent-induced ARF. Currently, nearly all clinicians empirically utilize some form of volume expansion before, during, and following contrast exposure if the patient's clinical condition permits such therapy (385,386) (see Chapter 45). However, there are no randomized, prospective trials that document effectiveness and compare the efficacy of any fluid administration regimens to "no therapy" (385). Similarly, early anecdotal clinical experience found that hydration decreased/prevented ARF that occurs occasionally in association with cisplatin (387,388). Currently, it is standard of care to hydrate all patients receiving cisplatin therapy if possible (387,388). As with contrast agents, there are no prospective randomized trials that either prove efficacy or hydration or compare various regimens. Amphotericin B often induces ARF (389–391). The nephrotoxicity associated with amphotericin B is directly related to cumulative dosage and worsened by concomitant diuretic administration (390). Although prospective, randomized trials are not available, case reports and anecdotal clinical experience strongly suggest that prophylactic volume expansion decreases the risk of amphotericin B-induced ARF (389–391). In patients with sepsis, the optimal form of volume replacement is unknown. Three meta-analyses have recently compared crystalloid versus colloid solutions: two showing no difference in mortality (392,393) and one showing that colloids increase mortality by 4% (394). A Cochrane review of albumin administration in systemically ill patients showed that albumin increased mortality in comparison to other intravenous solutions (395). In patients with severe sepsis, hydroxyethylstarch was associated with a higher risk of ARF than gelatin (396). Boldt and associates (397) found no difference between hydroxyethylstarch and albumin in a trial of volume replacement in critically ill patients. Finfer and colleagues (398) performed a multicenter randomized controlled trial of resuscitation fluids comparing albumin and saline in critically ill patients. There was no difference in 28-day mortality, organ failure, hospital stay, days on mechanical ventilation, or days on renal replacement therapy (398).

Another setting in which experimental (399–401) and retrospective clinical studies (229,232,236,240,243) suggest a role of volume expansion and maintenance of urine flow to potentially protect the kidneys from development of ARF is in pigmenturic states. In one study, seven individuals were given fluid resuscitation (1.5 to 3.0 L) during extrication and evacuation from crush injury (236). Subsequently, these patients were subjected to massive forced alkaline diuresis, and none developed ARF. The authors of this report have extensive experience dealing with crush injuries and felt that ARF would have been inevitable in these patients in the absence of forced alkaline diuresis. In another series of 21 patients admitted with nontraumatic rhabdomyolysis, 45% of patients responded to volume expansion with an increase in urine output (232). All patients who responded became nonoliguric, did not require dialysis, and survived. In patients who remained oliguric, 9% died, and all required dialysis. The timing of initiation of fluid resuscitation is also important. Gunal and colleagues (242) described a case series of 16 crush victims from the 2003 earthquake in Bingol, Turkey. Early and vigorous fluid resuscitation was performed as described by Better and Stein (402). Duration between rescue and initiation of fluids was significantly longer in the dialyzed victims as compared with nondialyzed ones (9.3 vs. 3.7 hours). To date, potential risks of forced alkaline diuresis in rhabdomyolysis, such as pulmonary edema, worsening muscle edema with neurovascular entrapment, and precipitation of calcium salts in damaged muscle, have not been observed. In view of controlled experimental data and the anecdotal human experience, it appears reasonable to attempt to maintain a carefully monitored diuresis in patients with myoglobinuria. Precise guidelines as to the magnitude of diuresis required to protect the kidneys are not available.

Deposition of relatively insoluble crystals within renal tubules with resulting tubular obstruction is another form of

TABLE 41-15

PHARMACOLOGIC AGENTS THAT HAVE BEEN ADVOCATED TO PREVENT/ATTENUATE CLINICAL ACUTE RENAL FAILURE IN SELECTED SETTINGS

	Prevention	Treatment	Reference
Renal vasodilators			
Dopamine[a]	Neutral	Neutral	(142,405,415,530, 533,534,536)
Fenoldopam	Beneficial (contrast nephropathy)	N/A	(425–428)
Atrial natriuretic peptides	Neutral	Neutral	(42,414,431,433, 434,542–547)
Sympathetic blocking agents			
Clonidine	Neutral	N/A	(178)
Calcium channel blockers	Neutral	N/A	(435–437,442,443)
Inotropic agents			
Dobutamine	Neutral	N/A	(444)
Dopexamine	Neutral	Neutral	(445–448,551)
Norepinephrine	N/A	Neutral	(536–538)
Vasopressin	N/A	Neutral	(539,540)
Diuretic agents			
Furosemide	Harmful	Neutral (beneficial as diuretic)	(406,459–461)
Mannitol	Neutral (beneficial in renal transplantation)	Neutral	(62,64,229,236,240, 242,295,411,413,414, 459,462–468,470, 472–476)
Growth factors			
Insulin-like growth factor	Neutral	Neutral	(479,480,548)
Growth hormone	N/A	Harmful	(549)
Thyroxine	N/A	Neutral	(550)
Theophylline	Beneficial (contrast nephropathy)	N/A	(412,481–484)
Albumin	Beneficial (hepatorenal syndrome)	N/A	(224)
Inflammation			
Endothelin antagonists	Harmful	N/A	(507)
Platelet activating factor	Neutral	N/A	(487–491)
Activated protein C	Beneficial (high APACHE score >25)	N/A	(485,486)
TNF blockade	Neutral	N/A	(493–495)
Nitric oxide synthase inhibitor	Harmful	N/A	(497)
Prostaglandin E1 antagonist	Neutral	N/A	(508)
Inhibitors of leukocyte adhesion	N/A	N/A	(498)
Tissue factor pathway inhibitor	N/A	N/A	(486)
Antithrombin	Neutral	N/A	(499)
Intensive insulin therapy	Beneficial	N/A	(518)
Steroid therapy	Neutral	N/A	(500,501)
N-acetylcysteine	Beneficial (contrast nephropathy)	N/A	(449–454)
Sodium bicarbonate	Beneficial (contrast nephropathy)	N/A	(477)

N/A, not applicable.
Beneficial = overall beneficial effect on renal function; harmful = overall harmful effect on renal function; neutral = no definite benefit or harm to renal function.
[a]See text for discussion of possible harmful effects.

ARF that may be preventable by maintenance of a high urine flow rate (103,104,111,296). Intratubular deposition of uric acid crystals has been clearly demonstrated to be prevented by maintaining a high rate of urine flow in the experimental setting (111,112). Although uric acid solubility is enhanced at an alkaline urinary pH, high urine flow appears to be significantly more protective against uric acid nephropathy than alkaline pH. Methotrexate, sulfadiazine, triamterene, indinavir, and other protease inhibitors, and acyclovir are therapeutic agents that, when administered in high doses, can occasionally be associated with ARF (104). Although the mechanisms of the ARF associated with these agents remain to be precisely defined, intratubular precipitation of insoluble parent drug or drug metabolite appears likely in many cases. Anecdotal experience suggests that maintenance of high urine flow can prevent the kidneys from the nephrotoxicity that occasionally follows high doses of these agents and is currently standard practice (104).

Several pharmacologic agents have been proposed to potentially protect the kidneys from ARF due to a variety of insults. Although much experimental data are available, few controlled clinical trials have evaluated the efficacy of the majority of these

agents (Tables 41-14 and 41-15). Two renal vasodilators—low-dose intravenous dopamine and intravenous atrial natriuretic peptide—have been tried as renoprotective agents in several clinical settings.

Dopamine

Dopamine, at doses of 0.5 to 5.0 μg/kg/minute, acts on two populations of dopamine receptors. In the kidney, this action usually results in vasodilation, natriuresis, diuresis, and perhaps an increase in GRF (403). Recently, dopamine has been demonstrated to antagonize the renal vasoconstrictor effects of norepinephrine in healthy men (403). Dopamine has a potential beneficial effect on splanchnic blood flow and oxygen consumption in patients with septic shock, provided the fractional splanchnic flow is not already high before treatment (404). To date, the data on use of dopamine to prevent ARF have generally been disappointing. Although dopamine appears capable of inducing a diuretic effect after major vascular surgery (405), it does not exert a clear-cut renoprotective effect in the settings of cardiac surgery (406,407), elective major vascular surgery (408), or oliguric patients with septic shock (323,409).

It is in the area of prevention of radiocontrast-associated ARF in which most data on dopamine prophylaxis have been obtained. In one study of high-risk patients (those with chronic renal insufficiency, 40% of whom had diabetes), dopamine appeared to exert a renoprotective effect in patients with a serum creatinine $\leq$2.0 mg/dL (410). Similar findings were reported by Hall and associates in patients with chronic renal insufficiency (411). By contrast, dopamine did not protect high-risk patients (mean age 74 years, mean serum creatinine 1.9 mg/dL, 60% diabetic patients) undergoing coronary angioplasty (412). Stevens and associates found no benefit in prevention of ARF by forced diuresis with mannitol, dopamine, and intravenous saline in patients (average serum creatinine 2.5 mg/dL) undergoing angiography (413). Weisberg found that prophylactic dopamine increased the rate of ARF in diabetic patients but perhaps protected nondiabetic patients undergoing coronary angioplasty (414). In summary, although suggestive, the current data do not clearly establish a renoprotective effect of dopamine to prevent contrast-associated ARF.

In a meta-analysis of randomized controlled trials using dopamine in prevention of ARF, Kellum and Decker examined 58 studies (N = 2,149), of which 24 were randomized controlled trials (N = 1,019) and 5 involved radiocontrast dye (N = 451). There was no benefit in favor of dopamine in terms of mortality, incidence of ARF, or requirement for hemodialysis (415).

Dopamine is not without side effects. Dopamine administration requires venous cannulation and local extravasation of dopamine adjacent to an artery may provoke distal ischemia and gangrene (416). Dopamine can depress respiratory drive and may increase cardiac output and myocardial oxygen consumption (even at "renal-doses"), and trigger tachyarrhythmias and myocardial ischemia (417,418). Dopamine may potentially induce or exacerbate hypovolemia and prerenal ARF through its natriuretic effects and trigger hypokalemia and hypophosphatemia. In a porcine model of hemorrhagic shock (419) low-dose dopamine hastened the onset of gut ischemia. The latter complication appeared due to shunting of blood away from the bowel mucosa rather than an absolute reduction in mesenteric blood flow. Dopamine may worsen pituitary dysfunction in critical illness (420). The effect of dopamine wears off after 48 hours, suggesting tolerance of dopaminergic receptors (421,422). There is evidence that in normal subjects, dopamine has unpredictable pharmacokinetic properties and so administration based on body weight may not give a predictable response (423).

Fenoldopam

Fenoldopam mesylate is a dopamine agonist that holds promise for prevention of ARF, particularly in the setting of radiocontrast nephropathy. Fenoldopam blocks reduction in renal plasma flow after radiocontrast (424). One prospective trial and three retrospective trials of fenoldopam in radiocontrast nephropathy support its efficacy (425–428). In elderly patients with severe vascular disease undergoing aortic aneurysmal repair, the use of fenoldopam in an open label uncontrolled trial was associated with a relatively rapid return of renal function to baseline values, despite profound decreases during aortic cross-clamping (429). A study of patients undergoing elective repair of a thoracoabdominal aortic aneurysm found that the use of fenoldopam was associated with reductions in mortality, dialysis requirements, and lengths of stay in the hospital and intensive care unit (430).

Atrial Natriuretic Peptide

Another vasodilator, atrial natriuretic peptide (ANP), or anaritide, has also been studied as a renoprotective agent. Like dopamine, ANP improves renal blood flow and GFR (431) and has also been shown to improve patient hemodynamics during cardiopulmonary bypass (432). Early studies provided no convincing effects for a renoprotective effect of atrial natriuretic peptide and perhaps some worsening of renal function in selected settings (414,433,434). Also an atrial natriuretic peptide did not appear to exert a renoprotective effect after cardiac transplantation in a small study (188).

Clonidine

Several clinical settings are associated with enhanced activity of the sympathetic nervous system. One such setting is cardiac surgery (178,179). A randomized controlled study found that clonidine reduced sympathetic activity and better preserved creatinine clearance than placebo in the immediate postcardiac surgery period (178). No data on the effect of inhibitors of sympathetic activity on the occurrence of ARF are available, to our knowledge.

Calcium Channel Blockers

A wealth of experimental data suggests a renoprotective effect of calcium channel blockers. However, limited clinical data are available. Two prospective studies found that either 20 mg of oral nitrendipine or 10 mg of sublingual nifedipine prevented mild decreases in GFR and renal blood flow after radiocontrast exposure in normal subjects compared with untreated controls (435,436). In a large study, no significant change in serum creatinine was seen in either control or nifedipine treated (20 mg) patients exposed to nonionic contrast (437). The intravenous administration of diltiazem was shown to attenuate the temporary decrease in creatinine clearance that occurs immediately following cardiopulmonary bypass (179). In patients who have undergone cardiac surgery, use of diltiazem postoperatively increases urine output and preserves markers of renal tubular integrity such as urinary alpha-glutathion *s*-transferase, alpha-1-microglobulin, and *N*-acetyl-*ß*-glucosaminidase (438). The addition of intrarenal gallopamil (a potent calcium entry blocking agent) to intravenous furosemide treatment enhanced the recovery of renal function after acute renal failure (439). Fewer patients develop acute tubular necrosis after kidney transplantation when treated with oral (440,441) or intravenous (442) diltiazem, however, graft function, rate of rejection, and graft loss were unchanged (443). These modest human data are inadequate to suggest a potential protective role for calcium channel blockers in clinical settings.

Dobutamine

In many critically ill patients at risk for renal failure, impaired cardiac index is present (23,56,124). Some of these patients are treated with β-adrenergic agonists such as dobutamine (444). In one comparative study of intensive care unit patients, dopamine induced a diuresis without any change in cardiac index or creatinine clearance, whereas in the same patients, dobutamine increased cardiac index and creatinine clearance without affecting urine output (444). To date there are no data on dobutamine relative to preventing ARF.

Dopexamine

Dopexamine is a dopamine-1 and less potent dopamine-2 agonist. It is also a $\beta 2$ agonist which acts on peripheral vessels and inhibits neuronal reuptake of norepinephrine. It reduces peripheral vascular resistance and increases cardiac output. Dopexamine does not protect against the development of ARF after abdominal surgery (445) or in the setting of cardiopulmonary bypass (446). A marginal benefit was seen in a small trial in patients undergoing aortic surgery (447). No benefit in renal vascular resistive index was seen in patients after coronary bypass surgery (448).

N-acetylcysteine

N-acetylcysteine has recently been advocated as a renoprotective agent, particularly in the setting of radiocontrast nephropathy (see Chapter 45). Tepel and associates (449) randomized 83 patients who underwent CT scanning with IV iopromide to receive *N*-acetylcysteine (600 mg twice daily) on the day prior to and day of the procedure, or placebo. He found a 90% reduction in radiocontrast nephropathy (defined as number of patients with 0.5 mg/dL rise in serum creatinine 48 hours postcontrast). Since then, several meta-analyses (450–454) have shown that *N*-acetylcysteine reduces the risk of radiocontrast nephropathy by about 50%. These meta-analyses were flawed by the heterogeneity of trials and variable definitions of what is "radiocontrast nephropathy." Those trials that showed lack of benefit of *N*-acetylcysteine in prevention of radiocontrast nephropathy may have done so for several reasons, including (a) negative results occurred in trials whose control group had less contrast nephropathy; (b) American studies which were negative used the liquid formulation which may be less efficacious; (c) in some negative studies acetylcysteine was not administered on the day prior to intervention—such a premedication may be required to induce sulfhydryl formation (451); (d) atheroemboli may be a more common cause than contrast nephropathy of ARF leading to dialysis following coronary angiography (455); (e) *N*-acetylcysteine is less effective in patients with moderate and severe renal impairment as shown in one study (456). The mechanism of action of *N*-acetylcysteine is unknown, but may include antioxidant effects and blockade of renal vasoconstriction (457). *N*-acetylcysteine may reduce serum creatinine (by increasing tubular secretion and/or enhancing metabolism) independent of its effect on prevention of radiocontrast nephropathy (458).

Furosemide

Diuretic agents such as furosemide and mannitol have long been used as possible renoprotective agents in high-risk settings for ARF. Recent controlled studies suggest that prophylactic furosemide exerts a deleterious effect on GFR in the setting of cardiac surgery (406). When administered in the setting of radiocontrast exposure, furosemide appears detrimental, even when care is taken to avoid concurrent extracellular fluid volume depletion (459–461). Furosemide is not effective in prevention of ATN due to cephalosporins and aminoglycosides (461).

Mannitol

Mannitol has long been utilized in clinical studies as a renoprotective agent (295,462–468). Early anecdotal experience suggested efficacy in several postoperative and other settings. For example, retrospective clinical experience suggests a beneficial effect of mannitol in some patients with rhabdomyolysis-associated ARF, although a randomized trial-documenting efficacy is not available (64,229,236,240,242,469). Mannitol has also been used in the setting of nephrotoxin exposure, including exposure to cisplatin, amphotericin B, and radiocontrast agents (414,464,466). With regard to amphotericin B, the only prospective randomized trial included 11 patients, and no benefit of mannitol was seen (468). With regard to radiocontrast exposure, mannitol appeared to increase the frequency of contrast nephropathy in one study and in diabetics with renal insufficiency in another study (411,459). In renal insufficiency in nondiabetics, mannitol may provide some renoprotective activity (414). Forced diuresis using a combination of mannitol, furosemide, and dopamine did not provide protection against radiocontrast nephropathy (413). In obstructive jaundice, mannitol does not protect against ARF (470,471). Mannitol does not reduce ARF but may reduce subclinical renal injury in individuals undergoing aortic aneurysm surgery (472). Mannitol in combination with moderate hydration administered prior to cross-clamp in cadaveric renal transplantation improved graft function postoperatively (473–476). In summary, clear-cut data supporting the widespread use of furosemide or mannitol as effective agents to prevent ARF are not available. Anecdotal observations suggest beneficial effects of mannitol in the setting of rhabdomyolysis. Mannitol is reported to cause ARF by inducing renal vasoconstriction and inducing vacuolar damage in renal tubular epithelial cells (62).

Sodium Bicarbonate

Intravenous sodium bicarbonate has been shown to be superior to sodium chloride in reducing the risk of radiocontrast nephropathy in a randomized controlled study of 119 patients with serum creatinine over 1.1 mg/dL (477). The postulated mechanism is alkalinization of urine within the renal medulla which inhibits contrast-induced free radical formation. Following the trial, a registry of 191 individuals receiving sodium bicarbonate for radiocontrast procedures demonstrated an incidence of radiocontrast nephropathy of 1.6% (477).

Growth Factors

Growth factors are among the most recent substances proposed to exert a protective influence to prevent ARF (478,479). Insulin-like growth factor was compared to placebo in 58 patients undergoing surgery of either the suprarenal aorta or the renal arteries (479). At 72 hours postoperatively, placebo-treated patients had decreased their creatinine clearance by 5 mL/minute, whereas growth factor-treated patients had increased their creatinine clearance by 8 mL/minute ($p < 0.05$). However, there were no differences in discharge serum creatinine or length of stay when treated and untreated patients were compared. In another study, 43 recipients of cadaveric renal allografts were randomized to receive subcutaneous insulin-like growth factor or placebo (480). Inulin clearance on day 7, nadir serum creatinines after 6 weeks, and need for dialysis did not differ between the two groups. Clearly more study is needed to ascertain if growth factors can act as significant renoprotective agents.

Theophylline

Theophylline has been suggested to protect against development of radiocontrast-associated ARF (412,481–483). Some of these studies suggest that theophylline attenuates the

nephrotoxic potential of radiocontrast agents; however, the effect appears to be modest in degree (1,88,481–483). Joachim Ix and colleagues (484) performed a meta-analysis of seven randomized controlled trials of theophylline and aminophylline in patients at risk of radiocontrast nephropathy (pooled sample size $N = 480$). The difference in mean change in serum creatinine was 11.5 μmol/L (95% confidence intervals 5.3–19.4 μmol/L, $p = 0.004$) lower in the theophylline- or aminophylline-treated groups than controls. One participant (0.6%) required dialysis.

Activated Protein C and Other Modulators of Sepsis

A large body of clinical and experimental data suggests that ARF in septic patients is driven by an intense inflammatory process, involving numerous regulatory mediators. Activated protein C (drotrecogin) has been shown in a large randomized controlled study of 1690 patients with sepsis and organ failure (485) to reduce 28-day mortality from 30.8% to 24.7%. Despite FDA approval, questions about study design have prevented universal acceptance of activated protein C as a therapy for patients with sepsis and organ failure (486). Inhibitors of platelet activating factor may decrease organ dysfunction in septic patients, decrease the need for dialysis (487) but do not significantly improve 28 day mortality (487–490). A large phase III trial was prematurely discontinued after interim data from >1250 patients failed to demonstrate improved 28-day all-cause mortality, the primary endpoint of the trial (491). In patients with septic shock, an elevated level of soluble tumor necrosis factor (TNF) receptors was shown to be an independent predictor for the development of ARF and death (492). However, TNF blockade had minimal benefit on mortality in septic patients (493) except in those whose serum interleukin-6 concentrations were elevated (494,495). The intricate relations between endothelial and epithelial cells, based in part on the relations between endothelial and inducible nitric oxide synthases, are perturbed in renal ischemia (496). An inhibitor of nitric oxide synthase was shown to increase mortality in a randomized controlled trial of 797 critically ill patients with septic shock (497). No clinical studies have yet been undertaken of inhibitors of leukocyte adhesion or endothelin (498). High-dose antithrombin III therapy had no effect on 28-day all-cause mortality in adult patients with severe sepsis and septic shock and did not reduce the risk of acute renal failure (499).

Steroid Therapy

Adrenal corticosteroid production is an important protective response during critical illness. Subnormal production of adrenal corticosteroids may result in hemodynamic instability and ongoing evidence of inflammation without an obvious source and lack of response to empirical therapy (498). The use of physiologic doses of steroids in critically ill patients, particularly those with adrenal hyporesponsiveness, has been shown to reduce 28-day mortality (500). However supranormal doses of corticosteroids may be harmful (501).

Minimizing Time of Mechanical Ventilation

Duration of time on mechanical ventilation is associated with increased mortality and renal failure in critically unwell patients (502). Daily interruption of sedation may reduce time on mechanical ventilation (503). Lower tidal volume on ventilation was shown to reduce mortality (504). Mechanical ventilation is associated with the disruption of pulmonary epithelium and endothelium, lung inflammation, atelectasis, hypoxemia, and the release of inflammatory mediators (504). These inflammatory mediators can cause injury to lung and other organs (504). In a rabbit model of acute respiratory distress syndrome, Imai (505) showed that low tidal volume ventilation reduced injurious epithelial cell apoptosis in the kidney *in vitro*, possibly due to a inhibition of Fas ligand.

Endothelin Antagonists

Endothelin antagonists can prevent experimental ARF (506), and also can block intrarenal vasoconstriction (507). However, the endothelin antagonist SB209670 exacerbated contrast nephropathy in a clinical trial comparing it against placebo in patients receiving 0.45% saline before and after radiocontrast (507).

Prostaglandin E1

Prostaglandin E1 (PGE1) has been studied in a phase I trial of 130 patients with chronic renal impairment undergoing radiocontrast administration (508). The parenteral administration of PGE1 immediately before radiocontrast exposure and continued for a period of 5 to 5.5 hours significantly reduced the elevation of serum creatinine poststudy. The most effective of the three PGE1 dosing regimens tested was 20 ng/kg/minute (508).

Albumin

Impairment of renal function occurs commonly in patients with cirrhosis and spontaneous bacterial peritonitis. In a prospective controlled trial, intravenous albumin (1.5 g/kg at diagnosis followed by 1 g/kg on day 3) decreased the frequency of ARF (defined as a 50% greater increase in pretreatment BUN or serum creatinine to levels >30 and 1.5 mg/dL, respectively) from 33% to 10% ($p = 0.002$) (224).

Preoperative Optimization

With regard to prevention of ARF in a postoperative setting, older studies suggested that "preoperative optimization" could reduce the frequency of ARF in selected high-risk settings (169,509). For example, Berlauk and colleagues (509) have provided data that preoperative "optimization" of cardiovascular hemodynamics—guided by Swan-Ganz measurements—can be helpful in selected patients undergoing limb-salvage arterial surgery. In this randomized prospective study, patients who were "optimized" had less mortality (1.5% vs. 9.5%), graft loss (2.9% vs. 19.0%), and ARF (1.5% vs. 4.8%). Gattinoni and associates found that volume expansion to supranormal cardiac indices and normal mixed venous oxygen saturation had no effect on mortality or incidence/severity of ARF (510). Early institution of treatment to increase central venous oxygen saturation to greater than 70% resulted in lower mortality and less severe organ dysfunction in patients with severe sepsis or septic shock (patients had a mean serum Cr 260 μmol/L) (511). Other retrospective studies suggest similar results in the setting of abdominal aortic aneurysm surgery (169). Confirmation of these results, with delineation of patient populations and optimization regimens of benefit, is needed.

Perfusion with Ringer's Lactate

In a study of patients undergoing thoracoabdominal aorta repair, perfusion of the kidneys with cold Ringer's lactate solution was associated with less renal failure (512).

Improvement of Cardiac Index

With regard to critically ill patients, it has been proposed that the combination of inadequate oxygen delivery and increased tissue oxygen need potentially results in generalized organ ischemia and multiple organ failure. Consequently, in selected patients, fluid volume and pharmacologic therapy manipulated to increase cardiac index and the delivery and consumption of oxygen to "supranormal levels" (cardiac index

>4.5 L/minute/m^2 body surface area; oxygen delivery >600 mg/minute/m^2) is sometimes performed. These supranormal levels are viewed by some as whole-body overcompensation necessary to survive severe critical illness. Does this therapeutic strategy of achievement and maintenance of supranormal cardiac index and oxygen delivery improve outcome and protect the kidney? Five prospective randomized trials have been undertaken to test this hypothesis in heterogeneous groups of seriously ill patients (513–517). Improved survival is seen in some but not all studies. In one study, this therapeutic approach significantly decreased survival (517). Similar results are seen with regard to ARF. It should be noted that the sample sizes of most of these studies are relatively small. Moreover, these catastrophically ill patients often represent an extremely heterogeneous patient population, rendering precise matching of control and experimental subjects difficult. However, to date, compelling evidence that maintaining supranormal values of hemodynamic and oxygen delivery parameters in all cases of catastrophic illness will improve survival and protect the kidneys is not available.

Intensive Glycemic Control

Insulin therapy with aggressive glycemic control has been shown in intensive care patients to reduce mortality and decrease (by 41%) the incidence of ARF requiring dialysis (518). The mechanism of action is not just due to its hypoglycemic effect. Insulin is antiapoptotic and antiinflammatory (519), may reduce the oxidative stress due to hyperglycemia (520), is mitogenic (521) and normalizes lipids (522).

Prophylactic Dialysis

An area of controversy is the use of prophylactic dialysis to prevent ARF. Marenzi and colleagues (523) studied 114 patients with chronic renal failure and a serum creatinine greater than 2 mg/dL who were to undergo coronary angiography. These individuals were randomized to either hemofiltration plus saline hydration or saline hydration alone. Hemofiltration was carried out in an ICU, commenced 4 to 6 hours prior to, stopped during, and continued for 18 to 24 hours after the angiographic procedure. Temporary renal replacement therapy (hemodialysis or hemofiltration) was required in 25% of the control patients and in 3% of the patients in the hemofiltration group. The rate of in-hospital events was 9% in the hemofiltration group and 52% in the control group ($p<0.001$). In-hospital mortality was 2% in the hemofiltration group and 14% in the control group ($p = 0.02$), and the cumulative 1-year mortality was 10% and 30%, respectively ($p = 0.01$). The benefit of dialysis may be due to the ability of the physician to give adequate hydration without fear of volume overload as well as the ability of dialysis to remove radiocontrast after angiography. However, it is not possible based on this study to determine if the benefit of hemofiltration was due to the dialysis procedure, the intensity of medical and nursing care or the use of anticoagulation. Its invasiveness and cost prevents this strategy from being used more widely in the prevention of radiocontrast nephropathy, at least until further studies are done.

Conservative Management

An approach to the overall management of the patient with ARF is presented in Table 41-16. Of primary importance is the exclusion of potentially treatable prerenal, postrenal, and renal parenchymal causes of ARF that may be amenable to specific therapeutic interventions. Once specifically treatable conditions are excluded, it is important to achieve a euvolemic state and to correct any abnormalities in cardiac index that are present. If the patient remains oliguric despite a euvolemic state, it has become common clinical practice to attempt to make the patient nonoliguric by administration of potent diuretic agents and/or renal vasodilators (524,525). The rationale for such therapy is the markedly lower morbidity associated with nonoliguric relative to oliguric ARF (38). Moreover, nonoliguric patients have higher GFRs and fewer biochemical and clinical complications than oliguric patients (38).

TABLE 41-16

CONSERVATIVE TREATMENT OF ACUTE RENAL FAILURE

1. Exclude reversible/treatable causes of acute renal failure.
2. Obtain and maintain euvolemic state.
3. Attempt to establish a urine output if patient remains oliguric.
4. Provide adequate nutrition.
5. Minimize use of invasive lines and procedures.
6. Monitor drug usage carefully, and modify dosage or dosing interval appropriately.
7. Monitor and treat for clinical and biochemical complications.
8. Institute renal replacement therapy when appropriate.

Diuretics and Renal Vasodilators

Substantial clinical experience allows several generalizations regarding diuretic/vasodilator therapy of patients who continue to be oliguric despite correction of prerenal and postrenal factors and exclusion of treatable renal parenchymal disorders. First, the greatest likelihood of success for converting an oliguric to a nonoliguric state occurs when the duration of oliguria is brief. Prospective randomized trials in oliguric patients in which furosemide was given late in the course of ARF demonstrate no benefit (525–527). In an analysis of 552 critically ill individuals, Mehta and colleagues found that those who were unresponsive to furosemide had a worse prognosis (528). Clinical observations clearly suggest that urinary chemical indices demonstrating more intact tubular function (i.e., lower spot urinary sodium concentration and FE_{Na}, and higher U/P osmolality) as well a briefer duration of oliguria predict a favorable response to furosemide (38,142,294,295). Second, the route of administration of the diuretic may be an important consideration because seriously ill patients with diminished renal function appear to respond more readily to a continuous infusion rather than a bolus of a loop diuretic (529). Third, a small controlled study and anecdotal experience suggest that dopamine may be synergistic with loop diuretics in converting an oliguric to a nonoliguric state (142,530). Although substantial controversy surrounds the issue as to whether dopamine exerts any beneficial effect in oliguric states (531,532), two recent studies clearly document that dopamine can increase urine flow in critically ill, oliguric patients (405,533). For example, Flancbaum and associates (533) used oliguric surgical intensive care unit patients as their own control and could clearly document a diuretic response to low-dose intravenous dopamine, which was reversible after cessation of dopamine. The combination of mannitol, furosemide, and dopamine has been shown to reduce the need for short term dialysis in patients with ARF after cardiac surgery (534). Finally, the complication rate of low-dose dopamine and continuous infusion of a loop diuretic appears to be low. Taken together, although no prospective, randomized controlled data document clinical outcome benefit, we continue to utilize a trial of a loop diuretic occasionally with low-dose dopamine in selected oliguric patients in whom

obstructive uropathy has been excluded and prerenal factors corrected.

Inotropic Support

Many experimental and clinical data suggest a beneficial effect of norepinephrine on the urine output in sepsis. A beneficial effect on renal function (glomerular filtration) is a less consistent finding suggesting that blood pressure may be partially responsible for inducing a diuresis (535). It has yet to be demonstrated convincingly that therapeutic doses of norepinephrine compromise renal function in human shock (536) or that dopamine is renoprotective in norepinephrine-treated patients (421,536). Some studies suggest that in patients with septic shock, norepinephrine induces a diuresis more effectively than other vasoactive drugs such as dopamine (537,538). In critically ill septic individuals unresponsive to catecholamines, an infusion of vasopressin may increase urine output, creatinine clearance, blood pressure, and systemic vascular resistance (539,540).

Atrial Natriuretic Peptide

In addition to loop diuretics and dopamine, other renal vasoactive agents have also been used in oliguric patients in an attempt to attenuate ARF. In this regard, experimental and small clinical trials suggested the potential utility of atrial natriuretic peptide (541,542). Rahman and associates (542), in a prospective randomized trial of 53 ARF patients, found that intravenous atrial natriuretic peptide and diuretics, when compared with diuretics alone, decreased the need for dialysis (23% vs. 52%, $p < 0.05$) and reduced mortality (17% vs. 35%, $p = 0.11$). Unfortunately, two large-scale multicenter studies failed to find benefit from intravenous infusion of atrial natriuretic peptides in established oliguric and nonoliguric ARF (42,543). Allgren and colleagues (42) examined 504 critically ill patients with ATN. They found that a 24 hour infusion of ANP (0.2 μg/kg/min) did not improve overall dialysis-free survival but did provide benefit in nonoliguric patients. Lewis (544) found that in 222 oliguric ARF patients there was a trend toward higher dialysis-free survival in patients on ANP. Sward (545) randomized 61 patients with nonoliguric ARF after cardiac surgery to ANP (lower dose 0.05 μg/kg/minute) or placebo. She found that ANP was associated with improved dialysis-free survival. Despite randomization, the placebo group had more diabetics ($N = 6$) than the ANP group ($N = 0$). Ularitide is a similar agent to ANP that showed no benefit in patients with ARF after abdominal surgery (546) but was beneficial in reducing the need for dialysis in a randomized study of 14 patients with ARF following cardiac surgery (547). One large-scale multicenter study failed to find benefit from intravenous infusion of ularitide in established oliguric and nonoliguric ARF (543). In summary, there is no consistent evidence for a beneficial role of atrial natriuretic peptides in the treatment of ARF.

Other Pharmacologic Agents

Insulin-like growth factor 1 has also been used in the setting of established ARF (548); however, a multicenter, randomized controlled trial found no benefit of this therapy (548). In patients with prolonged critical illness, high doses of growth hormone were associated with increased morbidity and mortality (549). Likewise, the thyroid hormone, thyroxine, has recently been demonstrated to be of no benefit in treating human ARF (550). Dopexamine (a dopamine agonist) did not improve creatinine clearance in critically ill patients (551).

Nutrition

Provision of adequate nutrition is another important therapeutic goal in the management of patients with ARF (552–556). The catabolic stress of infection and injury often seen in patients with ARF fosters the development of malnutrition with its potential consequences of immunosuppression, infection, poor wound healing, and skeletal muscle weakness (554). The goals of nutritional support in the setting of ARF include restoration of metabolic homeostasis with maintenance of fluid, electrolyte, and acid–base balance; preservation of lean body mass; maximization of protein synthesis; and prevention of vitamin, mineral, and trace element depletion. Obviously, accomplishment of these goals in critically ill, oliguric ARF patients represents a significant clinical challenge. Specific details on guidelines of nutritional assessment, calculation of protein and energy requirements, the products available, and means of administration of these products to meet the protein and energy requirements as well as the standards of care for monitoring nutritional therapy are beyond the scope of this chapter and can be found in readily available sources (552–556).

Monitoring Medication Use

Another aspect of the medical management of ARF that demands attention is careful monitoring of medication and drug usage. Nearly all pharmacologic agents are eliminated, at least in part, by the kidneys. Moreover, the presence of renal failure may impair hepatic metabolism and excretion of selected pharmacologic agents. Also, the consequences of the ARF state may potentiate end-organ responses to several pharmacologic agents, resulting in enhanced activity and possibly toxicity. Finally, the administration of some pharmacologic agents results in concomitant administration of "metabolic loads" (e.g., sodium and potassium) that are often poorly eliminated in patients with ARF. For these reasons, frequent, continuing scrutiny of the medication list is mandatory in all patients with ARF.

Complications of ARF

Clinical and laboratory monitoring are needed to detect the multiple potential complications of ARF. The frequency of this monitoring depends on several factors including the type and severity of underlying illness, whether the patient is oliguric, whether the patient is catabolic, and associated medical conditions.

Three of the most important complications of ARF are volume overload, hyperkalemia, and infection. All of these complications are associated with morbidity and are potentially fatal. All are preventable and/or treatable. With regard to volume overload, daily weight, intake and output, vital signs, oximetry, clinical assessments, and sometimes invasive monitoring are often necessary to evaluate extracellular fluid volume status and cardiac output. Occasional chest x-rays may also help. The most feared consequence of volume overload, pulmonary edema, is particularly difficult to manage in patients with ARF, especially if the patient is oliguric. Two studies emphasize the potential adverse consequences of volume overload in the postoperative setting (557,558). In 21 consecutive cases of postoperative ARF, Murkau and Latimer (557) found that 90% of these patients were grossly fluid overloaded at the time of institution of dialysis by an average of 10 to 11L. A study of Lowell and associates (558) suggested that volume overload per se is an important contributor to postoperative morbidity and mortality. In this study, 40% of 48 consecutive postoperative intensive care unit patients gained more than 10% body weight. Mortality in this group of patients was 32% versus 10% in those gaining <10% body weight. As noted previously, renal response to diuretic agents is markedly impaired in ARF, and the presence of volume overload and pulmonary edema often requires institution of either renal replacement or ultrafiltration therapy (557,558).

TABLE 41-17

TREATMENT OF HYPERKALEMIA IN ACUTE RENAL FAILURE

Modality	Onset	Duration	Mechanism
Calcium (10 mL of 10% calcium gluconate IV, 1–3 doses)	Immediate	30–60 min	Increases threshold potential
Sodium bicarbonate (1–2 ampoules; 45–90 mEq)	5–10 min	1–2 hr	Intracellular shift of potassium
Glucose–insulin (25–50 mL of 50% glucose with 5–10 U of insulin administered as intravenous push)	2–4 hr	30–60 min	Intracellular shift of potassium
Potassium exchange resin (25–50 g of kayexalate plus 70% sorbitol; 20–30 mL orally or 50–100 mL rectal enema)	1–4 hr	Few hours	Removal of potassium
Hemodialysis	2–3 hr	Several hours	Removal of potassium

Hyperkalemia is a frequent accompaniment of ARF and is due to continued potassium release from tissue with decreased renal potassium excretion. In some cases, such as in the presence of rhabdomyolysis, tumor chemotherapy, or extensive hemolysis, the endogenous potassium load results in profound hyperkalemia. Sometimes, exogenous potassium loads such as those occurring with inadvertent administration of potassium intravenously or blood transfusions contribute to hyperkalemia. Hyperkalemia exerts its deleterious effect by raising the resting electrical potential of excitable tissue toward the threshold potential. As the resting potential nears the threshold potential, there is danger of repolarization block with lack of electrical activity and cardiac arrest with asystole. The electrical effects of hyperkalemia are dependent on the rate and magnitude of rise in serum potassium concentration. Abrupt (only a few hours) increases to concentrations of 6.0 mEq/L or greater are often associated with electrocardiographic (ECG) and clinical manifestations, whereas slower (over several days) increases to this level may not be associated with either ECG or clinical findings. The ECG manifestations of hyperkalemia include a symmetrical increase in amplitude with peaking of the T waves (often seen only in leads V2 to V3), a decrease in P waves, widening of the QRS complex, prolongation of the PR interval, and development of left-axis deviation and left bundle branch block. Ultimately, a sine wave pattern and cardiac arrest occur. Rarely, paresthesias, muscular weakness, and flaccid paralysis can be seen with hyperkalemia.

Treatment of hyperkalemia (Table 41-17) is needed whenever ECG changes are present. The presence of neuromuscular signs and symptoms also mandates therapy. Some form of therapy should also be considered when the serum potassium exceeds 5.5 to 6.0 mEq/L. Available therapies with their mode of action, speed of onset, and duration are presented in Table 41-17.

As noted previously, infectious complications commonly complicate the course of patients with ARF and are a frequent cause of death. Minimization of use and duration of invasive catheters is important in an attempt to decrease nosocomial infection. Not only are infections common complications, but also they may be especially difficult to detect in the ARF setting. For example, renal failure can dampen the febrile response to bacteremia (559). Moreover, the leukocytosis that often accompanies an acute bacterial infection may be absent in patients undergoing dialysis (560). A high index of suspicion, aggressive culturing, and early use of specific antimicrobial agents in appropriately modified doses should be the standard of care.

A bleeding tendency can complicate the course of some patients with ARF (40,42,561). This coagulopathy rarely results in spontaneous hemorrhage but can be of potential importance in the setting of operative and invasive procedures. Therapeutic options for the treatment of ARF-related bleeding are outlined in Table 41-18. Intravenous desmopressin rapidly shortens the bleeding time in renal failure. However, its duration of action is brief, and repeated doses lose effectiveness. Cryoprecipitate can shorten the bleeding time for 12 to 24 hours. Conjugated estrogens require 12 to 24 hours to exert their effect to reduce bleeding time in chronic renal failure. Estrogens have a long

TABLE 41-18

TREATMENT OPTIONS FOR HEMORRHAGIC DIATHESIS IN ACUTE RENAL FAILURE

Treatment	Onset	Duration	Comment
Desmopressin (0.3 U/kg body weight IV over 30 min)	1–4 hr	<8–12 hr	Tachyphylaxis with repeated doses
Cryoprecipitate (10 U IV over 10–30 min)	1–4 hr	12–24 hr	Possible risk for hepatitis
Conjugated estrogens (0.6 mg/kg body weight IV over 40 min)	12–24 hr	Several days with repetitive doses	Not well studied in acute renal failure
Elevation of hematocrit (packed RBC sufficient to elevate hematocrit to >30%–35%)	Few hours	Few days	Complications of transfusion

RBC, red blood cells.

duration of action but have not been studied in the setting of ARF. Elevation of the hematocrit to >35% can shorten the bleeding time in ARF.

The anemia of ARF is managed initially by excluding and treating reversible causes (for example hemolysis and blood loss). The anemia of ARF is usually modest and rarely symptomatic. Thus, therapy is reserved for either symptomatic patients (e.g., angina) or for large reductions in hematocrit/hemoglobin, which can usually be attributed to other conditions such as gastrointestinal blood loss. Blood transfusion is indicated in severe cases of anemia. Hebert and colleagues showed that a restrictive policy in transfusing patients if the hemoglobin was less than 7 g/dL rather than 10 g/dL was not associated with increased mortality and was probably superior (562). Recombinant human erythropoietin is effective at reducing transfusion requirements in critically ill patients not requiring dialysis (563). No studies specifically in ARF have been performed (564).

With regard to other biochemical complications of ARF, the development of hyponatremia usually implies administration of excess water, whereas hypobicarbonatemia that can be attributed to renal failure per se rarely requires therapy. In fact, rapid alkanization can, by decreasing ionized calcium, precipitate enhanced neuromuscular activity with carpopedal spasm, cramps, stridor, and seizures.

Usually the mild hypocalcemia that accompanies ARF remains asymptomatic and does not require therapy. The hypercalcemia that rarely occurs in the ARF setting can usually be best managed by dialysis using either a very low or calcium-free dialysate. The hyperphosphatemia of ARF is usually not specifically treated. Although oral aluminum hydroxide can reduce the serum phosphorus in ARF, this agent often leads to constipation. If the serum phosphorus exceeds 7 to 8 mg/dL, then short-term calcium citrate, calcium carbonate, or aluminum hydroxide therapy and/or some form of dialysis can be considered. The modest hypermagnesemia that is seen in ARF can best be managed by removal of all sources of magnesium intake.

The modest hyperuricemia of ARF rarely leads to clinical complications. Because of low GFR, little uric acid is filtered and available for precipitation within the renal tubules. Also, acute gouty arthritis rarely complicates the course of the secondary hyperuricemia of ARF. In cases of either exceptionally high uric acid (>20 to 30 mg/dL) or where it is not clear whether hyperuricemia-induced ARF is present, then dialytic therapy and reduced dosage allopurinol may be therapeutic considerations.

Renal Replacement Therapy

The issues regarding renal replacement therapy in ARF are currently the source of much debate and investigation. The areas of debate include when to start, what modality to use, and how much is enough (36,565–617).

When to Start

Generally agreed-on indications for institution of renal replacement therapy for ARF include persistent hyperkalemia, fluid overload unresponsive to conventional treatment, ongoing marked acidemia, symptoms of uremia, and occasionally, bleeding. Based on substantial retrospective data and historic data, most nephrologists also feel that "prophylactic" dialysis to keep the BUN and creatinine 90 to 100 and 9 to 10 mg/dL, respectively, can be readily justified (565,578,606). There are, however, no definitive data supporting these numbers. Moreover, a general trend to even earlier renal replacement therapy has occurred over the past decade based on recent studies. A prospective study of cardiac surgical patients randomized 61 patients to early (oliguria unresponsive to furosemide) or late dialysis (dialysis when serum creatinine greater than 5 mg/dl or potassium greater than 5.5 mmol/L) (577). The "early" group started dialysis earlier (0.9 vs. 2.5 days), stayed less in ICU (7.9 vs. 12 days) and had lower mortality (23 vs. 55%) (577). A retrospective analysis of 100 trauma patients with ARF found that, after controlling for injury severity score, a lower BUN at initiation of dialysis was associated with earlier commencement of dialysis and better survival (578). In a prospective trial of 425 patients comparing different rates of ultrafiltration on continuous hemofiltration, Ronco (579) found that a lower BUN before commencement of dialysis was associated with lower mortality. However, one study of 132 critically ill ARF patients found an inverse relationship between serum creatinine at initiation of hemodialysis and increased mortality (601). A high percentage of patients with ARF, particularly those with mild, nonoliguric ARF, do not require renal replacement therapy. By contrast, intensive and often continuous renal replacement therapy is frequently needed in catabolic intensive care unit patients with severe, oliguric ARF. Moreover, the fluid-volume requirement to maintain nutritional status often dictates early institution of some type of renal replacement therapy in many ICU patients with ARF.

What Modality to Use

There is debate about the optimal modes of renal replacement therapy for patients with ARF. Generally available modes of therapy include conventional intermittent hemodialysis (IHD), intermittent and continuous peritoneal dialysis (PD), continuous arteriovenous hemofiltration (CAVH), continuous arteriovenous hemodialysis (CAVHD), continuous venovenous hemofiltration (CVVH), and continuous venovenous hemodialysis (CVVHD). A survey conducted several years ago in the United States indicated that IHD followed by types of continuous renal replacement therapies and then, infrequently, PD were the modes most often used to treat ARF (576). There has been a widespread, increasing trend to use more continuous therapy, especially CVVHD in critically ill patients.

Several non–patient-related factors must be considered when selecting a mode of renal replacement therapy. Such factors include the availability of the necessary equipment, the expertise demanded by each modality, and the cost of each modality. Patient-related factors that must be considered include the patient's hemodynamic status, the indication for therapy and speed with which the indication must be corrected, the patient's catabolic state, the availability of vascular access, the requirement for patient mobility, the anticipated fluid-volume load that will be given to the patient, the associated clinical conditions of the patient, and the anticipated duration of therapy.

Peritoneal dialysis is rarely performed currently in adults as a treatment modality for ARF (576). However, this modality can be used in adults, especially those with a strong contraindication to anticoagulation, and peritoneal dialysis is more frequently used in children with ARF (602). Peritoneal catheter placement may be either temporary (percutaneous with maximal longevity generally limited to 48 to 72 hours because of infection) or indwelling (can be inserted under local anesthesia and maintained indefinitely). Peritoneal dialysis does not require anticoagulation, can be done on a continuous basis allowing for constant removal of volume and solute, and can be performed in patients with hemodynamic instability. However, the relative efficiency of peritoneal dialysis is such that removal of solute and fluid is only 10% to 20% that of hemodialysis. This inefficiency generally limits usage in patients with ARF who require significant volume and solute removal, although peritoneal dialysis has been used successfully to manage posttraumatic ARF (607). Additionally, the frequency of

intraabdominal surgery and acute respiratory failure make PD impractical in critically unwell patients (580). Absorption of dextrose may lead to hepatic steatosis, increased carbon dioxide production causing worse respiratory failure, and hyperglycemia (581). Survival of PD patients was no different to hemodialysis patients in one small study (36).

The IHD modality has been in widespread use for the past four decades for the treatment of ARF (576). In recent years, the development of bicarbonate-based dialysate and volumetrically controlled machines for precise regulation of ultrafiltration has made IHD a safer procedure in the hemodynamically unstable ICU patient with multiorgan failure. In most centers in the United States, the standard approach to IHD for ARF uses moderate blood flow rates (200 to 250 mL/minute) and dialysate flow rates of 500 mL/minute. The most common reasons cited for preferential choice of IHD are efficacy, ease of use, familiarity with the modality, ready availability of nursing personnel familiar with the procedure, and ability to rapidly correct life-threatening fluid volume, electrolyte and acid–base disorders. Concerns about use of IHD include the fact that a reasonably good and stable level of mean arterial pressure is needed to safely and effectively use this modality. Moreover, any IHD-associated hemodynamic instability, combined with potentially impaired ability of the injured kidney to autoregulate blood flow, could either induce new ischemia or perpetuate previous ischemia (608).

Most recently, continuous modes of renal replacement therapy, especially CVVHD, have been increasingly utilized (565,572,574,575,602,603,605). Continuous modes of therapy such as CVVHD are especially useful for patients who have low mean arterial pressure, are hemodynamically unstable, are hypercatabolic, or have a requirement for large fluid volumes. Measurement of clearance and solute removal using CVVHD suggests treatment comparable to five quality IHD sessions per week (574,575).

The major disadvantage of CRRT is the need for well-trained ICU nursing personnel to perform the procedure. A lack of detailed understanding of the CRRT flow sheets and the computations necessary to determine replacement fluid volumes can lead to significant complications regarding significant volume depletion or excess. Occasionally, lactate-based replacement fluid may result in lactate accumulation and worsening acid–base status if the patient has liver disease and is unable to metabolize lactate as a source of base. On-site formulation of custom bicarbonate-containing replacement fluid is costly and time consuming. Ambulation and physical therapy is difficult while the patient is receiving CRRT.

CRRT is associated with activation of the inflammatory and coagulation pathways, and removal of antioxidants and cytokines (584,585). Removal of cytokines appears to be mediated by adsorption onto the dialysis membrane (586). Two recent studies suggest a hemodynamic and metabolic benefit of dialysis in patients with intractable circulatory shock without renal failure (587,588). It is uncertain if any such benefit is due to removal of cytokines or another mechanism (e.g. cooling, fluid removal) (589,590).

Because of difficulties precisely matching seriously ill patients with ARF, a clear-cut consensus as to whether IHD or CVVHD is a preferable mode of renal replacement therapy has not emerged. It is likely, however, that ARF patients are best served by considering these modalities as complementary rather than as competing therapeutic options. Martin and colleagues (582) have shown that continuous therapies are preferentially given to patients with marked hemodynamic compromise. The choice of modality may also be limited by availability of equipment and trained nursing staff (583). In this regard, the recent careful analysis of Swartz and associates is of interest (575). In this retrospective analysis of over 300 patients, the higher mortality seen with CVVH appeared clearly related to severity of case mix and comorbidity rather than modality of treatment. When matched populations were analyzed retrospectively, no clear-cut outcome differences by modality of renal replacement therapy were apparent. A prospective randomized study of 166 patients compared continuous renal replacement therapy with intermittent hemodialysis after excluding individuals with severe hypotension (591). Despite randomization there were differences between groups that favored the intermittent hemodialysis group. After adjusting for confounders, there was no significant difference between modalities in terms of mortality or renal survival (591). Two systematic reviews (592,593) showed no difference between intermittent and continuous therapies, but after adjusting for study quality and severity of illness, Kellum found a decreased relative risk of death with continuous therapies. Different methodologies, small numbers of patients, study flaws, and lack of standardized approaches to timing and indications of dialytic intervention make accurate comparisons between modalities problematical (594,595).

New modalities of dialysis incorporating the advantages of hemodynamic stability of CRRT coupled with high rates of solute and fluid removal of intermittent hemodialysis have emerged. In slow low efficiency daily dialysis, or SLEDD, near conventional dialysis equipment is used at low blood and dialysate flow rates, for prolonged periods of time (6 to 12 hours/day). This modality offers more hemodynamic stability, better correction of hypervolemia, and more adequate solute removal, compared with IHD (596) and compares favorably with CRRT in terms of patient outcomes (597). SLEDD also uses less nursing resources and does not require intensive anticoagulation (600). The single-path batch dialysis system uses a closed-loop system comprising a 75-L dialysate tank which lasts for 18 hours of extended single-path high-flux hemodialysis. It was well tolerated by critically ill patients in two small series (598,599).

Another controversial issue with regard to RRT in ARF is selection of dialysis membrane (565,566,570,571,609–612). The polysaccharide structure of cellulosic (bioincompatible) membranes provides a trigger for complement activation via the alternative pathway, which leads to the liberation of anaphylotoxins and activation of leukocytes. The potential induction of a systemic inflammatory reaction during such dialysis treatment with bioincompatible dialysis membranes could conceivably cause further ischemia or inflammatory changes within the previously injured renal microcirculation.

In a recent study of 72 patients with ARF, patients were randomized to intermittent dialysis treatment with either bioincompatible Cuprophane dialysis membranes, which activate the complement system and leukocytes, or to dialysis with a biocompatible membrane composed of polymethyl methacrylate, which has a less marked effect on complement and leukocytes (610). The two dialysis membranes chosen for the study had similar clearance and ultrafiltration characteristics and the patient groups were similar. Fifty-seven percent of patients on dialysis with biocompatible membranes survived, compared with 37% of these dialyzed with Cuprophane membranes ($p = 0.11$). Recovery of renal function occurred in 62% of those dialyzed with a biocompatible membrane, compared with 37% of those who underwent dialysis with Cuprophane membranes ($p = 0.04$). The time to recovery of renal function after initiation of dialysis was also significantly shorter in the biocompatible membrane group compared with the Cuprophane group: five dialysis treatments over 11 days versus 17 dialysis treatments over 33 days, respectively. Subgroup analysis revealed that the benefits of biocompatible membrane dialysis were evident only in patients who were nonoliguric before the initiation of dialysis. These results suggest that use of the biocompatible dialysis membrane increases the likelihood of recovery of renal function and survival of patients with ARF. Other studies have confirmed and extended these observations to include other

biocompatible dialysis membranes, including polysulfone, and polyacrylonitrile membranes (571); however, this issue may not be quite a clear-cut as those two studies suggest. For example, in recent prospective studies a clear-cut advantage of biocompatible membranes has not emerged (566,609,612). A meta-analysis by Jaber (567) demonstrated that the use of a biocompatible membrane did not significantly affect mortality among patients with ARF who require IHD. However, a meta-analysis by Subramanian (568) (which included an additional 169 patients from one trial) found a relative risk of mortality for cellulosic (bioincompatible) membranes of 1.37 (CI, 1.02–1.83). Given the known proinflammatory effects of these membranes in experimental models, the shift in clinical practice toward increased use of synthetic membranes in the setting of ARF, and the now reasonable cost of biocompatible membranes (in comparison to cellulose membranes), it is unlikely that this question will be resolved (569).

How Much Is Enough?

A final area of controversy with regard to RRT in ARF is how much is enough? Schiffl (613) randomized 160 patients with ARF to receive either daily hemodialysis or intermittent (second-daily) dialysis. Patients perceived to require CRRT because of hemodynamic instability were excluded. Daily hemodialysis resulted in better control of uremia, fewer hypotensive episodes during hemodialysis, and more rapid resolution of ARF (9 vs. 16 days) than did conventional hemodialysis. More patients in the conventional-hemodialysis group than in the daily-hemodialysis group had the systemic inflammatory response syndrome or sepsis, respiratory failure, changes in mental status, or gastrointestinal bleeding. The mortality rate, according to the intention-to-treat analysis, was 28% for daily dialysis and 46% for alternate-day dialysis. The mean time averaged urea concentration in the conventional hemodialysis group was 104 mg/dL, suggesting that this group was inadequately dialyzed and that the difference in outcomes was therefore more marked. Ronco (579) studied 425 patients with ARF commencing continuous venovenous hemofiltration, randomly assigning ultrafiltration at 20 mL/hour/kg, 35 mL/hour/kg, or 45 mL/hour/kg. Survival (calculated at 15 days after stopping dialysis) in the lowest ultrafiltration group was significantly lower (41%) than in the other groups respectively (57% and 58%). Renal survival was nonstatistically worse in the lowest ultrafiltration group. Increasing the dose of dialysis in these two studies improved both patient and renal survival. Measurement of dose in dialysis in ARF patients is not possible with the same techniques as in stable chronic hemodialysis patients due to lack of steady-state of urea generation and fluid compartments; however models have been developed (615–617).

Outcome and Prognosis of Acute Renal Failure

The outcome of ARF is highly dependent on the definition of the disorder and the patient population studied. In epidemiologic studies undertaken in general medical-surgical hospitals, ARF has often been defined as an increase of 0.25 to 1.0 mg/dL in serum creatinine. Although this appears to be a very mild increase in serum creatinine, it likely reflects at least a 30% to 60% decrement in GFR. In such ARF patients, mortality rates of 20% to 30% have been reported (37,55). These mortality rates exceed those for comparable hospitalized patients who do not develop ARF by a factor of 6 to 10 (55). Only 10% to 20% of these patients require dialytic therapy. When series of patients with ARF are identified by either reviewing consultations to a renal service or by retrospective reviews of discharge diagnoses, the selection bias generally results in a more seriously ill group of patients with more pronounced renal failure. In such patients, contemporary mortality rates of 30% to 60% are reported, with 30% to 50% of patients requiring dialytic therapy (35,39–44,312,618,619). Finally, many series report outcome and prognostic variables only in patients with ARF who have undergone dialytic therapy. In these patients, current mortality rates range from 50% to 90% (35,39–44,312,619).

A case-control study emphasizes some of the important outcome aspects of contemporary ARF (49). In this study, 183 patients with radiocontrast-associated ARF (defined as an increase in serum creatinine of at least 25% to at least 2 mg/dL) were matched to comparable patients exposed to radiocontrast that did not develop ARF. Mortality was 7% in control and 34% in ARF patients. After adjustment for comorbidity, the adjusted odds ratio for death with ARF was 5.5. The development of ARF was not only associated with death, but also with development of respiratory failure, sepsis, and bleeding (Fig. 41-9).

As alluded to previously, a major prognostic factor in patients with ARF is the severity and duration of the resultant renal dysfunction (Table 41-19, Figs. 41-8 and 41-9). Hou and collaborators (37), in a large prospective study, found that the presence of urinalysis abnormalities in patients with ARF had a marked influence on outcome. Death occurred in 31% of ARF patients with normal urine sediment and in 74% ($p < 0.025$) of patients with abnormal urine sediment (37). These observations have also been reported in other studies and probably

TABLE 41-19

PROGNOSTIC FACTORS IN ACUTE RENAL FAILURE

Severity of renal dysfunction
Magnitude of rise in serum creatinine concentration
Urinalysis
Fractional excretion of sodium
Presence of oliguria or anuria
Requirement for renal replacement therapy
Duration of renal dysfunction
Underlying health of the patient
Age
Presence, severity, and reversibility of underlying disease
Clinical circumstances
Cause of the renal failure
Severity and reversibility of acute process(es)
Number and type of other organ systems failed
Development of sepsis and other complications

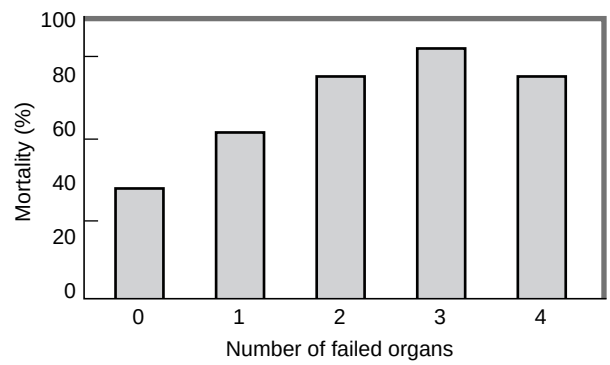

FIGURE 41-8. Acute renal failure mortality relative to number of organ systems failed. (From: Star RA. Treatment of acute renal failure. *Kidney Int* 1998;54:1817.)

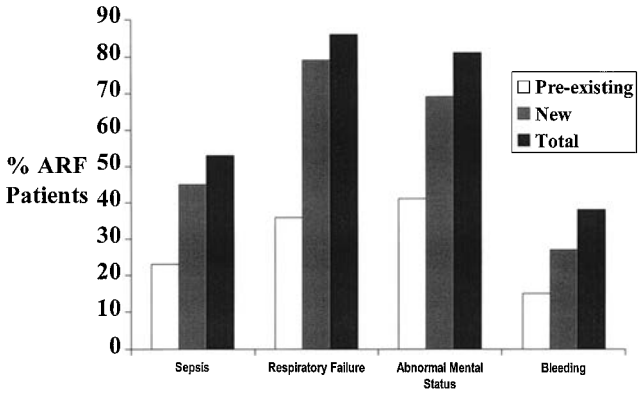

FIGURE 41-9. Morbidity occurring before and after radiocontrast-associated acute renal failure. (From: Levy EM, Viscoli CM, Horwitz RI. The effect of acute renal failure on mortality. *JAMA* 1996;275:1489, with permission.)

reflect outcome differences between prerenal azotemia (normal sediment) and renal azotemia (abnormal sediment). Hou and collaborators (37) also found a direct correlation between mortality and the magnitude of rise in serum creatinine in patients with ARF. The mortality rate was less than 20% to 30% until the increment in serum creatinine exceeded 3 mg/dL, at which time the mortality rate was 64%.

Other likely indicators of severity of renal insult in patients with ARF are the quantity and quality of urine output. Virtually all contemporary studies of patients with ARF find mortality rates of nonoliguric patients to be less than half of those of oliguric patients (Fig. 41-2).

It is unclear whether or not patients with acute renal failure due to sepsis appear to have a worse prognosis than those with non-septic acute renal failure. Confounding variables make an independent association among sepsis, ARF, and mortality difficult to tease out. In a prospective multicenter study of 345 patients with ARF, Levy (35) found that sepsis was an independent predictor of hospital mortality (OR, 2.51) and need for mechanical ventilation. Cytokine levels are also predictive of greater mortality in patients with septic ARF (620). Hoste (324) found that there was a high incidence of nosocomial bloodstream infection in a subgroup of severely ill patients with ARF who were treated with RRT in the ICU. However, nosocomial bloodstream infection did not lead to a statistically significant increased length of stay, costs, or mortality (324).

One advance in the study of ARF that has occurred over the past decade has been in the area of development of indices that predict ARF outcome (621–634). The issue of predicting outcome and estimating the probability of survival for individual patients with ARF is important with regard to cost-effective allocation of resources. Also, determination of severity of illness and likelihood of survival is important with regard to quality assurance assessment and comparability of patient populations for controlled prevention and treatment trials.

Most patients with ARF are seen in an ICU setting. Several multipurpose scoring systems for ICU patients have been designed to provide estimates of hospital mortality (621). These systems are all based on rigorous research involving several thousand patients from multiple sites for both development and validation studies (621). Some of three widely used systems (Acute Physiology and Chronic Health Evaluation or APACHE, Mortality Probability Models or MPM, and the Simplified Acute Physiology Score or SAPS) use logistic regression techniques and have evolved through three generations of development. These systems appear capable of assessing prognosis, comparing different ICU performances, and stratifying patients for clinical studies. However, the number of patients with ARF included in the development of these ICU scoring systems was modest at best. Thus, it is perhaps not surprising that these general ICU scoring systems perform suboptimally with regard to ARF, inasmuch as they often underestimate the risks of death when applied to an exclusive ARF population (459). A study has validated the APACHE system for 153 patients with ARF (635). This has led to the development of "ARF specific" prognostic indices (622–634).

Perhaps the best of the ARF specific prediction tools is the one developed by Liano and colleagues (56,565,628,629). This tool was initially based on retrospectively obtained data and has been validated, prospectively. When applied to ARF mortality in both the ICU (actual 72%, predicted 65%) and the non-ICU (actual 32%, predicted 32%) setting, this instrument performed well (56). Renal failure accounts for 21% of this index, whereas comorbid conditions such as hypotension, jaundice, and assisted ventilation account for the remainder. Inclusion of comorbid conditions as a key aspect of ARF outcome is important. For example, in one large series, the cause of death could be directly attributed to underlying disease in 61% of the ARF deaths (56). The performance of the Liano scale and other scales in prediction of ARF mortality are discussed in Star (565). Recently, the observation that blacks may have especially high ARF morbidity and mortality has been emphasized (3).

One simple but powerful method to assess ARF mortality is in the context of the number of associated failed organs (565). An estimated association is depicted in Figure 41-8. In general, associated failure of more than two organ systems places ARF outcome in the vicinity of 10% to 20% likelihood of survival.

There has been recent discussion whether the prognosis of patients with ARF has improved. Improving general medical care and supportive therapy and changes in case-mix do not allow for a definitive answer to this issue. However, a Mayo Clinic study provides a qualified yes to the suggestion that modern therapy has been associated with improved ARF outcome (43). In this analysis of dialyzed patients with ARF, overall mortality declined from 68% to 48% when the years 1977 to 1979 were compared with 1991 to 1993. Stratification by APACHE II revealed markedly improved mortality in the two compared time frames in patients with either low (36% to 11%), or moderate (75% to 35%) but not high scores (100% to 85%). Modern medical management does not appear to be helping those with the greatest chance of dying.

In general, recovery from oliguric ARF due to acute tubular necrosis occurs relatively quickly (636). In our experience, the majority of patients with oliguric ATN require renal replacement therapy for roughly 5 to 15 days. Notable exceptions occur, particularly in elderly patients, patients with multiple organ failure or patients in whom ATN complicates the course of solid organ transplantation. In the experience of Kjellstrand and associates (636), the median time from initiation of dialysis to recovery of renal function in survivors was 12 days. Usually, renal function has reached maximal levels within 4 to 6 weeks after the last dialysis for ARF. However, there are notable published exceptions of protracted, slow, continuous recovery of renal function (637–639). There is also a small but significant group of patients with ARF that do not recover renal function. For example, Bhandry and Turney recently reported that 16% of 1095 patients with severe ARF failed to recover renal function and needed chronic RRT (639). Only six patients recovered sufficient renal function to become independent of dialysis after 3 to 18 months on regular dialysis therapy (6 to 21 months after onset of ARF) (639). The frequency of lack of renal recovery from ARF was highest for ARF attributed to renal parenchymal disease (35%) and less high

for ARF occurring in either a medical (9%) or surgical (6%) setting. These latter settings likely reflected ATN as a cause of the ARF.

CONCLUSION

Acute renal failure is commonly encountered in contemporary medical practice. Acute renal failure is the end product of a variety of insults operating via several pathophysiologic mechanisms. At present, an incomplete understanding of the pathophysiology of human ARF hampers effective therapy to uniformly prevent, attenuate, and hasten recovery from ARF (640,641). However, effects directed toward prevention, early detection, timely diagnosis of the cause/predisposing factor(s), and specific intervention remain the keys to reducing the significant mortality and morbidity of ARF.

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CHAPTER 42 ■ ANTIBIOTIC- AND IMMUNOSUPPRESSION-RELATED RENAL FAILURE

JEAN-LOUIS BOSMANS AND MARC E. DE BROE

AMINOGLYCOSIDE ANTIBIOTICS

Nephrotoxic injury is a common complication of aminoglycoside antibiotic therapy. Studies that have used well-defined measures of nephrotoxicity indicate an incidence rate of 7% to 36% (1–9). This variability reflects differences with respect to the nephrotoxicity potentials of aminoglycoside antibiotics in clinical use as well as differences among patients receiving these drugs. A survey of clinical studies published between 1975 and 1982 reveals that the average incidence of nephrotoxicity caused by specific aminoglycoside antibiotics was gentamicin, 14%; tobramycin, 12.9%; amikacin, 9.4%; and netilmicin, 8.9% (10). In critically ill patients, the incidence of aminoglycoside nephrotoxicity may rise twofold (11).

Clinical Aspects

The clinical expression of aminoglycoside nephrotoxicity has been well described (12–16). The earliest and most common expression of aminoglycoside renal tubular cell alterations is increased urinary excretion of low-molecular-weight proteins (17,18) and of lysosomal and brush-border membrane enzymes (17–20). These changes may be detected within 24 hours of initiating drug therapy, and the frequency and magnitude of these changes increase as a function of dose and duration of therapy. Unfortunately, these changes do not predict which patients will progress to acute renal failure (ARF). This probably reflects the fact that several mechanisms underlie the expression of the enzymuria and proteinuria (13). With repeated dosing, the amount of enzymes and low-molecular-weight proteins excreted in the urine may increase quite sharply, which may signify the onset of proximal tubular cell necrosis (13).

Nonoliguric renal failure is a common expression of aminoglycoside nephrotoxicity (21) and may reflect a direct inhibitory effect on solute transport along the thick ascending limb of Henle's loop (22) or possibly tubulointerstitial cell injury (23), which results in impaired ability to maintain a hypertonic medullary interstitium. Inhibition of adenylate cyclase may also contribute to the polyuria (24). Neither mechanism, however, adequately explains the maintenance of normal to high urine output, even in the face of severe depression of whole-kidney glomerular filtration rate (GFR). The slow evolution of ARF, which has been attributed to a variable susceptibility of renal proximal tubular cells to aminoglycoside toxicity (12,25), may allow for the development of maximal compensatory adaptation by residual intact nephrons. In addition, micropuncture experiments (26) implicate a marked depression of solute and water transport along the proximal tubule such that the large increase in the fraction of filtrate escaping reabsorption along the proximal tubule may overwhelm the reabsorptive capacity of the distal nephron and contribute to the pattern of nonoliguric renal failure. When oliguria occurs, it usually signifies the influence of one or more complicating factors, for example, ischemia or another nephrotoxin, especially if the oliguria appears early in the course of aminoglycoside administration. Studies in animals have shown that aminoglycoside therapy sensitizes the kidney to a subsequent ischemic or nephrotoxic insult (27–36), such that the severity of the ARF is substantially greater than that predicted by the sum of the individual insults. Deterioration of other proximal tubular transport processes may occur during aminoglycoside toxicity and in rare cases may mimic a Fanconi-like syndrome (37). Hypokalemia and hypomagnesemia secondary to renal potassium and magnesium wasting may also appear (38,39).

Depression of GFR is a relatively late manifestation of aminoglycoside nephrotoxicity. In humans, depression of GFR typically does not occur before 5 to 7 days of therapy have been completed (15) unless there has been a major complicating factor such as renal ischemia. Studies in animal models of aminoglycoside nephrotoxicity have implicated activation of the renin–angiotensin system (40), reduction in the size and density of glomerular endothelial fenestrae (41), tubular obstruction (42), tubular back leak (26), and release of platelet activating factor from mesangial cells (43) as pathogenic factors causing depression of GFR.

The majority of patients with aminoglycoside nephrotoxicity recover renal function clinically, although in some cases the time to recovery may be prolonged (16). Chronic renal failure is a distinctly uncommon complication of pure aminoglycoside nephrotoxicity in humans, so that when it occurs, it usually signifies the contribution of some additional factor. Animal studies indicate, however, that incomplete regeneration with interstitial fibrosis does occur (44), and the same may be true for humans (45).

Morphologic Alterations

Aminoglycosides cause tubular cell necrosis that in animal models is largely confined to the proximal convoluted tubule and pars recta (46–48). In humans, the renal tubular site of injury is less well established (23,49), due in part to the fact that little human biopsy material has been available for study. Moreover, in human subjects, the development of ARF in conjunction with aminoglycoside administration typically occurs in association with other insults such as sepsis and renal ischemia (23,50,51), and each of these insults has been shown to interact synergistically with aminoglycoside antibiotics to magnify the severity and sites of tubular cell injury (30–36).

The earliest lesion seen by electron microscopy is an increase in the number and size of secondary lysosomes, also

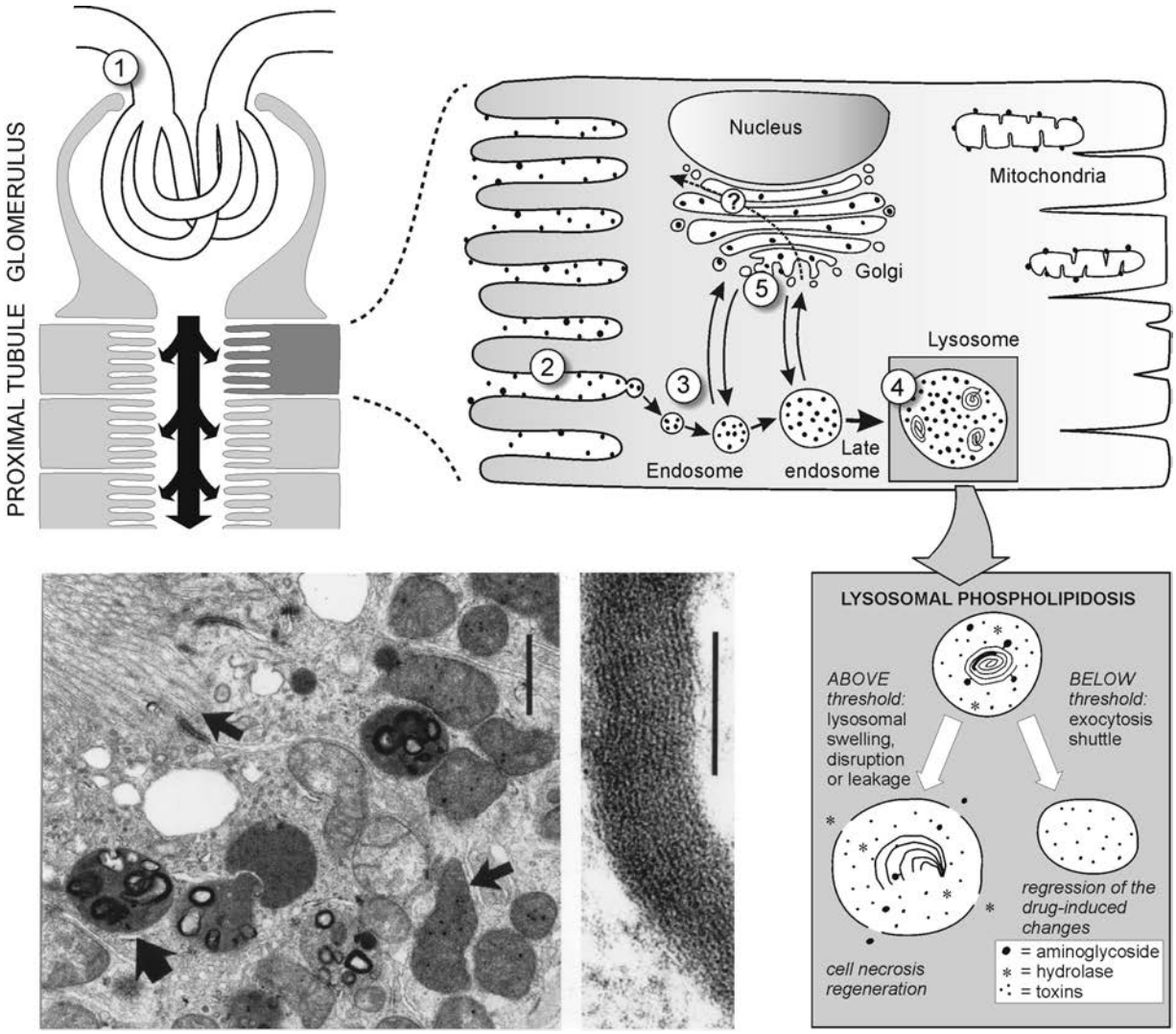

FIGURE 42-1. Above: Binding uptake and intracellular trafficking of gentamicin in renal proximal tubular cells. After glomerular filtration (1), gentamicin (•) is shown binding to the surface membrane (2) and being internalized by a receptor (megalin) mediated endocytic process (3). Gentamicin also enters the cell through fluid phase endocytosis. It moves through the endocytic system into late endosomes and from there into lysosomal structures (4). A small but quantifiable fraction (5%–10%) of gentamicin directly traffics from the surface membrane into the trans-Golgi network (5) and from there throughout the Golgi apparatus. **Below left**: Ultrastructural appearance of proximal tubular cells after 4 days of gentamicin treatment, showing lysosomes containing dense lamellar and concentric structures (*large arrow*), while brush border, mitochondria (*small arrow*) and peroxisomes are unaltered. Upon higher magnification, the structures in lysosomes show a periodic pattern. *Bar left* = 1 μm, *middle* = 0.1 μm. **Below right**: Internalization and lysosomal sequestration of gentamicin. (Adapted from: Verpooten GA, Tulkens PM, Molitoris BA. Aminoglycosides and vancomycin. In: DeBroe ME, Porter GA, Bennett VM, et al., eds. *Clinical nephrotoxins: renal injury from drugs and chemicals,* 2nd ed. Dordrecht, The Netherlands: Kluwer Acadmic Publishers, 2003:151.)

called cytosegrosomes or phagosomes (46–48). Examples of this lesion are shown in Figure 42-1. Secondary lysosomes are primary lysosomes that have coalesced with endocytic or autophagic vacuoles. Many of these lysosomes contain myeloid bodies, electron-dense lamellar structures of concentrically arranged and densely packed membranes. These lysosomal alterations probably represent autophagic vacuoles arising from sequestration of fragments of membranes and organelles damaged in the early phase of toxicity and are undergoing lysosomal processing. In experimental animals receiving single parenteral drug doses or continuous drug infusion, these changes have been observed as early as 6 to 12 hours posttreatment (52). Both the number and size of lysosomal myeloid bodies increase as a function of dose and duration of drug therapy and are accompanied by progressive expansion of the volume of the cell occupied by engorged lysosomes (47,52,53). These morphologic alterations also have been convincingly demonstrated in human kidney material (54,55). Studies in experimental animals and in cultured cells have demonstrated that the myeloid bodies are composed of membranes rich in phospholipids (56,57) and form as a consequence of the lysosomal accumulation in high concentration of aminoglycosides. This lysosomal accumulation of aminoglycosides inhibits lysosomal phospholipases (58,59) and possibly other lysosomal enzymes and impairs the degradation of cell membranes (57,60). Similar alterations have been induced by a variety of compounds that

accumulate within the lysosomal compartment and interfere with the activity of lysosomal enzymes (61–63).

Following lysosomal alterations, there occurs a decrease in the density and height of brush-border microvilli, dilation of the cisternae of rough endoplasmic reticulum, and the appearance of cytoplasmic vacuolization in tubular epithelial cells (48,52). As injury progresses, brush-border membrane fragments and extruded myeloid bodies, membrane vesicles, and cytoplasmic debris begin to be seen within tubular lumina (48,64). Later in the course of nephrotoxicity, mitochondrial swelling becomes evident, and patchy, but extensive tubular epithelial cell necrosis and desquamation occur. Many tubules, both proximal and distal, are filled with eosinophilic, granular material that, by electron microscopy, is composed of cytoplasmic debris, membrane fragments, and myeloid bodies. Transmission electron microscopy of the urine reveals the presence of myeloid bodies and fragments of brush-border membranes (64–66).

Proximal tubular cells manifest an apparent variable susceptibility to aminoglycoside toxicity evident by the appearance of cell regeneration simultaneously with ongoing cell necrosis (25,44,47,53,67). In several animal studies virtually complete recovery of renal structure and function has been observed during continued aminoglycoside administration (68,69). One explanation for these observations is that the renal tubular epithelium had acquired resistance to the nephrotoxic effects of the aminoglycoside antibiotic. Sundin and colleagues (70) report that the "acquired resistance" reflects selective inhibition of aminoglycoside uptake by renal proximal tubular cells, the mechanism of which does not involve a reduction in the membrane content of phosphatidylinositol or megalin. In animal models, cell regeneration can be detected by [^{3}H]thymidine incorporation into DNA after only 4 days of low-dose aminoglycoside administration and before cell necrosis is evident by light microscopy (53,70). The magnitude of DNA labeling correlates with the dose and duration of drug administration (71). Of particular interest is the observation that quantitatively similar labeling is observed in renal cortical interstitial cells as in tubular epithelial cells (53,71,72). This finding raises the question of the role of these interstitial cells in the pathogenesis of aminoglycoside toxicity. Eventually most areas of the affected kidney regain normal architecture and function, but residual scarring containing collections of collapsed, atrophic tubules may occur focally in the cortex (44,45,48). In animal models of aminoglycoside nephrotoxicity, the degree of tubular cell necrosis correlates reasonably well with the decline in renal excretory function. A similar correlation is lacking in human material (23,49,54).

Pathogenesis

The pathogenesis of aminoglycoside nephrotoxicity is intimately linked to the renal pharmacology of these drugs (73–76). Aminoglycoside antibiotics are organic polycations with a net cationic charge that, at pH 7.4, ranges from +4.47 in the case of neomycin to +2.39 for amikacin. Because these compounds are highly hydrophilic, they are poorly absorbed across the intestinal tract and therefore must be given parenterally. They are distributed in a volume slightly greater than extracellular volume and are eliminated from the body without metabolic transformation. The route of elimination is almost exclusively by the kidneys, and the principal mechanism of excretion is glomerular filtration. Of toxicologic significance is the fact that small amounts of aminoglycoside antibiotics are selectively transported into proximal tubular cells by adsorptive endocytosis (77–79), which has been shown to occur across the basolateral as well as the apical membrane (79). Several lines of evidence have implicated anionic phosphatidylinositol as a membrane binding site for aminoglycosides (80,81). More-recent studies also suggest a role for megalin, an endocytic receptor for cationic ligands, in the uptake of aminoglycoside antibiotics across the brush-border membrane of renal proximal tubular cells (82). Indeed, by using the specific antagonist receptor-associated protein, blocking the activity of megalin in perfused rat proximal tubules, a reduction of 20% in gentamicin clearance ensued. Nagai demonstrated similar results in rats treated with maleate, impairing the receptor-mediated uptake of megalin ligands (83). Megalin knockout mice are protected against aminoglycoside nephrotoxicity (84).

Following endocytosis, the aminoglycosides are translocated into the lysosomal compartment, where they accumulate in millimolar concentrations and reside with a half-life measured in days (74). As noted, the lysosomal compartment is the site of myeloid body formation consequent to aminoglycoside-induced inhibition of lysosomal enzymes such as phospholipase, sphyngomyelase, etc. When the concentration of drug and/or the amount of lysosomal phospholipid reaches a critical threshold, an injury cascade is triggered that eventuates in irreversible cell injury with progression to necrosis (52). However, neither the sequence nor the specific mechanisms involved in the progression to cell death have been clearly established. Sandoval and colleagues report that within 15 minutes of endocytosis gentamicin traffics to the Golgi complex as well as to the lysosomal compartment of LLC-PK1 cells (85,86) and rat renal proximal tubular cells (87). These observations raise the possibility that the Golgi complex may provide a pathway for the redistribution of aminoglycoside antibiotics to other intracellular compartments and thereby broaden the potential for these drugs to disrupt a variety of organellar functions. For example, the depression of protein synthesis observed early in the course of gentamicin administration may signify retrograde transport of gentamicin to the endoplasmic reticulum (87). The reason gentamicin and presumably other aminoglycoside antibiotics are transported from the endosomal compartment to the Golgi complex is not known; but, it may reflect an effect of these agents to perturb endosomal fusion (88) possibly as a consequence of binding to megalin (88) or to membrane-acidic phospholipids (89,90).

A growing body of evidence supports the view that the pathogenesis of aminoglycoside toxicity is causally related to the capacity of these cationic drugs to bind to and perturb the function and structure of biologic membranes. Aminoglycosides have been shown to bind to anionic (58,80,91–98) but not to neutral phospholipids (58,80,92,94). Among the anionic phospholipids, aminoglycosides bind most avidly to phosphatidylinositol 4,5-bisphosphate (PIP_2) (80,93,99–101). Several approaches have been used to gain insight into the molecular interaction between aminoglycosides and anionic phospholipids (80,91,94,97,98,102–104). All models indicate an electrostatic interaction between a protonated amino group and the anionic phosphate group. Ramsammy and Kaloyanides (103) propose a model, that in addition to an electrostatic interaction between a protonated amino group and the phosphate group, also involves formation of hydrogen bonds between an amino group of gentamicin and the carbonyl groups of glycerol. This model explains aminoglycoside-induced changes in the biophysical properties of artificial membranes (i.e., an increase in the transition temperature and a decrease in glycerol permeability of phosphatidylinositol [PI]-containing liposomes) (96). Both changes signify that gentamicin induces a decrease in membrane fluidity, and this finding has been confirmed in brush-border membranes as assessed by changes in the fluorescence polarization of membrane probes (92) and by electron spin resonance spectroscopy (105). Aminoglycosides also have been shown to promote membrane aggregation (102,106), a process that requires neutralization of surface charge. In a comparative study of aminoglycoside-induced aggregation of

PI-containing liposomes (102), it was observed that the rank order with respect to efficacy in neutralizing membrane surface charge was neomycin > gentamicin = tobramycin = netilmicin = spermine. The rank order for inducing aggregation of liposomes was neomycin > gentamicin > tobramycin > netilmicin = spermine and was identical to the rank order of these agents with respect to depressing glycerol permeability (102). This rank order also coincides precisely with the established clinical nephrotoxicity potentials of these drugs. Since depression of glycerol permeability was shown to be dependent on hydrogen bonding between one or more amino groups of the drug and carbonyl groups of the glycerol backbone (103), these data suggest that the membrane toxicity of aminoglycosides is closely linked to their potentials to engage in hydrogen bonding. Importantly, the rank order in terms of nephrotoxicity potentials does not coincide with the net cationic charge of these agents (102). This observation emphasizes that spatial orientation of charge rather than net charge is a critical determinant of toxicity.

Schacht and colleagues (93,95,100,107) utilize a variety of methods to assess aminoglycoside-induced perturbations of PIP_2-containing membranes as a measure of the ototoxicity potentials of these antibiotics. Increased fluorescence of 1-anilino-8-naphthalenesulfonate (95), increased permeability to carboxy fluorescein (100), and increased surface tension of monomolecular film of phosphatidylcholine (PC)/PIP_2 (107) were shown to correlate precisely with the ototoxicity potentials of aminoglycoside antibiotics. These studies have led to the hypothesis that the ototoxicity of aminoglycosides is causally related to their binding to PIP_2 and disruption of this signaling mechanism (108).

The studies cited here provide the foundation for the hypothesis that the toxicity of aminoglycoside antibiotics is causally related to their capacity to interact electrostatically and by hydrogen bonding to membrane anionic phospholipids and, thereby, to perturb the biophysical properties and function of cell membranes. It is well established that these drugs interact with and perturb the function of plasma membranes (13,109–112), lysosomes (13,52,53–60,113–118), mitochondria (48,119–122), and microsomes (123–125). It remains unclear, however, whether toxicity results from disruption of a single critical membrane function or multiple membrane functions. It is possible that the injury cascade is triggered by the rupture of lysosomes engorged with aminoglycoside antibiotic and with myeloid bodies. The resultant release of potent acid hydrolases and high concentrations of drug into the cytoplasm might cause disruption of a number of critical intracellular processes including mitochondrial respiration (48,119–122), microsomal protein synthesis (123–125), intracellular signaling via the PI cascade (126–129) as well as generation of hydroxyl radicals (130–132), all of which have been observed in experimental models of aminoglycoside toxicity. However, the observation that gentamicin is transported to the Golgi complex shortly after endocytic uptake (85,86), provides an alternate mechanism by which these drugs gain access to other organelles. Recently, proteomic analysis following gentamicin administration indicated energy production impairment and a mitochondrial dysfunction occurring in parallel to the onset of nephrotoxicity (133).

Further insight into the pathogenesis of aminoglycoside nephrotoxicity has been gleaned from studies of interventions that modify the severity of this disorder in experimental animals. Williams and colleagues (134–136) first reported that polyasparagine and polyaspartic acid (PAA) inhibited binding of gentamicin to rat renal brush-border membrane *in vitro* and when injected *in vivo* conferred protection against the development of aminoglycoside nephrotoxicity without inhibiting the renal cortical accumulation of drug. These findings have been confirmed and extended by three groups of investigators (137–145). The mechanism by which PAA protects against aminoglycoside nephrotoxicity was shown to be related to the ability of PAA, a polyanion, to form electrostatic complexes with the polycationic aminoglycoside antibiotics (142,146,147) presumably within the endocytic compartment (144), thereby preventing aminoglycosides from binding to anionic phospholipids, from inhibiting lysosomal phospholipase degradation of phospholipid, from forming lysosomal myeloid bodies, and from disrupting the PI cascade (146). Additional support for this theory is provided by the observation that PAA prevented gentamicin from depressing glycerol permeability or aggregating PI-containing liposomes (146), effects previously shown to be dependent on gentamicin binding electrostatically and by hydrogen bonding to PI (96,103). Subsequently, other compounds capable of forming electrostatic complexes with aminoglycosides have been reported to protect against nephrotoxicity (148–151).

Recently, an analog of pentoxifylline, HWA-448, was shown to protect against gentamicin toxicity in a cell culture model (152). Similar to PAA, HWA-448 did not depress the membrane binding or cellular uptake of gentamicin. It remains unknown whether HWA-448 forms a complex with gentamicin within the endosomal compartment.

Treatment and Prevention of Aminoglycoside Nephrotoxicity

The efficacy of PAA and other anionic compounds in preventing nephrotoxicity in humans has yet to be established. Therefore the primary focus of treatment is prevention, and this can be accomplished by understanding and modifying, when possible, the risk factors (Table 42-1) for this complication (153–155). Risk factors may be categorized into those that are determined by the individual patient and not easily influenced, if at all, and those that are determined by the clinician and potentially controllable (Table 42-1).

Prominent among the risk factors peculiar to the patient and not modifiable is advanced age (153). The mechanism is probably multifactorial and includes age-related decline of renal function that if not appreciated and corrected for results in excessive dosing (156). Animal studies suggest that aging is associated with altered renal pharmacokinetics accompanied by increased renal cortical accumulation of drug (157). Increased susceptibility of the aging kidney to aminoglycoside toxicity has also been suggested (158), possibly on the basis of an age-related impaired capacity for cellular repair and regeneration. Male gender has been shown to carry increased risk for aminoglycoside nephrotoxicity in the rat (159), whereas female gender has been identified as a risk factor in humans (153). The reason for this difference has not been established.

Obesity carries increased risk for aminoglycoside nephrotoxicity that is unexplained by differences in the volume of distribution or renal clearance of drug (160). The increased risk associated with chronic liver disease (153) may be related to the alterations in extracellular volume, hemodynamics, and electrolyte balance commonly observed in this disorder, all of which are known to promote renal cortical accumulation of drug (74). Preexisting chronic renal insufficiency is associated with increased risk primarily due to failure to adjust appropriately the dose of aminoglycoside for the level of impaired kidney function (161). Renal hypoperfusion from any cause carries an increased risk of aminoglycoside nephrotoxicity whether the renal ischemic insult occurs before (81), during (33), or after drug administration (31). The latter observation is particularly worthy of note because it implies that the increased risk of nephrotoxicity persists even after the drug has been discontinued. The prolonged half-life of aminoglycosides in renal cortex

TABLE 42-1

RISK FACTORS FOR AMINOGLYCOSIDE NEPHROTOXICITY

Patient factors
Older patients[a]
Preexisting renal disease
Magnesium, potassium, calcium deficiency[a]
Intravascular volume depletion,[a] hypotension[a]
Hepatic syndrome
Sepsis syndrome[a]
Aminoglycoside factors
Recent aminoglycoside therapy
Larger doses[a]
Treatment of 3 or more days[a]
Drug choice: e.g., gentamicin,[a] amikacin[a]
Frequent dosing interval[a]
Concomitant drugs
Amphotericin B
Cephalosporines
Cisplatin
Clindamycin
Cyclosporine
Foscarnet
Forosemide
IV radiocontrast agents
Piperacillin
Vancomycin

[a] Concurrent with experimental nephrotoxicity data.
(Adapted from: Verpooten GA, Tulkens PM, Molitoris BA. Aminoglycosides and vancomycin. In: De Broe ME, Porter GA, Bennett VM, et al., eds. *Clinical nephrotoxins—renal injury from drugs and chemicals,* 2nd ed. Dordrecht, The Netherlands. Kluwer Acadmic Publishers, 2003:151.)

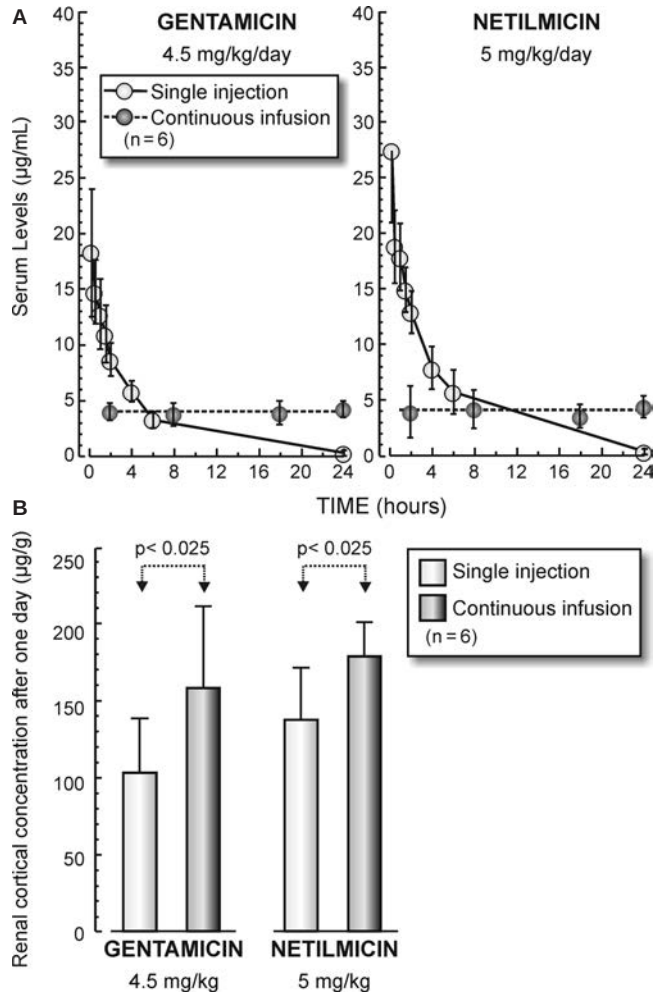

FIGURE 42-2. A: Course of serum concentrations of gentamicin and netilmicin after administration of the dose by a 30-minute intravenous injection or by continuous infusion of 24 hours. **B:** Cortical concentration of gentamicin and netilmicin after administration by the previously mentioned administration schedules. (Reprinted from: Verpooten GA, et al. Once-daily dosing decreases renal accumulation of gentamicin and netilmicin. *Clin Pharmacol Ther* 1989;45:22, with permission.)

(74) may contribute to this risk. Three components of the septic state—renal hypoperfusion, endotoxemia, and hyperthermia—have been identified as factors contributing to the heightened risk of nephrotoxicity during aminoglycoside therapy (34–36). Renal hypoperfusion (34,81) and endotoxemia (32,162) are associated with increased accumulation of drug in renal cortex; however, this factor alone does not explain the increased risk.

Of those risk factors that are potentially modifiable by the clinician, the most important are daily drug dose, interval of dosing, and the duration of therapy. A direct relationship between total dose (daily dose plus duration of therapy) and nephrotoxicity has been consistently found in experimental animals (25,44,48,52,67) and in humans (9,15,153,154,161). Animal studies have shown that the same dose of a drug administered in two or three divided doses leads to greater renal accumulation of the drug and greater nephrotoxicity than if it was given as a single dose (163,164). Two trials in humans found that the dosage schedule had a critical effect on the renal uptake of gentamicin, netilmicin (165), amikacin, and tobramycin (166). The study was carried out in patients with normal renal function (serum creatinine between 0.9 and 1.2 mg per dL, proteinuria lower than 300 mg per day) who had renal cancer and submitted to nephrectomy. Before surgery patients received gentamicin (4.5 mg per kg per day), netilmicin (5 mg per kg per day), amikacin (15 mg per kg per day), or tobramycin (4.5 mg per kg per day), as a single injection or as a continuous intravenous infusion over 24 hours. The single-injection schedule resulted in a 30% to 50% lower cortical drug concentration of netilmicin, gentamicin, and amikacin compared with administration by continuous infusion (Figs. 42-2 and 42-3). For tobramycin, in humans as well as in rats, no difference in renal accumulation could be found, indicating the linear cortical uptake of this particular aminoglycoside. Administration of drug by continuous IV infusion carries the highest risk of nephrotoxicity with respect to gentamicin, tobramycin, and netilmicin but not amikacin (76,164,167). These observations have stimulated studies in humans to assess the antimicrobial efficacy of once per day dosing with an aminoglycoside administered alone or in combination with a β-lactam antibiotic (168–171).

Several meta-analyses pooled the data of individual randomized control trials (RCT) (Table 42-2) (172–181), including a meta-analysis specifically of the studies in immunocompromised patients (181). It is apparent that only the meta-analyses that combine the results of the individual RCT by means of a fixed-effects model yielded significant results in favor of less nephrotoxicity in the single daily dose regimens. However, given the inhomogeneity of the study designs and the different aminoglycoside used, it seems prudent to use the random-effects model to combine the individual studies. The

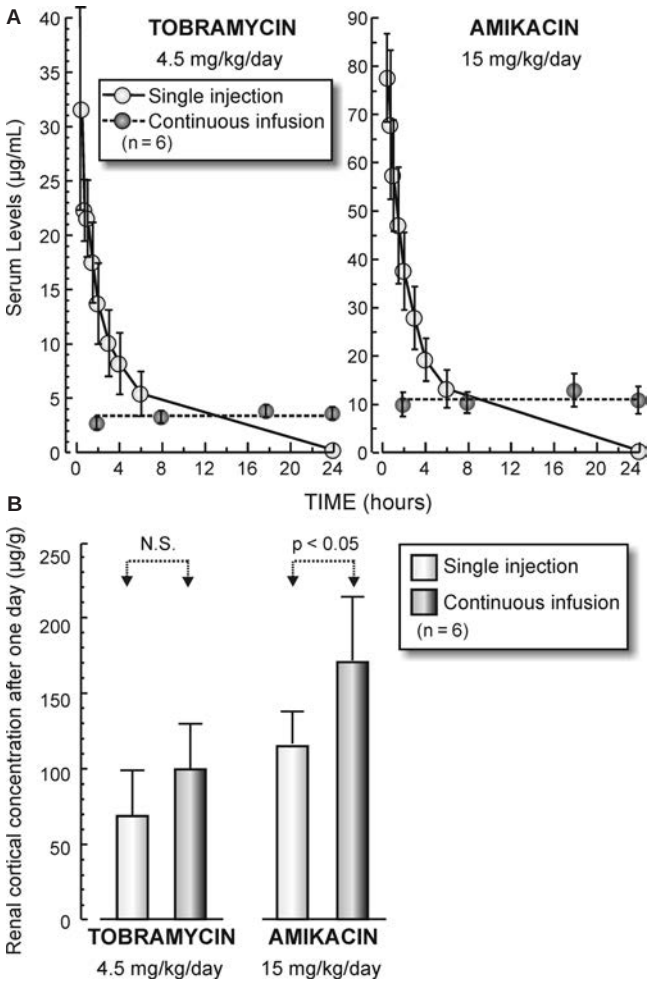

FIGURE 42-3. A: Course of serum concentrations of tobramycin and amikacin after administration of the dose by a 30-minute intravenous injection or by continuous infusion of 24 hours. **B:** Cortical concentration of tobramycin and amikacin after administration by the previously mentioned administration schedules. (Reprinted from: De Broe ME, Giuliano RA, Verpooten GA. Influence of dosage schedule on renal cortical accumulation of amikacin and tobramycin in man. *J Antimicrob Chemother* 1991;27[Suppl C]:41, with permission.)

meta-analyses that used this technique did not show a significant difference in the two dosing regimens. Nevertheless, in all analyses the single daily dose regimen was associated with a decrease in nephrotoxicity. Even the most recent prospective study (182) evaluating the efficacy and nephrotoxicity of once-daily administration of gentamicin versus multiple-daily administration in 52 children could not show a difference in incidence of nephrotoxicity in both groups. Although a decrease in nephrotoxicity rates in once-daily dose regimens has not been established, extended interval dosing strategies have never been associated with an increased risk of nephrotoxicity. The main reason why the majority of acute care hospitals (183) have adopted this strategy is that once-daily dosing provides a cost-effective method for administration of aminoglycosides by reducing workload among service personnel and by reducing or even eliminating the need for therapeutic drug monitoring (184,185).

Volume depletion (187), hypokalemia (188), hypomagnesemia (189), and metabolic acidosis (190) all carry increased risk for aminoglycoside nephrotoxicity. In the case of volume depletion and hypokalemia, the increased risk appears to be related to increased accumulation of drug in renal cortex (74). The mechanism underlying the increased risk associated with hypomagnesemia has not been definitively established but may relate to the competition between divalent cations and the cationic aminoglycoside antibiotics critical for membrane-binding sites (191). In the case of metabolic acidosis, the reduced pH promotes increased protonation of aminoglycoside antibiotics and augments the reactivity of these organic polycations with membrane anionic phospholipids (58,97,115).

Finally, the risk of nephrotoxicity has been shown to be augmented when aminoglycoside antibiotics are administered in conjunction with certain drugs and pharmaceutical agents, some of which have intrinsic nephrotoxicity potential. These include amphotericin B (171), cephalothin but not third-generation cephalosporins (192), vancomycin (195,194), cisplatin (195), furosemide (196), calcium channel blockers (197), radiocontrast agents (198), and nonsteroidal antiinflammatory drugs (199). Many of these synergistic interactions have been identified in animal studies so that the relevance of these observations to humans remains to be established. Nevertheless, prudence dictates that potentially nephrotoxic drugs should be avoided if possible in patients who are receiving or have recently completed therapy with aminoglycoside antibiotics.

The prevention of aminoglycoside nephrotoxicity requires that these drugs be used only for well-defined indications and that they be prescribed in the appropriate dose and for the appropriate duration to achieve the therapeutic goal. Dosing based on individualized drug pharmacokinetics derived from measurements of serum drug concentration would appear to be a rational approach. Unfortunately, prospective studies have failed to demonstrate that dosing based on drug pharmacokinetics reduces the incidence of nephrotoxicity (200). Indeed, eight prospective, randomized controlled trials specifically designed to investigate the effect of pharmacokinetic dosing (201) on aminoglycoside expression of nephrotoxicity could be identified from the literature (202–209). These individual studies have been unable to detect any change in the incidence of this adverse event. Nevertheless, close monitoring of serum drug concentration is still warranted, especially in high-risk patients to ensure that therapeutic concentrations are achieved. Even when those factors known to influence risk are absent, or have been minimized or eliminated, aminoglycoside nephrotoxicity will still occur in a certain percentage of appropriately dosed patients. These patients exhibit excessive renal accumulation of drug or increased sensitivity to a given level of drug accumulation (210). The clinician must be constantly alert to the possibility of aminoglycoside nephrotoxicity and monitor all patients on aminoglycoside therapy for this potential complication. The intensity of monitoring is dictated in part by the relative risk factors present. At a minimum, frequent measurements of serum creatinine concentration, generally every 2 to 3 days, should be performed. In high-risk patients, daily creatinine clearances and urinalysis may be required to detect early signs of toxicity before a rise in serum creatinine concentration or serum trough level of drug becomes evident. If renal injury occurs, then the drug should be stopped if possible or dosage should be reduced to prevent the accumulation of drug in serum and further toxic injury related thereto. Careful attention must be paid to maintaining fluid and electrolyte balance and avoiding potential insults to the kidney related to renal hypoperfusion or exposure to other potential nephrotoxins. Even when nephrotoxicity is recognized early and the drug is discontinued, renal failure may progress over the next 5 to 10 days, with the serum creatinine and blood urea nitrogen (BUN) rising to disturbingly high levels, where they may remain for a number of days before renal function slowly begins to improve. No specific therapy for hastening recovery has been identified to be effective in humans. In an animal model, epidermal growth factor was shown to accelerate recovery (211). The prognosis for recovery of renal function is generally good except in those

TABLE 42-2

META-ANALYSIS OF THE INCIDENCE OF NEPHROTOXICITY IN SINGLE DAILY DOSING VERSUS MULTIPLE DOSING OF AMINOGLYCOSIDES

Author	N of RCT	Method	Results (95% CI)
Blaser and König, 1995 (172)	24	Summation	RR 0.82
Galloe et al., 1995 (173)	16	Not given	RR 1.00 (0.98–1.02)
Barza et al. 1996 (174)	21	Random-effects model	RR 0.78 (0.57–1.07)
Munckhof et al., 1996 (175)	15	Random-effects model	RD −1.3% (−5%−3.1%)
Ferriols-Lisart and Alos-Alminan, 1996 (176)	18	Fixed-effects model	OR 0.60 (0.40–0.86)
Freeman and Strayer, 1996 (177)	15	Fixed-effects Peto	OR 0.70 (0.51–0.94)
Hatala et al., 1996 (178)	13	Random-effects model	RR 0.87 (0.60–1.26)
Ali and Goetz, 1997 (179)	26	Random-effects model	RD −0.18% (−0.99%–3.75%)
Bailey et al., 1997 (180)	22	Random-effects model	RD −0.6% (−2.4%–1.1%)
Hatala et al., 1997 (181)	4	Random-effects model	RR 0.78 (0.31–1.94)

OR, odds ratio; *RD*, risk difference; *RR*, risk ratio.
(Adapted from: Verpooten GA, Tulkens PM, Molitoris BA. Aminoglycosides and vancomycin. In: De Broe ME, Porter GA, Bennett VM, eds. *Clinical nephrotoxins—renal injury from drugs and chemicals,* 2nd ed. Dordrecht, The Netherlands: Kluwer Acadmic Publishers, 2003:151.)

cases where the underlying disease exposes the kidney to persisting or recurrent insults related to sepsis, hypotension, and hypoperfusion.

β-LACTAM ANTIBIOTICS

The β-lactam antibiotics comprise the penicillins, cephalosporins, and carbapenems. ARF has been observed with this class of antibiotics as a result of acute proximal tubular cell necrosis or allergic interstitial nephritis. Studies in animals have established the relative nephrotoxicity potentials of β-lactam antibiotics as cephaloglycin > cephaloridine >> cefaclor > cefazolin > cephalothin >>> cephalexin, ceftazidime, and penicillins, which do not exhibit clinical nephrotoxicity (212). The selective toxic potential of β-lactam antibiotics toward renal proximal tubular cells appears to be causally linked to their concentrative uptake by the organic anion transport system and their intrinsic reactivity toward sensitive intracellular target proteins (212,213). The importance of the organic anion transport system to the nephrotoxic potential of these agents is supported by the observations that (a) toxicity is restricted to β-lactams that are secreted by this transport system, (b) toxicity can be prevented by inhibition of organic anion transport, and (c) maneuvers that increase the intracellular uptake of drug augment toxicity (212,213). The product of intracellular drug concentration and time, defined as the area under the curve (AUC), is an important determinant of toxicity. Among the cephalosporins, the greatest AUC is observed with cephaloridine (212,213). This agent is readily transported into proximal tubular cells across the basolateral membrane; however, its egress across the apical membrane is retarded due to the fact that cephaloridine is a zwitterion and the cationic moiety impedes its permeation across the luminal membrane (214). Therefore, at equivalent doses, the AUC for cephaloridine is significantly higher than that of other cephalosporins. Cephaloglycin, the most nephrotoxic of the cephalosporins released for clinical use, has a renal cortical AUC only one-fifth that of cephaloridine (215). The greater nephrotoxicity of cephaloglycin reflects the fact that it is far more reactive than cephaloridine toward sensitive intracellular target proteins (212,216). Three molecular mechanisms have been implicated in the pathogenesis of cephaloridine nephrotoxicity: (a) lipid peroxidation (217), (b) competitive inhibition of mitochondrial carnitine transport and fatty acid oxidation (218,219), and (c) inhibition of mitochondrial respiration consequent to inactivation by acylation of mitochondrial anionic substrate transporters (220,221).

In the case of the other nephrotoxic β-lactam antibiotics, the pathogenesis of toxicity appears to be linked primarily to depression of mitochondrial respiration. This conclusion is supported by the following observations from *in vivo* animal studies (212,213,215,220,221):

1. The nephrotoxic potential of β-lactams correlates with the magnitude of inhibition of mitochondrial respiration.
2. Irreversible inhibition of mitochondrial respiration occurs within 1 hour after administration of a nephrotoxic dose.
3. Inhibition of respiration precedes the appearance of ultrastructural mitochondrial damage that resembles ischemic and cyanide injury.

Although only a limited number of β-lactam antibiotics cause toxic injury after *in vivo* exposure, many of these agents exhibit the capacity to inhibit *in vitro* mitochondrial respiration, especially that component supported by succinate (222). Inhibition of mitochondrial respiration is observed within 5 minutes of *in vitro* drug exposure. Increasing the concentration of succinate reverses the inhibition, presumably as a consequence of competitive displacement of drug from the mitochondrial membrane anionic carrier. However, as the exposure of mitochondria to drug is augmented by raising the product of drug concentration and time, inhibition of mitochondrial respiration becomes progressively irreversible, which has been attributed to drug-induced acylation and inactivation of

the transporter (212,213). The rank order of cephalosporins with respect to their potential to acylate target proteins *in vitro* is ceftazidime > cefaclor > cephaloglycin > cephalothin > cephaloridine > cefazolin >> cephalexin and several penicillins (212,213). This order is at variance with their *in vivo* nephrotoxicity potential, which is cephaloglycin > cephaloridine >> cefaclor > cefazolin > cephalothin >>> cephalexin, ceftazidime, and the penicillins. The explanation for the differences between the *in vitro* and *in vivo* toxicity potentials of these drugs resides in the important role of concentrative uptake of these drugs into intact proximal tubular cells by the organic anion transport system. Although ceftazidime and cefaclor exhibit high acylation activity *in vitro*, the AUC of these agents is low (only 7% that of cephaloridine and only 37% that of cephaloglycin), and this severely restricts their interaction with the mitochondrial anion transporter (212,213). Mitochondrial injury also has been implicated as the major mechanism of nephrotoxicity caused by imipenem (223,224). This drug is marketed in combination with cilastin, which inhibits the enzymatic breakdown of imipenem by cytoplasmic and brush-border dihydropeptidase and also inhibits its nephrotoxicity.

The therapeutic–nephrotoxic ratio of these agents is much more favorable than that of aminoglycoside antibiotics. The incidence of serum creatinine elevations is difficult to say with certainty, but severe nephrotoxic ARF is uncommon (225,226). Similar to other antibiotics, high doses and prolonged therapy elevate the risk of nephrotoxicity. In animal studies, the incidence and severity of toxicity associated with β-lactam antibiotics were augmented by combined therapy with aminoglycoside antibiotics (227), by renal ischemia (228), and by endotoxemia (229). In three prospective studies in human subjects, the combination of an aminoglycoside antibiotic with cephalothin was associated with a significantly higher incidence of nephrotoxicity (230–232). Early reports suggested a possible interaction between several second-generation cephalosporins and aminoglycoside antibiotics (233). In contrast, a recent prospective study provides no evidence that combination therapy with third-generation cephalosporins and an aminoglycoside antibiotic potentiates the risk of nephrotoxicity (171).

The diagnosis of nephrotoxic ARF secondary to β-lactam antibiotics is suggested by the appropriate clinical setting in combination with a urine sediment and urinary indices typical of acute tubular cell necrosis. Establishing the precise diagnosis may be difficult in the presence of septicemia, hypotension, or other nephrotoxic drugs. It should be kept in mind that β-lactam antibiotics also cause ARF secondary to allergic interstitial nephritis (234). The pattern of the rise in the blood urea nitrogen (BUN) and serum creatinine may be indistinguishable from that seen with acute tubular cell necrosis. The presence of large numbers of red and white blood cells in the urinary sediment, especially if associated with eosinophiluria and systemic signs of hypersensitivity (rash, fever, and eosinophilia), strongly suggests the diagnosis of allergic interstitial nephritis. However, in many patients, these clues are equivocal so that it may be necessary to perform a kidney biopsy to establish the correct diagnosis.

VANCOMYCIN

Vancomycin use in clinical medicine has increased significantly in recent years as a consequence of the rise in the incidence of methicillin-resistant staphylococcal infections. Because this antibiotic is poorly absorbed from the gastrointestinal tract, it is usually administered intravenously for the treatment of systemic infections. Vancomycin is not appreciably metabolized, and it is excreted essentially entirely by the kidneys primarily by glomerular filtration, as there is no evidence that the drug undergoes tubular absorption or secretion (235). Therefore, drug dosing must be modified in subjects with renal failure. Animal studies demonstrated that vancomycin had nephrotoxic and ototoxic potential (236). Early clinical experience in human subjects revealed a significant incidence of nephrotoxicity, which in retrospect may have been due to impurities generated during the initial manufacturing process (237). More recent reports indicate that the incidence of nephrotoxicity associated with vancomycin ranges between 0% and 7% when given as sole therapy (238). Animal studies initially suggested that vancomycin and aminoglycoside antibiotics interacted synergistically to cause ARF (239). Recent reports indicate that a similar interaction occurs in humans (194,195). Indeed, in a meta-analysis the incidence of nephrotoxicity associated with combination therapy was 13.3% greater than therapy with vancomycin alone. In a prospective study, comparing continuous versus intermittent infusion of vancomycin in severely ill patients, Wysocki et al. (240) found a significant rise in serum creatinine during treatment only in those patients who received vancomycin with other antibiotics including aminoglycosides. Monitoring vancomycin serum concentrations is not cost-effective in preventing vancomycin-induced nephrotoxicity in patients with normal renal function, since the correlation between serum levels and antibacterial efficacy or toxicity remains controversial (241). It should be noted that vancomycin has been reported to cause allergic interstitial nephritis (242); however, this appears to be an uncommon complication. Teicoplanin, a glycopeptide antibiotic similar to vancomycin, is devoid of nephrotoxicity.

SULFONAMIDE ANTIBIOTICS

The sulfonamide antibiotics and their metabolites are excreted primarily by the kidneys by a process involving glomerular filtration, tubular absorption, and tubular secretion (243). The high incidence of nephrotoxic ARF observed with the first-generation sulfonamides was due to their low solubility and the resultant precipitation of drug in the form of crystals that caused intratubular obstruction (244). Sulfadiazine, a poorly soluble sulfonamide, continues to be used today in combination with pyrimethamine for the treatment of *Toxoplasma encephalitis*; nephrotoxicity manifested as hematuria, crystalluria, renal colic, and ARF may complicate therapy in 5% of cases (245,246). These abnormalities usually subside with hydration and alkalinization of the urine.

Trimethoprim-sulfamethoxazole is administered intravenously in high concentrations as therapy for *Pneumocystis carinii* pneumonia. Although the solubility of sulfamethoxazole is high, ARF secondary to crystal deposition of the parent drug or a metabolite has been reported (247,248). More commonly, the elevation of serum creatinine observed in patients treated with this combination drug reflects inhibition of tubular secretion of creatinine by trimethoprim (249,250). This effect is more pronounced in subjects with baseline elevation of the serum creatinine secondary to underlying chronic renal insufficiency. Failure of the BUN to rise in proportion to the rise in serum creatinine should call attention to the correct diagnosis.

Sulfonamides including sulfamethoxazole also have been implicated in causing acute hypersensitivity reactions and ARF secondary to allergic interstitial nephritis (234).

ANTIFUNGAL AGENTS

Amphotericin B is widely used as the drug of choice for the therapy of systemic fungal infections, especially in immunocompromised patients (251,252). Unfortunately, the clinical application of this drug is accompanied by a number of

dose-dependent toxic side effects, the most serious of which is ARF (253,254). Amphotericin B is a polyene that consists of a large lactone ring with seven conjugated double bonds, seven hydroxyl groups, and a sugar moiety. It exhibits the propensity to bind to membrane sterols and form membrane pores, which in mammalian cells are estimated to be composed of eight molecules of cholesterol alternating with eight molecules of drug (255). The resultant increase in membrane permeability to small electrolytes is thought to be a dominant factor in the toxicity of the drug. Amphotericin B binds preferentially to ergosterol, the major sterol of fungi, and this presumably explains the selective toxicity of this and similar drugs for fungi (256).

The reason amphotericin B causes nephrotoxicity in humans and experimental animals is not apparent from its pharmacokinetics (257,258). Because amphotericin B is poorly transported across the gastrointestinal tract, it must be administered intravenously. Its volume of distribution is about 4 L per kg. Up to 95% of drug in serum is bound, primarily to β-lipoproteins. The major depot site for amphotericin B is the liver, where up to 41% of administered drug can be recovered compared to 6% in the lung and 2% in the kidney. The elimination of amphotericin B from serum can be described by a triexponential curve, the half-lives of which are 24 hours, 48 hours, and 15 days, respectively. Less than 10% of administered drug is recovered in the urine, and there are no known metabolites.

Although the kidney is not a major route of amphotericin B elimination, it is the major site of toxicity, the incidence of which is influenced by daily drug dose, duration of therapy, and the presence of potentiating factors (259,260). The clinical expression of amphotericin B nephrotoxicity is dominated by the appearance of azotemia and creatininemia, which may occur early in the course of drug therapy (260–262) and reflects depression of renal blood flow and GFR secondary initially to a reversible rise in renal vascular resistance. With prolonged therapy, depression of renal function may persist as a consequence of injury to tubular epithelium (263) and possibly the renal vasculature (264). A variety of abnormalities of tubular function may be seen as well. These include incomplete distal renal tubular acidosis (265), hypokalemia and hypomagnesemia secondary to renal tubular wasting of these cations (266,267), and loss of urine concentrating capacity (268). The urinary sediment frequently contains evidence of microscopic hematuria, pyuria, and cylinduria. While most of these abnormalities are reversible after the drug is discontinued, full recovery may be delayed for a number of months. Chronic renal insufficiency may occur with prolonged or multiple courses of therapy.

Insight into the pathogenesis of amphotericin B nephrotoxicity has been gleaned from studies in experimental animals (269). It has been shown that intravenous administration of amphotericin B elicits an acute depression of renal blood flow and GFR in association with an increase in renal vascular resistance that is not mediated by the renal nerves, by angiotensin II, by endothelium-dependent factors, or by tubular glomerular feedback (270–273). These hemodynamic alterations have been shown to be modifiable by a variety of interventions including administration of calcium channel blockers (274), a selective dopamine-1 receptor agonist (275), saline loading (276,277), atrial natriuretic peptide (273,278), and theophylline suggesting its direct vasoconstrictive effect (273). Depolarization of vascular smooth muscle consequent to the formation of membrane pores was postulated as the basic mechanism by which amphotericin B augmented renal vascular resistance (269,273). Amphotericin B also induces tubular dysfunction in the rat that mimics alterations observed in humans (278). The dominant site of tubular injury in the rat is the inner stripe of the outer medulla (279), a zone that functions on the verge of hypoxia even under physiologic conditions. Investigators have postulated that hypoxic injury to this zone results from the demand for increased oxygen to support increased sodium transport stimulated by the heightened influx of sodium across the apical membrane made permeable by amphotericin B at a time when the supply of oxygen is reduced as a consequence of amphotericin B–induced reduction in renal blood flow (279,280).

TABLE 42-3

RISK FACTORS FOR AMPHOTERICIN B NEPHROTOXICITY

Daily drug dose
Duration of therapy
Chronic renal insufficiency
Sodium depletion
Renal hypoperfusion
Concomitant drug therapy/exposure
Diuretics
Aminoglycosides
Cisplatin
Radiocontrast agents
Cyclosporine

A contributory factor to the toxicity of amphotericin B is deoxycholate, the vehicle in which the drug is suspended. Deoxycholate was shown to be cytotoxic to renal tubular cells *in vitro* (281). Various alternate vehicles and formulations for suspending amphotericin have been investigated in an attempt to reduce toxicity. Administration of amphotericin B in liposomes (257,282) or with other lipid preparations (283) has been reported to reduce the nephrotoxicity of this agent without compromising its therapeutic efficacy.

A number of factors have been identified as potentiating the risk of amphotericin B nephrotoxicity (Table 42-3), and the physician should strive to eliminate or minimize these risk factors whenever possible. Fisher et al. (259) observed a 1.8-fold increase in risk of nephrotoxicity for each 0.1 mg per kg increment in the daily dose of amphotericin B. The risk of nephrotoxicity was increased 15.4-fold in patients who had an elevated serum creatinine prior to the start of amphotericin B therapy and 12.5-fold in patients who received diuretics during the course of amphotericin B therapy. The latter observation may reflect the powerful influence of sodium depletion on this complication. Sodium loading has been shown to minimize amphotericin B nephrotoxicity (260) so that special attention should be paid to ensure that the patient is optimally volume-repleted prior to the initiation of therapy with this agent (Fig. 42-4).

ANTIVIRAL AGENTS

Acyclovir is a potent antiviral agent effective in the treatment of infections caused by herpes simplex viruses (285). Its major route of excretion is the kidney, which accounts for approximately 80% of total body clearance (286). Given the fact that the renal clearance of acyclovir exceeds the creatinine clearance by severalfold, it follows that a substantial fraction of drug must be eliminated by tubular secretion, which promotes the attainment of tubular fluid concentrations in excess of the drug's estimated solubility of 1.3 mg per L (286). Approximately 85% of the drug recovered in the urine is unchanged; the remainder is recovered as the principal metabolite, 9-carboxymethoxymethylguanine (286). In several large series, acyclovir has been reported to cause elevation of the BUN and serum creatinine in 10% to 15% of cases (287,288). In one series of 23 patients, an incidence of acute renal

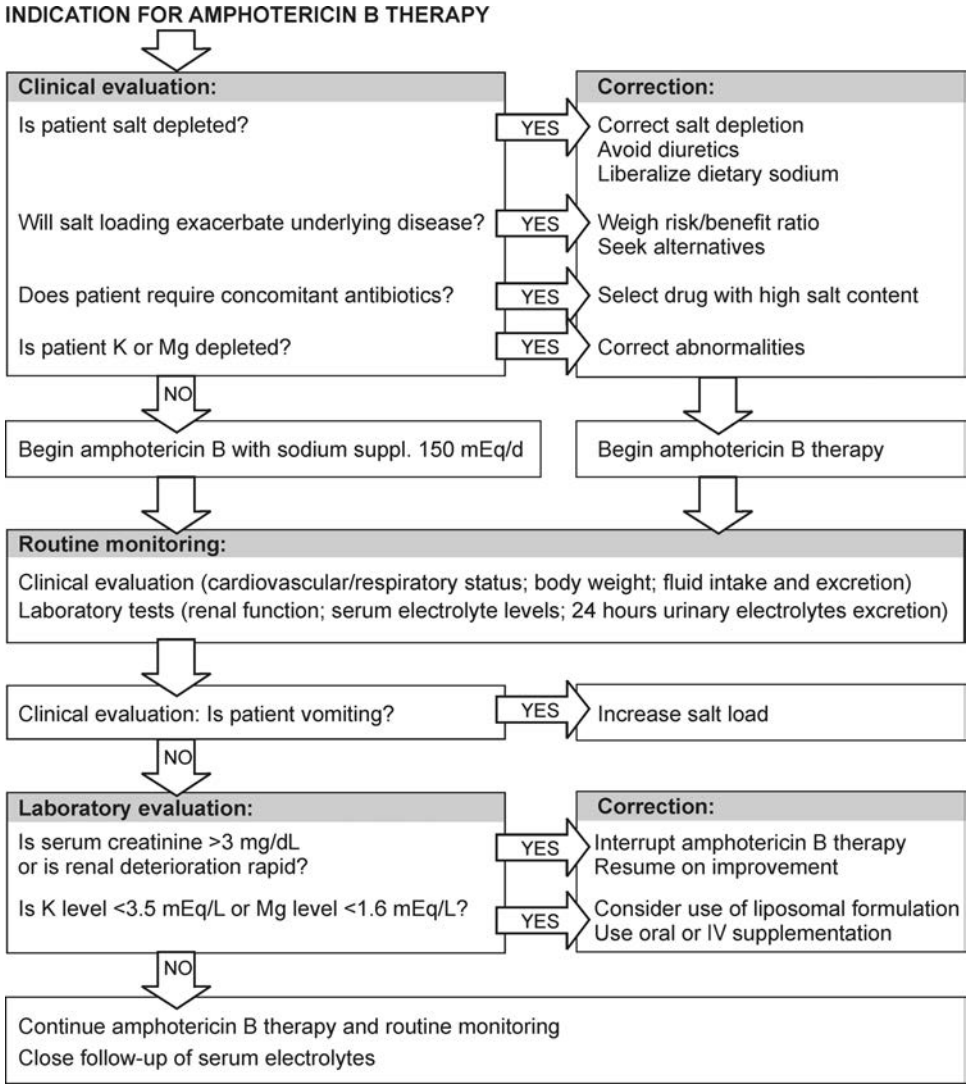

FIGURE 42-4. Proposed approach for management of amphotericin B therapy. (Reprinted from: Bernardo JF, Sabra R, Vyas SJ, et al. Amphotericin B. In: DeBroe ME, Porter GA, Bennett VM, eds. *Clinical nephrotoxins: renal injury from drugs and chemicals*. Dordrecht, The Netherlands: Kluwer Acadmic Publishers, 2003:199, with permission.)

insufficiency of 48% was reported (289). The clinical expression of nephrotoxicity may range from asymptomatic azotemia to renal colic with nausea and vomiting. Examination of the urinary sediment may reveal microscopic hematuria, pyuria, and birefringent crystals. The pathogenesis of acyclovir-induced ARF has been attributed to intratubular obstruction caused by precipitation of drug (288) as well as direct tubular cell toxicity (290,291). High drug dose, rapid drug infusion, and low urine volume predispose to the development of ARF. In about half the cases, the onset of azotemia occurs during the first few days of therapy; it is usually transient and frequently resolves in response to increased fluid intake even when drug therapy is continued. Severe renal failure has been reported, however, even in patients who were prehydrated (292). Fortunately, even in these cases, renal function usually recovers. In the rat infusion of acyclovir caused a decrease in whole kidney and single nephron GFR and renal plasma flow in association with an increase in renal vascular resistance (293).

Foscarnet is an antiviral agent that is being used with increasing frequency for the treatment of cytomegalovirus infections and acyclovir-resistant herpes virus infections, particularly in immunocompromised individuals (294,295). This agent is excreted unchanged in the urine by glomerular filtration and tubular secretion (296). Major complications of therapy include ARF, often severe and of uncertain pathogenesis (297,298), and electrolyte abnormalities that include hypercalcemia, hypocalcemia, hypophosphatemia, hypomagnesemia, and hypokalemia (246,299). ARF secondary to crystal deposition has been described as well (300). Volume expansion by infusing saline has been reported to greatly reduce the incidence and severity of ARF (298,301).

Cidofovir is an antiviral nucleotide analog indicated for the treatment of cytomegalovirus retinitis in patients with acquired immunodeficiency syndrome (AIDS) (302). The drug is eliminated primarily by the kidneys by glomerular filtration and tubular secretion via the organic acid transport system (303). The major complication of therapy with this agent is nephrotoxic injury to proximal tubular cells; but, this complication can be significantly reduced by the coadministration of probenecid, which presumably blocks the renal tubular uptake of cidofovir and decreases the renal elimination of the agent (304).

PENTAMIDINE

Pentamidine has been used for the treatment of *P. carinii* pneumonia since the 1950s. In the preacquired immunodeficiency syndrome (AIDS) era, pentamidine therapy was complicated by ARF in about 25% of cases (305). The incidence of ARF in patients with AIDS treated with pentamidine appears to be substantially higher than this figure, and it is unexplained by greater drug dose, longer duration of therapy, or concomitant therapy with other potentially nephrotoxic agents

(306). The mechanism of pentamidine-induced ARF has not been established. Although pentamidine is concentrated in the kidney (307–309), pharmacokinetic studies utilizing a high-performance liquid chromatography assay indicate that <5% of the drug is excreted in the urine each day (308,310). The mechanism of renal elimination is not known.

Pentamidine nephrotoxicity presents as nonoliguric ARF beginning 7 to 10 days after the start of therapy. Urinalysis reveals mild proteinuria, microscopic hematuria, pyuria, and cylinduria. Most patients experience mild to moderate ARF, but occasionally severe renal failure necessitating dialysis therapy occurs. In one series, azotemia was accompanied by hyperkalemia in association with a picture of hyperchloremic metabolic acidosis (306). Renal magnesium wasting has been observed in several cases (311). Recovery of renal function usually begins within a week after stopping drug therapy and in most cases returns to baseline within several weeks.

Chronic renal insufficiency, volume depletion, cumulative dose, and concurrent use of other nephrotoxic drugs heighten the risk of pentamidine nephrotoxicity in humans (250,312). In the rat, pentamidine nephrotoxicity was potentiated by amphotericin B, tobramycin, and cyclosporine, whereas it was ameliorated by fosfomycin, D-glucaro-1,5-lactam, verapamil, and enalapril (313).

NEPHROTOXICITY OF CYCLOSPORINE

Since its clinical use as an immunosuppressant drug in the early 1980s, cyclosporine A (CsA) has tremendously improved the outcome of solid organ (kidney, heart, liver, lung, and pancreas) and bone marrow transplants (314,315). In more recent years, the immunosuppressive properties of CsA have also been used in the treatment of autoimmune diseases (psoriasis, uveitis, and severe rheumatoid arthritis) as well as steroid-resistant nephrotic syndrome.

The major side effect of CsA is its renal toxicity. Although in preclinical animal studies, renal side effects were not observed (316–318), early reports from clinical practice revealed the nephrotoxicity of CsA (319–321). Since that time, numerous observations have added to the overwhelming evidence of three different forms of cyclosporine nephrotoxicity (322–330). This toxicity is not restricted to only the field of kidney transplantation but has also unequivocally been documented in heart (331,332), bone marrow (332), liver (333,334), and pancreas transplantation (335), as well as in a variety of autoimmune diseases (336–339), in which *a priori* rejection of the kidney graft is absent.

Based on experimental data and clinical experience, this chapter intends to summarize our present knowledge about the three different forms of cyclosporine nephrotoxicity: ARF (with sometimes protracted course evolving to chronicity); the hemolytic–uremic-like syndrome; and chronic irreversible nephrotoxicity.

Clinical Pharmacology of Cyclosporine A

The selective immunosuppressive effects of CsA were described for the first time in 1976 (340). CsA is a lipophilic fungal peptide with a molecular weight of 1.203 daltons, consisting of 11 amino acids (Fig. 42-5). As a consequence of its high hydrophobicity, CsA interacts easily with phospholipid bilayer membranes, while some CsA amino acids form a hydrophilic active immunosuppressive site (341).

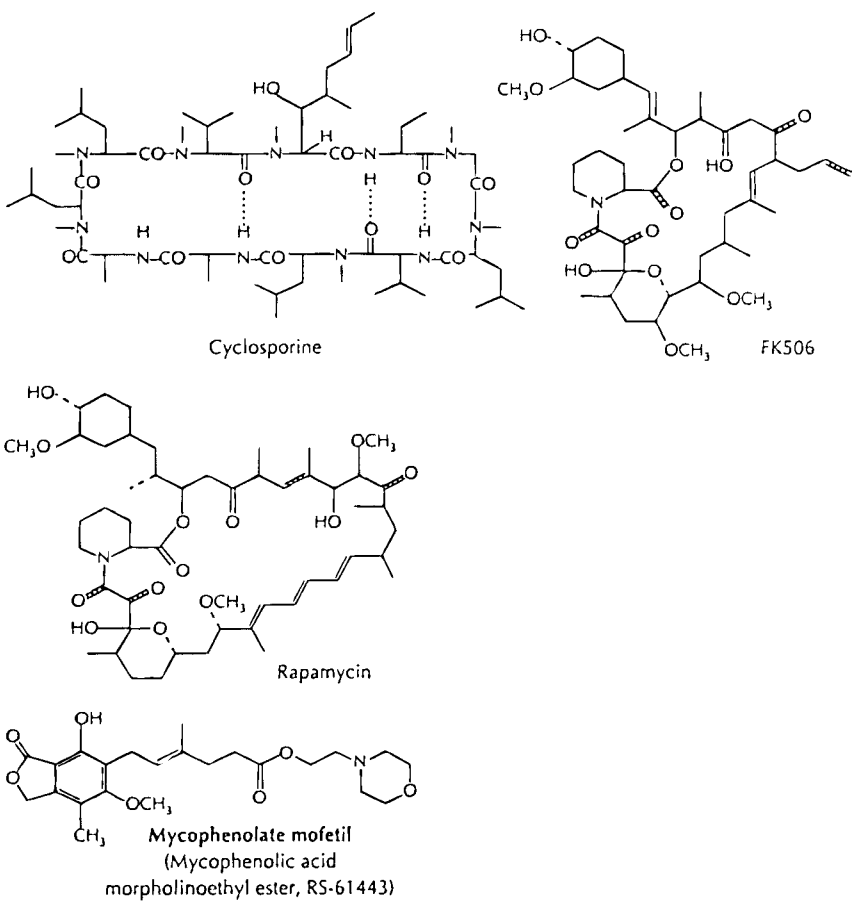

FIGURE 42-5. Structure of new immunosuppressive drugs.

CsA is available for clinical use in three formulations: one stabilized in castor oil (Cremophor) for intravenous injection, the second as a microemulsion formulation (Neoral), and a third formulation as soft gelatin capsules. The pharmacokinetic profile of the conventional CsA formulation (Sandimmune) exhibits a high degree of interpatient and intrapatient variability (342–344). Pharmacokinetic studies in healthy subjects and renal transplant recipients have shown that the more recent microemulsion formulation of CsA possesses superior pharmacokinetic characteristics, with more complete and predictable absorption of the drug from the gastrointestinal tract, resulting in less pharmacokinetic variability (345,346). In clinical trials, the microemulsion formulation of CsA increased drug exposure and reduced the incidence of acute rejections, without incremental toxicity (347–349).

In the circulation, CsA is mainly bound to high-, low-, or very low-density lipoproteins and to chylomicrons (350). Only a small fraction of CsA circulates unbound. The volume of distribution ranges from 4 to 8 L per kg of body weight (351). Due to its hydrophobicity, CsA dissolves extensively in cell membranes and tissue lipids (352). CsA accumulates in lymphocytes, liver, kidney, heart, lung, and neural and muscle cells (353).

CsA has a median half-life of 6.4 to 8.7 hours and is predominantly eliminated by hepatic metabolism through specific isoenzymes of the cytochrome P-450 superfamily (354). More than 90% of the parent compound and the metabolites are excreted in the bile, while only 6% is eliminated by the kidneys (352). Significant individual differences in CsA clearance rates (354,355), with a median value of 12 mL per minute per kg, can be explained by wide genetic differences among individuals in the content of cytochrome P-450 isoenzymes, as well as a variety of other factors such as patient age (356), the functional status of the liver (357), and interactions with other drugs (358). Independent of the intrinsic nephrotoxicity of CsA, its complex clinical pharmacokinetic profile entails the potential hazard of incorrect dosing, ultimately resulting in irreversible renal damage or acute rejection of the graft.

To optimize CsA therapy, monitoring of CsA trough levels in serum, plasma, or whole blood by means of radioimmunoassays or high-performance liquid chromatography is common clinical practice (359). Monitoring CsA trough levels has limited value, however, for the assessment of adequate immunosuppression or predicting protection from nephrotoxicity (360,361). The AUC is more informative and a better indicator of drug exposure (362) but is expensive and time consuming. Large-scale clinical trials using Neoral C_2 monitoring in renal and liver transplant recipients have demonstrated low acute rejection rates and good tolerability with a low adverse event profile to at least 1 year posttransplant (363–367). Neoral C_2 monitoring provided a more accurate assessment of delayed and/or low absorbers of CsA in these studies. Neoral C_2 monitoring in maintenance renal transplant recipients, showed that 26% to 49% of the patients, managed by monitoring of cyclosporine trough levels, were treated with excessive doses of CsA, adversely affecting graft function (368–370). Dose reduction to optimal C_2 levels, between 600 and 800 ng per mL, in these patients, resulted in improvement of graft function, without increased risk for rejection (368). These data provide evidence that monitoring of C_2 levels may result in more adequate dosing of cyclosporine.

Immunosuppressive Mechanism of Cyclosporine A

CsA blocks the activation of T cells, mainly through inhibition of transcription of lymphokines, most notably interleukin-2 (IL-2), the main growth factor for T cells (371). By inhibiting IL-2 expression in T cells, CsA prevents helper T cells from orchestrating a response to foreign antigens.

The immunosuppressive effect of FK506 (tacrolimus) is similar to that of CsA (372), as a logical consequence of a similar molecular mechanism of action of both drugs (373) (Fig. 42-6). CsA and FK506 bind with high affinity to intracellular target proteins, called immunophilins, which possess *cis–trans* isomerase activity. These immunophilins have been identified respectively as cyclophilins in the case of CsA (374) and FK-binding proteins in the case of FK506 and rapamycin (375). The binding of CsA or FK506 is a prerequisite of their immunosuppressive potential because it has been demonstrated that the CsA- or FK506-immunophilin complex competitively binds directly to the serine–threonine phosphatase calcineurin (376,377) in the presence of Ca^{2+}. The inhibition of calcineurin by the drug–immunophilin complex results in an altered modification pattern of the cytoplasmic components of transcription factors, thereby disturbing their nuclear translocation (373).

Potential substrates for calcineurin are the nuclear factor of activated T cells (NF-AT) and the nuclear factor of immunoglobulin (light chain in B cells (NF-κB), which were both reported as being affected in their IL-2 promoter binding activity by CsA and FK506, but not by rapamycin. This could explain the similarity of the immunosuppressive effect of CsA and FK506, in contrast to rapamycin, although rapamycin shares the same affinity as FK506 for the FK-binding protein (378,379).

Experimental Nephrotoxicity of Cyclosporine A

Much of our knowledge of cyclosporine nephrotoxicity derives from experimental studies in animal models. The understanding of the pathogenesis of chronic cyclosporine nephrotoxicity has long been hampered, however, by the lack of an experimental model until a number of investigators developed a suitable animal model of chronic cyclosporine nephrotoxicity (380–384).

Functional Alterations Induced by Cyclosporine A

It has been unequivocally established that CsA profoundly alters renal and glomerular hemodynamics. Acute and/or chronic CsA administration induces a decline in GFR, with a concomitant reduction in renal blood flow and an increase in renal vascular resistance as a consequence of vasoconstriction (385–388), preferentially at the level of afferent arterioles (324). These hemodynamic effects are mainly responsible for the acute cyclosporine nephrotoxicity and probably contribute also to the pathogenesis of chronic nephrotoxicity by inducing chronic renal ischemia. The possible underlying mechanisms of this vasoconstrictor effect of CsA are discussed under Pathophysiologic Studies of Cyclosporine Nephrotoxicity, below.

Although early pathologic studies suggested that CsA is a primary tubular toxin (389), the results of clearance experiments conflict with that premise (390). CsA reduces the end proximal tubular flow rate (391) and increases proximal fractional reabsorption (392). The latter is due to an inadequate adaptive reduction in the absolute rate of proximal reabsorption (393). CsA causes a resetting of the tubuloglomerular feedback with onset at lower tubular flow rates and greater maximum response (394).

CsA-treated rats show a reduction of sodium chloride reabsorption in the distal nephron, including Henle's loop (391,392), most likely as a secondary response to the decreased

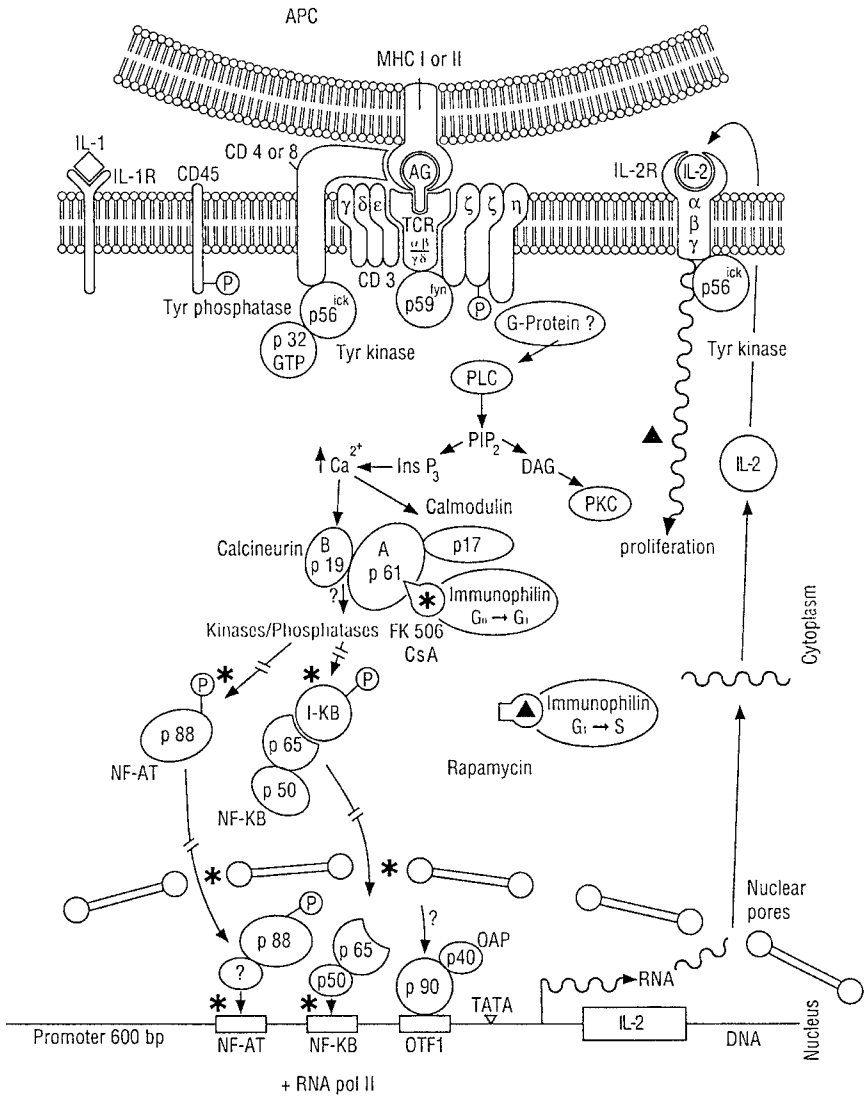

FIGURE 42-6. Cyclosporine (CsA) and FK506 both interfere, by binding to their respective immunophilins, with the function of intracellular molecules that transmit calcium-associated signals between the T-cell receptor (TCR) and the activation of lymphokine genes (interleukin-2) in the nucleus. Transcriptional regulation of interleukin-2 (IL-2) gene expression is modulated by the combination of transcription factors (e.g., NF-AT, NF-κ B, OTF-1) interacting with their corresponding recognition sites at the IL-2 promoter. These DNA/protein complexes, together with RNA polymerase II (RNA pol II), result in the antigen-inducible transcription of IL-2. Potential intervention sites for the pentameric complex (calcineurin A [p61], calcineurin B [p19], calmodulin [p17], immunophilin, drug), involving (e.g., modification and translocation) of antigen-inducible transcription factors (NF-AT [p88], NF-κ B [p50, p65]), are indicated by *asterisks*. CyA and FK506 interfere with the G_0 to G_1 transition of the cell cycle, whereas rapamycin interferes with the G_1 to S transition (indicated by a *triangle*). *AG,* antigen; *APC,* antigen presenting cell; *DAG,* diacylglycerol; *Ins* P_3, inositoltrisphosphate; *MHC,* major histocompatibility class; *Pi*P_2, phosphatidylinositolbiphos-phate; *PKC,* protein kinase C; *PLC,* phospholipase C. (Reprinted from: Baumann G. Molecular mechanism of immunosuppressive agents. *Transplant Proc* 1992;24[Suppl 2]:4, with permission.)

proximal tubular fluid delivery. This reduced sodium delivery to and reabsorption from the distal nephron results in reduced distal acidification and potassium secretory capacity through a decreased generation of a negative transmembrane potential. This explains the observed hyperkalemic metabolic acidosis in CsA-treated rats (395) as well as in CsA-treated kidney transplant recipients (396).

In summary, CsA reduces renal blood flow and GFR predominantly through vasoconstriction of the afferent arterioles. Effects of CsA on tubular function consist of increased proximal tubular reabsorption and decreased distal sodium delivery, which induce hyperkalemic metabolic acidosis.

Morphologic Alterations Induced by Cyclosporine A

The renal pathology induced by CsA is largely dose-related and time-related. For clarity, we will focus on two distinct pathologic patterns, the acute (potentially reversible) toxic injury and the chronic (essentially irreversible) nephrotoxicity induced by CsA.

At supratherapeutic levels (100 mg per kg per day PO), CsA induces mainly tubular pathology consisting of isometric vacuolization of tubular cells, accumulation of eosinophilic bodies often representing giant mitochondria, and microcalcifications in proximal tubules by 21 days (389,397–399). The effects on the proximal tubule tend to be most prominent in the S3 segment (400) and become more widespread at very high doses. These pathologic alterations are reversible after dose reduction or withdrawal of CsA. In contrast to the acute toxic injury, chronic administration of CsA (12.5 mg per kg per day) for 3 to 10 weeks induces striking morphologic alterations in the medullary rays of the cortex of the rat (380). These changes progress in time and result in areas of focal or striped interstitial fibrosis with foci of atrophic proximal tubules, which are most prominent in the subcapsular cortex (401,402). The severity of the lesion progresses with treatment and is exacerbated by salt depletion (384). Withdrawal of CsA does not reverse the observed structural changes (401).

Besides the striped interstitial fibrosis, mild glomerular endothelial damage, glomerular hypercellularity, and mesangial matrix expansion (381) are observed after long-term CsA administration. Morphometric analysis with three-dimensional reconstruction of individual glomeruli (403) shows subsets of glomeruli with small volume with significant reduction in GFR (404), alternating with hypertrophic glomeruli.

At the vascular level, scanning electron microscopy shows focal narrowing of the afferent arteriolar diameter that progresses with time of CsA treatment and parallels the decrease in inulin clearance (326). CsA nephropathy is associated with degenerative hyaline changes in the walls of afferent arteriolar-sized blood vessels (405), which can disappear after discontinuation of CsA (406,407).

Pathophysiologic Studies of Cyclosporine Nephrotoxicity

Numerous studies have focused on different pathophysiologic mechanisms of cyclosporine nephrotoxicity. We will sequentially discuss mechanisms of renal vasoconstriction, cellular and molecular mechanisms, mechanisms of matrix protein accumulation, and studies on lipid peroxidation.

Mechanisms of Cyclosporine A–Mediated Renal Vasoconstriction. As mentioned previously, administration of CsA induces a marked afferent arteriolar vasoconstriction resulting in decreased renal blood flow and GFR. The renal sympathetic nervous system has been implicated in the renal functional effects of CsA because the α-adrenergic antagonists phenoxybenzamine (385) and prazosin (408) prevent a CsA-induced fall in renal blood flow and GFR. Moreover, a significant increase in renal afferent and efferent nerve activity has been demonstrated in CsA-treated rats (409). The relevance of the activated sympathetic nervous system to the pathophysiology of cyclosporine nephrotoxicity in kidney transplantation is questionable, however, because the renal allograft is denervated. Nevertheless, increased sensitivity of the denervated organ to circulating catecholamines (409) or significant reinnervation of renal allograft after transplantation (410) is a possible explanation.

Rodent models of cyclosporine nephrotoxicity consistently show activation of the renin–angiotensin–aldosterone axis, in contrast to results in humans. Besides increased plasma renin activity in CsA-treated rats (398,411), hyperplasia of the juxtaglomerular apparatus (383,412,413), as well as elevated renin synthesis and release in juxtaglomerular cells (414,415), has been documented in experimental animals during CsA therapy. However, angiotensin-converting enzyme inhibitors show conflicting effects on renal blood flow in CsA-treated rodents, with improvement in some studies (385,416) but not in others (386,417). More recent experimental studies suggest that CsA-related chronic interstitial injury is mediated by angiotensin II, since renin–angiotensin blockade prevents CsA-induced tubulointerstitial fibrosis (418,419). However, in human cardiac and renal allograft recipients treated with CsA, plasma renin is suppressed (331,420), suggesting that the renin–angiotensin system is not of primary importance in human cyclosporine nephrotoxicity.

Hypovolemia could contribute to renal vasoconstriction with CsA therapy because CsA-treated rats have reduced plasma volume and saline expansion reverses the deficits in renal blood flow and GFR (421). Studies with furosemide (422), mannitol (423), and chronic sodium depletion (380) have demonstrated that hypovolemia potentiates cyclosporine nephrotoxicity. Sodium depletion enhanced fibrosis and the expression of TGF-β1 and matrix proteins in experimental CsA nephropathy (424). However, there is evidence implicating hypovolemia and sodium-depletion as an exacerbating rather than as a causative factor of human cyclosporine nephrotoxicity.

Much attention has been paid to the potential role of an altered eicosanoid metabolism in cyclosporine nephrotoxicity. In animal models, CsA consistently increases the generation of thromboxane A_2 (TxA_2), a potent renal vasoconstrictor (425–427), while its effects on vasodilatory prostaglandins are controversial (421,428,429). Pharmacologic manipulation of thromboxane metabolism with a specific TxA_2 receptor antagonist (430,431) or a TxA_2 synthase inhibitor (432) partially prevented the CsA-induced acute decline in GFR and renal blood flow in normal rats. The TxA_2 receptor antagonist also attenuated chronic cyclosporine nephrotoxicity in rats with renal isograft (433). The relevance of these data to human cyclosporine nephrotoxicity, however, is controversial (434,435).

Another potential mediator of CsA-induced vasoconstriction is the platelet activating factor (PAF) because it has been demonstrated that rat mesangial cells release increased quantities of PAF when incubated with CsA, and CsA-stimulated cell contraction is abolished by the PAF antagonists, BN52021 and alprazolam (436). Chronic cyclosporine nephrotoxicity is also attenuated in rats treated with the PAF antagonist BN52063 (437).

More recently, the role of endothelin, the most potent vasoconstrictor yet identified, has been advocated in CsA-induced vasoconstriction. CsA treatment has been shown to stimulate endothelin production (438,439) and promote glomerular endothelin binding *in vivo* (440). Endothelin appears to mediate CsA-induced renal vasoconstriction in the rat (441). The resulting reduced single-nephron GFR and glomerular plasma flow rate, as well as the decreased glomerular capillary pressure, were attenuated by an antiendothelin antibody (442). Similarly, the endothelin receptor antagonist BQ123 has the potential to prevent hypoperfusion and hypofiltration induced by CsA (443). Recent work additionally demonstrated that CsA selectively modulates renal messenger RNA (mRNA) expression for endothelin peptide and one of its receptor subtypes in a site-specific way (442). In humans, the endothelin receptor antagonist bosentan markedly blunted the renal hypoperfusion effect of CsA (444).

Experimental data indicate that an enhanced 5-HT2 (serotonin)-mediated vasoconstriction plays an important role in the suppression of renal blood flow (RBF) autoregulation induced by CsA, since the administration of ritanserin, a pure 5-HT2 antagonist, restored the RBF autoregulation (445). *In vivo* studies in humans demonstrated reduced basal and stimulated nitric oxide (NO) production from the endothelium of forearm resistance vessels in cyclosporine-treated renal transplant recipients (446). This suggests endothelial dysfunction, and may provide a potential mechanism to explain cyclosporine-induced hypertension.

Elevated cytosolic calcium is yet another attractive candidate to explain the CsA-induced vasoconstriction. This has been demonstrated in cultured rat mesangial cells (447) as well as in vascular smooth muscle cells (448). The augmented transmembrane Ca^{2+} influx and intracellular Ca^{2+} mobilization could account for the protective effects of calcium channel antagonists in acute (449–451) as well as chronic (452,453) cyclosporine nephrotoxicity.

In summary, the acute or subacute effects of CsA on renal hemodynamics are likely mediated by a number of vasoactive substances such as endothelin, serotonin, impaired NO production, TxA_2, and PAF. At the cellular level, CsA induces increased intracellular Ca^{2+}, resulting in contraction of vascular smooth muscle cells as well as mesangial cells. Calcium channel blockers are able to protect against these effects.

Cellular and Molecular Mechanisms of Cyclosporine Nephrotoxicity. CsA modulates mitochondrial calcium fluxes, resulting in reduced mitochondrial swelling, respiration, and calcium discharge (454,455). Additionally, CsA modulates cytosolic calcium regulation in mesangial cells (447,456).

In T lymphocytes, CsA only affects calcium-dependent pathways on T-lymphocyte activation. As stated earlier under Immunosuppressive Mechanism of Cyclosporine A, the immunophilin-ligand complex inhibits the Ca^{2+}-dependent phosphatase calcineurin, which is an important step in signal transmission pathways. The analogy of immunosuppressive effect of FK506, as well as its nephrotoxicity, has led to an attractive hypothesis stating that cyclosporine nephrotoxicity could be inherent to its immunosuppressive effect (457); a similar hypothesis was formulated with regard to FK506 (458).

The mechanisms underlying the linkage of nephrotoxic effects to immunosuppressive effects of CsA or FK506 are still

unknown. However, the blocking of T-cell activation by CsA or FK506 is an attractive explanation because it has been shown that a mononuclear cell infiltrate is part of cyclosporine nephrotoxicity (459). Alteration of the repair function of these cells could therefore be a possible mechanism inducing interstitial fibrosis.

Administration of CsA *in vivo* to rats causes a marked impairment of microsomal protein synthesis (460). Additional studies have shown a dose-dependent and time-dependent translational alteration of intracellular protein synthesis produced by cyclosporine (461).

The role of this decreased renal microsomal protein synthesis induced by CsA is speculative but could, if persistent with long-term cyclosporine treatment, alter renal cell matrix and interstitial cell interactions favoring fibrosis (461).

Recent *in vitro* studies showed that CsA induces apoptosis in tubular epithelial cells in a dose-dependent and time-dependent manner (462–465). This effect was mediated by the induction of inducible nitric oxide synthase (iNOS) (462), caspases (463), or TNF receptor super family member 6 (Fas) (464).

Mechanisms of Matrix Protein Accumulation. Interstitial fibrosis, the end point of chronic cyclosporine nephrotoxicity, results from an excessive extracellular matrix accumulation, which represents an imbalance between rates of extracellular matrix production and degradation. Cyclosporine has been shown to enhance the production of specific extracellular matrix components in mouse and rat kidney (466,467), as well as in renal cells in culture (468). In the presence of CsA, angiotensin II is known to induce interstitial collagen formation (469). Blockade of the renin–angiotensin system with angiotensin II receptor antagonists or angiotensin-converting enzyme inhibitors markedly abrogated the tubulointerstitial fibrosis without improving renal hemodynamics (418,419,470). The location of the angiotensin receptor type 1 mRNA in the outer medulla and medullary rays might explain the peculiar striped pattern of fibrosis noted in an experimental model of chronic cyclosporine nephrotoxicity (471). All together, these data strongly suggest that CsA-induced interstitial fibrosis could be mediated by angiotensin II, independent of its hemodynamic effects.

Several recent studies also implicated transforming growth factor-β1, a potent immunosuppressive and fibrogenic cytokine, as a potential mediator of CsA-induced interstitial fibrosis (472–478). Enhanced intragraft expression of TGF-β was associated with interstitial fibrosis in patients treated with CsA (472). In animals, CsA induced an increased expression of TGF-β1, both at the mRNA and the protein level, again associated with tubulointerstitial fibrosis (473–475,478). Similarly, CsA stimulated expression of TGF-β1 in renal cells (476,477). The fibrogenic effects, induced by CsA, were abrogated by a neutralizing anti-TGF-β1 antibody (477,478).

In contrast to the already mentioned enhanced collagen formation induced by CsA, recent work demonstrates an increased expression at both the transcriptional (mRNA) level and protein level of tissue inhibitor of metalloproteinases (TIMP-1) in a rat model of chronic cyclosporine nephrotoxicity (479,480). Moreover, Duymelinck and associates show that cholesterol feeding accentuates the cyclosporine-induced elevation of renal plasminogen activator inhibitor type 1 (PAI-1) (481). This increased expression and production of TIMP-1 and PAI-1, induced by CsA, could result in a decreased degradation of extracellular matrix, which would in turn lead to progressive extracellular matrix accumulation and interstitial fibrosis.

In summary, CsA-induced interstitial fibrosis results from a combination of increased synthesis of matrix components, as well as decreased degradation of extracellular matrix. Angiotensin II and transforming growth factor-β1 may play a role in the process of increased collagen formation induced by CsA, while the TIMP-1 and the PAI-1 likely mediate the decreased degradation of extracellular matrix induced by CsA.

Studies on Lipid Peroxidation. It has been shown that *in vitro* incubation of rat renal microsomes or human liver microsomes with CsA induces dose-related lipid peroxidation (482,483). Lipid peroxidation seems to be the main mechanism of free-radical toxicity (484–486). Reactive oxygen species through a peroxidative process may increase the availability of arachidonate metabolites and enhance prostanoid production (487–489). Recent *in vivo* studies in the rat indicated that cyclosporine nephrotoxicity is accompanied by dose-related systemic and renal lipid peroxidation (490), preceding the fall in GFR (491). Concurrent treatment with antioxidants (i.e., Vitamin E [490], melatonin [492], or N-acetylcysteine [493]), suppressed CsA-induced lipid peroxidation and reduced functional and structural damage. The mechanism by which CsA-induced lipid peroxidation could contribute to cyclosporine nephrotoxicity is putative, including direct cellular toxicity (494), thromboxane-mediated ischemia (495), or peroxidation-linked excess extracellular matrix production (496). Several reports suggest that calcineurin inhibitors, CsA and tacrolimus, have prooxidant activity, and they increase the susceptibility of low-density lipoprotein to oxidation in humans (497–499).

Clinical Nephrotoxicity of Cyclosporine A

CsA can cause a wide spectrum of renal functional and morphologic impairments, including a marked and rapidly reversible decrease in GFR and renal plasma flow (323) and a chronic form of renal damage in patients treated for more than 6 months with a potential evolution to end-stage renal disease (328,331). Thrombotic microangiopathy is another, although relatively uncommon, nevertheless serious, adverse effect of cyclosporine (326,500).

Acute Renal Failure Induced by Cyclosporine A. Acute cyclosporine renal dysfunction is not infrequent in clinical practice and occurs not only in patients with kidney transplantation (319) but also in heart (501), liver (322), and bone marrow (320) transplant recipients. This acute form of nephrotoxicity may occur within weeks following initiation of CsA therapy and can also be observed after years of drug therapy (502). The incidence of this acute renal injury can be enhanced by extended graft preservation (325), preexistent renal histologic lesions (503), donor hypotension, and perioperative complications (504).

Acute cyclosporine nephrotoxicity has clinical features similar to those of acute renal allograft rejection, including an abrupt fall in GFR, impaired urinary concentrating capacity, and sodium retention (505). Hypertension is observed in up to 50% of patients, whereas metabolic acidosis, hyperkalemia, and hyperuricemia are less frequent (506). Characteristic of this syndrome of acute reversible renal dysfunction induced by CsA is the rapid recovery of renal function on reduction of the CsA dose (323,507).

Delayed kidney graft function is a less frequent severe form of protracted ARF with oliguria induced by CsA (504). Its incidence varies largely between centers (508,509), presumably reflecting different strategies of immunosuppressive treatment or variations in time of ischemia of the kidney before transplantation (510,511).

Although nephrotoxicity due to cyclosporine alone is rarely observed with CsA trough blood levels below 200 ng per mL per (344,512), blood level monitoring has proved unreliable in the differential diagnosis between acute cyclosporine nephrotoxicity and acute rejection of kidney allografts (344).

The difficulty in differentiating acute rejection from cyclosporine nephrotoxicity in the setting of kidney transplantation often compels performance of a kidney biopsy (513,514).

On the histologic basis, cyclosporine nephrotoxicity is often a diagnosis of exclusion with the absence of definite signs of acute rejection, such as intimal arteritis (515,516) or intratubular lymphocytes (517). Histologic features of cyclosporine nephrotoxicity are nonspecific and include arteriolar hyalinosis (518,519), as well as isometric vacuolization of proximal tubular cells (520).

Analogous to experimental data obtained in animal models, CsA causes a dose-related and time-related fall in GFR and renal plasma flow in humans induced by renal vasoconstriction (521,522). In two studies, the intrarenal blood flow was significantly reduced after oral cyclosporine intake, but hypoperfusion could not be elicited by tacrolimus (523,524). The beneficial effects of different calcium channel blockers on this CsA-induced renal hypoperfusion (525–530) suggest this vasoconstriction is mainly effected at the afferent arteriolar level because it has been demonstrated that calcium antagonists preferentially reduce glomerular afferent arteriolar tone (531).

In contrast, coadministration of indomethacin unmasks CsA-induced renal vasoconstriction and potentiates cyclosporine nephrotoxicity by reducing the intrarenal prostaglandins (532). This suggests a role for the eicosanoids in the CsA-induced vasoconstriction. Further arguments in favor of this possibility are the partial beneficial effects observed with a specific TxA_2 synthase inhibitor (435) and with dietary regimens with omega-3 polyunsaturated fatty acids (533).

Although a role for increased vascular renin activity in cyclosporine-induced renal and peripheral vasoconstriction has been suggested (534,535), investigators have never detected any significant preventive effect of angiotensin-converting enzyme inhibition on the decline in renal blood flow and the increase in renal vascular resistance induced by CsA.

Unlike in animal models, prazosin did not significantly affect GFR, renal plasma flow, or renal vascular resistance in patients who had undergone transplant and were treated with CsA (536), thus questioning the role of the sympathetic nervous system in cyclosporine nephrotoxicity.

Endothelin has been implicated as a causative agent in CsA-induced vasoconstriction (see Experimental Nephrotoxicity of Cyclosporine A, above). Although intrarenal injections of antiendothelin antibodies protected against the effects of cyclosporine (438), administration of specific endothelin receptor antagonists has shown conflicting results (443,537).

Chronic Cyclosporine Nephrotoxicity. The main clinical issue associated with CsA treatment is, however, the chronic nephrotoxicity (331) that is clinically defined by progressive renal dysfunction with hypertension. Histologic lesions can already appear after 6 months of CsA therapy (328,538), with progression over time, even after CsA dose reduction (335). As mentioned previously, chronic cyclosporine nephrotoxicity has been documented in other clinical settings besides kidney transplantation (331–339). Chronic cyclosporine nephrotoxicity is related to the cumulative CsA dose (335,539) and may be irreversible even after CsA discontinuation (540).

The clinical features of chronic cyclosporine nephrotoxicity are nonspecific, including a slowly progressive decline of renal function over months or years, severe arterial hypertension, mild proteinuria, and tubular dysfunction (505). In renal allografts, differential diagnosis with chronic rejection is often impossible on clinical grounds alone, thus necessitating the performance of a kidney biopsy (541).

The histopathologic lesions of chronic cyclosporine nephrotoxicity have been extensively studied and are now well known (329,330,542,543). Histopathologic findings in 2-year protocol biopsies from a randomized study showed comparable lesions in renal allografts under cyclosporine and tacrolimus treatment (544). They include renal arteriolar damage (the so-called CsA-associated arteriolopathy), tubular atrophy, and (striped) interstitial fibrosis, as well as glomerular sclerosis. These lesions are nonspecific, however, except for the CsA-associated arteriolopathy.

The vascular lesions are located almost exclusively in the arterioles and arteries, with up to two layers of smooth muscle cells, and usually consist of circular nodular protein deposits or mucoid thickening of the intima, which contributes to narrowing or occlusion of the lumen (329). CsA-associated arteriolopathy affects a limited number of arterioles in a dose-related manner (543).

Tubulointerstitial changes may be nearly diffuse, but usually there are narrow stripes of atrophy and fibrosis, apparently corresponding to areas of cortex with afferent arteriolar lesions (329). This interstitial fibrosis progresses over time (335). Tubular atrophy is nearly always found in areas with interstitial fibrosis (545) and likewise progresses with time (335). CsA-induced glomerulopathy consists of global or focal and segmental sclerosis (330,546). Again, the number of affected glomeruli increase with time (335).

Although the histologic features of chronic cyclosporine nephrotoxicity have been well characterized, the differential diagnosis with chronic rejection of the renal allograft in kidney transplantation still often remains difficult (547).

A great matter of debate is whether prolonged therapy with CsA can result in progressive, irreversible renal damage, ultimately leading to end-stage renal disease. This was advocated by some authors (548,549) but denied by others (536,550). Multicenter studies in renal transplant patients showed reduced but stable renal function after up to 3 to 5 years of CsA treatment (551,552). Conversion from CsA to azathioprine in kidney transplant recipients after 3 months significantly improved the creatinine clearance at 5 years' posttransplantation (553). In patients who have undergone pancreas transplant, a sequential functional and morphologic study has unequivocally shown the progressive character of the histologic lesions due to cyclosporine nephrotoxicity (335). This was strongly correlated with CsA blood levels, CsA dose, and magnitude of the decline in creatinine clearance during the first posttransplant year (335). Analysis of sequential protocol biopsies of renal allografts over a period of 10 years, in a prospective study of 120 kidney-pancreas transplant recipients, confirmed this progressive character of renal histologic lesions, induced by calcineurin-inhibitors (554). In this study, severe histologic damage was present in 58.4% of the renal allografts by 10 years.

Altogether, these data point out that chronic cyclosporine nephrotoxicity has a progressive and irreversible character once the histologic lesions have arisen. Assessment of the renal function, be it by means of serum creatinine or creatinine clearance, underestimates the magnitude of the problem due to the relatively low sensitivity of those methods and to the slow progression of the renal damage induced by cyclosporine.

The pathophysiology of chronic cyclosporine nephrotoxicity in humans is a matter of extensive investigation, mainly through experimental models (see Pathophysiologic Studies of Cyclosporine Nephrotoxicity, above).

Hemolytic–Uremic-like Syndrome Induced by Cyclosporine A. Thrombotic microangiopathy is a relatively uncommon but serious adverse effect of cyclosporine in renal (326) and nonrenal (500) transplant recipients, with an overall 43% graft survival rate (555,556). The most striking morphologic changes are an extensive thrombotic process in the renal microcirculation, with several glomerular capillaries occluded by thrombi extending from the afferent arterioles and containing platelet aggregates (329). Laboratory anomalies include thrombocytopenia, hemolytic anemia, and deterioration of the renal function (326). In the setting of kidney transplantation, the differential diagnosis of hemolytic–uremic syndrome and vascular rejection is not obvious (555). According to a retrospective

study of 29 patients with calcineurin-inhibitor induced thrombotic microangiopathy, repeated plasma-exchange induced a recovery of the renal allograft function in 80% of the patients (557).

This hemolytic–uremic-like syndrome induced by CsA reinforces the concept that the vascular endothelium is the main target in this form of CsA toxicity. That CsA can damage vascular endothelium is confirmed by the high plasma concentration of factor VIII-related antigen, found in recipients of renal allograft given CsA and having clinical signs of nephrotoxicity (558). Recent work shows significantly higher plasminogen-activator inhibitor (PAI-1) levels in patients treated with CsA who underwent renal transplant, compared to patients who were not treated with CsA, suggesting a decreased fibrinolytic activity in the former patients (559). This could account for the increased risk of hemolytic–uremic syndrome induced by CsA.

Summary

CsA is a potent immunosuppressive drug with nephrotoxic side effects. Independent of the intrinsic nephrotoxic properties of CsA, its complex clinical pharmacokinetic profile could cause incorrect dosing, ultimately resulting in irreversible renal damage.

The clinical nephrotoxicity of CsA consists of three entities with different expressions of renal damage induced by CsA (i.e., ARF, hemolytic–uremic-like syndrome, and chronic cyclosporine nephrotoxicity). The ARF is essentially reversible and mainly hemodynamically mediated through afferent arteriolar vasoconstriction. Dosage reduction of CsA reverses the nephrotoxic effects. The hemolytic–uremic-like syndrome consists of an extensive thrombotic process at the level of the glomerular capillaries, causing loss of kidney function in more than half of the cases. Chronic cyclosporine nephrotoxicity is an irreversible renal damage characterized by a specific arteriolopathy and striped interstitial fibrosis, resulting in slow progressive decline of renal function. Although the pathophysiology of this process is extensively studied in experimental models, many questions still remain unanswered.

TACROLIMUS (FK506)

Tacrolimus (FK506) is a fungal product, a new macrolide immunosuppressant agent, which has shown important potential in transplantation and in the treatment of autoimmune diseases (560–562). Although it is many times more potent than cyclosporine, allowing the use of lower doses, both drugs have similar nephrotoxic properties (563,564). In 1991, the first international congress of FK506 was held in Pittsburgh, Pennsylvania (565).

Molecular Action

Cyclosporine and FK506 have dissimilar chemical structures (Fig. 42-5), nevertheless both agents bind to a similar class of ubiquitous intracellular receptors: immunophilins, molecules that are *cis–trans* prolyl isomerases. These intracellular binding proteins are well conserved through evolution and change the confirmation of cyclosporine and FK506. The cytosolic receptor for FK506 (FKBP) has been well characterized (566). This drug–immunophilin complex must bind to calcineurin, a calcium-dependent protein phosphatase, to allow the immunosuppressant actions of the drugs in lymphocytes (567,568) (Fig. 42-6). Similar calcineurin-mediated dephosphorylation of cyclosporine and FK506 may lead to inhibition of signal transduction in other cell types and organs, which mediates both the desirable immunosuppressant effects and the possibly toxic effects.

Cyclosporine and FK506 are powerful immunosuppressive drugs that inhibit the calcium–calmodulin-dependent phosphatase calcineurin in T cells, thereby preventing the activation of T cell-specific transcription factors such as NF-AT involved in lymphokine gene expression (Fig. 42-6). While this may, at least in part, explain the mechanism of cyclosporine and FK506 immunosuppression, additional mechanisms have to be invoked to explain the pharmacologic properties and toxic effect of these drugs such as nephrotoxicity and neurotoxicity. Schwaninger et al. (569) studied the effect of cyclosporine and FK506 on calcineurin phosphatase activity and gene transcription mediated by the cyclic adenosine monophosphate-responsive element (CRE), a binding site of the ubiquitous transcription factor CREB. An imported gene was placed under the transcriptional control of the CRE of the rat glucagon gene and transiently transfected into the glucagon expressing cell line αTC2. Cyclosporine and FK506 inhibited depolarization-induced gene transcription in a concentration-dependent manner. Both cyclosporine and FK506 inhibited calcineurin phosphatase activity at the drug concentrations that inhibited gene transcription. The FK506 analog rapamycin had no effect on calcineurin activity and gene transcription, but excess concentrations of rapamycin prevented the effect of FK506 on both calcineurin activity and gene transcription. These results further support the notion that the interaction of drug-immunophilin complexes with calcineurin may be the molecular basis of cyclosporine- and FK506-induced inhibition of CREB/CRE-mediated gene transcription. The ability to interfere with CREB/CRE-mediated gene transcription represents a new mechanism of cyclosporine and FK506 action that may underlie pharmacologic effects and toxic manifestations of these potent immunosuppressive drugs (569).

A recent report demonstrated that *in vivo* FK506 treatment eliminated antigen-stimulated T cells through DNA fragmentation (apoptosis), representing one of the mechanisms of immunologic tolerance (570).

Experimental Studies

Cell Culture

McCauley and colleagues (571) demonstrated a cyclosporine- and FK506-mediated, dose-dependent inhibition of renal cell proliferation using LLC-PK1 cells (an established cell line derived from the pig proximal tubule) in culture. Although FK506 inhibited renal cell proliferation to a greater degree than cyclosporine at the same concentration, when clinically relevant concentrations were compared, FK506 was significantly less inhibitory than cyclosporine. Moutabarrik and associates (572) observed similar effects in the same cell line but could not make a clear distinction between the FK506 and the cyclosporine effects on release of 3_H thymidine from prelabeled cells, *N*-acetyl-β-D-glucosaminidase release, and cell detachment. Ultrastructural changes such as vacuolization, swelling, and mitochondrial enlargement and inhibition of the growth of the cultured tubular cells were also observed at high concentrations of FK506 and cyclosporine. Low concentrations of FK506 and cyclosporine were not cytotoxic and induced only a minimal inhibitory effect on the growth of tubular cells *in vitro*. Cyclosporine and FK506 also induced a time-dependent stimulation of the secretion of endothelin by cultured tubular cells. The concentration of cyclosporine that induced these effects was 10 to 100 times higher than that required for FK506. The concentrations of FK506 and cyclosporine inducing endothelin secretion were not cytolytic for tubular cells *in vitro*. Yatscoff et al. (573) compare the effect of rapamycin and FK506 on

the release of prostacyclin and endothelin *in vitro* using cultured rabbit mesangial and endothelial cells. The effects of both rapamycin and FK506 on the basal or stimulated release of prostacyclin or endothelin from mesangial cells and endothelial cells are similar with the following exceptions: Rapamycin results in a significant increase in the release of prostacyclin, while in contrast FK506 results in a significant decrease in the release of prostacyclin from the endothelial cells. Benigni and colleagues (574) review the vascular effects of FK506 as compared to cyclosporine in endothelial cell culture and intact organ. FK506, unlike cyclosporine, is without significant effect on thromboxane B_2, 6-ketoprostaglandin $F1_{1\alpha}$, or endothelin release in bovine aortic endothelial cells grown in culture and does not alter the renal vascular resistance *in vivo*. These findings suggest that FK506 causes much less pronounced endothelial cell injury, at least *in vitro*.

Atcherson and Trifillis (575) examine *in vitro* cytotoxicity of FK506 on normal human proximal tubule cells. They find that FK506 is reversibly and mildly toxic to monolayers of human renal proximal tubule cells.

Edkins and associates (576) compare the effect of FK506 (2.5 mg per kg for 7 days) and cyclosporine (50 mg per kg per day) on renal, hepatic, brain and cochlear-reduced glutathione content. Both cyclosporine and FK506 increase glutathione levels in kidney to approximately equivalent levels after 5 days of treatment. Only FK506 increases glutathione levels in liver, and neither drug changes levels in other tissues.

Ali Shah et al. (577) show that FK506 exhibits a broad, powerful inhibitory effect on human hepatic microsomal cytochrome P-450-dependent drug metabolism. However, the full potential for drug interactions can only be determined by investigating its effects on other P-450 families using both *in vivo* and *in vitro* studies. On the other hand, Yoshimura et al. (578) recently report that, in rats, both FK506 and rapamycin are without significant effects in contrast to cyclosporine on renal microsomal P-450–dependent drug metabolism.

Yoshimura et al. (579) review the effect of FK506 and rapamycin on renal P-450 systems in rat models. They find that although cyclosporine has a strong effect on renal P-450 systems and induces such a system in kidney cortex (microsomal P-450), FK506 and rapamycin have no substantial effect on the induction of renal P-450.

The role of intracellular calcium in the pathogenesis of cyclosporine nephrotoxicity has received great attention (580,581) and has resulted in therapeutic implications to prevent nephrotoxic effects of the drug. The effect of cyclosporine and FK506 on microsomes and mitochondria of rabbit renal cortex tissue has been studied by Prasad and associates (582). Both drugs decrease calcium uptake and A23187-induced calcium release from microsomes and mitochondria in a dose-dependent manner (0.5 to 10.0 μg per mL). The effect of FK506 is significantly less at equivalent concentrations, and microsomal calcium-stimulated ATPase is not changed by either drug.

The potential role of the FK506 binding protein (FKBP12) in cellular calcium homeostasis has been suggested. Indeed, Jayaraman and colleagues (583) find that a 12-kd protein tightly bound to the calcium release channel in skeletal muscles of rabbit is FKBP12. Obviously, if this observation can be confirmed in vascular smooth muscle, it may explain the mechanism of FK506-induced vasoconstriction in renal vasculature. This process also is probably calcineurin-drug complex mediated. A further role of calcineurin in α-adrenergic stimulation of Na^+-K^+-ATPase activity in renal tubular cells is illustrated by Aperia et al. (584). They demonstrate that FK506 inhibited Na^+-K^+-adenosine triphosphatase (ATPase) activity induced by oxymetazoline, an α-adrenergic agonist. This study may suggest a role for FK506-mediated renal nerve changes in sodium and potassium homeostasis. In this context, Palevsky and colleagues (585) report a resistance to the effect of aldosterone on renal cells in cultures exposed to FK506.

Animal Studies

Animal studies have shown both acute and chronic nephrotoxicity produced by FK506 (586). Somewhat different than cyclosporine, FK506 produces toxicity at blood levels that are clinically relevant; however, the doses necessary to achieve these blood levels on a weight basis are at least 10-fold larger than those used clinically. This contrasts with cyclosporine, with which acute and chronic nephrotoxicity can be produced with doses on a weight basis that are very close to those clinically used, particularly in the salt-depleted rat model; however, the blood levels achieved with these doses are at least three to four times those achieved clinically (587).

Preclinical animal studies gave few hints of nephrotoxicity (588). However, a troubling series of side effects soon appeared including vasculitis, myocardial necrosis, and severe weight loss. Fortunately, most of these side effects turned out to be species-specific. Nephrotoxicity of FK506 became apparent from the initial series of rescue patients treated with the drug (589).

Several studies have documented reduction in effective renal plasma flow and GFR in animal models. Ueda and colleagues (590) have measured renal cortical blood flow, using a hydrogen ion clearance method, serum creatinine, and juxtaglomerular cell cross-sectional area in mice treated with FK506, 3 mg per kg per day given subcutaneously as compared with saline-treated control animals. Cortical blood flow is significantly reduced in FK506-treated animals as compared with control animals, as is juxtaglomerular cell area. Kumano et al. (591) also note a reduction in GFR and effective renal plasma flow using inulin and *p*-aminohippuric acid in a heminephrectomized rat model in response to an acute infusion of FK506 and after 21 days of treatment. Proximal tubular vacuolization typical of cyclosporine nephrotoxicity is noted, and diltiazem improves both the functional and morphologic changes caused by FK506. Lieberman and associates (592) note a significant volume reduction in both cyclosporine-treated and FK506-treated glomeruli that are inhibited by verapamil. Mitamura and colleagues (593) review the FK506-induced nephrotoxicity in spontaneous hypertensive rats. These results indicate that the acute nephrotoxicity of FK506 is derived from impaired glomerular function associated with renal arteriolar constriction brought about by the drug. All of these renal disorders induced by FK506 recover completely or partially when the drug is withdrawn for 2 or 4 weeks. Thus, the acute nephrotoxicity of FK506 in spontaneous hypertensive rats is reversible.

Ryffel and colleagues (594) explore the nephrotoxicity of immunosuppressants in rats. Specifically, they compare the nephrotoxic effects of FK506 and rapamycin with that of cyclosporine in male Wistar rats. FK506 causes proximal tubular epithelial changes consisting of atrophy, vacuolization, inclusion bodies, microcalcification, and focal mononuclear interstitial infiltrate as described for cyclosporine. The most striking alteration is hypertrophy of the juxtaglomerular apparatus. The percentage of renin-containing juxtaglomerular apparatus and the extent of renin immunoreactivity along afferent vessels are significantly increased in FK506-treated and CsA-treated rats. By contrast, no renal morphologic lesions are found in rapamycin-treated animals. Renal cortical extracts contain abundant cyclophilin and FK506-binding protein, the main intracytoplasmic receptors for cyclosporine and FK506, respectively. The authors hypothesize that both the immunosuppressive and toxic effects of FK506 and cyclosporine, but not of rapamycin, are mediated through an immunophilin–drug–calcineurin complex. The renal substrate of calcineurin, which mediates renal vasoconstriction, is yet to be identified.

Andoh and associates (595) also compare the acute rapamycin nephrotoxicity with cyclosporine and tacrolimus. They find that cyclosporine and FK506 strikingly decrease urinary excretion of nitric oxide, renal blood flow, and GFR, whereas rapamycin does not. In contrast, all three of these drugs cause significant hypomagnesemia associated with inappropriately high fractional excretion of magnesium, suggesting renal magnesium wasting. In addition, with all three drugs there are lesions in the rat kidneys consisting of tubular collapse, vacuolization, and nephrocalcinosis. These researchers show that only the calcineurin inhibitors produce glomerular dysfunction in an acute experimental model of nephrotoxicity.

Of interest is the experiment of Hara and colleagues (596) that shows that FK506 is effective in the prevention of the development of rapid glomerular injury in rats with accelerated nephrotoxic serum glomerulonephritis.

Clinical Studies

Since the initial reports on the use of tacrolimus in clinical transplantation by Starzl and Shapiro (597,598), numerous large-scale trials compared the efficacy and safety of tacrolimus and cyclosporine mainly in liver (599,600), and kidney (601–603) transplantation. According to five out of six of these large trials, renal function and the incidence of renal impairment were comparable in both treatment arms at 1 year posttransplantation. Similar results were reported in a long-term comparison of nephrotoxicity between tacrolimus and cyclosporine in pediatric heart transplant recipients (604). In contrast, Ashan et al. reported a significantly better renal allograft function at 2 and 3 years, under tacrolimus compared to cyclosporine, both in combination with steroids and mycophenolate mofetil (603,605). However, graft survival at 2 and 3 years was comparable in both groups. In an intention-to-treat analysis, graft survival at 5 years was comparable between patients, initially randomized to tacrolimus or cyclosporine in the large U.S. multicenter trial (601,606).

In all these large trials, the incidence of acute rejection was significantly lower in the tacrolimus treated patients, compared to the cyclosporine treated patients. Similarly, there was a different profile of adverse effects with higher incidence of post-transplant diabetes mellitus and neurotoxicity under tacrolimus, compared to a higher incidence of hypertension, hyperlipidemia, hirsutism, and gum hyperplasia in cyclosporine treated patients.

According to three studies, the intrarenal hemodynamics are less affected by tacrolimus compared to cyclosporine, with better preservation of the renal plasma flow (523,524,607).

The histopathological changes, induced by tacrolimus, in the (transplanted) kidney, are entirely comparable to those induced by cyclosporine, that is, arteriolar hyalinosis and striped interstitial fibrosis (544,608-611). The intrarenal expression of TGF-beta, collagen, fibronectin, MMP-2, TIMP-1, and osteopontin was assessed by RT-PCR, and proved to be similar in kidneys treated with either tacrolimus or cyclosporine (612).

In conclusion, the nephrotoxicity of tacrolimus is functionally and morphologically comparable to the nephrotoxicity of cyclosporine as well in recipients of a renal allograft, as in recipients of a solid nonrenal organ. However, there is evidence that tacrolimus is a more powerful immunosuppressive agent, with a different toxicity profile.

OKT3

OKT3 is a murine monoclonal antibody directed against the epsilon-moiety of the CD3 receptor that comodulates with the antigen T-cell receptor (TCR) on the surface of T lymphocytes. The immunosuppressive properties of OKT3 are related to its ability to deplete CD3+ cells from the circulation, to induce the internalization of CD3/TCR complexes, and to sterically inhibit residual CD3/TCR complexes (613). Since the CD3/TCR complex is a signal transducing system, binding of the OKT3 monoclonal antibody to this CD3 receptor activates the T lymphocytes before internalization or stripping of the CD3/TCR complex from the surface of the cell. In other words, before exerting immunosuppressive effects, OKT3 induces a transient activation of lymphocytes. In addition, monocyte activation is induced by the multivalent cross-linking of the CD3/TCR complex and the monocyte Fc receptor induced by OKT3 (614). This activation is accompanied by the release of several proinflammatory cytokines including tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), IL-2, and interleukin-6 (IL-6) into the circulation within hours after the initial OKT3 injection (615–620). T cells are the main source of TNF-α in this clinical setting, although monocytes can also be activated, as shown by their production of IL-1 and IL-6 (621). That TNF-α and IFN-γ play a key role in this inflammatory reaction is suggested by the prevention of the hypothermia, hypomobility, diarrhea, and piloerection using monoclonal antibodies directed against TNF-α and IFN-γ (622–624).

Complement activation seems to play a minor role in the pathogenesis of OKT3-associated cytokine release syndrome. Indeed, Mackie and colleagues (625) describe a syndrome presenting all the clinical symptoms of a classic anti-CD3-induced toxicity in complement-deficient mice. Furthermore, Waid and associates (626) demonstrate the lack of toxicity of a complement-binding nonmitogenic anti–T-cell receptor immunoglobulin M (IgM) monoclonal antibody.

Clinical Observations

The clinical syndrome of this post-OKT3 inflammatory reaction is characterized by generalized capillary leak, fever, chills, rigors, diarrhea, headaches, aseptic meningitis, noncardiogenic pulmonary edema, cerebral edema/convulsions, and the so-called cytokine nephropathy.

OKT3 has been used to treat allograft rejection and has also been used prophylactically to delay the use of cyclosporine in the immediate postoperative period when the allograft is ischemic and most vulnerable to the use of vasculopathic and nephrotoxic effect of cyclosporine. However, Toussaint et al. (627) observe a higher rate of delayed graft function among OKT3 patients than those treated initially with cyclosporine immediately after transplantation. These investigators use low doses of steroid (1 mg per kg of methylprednisolone) before the first OKT3 injection in contrast with other centers where 4 to 8 mg per kg is used (628). The critical role of steroid pretreatment at sufficient dose in the partial protection of the late graft function among OKT3 patients is confirmed when 8 mg per kg of methylprednisolone is used (629). This increased dose of steroid is associated with a smaller increase of TNF and IFN-γ (629). Simpson et al. (630) report signs of tubular alterations as demonstrated by investigating the urinary sediment in renal allograft recipients and a striking increase in serum creatinine during the first 3 days of OKT3 therapy. Batiuk and associates (631) report a syndrome of reversible renal dysfunction associated with OKT3 induction therapy after renal transplantation and with OKT3 rejection after cardiac transplantation. Indeed, 7.5% of renal allograft recipients with immediate graft function developed a rising serum creatinine and decline in urine output not attributable to cyclosporine because cyclosporine therapy had not yet been initiated. The renal biopsies obtained in all of the patients showed no histologic abnormalities or mild interstitial edema. Four of sixteen cardiac transplant patients developed an increase in serum creatinine following the second

dose of OKT3 given for rejection. However, two of the patients also received indomethacin. Renal dysfunction resolved spontaneously in all cases.

Goldman and colleagues (632) analyze the changes of serum creatinine levels in the first days of antirejection therapy with OKT3. Although initial serum creatinine levels were similar, the OKT3-treated group reached a mean peak increase in serum creatinine of 76% on day 4 as compared to the mean peak increase of 57% on day 3 in the pulse steroid treatment group. Chatenoud and others (616) examine the release of cytokines in the systemic circulation in 35 renal allograft recipients who received 5 mg per day of OKT3 starting at the time of transplantation. All but three patients were pretreated with a 1-g bolus of methylprednisolone from 1 to 3 hours before administration of OKT3 to ameliorate the clinical reactions. TNF reached a peak at 1 hour after OKT3 injection and remained elevated at 4 hours. Peak TNF was higher in the three patients who did not receive the steroid bolus and was higher in those who received the bolus concomitantly rather than 1 hour before OKT3. IFN-γ reached a maximum at 4 hours following the OKT3 injection and was highest in those not receiving the steroid bolus or who received it at the time of OKT3 injection. IL-2 increased in only five patients, three of whom did not receive the steroid bolus. IL-1β, IFN-α, and granulocyte–macrophage colony-stimulating factor levels did not increase with OKT3 injection. Fifteen to twenty hours after injection, TNF, IFN-γ, and IL-2 levels had returned to baseline.

The massive leukocyte activation resulting in cytokine release probably plays an important role in the acute renal dysfunction post-OKT3 administration. IL-1, IFN-γ, IL-2, IL-6, and TNF-α are involved in the capillary leak syndrome (633,634), which explains the clinical symptomatology of lung edema, reducing circulating volume, and hence compromising renal perfusion. Of note is the demonstrated release of endothelin after the first dose of OKT3 in renal transplant recipients (635). A direct cytotoxic effect of particular cytokines in kidney parenchyma has been put forward as a possible mechanism of allograft destruction (636). In addition, activation of leukocytes by cytokines induces an enhanced expression of adhesion molecules (637). The increased expression of adhesion molecules on endothelial and parenchymal cells may result in margination, diapedesis, migration, and appearance of those activated cells in the interstitium of the kidney. Indeed, activated neutrophils can play a mediator role by releasing oxygen radicals in the physiopathology of ischemic kidney injury. A recent report by Kelly et al. (638) shows that prohibiting neutrophils from infiltrating into the interstitium protects the kidney against ischemic injury, as is the case in other models (639).

Abramowicz and associates (640) demonstrate that OKT3 exerts procoagulant effects that can precipitate intragraft thromboses and result in transplant loss. The thrombotic events took place during the prophylactic administration of OKT3 occurring between day 1 and 11 postoperatively, always before the use of cyclosporine. The most striking finding in the renal biopsies was the formation of thrombi in some glomerular capillary loops and in afferent arterioles. Endothelial cells were swollen, causing diminished patency of capillary lumens. The interstitium was only slightly enlarged by edema without significant influx of inflammatory cells or tubular damage. Increased numbers of polymorphonuclear cells were present in the peritubular and glomerular capillaries. The authors look carefully for additional risk factors by comparing the clinical parameters of the 13 recipients with thrombosis to those of 218 patients free of this complication. The only relevant parameter appeared to be the methylprednisolone dose given as pretreatment before the first OKT3 injection. Six out of forty-two patients (14%) receiving 30 mg per kg of methylprednisolone experienced a thrombotic event, as compared to seven of the 189 patients (3.7%) who received the regular 8 mg per kg of methylprednisolone. The latter incidence compares to the 2% to 3% of vascular complications in renal transplantation reported in the absence of OKT3 prophylaxis (641,642), suggesting that the risk of thrombotic events related to OKT3 itself is rather low.

This clinically important side effect is related to the capacity of the first OKT3 dose to trigger the extrinsic pathway of the coagulation system and may precipitate early intragraft thrombosis, resulting in early transplant loss (641,643–645). Pradier and colleagues (643) find that all patients receiving OKT3 in the context of a kidney transplant have an increased plasma level of prothrombin fragments, peaking 4 hours after the first injection. There is no difference between the patients who receive 5 mg or 10 mg, neither when OKT3 is given as prophylaxis nor when given for treatment of rejection. Fibrin degradation products, indicative of a fibrinolytic process, are already above baseline values 4 hours after the first injection and continue to increase until 24 hours. The von Willebrand factor levels, an indicator of endothelial cell damage, are also significantly increased after OKT3 injection. The study of Raasveld and associates (644) confirms the previous findings, as they report an increased level of thrombin–antithrombin III complex, indicative of activation of the coagulation, as well as increased levels of tissue-type plasminogen activator and plasmin-α_2–antiplasmin complexes, indicative of fibrinolysis after the first injection of OKT3. According to the latter report, activation of the coagulation occurs via the extrinsic rather than the intrinsic pathway.

In vitro/Animal Studies

Pradier and associates (643) and Abramowicz and colleagues (645) studied this phenomenon *in vitro* and find that the activation of the coagulation by OKT3 is related to the induction of procoagulant activity of the tissue factor type on both endothelial cells and monocytes and that the high doses of methylprednisolone potentiate OKT3-induced tissue factor expression and activity on monocytes (646).

Indeed, using human umbilical vein endothelial cells, they could show that OKT3 and supernatants of unstimulated peripheral blood mononuclear cells are inactive. However, supernatants of OKT3-stimulated peripheral blood mononuclear cells induce a massive increase of tissue factor activity at the endothelial cell surface measured by thrombin generation. The inhibition of this procoagulant activity by anti-TNF monoclonal antibodies demonstrates that TNF is an important mediator of this effect of OKT3 at the endothelial cell membrane level. This is compatible with the well-known TNF effect on the expression of tissue factor on endothelium (647).

Itaka and colleagues observe (648) that monocytes also display increased procoagulant activity after culture in the presence of OKT3 antibody. This has been confirmed by other authors (645,646) as well. Again, this is due to an increased tissue factor expression by not clearly identified mechanisms. Indeed, TNF does not seem to play an important role (649). To what extent the monocyte procoagulant inducing factor (650), an as yet incompletely characterized cytokine (651), plays a role is still to be demonstrated. Furthermore, interactions between activated T cells and monocytes appear to be necessary to induce the monocyte procoagulant activity (651,652). The question of why only a small number of patients developed intragraft thrombosis after OKT3 was nicely addressed in a recent paper by Abramowicz and colleagues (645). They report that the procoagulant activity of unstimulated peripheral blood mononuclear cells reaches 3.0 $\pm$ 0.7 mU per mL after OKT3 stimulation and further increases to 7.4 $\pm$ 2.0 mU per mL when

those cells are first preincubated overnight with methylprednisolone before OKT3 stimulation. They show that this process involves a tissue factor–factor VII pathway; specifically, they find increased membrane expression of tissue factor on monocytes and a marked reduction of the induced procoagulant activity when the clotting assay is performed with factor VII-deficient plasma (645). Furthermore, corticosteroids increase plasminogen activator inhibitor-1 (PAI-1) secretion by hepatocytes (653), while at the same time they decrease tissue-type plasminogen activator (654) as well as prostacyclin (655,656) and nitric oxide production (657,658). In addition, one has to remember that OKT3 induces the release of proinflammatory cytokines such as TNF, IL-1, and IFN-γ, which also contributes to the decrease of endothelial cell expression of thrombomodulin (622,647,659), markedly inhibiting the anticoagulant effects of protein C and S (659,660).

Administration of OKT3 induces increased group II secretory phospholipase A2 levels inducing the biosynthesis of vasoconstrictive prostaglandins, vasoactive lipid mediators that influence glomerular hemodynamics, and renal function (661).

Alegre et al. (662) investigate the possible direct renal damage of anti-CD3 monoclonal antibody in a murine model. In this study hamster anti-mouse CD3 monoclonal antibody 145-2C11 (663) is injected and induces an important transient release of several cytokines in the circulation (662,664). Tubular lesions are histologically identified mainly at the S3 segment of the nephron and are also evidenced by discrete renal function impairment. Pretreatment with steroids almost completely abolishes the cytokine release, and no changes in histology and renal function can be observed.

Finally, the kidney graft is by all means susceptible to the development of ARF. Warm and protracted cold ischemia associated with organ procurement and the immunosuppression administered may present additional risk factors to develop and maintain ARF after OKT3 administration.

Abramowicz and colleagues (665) performed a study in order to prevent as much as possible the nephrotoxic effects seen in the context of OKT3 administration. According to these authors, combined strategy of appropriate dosage of steroids before the first OKT3 injection, administration of a calcium-channel blocker, and optimalization of volume status is safe and efficiently prevents OKT3 nephrotoxic effects.

OKT3 induces activation of both mononuclear cells and the common pathway of coagulation responsible for the first dose reactions and transient or severe nephrotoxic effects. Patient-related or donor-related risk factors and inappropriate doses of steroids contribute substantially to the severity of those side effects. New anti-CD3 agents such as humanized OKT3 and OKT3 $F(ab')_2$ are being developed and will contribute to a better clinical tolerance and comparable immunosuppression (666).

Daclizumab and basiliximab are new monoclonal antibodies approved for the prophylaxis of acute organ rejection in renal allograft recipients (667–671). The efficacy of these agents has been established in a number of well-controlled clinical trials. These multicenter studies assessed the efficacy of daclizumab and basiliximab versus placebo when used as part of a regimen of double therapy (cyclosporine and corticosteroids) or triple therapy (cyclosporine, corticosteroids, and azathioprine) to prevent acute allograft rejection. These studies demonstrate that daclizumab significantly decreases the incidence of acute rejection at 6 months. Daclizumab, when given in combination with other immunosuppressants, does not show an increase in the number of serious adverse events compared with placebo (670). With basiliximab, the incidence of acute rejection is 34% with double therapy in the basiliximab group and 52% in the placebo group. The incidence and types of adverse events are similar to those in placebo-treated patients. No drug interactions have been observed when daclizumab and basiliximab are administered with other immunosuppressive agents (667).

MYCOPHENOLATE MOFETIL

Mycophenolate mofetil (MMF), the morpholinoethyl ester of mycophenolic acid (Fig. 42-5), has been developed as an immunosuppressant for prevention of rejection in renal transplantation. *In vivo*, MMF is deesterified to mycophenolic acid (the active immunosuppressive component), which is a potent and specific inhibitor of the synthesis of guanosine nucleotides and thus a selective suppressor of proliferation of both T and B lymphocytes. MMF, given alone or with corticosteroids or cyclosporine, lowers the frequency of acute rejection after allogeneic organ transplantation in animals (672,673).

The immunosuppression of MMF appears to be additive with that of cyclosporine and tacrolimus, and MMF does not promote nephrotoxicity (674). Initial studies indicated that MMF, in combination with cyclosporine and steroids, reduces the incidence of acute rejection in renal transplantation (675–677).

In the meantime MMF has been used under several clinical conditions. In cardiac, liver, lung, and pancreas transplantation the use of MMF in association with reduced doses of cyclosporine has resulted in improved renal function and maintained immunosuppression (678–681). In renal transplantation, Halloran and others (682) summarize the three multicenter trials that confirm at 1-year posttransplant MMF is effective in preventing acute renal allograft rejection (675–677). Two studies address patients with chronic kidney graft dysfunction in whom MMF is introduced and cyclosporine exposure reduced (683,684). The rationale is that the increased, immunosuppressive potency of MMF would allow for a safe reduction of CsA doses. The conclusion from these two studies is that cyclosporine dose reduction in patients on MMF results in an improved graft function with no increased risk of rejection. It must be noted, however, that follow-up was short—less than 1 year—in both studies. In addition, whether MMF does better than azathioprine (AZA) in this setting remains an open question. Indeed, a similar improvement of chronic graft dysfunction is reported when AZA is introduced and cyclosporine doses reduced (685).

Is there a role for MMF in the attempt to withdraw calcineurin inhibtors in patients with stable renal transplantation? Several randomized (686–688), as well as nonrandomized (689), clinical trials in renal transplantation examined the efficacy and safety of calcineurin inhibitors in patients with stable graft function under triple immunosuppressive regimen, consisting of prednisolone, cyclosporine or tacrolimus, and MMF. All these studies reported a significant improvement of the graft function, as well as lower blood pressure, and an improved lipid profile after calcineurin inhibitor (CNI) withdrawal. In contrast, the incidence of acute rejection was higher in the CNI withdrawal group, without any impact on graft survival. Therefore, these studies provide evidence that CNI withdrawal is achievable in renal transplant recipients with stable graft function. CNI withdrawal appears to improve graft function, hypertension, and hyperlipidemia. However, caution should be paid to the increased incidence of acute rejection after CNI withdrawal, and the short term of follow-up, reported in these studies (6 to 32 months).Whether CNI withdrawal will result in improved graft survival, is not yet known.

Since MMF has multiple immunosuppressive and anti-inflammatory modes of actions including the inhibition of humoral and cellular immunity, antimutogenesis, reduction of mononuclear cell infiltration, and inhibition of vascular smooth muscle and mesangial cell proliferation, it is not surprising that the drug is now used in autoimmune-mediated

renal disease (690). Briggs and colleagues (691,692) report their limited experience in eight patients with different types of nephrotic-type glomerulonephritis and unsatisfactory response to steroids as cyclosporine. Controlled prospective studies are underway to clarify the potential advantages of MMF compared with other immunosuppressive agents in these disease entities.

In conclusion, MMF is a potent, nonnephrotoxic immunosuppressive drug, which significantly reduced the incidence of acute rejection under triple immunosuppressive regimens with prednisolone and cyclosporine or tacrolimus. In addition, MMF appears to allow dose reduction or even withdrawal of CNI in renal transplant recipients with stable graft function, thereby avoiding the long-term nephrotoxicity induced by calcineurin inhibtors. Because immunosuppressant-induced nephrotoxicity has been associated with significant financial costs, cyclosporine-sparing and FK506-sparing regimens should result in substantial savings in health care costs (693) (Fig. 42-5). It is important to emphasize here that there are no long-term data for these experimental regimens, but they offer a new direction if these short-term results are confirmed over a more sufficient period of time.

RAPAMYCIN

Rapamycin (sirolimus, or SRL) is a macrocyclic fermentation product of *Streptomyces hygroscopicus*, and was first isolated in 1975 (694). SRL has a similar molecular structure to FK 506 and also binds to FKBP12 (695). However, the SRL-FKBP12 complex does not affect the calcineurin phosphatase, but instead binds to a protein, called the mammalian target of rapamycin (mTOR) (696). This binding of the SRL-FKBP12 complex to mTOR inhibits both DNA and protein synthesis, resulting in arrest of the cell cycle in late G1, as it progresses to the S phase (697).

SRL blocks T-cell proliferation, induced by cytokines, alloantigens, and mitogens in a dose-dependent manner (698). In addition, SRL acts on B-cells, causing an inhibition of antigen and cytokine driven B-cell proliferation (699). *In vitro* studies have demonstrated the synergistic immunosuppressive interaction of CsA and SRL (700), in contrast to the combination of FK506 and SRL, which produced an antagonistic effect at low doses (701). Animal studies have confirmed the immunosuppressive potential of SRL (702), as well as its synergistic interaction with CsA (703). In contrast to the *in vitro* studies, FK506 interacted synergistically with SRL in animal studies (704).

Clinical Efficacy of Sirolimus in Renal Transplantation

Based on the results from several multicenter, prospective, randomized trials, including a U.S. study, a global study, a combined European-U.S. study, and two European studies, SRL was approved in 1999 by the Food and Drug Administration (FDA) for the prevention of acute rejection in renal transplant recipients. SRL was used in combination with CsA and prednisolone in these studies and reduced the incidence of acute rejection at 1 year to 10% (705).

Three prospective, randomized trials in renal transplant recipients compared CsA to SRL in combination with prednisolone and azathioprine or mycophenolate mofetil (706–708). All these trials showed a comparable incidence of acute rejection in both treatment arms, and more importantly, a superior graft function at one year in the SRL-treated patients. In one study, graft function remained significantly better at three years in the SRL-treated patients (709). In addition, the incidence of normal histology in protocol biopsies at 2 years was significantly higher in SRL-treated patients, compared to CsA-treated patients (66.6% vs. 20.8%) (709). These results from three trials provide evidence that avoidance of the long-term nephrotoxicity, induced by calcineurin inhibitors, is achievable in renal transplant recipients.

Several randomized trials in renal transplantation investigated the feasibility and the outcome of early calcineurin inhibitor withdrawal, in triple regimens with SRL and prednisolone (710–713). Overall, patient and graft survival, as well as the incidence of acute rejection at 1 year, were comparable in both treatment arms (CsA + SRL + P vs. SRL + P). In contrast, graft function was superior, and the incidence of hypertension was reduced in the patients weaned from CsA. Similarly, the incidence of chronic allograft nephropathy was significantly lower in protocol biopsies at 1 year from patients on SRL + P alone (713). Again, these data provide strong evidence that early withdrawal of calcineurin inhibitors can safely be achieved in renal transplant recipients under SRL, and may avoid long-term nephrotoxicity.

Nephrotoxicity of Sirolimus

Studies in pigs and rats have shown that sirolimus has no deleterious effects on glomerular filtration rate or renal blood flow, and caused minimal morphologic signs of toxicity (714,715). Sirolimus reduced medullary concentrating ability and increased tubular enzymuria in rat kidneys, suggesting that mild tubular injury may occur (716). In a salt-depleted rat model of CsA toxicity, the combination of CsA with SRL produced a functional and morphologic deterioration (717).

In clinical studies in renal transplant recipients, sirolimus proved to be an effective immunosuppressive drug, devoid of intrinsic nephrotoxicity (see Clinical Efficacy of Sirolimus in Renal Transplantation, above). However, sirolimus may prolong delayed graft function in renal transplant recipients (718). In addition, thrombotic microangiopathy has been described under the combination of sirolimus and tacrolimus, after intestinal transplantation (719). Of concern are the reports on de novo proteinuria, occurring after conversion from a calcineurin inhibitor to sirolimus in renal transplant recipients (720). Although the exact mechanism for the development of this proteinuria is currently putative, the increased intraglomerular pressure, resulting from the withdrawal of the intrarenal vasoconstriction, induced by CNIs, could be one reasonable explanation. However, proinflammatory effects of a rapamycin derivative, SDZ RAD, were described as well in experimental mesangial proliferative glomerulonephritis (721).

In conclusion, there is large evidence that sirolimus is a nonnephrotoxic immunosuppressive drug. Studies, performed in renal transplantation, have shown that sirolimus may allow the avoidance of the nephrotoxicity induced by calcineurin inhibitors. Of concern are the proteinuria, recently reported after conversion to sirolimus, and the side effects induced by this drug, such as myelosuppression and hyperlipidemia.

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CHAPTER 43 ■ RENAL DISEASES INDUCED BY ANTINEOPLASTIC AGENTS

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Treatment of previously resistant tumors with potent cytoreductive therapies for cure is now common clinical practice. Often this entails the use of nephrotoxic chemotherapeutic agents at high doses and in combinations that potentiate their ability to damage the kidney. In this setting, acute renal failure (ARF) has become an anticipated side effect of treatment along with the depressed hematopoiesis. Recent surveys of the incidence of ARF within hospitalized patients note that nearly 20% of such cases are due to antineoplastic therapy (1). This chapter reviews what is known about the nephrotoxicity of antineoplastic drugs, used alone or in combination with other nephrotoxic agents. The goal of this review is to rationalize therapy to prevent as much damage to the kidney as possible because, in almost all studies to date, acute renal failure, when it occurs, carries a grave prognosis for the patient with cancer.

FACTORS THAT POTENTIATE RENAL FAILURE IN PATIENTS WITH CANCER

Extracellular Volume Depletion and Diminished Effective Arterial Circulating Volume

The clinical context in which an anticancer drug is given contributes greatly to its nephrotoxic potential. Extracellular volume depletion as a consequence of nausea, vomiting, or diarrhea should always be suspected in patients with cancer. Fluid sequestered outside the vascular space, as might occur in peritonitis, bowel obstruction, and malignant effusion may also be present. Hepatobiliary disease and heart failure, both of which may be a consequence of the cancer or its treatment, also seem to potentiate nephrotoxicity. The ability of the renal circulation to respond normally to volume depletion may also be significantly impaired by the concurrent use of drugs that inhibit renal cyclooxygenase activity to achieve pain relief or diminish fever. To the extent that it is possible, steps should be taken to restore the circulation prior to the introduction of nephrotoxins and to stop these agents before antineoplastic treatment is instituted.

Patients with Cancer and Intrinsic Renal Disease

As will be seen, any preexisting decrease in renal function, even minor, contributes greatly to the incidence and severity of renal failure during antineoplastic therapy. In some cases, this may be a consequence of altered pharmacokinetics of drugs excreted primarily by the kidney, but in other circumstances, the reason for the potentiation is less clear. Recognition of underlying renal disease, therefore, is important in cancer patients. Indeed renal failure may be provoked by characteristics unique to the specific nature of the cancer. For example, the invasion of the ureters by tumors of the ovary and cervix or the obstruction of the ureters caused by retroperitoneal fibrosis provoked by breast cancer is not uncommon.

Minor degrees of renal dysfunction, often recognized only on retrospective analysis of treatment-related nephrotoxicity, are a risk factor in the development of renal failure following chemotherapy (2). Prior chemotherapy and exposure to radiocontrast dyes, which provoke minor, and sometimes overlooked, renal injury are often the culprits. Prior radiation therapy is an important contributing factor because the dose of radiation necessary to damage kidneys is less when administered together with other cytoreductive drugs (3). Hypercalcemia, hyperphosphatemia, and hyperuricemia, as well as high blood concentrations of myoglobin, hemoglobin, and light chains, may all provoke renal failure. The clinical status of the patient also impacts on outcome. Infection and prolonged immobilization and their relationship to rhabdomyolysis, the use of hyperalimentation and subsequent hypophosphatemia, incompatible blood transfusions, and marrow infusion injury, all may occur during the course of therapy and impact negatively on renal function. Invasion of the kidney parenchyma with tumor is rarely a cause of renal failure, but renal failure has been reported with massive invasion of lymphomatous cells (4). The immunocompromised cancer patient is frequently infected and exposed to nephrotoxic antibiotics that can damage the kidney, and pyelonephritis-induced renal failure has been reported in such cases even in the absence of nephrotoxic drugs (5).

Urinary Tract Obstruction and Patients with Cancer

Ureteral and bladder outlet obstruction always need to be considered in the cancer patient, not only under the obvious circumstances of tumor invasion and obstruction in cancer of the urinary bladder, prostate, and cervix, but also ureteral obstruction due to stones and blood clot or to retroperitoneal fibrosis associated with malignancy locally and elsewhere (6). These complications often go unrecognized because of the asymmetric nature of the involvement.

Chronic Renal Failure and Patients with Cancer

Glomerular abnormalities are common in patients with cancer. Principal among them is membranous glomerulopathy, which may be associated with cancer in 10% of all patients with such nephropathy (7) and as high as 22% in older patients (8). As many as 50% of patients with multiple myeloma have renal

TABLE 43-1

NEPHROTOXIC ANTINEOPLASTIC AGENTS

Alkylating agents	Antimetabolites	Antitumor antibiotics	Biologic agents
Cisplatin	High-dose methotrexate	Mitomycin	Recombinant interferon-α and interferon-γ
Carboplatin	Cytosine arabinoside	Mithramycin	Interleukin-2
Cyclophosphamide	5-Fluorouracil		
Nitrosoureas	5-Azacytidine		
Carmustine (BCNU)			
Lomustine (CCNU)			
Semustine (methyl CCNU)			
Streptozocin			
Ifosfamide			

failure and nephrotic syndrome with κ or λ light chains in the urine, frequently with primary amyloidosis in the kidney (9). The paraproteinemic syndromes associated with cancers of the hematopoietic system, such as macroglobulinemia and cryoglobulinemia, may also be associated with glomerular abnormalities (10). These paraproteins may be produced in such large quantities that they exceed the ability of the tubules to reabsorb them sufficiently to prevent the formation of proteinaceous casts that obstruct the flow of urine and cause further decline in glomerular filtration. This so-called "cast nephropathy" is also associated with glomerular abnormalities.

Interstitial renal disease is also common in patients with cancer. Recurrent serious infections in the immunocompromised host requiring chronic exposure to potentially nephrotoxic antibiotics can cause damage to the kidney. Treatment with high-energy radiation may cause renal failure with hypertension, proteinuria, and profound anemia (11). The renal failure may not develop for years after exposure and occurs in patients exposed to greater than 20 Gy (2,000 rads) of high-energy radiation to both kidneys.

Fluid and Electrolyte Disorders Associated with Cancer

A variety of electrolyte disorders occur in the background of cancer. Hypophosphatemia with renal phosphate loss and osteomalacia can occur with mesenchymal tumors (12). The syndrome remits when the tumor is removed, which suggests the production of a phosphaturic substance by the tumor. Hyponatremia is associated with a number of tumors including small cell cancer of the lung (13) and various head and neck tumors (14). Hypokalemia and hypophosphatemia may occur together in cancer patients and, although rare, may be so severe that they induce rhabdomyolysis and acute renal failure. In a recent report, 16% of patients with small cell lung cancer had hyponatremia and had shorter survival than patients with the same stage of the disease who did not have hyponatremia (15). Although there is a relationship between hyponatremia and tumor cell production of arginine vasopressin (AVP) in some patients (15), there are some patients in whom hyponatremia is not associated with high levels of AVP (16). Interestingly, a patient with leiomyosarcoma was recently described who presented with hyponatremia and hypertension and very high levels of prorenin and renin who responded to both angiotensin-converting enzyme inhibitors and tumor removal (17). Central diabetes insipidus as a result of metastatic disease, especially breast cancer (18), may also occur.

Hypercalcemia is perhaps the most common electrolyte abnormality of cancer and can be divided into two forms (19). One is related to local osteolytic lesions, and the other is associated with a humoral factor, a parathyroid hormone-related protein, which is the cause of enhanced bone resorption. Hypercalcemia can reduce glomerular filtration itself, but it may cause renal failure by potentiating the nephrotoxic effects of other drugs or require treatment by drugs that are nephrotoxic themselves (see next section). Thus hypercalcemia is an important risk factor for the development of renal failure in cancer patients.

ANTINEOPLASTIC AGENTS THAT CAUSE ACUTE RENAL FAILURE

Several antineoplastic agents have predictable dose-related nephrotoxicity. Included in this group (Table 43-1) are the alkylating agents cisplatin and streptozotocin, the antimetabolite methotrexate, the antitumor antibiotic mithramycin, and the cytokine interleukin-2 (IL-2). These agents induce a fall in glomerular filtration that is usually dose-related and predictable. Others have nephrotoxic potential after chronic repeated exposure to the drug, especially in combination with other drugs, as single exposure rarely causes renal failure. Taxol (paclitaxel), a microtubule-binding agent recently introduced into multidrug cytoreductive regimens, has shown potentiation of the nephrotoxicity of cisplatin (20) and in the setting of bone marrow transplant (21). When recognized, the decline in renal function with some chemotherapeutic drugs is sometimes irreversible. Examples of these agents are the alkylating agents lomustine (CCNU) and semustine (methyl CCNU) and the anti-tumor antibiotic mitomycin. Chronic and irreversible decline in renal function has also been documented after cisplatin therapy and after bone marrow transplantation, perhaps as a result of repeated, but subclinical damage to the kidney.

ALKYLATING AGENTS

Cisplatin

Cisplatin, perhaps the most commonly used antineoplastic agent for the treatment of solid tumors, is the best-studied antineoplastic nephrotoxin. Long-term survivors have provided the opportunity to follow cisplatin nephrotoxicity over longer periods of time than has been possible with other agents.

Preclinical studies identified nephrotoxicity as cisplatin's major dose-limiting side effect early (22), and initial protocols not employing aggressive hydration prior to administration of cisplatin produced severe and frequently irreversible renal failure (23). Other significant side effects, such as ototoxicity and severe nausea and vomiting, may also limit its use.

Pathogenesis

The kidney accumulates and retains platinum to a greater degree than other organs and is the principal excretory organ for injected cisplatin (24). In the rat, the kidney excretes the drug rapidly within the first hour of its administration by a process consisting predominantly of glomerular filtration, with a minor component of secretion (25–27). There is no evidence of tubular reabsorption, suggesting that the kidney accumulates cisplatin by peritubular uptake (26). The uptake of cisplatin by the kidney is dependent on temperature and the normal consumption of oxygen and can be inhibited by drugs that participate in the organic base transport system, suggesting that at least some portion of renal cisplatin uptake is facilitated (26). Consistent with the view that cisplatin nephrotoxicity may be linked to how the kidney transports cisplatin is the high cisplatin content of proximal straight tubules after [^{195m}Pt] cisplatin injection (28), the principal site of necrosis following cisplatin (29). Changes in the distal nephron including apoptosis, if not frank necrosis, have also been described (23,30). The glomerulus is spared of obvious morphologic changes.

Excreted platinum is predominantly cisplatin, but inside the cell cisplatin is converted to another species (31). Once gaining access into the cell, cisplatin is thought to undergo aquation reactions in which the labile chloride ligands are replaced by water molecules, resulting in a positively charged and highly reactive electrophilic product (32). The primary lethal lesion produced by cisplatin in cancer cells is its intrastrand binding to adjacent purine bases (33), which alters the secondary structure of DNA, inhibiting its template function and inhibiting DNA replication (34,35). This lesion is not produced by the transplatin isomer, which is neither antineoplastic nor nephrotoxic. Whether the nephrotoxicity of cisplatin also depends on such damage to DNA is unknown. Cisplatin may also damage the kidney by depletion of critical sulfhydryl centers, including glutathione, and provoke damage to the cell in much the same manner as oxidant stress. Indeed the administration of manganese superoxide dismutase attenuates cisplatin-induced renal failure (36).

CELLULAR RESPONSES TO CISPLATIN TREATMENT

Role of the Protein Kinase Cascades in Cisplatin Nephrotoxicity

Cellular stress, including DNA-damaging chemotherapeutic drugs, activates several signaling pathways: the stress-activated protein kinase (SAPK), including the Jun N-terminal kinases (JNKs), p38 MAPK (mitogen-activated protein kinase), and the extracellular regulated kinases or (ERKs) (37). Cisplatin-mediated activation of JNK and concomitant cell death are related, since expression of a dominant negative expression vector of the JNK (Jun N-terminal kinase) MKK4 (mitogen-activated protein kinase kinase 4) blocked cell death (38). Similarly, cells derived from animals in which the gene for c-jun was deleted, a specific protooncogene target of JNK, were more resistant to cisplatin-induced cell death than normal cells (39). A balance between the JNK and ERK pathways had been proposed to dictate the cellular decisions of death and survival: transient activation of JNK when also accompanied by activation of prosurvival ERK or AKT (v-akt kinase/protein kinase B) pathways may not be associated with cell death. Indeed, inhibition of JNK by dominant-negative mutants proved to be protective of cisplatin-induced injury (38). Pharmacologic inhibition of the cisplatin-induced ERK activity in ovarian carcinoma cells caused enhanced cisplatin cytotoxicity (40), and inhibition of the Akt/PKB pathway enhanced activation of caspase-3 and caspase-9 in proximal tubular cells (41).

However, inhibition of cisplatin-induced activation of ERK protected rabbit (42) and mouse proximal tubule (43) cells, suggesting that the interaction of cisplatin with such cells is fundamentally different from that seen in cancer cells. As these observations have important clinical implications, it will be necessary to explore these approaches *in vivo* to see whether the discrepancies are more a function of the limitations of *in vitro* models of tissue injury or signal important differences in cellular response.

Role of Casplase Activation in Cisplatin-induced Cell Death

Cisplatin induces apoptosis of renal proximal tubular cells (LLCPK) *in vitro* via mitochondria-dependent and –independent pathways (41), and the activation of the caspases is crucial to this process. Studies in renal tubular epithelial cells demonstrate that cisplatin induces selective and differential activation of caspases including executioner caspase-3 and initiator caspase-8, -9, and -2 but not proinflammatory caspase-1 (41). The translocation of Bax to the mitochondria and release of cytochrome c from the mitochondria to the cytosol further support the activation of caspase-9 (42). Cisplatin–induced activation of caspase-8 and apoptosis has also been reported in freshly isolated proximal tubular epithelial cells, which involves the TNF- and Fas pathways (44). DEVD-CHO (acetyl-Asp-Glu-Val-Asp-aldehyde) or LEHD-CHO (acetyl-Leu-Asp-Glu-Asp-aldehyde), inhibitors of caspase-3 and caspase-9, respectively, provided only partial protection against cisplatin-induced cell death and DNA damage in LLC-PK1 cells (41) indicating mechanisms other than caspase activation are also involved in cisplatin-induced cell death. Thus, cisplatin-induced activation of caspase-8 and caspase-9 in renal proximal tubules indicate that both receptor and mitochondrial signaling pathways participate in the activation process. In a recent study p53 inhibition was shown to partially protect cisplatin-induced cell death (45) indicating that p53 may also be involved in cisplatin -induced activation of caspases in renal tubular epithelial cells. Working out the precise details of renal cisplatin-induced caspase activation is likely to reveal additional means to ameliorate its nephrotoxicity.

Cytokines and Cisplatin-induced Cell Death

Cisplatin administration significantly upregulates several cytokines and chemokines (tumor necrosis factor-α [TNF-α], transforming growth factor-β [TGF-β], regulated upon activation, normal T cell expressed and secreted [RANTES], macrophage inflammatory protein [MIP-2], monocyte chemotactic protein-1 [MCP-1]) in the kidney (46). Several antiinflammatory strategies have yielded positive protective results. Anti-ICAM I (intercellular adhesion molecule-1) antibodies reduce cisplatin toxicity (47), as does salicylate treatment (48) and interleukin-10 (IL-10) administration (49). These studies provide convincing evidence that cisplatin cytotoxicity is

mediated in part by inflammatory mediators. Interestingly, TNFα itself provokes oxidant dependent damage to cells so that the possibility that the TNF-α–related toxicity is direct and unrelated to recruitment of inflammatory cells (50) cannot be ruled out.

Cell Cycle Events in Cisplatin Nephrotoxicity

Genotoxic damage either by intercalation of DNA or by oxidant-induced damage may be an important component of the stress imposed by cisplatin. Cisplatin provokes increased expression of the cyclin-dependent kinase inhibitor $p21^{WAF1/CIP1}$, which inhibits completion of the cell cycle, and promotes DNA repair. Interestingly, mice with deletion of the p21 gene are more susceptible to cisplatin nephrotoxicity (51), suggesting that it may be critical to the repair of cisplatin-induced damage to renal cells. Confronted with a hostile environment, the kidney mounts a response that is initiated by signaling molecules that engage multiple pathways including those that regulate the cell cycle. The cell undergoing these changes may decide to check the progression of the cycle and repair damage before proceeding or enter a pathway destined to cell death. This decision point is carefully regulated and cyclin-dependent kinase inhibitors, especially p21, are important in this decision. The interface between these pathways and the cell death pathways are first emerging but phosphorylation events critical to cell function reside in the cyclin dependent kinases and the kinases, phosphatases, inhibitors, and activators that regulate their activities. The identification of the precise pathways engaged in this process is an area of active research not only in acute renal failure but also in the field of cell biology in general.

Clinical Manifestations

Cisplatin predictably lowers glomerular filtration rate (GFR) in a dose-dependent manner (52) after even single drug exposure, and its nephrotoxicity is augmented by coadministration of drugs used in aggressive chemotherapy (53). The onset of the renal failure is gradual, usually occurring 3 to 5 days after its administration. Early proteinuria is mild (>500 mg/day), as is glycosuria. Enzymuria is common, even in the mildest forms of ARF. In a few patients, significant urinary electrolyte wasting may be provoked by cisplatin (54), including severe sodium, divalent cation excretion, and phosphate wasting, but this presentation is uncommon and seen primarily with high-dose therapy. Most common is the gradual onset of nonoliguric renal failure with water excretion in excess of solute (28). In micropuncture experiments, the site of altered water reabsorption is beyond the late distal tubule, probably in the apparently morphologically intact collecting duct (28). In rats, the cause of the fall in glomerular filtration is afferent vasoconstriction and possibly an altered ultrafiltration coefficient, before evidence of tubule obstruction (55). The mediators for these changes in segmental water transport and vascular resistance are unknown.

Cisplatin also produces hypomagnesemia (55) in a large percentage of patients receiving the drug. Although it may occur acutely, it is usually observed after repeated exposure over longer periods of time (56). It is often found when additional drugs with significant potential for magnesium wasting are used in conjunction with cisplatin, such as nephrotoxic antibiotics like gentamicin or amphotericin (57), or when cisplatin is used in combination with other chemotherapeutic agents (56). In the normomagnesemic animal ingesting a diet containing adequate magnesium, cisplatin does not induce hypomagnesemia. However, in the rat kept on a magnesium-deficient diet, cisplatin induced a defect in maximal reclamation of an administered magnesium load, suggesting that hypomagnesemia is a consequence of diets deficient in magnesium in combination with a mild defect in magnesium reabsorption (58). This is consistent with the relative ease of treating the hypomagnesemia with oral magnesium supplementation. Hypomagnesemia may persist even when cisplatin is withdrawn (59) but usually remits when cisplatin administration is discontinued (53). Serum calcium is usually normal in such cases.

In almost all studies, repeated cisplatin administration has been shown to reduce GFR chronically in a dose-related manner (52,60–66). Patients receiving up to 850 mg in multiple courses had a 9% reduction in hippuran clearance, while patients receiving more than 850 mg of cisplatin had a 40% reduction in GFR over a 5-year period (52). This study also found significant potentiation of cisplatin nephrotoxicity in those patients receiving cisplatin combined with radiotherapy. In each of these studies, GFR remained stable after discontinuation of the therapy for up to 3 to 5 years of follow-up.

Protective Measures

The wide therapeutic application of cisplatin depends not only on its clinical efficacy but also on the development of strategies that diminish the severity of the ARF it produces. The most commonly used protective measure is to establish a solute diuresis (67). A commonly applied protocol is to establish a diuresis before (12 to 24 hours) administering cisplatin, which is then given in isotonic saline by a 3-hour infusion, followed by infusion of isotonic saline or mannitol for 24 hours after the drug is infused. Cisplatin is usually administered in daily divided doses for 5 days until the maximum dose is reached, usually not to exceed 120 mg per m^2 body surface area (BSA). Beyond this dose cisplatin provokes an unacceptable degree of renal failure, even in the presence of a diuresis.

The mechanism of the protection is not known. Although the concentration of cisplatin is lower in the urine, neither the tissue concentration of the drug nor the degree of cytotoxicity is altered by such maneuvers (67). Alternatively, the high chloride content of the urine might reduce its speciation into toxic metabolites, but mannitol is equally effective in diminishing the degree of renal failure where urine chloride concentration is reduced, so this explanation seems inadequate as well.

Additional maneuvers have been attempted to reduce cisplatin nephrotoxicity. Diethyldithiocarbamate (DDTC), which has been shown to compete for platinum binding to DNA (68), has shown promise experimentally (69) but has been much less successful in human trials due to its toxicity and failure to modify cisplatin ototoxicity (70). Inorganic thiophosphates like WR2721 (amifostine) has been shown to be effective in preventing renal failure even after repeated exposure (71). The mechanism of protection may involve promotion of better DNA repair and synthesis by amifostine (72) and/or by the release of free thiols after dephosphorylation with alkaline phosphatase, which then act as free radical scavengers and metal binding centers (73).

Erythropoietin improves renal function when co-administered with cisplatin and the improvement seems to be mediated by improved regeneration rather than an inherent reduction in toxicity (74).

Promising future strategies will be based on a better understanding of the pathophysiology of cisplatin-induced kidney injury. In Figure 43-1 are outlined likely therapeutic targets for intervention. Cisplatin by its pro-oxidant effects and direct interaction with proteins and DNA initiates a stress response characterized by activation of pro-death and pro-survival transduction and molecular pathways. In each instance the balance between these pathways can be manipulated to ameliorate

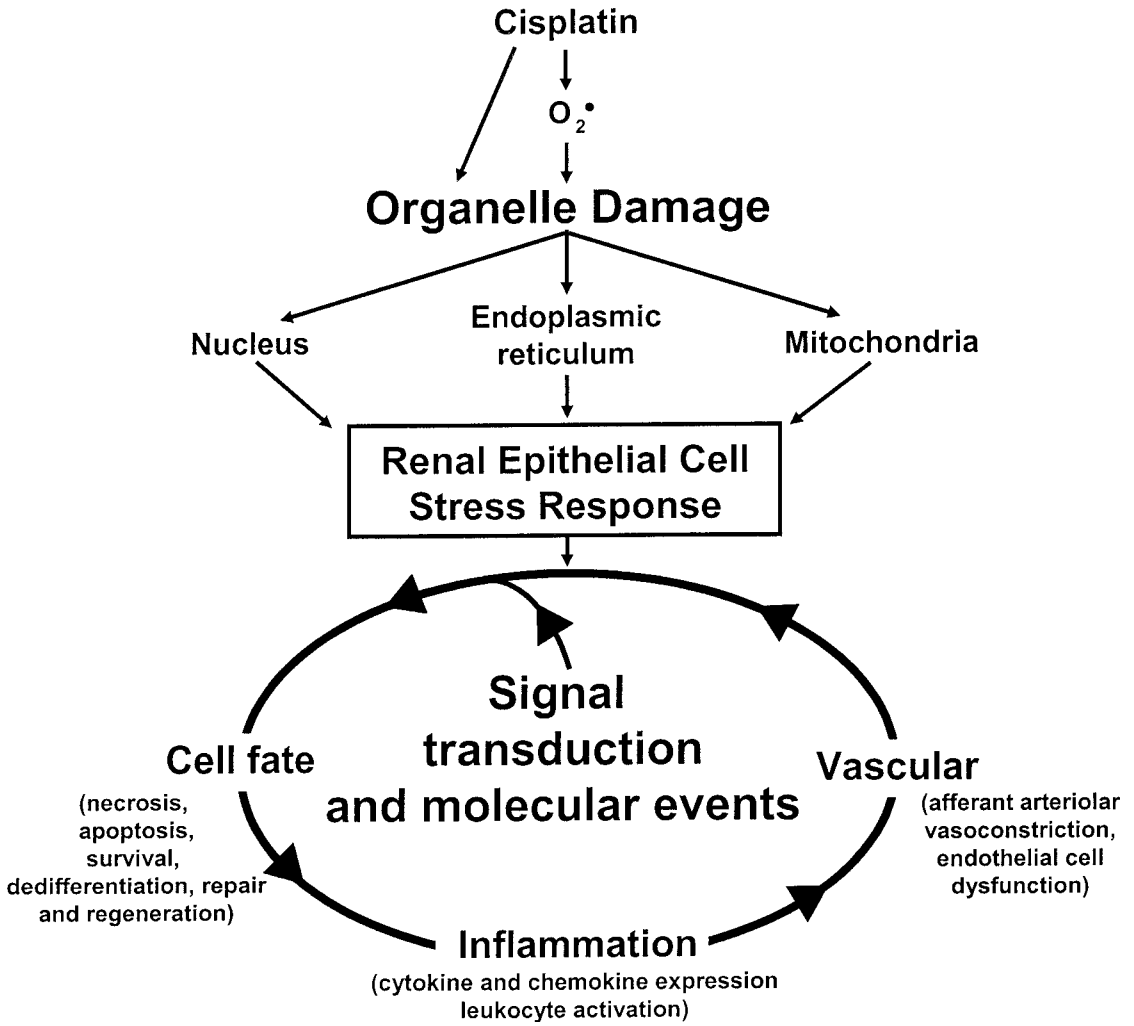

FIGURE 43-1. Cisplatin and the renal stress response. Sites of potential cisplatin-macromolecular interaction that initiates the renal stress response consisting of signal transduction and molecular pathways. These responses lead to the phenotypic changes characteristic of cisplatin-induced kidney injury including necrosis, apoptosis, survival, and repair, as well as inflammatory and vascular effects that may mediate and ameliorate these effects. The balance between the responses that promote repair and survival and those that cause cells to die can be manipulated by upregulating the former and downregulating the latter (see text).

the consequences of cisplatin exposure on kidney function and structure. Thus inhibitors of caspase activity, TNF-α inhibitors, inhibition of specific components of the MAPKs, upregulation of cell-cycle regulators, and antioxidants each show promise in ameliorating experimental cisplatin nephrotoxicity. Translating these observations to the bedside should be accomplished in the near future.

New platinum compounds with antineoplastic effects and less nephrotoxicity have been identified and are being increasingly applied. Prominent among them is carboplatin (*cis*-diaminecyclobutanedicarboxylplatinum II). While the kidney remains the primary excretory organ and dose modification must be applied in the presence of diminished renal function, the compound has much less nephrotoxicity than cisplatin (75). The dose-limiting side effect is myelosuppression, especially thrombocytopenia (76). Because of its overlapping toxicity with other antineoplastic agents and experience with such toxicity, carboplatin is increasingly included in protocols for lung, head and neck, and cervical cancer. Carboplatin and other similar compounds will almost certainly be increasingly used in patients with prior chemotherapy-induced renal dysfunction, especially in those patients with tumors expected to respond to anti-tumor platinum analogs. The full impact of the use of this drug on the occurrence of renal failure, however, is as yet unknown.

Cyclophosphamide

Cyclophosphamide is converted by hepatic mixed-function oxidases into an active alkylating agent with wide-ranging antitumor activities against many hematologic and solid tumors. Its primary toxicity is myelosuppression, but important toxicity involving the gastrointestinal tract and urinary bladder may also occur.

Cyclophosphamide diminishes the ability of the kidney to excrete water (77). Impaired water excretion occurs acutely and resolves after discontinuation of the drug. The water retention may involve a direct effect of cyclophosphamide on the collecting duct epithelium, as elevated serum AVP (arginine vasopressin)levels have not been detected (78). Recognition of this effect is important since hydration protocols with hypotonic solutions prior to the administration of cyclophosphamide are likely to provoke marked hyponatremia. Thus

hydration with normal saline is preferable. It is important to remember, however, that any detectable AVP is inappropriate in the presence of hypo-osmolality. While the effect of cyclophosphamide is self-limited and rarely of clinical significance, the concentrated urine predisposes to hemorrhagic cystitis and uric acid nephropathy. Cyclophosphamide is increasingly included in high-dose treatment regimens combined with autologous bone marrow or stem cell transplants, and possible new potentiating patterns of renal failure may emerge (79).

Nitrosoureas

Semustine (methyl-CCNU), carmustine (BCNU), and lomustine (CCNU) have demonstrated efficacy against brain tumors, Hodgkin's disease and other lymphomas, and multiple myeloma (80). Their high lipid solubility and high permeability across the blood–brain barrier make them ideal for the treatment of such tumors. Carmustine, which is the most commonly used nitrosourea, is given intravenously at a dose of 150 to 200 mg per m^2 infused over 1 to 2 hours. Nephrotoxicity presents the main limitation to their greater efficacy at these doses, while hepatic failure and pulmonary fibrosis are common at higher doses. The nitrosoureas are rapidly metabolized (81), and their metabolites appear in the urine for up to 72 hours following administration (82). These metabolites are thought to be responsible for the nephrotoxicity, as the parent compound is not detected in the urine. The mechanism of the nephrotoxicity of the nitrosoureas is unknown.

Streptozotocin

Streptozotocin is a nitrosourea that is used for the treatment of metastatic islet cell carcinoma of the pancreas and carcinoid tumors. Nephrotoxicity is its chief limiting side effect and is present in 75% of patients who receive prolonged streptozotocin administration (83). Nephrogenic diabetes insipidus and obstructive uropathy from uric acid nephrolithiasis caused by its uricosuric effect have also been described.

The major excretory route of streptozotocin is the kidney, which accumulates the drug to high concentrations (84). Proximal tubules are the principal site of damage (85). Although there are no definitive data relating cumulative dose with nephrotoxicity, renal damage often occurs with a cumulative dose of 4 g per m^2 body surface area (BSA) (87). Clinical streptozotocin-induced nephrotoxicity includes tubular reabsorptive abnormalities similar to Fanconi's syndrome including hypophosphatemia as well as proteinuria (86); even after a single exposure to the drug (87), these tubular effects may precede the reduction in GFR. Extreme caution should be used before introducing it in patients with preexisting renal disease. A related analog, chlorozotocin, also causes renal failure in patients with metastatic islet cell carcinoma of the pancreas treated with total doses exceeding 1,500 mg (88).

Ifosfamide

Ifosfamide has been shown to be active against testicular carcinoma and pediatric soft tissue sarcomas, osteosarcoma, and Ewing's sarcoma (89). Used in conjunction with mesna, the dose-limiting side effect of hemorrhagic cystitis has been eliminated (90). The incidence of severe nephrotoxicity, which is dose related, has been significantly reduced by administering ifosfamide in divided daily doses rather than by bolus infusion (89). Reabsorptive defects have been reported even under these circumstances (91–93) and consist of aminoaciduria, bicarbonaturia, phosphaturia, and proteinuria. Small but consistent reductions in GFR and persistent proximal tubule defects have been documented in patients receiving multiple courses of nonplatinum-containing ifosfamide protocols (94–95). Previous renal disease, perhaps as a consequence of cisplatin exposure, has proved to be an important risk factor for the development of ifosfamide nephrotoxicity (2,96–98). A severe irreversible Fanconi-like syndrome with rickets may appear in the course of therapy with ifosfamide, especially when combined with cisplatin (97).

ANTIMETABOLITES

Methotrexate

Methotrexate (MTX), an analog of folic acid, is used in combination chemotherapy of embryonic hematologic and solid tumors, as well as in cytoreductive protocols in bone marrow transplant. It is given in two dose ranges, low and high, with clearly different degrees of nephrotoxic risk. At conventional doses, nephrotoxicity is rarely a problem (99), but at the doses given in leucovorin rescue therapy (1 to 7 g per m^2 BSA), high rates of nephrotoxicity and drug-related mortality can occur unless precautions are taken to minimize the nephrotoxicity.

Primarily the kidney excretes MTX by glomerular filtration and tubular secretion so that 90% of the drug appears unchanged in the urine after conventional intravenous doses (100). Thus reduced glomerular filtration raises the concentration of MTX in body fluids for a prolonged period of time. Under these conditions, marked bone marrow, gastrointestinal, and renal toxicity is observed (101). Removal by hemodialysis and peritoneal dialysis is poor, as is the efficacy of plasma exchange (102–104).

The mechanism of MTX nephrotoxicity is unclear. A commonly invoked mechanism is its limited solubility at acid urine pH, which provokes intratubular precipitation especially during high-dose infusion (104). According to this hypothesis, dehydrated patients who excrete acid urine are more at risk for this complication. Consistent with this notion is the decline in MTX nephrotoxicity observed since the institution of urinary alkalinization and hydration associated with high-dose MTX therapy (105). It should be pointed out that hydration alone ameliorates the nephrotoxicity of a wide variety of toxic and physical insults as discussed previously. MTX may be directly toxic to the tubule epithelium, as proximal tubule necrosis without intraluminal precipitation has been noted (106). Finally, infusion of high-dose MTX reduces glomerular filtration without changing blood pressure, suggesting a direct renal hemodynamic effect (107,108). Thus MTX nephrotoxicity is probably multifactorial.

Clinical Course

Typically symptoms of renal colic sometimes precede overt renal failure, which is nonoliguric. Since the onset of the renal failure is rapid, often only hours after administration, thus further limiting its excretion, it is often associated with severe organ toxicities including gastroenteritis, hepatitis, mucositis, and pancytopenia. Plasma levels exceeding 0.5 μM for 48 hours following MTX infusion are associated with a 40% incidence of severe systemic toxicity (103). Expansion of the extracellular fluid with 150 mM $NaHCO_3$ should precede use of drug. Removal of drug by dialysis is problematic because of its high volume of distribution and protein binding, but high-flux dialyzers show improved drug removal (109).

Treatment

When acute renal failure intervenes, the goal of therapy is to reduce MTX concentration as rapidly as possible. A newly introduced treatment of high-dose MTX nephrotoxicity with carboxypeptidase-G2 (CPDG-2), a recombinant bacterial enzyme that hydrolyzed MTX to an inactive metabolite shows early promise. In a recent study CPDG-2 reduced MTX blood levels by 98% following infusion. While early concern for delay in recovery due to the potential for the metabolite to crystallize in urine, this has not proven to be the case. Given the ease of administration and its greater efficacy in reducing MTX concentration, this treatment would appear to have definite advantages over dialytic approaches (110).

MTX is routinely used in chemoprevention of graft-versus-host disease (GVHD) following bone marrow transplant, and its participation in the renal failure induced on that background is discussed in the section Renal Failure Associated with Bone Marrow Transplant, below.

Cytosine Arabinoside

Cytosine arabinoside, also known as cytarabine and ara-C, is a pyrimidine nucleoside that inhibits DNA synthesis when activated by the enzyme deoxycytidine kinase (111). It is used in multidrug protocols in the treatment of acute leukemia, in the blast phase of chronic myelocytic leukemia, and non-Hodgkin lymphoma (112). Deamination of free cytosine arabinoside yields uracil arabinoside, which is the principal urinary metabolite of cytosine arabinoside (113).

Renal failure occurs in 50% of patients treated with multidrug regimens including cytosine arabinoside, even if used with drugs not known to produce renal failure (114). Potentiation of nephrotoxicity was especially common in a drug regimen using cytosine arabinoside in combination with cisplatin and hydroxyurea (115). High-dose cytosine arabinoside leads to even greater nephrotoxicity and neurotoxicity, especially in patients with prior renal injury (116). Rarely acute rhabdomyolysis may be a causative factor in cases exposed to cytosine arabinoside (117). Prominent apoptosis of muscle cells has been demonstrated in such cases secondary to cytochrome-c release. All other drugs that initiate muscle damage, such as cyclophosphamide, 5-azacytidine, interferon, and interleukin-2 should be discontinued.

5-Fluorouracil

The active metabolite of 5-fluorouracil (5-FU), fluorodeoxyuridine monophosphate, inhibits DNA synthesis by inhibiting thymidylate synthase. While not nephrotoxic when given as a single agent, renal insufficiency has been observed when it is combined with other agents of known nephrotoxic potential. When given with mitomycin-C, which provokes renal failure in a minority of patients (discussed later in this chapter in the section Mitomycin-C), fluorouracil provoked severe and fatal renal insufficiency in 10% of patients undergoing such treatment for carcinoma of the gastrointestinal tract and pancreas (118,119). In these reports, either an acute or chronic syndrome of microangiopathic hemolytic anemia with thrombocytopenia emerged. Histologic examination of the kidney revealed fibrin thrombi in arterioles, which also demonstrated intimal hyperplasia. Interstitial fibrosis, tubular atrophy, and glomerular necrosis were observed in the chronic forms. Renal failure was also noted when the drug was given in combination with cisplatin (120). This combination is increasingly applied in otherwise resistant advanced solid tumors, especially of the head and neck (121) and invasive cervical cancers (122).

5-Azacytidine

A similar circumstance exists for azacytidine because there has been no nephrotoxicity described when this drug is used alone (123), but a high rate of tubular reabsorptive abnormalities has been noted when the drug is used in combination with others (124). Nephrotoxicity is manifested by glycosuria, acidemia, phosphaturia, and polyuria and with mild azotemia. The defects were compatible with an acquired renal tubular acidosis, which appears early in the course of treatment and resolves rapidly when the drug is discontinued.

ANTITUMOR ANTIBIOTICS

Mitomycin-C

Mitomycin-C is used in combination protocols, most often with 5-fluorouracil and doxorubicin, in the treatment of solid tumors of the genitourinary and gastrointestinal systems. In preclinical studies, acute tubular necrosis was induced by single intravenous injection (125). Although it is known that the drug is metabolized in the liver, the specifics of its renal handling have not been adequately addressed. The mechanism of the nephrotoxicity is also unknown.

The available data would seem to indicate that mitomycin-C occasionally induces a dose-related, mild form of ARF when administered alone (126). However, its nephrotoxicity is more frequent and severe when used in combination with other drugs. Clinically, the renal failure is accompanied by a microangiopathic hemolytic anemia, usually occurring late in the course of therapy (127). The incidence is reported to be between 2% and 10% of cases and patients receiving a cumulative dose of 30 to 50 mg per m^2 should be carefully monitored for fragmentation of red blood cells if unexplained anemia or thrombocytopenia appear. Histopathologically, the findings are consistent with a thrombotic microangiopathic process with fibrin deposition within glomeruli and interstitial blood vessels. Glomerular sclerosis and necrosis and interstitial scarring are prominent. While these changes are reminiscent of the hemolytic-uremic syndrome, plasma exchange does not improve renal function (127), nor is the experience with glucocorticoids and anti-platelet drugs good. Anecdotal evidence for improvement of renal function after immunoadsorbtion with staphylococcal protein-A columns suggest that immune complexes may play a role in the pathogenesis of the renal failure. A recent animal study of its nephrotoxicity, however, found a direct tubulotoxic effect only (128) and no good animal model exists that mimics the clinical situation.

Mithramycin

Mithramycin inhibits RNA synthesis and has some antineoplastic efficacy in testicular carcinomas and glioblastomas (129,130), but it is most frequently used to treat malignancy-associated hypercalcemia. The calcium-lowering effect of the drug seems to be due to its inhibition of osteoclast function (131) and is linked to its inhibition of messenger RNA (mRNA) synthesis (132). A single 25 μg per kg IV dose, which normalizes serum calcium in a large majority of cases, is usually not nephrotoxic. However, repeated daily injection of the drug has been reported to cause significant nephrotoxicity in up to

40% of patients in one study (129). Renal failure persists if it occurs on the background of preexistent renal disease and is the cause of death in 10% of the patients studied. Histologically, there is a picture typical of acute tubule necrosis with apparently normal glomeruli. The mechanism of the nephrotoxicity is unknown.

KIDNEY INJURY AND THE TREATMENT OF HYPERCALCEMIA OF MALIGNANCY

Zolendronnate

Zolendronnate, a highly effective bisphosphonate that is increasingly used to treat hypercalcemia in cancer patients, can also cause acute renal failure (133). The renal toxicity, which is characterized by proximal tubule necrosis and apoptosis but spares the glomerulus, is dose related and occurs in about 10% of cases receiving the usual 4 mg dose. Prolonging infusion time and reducing exposure dose have been found to reduce nephrotoxicity. Even under these circumstances, renal failure emerges slowly and requires cessation of drug. The renal failure, when recognized early, is partially reversible but is somewhat slow to resolve. The mechanism of its nephrotoxicity is unknown, but, like cisplatin, is almost certainly related to its active uptake and concentration in proximal tubule cells.

BIOLOGIC AGENTS

Interferons

The interferons are glycoproteins that have antiviral (134), as well as antitumor activity (135,136). Renal insufficiency has been produced by both interferon-α (IFN-α) and interferon-γ (IFN-γ), two of the three major classes of the interferons. IFN-α produces proteinuria, sometimes massive with nephrotic syndrome, and histopathologically a picture consistent with minimal-change nephropathy (137). The occurrence of ARF, however, is relatively rare (138). ARF with renal biopsy evidence of acute tubular necrosis has been described with IFN-γ treatment of acute lymphoblastic leukemia (139). Divided doses of up to 1×10^7 U per m^2 BSA total administered IFN-α and 1,000 μg per m^2 BSA IFN-γ are usually tolerated without significant nephrotoxicity (140).

Interleukin-2

IL-2 is a polypeptide cytokine that activates the natural killer function of lymphocytes. Infusion of IL-2 concurrently with such lymphokine-activated killer (LAK) cells is associated with regression of several solid tumors including malignant melanoma, renal cell cancer, and colorectal cancer (141). Profound reductions in blood pressure, GFR, and sodium excretion occur in patients during the period of drug infusion, which resolve fairly rapidly when the infusion is stopped (142).

A more comprehensive study of IL-2 nephrotoxicity documented falls in GFR in 90% of cases (143). Although the syndrome of hypotension, oliguria, salt retention, and azotemia suggests a prerenal component, massive infusion of fluid does not abrogate the fall in GFR (144). In Figure 43-2 is shown the time course of changes in salt excretion, GFR, and cardiac output in a patient receiving IL-2 infusion. It can be seen

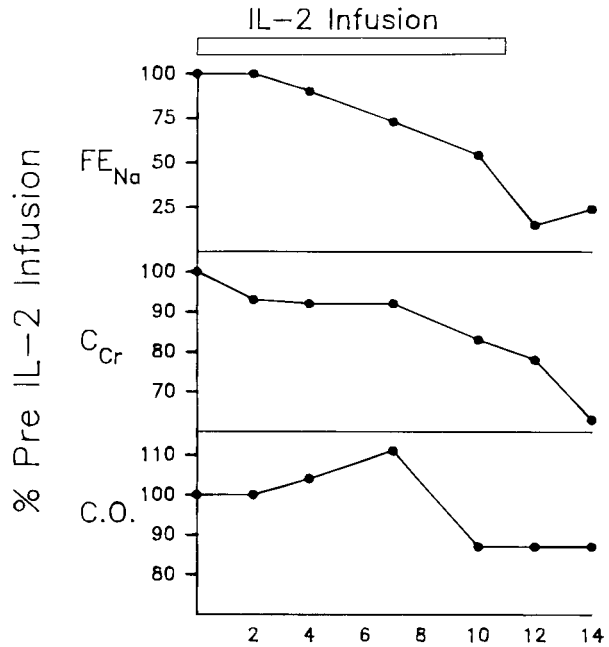

FIGURE 43-2. Hourly fractional sodium excretion (FE_{Na}), creatinine clearance (C_{Cr}), and cardiac output (CO) as a percent of preinfusion levels during the infusion of interleukin-2 (IL-2), 500,000 U in 12 hours, in a patient undergoing treatment for renal cell carcinoma. The patient also received mannitol and isotonic saline infusion prior to the infusion of IL-2.

that the fall in GFR and salt excretion precedes the period of reduced cardiac output, suggesting early effects of IL-2 on kidney function before effects on central hemodynamics are obvious. In a study of renal hemodynamics during high-dose IL-2 and IFN-α adoptive immunotherapy of renal cell carcinoma, GFR fell significantly following repeated infusion and was not accompanied by a fall in renal plasma flow, suggesting a fall in the net glomerular ultrafiltration pressure or permeability coefficient (145). The fall was accentuated by use of nonsteroidal anti-inflammatory drugs. Whether IL-2 mediates these effects directly or indirectly is not clear.

CHEMOTHERAPY-INDUCED TUMOR LYSIS AND RENAL FAILURE

The release of potential nephrotoxic products into the circulation from cancer cells lysed by chemotherapy is common. The blood concentration of uric acid, xanthine, phosphate, and potassium may increase to levels that impact on kidney function. This is especially true in the setting of prior renal insufficiency. This syndrome of metabolic derangements associated with renal failure during chemotherapy has been termed the "tumor lysis syndrome" and is not infrequently observed after the treatment of acute leukemia, malignant lymphoma, and bulky solid tumors (146,147).

Hyperuricemia and Acute Renal Failure

Renal insufficiency associated with acute hyperuricemia is most frequently associated with the therapy of leukemias and lymphomas (148), although it has been reported in disseminated carcinoma (149,150) and may even occur spontaneously (151).

The incidence in one series of patients treated for non-Hodgkin lymphoma was 6% of all patients but was highest in patients with high-grade tumors and renal insufficiency (152). Hyperuricemia-induced crystalluria and renal failure are discussed in Chapter 49, but pretreatment with allopurinol and establishing an alkaline diuresis have significantly reduced the incidence of this complication.

Xanthine Nephropathy

Although rare, xanthine nephropathy can occur during tumor lysis when allopurinol is used to prevent the production of uric acid. Xanthine concentration in the blood increases consequent to xanthine oxidase inhibition by allopurinol, and like uric acid, xanthine is poorly soluble in acid urines. Xanthine crystalluria can occur when its urinary excretion rises beyond its solubility (153). This is especially likely in patients who have minor degrees of hypoxanthine–guanine phosphoribosyltransferase deficiency (154).

Hyperphosphatemic Nephropathy

Hyperphosphatemia may occur as part of the tumor lysis syndrome and, when extreme, may result in ARF (155,156). This is especially true in patients with prior renal insufficiency (155). The cause of the reduced GFR seems directly related to the hyperphosphatemia itself because oral phosphate overdose also lowers GFR (157), and reduction in serum phosphate level reverses the renal failure (158,159). The mechanism of reduced GFR may be the intrarenal precipitation of calcium phosphate (155,160), which may be exacerbated by excessive alkalinization of the urine. Hemodialysis may be required to reduce plasma phosphate and to restore renal function under these circumstances.

Retinoic Acid Syndrome

Tretinoin (all-*trans*-retinoic acid) is a vitamin A derivative that is used in the treatment of acute promyelocytic leukemia. Tretinoin enhances the morphologic and functional maturation of leukemic promyelocytes and may provoke apoptosis in these cells. The appearance of a syndrome consisting of fever, respiratory distress, pulmonary infiltrates, and pleural and pericardial effusions occurs in as much as 25% of treated patients unless preventive measures are taken (161). Since the syndrome can be reproduced by the infusion of activated leukocytes, it is speculated that cytokine production by such stimulated white cells is responsible for its manifestations. The addition of intravenous dexamethasone and the reduction of the white cell count by prior chemotherapy usually before Tretinoin is added have decreased the occurrence of the syndrome markedly.

RENAL FAILURE ASSOCIATED WITH BONE MARROW TRANSPLANT

Bone marrow transplant is becoming an increasingly common modality for treatment of cancer and other disorders (162). Current indications include aplastic anemia, hematologic and nonhematologic malignancies, hereditary enzyme deficiencies, immunodeficiency states, and selected hemoglobinopathies. Renal failure occurs frequently in this group of patients, in most series as high as 40% (163,164) or higher (165).

It is useful to consider the frequency of ARF as it relates to the time of bone marrow transplant because it varies during the course of transplant (166). Also, the likelihood of the occurrence of ARF, especially its severity, appears to be related to whether the transplanted hematopoietic cells are derived from the patient (autologous) or not (allogeneic). In a recent study of autologous bone marrow transplants in high-dose patients with breast cancer (21), the incidence of ARF was significantly lower than that reported in an earlier report of predominantly allogeneic transplant patients (164). Thus the occurrence of graft-versus-host reactions and the use of immunosuppressive drugs with nephrotoxic potential may impact on the occurrence of ARF.

Up to 10 days following completion of conditioning with cytoreductive drugs and at the time of bone marrow infusion, a small percentage of patients (<5%) present with renal failure that is most often due to the tumor lysis syndrome or the infusion of the bone marrow. The latter would seem to be a result of toxic products that accumulate during cryopreservation of bone marrow cells or due to the cryopreservative that is used (167). Hemoglobin appears in the urine of 75% to 100% of such patients and may be due to disrupted red blood cells within the infusate or due to in vivo hemolysis induced by the dimethyl sulfoxide in the cryopreservation solution (168). This form of ARF is relatively uncommon, perhaps as a consequence of the induction of a solute and bicarbonate diuresis that is routinely induced prior to marrow infusion (167).

The most common time frame for ARF to develop after a bone marrow transplant is between 10 and 21 days after infusion (166), when as many as 20% of patients who survive the initial period of bone marrow transplant develop ARF. Whether the exposure of ordinarily nontoxic amounts of heme proteins might predispose the kidney to later injury has recently been the matter of some debate in the literature (169,170), since heme itself may be cytoprotective. During this time, almost all of the complications of prior cytoreductive therapy become most evident, including marrow aplasia, infection, and gastrointestinal and hepatic toxicity. This is also the period where dialysis will be most required (163,171). Mortality increases markedly if dialysis is required, approaching 90% in some studies (163,171). Despite the many possible causes of ARF, Zager and co-workers (163) find that the large majority of such patients develop renal failure in the background of liver failure reminiscent of the hepatorenal syndrome. The syndrome is characterized clinically by hyperbilirubinemia, hepatomegaly, usually with right upper quadrant pain, and sudden weight gain, often with peripheral edema and ascites. The urinary sodium remains low despite diuretic therapy, and in the presence of large requirements of fluid for administration of medications and blood products, there is the potential to develop significant degrees of circulatory congestion. Dialysis is most often required to relieve such congestion, rather than uremia.

The liver most often shows prominent changes of veno-occlusive disease characterized by venular thrombosis, variable degrees of hepatocellular necrosis, and portal hypertension (173,174). The cause of the syndrome is unknown, but it bears some resemblance to the veno-occlusive disease induced by radiochemical-induced endothelial cell damage. The severity of the liver disease, as assessed by serum bilirubin levels, correlates with the occurrence of renal failure. Also, preexisting liver disease, concurrent administration of estrogen–progestin, amphotericin, or MTX, and preexisting kidney disease all increase the risk of ARF (163,173,175). Zager et al. (163) point out the important role that sepsis plays in initiating the syndrome, either directly or by virtue of the exposure to additional toxins, especially amphotericin. Attempts to limit venous occlusion with heparin, prostaglandin E1 infusion, and tissue plasminogen activator have not been uniformly successful,

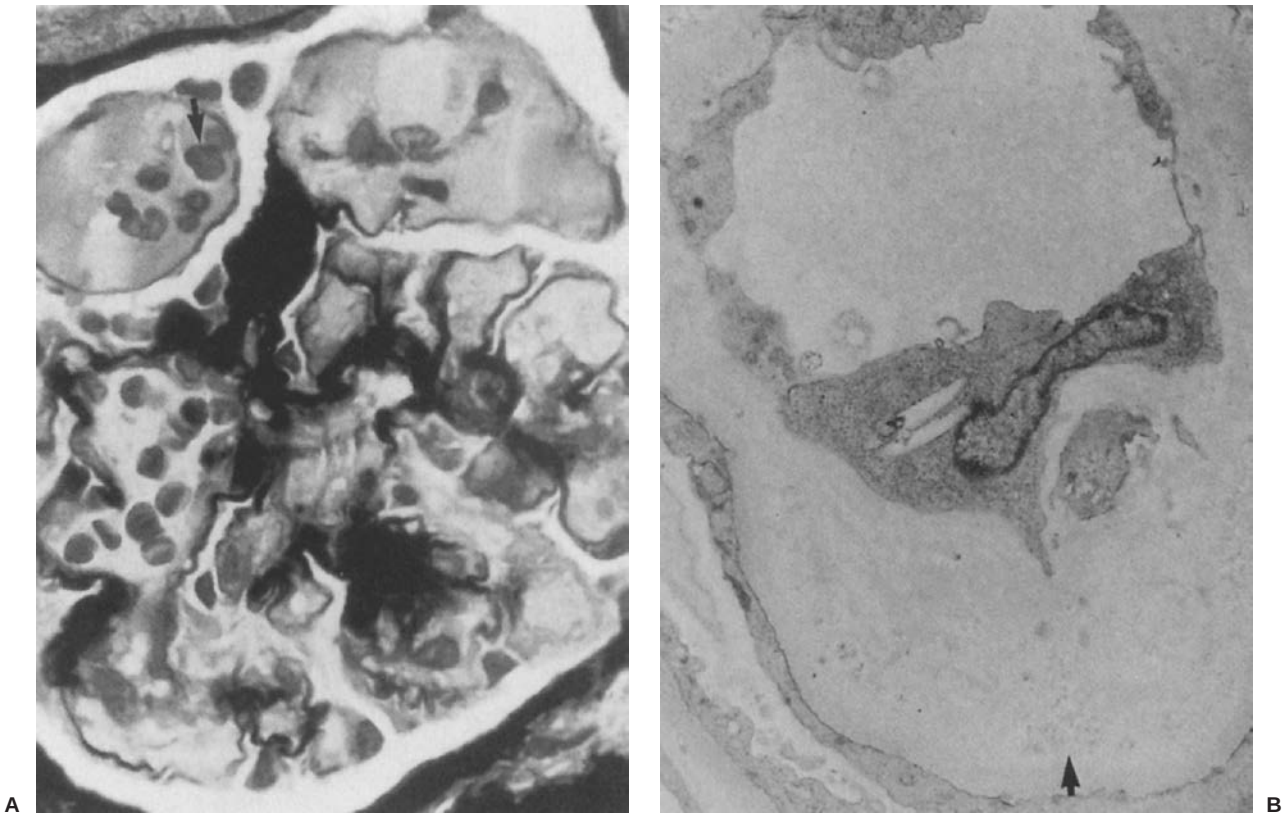

FIGURE 43-3. A: Light-microscopic appearance of a glomerulus from a patient who received a bone marrow transplant 6 months before and had a plasma creatinine of 2.1 mg/dL. Glomerular capillary loops are distended by the subendothelial deposition of proteinaceous material and formed elements of the blood including erythrocytes (*arrow*). There is loss of endothelial and mesangial cells. Focal sclerosis of the mesangium is present. (Jones silver methenamine, ×1,100.) **B:** Electron-microscopic appearance of a glomerular capillary from a patient 10 months after bone marrow transplant with a plasma creatinine of 4.3 mg/dL. Proteinaceous deposits, sometimes mineralized (*arrow*), lift the endothelium away from the glomerular basement membrane, tending to narrow the capillary lumen. (Magnification ×6,300.) (From: Cohen EP, et al. Clinical course of late-onset bone marrow transplant nephropathy. *Nephron* 1993;64:626, with permission.)

as is the attempt to reduce TNF-α by pentoxifylline (176). Recent studies have focused attention on the deficiency of the naturally occurring anticoagulants protein C and antithrombin III as causes of the syndrome (177), but preexisting liver and renal disease, the use of estrogens and progestin, amphotericin, MTX, mismatched grafts, and age greater than 25 years have each been shown to be risk factors. Once renal failure occurs, dialysis is the treatment of choice, as the administration of diuretics and dopamine infusion has not been successful in reversing it (166).

HEMOLYTIC-UREMIC SYNDROME ASSOCIATED WITH BONE MARROW TRANSPLANT

Renal failure may emerge as long as 2 years following bone marrow transplant in a manner that may be identical to the hemolytic-uremic syndrome (HUS). HUS was first reported by Shulman and colleagues (178) who described three patients who developed renal failure 6 weeks after bone marrow transplant with hypertension, congestive heart failure, and a severe microangiopathic hemolytic anemia. It is now a recognized complication in as many as 25% of transplant patients who survive 2 years following successful engraftment (179). The syndrome is variable and may occur abruptly or take on a more chronic course (180). The microangiopathic hemolytic anemia may be clinically obscure, and the diagnosis may require careful and sequential analysis of the blood and urine and finally a renal biopsy. In both forms of presentation, it is common for patients to enter a more chronic phase with severe, but stable renal failure. Renal histopathology reveals enlarged, hypocellular glomeruli with deposition of spongy material along the inner aspect of the glomerular basement membrane extending into the glomerular capillary loops and producing a "double contour" appearance (Fig. 43-3). Arterioles also show thickening of the wall with expansion of the subendothelial space with mucoid deposits. These changes, consistent with severe mesangiolysis (181) and arterionecrosis, are similar to those seen in acute radiation nephritis (182). Deposition of fibrin, C3 (Complement 3), Clq (Complement 1q), and immunoglobulin M (IgM) can also be demonstrated. In the few patients who have been biopsied in the chronic phase of the disease, glomerular and interstitial fibrosis and arteriolar nephrosclerosis predominate (180).

The mechanism of the nephrotoxicity is unknown, but the role of cyclosporin A (CsA) is not thought to be primary, as the syndrome occurs in its absence (180,183,184). Total body radiation is thought to be a principal cause of the syndrome, as syngeneic transplants not receiving radiation do not have the disease and partial shielding of the kidney results in a lower

incidence of HUS following bone marrow transplant (185). Interesting in this regard is that the radiation dose necessary to induce the damage is well below the known threshold of 20 Gy (11). Conditioning protocols used prior to transplantation that usually include cyclophosphamide and cytosine arabinoside, which may be radiomimetic, and the need to introduce CsA treatment to prevent GVHD, all probably potentiate the nephrotoxicity of ionizing radiation.

The similarity of post-transplant HUS and non–transplant-related HUS prompted attempts to treat patients with plasma exchange but without success (186). Another major treatment decision is whether to continue CsA. As already indicated, it does not seem that the role of CsA is primary, and the gravity of GVHD would dictate that patients should remain on the drug. Nonetheless, most centers attempt to reduce the CsA dose. Treatment includes aggressive management of the hypertension, as there is evidence that this slows the progression of the renal disease (179). Recently, there is some evidence to suggest that angiotensin-converting enzyme inhibition and angiotensin-receptor blockade can be used to prevent post-transplant HUS (187,188).

HEMOLYTIC-UREMIC SYNDROME SECONDARY TO CANCER AND CHEMOTHERAPY

HUS often occurs on the background of cancer and chemotherapy but may rarely occur in disseminated cancer even before the initiation of chemotherapy. In the latter circumstance, HUS may actually respond to chemotherapy (189). However, it is most often associated with mitomycin therapy as discussed above, but other agents have been implicated including bleomycin and cisplatin (190). As in autoimmune and familial forms of HUS, large molecular weight complexes of von Willebrand factor (vWF) trigger the characteristic microaggregation of platelets. But in contradistinction to these forms, the cause of the increase in the levels of circulating vWFs is not due to a deficiency of vWF-cleaving protease (ADAMTS13) but is due to release of these complexes from damaged endolethial cells (191,192). Standard plasma exchange therapy is less successful in cases of cancer chemotherapy-associated HUS and alternate therapy with plasma perfusion over staphylococcal protein-A is being advocated.

Cyclosporine Nephrotoxicity

GVHD is a serious complication of bone marrow transplant, carrying with it mortality rates of up to 50% (193). CsA, because of its lack of bone marrow toxicity and proven efficacy (193) over MTX alone in preventing GVHD, is the preferred drug to use in combination with MTX or prednisone. The use of CsA has been associated with a greater incidence of renal failure than the use of MTX alone (194) or MTX plus prednisone (195), and the risk of renal failure has correlated reasonably well with CsA levels in the blood (196), so that monitoring of CsA levels is recommended (166). However this is still a controversial point because the development of renal failure did not correlate with blood CsA levels in at least one large series (164). Currently the risk of CsA-induced renal failure is relatively low, perhaps as a consequence of close drug monitoring and the relatively brief period of full-dose therapy that is employed when no active GVHD is present (196). However, with the increasing use of unrelated donor grafts, it is likely that more chronic GVHD and prolonged CsA dosage will be accompanied by greater nephrotoxicity.

CONCLUSION

It is clear that several factors stand out as potentiating the nephrotoxicity of these agents. First is the presence of preexistent renal disease. Recognition of this and reduction of the administered dose of the nephrotoxin should lead to lower nephrotoxicity. Second is the fullness of the intravascular arterial circulation, and hydration of the patient prior to cytoreductive therapy is an important element in prevention of the most severe forms of renal disease. Data to support the notion that a full extracellular fluid space at the time of exposure ameliorate the subsequent course of the ARF are extensive and reviewed in Chapters 39 and 40. The mechanism of this protection is unknown, but several urinary indices have been identified to predict the outcome of exposure. Thus a high-solute excretion rate and increased renal blood flow are thought to be crucial elements in this regard (197). Interestingly, many different renal stresses respond to this regimen, including ischemic and nephrotoxic insults, and, experimentally at least, do not depend on altering the extent of the cytotoxic insult (34,197) or the pharmacodynamics of the drug (34), suggesting that the mechanism of protection depends on some interruption of the impact of cell injury on kidney function.

It is important to note that the coexistence of cardiovascular disease as well as pulmonary and liver disease will limit the kidney's ability to excrete salt so that monitoring the response of the kidney to salt infusion is crucial. Introduction of a diuretic may be necessary under conditions where there is no diuresis in response to the infusion of fluids alone. While there are advocates of one hydration protocol over another, there are very few randomized, prospective studies to demonstrate benefits of one protocol over another. One recent prospective trial found no benefit of the addition of either mannitol or furosemide to the infusion of 0.45% sodium chloride at a rate of 1 mL per kg body weight per hour for 12 hours before and 12 hours after exposure to radiocontrast dye (198). Adjustments to any standard protocol of fluid administration by careful monitoring of the urine output and assessment of solute excretion are crucial to achieve maximum benefit. If weight gain and peripheral or central edema are provoked, fluid administration may actually be deleterious to the outcome.

The full impact of the antineoplastic drugs on long-term renal function is more difficult to determine, but recent data with cisplatin and bone marrow transplant would indicate that significant degrees of renal dysfunction are to be expected. The challenge for the future is to uncover the mechanism of the permanent renal failure and prevent it.

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CHAPTER 44 ■ NEPHROTOXICITY OF NONSTEROIDAL ANTIINFLAMMATORY AGENTS, ANALGESICS, AND ANGIOTENSIN-CONVERTING ENZYME INHIBITORS

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NEPHROTOXICITY OF NONSTEROIDAL ANTIINFLAMMATORY DRUGS

Nonsteroidal antiinflammatory drugs (NSAIDs) are some of the most widely utilized therapeutic agents in clinical practice today. While the gastrointestinal toxicity of these medications is well known, it has become increasingly apparent that the kidney is also an important target for untoward clinical events. The renal toxicity associated with the use of NSAIDs can be divided into one of several distinct clinical syndromes. These include a form of vasomotor acute renal failure, nephrotic syndrome associated with interstitial nephritis, chronic renal injury, and abnormalities in sodium, water, and potassium homeostasis. The common link in these syndromes is a disruption in prostaglandin metabolism, the class of compounds whose synthesis is inhibited by these agents.

Prostaglandin Biosynthesis and Compartmentalization

Prostaglandins are members of a class of compounds termed eicosanoids. Eicosanoids are biologically active fatty acids that are all derived from the oxygenation of arachidonic acid. The particular enzyme involved in the oxygenation process dictates which class of eicosanoid will be synthesized. Oxygenation of arachidonic acid by the enzyme cyclooxygenase is responsible for prostaglandin and thromboxane synthesis (Fig. 44-1). The enzyme lipoxygenase converts arachidonic acid to leukotrienes, lipoxins, and eventually to hydro fatty acid derivatives such as hydroxyeicosatetraenoic acid (HETE). Finally, oxygenation by the cytochrome P-450 system generates epoxyeicosatrienoic acids (EETs).

The availability of free arachidonic acid is the rate-limiting step in eicosanoid biosynthesis. Normally, arachidonic acid is found esterified to membrane phospholipids, where it undergoes deacylation primarily under the influence of phospholipase A_2. Phospholipase A_2-mediated arachidonic acid release is a calcium–calmodulin-dependent step that is stimulated by vasopressin, bradykinin, angiotensin, and norepinephrine (1). Corticosteroids inhibit this reaction by inducing the formation of an inhibitor of phospholipase A_2 called macrocortin or lipomodulin. Once released, free arachidonic acid is either reesterified back into membrane lipids or is converted into one of the biologically active eicosanoids.

The first step in the synthesis of prostaglandins and thromboxanes is a cyclooxygenase reaction in which arachidonic acid is converted into the cyclic endoperoxide prostaglandin G_2 (PGG_2). PGG_2 then undergoes a peroxidase reaction to form a second endoperoxide called PGH_2, which is accompanied by the formation of a superoxide radical. Both of these reactions are catalyzed by the enzyme cyclooxygenase (COX), also known as prostaglandin endoperoxide H synthase (2,3). The cyclooxygenase and peroxidase reactions occur on distinct but neighboring sites on the COX enzyme. Once formed, PGH_2 has a short half-life and is rapidly acted on by a series of enzymes that produce biologically active prostaglandins or thromboxane. Prostacyclin synthase acts to form prostacyclin (PGI_2), thromboxane synthase forms thromboxane A_2, and isomerases are responsible for the formation of PGE_2, PGD_2, and PGF_{2a}. PGE_2 can be converted to $PGF_{2\alpha}$ by 9-ketoreductase, an enzyme that is stimulated by high-salt diet and inhibited by furosemide (4).

Prostaglandins are synthesized on demand and exert physiologic effects in discrete microenvironments along the nephron in close proximity to their points of synthesis (Table 44-1). Due to the virtual absence of distant effects, these compounds are best regarded as autacoids rather than hormones. Variations in the synthetic and degradative machinery along the length of the nephron account for the differing types and amounts of prostaglandins found in any given segment (5). PGI_2 is the most abundant prostaglandin produced in the cortex and is primarily synthesized in cortical arterioles and glomeruli (6). This location corresponds to the known effects of PGI_2 in regulating renal vascular tone, glomerular filtration rate (GFR), and renin release. PGE_2 and thromboxane A_2 are also produced in the glomerulus and therefore may exert effects at this site.

The most abundant prostaglandin found in the tubules is PGE_2 (5,6). The cortical and especially the medullary portion of the collecting duct is the dominant site of PGE_2 synthesis. Lesser amounts are found in the thin descending and thick ascending limb with the least amount of synthesis found in the proximal tubule. Medullary interstitial cells are also a rich source of PGE_2 production. This distribution provides the anatomic basis for PGE_2 to modulate sodium and chloride transport in the loop of Henle, regulate arginine vasopressin-mediated water transport, and control vasa recta blood flow. $PGF_{2\alpha}$ is synthesized primarily by medullary interstitial cells and less by the papillary collecting tubule and glomeruli. Prostaglandin-degradative enzymes are found in both the cortex and medulla but are most abundant in the cortex. Except for PGI_2, which undergoes spontaneous hydrolysis to 6-keto-$PGF_{2\alpha}$, prostaglandins are rapidly metabolized into inactive products by a 15-prostaglandin dehydrogenase (7). Increased concentration of this enzyme in the proximal nephron may facilitate degradation of prostaglandins delivered to the proximal tubule by glomerular filtration (8).

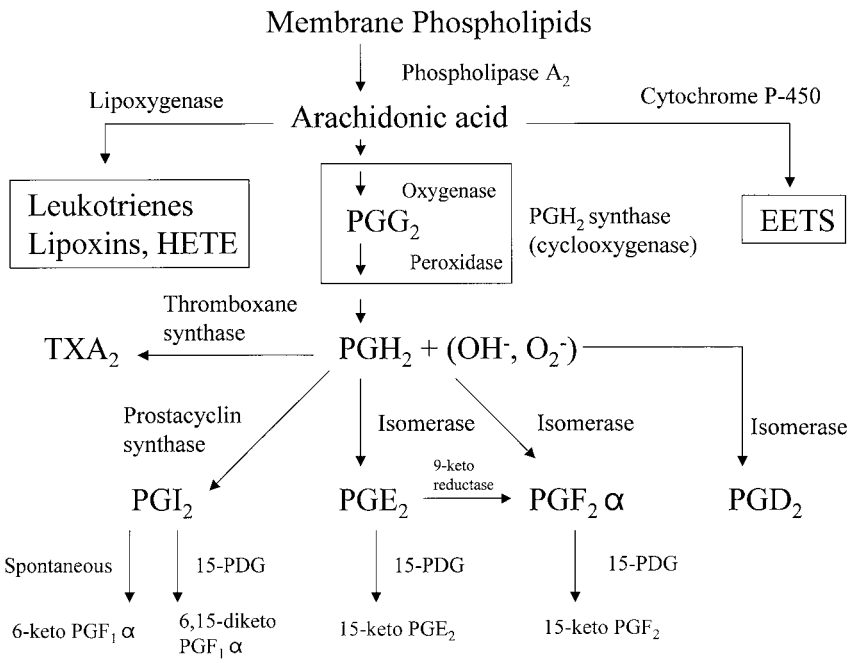

FIGURE 44-1. Synthetic and degradative pathways for the different types of eicosanoids. *15-PDG*, 15-prostaglandin dehydrogenase; *EETs*, epoxyeicosatrienoic acids; *HETE*, hydroxyeicosatetraenoic acid; *TXA_2*, thromboxane A_2.

Biologic Actions of Prostaglandins in the Kidney

Under baseline euvolemic conditions, prostaglandin synthesis is negligible, and as a result, these compounds play little to no role in the minute-to-minute maintenance of renal function. Where these compounds come to serve a major role is in the setting of a systemic or intrarenal circulatory disturbance. This interaction is best illustrated when examining renal function under conditions of volume depletion (Fig. 44-2). In this setting, renal blood flow is decreased while sodium reabsorption, renin release, and urinary concentrating ability are increased. To a large extent, these findings are mediated by the effects of increased circulating levels of angiotensin II (AII), arginine vasopressin (AVP), and catechols. At the same time, these hormones stimulate the synthesis of renal prostaglandins, which in turn act to dilate the renal vasculature, inhibit salt and water reabsorption, and further stimulate renin release. Prostaglandin release under these conditions serves to dampen and counterbalance the physiologic effects of the hormones that elicit their production. As a result, renal function is maintained near normal despite the systemic circulation being clamped down. Predictably, inhibition of prostaglandin synthesis will lead to unopposed activity of these hormonal systems, resulting in exaggerated renal vasoconstriction and magnified antinatriuretic and antidiuretic effects. In fact, many of the renal syndromes that are associated with the use of NSAIDs can be explained by the predictions of this model.

TABLE 44-1

COMPARTMENTALIZATION AND FUNCTION OF RENAL PROSTAGLANDINS

Site	Eicosanoid	Action
Arterioles	PGI_2, PGE_2	Vasodilation
Glomeruli	$PGI_2 > PGE_2$ (human) $PGE_2 > PGI_2$ (rat) TXA_2	Maintain GFR Vasoconstriction
Tubules	PGE_2, $PGF_{2\alpha}$	Enhance NaCl and water excretion
Interstitial cells	PGE_2	Enhance NaCl and water excretion, influence regional blood flow
Juxtaglomerular apparatus	PGI_2, PGE_2	Stimulate renin release

PGI, prostaglandin I; *PGE*, prostaglandin E; *PGF*, prostaglandin F; *TXA*, thromboxane A; *GFR*, glomerular filtration rate.

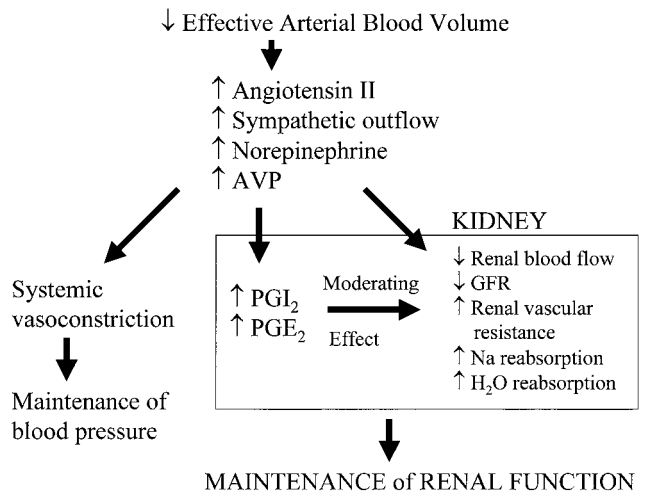

FIGURE 44-2. In the setting of absolute or effective volume depletion, a number of effectors are activated that serve to defend the circulation and at the same time stimulate the synthesis of renal prostaglandins. In turn, renal prostaglandins function to moderate the effects of these hormonal systems such that renal function is maintained in the setting of systemic vasoconstriction. *AVP*, arginine vasopressin; *GFR*, glomerular filtration rate.

Expression and Regulation of Cyclooxygenase (COX)-1 and COX-2 in the Kidney

Aspirin and other NSAIDs exert their prostaglandin-inhibitory effects by inhibiting the COX enzyme. The COX enzyme exists as two isoforms termed cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). These enzymes are encoded by two different genes and differ significantly in their regulation. The COX-1 enzyme is constitutively expressed in most tissues and is responsible for producing prostaglandins involved in maintaining normal tissue homeostasis. The COX-2 enzyme is principally an inducible enzyme rapidly upregulated in response to a variety of stimuli such as growth factors and cytokines typically found in the setting of inflammation (3). With the discovery of COX-2, a great deal of effort was put forth to develop compounds to selectively block the activity of this isoform without affecting the activity of COX-1. The availability of a COX-2–specific inhibitor would provide a therapeutic tool to inhibit the synthesis of arachidonic acid metabolites at sites of inflammation and yet leave unperturbed COX-1 derived prostanoids involved in normal homeostasis. In this manner the analgesic, antiinflammatory, and antipyretic effects of an NSAID could be obtained with minimal to no side effects. While initial experience with specific COX-2 inhibitors has been associated with a reduction in gastrointestinal complications, recent information suggests this paradigm is not applicable to the kidney.

COX-1 and COX-2 are both constitutively expressed in the kidney. COX-1 is localized to mesangial cells, arteriolar endothelial cells, parietal epithelial cells of Bowman's capsule and throughout the cortical and medullary collecting duct (9). COX-2 is primarily expressed in the macula densa and adjacent cells in the cortical thick ascending limb with lesser amounts in the podocytes and arteriolar smooth muscle cells (10–13). COX-2 is also abundantly expressed in interstitial cells in the inner medulla and papilla (Table 44-2).

The expression of COX-2 in different regions of the kidney varies in response to alterations in intravascular volume. This variation is particularly evident in the macula densa where studies show COX-2 plays an important stimulatory role in the release of renin via the tubuloglomerular feedback mechanism. Under conditions of low renal perfusion when the chloride concentration at the level of the macula densa is low, renin release is inhibited by a COX-2 selective inhibitor but unaffected by a COX-1 inhibitor (14). In genetically engineered mice lacking COX-2 there is a failure of renin release in response to a low salt diet whereas renin release is intact in animals lacking COX-1 (15,16).

Stimulation of renin with subsequent formation of angiotensin II is part of a feedback loop since angiotensin II exerts an inhibitory effect on COX-2 synthesis in the macula densa via the angiotensin type 1 (AT_1) receptor (17). In contrast to effects at the macula densa angiotensin II upregulates COX-2 and prostaglandin synthesis in vascular smooth muscle cells and mesangial cells (18,19). This latter effect provides a mechanism for COX-2 to both facilitate the tubuloglomerular feedback response to low salt delivery to the macula densa by increasing angiotensin II levels and preserve the glomerular filtration rate through generation of vasodilatory prostaglandins to antagonize the vasoconstrictive effect of angiotensin II (20).

The expression of COX-2 is also responsive to changes in volume in the medulla of the kidney. COX-2 expression decreases with salt depletion and increases with high salt diet and dehydration (13). COX-2–derived prostaglandins may play an important role in facilitating a natriuretic response to salt loading and help protect against volume overload. The increase in COX-2 in response to dehydration is thought to provide a cytoprotective effect in the setting of hypertonic stress (21,22). Treatment of water-deprived animals with a selective COX-2 inhibitor is associated with apoptotic patches of renal medullary interstitial cells. By contrast, no such changes are seen in animals treated with the inhibitor alone or in animals undergoing water deprivation without pharmacologic treatment.

In summary, COX-2 is constitutively expressed in the kidney and is highly regulated in response to physiologic perturbations in intravascular volume. As discussed in the remainder of this chapter the majority of experimental and clinical studies to date suggest that the specific COX-2 inhibitors may not offer any distinct advantage over traditional NSAIDs with regard to renal toxicity. In fact, most of the renal syndromes that have been linked to nonselective COX inhibitors have now been described with the selective COX-2 inhibitors (Table 44-3). The only exception is the development of chronic kidney disease and papillary necrosis. The failure to link COX-2 inhibitors use to these complications is not surprising since these agents have only been available for clinical use for a relatively short period

TABLE 44-2

INTRA-RENAL LOCALIZATION OF COX-1 AND COX-2

Localization of COX-1
Mesangial cells
Arteriolar endothelial cells
Parietal epithelial cells of Bowman's capsule
Cortical and medullary collecting duct cells
Localization of COX-2
Macula densa and adjacent cells of cortical thick ascending limb of Henle
Interstitial cells in inner medulla and papillae
Low-level expression in podocytes and arteriolar smooth muscle cells

TABLE 44-3

CLINICAL SYNDROMES ASSOCIATED WITH NONSELECTIVE COX INHIBITORS AND SELECTIVE COX-2 INHIBITORS

Syndrome	Nonselective COX inhibitor (traditional NSAID)	Selective COX-2 inhibitor
Vasomotor acute renal failure	Yes	Yes
Tubulointerstitial nephritis with nephrotic syndrome	Yes	Yes
Tubulointerstitial nephritis without nephrotic syndrome	Yes	Yes
NaCl retention and edema	Yes	Yes
Hypertension	Yes	Yes
Hyperkalemia	Yes	Yes
Decreased free water clearance and hyponatremia	Yes	Yes
Papillary necrosis	Yes	Not reported
Chronic kidney disease	Yes	Not reported

of time. As with traditional NSAIDs, the COX-2 inhibitors need to be used cautiously and require close monitoring of renal function in patients at high risk for adverse renal outcomes.

The overall clinical utility of selective COX-2 inhibitors has recently been called into question as a result of rofecoxib being removed from clinical use due to an increase risk of cardiovascular events. Whether such a risk applies to all COX-2 inhibitors is still a matter of debate.

Effects of Prostaglandins on the Renal Circulation

Prostaglandins primarily exert a vasodilatory effect on the renal vasculature (23). This vasodilatory effect alters the renal circulation in two major ways. First, these compounds influence the distribution of renal blood flow to different regions of the kidney. Prostaglandin stimulation results in a preferential increase in blood flow to the more juxtamedullary nephrons (24). By contrast, inhibition of prostaglandin synthesis results in a selective reduction of flow to inner cortical nephrons while flow remains well preserved in the outer cortex (25). Second, prostaglandins exert a vasoregulatory effect on the renal microcirculation to include the interlobular, afferent, and efferent arterioles as well as the glomerular mesangium. In isolated renal arterioles, both PGE_2 and PGI_2 attenuate AII-induced and norepinephrine-induced afferent arteriolar vasoconstriction. On the efferent side of the circulation, PGI_2 similarly antagonizes AII-induced and norepinephrine-induced vasoconstriction, but PGE_2 is without effect (26). In addition to local production, vascular reactivity of the efferent arteriole appears to be influenced by prostaglandins produced in the upstream glomerulus (27). In this regard, Arima and associates (27) find that orthograde infusion of AII (afferent arteriole-glomerulus-efferent arteriole) results in less vasoconstriction of the efferent arteriole as compared to when infused in a retrograde fashion (efferent arteriole-glomerulus-afferent arteriole). Pretreatment with indomethacin markedly increases the vasoconstrictive effect during orthograde infusion but is without effect during the retrograde infusion.

Prostaglandins have also been shown to attenuate mesangial cell contraction induced by AII, endothelin, AVP, and platelet-activating factor (28–31). Contraction of these cells will normally cause a decrease in the total glomerular capillary surface area and result in a fall in the GFR. Mesangial cell synthesis and release of PGI_2 in humans and PGE_2 in rats dampen the constrictor effects of these hormones such that the glomerular capillary surface area is maintained, thereby minimizing any fall in GFR. Thus, in the setting of enhanced hormonal constrictor activity, prostaglandins play a major role in maintaining glomerular hemodynamics by exerting a vasodilatory effect at the level of the afferent and efferent arteriole as well as within the glomerular mesangium.

Renal Syndromes Associated with Nonsteroidal Antiinflammatory Drugs

Vasomotor-Induced Acute Renal Failure

Prostaglandins appear to play a negligible role in maintenance of renal function under normal circumstances. This conclusion is based on studies in both experimental animals as well as humans. In conscious, sodium-replete dogs and rats, inhibition of renal prostaglandin synthesis with a variety of NSAIDs does not alter baseline renal blood flow or GFR (32–35). Similarly, renal hemodynamics are unaffected in healthy humans after both short-term (36,37) and long-term administration of aspirin (38). In related studies, administration of indomethacin to healthy volunteers was also found to produce no change in renal hemodynamics (39,40).

A sharply different effect of cyclooxygenase inhibition is observed when systemic hemodynamics are compromised. Under conditions of circulatory distress, renal blood flow represents a balance between vasoconstrictor influences on the one hand and vasodilatory prostaglandins on the other. Predictably, administration of NSAIDs in this setting will shift this balance toward unopposed vasoconstriction and potentially result in a precipitous decline in renal function.

This interplay between vasoconstrictive effectors and vasodilatory prostaglandins is particularly well illustrated in a series of studies utilizing a model of hemorrhage in dogs (41,42). In animals subjected to hemorrhage, prostaglandin synthesis inhibition was associated with a marked reduction in renal blood flow as compared to prostaglandin-intact dogs. This renal ischemic response was found to be partly reversed after infusion of an AII antagonist or after renal denervation. When renal denervation was combined with the AII antagonist, renal blood flow was restored to values comparable to that in the nonprostaglandin-inhibited animals. Clearly, these findings illustrate the pivotal role that prostaglandins play in opposing the renal ischemic effects of AII and renal nerves.

The modulating effect of vasodilatory prostaglandins on renal hemodynamics can be expected to roughly parallel the extent to which vasoconstrictor effectors are activated. In turn, the activity of these effectors will reflect the degree of circulatory distress. With only mild perturbations in the circulation, one can begin to detect a discernible effect of prostaglandins on renal blood flow. For example, unlike subjects ingesting an ad lib sodium diet, normal subjects placed on a salt-restricted diet will demonstrate a modest fall in creatinine clearance and renal blood flow following the administration of aspirin or indomethacin (43,44).

Diuretic therapy is a common clinical situation where NSAIDs may exert a deleterious effect on renal function in otherwise healthy subjects (45). Like sodium restriction, diuretics increase the dependence of renal blood flow and GFR on vasodilatory prostaglandins and potentiate the deleterious effects of prostaglandin inhibition with cyclooxygenase inhibitors (46). The degree to which renal function is disturbed, however, appears to vary depending on which diuretic–NSAID combination is used. In this regard, Favre and colleagues (45) find that the combination of triamterene and indomethacin given to healthy subjects results in a marked decline in creatinine clearance. By contrast, only a mild decrease in creatinine clearance is found when indomethacin is given in combination with furosemide, hydrochlorothiazide, or spironolactone. Interestingly, triamterene is the only diuretic associated with a marked increase in urinary prostaglandin secretion. Although there is little evidence to suggest that the renal failure patients in this study were volume-depleted, it appears that triamterene by some unknown mechanism renders the renal circulation critically dependent on vasodilatory prostaglandins. As a result, triamterene in combination with an NSAID should only be used with extreme caution.

As alterations in the circulation become more pronounced, rendering the renal circulation more dependent on vasodilatory prostaglandins, cyclooxygenase inhibition can be expected to result in more profound changes in renal hemodynamics. In congestive heart failure, a decrease in effective arterial circulatory volume is the proximate cause for activation of neurohumoral vasoconstrictor forces that participate in the maintenance of systemic arterial pressure and result in increased total peripheral vascular resistance. Important to note, the rise in renal vascular resistance is less than that seen in the periphery (47). Vasodilatory prostaglandins function in a counterregulatory role, attenuating the fall in renal blood flow and GFR

that would otherwise occur if vasoconstrictor forces were left unopposed (48).

Cirrhosis is another clinical condition in which the integrity of the renal circulation can become critically dependent on vasodilatory renal prostaglandins. Cirrhotic patients with a low urinary sodium concentration tend to be the most susceptible to develop acute decrements in renal function following the administration of NSAIDs (49). These patients have a more marked decrease in effective arterial circulatory volume, primarily due to splanchnic vasodilation, which in turn leads to higher levels of circulating catechols, AII, and AVP (50,51). As a result, the renal circulation in this subset of patients is more critically dependent on the effect of vasodilatory prostaglandins. As seen in patients with congestive heart failure, these patients have high urinary concentrations of PGE_2, which decline in parallel with the fall in GFR (49).

Renal prostaglandins may play an important role in maintenance of renal hemodynamics in nephrotic syndrome. GFR and filtration fraction are moderately decreased in most patients with the nephrotic syndrome (52,53). Micropuncture studies in an experimental model of the nephrotic syndrome have indicated that relative preservation of renal plasma flow may serve an important role in attenuating the fall in GFR that would otherwise occur due to a reduction in the ultrafiltration coefficient (54). In this setting, locally produced vasodilatory prostaglandins may serve to reduce afferent arteriolar resistance, thereby increasing renal plasma flow and increasing filtration pressure (55,56). Administration of NSAIDs in this setting would lead to increased afferent arteriolar tone. The resulting fall in renal plasma flow and filtration pressure combined with the already decreased ultrafiltration coefficient would result in a dramatic fall in GFR (55). Indeed, administration of prostaglandin synthesis inhibitors to nephrotic subjects is commonly associated with a fall in GFR and may precipitate acute renal failure in some patients (57,58). Other settings in which there is increased vasoconstrictive input focused on the kidney rendering it particularly vulnerable to the deleterious effects of NSAIDs include endotoxic shock (59) and anesthesia (60).

Risk factors for the development of NSAID-induced ARF are not necessarily confined to conditions characterized by decreases in absolute or effective arterial circulatory volume (Table 44-4). One such example is the presence of underlying chronic kidney disease. In this setting, increased vasodilatory prostaglandins are thought to play an adaptive role in minimizing the decline in global renal function by increasing GFR in surviving nephrons through increased renal blood flow. The signal for increased prostaglandin production is generally not a disturbance in the systemic circulation leading to increased circulating levels of AII and catecholamines but rather intrarenal mechanisms leading to generation of vasoactive compounds within the glomerular microcirculation (61).

TABLE 44-4

RISK FACTORS FOR NSAID-INDUCED ACUTE VASOMOTOR RENAL FAILURE

Decreased EABV	Normal or increased EABV
Congestive heart failure	Chronic renal failure
Cirrhosis	Glomerulonephritis
Nephrotic syndrome	Elderly
Sepsis	Contrast-induced nephropathy
Hemorrhage	Obstructive uropathy
Diuretic therapy	Cyclosporin A
Postoperative patients with "third space" fluid	
Volume depletion/ hypotension	

EABV, effective arterial blood volume.

TABLE 44-5

PREDISPOSING FACTORS FOR NSAID-INDUCED NEPHROTOXICITY IN THE ELDERLY

Age-related changes in renal function
↓ In glomerular filtration rate
↓ In renal blood flow
↑ In renal vascular resistance

Age-related changes in pharmacokinetics
↑ Free drug concentration
−Hypoalbuminemia
−Retained metabolites
↓ Total body water
↓ Hepatic metabolism with longer drug half-life

Increasing age is a risk factor for the development of nephrotoxicity when using NSAIDs (62,63). This susceptibility in part may be related to changes in renal function that normally accompany the aging process (64) (Table 44-5). Aging is associated with a progressive decline in the GFR and total renal blood flow. In addition, there is an increase in renal vascular resistance. Important to note, the renal vasculature becomes less responsive to vasodilators, while the response to vasoconstrictors remains intact. In an analysis of 1,908 patients treated with ibuprofen, renal impairment was found to occur in 343 (18%) patients (62). The two most important risk factors identified for the development of toxicity is age greater than 65 years and preexisting renal insufficiency. In a prospective study of 114 older patients (mean age 87 years) started on NSAID therapy, a greater than 50% increase in the serum urea nitrogen concentration was found in 15 (13%) patients (63). In this study, concurrent use of a loop diuretic and large doses of NSAIDs were found to be predictive of those who developed significant azotemia.

In addition to age-related changes in renal function, age-related changes in the pharmacokinetics of NSAIDs may also make this population more susceptible to renal toxicity (65,66). Older patients, particularly those with chronic illness, often have lower albumin levels, which reduce protein binding of the drugs and result in higher free-drug concentrations. This binding of the parent compound to circulating albumin is further impaired by retained metabolites, which accumulate as a result of the normal age-related impairment in renal function. Increased drug levels also occur as a result of the age-related decrease in total body water. Finally, decreased hepatic metabolism, which is often present in older adults, contributes to a longer half-life of the parent compound and can result in unexpectedly high drug levels.

Other conditions in which effective arterial circulatory volume is normal or increased and yet renal function is critically dependent on increased synthesis of prostaglandins include immune mediated glomerular injury, urinary obstruction (67), radiocontrast-induced injury (68), and administration of cyclosporin A (69). In these conditions, increased production of vasodilatory prostaglandins has been shown to counterbalance the effects of intrarenally generated vasoconstrictors such as thromboxane, leukotrienes, platelet-activating factor, and endothelin. Administration of NSAIDs in each of these settings can be expected to result in an exaggerated fall in renal function.

TABLE 44-6

CLINICAL FEATURES OF NSAID-INDUCED VASOMOTOR RENAL FAILURE

Oliguria
Usually occurs within a few days of beginning medicine
Hyperkalemia out of proportion to renal failure
Low fractional excretion of Na
Usually does not require dialysis
Usually reversible

NSAID-induced acute renal failure is most commonly an oliguric form of renal failure that begins within several days after initiation of the drug (Table 44-6). The urinalysis is unremarkable in the majority of cases. Unlike other causes of acute oliguric renal failure, the fractional excretion of sodium is often less than 1%. This low fractional excretion of sodium reflects the underlying hemodynamic nature of the renal failure. Hyperkalemia out of proportion to the decrement in renal function is also a typical feature of this lesion. If recognized early, the renal failure is reversible with discontinuation of the NSAID. As a result, dialysis is usually not required.

Are Certain Nonsteroidal Antiinflammatory Drugs Renal Sparing? An NSAID with effective antiinflammatory properties but associated with less nephrotoxicity would be highly desirable because there is a sizable population at risk for renal toxicity. This issue has been most commonly discussed with sulindac. It has been postulated that sulindac may have renal-sparing properties as a result of its unique metabolism. Sulindac is administered as a prodrug (sulindac sulfoxide), which must be first converted to sulindac sulfide to exert cyclooxygenase inhibitory effects. The sulfide metabolite can be converted back to the parent sulfoxide compound or be metabolized to an inactive sulfone metabolite (70). The liver and kidney are thought to be the major sites at which each of these biotransformations takes place. Based on the ability of the kidney to inactivate sulindac sulfide, renal prostaglandin synthesis should remain relatively intact (71). In fact, unlike other NSAIDs, the active metabolite of sulindac does not appear in the urine after oral administration of sulindac sulfoxide but rather is excreted as the parent compound or as the sulfone metabolite (72).

Despite the potential for less renal toxicity, studies examining this issue have produced conflicting results, some showing renal sparing effects (73,74) and others not (75–77). This discrepancy may in part be explained by changes in the pharmacokinetics of sulindac that occur as a function of duration of therapy and severity of underlying disease. Sulindac has a long half-life (16 to 18 hours) which is prolonged in the setting of renal or liver disease. In either circumstance, the active compound, sulindac sulfide, can progressively accumulate in the plasma over several days to weeks. In the setting of chronic renal failure, steady-state concentrations of ibuprofen are achieved within 24 to 48 hours, whereas concentrations of sulindac sulfide may not achieve a steady state even after 11 days of therapy (75,78,79). With regard to patients with baseline renal insufficiency, an adverse effect of sulindac on renal function has mostly been reported in studies of longer duration that included patients with more advanced degrees of renal insufficiency (75,78). In this setting, levels of sulindac sulfide up to 10 times normal have been reported (78). Such levels may exceed the capacity of remaining renal tissue to convert sulindac sulfide to inactive metabolites.

Similar observations have been noted in patients with cirrhosis and ascites (74). In patients who demonstrate a significant decline in both urinary prostaglandin excretion and GFR following the administration of sulindac, circulating levels of sulindac sulfide are much higher in comparison to patients with cirrhosis who fail to develop renal toxicity (74).

Thus, while there appears to be some reduced risk for vasoconstriction-induced renal insufficiency in patients who take sulindac versus other NSAIDs, this agent still needs to be given with caution. It is likely that the renal-sparing effect of sulindac may simply reflect that common clinical doses are at the low end of the dose-response curve in terms of inhibiting renal prostaglandins. By contrast, similar doses administered to high-risk patients, particularly those with renal insufficiency or liver disease, may result in much higher sulindac sulfide levels which may be capable of inhibiting renal prostaglandin synthesis. In addition to disease-induced changes in the pharmacokinetics of sulindac, there may be individual variability determined by genetic and/or environmental factors in the capacity of the kidney to convert sulindac sulfide to inactive metabolites (80). In this regard, a recent report found that 25 of 70 patients who were chronically ingesting sulindac for treatment of arthritis had detectable sulindac sulfide levels in the urine. It is interesting to speculate that this subgroup may be at risk for renal toxicity in the appropriate setting.

Nabumetone has also been postulated to have renal sparing properties due to modest selectivity of the drug toward the COX-2 enzyme. However, critical review of clinical studies examining this issue does not allow a definite answer to this question (81). As with sulindac, the use of nabumetone in patients at risk for adverse renal effects must be given cautiously with frequent monitoring of renal function.

COX-2 Inhibitors and Acute Renal Failure. Clinical studies comparing the specific COX-2 inhibitors with traditional NSAIDs have generally shown similar effects on renal function. One possible exception based on studies in healthy volume replete adults suggest prostaglandins derived from COX-2 may be more important in modulating urinary sodium excretion while COX-1 derived prostaglandins are more important in regulating renal blood flow and GFR. In one study 24 elderly subjects on a nonrestricted diet received either celecoxib or naproxen for a total of 10 days (82). A small but statistically significantly decrease in GFR developed only in the naproxen group. By contrast both drugs caused a similar decrease in urinary sodium excretion that was transient in nature. Urinary sodium levels returned to baseline after 72 hours and remained stable for the remainder of the study. Similar findings were found in 36 subjects maintained on a high-sodium diet and randomized to receive rofecoxib, indomethacin, or placebo (83). Both drugs caused a transient fall in urinary sodium excretion as compared to placebo while only the indomethacin-treated group demonstrated a decline in GFR.

Differing effects on GFR noted between selective and nonselective COX inhibitors in euvolemic patients are no longer apparent under conditions where renal function is more dependent upon prostaglandins. In elderly subjects placed on a low-sodium diet, rofecoxib lowers GFR to a similar extent as indomethacin (84). Similarly, treatment with celecoxib decreases GFR and renal plasma flow to the same extent as naproxen in young sodium-restricted healthy young men (85). Patients with stable chronic kidney disease treated for 7 days with either celecoxib or naproxen exhibit significant declines in GFR that are similar in magnitude (86).

There are now numerous reports of clinically relevant acute renal failure occurring in association with specific COX-2 inhibitor therapy (87). This complication has typically developed in patients with multiple risk factors for NSAID-induced nephrotoxicity to include decreases in absolute or effective circulatory volume. As with traditional NSAIDs, the renal failure is frequently accompanied by hyperkalemia and may be severe enough to require dialysis but is reversible following the

discontinuation of the drug and restoration of extracellular fluid volume.

Glomerular and Interstitial Disease

The use of NSAIDs can be associated with the development of a distinct syndrome characterized by the development of interstitial nephritis and nephrotic range proteinuria. The incidence of this lesion is unknown but is thought to be rare. One estimate for fenoprofen-induced interstitial nephritis was one case per 5,300 patient-years of treatment (88). While virtually all NSAIDs have been reported to cause this syndrome, the vast majority of cases have been reported in association with use of the propionic acid derivatives (fenoprofen, ibuprofen, and naproxen) (133–142). Of these, fenoprofen has been implicated in greater than 60% of cases (89). Interstitial nephritis with and without nephrotic syndrome has also been reported with the COX-2 inhibitors, rofecoxib and celecoxib (90–94).

Unlike hemodynamically mediated ARF, there are no clear-cut risk factors that serve to identify those at risk for development of this syndrome. The mean age of patients is 65 years (89). The presence of an underlying renal disease prior to exposure of the NSAID has been notably absent. This syndrome has generally been referred to as an example of acute interstitial nephritis. There are, however, a number of features that distinguish this form of interstitial renal disease from that observed with other pharmacologic agents (89,95) (Table 44-7). First, the average duration of exposure prior to the onset of disease is typically measured in months and can be as long as a year. By contrast, allergic interstitial nephritis due to other drugs usually presents within several days to weeks after exposure to the drug. Second, nephrotic range proteinuria is found in >90% of cases of NSAID-induced interstitial disease, a degree of proteinuria that is distinctly uncommon in acute allergic interstitial nephritis due to other drugs. Third, symptoms of hypersensitivity that are commonly seen in acute allergic interstitial nephritis such as rash, fever, arthralgias, or peripheral eosinophilia are uncommon in NSAID-associated disease. Fourth, the vast majority of cases associated with NSAIDs have been reported in older patients. On the other hand, allergic interstitial nephritis is seen in all age groups.

Renal biopsy findings typically show a diffuse or focal lymphocytic infiltrate. The number of eosinophils in the infiltrate is variable but generally is not marked. The glomerular changes are most commonly those seen in minimal change disease. In particular, the glomeruli are normal by light microscopy, while fusion of the podocytes is seen with electron microscopy. In some cases there is evidence of glomerulosclerosis. Since most patients who develop this syndrome are older, this latter finding may simply represent the normal age-related increase in glomerulosclerosis. Immunofluorescent studies are typically nonspecific. There has been an occasional report of weak and variable staining for immunoglobulin G and C_3 along the tubular basement membrane. Electron microscopy typically shows diffuse fusion of the podocytes in cases with heavy proteinuria. Mesangial, electron-dense deposits have been observed in only three patients, suggesting that this is not an immune-mediated disease (96–98).

While the combination of interstitial nephritis and nephrotic syndrome is the most common clinical manifestation, a second presentation is the development of nephrotic syndrome without evidence of interstitial renal disease (95,99). Once again, the glomerular histology is typical of minimal change disease, although a few patients have been described with changes typical of membranous glomerulopathy (90,95,100). It is likely that the pathophysiologic mechanism that underlies the development of glomerular disease in the absence of interstitial disease is similar, if not identical, to the more common finding of combined nephrotic proteinuria and interstitial nephritis.

A third presentation that has uncommonly been reported is the development of interstitial nephritis without nephrotic proteinuria (95,101–103). The onset of disease following the initiation of drug therapy tends to be much shorter and in this respect resembles the more common form of drug-induced allergic interstitial nephritis. In addition, these patients are more likely to exhibit a systemic hypersensitivity reaction. Given the closer temporal relationship between the administration of the offending NSAID and the development of renal insufficiency, one may confuse this latter presentation with that of NSAID-induced vasomotor ARF. Symptoms of hypersensitivity as well as histopathologic changes typical of interstitial renal disease should allow one to distinguish this lesion from hemodynamically mediated ARF.

Finally, NSAID toxicity may present as an exacerbation of an underlying disease. In a case report of a patient with systemic lupus erythematosus, the development of interstitial nephritis and nephrotic syndrome after administration of naproxen clinically appeared as a rapidly progressive lupus glomerulonephritis (104).

The clinical course of patients who present in any one of these manners is to develop a spontaneous remission after removal of the offending NSAID. The time until resolution is variable but can range from a few days to several weeks. In some patients, the degree of renal insufficiency can be severe enough that dialytic support is required. Steroid therapy has been used in many of the reported cases; however, the efficacy and necessity of this therapy are unknown. It should be noted that relapses have been reported after inadvertent exposure to the same NSAID or after exposure to a different NSAID (105–107).

TABLE 44-7

CLINICAL CHARACTERISTICS OF NSAID-INDUCED TUBULOINTERSTITIAL NEPHRITIS (TIN) VERSUS TYPICAL DRUG-INDUCED TIN

Characteristic	NSAID-induced TIN	Typical drug-induced TIN
Duration of exposure	5 days –>1 year	5–26 days
Hypersensitivity symptoms	7%–8%	80%
Eosinophilia	17%–18%	75%–80%
Proteinuria >3.5 g/24 hr	>90%	<10%
Eosinophiluria	0%–5%	80%–85%
Peak serum creatinine	1.5–>10 mg%	1.5–>10 mg%

Renal Sodium Retention

Sodium retention is a characteristic feature of virtually all NSAIDs, occurring in as many as 25% of patients who use them. The physiologic basis of this effect is directly related to the natriuretic properties of prostaglandins. Prostaglandins increase urinary sodium excretion by both indirect and direct mechanisms (Fig. 44-3). Through their activity as renal vasodilators, prostaglandins may cause an increase in the filtered load of sodium. In addition, these compounds preferentially shunt blood flow to the inner cortical and medullary regions of the kidney (24,25). As a result of increased medullary blood

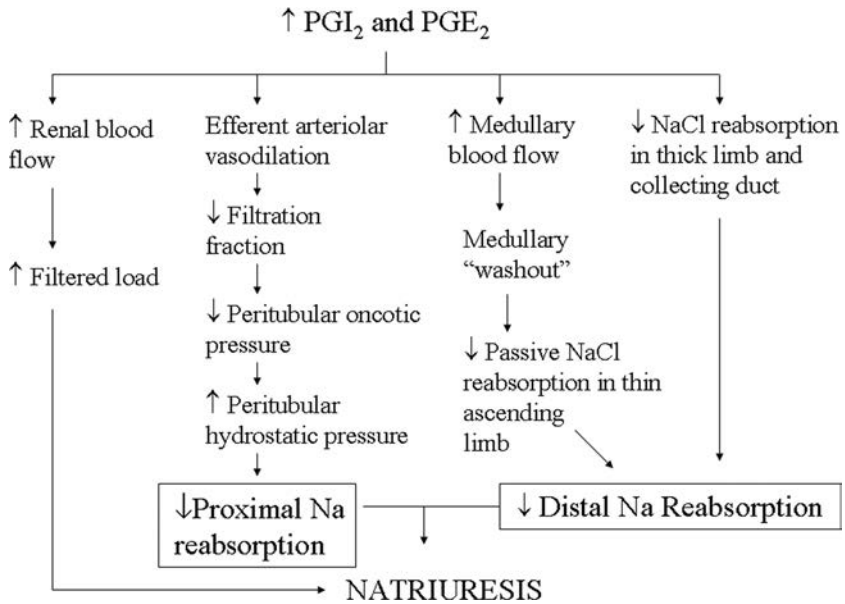

FIGURE 44-3. The direct and indirect mechanisms by which renal prostaglandins exert a natriuretic effect.

flow, there is a fall in the medullary interstitial solute concentration. Processes that reduce the degree of medullary hypertonicity lead to a concomitant reduction in the osmotic withdrawal of water from the normally sodium-impermeable thin descending limb of Henle. This in turn decreases the sodium concentration of fluid at the hairpin turn. The net effect is less passive reabsorption of sodium across the normally water-impermeable thin ascending limb of Henle. Consistent with this mechanism, infusion of PGE_1 lowers and prostaglandin synthesis inhibition raises sodium chloride and total solute concentration in the medulla (108–110).

Finally, prostaglandins can affect sodium reabsorption in the proximal tubule by virtue of their ability to influence the tone of the efferent arteriole. Changes in the tone of this vessel play a central role in determining the Starling forces that govern fluid reabsorption in this nephron segment. Increased resistance of this vessel, as that which occurs in the setting of high concentrations of AII, leads to a decrease in the downstream peritubular hydrostatic pressure. In addition, efferent constriction increases the filtration fraction by reducing glomerular plasma flow and increasing the upstream glomerular pressure. The increased filtration fraction leads to an increase in the peritubular oncotic pressure. A decrease in hydrostatic pressure and increase in oncotic pressure in the peritubular vessel favor fluid reabsorption in the proximal tubule. By modulating the degree to which the efferent arteriole is constricted and thus altering peritubular Starling forces acting on the proximal tubule, prostaglandins can decrease proximal tubular sodium reabsorption. Predictably, in a model of high circulating levels of AII induced by suprarenal aortic constriction, inhibition of prostaglandin synthesis was found to increase efferent arteriole oncotic pressure and decrease peritubular hydrostatic pressure, resulting in a significant increase in proximal fluid reabsorption (111).

In addition to these hemodynamically mediated changes in renal sodium handling, prostaglandins have direct effects on tubular sodium transport. In the isolated perfused tubule, PGE_2 has been shown to inhibit sodium transport in the cortical and outer medullary collecting duct (112,113). Using the same technique, PGE_2 has also been shown to decrease chloride transport in the thick ascending limb of Henle (114). *In vivo* studies also support a direct inhibitory effect of prostaglandins on sodium transport in the loop of Henle, distal nephron, and collecting duct (115,116). The mechanism of this direct inhibitory effect is unclear but may involve decreased activity of the Na–K–ATPase pump (117). Prostaglandins have also been shown to mediate the natriuretic response to increased renal interstitial hydrostatic pressure that occurs during renal interstitial volume expansion (118,119). In addition, these compounds play a permissive role in the sodium excretion that follows volume expansion and an increase in renal perfusion pressure (120,121).

It would at first seem paradoxical that under conditions of volume depletion the kidney would elaborate a compound that would have further natriuretic properties. The role of prostaglandins in this setting, however, is to moderate the avid salt retention that would otherwise occur in the setting of unopposed activation of the renin–angiotensin–aldosterone and adrenergic systems. By virtue of their natriuretic properties, prostaglandins play a role in ensuring adequate delivery of filtrate to more distal nephron segments under conditions in which distal delivery is threatened (e.g., renal ischemia, hypovolemia). In addition, diminished NaCl reabsorption in the thick ascending limb of Henle reduces the energy requirements of this segment. This reduction in thick ascending limb workload in conjunction with a prostaglandin-mediated reallocation in renal blood flow helps to maintain an adequate oxygen tension in the medulla under conditions that would otherwise have resulted in substantial hypoxic injury (122,123).

NSAIDs are thought to cause salt retention primarily by inhibiting prostaglandin synthesis and therefore disrupting the foregoing mechanisms. The extent to which salt retention becomes clinically manifest depends on the degree of baseline prostaglandin production. In normal healthy humans, baseline prostaglandin production is minimal. As a result, NSAID-induced positive sodium balance is transient and usually of no clinical importance. By contrast, NSAID administration in clinical conditions such as congestive heart failure, cirrhosis, or nephrotic syndrome can result in marked sodium retention and potentially adverse clinical consequences.

In addition to causing sodium retention, NSAIDs have been shown to attenuate the natriuretic effect of diuretics (124–126). The mechanism of this resistance is multifactorial. The natriuretic effects of loop diuretics have, in part, been linked to the ability of these drugs to increase renal blood flow, an effect mediated by the stimulation of vasodilatory prostaglandins (127–129). By inhibiting prostaglandin synthesis, NSAIDs limit sodium excretion by preventing the increase in renal blood flow normally seen after the administration of the diuretic (128). In

addition to this hemodynamic effect, micropuncture and microperfusion studies have shown that prostaglandin inhibition also blunts the effect of furosemide at the level of the thick ascending limb of Henle (130–132). This latter effect may be related to inhibition of furosemide-induced stimulation of natriuretic prostaglandins that act within this tubular segment. Finally, NSAIDs may limit the diuretic response to loop diuretics by competing for tubular secretion, thereby limiting the delivery of the drug to the luminal surface of the thick ascending limb (133).

Indomethacin has also been shown to attenuate the diuretic response to hydrochlorothiazide (134). The mechanism of this interaction may result from enhanced salt absorption in the loop of Henle, which would then limit the delivery of chloride to the site of the thiazide action in the distal nephron. A similar explanation may underlie the resistance that has been described with NSAIDs and spironolactone (135).

Sodium Balance and Hypertension. In considering the natriuretic and vasodilatory properties of prostaglandins, it is not surprising that administration of NSAIDs has been shown to interfere with blood pressure control. In pooled studies, administration of NSAIDs has been associated with an average increase in blood pressure of between 5 and 10 mm Hg (136–138). Of the various subgroups examined, this effect is most pronounced in patients who are already hypertensive and much less so in those who are normotensive. Of the hypertensive patients, those treated with β-blockers seem to be the most vulnerable to the hypertensive effect of NSAIDs (136,138). In this regard, it is particularly interesting to note that propranolol has been shown to increase prostacyclin formation (139). There is less of an interaction with diuretics and angiotensin-converting enzyme inhibitors, while no effect is seen with calcium channel blockers (136).

Subgroup analysis shows that patients with low renin hypertension (older adults and blacks) are at higher risk for worsening hypertension in association with NSAID use. Older hypertensive patients have reduced urinary PGE_2 excretion when compared to younger hypertensive patients (140). The pathogenesis of NSAID-induced hypertension is not known with certainty. In a recent metaanalysis, NSAIDs were found not to alter body weight or urinary sodium excretion significantly, implying that mechanisms other than salt retention were responsible for the increased blood pressure (138). In this regard, elimination of the vasodilator prostacyclin from the resistance blood vessels is believed to play some role in the development of hypertension in individuals at risk (141–143).

Sodium Balance, Edema, and Hypertension with COX-2 Inhibitors. The use of COX-2 inhibitors is complicated by the development of peripheral edema with a frequency similar to that seen with traditional NSAIDs. In approximately 6,000 patients with osteoarthritis lower extremity edema developed in 3.6% and 3.8% of patients treated with rofecoxib at 12.5 and 25 mg doses, respectively (87). These rates of edema were no different than the comparator NSAIDs ibupofren and diclofenac and greater than placebo. In over 5,000 patients with osteoarthritis treated with celecoxib the incidence of peripheral edema was 1.6% and 3.0% in patients receiving 100 or 200 mg celecoxib twice daily as compared to 2.2% for the NSAID comparator and 1.3% for placebo (144).

Increased blood pressure has also been reported to occur in a minority of patients in large trials designed to investigate the safety of rofecoxib and celecoxib. Conflicting results have been noted in smaller trials directly comparing these two drugs. In the Successive Celecoxib Efficacy and Safety Studies (SUCCESS) VI and VII only a minority of patients developed increased blood pressue but did so to a greater extent on rofecoxib as compared to celecoxib (145,146). These trials have been criticized for using doses of the drugs that were not equivalent based on efficacy data, different baseline patient characteristics, and timing of blood pressure measurements favoring the shorter acting celecoxib. Subsequent studies using equipotent doses of the drugs and measuring blood pressure at intervals that would capture the peak effect of the drugs have shown no difference between these drugs with regards to increasing blood pressure (147,148). While more rigorously designed prospective trials would be useful, the majority of data suggest no difference between the various COX-2 inhibitors and the tendency to develop increased blood pressure. This complication is a class effect that is dose related and develops with the same frequency as traditional NSAIDs. As previously mentioned, rofecoxib is no longer available for clinical use.

Potassium Metabolism

The use of NSAIDs has been associated with the development of hyperkalemia in the setting of chronic renal insufficiency as well as normal renal function (149–154). The physiologic basis for this effect is inhibition of prostaglandin-mediated renin release with subsequent development of hypoaldosteronism. Both *in vivo* and *in vitro* studies have shown a direct stimulatory effect of prostaglandins (primarily PGI_2 and PGE_2) on renin release from the juxtaglomerular cells (155,156). Clinical studies in salt restricted subjects have shown the COX-2 selective inhibitors reduced urinary potassium excretion to a similar extent as traditional NSAIDs (84,85). Both celecoxib and rofecoxib have been reported to cause significant hyperkalemia in case reports. These finding are consistent with COX-2 in the macula densa playing an important role in stimulating renin release.

In addition to direct effects, these compounds play an essential intermediary role in those pathways that are of primary importance in the regulation of renin release. In particular, renin release stimulated by both decreased perfusion pressure and decreased delivery of filtrate to the macula densa is dependent on an intact cyclooxygenase system (157,158). By contrast, β-adrenergic stimulation of renin release can occur independently of prostaglandin synthesis (159,160).

NSAID-induced suppression of renin release with subsequent development of a hyporenin–hypoaldosterone state is thought to be the primary mechanism of hyperkalemia. Decreased renin release leads to decreased circulating levels of angiotensin I, which in turn results in low levels of AII. Since AII normally stimulates aldosterone release from the zona glomerulosa cells in the adrenal gland, serum aldosterone levels fall. In addition to low circulating levels, the effect of any given level of AII on aldosterone release is impaired because prostaglandins have been shown to play an intermediary role in this stimulatory effect (161). Low circulating levels of AII further contribute to the development of hypoaldosteronism because adequate levels of AII are required for the stimulatory effect of hyperkalemia on aldosterone release (162). In addition to interfering with the renin–angiotensin–aldosterone cascade, NSAIDs favor positive potassium balance in other ways. As discussed earlier, inhibition of prostaglandin synthesis is associated with increased sodium reabsorption in the loop of Henle and thus decreased distal delivery. A reduction in sodium delivery to the aldosterone-sensitive cortical collecting tubule is a known factor impairing potassium excretion. In addition, tubular flow rates are an important determinant of potassium excretion. Since NSAIDs increase the hydroosmotic effect of AVP, flow rates can fall, further impairing potassium excretion. Finally, decreased synthesis of prostaglandins may have effects of decreasing potassium secretion at the level of the potassium channel (163).

The development of hyperkalemia in patients receiving an NSAID is most likely to occur in the setting of renal

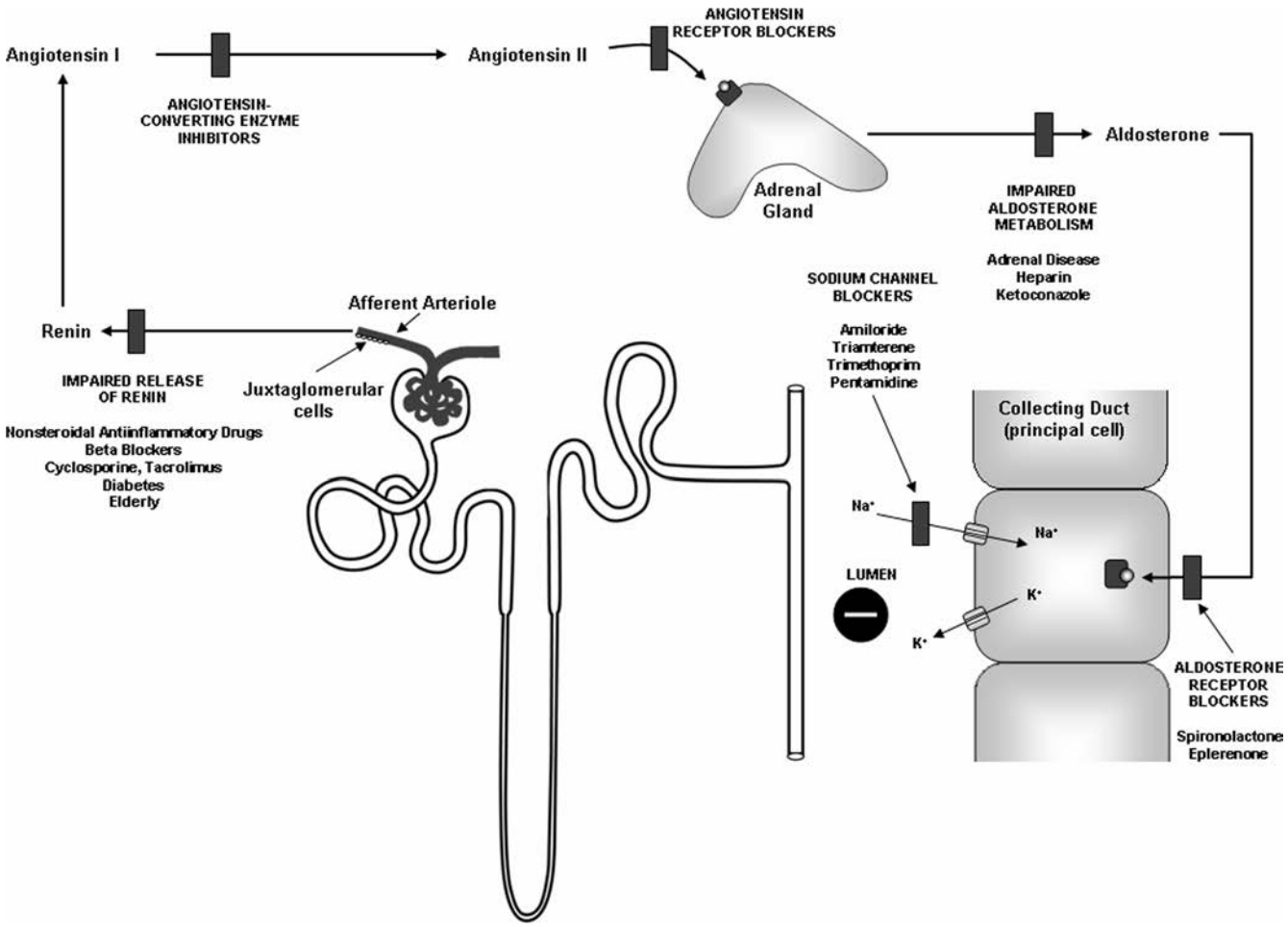

FIGURE 44-4. The renin-angiotensin-aldosterone system and regulation of renal potassium excretion. Aldosterone binds to a cytosolic receptor in the principal cell and stimulates sodium reabsorption across the luminal membrane through a well-defined sodium channel. As sodium is reabsorbed the electronegativity of the lumen increases thereby providing a more favorable driving force for potassium secretion through an apically located potassium channel. Disease states or drugs that interfere at any point along this system can impair renal potassium secretion and increase the risk of hyperkalemia when using angiotensin-converting enzyme (ACE)-inhibitors or angiotensin receptor blockers. In many patients, this risk is magnified due to disturbances at multiple sites along this system.

insufficiency or those with baseline abnormalities in the renin–angiotensin–aldosterone system (155). Diabetic patients are at risk due to the increased incidence of hyporeninemic hypoaldosteronism that occurs in this patient population (164,165). Similarly, older adults are at higher risk by virtue of the normal age-related decrease in circulating renin and aldosterone levels (166). Particular caution should be used when NSAIDs are combined with other pharmacologic agents known to interfere with the renin–angiotensin–aldosterone cascade (167) (Fig. 44-4). Examples would include β-blockers, cyclosporin A, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, heparin, ketoconazole, high-dose trimethoprim, and potassium-sparing diuretics.

Water Metabolism

Prostaglandins have important modulatory effects on renal water metabolism. Their primary effect is to impair the ability to maximally concentrate the urine. In doing so, two processes that are central in the elaboration of a concentrated urine are interfered with, namely, the generation of a hypertonic interstitium and maximal collecting duct water permeability. The decrease in interstitial hypertonicity is due to a washout effect that results from the ability of prostaglandins to shunt blood flow to the inner cortical and medullary regions of the kidney. In addition, prostaglandins decrease sodium absorption in the thick ascending limb and decrease AVP-induced urea permeability in the medullary collecting duct. Decreased accumulation of sodium and urea in the interstitium further reduces the interstitial osmolality. The impairment in collecting duct water permeability is the result of prostaglandins opposing the hydroosmotic effect of AVP (168–170). Interesting to note, AVP is known to stimulate PGE_2 synthesis in collecting duct cells; by doing so, AVP induces its own antagonist. This interaction is another example in which prostaglandins exert a moderating effect on an effector mechanism that elicited their synthesis. In this case, prostaglandins play an important role in minimizing the water retention that would otherwise occur if the activity of AVP was unopposed (171). By opposing the vasoconstrictive action of AVP, prostaglandins also contribute to the maintenance of glomerular perfusion and filtration (172).

Based on the foregoing discussion, administration of NSAIDs would predictably impair solute-free water excretion by increasing the hydroosmotic effect of any given level of circulating AVP and increasing the degree of interstitial

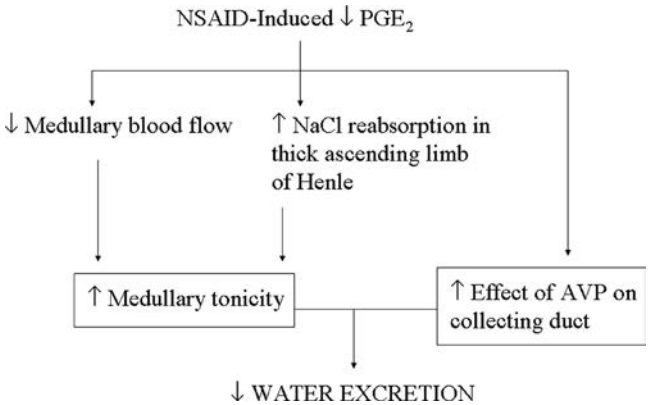

FIGURE 44-5. The mechanisms by which nonsteroidal antiinflammatory drugs lead to decreased renal water excretion. *AVP*, arginine vasopressin.

hypertonicity (Fig. 44-5). In most circumstances, however, hyponatremia is not associated with the use of NSAIDs. Under normal conditions, any decrease in serum osmolality would be sensed in the hypothalamus and result in inhibition of AVP release. As a consequence, excess solute-free water would be promptly excreted, restoring the serum osmolality back to normal. On the other hand, administration of NSAIDs in the setting of nonsuppressible AVP release may result in dramatic falls in the serum sodium concentration. Patients at risk for this complication would include those with high-circulating levels of AVP driven by a decreased effective arterial circulatory volume such as congestive heart failure or cirrhosis (173). Patients with syndrome of inappropriate antidiuretic hormone secretion (SIADH) or those taking medications capable of stimulating AVP secretion or impairing urinary dilution by other mechanisms are also at risk for the development of hyponatremia (60,174).

In this regard, a recent study examined the effects of ibuprofen and a thiazide diuretic on renal water handling in otherwise healthy young and older volunteers subjected to a water load (175). Three days of hydrochlorothiazide (100 mg per day) was found to impair both solute-free water clearance and the ability to elaborate a maximally dilute urine. A delay in the recovery of serum osmolality was noted in both the young and older subjects but to a significantly greater extent in the older subjects. When the young subjects were then given a water load after treatment with ibuprofen together with the thiazide, solute-free water clearance and serum osmolality were reduced further and to a degree similar to that seen in the older subjects on the thiazide regimen alone. It was postulated that the susceptibility to thiazide-induced hyponatremia known to occur in some elderly patients may, in part, be related to lower renal prostaglandin production. It can be expected that a greater number of older patients will be taking a combination of NSAIDs and hydrochlorothiazide given the efficacy of thiazide diuretics in the treatment of systolic hypertension.

NONSTEROIDAL ANTIINFLAMMATORY DRUG-INDUCED CHRONIC KIDNEY DISEASE AND ANALGESIC NEPHROPATHY

The most common form of drug-induced chronic kidney disease is analgesic nephropathy. This lesion has most commonly been linked to the chronic ingestion of compound analgesics containing aspirin, phenacetin, and caffeine (176). A still unresolved question is whether long-term use of NSAIDs alone can similarly result in a progressive and irreversible form of chronic kidney disease. In this regard, a number of observations have emerged that would appear to substantiate the belief that long-term use of NSAIDs can lead to a chronic form of renal injury. Furthermore, the clinical characteristics of NSAID-induced chronic kidney disease are sufficiently different from those in analgesic nephropathy to suggest that this is a distinct clinical entity. Before reviewing the data linking chronic NSAID use and renal insufficiency, a brief description of analgesic nephropathy will be provided.

Analgesic nephropathy is a chronic kidney disease characterized by renal papillary necrosis and chronic interstitial nephritis (176–178). The early reports linking analgesics and renal disease were generally found in patients who consumed combination products containing phenacetin. This fact focused attention on phenacetin as the primary cause of the syndrome and prompted many countries to officially remove the drug from nonprescription analgesics. Significantly, the removal of phenacetin has not been uniformly followed by the expected reduction in the incidence of the syndrome (179). Given that other agents such as acetaminophen or salicylamide have been substituted for phenacetin in many combination products, the lack of decline in incidence of analgesic nephropathy suggests that the use of combination products is as important as whether the compound contains phenacetin (179,180). This conclusion is further supported by the experience in Belgium where a strong geographic correlation exists between the prevalence of analgesic nephropathy and sales of analgesic mixtures containing a minimum of two analgesic components (181,182).

Numerous epidemiologic studies performed in the past demonstrated a wide variation in the geographic incidence of analgesic nephropathy (179,180,183–186). Much of this variability could be explained by differences in the annual per capita consumption of phenacetin (178,179). In those countries with the highest consumption such as Australia and Sweden, analgesic nephropathy was found responsible for up to 20% of cases of end-stage renal disease. In Canada, which had the lowest per capita consumption, analgesic nephropathy accounted for only 2% to 5% of end-stage renal disease patients. It has been estimated that between 2% and 4% of all end-stage renal disease cases in the United States can be attributable to habitual analgesic consumption. Within the United States, there are also regional differences in the reported incidence of analgesic nephropathy, which are thought to be reflective of differences in analgesic consumption (179,180,183,184). For example, the use of combination analgesics is more common in the southeastern United States, and the incidence of analgesic nephropathy is three to five times as common a cause of end-stage renal disease in North Carolina compared to Pennsylvania (179,180,183,184).

The development of analgesic nephropathy is associated with a number of well-defined clinical characteristics (187). The disease is more common in women by a factor of 2 to 6 and has a peak incidence at age 53 years. Patients typically consume compound analgesics on a daily basis, often for chronic complaints such as headache, dyspepsia, or to improve work productivity. It has been estimated that nephropathy occurs after the cumulative ingestion of 2 to 3 kg of the index drug. Often patients will exhibit a typical psychiatric profile characterized by addictive behavior. Gastrointestinal complications, such as peptic ulcer disease, are common. The patients are frequently anemic as a result of gastrointestinal blood loss as well as renal insufficiency. Ischemic heart disease and renal artery stenosis have both been reported to occur with higher frequency in these patients (178). In fact, regular use of analgesic drugs containing phenacetin is associated with an increased risk of

hypertension and mortality and morbidity due to cardiovascular disease (186,188). Finally, long-term use of analgesics is known to be a risk factor for the subsequent generation of uroepithelial tumors (189).

Patients with analgesic nephropathy have predominantly tubulomedullary dysfunction characterized by impaired concentrating ability, acidification defects, and occasionally a salt-losing state. Proteinuria tends to be low to moderate in quantity. Interesting to note, the pattern of proteinuria is typically a mixture of glomerular and tubular origin. Pyuria is common and is often sterile. Occasionally hematuria is noted, but if persistent should raise the possibility of a uroepithelial tumor.

There are several features of analgesic nephropathy that make it difficult to diagnose. The disease is slowly progressive and the symptoms and signs are nonspecific. Patients are often reluctant to admit to heavy usage of analgesics and therefore are either misdiagnosed or not diagnosed at all until the renal failure is far advanced. In addition, the lack of a simple and noninvasive test that reliably implicates analgesics as the cause of the renal injury has been an important limiting factor. Noncontrast abdominal computed tomography (CT) may emerge as a useful diagnostic tool in this setting given its usefulness in the diagnosis of papillary necrosis (190). Characteristic findings by computerized tomography suggesting the diagnosis include small kidneys with an irregular contour and intrarenal calcifications particularly in the medulla.

As mentioned earlier, there are a number of reports that suggest that chronic use of NSAIDs alone may also lead to renal injury. In this regard, several NSAIDs have been associated with the development of papillary necrosis either when administered alone or in combination with aspirin (191). In addition to inhibiting prostaglandin synthesis, the ability of these agents to redistribute blood flow to the cortex, rendering the renal medulla ischemic, may underlie this association. While the reports linking papillary necrosis and NSAIDs are predominantly anecdotal in nature, more recent observations would suggest that chronic renal failure resulting from long-term use of NSAIDs may be more prevalent than once thought (192,193). In a multicenter case-control study, Sandler and associates (192) report a twofold increase in the risk for chronic kidney disease associated with the previous daily use of NSAIDs. Chronic kidney disease in these patients was newly diagnosed and was defined as a serum creatinine concentration of 1.5 mg/dL or greater. This increased risk was primarily limited to older men. An additional report linking chronic use of NSAIDs with development of chronic kidney disease described 56 patients from Australia (178). These patients had taken only NSAIDs over a period of 10 to 20 years for treatment of varying rheumatic diseases. In 19 patients (34%), radiographic evidence of papillary necrosis was found. In 37 patients, renal biopsy material was available that disclosed evidence of chronic interstitial nephritis. The clinical characteristics of these patients were quite different from those with analgesic nephropathy, suggesting that NSAID-induced chronic kidney disease is indeed a distinct entity. In particular, patients with NSAID-associated renal disease were older, had an equal female-to-male ratio, a lower incidence of papillary necrosis, less severe renal insufficiency, and a lower incidence of urinary tract infections (178). In addition, an increased risk of uroepithelial tumors has not been described in these patients.

Further evidence of chronic toxicity has been reported in a preliminary communication in which patients treated with NSAIDs for rheumatoid arthritis and osteoarthritis were compared to a matched control arthritis population (194). In this study, the NSAID-treated patients had a rise in the serum creatinine concentration from 1.28 to 2.58 mg/dL over a mean period of 47.5 months. The control group not taking NSAIDs had stable renal function. Finally, Segasothy and colleagues (193) report on the risk of chronic renal disease in a prospective study of 259 heavy analgesic abusers. In this study, 69 patients developed radiographic evidence of papillary necrosis. Of these, 29 used NSAIDs either singularly (17 patients) or in combination with another NSAID (12 patients). Another nine patients used NSAIDs in combination with paracetamol, aspirin, caffeine, or a traditional herbal medicine. Renal insufficiency (serum creatinine concentration 1.4 to 8.8 mg/dL) was noted in 26 of the 38 patients who had used an NSAID chronically. Similar to the patients from Australia (178), this disorder was more common in males (1.9:1.0), distinguishing this disorder from classic analgesic nephropathy, which typically occurs in females. Similarly, these patients did not exhibit the usual psychological profile associated with analgesic abuse.

Thus, while further studies are needed to definitely assess the question of cumulative toxicity, it appears that some chronically treated patients may develop a change in renal function over a long-term period. Given the abuse potential of powerful NSAIDs and the fact that ibuprofen, naproxen, and ketoprofen are now available on an over-the-counter basis, it is possible that chronic NSAID abuse may become a more common cause of chronic kidney disease in the future.

In considering the definite association of compound analgesic abuse and the possible linkage of chronic NSAID use to the development of chronic kidney disease, it has become common clinical practice to recommend acetaminophen whenever possible for analgesia. In this regard, a recent case-control study examining the use of over-the-counter analgesics as a risk factor for end-stage renal disease found that acetaminophen may also cause chronic kidney disease when used on a continual basis (195). In this study, heavy average use of acetaminophen (>1 pill per day) and medium- to high-cumulative intake (1,000 or more pills in a lifetime) each doubled the odds of end-stage renal disease. These authors conclude that reduced consumption of acetaminophen could decrease the overall incidence of end-stage renal disease approximately 8% to 10%. The findings in this study confirmed an earlier report that also concluded that long-term daily use of acetaminophen is associated with an increased risk of chronic kidney disease (183). While these studies do not establish a cause-and-effect relationship between acetaminophen ingestion and chronic kidney disease, the data do suggest that ingestion of acetaminophen on a continual and chronic basis should be discouraged.

A recent organized review by a consensus panel of the National Kidney Foundation (NKF) surveyed over 600 articles and studied the implications of several different kinds of analgesic ingestion and renal failure risks (196,197). The highlights of the recommendations from the NKF consensus panel based on this review are that:

1. Ingestion of aspirin and nonsteroidal combinations are not encouraged because of an increased risk of renal failure when those combinations are ingested together.
2. Habitual consumption of analgesics is discouraged, and monitoring is recommended when such use is mandatory.
3. Combination analgesics are recommended to be available by prescription only with an explicit warning to physicians that the habitual consumption of these combination products could lead to the insidious development of chronic kidney disease.
4. There should be an explicit warning to consumers regarding NSAID ingestion.

The panel concluded that there is negligible clinical evidence that suggests habitual use of acetaminophen alone causes the clinical entity of analgesic nephropathy and that there is no evidence that occasional use of acetaminophen causes renal injury. Finally the panel points out that there is no risk from the

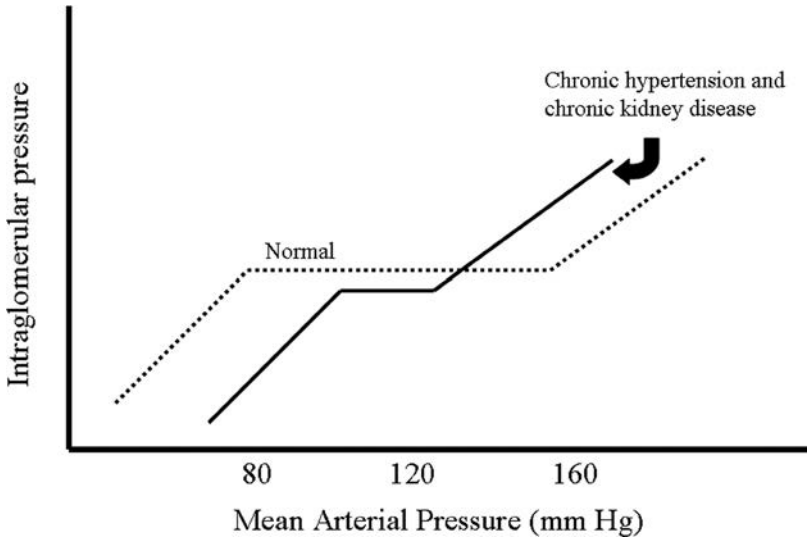

FIGURE 44-6. Renal autoregulation normally maintains intraglomerular pressure relatively constant despite variations in renal perfusion pressure. In patients with chronic hypertension or mild chronic kidney disease renal autoregulation changes in such a way that intraglomerular pressure begins to vary more directly with changes in mean arterial blood pressure. One can conceptualize this change as if the normal sigmoidal relationship between systemic blood pressure and intraglomerular pressure becomes progressively more linear. As a result, increases in mean pressure cause exaggerated rises in intraglomerular pressure while declines in mean pressure will cause exaggerated falls in intraglomerular pressure. The decline in intraglomerular pressure accompanying more stringent levels of blood pressure control will manifest itself by an increase in the serum creatinine concentration. Renal dysfunction that occurs in this setting is hemodynamic in origin and is reflective of a lower intraglomerular pressure.

regular use of aspirin in the relatively small doses recommended for prevention of cardiovascular events.

NEPHROTOXICITY OF ANGIOTENSIN-CONVERTING ENZYME INHIBITORS AND ANGIOTENSIN RECEPTOR BLOCKERS

The most common form of nephrotoxicity associated with angiotensin-converting enzyme (ACE) inhibitor therapy is an increase in the serum creatinine concentration occurring in the setting of antihypertensive therapy (198). This complication is becoming more common in clinical practice since guidelines governing adequate blood pressure control have been made more stringent. This decline in renal function is hemodynamic in origin and not secondary to structural injury to the kidney and can be traced to changes in renal autoregulation that accompany chronic kidney disease.

Normal renal autoregulation enables the kidney to maintain fairly constant renal blood flow and glomerular filtration rate in the setting of varying systemic blood pressure. One component of this process is an intrinsic property of the afferent arteriole called the myogenic reflex. The myogenic reflex causes this vessel to either constrict or dilate in response to changes in intraluminal pressure. An increase in arterial pressure elicits a vasoconstrictive response, whereas a decreased arterial pressure results in vasodilation. These changes in afferent tone provide an immediate response to maintain intraglomerular pressure and glomerular filtration rate relatively constant in the face of everyday fluctuations in systemic blood pressure (199).

In the setting of chronic hypertension, the small arteries of the kidney to include the afferent arteriole undergo a number of pathologic changes that give rise to alterations in the way the kidney autoregulates (200). As with vessels elsewhere, the afferent arteriole initially demonstrates evidence of endothelial dysfunction leading to impaired vasodilation. Over time this impairment is exaggerated by histologic changes of hyaline arteriosclerosis and myointimal hyperplasia. These changes lead to a blunted ability of the preglomerular circulation to either constrict or dilate in response to changes in renal perfusion pressure. In essence these vessels take on the characteristics of a pressure-passive vasculature where changes in mean arterial pressure are matched by proportional change in GFR (201,202) (Fig. 44-6).

A blunted ability of the preglomerular circulation to dilate in response to a drop in the mean arterial pressure will cause an exaggerated decrease in the intraglomerular pressure and GFR. This impairment in autoregulation explains why patients with hypertension and chronic kidney disease are more likely to have an increase in the serum creatinine concentration when blood pressure is lowered. Any drug that lowers blood pressure can cause an increase in the serum creatinine concentration through this mechanism but ACE inhibitors are more commonly associated with this complication. ACE inhibitors will exaggerate the decline in intraglomerular pressure due to blood pressure reduction by concomitant vasodilation of the efferent side of the glomerular circulation.

As long as the increase in serum creatinine concentration is not excessive (>30% above the baseline value), or progressive, discontinuation of the ACE inhibitor is not necessarily warranted particularly considering the potential benefit, these agents have in slowing the progression of chronic renal failure. Long-term trials in both diabetic and nondiabetic patients have shown that the initial decline in renal function reaches a plateau within several weeks and is reversible with discontinuation of the ACE inhibitor even after several years of therapy (203,204). Thus a small, stable increase in the serum creatinine concentration after the start of an ACE inhibitor is hemodynamic in nature and reflects a fall in intraglomerular pressure.

If the rise in serum creatinine is >30% or the repeat value shows a progressive rise then the appropriate response is to discontinue the ACE inhibitor and initiate a search for other causes of renal dysfunction. There are several conditions in which use of ACE inhibitors may cause exaggerated or progressive declines in renal function (Table 44-8). The first

TABLE 44-8

RISK FACTORS FOR ACEI- OR ARB-INDUCED ACUTE RENAL FAILURE

Renal artery stenosis
Polycystic kidney disease
Decreased absolute or effective arterial blood volume
Nonsteroidal antiinflammatory drugs
Cyclosporin A, FK-506 (tacrolimus)
Sepsis

setting involves significant (usually >70%) bilateral renal artery obstruction or unilateral renal artery obstruction to a solitary functioning kidney. Under these conditions increased tone of the efferent arteriole acts to attenuate the decline in intraglomerular pressure that results from the arterial obstruction. The trade-off is that renal function and glomerular filtration rate become dependent upon sustained constriction of the efferent vessel by AII. A similar physiology can develop in patients with polycystic kidney disease where the renal arteries become extrinsically compressed by large cysts (205). Unless the underlying obstruction can be treated then other classes of antihypertensive agents will have to be utilized.

ACE inhibitors can also cause an azotemic response under conditions of an absolute (gastroenteritis, aggressive diuresis, poor oral intake) or effective reduction in arterial circulatory volume (moderate to severe congestive heart failure). In these settings angiotensin II-mediated constriction of the efferent arteriole serves to minimize the decline in glomerular filtration rate that would otherwise occur as a result of the fall in renal perfusion pressure. In the volume-contracted patient, the appropriate response to hold the ACE inhibitor and restart the drug once the extracellular fluid volume has been replenished. In a patient with congestive heart failure, ACE inhibitors will increase the serum creatinine when the decrease in intraglomerular pressure resulting from efferent vasodilation is not offset by an increase in renal perfusion. This can occur in patients with severely depressed cardiac function in which afterload reduction can no longer increase cardiac output or in the setting of aggressive diuresis.

A similar mechanism is responsible for renal dysfunction that occurs in patients given ACE inhibitors in the setting of NSAIDs, cyclosporin A, or early sepsis (206–208). In these settings, there is increased vasoconstriction of the renal vasculature. ACE inhibitor-induced efferent vasodilation in the face of decreased perfusion pressure accounts for the fall in GFR.

Angiotensin receptor blockers can cause renal dysfunction through the same mechanisms described with ACE inhibitors. Based on the biology of angiotensin II and its receptors there are reasons to suggest that such adverse effects on renal function may be slightly less common. These drugs block the AT_1 receptor, which is located primarily on the efferent vessel resulting in a vasodilatory response. AT_1 blockade is also associated with higher levels of angiotensin II that can then stimulate AT_2 receptors that are located to a greater extent on the afferent side of the glomerular circulation (209–211). Stimulation of the AT_2 receptor causes vasodilation (210). Efferent vasodilation (from AT_1 blockade) combined with afferent vasodilation (from AT_2 stimulation) might allow GFR to be better maintained in the setting of decreased blood pressure. In animal models of volume depletion, angiotensin receptor blockers have been shown to better preserve GFR as compared to ACE inhibitors (212,213). Whether such differences between angiotensin receptor blockers and ACE inhibitors are sufficient to be of clinical relevance in maintaining renal function has not been well studied (214).

A few cases of membranous nephropathy have been attributed to the use of ACE inhibitors (215,216), but the overall incidence of this complication and that of tubulointerstitial nephritis are believed to be low. To date there are no such reports linking angiotensin receptor blockers to the development of glomerular or interstitial renal disease.

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CHAPTER 45 ■ RADIOCONTRAST MEDIA-INDUCED ACUTE RENAL FAILURE

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Since the introduction of the first iodinated contrast medium in 1954, the use of iodinated radiocontrast media has markedly expanded, in parallel with the increasing use of imaging procedures, including computerized tomography and angiographic interventions. Some 80,000,000 imaging studies with iodinated contrast agents were performed worldwide during 2003, corresponding to about 8 millions liters of radiocontrast material (Idee JM, personal communications). This explains why contrast-induced nephropathy (CIN) remains an important iatrogenic cause of acute renal failure (1,2), despite its recognition, the implementation of preventive strategies and the use of safer agents. Hou reported in 1987 that of 129 cases of hospital-acquired renal insufficiency, 12% followed the use of radiocontrast (1), putting contrast media as the third leading cause of new-onset acute renal failure (ARF) in hospitalized patients. Fifteen years later, CIN remains an important cause of ARF acquired in a tertiary care hospital, together with reduced renal perfusion, medications, and surgery (3).

This chapter will first provide a brief review of the radiocontrast materials presently used. This will be followed by the important clinical features of CIN, and by an evaluation of laboratory investigations that provide insights into the pathogenesis of this disorder. The last section deals with methods currently used to prevent the development of CIN, and the search for additional preventive strategies.

TYPES OF CONTRAST AGENTS

The first widely used radiocontrast media were high-osmolar (≈1,600 mOsm) ionic derivatives of triiodobenzene, such as diatrizoate, meglumine, or metrizoate (Table 45-1, Fig. 45-1). Since the early 1990s their use has gradually been replaced by safer "low-osmolar" (600 to 800 mOsm) agents, such as the nonionic iohexol, ioversol, iopromide, or iopamidol, and the ionic dimer ioxaglate, particularly for high-risk patients. Third-generation isoosmolar (≈300 mOsm) nonionic agents, such as iodixanol and iotrolan have been introduced during the late nineties and their nephrotoxic potential as compared with the low-osmolar contrast media is currently being investigated.

The basic structure of high-osmolar agents, such as sodium-diatrizoate, is a single benzene ring that contains three iodine atoms and a short ionic residue that facilitates water solubility. The nonionic monomers, such as iohexol, do not have an ionic residue, and thus are only moderately hypertonic. Dimeric ionic compounds, for instance ioxaglate, are constructed of two linked benzene rings that together contain six iodine atoms and a cationic residue of either sodium or methylglucamine. Because the iodine content of these dimeric compounds is twice that of either the ionic or the nonionic agents, similar amounts of iodine can be given with only half the osmotic load. The recently developed nonionic dimers resemble the dimeric ionic compounds but are devoid of the ionic site. This structural modification further reduces the osmolarity to a near-physiologic value, but at the price of a substantial increase in viscosity (Table 45-1). As outlined below, the new generation low- and isoosmolar agents are considered safer, as compared with the high-osmolar radiocontrast media. Indeed, the median lethal dose of intravenously administered iohexol, is three times greater than that of the ionic high osmolar agents (4). Nonionic contrast media seem to have a particular advantage in myelography, digital subtraction angiography, leg phlebography, arteriography and renal tomography. Systemic effects, such as a feeling of warmth (especially after intravenous use), that depend on iodine content, osmolality, and sodium ion concentration are less frequent with these agents (5). Unlike the ionic agents that are minimally reabsorbed by the nephron, the nonionic agents are to some extent reabsorbed by the proximal tubule, but there is species specificity to this property (6). Radiocontrast agents cause mild proteinuria when injected directly into the renal artery, but the magnitude of this effect with nonionic agents is smaller than with ionic radiocontrast (7). The nonionic agents also have significantly less cardiac effects, causing less depression of ventricular contractility and less reduction in coronary sinus calcium concentration (5). They also appear to have less effect on complement consumption, cause fewer hypersensitivity reactions, and have a less disruptive effect on the endothelial wall of blood vessels (8).

CLINICAL FEATURES OF CIN

Historical Perspective

In the early 1960s, radiocontrast material was suspected of causing acute renal failure (ARF), but controversy surrounded many of the early case reports. Ten years later, CIN was recognized as one of the leading causes of ARF in patients with renal insufficiency, diabetes mellitus, volume depletion, and low cardiac output. Soon after this recognition, however, the incidence of ARF in high-risk patients declined sharply, simply because physicians learned to volume expand these patients before administering radiocontrast (Fig. 45-2). This bell-shaped curve, which characterizes the history of clinical drug toxicities in general, disqualifies historical controls. For instance, the use of mannitol to prevent CIN was based on historical controls; more recent reports, using concurrent controls, suggest that mannitol may in fact be detrimental, especially in diabetic patients (9,10). Moreover, in prospectively studied, carefully prepared patients, the incidence of clinically significant ARF is very low; only about 1% of high-risk patients need dialysis following radiocontrast administration (9,10). Similar observations have led some investigators to suggest that following imaging studies, ARF may not occur much more often when radiocontrast medium is administered than when it is not (11). Prospective studies that focus on patient selection and optimal treatment often miss one of the most important factors

TABLE 45-1

TYPES OF RADIOCONTRAST AGENTS IN USE

Class of radiocontrast agent	Contrast agents	Osmolality (mosm)	Viscosity (mPa*s, 37°C)
High-osmolar	Iothalamate	1,400–2,000	~5–6
Ionic monomer	Diatrizoate		
"Low-osmolar"	Iohexol, ioversol	600–800	~4.5–6.5
Nonionic monomer	Iopamidol, iopromide		
	Iomeprol		
Ionic dimer	Ioxaglate		
Isoosmolar	Iotrolan	~300	~9
Nonionic dimer	Iodixanol		

involved in CIN—lack of awareness leading to the administration of large doses of radiocontrast to the sickest and least prepared patients (12).

Incidence of CIN

The incidence of CIN is highly dependent on the population studied and the criteria used to diagnose renal injury. CIN has been defined as a rise in serum creatinine of 0.3 mg/dL (13,14), 0.5 mg/dL (9,15–19), 0.6 mg/dL (20), 1.0 mg/dL (17,21–26), or 2.0 mg/dL (27,28), or a 10% (29), 25% (10,29–31) or a 50% increase in baseline serum creatinine 1 to 5 days following exposure to radiocontrast material (11,32). D'Elia and coworkers (22) report that 0.68% of nonazotemic patients and 17.4% of azotemic patients had a 1 mg/dL rise in serum creatinine following nonrenal angiography. A 12% incidence of ARF was reported in seriously ill, hospitalized patients, using a rise in serum creatinine of at least 1 mg/dL within 48 hours of the study as the criterion for nephrotoxicity (33). A prospective study of 537 consecutive patients undergoing major angiography, that used somewhat more stringent criteria for nephrotoxicity (i.e., 1 mg/dL serum creatinine rise within 24 hours), uncovered no episodes of ARF (23). Nevertheless, these patients received saline expansion during the angiographic procedure. The reported incidence of CIN seems to be declining (16, 34–36). In a group of patients with few risk factors other than an age >70 years, the incidence of ARF after angiography was as low as 1.2% (36). Mueller reported a comparable incidence of 0.7% to 2% of CIN in 1,620 patients undergoing coronary angiography, the majority without predisposing risk factors (18). Rudnick and colleagues (37) have emphasized that studies examining the incidence of CIN also report substantial ARF in the control patients not receiving radiocontrast. This suggests that earlier reports probably overestimated the true incidence of CIN in the general population. However, the reported incidence of CIN in high-risk patients remains much higher, at the range of 10% to 45% (21,26,36,38–43), despite the use of newer contrast media and the implementation of preventive measures, though only a small fraction of these patients require

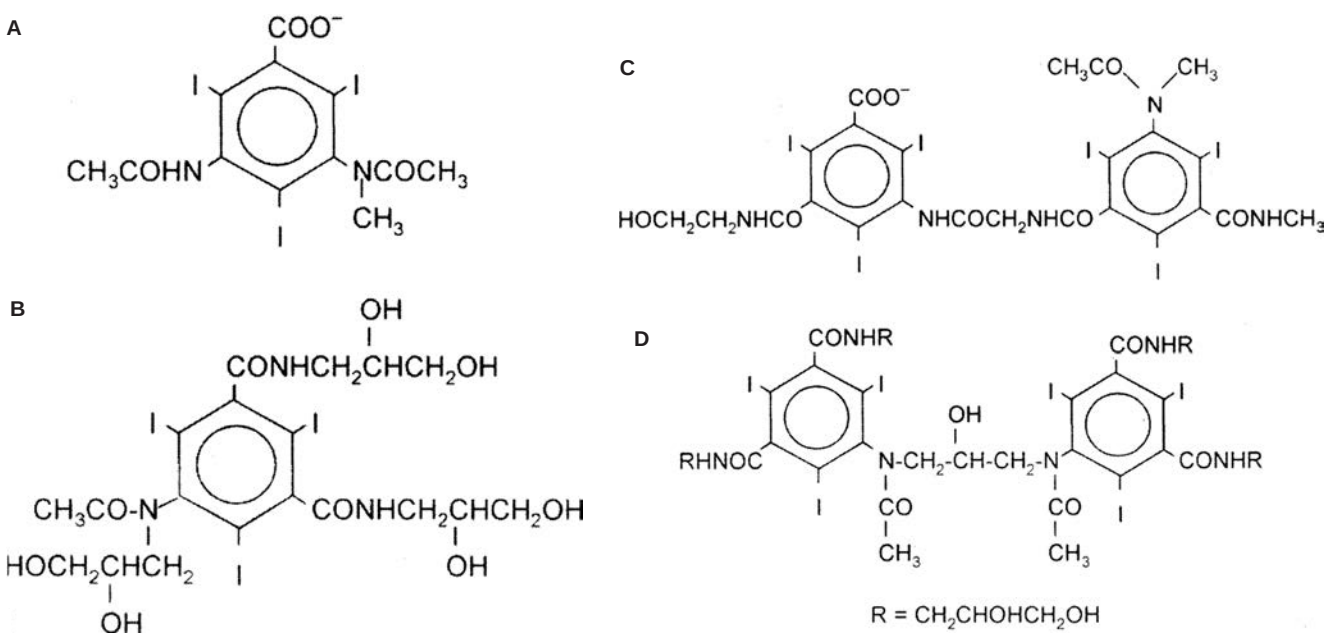

FIGURE 45-1. A: High-osmolar ionic radiocontrast agents are triiodinated benzoic acid anionic derivatives and a cation (metrizoate). **B:** Nonionic "low-osmolar" radiocontrast agents are also triiodinated benzoic acid derivatives (iohexol). **C:** Ionic "low-osmolar" dimeric radiocontrast agents contain two benzene rings, with a total of six iodine atoms and a cation (ioxaglate). **D:** Isoosmolar agents are nonionic dimmers also consisted of two iodinated benzene rings (iodixanol).

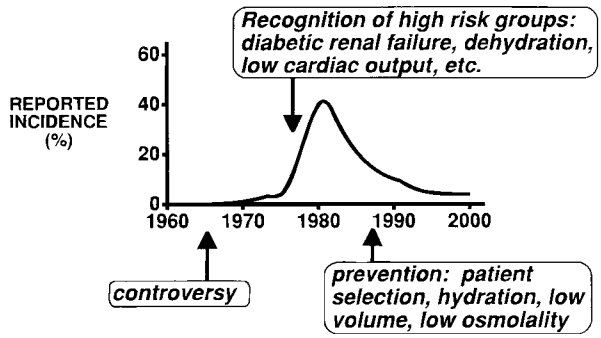

FIGURE 45-2. Schematized history of radiocontrast nephropathy. (From: reference 179, Heyman SN, Rosen S, Brezis M. Radiocontrast nephropathy: a paradigm for the synergism between toxic and hypoxic insults in the kidney. *Exp Nephrol* 1994;2:153, with permission.)

renal replacement therapy. Thus, the incidence of CIN and the assessment of the efficacy of preventive measures should be regarded in the perspective of predisposing risk factors, type and dosage of the radiocontrast agent, and the implementation of protective strategies. Indeed, diversity in the definition of CIN and the marked heterogeneity regarding patients' characteristics, the type and volume of administered radiocontrast, the type of imaging procedure and the applied protective measures, interfere with comparisons of clinical studies and in the assessment of efficacy of various interventions (44).

Risk Factors for CIN

Factors linked to the development of CIN can be divided into patient-related and procedure-related factors (Table 45-2). The former group include preexisting renal insufficiency (22,26,27,45–53), diabetes mellitus (21,24,26–28,33,46,47, 49,54–60), advanced age (13,27,28,33,45,46,54,55,61), multiple myeloma (62–65), volume depletion (45,49,53,66), presence of cardiovascular disease (13,14,28,46,49,53,56,59,67), hypertension (13,14,28,48,67,68), dehydration (54,57,59, 67,69), concomitant exposure to other nephrotoxins (56,57, 59,69), anemia (49,70), proteinuria (13,28,33,71), abnormal liver function (33,71), hyperuricemia (24,27,68), male sex (13,18), and renal transplantation (72,73). Hyperlipidemia also appears to be a risk factor in experimental CIN (74). Among procedure-related risk factors are the type of radiocontrast material used (i.e., high- vs. low- or isoosmolar agents) (26), volume of contrast media (19,21,45,49,56,57,66,75,76), repeated exposure to radiocontrast media over a few days (13,14,67,68,71), and injection site (i.e., intraarterial vs. intravenous) (27,49).

Many of these risk factors may be covariate rather than be independent variables. This probably accounts for reports that fail to confirm many of these as risk factors. The incidence of CIN is proportional to the number of coexisting clinical risk factors (36). As shown in Figure 45-3, among aged patients it was almost inevitable in the presence of three or more risk factors, while at their absence the chance to develop CIN became negligible.

Underlying renal insufficiency is the risk factor most commonly associated with CIN. Factors that may be involved in this predisposition include (a) a reduced glomerular filtration rate (GFR) that obligates each remaining nephron to excrete a proportionately greater load of contrast material, hence exposure to a greater amount of contrast, and (b) the lack of functional renal reserve in chronic stable renal insufficiency to buffer acute losses in glomerular filtration. Thus, any nephron

TABLE 45-2

RISK FACTORS FOR DEVELOPMENT OF RADIOCONTRAST AGENT-INDUCED ACUTE RENAL FAILURE

Patient-Related Factors	Procedure-Related Factors
Renal insufficiency	**Type of radiocontrast medium** (High-osmolar > low or isoosmolar)
Diabetes mellitus	**Dose of radiocontrast medium**
Age	**Repeated exposures to radiocontrast material within 72 hr**
Effective volume depletion Dehydration Congestive heart failure Chronic liver disease Nephrotic syndrome Concomitant hypotension	**Mode of administration** (Intraarterial > intravenous) Primary coronary intervention for acute MI*
Concomitant exposure to nephrotoxins Medications Other exogenous nephrotoxins Sepsis	
Myeloma	
Male gender	
Hypertension	
Transplanted kidney	
Hyperuricemia	
Proteinuria	
Anemia	

(*Marenzi, et al. N-acetylcysteine and contrast-induced nephropathy in primary angioplsty. *N Engl J Med* 2006;354:2773.)

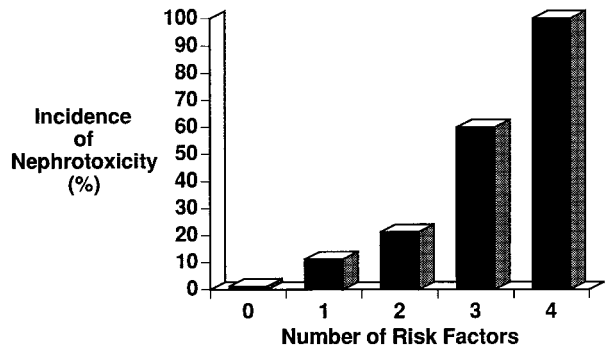

FIGURE 45-3. Clinical incidence of radiocontrast nephrotoxicity as a function of the number of preexisting risk factors present in the patient. (Redrawn from: Rich MW, Crecelius CA. Incidence, risk factors, and clinical course of acute renal insufficiency after cardiac catheterization in patients 70 years of age or older. *Arch Intern Med* 1990;150:1237, with permission.)

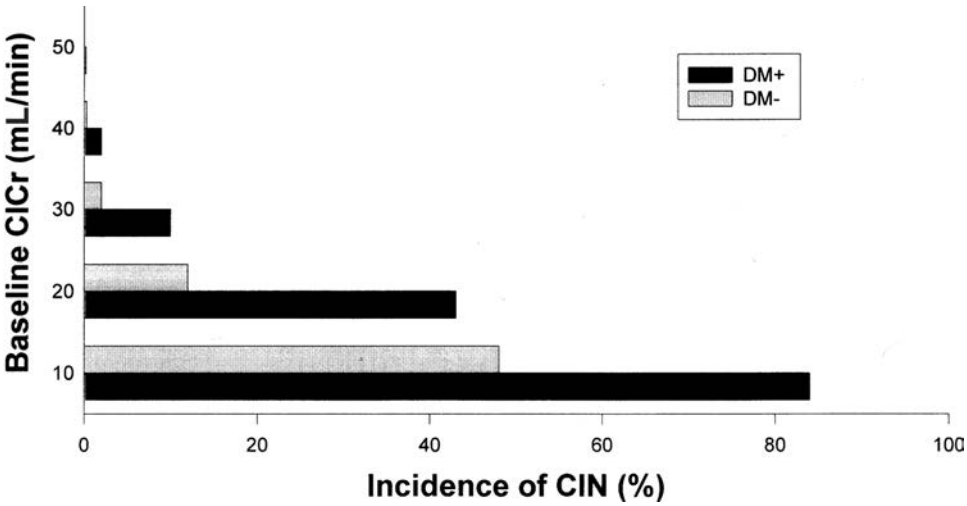

FIGURE 45-4. The impact of preexisting renal dysfunction and diabetes on the incidence of CIN. (Modified with permission from: McCullough et al. Acute renal failure after coronary intervention: incidence, risk factors, and relationship to mortality. *Am J Med* 1997;103:368.)

damage or loss is translated immediately into a rise in serum creatinine. D'Elia and co-workers (22) have studied 378 hospitalized patients undergoing nonrenal angiography and identified the presence of preexisting azotemia as the only risk factor predisposing to nephrotoxicity (defined as a rise in serum creatinine of ≥1 mg/dL following the procedure). McCullough, evaluating the incidence and risk factors for CIN in 3,700 patients undergoing coronary intervention (75), reports that the lower the baseline creatinine clearance, the larger was the risk for the development of CIN (Fig. 45-4).

Taliercio and co-workers (77) show that class IV heart failure is an independent risk factor for CIN, particularly when the amount of contrast exceeds 125 mL. They report a 23% incidence of contrast nephropathy (>1 mg/dL rise in serum creatinine) in 139 patients with preexisting abnormal renal function (serum creatinine ≥2 mg/dL) who underwent cardiac angiography. The mean increment in serum creatinine was 2.6 mg/dL, the time to peak serum creatinine was 2.8 days, and 9% developed anuria or oliguria. Similarly, Gomes and colleagues (78) show an increased incidence of CIN in patients receiving digoxin for congestive heart failure or arrhythmias, and Rihal shows an increased risk for CIN in patients following acute myocardial infarction and hypotension (53).

Volume depletion and dehydration, often the result of purging enemas and restricted food and fluid intake prior to the radiographic study, appear to be less important risk factors nowadays, as current practice encourages volume expansion prior to most contrast requiring procedures. Nevertheless, effective volume depletion may still exist during contrast studies despite fluid administration in patients with poor cardiac output, cirrhosis or hypoalbuminuria, or in critically ill patients with sepsis syndrome or shock.

Whether diabetes mellitus per se predisposes to radiocontrast-induced ARF was for years a matter of debate. The incidence of CIN in diabetic patients rises sharply if the baseline serum creatinine is elevated. A serum creatinine >1.5 mg/dL increases by approximately 50% the likelihood of developing ARF (24,46). Harkonen and Kjellstrand (24) report that 22 of 29 (76%) of all diabetic patients with a serum creatinine >2 mg/dL developed ARF following intravenous pyelography. Diabetic patients with a baseline serum creatinine >5 mg/dL and those developing diabetes before the age of 40 years had an even greater risk of CIN. In 56% of these latter patients, renal failure was irreversible. The experience of 13 patients with type I diabetes with advanced nephropathy (mean serum creatinine of 6.8 mg/dL) undergoing coronary angiography was equally stark, with 12 of the 13 developing CIN (49). When renal insufficiency is advanced at the time CIN develops, it is often irreversible (49,58,79). The age of onset of diabetes also seems to affect the severity and likelihood of developing CIN. It has been suggested that Type II diabetic patients are less likely to develop CIN than type I diabetic patients (24,80). Nonetheless, the risk of developing CIN in type II diabetes is not trivial. Shieh and others (80) report a 6% incidence of ARF after excretory urography in 49 type II diabetic patients who, as a group, had only minor renal impairment (mean baseline serum creatinine of 1.3 mg/dL and mean creatinine clearance of 79.6 ml/minute). Several reports suggest that diabetes in the absence of renal insufficiency does not predispose to CIN (11,22,26,32,35). By contrast, Rihal, reports that diabetes significantly predisposes to CIN after coronary intervention when baseline creatinine is below 2 mg/dL, but not above it (53). Other studies using sensitive measures to detect toxicity or using multivariate analysis of risk factors also suggest that diabetic patients with normal renal function are at increased risk for CIN (51,52). In summary, it seems most prudent to view the presence of diabetes as a synergistic factor in causing radiocontrast-induced ARF, although renal function may appear normal when insensitive measures such as the serum creatinine are used. This is well illustrated in McCullough's large cohort of patients undergoing coronary interventions (75): among patients with preexisting renal failure, the risk of CIN was doubled in diabetic patients at any given baseline renal function (Fig. 45-4).

Multiple myeloma historically has been singled out as a high-risk disease for the development of CIN (62–64). A central event in the pathogenesis of myeloma-induced renal failure is the co-precipitation of Bence-Jones proteins with Tamm-Horsfall protein in the renal tubule (81,82). However, an analysis of 476 patients with myeloma exposed to radiocontrast revealed an incidence of ARF of 0.6% to 1.25% (65). This low value is similar to the reported incidence of CIN found in patients who served as controls for radiocontrast-administered subjects (11) or in well hydrated low-risk population (18). The early reports of ARF following contrast exposure in myeloma patients may have underestimated the role of comorbid factors such as sepsis, hypercalcemia, and volume depletion. Nevertheless, it seems prudent in patients with unexplained renal disease undergoing contrast studies, particularly older patients, to screen the urine for Bence-Jones proteins with *p*-toluene sulfonic acid (TSA) or, if unavailable, the dipstick and sulfosalicylic acid (SSA) test (83). A positive TSA test, or a positive SSA test (which checks for all urinary proteins) with a negative dipstick (albumin), may be considered presumptive evidence of Bence Jones proteinuria, and radiocontrast studies should then be undertaken only with caution if no alternative imaging procedure is suitable.

As discussed below (under Prevention of CIN), the type of radiocontrast should be considered a renal risk factor, when the use of high-osmolar agents is to be considered. It is now clear that low-osmolar contrast media are significantly safer, as compared with the old generation high-osmolar agents, with an estimated 50% risk reduction for CIN (26,30). By contrast, the use of the more recently developed isoosmolar contrast materials is probably not advantageous over low-osmolar agents, regarding the risk of nephrotoxicity (42,43).

The importance of the volume of radiocontrast in the etiology of ARF was also debated for a while, with some studies showing a positive relationship (45,53,74,77,78) and others showing no relationship (22,24,84). Miller and colleagues (84) have studied prospectively 200 patients requiring intravenous or intraarterial contrast material and found no consistent change in renal function with increasing doses of contrast material. However, Cigarroa and associates (21) showed that careful limitation of the volume of contrast material used during cardiac catheterization could reduce the incidence of nephropathy (see Prevention of CIN, below). Recent studies, using much larger volumes of radiocontrast material for complicated angiographyic interventions, settled this debate, clearly defining contrast dose as an independent risk factor for CIN (19).

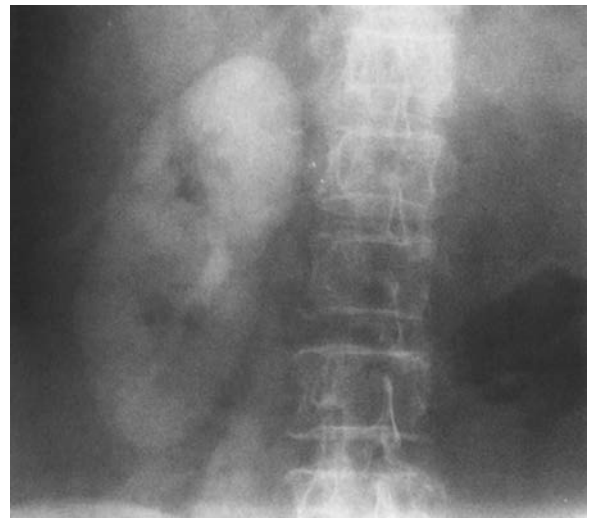

FIGURE 45-5. Persistent nephrogram in solitary kidney in a 73-year-old woman 8 hours following excretory urography performed to evaluate hematuria. The contralateral kidney had been removed previously because of nephrolithiasis. Baseline serum creatinine was 1.6 mg/dL. (Photograph courtesy of Dr. Thomas Curry.)

Clinical Presentation of CIN

CIN, manifested as an acute decline in renal function, has been reported following virtually every radiographic procedure using intravascular injection of iodinated radiocontrast. Renal toxicity appears to be favored in the presence of the risk factors outlined above. Nonoliguric CIN is far more common than the oliguric form, and is generally more common in patients initially having a lower serum creatinine prior to receiving the contrast. In oliguric ARF, the time course of the oliguria and the rise in serum creatinine depend on the baseline serum creatinine prior to receiving the contrast agent. Patients with normal or mild renal functional impairment prior to receiving radiocontrast agents usually have oliguria lasting 2 to 5 days, with recovery to baseline urine volumes and serum creatinine by day 7. When more serious impairment of underlying renal function is present, recovery is generally prolonged, and up to 30% of patients may end up with various degrees of residual renal impairment (85). Renal failure may even be irreversible, requiring long-term hemodialysis (22,23,41,45,49,51,77,78,86,87). As with ARF of other etiologies, protracted CIN is associated with a prolonged hospitalization course (88) and over fivefold increase in mortality (89).

Diagnosis of CIN

The most easily diagnosed case of CIN is that in which oliguria develops within 24 to 48 hours following a radiocontrast study. More often, a subtle, nonoliguric episode of ARF is diagnosed in retrospect by demonstrating a reversible 0.5 to 3.0 mg/dL rise in serum creatinine. In most situations, the serum creatinine remains the most practical test for detecting the presence of CIN. However, differential diagnoses should be taken into account for this indication of declining glomerular filtration, including pre- and postrenal causes, comorbidities such as sepsis syndrome or advanced liver failure, exposure to other nephrotoxins, or cholesterol emboli in the case of angiographic studies performed through the aorta.

One feature that characterizes many patients with CIN is the paradoxical and, as yet, poorly explained propensity for the urinary sodium concentration and the fractional excretion of sodium to be low (28,46,47,90). Fang and colleagues (47) report 12 patients with radiocontrast-induced ARF who had a low fractional excretion of sodium (mean 0.36%), which persisted for up to 5 days during the oliguric phase. Reduced renal perfusion and acute tubular obstruction have been offered as explanations for this phenomenon (47).

The urinalysis may occasionally show features of ATN (renal tubular cellular casts, muddy brown or coarsely granular casts), but these may often be absent (22,49). Conversely, in the absence of a rise in serum creatinine, radiocontrast agents may still alter the urinary sediment. Gelman and associates (91) demonstrate more formed elements (cells, casts, and debris) in the urine of 12 of 14 patients following angiography, although none of the patients experienced a reduction in GFR. All patients showed amorphous urate crystals, and two patients had a heavy shower of calcium oxalate crystals.

A persistent nephrogram 24 to 48 hours after the contrast study is a characteristic but not pathognomonic feature of CIN (20,22,92) (Fig. 45-5). In a healthy subject given a bolus of radiocontrast material, renal opacification is the densest immediately after the end of the injection and then fades rapidly, while it appears in the urinary collecting system. A very little nephrogram effect remains detectable at 6 hours (93). In most patients with radiocontrast-induced ARF as well as in patients with other forms of ARF, the nephrogram develops quickly but fails to disappear with time. A persistent nephrogram is a sensitive indicator of the presence of renal failure (83% of patients with renal failure had a positive nephrogram) with high specificity (93% of patients without renal failure lacked the persistent nephrogram) (22). The likelihood ratio of ARF being present, then, following a positive result is equal to 12 (sensitivity/[100 – specificity]). In other words, a positive nephrogram increases by 12 times the likelihood that ARF has occurred over baseline incidence (i.e., if it was 1.5%, it is now about 18%). The likelihood ratio following a negative test is equal to 0.18 ([100 – sensitivity]/specificity), or about one-fifth. Thus, the absence of a nephrogram decreases the likelihood of contrast nephropathy to one-fifth from baseline incidence (e.g., if it was 1.5%, it is now about 0.3%).

Detection of urinary biomarkers, released from tubular cells, such as gamma-glutamyltranspeptidase, alanine aminopeptidase, alkaline phosphatase, or *N*-acetyl-beta-glucosaminidase (NAG) has also been suggested in the diagnosis of CIN (94), but current technologies are considered to suffer from large basal variations and low specificity of increased

urinary enzymes. For instance, increased urine volume per se may enhance enzymuria (95). However, protracted enzymuria five days after the exposure to the radiocontrast agent may be more indicative for tubular injury (96). Another limiting factor for the usefulness of urinary biomarkers is the selective or predominance of their expression by specific segments of the nephron, as with kidney injury molecule (KIM)-1, that serves as a sensitive marker of proximal tubular damage (97).

Human Pathology of CIN

The reportedly characteristic renal lesion with CIN is an intense vacuolization of proximal tubular cells, often called osmotic nephrosis (98,99). A review of 211 renal biopsies obtained within 10 days of urography or renal arteriography revealed osmotic nephrosis in 47; a diffuse form was found in patients with severe preexisting renal disease, while a milder focal form was seen in patients with less severe renal impairment or in patients with previously normal kidneys. However, the presence of the focal or even the diffuse form did not necessarily predict the presence of renal functional impairment. Conversely, virtually normal proximal tubular cells were found in patients who developed oligoanuric ARF after urography (99). Hyperosmolality or high ionic medium does not seem to be required for the development of these lesions because the newer low- and iso-osmotic contrast media are capable of inducing it as well (100–102). Notably, in 13 patients with histologically normal kidneys, vacuoles were not found in any of the tubular cells, implying that an underlying nephropathy was required to induce this histologic lesion. Iodine cannot be demonstrated in these vacuoles (103,104), which develop rapidly within 5 to 15 minutes under experimental settings (102,103). Heyman and co-workers (103) suggest that the origin of the vacuoles is not from endocytosis but from invagination of membranes of lateral cellular interdigitations. They suggest that the contrast media in the paracellular space may have damaged these membranes, leading to the vesicular outpouchings. Ultrastructural histochemistry, however, reveals that these vacuoles contain acid phosphatase activity (102), suggesting merging with lysosomes. Noteworthy, KIM-1, a marker of proximal tubular damage, was not found in the urine of patients with CIN (97), supporting the concept that proximal tubular vacuolization not necessarily reflects tubular cell injury. In summary, most human and animal studies indicate that proximal tubular vacuolization appears after radiocontrast exposure irrespective to changes in kidney function. In that perspective, and given the very limited (cortical) sampling of human material, that is often obtained at a very late phase of established ATN, we believe that proximal tubular vacuolization should serve as marker of radiocontrast exposure, rather than an indicator of CIN (105).

PATHOGENESIS OF CIN

Overview

Though recognized for some 40 years, the pathophysiology of CIN remains an issue of controversy. It is classified as "acute tubular necrosis" (ATN), or "toxic nephropathy," but, in fact, very little is known about its true nature and morphologic characteristics. Indeed, the low fractional excretion of sodium noted in many cases of CIN is not a typical finding in ATN. As detailed above, cortical tubular vacuolization, also present after radiocontrast administration under experimental settings, should better be regarded as a marker for contrast medium exposure, rather than an indicator of tubular damage. Unfortunately, human morphology of deeper kidney structures is lacking, and urinary biomarkers of tubular injury are of limited value.

Typical toxic ATN, such as *cis*-platinum or gentamicin nephropathies are believed to be mediated primarily through well-characterized direct tubular-cell toxicity, as also documented with heavy metals in experimental and clinical settings. Renal handling of the toxin predominantly determines the distribution pattern of tubular damage. The injury is dose-dependent, and the contribution of perturbations is relatively marginal, with the exception of the hydration state (106). On the other hand, data regarding direct tubular toxicity of radiologic contrast agents is quite limited, and is predominantly based on *in vitro* prolonged incubation of tubular cells or tubular segments with iodinated dye. Contrast agents, once filtered through the glomerulus, are not reabsorbed or metabolized by tubular cells (perhaps with the exception of traces of nonionic agents) (6). Furthermore, although the dose of radiocontrast is considered proportional to the risk for renal dysfunction, CIN is rarely encountered in the absence of predisposing factors. These data suggest that factors other than direct tubular toxicity are predominantly responsible for CIN.

The current knowledge regarding the pathophysiology of CIN predominantly stems from animal studies that examined the effect of radiocontrast media upon renal function, hemodynamics and oxygenation. In complementary studies, the impact of contrast agents upon various mediators that could govern these changes was examined, and ultimately, adequate animal models of CIN were developed. Together with *in vitro* research and experiments in kidneys perfused *ex vivo*, these studies have led to the recognition that CIN represents the outcome of a unique combination of interactive pathogenic processes, including regional endothelial dysfunction, renal tissue hypoxia, and subsequent oxygen free radical-induced cytotoxicity (Table 45-3, Figure 45-6).

In this section we shall describe the intensification of the outer medullary hypoxemia that follows the administration of iodinated radiocontrast agents, review the various components believed to be involved in this process and their potential role in CIN, analyze related cytotoxic processes, and appraise the lessons learned from the development of animal models of CIN.

Physiologic Medullary Hypoxia

While oxygen is abundant in the renal cortex, the medulla is poorly oxygenated, working normally on the verge of anoxia. Medullary partial pressure of oxygen (PO_2) as low as 30 mm Hg has been detected with oxygen microelectrodes under normal physiologic conditions in rats, dogs, and humans, reflecting relatively low regional blood flow and countercurrent oxygen diffusion from decending to ascending vasa recta. The limited medullary oxygen delivery of some 8 mL/minute/100 g tissue is hardly sufficient for the metabolic needs for tubular transport, predominantly carried out in the outer medulla by medullary thick ascending limbs (mTALs) and S3 (straight) segments of the proximal tubule. Consequently, oxygen extraction by the renal medulla is near-maximal, reaching 79% of regional oxygen supply, leaving a very marginal oxygen reserve (107,108). This critical medullary oxygen balance is manifested by high levels of cytochrome AA3 in its redox state, and by the high levels of unsaturated hemoglobin, detected within the renal medulla by blood-oxygen-level dependent (BOLD) magnetic resonance imaging (MRI) (109,110). The kidney appears to have designed regulatory mechanisms to allow urinary concentration without medullary hypoxic injury. Optimal urinary concentration requires a perfect match between oxygen supply and demand, an effect achieved through a precise regulation of blood flow and tubular work in the outer medulla. Distal tubular reabsorption is affected by the control of GFR and

TABLE 45-3

CONTRAST-INDUCED PHYSIOLOGIC CHANGES

Extrarenal
- Decreased systemic oxygenation
  - Altered pulmonary function
  - Leftward shift of oxygen-hemoglobin dissociation curve
- Hemodynamic changes leading to transient renal hypoperfusion
- Rheologic alterations of the blood

Renal: physiologic changes
- Diuresis
- Altered GFR: biphasic response ↑→↓
- Intrarenal blood viscosity ↑
- Urine viscosity ↑
- Renal volume and interstitial pressure ↑
- Altered renal circulation
  - Cortex: biphasic response ↑→↓
  - Outer medulla: ↑↑↑
  - Papilla: ↓↓↓
- Reduced renal oxygenation (cortex ↓; medulla ↓↓↓)
  - Oxygen delivery ↓
  - Oxygen consumption ↑
- Uric acid and oxalate excretion ↑
- Enzymuria

Renal: altered mediators regulating renal hemodynamics and function
- Adenosine ↑
- Nitric oxide: ↓ in cortex; ↑ in outer medulla
- Prostaglandins: PGE_2 ↑, PGI2 ↑ or ↓
- Atrial natriuretic peptide ↑
- Endothelin ↑
- Vasopressin ↑
- Histamine ↑
- Reactive oxygen species ↑

Renal: additional major changes at cellular level
- Free radical species ↑
- Hypoxia-inducible factors (HIF) and related stress-response genes ↑
- Poly-(ADP-ribose) polymerase (PARPP) ↑ (probably)

proximal tubular reabsorption (both governing solute delivery to the distal nephron) and by the regulation of distal tubular reabsorption. Locally produced prostaglandins and nitric oxide (NO), and the generation of adenosine from the breakdown of adenosine triphosphate (ATP) are major participants in the regulation of medullary oxygenation. All three induce medullary vasodilation and directly inhibit tubular reabsorptive activity. Adenosine can further improve medullary oxygenation by the induction of cortical vasoconstriction with the reduction of GFR, and subsequently diminished solute delivery for reabsorption by the distal nephron (107,108). The location were the mediator is released and the distribution and density of its receptors are important in maintaining medullary oxygenation. For instance, both the synthesis and the density of receptors for prostaglandine E_2 (PGE_2) are abundent in the outer medulla. The effect of various mediators upon cortical and medullary microcirculation may be diverse, depending on receptor types. Adenosine exerts cortical vasoconstriction through adenosine-A_1 receptors, while it induces medullary vasodilation through adenosine-A_2 receptors (111). Comparably, endothelin-1 exerts cortical vasoconstriction through ET_A receptors, but enhances medullary blood flow activating ET_B receptors (112). Angiotensin II also selectively induces cortical vasoconstriction, while medullary flow is maintained (113). Thus, in the normal intact kidney medullary blood flow is usually maintained, even during systemic and local vasoconstrictive stimuli, by the combined effects of locally produced vasodilators (NO, PGE_2) and a unique regional vasodilatory effect of renal vasoconstrictors. Corticomedullary redistribution of blood flow and the activation of the tubuloglomerular feedback mechanism may, therefore, be regarded as measures designed to maintain medullary oxygenation and prevent tubular hypoxic damage. Most risk factors for CIN are characterized by defective nitrovasodilation or prostaglandin synthesis (see below), major protectors of medullary oxygenation. Indeed, renal medullary PO_2 markedly declines during experimental inactivation of these mechanisms (114,115).

Ample experimental data indicate that enhancement of distal tubular reabsorptive workload, unmatched by adequate oxygen supply, or altered regional blood flow, may lead to ATN with a rather selective outer medullary hypoxic damage, predominantly affecting mTALs, and to a lesser extent S3 segments. Papillary injury may develop as well. The reader is referred to detailed reviews of the role medullary hypoxic injury plays in the evolution of ATN and in chronic renal parenchymal disease (107,108).

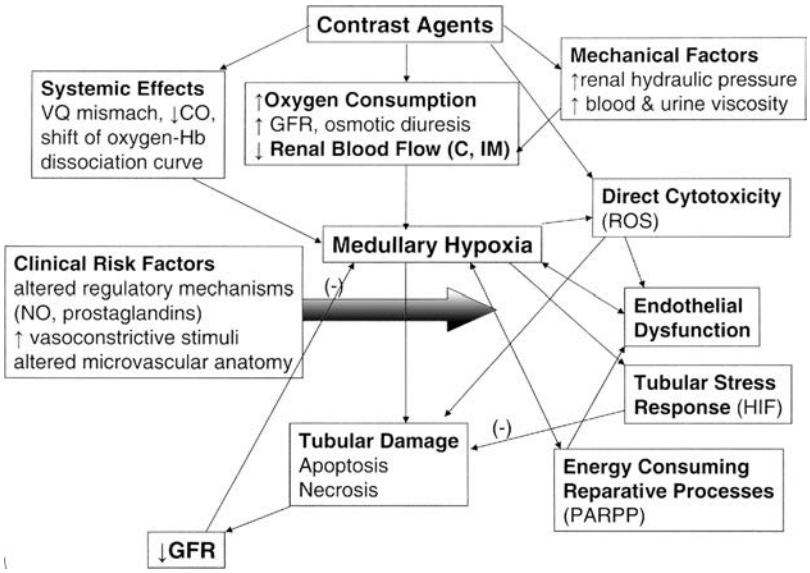

FIGURE 45-6. Schematic algorithm illustrating putative hypoxic and toxic mechanisms involved in the pathophysiology of CIN, and their interrelations.

Aggravation of Medullary Hypoxia by Radiocontrast Agents

Using Clark-type oxygen microelectrodes, Brezis found that injection of radiocontrast markedly affects renal parenchymal oxygenation (113). Following the administration of the high-osmolar ionic agent sodium iothalamate, cortical PO_2 declined from 40 to 25 mm Hg. More impressive was the change observed in outer medullary PO_2 which fell from 26 mm Hg at baseline to mean levels as low as 9 mm Hg (Figure 45-7). Comparable studies with oxygen microelectrodes were repeated by Liss and colleagues, showing a fall in medullary oxygenation from about 30 mm Hg to 15 mm Hg after the administration of ionic, as well as nonionic, low-osmolar and isoosmolar contrast agents (116). Radiocontrast-induced intensification of medullary hypoxemia has also been suggested by non-invasive BOLD MRI (110), which detects increased unsaturated hemoglobin concentration within the renal medulla, and by the detection of hypoxia inducible factors (HIF) shortly after contrast administration (117).

Systemic effects of the radiocontrast medium may contribute to the decline in renal tissue oxygenation, including the induction of pulmonary ventilation-perfusion mismatch (118), reduced cardiac output and renal perfusion pressure (119), rheologic alterations of the blood (120,121), and a leftward shift of the oxygen-hemoglobin dissociation curve (122). Systemic hypoxemia is less likely to occur with the use of nonionic agents (123), yet it may aggravate regional ischemic injury (124). However, the greater part of the decline in renal parenchymal oxygenation is attributed to altered intrarenal balance of oxygen supply and demand.

Radiocontrast-Induced Change in Oxygen Demand

Administration of radiocontrast media induces an abrupt transient increase in glomerular filtration, and urinary output (125). This response, comparable to the osmotic diuretic effect of mannitol, occurs despite a rise in vasopressin (126,127), and is mediated in part by an increase in plasma volume and the release of natriuretic peptides (128,129). Natriuresis and diuresis may also be related to an ET_B- mediated effect of endothelin (130), released in response to radiocontrast injection (128,131). All these factors, perhaps in addition to the substantial osmotic load provided by many contrast media, lead to enhanced solute delivery to the distal nephron, with subsequent increased oxygen consumption for tubular reabsorption. The decline in outer medullary PO_2 despite enhanced regional blood flow (see below) only emphasizes the important role for increased reabsorptive activity in the ensuing regional hypoxia. Indeed, the inhibition of tubular transport with the loop diuretic furosemide abruptly reverses radiocontrast-induced medullary hypoxemia (114,132). The improvement of medullary hypoxemia with furosemide takes place even though it induces profound regional vasoconstriction (133), further emphasizing the central role of regulated tubular transport in the maintenance of medullary oxygen balance.

Radiocontrast-Induced Intrarenal Changes in Oxygen Supply

The decline in renal parenchymal oxygenation may also reflect radiocontrast-induced altered renal microcirculation. Indeed, it has been known for over 30 years that renal blood flow briefly and transiently increases following radiocontrast injection, with a prolonged subsequent decline to about 25% below baseline (134). This effect occurs whether the radiocontrast is administered intravenously or intra-arterially and is an intrinsic response of the kidney because it can be reproduced in isolated perfused kidneys (103). Weisberg and colleagues directly measured renal blood flow during cardiac catheterization in patients with chronic renal failure and found no association between reduced renal blood flow and the development of CIN (135). However, alterations in renal blood flow during contrast injection, predominantly reflecting changes in cortical flow, conceal very important changes in medullary flow, that constitute of only 10% of total renal perfusion. Thus, changes in renal blood flow cannot predict alterations in medullary oxygen supply (107,108). Moreover, medullary flow is usually preserved during a moderate decline in renal blood flow (within the "autoregulatory" range), despite a remarkable fall in cortical blood flow (136), a phenomenon called "corticomedullary redistribution of blood flow." Furthermore, a fall in cortical blood flow alone is expected to increase medullary oxygenation, the outcome of diminished glomerular filtration rate (GFR) and solute delivery for reabsorption by the distal nephron (136).

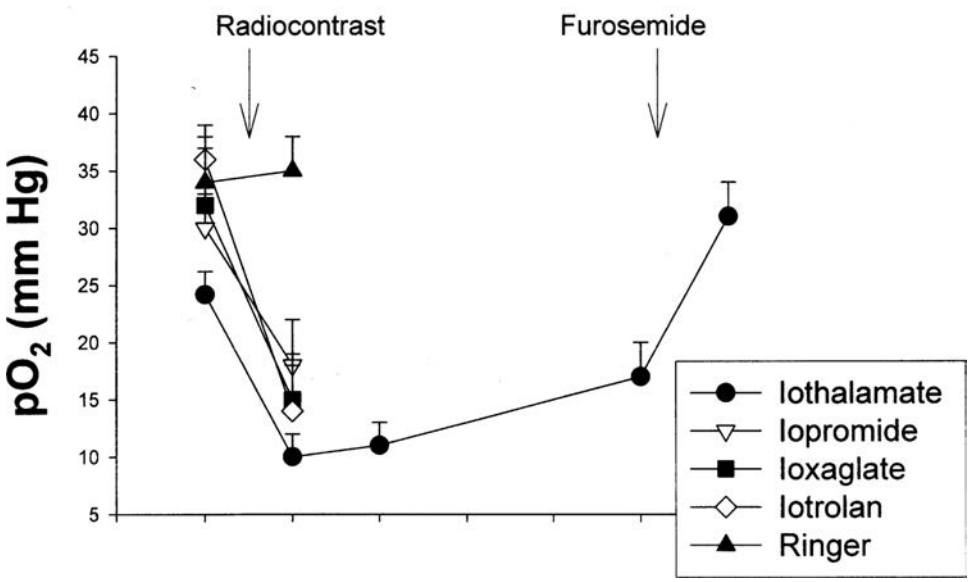

FIGURE 45-7. Changes in outer medullary partial pressure of oxygen (PO_2) following the administration of high-osmolar ionic agent (iothalamate), low-osmolar nonionic (iopromide), low-osmolar ionic (ioxaglate) and isoosmolar nonionic contrast medium (iotrolan), compared with Ringer's solution. Also illustrated is the reversal of iothalamate-induced decline in medullary oxygenation with furosemide. (Modified from: Heyman SN, et al. Early renal medullary hypoxic injury from radiocontrast and indomethacin. *Kidney Int* 1991;40:632, and from: Liss P, et al. Injection of low and isoosmolar contrast medium decreases oxygen tension in the renal medulla. *Kidney Int* 1998;53:698).

The possibility that radiocontrast-related medullary hypoxia reflects altered medullary microvasculature has therefore been explored by direct determination of the local microcirculation. Nygren (137) and subsequently Liss and colleagues (138) recorded papillary blood flow with laser-Doppler probes after the exposure of the papilla by the dissection of the renal pelvis. Indeed, they found that ionic high-osmolar, as well as nonionic and low-osmolar radiologic contrast media markedly reduced papillary blood flow. Using video microscopy of trans-illuminated papillary vasa recta and dual-window cross-correlation technique, they also documented near cessation of red blood cell movement in papillary blood vessels, associated with red cell aggregation (138).

At the outer medulla, however, microcirculatory response to radiocontrast was found to be quite different. Using needle laser-Doppler probes inserted through the cortex following partial renal decapsulation, Agmon found that outer medullary regional microcirculation markedly increases after the injection of the high-osmolar agent iothalamate, as long as NO- or prostaglandin-synthesis are intact (139). Increased outer medullary flow in response to radiocontrast was also reported by Heyman (140) and by Palm (141), using the nonionic low-osmolar iopromide, while Liss found a dose-related response, with a decline in regional flow at low and intermediate volumes of contrast, but enhancement at high volumes (142).

Altogether, these findings indicate that radiocontrast-induced accentuation of inner medullary hypoxia is mediated to large extent by a decline in regional blood flow and oxygen supply. By contrast, intensification of outer medullary hypoxia predominantly represents enhanced oxygen consumption, not fully compensated by increased regional oxygen delivery. The cause for the disparate papillary and outer medullary microcirculatory response to radiocontrast agents is unknown, but it may reflect structural and functional differences between regional pericytes (143), or diverse distribution of vasoactive mediators or their receptors. An additional artificial effect related to the technical procedures (i.e., papillary exposure and removal of the renal capsule, respectively), cannot be excluded with certainty.

Radiocontrast-Triggered Neurohumoral Responses and Their Potential Role in CIN

Numerous mediators are involved in the changes in renal microcirculation, associated with radiocontrast injection, some inducing renal vasoconstriction (vasopressin, histamine), others exerting renal vasodilation (nitric oxide [NO], natriuretic peptides, prostaglandins [PGE_2, PGI_2]), and others producing varying responses at different renal vascular beds (endothelin, adenosine).

The role of the renal sympathetic system is yet to be defined, though radiocontrast-induced constriction of innervated renal artery is not mediated through alpha receptors (144). Vasopressin levels rise after radiocontrast injection in response to the increase in plasma osmolality, and probably due to contrast-induced hypotension (126,127). Endothelin synthesis and release from endothelial cells is triggered by high- and low-osmolar ionic and nonionic agents, both *in vitro* and *in vivo* (128). This response is dose-related, and is not mediated through hypertonicity. Plasma and urinary endothelin increases in humans following radiocontrast studies (131). In a rat model of CIN, the low-osmolar agent ioversol stimulated endothelin remarkably less than iothalamate, and was less nephrotoxic, suggesting a role for endothelin in CIN (145). The renal microcirculatory response to endothelin-1 closely resembles radiocontrast-induced changes in renal hemodynamics (112). Total renal and cortical flows decline, an effect blocked by endothelin-ET_A antagonist (146), and outer medullary flow increases, an effect triggered by ET_B-mediated release of NO (112).

Intrarenal adenosine also rises following the administration of radiocontrast media (125,147), reflecting ensuing renal hypoxia and/or increased tubular workload, with consequent breakdown of ATP. Like endothelin, and in resemblance to renal hemodynamic response to radiocontrast agents, released adenosine exerts dual effects on the renal vasculature, with A1-mediated cortical vasoconstriction and A2-dependent outer medullary vasodilation (111), with an overall decline in total renal blood flow and GFR (111,148). The administration of nonselective- or selective adenosine A1-receptor blockers did not attenuate the contrast-associated decline in renal blood flow and GFR in healthy rats (149), suggesting that under normal physiologic conditions renal hypoxia leads to adenosine release, rather than the other way around. By contrast, severe renal hypoxia induced by chronic inhibition of NO synthase increases renal vascular sensitivity to the radiocontrast-induced adenosine release, generating renal vasoconstriction and reduced GFR, both ameliorated with adenosine receptor antagonists (149). In the same fashion, theophylline blocks the decline in renal blood flow in volume-depleted anesthetized dogs (148). Adenosine breakdown generates oxygen free radicals that may lead to cytotoxic epithelial and endothelial injury (see below). Iodinated contrast agents also trigger the release of histamine that may also participate in the decline in renal blood flow (150).

Contrast media also alter renal vasodilatory mechanisms. Plasma levels of atrial natriuretic peptide (ANP) rapidly rise after the injection of the contrast material, in parallel with the abrupt transient rise in renal blood flow and diuresis (128). Intrarenal NO concentration is modified as well (140). Cortical NO declines, in parallel with the gradual fall in cortical blood flow. Cortical NO-synthase activity also tends to fall, and markedly declines in response to nonselective endothelin-receptor blockade, illustrating the cross-talk between these mediators. By contrast, medullary NO-synthase activity is unaffected by the contrast medium, though NO tracings with a selective electrode markedly increase, in parallel with the rise in regional flow (140). This might be explained by the evolving decline in regional PO_2 and extended local bioavailability of NO (151). Iodinated radiocontrast agents also affect vasoactive cyclooxygenase metabolites. Urinary PGE_2 increases sevenfold in the rat (146), indicating substantial upregulation of this important medullary vasodilator in response to ensuing hypoxia. The effect of radiocontrast agents on prostacyclin (PGI_2) production by endothelial cells is debated, with one report showing declining levels of 6-keto-PGF1-alpha in renal veins in dogs (134), and another study demonstrating rising plasma levels in humans with ionic as well as nonionic agents (152).

Intact nitrovasodilation and prostaglandin synthesis are essential for the maintenance of adequate medullary oxygenation during contrast administration. As shown in Figure 45-8, the outer medullary vasodilatory response to radiocontrast is blocked by the inhibition of each of these systems, and is replaced by intense vasoconstriction. This leads to a critical reduction in ambient PO_2 to levels as low as 8 mm Hg (115,139) and increases medullary hypoxia-induced factor (HIF) expression (117).

Mechanical Factors

Blood viscosity may be substantially influenced by contrast media (153,154) and may contribute to the reduction in papillary blood flow, noted by Liss (138). This may be related to the intrinsic physical properties of the dye (Table 45-1), or

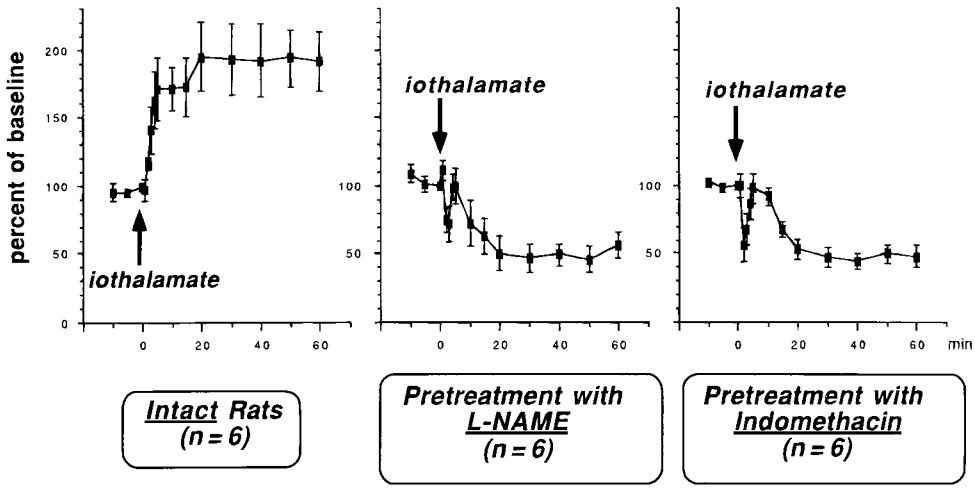

FIGURE 45-8. Reversal of outer medullary hyperemic response to radiocontrast administration after inhibition of nitric oxide or prostaglandin synthesis. (From: Agmon Y, et al. Nitric oxide and prostanoids protect the renal outer medulla from radiocontrast toxicity in the rat. *J Clin Invest* 1994;94: 1069, with permission.)

due to erythrocyte rigidity, endothelial injury or microthrombi (121,155,156). Additionally, the early radiocontrast-induced enhanced diuresis is associated with swelling of the renal parenchyma, presumably due to tubular luminal expansion and increased interstitial volume (157). It is conceivable that this could increase renal interstitial pressure with subsequent compression of the vasa recta and peritubular capillaries, compromising regional oxygenation. Tubular intraluminal urine viscosity also increases following radiocontrast administration, particularly at the distal nephron, as the result of water reabsorption with increasing concentration of the contrast medium (158). Rising urine viscosity, noted with high- and low-osmolar agents is markedly more pronounced with the new isoosmolar agents (154), reflecting their physical properties (Table 45-1). This could contribute to the increased intraluminal hydraulic pressure that might directly diminish GFR. Radiocontrast agents may also induce intratubular obstruction by precipitation of uric acid (76,91) or oxalate crystals, or co-precipitate with Tamm-Horsfall protein, synthesized and released by medullary thick ascending limbs following radiocontrast exposure (159). Radiocontrast agents may also bind with Bence-Jones protein in patients with multiple myeloma (160,161).

Toxic-Hypoxic Interactions and Endothelial Dysfunction

Increased urine viscosity and cast formation by cell debris and precipitation of contrast medium with urinary proteins could lead to prolonged exposure of tubular cells to the dye, as evidently happens during the persistent nephrogram phenomenon (20,62), increasing the risk for a direct cytotoxic effects on tubular cells. Indeed, *in vitro* studies show independent direct tubular damage caused by radiocontrast agents (162), with tubular membranal oxidative injury (163,164). *In vivo* studies also confirm the existence of oxygen free radical-mediated membranal damage (159,164–166), though this could indirectly result from reperfusion of critically hypoxic regions. Reactive oxygen species may cause DNA damage, activating high-energy consuming reparative processes such as the DNA mending poly-(ADP-ribose) polymerase (PARP). The induction of PARP may, in turn, initiate a vicious circle of additional intracellular energy store depletion and tubular damage (167,168).

Most importantly, endothelial cells may also be injured by the evolving hypoxic stress. Indeed, accumulation of HIF-2α can be detected in medullary endothelial capillaries following exposure to contrast media (117). Subsequent endothelial damage, induced by reactive oxygen species (149,169) and energy consuming reparative mechanisms, such as PARP, may lead to endothelial dysfunction, which may further aggravate regional oxygen insufficiency (164,170). Finally, contrast studies are often carried out in critically ill patients, treated with other nephrotoxins or displaying co-morbid states such as sepsis or myohemoglobinuria that may exert additional tubulotoxic and hypoxic insults.

Thus, synergism exists between hypoxic, hemodynamic, toxic, and obstructive components of CIN. Renal dysfunction with minimal or absent tubular damage, noted in some of the animal models detailed below, implies that reversible cell injury has been restored, or that renal microvascular response predominates. Altered glomerular hemodynamics, mediated by tubuloglomerular feedback, helps in restoring medullary oxygenation by decreasing reabsorbtive workload (108). Yet, it is manifested as diminished GFR with a low fractional sodium excretion.

Risk Factors for CIN: Predisposition to Medullary Oxygen Insufficiency

As detailed above, CIN seldom develops among patients without risk factors. This highlights the value of protective mechanisms, designed to maintain medullary oxygen sufficiency, including vasodilating prostaglandins, NO, and adenosine. By adjustment of local transport activity to the limited available oxygen supply, these mechanisms enhance outer medullary blood flow and suppress tubular reabsorptive activity. Their inactivation in rats reverses the increase in outer medullary blood flow induced by radiocontrast, and aggravates regional hypoxia (115,139).

Dysregulation of medullary oxygen balance is also encountered in most clinical circumstances considered to predispose to CIN (105). As shown in Table 45-4, most risk factors for contrast nephropathy are characterized by structural or functional changes that can aggravate hypoxic stress during contrast administration. Diabetes, hypertension, aging, atherosclerosis, and hyperlipidemia are all characterized by defective nitrovasodilation, while defective prostaglandin synthesis is encountered in aging and in patients receiving nonsteroidal anti-inflammatory agents. Indeed, intensified medullary hypoxia has been encountered is some of these conditions (114,171–173), associated with regional HIF expression (173,174),

TABLE 45-4

RISK FACTORS FOR CIN PREDISPOSE TO MEDULLARY OXYGEN INSUFFICIENCY

Defective protective mechanisms
Altered nitrovasodilation
Diabetes, hypertension, aging, hyperlipidemia, atherosclerosis
Altered renal prostaglandin synthesis
Aging, NSAIDs
Enhanced systemic vasoconstrictive stimuli
Volume depletion, heart failure, cirrhosis, nephrosis
Increased reabsorptive workload
Diabetes, chronic renal disease
Structural changes of the renal microvasculature
Chronic renal disease
Blood hyperviscosity
Myeloma

and a defective medullary vasodilatory response (169,175). Consequently, the increase in outer medullary blood flow in response to radiocontrast is lost and even reversed, as shown by Palm in diabetic rats (141). Arginine depletion in these clinical conditions may also contribute to endothelial dysfunction (74). As reviewed elsewhere (107,108), enhanced systemic vasoconstrictive stimuli accompany effective arterial volume depletion, for instance in patients with heart failure, cirrhosis, hypovolemic shock, dehydration, or hypoalbuminemia. Preexisting renal parenchymal disease is characterized by deformed medullary microcirculation, the result of structural parenchymal changes, and local oxygen insufficiency may be intensified by increased reabsorbtive workload in hypertrophic remnant nephrons. Enhanced tubular reabsorption and oxygen consumption may accompany early or uncontrolled diabetes, associated with increased GFR, osmotic diuresis, or upregulation of tubular transport (171). Myeloma proteins may increase blood viscosity and alter the medullary microcirculation. Exposure to additional nephrotoxins may intensify medullary hypoxia by the induction of renal vasoconstriction (cyclosporine), by the increase in metabolic workload (mannitol) or by their combination (amphothericin, hypercalcemia). Finally, rhabdomyolysis, urine outflow obstruction, and sepsis are among other renal perturbations characterized by altered renal microcirculation and predisposition to medullary hypoxic damage (107,108).

Experimental Models of CIN: The Concept of Combined Insults

In isolated rat kidneys, contrast agents hasten the decline in kidney function and extend hypoxic tubular damage, which selectively involves mTALs and S3 segments in the outer medulla (103). *In vivo*, resembling humans, normal animals subjected to contrast media do not develop CIN (176), so much so that the intrinsic nephrotoxicity of radiocontrast agents has even been questioned (177). They either retain kidney function, or die when administered extremely large volumes of the dye. As in humans, proximal tubular vacuolization is a hallmark of radiocontrast exposure, rather than an indicator of CIN (103,178). In intact rats, the radiocontrast-associated decline in medullary PO_2 invokes adaptive cellular hypoxic stress response, initiated by post-transcriptional medullary accumulation of HIF, with preservation of renal integrity and function (117). By contrast, the induction of other insults that

TABLE 45-5

ANIMAL MODELS OF CIN: INDUCTION OF PREDISPOSING FACTORS

Altered endothelial nitrovasodilation
Congestive heart failure (182)
Diabetes (256)
Hypertension (184)
Hypercholesterolemia (74)
Transient global renal ischemia (189,191)
Inhibition of nitric oxide synthase (115,139,149,186,227)
Altered prostaglandin synthesis
Aged animals (184)
Inhibition of cyclooxygenase (103,115,139,181)
Increased single nephron GFR
Preexisting renal disease: prior reduction of renal mass (103,185)
Acute-on-chronic renal failure (192)
Amino-acid infusion (185)
Diabetes (256)
Aged animals (184)
Vasoconstrictive stimuli
Congestive heart failure (182)
Salt depletion (103,148,181)
AII infusion (183)
Endothelin infusion (184)
Increased interstitial pressure
Urinary outflow obstruction (186)

mimic predisposing clinical conditions is a prerequisite for the development of CIN with tubular damage (106,179,180). As illustrated in Table 45-5, some perturbations were applied to generate vasoconstrictive stimuli, like volume depletion in rats and rabbits (163,181), heart failure in dogs (182), or the infusion of angiotensin II (183) or endothelin (184) in rats. Others were added to enhance oxygen requirements, by hypertrophy of single remnant kidney (103,185) or increased glomerular filtration (185). In additional models, renal medullary protective mechanisms were altered, such as the direct inhibition of prostaglandin- (103,115,139,181) or NO-synthesis (115,139,149,186). In others, endothelial dysfunction was reproduced by the induction of hypercholesterolemia (74) or with short- (187–190) or long-term ischemia (191). Ureteral obstruction served to enhance interstitial hydraulic pressure (186), while residual chronic damage produced by ischemia-reflow served to mimic preexisting tubulointerstitial disease (192). Unlike the consistantly high reproducibility of other models of toxic nephropathies, most of these experimental models produce a rather heterogenous response with a wide spectrum of renal dysfunction and tubular injury, in reminiscence with the inconsistent occurence and pattern of CIN in humans.

We found the models of radiocontrast administration following the combined inactivation of prostaglandin- and NO-production (139) most convenient and clinically relevant, with respect to the characteristics of most risk factors, displaying altered renal microvascular regulation. In these rat models, renal sodium gradient, an indicator of an intact countercurrent exchange system and urinary concentrating capacity, is disrupted shortly after the administration of the radiocontrast medium (193), representing medullary functional failure, and subsequently diminished glomerular filtration (103,115,139). The severity of renal dysfunction is proportional to the number of the applied coperturbations (Figure 45-9A), resembling observations in humans (36). Renal dysfunction correlates with

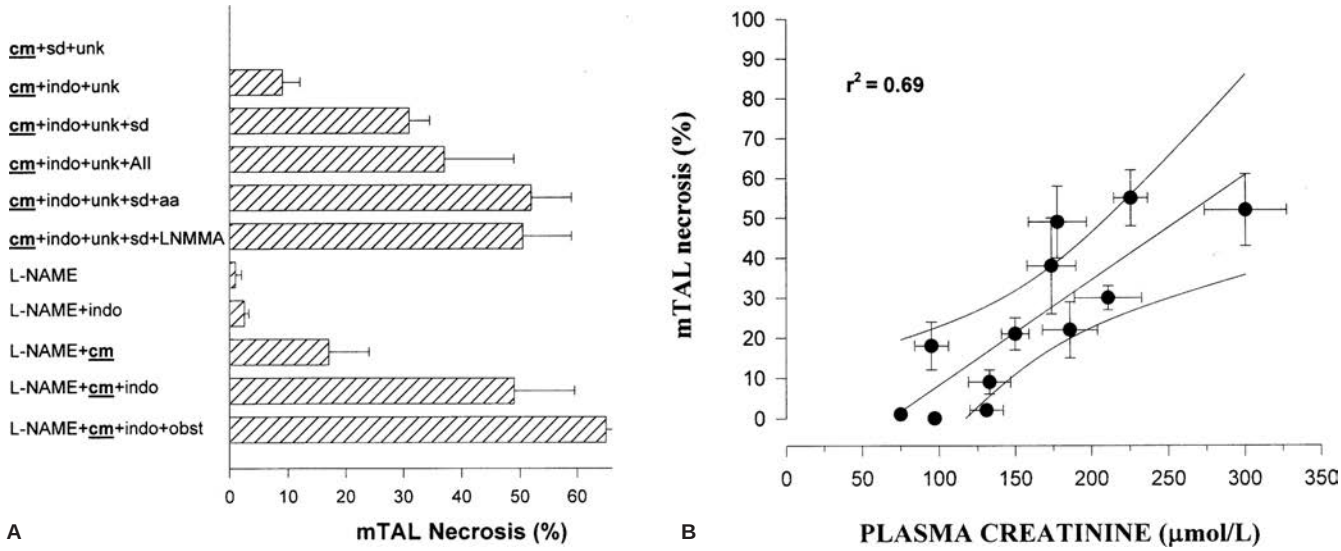

FIGURE 45-9. Rat models of CIN. **A:** the extent of medullary thick ascending limb (mTAL)-necrosis, induced by radiocontrast medium (CM), in animals preconditioned by prior uninephrectomy (UNK) salt depletion (SD), angiotensin II infusion (AII), amino acid infusion (AA), indomethacin (INDO), inhibition of NO synthase with NG-monomethyl-L arginine (L-NMMA) or NG-nitro-L-arginine methyl ester (L-NAME), and ureteral obstruction (Obst). **B:** Functional-morphologic correlations in these models. (Modified with permission from: Heyman SN, Reichman J, Brezis M. The pathophysiology of contrast nephropathy: a role for medullary hypoxia. *Invest Radiol* 1999;34:685.)

structural damage in the more severe protocols (Fig. 45-9B), but less so with the more moderate models of "partial" protocols, that cause limited tubular damage, underscoring a potential role for ensuing altered renal hemodynamics as the cause for the decline in kidney function (180).

Careful assessment of perfusion-fixed kidneys in our rat models reveals that morphologic tubular damage rapidly develops in the outer medulla as early as 15 minutes after the administration of the radiocontrast (114), predominantly affecting medullary thick ascending limbs and to a lesser extent S3 segments in the outer stripe of the outer medulla and in medullary rays. (103,114,115,139). These morphologic findings range from reversible injury (mitochondrial swelling with maintained cellular integrity) to a more severe damage pattern (nuclear pyknosis and disruption of cell membranes). A gradient of damage is noted, maximal among mTALs at the mid-interbundle zone, most remote from vasa recta. By 24 hours tubular necrosis is maximal, affecting about 50% of mTALs in the most severe models, occasionally associated with injured collecting ducts. Damaged inner medullary structures with necrosis of the papillary tip may develop as well (186). Apoptotic cell death is also noted with a pattern of distribution comparable to that of tubular necrosis (194). An additional morphologic hallmark of radiocontrast administration is outer medullary vascular congestion that appears as early as 15 minutes after the administration of contrast (114), representing altered microcirculation due to hypoxic endothelial dysfunction. Indeed, two-photon microscopy reveals renal endothelial injury with vascular leak, white blood cell adhesion, and rouleaux formation of red blood cells (195).

Conclusions

In summary, as schematically shown in Figure 45-6, the osmotic and volume loads of radiocontrast agents induce a host of systemic and renal physiologic responses that include diuresis and hemodynamic alterations, associated with profound medullary hypoxia (105,179,180). Numerous neurohumoral and physical mechanisms are involved in these reactions. In healthy subjects they are balanced by protective regulatory systems, structured to maintain renal parenchymal oxygenation, function, and integrity. These protective regulatory systems are characteristically altered in clinical risk factors that predispose to CIN. Consequently, renal parenchymal injury develops during contrast administration, the outcome of synergic hypoxic and toxic insults to tubular epithelial cells and to the renal microcirculation. In adequate models of CIN (106) animals are subjected to perturbations designed to mimic clinical risk factors. These models may improve our understanding the pathophysiology of CIN, and enable the exploration of potential preventive strategies.

PREVENTION OF CIN

In generally healthy individuals, without risk factors, the threat of CIN is very low, probably less than 1%, and the possibility of severe and protracted renal dysfunction requiring renal replacement therapy is negligible (18,26,36,75). Among high-risk patients, a few strategies were found to be effective in reducing the incidence of CIN, including selection of patients, the choice of low- or isoosmolar radiocontrast materials rather than high osmolar agents, given at the lowest required dose, and proper hydration protocols (Table 45-6). Since the incidence of CIN remains substantial in high-risk individuals despite these precautions, a variety of additional measures, including vasodilators, antioxidants, and attenuators of tubular metabolism, are continually tested in animal models as well as in clinical trials, so far without unequivocal clinical success.

Selection of Patients

The first and most important step in the prevention of CIN is to identify those patients at risk and to consider the use

TABLE 45-6

CIN: PREVENTIVE STRATEGIES

Patients' selection
Identification of patients at risk
Consideration of alternative imaging procedures without radiocontrast
Risk benefit assessment[a]
Avoidance of concomitant nephrotoxins
NSAIDs in particular
Prophylactic hydration[a]
Saline
Bicarbonate
Type of Radiocontrast medium[a]
The use of low- or nonionic agents rather than high-osmolar contrast material
Radiocontrast dosage/removal[a]
The use of the smallest doses required
Dose adjustment to renal function
Prophylactic hemodialysis (failed)
Prophylactic hemofiltration
Additional experimental approaches[a]
Vasodilators
Adenosine receptor antagonists
Dopamine/fenoldopam
Atrial natriuretic peptide
Endothelin receptor antagonists (nonselective, selective ETA)
Calcium-channel blockers
Acetylcysteine
L-Arginine
Prostaglandins
Angiotensin-converting enzyme inhibitors
Attenuation of metabolic requirements
Loop diuretics
PARPP inhibition
Hypothermia
Prostaglandins
Attenuation of free radical formation/attack
Allopurinol
Acetylcysteine
Alkaluria: bicarbonate, acetazolamide
Cellular adaptation to hypoxic/toxic stress
Induction of heme-oxygenase
Others
Mannitol

[a]Strategies applied in high-risk patients/animal models of CIN. All strategies classified as "additional experimental approaches" have failed in human trials, exert debatable effects, or are under clinical or laboratory investigation.

of alternative imaging technologies, such as sonography, nuclear imaging procedures, magnetic resonance imaging (MRI), or positron emission tomography (PET). The availability of these techniques, and their appropriateness in respect to convenience, costs, and their sensitivity and specificity should be taken into account and weighed against the risk of iodinated radiocontrast study. Obtaining medical history and physical examination is therefore a mandatory requirement before such procedures, in order to identify patients at risk, to correct reversible risk factors, to initiate effective protective measures and to consider alternative imaging approaches (196). Concurrent administration of drugs with potential nephrotoxicity should be avoided, if possible. The concomitant use of overlooked over-the-counter nonsteroidal antiinflammatory agents is of particular concern, as are other medications that adversely affect renal parenchymal oxygenation, such as cyclosporine or amphothericin. For the above reasons, hospitalization prior to ambulatory imaging procedures is highly recommended for very high-risk patients. Monitoring of renal function over 48 to 72 hours following the imaging procedure should be scheduled as well.

Reports of lactic acidosis following contrast administration in diabetic patients taking metformin have raised concern about performing radiocontrast studies in patients on this drug (197,198). However, there is little evidence that this complication develops in patients taking metformin who have normal renal function before receiving contrast. It is more likely that the diabetic patient with prior renal insufficiency and superimposed radiocontrast nephropathy experiences delayed excretion of the drug that predisposes to the development of lactic acidosis. Rasuli (199) recommend that metformin be held for 48 hours after radiocontrast administration to determine whether renal function has been impaired. If renal function remains stable, the prior dose of metformin may be resumed. There is no pharmacologic reason to withhold metformin for 48 hours prior to the study in these patients, as is sometimes suggested.

Hydration Protocols

The administration of intravenous fluids has long been the standard treatment in the prevention of CIN in high-risk patients. This approach, based on retrospective and uncontrolled observations during the eighties (23,200) became widely accepted, though randomized controlled trials comparing hydration protocols with no fluids have never been performed. The rationale for fluid administration is to attenuate preexisting vasoconstrictive stimuli in patients with effective volume depletion and to compensate for fluid loss induced by the osmotic diuresis. In addition, fluid administration may decrease the tubular intraluminal concentration of the radiocontrast and hasten its clearance, reducing urine viscosity and minimizing the potential for direct tubular toxicity.

A routine protocol in hospitalized adult patients was for years the intravenous administration of 0.45% saline, applied at the rate of 1 mL/kg/hour for 24 hours and initiated 6 to 12 hours before the radiocontrast study (12). This simple routine is well tolerated even among patients with congestive heart failure (9), though minor adjustments may be needed to address the patient's clinical status.

For outpatient procedures this protocol was substituted with short oral hydration before the procedure, followed by intravenous 0.45% saline for 6 hours. This regimen was as successful as the inpatient intravenous hydration for the prevention of CIN following angiography in patients with mild-to-moderate renal failure (201). The choice of 0.45% saline has recently been challenged by a large study, in which 1,620 unselected patients, undergoing elective or emergency cardiac catheterization were randomized to hydration protocols using 0.45% versus 0.90% saline (18). The incidence of CIN was 2% and 0.7%, respectively, for the entire cohort ($p = 0.04$), indicating that normal saline may be better. However, most of the enrolled patients were at low risk for CIN. Subgroup analysis disclosed that in a subset of 286 patients with preexisting renal failure, the corresponding incidence of CIN was 4% and 2%, respectively (NS), but diabetic patients and those administered with large amounts of the contrast medium had a statistically significant favorable outcome with the 0.9% saline preparation (0% vs. 5.5%, and 0% vs. 3%, respectively). For obscure reason, women also had better results with the

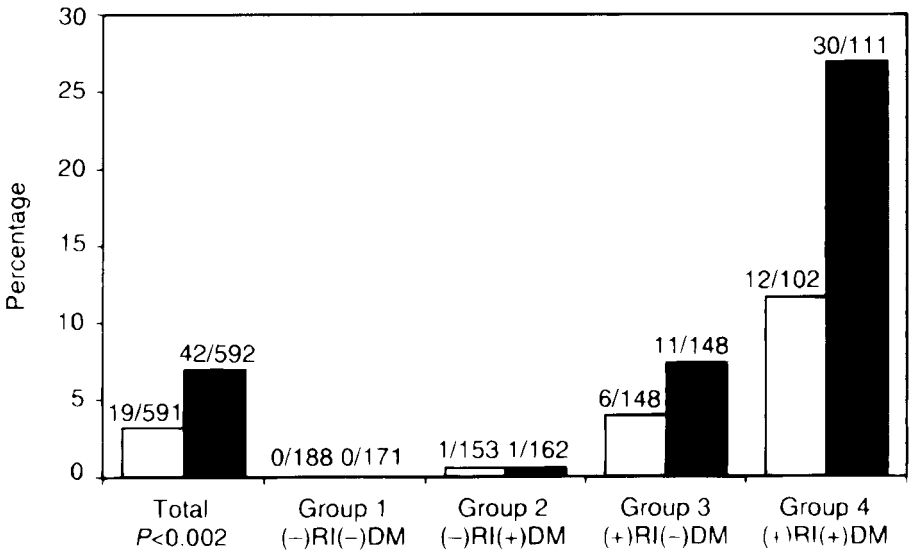

FIGURE 45-10. Percentage of patients able to be evaluated who developed nephrotoxicity for each treatment and stratification group following cardiac angiography. *DM,* diabetes mellitus; □, iohexol; ■, meglumine/sodium diatrizoate; RI, renal insufficiency. (From: Rudnick MR, et al. Nephrotoxicity of ionic and nonionic contrast media in 1,196 patients: a randomized trial. *Kidney Int* 1995;47:254, with permission.)

0.9% solution (0.6% vs. 5.1%). In another recent clinical trial, 119 patients with preexisting renal failure were randomized to a saline infusion protocol or equivalent load of bicarbonate solution (31). The rationale here was that alkaluria attenuates the generation of oxygen free radicals and may reduce subsequent tubular toxicity. CIN developed in 14% of patients on the saline hydration protocol, as compared with 2% only in the bicarbonate group. This finding was conceptually supported by a recent report that acetazolamide attenuates tubular damage and renal dysfunction in rats subjected to CIN (195). Bicarbonate infusion, however, may require a closer clinical monitoring to prevent clinically significant metabolic alkalosis. In conclusion, though additional large studies are required to confirm the above findings and to establish the superiority of isotonic saline over half-normal saline in patients with preexisting renal disease, it is reasonable to advocate the use of isotonic saline in high-risk patients, and to consider the alternative use of iso-equivalent bicarbonate solution.

The Choice of Radiocontrast

The development of low-osmolar agents was aimed chiefly to reduce the incidence of adverse effects, including CIN. Indeed, as outlined above, these agents produce more moderate systemic hemodynamic effects, are given at smaller volumes (dimeric agents), produce smaller osmotic and sodium ionic loads, have a less disruptive effect on the vascular endothelium and, in the case of ioversol, do not stimulate endothelin release (145). Nevertheless, whether the use of low-osmolar contrast media causes a clinically important reduction in nephrotoxicity remained controversial for a long time, since their introduction in the late 1980s. Several well-designed studies were unable to demonstrate a lower incidence of nephrotoxicity when compared to high-osmolality contrast media (16,32,35,51,86). Others found a significant advantage of low-osmolality contrast medium over a conventional high-osmolality agent (30,202). Because the relatively small number of high-risk patients in the studies showing no benefit may have obscured a positive effect, a type II error, the Iohexol Cooperative Study enrolled 1,196 patients to compare the low-osmolar iohexol with the high osmolar agent meglumine/sodium diatrizoate in healthy and high-risk patients (26). This prospective, multicentered trial showed that individuals with normal renal function, in the presence or absence of diabetes, had a very low risk of renal injury with either contrast agent (Fig. 45-10). However, patients with renal insufficiency who received diatrizoate were 3.3 times more likely to develop renal failure than those receiving iohexol. A metaanalysis of 45 controlled trials comparing high- and low-osmolality contrast media was consistent with this multicentered trial, showing that low-osmolality media is associated with reduced nephrotoxicity (203). Thus, for patients with normal renal function, there was no statistical benefit from the use of low-osmolality agents. By contrast, in patients with preexisting renal failure, the risk of developing ARF was reduced by 50%. Irrespective to these issues, the extensive use of cheap high-osmolar radiocontrast agents have been replaced to a large extent by the less toxic low-osmolar agents over the last decade, in parallel with the declining costs of the newer agents. Evidently, economic considerations in the long-lasting debate regarding the use of low-osmolar contrast media in all radiographic studies, or selectively for high-risk patients (26,204), are no longer valid.

A further reduction of the incidence of CIN in high-risk patients has been anticipated with the recent introduction of isoosmolar contrast media. Aspelin et al. (17) randomized 129 patients with chronic renal failure (mean plasma creatinine 1.5 mg/dL), undergoing cardiac catheterization, to the isoosmolar agent iodixanol or to the low-ionic material iohexol. The incidence of CIN (peak increase in serum creatinine >0.5 mg/dL) was significantly lower with the isoosmolar agent—2% versus 17%, respectively. Comparable results were also shown in a previous study by Chalmers and Jackson, who found that the incidence of CIN was 50% less in patients with chronic renal impairment given iodixanol, as compared to iohecol (29). However, in subsequent studies the incidence of CIN following iodixanol in comparable high-risk patients ranged from 12% to 33%. Indeed, in recent review and a meta-analysis, iso- and low-osmolar agents were found to be comparable regarding the risk for CIN (42,43). Possibly, the advantage of reducing radiocontrast volume and osmolar loads with the new isoosmolar agents is offset by the adverse effects of increased viscosity (154), as reflected by a comparable decline in medullary PO_2 (116). In conclusion, for high-risk patients, particularly those with renal dysfunction and diabetes, the use of low- or isoosmolar radiocontrast media is justified to minimize the risk of clinically significant CIN. By contrast, in patients with

normal renal function the risk of CIN is extremely low and equal for low-osmolar and high-osmolar agents. The new isoosmolar contrast materials are not safer than low-osmolar agents, regarding nephrotoxicity.

Radiocontrast Dose

Since the dose of the iodinated radiocontrast agent is considered a risk factor for CIN, high-risk patients should be given the smallest loads of contrast material possible. There is a dose of radiocontrast below which severe radiocontrast nephropathy is very unlikely to occur. McCullough and colleagues (75), in a cohort of more than 3,600 patients undergoing coronary contrast intervention studies, reported that acute renal failure requiring dialysis does not occur in any patient who receives 100 mL of contrast or less. Cigarroa suggests adjusting the maximal permitted radiocontrast volume to the degree of renal dysfunction (21). Controlled dosing of the contrast medium using the following formula resulted in a 90% decline in the incidence of CIN.

$$\text{contrast medium dose (mL)} = \frac{5\ \text{mL} \times \text{body weight (kg)}}{\text{serum creatinine (mg/dL)}}$$

Radiocontrast Removal

The load of contrast material per functioning nephron inversely correlates with their numbers. Since iodinated contrast agents are effectively removed by hemodialysis (205), this procedure was expected to alleviate the risk of CIN when performed immediately after contrast studies in patients with advanced renal failure, given large volumes of the radiocontrast material. However, when hemodialysis was initiated in 30 patients with advanced renal failure one hour after the contrast study, the incidence of CIN was not reduced: 53% inpatients on hemodialysis vs. 40% in patients treated conservatively (206). In another larger cohort of 113 patients with advanced renal failure (creatinine above 3 mg/dL), prophylactic hemodialysis, started immediately after the contrast injection, also did not reduce the incidence of CIN. Moreover, subsequent dialysis was more than doubled among patients assigned to prophylactic hemodialysis, as compared with the control group (41). By contrast, Marenzi has recently reported favorable outcome with prophylactic venovenous hemofiltration among 114 patients with comparably advanced renal failure, undergoing coronary interventions with high volume (250 mL) of contrast (87). The need for subsequent renal replacement therapy was reduced eightfold (3% as compared with 25% in controls) and in-hospital mortality rate was 2% and 14%, respectively. Of the remaining patients, only one in the treatment group ended up on permanent hemodialysis, as compared with three patients in the control group. These study groups illustrates the results of angiographic interventions using large volumes of radiocontrast medium, often in hemodynamically unstable cardiac patients. The advantage of continuous venovenous hemofiltration over hemodialysis seems to be the hemodynamic stability, avoiding hypovolemia and hypotension. In addition, it enables vigorous hydration without causing pulmonary congestion. Thus, the study by Marenzi indicates that hemofiltration is probably the procedure of choice under such circumstances. As outlined by Asif (196), the associated increased costs must be viewed in the context of short- and long-term benefits obtained, that may justify its use in very high-risk patients with advanced renal failure.

Vasodilators—A Conceptual Debate

As shown in Table 45-6, numerous types of renal vasodilators were tried for the prevention of CIN. The late and protracted decline in renal blood flow and GFR after radiocontrast administration (132) provides the rationale for these trials. Increased GFR and subsequent urine generation may also reduce the intraluminal concentration of the radiocontrast medium, shorten its transit time, and remove sediments. However, increased renal blood flow predominantly reflects augmented cortical, rather than medullary blood flow, and endothelial dysfunction is not restored. Radiocontrast-induced reduction of cortical blood flow and GFR are mediated in part by renal defense mechanisms, designed to maintain medullary oxygen sufficiency (105). Their neutralization may enhance GFR and increase distal tubular reabsorption and oxygen consumption. Thus, a conceptual debate exists regarding the usefulness of induction of renal vasodilation in CIN. Should we, or should we not improve GFR at the price of intensification of medullary hypoxic injury? In the absence of unequivocal efficacy of vasodilators or available clinical tools for the noninvasive assessment of medullary hypoxic stress and injury, this debate remains open.

Theophylline

Since adenosine has been associated with the renal hemodynamic changes induced by the contrast material, the effect of the adenosine receptor antagonist, theophylline, was explored in few clinical trials, with small numbers of patients included, many at low risk for CIN (207,208). In these studies theophylline was not protective. Abizaid compared 20 high-risk patients (creatinine $\geq$1.5 mg/dL), half of them diabetics, treated with intravenous theophylline, with patients given fluids, only, ending with similar results (209). Early studied the effect of oral theophylline in a placebo-controlled study in 80 comparably high-risk patients, and found that theophylline provided no functional benefit over hydration, though it prevented enzymuria (210).

Most other studies, however, found that theophylline reduced the risk of CIN. Katholi studied its effect in 93 hydrated patients who were also treated with calcium channel blockers and randomized to low- and high-osmolar contrast agents (211). Theophylline totally prevented or attenuated by half the decline in creatinine clearance in patients receiving low- or high-osmolar agents, respectively. Kapoor studied 70 diabetic patients with a relatively preserved renal function, undergoing coronary angiography with high-osmolar agents. CIN developed in 3% of patients treated with theophylline, as compared with 31% in the control group (212). In intensive care unit (ICU) patients on aminophylline prophylaxis Huber reported CIN in 2% of 78 patients, as compared with 14% of 565 matched control patients (213). In a randomized, double-blind placebo-controlled study in 100 patients given low-osmolar contrast medium, the same group report corresponding incidence of 4% and 16% of CIN, respectively, with a significant protective impact of theophylline shown by multiple regression analysis (214).

As with most clinical studies of CIN, comparison of the different studies is difficult in the perspective of varied patients' characteristics, radiologic procedures, type and dose of the radiocontrast agent used, dosing and mode of theophylline administration, other interventions, and the definition of CIN. Nevertheless, it seems that the decline in GFR following CIN may be attenuated by adenosine-receptor antagonists. These findings underscore the role of adenosine in the

contrast-related reduction of renal blood flow (149,208), but as discussed above, blocking its effect harbors a danger of enhancement of medullary hypoxia (111). Thus, the true impact of adenosine-receptor antagonists on the prevention of clinically significant CIN is yet to be proven.

Dopamine and Fenoldopam

Low-dose dopamine is a potent renal vasodilator and enhances GFR even in patients with renal impairment. In intact animals, dopamine increases outer medullary blood flow. However, physiologic medullary hypoxia is not improved, possibly as the result of enhanced distal tubular transport (215). Clinical trials with dopamine in the prevention of CIN provide mixed results. In a randomized study of 55 patients with chronic renal dysfunction, dopamine was not beneficial, though in a subgroup of patients with advanced renal failure (baseline creatinine ≥2 mg/dL) creatinine clearance was maintained, while in control patients it significantly declined (216). Weiseberg (10) randomized 50 patients with chronic renal failure, half of them with diabetes, to hydration alone, or together with dopamine, ANP, or mannitol. In nondiabetic individuals dopamine slightly reduced the plasma creatinine following the contrast study. By contrast, in diabetic patients, while renal blood flow was significantly improved, plasma creatinine paradoxically rose (10). Similar findings were noted with ANP and mannitol (see below), suggesting that in diabetic patients with endothelial dysfunction, improving cortical blood flow and GFR may adversely affect medullary oxygenation and intensify regional hypoxic damage (105).

Fenoldopam is a recently developed selective DA-1 agonist, devoid of DA-2 or adrenergic effects. It induces renal vasodilation and increases GFR, diuresis and natriuresis. In anesthetized dogs fenoldopam prevented the decline in renal blood flow, induced by radiocontrast (217). In a prospective study Kini compared 110 high-risk patients with baseline creatinine ≥1.5 mg/dL, undergoing coronary interventions, with historic controls. The incidence of CIN was 4.5% versus 19%, respectively (218). A small randomized multicenter study of 45 patients ended with comparable results (219). However, in a large, double-blind, placebo-controlled, randomized multicenter study, involving 315 high-risk patients (creatinine clearance ≤60 mL/min, half of them diabetics), the incidence of CIN among fenoldopam-treated patients was 34% as compared with 30% in the control group (40). Doses of fenoldepam higher than those studied so far seem impractical due to the risk of hypotension. Thus, currently both dopamine and fenoldopam are not recommended for the prevention of CIN in high-risk patients.

Natriuretic Peptides

Weisberg studied the effect of atrial natriuretic peptide (ANP) administered during contrast studies in patients with chronic renal failure, in addition to hydration (10). In nondiabetic patients ANP induces some decline in plasma creatinine. In diabetic individuals, however, as with dopamine and mannitol, ANP enhanced renal blood flow but paradoxically reduced kidney function, with plasma creatinine increasing twice as high as in the hydration-only group. Possibly, enhancing renal blood flow and GFR during contrast administration in patients with altered medullary microcirculation may intensify regional hypoxia and exacerbate medullary hypoxic damage. Comparable findings were noted in a more recent large-scale study, enrolling 247 patients with chronic renal failure undergoing contrast studies, half of them diabetics (220). Aneritide in addition to hydration did not prevent CIN in nondiabetic patients (8% vs. 9% in the saline-only group). Once again, in diabetic patients the incidence of CIN tended to be higher in the ANP group (39% vs. 26%, NS). Thus, ANP analogs are ineffective in the prevention of CIN in nondiabetic high-risk patients, and may be harmful in diabetic individuals.

Endothelin-Receptor Antagonists

Since radiocontrast media exert endothelin release that participates in contrast-related alterations in the renal microvasculature, the potential use of endothelin antagonists in the prevention of CIN was explored. In a recent placebo-controlled study, 158 hydrated patients with renal failure (mean serum creatinine 2.7 mg/dL) undergoing cardiac catheterization were randomized to receive the nonselective ET_A/ET_B receptor antagonist bosentan (39). The mean increase in serum creatinine 48 hours after the angiographic study was significantly higher in the treatment group (0.7 vs. 0.4 mg/dL in the placebo group) and the incidence of CIN was higher (56% vs. 29%, respectively). Endothelin ET-1 exerts cortical vasoconstriction by binding to endothelin ET_A receptors, while it induces medullary nitrovasodilation, mediated by ET_B receptors (112), with both actions conceivably leading to the amelioration of medullary hypoxemia. Nonselective inhibition of endothelin receptors is, therefore, likely to intensify medullary hypoxemia and to enhance CIN. Future research may therefore be directed to the evaluation of selective endothelin ET_A antagonists and their possible potential to prevent CIN.

Other Vasodilators

The prophylactic use of calcium channel blockers has been studied in few small studies (221–223), predominantly in patients without renal failure. Nifedipine acutely increased renal plasma flow when given before the radiocontrast, as compared with a decline or no change, noted in control patients given the high- and low-osmolar agents, respectively (222). Nevertheless, with the exception of one study (221) this treatment was found to be ineffective, and has not been explored further.

The protective potential of the vasodilating prostaglandin PGE_1 seems more promising. In a randomized, double-blind, placebo-controlled study, 130 patients with renal dysfunction (serum creatinine ≥1.5 mg/dL) were randomized to hydration alone or with the addition of PGE_1 infusion at 3 different doses. The mean increase in serum creatinine in the placebo group 48 hours following the radiographic study was 0.72 mg/dL, significantly higher than the 0.12 to 0.30 mg/dL increment in the three treatment groups (224). Comparable findings were achieved in a repeated experiment (225), establishing a potential protective effect of PGE_1 at a rate of 20 ng/kg/minute, administered 30 to 60 minutes prior the contrast study. This approach is compelling, in the perspective of the central role of prostaglandins in the maintenance of medullary oxygenation. This strategy may be particularly important in the aged population, characterized by reduced renal prostaglandin synthesis.

So far, a single randomized study was published in India, looking at the prophylactic use of angiotensin-converting enzyme inhibitors during contrast studies (226). Among 71 diabetic patients, the incidence of CIN was 29% in the control group, as compared with 6% in patients receiving captopril. The better outcome with captopril might reflect antioxidant properties of the sulfhydryl-containing compound, rather than, or in addition to, the class effect. Unfortunately, the wide use of these agents in the high-risk population precludes the conduction of comparable wide-scaled placebo-controlled studies. This report, however, possibly illustrates the safe use of

angiotensin-converting enzyme inhibitors during contrast studies, and suggests a possible role for the renin-angiotensin system in the pathogenesis of CIN.

Finally, the NO precursor arginine may restore endothelial dysfunction and improve outer medullary vasodilatory response to contrast media. This is indirectly implicated from an experimental model of CIN in hypercholesterolemic rats, where L-arginine, but not D-arginine, prevented the contrast-mediated decline in renal blood flow and GFR (74). L-arginine also improved renal blood flow and GFR in salt-depleted rats given radiocontrast (227). However, it was ineffective in a small randomized clinical trial in patients undergoing coronary angiography, and may have caused a trend for larger deterioration in renal function. However, data presented is not stratified for patients with or without a propensity for renal endothelial dysfunction (228).

Loop Diuretics

While renal vasodilators may increase medullary tubular reabsorption and deepen medullary hypoxia, potentially intensifying CIN, inhibition of tubular transport seems a reasonable intervention in the attenuation of medullary hypoxemia and in the prevention of this disorder. Indeed, furosemide reverses the decline in medullary oxygenation caused by radiocontrast (114), even though it decreases regional blood flow (229). When given prior to the radiocontrast agent, furosemide does not prevent the decline in medullary PO_2, but since the starting point is higher, critical medullary hypoxia is prevented (230). In a rat model of CIN, furosemide averts the development of tubular necrosis in the outer medulla, though function is only partially restored, conceivably due to effective volume depletion (231). In young human subjects medullary hypoxia is improved with furosemide, as shown by BOLD MRI (109).

Solomon et al. (9) prospectively assigned 78 patients with chronic renal insufficiency undergoing cardiac catheterization, half of them with diabetes, to prior treatment with saline, saline plus mannitol, or saline plus furosemide. Eleven percent of patients treated with saline, 28% treated with mannitol, and 40% treated with furosemide developed ARF (i.e., 0.5 mg/dL rise in serum creatinine within 48 hours). Urine output and sodium excretion were unexpectedly similar in all three groups 24 hours postangiography, making volume depletion an unlikely explanation for the results. The worst outcome of patients receiving furosemide was consistent with an earlier report also showing that furosemide enhances radiocontrast-induced ARF (232). However, in the later study, substantial weight reduction in the furosemide group, as opposed to weight gain in the hydration-only group indicates that the decline in kidney function could result from volume depletion. Furosemide may also be deleterious in human CIN because of ensuing medullary vasoconstriction (151,229,233), and its failure to attenuate medullary hypoxemia in the aged (109).

Other Diuretics

Mannitol was one of the first agents used in an attempt to prevent radiocontrast-induced ARF. It reportedly decreased the incidence of ARF in a small group of patients when given within 1 hour of the radiocontrast (234,235). The precise interpretation and weight that should be given to these reports are unclear because in one report only five patients each were studied in the experimental and control groups (235). Mannitol-induced osmotic diuresis could have a beneficial effect in ARF by decreasing tubular obstruction. However, radiocontrast agents also promote a vigorous osmotic diuresis, making it unclear what additional benefit mannitol might afford in protecting renal function. Mannitol, like dopamine and atrial natriuretic peptide, was superior to saline in preventing ARF in nondiabetics, but paradoxically, it worsened the severity of ARF in the diabetic patients when compared to saline alone (10). Other reports fail to demonstrate a protective effect from mannitol and suggest that saline expansion is the preferred agent to prevent contrast toxicity (9,25). Mannitol may be deleterious by increasing the osmotic load to the kidney and aggravating medullary hypoxia (229). In fact, mannitol itself, when given in large doses, can induce ARF (236).

The carbonic anhydrase-inhibitor acetazolamide was renoprotective in a rat model of CIN (195), perhaps by the attenuation of oxygen free radical formation through alkaluria.

Antioxidants: *N*-Acetylcysteine (NAC)

Evidence for renal parencymal injury by oxygen free radicals led to the evaluation of effectiveness of antioxidants in the prevention of CIN (164). In addition to a direct protection of tubular epithelial cells, this approach repairs vascular endothelial dysfunction, typical of clinical conditions that predispose to CIN (171,237), and may reduce the propensity to develop medullary hypoxic damage. NAC is a scavenger of reactive oxygen species, widely used in the prevention of acetaminophen liver toxicity. Being cheap and almost devoid of side effects, NAC became the subject of numerous clinical trials around the world since Tepel's report in 2000 (238), given orally or intravenously, before and after the radiocontrast study.

As underscored by Safirstein (239), NAC directly prevents cytotoxicity by neutralizing free radical species (162), and may replenish cellular glutathione levels. In addition, it might exert regional vasodilation through nitric oxide production, and may restore endothelial dysfunction and improve oxygen supply. Indeed, NAC was found to improve renal medullary microcirculation induced by reperfusion injury (240) and to attenuate renal vasoconstriction induced by radiocontrast media (241). Tepel randomized 83 hydrated patients with chronic renal failure, given a small intravenous dose (75 mL) of nonionic low-osmolar radiocontrast agent, to receive placebo or NAC orally, 600 mg every 12 hours, a day before, and a day after the procedure. The incidence of CIN after 48 hours was 2% in NAC-treated patients, significantly lower than 21% in the control group (238). Diaz Sandoval conducted a comparable study in 54 patients undergoing coronary angiography (38) with more than twice the volume of the contrast medium, showing a similarly striking difference in the incidence of CIN between the treatment and control groups (8% vs. 45%). In a study by Shyu (242) in patients with more advanced renal failure (serum creatinine $\geq$2.8 mg/dL) undergoing coronary intervention findings were similar (3% vs. 25%). Baker reported that high-dose intravenous NAC shortly before the contrast study was also highly effective in the prevention of CIN (5% vs. 21%) (243).

However, additional studies provide different results. Durham studied 79 patients with chronic renal failure (serum creatinine $\geq$1.7 mg/dL), undergoing cardiac studies with low mean volumes of contrast medium (85 mL). The incidence of CIN was 26% in the treatment group, as compared with 22% in the controls. Noteworthy, NAC oral administration was modified, given at two doses 1,200 mg each, 1 hour before and 3 hours after the procedure (244). However, NAC was non-protective also in other trials, such as Boccalandro's (245), that exactly followed Tepel's protocol. Briguori also conducted a randomized study in 183 consecutive patients with renal dysfunction, undergoing coronary or peripheral arterial procedures, adopting Tepel's dosing regimen of NAC (19). In this study the incidence of CIN was not statistically different (6.5% and 11% in the treatment- and control-groups, respectively). Subgroup analysis revealed that while in patients given

contrast in excess of 140 mL NAC was not protective, at lower volumes of the contrast medium NAC treatment was significantly favorable (0% vs. 8%, respectively). However, logistic regression analysis showed that the amount of the radiocontrast agent and not the treatment strategy predicted the occurrence of CIN. The same Italian group conducted most recently two additional studies in large cohorts of patients with chronic renal failure, undergoing coronary or peripheral arterial procedures (246,247), and report that NAC administration at a double dose (1,200 mg twice a day) is more protective than Tepel's regimen in the prevention of CIN (3.5% vs. 11%), and more effective that fenoldopam infusion (4.3% vs. 14%, respectively). The double dose of NAC was this time protective, particularly when large volumes of the radiocontrast (>140 ml) were used (247).

In the perspective of additional studies with inconsistent results, Birck (248) and Alonso (249) performed meta-analysis of 7 and 12 well-conducted, randomized controlled reports, respectively, evaluating the outcome of over 800 patients. Both studies acknowledge the heterogeneity of patients' characteristics and of the variables related to the radiologic studies and NAC administration that were not controlled for in the meta-analysis. The potential bias of not reporting negative findings has also been addressed, as was the mixed outcome, with some reports showing clear benefit, while others concluded with no demonstrable efficacy. Birck and Alonso both reached the conclusion that NAC treatment was effective in the prevention of CIN, with an overall calculated risk reduction of 41% to 56%. A most recent meta-analysis by Kshirsagar (44), however, reaches a different conclusion, predominantly addressing the heterogeneity of the various study groups, regarding the definition of CIN, the patients' characteristics, the sort of radiologic procedure, the type and dose of radiocontrast studies, the hydration protocols used, and the dosing and timing of NAC administration. Comparing 16 well-designed studies, Kshirsagar concludes that research on NAC and the incidence of CIN is too inconsistent at present to warrant a conclusion on efficacy or a recommendation for its routine use (44). Hoffmann further questions the true value of NAC, showing that it may directly hasten creatinine excretion, irrespective to changes in GFR (250). Fishbane and colleagues (251) propose that currently the use of NAC should be recommended, in the perspective of the overall suggested benefit, the lack of effective alternatives, the safety and tolerability of the drug and its low costs. Thus, large-scale multicenter studies with strict definitions and control of variables are required, to confirm efficacy of NAC in the prevention of CIN (44). The use of intravenous NAC (243) or larger oral NAC doses, as suggested by Briguori, should be further explored (247).

Other Potential Strategies

Adenosine breakdown by xanthine oxidase elaborates free radical species, believed to exert tubular toxicity, as well as endothelial dysfunction. Bakris reported that the xanthine oxidase inhibitor allopurinol effectively prevented the fall in renal blood flow and GFR, and reduced urinary malondialdehyde, a marker of membranal injury, produced by oxygen free radicals (164). Katholi studied 39 patients with mild renal failure given ionic contrast agents (252). Allopurinol-treated patients with low plasma Mg^{2+} had somewhat milder decline in creatinine clearance as compared with control, low Mg^{2+}patients (33% vs. 79%, respectively). However, in nonhypomagnesemic patients allopurinol did not affect the decline in kidney function. PARP inhibition is another potential therapeutic approach. In a rat model of CIN, PARPP inhibition attenuated renal dysfunction, though the extent of tubular damage was unaffected (167). Activation of stress response genes is another alternative, as shown in another rat model of CIN in rats, pretreated by the induction of heme-oxygenase (253). Other related potential options are erythropoietin or HIF stabilizers (254). Hypothermia was also found to prevent CIN in rabbits (255), supposedly through attenuation of renal oxygen consumption.

In summary, in high-risk patients attempts to reduce the incidence of CIN below 10% to 20% were futile, so far. A basic conceptual debate is whether deactivation of renal vasoconstrictive mechanisms is the appropriate mode of intervention, or is it the amelioration of medullary hypoxia. This debate calls for consideration of combined strategies that in addition to vigorous hydration will concomitantly enhance renal oxygen supply, reduce medullary oxygen consumption, and restore endothelial dysfunction.

CONCLUSIONS

Radiocontrast ARF may be a paradigm for the synergism between toxic and hypoxic insults to the kidney, illustrating the renal vulnerability to disruption of the delicate medullary oxygen balance and the pathogenic implications of endothelial dysfunction within the kidney. It develops almost exclusively in patients with risk factors, characterized by the propensity to develop medullary hypoxia. Prevention of CIN is initiated by an appropriate selection of patients and the identification of patients at risk, substitution of contrast studies with alternative imaging procedures, if possible, in high-risk individuals, and avoiding concomitant administration of other nephrotoxins. High-risk patients undergoing contrast studies should be given hydration regimen with normal saline, and receive low- or isoosmolar agents at the smallest dose possible. Hemofiltration may be used for very-high-risk patients that require large volumes of contrast medium for radiographic interventions. Regardless of all these measures, CIN develops in about 10% to 45% of high-risk patients, and remains a significant cause of iatrogenic acute renal failure. Measures to further reduce the incidence of CIN with additional preventive strategies are currently being explored.

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CHAPTER 46 ■ NEPHROTOXICITY SECONDARY TO DRUG ABUSE AND LITHIUM USE

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DRUG ABUSE

Abuse of narcotics, cocaine, and other illicit substances may be associated with a variety of renal disorders as listed in Table 46-1. In the 1970s and early 1980s, glomerulosclerosis associated with intravenous heroin abuse in young African American adults emerged as an important correlate of nephrotic syndrome and end-stage renal disease in urban areas and accounted for approximately 10% of patients aged 18 to 45 years undergoing dialysis (1,2). Also, in the early 1980s, secondary amyloidosis became an important cause of renal failure in heroin abusers who injected drugs via the subcutaneous route ("skin poppers") and developed chronic suppurative skin lesions (3–6). Later, glomerulosclerosis in intravenous drug abusers infected with the human immunodeficiency virus (HIV) was recognized as a clinicopathologic entity distinct from heroin-associated nephropathy in HIV-negative heroin addicts (7,8). HIV-associated nephropathy rapidly supplanted heroin-associated nephropathy as the major renal disorder affecting intravenous drug abusers. Concurrently, the incidence of heroin-associated nephropathy and of "skin poppers' amyloidosis" decreased dramatically (9–11). Friedman and Rao (9) reported on the sharp decline in the incidence of heroin-associated nephropathy since 1989 and the complete absence of new cases of end-stage renal disease due to this disease for the years 1991 to 1993. Friedman and Rao (9) suggested that heroin-associated nephropathy may be due to the adulterants contained in "street" heroin rather than the heroin itself, and attributed the virtual disappearance of heroin nephropathy to an increase in purity of "street" heroin leading to reduced exposure to nephrotoxic adulterants. However, the changing epidemiology of renal disease in drug abusers may instead reflect the high prevalence, ranging up to 50% to 60%, of HIV infection among drug abusers (12). The presence of HIV infection in such a large proportion of the addict population has greatly reduced the number at risk for heroin-associated nephropathy, which has been pre-empted by the occurrence of HIV infection and its nephropathy.

Focal Glomerulosclerosis in Intravenous Heroin Abusers

Nineteenth-century clinicians appreciated the occurrence of proteinuria among opium addicts. Early investigators found albuminuria in 7% to 17% of addicts (13,14). Later studies attempted to define the nature of renal disease in narcotics abusers (15,16–20). In cross-sectional studies, investigators determined the prevalence of glomerular abnormalities in postmortem specimens from heroin addicts and the prevalence of proteinuria and renal sediment abnormalities in populations of addicts attending drug rehabilitation centers (15–20). Markedly disparate data were reported. However, these studies probably have little relevance to renal disease in heroin addicts as we now understand it. The finding of nonspecific urinary abnormalities in surveys of the addict population cannot be accepted as representative of the incidence of heroin-associated nephropathy. The rarity with which cases could be detected in cross-sectional or prospective studies of heroin abusers is readily apparent when one considers that data available from the early 1980s, at the height of the epidemic of heroin-associated nephropathy, placed its prevalence at well below 1%, with an annual incidence of less than one new case per 1,000 heroin addicts (21,22).

In the early 1970s several investigators began to recognize a clinicopathologic entity in heroin addicts characterized by the nephrotic syndrome and glomerulosclerosis (16,19, 23,24). Rao and colleagues (25) identified focal and segmental glomerulosclerosis that progressed to global sclerosis as heroin-associated nephropathy. These authors described 14 African American intravenous heroin users in whom the nephrotic syndrome was present in all but one. Renal biopsy specimens showed focal and segmental or global glomerulosclerosis (FSGS) in 11 of 13 patients, associated with focal and segmental glomerular deposition of immunoglobulin M and complement. Numerous other investigators confirmed these characteristic morphologic features (3,4,26–33). A similar syndrome of glomerulosclerosis associated with the nephrotic syndrome and progressive renal insufficiency has been observed rarely in abusers of intravenous drugs other than heroin, among them cocaine and pentazocine with tripelennamine (1,23,29).

Clinical Features of Heroin-Associated FSGS

Detailed descriptions of the course of nephropathy in 298 intravenous drug abusers, most described prior to the advent of HIV, with biopsy-proven focal and segmental glomerulosclerosis are available in the literature (1–4,16,17,20,25–28,34–37); incomplete information is available in an additional 168 cases (Table 46-2). Over 90% were African American males. The mean age was 29 years. Intravenous heroin, often in conjunction with cocaine, was used in all but a few cases (1,23,29). The mean duration of drug abuse prior to presentation with renal disease was 6 years, ranging widely from 6 months to 24 years but generally exceeding 1 to 2 years.

Two-thirds of patients presented with edema due to the nephrotic syndrome, an additional one-fourth with an abnormal urinalysis, and most of the remainder with uremia. Hypertension was present in nearly two-thirds on initial presentation and developed in all those who progressed to uremia (1). Microscopic hematuria was described in one-third and pyuria in one-half of the cases, but nearly one-fourth had no documented sediment abnormalities. Renal function on presentation varied widely but was reduced in three-fourths of cases.

TABLE 46-1

RENAL DISEASE ASSOCIATED WITH DRUG ABUSE

1. Focal glomerulosclerosis in intravenous heroin users
2. Amyloidosis in subcutaneous heroin abusers
3. Endocarditis-associated glomerulonephritis in intravenous drug users
4. Acute renal failure due to nontraumatic rhabdomyolysis
5. Cocaine–associated nephropathy
6. Systemic necrotizing vasculitis
7. Nephropathy in glue and solvent "sniffers"
8. Hepatitis-related glomerulonephritis in drug abusers
9. Focal glomerulosclerosis in drug abusers infected with the human immunodeficiency virus

Ten percent presented with uremia. Urinary protein excretion averaged 9.3 g/24 hours and was less than 2 g/24 hours in only 6% of cases. Proteinuria exceeded 10 g in 43%. Serum complement levels were normal and rarely erythrocytosis was reported (28).

Follow-up observations demonstrated that heroin-associated nephropathy regularly progressed inexorably to uremia. Patients presenting with a serum creatinine level below 2.2 mg/dL or a creatinine clearance exceeding 50 mL/min advanced to end-stage renal failure (ESRD) over a mean period of 43 months (range 6 to 148 months). As would be expected, ESRD occurred over a commensurately shorter period in those who presented with more advanced renal insufficiency.

The effect of abstinence from drug use on the course of established nephropathy is not known. Several patients with heroin-associated nephropathy have been described who underwent sustained remission of proteinuria and/or stabilization or improvement in renal function following abstinence (4,35,37). Eleven other patients with heroin-associated FSGS progressed to ESRD despite discontinuation of drugs. It appears, however, that patients who abstained may have followed a more protracted course. Corticosteroid and immunosuppressive therapy have generally proved to be ineffective. Despite reported responses to such therapy (26), there is no convincing evidence that treatment induces remission of proteinuria or prevents progression to end-stage renal failure.

TABLE 46-2

FOCAL GLOMERULOSCLEROSIS IN INTRAVENOUS HEROIN ABUSERS

Clinical features	
African American	95%
Male sex	92%
Age	29 yr
Duration of drug abuse	6 yr
Hypertension on presentation	64%
Serum creatinine on presentation	3.6 mg/dL
Nephrotic syndrome	80%
Time to uremia:	
Initial creatinine clearance >50 mL/min	43 mo
Initial creatinine clearance 20–50 mL/min	23 mo
Initial creatinine clearance <20 mL/min	7 mo

Comparison with Other Forms of Focal Glomerulosclerosis

In studies performed prior to the recognition of HIV-associated nephropathy, we found that heroin-associated FSGS and idiopathic FSGS showed similar histology on presentation (39). Both frequently displayed segmental glomerulosclerosis with foam cells and hyalinosis as well as varying numbers of globally sclerotic glomeruli. In contrast, HIV-associated nephropathy is characterized by segmental and/or global collapse of tufts together with prominent visceral epithelial cell hypertrophy. In addition, patients with HIV-associated FSGS often present with severe renal failure even though they lack advanced stages of diffuse global glomerulosclerosis. In this respect, HIV-associated FSGS differed from idiopathic and heroin-associated FSGS. Furthermore, interstitial inflammation and distinctive cystic tubular dilatation was noted to be more prominent in HIV-associated FSGS than in idiopathic or heroin-associated FSGS.

D'Agati and co-workers (7,38) compared renal biopsy specimens from patients with idiopathic FSGS, heroin-associated FSGS, and HIV-associated FSGS. They found the specimens to be qualitatively similar but noted that the HIV group had more globally "collapsed" glomeruli, less hyalinosis, more severe visceral epithelial cell swelling, more prevalent and severe tubular microcytic dilation, and more severe tubular cell degenerative changes (7,38). The severity of tubular atrophy and interstitial fibrosis, edema, and inflammation did not differ among the three groups (7). Ultrastructural examination in the HIV group showed numerous tubuloreticular inclusions involving glomerular endothelium, interstitial capillary endothelium, and interstitial leukocytes (7). These inclusions were only rarely observed in biopsies from patients with idiopathic or heroin-associated FSGS and when present were seen only in glomerular capillaries (7,38). These authors concluded that, while no single morphologic feature distinguishes HIV-associated FSGS from idiopathic or heroin-associated FSGS, the constellation of clinical and pathologic features that characterize HIV-associated nephropathy is diagnostic (7,38).

Epidemiology

Epidemiologic studies performed during the 1970s and 1980s, prior to the HIV epidemic, suggested the existence of a form of FSGS that occurred in intravenous heroin abusers, predominantly young African American males. FSGS in nonaddicts is likewise more prevalent in African Americans than in Caucasians, but this race disparity is more marked in parenteral drug abusers (21). Intravenous drug abuse may unmask a genetic predisposition in blacks to develop glomerulosclerosis (1,21). The emergence of this latent tendency may be triggered by exposure to heroin itself or adulterants in "street" drugs, bacterial or viral contaminants, or other environmental factors related to heroin use. In this regard, Haskell and colleagues (40) have demonstrated an increased frequency of HLA-BW53 among African American intravenous drug abusers with heroin-associated nephropathy, and suggest a genetic predisposition to the development of FSGS in this population (40,41).

The epidemiology of nephropathy in intravenous heroin users has changed radically in the last two decades. HIV-associated collapsing FSGS and hepatitis C-related glomerulonephritis have supplanted heroin-associated FSGS as the major causes of glomerular disease among intravenous drug abusers. The virtual disappearance of heroin-associated FSGS may be due to an increase in purity of "street" heroin leading to reduced exposure to nephrotoxic adulterants, or the disease

may have been pre-empted by the occurrence of HIV infection with its distinctive collapsing glomerulopathy.

Studies performed in the late 1980s and 1990s described renal histopathology in European intravenous heroin users who were selected for evaluation of proteinuria or examined postmortem (42,43,44,45). Glomerular lesions in some were classified as membranoproliferative glomerulonephritis and may have been due to infection with hepatitis C. However, serum antibody to the hepatitis C virus when sought postmortem could not be detected (42).

Perneger et al. (46) performed a case control study of 716 illicit drug users and 361 controls. Those who had ever used heroin or other opiates were at increased risk for end stage renal disease, with an adjusted odds ratio of 19.1. The association showed a dose-dependent effect. The use of cocaine, crack, or psychedelic drugs was also associated with ESRD but these associations could not be separated from the effects of heroin use. The authors estimated that heroin-associated renal disease accounts for 5.6% of all treated ESRD patients aged 20 to 64 and for 5% to 6% of new patients starting renal replacement therapy in this age group. However, these investigators failed to distinguish between HIV- or hepatitis C-related renal disease in heroin addicts and heroin-associated FSGS. In this regard, the United States Renal Data System (47) attributed 0.1% of new cases of ESRD from 1998–2002 to heroin/related abuse. The median age was 45 years, 75.5% were male, and 59.2% were black.

Pathogenesis

Early investigators suggested that FSGS in heroin abusers was immunologically mediated (22,25). The finding of immunoglobulin M (IgM) and C3 in segmental lesions suggested an immunologic mechanism of damage due to deposition of immune complexes; the putative antigen being a self-injected bacterial or viral contaminant, an adulterant used to dilute heroin, or the heroin itself (1). Although electron-dense material may be seen in areas of sclerosis, they are not found in uninvolved peripheral capillary walls in kidney biopsies of addicts with FSGS. The appearance and location in the lumens of sclerosing tufts suggest that these accumulations represent nonspecific trapping of immunoglobulins rather than immune deposits. Immunofluorescence findings are consistent with this interpretation.

Administration of morphine to rats by Marchand and co-workers (48) did not result in glomerular disease but instead caused tubulointerstitial lesions in the cortex, medulla, and papilla consisting of basement membrane thickening, loss of microvilli, and dense cytoplasmic inclusions in proximal, distal, and collecting duct tubules. However, other investigators have demonstrated increased glomerular podocyte microprojections in morphine-treated rats (49). Clearly, administration of morphine sulfate to rats is not analogous to the nonsterile self-injection of heroin and its adulterants by addicts.

Studies by Singhal and co-workers (50–58) suggest that morphine, the active metabolite of heroin, may directly mediate heroin nephrotoxicity. Exposure of mesangial cells to morphine stimulates cellular proliferation and synthesis of collagen and laminin and suppresses collagenase activity (50–56). Morphine enhances accumulation of macromolecules within the mesangium and stimulates production of pro-proliferative and fibrogenic cytokines (52–56). These alterations in mesangial cell function may contribute to the development of cell proliferation and matrix abnormalities observed in cultured mesangial cells after long-term exposure to morphine (50). In addition, morphine increases renal medullary interstitial cell and renal fibroblast proliferation and enhances the release of proinflammatory macrophage secretory products (57,58). However, the relevance of these *in vitro* experimental observations to the development of heroin-associated nephropathy in humans remains to be established.

Deposition of talc granules has been observed in arterioles and glomeruli of intravenous drug abusers who inject drugs compounded for oral or rectal use (59–63). Granulomatous interstitial nephritis with particulate deposits in the renal interstitium has also been reported (60,62,64). In addition, a syndrome of microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure has also been observed with intravenous heroin use (65).

Amyloidosis in Subcutaneous Heroin Abusers ("Skin Poppers' Amyloidosis")

In the late 1970s and early 1980s, systemic amyloidosis emerged as a major cause of nephropathy in heroin abusers (3–6). The association was first reported in small numbers of addicts with proteinuria between 1975 and 1979 (66–72). By the early 1980s, approximately one-half of heroin abusers who underwent renal biopsy for proteinuria in New York City were found to have amyloidosis (3,4,6). However, with the advent of HIV infection among drug users, amyloidosis has become an infrequent cause of nephrotic syndrome and renal failure in drug abusers, paralleling the decline in heroin-associated FSGS.

Pathogenesis

We performed a prospective survey of 150 predominantly African American male intravenous drug addicts who were examined consecutively at postmortem in the early 1980s (5). We demonstrated that addicts with suppurative skin infections were at high risk for the development of amyloidosis (5). Twenty-six percent of the 23 addicts with chronic suppurative skin infections due to subcutaneous injections had renal amyloidosis. The skin infections, usually in the extremities, were generally extensive, involving 10% to 20% of the body surface area. The amyloid was amyloid A protein-related in all cases. All but one of those found to have renal amyloidosis had chronic skin infections. The near universal association of renal amyoidosis with chronic suppurative cutaneous lesions in subcutaneous drug abusers strongly implicates skin infection in the pathogenesis (3–6,70,73,74). Thus, to the extent examined, amyloidosis in addicts does not differ from amyloidosis secondary to chronic infections and inflammatory diseases in nonaddicts associated with chronic cutaneous dermatoses, secondarily infected burns, or decubitus ulcers (5).

The average duration of drug abuse in addicts with renal amyloidosis was 18 years, significantly longer than the average 6 years in addicts with FSGS. The mean age of addicts with amyloidosis was 41 years, while those with focal sclerosis averaged 29 years of age. In general, intravenous drug abuse antedated the subcutaneous route in those with amyloidosis. With advancing age and prolonged addiction, intravenous sites for injection of drugs become exhausted, necessitating a shift to the subcutaneous route. Cutaneous ulcerations that develop in response to local reactions to the injected heroin, bacterial contaminants, and/or diluents are followed by suppurative complications (72,75). Longer survival of addicts in the late 1970s and early 1980s due to improved management of bacterial endocarditis, hepatitis, and other narcotic abuse-related medical illnesses likely allowed the emergence of renal amyloidosis. However, there has been a dramatic decline in the incidence of "skin poppers' amyloidosis" in the HIV era.

Pathology

Deposits of amyloid are typically present in all renal compartments. Glomeruli typically exhibit prominent mesangial expansion or solidified lobules, which may be misinterpreted as sclerosis in hematoxylin–eosin-stained sections. However, routine stains readily differentiate the two processes because amyloid is silver-negative with periodic acid-silver methenamine stain, whereas the opposite is true of sclerosis. Peripheral capillary walls are often thickened and have a "spicular" deformity in silver-stained sections produced by the penetration of amyloid fibrils through the glomerular basement membrane. Tubular basement membranes, especially those of the distal segments, are thickened due to deposits of amyloid, and the degree of tubular atrophy appears to parallel the amount of amyloid deposited. While amyloid is seen focally in tubular basement membranes of some proximal tubules, the amount and distribution are far greater in distal segments.

All of the 17 kidneys with skin poppers' amyloidosis that we examined by immunofluorescence showed predominant staining for AA protein. Amyloid was isolated from the kidney and biochemically characterized in two instances. The amyloid protein was AA protein–related and did not differ from AA amyloid secondary to other chronic infections and inflammatory diseases (5,76).

Clinical Features

The following description of the clinical features of skin poppers' amyloidosis is based on data from 60 cases reported in the literature, (3,4,6,66–74,77–79) (Table 46-3). The mean age of these patients was 41 years (range, 5 to 57 years). When specified, nearly all patients were African American, and all but 10 were men. Heroin was abused in nearly all cases, often in association with cocaine; however, in two patients, crushed tablets of pentazocine and tripelennamine were the sole drugs abused. The vast majority injected drugs by the subcutaneous route and invariably had extensive skin ulcerations, usually with active chronic suppuration at the time of presentation. Chronic skin infections at intravenous injection sites or systemic illnesses known to be associated with systemic amyloidosis were seen in those who had not abused drugs by the subcutaneous route. The mean total duration of drug abuse was 18 years, and the average time from initiation of the subcutaneous route to presentation with amyloidosis was 3 years, with a range of 1 to 8 years.

Addicts with amyloidosis typically present with the nephrotic syndrome. Urinary protein excretion averaged 13 g/24 hours and was in the nephrotic range in over 80%. Microscopic hematuria and pyuria were uncommon. Hypertension on initial presentation was reported in 17%. Average serum creatinine levels did not differ between hypertensive and normotensive patients. Dubrow and colleagues (3) comment on the infrequency of hypertension in heroin abusers with systemic amyloidosis in contrast to its presence at presentation in two-thirds of heroin abusers with FSGS. Our own experience differs insofar as we frequently observed hypertension at presentation among heroin addicts with systemic amyloidosis. Kidney size was normal in two-thirds of cases and was increased in the remainder. Serum creatinine on presentation varied widely and averaged 2.5 mg/dL. Only 4% were uremic at presentation.

Follow-up data are available in a limited number of patients who generally progressed to uremia over 2 to 3 years after presenting with serum creatinine levels ranging from 1.5 to 4.7 mg/dL. Abstinence from subcutaneous drug use and clearance of infection has been associated with remission of the nephrotic syndrome and improvement or stabilization of renal function in several patients (73–80). However, repeat renal biopsy showed persistent glomerular deposits of AA amyloid in one of these (74). In contrast, others have described persistent nephrotic syndrome, progressive loss of renal function and persistent renal amyloid deposits despite discontinuation of drug use and clearance of infection (72). Tan and colleagues (78) treated one patient with colchicine. Although renal function improved and the nephrotic syndrome remitted, repeat renal biopsy after 12 months failed to show any improvement in the amyloid burden (78).

TABLE 46-3

AMYLOIDOSIS IN SUBCUTANEOUS HEROIN ABUSERS

Clinical features	
African American	98%
Male sex	86%
Age	41 yr
Duration of intravenous route	18 yr
Duration of subcutaneous route	3 yr
Suppurative skin lesions	88%
Nephrotic syndrome on presentation	86%
Hypertension on presentation	17%
Serum creatinine on presentation	2.5 mg/dL

Tubular Disorders

We assessed renal tubular function in 13 addicts with renal amyloidosis (6). Renal tubular acidosis was found in eight patients. Polyuria and polydipsia, glycosuria, and phosphaturia were additional clinical features. Nephrogenic diabetes insipidus insensitive to exogenous vasopressin administration was documented in one patient in whom amyloid deposits were demonstrated in renal tubular basement membranes, predominantly involving distal segments.

In summary, systemic amyloidosis emerged in the late 1970s and early 1980s as an important cause of nephropathy in drug abusers in urban areas related to use of the subcutaneous route of drug injection. However, with the advent of HIV infection, renal amyloidosis in the addict population has experienced a dramatic decline.

Endocarditis-Associated Glomerulonephritis in Heroin Abusers

In a review of the epidemiology and clinical features of glomerulonephritis during the course of bacterial endocarditis, we noted that patients were frequently parenteral drug abusers infected with *Staphylococcus aureus* (81,82). Others have reported a similarly high incidence of glomerulonephritis in parenteral drug abusers with bacterial endocarditis due to *S. aureus* (83–91). The predisposition of parenteral drug abusers to develop glomerulonephritis during the course of bacterial endocarditis may be related to preformed serum antibodies to bacterial antigens.

Pathology

Glomerulonephritis encountered in the course of endocarditis can be classified as diffuse or focal (81). Diffuse glomerulonephritis, as it occurs in addicts with acute bacterial endocarditis due to *S. aureus,* is generally indistinguishable from acute post streptococcal or other post infectious glomerulonephritis. Proliferation in glomeruli is global and regular, and electron-dense deposits are present in a subepithelial distribution. This contrasts with the morphologic features of diffuse glomerulonephritis seen with subacute bacterial endocarditis

due to *Streptococcus viridans*. In this latter setting, deposits are predominantly subendothelial and mesangial, and proliferation in glomeruli is irregular with coexistence of segmental and global lesions (81). Focal glomerulonephritis in infective endocarditis is characterized by varying degrees of segmental glomerular hypercellularity, sclerosis, or capsular adhesions. Epithelial and fibroepithelial crescents, either small or circumferential, may be present.

Clinical Features

Clinical manifestations of focal glomerulonephritis are usually mild (82). Microscopic hematuria, pyuria, or modest proteinuria may be observed; heavy proteinuria and hypertension are rare. Renal functional impairment is uncommon, but when segmental glomerular involvement is severe and extensive, renal insufficiency may supervene (81). Occasionally, focal glomerulonephritis may be present in the absence of clinical abnormalities.

We reported our experience with the course of diffuse glomerulonephritis during bacterial endocarditis as seen predominantly in heroin abusers (81,82). Renal functional impairment on initial presentation ranged from mild to advanced renal failure. No correlation existed between the duration of symptoms prior to presentation and the initial and peak serum creatinine level. In the majority of patients, serum creatinine reached maximal levels prior to initiation of antibiotic therapy or during the early phase of treatment. Advanced renal insufficiency on initial presentation was associated with both failure of bacterial cure and failure to recover renal function. It is unclear whether the presence of severe renal insufficiency hindered the eradication of infection or whether more "severe" endocarditis gave rise to more severe glomerulonephritis. By contrast, effective antibiotic therapy led to recovery of renal function, normalization of the serum complement level, and remission of the clinical features of glomerulonephritis. Renal recovery occurred consistently in those patients in whom renal functional impairment was mild or moderate.

Microscopic hematuria and proteinuria are universal in endocarditis-associated diffuse glomerulonephritis (81). Gross hematuria may also be observed or may result from renal infarction or concurrent drug-induced interstitial nephritis. It is generally thought that the nephrotic syndrome is a rare feature; however, the nephrotic syndrome has been reported in 16% of histologically confirmed cases of glomerulonephritis in patients with bacterial endocarditis (81). Fifty-six percent of those with nephrotic syndrome were parenteral drug abusers with *S. aureus* bacterial endocarditis (81). Diffuse glomerulonephritis was present in most (81). These data, however, may overestimate the true incidence of nephrotic syndrome because patients with heavy proteinuria are more likely to be subjected to renal biopsy. Hypertension is rarely described despite its frequent occurrence in other forms of post infectious endocapillary glomerulonephritis (81). Because of the difficulty in differentiating cardiac, nephrotic, and "nephritic" edema in patients with endocarditis and renal disease, the contribution of primary renal sodium retention cannot be determined with certainty.

Outcome

With control of infection by antibiotic therapy, the urinary abnormalities normalize in most patients within several days to several weeks (81). Occasionally, microscopic hematuria or proteinuria may persist for months to years after bacteriologic cure (81). In the majority of cases, renal insufficiency of mild to moderate severity resolves promptly with successful antibiotic therapy. Infrequently, transient worsening of renal function may occur prior to recovery (81). Despite the worst prognosis in patients who present with uremia, even severe renal failure may resolve with successful antibiotic therapy, sometimes with improvement continuing over weeks or months. Serial renal biopsies may reveal almost complete histological resolution of glomerulonephritis in association with clinical recovery (81).

Renal failure requiring dialysis may persist despite bacteriologic cure (81,82). This might occur more frequently were it not for the high mortality associated with uremia due to failure of antibiotic therapy. Among those cured of endocarditis, later deaths due to chronic renal failure secondary to glomerulonephritis acquired during the active phase of endocarditis have occasionally been reported (81). Persistent renal insufficiency, nephrotic syndrome, and sediment abnormalities have been described in parenteral drug abusers after bacteriologic cure of *S. aureus* endocarditis (92,93). Our own studies demonstrate that glomerulonephritis acquired during the course of endocarditis may occasionally prove to be irreversible despite bacteriologic cure and may result in persistent renal insufficiency or sediment abnormalities (82). A role for corticosteroids, cytotoxic agents, or plasmapheresis has been suggested in patients with bacterial endocarditis and diffuse proliferative glomerulonephritis with extracapillary proliferation in whom renal function fails to recover with antibiotic therapy alone (94). However, these recommendations are based solely on isolated case reports and require confirmation with controlled, randomized studies.

Acute Renal Failure Due to Nontraumatic Rhabdomyolysis in Drug Abusers

Nontraumatic rhabdomyolysis in drug users is a well-recognized clinical syndrome. Although cocaine (95–129) and heroin (122,130–161) are the agents most frequently responsible, the syndrome has also been described after intravenous or oral administration of amphetamine, methamphetamine, phencyclidine, methadone, and barbiturates and other sedatives singly or in combination (138,162–174). In particular, phencyclidine may cause rhabdomyolysis during periods of involuntary isometric activity against mechanical restraints (168,169). Intra-arterial injection of temazepan formulated as a gelatin capsule or hard gel may cause acute renal failure due to particulate embolization with rhabdomyolysis (173). However, temazapan is no longer available in these formulations. Tetanus or self-injection of water may rarely cause nontraumatic rhabdomyolysis in drug abusers (30,37).

Prolonged pressure on dependent muscles during extended periods of depressed consciousness and immobilization resulting in vascular compromise, is generally believed to be responsible for heroin-associated rhabdomyolysis (36,37,130–137). This sequence results in compression myonecrosis and ischemic injury. However, a number of cases of heroin-associated rhabdomyolysis have occurred in the absence of depressed consciousness or muscle compression (134,145–152). Rhabdomyolysis in these instances has been attributed to a direct toxic effect or an allergic reaction to heroin or a contaminant. Instead of the typical localized muscle involvement seen with pressure myonecrosis, generalized and symmetric muscle swelling may be observed in these cases (150–152). Lending further credence to a toxic or allergic pathogenesis is the occasional occurrence of swelling of an entire extremity after local injection of heroin (150). In several cases, myocarditis appeared to accompany nontraumatic rhabdomyolysis due to heroin (148,152,153); however, in only one case was myocardial involvement confirmed histologically (152).

Most heroin abusers with nontraumatic rhabdomyolysis are young men between the ages of 18 and 41 years (mean, 25 years). Prolonged muscle compression during extended periods of stupor and immobilization was present in the vast

majority. The degree and duration of stupor usually determined the severity of rhabdomyolysis and the occurrence and severity of acute renal failure (37). Hypoxia, volume depletion, acidosis, hypotension, and disturbances in temperature regulation, which frequently accompany drug overdose, contributed to the occurrence of renal failure in this setting (134,154,155). Clinical evidence of muscle injury such as swelling and tenderness of involved muscles or localized pain and weakness was frequent; however, these findings may be absent or overlooked. Elevation of serum levels of muscle enzymes and myoglobinuria were characteristic features. Acute renal failure occurred in two-thirds of cases and was frequently oliguric. Prognosis was excellent, with full recovery in all but several patients who died during oliguric acute renal failure (150,152).

The first reports calling attention to the association between cocaine abuse and nontraumatic rhabdomyolysis appeared in 1987 (95–97). Well over 100 cases have been described to date (95–129,174–180). The vast majority were male, and their average age was 30 years. Ischemic myonecrosis due to cocaine-induced vasoconstriction may be primarily responsible for rhabdomyolysis; however, cocaine has also been shown to have direct toxic effects on muscle *in vitro* (181). Most patients experienced complications of cocaine use that may have contributed to the development of rhabdomyolysis or may have been primarily responsible for muscle injury. These complications included hypotension, seizures, hyperpyrexia, and muscle compression following prolonged immobilization. Others abused cocaine together with heroin or alcohol—drugs that are themselves associated with muscle injury. Cocaine may be contaminated with arsenic, strychnine, amphetamine, and phencyclidine, which may cause seizures and rhabdomyolysis (173). Myalgia or muscle tenderness accompanied cocaine-induced rhabdomyolysis in one-half of cases. Cocaine was most frequently administered by intravenous injection or by smoking of the cocaine alkaloid. It has been suggested that renal failure is unrelated to the route of administration (155); however, we found that intravenous cocaine was associated with more severe muscle injury and a greater incidence of renal failure (105,107).

Urinalysis typically showed a positive orthotolidine reaction for heme. Proteinuria was detected by dipstick in two-thirds of cases. Renal failure developed in nearly one-half of the patients. Of those who developed renal failure, one-half were oliguric and an equal number required dialysis. Roth and associates (104) found evidence of more severe muscle injury in patients with cocaine-induced rhabdomyolysis who developed acute renal failure. Mean serum creatine phosphokinase levels in those who developed renal failure were 150 times the upper limit of normal and twice as high as in the group as a whole (104). In our own series, mean peak serum creatine phosphokinase levels exceeded 90,000 IU/L in cocaine addicts with rhabdomyolysis who developed acute renal failure as compared to a mean peak value of approximately 1,000 IU/L among nonazotemic addicts (107). Hypotension, hyperpyrexia, volume contraction, diffuse intravascular coagulation, and severe hepatic dysfunction were associated with the development of acute renal failure in both series as well as in many individual case reports. Of the 13 patients with renal failure reported by Roth and associates (104), 6 died. In contrast, we observed an excellent renal prognosis (107). In view of the rapid proliferation of cocaine addiction among young adults, cocaine-induced rhabdomyolysis has become an increasingly frequent cause of acute renal failure in urban areas.

Cocaine-Associated Nephropathy

Although the most frequent renal manifestation of cocaine abuse is acute renal failure due to nontraumatic rhabdomyolysis, cocaine may induce nephropathy by other mechanisms (182–209). In several cases, cocaine use was associated with acute renal failure in the absence of rhabdomyolysis or accelerated hypertension (207–209). Renal failure was attributed to cocaine-induced vasoconstriction leading to acute tubular necrosis. Cocaine may also precipitate malignant hypertension with rapid progression to end-stage renal failure (175,182,183,210).

Cocaine may unmask, exacerbate, or induce scleroderma and has been responsible for precipitating scleroderma renal crises (184,186). Cocaine is a potent arterial vasoconstrictor and may contribute to thrombosis by its ability to increase platelet aggregability, enhance thromboxane production, and induce endothelial injury. Renal infarction has been attributed to cocaine-induced renal artery vasospasm or arterial thrombosis (200,204,211,213). Cocaine use has also been associated with the development of microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure due to thrombotic microangiopathy in the absence of hypertension (187,188). Isolated case reports have linked cocaine with a variety of glomerular and tubulointerstitial renal diseases, including anti-glomerular basement membrane disease, acute interstitial nephritis, and systemic necrotizing vasculitis; however, these associations have not been clearly established (189–191,205).

In the rat kidney, cocaine has been shown to enhance mesangial cell uptake of macromolecules, indirectly stimulate mesangial cell proliferation, and induce release of macrophage secretory products, including interleukin-6 and transforming growth factor β (192,193). In addition, chronic administration of cocaine to rats causes renal injury leading to glomerular sclerosis and chronic tubulointerstitial damage (194). However, these experimental observations may not translate to human disease. It has been suggested that cocaine use may be a risk factor for hypertensive end-stage renal disease in African Americans (10,183,214–216). However, convincing evidence of a cause and effect relationship remains to be established.

Vupputuri et al. (215) found that the relative risk for mild renal functional impairment among 637 men enrolled in a hypertension clinic was increased to 2.3 with the use of any illicit drug, to 3.0 with cocaine use and to 3.9 with psychedelic drug use. Use of marijuana, amphetamines, or heroin did not lead to a significant increase in risk. In a cross-sectional study of 193 ESRD patients, Norris et al. (216) found that a clinical diagnosis of hypertensive nephrosclerosis was strongly associated with a history of cocaine use. Cocaine users had an earlier onset of ESRD despite only a brief history of hypertension. In contrast, Breclin and colleagues (195) studied blood pressure measurements in 301 predominantly African American males hospitalized for cocaine addiction treatment and concluded that cocaine use is associated with acute but not chronic hypertension. There was no significant difference in age-adjusted blood pressure in the cocaine users compared to National Health and Nutrition Examination Survey groups. In addition, microalbuminuria was not detected in these patients who had used cocaine for a mean of 12 years.

Several reports have attributed accelerated arteriosclerosis of the renal vasculature to cocaine use even in the absence of hypertension (196–199). DiPaolo and associates (197) compared 40 renal autopsy specimens from cocaine-related deaths with 40 specimens obtained from road accident victims. Kidneys from cocaine addicts showed extensive arteriosclerosis with medial thickening, luminal narrowing, and vessel obstruction, which were absent in control cases. In association with these vessel changes, cocaine addicts showed significantly more glomerular hyalinosis, periglomerular fibrosis, and interstitial cellular infiltration. The pathogenesis of accelerated hyperplastic atherosclerotic damage in cocaine addicts is speculative but may relate to repeated episodes of cocaine-induced vasospasm,

mast cell activation, activation of the renin-angiotensin system, or cocaine-stimulated thromboxane production, platelet aggregation, or collagen synthesis. In addition, cocaine may modulate mononuclear cell infiltration of atherosclerotic plaques and may adversely affect lipoprotein metabolism (196).

Teratogenicity

Exposure to cocaine *in utero* due to maternal use has been associated with a more than four-fold risk of developing congenital urinary tract abnormalities (217–226). Hydronephrosis, horseshoe kidney, prune belly syndrome, renal agenesis, prominent renal pelvis, nephromegaly, unilateral small kidney and genital organ abnormalities have been described in association with *in utero* cocaine exposure (218,221). Reduced urine output has been observed in fetuses exposed to cocaine (223,225). Stillborn fetuses exposed to cocaine show thickening of the renal interlobular artery walls with narrowing of the lumen (224). These vascular abnormalities are reflected in higher renal artery resistance indices (227). Severe hypertension due to renal artery stenosis and persistent postnatal hypertension have been described after prenatal cocaine exposure (226,228). Renal vascular disease and congenital urinary tract abnormalities are also observed in experimental models of in utero cocaine exposure (220). In addition, an increased incidence of urinary tract infections has been observed in infants after prenatal cocaine exposure (229). It has been postulated that cocaine-induced vasoconstriction of uterine and fetal blood vessels may lead to developmental abnormalities. Alterations in calcium metabolism may also contribute. Persistent postnatal hypertension in the absence of renal anomalies has been attributed to dysfunction of the sympathetic nervous system (228).

Ecstasy (3–4 methylenedioxymethamphetamine) (MDMA) has been associated with acute renal failure, accelerated hypertension and electrolyte disturbances (173,230–239). A syndrome of hyperthermia, coagulopathy, rhabdomyolysis with acute renal failure, and hepatic failure may complicate ingestion of this agent (230,231,234,235). Acute renal failure may also arise as a result of accelerated hypertension (237). MDMA may cause inappropriate release of antidiuretic hormone with resultant hyponatremia and has also been reported to cause urinary retention due to bladder neck closure (173).

Systemic Necrotizing Vasculitis in Parenteral Drug Abusers

Citron and co-workers (240) described systemic necrotizing vasculitis in parenteral drug abusers, which was clinically similar to classic polyarteritis nodosa, and attributed this syndrome to abuse of amphetamines. Although a variety of drugs were involved, methamphetamine had been abused by all but 2 of their 14 patients and was used exclusively by one of them. An experimental model of amphetamine-induced cerebral vasculitis was developed in the rhesus monkey (241). Cerebral angiography demonstrated vessel spasm, with occasional microaneurysm formation, while histology showed generalized cerebral edema, hemorrhage, and infarction. However, the validity of this model has been questioned because severe hypertension is produced, which itself may be responsible for the histological and angiographic features observed (242).

An association exists between classic polyarteritis nodosa and hepatitis B surface antigenemia (243). Hepatitis B surface antigen has been found in the serum of 4% to 13% of parenteral drug abusers (244–247). Accordingly, some investigators have proposed that systemic necrotizing vasculitis in parenteral drug abusers may be related to hepatitis B surface antigen, thus discounting a pathogenetic role for amphetamine (248). When sought, hepatitis B surface antigen has been found in the serum of some, but not all, drug abusers with systemic necrotizing vasculitis (242,248–252). In one study, hepatitis B surface antigen was demonstrated in fewer than one-third of cases (250). However, Koff and colleagues (248) describe one abuser of heroin and amphetamine with systemic necrotizing angiitis in whom hepatitis B surface antigenemia was transient. More recently, systemic necrotizing vasculitis has been associated with hepatitis C infection and cryoglobulinemia. It is possible that hepatitis B surface antigen-negative drug users with systemic necrotizing vasculitis reported in earlier series may have been infected with hepatitis C. Thus, it is unclear what role methamphetamine use, hepatitis B infection, and hepatitis C infection play in the pathogenesis of systemic necrotizing angiitis in drug abusers.

The clinical course and angiographic features of systemic necrotizing vasculitis have been described in 39 parenteral drug abusers, and in addition, isolated cerebral angiitis has been described in 20 others (36,240,242,248–259). Patients with widespread vasculitis ranged in age from 18 to 47 years (mean, 24 years); one-half were male. The duration of parenteral drug abuse invariably exceeded 3 months. When specified, amphetamines were among the agents abused in all but two patients, usually in association with multiple other drugs. Hepatitis B surface antigen was demonstrated in the serum of fewer than one-third of the 30 patients evaluated by Citron and Peters (250). Hepatitis B surface antigen was sought in five additional patients and was present in all (242,248,249,251,252). In five asymptomatic patients, the diagnosis was made on the basis of typical angiographic findings consisting of arterial aneurysms and sacculations involving multiple organs (240). In symptomatic cases, the duration of illness prior to presentation ranged widely from several days to 1 year. The severity of hypertension observed in this syndrome and its refractoriness to therapy has been emphasized (249). Hypertension was present in 13 of 17 patients. Malignant hypertension associated with papilledema, encephalopathy, or rapidly progressive renal failure occurred in six patients.

Renal manifestations were present in 4 of the 14 patients reported by Citron and co-workers (240). Uremia developed in two cases and hematuria, proteinuria, or azotemia were present in the other two. Renal involvement was seen in a total of 11 patients; 7 of whom were uremic. Severe or accelerated hypertension was characteristic of all but one of those who developed renal failure. Small kidneys with segmental ischemic atrophy, reflecting old as well as more recent renal infarctions, were characteristic findings at postmortem examination. Necrotizing arteriolitis frequently involved the interlobular and arcuate arteries. Vascular changes of different ages often coexisted. Glomerulonephritis was not observed.

Of the 11 patients with renal involvement, 3 died in uremia and 4 others died due to visceral infarction, cardiovascular complications, or aneurysmal rupture. One patient underwent renal transplantation. Follow-up was not available in the remaining three uremic patients. The response to immunosuppressive therapy has been inconsistent. Four patients responded favorably to therapy with steroids or cytotoxic agents. In one of these, cytotoxic therapy induced a remission despite an equivocal response to steroids. However, relapse occurred after discontinuation of therapy. Two patients died and a third developed accelerated hypertension and renal failure during corticosteroid therapy.

Nephropathy in Glue and Solvent "Sniffers"

Inhalation of the fumes of toluene or toluene-containing compounds (spray paint, household and model glue, lacquer and paint thinners) may be associated with a variety of electrolyte

and acid–base disturbances (260–281). Taher and co-workers (260) first recognized nonanion gap hyperchloremic metabolic acidosis in association with toluene "sniffing" in two patients, one of whom was studied in detail. Urine pH was inappropriately high, and studies of urinary bicarbonate excretion suggested a distal renal tubular acidosis. Azotemia was absent, and proximal tubular reabsorption of solutes was unimpaired. The acidifying defect was rapidly reversible but recurred with repeated episodes of toluene abuse. Renal potassium wasting was attributed to hyperaldosteronism, and generalized muscle weakness and flaccid quadriparesis resulted from severe hypokalemia. Numerous other cases of hyperchloremic metabolic acidosis due to toluene inhalation have subsequently been reported (261–280). In addition, transient congenital renal tubular dysfunction with hyperchloremic metabolic acidosis due to maternal toluene abuse has been described (267,268,270,281).

Moss and colleagues (263) reported proximal tubular dysfunction with toluene abuse. They describe a patient with hyperchloremic metabolic acidosis, hypokalemia, hypouricemia, hypophosphatemia, and hypocalcemia. Proximal reabsorption of uric acid, glucose, phosphate, calcium, and amino acids was impaired. The high fractional excretion of bicarbonate suggested a proximal defect, while submaximal urinary acidification during ammonium chloride loading suggested an additional distal defect. Microscopic hematuria and pyuria were present. Despite abstinence from toluene, hyperchloremic metabolic acidosis persisted, whereas the other tubular defects reversed, only to recur with repeated exposure. Two additional cases with hyperchloremic metabolic acidosis due to proximal bicarbonate wasting were described in this report (263).

Streicher and associates (265) reported 25 cases of toluene abuse associated with hypokalemia in 13 patients, hypophosphatemia in 10 patients, and hyperchloremic metabolic acidosis in 19 patients. Hypokalemia and hypophosphatemia contributed to muscle weakness, which was a frequent presenting complaint and which progressed in some to quadriparesis. Several patients developed nontraumatic rhabdomyolysis, perhaps related to the combined effects of hypokalemia and hypophosphatemia on skeletal muscle. In other cases, rhabdomyolysis occurred in the absence of these electrolyte disturbances, leading the authors to suggest that toluene may have direct toxic effects on skeletal and cardiac muscle. Hypokalemia was attributed to a redistribution of extracellular potassium. Despite normal values at presentation, serum calcium levels declined during fluid repletion, leading to symptomatic hypocalcemia in some patients.

Batlle and colleagues studied the mechanisms underlying toluene-induced distal renal tubular acidosis (274). Toluene abusers showed normal bicarbonate reabsorption but were unable to maximally acidify their urine. Inability to normally increase urinary partial pressure of carbon dioxide (P_{CO_2}) in a highly alkaline urine excluded hydrogen ion back-diffusion as a cause of the acidification defect. Toluene decreased the rate of proton secretion in a turtle urinary bladder preparation, leading the authors to conclude that the defect in distal acidification observed in toluene abusers is due to impaired active hydrogen ion transport.

An elevated anion gap was found in approximately one-third of reported cases of toluene-induced metabolic acidosis (273). The elevated anion gap is due to the accumulation of products of toluene metabolism (benzoic acid and hippuric acid). These metabolites are more likely to accumulate and cause anion gap acidosis in those with impaired renal function. In contrast, in individuals with normal renal function, overproduction and urinary excretion of the anions of these organic acids may contribute to the development of nonanion gap hyperchloremic metabolic acidosis (261,273).

Heavy toluene exposure has been associated with pyuria in nearly one-third of reported cases, while microscopic hematuria and proteinuria were each observed in approximately one-fifth (264,282–284). In the series reported by Streicher and associates (265), abnormalities of the urinary sediment were particularly frequent, occurring in 18 of 21 patients, and included hematuria, pyuria, and proteinuria ranging up to 1 g/24 hours. Similarly, pyuria was universal, and hematuria and proteinuria each occurred in three-fourths of the 16 episodes of toluene intoxication reported by Voigts and Kaufman (264).

Acute renal failure has been observed in association with toluene inhalation (227,261,264,285–292). Transient renal insufficiency was observed in only one of the 25 cases reported by Streicher and associates (265). By contrast, Voigts and Kaufman (264) describe transient renal insufficiency in 38% of 16 episodes of toluene intoxication. Renal failure may be caused by nontraumatic rhabdomyolysis, hypotension-induced acute tubular necrosis, or in association with hepatic failure (261,285–286,289,290). Serial renal biopsies in one patient with irreversible acute renal failure demonstrated progressive tubulointerstitial injury (287). Of interest is a report of a long-term regular toluene abuser who developed recurrent nephrolithiasis due to toluene-induced distal renal tubular acidosis causing bone calcium mobilization and hypercalcuria (262). Renal calculi have also been observed in other chronic toluene abusers (269,292,293). Anti-glomerular basement membrane–mediated glomerulonephritis, focal glomerulosclerosis, and membranoproliferative glomerulonephritis have been associated with glue sniffing, however, a causal relationship has not been established (294–301).

Acute renal failure has been reported in inhalation abusers of trichloroethylene and other compounds contained in cleaning fluid and other solvents ("solvent sniffers") (302,303). Oliguria and renal insufficiency, with or without associated acute centrilobular necrosis of the liver, were reversible in all cases. Modest proteinuria was a universal feature, whereas microscopic hematuria was infrequent. Similarly, occupational inhalation exposure to trichloroethylene has been associated with reversible acute oliguric renal failure, abnormalities of the urinary sediment, and fatal combined hepatic and renal failure (304–308). Examination of renal tissue in one case showed acute tubular necrosis. Anti-glomerular basement membrane–mediated glomerulonephritis and hemolytic—uremic syndrome have also been associated with solvent abuse (309,310).

Hepatitis-Related Glomerulonephritis in Drug Abusers

Parenteral drug abusers are frequently infected with hepatitis B or hepatitis C (244–247). The association of these infections with glomerular disease is discussed in Chapter 59.

Human Immunodeficiency Virus-Associated Nephropathy in Drug Abusers

This subject is discussed in Chapter 59.

LITHIUM AND THE KIDNEY

Early histopathologic studies of the kidney in lithium-treated psychiatric patients raised great concern about the potential for serious nephrotoxicity (311,312). However, these disturbing estimates of the frequency and severity of lithium-induced renal injury were based on uncontrolled studies of patients who were selected because of prior acute lithium intoxication or

severe polyuria (311,312). Clearly, lithium intoxication may be associated with acute deterioration of renal function, which can be followed by irreversible renal damage (313–316). However, the key question is whether maintenance therapy with doses in the therapeutic range may cause chronic renal injury and if so, how often this occurs. Based on the publication of more rigorous studies, a more accurate picture of lithium nephrotoxicity has emerged. However, even these studies have failed to fully define the spectrum of lithium nephrotoxicity.

Adequacy of Clinical Studies

Results of studies evaluating renal function in lithium-treated patients must be interpreted cautiously, with attention to differences in methodology and experimental design. Published reports differ with respect to sample size, criteria for patient selection, treatment duration, dose and type of lithium preparation, plasma lithium levels, concurrent therapy with other psychotropic agents, and the reference range used to determine normality. Exclusion of preexisting renal disease by pretreatment evaluation has not been a consistent feature.

Studies lacking a suitable control group are of limited informational value. An ideal control group would consist of patients suffering from affective disorders who have never received lithium and who are well matched for demographic factors including use or other psychotropic agents. In addition, behavioral factors such as psychogenic water drinking cannot be controlled. Prospective longitudinal studies, as opposed to the cross-sectional approach, provide the most reliable assessment of the effects of lithium on renal function. However, no longitudinal study has included a matched psychiatric group to control for the effects of aging and of other psychotropic agents on renal function.

Clinical Features of Lithium Nephrotoxicity

Acute Renal Failure

Acute lithium intoxication severe enough to require hospitalization is associated with renal insufficiency in up to 75% of cases (313,314,317–325). Circulatory collapse contributes to acute renal failure in some cases (319). Saline loading may improve renal function, suggesting that volume depletion due to lithium-induced natriuresis contributes to impaired renal function. However, a reduced glomerular filtration rate (GFR) frequently persists even after volume expansion (313,314,320). Examination of renal tissue in patients with acute renal failure associated with lithium intoxication has demonstrated proximal tubular necrosis, minimal histologic abnormalities, or nonspecific findings of distal tubular injury (319,321,325). In some, chronic tubulointerstitial nephritis has been found in the absence of acute tubular injury (313,314). Renal insufficiency may persist after recovery from an episode of lithium intoxication (313,314). In addition, rare cases of nontraumatic rhabdomyolysis have been described in association with lithium-induced hyperosmolar dehydration or neuroleptic malignant syndrome (326,327).

Chronic Renal Insufficiency

Whether or not lithium therapy may lead to progressive renal failure in the absence of clinical episodes of intoxication has been debated for decades. Identification of occasional patients with severely impaired renal function in cross-sectional or prospective studies of lithium-treated patients has limited informational value because renal disease unrelated to lithium cannot be excluded. The failure to identify patients with end-stage renal failure in longitudinal studies does not necessarily exclude the possibility that a small subgroup may progress to uremia or that the duration of follow-up was insufficient. Until recently, remarkably few patients have been reported in whom advanced renal functional impairment or end-stage renal failure was attributed to lithium therapy (319,328–334).

Thirteen longitudinal studies measured serum creatinine or GFR prior to initiation of lithium therapy and again after periods of treatment ranging from 1 to 10 years (337–349). In addition, 13 longitudinal series measured serum creatinine or GFR on repeated occasions during the course of lithium therapy (315,334,335,338,346,350–357). No significant change in serum creatinine was observed in 16 of these reports after an average follow-up period of 4.5 years (315,335,340,343–348,351–356). A modest increase in serum creatinine, on the order of 10% to 20%, was reported in four studies over a follow-up period averaging 7 years (341,342,347,349). The rise in serum creatinine was not a general phenomenon but was restricted to a small number of patients. When filtration rate was measured by clearance techniques, GFR was unchanged in several series after an average follow-up period of 5 years (339–342,344,345). Other studies showed a 6% to 20% decline in GFR after a follow-up period that averaged 4 years (315,335,337,338,347,350,355–358). However, none of these studies was properly controlled for the effects of aging on renal function. Lokkegaard (347) evaluated 142 lithium-treated patients and found only a modest decline in GFR after 5 to 17 years, with none falling below the range of normal until after 7 years of therapy. It has been suggested that GFR only begins to decline after a decade or more of therapy (359,360). A prospective, longitudinal study of 14 lithium-treated patients performed by Presne et al. (334) found that creatinine clearance declined 1.93 mL/min/year over a mean follow-up period of 18.9 years (discussed in greater detail below).

Ten cross-sectional series compared GFR or serum creatinine levels in lithium-treated patients with a control group of patients suffering from affective disorders who had never received lithium (nine studies), with healthy controls (one study), or with both (one study) (316,351,355,361,370). In four of these studies, serum creatinine did not differ between lithium-treated patients and controls. In three others, a modest increase of serum creatinine, on the order of 10%, was observed in lithium-treated patients. Creatinine clearance was measured in six studies in which the average duration of lithium therapy was 6 years and was found not to differ between lithium-treated patients and controls (316,350,353,355,361,369). In another study, creatinine clearance was 42% lower in patients treated with lithium for 80 months than in lithium-naïve controls (370). In two studies, GFR was measured isotopically after an average duration of 7.5 years on lithium and was found to be modestly reduced (363,364).

Renal function has been assessed in lithium-treated patients in 24 cross-sectional reports that lacked any control group (329,330,359,366,371–391). Serum creatinine was found to be modestly elevated above the normal range in approximately 5% of patients. Five observers failed to demonstrate any reduction in GFR below the range of normal in lithium-treated patients (374,381,383,387,389). Other investigators found that GFR measured by creatinine clearance, isotopically, or by inulin clearance was modestly reduced below the range of normal in 3% to 36% of lithium-treated patients (359,366,367,371,372,376–380,382,384,385,388,390,391).

Data are inconsistent with respect to the relationship between various therapeutic variables and renal function. Several studies have found an inverse correlation between GFR and duration of lithium therapy (334,357,375,389,390,392). However, numerous other investigators have failed to confirm this observation (315, 351, 364, 368–370, 373, 377, 380, 382, 384, 385,389,391). Most have failed to find any correlation between GFR and average serum lithium level or total cumulative

dose (334,341,345,347,368–371,379,380,382,384); however, contrary data exist (334,347).

In summary, in most studies, adverse effects of lithium on GFR were modest and of little clinical significance. However, two recent studies suggest that chronic administration of lithium over decades may lead to end-stage renal failure (332,334).

Markowitz et al. (332) describe 24 lithium-treated patients who underwent renal biopsy due to renal insufficiency. The patients ranged in age from 26 to 57 and were predominantly white; 50% were men. The duration of lithium therapy ranged from 2 to 25 years (mean 13.6 years). Serum creatinine ranged from 1.3 to 8.0 mg/dL (mean, 2.8 mg/dL). Proteinuria exceeded 1 g/day in 10 patients, 6 of whom manifested the nephrotic syndrome. Only two patients had experienced prior episodes of acute lithium intoxication. Renal biopsy showed chronic tubulointerstitial nephropathy, associated in all but one case with cortical and medullary tubular cysts or dilation. Focal segmental glomerular sclerosis was seen in 50% and global glomerulosclerosis in 100% of biopsies. Despite discontinuation of lithium therapy in 19 of 20 patients, 8 progressed to end-stage renal failure over a mean of 30.6 months. A serum creatinine of 2.5 mg/dL or greater predicted progression to ESRD despite discontinuation of lithium. Renal function improved in only three patients, all of whom discontinued lithium before the serum creatinine exceeded 2.5 mg/dL. These authors conclude that renal dysfunction due to lithium is often irreversible, may be associated with proteinuria due to glomerular involvement, and may progress to end-stage renal failure.

Presne et al. (334) studied 54 patients with lithium-induced renal insufficiency and an additional 20 lithium-treated psychiatric patients, 14 of whom were studied prospectively. Eighteen percent had proteinuria, which exceeded 1 g/day approximately 10%. Microscopic hematuria was present in 24% and leucocyturia in 31%. After a mean of 10 years, calculated creatinine clearance fell from 62.6 to 41.4 mL/minute at a rate of 2.29 mL/min/year. Heavy proteinuria, which exceeded 750 mg/24 hours, was associated with a more rapid decline in renal function. Twelve patients reached end stage renal failure. They had higher serum creatinine levels at entry and two-third had proteinuria. Few had a history of lithium intoxication. Renal functional impairment generally required a duration of lithium therapy exceeding 20 years. Discontinuation of lithium when the calculated creatinine clearance exceeded 40 mL/min was usually associated with improvement in renal function, whereas discontinuation of therapy when renal function was more impaired was usually associated with continued deterioration. In the 14 patients studied prospectively, calculated creatinine clearance fell at a rate of 1.93 mL/min/year from 77.8 to 40.8 after a mean follow-up of 18.9 years. Presne et al. (334) performed renal biopsies in 29 patients (334). Interstitial fibrosis and tubular atrophy was present in 85%. Interstitial fibrosis was predictive of the final creatinine clearance and correlated with the duration of lithium administration and the cumulative dose. Tubular cysts were present in 28% and tubular dilation in 66% and correlated with the duration of lithium administration but not with renal function. The median percentage of sclerotic glomeruli was 10% (range 0% to 90%); only one patient had focal glomerulosclerosis.

These investigators surveyed dialysis centers in France and identified 24 patients in whom end-stage renal failure was attributed to lithium (0.22% of the dialysis population). The average duration of lithium administration prior to the development of end-stage renal failure was 20 years. By comparison, the incidence rate of end-stage renal disease attributed to lithium in Australia is 0.7% and in New Zealand, 0.2%.

In the series reported by Presne et al. (334), it is difficult to evaluate the relative contributions of concomitant neuroleptic therapy, hypercalcemia, and hypertension to progressive renal injury. Only 1 of the 12 patients who progressed to end-stage renal disease underwent renal biopsy. Segmental glomerulosclerosis was present in only 1 of the 29 renal biopsy specimens, including nine cases selected for lithium-induced renal insufficiency. It is difficult to reconcile the fact that focal and segmental glomerulosclerosis was common among the patients reported by Markowitz et al. (332), but rare in the series reported by Presne et al. (334). Nevertheless, prudence dictates an awareness of the potential for nephrotoxicity, as well as periodic monitoring of renal function and utilization of the lowest serum lithium levels capable of achieving a therapeutic response.

Urinary Concentrating Ability

Six longitudinal studies evaluated urinary concentrating ability before initiation of lithium therapy and again after treatment periods ranging up to 8 years (337,338,341,342,393,394). Most showed a 10% to 15% decline in maximum urinary osmolarity (Uosm) within weeks to months after initiation of therapy (338,393,394). Schou and Vestergaard (341) report a large cohort of patients before and up to 7 years after initiating lithium therapy. The investigators maintained a mean serum lithium level of 0.6 mmol/L, which was lower than previously recommended therapeutic serum levels (0.8 to 1.2 mmol/L). Maximum Uosm declined 7% from pretreatment levels and was abnormal in only 6% of patients (341). Similarly, two other reports in which serum lithium levels were maintained below 0.7 mmol/L found little decline in maximum Uosm after 3 and 8 years of treatment (337,342). In three studies, urine volume increased only modestly from prelithium levels after periods of treatment ranging up to 8 years, while in a fourth, urine volume increased by 23% (338,341,342,394). In patients maintained on a low therapeutic serum lithium level treatment regimen by Schou and Vestergaard (341), mean urine volume rose by only 10% to 20%, and the number of patients with severe polyuria did not increase.

Six longitudinal studies performed serial measurements of urinary concentrating ability in patients treated with lithium for 3 to 10 years and again after an additional treatment period ranging up to 10 years (315,335,338,355,356,357,363,395). Two failed to find any interval decline in urinary concentrating ability when first assessed 5 to 10 years after initiation of treatment and again after an additional treatment period of 2 to 10 years (315,335,363). By contrast, in three other studies, maximum Uosm declined by 7% to 20% between measurements first made 4 to 6 years after initiation of treatment and again after an additional treatment period of 2 to 8 years (355,356,357,395). Urine volume increased by 10% to 20% in two reports but showed no significant interval change in another (335,350,355,363).

Five longitudinal studies measured urinary concentrating ability in unselected patients during long-term lithium treatment and again after discontinuation of therapy (335,345,355,392,396,397). Maximum Uosm increased 10% to 30% within weeks to months after withdrawal of lithium but remained subnormal in all but one study. Bucht and Wahlin (392) examined a large cohort of lithium patients for periods up to 1 year after discontinuation of lithium. Maximum Uosm increased by nearly 30% in the first 8 weeks after lithium withdrawal, but no further improvement was observed thereafter (392). The percentage of patients with abnormal concentrating ability decreased from 96% while on lithium to 63% 1 year after discontinuation of lithium (392). Three studies reported urine volume measurements, which normalized after discontinuation of lithium in two studies and never increased in the third (335,355,396).

Sixteen cross-sectional studies compared concentrating ability in lithium-treated patients with control subjects suffering from affective disorders or with healthy controls (316,339, 351,355,360,361,363–367,369,392,395–400). In 10, urinary concentrating ability was reduced in lithium patients compared to psychiatric controls (316,339,355,360,363–365, 369,370,392,395–397). The average maximum Uosm was 589 mOsm/kg H_2O on lithium (range, 406 to 737 mOsm/kg H_2O) versus 818 mOsm/kg H2O in psychiatric controls (range, 683 to 850 mOsm/kg H2O). On average, 67% of patients treated with lithium for 5 to 10 years (range, 36% to 85%) showed abnormal urinary concentrating ability compared to 34% of psychiatric controls (range, 10% to 48%). By contrast, in three other studies, urinary concentrating ability did not differ between patients treated with lithium for 3 to 8 years and psychiatric controls (351,361,366,367,400). One of the latter reports maintained patients on low therapeutic serum lithium levels, while another utilized a single-dose schedule. Urine volume was increased in lithium-treated patients compared to psychiatric controls in nearly all studies (278,316, 339,360,361,363,364,366,367,369,392,396–398,400). Urine volume averaged 2.6 L/24 hours in lithium-treated patients compared to 1.8 L/24 hours in psychiatric controls. In the one study that failed to show any difference in urine volume between lithium-treated patients and psychiatric controls, patients were maintained on low therapeutic serum lithium levels (366,367).

Twenty-eight investigations evaluated concentrating ability in unselected lithium-treated patients (315,345,347–349,359, 366,367,371,372,374,375,377–379,381,383–391,393,401–405). When compared to the accepted range of normal, the prevalence of impaired urinary concentrating ability among patients treated with lithium for an average of 2 to 19 years ranged from 16% to 85% (mean, 53%), while the prevalence of increased urine volume ranged from 6% to 70% (mean, 24%). Only three groups failed to find any impairment in urinary concentrating ability in lithium-treated patients (366,367,374,381). Of note, two of these maintained patients on low therapeutic serum lithium levels.

Data are inconsistent with respect to the relationship between various therapeutic variables and concentrating ability. An inverse correlation between maximum Uosm and duration of lithium therapy or cumulative lithium dose has often been reported (316,345,347,349,355,357,371,378,379,385,390,392, 395–397,406). A positive correlation between urine flow rate and duration of lithium therapy has also been described (347, 348,374,375,385,389,396,407,408). However, many other studies have failed to confirm one or both of these observations (315,339,341,347,356,369,370,372,378,381,384,389, 391,406). Serum lithium levels correlated inversely with urinary concentrating ability or positively with urine flow rate in some reports (315,339,341,355,378,387,392,407), but not in others (341,347,357,369,370,372,381,384,393,406). Notwithstanding these inconsistent data, the preponderance of evidence suggests that maintenance of low serum lithium levels limits the adverse impact of lithium therapy on urinary concentrating ability and urine flow rate.

Polyuria and Polydipsia

Polyuria and impairment of urinary concentrating ability develop rapidly after initiating therapy and improve after lithium withdrawal (392,409,410). Hypernatremic dehydration may result if access to water is restricted (411–414). Inhibition of vasopressin-stimulated water flow in the collecting duct is primarily responsible for the concentrating defect (254,415–419). However, factors other than resistance to vasopressin may also contribute to lithium-induced polyuria. Typically the defect in concentrating ability is not severe enough to prevent elaboration of a hypertonic urine, and in most patients polyuria cannot be explained solely by the degree of reduction in maximum Uosm. Those who show only a modest impairment in maximum Uosm may nevertheless produce large 24-hour urine volumes. Polyuria may be observed as well, even in patients with normal urinary concentrating ability. This constellation of observations can only be explained if excessive water drinking contributes to the polyuria in many lithium-treated patients. Although lithium clearly stimulates thirst in rat models, it is generally claimed that lithium-induced stimulation of thirst does not play a significant role in the development of polyuria in humans (319,420–424). The finding of normal or elevated plasma levels of vasopressin in lithium-treated polyuric patients is cited as evidence against a prominent role for primary polydipsia (313,366,398,415,425–427). However, since lithium induces resistance to vasopressin, it is reasonable to suggest that these patients operate at an elevated set point and that finding normal or mildly raised plasma vasopressin levels in this setting may still reflect some suppression by excessive water drinking. Increased fluid intake may be psychogenic in origin or due to dry mouth resulting from other psychotropic agents (424).

Thiazide diuretics may ameliorate lithium-induced polyuria through a reduction in solute-free water excretion (420,428–430). Thiazide diuretics induce a natriuresis, which results in a compensatory increase in sodium and lithium reabsorption in the proximal tubule (431). Thiazide diuretics may raise serum lithium levels and precipitate lithium intoxication by inducing extracellular volume depletion (420,429,430). Nonsteroidal antiinflammatory agents may also be used to treat lithium-induced polyuria, albeit, at the risk of precipitating lithium toxicity due to increased tubular reabsorption of lithium (432–443). Inhibition of prostaglandin synthesis increases cyclic adenosine monophosphate through increased production or inhibition of degradation (438). Nonsteriodal antiinflammatory agents also enhance sodium reabsorption leading to a secondary increase in passive water absorption (236). Both these factors contribute to antagonism of lithium-induced polyuria. Amiloride is the drug of choice to raise maximum urinary osmolarity and ameliorate lithium-induced polyuria (444–446). Amiloride antagonizes the inhibitory effect of lithium on vasopressin-induced water transport by blocking the cellular uptake of lithium through the amiloride-sensitive sodium channel (444).

Proteinuria

The effect of long-term lithium therapy on urinary protein excretion is controversial. However, it is clear that in the vast majority of patients any observed effects are modest and of little clinical significance. Administration of lithium to rats increases urinary protein excretion (447). In six longitudinal investigations of lithium-treated patients extending from 90 days to 7 years, no increase in the urinary excretion of protein, albumin, or β2-microglobulin was demonstrated (315,337,341,343,356,448). Similarly, in 11 cross-sectional reports of lithium-treated patients, albuminuria and low molecular-weight proteinuria were absent (361, 365–367,374,381–383,390,391,448). In contrast, one longitudinal and five cross-sectional studies showed modest proteinuria or albuminuria in lithium-treated patients (332,334,365,379,408,449). Markowitz et al. (332) reported proteinuria in 10 of 24 lithium-treated patients undergoing renal biopsy for renal insufficiency (six in the nephrotic range) (332). Similarly, Presne et al. (334) reported proteinuria in 11 of 62 lithium-treated patients; proteinuria exceeded 1 g in 10%. In six other studies, urinary excretion of low–molecular-weight proteins including β2-microglobulin and *N*-acetyl-β-glucosaminidase was increased (369,379,383,408,450,451).

At least 39 cases of the nephrotic syndrome have been described in association with lithium therapy (332,334,359,452–473). The duration of lithium therapy prior to the development of nephrotic syndrome ranged widely from weeks to 14 years. Serum lithium levels were in the therapeutic range in nearly all cases. Most renal biopsies showed minimal glomerular changes. Mesangial hypercellularity or focal or global glomerular sclerosis was observed in other cases. Resolution of the nephrotic syndrome after discontinuation of lithium and recurrence upon rechallenge in several cases strongly support an association between lithium and minimal change disease (452,454,462). In one case, the nephrotic syndrome due to focal glomerular sclerosis remitted after discontinuation of lithium (467), but failed to remit in other cases (332,453,463). Markowitz and colleagues (332) describe the nephrotic syndrome in six lithium-treated patients, all of whom showed focal or global glomerulosclerosis on renal biopsy. These investigators suggest that lithium may be responsible for a podocytopathy.

Pathophysiology

Concentrating Ability

Primary among the factors that contribute to impaired urinary concentrating ability is lithium-induced inhibition of vasopressin-stimulated water flow in the collecting duct (353,415–419). This effect has been attributed to inhibition of adenylate cyclase activity (353,415–419). As a result, lithium blunts vasopressin-stimulated synthesis of cyclic adenosine monophosphate (cAMP), which mediates the hydroosmotic actions of vasopressin in the collecting duct (415,419). Although the primary site of action of lithium on renal concentrating ability is at the luminal surface of the cortical collecting duct, lithium also influences vasopressin-stimulated cAMP synthesis in the medullary thick ascending limb and in medullary collecting tubules (416,484).

Vasopressin binds to the V2 receptor and induces a conformational change, which enables a stimulatory guanylnucleotide regulatory protein to be activated by intracellular guanosine triphosphate (438). Once activated, the stimulatory G protein in turn activates the catalytic subunit of adenyl cyclase to enhance cAMP generation (438). A study of the effects of lithium on medullary collecting tubules in a rat model of chronic oral lithium administration suggests that lithium-induced inhibition of vasopressin-sensitive adenylate cyclase activity is mediated via activation of an inhibitory G protein (475). In contrast, earlier studies suggested that lithium acts by inhibiting activation of the stimulatory G protein (353,476). Magnesium plays an essential role in transducing vasopressin-induced activation of the stimulatory G protein (476). Antagonism of magnesium's actions by lithium may explain the ability of magnesium loading to reverse the inhibitory effects of lithium on adenyl cylase activity.

Studies in a variety of vasopressin-responsive tissues demonstrate that lithium acts at points both proximal and distal to the generation of cAMP (353,393,416–418,477–482). In some vasopressin-responsive tissues, lithium fails to inhibit cAMP–analog-stimulated water flow, suggesting a site of action proximal to the generation of cAMP (481). However, in other tissues, including cortical collecting tubules from lithium-treated rabbits, lithium inhibits the hydroosmotic actions of cAMP analogs, suggesting a site of action beyond the generation of cAMP (393,417,480). Lithium does not appear to enhance degradation of cAMP via activation of phosphodiesterase; however, contrary data exist (418,483,484).

Aquaporin-2 is the apical water channel of the principal cell and the primary target for regulation of collecting duct water permeability by vasopressin (485). Cyclic AMP and protein kinase A are involved in the regulation of acquaporin-2 expression as well as vesicular trafficking of aquaporin-2 (486,487). Lithium-fed rats show decreased expression of aquaporin-2 protein and reduced mRNA levels in cortical and inner medullary collecting duct principal cells (485,488–493). Levels of acquaporin-3 levels are also decreased in the kidney of lithium-treated rats, whereas acquaporin-6 levels are increased and acquaporin-1 and acquaporin-4 levels are unchanged (485,488,492,494,495). In addition, the fraction of intercalated collecting duct cells is increased at the expense of a reduced fraction of aquaporin-2-expressing principal cells (488,496).

Lithium also inhibits delivery of aquaporin-2 to the plasma membrane (494). The presence of a cAMP response element in the 5′ untranslated region of the aquaporin-2 gene suggests that the effects of lithium on aquaporin-2 expression and targeting may be due to inhibition of cAMP production (497). However, lithium's downregulation of aquaporin-2 expression is only partially reversed by 1-desamino-8d-arginine-vasopressin, whereas targeting to the apical plasma membrane is restored (494).

Lithium also decreases vasopressin V2 receptor density but has no effect on binding affinity (498). Some investigators suggest that enhanced synthesis of cyclooxygenase products may also play a role in mediating the effects of lithium on water transport; however, other studies fail to support this hypothesis (475,499–501). Carney and co-workers (502) have suggested that impaired urinary concentrating ability may result from lithium-induced upregulation of circulating parathyroid hormone, which competitively inhibits the hydroosmotic actions of vasopressin. Lithium-induced hyperparathyroidism may also impair concentrating ability via hypercalcemia-induced downregulation of aquarin-2 (485).

Factors other than resistance to vasopressin also contribute to the impairment of concentrating ability induced by lithium. In rare patients, central diabetes insipidus may coexist (319,362,393,399,444,503). Lithium-induced suppression of proximal tubular sodium and water reabsorption increases delivery of filtrate to the ascending limb, thereby increasing solute-free water generation (420,482). This increased delivery of tubular fluid to collecting tubule cells that are resistant to the hydroosmotic action of vasopressin may contribute to polyuria in those with excessive water intake (420,482). In patients with renal functional impairment and a reduced number of functioning nephrons, an osmotic solute diuresis in remaining nephrons may also contribute to impaired urinary concentrating ability. In addition, high urine flow rates and disturbances in sodium transport may impair medullary urea trapping and disrupt the medullary osmotic gradient (418). Whereas earlier direct measurements of the corticopapillary gradient have yielded conflicting results (418,420,504,505), Klein et al. (491) suggest that chronic administration of lithium reduces inner medullary interstitial osmolarity due to reduced interstitial urea and sodium chloride concentration. When administered to rats, lithium down-regulated expression of the urea transporters UT-A1 and UT-B in the renal inner medulla and inhibited vasopressin-stimulated UT-A1 phosphorylation in inner medullary collecting duct suspensions (491). Thus, reduced UTA-1 and UT-B protein and/or reduced sensitivity of UT-A1 to phosphorylation by vasopressin contribute to a decrease the in inner medullary interstitial urea concentration in lithium-treated rats (491).

Structural injury to tubules and interstitial tissue may contribute to reduced maximal Uosm after prolonged lithium administration. If this were the case, then the urinary concentrating defect might progress with increasing duration of therapy and persist in some patients after withdrawal of lithium. Although polyuria frequently corrects within several

weeks after discontinuation of lithium, several longitudinal observations have shown a failure to normalize urinary concentrating ability up to 12 months after lithium withdrawal (311,345,392,396,397,506). Although controversial, several investigators have found a correlation between polyuria or impaired urinary concentrating ability and duration of lithium therapy, lithium dose, or histopathologic lesions (335,349,371,377,383,392,397,403,507). In addition, several patients have been reported in whom polyuria and impaired urinary concentrating ability persisted up to a decade after discontinuation of lithium (396,415,420,508–516). Persistence of impaired urinary concentrating ability was frequently associated with reduced GFR and correlated with chronic tubulointerstitial nephritis (510,511,513).

Normal or elevated plasma levels of vasopressin have been demonstrated in lithium-treated patients with impaired urinary concentrating ability (313,366,398,415,425–427). Hypothalamic responses to water loading and water deprivation are normal in most but not all cases (313–316,370). Lithium-induced central diabetes insipidus has been described in a small number of patients (370,393,399,481,503). Of note, diminished renal response to vasopressin and increased serum levels of vasopressin has been demonstrated in some lithium-treated patients with normal urinary concentrating ability (366). Thus, despite lithium-induced renal resistance to vasopressin, it appears that water balance may be maintained by compensatory increases in serum vasopressin levels in some patients (366).

Sodium Handling

Lithium inhibits reabsorption of sodium by the proximal tubule and by nephron segments beyond the loop of Henle (319,521,522). Although data are conflicting, lithium does not appear to impair sodium transport in the thick ascending limb of the loop of Henle (319,521–525). Lithium directly competes with sodium for tubular transport; however, the importance of this effect in humans is controversial (319,521,522). In addition, lithium antagonizes the tubular sodium-retaining properties of mineralocorticoids (319,526–530).

Lithium induces an immediate sodium and water diuresis, which lasts 1 to 2 days (531,532). Transient natriuresis is followed by a period of sodium and water retention lasting 4 to 5 days, associated with increased serum levels of aldosterone (531,533). This is followed by a return of serum aldosterone to pre-lithium levels and a return to sodium balance (319,533).

Downregulation of the amiloride-sensitive sodium channel (ENaC) contributes, at least in part, to the sodium wasting associated with lithium therapy (492,534). Nielson et al. (492) found a marked reduction in the β and γ subunits of ENaC in the principal cells of cortical and outer medullary collecting ducts of lithium-fed rats. These finding help explain the observation that lithium-treated animals show an impaired renal responsive to amiloride and mineralocorticoids (530). Downregulation of the Na-Cl cotransporter in the distal convoluted tubule may also contribute to lithium-induced sodium wasting, however this finding is controversial (485). In contrast, other major sodium transporters, including the type 3 Na/H exchanger, the α subunit of Na-K-ATPase and the bumetanide-sensitive Na-K-2Cl cotransporter do not contribute to the development of lithium-induced polyuria or increased sodium excretion (485,492). The expression of these transporters after administration of lithium to rats has generally (but not universally) been found to be unchanged or increased, presumably as a compensatory response to sodium wasting (490,493).

Acute administration of large doses of lithium raises plasma renin and aldosterone levels, secondary to lithium-induced natriuresis and perhaps to a direct effect of lithium on the juxtaglomerular apparatus (319,502,531,535,536). Most studies suggest that chronic administration of lithium is associated with normal plasma levels and appropriate responses of the renin–angiotensin axis to various stimuli (319,394,536–538). In contrast, other studies have demonstrated activation of the renin–angiotensin system in patients on long-term lithium therapy, possibly as a compensatory response to lithium-induced mineralocorticoid resistance (539–541).

Renal Lithium Handling

Lithium is not protein-bound and is freely filtered at the glomerulus (313,336,542,543). Approximately 80% of filtered lithium is reabsorbed by the nephron, primarily in the proximal tubule (313,336,542,543). Proximal reabsorption of lithium is enhanced by dietary sodium restriction or extracellular volume depletion leading to reduced urinary lithium excretion and elevated serum lithium levels (320,544). Thus, lithium intoxication may be precipitated by volume depletion induced by diuretic therapy (320,544). Nonsteroidal antiinflammatory agents may also raise serum lithium levels by increasing reabsorption of lithium by the proximal tubule (432–443). Toxic serum lithium levels may in turn exacerbate volume depletion by inducing a salt and water diuresis, which further elevates serum lithium levels (320,544). In addition, combined therapy with lithium and angiotensin-converting enzyme inhibitors has been associated with the development of renal insufficiency and lithium intoxication (545–556). Experimental studies have shown that angiotensin-converting enzyme inhibitors decrease renal lithium clearance by reducing the filtered load and increasing fractional reabsorption of lithium (557). It has been reported that losartan does not alter renal lithium handling or serum levels in the rat (558).

Calcium Metabolism

Lithium therapy is frequently associated with alterations in parathyroid hormone and calcium homeostasis (359,559–596). Therapeutic concentrations of lithium increase the concentration of calcium required for half-maximal inhibition of parathyroid hormone release by parathyroid cells in-vitro (561,562,570,571,585,591). The molecular mechanism responsible for this increase in the set-point for calcium is unclear. It has been suggested that lithium blunts the rise in intracellular calcium in response to an increase in extracellular calcium (570,585), however, other investigators discount any role for intracellular calcium, cAMP generation, or calcium receptor-mediated signal transduction (571,591).

Chronic treatment with lithium is associated with elevated serum calcium levels in 0% to 36% of patients, elevated ionized calcium levels in 25% to 42% of patients and elevated parathyroid hormone (PTH) level in 3% to 42% of patients (334,359,564,573,574,575,586,590,593,594). An even greater number of patients exhibit evidence of hyperparathyroidism as reflected by shift to the right in the relationship between PTH and ionized calcium (574). Serum magnesium levels are also frequently elevated (564–566).

The time course of lithium's effect on calcium and PTH are not entirely consistent (572,573,587,588). One longitudinal study found that ionized calcium rose after several weeks of lithium therapy, but PTH levels rose only after prolonged therapy (572). Other studies indicate that PTH levels rise shortly after initiation of lithium therapy (587,593). Another longitudinal study found that serum calcium and PTH levels rose in 80% of patients but remained within the normal range in the first few weeks after initiation of lithium therapy (573).

In thyro-parathyroidectomized rats, lithium inhibited PTH-stimulated renal resorption of calcium and magnesium and blunted PTH-stimulated phosphaturia (563). In lithium-treated patients, urinary calcium reabsorption is increased, whereas plasma phosphorus levels, urinary phosphorus

excretion, and urinary cAMP levels are normal and renal stones are rare (569,572,583,593–595).

Lithium therapy has been associated with parathyroid adenomas and hyperplasia (336,559,584,592,595,597). Although parathyroid adenomas are more common than parathyroid hyperplasia in lithium-treated patients, the latter are overrepresented compared with the general population (596). Long-term administration of lithium leads to an increase in parathyroid gland volume (572). *In vitro* studies show that lithium stimulates cellular proliferation of hyperplastic and adenomatous parathyroid tissue but has no effect on the growth of normal parathyroid tissue (586).

In a cross-sectional study, Bendz et al. (596) examined calcium and parathyroid hormone homeostasis in 142 psychiatric patients on long-term lithium therapy compared to age and sex-matched psychiatric controls. Persistent hypercalcemia was found in 3.6% of lithium-treated patients. Serum calcium level was inversely correlated with glomerular filtration rate and urinary concentrating ability but not with serum lithium levels or the dose or duration of lithium therapy. The incidence of hyperparathyroidism over 19 years was 6.3%. Surgically-verified hyperparathyroidism was present in 2.7%. Despite withdrawal of lithium for 8.5 weeks, serum calcium levels remained higher than in controls. By contrast, other studies show that serum calcium levels normalize within several weeks after discontinuation of lithium therapy, however the duration of lithium therapy was shorter in those reports (559).

Mak et al. (594) prospectively studied 53 lithium-treated patients for 2 years. There was no change in serum calcium, serum phosphorus or tubular reabsorption of phosphorus. PTH levels rose by 1 month and were significantly elevated at 6 months. In the fasting state, renal calcium reabsorption was increased and calcium excretion decreased, suggesting that bone resorption was reduced. By contrast, bone mineral density was found to be reduced in lithium-treated patients by other investigators (559,564,565,569,583).

Urinary Acidification

Lithium induces a distal tubular acidification defect (316,337, 364,394,404,405,435,597–606). Although hyperchloremic metabolic acidosis develops in experimental animals treated with large doses of lithium, an incomplete form of distal renal tubular acidosis is usually observed in humans. Longitudinal studies have documented the development of a urinary acidification defect after initiation of lithium therapy in humans (316,364,396), which is reversible after lithium withdrawal (404). A correlation between reduced urinary acidification and duration of therapy has been suggested (316,364). The fractional excretion of bicarbonate is not markedly elevated in lithium-treated patients (304), and most are unable to achieve a normal urine-to-blood PCO_2 gradient in a maximally alkaline urine, suggesting a distal acidification defect (405,602). Some studies have found that lithium-treated patients are able to maximally acidify their urine and excrete normal amounts of ammonia and titratable acid after acid loading (405,602). However, these findings have not been universal (361,381,388,394,435,601). Studies carried out in isolated rabbit cortical collecting tubules and in the turtle bladder suggested that the impaired urinary acidification seen with lithium administration results from a voltage dependent defect (598,605,606). In this context, lithium-induced downregulation of the amiloride-sensitive sodium channel reduces sodium reabsorption in the collecting duct, which in turn decreases hydrogen ion secretion by decreasing lumen negative transepithelial potential (496). The expression of other renal acid transporters is unchanged or increased (496). Infusion of sodium sulfate corrected the acidification defect in several human studies (435,559,601). In addition, lithium may directly inhibit hydrogen ion secretion by inhibiting the activity of H-adenoisine triphosphatase (H-ATPase) despite increased protein expression (496,498).

Histopathology

Concern over irreversible renal damage following long-term lithium therapy led to the first systematic study of renal histopathology (312). This study was undertaken in selected patients with a history of lithium intoxication or with nephrogenic diabetes insipidus, many of whom showed marked reductions in GFR (312). This report described focal interstitial fibrosis, tubular atrophy and dilation, microcysts formation in distal and collecting tubules, and focally sclerotic glomeruli (312). The glomerular lesions appeared to be secondary to tubulointerstitial disease.

Although we now believe that lithium-induced inhibition of vasopressin-stimulated water flow is primarily responsible for impaired urinary concentrating ability, structural injury to tubules and interstitial tissue contribute to the reduced maximal Uosm. In this regard, early reports suggested that the degree of impaired concentrating ability correlated with the severity of tubular damage and the duration of lithium treatment (312,349).

The causal relationship between chronic tubulointerstitial abnormalities and lithium treatment has since been challenged because similar changes have been observed in psychiatric patients who have not received lithium (316,364,607,608). In a comparison of renal biopsies from 44 patients treated with lithium and 25 patients with affective disorders who had never received lithium, there was no difference in the degree of interstitial fibrosis (364). The only distinguishing feature was the presence of microcysts in lithium-treated patients (Fig. 46-1). In the same study, interstitial fibrosis did not differ between psychiatric patients who had never received lithium and 25 age-matched transplant kidney donor controls, but was statistically greater in lithium-treated patients than in the transplant donor controls. These observations suggest that features shared by patients with affective disorders, such as the use of other psychotropic medications, may be partly responsible for the tubulointerstitial changes that have been attributed to lithium. Studies by other investigators have shown that renal abnormalities were more pronounced in patients treated with combinations of lithium and neuroleptics than with lithium alone, raising further questions concerning attribution of the nephropathy exclusively to lithium (507).

Chronic tubulointerstitial nephropathy is a nonspecific pattern of renal injury; however, specific renal lesions have been attributed to lithium (332,349,364). Lithium increases the mitotic rate of collecting duct cells both *in vitro* and *in vivo* (488) and causes marked principal cell hypertrophy (485). Tubular cysts have been observed in approximately 50% of lithium-treated patients with interstitial nephritis (332,349). These cysts show characteristics suggesting distal tubule and collecting duct origin (332). In addition, lesser degrees of tubular dilation are frequently observed (332). New Zealand white rabbits develop progressive chronic tubulointerstitial nephritis, identical tubular cysts, and progressive renal insufficiency with increasing duration of lithium exposure (609). The fact that tubular cysts are observed in lithium-treated psychiatric patients with chronic tubulointerstitial nephritis, but are not seen in psychiatric patients with chronic tubulointerstitial nephritis who were not treated with lithium, supports the specificity of these lesions (364). Farres et al. (610) utilized gadolinium-enhanced magnetic resonance imaging to evaluate patients with lithium-induced renal insufficiency. They identified abundant, uniformly and symmetrically distributed renal microcysts in both the cortex and medulla.

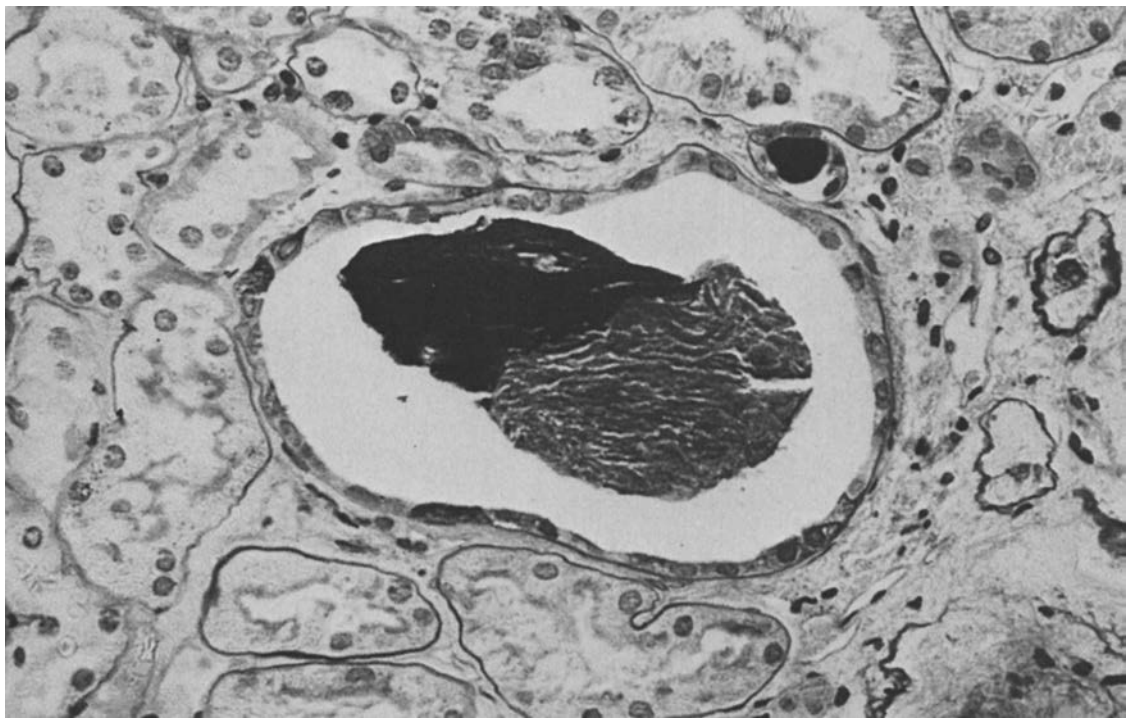

FIGURE 46-1. Cystic dilation of tubules, or "microcyst," formation, the only histologic feature suggesting chronic damage, which distinguished biopsies from patients receiving lithium from those of patients with manic-depressive psychosis who had not received lithium. (Periodic acid-Schiff, ×900.)

Reversible cytoplasmic lesions have been described in the distal tubules of lithium-treated patients, which are absent in non–lithium-treated psychiatric controls (316,449,611) (Fig. 46-2). These lesions, which consist of vacuolar swelling and glycogen accumulation, have been reproduced in the distal convoluted tubules and collecting ducts of lithium-treated rabbits (609). Glycogen accumulation has been attributed to lithium-induced decreases in intracellular cAMP (609). However, other investigators have only rarely observed these lesions (332).

Markowitz and colleagues (332) studied 24 renal biopsy specimens from lithium-treated patients referred for renal insufficiency. The predominant findings were tubular atrophy and interstitial fibrosis that tended to be patchy in early cases. A sparse, predominantly lymphocytic, interstitial infiltrate was often seen in association with interstitial fibrosis. Tubular cysts were seen in two-thirds of biopsies. The cysts were sparse and did not exceed 1 to 2 mm in diameter. All biopsies showed global glomerulosclerosis, whereas focal segmental glomerulosclerosis was present in half of the biopsies, involving 6% to 20% of glomeruli. Glomerulomegaly was frequent. Immunofluorescence showed focal nonspecific binding of IgM and C3. Electron microscopy showed variable podocyte effacement and no electron-dense deposits.

Dosing Regimen and Toxicity

It has been suggested that the type of lithium preparation and the dosing pattern may influence nephrotoxicity. However, results are conflicting. Slow-release lithium preparations have been said to be less nephrotoxic than rapidly dissolving preparations that deliver higher peak serum lithium levels (390,406,612,613). Although several cross-sectional studies show more severe impairment of urinary concentrating ability in patients treated with rapidly dissolving preparations, others find no difference between the preparations (341,347,372,389,390,406,613). It has been proposed that trough serum lithium concentrations are crucial in determining the severity of renal functional impairment and structural injury (612,614–616). This observation could be explained if tubular cell regeneration occurs only during periods of low serum lithium concentration (384,616). In several cross-sectional studies, multiple-dose regimens, which achieve relatively high trough but low peak serum lithium levels, were associated with greater histologic injury, lower GFR, or more pronounced impairment of urinary concentrating ability (335,363,384,403,615–621). Urine volume was correlated with trough serum lithium levels but not with peak levels or with lithium dose (614,616). In a prospective longitudinal study extending over 10 years, a multiple-dose regimen was associated with greater urine volumes and higher serum creatinine levels (335). In another report, urine volume decreased after lithium-treated patients were switched from a multiple-dose to a single-dose schedule in the absence of any difference in steady-state serum lithium concentration (363,615). However, treatment regimens were not randomly allocated, and in some of the studies cited, the lithium dose was greater in the multiple-dose group (614,618). Moreover, other investigators found no change in urine volume when lithium-treated patients were switched from a multiple- to a single-dose regimen or vice versa (622–625). In a randomized, cross-over study, Waldron et al. (626) failed to find any difference in urine volume with twice a day versus single daily dosing. In addition, several cross-sectional studies found no correlation between urine volume, renal concentrating ability, or GFR on the one hand and the number of lithium doses on the other (341,347,385,389,393). These discrepant data may be reconciled in part by the observation of Kusalic and Engelsmann (627) that conversion from a multiple- to single-dosing regimen was associated with an improvement in renal function only in those patients who had been treated with a multiple-dosing regimen for less than 5 years.

There is convincing evidence to suggest that maintenance of lower average serum lithium levels is associated with less nephrotoxicity. In prospective observations extending over

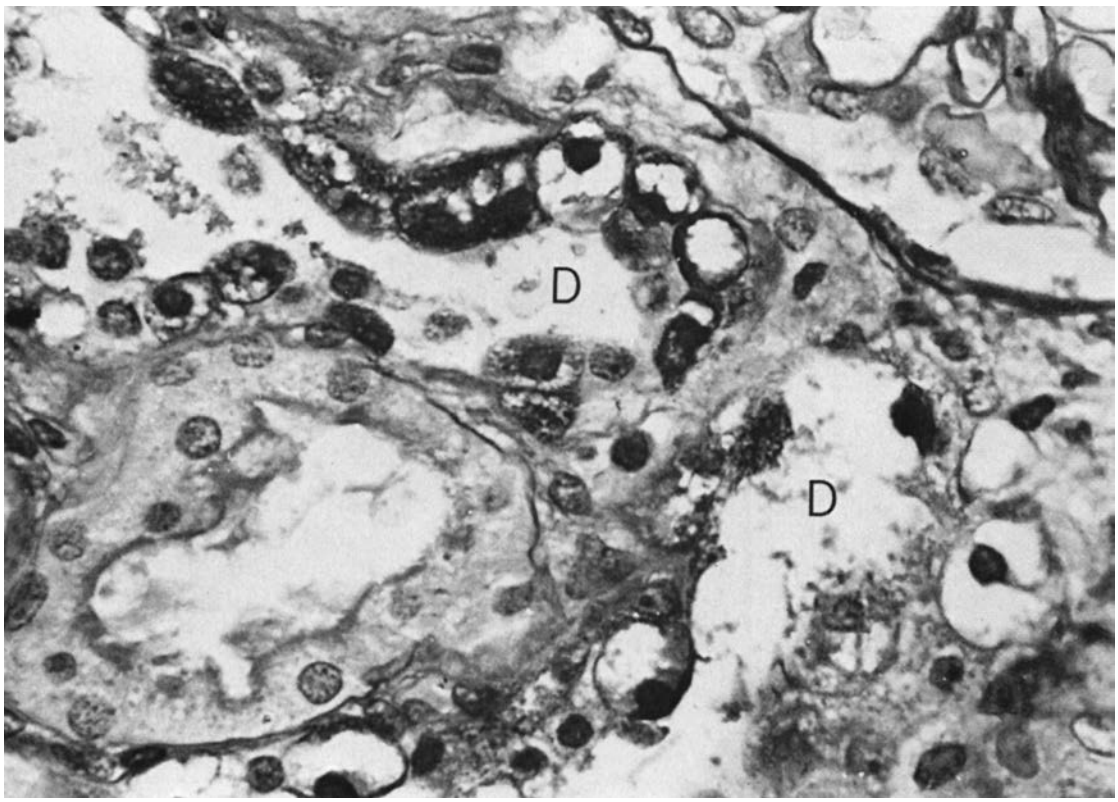

FIGURE 46-2. Ballooning, vacuolation, and accumulation of periodic acid-Schiff (PAS)–positive glycogen in epithelial cells in distal tubules (*D*) in a patient taking lithium. These changes appear within days of starting lithium and disappear soon after lithium is ceased. (PAS, ×900.)

7 years in which the average serum lithium concentration was maintained at a low level (0.68 mmol/L), GFR was unchanged, while urinary concentrating ability and urine flow rate showed only modest changes (341). In two cross-sectional series of patients maintained on low-dose regimens (0.4 to 0.6 mmol/L), renal function did not differ from controls suffering from affective disorders (302,303). Similar results have been obtained in uncontrolled cross-sectional studies (315,374), but contrary data do exist (357).

It has also been suggested that lithium nephrotoxicity may be enhanced by concurrent administration of neuroleptic agents. While several reports indicate that therapy with neuroleptic agents alone is associated with reduced urinary concentrating ability or increased urine volume, others have failed to confirm these observations (341,365,392,397). In several studies, concurrent neuroleptic therapy has been associated with more severe impairment of urinary concentrating ability, increased urine flow rate, worse renal histology, or lower GFR (341,344,348,351,362,365,371,379,392,395,397, 507,628). However, in some of these studies, the dose of lithium was greater in those who also received neuroleptic agents (351,392,507). Moreover, others investigators failed to find any differences in lithium nephrotoxicity between patients treated with lithium alone versus those also receiving other psychotropic agents (338,347,356,382,389,390,618).

Summary

In summary, in most patients the adverse effects of lithium on renal function are modest and of little clinical significance if maintenance serum levels are kept at the lowest effective level. However, it appears that advanced renal insufficiency may develop on maintenance lithium therapy after decades of treatment. Much more commonly, patients develop distressing polyuria. The relative contributions of lithium nephrotoxicity, behavioral factors (polydipsia), and the effects of other psychotropic agents in the development of renal symptoms vary among individual patients. Because nephrotoxicity may be associated with clinical episodes of lithium intoxication or high serum lithium levels, it is prudent to regularly monitor renal function and maintain the lowest effective serum lithium levels. Isolated polyuria due to impaired concentrating ability may respond to amiloride. In general, modest reduction of filtration rate in association with mildly impaired urinary concentrating ability may not be a harbinger of progressive renal failure. However, the risks of nephrotoxicity must be weighed in individual patients against the therapeutic benefit from continued lithium therapy.

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CHAPTER 47 ■ NEPHROTOXICITY SECONDARY TO ENVIRONMENTAL AGENTS AND HEAVY METALS

RICHARD P. WEDEEN

Environmental kidney diseases are usually recognized when they result from occupational exposure, a special case of environmental disease. Toxic effects detected in a few highly exposed workers suggest the diseases that can be anticipated from the lower exposures encountered by large populations from environmental pollution. Recognized chronic occupational renal diseases include those arising from exposure to heavy metals, organic solvents (aliphatic, aromatic, and halogenated hydrocarbons), and silica. Itai-itai disease from cadmium and Minimata disease from mercury are two environmental diseases arising from industrial pollutants first identified in Japan. To this group can be added Balkan nephritis, a disease of unknown etiology presumed to be of environmental origin and nephropathy caused by the ingestion of germanium compounds.

More than 45 naturally occurring elements are classified as heavy metals by virtue of their metallic qualities and specific gravities >5. Seven of these metals are generally recognized as nephrotoxic following environmental or occupational exposure: lead, cadmium, mercury, uranium, chromium, copper, and arsenic, although chronic renal failure has been described for only lead, mercury, cadmium, uranium, and arsenic. Therapeutic forms of platinum (Chapter 43, Renal Diseases Induced by Antineoplastic Agents), gold (Chapter 66, Renal Involvement in Systemic Sclerosis, Rheumatoid Arthritis, Sjögren's Syndrome, and Polymyositis-Dermatomyositis), lithium, and bismuth may also induce kidney damage. Although other heavy metals are potentially damaging to the kidneys, too little evidence of the clinical importance of these renal effects is available to warrant inclusion here. The potentially nephrotoxic heavy metals include barium, cobalt, manganese, nickel, silver, thallium, thorium, tin, and vanadium. The paucity of incriminating evidence against these elements may be more a testimony to our ignorance of the etiologic factors that lead to end-stage renal disease than to the benign nature of the metals.

Cause and effect are relatively easy to demonstrate when renal damage is acute. Establishing the contribution of an environmental toxin to kidney disease, however, is considerably more difficult if the toxicity is delayed. When renal disease is a consequence of long-term, low-dose, asymptomatic exposure modulated by complex interactions with other toxins, nutritional factors, other diseases, and genetic susceptibility, etiology remains difficult to prove. Kidney disease arising from exposure to environmental agents and heavy metals plays a special role in nephrology because of the potential for prevention.

URINARY BIOMARKERS

More than 25 urinary proteins and biochemical markers (eicosanoids) have been measured in urine using sensitive, specific assays to characterize early stages of toxic renal injury. We hope to detect specific causes of toxic nephropathy before the reduction in glomerular filtration rate (GFR) is sufficient to be manifest clinically by an increase in serum creatinine. The goal of establishing "fingerprints" for specific environmental nephrotoxins has been partially achieved. Urinary biomarkers are selected because they reflect specific sites of renal injury: (a) low-molecular-weight proteins and intracellular enzymes—proximal tubule damage, (b) Tamm-Horsfall glycoprotein and kallikrein—distal tubule injury, (c) high-molecular-weight proteins—increased glomerular permeability (if >200 mg/g creatinine), and (d) biochemical markers—eicosanoids suggesting vascular injury. Comprehensive urinary profiles have been evaluated in cooperative studies conducted in Europe (1–5) (Table 47-1). To achieve broad diagnostic value, the urinary markers are examined in subjects with known toxic exposures, over a wide range of dose rates and exposure times. Patients with known clinical renal failure, glomerular disease, multinephrotoxin or drug exposure, or systemic diseases that predispose to kidney damage (e.g., hypertension, diabetes mellitus, and gout) are excluded from these studies to reduce confounding variables. The specificity of tubular injury tends to disappear once renal damage is sufficient to cause an increase in the serum creatinine.

A sampling of 11 urinary markers (human intestinal alkaline phosphatase [HIAP], total nonspecific alkaline phosphatase [TNAP], *N*-acetyl-*β*-D-glucosaminidase [NAG], retinol binding protein [RBP], Tamm-Horsfall glycoprotein [THG], β_2-microglobulin, microalbumin, thromboxane B_2 [TBX_2], and three prostaglandins [prostaglandin E_2, PGE_2; prostaglandin $F_{2\alpha}$, $PGF_{2\alpha}$; 6-keto-prostaglandin $F_{1\alpha}$, 6-keto-$PGF_{1\alpha}$]) are presented to illustrate how differentiation of the toxic nephropathies can be accomplished (Table 47-1). The excretion patterns illustrated in Table 47-1 represent occupational exposure levels indicated by the mean blood or urine concentrations specified. The isoenzyme HIAP is a sensitive and specific indicator of injury to the S3 segment of the proximal tubule owing to occupational exposure to mercury and cadmium (3–5). Along with other markers of tubular and glomerular injury, HIAP is increased in the ischemic kidney and hypertension. Total nonspecific alkaline phosphatase is not increased in analgesic abuse or lead exposure, but it is increased after perchlorethylene exposure (6,7). *N*-acetyl-*β*-D-glucosaminidase is not elevated in the urine of workers exposed to perchlorethylene, or mercury (1,6); however, NAG and RBP are elevated after cadmium exposure (3,8). Tamm-Horsfall glycoprotein appears to be a marker of distal tubular injury in contrast to HIAP, TNAP, NAG, and RBP, which reflect proximal tubule injury. Although the pathophysiologic significance of the urinary eicosanoids is unclear, measurement of urinary PGE_2, $PGF_{2\alpha}$, and 6-keto-$PGF_{1\alpha}$ may provide insight into the mechanisms of hypertension and injury to the glomerulus or renal medulla.

TABLE 47-1

URINARY MARKERS IN TOXIC NEPHROPATHIES—EUROPEAN COOPERATIVE STUDY

	HIAP	TNAP	NAG	RBP	THG	β_2-M	mAlb	TXB_2	PG
Pb	−	−	+	−	−	−	−	++	6F−
Cd	+++	−	+++	+	−	+	++	−	6F++
Hg	+++	+	+	−	−	−	−	−	F−
					−			−	E−
PCE	−	+++	−	−	±	−	+		F−
									E−

HIAP, human intestinal alkaline phosphatase; *TNAP*, total nonspecific alkaline phosphatase; *NAG*, *N*-acetyl-β-D-glucosaminidase; *RBP*, retinol binding protein; *THG*, Tamm-Horsfall glycoprotein; β_2-*M*, β_2-microglobulin; mAlb, microalbumin; TXB_2, thromboxane B_2; *PG*, prostaglandin; *Pb*, lead; *Cd*, cadmium; *Hg*, mercury; *PCE*, perchloroethylene; *6F*, 6-keto-$PGF_{1\alpha}$; *F*, $PGF_{2\alpha}$; *E*, PGE_2.

The ability of these urinary markers to discriminate between various nephrotoxins increases with increasing exposure levels.

Low levels of urinary albumin may reflect either glomerular or tubular injury. Although generally considered to indicate early glomerular injury in diabetic patients, the appearance of small quantities of albumin in the urine may sometimes represent the failure of the tubule to reabsorb or metabolize albumin that passes through the glomerular filter in minute quantities but does not normally reach the bladder. Thus, low levels of urinary albumin may represent proximal tubular dysfunction rather than increased glomerular permeability.

A number of alternatives to the 11 urinary markers shown in Table 47-1 are available. For the present, they appear either to be redundant (e.g., alanine aminopeptidase, α_1-microglobulin, γ-glutamyltransferase) or to offer few advantages because their functional significance is unclear (e.g., fibronectin, glycosaminoglycans, kallikrein, or sialic acid). Reports by Mutti and associates (9) and the European Cooperative Study Group (1–3,6,8) suggest that the proximal tubule brush-border antigens designated BB50, BBA, and HF5 may be sensitive indicators of proximal tubular injury. However, standardization of these immunoassays has not been accomplished.

Urinary markers are determined in fresh-voided specimens (spot urines) and expressed relative to the creatinine concentration. Urine collections should be made at 8 AM when both GFR and protein excretion are highest. Circadian rhythm has been shown to occur for both GFR and low-molecular-weight proteinuria, with both being lowest during sleep (10).

LEAD NEPHROPATHY

Occupational exposure to lead began over 10,000 years ago in the region of the Aegean sea. The earliest description of lead poisoning is found in a poem dating from about 200 BC by the Greek philosopher Nikander of Colophon (11). Although possible recognition of renal effects of lead can be traced to the 17th century, Lancereaux provided the first description of lead nephrotoxicity in modern terms in 1862. Lancereaux's patient had saturnine (lead-induced) gout; his kidneys showed interstitial nephritis at postmortem examination. Controversy concerning the renal effects of lead stems from this 19th century description compounded by the recurrent failure to recognize the late sequelae of chronic absorption of relatively low levels of lead or to distinguish glomerular from extraglomerular renal disease. Additional confusion has been created by the failure to distinguish the transient Fanconi's syndrome of acute childhood lead poisoning from the chronic interstitial nephritis characteristic of lead nephropathy in adults. In addition to the difficulty in assigning cause when the effect is delayed in time, identification of the renal effects of lead was further obscured because the late complications of excessive lead absorption, namely, gout and hypertension, can themselves produce renal damage unrelated to lead.

Diagnosis

In the past, lead nephropathy was identified in individuals who had repeated episodes of acute lead intoxication (11). The classic symptoms of inorganic lead poisoning (abdominal colic, extensor muscle weakness, and encephalopathy) in patients known to have excessive lead absorption made the diagnosis straightforward. In recent decades, the diagnosis was confirmed in the clinical laboratory by finding anemia in association with excessive urinary excretion of lead, coproporphyrins, or δ-aminolevulinic acid. Following the extensive studies of lead metabolism by Kehoe beginning in the 1930s (11), the mainstay of laboratory diagnosis was the blood lead concentration. Until 1978, a whole blood lead concentration of up to 80 μg/dL was considered "acceptable" in occupationally exposed adults. Blood lead levels below 80 μg/dL were considered incompatible with the diagnosis of lead poisoning in adults, although levels above 40 μg/dL were considered unacceptable in children. The battle over the determination of the "safe" blood lead level continues despite a growing consensus that blood levels over 10 μg/dL may be associated with lead-induced organ damage (12).

The blood lead concentration is relatively insensitive to cumulative body stores acquired over many years of moderate exposure (i.e., exposure insufficient to produce classic symptoms of acute poisoning). Blood lead concentrations tend to fall markedly within weeks of removal from exposure. Alternative approaches to the detection of excessive lead absorption have been examined because approximately 95% of the body stores of lead are retained in bone with a mean residence time approximating 20 years (13). At present, cumulative past lead absorption is best assessed by the calcium disodium edetate ($CaNa_2EDTA$) lead-mobilization test.

The ethylenediamine tetraacetic acid (EDTA) test is performed in adults by parenteral administration of 1 to 3 g of $CaNa_2EDTA$ over 4 to 12 hours with subsequent collection of 24-hour urine samples over 1 to 4 days. A dosage of 20 to 30 mg of $CaNa_2$ EDTA per kg is generally used in children. Adults without undue prior lead absorption excrete up to 650 μg of lead-chelate in the urine. Neither the dose (1 to 3 g) nor the route of administration (intravenous or intramuscular) appears to critically modify the normal response to chelation testing (14), but in the presence of renal failure (serum creatinine >1.5 mg/dL) urine collections should be extended to

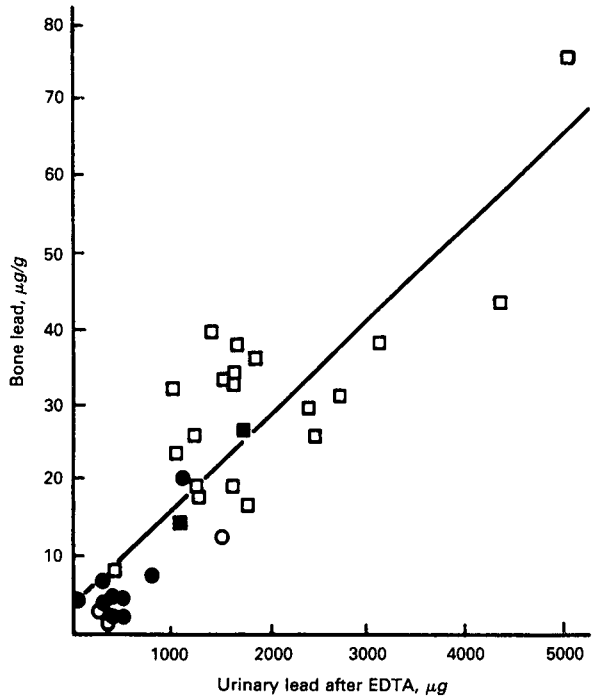

FIGURE 47-1. Relationship of bone lead to chelatable lead in 35 Belgians including 22 lead workers (*squares*). Lead was measured in transiliac bone biopsy specimens by atomic absorption spectroscopy and chelatable lead by the EDTA lead-mobilization test. The linear regression correlation coefficient (*r*) is 0.87. *Open symbols* represent subjects with normal glomerular filtration rates; *closed symbols*, those with reduced glomerular filtration rates. (From: Van de Vyver FL, et al. Bone lead in dialysis patients. *Kidney Int* 1988;33:601, with permission.)

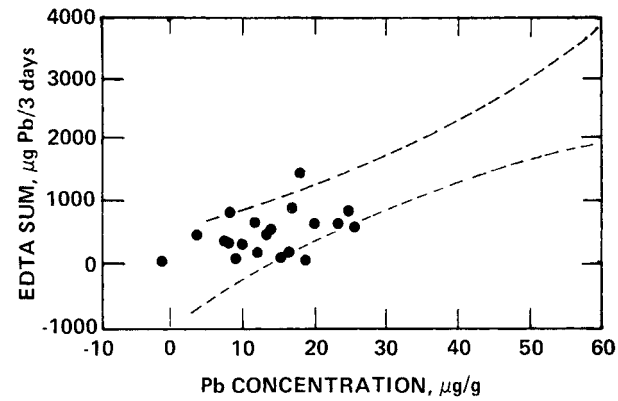

FIGURE 47-2. Bone lead determined by *in vivo* tibial K x-ray fluorescence compared to chelatable lead in American armed service veterans without known excessive exposure to lead. *Dotted lines* represent 95% confidence limits of data presented in Figure 47-1. Transiliac bone lead values in Figure 47-1 were multiplied by 1.75 to convert to tibial bone values. Pb, lead. (From: Wedeen RP. Bone lead, hypertension, and lead nephropathy. *Environ Health Perspect* 1988;78:57, with permission.)

at least 3 days. The intramuscular administration of 2 g of $CaNa_2EDTA$ (1 g of EDTA mixed with local anesthetic in each of two injections, 12 hours apart) may be the preferable method of performing the chelation test because it has been well standardized in both normal subjects and patients with renal failure (14–19). In the presence of reduced GFR, urinary excretion of lead chelate is measured for 3 consecutive days and the adequacy of collection checked by simultaneous measurement of urinary creatinine excretion (1.3 g of creatinine per day is an acceptable lower limit in normal adult males).

Because lead in bone has a biologic half-life measured in decades, compared to a biologic half-life of lead in blood of only 4 weeks (20), the bone more closely reflects cumulative body lead stores. Chelatable lead correlates well with bone lead (21,22) (Fig. 47-1). Diagnostic monitoring of the body lead burden can be accomplished by *in vivo* tibial K x-ray fluorescence, a new noninvasive technique that is both safe and accurate at bone lead concentrations associated with interstitial nephritis caused by lead (23–25) (Fig. 47-2). The characteristic K x-rays of lead are stimulated by the 88-keV gamma emissions from a ^{109}Cd radioactive source. The fluorescent x-rays are measured with a high-purity, liquid nitrogen-cooled, germanium detector and recorded in a computer equipped with a multichannel pulse height analyzer. The characteristic K x-rays differ from the characteristic L x-rays of lead in that the higher-energy K photons penetrate 2 cm of cortical bone (26). L x-rays only detect lead within the outermost 0.5 mm of subperiosteal bone. Calibration of the L x-rays is problematic because of major soft tissue absorption. K x-rays, on the other hand, can be accurately calibrated and normalized to the bone calcium content. The K x-ray fluorescence technique records the calcium-phosphorus content of the bone region under study (elastic scatter) and thus permits measurement of the lead-calcium atomic ratio. This ratio is largely independent of target-source geometry and therefore permits calibration by either plaster-of-Paris phantoms or absolute physical properties (25). Whole-body radiation during the 30-minute K x-ray fluorescence test is 0.3 mrem, equivalent to background cosmic radiation absorbed over about 10 hours.

Although the blood lead reflects absorption of both organic and inorganic lead, the clinical symptoms of organic lead are primarily cerebral. Colic, peripheral neuropathy, and anemia are not seen in acute organic lead poisoning. Chelation therapy is ineffective in acute organic lead poisoning (27). DuPont's Chambers Works in Deepwater, New Jersey, became known as the "House of Butterflies" because of the frequency of hallucinations among workers producing tetraethyl lead shortly after discovery of the antiknock gasoline additive in 1923 (28). Renal disease has not been found following tetraethyl lead absorption (27).

Acute Lead Nephropathy

In children with lead encephalopathy, a proximal tubule reabsorptive defect characterized by aminoaciduria, phosphaturia, and glycosuria (Fanconi's syndrome) has been observed (29). Fanconi's syndrome is found in the presence of blood lead levels usually in excess of 150 μg/dL and appears to be rapidly reversed by chelation therapy designed to treat the far more dangerous encephalopathy. The proximal tubule reabsorptive defect has been induced experimentally in rats fed dietary lead (30). In both children and experimental animals, acute lead nephropathy is consistently associated with acid-fast intranuclear inclusions in proximal tubule epithelial cells (31). The intranuclear inclusion bodies consist of a lead–protein complex and may be seen in tubular epithelial cells in the urinary sediment during acute poisoning (32). Lead-containing intranuclear inclusions have been observed in liver, neural tissue, and osteoclasts as well as kidney. Acute poisoning is also associated with morphologic and functional defects in mitochondria.

Chronic Lead Nephropathy

The phrase *chronic lead nephropathy* refers to the slowly progressive interstitial nephritis occurring in adults following prolonged lead exposure. Occupational lead nephropathy has

developed after as little as 3 years of intense exposure (18). Analysis of death certificates of 601 men employed at the Bunker Hill Lead Mine and Smelter in Kellogg, Idaho, up to 1977 indicated a twofold-increased risk of dying from chronic renal disease (33). The increased risk approached fourfold after 20 years of occupational exposure. Although most frequently recognized in lead workers after decades of occupational exposure, chronic lead nephropathy also has been recognized among young adults in Australia who sustained acute childhood lead poisoning (34) and among illicit whiskey ("moonshine") consumers in the southeastern United States. Chronic interstitial nephritis owing to lead has also been seen among American workmen whose exposure was never severe enough to produce acute symptoms of lead poisoning (18,19), and in U.S. armed service veterans suffering from renal failure attributed to gout or essential hypertension (16,17). In the veterans, exposure to lead had never produced acute symptoms of poisoning, and the source of exposure had never been recognized. The diagnosis was only established by performance of the $CaNa_2EDTA$ lead-mobilization test after renal failure was apparent. Medical histories were often misleading; patient recall frequently contradicted the objective evidence of chelation testing. Sporadic case reports of lead nephropathy arising from unusual accidental exposure such as geophagia (35) or Asian folk remedies and cosmetics continue to appear in the medical literature (11).

"Queensland nephritis" appears to represent the transition from the acute disease of childhood to the chronic nephropathy of adults (34). This evolution has been observed in experimental animals but has not been reported in American children. The difference between the American and Australian experience may well owe to the fact that the American children with pica who had long-term follow-up received chelation therapy in childhood. In an early follow-up study of untreated childhood lead poisoning, diagnostic criteria for both lead poisoning and renal disease were unacceptably vague (36). A 50-year follow-up of untreated lead-poisoned children in the United States found evidence of increased renal disease (37).

Chronic lead nephropathy from moonshine came to medical attention because of the dramatic symptoms of acute lead poisoning. As in severely exposed industrial workers, lead colic and anemia were associated with reduced GFR, which often improved following chelation therapy. Transient renal failure, apparently the result of renal vasoconstriction (38), was superimposed on more chronic renal damage that appeared to be less responsive to chelating agents. The chronic lead nephropathy of the moonshiners, more often than not, was accompanied by gout and hypertension, in accord with 19th century descriptions of plumbism and contemporary reports from Australia (11). A statistically significant odds ratio of 2.4 has been reported for moonshine consumption and end-stage renal disease, suggesting a causal association in the absence of acute lead poisoning (39).

Evaluation of renal function in workmen with excessive body lead stores has revealed previously unsuspected reductions in GFR (i.e., <90 mL/minute/1.73 m^2 body surface area) before renal dysfunction was clinically evident (19). In these occupationally exposed individuals, minimal (about 30%) reductions in GFR were restored to normal by long-term, low-dose chelation therapy (1 g of $CaNa_2EDTA$ with local anesthetic three times weekly until the chelation test returned to normal). This therapeutic response in preazotemic lead nephropathy may reflect reversal of functional impairment rather than reversal of established interstitial nephritis. Both the renin-angiotensin system and Na^+-K^+-ATPase are inhibited by lead (40), and these effects may have been modified by chelation therapy. Considerable epidemiologic evidence suggests that modest azotemia is significantly more prevalent among lead-exposed workers than among nonexposed counterparts, presumably owing to both morphologic and functional changes (11,41).

Renal biopsies in chronic lead nephropathy show nonspecific tubular atrophy and interstitial fibrosis with minimal inflammatory response as well as mitochondrial swelling, loss of cristae, and increased lysosomal dense bodies within proximal tubule cells (18,21) (Fig. 47-3). Arteriolar changes indistinguishable from nephrosclerosis are found, often in the absence of clinical hypertension. Intranuclear inclusion bodies are often absent when the renal disease is long-standing or following the administration of chelating agents. Clumped chromatin and nuclear invaginations of cytoplasmic contents may be found even in the absence of intranuclear inclusions. Morphologic alterations are minimal in glomeruli until the reduction in GFR is advanced.

The hypothesis derived from acute lead nephropathy—that proximal tubular injury accounts for the pathogenesis of chronic lead nephropathy—has gained little support. The appearance of arteriolar nephrosclerosis before hypertension develops and the relatively short duration of hypertension before renal failure supervenes suggest that the initial renal injury from lead may be in the microvascular endothelium (42,43). At the cellular level, lead metabolism mimics that of calcium (44). Proximal tubular transport defects in excess of that expected in comparable chronic renal failure have not been convincingly demonstrated in chronic lead nephropathy. The mechanism of microvascular injury remains speculative. Interference

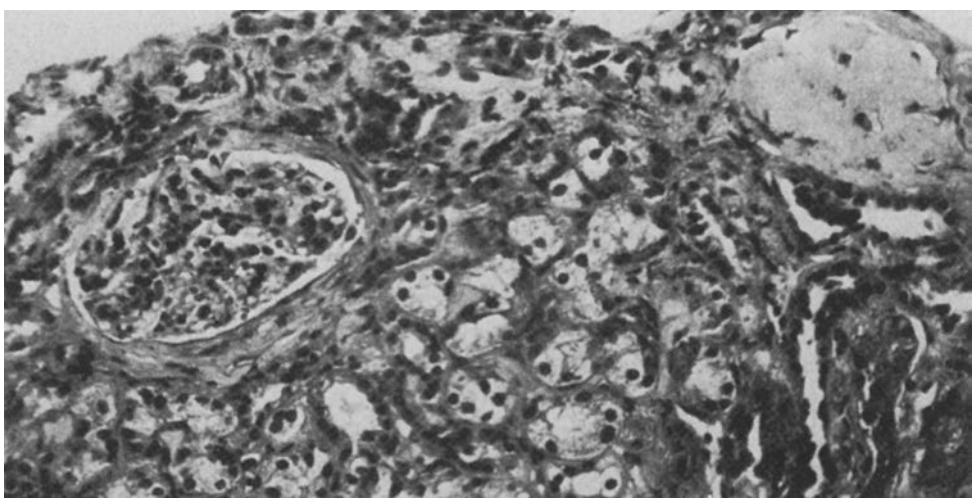

FIGURE 47-3. Renal biopsy obtained from a 28-year-old man who had prepared lead solder for 5 years. His ^{125}I-iothalamate clearance was 52 mL/minute/1.73 m^2; hemoglobin, 9.6 g/dL; uric acid, 13.2 mg/dL; and blood lead, 48 μg/dL when he was initially seen. Lead-chelate excretion following 2 g of $CaNa_2EDTA$ intramuscularly was 5.2 mg/24 hours. Light microscopy shows periglomerular fibrosis, a sclerotic glomerulus, and tubular atrophy. (Trichrome stain; magnification ×304.) (From: Wedeen RP, et al. Occupational lead nephropathy. *Am J Med* 1975;59: 630, with permission.)

with cation transport is a reasonable hypothesis because the metabolism of lead is similar to that of other cations. The paradox that increased dietary calcium reduces vascular smooth muscle tone, whereas increased free intracellular calcium increases it could, in part, be explained by lead–calcium interactions (15). Inhibition of red blood cell Na^+-K^+-ATPase in lead workers correlates with membrane-bound lead (45), and lead increases red blood cell Na^+-Li^+ countertransport *in vitro* (46). Similarly, lead interactions with vasoactive substances may modulate blood pressure and induce endothelial injury (47,48).

The functional changes in chronic lead nephropathy appear to be less specific than those observed in acute poisoning. As in other forms of interstitial nephritis, proteinuria and glycosuria are initially absent. In contrast to cadmium nephropathy, the excretion of urinary marker proteins such as HIAP, TNAP, THG, RBP, lysozyme, and β_2-microglobulin (2,8) is not increased in the absence of a reduced GFR (Table 47-1). The increase in urinary NAG with increasing blood lead reflects Fanconi's syndrome of acute lead poisoning rather than the chronic interstitial nephritis associated with occupational lead exposure (49,50). *N*-acetyl-β-D-glucosaminidase excretion correlates positively with the blood lead concentration but not with the bone lead concentration (50). Exhibiting a pattern of eicosanoid excretion noted in essential hypertension, lead-exposed workers showed an increase in TXB_2 and a decrease in PGE_2 and 6-keto-$PGF_{1\alpha}$ in the urine (2,50). In contrast to the reabsorptive defect of acute lead nephropathy, saturnine gout is characterized by renal retention of uric acid (34). The clearance (C_{PAH}) and maximal secretion rate (Tm_{PAH}) for *p*-aminohippurate (PAH) have been found to be variable in patients with occupational lead nephropathy.

The relationship of lead to gout nephropathy has provoked controversy for over a century (11,51). Hyperuricemia and gout are common among individuals with excessive exposure to lead, apparently the result of decreased excretion and increased production of uric acid. Similarly, although hyperuricemia invariably accompanies azotemia, gout is rare in patients with renal failure except in those with lead nephropathy. Half of uremic patients with lead nephropathy have clinical gout (34), but in the absence of renal failure, gout cannot usually be attributed to lead despite coexisting hypertension (52,53).

There is substantial evidence that renal failure in gout is sometimes secondary to overt or unsuspected lead poisoning. In Queensland, Australia, as many as 80% of gout patients with renal failure have elevated EDTA lead-mobilization tests (34). In New Jersey, chelatable lead was found to be significantly greater among gout patients with renal failure than among gout patients with normal renal function (16). Because patients with comparable renal failure owing to known causes other than lead show no increase in chelatable lead, the excessive mobilizable lead in these gout patients appears to be the cause rather than consequence of their renal failure. Measurement of lead levels in transiliac biopsy specimens from patients with end-stage renal disease confirms the fact that renal failure per se does not cause increased bone lead levels (22). Unrecognized lead poisoning, therefore, may explain the occurrence of renal failure in some gout patients who have neither urinary calculi nor intratubular uric acid deposition disease. Similarly, overt lead poisoning may explain the protean manifestations of gout in past centuries, the so-called irregular gout, as well as the long but almost forgotten association of gout with wine (11). Sporadic contamination of alcoholic drinks with lead throughout history may have been responsible for gout that terminated in cerebral disease (e.g., uremia, stroke, or encephalopathy).

The association between lead and hypertension has also been a subject of controversy since the first use of the sphygmomanometer. The early view that renal injury induced by lead causes hypertension has gained increasing support. The EDTA lead-mobilization test can sometimes indicate that lead is the most probable cause of hypertension with renal failure when lead exposure has not been previously suspected. Patients believed to have "essential hypertension with nephrosclerosis" may be identified as having lead nephropathy by the EDTA lead-mobilization test (17). The duration of hypertension in patients with lead nephropathy tends to be shorter than that in hypertensives without renal failure, suggesting that lead-induced renal injury precedes and therefore causes the hypertension. This view is consistent with the finding that creatinine clearance decreases with increasing blood lead in the general population, an effect that is independent of blood pressure (41,54).

Lead nephropathy does not account for renal failure in all hypertensives with kidney disease any more than it accounts for renal failure in all gout patients with kidney disease. The heavy metal may, however, contribute to the long and controversial association of gout with hypertension, as well as to the variable incidence of renal failure in each of these conditions. Mortality data show that death from hypertensive cardiovascular disease is more frequent among lead workers than the general population (33).

A role for lead in hypertension gains further credence from epidemiologic studies of low-level lead exposure (i.e., exposure too low in intensity to produce the classic symptoms of acute lead poisoning). The Second National Health and Nutrition Examination Survey (NHANES II) performed between 1976 and 1980 included blood lead and blood pressure measurements in almost 10,000 noninstitutionalized Americans aged 6 months to 74 years (55). The correlation between blood lead and blood pressure was robust even when both measurements were within the accepted "normal" range (56,57). Similar conclusions have been drawn from studies performed throughout the world, although statistically nonsignificant findings in small studies have also been reported. A longitudinal study of 590 men with a low mean blood lead concentration (6.3 μg/dL) found that bone lead was higher in those who developed hypertension than in those who did not, although the blood lead was not significantly different (58). Although some doubts have been raised about the magnitude of the dose-response relationship, there is a growing consensus that lead contributes to hypertension in the general population, particularly in the presence of renal dysfunction. Lead may also contribute to the disproportionate representation of black men with hypertensive nephrosclerosis and diabetic nephropathy in end-stage renal disease programs in the United States (15).

Treatment

Lead nephropathy is important because it is one of the few renal diseases that is preventable. Moreover, lead nephropathy can sometimes be reversed or its progression retarded by judicious use of chelation therapy (11,19,38,59). Lead nephropathy is unusual for a nonsystemic disease involving the kidneys in that an etiologic diagnosis can be established even after renal failure supervenes. The observation that chelation therapy improved renal function in renal failure patients with low body lead stores suggests that unrecognized low-level lead absorption contributes to renal failure owing to other causes (60).

Although chelation therapy effectively reverses acute lead nephropathy and the preclinical renal dysfunction of occupational lead nephropathy (19), there is no evidence that such therapy reverses established interstitial nephritis. The partial remissions achieved among moonshiners and symptomatic lead workers may represent reversal of acute poisoning superimposed on chronic lead nephropathy. No improvement in renal function can be expected once advanced interstitial nephritis is present and the steady-state serum creatinine concentration

exceeds about 3 mg/dL (61). Chronic volume depletion and hyporeninemic hypoaldosteronism may contribute to the reversible component of renal dysfunction. The effect of lead on the renin–angiotensin system, however, remains controversial because increased renin responsiveness has also been observed in lead-exposed experimental animals and humans (40).

Before chelation therapy is undertaken, the diagnosis should be clearly established. It may be necessary to perform the EDTA lead-mobilization test, and other possible causes of renal disease must be rigorously excluded. For the present, attempts at chelation therapy probably should be avoided when the cause of the renal disease is unclear (e.g., in the presence of heavy proteinuria). Moreover, long-term, low-dose EDTA therapy should only be undertaken with a clear end point in mind, such as reversion of the EDTA test to normal and restoration of renal function. Although the EDTA test has been shown to be safe even in the presence of renal failure (62), the cumulative nephrotoxicity of prolonged EDTA therapy in patients with markedly reduced GFR is unknown. Reports that $CaNa_2EDTA$ therapy has been followed by deterioration of renal function warrant careful follow-up of treated patients (61,63). Despite these caveats, it may be appropriate to perform EDTA lead-mobilization tests in individuals with gout or hypertension and renal failure or interstitial nephritis of unknown etiology, because a positive test may provide the best available indication of etiology. Knowledge of etiology may permit identification and removal of the source of lead and prevention of lead nephropathy in others.

CADMIUM NEPHROPATHY

Industrial use of cadmium has increased steadily since its discovery by Stromyer in 1817. Cadmium-containing compounds are widely used in the manufacturing of pigments, plastics, glass, metal alloys, and electrical equipment. Acute absorption of as little as 10 mg as dust or fumes may induce severe gastrointestinal symptoms and, after a delay of 8 to 24 hours, fatal pulmonary edema (64,65). Chronic low-dose exposure, on the other hand, causes slowly progressive emphysema, anosmia, and proximal tubular reabsorptive defects characterized by low-molecular-weight proteinuria, enzymuria, aminoaciduria, and renal glycosuria (66). Hypercalciuria (with normocalcemia), phosphaturia, and distal renal tubular acidosis result in clinically important osteomalacia, pseudofractures, and urinary tract stones (67,68). Interstitial nephritis resulting from parenteral administration of cadmium to experimental animals was recognized in the 19th century (69), but only recently has the progression of early proximal tubular dysfunction to chronic renal failure been documented.

Metabolism

Nonoccupationally exposed individuals accumulate cadmium throughout their lives through food and cigarettes. The biologic half-life of cadmium in humans exceeds 15 years, and one-third of the total body stores (10 to 20 mg) is retained in the kidneys. Tubular proteinuria has resulted from low-level environmental exposure to cadmium as well as to the higher exposure levels encountered in industry (70).

Absorbed cadmium is initially sequestered in liver and kidney, where it is bound to a cysteine-rich apoprotein, metallothionein (64,66). Zinc, copper, mercury, and iron, as well as cadmium, induce metallothionein synthesis in these organs. With a molecular mass of 6,500 daltons, the cadmium–thionein complex is filtered at the glomerulus, taken up in the proximal tubule by endocytosis, and transferred to lysosomes, where it is rapidly degraded. Cadmium–thionein is considerably more nephrotoxic than cadmium or metallothionein alone, but cytoplasmic cadmium ions released from lysosomes may be the mediator of tubular cell injury (71). Although uptake in liver initially exceeds that in kidney, most of the cadmium is eventually bound to protein in the proximal tubules, where it is accumulated until a "critical concentration," approximately 200 μg/g of renal cortex, is achieved. At this tissue level, renal effects become evident, including tubular proteinuria and increased cadmium excretion (66). Although urinary cadmium excretion is normally less than 2 μg/day, after the critical concentration has been exceeded, urinary cadmium in excess of 10 μg/day is usual. Significant abnormalities of proximal tubular function are associated with urinary cadmium excretion in excess of 30 μg/day (72). The blood cadmium concentration is less reliable as an indicator of health effects. Although the blood cadmium level rises promptly following occupational exposure, blood is a poor indicator of cumulative absorption. Nevertheless, blood levels greater than 1 μg/dL, as well as urine concentrations of over 10 μg/g of creatinine, are considered evidence of excessive exposure.

Increased urinary excretion of low-molecular-weight proteins such as β-2-microglobulin or RBP is an early renal effect of cadmium (64). β-2 Microglobulin has been the most extensively examined urinary protein in cadmium nephropathy, but because of its instability in acid urine, measurement of urinary RBP or NAG is probably more reliable (3,8,50,72). Low-level increases in the excretion of albumin and transferrin in cadmium workers with low-molecular-weight proteinuria and enzymuria (3) raise the possibility of glomerular injury but also could be explained by impaired tubular reabsorption or metabolism of these proteins that are normally filtered in small quantities. Proteinuria in cadmium workers rarely exceeds a few hundred milligrams per day and does not approach nephrotic levels (>3.5 g/day). The usual techniques for detecting albuminuria such as Albustix, heat and picric acid, or nitric acid are not sufficiently sensitive or specific to reliably detect tubular proteinuria. Although phosphotungstic, trichloroacetic, and sulfosalicylic acids are more sensitive, immunologic techniques are required for specific protein identification. The pathophysiologic implications for increased excretion of 6-keto-$PGF_{1\alpha}$ and sialic acid in cadmium workers remain to be determined (3).

Calcium Wasting

Clinical symptoms associated with cadmium nephropathy derive primarily from the increased calcium excretion that accompanies the renal tubular dysfunction. Hypouricemia, hypophosphatemia, intermittent renal glycosuria, or elevated serum alkaline phosphatase (in the absence of renal failure or hyperparathyroidism) may bring the acquired Fanconi's syndrome to the clinician's attention, but ureteral colic is more likely to be the cadmium worker's chief complaint (64,67). Although both osteomalacia and renal failure are distinctly uncommon in cadmium workers (62), urinary calculi have been reported in up to 40% of those subjected to industrial exposure (66,67,74). Osteomalacia is associated with diminished renal tubular reabsorption of calcium and phosphate, elevated circulating parathormone levels, and reduced hydroxylation of vitamin D metabolites (76).

Itai-Itai Disease

In Japan, a painful bone disease associated with pseudofractures caused by cadmium-induced renal calcium wasting was recognized in the 1950s. Attributed to local contamination of

food staples by river water polluted with industrial effluents, particularly cadmium, the syndrome known as itai-itai or "ouch-ouch" disease afflicted postmenopausal, multiparous women. Sustained deficiencies in iron, zinc, calcium, and vitamin D rendered these women particularly vulnerable to cadmium toxicity. The women with itai-itai disease tended to have reduced GFR, anemia, lymphopenia, and hypotension as well as osteomalacia. They exhibited a waddling gait, short stature, anemia, glucosuria, and elevated serum alkaline phosphatase levels. Hypertension was absent. β_2-Microglobulin excretion exceeded the normal maximum (1 mg/g of creatinine) 100-fold, and GFR was substantially reduced in the most severely affected individuals. An increase in mean serum creatinine concentration from 1.19 to 1.68 mg/dL in 21 individuals averaging 65 years of age who were followed for 9 to 14 years after β_2 microglobulin was first detected demonstrated the progression of renal failure (76). The long-term follow-up demonstrated that β_2-microglobulin predicts the later development of renal failure in patients with itai-itai disease and that renal damage progresses even after exposure has ceased.

Chronic Interstitial Nephritis

Until recently, the role of cadmium in the induction of chronic interstitial nephritis has been controversial (Fig. 47-4). Epidemiologic studies do not show a consistent correlation between blood or urinary cadmium levels and blood pressure (77). Mortality studies designed to evaluate the long-term implications of industrial cadmium exposure have yielded conflicting results (78). Nevertheless, tubulointerstitial nephritis was found in 23 occupationally exposed and 26 environmentally exposed individuals in whom postmortem tissue or renal biopsy specimens were examined (79). These findings in conjunction with recent epidemiologic studies in the United States (74) and Belgium (80,81) and the long-term follow-up of itai-itai disease in Japan (76) leave little room for doubt that cadmium can induce chronic interstitial nephritis.

In the United States, Thun and coworkers (74) examined 45 current and retired nonferrous smelter workers exposed to cadmium for a mean of 19 years. Their blood cadmium levels averaged 7.9 μg/dL and urinary cadmium 9.3 μg/g of creatinine. Cumulative cadmium dose estimated from air measurements correlated with low-molecular-weight proteinuria and with decreased fractional calcium and phosphate reabsorption. The cadmium workers had significantly more kidney stones than did controls. Correlations between renal function and cadmium exposure were independent of other diseases in the workmen. A strong association between cumulative cadmium exposure and the later increase in serum creatinine supported the notion that cadmium-induced renal disease progresses slowly after a latent period of several decades. Roels and colleagues (80) provided further evidence that clinical renal disease results from cadmium. Workers who were exposed to cadmium in a nonferrous smelter in Belgium for up to 5 years and who had tubular proteinuria were examined annually for 5 years after exposure had ceased. Cadmium levels in liver ranged from 24 to 158 μg/g and from 133 to 355 μg/g in the kidney. Serum creatinine concentrations increased from a mean of 1.2 to 1.5 mg/dL over 5 years (Fig. 47-5). The reduction in GFR was accompanied by an increase in mean serum β_2-microglobulin from 0.189 to 0.300 mg/dL and an increase in mean urinary β_2-microglobulin excretion from 1.770 to 2.500 mg/L. The loss of glomerular filtration over the 5-year period was estimated to be 30 mL/minute, 30 times the predicted loss of kidney function for these elderly men. In contrast to the progression of renal disease in these cadmium workers, the same laboratory found that environmental exposure leading to urine cadmium concentrations of less than 1 μg/24 hours did not result in progressive renal failure over 5 years (81). Despite the low levels of cadmium absorption, the environmentally exposed group exhibited diminished bone density and increased risk of fractures (82).

Diagnosis

Most commonly, the diagnosis of cadmium nephropathy is established by the history of exposure in conjunction with laboratory tests indicative of proximal tubule dysfunction

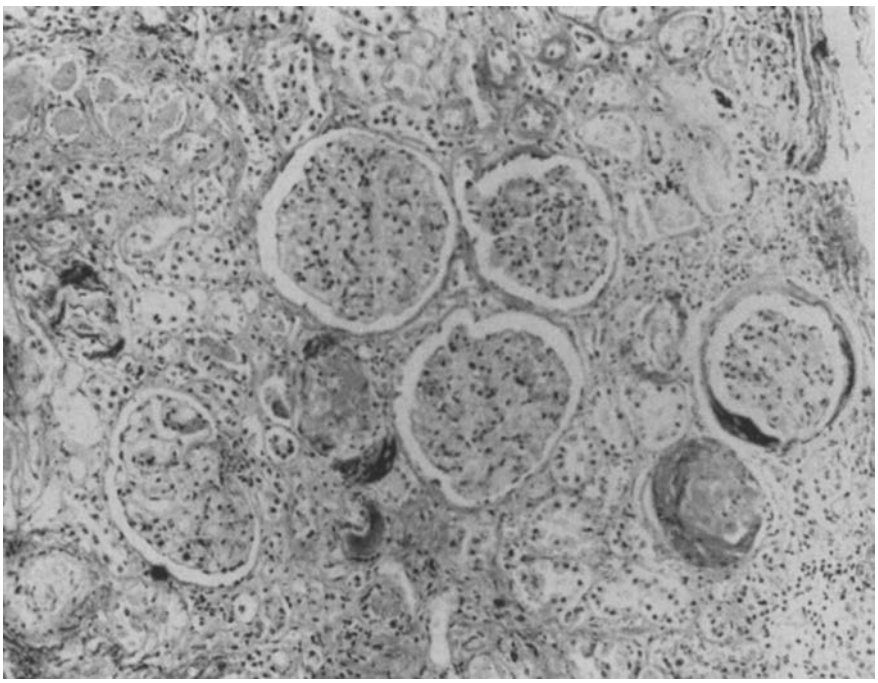

FIGURE 47-4. Autopsy section of kidney from a 46-year-old man who had manufactured cadmium pigments for 28 years. He was found to have renal glycosuria, proteinuria, mild hypertension, and severe pulmonary emphysema. Death was attributed to cor pulmonale. His kidneys contained 55 μg Cd/g wet wt and showed extensive interstitial fibrosis, tubular atrophy, and glomerular sclerosis. (From: Kazantzis G, et al. Renal tubular malfunction and pulmonary emphysema in cadmium pigment workers. *Q J Med* 1963;32:165, with permission.)

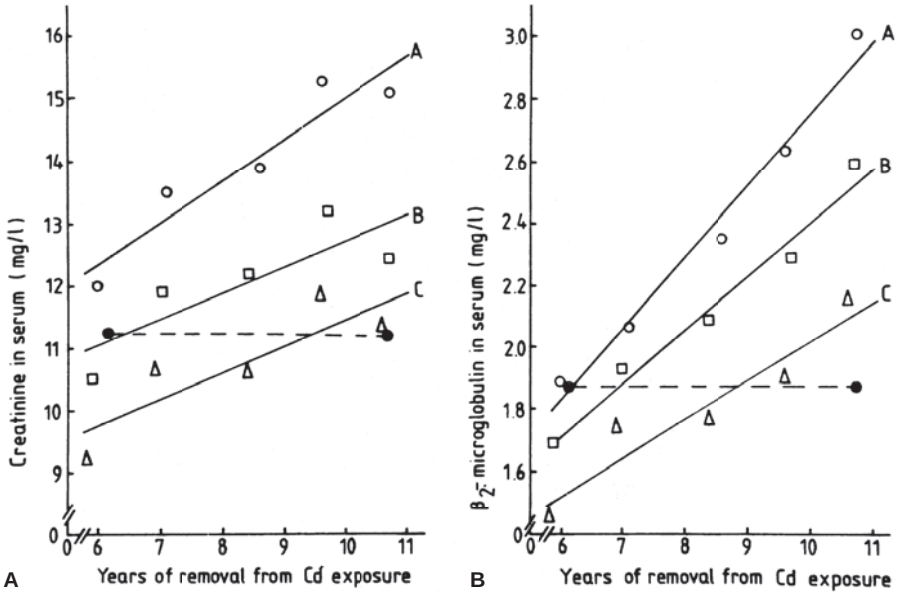

FIGURE 47-5. Progression of renal dysfunction in cadmium (Cd) workers over 5 years after removal from exposure. **A:** Total population of cadmium workers (N = 23). The mean serum creatinine increased from 1.2 to 1.5 mg/dL. **B:** Cadmium workers with serum creatinines <1.5 mg/dL (N = 21). **C:** Cadmium workers with tubular proteinuria and serum creatinine <1.3 mg/dL (N = 11). *Dashed line* represents two age-matched control groups of 23 subjects each.

(e.g., increased excretion of urinary biomarkers, hypercalciuria, or renal glycosuria). Suspicions are confirmed if the urinary cadmium concentration exceeds 10 μg/g of creatinine. Renal and hepatic accumulation of cadmium has been exploited for diagnostic purposes by *in vivo* neutron-activation analysis. This noninvasive technique for assessing organ cadmium content correlates well with tissue and urinary cadmium levels and β_2-microglobulin excretion. It should prove valuable for monitoring industrial exposure before toxic levels have been attained (80). In individuals without occupational exposure, renal cadmium content reaches a maximum of about 50 μg/g of cortex at about 45 years of age and thereafter declines as urinary elimination increases (66). Renal cadmium concentration tends to fall with the development of renal failure, so that the diagnostic value of neutron-activation analysis of kidney is diminished in azotemic or uremic patients. *In vivo* neutron-activation studies indicate that when the hepatic cadmium level exceeds about 60 ppm, and renal cortical content exceeds 200 ppm (20 mg/kidney), tubular proteinuria is likely to occur (80).

Treatment

Although $CaNa_2EDTA$ given to experimental animals simultaneously with cadmium results in prompt excretion of the cadmium chelate and protection from nephrotoxicity, the chelating agent has little effect after cadmium has been complexed with metallothionein (83). Progression of renal disease has been described despite removal from exposure (76). Although osteomalacia may be arrested by calcium and vitamin D replacement (72), urinary tract stones represent a relative contraindication to such therapy.

MERCURY

The toxicity of mercury depends on both its chemical form and the route of absorption. Elemental mercury is virtually harmless when ingested (84), but inhalation of the metallic vapor can produce bronchial damage and later neurologic disease (85,86). After *in vivo* oxidation of metallic mercury to the ionic form, the inhaled vapor readily crosses the blood-brain barrier and is retained in the cerebrum and cerebellum. Although preferentially accumulated in the kidney, neurologic but not nephrologic disease regularly follows exposure to elemental mercury; paresthesias, tremor, ataxia, visual impairment, erythrism, and ultimately stupor and death are the clinical course.

Once in the environment, elemental mercury undergoes biotransformation to both organic and inorganic salts that are absorbed by living organisms and thus enter the food chain. Methyl, ethyl, and phenoxyethyl mercury are important organomercurial contaminants arising from industrial and agricultural processes. Methyl and ethyl mercury compounds are widely used as fungicides but also enter the environment as industrial wastes and through biotransformation of less toxic mercurials. Although a diuretic effect of inorganic mercury was documented in the late 19th century, diuresis following therapeutic administration of organomercurials was first noted by a medical student in Vienna in 1920 as a side effect of Novasurol used in antisyphilitic therapy (86). Organomercurials subsequently became the mainstay of diuretic therapy until the advent of ethacrynic acid and furosemide in the 1960s. The inorganic mercurous salt, calomel (Hg_2Cl_2), is relatively nontoxic and was widely used as a medicinal agent until the twentieth century. The mercuric salt, corrosive sublimate ($HgCl_2$), on the other hand, is highly nephrotoxic and continues to be used to create animal models of acute tubular necrosis (ATN) (86). Phenyl and methoxy methyl mercuric salts produce similar nephrotoxicity.

Although all chemical forms of mercury are accumulated in the cells of proximal tubules, the cellular concentration by itself provides little insight into pharmacologic or toxic mechanisms. Inorganic mercury is retained in the kidney with a biologic half-life approximating 2 months (87). While $HgCl_2$, chlormerodrin, and *p*-chloromercuribenzoate (*p*-CMB) are each selectively concentrated in the proximal tubule, the inorganic $HgCl_2$ salt produces tubular necrosis, chlormerodrin is a diuretic, and *p*-CMB blocks the diuretic effect of chlormerodrin (87–89).

The inorganic and organic mercurials bind avidly to sulfhydryl groups in circulating proteins and amino acids as well as intracellular glutathione, cysteine, and metallothionein. Selective accumulation in the pars recta of proximal tubules is accomplished by transport primarily from the luminal side of mercury bound to amino acids or proteins. Mercury-ligand reaches lysosomes by endocytosis (88) with subsequent release into the cytosol by intralysosomal enzymatic degradation. It is less clear if mercury moves in the secretory direction, entering proximal tubular cells from the peritubular surface. Excretion is primarily through bile in the feces.

Diagnosis

Although blood mercury levels over 3 μg/dL or urine levels above 50 μg/g of creatinine are considered abnormal, the correlation of blood and urine concentrations with renal disease is poor (90). Workers exposed to mercury vapors frequently excrete more than 200 μg of mercury per liter of urine, but very few have renal disease. Consequently, the diagnosis of mercury-induced renal disease is usually dependent on known exposure in the presence of renal dysfunction. Case reports of ATN or the nephrotic syndrome attributed to occupational, accidental, or intentional exposure to mercury are necessarily limited to situations in which the toxic agent has been identified. Occupational exposure to elemental mercury for a decade with urinary concentrations exceeding 50 μg/dL is associated with increased HIAP excretion but little increase in urinary TNAP, NAG, RBP, THG, β_2 microglobulin, or microalbumin (1,5) (Table 47-1). There is no evidence that enzymuria from mercury exposure predicts the development of renal failure.

Acute Tubular Necrosis

Ingestion of as little as 0.5 g of $HgCl_2$ produces ATN in humans. Over the first few days, the clinical picture is dominated by gastrointestinal symptoms including erosive gastritis with hematemesis and melena. Before oliguria supervenes, chelation therapy with intravenous BAL (British antilewisite; dimercaprol) may limit renal damage. Initially, oliguria should be treated by volume expansion with close monitoring of central venous or pulmonary wedge pressure. Not only may diuresis induced by hydration, mannitol, and furosemide prevent the development of oliguric acute renal failure, but also persisting oliguria in the face of adequate therapy indicates renal parenchymal damage. An elevated urinary sodium concentration (>40 mEq/L) and diminished concentrating capacity (Uosm <450 mOsm/L) in association with failure to obtain diuresis in an acutely oliguric patient with adequate circulatory status confirm the diagnosis of ATN. Once oliguria has become established, rigid fluid restriction is mandatory (<500 mL/day). Acute oliguric renal failure may rapidly leads to death unless dialysis is provided.

Histologic examination of the kidneys reveals necrosis of the proximal tubules, particularly the pars recta. In the experimental model, the tubular basement membrane is spared compared with the damage incurred during ischemic tubular necrosis (87). However, backleak of inulin from the tubular lumen to peritubular capillaries has been observed in experimental animals, indicating loss of integrity of the proximal tubule. Such backleak contributes to the reduction in inulin or creatinine clearance induced by outer cortical ischemia. In the rat, $HgCl_2$-induced ATN is accompanied by sequestration of mercury sulfide within lysosomes of surviving proximal tubule cells (88). The extent of damage to individual nephrons is highly variable, although tubular necrosis extends to more proximal segments after larger doses (87).

During the recovery phase, oliguria is replaced by polyuria while the GFR is still low. Urine flow rates may double daily, reaching a maximum of about 5 L/day while the serum creatinine continues to rise, albeit more slowly than the initial rate of 1 to 2 mg/dL/day. During the diuretic phase, salt and water must be vigorously replaced, but potassium restriction and reduced doses of toxic drugs normally excreted by the kidneys should be maintained. If death from hypervolemia, hyperkalemia, hemorrhagic complications, uremia, and infection is avoided, spontaneous regeneration of tubular epithelium occurs with subsequent recovery. Dystrophic calcification of necrotic tubules may, however, limit restoration of function, and the kidneys may show residual interstitial nephritis (88). The entire process from acute oliguria through polyuria and recovery may last from a few days to many months.

Nephrotic Syndrome

Sporadic case reports of nephrotic syndrome following exposure to elemental or organic mercury have appeared since the middle of the twentieth century. Proteinuria in children with acrodynia ("pink disease") following external use of mercurial ointments or powders has been attributed to allergic reactions. In the occupational setting, the causal relationship of mercury exposure to proteinuria and the nephrotic syndrome has been less compelling (84), largely because the dose-response is unpredictable and, in addition, the etiology of nephrotic syndrome unrelated to mercury is rarely known.

Renal biopsies have most often shown deposits within glomerular capillaries consistent with membranous nephropathy (92) (Fig. 47-6), but normal glomeruli (e.g., nil disease) and antiglomerular basement membrane (anti-GBM) antibody deposition also have been described. In most instances, proteinuria owing to mercury appears to be self-limited and disappears spontaneously when the source of exposure is removed.

Observations in rats may provide a framework for understanding mercury-induced glomerular disease in humans. In 1971, Bariety and associates (93) reported that multiple subcutaneous injections of $HgCl_2$ in rats, in doses too small to produce ATN, induced membranous nephropathy. Renal disease characterized by glomerular deposition of immune complexes and heavy proteinuria developed in about 2 months. Subsequent studies showed that the immune response is actually biphasic; immune complex deposition is preceded by anti-GBM antibody and complement deposition (94). The response to mercury in the rat is under precise genetic control and is dose

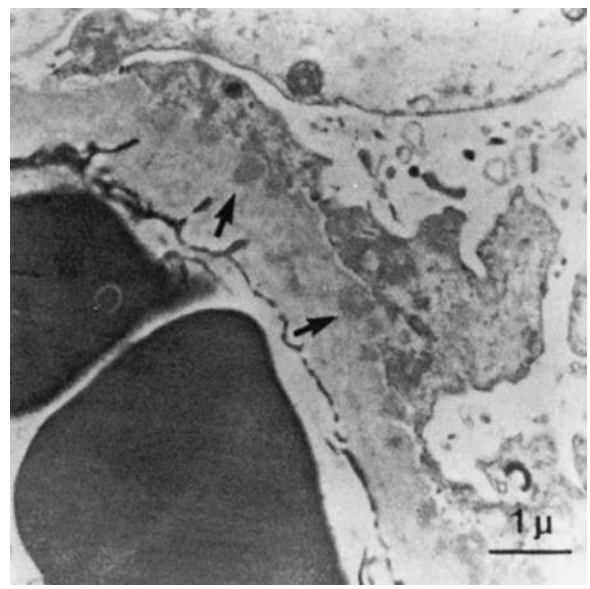

FIGURE 47-6. Electron micrograph of kidney from a 24-year-old man exposed to mercury vapor in an industrial electrolysis unit. The urinary mercury was 174 μg/24 hours; urinary protein, 3.11 μg/24 hours. Creatinine clearance was 116 mL/minute/1.73 m^2. Subepithelial electron-dense deposits (*arrows*), presumably immune complexes, overlie the glomerular basement membrane. (Lead citrate and uranyl acetate; magnification ×11,000.) (From: Tubbs RR, et al. Membranous glomerulonephritis associated with industrial mercury exposure. *Am J Clin Pathol* 1982;77:409, with permission.)

dependent (95,96). As little as 0.005 mg/100 g of body weight elicit immunologically mediated glomerular disease in selected strains. Metallic mercury vapor (1 mg/m^3) is as effective as $HgCl_2$ for inducing autoimmune disease in susceptible rats. Similar renal disease has been induced in other rodents and with alternate forms and routes of exposure, including organomercurials and inhalation. As in humans, mercury-induced glomerular disease in rats is self-limited. Immunoglobulin localization in the glomeruli is associated with heavy proteinuria, circulating immune complexes, and polyclonal B-cell activation owing to antiself Ia autoreactive T cells. The glomerular disease can be transferred to T cell-depleted rats by T cells and helper T cells taken from $HgCl_2$-treated rats of the same strain (97). Weening and co-workers found evidence that the autoimmune process is initiated by mercury inhibition of suppressor T-cell functions (97).

Minimata Disease

In 1956, endemic methyl mercury poisoning was recognized in Japan arising from the contamination of food by industrial effluents in the area of Minimata Bay (98). Severe neurologic defects including visual, speech, and gait disturbances afflicted several hundred adults whose diet consisted largely of contaminated fish. The mercury pollution had been going on for a decade before the health consequences were recognized. Fish from Minimata Bay contained up to 36 mg of mercury per kg. Cerebral palsy was common among the children of affected mothers. Similar clusters of cases were subsequently identified in Nigata, Japan, and in Iraq, where the disease was the result of bread prepared from grain that had been treated with methyl mercury fungicide.

Although methyl mercury is more avidly accumulated by renal than by neural tissue, the kidney manifestations of Minimata disease are minor. Tubular proteinuria occurs (99), but clinically important albuminuria and azotemia have not been reported. Postmortem examinations have shown minimal nonspecific renal abnormalities, although rats treated with methyl mercury show increased numbers of mercury-containing lysosomes in proximal tubule epithelial cells. In more heavily exposed rats, lysosomal and mitochondrial dysfunction has been observed and may account for the tubular proteinuria in patients with Minimata disease.

Treatment

British antilewisite is an effective chelator for acute inorganic mercury poisoning. Up to 5 mg/kg is given initially by the intramuscular route, followed by 2.5 mg/kg twice daily for 10 days. In the presence of acute renal failure, the mercury chelate can be removed by hemodialysis. BAL is of doubtful value in chronic poisoning, which is effectively treated by removing the patient from the source of exposure. Succimer (DMSA), an oral chelating agent, approved for the treatment of lead poisoning in children, is an effective chelator of mercury and may play an important role in the treatment of mercury toxicity in the future (89).

OTHER HEAVY METALS

As long as the etiology of most end-stage renal disease is unknown, the contribution of environmental and industrial toxicants to the induction of chronic renal disease will remain unclear. Uranium is used to induce experimental ATN and, like lead, is stored in bone. Uranium is selectively accumulated in the proximal tubule with a biologic half-life approximating 1 week for 95% of the renal stores (100). After inhalation, the uranyl ion binds to circulating transferrin and to proteins and phospholipids in the second and third segments of the proximal tubule. Rats given uranyl nitrate subcutaneously develop intrarenal microcysts 8 weeks after injection (101). Acute tubular necrosis occurred in men working on the atomic bomb during the Manhattan Project in the 1940s (102), but chronic renal failure caused by uranium has not been reported. A workplace hazard evaluation performed by the National Institute of Occupational Safety and Health revealed significantly increased $ß_2$-microglobulin excretion compared to controls in workmen exposed to uranium dust (103). The clinical significance of this finding is doubtful, however, because the urinary β_2-microglobulin levels were well within the normal range and there was no evidence of reduced renal function. Many of these men had urinary uranium levels in excess of the upper acceptable limit of 30 μg/L.

Acute tubular necrosis has been reported after voluntary ingestion of copper sulfate in young female science students attempting suicide in Delhi, India (104).

Minimal tubular proteinuria in the absence of reduced glomerular filtration has also been reported in chrome platers, but the implications of this finding for clinically important renal disease also remain speculative (105). Like other heavy metals, chromium is selectively accumulated in the proximal tubule. The hexavalent form induces ATN, but there is no convincing evidence of tubular injury from usual occupational exposure (9,106–109). Chromium has been recognized as an important cause of lung cancer for over 100 years, but chronic renal disease from occupational or environmental exposure has not been reported.

Bismuth compounds prepared as therapeutic agents have produced unequivocal ATN. Lower dosage induces Fanconi's syndrome with reduced glomerular filtration and bismuth-containing intranuclear inclusions in proximal tubule cells that are similar to, but distinguishable from, lead inclusions (110).

The acute cardiovascular collapse and hemolysis accompanying arsenic poisoning are associated with renal failure, but whether the mechanism of injury involves distinct cellular toxicity is unclear (111). Moonshine contaminated with arsenic produces painful polyneuropathy that is distinct from the isolated motor neuropathy of lead poisoning. Patchy cortical necrosis with persistent residual renal failure has been reported following consumption of arsenic-contaminated liquor (111,112). When arsenicals react with acid, the deadly, colorless, odorless gas arsine (AsH_3) evolves. Almost invariably the result of an industrial accident, arsine inhalation produces hemolysis, hematuria, and abdominal pain within a few hours, followed by acute oliguric renal failure and jaundice within 2 days (113). Reticulocytosis, basophilic stippling, bilirubinemia, and free hemoglobin in the plasma may assist diagnosis, which is established by detecting arsenic in the urine. Acute tubular necrosis followed by residual interstitial nephritis 2 years after exposure has been documented (Fig. 47-7). BAL is ineffective once renal failure is present. In addition to hemodialysis, exchange transfusion may be necessary to eliminate hemoglobin-bound arsenic from the body.

SOLVENTS

Halogenated hydrocarbons have often been implicated in the induction of ATN or Fanconi's syndrome in both humans and experimental animals. Low-level occupational absorption by inhalation of volatile hydrocarbons or absorption through the skin may also induce tubular proteinuria, which does not necessarily signify the presence of clinically important renal disease, glomerular damage, or immune system activation (114,115).

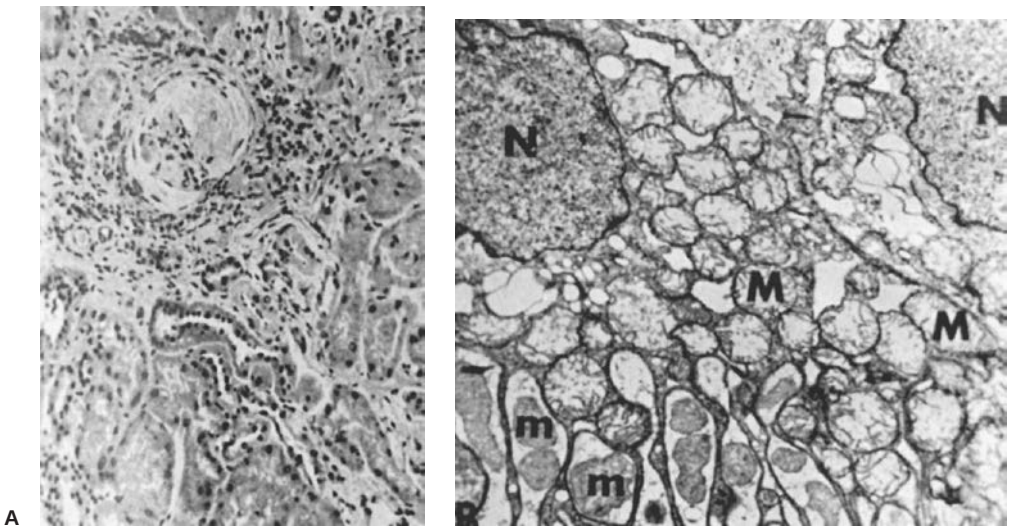

FIGURE 47-7. Renal biopsy obtained 2 years after acute renal failure from arsine poisoning in an industrial accident. The patient required peritoneal dialysis and hemodialysis for 6 weeks. The initial renal biopsy showed acute tubular necrosis. At the time of this biopsy the creatinine clearance was 24 mL/minute. **A:** Light microscopy shows local interstitial fibrosis, tubular atrophy, and sclerosis in 13 of 17 glomeruli. (Hematoxylin and eosin; magnification ×300.) **B:** Electron microscopy shows normal and damaged proximal tubule cells. In this illustration, normal and distorted mitochondria are present in a single cell adjacent to a normal nucleus. (Uranyl acetate and lead citrate; magnification ×9,000.) *m,* probably newly formed mitochondria; *M,* normal mitochondria; *N,* nuclei. (From: Muehrcke RO, Pirani CL. Arsine-induced anuria: a correlative clinicopathological study with electron microscopic observations. *Ann Intern Med* 1968;68:853, with permission.)

Light Hydrocarbon Nephropathy

Toxicologic studies of the effects of gasoline distillates performed over the past two decades under the auspices of the American petroleum industry have identified an effect of gasoline constituents on the renal tubule of male rats. Referred to as light hydrocarbon nephropathy, the tubular injury is induced by exposing Fischer 344 male rats to petroleum hydrocarbon vapors from a few hours up to a few years (116,117). Mice, guinea pigs, dogs, primates, and female rats do not develop the lesion. It is not known if similar morphologic tubular damage occurs in humans exposed to gasoline vapors. However, acute renal failure following intense exposure to diesel fumes has been reported in humans (118). The hydrocarbons studied in animal models include *n*-nonane, C_8, C_{10}–C_{11} isoparaffinic solvent, jet fuels, methylisobutyl ketone, varnish, unleaded gasoline, naphthas, and a variety of complex organic solvents and distillates. These volatile hydrocarbons are cytotoxic to proximal tubules, where they and their metabolic products are selectively accumulated. The most prominent lesion is hyaline droplet formation within epithelial cells of proximal tubules. Sustained renal failure with permanently reduced GFR has not been reported in light hydrocarbon nephropathy in humans or experimental animals.

A number of investigators have attempted to identify solvent-induced glomerulonephritis by assessing the urine of exposed workers for low-molecular-weight proteins and enzymes, markers for tubular, rather than glomerular disease. Light hydrocarbon nephropathy should not be confused with the relatively rare glomerular disease in humans referred to as solvent nephropathy (115). Increased excretion of TNAP has been reported in workers exposed to perchlorethylene in dry cleaning establishments (50). Although such tubular proteinuria is common, the massive albuminuria of solvent nephropathy is distinctly rare in association with perchlorethylene exposure.

Severe neurologic toxicity has been reported following glue sniffing among teenagers seeking intoxication. Permanent as well as transient neurologic and hepatic damage has resulted. Fanconi's syndrome caused by proximal tubular reabsorptive defects has been observed following recreational glue sniffing, apparently the result of toluene, mixed with acetone, isopropyl alcohol, ethyl acetate, and trichloroethylene. Immunologically mediated glomerulonephritis and the hemolytic–uremic syndrome have also been reported following glue sniffing (14). In addition, distal renal tubular acidosis and myoglobinuria have been reported with acute renal failure and residual chronic tubulointerstitial nephritis.

Toluene is metabolized to benzoic acid and then to hippuric acid, which are selectively accumulated in proximal tubules. These organic acids may contribute to "high anion gap metabolic acidosis" because they are "unmeasured anions" in the blood. Significant renal disease, however, has not been found in cross-sectional studies of industrial workers regularly exposed to styrene, toluene, or xylene or oil refinery workers (119). The failure to find increased tubular proteinuria in cohorts exposed to solvents, oils, or hydrocarbons, of course, has no bearing on the question of solvent-induced glomerulonephritis.

Solvent Nephropathy

At least 40 clinical epidemiologic studies have examined the relationship between glomerulonephritis and exposure to organic solvents (120–122). A number of these studies concluded that patients with chronic glomerulonephritis have been exposed to organic solvents (aliphatic and aromatic) more frequently than patients with other diseases. Initially, solvent nephropathy was associated with anti-GBM antibody-mediated glomerulonephritis and pulmonary hemorrhage (e.g., Goodpasture's syndrome) (114). As of 1992, at least 31 cases of Goodpasture's

syndrome following solvent exposure had been reported (120). Organic solvents may expose the otherwise cryptic Goodpasture antigen (type IV collagen $\alpha 3$ chain) to the immune response system in susceptible individuals (123). On the other hand, inhaled hydrocarbons may alter alveolar immune reactivity, making lung a preferential target for antialveolar basement membrane antibody deposition (124).

Following the initial description of Goodpasture's syndrome resulting from solvent exposure, reports of solvent nephropathy have included many different types of glomerulonephritis. Although a graded dose-response to solvents has not been documented, this caveat may not be important for immunologically mediated disease. Progression of glomerulonephritis has been associated with continued solvent exposure (124,125). The huge number of chemically distinct compounds contained in petroleum may preclude identification of a specific toxic agent. Avoidance of exposure to volatile hydrocarbons and their derivatives remains an essential preventive approach.

The existence of solvent nephropathy has met with some skepticism. Indeed, caution is justified because of the absence of a good experimental model of immunologically mediated glomerulonephritis, combined with concern that the correlation between solvent exposure and chronic glomerulonephritis may be influenced by patient recall bias. The etiologic role of solvents remains controversial because the dose and composition of industrial solvents are usually unknown. Moreover, of the thousands of workers exposed, very few develop kidney disease. The genetic and environmental factors that make specific individuals susceptible to solvent nephropathy have not been delineated. The inability to obtain reliable measurements of solvent exposure makes it likely that the etiologic role of solvents in the induction of glomerular disease will remain controversial.

SILICON

Silicon is a semimetal found as the dioxide silica (SiO_2), in 28% of the earth's crust. Because it has a specific gravity of only 2.3 it is not a heavy metal. Silica is present in the serum at concentrations of 20 to 50μg/dL in an unbound form as silicic acid and is cleared in the urine at the rate of glomerular filtration (126). Silica is believed to induce renal disease by direct deposition of crystalline material in the renal parenchyma (127) and by immunologic mechanisms acting as an adjuvant to stimulate the immune response. Tubular proteinuria is found in workers exposed to silica dust (128,129). The odds ratio to develop end-stage renal disease for a patient who worked as a sandblaster is 3.8 compared to matched controls (39). In the accelerated form of silicosis known as silicoproteinosis, rapidly progressive, immune complex-mediated focal glomerulosclerosis may appear (129). In addition to the severe nodular pulmonary fibrosis, these patients develop an overwhelming autoimmune response that simulates lupus erythematosus (Caplan's syndrome). Glomerular disease that is independent of silicosis also has been described as a result of exposure to silica (128–131). Antineutrophil cytoplasmic antibody (c-ANCA) positive Wegener's granulomatosis has been associated with exposure to silica dust as well as to silicon containing compounds such as grain dust (132).

GERMANIUM

Falling just below silica in the periodic table group IVA, which includes tin (Sn) and lead (Pb), germanium has a specific gravity of 5.23. Like silicon, it has semiconductor properties. It has been used in the treatment of cancer, a variety of medical ailments, and in unproved remedies for conditions such as arthritis, acquired immunodeficiency syndrome (AIDS), and the chronic fatigue syndrome. Germanium-containing elixirs and health foods were reported to cause chronic tubulointerstitial nephritis first in Japan in the 1980s and more recently in Europe and the United States (133–137). Most cases have resulted from the ingestion of germanium oxide (GeO_2), but germanium-lactate-citrate and the organic germanium compound carboxyethyl germanium sesquioxide (Ge-132) have also been implicated. The renal disease differs from that induced by other heavy metals in that widening of the interstitium and tubular atrophy (apparently distal tubular) has been evident after prolonged (6 to 36 months), high-dose (16 g to hundreds of grams) consumption. The tubulointerstitial disease is slowly progressive even after exposure has been terminated. Fatal outcomes have been reported. The pathophysiologic mechanism of tubular damage is unclear because selective accumulation in the kidney does not occur and immunologic mechanisms have not been implicated. There is no evidence of primary proximal tubular injury. Proteinuria is absent. Electron-dense, periodic acid-Schiff (PAS) reagent-positive granules (containing germanium in the experimental rat) are found in distal tubular mitochondria (136). Vacuolization is present in proximal and distal tubular epithelia (133,134). Prolonged consumption of germanium compounds also induces severe hepatic steatosis, polymyositis, and peripheral neuropathy. Acute lactic acidosis has been reported from germanium-lactate-citrate (133,137).

BALKAN NEPHROPATHY

Balkan endemic nephropathy is a slowly progressive tubulointerstitial nephritis of unknown etiology described about 40 years ago among middle-aged men and women living in farming villages along the Danube River in Croatia, Serbia (the former Yugoslavia), Rumania, and Bulgaria (138). The disease occurs in families and is associated with urinary tract transitional cell carcinomas and tubular proteinuria (β_2-microglobulin). Heavy proteinuria, fluid retention and hypertension are absent. Unfortunately, control groups with renal disease of known etiology and comparable severity have not been studied in this region. A variety of environmental factors have been suspected including lead, cadmium, and mycotoxins, but no single etiologic agent has been incriminated. The possibility of low-level toxicity from suspect environmental nephrotoxins or a multifactorial etiology has not been excluded. The prevalence of generally recognized renal diseases has not been established in the Danube region, making differentiation of Balkan nephropathy from known renal diseases identified in other regions of the world problematic.

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CHAPTER 48 ■ ACUTE TUBULOINTERSTITIAL NEPHRITIS

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When first introduced, the pathologic diagnosis of "acute interstitial nephritis" was used to identify the morphologic features observed at postmortem in the kidney of patients with a disparate group of infectious diseases in which the kidney was sterile and free of the infectious organism causing the systemic illness (1). The earliest description of the lesion was reported in 1860 in a fatal case of scarlet fever that at postmortem examination revealed infiltration of the kidney by lymphocytic cells, in the absence of suppurative bacterial infection; the condition was termed *lymphomatous nephritis* (2). After the report of several similar cases, Councilman (1) in 1898 published his now classic paper in which he described the lesions of acute interstitial nephritis (AIN), based on a review of the literature and his observations made at postmortem examination of 42 cases. He found the lesion to occur with approximately equal incidence in patients with scarlet fever (25%) and diphtheria (23%), and less frequently in patients with other infectious diseases. Councilman summarized the pathologic process as "an acute inflammation of the kidney characterized by cellular and fluid exudation in the interstitial tissue, accompanied by, but not dependent on, degeneration of the epithelium; the exudation is not purulent in character, and the lesions may be both diffuse and focal," and identified the infiltrating cells as "plasma cells that had migrated from the blood vessels and multiplied locally by mitotic division." He localized the foci of cellular infiltrates to three sites: the boundary zone of the pyramids, the subcapsular region of the cortex, and around the glomeruli. These meticulous observations, lucid description, and insightful analysis are just as valid today as they were then and encapsulate the lesions of this disease of the kidneys more succinctly and thoroughly than anything that has been written over the century since they were first reported (1).

Subsequent reports confirmed Councilman's observation that nonsuppurative lesions of the kidney interstitium appeared after short but variable periods after the onset of acute infections that were primarily streptococcal in origin. These were most common in the cortex of the kidney and consisted mostly of focal infiltrates comprising for the most part lymphocytes and plasma cells, with only occasional polymorphonuclear leukocytes (3,4). The concept of acute tubulointerstitial injury after acute infection was so well accepted that several of the initial reports of acute tubular necrosis (ATN), which appeared immediately before the proper characterization of acute renal failure (ARF) due to tubular necrosis during the first part of World War II, called the lesion *acute hematogenous interstitial nephritis* and argued for the similarity of the lesions of ATN to those noted after streptococcal infections (5,6). In fact, there was a brief period during which the lesions of ATN and AIN were confused because they were considered variants of the same entity. This difficulty persists to this day if the kidney tissue is not examined carefully and is interpreted without benefit of the clinical history (7). The pathologic lesions of ATN and AIN now are classified as variants of tubulointerstitial disease in general (8).

The introduction of antibiotics during World War II and the eradication of serious and fatal streptococcal infections resulted in loss of interest in the entity described by Councilman, while attention focused on ischemic or nephrotoxic ATN as the predominant cause of ARF. It therefore is ironic that interest in AIN was revived in the 1960s when the very antibiotics used to treat streptococcal infections came to be recognized as a cause of AIN (9), after the lesions were described in a case of penicillin hypersensitivity in 1958 (10). In fact, the bulk of the current reports in the literature are on drug-induced AIN, and the number of drugs implicated as causing AIN continues to increase, as does that of the variations in their clinical and laboratory manifestation (11,12). AIN has since come to define the clinicopathologic syndrome that develops in diverse conditions, including infections, and is characterized by an acute deterioration of kidney function, the pathologic features of which remain those first described by Councilman in 1898 as "characterized by cellular and fluid exudation in the interstitial tissue, accompanied by, but not dependent on, degeneration of the epithelium; . . . and the lesions may be both diffuse and focal" (1).

Although this clinicopathologic syndrome initially was referred to as *acute interstitial nephritis* (1,9,13), the more inclusive and precise descriptive terms *acute tubulointerstitial nephritis* (ATIN) and *acute tubulointerstitial nephropathy* are used now (12). The preferential use of the term *tubulointerstitial* stems from the fact that although the dominant morphologic features are those evident in the interstitium, the tubules also are affected, albeit to a degree that may be difficult to detect on light microscopy. However, the structural injury to the tubules can be severe; more important, and independent of the severity of their structural injury, it is disorders of tubular function that constitute a characteristic component of the disordered kidney function and that differentiate this entity from other forms of ARF due to glomerular or vascular disease (14–17). In fact, tubular dysfunction is an invariable accompanying feature of the reduction in glomerular filtration rate (GFR) and, as a rule, can be documented to precede a clinically evident decrease in GFR (15,18). In addition, although the interstitial cellular infiltrates contribute significantly to the pathogenesis of the disease, there is increasing evidence that the tubules play an important role in the processing and presentation of the antigenic stimulus and the immunopathogenesis of the disease process (19–22). Stimulated tubular epithelial cells also differentiate into infiltrating fibroblasts and are responsible for the release of cytokines that lead to recruitment of the infiltrating cells responsible for progression of the injury and its potential progression to irreversible damage (22–25).

Although ATIN may be suspected clinically, the diagnosis depends on the presence of characteristic morphologic changes of the kidney noted on biopsy specimens or at postmortem examination. The adjective *acute* refers to the sudden onset and rapid progression of the clinical features of this syndrome and not to its pathologic features, which are notable for

mononuclear cell infiltration rather than the polymorphonuclear leukocytes characteristic of an acute inflammatory reaction (9).

PATHOLOGIC FEATURES

Independent of the causative agent or clinical condition responsible for ATIN, the principal morphologic features that characterize it are an increase in the interstitial volume, mainly because of edema; mononuclear cell infiltrates of varying degrees and distribution; and tubular injury and degeneration of differing severity, which in general are localized to the sites of the greatest cellular infiltrates. The glomeruli are spared, but many show some degree of periglomerular infiltrates and ischemic change, except in ATIN associated with nephrotic syndrome, where the lesions of minimal-change disease are seen on electron microscopy. Although the extent and severity of each of the tubulointerstitial lesions show some correlation with the level of reduction in GFR, the closest correlation is found with the infiltrative process (11,26). The interstitial infiltrative lesions may be diffuse but usually are patchy in distribution and most evident in the cortex and outer medulla of the kidney. When the infiltrating cells are meager, they show a focal peritubular distribution that is most evident in the corticomedullary region; when profuse, they literally obscure the normal appearance of the tubules, are even periglomerular, and may extend into the medulla, where they are always much less prominent. As first noted by Councilman (1), even in cases in which the infiltrates are diffuse they always are more prominent at certain foci. A diffuse infiltrative process has been shown to be associated with higher levels of serum creatinine and a poorer prognosis for recovery than patchy infiltrations. The infiltrating cells are composed mostly of mononuclear cells, lymphocytes, and plasma cells, and only rarely polymorphonuclear cells and macrophages. The prognosis appears to be less favorable when 1% to 6% of the interstitial infiltrating cells are composed of neutrophilic granulocytes (26). By the same token, an increase in the number of macrophages (usually <10%) and the presence of granulomatous reactions are associated with a prolonged course of ARF and varying degrees of residual impairment of kidney function (11,27–29). When present, eosinophils are sparse and constitute only a small component of the infiltrating cells, except in occasional cases of antibiotic-induced AIN, where they may be marked. The presence of infiltrating eosinophils shows no relation to the increased eosinophilia or the presence of urinary eosinophils that also occur in drug-induced ATIN. A role for a specific chemoattractant for eosinophil cells, eotaxin, which is expressed in the kidney, has been proposed as a mechanism for tissue eosinophilia (30). The production of eotaxin is upregulated in various forms of allergic and hypersensitivity inflammation such as asthma, conjunctivitis and drug-induced exanthems (31,32). Monoclonal antibodies to eotaxin have shown the presence of eotaxin mainly in the endothelial cells, infiltrating mononuclear cells, and in a small number of tubular epithelial cells in a case of renal interstitial eosinophilia with membranous glomerulopathy (33). Whether the same mechanism is operative in all cases of ATIN remains to be determined and deserves to be examined.

Immunologic characterization and analysis of the interstitial cellular infiltrates reveals that most (up to 80%) of the mononuclear cells are activated T lymphocytes. B lymphocytes also are present but constitute a much smaller portion of the interstitial cellular infiltrates, being highest in cases due to nonsteroidal antiinflammatory drugs (NSAIDs) (34). The infiltrating T cells are predominantly CD3+, with relatively smaller numbers of CD4+ cells of the helper/inducer subset and CD8+ cells of the cytotoxic/suppressor subset. In most cases, the CD4 variety predominates over the CD8 variety (34–38). Natural killer lymphocytes are rare and, when present, constitute only a small proportion of the infiltrating cells (35). A slight prevalence (just over 50%) of the CD8+ suppressor/cytotoxic subset has been noted in cases of ATIN associated with massive proteinuria after exposure to NSAIDs (34) or antibiotics (38). The relevance of this observation to massive proteinuria as a feature of ATIN is unknown (34–36). Convincing evidence has been advanced that the infiltrating cells are antigenically activated (39,40) and immunologically engaged in the pathogenesis of the lesions (41–43). However, no diagnostic pattern of markers or cell types has come to be identified with the lesions associated with any specific form of ATIN.

Variable degrees of tubular injury usually are present, but tubular atrophy is absent. The tubular lesions are most evident at the site of greatest concentration of infiltrating cells. They usually are focal and are more severe in the proximity of infiltrating cells (26,44). A distinguishing lesion that results from focal infiltrating lymphocytes is disruption of the tubular basement membrane (TBM) and its epithelial cell lining, so-called "tubulitis," which is characteristic of ATIN. Granulomatous reactions, multinucleated epithelioid cells, and polymorphonuclear leukocytes are detected in most cases with marked tubular injury. Rupture of the TBM and "tubulitis" may be seen in these severe cases. Even in the absence of evident epithelial cell injury on light microscopy, electron microscopy reveals structural cell changes, loss of the brush border, and fragmentation or lamination of the TBM (44,45). Occasional mitotic nuclei of the epithelial cells may be observed. As a rule, immunofluorescent studies are not revealing. Scant and nonspecific granular staining for immunoglobulins, usually without complement, may be detected along the TBM. In rare drug-induced cases, linear deposits of immunoglobulin G (IgG) and complement (C3) may be present, indicating antibodies directed against tubular membrane antigens. There is increasing evidence for a participatory role of the epithelial cells in the pathogenesis of ATIN (21,22). Enhanced expression of the human leukocyte antigen (HLA) class II antigens (HLA-DR) of injured tubular epithelial cells has been demonstrated (46,47), but shows no correlation with the intensity or phenotype of the infiltrating interstitial cells (37). The basilar vascular adhesion molecule (VCAM-1) and the luminal intercellular adhesion molecule (ICAM-1) have been shown to be induced (48). Both of these molecules are ligands to the integrin expressed on memory-activated T cells and their expression may serve to promote T-cell recruitment, adherence, and interaction with the tubule at sites of tissue injury.

The overall profile of immunocompetent cells identified in ATIN does not differ from that of chronic tubulointerstitial nephritis and suggests an important pathogenetic role for cell-mediated immunity in which both the epithelial and infiltrating cells are active and participating components. The role of mononuclear cells in lesions that pursue an acute time course is not unexpected. Activated lymphocytes are the dominant cell types observed, within the first 12 to 24 hours, in experimental models of delayed-type hypersensitivity (49). Their reversible presence over a limited time course in ATIN may reflect the balance of the mechanisms that regulate the immune response (50,51).

PATHOGENESIS

The immune response implicated in the pathogenesis of ATIN has been categorized into three phases: an antigen expression and recognition phase, an integrative or regulatory phase, and an effector or mediator phase (50). In the first phase, either

the resident interstitial cells expressing major histocompatibility complex (MHC) class II or the tubular epithelial cells may function as antigen presenters. Tubular injury has been shown to stimulate MHC class II antigen expression by the tubular epithelial cells, providing a basis for a central role of the tubules in the initiation of the disease process (19,20,46).

The subsequent integrative or regulatory phase may suppress or intensify the reaction to the stimulus provided by the antigen-presenting cells. This is a rather complicated and still being deciphered as a phase of the immune response, in which T cells and antibodies directed at the presented antigen play a central role. Locally produced cytokines implicate a participatory role for the infiltrating, epithelial, and endothelial cells in this regulatory phase (21,22,52). The role of tubular epithelial cells (TECs) in this process has been examined. Exposure to selected insults or toxins result in activation of the TECs and their expression of various growth factors and chemokines, such as interleukins (IL), monocyte chemoattractant proteins (MCP), major histocompatibility complex (MHC), vascular endothelial growth factor (VEGF), transforming growth factor (TGF) and regulated on activation, normal T expressed and secreted (RANTES) (21,22). The subsequent bidirectional cross-talk between the TECs and recruited infiltrating inflammatory cells, either by soluble factors or direct contact, in this phase ultimately modulate the course of renal involvement and its potential for reversibility (32,50,51).

The final effector or mediator phase is mediated primarily by humoral factors released by the infiltrating cells, with the TECs playing a participatory role (Fig. 48-1). The cytotoxic cells may induce injury by the release of proteases, and the inducer cells by the release of lymphokines, which in addition to a direct detrimental action augment the role of the macrophages (50,53–55). In turn, the release of collagenases, elastases, and reactive oxygen species by the macrophages magnifies the injury initiated by the lymphocytes. Experimental studies indicate that the activated macrophages are responsible for the formation of a multinucleated epithelioid and granulomatous reaction (54,55). Lymphokines also promote fibroblast proliferation and alter the balance in favor of increased matrix synthesis rather than removal (21,22,25,50,56).

In the final analysis, it is the presence of a rather wide range of activated mononuclear cells and their interaction with each other and with parenchymal cells, during the integrative and regulatory phases, which is potentially damaging to the kidney (Table 48-1). Several of the cellular (epithelial, endothelial, lymphocytes, macrophages) signals have varying, often overlapping, functions that interact to modulate or amplify the inflammatory reaction of ATIN. Some of the ones that have been identified include cell surface markers that have either *antigenic* (MHC, HLA class II, secreted protein acidic and rich in protein [SPARC]) or *adhesive* (ICAM-1, VCAM-1, integrins; selectins) properties. Others are *chemoattractant* MCP-1; osteopontin; macrophage inflammatory protein-1 (MIP-1); eotaxin; RANTES, *proinflammatory* (interleukin [IL]-6 and IL-8; TGF-β; platelet-derived growth factor-β [PDGF-β]; granulocyte–monocyte colony-stimulating factor [GM-CSF]; tumor necrosis factor-α [TNF-α]), *vasoactive* (adenosine; nitrous oxide [NO]; endothelin-1 [ET-1]), *cytotoxic* (metalloproteinases [MP]; tissue inhibitor metalloproteinases [TIMP-1]; reactive oxygen radicals; ferric ion), or *fibrogenic* [TGF-β, PDGF, IL-1, IL-6, TNF, plasminogen activator inhibitor (PAI)]. Evidence also exists for possible protective cytokines that can favorably modify the proinflammatory sequence of events (57). The resultant integration, during the regulatory and effector phases, either suppresses the effector phase, as in mild forms of ATIN, or amplifies it, as in severe forms of ATIN. Ultimately, feedback mechanisms and removal of the inciting agent restore the response to injury to its baseline dormant state with consequent

TABLE 48-1

IMMUNOMODULATORY SIGNALS INVOLVED IN THE PATHOGENESIS OF ACUTE TUBULOINTERSTITIAL NEPHRITIS

Antigenic cell surface markers
MHC, major histocompatibility complex
HLA Class II
SPARC, secreted protein acidic and rich in protein
Adhesive cell surface makers
ICAM-1, intracellular adhesion molecule 1
VCAM-1, vacular adhesion molecule 1
Integrins
Selectins
Chemoattractants
MCP-1, monocyte chemoattractant protein 1
RANTES, normal T cell expressed and secreted
MIP-1, macrophage inflammatory protein-1
Eotaxin
Proinflammatory cytokines
IL-6, interleukin-6
IL-8, interleukin-8
TGF-α, transforming growth factor α
GM-CSF, granulocyte monocyte-colony stimulating factor
PDGF-β, platelet derived growth factor β
TNF-α, tumor necrosis factor α
Vasoactive substances
Adenosine
NO, nitric oxide
ET-1, endothelin 1
Cytotoxic substances
MP, metalloproteinases
TIMP-1, tissue inhibitor metalloproteinases
ROS, reactive oxygen species
Ferric ion
Profibrotic cytokines
TGF-β
PDGF
IL-1
IL-6
TNF
PAI, plasminogen activator inhibitor

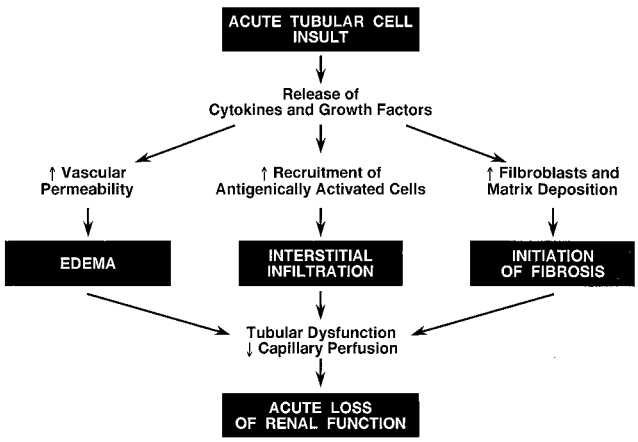

FIGURE 48-1. Pathogenetic pathways implicated in acute tubulointerstitial nephritis.

recovery of normal kidney function or residual permanent damage (31–33,52,58–62).

The bulk of the available information about the phases of the immune response and interstitial inflammatory reaction in ATIN derives from studies in experimental animals, particularly in models of recombinant rats and mice (43,51,56,63). Unfortunately, there are no animal models that correspond to the reaction that occurs in human ATIN. A principal limitation of human studies is that the data derived from kidney biopsies provide information only at fixed moments in the course of a lesion that is an evolving process. The same limitation applies to the serologic data provided in clinical studies that are obtained at a fixed period, usually when the lesion is well established and often at its worst, with limited or no data available on the onset or resolution phases of the lesion. These limitations notwithstanding, considerable evidence has accrued for a role of the principal immunologic mechanisms—cell-mediated injury, anti-TBM antibodies, immune complex deposition—in the pathogenesis of ATIN. Although there is evidence that all three mechanisms may be operative to varying degrees in different patients, the bulk of the available information favors a predominant role for cell-mediated injury.

Contrary to the extensive experimental data in support of anti-TBM disease from animal models of interstitial disease, anti-TBM antibodies have been detected only rarely in human ATIN (64,65). The evidence presented in favor of anti-TBM antibodies as a cause of ATIN derives from cases attributed to methicillin. The deposition of a metabolite of methicillin, dimethoxyphenylpenicilloyl, along the TBM was initially implicated as the antigenic stimulus but subsequently questioned to have a pathogenetic role when the deposits were found in the kidneys of totally asymptomatic patients exposed to the drug (66,67). By the same token, patients with a transplanted kidney who have anti-TBM antibodies that are shown to bind to the TBM of the allograft do not manifest the clinical features of ATIN (68). In two cases of ATIN attributed to phenytoin in which linear deposits of IgG were detected, the lesion could not be induced in rats by passive transfer, although binding of the antibodies to TBM could be demonstrated *in vitro* (69,70). Thus, the dearth of evidence for anti–TBM-mediated disease is far from convincing for an important role, if any, of this mechanism in human ATIN.

The same limitations apply for a role of immune complex–mediated ATIN. The cases in which granular deposits of IgG have been detected have been in patients with Sjögren's syndrome (71,72) and systemic lupus erythematosus (73–76), whose underlying disease mechanism accounts for the deposition of immune complexes in the kidneys as well as other body organs. Their presence in other, more common forms of ATIN is extremely rare and never a dominant feature.

By contrast, the evidence in favor of cell-mediated disease is overwhelming. The infiltrating cells are antigenically activated. T-cell reactivity has been demonstrated from *in vitro* studies of lymphocyte stimulation (52,63,77,78). Infiltrating cells have been analyzed by immunoperoxidase studies using monoclonal antibodies. Most are T lymphocytes that express the T-cell antigen–receptor complex (39,48,79). As a rule, the infiltrating lymphocytes are CD4+ and CD8+, with a slight preponderance of the former (46,80), except in cases due to NSAIDs (34,52) and on occasion in cases due to cimetidine (81) or antibiotics (79), in which CD8+ cells may show a slight preponderance. Comparative studies of ATIN due to NSAIDs or β-lactam antibiotics have failed to reveal significant differences in the CD4+/CD8+ ratio or in the overall percentage composition of T cells, B cells, and monocytes. The reported differences in infiltrating cell subtypes can be due to individual genetic background, nature of the insult, and the point in time during the course of the disease when biopsies were obtained. Nevertheless, it is clear that where sought, activated antigens have been demonstrated on the surface of the infiltrating cells (36,39), and plasma cells containing IgE (41,42,81), IgA (34), IgG, and IgM (42) have been detected. However, no diagnostic pattern of markers or infiltrating cells has emerged.

FUNCTIONAL MANIFESTATIONS

The principal manifestations of ATIN are those of tubular dysfunction, which is one reason that the term *tubulointerstitial nephritis* was introduced in preference to the initial appellation of *interstitial nephritis*. As a rule, tubular dysfunction precedes the onset of azotemia, which in turn precedes that of oliguria. As such, vigilance to clinically evident abnormalities of tubular function, including polyuria, are essential to the early detection of ATIN during its initial reversible stages.

Because of the focal nature of the lesions and the segmental nature of normal tubular function, the pattern of tubular dysfunction that results varies depending on the major site of injury, whereas the extent of damage determines the severity of tubular dysfunction. The principal hallmarks of glomerular disease, such as salt retention, edema, and hypertension, characteristically are absent (15,16,78,82). However, massive proteinuria does occur in ATIN due to NSAIDs and, occasionally, antibiotics (16,34,83). The magnitude of reduction in GFR that occurs seems to correlate with the extent of the interstitial cellular infiltrates (11,26). This is in contrast to chronic forms of tubulointerstitial nephritis, in which it is the magnitude of interstitial fibrosis that correlates with the severity of decreased GFR, whereas the enlargement of the interstitium by cellular infiltrate has less bearing on GFR (81,85). Under any circumstance, the structural changes of ATIN account for the functional changes observed in this clinicopathologic syndrome. In addition to the direct injury to epithelial cells that may account for tubular dysfunction, changes in the interstitial volume and composition contribute to the functional abnormalities that develop. A major part of the reabsorbed or secreted tubular fluid has to traverse a true interstitial space (86). The structure, composition, and permeability characteristics of the interstitial space must, of necessity, exert an effect on such an exchange. A change in any of these parameters of the interstitium or those of the epithelial cell tight junctions, by delaying equilibration or exchange processes, could well account for the functional alterations of the renal tubule that develop in ATIN. It also is possible that changes in the pressure of the supporting interstitium, which are transmitted to the periarterial space, could affect the blood flow to the adjacent tubule and thereby cause tubular dysfunction. In addition, the expansion of interstitial space would increase the distance between tubular epithelium and the peritubular capillary plexus, where the reabsorbed fluid is delivered.

Tubular alterations also may be reflected in reductions of the GFR through tubuloglomerular feedback as the function of the affected proximal tubular segments becomes compromised and both the rate and load of solute delivery to the macula densa are altered (87–89). Furthermore, the increase in hydrostatic pressure of the edematous interstitium, as has been demonstrated in allograft rejection (15), may adversely affect the intraarteriolar pressure and cause a reduction of the hydrostatic pressure transmitted to the glomerular capillaries (Fig. 48-1). In addition, a vasoactive effect of the cytokines, eicosanoids, and reactive oxygen species elaborated by the interstitial infiltrating cells and injured epithelial cells may affect renal hemodynamics through the increased production of vasoconstrictive substances, such as thromboxane A_2 and leukotrienes, and decreased production of vasodilators, such as NO, brought about by reduced production of its substrate L-arginine by the injured proximal tubule cells (90–92). Evidence for a role for

TABLE 48-2

CAUSES, PRINCIPAL SITES OF INJURY, AND PATTERNS OF TUBULAR DYSFUNCTION IN ACUTE TUBULOINTERSTITIAL NEPHROPATHIES

Site of injury	Causes	Tubular dysfunction	Clinical manifestation
Cortex			
Proximal tubule	Antibiotics Multiple myeloma Immunologic diseases Neoplastic diseases Idiopathic	↓ Reabsorption: Na^+, glucose, HCO_3^-, urate, PO_4, amino acids	Glucosuria, hypouricemia, hypophosphatemia, aminoaciduria, alkaline urine, acidemia
Distal tubule	Antibiotics Immunologic diseases Nonsteroidal antiinflammatory drugs Idiopathic	↓ Secretion: H^+, K^+ ↓ Reabsorption Na^+	Alkaline urine, acidemia, hyperkalemia, inability to preserve sodium
Medulla and papilla	Infections Analgesics Nonsteroidal antiinflammatory drugs Disorders of uric acid, calcium, oxalate Immunologic diseases Idiopathic	↓ Reabsorption: Na^+ ↓ Concentrating ability	Polyuria, nocturia, inability to preserve sodium

cytokines released by the infiltrating cells in the reduction in renal blood flow and GFR has been advanced from studies of an experimental model of acute obstructive nephropathy that is characterized by acute mononuclear cell infiltrates (93). Certainly, the correlation between the severity and extent of cellular infiltrates and increments in serum creatinine attests to a role of the infiltrating cells in the reduction of GFR in ATIN (Fig. 48-1).

The tubulointerstitial lesions usually are localized either to the cortex or the medulla. Cortical lesions predominantly affect either the proximal tubule or the distal tubule. Medullary lesions affect the loop of Henle and the medullary collecting duct. The change in the normal function of each of the affected segments then determines the manifestations of tubular dysfunction (Table 48-2). Lesions principally affecting the proximal tubule result in bicarbonaturia (proximal renal tubular acidosis), glucosuria (renal glucosuria), aminoaciduria, β_2-microglobinuria, phosphaturia, and uricosuria (15,16). The latter two events can be valuable in suggesting the possibility of tubulointerstitial disease when the serum phosphate and urate levels are lower than expected in any patient with reduced kidney function. The presence of glucosuria when the blood sugar levels are normal should always lead to a consideration of ATIN. The distal nephron segment secretes hydrogen and potassium and regulates the final amount of sodium chloride excreted. Lesions primarily affecting the distal tubule result in the distal form of renal tubular acidosis, hyperkalemia, and salt wasting (15,16). Lesions that primarily involve the medulla and papilla disproportionately affect the loops of Henle, collecting ducts, and other medullary structures essential to achieving and maintaining medullary hypertonicity. Disruption of these structures therefore results in different degrees of nephrogenic diabetes insipidus, polyuria, and nocturia. A reduction in collecting duct aquaporins has been shown in an experimental model of acute renal injury (82). Actually, a polyuric phase almost always precedes the onset of oliguria in ATIN.

Although this general framework is useful in localizing the site of injury, considerable overlap is encountered clinically, with different degrees of proximal, distal, and medullary dysfunction present in any one patient, all of which usually precede any clinically detectable changes in GFR. In most cases, however, the reduction in GFR and consequent azotemia are the presenting clinical abnormalities calling attention to the acute kidney injury. Preexisting or coexistent evidence of tubular dysfunction, unless specifically sought, may go undetected and either delay the diagnosis or result in the wrong clinical diagnosis. One can only speculate on the number of cases of ATIN that go undetected clinically because of the presence of tubular dysfunction alone in the absence of frank azotemia or oliguria—hence the importance of monitoring for tubular dysfunction in patients exposed to agents known to be associated with ATIN, and the necessity of documenting tubular dysfunction in those with azotemia to marshal evidence for the diagnosis of ATIN.

CLINICAL FEATURES

The clinical presentation of individual cases of ATIN is diverse and varies to some degree depending on the causative factor. The manifestations best characterized in methicillin-induced ATIN can be considered the prototypically classic clinical manifestations, around which general variation occurs depending on the causative agent and individual variation depending on the particular case encountered clinically.

In most cases, symptoms develop several days or even weeks after exposure to the inciting agent, which in the case of drugs is not dose dependent. The classic triad of low-grade fever (70% to 100%), fleeting skin rash (30% to 50%), and mild eosinophilia (80%) described with methicillin-induced ATIN is not invariably present, and certainly it is less common for all three to occur together. Their documentation depends to some degree on the vigilance with which they are sought because they may be mild and transient. The full triad was noted to be present in approximately one-third of cases of methicillin-induced ATIN (94–97), but only in 5% of cases of ATIN induced by other drugs in which any one of them (fever, rash, or eosinophilia) is less likely to occur (11,12,83,98). The skin rash consists of erythematous maculopapular lesions, which often are pruritic, and affect preferentially the trunk and proximal portion of the extremities. Flank pain, reflecting

edema-induced distention of the renal capsule, may be detected in over one-third of the cases when queried and may be the presenting symptom in some cases (99). Gross hematuria, another presenting feature, may be present in 10% to 15% of drug-induced ATIN cases when specifically sought. Gross hematuria was a distinct feature of the first reported case of methicillin-associated ATIN in a child with cystic fibrosis treated intermittently with methicillin, who experienced gross hematuria each time she was exposed to the drug (100), and occurs in more than half the cases of methicillin-induced ATIN (11). Although a transient eosinophilia often (40% to 60%) is present, it may go undetected unless specifically sought, which accounts for its inconsistency in reported cases.

Urinalysis can be helpful in the diagnosis. Proteinuria, hematuria, and pyuria are present in most cases. The proteinuria is mild, seldom exceeds 2 g/day, and only rarely is in the nephrotic range, except in those cases due to NSAIDs (95–97,101,102). Microscopic hematuria is detected in up to 90% of cases and gross hematuria in 15%; rarely, red blood cell casts may be detected (103). The pyuria is nonspecific except when eosinophils are detected in an appropriately prepared and carefully examined urinary sediment (104–106). Although eosinophils may be observed with Wright's stain of a well-spun urinary sediment, the use of Hansel's stain on the sediment of an alkalinized urine sample is superior in detecting eosinophils (104). The mere detection of eosinophiluria is not specific for ATIN (107,108). The sensitivity of eosinophiluria for the diagnosis of ATIN has been estimated to be 40% to 60%. Its specificity has not been well established, but its positive predictive value has been estimated to be only 38% (108). Eosinophiluria is present in approximately 15% of hospitalized patients, in whom it usually is caused by a variety of other inflammatory diseases of the urinary tract, and in only 14% of those with eosinophiluria is it due to ATIN (105). The presence of eosinophiluria is a better predictor of ATIN when more than 5% of the leukocytes are eosinophils, in which case 40% of the eosinophiluric patients have ATIN, as opposed to an incidence of 3.5% in those with less than 1% are eosinophils. Other conditions in which an eosinophiluria exceeding 5% may be present are urinary tract obstruction, cystitis, contrast-induced ARF, IgA nephropathy, and cholesterol emboli. Thus, the detection of selective eosinophiluria, although useful, is neither necessary nor sufficient for the diagnosis of ATIN. Equally useful urinary findings in the diagnosis of ATIN are the detection of renal glucosuria, increased fractional excretion of uric acid, and inability to concentrate the urine maximally and to conserve sodium, as reflections of tubular dysfunction detailed in the previous section.

The impairment in kidney function varies, ranging from discrete selective abnormalities of tubular function to frank kidney failure, with or without oliguria (109,110). As a rule, increments in blood urea nitrogen and serum creatinine develop after tubular dysfunction is detectable and while the patient is still nonoliguric or even polyuric. Oliguria develops if the presenting systemic symptoms, modest azotemia, and evidence of tubular dysfunction go undetected and if exposure to the causative agent or factor continues. Kidney failure is more likely to occur in older patients and is more severe in those who become oliguric. The duration of the oliguric period is variable, ranging from a few days to several weeks. Supportive renal replacement therapy may be required in approximately one-third of those patients. Reversal of kidney failure and return to baseline kidney function is the rule in the majority of cases (about 60% to 65%). Irreversible kidney failure can occur but is rare (about 5% to 10%), while partial recovery with persistent impairment of kidney function is relatively more common (10% to 20%) in cases where interstitial fibrosis and granulomas are present in biopsy specimens (11,12.83,111,112).

Increased kidney size on ultrasonography, reflecting interstitial edema, is common but nondiagnostic. Radioactive gallium uptake by the kidney, reflecting interstitial cellular infiltration, can be detected in one-third of cases, but lacks diagnostic specificity (78,113). However, when the gallium scan is negative it can be useful to rule out ATN (78,95,113). The lymphocyte stimulation test can be valuable in the diagnosis of drug-induced ATIN, especially in determining the responsible agent in cases of multiple-drug therapy (78,93). The serum level of IgE can be elevated and IgE-containing plasma cells have been demonstrated among the renal interstitial cellular infiltrates, providing further evidence for a hypersensitivity reaction (41,101,114).

There is suggestive evidence that a short course of treatment with steroids improves the kidney lesions and expedites recovery from drug-induced ATIN (115–117), although this is not a uniformly observed response (83,118,119). The use of steroids should be considered in patients with protracted kidney failure, especially when the failure persists after the inciting agent has been discontinued (26,94–96), and in those whose biopsy reveals granulomatous lesions that are associated with increased risk of permanent kidney injury (117,120,121). Reversal of apparently permanent kidney failure after steroid therapy in a patient on maintenance hemodialysis has been reported (122). However, in a retrospective evaluation of biopsy-proven cases of ATIN, the routine use of steroids was not associated with a statistical difference in kidney function or outcome (123). Unfortunately, no controlled clinical trials are available, and controversy over the use of steroids persists. If steroids are used, a response usually is evident relatively early after initiation of treatment. The course of treatment should be brief, and steroids should be discontinued if no response is observed after 3 to 4 weeks of therapy (124,125).

INCIDENCE AND DIAGNOSIS

The frequency with which ATIN accounts for cases of clinically encountered ARF is difficult to establish. The diagnosis of ATIN is based on the finding of characteristic morphologic features on kidney biopsy and on the identification of the causative factor. Both of these requirements for a correct diagnosis are fraught with difficulties and limitations.

Part of the difficulty associated with the diagnosis of ATIN stems from the relatively low index of suspicion with which its possibility is considered clinically and the general reluctance to perform a kidney biopsy in cases of ARF. Even if a biopsy is performed, there are difficulties in differentiating the lesions of ATIN from those of ATN, a distinct possibility in most cases of ARF in which the prevailing clinical condition could be conducive to either ATIN or ATN (7,126). An essential differential feature between the two entities is the magnitude of interstitial edema and cellular infiltrates, which are more prominent in ATIN, and the magnitude of tubular cell injury, which is more prominent in ATN (Fig. 48-2). However, given the variable degree to which each of these features may be present in each entity (7,126), there is sufficient overlap between the extent of edema and the severity of tubular injury in ATIN and ATN to make it difficult to differentiate among them on morphologic features alone, at least in some cases. This is best illustrated in the literature before World War II, when ATN came to be recognized as a distinct entity. In contemporary reports, classic ATN cases due to transfusion reactions were labeled *acute hematogenous interstitial nephritis,* emphasizing the similarity of the lesions to those reported by Councilman as being associated with systemic infections (5,6). Ironically, the subsequent focus on tubular cell injury in ATN has deviated attention from the interstitial infiltrates present in ATN and the

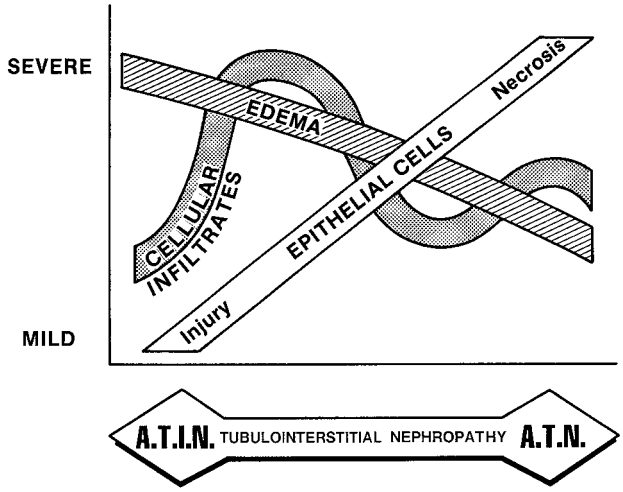

FIGURE 48-2. Schematic representation of the spectrum of structural changes associated with cases of acute renal failure due to acute tubulointerstitial nephritis (ATIN) and acute tubular necrosis (ATN). (Reproduced from: Eknoyan G. Acute renal failure associated with tubulointerstitial nephropathies. In: Brenner BM, Lazarus JM, eds. *Acute renal failure,* 2nd ed. New York: Churchill Livingstone, 1988:491, with permission.)

emerging evidence for their role in the pathogenesis of kidney injury in ATN (127). Broadly defined, the morphologic features of tubulointerstitial nephritis can be considered a spectrum of lesions characterized by interstitial edema, cellular infiltrates, and tubular injury (Figs. 48-2 and 48-3). Depending on the magnitude and severity of each of these structural features, at one end of the spectrum would be clear-cut cases of ATIN, and at the other end, ATN. Difficulty is encountered in cases that fit in the middle of this spectrum, and is further magnified in those with focal rather than diffuse lesions. In such cases, the history of exposure to an inciting agent and its identification become important in establishing a correct diagnosis.

There also are difficulties associated with identifying the causative factor. Clinically, removal of a suspected agent followed by reversal of the lesion strongly suggests the diagnosis. This can be particularly convincing in the presence of systemic manifestations of a hypersensitivity reaction, such as fever, skin rash, and eosinophilia, in addition to ARF, all of which subside on removal of the inciting factor or agent. Difficulties arise when the only manifestation is ARF, which is potentially reversible, and when a number of corrective measures are instituted simultaneously. Moreover, most patients are on several drugs, and there is the expected tendency to incriminate the most common and better known agents that have been associated with ATIN. When the wrong drug is discontinued, the acute kidney injury continues unabated because the causative agent continues to be administered. The diagnostic limitations of removing agents wrongly surmised as a cause of ATIN have been shown by studies in which lymphocyte stimulation testing revealed causative agents that had not been suspected clinically (78). Proof depends on recurrence with reexposure to the same agent, as has occurred accidentally with some antibiotics (128).

Most of the figures quoted in the literature on the incidence of ATIN stem from retrospective studies based on kidney biopsies. Review of unselected kidney biopsy specimens reveals a low incidence of ATIN (26). Review of biopsy specimens from patients with unexplained ARF reveals a frequency that ranges from 8% to 22% (118,123,129–133), which generally is higher in the elderly population (134). The problem in deriving conclusions on incidence from kidney biopsies is illustrated by the following two studies. In a report of 976 patients with ARF, 218 of whom underwent kidney biopsy, a diagnosis of ATIN was made in 29 cases, an incidence of 14% of patients sampled for biopsy, but only 3% of all patients with ARF (135). The question remains whether such a report can be considered a cross-sectional study and whether the incidence of ATIN in the 758 patients who did not undergo biopsy was the same. In another study, 13% of the biopsies of patients with ARF revealed ATIN; the diagnosis was not suspected clinically in any of them (129). Thus, given the very small number of patients with ARF who are subjected to kidney biopsy, the necessity of kidney biopsy to diagnose ATIN, and the failure to suspect the diagnosis of ATIN clinically, all render conclusions derived from biopsy reports of limited value in estimating the true incidence of ATIN (135). Furthermore, because of the variable and often subjective reasons for which kidney biopsies are done in different centers, any attempt to compare or combine results from different reports would not be useful. Coupled with the fact that cases of ATIN with mild azotemia and only some

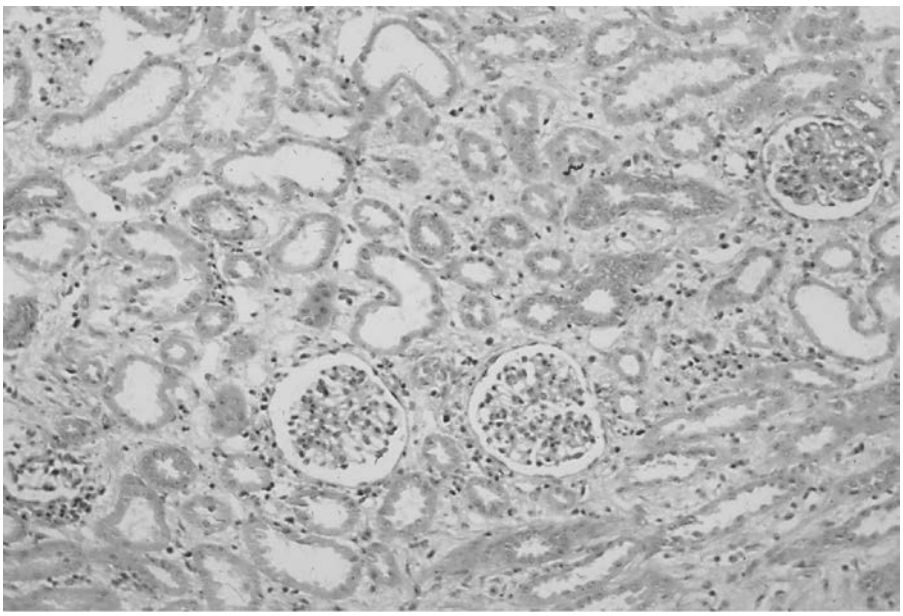

FIGURE 48-3. Kidney biopsy from a case of NSAID-induced acute tubulointerstitial nephritis. Note the increased interstitial space between the tubules showing edema and focal infiltration my mononuclear inflammatory cells. The glomerular structure is preserved, but there are periglomerular clusters of mononuclear cells.

TABLE 48-3

PRINCIPAL CONDITIONS ASSOCIATED WITH ACUTE TUBULOINTERSTITIAL NEPHROPATHY

Infections
Invasive of renal parenchyma
Reactive to systemic infections
Drugs
Antibiotics
Sulfonamides
Nonsteroidal antiinflammatory drugs
Other drugs
Systemic diseases
Metabolic disorders
Immune diseases
Neoplastic disease
Idiopathic with uveitis

degree of tubular dysfunction may go entirely undetected, it is impossible to determine the true incidence of ATIN from biopsy reports. In cases of clinically evident ARF, ATIN probably accounts for approximately 15% to 20% of them. Among the causes of ATIN, infections and drugs are the most common (Table 48-3).

INFECTIONS

Infections as a possible cause of ATIN often are overlooked, and as a consequence their diagnosis is missed and their incidence as a cause of ATIN is underestimated. The eradication of serious streptococcal infections that were first associated with ATIN after the introduction of antibiotics and the recognition of antibiotics as a cause of ATIN have led to the preferential consideration of drugs used in the treatment of infections as a principal cause of ATIN. In addition, the hemodynamic changes associated with severe infections and the use of antibiotics with a direct nephrotoxic effect in their treatment usually are implicated as a cause of any deterioration of kidney function without adequate investigation of ATIN as a possible cause of ARF. Another reason that hinders the diagnosis of ATIN is the commonly held but questionable notion that postinfectious acute glomerulonephritis is the most common kidney lesion associated with infections. Finally, the modest reduction in kidney function and its return to baseline as the infection is treated limits the clinical consideration given to the observed changes in kidney function. Thus, unless the possibility of infection-induced ATIN is considered seriously, without undue bias toward coexistent and confounding conditions that may account for an acute deterioration of kidney function, and the diagnosis pursued actively, cases of infection-induced ATIN will go undetected, and its true incidence will remain underestimated. Nevertheless, several new causes of infection-induced ATIN have come to be identified since Councilman described their association.

Tubulointerstitial lesions of the kidney may develop in the kidney either because of direct invasion of the kidney parenchyma by the infective microorganisms or because of reactive changes to a nonrenal systemic infection.

Invasive Infections

The classic and by far the most common example of ATIN is due to direct bacterial invasion of the kidney, although fungal, viral, and parasitic infections also may account for it. The usual forms of pyelonephritis are considered in Chapter 34, Infections of the Upper Urinary Tract, and are not detailed here, except to highlight their differential features from ATIN reactive to a systemic infection. Apart from the classic local and systemic symptoms of acute pyelonephritis that clinically differentiate direct infection of the kidney from reactive ATIN and culture of the invasive organism in the urine, there are distinct morphologic differences between the two entities that deserve emphasis. Acute bacterial pyelonephritis is a focal rather than diffuse lesion, which usually is limited to an individual pyramid and rarely affects all the pyramids of the kidney. The areas of infection are characteristically wedge shaped, with the apex directed to the medulla. The infected foci are focal and sharply demarcated from the adjoining uninfected parenchyma, and lack the tendency to spread laterally (9,136). Contrast-enhanced computed tomography reveals the typical wedge-shaped, nodular, hypodense areas corresponding to the distribution of the lesions and heavy cellular infiltrates in pyelonephritis (137). These are quite distinct from the generalized changes of ATIN (138). In the absence of obstruction, the infection tends to be confined to the originally affected lobule and to resolve gradually over a period of weeks. An acute reduction in kidney function is rare except in severe cases and those with obstruction, or when the infection is superimposed on preexisting chronic kidney disease (139). As recovery occurs, the initial polymorphonuclear leukocytic infiltrate is replaced by mononuclear cells and the development of streaks of fibrous tissue that account for the cortical scars encountered in such cases (140–144).

Occasional cases of acute ATIN have been reported in association with otherwise invasive infections such as mycoplasmal pneumonia (145), toxoplasmosis (146), cytomegalovirus infection (113,147,148), infectious mononucleosis (149–153), polyomavirus infection (154), hantavirus infection (155), Rocky Mountain spotted fever (156), leptospirosis (157,158), Legionnaires' disease (159,160), candidiasis (161,162), *Yersinia* pseudotuberculosis (163), *Ascaris lumbricoides*(164), and adenoviral infection (165,166). A greater number of such cases are now encountered clinically and recorded in the literature because of the increasing number of immunocompromised hosts [patients with acquired immunodeficiency syndrome (AIDS), transplant recipients, the elderly] in the general population. Of those, patients with AIDS constitute a special challenge in the diagnosis of ATIN, not only because of their propensity to contract unusual infections (167), but because of the number of drugs they consume, such as protease inhibitors, which not only are nephrotoxic but cause ATIN (168–172). Although the passage of the of implicated organisms through the renal parenchyma and their isolation from the urine have been demonstrated, their localization in the kidney is less well substantiated. The cellular infiltration is mononuclear rather than polymorphonuclear, and evidence has been advanced for an immunologic mechanism responsible for the renal reaction elicited by some of them (151). Antigenic material has been demonstrated in the renal parenchyma of humans with ATIN associated with some of these infectious agents, such as *Mycoplasma* (145), polyomavirus (154), the rickettsiae responsible for Rocky Mountain spotted fever (156), and leptospira (173–181). The complex nature of the lesions encountered in some of these infections can be better appreciated from a specific consideration of some of them.

Leptospirosis

Acute renal failure occurs in almost half the cases of leptospirosis, and the degree of kidney involvement often determines the gravity of the disease (173). In fact, in endemic areas of the disease, leptospirosis is one of the most common causes of ARF

(174). Although there might be evidence of renal involvement in the preicteric phase of the disease, it is in its icteric phase that ATIN develops in conjunction with a rapid deterioration of kidney function. Rodents are the principal reservoir of the organisms, which enter the host through the skin or mucosa and gain access to the kidney by the bloodstream. At first, they transiently invade the glomeruli, then pass to the peritubular capillaries, and ultimately penetrate the interstitium and tubules (175,176). The renal lesions of leptospirosis are limited exclusively to the tubules and interstitium. They are focal in distribution and are most pronounced at the corticomedullary junction, a structural feature characteristic of ATIN lesions that are reactive to infections. The tubular changes consist of degeneration, epithelial necrosis, and rupture of the basement membrane (174–177). The interstitial changes are restricted to the region of the affected tubules. Endothelial cell injury and capillary thrombosis are demonstrable in the affected foci. Initially, interstitial edema and cellular infiltration are minimal but ultimately become prominent, such that the whole kidney becomes enlarged, congested, and soft. The infiltrates consist of mononuclear cells, plasma cells, and eosinophils. The interstitial lesions may be present even in the absence of clinically detectable kidney failure (178). The tubular lesions occur during leptospiral migration through the renal parenchyma, and during this stage the spirochetes can be detected in the urine (176–179). Thus, direct tubular injury by *Leptospira* may account for the renal lesions. Leptospiral antigens have been demonstrated in the renal interstitium of a canine model of the disease, suggesting a role for immunologic mechanisms (180). Leptospiral outer membrane proteins and lipoproteins have been implicated in causing tubular dysfunction and initiating the immunologic injury (181–183). They have been shown to inhibit Na^+-K^+-Cl^- cotransporter activity and messenger RNA (mRNA) synthesis (182–183) in the thick ascending limb. This could account for the polyuria and hypokalemia frequently seen in these cases. Furthermore, the exposure of cultured proximal and thick ascending limb cells to the outer membrane proteins results in activation of a pro-inflammatory cascade, with increased induction gene expression of NF-?B, iNOS, MCP-1, and TNF-α (181,182) The superimposition of shock and volume depletion in severe cases of the disease may further contribute to the tubular lesions, and the structural changes observed may represent the cumulative effect of both direct invasion and ischemia, possibly coupled with an immune injury (178). Anti-glomerular basement membrane antibodies with linear, immunofluorescent deposits along the glomerular and TBM have been reported in one case (184) and diffuse nodular complement deposits in another (185).

Although the interstitial changes may persist for several months in an occasional patient, usually with persistent spirochetes in the urine, in most cases the renal changes subside within a few weeks after resolution of the disease with appropriate antibiotic therapy (140,152,157). In these usual cases, kidney function returns to normal, whereas in those who develop a chronic carrier state the leptospira localize in the kidney, fibrosis and tubular atrophy develop, and permanent impairment of kidney function ensues (157,177).

Hantavirus Infection

The once mysterious entities of "hemorrhagic fever with renal syndrome" in Asia and "nephropathia epidemica" in Europe are now recognized as due to infection with one of the related RNA viruses of the Hantavirus genus, which belong to the family of Bunyviridae (186–188). The availability of serologic tests for the specific antibodies, which appear early in the course of the infection and may persist indefinitely, now allows for the easy diagnosis of this once enigmatic disease that mimics several of the features of leptospirosis—fever, petechiae, thrombocytopenia, and liver and kidney dysfunction (189–191).

The renal lesions consist of the classic changes of ATIN, with tubular injury, interstitial edema, and predominantly mononuclear cellular infiltrates with occasional polymorphonuclear leukocytes and eosinophils. They are most prominent in the medulla and corticomedullary junction, together with the hallmarks of Hantavirus nephropathy: vascular congestion and interstitial hemorrhage (155,188). This accounts for the microhematuria and, together with the enlarged kidneys, for the back pain present in most cases. The transient heavy proteinuria (>2 g/d) noted at the onset of the disease correlates with the urinary excretion of IL-6 (192). The severity of the lesions and frequency of renal failure are higher and the prognosis worse in the Asian than in the European and American varieties of the infection (193–197).

Polyoma Virus

Polyoma is a double-stranded DNA virus family, one strain of which, BK virus, commonly causes ATIN. Typically, the virus is acquired in childhood but remains latent as over 70% of adults are seropositive (198). However, when the immune system is compromised, as in AIDS and transplant recipients, the virus reemerges and causes ATIN (199,200). Its incidence in kidney transplant recipients is reported to be 3% to 5%, with an associated 50% graft loss among those affected (210,202). It is considered to result from over immunosuppression, and in transplant recipients its incidence has increased with the use of newer more potent immunosuppresants. Decreasing immunosuppression has been effective in the management of some cases but is associated with an increase in acute rejection episodes (201).

There are no specific clinical features the kidney disease except for increasing levels of serum creatinine. Diagnosis is based on findings on kidney biopsy that are distinct from those of acute rejection. The finding of cells in the urine that have viral inclusions, so-called decoy cells, are indicative of viral replication in the urinary tract but are not specific of ATIN. Detection and quantification of viral DNA load in plasma by PCR is potentially useful for the detection of significant viral reactivation, and can serve as a quantifiable surrogate marker of the course of the infection (201,202).

Brucellosis

Renal involvement is rare in brucellosis, although the organisms frequently are recovered from the urine of infected patients. By contrast, in those with renal involvement, it is unusual to recover the organisms from the urine (203). In the acute form of the disease, fever, proteinuria, hematuria, and pyuria are presenting features. The renal lesion consists of a focal glomerulitis coupled with a severe, sometimes diffuse, interstitial inflammatory reaction caused by infiltrating polymorphonuclear leukocytes (203). In the chronic form of the disease, extensive fibrosis develops that clinically and radiologically mimics the findings of tuberculosis (204).

Candidiasis

Disseminated candidiasis almost always affects the kidney (205,206). *Candida albicans* causes two types of lesions in the kidneys. The first type, which is relevant to this chapter, occurs during the candidemic phase with the initial seeding of the kidney, which rapidly elicits an inflammatory reaction with polymorphonuclear infiltration limited to the renal cortex. Depending on the severity and extent of the lesions, pyuria and candiduria may be the only findings, with varying degrees of renal insufficiency in approximately half the cases (207). When multiplication extends to the tubular lumen, they may invade

the interstitial tissue and elicit a severe interstitial inflammatory reaction in the medulla. Based on experimental studies, it would seem that when smaller doses of *Candida* are injected, the mycelia pass down the tubules and enter the collecting system without eliciting the cortical lesions, which are seen only when larger doses of *Candida* are injected (208).

The second type of lesion develops in the more chronic form of the infection and results from the multiplication of the organisms in the urinary tract, where no limiting inflammatory reaction can be elicited. This can result in the proliferation of the organisms into mycelial filaments that form bezoars of *Candida* in the renal pelvis and calices (207,208). The large bezoars or fungus balls can completely fill and occlude the renal pelvis and calices and result in obstructive nephropathy and hydronephrosis (208,209).

Noninvasive Infections

Acute tubulointerstitial nephritis may complicate infections in the absence of bacteremia and parenchymal invasion of the kidneys. Before the widespread use of antibiotics, ATIN was a relatively common complication observed at autopsy of cases of scarlet fever, β-hemolytic streptococcal infections, and diphtheria (3,4,210). Although the lesions of ATIN were noted to be more common in cases of scarlet fever (3), the more severe destructive lesions occurred in cases of diphtheria (4). The availability of specific vaccines has eradicated diphtheria, whereas specific antibiotic therapy has ameliorated the severity of streptococcal infections such that, although renal lesions continue to occur, their clinical outcome is now more favorable (211–213). Unlike the relatively later renal changes of streptococcal infections, which occur in the second or third week after onset of the infection and cause typical postinfectious acute proliferative glomerulonephritis, the lesions of ATIN occur early in the course of infection, usually during the first few days of onset of the infection (3,4, 210–214), often between the 9th and 12th days of the disease (13,214), and in the absence of edema or hypertension.

The renal lesions may be focal or diffuse. Interstitial edema is more prominent than the cellular infiltrates, which are rather diffuse and localized to the corticomedullary junction. The cellular infiltrates are especially prominent in the perivascular areas and, on occasion, may be dense enough to mimic leukemic infiltration. The initial infiltrates consist of plasma cells, histiocytes, and lymphocytes, whereas in the later stages of the disease the number of plasma cells decreases and that of lymphocytes increases. The lesions can easily be differentiated from those of pyelonephritis by their distribution. In pyelonephritis, the inflammatory foci are arranged radially, whereas in noninvasive ATIN they form a circular band around the vessels at the corticomedullary region. Occasional eosinophils may be present (211–213). The proximal tubules may be dilated, with swelling and occasional necrosis of the lining cells (13). The interstitial ground substance may stain heavily with periodic acid-Schiff stain, suggesting depolymerization of the interstitial mucopolysaccharides (13).

In contrast to the detailed morphologic changes reported in the early literature describing this entity, the clinical information provided is sparse. Where noted, proteinuria and pyuria were said to be common (4). In a careful study of the urinary sediment of 14 children with scarlet fever, an abnormal sediment was reported to be present in all (215). In larger series, the urinary sediment is noted as being abnormal in only 50% to 60% of the cases (212).

The availability of antibiotics and supportive therapy has altered the picture of infection-associated ATIN, not only through the emergence of antibiotics as a more common cause of ATIN but by prolonging the course of the infective disease in severe cases that were once rapidly fatal and revealed ATIN at postmortem. The use of potentially nephrotoxic antibiotics allows time for tubular necrosis to develop and become the lesion most evident at autopsy. This is in contrast to the preantibiotic era, when patients died soon after the onset of severe infection, probably before epithelial cell necrosis had set in (216). Certainly, the lesions described by Councilman (1) are much less rarely noted in the current literature, and most of the available information on them dates back to the preantibiotic or early antibiotic periods. Nevertheless, they continue to be encountered, particularly in children (212,215,217), and must be considered in streptococcal infections with ARF in the absence of hypertension and edema, and with normal complement levels (218). In one series of 13 kidney biopsies of ATIN in children, 10 were attributed to systemic infections, of which 7 were considered to be streptococcal in origin (45).

Drugs

Drugs have emerged as the most common cause of ATIN, with most cases attributed to antimicrobials (219,220). First described in conjunction with the sulfonamides used in the 1940s (185,186), the lesion was next reported to occur during penicillin therapy in the 1950s (10) and was ultimately best characterized in patients treated with methicillin in the 1960s (100,109,223). Since then, the number of antimicrobials, as well as other drugs, implicated as a cause of ATIN continues to increase. Although the implicated mechanisms and general morphologic feature on kidney biopsy are the same as those described with methicillin, their clinical presentations differ with other drugs. Essentially, nonoliguric ARF is more common, while those of fever, eosinophilia and skin rash are less common. Other subtler differences are the less frequent occurrence of gross hematuria clinically, and of tubulitis on kidney biopsy (11,12,206).

The association of ATIN with most of the incriminated drugs is quite rare, often based on individual case reports, and the evidence for a causative role of the drug merely circumstantial, particularly in the absence of a kidney biopsy. Even in cases where a kidney biopsy is available, the possibility of ATIN due to the underlying infection or of ATN due to the hemodynamic instability of infected patients could make interpretation of the biopsy difficult and the attribution of ATIN to the implicated drug suspect, especially in instances where only a single case is reported in the literature. In cases where kidney biopsy is not available for documentation of interstitial lesions, the acute deterioration of kidney function and abnormal urinary sediment, even in the presence of the eosinophiluria used to implicate ATIN, may be due to any of the other possible causes, including preexisting kidney disease, ATN, postinfectious glomerulonephritis, or a direct nephrotoxic effect of the implicated agent or one of the coadministered drugs. The latter possibility is particularly confounding because any one of the multiple agents used in the treatment of most patients can be responsible for ATIN, even when least suspected (78,115). By contrast, the possibility that ATIN may be missed clinically because acute deteriorations of kidney function, particularly when only modest, are attributed to other possibilities and their diagnosis not pursued by kidney biopsy may occasionally prove detrimental to patient care, but it certainly limits any estimation of the true prevalence of drug-induced ATIN and its incidence in cases of clinical ARF (115).

Drug-induced ATIN is due to a hypersensitivity reaction, as evidenced by several facts: It occurs in only a small number of people exposed to the drugs; it is not dose related; it often is associated with other systemic manifestations of hypersensitivity such as fever, skin rash, eosinophilia, and arthralgia; the reaction recurs on reexposure to the same drug or one of its

congeners; circulating antibodies to some of the incriminated drugs have been demonstrated in some instances; and laboratory evidence for a hypersensitivity reaction has been documented in some cases. In addition, the structural features of individual drugs (propionic acid derivatives) or those common to some groups of drugs (β-lactams) do seem to predispose to the development of ATIN. The most common causes of drug-induced ATIN are antibiotics and NSAIDs. The lesion is more commonly encountered with methicillin than penicillin (224) and with fenoprofen than other NSAIDs, including others that are propionic acid derivatives (225).

Antibiotics

Of the several antibiotics that have been incriminated as a cause of ATIN, most share the β-lactam structural ring—penicillin and its derivatives and the cephalosporins.

Methicillin

Methicillin is the agent with the highest incidence of drug-induced ATIN and the one in which the clinical features of this side effect of antibiotics are best characterized (11,12,226). The mean period of drug administration before the renal lesions occur is approximately 15 days, with a reported range of 10 to 45 days (227). The incidence of ATIN has been estimated to be approximately 2% of those exposed to the drug and increases to 15% in those who are exposed to it for over 2 weeks (53,224,228–230). In those who experience a reaction, subsequent exposure to methicillin results in recurrence after a much shorter period of exposure and at lower doses of the drug (229–233). With the reduced clinical use of methicillin, there has been a reduction in the number of cases encountered clinically and reported in the literature (28,98,233).

Systemic manifestations are a dominant feature of methicillin-induced ATIN. Characteristically, drug-induced fever appears after the initial fever caused by the infection has subsided. The recurrent episode of fever usually is accompanied by skin rash, eosinophilia, and arthralgias. The skin rash is fleeting and consists of erythematous, often pruritic, maculopapular lesions that are present in 30% to 50% of cases. Eosinophilia is common (80%) but transient, and, unless noted and specifically sought, goes undetected (11,94–97). Arthralgias are nonspecific and less common (10% to 20%) than the fever, skin rash, and eosinophilia. Eosinophiluria appears to be more common and more evident than in other forms of drug-induced ATIN (11,12,94).

As a rule, patients are polyuric as kidney function begins to deteriorate, and the ARF, when first detected, is nonoliguric in two-thirds of patients (95–97,101,234). Independent of the severity of the ARF, the outcome is favorable after withdrawal of methicillin in 90% of cases (95–97,227). The initial improvement, which is rapid over the first few weeks of discontinuing methicillin, is followed by a slower return of kidney function to normal in most (>2/3), but only to near normal in a some 20% of cases (12,26,235). The worst prognosis is in those with granulomatous lesions. Instances of persistent kidney failure are rare (<10%), and are more likely in those whose kidney biopsy reveals diffuse infiltrates, interstitial fibrosis, granulomatous reaction, and tubular injury (234,236,237). Recovery is faster and more likely in those whose dominant kidney lesions consist of interstitial edema rather than cellular infiltrates, and when the cellular infiltrates are focal rather than diffuse (26).

A metabolic derivative of methicillin, dimethoxyphenylpenicilloyl, has been implicated as the inciting antigen on the basis of its demonstration by immunofluorescence techniques along the TBM (65,66) and the detection of circulating antibodies to dimethoxyphenylpenicilloyl in cases of ATIN (109). However, neither of these findings appears to be specific to the incidence of ATIN because both have been shown to be present in methicillin-treated cases with no evidence of acute kidney injury (67,238).

Rifampin

Since the introduction of rifampin (rifampicin) as an antituberculous agent in 1976, over 100 cases of ARF have been ascribed to it in the literature. As a rule, ARF has occurred during intermittent administration of the drug (once or twice weekly), usually appearing several months after institution of therapy (239–242). A more abrupt onset occurs with rifampin readministration after a hiatus in its previous daily, uneventful use (243). ARF during continuous daily rifampin therapy also has been noted (18). However, most cases reported have occurred during intermittent therapy, and the number of cases noted has decreased since intermittent use of the drug has been abandoned (234,244).

Circulating antibodies to rifampin have been detected in most, but not all, affected patients, as well as in the serum of those without any adverse reaction to the drug (245–248). The suggestion has been made that the kidney lesions develop as a response to a critical level of antigen–antibody complexes that accumulate during intermittent rifampin therapy (245, 246,249). However, immunofluorescence staining of kidney biopsy specimens for deposits of antibodies to rifampin has been negative in most cases (18,239,243–246), although it has been positive in an occasional case (243–250). A role for cell-mediated immune injury appears more likely based on *in vitro* studies of lymphocyte activation on exposure to rifampin (234,251).

Accompanying and preceding symptoms of patients in whom renal involvement develops include fever, chills, nausea, vomiting, diarrhea, dizziness, lumbar or abdominal pain, and myalgias, which occasionally are accompanied by abnormalities of liver function, hemolysis, and thrombocytopenia (18,239–244). The symptoms are dramatic in their sudden appearance and severity in those patients exposed to the drug for a second time and sometimes are associated with hypotension and hemodynamic compromise. As a result, the initial reports of ARF attributed to rifampin were ascribed to ischemic tubular injury. A direct nephrotoxic effect that is slower in onset has been reported with rifampin. Kidney biopsy in such cases reveals marked proximal tubular injury but very little interstitial infiltration (249). Actually, the majority of lesions of rifampin-induced ARF are those of ATIN and consist of interstitial edema and focal infiltration with mononuclear cells and occasionally eosinophils, with focal segments of tubular epithelial cell injury and necrosis (18). In some cases, the necrotic tubular lesions may be severe and extensive (240–243,252). Whether this reflects the severity of ATIN or is due to ischemic injury secondary to vasomotor phenomena is difficult to establish. Certainly, the accompanying systemic manifestations—eosinophilia, recurrence on challenge, the reported humoral studies, as well as the morphologic features seen on kidney biopsy—are strongly supportive of ATIN due to an allergic hypersensitivity reaction to the drug. While ATIN is the most common lesion of rifampin-induced ARF, glomerular lesions have been reported also (242). As a drug that induces cytochrome P-450, the consequent reduced levels of tacrolimus and cyclosporine can result in ARF due to acute rejection in kidney transplant recipients (241,242)

The severity of ARF due to ATIN is variable. Dialysis may be required in some patients in whom prolonged oliguria develops (253). The course of renal failure usually is favorable, with return of kidney function to normal in most cases after withdrawal of rifampin, although residual impairment of renal function may occur in some (239,256,257). The role of

steroids in accelerating recovery from rifampin-induced ATIN is undetermined. The lesion has occurred in a patient who was receiving steroids for other reasons (18).

Penicillin and Congeners

Acute tubulointerstitial nephritis clinically and pathologically undifferentiable from that described with methicillin also occurs with penicillin and its congeners, but is much less common than with methicillin. The dose of penicillin used has been fairly large, 12 to 60 million U/day, although ATIN has been reported at lower doses (16,109,113,146,234) and even after a single dose (258).

Other penicillin derivatives that have been associated with the lesions of ATIN are, in descending order of frequency, ampicillin (16,26,95,259–262), oxacillin (263), carbenicillin (264,265), nafcillin (102), and oxacillin and amoxicillin (102,234,237,265–268). The systemic manifestations of a hypersensitivity reaction in these cases have been less common than in ATIN due to methicillin and penicillin.

Cephalosporins

Acute tubulointerstitial nephritis also has been noted with the cephalosporins. It is more common with cephalothin (231,234,269–272), followed in descending order of frequency by cephalexin (95,273), cephradine (274), cefoxitin (275), cefazolin (276), cefaclor (102), cefotaxime (28,277), and cefotetan (278), cefoperazone (279), cephaloridine, cefamandole, and cephapirin (11).

Other Antibiotics

The incidence of ATIN with other antibiotics is much less than that encountered with methicillin, penicillin congeners, and rifampin, except for ciprofloxacin, with which an increasing number of cases continue to be reported (117,234,280–283). Isolated instances of ATIN have been reported with minocycline (284), doxycycline (26), gentamicin (26,230,285), polymyxin (286), vancomycin (287,288), lincomycin (287), mezlocillin (289), chloramphenicol (26), erythromycin (290–293), flurithromycin (294), netilmicin (291), norfloxacin (295,296), tetracycline (297), ethambutol (298), telithromycin (299), and levofloxacin (300).

Sulfonamides

The introduction of sulfonamides in the 1930s heralded a new and welcome era in the specific treatment of infectious diseases. Despite their utility and general safety, severe adverse reactions were quite common with these agents and often necessitated cessation of their use (301,302). The introduction of antibiotics during the following decade led to a consistent decline in the use of sulfonamides until the introduction of trimethoprim–sulfamethoxazole (TMP/SMX) (170), when a resurgence of adverse reactions attributed to sulfonamides was observed, necessitating modification of the warnings in the labeling of TMP/SMX (303). The advent of AIDS with the associated prophylactic use of TMP/SMX (170), as well as its use in transplant recipients (304), has led to the increased incidence of these side effects.

The renal effects observed with the initially available, less soluble sulfonamides (sulfathiazole, sulfapyridine, sulfadiazine) were due to the precipitation of sulfa crystals in the renal tubules, with consequent hematuria, obstructive uropathy, and kidney failure. The renal lesions consisted of focal tubular necrosis, sometimes with sulfa crystals noted in the lumen, and with peritubular interstitial infiltration and edema in the affected segments (305,306). The introduction of more soluble sulfonamides (sulfisoxazole, sulfamethoxazole, sulfamerazine, sulfasalazine) made this complication much less common, but by no means eliminated it (301,305–309). An idiosyncratic hypersensitivity-type reaction as a cause of the acute renal injury, which was recognized from the outset, now is the principal cause of the renal adverse effects of sulfonamides (301,305–310). The symptoms develop at a mean of 14 days after initiating therapy. Fever followed by skin rashes of varying severity usually precede the onset of ARF. Eosinophilia has been noted as an accompanying finding in most cases. Unlike antibiotic-induced ATIN, there is evidence of multiple organ involvement (heart, liver, lungs) in patients who have sulfonamide-induced ATIN (301,310,311). The renal lesions noted in kidney biopsy specimens consist of interstitial edema and focal areas of mononuclear cell infiltration that are most pronounced around the segments of tubular injury and necrosis (306,310). The kidney failure is reversible after withdrawal of the drug and institution of steroid therapy (306), although residual fibrosis and tubular atrophy with persistent impairment of renal function may occur (310).

The predisposition of patients to idiosyncratic reactions has been attributed to individual differences in the metabolism of sulfonamides. Those who have idiosyncratic reactions are slow acetylators of sulfonamide, which leads to the accumulation of drug metabolites that can covalently bind to cell macromolecules and cause cell injury, or elicit a secondary immunologic phenomenon (311,312).

Acute renal failure due to a hypersensitivity reaction also occurs with TMP/SMX (26,232,234,313–317). The increase in serum creatinine that occurs with this agent does not necessarily indicate renal injury because trimethoprim interferes with the tubular secretion of creatinine, with a consequent increase in the serum creatinine level, without necessarily affecting true glomerular filtration rate (318). The clinical and morphologic features of ATIN induced by TMP/SMX are identical to those induced by other sulfonamides and are due to the sulfamethoxazole content. Multinucleated giant cells and granulomatous reactions, reflecting the severity of the reaction, have been described and are associated with only partial recovery of kidney function after withdrawal of the drug, despite steroid therapy (234).

Most of the reported cases of TMP/SMX-induced ATIN have been in patients with reduced kidney function, or transplant recipients (313,315,316). It has been suggested that the propensity of patients with reduced kidney function to ATIN may be due to the accumulation of a toxic metabolite, which predisposes them to the adverse effects of the drug (313,316), much like that noted in the genetically predisposed slow acetylators (311). Crystalluria occurs with the use of high doses of TMP/SMX. In patients with severe renal insufficiency, this could cause further deterioration of kidney function without necessarily eliciting an allergic reaction (319), as has been documented experimentally (320,321).

Nonsteroidal Antiinflammatory Drugs

The NSAIDs provide a relatively effective approach to the treatment of musculoskeletal pain and inflammation, which are among the most common complaints of an aging population. As a result, NSAIDs have become the most widely prescribed category of drugs. The impact of recent reports of increased relative risk for cardiac death associated with NSAID use remains to be determined, but is bound to have a significant dampening effect on their widespread level of utilization heretofore (322).

The most serious untoward effects of their use have been noted in the gastrointestinal tract and the kidney (323,324). The renal effects include reduction in GFR and renal blood flow, acute and chronic renal injury, and abnormalities of

sodium and potassium homeostasis (323–326). By far the most common of these side effects is the hemodynamically mediated, reversible ARF related to the prostaglandin-inhibitory action of these agents, which occurs principally in patients with congestive heart failure, cirrhosis of the liver, nephrotic syndrome, septicemia, and volume depletion from any cause (326–328). Less common has been the development of another form of ARF due to tubulointerstitial nephritis, which, unlike other drug-induced ATINs, is frequently associated with massive proteinuria (215,326,329–331). A necrotizing vasculitis also has been reported in a few cases in association with fenoprofen, indomethacin, and diclofenac (331–335).

The duration of exposure to NSAIDs before the onset of ATIN is variable, ranging from 2 weeks to 18 months; as a rule, ATIN develops after prolonged exposure (mean, 5.4 months) to the incriminated agent (225,234,331). The propionic acid derivatives fenoprofen, ibuprofen, and naproxen account for approximately three-fourths of the cases reported (225, 331–336). Fenoprofen alone has been incriminated for over one-half of the reported cases. The lesions have been noted after the self-administration of over-the-counter ibuprofen (337,338) and of mefenamate in drug mixtures (339), and to recur after exposure to different NSAIDs of the same group (330,334) or on reexposure to the same agent (225). Other NSAIDs that have been incriminated include, in decreasing order of frequency, tolmetin (340–342), zomepirac (343–346), indomethacin (225,347,348), diclofenac (349–351), and diflunisal (3352,353). Isolated cases have been reported with the use of phenylbutazone (354,355), mefenamate (356), phenazone (357), sulindac (358), noramidopyrine (234), and piroxicam (259,260). Contrary to initial expectations they also occur with the selective cyclooxygenase-2–inhibitory agents such as celecoxib (361–363), rofecoxib (364,365) and nimesulide (366,367).

The proteinuria and clinical manifestations of the nephrotic syndrome usually are of insidious onset and precede the onset of kidney failure. Nephrotic syndrome, without renal insufficiency, may be the only manifestation in approximately 10% of cases. On the other hand, kidney failure without nephrotic syndrome occurs in approximately 15% of cases (11,12,83,225,331). The variation in clinical presentation has been attributed to the severity of the allergic hypersensitivity reaction, with a more rapid course presenting with reduced kidney function and nephrotic syndrome in hyperreactors (332). β_2-Microglobulinuria is a common and prominent feature. Flank pain has been a notable complaint in several cases. The clinical features of hypersensitivity reaction (fever, rash, eosinophilia) occur in only 20% of cases, compared with an incidence of over 85% in patients with methicillin-induced ATIN. The serum complement and IgE levels usually are normal, and anti-TBM antibodies are absent. Most of the patients reported have been older than 60 years of age, which is more a reflection of the greater use of NSAIDs in this age group than of age as a predisposing factor (333). The urine sediment reveals microscopic hematuria and pyuria and only rarely eosinophiluria (83). In the diagnosis of proteinuria due to NSAIDs, it should be kept in mind that tolmetin can produce pseudoproteinuria. This is due to the effect of its dicarboxylic metabolite on the detection of proteinuria by the acid precipitation (sulfosalicylic acid and trichloroacetic acid) methods. In such cases, no proteinuria is detected with dye-impregnated reagent strips, electrophoresis, or nephelometry (368,369).

Histologic examination of the kidney reveals diffuse edema, focal areas of interstitial infiltrates consisting predominantly of lymphocytes, and vacuolar degeneration of the tubules (Fig. 48-3). In some cases, the proportion of infiltrating B lymphocytes and CD8+ cells has been more than that noted in other forms of ATIN. Eosinophils have been observed in one-third of the cases reported and may constitute up to 20% of the infiltrating cells, even when eosinophiluria is absent. The glomeruli are minimally altered, if at all, except for the effacement of the epithelial foot processes consistent with minimal-change disease in nephrotic cases. Mesangial electron-dense deposits have been noted in rare instances in association with modest membranous and subendothelial deposits (370,371). Membranous glomerulonephritis, reversible after drug withdrawal, has been reported with sulindac and other NSAIDs (332,372). Immunofluorescent microscopy is negative, although a slight, nonspecific focal immunofluorescence of the glomeruli and TBM occasionally has been noted (334). Granulomatous reactions occur in severe cases (373).

The kidney failure, although insidious in onset, can be severe enough on clinical presentation to require supportive dialytic therapy in approximately a third of the patients. The severity of the kidney failure may stem from the additive effects of ATIN, reduced renal blood flow due to inhibition of prostaglandin, altered glomerular permeability, and absence of systemic effects, the latter of which leads to delayed diagnosis early in the course of the disease process until patients finally present with nephrotic syndrome and kidney failure. The response to discontinuation of the offending NSAIDs has been favorable in most cases, with improvement of kidney failure occurring within a few days, but with a slower subsidence of proteinuria over weeks or months. Cases of persistent kidney failure have been reported (225,374). Progression of the kidney lesion to focal glomerular sclerosis has been noted on repeat kidney biopsy in some patients (332,375,376). Steroids have been used in the treatment of severe cases. The response to steroid therapy, if any, appears to be slow, and the value of corticosteroids in this entity is questionable (83,377).

The pathogenesis of NSAID-associated ATIN remains undetermined. The rarity of the lesion despite the widespread use of these agents (estimated at 1 in 5,300 patient-years of treatment with fenoprofen) supports an idiosyncratic reaction (329). The clinical features of a hypersensitivity reaction, although present in only one-fifth of the cases, support an allergenic reaction. Their absence from most cases has been construed as an argument against a hypersensitivity reaction, but may actually reflect an amelioration of the hypersensitivity symptoms because of the antipyretic and antiphlogistic actions of NSAIDs. The presence of a diffuse interstitial eosinophilic infiltrate, in the absence of peripheral eosinophilia and eosinophiluria, also is compatible with the antiinflammatory effect of the NSAIDs. Evidence for a humoral antibody reaction is lacking. There is a slight preponderance of cytotoxic/suppressor (CD8+) T cells in the infiltrates, especially early in the course of the disease, which has been advanced as an argument for cell-mediated immunity (55,83,378,379). Potentially, the metabolism of these agents could yield metabolites that are injurious to the tubules and podocytes. Moreover, cyclo-oxygenase blockade can affect the arachidonic acid lipo-oxygenase pathways leading to the formation of proinflammatory and vasoactive compounds (313a). Whether the prostaglandin-inhibitory effect of these agents plays a role in the unique proteinuric features of this form of ATIN has not been ascertained but certainly is an attractive conceptual possibility that cannot be neglected (225,380).

Phenindione

An increasing number of severe sensitivity reactions were noted with the anticoagulant phenindione after its introduction in 1947. The more common reactions were skin rash, fever, diarrhea, blood dyscrasias, stomatitis, and hepatitis, in descending order of frequency. The reactions appeared after 3 to 5 weeks of therapy and continued to progress even after discontinuation of the drug, proving fatal in one-half of the cases. ARF due

to ATIN, which was granulomatous in some cases, also was reported in approximately 30 cases, usually coincident with the development of jaundice and hepatotoxicity (381–388). ATIN was accompanied by the classic triad of skin rash, fever, and eosinophilia, usually with a prolonged period of oliguria and death in one-third of the cases reported (16,385), chronic kidney disease in some (358), and massive proteinuria in others (387).

Because of the high incidence (10%) of these adverse side effects and their severity, this agent was prescribed much less over the years, leading to the cessation of its manufacture in 1983 for economic reasons. It is, however, still available in some countries, or as an investigational drug in the United States. Phenindione is an indanedione derivative and since then other analogs (fluindione, anisoindione, diphenadione) have been introduced but ATIN has not been reported with any of them.

A metabolic product of phenindione that is excreted by the kidneys imparts a red-orange color to the urine. This is of no clinical importance except in the differential diagnosis of hematuria. Acidification of the urine eliminates the color and differentiates it from the true hematuria that develops in ATIN (389).

Allopurinol

Severe hypersensitivity reactions to allopurinol have been reported since its introduction in 1963 (337). They have the features of a diffuse vasculitis involving multiple organs (338). The symptoms appear approximately 4 weeks after initiation of therapy and consist of fever, rather severe generalized skin rashes that may be desquamative, eosinophilia, hepatic failure, and prolonged oliguric kidney failure (390–394).

The structural changes in the kidney are characteristic of ATIN, including those of a granulomatous reaction in severe cases (395). Although the renal lesions have been reported in patients with normal kidney function, most cases have occurred either in patients with preexisting kidney disease and reduced kidney function, or in those receiving other drugs that might themselves cause ATIN. The decreased excretion of a toxic metabolite of the drug, possibly oxypurinol, has been implicated as the inciting agent in those with reduced kidney function. A predisposing role of thiazides, furosemide, and ampicillin has been implicated in those who have experienced a reaction while receiving other drugs (390,396).

The finding of circulating anti-TBM antibodies, together with granular deposits of C3 along the TBM of a single case, has been advanced as evidence for the role of an immune-mediated mechanism (393). However, the absence of linear deposits of anti-TBM antibody in the kidney biopsy specimen and the fact that the patient had IgA nephropathy as the underlying primary kidney disease make the interpretation of the results from this individual case impossible, unless these findings are substantiated in other cases.

Proton Pump Inhibitors

Cases of classic ATIN have been reported with the antiulcer agent cimetidine (397–404). The renal lesions are patchy, with focal areas of tubule cell injury and interstitial inflammatory infiltrates consisting of lymphocytes and a few eosinophils.

In one carefully studied case, IgE-producing plasma cells were identified as part of the infiltrating cells, most of which were identified as being cytotoxic/suppressor T lymphocytes, and activated T cells were demonstrated in the circulation, strongly implicating a cell-mediated immune reaction as the pathogenetic mechanism of cimetidine-induced ATIN (81). In addition to its inhibitory action on gastric acid secretion, there is evidence that cimetidine might have a role as an immunomodulator (81). It is possible that this action plays a role in cimetidine-induced ATIN; it seems unlikely, however, given that there are only a few case reports of ATIN related to this widely prescribed agent, now available over the counter. Hence, the more likely possibility is that the kidney injury is idiosyncratic. In addition, the clinical and morphologic features of the ATIN are characteristic of a hypersensitivity reaction: eosinophilia, eosinophiluria, fever, and IgE-containing plasma cells in the infiltrate, with recurrence of the symptoms on rechallenge (397–404). The lesions of ATIN have been reported also in association with other proton pump inhibitors such as ranitidine (98,405–407), lansoprazole (408), omeprazole (290,409–411), and pantoprazole (412,413).

Phenytoin

One of the adverse side effects associated with the anticonvulsant phenytoin, formerly known as diphenylhydantoin, is a delayed hypersensitivity reaction characterized by a systemic reaction and ARF. The systemic manifestations include fever, hepatitis, myositis, lymphadenopathy, skin rashes that may be exfoliative, eosinophilia, and anemia (69,70,414–416). The kidney injury is due to ATIN characterized by edema and focal inflammatory infiltrates consisting of mononuclear cells and occasional eosinophils. Nephrotic syndrome has been observed in some patients (69). The lesions are reversible on withdrawal of the drug, but steroid treatment has been successfully used in most reported cases (69,414–417).

In two cases, circulating antibodies to human TBM were detected and linear deposits of anti-TBM antibodies demonstrated along the renal TBM (69,70). In one case, deposits of phenytoin were demonstrated along the TBM, leading to the suggestions that the initial alteration of the host TBM is due to phenytoin deposition with secondary immune reaction directed at the phenytoin–TBM complex or to the phenytoin-altered TBM (69). Passive transfer of the anti-TBM from one patient failed to produce the disease in experimental animals (70), with increased DNA incorporation, on exposure to phenytoin could be induced only in the lymphocytes from patients demonstrating the hypersensitivity reaction (65,414).

Diuretics

Acute renal failure often is associated with the use of diuretics. The renal insufficiency that results usually is prerenal in origin and a result of the systemic and renal compensatory mechanisms mediated by extracellular fluid volume depletion induced by the diuretics (418). Less commonly, deterioration of kidney function is the result of other mechanisms, including direct renal toxicity, such as with mercurial diuretics (418,419); obstruction, such as with triamterene nephrolithiasis (420); necrotizing vasculitis, such as with thiazides (421); and occasionally ATIN that has been noted to occur with furosemide, thiazides, triamterene, chlorthalidone, ethacrynic acid, tienilic acid, and indapamide (422–428). Most of the reported cases have been associated with the use of thiazides and furosemide, both of which are structurally related to sulfonamides, another group of drugs associated with ATIN. A potentiating role of triamterene used in conjunction with thiazides or furosemide has been suggested (423).

Several of the patients with diuretic-induced ATIN had preexisting kidney disease (26,422), although the lesions also are noted in patients with normal kidney function (368,371). The deterioration of kidney function usually is insidious in onset but may be rapid and lead to oliguric ARF. Systemic symptoms

of drug-related hypersensitivity reaction (fever, skin rash, and eosinophilia) were present in almost all the patients reported. The kidney biopsy findings were those of classic ATIN, including an eosinophilic infiltrate in one-half of the reported cases and a granulomatous reaction in an occasional case (422–430). Immunofluorescent studies have been negative. Abatement of symptoms and return of kidney function to baseline have occurred in all patients reported after removal of the incriminated diuretic and the administration of steroids to some patients.

Given the frequency with which diuretics are prescribed, the fact that less than 20 cases of ATIN have been reported with their use reflects the relative rarity of diuretic-induced ATIN.

Other Drugs

The list of other drugs associated with ATIN continues to expand. Most of these have been isolated, single case reports of patients who often were using more than one drug.

By far the most common among these to be associated with ATIN are glafenin (431,432) and its derivatives, antrafenin (433,434) and floctafenin (434). Although direct drug toxicity, with lesions of ATN, has been incriminated as the cause of ARF in some of these patients (434,435), clinical and laboratory evidence of an immunologically mediated hypersensitivity reaction causing ATIN has been sufficiently well documented to justify incriminating these agents as a cause of ATIN.

Other drugs that have been implicated are sulfinpyrazone (95,436,437), aminopyrine (11), azathioprine (438,439), quinine (440), clofibrate (441), propylthiouracil (442), carbamazepine (443,444), amidopyrine (445), amphetamine (446), phenobarbital (114,447), *p*-aminosalicylic acid (448), captopril (449–451), diltiazem (452), methyldopa (453), acyclovir (454,455), interferon (456), indinavir (457), griseofulvin (458), ifosfamide (459), intravenous immunoglobulin (446), phentermine and phendimetrazine (461), foscarnet (462), interleukin (463), and even aspirin (464,465).

SYSTEMIC DISEASES

Although most cases of ATIN are due to a hypersensitivity reaction to drugs or infections, whether renal or extrarenal, an acute deterioration of kidney function with the structural changes of ATIN also occurs in a variety of systemic diseases. In these, the renal involvement is due either to the metabolic disturbances associated with the underlying disease, the immunologic basis of the primary disease process, or the infiltrative nature of the disease (Table 48-3).

Metabolic Disorders

The metabolic disorders in which ATIN develops are those due to abnormalities in the metabolism of urate, oxalate, calcium, potassium, and heavy metals (466,467).

Uric Acid

The kidney, as the major organ responsible for uric acid excretion, becomes the principal target organ affected by disorders of urate metabolism. Renal involvement results from the precipitation of uric acid in the renal parenchyma and the urine outflow tract. Depending on the load of uric acid presented to the kidney and the duration of exposure, the parenchymal lesions that develop are those of either an acute or a chronic urate nephropathy. In the acute form of urate nephropathy, the renal changes are those of an ATIN (466–468). The most common condition in which this is encountered is in ARF associated with the tumor lysis syndrome (469,470) and occasionally in massive tissue destruction such as rhabdomyolysis (471).

Oxalate

The increased metabolic production or intestinal absorption of oxalate and its consequent excretion by the kidney almost invariably results in the precipitation of calcium oxalate in the urine outflow tract (466). Microcrystallization first occurs in the proximal tubule, where oxalate is secreted. However, the lesions that develop are more prominent in the medulla, where the increased concentration of oxalate in the tubular fluid, as water is reabsorbed, and the acidification of the fluid, as hydrogen is secreted, favor the precipitation of calcium oxalate. This results in injury and atrophy of the affected tubules, with interstitial edema and inflammatory cell infiltration around the injured tubular segments (466,467,472,473). When the hyperoxaluria is sudden and massive, such as after ethylene glycol ingestion (474,475), methoxyflurane anesthesia (476) or the ingestion of star fruit (477), acute deterioration of kidney function occurs, the morphologic features of which are those of ATIN. Otherwise, when the hyperoxaluria is modest and chronic, such as in inflammatory bowel disease, the onset of kidney failure is insidious and the lesions are those of chronic tubulointerstitial nephritis (466,478), although ARF associated with enteric hyperoxaluria can occur (479,480).

The treatment of acute oxalate nephropathy consists of maintaining a high urine flow rate, hemodialysis, and supportive measures directed at elimination of the oxalate precursors.

Immune Diseases

Diseases due to an immune mechanism affect primarily the glomeruli, with secondary involvement of the tubules and interstitium. Primary tubulointerstitial nephritis mediated by an underlying altered immune mechanism is extremely rare in humans. The two conditions in which an immune mechanism may result in ARF primarily due to tubulointerstitial lesions, with only limited glomerular involvement, are the transplanted kidney (481,482) and systemic lupus erythematosus (73).

The diagnosis of ATIN in kidney transplant recipients can be challenging (483–490). Despite the numerous medications these patients receive, drug-induced ATIN is an uncommon cause of ARF in them. Drug-induced ATIN lesions can resemble acute cellular rejection morphologically, and both entities can show improvement after steroid therapy. While infiltrating eosinophils can be seen in acute cellular rejection, their presence and localization at the cortico-medullary junction and the presence of granulomatous lesions are suggestive of drug-induced ATIN (486–488), while that of tubulitis is a defining feature of allograft rejection (489). Clinically, the diagnosis is compounded by the fact that allograft recipients are prone to infection-induced ATIN, such as cytomegalic virus (483), adenovirus (484), and polyoma virus (485).

ARF with prominent tubulointerstitial lesions may occur in other diseases due to altered immune mechanisms, but very rarely in the absence of glomerular lesions. These diseases are Sjögren's syndrome (71), mixed cryoglobulinemia (71), Wegener's granulomatosis (491,492), crescentic glomerulonephritis (65), sarcoidosis (493), IgA nephropathy (494,495), and autoimmune pancreatitis (496).

Neoplastic Diseases

An acute deterioration of kidney function may occur in any neoplastic disease, either because of direct invasion of the kidney or the urinary tract by malignant cells or because of the

metabolic disorders (hypercalcemia, hyperuricemia) that may be caused by the malignancy. The institution of antineoplastic therapy, which may itself be nephrotoxic or can magnify the existing tumor-related metabolic disorder (hyperuricemia), is another mechanism of the acute renal injury experienced by these patients (497). Lymphoproliferative disorders and plasma cell dyscrasias are the two neoplastic diseases in which ATIN due to neoplastic cellular infiltrates is a principal cause of the ARF (498–500).

Lymphoproliferative Disorders

The kidney is one of the most common extranodal sites of metastatic lymphomas (501–504), although primary lymphoma in the kidneys without evidence of extrarenal lesions also can occur (505–507). Infiltration of the kidney is more common in non-Hodgkin's lymphomas and acute lymphoblastic leukemia than in Hodgkin's disease (498,499). Leukemic infiltration of the kidney has been noted in two-thirds of patients with leukemia, usually in those with the acute form of the disease (495,508,509). Renal infiltrates usually are bilateral and symmetrical and may go undetected unless extensive parenchymal infiltration occurs, in which case flank pain, palpable, tender kidneys, gross hematuria, and ARF are the presenting findings (499,502–511). The infiltrates are localized to the interstitium, compressing the tubules but sparing the glomeruli, thereby mimicking the classic morphologic features of an ATIN. Irradiation of the kidneys or systemic chemotherapy can result in a dramatic reversal of the structural and functional abnormalities (504,512,513).

Plasma Cell Dyscrasias

The renal complications of plasma cell dyscrasias are a major cause of morbidity and mortality in patients with multiple myeloma (499,501,514). ARF occurs in approximately 10% of these patients, and chronic kidney disease is present in more than two-thirds of them (499,500,515–517).

The pathogenesis of ARF is multifactorial (517–523). The acute lesions directly related to multiple myeloma, the so-called myeloma cast nephropathy, are those that result from the excessive production of light chains and their precipitation as dimers in the distal tubules. As a result, varying degrees of injury, necrosis, and regeneration of the tubular epithelial cells occur, with interstitial edema and an inflammatory reaction, including polymorphonuclear, mononuclear, and multinucleate giant cells, around the affected tubules. Immunofluorescent study of the kidney tissue can provide the definitive diagnosis when fluorescence is positive for specific sera for either κ or λ light chains (499,518,519).

The propensity of light chains to lead to myeloma cast nephropathy depends on their concentration in the tubular fluid, the tubular fluid pH, and the intrinsic physiochemical properties of the light chains (521,522). These factors account for the fact that the patients who are dehydrated, those who are producing an acid urine, and those with λ light chains rather than κ chains are more prone to development of acute lesions of myeloma-induced kidney failure. They also provide a physiologic basis for treatment consisting of maintaining a high rate of urine flow and its alkalinization, which can prevent and reverse the lesions in their early stage of formation (514,522,523).

IDIOPATHIC ACUTE TUBULOINTERSTITIAL NEPHRITIS

Since the entity of idiopathic ATIN was first noted in the literature in 1972 (524), an increasing number of cases of ATIN in the absence of exposure to drugs, infections, or any of the other conditions usually associated with ATIN have been reported (26,36,101,234,524–530). In fact, in most reports of ATIN, where a series of cases from a single institution are presented, a varying number of cases included have been of unknown cause (26,45,101,528,529).

The only common feature of these cases has been the presence of reversible ARF and the finding of edema and a mononuclear inflammatory infiltration of the interstitium on kidney biopsy. Although certain of the laboratory features of a hypersensitivity reaction (elevated IgE levels, eosinophilic infiltrate, and eosinophiluria) are present in some of the cases (101,524,529), the systemic manifestations of a hypersensitivity reaction (fever, arthralgia, rash) have been characteristically absent. Linear deposits of IgG, C3, and anti-TBM antibodies have been detected on immunofluorescent study of the kidney biopsy in some cases (527,528), granular deposits of C3 and IgG were seen in others (524–526), but no deposits were present in the rest (36,524)

The clinical presentation of most has been nonspecific, except for the acute deterioration of kidney function. The renal failure has been severe enough in a third of the cases to necessitate supportive dialytic therapy. The outcome has been favorable in most cases, with recovery occurring spontaneously or after the institution of steroid therapy (45,122,525–532). There have been cases of irreversible kidney failure that have failed to respond to steroid therapy (525–527). Clinically, the nonspecific presentation of idiopathic ATIN coupled with its favorable prognosis and response to steroids, at least in some cases, mandates that its possibility be considered in the differential diagnosis of every patient with ARF in whom the cause of the kidney dysfunction is uncertain.

The number of cases reported is too low to formulate a common pattern for idiopathic ATIN. Idiopathic ATIN constitutes 15% to 25% of cases of ARF that are sampled for biopsy (132,133). Systemic diseases, such as chronic active hepatitis or ulcerative colitis, have been present in an occasional case (528). Uveitis, on the other hand, has been a feature of several patients with idiopathic ATIN. This group constitutes a variant that deserves separate consideration.

Idiopathic Acute Tubulointerstitial Nephritis with Uveitis

An acute eosinophilic interstitial nephritis that occurred in association with an anterior uveitis of unknown cause was first described in 1975 in two patients who also had bone marrow and lymph node granulomas (533). The syndrome of ATIN with uveitis has since been noted to occur rarely in other granulomatous conditions (534), and has been identified in patients with no bone marrow granulomas in most of the reported cases (535–545). Most of the patients have been adolescent girls of pubertal age, although the lesion occurs in adults (534,542,546). Familial occurrence and an association with certain HLA serotypes have been suggested (547,548), with a strong association with the HLA-DQ and HLA-DR alleles (549). Anorexia, asthenia, nocturnal fever, and weight loss may be present for several months before the onset of the ocular complaints. A moderate anemia is common and hyperglobulinemia usually is present (550,551). There also is evidence of tubular dysfunction, such as glucosuria, proteinuria, aminoaciduria, β_2-microglobulinuria, impaired urinary concentrating ability, and azotemia (530,546,548–551). Uveitis may occur as a manifestation of a variety of systemic diseases (552). It is unusual for any of these systemic disorders to be complicated by ATIN, and its occurrence with uveitis in those in whom ATIN develops has been termed *renal ocular*

syndrome (535) or *tubulointerstitial nephritis and uveitis syndrome* (TINU syndrome) (536).

The cause of this syndrome complex remains undefined but has been presumed by some to be the result of an autoimmune process or reaction of an as yet undetermined viral infection. It has been described in individual cases of toxoplasmosis, giardiasis, chlamydiosis, and Epstein-Barr virus infection (553,554). No cause has been identified despite extensive investigation. The demonstration of circulating immune complexes in some cases has led to the suggestion of a role for an immune-mediated process (536). Mast cells may play a role in the development of interstitial injury in these cases (555). Both the ocular and renal changes respond to a brief course of steroid treatment (535,536) but can recur; spontaneous remission without steroid treatment also has been reported especially in children (536,551–556). The uveitis may be asymptomatic indicating the need for ophthalmologic examination in all cases of idiopathic ATIN (557). The uveitis can precede the onset of ATIN (558) or relapse without any renal manifestations, in which instances it responds to topical steroid treatment. In children and adolescents, the long-term prognosis is good, with recovery of kidney function and no documented visual loss. In adults, the prognosis is less favorable and kidney failure requiring dialysis may develop, particularly if steroid therapy is withheld (559,560).

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CHAPTER 49 ■ ACUTE RENAL FAILURE ASSOCIATED WITH PIGMENTURIA OR CRYSTAL DEPOSITS

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ACUTE RENAL FAILURE RESULTING FROM HEME PIGMENTS

Myoglobinuric Acute Renal Failure

The destruction of skeletal muscle and the release of muscle cell contents, notably myoglobin, into the circulation can cause an acute deterioration in renal function. The seminal description of the consequences of traumatic muscle injury on kidney function is attributed to Bywaters and Beall (1), who vividly documented the brown-black granular casts, the reduction in urinary output, hyperkalemia, and ultimately death in the victims of crush injuries at the time of the German blitz of London during World War II. In addition, Bywaters et al. (2,3) were the first to establish a definite pathophysiologic relationship between crush injury, myoglobinuria, and acute tubular necrosis. Since that time, it has become evident that muscle damage from nontraumatic, (4) as well as traumatic, causes can produce the syndrome of myoglobinuric acute renal failure, thus establishing rhabdomyolysis as a common cause of acute renal failure on medical and surgical services of large hospitals.

Causes of Rhabdomyolysis and Myoglobinuria

A variety of conditions and diseases can lead to rhabdomyolysis and acute renal failure, and the list of causes is constantly being expanded with new case reports (Table 49-1). Although the list is lengthy, it can be divided into eight basic categories: (a) direct muscle injury, (b) drugs and toxins, (c) genetic disorders (decreased energy production), (d) infections, (e) excessive muscular activity, (f) ischemia, (g) electrolyte and endocrine/metabolic disturbances, and (h) immunologic diseases. The common denominator for all the causes is a disruption of normal skeletal muscle cell structure or metabolism that leads to influx of Ca^{2+} across the sarcolemmal membrane and impairment of the normal sequestration of calcium in the sarcoplasmic reticulum. Adenosine triphosphate (ATP) depletion further interferes with Ca^{2+} sequestration, leading to lethal intracellular Ca^{2+} overload. The high intracellular Ca^{2+} concentration activates neutral proteases, phospholipases, and other degradative enzymes that cause myofibril and membrane damage (5). The subsequent death and lysis of the skeletal muscle cells results in the release of intracellular contents into the circulation. In the United States, the three most common causes of rhabdomyolysis are drug abuse (with a substantial percentage related to ethanol use), muscle compression, and seizures (6,7).

Crush injuries (8–12) and prolonged compression of the limbs (13) can lead to massive rhabdomyolysis and its sequelae, including acute renal failure. Related to such injuries are hypovolemia, hypocalcemia, and shock as sodium chloride, calcium, and water flow from the extracellular compartment into the damaged muscle cells. In addition, hyperkalemia and hyperphosphatemia commonly are seen as a result of the efflux of these solutes from injured cells. Prolonged compression of a limb has been shown to increase intramuscular pressures to a point sufficient to cause muscle and capillary ischemia and necrosis by local obstruction of the circulation (13).

Drugs and toxins that have been implicated in causing rhabdomyolysis are legion (14–16). Several mechanisms have been implicated for drug- and toxin-induced rhabdomyolysis, including (a) drug-induced coma leading to compression of a limb (13); (b) excessive muscular activity [e.g., phencyclidine (17), LSD, hemlock (18)]; (c) drug-induced hyperthermia (19); (d) drug-induced vasoconstriction with muscle ischemia [e.g., cocaine (20)]; (e) impaired ATP formation (e.g., cyanide, salicylates); (f) induction of potassium or phosphorus depletion (e.g., diuretics); (g) hypersensitivity reaction resulting in myositis; and (h) a direct toxic effect on skeletal muscle cell, as has been attributed to ethanol (21). Although certain drugs such as heroin (22) or ethanol may have a direct toxic effect on skeletal muscle cells, a more important factor in causing rhabdomyolysis is the occurrence of coma after their use, which leads to muscle compression and ischemia. In addition, drug use may be associated with other conditions that predispose to rhabdomyolysis. For example, in the alcoholic patient, concomitant hypokalemia (23), hypophosphatemia (24–26), starvation (27), and ethanol-induced enzyme defects (28) may contribute to rhabdomyolysis. The presence of multiple etiologic factors may be a common scenario, as noted in a large clinical series by Gabow et al. (29) in which more than one factor capable of injuring muscles was present in 51 of 87 episodes of rhabdomyolysis.

Various hereditary enzyme deficiencies and defects have been associated with rhabdomyolysis and myoglobinuria. In patients with hereditary deficiency of myophosphorylase (McArdle's syndrome) (30), phosphofructokinase (31), and carnitine palmityl transferase (32), physical exercise can induce rhabdomyolysis. The common denominator of these disorders is decreased energy production on the part of skeletal muscle cells due to impaired substrate utilization. In the setting of increased energy demands (exercise), the inability of muscle cells to generate sufficient ATP leads to cell damage and rhabdomyolysis.

The myositis occasionally associated with infectious diseases such as influenza (33) and leptospirosis can lead to disruption of skeletal muscle cells and thus rhabdomyolysis and myoglobinuria. In addition, infections like gas gangrene produce a clostridial toxin that is directly myotoxic (34).

Excessive muscular activity has been increasingly recognized as a common and preventable cause of rhabdomyolysis (35). Strenuous and exhaustive exercise, especially in

TABLE 49-1

CAUSES OF RHABDOMYOLYSIS

Traumatic muscle injury
Crush injuries
Compression/pressure necrosis
Severe burns
Contact sports
Direct muscle trauma
Drugs and toxins (partial list)
Ethanol
Heroin
Barbiturates
Cocaine
Amphetamines
Benzodiazepines
Phencyclidine
HMG-CoA reductase inhibitors (statins)
Fibric acid derivatives (clofibrate, gemfibrozil)
Hemlock
Salicylates
Carbon monoxide
Ethylene glycol
Isopropyl alcohol
Snake and insect venoms
Succinylcholine
Colchicine
Propofol
Para-phenylenediamine
Colchicum autumnale (autumn crocus)
Monensin
Genetic disorders
Phosphorylase deficiency (McArdle's disease)
Phosphofructokinase deficiency
α-Glucosidase deficiency
Carnitine palmityltransferase deficiency
Amylo-1,6-glucosidase deficiency
Phosphohexoseisomerase deficiency
Infections (partial list)
Influenza
Tetanus
Gas gangrene
Legionnaires' disease
Shigellosis and salmonellosis
Coxsackievirus
Leptospirosis
Streptococcus
HIV
Excessive muscular activity
Vigorous exercise
Seizures/status epilepticus
Delirium tremens
Status asthmaticus
Psychotic muscle contractions
Tetany
Ischemia
Arterial occlusion
Compression
Electrolyte and endocrine/metabolic disorders
Hypokalemia
Hypophosphatemia
Hypothyroidism
Diabetic ketoacidosis
Diabetic hyperosmolar nonketotic coma
Hypothermia and hyperthermia
Immunologic disease
Polymyositis
Dermatomyositis

deconditioned men (so-called "white collar" rhabdomyolysis), can result in major morbidity from hyperkalemia, metabolic acidosis, disseminated intravascular coagulation (DIC), acute respiratory distress syndrome, and rhabdomyolysis (36). Contributing factors to this syndrome include exercising in a hot or humid environment, volume depletion, fasting, eccentric muscle contractions (running downhill), preexistent muscle injury (alcoholic myopathy), and male sex (36). Intense muscle contractions deplete energy reserves, thus disrupting normal cellular transport processes and permitting calcium to accumulate in the cell, resulting in activation of proteolytic enzymes and cell death. Based on a number of studies reviewed by Knochel (36), physical training raises the threshold and induces a degree of resistance to the development of exertional rhabdomyolysis. Training may induce this adaptation by increasing blood flow and oxygen delivery to muscles, increasing muscle glycogen content (improved fuel storage), and increasing the capacity for oxidative metabolism (improved fuel utilization). Other conditions associated with excessive muscle contractions and significant rhabdomyolysis include seizures, tetanus, and delirium tremens.

Severe potassium deficiency can lead to rhabdomyolysis, myoglobinuria, and acute renal failure. During exercise, there is an increase in blood flow to the contracting muscles that is mediated by potassium released from the contracting muscle fibers. In the setting of potassium depletion, the potassium release from contracting muscle fibers is suboptimal, leading to blunted vasodilation and thus muscle ischemia (cramps) and necrosis (23). Hypophosphatemia, especially in the setting of severe alcoholism, has been associated with muscle cell injury and rhabdomyolysis (24–26). Other metabolic conditions that have been reported to cause rhabdomyolysis include hypothyroidism (37) and both hyperthermia (19) and hypothermia (38).

Myoglobin Metabolism

Myoglobin is composed of a folded polypeptide portion (globin) and a prosthetic group, heme, which contains an atom of iron. The molecular weight of myoglobin is 17,800 daltons, which is approximately one-fourth that of the other major heme pigment, hemoglobin (39). Based on tracer studies, the half-life of myoglobin in the circulation varies from 1 to 3 hours; after 6 hours, it has completely disappeared (40). Small quantities of myoglobin (milligram amounts) released during normal conditions are probably cleared by the reticuloendothelial system (41). Because of its relatively small molecular weight and size, larger quantities of myoglobin released from muscle in states of injury or disease are readily filtered at the glomerulus and thus can be cleared by renal mechanisms.

In the human circulation, myoglobin appears to be bound to an α_2-globulin that has a binding capacity of 23 mg/dL. Because myoglobin is loosely bound to α_2-globulin, at concentrations below 23 mg/dL, approximately 15% to 50% of the myoglobin

is in an unbound state and is filtered (fractional clearance relative to inulin, 0.75) and excreted in the urine. This interesting kinetic relationship between myoglobin and its binding protein probably explains why myoglobin is detected in the urine when plasma levels are less than 23 mg/dL (39). According to Kagen (42), the effective renal threshold for myoglobin occurs when the plasma concentration exceeds 0.5 to 1.5 mg/dL. Based on a distribution volume of myoglobin of 28.5 L and a muscle myoglobin content of 4 mg/g, Knochel (43) has calculated that injury of approximately 102 g of muscle would be required to exceed a renal threshold of 1.5 mg/dL. Beyond this threshold, the factors that determine the urinary concentration and excretion rate of myoglobin include (a) the plasma concentration of myoglobin, (b) the extent of myoglobin binding in plasma, (c) glomerular filtration rate (GFR), and (d) urine flow rate.

Myoglobin is visible in plasma or urine to the unaided eye when the concentration exceeds 100 mg/dL. Because of relatively rapid renal clearance of myoglobin, visible plasma levels of myoglobin have never been reported. Knochel (43) has estimated that a visible plasma level of myoglobin would require the destruction of 7.1 kg of muscle in an anephric patient. In contrast, because myoglobin is cleared rapidly in patients with normal renal function, visible myoglobinuria is achieved with far less muscle necrosis. For example, necrosis of only 178 g of muscle, achieving a plasma myoglobin level of only 2.5 mg/dL, is sufficient to produce visible myoglobinuria in a patient with normal renal function excreting concentrated urine (43). However, reduced renal function or a high urine flow rate decreases the concentration of myoglobin in urine, diminishing the utility of a visual inspection of the urine to detect myoglobinuria for a given amount of muscle necrosis. In these situations, benzidine, guaiac, or orthotoluidine (dipstick) tests detect levels as low as 0.5 mg/100 mL. These tests, however, do not distinguish between myoglobin and hemoglobin. This can be accomplished either by immunodiffusion or spectrophotometry (39).

Although the presence of myoglobinemia or myoglobinuria is indicative of skeletal or cardiac muscle injury, it may not be the most sensitive method to detect rhabdomyolysis. Given that myoglobin has a relatively rapid renal clearance (1 to 6 hours), a patient with rhabdomyolysis may have a normal plasma level by the time he or she is hospitalized. In the series by Gabow et al. (29), 26% of the patients with documented rhabdomyolysis had a negative orthotoluidine reaction for myoglobin in the urine. In contrast, creatine kinase, an intracellular muscle enzyme, appears to be a more sensitive plasma marker for rhabdomyolysis because of its slower clearance (serum half-life, 1.5 days) (44). Thus, at initial clinical evaluation, patients with rhabdomyolysis have increased serum creatine kinase levels, whereas urine myoglobin levels may or may not be detected.

Pathophysiology of Myoglobinuric and Hemoglobinuric Acute Renal Failure

The exact pathophysiology of pigment-induced acute renal failure is unclear and probably is multifactorial. Given the biochemical similarity between myoglobin and hemoglobin, most investigators suspect that both myoglobinuric and hemoglobinuric acute renal failure share a common pathogenesis. In fact, the major animal model used to study pigment-induced oliguric acute renal failure is produced by subcutaneous or intramuscular injections of glycerol, and results in both intravascular hemolysis (hemoglobinuria) and rhabdomyolysis (myoglobinuria). Thus, the pathogeneses of myoglobinuric and hemoglobinuric acute renal failure are considered together in this discussion.

The proposed mechanisms by which myoglobinuria or hemoglobinuria causes acute renal failure include (a) hypovolemia and renal ischemia, (b) direct tubular toxicity of myoglobin/hemoglobin, (c) tubular obstruction from heme pigment casts or uric acid crystals, and (d) glomerular fibrin deposition. As in many clinical syndromes, it is probably the interplay of these proposed mechanisms that results in acute renal failure, rather any one single factor; these interactions are schematized in Figure 49-1.

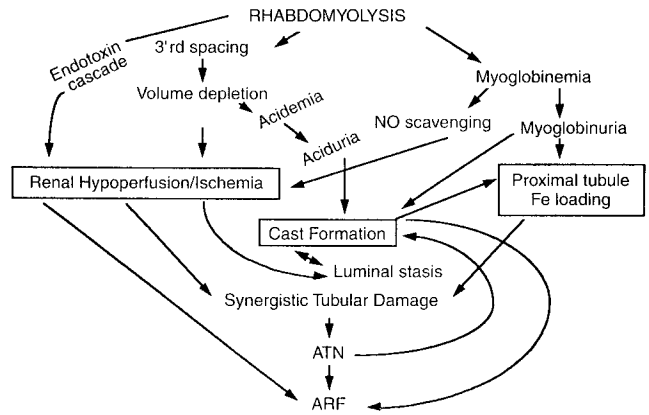

FIGURE 49-1. Pathophysiologic process of myoglobinuric and hemoglobinuric acute renal failure. (Reprinted from: Zager RA. Rhabdomyolysis and myohemoglobinuric acute renal failure. *Kidney Int* 1996;49:317, with permission.)

Hypovolemia and Renal Ischemia

During the initial phase of glycerol-induced acute renal failure, there is a marked reduction in cardiac output (36%) and renal blood flow (20%) and an increase in renal vascular resistance (45). Subcutaneous or intramuscular (but not intravenous) glycerol not only produces muscle injury but causes sequestration of fluid into the injection site (46). Thus, the hemodynamic changes are due, in part, to the migration of plasma water into the site of injury, with consequent severe intravascular volume contraction that occurs in this model of myohemoglobinuric acute renal failure. Comparable events occur in the clinical setting inasmuch as muscle injury that occurs in the crush syndrome causes a shift of fluid from the vascular space into the injured muscle, leading to intravascular volume depletion (12). Moreover, the conditions that predispose a person to rhabdomyolysis, such as drug-induced coma with accompanying poor oral intake or excessive insensible fluid losses from exhaustive exercise, contribute to intravascular volume depletion and compromise of renal function.

In the initial phases of glycerol-induced acute renal failure, the reduction in renal blood flow is associated with a redistribution of regional blood flow from the outer to the inner cortex (47) and vasoconstriction of the afferent and efferent arteriole. As reviewed by Honda (48), the proposed mediators of this initial renal vasoconstriction include (a) increased sympathetic nerve activity, (b) augmented activity of the renin–angiotensin system, (c) reduced nitric oxide production, (d) suppressed renal prostaglandin production, (e) increased plasma vasopressin concentration, and (f) glomerular microthrombi and reduced nitric oxide activity. The reduction in nitric oxide may be due to the fact that heme proteins can scavenge this important endogenous vasodilator. Marlee et al. (49) have shown that nitric oxide synthase inhibition worsens and nitric oxide supplementation protects against glycerol-induced acute renal failure, lending support to the importance of nitric oxide in the pathogenesis of myoglobin-induced acute renal failure. The exact mediators have not been established and the renal vasoconstriction may be due to the interplay among a number of vasoconstricting/vasodilating systems.

The critical role that intravascular volume depletion plays in the pathogenesis of myohemoglobinuric acute renal failure is demonstrated by studies in which volume status is manipulated in the glycerol-treated rat. In initial studies, Oken et al. (50,51) noted that renal damage was ameliorated if the rats ingested adequate quantities of water before the administration of glycerol. Similarly, Hsu et al. (45,52) found that the reduction in renal blood flow and function in response to the administration of glycerol was attenuated in rats chronically drinking saline compared with rats drinking water. The study by Reineck et al. (53) provided a better understanding of the important temporal relationship between volume expansion and improvement of renal function in the glycerol-treated rat. Like other investigators, they noted that there was a significant reduction in renal blood flow and GFR after the administration of glycerol. These variables could be restored to normal levels by volume expansion with Ringer's solution at 3 and 6 hours after administration of glycerol. They concluded that the initial decrease in GFR and low fractional excretion of sodium in this model of myohemoglobinuric acute renal failure was due to a decrease in renal blood flow (renal hypoperfusion). Volume expansion, however, was not successful in restoring GFR when given 18 hours after the administration of glycerol, suggesting that other events (e.g., tubular necrosis) had occurred that were not amenable to volume therapy. These studies in the rat support the clinical observation that, initially, patients with myoglobinuric acute renal failure have features of prerenal azotemia, including a low urinary fractional excretion of sodium (54). In addition, they provide the rationale for early use of volume expansion in patients with significant rhabdomyolysis and hemoglobinuria.

As noted above, renal ischemia as well as oxygen metabolites play an important role in the renal injury during myoglobinuric acute renal failure. Recent studies in experimental animal models of myoglobinuric-induced acute renal failure have shown that antioxidant bioflavonoids, such as polyphenols found in red wine, attenuate the severity of the renal damage (55–57). Rodrio et al. noted that red wine reduces both biochemical and morphologic renal damage caused by rhabdomyolysis in rats given Chilean red wine. This attenuation of renal damage is less impressive when the animals were treated with alcohol-free red wine suggesting a synergy between ethanol and nonalcoholic components of red wine in reducing renal damage (56).

Myoglobin and Hemoglobin Nephrotoxicity. Bywaters and co-workers expanded on their original description of the clinical syndrome of rhabdomyolysis-induced acute renal failure (1) to examine the role of myoglobin as a direct nephrotoxin. In a study published in 1942 (2), they noted that rabbits ingesting an acid diet with a urine pH below 6.0 had acute renal failure after infusion of human myoglobin, whereas rabbits ingesting a normal diet were spared from renal injury. Other investigators (58–61) have confirmed this observation that intravenous infusions of myoglobin are relatively benign, but can become highly nephrotoxic in the setting of acidemia/aciduria and volume depletion. Vetterlein et al. (62) demonstrated that infusions of myoglobin had no effect on renal blood flow in normal rats, but worsened renal blood flow in hypotensive animals. Thus, it appears that heme proteins can intensify the degree of vasoconstriction in the setting of hypovolemia. This may explain the clinical observation that the presence merely of myoglobinuria or a markedly elevated creatine kinase at the time of hospital admission had little predictive value in determining who experiences acute renal failure (29), suggesting that other conditions (i.e., volume depletion, acidemia) are required for renal injury to occur.

To address the question of why heme pigments are nephrotoxic only in certain metabolic conditions, Braun et al. (46) performed a detailed study investigating the effect of breakdown products of heme pigments on renal tubular transport. First, they noted that, 4 hours after subcutaneous glycerol administrations to rats, there was both swelling and pallor of the proximal tubule and depression of normal tubular uptake of hippurate and tetraethylammonium. Then, the investigators measured the uptake of hippurate in renal cortical slices incubated with various specific heme proteins or their derivatives. Incubation with hemoglobin did not depress uptake if the pH of the medium was kept at 7.4. However, uptake was depressed when the pH was lowered to 5.4 or during hypoxic conditions. In an acidic medium (pH <5.6), both myoglobin and hemoglobin dissociate into ferrihemate (hematin; molecular weight, 670 daltons) and their respective globin moieties (63). Incubation with ferrihemate, regardless of the pH of the medium, depressed the uptake of hippurate in the renal cortical slices, whereas incubation with either globin or albumin alone had no significant effect on transport. The inhibitory action of ferrihemate on hippurate transport could be mitigated if the incubation medium also contained albumin, which presumably bound the ferrihemate. Intravenous injection of ferrihemate has been shown to cause glomerular and tubular damage in the dog (64). Therefore, it has been proposed that after filtration by the glomerulus, myoglobin or hemoglobin is converted to ferrihemate in the presence of an acid tubular fluid, or after exposure to the acid pH of cellular lysosomes, and it is this metabolite that is directly nephrotoxic.

These early studies implicated the heme moiety as the nephrotoxic factor in myoglobinuric and hemoglobinuric acute renal failure. More recent studies have suggested that the iron component of heme may be the specific culprit in causing renal injury. Iron can promote hydroxyl free radical formation by the Fenton/Haber/Weiss reactions (65), leading to lipid peroxidation and cell necrosis. Both myoglobin (66) and hemoglobin (67) can act as Fenton reagents. Shah and Walker (68) and Paller (69) have proposed that the iron in these heme pigments could cause renal injury *in vivo* through the damaging effects of free radicals. Administration of hydroxyl free radical scavengers such as dimethylthiourea and sodium benzoate attenuated the renal injury in glycerol-treated rats (68). In addition, both laboratories demonstrated that the iron chelator, deferoxamine, ameliorated both myoglobinuric and hemoglobinuric acute renal failure and lipid peroxidation. Zager (70) has also shown that deferoxamine attenuates renal damage in the glycerol-induced model of acute renal failure, but concluded that iron toxicity is mediated by factors other than free radical generation. For example, it has been suggested that heme protein endocytosis in the proximal tubule sensitizes the tubular cell membranes to the damaging effects of phospholipase A_2 (71). In addition, heme proteins appear to deplete cellular ATP stores and thus have an adverse effect on cellular energetics (5). A more recent study suggest that the iron toxicity may be due to a redox cycling of the heme moiety from ferrous to ferric and to ferryl oxidation states (72).

The kidney may have its own system to contend with exposure to iron-containing heme pigments. Normally, iron is released from heme by the rate-limiting enzyme heme oxygenase, which acts by opening up the heme ring, generating iron and biliverdin. In turn, the liberated iron molecule can be taken up by ferritin, the major cellular repository for iron. Nath et al. (7) have demonstrated that the renal production of both heme oxidase and ferritin is increased in the glycerol-induced model of myohemoglobinuric acute renal failure. The increase in heme oxygenase and ferritin production is proposed to be an adaptive response on the part of the kidney on exposure to heme pigments and is a mechanism by which the kidney normally degrades heme and sequesters the potentially nephrotoxic iron. They noted that prior stimulation of the activity of heme oxygenase coupled with increased ferritin synthesis attenuated

renal damage, whereas inhibition of the enzyme worsened renal function. This increased activity of heme oxygenase or possibly a broad-based proximal tubular cytoresistance (73) in the kidney may explain the experimental observation that, after induction of myohemoglobinuric acute renal failure, rechallenging the animals with a second dose of glycerol does not result in acute renal failure (74). One speculation is that in the setting of clinical myoglobin-induced acute renal failure, there may be factors contributing to inhibition of heme oxygenase and ferritin synthesis or a diminution in proximal tubular resistance, resulting in both accumulation of nephrotoxic iron and tubular necrosis.

Tubular Obstruction. Filling of the tubular lumen by pigmented casts that become inspissated and obstruct urinary flow with subsequent injury to tubular epithelium is one of the earliest mechanisms proposed to explain the nephrotoxicity of the heme pigments (75). In their original clinical description of rhabdomyolysis-induced acute renal failure, Bywaters and Beall (1) described the prominent histologic features, including the appearance of tubular obstruction by cellular debris and pigmented casts. It has been suggested that hypovolemia and acidemia and the concomitant acidic concentrated urine facilitate precipitation of filtered myoglobin or hemoglobin, leading to obstructive cast formation (76). The presence of Tamm-Horsfall protein in the tubular lumen is critical for heme protein cast formation in the distal nephron. Moreover, an obstructing cast induces urinary stasis, providing for an extended time for proximal tubular heme reabsorption and its attendant tubular toxicity, as noted previously (77). In addition, it has been speculated (43) that the increased urinary uric acid concentration observed in rhabdomyolysis (especially that caused by exertion) may result in uric acid precipitation in the tubules.

Tubular obstruction can decrease GFR either by increasing the tubular pressure and thus decreasing the glomerular transcapillary hydraulic pressure, or by inducing the release of factors (e.g., thromboxane) that cause renal vasoconstriction, thereby reducing glomerular blood flow. The importance of tubular obstruction as a possible mechanism of heme pigment–induced acute renal failure is suggested by the studies of Zager and colleagues (70,78) that explored the reasons why mannitol exerts a protective effect against this syndrome. They evaluated three hypotheses: (a) mannitol attenuates renal hypoperfusion, (b) mannitol scavenges hydroxyl free radicals, and (c) mannitol prevents intrarenal heme trapping and cast formation. The major beneficial effect of mannitol was attributed to its diuretic effect, which presumably decreased cast formation and proximal tubular uptake of heme proteins. Similarly, alkalinization of the urine may mitigate against myoglobinuric acute renal failure by increasing the solubility of myoglobin (reduced cast formation) and inducing a solute diuresis (76).

Although there is evidence that tubular obstruction may be a factor in the pathogenesis of the acute renal failure, it probably is not the primary cause of the initial decrease in GFR in myohemoglobinuric acute renal failure. Rather than high intratubular pressures from obstructing casts, intratubular pressures were found to be low in the glycerol-induced model of acute renal failure (50). This observation was interpreted to indicate that the presence of casts is the result, rather than the cause, of the decrease in GFR and urine flow. Instead of causing the initial decrease in renal function, cast formation may play a role in the maintenance of the renal failure once it develops (79).

Glomerular Fibrin Deposition. Because of the liberation of tissue factors, both rhabdomyolysis and intravascular hemolysis can initiate DIC (43). Fibrin strands have been demonstrated in glomeruli from patients (80) and experimental animals (81) with rhabdomyolysis-induced acute renal failure. Intravenous infusion of a muscle extract in rabbits resulted in DIC, renal dysfunction, and glomerular microthrombi, whereas an intravenous infusion of pure myoglobin had no untoward effect (82). This led to the conclusion that myoglobin, *per se,* is not the cause of the renal damage in the crush syndrome, but rather it is the release of other muscle constituents that induces DIC and the subsequent deposition of glomerular microthrombi that is responsible for rhabdomyolysis-induced acute renal failure.

Clinical and Laboratory Features of Rhabdomyolysis and Acute Renal Failure

The diagnosis of myoglobinuria can be suspected from history and physical examination but must be confirmed by laboratory testing. The diagnosis should be entertained in any patient with obvious muscle damage from trauma or ischemia in whom the involved region, usually an extremity, shows reddened overlying skin with local swelling and induration. In patients with rhabdomyolysis resulting from a drug overdose, these findings also are common. Less obvious is muscle involvement in cases of diffuse rhabdomyolysis, as from exertion, hypophosphatemia, or viral infections. In such patients, mild, diffuse muscle tenderness, or no tenderness at all, may mask the rhabdomyolysis and lead the examiner to dismiss the symptoms as resulting from viral myalgias (4). In one series (29), 50% of patients with rhabdomyolysis did not admit to the presence of muscle pain and only 5% had obvious muscle swelling on admission, thus emphasizing the importance of continued clinical inquiry, observation, and laboratory follow-up.

Risk Factors for Acute Renal Failure. The frequency of acute renal failure in the setting of rhabdomyolysis is unknown, and reports of frequency have ranged from 5% (4,83) to 33% (29). Gabow and colleagues (29) emphasized that no single laboratory value could predict which patients are at high risk for the development of acute renal failure. However, using discriminant analysis, patients could be separated into high- and low-risk groups, with the high-risk group (elevated serum potassium and creatinine and reduced serum albumin concentrations) having a 41% prevalence of acute renal failure.

Based on a large historical cohort (157 patients), Ward (84) identified clinical and laboratory differences between those patients in whom renal failure did or did not develop, and factors predictive of progression to renal failure. As shown in Table 49-2, patients with rhabdomyolysis and renal failure were older, had a higher incidence of hypertension, and were more hypotensive and dehydrated. A significantly greater proportion of them had a creatine kinase level greater than 16,000 IU/L, although as noted in the study, elevations to this degree were seen in 10.7% of patients in whom renal failure did not develop (Table 49-3). The renal failure group also had significantly higher serum potassium and phosphorus levels and lower serum calcium and albumin concentrations, and was more acidemic with a concomitant lower urinary pH. Sepsis, burns, and drug ingestion were the causes of rhabdomyolysis more closely associated with the development of renal failure. Using multiple logistic regression analysis, a scoring system was developed predicting the risk of renal failure in patients with rhabdomyolysis based on the variables of serum phosphorus, potassium, albumin, and creatine kinase concentrations, and the presence of dehydration and sepsis. A point score of 7 or greater predicted a greater than 50% likelihood for development of renal failure. In a more recent multivariate analysis of 72 consecutive patients with rhabdomyolysis due to illicit drug use, patients with a creatine kinase greater than 25,000 U/L, hypotension, and leukocytosis were at a greater risk of developing acute renal failure, whereas hyperthermia (temperature $>38.5^{\circ}$C) was associated with a reduced risk (85).

TABLE 49-2

UNIVARIATE ANALYSIS OF CLINICAL VARIABLES IN PATIENTS WITH RHABDOMYOLYSIS DEVELOPING AND NOT DEVELOPING RENAL FAILURE

Variables	Group: Renal failure ($N = 26$)	Group: Nonrenal failure ($N = 131$)	p^a
Age, yr (SD)	53.7 ± 20.6	41.4 ± 18.1	0.002
Male (%)	69.2	61.1	0.418
Hypertension (%)	46.2	22.9	0.026
Diabetes mellitus (%)	11.5	7.6	0.562
Previous renal disease (%)	19.2	3.8	0.051
Dehydration (%)	38.5	4.6	0.001
Hypotension (%)	34.6	14.5	0.040
Nephrotoxin exposure (%)	19.2	39.7	0.020
Diuretic use (%)	30.8	16.8	0.147
Nonsteroid drug use (%)	19.2	6.1	0.101
IV hydration (%)[b]	80.7	54.2	0.289
Osmotic treatment (%)	26.9	22.9	0.674
Bicarbonate treatment (%)	50.0	12.2	0.001

[a] p value for difference in means or proportions between renal failure and nonrenal failure groups.
[b] >150 mL/hr averaged over the first 24 hr after admission. IV indicates intravenous.

Urinalysis. Examination of the urine provides the first laboratory clue to the presence of myoglobinuria. Classically, the initial urine is dark (Table 49-4) and usually with an acid pH; the benzidine or orthotoluidine reagent gives a positive reaction for blood, (3+ to 4+), but microscopic examination of the urinary sediment fails to reveal any red blood cells (RBCs) or, at best, only a few (less than five per high-power field), indicating the presence of a heme pigment not contained in RBCs. This could result from hemoglobin released from RBCs lysed in the urine, but more likely reflects myoglobin excreted by glomerular filtration. Specific tests for urine myoglobin determination are available in some clinical laboratories but, as noted earlier, urine myoglobin levels are not the most sensitive clinical markers for rhabdomyolysis. Although the strongest clinical clue for myoglobinuria is the presence of a strongly heme-positive urine and the absence of RBCs, in one major series (29), hematuria was present in 32%, and the dipstick was heme negative in 18% of the patients with rhabdomyolysis. In addition, proteinuria was detected by dipstick in 45% of patients (29), which may be attributed to altered glomerular permeability or tubular transport of small proteins (86). The urinary sediment demonstrates brown "debris" and, with the evolution of renal injury, pigmented brown granular casts and renal tubular epithelial cells are seen with great frequency.

Serum Potassium Concentration. The most life-threatening consequence of rhabdomyolysis is the release of large amounts of intracellular potassium into the circulation. Because more than 98% of total body potassium resides in cells, and skeletal muscle represents 60% to 70% of the total cellular mass, breakdown of even a small area of skeletal muscle releases a considerable potassium load. The presence of acidosis may shift more potassium extracellularly and worsen the hyperkalemia. As noted in the section on Risk Factors for Acute Renal Failure, above, admission serum potassium levels tend to be higher in the patients who go on to experience acute renal failure (84). Approximately half of an acute potassium load is handled by renal excretion (87,88); therefore, in acute renal failure, serious

TABLE 49-3

UNIVARIATE ANALYSIS OF LABORATORY VARIABLES IN PATIENTS WITH RHABDOMYOLYSIS DEVELOPING AND NOT DEVELOPING RENAL FAILURE

Variables	Group: Renal failure ($N = 20$)	Group: Nonrenal failure ($N = 131$)	p
Peak creatine kinase >16,000 U/L, %	57.7	10.7	<0.001
Serum bicarbonate (mmol/L)	21.4 ± 7.2	23.7 ± 4.0	0.1306
Serum potassium (mmol/L)	4.73 ± 1.2	3.92 ± 0.6	0.0018
Serum phosphorus (mmol/L)	1.85 ± 1.08	0.06 ± 0.35	0.0006
Serum calcium (mmol/L)	2.02 ± 0.4	2.14 ± 0.2	0.1452
Serum albumin (g/L)	30.8 ± 10.0	35.9 ± 8.0	0.0107
Arterial pH	7.33 ± 0.10	7.38 ± 0.11	0.0495
Urinary pH	5.19 ± 0.06	5.75 ± 1.0	0.0009

All values are mean SD except peak creatine kinase.

TABLE 49-4

DIFFERENTIAL DIAGNOSIS OF PIGMENTURIA

Factors	Myoglobinuria	Hemoglobinuria	Porphyria
Urine color	Brown	Reddish-brown	Dark red
Serum color	Clear	Pink	Clear
Orthotoluidine reaction	Positive	Positive	Negative
Watson-Schwartz porphobilinogen	Negative	Negative	Positive
Muscle pain/tenderness	Present	Absent	Absent
Serum creatine kinase level	Elevated	Normal	Normal
Serum haptoglobin	Normal	Decreased	Normal

(Reprinted from: Schultze VE. Rhabdomyolysis as a cause of acute renal failure. *Postgrad Med* 1982;72:145, with permission.)

hyperkalemia can result and is usually the major indication for dialysis.

Creatine Kinase. The relatively slower clearance of creatine kinase compared with myoglobin makes this enzyme level a more sensitive marker of muscle injury (44). Although no correlation has been established between the absolute level of the creatine kinase and the risk for development of acute renal failure, creatine kinase levels are significantly higher in the patients in whom renal failure develops (89). It has been stated anecdotally that it is unusual to see acute renal failure develop in patients with serum creatine kinase levels less than 3,000 IU/L (90,91). Following serial serum creatine kinase levels is crucial to monitoring patients with rhabdomyolysis, inasmuch as the concentration of creatine kinase may continue to increase after admission to the hospital, reflecting ongoing or worsening muscle necrosis. Increasing or decreasing creatine kinase concentrations provide some insight into whether the rhabdomyolysis is worsening or resolving, and following these levels is essential to observe for the "second wave" phenomenon (see later).

Acid–Base Balance. The conditions that cause rhabdomyolysis involve tissue trauma or ischemia and predispose the patient to an augmented acid load. In the study by Ward (84), the patients with rhabdomyolysis who progressed to renal failure tended to be significantly more acidemic. An elevated serum anion gap is usual in patients with rhabdomyolysis and is due to impaired renal excretion of intracellular organic acids released from damaged muscles (92), as well as retention of inorganic anions such as phosphate.

Uric Acid. Hyperuricemia is expected in patients with rhabdomyolysis, especially when the muscle injury is due to strenuous exercise or exertion. The increase in uric acid levels is due to the release of intracellular purines from damaged muscle cells, which are converted to uric acid in the liver (43).

Blood Urea Nitrogen: Creatinine Ratio. The rate of rise of serum creatinine relative to the rise in blood urea nitrogen (BUN) is often disproportionately greater in rhabdomyolysis-induced acute renal failure compared with other causes of acute renal failure. This phenomenon has been attributed to the release of large quantities of creatine from damaged muscles and the subsequent conversion of the creatine to creatinine, resulting in a more rapid increase in the serum creatinine concentration (4,48). Based on creatine:creatinine kinetics and their respective concentrations in skeletal muscle, Oh (93) has challenged this conventional view. He has pointed out that the patient population in which rhabdomyolysis develops tends to have a larger percentage of younger men with a greater muscle mass, whereas other forms of acute renal failure are more often associated with older and more cachectic patients who have less muscle mass and thus reduced creatinine production rates.

Calcium–Phosphorus Metabolism. The perturbations of calcium and phosphorus metabolism usually seen in most types of acute renal failure appear to be exaggerated in rhabdomyolysis-induced acute renal failure (94). Muscle damage leads to breakdown of intracellular phosphate compounds and release of large quantities of inorganic phosphorus into the circulation, resulting in hyperphosphatemia. This abnormality is accentuated when acute renal failure develops owing to impaired urinary excretion. Hypocalcemia also occurs early in the course of myoglobin-induced acute renal failure (29); its pathogenesis has been attributed to a number of mechanisms. Early observations by Meroney et al. (95) suggested that the hypocalcemia may be due to the deposition of calcium salts in the damaged muscles. Using electron radiography and technetium-99m diphosphonate scans, Akmal et al. (96) provided evidence of deposits of calcium in the damaged muscles of patients during the oliguric phase of myoglobinuric acute renal failure. When renal function improved, the calcium deposits disappeared. They concluded that, initially, the damaged muscles serve as a nidus for calcium deposition (dystrophic calcification), which is accentuated by the concomitant hyperphosphatemia and elevated calcium–phosphorus product. Subsequently, during the recovery phase of acute renal failure as GFR increases, the concentration of phosphorus and resultant calcium–phosphorus product decrease. This permits the release of the sequestered calcium deposits back into the circulation, leading to an increased serum calcium concentration, sometimes to the point of hypercalcemia.

More recently, it has been suggested that the hypocalcemia may be due to abnormalities of vitamin D and parathyroid hormone metabolism. Very low levels of 1,25-dihydroxycholecalciferol ($1,25[OH]_2D_3$) and high levels of parathyroid hormone have been noted during the oliguric phase of myoglobinuric acute renal failure (97,98). This may be due, in part, to the hyperphosphatemia associated with rhabdomyolysis, inasmuch as hyperphosphatemia has been demonstrated to reduce the renal synthesis of $1,25(OH)_2D_3$ and stimulate the production of parathyroid hormone (99). Thus, the hypocalcemia of myoglobin-induced acute renal failure may be due both to a deficiency of $1,25(OH)_2D_3$ and to concomitant skeletal resistance to parathyroid hormone (97). Regardless of the mechanism, in the absence of frank tetany, hypocalcemia

usually does not require treatment. In fact, correction of the hypocalcemia with vigorous intravenous calcium replacement may increase both dystrophic (calcium deposition in the damaged muscle) and metastatic calcification.

Approximately 20% to 30% of patients with myoglobinuric acute renal failure experience transient hypercalcemia during the recovery (diuretic) phase of their acute tubular necrosis (4,83). Early studies (4,83,96) suggested that the hypercalcemia was due to an augmentation of the normal remobilization of the calcium deposits in the injured muscle that occurs during the recovery phase of acute renal failure. Alternatively, it has been proposed that as renal function improves, the combination of a decreasing serum phosphorus concentration and the ambient secondary hyperparathyroidism stimulates the synthesis of $1,25(OH)_2D_3$, resulting in an "overshoot" hypercalcemia (97,100,101). This augmented $1,25(OH)_2D_3$ production may be due, in part, to release of vitamin D from damaged muscle tissue (102).

Urinary Sodium Excretion. Impaired renal tubular reabsorption of sodium is typically seen in most types of oliguric acute renal failure as manifested by a high fractional excretion of sodium. However, in both myoglobinuric and hemoglobinuric acute renal failure, a low fractional excretion of sodium (<1%) has been observed (54). As noted earlier in this chapter, hypovolemia and renal ischemia are important factors in the development of renal failure in heme pigment–induced acute renal failure, and thus the increased sodium avidity may be due to renal hypoperfusion. In addition to this mechanism, it is known that tubular obstruction and urinary tract obstruction are associated transiently with a low fractional excretion of sodium; therefore, this also may be a factor contributing to the augmented sodium reabsorption (103,104). A low fractional excretion of sodium is not seen in all cases of myoglobinuric and hemoglobinuric acute renal failure, however, and the presence of a low fractional excretion of sodium does not indicate less severe renal injury.

Disseminated Intravascular Coagulation. Disseminated intravascular coagulation is commonly present in patients with rhabdomyolysis and may be due to the release of intracellular thromboplastins that activate the clotting cascade (43). Moreover, DIC may be an important factor in the pathogenesis of the acute renal failure (see section on Glomerular Fibrin Deposition, above).

Differential Diagnosis

Myoglobin-induced acute renal failure should be suspected in patients subjected to trauma presenting with the classic triad of heme-positive urine, an elevated serum creatine kinase level, and dark (pigmented) urine containing dirty-brown granular casts. More subtle cases, usually associated with diffuse, nontraumatic rhabdomyolysis, may be more difficult to detect. The differential diagnosis of pigmenturia is limited (Table 49-4). Although certain drugs may impart an orange, red, or brown hue to the urine, they do not react with the benzidine or orthotoluidine reagent on the urine dipstick. Among such agents are rifampin, phenazopyridine (Pyridium), nitrofurantoin, and some sulfa compounds. Porphyrins also color the urine brown but do not react to give a positive test for occult blood. The most difficult challenge is to discriminate myoglobin from hemoglobin in the urine. Because these are heme compounds, they both react with the benzidine or orthotoluidine reagent and both are associated with the absence of RBCs in the urine sediment. One helpful clue may be the color of the serum in these two conditions. Because myoglobin is relatively rapidly cleared by the kidney, serum levels of myoglobin are not sufficiently elevated to alter the color of the serum in patients with rhabdomyolysis. In contrast, because of its much larger size and its avid binding to haptoglobin, hemoglobin is not as rapidly cleared by the kidney, and serum levels may be high enough to result in a pink discoloration of the serum in patients with hemoglobinuria.

Clinical Course and Complications of Myoglobinuric Acute Renal Failure

Myoglobinuric acute renal failure can run a course ranging from mild renal dysfunction with only transient oliguria and quick recovery to a much more catastrophic disease requiring frequent dialysis for periods of 2 or 3 weeks. Typically, the duration of oliguria is 7 to 10 days; during this interval, patients may excrete virtually no urine for up to 3 days at a time. Resumption of more normal urine formation heralds the recovery of renal function as patients enter the diuretic phase, with subsequent clearing of azotemia and a cessation of the requirement for hemodialysis.

In addition to muscle injury and acute renal failure, patients with rhabdomyolysis may have peripheral neuropathies. These can result from compartment syndromes in which involved muscle becomes edematous in confined tissue spaces with compromise of blood supply to both muscle and nerves in the area (96). Measurement of tissue pressure has been advocated as a tool in identifying those areas of damaged muscle at risk; surgical fasciotomy may be required to avoid this complication (13). The swelling of the muscles can lead to an impairment in the blood supply of the muscles, resulting in a recurrence or "second wave" of muscle necrosis as reflected by a second rise in the serum creatine kinase concentration (43). Neuropathy also can result from traction if rhabdomyolysis is caused by prolonged coma, as from drug overdose (13).

Prevention and Treatment of Myoglobinuric Acute Renal Failure

In addition to their landmark description of the medical consequences of the "crush syndrome" and implication of myoglobin as a nephrotoxin, Bywaters and colleagues in 1941 (1) were one of earliest groups to recognize the benefits of brisk alkaline fluid resuscitation in victims of crush injuries to mitigate the development of acute renal failure. They demonstrated that prompt and early oral and intravenous administration of fluids containing sodium salts of bicarbonate, lactate, and citrate dramatically reduced mortality rates and the development of renal injury in victims of the blitz of London (6). The rationale for this therapy was based on their notion that both an augmentation of urinary flow and an increase in the urine pH would facilitate the clearance and minimize the nephrotoxicity of myoglobin. As is discussed later, the current recommended therapy to prevent acute renal failure in the setting of rhabdomyolysis has not changed appreciably since Bywaters and colleagues' original recommendations.

Understanding the possible mechanisms by which rhabdomyolysis causes acute renal failure can provide the basis for the various therapies that have been advocated for this disorder. Because hypovolemia and renal ischemia are important factors in the pathogenesis of myoglobinuric acute renal failure, it has long been recognized that early and vigorous intravenous fluid therapy is important in attenuating renal injury. Based on the notion that myoglobin is more nephrotoxic at an acid pH (increased formation of ferrihemate; see earlier), most groups advocate the addition of sodium bicarbonate to the intravenous fluids for alkalinization of the urine (8–11,91). By correcting cellular acidosis, bicarbonate therapy may reduce renal tubular epithelial swelling and attenuate renal tubular and vascular collapse (105). Further, this therapy may ameliorate the hyperkalemia commonly seen in rhabdomyolysis. There is a theoretical concern that inducing a metabolic alkalosis with

such treatment may enhance metastatic calcification, but the salutary benefit of bicarbonate therapy probably outweighs any untoward effect (9).

Mannitol has long been recognized to be an effective agent in the prophylaxis against the development of experimental and clinical acute renal failure, and has been used in combination with fluid/alkaline therapy to prevent renal injury in patients with rhabdomyolysis. Potential beneficial effects of mannitol include (a) a decrease in blood viscosity and oncotic pressure across the glomerulus, causing an increase in GFR; (b) dilatation of glomerular capillaries and stimulation of prostaglandin release; (c) increase in urine flow and prevention of obstructing cast formation; (d) reduction in renal tubular epithelial swelling and injury; and (e) scavenging of oxygen free radicals (8). In addition, mannitol may have extrarenal benefits, including (a) extracellular volume expansion, (b) increased cardiac contractility, (c) increased release of atrial natriuretic factor, and (d) reduction in skeletal muscle cell edema and decompression of muscle tamponade (8,106).

Furosemide, a loop-acting diuretic, has the theoretic advantage of inhibiting sodium transport in the thick ascending limb of Henle's loop. Oxygen consumption is dictated primarily by the rate of sodium transport, and a precarious balance exists in this segment between the rate of oxygen delivery and its consumption (107). By inhibiting sodium transport, furosemide may reduce oxygen consumption in the face of limited delivery and thereby preserve cell viability. In addition, the augmented urinary flow induced by the diuretic may reduce the risk of tubular obstruction. Loop diuretics, however, have the theoretic disadvantage of increasing acidification of the urine, worsening intravascular volume depletion, and inducing ototoxicity, and thus the use of these agents has not been generally recommended (8,9,90).

Plasma exchange therapy (108) and continuous hemodiafiltration (109) have been performed in patients with rhabdomyolysis for the purpose of removing myoglobin to prevent renal failure. These studies are anecdotal and have not shown a demonstrable benefit.

Although there are no controlled trials to show a direct benefit of a forced alkaline–mannitol diuresis in the prevention of acute renal failure in rhabdomyolysis, there are many case reports suggesting such therapy was instrumental in averting renal injury (11,91,110). Adequate fluid hydration and bicarbonate therapy, however, did not ameliorate the development of renal failure in a large retrospective study (84). Moreover, in a recent retrospective evaluation of 382 ICU trauma admissions with a creatine kinase of >5,000 U/L, the use of bicarbonate and mannitol in 40% of this group had no effect on rates of renal failure, need for dialysis, and mortality, although there was a trend to lower mortality rate in patients with creatine kinase greater than 30,000 I/U treated with bicarbonate and mannitol (111). The Divisions of Nephrology at San Francisco General Hospital and at the University of California, Davis Medical Center recommend the infusion of both mannitol and sodium bicarbonate in most cases of myoglobinuria. Initially, optimization of intravascular fluid volume deficits should be carried out with dispatch using isotonic crystalloid solutions, usually normal saline. Variables useful in following this course of therapy include physical examination of the state of the circulation, hematocrit, and recording of external fluid balance. If the clinical assessment suggests that a euvolemic state has been achieved but no improvement in oliguria has occurred, the decision must be made about further intervention. Usually by this time, laboratory results offer further support for the diagnosis of myoglobinuria and acute renal insufficiency, and we recommend the prompt infusion of a mannitol–bicarbonate solution. This is made by adding two ampules, each containing 12.5 g mannitol in 50 mL, and two ampules of 50 mEq $NaHCO_3$ in 50 mL to 800 mL of 5% dextrose in water for intravenous infusion. This reconstituted liter is roughly isosmotic with plasma once the glucose is metabolized and contains both mannitol and 100 mEq $NaHCO_3$. It should be infused at 250 mL/hour; urine flow rate should increase by the end of the 4-hour infusion if the treatment is successful. If this is the case, the solution should continue to be administered at a rate equal to urine output and sufficient to achieve a urine pH greater than 6.5 until such time as azotemia has started to clear and all evidence of myoglobinuria has disappeared. If urine flow does not increase after the 4-hour infusion, the patient has entered the established phase of oliguric acute renal failure and should be treated conservatively until dialysis can be arranged, based on conventional criteria. This approach corrected oliguria, hastened the clearing of azotemia, and avoided the need for dialysis in roughly half of patients with myoglobinuric acute renal failure (91). As a group, these patients had somewhat lower indices of muscle damage and somewhat better preservation of renal function than the half that did not respond. Whether this reflects earlier intervention or a less severe degree of muscle injury, or both, is not known, and it also is possible that vigorous volume expansion with normal saline alone might have caused the same result in some patients. Given that complications from the mannitol–bicarbonate infusion are few, even in those patients who do not respond, its use should be seriously entertained in patients with myoglobin-induced acute renal failure.

When acute renal failure has become established, dialysis must be used. Early and intensive hemodialysis may be associated with significantly lower morbidity and mortality rates (112). Experience with peritoneal dialysis indicated that solute clearance using this modality was inadequate to keep pace with the rapid rate of solute appearance in these highly catabolic patients (113) and, thus, hemodialysis should be the modality of treatment. Even so, daily hemodialysis often is required for the first several days until the consequences of extensive muscle injury have abated and rates of urea and potassium accumulation have fallen. Thereafter, a schedule of thrice-weekly dialysis usually is adequate unless other factors, such as continued catabolism from infection or surgical wound debridement, or volume overload from parenteral nutritional therapy demand more frequent treatments. The prognosis for the renal failure is good, and full recovery of function is the rule. However, the ultimate prognosis for the patient probably depends more on other coexisting conditions such as sepsis, bleeding, and respiratory failure.

Hemoglobinuric Acute Renal Failure

Compared with the frequency of myoglobinuric acute renal failure, hemoglobinuric acute renal failure is an uncommon event. This is because the most common cause of hemoglobinuric acute renal failure is intravascular hemolysis from mismatched blood transfusions, and with modern blood banking techniques, such untoward events are rare. An additional point to emphasize in this regard is that significant hemoglobinuria and renal failure are seen primarily in the setting of major intravascular hemolysis. During most types of extravascular hemolysis, the released hemoglobin is quickly taken up by the reticuloendothelial system and metabolized to bilirubin. Thus, extravascular hemolysis rarely results in acute renal failure.

Causes of Hemoglobinuria

Hemoglobinuria results from filtration of free hemoglobin in plasma, due almost exclusively to intravascular hemolysis, which occurs in a variety of conditions (Table 49-5). Although each of the listed causes may be associated with acute

TABLE 49-5

CAUSES OF HEMOGLOBINURIA AND ACUTE RENAL FAILURE

Genetic defects
Glucose-6-phosphate dehydrogenase deficiency
Paroxysmal cold hemoglobinuria
March hemoglobinuria
Infection
Malaria
Clostridia
Transfusion reactions
Chemical agents
Arsine
Glycerol
Quinine sulfate
Analine
Benzene
Hydralazine
Fava beans
Cresol
Sodium chlorate
Methyl chloride
Coal tar products
Venoms
Rattlesnake, copperhead, water moccasin, coral snake
Tarantula
Brown recluse spider
Traumatic/mechanical destruction
Prosthetic valves
Disseminated intravascular coagulation
Extracorporeal circulation
Miscellaneous
Heat stroke
White phosphorus
Hemoglobin infusions

(Reprinted from: Dubrow A, Flamenbaum W. Acute renal failure associated with myoglobinuria and hemoglobinuria. In: Brenner BM, Lazarus JM, eds. *Acute renal failure,* 2nd ed. New York: Churchill Livingstone, 1988:285, with permission.)

renal dysfunction, hemoglobinuria is more likely to occur in only a few settings. These are hemolytic transfusion reactions (114–116), DIC (117), march hemoglobinuria (118), and infections with clostridia (119,120) and *Plasmodium falciparum* malaria, the latter causing blackwater fever (121–124). In addition, infusion of hemoglobin solutions as a plasma volume expander has been noted to cause acute renal dysfunction owing to associated hemoglobinuria (125).

Hemoglobin Physiology and Metabolism

Hemoglobin has a molecular weight of 68,000 daltons and is a tetramer of two α and two β globin chains surrounding a ferrihemate core. As noted earlier, free hemoglobin in plasma is tightly bound to haptoglobin, and the hemoglobin–haptoglobin complex is too large to be filtered by the glomerulus. Thus, free hemoglobin appears in the urine only after the plasma concentration of hemoglobin exceeds the maximum binding capacity of haptoglobin, which is approximately 100 mg/dL (in contrast to 1.5 mg/dL for myoglobin). In the setting of intravascular hemolysis, the relatively low renal clearance of hemoglobin (fractional clearance relative to inulin, 0.03) results in an increase in plasma hemoglobin levels sufficient to be visible to the naked eye as pink-colored plasma, whereas with rhabdomyolysis, the rapid renal clearance of myoglobin (fractional clearance, 0.75) prevents myoglobin retention in the plasma and the plasma color is not visibly altered. The color of the plasma is an important "bedside" clue that helps to distinguish between these two forms of pigmenturia.

Clinical and Laboratory Features of Hemoglobinuric Acute Renal Failure

As with myoglobin, the mechanisms by which hemoglobin causes acute renal failure are not clearly understood. Because both are heme-containing compounds and the heme moiety has been implicated as a major factor in inducing renal injury, it is generally accepted that the mechanisms by which they both cause nephrotoxicity are similar, if not the same. As with myoglobinuric acute renal failure, clinical experience indicates that coexistent compromise of renal function from volume depletion, acidosis, or hypotension must be present for hemoglobinuric acute renal failure to develop. Moreover, in the clinical settings most commonly associated with it, other pathogenetic mechanisms have been proposed to account for the acute renal failure. For example, with hemolytic transfusion reactions, the interaction of antigens on the red cell stroma with preformed antibodies may be responsible for adverse effects on kidney function (116). In DIC, afferent arteriole and glomerular capillary fibrin deposition are the events most directly related to acute renal failure (117). March hemoglobinuria occurs from traumatic hemolysis of RBCs, most likely in people with a genetic susceptibility (118); acute renal failure in this setting results from volume depletion as well as hemoglobinuria. In blackwater fever, hemolysis is caused by the abrupt release of *falciparum* trophozoites and perhaps also from the quinine used to treat it (121–124). These patients are dehydrated and volume depleted from sweating and high fevers. Clostridial sepsis also has multiple effects on renal function, including hypotension, acidosis, and DIC, as well as hemolysis (119,120).

Laboratory features of intravascular hemolysis and hemoglobinuria include (a) increased serum lactate dehydrogenase (LDH) levels, (b) low serum haptoglobin levels, (c) increased unconjugated (indirect) serum bilirubin, (d) increased reticulocyte count, and (e) hyperkalemia. As with myoglobinuria, hemoglobinuria and hemoglobinuric acute renal failure are associated with pigmented urinary casts. The differential diagnosis and clinical course of hemoglobin-induced acute renal failure are similar to those described for myoglobinuria.

Prevention and Treatment of Hemoglobinuric Acute Renal Failure

Prevention of hemoglobinuric acute renal failure involves many of the same preventative measures for myoglobinuric acute renal failure, such as correcting volume depletion and the administration of mannitol and bicarbonate. In fact, Bywaters' therapy to treat crush injuries using saline and bicarbonate in the 1940s was based on earlier reports demonstrating such therapy was beneficial in preventing renal failure in mismatched blood transfusion reactions (6). In an experimental animal model of hemoglobinuric acute renal failure, the simultaneous administration of the amino acid, lysine, prevented the development of acute renal failure. This was attributed to the ability of lysine to inhibit proximal tubular reabsorption of hemoglobin or its heme moiety (126). The clinical utility of such therapy remains to be determined.

Management of sustained acute renal failure usually requires hemodialysis. These patients in general are less catabolic than patients with rhabdomyolysis. The acute renal failure usually lasts 1 to 2 weeks, but full recovery of renal function can be expected.

CRYSTALLINE ACUTE RENAL FAILURE

Uric Acid Nephropathy

Acute uric acid nephropathy is the term given to the development of acute renal failure caused by renal tubular obstruction by urate and uric acid crystals. The main clinical setting in which uric acid nephropathy occurs is the treatment of malignancy, especially of leukemia and lymphoma. Treatment of these malignancies results in cell death and release of large amounts of uric acid precursors. Some patients with these malignancies also have renal insufficiency and high serum uric acid levels before chemotherapy, possibly because of early uric acid nephropathy on the one hand, and the rapidly dividing cell population on the other (127).

Properties of Uric Acid

The final breakdown product of purine degradation in humans is uric acid (Fig. 49-2). Most other mammals degrade purines to the soluble end product allantoin, but humans lack the enzyme uricase. Uric acid (2,6,8-trioxypurine) is a weak acid with a pK_a of 5.75. Urates are the ionized form of uric acid, and at a physiologic pH of 7.4, over 95% of uric acid dissociates into urates, with 98% existing as monosodium urate. However, uric acid predominates in an acid urine. Although initial *in vitro* and *in vivo* studies had shown urate binding to plasma proteins, urate binding to human serum proteins probably is not significant (128–130).

At a temperature of 37°C and a plasma pH of 7.40, the saturation point of urate is at a concentration of 8.8 mg/dL, which is only slightly above the normal physiologic range in humans (131). However, urate crystal precipitation in the bloodstream does not occur even with concentrations much higher than the saturation point. Urates form stable supersaturated solutions in plasma, possibly because of solubilizing substances (128,132). On the other hand, precipitation of urate occurs in extracellular fluid when the solubility concentration is exceeded. The most important factor affecting the solubility of uric acid is pH. For example, in a buffer medium at a pH of 5.0, saturation with uric acid occurs at a concentration below 10 mg/dL, whereas at a pH above 7.0, saturation occurs at a concentration above 150 mg/dL (131).

There are four components to the renal handling of urate. First, urate is filtered freely at the glomerulus. Virtually all of this filtered urate is then reabsorbed in the proximal tubule. An amount equal to 50% is then secreted, and after further absorption, 10% is finally excreted (133,134). Animal data have provided evidence for this bidirectional transport of urate, although net transport differs among species. In humans, there is net reabsorption of urate with the fractional excretion of urate being approximately 10%, whereas in rabbits there is net secretion with the fractional excretion of urate being over 100% (135). Animal micropuncture studies have localized the primary nephron site of urate absorption and secretion to the proximal tubule (135–137). An anion exchanger and a

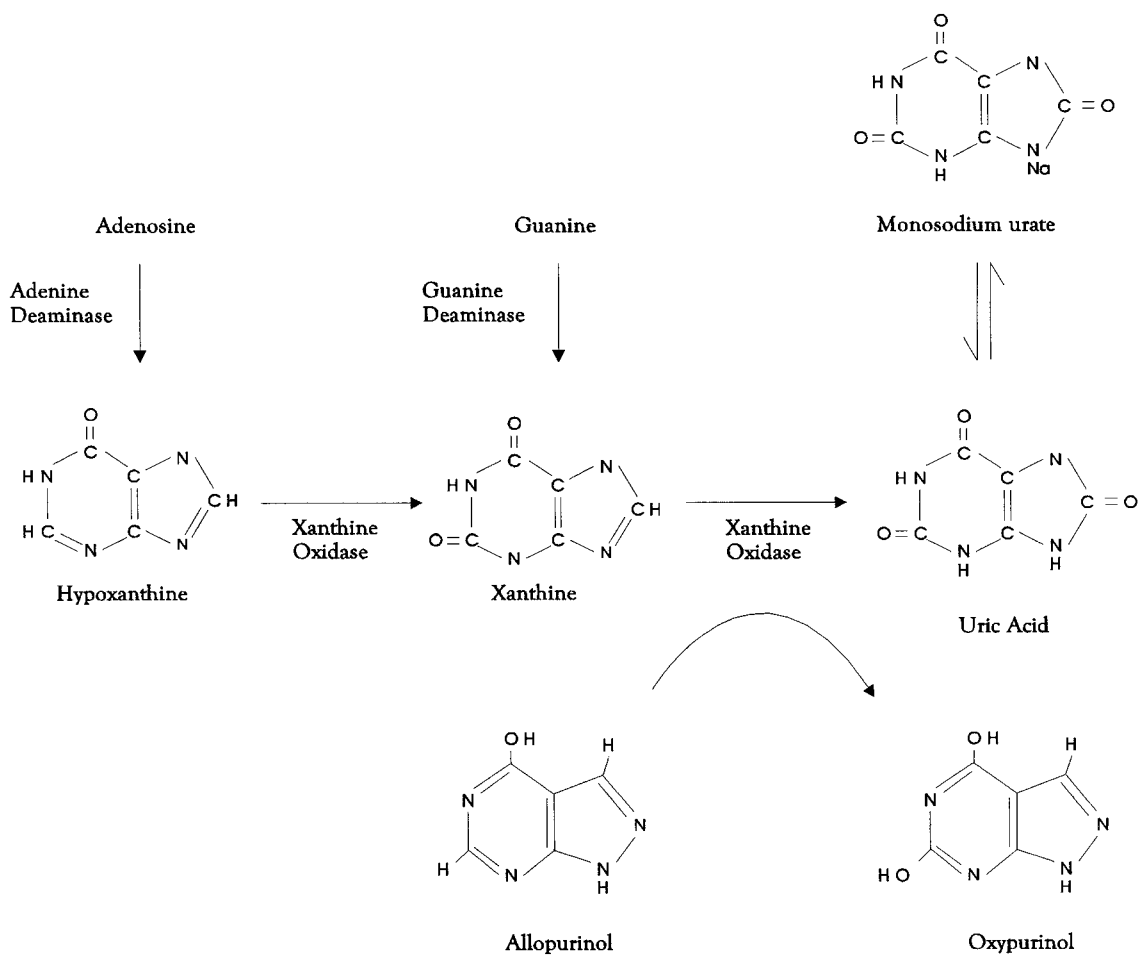

FIGURE 49-2. Pathway of purine degradation showing the competitive inhibition of urate formation by allopurinol and the site of action of rasburicase.

voltage-dependent pathway seem to be the mechanisms involved in urate transport (138,139).

Pathogenesis of Uric Acid Nephropathy

Experimental animal models of uric acid nephropathy are characterized by hyperuricemia, hyperuricosuria, and uric acid deposits in and dilation of the kidney tubules, as observed in the clinical entity (140). Along with the presence of extensive distal tubule deposits of uric acid and urate, micropuncture studies in the rat have shown increased proximal and distal tubular pressures. The vasa recti also show deposits, and efferent arteriolar and peritubular capillary pressures also are increased (141). In this model, uric acid nephropathy was prevented by maintaining a high tubular fluid flow, and only a modest benefit was shown by alkalinization of the urine (142).

Humans with malignancies and hyperuricemia have an increased urinary excretion rate and urinary concentration of uric acid both in the presence and absence of renal insufficiency (143). Autopsy studies have documented the presence of uric acid crystals in patients with leukemia. The uric acid crystals were found only within the lumens of renal tubules, in contrast to patients with gout, in whom no uric acid crystals were found in tubular lumens (144,145). In addition, internal hydronephrosis has been described in association with the intraluminal uric acid crystals, but the glomeruli and tubules usually are intact (146). Supporting the concept that mechanical intraluminal obstruction causes uric acid nephropathy are the observations that the renal failure reverses after a short time and that there is earlier and greater depression of inulin clearance compared with *p*-aminohippurate clearance (146). This evidence is consistent with the concept that uric acid nephropathy occurs because of uric acid crystals obstructing the renal tubular segments with maximum acidifying and concentrating abilities, namely, the distal tubule and collecting duct. The obstruction then leads to increased intraluminal pressure, decreased filtration pressure, and a reduction in GFR.

Clinical and Laboratory Manifestations

The initial reports of uric acid nephropathy focused on patients treated for acute lymphoblastic leukemia, 10% of whom had uric acid nephropathy (146). In these early patient series, risk factors for uric acid nephropathy included urine pH less than 5.0, dehydration, rapid response to chemotherapy, elevated serum uric acid, increased urinary excretion of uric acid, and preexisting renal insufficiency (145–147). Tumor lysis syndrome and acute uric acid nephropathy develop primarily during the treatment of leukemia and lymphoma, but can also occur in association with the treatment of other types of malignancies or in other situations associated with elevated plasma level and urinary excretion of uric acid. Metastatic breast carcinoma, bronchogenic carcinoma, disseminated adenocarcinoma, pancreatic carcinoma, and other nonhematopoietic neoplasms have been reported to cause acute uric acid nephropathy and tumor lysis syndrome (148–154). In addition to myoglobinuria, elevated serum uric acid concentration and increased renal excretion of uric acid are thought to play a role in the etiology of the renal failure caused by heat stress (155). Hyperuricemic acute renal failure also has been reported after epileptic seizures, during pregnancy, and in the setting of cyclosporine use and renal transplantation (156–158).

Uric acid nephropathy during tumor lysis syndrome is characterized by elevations in serum urea nitrogen, creatinine, potassium, uric acid, and phosphate concentrations, and by a decrease in the serum calcium concentration. Hyperuricemia before chemotherapy occurs in 30% to 50% of patients with leukemia and lymphoma, and renal insufficiency seems to be more common in patients with hyperuricemia before chemotherapy (127,147). Before the routine use of aggressive prophylactic measures, patients with uric acid nephropathy presented with serum uric acid levels of 20 to 80 mg/dL, but although most patients still present with uric acid levels greater than 8.0 mg/dL, uric acid concentrations in most patients can be normalized before chemotherapy with allopurinol, alkalinization, and diuresis (127,159). Patients receiving chemotherapy are at risk for other forms of renal failure in addition to acute uric acid nephropathy, and the urinary uric acid-to-creatinine ratio is a useful test to differentiate these various forms of renal failure. A ratio greater than 1 is consistent with acute uric acid nephropathy (160). This is supported by the observation that both the serum uric acid concentration and the urinary excretion of uric acid are elevated in acute uric acid nephropathy as opposed to other forms of renal failure, where serum uric acid concentrations may be high but urinary excretion is not elevated (161). The urinary uric acid-to-creatinine ratio is more helpful in the diagnosis of uric acid nephropathy than urinalysis, which usually is nondiagnostic. The urine sediment may be normal or occasionally reveal amorphous material containing uric acid crystals (150,160). Uric acid crystals appear as rhomboid crystals or as microcrystallites (Fig. 49-3).

Elevations in BUN and serum creatinine typically develop 2 days after the initiation of chemotherapy, with return to baseline after 7 to 10 days. Prior renal insufficiency seems to predispose to the development of uric acid nephropathy (162,163). The acute renal failure is usually of the oliguric variety, even when treated with diuretics (163). When indicated, dialysis usually is initiated within the first week from the start of chemotherapy, but only one to four dialysis treatments usually are required before the spontaneous return of renal function (159,163).

As noted previously, the electrolyte abnormalities associated with the tumor lysis syndrome and uric acid nephropathy are hyperkalemia, hyperphosphatemia, and hypocalcemia. These abnormalities result from the release of intracellular contents after tumor necrosis, and patients with large tumor burdens are at higher risk for tumor lysis syndrome (127). Hyperkalemia actually occurs in less than 5% of patients after chemotherapy, but if it develops, it can occur within 24 hours of initiation of chemotherapy and can be severe enough to necessitate emergent dialysis. In fact, sudden death has been reported as a consequence of tumor lysis–induced hyperkalemia, occurring within 48 hours of chemotherapy (164). Hyperkalemia also is more likely to occur in patients with preexisting renal insufficiency (127,162,163).

Hyperphosphatemia, on the other hand, is very common in the tumor lysis syndrome, and occurs in virtually all patients in whom acute renal failure develops and in 30% of patients with normal renal function (127). The development of hyperphosphatemia also is correlated with the tumor burden (127). In patients with renal failure, the phosphorus concentrations average 12 mg/dL, with a range of 7 to 22 mg/dL (127,162,163). Hypocalcemia also is common, and development of hypocalcemia correlates with hyperphosphatemia (127,159). In the presence of hyperphosphatemia, the etiology of hypocalcemia may be the precipitation of calcium phosphate salts, as discussed in the section on Myoglobinuric Acute Renal Failure, above; when this occurs in the kidney, it may contribute to the acute renal failure seen in tumor lysis syndrome (165,166). Hyperphosphatemia also may contribute to the development of hypocalcemia by depressing the production of $1,25(OH)_2D_3$, as was discussed earlier. Hyperphosphatemia has been shown to worsen experimental acute renal failure, but the mechanism is not clear because calcium phosphate deposition could not be demonstrated in the rat kidneys (167). Calcium deposits have been found at autopsy in the calices and tubules of a patient who had acute renal failure in association with tumor lysis syndrome, hyperphosphatemia (20 mg/dL), and hypocalcemia (168).

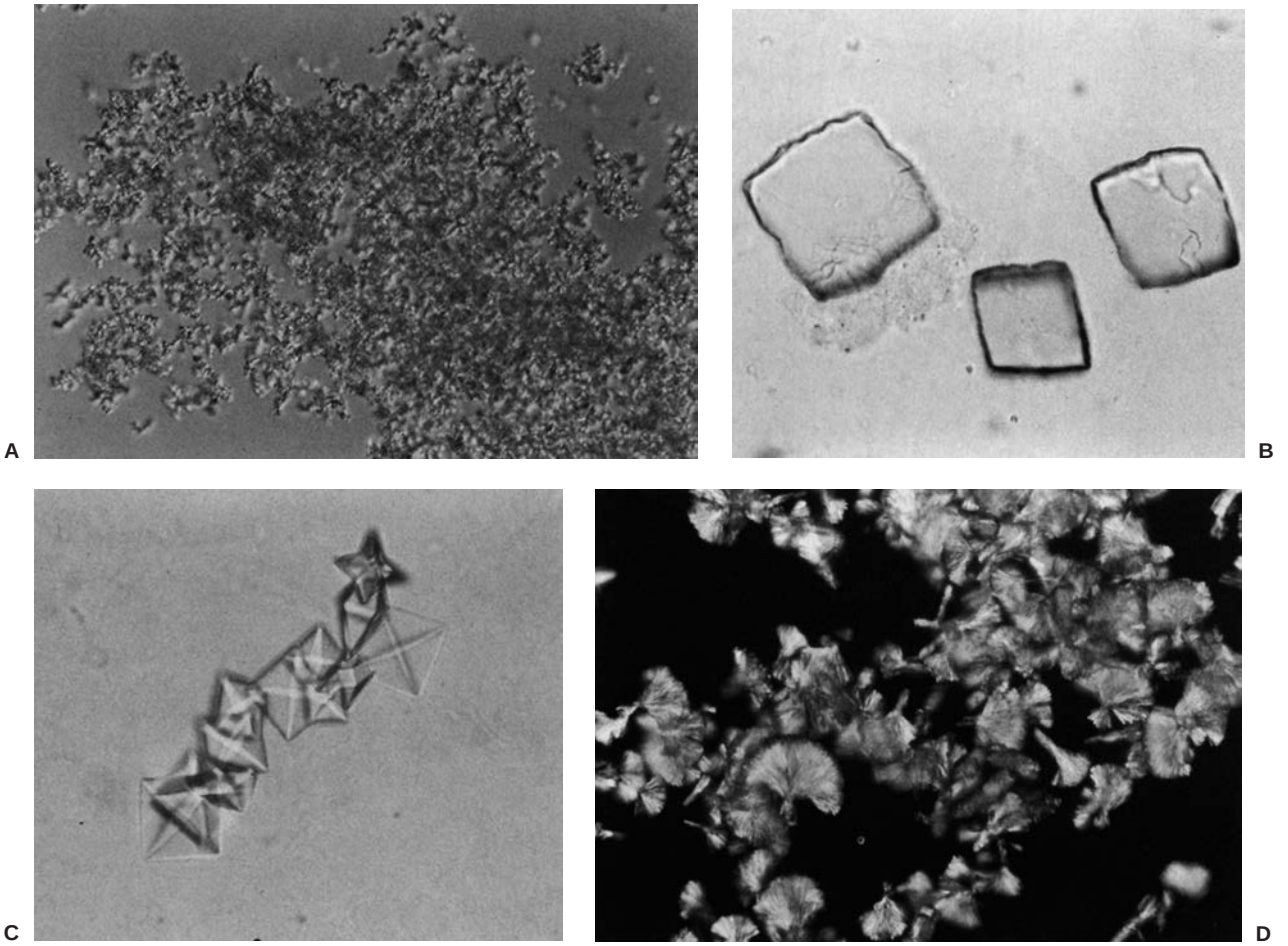

FIGURE 49-3. A: Amorphous urate crystals in urine. (Interference contrast microscopy, magnification ×200.) **B:** Uric acid crystals, cuboidal shape. (Magnification ×160.) **C:** Envelope-shaped calcium oxalate dihydrate form of crystal. (Note: needle-shaped monohydrate calcium crystals are not pictured.) **D:** Sulfadiazine crystals in urine. (Polarized microscopy, magnification ×40.) These birefringent crystals often assume a "fan" or "shock of wheat" shape. (Courtesy of Professor M. H. Haber, Department of Pathology, Rush Medical College, Chicago, IL.)

Despite these characteristic manifestations of the tumor lysis syndrome, it is difficult to predict in which patients acute uric acid nephropathy will develop. Even when appropriate prophylactic measures are taken with hydration, alkalinization, and allopurinol, the following still may develop: hyperuricemia in 9%, hyperphosphatemia in 25% to 50%, hypocalcemia in 10% to 60%, and hyperkalemia in 8%. However, the incidence of clinically significant tumor lysis syndrome after chemotherapy is only 5% (127,162). The likelihood for development of acute uric acid nephropathy is increased in the presence of renal insufficiency, in patients with oliguria before therapy, and in patients with lymphoma with high serum LDH levels (127,162,163).

Differential Diagnosis

The diagnosis of acute uric acid nephropathy should be suspected when acute renal failure in concert with tumor lysis syndrome develops within the first 1 to 3 days after initiation of chemotherapy for lymphoma or leukemia. The diagnosis sometimes is made difficult by the variety of drugs, radiographic studies, and associated clinical problems common during the early presentation of malignancies. Renal complications associated with malignancies include direct lymphomatous or leukemic renal infiltration, obstruction due to stones, and obstruction due to malignancy. However, despite the common occurrence of direct lymphomatous or leukemic renal infiltration, it rarely causes acute renal failure (146,163). Although early reports suggested an association of tumor lysis and ureteral obstruction due to calculi, this also occurs only rarely (159,166). Contrast nephropathy, dehydration, myeloma kidney, light-chain disease, ureteral obstruction due to tumor compression or invasion, sepsis-related renal failure, and acute tubular necrosis due to nephrotoxic drugs all should be considered in this high-risk population. The diagnosis also is made difficult by the common occurrence of hyperuricemia unrelated to tumor lysis syndrome, but caused by any of the following: preexisting renal failure, volume depletion, diuretics, salicylates, levodopa, cyclosporine, nicotinic acid, pyrazinamide, or ethambutol.

The diagnosis of uric acid nephropathy also should be considered when patients present with renal insufficiency before treatment of the malignancy. Spontaneous hyperuricemia occurs more commonly with lymphomas, and some patients present with renal insufficiency before treatment, possibly because of uric acid nephropathy (149,159,162). Ultimately, the diagnosis of acute uric acid nephropathy is made in the presence of tumor lysis syndrome, with the urinary uric

acid-to-creatinine concentration ratio greater than 1, and exclusion of other causes acute renal failure.

Prophylactic Measures and Treatment

Before the dialysis era, the mortality rate from acute renal failure associated with uric acid nephropathy was 50%, but with modern treatment, including proper prophylaxis and dialysis, uric acid nephropathy is rare, and when it does occur, the prognosis for the acute renal failure is excellent (159). The treatment approach to uric acid nephropathy is divided into two stages. The first is to prevent or minimize the metabolic consequences of the tumor lysis syndrome, and the second is to treat these consequences when they do occur. The approach to both prophylaxis and treatment of tumor lysis syndrome includes inhibition of xanthine oxidase, forced diuresis, and urinary alkalinization. When these measures fail to prevent the consequences of tumor lysis, and acute renal failure from uric acid nephropathy develops, dialysis must be initiated to treat uremia and severe electrolyte problems, and control hyperuricemia (Table 49-6). However, in the future, rasburicase, a recombinant form of urate oxidase, may be an additional option for adult patients with acute renal failure from uric acid nephropathy (169) (see below). In July 2002, the U.S. Food and Drug Administration approved rasburicase for the initial management of plasma uric acid levels in pediatric patients with leukemia, lymphoma, and solid tumor malignancies who are receiving anti-cancer therapy expected to result in tumor lysis.

TABLE 49-6

THE APPROACH TO URIC ACID NEPHROPATHY AND TUMOR LYSIS SYNDROME

Approach to prophylaxis for tumor lysis syndrome and uric acid nephropathy

Patients presenting prior to chemotherapy with hyperuricemia and evidence of a large tumor burden: allopurinol, 300–600 mg

4–5 L/24 hr of normal saline. Add diuretics if the patient is well hydrated and not maintaining an adequate urine output. If there is no response to diuretics, match fluid input with urine output.

Urinary pH should be maintained above 7.0 by titrating intravenous bicarbonate therapy. Start with 100 mEq/L of sodium bicarbonate in D5W per hour. Bicarbonate therapy should be discontinued after serum uric acid is normalized.

If clinically feasible, postpone chemotherapy until uric acid is normalized along with any other electrolyte abnormalities.

Patients presenting prior to chemotherapy and with a normal uric acid level but still at risk for tumor lysis syndrome:

A llopurinol, 300–600 mg

4–5 L/24 hr of normal saline as described above.

If clinically feasible, postpone chemotherapy until 2 days after start of allopurinol.

Treatment of uric acid nephropathy and tumor lysis syndrome

Hemodialysis initiated per the routine indications including hyperkalemia, acidosis, hyperphosphatemia, volume overload, or uremia.

Hemodialysis for control of hyperuricemia unresponsive to the above measures. Adjust allopurinol doses for renal failure:

Creatinine clearance	Allopurinol dose
0	100 mg q3d
10	100 mg q2d
50	200 mg qd

After hemodialysis, supplement with 50% of allopurinol dose.

Inhibition of Xanthine Oxidase. Allopurinol is a substrate for and a competitive inhibitor of the enzyme xanthine oxidase (Fig. 49-2). It blocks the conversion of hypoxanthine and xanthine to uric acid, resulting in a reduction in both serum uric acid concentration and urinary excretion of urates. In the presence of allopurinol, hypoxanthine and xanthine accumulate instead of uric acid, and the urinary excretion of these precursors also increases (170). Hypoxanthine is highly soluble, and even with increased renal excretion, does not cause clinical problems. Xanthine, on the other hand, is less soluble than uric acid. Precipitated xanthine can be found in the urine of patients receiving allopurinol, but these precipitates do not correlate with the development of renal failure (170). However, well documented cases of xanthine nephropathy and xanthine calculi associated with allopurinol use have been reported (170–173).

The half-life of allopurinol is less than 2 hours owing to prompt renal elimination and rapid conversion to its chief metabolite, oxypurinol. Oxypurinol is an active metabolite, and reduces serum uric acid concentration and urinary uric acid excretion half as much as allopurinol (174). Unlike allopurinol, oxypurinol is eliminated solely by the kidney and its half-life is approximately 24 hours (174). Renal clearance of oxypurinol is approximately 30 mL/minute, and its clearance correlates directly with creatinine clearance such that with a creatinine clearance of less than 10 mL/minute, the half-life of oxypurinol is approximately 1 week.

In patients with normal renal function and hyperuricemia associated with malignancy, allopurinol decreases serum uric acid within 48 hours with a peak effect at 5 days (175). The clinical effects of allopurinol probably are mediated by oxypurinol because the half-life of allopurinol is short. Despite the use of allopurinol, hyperuricemia and acute uric acid nephropathy sometimes cannot be avoided, and reasons for this failure include a large tumor burden, aggressive chemotherapy, and the inability to delay chemotherapy until allopurinol has decreased the serum uric acid concentration (159,162,163).

For optimal prophylaxis, allopurinol should be administered at least 3 days before chemotherapy. The level of existing renal function also must be considered when dosing the drug. Allopurinol can lead to a life-threatening toxicity syndrome that is characterized by a diffuse, desquamative skin rash, fever, hepatic dysfunction, eosinophilia, and worsening renal function of unknown etiology, although consistent with a diffuse vasculitis. Eighty percent of patients reported with this toxicity had renal insufficiency (176–178). Improper dosing of allopurinol also can lead to xanthine nephropathy (171–173,176).

Optimal allopurinol dosing is reflected by a therapeutic serum oxypurinol concentration, which ranges from 30 to 100 μmol/L (178). Patients with end-stage renal disease achieve therapeutic levels of oxypurinol after one dose of allopurinol (300 to 600 mg) and maintain this level until the next dialysis, at which time the serum level is reduced by 40% (176,179). Therefore, the maintenance dose must be reduced in patients with renal insufficiency to avoid accumulation of oxypurinol. For example, plasma oxypurinol levels greater than 300 μmol/L can be achieved after the usual dose of allopurinol (300 mg) in the presence of mild renal insufficiency, and these potentially toxic levels persist for days after cessation of the drug (179). The oral route is equivalent to intravenous dosing of allopurinol; therefore, intravenous dosing should be considered only in patients unable to take anything by mouth. Rectal administration of allopurinol is not effective and should not be used (180,181). Allopurinol started at 300 to 600 mg is safe and achieves therapeutic levels of oxypurinol, but the peak clinical effect on uric acid production is not seen for 3 days. Optimally, allopurinol should be started 3 days before chemotherapy, and dose adjustments must be made in the presence of renal insufficiency (Table 49-6).

Rasburicase

As mentioned above, most mammals degrade purines to the soluble end product allantoin utilizing the enzyme uricase (Fig. 49-2) which humans lack. Rasburicase, the recombinant form of urate oxidase, has several advantages over allopurinol. Rasburicase has a rapid onset of action and has been shown to return uric acid to normal levels with hours (182). Unlike allopurinol which inhibits the production of uric acid, rasburicase quickly reduces the existing uric acid levels and does not rely on the renal clearance of existing uric acid or alkalization of the urine. In one compassionate use trial, rasburicase (0.20 mg/kg) was administered intravenously once a day for 1 to 7 days. The mean uric acid level in 29 hyperuricemic children decreased from 15.1 to 0.4 mg/dL, and in 27 hyperuricemic adults, the mean level decreased from 14.2 to 0.5 mg/dL (183). Rasburicase is a very expensive drug and although clinical trials have compared rasburicase to allopurinol, the outcomes have been a decrement in uric acid level rather than important metabolic outcomes or acute renal failure (184). Additional clinical trials are necessary before rasburicase will be approved for use in adults.

Forced Diuresis. Animal data have suggested that high renal tubular fluid flow induced by a solute or water diuresis is important in the prevention of acute urate nephropathy. In fact, rats treated with high-dose furosemide, and Brattleboro rats with central diabetes insipidus and water diuresis both had complete protection from uric acid nephropathy, whereas rats treated solely with urine alkalinization had only partial protection (142). Diuresis probably imparts protection by lowering the urate concentration in the collecting duct where uric acid precipitation occurs, or by effects on tubular urate handling. Whether these results can be applied to humans is not known because species differences in urate handling exist.

Despite efforts to maintain high urine flow with hydration and diuretics, a lower urine flow rate preceding chemotherapy is more common in patients who have renal failure than in those who do not (163). Although this observation probably reflects the existence of mild spontaneous uric acid nephropathy before chemotherapy, it is reasonable to assume that increased urine flow would add protection from uric acid nephropathy. Patients should be hydrated with 4 to 5 L of normal saline per 24 hours. If the patient is well hydrated and not maintaining the expected urine output, diuretics should then be initiated. If urine output remains low, fluid intake should be adjusted to match output in the effort to avoid fluid overload.

Urinary Alkalinization. Although evidence is lacking to confirm its theoretic role in preventing uric acid nephropathy, urinary alkalization remains a prominent component in prophylactic regimens. The theoretical benefit of urinary alkalization is to increase the solubility of uric acid. However, in animal studies, the most important intrarenal dynamic in prevention of acute uric acid nephropathy was high urine tubular flow (142). In this study, the use of acetazolamide achieved only partial protection, which was likely due to the drug's diuretic effect and not its effect on urine pH (142). Along with the inherent risk of causing a severe metabolic alkalosis when attempting to alkalinize the urine with sodium bicarbonate administration, other potential disadvantages include increasing the risk of symptomatic hypocalcemia and calcium phosphate precipitation, which can cause acute renal failure by itself in this setting (165,166,168,185). Urinary alkalinization also does not have an effect on xanthine precipitation because the pK_a of xanthine is 7.4, as opposed to 5.6 for uric acid.

Bicarbonate therapy should be included in the prophylactic regimen only when attempting to correct hyperuricemia. If hyperuricemia is present before chemotherapy, bicarbonate should be added to intravenous fluids with the aim of keeping the urine pH above 7.0. Once hyperuricemia has been corrected, bicarbonate therapy should be discontinued.

Hemodialysis. Dialysis assists in the management acute uric acid nephropathy in two ways. First, dialytic therapy is initiated for the typical indications common in acute renal failure such as hyperkalemia, severe hyperphosphatemia, azotemia, and fluid overload, although these indications may be more severe and occur more rapidly than in other forms of acute renal failure. Cases of fatal hyperkalemia have occurred within hours after initiation of chemotherapy (163,164). Second, dialysis is an effective way to reduce the serum uric acid level. This is an important role for dialysis because patients usually do not recover from acute uric acid nephropathy until the serum uric acid level is reduced (186). Once this occurs, usually after only one to four dialysis treatments, recovery of renal function is signaled by a brisk diuresis (159).

Depending on the dialyzer and blood flow used, hemodialysis has a uric acid clearance rate of 90 to 150 mL/minute, whereas the peritoneal dialysis clearance is only 10 to 20 mL/minute (159,186,187). The superior clearance of hemodialysis compared with peritoneal dialysis is evidenced by the typical total uric acid cleared on hemodialysis versus peritoneal dialysis: 7 to 8 g versus 4 g. When starting a patient on hemodialysis, caution should be taken not to use a high-calcium bath if severe hyperphosphatemia is present because of the risk of increasing the calcium–phosphorus product. Selected patients may benefit from continuous forms of dialytic therapy such as continuous arteriovenous hemodialysis. On continuous arteriovenous hemodialysis with a dialysate flow of 4 L/hour, the uric acid clearance is 40 mL/minute, and the possible advantage over the intermittent nature of dialysis is the continuous clearance and control of phosphorus and potassium (188).

Ethylene Glycol Toxicity

Acute ethylene glycol intoxication is a medical emergency that, if not treated aggressively, leads to serious neurologic, cardiopulmonary, and renal dysfunction and may result in death. In 1993, 501 ethylene glycol exposures, including 254 hospitalizations and 11 deaths, were reported to the American Poison Control Centers (189). Ethylene glycol, an odorless and clear liquid, is the major ingredient in antifreeze, and is most commonly consumed either intentionally by alcoholics seeking an ethanol substitute or accidentally by children. An ingestion of 100 mL is considered the minimal lethal dose of ethylene glycol (190). Diethylene glycol is a condensation product of ethylene glycol production, and ingestion causes the same toxicities as ethylene glycol. Diethylene glycol was used as the diluent in the first sulfa antibiotic, sulfanilamide, and consequently led to mass poisonings in 1937. One hundred five patients died from the therapeutic use of Elixir Sulfanilamide, and one important consequence of this tragedy was the 1938 Federal Food, Drug and Cosmetic Act, requiring proof of product safety before release of a drug (191,192). Unfortunately, this kind of governmental supervision of pharmaceutical companies does not exist in other countries such as Nigeria and Haiti, where 47 and 85 children, respectively, died when diethylene glycol was used as a solvent in a preparation of cough syrup (193,194).

Metabolism of Ethylene Glycol

The metabolism of ethylene glycol is complex and incompletely understood. As is the case with other alcohols such as ethyl and methyl alcohol, nicotinamide adenine dinucleotide (NAD)-dependent alcohol dehydrogenase is responsible for the first

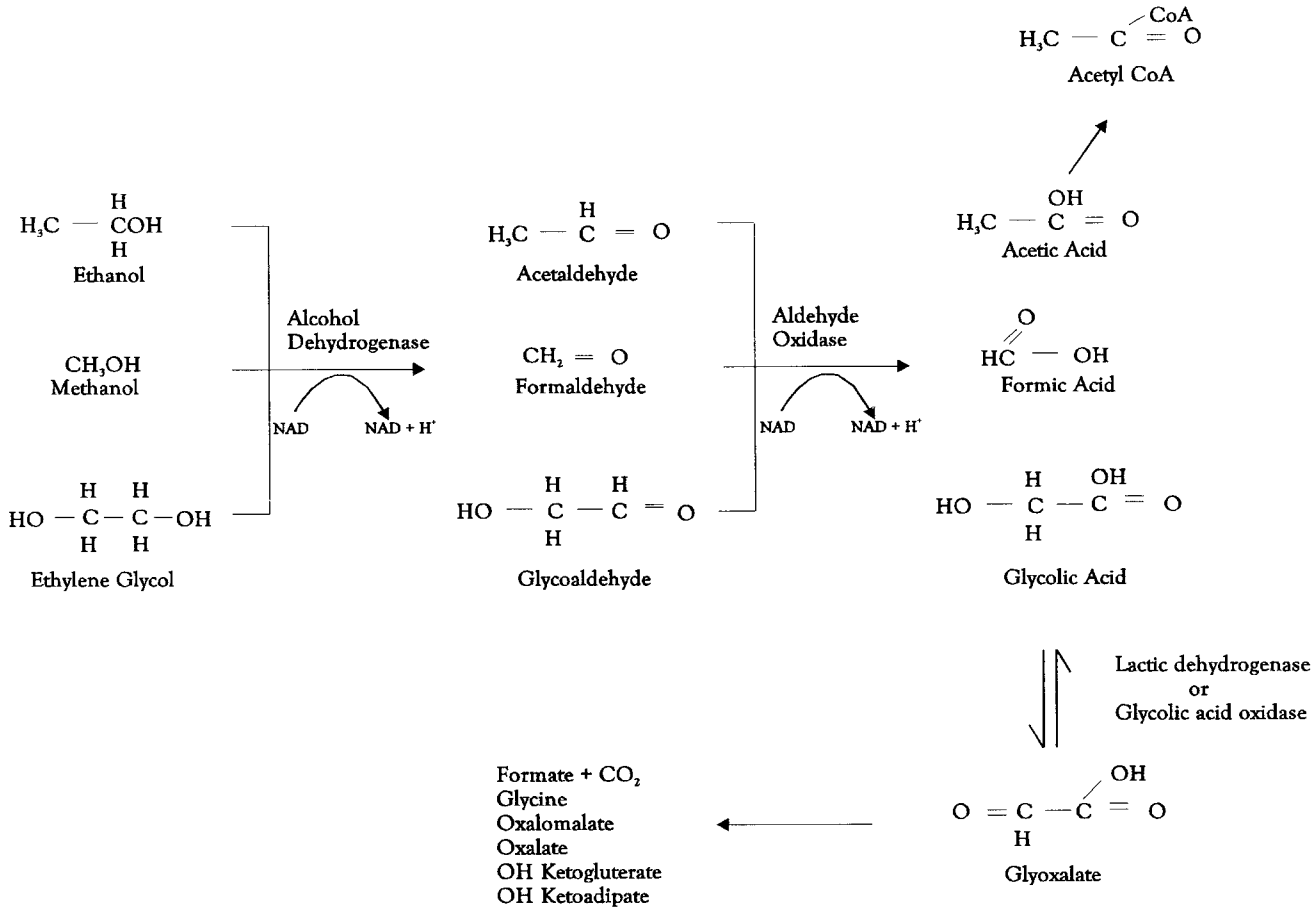

FIGURE 49-4. Pathway of ethylene glycol degradation.

oxidative step, converting ethylene glycol to glycoaldehyde (Fig. 49-4). After this first step, the pathways have not been well elucidated in humans, but are thought to include the following: glycoaldehyde oxidized to glycolic acid by aldehyde oxidase, glycolic acid to glyoxylate by glycolic acid oxidase or LDH, and then numerous subsequent pathways for glyoxylate metabolism, including one to oxalate by LDH and glycolic acid oxidase (190) (Fig. 49-4). Glycolate is converted to glyoxylate very slowly and is probably the rate-limiting step in the metabolism of ethylene glycol, whereas glycoaldehyde and glyoxylate have very short half-lives (195,196). Lactic acid production had been thought to be increased in ethylene glycol intoxication because of the altered NADH:NAD ratio resulting from increased NADH formation from alcohol dehydrogenase, but lactic acidosis is not a consistent finding and may be associated with alcoholism or hemodynamic instability (190,195,197).

Ethylene glycol metabolites are thought to mediate the toxicity seen with ethylene glycol ingestion, and ethylene glycol itself is not toxic. In fact, inhibition of ethylene glycol metabolism with ethyl alcohol or pyrazole prevents toxicity (198,199). The observation that the mortality rate in rats is reduced by performing a partial hepatectomy before administration of ethylene glycol and glycolate illustrates the importance of ethylene glycol metabolites on toxicity: The partially hepatectomized rats metabolized ethylene glycol more slowly to its toxic byproducts, which allowed more time for renal excretion of the nontoxic and unchanged ethylene glycol (200). Glycolate and oxalate are thought to be important mediators of ethylene glycol toxicity.

The pathophysiologic process of ethylene glycol toxicity is multifactorial and is thought to include accumulation of toxic ethylene glycol metabolites, calcium oxalate crystal deposition in tissues, and the effects of severe acidosis. After administration of a lethal dose of ethylene glycol in rats, profound renal oxalosis is produced, and the same occurs with administration of glycolic acid and glyoxylic acid. Renal pathology in these rats demonstrated calcium oxalate crystals in the proximal and distal convoluted tubules, with smaller amounts in the collecting tubule and none in glomeruli or renal interstitium (201). The degree of crystal formation correlated with diffuse convoluted tubular dilatation, but occasional epithelial necrosis seemed to bear a relation to the degree of crystal formation. Administration of glycoaldehyde did not produce significant crystal formation or the same degree of microscopic changes, but did lead to pronounced tubular epithelial swelling. In addition to the renal findings, oxalate crystals were found in brain tissue (201). In dogs given nonlethal doses of ethylene glycol, renal biopsy specimens revealed interstitial edema, tubular dilatation, hydropic degeneration, and tubular cell necrosis even in areas free of crystals. Electron microscopic findings were most prominent in proximal tubule cells and included vacuolization, cellular rupture, cytoplasmic buds, and increased density of mitochondria (202). This pattern of proximal tubule damage is similar to other models of ischemic and nephrotoxic forms of renal failure (202).

These findings are similar to human autopsy series that also have found calcium oxalate crystals in renal tubules. In these studies, renal epithelial cells appear either normal or extensively necrotic, depending on the interval between ingestion

and death; minimal if any damage of the glomeruli has been reported (203–205). Despite severe clinical neurologic disturbances, the brain characteristically has only mild perivascular and meningeal deposition of calcium oxalate crystals, but edema, capillary engorgement, hemorrhage, and infarctions also have been described (205,206). Although crystal deposition usually is not reported, myocardial tissue findings are consistent with myocarditis (205).

Renal biopsies in survivors of ethylene glycol intoxication helped clarify the renal pathologic process because most reported autopsy subjects had died within 48 hours of ingestion of ethylene glycol. Survivors consistently show widespread renal epithelial cell necrosis with preservation of the basement membrane; calcium oxalate deposition is present but in a scattered distribution, with preservation of epithelial cells at the point of contact (207). Crystals are prominent in proximal tubules and sparse in the distal tubules, and tubular dilatation and obstruction are not consistently observed (208,209). Serial biopsies have shown a clear sequence from initial severe tubular damage to regeneration of tubular epithelium over a 60-day period (209). In aggregate, the autopsy and renal biopsy studies do not support the hypothesis that calcium oxalate crystallization is the primary cause of ethylene glycol toxicity; despite widespread renal tubule damage, calcium oxalate crystal deposition is patchy; and despite the presence of crystals, there is no tubule obstruction or dilation.

High–anion-gap metabolic acidosis is a major feature of ethylene glycol intoxication, and also is thought to contribute directly to the clinical toxicity. In rats poisoned with ethylene glycol, survival rates are five times greater after treatment with sodium bicarbonate alone or with ethanol alone compared with no treatment, and giving ethanol and sodium bicarbonate together improved the survival rate to six times that seen in rats with no treatment (210). Also possibly illustrating the relationship between acidosis and ethylene glycol toxicity is a report of a patient who ingested a large amount of ethylene glycol and lithium carbonate, a potential source of 320 mEq of bicarbonate. Despite an ethylene glycol level of 500 mg/dL on admission, the patient did not have an anion gap metabolic acidosis or renal failure (211). The major determinant of the metabolic acidosis is glycolic acid; in dogs and monkeys, administration of ethylene glycol produces severe metabolic acidosis, and the depressed bicarbonate is matched by the increase in glycolic acid production (212). Oxalic acid is very toxic to kidneys and is lethal in doses much lower than toxic doses of ethylene glycol, but in rats and monkeys, only 0% to 2.5% of the original dose of ethylene glycol is excreted as oxalic acid (190,196,213). In humans with ethylene glycol poisoning, plasma glycolate concentration correlated with the increased anion gap, and the serum concentrations of oxalate and glyoxylate were negligible in these patients (195,214). Although studies have suggested that organic acids such as lactic acid contribute to the severe metabolic acidosis of ethylene glycol intoxication, glycolic acid seems to be the main cause of the acidosis, with lactic and β-hydroxybutyric acids being elevated in special circumstances such as associated hypotension or alcoholic ketoacidosis (197,214).

It has been postulated that production of glycoaldehyde, glyoxal, glycolate, and glyoxylate from ethylene glycol metabolism is important in the pathophysiologic process of the toxicity. Aldehyde production is greatest 6 to 12 hours after ethylene glycol ingestion, and this is when cerebral symptoms are most severe (190). However, as mentioned, glycolate is the only metabolite that accumulates; its direct toxicity has not been well studied, but it is known to be toxic in animals (196). For example, glycolic acid given to rats is lethal and also causes renal tubular oxalosis (201). The role that glycolic acid plays in the human renal, cerebral, and cardiac toxicity remains to be proven, but it probably is one of the multifactorial causes along with acidosis and calcium oxalate crystals.

Clinical and Laboratory Manifestations

The initial reports of ethylene glycol poisoning in the 1940s and 1950s noted that the clinical manifestations of acute ethylene glycol poisoning could be divided into three stages (207). During the first stage, occurring 30 minutes to 12 hours after ethylene glycol ingestion, the central nervous system manifestations predominate. During the second stage, occurring over the next 12 hours, cardiopulmonary dysfunction develops, and includes tachypnea, pulmonary edema, and cardiac failure. In patients who survive past the first 24 hours, the third stage is characterized by prolonged renal failure. Before the advent of aggressive treatment with hemodialysis and intravenous ethanol, these stages were very typical of the clinical course of most patients, but with modern treatment and depending on the amount of ethylene glycol ingested, the sequence and occurrence of these clinical features and stages vary considerably (206,215).

In addition to apparent inebriation but without an alcoholic odor, central nervous system manifestations include nystagmus, depressed reflexes, seizures, and coma (190,216,217). Delayed appearance of multiple cranial nerve deficits also has been reported, and the deficits have not always been reversible (218). Ocular effects are a main feature of methanol ingestion, but ophthalmoplegia, papilledema, loss of visual acuity, and eventual optic atrophy also have been reported with ethylene glycol ingestion (219). Abdominal signs and symptoms, including nausea, vomiting, and pain, are very common (215). For unexplained reasons, mild hypertension, tachycardia, and a low-grade fever sometimes are present, and one study noted a poor prognosis in patients with hypertension. Three of 6 patients with hypertension died in a series of 36 patients in which the total deaths were 6 (190,206).

High–anion-gap metabolic acidosis with a high osmolar gap is the most striking initial laboratory finding and is the main diagnostic feature. The severity of the clinical presentation depends on the quantity of ingested ethylene glycol and elapsed time since its ingestion, but typically patients present with a pH of less than 7.2, bicarbonate less than 10 mEq/L, anion gap greater than 20, mean osmolal gap of 35, measured osmolality greater than 300 mOsm, and hyperkalemia (206,220,221). Hypocalcemia is a frequent finding and can be severe and symptomatic, leading to tetany or cardiac arrhythmia (206,217). The onset of hypocalcemia is usually within the first 12 hours, and the serum calcium usually remains low despite treatment (222,223). The hypocalcemia probably is caused by a combination of chelation of calcium by oxalate and an abnormal parathyroid hormone response (222).

Lumbar puncture frequently is performed because of the mental status changes, and the cerebrospinal fluid sometimes reveals pleocytosis with a sterile culture (203,205,215). A normal hematocrit and platelet count but a moderate leukocytosis of 10,000 to 40,000/mm^3 with a predominance of polymorphonuclear cells is seen commonly in the initial complete blood count (190,205).

The urinalysis typically includes a low specific gravity, mild proteinuria, microscopic hematuria, and pyuria (215,217,224). Crystalluria is not invariably present, but usually is seen on presentation. The envelope-shaped calcium oxalate dihydrate form of crystals (octahedral dihydrate) traditionally has been thought of as the most commonly seen crystal in ethylene glycol intoxication, but in fact the needle-shaped monohydrate calcium oxalate crystals predominates in ethylene glycol intoxication (224–226) (Fig. 49-3). Monohydrate calcium oxalate crystals are thermodynamically stable, and with time the dihydrate form transforms to the monohydrate form (227). *In vitro,* the dihydrate form is seen only at high

TABLE 49-7

DOSING GUIDELINES FOR ETHANOL TREATMENT

Ethanol solution and route of administration	Specific gravity of ethanol (g/dL)	Loading dose (100 mg/dL of ethanol × 0.6 L/kg) (mL/kg)	Maintenance dose in nondrinkers 66 mg/kg/hr (mL/kg/hr)	Maintenance dose in drinkers 154 mg/kg/hr (mL/kg/hr)	During dialysis add the following to the maintenance dose (mL/hr)
5% IV	3.9	15.4	1.7	3.9	185
10% IV	7.8	7.7	0.84	2.0	90
20% PO	15.8	3.8	0.42	1.0	45
50% PO	39.5	1.5	0.17	0.4	18

IV, intravenous; *PO*, orally.
Oral ethanol dose should be increased by 50% after charcoal therapy.

concentrations of both calcium and oxalate (227). The pattern of oxalate crystals in individual patients transforms from the envelope-shaped crystals to the needle-shaped crystals in a matter of hours (214).

The cardiopulmonary consequences of ethylene glycol intoxication now are rarely seen with prompt, aggressive treatment. However, early reports described rapidly progressive tachypnea, cyanosis, pulmonary edema, hypotension, and cardiac failure in most cases (203,204,207). In a recent report from an ethylene glycol poisoning epidemic in Sweden, pulmonary edema occurred in only 1 of 36 patients and circulatory failure and hypotension in 2 of 36 patients (206). The etiology of the pulmonary dysfunction is not known, but is probably adult respiratory distress syndrome (228). However, this is not necessarily due to direct ethylene glycol toxicity because these patients are at risk for aspiration and pneumonia (206). Myocarditis, with focal muscle cell necrosis and diffuse infiltrates of mononuclear cells, has been reported as a consequence of ethylene glycol poisoning (206,229).

After ingestion of ethylene glycol sufficient to cause metabolic acidosis, oliguric renal failure develops in most patients (206,215). If aggressive treatment, including dialysis and ethanol, is provided soon after the ethylene glycol is ingested, renal failure can be avoided. However, most patients do not seek medical attention until symptoms develop, which usually is many hours after ingestion. Thus, renal failure is common, and may develop as soon as 24 hours after ingestion (203,214,215,230). The course of the renal failure is typical of oliguric acute tubular necrosis. The oliguria lasts 4 to 5 days and is followed by a diuretic phase. The BUN and serum creatinine usually peak at 7 to 10 days, and most patients require only 1 to 2 weeks of dialytic support (199,206,207,215). However some patients require dialysis for many months, and despite the return of sufficient renal function to stop dialysis, renal function does not always return to baseline values (214).

Diagnosis

In the absence of ketoacidosis and in the presence of the characteristic signs and symptoms, it should be assumed that all patients presenting with metabolic acidosis combined with increased anion and osmolal gaps have either methanol or ethylene glycol poisoning (221). The prognosis of both these poisonings is improved with early diagnosis and treatment, and therefore, if diagnosis cannot be confirmed with serum levels of methanol or ethylene glycol, treatment with bicarbonate and ethanol infusion and hemodialysis should be initiated. Screening tests for the identification of ethylene glycol are being developed, but are not commonly used (231). Determination of ethylene glycol in serum is best performed with gas chromatography, but this method is not routinely available at most hospitals (232). Sodium fluorescein is a fluorescent dye that is added to some commercial antifreeze preparations, and a Wood's lamp can be used to detect fluorescence visually in urine, a finding that supports the diagnosis of ethylene glycol ingestion (233).

Once the diagnosis of ethylene glycol poisoning has been confirmed and the blood concentration of any concomitantly ingested ethanol determined, the serum ethylene glycol level can be estimated using the osmolal gap (234–236) (Table 49-7). Ethylene glycol levels above 20 mg/dL can be lethal if not treated aggressively (235).

Clinical Course and Treatment

Initial Emergency Department Treatment. If the patient is seen in the first few hours after ethylene glycol ingestion, gastric lavage and oral charcoal should be initiated to reduce further drug absorption. Ethylene glycol is metabolized quickly to acid intermediaries, and thus the high–anion-gap acidosis develops rapidly after ingestion of ethylene glycol. Hemodialysis to provide a source of bicarbonate and to clear ethylene glycol and its metabolites is the therapy of choice for treatment of the acidosis. However, hemodialysis usually is delayed, and during this waiting period patients usually require hundreds of milliequivalents of sodium bicarbonate. Despite intravenous administration of 300 to 500 mEq of sodium bicarbonate in the first 6 hours, severe metabolic acidosis usually persists and is not corrected until hemodialysis is initiated (214,237).

Correction of the acidosis may increase the likelihood of symptomatic hypocalcemia such as seizures, tetany, and cardiac dysfunction. Intravenous calcium supplementation should be given cautiously because of the potential risk of further calcium oxalate precipitation. Calcium should be given if clinical signs or symptoms of hypocalcemia develop, but not prophylactically (217). Thiamine and pyridoxine are cofactors required in the nontoxic metabolic pathways of ethylene glycol (away from oxalate), and early replacement of these cofactors is advocated to prevent potential depletion (190,216).

Administration of ethanol and hemodialysis have traditionally made up the definitive treatments for ethylene glycol intoxication. Compared with ethylene glycol, ethanol has a higher affinity for alcohol dehydrogenase and therefore inhibits the metabolism of ethylene glycol to the toxic metabolites, permitting the ethylene glycol to be renally excreted or dialyzed. With a blood ethanol level of 100 mg/dL, liver alcohol dehydrogenase is saturated, and the half-life of ethylene glycol increases from 3 to 17 hours (238). In rats, the median lethal dose of ethylene glycol is doubled when ethanol also is given, and in monkeys, a dose of 3.2 mL/kg of ethylene glycol was lethal

except for those also receiving ethanol. The urinary excretion of ethylene glycol was 10 times greater in ethanol-protected monkeys than in those receiving ethylene glycol alone (239). Since the first report of ethyl alcohol treatment in humans, ethyl alcohol has been used in conjunction with dialysis in the treatment of ethylene glycol poisoning, and ethanol is not recommended as a sole treatment (199). Although there have been reports of successful treatment with ethyl alcohol without dialysis, these were isolated cases in which ingestion only of small amounts of ethylene glycol occurred (206,215).

For maximal inhibition of ethylene glycol metabolism, the plasma ethanol concentration should be maintained between 100 and 200 mg/dL. This is achieved with a loading dose of 0.6 g/kg, followed by a maintenance dose of 66 mg/kg in nondrinkers, and 154 mg/kg in regular alcohol consumers. During dialysis, 7.2 g/hour should be added to the maintenance dose (238,240). Oral ethanol also can be used, but the dose should be increased by 50% if given soon after the administration of charcoal (238). Intravenous ethanol comes in 5% and 10% solutions diluted in dextrose and water, whereas a 20% or 50% solution usually is used for oral or nasogastric administration. The specific gravity of ethanol is used in calculating the correct dose (234,238) (Table 49-7). Until the correct dose to achieve a level between 100 and 200 mg/dL has been ascertained, hourly ethanol concentrations should be checked.

4-Methylpyrazole. An alternative to ethanol therapy is fomepizole (4-methylpyrazole; Antizol) which is a potent inhibitor of alcohol dehydrogenase. It is now considered by many to be the first line therapy over ethanol for treatment of ethylene glycol toxicity (241,242). Animal studies have shown that fomepizole prevents ethylene glycol–related mortality and toxicities, and increases the urinary excretion of ethylene glycol by preventing its metabolism (198,212). In humans, fomepizole has been studied in a small number of patients, and has been shown to normalize acidosis within hours, prevent decreases in renal function if used early, and decrease serum levels of ethylene glycol toxic metabolites (243). In humans without renal failure, treatment results in an increase in the ethylene glycol half-life from 3 to 14 hours, an increase in urinary excretion of ethylene glycol, and prevention of clinical toxicity (244,245).

Fomepizole offers advantages over ethanol treatment, including predictable pharmacokinetics, avoiding the need to achieve and maintain the desired blood ethanol level, and avoiding the ethanol-induced central nervous system depression. Fomepizole is available as a parenteral solution. The loading dose is 15 mg/kg intravenously, followed by 4 more doses of 10 mg/kg every 12 hours, after which it is continued at a rate of 15 mg/kg every 12 hours until the ethylene glycol concentration is undetectable or the patient is asymptomatic with a resolution of the high–anion-gap metabolic acidosis. Like ethanol, the dose of fomepizole is adjusted during dialysis therapy. At the start of dialysis, the next scheduled dose is given if it has been longer than 6 hours since the last dose, but if it has been less than 6 hours, the next scheduled dose is held. Fomepizole is then given every 4 hours during dialysis. At the completion of dialysis, no additional dose is given if it has been less than 1 hour since the last dose, one-half of the next scheduled dose is given if it has been 1 to 3 hours since the last dose, and the next scheduled dose is given if it has been longer than 3 hours since the last dose. The maintenance dose off dialysis is continued 12 hours after the last dose (246).

Fomepizole has been used to treat ethylene glycol poisoning successfully without hemodialysis or ethanol, but these patients had normal renal function and fomepizole treatment was initiated soon after ethylene glycol ingestion (244,245). In mild cases of ethylene glycol poisoning as evidenced by normal renal function and no high–anion-gap acidosis, ethanol or fomepizole is used by some as sole therapy without dialysis, but in these cases forced diuresis with intravenous fluids and mannitol or furosemide should be used to avoid dehydration, minimize renal calcium oxalate crystal formation, and maintain renal clearance of ethylene glycol (223,230,245). However, recent data suggest that an abnormal presenting serum creatinine concentration ($\geq$1.5 mg/dL) predicts significantly prolonged ethylene glycol elimination during fomepizole therapy, and in the presence of metabolic acidosis, patients should undergo hemodialysis (247).

Hemodialysis is indicated in all cases of confirmed or strongly suspected ethylene glycol poisoning presenting with renal failure, metabolic acidosis, and/or deteriorating clinical status. Ethylene glycol and glycolate have low molecular weights, no protein binding, and a volume of distribution of 0.8 and 0.55 L/kg, respectively, which make them easily dialyzable (195,238,248). Large surface-area dialyzers (>2 m^2) can achieve clearance of ethylene glycol of greater than 200 mL/minute, and with smaller surface-area dialyzers (1.1 to 1.6 m^2) clearance of ethylene glycol and glycolate typically ranges from 150 to 190 mL/minute and from 140 to 170 mL/minute, respectively (195,214,238,248,249). The renal clearance of ethylene glycol can be as high as 30 mL/minute in patients with preserved renal function, but the importance of hemodialysis is illustrated by the fact that most patients present with renal insufficiency, and in these patients the renal clearance of ethylene glycol and glycolate is negligible (214,249). The length of the hemodialysis session should be determined by the quantity of ethylene glycol ingested, but this rarely is known. Although blood ethylene glycol levels are helpful, they do not necessarily reflect the total quantity ingested because the blood ethylene glycol level is influenced by time since ingestion and amount metabolized. Dialysis should be continued for 8 hours if ethylene glycol levels are not available, and when levels are available, the dialysis prescription should be calculated using the total body water, blood ethylene glycol level, and the manufacturer-specified dialyzer urea clearance (mL/minute) at the initial observed blood flow rate (250). Bicarbonate-based dialysate is probably optimal compared with acetate dialysate, which is associated with greater hemodynamic instability, more central nervous system symptoms, and more oscillations in plasma bicarbonate (216,251). Although peritoneal dialysis clears ethylene glycol and oxalate, it should not be used over hemodialysis because of the high efficacy of hemodialysis (211).

Sulfonamide Antibiotics, Indinavir, and Acyclovir

Crystalline acute renal failure also can be caused by drugs used for therapeutic purposes. If the solubility limit of a given drug is exceeded in the renal tubules, the drug can then crystallize and possibly cause obstructive nephropathy. Certain sulfonamide antibiotics and acyclovir are the most common drugs that can cause crystalline acute renal failure, but other drugs such as methotrexate, triamterene, and high-dose vitamin C potentially can crystallize and cause stones or obstructive nephropathy (252–255,256). Before the acquired immunodeficiency syndrome (AIDS) era, crystalline acute renal failure had become fairly rare, but with the frequent use of high-dose sulfadiazine, sulfamethoxazole, indinavir, and acyclovir in this population, it is again an important cause of acute renal failure (257).

The sulfonamides were introduced into medical practice in 1936, and early animal experiments recognized that sulfonamides of low solubility were able to crystallize in the urinary

tract and renal parenchyma, causing obstructive nephropathy (258,259). Reports of patients with hematuria, crystalluria, renal colic, and renal failure were common until the 1950s, when sulfonamides with greater solubility became available (258,260). In patients with AIDS, high-dose sulfadiazine is again being used commonly in conjunction with pyrimethamine for the treatment of toxoplasmosis. After an oral dose, sulfadiazine is rapidly absorbed and then partially acetylated in the liver. The half-life of sulfadiazine is 8 to 17 hours in patients with normal renal function and 22 to 34 hours in patients with severe renal insufficiency (257). Renal crystal formation in the nephron is promoted as the filtrate is concentrated and acidified. The solubility of sulfadiazine is almost 10-fold higher at a pH of 7.5 than a pH of 6.5.

Patients with sulfonamide-induced renal failure classically present with renal colic, hematuria, acute renal failure, and oliguria or anuria (261,262). Although renal failure develops in most patients in the first week after the start of the sulfadiazine, patients also can present months after the start of the medication. Delayed presentation of acute renal failure usually occurs with the concurrent development of volume depletion, often due to diarrhea, and these patients can be managed with hydration without stopping the sulfadiazine (263,264). The urinalysis usually shows hematuria, mild pyuria, and "shock of wheat" crystals (Fig. 49-3). Renal ultrasonography may reveal multiple echogenic foci in the renal parenchyma, but occasionally shows frank hydronephrosis with ureteral stones (261,265,266).

The acute renal failure should be managed with intravenous fluids containing sodium bicarbonate with the aim of maintaining urine pH over 7.15 and urine output over 1 L/day. Urologic intervention sometimes is required in patients who remain anuric. Bilateral retrograde ureteral catheterization with warm 5% sodium bicarbonate solution, ureteral stents, and stone extraction with a stone basket all have been used in cases of ureteral obstruction with stones (267–270). Although temporary hemodialysis sometimes is necessary, recovery of renal function to baseline is the rule within 7 days (262,266).

Patients starting sulfadiazine therapy should receive prophylaxis against renal toxicity. To minimize crystal formation, patients should be encouraged to maintain fluid intake over 2 to 3 L/day and should be started on sodium bicarbonate (6 to 12 g/day) to maintain urine pH higher than 7.15 (262). Patients with renal insufficiency, diarrhea, or volume depletion should be monitored closely with urinalyses, looking for hematuria and crystalluria, and sulfadiazine levels should be considered in patients with renal insufficiency (262).

Indinavir is one of the most common protease inhibitors used in patients with AIDS as part of highly active antiretroviral therapy. Indinavir causes nephrolithiasis in 3% to 4%, and symptomatic urinary tract disease, including nephrolithiasis with renal colic, flank pain without evidence of stones, and dysuria or urgency, in 8% of patients taking the drug (271,272). Most patients presenting with symptomatic urinary tract disease have crystalluria, and many have radiographic evidence of either stones or renal parenchyma filling defects. However, only a minority have mild to moderate renal insufficiency. Hydration can prevent symptomatic urinary tract disease, but permanent discontinuation of indinavir is necessary in some because of recurrence of symptoms. Asymptomatic indinavir crystalluria is found in 20% of patients receiving the drug in the normal dosage of 800 mg orally, three times a day, and the drug should not be discontinued for asymptomatic crystalluria. *In vitro,* indinavir is more soluble at a pH of 4.5, but this is below the *in vivo* potential of the kidney (272). The presence of crystalluria and pyuria may signal the presence of interstitial nephritis, which may not reverse with conservative treatment with hydration alone (273).

High-dose acyclovir also is associated with acute renal failure and crystalluria. Early preclinical toxicology studies in animals clearly demonstrated that high-dose acyclovir given to rats resulted in precipitation of drug crystals in the distal nephron and also caused reversible obstructive nephropathy (274,275). Although it has been assumed that intratubular acyclovir crystallization also is responsible for the renal failure observed in humans, the pathophysiologic process is not entirely clear. Most reported kidney biopsy or autopsy specimens have not demonstrated intrarenal crystals, but typically show normal glomeruli, no obstruction, occasional ruptured tubules, and minimal focal areas of interstitial hemorrhage, congestion, and inflammatory infiltrates (276,277). In one case report of acyclovir nephrotoxicity, the renal biopsy was consistent with acute tubular necrosis without any evidence of intratubular crystals (278). Crystal dissolution during tissue fixation or the time interval between discontinuation of acyclovir and obtaining the renal biopsy could account for the inconsistent demonstration of crystals in renal tissue (278).

Renal impairment after intravenous acyclovir was commonly observed when bolus injections were used instead of slow infusions; one series reported that increased BUN or serum creatinine developed in 58 of 354 (16%) of patients 24 to 48 hours after administration of acyclovir (275,279). Unlike all other subsequent reports, one infant in this series with renal failure did show birefringent crystals in the renal tubules in the postmortem examination (275). In contrast to bolus injections, renal failure after slow intravenous infusions or oral acyclovir is less common, but does occur, especially in patients with renal insufficiency or volume depletion (276,280–282).

The acute renal failure caused by acyclovir typically develops 24 to 72 hours after the first dose of intravenous acyclovir. Unlike with the sulfonamide antibiotics, most patients do not have renal colic, stones, or ultrasonographic findings of obstruction. Many patients also have neurotoxicity, including headache, irritability, tremulousness, ataxia, nystagmus, lethargy, dysarthria, confusion, and coma (276,283,284). The urinalysis usually reveals both mild hematuria and pyuria, and examination of the urine with a polarizing microscope may show birefringent, needle-shaped crystals within leukocytes (276,283). Despite the development of massive acyclovir crystalluria in some patients, renal function may remain unaffected (277,285).

Risk factors for the development of acyclovir-induced acute renal failure include dehydration, bolus dosing, chronic renal failure, and an acyclovir serum level of greater than 25 μg/mL (275,282,286). Renal function in patients with acute renal failure usually normalizes within 4 to 9 days after drug discontinuation (276,286). Conservative management, with hydration and discontinuation of acyclovir, is sufficient in most patients, but in patients with combined severe neurotoxicity and nephrotoxicity, hemodialysis can be used to reduce serum acyclovir levels. This results in prompt reversal of acyclovir-associated neurologic symptoms (283). In cases of mild renal failure, acyclovir nephrotoxicity can be managed by hydration and dose reduction of acyclovir (282).

The half-life of acyclovir is 3 hours, and renal excretion is the major route of elimination. For example, over 90% of a given dose of acyclovir can be recovered unchanged in the urine of subjects with normal renal function 12 hours after dosing (287). There is a linear relationship between creatinine clearance and the renal clearance of acyclovir. The renal clearance of acyclovir is three times that of a given creatinine clearance, indicating significant tubular secretion (287). In subjects with preexisting renal insufficiency, the half-life of acyclovir can be as high as 20 hours, and dosing in renal insufficiency should be adjusted according to the level of renal function (287,288). Hemodialysis effectively removes acyclovir, reducing the

half-life to 5 hours, and can effectively remove 40% of acyclovir in body stores (283,287,289).

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SECTION VIII
HYPERTENSION

CHAPTER 50 ■ BLOOD PRESSURE AND THE KIDNEY

HUGH E. DE WARDENER AND GRAHAM A. MACGREGOR

Ten to fifteen percent of the world's population suffers from hypertension, but the cause can be identified in only 5%. Absurdly, hypertension is referred to as essential hypertension when the cause cannot be identified, as if other causes were nonessential. It now seems increasingly likely that the initiating fault underlying hypertension of unknown cause (essential hypertension) is a covert abnormality of the kidney. It appears therefore that nearly all patients with high blood pressure have a renal abnormality for the cause of hypertension, when known, usually is an overt abnormality of the kidney.

HISTORICAL BACKGROUND

Bright, Hypertension, and Advanced Renal Disease

Bright first proposed the notion in 1841 that hypertension is in some way connected with the kidney (1). He performed postmortem examinations on patients who had died from "dropsy and coagulable urine" in whom he stated there was always "some obvious derangement of the kidneys." He also noted that the left ventricle was enlarged and the wall hypertrophied. "What is most striking," he pointed out was that, " . . . there were twenty two (patients) without any probable organic cause (e.g., a valvular lesion) for the marked hypertrophy." In trying to find an explanation for the association of disease of the kidney and left ventricular hypertrophy, Bright is credited with a proposal that, after many vicissitudes, appears to be increasingly relevant today.

Bright had noted that the radial pulse of these patients was hard. Although he could not measure the arterial pressure, fluctuating hydrostatic pressure within the artery was a well-accepted phenomenon; therefore, a hard pulse denoted raised arterial pressure. Harvey (2) had demonstrated the presence of arterial pressure by cutting an artery, and Hales had measured the arterial pressure of a horse (3) with a glass tube inserted into the femoral artery (Fig. 50-1). Bright (1), searching for a cause for cardiac hypertrophy and hypertension, concluded that,

> This leads us to look for some less local cause for the unusual effects to which the heart has been impelled; and the two most ready solutions appear to be; either that the altered quality of the blood affords irregular and unwanted stimulus to the organ (the heart) immediately; or that it so effects [sic] the minute and capillary circulation as to render greater action necessary to force the blood through the distant subdivisions of the vascular system.

The "altered quality of the blood" referred to those substances he rightly assumed severely diseased kidneys could no longer excrete. The idea that an "unwanted stimulus" to the heart (i.e., an increase in cardiac output) might raise blood pressure was not taken up until the 1960s (4), when it was put forward as a possible factor for the onset of a pressure rise in some patients (see later discussion). The second proposal, that a greater action of the heart is necessary to force blood through the vascular system, indicates that Bright considered that raised arterial pressure might cause ventricular hypertrophy.

Bright did not specify which "distant sub-division of the vascular system" he had in mind. One reads the word "distant" and assumes that he was referring to all the vessels in the body. Bright's contemporaries, however, clearly thought he was referring " . . . solely to the resistance of the small renal arteries" (5); in other words, the rise in blood pressure was necessary to force the blood through the kidneys. It is possible that this interpretation was derived from some anatomic dissections of kidneys made by Toynbee (6) working in collaboration with Bright. He found that in the final stages of Bright's disease when the kidneys were contracted, the renal arteries and vessels in the "malpighian tubules" (the glomeruli) were so contracted that they were very difficult or impossible to inject via the renal artery. Subsequently, Johnson in 1868 (5) drew attention to the fact that, in contrast to Bright's opinion, all the minute arteries of the body were involved in that the walls of the very small arteries of the brain and pia mater, as well as those in the kidneys, were hypertrophied. Like Bright, he thought this change directly resulted from an abnormality of the blood that was "contaminated by urinary excreta and otherwise deteriorated."

Traube (7) made a fascinating suggestion in 1871. He pointed out that "the shrinking of the renal parenchyma will . . . act by decreasing the amount of liquid which is . . . removed from the arterial system by urinary excretion. As a result . . . the mean pressure of the arterial pressure must increase." This inspired teleologic hypothesis anticipated Fahr (8), Borst and Borst-de-Gues (9), and Guyton's (4) identical (although more detailed) proposals by about 100 years, even though it leaves unexplained the mechanisms that cause blood pressure to rise. Traube's assertion that hypertension is a compensatory phenomenon to increase an impaired urinary excretion toward normal is now considered to be relevant to most forms of hypertension, including those in which there is no overt renal disease such as essential hypertension. Borst and Borst-de-Gues's (9) summing up of this idea and its overall effect was that the arterial pressure rises to correct the kidney's "unwillingness to excrete sodium. Thus a seemingly normal sodium output is maintained at the expense of the hypertension."

Essential Hypertension and Nephrosclerosis

The next step forward was that of Mahomed in 1879 (10). He observed that, "it is very common to meet people, apparently in good health, who have no albumin in the urine or any other sign of organic disease, who constantly present a condition of high arterial tension." He found at postmortem examination that the kidneys of such patients, who had not had albuminuria, were "red" rather than "yellow," like those who had died with

FIGURE 50-1. Stephen Hales and assistant measuring blood pressure of a horse. (From: Pickering GW. Systemic arterial hypertension. In: Fishman AP, Richards DW, eds. *Circulation of the blood, men and ideas*. New York: Oxford University Press, 1964:487, with permission.)

albuminuria and dropsy; that is, they did not look very abnormal, although they were somewhat contracted. Patients without proteinuria had come under treatment for "symptoms of cerebral haemorrhage, heart disease, lung disease and sundry medical and surgical disease." This is the first description of essential hypertension, a condition of sustained hypertension not due to overt renal disease. Mahomed concluded that

> high pressure is a constant condition in the circulation of some individuals and that this condition is a symptom of a certain constitution or diathesis. . . . These persons appear to pass through life much as others do and generally do not suffer from their high blood pressure except in their petty ailments upon which it imprints itself. . . . As age advances the enemy gains accession of strength. . . . The individual has now passed 40 years of age, his lungs begin to deteriorate, he has a cough in the winter time, but by his pulse you will know him. Alternatively headache, vertigo, epistaxis, a passing paralysis, a more severe apoplectic seizure, and then the final blow. . . . Of this I feel sure, that the clinical symptoms and the pathological changes resulting from high arterial pressure are frequently seen in cases in which slight, if any, disease is discoverable in the kidney.

Mahomed's proposals overlapped certain conclusions put forward a few years earlier by Huchard (11) in Paris, who had written that: "It has been wrongly assumed that chronic hypertension only appears following interstitial nephritis (glomerular nephritis). The opposite is true; arterial hypertension is the cause of arteriosclerosis; it precedes by a varying time interval the evolution of different diseases (heart disease, arterial nephritis, etc.) which are in turn secondary to vascular sclerosis."

In addition to the suggestion attributed to Bright that occlusive changes in the renal arteries are responsible for the associated rise in arterial pressure, Huchard put forward the concept that hypertension itself could cause widespread occlusive arterial changes (including in the renal arterial tree) that he called "arterial nephritis," what we now call hypertensive vascular changes or nephrosclerosis, when present in the kidney.

Malignant Hypertension

These seminal observations were confirmed, elaborated, forgotten, and revived during the next 100 years. The next step forward was Volhard's (12) demonstration in 1931 that primary hypertension could have either a benign or malignant course. The benign form of hypertension, although accompanied by enlargement of the heart, might remain unchanged for years (as Mahomed had noted), the patient eventually dying of heart failure or a cerebrovascular accident while renal function remained relatively unimpaired throughout. Malignant hypertension was heralded by acute retinal changes and then increasing renal functional deterioration, the patient rapidly dying of renal failure. Volhard considered that benign essential hypertension was caused by a primary thickening of the systemic arteriolar walls that he called elastosis (i.e., full of elastic tissue), which he suggested was due to the effects of age on a "genetic constitution." He thought that malignant hypertension resulted from the action of a renal pressor substance released because of renal ischemia, which in turn resulted from severe "elastosis" of the renal arteries or glomerular nephritis that reduced renal blood flow by contracting the kidneys. He was also the first to suggest that vascular spasm of the renal vasculature produced by a pressor substance released by the kidney could lead to a vicious cycle effect that released more pressor substance. The concept of a circulating pressor substance resulted from the work of Tigerstedt and Bergman (13), who showed in 1898 that saline extracts of fresh rabbit kidney or an alcohol-dried powder produced a prolonged rise of pressure when injected into rabbits that were lightly anesthetized with urethane. They named this substance renin.

Volhard's hypothesis about the origin of malignant hypertension was supported first by the observation of Goldblatt et al. (14) in 1934 that renal ischemia in dogs, induced by partially constricting renal arteries, induced persistent hypertension that was caused by some substance released by the kidney. However, it took another 56 years before Tigerstedt and Bergman's observations led to the identification and structure of horse and ox angiotensin II by Skeggs et al. (15) and Peart (16), respectively. Volhard's vicious cycle hypothesis was supported by Wilson and Byrom's (17) experiments in rats, in which hypertension was induced by a partially occluding renal artery clip placed on one renal artery while the other kidney was left intact. In the first few weeks thereafter, removal of the "clipped" kidney was associated with a return of arterial pressure to normal, but removal of the clipped kidney did not lower arterial pressure if the clip was removed later (after the unclipped kidney had suffered irreversible vascular damage). Rettig et al. (18) confirmed, by transplanting the unclipped kidney into a normal rat that then develops hypertension, that the persistence of hypertension after the clip is removed is caused by the hypertensive vascular changes in the unclipped kidney.

Renal Origin of Hypertension

Mahomed's notion that the kidney in nonmalignant essential hypertension is normal before being damaged by the rise in pressure was continuously reaffirmed (19–21). Bell (19), a pathologist, concluded in 1946 that "hypertension may develop in persons whose renal vascular lesions are no greater than is found in non-hypertensive controls." It was not until the emergence of hereditary strains of hypertension in the rat

that Borst and Borst-de-Gues (9) and Guyton's (4) claim began to be accepted that the kidney is abnormal in essential hypertension. This followed the finding that hereditary hypertension in the rat strongly resembles essential hypertension in humans, and that the kidneys in these strains, as in essential hypertension, are abnormal before the onset of hypertension, thus excluding the possibility that the high blood pressure itself is responsible for the renal abnormality.

Renal Origin of Hypertension in Hereditary Strains in Animals

The principal evidence that the initiating hypertensive trigger in hereditary rat strains of hypertension resides in the kidney has been obtained in renal cross transplantation experiments (22,23) (Fig. 50-2). This experiment was carried out from 1960 on by many different groups of research workers using four different strains of rat—the Dahl salt-sensitive rat, spontaneously hypertensive rat, stroke-prone spontaneously hypertensive rat, and Milan hypertensive rat (24–30). A unilateral nephrectomy is performed on both rats, and the remaining kidney from each is then cross-transplanted. Transplanting a kidney from a hypertensive to a normotensive strain rat raises blood pressure of the normotensive rat. To avoid the possibility that preexisting hypertensive vascular changes in the graft induce pressure rises in recipients, some of the kidneys from hypertensive strain rats were taken from prehypertensive 7-week-old or 20-week-old rats, blood pressure of which was kept within normal limits from the age of 4 weeks with a converting enzyme inhibitor. Nevertheless, blood pressure of the rat that received the kidney still rose to the same extent. In the reverse set of experiments, a kidney from a normotensive strain rat, when placed into a young hypertensive strain rat before the onset of hypertension, prevented the onset of hypertension. If placed into an older rat with established hypertension, it lowered the blood pressure. These experiments clearly demonstrate that an underlying genetic defect in the kidney is responsible for high blood pressure in these rats with hereditary hypertension.

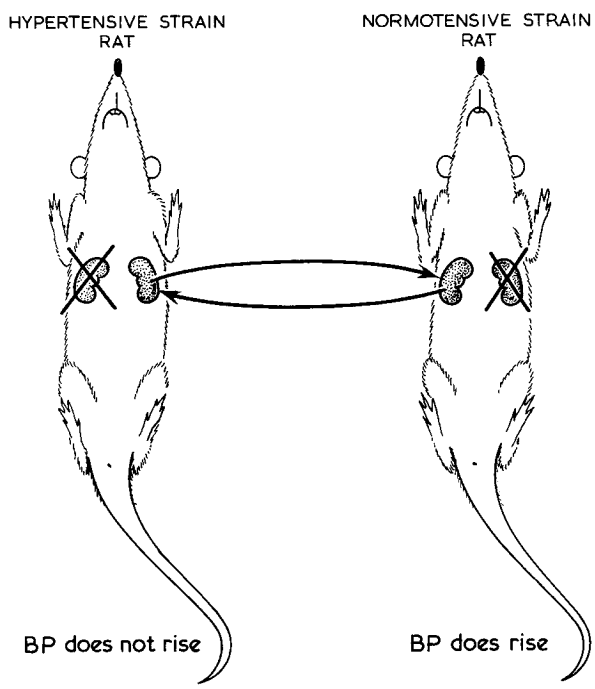

FIGURE 50-2. Cross-transplantation experiment between an inherited hypertensive strain rat and a control normotensive strain rat demonstrating that the kidney carries a genetic abnormality that causes blood pressure (BP) to rise. One kidney is removed from each rat, and the remaining kidney is then cross-transplanted into the other rat. The experiments are performed before the arterial pressure has risen in the hypertensive strain rat. (From: de Wardener HE. *The kidney,* 5th ed. Edinburgh: Churchill Livingstone, 1985:260, with permission.)

Renal Origin of Essential Hypertension

In essential hypertension, the nearest equivalent to the cross transplantation experiments in animals is the relation of the recipient's blood pressure, after renal transplantation, to blood pressure of the donor's family. In one study of 50 recipients, those from normotensive families who received a kidney from a donor with a hypertensive parent needed significantly more hypertensive therapy than those who received a kidney from a normotensive family (31). In addition, there is a study of six black hypertensive patients with terminal renal failure owing to nephrosclerosis from severe essential hypertension (32). Their blood pressure fell to normal without the need for hypertensive treatment after they received a kidney from young normotensive donors. The average follow-up was 4.5 years.

Nature of the Renal Abnormality Responsible for Hypertension in Inherited Hypertension

The principal overall abnormality appears to be an impaired ability to excrete sodium. In cross transplantation experiments in the rat, sodium is retained as blood pressure rises by the recipients of hypertensive strain kidneys (33,34), which suggests that the rise in blood pressure is directly related to an impaired ability of the renal graft to excrete sodium. Recent observations in the spontaneously hypertensive rat (SHR) demonstrated that the impaired ability to excrete sodium is present at 3 to 7 weeks of age and that such kidneys have a blunted natriuretic response to arterial pressure associated with an increase in renal vascular resistance owing to a narrowing of the afferent arterioles. The most marked changes occur in the papillae, where there is a reduction in blood flow and interstitial hydrostatic pressure. Such vascular changes certainly could contribute to the impaired ability of the kidneys to excrete sodium (35–39). In addition, the kidney in essential hypertension and several strains of hypertensive rats is the site of several other, inherited abnormalities that could alter the ability of the kidney to excrete sodium (see the following).

In humans renal vascular resistance is raised in normotensive children of hypertensive parents (40) and there is considerable evidence that they also have an impaired ability to excrete sodium. In some patients with essential hypertension who have an exaggerated increase in blood pressure following an intravenous infusion of saline (41), there is a detectable impairment in their ability to excrete an acute sodium load (42).

Urinary sodium excretion is closely related to dietary intake. When the kidney has difficulty excreting sodium, the consequences that follow are more severe, that is, the greater the amount that has to be excreted. Thus, as the evidence accumulates, studies demonstrate that hypertension is closely related to the dietary intake of sodium.

Summary

Bright proposed (or at least is given credit for proposing) that hypertension associated with severe renal disease is caused by occlusive changes to the distant subdivisions of the vascular system of the kidney (1). Subsequently it was found that hypertension without severe renal disease (i.e., essential hypertension), can occur without microscopic vascular abnormalities in the kidneys; therefore, it was deemed that essential hypertension is unrelated to any abnormality of the kidney, in contrast to hypertension with severe renal disease.

In the past 40 years, however, evidence has accumulated that although the renal vessels in an animal model of hypertension that closely resembles essential hypertension appear to be normal on microscopy before the rise in pressure, they are functionally abnormal in that they have an increased tone. Such an increase, together with several other documented renal abnormalities in both essential hypertension and hypertensive strains of rats, could be responsible, in part, for the "unwillingness" of these kidneys to excrete salt. There is now evidence that even a single monogenetic lesion, intrinsic to the kidney, such as Liddle's syndrome, which impairs sodium excretion, is sufficient itself to cause blood pressure to rise (43). Thus, both hypertension associated with severe renal disease, first described by Bright, and essential hypertension are renal diseases, and the principal functional abnormality appears to be an impaired ability to excrete sodium.

SALT INTAKE AND BLOOD PRESSURE

If essential hypertension is caused by an impaired ability to excrete sodium, then there should be some relation between salt intake and hypertension.

History of Salt in Human Affairs

The human race is considered to have been on the earth for about 3 million years, and those living inland had to eat a low-salt diet (approximately 10 to 15 mmol/day) until about 5,000 years ago. In other words, the human race is genetically programmed to eat a low-salt diet. This was accomplished by increasing the tongue's exceptional ability to detect minute quantities of salt, and by a remarkable capacity to conserve sodium by reducing sodium lost in sweat and urine. (It is possible to reduce 24-hour urine loss to less than 1 mmol.) Salt intake rose when humans discovered that salt had the "magical" property of being able to preserve food. Initially, salt was obtained with great difficulty from the sea and was one of the precious cargoes carried inland by camel caravans across the Arabian and African deserts. The Roman army, which at first issued a monthly ration of salt to each man, eventually found it more convenient to pay each man a monthly lump sum (a salary) with which to purchase salt. Salt's ability to preserve food was of vital economic importance. It is probable, however, that the consumption of highly salted preserved food meant that fresh food tasted bland, and not surprisingly, those who could afford it started to add salt to unsalted food. The increasing use of salt made it an important object of commerce, especially for those countries that bordered on the sea or had salt mines. The salt trade was the underlying foundation of the Venetian empire that lasted nearly 800 years (44). The Chinese had a salt tax circa 2000 BC. Governments used the desire for salt as a source of revenue, much as they now use that for alcohol. The demand for salt was so powerful and pervasive that smugglers took enormous risks to bypass the tax. In the 17th century, about one-third of the galley slaves in France had been sentenced for violation of the salt tax. It was the most hated of all taxes, and in the next century was one of the major causes of the French Revolution (45).

By 1850, salt was no longer expensive, and it was estimated that salt consumption in England and France per capita was about 20 g/day (330 mmol), half added to the food at table, the other half present in food. The introduction of refrigeration reversed this trend, and during the 20th century salt consumption tended to fall. Nevertheless, in Western communities, it is still 100 to 400 mmol/day, with an average intake of 150 mmol in Europe and the United States, at least six times more than late Paleolithic man (46). Now, less is added at the table than in 1850, but processed foods are responsible for the 70% to 80% of the total intake.

THE INFLUENCE OF SALT INTAKE ON BLOOD PRESSURE

The relation of salt intake to blood pressure has been revealed by (a) epidemiologic studies, (b) measuring the effect of increasing and decreasing the intake of salt on the normal blood pressure, and (c) decreasing salt intake in patients with hypertension.

Epidemiologic Studies

There have been nearly 40 accounts of certain unacculturated populations in which blood pressure did not rise with age—in other words, such populations did not have essential hypertension (47). Their dietary intake of salt was below 3 g/day, in a few it was below 1 g/day, and in one it was about 0.05 g/day. At the other end of the scale, there have been studies among Japanese and Portuguese communities with a high prevalence of hypertension in one of which the salt intake averaged 26 g/day. The bulk of Westernized societies, which consume around 7 to 12 g of salt/day with an average of 9 g/day, are in between. The connection between salt intake and hypertension in these intermediate populations is evident but more difficult to discern mainly because of the relatively narrow range of salt intake (approximately 6 to 10 g/day) and the wide fluctuations of 24-hour urinary salt excretion (used to measure salt intake), which is owing to the variable day-to-day salt intake of individuals in most developed countries.

Unacculturated Populations

Low-salt-eating unacculturated populations used to inhabit the tropics of Africa, South America, and the Pacific and Arctic regions. The outstanding example is the Yanomamo Indians in South America. Their staple foods are cooked bananas and manioc; they have little access to salt. Their mean 24-hour urinary excretion of salt is around 0.05 g/day and their potassium intake is about 3 g/day. The Yanomamo culture encourages chronic warfare and violence (48), yet there is no rise in blood pressure with age.

Nigeria

In two related rural tribes in Nigeria only one had access to salt. There was a highly significant difference in blood pressure between the two tribes, the one with access to salt having the higher arterial pressure. The two tribes were identical in all other respects.

The Iranian Qash' qai

The relation of salt intake to blood pressure in this primitive nomadic tribe complements the others. Throughout the area that this tribe has inhabited for approximately 400 years are natural surface deposits of salt, which the tribe uses liberally in baking and cooking as well as at the table. The average urinary sodium excretion is 186 mmol/day in the men and 141 mmol/day in the women. The prevalence of hypertension in those over 30 years of age is 12% and 18%, respectively, with no tendency for increased weight with age.

Thus, whatever subsidiary factors may be suggested, other than the dietary intake of salt to account for the stability in blood pressure with age in unacculturated people on low-salt intakes, they did not prevent a rise in blood pressure in the unacculturated tribe, which consumes a high-salt diet.

Migratory Studies

There are examples of groups of individuals from low-salt-eating communities whose blood pressure rises when a change in their circumstances causes them to eat more salt. The best of these was a carefully controlled study from Kenya where subsistence farmers ate a low-salt/high-potassium diet. Some of the farmers migrated to an urban community where there was a marked increase in salt intake and a fall in potassium intake to levels similar to those in Western countries. Blood pressure in these migrants rose, whereas there was no change in blood pressure in a control group that did not migrate. It was concluded that a major part of blood pressure rise that occurred on migration resulted from the increase in salt intake (49).

High-Salt-Intake Societies

There are several societies that continue to have a high salt intake, comparable to that of most Western societies during the 19th century, before the introduction of refrigeration and canning. These include Portugal, northern China, and Japan. Japan has been studied the longest.

Japan

In the late 1950s, the Japanese became aware that certain northeastern parts of the country, which had a very high consumption of salt, had the highest incidence of death from stroke (owing, at that time, to brain hemorrhage from a ruptured cerebral artery), and that in these areas the prevalence of high blood pressure also was greater than in the rest of the country (Fig. 50-3). For instance, the number of hypertensive individuals aged between 50 and 60 years in one community in the northeast in which the salt intake was 26 g/day was 70%, whereas it was only 10% in the south where the salt intake was 14 g/day.

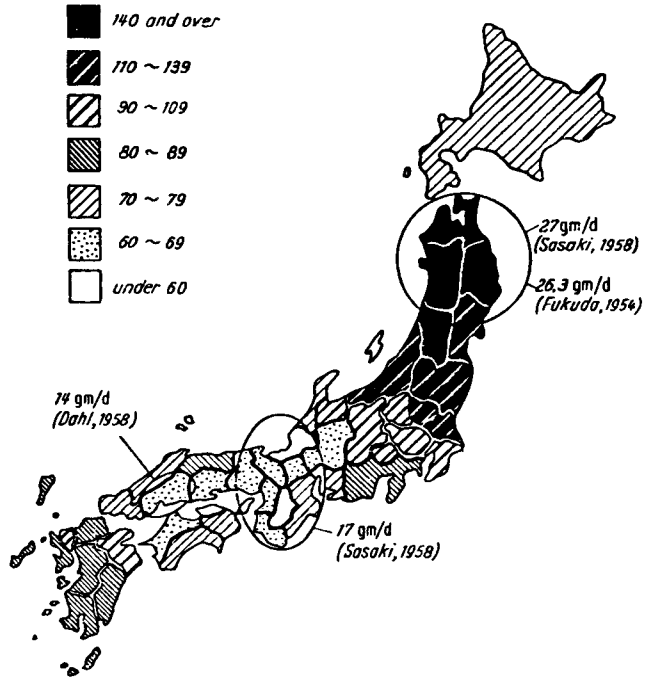

FIGURE 50-3. Regional distribution of cerebral hemorrhage in Japan in 1957–highest in the north and lowest in the south. The intake of sodium in four areas is also indicated, 14 g/day in the south and 27 g/day in the north. (Reprinted from: Takahashi E, et al. The geographic distribution of cerebral hemorrhage and hypertension in Japan. *Hum Biol* 1957;29[2]:139, with permission.)

THE RELATIONSHIP OF BLOOD PRESSURE TO SALT INTAKE WITHIN AND BETWEEN POPULATIONS

Studies of rural Asian communities in which there is a wide range of salt intake but each individual's intake is relatively constant have repeatedly shown a direct within-center relationship between 24-hour urinary sodium excretion and blood pressure (50–54). In Western cultures, however, in which the within-individual daily salt intake varies considerably, it has been difficult until recently to detect a significant relationship within populations. In addition, early attempts to correlate data among several international centers were criticized in that the various centers from which the data were obtained had used different criteria and there had been a failure to take into account many confounding variables. Thus, interesting cross-center linear relationships between blood pressure and sodium intake, which were repeatedly demonstrated from data collected worldwide (55,56), consistently failed to be universally convincing.

An extensive and very well controlled international epidemiologic study (the Intersalt Study) that avoided the weaknesses of previous investigations was carried out in 10,079 men and women aged 20 to 59 years sampled from 52 centers around the world. They were studied using a standardized protocol, central training, and a central laboratory (57,58). After standardization for age, sex, body mass index, alcohol intake, and potassium excretion, within-center analyses showed that sodium excretion was significantly related to systolic pressure among individuals in a population, and cross-center analysis showed that sodium excretion is significantly related to the rise in systolic and diastolic pressure with age (Fig. 50-4). A conservative estimate of the size of this effect is that, on average, a reduction in sodium intake of 100 mmol/day between the ages of 25 and 55 years corresponds to a 9 mm Hg lower rise in systolic pressure. This is an underestimate because only one 24-hour urine sample was obtained from each person to measure the sodium excretion. Within-individual variations in daily sodium excretion, however, may be considerable (e.g., more than fivefold). When multiple regression analyses are calculated, factors that fluctuate to a large degree in this way tend to appear less important than those, such as weight, that fluctuate much less (an effect known as regression dilution bias). In order to obtain a correct regression coefficient in Western communities in which daily urinary sodium excretion fluctuates most widely, it would be necessary to sample five to 14 24-hour samples (59–62). This is impractical in a large survey. In the Intersalt Study, a partial adjustment was attempted by calculating a coefficient of reliability from the results of two 24-hour urine samples collected from 807 participants at random.

Once again, the Intersalt Study demonstrated that in geographically isolated centers where urinary sodium excretion rates were below approximately 60 mmol/day, of which there were four in the Intersalt Study, blood pressure does not rise with age, and hypertension is rare. In the past, the applicability

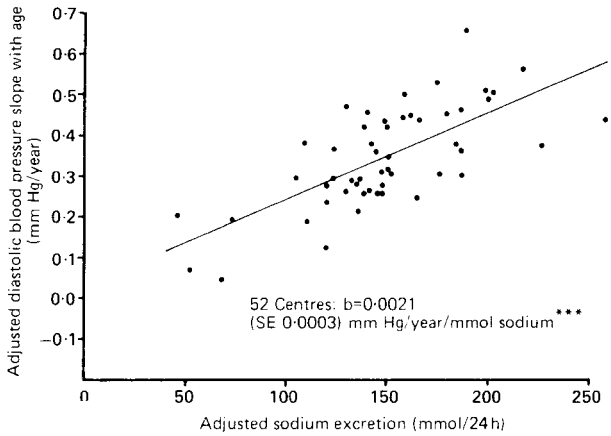

FIGURE 50-4. Cross-center plots of diastolic blood pressure slope with age, and median sodium excretion and fitted regression lines standardized for age, sex, body mass index, and alcohol consumption for 52 centers. (From: Intersalt Cooperative Research Group. Intersalt: an international study of electrolyte excretion and blood pressure. Results for 24-hour urinary sodium and potassium excretion. *Br Med J* 1988;297:319, with permission.)

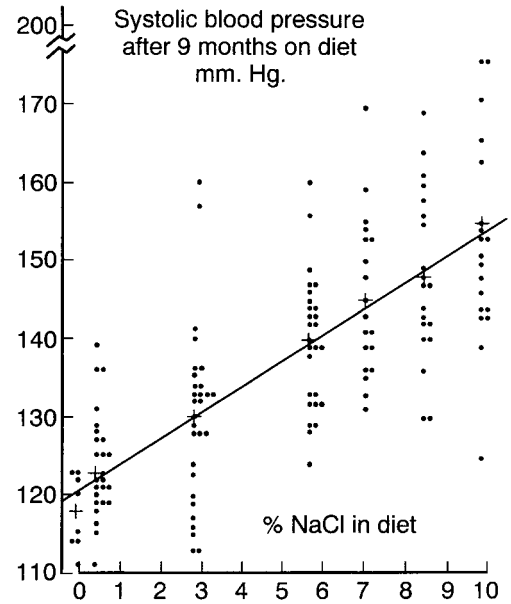

FIGURE 50-5. The effect of various intakes of salt for 9 months on the systolic blood pressure of normal rats. (From: Bell CD, Meneely GR. Observations on dietary sodium chloride. *J Am Diet Assoc* 1957;33:366, with permission.)

of such observations to other societies, particularly Western societies, tended to be dismissed on the assumption that the general health and daily life patterns of such isolated unacculturated societies was probably more relevant to their low blood pressure than their intake of sodium (63). It appears that this assumption is unwarranted, at least regarding their physical features. The local organizers at each of the four Intersalt centers with sodium intakes below 60 mmol/day reported that the participants were physically active, appeared healthy, and showed no sign of malnutrition or protein deficiency (64).

The Effect of Increased Salt Intake on Normal Blood Pressure

Normal Animals

The prolonged ingestion of increased quantities of salt causes hypertension in the normal dog (65,66), chicken (67), rabbit (68), baboon (69), rat (70–72), and chimpanzee (73). The rate of rise in blood pressure depends on the amount ingested. Hypertension usually comes on within a few weeks, is sustained thereafter, and is proportional to the intake. The effect is more marked in animals exposed just after birth (69,70,74) and in males (69,75).

Salt intake was first unequivocally demonstrated in animals as an initiator of hypertension by Meneely and associates (71), who fed groups of ordinary stock rats a range of dietary sodium intakes. At the end of 9 months, the mean blood pressure of each batch was directly related to the intake of sodium (Fig. 50-5). Dahl (76) confirmed these findings and made an additional observation of immense importance. He found that in each batch of rats' blood pressure rose in some rats but not others, and that this "sensitivity" and "resistance" of blood pressure to a high-salt diet was genetically determined. At about the same time, Smirk and Hall (77) bred a strain of rats that became hypertensive with age on a normal sodium intake (the first strain of "spontaneously hypertensive" rat) and another strain that did not develop hypertension with age.

A recent study in chimpanzees (73) phylogenetically our closest relation (98.4% genetic identity) is the most convincing testimony that excess sodium probably causes hypertension in humans. Normally chimpanzees, which live up to 50 years and weigh up to 50 kg, eat a diet of fruit and vegetables with sodium content around 10 mmol/day. A gradual increase in sodium intake in a colony of chimpanzees over 20 months to 200 mmol/day increased blood pressure by 33/10 mm Hg. Blood pressure returned to values present at the beginning of the study when the salt intake was lowered to 10 mmol/day (Fig. 50-6). This experiment shows that if the animal species most closely related to humans (which normally consumes a low-salt diet, as both they and humans are genetically programmed to do) increases its salt intake into the same range as present-day humans, then they will develop hypertension, just as humans do.

Normal Humans

There do not appear to be any studies comparable to those made in animals of the effect of an imposed, prolonged increase in salt intake on blood pressure of normotensive humans previously accustomed to a low salt intake; that is, a situation comparable to the chimpanzees. Salt intake has been increased for relatively short periods in the normal high-sodium diet of young adults (150 mmol/day), and this has induced only small changes in blood pressure. For instance, in four out of five studies (78–82), increases in sodium intake up to 420 mmol/day for up to 4 weeks in young or middle-aged (<47 years) normotensive subjects did not cause a rise in arterial pressure.

Normotensive Offspring of Hypertensive Parents

Observations in monozygotic and dizygotic twins have established that in normal subjects there are "strong heritable influences on the renal excretion of sodium which are most readily identified in the volume expanded state" (83); and that following a relatively slow infusion of saline (2 L in 4 hours), normotensive, first-degree relatives of patients with essential hypertension excrete less sodium than control subjects do (84). The relation of habitual salt intake to blood pressure, as measured by 24-hour urinary sodium excretion, has also been studied in normotensive offspring of two hypertensive parents (n = 41), normotensive offspring of one hypertensive parent (n = 52), and normotensive offspring of two normotensive

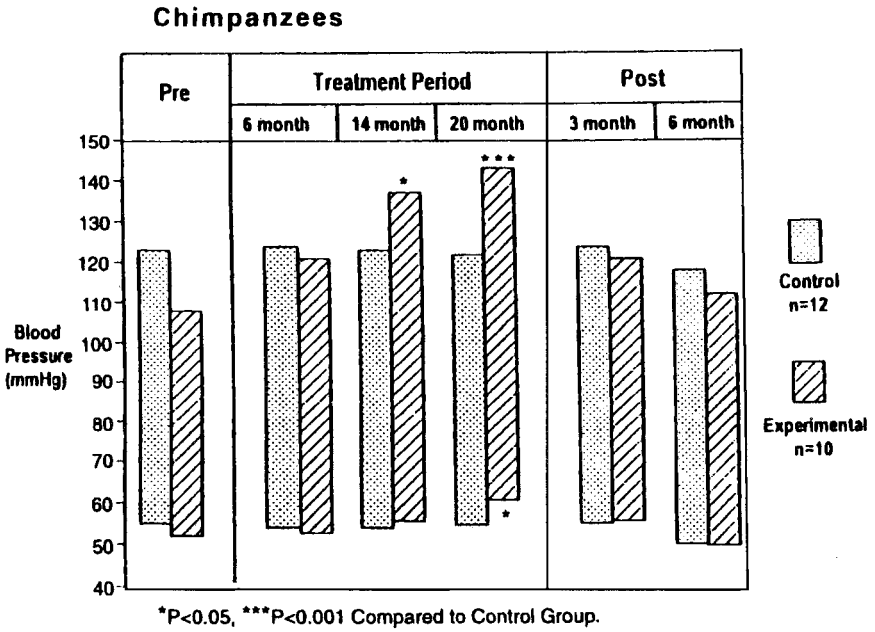

FIGURE 50-6. Blood pressure in chimpanzees who either continued on their usual diet (sodium 10 mmol/day) or were given an increased salt intake (sodium 200 mmol/day). At the end of the 20-month study, the salt supplements were stopped, and blood pressure declined to that of the control group. (From: Denton D, et al. *Nat Med* 1995;I:1009, with permission. Figure reproduced from: MacGregor GA. Salt: blood pressure, the kidney, and other harmful effects. *Nephrol Dial Transplant* 1998;13:2471, with permission.)

parents (n = 61). The mean age of the three groups was 21, 22, and 23 years, respectively. The 24-hour urinary sodium excretion was similar in the three groups, but although there was a positive association between 24-hour urinary sodium excretion and systolic blood pressure in the offspring of two hypertensive parents, no such association was apparent in the offspring of two normotensive parents (85).

In contrast to a group of normotensive children of normotensive parents, a high dietary intake of sodium (270 mmol/day for 7 days) by normotensive children (mean age 25 ± 3 years) of hypertensive parents causes an increase in blood pressure (86). The effect of a relatively slow intravenous infusion of saline (2 L in 4 hours) has been studied frequently (87–89). Again in contrast to normotensive children of normotensive parents, an intravenous infusion to normotensive offspring of hypertensive parents causes a rise in blood pressure associated with a lesser rise in sodium excretion. The reduced excretion of sodium is consistent with the evidence that the kidneys in essential hypertension have an inherited abnormality of their ability to excrete sodium.

The Effect of Reducing Salt Intake on the Blood Pressure of Normal Humans

The effect of a reduction in salt intake on the arterial pressure of normal subjects has been studied in neonates, school-age children, and adults.

Neonates

The blood pressure in normal newborn babies has been found to be particularly sensitive to a prolonged small reduction in sodium intake. Hofman et al. (90) allocated 476 babies into two groups, one of which had a normal sodium intake and the other a lower intake for 6 months. The normal intake was three times greater than the lower. There was a progressive and increasing difference in systolic pressure between the two groups, so that the mean systolic pressure at 6 months was significantly higher in those on the higher sodium intake. Of the original 476 subjects, 167 were reinvestigated when they were 15 years old; blood pressure difference persisted in spite of the same salt intake in both groups in the interval (91) (Fig. 50-7). This apparent prolonged effect on blood pressure of an increase in salt intake early in human life is consistent with many examples in animals that there is a period of high susceptibility to the effects of increased salt intake in youth (74).

Children

In four studies in children, the significance of the results was greatly influenced by the number of participants. In one study

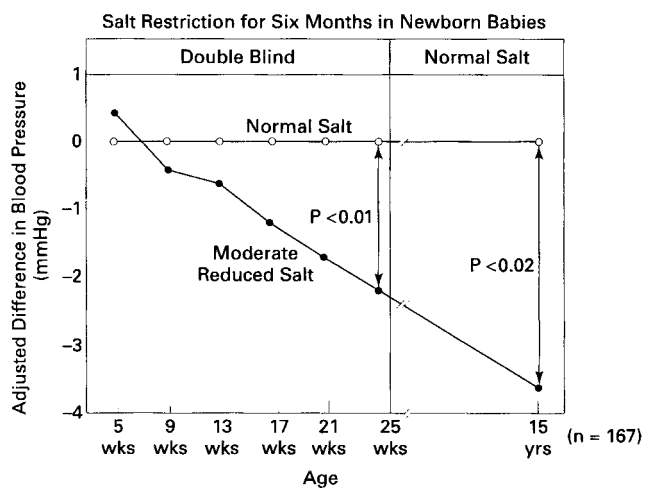

FIGURE 50-7. This study began with 476 babies. The figure illustrates the differences in blood pressure between two subgroups who were studied for the first 6 months of life and 15 years later. The heading "Double Blind" covers the first 6 months during which half the babies consumed a normal intake of salt and the other half a lower intake. At the end of 6 months blood pressure of the group that received the lower intake of salt was lower than that of the group that received a normal intake. For the next 14.5 years both groups ate a diet with a normal intake of salt. The blood pressure difference between the two groups, as measured in 167 of the original 476, in the 15th year is illustrated under the heading "Normal Salt." It can be seen that the difference in blood pressure that was present at 6 months is still present, although both groups have been eating the same amount of salt since that time. (Drawn from data in Hofman A, et al. *JAMA* 1983; 250:370, and Geleijnse et al. *Hypertension* 1996;29:913, with permission.)

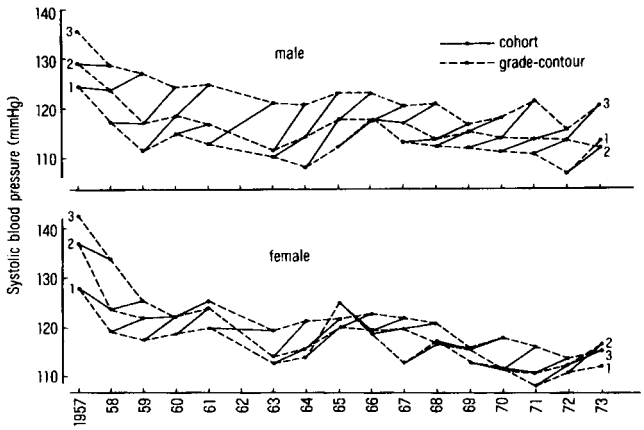

FIGURE 50-8. Mean systolic blood pressure levels in school children in grade 1 (12 to 13 years old), grade 2 (13 to 14 years old), and grade 3 (14 to 15 years old). The blood pressure of each new intake into these grades was measured at the beginning of the year. (From: Sasaki N. In: Yamori Y, et al., eds. *Prophylactic approach of hypertensive diseases*. New York: Raven Press, 1979:467. Reprinted by permission of Lippincott Williams & Wilkins.)

with a total of only 80 children, a reduction in sodium intake from 86 mmol/day to 56 mmol/day had no effect on blood pressure at the end of 1 year (92). In the second study with a total of 124 adolescents, a reduction in sodium intake of 65 mmol/day to an intake of 45 mmol/day for 24 days induced a nonsignificant fall in blood pressure (93). In a third group of 149 children, a reduction in sodium intake from 105 mmol/day to 50 mmol/day induced a significant fall in blood pressure after 12 weeks but only after adjustment for age and weight (94). With a total group of 750 children in the fourth study, however, a reduction in sodium intake from 166 mmol/day to 127 mmol/day induced a significant fall in blood pressure at 24 weeks (95). Records from one school in Japan between 1957 and 1972, during which there was a national reduction in dietary salt intake, show a steady decline in blood pressure in children between of 12 and 14 years of age (96) (Fig. 50-8).

Adults

There are over 100 trials in adults on the effect of a reduction in salt intake on blood pressure of a relatively small number of normal subjects. Four older meta-analyses limited to random controlled trials (97) agree that there is a significant fall in blood pressure of 2/1 mm Hg. It is difficult to know what to conclude from such trials because most of them lasted for less than 2 weeks and some involved very large acute reductions in salt intake that transiently stimulate the sympathetic nervous system. A more recent meta-analysis of studies of 1 month or more did show highly significant falls in blood pressure and a dose response—i.e. the greater the reduction in salt intake, the larger the fall in blood pressure. Indeed, a 6 gram reduction in salt intake would cause a fall in blood pressure of 7/4 mm Hg in hypertensives and 4/2 mm Hg in normotensives (98).

There is some evidence of the effect of prolonged reductions in salt intake, but it was obtained by studying whole communities, which precluded it being randomly controlled.

The Effect of Reducing Salt Intake on the Blood Pressure of Populations

The most vigorously controlled trial was carried out in Portugal, which is notorious for its high salt consumption (99). The trial was carried out in two communities within the same district, each with about 800 inhabitants, who had a very high salt intake of about 21 g/day, and in which 30% had high blood pressure. Each community was close-knit and rural, which was particularly favorable for health education. One village was used as the control for the other in which the intake of salt was reduced. Community awareness of stroke and hypertension probably enhanced cooperation and the acceptance of guidance. Active intervention lasted 2 years and involved the whole community, but the responses were assessed in random samples. About half the total salt intake was added in the kitchen, which was easy to influence; another 30% came from salted codfish; the rest was in bread. Those responsible for cooking were advised to add less salt and instead to use herbs and other alternative flavorings (often previously unknown in the village), which became popular. The community as a whole was advised to eat less cod and fewer sausages, which also contained much salt, and the bakers were asked to reduce the salt added to bread by about 50% during the 2 years of the trial (Fig. 50-9). The reduction in salt intake in the trial village was associated with a highly significant difference in blood pressure. At the end of the second year the difference in blood pressure between the two villages was 13 mm Hg in systolic and 6 mm Hg in diastolic pressure. The fall was maintained in the second year. There was an upward trend in blood pressure of the control village over the 2-year period. The fall in blood pressure involved the whole community, not just those with hypertension, and the response was similar between young and old or men and women. It was estimated that a downward shift of blood pressure by 5 mm Hg, as observed in this trial, could reduce the prevalence of hypertension by about one-fourth.

Japan

The most comprehensive and wide-ranging endeavor to reduce the salt intake of whole communities took place in Japan. As a result of much public health activity to reduce salt intake the natural average salt consumption was reduced from 13.5 to 12.1 g/day. The change was greatest where the salt intake was highest. For instance, between 1960 and 1989, the salt intake in the province of Akita was reduced from 18 to 14 g/day. This reduction was accompanied by a gradual fall in average blood pressure and hypertension, and a marked decline in stroke mortality (100).

The Effect of Reducing Salt Intake in Essential Hypertension

Historical Background

Ambard and Beaujard first demonstrated the connection between salt and hypertension in humans in 1904 (101). They varied the intake of salt in patients with hypertension by means of three diets: 2 L/day of milk only, milk plus meat and eggs without the addition of salt, or 2 L of salty broth (containing approximately 10.6 g of salt/L). The urinary output of chloride was measured. Six hypertensive patients were studied for a period of about 3 weeks. It was found that a negative chloride balance lowered the blood pressure, whereas a positive chloride balance increased the blood pressure. One patient's blood pressure was relatively unaffected. They concluded: "We have shown that there is a close relationship between chloride balance and the arterial pressure. Does this mean that chloride is the initiator of hypertension or is it no more than yet another index that certain putative poisons are being retained though they have never been demonstrated?"

In the next 20 years, salt deprivation was occasionally used to lower blood pressure, mainly in patients with renal disease,

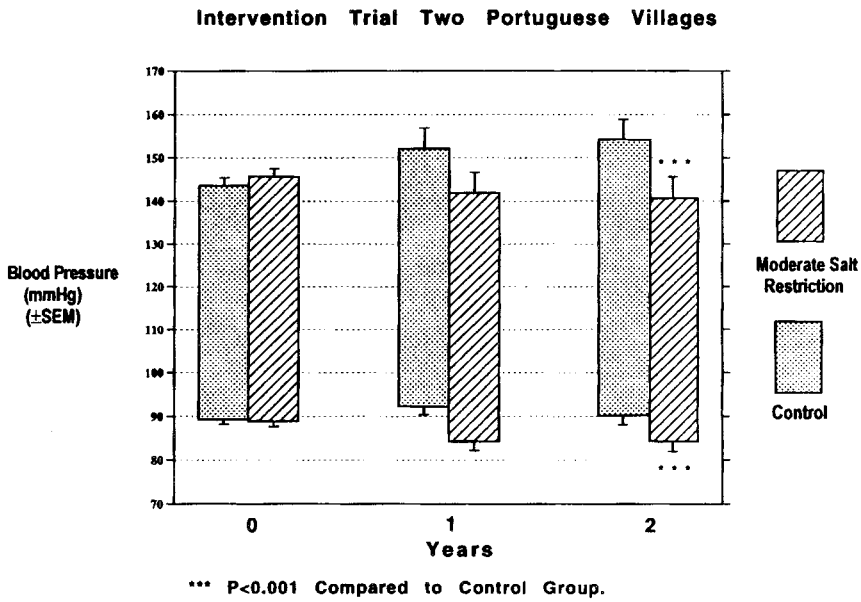

FIGURE 50-9. Blood pressure changes in two Portuguese villages, in one of which the dietary intake of salt was reduced. (Drawn from: Forte JG, et al. Salt and blood pressure: a community trial. *J Hum Hypertens* 1989;3:179.)

but the poor success reported, particularly by German workers (102), was sufficient to discourage its general use. The notion that there was a connection between salt intake and hypertension in humans was discredited. The "protein intoxication" theory of hypertension, referred to by Ambard and Beaujard (101), undoubtedly dominated the scene. This idea had originally been put forward by Bright (1) in relation to hypertension associated with severe renal disease when it had some justification. Its extension to essential hypertension, particularly at a time when it was considered that the kidneys were normal, is difficult to understand. Allen scathingly pointed out in 1920 (103) that treatment for hypertension at this time consisted "chiefly in low protein diets, the elimination of supposed toxins or the artificial reduction of pressure by drugs, bleeding, electricity and the like. Mental and bodily rest is advised to a degree which largely terminates usefulness of life." Houghton (104) and Allen and Sherrill (105) deplored this approach and published detailed accounts of their experience of reducing salt intake to patients with essential hypertension. Both groups agreed that the effective hypotensive level of sodium intake might vary from patient to patient; it might be necessary for some patients to lower the intake of sodium to less than 2 g/day. Allen and Sherrill (105) described the effect of a low-sodium diet in 180 severe cases of hypertension. All patients had essential hypertension and were given a normal protein intake. The blood pressure returned to normal in 19%. The relief of hypertension and other symptoms was sufficient to be regarded as a distinct therapeutic success in 42%. Complete failure occurred in 30%. Allen and Sherrill concluded that pure hypertension is "essentially a salt nephritis." Houghton (104), discussing all the effects of salt reduction in several forms of hypertension, concluded that arterial hypertension is a "tertiary condition of which the immediate cause is a larger sodium chloride intake than the damaged kidneys can excrete," a very modern view. The nature of the renal abnormality that might impair the kidney's ability to excrete salt in primary hypertension (i.e., essential hypertension) was not discussed. Fahr (8) in 1919 was the first to suggest that essential hypertension might result from a primary renal disturbance, but he did not elaborate on its nature.

The connection between salt and hypertension continued to be denied in spite of Ambard and Beaujard, Allen and Sherrill, Houghton, and others. Kempner (106) finally clarified the position in 1948, although somewhat against his own inclinations. He studied patients with and without renal "involvement" (nephrosclerosis) who were given a low-fat, low-protein (20 g/day), low-sodium (180 mg/day) rice diet for months and years. Kempner claimed that he had devised this diet because of his findings on "protein, fat and carbohydrate metabolism of isolated kidney cells under various pathologic conditions (cell injury and or changes in pH, sodium bicarbonate concentration, oxygen tension, and metabolizable substrate)," a non sequitur that many, including Pickering (107), found incomprehensible. Kempner was clearly more interested in the low protein content of the diet and was reluctant to admit that it might be the low sodium alone that lowered blood pressure. He attributed such an assertion to others who used his diet. He stressed that what he called the "active principle" of the rice diet was its rigid restriction of protein, fat, sodium, and chloride. His only acknowledgment that others before him had observed that a low-salt diet alone lowered blood pressure was to use derogatory quotes by some contemporary prominent hypertension experts: (a) Fishberg (108) in 1939, who had stated that "No dietary treatment is known which has a specifically favorable effect on essential hypertension;" (b) Goldring and Chasis (109) in 1944, who had concluded that in the absence of edema and paroxysmal dyspnea the restriction of salt was unwarranted; and Page and Corcoran (110) in 1945, who had written that the hypotensive results obtained were owing not to salt restriction but to "rest in bed and the psychotherapy of constant attention." It is ironic, therefore, that Kempner is now remembered as the person who brilliantly demonstrated that high blood pressure can often be lowered by a low-salt diet.

The sodium content of Kempner's rice diet was so low that the 24-hour urinary excretion of sodium at the end of 2 months usually fell to below 100 mg. The effect of the diet in 500 patients with hypertensive vascular disease as opposed to glomerular nephritis (i.e., intrinsic overt renal disease) with and without renal "involvement" was reported. The probable reason that Kempner's paper had such an impact was that it was visually so compelling and that it came 44 years after Ambard and Beaujard's initial seed had been sown (101). The paper contained (a) blood pressure charts showing the relentless fall of blood pressure (Fig. 50-10), (b) chest x-rays showing pronounced reductions in heart size, (c) electrocardiograms showing T-wave inversions reverting to normal, and (d) photographs of the retinae showing loss of papilledema,

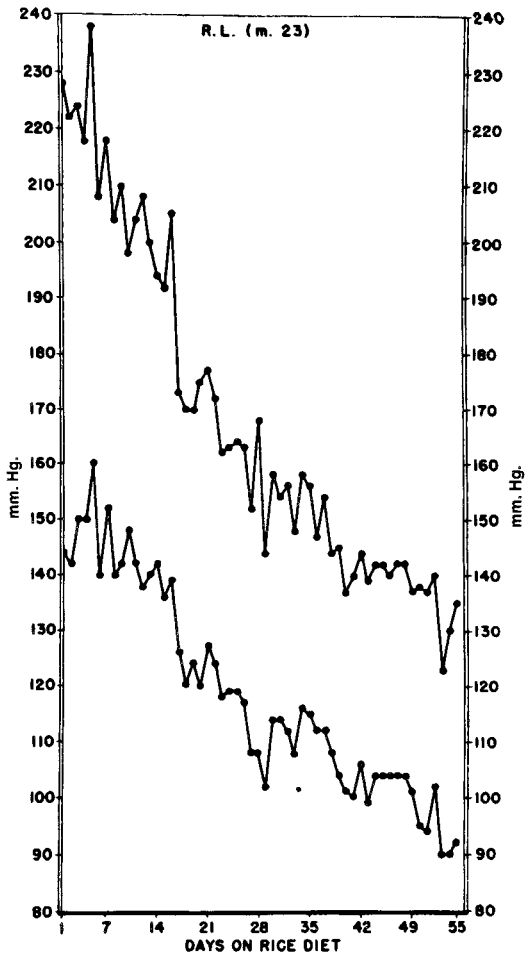

FIGURE 50-10. The fall in blood pressure in a patient with severe hypertension who was placed on Kempner's rice diet. (From: Kempner W. Treatment of hypertensive vascular disease with rice diet. *Am J Med* 1948;4:545, with permission.)

hemorrhages, and exudates. There is no doubt that in his hands the rice diet achieved remarkable and sustained results; however, Kempner made no mention of the overwhelming difficulty of trying to get patients to follow the rice diet or of the complications associated with such severe reductions in sodium intake, although he did admit that the rice diet was contraindicated unless frequent checks of the patient's blood and urine were possible. Nor did Kempner discuss the role of the kidney in the etiology of essential hypertension. Nevertheless, the paper irrevocably linked the intake of sodium to hypertension and therefore reinforced the suggestion that the connection between the two was the abnormal handling of sodium by the kidney.

The use of severe salt restriction as recommended by Kempner had a temporary vogue but ceased with the introduction of oral diuretics in the 1950s. In the 1970s and subsequently numerous studies have shown theeffect of much more moderate restrictions in salt intake.

There has been a slow acceptance that lowering the salt intake of hypertensive patients lowers blood pressure and increases the effectiveness of hypotensive drugs. There is no doubt that part of the reluctance to reduce salt intake has stemmed from the difficulty in getting patients to change their dietary habits. Even when the patient is cooperative, it is difficult because of the high salt content of bread, and nearly all processed foods, as well as fast food, restaurant food, and so on. For instance, in 1997 Liebman and Jacobson ascertained

TABLE 50-1

SODIUM CONTENT OF VARIOUS FOODS SOLD AT RESTAURANTS IN UNITED STATES

Item	Sodium (mg)
Tuna salad sandwich	1,320
Lasagna	2,055
Ham sandwich	2,200
Spaghetti with sausage	2,435
House fried rice	2,680
General Tso's chicken	3,150
House lo mein	3,460
Beef burrito platter	3,920
Fried seafood platter	4,405

the sodium content of various foods sold in restaurants in the United States (Table 50-1) (111). Thus, in a single meal many customers at that time were exceeding the 2,400 mg of sodium (6 g of sodium chloride) recommended for an entire day.

In one double-blind randomized study in 20 patients with a mean age of 57 and a blood pressure of 164/101 mm Hg in which salt excretion was reduced to 3 g/day for 1 year, 1 patient was lost to follow-up, and 3 required the addition of drug therapy to control blood pressure, but blood pressure fell to 142/85 mm Hg in the remaining 16 patients (112). There are several other studies that agree that reducing the salt intake of elderly hypertensive patients induces a substantial fall in blood pressure. Cappuccio et al. reported the response of a group of elderly patients in whom a reduction in salt intake of 83 mmol/day for 1 month induced a fall in blood pressure of 7.2/3.2 mm Hg. In another study in 975 elderly patients aged 60 to 80 who were being successfully treated with antihypertensive drugs, treatment was stopped and they were randomly allocated to one of four regimens: (a) sodium restriction, (b) weight reduction, (c) both sodium restriction and weight reduction, and (d) the control group. They were monitored over the next 30 months to detect those whose blood pressure returned to hypertensive levels. There was a 50% decrease in patients whose blood pressure returned to hypertensive level in the group on 40 mmol sodium/day (113).

A meta-analysis of studies of sodium restriction of a month or more confirmed that for a reduction in salt intake of 6 grams/day in untreated patients with essential hypertension, blood pressure fell by 7/4 mm Hg (98).

There was also a successful mass campaign in Belgium against salt in 1969. A total of 3,328 elderly subjects between the age of 70 and 81 were studied. Between 1967 and 1986 the prevalence of hypertension in these subjects decreased substantially and severe hypertension disappeared. The reduction in salt intake was from 16 to 11 g/day in the men and from 12 to 9 g/day in the women (114).

CONSEQUENTIAL THERAPEUTIC AND PUBLIC HEALTH IMPLICATIONS OF SALT INTAKE AND HYPERTENSION

Both the epidemiologic evidence and the rise in blood pressure that accompanies a rise in salt intake in normal animals and humans demonstrate that salt intake is a major determinant of blood pressure. Reducing the salt intake of patients with essential hypertension induces a fall in blood pressure; therefore, all such patients should reduce their salt intake. Furthermore,

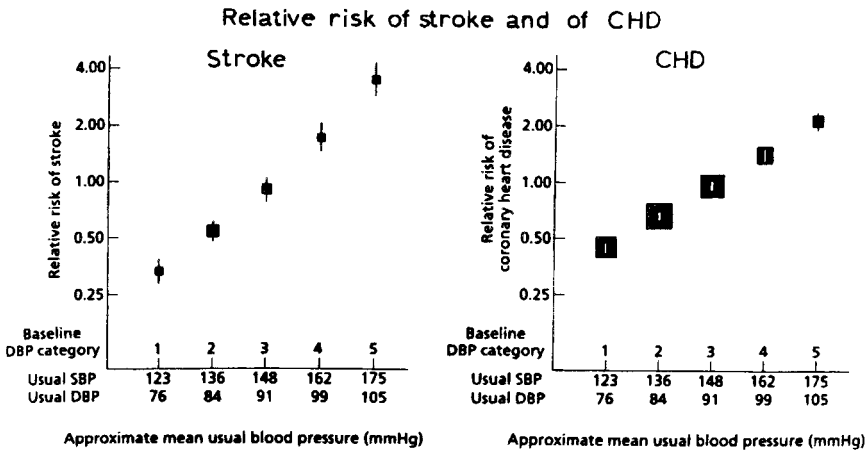

FIGURE 50-11. This figure summarizes studies that had been performed up to 1990, in which blood pressure had been measured and the subjects followed up subsequently to find out how many went on to develop a stroke or coronary heart disease (CHD). The *horizontal axis* shows that blood pressure has been arranged in five ascending groups: both the systolic (SBP) and diastolic (DBP) pressures are shown. The *vertical axis* on the *left* shows the relative risk of developing a stroke, and on the *vertical axis* on the *right* the risk of developing CHD. It is clear that the risk of developing a stroke (left) or CHD (right) is directly related to the level of blood pressure throughout the normal and hypertensive range. Note that the risk of developing a stroke is 16 times less with a low normal blood pressure than with a moderately raised blood pressure. The size of the *black boxes* denotes the number of persons who died during the period of observation: The boxes for CHD are much larger, indicating the larger number who died from CHD compared to stroke. (From: MacMahon S, et al. Blood pressure, stroke, and coronary heart disease. Part 1: Prolonged differences in blood pressure: prospective observational studies corrected for the regression dilution bias. *Lancet* 1990;335:765, with permission.)

the close connection between blood pressure and salt intake in normal subjects and animals and the blood pressure lowering effect of salt reduction all indicate that it should be possible to prevent a rise in arterial pressure by reducing the population's salt intake.

However, the slight fall in arterial pressure achieved after a few days of salt reduction in normotensive subjects has led some (115) to oppose the proposal that the nation's salt intake should be reduced (97). Although the effects of a few days of salt reduction are irrelevant to the proposal that the nation's salt intake should be permanently reduced, it is incorrect to suggest that the smallness of such a reduction is unimportant because it neglects the fact that the relation of salt intake to strokes and myocardial infarctions is continuous not only in the hypertensive but also in the average range starting at a systolic pressure of 115 mm Hg (Fig. 50-11). In other words, although patients with hypertension have a much greater risk of developing a stroke, the greater number of subjects in the upper range of normal blood pressure have a greater number of strokes (Fig. 50-12). A population-based reduction in salt intake of 6 grams/day would lower blood pressure in adults by an average of 5/3 mm Hg. This would result in an 24% in stroke deaths and an 18% reduction in coronary heart disease deaths (116).

The principal overt objections to the widespread agreement among scientific bodies that the population's salt intake should be lowered from 10 to 12 g/day to 5 to 6 g/day are that the long-term effects of such a maneuver are not known and that they might be dangerous. Taking the second point first there is the U.S. Food and Drug Administration's (117) conclusion that "no convincing evidence has been presented that a moderate but significant reduction in salt intake would have any adverse health effects." Nevertheless, a myth continues to be disseminated which is encapsulated in Muntzel and Drüeke's (118) assertion that "lowering salt intake may result in health risks that outweigh the benefit of blood pressure reduction." This remarkable assertion is founded in part on the results of experiments carried out in animals subjected to intense sodium deprivation or massive intravenous injections of angiotensin. It is also based on interpretations of some observations in humans that have been repeatedly exposed as irrelevant or based on data that turn out not to support the assertion (119,120).

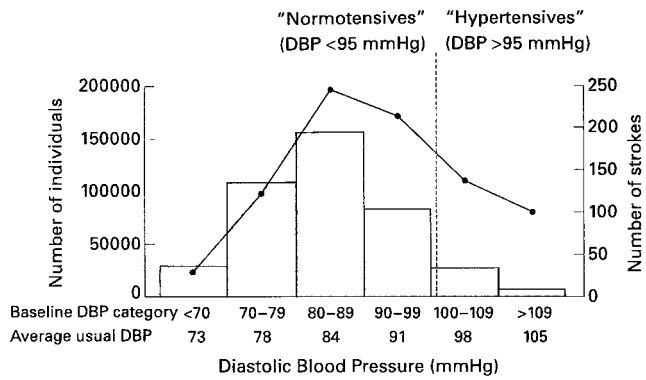

FIGURE 50-12. This figure shows how blood pressure of a large number of persons is distributed. The distribution of the population has been divided into six solid blocks. The tallest and largest block contains the greatest number of people and their diastolic pressure lies between 80 and 89 mm Hg. The line that connects the *small black circles* indicates the number of strokes that occurred in each block. What is clearly illustrated is that the greatest total number of strokes occurred in those subjects with a diastolic pressure of 80 to 89 mm Hg, the upper range of normal, and that a slightly lower number occurred in those with a diastolic pressure of 90 to 99 mm Hg, which is a mild elevation of blood pressure. Patients with severe hypertension, however, with a diastolic pressure above 100 mm Hg have far fewer strokes in total (although individually they have a much greater risk of developing a stroke) because there are far fewer patients with severe hypertension. (Redrawn from: MacMahon S, Rogers A. *J Hypertens* 1994;12:S5.)

The long-term effects of salt intake reduction are not known. In order to find out unequivocally it would be necessary to have a randomized trial in several thousand subjects, starting at birth and lasting about 50 years. There is no support for such a trial, which would be unethical, an administrative nightmare, prohibitively expensive, and that would have to rely on those control participants who were consuming a high (normal) sodium intake being willing to continue on such an intake when they grew up and understood the dangers. In the absence of such a trial decisions have to be made on what evidence there is, keeping Geoffrey Rose's words in mind (121): "The level of evidence approximate to a particular decision depends on the consequences of making the wrong decision. For example, there is substantial evidence, but still well short of proof, that a reduction in national salt consumption leads to a somewhat lower mean blood pressure, with important expected health benefits. The change is safe and its cost minimal (except to a small but noisy section of the business community). The evidence for this policy is important, but one may judge it to be sufficient."

Worldwide there is now a scientific consensus that the population's salt intake should be reduced. The World Health Organization (WHO) has recommended that all adults should eat less than 5 g of salt per day (122). In the United States similar recommendations have been put forward by the National Heart Lung and Blood Institute, the National Academy of Sciences, the National Research Council, the Department of Health and Human Services, the Department of Agriculture, the American Dietetic Investigation, the American Public Health Association, the American Society of Clinical Investigation, and the American Medical Association. Similar views have been expressed in the United Kingdom by government appointed nutritional committees of independent experts (123). The UK Food Standards Agency through its Specialist Advisory Committee (SACN) recommended in 2003 that all adults should eat less than 6 g of salt/day and set much lower targets for children depending on age. Currently in the United Kingdom salt concentrations of many processed, canteen, and fast foods are gradually being reduced so that salt intake will fall below 6 g/day by 2110 (124).

It has to be pointed out that the case for a general reduction in salt intake is much wider than simply preventing a rise in blood pressure (125). There is evidence in humans that a decrease in sodium intake provides the following additional benefits, which are largely independent of any fall in blood pressure: (a) regression of left ventricular hypertrophy (126,127), (b) reduction of proteinuria (128), (c) reduction of urinary calcium (129) with a decrease in osteoporosis (129), (d) reduction in the risk of renal stones (130), (e) protection against cancer of the stomach (131), (f) reduction in the incidence of stroke (114,132), (g) enhancement of the antihypertensive effect of antihypertensive agents (133), and (h) decrease in diuretic-induced potassium loss (134). In addition, reducing salt intake may influence the genesis of nephrosclerosis and renal failure because it has been shown in normotensive and hypertensive rats that a high dietary salt intake leads to widespread fibrosis and increase in transforming growth factor-beta (TGF-$_{\beta 1}$) (135) in the heart and kidney.

RENAL ABNORMALITIES THAT DIMINISH AND ACCOMPANY THE KIDNEY'S ABILITY TO EXCRETE SODIUM

The effect of a change in salt intake on the blood pressure of normal subjects and hypertensive patients, the epidemiologic studies on the prevalence of hypertension in relation to salt intake, the influence of cross-transplantation of kidneys between normal and hypertensive animals or humans, and the finding that the rise in blood pressure takes place during a period of salt retention in hereditary forms of hypertension in the rat suggest that the hypertensive effect of sodium on blood pressure is related to a renal impairment in sodium excretion.

There is considerable evidence for the presence of multiple inherited renal abnormalities, each of which could interfere with sodium excretion. In essential hypertension and the hypertensive strains of rats it is probable that to have an overall impairment in the ability to excrete sodium, it is necessary for the kidney to have several functional disturbances, each of which has this effect. But there is no doubt that, as was stated earlier, an isolated impairment in sodium transport limited to the kidney can cause hypertension. In the rare human monogenic condition known as Liddle syndrome, mutations of the gene-encoding subunits of the sodium channel cause an increase in sodium reabsorption and hypertension. The effect of these mutations in raising blood pressure appears to be limited to the kidney in that renal transplantation lowers blood pressure (136). It is important to distinguish an inherited functional abnormality from one that has been acquired as a result of the rise in arterial pressure. The arterial pressure in inherited hypertensive rats tends to rise steeply around the sixth week of life; therefore, the distinction between an inherited lesion and a lesion secondary to the rise in pressure has to be made in young rats. In essential hypertension, the studies have to be made in young normotensive relatives of patients with hypertension.

Renal Blood Flow, Glomerular Filtration Rate, and Patterns of Urinary Sodium Excretion

Changes in renal hemodynamics in the normotensive children of hypertensive parents have yielded inconsistent results. Bianchi and Barlassina (137) reported an increase in renal blood flow and glomerular filtration rate (GFR). In contrast, some investigators (40,138) have found that renal blood flow and GFR rate are normal but that there is an increase in vascular resistance, whereas others (139) have reported that the renal blood flow is reduced and renal vascular resistance is raised. This variability has been claimed to be artificial because of differences in the techniques used to measure the renal plasma flow and filtration rate (140); there is probably some increase in renal vascular resistance overall.

Approximately half of the patients with essential hypertension have a group of abnormalities that consist of: (a) a failure of renal blood flow to increase normally in response to a high salt intake, (b) a failure of renal blood flow to decrease normally in response to an infusion of angiotensin II, and (c) a lower than normal rise in plasma aldosterone in response to an infusion of angiotensin II when on a low sodium diet (141). Eighty-five percent of hypertensive patients who have some features of this characteristic set of abnormalities have a positive family history of hypertension (142), and such factors are more likely to be present in both of hypertensive sibling pairs than would be expected from the frequency of their presence in the general hypertensive population (143). These abnormalities also occur in normotensive offspring of parents with hypertension (144).

Renal blood flow and GFR in hypertensive strains of rats are reduced before the rise in pressure (145,146). The glomerulus of young SHR and Milan hypertensive rats has a reduction in glomerular ultrafiltration coefficient, GFR, and single-nephron filtration rate. The Dahl salt-sensitive hypertensive rat has an impaired ability to excrete a high intake of sodium and a

persistently low plasma renin value. On a normal sodium intake, however, blood pressure tends to be normal. In the Milan (147) and Heidelberg (148) hypertensive strain rats, in which blood pressure rises on a normal intake of sodium, there is a transient period ending about the sixth to the seventh week of life when the fractional excretion of sodium is lower, the cumulative retention of sodium is greater, and plasma renin activity is lower in the hypertensive strain than in the control normotensive rat. At the end of this period, blood pressure of the hypertensive strain rat has risen considerably.

The phenomenon of accelerated natriuresis in response to an infusion of saline is prominent in inherited hypertensive strains of rats and occurs, as in humans, both before and after the rise in arterial pressure (149,150). As in humans, it has been shown that the phenomenon of accelerated natriuresis in rats is unrelated to changes in peritubular capillary hydrostatic or colloid osmotic pressure (150).

Structural Glomerular Changes

There is a scanning and electron microscopic study in the 6-week-old SHR in which the arterial pressure was 131 mm Hg, as opposed to 121 mm Hg in control Wistar-Kyoto (WKY) rats (151). The fenestrae of the glomerular capillary endothelium of the SHR were already significantly smaller with an increased number of cytoplasmic ridges and bulbous projections lying across the endothelial surface. These changes should reduce the filtration surface per unit area of endothelium. At 12 weeks, when the arterial pressure of the SHR was 175 mm Hg and that of the WKY rat was 138 mm Hg, these abnormalities were more pronounced, whereas the endothelium of the WKY rat was unchanged (in spite of the WKY rat having an arterial pressure slightly higher at 12 weeks than that of the SHR at 6 weeks). Not only is the filtration surface per unit area reduced, some workers have also found that the SHR has a diminished number of nephrons (152).

Proteinuria

In a study of microalbuminuria, Fauvel et al. (153) found that normotensive individuals with a genetic risk of hypertension had a mean albuminuria of 36 ± 37 μ/min, whereas a control group of normotensive individuals without a genetic risk of hypertension had a mean proteinuria of 6 μ/min.

Tubular Sodium Reabsorption

Fractional lithium and uric acid clearance are considered to be a measure of proximal tubule sodium reabsorption. A decrease in renal fractional lithium clearance (i.e., an increase in reabsorption) has been observed in the normotensive offspring of hypertensive parents by one group (154) but not by three others (155–157). On the other hand, the two groups who have studied fractional uric acid excretion agree that it is reduced in the offspring of two and one hypertensive parents (85,158). There is increased sodium reabsorption in the proximal tubule in both the SHR and Milan hypertensive rat (145,146). The amount of sodium delivered to the distal tubule is reduced, but to keep in balance this should normally be adjusted by a diminished reabsorption from the distal tubule and collecting duct. The inability of the distal nephron to adjust sodium excretion to the reduced filtration rate therefore must be owing to an additional functional abnormality of the distal and collecting tubule.

Vascular Changes

Altered Relation of Arterial Pressure to Urinary Sodium Excretion

The urinary sodium excretion of an isolated kidney is directly related to the perfusion pressure (159–161). Changes in pressure alter sodium excretion by changing the hydrostatic pressure of the venous capillaries and the interstitial pressure surrounding the tubules, an effect that is independent of the GFR or renal blood flow, both of which tend to remain unchanged throughout the autoregulatory range of perfusion pressure. The relation between urinary sodium excretion and renal perfusion pressure is sometimes referred to as a "pressure natriuresis" curve. Some isolated kidneys need greater pressure to promote the same rate of sodium excretion, a change that can be referred to as a right shift of the pressure natriuresis curve. If, therefore, the pressure natriuresis curve of an isolated kidney, perfused with blood from a normal animal, has shifted to the right, it is probable that there is a rise in resistance in a vascular site between the arterial perfusion pressure and the peritubular venous capillaries. It is probable that such a site of raised resistance is present in kidneys *in situ* in all forms of hypertension; otherwise, hypertension would be accompanied by severe sodium depletion.

In essential and inherited hypertension in the rat, there is a primary shift of the natriuresis curve to the right. Tobian and colleagues (162) and Roman and Cowley (163) have shown that isolated kidneys of young prehypertensive salt-sensitive and spontaneously hypertensive Dahl rats have a shift to the right of the pressure natriuresis curve (Fig. 50-13). In the SHR, there is evidence that the increased vascular resistance is owing to narrowing of the afferent arteriole; these vessels are already smaller than control at 6 to 7 weeks of age (164,165) because of an increase in tone rather than medial hypertrophy. The increase in resistance that results is particularly marked in the papillary circulation and is evident between 4 and 10 weeks of age, when the arterial pressure is rising (37). There is an accompanying reduction in papillary blood flow and interstitial pressure (38), which tends to enhance sodium reabsorption. In the Dahl salt-sensitive rat, in contrast to the SHR, the shift

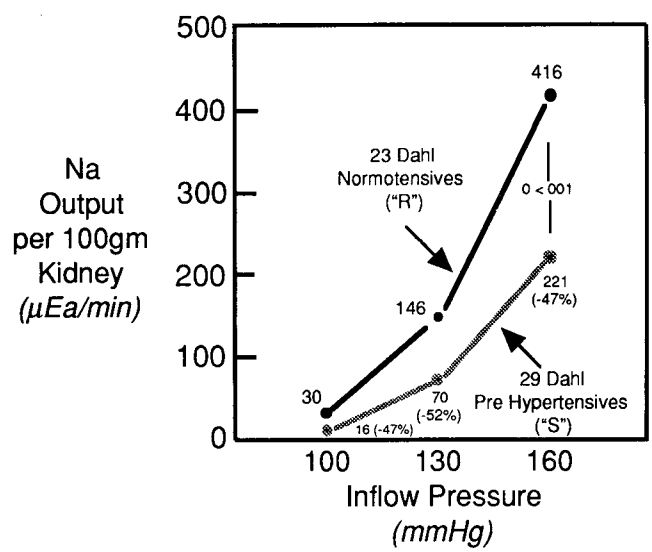

FIGURE 50-13. Sodium excretion of isolated kidneys from salt-sensitive prehypertensive and salt-resistant normotensive Dahl rats. (From: Tobian L. The relationship of salt to hypertension. *Am J Clin Nutr* 1979;32:2739, with permission.)

to the right of the pressure natriuretic curve does not appear to be associated with a particular reduction of the papillary interstitial pressure (166), but to a reduced sensitivity of the renal tubules to the interstitial pressure (167). These observations are consistent with the finding of increased renal vascular resistance in the normotensive children of hypertensive parents.

Abnormalities of Arachidonic Acid Metabolism

The increased renal vasoconstriction of the young SHR may result from one or more abnormalities of arachidonic acid metabolism.

Cyclooxygenase Activity

At 6 weeks of age, bolus injections of arachidonic acid into the renal artery of isolated kidneys perfused at constant flow cause a greater rise in the perfusion pressure of the SHR kidney than in the WKY rat (168). This is accompanied by a greater release of the vasoconstrictor, thromboxane B_2. This difference is no longer evident at 18 weeks. These changes do not occur in kidneys from rats made hypertensive with deoxycorticosterone acetate (DOCA) or a clip on the renal artery of one kidney.

Cytochrome P-450-Dependent Monooxygenase Activity

There are two forms of these enzymes of particular interest, epoxygenases and hydroxylases (169). Epoxygenase activity leads to the production of 11,12-epoxyeicosatrienoic acid (EET), which can be hydrolyzed to 11,12-dihydroxyeicosatrienoic acid (DHT). Hydroxylase activity leads to the production of 20-hydroxyeicosatetraenoic acid (20-HETE) and 19-hydroxyeicosatetraenoic acid (19-HETE). These products of cytochrome P-450 metabolism of monooxygenase activity are present in greater amounts in SHR microsomes from rats aged 5 to 13 weeks (170). Tin ($SnCl_2$), which reduces the concentration of cytochrome P-450 monooxygenase when given for 4 days to 7-week-old SHR, prevents the customary rise in blood pressure, an effect that lasts for 7 weeks after the administration of tin. Tin has no effect on the blood pressure of 20-week-old rats (171).

It has been found that from birth until the ninth week, hydroxylase activity in cortical microsomal fractions is greater in the SHR than the WKY rat, whereas there is no difference in epoxygenase activity (172). The production of 20- and 19-HETE is greater in the SHR. In addition, the conversion of the hydroxylase metabolite 20-HETE by cyclooxygenase is much greater in the SHR from birth until the seventh week. The production of the resultant metabolite 20-OH prostaglandin (P5) and 20-OH (PGH_2) in the SHR rises substantially. Because these are potent vasoconstrictors, it is possible that in the SHR during the early weeks when the pressure is rising (173), these metabolites are responsible in part for the observed renal vascular constriction. In addition, although epoxygenase activity is the same in the SHR and WKY rat, the production of EET in the SHR is less than in the WKY because its conversion to DHT is greater in the SHR (174). Thus, in the SHR the increased presence of 19-HETE, which stimulates Na-K-ATPase, and the diminished presence of ETE, which inhibits Na-K-ATPase (175), may be responsible for the documented increase in renal Na-K-ATPase activity in the young SHR and in part for the sodium retention.

Urinary Kallikrein Excretion

Urinary kallikrein influences urinary sodium excretion. Its excretion is reduced in essential hypertension (176) in the SHR and the Dahl salt-sensitive rat (177). It is also reduced in normotensive children of hypertensive parents (178). The lower kallikrein excretion in essential hypertension extends through a range of 9 to 259 mmol/day of urinary sodium excretion (179). Urinary kallikrein is synthesized in the distal tubule and secreted into its lumen, where it acts on kininogen to release kinins (180). Kinins are natriuretic substances (181) closely linked to several other intrarenal substances that have an effect on sodium excretion, such as angiotensin II, prostaglandins, and antidiuretic hormone (182). Plasma aldosterone is the major regulator of urinary kallikrein excretion (183). A decrease in sodium intake raises and an increase lowers urinary kallikrein excretion (184,185). Kallikrein induces a loss of amiloride-sensitive sodium current and an increase in amiloride-insensitive "leak" current on the mucosa of the mammalian bladder (186). The net effect is to reduce sodium transport, from which it is concluded that kallikrein may hydrolyze amiloride-sensitive channels, thus diminishing the rate of entry of sodium into the cell. If this takes place in the apical membranes of the distal tubules there is diminished sodium reabsorption so that a reduction in urinary kallikrein may be an additional factor, together with the reduced presence of the natriuretic kinins, which could increase sodium reabsorption and thus impair the ability of the kidney to excrete sodium. There is some fragmentary information on the genetic origin for the reduced urinary kallikrein in essential hypertension (187) and hereditary hypertension (188).

Tubuloglomerular Feedback

An increase in GFR increases the rate of delivery of tubular fluid to the macula densa, the cells of which then signal the afferent arteriole to constrict. This reduces the filtration rate and the delivery of tubular fluid to the macula densa, which results in a reduction of urinary sodium excretion (189). In the normal animal, the sensitivity and reactivity of this adjustment increase when there is a need to conserve sodium (hemorrhage and dehydration) (190,191) and diminish when there is a prolonged need to increase sodium excretion (chronic salt loading and DOCA administration) (192–195). When dietary sodium is increased, the depression in tubuloglomerular feedback activity results from the presence of an inhibitory factor in the tubular fluid (192), whereas with prolonged DOCA administration (196) the fall in tubuloglomerular feedback activity is associated with a change in the characteristics of the juxtaglomerular apparatus.

In the young SHR (196,197) and Milan hypertensive rat (198) at a time when there is most evidence of sodium retention and blood pressure is beginning to rise, there is a paradoxical increase in tubular feedback activity that would tend to aggravate the sodium retention. This increase in tubuloglomerular activity instead of a compensatory fall, which would increase sodium excretion, appears to be owing to a relative lack of the humoral inhibiting factor in the tubule fluid (199). By the 12th week, however, when blood pressure has risen considerably, the tubuloglomerular feedback has almost returned to normal. The Milan hypertensive rat has been studied at 3.5 to 5 weeks and at 5 to 7 weeks, just before and after blood pressure begins to rise (198). At 3.5 to 5 weeks, tubuloglomerular feedback activity is absent, and 2 weeks later, when the blood pressure has started to rise, tubuloglomerular feedback increases inappropriately to supranormal levels as in the SHR, thus diminishing the kidney's ability to excrete sodium.

Dopamine Metabolism

Urinary dopamine is formed in the kidney. The renal actions of dopamine include a substantial increase of regional blood flow, a less marked rise in GFR, inhibition of proximal tubule sodium reabsorption, and inhibition of renin release by the juxtaglomerular apparatus. These are all natriuretic mechanisms, but the effect on the proximal tubule is by far the most prominent (200).

Basal dopamine excretion in essential hypertension may be normal or even increased (201). Nevertheless, although the sodium loading of normal Whites increases urinary dopamine excretion, in patients with essential hypertension it either induces a smaller increase or a reduction in dopamine excretion (202). In a group of Japanese patients with hypertension who were divided into sodium-resistant and -sensitive, depending on the response of their blood pressure to a sodium load, the salt-resistant patients (who showed a mean rise of blood pressure of only 0.5 mm Hg) had a prompt increase in dopamine excretion, whereas the sodium-sensitive patients (in whom there was a mean rise in blood pressure of 10.5 mm Hg) had no increase in dopamine excretion (203). Other Japanese workers (204) have found that dopamine excretion in hypertensive patients in response to salt loading is lower than in normal subjects, particularly in the low renin group (205,206). Normotensive relatives of patients with hypertension also have a lower urinary dopamine excretion and do not have a normal regression between urinary sodium and dopamine excretion. There is also some evidence that this renal dopamine abnormality is present in hypertensive black Americans, but there are important ethnic differences. The increasing dopamine abnormalities detected in the Japanese hypertensive patients and their normotensive offspring are also present in normotensive American blacks, Thais, and Zimbabweans but not Ghanaians and Iranians. Various studies suggest that in essential hypertension it is the intrarenal generation of dopamine that is defective (201,206). The carboxylation of exogenous L-dopa is reduced (207), and the intravenous administration of dopamine induces a greater increase in urinary sodium excretion and fractional excretion, which suggests an enhanced dopamine receptor activity. These renal abnormalities of dopamine metabolism are also present in normotensive relatives of hypertensive patients (202).

Abnormalities of dopamine metabolism also have been detected in the Dahl (208) and SHR (209) forms of hypertensive strain rats, but they are not the same as those in essential hypertension. Although the overall excretion of a salt load in the SHR is less than normal, the initial rise in urinary sodium excretion, in contrast to the response in essential hypertension, is *greater* than in the WKY; and, instead of an impairment of dopamine generation, there appears to be a defect of the dopamine A_1 receptor in both the SHR and the Dahl salt-sensitive rat. For instance, the natriuretic effect of a dopamine A_1 receptor agonist in the SHR is less than in the WKY rat. Furthermore, there is a dopamine A_1 receptor coupling defect to adenyl cyclase in the proximal tubules of the SHR kidney, even at 3 weeks of age, well before the onset of hypertension (210).

Atrial Natriuretic Peptide

There is diminished atrial natriuretic peptide (ANP) immunoreactivity at distal tubule segments in essential hypertension, which suggests a diminished binding capacity (211). A continuous low dose of ANP induces a similar natriuretic response in normotensive subjects as it does in hypertensive patients, whereas there is a greater rise in solute-free water excretion in the SHR (212). As in a normal animal, the natriuretic effect of ANP is directly related to blood pressure (213), the absence of a greater natriuretic effect in the SHR and the increase in solute-free water suggest that the distal tubules in the SHR have an increased sodium reabsorption.

There is a lowered renal response to ANP in the mature hypertensive SHR (214). This is associated with a maximum binding capacity of SHR glomeruli for I^{131}-ANP that is reduced by about 50% (215) and similarly diminished immunoreactivity at distal tubule segments (216). Urinary cyclic guanosine monophosphate (cGMP) excretion response to intravenous synthetic ANF (synthetic 26 amino acid peptide contained with rat ANP), however, is increased and is associated with an increase in affinity (217,218).

The intravenous injection of atrial extracts to Dahl rats has also shown that the mature Dahl salt-sensitive hypertensive rat has a natriuretic response that is about 50% less than that of the Dahl salt-resistant strain (219). This is associated with a defect of intracellular cGMP synthesis. There is a similar hyporesponsiveness to sodium nitroprusside, but cyclic adenosine monophosphate response to arginine vasopressin (AVP) of the Dahl sensitive rat is the same as from the Dahl resistance rat (220). These changes are present in the 5- to 6-week prehypertensive rat and appeared to be specific to the medullary structure in that the response of mesangial cells is the same in the Dahl-sensitive and resistant rats.

The Effect of Age

Blood pressure rises with advancing age (Fig. 50-14). It is probable that this relationship owes in part to a gradual involutional diminution in renal function (221), which aggravates the overall effect of any inherited difficulty in excreting sodium. After the age of 30 years there is a gradual decline in renal blood

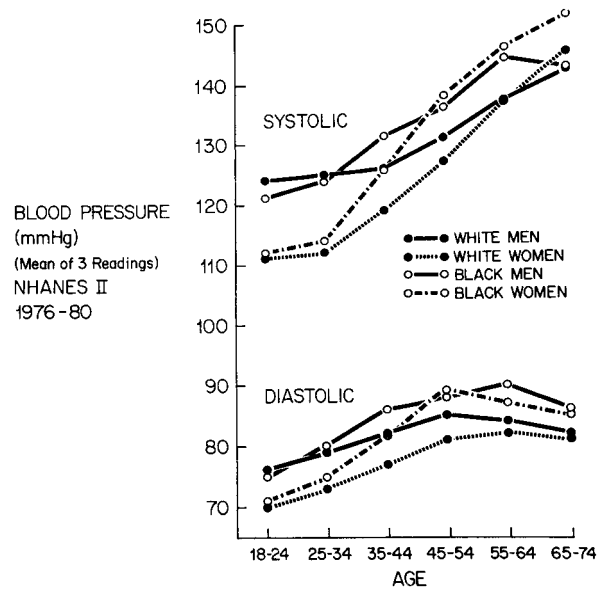

FIGURE 50-14. Mean systolic and diastolic blood pressures for white and black men and women in various age groups in the 1976 to 1980 National Health and Nutrition Examination Survey (NHANES II). (From: Rowland M, Roberts J. *Blood pressure levels and hypertension in persons aged from 6–74 years.* United States 1976–80 NCHS, Advance Data No. 84, Vital and Health Statistics of the National Center for Health Statistics. Washington, DC: U.S. Department of Health and Human Services, Oct. 8, 1982.)

flow, GFR, secretion tubular maximum (T_m), and the ability to concentrate the urine (222). By the age of 90 years, these functions have decreased to approximately half their value at the age of 30 years.

The Renal Contribution to the Fetal Origins of Essential Hypertension

At all ages beyond infancy, people who had lower birth weights have a higher blood pressure (223,224). This relationship becomes larger with increasing age. Measuring renal growth by counting the number of glomeruli has raised the possibility that the principal cause of this phenomenon is impaired intrauterine renal development. Sixty percent of nephron growth develops in the third trimester (225). Severe intrauterine growth retardation in human fetuses has been shown to exert a profound effect on renal development (226). This effect is magnified thereafter by the lack of any compensatory increase in either nephron numbers or size during the early phase of postnatal life. There is one report, however, that maternal blood pressure is inversely correlated, though weakly, with infant birth weight, which suggests that the link between birth weight and hypertension may in part result from an inherited tendency to hypertension (227).

A similar phenomenon can be produced experimentally in the pregnant guinea pig, which has a bicornuate uterus, by tying the uterine artery on one side of the uterus while allowing the fetuses on the other side to grow normally. The animals on the ischemic side have low birth weight and develop higher blood pressure in later life. In rats, maternal low-protein diets, which have been shown to impair renal function of the fetus (228) if maintained throughout pregnancy, also lead to an inverse relationship between blood pressure and the low protein intake at 9 weeks (229).

Conclusion

Many of the renal functional abnormalities in the various forms of hereditary hypertension are the same, for instance, there are defects of dopamine and renin–angiotensin metabolism that could impair urinary sodium excretion in essential hypertension, the spontaneously hypertensive rat, and the Dahl salt sensitive rat but the molecular disturbances and thus, presumably the genetic faults that gives rise to them are usually different. In other words, although patients with essential hypertension share several functional abnormalities with hypertensive strains of rats, the precise nature of the few genetic disturbances that have been detected in essential hypertension, and the many detected in the hypertensive strains of rats are different, with the exception of the adducin gene.

GENETIC ABNORMALITIES AND HYPERTENSION

Polygenic Changes and Hypertension

The following account demonstrates that, although many genetic abnormalities have been detected in hypertensive strains of rats thus far, only three have been identified in essential hypertension and they appear to play a minor part in raising blood pressure.

Essential Hypertension

Angiotensinogen

A variant of the angiotensinogen (AGT) gene locus, so-called AGT 235T, has been found to be more frequent in hypertensive patients than in controls in Europe, the United States, Japan (230), and the Afro-Caribbean (231). There is no evidence that this variant confers a similar risk in African Americans and Nigerians (232). The variant is associated with high AGT levels, which suggests that the mutation results in an increase in activity with a higher basal transcription rate. "The AGT gene has been the most scrutinized and the most promising finding of the primary hypertension genes thus far, however the AGT 235T variant explains a relatively small part of blood pressure variance" (233). An increase in angiotensinogen activity in the kidney could increase sodium reabsorption.

The Epithelial Sodium Channel

A variant (the T594m mutation) of the β subunit of the epithelial sodium channel has been found in about 8% of black London residents with a raised blood pressure as opposed to 2% of those with normal blood pressure (234). Plasma renin activity was also significantly lower in those with hypertension and the T594m variant. In addition, lymphocytes with the variant show increased sodium transport, suggesting that the variant may increase sodium reabsorption in the renal tubules, thus reducing renal sodium excretion.

Adducin

The membrane-skeleton protein adducin was originally chosen for genetic studies in the Milan hypertensive rat because it was the only membrane skeletal protein eliciting an immunologic response in cross-immunization experiments between Milan hypertensive strain rats and Milan normotensive controls. It is an $a\beta$ heterodimer that promotes the organization of a spectrin-actin lattice. In the Milan hypertensive rat there is a mutation in each of the two genes that codes for adducin. There is a high degree (94%) of amino acid homology between rat and human adducin, and many of the renal cell membrane ion transport alterations in the Milan hypertensive strain rat are shared by a subset of patients with essential hypertension. Linkage and association studies were performed in hypertensive patients and controls in Italy and a point mutation (G460W) was found in the human α-adducin gene. The 460W variant was shown to be more frequent in hypertensive patients than in controls (235). But no association between α-adducin allele variants was found in a group of patients with essential hypertension in Japan (236) or Scotland (237). Experiments in patients with essential hypertension with the α-adducin variant have shown them to flatten pressure natriuresis relationships (235). This is consistent with the findings in renal cell transfection experiments (238) that the α-adducin variant increases Na-K-ATPase activity, which increases tubular sodium reabsorption, and decreases fractional excretion of lithium and uric acid in hypertensive patients exhibiting the variant, which is consistent with an increase in proximal tubule sodium reabsorption (239).

Hypertensive Strains of Rats

An identified genetic locus influencing a quantitative trait such as blood pressure is known as a quantitative trait locus (QTL)

(240). Many QTLs that influence blood pressure have been identified in hypertensive strains of rats. On chromosome 1 there is the SA gene responsible for a protein of unknown function that is expressed 10 times more in the proximal tubules of SHR than in WKY, and the kallikrein gene that is also linked to hypertension in the SHR. On chromosome 2 there is the ANP receptor A gene, which is more expressed in the glomeruli of SHR. On chromosome 4 of the SHR there is a QTL associated with the neuropeptide Y gene and on chromosome 8 a significant QTL with the dopamine receptor and potassium channel genes, which explain about one-eighth of the genetic variance in blood pressure in the SHR. On chromosome 10 there are strong associations between blood pressure and some markers on a region near the ACE gene in the SHR and Dahl salt-sensitive strain. On chromosome 13 the renin locus influences blood pressure but appears to be expressed only in conjunction with as yet unknown alleles. The interaction of the missense mutations found in the coding regions of the α-adducin genes on chromosome 14 and α-adducin on chromosome 4 explain up to 50% of blood pressure difference between the Milan hypertensive and normotensive rats. And there are other genes on chromosome 17 and 20, the testis-specific histone locus and the heat-shock protein 70 and 27, respectively, that are significantly related to blood pressure but their relation to renal function is not apparent. There is also a linkage of hypertension to the Y chromosome in some strains of SHR but not in others.

Monogenic Changes and Hypertension

In contrast to the apparently diffuse, uncertain, and subsidiary position of the polygenic contribution in essential hypertension and hypertensive strains of rats, there are rare monogenic abnormalities in humans, which are entirely responsible for raising blood pressure, and others that lower it (241). In view of the extent of the evidence that links sodium and the kidney to hypertension it is not surprising that all the monogenic mutations identified so far, which induce changes in blood pressure, both up and down, do so by interfering with sodium reabsorption in the kidney.

The most relevant mutation regarding essential hypertension is Liddle's syndrome, in which the patient presents early with moderate to severe hypertension. It is caused by mutations of either the β or γ subunits of the amiloride-sensitive sodium channel on chromosome 16 that increase the passage of sodium into the cell and therefore increase sodium reabsorption in the kidney. Renal transplantation corrects the rise in blood pressure, which suggests that, as regards the origin of the hypertension, the defect is intrinsic to the kidney, and that therefore impairment in the kidney's ability to excrete sodium alone can cause hypertension.

There are two other monogenic causes of hypertension, glucocorticoid-remediable aldosteronism (GRE) and the syndrome of apparent mineralocorticoid excess (AME). In GRE, mutations of two genes on chromosome 8, the products of which are involved in adrenal biosynthesis cause aldosterone synthesis to be brought under the control of ACTH as well as angiotensin II. This leads to a rise in plasma aldosterone and therefore increases sodium reabsorption and the blood pressure. AME patients also have excess stimulation of mineralocorticoid receptors, although the plasma aldosterone is low. Normally the cells that contain mineralocorticoid receptors contain an enzyme, 11 β hydroxycorticoid dehydrogenase, that metabolizes cortisol to cortisone and prevents cortisol from binding onto the mineralocorticoid receptors. Patients with AME have a mutation of the gene responsible for 11ßhydroxycorticoid dehydrogenase so that cortisol can now bind onto aldosterone receptors (as well as aldosterone), but as cortisol circulates at concentrations that are orders of magnitude greater than aldosterone the net effect is one of mineralocorticoid excess. Glycyrrhetinic acid, which is a constituent of liquorice, inhibits 11β hydrocorticoid dehydrogenase and is the cause of the hypertension that is associated with the excessive consumption of licorice particularly in Holland.

In contrast to the monogenic lesions that increase sodium reabsorption and raise the blood pressure, there are monogenic lesions that diminish sodium reabsorption, and lead to sodium loss and hypotension (e.g., Gitelman's syndrome and pseudohypoaldosteronism). Patients with Gitelman's syndrome have a low blood pressure, neuromuscular abnormalities, and hypokalemia. There is an array of mutations of a gene on chromosome 16 that encodes the renal thiazide-sensitive Na-Cl cotransporter on the distal convoluted tubule. These result in loss of function and an increase in sodium excretion. Pseudohypoaldosteronism type I (PHA-1) is characterized by severe dehydration in the neonatal period, marked by hypotension, metabolic acidosis, and a high serum potassium concentration. These patients have mutations in chromosome 12 or 16, which each encodes different subunits of the same amiloride-sensitive sodium channel as that which is affected in Liddle's syndrome. In PHA-1, however, the mutations instead of activating the sodium channel impair its function and thus diminish sodium reabsorption and increase sodium excretion, this is accompanied by secondary defects in the secretion of hydrogen and potassium ions.

The Mechanisms Responsible for the Rise in Blood Pressure Resulting from Impaired Ability to Excrete Sodium

The renal cross-transplantation experiments described earlier and the demonstration that there is a connection between the kidney's ability to excrete sodium and the onset of hypertension are among the most significant advances made in the pathogenesis of essential hypertension in the past 80 years. However, they beg the question, how does an "unwillingness" to excrete sodium cause hypertension? It is not a question that is very often addressed. Two disconcerting but logical conclusions emerge from the cross-transplantation experiments in rats. The first is that in hereditary strains of hypertension the rise in arterial pressure stems from one or more genetic abnormalities of the kidney. It does not follow that the genetic faults that lead to the functional hypertensinogenic renal disturbance are only expressed in the kidney. There is much evidence that there are generalized membrane abnormalities in hereditary hypertension in both rats and humans (242–245). Nor does it follow that the same genetic abnormalities are responsible for all forms of hereditary hypertension. The second conclusion that emerges from cross-transplantation experiments is that if there are genetic abnormalities expressed in vascular smooth muscle, the brain, or sympathetic system in hereditary forms of hypertension, they do not, per se, cause hypertension, because blood pressure of a hypertensive strain rat does not rise in the presence of a normal kidney. Conversely, an imposed surgical procedure on a kidney can induce hypertension in an animal with no genetic abnormalities of its vascular smooth muscle or nervous system.

In other words, the mechanisms that cause blood pressure to rise as a consequence of a primary abnormality in the kidney are secondary. It is useful to divide these into afferent and efferent mechanisms. The evidence suggests that the kidney's impaired ability to excrete sodium stimulates cardiothoracic (or renal) afferents to the hypothalamus that induce a multiplicity of pressor changes; these increase sympathetic activity and thus arterial tone, which raises the blood pressure, a rise buttressed by rises in plasma noradrenaline (246) and ouabain

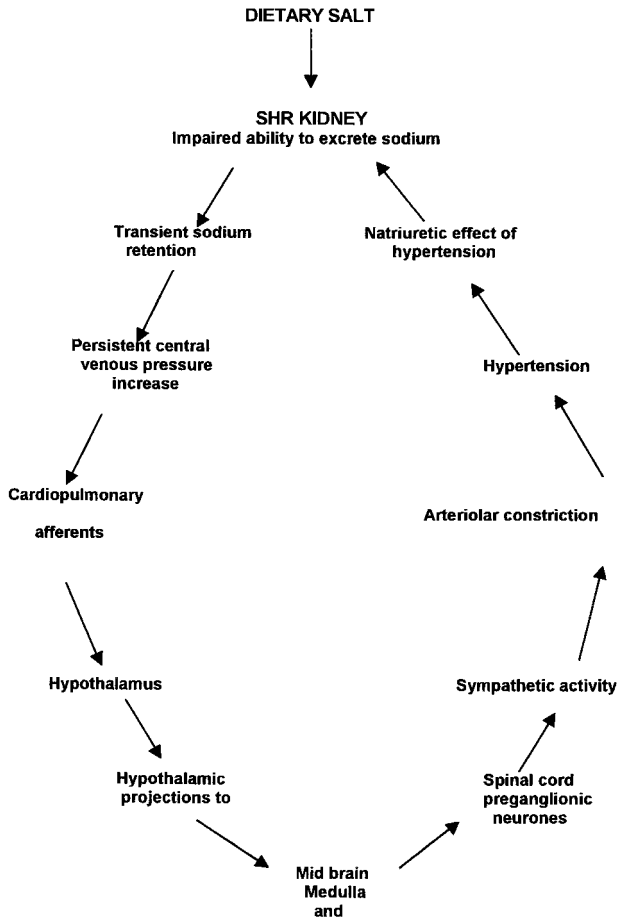

FIGURE 50-15. Flow diagram of a hypothesis that illustrates a chain of possible links between an impaired ability to excrete sodium and hypertension. (From: de Wardener HE. The hypothalamus and hypertension. *Physiol Rev* 2001;81:1599, with permission.)

(247,248); the natriuretic effect of a rise in pressure then tends to correct the kidney's impaired ability to excrete sodium (Fig. 50-15), which is also countered by the well-documented decrease in plasma renin and rise in plasma atrial natriuretic peptide (249).

Afferent Mechanisms

Theoretically, the immediate effect of an impaired ability to excrete sodium should be to alter hydrostatic pressure or change sodium concentration. Cardiopulmonary hydrostatic pressure changes in hereditary strains of hypertensive rats and essential hypertension do occur, and it has been demonstrated in normal humans that it is the intrathoracic blood volume that controls urinary sodium excretion, not total blood volume (250). Blood volume in established essential hypertension and the inherited forms of hypertension is usually normal or low, although it is raised in a minority of patients (251). This appears inconsistent with the proposal that the kidneys have a persistent difficulty in excreting sodium in essential hypertension, because one would intuitively suppose that such a persistent tendency toward volume expansion would lead to volume expansion. In primary hyperaldosteronism, however, a parallel situation in which hypertension is certainly associated with a persistent tendency to retain sodium, the blood volume in the majority of patients is either normal or low, as in essential hypertension (252)

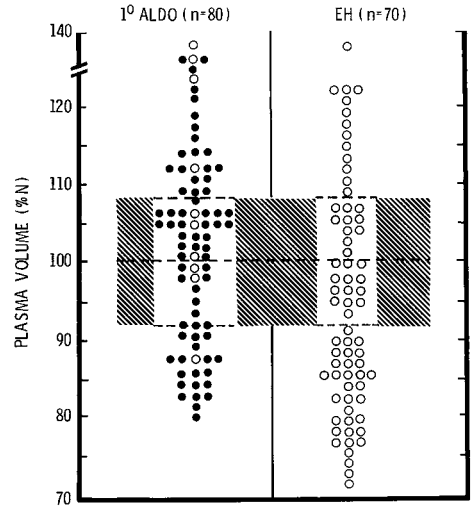

FIGURE 50-16. Plasma volume values during normal dietary sodium intake in patients with primary hyperaldosteronism (1° Aldo) and essential hypertension (EH). Values are expressed as percent of normal. The *crosshatched area* represents the variability of the method (± 8%). The *open circles* are primarily aldosteronism owing to hyperplasia; the *closed circles*, aldosteronism owing to adenomas. In primary aldosteronism, there were as many patients who were hypovolemic (25%) as there were those who were hypervolemic (30%). (From: Bravo EL, et al. The changing clinical spectrum of primary aldosteronism. *Am J Med* 1983;74:641, with permission.)

(Fig. 50-16). It is raised in only a minority of patients; nevertheless, patients with essential hypertension and those with primary hyperaldosteronism behave as if they were volume expanded. The phenomenon of accelerated natriuresis (253,254) occurs in both, and the plasma of both has an increased concentration of mammalian ouabain (255). The rise in plasma ANP in essential hypertension is also consistent with a central blood volume disturbance, because the stimulus for the release of ANP is distention of the atria (256).

Venous Tone and Central Venous Pressure

The discrepancy between having a normal or low total blood volume and yet responding to certain stimuli as if the blood volume were expanded is perhaps explained by the observation that in essential hypertension and in hypertensive strains of rats, there is an increase in tone of the venous vasculature with a diminished venous compliance that causes a shift of blood from the periphery to the thorax (257,258). These changes are accompanied by a rise in left atrial pressure both in humans (259) and the rat (260,261). It appears, therefore, that a normal or even low blood volume in hereditary forms of hypertension is associated with such a shift of blood to the chest that the intrathoracic venous pressure rises in spite of an overall fall in blood volume. That this rise in central venous pressure leads to a persistent increase in neural traffic from the chest to the hypothalamus is a supposition that appears reasonable. There is evidence that acute afferent neural stimulation from the chest can induce certain hypothalamic changes. The proposal that cardiothoracic afferents might be responsible for pressor hypothalamic changes in essential hypertension and hypertensive strains of rats mirrors the well-documented phenomenon that renal afferents appear to induce pressor hypothalamic changes in certain forms of experimental renal failure (262–264). Renal deafferentation by dorsal root section prevents the rise in arterial pressure after 5/6 removal of renal mass (264) and lowers the arterial pressure by about 50% in the one-kidney, one-clip hypertensive rat but it has no effect on the development of

hypertension in the SHR—one form of hereditary hypertension (265). There also is some evidence that renal afferents are involved in the hypertension that accompanies chronic renal failure in humans (266).

Efferent Mechanisms

In most forms of hypertension, particularly early on, there is an increase in sympathetic activity that is owing to the extensive functional changes in the hypothalamus.

Sympathetic Activity

The increase in sympathetic nervous activity in essential hypertension has been recorded by measuring noradrenaline "spillover" (i.e., the amount that is released from the nerve endings that does not return into the sympathetic nerves and is not metabolized), and by measuring neuronal activity of the posterior tibial or peroneal nerve. Both methods reveal that in patients with essential hypertension below the age of 30 to 40 there is an increase in activity that diminishes thereafter (267–269). Direct evidence for an increase in sympathetic nervous activity in the spontaneously hypertensive rat, the Dahl salt-sensitive rat, renovascular hypertension, chronic renal failure from reduction of renal mass, and mineralocorticoid hypertension has been obtained by measurement of electrical activity in sympathetic nerves (270–272).

Hypothalamic Changes

Experiments in hypertensive strains of rats and certain experimental forms of hypertension have shown that the particular site in the hypothalamus where a functional change takes place, and the direction of the change, determine the direction of the alteration in arterial pressure. The same neurotransmitter is able to have either pressor or depressor activity depending on the neuronal system targeted (273). Hypothalamic abnormalities include increases in neuronal firing and the secretion and production of neurotransmitters and neuromodulators such as catecholamines, acetylcholines, angiotensin, natriuretic peptide, vasopressin, nitric oxide synthase, ouabain, serotonin, γ-aminobutyric acid, neuropeptide Y, tachykinin, opioids, thyrotropin-releasing hormone, bradykinin, vasoactive intestinal peptide, histamine, corticotrophin-releasing factor, and their receptors and associated enzymes (249). Increased gene expression in the hypothalamus of the SHR has been detected for angiotensinogen (274), angiotensin receptors (275), vasopressin (276), atrial natriuretic peptide (277), nitric oxide synthase (278), pre-proneuropeptide (279) pre-proenkephalin (280), vasoactive intestinal peptide (281), and thyrotrophin releasing hormone precursor (282). The type and direction of most of the changes found in hypertensive animals are pressor and they appear to be, both individually and collectively, responsible for the increase in sympathetic activity and rise in arterial pressure. A few are probably depressor, effectively compensatory adjustments (278).

Most of the changes in hypothalamic activity that occur in hypertension take place in the rostral hypothalamus and the forebrain. The effect of the anesthetic used is critical and the age of the rat at the time of observation is also of paramount importance. A few changes can be detected before the onset of the rise in pressure and others only appear as the pressure is rising or thereafter. Overall the evidence suggests that with age there is a progressive pattern of change. Early pressor increases in catecholamine (283) and serotonin (284) activity become less pronounced with time and, as a sustained pressor increase is seen in cholinergic activity (285) and diminution in gamma-aminobutyric acid (GABA) activity (286) take over. The earlier diminution in nitrate oxide synthase (NOS) activity changes to a depressor increase. The lessening with age, of the early increase in sympathetic activity (see the preceding) makes it likely that overall there is an evolutionary decrease in the intensity of the initial pressor associated hypothalamic change. The functional changes in the various forms of hypertension are similar but there are differences. In DOCA + salt hypertension there is no increase in catecholamine activity and there is the pressor effect of the circulating DOCA on hypothalamic mineralocorticoid receptors (287). In the Dahl salt-sensitive rat there is greatly increased permeability of the blood-brain barrier and an associated increase in water content of the brain (288).

Summary

It is proposed that in patients with essential hypertension and inherited forms of hypertension in rats there is an initial retention of sodium that stimulates compensatory mechanisms. They not only cause an increase in arteriolar tone, but also an increase in venous tone that tends to shift blood centrally; they also are responsible for a wide variety of central nervous and humoral changes, including abnormalities in the hypothalamus, sympathetic nervous system, and the concentration of a variety of substances that circulate in the plasma. Collectively, these changes raise blood pressure, which overcomes the impaired ability of the kidneys to excrete sodium.

ACQUIRED HYPERTENSION AND THE KIDNEY

The diseases that afflict and destroy the kidney cause acquired hypertension in humans. Many of the same conditions can be imposed experimentally in animals. A variety of glomerular nephritides can be induced. Hyperaldosteronism is usually reproduced in animals by administration of DOCA; and increasing the salt intake speeds and accentuates the process. Coarctation of the aorta in humans can be surgically reproduced in dogs by an operation that narrows the aorta. The relatively uncommon human condition of renal artery stenosis can occur with a normal contralateral kidney. This condition can be reproduced in animals by placing a partially constricting clip on the renal artery of one kidney and either leaving the other kidney intact or removing it. Salt intake is often increased to accelerate the onset and increase the severity of the hypertension in such experimental maneuvers. The nearest experimental equivalent to a renin-producing tumor of the kidney is a slow chronic infusion of angiotensin II. Acromegaly is another form of hypertension with salt and water retention.

Mechanisms that Raise Blood Pressure

The mechanisms responsible for the rise in arterial pressure in the acquired forms of hypertension are much the same as those discussed for essential hypertension. Most forms of acquired hypertension involve some form of renal abnormality that gradually distorts and destroys the kidney and consequently impairs sodium excretion. This sets in motion similar mechanisms that raise blood pressure in essential hypertension and hereditary forms of hypertension in the rat. And these are reinforced by increased renal afferent stimulation to the hypothalamus (see the following). In addition, the reduction in renal mass appears to bring in to operation three other mechanisms: (a) the renal control of interstitial space compliance, (b) the secretion of certain hypotensive hormones from the renal medulla, and (c) the reciprocal relationship

between fluid volumes and the concentration and effectiveness of angiotensin II.

Renal Control of Interstitial Space Compliance

The compliance of the interstitial space determines the proportion of the extracellular fluid volume that is apportioned between the plasma and the interstitial space. Interstitial space compliance, therefore, is one determinant of blood volume. Blood volume, more particularly its distribution, is an important factor in the control of arterial pressure.

An index of the compliance of the interstitial space can be obtained by measuring the ratio of the plasma volume (PV) to the portion of the extracellular fluid volume (ECFV) that lies in the interstitial space (i.e., the interstitial fluid volume [IFV]); thus IFV = ECFV − PV. Measuring changes in interstitial fluid pressure, particularly during an acute infusion of saline, can also assess interstitial space compliance. To maintain constant extracellular fluid osmolality, it is probable that changes in interstitial space compliance (i.e., its swelling to pressure characteristics) are accompanied by a change in the capacity of the space to bind sodium. Hyaluronic acid molecules can bind large amounts of sodium ions (289). Changes in the compliance of the interstitial tissues and their ability to bind sodium probably represent a physiologic way to store water and sodium. These mechanisms can be demonstrated in some animals adapted to withstand heat and dehydration. There are studies of changes in body water and electrolytes after water deprivation and heat stress in Somali donkeys and Zebu cattle, animals that live under desert conditions for several days without drinking (290). Donkeys lose up to 21% of body water and 29% of extracellular fluid but up to only 14% of plasma volume, whereas serum sodium concentration increases by only 7%. They can then drink large amounts of water and restore their total body water deficiency in a few minutes, whereas serum sodium falls by only 5% (291).

The following observations suggest that the kidney controls the compliance of the interstitial space. Plasma volume: extracellular fluid volume ratio increases significantly more after bilateral nephrectomy than after unilateral nephrectomy and placement of the remaining ureter in the inferior vena cava. The interstitial pressure rises more after bilateral nephrectomy, although there is greater expansion of the interstitial fluid volume after unilateral nephrectomy and placement of the remaining ureter in the vena cava (290,292,293). Interstitial space compliance has been measured by measuring changes in interstitial fluid volume and tissue pressure (TP) 10 minutes after a saline infusion (compliance = IFV/TP). After unilateral nephrectomy and placing the remaining ureter in the vena cava, there was little change in compliance from values obtained after unilateral nephrectomy alone; following bilateral nephrectomy, however, compliance falls severalfold. Similar observations have been made 60 days after partial constriction of one renal artery with a clip and removal of the opposite kidney. Tissue pressure and venous pressure are significantly higher in the rats that develop hypertension; in these animals, plasma volume also increases, but interstitial fluid volume remains unchanged (i.e., the plasma volume: interstitial fluid volume [PV/IFV)] ratio is higher). After removal of the clip, tissue pressure falls despite a rise in interstitial fluid volume, and there is a fall in PV/IFV.

A study of patients with chronic renal failure has yielded similar results (294). Patients were divided into two groups, one with GFR >32 mL/minute and the other with GFR <20 mL/minute. The PV/IFV ratio of the group with the lower GFR was significantly higher than that in the group with the higher GFR. On increasing salt intake, the PV/IFV ratio of the group with the lower GFR rose still higher, whereas in the patients with the higher GFR the ratio did not change. It was also noted that the associated rise in blood pressure induced by the increase in salt intake was directly related to the change in the PV/IFV ratio. In other words, the lower the renal mass, the less the compliance of the interstitial fluid space, so that, with the increase in sodium intake and the tendency to retain sodium (and water) that are associated with reduced renal mass, a large proportion of the extracellular fluid volume is apportioned to the plasma volume. The greater the plasma volume, the higher the arterial pressure, possibly through the mechanisms discussed earlier.

The nature of the substance responsible for the change in interstitial space compliance is not known. The experiments demonstrating its presence make it likely that the concentration of the substance is dependent on the excretory function of the kidney or on the kidney's ability to secrete renin. It is probably a hormone, the rate of release of which is dependent on the amount of renal mass and its effective perfusion with blood.

Hypotensive Hormones from the Renal Papilla

If fragments of renal papilla (0.5 to 1.0 mm in size) from a healthy donor animal are transplanted subcutaneously or intraperitoneally into an animal suffering an experimentally induced form of hypertension, blood pressure falls toward normal (295–297). This observation has been made in renoprival hypertension in the dog and the rabbit and in one-clip, one-kidney hypertension in the rat (295,298). The hypotensive effect is reversed by removal or rejection of the papillary transplant. In time there is a change in the morphologic characteristics of the transplant. The tubules disappear, and the transplant consists mostly of renal interstitial cells embedded in a network of capillaries. Similar results have been obtained by injecting live monolayer cultures of renomedullary interstitial cells subcutaneously. In contrast, chemical ablation of the papillae in a rat on a normal diet leads to hypertension (299). It has been concluded that the renal medullary cells induce this hypotensive effect by releasing into the circulation a substance or substances that cause vasodilation (300).

Renal medullary cells lie alongside and between Henle's loop, the vasa recta, and the collecting ducts (295,301). They are surrounded by proteoglycans, which they secrete, and they extend cytoplasmic processes that touch adjoining structures. Renal interstitial cells have lipid-containing granules that are a source of substrate for conversion into membrane lipid, prostaglandins, and possibly antihypertensive lipids. The granules per se, either intact or disrupted, however, do not have a hypotensive effect on their own when injected intravenously. Many forms of experimental hypertension are associated with a significant decrease in the lipid granules, which also show several degenerative changes. Such changes also occur in a papillary renal transplant or in cultured renal medullary cells when they are no longer able to lower the blood pressure.

The renomedullary vasodepressor hormone secreted by the renomedullary interstitial cells in the renal papillae is a lipid named medullolipin I. It is conveyed to the liver, where it is converted into its active form, medullolipin II, a vascular dilator that suppresses sympathetic activity and causes natriuresis. Its action also opposes that of angiotensin II (302). The secretion and conversion of medullolipin in both the kidney and liver are related to cytochrome P450-dependent enzyme systems.

The main factor that controls the secretion of medullolipin is renal arterial perfusion pressure. Renal venous blood medullolipin I has been obtained in greatly increased amounts after suddenly raising renal arterial perfusion pressure by unclipping a one-kidney, one-clip hypertensive rat or rabbit preparation (303). The effect of this sudden release of medullolipin I has been demonstrated by an extracorporeal pump perfusion

of an isolated clipped kidney in circuit with a conscious normotensive rat (303). On removing the renal artery clip, there is a significant fall in pressure of the normotensive rats from 125 to 70 mm Hg within 1 hour without an associated rise in heart rate. The sudden appearance of medullolipin in the renal venous blood as blood pressure is falling is associated with an acute degranulation of the renomedullary interstitial cells of the kidney that has just been unclipped. It is concluded that the medullolipin acutely released into the general circulation after unclipping the renal artery is an important factor in the fall of arterial pressure, which occurs for about 24 hours after the clip is removed. Maintenance of the low arterial pressure subsequently appears to be caused by other mechanisms. In contrast therefore with the effect of lowered perfusion pressure on renin secretion, elevation of the renal arterial perfusion pressure causes a compensatory increase in medullolipin I secretion, and lowering the perfusion pressure shuts off secretion.

A deficiency of medullolipin that contributes to an associated hypertension can be caused by: (a) the decrease in the number of and damage to renomedullary cells that occurs in accelerated experimental hypertension and malignant hypertension in humans, (b) inhibition of medullolipin secretion by angiotensin II, sympathetic stimulation, and nitric oxide synthase inhibition, and (c) removal of renomedullary cells by bilateral nephrectomy, surgical and chemical papillectomy, and papillary atrophy or necrosis (295,302,304,305).

Medullolipin I by mouth lowers blood pressure of the SHR without altering cardiac output or heart rate (306). One case of hypotension resulting from hypermedullolipinemia, which in turn is caused by a lipinomedullolipinoma of renal medullary interstitial cells, has been described in a 46-year-old woman (307).

Fluid Volumes and Angiotensin II

There is a relationship between the concentration of plasma angiotensin II and the state of fluid volumes that is particularly relevant in most forms of acquired hypertension. In normal humans and animals, there is a reciprocal relationship between plasma angiotensin II and the effectiveness of angiotensin II. Normally, the effects of changes in blood volume or sodium intake on the arterial pressure are nicely balanced. Although moderate blood volume expansion induces a fall in angiotensin II, it increases its effectiveness; although a contraction of the blood volume increases angiotensin II, it reduces its effect; thus, in both instances the resultant change in blood pressure is buffered.

These relationships may become distorted with renal disease; therefore, serious disturbances in blood pressure usually occur. Eighty percent of patients with diseased kidneys have a tendency to volume expansion, whereas the distortion and architectural compression of the renal vascular tree causes a simultaneous rise in plasma renin activity and angiotensin II. This is the worst of all worlds. There is an increased vascular response to the raised levels of angiotensin II because of the tendency toward volume expansion. This is presumably why blood pressure rises early, quickly, and often to heights that cause severe complications in most patients with chronic renal failure.

There is a persistent restraint on urinary sodium excretion in primary hyperaldosteronism. Similarly, it can be shown in experimental animals given DOCA or fludrocortisone, or following renal artery stenosis, that there is a brief initial period of positive sodium balance with a gain in weight; however, the weight gain usually levels out, then the weight is restored to near its control value (308–310). As in essential hypertension, systemic venous compliance diminishes, and there is a tendency for the central blood volume to rise (297,310,311). Edema does not form. Blood volume, like that in essential hypertension, tends to be normal or low or is raised in only a minority of patients (308,310). Total exchangeable sodium tends to be raised (308,309). Plasma renin is reduced, and it is possible that the hypertension is due in part to the raised level of plasma ouabain (312) and vascular reactivity factor (313,314), but there is still insufficient information on this point.

In unilateral renal artery stenosis, with the other kidney *in situ,* plasma renin activity rises, and there is initial sodium retention. Then, as mentioned, blood pressure rises, and sodium balance is adjusted by increasing the excretion of sodium through the unclipped kidney, presumably because of the rise in arterial pressure and an increase in plasma natriuretic activity. Then there is a continued rise in plasma renin that can be demonstrated to be an important cause of the rise in arterial pressure. The eventual outcome with renal artery stenosis of a sole remaining kidney is a tendency toward salt and water retention and a fall in plasma renin, so that the main hypertensive mechanisms are related to volume retention.

Hypertension and Dialysis

Most patients on maintenance hemodialysis continue to fight a relentless battle against hypertension (315). The mechanisms responsible for the rise in pressure are the same as those that caused the rise in pressure before dialysis, but now they are magnified and tend to be glaringly obvious. This is the reason why nephrologists have no difficulty in accepting the role of salt and water in the control of blood pressure. On the one hand, a normal intake of fluids and the gross reduction in daily urine volume inevitably cause a rapid increase in extracellular fluid volume between each dialysis (easily monitored as a gain in weight), and on the other hand, severely diseased kidneys tend to secrete large amounts of renin. Furthermore, the need to lower the patient's weight at each dialysis by rapidly removing a considerable amount of fluid may cause a further brisk rise in plasma renin activity (316). If this recurring seesaw reciprocal movement of extracellular fluid volume and renin release is sufficiently violent, it eventually seems to resonate. Then the more one attempts to control blood pressure by fluid removal, the greater the rise in renin and angiotensin II so that at each dialysis the blood pressure, instead of falling, rises and becomes uncontrollable. In one patient, in an attempt to control the hypertension, the weight was lowered from 60 kg to 40 kg in about 2 months (316) (Fig. 50-17). This so reduced the extracellular fluid and blood volume that the patient fainted when he stood up, but his renin secretion was now so great that when he lay down he had severe hypertension with intolerable headaches and a tendency to develop acute pulmonary edema. The problem was resolved by bilateral nephrectomy, an operation that is now rarely performed to control hypertension in patients on maintenance hemodialysis. Weight reductions are performed now with more circumspection, and there are better hypotensive agents. By using measurements of blood volume, extracellular fluid volume, and plasma renin activity before and immediately after a dialysis and administering substances that nullify the pressor effect of angiotensin II, it is possible to show that hypertension in patients on maintenance hemodialysis are either dependent on an increased volume or a raised plasma angiotensin II, or sometimes a combination of both.

In Figure 50-18 are illustrated the findings in a patient whose blood pressure was volume dependent. Before dialysis when blood pressure was 200/100 mm Hg, the blood volume was obviously high, and there was a gross expansion of extracellular water, whereas plasma renin activity was abnormally low. And the lack of effect of an intravenous infusion of saralasin

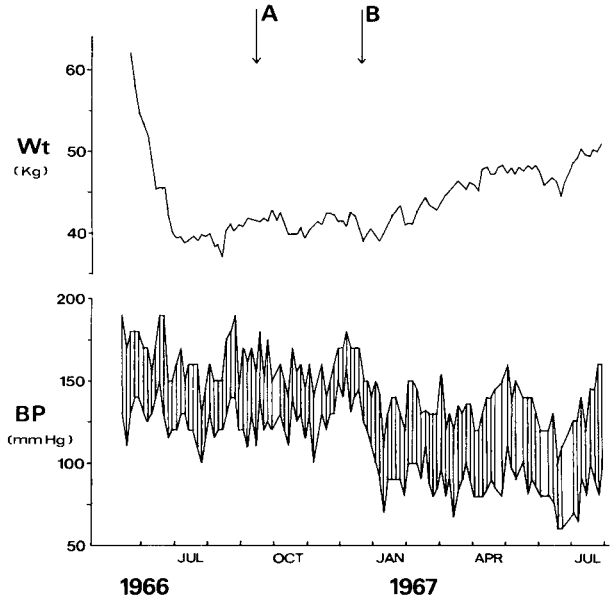

FIGURE 50-17. The effect of bilateral nephrectomy (*B*) on uncontrollable hypertension in a patient on maintenance hemodialysis with a very high plasma renin. There is an immediate fall in blood pressure, which is maintained although there is a rise in weight. At point **A** the patient had an attack of acute infectious hepatitis. (From: Brown JJ, et al. Plasma renin concentration and the control of blood pressure in patients on maintenance haemodialysis. *Nephron* 1969;6:329.)

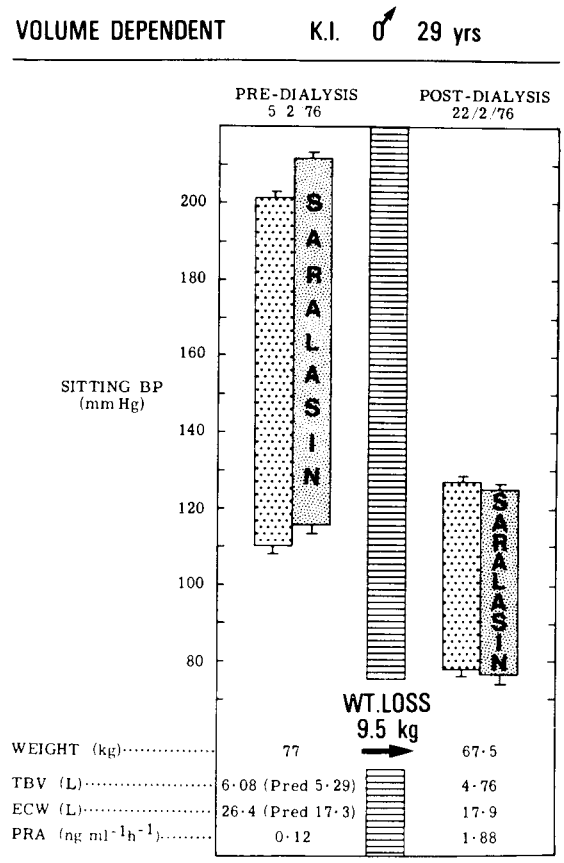

FIGURE 50-18. Patient on maintenance hemodialysis with hypertension caused by excess volume. Blood pressure (BP), weight, total blood volume (TBV), extracellular water (ECW), plasma renin activity (PRA), and the effect of an infusion of saralasin on the blood pressure, measured before and after the loss of 9.5 kg of weight in 17 days, are shown. Saralasin, a competitor of angiotensin II, was infused to gauge the dependency of blood pressure on angiotensin II. On the first occasion, when blood pressure was 200/100 mm Hg, the blood volume obviously was high, and there was a gross expansion of extracellular water, plasma renin activity was abnormally low, and the lack of effect of the infusion of saralasin on blood pressure indicated that blood pressure was not dependent on the plasma level of angiotensin II. On the second occasion, after the loss of weight, when blood pressure was 128/78 mm Hg, blood volume, extracellular water, and plasma renin activity were now normal, and the saralasin infusion again demonstrated that blood pressure was not dependent on angiotensin II.

(a competitor of angiotensin II) on blood pressure indicated that blood pressure was not dependent on the plasma level of angiotensin II. After several dialyses, the patient lost 9.5 kg when blood pressure was 128/78 mm Hg, and the blood volume, extracellular fluid volume, and plasma renin activity became normal; the saralasin infusion again demonstrated that blood pressure was not dependent on angiotensin II.

In Figure 50-19 are illustrated the findings in a patient in whom blood pressure was dependent on a raised level of angiotensin II. Before dialysis, when blood pressure was 180/100 mm Hg, the blood volume was normal, plasma renin activity was greatly raised, and the fall in pressure induced by saralasin indicated that blood pressure was under the influence of angiotensin II. After a dialysis, the patient lost 2.2 kg, blood pressure fell to 170/95 mm Hg, and the blood volume was still "normal," but the plasma renin activity was now extremely high and the greater fall in blood pressure induced by the saralasin infusion indicated that blood pressure was even more dependent on angiotensin II.

Renal Hypotension

Renal hypotension is an infrequent complication of some forms of renal disease and usually follows bilateral nephrectomy in a patient on maintenance hemodialysis. The renal diseases that are most prone to this unusual complication are those that affect predominantly the renal medulla (e.g., the reflux nephropathy, obstructive uropathy, and phenacetin nephropathy). Although the condition is sometimes referred to as salt-losing nephritis, it is rarely owing to glomerular nephritis. The fall in arterial pressure is due to a urinary leak of sodium. It occurs only with bilateral renal disease and after the onset of renal failure. It is associated with a gross generalized hypertrophy of the juxtaglomerular apparatus and the adrenals. Plasma renin activity and aldosterone are raised. Often there is hypokalemia caused in part to the hyperaldosteronism and in part to the renal disease. The condition tends to fluctuate wildly. Exacerbations often are caused by an upper urinary tract infection when the combination of infection, hypotension, a fall in extracellular fluid volume, and hypokalemia may cause severe but usually entirely and easily reversible reductions in renal function. In spite of these alarming episodes, these patients survive longer than other patients with renal failure whose progress is calmer but who develop hypertension. Survival of patients with renal hypotension depends on the skill and speed of reaction of those who look after them. The continuous administration of oral supplements of sodium chloride such as Slow Sodium (Novartis), repeated monitoring of the urine for infection, and prompt treatment of a relapse may prevent any progressive deterioration in renal function.

Renal hypotension is such a serious and frequent complication of the anephric state that bilateral nephrectomy is no longer used for the treatment of intractable hypertension in

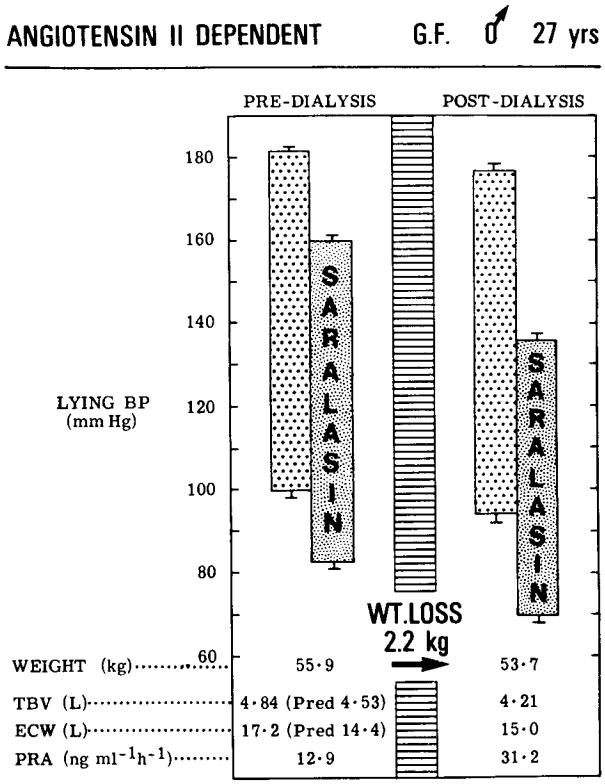

FIGURE 50-19. Patient on maintenance hemodialysis with hypertension caused by high plasma renin activity. Blood pressure (BP), weight, total blood volume (TBV), extracellular water (ECW), plasma renin activity (PRA), and the effect of an infusion of saralasin on blood pressure measured before and after a loss of 2.5 kg of weight during a single dialysis of 7 hours are shown. Saralasin, a competitor of angiotensin II, was infused to gauge the dependency of blood pressure on angiotensin II. Before dialysis, when blood pressure was 180/100 mm Hg, the blood volume was normal, although the extracellular water was 2.5 kg greater than predicted (a not unusual gain in weight between dialyses), plasma renin activity was greatly raised, and the fall in blood pressure induced by the infusion of saralasin indicated that blood pressure was under the influence of angiotensin II. After dialysis, having lost a "normal" amount of weight, blood pressure was 170/95 mm Hg, the blood volume and extracellular water were normal, but the plasma renin activity was now extremely high, and the greater fall in blood pressure induced by saralasin infusion indicated that blood pressure was even more dependent on angiotensin II.

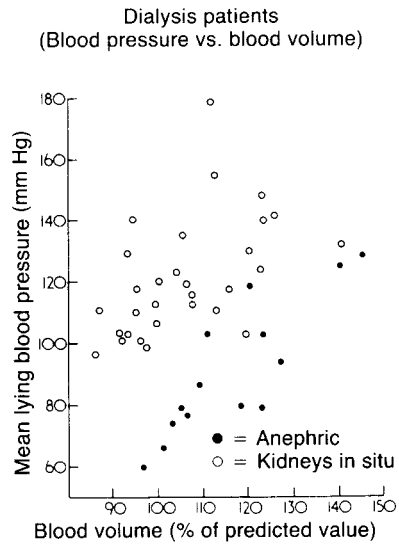

FIGURE 50-20. Blood volume versus mean lying blood pressure in patients on maintenance hemodialysis. At normal blood volumes blood pressure of anephric patients (•) was lower than in patients whose kidneys were still *in situ* (○).

patients on maintenance hemodialysis. After bilateral nephrectomy and the fall in plasma angiotensin II, the relation between the arterial pressure and the blood volume is such that the volume has to be greater than normal to maintain a normal blood pressure (317) (Fig. 50-20). Many anephric patients therefore live precarious lives of fluctuating hypotension in spite of an overexpanded extracellular fluid volume, sometimes to the point of demonstrable clinical edema and breathlessness. The low arterial pressure presumably is due primarily to the almost total absence of renin and angiotensin II, but there is evidence that it may also result in part from the associated reduction in plasma aldosterone.

These two forms of hypotension beautifully illustrate the interdependence of angiotensin and extracellular fluid volume in the control of blood pressure. On the one hand, in "salt-losing nephritis," the urinary leak of sodium and the decrease in extracellular fluid volume are so great that the arterial pressure falls in spite of enormous rises in plasma renin activity. On the other hand, after bilateral nephrectomy in the absence of angiotensin, gross overexpansion of the extracellular fluid volume does not prevent the arterial pressure from being intolerably low. Some workers have claimed that the relationship between fluid volume and arterial pressure after bilateral nephrectomy depends on whether the patient suffered from hypertension before the operation. Occasionally, amyloidosis of the sympathetic chains causes hypotension in a patient on dialysis. Until now this has been an intractable and irreversible condition that leads to endless difficulties and death.

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CHAPTER 51 ■ HYPERTENSION ASSOCIATED WITH RENAL PARENCHYMAL DISEASE

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In 1836, Richard Bright (1) reported that the presence of small granular kidneys at postmortem examination was associated with cardiac enlargement and theorized that the kidney was the "chief promoter" of left ventricular hypertrophy. Subsequently, the discovery of renin in 1898 (2) and the classic experiments by Goldblatt et al. in 1934 (3) established the central role of the kidney in the control of arterial blood pressure. Multiple neurohumoral factors increase blood pressure by limiting salt excretion and shifting the renal function curve to the right (4–9). In addition, not only can renal parenchymal disease initiate and sustain hypertension (8), but also the consequent elevation of blood pressure exposes the remaining renal tissue to further hydraulically mediated damage (10).

This chapter reviews the important pathophysiologic mechanisms, clinical features, and therapy of hypertension caused by both unilateral and bilateral renal parenchymal disease. Although relevant models of experimental renal parenchymal hypertension are discussed, the primary focus of this chapter is to emphasize key advances in the understanding of hypertension in human renal disease.

UNILATERAL KIDNEY DISEASE

Unilateral Kidney Disease

Unilateral renal parenchymal disease resulting from reflux nephropathy, congenital malformations, or bacterial pyelonephritis can cause hypertension. Butler first described the association between unilateral kidney disease and hypertension (11). He reported that unilateral nephrectomy could cure hypertension in patients with unilateral chronic pyelonephritis. However, the exact prevalence of this form of secondary hypertension is unclear (12). Extensive early experience with unilateral nephrectomy in hypertensive patients with presumed unilateral parenchymal disease produced a disappointing cure rate of only 26% (13). Additional investigation, however, has clearly shown that with proper selection and careful preoperative evaluation a subset of patients benefits substantially from surgical intervention. Although initial investigations utilized split renal function studies (14,15) or clinical characteristics (16) to predict response to unilateral nephrectomy, these criteria have been abandoned because of poor predictive value and have been replaced by renal vein renin ratios.

Vaughan et al. (17) and Delin et al. (18) evaluated hypertensive patients with unilateral renal parenchymal disease of diverse etiologies. Both groups of investigators found that cure or improvement of hypertension was limited to patients with preoperative lateralizing renal venous renins. Additional data have confirmed and extended the positive predictive value of renal venous renins by demonstrating amelioration of hypertension in 90% of patients with lateralizing renal venous renin ratios of 1.5 or greater (19,20). In an important study, Gordon et al. (21) evaluated 20 hypertensive patients with unilateral renal parenchymal disease. All patients had measurements of supine and stimulated renal venous renins and underwent unilateral nephrectomy without regard to the renin values. These investigators found that recumbent lateralizing renal venous renin ratios of 1.5 or more identified patients likely to benefit from nephrectomy with a sensitivity of 100%, a specificity of 84%, and a positive predictive value of 100%. Despite the fact that not all studies support the utility of renal venous renins in predicting response to surgery (22), we believe it is reasonable to restrict operative intervention or renal artery embolization to hypertensive patients, with uncontrolled blood pressure, who demonstrate evidence of functional ischemia. Hypertension in patients with unilateral kidney disease and nonlateralizing renins probably reflects occult bilateral disease or essential hypertension.

A note of caution is warranted, however, in the evaluation of hypertensive patients with apparent unilateral kidney disease. DeJong et al. (23) reported eight young women with hypertension and unilateral renal parenchymal disease. Renal venous renins were measured in four and did not lateralize to the side with the apparent unilateral parenchymal disease. Renal angiography revealed fibromuscular disease of the contralateral renal artery in all eight patients. Surgical revascularization of the stenotic kidney normalized blood pressure in all patients. The fact that unilateral renal parenchymal disease can coexist with contralateral fibromuscular dysplasia underscores the importance of this diagnostic consideration, especially in patients with nonlateralizing renal venous renins. In this latter group of patients, nephrectomy should not be considered until renal artery stenosis has been excluded by angiography.

Hypertension in Patients with Solitary Kidneys

Hypertension, proteinuria, focal glomerulosclerosis (FGS), and progressive loss of renal function occur in experimental renal ablation in rats (24–26) and frequently in humans with unilateral renal agenesis (26–32). Because of these observations, some authorities have raised the issue of whether patients undergoing unilateral nephrectomy for renal disease or transplant donation are at risk for the development of hypertension, loss of renal function, or both (30,33–35). Renal ablation by five-sixths nephrectomy in rats consistently results in hypertension, proteinuria, and FGS (24,36,37). Moreover, in the same model, supplementation of renal mass by isograft transplantation reduces blood pressure, attenuates proteinuria, and ameliorates glomerulosclerosis (38). Glomerulosclerosis and progressive renal insufficiency have been associated with characteristic intraglomerular hemodynamic alterations consisting

TABLE 51-1

HYPERTENSION IN PATIENTS WITH SOLITARY KIDNEYS

	No. of patients	No. with hypertension	Percent	References
Unilateral agenesis	193	96	50	27–29,31,32,49–54
Unilateral nephrectomy	156	22	14	34,56,57,59
Kidney donation	833	137	16	35,58,60–65

of an increased single-nephron glomerular filtration rate (GFR), an increment in glomerular capillary flow rate (Q_A), and elevated glomerular capillary pressure (P_{GC}) by most (37,39,40), but not all (41,42) investigators. On the other hand, some studies have suggested a link between glomerular hypertrophy (41,42), manifest as increments in glomerular volume (V_G) or glomerular capillary radius (R_{GC}), or both glomerular hypertrophy and altered intrarenal hemodynamics (26,43,44) and progressive kidney disease in this model. Therapeutic maneuvers that reduce P_{GC}, such as dietary protein restriction or angiotensin-converting enzyme (ACE) inhibition, decrease proteinuria and retard progressive renal injury (25,26,37). Nevertheless, the relation among altered intrarenal hemodynamics, glomerular hypertrophy, and progressive FGS in experimental renal ablation is unsettled and requires further investigation (45,46). It is important to note, however, that three-fourths reduction in renal mass in a canine model does not lead to inexorable loss of renal function (47). Thus, although these observations in experimental models of renal ablation are provocative, their relevance to human renal disease is not established.

Table 51-1 depicts the prevalence of hypertension in patients with unilateral renal agenesis and in those who underwent unilateral nephrectomy for renal disease or transplant donation. In 1960, Ashley and Mostofi (48) reported the first association between unilateral renal agenesis and progressive renal insufficiency. They noted that, of 232 patients with unilateral renal agenesis, 16% developed chronic renal failure and that one-third of these had "chronic glomerulonephritis;" however, the prevalence of hypertension was not reported. Since then, the association between unilateral renal agenesis and the development of proteinuria, hypertension, and chronic kidney disease (CKD) owing to FGS has been emphasized repeatedly (27–29,31,32,49–54). Hypertension is more prevalent in patients with unilateral renal agenesis compared with uninephrectomized individuals (Table 51-1). Kiprov et al. (27) found that four of eight patients with unilateral renal agenesis and histologically proven FGS were hypertensive. In addition, in a series of 586 cases of renal biopsy or nephrectomy specimens, they found 29 cases of FGS; there were 24 cases of FGS in the 581 patients with two kidneys (4.1%), whereas all 5 patients with unilateral agenesis demonstrated FGS ($p < 2 \times 10^{-7}$) (27). These findings have been confirmed by other investigators (28–32) and suggest a clear relation among an increased prevalence of hypertension, FGS, and unilateral renal agenesis. Argueso et al. confirmed this increased risk for proteinuria, hypertension, and CKD in a study of 159 patients with unilateral renal agenesis (54). It is possible that the number of remaining nephrons in the contralateral kidney is reduced in patients with unilateral renal agenesis. Alternatively, an *in utero* reduction in renal mass could subject the remaining nephrons to abnormal hemodynamic forces or neurohumoral factors with the consequent development of glomerulosclerosis (26,55). Occasionally, the hypertension is renin dependent and subsides with removal of the dysplastic kidney (53), whereas in other cases proteinuria and renal insufficiency antedate the increase in blood pressure (27,28). In most instances of unilateral renal agenesis, however, the pathophysiologic mechanisms relating hypertension, proteinuria, and FGS are speculative but unproved (30,32).

The prevalence of hypertension is lower in patients subjected to unilateral nephrectomy for renal disease or transplant donation (Table 51-1). Despite periods of prolonged observation, multiple studies have shown that high blood pressure was not more common overall in patients undergoing nephrectomy for unilateral kidney disease compared with the general population (28,29,34,50–52,56,57). A more important issue, and the subject of considerable debate, is whether kidney donors are at increased risk for the development of hypertension or CKD. One group of investigators noted a 47% prevalence of hypertension in 38 renal transplant donors followed for more than 10 years (58); however, this prevalence was not different from that of an age- and gender-matched control group. Other authorities have made similar observations (59). Despite the fact that one report suggests that kidney donors demonstrate an accelerated rate of development of hypertension (60), the majority of studies show no increase in the risk for the development of proteinuria, hypertension, or CKD (61–65). With an average follow-up ranging from 8 to more than 15 years, the prevalence of hypertension does not exceed that of the general population (Table 51-1). In a meta-analysis of 48 studies with 3,124 patients who had undergone a nephrectomy and 1,703 controls, Kasiske et al. (66) demonstrated that there was a small rise in systolic blood pressure (2.4 mm Hg) that increased further (1.1 mm Hg/decade) with longer duration of follow-up in the nephrectomized group but there was no increase in the overall prevalence of hypertension. Thus, in normal individuals, unilateral nephrectomy does not cause progressive renal dysfunction, but may be associated with a small increase in blood pressure. However, patients with a family history of hypertension may be more likely to develop hypertension following uninephrectomy (67).

Taken together, the available data suggest that although the findings from experimental models of renal ablation might be applicable to patients with more than 50% reductions in renal mass (68,69), they cannot be extrapolated to all patients with solitary kidneys. Although unilateral renal agenesis is definitely associated with an increased risk for the development of proteinuria, hypertension, and FGS (70), unilateral nephrectomy for renal disease or transplant donation does not increase the risk for developing systemic hypertension or CKD in the remaining kidney. The reason for these apparently contrasting observations is unclear but might relate to unrecognized contralateral renal disease, the developmental stage at which the reduction in renal mass occurs, or other unrecognized factors (26).

Miscellaneous Lesions

Hypertension has been described in association with several lesions that could potentially stimulate renin secretion from the unilaterally involved kidney. Renin secreting tumors arising

from the juxtaglomerular cells are rare. Patients present with hypertension, hypokalemia, and hyperreninemic hyperaldosteronism (71). Control of blood pressure usually requires ACE inhibition and definitive treatment is surgical resection. Most patients with Wilms' tumor also develop a renin dependent form of hypertension that resolves after excision of the tumor (72,73). Simple cysts (74,75), renal tuberculosis (76), renal infarction (77,78), and segmental hypoplasia (79,80) have been linked to the development of hypertension. In the majority of cases, unilateral hypersecretion of renin was demonstrated and blood pressure normalized after surgical intervention (74–76,78–80). Some reports, however, have not documented lateralizing renal venous renins in all patients (79). These findings are reminiscent of similar observations in renovascular hypertension and might reflect the effect of dietary salt intake or hypertension maintained by increased activity of the sympathetic nervous system.

Bilateral Kidney Disease

Hypertension is common in both acute and chronic renal disease. In acute renal parenchymal disease, hypertension is chiefly mediated by expansion of the extracellular fluid volume (ECFV). Volume depletion usually normalizes blood pressure, and hypertension resolves with improvement of renal function. Because hypertension is often transient in acute kidney disease, its pathogenesis has not been studied extensively. On the other hand, in CKD, not only is hypertension more frequent, but its pathogenesis is more complex and its long-term cardiovascular effects are of greater significance. Consequently, the following sections focus largely on the pathogenesis, sequelae, and management of hypertension in patients with CKD.

Acute Renal Disease

Hypertension occurs more frequently in acute glomerulonephritis than in acute tubular necrosis (81–83). In the largest analysis to date, Bonomini et al. (81) retrospectively reviewed 262 patients with acute renal failure and serum creatinine concentrations >5 mg/dL. Hypertension developed at some time during the course of acute renal failure in 103 patients (39%) and was more common in patients with acute renal failure due to glomerular or vascular disease (73%) compared with those whose renal insufficiency was the result of tubulointerstitial processes (15%). High blood pressure was consequent to expansion of the ECFV from salt and water retention and responded to salt subtraction during dialysis in hypertensive patients with acute renal failure, especially in the subset with acute tubular necrosis (81). These investigators found that overall 17% of patients who experienced an episode of acute renal failure were hypertensive at 3 months; however, the prevalence of hypertension was 30% after 3 years of follow-up. Hypertension was more common in patients who developed acute renal failure consequent to glomerular disease compared with those with acute tubulointerstitial disease after both 3 months (42% vs. 3%) and 3 years (53% versus 17%) of observation.

The pathogenesis of hypertension in acute renal failure due to clinically apparent acute poststreptococcal glomerulonephritis has been studied extensively (82–85). Hypertension occurs in 80% of patients with epidemic poststreptococcal glomerulonephritis and is thought to be primarily volume mediated because of consistent findings of suppressed plasma renin activity (PRA) and reduced aldosterone levels (82–84). Rodriguez-Iturbe et al. (85) studied 13 children with acute poststreptococcal glomerulonephritis during the acute illness and after resolution of the disease. Reduction in blood pressure from a mean of 121/80 mm Hg to 90/54 mm Hg was associated with an average weight loss of 1.4 kg and an increase in creatinine clearance from 47 to 123 mL/minute. Initially, PRA and aldosterone were suppressed but increased to normal values within 4 to 6 weeks. Because the elevation of blood pressure during the acute illness was significantly correlated with fluid retention, estimated by changes in weight ($R = 0.74$, $p < 0.01$), these investigators concluded that hypertension in poststreptococcal glomerulonephritis was chiefly volume dependent. They postulated that an acute decline in glomerular filtration rate (GFR) causes salt and water retention, expands the ECFV, suppresses the renin-angiotensin-aldosterone system (RAAS) and results in hypertension. Further investigations by the same group (86) confirmed their initial observations and also demonstrated reduced urinary excretion of prostaglandin E_2 (PGE_2) and $PGF_{2\alpha}$ as well as kallikrein in 14 patients with acute poststreptococcal glomerulonephritis. They found no correlation, however, between decreased prostanoid or kallikrein excretion and activity of the RAAS, or changes in weight or blood pressure. Converting enzyme inhibition with captopril decreased blood pressure in hypertensive patients with poststreptococcal glomerulonephritis, reduced plasma aldosterone, and increased urinary PGE_2 and kallikrein (87). The unexpected antihypertensive efficacy of ACE inhibition in this setting of presumably volume-dependent hypertension could be the result of a further decrease in an inappropriately elevated angiotensin II level, interruption of the vascular RAAS, or increased synthesis of vasodilator and natriuretic prostaglandins and kinins.

Twenty percent to 30 percent of adults with minimal change disease are hypertensive at the time of initial presentation (88). Both vasoconstrictor (83,89) and volume-dependent (90) forms of hypertension have been described. Some investigators have shown hypertension and increased PRA in minimal change disease that are ameliorated by volume expansion or steroid-induced remission (83,89). These observations support the notion that hypertension in this setting is the result of angiotensin II–induced vasoconstriction consequent to underfilling of the systemic vasculature that results from hypoalbuminemia. On the other hand, some studies have clearly shown a volume-dependent component of hypertension in some patients with minimal change disease. Dorhout Mees et al. (90) measured plasma albumin, blood pressure, plasma volume, and blood volume in 10 patients, 5 of whom were hypertensive, with minimal change disease before and after treatment with corticosteroids (Fig. 51-1). Steroid therapy resulted in a substantial increase in plasma albumin and significant reductions in blood pressure (17/14 mm Hg), plasma volume, and blood volume. Blood pressure normalized in the 5 hypertensive patients, and GFR increased an average of 42 mL/minute in all 10 subjects. There were no consistent changes in the normal or decreased PRA levels with steroid administration.

Consequently, the evidence to date suggests that the majority of hypertensive patients with acute renal disease exhibit volume-dependent hypertension that improves with salt removal during dialysis or with spontaneous or steroid-induced natriuresis. However, vasoconstrictor-mediated hypertension is evident in some. It is also likely that, given the multiple etiologies of acute renal disease, both volume and vasoconstrictor mechanisms are responsible for hypertension in many patients in a fashion similar to the development of hypertension in chronic renal disease.

Chronic Kidney Disease

Hypertension associated with renal parenchymal disease is the most common form of secondary hypertension; 5% of all hypertensive patients have underlying kidney disease (91). Renal parenchymal hypertension is important for several reasons. First, renal parenchymal hypertension increases the risk of

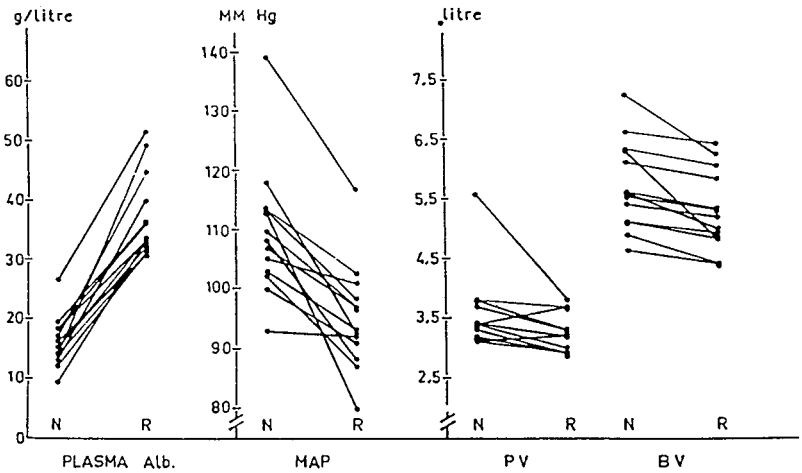

FIGURE 51-1. Plasma albumin (Alb), mean arterial pressure (MAP), plasma volume (PV), and blood volume (BV) in 10 patients with minimal change disease before *(N)* and after *(R)* steroid-induced remission. (Reprinted with permission from: Dorhout Mees EJ, et al. Observations on edema formation in the nephrotic syndrome in adults with minimal lesions. *Am J Med* 1979;67:378.)

cardiovascular morbidity and mortality to an equal or even greater extent compared with the risk attributable to essential hypertension (91,92). Second, hypertension secondary to kidney disease is associated with a greater prevalence of retinopathy, especially malignant retinopathy, compared with essential hypertension at all levels of blood pressure (93), suggesting that neurohumoral factors involved in its pathogenesis are particularly vasculotoxic. Finally, hypertension in kidney disease accelerates the loss of renal function in both diabetic and nondiabetic nephropathy (94,95). Renal parenchymal hypertension can occur even with a normal serum creatinine concentration and normal GFR (94,96) and increases in prevalence as renal function decreases (94,95,97–99). More than 80% of patients with CKD are hypertensive (98), and hypertension is present in up to 90% of patients by the time end stage renal disease (ESRD) supervenes (100,101).

Similar to hypertension in acute renal disease, the prevalence of hypertension in CKD varies depending on the underlying pathology. Table 51-2 summarizes the approximate prevalence of hypertension in different chronic renal diseases. The data are derived from series in the recent literature and include patients with various levels of renal function (83,97–99,102–108). It is apparent that hypertension is more frequent in patients with chronic glomerular disease compared with those with polycystic kidney disease or chronic interstitial nephritis (99,102,109). Moreover, among the glomerular diseases, hypertension is more common in FGS and membranoproliferative glomerulonephritis compared with mesangioproliferative glomerulonephritis or immunoglobulin A (IgA) nephropathy (108).

TABLE 51-2

PREVALENCE OF HYPERTENSION IN CHRONIC RENAL PARENCHYMAL DISEASE

Disease	Percent with hypertension	References
Glomerular disease		83,97,98, 99,102,103, 104,105,106, 107,108
Focal glomerular sclerosis	75–80	
Membranoproliferative glomerulonephritis	70–75	
Diabetic nephropathy	70–75	
Membranous nephropathy	50–60	
Mesangioproliferative glomerulonephritis	40–45	
IgA nephropathy	40	
Polycystic kidney disease	70	
Chronic interstitial nephritis	50–60	

Pathophysiology

Discussion of hypertension in CKD requires a brief examination of the central role of the kidney in the regulation of arterial blood pressure (110). This critical function is primarily the result of the kidney's unique position in regulating salt balance. Blood pressure is expressed by the equation

$$BP = CO \times TPR$$

where *BP* is blood pressure, *CO* is cardiac output, and *TPR* is total peripheral resistance. Although cursory examination of this equation could lead to the conclusion that increments in cardiac output, TPR, or both can result in hypertension, its simplicity obscures two important observations. First, isolated elevation in TPR will not cause sustained hypertension without a simultaneous constraint on renal sodium excretion. Second, a primary defect in salt excretion can result ultimately in an increased blood pressure maintained by an elevated TPR with a normal cardiac output. Both of these critical concepts have been developed and refined by Guyton et al. in a series of elegant experiments over the last 20 years (9,111–114).

In an isolated perfused kidney, Guyton et al. (111) developed a renal function curve by plotting arterial pressure as a function of urinary sodium excretion (Fig. 51-2). The solid line in Figure 51-2 depicts sodium excretion over a wide range of perfusion pressures in the isolated perfused kidney free of any neurohumoral constraints on salt excretion. The curve intersects the line at a point (a) where sodium intake and excretion are in equilibrium. Utilizing a computer-generated model of arterial pressure regulation, Guyton et al. (111,113) found that alterations in TPR alone would not change long-term arterial pressure in the presence of a constant sodium intake and an unchanged renal function curve. For example, if an increase in TPR raised arterial pressure to 150 mm Hg (b), sodium excretion would increase substantially (c) until a sufficient decrease in ECFV returned arterial pressure to the original set point (a). On the other hand, a decrease in arterial pressure owing to a reduction in TPR (d) would lower sodium excretion below sodium intake, eventually increasing ECFV and returning arterial pressure to baseline (a). Therefore, according to this model, changes in salt intake or salt excretion (i.e., the position of the

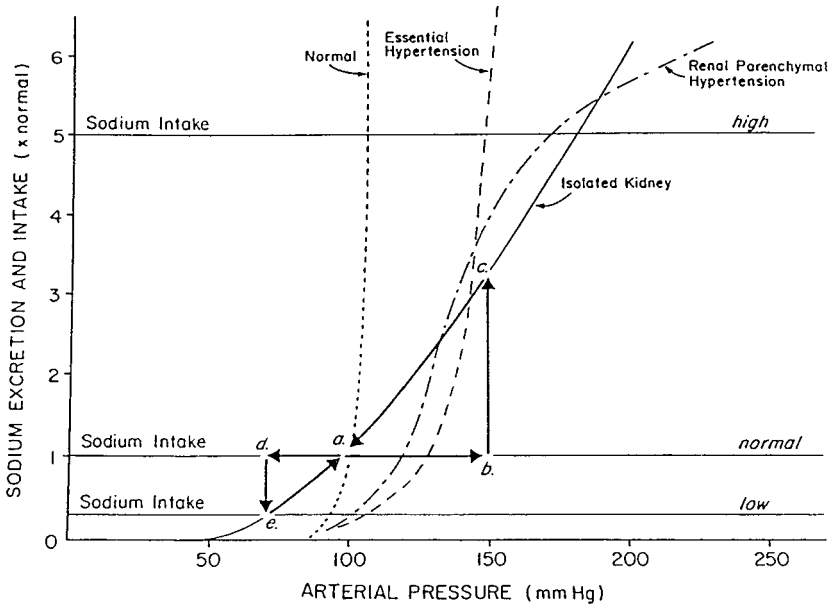

FIGURE 51-2. Renal function curves plotting sodium intake and excretion against mean arterial pressure. Curves for the isolated perfused kidney and normal individuals are shown along with hypothetical renal function curves for salt-sensitive essential hypertension and renal parenchymal hypertension. (Modified with permission from: Guyton AC. Dominant role of the kidneys and accessory role of whole-body autoregulation in the pathogenesis of hypertension. *Am J Hypertens* 1989;2:575.)

renal function curve) are required for the development of sustained hypertension.

Renal function curves can be developed in humans by determining arterial blood pressure, once salt balance is achieved, over a range of dietary salt intake. The series of dashed lines in Figure 51-2 are examples of renal function curves in normotensive subjects, patients with salt-sensitive essential hypertension, and patients with renal parenchymal hypertension. The curve from the normotensive subjects was derived at extremes of dietary salt intake (115) and underscores the fact that wide variation in sodium intake has little or no effect on blood pressure in normal individuals, presumably because of the integrated neurohumoral response to changes in salt intake. The curves for essential hypertension and renal parenchymal hypertension are theoretic and are shifted to the right of the normal renal function curve. In both conditions, salt balance occurs at higher than normal arterial pressure with either normal or high salt intake. Salt restriction, however, reduces arterial pressure to the normotensive range at the equilibrium point.

Despite the fact that hypertension is sustained by an elevated TPR in the majority of patients with renal parenchymal hypertension (see Hemodynamic Patterns, below) (116–120), initiation of hypertension is probably the result of a positive salt balance and expanded ECFV. Guyton et al. (111,113) examined the effect of salt loading on circulating hemodynamics in a canine model of renal ablation. Substantial hypertension developed in this model after 4 days of a high-salt diet at a time when TPR was normal. This early increase in arterial pressure was consequent to an increased ECFV and elevated cardiac output. Over the next 2 weeks, blood pressure remained increased, and ECFV and cardiac output decreased toward, but not quite to, baseline (113). These observations are consistent with longitudinal studies in patients with mild to moderate renal insufficiency (121,122), and suggest that whereas hypertension is initiated by volume expansion in this model, it is maintained primarily by vasoconstriction. It is not clear how the transition from a volume-mediated to vasoconstrictor form of hypertension occurs. Possible explanations include local autoregulation of tissue blood flow (111) or the production of an endogenous digitalislike substance (EDLS) (123–125) that simultaneously increases TPR and enhances renal sodium excretion.

Eventually, renal parenchymal hypertension results from the integrated action of elements that influence cardiac output, TPR, or both. Table 51-3 lists some of the factors implicated in the pathogenesis of hypertension in CKD and their effect on cardiac output and TPR. It is important to note that each has a dual effect; both vascular resistance and salt balance are affected. Moreover, the effects are generally concordant with regard to the potential influence on blood pressure; vasoconstrictor compounds are antinatriuretic, whereas vasodilator substances are natriuretic; hence, it is difficult, if not impossible, to selectively alter TPR without simultaneously affecting renal salt excretion. The development of hypertension in a given patient with CKD results from the complex interplay of several or more of these elements in addition to the absolute level of GFR, underlying renal disease, genetic predisposition to hypertension, and dietary salt intake. Despite the fact that

TABLE 51-3

FACTORS THAT REGULATE ARTERIAL BLOOD PRESSURE

Affect cardiac output	Affect TPR
Extracellular volume (stroke volume)	Vasoconstrictor
Antinatriuretic	Angiotensin II
Angiotensin II	Arginine vasopressin
Norepinephrine	Endogenous digitalis-like substance
Antidiuretic	Endothelin
Arginine vasopressin	Norepinephrine
Natriuretic	Thromboxane A2
Atrial and brain natriuretic peptides	Vasodilator
Endogenous digitalis-like substance	Atrial and brain natriuretic peptides
Kinins	Kinins
Nitric oxide	Nitric oxide
PGE_2, PGI_2	PGE_2, PGI_2
Sympathetic nervous system (heart rate)	

TPR, total peripheral resistance.

multiple factors (Table 51-3) are conceivably involved in the pathogenesis of renal parenchymal hypertension, only those for which there is substantial support are discussed.

Salt Balance

Renal ablation in both rats (126) and dogs (113) clearly demonstrates the central role of salt balance in the development of hypertension under circumstances of reduced nephron mass. Ylitalo et al. (126) subjected rats to subtotal nephrectomy and found that a high-salt diet suppressed the RAAS and consistently produced severe hypertension in animals with a 70% reduction in GFR. In contrast, in the same experimental model, a normal salt intake caused only mild hypertension, and a low-salt diet resulted in no increase in blood pressure. Similarly, Guyton et al. (113) showed that dogs with renal ablation fed a high-salt diet develop hypertension initiated by expansion of the ECFV and an elevated cardiac output but maintained by an increased TPR.

Although hypertension produced by renal ablation in animals is clearly dependent on a positive salt balance, the situation with regard to human renal parenchymal hypertension is more complicated. Human renal disease often causes hypertension that not only is related to a positive salt balance consequent to a decreased GFR but also is the result of the production of hormones and autacoids that increase TPR, reduce sodium excretion, and shift the renal function curve to the right (Fig. 51-2). However, despite the complexity of the situation in an individual patient with a specific renal disease, there is persuasive evidence that suggests that overall a positive salt balance is critical to the development of hypertension in human renal disease. Many (127–129), but not all (130) studies have shown that hypertensive subjects with mild to moderate renal impairment have increased total exchangeable sodium compared with normotensive subjects, patients with essential hypertension, or normotensive patients with similar levels of renal function. These subtle alterations in total body sodium antedate frank hypertension or an increase in serum creatinine concentration. Harrap et al. (131) compared young adults with polycystic kidney disease to unaffected subjects from the same kindreds. They found that the affected subjects had a significant increase in total exchangeable sodium, PRA, and systolic blood pressure compared with unaffected offspring despite exhibiting similar, normal GFRs. Consequently, these investigators theorized that the increase in total exchangeable sodium, presumably owing to activation of the RAAS, could cause the increase in systolic pressure possibly mediated by elaboration of an EDLS (131).

Plasma volume, blood volume, or ECFV are often normal (130,132) or expanded (133,134) early in the course of CKD, but are consistently elevated by the time ESRD supervenes (135–138). In addition, salt loading in patients with ESRD results in a disproportionate increase in plasma volume and blood volume compared with interstitial fluid volume, further exacerbating hypertension (139). Last, blood pressure directly correlates with plasma volume (132) and exchangeable sodium (140) in patients with mild to moderate renal insufficiency and with ECFV (136,141) and exchangeable sodium (142) in those with ESRD; however, not all studies have confirmed these findings. Some have shown no correlation between blood volume or ECFV and blood pressure after the institution of maintenance dialysis (138,143) and only detected a relation between sodium and blood pressure in renoprival hypertension (143). Nevertheless, the bulk of the data support the concept that an increase in total exchangeable sodium occurs early in the course of renal disease when most measurements of plasma volume or ECFV are normal. As kidney function declines, an increase in ECFV is more readily detected and correlates significantly, although often weakly (140), with arterial pressure.

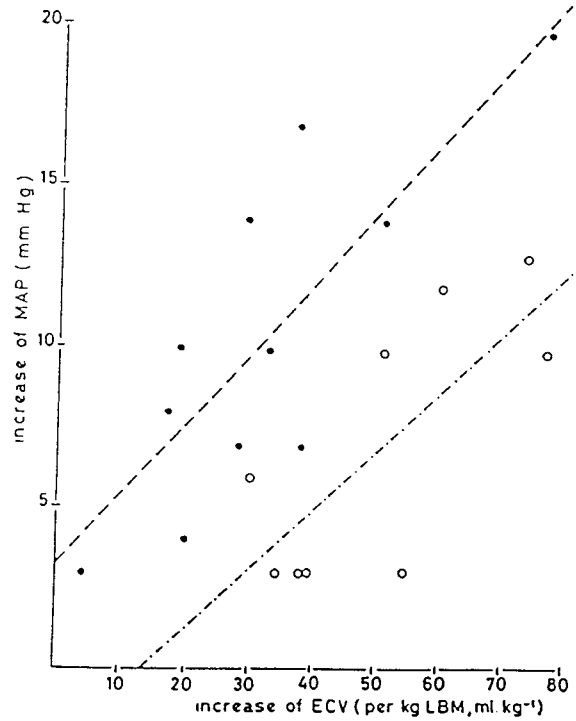

FIGURE 51-3. The relation between increments in mean arterial pressure (MAP) and extracellular fluid volume (ECV) per kilogram of lean body mass (LBM) in patients with chronic renal failure subjected to a high salt intake. For any given increase in ECV, there was a larger increment in MAP ($p < 0.005$) in group 2 (• creatinine clearance <22 mL/minute) compared with group 1 (○ creatinine clearance >32 mL/minute). (Reprinted with permission from: Koomans HA, et al. Salt sensitivity of blood pressure in chronic renal failure: evidence for renal control of body fluid distribution in man. *Hypertension* 1982;2:190, with permission of the American Heart Association, Inc.)

Studies that have examined the effect of changes in salt balance on blood pressure in patients with chronic renal disease provide additional support for the importance of salt in the genesis of renal parenchymal hypertension. Koomans and et al. (141) measured arterial pressure, blood volume, and ECFV in patients with stage 3 (creatinine clearance >32 mL/minute) or stage 4 (creatinine clearance <22 mL/minute) CKD at two levels of salt intake. Institution of a high-salt diet increased ECFV and arterial pressure in both groups. The increment in arterial pressure, however, was larger for any given increase in ECFV in the group with stage 4 disease (Fig. 51-3) compared with those with stage 3 CKD. These data suggest that a positive salt balance consequent to an increase in dietary salt augments both ECFV and blood pressure; this hypertensive effect is inversely related to GFR and is most pronounced in severe renal insufficiency. In patients with ESRD, the prohypertensive effect of a positive salt balance is the result, not only of an increase in plasma volume and cardiac output (144), but also of an enhanced vasoconstrictor response to angiotensin II (100,145).

Just as a positive salt balance increases blood pressure in patients with renal disease, reduction of salt intake or administration of diuretics decreases exchangeable sodium, plasma volume, ECFV, and arterial pressure in hypertensive patients with CKD (134,146–148). Vasavada and Agarwal recently demonstrated that daily administration of loop diuretics to patients with stage 2 and 3 CKD significantly decreased ECFV and ambulatory systolic blood pressure over a 3-week period (134). Further support for the dependence of blood pressure on salt balance is the fact that salt subtraction with reduction

of dry weight by hemodialysis (144,147,149–152) or peritoneal dialysis (153,154) normalizes blood pressure in the majority of ESRD patients without the need for pharmacologic therapy. Vertes et al. (149) initially showed that hypertension could be controlled in 35 of 40 patients undergoing maintenance hemodialysis with salt removal during ultrafiltration. Recently, Ozkahya et al. confirmed these findings by documenting substantial improvements in blood pressure in both hemodialysis and peritoneal dialysis patients with aggressive ultrafiltration and sodium restriction (154,155). Further, in a large cohort of patients undergoing chronic hemodialysis, interdialytic weight gain, reflecting salt and water intake, was an independent predictor of blood pressure (156). Finally, in the Hemodialysis Study, there was a strong, statistically significant correlation among intradialytic changes in blood pressure, body weight, and plasma volume (152). These important observations have been confirmed and extended by other investigators (150–154) and reinforce the concept that adequate fluid removal is the cornerstone of therapy in the hypertensive patient with ESRD.

The preceding data strongly support the central role that salt balance plays in renal parenchymal hypertension. However, salt balance is a major, but not exclusive, determinant of blood pressure in patients with CKD. Even in patients whose blood pressure is well controlled by salt removal during dialysis, binephrectomy reduces blood pressure without significant alterations in exchangeable sodium or ECFV (100,146). This latter observation might result from a decrease in the RAAS or a reduction in central sympathetic outflow and emphasizes the important relation between volume and vasoconstrictor factors in the control of arterial pressure even under circumstances where hypertension appears to be primarily sodium dependent (100,144,145).

Sympathetic Nervous System

Increasing evidence suggests that the sympathetic nervous system plays an important role in renal parenchymal hypertension. Activation of the sympathetic nervous system directly increases cardiac output and TPR and indirectly elevates vascular resistance by stimulation of the RAAS (157–159). Angiotensin II enhances both peripheral and central sympathetic activity reciprocally; the increment in central sympathetic outflow may be consequent to an angiotensin II-induced decrease in nitric oxide content in the posterior hypothalamus (160). Further, elevated renal sympathetic nerve traffic directly increases tubular reabsorption of sodium, reduces renal blood flow, and decreases GFR (160). Taken together, there are multiple mechanisms by which increased adrenergic activity contributes to hypertension through both vasoconstrictor and volume-dependent mechanisms.

The sympathetic nervous system plays an important role in several models of experimental renal hypertension. Vari et al. (161) studied the effect of renal denervation on the development of hypertension in uninephrectomized rats maintained on a low-salt diet. Simultaneous renal denervation and contralateral nephrectomy decreased renal norepinephrine stores and prevented the development of hypertension. When denervation was delayed until 6 weeks postnephrectomy, both renal norepinephrine content and arterial pressure declined (161). These investigators concluded that both the development and maintenance of hypertension in this experimental model were dependent on intact renal nerves. Theoretically, this beneficial effect of renal denervation might be consequent to elimination of the decrements in GFR and sodium excretion mediated by increased renal efferent sympathetic nerve activity. However, accumulating evidence suggests that renal afferent sympathetic neurons are important in renal hypertension. Campese et al. studied the role of the afferent renal nerves in the development of hypertension in rats that had undergone 5/6 nephrectomy (162). Endogenous norepinephrine concentrations and norepinephrine turnover in the hypothalamus were significantly increased in rats with intact renal afferent nerves. However, in rats subjected to dorsal rhizotomy, which interrupts the renal afferent nerves, the increment in hypothalamic norepinephrine was blunted and norepinephrine turnover was similar to control rats. Importantly, the increase in blood pressure induced by 5/6 nephrectomy was substantially attenuated by renal afferent denervation. The same group of investigators (163) repeated a similar set of experiments after the induction of acute renal injury by intrarenal injection of phenol. There was a significant increase in blood pressure and secretion of norepinephrine from the hypothalamic nuclei in rats with intact renal afferent nerves. As in their previous model, in rats that underwent renal denervation, renal injury by phenol did not induce a rise in blood pressure or norepinephrine secretion. These data provide persuasive evidence that afferent signaling from diseased kidneys is important in mediating central sympathetic outflow and hypertension in experimental renal disease. Whether the proximate cause of the increased afferent traffic is local ischemia or cytokine production remains to be determined (164,165).

Most studies (130,166,167), but not all (171), have found increased plasma concentrations of norepinephrine in hypertensive patients with mild to moderate renal dysfunction. Ishii et al. (166) found significantly increased plasma norepinephrine in hypertensive patients with chronic glomerulonephritis compared with normotensive patients with similar renal function or normal subjects. Further, they noted a significant correlation between arterial pressure and plasma norepinephrine concentration. Ligtenberg et al. demonstrated that muscle sympathetic nerve activity (MSNA) and serum norepinephrine levels were higher in patients with hypertension and CKD compared to normal controls (167). Treatment with enalapril for 4 to 6 weeks resulted in reduction of blood pressure and improvement but not normalization of MSNA (Fig. 51-4) and the baroreflex curve. In contrast, amlodipine lowered blood pressure but MSNA remained elevated. These important observations have been confirmed and extended by other investigators (168–170). Klein et al. showed that MSNA is increased in hypertensive patients with polycystic kidney disease irrespective of renal function (168). In patients with CKD, the enhanced MSNA is due to intrinsic renal disease and is not solely a function of reduced nephron mass. Moreover, the elevated MSNA in CKD that is ameliorated by interruption of the RAAS can be completely normalized by inhibition of central sympathetic outflow with monoxidine (170). Finally, hypertensive patients with renal disease demonstrate an increased pressor response to exogenous norepinephrine (171).

Accumulating data suggest that increased activity of the sympathetic nervous system contributes to hypertension in patients undergoing long-term dialysis. Plasma norepinephrine levels are usually increased in hypertensive patients with CKD before initiation of dialysis (166,167,172) and normal (173) or elevated (174–176) in hypertensive dialysis patients. Selective postganglionic sympathetic blockade reduces blood pressure in patients on maintenance hemodialysis. Schohn and et al. (174) gave debrisoquine, a postganglionic sympathetic blocker, to normal subjects and both normotensive and hypertensive patients undergoing long-term dialysis. Blood pressure declined by an average of 33/19 mm Hg in the hypertensive patients but did not change significantly in the two former groups. Further, Converse et al. (175) measured MSNA to skeletal muscle in 11 normal subjects and 23 patients receiving long-term hemodialysis. Five patients on dialysis had undergone bilateral nephrectomy, but 18 retained their native kidneys; 14 of the latter group were hypertensive. Sympathetic nerve discharge was significantly higher in the dialysis patients who retained their kidneys compared with their nephrectomized counterparts or

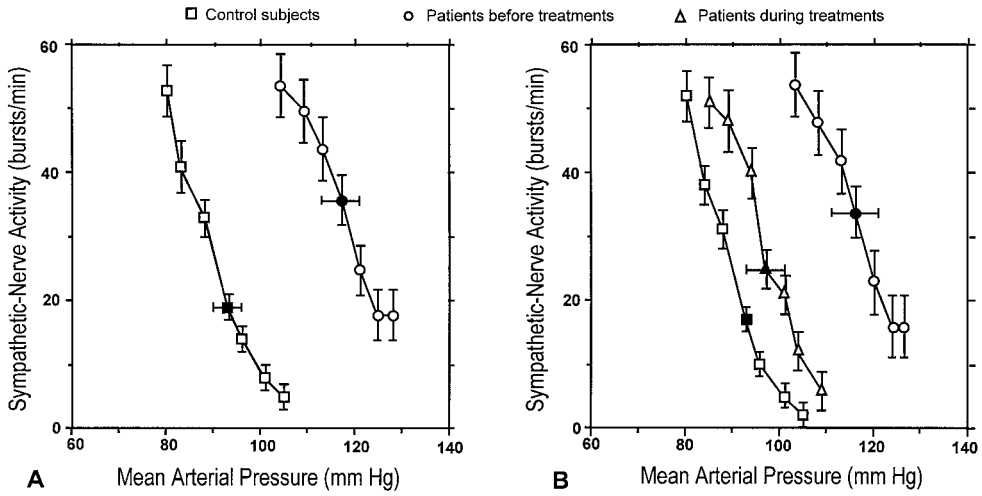

FIGURE 51-4. Baroreflex response to changes in mean arterial pressure (MAP) in patients with chronic renal insufficiency (average creatinine 3.9 ± 1.9 mg/dL) before and after treatment with enalapril and in control subjects. Sodium nitroprusside and phenylephrine were infused to provide gradations in MAP. Results are shown at baseline (**A**) in 14 patients with renal insufficiency and 14 control subjects and before and during treatment with enalapril (**B**) in 11 patients and 11 controls. Values are means ± SE. (Reproduced with permission from: Ligtenberg G, et al. Reduction of sympathetic hyperactivity by enalapril in patients with chronic renal failure. *N Engl J Med* 1999;340:1321, with permission of the Massachusetts Medical Society.)

normal subjects. Mean arterial pressure and vascular resistance in the calf were significantly lower in the nephrectomized dialysis patients compared with the patients who had not undergone nephrectomy. These and other data (177) suggest that retention of diseased kidneys, and not the uremic environment, is responsible for the increase in MSNA. Taken together, these observations provide persuasive support for the concept that afferent sympathetic nerve traffic from diseased kidneys signals the anterior hypothalamus with a consequent increase in peripheral sympathetic nerve activity (159,162,163,176).

Hence, there is abundant evidence that sympathetic overactivity contributes importantly to renal parenchymal hypertension. Early in the course of renal disease, circulating norepinephrine is often elevated, MSNA is increased, and the vascular response to exogenous norepinephrine is augmented. Increased sympathetic activity results in hypertension in patients with pre-ESRD not only by increasing TPR but also by directly stimulating renal sodium reabsorption and indirectly limiting salt excretion through activation of the RAAS. In ESRD, because urine volume is minimal, sympathetic overactivity raises blood pressure primarily, if not solely, by increasing TPR.

Pressor Compounds

Arginine Vasopressin. Despite extensive investigation, the exact role of arginine vasopressin (AVP) in renal parenchymal hypertension remains to be determined (178). Cowley et al. (179) infused AVP into normal dogs with an *ad libitum* intake of water; serum sodium decreased, weight increased, but blood pressure was unchanged. Any pressor effect of AVP, however, under these circumstances is difficult to interpret because any increase in TPR is offset by dose-dependent decrements in cardiac output with no net change in mean arterial pressure (180). On the other hand, in dogs with subtotal renal ablation, chronic infusion of subpressor doses of AVP resulted in hyponatremia, an increased blood volume, suppression of the RAAS, and hypertension (181). These data suggest that, in the face of substantial reductions in renal mass, AVP can contribute to hypertension by stimulation of V_2 receptors in the collecting duct with consequent hypotonic expansion of the ECFV. In rats subjected to bilateral nephrectomy (182) or subtotal renal ablation (183) and later rendered hypernatremic, administration of an AVP antagonist significantly reduced mean arterial pressure. In one study (182), there was an inverse correlation between blood pressure and plasma volume indicating that, in rats with decreased renal mass, AVP increases arterial pressure by stimulating V_1 receptors, inducing vasoconstriction, and increasing TPR.

Studies in humans have confirmed and extended these observations in experimental models. Padfield et al. (184) studied normal volunteers, patients with malignant hypertension, and patients with the syndrome of inappropriate antidiuretic hormone secretion. They found no correlation between the plasma concentration of AVP and blood pressure in either group of patients. In addition, an infusion of AVP in normal subjects did not increase arterial pressure. In normally hydrated volunteers, AVP antagonism had no significant effect on blood pressure, heart rate, or cutaneous blood flow (185). In contrast, application of an orally active V_1 antagonist to patients with essential hypertension reduced the peak blood pressure response and increased heart rate after the administration of hypertonic saline (186). These data suggest that AVP has a limited role in blood pressure homeostasis in normal individuals or those with essential hypertension; however, in patients with chronic renal failure, AVP might play a greater part in control of blood pressure. Plasma concentrations of AVP are increased in hypertensive patients with severe chronic renal insufficiency compared with normal volunteers and exhibit an enhanced response to an infusion of hypertonic saline (187). In patients with ESRD undergoing long-term hemodialysis (188,189) or continuous ambulatory peritoneal dialysis (190), plasma AVP levels are also increased and correlate significantly with plasma osmolality in some studies (189), but not in others (188). These observations, although provocative, shed no light on the role of AVP in the control of arterial blood pressure in patients with chronic renal failure.

Investigations that utilized specific AVP antagonists (191,192) provide more direct evidence of a role for AVP in renal parenchymal hypertension. Gavras et al. (193) infused

an AVP inhibitor in salt-loaded patients with ESRD receiving maintenance hemodialysis and demonstrated a modest decline (13/8 mm Hg) in blood pressure. Further, Miura et al. (194) administered an orally active V_1 receptor antagonist to a fluid-overloaded, hypertensive patient with diabetic nephropathy and a serum creatinine concentration of 5.5 mg/dL. Blood pressure decreased markedly without any change in heart rate, suggesting a substantial reduction in TPR owing to V_1 blockade. These preliminary but important observations suggest that, although in the presence of euvolemia and normal renal function AVP might not contribute significantly to control of arterial pressure, its vasoconstrictor effect is revealed in the setting of CKD. Additional investigation is required to determine whether AVP is consistently involved in the initiation or maintenance of renal parenchymal hypertension and to elucidate whether its effect is primarily mediated through expansion of the ECFV or an increment in TPR (178).

Renin-Angiotensin-Aldosterone System. Multiple lines of evidence suggest an important role for the RAAS in the initiation and maintenance of hypertension in patients with renal parenchymal disease. Plasma renin activity (131,166,195) and angiotensin II concentrations (130,196) are increased and correlate with arterial pressure in hypertensive patients with pre-ESRD. In addition, angiotensin II blockade with saralasin in hypertensive patients with early renal disease results in a decrease in blood pressure that is inversely proportional to baseline PRA (195). Some of the most persuasive evidence linking the RAAS system to renal parenchymal hypertension derives from studies in early polycystic kidney disease. Many (131,197,198), but not all (199) reports have found increased PRA in hypertensive patients with polycystic kidney disease and normal or only slightly impaired renal function. Filtration fraction is increased (131,199), reflecting the fact that angiotensin II preferentially constricts the efferent arteriole (200). Finally, administration of lisinopril decreases mean arterial pressure, renal vascular resistance, and filtration fraction (200). These data, along with the observation that renal volume is increased in hypertensive patients with polycystic disease compared with their normotensive counterparts, have led some authorities to speculate that enlarging cysts compress intrarenal arterioles, producing ischemia and ultimately activating the RAAS (197).

Not all studies, however, have supported a significant role for the RAAS in the hypertension of pre-ESRD. Vascular reactivity to exogenous angiotensin II is not increased (130,171), and PRA is often normal (199) or decreased (201). Recently, Wagner et al. (202), utilizing the quantitative polymerase chain reaction, demonstrated that renin gene expression in human renal biopsy specimens was decreased early in the course of glomerulonephritis. Consequently, hypertension in early stage kidney disease might be due to increased activity of the RAAS in some circumstances, but not others. Analysis of the relation is complicated by the fact that most studies have measured PRA and not assessed the role of the vascular renin-angiotensin system (203). Moreover, the involvement is complex because angiotensin II directly and indirectly stimulates renal sodium absorption, increases total exchangeable sodium, regulates GFR, and potentiates the activity of the renal sympathetic nerves (204). Finally, the role of aldosterone, per se, in the pathogenesis of renal parenchymal hypertension is incompletely understood. Aldosterone not only increases sodium reabsorption with consequent expansion of plasma volume but also enhances vasoconstriction in response to adrenergic stimulation (205). Administration of exogenous aldosterone to rats with remnant kidneys treated with losartan increased blood pressure, proteinuria, and glomerulosclerosis compared with rats that did not receive aldosterone (206). These data suggest a possible role for aldosterone in renal parenchymal hypertension, independent of angiotensin II. Hence, activation of the RAAS can mediate hypertension in CKD through both vasoconstrictor and volume-dependent mechanisms.

The majority of patients with ESRD demonstrate normal PRA and angiotensin II levels (144), are salt sensitive, and remain normotensive with reduction to dry weight during dialysis (149,150,155). Ten to twenty percent of hypertensive patients with ESRD, however, clearly exhibit renin-dependent hypertension. Blood pressure in this latter group does not respond to salt removal, and most patients (147,149,207,208), but not all (209) have elevated PRA. Arterial blood pressure and PRA correlate significantly in this subset of patients (143). Weidmann and et al. (207) examined the relation between blood pressure and PRA in 51 patients undergoing long-term dialysis. Thirty-one of 33 patients with normal blood pressure or hypertension that was easily controlled by ultrafiltration demonstrated normal PRA. Moreover, 18 patients demonstrated hypertension that was resistant to salt removal and eventually required bilateral nephrectomy to control blood pressure; 17 had elevated PRA. Before the availability of minoxidil and ACE inhibitors, bilateral nephrectomy was often required to control blood pressure in patients with increased PRA and resistant hypertension (207,208). Lifschitz et al. (208) found that hypertensive patients whose blood pressure normalized after bilateral nephrectomy could be identified by their preoperative decrease in blood pressure in response to angiotensin II blockade and their increased PRA. Decrements in arterial pressure in response to binephrectomy have been associated with a reduction in TPR but no change in cardiac output (210,211). Taken together, the humoral and hemodynamic response to bilateral nephrectomy has been interpreted by some investigators to further implicate the RAAS in this form of hypertension. However, these observations are also consistent with the important role of the sympathetic nervous system in the hypertension of ESRD (175). Predictably, ACE inhibition normalizes arterial pressure in patients with increased PRA and dialysis-resistant hypertension (100,212,213). Whether this owes exclusively to interruption of the RAAS or is mediated in part by reduced sympathetic nervous system (SNS activity) (167) remains to be determined.

Hence, there is substantial support for the pathogenetic role of the RAAS in the hypertension of CKD. Early in the course of some forms of renal disease, activation of the RAAS causes hypertension not only by increasing TPR but also by limiting renal sodium excretion. Patients with chronic interstitial nephritis are often normotensive with normal or only slightly increased PRA (207,214). When hypertension develops, it is usually responsive to salt depletion. At the other end of the spectrum, hypertensive patients with chronic glomerular disease, especially those with severe hypertension, often demonstrate markedly elevated PRA, respond poorly to salt removal, and require interruption of the RAAS for control of blood pressure (207). In the 10% to 20% of hyperreninemic hypertensive patients with ESRD, the vasoconstrictor action of angiotensin II is dominant, and hormonally mediated changes in salt balance are insignificant. However, the lack of consistent relation between blood pressure and either ECFV or PRA in patients with ESRD clearly suggests that multiple other factors are involved in the pathogenesis of hypertension in this setting (211,215).

Endothelin. Yanagisawa and et al. (216) initially identified endothelin, a 21 amino acid, vasoactive, mitogenic peptide, in 1988. Endothelin exists in three distinct isoforms and, although originally thought to be produced solely by vascular endothelium, is secreted by various cells in multiple tissues (217,218) including glomerular endothelial, epithelial, and mesangial cells. Synthesis of endothelin is increased by shear stress and a variety of agonists such as thrombin, angiotensin II, AVP, and transforming growth factor-β (219,220). Endothelin is the most potent vasoconstrictor yet described (218,221) and exerts

a wide range of physiologic effects including increasing the secretion of atrial natriuretic peptide (ANP), nitric oxide (NO), renin, and prostaglandins as well as stimulating mitogenesis in diverse cell lines (218,222). Infusion of endothelin in dogs increases blood pressure, TPR, and PRA and simultaneously decreases renal blood flow, GFR, and urinary sodium excretion (223,224). Therefore, it is possible that endothelin could be pathogenetically related to renal parenchymal hypertension consequent to hormonal, paracrine, or autocrine actions. Actions of endothelin are mediated through two different receptors. The ET_A and ET_B receptors in vascular smooth muscle mediate vasoconstriction, whereas ET_B receptors on endothelial cells mediate vasodilatation. However, there are species differences in receptor actions since in rats, but not dogs, renal vasoconstriction is due to endothelin binding to the ET_B receptor (225). Therefore, endothelin provides a counterregulatory mechanism serving the maintenance of normal renal function (226) depending on the differential effects of endothelin on various receptor subtypes.

Administration of endothelin at pathophysiologic doses in dogs markedly decreases renal blood flow (RBF) and GFR and increases PRA and plasma aldosterone (224). Experiments that have examined the effect of inhibition of endothelin converting enzyme or blockade of endothelin receptors, however, provide greater insight into the potential pathophysiologic role of endothelin in hypertensive renal disease. Erythropoietin-induced hypertension is ameliorated by ET_A blockade but not ET_B blockade (226). Inhibition of endothelin converting enzyme reduces blood pressure in both spontaneously hypertensive rats (SHR) and rats with renovascular hypertension (227). On the other hand, administration of an endothelin receptor antagonist decreases arterial pressure in the SHR (228,229) and in some (228), but not all (229) models of renovascular hypertension. Benigni and et al. (230) treated rats with subtotal renal ablation with a specific ET_A receptor antagonist and found that endothelin receptor blockade not only decreased blood pressure and proteinuria but also ameliorated the biochemical and histologic progression of renal disease. However, more recent studies utilizing both ET_A and $ET_{A/B}$ antagonists have been unable to confirm these earlier observations (231,232). Nevertheless, these preliminary observations suggest possible application of endothelin receptor antagonists in human renal parenchymal hypertension.

Infusion of endothelin into normal subjects at low doses that double the normal plasma concentration significantly decreases urinary sodium excretion without affecting RBF, GFR, or blood pressure (233). Higher infusion rates that achieve the pathophysiologic plasma endothelin concentrations found in renal failure result in significant increments in renal vascular resistance and systemic blood pressure while preferentially reducing RBF compared with GFR (234). The resultant increase in filtration fraction is associated with a significant decrease in urinary sodium excretion. Two reports provide additional support for a contributory role of endothelin in some forms of human hypertension. In one patient with carcinoma of the lung, episodic hypertension during bouts of disseminated intravascular coagulation correlated with plasma endothelin levels (235). Yokokawa and et al. (236) described two patients with malignant hemangioendothelioma, increased plasma endothelin levels, and hypertension. Removal of the tumor, which contained increased concentration of endothelin and endothelin messenger RNA, normalized both blood pressure and plasma endothelin concentration. Recurrence of the tumor in one patient was again associated with hypertension and increased plasma levels of endothelin. Multiple studies have compared radioimmunoassayable plasma concentrations of endothelin in normotensive subjects, patients with essential hypertension, and hypertensive patients with renal disease. Endothelin levels have been increased in patients with essential hypertension compared with normal control subjects in some studies (237,238), but not in others (239,240). Although no consistent correlation has been found between plasma concentrations of endothelin and arterial pressure in essential hypertension (237,238), administration of bosentan, a mixed $ET_{A/B}$ receptor antagonist, to essential hypertensives, decreased blood pressure to a level similar to that achieved with ACE inhibition (240). Plasma concentrations (239,241,242) and urinary excretion (243) of endothelin are more consistently elevated in hypertensive patients with renal disease compared with normal volunteers but still correlate poorly with blood pressure (241,243). These inconsistent findings are not surprising, however, considering that endothelin probably functions as an autocrine or paracrine substance and not as a circulating hormone (217,222). Vajo and coworkers demonstrated that endothelin levels were elevated in an anephric patient with severe hypertension, implying a role for endothelin in the development of hypertension in this patient (244). However, methods to exclude volume overload contributing to hypertension were not well described in this study (245). In contrast, Hand et al. administered a selective ET_A antagonist to patients with pre-ESRD and healthy control subjects (246). Forearm blood flow increased in both groups but the increment was significantly greater in the healthy subjects than in the patients with chronic renal failure, suggesting a reduced role of endothelin in the maintenance of resting vascular tone in patients with pre-ESRD. Hence, the role of endothelin in renal parenchymal hypertension remains to be elucidated.

Endogenous Digitalis-like Substance (EDLS). The concept that an inhibitor of Na^+-K^+-ATPase, an EDLS, might be involved in the pathogenesis of renal parenchymal hypertension was initially postulated by several groups of investigators (123,247,248) more than 20 years ago. Despite intensive study, the precise identification of this compound(s) remains elusive (125,249,250). The EDLS is clearly distinct from ANP, brain natriuretic peptide (BNP), and steroidal natriuretic compounds (249). Candidate factors share several common features; they inhibit Na^+-K^+-ATPase, are natriuretic, and cross-react with antibodies to digitalis (249). The theoretic mechanism by which an EDLS causes renal hypertension has been debated but has received increasing support. According to this hypothesis, an intrinsic or acquired defect in renal sodium excretion causes an increase in the ECFV that signals the release of an EDLS from the hypothalamus that inhibits Na^+-K^+-ATPase (250). Inhibition of Na^+-K^+-ATPase in renal tubular epithelia decreases sodium chloride reabsorption and increases urinary sodium excretion, thus attenuating the expansion of the ECFV. On the other hand, inhibition of Na^+-K^+-ATPase in vascular smooth muscle increases intracellular sodium concentration, reduces the transplasmalemmal sodium gradient, and raises the intracellular calcium content because of decreased sodium-dependent calcium efflux (123,125,250). The eventual increment in intracellular calcium results in an increase in vascular tone and TPR. Hence, salt balance is returned toward baseline at the expense of an elevated TPR and increase in arterial pressure.

Rats subjected to subtotal renal ablation and maintained on a high-salt diet develop an increase in ECFV and hypertension and demonstrate decreased Na^+-K^+-ATPase activity in cardiac microsomes and arteries (251). In addition, diabetic rats with a 25% reduction in renal mass demonstrate an increase in ECFV, suppressed PRA, and hypertension (124). Plasma levels of EDLS are increased, and cardiac and renal medullary microsomal Na^+-K^+-ATPase activity is decreased. Further, EDLS significantly correlates with mean arterial pressure (124). Gomez-Sanchez et al. (252) immunized salt-sensitive Dahl rats with an ouabain conjugate and ameliorated, but did not completely prevent, the development of hypertension when the animals

were fed a high-salt diet. Finally, Digibind significantly reduced mean arterial pressure in rats with reduced renal mass (253).

Since the initial report by Graves et al. (254) of an EDLS in the plasma of patients with CKD, increased levels of EDLS have been found in most (131,255–259), but not all (260), studies of patients with essential hypertension and hypertensive patients with CKD. Young adults with polycystic kidney disease and normal GFR have significantly increased total exchangeable sodium, higher systolic blood pressure, decreased RBF, and reduced ouabain-sensitive sodium efflux that is inversely related to total exchangeable sodium compared with their unaffected siblings (131). These observations are consistent with the presence of a circulating EDLS. Similar results have been reported in hypertensive patients with mild to moderate renal insufficiency (256). Other investigators have demonstrated radioimmunoassayable evidence of digoxin-like activity in patients with renal insufficiency who were not receiving digitalis (257). Although digitalis-like activity correlated inversely with GFR, there was no relation to blood pressure. Kelly et al. (258) demonstrated both increased levels of digoxin-like immunoreactivity and greater Na^+-K^+-ATPase inhibitory activity in hypertensive patients with moderate renal dysfunction compared with hypertensive patients with normal renal function or with normal subjects. However, in patients receiving maintenance dialysis, digoxin-like immunoreactivity was normal. There was no correlation between digoxin-like immunoreactivity and blood pressure in any group. On the other hand, in patients undergoing continuous ambulatory peritoneal dialysis, Na^+-K^+-ATPase increased with sustained volume expansion and significantly correlated with both mean arterial pressure and change in body weight (259). Finally, Bisordi and Holt found elevated levels of an EDLS in 15 hemodialysis patients (261). Plasma concentrations of EDLS correlated with both pre- and post-dialysis ECFV and were paralled by similar changes in ANP levels. Taken together, increasing evidence suggests that an EDLS is elaborated in response to increments in salt balance in patients with normal or reduced renal function, but its role in the pathogenesis of renal parenchymal hypertension requires further clarification.

Erythropoietin. Recombinant human erythropoietin (r-HuEPO) was introduced two decades ago for the treatment of the anemia of chronic renal failure (262). It was soon apparent that the development or exacerbation of hypertension was a frequent adverse effect of therapy with r-HuEPO (263,264). This prohypertensive effect of r-HuEPO occurs in patients with mild to moderate renal dysfunction as well as in those with ESRD (265,266). Increments of blood pressure can occur in normotensive patients with ESRD, but elevations are more evident in hypertensive individuals. Although early reports (262,263) occasionally noted the development of severe hypertension with seizures or encephalopathy, this rarely occurs now, given the increased awareness of this potential complication. The incidence of reported hypertension in patients with renal disease treated with r-HuEPO ranges from 17% to 48% (265–268). However, some studies have shown no correlation between the dose of r-HuEPO and blood pressure (269). On the other hand, an analysis of placebo-controlled multicenter trials (268) found that 21% of patients on chronic hemodialysis treated with r-HuEPO developed clinically significant increases in blood pressure. This latter estimate is a more accurate reflection of the prohypertensive effect of r-HuEPO because some previous trials were uncontrolled.

Although endogenous erythropoietin concentrations correlate with blood pressure in patients with essential hypertension (270), the mechanism(s) by which r-HuEPO results in hypertension remains uncertain (271). Since blood transfusion to correct uremic anemia increases arterial pressure in patients on chronic dialysis (272), some investigators have examined the relation between either red cell mass or hematocrit and blood pressure in patients with chronic renal failure. Treatment with r-HuEPO increases red cell volume, but because of a concomitant decrease in plasma volume, blood volume remains unchanged (265,273). Moreover, despite a significant increase in hematocrit, increments in blood pressure do not correlate with the net change in hematocrit, the rate of increase in hematocrit, or the dose of r-HuEPO (265,267,269). Finally, Kaupke et al. (274) studied the effect of an increase in red cell mass owing to iron repletion in anemic iron-deficient patients with ESRD who were receiving a constant dose of r-HuEPO. In 15 patients, repletion of iron stores resulted in an increase of hematocrit from 25% to 32% ($p < 0.001$) but did not significantly change either systolic or diastolic blood pressure. Hence, therapy with r-HuEPO causes hypertension by additional mechanisms other than an increase in red cell mass.

Although r-HuEPO is not a direct vasoconstrictor, incubation of r-HuEPO with rabbit aorta or carotid artery (275) increases endothelin production. Incubation with a selective ET_A antagonist blocks the vasoconstrictor response induced by r-HuEPO (275). These observations have led some investigators to speculate that endothelin might play a role in the hypertension associated with therapy with r-HuEPO (275,276). Carlini et al. (276) found increased plasma levels of endothelin in patients on chronic hemodialysis treated with intravenous r-HuEPO compared with their counterparts receiving subcutaneous r-HuEPO. There was a significant correlation between mean arterial pressure and plasma levels of endothelin only in the patients receiving intravenous erythropoietin. Other investigators have also been unable to demonstrate any change in plasma concentration of endothelin during the subcutaneous or intraperitoneal administration of r-HuEPO (268). Hence, if endothelin is causally related to r-HuEPO-induced hypertension, the route of administration might be important.

Correction of the anemia of ESRD with r-HuEPO is associated with an increase in blood pressure mediated by an increased TPR and a decrease in cardiac output (265,268,277). The decrement in cardiac output, however, is proportionately less than the increment in TPR, resulting in an increase in blood pressure. Several mechanisms potentially contribute to the increased TPR. Correction of anemia increases whole blood viscosity (264,277), which increases TPR (264,265). Further, an increase in red cell mass improves tissue oxygenation and could reverse hypoxic vasodilation, thereby increasing vascular tone (265). Hence, enhanced production of endothelin, increased blood viscosity, and reversal of hypoxic vasodilation together with a genetic predisposition to hypertension (278), an augmented vascular responsiveness to norepinephrine (275), and possibly other undefined factors (265,271) increase blood pressure during therapy with r-HuEPO. The observation that hypertension does not develop to a significant extent in normal volunteers, patients with multiple myeloma, or individuals with the acquired immune deficiency syndrome (279,280) treated with r-HuEPO underscores the concept that other factors associated with renal disease are required for therapy with r-HuEPO to increase blood pressure (281). Hypertension associated with the treatment of the anemia of chronic renal failure with r-HuEPO should be anticipated. Blood pressure must be carefully monitored, and if hypertension develops or worsens, it should be treated in the same fashion as in patients not receiving r-HuEPO (see Treatment of Hypertension, below).

Depressor Compounds

In addition to producing vasoconstrictor and antinatriuretic compounds, the kidney also synthesizes vasodilator and natriuretic factors (7,201,283,284). Therefore, it is theoretically possible that deficient production of vasodepressor medullary

lipids, nitric oxide, kinins, or renal prostaglandins could contribute to the generation or maintenance of renal parenchymal hypertension. However, despite the important antihypertensive function of nonprostaglandin renal medullary lipids in some forms of experimental hypertension (6,284,285) and the prohypertensive effect of chemical renal medullectomy (286), there is no substantial evidence linking a deficiency of these compounds to the genesis of hypertension in human renal disease. On the other hand, considerable evidence suggests that alterations in the production of nitric oxide, kinins, ANP, and prostaglandins occur in renal parenchymal hypertension.

Nitric Oxide. Nitric oxide is produced by the vascular endothelium from L-arginine by the enzyme NO synthase (NOS) and functions as an endogenous nitrovasodilator (287–289). To date, three isoforms of NOS have been described: neuronal NOS (nNOS); inducible NOS (iNOS); and endothelial NOS (eNOS). Within the kidney, NO mediates the vasodilator action of kinins, participates in pressure-mediated natriuresis, exerts a tonic vasodilator effect on the afferent arteriole, decreases tubular sodium reabsorption, and opposes endothelin-induced vasoconstriction (283,288,289). Accumulating data suggest that impaired production of NO might be related to both experimental and human renal parenchymal hypertension. For example, in rats, stimulation of endogenous NO production increases RBF and salt excretion, whereas inhibition of NO synthesis produces the opposite effect (288,290,291). Increased dietary salt stimulates renal NO production, and inhibition of NOS under conditions of salt loading results in increased renal vascular resistance, decreased RBF and GFR, increased urinary protein excretion, systemic hypertension, and chronic progressive nephropathy (290–294). Angiotensin II receptor blockade or ACE inhibition ameliorate the deleterious renal effects of chronic inhibition of NOS (294), implying a particularly noxious effect of angiotensin II in the face of reduced NO production. In rats subjected to subtotal renal ablation, systemic NO production is augmented whereas renal synthesis is decreased (295,296) and chronic NOS inhibition aggravates both systemic and glomerular hypertension, resulting in both functional and histologic renal deterioration (297).

Decreased NOS activity and reduced NO production have recently been demonstrated in an experimental model of immunologically mediated glomerulonephritis (298). Data in humans provide some support for these experimental observations. Some (299) but not all (300) studies suggest that NO synthesis is reduced in essential hypertension. Further, NOS inhibition in normotensive volunteers resulted in a marked increase in blood pressure (301). Endogenous inhibitors of NOS that are normally excreted in the urine accumulate in the plasma of patients with CKD and ESRD (302,303). Infusion of one of these compounds, asymmetric dimethyl arginine (ADMA), results in systemic hypertension in guinea pigs and decreases forearm blood flow in normal volunteers (304). Schmidt and Baylis (305) found that 13 patients with stage 3–5 CKD demonstrated increased ADMA levels, reduced total NO production and higher blood pressure compared with normal control subjects. However, other investigators have found no difference in plasma ADMA concentrations between normotensive and hypertensive patients with CKD (302). There are conflicting data with regard to NO production in patients with ESRD. Both increased (306) and decreased (307,308) NO synthesis have been reported. Hence, at this juncture no firm conclusions can be drawn regarding the role of NO in the initiation or maintenance of human renal parenchymal hypertension.

Kinins. Kinins stimulate the release of NO and PGI_2 from vascular endothelium and thereby cause vasodilation (283,309). However, their role in the regulation of arterial pressure under physiologic or pathophysiologic circumstance is uncertain. Intrarenal infusion of bradykinin in conscious dogs for 7 days had no significant effect on urinary sodium excretion, GFR, or arterial blood pressure (310). These observations suggest that circulating kinins do not exert an important long-term effect on blood pressure but do not exclude a potentially significant vasodepressor role of local intrarenal kinin production. In this regard, rats subjected to chronic NOS inhibition or 5/6 renal ablation, both models of salt-sensitive hypertension, demonstrate decreased renal kallikrein expression and kinin excretion (311). Levy et al. (312) demonstrated a direct relation between RBF and urinary kallikrein excretion in patients with essential hypertension. Further, excretion of both active and inactive urinary kallikrein is decreased in salt sensitive subjects with essential hypertension (313,314) and in Japanese (314) and whites, but not blacks (315), with renal parenchymal hypertension. Almeida and et al. (316) demonstrated reduced plasma kininogen concentration in patients with malignant hypertension and an average serum creatinine concentration of 4 mg/dL compared with essential hypertensives or normal subjects. After 3 months of adequate control of blood pressure, plasma kininogen concentrations were still decreased, suggesting that the reduced levels were not the result of the severe hypertension but might have contributed to its pathogenesis.

Natriuretic Peptides. The natriuretic peptide system consists of ANP, brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP) (317). Of the three peptides, ANP and BNP have been best characterized. Multiple studies have shown that both ANP and BNP increase GFR and urinary sodium excretion, inhibit the release of renin, aldosterone, and AVP, and directly relax vascular smooth muscle (317–319). Hence, it is theoretically possible that a deficiency of ANP, or BNP, or both is related to the development of hypertension because all of the actions of these natriuretic peptides tend to reduce blood pressure and administration of synthetic ANP to patients with essential hypertension decreases arterial pressure and increases sodium excretion (320). Basal levels of ANP and BNP, however, are significantly higher in patients with essential hypertension compared with normotensive individuals and correlate with blood pressure and left ventricular mass index (130,318,321). Concentrations of both peptides are elevated in pre-ESRD CKD (322,323), and in patients with ESRD (323–325). Suda and et al. (322) measured plasma ANP concentrations in hypertensive patients with an average creatinine clearance of 39 mL/minute and in normal subjects. They found that ANP levels in the hypertensive patients with renal disease were significantly greater than in the normotensive controls and that plasma ANP correlated with blood pressure. Similarly, Takami et al. (323) demonstrated that plasma BNP concentrations were five-fold greater in hypertensive patients with pre-ESRD than in hypertensive controls. Brain natriuretic peptide levels correlated with echocardiographic estimates of left ventricular overload independent of the degree of renal dysfunction (323). In ESRD, the concentrations of both natriuretic peptides are elevated to an even greater extent, increase with volume expansion, decrease significantly with fluid removal during dialysis, and directly correlate with the degree of volume expansion (324,326). Changes in natriuretic peptide concentrations with salt removal during dialysis undoubtedly reflect decreases in secretion because they are not cleared significantly across conventional dialysis membranes (324,325). Taken together, these observations suggest that both ANP and BNP are increased in hypertensive patients with renal disease and respond appropriately to changes in cardiac filling pressure. Hence, there is no deficiency of these natriuretic peptides; rather, their levels are increased, and they modulate the salt-retaining and vasoconstrictor actions of other neurohumeral influences (Fig. 51-5).

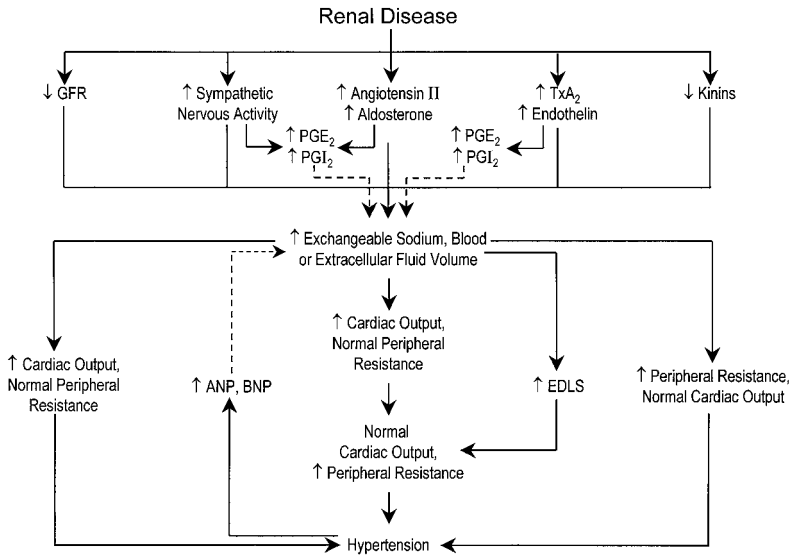

FIGURE 51-5. Pathophysiologic mechanisms responsible for the initiation of renal parenchymal hypertension and the hemodynamic patterns that sustain the elevated arterial pressure. Direct effect (–); decreases or attenuates (—). *ANP,* atrial natriuretic peptide; *BNP,* brain natriuretic peptide; *EDLS,* endogenous digitalis-like substance; *GFR,* glomerular filtration rate; PGE_2, prostaglandin E_2; PGI_2, prostacyclin; TxA_2, thromboxane A_2. (Modified from: Smith MC, Dunn MJ. Hypertension in renal parenchymal disease. In: Laragh JH, Brenner BM, eds. *Hypertension: pathophysiology, diagnosis, and management.* New York: Raven Press, 1995:2091.)

Prostaglandins. Renal and extrarenal prostaglandin synthesis exerts an important influence on vascular tone and salt excretion. Prostacyclin (PGI_2), PGE_2, and PGD_2 are vasodilator and natriuretic, whereas thromboxane A_2 and $PGF_{2\alpha}$ are vasoconstrictor and antinatriuretic (7,282,327). Not only do prostaglandins directly modulate vascular tone and sodium chloride excretion, they also indirectly affect vascular resistance by regulating renin secretion and sympathetic tone (282). However, because stimulation of endogenous prostaglandin production tends to decrease blood pressure (328) and administration of both nonselective and selective nonsteroidal antiinflammatory drugs (NSAIDs) increases blood pressure in treated hypertensives (329–332), a deficiency of systemic or renal prostaglandin synthesis could conceivably contribute to hypertension in patients with renal disease.

This notion is supported by the observation that urinary excretion of PGE_2 is reduced in about a third of patients with essential hypertension (333) and that plasma concentrations of 6-keto-$PGF_{1\alpha}$, a stable metabolite of PGI_2, are reportedly decreased in some populations with essential hypertension as well (334). In contrast, urinary excretion of PGE_2 is normal (335) or increased (336) in hypertensive patients with CKD and increases further with salt loading (337). These latter data imply that deficient prostaglandin synthesis is not etiologically related to human renal parenchymal hypertension but rather that normal or enhanced prostaglandin production modulates the vasoconstrictor and salt-retaining effects of angiotensin II, the sympathetic nervous system, and other pressor compounds (Fig. 51-6). The fact that virtually all NSAIDs, except aspirin and sulindac, increase blood pressure in treated patients with essential hypertension (282,327,329–332,338,339) and in hypertensive patients with renal disease (335,339) further supports this contention. Ruilope and et al. (335) treated hypertensive patients with stage 2–3 CKD with indomethacin at a dosage of 2 mg/kg for 3 days and demonstrated a simultaneous decrease in urinary excretion of PGE_2 and creatinine clearance together with a significant increment in weight and diastolic blood pressure. Thus, inhibition of renal prostaglandin production by NSAIDs in hypertensive patients with renal disease can further reduce GFR and salt excretion in addition to exacerbating hypertension (327,335,338–341). Sulindac and piroxicam are less nephrotoxic than ibuprofen (341) but should still be used cautiously in patients with renal parenchymal hypertension. On the other hand, selective cyclooxygenase-2 inhibitors can also exacerbate hypertension and cause acute on chronic renal failure (330,342) and do not offer distinct advantages over nonselective NSAIDs in this patient population (343).

Hemodynamic Patterns

In hypertensive patients with renal disease, the increased arterial pressure must be caused by an elevated cardiac output or TPR, or both, consequent to expansion of the ECFV owing to salt retention or as the result of vasoconstriction from increased sympathetic tone or increased synthesis of pressor compounds (Fig. 51-5). Both cross-sectional and longitudinal studies have examined this issue. Cross-sectional studies in stage 2–3 CKD (116–118) and ESRD (119,120,344) show that hypertension is maintained chiefly by an increase in TPR and bilateral nephrectomy or salt subtraction during hemodialysis reduces both blood pressure and TPR without changing cardiac output (119,120). Longitudinal observations in patients with early stage kidney disease (121,122) in addition to

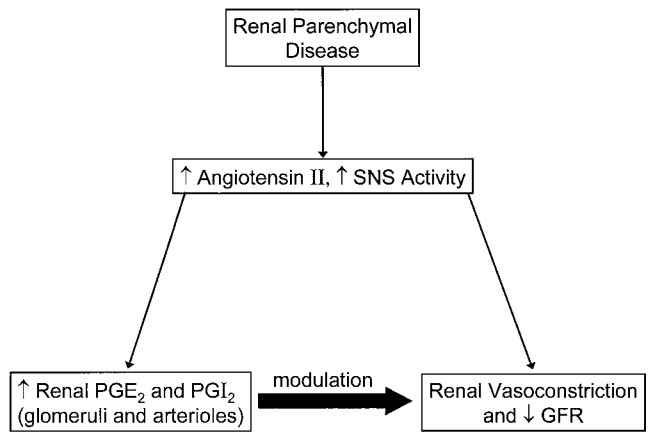

FIGURE 51-6. Renal parenchymal disease is often associated with augmented production of angiotensin II and increased activity of the sympathetic nervous system (SNS). The effect is twofold: first, a direct intrarenal vasoconstrictor effect that decreases renal blood flow (RBF) and GFR and second, an increased synthesis of vasodilator and natriuretic prostaglandins (PG). The latter modulates intrarenal vasoconstriction. Inhibition of intrarenal PG production by nonsteroidal antiinflammatory drugs results in unopposed intrarenal vasoconstriction with a further decline in RBF and GFR.

hemodynamic measurements in patients with ESRD subjected to salt loading (9,113,345), however, suggest that alternative hemodynamic patterns exist. For example, some data imply that an elevated cardiac output can precede an increase in TPR or, under certain circumstances, maintain hypertension. Brod and et al. (121,122) repetitively measured hemodynamic parameters over a period of 2 to 8 years in 97 patients with stage 1 CKD (GFR 90–117 mL/minute). Initially 12 of 32 normotensive patients demonstrated an increased cardiac output, expanded blood volume, and reduced TPR. During the follow-up period, 11 of these 12 patients eventually developed hypertension. Of the group of patients with initially mild to moderate hypertension, one-third had an increased cardiac output and normal vascular resistance. On the other hand, patients with severe hypertension uniformly had an increased TPR. Taken together, these data support the hypothesis that early in the development of renal parenchymal hypertension the elevated blood pressure is consequent to salt retention, an expanded ECFV, and an increased cardiac output. In the established phase, especially with moderate to severe hypertension, the increased blood pressure is the result of an augmented TPR.

The sequential hemodynamic changes subsequent to volume expansion observed in experimental models of renal ablation (9,113) also have been demonstrated in humans with renoprival hypertension and nonnephrectomized patients with ESRD (345,346). Coleman et al. (346) studied three nephrectomized patients before and after volume expansion. During expansion of the ECFV, blood pressure increased as a result of an elevated cardiac output, whereas TPR remained constant. Established hypertension, however, was associated with an elevated vascular resistance and normal cardiac output. Similar maneuvers in a larger group of nonnephrectomized patients with ESRD have confirmed and extended these findings and have indicated that an increase in cardiac output is not a necessary initial event in the development of hypertension. Kim et al. (345) found four hemodynamic patterns consequent to salt loading in 10 patients undergoing chronic hemodialysis: Two showed no increase in blood pressure; two developed an increment in blood pressure along with an increase in cardiac output; one patient developed hypertension that was initiated by an increase in cardiac output and sustained by an elevated TPR; and five had increases in blood pressure and vascular resistance without a measurable change in cardiac output. However, in the latter group, it is possible that transient increments in cardiac output occurred but were missed before the new steady state developed.

The relation between the factors that initiate hypertension in renal disease and the hemodynamic patterns that maintain the increased blood pressure is complex and multidirectional (Fig. 51-5). A decline in GFR together with increased activity of the sympathetic nervous system, the RAAS, or other vasoconstrictor or salt-retaining compounds causes an increase in exchangeable sodium and ECFV or an abnormal volume–renin relationship. These phenomena trigger a sequence of hemodynamic events culminating in hypertension. In anephric patients or those with a decreased PRA, hypertension is initiated or sustained by an elevated cardiac output. On the other hand, if the underlying renal disease increases the activity of the sympathetic nervous system or is associated with the elaboration of vasoconstrictors (e.g., angiotensin II, endothelin), hypertension is mediated chiefly by an increase in TPR. Under certain circumstances, however, an increment in cardiac output antedates the sustained elevation of TPR and is reminiscent of the autoregulation theory of blood pressure control espoused by Guyton et al. (113). The factors that mediate the transition from hypertension maintained by an increased cardiac output to elevated blood pressure sustained by an increment in TPR remain elusive. Increased sodium and calcium content of vascular smooth muscle consequent to a circulating EDLS that inhibits Na^+-K^+-ATPase could mediate the increase in vascular resistance (125,261).

Effect of Systemic Hypertension on Renal Function

The concept that systemic hypertension accelerates the decline in renal function has become well accepted since the initial reports by Wilson and Byrom (347) and Ellis (348) more than 50 years ago (349). Progressive renal dysfunction is uncommon in benign essential hypertension (350); however, GFR declines more rapidly in hypertensive patients with renal disease compared with their normotensive counterparts. Multiple epidemiologic studies and clinical trials have convincingly demonstrated by multivariate analysis that elevated systolic blood pressure (351,352) or mean arterial pressure (353) strongly predict progression of renal disease and rate of decline in GFR. Poorly controlled blood pressure contributes to progressive loss of kidney function in both diabetic nephropathy (354) and nondiabetic renal disease (10,349,353,355–358). The exact mechanism whereby elevated systemic blood pressure aggravates renal injury has not been firmly established. Initially, it was speculated that hypertension caused arteriolar nephrosclerosis with consequent ischemic glomerular sclerosis superimposed on primary renal disease (359). Investigators theorized that luminal obliteration owing to arteriolar nephrosclerosis further decreased renal blood flow (RBF) and GFR and contributed additionally to the decline in renal function. Few experimental data, however, support this notion. Rather, accumulating evidence over the past two decades suggests that multiple interrelated mechanisms, including impaired autoregulation of RBF and GFR with consequent glomerular capillary hypertension, glomerular hypertrophy, and the production of fibrogenic cytokines cause the accelerated loss of kidney function in hypertensive patients with renal disease.

Azar and et al. (360,361) first demonstrated that in some experimental models of hypertension, increments in systemic blood pressure were transmitted directly to the glomerulus. Utilizing micropuncture, they found that rats with one-kidney post-salt hypertension had significantly greater P_{GC} compared with normotensive controls. The higher P_{GC} was consequent to reduced afferent arteriolar resistance (R_A) and was associated with increased transcapillary hydraulic pressure differences (ΔP). Histologically, the animals developed glomerulosclerosis rather than arteriolar sclerosis (361). Olson et al. (362) confirmed and extended these observations. They demonstrated greater glomerular damage in experimental models of hypertension in rats that showed a reduced R_A (deoxycorticosterone-salt, renal ablation) compared with models that had a normal or increased R_A (aortic ligature, stroke-prone spontaneously hypertensive rats). These data imply that in experimental hypertension, unassociated with primary renal disease, impaired auto-regulation of RBF and GFR is decreased R_A causes hydraulically mediated renal damage and progressive glomerular injury.

Substantial evidence suggests that similar intrarenal hemodynamic abnormalities are linked to progressive renal dysfunction when hypertension develops in conjunction with renal disease or is superimposed on preexisting renal disease. Dogs with a marked reduction in renal mass exhibit significant loss of autoregulation of RBF and GFR in response to changes in renal perfusion pressure (363). In rats, nephrotoxic serum nephritis (364), aminonucleoside nephrosis (365), or subtotal renal ablation (366–368) disturbs renal autoregulation with a consequent increment in Q_A and GFR as systemic blood pressure increases. Bidani and et al. (366) showed that rats subjected to 5/6 nephrectomy and fed a normal protein diet demonstrated impaired autoregulation in response to graded changes in renal perfusion pressure and greater glomerular injury compared with their counterparts fed a low-protein diet. In contrast, they

found no significant short-term loss of renal function in normotensive rats with subtotal renal ablation (368). However, recent studies from the same group (369) in a "normotensive" rat remnant kidney model showed that the percent glomerulosclerosis correlated significantly with average systolic blood pressure. These investigators speculated that impaired autoregulation of RBF and GFR antedates morphologic injury and explains the observation that progressive glomerular damage occurs in this model despite no significant increase in blood pressure. Their conclusions are further strengthened by the fact that superimposition of hypertension, by clipping a renal artery (370) or institution of a high-salt diet (371), in experimental immunologically mediated renal disease results in increasing proteinuria and accelerated glomerulosclerosis. Finally, in rats with streptozotocin-induced diabetes and two-kidney, one-clip hypertension, the clipped kidney exhibits fewer morphologic manifestations of diabetic nephropathy as compared with the unclipped kidney (372).

These indirect observations are bolstered by direct measurements of Q_A and P_{GC} by micropuncture in experimental models of hypertensive renal disease. In rats with subtotal renal ablation or in uninephrectomized spontaneously hypertensive rats, increases in systemic pressure are transmitted directly to glomerular capillaries as the result of a greater reduction in R_A compared with efferent arteriolar resistance (R_E) (37,43,373). The consequent increment in P_{GC} is associated with progressive proteinuria and glomerular injury. Similarly, in diabetic rats, glomerular hypertension with increments in ΔP and P_{GC} occurs in the absence of systemic hypertension because of relatively greater reductions in R_A compared with R_E (374). Moreover, in fawn-hooded rats that spontaneously develop proteinuria, hypertension, and progressive glomerulosclerosis, proteinuria and glomerular damage are linked to an increase in P_{GC} and aggravated by uninephrectomy with its attendant preferential reduction in R_A (39,375,376). Hence, in diverse models of renal parenchymal disease, renal morphologic and functional deterioration occurs together with impaired renal autoregulation owing to a greater decrease in R_A compared with R_E with a resultant increase in P_{GC} (46,377).

The relation between intraglomerular hemodynamic abnormalities and progressive glomerulosclerosis in experimental renal disease is not straightforward. For example, Yoshida et al. (41,43) demonstrated that glomerulosclerosis correlated with glomerular hypertrophy and not P_{GC} in a rat remnant kidney model. Similarly, other investigators have dissociated progression of experimental renal disease from increments in P_{GC} and have suggested that measures of nephronal hypertrophy such as V_G and R_{GC} or the production of growth factors are more tightly linked to ongoing glomerular injury (26,378–382). In this regard, mesangial cells in culture proliferate (383) and produce excess type I and IV collagen (384) in response to mechanical stretching. Further, both angiotensin II (385,386) and endothelin (387,388) stimulate mitogenesis of cultured mesangial cells. The degree of glomerular sclerosis correlates significantly with urinary excretion of endothelin in rats with subtotal renal ablation (388). Moreover, emerging data suggest that aldosterone has an important role, independent of angiotensin II, to mediate glomerulosclerosis and production of TGFβ (206). Finally, in a rat model of Heymann nephritis the combination of an ACE inhibitor and an ET_A receptor antagonist reduces glomerular sclerosis and interstitial damage to a greater extent than either drug alone (381). Consequently, the above data imply that in various experimental models renal disease progresses by different mechanisms; increments in P_{GC} or V_G or augmented production of angiotensin II, aldosterone or endothelin stimulate the production of growth factors that results in progressive glomerular obsolescence.

Both salt restriction (42,378) and pharmacologic therapy (26,37,38,374,376,389–391) limit proteinuria, preserve renal function, and retard glomerular injury in experimental renal parenchymal hypertension. Salt restriction exerts its beneficial effect by reducing renal hypertrophy without significantly decreasing P_{GC} (42,378). Pharmacologic treatment, on the other hand, has ameliorated progressive renal disease while decreasing P_{GC} (37,44,374,376,389,392), minimizing glomerular hypertrophy (43,44,380), and limiting (381,393,394) or reversing (395) glomerular and interstitial injury. Although the protective effect of drug treatment differs according to the experimental model (37,391), stage of the disease at which therapy is instituted (396), and probably other unidentified variables, some classes of antihypertensive agents might be more renoprotective than others. For example, ACE inhibitors and angiotensin II antagonists consistently ameliorate progressive renal injury (37,43,44,374,376,380,389–393,397,398), whereas calcium channel blockers or combinations of other drugs have prevented glomerular damage in some (30,43,44,380,391,392,399), but not all (37,389,390,397,398) studies. Anderson and et al. (37) compared ACE inhibition by enalapril with a combination of reserpine, hydralazine, and hydrochlorothiazide with regard to development of proteinuria and glomerular sclerosis in rats subjected to subtotal renal ablation. They found that enalapril reduced P_{GC} by preferentially decreasing R_E relative to R_A, decreased systemic blood pressure, minimized proteinuria, and prevented progressive glomerular damage. In contrast, triple drug therapy did not decrease P_{GC} or R_E, despite similar effects on systemic blood pressure, and failed to ameliorate progressive renal injury. Based on these and similar data (374,376,389,393,395), many authorities have concluded that ACE inhibitors and angiotensin II receptor antagonists are especially renoprotective because of their unique ability to modulate hydraulically mediated glomerular damage, decrease glomerulosclerosis, and minimize interstitial damage. On the other hand, Dworkin et al. (391), in the uninephrectomized spontaneously hypertensive rat, demonstrated that higher doses of triple drug therapy decreased P_{GC} and ΔP to a similar extent compared with ACE inhibition and that both retarded the progression of renal disease. In addition, these same investigators (44) showed that nifedipine and enalapril were equally renoprotective in rats with subtotal renal ablation. However, all investigators have not substantiated the latter observation (37,389,390,397). The lack of calcium channel blockade to consistently ameliorate renal injury in experimental models of renal disease could be due to further impairment of renal autoregulation or inconsistent effects on systemic blood pressure. For example, Griffin and et al. (390,397) showed that dihydropyridine calcium channel blockers (DHPCCBs) caused a further loss of renal autoregulation in the rat remnant kidney model associated with increased proteinuria and glomerular sclerosis. In addition, radiotelemetric blood pressure monitoring in this model demonstrates marked lability of blood pressure that is incompletely ameliorated by calcium channel blockade compared with ACE inhibition (369,390,394,397). The finding that glomerular damage in the renal ablation model is tightly linked to achieved blood pressure reduction (369,389,394) could explain some of the inconsistent experimental data with regard to the renoprotective effects of calcium channel blockade. In addition, combination therapy with both an ACE inhibitor and an angiotensin II receptor blocker, despite similar control of blood pressure, is more renoprotective with regard to limiting proteinuria and renal injury than monotherapy with either drug alone (400). Taken together, the available data indicate that ACE inhibitors and angiotensin II antagonists consistently limit renal injury in experimental renal parenchymal disease through both hemodynamic (37,374,376,389,393) and nonhemodynamic (392) mechanisms. Other antihypertensive regimens are less effective. It is uncertain whether this is the result of inconsistent control of systemic blood pressure, adverse hemodynamic effects

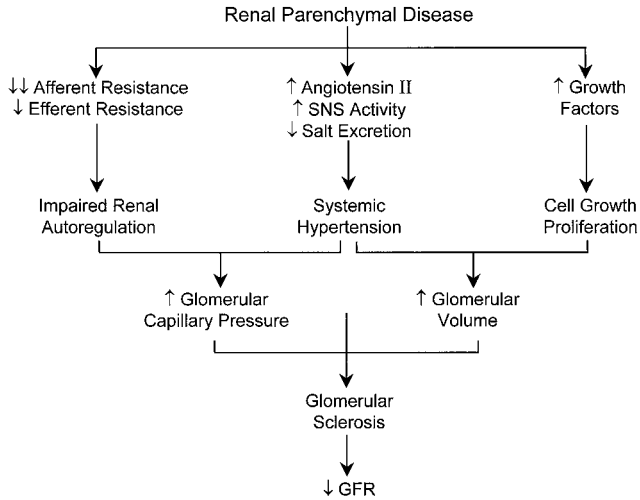

FIGURE 51-7. Mechanisms responsible for the progression of renal disease when systemic hypertension coexists with renal parenchymal disease. GFR, glomerular filtration rate; SNS, sympathetic nervous system. (Reprinted with permission from: Smith MC, Dunn MJ. Hypertension in renal parenchymal disease. In: Laragh JH, Brenner BM, eds. *Hypertension: pathophysiology, diagnosis, and management.* New York: Raven Press, 1995:2094.)

in the renal microcirculation, or incomplete suppression of fibrogenic cytokines (401).

Figure 51-7 summarizes our current understanding of the pathophysiologic mechanisms responsible for the accelerated loss of renal function in renal parenchymal hypertension. Hypertension results from decreased excretion of salt, increased production of vasoconstrictor compounds, or both. The preferential reduction in R_A relative to R_E owing to increased intrarenal prostaglandin (367) or nitric oxide (289) synthesis impairs autoregulation of renal blood flow. Consequently, even small increments in systemic blood pressure are transmitted to the glomerulus and increase P_{GC}. The mitogenic effects of angiotensin II, aldosterone, endothelin, tumor growth factor β (TGFβ), and other growth factors result in mesangial cell proliferation and glomerular hypertrophy (206,386,387,401,402). Increased P_{GC} and nephronal hypertrophy alone or together are associated with progressive glomerular injury and fibrosis. Sustained reduction of systemic blood pressure limits glomerular damage.

Beneficial Effects of Treatment of Hypertension in Chronic Kidney Disease. Both systolic and diastolic blood pressures are continuous, strong, independent risk factors for the development of cardiovascular morbidity and mortality in the general population (403). The Prospective Studies Collaboration Group (404) developed a meta-analysis of individual data for one million adults in 61 prospective studies to examine the relationship between blood pressure and mortality. They found that, at ages 40–69 years, each difference of 20 mm Hg usual systolic blood pressure or 10 mm Hg usual diastolic blood pressure, was associated with more than a twofold difference in the death rate from stroke and a twofold difference in the death rates from ischemic heart disease and other vascular causes. The relationship existed down to a blood pressure of 115/75 mm Hg. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC VII) embraced these data in their 2003 recommendations (405). Over the past 40 years, numerous studies have clearly demonstrated that effective pharmacologic therapy significantly decreases cardiovascular morbidity and mortality in patients with essential hypertension. Despite the fact that upward of 80% of CKD patients will have hypertension as their GFR declines (98,406), similar data pertaining to patients with CKD are scant (92). Nevertheless, it is logical to assume that existing data for the general population support an aggressive approach to blood pressure control in the CKD population. Unfortunately, the Third National Health and Nutrition Examination Survey (NHANES III) data indicate that only 11% of individuals with hypertension and an elevated serum creatinine had a blood pressure <130/85 and only 27% had a blood pressure <140/90 mm Hg (407). Patients with ESRD are at a 500-fold risk of a cardiovascular event in some age ranges (408) and the cardiovascular risk increases at the earliest stages of CKD (409–412). In fact, a patient with early stage CKD is more likely to die from a cardiovascular event than progress to ESRD (413). Thus, the ultimate goals of the treatment of hypertension in CKD are reduction of cardiovascular events and slowing the progression of CKD to ESRD. The following sections will use evidence primarily from studies of nondiabetic kidney disease to discuss the concepts of: (a) the racial disparity in ESRD, especially hypertensive renal disease; (b) the importance of dietary sodium restriction and the use of diuretics to ameliorate hypertension; (c) the appropriate blood pressure goal; (d) proteinuria as a contributor to renal disease progression and a target for therapy; (e) the primary role of ACE inhibitors, angiotensin receptor blockers, and their combination; (f) add-on therapy with calcium channel blockers and sympatholytics; and (g) emerging agents such as aldosterone antagonists in the treatment of hypertension in CKD patients.

Racial Differences in ESRD. Blacks have a sixfold greater risk of developing hypertensive ESRD compared with whites, a 3.8-fold increase in risk for ESRD owing to diabetic nephropathy, and a 2.6-fold greater risk for renal failure from chronic glomerulonephritis (414). Blacks have the highest incidence rates for ESRD but the rate of rise may be slowing (410). There are multiple potential reasons for the marked difference in the occurrence of hypertensive renal disease in blacks compared with whites. Several investigators postulate that lower socioeconomic status and reduced access to preventive health care are important determinants of the higher prevalence of ESRD, particularly hypertensive ESRD, in the black population (415,416). However, one study (350) showed that neither accurately predicted the development of renal disease, and others (417,418) contend that controlling for these factors does not eliminate the influence of race on the development of ESRD. In addition, the availability of effective antihypertensive therapy over the past two decades has decreased the occurrence of stroke and congestive heart failure in blacks as well as whites but has not reduced the incidence of hypertensive ESRD (419). Although the prevalence of hypertensive in blacks is 1.3- to 2-fold greater than whites (420,421), this fact alone does not explain the greater than six-fold increase in the occurrence of hypertensive ESRD in blacks (416,420,421). Misclassification bias could contribute to the higher prevalence of hypertensive ESRD in blacks compared with whites (420). In this regard, Perneger et al. (422) have shown that, given the same written case histories, black patients were twice as likely to receive a diagnosis of hypertensive ESRD compared with their white counterparts. However, the African American Study of Kidney Disease (AASK) Trial showed that renal biopsies in nondiabetic hypertensive blacks with mild to moderate renal insufficiency and minimal proteinuria revealed changes consistent with hypertensive renal disease in over 90% of the specimens (423). Treated hypertensive blacks have significantly elevated nocturnal blood pressures compared with their white counterparts (424), an observation that may explain their higher prevalence of hypertensive ESRD. Finally, it is possible that an inherent

genetic susceptibility of certain black kindreds to develop ESRD contributes to the observed racial disparity (425,426).

Blacks with essential hypertension demonstrate greater arteriographic (427) and histologic (428) evidence of arteriolar nephrosclerosis compared to whites with essential hypertension. There are several functional correlates of these anatomic differences. Both normotensive (429,430) and hypertensive (431,432) blacks excrete less of a sodium load compared with their white counterparts. This sluggish response to salt loading is associated with a higher prevalence of salt-sensitive hypertension and lower plasma renin activity compared with white hypertensives in blacks with essential hypertension (421,431,432). Hypertensive blacks exhibit an increase in filtration fraction under basal conditions (427) and in response to dietary salt loading (432,433). Campese et al. (432) examined the renal hemodynamic response to a high dietary salt intake in 26 subjects with essential hypertension. Fifteen patients (nine white, six black) were salt resistant; 11 patients, all of whom were black, were salt-sensitive. Renal blood flow increased and GFR was unchanged with a consequent decline in filtration fraction from 21% to 19% in the salt-resistant group. On the other hand, in the salt-sensitive patients, GFR remained stable whereas renal blood flow decreased, resulting in an increase in filtration fraction from 19% to 23%. Calculated P_{GC} decreased from 58 to 52 mm Hg in the salt-resistant subjects but increased from 48 to 58 mm Hg in the salt-sensitive group. These intrarenal hemodynamic abnormalities in the latter group were due to a disproportionate increase in R_E compared with R_A in response to salt loading. Interestingly, nifedipine reversed the abnormal renal adaptation to a high-salt diet in the salt-sensitive patients (432). Hence, the renal hemodynamic response of salt-sensitive blacks to an augmented salt intake (decreased R_A with respect to R_E and a consequent increment in P_{GC}) is reminiscent of the micropuncture data in experimental models of renal hypertension and offers a plausible explanation for the enhanced susceptibility of blacks to develop hypertensive ESRD as well as chronic renal failure from diabetes or other glomerular diseases.

Salt Restriction and Diuretic Use. The pathophysiology of hypertension in patients with CKD is largely consequent to a positive sodium balance and ECFV expands as GFR decreases (434). In addition, the magnitude of increase in blood pressure in response to ECFV expansion is greater as renal function declines (141,434,435) (Fig. 51-3), but the impairment in ECFV control antedates the development of hypertension and reduction in GFR (436,437). These observations underscore the important therapeutic implications of dietary salt restriction and diuretic administration in the treatment of renal parenchymal hypertension. Sodium retention not only causes hypertension in CKD, but it also reduces the antihypertensive response to nondiuretic antihypertensive agents such as ACE inhibitors, angiotensin receptor blockers, vasodilators, and centrally acting sympatholytics but not calcium channel blockers (438–441). Heeg et al. (442) found that the antiproteinuric effect of lisinopril was abolished by increasing sodium intake but that it was restored by dietary salt restriction. Other investigators (443) demonstrated that diuretic therapy could restore the beneficial effect of ACE inhibition or nondihydropyridine CCBs (444) on proteinuria and blood pressure control that had been blunted by a high-sodium diet.

Despite the data supporting the pathophysiologic role of a positive salt balance in renal parenchymal hypertension, large, controlled, prospective studies on the effects of a low-salt diet have not been conducted with CKD patients. Although Koomans et al. (435) found that lower sodium intake markedly diminished blood pressure in patients with advanced CKD, these data have not been rigorously validated. Further, Kumagai et al. (445) showed that restriction of daily salt intake decreased mean blood pressure by 16 mm Hg in 10 hypertensive patients with chronic glomerulonephritis and an average creatinine clearance of 50 mL/minute, but did not change blood pressure in normotensive patients with glomerular disease or in normal volunteers. Nevertheless, it is reasonable to recommend that hypertensive patients with CKD limit their daily sodium consumption to 80–100 mmol/day. Early in the course of renal disease, modest salt restriction alone can normalize blood pressure in a minority of hypertensive patients. However, diuretic therapy is required in the majority of cases and is the cornerstone of antihypertensive therapy, especially as renal function deteriorates (405,406). In the AASK trial, the importance of diuretic use, and dose, was confirmed. Patients required higher doses of diuretics at lower estimated GFR to achieve the target blood pressure for the trial (on average, 50 mg/day for GFR of 50–65 mL/minute, 60 mg/day for GFR of 50–65 mL/minute, and 70 mg/day for GFR <35 mL/minute) (446). The requirement for a diuretic to achieve goal blood pressure and optimize the antiproteinuric and antihypertensive effect of ACE inhibitors, may explain the lack of superiority of the ACE inhibitor in the ALLHAT CKD subgroup since diuretics were prohibited in the ACE inhibitor group (413).

Thiazide diuretics are effective in patients with stage 1–3 CKD (447). Loop-blocking diuretics, often in a multiple daily dosing regimen, are required to achieve an adequate natriuresis with more severe renal impairment (447,448). It is important to emphasize that the diuretic regimen should be advanced until the patient is free of edema, because even small increments in ECFV can contribute to poor blood pressure control (449). In patients with stage 5 CKD (i.e., creatinine clearances <15 mL/minute), the combination of a loop diuretic with a thiazide minimizes diuretic resistance and has a synergistic effect on salt excretion (450,451). Effective diuretic therapy alone will normalize blood pressure in approximately one-third of hypertensive patients with chronic renal insufficiency (148); the remainder require additional pharmacologic treatment. Nevertheless, dietary or pharmacologically induced salt depletion is crucial both from a pathophysiologic perspective and to limit the development of pseudotolerance if short-acting vasodilators or centrally acting sympatholytics are utilized as antihypertensive agents (452,453).

Blood Pressure Goal. Recently, the JNC VII guidelines (405) and the Kidney Disease Outcomes Quality Initiative (K/DOQI) Clinical Practice Guidelines on Hypertension in Chronic Kidney Disease (406) recommended a blood pressure goal of less than 130/80 mm Hg for patients with CKD. The stated goals of aggressive blood pressure management in these guidelines are to slow deterioration of renal function and prevent cardiovascular events. These recommendations are based on data from several randomized prospective trials. Schrier et al. (454), in the normotensive Appropriate Blood Pressure Control in Diabetes (ABCD) trial, demonstrated that an achieved blood pressure of 128/75 mm Hg versus 137/81 mm Hg significantly reduced the progression of incipient to overt diabetic nephropathy. A secondary analysis of the Reduction of Endpoints in Non–Insulin-Dependent Diabetes Mellitus (NIDDM) with the Angiotensin II Antagonist Losartan (RENAAL) study of 1,513 type 2 diabetics with overt nephropathy found an increased risk of progression of kidney disease among those with a treated systolic blood pressure of greater than 140 mm Hg compared with values less than 130 mm Hg (455). Thus, there are reasonably good data to support a blood pressure goal of less than 130/80 mm Hg in the setting of diabetic nephropathy.

Emerging data also support lower goal blood pressures for patients with nondiabetic renal disease. Schrier et al. (456) randomized 72 patients with polycystic kidney disease to either rigorous blood pressure control (<120/80 mm Hg) or standard control (<135–140/85–90 mm Hg). Primary end points were

change in creatinine clearance and change in LV mass index (LVMI) as a cardiovascular surrogate. At seven years, the rigorous control group achieved a blood pressure of 119/77 mm Hg compared with 130/82 mm Hg for the standard control group. Although there was no significant difference in change in creatinine clearance between the two groups, the patients with the lower goal blood pressure had a significantly greater decrease in LVMI. A lower blood pressure was achievable, safe, and theoretically could prevent cardiovascular events since LVH is a significant risk factor for cardiovascular morbidity and mortality (457).

In the Modification of Diet in Renal Disease (MDRD) (458,459) trial, 585 patients with nondiabetic renal disease and a mean GFR of 39 mL/minute were randomized to usual blood pressure control (MAP = 107 mm Hg) or aggressive control (MAP ≤92 mm Hg). Achieved blood pressures were approximately 130/80 mm Hg versus 125/75 mm Hg, respectively. When patients were stratified according to baseline proteinuria, those with >3 g/day showed significantly slower progression of renal disease in the lower blood pressure group compared with those randomized to the usual blood pressure goal. Patients excreting between 1 and 3 g of protein per day demonstrated a modest benefit from the lower blood pressure goal while those with <1 g/day of proteinuria had the slowest rate of progression and derived no benefit from the lower blood pressure goal (459).

The African American Study of Hypertension and Kidney Disease (AASK) trial enrolled nearly 1,100 African-Americans with hypertension, minimal proteinuria (mean of approximately 500 mg/day), mean serum creatinines of approximately 1.7–2.7 mg/dL, and a clinical diagnosis of hypertensive nephropathy (460). Patients were randomized to ramipril, metoprolol, or amlodipine and to one of two blood pressure goals, 125/75 (MAP <92 mm Hg) or 140/90 mm Hg (MAP 102–107 mm Hg). At four years the groups achieved blood pressures of 128/78 mm Hg and 141/85 mm Hg. Patients randomized to ramipril had a significantly decreased risk of reaching the composite end point of a 50% reduction in GFR, decrease in GFR of 25 mL/minute, the onset of renal failure, or death (461). However, there was no difference between the two goal blood pressure groups with respect to the primary outcome of rate of change in GFR.

A meta-analysis by Jafar et al. for the Ace Inhibition in Progressive Renal Disease (AIPRD) study group (462) analyzed 1,860 nondiabetic patients to assess the relationship between systolic and diastolic blood pressures, urinary protein excretion, and progression of kidney disease. A systolic blood pressure of 110 to 129 mm Hg and urine protein excretion of less than 2 g/day were associated with lowest risk for kidney disease progression. In patients with proteinuria >1 g, the risk for kidney disease progression decreased as systolic blood pressure declined from 160 to 110 mm Hg. In contrast, patients with <1 g of proteinuria showed no significant benefit with regard to loss of kidney function over the same range of blood pressure. Finally, a long-term follow-up (mean 10.6 years) of the MDRD study found that patients assigned to the low target blood pressure (MAP <92 mm Hg) had a significantly decreased risk of progression to ESRD or a composite end point of ESRD or all cause mortality compared with those assigned to the usual blood pressure goal (MAP <107 mm Hg) (463). Collectively, the MDRD, AASK, and AIPRD study group data support a more aggressive blood pressure goal of <125/75 mm Hg among patients with greater than 1 g of proteinuria per day.

Taken together, the available data suggest that a blood pressure of <130/80 mm Hg is appropriate for most CKD patients; however, for patients with greater than 1g/day of proteinuria, <125/75 should be the goal. If a blood pressure of <125/75 mm Hg can be achieved without unacceptable side effects, all CKD patients may derive cardiovascular and renal benefit.

Reduction of Proteinuria as a Treatment Goal. Both observational studies and prospective trials support the concept that the extent of urinary protein excretion is the best predictor of future progression to ESRD (464). A 20-year observational study in a large white population found that dipstick-positive proteinuria was a powerful predictor of both ESRD and overall mortality (465). A Japanese screening study of over 100,000 people found that the risk of ESRD over a 7-year follow-up was almost entirely restricted to subjects with dipstick-positive urine at enrollment, regardless of their initial renal function (466). Whether proteinuria is a reflection of the kidney disease, or plays a role in its progression is controversial. There are data to suggest, however, that proteinuria itself propagates renal injury.

Chronic nephropathies share common pathogenetic mechanisms that contribute to disease progression that are independent of the original etiology of the renal disease. A reduction in renal mass causes remaining nephrons to undergo hypertrophy with concomitant decrease in R_A, increase in glomerular plasma flow, and increase in P_{GC} (467). This adaptation initially maintains GFR but is ultimately detrimental, causing an increase in protein trafficking (468). Filtered proteins are reabsorbed by proximal tubules and degraded by lysosomes which causes gene activation encoding NK-kappa β, MCP-1, RANTES and other proinflammatory mediators (469,470). These pathways, along with increased TGF-β upregulation by angiotensin II (471), may cause fibrogenesis and subsequent scarring. Hence, reduction of proteinuria has become an important treatment goal in hypertensive patients with CKD.

Studies of ACE inhibition and angiotensin receptor blockade in patients with proteinuric renal disease support the concept that the reduction of proteinuria, itself, should be a primary goal of therapy for these patients. Table 51-4 summarizes the salient features of recent trials that examined the effect of pharmacologically induced reduction of blood pressure, or the addition of ACE inhibition, or both on the progression of nondiabetic renal disease. Zucchelli et al. (472) conducted a prospective, randomized trial assessing the effect of captopril compared with slow-release nifedipine on the progression of nondiabetic renal disease. After a 1-year prerandomization period with conventional antihypertensive therapy during which blood pressure averaged 165/100 mm Hg, 121 patients were randomly allocated to treatment with captopril or slow-release nifedipine. After 3 years of follow-up, both groups demonstrated significantly lower blood pressure and slower decline in renal function compared with the conventional antihypertensive treatment administered during the prerandomization period. There was no difference between the captopril and nifedipine groups with regard to progression of renal disease assessed by the reciprocal of the serum creatinine, creatinine clearance, or radionuclide clearance. Urinary protein excretion was unchanged over the duration of the study in both the ACE inhibitor group and the calcium channel blocker group. The authors concluded that improved control of arterial pressure slows the rate of decline of renal function in nondiabetic renal disease and that ACE inhibitors and calcium channel antagonists are equally renoprotective. The primary results of the MDRD study (458), based on an intention to treat analysis, showed no significant difference over a 3-year period with respect to change in GFR between subjects randomized to a usual (107 mm Hg) mean arterial pressure (MAP) goal compared with a low (92 mm Hg) MAP. However, the relatively short mean duration of follow up (2.2 years), small separation of MAP (4.7 mm Hg) between the two blood pressure goals, and the greater than anticipated initial decline in GFR in the low MAP group obscured important observations. In this

TABLE 51-4

LONG-TERM OUTCOME IN RANDOMIZED CONTROLLED TRIALS EXAMINING THE EFFECT OF PHARMACOLOGICALLY INDUCED REDUCTION IN BLOOD PRESSURE ON THE PROGRESSION OF NONDIABETIC RENAL DISEASE

Study (reference)	Mean follow-up (Y)	Patients (*N*)	Primary outcome measure(s)	Intervention group: Change in BP and MAP (mm Hg)				Comparitor	Comparitor group: Change in BP and MAP (mm Hg)			Conclusion
				Intervention	Baseline	Δ	Follow-up		Baseline	Δ	Follow-up	
Zucchelli (472)	3	121	Change in renal function[a]	Captopril	166/101 121	NA −21	NA 100	Nifedipine	164/99 121	NA −18	NA 103	Both slow progression; no difference between ACEI and CCB
Kamper (515)	2.2	70	Change in renal function	Enalapril	151/92 112	−18/10 −13	133/82 99	Conventional therapy[b]	140/90 107	−4/4 −4	136/86 103	Enalapril slows progression of renal disease
Klahr (458)	2.2	840	Change in renal function	MAP 92	133/81 98			MAP 107 ← Δ MAP −4.7→	133/80 98			MAP goal of 92 not more renoprotective
Hannedouche (516)	3	100	Progression to ESRD, change in renal function	Enalapril	167/103 124	−20/15 −16	147/88 108	Conventional therapy[c]	166/101 121	−13/11 −11	153/90 111	Enalapril slows progression of renal disease
Bannister (511)	1	34	Change in renal function	Enalapril	NA 116	NA −14	NA 102	Nifedipine	NA 114	NA −8	NA 106	No difference between ACEI and CCB
Ihle (517)	2	70	Change in renal function	Enalapril	147/87 107	−8.9/4.4 −6		Placebo + conventional therapy[d]	154/88 110	−0.7/1.4 −1	NA NA	Enalapril slows progression of renal disease
Maschio (474)	3	583	Change in renal function, Progression to ESRD	Benazepril	142/87 105	−4.5–8.0/ −3.5–5.0 NA	NA NA	Placebo + conventional therapy	144/88 107	+0.2–1.5/ +1.0–3.7 NA	NA NA	Benazepril slows progression of renal disease

Vanessen (518)	3.9	89	Change in renal function	Enalapril	151/89 110	−16/11 −13	135/78 97	Atenolol	153/91 112	−16/11 −13	137/80 99	No difference between ACEI and β-blocker
REIN Stratum 2 (476)	1	166	Change in renal function	Ramipril	150/92 111	−6/4 −4	144/88 107	Placebo + conventional therapy[d]	148/91 110	−3/2 −2	145/89 108	Ramipril reduced rate of decline in GFR
REIN Stratum 1 (477)	2.6	186	Change in renal function, Progression to ESRD	Ramipril	142/87 106	NA NA	NA NA	Placebo + conventional therapy[d]	145/90 108	NA NA	NA NA	No difference in ΔGFR; less ESRD with ramipril
SHEP-CKD (634)	5	4,336	Cardiovascular event	Chlorthalidone	172/77	−27/7	145/70	Placebo + conventional therapy[e]	172/77	−14/5	158/72	Fewer CV events in diuretic group; lower BP achieved
Cinotti (635)	1.9	131	Change in renal function	Lisinopril	141/84	−3/0	138/84	Conventional therapy	142/85	−1/1	141/84	Lisinopril slowed decline in GFR
ALLHAT (413) GFR <60 mL/min	4.9	5,662	ESRD and/or 50% decrease in GFR	Chlorthalidone	147/83	−11/9	136/74	Lisinopril + conventional therapy	147/83	−12/9	134/73	No difference in renal outcomes
ALLHAT (413) GFR <60 mL/min	4.9	5,662	ESRD and/or 50% decrease in GFR	Chlorthalidone	147/83	−11/9	136/74	Amlodipine + conventional therapy	146/82	−12/9	134/73	No difference in renal outcomes

NA, Not available; *ESRD,* end-stage renal disease.
[a]Change in renal function, assessed by change in creatinine clearance, 1/SCR, GFR, or plasma creatinine.
[b]Conventional, α-blockers, diuretics, vasodilators.
[c]Conventional, β-blocker, furosemide, calcium channel blocker, or α_2-agonist.
[d]Conventional, α_1-blocker, β-blocker, diuretics, calcium channel blockers, or α_2 agonists.
[e]Conventional, atenolol, or reserpine.

regard, secondary analyses of the MDRD data demonstrated a faster decline in GFR in patients with a higher achieved blood pressure ($p < 0.001$), particularly in blacks and subjects with proteinuria >1 g/24 hours (459,473).

The Angiotensin-Converting-Enzyme Inhibition in Progressive Renal Insufficiency (AIPRI) study, a trial of ACE inhibition in nondiabetic renal disease patients, included 563 patients followed for an average of 3.5 years (474). The primary end point was a doubling of baseline creatinine or the need for dialysis. Patients receiving benazepril had a 53% reduced risk of reaching the primary endpoint; however, the benefit was seen primarily in those patients with >3 g of proteinuria per day. Unfortunately, there was a significant difference in final blood pressures between the benazepril and placebo group making it difficult to assign a unique renoprotective effect to ACE inhibition independent of a reduction in blood pressure.

In 1997, Giatras et al. (475) published a meta-analysis for the AIPRD study group assessing the effect of ACE inhibitors on the development of ESRD in nondiabetic kidney disease. Among 1,594 patients from 10 studies, patients receiving ACE inhibitors had a 30% reduction in the risk of developing ESRD. Again, blood pressures were lower in the ACE inhibition group casting doubt on whether ACE inhibitors themselves, or better blood pressure control, were to be credited with lowering the risk of ESRD.

Results from the Ramipril Efficacy in Nephropathy (REIN) study (476) provide the most persuasive evidence for a uniquely renoprotective effect of ACE inhibition. Three hundred fifty-two patients with chronic nondiabetic renal disease were stratified according to baseline proteinuria (stratum 1: 1 to 3 g/24 hours; stratum 2: ≥3 g/24 hours) and randomly assigned to ramipril or placebo plus conventional antihypertensive therapy to achieve a goal diastolic blood pressure less than 90 mm Hg. By the time of the second interim analysis, there was already a significant difference in the rate of decline of GFR between the two groups despite similar blood pressure control. Glomerular filtration rate decreased by 0.53 mL/minute per month in the ramipril-treated group compared with 0.88 mL/minute per month in the placebo group ($p = 0.03$). Moreover, reduction of proteinuria predicted the decrease in risk of doubling the serum creatinine or progressing to ESRD. The renoprotective effect of ACE inhibition was attenuated, however, in patients with only 1 to 3 g of proteinuria (stratum 1). In REIN stratum 1 the decline in GFR was not significantly different between the ramipril group (0.26 mL/minute per month) and the placebo group (0.29 mL/minute/month); however, progression to ESRD was significantly decreased with ACE inhibition (477). In the most recent analysis of the REIN data, both proteinuria >2 g and hypertension were independent predictors of a more rapid decline in GFR (478). Not surprisingly, hypertensive patients with proteinuria >2 g benefited most from ramipril. On the other hand, ACE inhibition did not confer a significant benefit to patients with <2 g proteinuria, non–insulin-dependent diabetes, or polycystic kidney disease.

With inclusion of the REIN data and the AIPRD study, a meta-analysis of 11 trials confirmed the role of proteinuria in the progression of renal disease (479). Among 1,860 nondiabetic patients with proteinuric renal disease, relative risk in the ACE inhibitor group was .69 for ESRD and .70 for the composite outcome of doubling of baseline creatinine or ESRD (479,480). Patients with greater baseline proteinuria benefited most from ACE inhibitor therapy. There was a trend toward benefit of ACE inhibition at baseline proteinuria of approximately 500 mg but the data were inconclusive. Moreover, the relative risk reduction for ESRD was greater at increasing levels of baseline proteinuria. Finally, in the AIPRD study, change in proteinuria during treatment predicted renal outcome. The 1,710 patients whose proteinuria declined had an improved renal outcome while the 638 patients without a decrease in proteinuria saw no improvement in renal outcome (481). In fact, the percent change of proteinuria at 3 months predicted the change in GFR at 31 months (482). This analysis further strengthens the concept that proteinuria is a modifiable risk factor for progression of renal disease.

In the AASK trial, subjects with >300 mg/d of proteinuria at baseline had more rapid decline in renal function and ESRD events (461). Importantly, participants with a urine protein:creatinine rate > 0.66 g/g assigned to amlodipine had a rate of decline in GFR double that of those assigned to ramipril. After controlling for randomization, baseline GFR, and change in GFR over the first 6 months of follow-up, the change in urine protein excretion rate from baseline to 6 months was an independent predictor of subsequent development of ESRD (461). Thus, the results of the AASK trial are in agreement with other important trials of proteinuric renal disease, suggesting that a primary goal of treatment should be reduction of proteinuria. Taken together, the data support the recommendation that ACE inhibitors and angiotensin II receptor blockers (ARBs) are first-line therapy in patients with CKD.

Combination ACE Inhibition and Angiotensin Receptor Blockade. Long-term ACE inhibition results in the accumulation of angiotensin I that may "escape" ACE inhibition and be converted to angiotensin II at the tissue level via alternative pathways including cathepsin G and chymase (483). Theoretically, an angiotensin II receptor antagonist would block the alternate pathway effects. Huang et al. have shown that chymase is upregulated in patients with CKD, particularly those with diabetic nephropathy (484). In addition, ACE inhibition may regulate important metalloproteinase activities in CKD patients, where ARBs are not effective (485). Thus, combination ACE inhibitor and an angiotensin II receptor blocker therapy could have additive renoprotective effects.

Short-term studies in patients with both diabetic nephropathy (486) and nondiabetic nephropathies (487–489) have demonstrated additive beneficial effects of ACE inhibition and angiotensin II receptor blockade on microalbuminuria, proteinuria, and serum creatinine. In the largest trial to date, Nakao et al., in the COOPERATE study (490), randomized 301 nondiabetic patients with a mean GFR of 38 mL/minute to losartan, trandolapril, or a combination of these medications. At 36 months patients treated with combination therapy, despite similar blood pressure control, had a 76% reduction in proteinuria versus 42% and 44% decreases for losartan and trandolapril, respectively. More importantly, the combination therapy group had a significantly lower risk (11%) of ESRD or doubling of creatinine compared with both the losartan and trandolapril groups (23%) ($p < 0.02$). Combination therapy was particularly efficacious among patients with greater than 1 g of proteinuria per day. These data provide strong support for the concept that combination therapy with an ACE inhibitor and an ARB more effectively retards the progression of nondiabetic kidney disease compared with monotherapy with either class of drugs.

Calcium Channel Blockers (CCB). In most instances, patients with CKD will require between two and four anti-hypertensives to achieve goal blood pressure (405). Thus, the role of CCBs as add-on therapy in the treatment of hypertensive patients with CKD is important. Recent studies have demonstrated the efficacy of both nondihydropyridine (491) and DHPCCBs (492,493) in reducing cardiovascular events in high-risk patients. However, data with regard to the effect of CCBs on the progression of CKD are conflicting. In the REIN study (494), patients with proteinuric nondiabetic nephropathies treated with DHPCCBs and placebo (no ACE inhibitor) had significantly greater proteinuria when compared with patients not

administered DHPCCBs. In the AASK trial (460), patients with proteinuria greater than 300 mg/day randomized to ramipril had a significantly slower decline in GFR over 3 years compared with patients treated with amlodipine. On the other hand, non-DHPCCBs reduce proteinuria and slow the rate of renal disease progression in diabetic nephropathy (495,496). However, in patients with nondiabetic nephropathies, Boero et al. (497) found no difference in the reduction of proteinuria between those treated with trandolapril and verapamil compared with those who received trandolapril and amlodipine.

Herlitz et al. (498) showed that the addition of felodipine to ramipril resulted in a slower progression of nondiabetic renal disease compared with felodipine alone without any suggestion of adverse effect from the DHPCCB. In the RENAAL study (499), patients with type 2 diabetic nephropathy who received a DHPCCB without losartan had a higher risk of reaching the primary composite end point of ESRD, death, or a doubling of serum creatinine. In contrast, patients receiving a DHPCCB with losartan showed a slower progression to ESRD than did the entire losartan arm.

In summary, CCBs are important add-on medications to diuretics and ACE inhibitors or angiotensin II receptor blockers in order to achieve goal blood pressure. For patients with significant proteinuria, non-DHPCCBs may decrease proteinuria further and thus slow progression of renal disease, while DHPCCBs are effective antihypertensive agents and may slow progression of renal disease when added to a regimen already containing a drug that interrupts the RAAS. Among CKD patients without significant proteinuria, either class of CCB is likely safe when added to an ACE inhibitor or angiotensin II receptor blocker.

Emerging Agents. Emerging data supporting an important role for aldosterone in the pathogenesis of progressive renal disease (500,501) have stimulated interest in the study of aldosterone antagonism in patients with CKD. Shiigai et al. (502) found that between 24% and 43% of patients had a loss of the antiproteinuric effects of ACE inhibition within months of treatment initiation. Sato et al. (503) studied 45 patients with diabetic nephropathy treated with an ACE inhibitor. Forty percent of the subjects who demonstrated an initial decline in microalbuminuria subsequently showed an increase in albumin excretion within several months. The loss of the antiproteinuric effect of ACE inhibition corresponded to an increase in plasma aldosterone. The addition of spironolactone at a dose of 25 mg/day reduced urinary albumin excretion rate and ameliorated the effect of "aldosterone escape." Rachmani et al. (504) compared the effect of spironolactone, cilazapril, and the combination on albuminuria in patients with hypertension and diabetic nephropathy. Despite a similar reduction in blood pressure, spironolactone was superior to cilazapril alone with regard to decreasing urinary albumin excretion, and the combination was more effective than either drug alone. Eplerenone, a selective aldosterone receptor antagonist, has less anti-androgenic effects than spironolactone (505). As monotherapy in essential hypertensives, eplerenone was as effective as enalapril but decreased albuminuria more effectively than the ACE inhibitor (506). Less than 1% of patients had adverse events such as hyperkalemia. Taken together, the data suggest that complete suppression of the RAAS might be an important goal in retarding the progression of CKD. However, hyperkalemia could be a limiting factor in a population that already has multiple constraints on potassium homeostasis. Thus, while the concept of aldosterone antagonism in patients with CKD appealing, randomized controlled trials are necessary to confirm the utility and safety of this approach.

Approach to Treatment. Available classes of antihypertensive drugs are shown in Table 51-5 along with general comments regarding their utility or special attributes in patients with renal

TABLE 51-5

CLASSES OF ANTIHYPERTENSIVE AGENTS

Class	Comments (References)
Thiazide diuretics	Usually not effective with creatinine clearances <25–30 mL/min (447). Additive or synergistic effect on salt excretion in severe renal insufficiency when combined with a loop diuretic (448,450,451).
Loop-blocking diuretics	Short duration of action often requires multiple daily dosing (BID or TID) in patients with avid salt retention (447,448). Continuous infusions more effective than bolus therapy in severe renal dysfunction when parenteral treatment required (448).
β-Adrenoreceptor antagonists	No significant effect on renal hemodynamics or salt excretion (519,520).
α_1-Adrenoreceptor antagonists	Salt retention (pseudotolerance) with short-acting agents can limit long-term efficacy in chronic renal failure (521). Improve lipoprotein profile.
Central α_2-adrenergic agonists	Can cause pseudotolerance. Concomitant diuretic therapy usually necessary in patients with renal insufficiency (452,513). Have therapeutic advantage because of important role of sympathetic nervous system in renal parenchymal hypertension (167,169,170,175,177).
α- and β-Adrenoreceptor antagonists	Logical choice from pathophysiologic perspective. Side effects might limit utility.
Vasodilators	Not suitable as monotherapy. Diuretics and sympatholytics required to limit salt retention and reflex tachycardia.
ACE inhibitors	Renoprotective effect independent of reduction in blood pressure (461,462,476). Cardioprotective in high-risk patients (507). Suitable as monotherapy. Can cause hyperkalemia (522). Exaggerated hypotensive effect with salt removal (522,523).
Angiotensin II receptor antagonists	Antihypertensive effects and renoprotective effects probably similar to ACE inhibitors (455,483). May be beneficial in combination with ACE inhibitors (487–490).
Calcium channel blockers	Antihypertensive effect not blunted by high salt intake (509,510). Verapamil might accumulate in severe renal insufficiency and produce acute toxicity (524).
Aldosterone antagonists	Spironolactone and eplerenone might have specific renoprotective effects (503–505). Can cause hyperkalemia alone or if added to ACE inhibitor or angiotensin II receptor antagonist (506).

parenchymal hypertension. In patients with pre-ESRD, an ideal antihypertensive agent should be logical from a pathophysiologic perspective, produce few or no adverse clinical or metabolic effects, exert a favorable effect on cardiovascular morbidity and mortality, and beneficially influence the progression of renal disease. In this regard, ACE inhibitors, ARBs, centrally acting sympatholytics, and calcium channel antagonists are particularly useful for several reasons. Because both increased central sympathetic activity and activation of the RAAS are important pathophysiologically in the initiation and maintenance of hypertension in renal disease, pharmacologic interruption of these systems is logical from a therapeutic standpoint. Angiotensin-converting enzyme inhibitors and ARBs not only inhibit the RAAS but also modulate activity of the SNS (167,170). They are rational agents from a pathophysiologic perspective, exert a beneficial effect on the progression of renal disease independent of their antihypertensive effect, and decrease cardiovascular morbidity and mortality in this high-risk population (507,508). Consequently, ACE inhibitors or ARBs should be considered as first-line antihypertensive therapy in the majority of patients with CKD and hypertension (462,478). Calcium channel antagonists effectively decrease blood pressure despite extremes of dietary salt intake (509,510) and consequently have proved effective antihypertensive agents in patients with CKD (472,511). Centrally acting sympatholytics such as clonidine or monoxidine are logical from a pathophysiologic and practical perspective (169,170,512); however, they are not effective as long-term monotherapy (452,513) and exhibit significant side effects that often limit long-term utility. Nevertheless, they are quite effective as adjunctive therapy in selected patients.

Based on the previous discussion, we recommend the following approach to the treatment of renal parenchymal hypertension. Limitation of sodium intake to 100 mEq/day or less is essential to achieve the blood pressure goal, derive full benefit from ACE inhibition or angiotensin II receptor blockade, and limit the development of pseudotolerance. An ACE inhibitor or angiotensin receptor blocker is recommended as first line therapy for all patients, particularly those with more than 30 mg/day of albuminuria or overt proteinuria. A diuretic will likely be necessary to achieve the blood pressure goal. Thiazide diuretics are effective in patients with mild to moderate renal insufficiency and creatinine clearances >30 mL/minute (447). Loop-blocking diuretics, often in a multiple daily dosing regimen, are required to achieve an adequate natriuresis with more severe renal impairment (447,448). It is important to emphasize that the diuretic regimen should be advanced until the patient is free of edema, because even small increments in ECFV can contribute to poor blood pressure control (449,452). In patients with advanced renal insufficiency (i.e., creatinine clearances <15 mL/minute), the combination of a loop diuretic with a thiazide minimizes diuretic resistance and has a synergistic effect on salt excretion (450,451). Effective diuretic therapy alone may normalize blood pressure in approximately one-third of hypertensive patients with chronic renal insufficiency (148). Dietary and/or pharmacologically induced salt depletion is crucial both from a pathophysiologic perspective and to limit the development of pseudotolerance if short-acting vasodilators or centrally acting sympatholytics are utilized as antihypertensive agents (452,453).

As necessary, a CCB can be added to help achieve blood pressure goal. A non-DHPCCB may be preferred for those with overt proteinuria. The use of a beta-blocker should be considered 'earlier' if patients have heart failure or another indication for its use. The addition of a beta-blocker may offer little additional antihypertensive effect when added to an ACE inhibitor if the pulse is greater than 84 beats/minute (514). A vasodilator or central sympatholytic agent may be necessary add-on therapy for some patients, as studies have found that as many as four or more drugs may be necessary to achieve goal. Klein et al. (169) and others (167,175) have shown that sympathetic nerve activity is inappropriately increased in chronic kidney disease. Agents such as clonidine, or the recently studied moxonidine (170), provide theoretical benefit to CKD patients with elevated sympathetic tone.

Hypertension is a common clinical problem in patients undergoing chronic hemodialysis (525–528); 60% to 90% of patients are hypertensive at the onset of ESRD. In a large, nationally representative sample from the U.S. Renal Data System, 63% of prevalent hemodialysis patients were hypertensive. Of these, 27% of patients had stage I, 25% had stage II, and 11% had stage III hypertension, respectively (525). Several studies show that blood pressure remains uncontrolled in the majority of hemodialysis patients in the Unite States (529–531). Hypertension is clearly established as a risk factor for cardiovascular disease morbidity and mortality in the general population; however, whether hypertension is an independent predictor of survival in patients undergoing chronic hemodialysis is uncertain. Several studies show the association between hypertension and adverse cardiovascular outcomes in hemodialysis patients. In a long-term follow up of 432 ESRD patients, after adjusting for age, diabetes, and ischemic heart disease, as well as hemoglobin and serum albumin levels measured serially, each 10 mm Hg rise in mean arterial blood pressure was independently associated with the presence of concentric LV hypertrophy, and the development of de novo cardiac failure and ischemic heart disease (532). Other studies have confirmed that hypertension is a risk factor for left ventricular hypertrophy (533,534), heart failure, coronary heart disease, and arrhythmias in hemodialysis patients (535). Conversely, reduction of arterial pressure is associated with regression of LV hypertrophy (536). In addition, hypertension has been associated with stroke and cerebral infarction (537,538) and progression of atherosclerosis (539) in patients on hemodialysis. Given this adverse impact on cardiovascular events, it is surprising that several epidemiologic studies show no association between hypertension and survival, and in fact have shown that low blood pressure is strongly associated with mortality (540–542). In a national random sample of 4,499 U.S. hemodialysis patients, no association with an elevated mortality risk could be observed for predialysis systolic hypertension (RR = 0.98 to 0.99, p value not significant) (540). Similarly, in a cohort of 5433 patients followed by a national dialysis provider (Dialysis Clinic, Inc.), predialysis hypertension was not associated with an increase in cardiovascular mortality (541). In a cohort of 932 patients, the lowest mortality was associated with predialysis systolic pressure of 160 to 189 mm Hg, whereas normal to low predialysis pressure values were associated with significantly increased mortality (543). Even in home hemodialysis patients, low and high blood pressure were associated with increased mortality and patients with mid-range blood pressure values survived the longest (544). Thus hypertension is associated with increased cardiovascular events, but not increased mortality in hemodialysis patients (545). The reasons underlying this apparently paradoxical relationship between low pressure and mortality in this population have not been clearly elucidated. It is possible that advanced cardiac disease may confound the interpretation of these data. Patients with ESRD who develop congestive heart failure have a very high mortality; they also have a decline in blood pressure after the onset of congestive heart failure that is well documented in the Canadian cohort study (546–548). In addition, advancing age is associated with lower blood pressure in ESRD patients (525). Therefore, the effects of age and congestive heart failure on mortality may mask the true relationship between blood pressure and mortality in these studies. Another limitation of previous studies may have been inadequate duration of follow up. When early mortality was analyzed in an Italian study, (in

the first 2 years) only low BP (diastolic BP <74.5 mm Hg) was significantly associated with mortality, a finding that is consistent with the literature. However, when late mortality was analyzed, only high BP (systolic BP >160 mm Hg) was significantly associated with mortality (549). Therefore, well-designed prospective studies of appropriate duration, carefully controlling for comorbid conditions, are needed to resolve this critical issue (550). A high pulse pressure is an independent predictor of adverse cardiovascular outcomes in dialysis patients, perhaps reflecting decreased arterial compliance in this population (551,552).

Measurement of blood pressure presents some unique problems in hemodialysis patients (553). Inaccurate readings may result from lack of standardization of blood pressure measurement (554,555). In addition, blood pressure readings vary considerably before and after dialysis and in the interdialytic period. Predialysis blood pressures correlate better with LV hypertrophy than postdialysis blood pressure readings (556). However, postdialysis systolic pressure, not predialysis systolic pressure, may be associated with an elevated mortality risk (540,541). Using multiple blood pressure readings, such as a 2-week average predialysis BP of greater than 150/85 mm Hg or a postdialysis BP of greater than 130/75 mm Hg increases the sensitivity in diagnosing hypertension compared to single readings (557,558). Despite these conflicting data, pending further definitive information, some authors recommend a target predialysis blood pressure of less than 140/90 mm Hg, unless the patient has symptomatic hypotension during or after dialysis (559). The use of ambulatory blood pressure monitoring (ABPM) has been gaining popularity in hemodialysis patients to overcome the difficulties of conventional measurements described above (560,561). Blood pressure is often low at the end of the dialysis treatment and gradually rises during the interdialytic period (562). The "nondipping" profile with the lack of normal nocturnal decline in blood pressure is common and contributes to the accelerated target organ damage in these patients. For example, Covic et al. demonstrated that reduced blood pressure diurnal variability was an independent risk factor for progressive left ventricular dilatation in hemodialysis patients (563). In addition, Amar et al., albeit in a small sample, demonstrated that nocturnal blood pressure and 24-hour pulse pressure were predictive of mortality in ESRD patients (564). Finally, the nondipping phenomenon is closely related to a high incidence of cardiovascular diseases and a poor long-term survival (565). While the overall reproducibility of the nondipper profile is variable (566), most patients diagnosed as nondippers will continue to demonstrate that profile over time (567). However, until larger studies comparing the predictive value of ABPM with conventional blood pressure measurements in predicting outcomes of cardiovascular disease in ESRD patients are available, the expense and inconvenience of ambulatory blood pressure monitoring in all hemodialysis patients may not be justifiable. Home blood pressure monitoring may be a useful adjunct in the management of hypertension (568).

While pathogenesis of hypertension in hemodialysis patients is clearly multifactorial, expansion of intravascular volume status due to sodium and water retention is thought to be the major determinant of blood pressure in this population (569–571). This concept is supported by the fact that blood pressure is directly correlated with total body water content (572), and that higher interdialytic weight gains are associated with higher blood pressure (525,573). Differences in interdialytic weight gain often contribute to seasonal variations in blood pressure (574,575). However, hypertensive dialysis patients have a higher rise in blood pressure for a given interdialytic weight gain than normotensive dialysis patients, suggesting that altered arterial compliance may contribute to the pressor response to a large interdialytic weight gain (529). The best clinical demonstration of the importance of the volume regulation in the pathogenesis of hypertension is by Charra et al. in Tassin, France. By performing long and slow dialysis, and meticulous ultrafiltration, this group has been able to achieve excellent volume control, blood pressure reduction with minimal antihypertensive medication use, and high survival rates in their patients (576,577). Similarly daily dialysis has been shown to have a beneficial impact on blood pressure control (555). Other factors that may contribute to hypertension in hemodialysis patients include increased or inappropriate response of the renin-angiotensin system (578), over activity of the sympathetic nervous system (579), and imbalance of factors such as endothelin (580), nitric oxide (581), and other vasoactive substances including asymmetric dimethylarginine (582–585). An elevation in blood pressure in association with erythropoeitin therapy, particularly given intravenously, has been well established; however, the precise mechanism of this action remains poorly defined (586). In addition, elevated levels of parathyroid hormone may cause an elevation in blood pressure (587,588). Unlike the general population, higher body mass index has not been associated with higher blood pressure in hemodialysis patients (589).

Management of Hypertension in Hemodialysis Patients

The primary focus of management of hypertension in hemodialysis patients should be to optimize intravascular volume status (590,591); this is accomplished by a strategy of restricting salt intake, limiting interdialytic weight gain, and adjusting utrafiltration and dialysate sodium concentration to consistently attain dry weight (592). Patients who do not become normotensive by this strategy will require antihypertensive drug therapy.

Dry Weight Estimation and Maintenance

The crucial role of achieving and maintaining patients at dry weight is illustrated by the Tassin experience discussed above. In addition, patients with "resistant hypertension" may still be volume expanded as demonstrated by Fishbane et al. who showed that atrial natriuretic peptide levels (ANP) were elevated in patients with refractory hypertension, suggesting that intravascular volume remained expanded (593). When these patients were subjected to more intensive ultrafiltration, blood pressure and ANP levels were reduced, suggesting that patients were now closer to their dry weight.

A practical problem in optimizing the intravascular status of a patient is the lack of a consistent definition of, and a reliable diagnostic tool for the determination of "dry weight." Dry weight is defined as "that body weight at the end of dialysis at which the patient can remain normotensive until the next dialysis despite the retention of salt and water" (594). Clinically, this is often estimated by the weight below which further fluid removal would produce hypotension, muscle cramps, nausea, and vomiting. Other diagnostic tools that have can be used include bioimpedance (595), estimation of inferior venacaval diameter (596), measurement of ANP (597), and online hematocrit (598) or blood volume monitoring (599); the utility of these techniques in usual clinical practice is not well established.

At the initiation of dialysis, ultrafiltration and restriction of dietary salt intake should be used to reduce excess extracellular fluid volume while antihypertensive drug therapy is being tapered. Reduction of blood pressure may be seen several weeks following optimization of intravascular volume status—this has been described as the "lag phenomenon" of blood pressure and may be due to gradual changes in peripheral

resistance (600–603). An individualized sodium concentration of the dialysate based on predialysis plasma sodium levels can decrease thirst, interdialytic weight gain and blood pressure (602). Once dry weight has been established, it should be reassessed every few months since lean body mass may change due to changes in nutritional status. Patients who remain hypertensive despite apparently reaching dry weight should be restarted on antihypertensive drug therapy.

Minimizing Interdialytic Weight Gain

Excessive interdialytic weight gain is independently associated with higher blood pressure (525,529). In addition, excessive intradialytic weight gains may result in intradialytic hypotension when large-volume ultrafiltration is attempted. This may result in symptoms of nausea and cramping and limit the amount of fluid that can be removed. Therefore, patients should be instructed not to gain more than 2.5% to 3% of their body weight in between dialysis sessions.

Daily and Prolonged Dialysis

Increased frequency and duration of dialysis, such as nocturnal dialysis, has been associated with improvement in blood pressure (604). Gentle and persistent ultrafiltration may allow time for refilling of the intravascular compartment and permits normalization of extracellular volume (605). It is also possible that intensive dialysis enables removal of pressor molecules and improves endothelial function (606). Thus, prolonged and frequent dialysis permits better control of hypertension via volume and volume-independent mechanisms (606). In addition, nocturnal dialysis has been shown to improve endothelial function (555), and induce regression of left ventricular hypertrophy (607).

Antihypertensive Drug Therapy

Antihypertensive drugs are frequently used as an adjunct to dialysis to control hypertension (526,608,609). Interestingly, Salem et al. have shown that antihypertensive treatment has a favorable effect on survival in the hemodialysis population irrespective of the level of blood pressure (610). This provocative study hints at perhaps beneficial effects of antihypertensive drug therapy at the end organ level, in addition to lowering blood pressure.

Other than diuretics, all classes of antihypertensive agents appear to be effective in lowering blood pressure in hemodialysis patients. High doses of individual medications and combination therapy are often required (611). No single class of antihypertensives appears to offer particular advantage over another with regard to lowering blood pressure (569). Observational data indicate that the use of calcium channel blockers (612), ACE inhibitors (613), and beta-blockers (614) is associated with lower total and cardiovascular-specific mortality in hemodialysis patients. Most calcium channel blockers are not removed by dialysis (615), and the use of calcium channel blockers has been associated with better graft patency (616). However, there are no randomized prospective clinical trials comparing the effects of different classes of antihypertensive agents on clinical outcomes. Pending such studies, it is reasonable that the recommendations of JNC-7 (other than choice of thiazide diuretic as initial therapy) may be used as a guideline with regard to the choice of antihypertensive drug therapy in specific situations (617). Several antihypertensive medications may require dosage modification in hemodialysis patients. The *Physicians Desk Reference* (PDR) or other sources of pharmacologic information should be consulted prior to prescription of antihypertensive drug therapy (618).

Several issues regarding ACE inhibitor therapy are unique to the hemodialysis population. ACE inhibitors can trigger an anaphylactic reaction in patients dialyzed with a polyacrylonitrile membrane dialyzer (619). In addition, ACE inhibitors have been associated with worsening of anemia and erythropoietin resistance in hemodialysis patients (620), though other reports have not confirmed this (621). However, long-term therapy with ACE inhibitors has been show induce regression of LV hypertrophy in this population (622), and may reduce interdialytic weight gain by minimizing the effect of angiotensin II on the thirst mechanism (623).

Refractory Hypertension. Several factors may contribute to resistant hypertension; these include inadequate fluid removal, excessive interdialytic weight gains, poor compliance with dialysis and medication regimens, and underprescription of antihypertensive drugs (529). In patients who are poorly compliant, three times a week supervised administration of atenolol or lisinopril may be a safe and effective technique of lowering blood pressure (624–626). Patients who remain refractory or have suggestive signs or symptoms should be evaluated for secondary hypertension (627,628). In the occasional patient who remains refractory to all conventional measures, bilateral nephrectomy may be considered (629).

Patients on peritoneal dialysis tend to have better blood pressure control, perhaps due to better ultrafiltration (630–632). Recent development of new solutions have enhanced the ability to achieve adequate volume removal in peritoneal dialysis (633).

Conclusion

In conclusion, hypertension remains a common and difficult clinical problem in patients undergoing chronic hemodialysis. Meticulous attention to optimizing intravascular volume status often results in improvement in blood pressure. Prospective clinical studies are needed to define treatment goals, and develop rational therapeutic strategies based on understanding of pathophysiology. Development of reliable and clinically useful tools to define intravascular volume may be invaluable in obtaining better control of hypertension in this population.

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CHAPTER 52 ■ RENAL ARTERY STENOSIS, RENAL VASCULAR HYPERTENSION, AND ISCHEMIC NEPHROPATHY

MARC A. POHL AND CHRISTOPHER STUART WILCOX

Since 1934, when Goldblatt and et al. first experimentally produced hypertension by clamping the renal artery, the relationship between renal artery stenosis and hypertension has intrigued countless investigators and clinicians (1). The physiology and pathophysiology of the renin-angiotensin system have been widely discussed, and much of our understanding of the role and regulation of the renin-angiotensin system has evolved from models of experimental hypertension produced by renal artery clamping. The development of pharmacologic agents that interfere with the activity of the renin-angiotensin system (e.g., beta-blockers, angiotensin-converting enzyme (ACE) inhibitors, and angiotensin II receptor antagonists), fascination with numerous diagnostic screening studies designed to predict a relationship between renal artery stenosis and hypertension, and controversies about renal revascularization versus medical management of renal artery stenosis may all be viewed, in one way or another, as emanating from the original Goldblatt experiment.

The detection of renal artery stenosis in a patient with hypertension typically raises the question as to whether the renal artery stenosis causes the hypertension. However, renal artery stenosis can be observed angiographically in normotensive individuals (2–4). In a postmortem study, Holley and associates found moderate or severe stenosis of the renal artery in nearly 50% of patients who had been normotensive (4). Dustan et al. reported that 39 (35%) of 111 normotensive patients had unsuspected renal artery stenosis in one or both renal arteries revealed by aortography that was performed to evaluate peripheral arterial occlusive disease (3). Thus, significant renal arterial stenosis in patients with normal arterial pressure or in patients with mild hypertension is well recognized. Conversely, many hypertensive patients with renal artery stenosis are not cured of their hypertension by renal revascularization (surgical or endovascular) procedures. Most patients with hypertension and atherosclerotic renal artery disease have essential or primary hypertension despite the anatomic presence of renal artery stenosis. Therefore, it is critical to distinguish between renal artery stenosis wherein a stenotic lesion is present, but not necessarily causing hypertension, and renovascular hypertension in which sufficient arterial stenosis is present to produce renal tissue ischemia and initiate the sequence of pathophysiologic events leading to elevated blood pressure.

The syndrome of renovascular hypertension may be broadly defined as the secondary elevation of the blood pressure (BP) produced by any of a variety of conditions interfering with arterial circulation to kidney tissue and causing renal tissue ischemia. Most of the time, the condition interfering with the arterial circulation is main renal artery stenosis. However, Page demonstrated persistent arterial hypertension in experimental animals in which one kidney was wrapped in cellophane; within 3 to 5 weeks of cellophane wrapping, severe hypertension occurred, and was later alleviated by removal of the wrapped kidney (5). Unilateral renal trauma, chronic subcapsular hematoma, perirenal hematoma, unilateral ureteral obstruction, unilateral chronic atrophic pyelonephritis, unilateral renal hypoplasia, and unilateral segmental hypoplasia (Ask-Upmark kidney) may be associated with hypertension and viewed as clinical counterparts of the experimental Page kidney, wherein one may observe the syndrome of renovascular hypertension without main renal artery stenosis. Thus, renovascular hypertension and renal artery stenosis are not synonymous.

Historically, clinical discussions of renal artery stenosis have focused on its role in the pathogenesis of hypertension, that is, renovascular hypertension. More recently, the notion that renal artery stenosis can produce renal insufficiency has generated considerable interest and enthusiasm. Surgical renal revascularization, percutaneous transluminal renal angioplasty (PTRA), and renal artery stent placement sometimes produce improvement in overall renal function in selected patients, and occasionally, patients can discontinue dialysis after renal revascularization. This syndrome of clinically significant reduction in renal function due to compromise of the renal circulation has been called ischemic renal disease or ischemic nephropathy. In comparison to the voluminous literature addressing the relationship between renal artery stenosis, renal ischemia, and hypertension (i.e., renovascular hypertension), the syndrome of ischemic nephropathy, and the notion that renal artery stenosis can produce renal failure have received relatively little attention.

This chapter reviews the types of renal artery disease producing renal artery stenosis; the pathophysiology of renovascular hypertension; clinical features and diagnostic approaches to renal artery stenosis, renovascular hypertension, and ischemic nephropathy; management of patients with renal artery stenosis; and therapeutic strategies for the clinical syndromes of renovascular hypertension and ischemic renal disease.

HISTORICAL ASPECTS OF RENOVASCULAR HYPERTENSION

The history of renal disease and hypertension can be traced to Richard Bright, who demonstrated in 1827 the association between hardness of the pulse, proteinuria, and dropsy with hardening of the kidneys (6). These observations suggested a link between renal disease and disturbances of the circulation. Bright also noted that the altered quality of blood so affects the minute and capillary circulation as to render greater action necessary to force the blood through the distal division of the vascular system (6). Mahomed reported in 1874 a high tension in the arterial system in patients with renal disease (7). Blood pressure measurements were not described in the observations of either Bright or Mahomed. In 1898, Tigerstedt and

Bergmann described a renal pressor substance in the rabbit that they called renin (8). The development of ideas on renovascular hypertension has been reviewed by Peart (9).

In 1934, Goldblatt et al. produced hypertension in dogs by constriction of the renal artery (1). They also noticed extensive renal vascular abnormalities at autopsy of patients with hypertension. About 40 years later, reflecting on his own work, Goldblatt stated, "Contrary, therefore, to what I had been taught, I began to suspect that vascular disease comes first, and when it involves the kidneys, the resultant impairment of the renal circulation probably, in some way, causes elevation of blood pressure" (10). Goldblatt's studies, investigating the relationship between hypertension and the kidney, largely preceded renal revascularization procedures. However, they strongly suggested that hypertension secondary to renal ischemia could be corrected by nephrectomy (11).

Although renin was first identified by Tigerstedt and Bergman in 1898, most of the information on it and the renin-angiotensin cascade have accumulated over the past 50 years. Tigerstedt's initial experiments proved difficult to reproduce by others, and renin lost credibility until Goldblatt's classic experiment. The biochemistry of the renin-angiotensin system was elucidated over the next 20 years, forming the basis for many physiologic and clinical studies that continue today (12,13). The complexity of the renin-angiotensin system in terms of its regulation, expression in different tissues, and sites of action has been widely studied over the past decade with application of modern molecular biology. Although much is currently known about the renin-angiotensin system and its role in the elevation of BP, fundamental questions about its mode of action in the pathogenesis of elevated BP and other diseases of the circulation remain unanswered.

PATHOLOGIC CLASSIFICATION OF RENAL ARTERY DISEASE

A variety of pathologic lesions (Table 52-1) cause renal artery stenosis, with or without associated renovascular hypertension. The two main types of renal arterial lesions forming the anatomic basis of renal artery stenosis are atherosclerotic renal artery disease and fibrous dysplasia or fibrous renal artery disease. Atherosclerotic renal artery disease is the most common cause of renal artery stenosis, accounting for about 80% of renal arterial lesions. Fibrous renal artery diseases, as a group, account for about 20% of renal arterial lesions (14–19). Recent reports of "resistant hypertension" have indicated figures of 84% for atherosclerotic renal artery disease and 16% for fibrous renal artery disease of any kind (20). Fibrous renal artery disease has been reported in 2% to 6% of potential renal transplant donors (usually normotensive individuals) (19,21–26). The vast majority of "incidental" renal artery lesions in angiographic series are atherosclerotic.

TABLE 52-1

CAUSES OF RENAL ARTERY STENOSIS

Atherosclerotic renal artery disease
Fibrous renal artery disease (fibromuscular dysplasia, fibrous dysplasia)
Medial fibroplasia
Perimedial fibroplasia
Intimal fibroplasia
Medial hyperplasia
Acute arterial embolism
Arterial trauma
Aortic dissection
Neurofibromatosis
Arterial aneurysm
Arteriovenous malformation
Cholesterol emboli
Systemic necrotizing vasculitis
Polyarteritis nodosa
Takayasu's arteritis

In addition to atherosclerotic and fibrous renal artery disease, a number of less common clinical entities can produce renovascular hypertension. These include acute arterial thrombosis or embolism, cholesterol emboli, aortic dissection, renal arterial trauma, arterial aneurysm, arteriovenous malformation of the renal artery, neurofibromatosis, polyarteritis nodosa, and Takayasu's arteritis. Renal artery thrombosis occurring as a complication of umbilical artery catheterization has been recognized as causing renovascular hypertension in infants (27). Transplant renal artery atherosclerosis, intimal hyperplasia, or vascular kinking may contribute to renal transplant renovascular hypertension (see Chapter 98).

Table 52-2 presents a classification of atherosclerotic and fibrous renal artery diseases with a description of their morphology and histology. These types of renal artery occlusive diseases represent a heterogeneous group of diseases, occurring in different age groups and behaving differently with regard to their individual natural history. An appreciation of these differences may be important for therapeutic decision making in patients with renal artery occlusive disease.

ATHEROSCLEROTIC RENAL ARTERY DISEASE

Atherosclerotic renal artery disease is the most common form of renal artery stenosis and the most common cause of renovascular hypertension in the Western world. Although on average it accounts for 70% to 80% of renal artery lesions, it may account for as many as 90% of all renal artery stenoses in certain populations. Atherosclerosis of the renal artery, frequently observed in conjunction with generalized atherosclerosis obliterans, predominantly affects men after the fifth decade of life, but is not uncommon in women of similar ages or in younger adults.

Renal artery atherosclerosis is often observed in patients with peripheral vascular disease and in patients with coronary artery disease. A prospective study by Olin et al. reviewed 395 consecutive patients who underwent arteriography for routine evaluation of abdominal aortic aneurysms, aortoocclusive disease, lower extremity occlusive disease, or suspected renal artery stenosis (28). Unilateral renal artery stenosis of more than 50% was observed in 38% of the patients with abdominal aortic aneurysm, in 33% of patients with aortoocclusive disease, and in 39% with lower extremity atherosclerotic-occlusive disease. Bilateral renal artery stenosis of more than 75% was noted in 13% of the patients studied. Fifty percent of patients with diabetes and claudication had unilateral renal artery stenosis of more than 50%. Other observers note significant atherosclerotic renal artery disease in up to 30% of patients with carotid and lower extremity atherosclerosis (29) and in patients with coronary artery disease (30,31).

The precise epidemiologic correlations between hypercholesterolemia or cigarette smoking and atherosclerotic renal artery disease are not clear, but one might presume that they are risk factors for renal artery atherosclerosis just as they are for atherosclerosis in other vascular beds. In one series of patients with atherosclerotic renal artery disease, 88% were smokers, compared with only 42% of patients with essential

TABLE 52-2

CHARACTERISTICS OF THE TWO MAIN TYPES OF RENAL ARTERY DISEASE

Lesion type	Frequency (%)	Sex	Age (yr)	Anatomic location	Morphology	Histology	Natural course
Atherosclerosis	70–80	M > F	>50	Proximal 2 cm of renal artery, ostial and adjacent aorta; branch disease uncommon; 50%–75% bilateral	Eccentric or concentric focal stenosis	Complex fibrous plaque with mural calcification, hemorrhage, cholesterol crystals, and luminal thrombus	Progression in 40%–50%, often to total occlusion
Fibrous dysplasias	20–30						
Medial fibroplasia	25 (65–85[a])	F	25–50	Mid and distal main renal artery and branches; 60% bilateral	Serial stenoses with intervening mural aneurysm; "string-of-beads" appearance	Areas of disorganized proliferation, medial fibroblasts surrounded by dense fibrous connective tissue (stenosis), alternating with areas of marked medial thinning (aneurysm)	Progression in one-third; dissection, thrombosis and complete occlusion rare
Perimedial fibroplasia	5 (15[a])	F	15–30	Mid and distal main renal artery or branches; 30% bilateral	Serial stenoses without mural aneurysm	Excess elastic tissue in subadventitial region	Progression in most cases; dissection and/or thrombosis common
Intimal fibroplasia and medial hyperplasia	5 (5–10[a])	F > M	Children and young adults	Mid main renal artery, occasionally proximal main renal artery; rarely bilateral	Focal or tubular	Subendothelial accumulation of mesenchymal cells within loose connective tissue; intact internal elastic lamina	Progression in most cases; dissection and/or thrombosis common

[a]Percent of fibrous dysplasia lesions.

FIGURE 52-1. Aortogram demonstrating severe nonostial atherosclerotic renal artery disease of the left main renal artery.

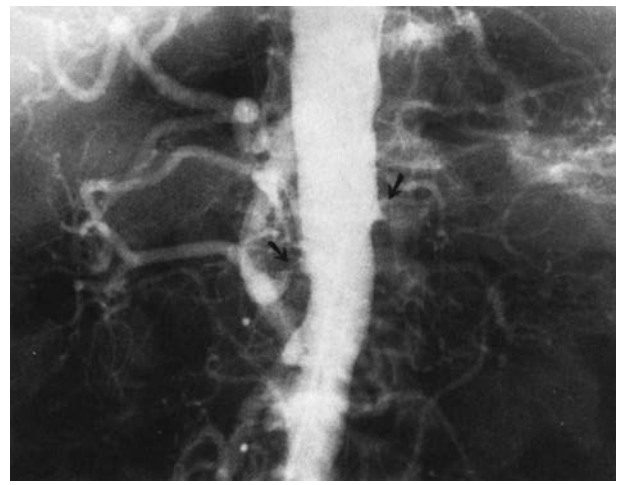


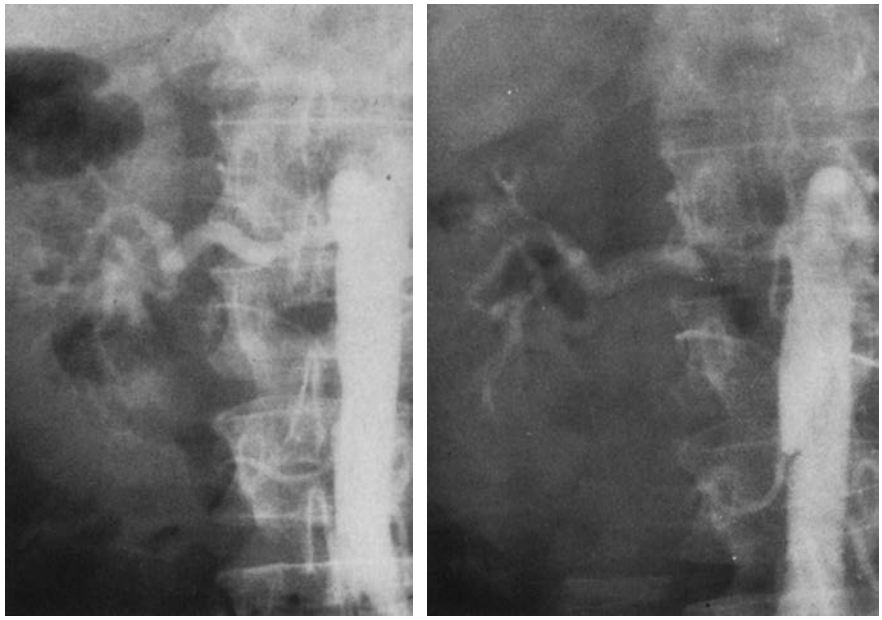
FIGURE 52-2. Abdominal aortogram demonstrating severe bilateral atherosclerotic renal artery disease with total occlusion of the left renal artery at the ostium and tight right orificial (ostial) stenosis.

hypertension (32). The investigators of this study also note a higher-than-expected prevalence of cigarette smoking in patients with fibrous renal artery disease. Atherosclerotic renal artery disease is infrequently observed in women younger than 50 years of age unless they have experienced premature menopause or are heavy cigarette smokers, or both. Men are affected twice as often as women, but this gender difference declines with advancing age. Recent series indicate a distinct shift toward women in series referred for revascularization (33–35). Several studies suggest that atherosclerotic renal vascular disease is associated with adverse coronary events (36) and increased mortality (37,38).

Renal artery atherosclerosis presents anatomically in two locations. The first appears as an eccentric or concentric lesion approximately 1 cm distal to the ostium of the renal artery ("nonostial" atherosclerotic renal artery disease) (Fig. 52-1). The second location for renal artery atherosclerosis is at the orifice (ostium) of the renal artery, occurring as an aortic plaque spillover lesion involving that orifice (ostial atherosclerotic renal artery disease) (Fig. 52-2). The histologic appearance at both locations of renal artery atherosclerosis is similar, with typical complex plaque features of mural hemorrhage and calcification, deposition of cholesterol crystals, and accumulations of luminal thrombus. Some 75% to 80% of patients with renal artery atherosclerosis and renovascular hypertension have ostial atherosclerotic lesions, and 25% to 30% of lesions are in the nonostial location (39–41). Differentiating between ostial and nonostial atherosclerotic renal artery disease is clinically important in planning interventive strategies for renal revascularization.

With recent interest in obstructive atherosclerotic renal artery disease as a threat to long-term renal function and the ability to improve kidney function with renal revascularization, an understanding of the natural history of atherosclerotic renal artery disease as a guide to the long-term management of such patients is important. Unfortunately, prospective angiographic studies on the natural history of atherosclerotic renal artery disease are not available. Four reports, retrospectively describing the natural history of atherosclerotic renal artery disease with serial arteriographic studies, suggest that atherosclerotic renal artery disease is a progressive disorder. Progressive stenosis occurs in 40% to 50% of patients with progression to total arterial occlusion in approximately 15% of patients (42–45). A contemporary prospective study reported a 16% progression to total occlusion in medically managed (no vascular intervention) patients over one year (46). It is important to distinguish here between the "natural history" of renal vascular lesions and the natural history of kidney function in these patients.

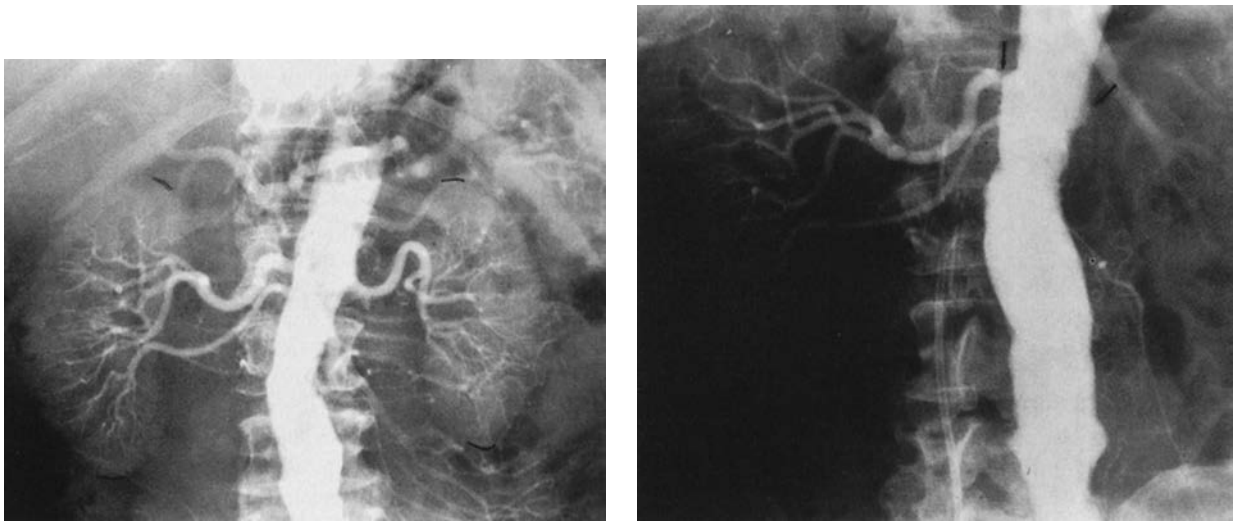
In the largest of these retrospective studies, Schreiber et al. (44) observed that progressive atherosclerotic lesions developed in 37 (44%) of 85 patients over a mean follow-up period of 52 months; progression to complete occlusion of the involved renal artery occurred in 14 patients (16%). Examples of progressive atherosclerotic renal artery disease are indicated in Figures 52-3 and 52-4. In patients in whom progressive disease developed, it occurred primarily within the first 2 years of angiographic follow-up. A careful analysis of the diseased renal arteries suggested that the rate of progression of atherosclerotic renal artery disease bore a relationship to the degree of stenosis on the initial renal angiogram. Most renal arteries with less than 50% stenosis on the initial angiogram demonstrated less than 50% stenosis on the follow-up angiogram (i.e., the lesion remained essentially unchanged). In contrast, 7 (39%) of 18 renal arteries initially demonstrating 75% to 99% stenosis progressed to total occlusion. Thus, progression to complete occlusion occurred more often and more rapidly in renal arteries initially demonstrating higher degrees of stenosis. Renal arteries with more than 50% stenosis on the initial angiogram that eventuated in complete occlusion did so within 2 years of the initial angiogram. The serum creatinine concentration increased in 54% of patients with progressive disease, but in only 25% of patients without evidence of angiographic progression. The size of the involved kidney decreased in 70% of patients with progressive renal arterial disease, but in only 27% of patients without progressive disease. There was no significant difference in how well BP was controlled between patients with and those without progressive renal artery disease. In a more recent study by Tollefson and Ernst, 48 patients with a minimum of two renal arteriograms at least 1 year apart were followed for a mean of 7.3 years (45). Stenosis progression was found in 42 (53%) of the 79 renal artery lesions, with 7 progressing to total occlusion.



A,B

FIGURE 52-3. A: Mild stenosis (less than 50%) due to atherosclerotic disease of the right main renal artery (**B**) progressed to high-grade (75% to 99%) stenosis, as shown on a later arteriogram. (From: Schreiber MJ, Pohl MA, Novick AC. The natural history of atherosclerotic and fibrous renal artery disease. *Urol Clin North Am* 1984;11:383, with permission.)

Some centers use renal duplex ultrasonography and color-coded duplex ultrasonography to noninvasively diagnose, grade, and monitor the progress of atherosclerotic renal artery lesions (47–53) The overall sensitivity and specificity of renal artery duplex ultrasonography (compared with renal arteriography—the gold standard) was 98% in one study (51). Another recent study reported a 92% sensitivity in identifying renal artery stenosis of more than 75%, with none of the high-grade stenoses missing detection (52). Prospective studies of progressive renal artery stenosis that used duplex ultrasonography also suggested that atherosclerotic renal artery disease is a progressive disorder; one report suggested a cumulative incidence of progression to total occlusion of 5% ± 3% at 12 months and 11% ± 6% at 24 months (53). A more recent report from the same institution, which provided longer follow-up on two-and-a-half times as many kidneys, prospectively monitored a total of 295 kidneys in 170 patients with atherosclerotic renal artery stenosis for a mean follow-up period of 33 months (54). Overall, the cumulative incidence of atherosclerotic renal artery stenosis progression was 35% at 3 years and 51% at 5 years. The 3-year cumulative incidence of renal artery disease progression stratified by baseline disease classification was 18%, 28%, and 49% for renal arteries initially classified as normal, less than 60% stenosis, and greater than or equal to 60% stenosis, respectively. The cumulative incidence of progression of lesions with less than 60% reduction in lumen diameter progressing to more than 60% reduction in lumen diameter was 30% at 1 year, 44% at 2 years, and 48% at 3 years. Progression to total occlusion occurred only in nine arteries all of which had a baseline reduction in lumen diameter of greater than 60%. The cumulative incidence of progression to total occlusion in patients with baseline stenosis



A B

FIGURE 52-4. A: Aortogram taken in 1974 demonstrates two normal right renal arteries and minimal atherosclerotic irregularity of the left main renal artery. **B:** Repeat aortogram taken in 1978 shows progression to moderate stenosis of the upper right renal artery and progression to total occlusion of the left main renal artery. (From: Pohl MA, Novick AC. Natural history of atherosclerotic and fibrous renal artery disease: clinical implications. *Am J Kidney Dis* 1985;5:A120, with permission.)

of 60% or more was 4% at 1 year, 4% at 2 years, and 7% at 3 years. Factors significantly associated with the risk of renal artery disease progression during the time of monitoring included: systolic BP greater than or equal to 160 mm Hg, diabetes mellitus, and high-grade (more than 60%) stenosis in either the ipsilateral or contralateral renal artery. Blood pressure control and serum creatinine did not predict progression. Further observations by these authors demonstrated that kidneys distal to high-grade renal artery stenosis and subjected to severe ischemia and poorly controlled hypertension are at increased risk of renal atrophy, and that loss of renal mass correlates with elevations in the serum creatinine concentration (55).

Cholesterol emboli and atheroembolic renal disease frequently occurs in patients with diffuse atherosclerosis. Renal cholesterol embolization may occur spontaneously, after abdominal aortography or selective renal angiography, after aortic surgery or following trauma (56–60). Chapter 70 provides a detailed discussion of atheroembolism. Clinically, cholesterol emboli through the renal arterial circulation are associated with deteriorating renal function, sudden onset of hypertension, or an exacerbation of hypertension (58,61,62). Malignant hypertension resulting from atheromatous embolization has also been described (63). The pathogenesis of hypertension in patients with renal cholesterol embolism has not been investigated extensively, but might be the result of activation of the renin-angiotensin system, producing a vasoconstrictor-type of hypertension. Bilateral renal vein renin sampling in one patient with malignant hypertension and papilledema demonstrated evidence of unilateral hypersecretion of renin; at autopsy, the ipsilateral renal artery was occluded by atheromatous debris and evidence of cholesterol embolism was found in the high renin-producing kidney. The authors speculate that cholesterol embolism causes increased renin release from the involved kidney, in turn causing renin-dependent hypertension (63).

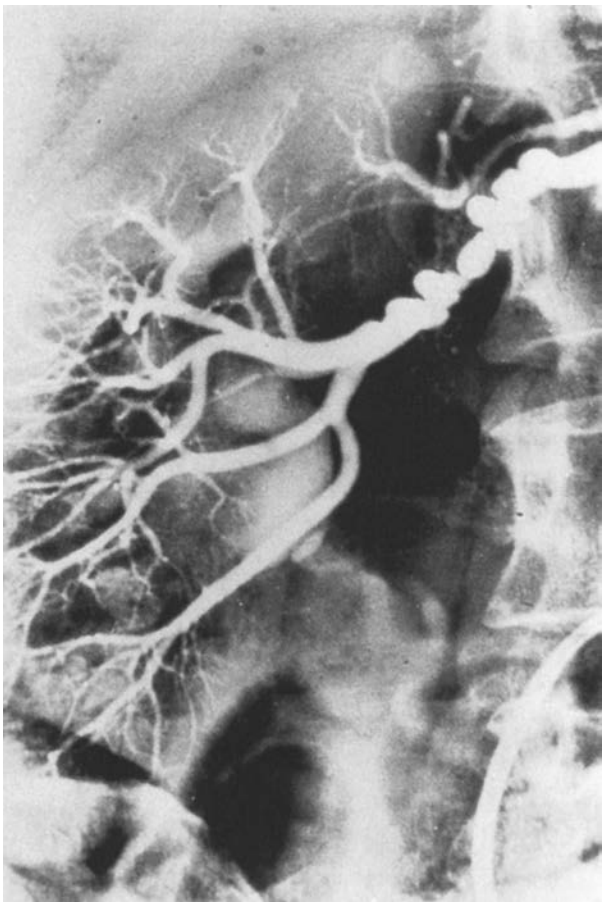


FIGURE 52-5. Right renal arteriogram demonstrating the weblike stenoses with interposed segments of dilation (large beads) typical of medial fibroplasia ("string-of-beads" lesion).

FIBROUS RENAL ARTERY DISEASE (FIBROMUSCULAR DYSPLASIA)

Fibrous diseases of the renal artery are the most common cause of renovascular hypertension in persons younger than 40 years (Table 52-2). These abnormalities of the renal artery are a heterogeneous group of nonatherosclerotic, noninflammatory vascular occlusive diseases. Fibrous dysplasias account for 20% to 30% of renal artery lesions producing renovascular hypertension, and are the most frequent cause of renovascular hypertension in children, accounting for 40% to 80% of cases. Fibrous renal artery disease producing renovascular hypertension is much more common in women than men and in white patients rather than African American or Asian patients with hypertension. It is only rarely observed in patients older than 60 years (64).

The etiology of the fibrous dysplasias is not completely understood. Genetic factors and cigarette smoking have been implicated as etiologic factors (19,65). The preponderance of fibrous renal artery disease in women suggests hormonal influences, but no proof is available and no animal models exist. An association between ptotic kidneys and fibrous dysplasia suggests that arterial stretching may be a factor in the development of fibrous renal artery disease (66,67). Mural ischemia resulting from functional defects in the vasa vasorum supplying the arterial wall has been implicated (68,69).

There are four different types of fibrous renal artery diseases: medial fibroplasia, perimedial fibroplasia, medial hyperplasia, and intimal fibroplasia (Table 52-2). Although the true incidence rates for these four types have not been clearly defined, medial fibroplasia is most common, accounting for approximately 75% of fibrous renal artery lesions. Medial fibroplasia affects the distal half of the main renal artery, frequently extends into the major branches, is often bilateral, and angiographically gives the appearance of multiple aneurysms (a "string of beads") wherein the diameter of the aneurysms is wider than the apparently unaffected portion of the main renal artery (Fig. 52-5). Most cases of medial fibroplasia are diagnosed in women between the ages of 30 and 50 years. Although medial fibroplasia progresses to higher degrees of stenosis in about one-third of patients, complete arterial occlusion and ischemic atrophy of the kidney ipsilateral to the renal artery stenosis are rare. The stenotic lesions in medial fibroplasia are secondary to thickened fibromuscular ridges replacing the normal structure of the intima and media of the artery. These areas alternate with thinned areas that do not have an internal elastic membrane, thereby becoming aneurysmal.

Perimedial fibroplasia (subadventitial fibroplasia) accounts for approximately 15% of fibrous renal artery lesions. This lesion also occurs predominantly in women, typically between the ages of 15 and 30 years. Angiographically, it is often characterized by a small string-of-beads appearance, with the beads being of similar or smaller diameter compared to the diameter of the apparently unaffected portion of the renal artery (Fig. 52-6). This lesion typically affects the distal half of the main renal artery, is frequently bilateral and highly stenotic, and may progress to total arterial occlusion. Collateral blood vessels and renal atrophy on the involved side are commonly observed (19,42,70,71).

Medial hyperplasia and intimal fibroplasia account for only 5% to 10% of fibrous renal artery lesions. Intimal fibroplasia

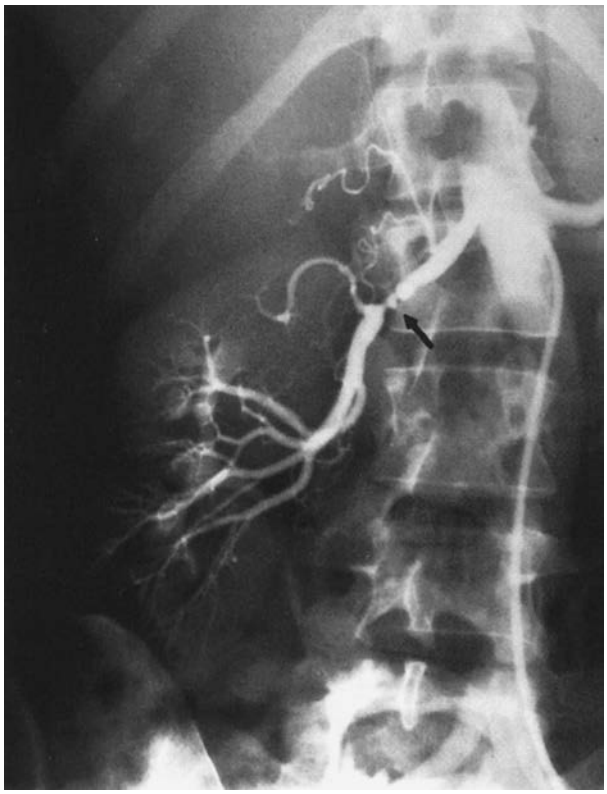


FIGURE 52-6. Selective right renal arteriogram shows tight stenosis in the midportion of the renal artery, with a small string-of-beads appearance typical of perimedial fibroplasia. Note that the beads are of a smaller diameter than the unaffected proximal portion of the renal artery. Note also the presence of collateral vessels often seen with perimedial fibroplasia. (From: Pohl MA, Novick AC. Natural history of atherosclerotic and fibrous renal artery disease: clinical implications. *Am J Kidney Dis* 1985;5:A120, with permission.)

FIGURE 52-7. Selective right renal arteriogram shows smooth tight stenosis of the distal right renal artery from intimal fibroplasia.

occurs primarily in children and teenagers and angiographically appears as a localized, highly stenotic, smooth lesion with poststenotic dilation. It may occur in the proximal portion of the renal artery and when it does, it can resemble atheroma. Intimal fibroplasia is progressive, and is occasionally associated with dissection or renal infarction, and renal atrophy (Fig. 52-7). Medial hyperplasia, also rare, is found predominantly in teenagers, and angiographically also appears as a smooth, linear stenosis, sometimes appearing as though a ligature were tied around the renal artery. There is considerable difficulty in distinguishing between intimal fibroplasia and medial hyperplasia by angiography, and in the literature, these two types of fibrous artery disease may be grouped together (70,71).

As emphasized by Goncharenko and associates, who reviewed the progression of fibrous renal artery disease in 42 patients studied by serial angiography, perimedial fibroplasia, medial hyperplasia, and intimal fibroplasia produce high-grade renal artery stenosis that eventually leads to renal atrophy, renal infarction, and loss of renal parenchyma ipsilateral to the stenosis (70). Because these disorders are generally encountered in children, adolescents, and young adults with significant hypertension, who would, without intervention, require lifelong antihypertensive therapy, surgical renal revascularization is recommended for relief of hypertension and preservation of renal function.

On the other hand, the most common form of fibrous renal artery disease, medial fibroplasia, rarely progresses to total arterial occlusion. Further, progressive arterial stenosis from medial fibroplasia does not adversely affect total renal function as measured by serum creatinine concentrations, nor is it associated with a significant reduction in kidney size (44). Despite the progressive nature of medial fibroplasia, the available data indicate that the risk of losing renal function over time due to progressive arterial occlusion is small. Accordingly, surgical renal revascularization or PTRA need not be considered for the goal of restoring or preserving renal function in patients with medial fibroplasia. In most centers, PTRA is the initial interventive maneuver for patients with medial fibroplasia and hypertension refractory to drug therapy or for hypertensive patients wishing to avoid antihypertensive drug therapy. Because medial fibroplasia and perimedial fibroplasia can produce a "beaded" appearance on angiography, differentiating between these two forms of fibrous renal artery disease may be difficult. Accordingly, prudence dictates careful monitoring of serum creatinine levels, kidney size, and BP control for patients thought to have medial fibroplasia.

Acute and chronic renal artery occlusion secondary to renal artery emboli, aortic or renal artery dissection, neurofibromatosis, arterial trauma, arteriovenous malformations, polyarteritis nodosa, and Takayasu's arteritis are discussed in conjunction with corresponding angiograms in Chapter 14. Rupture of a renal artery aneurysm is a rare but serious complication that requires urgent surgery. Novicki et al. reported 200 cases of perirenal hematoma, 20% due to renal artery aneurysm rupture (72). Stanley et al. reported a series in which the incidence of rupture of renal artery aneurysm was 5.6% (73). There is disagreement about the natural history and factors that predispose to rupture. Hageman and associates followed 25 patients with renal artery aneurysms less than 2 cm in diameter for periods of 1 to 17 years without demonstration of rupture or increase of aneurysm size (74). Rupture

of a renal artery aneurysm tends to occur in noncalcified or incompletely calcified aneurysms larger than 1.5 to 2.0 cm in diameter (75–78). Pregnancy also predisposes to rupture of renal artery aneurysms (77).

Takayasu's arteritis, a nonspecific inflammatory disease affecting large arteries such as the aorta and its main branches, including the renal arteries, mainly affects young women. It can cause discrete stenosis of the aorta and its main branches, including the common carotid, subclavian, and renal arteries. Renal artery involvement is not uncommon in Asian and African patients and is a major cause of renovascular hypertension in these countries. Koide reports renovascular hypertension in 278 (18.8%) of 1,475 patients in a Japanese nationwide survey of Takayasu's arteritis (79). Takayasu's arteritis is the most common cause of renovascular hypertension in India and China, accounting for as many as 60% of all cases of renovascular hypertension (80–82).

THE ATROPHIC KIDNEY IN RENOVASCULAR HYPERTENSION

A variety of imaging techniques (see Chapters 12–14) are employed in clinical medicine to evaluate diseases of the kidney and urinary tract and pathologic processes in the abdominal and pelvic cavities. Not infrequently, an atrophic kidney or a substantial discrepancy in renal size is observed. Although the relationship between an atrophic kidney and its possible role in hypertension has long been appreciated, in the hypertensive patient with unilateral renal atrophy, the renal atrophy is not necessarily due to main renal artery stenosis.

In a postmortem study of 84 cases of unilateral atrophy reported by Baggenstoss and Barker, ischemic atrophy from occlusive disease of the renal artery was not mentioned as a cause of renal atrophy (83). In 1944, Sensenbach reviewed the literature and found that 48 of 75 (64%) diseased (but not necessarily atrophic) kidneys removed for relief of hypertension showed chronic pyelonephritis (84). These investigators noted only one instance each of renal infarction and occlusion of the renal artery. Subsequent reviews by Smith (1948) (85), Sabin (1948) (86), and Barker (1951) (87) attributed most cases of an atrophic kidney, wherein the atrophic kidney was presumably related to hypertension, as due to either unilateral pyelonephritis, post obstructive atrophy, or congenital hypoplasia.

With the advent of renal angiography in the late 1950s and early 1960s for the evaluation of patients with hypertension, renal artery occlusive disease became more evident as a cause of the atrophic kidney. In 1965, Gifford and associates found that in 53 (71%) of 75 patients with an atrophic kidney, the renal atrophy was due to stenosing atherosclerotic renal artery disease (88). In 22 (29%) of these 75 patients, the atrophic kidney was associated with a patent renal artery and the renal atrophy was thought to be secondary to pyelonephritis or congenital hypoplasia. Of special interest in that report was the observation that of the 53 patients with atherosclerosis of the renal artery supplying the atrophic kidney, 17 (32%) had unsuspected atherosclerotic disease involving the renal artery of the contralateral normal-sized kidney. Lawrie and associates reviewed 40 patients with renal atrophy due to total arterial occlusion and observed contralateral renal artery stenosis in 31 patients (78%) (89). An example (Case 1) is included in the section Ischemic Renal Disease (Ischemic Nephropathy) later in this chapter.

In children, the observation of hypertension in conjunction with an atrophic kidney raises the possibilities of congenital hypoplasia, segmental hypoplasia of the kidney (Ask-Upmark kidney) (90–93), postobstructive atrophy (88), chronic pyelonephritis, and fibrous renal artery disease as the basis for the small kidney. With advancing age, and certainly for patients older than 45 to 50 years, particularly in the setting of generalized atherosclerosis, atherosclerotic renal artery stenosis is the leading cause of an atrophic kidney.

THE RENIN-ANGIOTENSIN SYSTEM IN RENOVASCULAR HYPERTENSION

Activation of the renin-angiotensin system by proteolysis of circulating angiotensinogen is summarized in Figure 52-8. Renin, an enzyme produced and released by the juxtaglomerular cells of the kidney, hydrolyzes the circulating peptide angiotensinogen or renin substrate to form angiotensin I. Angiotensin I, a decapeptide, reaches an active form after two additional amino acids are cleaved by a carboxypeptidase, angiotensin-converting enzyme (ACE), yielding angiotensin II. Many details concerning the renin-angiotensin cascade have been established by pharmacologic blockade of the action or formation of angiotensin II (94–97). These sites of intervention are illustrated on the right side of Figure 52-8. A non-ACE pathway is an alternative route for the generation of angiotensin II (98–100).

The renin-angiotensin-aldosterone system (RAAS) has a central role in renovascular hypertension(101). Angiotensin II is among the most potent, naturally occurring vasoconstrictors interacting with specific vascular receptors in nearly all vascular beds. At high levels, angiotensin II produces elevations in vascular resistance, thereby raising BP directly. Another major role of angiotensin II is the regulation of aldosterone production by the adrenal glomerulosa. Although not the only factor stimulating aldosterone production, angiotensin II is a potent stimulus for the secretion of aldosterone, thereby producing sodium and volume retention and "secondary aldosteronism," clinically manifested by hypokalemia. A more detailed discussion of the biochemical pathways that generate angiotensin II, actions of angiotensin at specific locations in the kidney, actions of angiotensin II on the central nervous system and on sympathetic nerve modulation, and interactions of angiotensin II with other hormones (e.g., bradykinin, prostaglandins, vasopressin) is beyond the scope of this chapter, but is explored more thoroughly in Chapters 2 and 9.

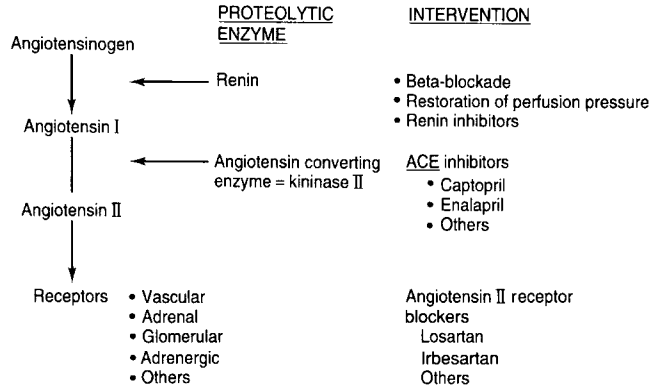


FIGURE 52-8. Progressive activation of the renin-angiotensin system initiated by proteolysis of circulating angiotensinogen. Sites of pharmacologic intervention are illustrated on the right side of the diagram. (Adapted from: Textor SC. Pathophysiology of renovascular hypertension. *Urol Clin North Am* 1984;11:373, with permission.)

PATHOPHYSIOLOGY OF RENOVASCULAR HYPERTENSION

Current understanding of the pathophysiology of renovascular hypertension has been derived largely from animal studies. The pioneering experiments of Goldblatt et al. in 1934 demonstrated that persistent hypertension could be produced in dogs by renal artery clamping (1). They proposed that a circulating substance might be responsible for the hypertension, and this concept was later confirmed by Page and Helmer (102), Pickering (103), and Braun-Menendez et al. (104). Subsequently, "Goldblatt hypertension" was extensively studied in animals, and it has been assumed that many of the experimental observations apply to human renovascular hypertension.

Critical Perfusion Pressure

The generation of elevated BP by constriction of a renal artery in experimental animals and in humans requires sufficient compromise of the renal arterial lumen to initiate the pathophysiologic sequence of events resulting in an increase in the BP. Studies of arterial lesions in several vascular beds indicated that compromise of the arterial lumen must reach an advanced level, exceeding 80% to 85% cross-sectional obstruction, before hemodynamic changes are evident (105,106). Renal blood flow and the pressure gradient across a graded stenosis of the artery supplying a dog's kidney are not changed until the degree of stenosis exceeds 70% to 80% (107). Renin secretion increases as renal perfusion pressure falls, and becomes maximal at the limit of autoregulation (101,108). These experimental studies demonstrated that a substantial reduction in both lumen diameter and cross-sectional area is necessary before any measurable decrease in arterial flow or pressure occurs (Fig. 52-9). In experimental animals, the Goldblatt clamp is manually tightened to produce a presumable reduction in lumen diameter of at least 80% to 85% and to induce hemodynamically significant renal artery constriction (109). In humans, the ability of currently available imaging techniques to visualize hemodynamically critical renal artery stenosis (i.e., greater than 75% to 80%) may be more problematic; renal artery stenosis is often present, but not sufficiently tight to be hemodynamically significant. It is notoriously difficult to establish the true dimensions of a stenotic lesion from two-dimensional arteriograms, but poststenotic dilation suggests hemodynamically significant main renal artery stenosis (see Chapter 14). Simon has concluded that an 80% stenosis of a renal artery is required to stimulate renin secretion and lead to renovascular hypertension in humans (110).

The feature common to all experimental models of renovascular hypertension and to clinical examples of the syndrome of renovascular hypertension is a reduction in renal perfusion pressure. Whether or not this reduced renal perfusion pressure predictably causes intrarenal ischemia is unclear. Although the clinical conditions producing human renovascular hypertension vary widely, these conditions all share the common denominator of rendering renal perfusion sufficiently low to activate the renin-angiotensin system. In the case of a main renal artery stenosis, it is important to recognize that detection of hemodynamic effects related to renal artery stenosis implies far-advanced (greater than 75% to 80%) reduction in lumen diameter. High-grade renal artery occlusion may result in loss of poststenotic perfusion and possibly, eventual total arterial occlusion. The concomitant renal hypotension injures the kidney and causes progressive loss of renal parenchyma (111). These concepts are explored in greater detail in Chapters 2 and 39.

Experimental Models

Although there are differences among species (rat, dog, rabbit) in experimental renovascular hypertension, two basic models of Goldblatt hypertension are recognized: the two-kidney, one-clip (2K-1C) model (in which one renal artery is constricted and the contralateral renal artery and kidney are left intact) and the one-kidney, one-clip (1K-1C) model (in which one renal

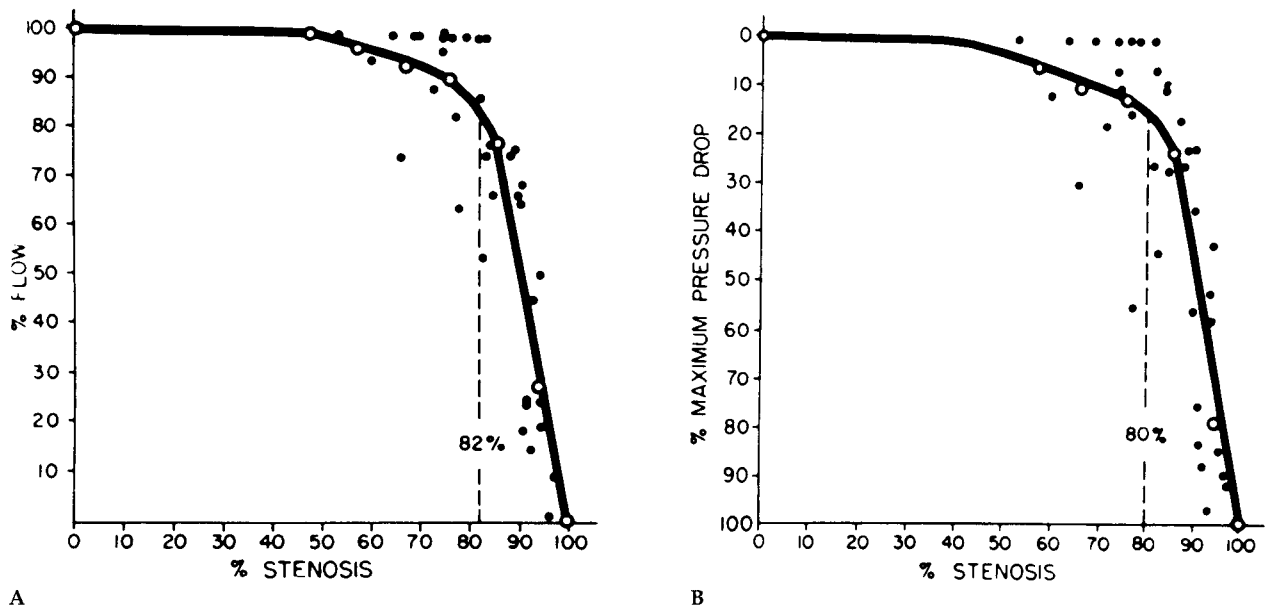


FIGURE 52-9. A: Changes in arterial flow and (**B**) pressure across a stenotic lesion during variation in the degree of stenosis in the dog. These studies demonstrate that blood flow and pressure gradients induced by vascular stenosis are negligible until the lumen narrows to more than 75% to 80% of total occlusion. Further stenosis produced abrupt loss in blood flow. (Adapted from: May AG, Van de Berg L, DeWeese JA, et al. Critical arterial stenosis. *Surgery* 1963;54:250, with permission.)

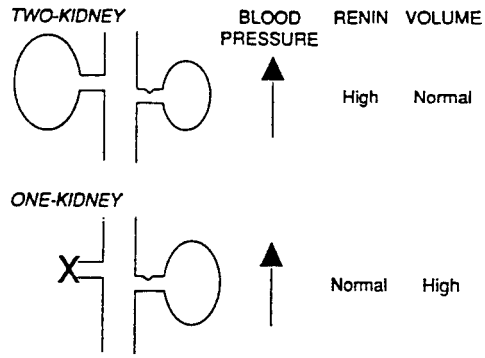


FIGURE 52-10. Two animal models of renovascular hypertension. In the two-kidney model the hypertension is mainly renin-dependent; in the one-kidney model, hypertension is mainly volume-dependent.

artery is constricted and the contralateral kidney is removed). These two experimental models of renovascular hypertension are diagrammed in Figure 52-10.

In 2K-1C ("two-kidney") Goldblatt hypertension, the renal artery on one side is partially occluded (clipped) and the renal artery to the contralateral kidney remains patent. Initially, elevated arterial pressure is associated with increased renal and plasma renin levels and normal plasma and extracellular fluid volumes. This form of experimental renovascular hypertension is mainly renin- or angiotensin II-dependent (112). In 1K-1C ("one-kidney") Goldblatt hypertension, the renal artery on one side is partially occluded and the contralateral kidney is removed. In this model, the elevated BP is associated with volume expansion and circulating renin levels which become normal over time. A renin-initiated vasoconstrictor state is implicated in the two-kidney model (112,113). The volume component is not a major initial mechanism because the intact contralateral kidney undergoes a pressure natriuresis (114,115). In the one-kidney model, pressure-induced natriuresis is absent owing to the removal of the contralateral kidney (96,116).

Human unilateral renovascular hypertension most closely resembles the 2K-1C model. Clinical counterparts of experimental two-kidney renovascular hypertension are listed in Table 52-3. The 1K-1C model may be comparable to human bilateral renal artery stenosis, renal artery stenosis in a solitary functioning kidney, hypertension in a transplanted kidney, and renovascular disease with coexisting bilateral renal parenchymal disease (Table 52-4). Nevertheless, most patients with apparently significant bilateral renal artery stenosis, or stenosis of a transplanted kidney have elevated levels of PRA (117,118).

TABLE 52-3

CLINICAL COUNTERPARTS OF EXPERIMENTAL 2K-1C ("TWO-KIDNEY") RENOVASCULAR HYPERTENSION (IMPLIES CONTRALATERAL [NONAFFECTED] KIDNEY IS PRESENT)

Unilateral atherosclerotic renal artery disease
Unilateral fibrous renal artery disease
Renal artery aneurysm, arterial embolus
Arteriovenous fistula (congenital or traumatic)
Segmental renal arterial occlusion
Pheochromocytoma compressing renal artery
Unilateral ureteral obstruction, atrophic pyelonephritis, or hypoplastic kidney
Unilateral metastatic tumor, perirenal hematoma, or subcapsular hematoma (compressing renal parenchyma)[a]

[a] Clinical analogs of experimental Page kidney.

TABLE 52-4

CLINICAL COUNTERPARTS OF EXPERIMENTAL 1K-1C ("ONE-KIDNEY") RENOVASCULAR HYPERTENSION (IMPLIES TOTAL RENAL MASS HYPOPERFUSED)

Stenosis to a solitary functioning kidney
Bilateral renal arterial stenosis
Aortic coarctation
Vasculitis (polyarteritis nodosa and Takayasu's arteritis)
Unilateral renal arterial disease (atherosclerotic or fibrous) with coexisting renal parenchymal disease
Atheroembolic renal disease

Most discussions of renovascular hypertension focus on two-kidney hypertension. In Figure 52-11 are schematically represented the classic model of two-kidney Goldblatt hypertension. In the presence of hemodynamically sufficient unilateral renal artery stenosis, the kidney distal to the stenosis ("stenotic kidney") is rendered ischemic, consequent to impaired renal perfusion, activating the renin-angiotensin system, generating high levels of angiotensin II, and producing a "vasoconstrictor" type of hypertension. Numerous studies established the causal relationship between angiotensin II–mediated vasoconstriction and hypertension in the early phase of experimental renovascular hypertension (94,112,113,115,119). The high levels of angiotensin II stimulate the adrenal cortex to elaborate large amounts of aldosterone (secondary aldosteronism), promoting sodium retention by the stenotic kidney. Moreover, angiotensin directly enhances renal NaCl and fluid reabsorption (120). Both the direct pressor effects of angiotensin II and sodium retention lead to restoration of renal perfusion pressure at the expense of producing systemic hypertension (119). Mild or moderate degrees of renal artery stenosis (less than 75% to 80%) do not initiate this sequence of events. The human correlate of experimental two-kidney hypertension assumes a similar sequence of events, with renal artery stenosis producing a hemodynamically significant reduction in perfusion, and activating the renin-angiotensin system. Secondary aldosteronism ensues, and hypokalemia is frequently observed.

The model of 2K-1C Goldblatt hypertension implies that the "contralateral" (unaffected) kidney is present and that its renal artery is patent or only minimally narrowed. As indicated in Figure 52-11, the contralateral kidney exhibits suppressed renin secretion and a pressure natriuresis. The suppressed renin from the contralateral kidney is explained by the exposure of this kidney to systemic arterial pressures that are higher than normal and higher than the pressure reaching the stenotic kidney. Accordingly, there should be no stimulus for renin release by the contralateral kidney, and renin content should be suppressed on this side. Renal venous renin levels are typically the same as arterial levels, indicating no net renin secretion from the contralateral kidney (121,122). In experimental studies, the contralateral kidney is devoid of renin content and juxtaglomerular cell granulation (123,124). Further, the contralateral kidney, if truly functioning normally and devoid of renal parenchymal damage, should undergo a "pressure natriuresis," excreting salt and volume at levels higher than normal (125). Numerous studies confirmed that excretion of sodium predominates in the contralateral kidney, but it has been difficult to establish in humans whether this pressure natriuresis is caused purely by exaggerated natriuresis of the contralateral kidney or by extreme sodium avidity by the stenotic kidney, or by both (126,127).

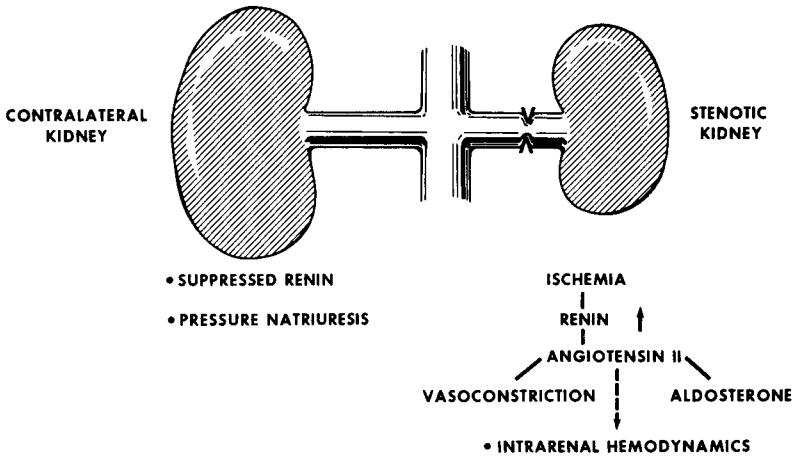


FIGURE 52-11. Schematic representation of two-kidney renovascular hypertension. The presence of a unilateral, hemodynamically significant renal arterial narrowing initiates a sequence of events within the affected kidney ("stenotic kidney") based on activation of the renin-angiotensin system. Initially vasoconstriction and sodium retention result, leading to the systemic elevation of blood pressure and suppression of renin and enhanced excretion of sodium by the "contralateral kidney."

Therefore, the hemodynamic, biochemical, and functional observations in two-kidney Goldblatt hypertension differ between the stenotic and contralateral kidneys. The kidneys effectively work against one another; the contralateral kidney excretes sodium and water continuously and thereby prevents the systemic arterial pressure from reaching levels sufficient to restore renal perfusion to the stenotic kidney adequate to suppress the release of renin (128). Based on this pathophysiology, two-kidney renovascular hypertension should be characterized by the following: (a) unilateral release of renin and contralateral suppression; (b) sodium avidity by the stenotic kidney and continuous excretion of sodium by the unaffected, contralateral kidney; (c) euvolemia or relative intravascular volume depletion, depending on the balance between sodium avidity in the stenotic kidney and the pressure natriuresis in the contralateral kidney; and (d) BP dependent on circulating angiotensin II (and marked decreases in BP with administration of angiotensin II antagonists or ACE inhibitors). These observations have been largely confirmed in humans, although not entirely (129–133).

A similar sequence of events probably produces elevated arterial pressure in one-kidney renovascular hypertension. The critical difference between one-kidney and two-kidney renovascular hypertension is that in one-kidney renovascular hypertension there is no contralateral kidney to offset the systemic BP elevation and sodium retention by the kidney distal to the renal artery stenosis. There is no contralateral kidney to undergo a pressure natriuresis. Renal perfusion pressures are thus able to reach levels sufficient to suppress renin to "normal" levels although these can be considered markedly elevated relative to the high BP. The elevated arterial pressure is sustained by sodium and volume excess and inappropriate, ongoing secretion of renin (113,134,135). BP elevation is less dependent on circulating angiotensin II (94,112,113). Under conditions of sodium depletion, however, the pressor role of angiotensin II may be demonstrated (136). The normal renin and angiotensin II levels in one-kidney renovascular hypertension might be viewed as being inappropriately normal for the level of BP elevation, arguing for a vasoconstrictor contribution of angiotensin II in one-kidney hypertension.

Evolution of Renovascular Hypertension

The notions that 2K-1C renovascular hypertension is renin-dependent and that 1K-1C renovascular hypertension is volume-dependent are oversimplifications. Considerable data in animals (mainly rats and dogs) indicate that the pathogenetic processes producing renovascular hypertension do not remain static. Operationally, the pathogenesis of both models of Goldblatt hypertension can be viewed as evolving through an acute phase (phase I), a transition phase (phase II), and a chronic phase (phase III) (114,116,137). In both the two-kidney and one-kidney models of experimental renovascular hypertension, induction of renal hypoperfusion produced by constriction of a renal artery produces prompt elevation of the BP in concert with activation of the renin-angiotensin system. Administration of ACE inhibitors or the angiotensin II receptor blocker saralasin prevents this rise in BP, indicating that this initial elevation of arterial pressure is renin- or angiotensin II-dependent (138,139). Cervenka demonstrated that mice with genetic knockout of the AT1 receptor failed to develop renovascular hypertension with the renal artery clipped (139). Removal of the renal artery clip or unilateral nephrectomy of the stenotic kidney promptly normalizes the BP in this acute phase of 2K-1C experimental renovascular hypertension (94,96,113,114,116,119).

Although one presumes that excess renin is released from the stenotic kidney, peripheral plasma concentrations of renin are not always increased (140,141). Removal of the stenotic kidney does not always reduce the BP (119,131,142,143). Further, widespread clinical experience supports the observation that surgical renal revascularization to the stenotic kidney or balloon dilation of the renal artery ipsilateral to the stenotic kidney often fails to lower the BP, particularly in patients with atherosclerotic renal artery stenosis (131,144–146).

These observations may be reconciled by separating two-kidney renovascular hypertension into sequential phases as depicted in Figure 52-12 (116). In phase I, renal ischemia and activation of the renin-angiotensin system are of fundamental importance, and the BP elevation is renin-dependent. Acute administration of angiotensin II antagonists or ACE inhibitors, removal of the renal artery stenosis (i.e., removal of the clip in the experimental animal), or removal of the stenotic kidney will promptly normalize the BP. In the absence of these maneuvers, a transition phase (phase II) subsequently develops, bridging the acute (phase I) and chronic (phase III) phases of experimental renovascular hypertension. This transition phase variably lasts from a few days to several weeks, depending on the experimental model and animal species. During this transition phase, plasma renin levels progressively fall, but the BP remains elevated. Salt and water retention are observed as a consequence of the effects of hypoperfusion of the stenotic kidney, augmented proximal renal tubular reabsorption of sodium and water, and secondary aldosteronism (137,147). In addition, the high levels of angiotensin II stimulate thirst, further contributing to an expansion of the extracellular fluid volume. The expanded extracellular fluid volume results in a progressive

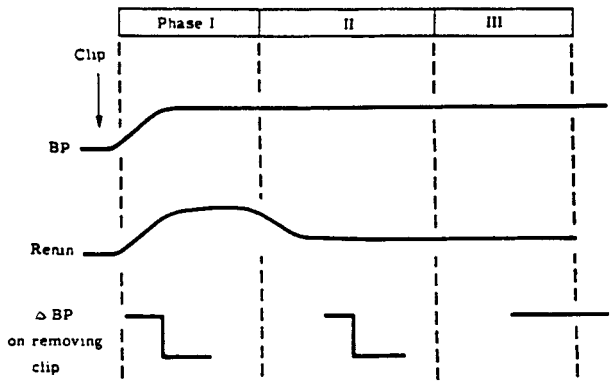


FIGURE 52-12. Sequential phases in two-kidney, one-clip (two-kidney) experimental renovascular hypertension and the effect on blood pressure and renin secretion. After the clip is put on one main renal artery with the contralateral kidney left untouched, blood pressure and renin secretion increase. In phase II, the blood pressure remains high, but renin secretion falls. In phase III, the blood pressure remains elevated, despite removal of the clip, presumably reflecting vascular damage in the contralateral kidney. (From: Brown JJ, Davies DL, Morton JJ, et al. Mechanism of renal hypertension. *Lancet* 1976;1:1219, with permission.)

suppression of peripheral plasma renin activity (PRA). During this transition phase, the hypertension is still responsive to removal of the unilateral renal artery stenosis, to angiotensin II blockade, or to unilateral nephrectomy, although these maneuvers do not normalize the BP as promptly and consistently as in the acute phase (137,148). These changes may depend on recruitment of additional mechanisms of angiotensin II action, including the generation of reactive oxygen species (149,150), quenching of nitric oxide (151), and generation of endothelium-derived substances such as endothelin (152,153) and thromboxanes (154,155). Some of these mechanisms depend upon slowly developing effects of angiotensin II at levels that do not reverse with direct angiotensin II blockade (156).

After several days or weeks, a chronic phase (phase III) evolves, wherein removal of the stenosis by unclipping the renal artery in the experimental animal or nephrectomy of the stenotic kidney fails to reduce the BP (116). The mechanism maintaining elevated arterial pressure, that is, the failure of "unclipping" to lower the BP in this chronic phase of 2K-1C hypertension, is almost certainly multifactorial but is in large part due to widespread arteriolar damage in the contralateral kidney consequent to elevated systemic pressure and the initial high levels of angiotensin II. In this chronic phase of 2K-1C renovascular hypertension, extracellular fluid volume expansion and systemic vasoconstriction are the main pathophysiologic abnormalities (112), The BP remains elevated even though the PRA has returned to a normal level. The pressure natriuresis of the contralateral kidney blunts the extracellular fluid volume expansion initially generated by the stenotic kidney (Fig. 52-11), but as the contralateral kidney suffers vascular damage from prolonged exposure to the increased BP, its excretory function diminishes and extracellular fluid volume expansion persists. In phase III of 2K-1C hypertension, acute blockade of the renin-angiotensin system fails to lower the BP. Sodium depletion may ameliorate the hypertension but does not normalize it (112,157).

Systemic vasoconstriction also contributes to the elevated BP in phase III of two-kidney hypertension, despite normalization of renin levels. Conceptually, the normal levels of renin and angiotensin II may be viewed as inappropriately high for the level of arterial pressure. In rat studies 9 to 12 months into 2K,1C hypertension, although there is no immediate response to an angiotensin receptor blocker (ARB), the BP gradually falls to near normal values over 3 days of ARB infusion (155). This suggests that angiotensin II remains a critical determinant of the hypertension, but that this is no longer due to direct effects of circulating angiotensin II. Interestingly, blockade of thromboxane-prostanoid (TP) receptors over 3 days also reduces BP in this model, suggesting a role for vasoconstrictor prostaglandins. Whether the peripheral renin level is elevated or not, this phase of renovascular hypertension is associated with increased sensitivity to angiotensin II (157), increased levels of vasopressin (137,158,159), and increased peripheral and central sympathetic nervous system activity (127,160–164). Experimental studies demonstrated that angiotensin II acts on both the central and the peripheral nervous system to activate sympathetic outflow. Plasma norepinephrine concentration and norepinephrine content in the hypothalamus are increased in 1K-1C hypertensive rats, and in the same model, central sympatholytic interventions such as creation of lesions in the posterior hypothalamus prevent or attenuate the hypertension. Further, renal denervation lowers BP, plasma norepinephrine levels, and hypothalamic norepinephrine content in the absence of changes in sodium or water intake, sodium excretion, PRA, or creatinine clearance (164). A recent study demonstrated elevated sympathetic nerve activity by means of intraneural recordings of muscle sympathetic nerve activity in patients with renovascular hypertension (165). Administration of clonidine lowers BP, PRA, and urinary norepinephrine excretion, providing further support for a role of central nervous system pressor mechanisms in the chronic phase of experimental renovascular hypertension. Hypertension-induced structural changes in the vascular wall may also contribute to the maintenance of elevated pressure in this chronic phase of 2K-1C renovascular hypertension. These changes may develop via mechanical, neural, humoral, or pressure-related factors (137,147). This may explain why it takes some days for an angiotensin II receptor blocker to reduce BP in chronic renovascular hypertension in rats (155).

In human 2K-1C renovascular hypertension, the duration of hypertension is probably a surrogate for the chronic phase (phase III) of experimental 2K-1C renovascular hypertension. Hypertension, lasting more than 3 to 5 years in patients with unilateral renal artery stenosis, particularly if poorly controlled, has a mediocre cure rate after renal revascularization or nephrectomy (131,144–146). This is illustrated in studies of the outcome of two sets of selected patients having tests indicating functionally important renal artery stenosis. Among 63 patients with atherosclerotic RAS, hypertension was cured by intervention in 21% (166) while it was cured by intervention in 52% with fibromuscular RAS (167). In both series, the strongest factor predicting cure was a short history of hypertension. These observations are particularly true for patients with unilateral atherosclerotic renal artery disease, and may be accounted for by the development over time of unrecognized nephrosclerosis in the contralateral kidney (168–171). As emphasized by Vertes and associates (170), many patients with unilateral atherosclerotic renal artery stenosis have the unequal development of bilateral, obliterative, intrarenal vascular disease. Detection of such pathologic changes in renal biopsy specimens from the contralateral kidney in patients with atherosclerotic renal artery stenosis was consistently associated with failure to achieve BP reduction by either renal revascularization of the stenotic kidney or its removal (171,172). However, the concept that the late stage of renovascular hypertension in humans is strictly analogous to phase III renovascular hypertension in animals may be an oversimplification because nephrectomy of the kidney ipsilateral to the renal artery stenosis usually leads to a significant fall in BP.

Thus, although in the early phase of 2K-1C Goldblatt hypertension the generation of the hypertension appears to be

TABLE 52-5

PATHOGENETIC MECHANISMS IN SEQUENTIAL PHASES OF RENOVASCULAR HYPERTENSION

- Acute phase
  - Renin dependency
- Transition phase
  - Sodium and water retention
  - Angiotensin II effects
    - Aldosterone
    - Intrarenal hemodynamics
    - Tubular function
    - Thirst
  - Progressive suppression of plasma renin activity
  - Changes in the contralateral kidney
    - Blunted pressure natriuresis
    - Impaired autoregulation
- Chronic phase
  - Volume expansion
    - Suppressed plasma renin activity
    - Reciprocation of sodium dependency with renin dependency
  - Systemic vasoconstriction
  - Renin-angiotensin system
    - Increased angiotensin II sensitivity
    - Local vascular renin-angiotensin system
    - Vasopressin
    - Peripheral and central sympathetic activity
    - Quenching of nitric oxide
    - Oxidative stress
    - Thromboxanes
    - Endothelin
    - Impaired sodium transport
    - Total body autoregulation
    - Structural changes in vascular wall
  - Development of nephrosclerosis

predominantly renin-dependent and the hypertension is ameliorated by interference with the renin-angiotensin system, revascularization, or nephrectomy, the maintenance of BP elevation in the chronic phase of experimental renovascular hypertension and in hypertensive patients with long-standing unilateral renal artery stenosis is multifactorial and less clearly defined. The complexities of the pathogenetic mechanisms in sequential phases of renovascular hypertension are summarized in Table 52-5.

Extrapolating these observations to patients, one might presume that the sooner an arterial lesion presumably causing renovascular hypertension is removed, the more likely the chance for relief of the hypertension. Admittedly, this clinical suggestion may be stretching the experimental observations too far. Nevertheless, clinical experience indicates that corrective surgery for unilateral renal artery stenosis as the presumptive cause for hypertension is far more successful in patients with a brief history of hypertension (e.g., less than 5 years) in contrast to patients with hypertension of a longer duration or with coexisting azotemia (131,144–146).

Thus far in this chapter, our discussion of the pathophysiology of renovascular hypertension has focused on the 2K-1C model of renovascular hypertension (Fig. 52-11). The most common clinical counterpart to this type of hypertension is unilateral renal artery stenosis due to atherosclerotic or fibrous renal artery disease. It should be appreciated, however, that several other clinical circumstances (Table 52-3) may be associated with this type of hypertension, including unilateral renal artery aneurysm (75,173,174), congenital and traumatic arteriovenous fistula (175,176), and segmental arterial occlusion. Unilateral renal trauma, with perirenal or subcapsular hematoma formation, and development of a calcified fibrous capsule surrounding the injured kidney causing compression of the renal parenchyma may produce the syndrome of two-kidney renovascular hypertension (177–182), a clinical situation analogous to the experimental Page kidney. Clinical counterparts of 1K-1C experimental renovascular hypertension (Table 52-4), wherein the total renal mass is implicitly underperfused and possibly ischemic, are common in clinical medicine. In polyarteritis nodosa, Takayasu's arteritis (81,183), and atheroembolic renal artery disease (61,63,184) the syndrome of renovascular hypertension is initiated by renal underperfusion, but without main renal artery stenosis. In patients with bilateral renal artery stenosis or renal artery stenosis of a solitary functioning kidney (native or transplanted kidney), multiple physiologic factors contribute to the hypertension; these include systemic vasoconstriction, volume expansion, and probable coexisting intrarenal ischemia due to nephrosclerosis or the vascular changes of chronic rejection.

In summary, the pathogenesis and pathophysiology of human renovascular hypertension are complex, and to a large degree incompletely defined. The relative contribution of various physiologic factors implicated in experimental models is uncertain. These uncertainties apply to human renovascular hypertension as well. Not surprisingly, predicting which patients might have renovascular hypertension is even more difficult. Nonetheless, compelling evidence exists for the important participation of the renin-angiotensin system as well as of volume-renin interrelationships in the pathogenesis of renovascular hypertension. The contribution of other factors and, in particular, a clearer understanding of intrarenal physiology and pathology distal to main renal artery stenosis are areas for future investigation.

Diagnosis of Renal Artery Stenosis and Renovascular Hypertension

There are two reasons to diagnose renal artery stenosis. First, renal artery stenosis in some hypertensive patients, if hemodynamically significant, might be the cause of the hypertension (i.e., renovascular hypertension). Second, renal artery stenosis has the potential to adversely affect renal function. Clinical concern over these two issues "hypertension and renal function" dictates the appropriate choice of diagnostic tests.

A number of epidemiologic observations, clinical features, laboratory tests, and diagnostic maneuvers are associated with the anatomic presence of renal artery stenosis or predict the likelihood that the renal artery stenosis is causing or contributing substantially to hypertension (Tables 52-6 to 52-8). Some clinical features and diagnostic tests (e.g., an atrophic kidney in a hypertensive patient with generalized atherosclerosis or an abnormal-appearing renal artery duplex ultrasound scan [34]) indicate main renal artery stenosis. Auscultation of a

TABLE 52-6

STEPS IN MAKING THE DIAGNOSIS OF RENOVASCULAR HYPERTENSION

1. Demonstration of renal arterial stenosis by angiography
2. Determination of pathophysiologic significance of the stenotic lesion
3. Cure of the hypertension by intervention (i.e., surgical revascularization, percutaneous transluminal angioplasty, or nephrectomy)

TABLE 52-7

DIAGNOSIS OF RENAL ARTERIAL STENOSIS

Clinical clues
Age at onset of hypertension <30 or >55 yr
Abrupt onset of hypertension
Acceleration of previously well-controlled hypertension
Hypertension refractory to an appropriate three-drug regimen
Accelerated retinopathy
Malignant hypertension
Systolic-diastolic abdominal bruit
Flash pulmonary edema
Evidence of generalized atherosclerosis obliterans
Acute renal failure with angiotensin-converting enzyme inhibitor treatment
Diagnostic tests
Duplex ultrasonography
Magnetic resonance angiography
Computerized tomography angiography
Radionuclide renography
Captopril renography
Captopril provocation test (captopril plasma renin activity)
Rapid-sequence intravenous pyelography
Intravenous digital subtraction angiography
Carbon dioxide angiography
Conventional arteriography

systolic/diastolic abdominal bruit in the abdomen of a young white woman not only suggests renal artery stenosis due to fibrous renal artery disease, but also predicts a cure for the hypertension with renal revascularization (185). Similarly, captopril renography is highly predictive of both renal artery stenosis and renovascular hypertension (28,186). Formal renal angiography detects renal artery stenosis but does not differentiate renal artery stenosis from renovascular hypertension. Confounding the interpretation of clinical clues and diagnostic tests suggesting renal artery stenosis and renovascular hypertension is the frequent occurrence of renal artery stenosis in patients with essential hypertension and the fact that the diagnosis of renovascular hypertension is only firmly established, retrospectively, by observing cure or marked amelioration of the hypertension following renal revascularization or nephrectomy. There is abundant literature devoted to clinical features and diagnostic maneuvers predicting that renal artery stenosis is causing the hypertension. On the other hand, clinical clues and diagnostic tests predicting that renal artery stenosis will eventuate in renal insufficiency are lacking.

TABLE 52-8

DETERMINATION OF PATHOPHYSIOLOGIC SIGNIFICANCE OF THE STENOTIC LESION (THE DECISION TO RECOMMEND INTERVENTION, i.e., OPERATION OR PERCUTANEOUS TRANSLUMINAL RENAL ANGIOPLASTY [PTRA], OR RENAL ARTERY STENT)

Duration of hypertension <3–5 yr
Positive findings on captopril renogram
Positive result on captopril test
Renal vein renin ratio >1.5
Hypokalemia
Systolic-diastolic bruit in abdomen
Abnormal rapid-sequence intravenous pyelogram
Appearance of lesion on angiogram (>75% stenosis)

Because the great majority of patients diagnosed as having renovascular hypertension have this syndrome consequent to main renal artery stenosis, the first step in making the diagnosis of renovascular hypertension is to demonstrate that renal artery stenosis is present. Until recently, the anatomic diagnosis of renal artery stenosis was confirmed only by conventional renal arteriography. Newer imaging modalities (see Chapters 12–14), including intravenous digital subtraction angiography, magnetic resonance angiography, computerized tomography angiography, and duplex ultrasound, have evolved in recent years, allowing for variable reductions in expense, risk, and invasiveness in diagnosing renal artery stenosis. The second step in making the diagnosis of renovascular hypertension is to determine the pathophysiologic significance of the renal artery stenosis. The third step in diagnosing renovascular hypertension is to cure or markedly ameliorate the hypertension with surgical or endovascular renal revascularization or removal of the involved kidney. Thus, hypertension presumed to be renovascular in origin is a retrospective diagnosis. Although renovascular hypertension is hypertension that is improved or cured by a technically successful intervention, at some point the hypertension may be analogous to phase III renovascular hypertension in animals when the hypertension becomes autonomous.

Issues of Prevalence, Epidemiology, Clinical Features, and Physical Examination

Epidemiology and Prevalence

The prevalence of renovascular hypertension is not known with precision, but available data suggest that it occurs in 0.5% to 5.0% of the general hypertensive population (187–196). Centers with special interest in hypertensive disorders have generated the available data on the prevalence of renovascular hypertension. Consequently, the incidence of renovascular hypertension in the community remains unknown. Many centers search for the presence of renovascular hypertension only when BP is poorly controlled. Screening procedures were less sensitive when these prevalence figures were obtained. It is difficult to determine whether prevalence figures in various reports are referring to renal artery stenosis, renovascular hypertension, or both. A negative result on a screening procedure at some point during a patient's clinical course does not exclude future development of renal artery stenosis, particularly from atherosclerotic renal artery disease.

Data from the Mayo Clinic revealed relative rarity of renovascular hypertension; only 0.18% of their hypertensive population underwent surgery for presumed renovascular hypertension. This low prevalence may be accounted for in part by the fact that not all the screened patients underwent arteriography (197). Although the small prevalence range for renovascular hypertension implies that this condition is rare, hypertension affects approximately 15% of the general population in Western countries, and it is estimated that more than 60 million people in the United States have hypertension (187,198). With the exception of hypertension due to oral contraceptive use, excessive alcohol ingestion, and the relief of hypertension by treatment of end-stage renal disease, renovascular hypertension is the most common correctable cause of secondary hypertension.

Although the prevalence of renovascular hypertension is probably less than 1% for patients with mild-to-moderate hypertension (187,193,195), the incidence of renovascular hypertension may be as high as 10% to 45% in white patients

with acute onset of hypertension or severe or refractory hypertension, even if worsening BP is superimposed on preexisting mild-to-moderate hypertension (199–202). Most studies suggested that renal artery stenosis and renovascular hypertension are less common in African American patients, in whom severe hypertension is usually due to essential hypertension (203–205). Grim and co-workers (205) found functional renal artery stenosis in 11.7% of 377 hypertensive whites, but in none of 87 hypertensive blacks. However, because hypertension affects a greater proportion of the black population, the number of blacks with renovascular disease may be underestimated. One study indicated that the prevalence of renovascular hypertension in selected whites and blacks was similar, but that blacks had a much higher prevalence of both renal artery stenosis and renovascular hypertension compared to rates in previous reports (206). Another report indicated that 30% of blacks with significant coronary disease had concomitant renal artery stenosis (207). These data are supported by population surveys in the elderly in North Carolina (208).

Clinical Signs and Symptoms

When sustained diastolic hypertension (with diastolic pressure greater than 110 mm Hg) appears before age 30 or after age 55, renovascular disease should be suspected. Renovascular hypertension may appear between the ages of 30 and 55 years, but the onset of hypertension between these years typically indicates primary (essential) hypertension. In the Cooperative Study of Renovascular Hypertension, the average age of onset for fibrous renal artery disease as the cause of renovascular hypertension was 33 years, and 16% of these patients were younger than 20 years. For atherosclerotic renal artery disease as the cause of renovascular hypertension, the average age at onset was 46 years, and 39% of these patients were older than 50 years. For essential hypertension, the average age at onset was 35 years, and only 7% were older than 50 years; 12% were younger than 20 years (133,209). The decision for or against renal angiography should not be based on gender, because atherosclerotic renovascular disease occurs with enough frequency in either sex to justify renal angiography if other clinical clues are present.

Virtually all discussions and definitions of renovascular hypertension have focused on persistent elevation of the diastolic BP or on elevations of both diastolic and systolic BP. Therapies directed at curing or improving presumed renovascular hypertension have always defined "cure" or "improvement" in terms of reduction in the diastolic BP or improved levels of both diastolic and systolic pressure. The notion that isolated systolic hypertension or wide pulse pressure hypertension (e.g., 180/70 mm Hg) could be renovascular in origin has been largely ignored. In the Cooperative Study of Renovascular Hypertension (209), no mention was made that renovascular hypertension might be a reflection of isolated systolic hypertension or wide pulse pressure hypertension. With the widespread employment of renal artery stenting, particularly in elderly patients with generalized atherosclerosis obliterans, new thoughts and data regarding degrees of improvement in systolic BP in patients with normal diastolic BPs could emerge. Conceivably, new definitions of renovascular hypertension could include reductions in systolic pressure alone or reductions in pulse pressure in addition to the time-honored definitions of renovascular hypertension which have required improvement in diastolic BP levels or both diastolic and systolic BP levels.

A strong family history of hypertension suggests primary (essential) hypertension and argues against renovascular hypertension. However, a negative family history is not always useful as evidence for renovascular hypertension unless one can ascertain that close relatives are normotensive. The Cooperative Study of Renovascular Hypertension reported a statistically significant difference in the presence of family histories of hypertension between patients with renovascular hypertension (46%) and a group of patients with essential hypertension (71%) matched for race, age, and sex (133,209). However, family history by itself has limited usefulness in guiding subsequent diagnostic studies for a specific hypertensive patient because the overlap is large and many patients with essential hypertension develop superimposed atherosclerotic renal artery stenosis later in life. Several reports suggest the familial occurrence of fibrous renal artery disease, the exact mode of transmission being unclear (210,211). Cigarette smoking has been proposed as an etiologic factor in fibrous renal artery disease (19,32,65), and one presumes that cigarette smoking is a risk factor for atherosclerosis of the renal artery as it is for atherosclerosis in other vascular beds.

In general, the duration of hypertension is shorter for patients with renovascular hypertension than for patients with essential hypertension (131,133,211,212). When the clinical characteristics of essential and renovascular hypertension (cured by surgery) were compared, a duration of hypertension less than 1 year was observed in 10% of patients with essential hypertension and 25% of patients with renovascular hypertension (212). Duration of hypertension is an important prognostic factor regarding the response to surgery and it has been suggested that the success of surgical treatment in reducing blood pressure is inversely proportional to the duration of renovascular hypertension (131,133). The likely pathophysiologic explanation for this clinical observation was discussed earlier in this chapter. Patients whose hypertension suddenly becomes more severe or resistant to a previously effective drug regimen, or both, are candidates for renal artery stenosis and renovascular hypertension; the possibility that renovascular hypertension has been superimposed on underlying essential hypertension should also be considered. Although the Cooperative Study of Renovascular Hypertension did not find that acceleration of hypertension occurred more frequently in patients with renovascular hypertension than in patients with essential hypertension, 133 many patients with renovascular hypertension demonstrate resistance to drug therapy (64,213).

Accelerated or malignant hypertension and hypertensive crisis may be manifestations of renovascular hypertension (133,199,214). Rapid onset or progression of hypertension associated with severe funduscopic changes is a strong clue to the presence of renal artery stenosis. Davis et al. reported renovascular hypertension in 23% of 123 patients with severe hypertension and grade III or IV funduscopic retinal changes (199). This figure is considerably higher than the prevalence of renovascular hypertension among the general hypertensive population and argues for a thorough evaluation for renovascular hypertension in patients presenting with accelerated or malignant hypertension.

Pulmonary edema ("flash pulmonary edema") has been suggested as a pattern of clinical presentation for patients with advanced renal artery stenosis (215–217). In a series of 55 consecutive patients who were also azotemic, pulmonary edema was present in 13 (25%). Most of these patients had high-grade renal artery stenosis bilaterally or involving the artery to a solitary kidney. Successful renal revascularization prevented further occurrence of pulmonary edema. Although pulmonary edema is frequently observed in the setting of severe hypertension and hypertensive heart disease, in this series (217) the occurrence of pulmonary edema was not related to the severity of the hypertension or coexisting renal failure. However, in a study of 90 patients with renal artery stenosis, 23 had bilateral renal artery stenosis and recurrent pulmonary edema (218). Ninety percent of this latter group had renal insufficiency; only 20% had moderate or severe reduction in ejection fraction. Therefore, the findings of hypertension, recurrent pulmonary edema, azotemia, and a well-preserved ejection

fraction should raise the possibility of bilateral renal artery stenosis.

Generalized vascular disease is closely associated with atherosclerotic renal artery stenosis (28,207,219). Of interest, atherosclerotic renovascular disease is not more common in diabetic patients despite the increased prevalence of vascular disease in this patient population (187,195).

There are no symptoms that are pathognomonic of renovascular hypertension. Flank pain does not occur more often in patients with renal vascular disease than in patients with essential hypertension (133,209). Flank pain or hematuria or both may indicate segmental renal infarction due to segmental renal artery occlusion (220), renal artery dissection associated with several of the fibrous dysplasias (221), or intimal dissection of the renal artery after selective angiography (222). Hypertension following traumatic injury is an indication to exclude traumatic renal artery occlusion. A delayed onset of hypertension following traumatic injury to the retroperitoneal area suggests subcapsular or perirenal hematoma (Page kidney). Takayasu's arteritis, frequently involving the renal artery and associated with renovascular hypertension, is characterized by nonspecific symptoms such as fever, myalgias, arthralgias, weight loss, general fatigue, vertigo, and cold extremities. Hypertension developing in a young woman, an abdominal bruit, increased erythrocyte sedimentation rate, and absent radial artery pulse are important clinical findings for establishing this diagnosis (223).

Rapid deterioration of renal function following BP reduction with conventional antihypertensive agents or particularly following BP reduction with ACE inhibitors strongly suggests the presence of bilateral renal artery stenosis, or stenosis in a solitary functioning kidney (162,224–229). In one series, more than one-half of patients demonstrating an acute elevation in the plasma creatinine concentration that was either unexplained or occurred shortly after institution of therapy with an ACE inhibitor had main renal artery disease, while the remainder presumably had disease of the intrarenal vessels due to nephrosclerosis (230). The mechanism of acute renal dysfunction after treatment with ACE inhibitors appears to be a combination of a fall in BP and a direct action of these compounds on intrarenal hemodynamics. It is presumed that the glomerular filtration rate (GFR) in the presence of renal artery stenosis is maintained, in part by the actions of angiotensin II on the efferent glomerular arterioles. Attenuation of this action of angiotensin II by converting enzyme inhibition may explain renal dysfunction occurring with ACE inhibitor therapy in the setting of bilateral renal artery stenosis, or stenosis of a solitary kidney.

In an instructive series of 104 patients at high risk for bilateral renal artery stenosis, all were given an ACE inhibitor for 2 weeks (although this was discontinued after 4 days if the serum creatinine concentration had increased >20%) (231). If after 2 weeks, the serum creatinine had not increased >20%, and the BP remained elevated, patients were given a diuretic to correct fluid retention. All patients subsequently had a renal arteriogram. Of the 52 patients with severe (>50%) bilateral renovascular disease or severe stenosis of the artery to a solitary functioning kidney, each had a rise in serum creatinine of >20% with ACE inhibitor therapy. The authors concluded that a controlled exposure to an ACE inhibitor is generally safe and that an ACE inhibitor-induced increase in serum creatinine is 100% sensitive and 70% specific for the detection of severe bilateral renovascular disease.

Physical Examination

The physical examination affords four clues to the presence of renovascular disease: the severity of the hypertension, an abdominal bruit, abnormalities of the optic fundi, and evidence of atherosclerotic disease of the aorta and peripheral arteries. As in essential hypertension, the hypertension in patients with renovascular disease can be of any degree. As alluded to earlier, however, severe hypertension and accelerated or malignant hypertension are not uncommon manifestations of renovascular hypertension (189,214,232,233). An acute rise in BP over previously controlled or stable baseline BP suggests renovascular disease.

Detection of an abdominal bruit, although not diagnostic, suggests renal artery disease and possible renovascular hypertension. A systolic/diastolic bruit in the epigastrium or one or both upper quadrants is virtually pathognomonic of renovascular disease and provides a strong indication for renal angiography even if other clinical clues to renovascular disease are lacking. Further, the abdominal systolic/diastolic bruit predicts a successful result from surgical renal revascularization (185), and hence is an index of renovascular hypertension as well as of renovascular disease. The absence of a systolic/diastolic bruit in the epigastrium does not exclude renovascular disease, but, particularly in the case of fibrous renal artery disease, makes it less likely that surgery or angioplasty will relieve the hypertension.

The diastolic component to an epigastric bruit is frequently difficult to appreciate; a soft, wide, pansystolic bruit also suggests renovascular disease. The helpfulness of an abdominal bruit in the diagnosis of renovascular hypertension has been stressed by several authors (133,209,234–236). Maxwell reported detecting an abdominal bruit in 41% of patients with renovascular hypertension due to atherosclerotic renal artery disease and in 57% of patients with renovascular hypertension due to fibrous dysplasia. Hunt et al. reported abdominal bruits with diastolic components in 25 (36%) of 69 patients with atherosclerotic renal artery disease and in 76 (74%) of 104 patients with fibrous renovascular disease (235). Eipper et al. found that 25 of 47 patients with fibrous renovascular lesions had systolic/diastolic bruits in the abdomen while such bruits were present in only 5 of 40 patients with atherosclerotic renovascular disease (185). Moser and Caldwell and the Renovascular Study Cooperative Group, although emphasizing the importance of abdominal bruits, were less impressed with the significance of the diastolic component (236).

Systolic bruits are frequently heard in the abdomen and it is often difficult to determine whether the systolic bruit is emanating from the renal artery, other intraabdominal arteries, or the aorta. In contrast to the importance of a systolic/diastolic bruit in patients with fibrous renal artery disease, systolic epigastric bruits, without a diastolic component, are of marginal clinical utility.

In the absence of an arteriovenous fistula, a diastolic component to an arterial bruit signifies stenosis of approximately 70% or more (237,238). A diastolic component to the arterial bruit, although highly suggestive of hemodynamic renal artery disease, may also reflect hemodynamically significant stenosis of other visceral arteries, particularly the celiac access or the superior mesenteric artery. Fibrous renal artery disease may affect these vessels, as well as the renal arteries, and occasionally the carotid arteries. The importance of a bruit in the back or flank is probably overstated and when present in these locations is almost certainly audible in the epigastrium or upper abdominal quadrants (214).

Severe hypertensive retinopathy (Keith-Wagener-Barker grades III and IV), detected on examination of the optic fundi, is a strong clue to renovascular hypertension (199,206). Davis et al. 199 reported that 41 (48%) of 85 hypertensive patients with retinal hemorrhages and exudates with or without papilledema had stenotic lesions on renal arteriograms and 26 (31%) had a strong likelihood of renovascular hypertension, which was defined by the presence of lateralizing differential renal function

or renal venous renin activity. Nearly 25% of these patients with severe hypertensive retinopathy turned out to have proven renovascular hypertension. In the Cooperative Study of Renovascular Hypertension (209), the prevalence of Keith-Wagener-Barker retinopathy of grades III and IV was twice as high among those having renovascular hypertension compared to those with primary or essential hypertension. The presence of malignant hypertension (diastolic BP greater than 140 mm Hg and papilledema) suggests renovascular disease, particularly when accelerated hypertension and accelerated retinopathy occur in a white patient with well-documented hypertension of less than 2 years, and is a strong indication for renal angiography.

In the absence of hemorrhages, exudates, and papilledema (Keith-Wagener-Barker grades III and IV), severe retinal arteriolar constriction with minimal or no retinal arteriolar sclerosis suggests recent onset of severe hypertension, and is a clue to the presence of renovascular hypertension. This funduscopic observation has been called *arteriospastic angiopathy*. Hunt et al. observed these changes in 15 (20%) of 81 patients with atherosclerotic renovascular disease and in 60 (45%) of 133 patients with fibrous renal artery disease (235).

Moderate to severe hypertension in patients with evidence of atherosclerotic disease of the aorta and peripheral arteries, particularly in patients older than 50 years, implies atherosclerotic renovascular disease as a possible cause of the hypertension. This is especially true for patients with abdominal aortic aneurysms or occlusive disease in the distribution of the aorto-iliac-femoral popliteal system (2,3,34,239). In one prospective study of 100 patients presenting with signs of peripheral vascular disease, bilateral renal artery stenosis was present in 24, of whom 7 had complete occlusion on one side (34). Others reported as much as a 50% incidence of at least 50% stenosis in one renal artery in patients with peripheral vascular disease, with or without hypertension (28,239). Some of these patients were normotensive and many had essential hypertension. Thus, although these atherosclerotic renal artery lesions may not be instrumental in the pathogenesis of hypertension, the existence of these stenoses may threaten long-term renal function.

Laboratory Tests, Radiologic Examinations, and Diagnostic Maneuvers

Laboratory Tests

Urinalysis is of little benefit in evaluating renal artery stenosis and renovascular hypertension. Mild-to-moderate proteinuria is common and probably reflects the severity of the hypertension or associated nephrosclerosis (133). Nephrotic range or heavy proteinuria has been described in association with renal artery stenosis and renovascular hypertension, usually in the setting of accelerated or malignant hypertension (prior to availability of effective antihypertensive drugs) (240). Several authors reported nephrotic-range proteinuria due to renal artery stenosis wherein the urinary protein excretion normalized after renal revascularization or nephrectomy (241–244). In these uncommon situations, excessive production of angiotensin II may be responsible for promoting high levels of protein excretion (245,246). Focal segmental glomerulosclerosis (FSGS) with heavy proteinuria has been described in association with renal artery stenosis (247,248). In a series of 24 patients with biopsy-proven FSGS who were over 50 years old, 8 had renovascular disease (249). These eight patients had typical glomerular lesions with focal segmental tuft collapse and synechiae. All patients had heavy proteinuria and developed a further decline in renal function at follow-up. The authors proposed that FSGS may be an unrecognized complication of renovascular disease in elderly patients and may contribute to irreversible changes in the kidneys. Nephrotic-range proteinuria can occur in adults with diabetic renal disease and atherosclerotic renal artery stenosis, wherein the proteinuria is likely due to the diabetic glomerular disease, rather than to main renal artery stenosis.

Unexplained azotemia in elderly patients with generalized atherosclerosis and minimal proteinuria suggests vascular occlusive disease of the kidneys, including bilateral renal artery stenosis, stenosis of an artery to a solitary kidney, or a combination of renal artery stenosis and nephrosclerosis. Uncontrolled BP itself may contribute to renal insufficiency, particularly if there is an underlying baseline impairment in GFR (233,239,250–252). In the Cooperative Study of Renovascular Hypertension, 15% of patients with atheromatous renal artery disease had an elevated serum creatinine concentration, compared with 11% of patients with essential hypertension; only 2% of patients with fibrous renal artery disease had an elevated serum creatinine concentration (209).

Hypokalemia (serum potassium level less than 3.4 mEq/L) is a marker of hemodynamically significant renal artery stenosis, reflecting stimulation of the renin-angiotensin system with secondary aldosteronism. Although the concomitant use of diuretics to treat the hypertension may confound the interpretation of hypokalemia in patients with renovascular disease, hypokalemia in a patient (not receiving a diuretic) with severe hypertension is highly suggestive of renovascular hypertension. In the Cooperative Study of Renovascular Hypertension, a serum potassium concentration of less than 3.4 mEq/L was observed in 16% of patients with renovascular hypertension cured by surgery, in contrast to 8% of patients with essential hypertension (133,209).

A significant discrepancy in kidney size in hypertensive adults, and particularly the observation of an atrophic kidney, strongly indicate renal artery stenosis as the basis for the smaller kidney. Not infrequently, the small kidney is the cause of the hypertension, which may be relieved by renal revascularization or nephrectomy (88). A number of currently available renal imaging techniques, including ultrasonography, scintigraphy, radiography of the kidneys, ureters, and bladder (KUB), intravenous pyelography, and computed tomography, all allow for easy detection of a unilateral small kidney. This observation, in conjunction with additional clinical clues suggesting renovascular hypertension, may be sufficient reason to proceed quickly to renal angiography to confirm that renal artery stenosis is the cause of the smaller kidney. Significant renal artery stenosis involving the artery to the contralateral kidney is observed frequently (30% to 50% of the time) in the setting of high-grade stenosis or total arterial occlusion of the artery supplying the atrophic kidney (88,89).

Plasma Renin Activity

Although unilateral hypersecretion of renin is an expected finding in hemodynamically significant renal artery stenosis when renovascular hypertension is eventually proved (247), baseline plasma renin activity (PRA) is elevated in only approximately 50% of patients with renovascular hypertension (213,229,253). Tabulation of the number of patients whose hypertension was cured or improved by surgical renal revascularization indicates that probably no more than 50% of patients with renovascular hypertension have elevated PRA (205,212,213,254-259).

There are several reasons for the relatively low specificity and sensitivity of peripheral PRA in the diagnosis of renovascular hypertension. Most high-renin hypertension is not renovascular in origin; increased renin substrate can be due to estrogen intake, pregnancy, cortisol excess, intrarenal ischemia

TABLE 52-9

SENSITIVITY AND SPECIFICITY OF THE CAPTOPRIL PLASMA RENIN ACTIVITY TEST

Investigators	No. of patients studied	No. of patients with renal artery stenosis	Sensitivity (%)	Specificity (%)
Muller, et al. (269)	152	49	100	95
Derkx, et al. (271)	179	89	93	84
Frederickson, et al. (117)	100	29	100	80
Gosse, et al. (272)	114	11	73	84
Hansen, et al. (273)	47	11	91	89
Postma, et al. (274)	149	44	38	93
Svetkey, et al. (275)	66	11	73	72
Thibonnier, et al. (276)	65	14	40	100
Elliott, et al. (277)	100	59	76	58

(From: Nally JV Jr, Olin JW, Lammert GK. Advances in noninvasive screening for renovascular disease. *Cleve Clin J Med* 1994;61:328, with permission.)

(parenchymal disease), or accelerated or malignant hypertension, and the 15% to 20% of patients with primary (essential) hypertension and high renin levels constitute the majority of high-renin hypertensives. Renin secretion fluctuates widely (260), and is also influenced by sodium intake and posture (261), a variety of antihypertensive drugs, age (262), sex, and race (263). The utility of peripheral PRA is reportedly enhanced when measured in the morning with the patient in the seated position and when indexed against urinary sodium excretion; when measured under these exacting circumstances, a high peripheral PRA is found in 75% to 80% of patients with proven renovascular hypertension (122,264). Other investigators, measuring peripheral PRA under similar circumstances, failed to demonstrate significantly increased sensitivity of the peripheral PRA in predicting renovascular hypertension, even under controlled circumstances (122,265,266). Complicating the interpretation of peripheral plasma renin values is the long list of coexisting medical conditions and drugs that either stimulate or inhibit renin release. A very low PRA (e.g., less than 0.3 ng/mL/hour) indexed against a normal urinary sodium excretion in the absence of drugs known to suppress renin argues strongly against renovascular hypertension (267–269).

Captopril Provocation Test, Captopril Plasma Renin Activity

The predictive value of the peripheral PRA can be increased by measuring the rise in PRA 1 hour after the oral administration of 50 mg of captopril (a rapidly acting ACE inhibitor) (118,229,253,269,270). Case and Laragh (270) first showed that the reactive rise of renin following the administration of captopril is greater in patients with renovascular hypertension than in those with essential hypertension. The criteria that this group recommended (269) for distinguishing patients with renovascular hypertension (i.e., criteria for a positive captopril test result) are: (a) a stimulated PRA of 12 ng/mL/hour; (b) an absolute increase in PRA of 10 ng/mL/hour; and (c) an increase in PRA of 150% or more if the baseline PRA is more than 3 ng/mL/hour, or a percent increase in PRA of 400% if the baseline PRA is less than 3 ng/mL/hour. These investigators emphasized that all three of these criteria should be satisfied and that blood samples should be drawn with the patient seated (not supine). It is important to realize that the renin assay to which these results relate was that used in Laragh and Sealey's laboratory. Other renin assays may provide very different values for cut off points as described in Frederickson et al. (117). A simpler criterion of a post-captopril PRA (PRA >5.7 ng/mL/hour) yields a test without any loss of sensitivity (117,118). In a series of more than 200 patients, the sensitivity and specificity of this test were better than 95% (269). Other groups, however, reported lower levels of sensitivity, in part using different criteria; the sensitivity and specificity of the captopril PRA test from nine separate series are summarized in Table 52-9 (117,186,269,271–277). The general utility of this test is further limited by: (a) the need to discontinue antihypertensive medications that can affect the PRA (e.g., beta-blockers and diuretics), (b) the need to standardize the procedure, (c) the variable sensitivity, and (d) the somewhat decreased predictive value when compared to captopril renography (102,201). When applied prospectively, the accuracy of renin-based measurements for predicting the outcomes of renal revascularization is limited to between 35 to 60% sensitivity and specificity (278).

In interpreting the captopril test, the poststenotic kidney is likely to have excess stores of renin and thereby could be considered to be primed to demonstrate a reactive rise of renin following administration of captopril, particularly with a concomitant fall in arterial pressure (270). This possible mechanism, however, may not be the major cause for the enhanced response to ACE inhibition, since equivalent reductions of BP produced by other agents, such as clonidine, do not elevate PRA excessively in patients with functionally significant unilateral renal artery stenosis (279,280). More likely, a positive captopril provocation test signifies the same intrarenal hemodynamic changes as a positive ACE inhibitor renogram, that is, a fall in GFR in the poststenotic kidney. This GFR decline will reduce NaCl delivery to the macula densa and stimulate renin secretion preferentially in a functionally stenotic kidney. A decrease in BP 1 hour after captopril administration may be expeceted to be greater for patients with renovascular hypertension than for patients with essential hypertension, and this assumption is supported by the observation in one study that patients with renovascular disease demonstrated an average decrease in BP of 18 mm Hg, whereas patients with essential hypertension had a decrease in BP of 7 mm Hg (202,275,281). However, considerable overlap exists in the decrease in BP in patients with renovascular versus essential hypertension that limits the utility of this observation as a reliable screening test for renovascular hypertension. A dramatic decrease in BP after captopril administration, however, may predict a good BP response to renal revascularization or angioplasty (281). The captopril-stimulated PRA test becomes less reliable in patients

with renal insufficiency, and it does not discriminate between patients with unilateral versus those with bilateral renal artery stenosis (269,271).

Three prospective studies showed that the BP of hypertensive subjects with renal artery stenosis during established monotherapy with ACE inhibitors correlates quite closely with the BP after reconstructive surgery (129,282), nephrectomy (129,282), or percutaneous transluminal angioplasty (PTRA) (283). The overall correlation coefficient in these three trials for BP during ACE inhibitor monotherapy and BP after intervention is 0.64 (229). Therefore, some insight into the effectiveness of a planned intervention to correct renal artery stenosis for reduction in BP can be obtained from the BP achieved during ACE inhibitor therapy.

Saralasin Infusion Test

Before captopril administration became popular to enhance the sensitivity of the peripheral PRA in the diagnosis of renovascular hypertension, the BP response to the angiotensin II antagonist saralasin was advocated both as a screening test for presumed renovascular hypertension and to identify patients who might be candidates for renal angiography (270,284,285). Saralasin, an angiotensin II analog, competitively binds to angiotensin II vascular receptors and inhibits the pressor effect of angiotensin II. In addition, saralasin has weak agonist (vasoconstrictor) properties. If renal artery stenosis is instrumental in causing the hypertension, angiotensin II levels are presumably high, and an infusion of saralasin should markedly lower the diastolic BP. When angiotensin II levels are low, saralasin either has a minimal response in reducing the BP or, via its agonist properties, could produce a pressor effect. This construct would then allow for differentiation between angiotensin II-dependent and angiotensin II-independent hypertension. Initial enthusiasm for this test waned because its performance requires careful adherence to an infusion protocol and discontinuation of antihypertensive medications other than diuretics 1 week before testing. Although mild volume depletion may augment the discriminatory potential of the test (285), several sizable series indicated a sensitivity of only 50% to 60% and a specificity of about 85% (205,286,287). For these reasons, the saralasin infusion test is not recommended as a screening procedure for renovascular hypertension (213,288).

Differential Renal Vein Renin Determinations

For almost 30 years, determination of the renal vein renin ratio (ipsilateral renal vein renin to contralateral renal vein renin) was used to assess the functional significance of renal vascular stenoses. The rationale of obtaining renal vein renin ratios is derived from the pathophysiology of classic two-kidney Goldblatt hypertension, wherein renal hypotension distal to the renal artery stenosis generates hypersecretion of renin by the affected kidney. Characteristics of renin-dependent unilateral renovascular hypertension are (a) increased renin secretion from the affected side and (b) suppression of contralateral renin release (122,259,268,289–293). Blood is sampled from each renal vein and from the inferior vena cava, and the plasma renin levels from the two renal veins are compared to each other and to the corresponding level in the inferior vena cava (below the level of the renal veins), which has been shown to be the same as the renin level in the main renal artery. Vaughan et al. (122,293) added a third criterion suggesting renin-dependent unilateral renovascular hypertension based on their analysis of renal vein and renal arterial renin relationships in patients with hypertension. These workers calculated that the mean renal venous renin is about 25% higher than the arterial renin, indicating that the hypoperfused kidney is truly overproducing renin (122,290,293). They derived a specific formula to estimate renin production from the two kidneys. Most centers simply employ a renal vein renin ratio of at least 1.5 to 2.0 (ipsilateral to contralateral) with suppression of renin production (i.e., renin from contralateral kidney is equal to or below the plasma renin level from the inferior vena cava below the renal arteries) as the criterion for "lateralizing" renal renin secretion and as a predictor of a beneficial surgical outcome for relief of hypertension.

Since the report of Helmer and Judson in 1960 (294), wherein a renal vein renin ratio of 2.0 or higher was used as a predictor of surgical cure of renovascular hypertension, the renal vein renin ratio has been widely accepted as a criterion to diagnose hemodynamically significant unilateral renal artery stenosis. In 1976, Marks et al. reviewed 21 previously published series encompassing 468 patients with unilateral renovascular disease who had been subjected to a broad spectrum of renin-stimulating maneuvers (e.g., sodium depletion, upright posture) (132). They concluded that a lateralizing renal vein renin ratio predicted surgical cure or substantial improvement in the BP in 93% of patients. Fifty-seven percent of patients with a nonlateralizing renal vein renin ratio (less than 1.5 ipsilateral to contralateral) also benefited from surgery (132). In another review of 58 studies, a ratio of 1.5 or more (ischemic kidney to contralateral kidney) as the diagnostic criterion predicting renovascular hypertension had a sensitivity of 80% and a specificity of 62% (295). Remarkably low renin levels sampled from the renal veins (less than 1 ng/mL/hour), particularly if equally low bilaterally, militate against renovascular hypertension (267,269,296).

The high predictive value of a lateralizing renal vein renin ratio as well as the high false-negative rate (i.e., nonlateralizing renal vein renin ratio) have been observed in many subsequent studies. An analysis of 10 reports between 1976 and 1986 (147) including 282 patients with renovascular disease subjected to various renin-stimulating maneuvers indicated an average false-positive rate of only 14% but an impressively high false-negative rate of 67%. Some of the false-negative results may be attributed to technical errors; nevertheless, the high false-negative rate of renal vein renin determination is a consistent finding from many centers. Possible explanations for this high false-negative rate include technical difficulties in placing the catheters far enough distally in the renal vein or failure to use two catheters so samples can be drawn simultaneously from the renal veins (297); renal vein sampling while the patient is taking antihypertensive drugs (298); branch lesions of the renal arteries or bilateral renal artery stenoses, which are notorious for minimizing differences in renal venous PRA between the two kidneys (268,299); dilution errors, or substances interfering with the assays; and non–renin-mediated renovascular hypertension (e.g., hypoplastic kidney, atrophic pyelonephritis) (88).

Several stimulatory maneuvers have been designed to increase the sensitivity of differential renal vein renin determinations (209,300). These include sodium depletion (low-sodium diet and/or diuretic presampling) (131,209,259,300,301), upright posture (tilt) (290,302–304), and a variety of pharmacologic agents. Saralasin (270,285), acute administration of captopril (270,276,285,305–307), intravenous hydralazine (308), and bolus furosemide (298,309) have been utilized. Aurell (303) and Michelakis (304) and their respective colleagues demonstrated a reduced incidence of false-negative renal vein renin results with upright posture stimulation wherein the disparity in renal vein renin secretion from the two sides was exaggerated by tilting during the collection of renal venous blood. Captopril administration, which results in a reactive rise in renin in renovascular hypertension, potentiates the disparity in the renin secretion from the two kidneys, reducing the false-negative rate of renal vein renin determination. The mechanism of augmented renin secretion with bolus furosemide at the time of renal venous sampling is not clear, but may be due to

inhibition of the macula densa NaCl reabsorption, intrarenal vasodilation, and prostaglandin secretion all of which can potentiate renin release. Assuming that the kidney ipsilateral to the stenosis is primed to release large amounts of renin, these stimulatory maneuvers should theoretically increase the renal vein renin value from the ipsilateral kidney more than from the contralateral kidney, thereby reducing false-negative renal vein renin results. In addition to augmenting the renal vein renin differential, captopril administration also obviates the need to discontinue previous antihypertensive drugs in preparation for renal vein renin sampling, except, possibly, diuretics (306,307). This maneuver is particularly pertinent, because stopping all antihypertensive medications in preparation for renal vein renin sampling may be hazardous in patients with presumed renovascular hypertension, who often have significant and dangerous elevations in the BP.

In patients with stenoses of both renal arteries, the pattern of renal vein renin levels often shows the same degree of asymmetry as in patients with unilateral stenosis, and the renin values usually lateralize to the kidney demonstrating the greater degree of stenosis on the arteriogram (268). The most marked asymmetry is seen in patients who have complete occlusion of one renal artery, wherein renal vein renin levels from the side of the occluded artery may represent low flow through the kidney rather than hypersecretion of renin. Contralateral suppression of renin secretion may occur even in the presence of severe stenosis of the contralateral kidney. This asymmetry is less pronounced in the presence of azotemia. Although less valuable in patients with bilateral renal artery stenosis, determination of renal vein renin levels may be useful for identifying which kidney is the "most ischemic," which can be useful in planning which kidney to subject to intervention (268).

Taken together, the data regarding renal vein renin determinations indicate that the renal vein renin ratio has a strong positive predictive value in forecasting a beneficial interventional outcome. On the other hand, many patients (at least 50%) demonstrating a nonlateralizing renal vein renin ratio have marked improvement or cure of their hypertension following intervention for the renal artery stenosis. This fact, and the ready availability of captopril scintigraphy, and the common practice of PTRA or renal artery stenting done at the time of diagnostic renal angiography, have led to a decline in the use of renal vein renin tests.

Intravenous Pyelography

The rapid-sequence (hypertensive) intravenous pyelogram (IVP) has historically been used as a test for renovascular hypertension. In 1964, Maxwell et al. described the radiographic features suggesting renal artery stenosis (310). In the 1960s and 1970s, the hypertensive IVP was the standard screening test for renal artery stenosis and renovascular hypertension, and its initial popularity was attributed to its simplicity and safety, as demonstrated in the renovascular cooperative study (209,310,311). Abnormalities in the IVP suggesting renovascular disease are (a) a difference in renal lengths of 1.5 cm or more (the left kidney is normally 0.5 cm longer than the right); (b) delayed appearance of contrast medium in the intrarenal collecting system on the early sequence films; and (c) prolonged concentration of contrast medium, that is, persistent nephrogram, on late sequence films. Of these features, the delayed appearance of contrast medium on early sequence films was the most discriminating between essential and renovascular hypertension in the Cooperative Study of Renovascular Hypertension (209,311). In this study, 78% of patients who had at least 50% stenosis of one renal artery had one or more abnormalities on the IVP, compared with 11.4% of patients with essential hypertension. The more severe the renal artery stenosis, the greater likelihood that the IVP was abnormal. In patients with a totally occluded renal artery, 96% of pyelograms appeared abnormal, readily demonstrating a nonfunctioning kidney ipsilateral to the totally occluded renal artery. Although 78% of patients with significant renal artery stenosis had an abnormal IVP, there was a 17% false-negative and an 11% false-positive rate in surgically proved unilateral renovascular hypertension (311).

Reanalysis of the same data by Thornbury et al. derived a false-negative rate of 22% and a false-positive rate of 13% (312). Havey et al., in a review of 20 studies, reported a sensitivity of 74.5% and a specificity of 86.2% for IVP in detecting renovascular hypertension (313). The IVP is insensitive in detecting bilateral or branch stenosis (187,200,253,313). The suboptimal sensitivity and specificity, sizable dye load, and radiation dosage have all contributed to the current unpopularity of the hypertensive IVP as a routine screening procedure for renal artery stenosis and renovascular hypertension.

Renal Scans

Isotope renograms or renal scans have been available for many years and are helpful in selecting hypertensive patients for renal angiography (209,314,315). However, routine renal scanning never gained widespread acceptance because of the high incidence of false-negative results (315,316). The most widely used isotopes have been iodohippurate sodium (hippuran) and diethylenetriamine pentaacetic acid (DTPA). Iodohippurate sodium gives an approximation of the renal flow rate, while DTPA gives an approximation of the GFR. The value of radionuclide imaging of the kidneys is that it is a noninvasive and relatively simple method of assessing renal ischemia. In addition, computer-assisted qualitative and quantitative measurements of renal function (renal blood flow and GFR) can be performed (317). Radionuclide renal scanning has comparable sensitivity to the rapid-sequence IVP in diagnosing renovascular hypertension, but lower specificity; false-positive rates approximating 25% have been reported (209,212). In selecting patients for renal angiography, radionuclide renal scanning has a marginal advantage over the rapid-sequence IVP in that it obviates the need for contrast medium, and entails less radiation exposure. A renal scan can also be utilized to estimate the contribution of one kidney to overall renal function prior to a planned nephrectomy. The IVP on the other hand provides much more information about the anatomy of the kidneys and urinary tract, which in selected situations is useful in evaluating hypertensive patients.

Captopril Renography

The sensitivity and specificity of the routine renal radionuclide scan have been improved by combining scintigraphy with the administration of captopril (captopril renography, captopril scintigraphy). The rationale for this maneuver is that the GFR of the ischemic kidney ipsilateral to the renal artery stenosis is dependent on the effects of angiotensin II on the efferent glomerular arterioles; captopril reduces the angiotensin II-mediated constriction of the efferent arteriole, thus lowering glomerular pressure (229,318). The resulting decrease in glomerular filtration of the hypoperfused kidney is demonstrated by renal handling of radioactive tracers with the characteristic observation of a decreased uptake of DTPA (reflecting the GFR) with a delayed time to peak and slowed, or absent, washout (226,319–322). Simultaneously, inhibition of the pressor effects of excess circulating angiotensin II might improve the function of the contralateral (nonischemic) kidney, thereby exaggerating the asymmetry in renal function between the two kidneys (143,323–326). Studies of the effects of diuretic administration have shown that the characteristic captopril-induced changes are little affected by furosemide, but are abolished by mannitol (229). This indicates that the

TABLE 52-10

SENSITIVITY, SPECIFICITY, AND PREDICTIVE VALUE OF CAPTOPRIL RENOGRAPHY

Investigators	No. of patients	No. of patients with RAS	Radionuclide used	Sensitivity (%)	Specificity (%)	Predicted blood pressure response
Geyskes, et al. (328)	34	15	OIH	80	100	Yes: 12/15
Sfakianakis, et al. (319)	31	16	OIH	67	100	—
			Tc-DTPA	48	—	—
Erbslöh-Möller, et al. (329)	40	28	OIH	96	95	Yes: 10/11
Svetkey, et al. (275)	61	11	Tc-DTPA	74	44	—
			OIH	71	41	—
Setaro, et al. (330)	90	44	Tc-DTPA	91	94	Yes: 15/18
Mann, et al. (331)	55	35	Tc-DTPA	94	95	—
			OIH	83	85	—
Fommei, et al. (332)	472	259	Tc-DTPA	83	91	—
			Tc-MAC_3	83	100	—
Dondi (333)	102	54	Tc-MAC_3	90	92	Yes
Elliott, et al. (242)	100	59	Tc-pentetate	92	80	Yes: 51/53

RAS, renal artery stenosis; *OIH*, iodine-131-orthoiodohippurate; *Tc-DTPA*, technetium 99m-diethylenetriaminepentaacetic acid; *Tc-Mag3*, technetium 99m-mercaptoacetyltriglycine.
(From: Nally JV Jr, Olin JW, Lammert GK. Advances in noninvasive screening for renovascular disease. *Cleve Clin J Med* 1994;61:328, with permission.)

captopril-induced reduction in renal clearance of the tracer is due to accumulation in the proximal nephron because of a reduced GFR leading to a reduced tubular fluid flow that can be reversed by mannitol (118).

Although protocols for performing captopril renography are complex, and diagnostic criteria are not well standardized (327), the accuracy of captopril renography is clearly an improvement over routine renal scans. As indicated in Table 52-10, the accuracy of captopril renography is quite acceptable, with a sensitivity of approximately 90% to 93% (range 48% to 94%) and a specificity of approximately 93% to 98% (range 41% to 100%) (186,275,277,319,328–333). Unlike the captopril PRA test, captopril renography can be conveniently done without discontinuing antihypertensive medications (330,331), with the exception of ACE inhibitors. Administration of furosemide during the procedure may improve sensitivity without compromising specificity (118,329,334). In performing captopril renography, a baseline renal scan is obtained. Subsequently, a second renal scan is obtained, usually on another day and after oral administration of captopril (25 to 50 mg), and the two scans are compared. Typical renographic time-activity curves with technetium 99m-DTPA at baseline and after captopril stimulation are depicted in Figure 52-13. Alternatively, a renal scan after oral administration of captopril can be performed initially, and if the initial scan is abnormal, a repeat renal scan without captopril is performed subsequently to ascertain if there are discrepancies in renal function observed after captopril administration disappear or if they persist in the absence of captopril provocation. The former scenario suggests hemodynamically significant unilateral renal artery stenosis, whereas the latter does not (325,327,330,331,335).

The isotopic label most widely used in captopril renography is DTPA, although some authors claim that iodohippurate sodium can be as sensitive (329,336). More recently, technetium 99m-mercaptopacetyltriglycine (99 mTc-MAG3) has been utilized as the labeling isotope to provide images superior to those made with hippurate, with less radiation dosage and better resolution than provided by DTPA (333,337,338). Nearly always, captopril is the converting enzyme inhibitor employed because of its rapid onset of action, but enalapril or intravenous enalaprilat have also been used (336).

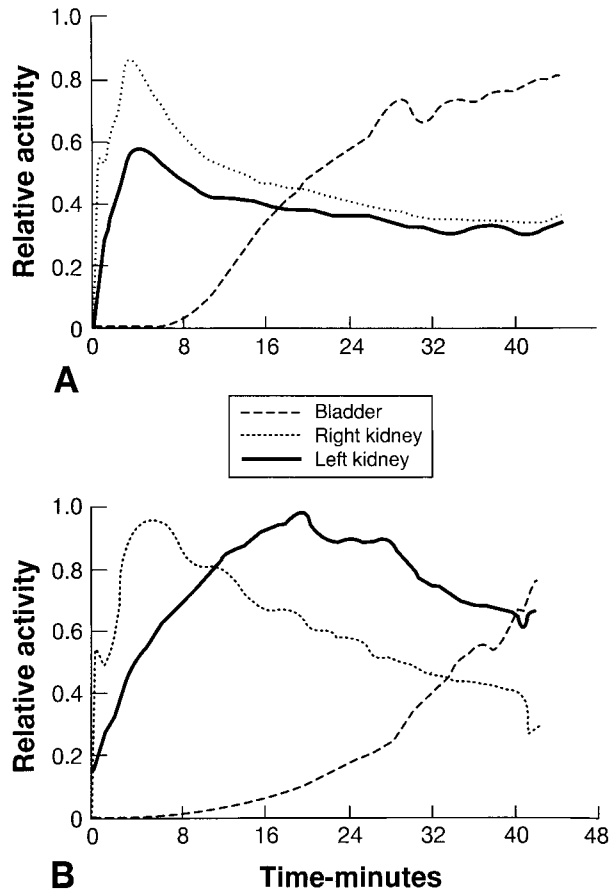


FIGURE 52-13. Renogram of a patient with unilateral left renal artery stenosis. **A:** Technetium 99m-diethylenetriamine pentaacetic acid (99mTc-DTPA)-timed activity curves during baseline. **B:** 99mTc-DTPA-timed activity curves following captopril administration. (From: Nally JV Jr, Olin JW, Lammert GK. Advances in noninvasive screening for renovascular disease. *Cleve Clin J Med* 1994;61:328, with permission.)

Diagnostic criteria in interpreting the captopril scintigram are based on a comparison of either (a) affected versus contralateral kidney (for asymmetry of uptake or excretion), or (b) pre– versus post–captopril administration scintigram (for captopril-induced changes) (253). Specific quantitative diagnostic criteria to distinguish essential from renovascular hypertension have not been firmly established; some investigators prefer the use of qualitative criteria (335).

Asymmetry of uptake or excretion or both may occur when uptake is measured between 1.5 and 2.5 minutes after injection. Asymmetric uptake has been defined as an uptake of less than 40% by the affected kidney and more than 60% of the total renal uptake by the contralateral kidney. A lesser degree of asymmetry occurs frequently and is not diagnostically helpful (330,331). The more severe the stenosis, the more reduced the uptake of the isotope (339). When stenosis is less severe, delayed excretion may be the only renographic abnormality. When stenosis approaches total occlusion, renal function is dramatically reduced, and renal function as assessed isotopically may cease (331).

Renal insufficiency reduces the sensitivity of captopril renography, and the dose of orthoiodohippurate that can be administered, as an index of effective renal plasma flow, is limited by the cumulative radiation exposure associated with renal insufficiency. MAG_3, because of its enhanced tubular secretion and lesser duration of radioactive exposure compared to orthoiodohippurate (338), probably has some advantages with better resolution than does DTPA. Patients with severe bilateral renal artery stenosis or renal artery stenosis of a solitary kidney are at risk of developing a transient but significant decline in renal function with captopril administration during this test (335), but this concern has not been a major clinical problem. Some studies indicate that captopril renography can discriminate between renovascular and renal parenchymal disease. In a study of 20 azotemic patients, all had abnormal baseline hippuran scans (329). However, a captopril-induced change in the scan predicted the presence of renal artery stenosis with a good BP response to intervention after renal artery angioplasty with a sensitivity and specificity >90%.

Taken together, experience with captopril renography to date is a major advance in diagnostic maneuvers designed to predict renal artery stenosis and renovascular hypertension (340). A positive finding by captopril renography strongly suggests the anatomic presence of renal artery stenosis and is an indication for renal angiography. A positive finding further indicates that renal artery stenosis is physiologically significant and instrumental in producing hypertension, thereby providing an indication for intervention (e.g., angioplasty, surgery) in an attempt to cure the hypertension. Whether captopril renography is a reliable probe for predicting progressive renal insufficiency due to renal artery stenosis is undetermined at this time.

Renal Arterial Pressure Gradient

One might assume that the more severe the renal artery stenosis, the greater the fall in arterial flow and pressure across the area of stenosis (105). The more severe the stenosis, the more likely that this lesion is generating the hypertension. Stewart et al. (341) confirmed this notion in humans by measuring the gradient across the stenotic lesion at surgery. Subsequent experience with this approach in the 1960s demonstrated that the preoperative measurement of a pressure gradient across a stenosis of the renal artery is associated with catheter-induced artifacts in the pressure distal to the lesion and the hazard of dissecting or perforating a highly stenotic artery. Even when measurements are made under direct vision on the operating table, the gradient varies considerably, depending on the state of the renal parenchymal vascular bed (342). If the intrarenal vascular bed is dilated by drugs or anesthetic agents, the gradient will be high; if the renal vascular bed is constricted, the pressure gradient across the renal artery stenosis will be low. Therefore, renal arterial pressure gradient measurements were rarely used to influence decision making regarding intervention.

The pressure gradient across a stenosis is proportional to the resistance of the stenosis, but inversely proportional to the downstream resistance in the renal microvessels. A successful outcome following an intervention requires that the stenosis be high grade (i.e., high stenosis resistance) and that the renal microvessels be compliant, reactive and not irreversibly fibrosed and remodeled (i.e., low intrarenal resistance). Accordingly, the degree of pressure gradient across a stenosis should be a useful predictor of functional severity of the stenosis and the degree of preservation of normal microvascular function within the affected kidney and should predict a good outcome after intervention. These assumptions have been validated in a recent treatment trial (343), using a group of patients with positive clinical criteria of renovascular disease and positive color-coded Doppler velicometry studies. All patients were investigated by angiography, and patients with renal artery stenosis >70% and transluminal pressure gradient >30 mm Hg were selected for PTRA and stenting. This selection procedure yielded a population that had favorable results from the intervention, suggesting that a combination of careful measurements of the degree of stenosis and preintervention trans-stenotic pressure gradient may be useful in selecting patients likely to benefit from intervention to correct renal artery stenosis.

In comparison, in a series of 231 patients with advanced atherosclerotic renal artery disease, mean serum creatinine concentration of 2.6 mg/dL, and a pre-intervention average pressure gradient of 109 mm Hg, which fell to 12 mm Hg after endovascular intervention, no relationship was apparent between severity of BP, number of antihypertensive medications, renal function and the gradients measured (344). Although many interventional angiographers obtain renal artery pressure gradients before and after PTRA or PTRA/stenting to predict the response to these interventional maneuvers, the relationship of the pressure gradient before and after endovascular intervention to later effects on BP and renal function is unclear.

Renal Biopsy

Vertes et al. (168–171) once advocated bilateral renal biopsy to predict the curability of hypertension in patients with renal arterial stenosis, the rationale being that if either kidney had severe arteriolar sclerosis, hypertension would probably not be relieved by surgery to repair the stenotic renal artery. Although, as previously discussed (see Pathophysiology of Renovascular Hypertension), there is substantial rationale for this maneuver, there are less invasive and much safer surrogates (duration of hypertension, duration of severe hypertension, renal insufficiency) to suggest bilateral renal parenchymal disease (i.e., nephrosclerosis) or unilateral renal artery stenosis with small-vessel disease in the contralateral kidney. Bilateral percutaneous renal biopsy and biopsy of the kidney ipsilateral to the renal artery stenosis, searching for significant arteriolar nephrosclerosis, are relatively formidable procedures such that this approach is virtually never employed today. There are more than enough clinical clues, laboratory suggestions, and noninvasive diagnostic maneuvers suggesting cure of hypertension following renal revascularization that renal biopsy to predict outcome of surgery or angioplasty is not recommended. Further, the observation of vascular changes in the renal parenchyma does not necessarily confer a poor outcome from surgical treatment of unilateral renal vascular disease (345). The role of renal biopsy in guiding renal revascularization

for improvement or preservation of renal function however is under continuing investigation (250).

Imaging of the Renal Vasculature

Several angiographic and nonangiographic imaging procedures are currently employed for the anatomic diagnosis of renal artery stenosis. The techniques, methodology, advantages, disadvantages, and complications of conventional renal arteriography, intravenous digital subtraction arteriography (IV-DSA), intraarterial digital subtraction angiography (IA-DSA), computed tomographic angiography, and magnetic resonance angiography (MRA) are detailed in Chapters 12–14.

Conventional Renal Arteriography

Although the need for conventional renal arteriograms for diagnosis of renal artery stenosis has decreased since the advent of IA-DSA, conventional arteriography gives the maximum information about vascular architecture, the intrarenal vasculature (e.g., segmental arterial branches), and collateral blood vessels (346). Selective renal arteriography is generally performed if there is no significant orificial stenosis and when the angiographer is searching for branch arterial lesions or better definition of main renal artery lesions viewed with abdominal aortography. If significant stenosis is present at the orifice of the renal artery, selective renal arteriography probably should be avoided because the catheter is advanced into the proximal renal artery and there is a risk of dissecting or occluding the artery or generating atheroemboli to the renal vascular bed. Renal angiography is generally performed when DSA is not available or when the DSA technique provides suboptimal images.

Digital Subtraction Angiography

DSA is a major advance in the radiologic evaluation of renal artery disease. IA-DSA has several advantages over conventional angiography: (a) there is significant time savings for the angiographer because the images can be recalled immediately on the monitor, and there is no need for the development of film before proceeding to the next injection of contrast medium; (b) only selected images are eventually recorded on film, resulting in significant cost savings; (c) digitalized images allow for computer processing such as enhancement of vessel edges, measurement of vessel diameters, and optimization of picture contrast and intensity; (d) IA-DSA requires less than half of the amount of contrast material needed for conventional angiography, and often no more than 50 mL (346).

There are two disadvantages to DSA: motion artifact and inferior spatial resolution on a digitally subtracted image compared to a conventional film. This latter disadvantage not infrequently results in suboptimal delineation of small distal arterial branches in the renal parenchyma and lessens the ability to detect the intrarenal changes of vasculitis and nephrosclerosis. The development of newer DSA equipment that uses smaller pixel sizes is quickly eliminating this disadvantage.

IV-DSA has been extensively used in the diagnosis of renovascular disease (347,348).

Renal IV-DSA is performed by injecting contrast material via a catheter placed in the antecubital vein or in the right atrium. During the imaging, the patient must remain motionless to avoid motion artifacts, a not insignificant disadvantage of IV-DSA. Although IV-DSA was quite popular in the early to mid-1980s because of its convenience as a simple outpatient procedure, its relatively low cost, and the fact that it made femoral artery cannulation with its attendant risks of atheroemboli unnecessary, recent experience suggests that its sensitivity is not be as high as originally proposed. In experienced hands, there is approximately 80% agreement between IV-DSA and either conventional arteriography or IA-DSA (347,349,350). Disadvantages of IV-DSA include opacification by the contrast bolus of all arteries within the field of view, resulting in considerable overlapping of mesenteric and renal arteries; a relatively long time gap between the injection of contrast material into the venous system and its arrival in the abdominal aorta; and artifact from patient motion, respiration, and bowel peristalsis. IV-DSA images are of poor quality in patients with impaired cardiac output, and the relatively large volume of contrast material required poses a relative contraindication in patients with renal insufficiency. Nevertheless, IV-DSA is a useful screening procedure for renovascular disease or as a follow-up procedure to surgical revascularization or balloon angioplasty. In addition, limited excretory urography can be performed concomitantly by obtaining a plain film of the abdomen with renal laminography after the injection of contrast material for IV-DSA.

Magnetic Resonance Angiography (MRA)

MRA (see Chapters 13 and 14) is entirely noninvasive and less operator-dependent than duplex ultrasound scanning. Several series examined the performance of MRA of the renal arteries with conventional angiographic confirmation, and demonstrated sensitivity and specificity rates for MRA of 80% to 90% (351–355). MRA exaggerates the severity of renal artery stenosis, particularly the distal half of the main renal artery, and its ability to detect branch stenosis is not clear. Technical improvements in MRA including gadolinium enhanced MRA (breath-hold MRA with perimagnetic contrast material [gadopentetate dimeglumine]), improve the ability to visualize some accessory renal arteries, but certainly not as clearly as conventional angiography or IA-DSA (356,357). The detection of hemodynamically significant renal artery lesions may be enhanced by using phase-contrast MRA (358). Although more expensive than duplex ultrasound scanning, MRA is increasingly reported to provide results as good as or better than duplex Doppler ultrasonography. Whereas most studies of the accuracy of MRA have been conducted retrospectively by radiologists, and have given very good results, a recent prospective trial evaluated MRA and CT angiography compared with IA-DSA in patients studied consecutively (359). This study yielded reduced values for both sensitivity and specificity (in. 70%) for both MRA and CT angiography in the detection of renal artery stenosis lesions that were deemed to be significant by angiography. MRA is contraindicated for patients with claustrophobia or metallic implants such as cardiac pacemakers, cranial aneurysm clips, cochlear implants, and older types of Starr-Edwards heart valves.

Carbon Dioxide Angiography

Carbon dioxide (CO_2) is currently being utilized not only for diagnostic imaging of the renal artery but also to guide percutaneous interventional vascular procedures. The use of CO_2 angiography was pioneered by Hawkins (360), and the potential for CO_2 to serve as a clinically useful arterial contrast agent has advanced over the past 15 years (310,361,362). In experienced centers, DSA using CO_2 as an intravascular contrast agent has proved to be both safe and clinically useful in the evaluation of patients with renovascular disease. In a dog study in which 5 to 10 times the maximum quantity of CO_2 normally used was injected directly into the renal artery, only minor reductions in blood flow, and no morphologic changes in the kidneys or vascular endothelium were detailed subsequently (363). In most centers, CO_2 angiography is not available, and research is continuing utilizing CO_2 for diagnostic arterial and venous imaging and as a guide to percutaneous vascular interventional procedures in selected centers.

Duplex Ultrasound Scanning

Duplex ultrasound scanning of the renal arteries is a noninvasive screening test for the detection of the anatomic presence of renal artery stenosis. It combines direct visualization of the renal arteries (B-mode imaging) with measurement of various hemodynamic factors in the main renal arteries and within the kidney (Doppler), thus providing both an anatomic and a functional assessment of renal arterial disease. Duplex scanning also allows measurement of kidney size at the same time. Unlike other noninvasive tests, duplex scanning is not affected by medications, the level of renal function, or whether the disease is unilateral, bilateral, or affecting a solitary functioning kidney (47,48,51,364–368). As indicated earlier in this chapter, increasing experience with duplex ultrasound scanning demonstrates an overall sensitivity and specificity when compared with renal arteriography of 98%, with a positive predictive value of 0.99 and a negative predictive value of 0.97 (51). Olin et al. (51) prospectively studied 102 consecutive patients by both duplex ultrasound scanning of the renal arteries and renal arteriography. All patients in the study had difficult-to-control hypertension, unexplained azotemia, or associated peripheral vascular disease, giving them a high pretest likelihood of renovascular disease. Sixty-two of 63 arteries showing stenosis of less than 60% by arteriography were correctly identified by duplex ultrasound scanning. Thirty-one of 32 arteries with 60% to 79% stenosis on arteriography were identified as having 60% to 99% stenosis on duplex ultrasound; 67 of 69 arteries with 80% to 99% stenosis on arteriography were identified as having 60% to 99% stenosis on ultrasound. Twenty-two of 23 arteries with total occlusion on arteriography were correctly identified by duplex ultrasound (Table 52-11) (51).

Of the arteries with an end-diastolic velocity of 150 cm/second or more, 81% (38/47) had 80% to 99% stenosis, while only 19% (9/47) had 60% to 79% stenosis by arteriography. At the present time, most centers experienced in the duplex ultrasound scanning technique are able to discriminate between renal arterial stenoses of less than 60% or of 60% or more by ultrasound. Gradations of stenosis by duplex ultrasound scanning are generally reported as 0% to 59% stenosis, 60% to 99% stenosis, and total occlusion. The ability to discriminate by duplex ultrasound renal arterial lesions causing 60% to 79% stenosis from those causing 80% or more stenosis (most likely the degree of stenosis that is hemodynamically significant) is approaching with advances in duplex ultrasound technology.

Radermacher et al. have validated the renal resistive index (RI) obtained by Doppler velicometry over the intrarenal vessels as a predictor of future renal outcomes in patients with renal transplant nephropathy (369), chronic renal insufficiency (370), and renal artery stenosis (371). The RI is calculated as: (1 − [end diastolic velocity] maximal systolic velocity) ×100. Of 138 patients with renal artery stenosis subject to angioplasty or surgery, the RI scanning procedure was technically successful in 131 patients. The results demonstrated that a RI of >80% reliably identifies patients with renal artery stenosis in whom intervention will not improve renal function, BP, or kidney survival. The index effectively quantitates the microvascular resistance within the kidneys, which is emerging as a critical factor in determining outcome in these patients.

The availability of high-quality duplex ultrasound scanning has dramatically altered the approach to renal artery stenosis in several centers. When the history and physical examination suggest renal artery stenosis, duplex ultrasound scanning may well be the procedure of choice for detecting the anatomic presence of renal artery disease. When duplex ultrasound scanning indicates 0% to 59% stenosis, hemodynamically significant renal artery stenosis is highly unlikely. However, the technique is technically demanding and has a steep learning curve. Each vascular laboratory needs to correlate the results obtained from duplex ultrasound of the renal arteries with arteriography to ensure a reasonable degree of internal correlation. It is important to examine the renal artery from the anterior, lateral decubitus, and at times, posterior approaches so that all segments of the renal artery can be visualized and adequate Doppler samples obtained. Accessory renal arteries are difficult to identify and remain a limitation of the test (51,368). After detecting renal artery stenosis by duplex ultrasound, an angiographic imaging procedure in concert with a maneuver designed to detect the physiologic significance of the renal artery stenosis (e.g., captopril renography, renal vein renin determination) is recommended.

Spiral Computed Tomography Scanning

Spiral (helical) CT scanning with intravenous contrast injection (CT angiography) combines the diagnostic accuracy of formal arteriography with the low-risk of intravenous digital subtraction angiography. In several reports, the spiral CT scan had a sensitivity and specificity for renal artery stenosis ranging from 87% to 98% (sensitivity) and 94% to 98% specificity, respectively (372–374).

In summary, the ideal approach to the choice of appropriate workup in this patient population is to select in advance the specific questions to be answered: (a) is significant renal artery stenosis present? (b) is it bilateral or unilateral? (c) is it associated with and suspected of causing hypertension? (d) is

TABLE 52-11

COMPARISON OF DUPLEX ULTRASONOGRAPHY WITH ARTERIOGRAPHY IN 102 CONSECUTIVE PATIENTS[a]

Percent stenosis by ultrasonography	Percent stenosis by arteriography				Total
	0–59	60–79	80–99	100	
0–59	62	0	1	1	64
60–99	1	31	67	0	99
100	0	1	1	22	24
Total	63	32	69	23	187

[a]Numbers refer to arteries visualized ($N = 187$). The sensitivity was 98%, specificity 98%, positive predictive value 99%, and negative predictive value 97%.
(From: Olin JW, Piedmonte MR, Young JR, et al. The utility of duplex ultrasound scanning of the renal arteries for diagnosing significant renal artery stenosis. *Ann Intern Med* 1995;122:833, with permission.)

it associated with and suspected of causing renal insufficiency? and (e) are we seeking information about the likely outcomes of revascularization? Answers to these questions help in selecting the most useful test in each individual circumstance. Generally, conventional renal angiography is reserved as a prelude to renal revascularization, for example, PTRA, PTRA/stenting or surgical renal revascularization.

ISCHEMIC RENAL DISEASE (ISCHEMIC NEPHROPATHY)

Ischemic renal disease and *ischemic nephropathy* are terms that imply gradual loss of renal function caused by vascular occlusion. Although interrelationships between main renal artery stenosis, arteriolar nephrosclerosis, hypertension, and renal insufficiency have, historically, been appreciated, the notion that main renal artery stenosis might threaten renal function, possibly independent of BP control, is relatively recent. The frequency of atherosclerotic renovascular disease, and in particular the appearance of atherosclerotic renal artery disease in patients with atherosclerosis in other vascular beds and the suggestion that atherosclerotic renal artery stenosis might be responsible for advanced renal failure, particularly in patients older than 50 years (34,239,251,375), and reports of patients coming off dialysis following renal revascularization (233,250,376–38) have contributed to current interest in ischemic nephropathy and enthusiasm for renal revascularization (surgical or endovascular) for preservation or restoration of renal function. Regardless of whether or not renovascular hypertension is likely, the anatomic presence of atherosclerotic renal artery disease as a threat to renal function has become a more pressing concern than that of atherosclerotic renovascular hypertension (28,30,34,381,382). Accordingly, in some centers, trends in surgical revascularization for renal artery disease have shifted from the issue of renovascular hypertension to the issue of preservation of renal function (383).

Ischemic renal disease or ischemic nephropathy may be defined as a clinically significant reduction in GFR due to hemodynamically significant obstruction to renal blood flow, or renal failure due to renal artery occlusive disease. The following case histories are examples of this clinical syndrome.

Case 1

A 62-year-old white woman presented in 1977 with the recent appearance of hypertension and a BP of 170/115 mm Hg. Three years previously, she had been found to have polycythemia vera, and an IVP at that time appeared normal. She had been followed closely until 1977 by her local internist and was always normotensive until the hypertension suddenly appeared. A rapid- sequence IVP demonstrated a reduction in the size of the left kidney from 14.0 to 11.5 cm. Further evaluation demonstrated a serum creatinine level of 2.6 mg/dL and an asymptomatic left carotid bruit. Renal arteriography disclosed a left kidney 11.5 cm in height and 100% stenosis of the left renal artery; the right kidney measured 14.5 cm in height and there was 95% stenosis of the right renal artery (Fig. 52-14). Renal vein renin determinations lateralized strongly to the smaller (left) kidney. The BP was well controlled with propranolol and a diuretic. Right aortorenal reimplantation was undertaken solely to preserve renal function. Postoperatively, the serum creatinine level fell to 1.5 mg/dL and remained at this level for the next 12 years. The BP remained controlled postoperatively with propranolol, 40 mg daily, and a small dose of chlorthalidone. The patient died in 1989 of complications of polycythemia vera, with a serum creatinine of 1.5 mg/dL.

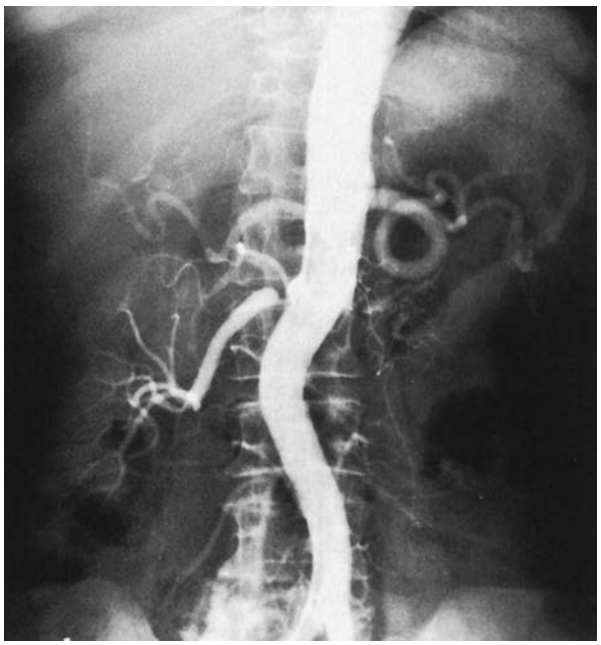


FIGURE 52-14. Case 1: Aortogram of a 62-year-old woman, demonstrating total occlusion of the left renal artery supplying an atrophic left kidney and high-grade ostial stenosis of the proximal right renal artery from atherosclerosis.

Case 2

A 67-year-old white woman, known to have a single (left) kidney and a serum creatinine level of 1.0 mg/dL, was in her usual state of good health until October 1988, when she observed a marked diminution in urine volume after 4 to 5 days of nausea, vomiting, diarrhea, and diminished food and fluid intake, all of which were attributed to viral gastroenteritis. The gastrointestinal symptoms subsided, but oliguria persisted. She sought medical attention and was observed to be severely azotemic. Over the next several days, oligoanuria developed, and acute hemodialysis was initiated. Over the next 6 weeks, there was no increase in urine volume, and regular hemodialysis was required. The patient's nephrologist presumed that she had sustained an episode of severe acute tubular necrosis, but when renal function failed to improve after 6 weeks of hemodialysis, open renal biopsy was performed. The biopsy specimen was surprisingly unremarkable, revealing only mild interstitial fibrosis, intact tubular basement membranes, absence of glomerular sclerosis, and retracted glomeruli consistent with ischemia (Fig. 52-15). There was no evidence of active glomerular proliferation or crescents, and no evidence of vasculitis. Six months later a renal arteriogram demonstrated tight left renal artery stenosis beginning just beyond the takeoff of the renal artery and extending to the first major bifurcation, with evidence of collateral circulation. The patient was referred for further evaluation in June 1989, at which time laminography indicated the left kidney to be 10.5 cm in height, with surprisingly good function demonstrated by DTPA isotope renal scanning. Left renal revascularization (bench repair and autotransplantation) was performed. During the first 4 postoperative weeks, the serum creatinine level gradually fell from the range of 12 to 16 mg/dL to 3 mg/dL, and the urine volumes increased to 1,500 to 2,000 mL/day. There have been no subsequent

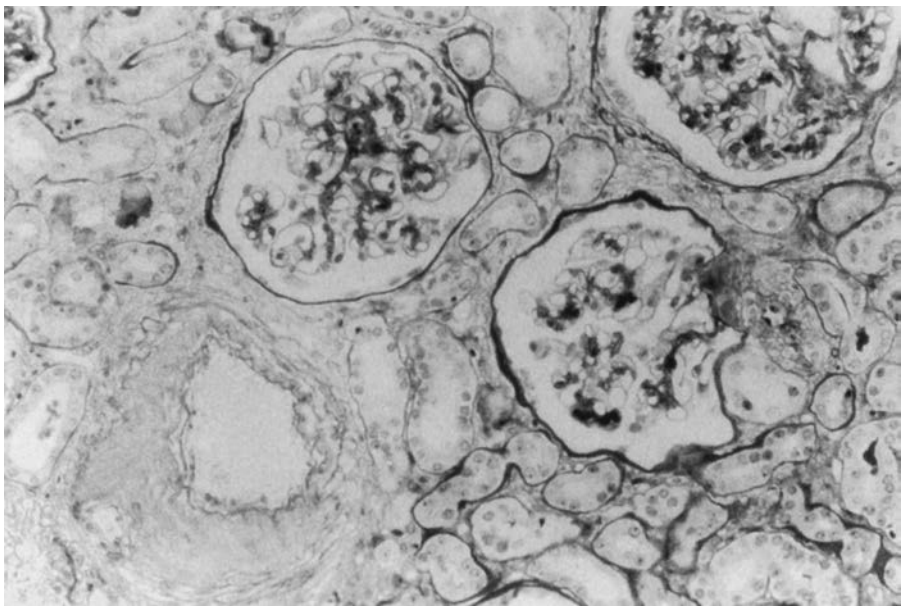


FIGURE 52-15. Case 2: Left renal biopsy specimen obtained after 6 weeks of hemodialysis from a 67-year-old woman who had been anuric. The biopsy specimen shows hypoperfused retracted glomeruli consistent with ischemia. There is no evidence of active glomerular proliferation or glomerular sclerosis. Note the intact tubular basement membranes, and negligible interstitial inflammation. Left renal revascularization resulted in recovery of renal function and discontinuance of dialysis.

dialysis treatments. The serum creatinine level 10 years postoperatively was 2.3 mg/dL.

Discussion

These two patients represent two ends of the spectrum of ischemic nephropathy. In the first patient, surgical revascularization of the right kidney was done solely to improve moderately severe renal insufficiency and preserve long-term renal function, ignoring the prediction that left renal revascularization would cure the hypertension. In the second patient, renal revascularization removed a patient from lifelong dialysis.

The observation of improved renal function after renal revascularization is not new. In 1962, Morris et al. (384) reported on eight azotemic patients with bilateral renal artery stenosis who experienced improvement in renal function following surgical renal revascularization. However, the major indication for operation in this report was the presence of severe associated hypertension—the concept of undertaking renal revascularization primarily to stabilize or improve renal function had not yet arisen. Additional reports in the 1960s and 1970s (133,385) described improvement in renal function in patients undergoing renal revascularization for presumed renovascular hypertension. Although the beneficial effect of surgery on renal function in these reports was in part due to improvement in BP control, the possibility that improved renal perfusion pressure promoted increased GFR was also entertained. Case 1 reflects an approach taken by Novick et al. (250), wherein 51 patients with significant renal artery stenosis and hypertension underwent surgery primarily to preserve or salvage renal function. In all patients, the BP was well controlled preoperatively with antihypertensive drug therapy, and hypertension was not considered an indication for surgery. In 24 of these 51 patients, progressive deterioration in kidney function, despite adequate BP control, was observed during the 6 months prior to surgery (250). Shortly thereafter, Ying et al. (233) described eight patients with bilateral atherosclerotic renal artery disease or unilateral arterial stenosis in a solitary functioning kidney, in whom antihypertensive drug therapy substantially worsened preexisting renal insufficiency despite enhanced BP control; surgical renal revascularization produced improvement or stabilization of renal function in these patients. These investigators concluded that refractory hypertension with renal insufficiency is a common problem and suggested that renal revascularization is warranted, particularly if renal function deteriorates with medical management.

The reports of Novick (1983) (250) and Ying (1984) (233) et al. in conjunction with the advent of PTRA fueled enthusiasm for detecting and correcting main renal artery stenosis to preserve renal function. Reports of the role of PTRA in improving renal function in patients with atherosclerotic renal artery disease were encouraging, and a number of centers reported improvement or stabilization in renal function following surgical renal revascularization or balloon angioplasty in patients with ischemic renal disease. More recently, atherosclerotic renal artery disease has been claimed to contribute to the end-stage renal disease population (251,375–378,380,386,387). Case 2 is representative of this type of patient.

Despite these rewarding anecdotes, most patients who progress to end-stage renal failure in conjunction with main renal artery stenosis have irreversible ischemic parenchymal damage (Fig. 52-16) that precludes recovery of renal function even if main renal arterial blood flow is restored. Many of these patients have associated atheroembolic renal disease (388), severe small-vessel disease (nephrosclerosis), or both, and many patients have secondary focal segmental glomerulosclerosis or global glomerulosclerosis (Fig. 52-17). These pathologic alterations in the renal parenchyma limit the effectiveness of surgical renal revascularization, PTRA, or stenting of the main renal artery in improving renal function (389). The studies of Radermacher utilizing measurement of renal resistance index (RI) demonstrated that a RI >80% predicted an unfavorable effect of renal artery stenting on post stent creatinine clearance, whereas a pre-stent RI <80% was associated with an improved creatinine clearance post renal artery stenting (369). In contrast to the voluminous literature devoted to predicting which patients might gain improvement in BP control following renal revascularization, a clear understanding is lacking about the pathogenesis of ischemic nephropathy on the basis of large-vessel occlusive disease and of which diagnostic tests might predict improvement in renal function following renal revascularization. The relative contributions of main renal artery stenosis, arteriolar nephrosclerosis, glomerular collapse, and interstitial fibrosis—all associated with main renal artery stenosis—await further definition. The renal RI studies of Radermacher (369) and the study of Cheung (390) in patients with total renal artery occlusion of one renal artery and varying degrees of stenosis

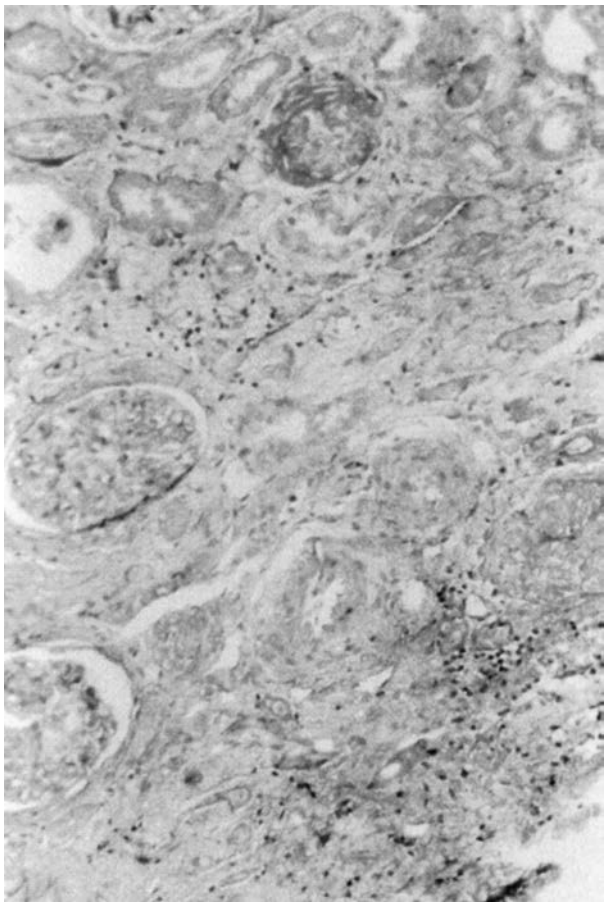


FIGURE 52-16. Pathologic specimen of a kidney from beyond a main renal artery occlusion demonstrating glomerular sclerosis, tubular atrophy, and interstitial fibrosis. The magnitude of glomerular and interstitial scarring is consistent with irreversible loss of kidney viability.

in the contralateral renal artery emphasize the importance of renal parenchymal damage as a major contributor to the overall impairment in GFR (i.e., renal function) in patients with main renal artery stenosis. Further, paradigms applied to acute renal ischemic injury may have limited applicability to chronic vascular injury.

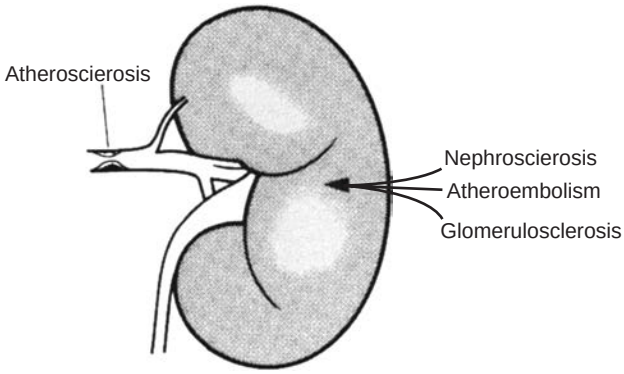


FIGURE 52-17. Schematic representation of ischemic nephropathy. Patients with atherosclerotic artery stenosis often have coexisting renal parenchymal disease with varying degrees of nephrosclerosis (small-vessel disease), atheroembolic disease, or glomerulosclerosis.

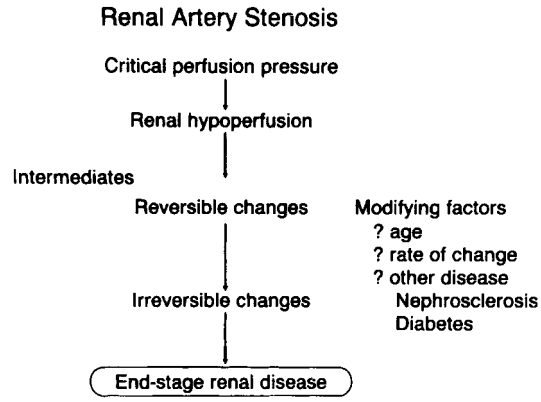


FIGURE 52-18. Theoretical scheme wherein renal artery stenosis leads to end-stage renal disease. Central to this model is the notion that vascular compromise produces intermediate events that injure the kidney. How these events promote "reversible" to "irreversible" tissue damage awaits further clarification. (Adapted from: Textor SC. Pathophysiology of renal failure in renovascular disease. *Am J Kidney Dis* 1994;24:642, with permission.)

The pathophysiology of ischemic nephropathy is incompletely understood. Presumably, a sufficiently severe vascular lesion reduces renal perfusion pressure below a "critical perfusion pressure" when the kidney is perfused at these reduced arterial pressures, a series of changes develop, some irreversible, eventuating in end-stage renal disease (Fig. 52-18). Textor proposes a sequence of events for chronic ischemic renal injury wherein repetitive episodes of renal hypoperfusion might produce irreversible renal parenchymal damage beyond a stenotic main renal arterial lesion (Fig. 52-19). Data to support the role of each of these steps have been obtained from models of acute ischemic renal failure (see Chapter 39), although their role in chronic renovascular disease is not yet established (109,111). Renal fibrosis induced by ischemia evolves with intrarenal ischemia increasing the generation of angiotensin II that is fibrogenic, owing to interaction with endothelin-1 and TGF-beta (391).

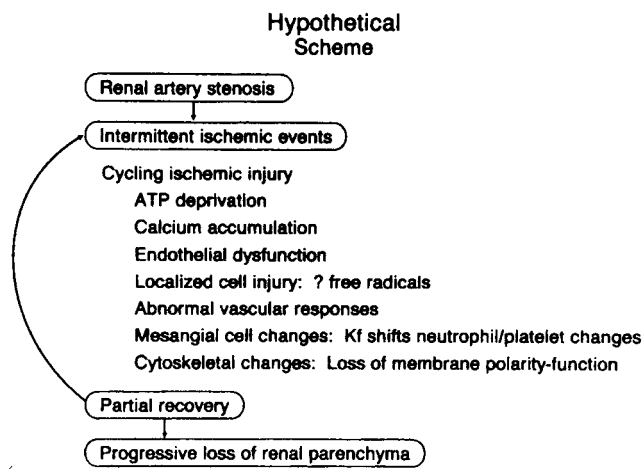


FIGURE 52-19. Proposed sequence of events by which repetitive episodes of renal hypoperfusion might produce irreversible renal parenchymal injury beyond a stenotic arterial lesion. Data to support the role of each step were obtained from models of acute ischemic renal failure, although their role in chronic renovascular disease is not yet established. (Adapted from: Textor SC. Pathophysiology of renal failure in renovascular disease. *Am J Kidney Dis* 1994;24:642, with permission.)

The pathologic changes in the kidney beyond an arterial occlusion include glomerular collapse with loss of tuft volume, tubular atrophy, and interstitial fibrosis. The fibrosis of Bowman's capsule can obstruct the origin of the proximal tubule, leading to atubular glomeruli and tubular atrophy, while fibrosis around tubules themselves likely impairs their function and viability. Loss of tubular structures, interstitial inflammation, and glomerulosclerosis are consistent with irreversible loss of kidney viability (109,392–395). Typical changes of arteriolar nephrosclerosis coexist (Fig. 52-16) (396,397). Experimental studies using progressive renal arterial occlusion in a swine model indicate a complex interaction between atherosclerosis and renal arterial disease in activating fibrogenic pathways of the kidney, potentiating the renal scarring (398,399).

Clinical Presentation

The clinical presentation of patients likely to develop progressive renal failure from atherosclerotic ischemic nephropathy is that of an older (more than 50 years) patient demonstrating progressive azotemia in conjunction with antihypertensive drug therapy, history of cigarette smoking, known renal artery disease, refractory hypertension, and generalized atherosclerosis obliterans (233,250,251). Men or women over the age of 50 who smoke and have unexplained azotemia with mild-to-moderate proteinuria, hypertension (usually), and a bland urinary sediment with few casts or cells are frequently found to have atherosclerotic renal artery disease. Clinically, ischemic renal disease is present in patients with high-grade (greater than 75%) arterial stenosis involving both kidneys or more than 75% arterial stenosis to a solitary kidney. The threat to overall kidney function in patients with high-grade (greater than 75%) unilateral renal artery stenosis in the setting of azotemia, wherein renal functional impairment is most likely due to a combination of nephrosclerosis and unilateral main renal artery stenosis, is less clear (250). Accordingly, an aggressive interventive approach with either surgical or endovascular renal revascularization appears to be most appropriate for patients with high-grade bilateral renal artery stenosis or high-grade renal artery stenosis to a solitary functioning kidney. Clinical clues to bilateral renovascular disease include (a) generalized atherosclerosis obliterans, (b) presumed renovascular hypertension, (c) a unilateral small kidney, (d) unexplained azotemia, (e) deterioration in renal function with BP reduction, particularly with ACE inhibitor therapy (225,251,400), and (f) recurrent episodes of flash pulmonary edema (216–218).

Preserving Renal Function

In considering patients as candidates for revascularization (surgical or endovascular) to preserve renal function, some determination should be made of the potential for salvable renal function. Total occlusion of the renal artery usually eventuates in irreversible ischemic damage of the involved kidney; however, in some patients with gradual arterial occlusion, the viability of the kidney can be maintained through the development of collateral arterial supply (401–404). Clinical clues suggesting renal function preservation include (a) kidney height more than 9 cm (by IVP or renal laminography); (b) evidence of function of the involved kidney, on either intravenous urogram or isotope renography; (c) angiographic filling of the distal renal arterial tree by collateral circulation in patients with total renal arterial occlusion proximally (Fig. 52-20); and (d) a specimen obtained by intraoperative renal biopsy demonstrating well-preserved glomeruli with minimal arteriolar sclerosis (see Case 2) (Fig. 52-15) (89,250,403–407). When these criteria are present, restoration of normal renal arterial flow can result in recovery of renal function.

As has been emphasized, the coexistence of arteriolar nephrosclerosis, secondary glomerulosclerosis, and in many patients atheroembolic renal disease, in conjunction with main renal artery atherosclerotic renal artery stenosis, makes predictions difficult as to which patients will show improvement in

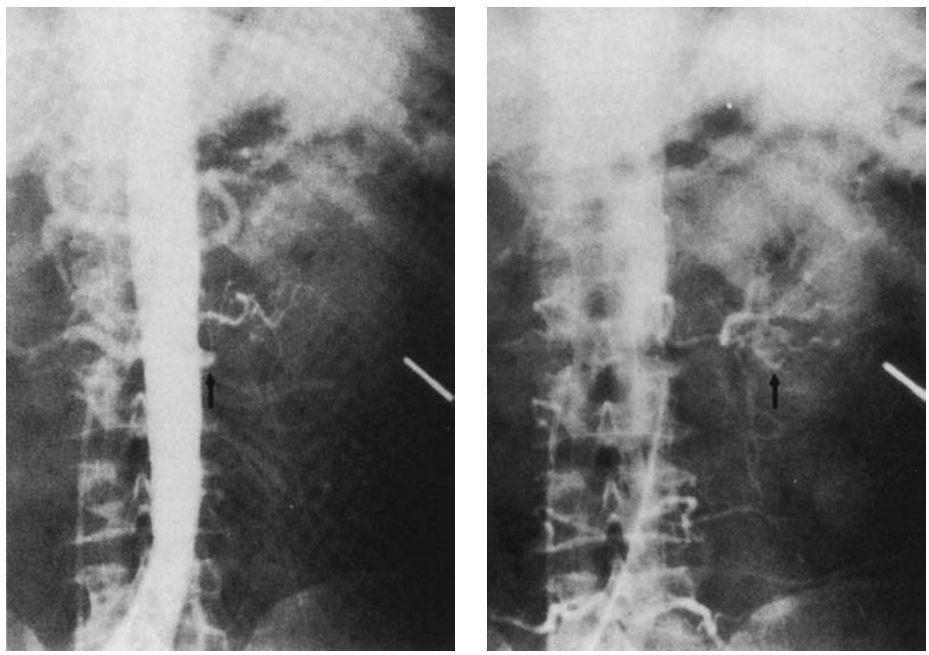


FIGURE 52-20. A: Abdominal aortograms reveal complete occlusion of the left main renal artery, (**B**) with filling of the distal renal artery branches from the collateral blood supply seen on a delayed film. The observation of collateral circulation when the main renal artery is totally occluded proximally suggests viable renal parenchyma.

renal function following surgical or endovascular renal revascularization. Data from the Mayo Clinic of 304 patients who underwent surgical renal revascularization (408) and 44 patients who underwent PTRA389 indicated that approximately 27% improved renal function (serum creatinine decreased more than or equal to 1 mg/dL), 53% had no change in renal function (change in serum creatinine less than 1 mg/dL), and 20% experienced worsened renal function (serum creatinine increased more than 1 mg/dL). Many of the patients who had worsening of renal function developed rapid deterioration in renal function within 1 year of the interventive procedure. Similar observations have been reported by Sandy et al. (409).

In general, surgical or endovascular renal revascularization to preserve renal function in patients with atherosclerotic renal artery stenosis is most likely to be useful in patients who have not yet demonstrated severe and permanent impairment of overall renal function. In a study from the Cleveland Clinic (410) baseline renal function predicted the outcome of surgical revascularization in older adult patients; a preoperative serum creatinine level of less than 3 mg/dL correlated with a stable or improved postoperative serum creatinine level. In contrast, patients with moderately severe azotemia (serum creatinine greater than 3.0 to 3.5 mg/dL) are likely to have severe renal parenchymal disease (Fig. 52-16), which renders improvement in renal function following surgical or endovascular revascularization unlikely (389,411,412). The study of Radermacher (369) using the renal RI measurement prior to endovascular renal revascularization is consistent with these observations. For patients over age 65, in whom serum creatinine concentrations are between 2 and 3 mg/dL indicating severe renal functional impairment, desired benefits on renal function from renal revascularization maneuvers are also frequently disappointing (413,414). Exceptions to these observations are patients with total main renal artery occlusion in whom renal viability is maintained via collateral circulation. In situations of uncertain renal viability, an intraoperative renal biopsy may be helpful. On occasion, we have used the findings by intraoperative kidney biopsy to help guide subsequent decision making regarding surgical revascularization for the goal of improving kidney function (250). Several groups reported their results in such patients, confirming that when criteria indicative of renal function preservation are present, successful revascularization can in fact lead to reversal of renal failure (89,250,404–406,415–422).

In addition to the absolute level of renal function as measured by serum creatinine as a criterion for renal function preservation, the rate of decline in overall renal function was recently suggested as an important determinant of outcome following renal revascularization in atherosclerotic ischemic renal disease. This was particularly true in dialysis-dependent patients for whom dialysis is presumed to be required for ischemic nephropathy (380,423). Dean et al. and Hansen et al. (380,423) suggested that predictors of recovery of renal function in dialysis-dependent patients with atherosclerotic renal artery stenosis are (a) bilateral (vs. unilateral) renal artery stenosis; (b) a rapid rate of deterioration in estimated GFR during the 6 months preceding surgical revascularization; and (c) less severe nephrosclerosis angiographically. Postoperative recovery of renal function was not associated with the severity of renal artery stenosis, preoperative length, or extent of extrarenal atherosclerosis. Other groups suggest that patients presumed to have end-stage renal disease from ischemic nephropathy may be removed from dialysis when there is chronic bilateral total renal arterial occlusion or total occlusion of the artery to a solitary kidney. In these patients, fortuitously, the viability of one or both kidneys has been maintained through collateral vascular supply (see Case 2) (376,421). Unfortunately this clinical presentation is rare, and a less favorable outcome of total arterial occlusion on renal viability is far more common.

End-Stage Renal Disease

The frequency with which main renal artery stenosis causes end-stage renal disease (ESRD) is unclear. Mailloux et al. (378) and Appel et al. (386) suggested that ESRD may be a relatively common consequence of atherosclerotic renal artery stenosis. Appel et al. reported that renovascular disease accounts for 11% of all ESRD patients more than 50 years of age, and 20% of ESRD cases among whites older than 50 years. These figures were rough estimates, however, and there have been no prospective studies carefully addressing the relationship between renal artery disease and ESRD. More likely, the incidence of renovascular disease as a cause of ESRD is much lower as supported by the United States Renal Data System 1997 Annual Data Report (424). In this report, renal artery stenosis or occlusion was listed as the primary disease in only 1.7% of all ESRD incident patients ($N = 305{,}876$) for the years 1991 to 1995. Fatica et al. reported an annualized percentage growth of ESRD attributable to atherosclerotic renal artery disease (ASO/RAD-ESRD) to 12.4% (vs. 8.4% for diabetes mellitus and 5.4% for all-cause ESRD) from 1991 to 1997 (USRDS database), and that the percentage of incident patients with ASO/RAD-ESRD increased from 1.4% to 2.1% over this time period (425).

Documentation of atherosclerotic renovascular disease in patients on dialysis is often plagued with coexisting comorbid conditions that in conjunction with the requirement for dialysis, predict a poor long-term survival (378,386,426). In these patients, at the initiation of dialysis, the most frequently occurring associated comorbid conditions were documented preexisting cardiac disease, peripheral vascular disease, hypertension, cerebrovascular disease and transient ischemic attack, and congestive heart failure. Patients with atherosclerotic renovascular disease had these risk factors at a significantly increased frequency (378) compared to patients on dialysis with renal diagnoses other than ischemic renal disease. Novick et al. described the survival of 25 patients with end-stage renal failure and coexisting atherosclerotic renovascular disease in whom it was presumed that the renal failure was due to main renal artery occlusion (426). Eight of these 25 patients underwent surgical revascularization. At the time of analysis, 6 of the 8 patients were alive (mean survival time 58.2 months) and 2 patients had died (mean survival time 96 months); of the 17 "chronic dialysis" patients who did not undergo surgical revascularization, 3 were alive (mean survival time 29.5 months) and 13 were dead (mean survival time 8.7 months) at the time of analysis.

In view of the increasing median patient age and associated comorbid cardiovascular risk factors that impact poorly on dialysis survival (427–431), one might consider detection and correction of atherosclerotic renal artery stenosis in patients requiring dialysis for the goals of discontinuance of dialysis as well as improved long-term survival. This suggestion warrants close scrutiny, however, because the precise etiology of the ESRD in these reports was frequently unclear, the assumption that coexisting renal artery stenosis produced the renal insufficiency was not proved, and in most cases, irreversible parenchymal damage was present at the time of dialysis, precluding removal of these patients from dialysis following surgical or endovascular renal revascularization. Most patients with ESRD and atherosclerotic renal artery stenosis without total arterial occlusion are not appropriate candidates for revascularization to restore function. In such patients, main renal arterial blood flow is preserved at a reduced level, but the supplied renal parenchyma is without function. The renal parenchymal involvement is, in most situations, severely diseased with

varying degrees of nephrosclerosis (170,171,395,432) or atheroembolic disease (388) in conjunction with severe interstitial fibrosis, tubular atrophy, and glomerular sclerosis. A more encouraging view has been reported by Hansen et al. who have performed surgical renal revascularization in 222 patients; 27 of these patients (12%) who had been dialysis-dependent were removed from dialysis (433).

Hemodynamic Probes

Hemodynamic probes may be helpful in confirming the functional significance of large-vessel occlusive disease. Clearly, such probes need to be designed and tested prospectively, to help identify patients at highest risk for loss of renal function prior to the development of moderate to severe azotemia (i.e., serum creatinine greater than 3 to 4 mg/dL) presumably consequent to main renal artery stenosis. In the 1980s, Textor and associates (224,434) described a test involving an intravenous infusion of sodium nitroprusside that was designed to define the hemodynamic limits posed by vascular disease on overall renal function in patients with main renal artery stenosis. Sixteen hypertensive patients underwent a graded reduction in BP with sodium nitroprusside. Eight patients with high-grade (greater than 70%) unilateral renal artery stenosis tolerated BP reduction (205/103 to 146/84 mm Hg) without a change in either renal plasma flow or GFR. Eight patients with high-grade bilateral renal arterial stenosis had similar BP reduction, but renal plasma flow fell (152 to 66 mL/minute) and GFR declined (38 to 16 mL/minute). Four patients with high-grade bilateral renal artery stenosis who initially experienced a decline in renal plasma flow and GFR were studied after unilateral renal revascularization; a reduction in BP with nitroprusside no longer reduced total renal blood flow or GFR (Fig. 52-21). Six of these 16 patients had been receiving captopril prior to referral, and 5 of these 6 patients developed severe acute renal failure while on captopril therapy. The data from these studies suggest that renal hemodynamic and functional evaluation during graded BP reduction with sodium nitroprusside provides a means of identifying a pathophysiologic limit on perfusion pressure required for renal function in patients with severe renal artery stenosis in whom the entire renal mass is threatened by renal artery occlusion. Despite these appealing observations, the invasive nature of this test and the time necessary to perform it have limited its clinical application in this patient population.

Another hemodynamic probe that might be predictive of loss of renal function is the simple observation of worsening renal function following BP reduction with any type of antihypertensive agent with or without the concomitant use of ACE inhibitor drugs (225,233,239,251,400). Deterioration in renal function with BP reduction, particularly with ACE inhibitor drugs or angiotensin receptor blocking agents, is not only an important clue to the presence of bilateral renovascular disease but also a marker for the hemodynamic significance of the lesion. Acute renal failure with converting enzyme inhibitor therapy may also be seen without main renal artery stenosis and may indicate intrarenal vascular disease (i.e., nephrosclerosis) or occasionally, other types of renal parenchymal damage (435). Whether or not captopril renography, widely employed as a means of detecting renal artery stenosis and predicting that renal artery stenosis is the cause of hypertension, can be utilized to predict which patients are at risk for losing renal function has not been tested. Serial captopril renography could theoretically become a sensitive, noninvasive means of following patients with high-grade renal artery stenosis over time, with recommendations regarding the timing of interventive therapy determined by changes in the captopril renogram in a given patient. This conjecture requires further study.

If a fixed increase in intrarenal microvascular resistance is critical for making ischemic nephropathy or renovascular hypertension irreversible, then how can this be assessed? Two strategies have been proposed, both depending on the demonstration of preserved diastolic blood flow into the kidneys as an index of microvascular function. The first strategy is the use of duplex Doppler velicometry estimates of the resistance index (RI) of the two kidneys. Resistance index can be quantitated by a skilled technician from the profile of blood flow velocities over time in the vessels within the kidneys. The index is calculated from the ratio of maximal systolic to diastolic blood flow velocity (see section on ultrasound testing for details). Using a cutoff RI of 80%, Radermacher et al. (371) investigated 130 patients with renal artery stenosis (RAS) prior to percutaneous transluminal angioplasty and stenting. The odds ratio for worsening of renal function or failure to reduce BP at follow up after 1 year was in excess of 100 for those with an RI >80%. The second strategy is the use of small, pediatric intraarterial catheters to measure the pressure gradient across a stenosis of the main renal artery >70%. The gradient is directly proportional to the resistance offered by the renal vasculature downstream. For example, the *reductio ad absurdum* would be that even a very tight stenosis of 95% would have no gradient

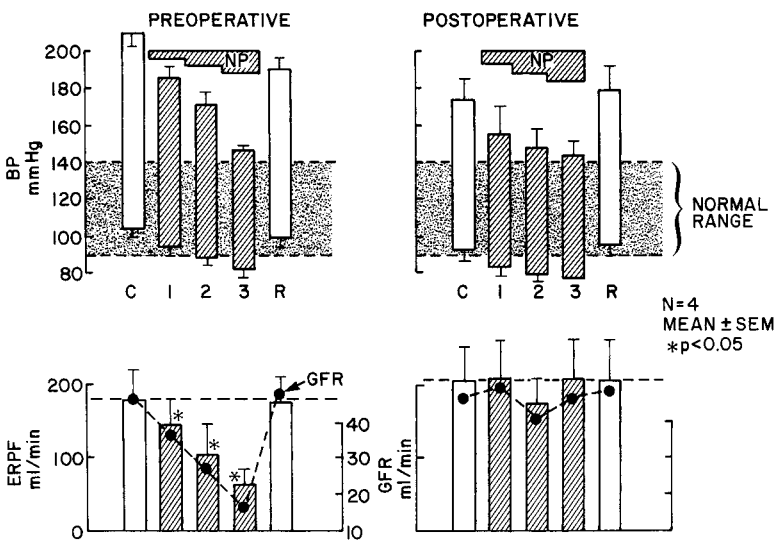


FIGURE 52-21. Blood pressure, renal plasma flow, and GFR in patients with bilateral renal artery stenosis undergoing nitroprusside (NP) infusion before and after renal revascularization. BP, blood pressure; ERPF, effective renal plasma flow. (From: Pohl MA, Novick AC. Natural history of atherosclerotic and fibrous renal artery disease: clinical implications. *Am J Kidney Dis* 1985;5:A120, with permission.)

if a ligature were placed downstream to provide infinite resistance, whereas even a modest stenosis would have a large gradient if the arterioles were widely patent and there was a brisk blood flow run off. The use of the gradient to select patients with preserved diastolic flow implies that the stenosis itself is hemodynamically sufficiently significant to reduce renal perfusion pressure and trigger autoregulatory vasodilatation of downstream renal resistance vessels. Ramos et al. (343) selected a group of patients with >70% stenosis of a main renal vessel due to atherosclerosis and >30 mm Hg maximum pressure gradient across the stenosis. One year after PTRA and stenting, this group experienced either a significant fall in BP if renal function was not impaired, or a significant improvement in renal function if it was initially impaired.

Both of these tests, that is, estimates of renal RI and measurement of pressure gradients, need to be validated by other investigators in prospective studies. The advantage of the RI is that it is entirely noninvasive, but it requires practice and skill. The advantage of the assessment of the stenosis and pressure gradient is that it can be done at the time of arteriography, but it also requires skill and practice and may increase the morbidity of the arteriography procedure.

Ischemic renal disease or ischemic nephropathy is an important clinical problem, particularly in older patients with generalized atherosclerosis obliterans. Noninvasive diagnostic maneuvers to screen for the anatomic presence of renal artery stenosis, particularly renal duplex ultrasonography, have made it possible to readily identify these patients. However, the importance of ischemic nephropathy as a major contributor to progressive renal insufficiency and ESRD remains to be rigorously determined. In some patients, severe renal artery stenosis poses a threat to renal function, and in others it does not. As detailed in this chapter, renal artery stenosis and renovascular hypertension are not synonymous. Similarly, renal artery stenosis and renal insufficiency are not synonymous.

Several factors must be considered in evaluating the renal functional benefit of intervention to relieve arterial obstruction. These include the severity and extent of renal artery obstruction, the level of renal function, the renal histopathologic information, the relative contribution of renal parenchymal damage and main renal artery stenosis to the renal insufficiency, the role of competing risks and high mortality from other cardiovascular diseases, and the merits of medical management of frequently associated hypertension versus renal revascularization in such patients (414,436–438). Although intervention to restore normal renal arterial blood flow may be indicated in selected patients to prevent deterioration of renal function that may culminate in the need for dialysis therapy, and although there is an occasional dialysis-dependent patient in whom renal revascularization might remove from dialysis, a large, prospective, randomized controlled study of patients assigned to receive medical (nonsurgical) therapy or to undergo renal revascularization (surgical or endovascular) is needed to test the hypothesis that atherosclerotic renal artery disease is a correctable cause of progressive renal failure. Several small, prospective, randomized studies of either endovascular or surgical renal revascularization showed no difference in either survival or renal functional outcomes as compared to medical management alone (439,440). A large multicenter study comparing optimal medical management plus stenting to optimal medical management alone is currently in progress (441).

A number of unresolved issues relative to ischemic nephropathy remain: Can we be certain that atherosclerotic renal artery disease is indeed a progressive disorder in a specific individual? For which patients does progression pose a hazard greater than the risks of vascular intervention? What is the optimal time to intervene to preserve renal function in patients with progressive azotemia? That is, is there a "window of opportunity in which one should intervene? And, at what point is it too early or too late to intervene? In patients with controlled hypertension and mild degrees of renal insufficiency, is an aggressive approach for preservation of renal function justified? How often does atherosclerotic renal artery disease cause end-stage renal failure? What is occurring in the renal parenchyma distal to the stenosis, metabolically and structurally? Although the information reviewed herein suggests that interventive therapy might favorably affect the natural course of atherosclerotic renal artery disease and prevent or stabilize ischemic nephropathy, much more information needs to be accumulated and analyzed about this subject.

TREATMENT OF RENOVASCULAR HYPERTENSION AND ISCHEMIC NEPHROPATHY

There are currently four therapeutic options available for patients with presumed renovascular hypertension or renal insufficiency, or both, resulting from renal artery disease. These are (a) medical management, (b) surgical revascularization, (c) PTRA, and (d) renal artery stents. Several factors must be weighed in determining whether medical or interventive management is more appropriate for a given patient. These include the causal relationship of renovascular disease to hypertension, that is, the likelihood that intervention will cure or markedly ameliorate the hypertension; the severity of the hypertension; the adequacy of BP control with medical antihypertensive therapy; the specific type of renal artery disease (fibrous or atherosclerotic); the natural history of untreated renovascular disease with regard for the risk of losing renal function; the general medical condition of the patient; and the known efficacy and risks of medical antihypertensive therapy, surgical revascularization, PTRA, and stents in various clinical subgroups.

The best method of treating patients with presumed renovascular hypertension remains an elusive goal. What constitutes appropriate management—medical, surgical revascularization, endovascular revascularization—is a matter of ongoing debate. Each therapeutic modality has its advocates, advantages, and disadvantages. In addition, factors influencing therapeutic decision making in patients with renal artery stenosis include the expertise and experience in performing surgical renal revascularization or endovascular revascularization (PTRA and stent placement) in an individual medical center. The goals of treatment are to cure or markedly improve the BP, to prevent target organ complications, to reverse or prevent impairment of renal function, and occasionally, to alleviate flash pulmonary edema. The anatomic presence of renal artery stenosis is not necessarily a mandate for intervention.

Surgical Treatment

Surgical treatment of presumed renovascular hypertension includes unilateral nephrectomy, partial nephrectomy (segmental hypoplasia, branch renal artery stenosis), endarterectomy, *in situ* aortorenal bypass procedures, alternative bypass procedures, atherectomy, and extracorporeal microvascular reconstruction and autotransplantation for branch renal artery disease. A detailed discussion of the surgical techniques involved, and results and complications of each of these procedures, is beyond the scope of this chapter.

The indications for primary nephrectomy are limited, and a procedure to preserve renal function as a secondary goal in patients with presumed renovascular hypertension is preferable. Nephrectomy is generally employed to relieve hypertension in the setting of a small (less than 8- to 9-cm pole-to-pole length

by laminography) hyperreninemic kidney or because of a congenitally hypoplastic kidney, atrophic pyelonephritis, surgically uncorrectable renovascular disease, occlusion after attempted surgical or endovascular revascularization, or a nonfunctioning kidney consequent to renal trauma (88,179,180,182,442,443). Partial nephrectomy can be attempted to cure hypertension if there is stenosis of one of multiple distal renal arteries or branches, or to excise a hypoplastic segment (Ask-Upmark kidney). Partial nephrectomy has variable results in improving BP, in part because ischemic zones may remain in nonexcised renal tissue (92,444. Laparoscopic nephrectomy reduces postoperative complications compared to an open major surgical procedure and certainly reduces postoperative recuperation time.

Endarterectomy was the first revascularization procedure described as an alternative to nephrectomy (445). Several techniques of endarterectomy (aortorenal endarterectomy, transaortic endarterectomy, and aortic endarterectomy by transection of aorta) have variable popularity, depending on the experience and preference of the vascular surgeon (446–448). Currently, however, there are several revascularization procedures, most of which employ aortorenal bypass using a saphenous vein or hypogastric artery (446,449–451). The choice of revascularization procedure is influenced by the position and anatomic characteristics of the lesion as well as by the available surgical expertise. Lesions involving the proximal or middle third of the renal artery can generally be revascularized satisfactorily by conventional *in situ* procedures. Lesions involving the distal third of the renal artery or its branches are optimally treated by *ex vivo* reconstructive procedures using microsurgical techniques (452–455). Polytetrafluoroethylene aortorenal bypass grafts have been successfully employed by some surgeons, usually when an autogenous graft is not available (456–458).

In older patients, severe atherosclerosis of the abdominal aorta may render an aortorenal bypass or endarterectomy technically difficult and potentially hazardous to perform. In such cases, some surgeons prefer alternative surgical approaches that allow renal revascularization to be safely and effectively accomplished while avoiding surgery on a badly diseased aorta. Splenorenal bypass for left renal revascularization (459,460) and hepatorenal bypass for right renal revascularization (461) have been effective alternative bypass techniques. The absence of occlusive disease involving the origin of the celiac artery is an important prerequisite for either splenorenal or hepatorenal bypass surgery. A recent study indicates the presence of significant celiac artery stenosis in 50% of patients with atherosclerotic renal artery stenosis (462). This information mandates the importance of preoperative lateral aortography in evaluating the celiac artery origin in patients who are being considered for hepatorenal or splenorenal bypass (Fig. 52-22).

Iliorenal bypass is occasionally used for revascularization in patients with severe aortic atherosclerosis, providing there is acceptable blood flow through the diseased aorta and no significant iliac disease (463,464). Mesenterorenal bypass is occasionally used in patients with a severely diseased aorta in whom a bypass to the kidney from the celiac or iliac arteries is not possible. Ideally, when this procedure is considered, an enlarged superior mesenteric artery, if present, may be employed for arterial bypass to either kidney. When the superior mesenteric artery is sufficiently enlarged to allow for mesenterorenal bypass, there is generally no compromise of intestinal blood flow (464,465).

Use of the supraceliac or lower thoracic aorta for renal revascularization is a new surgical alternative in patients with significant atherosclerosis of the abdominal aorta and its major visceral branches (466–468). The supraceliac aorta is often relatively disease-free in these patients and can be used to achieve renal vascular reconstruction with an interposition saphenous vein graft. Simultaneous aortic replacement and renal revascularization may be recommended for patients with a fixed indication for aortic replacement such as a significant abdominal aortic aneurysm or symptomatic aortoiliac occlusive disease in conjunction with renal artery occlusive disease (469). Several centers reported an increased operative mortality rate with simultaneous aortic replacement and renal revascularization (469–471).

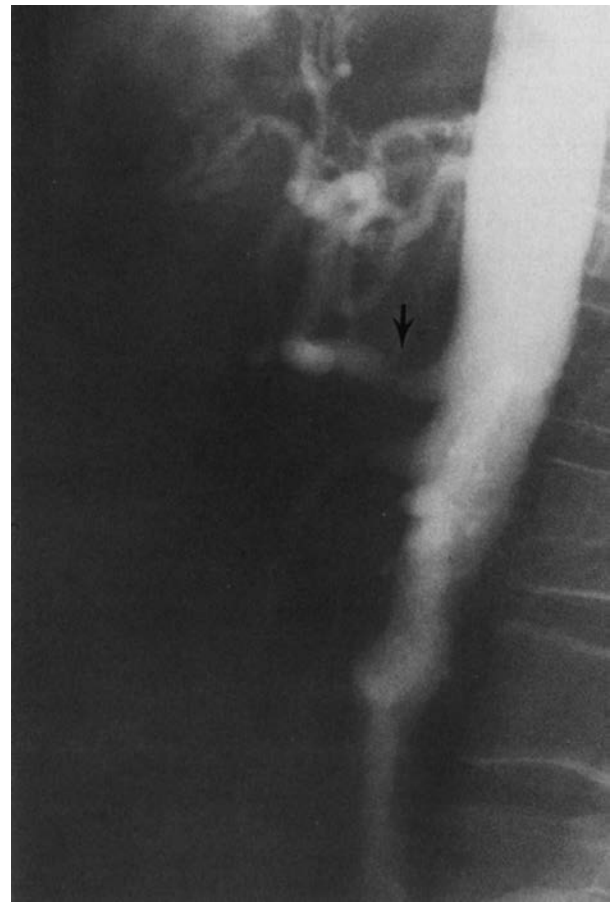


FIGURE 52-22. Lateral aortogram demonstrating a patent celiac artery, a prerequisite for employing hepatorenal or splenorenal bypass procedures.

Reports from several centers indicated that the techniques described above for surgical renal vascular reconstruction can be safely performed with a high technical success rate (383,469,472). The choice of surgical technique depends, to a large degree, on the experience and preference of the surgeon. Patients with fibrous dysplasia are generally healthy, without comorbid risk factors for major visceral vascular surgery; operative morbidity and mortality rates following revascularization in this patient group have been minimal (383,472). On the other hand, operative mortality rates of 2.1% (383), 3.1% (472), 3.4% (473), 5.5% (469), and 6.1% (474) have been reported following surgical revascularization in patients with atherosclerotic renal artery disease. An increased risk of operative mortality has been observed with bilateral simultaneous renal revascularization (475) or when renal revascularization is performed in conjunction with another major vascular surgery such as aortic replacement (469,471).

Most studies have indicated a high technical success rate for surgical vascular reconstruction, with postoperative thrombosis or stenosis rates of less than 10% (383,455,474). In analyzing the results of surgical revascularization for renovascular hypertension and in comparing these results to medical management, most studies considered patients as surgically cured

TABLE 52-12

RESULTS OF SURGICAL SERIES FOR TREATMENT OF RENOVASCULAR HYPERTENSION

Type of renal arterial disease (reference numbers)	No. of patients	Cured (%)	Improved (%)	Failed (%)
Fibromuscular dysplasia (40,431, 460–463,465,466,467,520)	663	59	30	11
Atheroma (focal) (460,463,465,468,575)	382	41	46	13
Atheroma (diffuse) (460,464,468,575)	435	24	45	36

(From: Pickering TG, Laragh JH, Sos TA. Renovascular hypertension. In: Schrier RW, Gottschalk CW, eds. *Diseases of the Kidney*. Boston: Little, Brown, 1993:1451, with permission.)

if the BP was 140/90 mm Hg or less postoperatively. Patients were considered improved if they showed either a reduction in diastolic pressure of 10 to 15 mm Hg or more or became normotensive with medication. Failures of surgical revascularization generally were viewed as those patients who did not qualify for either of the aforementioned criteria for "cured" or "improved."

The results of surgical treatment for presumed renovascular hypertension vary according to the underlying nature of the renal artery disease (Table 52-12) (64,209,228,383,451,472, 476–484,485–487). For patients with fibrous renal artery disease, 50% to 60% are cured, 30% to 40% are improved, and the failure rate is approximately 10%. For example, the Cooperative Study of Renovascular Hypertension, a study of 502 patients who underwent 577 surgical procedures in various centers in the United States published in 1975, reported a favorable BP response in 80% of patients with unilateral fibrous dysplasia, with an operative mortality of 3.4% (209,476). In patients undergoing revascularization for atherosclerotic renovascular disease, the failure rate is somewhat higher, fewer patients are cured, and fewer improve postoperatively. For example, in the Cooperative Study of Renovascular Hypertension, a favorable BP response was observed in 63% of patients with unilateral atherosclerotic stenosis and in 56% of patients with bilateral atherosclerotic stenosis (209,476). The operative mortality rate was 9.3% for atherosclerotic renovascular disease. In the patients with atherosclerotic renal artery disease, the presence of coronary disease or preoperative renal insufficiency (defined by a serum creatinine concentration greater than 1.4 mg/dL) raised the mortality rate to about 23%. Concurrent extrarenal surgery was associated with mortality in the range of 16% to 25% (209,476).

Clearly, fewer patients with atherosclerotic renal artery stenosis are cured of their hypertension in comparison to patients with fibrous dysplasia (Table 52-12). The presumed renovascular hypertension in patients with atherosclerotic renal artery disease either is superimposed on existing essential hypertension or is entirely due to primary (essential) hypertension with coexisting anatomic atherosclerotic renal artery stenosis. On the other hand, a recent study by van Bockel et al. demonstrates excellent long-term results following renal revascularization for atherosclerotic renal artery stenosis; with a mean follow-up of 8.9 years, postoperative hypertension was cured or improved in 83 (79%) of 105 patients (455).

The benefits of surgical treatment should also be assessed by long-term follow-up of BP control, renal function, and patient survival. In over 300 revascularization procedures performed during a 10-year period at the Cleveland Clinic, the operative mortality rate was 2.1% in patients with atherosclerotic disease and 0% in patients with fibrous dysplasia. Long-term cure or improvement in BP was observed in 72.4% of patients, but cured in only 35.7% with atherosclerotic renal artery disease. Hypertension was cured in 93% of patients with fibrous dysplasia. Renal function was preserved or improved in 89% of patients who underwent surgery primarily for improving renal function (383,488). Starr et al. report that during a 20-year follow-up period, the actuarial patient survival rate was 93% at 5 years, 80% at 10 years, and 70% at 20 years (489). Seventy-four percent of these patients remained normotensive at 15 years. Even in elderly patients who are generally considered to be at increased operative risk, surgical revascularization can be successfully accomplished in those properly selected (383,488,490). These results are consistent with an earlier report from the Mayo Clinic (64) indicating the value of operative treatment in properly selected patients with presumed renovascular hypertension. The surgical benefits are inversely proportional to the duration of hypertension, and for patients with atherosclerotic renal artery disease in particular, long-term patient survival appears to be somewhat better for surgically treated patients than for medically treated patients (64).

Taken together, with proper patient selection, that is, high likelihood that the hypertension is renovascular in origin, and in experienced surgical centers, excellent long-term benefits relative to BP control can be achieved from surgical renal revascularization. Further, surgical morbidity has been considerably reduced over the past 30 years with negligible mortality (18,383,479,491–493).

The past 30 years have witnessed more centers performing surgical renal revascularization for the goal of preserving renal function (i.e., to correct or prevent ischemic nephropathy) in patients with high-grade atherosclerotic arterial occlusive disease affecting both kidneys or a solitary kidney. These are generally older patients with diffuse atherosclerosis, ostial renal artery lesions, and varying degrees of renal functional impairment. In 1983, Novick et al. (250) described improved renal function in 34 of 51 (67%) patients in whom BP was controlled medically preoperatively. The indication for surgery in this study was solely to improve or preserve renal function. Renal function improved most dramatically in patients with chronic bilateral total renal arterial occlusion.

In analyzing their data over a 10-year period, Novick et al. reported that indications for surgical renal revascularization fell substantially (41% to 26%) when the goal of surgery was for relief of hypertension alone, and increased substantially (14% to 36%) when the goal of surgery was only to preserve renal function, when this 10-year period was divided into two time segments (1975 to 1980 vs. 1981 to 1984) (378). Studies from several centers (Table 52-13) indicate improvement or stabilization of renal function postoperatively in 75% to 89% of patients (216,239,383,473,474,494–496). A more contemporary series of surgical renal revascularization in 96 patients operated on between 1990 and 2001 describes long-term

TABLE 52-13

RESULTS OF SURGICAL SERIES FOR TREATMENT OF ISCHEMIC NEPHROPATHY

Investigators, year	No. of patients	Surgical outcome, n (%)			
		Improved	Stable	Worse[a]	Death
Luft, et al., 1983 (494)	12	8 (67)	2 (17)	2 (17)	2 (17)
Jamieson, et al., 1984 (495)	23	15 (65)	0 (0)	8 (35)	4 (17)
Novick, et al., 1987 (383)	153	93 (61)	50 (33)	10 (6)	5 (3)
Hansen, et al., 1989 (496)	25	12 (48)	11 (44)	2 (8)	2 (8)
Messina, et al., 1992 (216)	17	12 (71)	2 (12)	3 (18)	1 (6)
Bredenberg, et al., 1992 (473)	25	9 (36)	12 (48)	4 (16)	NA
Libertino, et al., 1992 (474)	97	45 (46)	31 (32)	21 (22)	6 (6)
Total	352	194 (55)	108 (31)	50 (14)	20 (6)

NA, not available.
[a]Includes all deaths.
(Adapted from: Rimmer JM, Gennari FJ. Atherosclerotic renovascular disease and progressive renal failure. *Ann Intern Med* 1993;118:712, with permission.)

clinical success in the preservation of renal function in 70% of patients, with prediction of long-term success being the initial postoperative response in renal function and bilateral renal artery repair (497). Considering the potential risks of progressive occlusive disease and renal failure (ischemic nephropathy) that have been associated with medical management in similar patients, these results demonstrate a favorable influence of surgical revascularization on the natural history of untreated atherosclerotic renal artery disease.

Before recommending surgical revascularization for patients with atherosclerotic renal artery disease, one must appreciate that these patients are at high risk because of age and frequently associated coronary, cerebrovascular, or peripheral vascular disease (37,437,438,469). Their aortas are often laden with extensive atherosclerotic plaque, making angiographic investigation hazardous. Technical difficulties and complications of angiographic investigation in these patients include contrast medium-induced acute renal failure and atheroembolic renal disease. Spontaneous atheroembolic renal disease or atheroembolic renal disease associated with angiography or PTRA is not uncommon in this patient population (388,498). It is well known that coronary heart disease is often the determining factor affecting early and late mortality after elective surgical reconstruction of the abdominal aorta for an aneurysm or for occlusive peripheral artery disease (499–501). Surgical morbidity and mortality in patients with atherosclerotic renal artery disease can be minimized by selective screening and correcting significant coexisting coronary and cerebrovascular disease before undertaking elective surgical renal revascularization, either for the goal of improving BP or for ischemic nephropathy in patients with atherosclerotic renal artery disease (383,501,502).

Percutaneous Transluminal Renal Angioplasty (PTRA)

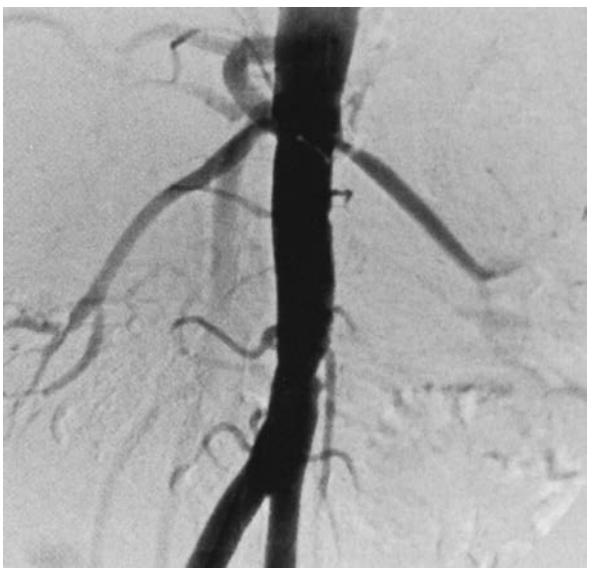
Since the introduction of percutaneous transluminal renal angioplasty (PTRA) by Dotter and Judkins in 1964 (503) and the subsequent development of a modified technique by Gruntzig et al. in 1978 (483), considerable interest has ensued in the application of this procedure to patients with renal artery stenosis. PTRA has the advantages of avoiding general anesthesia, the ability to be repeated, and a shorter duration of hospitalization. Many reports have reviewed the role of PTRA in patients with renal artery disease, and some have suggested that PTRA should be the initial treatment of choice for all patients with renal artery stenosis, atherosclerotic or fibrous, whether the indication for intervention is for presumed renovascular hypertension or ischemic nephropathy or both. The technique of PTRA, efficacy in the treatment of renovascular hypertension due to fibrous dysplasia or atherosclerotic renal artery disease, experience in transplant renal artery stenosis, and complications of PTRA are reviewed in Chapter 14.

In many instances, it is difficult to interpret and compare information from various published series, owing to differences in methodology and reporting of data. Many studies employed different patient selection criteria in terms of the degree of stenosis necessary for treatment. They used different definitions of BP response to treatment and variable post-PTRA follow-up intervals to categorize BP response to PTRA. Many reports did not provide pre- and post-PTRA renal function data. The distinction between technical success rates (e.g., residual stenosis, reduction of pressure gradient before versus after PTRA) and clinical success (improvement in BP, improvement in serum creatinine) following PTRA is not always clear. Few studies provided follow-up angiographic results on all patients treated with PTRA, and long-term results regarding BP control and renal function are often lacking. Results in specific patient subgroups such as those with ostial versus those with nonostial atherosclerotic lesions have not always been reported. For these reasons, including the lack of prospective randomized comparative data, it is difficult to compare the efficacy of PTRA with that of surgical vascular reconstruction for renal artery disease.

In general, data on technically successful dilation in patients with atherosclerotic renal artery disease have been excellent (70% to 90%) for nonostial plaques located exclusively within the renal artery (Fig. 52-23) (39,40,485). On the other hand, ostial lesions have responded poorly to PTRA, with an average technical success rate of 30% to 50% (39,485,504). A higher incidence of recurrent renal artery stenosis after PTRA is observed in patients with ostial lesions than in those with nonostial lesions (74% vs. 55%) (504). An earlier study by Cicuto et al. emphasized that PTRA provides less effective treatment for ostial atherosclerotic lesions than for nonostial atherosclerotic lesions (505).

Canzanello et al. reported a large experience with PTRA in the management of 100 consecutive patients with presumed renovascular hypertension due to high-grade (greater than 75%) atherosclerotic renal artery stenosis (40). Technical success was determined from an immediate post-PTRA arteriogram, and clinical success was determined from the BP response at a mean follow-up of 35 months. In the overall group

A



B

FIGURE 52-23. A: Intraarterial digital subtraction aortogram showing severe proximal right renal artery stenosis and moderately severe narrowing of the left renal artery due to atherosclerosis. **B:** Balloon angioplasty of the right renal artery was successfully performed, with reduction in the pressure gradient across the stenotic lesion from 150 mm Hg before to 10 mm Hg after the procedure. Repeat aortogram 2 years later demonstrated patency of the right renal artery.

of 100 patients, the technical success rate of PTRA was 73% (nonostial 72%, ostial 62%), and the clinical success rate (BP cured or improved) was 43% (nonostial 53%, ostial 25%). Post-PTRA angiographic studies indicated that restenosis was the main cause of failure in patients with ostial lesions.

Ramsay and Waller (506) summarized the results of PTRA as treatment for renovascular hypertension in 10 large studies published from 1981 to 1987 (Table 52-14) (39,485,507–514). PTRA was technically unsuccessful in 84 (12%) of 691 patients. Excluding technical failures and patients lost to follow-up, BP response data were available for 391 patients with atheromatous renal artery stenosis and 175 patients with fibrous renal artery disease. These aggregate data indicated post-PTRA cure, improvement, and failure rates of 50%, 42%, and 9%, respectively, in patients with fibrous disease; the aggregate post-PTRA cure, improvement, and failure rates in patients with atheromatous renal artery lesions were 19%, 52%, and 30%, respectively. The pooled analysis of these 10 studies supports the notion that the outcome of PTRA depends on the type of renal artery disease treated. In all studies, cure rates were higher for fibromuscular disease than for atherosclerotic disease. In patients in whom angioplasty was technically successful, the overall cure rate for fibrous renal artery disease was 50%. Accordingly, for fibrous renal artery disease, many centers view PTRA as the treatment of choice when intervention is pursued for presumed renovascular hypertension. For atheromatous disease, the cure rate after technically successful angioplasty was only 19%.

Brawn and Ramsay indicated that in completely unselected patients, atherosclerotic lesions were associated with a very high rate of technical failure, as high as 60% (515). The true cure rate might, therefore, be substantially lower than 19% for atheromatous renal artery disease. A more current review from the Mayo Clinic described the technical results and clinical outcome following renal artery angioplasty in 320 patients (389). The investigators observed significant reductions in mean arterial pressure and in antihypertensive medications after PTRA. The percentage of patients who benefited after renal artery angioplasty was 70% for patients with atherosclerotic renal artery disease (8.4% cured) and 63% for patients with fibrous renal artery disease (22% cured). No significant overall change in serum creatinine level was noted after the procedure in any group. The authors concluded that PTRA rarely leads to a "cure" of hypertension, but provides effective control of BP and decreases the medication requirements in selected patients.

Three prospective comparisons of PTRA versus medical therapy for hypertensive patients with atherosclerotic renal artery stenosis failed to demonstrate substantial benefits in BP control following PTRA (46,439,516). Webster randomized 55 patients to either PTRA or medical management; there was no benefit of PTRA in patients with unilateral renal artery stenosis in comparison to the medically treated patients. A modest benefit on BP was observed in patients with bilateral renal artery stenosis, particularly a reduction in systolic BP (439). In the DRASTIC study, van Jaarsveld randomly assigned 106 hypertensive patients with atherosclerotic renal artery disease to medical antihypertensive drug therapy versus PTRA. According to intention to treat analysis, at 12 months there were no significant differences between the PTRA and drug therapy groups in systolic and diastolic BPs, daily drug doses, or renal function (46). The results of this study were confounded by about 40% of patients initially allocated to medical treatment who eventually received PTRA. The Plouin study was particularly notable for randomizing patients who, by clinical criteria, had a high likelihood of substantial improvement in BP following renal artery intervention (516). Taken together, these studies indicate that renal angioplasty is no more effective for control of BP than antihypertensive drug therapy alone in patients with atherosclerotic renal artery stenosis.

The limited benefit of PTRA on BP in these studies was probably not attributable to restenosis. Restenosis did occur in about half of the patients who underwent PTRA, but there was no difference in BP control or renal function between patients who had restenosis and in those who did not (46,516). Currently available potent antihypertensive drugs, not available in the early days of PTRA, might have contributed to the negative results of these randomized trials. The tendency toward

TABLE 52-14

SUMMARY OF OUTCOMES AFTER ANGIOPLASTY IN 10 PUBLISHED SERIES OF HYPERTENSIVE PATIENTS ACCORDING TO TYPE OF RENAL ARTERY DISEASE (ATHEROMATOUS OR FIBROMUSCULAR RENAL ARTERY STENOSIS)[a]

	Atheromatous renal artery stenosis				Fibromuscular renal artery stenosis			
	Technically successful angioplasty	Blood pressure response			Technically successful angioplasty	Blood pressure response		
Investigators		Cured	Improved	Failure		Cured	Improved	Failure
Martin, et al. (513)	13	2 (15)	4 (31)	7 (54)	8	5 (63)	1 (13)	2 (25)
Colapinto, et al. (510)	44	8 (18)	29 (66)	7 (16)	9	4 (44)	5 (56)	0 (0)
Geyskes, et al. (511)	44	4 (9)	19 (43)	21 (48)	21	10 (48)	10 (48)	1 (5)
Sos, et al. (39)	34	7 (21)	10 (29)	17 (50)	27	16 (59)	9 (33)	2 (7)
Tegtmeyer, et al. (485)	61	15 (25)	46 (75)	0 (0)	27	10 (37)	17 (63)	0 (0)
Miller, et al. (512)	34	5 (15)	15 (44)	14 (41)	13	11 (85)	2 (15)	0 (0)
Martin, et al. (513)	60	9 (15)	30 (50)	21 (35)	20	5 (25)	12 (60)	3 (15)
Kaplan-Pavlohcic, et al. (514)	48	11 (23)	21 (44)	16 (33)	21	10 (48)	8 (38)	3 (14)
Kuhlmann, et al. (507)	31	9 (29)	15 (48)	7 (23)	22	11 (50)	7 (32)	4 (18)
Bell, et al. (508)	22	3 (14)	13 (59)	6 (27)	7	5 (71)	2 (29)	0 (0)
Totals	391	73 (19)	202 (52)	116 (30)	175	87 (50)	73 (42)	15 (9)
Range (%)		9–29	29–75	0–54		25–8	13–63	0–25

[a]Results are numbers (percentages) of patients.
(From: Martin EC, Mattern RF, Baer L, et al. Renal angioplasty for hypertension: predictive factors for long-term success. *AJR Am J Roentgenol* 1981;137:921, with permission.)

improvement in systolic BP compared to medical therapy alone in patients undergoing PTRA in the Webster study raises the question of which BP level (systolic or diastolic) should be treated, or treated preferentially, in hypertensive patients with atherosclerotic renal artery stenosis. Consistent with this thought are the observations of Burket et al. (35) who observed significant improvement in systolic BP among patients with highest baseline systolic BP treated with renal artery angioplasty and stent placement.

There are relatively few published reports on the results of PTRA undertaken primarily for preservation of renal function in patients with atherosclerotic renal artery disease. Donovan and coauthors reported stabilization or improvement of renal function in 11 of 17 patients, most of whom had nonostial lesions (517). Pickering et al. and Sos et al. reported improvement or stabilization of renal function in 45 (82%) of 55 patients who underwent PTRA for atherosclerotic renovascular hypertension and azotemia (518,519). The outcome of angioplasty in ischemic renal disease is summarized in Table 52-15 (494,508,517,518). Paulsen et al. described their results with 227 PTRA procedures of 223 stenoses in 135 patients performed between 1982 and 1993 at a single center; improved renal function was achieved in 23% of patients, stabilization in 56%, and failure in 21%. Stabilized or improved renal function was higher when baseline serum creatinine was less than or equal to 250 μmol/L than when it was greater than 250 μmol/L (520).

The potential benefits of angioplasty must be assessed in comparison to the risks. *Complications* of transluminal angioplasty of the renal arteries are listed in Table 52-16. The most common complications have been hematoma (4% of patients) and transient worsening of renal function from contrast media (518). With angioplasty done in conjunction with IA-DSA, the dye load is reduced and contrast medium-induced acute renal failure is observed less frequently. Dissection of the intima of the renal artery occurs in 5% of patients, and usually resolves spontaneously, although it may require emergency surgery (508).

TABLE 52-15

ISCHEMIC RENAL DISEASE: OUTCOME OF ANGIOPLASTY

		Angioplasty outcome, *N* (%)			
Investigators, year	No. of patients	Improved	Stable	Worse[a]	Death
Luft, et al., 1983 (494)	12	3 (25)	5 (42)	4 (33)	0 (0)
Pickering, et al., 1986 (518)	55	26 (47)	19 (35)	10 (18)	NA
Bell, et al., 1987 (508)	20	7 (35)	10 (50)	3 (15)	0 (0)
O'Donovan, et al., 1992 (517)	17	9 (53)	2 (12)	6 (35)	5 (29)
Total	104	45 (43)	36 (35)	23 (22)	5 (5)

NA, not available.
[a]Includes all deaths.
(Modified from: Rimmer JM, Gennari FJ. Atherosclerotic renovascular disease and progressive renal failure. *Ann Intern Med* 1993;118:712, with permission.)

TABLE 52-16

COMPLICATIONS OF TRANSLUMINAL ANGIOPLASTY OF THE RENAL ARTERY OR RENAL ARTERY STENTING

Contrast medium-induced acute renal failure (mild or severe)
Atheroembolic renal failure
Rupture of the renal artery
Dissection of the renal artery
Thrombotic occlusion of the renal artery
Occlusion of a branch renal artery
Balloon malfunction (may lead to inability to remove balloon)
Balloon rupture
Puncture site hematoma, hemorrhage, or vessel tear
Median nerve compression (axillary approach)
Renal artery spasm
Mortality (<1%)

The most worrisome complication of angioplasty of the renal arteries is that of cholesterol emboli. Cholesterol emboli are seen mainly in patients with diffuse atheroma and may be the cause of deterioration in renal function, with or without contrast medium-induced acute renal failure. The mortality rate related to angioplasty of the renal arteries is exceedingly low. In the series of Pickering et al., in 55 high-risk patients, the mortality rate was 0% (518); in the series of Canzanello et al., the mortality rate was 2% (40). In the Mayo Clinic report of 320 patients, the 30-day all-cause mortality rate was 2.2%; all deaths occurred in patients with atherosclerotic renal artery disease (389).

The published data on PTRA in patients with renal artery stenosis due to fibrous dysplasia are excellent and in general, equivalent to those obtained with surgical revascularization. Therefore, PTRA appears to be the treatment of choice, particularly for medial fibroplasia. However, as many as 30% of patients with medial fibroplasia or perimedial fibroplasia have disease in the distal main renal artery, often extending into renal artery branches, which increases the technical difficulty of PTRA and may render this procedure technically difficult or hazardous to perform. Some focal fibrodysplastic lesions that are confined to one or two accessible branches may be managed successfully with PTRA. However, surgical renal revascularization is probably the primary interventive treatment for most patients with branch renal artery disease.

In patients with nonostial atherosclerotic renal artery lesions, the technical and clinical success rates of PTRA have been excellent and comparable to those for surgical revascularization in patients with nonostial lesions. Unfortunately, nonostial lesions comprise only 15% to 25% of all atherosclerotic renal artery lesions. For the more commonly encountered ostial atherosclerotic lesions, the efficacy of PTRA is less favorable, and as indicated previously, restenosis rates are substantial. Whether patients with ostial atherosclerotic disease should undergo attempted PTRA versus surgical revascularization versus renal artery stenting has been a matter of local expertise and preference. Currently, most patients with ostial atherosclerotic lesions that undergo endovascular intervention will undergo primary renal artery stenting (PTRA plus stent) as opposed to PTRA alone.

Controlled trials comparing PTRA versus surgical revascularization are sparse. Weibull and co-workers reported the results of a recent single-center randomized prospective study comparing PTRA with surgical revascularization as treatment for unilateral ostial atherosclerotic renal artery stenosis in 58 patients with severe hypertension (521). Detailed clinical and angiographic follow-up data were available on all patients in this study. PTRA was technically successful in 24 (83%) of 29 patients. Primary renal artery patency was achieved in 18 of these patients after 2 years, and 5 additional patients with recurrent stenosis underwent successful repeat PTRA. There was no difference in follow-up BP response between patients treated with PTRA and those treated with surgery. This report suggested a better outlook for PTRA in patients with ostial atherosclerotic renal artery lesions than has been the experience from other centers.

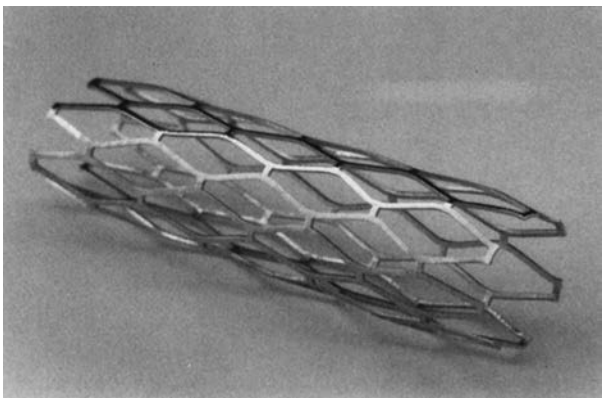


FIGURE 52-24. Palmaz stent, expanded.

Renal Artery Stents

Much of the current work in the area of renal angioplasty is focused on catheter-delivered expandable metallic stents that mechanically hold open the vascular lumen. Metallic stents for intravascular use were first proposed by Dotter in 1969, employing simple stainless-steel coils (522). Subsequently, many other stent devices have been created, with variable success. Currently, there are three basic categories of stent designs: shape-memory alloy stents, balloon-expanded stents, and self-expanding stents. The Palmaz stent (Fig. 52-24) is a balloon-expandable stent developed in the 1980s by Julio Palmaz (523). This stent was initially approved for use in the United States by the Food and Drug Administration (FDA), specifically for the iliac and coronary arteries. Trials on use of the Palmaz stent in renal arteries have been in progress for the past decade (524).

The basic concept of stents (Palmaz stent, Strecker stent, Wallstent) is similar. They are composed of metallic wires or struts that can be collapsed and affixed to a catheter for the purpose of insertion. In most models, the unexpanded stent is mounted on the uninflated balloon of an angioplasty catheter. The stent mounted on the balloon is then positioned across the lesion, and the balloon is inflated. This maneuver expands and deploys the stent. The balloon is then deflated and removed, with the expanded stent left in place (Fig. 52-25). Over the next several weeks, endothelialization occurs, and the stent struts become covered with intima (525,526).

The rationale for renal artery stent placement relates to the generally poor technical results of balloon angioplasty (PTRA), particularly for ostial atherosclerotic lesions, with significant residual stenosis and high short-term restenosis rates characteristic of balloon angioplasty of orificial atherosclerotic renal artery lesions. Ostial renal artery stenoses consist of two entities: (a) aortic plaque encroaching on the renal artery orifice, and (b) disease of renal artery origin. Balloon angioplasty works by "controlled trauma." The atheroma is fractured longitudinally and circumferentially. Soon after deflation of the balloon, the atheroma recoils, and stenosis recurs. Stents are useful in preventing this recoil (527).

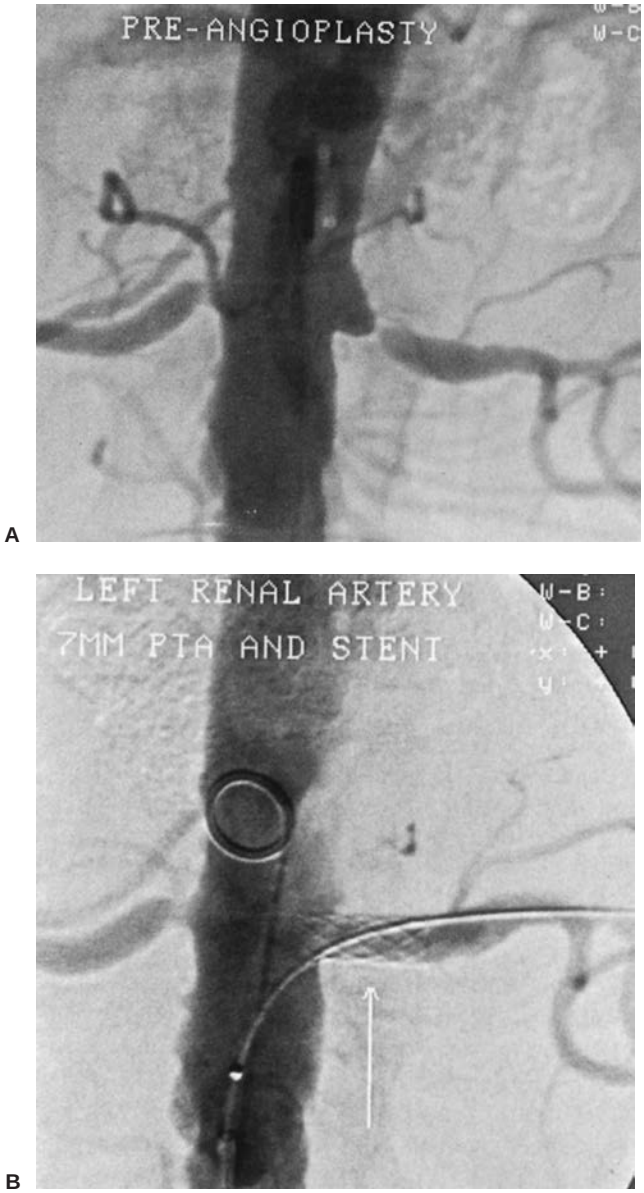


FIGURE 52-25. A: Intraarterial digital subtraction aortogram (before angioplasty) showing bilateral orificial renal artery stenosis, more severe on the left, due to atherosclerosis. **B:** Balloon angioplasty of the left renal artery was performed but significant stenosis remained. A Palmaz stent restored luminal caliber to normal.

Current clinical trials on the placement of stents in renal arteries are focused on lesions where the technical results of balloon angioplasty alone are unsatisfactory; that is, there is a significant residual stenosis commonly occurring in orificial lesions or significant postangioplasty dissection. Renal artery stents are therefore best suited when there are ostial stenoses, restenosis following PTRA, and complications of PTRA. Accumulating data suggest that use of stents for ostial stenoses has a higher initial technical success rate, a greatly improved immediate patency rate, and lower restenosis rate in comparison to balloon angioplasty alone (528–532). Most angiographers now consider the accumulating information sufficiently impressive to justify primary placement of stents for ostial or nonostial disease regardless of the results of angioplasty (38,533).

Several other factors affect the decision to utilize balloon angioplasty or primary renal artery stent placement as treatment for ostial atherosclerotic disease. Balloon angioplasty does not usually affect subsequent surgical renal artery revascularization, but a stent in the ostial location prevents the performance of aortorenal endarterectomy, a procedure that is popular in some centers. A proximally placed renal artery stent will not usually interfere with most surgical bypass procedures, unless the stent extends near the branching of the main renal artery. Placement of stents in the distal main renal artery will severely limit the options for subsequent surgical reconstruction.

The complications of stent placement are the same as those of PTRA (Table 52-16), most importantly atheroembolic renal disease and contrast medium-induced acute renal failure. Additional problems with stent placement include malpositioning, migration, embolization of the stent, and difficulty removing the balloon from the stent (534).

Results of stent placement in the renal arteries are currently being generated and are encouraging. Data presented in 1994 by Rees et al. in the United States Multicenter Trial reported on 304 stents placed in 296 renal arteries in 263 patients (535). The technical success rate was 95%, with 80% of the lesions in the ostial location and 98% of the lesions atherosclerotic. At 6-month follow-up, 64% of patients had improvement or cure of hypertension. In 123 patients with renal insufficiency (serum creatinine greater than 1.5 mg/dL), renal function improved in 34%, remained stable in 39%, and deteriorated in 27%. Angiographic follow-up at 6 months was performed in 150 patients, unfortunately showing restenosis in 32.7%.

Olin et al. and Bacharach et al. deployed renal artery stents for ostial atherosclerotic renal artery stenosis in approximately 120 patients (490,536). The mean clinical follow-up period was 13.8 months, with a maximum follow-up of 36.5 months. The 6-month patency rate by angiography and duplex ultrasonography was 95%, with a 12-month patency rate of 82%. When restenosis was identified by routine duplex ultrasound surveillance studies, the patients underwent repeat arteriography and the stent was reexpanded if significant stenosis was present. The primary assisted patency rate was 100%. The BP was improved in approximately 85% of patients. Renal function improved in 35% of patients, remained stable in 35%, and worsened in 30% of patients. Complications included nine pseudoaneurysms, four episodes of acute tubular necrosis, and three episodes of clinically significant atheromatous embolization. Additional results reported by Dorros et al. in a multicenter Palmaz stent renal revascularization registry describe a 4-year follow up of more than 1000 stented patients (537).

A recent study selected 105 patients for intervention on the basis of clinical criteria, positive Doppler flow studies, a transstenotic pressure gradient of >30 mm Hg with >70% renal artery narrowing at arteriography (343). At a mean follow-up of 1 year, systolic and diastolic BP were reduced significantly (p <0.0001) in those with normal or mildly reduced renal function (GFR >50 mL/min). In patients with an initial reduction in GFR, the calculated GFR increased significantly (p <0.007) from 33 ± 10 to 54 ± 24 (mean ± SD) mL/minute. The authors concluded that in patients with atherosclerotic RAS fulfilling these strict criteria for severity, there may be a favorable BP response following angioplasty and stenting in those with normal renal function prior to stenting and a favorable renal function response in those with impaired renal function prior to stenting.

A review of renal artery stenting in 10 studies is presented in Table 52-17 (538). This report did not offer comparisons of stenting with angioplasty or with surgery. Overall, stents were placed in 416 renal arteries in 379 patients, mean age 64 years. Of the stenoses, 97% were atheromatous with 80% being ostial lesions. Technical success was reported in 96% to 100% of procedures. Restenosis (greater than or equal to 50% narrowing), evaluated in 312 of 416 (75%) arteries, generally between 6 and 12 months, was 16% overall. Hypertension was

TABLE 52-17A

RENAL ARTERY STENTING—BASELINE CLINICAL DATA IN 10 STENT STUDIES

Investigator, year	No. of patients/ no. of arteries	Age (range)	Male (%)	Atheromatous (%)	Ostial (%)	Bilateral (%)
Rees, et al., 1991 (524)	28/28	66 (48–80)	46	100	100	21
Hennequin, et al., 1994 (611)	21/21	55 (27–74)	52	71	33	24
Raynaud, et al., 1994 (612)	18/18	58 (31–77)	56	83	22	44
Dorros, et al., 1995 (613)	76/92	67 (37–84)	53	100	100	21
van de Ven, et al., 1995 (614)	24/28	66 (45–81)	54	100	100	87
Rundback, et al., 1996 (615)	20/24	70 (57–84)	45	100	92	20
Henry, et al., 1996 (616)	59/64	65 (27–84)	66	93	53	12
Blum, et al., 1997 (617)	68/74	60 (31–80)	65	100	100	18
Boisclair, et al., 1997 (618)	33/35	63 (37–77)	42	100	54	45
Harden, et al., 1997 (619)	32/32	67 (49–79)		100	75	78
Total (all 10 studies)	379/416	64 (27–84)	97	56	80	31

(Adapted from: Isles CG, Robertson S, Hill D. Management of renovascular disease: a review of renal artery stenting in 10 studies. *QJM* 1999;92:159, with permission.)

cured by stenting (diastolic BP less than or equal to 90 mm Hg without drugs) in 34 of 379 (9%) patients overall and in 34 of 207 (16%) patients whose renal function was normal initially. Renal function, as judged by serum creatinine concentration improved in 26%, stabilized in 48%, and deteriorated in 26% of patients whose renal function was impaired initially (serum creatinine greater than 133 μmol/L).

Similar results were reported by Leertouwer and associates in a meta-analysis of 14 studies dealing with renal artery stent placement involving 678 patients (532). This report also compared, by meta-analysis, PTRA (10 articles, 644 patients) with the stent meta-analysis. Studies published well into 1998 were selected. Most articles reviewed described a decrease in systolic and diastolic pressure after stent placement. Because of large variation in definitions for "cure" and "improvement" of hypertension, PTRA and stents could not be critically compared regarding their effect on BP. The renal arterial stent meta-analysis indicated an overall improvement in renal function in 30% of patients, stabilization in 38%, and worsening renal function in 32%. The restenosis rate at a follow-up of 6 to 29 months was 17% after renal artery stenting versus 26% following PTRA. The cure rate for hypertension was higher and the improvement rate for renal function was better after stent placement than after PTRA (20% vs. 10%, and 30% vs. 38%, respectively; $p < 0.001$).

Taken together, current reports are encouraging with regard to immediate and intermediate results of renal artery stent placement. Longer follow-up is needed to test the patency of stents in comparison with surgical revascularization and to compare long-term effects on BP, renal function, and patient survival with surgical revascularization and/or medical management alone. Burket et al. have emphasized the improvement in systolic BP, but not diastolic BP, after renal artery stenting (35). Dorros et al., in a follow-up of Palmaz stent

TABLE 52-17B

TECHNICAL ASPECTS IN 10 STENT STUDIES[a]

Investigator, year	Inclusion stenosis (%)	Indications for stent				Stents evaluated (% total stents)	Restenosis (% stents evaluated)	Average[b] time to evaluation (months)
		Rec	Dis	Res	Pri			
Rees, et al., 1991 (524)	≥40	71	0	29	0	18 (64)	7 (39)	8
Hennequin, et al., 1994 (611)	≥70	38	0	62	0	20 (95)	4 (20)	29
Raynaud, et al., 1994 (612)	≥60	66	11	6	17	18 (100)	2 (11)	11
Dorros, et al., 1995 (613)	≥70	0	0	0	100	56 (61)	14 (25)	7
van de Ven, et al., 1995 (614)	≥50	58	0	0	42	23 (82)	3 (13)	6[c]
Rundback, et al., 1996 (615)	≥60	83	0	17	0	16 (67)	3 (19)	6
Henry, et al., 1996 (616)	≥70	66	3	31	0	54 (84)	5 (9)	14
Blum, et al., 1997 (617)	≥50	85	1	14	0	74 (100)	8 (11)	24
Boisclair, et al., 1997 (618)	≥60	83	0	17	0	9 (23)	0 (0)	8
Harden, et al., 1997 (619)	≥50	94	6	0	0	24 (75)	3 (13)	6[a]
Total (all 10 studies)	≥50 in 9 studies	58	2	15	25	312 (75)	49 (16)	6–12 in 7 studies

Rec, elastic recoil; *Dis*, dissection; *Res*, restenosis; *Pri*, primary procedure.
[a]Palmaz stent used in all studies except Hennequin and Raynaud (Wallstent).
[b]Follow-ups are means except[c] medians.
(Adapted from: Isles CG, Robertson S, Hill D. Management of renovascular disease: a review of renal artery stenting in ten studies. *QJM* 1999;92:159, with permission.)

revascularization as treatment for atherosclerotic renal artery stenosis, found that 38 patients indicated a 3-year cumulative probability of survival which worsens with increasing baseline serum creatinine concentrations and extent (bilateral vs. unilateral renal artery stenosis) of atherosclerotic disease. In this report, the survival probability at 3 years was only 46 ± 15% for patients with bilateral atherosclerotic renal artery disease and baseline serum creatinine concentrations equal to or greater than 2.0 mg/dL. Kennedy et al. reported similar results in a series of 230 patients (539).

Restenosis of stents tends to be secondary to intimal hyperplasia and located at or just beyond the end of the stent (525). Active research is ongoing to improve on current stent design and possibly the use of covered, biodegradable, and chemically impregnated stents. Based on current experience, renal artery stent placement is recommended for endovascular revascularization of atherosclerotic ostial lesions, either as an adjunct to PTRA or as a primary procedure.

Renal Artery Disease in Children

The most common cause of renal artery disease in children is fibrous dysplasia. Other lesions in this age group include arterial aneurysm, arteriovenous malformation, Takayasu's arteritis, neurofibromatosis, thromboembolic disease, and renal trauma (540). Bilateral or branch renal artery stenosis, particularly in fibrous dysplasia, is not infrequently present, nor is extrarenal vascular disease. As reviewed earlier, many renal artery lesions in children, such as intimal and perimedial fibroplasia, cause progressive vascular obstruction. Accordingly, treatment is directed not only at relief of hypertension but also at preserving renal function.

Although PTRA is effective in some children, angioplasty is often not technically feasible because of the small size of the diseased vessels, involvement of renal artery branches, or the presence of multiple lesions, aneurysmal disease, or perivascular scarring in cases of arteritis. Medical antihypertensive therapy is generally not recommended as the definitive treatment in young children with renovascular hypertension because it would be lifelong and there is a risk of losing renal function from progressive renal arterial disease. Accordingly, surgical revascularization is probably the best option.

The results of surgical revascularization in children have improved, owing to the development of microvascular techniques and a better appreciation of specific operative approaches most likely to be effective in this group (541). Aortorenal bypass grafting with an autogenous hypogastric artery is the usual revascularization technique. Saphenous vein grafts are generally avoided in the small child, in whom postoperative aneurysmal graft expansion may occur. When aortorenal bypass is not possible because of peripheral renal artery disease or aortic hypoplasia, renal autotransplantation is the treatment of choice. Extracorporeal microvascular arterial reconstruction with autotransplantation is recommended for children with branch renal artery disease (245,542).

MEDICAL MANAGEMENT

The issue of medical versus surgical treatment of presumed renovascular hypertension has generated intense interest and discussion for many years. The advent of balloon angioplasty and renal artery stents has amplified this discussion. To date, no truly randomized study has addressed the benefits and risks of medical versus surgical versus angioplasty therapy. Until recently, few reports assessed the natural history of renal artery stenosis without surgical intervention. The search for the presence of renal artery stenosis and assessing its likelihood in causing hypertension implies an intent to intervene by either surgical or endovascular renal revascularization. The major concerns with medical management (antihypertensive drug treatment) of these patients are the progression of renal artery stenosis, the hemodynamic effects of BP reduction on renal function, and the diminished likelihood of success of renal revascularization in curing the hypertension the longer one waits to intervene.

There is little question that the more severe the hypertension, the greater the likelihood that it is renovascular in origin. Further, in general, younger patients with fibrous renal artery disease respond well to intervention with either surgery or angioplasty. However, the results of renal revascularization in patients with atherosclerotic renal artery disease are less favorable, as many of them are older and almost certainly have coexisting primary or essential hypertension (46,131, 187,232,439,516,543,544).

Several scenarios should be considered in the care of patients with renal artery stenosis and hypertension: (a) true renovascular hypertension, in which atherosclerotic or fibrous renal artery disease is the sole cause of the hypertension; (b) pure essential hypertension, in which atherosclerotic or fibrous renal artery disease is present but does not contribute to the hypertension at all; (c) essential hypertension with superimposed renal artery stenosis producing a renovascular contribution to the underlying essential hypertension; and (d) the hypertension of renal parenchymal disease, that is, chronic renal insufficiency (see Chapter 51), with superimposed renal artery stenosis contributing to the hypertension. Accordingly, the medical management of patients with presumed renovascular hypertension and renovascular disease is really an effort to control the BP. Despite the uncertainty in knowing whether a patient with hypertension and coexisting renal artery stenosis has renovascular hypertension, the goals of medical therapy in such patients similar to those with hypertension in general. They are to control the BP, minimize target organ damage, prolong life, avoid adverse drug side effects, and maintain renal function.

In 1974, Hunt et al. reported results comparing medical versus surgical treatment in 214 patients followed prospectively for 7 to 14 years (235). This was not a randomized study because of 100 patients selected for surgical therapy, 82 had failed to respond to 3 months of medical treatment. During the subsequent 7 to 14 years, 16% of the surgically treated and 40% of the medically treated patients with atherosclerotic renal artery disease died. Fifty-one of the 84 surviving patients treated surgically were normotensive without medication at latest follow-up. Satisfactory BP control (diastolic BP less than 100 mm Hg) was maintained for 7 years or longer in 52 of 98 patients managed medically. Most late deaths were caused by cardiac or cerebral events or uremia. Survival rates for a younger group of patients with fibromuscular disease undergoing surgical or medical therapy were 92% and 83%, respectively.

Dean et al. reported on 41 patients with atherosclerotic renal arterial disease and presumed renovascular hypertension who were treated medically (545). Progression of the renal arterial stenosis to total occlusion was observed in 4 (12%) of 31 patients who underwent serial angiographic studies. Seventeen patients (41%) had deterioration of renal function or loss of renal size that led to surgery. Despite deterioration, most patients had acceptable BP control. Sheps et al. reviewed their experience with medical management in 54 patients for an average follow-up of 20 months (546). The effectiveness of medical therapy was emphasized by excellent control of BP in 32 (65%) of 49 surviving patients. Funduscopic changes improved in half the patients. Despite BP control, additional cardiovascular complications developed in 12 patients with atherosclerotic disease, and 5 patients died. Three patients had progressive deterioration in renal function despite good BP control.

Shapiro et al. reported on 72 medically treated patients (543). Compared with a surgical subgroup at the same institution, the medically treated patients were older and had more evidence of significant target organ involvement, indicating that they were at greater long-term risk. In a follow-up period of 1 to 6 years, 29 (40%) of the 72 nonsurgical patients had died. Similarly, Dustan et al. studied 32 medically managed patients (547). Coronary and cerebrovascular disease were common in these patients, and although 12 (37%) apparently achieved normal BPs over 5 years of follow-up, 10 (31%) had died at the end of the follow-up period.

Altogether, the aforementioned reports suggest that the most dependable treatment for presumed renovascular hypertension, particularly when the likelihood of renovascular hypertension is high, is to relieve the ischemia by means of surgery. In this context, conceptually, indications for intervention on the renal artery stenosis should be similar and applied equally to either surgical or endovascular renal revascularization. Assessment of operative risk for postoperative morbidity and mortality is standard medical practice for patients being considered for surgical renal revascularization. Similar comorbid risk factor assessment (advanced age, increased serum creatinine, i.e., >2 mg/dL, history of congestive heart failure or myocardial infarction) has been emphasized in predicting morbidity and subsequent cardiovascular mortality post–renal artery stenting (438,537,539).

Although hypertension in patients with true renovascular hypertension is more severe than in most patients with primary or essential hypertension (131,148,546,548,549), renovascular hypertension does, indeed, respond to standard antihypertensive drug therapy. The degree of hypertension and the response to drug therapy are highly variable in patients with presumed renovascular hypertension, and one should not assume that every patient with renal artery stenosis and coexisting hypertension will need surgical revascularization, angioplasty, or nephrectomy to control their BP. This is particularly true now with the availability of a number of potent antihypertensive agents with a variety of pharmacologic properties that, when used appropriately and in proper combinations, can successfully control the BP. In addition, medical antihypertensive therapy is necessary to optimize BP control before planned surgery or angioplasty and for patients whose BP is not normalized by surgery or angioplasty.

The main indications for antihypertensive drug therapy (medical management) of presumed renovascular hypertension include advanced age, poor surgical risk, atherosclerotic lesions, situations wherein renal revascularization or angioplasty is not feasible owing to technical difficulties of these treatment modalities, irreversible atrophy of the kidney distal to the stenosis, doubtful significance of the renal artery stenosis lesion by angiographic criteria, hypertension of long duration (suggesting concomitant essential hypertension or arteriolar nephrosclerosis or both), and patients who simply choose medical management (131,232,544,546,548,549). Before the early 1970s, pharmacologic therapy for presumed renovascular hypertension was empirical and quite similar to that of essential hypertension. The degree of hypertension and response to drug therapy are, not unexpectedly, highly variable for patients with renal artery stenosis, and one should not assume that every patient with renal artery stenosis will respond readily to antihypertensive treatment or to treatment with only one or two drugs. Neither can the physician assume that patients with hypertension and renal artery stenosis will necessarily need surgery, angioplasty, stenting, or nephrectomy to control the BP, even when clinical clues predicting that such intervention will cure or markedly improve the hypertension are present.

Patients undergoing medical treatment of presumed renovascular hypertension must be closely monitored for continuing or worsening hypertension, intolerable drug side effects, progressive atrophy of the kidney distal to the stenosis, and the development of azotemia or worsening azotemia. Surgical therapy, angioplasty, or renal artery stenting for presumed renovascular hypertension should, therefore, be strongly considered for patients whose hypertension is refractory to medical management, younger patients with fibrous renal artery disease, patients of any age who are good surgical risks, and when progressive arterial stenosis poses a threat to renal function.

Responses to Medical Treatment

There is considerable successful experience in the medical treatment of hypertension associated with renovascular disease (550). The first substantial series of patients with presumed renovascular hypertension, treated medically, was reported by Dustan et al. in 1963 (547). These investigators used a thiazide diuretic and either hydralazine or guanethidine or both in 32 patients followed for 1 to 6 years. Treatment successfully controlled the hypertension in 13 (41%) and failed in 9 (28%); 10 (31%) of the patients died during follow-up.

Results of antihypertensive drug therapy during the late 1960s and the 1970s yielded somewhat better results, that is, controlled or much improved BPs (543,551–555). Dramatic improvements in the medical treatment of presumed renovascular hypertension evolved with the development of beta-adrenergic blocking agents (beta-blockers) and subsequently with the use of ACE inhibitors and angiotensin receptor blockers (128,129,228,261,555–564 565,566). These classes of antihypertensive agents are particularly effective because they interfere with specific steps in the renin-angiotensin-aldosterone system, which plays a pivotal role in the pathogenesis of hypertension.

As depicted in Figures 52-8 and 52-11, the pathophysiology of classic two-kidney Goldblatt hypertension produces hypoperfusion of the stenotic kidney, activating the renin-angiotensin-aldosterone axis and producing a vasoconstrictor type of hypertension. Beta-adrenergic blockade, by suppressing renin release (261,561,562,564,567), blunts the generation of angiotensin I and subsequently, angiotensin II, the primary vasoconstrictor substance operative in the early stages of classic 2K-1C Goldblatt hypertension. In addition, beta-blockers also lower BP by reducing cardiac output, suppressing the central nervous system, reducing peripheral vascular resistance, resetting the baroreceptor levels, and preventing the pressor response to catecholamines with exercise and stress (548,563,564,568).

Angiotensin-Converting Enzyme Inhibitors and Beta-Adrenergic Blockers

As shown in Figure 52-8, ACE inhibitors interfere with the conversion of angiotensin I to angiotensin II, again reducing the concentration of this potent endogenous vasoconstrictor. Improved responses to medical management with beta-blockers and ACE inhibitors are not surprising, at least in the early stages of renovascular hypertension, because these two classes of antihypertensive agents directly affect the renin-angiotensin system. Although experience with the angiotensin II receptor blockers is limited in this patient population, one would anticipate them to be effective in controlling BP in the early stages of renovascular hypertension when the elevated arterial pressure is driven by angiotensin II. Interference with the generation of angiotensin is not, however, the entire explanation for the BP-lowering effect of ACE inhibitors; they also potentiate bradykinin, and some ACE inhibitors are associated with increased levels of vasodilatory prostaglandins (565).

TABLE 52-18

REPORTED RESPONSES TO MEDICAL THERAPY FOR RENOVASCULAR HYPERTENSION

Investigators, year	N	Follow-up	Agents[a]	Results (%)			
				Controlled or much improved	Intermediate	Failure	Deaths
Dustan, et al., 1963 (547)	32	1–6 yr	Hydralazine, guanethidine	41	31	28	31
Peart, 1967 (551)	42	0–8 yr	—	45	36	19	—
Shapiro, et al., 1969 (543)	72	34 mo	—	—	—	—	40
Kjellbo, et al., 1970 (552)	165	0.5–10 yr	—	46	30	24	30
Owen, 1973 (553)	83	5 yr	—	35	34	31	34
Buhler, et al., 1973 (556)	11	1–180 days	Propranolol	45	27	18	—
Hunt and Strong, 1976 (554)	114	1–8 yr	Methyldopa	81	19	3	—
	59	7–14 yr		46	—	39	—
Streeten and Anderson, 1979 (555)	4	6 mo	Propranolol	9	3	8	—
			No propranolol	9	8	3	—
Struder, et al., 1981 (557)	2	6 mo	Captopril	9	38	0	—
Atkinson, et al., 1982 (129)	15	6 wks	Captopril	62	23	15	—
Hollifield, et al., 1982 (558)	53	2–3 yr	Captopril	68	20	17	—
Case, et al., 1982 (559)	21	28 ± 3 mo	Captopril	90	10	0	—
Hollenberg, 1983 (570)	269	0.3–2 yr	Captopril	77	18	0.37	—

[a]In most patients, a thiazide diuretic was also used.
(Adapted from: Hollenberg NK. Treatment of renovascular hypertension: surgery, angioplasty, and medical therapy with converting enzyme inhibitors. *Am J Kidney Dis* 1987;10[Suppl 1]:52, with permission.)

Despite the physiologic rationale for the use of beta-blockers, ACE inhibitors, and angiotensin II receptor blockers in patients with presumed angiotensin II-dependent hypertension, widespread experience indicates that medical antihypertensive therapy even with these agents becomes more difficult if hypertension has lasted longer than 3 to 5 years (particularly for patients with atherosclerotic renal artery disease), if a patient has coexisting chronic renal insufficiency with concomitant extracellular fluid volume expansion, or if there is a major element of essential hypertension or arteriolar nephrosclerosis or both. Treatment with a beta-blocker or an ACE inhibitor alone (or in combination) is often ineffective in controlling the BP in these circumstances. The same observations are true for patients with bilateral atherosclerotic renal artery stenosis.

As reviewed earlier in this chapter, the pathophysiology of 2K-1C hypertension indicates that the high pressure initially generated by the stenotic kidney is transmitted to the contralateral kidney via the contralateral patent renal artery, producing substantial small-vessel disease or augmenting preexisting small-vessel disease (i.e., arteriolar nephrosclerosis). Therefore, over time, and especially in the presence of uncontrolled BPs, the 2K-1C hypertension escalates to an analog of one-kidney hypertension, wherein the pathophysiologic features are similar to those of chronic renal failure associated with extracellular fluid volume expansion (reviewed in Chapter 51) (128). Volume control is necessary for such patients, usually with dietary salt restriction and diuretics, and it has been well documented that the effectiveness of beta-blockers and ACE inhibitors is markedly augmented when they are used in conjunction with diuretics (556,557,559). Thus, in contrast to the choice of antihypertensive drugs in initiating therapy for patients with essential hypertension, wherein the pathogenesis and pathophysiology are multifactorial and poorly understood, the pathophysiology of renovascular hypertension allows for a more mechanism-oriented approach to drug selection and focused treatment interfering with elements of the renin-angiotensin system (569).

Reports of the effectiveness of captopril in patients with renovascular hypertension are especially encouraging, particularly because some of these patients were resistant to alternative medical regimens that included beta-blockers (550,559,570). In a series of 269 patients, the efficacy of captopril was unequivocal, even though 40% of the patients were azotemic and 51% (136/269) had either a solitary kidney or advanced bilateral renal arterial disease (570). Captopril was ineffective in only 5% of patients, and more than 80% of the patients achieved marked improvement or goal BP. Like captopril, enalapril has also been shown to be useful in the medical management of patients with renovascular hypertension (571). Reported responses to medical therapy in patients with presumed renovascular hypertension are summarized in Table 52-18 (129,543,547,551–559,570).

Although certain pharmacologic differences exist between captopril and enalapril, both drugs exert equal antihypertensive effects in patients with renovascular hypertension. Franklin and Smith compared the results of enalapril plus hydrochlorothiazide therapy with standard triple therapy in 75 patients with presumed renovascular hypertension (571). Blood pressure control was better in the enalapril group. Poor response to therapy occurred in patients with bilateral renal artery stenosis or impaired renal function. Renal function deteriorated (increase in serum creatinine level of 0.3 mg/dL or more from pretreatment value) in 10 (20%) of the 49 enalapril-treated patients compared to 1 (3%) of 39 patients receiving triple therapy (571). In the enalapril-treated patients demonstrating an increase in serum creatinine, this increase occurred during the first 2 to 3 weeks of therapy, after which point the level plateaued without any evidence of progressively worsening renal function. No occurrence of acute oliguric renal failure was observed in any of the patients receiving enalapril-plus-hydrochlorothiazide therapy, even among the 18 patients with bilateral renal artery stenosis.

The pharmacodynamic and pharmacokinetic properties of beta-blockers (564,565,567) and ACE inhibitors (183) are detailed in standard pharmacology reviews. Propranolol is the

prototype of a number of beta-adrenergic blocking agents (556,572). Regardless of the etiology of the hypertension, the initial antihypertensive effects of propranolol and ACE inhibitors (573) relate to the preexisting level of the plasma renin activity considered in relation to the urinary sodium excretion. The antihypertensive action of propranolol and other beta-blockers affects both the vasoconstrictor and the volume components of hypertension because inhibition of renin secretion also retards aldosterone secretion, thus preventing sodium and water retention. The beta-blockers are particularly effective in combination with a diuretic and occasionally with a vasodilating agent such as hydralazine. The available beta-blockers have different effects on renal function (564,567,573). There is little evidence to suggest that administration of beta-blockers in patients with preexisting renal disease or renal artery stenosis results in a deterioration of renal function, or carries the risk of irreversible renal failure.

ACE inhibitors are excreted mainly by the kidneys. Because many of their side effects are dose-dependent, the dosage should be reduced in patients with impaired renal function. Important adverse effects with captopril include skin rash, dysgeusia, and neutropenia. Undesirable reactions to ACE inhibitors as a class are cough, the prevalence of which varies from 45% to 16% (183); functional renal insufficiency; and hyperkalemia. Angioedema is a rare, but potentially serious complication of ACE inhibitors. Cough and angioedema are most likely attributable to decreased degradation of bradykinin (565).

Angiotensin II Receptor Blockers

Because of the potential of interfering more specifically with the actions of angiotensin II at the receptor level (574), angiotensin II receptor blockers are now widely used in the treatment of hypertension. Of historical interest, the first angiotensin II receptor blocker, saralasin, was used in the early 1970s, mainly to explore the effects of inhibition of the renin-angiotensin-aldosterone system (575). Although widely used in clinical investigation, its complex peptide structure and quick hydrolysis after oral administration made it impractical as an antihypertensive drug (270). Several nonpeptide molecules that interfere with angiotensin II receptors and lower BP have now been synthesized (576). The first blocker of this type to become available for clinical use was losartan. Currently, many other angiotensin II receptor blockers are now available. There is hope that since these agents interfere farther down the renin-angiotensin system cascade than do the ACE inhibitors (Fig. 52-8), they may not cause some of the troublesome side effects associated with ACE inhibition (e.g., cough and angioedema). Whether these agents will be as effective as currently available ACE inhibitors in patients with presumed renovascular hypertension remains to be determined. At present, there is no unequivocal information as to whether the angiotensin receptor blockers differ significantly from the available ACE inhibitors in causing hyperkalemia, mild reduction in GFRs (577), or frank acute renal failure, but at least one report suggests they have less adverse effects on renal function (578).

Calcium Antagonists

Calcium antagonists have been found to be quite effective in patients with presumed renovascular hypertension (579,580). Their antihypertensive effect is primarily due to direct arteriolar vasodilatation. The calcium antagonists are a chemically, pharmacologically, and therapeutically heterogeneous group of agents. Their clinical effects are related to blocking the L class of voltage-gated calcium channels in a variety of tissues. In general, the calcium antagonists act by selective inhibition of calcium influx through the cell membrane. This interference appears to interact with selective blockade of the slow channels for transmembrane calcium influx. Because calcium ions play an important role in the contractile process of smooth muscle, calcium antagonists, by preventing this influx, produce arteriolar relaxation (581,582).

The precise mechanisms of action of the calcium antagonists are under active investigation, but the effects of calcium antagonists on the elements of the renin-angiotensin system are complex. In general, an increase in intracellular calcium produces an increase in hormone release and synthesis, and blockage of calcium entry might be expected to blunt this response. However, calcium antagonists stimulate the synthesis and release of renin in juxtaglomerular cells (583,584). Therefore, the antihypertensive effect of calcium antagonists in renovascular hypertension is probably not due to decreasing renin release, but is more likely secondary to direct peripheral arteriolar vasodilation.

One potential benefit of calcium antagonists in the treatment of hypertensive patients with renovascular disease is their favorable effect on renal function in comparison to ACE inhibitors (585–587). Although there have been occasional reports of calcium antagonists adversely affecting renal function, particularly in patients with renal insufficiency (588), calcium antagonists are generally safe in patients with renal artery stenosis. In patients with bilateral renal artery stenosis, calcium antagonists lower BP effectively without causing as much deterioration of GFR as ACE inhibitors (585–587).

Calcium antagonists have had a major impact on the treatment of all types of hypertension and of ischemic heart disease. Their well-documented effectiveness in lowering BP has resulted in their widespread use (258,585–587). The pharmacodynamic, pharmacokinetic, cardiac, and hemodynamic effects of calcium antagonists are detailed in a number of reviews (258,579,581,582,589,590). Three categories of calcium antagonists are clinically available: dihydropyridines (e.g., nifedipine, felodipine, amlodipine), benzothiazepines (e.g., diltiazem), and phenylalkylamines (e.g., verapamil). All three categories are useful in hypertensive patients with or without renal artery stenosis and in the presence of a wide variety of concomitant conditions, including ischemic heart disease, peripheral vascular disease, diabetes mellitus, and chronic renal disease.

Nifedipine, a dihydropyridine calcium antagonist, has little suppressive effect on myocardial tissue, having its major effects in blocking vasoconstriction in large to medium-sized arteries. Verapamil is less directly vasodilatory than the dihydropyridines. Verapamil depresses sinoatrial and atrioventricular nodal activity and should be used with caution in patients with heart block. Diltiazem, with properties closely related to those of verapamil, has a more variable and evidently dose-dependent effect on heart rate (579,581,582,589,590). Whether the three classes of clinically available calcium antagonists have differing BP-lowering capabilities or different side-effect profiles in patients with renal artery stenosis has not been determined.

A detailed discussion of the many additional types of antihypertensive agents (e.g., diuretics, alpha1-agonists, alpha-blockers, peripheral neurogenic inhibitors, and the vasodilators hydralazine and minoxidil) is beyond the scope of this chapter. In general, the use of these agents in the medical management of patients with renovascular disease and presumed renovascular hypertension follows the usual guidelines for these agents when used for essential hypertension. However, when renovascular hypertension is suspected, the first step should be to use either a beta-blocker, ACE inhibitor, angiotensin II receptor blocker, or calcium antagonist. The combination of an ACE inhibitor and a calcium antagonist is extremely effective. The conjoint use of an ACE inhibitor and a beta-blocker is also effective.

Caution must be exercised in using a beta-blocker in combination with verapamil or diltiazem, especially in patients prone to bradycardia or with any electrocardiographic evidence of heart block, because the conjoint use of these two drug classes might dangerously slow the heart rate or precipitate congestive heart failure.

The next drug to be added would ideally be a diuretic agent, especially in conjunction with an ACE inhibitor, beta-blocker, or angiotensin II receptor blocker. Clonidine is also effective, often as a third drug in patients with severe hypertension in association with renovascular disease. Clonidine administration may produce an acute reduction in BP in patients with renovascular hypertension; the reduction in BP occurs without a significant change in plasma renin activity, but is associated with a decrease in plasma norepinephrine levels (591). Clonidine is especially effective in lowering the systolic BP in older adult hypertensive patients.

Complications and Liabilities of Medical Management of Renovascular Hypertension and Renovascular Disease

The side effects of the many antihypertensive drugs used in treating renovascular hypertension are well known and beyond the scope of this chapter. However, several untoward metabolic or physiologic effects of medical antihypertensive therapy are of particular interest in managing patients with renovascular disease including, but not limited to, hypokalemia, hyperkalemia, acute renal failure, reduced renal perfusion pressure, flash pulmonary edema, and ischemic shrinkage of the kidney distal to the stenosis.

In two-kidney Goldblatt hypertension, aldosterone levels increase as a consequence of activation of the renin-angiotensin system. Secondary aldosteronism ensues, and hypokalemia is a cardinal feature of true renovascular hypertension. Diuretics may further activate the renin-angiotensin-aldosterone cascade and increase sodium delivery to the site of aldosterone action in the nephron, and produce severe hypokalemia. The clinical syndrome of hyporeninemic hypoaldosteronism is not uncommon in older adult diabetic patients, many of whom have renovascular disease and underlying essential hypertension (not true renovascular hypertension); these patients may develop hyperkalemia, which can be aggravated to dangerous levels with the use of ACE inhibitors and beta-blockers, both of which increase the serum potassium concentration.

Acute renal failure in conjunction with ACE inhibition in patients with bilateral renal artery stenosis or high-grade stenosis of an artery to a solitary kidney is well described (225,228,230,434,435,592–596). This drawback is often the limiting factor in drug treatment of patients with presumed renovascular hypertension. Acute deterioration in renal function in patients undergoing medical treatment for presumed renovascular hypertension is frequently the clinical observation that prompts the physician to discontinue medical therapy and consider angioplasty or surgical revascularization in suitable candidates. This phenomenon has been observed in up to 23% of patients with high-grade renal artery stenosis who take ACE inhibitors (597). Fortunately, renal function usually recovers when the ACE inhibitor is discontinued (435,594–596). Although clear evidence of permanent structural damage in the kidney distal to the stenosis in such situations is lacking in humans, renal artery thrombosis has been described (597–600). Whether shrinkage of the kidney distal to the stenosis in patients treated with ACE inhibitors for a long time is due to ACE inhibition per se or to progressive arterial stenosis (or to both) is unclear, but progressive renal atrophy is an indication to abandon medical management and proceed with angioplasty or surgery if technically feasible.

Calcium antagonists appear to induce less impairment of renal function in patients with renovascular hypertension than do ACE inhibitors (585,586). In two studies comparing the effects of captopril and nifedipine, both agents demonstrated excellent antihypertensive efficacy (585,586). Nifedipine, however, produced a smaller decrement in the GFR than did captopril in patients with unilateral, bilateral, or solitary kidney renal artery stenosis. Dihydropyridine calcium antagonists may better maintain renal blood flow and glomerular filtration because of their preferential afferent arteriolar vasodilatory effect (579).

Any type of antihypertensive therapy that effectively lowers the BP may decrease renal blood flow and glomerular filtration if the BP is lowered below a "critical" renal perfusion pressure (224,233). Diuretic-induced extracellular fluid volume contraction would potentiate this untoward effect of reduced perfusion pressure on renal function.

Flash pulmonary edema has been discussed as a clinical clue to unilateral or bilateral renal artery stenosis (216,217), or in association with uncontrolled BP. Episodes of acute pulmonary edema in the absence of obvious overt cardiac disease in patients with a reasonable left ventricular ejection fraction are being observed more frequently in the older adult population with disseminated vascular disease involving the renal arteries. These episodes occur more frequently in patients with bilateral renal artery stenosis or high-grade stenosis of an artery to a solitary kidney than in patients with unilateral renal artery stenosis (218). This clinical syndrome complicates the medical management of patients with renal artery stenosis and may be the critical factor prompting an intervention procedure on the renal artery (218,536).

Some authors have suggested that atherosclerotic RAS is associated with increased cardiovascular events and decreased survival in comparison to patients without RAS (36–38). Edwards et al. conducted a prospective, multicenter cohort study of cardiovascular disease risk factors, morbidity, and mortality among Americans older than 65 years (36). Renovascular disease was associated with an increase in the risk of adverse coronary events in this study population. The increment in risk was not dependent on the effects of associated atherosclerotic risk factors, other prevalent cardiovascular disease, or increased BP. Whether these authors' findings relate specifically to the anatomic presence of atherosclerotic RAS versus other comorbid cardiovascular risk factors associated with generalized atherosclerosis merits continued examination. Such reports could, however, fuel enthusiasm for renal revascularization to prevent adverse coronary outcomes.

An explosive increase in endovascular renal revascularization procedures over the past decade has been emphasized recently by Murphy et al. (601). Whether or not this explosion in endovascular intervention is due to a substantial increase in renovascular hypertension, increased concern that systolic hypertension in an increasingly aging population may be helped by renal artery stenting, that more patients are developing end-stage renal disease (ESRD) as a consequence of atherosclerotic renal artery disease (ASO-RAD), concerns about chronic kidney disease (CKD) (elevated serum creatinine concentrations) as promoting cardiac disease and adversely affecting patient survival, or other considerations, is unclear. What is clear, however, is that despite the widespread occurrence of ASO-RAD, there is no consensus on the best treatment approach.

The discussion of medical management has focused on a pathophysiologic approach to the drug treatment of renal artery stenosis and presumed renovascular hypertension. Regardless of the pharmacologic regimen employed to control BP in such patients, long-term adherence to therapy remains a major problem, as it does for patients with essential hypertension.

Failure to control BP leaves such patients at risk for the cardiovascular complications of stroke, congestive heart failure, and progressive renal insufficiency.

Which drugs to use and the choice of medical or surgical treatment or angioplasty or stent placement must be considered carefully for each individual. In general, medical management is preferred for older patients, especially those with serious cardiovascular risk factors for surgery. Older patients with tight ostial renal artery atherosclerosis in conjunction with severe aortoiliac disease are at high risk during angiography, angioplasty, renal artery stent placement, and surgery, and probably should be managed medically if at all possible. Balloon angioplasty or surgical renal revascularization is preferred for patients with fibrous renal artery disease and for younger patients with atherosclerosis, particularly if they have acceptable surgical risk factors.

For all patients managed medically, meticulous follow-up is required to ensure that the systolic BP remains less than 140 mm Hg and the diastolic pressure less than 90 mm Hg. Optimal medical management of such patients, particularly those with atherosclerotic renal artery disease, demands modification of cardiovascular risk factors such as smoking and serum lipid levels. Whether current or future antihyperlipidemic drugs (e.g., hepatic hydroxymethylglutaryl coenzyme A reductase inhibitors) will have a meaningful impact on atherosclerotic renal artery stenosis (602,603) remains to be determined, but their benefits in promoting regression of atherosclerosis in other vascular beds are promising (604–609).

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CHAPTER 53 ■ HYPERTENSION AND PREGNANCY

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In the Western world, hypertension, preeclampsia, and eclampsia are the most common causes of morbidity and mortality in the mother (1,2) and child (3). This chapter presents an overview of

- Physiologic changes in renal hemodynamics, blood pressure, and water metabolism in normal pregnancy
- Hypertensive disorders in pregnancy
- Pathophysiologic mechanisms in pregnancy-induced hypertension and preeclampsia and eclampsia
- Management of hypertensive disorders in pregnancy

PHYSIOLOGIC CHANGES IN SYSTEMIC AND RENAL HEMODYNAMICS AND WATER METABOLISM IN NORMAL PREGNANCY

Changes in Systemic Hemodynamics

Normal pregnancy is characterized by decreased systemic vascular resistance with decreased mean arterial pressure and increased cardiac output. In pregnant women, alterations in systemic hemodynamics are detectable by 6 weeks of gestation (4,5) (Figs. 53-1, 53-2). A decrease in peripheral vascular resistance occurs prior to blood volume expansion, thus supporting the conclusion that arterial vasodilation is the primary event and both the increase in cardiac output and volume expansion are secondary events (6) (Fig. 53-3). There are several mediators that may cause the early peripheral vasodilation in pregnancy. The plasma concentration of the vasodilating prostacyclin (PGI_2) and urinary excretion of its major metabolites are increased in pregnancy (7,8). Several hormones known to relax vascular smooth muscle such as estrogens, progesterone, and prolactin are increased in pregnancy; however, their administration does not cause hypotension. The mature placenta may act as an arteriovenous fistula. However, early in pregnancy when the vascular resistance falls and reaches the nadir, blood flow in the placenta and uterus is still rather small. Another vasodilating substance, is nitric oxide (NO). NO is a potent relaxant of vascular smooth muscle cells and also stimulates vasodilation in the presence of vasoconstricting peptides (9). In pregnant rats, plasma levels of NO (10,11) and plasma levels and urinary excretion of the second messenger of NO, cyclic guanosine monophosphate (cGMP), are increased (12–14). Upregulation of endothelial NO synthase in the resistance vessel has been documented in animal pregnancies (15). The physiologic decrease in blood pressure in pregnant spontaneously hypertensive rats has been shown to depend completely on NO release (16). The production of cGMP may result from stimulation of guanylate cyclase by NO but also by cytokines such as tumor necrosis factor-α (TNF-α).

Changes in Renal Hemodynamics

There are considerable alterations in renal hemodynamics during pregnancy. Some of these reflect cardiovascular changes such as peripheral vasodilation and an increase in cardiac output (4,5). An increase in fluid volumes and endocrine changes also occur. Renal blood flow (RBF) increases progressively until it peaks in the second trimester at about 180% of the preconception value (4,5,17–19). In parallel, glomerular filtration rate (GFR) rises beginning 4 weeks after conception (Fig. 53-1). Creatinine clearance is enhanced by about 50% in the first trimester and remains elevated until the last month of gestation (20,21). There is little change in creatinine production during pregnancy; therefore, changes in creatinine clearance result in a fall in plasma levels of creatinine on average to about 0.45 mg/dL as compared to 0.67 mg/dL in nonpregnant controls (22). Studies in rats suggest that the pregnancy-associated increase in GFR is due solely to renal vasodilation, with parallel relaxation of afferent and efferent arteriolar resistance leading to increased renal plasma flow (RPF) without change in glomerular blood pressure (23). The potent vasodilator responsible for the rise in GFR by 30% to 50% during pregnancy is not yet known. However, a major contribution to the increase in GFR may derive from enhanced NO production during pregnancy (24). Studies in rats demonstrate a reversal of glomerular hyperfiltration and renal vasodilation in pregnant rats during chronic inhibition of NO synthase (11).

After delivery, GFR returns to the pregestation range (18). Neither volume expansion, high-protein diet, nor unilateral nephrectomy has been shown to enhance GFR to the same level as occurs with pregnancy. Due to a lesser increase in GFR as compared to RBF, filtration fraction falls in early pregnancy. However, RBF decreases in the last weeks of gestation, and filtration fraction may rise. In pregnant rats with glomerulonephritis and superimposed pregnancy, GFR also increases (25). Pregnancy-induced changes in RBF and GFR also occur in women with hypertrophied single kidneys or transplant recipients (26,27) but may be attenuated in women with underlying renal insufficiency. In a study by Cunningham et al. only 50% of women with moderate and none of the women with severe chronic renal disease demonstrated any increase in GFR during pregnancy (28).

Urinary protein excretion may be increased in normal pregnancy to the upper limit of 300 mg/24 hours (29). The majority of normal pregnant women, however, will have a protein excretion of less than 150 mg/24 hours. Albumin excretion in nonpregnant women is less than 40 mg/24 hours. A rise in the ratio of urinary albumin to creatinine may demonstrate impaired glomerular permeability (30). This ratio method is accurate and avoids 24-hour urine collections.

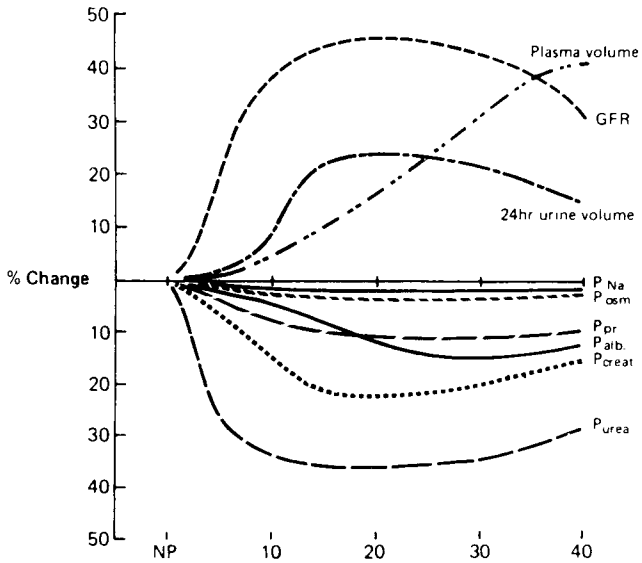

FIGURE 53-1. Physiologic hemodynamic and biochemical changes induced by pregnancy beginning at the nonpregnant (NP) state and through 40 weeks of pregnancy. In pregnant women, glomerular filtration rate (GFR) rises before blood volume expansion occurs. Increments and decrements of the various parameters are shown in percentage terms with reference to nonpregnant state. *GFR*, glomerular filtration rate; P_{alb}, plasma albumin; P_{creat}, plasma creatinine; P_{Na}, plasma sodium; P_{osm}, plasma osmolality; P_{pr}, plasma protein; P_{urea}, plasma urea. (From: Davison JM. Renal hemodynamics and volume homeostasis in pregnancy. *Scand J Clin Lab Invest* 1984;169(Suppl):15, with permission.)

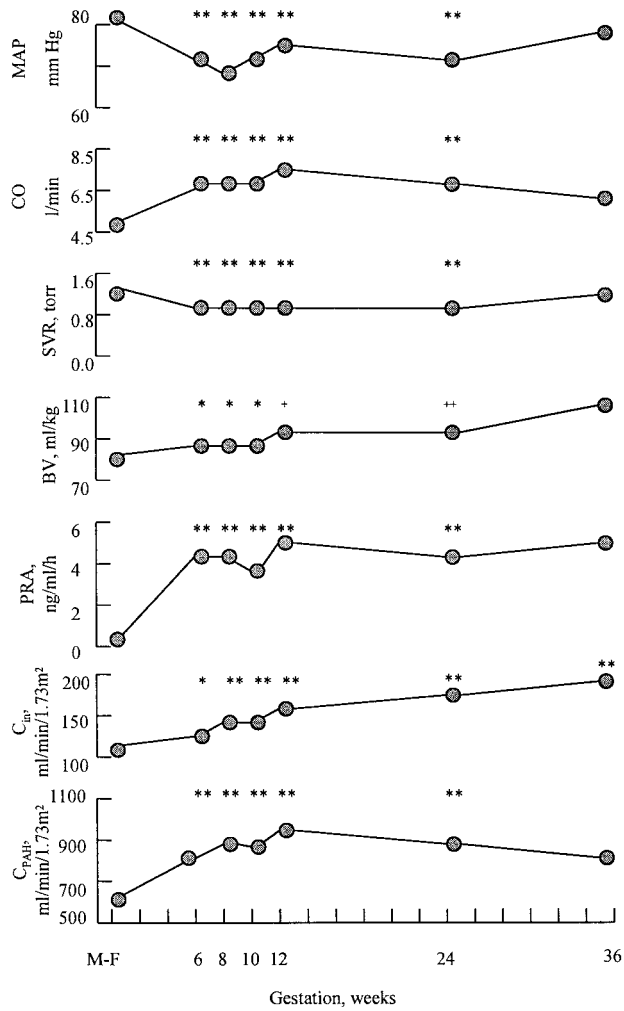

FIGURE 53-2. Physiologic hemodynamic alterations induced by pregnancy in women beginning at the midfollicular (M-F) phase of the menstrual cycle and through 36 weeks' pregnancy. Decreased mean arterial pressure (*MAP*), systemic vascular resistance (*SVR*), increased cardiac output (*CO*), blood volume (*BV*), plasma renin activity (*PRA*), inulin clearance (C_{in}), and paraaminohippurate clearance (C_{PAH}) are detectable by 6 weeks of gestation. $^{*}p < 0.05$, $^{**}p < 0.001$, $^{+}p < 0.0001$. (From: Chapman AB, et al. Temporal relationships between hormonal and hemodynamic changes in early human pregnancy. *Kidney Int* 1998;54:2056, with permission.)

Changes in Sodium and Water Homeostasis

Volume expansion is extraordinary during pregnancy and results in a total body water increase by 6 to 8 L (31). The mean plasma volume in pregnancy increases progressively from conception to term by almost 50% (32) (Fig. 53-1). The mechanism controlling volume expansion is poorly understood, but sodium retention is an important factor. An increase in GFR augments the filtered load of fluid and electrolytes; therefore, changes in tubular function are required to prevent urinary loss of these substances in the glomerular filtrate (33). The filtered sodium load increases from 20,000 to 30,000 mmol/day; however, due to increased sodium reabsorption in the proximal and distal renal tubules, sodium depletion is prevented. In fact, sodium retention during pregnancy totals nearly 1,000 mEq (32). This sodium retention may be induced or enhanced by estrogens, deoxycorticosterone, and aldosterone (33). It has been suggested that the increased aldosterone production in pregnancy exceeds the physiologic requirements. In nonpregnant humans, the continued administration of mineralocorticoids causes sodium retention of about 400 mEq of sodium; however, despite continuation of the hormone administration, no further sodium retention occurs. An adaptive mechanism known as mineralocorticoid escape thus prevents excessive sodium retention. Although basal plasma concentrations of aldosterone are high in pregnancy, changes in sodium intake, administration of diuretics, and changes in postural position lead to a similar pattern of changes in aldosterone production and plasma levels as occurs in normal nonpregnant humans (34). Thus, despite high levels of plasma aldosterone, adaptive mechanisms to various stimuli are qualitatively preserved in pregnancy.

Plasma osmolality decreases at 1 month after conception and plateaus at 10 to 12 weeks of pregnancy to levels about 10 mOsm/kg below normal (35). Normally, a decrease of osmolality of this degree would cause inhibition of arginine vasopressin (AVP) secretion and a water diuresis. However, studies suggest that plasma AVP concentrations are either normal or increased in pregnancy as compared to nonpregnant controls (15,36). Furthermore, metabolic clearance of AVP in rats is increased in mid- to late pregnancy (37), coinciding with increased trophoblastic mass and hence increased levels of placental and plasma vasopressinase. Although once thought to be an *in vitro* phenomenon, abnormally high circulating vasopressinase activity can produce diabetes insipidus in human pregnancy (38).

Previous reports suggested that the threshold for vasopressin secretion and thirst is decreased in normal pregnancy and corresponds to the decrease in osmolality (39) (Fig. 53-4). Thus, gravidas maintain their new plasma osmolality, and neither fluid loading nor fluid restriction alters this "reset" osmolality. More recent results, however, suggest that pregnancy

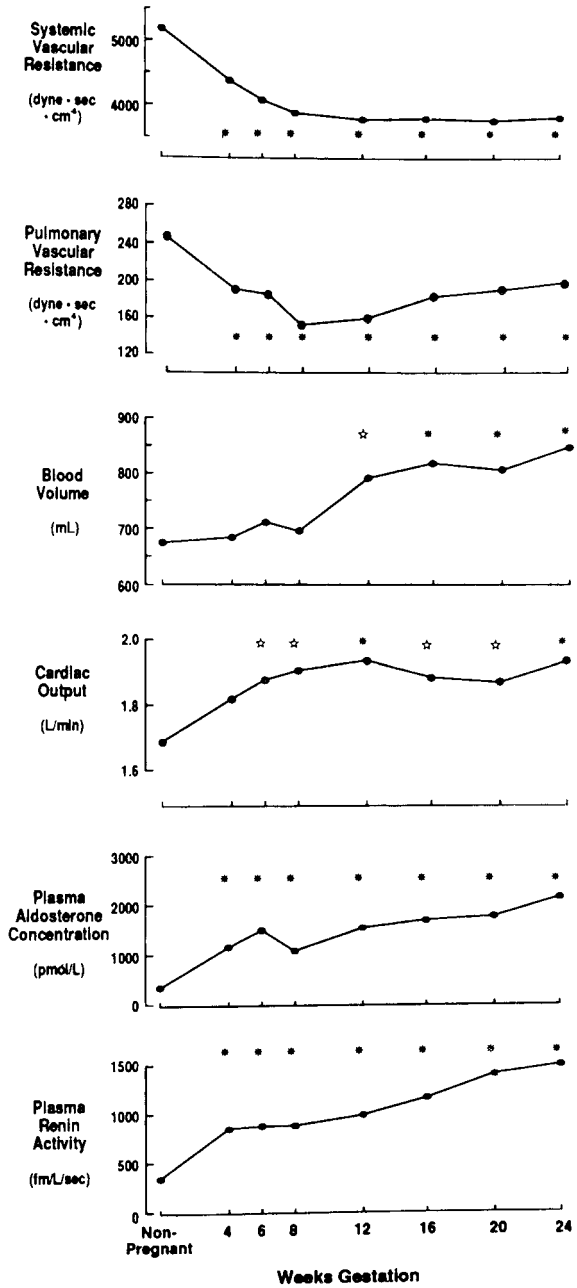

FIGURE 53-3. Early hemodynamic and humoral profile for pregnant baboons. A significant rise in cardiac output, plasma renin activity, and plasma aldosterone concentration occurs at 4 to 6 weeks of pregnancy and parallels a decrease in pulmonary and systemic vascular resistance. (From: Phippard AF, et al. Circulation adaptation to pregnancy: serial studies of hemodynamics, blood volume, renin and aldosterone in the baboon. *J Hypertens* 1986;4:773, with permission.)

is associated with increased expression of collecting duct aquaporin-2 water channels (36). As described in the "shuttle hypothesis" (40), circulating vasopressin binds to its V_2 receptor on the basolateral membrane of the principal cell of the renal collecting duct. This initiates cyclic adenosine monophosphate-mediated and protein kinase-mediated translocation of AQP2 water channels from cytosolic storage vesicles to the apical membrane, thus allowing reabsorption of water to occur. Upregulation of AQP2 water channels is evident in the first trimester of rat pregnancy and persists throughout pregnancy in association with hypoosmolality (36). Thus vasopressin release and AQP2 mediated with retention can explain the hypoosmolality of pregnancy.

Injection of estrogen or progesterone has failed to reduce plasma osmolality in nonpregnant rats (41). Although chronic NO synthase inhibition in pregnant rats returns systemic and renal hemodynamics to nonpregnant levels, hypoosmolality is maintained due to increased thirst (11). Alternatively, prolactin injection into rats (42) and human chorionic gonadotropin injection into nonpregnant women (but not men) may reduce the osmotic threshold (43). Although prolactin production is enhanced during lactation, serum osmolality increases after delivery in lactating mothers, suggesting little contribution to the reset osmolality of this hormone during pregnancy. In molar pregnancies, plasma osmolality and the threshold level for the release of vasopressin is reduced as long as human chorionic gonadotropin is increased (43). As noted previously, plasma osmolality may also be affected by changes in the metabolism of vasopressin (37,38).

Hormonal Changes in Pregnancy

Plasma concentrations of a number of hormones change substantially during pregnancy. Some of these hormones have effects on the kidney and the blood vessels to modulate hemodynamics. No individual hormone, however, is known to cause hemodynamic changes as large as those seen during pregnancy. In this section, we will discuss some of the hormones with rising blood concentrations during pregnancy that may contribute to the complex and diverse changes in salt and water homeostasis and hemodynamics associated with gestation.

Renin-Angiotensin-Aldosterone System

The renin-angiotensin-aldosterone (RAA) system has direct effects on vascular tone, and by causing sodium retention, may also increase plasma volume. The RAA system is stimulated in pregnancy, and most likely this is secondary to the peripheral arterial vasodilation (Fig. 53-3). Plasma renin concentration, renin substrate, and angiotensin II are increased throughout pregnancy, reaching peak levels close to term (44). In spite of increased angiotensin II levels, blood pressure declines during pregnancy. Vascular insensitivity to angiotensin II during normal pregnancy is maximal in the second trimester (45) (Fig. 53-5). Part of this loss of the vascular effect of angiotensin II may be the result of a decrease in angiotensin II receptors (46). In studies of pregnant baboons, the blood volume expansion occurs after stimulation of the RAA axis (6) (Fig. 53-3). If the blood volume expansion were primary, rather than secondary to the arterial vasodilation, suppression, not stimulation, of the RAA system would be expected. Basal renin substrate and plasma renin are both enhanced during pregnancy; these effects cannot be demonstrated in nonpregnant patients treated with progesterone and estrogens (47). Renin is produced in uteroplacental tissue (48), and that renin is indistinguishable from that found in the kidney. A role of uteroplacental renin in modulating uterine blood flow has been suggested (49). The administration of saralasin, an angiotensin II antagonist, or captopril, an angiotensin converting enzyme (ACE) inhibitor, attenuates uterine blood flow (50,51). The regulation of the RAA system in normal pregnancy, although it is activated, remains under similar control mechanisms as in the nonpregnant state, with activation and suppression occurring with sodium depletion and sodium loading, respectively (52).

As noted earlier, in normal pregnancy, vascular tone and blood pressure are resistant to an average dose of angiotensin II infusion (53) (Fig. 53-5). This angiotensin II refractoriness may be the result of mechanisms involving several intracellular pathways in vascular smooth muscle. The increase in prostacyclin

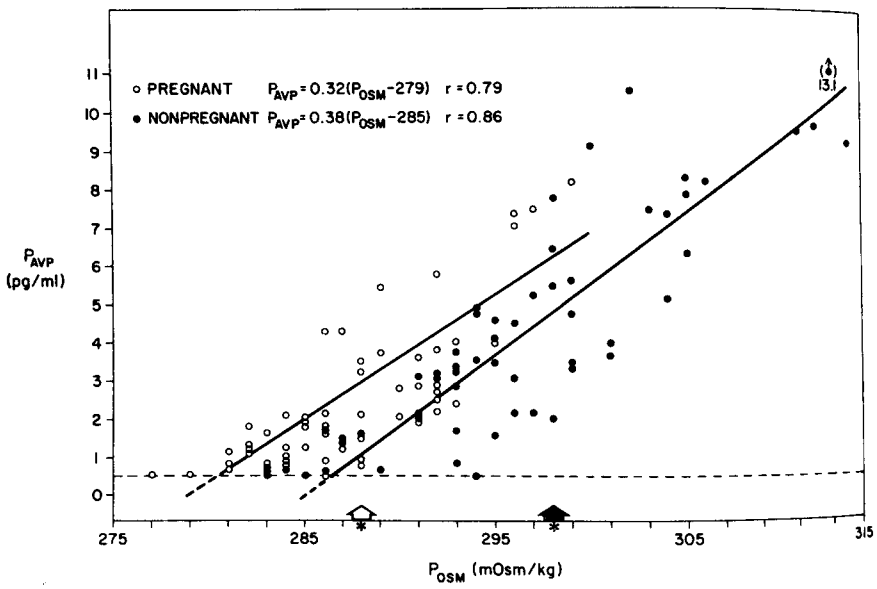

FIGURE 53-4. Relationship between plasma osmolality (P_{OSM}) and plasma vasopressin (P_{AVP}) during a 2-hour infusion of 5% saline in first trimester pregnant women (*open circles*) and 6 to 8 weeks' postpartum. The *arrow* indicates the thirst threshold, which is reduced during pregnancy. (From: Davison JM, et al. Altered osmotic threshold for vasopressin secretion and thirst in human pregnancies. *Am J Physiol* 1984;246:F105, with permission.)

concentrations during pregnancy stimulates adenylate cyclase to produce cyclic adenosine monophosphate (cAMP). An increase in cAMP attenuates the intracellular signaling of pressor hormones in vascular smooth muscle and inhibits vasoconstriction. The administration of a low dose of aspirin to block the production of the vasoconstricting prostaglandin thromboxane A_2 enhances the effects of prostacyclin and thus lessens angiotensin II-induced rise in blood pressure (54). In contrast, a dose of aspirin that also blocks prostacyclin production enhances the pressor effects of angiotensin II (54). Another cyclic nucleotide, cGMP, has effects similar to cAMP in smooth muscle. Plasma levels and urinary excretion of cGMP have been reported to be elevated or unchanged during pregnancy (12–14,55). There are also data to support the view that NO, a major stimulator of cGMP production, participates significantly in vasodilation in pregnancy. The peripheral arterial vasodilation during pregnancy stimulates the RAA system in a compensatory manner and thus explains why blood levels of renin, angiotensin, and aldosterone are elevated despite volume expansion during normal pregnancy. The rise in plasma aldosterone may counterbalance the increased amount of urinary sodium reaching the distal tubules as a result of the profound rise in GFR during pregnancy (56). Aldosterone thus may prevent excessive sodium loss and volume depletion during pregnancy. In addition to the RAA system, progesterone has also been shown to enhance aldosterone excretion (57). In nonpregnant humans, the administration of mineralocorticoids induces sodium retention with resultant water retention and a weight gain of about 2 to 3 kg. Continuation of the mineralocorticoid treatment does not cause any further sodium and water retention. This phenomenon is called the "mineralocorticoid escape" mechanism. As noted earlier, in spite of the increased basal concentration of aldosterone in pregnancy, there is a rise in plasma aldosterone levels in response to sodium restriction (58). These observations suggest a new set point in the RAA system during pregnancy. Plasma levels of the potent mineralocorticoid deoxycorticosterone also increase in pregnancy (59). It is suggested that this increment results mainly from the hydroxylation of progesterone (60).

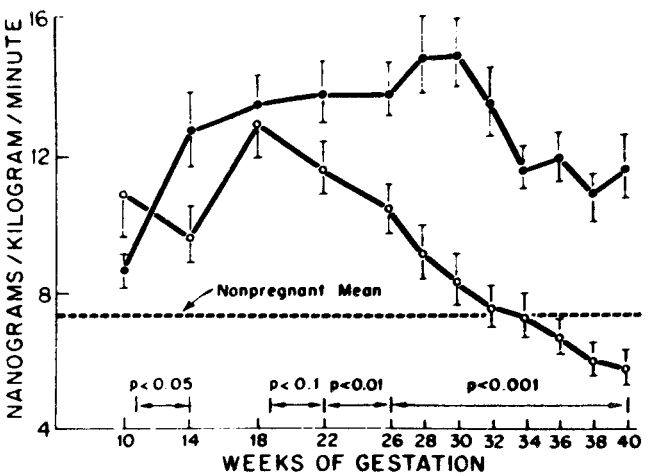

FIGURE 53-5. Angiotensin II response in 192 primigravidas. *Closed circles* demonstrate reduced sensitivity to angiotensin II and requirement of a greater dose to enhance diastolic blood pressure by 20 mm Hg compared with nonpregnant controls. *Open circles* demonstrate rising sensitivity to angiotensin II in women who develop pregnancy-induced hypertension. (From: Gant NF, et al. A study of angiotensin II pressor response throughout primigravid pregnancy. *J Clin Invest* 1973;52:2682, with permission.)

Progesterone, Estriol, and Prolactin

Plasma progesterone, estriol, and prolactin concentrations markedly increase during pregnancy (61). The effects of progesterone are not restricted to the uteroplacental tissue. Progesterone and prolactin may both contribute to the rise in GFR during pregnancy (42,62). Independent of pregnancy, progesterone causes a natriuresis by a mechanism that in part is independent of the inhibition of aldosterone-induced sodium retention (63). Estrogen replacement therapy has been shown to promote vasodilation by decreasing plasma concentrations of renin (64), ACE (65), and endothelin-1 (66) and by increasing the ratio of NO to endothelin-1 (67). Vascular tone is most reduced during pregnancy when the concentrations of estrogen and progesterone are the highest (68). There is evidence that estrogens modulate NO during pregnancy. Pregnancy and estradiol administration cause an increase in the activity of calcium-dependent NO synthase in uterine artery and also in other tissues such as the kidney in guinea pigs (69). It has recently been suggested that estrogen mediates this effect via a mitogen-activated protein (MAP) kinase mechanism (70).

Calcium-independent NO synthase is not altered by pregnancy or female sex hormones. Progesterone does not significantly increase NO synthase (71). NO is therefore a vasodilator that has the potential to decrease vascular resistance, modulate renal hemodynamics (72), and antagonize vasoconstrictor-mediated smooth muscle cell contraction (73) in pregnancy.

Relaxin

There are several lines of evidence implicating the peptide hormone relaxin in the regulation of the altered renal hemodynamics and water metabolism in pregnancy (74). Relaxin is composed of two peptide chains that are connected by two interchain disulfide bonds, resembling the structure of insulin. Production of relaxin occurs in the corpus luteum of the ovary, the placenta, and the endometrium under the stimulation of human chorionic gonadotropin. Blood levels of relaxin rise in the first trimester of pregnancy (75), coincident with the pregnancy-associated rise in GFR and RPF. Circulating relaxin concentrations are also increased in the luteal phase of the menstrual cycle, when there is a transient 20% increase in GFR and RPF (76). Chronic administration of relaxin to nonpregnant rats results in elevations in GFR and RPF similar to the pregnant state (77); these effects appear to be mediated via NO. Moreover, relaxin administration in animals attenuates the renal vasoconstrictor response to angiotensin II infusion (77). Relaxin administration also increases water intake, reduces plasma osmolality, and alters the threshold for vasopressin release in rats (77,78). There seems to be an essential role of gelatinase in the relaxin-mediated renal effects of pregnancy and it is involved in the endothelial ET_B–NO pathway (79).

Endothelin 1

Endothelin-1 (ET-1) is an endothelium-derived peptide which acts as a potent vasoconstrictor through direct effects and via stimulation of the RAA and the sympathetic nervous system (reviewed in reference 80). ET-1 has complex effects on vascular tone. Although endothelin induces vasoconstriction in vascular smooth muscle cells via activation of ET_A receptors, activation of endothelial ET_B receptors is associated with transient hypotension and increased NO synthesis (81). Several studies have suggested a role for ET-1 in the regulation of blood pressure, renal and spiral artery hemodynamics in pregnancy. Urinary ET-1 excretion has been shown to be elevated in pregnancy (82). ET_B receptor antagonism produced a significant decrease in both GFR and RPF in pregnant rats (83). The magnitude of decline was greater in pregnant as compared to nonpregnant rats, suggesting a role for ET_B receptor activation in the maintenance of pregnancy-associated GFR and RPF elevations. Moreover, concomitant NO inhibition did not further decrease renal hemodynamics, implicating NO as a mediator of ET-1 action.

Adrenomedullin

Adrenomedullin is a 52-amino acid peptide that produces natriuresis and blood pressure reduction. Its concentration rises as pregnancy proceeds (84). Although adrenomedullin levels did not differ in preeclamptic patients, levels in the amniotic fluid and blood of the umbilical vein were several fold higher in eclampsia than in controls (85).

Pregnancy and Changes in Glucose Tolerance

Physiologic changes of the metabolic environment initially favor maternal fat deposition, and later fetal growth. By the eighth week of pregnancy, the fasting blood glucose level falls and reaches its nadir around the twelfth week. Postprandial blood glucose level rises to peak at around 30 weeks of gestation. Fasting insulin level rises during pregnancy as does glucose-stimulated insulin secretion. Insulin sensitivity is reduced by about 50% during normal pregnancy while insulin requirement rises about threefold. This metabolic change is most prominent in the second and third trimester and resembles changes comparable to that in noninsulin-dependent diabetes mellitus. Insulin resistance in pregnancy appears to resolve immediately after delivery (86). Sex hormone binding globulin (SHBG) is a glycoprotein synthesized by the liver and binds circulating estrogens. Insulin inhibits the production of SHBG and therefore has been shown to be a marker of hyperinsulinemia and insulin resistance (87). Compared with controls, women who develops preeclampsia had significantly reduced first trimester SHBG levels (88). In addition, for every 100 nmol/L increase in SHBG there was a 31% reduction of the risk of preeclampsia ($p < 0.01$). The independent association between SHBG levels and preeclampsia was seen in lean women, suggesting that in obesity SHBG is only one of the several contributing factors.

Insulin resistance leads to a variety of biochemical abnormalities. The capacity of the pancreatic β cells is important for compensation of impaired insulin sensitivity. About 5% of pregnancies are complicated by an abnormal glucose tolerance test and less than 5% of these develop gestational diabetes mellitus (89). Gestational diabetes is a harbinger of subsequent noninsulin-dependent type II diabetes mellitus (90), and type II is associated with a high rate of essential hypertension (91). Furthermore, it has been shown that pregnancy-induced hypertension and preeclampsia are more common in the presence of gestational diabetes (92). Even within the normal range, the level of plasma glucose elevation 1 hour following an oral glucose challenge is correlated with risk of preeclampsia (92). Hyperleptinemia has been suggested to play a role in insulin resistance. In pregnant women, leptin is synthesized in and secreted from placental trophoblasts into the maternal circulation at a considerable amount, with increases of 100% to 200% over prepregnancy values. Patients with preeclampsia exhibit serum leptin levels which are significantly higher than those of normal pregnant controls (93). Increased serum leptin concentration may be a marker of placental hypoxia in severe preeclampsia (94). TNF-α is another marker for insulin resistance (95) and circulating TNF-α levels correlate with microvascular permeability in preeclampsia (96). If evidence accumulates that pregnancy-induced hypertension and preeclampsia are at least partially mediated by insulin resistance, there may be a possibility to intervene with agents that enhance insulin sensitivity.

HYPERTENSIVE DISORDERS IN PREGNANCY

To understand gestational hypertension, it is important to know the physiologic changes in hemodynamics and blood pressure that occur during normal pregnancy. Soon after conception and during the first trimester of pregnancy, blood pressure decreases significantly despite increased plasma concentrations of angiotensin II. Decrements in diastolic blood pressure are in the range of 10 mm Hg (97). Women who are hypertensive before conception may demonstrate an even greater decrease in blood pressure (98). Subsequently in the second and third trimesters, blood pressure rises to reach near pregestation values at term (99). Thus, the definition of normal range of blood pressure differs in the pregnant from the nonpregnant state. The World Health Organization (100) classified hypertensive disorders of pregnancy in 1987 in a detailed manner, while the American College of Obstetricians and Gynecologists (101) and the National High Blood Pressure Education

Program Working Group (102) classified them in a more practical fashion and defined only four categories:

1. Chronic hypertension
2. Transient hypertension was changed to gestational hypertension in 2000 (102)
3. Preeclampsia and eclampsia
4. Preeclampsia and eclampsia superimposed on chronic hypertension

It is important to differentiate between these four disorders because prognosis and treatment vary. Despite the different classification of hypertensive disorders in pregnancy, hypertensive levels of blood pressure and increase in blood pressure thought to be critical for the development of preeclampsia are similar, namely systolic blood pressure ≥140 mm Hg and diastolic blood pressure ≥90 mm Hg, a rise in systolic blood pressure by ≥30 mm Hg or diastolic by ≥15 mm Hg. Early in pregnancy, blood pressure declines, with average values of 103 ± 11 mm Hg systolic and 56 ± 10 mm Hg diastolic in the first trimester (103); thus, before 20 weeks of gestation, a significant rise in blood pressure may still remain below designated preeclamptic levels and yet cause preeclampsia, especially when the increase in blood pressure occurs rapidly. A significant rise in blood pressure, especially in primiparas, is a suspicious sign of preeclampsia, and hospitalization should be considered. Although it may be difficult to make the diagnosis, overdiagnosing rather than underdiagnosing preeclampsia is preferred because the development of preeclampsia may be rapid and fulminant.

It may be very difficult to distinguish clinically between essential hypertension, secondary hypertension due to kidney disease, and pregnancy-induced hypertension or preeclampsia and combinations of these disorders. Preexisting hypertension may be suggested by microvascular changes in the retina. Preexisting renal disease may be suggested in women demonstrating reduced kidney size or proteinuria occurring without hypertension or occurring very early in pregnancy. However, glomerular diseases may occur in more than 20% of primipara pregnancies presenting with hypertension (104). Acceleration of renal diseases typically occurs during pregnancy in active lupus erythematosus. Pregnancy-associated changes in the thymus (105) and B-cell lymphopoiesis (106) may modulate autoantibody production and contribute to the flares observed in pregnant lupus patients.

Chronic Hypertension

Women with chronic hypertension have elevated blood pressures (≥140/90 mm Hg) prior to pregnancy. Hypertension is usually well tolerated when diastolic blood pressure remains less than 100 mm Hg (102). In general, antihypertensive therapy should be continued during pregnancy. When hypertension is diagnosed during pregnancy and persists for more than 42 days after delivery, it is also termed chronic hypertension (102). Some drugs may have side effects that may require a change in medication during pregnancy. There may exist an underlying secondary cause for the hypertension such as renal parenchymal or renal vascular disease, hypercorticism, or hyperaldosteronism; however, the presence of primary essential hypertension is not unusual. Patients with preexisting hypertension demonstrate a decrease in blood pressure early in pregnancy to a similar or even greater degree than normotensive women (107). In the third trimester, blood pressure rises to levels approximating the nonpregnant state. If uric acid and antithrombin III levels (108) are normal in this state, it is likely that the patient still has chronic hypertension; thus weekly reexamination is indicated. Chronic hypertension is associated with adverse pregnancy outcome, regardless of superimposed preeclampsia. It predisposes to preeclampsia by about a fivefold risk and small-for-gestational age babies by about a twofold risk (109,110). The risk for perinatal death also increases (111). Furthermore, women with chronic hypertension and proteinuria greater than 300 mg/24 hours before 26 weeks of gestation have a threefold increased risk of preterm delivery and small-for-gestational age babies as compared to women with chronic hypertension and no proteinuria (112). Chronically hypertensive African American women are more likely to develop preeclampsia (ca. 32%) than white women (ca. 15%)(113).

If blood pressure rises further in the third trimester and proteinuria and edema develop, it is suggestive of superimposed preeclampsia. The mechanism of inducing superimposed preeclampsia in women with chronic hypertension is not well known. Hypertension-induced vascular lesions and subsequent impairment of hemodynamics in the spiral arteries of the decidua and reduction of the perfusion of the uterus may result.

Gestational Hypertension

There are some women who demonstrate a mild to moderate rise in blood pressure to the hypertensive range in the third trimester, but without developing edema or proteinuria. This form of hypertension does not carry the perinatal and maternal morbidity of preeclampsia and does not progress to preeclampsia. Blood pressure normalizes within the first few days postpartum. Gestational hypertension has a high rate of recurrence in subsequent pregnancies (114,115), and it is believed that women of this group may develop essential hypertension later in life (116).

Preeclampsia and Eclampsia—Clinical Aspects

The syndrome of preeclampsia/eclampsia was recognized more than 100 years ago, when convulsions that occurred late in pregnancy and disappeared after delivery were reported. Later it was recognized that proteinuria and increased blood pressure frequently antedated these seizures associated with pregnancy (117). Circadian blood pressure measurements revealed a changing pattern in eclampsia demonstrating the highest pressure at midnight (118). Thus, it was suggested to classify this syndrome on arbitrary grounds of blood pressure levels and on the presence of proteinuria. It was hoped that these clinical criteria would allow recognition early in pregnancy of the group of women prone to subsequently develop preeclampsia and pregnancy-induced convulsion. However, although dramatically increased blood pressures may occur in women who progress to have convulsions, evaluation of blood pressure is only one component of the pathophysiology of eclampsia. For example, as many as 20% of pregnant women with eclampsia have blood pressure levels below 140/90 mm Hg just prior to the onset of convulsions (119,120), and up to 40% have little or no proteinuria (121). In 201 pregnant women, the blood pressure was measured within 1 hour of the onset of convulsion, and the mean diastolic pressure was 97 ± 14.6 mm Hg (119,122).

The diagnosis of preeclampsia is now based on criteria suggested by Chesley (123) and the National High Blood Pressure Education Program Working Group (102). These include: (1) proteinuria of ≥300 mg/day and often in the nephrotic range (124) associated with puffy edema; and (2) a rise in blood pressure of at least 15 mm Hg diastolic, 30 mm Hg systolic, or an increased mean blood pressure level of 20 mm Hg as compared to the levels before 20 weeks of pregnancy. In the absence of previous blood pressure measurements, blood pressure ≥140/90 mm Hg after 20 weeks of gestation is considered

sufficient to fulfill the blood pressure criteria of preeclampsia (102). More recently, Redman and Jeffries (125) suggested that in addition to this rise in blood pressure, the diastolic blood pressure level first has to be less than 90 mm Hg. With this selection criterion, patients with chronic or essential hypertension could be excluded from those with real preeclampsia. A modest amount of edema develops very frequently in pregnancy; however, some women destined to develop preeclampsia may rapidly gain several kilograms before developing the critical stigmata of preeclampsia (126). Although nephrotic proteinuria in preeclampsia is associated with more severe lesions in the biopsy specimen, nephrotic proteinuria does not correlate with the severity of clinical findings when compared to preeclamptic women with proteinuria less than 3 g/day (104). Although the clinical features of preeclampsia and eclampsia are similar to the characteristics of malignant hypertension, there is usually no papilledema or retinal hemorrhages and no malignant nephrosclerosis in eclampsia. Thus, eclampsia is not pregnancy-induced malignant hypertension. The blood pressure level does not correlate as well with perinatal mortality as does the elevated uric acid concentration (127) and activation of the coagulation cascade (128). Thus, classification of preeclampsia on blood pressure alone may overemphasize the importance of hypertension in the pathophysiology and result in mismanagement of those women with preeclampsia without hypertension. There are no strict values and parameters for detecting women at risk of developing preeclampsia and eclampsia. There are, however, some rules of thumb that may be helpful. Patients with a significant rise in blood pressure, occurrence of proteinuria, or nausea and abdominal pain (129) or having headaches and feeling ill during the second trimester should be considered as developing preeclampsia. Patients with blood pressure ≥140/90 mm Hg and the new occurrence of proteinuria of 1+ or greater require hospitalization (1120).

Preeclamptic women may demonstrate abnormal left ventricular function (130), and visual disturbances such as blurred vision, scotomata, amaurosis, and cortical blindness also may occur (131). Pregnant women are at increased risk of thromboembolic accidents due to changes in the fibrinolytic activity and coagulation factors (132). All cases of perinatal death associated with preeclampsia/eclampsia have abnormal intravascular coagulation abnormalities suggesting activation of the coagulation cascade (128). Mild thrombocytopenia is the most commonly found hematologic disturbance associated with preeclampsia (133), and it often antedates the clinical disease (134). Bone marrow studies in women with preeclampsia show that increased peripheral consumption and platelet turnover cause the decreased number of circulating platelets (133). Platelets from humans with essential hypertension (135) or women with preeclampsia show an exaggerated calcium response to vasopressin (136). The activation and impaired calcium response of platelets antedate the increased vascular sensitivity of preeclampsia. In contrast to the vasopressin response, adenosine diphosphate-induced and serotonin-induced calcium rise in platelets is reduced in preeclampsia (137). A decrease of platelets below 100×10^9/L is an ominous sign (138) (Table 53-1) and is a cause for hospitalization.

Activation of the coagulation cascade and vasospasm are consistent early findings in preeclampsia and may cause reduced organ perfusion. Furthermore, fibrin thrombi are demonstrated in glomerulus (139), cerebral (140) and hepatic (141) capillaries of women suffering from eclampsia, and these thrombi may contribute to the impairment of microcirculation in the presence of enhanced vasoconstriction (142). Depending on the severity of occlusion of capillary lumens, renal, cerebral, or hepatic manifestations of eclampsia may dominate. At autopsy, severe cases of preeclampsia/eclampsia demonstrate features of disseminated intravascular coagulation (DIC). An increased level of fibrin degradation products (143) *in vivo* is another sign of this coagulation disorder. By using sensitive assays, activation of the coagulation cascade can be demonstrated weeks to months before the clinical symptoms occur (144). A coagulation index based on the platelet count, factor VIII concentration, and serum fibrinolytic degradation products has been shown to correlate with the signs and symptoms of preeclampsia and also with perinatal death (128). Thrombocytopenia can occur weeks before clinical symptoms of preeclampsia and eclampsia are present (138) and even when there is no liver involvement (145). Spasm of the retinal arterioles is another prominent feature in patients with preeclampsia (146). The severity of spasm of retinal arteries correlates with the severity of glomerular lesions seen in kidney biopsies (146) (Fig. 53-6) and also with the occurrence of preeclampsia. Despite the capillary obstructions, intravascular coagulation is not necessarily the primary event that induces preeclampsia; however, DIC may be a major factor contributing to the impairment of organ perfusion and function.

TABLE 53-1

OMINOUS SIGNS OF PREECLAMPSIA

Blood pressure ≥160 mm Hg systolic, ≥90 mm Hg diastolic
Proteinuria (occurrence for the first time) ≥2 g/24 hr
Serum creatinine (normal before pregnancy) ≥1.2 mg/dL
Platelet count ≤100,000/μL
Microangiopathic hemolytic anemia (haptoglobin ↓, schistocytes)
Liver enzymes ↑
Headache
Cerebral disturbances
Visual disturbances
Pulmonary edema
Epigastric pain

(From: National High Blood Pressure Education Program Working Group Report on High Blood Pressure in Pregnancy. *Am J Obstet Gynecol* 1990;163:1691, with permission.)

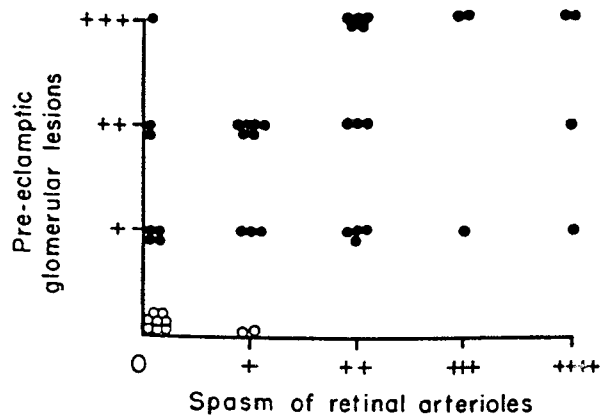

FIGURE 53-6. Funduscopic evaluation of retinal blood vessels demonstrates correlation of severity of spasm of retinal vessels and severity of preeclampsia-associated glomerular endotheliosis in kidney biopsies. Severe retinal arteriolar spasm was only seen in women with preeclampsia (*closed circles*) but not in hypertensive vascular disease (*open circles*). (From: Pollak VE, Nettles JB. The kidney in toxemia of pregnancy: a clinical and pathologic study based on renal biopsies. *Medicine* 1960;39:469, with permission.)

Genetic Factors Predisposing to the Development of Preeclampsia and Eclampsia

Genetic factors may predispose to the development of preeclampsia and eclampsia. Epidemiologic data suggest a familial tendency to preeclampsia (147). An analysis of the family history of patients with eclampsia demonstrated a fivefold increased risk in first-degree relatives and a twofold increased risk in second-degree relatives as compared to the general population (148). Chesley (149) suggests the existence of an autosomal-recessive gene defect that predisposes to preeclampsia. Studies of 26 Icelandic and 18 Australian families with a high rate of miscarriages, specifically 22 cases of eclampsia and 116 cases of preeclampsia, suggest that a dominant gene defect with low penetrance may be involved (150,151). A genome-wide scan of 72 Icelandic families, including 186 women with preeclampsia or eclampsia, suggests a maternal susceptibility locus for preeclampsia on chromosome 2p13 (152). In contrast, no linkage is found with angiotensinogen or endothelial NO synthase genes. Retrospective analysis of the records of 14 primigravidas giving birth to babies with trisomy 13 all demonstrated severe preeclampsia (153). In contrast, the first pregnancy was normal when the baby had trisomy 18 or trisomy 21. This may suggest a role for chromosome 13 in preeclampsia.

Incidence of Preeclampsia and Eclampsia

The incidence of eclampsia is quite similar in Western countries. In Great Britain, it is 4.9 per 10,000 pregnancies but may occur in up to 10% of primigravidas (119). In the United States, the incidence of eclampsia is 4.3 per 10,000 pregnancies (154). Analysis of regional incidences suggests that there has been a decline in incidence since introducing antihypertensive treatment. However, since then, no significant further decrease in incidence of eclampsia has occurred in the past 20 to 30 years (119,155,156). Douglas and Redman (119), Corkill (155), and Hibbard and Rosen (157) suggest that classic eclampsia presenting with high blood pressure and proteinuria may be prevented by screening and antenatal control. However, atypical presentation of eclampsia still has an unchanged incidence. In this regard, it is important to note that one-third of women have only mild or no hypertension before the onset of eclampsia, and therefore the diagnosis and treatment may be delayed. The incidence of preeclampsia is highest in primigravidas (156), especially in primigravidas older than 40 years (158) or younger than 20 years (98). Twin pregnancy increases the risk about five times (159). Women with preexisting hypertension (121); microangiopathy, such as diabetic angiopathy; moderate renal insufficiency due to pregestational renal disease (160); and patients who have had preeclampsia with proteinuria are at risk to develop preeclampsia (161) (Table 53-2).

Two-thirds of cases of eclampsia develop in women with the first pregnancy. In a multicenter study in Great Britain, 383 pregnancies were complicated by eclampsia, and 18% of cases were multiparous women with no previous history of preeclampsia (119). Eclampsia has not only been documented to occur antepartum but also during labor and up to 3 weeks' postpartum (162–164). In the British multicenter analysis (119), 38% of women had antepartum eclampsia, 18% intrapartum eclampsia, and 44% postpartum eclampsia. Preterm and antenatal eclampsia appear to be more dangerous to mother and child (119). Women with antepartum eclampsia are reported to have a higher incidence of abruptio placentae and *h*emolysis, *e*levated *l*iver enzyme levels and a *l*ow *p*latelet count (HELLP) syndrome (microangiopathic hemolytic anemia, elevated liver enzymes, and low platelets) as compared to those with postpartum eclampsia (165). Multiparous women who have preeclampsia will usually demonstrate a predisposing factor and will develop preeclampsia in about 70% of subsequent pregnancies (147). Patients with preeclampsia early in pregnancy very likely have underlying renal disease (165) or hydatidiform mole (166). Hydatidiform moles increase the risk of preeclampsia by about 10 times.

TABLE 53-2

RISK FACTORS FOR DEVELOPING PREECLAMPSIA

Family history of preeclampsia, hypertension (159)
Diabetes mellitus, insulin resistance
Renal disease
Nulliparity (159)
Uric acid level ↑ (146)
β_2-microglobulin ↑ (381)
Platelet count ↓ (131)
Antithrombin III ↓ (105)
Blood pressure ↑ (99)
Plasma fibronectin ↑ (269)
Proteinuria new or ↑ (99)
Urinary calcium excretion ↓ (384)
Urinary 6-keto-prostaglandin $F_{1\alpha}$ ↓ (302)
Angiotensin II sensitivity test abnormal (47)
Rollover test abnormal (47,175)
Retroaortic left renal vein (370)

In patients with preeclampsia and eclampsia, total plasma volume is decreased as compared to pregnant women with normal pregnancies (167). There is a significant relationship between plasma volume at 38 weeks of gestation and birth weight of the neonate (32). Atrial natriuretic peptide (ANP) concentrations are increased in pregnancy-induced hypertension and may reflect a compensatory mechanism to attenuate blood pressure (168). There is good correlation of ANP levels and severity of hypertension and eclampsia. In this setting, ANP secretion is probably not stimulated by increased atrial pressure but rather by a different mechanism. Animal studies suggest that ANP is involved in the regulation of placental blood flow. Pregnant rats with hypertension may normalize impaired placental blood flow by increasing ANP concentrations (169). Hemoconcentration is a common finding in women with preeclampsia and eclampsia. Although fluid administration seems logical in such a situation, a state of increased afterload due to volume expansion may facilitate the development of pulmonary edema.

Preeclampsia usually becomes symptomatic after 20 weeks of gestation. In women who develop preeclampsia, blood pressure may rise early in the second trimester, although it may still be within the normal range. A characteristic finding in preeclampsia is the lability of blood pressure. In parallel to this lability, vascular resistance to the vasoconstricting effect of angiotensin II is lost by 14 to 16 weeks (45). The required dose of angiotensin II to increase diastolic blood pressure by 20 mm Hg decreases significantly (Fig. 53-5). Repetitive tests in preeclampsia-prone women will demonstrate that the increased sensitivity to angiotensin II-induced vasoconstriction antedates clinical symptoms and increases progressively (45). Wallukat et al. report that serum from a study of 25 preeclamptic patients contained stimulatory autoantibodies against the angiotensin-II type I receptor (170); it is believed that these autoantibodies may participate in the hypertension observed in preeclampsia. Despite the good correlation between changes in sensitivity to angiotensin II and the development of preeclampsia, the test is not practical as a screening method in large populations. The response of women with preeclampsia to other vasopressor hormones such as norepinephrine (171) or vasopressin (172) is less consistent. The rollover test, another test to screen for high-risk pregnant women, was introduced by Gant et al (173).

In this test, the pregnant woman is stabilized in the lateral position and her blood pressure recorded; thereafter, she is turned to the supine position, and the diastolic blood pressure is immediately measured. The test is considered positive when there is an increase of 20 mm Hg in the supine position. Women with a positive test have a twofold to fourfold increase in their rate of preeclampsia. A later evaluation of this test, however, demonstrated less optimistic predictive results (174).

Eclampsia

When preeclampsia progresses, nonspecific signs may occur such as hyperreflexia, restlessness, headaches (approximately 50%), epigastric pain (approximately 20%), and visual disturbances (approximately 20%) (119). However, whenever convulsions occur, the symptom complex is termed eclampsia. Some patients may have only minor systemic organ dysfunction and still demonstrate convulsions, while others are severely ill with progressive renal failure, microangiopathic hemolysis, and DIC without convulsions. Thus, some preeclamptic patients are equally or even more ill than some patients with overt eclampsia. Despite the improvement in treating eclampsia, maternal complications are high with a 1% to 2% mortality rate. More than one-third of eclamptic women have at least one major complication (119) (Table 53-3). Fetal outcome in the British multicenter eclampsia study demonstrated that 29% of 411 fetuses were small for their gestational age and there were 7.3% deaths. More recent data suggest that a convulsion is just one symptom of a broad range of signs that are the result of widespread endothelial cell damage (175). Therefore, the initial site of symptoms depends on the site of endothelial damage. On the other hand, the seizures of eclampsia may occur without other signs of preeclampsia but may still result from the same underlying endothelial injury mechanism.

Postmortem studies in women who have died from eclampsia have confirmed that eclampsia is a systemic disease. Fibrin deposition, petechial or gross hemorrhages, thrombosis, and fibrinoid necrosis were shown in blood vessels in several organs such as the brain, kidney, and liver (140). Reduced organ perfusion was the predominant anomaly. Evidence of vasoconstriction has been observed on funduscopic examination by demonstrating segmental narrowing and spasms of retinal arterioles (146). When using sensitive computed tomographic (CT) scanning equipment, nearly 50% of women with eclampsia demonstrate *in vivo* abnormal cerebral results (176) and some degree of edema (177). Eclampsia is a life-threatening complication of pregnancy, and urgent delivery is demanded. Even so, mortality is high in these patients (178). At necropsy, more than 50% of patients have cerebral hemorrhages as the lethal event (178). Although eclampsia is a disease of primigravidas, those who die tend to be older and multiparous (178). Placental abruption may occur in the setting of eclampsia and cause fetal death and contribute to the high mortality of the mother (179). Since the introduction of antihypertensive medications to lower blood pressure and magnesium sulfate to control convulsions, the frequency of developing overt eclampsia has decreased and is a rather rare complication in industrialized countries (180).

TABLE 53-3

MAJOR MATERNAL COMPLICATIONS IN 382 CASES OF ECLAMPSIA

Complication	No. of patients
Death	7 (1.8%)
Persistent vegetative state	1 (<1%)
Cardiac arrest	6 (1.6%)
Cerebrovascular accident	7 (1.8%)
Adult respiratory distress syndrome	7 (1.8%)
Required ventilation	87 (23%)
Pulmonary embolism	5 (1.3%)
HELLP syndrome	27 (7%)
Disseminated intravascular coagulopathy (without HELLP)	33 (9%)
Renal failure	24 (6%)
Septicemia	2 (<1%)

HELLP, microangiopathic hemolytic anemia, elevated liver enzymes, and low platelets.
(From: Douglas KA, Redman CW. Eclampsia in the United Kingdom. *Br Med J* 1994;309:1395, with permission.)

Renal Findings in Preeclampsia and Eclampsia

The majority of women with preeclampsia and eclampsia have mild to moderate impairment of kidney function as compared to normal pregnant women. As the creatinine clearance decreases, the rise in serum creatinine levels may still be less than 1 mg/dL, thus within the normal range for nonpregnant women. Characteristic changes in renal morphology occur in preeclampsia and eclampsia. The capillary wall in the glomerulus is thickened as a result of characteristic changes of glomerular endothelial swelling, termed *endotheliosis* (181,182). Glomerular endotheliosis has been observed in all primiparous women with preeclampsia (104). Narrowing of the glomerular capillaries frequently causes obliteration of the lumen. Deposits may be demonstrated in the mesangium that are consistent with fibrinogen. Deposition of immunoglobulin, most frequently IgM, in preeclamptic women (183) may reflect another underlying renal disease (104). In preeclampsia, the glomeruli are enlarged, and in severe cases, Bowman's capsule may be distended. Herniation of an enlarged glomerulus into the proximal tubule may occur in some cases (146). Glomerular epithelial cells may be enlarged with vacuolization and may contain hyaline deposits (184). There is, however, no glomerular crescent formation. Subendothelial depositions of fibrin and fibrinoid may be present and particularly prominent on electron microscopic examination (182,185). The glomerular basement membrane seems thickened and has a double contour (184). Electron microscopic examination has identified deposition of fibrils beneath the endothelial cells. The glomerular histology does not resemble findings of malignant hypertension but rather resembles the findings of hemolytic–uremic syndrome and DIC (186). Depending on the time point of performing a biopsy, the renal lesions may differ greatly. The subendothelial depositions resolve soon after delivery. Postdelivery, foam cells may be present in the glomerulus (184); it has been suggested that these are due to resolution of fibrinoid deposition. The changes in the basement membrane resolve after delivery, but this may take several weeks to months (187).

Gaber and Spargo (188) demonstrate focal glomerular sclerosis and suggest these findings are the result of an underlying renal disease, while Kincaid-Smith and Fairley (117) favor the idea that these renal changes are due to preeclampsia and eclampsia. Fisher et al. (104) observe that 74% of pregnant women with nephrosclerosis on renal biopsy later became hypertensive. Peyser and associates (189) show that 10 of 13 preeclamptic women with nephrosclerotic lesions became hypertensive within 2 to 7 years. Fisher et al. (104) report that on the basis of clinical findings the diagnosis of preeclampsia was made correctly in 54% of 176 pregnant women; however, the diagnosis was greater than 80% in primiparous women using the histology of renal biopsy as the "gold standard." Nochy et al. (183) biopsied 114 pregnant women after delivery and found that those women with clinical signs of preeclampsia also showed classic endotheliosis on renal biopsy. In addition,

patients with isolated pregnancy-induced hypertension, hyperuricemia, and no proteinuria also demonstrated the typical glomerular lesions of preeclampsia. The preeclamptic lesions could be superimposed in patients with preexisting nephropathy. Rarely, preeclampsia may cause acute tubular and cortical necrosis. Autopsy of seven infants born to mothers with preeclampsia or eclampsia demonstrated no lesions in the kidney (146).

After delivery, clinical pathology and laboratory abnormalities return to normal within days to weeks. However, organ function in some patients with preeclampsia and eclampsia may not recover immediately or recover incompletely due to severe tissue injury initiated during preeclamptic state. Proteinuria greatly resolves within 3 months (190). In most patients, nonspecific cerebral disorders resolve within weeks after delivery. In two patients suffering from eclampsia, Fredriksson and associates (191) demonstrated disseminated cerebral microvascular occlusion by CT and magnetic resonance imaging. These lesions disappeared completely without residua. In women without underlying disease, the long-term prognosis is excellent. The recurrence of the preeclampsia/eclampsia syndrome in subsequent pregnancies is 10 to 20 times less as compared to the risk for the first pregnancy (192). The remote prognosis of preeclampsia is a matter of debate. The discrepancy may in part be the result of different definitions of preeclampsia. Some cases of transient hypertension may have been misdiagnosed as preeclampsia. Some authors believe that in primiparous women the remote prognosis is identical to that of women with normal pregnancy (104,114,193). When the diagnosis was made on clinical and laboratory findings and confirmed by renal biopsy, the rate of later development of hypertension was identical to the expected rate of the overall population (104). In a more recent cohort study of 626, 272 births in Norway found that in preeclampsia a 1.2 fold higher long- term risk of all cause of death and eightfold risk of cardiovascular causes in preeclamptic women with a child of low birth weight (194). In contrast, a follow-up in 206 women who suffered from preeclampsia as a primigravida showed no increased incidence in hypertension more than 30 years later (114). Women who suffered from eclampsia with convulsions during the first pregnancy as long ago as 40 years did not demonstrate a different mortality rate in the follow-up period. In contrast, 64 women who developed eclampsia during the second or subsequent pregnancies more often had hypertension later in life and demonstrated an increased mortality rate as compared to controls without this complication (114) (Fig. 53-7). The best correlation of the occurrence of hypertension as a late complication of preeclampsia and eclampsia was seen with systolic blood pressure in the early phase of pregnancy (195). However, this higher systolic blood pressure during early pregnancy may have reflected an early sign of essential hypertension.

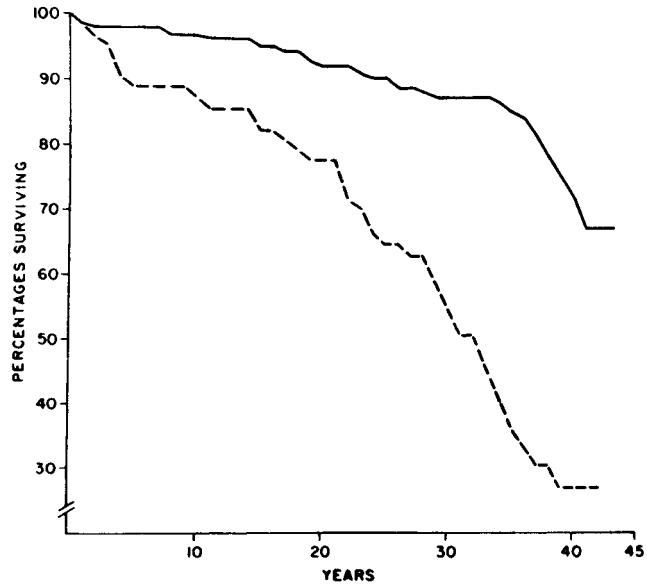

FIGURE 53-7. Survival of patients who had eclampsia as primigravidas (*solid line*) as compared to survival of women who had eclampsia as multigravidas (*dotted line*). Mortality of primigravidas was not different than matched controls. (From: Chesley LC, Annitto JE, Cosgrove RA. The remote prognosis of eclamptic women. *Am J Obstet Gynecol* 1976;124:446, with permission.)

Microangiopathic Hemolytic Anemia, Elevated Liver Enzymes, and Low Platelets (HELLP Syndrome)

Hemolysis with red blood cell fragmentation, abnormal liver function, and thrombocytopenia have long been recognized as complications in preeclamptic women with hypertension (138,196), but these symptoms may occur in a great number of pregnant women without a rise in blood pressure (197). In 1982, Weinstein (190) described 29 women with these symptoms in association with preeclampsia or eclampsia and termed the disease the HELLP syndrome. The acronym HELLP stands for microangiopathic hemolytic anemia, elevated liver enzymes, and low platelets. While extremely high liver enzyme levels and thrombocytopenia of less than 100×10^9/L are usually demonstrated, these findings are not pathognomonic for the HELLP syndrome. These parameters may also be altered in patients with preeclampsia and eclampsia. The ratio of preeclampsia to HELLP syndrome is 1.2:1.0 when mild cases are included. Some authors consider the HELLP syndrome an early form of preeclampsia, while others believe it to be mild DIC that was misdiagnosed. Weinstein's clotting studies in these women were normal by measuring prothrombin time, partial thromboplastin time, and serum fibrinogen. Others suggest that hemolysis and low platelet counts reflect a low grade of intravascular coagulation, which is not detected by these insensitive tests (198). HELLP cases have been reported with the full signs of DIC (199). The syndrome may also develop during conservative management of patients with documented preeclampsia (190). The most reliable parameters to recognize microangiopathic hemolysis are schistocytes on peripheral smear, low plasma haptoglobin levels, reticulocytosis, and an increase in indirect plasma bilirubin (200). Serum concentration of total lactate dehydrogenase (LDH) is less frequently enhanced, while isoenzyme analysis has been shown to detect a rise in LDH_4 and LDH_5 early in the development of the syndrome. Liver enzymes and bilirubin are increased as well as serum uric acid levels. There are other liver diseases unique to pregnancy that may increase liver enzymes but are not associated with the HELLP syndrome, preeclampsia, or eclampsia (201). Classic hepatic lesions associated with the HELLP syndrome are periportal and/or focal parenchymal necrosis in which fibrin-like material can be seen.

Most common symptoms of the HELLP syndrome are nausea, headaches, right upper quadrant tenderness, or epigastric or right hypochondrial pain, which may easily be misdiagnosed as dyspepsia, gastroenteritis, hiatal hernia, viral hepatitis, or cholecystitis. None of these symptoms and signs is diagnostic for the HELLP syndrome. Regardless of the blood pressure, the patient requires hospitalization. Most often prompt delivery is indicated (102,141), although conservative management has been successful (197). In the absence of evidence of DIC, steroids may be given to accelerate fetal lung maturation; however, maternal and fetal conditions should be assessed continuously. Maternal mortality ranges between 1% and 20%, and fetal mortality is as high as 50% (190,197,198,200,202). Mothers are at risk for abruptio placentae, pulmonary edema,

acute renal failure, and subcapsular liver hematomas, which may detach and eventually tear the capsule of the liver with liver rupture and intraabdominal bleeding (203). Hepatic failure may be caused by microemboli in the hepatic vasculature, leading to ischemia and tissue damage. Vasospasm causing endothelial injury may predispose to platelet aggregation and thrombocytopenia in the same manner as described for preeclampsia (198). The fetus may suffer perinatal distress and asphyxia; overall, however, neonatal survival is primarily dependent upon gestational age and birth weight at the time of delivery (204). The majority of these women have evidence of preeclampsia before delivery. Sibai (205) followed 59 women with the HELLP syndrome through 80 subsequent pregnancies. Only two patients (3.4%) had recurrence. In an analysis of this study, the syndrome developed in 62 of 206 (30%) cases a few hours to 6 days after parturition, and the remainder (70%) occurred before delivery (205).

PATHOPHYSIOLOGIC MECHANISMS IN PREGNANCY-INDUCED HYPERTENSION AND PREECLAMPSIA

The Role of Trophoblast and Spiral Arteries in the Development of Preeclampsia and Eclampsia

There is considerable evidence that preeclampsia/eclampsia is the result of impaired trophoblastic invasion and perfusion of the uterus. In normal pregnancies, trophoblastic invasion causes morphologic and functional changes in the spiral arteries but not the arcuate and radial arteries of the uterus (206). Invasive trophoblasts alter their adhesion molecule expression from epithelial to those of endothelial cells, a process of remodeling called "pseudovasculogenesis" (207). Yet little is known about the mechanism that promotes and controls this invasion of maternal tissue. A variety of cells such as natural killer (NK) cells, lymphocytes and macrophages are involved in the production of mediators in the decidua. Insulinlike growth factor, fibroblast and placental growth factor, vascular endothelial growth factor (VEGF), and cytokines such as TNF-α, transforming growth factor-β (TGF-β), epidermal growth factor, interleukin-1 (IL-1) and -6, tissue metalloproteinase inhibitors have been implicated in this process. During normal pregnancy, the spiral vessels increase their caliber markedly, lose muscular and elastic layers, and become low-resistance vessels that open into the intervillous space (207a). This process is completed by about 20 weeks of gestation (208). The changes extend through the decidua into the inner third of the myometrium (Fig. 53-8). Spiral arteries of women with preeclampsia do not demonstrate these structural changes (209), and trophoblast invasion is limited to the superficial decidua. Studies of trophoblast tissues show that cell differentiation and invasion in preeclamptic pregnancies are interrupted (210) and expression of adhesion molecules is abnormal (211). In addition, the lumens of many vessels are occluded by changes called acute atherosis and characterized by the presence of large foam cells, fibrinoid necrosis in the media of the vessel wall, and narrowed segments of the spiral arteries due to a lack of the physiologic adaptation (Fig. 53-8). Reduced spiral artery development and acute atherosis attenuate trophoblastic perfusion (212). Injection of ^{22}Na into the intervillous space is removed more slowly in women with preeclampsia than in women with normal pregnancy (213).

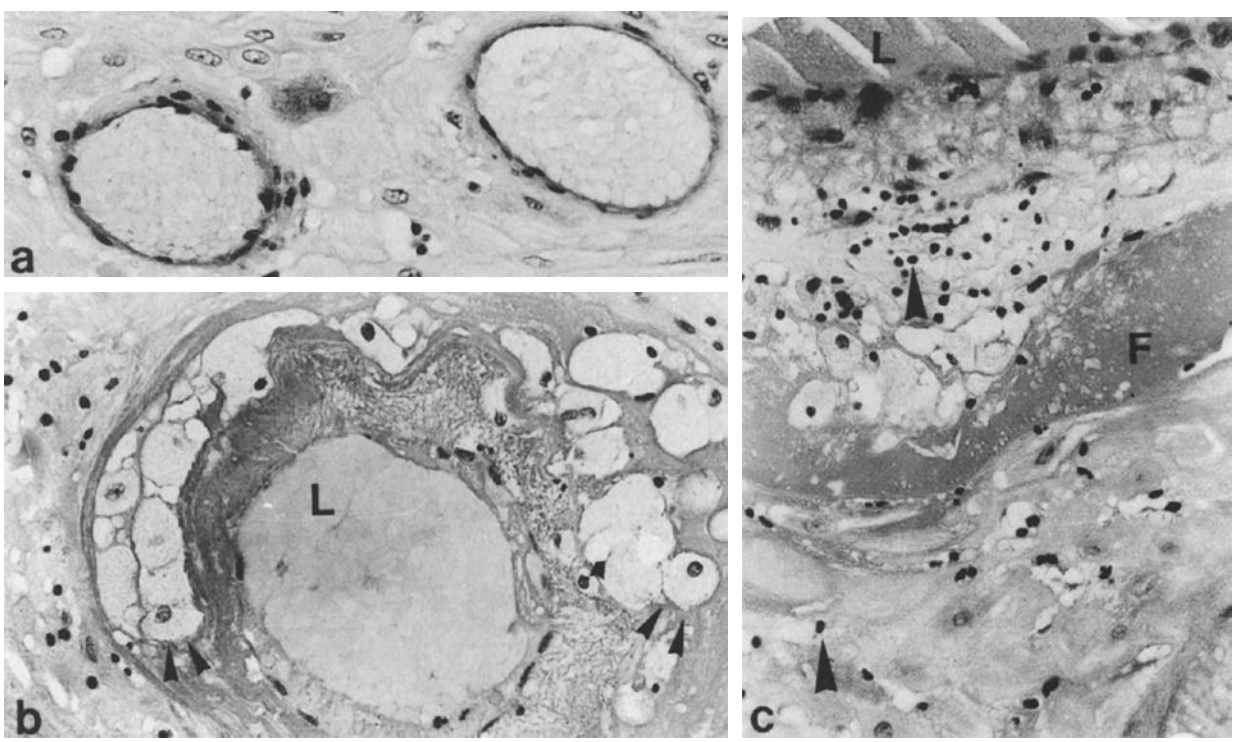

FIGURE 53-8. Acute decidual atherosis. **A:** Decidual tissue with normal blood vessel. Absence of inner elastic lamina and smooth muscle. **B,C:** Decidual arteriopathy. Acute atherosis characterized by thickening of the vessel wall. Accumulation of foamy macrophages (*double arrows*). **C:** Fibrin depositions (*F*) in the intima and sparse lymphocytic infiltrates (*arrows*) are observed. *L*, lumen of decidual vessel. (Hematoxylin and eosin ×350.) (Photographs courtesy of Dominique Gaeng, Pathology, University Hospitals, Bern, Switzerland.)

Women giving birth to small-for-gestational age babies have also demonstrated impaired trophoblast invasion (214). Vascular lesions in placentas derived from eclamptic patients are similar to vascular rejection in transplant grafts (215,216).

Other conditions that reduce trophoblast perfusion and predispose to preeclampsia independent of parity include increased placental mass as seen in twin pregnancies (159), trophoblastic tumors, hydatidiform moles (217), preexisting diabetes mellitus, collagen vascular diseases, and antecedent hypertension (206,209,218). Even without preeclampsia, diabetic women demonstrate atherosis in spiral arteries (206). This vasculopathy may lead to vascular thrombosis and vessel rupture with subsequent placental infarction, abruptio placentae, and retroplacental hemorrhage. The occurrence of preeclampsia in abdominal and molar pregnancies indicates that neither uterine nor fetal factors are required for the development of this syndrome. Experimental reduction of uterine perfusion by ligation of the abdominal aorta below the renal arteries causes the clinical picture of glomerular endotheliosis and preeclampsia in monkeys (219) and other mammals (220,221). In humans, those medical conditions that increase the risk of preeclampsia are all associated with microvascular diseases and thus the potential to reduce placental perfusion.

A number of other mediators such as hepatocyte growth factor (HGF), TGF-β, epidermal growth factor, and IL-1 have been shown to regulate trophoblast invasion. Thus, HGF knockout mice are lethal to the embryo due to abnormal placental development (222). The vascular endothelial growth factor (VEGF) plays a role in promoting angiogenesis. Its activity is modulated by the soluble fms-like tyrosine kinase 1 (sFlt 1), expressed on vascular endothelial cells. The sFlt 1 is a naturally occurring blocker of VEGF in patients with preeclampsia. The administration of sFlt 1 to rats has been shown to induce changes with albuminuria, hypertension and glomerular endotheliosis (223). In preeclamptic women sFlt 1 level increases as early as 13 weeks, several weeks before the onset of symptoms (preeclampsia 4382 pg/mL; control 1643 pg/mL $p < 0.001$)(224). In contrast, the level of VEGF decreases before the onset of symptoms (225). Furthermore, VEGF knockout mice also demonstrate glomerular endotheliosis and proteinuria (226). However, sFlt 1 may play a critical role in some patients but does not induce alteration of clotting, suggesting additional factors contributing to the full picture of preeclampsia. Disturbance of the balanced interplay of these mediators may therefore impair trophoblast invasion. Under experimental conditions, fibrin deposition is initiated by injecting an extract from placental tissue into experimental animals; this results in fibrin deposition with hepatic lesions similar to those observed in eclampsia (227).

It is suggested that as a consequence of reduced uteroplacental blood flow, aberrant expression of genes occurs that may contribute to the pathophysiology of preeclampsia (228). In this context, it is interesting to note that preeclampsia/eclampsia is associated with a significant increase in embolic trophoblastic fragments in the venous circulation (229). These data further demonstrate the significance of trophoblasts in the induction of pregnancy-induced hypertension and preeclampsia and eclampsia.

Immunologic Factors Predisposing to Preeclampsia and Eclampsia

There is evidence that the maternal immune response may be changed from cellular responses toward humoral immunity (230). There is clinical evidence that in pregnancy cell-mediated immunity is weakened and humoral immunity is strengthened. The majority of women with rheumatoid arthritis, a cell-mediated disease, show remission of their symptoms during pregnancy (231), while systemic lupus erythematosus, a disease resulting from excessive autoantibody production, may flare during gestation (232). The lack of human leukocyte antigens (HLA) on the syncytiotrophoblast and the presence of only the nonclassic HLA-G antigen on the cytotrophoblast cells preclude the fetal trophoblasts from playing a major part in currently recognized types of allogeneic immune reactions (233,234). The trophoblast produces a factor that inhibits T-cell proliferation and that may be a paracrine mechanism that contributes to fetal survival (235). Cytotoxicity, mediated by nonspecific NK cells, seems to be dampened by inhibition by the T_H1 helper cells, which produce cytokines such as interleukin-2 (IL-2) and interferon-γ (IFN-γ). In normal pregnancy, IL-2 levels are increased (236). On the other hand, activation of NK cells is enhanced in women with preeclampsia (237). In mice, the activation of these cells has been shown to result in fetal resorption (238).

Dudley et al. (238) further suggest that the regulation of cytokine production changes during normal pregnancy by a mechanism not resulting from specific fetal antigens. Nonspecific complement-mediated damage to the trophoblast may be inhibited by membrane-bound complement regulatory proteins, which protect the trophoblast from maternal complement-mediated damage arising from classic or alternate pathways and also systemic complement activation in response to microbial infections (239). However, pregnancy is not an immunodeficient state. Viral infections are not more common in pregnancy and are not more likely to become generalized as occurs in immunodeficient patients. The reason why falciparum malaria is more severe during the first pregnancy is not known (240) and why the resistance to the intracellular pathogens *Listeria monocytogenes* and *Toxoplasma gondii* is lowered is not known (241). Even more interesting is that women receiving malaria chemoprophylaxis during their first pregnancy have similar clinical course and prevalence of malaria infection of the placenta during the second pregnancy as do women not having received antimalaria medication during the first pregnancy (242). Current data do not suggest that pregnancy affects the course of human immunodeficiency virus infection in women (243). Immunologic tolerance between two genetically dissimilar tissues, maternal and fetal, is required for successful pregnancy. The current concept suggests that fetal trophoblast is not susceptible to attack by maternal T cells but suggests that other aspects of the immune system may be modulated during pregnancy as part of the maternal adaptations necessary for successful development of the fetus.

The thymus changes dramatically during pregnancy (105). In the cortex, the thymocytes die, and the medulla enlarges. Medullary epithelial cells become rearranged and proliferate to form the medullary epithelial rings. Estradiol is the most effective of the sex steroids at depleting immature CD4+ CD8+ thymocytes. Under experimental conditions, antithymocyte antibodies prevented implantation of the conceptus (244). Thus, cell death in the cortex may shut down the production of clones reactive with fetal antigens. However, the thymus is not necessary for a successful pregnancy (245). In addition to T cells, B cells are also affected by pregnancy. Estradiol pellets implanted in male or female mice demonstrated selective depression of B-cell precursors, and progesterone potentiated this effect (106,246).

That the etiology of preeclampsia/eclampsia has an immunologic component derives from the idea that normal mechanisms to protect fetoplacental tissue from rejection may be impaired (247). The following indirect evidence supports this hypothesis: (a) The risk to develop preeclampsia/eclampsia increases with a new partner, and (b) contraceptive methods that prevented exposure to sperm and seminal fluid prior to pregnancy enhance the risk of preeclampsia/eclampsia by 2.4-fold (248). Moreover, the risk of preeclampsia is reduced in the second pregnancy with the same partner but not with a new

partner (249,250). The risk of preeclampsia in women whose partner had already a child with another women with eclampsia is twice as high as the risk with no family history of any side effect (251). However, the protective effects of previous pregnancy against preeclampsia is transient. When the inter birth interval was 10 years or more, the risk approximated that of nulliparous women (252). As suggested by Rebiller et al. (253), pregnancy-induced hypertension may be a problem not only of primi gravidity but also of primipaternity. The incidence of pregnancy-induced hypertension was 11.9% among primigravidas, 4.7% among same-paternity multigravidas, and 24% among new-paternity multigravidas. Furthermore, the overall incidence of pregnancy-induced hypertension of 11.9% decreased in their cohort to 3.3% in primigravidas who had more than 12 months of sexual contact with the same partner before conception. In pregnancies with artificial donor insemination, the incidence of preeclampsia/eclampsia is elevated twofold for primigravidas and ninefold for multigravidas (254). Five out of 10 women treated for infertility with oocyte implantation developed preeclampsia (255). In contrast, regular inoculation of the female genital tract with allogenic spermatozoa bearing histocompatibility antigens of the male reduces the incidence of subsequent preeclampsia (256). Thus, it is suggested that the dose of antigen presented by the trophoblast overwhelms the immune system of the mother, or factors responsible for placental immunoprotection may be produced in insufficient levels to produce an immunologic blockade to the fetal antigens. Recently it has been suggested that primipaternity in some cases may be a relevant risk factor rather than primiparity. In women with no history of preeclampsia a new father increased the risk of preeclampsia by 30% (257). In some patients with preeclampsia a new father was associated with a non-significant decrease in risk. First pregnancy may induce tolerance to the paternal antigen during the second pregnancy. Data from the Medical Birth Registry of Norway including more than 1.8 million births demonstrated that the risk of preeclampsia in the subsequent pregnancy was related to the time that had passed since the prior pregnancy ending with preeclampsia and not the change of the partner (258). The resulting vascular changes pathognomonic of preeclampsia caused a disruption of the balance between vasodilating and vasoconstricting mediators, thus resulting in impaired hemodynamics and hypoperfusion of the fetoplacental unit. Some mediator(s) are produced and released from the trophoblast and seem to cause activation or damage to the vascular endothelium in the circulation of the mother. Inhibin A and activin A are released from the preeclamptic placenta with subsequent rise in its blood level. In 71 patients of 1,496 nulliparous women preeclampsia developed. Still, the potential of this marker for identifying pre-symptomatic women at risk of developing the disease is less clear (259). During the first exposure to paternal antigens, the mother acquires protection for subsequent pregnancies. The role of the decidial NK cells are considered the endometrial memory of paternal antibodies. In experimental animals undergoing spontaneous fetal resorption, immunization with allogeneic lymphocytes leads to enhanced fetoplacental survival and growth (260). Maternal immunity may also affect human pregnancy, since it has been shown that women with recurrent spontaneous abortion can deliver a healthy baby following immunization with paternal white blood cells (230).

T cells induce many of their effects by secretion of cytokines. Cytokines such as granulocyte–macrophage colony-stimulating factor (GMCSF) can enhance fetal growth, and cytokines such as TNF-α and IFN-γ can impair pregnancy (260). However, TNF-α and IFN-γ may also be required in the invasion of the trophoblast and development of the fetoplacental unit. Placentas from abortion-prone pregnancies induce predominantly TNF-α, IFN-γ, and IL-2 (230). These cytokines may be important for the remodeling of the uterus. Uteroplacental TNF-α production increases during gestation (261) but may be neutralized by a soluble TNF-α receptor produced by the trophoblast (262). Wegmann and associates (230) suggest that a decrease in the ratio of TNF-α to TNF-β receptor may initiate contraction and labor. The second pregnancy with the same partner would be less likely to stimulate the same immunologic processes because long-lived blocking immunoprotective factors were formed during the first pregnancy. Sufficient evidence therefore exists that some cases of preeclampsia may be the result of inadequate immunoprotection of the fetoplacental unit.

Endothelial Dysfunction Prior To and During Preeclampsia and Eclampsia

Serum of preeclamptic women is cytotoxic. Moreover, there are several clinical and laboratory findings suggesting maternal endothelial dysfunction as an important component in the development of preeclampsia and eclampsia. The pathologic findings in tissue histology of women with preeclampsia/eclampsia are not the changes of blood vessel disruption typically occurring in patients with markedly elevated blood pressure. Rather, the most common findings are subendothelial necrosis and hemorrhages resembling ischemic injuries similar to patients with hypovolemic shock (201). As already discussed, the typical renal changes are swelling of glomerular endothelial cells, or glomeruloendotheliosis (263). No other form of hypertension demonstrates similar histologic findings except hemolytic–uremic syndrome, which preferentially presents with glomerular endothelial changes and fibrin thrombi (264). The increased rate of disappearance of Evans blue dye in preeclampsia is compatible with loss of endothelial cell integrity (265). *In vitro* studies also support the notion of endothelial damage as an important factor in the development of preeclampsia/eclampsia. Biochemical indicators of endothelial cell activation and injury are enhanced in the blood of women with preeclampsia/eclampsia. Serum from preeclamptic women added to the culture medium of human umbilical vein endothelial cells (HUVEC) enhanced ^{51}Cr release, an index of endothelial cell activation (266). In contrast, however, ^{51}Cr release induced with serum derived from the same women shortly after delivery was not different from controls (Fig. 53-9). When similar amounts

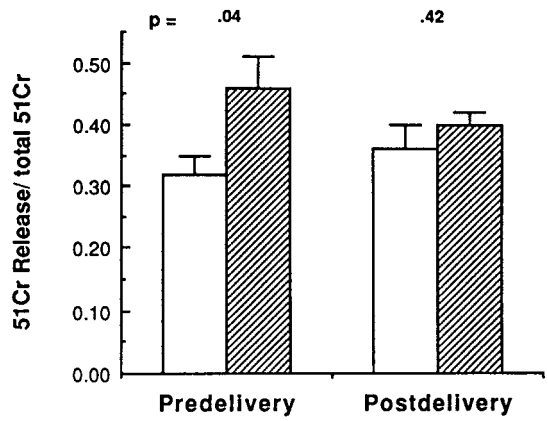

FIGURE 53-9. Cytotoxicity of serum derived from preeclamptic women (*hatched bars*) is enhanced compared to serum derived from healthy pregnant women (*open bars*) and causes increased ^{51}Cr release from prelabeled human endothelial cells. After delivery, ^{51}Cr release normalized and was not different from that in women with normal pregnancy. (From: Rodgers GM, Taylor RN, Roberts JM. Preeclampsia is associated with a serum factor cytotoxic to human endothelial cells. *Am J Obstet Gynecol* 1988;159:908, with permission.)

of serum from women with preeclampsia and normal pregnancy were added to HUVEC, ^{51}Cr release with the preeclamptic serum was not prevented, thus suggesting a toxic mediator in the serum rather than a deficiency of some factor(s) triggering this injury of endothelial cells. Recent studies suggest that the factor of preeclamptic serum that increases endothelial cell permeability acts by protein kinase C activation (267). Preeclampsia has histologic findings in common with hemolytic–uremic syndrome and thrombotic thrombocytopenic purpura, syndromes in which impairment of endothelial function has been suggested. The findings of unusually large von Willebrand multimers (268), cellular fibronectin (269) and thrombomodulin blood levels (270) in women with preeclampsia/eclampsia provide further biochemical evidence for disturbed endothelial cell function. An increase in endothelium-derived fibronectin levels predicted the development of gestation-induced hypertension 6 to 13 weeks before clinical abnormalities were obvious. On the other hand, women with transient hypertension had plasma fibronectin concentrations indistinguishable from those of normal controls (271) (Fig. 53-10).

Maternal Serum Markers of Preeclampsia and Eclampsia

Blood levels of a variety of substances have been shown to change during pregnancy and in preeclampsia and eclampsia. However, it is less clear whether these changes are the cause or solely the result of preeclampsia and eclampsia.

The fibronectins are a family of glycoproteins. In a longitudinal study, elevated fibronectin levels were measured at 2 to 3 months' gestation in women who develop preeclampsia (272). Adhesion molecules are receptors that modulate inflammatory responses and may be important in the placental bed during trophoblast invasion. Although intercellular adhesion molecule (ICAM) and vascular cell adhesion molecule (VCAM) are expressed in the deciduus (273) there seems no use of soluble forms of these molecules for prediction of subsequent preeclampsia (274). Endothelial dysfunction has been demonstrated in 10 of 43 women who eventually developed preeclampsia (275). Flow mediated dilation of the brachial artery was 3.58% versus 8.59% (p <0.0001). There was also a strong correlation between asymmetric dimethylarginine, the endogenous inhibitor of endothelial NO synthase. Still, it is not clear whether endothelial dysfunction is the cause or the consequence of preeclampsia.

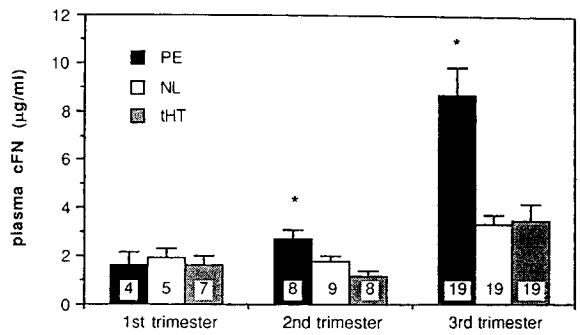

FIGURE 53-10. Prospective analysis of plasma levels of endothelium-derived fibronectin was performed throughout pregnancy. Average concentrations were determined for each trimester in the same group of patients. Fibronectin levels from women developing preeclampsia (PE) later in pregnancy were higher than in women with normal pregnancy (NL) or transient hypertension (tHT) during the second trimester. cFN, concentration of fibronectin. (From: Taylor RN, et al. High plasma cellular fibronectin levels correlate with biochemical and clinical features of pre-eclampsia but cannot be attributed to hypertension alone. *Am J Obstet Gynecol* 1991;165:895, with permission.)

Peroxidation Products During Preeclampsia and Eclampsia

In preeclamptic women, increased blood levels of lipid peroxidation products have been demonstrated and may contribute to the disorder by disruption of membrane lipids, inhibiting prostacyclin and NO production and stimulating endothelin synthesis (276–278) and platelet aggregation (279). Free radicals are produced during normal physiologic processes; however, their release is exaggerated during ischemia (280). These free radical species may induce endothelial cell toxicity and facilitate coagulation by oxidative conversion of unsaturated fatty acids to lipid peroxides (281). During normal pregnancy, free radical production is enhanced, and in preeclampsia, this process is even more exaggerated (282). High levels of oxygen free radicals correlated well with increased blood pressure levels (283). Superoxide anions scavenge NO and thus contribute to the removal of this very important vasodilator in the regulation of blood flow and hemodynamics (277). Increased endothelial NO synthase with decreased superoxide dismutase and increased nitrotyrosine immunostaining in the vasculature of women with preeclampsia is consistent with increased peroxynitrite formation (284). Free radicals may also be derived from activated monocytes, which have been demonstrated in the circulation of hypertensive pregnant women (285). Thromboxane synthesis in platelets is not affected by lipid peroxides. Lipofuscin, which derives from lipid peroxide, has been found more frequently in term placentas than in those less than 32 weeks of gestation. This finding suggests that peroxidation activity increases with gestation. When uncontrolled peroxidation occurs, membrane damage may be initiated. Under experimental conditions, elevation of circulating lipid peroxides or deprivation of vitamin E, an antioxidant, can reproduce the signs of endothelial cell dysfunction similar to those seen in preeclampsia and clinical signs and symptoms of typical preeclampsia (286). Reduced vitamin E levels have been observed in preeclamptic women (287). It is not known if the imbalance between lipid peroxides and vitamin E is a primary or secondary phenomenon; nevertheless, it may contribute to the impairment of endothelial cell function in preeclampsia.

Vasoactive Mediators in Preeclampsia and Eclampsia

The production and secretion of endothelin, a potent peptide hormone that may lead to sustained vasoconstriction, is stimulated in endothelial cells exposed to injuries, such as ischemia (286). The production of endothelin is also enhanced in preeclampsia and pregnancy-induced hypertension (288,290), and endothelin receptor sites are enhanced in the placenta of preeclamptic women (291). The production of another vasoconstrictor, platelet-derived growth factor (PDGF), is enhanced in the supernatant of human umbilical vein endothelial cells (HUVEC) incubated with medium supplemented with serum of preeclamptic women (292). In addition to the effect on vascular tone, PDGF is also mitogenic. Serum of preeclamptic women is selectively mitogenic for fibroblasts but not endothelial cells (293,294). Endothelium-dependent relaxation is impaired in arteries of women with preeclampsia as compared to arteries from normotensive pregnant women, while endothelium-independent vasodilation as assessed by sodium nitroprusside is not altered (295). Animal experiments suggest a major role of NO in the regulation of the physiologic decrease in blood pressure (16). Perfusion of rabbit arteries with serum from preeclamptic women but not from normal pregnant

women increased the sensitivity of the vessel to angiotensin II and norepinephrine (296). As described previously, preeclamptic women have been shown to produce stimulatory autoantibodies against the angiotensin-ATI receptor (170). These autoantibodies may participate in the angiotensin II–induced exaggerated vasoconstriction in preeclampsia and contribute to impaired placental blood flow and infarction.

Prostaglandins in Preeclampsia and Eclampsia

Platelet activation (297) occurs in preeclampsia and enhances thromboxane release, which favors platelet aggregation, especially in the presence of factor VIII antigen (267), and stimulates uterine contraction (298). Platelet aggregation is followed by the release of thromboxane A_2 (TXA_2), serotonin, adenosine triphosphate (ATP), and adenosine diphosphate (ADP); the latter three of these are mediators that induce endothelium-dependent vasodilation. In the absence of the endothelium, paradoxical vasoconstriction and platelet aggregation occur (299). In the plasma and placenta of preeclamptic women, TXA_2 concentrations (212) are increased. Inhibition of the prostacyclin production (300) may be caused by endothelial cell injury and may antedate clinically overt disease (132). A multicenter prospective study has shown that reduced prostacyclin I_2 (PGI_2) production but not TXA_2 production occurs many months before the clinical onset of preeclampsia (302). Deficiency of PGI_2 results in an early increase in the vasoconstrictor to vasodilator ratio (TXA_2/PGI_2). Fitzgerald et al. (302) demonstrated reduced excretion of endothelium-derived prostacyclin metabolite, 6-keto-$PGE_{1\alpha}$, in pregnancy-induced hypertension. Furthermore, serial measurements of urinary 6-keto-$PGE_{1\alpha}$ could be used to predict the development of pregnancy-induced hypertension. In addition to the reduction in vasodilating prostaglandins, the synthesis of the vasoconstrictor TXA_2 is enhanced in pregnancy-induced hypertension (302) (Fig. 53-11). As discussed, the major sources of TXA_2 are activated or aggregated platelets.

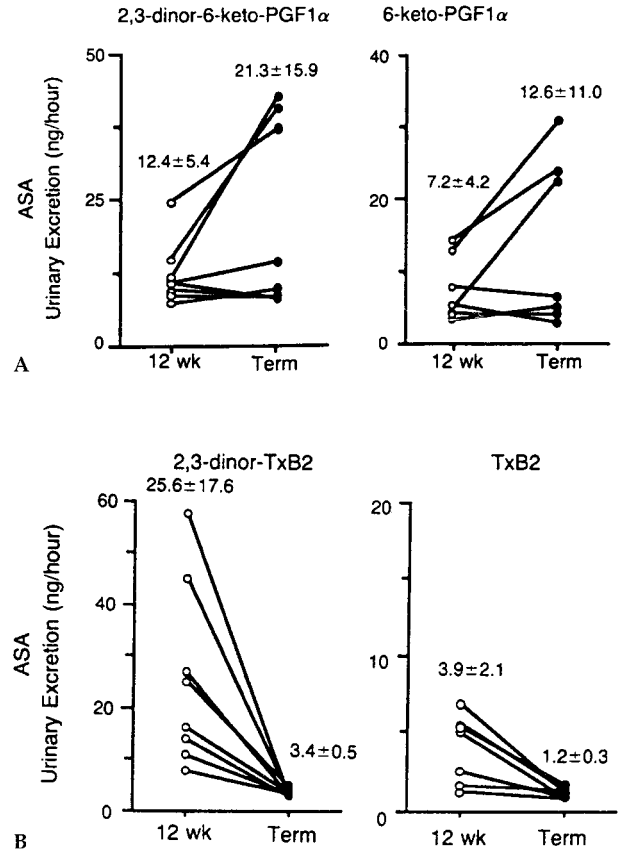

FIGURE 53-11. Effect of 60 mg/day of aspirin on prostaglandin excretion in pregnant women at risk for pregnancy-induced hypertension. **A:** Urinary excretion of 6-keto-prostaglandin $F_{1\alpha}$ (**right**) and its metabolite 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ (**left**) did not change significantly in aspirin-treated and nontreated (not shown) women. **B:** Urinary excretion of the major thromboxane A_2 metabolite 2,3-dinor-thromboxane B_2, which largely derives from platelets, was reduced by 80% in women at risk for pregnancy-induced hypertension (**left**) and by 59% of mainly kidney-derived thromboxane B_2 excretion (**right**). (From: Benigni A, et al. Effect of low-dose aspirin on fetal and maternal generation of thromboxane by platelets in women at risk for pregnancy-induced hypertension. *N Engl J Med* 1989;321:357, with permission.)

Intravascular Coagulation in Preeclampsia and Eclampsia

Although the DIC associated with preeclampsia can rarely be demonstrated by measuring prothrombin time and partial thromboplastin time (199), more sensitive tests demonstrate abnormal results in a high percentage of preeclamptic women. For example, reduced concentrations of antithrombin III (108,132), an indicator of activation of coagulation, are a useful and sensitive indicator to differentiate preeclampsia from other causes of hypertension (108). Endothelial cells can attenuate the coagulation property by the ectoenzymes that rapidly metabolize platelet-derived ATP and ADP to adenosine, a strong inhibitor of platelet aggregation, and by endothelium-derived NO, which inhibits platelet adhesion. Fibrinolysis is stimulated by the release of plasminogen activator, which converts plasminogen to plasmin and thereafter splits fibrin into soluble degradation products. In normal pregnancy, fibrinolysis property is decreased, and in preeclampsia, this decrease is obvious much earlier (303). TNF-α has been shown to promote procoagulatory conditions (304), adhesion molecules, and secretion of other cytokines (305), which may perpetuate this process. To balance the coagulation process, endothelial cells may also release a plasminogen activator inhibitor (306). Changes in blood viscosity may contribute to the attenuation of the microvascular circulation in preeclampsia (307). In 50% of women with preeclampsia and also in 15.4% of women with normal pregnancy, antiendothelial antibodies have been isolated, which have shown increased staining of renal cortical arterioles (308). The functional significance of this finding is not known yet. The rapid disappearance of cytotoxicity after delivery suggests a short half-life of the cytotoxic mediator(s) and makes it unlikely that antiendothelial antibodies induce endothelial injury because immunoglobulins have a half-life of days to weeks. The long duration of impaired organ function in some patients with preeclampsia and eclampsia compared to the short time of pathologic effects induced *in vitro* by serum derived from preeclamptic women may reflect severity of tissue injury initiated during the preeclamptic state.

Thrombocytopenia may be present in the newborn of a preeclamptic mother (309,310). This finding may suggest that a toxic, humoral factor is transferred transplacentally to the fetus. Clinically, the thrombocytopenia is generally no problem to the newborn (202). There seems, however, to be an association between the severity of hypertensive disorders in the mother and the thrombocytopenia in the newborn (311).

Conclusion

Preeclampsia is the result of very early events in pregnancy when angiogenesis is impaired. Narrowed spiral arteries and subsequent placental ischemia may be important in triggering the production of mediators that enter the maternal circulation to induce endothelial cell injury early in the development of preeclampsia and eclampsia. Many of the pathophysiologic changes of preeclampsia can be explained by derangement of endothelial cell function. Under physiologic conditions, endothelial cells produce substances to regulate tone of vascular smooth muscle cells to maintain adequate organ perfusion; endothelium-derived mediators inhibit coagulation; the endothelium produces growth factors, expresses adhesion molecules to capture blood cells such as leukocytes, produces extracellular matrix, and controls fluid and electrolyte transfer into the interstitial space (312). When endothelial cells are activated or injured, they may lose their normal responses and diminish the production of vasodilators and thus increase the sensitivity to normally circulating vasoconstrictors. In addition, endothelial cells may also demonstrate new activities such as synthesis of vasoconstrictor hormones, mitogens, and production of procoagulants to activate the coagulation cascade. Increased sensitivity to vasoconstrictor hormones, vasospasm, proteinuria, edema, activation of the coagulation cascade, and production of microthrombi may all be explained by endothelial dysfunction (Fig. 53-12). These processes impair maternal organ and placental perfusion. Placental perfusion is further impaired and the production of "trophotoxin(s)" sustained. A vicious cycle is thus established that often may be broken only by delivery of the fetus.

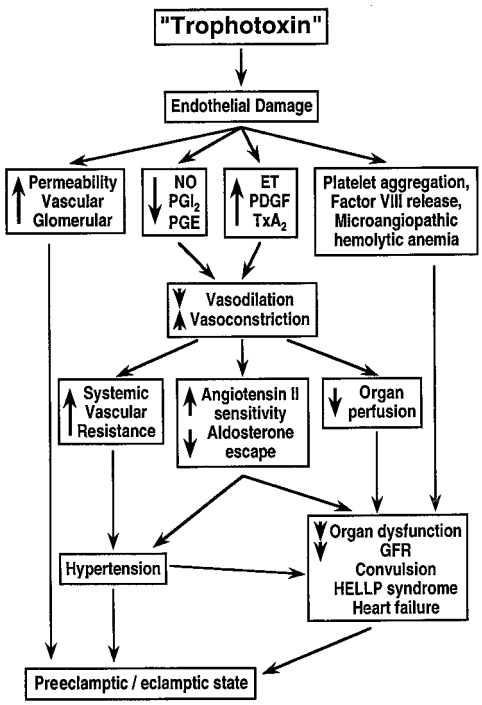

FIGURE 53-12. Endothelial damage attenuates vasodilation, enhances vasoconstriction, and promotes coagulation, which leads to the events characteristic for preeclampsia and eclampsia. *ET*, endothelin; *NO*, nitric oxide; *PDGF*, platelet-derived growth factor; *PGE*, prostaglandin E; PGI_2, prostacyclin; TxA_2, thromboxane A_2.

MANAGEMENT OF HYPERTENSIVE DISORDERS IN PREGNANCY

Although in most cases of pregnancy-induced hypertension the rise in blood pressure is transient, there is good reason to treat hypertension. Hypertension of the mother increases morbidity and mortality in both mother and child (110). In 24,000 women, increased perinatal mortality and increased risk of small-for-gestational age babies were demonstrated when the blood pressure of the mother increased to 125/75 mm Hg prior to the 32nd week of gestation and greater than 125/85 mm Hg thereafter (313). When mean arterial blood pressure was ≥90 mm Hg in the second trimester, there was greater risk of stillbirth, fetal growth retardation, and preeclampsia (111).

Treatment of Hypertension

The American College of Obstetricians and Gynecologists recommends treatment for acute hypertension with diastolic levels of ≥100 mm Hg (102). A lower threshold of diastolic blood pressure, namely ≥90 mm Hg, is believed by other experts to require therapy because many women may have diastolic blood pressure below 90 mm Hg during the second trimester and still develop symptoms of hypertension. In addition to the absolute level, the increase in blood pressure should also be considered important in the development of preeclampsia. Thus, diastolic blood pressure exceeding 75 mm Hg in the second trimester or 85 mm Hg in the third trimester may require surveillance at weekly intervals and may need treatment. Treating women with chronic or pregnancy-induced hypertension must take into account that antihypertensive drugs may have teratogenic effects, impair placental and uterine blood flow, or affect fetal development and organ function. Several groups also demonstrated that treating mild hypertension does not prevent the development of superimposed preeclampsia (314,315). In contrast, evidence strongly suggests that treating severe hypertension with antihypertensive medications may reduce the perinatal death rate and might prevent preeclampsia (316–318). Excessive reduction in blood pressure, however, has the risk of impairing maternal organ and fetal–placental perfusion and should therefore be avoided when antihypertensive medication is initiated. Blood pressure monitoring at weekly intervals is required when blood pressure is ≥130/80 mm Hg. Continuation of antihypertensive treatment after conception improves fetal growth and lowers perinatal mortality (167).

Diuretics

The use of diuretics in pregnancy is controversial. In contrast to women with normal pregnancy (319), preeclamptic women demonstrate contracted plasma volume (167). In addition, systemic vascular resistance is increased, and cardiac output may be normal or reduced (320). In a setting of reduced intravascular volume, as is seen in preeclampsia, it seems rather inappropriate to diminish plasma volume further with diuretic therapy. However, Collins et al. (321) conclude from the data of nine randomized trials, including more than 10,000 women, that preeclampsia could be prevented by therapy with diuretics. The incidence of stillbirths was reduced, and no increase in perinatal mortality was observed. Diuretics do not appear to affect fetal growth (322). Thus, if treatment of hypertension with diuretics has been initiated prior to pregnancy, this therapy probably does not require cessation if the patient becomes pregnant.

Agents Affecting the Adrenergic System

Central-acting adrenergic agonists, such as methyldopa and clonidine, have been most widely used and studied in pregnancy (323). Methyldopa given to pregnant women with essential hypertension has been demonstrated to reduce the number of midterm spontaneous abortions (324). No significant adverse maternal or fetal effects have been reported with methyldopa treatment (325). Children with intrauterine fetal exposure to methyldopa were followed for up to 7 years and did not show any adverse mental or physical effects (326). However, it has to be taken into account that methyldopa may cause somnolence. Methyldopa still remains the drug of choice for treating hypertension in pregnant women as recommended by the National Institutes of Health (NIH) working group (102).

Intrauterine growth retardation (327) and neonatal bradycardia have been reported with β-adrenergic antagonists (328). Attenuation of fetal growth seems to occur when the β-adrenergic antagonist propranolol is used for longer periods. By using oxprenolol, however, there was no difference in perinatal mortality in women with preeclampsia as compared to normotensive controls (329). In a prospective, randomized double-blind study of 120 women with preeclampsia, premature labor occurred in no patient treated with atenolol but in five women on placebo (330). Labetalol, a combined α- and β-adrenergic antagonist with more β-adrenergic antagonistic activity, has been found useful in treating both hypertension throughout pregnancy and an acute rise in blood pressure without affecting uteroplacental blood flow (315,331,332). The National High Blood Pressure Education Program Working Group has not recommended β-adrenergic antagonists as drugs of first choice in the treatment of hypertension during pregnancy, although they may be acceptable and safe agents for treating preexisting hypertension during pregnancy (102). Beta-adrenergic antagonists also cause less somnolence than methyldopa. They should however, whenever possible, be confined to short-term administration before the delivery. Pregnant patients with hypertension due to pheochromocytoma are at high risk of death. Immediate treatment with an α- and β-adrenergic blocker is warranted, and tumorectomy should be performed whenever possible during pregnancy (333).

Vasodilators

The vasodilator hydralazine is frequently used to treat hypertension in pregnancy. The drug seems quite safe; however, it may cause reflex tachycardia and a rise in cardiac output. Moreover, even used alone it may not be effective enough. Beta-adrenergic blockers prevent tachycardia and are efficient in reducing blood pressure when given in combination with vasodilators. In patients with essential hypertension, hydralazine may also be given with methyldopa during pregnancy. Hydralazine has been reported to induce neonatal thrombocytopenia (334). Vasodilators, like other smooth muscle relaxants, cause cessation of labor in a significant number of patients. Magnesium sulfate also suppresses myometrial and myocardial contractility.

Calcium Channel Blockers

Nifedipine has been used without major side effects in women with acute hypertension in pregnancy (335). Calcium channel blockers, however, may cause cessation of uterine contraction. When hypertensive ewes were treated with nicardipine, fetal acidemia was frequent, and 5 of 15 fetuses died within 1 hour after drug administration (336). Calcium channel blockers have teratogenic effects in rat studies when given in a pharmacologic dose 30 times the maximum recommended human dose. The drug is not recommended as first-line medication throughout pregnancy; however, it may be used for short-term treatment during pregnancy (337).

Angiotensin-Converting Enzyme (ACE) Inhibitors and Angiotensin II Receptor (A II) Blockers

ACE inhibit the conversion of angiotensin I to angiotensin II and decrease bradykinin degradation. ACE inhibitors are effective in reducing blood pressure during pregnancy; however, in 22 women with essential hypertension, there were two miscarriages, and in four patients with preeclampsia, there were two stillbirths (338). Acute renal failure has been reported in neonates of women treated with ACE inhibitors (339). Additional reported fetal and neonatal complications of ACE inhibitor use in late pregnancy include oligohydramnios, intrauterine growth retardation, premature labor, bony malformations, limb contracture, persistent patent ductus arteriosus, prolonged hypotension, and neonatal death (340,341). Although retrospective studies of ACE inhibitor use at conception or during early pregnancy were not associated with adverse perinatal outcomes (342), ACE inhibitors should not be prescribed in pregnancy. There are limited reports of side effects of angiotensin II receptor blockers inducing fetal toxicity. Oligohydramnion, incomplete skull formation and oliguria occurred (343,344).

Treatment of Preeclampsia and Eclampsia

The preeclampsia/eclampsia syndrome is characterized by a phase of no or little clinical symptoms while biochemical markers in the maternal blood may already suggest alteration of endothelium function. However, it is not easy to predict the time point of the development of preeclampsia and eclampsia in these women. Bed rest has been recommended to prevent progression of the syndrome, but the beneficial effects have been questioned (345). Proteinuric preeclampsia requires hospitalization. Early delivery is often mandatory to remove the site of production of mediators that stimulate the progression of symptoms of preeclampsia and eclampsia. Conservative therapy (without delivery) of severe preeclampsia occurring in the second trimester may lead to a perinatal mortality of greater than 80% and a high rate of maternal morbidity (346). Interruption of the pregnancy therefore may be recommended in these settings (102). In contrast, isolated mild hypertension without other complicating factors can be followed on an ambulatory basis (347).

Antihypertensive Medication for Preeclampsia and Eclampsia

In hypertensive patients suffering from preeclampsia or eclampsia and especially in patients demonstrating intracerebral hemorrhage, blood pressure should be reduced rapidly. In this situation, blood pressure control is best by intravenous administration of drugs such as the vasodilator hydralazine (348). When there is no response, other drugs are necessary such as the α- and β-adrenergic blocker labetalol (348). Sublingual administration of the calcium channel blocker nifedipine has also been used successfully (349). Women with eclampsia receiving magnesium sulfate may have a precipitous fall in blood pressure. An interaction between nifedipine and magnesium sulfate has been reported to produce profound maternal muscle weakness. Treatment with intravenous sodium nitroprusside, another very potent vasodilator, may cause accumulation of cyanide (350) and should therefore be avoided, although a case report did not confirm this fear of intoxication (351). Reduction in systemic blood pressure may also affect uterine hemodynamics and lower uterine, placental, and subsequently

fetal blood flow. It is not known if there is sufficient autoregulation of the uteroplacental vasculature to maintain sufficient perfusion. A number of antihypertensive agents are excreted in breast milk (352). Severe sustained hypertension postpartum requires treatment, and therefore breast-feeding is not recommended.

In the preeclampsia/eclampsia state with reduced intravascular volume, treatment with diuretics should be avoided unless signs and symptoms of pulmonary edema are present. Some observations indicate that volume expansion may improve peripheral vascular resistance and decrease blood pressure (353); however, pulmonary and cerebral edema may develop with this treatment. The significance of serotonin in the pathophysiologic events of preeclampsia is supported by the beneficial effect of receptor blockade with ketanserin. Attenuation of both blood pressure and platelet aggregation in preeclampsia has occurred with this medication (354).

Anticoagulation

The discovery of DIC, fibrin deposition, and thrombosis in preeclampsia and eclampsia suggests the possible treatment of these women with heparin (355). However, such a treatment may be deleterious in the presence of petechiae or overt bleeding and should therefore be omitted.

Anticonvulsant Therapy

Eclampsia by definition is associated with convulsions. Neither blood pressure level nor proteinuria is a good indicator of impending seizures. Chua and Redman (356) therefore suggest restricting anticonvulsant treatment to patients demonstrating overt convulsions. Magnesium sulfate has been used to prevent convulsions in women with peripartum hypertension and has been demonstrated to be superior to phenytoin as a prophylaxis against eclampsia (357). Evidence from the Magpie Trial has demonstrated in 10 out of 110 women a very important role of magnesium sulfate in preventing as well as controlling eclampsia (358). Although it is not an anticonvulsant or antihypertensive drug, magnesium sulfate inhibits calcium uptake in vascular smooth muscle cells, thereby lowering plasma endothelin-1 levels (359). Magnesium sulfate may also block the constrictor effect of endothelin, angiotensin II, and neuropeptide Y (360) and increase the production of cyclic guanosine monophosphate (cGMP), the second messenger of several vasodilators (361). Thus, attenuation or reversal of vasoconstriction (362) and improvement of cerebral perfusion may occur. Magnesium sulfate does reduce seizures in women with preeclampsia, however, does not improve electroencephalographic pathology (363) and overall mortality and morbidity (364). Therefore, its use in eclampsia is still somewhat a matter of debate. Other common anticonvulsants such as diazepam or distraneurin have been effective in treating eclampsia (365).

Epidural Anesthesia

In spite of antihypertensive medication and bed rest, there are still preeclamptic patients who are not responsive to this treatment. Lumbar epidural anesthesia blocks the abdominal sympathetic nerves and improves hypertension during preeclamptic labor. In preeclampsia, long-term epidural anesthesia for more than 3 weeks has been shown to improve the preeclamptic condition, with decreased blood pressure and increased platelet count and serum protein level (366). In addition, infants in the epidural group had a higher body weight of 2,240 $\pm$ 310 g compared to 1,590 $\pm$ 380 g in the control group treated with antihypertensives and bed rest.

Timing of Delivery

The therapy of eclampsia includes supportive care for the mother and the fetus and the need for a prompt decision regarding the time and mode of delivery. Delivery is always the best therapy for the mother with preeclampsia and eclampsia; however, this may not be the case for the baby. When the gestational age is between 25 and 30 weeks, fetal survival is a serious consideration. Thus, the delivery may be postponed in women with mild signs of preeclampsia and a premature fetus. Clinical and laboratory monitoring, however, are required in these cases. Deterioration in maternal condition with organ dysfunction (e.g., brain, kidney, liver) or fetal deterioration should be an indication for delivery. If maternal and fetal well-being remain stable, pregnancy should be continued, and spontaneous labor may be possible. Epidural anesthesia for cesarean section is a safe technique (367). With epidural anesthesia, peripheral vasodilation may cause a severe fall in blood pressure and diminished uteroplacental perfusion. This complication can be prevented with means such as volume expansion as used in nonpregnant patients with epidural anesthesia. General anesthesia is recommended when there is high risk of epidural hematoma with epidural anesthesia in patients with severe coagulation alterations (190,368,369). The risk of aspiration of gastric contents during intubation, however, is increased. Glucocorticoids may hasten fetal lung maturation; however, they have no effect on maternal outcome.

Prevention of Preeclampsia and Eclampsia

Since the mechanisms underlying preeclampsia are not well understood, prevention of this disorder focuses on intensive screening for women with high risk or early signs of preeclampsia and eclampsia. Taking into account that in experimental conditions impaired uterine blood flow may induce preeclampsia, there was great hope that ultrasound measurements of changes in placental and uterine circulation may predict the development of early preeclampsia (370,371). However the results of uterine arterial waveform by ultrasound and the risk of hypertension and eclampsia are conflicting. There are also many biochemical maternal serum parameters such as hyperuricemia, thrombocytopenia, increased liver enzymes, low levels of antithrombin III, and increases in β_2-microglobulin and fibronectin, that may be prodromal signs of preeclampsia; however, their absence does not exclude the syndrome (Table 53-2). Preeclampsia may progress rapidly to severe disease within days to weeks. Antenatal visits in primiparous women should be performed every 2 weeks (372), and when clinical or biochemical signs or symptoms of preeclampsia occur, hospitalization for more intensive monitoring or delivery can then be performed. While preeclampsia cannot be prevented, with this strategy the risk of severe maternal and fetal morbidity can be diminished.

A good indicator for monitoring the occurrence of preeclampsia is serum uric acid concentration (373). Normal pregnancy induces a 25% decrease in plasma uric acid concentration early in pregnancy (374). In the third trimester, uric acid levels may rise close to the normal range in healthy women but also in women with hypertensive disease without preeclampsia (146) (Fig. 53-13). In contrast, in preeclampsia, serum uric acid levels are consistently increased in the first trimester and subsequently continue to rise. Furthermore, there was a statistically significant correlation of the serum uric acid level with perinatal prognosis (373) and the severity of glomerular changes of preeclampsia and eclampsia (146) (Fig. 53-13). The mechanism of the hyperuricemia remains obscure. Renal insufficiency cannot be the responsible factor because many patients have no or little increase in serum creatinine levels. Plasma β_2-microglobulin levels also parallel the rise in uric acid concentrations in preeclampsia (375).

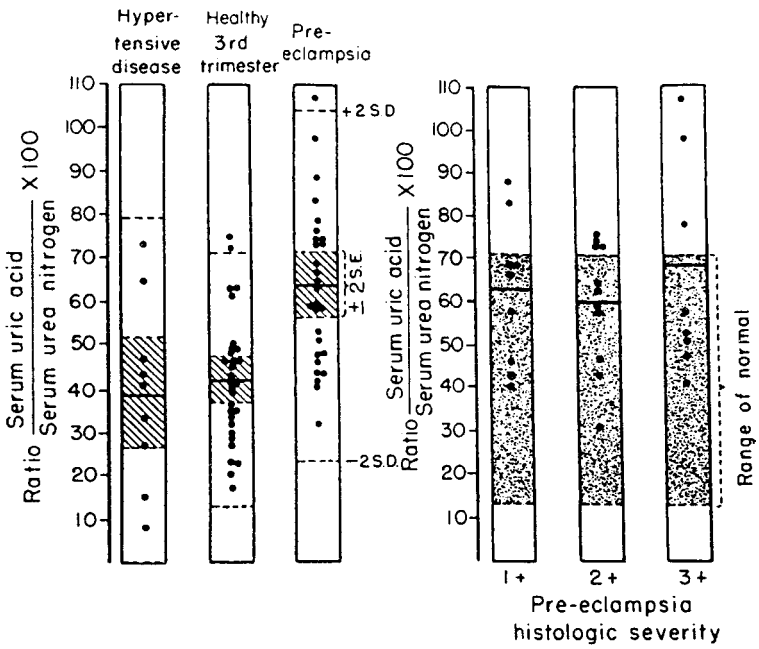

FIGURE 53-13. The diagram shows the distribution of serum uric acid in pregnant women. **Left three columns:** Uric acid levels do not differ in hypertensive compared to normotensive pregnant women, while preeclamptic patients demonstrate great increase in uric acid concentration. **Right three columns:** Severity score of glomerular histology ranging from mild (1+) to severe (3+) correlates with serum levels of uric acid. (From: Pollak VE, Nettles JB. The kidney in toxemia of pregnancy: a clinical and pathologic study based on renal biopsies. *Medicine* 1960;39:469, with permission.)

If endothelial cell injury were the cause of hypertension, proteinuria, and preeclampsia and eclampsia, one would expect that evidence of such injury would antedate these clinical disorders. As mentioned earlier, this is the case. Preventive means, therefore, need to start early in pregnancy, perhaps around 12 to 14 weeks of gestation. Screening tests to detect women who very likely will develop preeclampsia should be accurate, noninvasive, easy to perform, and inexpensive. So far the most reliable clinical test is the angiotensin II infusion test. However, it is not suitable as a routine method. A rise in blood pressure, proteinuria, or facial edema and rapid weight gain may be an early sign of the development of preeclampsia; however, these are not pathognomonic features of preeclampsia. Nevertheless, they are of concern for closer monitoring of the patient. Since preeclampsia and eclampsia are high-risk states for maternal and fetal morbidity, overdiagnosis of impending preeclampsia is preferable to underdiagnosis. If the condition is stable, weekly observations may be appropriate (102); however, if the disease progresses, more frequent visits are necessary, and hospitalization should be considered.

Antiplatelet Drugs

Activation and aggregation of platelets have been shown to occur in preeclampsia. The significance of platelets in preeclampsia has further been suggested by the finding of reduced numbers in this state (376). As discussed earlier, there is evidence that preeclampsia and eclampsia are associated with an imbalance in the ratio of vasoconstricting to vasodilating prostaglandins. Thus, it was believed that inhibition of the synthesis of vasoconstricting prostaglandins, for example, thromboxane, might shift the ratio toward the more physiologic range. Initial studies using platelet inhibitors such as aspirin or dipyridamole for prevention of preeclampsia showed promising results, including decreased incidence of proteinuric hypertension, preterm birth, infants small-for-gestational age, and perinatal death (377–379). However, a multicenter prospective study of women at risk for preeclampsia showed no benefit (380) However, women demonstrating a reduction in flow in the uterine artery measured by doppler may benefit of aspirin (381). Caritis et al. reported a significant decrease in urinary prostacyclin excretion and no change in urinary thromboxane excretion in 134 preeclamptic women as compared with 139 matched normotensive controls (382). This study suggests that prostacyclin deficiency, rather than increased thromboxane production, may contribute to the development of preeclampsia and thus may provide an explanation for the lack of efficacy of aspirin therapy in most of these patients.

Antioxidants

Oxidative stress has been implicated in the pathogenesis of preeclampsia. As noted previously, oxygen free radicals have been demonstrated to be increased in preeclampsia. Ascorbic acid is a scavenger of superoxide radicals and thus might potentially help preserve NO. Alphatocopherol and ascorbic acid also decrease low-density lipoprotein (LDL) oxidation. Therefore, it was suggested that antioxidant therapy with vitamins C and E could be beneficial in the prevention of preeclampsia. A double-blinded, randomized, placebo-controlled trial of vitamins C and E supplementation in 238 women at increased risk of preeclampsia demonstrated a decrease in the incidence of preeclampsia in the treated group (383). However, no differences in perinatal outcome were found. Further studies with large numbers of patients are needed before antioxidant supplementation can be recommended for prevention of preeclampsia. Other supplement of the nutrients such as calcium in 4,589 primiparae did not confirm earlier findings of a lower incidence of preeclampsia compared to controls (384).

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CHAPTER 54 ■ PATHOGENESIS AND TREATMENT OF HYPERTENSION IN THE DIABETIC PATIENT

RAYMOND O. ESTACIO AND ROBERT W. SCHRIER

INTRODUCTION

The prevalence of diagnosed diabetes in the United States continues to rise and has increased from 4.9% in 1990 (1) to 7.3% in 2000 (2). There were approximately 15 million U.S. adults aged 18 years or older who had a diagnosis of diabetes (6.3 million men and 8.7 million women) (2). In the United States, the prevalence of diabetes, specifically type 2, in African Americans, Native Americans, Asian Americans, and Hispanic Americans is two- to sixfold greater than white non-Hispanic American diabetic patients (3,4). Worldwide diabetes is expected to rise from 171 million in 2000 to an estimated 366 million by 2030 (5). This is alarming because diabetes places a tremendous burden on the current tenuous medical economy and, more importantly, its effects on human suffering. Epidemiologic data exist to suggest that the vascular complications associated with diabetes mellitus are substantially aggravated in the presence of hypertension which occurs in both type 1 and type 2 diabetes. Mortality among diabetic subjects is increased as much as fivefold when hypertension is present (6,7). Risks for cardiovascular disease (8–12), renal disease (13–17), retinopathy (18–20), and neuropathy (21) are also significantly increased when hypertension is found in association with diabetes.

One of the major differences between type 1 and type 2 diabetes is the onset of hypertension. The prevalence of hypertension in type 1 diabetes is similar to that of the general population until the onset of diabetic nephropathy (22). In comparison, nearly 50% of type 2 diabetic patients have hypertension at the time of diagnosis of their diabetes (23). Along with hypertension, type 2 diabetic patients often present with other potent risk factors for vascular disease, specifically obesity, hyperinsulinemia and abnormal lipid profiles which place them at a higher risk for cardiovascular disease. To compound the problem, the diagnosis of type 2 diabetes is often delayed resulting in prolonged exposure to these risk factors before any intervention is implemented (24). Thus it is paramount to identify these patients and aggressively treat them with interventions proven to decrease the morbidity and mortality. Recently, there have been a considerable number of prospective interventional studies demonstrating the effectiveness of treating hypertension in diabetes.

The purpose of this chapter is to review some of the potential pathogenic mechanisms by which hypertension develops in type 1 and type 2 diabetes and to provide an overview of recent clinical trials in order to guide the treatment of hypertension in diabetes.

Pathogenesis of Hypertension in Diabetes

Although not all diabetic patients will develop overt nephropathy leading to end-stage renal disease (ESRD), most patients who develop overt nephropathy and all patients who develop ESRD will have hypertension. In fact, the prevalence of hypertension increases with albuminuria stage. In type 1 diabetic patients, the prevalence of hypertension was 42%, 52%, and 79% in patients with normo-, micro- and overt albuminuria, respectively. The corresponding prevalences in type 2 diabetic patients were from 71%, 90%, and 93%, respectively (25). It is believed that there are several factors that contribute to the pathogenesis of hypertension in the diabetic patient. These include (a) genetic predisposition, (b) sodium retention, (c) metabolic factors such as insulin resistance that contribute to the genesis of hyperinsulinemia, and (d) accentuated neurohumoral responses. (Table 54-1).

Genetic Predisposition

It appears that genetics plays a significant role in the development of hypertension in both type 1 and type 2 diabetes. The notion of genetic susceptibility is supported by the difference of prevalence rates of hypertension in certain racial and ethnic groups such as in African Americans (63%) versus non-Hispanic whites (38%) and Hispanic whites (34%) (26), especially in type 2 diabetes. As mentioned earlier, the prevalence of hypertension increases in type 1 diabetes with increasing albuminuria stage. Thus it is not surprising to observe that the genetic susceptibility to hypertension in type 1 diabetes appears to be related to the progression of diabetic nephropathy (27–29). In a case-control study, Fagerudd et al., assessed the prevalence of hypertension among parents of type 1 diabetic patients with and without diabetic nephropathy. Arterial hypertension was present in 57% of parents of patients with diabetic nephropathy compared with 41% of parents of patients without diabetic nephropathy ($p = 0.034$) (29). In addition, the cumulative incidence of hypertension was higher among parents of patients with diabetic nephropathy, with a shift toward younger age at onset of hypertension in this group (29). The authors concluded that familial predisposition to essential hypertension increases the risk of diabetic nephropathy and may also contribute to the development of systemic hypertension in patients with type 1 diabetes and diabetic nephropathy. Other genetic associations that may increase the risk for the development of hypertension include increases in sodium-lithium (30–32) and sodium-hydrogen counter-transport (33) and insulin resistance (34–36).

Sodium Homeostasis

Diabetic patients are noted to have an increase in total body sodium which contributes to the development of hypertension. It appears that both hyperglycemia and hyperinsulinemia can independently contribute to this process. Sodium (Na^+) retention occurs as a characteristic alteration in both type 1 and type 2 diabetes, Weidmann et al. demonstrated that exchangeable total body Na^+ on average was increased by 10% when

TABLE 54-1

FACTORS INFLUENCING THE DEVELOPMENT OF HYPERTENSION IN DIABETES

	Factors
Genetic	African American race Predisposition Na^+/Li^+ Transport Insulin resistance
Electrolyte abnormalities	Increased vascular smooth muscle calcium Increased sodium retention Sodium/hydrogen cotransport Decreased magnesium
Neurohumoral	Renin-angiotensin-aldosterone system Plasma catecholamines Increased pressor response to norepinephrine Decreased β-receptor response

compared to healthy controls and hypertensive patients without diabetes (Fig. 54-1) (37). This abnormality which develops in the uncomplicated stage (no nephropathy) of both type 1 and type 2 diabetes differentiates diabetic from nondiabetic essential hypertensive subjects. This is further supported by Roland et al. who demonstrated a reduction of Na^+ excretion in diabetic patients versus nondiabetic patients when presented with a sodium load (38). Roland theorized this was secondary to enhance tubular absorption rather than impaired filtration as the glomerular filtration rate of the diabetic patients were higher than the nondiabetic patients.

Enhanced sodium reabsorption appears to occur by a number of mechanisms in diabetic patients. Hyperglycemia has been noted to increase total body sodium by enhancing the glucose–Na^+ cotransporter in the kidney (39). Na^+ retention is maintained as long as the hyperglycemia is not severe enough to induce an osmotic diuresis. DeFronzo et al. demonstrated that the administration of insulin in nondiabetic subjects resulted in a reduction in tubular Na^+ excretion in the absence of changes in the filtered load of glucose, glomerular filtration rate, renal blood flow, and plasma aldosterone concentration (40). DeFronzo suggested that insulin's effect was due to enhancement of Na^+ reabsorption in the diluting segment of the distal nephron. The mechanisms involve may include stimulation of the renal tubular Na^+-K^+ ATPase, Na^+-H^+ antiporter

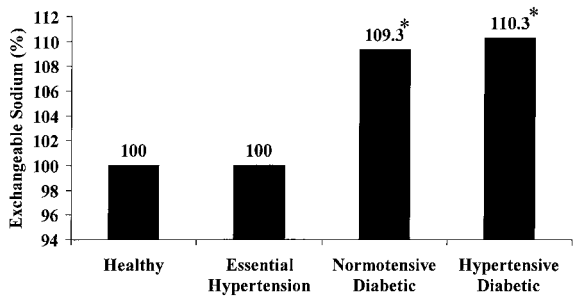

FIGURE 54-1. Comparison of exchangeable sodium after a sodium load in healthy nondiabetic patients, nondiabetic patients with essential hypertension, and diabetic patients without and with hypertension. *P<0.05. (From: Herman WH, Prior DE, Yassine MD, et al. Nephropathy in NIDDM is associated with cellular markers for hypertension. *Diabetes Care* 1993;16:815.)

and amplifying the action of aldosterone on Na^+ and K^+ transport (39).

It is clear once diabetic nephropathy occurs in either type 1 or type 2 diabetes, that one of the major mechanisms of hypertension is sodium retention. In subjects who were studied as inpatients receiving unrestricted sodium intake and in stable metabolic control, O'Hare et al. demonstrated that total exchangeable Na^+ was higher in diabetic patients with nephropathy than diabetic patients without nephropathy (41). Furthermore, the value for exchangeable Na^+ correlated with blood pressure only in diabetic patients with nephropathy ($r = 0.61$, $p < 0.01$). Thus, once diabetic nephropathy develops, hypertension becomes a consequence and a cause of further progression of renal disease in the diabetic patient (42,43).

Ion Transport Homeostasis

Electrolyte abnormalities associated with hypertension appear to involve not only alterations in ion transport of sodium but also in transport of calcium and magnesium. But what is not clear is whether the alterations occur prior to or a result of the milieu associated with the presence of diabetes and hypertension. One of the most studied disturbances in electrolyte abnormalities deal with sodium transport homeostasis. Sodium (Na^+)/lithium (Li^+) countertransport activity has been demonstrated to be strongly associated with the development of hypertension in the nondiabetic populations (44–47). Increased activity of Na^+/Li^+ CT strongly correlates with the development of hypertension in certain families (30–32).

In type 1 diabetic patients, Krolewski et al. demonstrated an association between the development of diabetic nephropathy and a predisposition to hypertension in subjects with an elevated maximal velocity of Na^+/Li^+ countertransport (30). In a review by Van Norren et al, Na^+/Li^+ countertransport activity was significantly higher in type 1 diabetic patients without nephropathy than in healthy control subjects (48). This prompted the authors to postulate that the etiology of the association is not clear but might be due to the association of Na^+/Li^+ countertransport activity with hypertension and hyperlipidemia, which are disorders frequently detected in diabetic patients.

In type 2 diabetes, it appears that insulin resistance may be associated with increased Na^+/Li^+ countertransport activity (49). Pinkney et al. measured Na^+/Li^+ countertransport activity in type 2 diabetic patients without nephropathy and found no correlation with fasting insulin levels ($r = 0.074$, $p = 0.28$), but did demonstrate a correlation between decreased insulin sensitivity and increased Na^+/Li^+ countertransport activity ($r = -0.37$, $p = 0.036$). Similar findings have been demonstrated by other investigators (50,51). Although the explanation for this association between increased Na^+/Li^+ countertransport activity and insulin resistance is not clear, it may be a marker or predictor for the development of hypertension and possibly diabetic nephropathy in insulin resistant, type 2 diabetes.

Another ion transport abnormality that has been studied is the sodium-hydrogen (Na^+/H^+) antiporter activity. Increased Na^+/H^+ antiporter activity has been shown to be increased in erythrocytes, leukocytes, and platelets from patients with essential hypertension and in lymphocytes and vascular smooth muscle cells from spontaneously hypertensive rat (52–54). In animal studies, induction of diabetes and increased blood glucose levels resulted in an increase in Na^+/H^+ antiporter activity which was then reversed with glycemic control (55). The mechanism by which this occurs appears to be via a glucose-induced protein kinase C–dependent mechanisms (56,57). The Na^+/H^+ antiporter has been shown to play a key role in the regulation of intracellular pH, cell volume, growth, differentiation,

and contractility (58–61). Additionally, increased Na^+/H^+ antiporter activity may explain the relationship between intracellular Ca^{2+} metabolism in essential hypertension leading to enhanced vascular contractility (62).

As noted above, accumulation of intracellular Ca^{2+} has been implicated as major facilitator of hypertension in both diabetic and nondiabetic subjects. Acute intracellular calcium overload of vascular smooth muscle cells increases contractility leading to a rise in peripheral resistance resulting in essential hypertension (63). Results from diabetic animal models and from human subjects with diabetes reveal that intracellular calcium levels are increased in most tissues through various mechanisms and may play an essential role in the pathophysiology of diabetic complications (64).

Renin-Angiotensin-Aldosterone System

The renin-angiotensin-aldosterone system (RAAS) in diabetes has been examined by several investigators and has been implicated as a major contributor in the pathophysiology of the diabetic vascular complications. However, the role of RAAS in the pathogenesis of hypertension in the diabetic patient is not conclusive. In most studies, plasma renin activity (PRA) has not been found to be increased in hypertensive diabetic subjects (65,66). However, the "normal" PRA in an environment of elevated total body sodium and increased blood pressure, both known to suppress PRA, may infer inadequate suppression of RAAS in diabetic hypertension. Recent studies suggest that this may be the case (67,68). Price et al. compared the PRA response to the upright position on a low-salt diet and on a high-salt diet in hypertensive diabetic patients compared to healthy controls and hypertensive patients without diabetes. On the high-salt (200 mmol) diet, healthy subjects showed the expected PRA suppression (0.3 ± 0.1), but in patients with type 2 diabetes the PRA was less suppressed (1.2 ± 0.3 ng AI/mL/hour; $p = 0.003$) (68). The mechanism for this relative stimulation of the RAAS system has not been elucidated in the diabetic patient. Recently, Miller demonstrated that in healthy type 1 diabetic patients without complications during hyperglycemic conditions without glycosuria, mean arterial pressure (MAP), renal vascular resistance (RVR) and filtration fraction (FF) were significantly higher compared with the euglycemic phase (69). After the administration of losartan, an angiotensin II type 1 receptor blocker, a significant renal and peripheral vascular depressor effect was noted, with decreases in MAP, RVR, and FF in the hyperglycemic phase. During the euglycemic phase the responses to losartan were minimal. Miller suggested that moderate hyperglycemia, at least of a short duration, results in the activation of the circulating, and possibly the intrarenal, RAAS with resultant increases in blood pressure (69).

Additionally, a number of investigators have demonstrated that the diabetic vasculature is more sensitive to the pressor effects of angiotensin II (37,70). Weidmann et al. demonstrated that a lower infusion rate of angiotensin II was required in both normotensive and hypertensive type 2 diabetic patients to increase diastolic blood pressure by 20 mm Hg than in control subjects (37). The authors suggest that the increased sensitivity may be a reflection of disturbance in the RAAS feedback system. Drury et al. demonstrated a similar finding in type 1 diabetes (70).

Insulin Resistance and Hyperinsulinemia: Influence on Hypertension

There have been a number of studies demonstrating a clear relationship between insulin resistance, hyperinsulinemia and hypertension (71–74). Most recently, Ferrannini et al. demonstrated in 333 nondiabetic, normotensive, and nonobese patients that both decreased insulin sensitivity and increased fasting plasma insulin levels were independently associated with increases in blood pressure (73). The authors demonstrated that systolic blood pressure was 1.7 mm Hg and diastolic blood pressure was 2.3 mm Hg higher for each 10-unit increase in insulin resistance (i.e., a 10 μmol/min^{-1}/kg^{-1} decrement in the M value). Insulin resistance/hyperinsulinemia appears to contribute to the development of hypertension in several ways.

As already mentioned, insulin plays a role in Na^+ homeostasis by affecting the Na^+ transport systems and promoting Na^+ retention. Although insulin is considered to have direct vasodilatory effects in normotensive and nondiabetic subjects (75,76), possibly by inducing nitric oxide release (77), a number of studies have demonstrated that this response is blunted in obese (78), hypertensive (79,80) and diabetic subjects (81). In addition to the vasodilatory effects, insulin is also known to stimulate the sympathetic nervous system. Rowe et al. have demonstrated that the administration of insulin with subsequent stimulation of carbohydrate metabolism leads to the activation of the sympathetic nervous system and an increase in circulating norepinephrine levels (82), findings corroborated by other investigators (83–86). Thus, these studies suggest that insulin can affect the cardiovascular system by direct vasodilatory actions on the peripheral vasculature and an indirect effect on the sympathetic nervous system. These results suggest that an imbalance between these insulin effects favoring sympathetic stimulation may play a role in the pathogenesis of hypertension in diabetic patients. The mechanism by which insulin mediates its vasoactive effects through the sympathetic nervous system appears to be through beta-adrenergic receptors (87,88). Gros et al. demonstrated that insulin enhancement of vascular beta-adrenergic responsiveness resulting in vasodilation in normotensive animals is blunted in hypertensive animals (88).

Pathogenesis of Vascular Complications: Role of Hypertension and Diabetes

As we have already noted, the combination of hypertension and diabetes is associated with an increase in vascular complications. This next section will discuss the possible mechanisms by which this combination contributes to the pathogenesis of micro- and macrovascular diabetic complications. Although the mechanisms by which diabetes and hypertension cause vascular complications have not been completely defined, alterations in hemostasis and vascular endothelium function and structure appear to be involved (Table 54-2).

Hemostasis

Coagulation Abnormalities. Diabetes causes an imbalance between coagulation and fibrinolysis in favor of the procoagulant state. Specifically, diabetes is associated with an increase of procoagulant factors and impairment of fibrinolysis. Fibrinolysis is induced by plasmin which is mediated plasminogen activators. Plasminogen activator inhibitor-1 (PAI-1) produced by the liver and endothelial cells neutralizes the activity of the plasminogen activators leading to a decrease in fibrinolytic activity and a propensity for thrombosis (89). In this regard, PAI-1 levels have been noted to be elevated in both type 1 and type 2 diabetes (90,91) and in hypertension (92,93). The increased PAI-1 levels appear to be related not only to hyperinsulinemia but also to occur in conjunction with hyperglycemia and increased triglyceride levels (94). Other procoagulant factors that have

TABLE 54-2

FACTORS INFLUENCING THE PATHOGENESIS OF VASCULAR COMPLICATIONS IN PATIENTS WITH HYPERTENSION IN DIABETES

	Diabetes	Hypertension
Hemostasis	Coagulation abnormalities Abnormal platelet aggregation and adhesion	Abnormal platelet aggregation and adhesion
Abnormal Vascular Endothelial Function and structure	Hypertrophy Impaired endothelium-mediated vasodilation	Hypertrophy Impaired endothelium-mediated vasodilation Leukocyte adherence

been noted to be increased in diabetes include endothelium-derived von Willebrand factor, factors VII and VIII, fibrinogen and thrombin-antithrombin complexes (95).

Platelet Abnormalities. Platelet adhesion and aggregation are enhanced in diabetic patients, further contributing to a procoagulant milieu in the diabetic individual (96). Increased platelet aggregation appears to be mediated by the high intracellular calcium and low magnesium reported to be present in diabetic and hypertensive subjects (96). Other abnormalities demonstrated in platelet function include an increase in the release of thromboglobulin and platelet factor 4, decreased levels of platelet-derived growth factor and serotonin, decreased platelet survival, increased platelet generation of vasoconstrictor prostanoids, reduced platelet generation of prostacyclin and other vasodilator prostanoids, and increased glycosylation of platelet proteins (97). Nitric oxide produced by platelets and endothelial tissue is decreased in diabetic and hypertensive states and thereby lead to an increase in platelet aggregation and adhesion to endothelial cells (98).

Vascular Endothelium Function and Structure. Both diabetes and hypertension have a number of functional and structural abnormalities in the vascular endothelium, which can precipitate the development of diabetic vascular complications. In the hyperglycemic state, protein kinase C can be activated and stimulate prostanoids, endothelin and angiotensin converting enzyme which can result in decreasing vascular relaxation (98,99). Hyperglycemia also increases endothelial cell collagen IV and fibronectin synthesis; increased activity of enzymes involved in collagen synthesis also may result in basement membrane thickening (100). Diabetes, specifically type 2 diabetes, is associated with lipid abnormalities which include higher triglyceride and very-low-density lipoproteins (VLDL) levels, lower high-density lipoprotein (HDL) levels, and increased levels of small dense low-density lipoprotein (LDL) particles (101). These specific lipid abnormalities have been associated with an increased risk for the development of vascular complications in the diabetic patient. Hyperinsulinemia may also mediate, through insulin-like growth factor-1 (IGF-1), their atherogenic effects on both vascular endothelial cells and vascular smooth muscle cells (VSMC) by increasing mitogenic signaling pathways and thymidine incorporation into DNA (96).

Panza et al. have demonstrated by strain-gauge plethysmography that nondiabetic patients with essential hypertension have impaired endothelium- mediated vasodilation (102). The primary mechanism by which hypertension affects endothelial function is by the elevation of shear stress resulting in structural changes which alter endothelial cell metabolism and even endothelial cell detachment (103). Hypertension also increases the accumulation of macrophages in the subendothelial space (104).

Treatment of Hypertension in Diabetes

It is clear that the combination of hypertension and diabetes increase the risk of micro- and macrovascular complications, and that blood pressure control as demonstrated in several trials can reduce the development and/or progression of these complications. A number of issues regarding blood pressure therapy need to be considered in the care of the diabetic patient: (a) when to start antihypertensive therapy, (b) the target of blood pressure level, and (c) the primary antihypertensive to be utilized. As opposed to glycemic control where increases in blood glucose levels can result in acute life-threatening complications such as diabetic ketoacidosis in type 1 diabetic patients or nonketotic hyperosmolar coma in type 2 diabetic patients, the deleterious effects of elevated blood pressure are often insidious. Currently, the consensus groups such as the Joint National Committee (JNC-7) on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (104) and the American Diabetes Association (105) recommend that blood pressure control in diabetic patients be at or below 130/80 mm Hg with the use of an angiotensin converting enzyme inhibitor or an angiotensin receptor blocker as the initial antihypertensive agent.

Diabetic Nephropathy

Diabetic nephropathy is one of the leading causes of ESRD in the United States and occurs in approximately 30% to 35% of both type 1 and type 2 diabetic patients (106). In addition, diabetic nephropathy not only leads to ESRD but is also one of the strongest predictors of cardiovascular morbidity and mortality (107).

Normotensive Diabetic Patients with Normoalbuminuria

Data are sparse with regard to the treatment of normotensive diabetic patients with normal albuminuria levels. Three studies in type 1 diabetic patients with normal albuminuria levels and normotensive blood pressures have been published (107–108). Tuominen et al. evaluated the effects of lisinopril versus placebo on exercise-induced albuminuria, which is considered a predictor of microalbuminuria, in normotensive normoalbuminuric type 1 diabetic patients (108). A total of 26 patients

with normoalbuminuria and an average blood pressure of approximately 118/81 mm Hg were randomized to lisinopril or placebo and followed over 2 years. Those randomized to lisinopril demonstrated a 40% decrease in exercise-induced urinary albumin excretion (UAE) rates in the first year ($p = 0.059$) and 66% decreased after the second year ($p < 0.01$), whereas exercise-induced UAE rates remained unchanged in the control group. Analyses of the systolic blood pressures (sBP) and diastolic blood pressures (dBP), however revealed significantly lower blood pressures in the lisinopril group at 2 years, 13 mm Hg ($p = 0.03$) and 9 mm Hg lower ($p = 0.052$), respectively. The authors concluded that lisinopril treatment reduces the exercise-induced UAE rate in normotensive normoalbuminuric type 1 diabetic patients and may have a protective effect against the development of microalbuminuria. Although not considered normotensive in the current guidelines, a multicenter study in Europe of type 1 diabetic patients with blood pressures <155/90 mm Hg were randomized to receive an angiotensin converting enzyme inhibitor (ACE-I), lisinopril, or placebo over a follow-up of 2 years (108). Although no difference was observed between those patients randomized to lisinopril versus placebo with regard to progression to microalbuminuria, those randomized to lisinopril had an overall lower urinary albumin excretion (UAE) rate (17.3%, $p = 0.05$) when adjusted for blood pressure and baseline UAE level. More recently, Kvetny et al. performed a randomized trial in normoalbuminuric type 1 diabetic patients with blood pressure <140/90 mm Hg and a mean blood pressure of about 90 mm Hg. Eighty-nine patients were randomized to perindopril versus placebo. After 36 months, the albumin/creatinine ratio (ACR) in the placebo group increased significantly to a value of 1.7 ± 1.1 mg/mmol ($p = 0.007$) versus the perindopril group whose ACR remained stable at 0.6 ± 0.2 mg/mmol. Although the group randomized to perindopril had lower mean blood pressures during the study, the difference was not statistically significant.

In type 2 diabetic patients, similar findings as in the type 1 diabetic studies were demonstrated by Ravid et al. (109). Randomization of patients with normoalbuminuria and a mean blood pressure <107 mm Hg to enalapril revealed a decrease in albumin excretion of 1.9 mg/24 hours at 2 years which was followed by a gradual increase of 4.2 mg/24 hours at 6 years. Whereas, those randomized to placebo had a larger increase in albumin excretion at 6 years (15.7 mg/24 hours, $p = 0.001$ for enalapril compared with placebo). The investigators also demonstrated lower incidence of microalbuminuria, absolute risk reduction of 12.5% (95% confidence interval [CI], 2% to 23%; $p = 0.042$), and a slower decline in creatinine clearance in the enalapril group ($p = 0.040$). Mean blood pressures in the treatment group were somewhat lower than those in the placebo group; this difference reached statistical significance during the fifth year of the study. Additionally, in the enalapril group, a significant correlation was found between the decrease in creatinine clearance and mean blood pressure ($r = 0.41$; $p = 0.05$), mean total plasma cholesterol level ($r = 0.42$, $p = 0.046$), and mean UAE ($r = 0.42$, $p = 0.044$). Therefore, it is difficult to discern whether the statistical benefit observed in the study was from the effect of the angiotensin converting enzyme inhibitor (ACE-I) and/or the effects associated with the decrease in blood pressure.

The results of the normotensive Appropriate Blood Pressure Control in Diabetes (ABCD) study evaluated the effects of intensive blood pressure control versus moderate blood pressure control with either enalapril or nisoldipine as the initial antihypertensive medication in 480 normotensive type 2 diabetic patients followed over a 5-year period (110). In a subanalysis of patients with normoalbuminuria at baseline, intensive blood pressure control (mean of 128/75 mm Hg) versus moderate blood pressure control (mean of 137/81 mm Hg) resulted in a lower incidence of microalbuminuria independent of the use of an ACE-I or a calcium channel blocker (CCB). As a follow-up to the ABCD study, the Appropriate Blood Pressure Control in Diabetes Part 2 with Valsartan (ABCD-2V) study, 129 type 2 diabetic patients with a blood pressure <140/80 to 90 mm Hg without overt albuminuria were randomized to either intensive BP control (diastolic BP goal 75 mm Hg) utilizing an angiotensin II receptor blocker (ARB), Valsartan, versus moderate BP control (diastolic BP 80 to 90 mm Hg with placebo) (Estacio et al., presented at American Society of Hypertension, New York, 2004). The primary end point evaluated was the change in creatinine clearance, proportion of patients with doubling of serum creatinine and the change in UAE from baseline. The majority of the patients, >70%, had normoalbuminuria (<20 μg/min). The mean entrance BP was 126 ± 8.8/84 ± 2.4 mm Hg with a follow-up period of 1.9 ± 1.0 years. During the follow-up period, the mean BP was 118 ± 10.9/75 ± 5.7 for the intensive versus 124 ± 10.9/80 ± 6.5 mm Hg for the moderate BP groups, $p < 0.001$. The study demonstrated that intensive blood pressure control led to a significant benefit in lowering UAE adjusting for baseline age, gender, race, duration of diabetes, hemoglobin A1cHbAc, and albuminuria status revealed a significant beneficial treatment effect ($p = 0.007$). The study suggests that even a lower blood pressure then 130/80 mm Hg with an ARB improved UAE in the early stages of nephropathy.

The data from these interventional studies suggest that treatment of blood pressure below the level of 130/80 mm Hg or even lower, as seen in the ABCD-2V study, may be beneficial with regard to progression to microalbuminuria. Does the type of antihypertensive medication matter? Although the EUCLID Study was not statistically significant for those with type 1 diabetic patients with normoalbuminuria, the 2-year follow-up period may have been inadequate to observe a difference. In the studies by Tuominen (108), Kvetny (110), and Ravid (111), an ACE-I was used against a placebo but, as noted above, statistically lower mean blood pressures were obtained in those patients randomized to the ACE-I. In the ABCD trial, intensive blood pressure control with either a dihydropyridine CCB or an ACE-I led to less progression to microalbuminuria (112). Although performed in hypertensive type 2 diabetic patients, the Bergamo Nephrologic Diabetes Complications Trial (BENEDICT) was designed to assess whether ACE-I and nondihydropyridine CCB, alone or in combination, would prevent microalbuminuria in subjects with normal UAE. Over 1,000 subjects were randomly assigned to receive at least 3 years of treatment with trandolapril (at a dose of 2 mg/day) plus verapamil (sustained-release formulation, 180 mg/day), trandolapril alone (2 mg/day), verapamil alone (sustained-release formulation, 240 mg/day), or placebo with a target blood pressure was 120/80 mm Hg. The study revealed that the use of trandolapril plus verapamil (5.6%, $p = 0.01$) or trandolapril alone (6%, $p = 0.01$) but not verapamil alone 11.9%, $p = 0.54$) decreased the incidence of microalbuminuria when compared to placebo. The data, while limited, suggest that the level at which the diagnosis of "hypertension" is made in diabetic patients may need to be redefined and that utilization of an agent that blocks RAAS would be most beneficial.

Patients with Microalbuminuria

Microalbuminuria may represent the "reversible" stage of diabetic nephropathy in which interventions can prevent the progression to overt nephropathy and inevitable renal failure. Thus, interventions at this stage may be crucial in preventing the progression to overt albuminuria.

In a type 1 diabetic study of six patients, Christensen et al. demonstrated the beneficial effect of treating patients with microalbuminuria with antihypertensive therapy utilizing a beta-blocker over a 5-year follow-up period (111). Subsequent studies in both type 1 and type 2 diabetes confirmed the effects of blood pressure control with various antihypertensive medications as monotherapy or in combination on slowing the progression to overt albuminuria (112–117). Angiotensin converting enzyme inhibitors consistently demonstrated beneficial effects with regard to retarding the progression of microalbuminuria when compared to placebo (118–124). As in the normotensive studies already cited, however, it is difficult to discern whether the blood pressure reduction, the specific properties of angiotensin converting enzyme inhibitors, or both result in the beneficial effects. Comparison studies evaluating the effects of angiotensin converting enzyme inhibitors with other antihypertensive medications on incipient diabetic nephropathy are few and are of short duration. A number of studies comparing the effects of CCBs with angiotensin converting enzyme inhibitors in patients with microalbuminuria have been performed but also are limited in size and study duration. When comparing "short-acting" CCBs to angiotensin converting inhibitors, angiotensin converting enzyme inhibitors demonstrated a clear advantage. In contrast, results of studies comparing angiotensin converting enzyme inhibitors to longer-acting formulations of CCBS are less clear (113,114). Similar findings were reported from the hypertensive ABCD study in which no difference was demonstrated between a long-acting CCB, nisoldipine, versus an angiotensin converting enzyme inhibitor, enalapril, with regard to the progression from microalbuminuria to overt albuminuria (115).

In a study of 590 hypertensive patients with type 2 diabetes and microalbuminuria, patients were randomized to receive irbesartan, at a dose of either placebo, 150 mg daily or 300 mg daily, and were followed for 2 years (116). The primary outcome was the time to the onset of diabetic nephropathy, defined by persistent albuminuria in overnight specimens, with a UAE rate that was greater than 200 μg/minute and at least 30% higher than the baseline level. Of the patients studied, 5.2% in the group that received 300 mg daily and 9.7% in the group that received 150 mg daily reached the primary end point, as compared with 14.9% in the placebo group (14.9%) (hazard ratios, 0.30 [p <0.001] and 0.61 [p = 0.081 for the two irbesartan groups, respectively]). The average blood pressure during the course of the study was slightly lower in the actively treated group, 144/83 mm Hg in the placebo group, 143/83 mm Hg in the 150 mg group, and 141/83 mm Hg in the 300 mg group (p = 0.004 for the comparison of systolic blood pressure between the placebo group and the combined irbesartan groups). In the Microalbuminuria Reduction with VALsartan (MARVAL) Study (117), valsartan was compared to amlodipine in patients with microalbuminuria. Although the follow-up period was only 24 weeks, the patients randomized to valsartan demonstrated a benefit with regard to lowering of UAE to 56% (95% CI 49.6–63.0) of baseline values as compared to 92% (95% CI 81.7–103.7) of baseline with amlodipine; there was a significant difference between the groups (p <0.001). Additionally, more patients regressed to normoalbuminuria with valsartan (29.9% vs. 14.5%; p = 0.001). Over the study period, BP reductions were similar between the two treatments (systolic/diastolic 11.2/6.6 mm Hg for valsartan, 11.6/6.5 mm Hg for amlodipine) and at no time point was there a between-group significant difference in BP values in either the hypertensive or the normotensive subgroup. Similar to trials with angiotensin converting enzyme inhibitors, ARBs at least compared to placebo demonstrated a benefit with regard to preventing or slowing the progression to overt nephropathy. Although the MARVAL study suggests that ARBs may be more efficacious than a dihydropyridine calcium channel blocker (CCB), more studies would be needed with a longer follow-up period when comparing to another agent.

Patients with Overt Nephropathy (Albuminuria)

In the study by Parving et al., antihypertensive treatment with metoprolol, hydralazine and furosemide or hydrochlorothiazide in type 1 diabetic patients with overt nephropathy led to an improvement in the rate of decline of the glomerular filtration rate over an 8-year period (118). During the study the mean treatment blood pressure was 129/84 mm Hg versus the pretreatment blood pressure of 143/96 mm Hg. Additionally, the investigators demonstrated a decrease in UAE of nearly 50% with treatment. Similar results were demonstrated with the use of a beta-blocker alone or in combination with hydralazine or furosemide (119). In 1993, Lewis et al. in a landmark study demonstrated the beneficial effects of an angiotensin converting enzyme inhibitor, captopril, versus placebo in decreasing the doubling of serum creatinine and the progression to death or ESRD in a group of type 1 diabetic patients with overt nephropathy and serum creatinine ≤2.5 mg/dL at baseline (120). These results confirmed earlier smaller reports demonstrating the beneficial effects of ACE-I versus placebo (121,122).

In type 2 diabetic patients with overt nephropathy, 2 studies utilizing ARBs, losartan (123) and irbesartan (124), demonstrated findings similar to those of Lewis's landmark study in type 1 diabetes. Both studies demonstrated that including an ARB in the antihypertensive regimen led to a decrease risk in the doubling of serum creatinine and progression to ESRD. In a study performed by Barnett et al., the use of an ARB, telmisartan, versus an ACE-I, enalapril, resulted in similar effects with regard to change in glomerular filtration rate and UAE over a 5-year follow-up period (125).

Studies comparing other antihypertensive medications with angiotensin converting enzyme inhibitors (ACE-I's)with regard to their effects on overt diabetic nephropathy have been conflicting (138–143). In a recent meta-regression analysis performed by Kasiske et al., including over 100 studies and nearly 2,500 patients, the effects of ACE-Is on diabetic nephropathy were compared to those of other antihypertensive medications (126). Whereas reductions in proteinuria from other antihypertensive agents could be entirely explained by changes in blood pressure, ACE-I's demonstrated an independent decrease proteinuria after adjusting for blood pressure, treatment duration, and the type of diabetes or stage of nephropathy, as well as study design (p <0.0001). Similar findings were demonstrated by Gansevoort et al. (127) and Weidmann et al. (128) in their meta-analyses comparing the effects of ACE-I's with other antihypertensive medications.

Two studies demonstrated no difference in the effects of an ACE-I with either a beta-blocker (129,130) and or a CCB (127) in hypertensive type 2 diabetic populations. In the United Kingdom Prospective Diabetic Study (UKPDS), no difference was demonstrated between the use of a beta-blocker, atenolol, with an ACE-I, captopril, with regard to urinary albumin concentrations (147). A slight difference in UAE was noted with tight blood pressure control (mean 144/82 mm Hg) versus less tight control (mean 154/87 mm Hg) at 6 years (p = 0.0085) but was not seen at 9 years (p = 0.33) (148). In addition, there was no difference in the occurrence of diabetic nephropathy in the UKPDS between the atenolol and captopril treated groups. In the hypertensive ABCD study, although treatment blood pressure for both the intensive (mean 132/78 mm Hg) and moderate (138/86 mm Hg) groups were lower than the UKPDS, no statistical difference was seen in treatment groups followed over 5 years on renal function as measured by creatinine clearance and UAE (127). ABCD Trial investigators also did not observe a difference with regard to the use of an ACE-I, enalapril, versus

a CCB, nisoldipine, as the initial antihypertensive medication with regard to the renal outcomes. During the study, there was a steady decline in creatinine clearance of 5–6 mL/minute/1.73 m^2/year in those patients who had diabetic nephropathy (UAE $\geq$300 mg/day) at baseline. These results for the overt albuminuria group were however limited by the relatively few patients with overt nephropathy at baseline.

Cardiovascular Disease

There have been a number of antihypertensive trials within the past few years confirming the advantage of BP control in diabetic populations with regard to cardiovascular events. As Haffner et al. demonstrated in a 7-year longitudinal study of type 2 diabetic subjects, the risk of developing a myocardial infarction for diabetic patients without a previous history of myocardial infarction is similar to that of nondiabetic patients with a previous myocardial infarction (131).

Two studies evaluating the treatment of isolated systolic hypertension in elderly diabetic patients with either diuretic therapy (132) or a long-acting CCA (133) demonstrated unequivocal advantage with active therapy than placebo with respect to cardiac disease. In both studies there were similar advantages with regard to overall cardiovascular disease and cardiovascular mortality in 5- and 2-year follow-up periods, respectively. In the UKPDS, tight blood pressure control demonstrated a clear advantage when compared to less tight control with regard to death related to diabetes and strokes (148). The result was independent of the use of captopril or atenolol although the lack of difference between the antihypertensive medications may have been due to inadequate dosing of captopril (147).

In the substudy of the Hypertension Optimal Treatment (HOT) study, 1,500 diabetic patients were randomized to three levels of diastolic blood pressure control ($\leq$90 mm Hg vs. $\leq$85 mm Hg vs. $\leq$80 mm Hg) with felodipine (long-acting CCA) as the initial antihypertensive medication (134). Those randomized to $\leq$80 mm Hg when compared to <90 mm Hg demonstrated an advantage with regard to the cardiovascular mortality and total cardiovascular events. The investigators estimated that the ideal blood pressure was 138.5/82.6 mm Hg and found no evidence consistent with a J-curve. During the study, additional antihypertensive medications were added to achieve the blood pressure goals with 45% of patients in the $\leq$80 mm Hg group being on an ACE-I versus 35% in the $\leq$90 mm Hg group. Beta-blockers and diuretics were also utilized during the study, but as with the ACE-I, a statistical comparison with regard to the use of the specific antihypertensive medications was not reported across the treatment arms.

In the Heart Outcomes Prevention Evaluation (HOPE) study, subgroup analyses were performed on the 3577 diabetic patients enrolled in the study (81 type 1 and 3,496 type 2 diabetic patients) who were followed for nearly 5 years. Low-dose ramipril was compared to placebo as "add on" therapy to evaluate the effects on combined cardiovascular (myocardial infarction, strokes, and cardiovascular death) events (124). The study was stopped 6 months early after 4.5 years of follow-up by the Data Safety Monitoring Board because of a significant advantage of ramipril with regard to the primary outcome. During the study, there was a minor but statistically significant difference in the blood pressures between the ramipril group and placebo which the investigators postulated was too small to account for the difference seen between ramipril and placebo treatment arms.

In addition to the UKPDS, three additional trials have compared the effects of an ACE-I versus a CCB (135,136) or beta-blocker (137). In the ABCD Trial, the use of the ACE-I demonstrated an advantage with regard to the incidence of myocardial infarctions when compared to the CCB nisoldipine (153). The difference was independent of blood pressure, smoking, total cholesterol, beta-blocker and past cardiovascular events. The investigators stressed that the study could not determine whether it was the beneficial cardiovascular effect of the ACE-I or a deleterious effect of the CCB or a combination of both. In another study, a similar result was obtained when a post hoc analysis revealed that fosinopril was associated with fewer cardiovascular events than amlodipine in a 2.8-year follow-up period (154). In the Captopril Prevention Project, the use of captopril was associated with less myocardial infarctions and total cardiovascular events in diabetic patients when compared to atenolol over a 5-year follow-up period (155). This result contrasts with the UKPDS findings which did not demonstrate a difference between the two antihypertensive medications (147). As stated earlier, the lack of difference seen in the UKPDS may be a result of the inadequate dosing of captopril during the study (25–50 mg twice a day [bid] rather than three times a day [tid]).

The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial, or ALLHAT, was a study designed to determine whether treatment with a CCB, amlodipine, or ACE-I inhibitor, lisinopril, lowers the incidence of coronary heart disease (CHD) or other cardiovascular disease (CVD) events versus treatment with a diuretic, chlorthalidone (138). Initially the study also included doxazosin, alpha-blocker, but this arm was discontinued early because increased risk heart failure admissions (139). The main results compared the three remaining antihypertensive medications which also included over 11,000 type 2 diabetic patients with a mean follow-up period of 4.9 years. The primary outcome, fatal CHD or nonfatal myocardial infarction (MI), occurred in 2,956 participants, with no difference between treatments. In the diabetic population, amlodipine was associated with higher incidence of heart failure when compared with chlorthalidone (Risk Ratio [RR] 1.45, 95% CI 1.23–1.64). When comparing lisinopril to chlorthalidone in the diabetic patients, lisinopril was associated with a higher rate of combined CVD (CHD, stroke, angina, heart failure and peripheral artery disease) (RR 1.08, 95% CI 1.00–1.17) and, surprisingly, a higher incidence of heart failure (RR 1.22, 95% CI 1.05–1.42). The results of the ALLHAT study, in light of previously published studies, were surprising and some cited the difference in blood pressures, especially in the lisinopril group, as a specific factor contributing to the findings.

In the Losartan Intervention for Endpoint reduction in hypertension study (LIFE) substudy with diabetic patients, 1,195 patients with diabetes, hypertension, and signs of left ventricular hypertrophy (LVH) on electrocardiograms, a losartan-based versus a atenolol-based treatment was compared with follow-up period of least 4 years (mean 4.7 $\pm$ 1.1 years) (140). The study compared the effects of the drugs on the primary composite end point of cardiovascular morbidity and mortality (cardiovascular death, stroke, or MI). Both arms had similar blood pressures, 146/79 mm Hg in losartan patients and 148/79 mm Hg (19/11) in atenolol patients. The results revealed that losartan was associated with a lower risk in the primary endpoint (RR 0.76; 95% CI 0.58–0.98; $p = 0.031$), cardiovascular death (RR 0.63, 95% CI 0.42–0.95; $p = 0.028$) and all-cause mortality (RR 0.61, 95% CI 0.45-0.84, p = 0.002).

Summary

Hypertension unfortunately is a frequent partner with diabetes. The prediabetic and diabetic state, especially in type 2 diabetes, lends itself to the development of hypertension. Once hypertension is present, there are numerous mechanisms by which both diabetes and high blood pressure contribute to the development of the vascular complications. Fortunately, it appears that blood pressure control can stall or even prevent the

development or progression of these complications, especially cardiovascular and renal disease. Review of the literature reveals that aggressive and early blood pressure control may be the key to antihypertensive therapy. In this regard, blood pressure therapy prior to the development of overt diabetic nephropathy or even microalbuminuria may be the most optimal strategy with regard to preventing ESRD. Although further studies are needed, results from studies evaluating the antihypertensive treatment of normotensive diabetic patients suggest that it may be even beneficial to treat normoalbuminuric patients with "normotensive" blood pressures below the current recommendations of 130/80 mm Hg. The ALLHAT study, although, demonstrated that a benefit with diuretic therapy with regard to cardiovascular outcomes, specifically heart failure, and inhibition of the RAAS with an ACE-I or ARB may have the added benefit of renal protection. Although we did not discuss the effects of combination antihypertensive therapy, it is clear that a majority of patients with diabetes, especially in the presence of even a mildly elevate UAE, will require at least two or even three antihypertensive medications. In the UKPDS, a study in which the target blood pressure were well above the current consensus guidelines of <130/80 mm Hg, 69% of patients required two antihypertensive medications and 29% of patients required more than two such medications (148).

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CHAPTER 55 ■ HYPERTENSION ASSOCIATED WITH ENDOCRINE DISORDERS

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The frequency of secondary forms of hypertension in an unselected population of patients is unknown because the application of the requisite highly sensitive and specific screening and diagnostic tests in such individuals is expensive and not always risk-free. Moreover, no comprehensive surveys of unselected hypertensives have been performed using screening or diagnostic tests sensitive enough to establish the presence of the most common forms of potentially curable hypertension and thus to provide accurate estimates of the prevalence of these disorders. However, there are several clues that are helpful in identifying patients in whom the likelihood of a secondary form of hypertension is enhanced and in whom screening efforts are more likely to be productive. When the blood pressure elevation is noted at a young age, a secondary cause should be considered because primary ("essential") hypertension typically manifests in the fifth decade of life and later. A sudden onset of hypertension in an individual previously known to be normotensive or one in whom minimal antihypertensive therapy has previously been effective and who then becomes difficult to control despite probable compliance with treatment may be further clues to the presence of an identifiable form of hypertension. Severe hypertension and drug-resistant hypertension are also reasons to consider further evaluation. In addition, the various causes of secondary hypertension have specific findings that, when present, should trigger further assessment. This chapter considers the most common endocrine forms of secondary hypertension from the perspectives of relative frequency, pathophysiology, screening tests to establish their presence, diagnostic techniques to separate subtypes, and therapeutic approaches based on current information.

PRIMARY ALDOSTERONISM

Prevalence and Pathophysiology

Primary aldosteronism is probably the most recently recognized endogenous secondary form of hypertension, having been described initially in the mid-1950s. Based on the experience of several large referral centers, primary aldosteronism is second only to renal vascular hypertension in its prevalence as an identifiable and thus potentially curable form of hypertension (1,2).

Two recent studies in large groups of Italian (3) and Australian (4) hypertensive patients indicate a prevalence exceeding 9%. Several adrenal abnormalities capable of producing the syndrome of hyperaldosteronism have been identified and will be differentiated subsequently. To date, there have been no demographic or geographic bases for excluding the diagnoses. In brief, this group of disorders is characterized by anomalous, and generally autonomous, hypersecretion of aldosterone or other mineralocorticoids by the adrenal gland, leading to enhanced renal reabsorption of sodium in exchange for potassium and hydrogen ions. The enhanced sodium reabsorption produces extracellular fluid volume expansion and thus raises blood pressure. Despite continued excessive production of mineralocorticoids, the kidney ultimately "escapes" from continued and excessive sodium reabsorption (and volume expansion) by a combination of increases in perfusion pressure, renal blood flow, glomerular filtration, and the actions of natriuretic factors. Several studies have now identified increased levels of natriuretic peptide, presumably from the atrium, but also reportedly of adrenal origin, in patients with primary aldosteronism (5). These findings confirm a role for this peptide in the "escape" phenomenon. Although the sample sizes of the studies have been small, a suggestion has been made that patients with primary aldosteronism due to unilateral adenoma have higher plasma levels of natriuretic peptide than do those with bilateral (hyperplastic) adrenal disease.

Because of the mineralocorticoid-induced exchange of sodium for potassium and hydrogen ions, hypokalemia and metabolic alkalosis are commonly, but not invariably observed. Intracellular potassium depletion is typical and may produce carbohydrate intolerance, electrocardiographic abnormalities suggestive of left ventricular hypertrophy as well as arrhythmias, muscle weakness, and polyuria. Magnesium loss is also observed in primary aldosteronism, although not often evaluated, and contributes to muscle symptoms and arrhythmias. Renal cysts are commonly found in patients with primary aldosteronism as well as proteinuria. A recent report of renal nephrocalcinosis in a patient with primary aldosteronism suggests that the increased urinary calcium excretion and reduced citrate excretion may be related to stone formation (6). Proteinuria may reflect the glomerular hyperfiltration commonly seen in patients with primary aldosteronism. Accompanying the alkalosis, a decreased ionized calcium level is usually found, contributing to the muscle weakness and cramps and accounting for the occasional occurrence of tetany and a positive Chvostek's sign.

The blood pressure elevation in patients with primary aldosteronism is usually moderate or severe, and some studies have suggested that this syndrome is a common cause of malignant hypertension. While the hypertension is usually responsive to diuretic therapy and volume depletion, it is difficult to avoid worsening of the alkalosis, hypokalemia, hypomagnesemia, and decreased ionized calcium when diuretics are used, despite the use of potassium-sparing agents. The consideration of medical therapy will be discussed later. While several cases of primary aldosteronism during pregnancy have been observed, a recent report of the development of severe postpartum hypertension developing in two women confirms the masking effects of pregnancy, presumably attributable to the antialdosterone

action of progesterone, on the clinical manifestations of this disorder (7).

Screening Tests

Measurement of serum potassium levels has traditionally been advocated as the most effective single screening test for primary aldosteronism. However, critical evaluation of the observations of several centers reporting studies in substantial numbers of patients with primary aldosteronism and with essential hypertension indicate that this may not be a very sensitive or specific screening test (1,2). One large study has recently reported normal plasma potassium levels in all patients with primary aldosteronism (3). Clues that would indicate a workup of a hypertensive patient for primary aldosteronism when the serum or plasma potassium levels are normal would be severe or refractory hypertension; onset of hypertension at a young age; or sudden increase in blood pressure in a previously normotensive or easily controlled hypertensive patient. At least 20% of patients with surgical confirmation of primary aldosteronism have been found to have serum (as opposed to plasma) levels of potassium that are within the normal range. Hemolysis of blood during venipuncture, which appears to be more common in patients with primary aldosteronism as is increased bruisability, may artifactually increase the potassium concentration. While the normal range for plasma potassium concentration may be as low as 3.5 mmol/L in some laboratories, serum potassium values rarely are below 4.0 mmol/L in normal subjects. Moreover, the majority of hypertensives manifesting hypokalemia have secondary aldosteronism from diuretic administration, the presence of renal vascular hypertension or other "high-renin" states leading to excessive stimulation of aldosterone production. Finally, when hypokalemia is found, repeat measurements, often after withdrawal of diuretics, and collection of 24-hour urine samples for measurement of sodium and potassium excretion have been advocated. This cumbersome and expensive series of screening maneuvers can be simplified by measuring plasma renin activity because in all cases of uncomplicated primary aldosteronism peripheral levels of renin are markedly suppressed by the elevated pressure, increased sodium balance, and expanded extracellular fluid volume associated with this syndrome. Thus the observation of "normal" or "elevated" plasma renin levels can effectively exclude primary aldosteronism when renal function is normal.

However, the finding of "low" or suppressed renin levels alone is not diagnostic of primary aldosteronism because as many as 40% of patients with "essential" hypertension may manifest suppressed plasma renin levels (e.g., the "low-renin essential hypertensive" subgroup). In addition, a variety of antihypertensive medications are known to influence renin levels. Most of these agents will raise renin, but antisympathetic drugs, acting centrally or peripherally, and β-adrenergic blocking agents can suppress plasma renin levels. Thus screening measurements of plasma renin activity should only be conducted when patients can safely be withdrawn from agents that are known to suppress renin release. Many of these, particularly the β-blocking drugs, have little or no effect on blood pressure in patients with primary aldosteronism.

We have found that the most sensitive and specific screening test for primary aldosteronism is the measurement of both plasma renin activity and plasma aldosterone concentration in peripheral venous blood obtained in ambulatory patients in whom agents that are known to suppress renin release have been withdrawn (8). Because renin is normally the primary stimulus to aldosterone production and in primary aldosteronism this relationship is essentially abolished, the sensitivity of these measurements can be enhanced by expressing them as a ratio of aldosterone to renin (disregarding the units of measurement) with the lowest value for the latter being 0.7 in our hands. Although absolute values vary depending on the laboratory, in general, a ratio of 30 or more is a clear indication of primary aldosteronism and usually suggests a unilateral adenoma. A ratio between 15 and 30 is suggestive, and usually includes most patients with primary aldosteronism due to bilateral adrenal hyperplasia. In our hands, this measurement completely separates patients with primary aldosteronism from those with all forms of essential hypertension and, theoretically, can even be conducted in patients receiving antihypertensive medications that would be expected to stimulate renin release, such as diuretics, angiotensin-converting enzyme (ACE) inhibitors, calcium channel entry blockers, and other vasodilators. Because a few patients with primary aldosteronism appear to be exquisitely sensitive to angiotensin II, the finding of a normal aldosterone-to-renin ratio while receiving ACE inhibitors may require repeat sampling after withdrawal of the agents. However, like β-blockers, ACE inhibitors are usually not effective in reducing blood pressure in patients with uncomplicated primary aldosteronism. In our hands, this ratio is not only useful as a sensitive and specific screening test but also serves to differentiate the two most common forms of the syndrome (8). Two recent large studies confirm the utility of this screening test by estimating the prevalence of primary aldosteronism as 9% to 15% among "essential" hypertensive subjects (3,4).

Diagnosis

Traditionally, the diagnosis of primary aldosteronism has depended on the demonstration of excessive aldosterone production plus suppression of the normal stimulus, angiotensin II (renin). This has required the application of suppressive maneuvers to demonstrate hyperaldosteronism. These have included several days of a high-salt diet, often augmented by administration of intramuscular mineralocorticoids, such as deoxycorticosterone acetate (DOCA) to ensure sodium retention and volume expansion or the rapid intravenous administration of normal (0.9%) saline for the same purpose (1). Excessive aldosterone production is then based on measurement of one of several aldosterone metabolites in a 24-hour urine collection or, in the case of the saline infusion test, measurement of plasma aldosterone concentration (1). To complete the diagnostic approach it has been necessary to document suppressed renin levels, which traditionally has required a stimulatory maneuver such as several days of a low-sodium diet, often augmented with diuretic administration, or the use of intravenous or rapid diuretic-induced sodium and volume depletion to demonstrate the failure of the renin system to respond normally (1). Thus the diagnosis of primary aldosteronism requires not only evidence of excessive or inappropriate aldosterone production, but also its autonomy from the primary stimulus, renin.

Our recent observations, confirmed by several other groups, indicate that the diagnosis can be made simply by measurement of plasma renin and aldosterone levels in peripheral blood in an ambulant patient not receiving drugs known to interfere with the renin-angiotensin-aldosterone axis previously described (8). Not only is this approach easier to accomplish, it is also less expensive because it can be performed on an outpatient basis and is not subject to the inaccuracy that may accompany incomplete collection of urine or errors induced by alterations in aldosterone metabolism, since any one of the 13 metabolites found in urine may be normal or abnormal in specific patients (1,2). Finally, hypokalemia itself, common in primary

aldosteronism, may alter the secretion and thus the urinary excretion of aldosterone, yielding false-negative results. After establishing the diagnosis of primary aldosteronism, the differentiation of several subtypes with important implications for treatment must be pursued.

Differentiation of Subtypes

The most common form of primary aldosteronism, accounting for 60% to 65% of cases in most series, is due to a unilateral adrenal adenoma. This form is most amenable to surgical intervention with a high rate of normalization of elevated blood pressure reported by most investigators. The adenomas are often small, frequently less than 1 cm in diameter, and thus localization techniques based on anatomy alone may not be effective. A recent report (9) of a group of 20 subjects with primary aldosteronism who were evaluated with magnetic resonance indicated a sensitivity of 60% with this technique, which is inadequate for use as a sole localizing procedure. In addition, several patients with apparent unilateral aldosteronism responsive to unilateral adrenalectomy have been found to have macro- and micronodular hyperplasia on microscopic examination. Yet long-lasting cure of hypertension has been produced by unilateral adrenalectomy (1). For these reasons, the use of adrenal venography, which may have substantial morbidity, computed tomography, magnetic resonance imaging, and isotopic scanning using a radioactive steroid precursor have been disappointing procedures, all fraught with unacceptably high rates of false-positive and false-negative results (1,10). The use of biochemical techniques or those based on the pathophysiology of primary aldosteronism have been of greater value in separating unilateral and bilateral forms of this syndrome.

Typically hyperaldosteronism resulting from a unilateral adrenal abnormality is modulated by adrenocorticotropic hormone (ACTH) but not by angiotensin II (11). Thus measurement of plasma aldosterone under conditions when the renin system would be relatively quiescent, such as early morning recumbency and again after activation of this system by upright posture for several hours, at noon, has been used to separate unilateral and bilateral forms (12). The rationale is that plasma levels in unilateral hyperaldosteronism would be expected to be higher early in the morning when the effect of ACTH is greater and to be relatively unresponsive to the increase in angiotensin II associated with assumption of the upright posture, producing lower values when plasma is sampled at noon. In contrast, normal subjects and those with bilateral forms of hyperaldosteronism would be more likely to have higher plasma aldosterone levels at noon compared to early morning levels. While this test has some usefulness, false-negative and false-positive results have been reported by several groups, including ours (10). This appears to be due to the fact that some of the adenomas have been reported to be angiotensin II responsive, accounting for the rise in plasma aldosterone levels with upright posture, and some patients with aldosteronism due to adrenal hyperplasia have demonstrated no increase or even a fall in plasma aldosterone levels when the noon value is compared to that obtained earlier during recumbency. Some investigators have advocated pharmacologic testing with a short-acting ACE inhibitor, captopril, based on the same pathophysiologic rationale. Again, false-positive and false-negative results have reduced the accuracy of this test.

Another biochemical measurement that has been helpful in the hands of some investigators has been the measurement of 18-hydroxycorticosterone in plasma or 18-oxocortisol in urine (13). Both of these steroids have been reported to be higher in patients with adenomas than in those with adrenal hyperplasia in studies of small groups of patients from two laboratories. While encouraging, more extensive observations by a variety of investigators will be required to provide confirmation of these promising preliminary findings.

Adrenal venous blood sampling for the measurement of steroids is the most sensitive and specific way of separating unilateral and bilateral forms of this disorder (1,10). Nevertheless, while this technique is one that has been used for a long time, it has often been inconclusive or misleading. The reasons for previous failure have been both technical and physiological. Often the right adrenal vein cannot be located. In other situations, the adrenal venous drainage is multiple, or adrenal effluent may be diluted by blood from other sources such as the phrenic vein or left renal vein. In addition, steroid secretion is episodic, influenced by ACTH and perhaps other factors, thus potentially introducing another source of error. We have attempted to reduce these limitations by sampling adrenal venous blood during continuous infusion of ACTH and by measuring both aldosterone and cortisol in blood from the adrenals and the inferior vena cava (1,10). By expressing the observations as a ratio of aldosterone-to-cortisol, we can then correct for dilution of adrenal venous blood, minimize errors resulting from episodic secretion of steroids and, if one adrenal is not able to be sampled, infer the location of a unilateral lesion to that gland if the contralateral adrenal has an aldosterone-cortisol ratio that is lower than that in the inferior vena cava because extraadrenal sources of aldosterone have not been reported except in cases of metastatic malignancy (1). We have found that this technique has the highest accuracy (91%) in separating unilateral from bilateral disease (1,10), as recently confirmed by other investigators. We are most comfortable in making therapeutic recommendations when the adrenal venous data are corroborated by one of the anatomic or physiologic observations. An added benefit of localization, beyond determining whether a patient with primary aldosteronism is best treated with medication or with unilateral adrenalectomy, is the option of utilizing the less morbid dorsal (flank) or laparascopic approach in the latter case.

Molecular genetics has improved our recognition and understanding of a rare form of hyperaldosteronism which is remediable with dexamethasone (14). While several familial cases indicated a genetic basis for this syndrome, only with the ability to identify the aldosterone synthase gene and its mutations were the mysteries of this disorder elucidated. The most frequently encountered mutation of this type causes ACTH-responsive aldosterone production, accounting for the ability of glucocorticoid administration to provide persistent reduction in aldosterone production, relief of the syndrome of hyperaldosteronism, and a reduction in blood pressure with continued administration. However, long-term treatment with glucocorticoid may not be desirable in such patients. Evidence indicates that they can be treated with potassium-sparing diuretic combinations in most cases (15). An Australian group has recently reported another form of familial hyperaldosteronism not responsive to glucocorticoid administration (16). These families have revealed both adenomatous and bilateral forms of hyperaldosteronism, and future studies may elucidate the etiology of this interesting observation.

Two cases have been reported of primary aldosteronism resulting from bilateral solitary adrenal adenomas (1) which obviously produced confounding localization observations. When unilateral adrenalectomy with only transient improvement in the syndrome prompted reevaluation and exploration of the remaining adrenal the situation was clarified. Adrenal cortical carcinoma has been a rare cause of primary aldosteronism, but the malignant nature of the disease and the presence of excessive production of ketosteroids have usually been clues.

Treatment

When the evidence indicates that the patient has a unilateral source of hyperaldosteronism, adrenalectomy is almost uniformly effective in eliminating the hyperaldosteronism and its biochemical consequences and leads to normalization of blood pressure with no, or minimal amounts of, antihypertensive medication in over 70% of most reported cases (1). In some series, the normalization of blood pressure without medication has been reported to occur in a higher proportion of patients. The discrepancy between reports appears to be related to both the duration of disease before diagnosis and the duration of follow-up after surgery. While some patients demonstrate a reduction in blood pressure immediately after surgery, in others months may be required before the nadir in blood pressure is seen. In these latter patients, continued antihypertensive therapy may be required with trial withdrawal or reduction in doses after several months have elapsed. Extensive experience with the laparoscopic technique for adrenalectomy has now been described in the literature. However, some caveats are indicated. In one case, it took 4.5 hours to accomplish this procedure. Another report indicates recurrence of malignant adrenal tumor 6 months after removal of what was thought to have been a benign adrenal adenoma causing primary aldosteronism (17).

In bilateral disease, medical therapy is preferred. The aldosterone antagonist spironolactone is the most effective agent; however, doses as high as 800 mg per day may be required. Moreover, the side effects of spironolactone (e.g., painful gynecomastia) often lead to withdrawal of the agent. Eplerenone, a new mineralocoticoid receptor blocker, has not been extensively evaluated in primary aldsoteronism. Alternatively, potassium-sparing diuretics such as triamterene or amiloride combined with hydrochlorothiazide may be used with careful attention to the need for additional potassium and magnesium replacement (15). Calcium channel entry blockers have been reported to be effective in primary aldosteronism, but long-term experience in such patients is quite limited. There are scattered reports of the efficacy of ACE inhibitors, but these are largely anecdotal and unconfirmed.

For glucocorticoid-remediable hyperaldosteronism, glucocorticoids or potassium-sparing diuretics have been reported to be effective. There are few additional data regarding therapeutic responses to other agents in this rare disorder.

PHEOCHROMOCYTOMA

Prevalence and Pathophysiology

While pheochromocytoma is acknowledged to be less frequent than primary aldosteronism, its presence was often unsuspected and manifested by catastrophic outcomes during anesthesia and surgical procedures in the era before the availability of biochemical tests. In the past 30 years, it has become much easier to identify patients with this disorder, and the diagnosis is being considered in increasing numbers of hypertensive subjects. Unfortunately, because of the protean nature of the symptoms associated with pheochromocytoma, many more patients with hypertension not due to pheochromocytoma are evaluated for it in proportion to the few who are actually found to harbor tumors producing excessive catecholamines. In short, the symptoms and the diagnosis of pheochromocytoma are directly related to overproduction of catecholamines and thus must be differentiated from syndromes in which such overproduction results from physiologic or pharmacologic sources, frequently described as pseudopheochromocytoma (18).

The overwhelming majority (over 85% in most large series) of cases of pheochromocytoma result from adrenal medullary tumors. Extraadrenal pheochromocytomas are usually located below the diaphragm, typically in the periaortic area, and are more commonly malignant than the adrenal tumors, although malignancy can also be found in the latter. Cases of pheochromocytoma or paraganglioma have been reported in the urinary bladder, the heart, the brain, and the carotid body. Pheochromocytoma is included in several syndromes featuring multiple endocrine neoplasms (MEN type IIa) including thyroid and parathyroid tumors, MEN type IIb with ganglioneuromas, the von Hippel-Lindau syndrome (retinal angiomatosis, cerebellar hemangioblastoma), and other neuroectodermal abnormalities such as neurofibromas or "café-au-lait" skin lesions.

The symptoms of pheochromocytoma are generally nonspecific and may include headache, diaphoresis, pallor, tachycardia, anxiety or tremulousness, nausea, weakness, chest pain, dyspnea, fever, weight loss, and a variety of other systemic complaints. Sustained elevation of blood pressure is the most frequent finding, with about one-third of patients exhibiting episodic or paroxysmal hypertension and even normal blood pressure being observed in 10% to 15% of the subjects (19). A recent review from Italy of 284 patients with pheochromocytoma indicates paroxysmal hypertension in 67%, hypertensive crises in 59%, and normal blood pressure in 21% of the cases (20). Recent observations suggest that nocturnal hypertension can be found in those with apparent normotension during the daytime hours (21). Severe hypertension is common, and orthostatic hypotension has been reported to occur. Grade III or IV hypertensive retinopathy is observed in over 50% of patients with pheochromocytoma, presumably owing to the intense vasoconstriction that is the result of excessive catecholamine production. Raynaud's phenomenon and tremor are other findings observed in some patients. Rarely, an abdominal pheochromocytoma can be felt on deep palpation of the abdomen. However, the risk of precipitating a hypertensive crisis by manipulation of the tumor is reason not to be overly vigorous in such examination.

The majority of signs, symptoms, and findings associated with pheochromocytoma can be attributed to the effects of increased catecholamine production and the resultant stimulation of α- and β-adrenergic receptors. It is easy to understand why excessive release of catecholamines can raise blood pressure by inducing both vasoconstriction and an increase in cardiac output. Paroxysmal hypertension is also understandable given the variety of stimuli of catecholamine release, with an exaggerated response occurring in pheochromocytoma cases. Thus the episodic occurrence of headache, pallor, tremulousness, anxiety, and cardiovascular symptoms (e.g., tachycardia, arrhythmias, dyspnea, chest pain) can also be explained by the episodic release of catecholamines and the accompanying increase in blood pressure. It is more difficult to understand the reason for occasional reports of orthostatic hypotension or normotension (22) in these patients. Some have suggested that the intense vasoconstriction and elevated blood pressure induced by release of catecholamines can suppress baroreceptor reflex behavior. In addition, the vasoconstriction is also associated with a relatively contracted intravascular volume. Thus when the tumor is quiescent and vasorelaxation occurs, the gravitational effect of upright posture may not be adequately compensated for by the suppressed baroreceptor response and may be aggravated by the relative volume depletion. An additional explanation has been the vasodilatory effects of some catecholamines such as dopamine, which may be produced in preferential excess by some tumors. Finally, a variety of vasodilatory compounds have been identified that could be released in increased amounts to counter the catecholamine-induced vasoconstriction in an attempt to maintain pressure homeostasis,

and thus could have a hypotensive effect when catecholamine release is abruptly diminished.

Pheochromocytoma has been identified in patients in whom it was not previously suspected when a paradoxical rise in blood pressure was observed following administration of a β-adrenergic blocking agent. This could even occur, theoretically, when β-blocker eyedrops are used because of the systemic absorption of these agents. The paradoxical rise in pressure occurs because of blockade of the β_2-receptor, typically associated with peripheral vascular vasodilation, leaving the vascular α-receptors, which are constrictor, unopposed.

Metabolic abnormalities are often seen in patients with pheochromocytoma. These include hyperglycemia and glucose intolerance as well as lipid abnormalities presumably related to the effects of catecholamines on glucose production (glycogenolysis) and/or uptake and α-adrenergic stimulation of triglyceride synthesis. Hypokalemia may occur because of catecholamine-induced shifts of potassium into skeletal muscle with β_2-receptor stimulation.

While unrecognized pheochromocytoma can be fatal, a high index of suspicion and the use of sensitive diagnostic tests provide a reasonable way of detecting most patients. One situation in which an unrecognized pheochromocytoma can be particularly catastrophic is in the pregnant patient (23). In this situation, high maternal and fetal mortality rates have been reported. Thus when hypertension or labile blood pressure is observed in pregnancy, it is important to rule out the presence of a pheochromocytoma. In patients in whom this has been recognized, surgical removal of the tumor has permitted the safe progression of the pregnancy to successful delivery.

A variety of factors, activities, and agents have been reported to trigger a paroxysm of blood pressure elevation or symptoms of pheochromocytoma in some patients. Such activities as defecation, sexual intercourse, bending over, Valsalva maneuver, coughing, sneezing, cigarette smoking, intake of red wine or hard cheeses (both of which contain tyramine), shaving, and a variety of invasive and surgical procedures have been incriminated. These and other normal stimuli of catecholamine production can trigger excessive release by a tumor and thus cause symptoms.

The presence of a pheochromocytoma can be mimicked by a variety of circumstances, frequently referred to as "pseudopheochromocytoma" (18). These events feature either excessive catecholamine production, excessive stimulation of adrenergic receptors, or both. Use of sympathomimetic agents (nasal sprays, cold remedies, diet aids, ephedrine and its congeners, amphetamines, and "street" drugs) or administration of drugs containing sympathomimetic substances, including agents such as imipramine and amitriptyline, to individuals receiving monoamine oxidase inhibitors, can produce symptoms suggestive of increased catecholamine production. Individuals undergoing addictive substance withdrawal also manifest evidence of a hyperadrenergic state. Further, abrupt or acute withdrawal from centrally acting α-adrenergic agonists such as clonidine, guanabenz, and guanfacine, particularly when used at higher doses, may precipitate a rebound hyperadrenergic response. Such exogenous causes of a hyperadrenergic state should be considered in the individual who presents with acute and/or severe symptoms suggestive of pheochromocytoma.

Screening Tests

The establishment of a diagnosis of pheochromocytoma requires a high index of suspicion because the symptoms and findings are not highly specific. The search for this disorder begins with studies to establish biochemical evidence of increased production of catecholamines. Before the advent of sensitive and specific screening and diagnostic tests, a variety of pharmacologic approaches were used, involving intravenous administration of substances known to stimulate catecholamine release (histamine, glucagon) or to block catecholamine-induced α-adrenergic stimulation (phentolamine). The stimulatory tests were fraught with catastrophic responses, including sudden death, in some patients with pheochromocytoma as well as false-positive responses in individuals in whom a tumor could not be found. The phentolamine test was also notoriously inaccurate, and thus these pharmacologic tests have been abandoned by most experts. The availability of sensitive and specific measurements of catecholamines and their metabolites in urine and in plasma has provided a safer, convenient, and reasonably accurate way to screen for the presence of a pheochromocytoma. However, these tests have also been fraught with inaccuracy for several reasons.

Plasma values for catecholamines can be in the normal range if the tumor releases catecholamines intermittently or can be elevated in patients without a pheochromocytoma for various reasons. The latter is not a major problem because additional screening or diagnostic tests can separate the false-positive responders from those harboring a tumor. A recent report of 35 patients with pheochromocytoma associated with von Hippel-Lindau disease or MEN-II indicates that plasma metanephrine and normetanephrine values had a sensitivity of 97% for the identification of tumors (24).

Urine studies are more frequently chosen to screen for pheochromocytoma. These can be influenced by several factors. The most obvious is the failure of the patient to collect a complete 24-hour sample. Some investigators have advocated "spot" urine samples, which may be influenced negatively by episodic secretion of catecholamines. Another source of error may be the choice of a specific metabolite for measurement that is not the major product of enzymatic action in a given patient. Thus it frequently becomes necessary to measure total catecholamines, norepinephrine, metanephrine, normetanephrine, vanillylmandelic acid, and/or homovanillic acid to be certain that the patient does not have a pheochromocytoma. The conventional techniques for screening for pheochromocytoma, therefore, may not be very useful. In addition, most antihypertensive drugs elevate catecholamines and thus may be a source of false-positive results. Exceptions to this are the centrally acting α-adrenergic agonists (clonidine, guanabenz, guanfacine) and reserpine when taken chronically.

We have developed a simple screening test that avoids many of these problems and has yielded a very high (over 98%) accuracy rate (25). We ask patients to collect the urine during the sleep period, discarding urine before retiring, saving any sample voided during the sleep period, and including the first sample upon arising. We then measure norepinephrine excretion in the sample by a specific radioenzymatic technique and express the results in units per hour, given the duration of the sleep period. This obviates the problems caused by incomplete 24-hour urine collection. In addition, since the period of sleep is a time of basal catecholamine release for normal subjects and hypertensives without pheochromocytoma, it becomes easier to identify even slight elevations in basal catecholamine production, which are invariably found in patients with pheochromocytoma. In our experience over the last 20 years with this approach, we have found the lowest value for a patient with pheochromocytoma to be seven times higher than the highest value for normal or essential hypertensive patients (25). These observations have recently been confirmed by other investigators, indicating that it has broad applicability. If elevated values are observed in hypertensives receiving antihypertensive medications, the study can be repeated after withdrawal of the offending drug(s). Obviously the finding of elevated values in a patient ingesting one of the agents known to suppress catecholamine release would presumably indicate a positive screening result.

Diagnosis

Some investigators have advocated the use of plasma catecholamines as a screening test with the response of plasma catecholamines to oral administration of 0.3 mg of clonidine used as a diagnostic test. In untreated essential hypertensives with elevated plasma catecholamines, this dose of clonidine should reduce plasma levels by 50% after 3 hours. Many investigators have found this to be neither a sensitive nor a specific procedure and thus have abandoned its use (26,27). After documentation of an abnormal "sleep" urinary norepinephrine value or elevated 24-hour urine values for catecholamines or their metabolites, the next step is localization of the tumor. This is necessary for several reasons, including decisions regarding the therapeutic approach, which will be discussed subsequently. Fortunately, the majority of the tumors are located in the adrenal and are relatively large, usually greater than 2 cm in diameter. Thus computerized tomographic (CT) scanning will usually reveal the site of the tumor (28). The large Italian study (20) indicates a sensitivity of 99% for intraadrenal and 91% for extraadrenal tumors while the MIBG isotopic scan had a slightly lower (88.5%) sensitivity. Since hemorrhagic necrosis and subsequent calcification of parts of the tumor are not uncommon, they can sometimes be seen on a plain abdominal radiograph. The recent development of an isotopic product, labeled metaiodobenzylguanidine (MIBG), has provided another approach to localization of pheochromocytoma that is often useful when a lesion in the adrenal cannot be identified by computerized tomography or when extraadrenal or metastatic pheochromocytoma is suspected. Much less experience has been obtained with magnetic resonance imaging (MRI) and positron emission tomography (PET) scanning techniques. A recent study of 29 patients with pheochromocytoma compared the MIBG scan to the PET scan using fluorodeoxy-D-glucose and found the latter much more accurate (88% vs. 56%) (29). Rarely is it necessary to perform adrenal venography for the purpose of visualizing pheochromocytoma or, more commonly, to obtain venous blood samples for catecholamine content. The latter procedure may be required when other localizing techniques are not fruitful or when ectopic, multiple, or malignant pheochromocytoma is suspected.

Treatment

The preferred treatment of pheochromocytoma is surgical removal of the tumor (see below). Rarely, surgical intervention cannot be performed because of debility, relative or absolute contraindications, the presence of metastatic malignancy, or patient refusal. In such instances, treatment with effective doses of peripheral α-adrenergic blocking agents (vide infra) or metyrosine (Demser) can be useful. Recently several new, nonsurgical approaches to treatment have been reported. 131-I-MIBG has been used as radiotherapy in some cases of malignant tumors or tumors in areas not amenable to surgery (usually in the head and neck areas) alone (30) or in combination with chemotherapy (31).

The proper preparation of the patient for surgery is mandatory and must consider a variety of factors. First, the lesion must be localized so that the surgeon can minimize excessive exploration and manipulation of the tumor. Fortunately, the large size of most tumors makes it possible to identify them prior to surgery and thus permits the surgeon to go directly to the site and concentrate on ligation of the major blood supply of the tumor to reduce the amount of catecholamines introduced into the circulation. Another important requirement is for a period of 7 to 10 days of effective α-adrenergic blockade prior to surgery. This is not necessary for blood pressure or arrhythmia control during the surgical procedure because intravenous administration of α- and β-adrenergic antagonists, respectively, can be used for that purpose, but rather to permit the contracted intravascular volume induced by the intense catecholamine-related vasoconstriction to expand before surgery is undertaken. Without such expansion of volume, the removal of a pheochromocytoma is associated with immediate vasodilation, which in the presence of a contracted extracellular fluid volume can produce rapid and irreversible shock and a state generally unresponsive to pressor agents. Effective α-adrenergic blockade can be assumed when the dose is titrated to a level at which the patient demonstrates a mild decrease in blood pressure on assumption of upright posture. Phenoxybenzamine (Dibenzyline), which chelates the α-receptor, may require a longer period of time to achieve effective doses than the use of the quinazoline class of α-blockers (prazosin, terazosin, and doxazosin). The doses of these agents required for effective α-blockade in pheochromocytoma are quite variable, ranging from 10 to 120 mg per day for phenoxybenzamine and as high as 40 mg per day for the quinazoline group. Beta-adrenergic blocking agents may also be required to decrease the tachycardia and arrhythmias during the preparatory phase. During the operative period, intravenous α-blockers (phentolamine) and short-acting parenteral β-blockers can be used. Several recent reports of successful removal of pheochromocytomas by the laparoscopic approach, occasionally utilizing laparoscopic ultrasound for localization, have been published (32,33). One such report described partial adrenal-sparing bilateral adrenalectomy (33).

Follow-up

Because multiple and malignant pheochromocytomas occur in about 10% of patients, it is prudent to evaluate catecholamine excretion periodically after removal of the tumor. If the patient becomes normotensive following surgery, assessment of urinary catecholamines at 6- to 12-month intervals is appropriate. Since familial pheochromocytoma is not uncommon, screening of family members with urinary catecholamine measurements is often pursued, and particular scrutiny should be given to any hypertensive family members.

CUSHING'S SYNDROME

Prevalence and Pathophysiology

Endogenous hypercortisolism is another endocrine form of hypertension that is potentially curable. The most frequent cause of this syndrome is exogenous steroid ingestion, but the signs and symptoms are the same for both. The typical observations are of truncal obesity with thin extremities because of the increased fat deposition in the abdomen and muscle wasting associated with the catabolic effects of glucocorticoids, increased cervical and dorsal fat pads manifesting as supraclavicular fullness and the "Dowager" or "buffalo" hump, increased facial fat distribution ("moon facies"), and increased capillary fragility often resulting in bruising of the skin, petechiae, and violaceous striae on the abdomen, inner thighs, and arms. The mechanisms responsible for the development of hypertension are not clear (34). There is some evidence that the renin-angiotensin system may play a role because glucocorticoids appear to increase the concentration of angiotensinogen, the renin substrate. In addition, high concentrations of glucocorticoids are capable of occupying the mineralocorticoid receptor of the kidney and inducing sodium and water retention and thus increasing extracellular fluid volume. Recent studies

indicate that cortisol-induced hypertension is associated with sodium retention and volume expansion, but this is not believed to be the primary mechanism for the increase in blood pressure since it is not prevented by administration of the mineralocorticoid receptor antagonist, spironolactone (35). Additional observations indicate no increase in sympathetic nervous system activity but do suggest a suppression of nitric oxide activity (35). Carbohydrate intolerance and diabetes are frequently found in Cushing's syndrome and appear to be due to insulin resistance. Inappropriate elevation of leptin has been reported in Cushing's syndrome and may contribute to the abnormalities in glucose and lipid metabolism (36). Glucocorticoids also can inhibit osteoblastic activity and lead to osteoporosis and vertebral fractures.

The symptom complex can arise from either primarily pituitary or adrenal abnormalities. In about two-thirds of the cases, excessive ACTH production occurs due to a microadenoma of the pituitary, leading to sustained and excessive stimulation of the adrenals. In some patients, ectopic production of ACTH may stem from a carcinoma, often in the lung. Cushing's syndrome may also be a feature of the multiple endocrine adenomatosis (MEA, MEN) syndrome. Roughly 20% to 25% of patients with Cushing's syndrome are found to have a primary adrenal abnormality, typically an adenoma but also including adrenal carcinoma.

Screening Tests

The diagnosis of Cushing's syndrome requires a modestly high index of suspicion and demonstration of elevated or inappropriate glucocorticoid production. Since cortisol typically has an ACTH-driven diurnal rhythm, being higher in the morning than in the afternoon or evening, measurement of plasma cortisol in the morning and evening has been used as a screening technique. However, the sensitivity and specificity of this approach have been relatively poor because many factors can raise cortisol levels, including anxiety, venipuncture itself, estrogen administration, and others. Another long-used approach was to collect urine for the measurement of the major metabolites of cortisol, 17-hydroxycorticosteroids and 17-ketosteroids. However, these measurements can also be influenced by a variety of factors and are often elevated in simple obesity, a condition that mimics Cushing's syndrome because it is often associated with hypertension and carbohydrate intolerance. A single-dose dexamethasone suppression test can also be used as a screening procedure. Dexamethasone, 1 mg, is given orally at 11 PM, and plasma cortisol is measured at 8 AM the following morning. In normal individuals, plasma cortisol levels should be less than 5 μg/dL. The measurement of urinary excretion of free cortisol in a 24-hour sample appears to be the screening test with the highest sensitivity. A value greater than 100 μg/24 hours can be considered to be abnormal.

Diagnosis and Localization

After finding elevated urinary free cortisol excretion, the next step is to differentiate pituitary, adrenal, and ectopic etiologies. Plasma ACTH levels can separate primary adrenal disease from the other two sources. Typically, dexamethasone suppression tests can also separate pituitary and adrenal sources. There are two such tests. The first requires administration of 0.5 mg of dexamethasone every 6 hours for 2 days with collection of 24-hour urine samples for urinary free cortisol and 17-hydroxycorticosteroid excretion before and on the last day of dexamethasone. Plasma cortisol can also be measured on the morning following the last dose and should be less than 5 μg/dL. Urinary free cortisol should be less than 20 μg/24 hours, and 17 hydroxysteroid excretion less than 4 μg/24 hours. The high-dose dexamethasone test is then performed to separate pituitary and adrenal etiologies. Dexamethasone is given as 2 mg every 6 hours for eight doses, and a 24-hour urine collection is obtained on the second day. The majority of patients with pituitary Cushing's evidence have at least a 50% reduction from the pre-dexamethasone values, while in adrenal Cushing's, minimal change is seen in the overwhelming majority of patients. With ectopic ACTH syndromes, the suppression is less consistent, and steroid production is quite variable. Usually, marked wasting and elevated 17-ketosteroid excretion are seen in these patients.

Pituitary tumors are frequently detected by CT scan or magnetic resonance imaging (MRI). Adrenal tumors are also often visible on CT scan because they are usually larger than 1.5 to 2.0 cm in diameter. Occasionally, when the CT scan is not useful, iodocholesterol isotopic scanning may be helpful.

Treatment

When a pituitary adenoma is detected, surgical removal is usually curative, particularly when a microadenoma is found. In addition, such a lesion can usually be removed without requiring total hypophysectomy with its attendant hypopituitarism. Occasionally, irradiation of the pituitary is required if surgical removal is not feasible or yields incomplete results with a recurrence of Cushing's syndrome. When the lesion is localized in the adrenal, surgical removal is almost invariably curative except in the instance of adrenal carcinoma with metastases. Cushing's syndrome due to ectopic ACTH production is the least amenable to treatment because of the aggressive nature of the underlying malignancy, which has usually produced metastases by the time the metabolic abnormalities of Cushing's syndrome become manifest. A few reports of adrenalectomy by laparoscopic technique have now been reported for Cushing's syndrome (37).

HYPERTENSION ASSOCIATED WITH THYROID DISORDERS

Prevalence and Pathophysiology

Hypertension is encountered in both hyperthyroidism and hypothyroidism, although the mechanisms and manifestations appear to be different. In hypothyroidism, the major hemodynamic abnormality is an increase in peripheral vascular resistance, and cardiac output is usually reduced (38). This is often manifest as a primary elevation of diastolic pressure, although systolic elevation can also be seen. The increase in resistance is most likely the result of increased sympathetic nervous system activity or increased β-adrenergic responsiveness (39). Thyroid replacement therapy typically restores blood pressure to normal unless excessive doses are given. Since hypothyroidism may occur more frequently in older patients who often are more likely to have hypertension than younger individuals, residual blood pressure elevation may be seen despite adequate thyroid replacement therapy.

In hyperthyroidism, the dominant hemodynamic abnormality is an increase in cardiac output, and peripheral resistance may be normal (38). Thus a primary increase in systolic blood pressure is typically seen in hyperthyroidism. The mechanism most commonly invoked for the increase in cardiac output in hyperthyroidism is also related to the increased activity of the sympathetic nervous system (38). In this case, it appears to be related to increased β-adrenergic activity or responsiveness,

particularly of cardiac β-receptors. Again, as with hypothyroidism, adequate treatment of the hyperthyroidism is usually associated with normalization of the elevated blood pressure except in those with background essential hypertension. It is unusual for hypertension to be the only presenting symptom of thyroid disease, but it can occur. This is particularly likely in older individuals in whom the symptoms of hypothyroidism may be subtle or masked by nonspecific complaints more frequently voiced by older patients such as constipation, coldness of the extremities, and the like. In older subjects, the "apathetic" form of hyperthyroidism is also more frequent, and thus hypertension may be the primary manifestation.

Screening Tests

Typically, measurements of circulating thyroxine (T_4) levels in plasma can establish the diagnosis of hyperthyroidism and, with thyroid-stimulating hormone (TSH) levels, of hypothyroidism. In some rare circumstances, T_4 levels may be normal in hyperthyroid individuals in whom the predominant production of triiodothyronine (T_3) may be excessive. Estrogen administration can be associated with increased plasma hormone binding proteins, and thus free hormone levels or assays that assess the unbound protein fraction ("uptake" assays) will provide a correct assessment of hormonal status. Since TSH is the dominant stimulus for thyroid hormone production it can be expected to be elevated in hypothyroidism in an attempt to increase thyroid hormone output from a diseased gland. TSH will be decreased in hyperthyroidism when the disorder is due to autonomous overproduction of thyroid hormone by the gland itself. Occasionally, hyperthyroidism is a disorder of excessive production of TSH, in which case both TSH and thyroid hormone levels will be elevated.

Diagnosis

The diagnosis of thyroid disorders after the abnormality is identified by the screening tests outlined above is based on determining the cause of the excessive or deficient thyroid hormone production. This is often simplified by the combination of T_4 (or T_3) and TSH measurements. When both are elevated, a pituitary or hypothalamic lesion is implied as the cause of hyperthyroidism. When hypothyroidism is accompanied by elevated TSH levels, primary thyroid deficiency is the usual cause. When hyperthyroidism occurs with low TSH levels, the lesion is usually in the thyroid itself, and an isotopic scan will reveal if it is due to diffuse overactivity or localized to one area of the thyroid, as with an adenoma or carcinoma, or to discrete multinodular lesions.

Treatment

The treatment of hypothyroidism is generally thyroid replacement therapy. In patients in myxedema coma, exquisite sensitivity to thyroid hormone is often encountered, and thus treatment is begun with very small doses, increased as tolerated based on the patient's cardiovascular and central nervous system responses. For most hypothyroid patients without myxedema, a small replacement dose of T_3 (0.1 mg per day) is initiated, with measurement of T_4 and TSH levels being performed after a period of 4 to 6 weeks. If abnormal, incremental increases or decreases in dose can be made. In some patients who are older and thus have decreased clearance of thyroid hormone, or those with cardiac disease or arrhythmias, a lower starting dose can be used.

In hyperthyroidism, several therapeutic options are available. Surgical removal of part or the entire gland is indicated only when malignancy is suspected, the patient is pregnant, or other treatment approaches have failed. Administration of radioactive iodine has become the preferred treatment for many patients. Both recurrent hyperthyroidism and hypothyroidism may occur with this approach. Continuing (lifelong) follow-up is necessary. Another option is for the use of antithyroid drugs such as methimazole and propylthiouracil, which inhibit thyroid hormone synthesis. After successful suppression of thyroid hormone production based on monitoring of thyroid function tests for a period of 4 to 6 months, it may be possible to taper the dose of antithyroid medication and, in some cases, withdraw it completely. In a substantial number of patients, recurrent hyperthyroidism may occur, but in others a state of normal thyroid function may be observed. Obviously, periodic monitoring of thyroid status will be required for these individuals as well. Often the initial clue to the recurrence of hyperthyroidism will be an increase in systolic blood pressure if this was observed before treatment was begun.

SUMMARY

Hypertension is often a manifestation of an underlying endocrinologic disorder. The initial medical contact for such a patient may be any of a variety of physicians with variable experience with such problems. A high index of suspicion coupled with an understanding of the pathophysiology, signs, and symptoms of the most frequent endocrine forms of hypertension as well as current information about effective screening tests for these disorders will enhance their identification. Definitive diagnostic tests and appropriate treatment will usually provide a means to relieve the hypertension and its sequelae in such individuals.

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CHAPTER 56 ■ MALIGNANT HYPERTENSION AND OTHER HYPERTENSIVE CRISES

CHARLES R. NOLAN AND STUART L. LINAS

THE CLINICAL SPECTRUM OF SEVERE HYPERTENSION

The vast majority of hypertensive patients are asymptomatic for many years until complications due to atherosclerosis, cerebrovascular disease, or congestive heart failure develop. In a minority of patients this "benign" course is punctuated by a hypertensive crisis.

A *hypertensive crisis* is defined as the turning point in the course of an illness at which acute management of the elevated blood pressure plays a decisive role in the eventual outcome. The haste with which the elevated blood pressure must be controlled varies with each crisis. However, the crucial role of hypertension in the disease process must be identified and a plan for management of the blood pressure successfully implemented if the outcome is to be optimal. The absolute level of blood pressure is not the most important factor in determining the existence of a hypertensive crisis. In children, pregnant women, and other previously normotensive individuals in whom moderate hypertension develops suddenly, a hypertensive crisis can occur at a diastolic blood pressure normally well tolerated by adults with chronic hypertension. Furthermore, in adults with only mild to moderate hypertension, a crisis can occur when there is concomitant acute end-organ dysfunction involving the heart or brain. It has been estimated that of the 50 to 60 million hypertensive patients in the United States, approximately 1% will experience a hypertensive crisis during their lifetime (1). Moreover, the incidence of hypertensive crisis appears to be increasing over time. In the period from 1983 to 1992, the number of hospital admissions with malignant hypertension or accelerated hypertension as the primary diagnosis doubled, from approximately 16,000 to 32,000 (2). It has been postulated that this trend of increasing incidence of hypertensive crises may be explained at least in part by medical economics. Since a significant portion of the population lacks health insurance, the lack of a consistent health care provider may lead to inadequate treatment of patients with essential hypertension thereby increasing the number of patients at risk for development of a hypertensive crisis (3). The spectrum of hypertensive crises and other categories of severe hypertension are outlined in Table 56-1.

Malignant hypertension is a clinical syndrome characterized by marked elevation of blood pressure with widespread acute arteriolar injury. Funduscopy reveals *hypertensive neuroretinopathy* with striate (flame-shaped) hemorrhages, cotton-wool (soft) exudates, and often papilledema. Regardless of the degree of blood pressure elevation, malignant hypertension cannot be diagnosed in the absence of hypertensive neuroretinopathy (4). Some authors have defined *malignant hypertension* based on the presence of papilledema and have used the term *accelerated hypertension* when hemorrhages and cotton-wool spots occur in the absence of papilledema (5). However, it is now accepted that the prognosis is the same in hypertensive patients with striate hemorrhages and cotton-wool spots whether or not papilledema is present (6,7). In this regard, the World Health Organization has recommended that accelerated hypertension and malignant hypertension be regarded as synonymous terms for the same disease (4). Hypertensive neuroretinopathy is thus an extremely important clinical finding that indicates the presence of a widespread hypertension-induced arteriolitis which may involve the central nervous system, heart, and kidneys. In patients with untreated malignant hypertension, there is a rapid and relentless progression to end-stage renal disease (ESRD) or death in less than 1 year. Mortality can result from hypertensive encephalopathy, intracerebral hemorrhage, congestive heart failure, and/or complications of uremia.

Hypertensive encephalopathy is a medical emergency in which cerebral malfunction is attributed to the severe elevation of blood pressure. It is one of the most serious complications of malignant hypertension. However, hypertensive encephalopathy can also occur in the absence of malignant hypertension (neuroretinopathy). Hypertensive encephalopathy can develop in the setting of severe hypertension of any cause, especially when acute blood pressure elevation occurs in previously normotensive individuals with eclampsia, acute glomerulonephritis, pheochromocytoma, or drug withdrawal hypertension. Clinical features include severe headache, blurred vision or blindness, nausea, vomiting, and mental confusion. If aggressive treatment is not initiated, stupor, convulsions, and death can ensue within hours. The *sine qua non* of hypertensive encephalopathy is the prompt and dramatic clinical response to antihypertensive therapy.

On occasion, hypertension that is not in the malignant phase (hypertensive neuroretinopathy is absent) may still qualify as a hypertensive crisis when acute end-organ dysfunction occurs in the presence of even moderate hypertension. The term *benign hypertension with acute complications* includes hypertension complicating acute pulmonary edema (acute diastolic dysfunction), acute myocardial infarction or unstable angina, acute aortic dissection, active bleeding, or central nervous system catastrophe (hypertensive encephalopathy, intracerebral or subarachnoid hemorrhage, or severe head trauma). In each case, control of the blood pressure is the cornerstone of successful therapy.

Catecholamine excess states such as pheochromocytoma crisis; monoamine oxidase inhibitor–tyramine interactions; use of sympathomimetic drugs (cocaine, amphetamines, phencyclidine, or high-dose phenylpropanolamine); and abrupt withdrawal of antihypertensive medications (clonidine, methyldopa, or guanabenz), can produce life-threatening hypertensive crises. The clinical presentation usually includes marked elevation of blood pressure with headache, diaphoresis, and

TABLE 56-1

THE CLINICAL SYNDROMES OF SEVERE HYPERTENSION

- Hypertensive crises
  - Malignant hypertension (hypertensive neuroretinopathy present)
  - Hypertensive encephalopathy
  - Benign hypertension with acute complications (acute organ system dysfunction but no hypertensive neuroretinopathy)
    - Acute hypertensive heart failure (acute diastolic dysfunction with pulmonary edema)
    - Atherosclerotic coronary vascular disease
      - Acute myocardial infarction
      - Unstable angina
    - Acute aortic dissection
    - Active bleeding including postoperative bleeding
    - Central nervous system catastrophe
      - Hypertensive encephalopathy
      - Intracerebral hemorrhage
      - Subarachnoid hemorrhage
      - Severe head trauma
    - Catecholamine excess states
      - Pheochromocytoma crisis
      - Monoamine oxidase inhibitor-tyramine interactions
      - Antihypertensive drug withdrawal syndromes
      - Phenylpropanolamine overdose
    - Preeclampsia and eclampsia
    - Poorly controlled hypertension in a patient requiring emergency surgery
    - Severe postoperative hypertension
    - Scleroderma renal crisis
    - Miscellaneous hypertensive crises
      - Severe hypertension complicating extensive burn injury
      - High-dose cyclosporine in children after bone marrow transplantation
      - Autonomic hyperreflexia in quadriplegic patients
      - Severe hypertension with acute rejection or transplant renal artery stenosis in renal allograft recipients
      - Hypoglycemia in patients receiving β-adrenergic receptor blockers
- Benign hypertension with chronic stable complications (chronic end-organ dysfunction but no hypertensive neuroretinopathy)
  - Chronic renal insufficiency due to primary renal parenchymal disease
  - Chronic congestive heart failure with diastolic dysfunction
  - Atherosclerotic coronary vascular disease
  - Stable angina
  - Previous myocardial infarction
  - Chronic cerebrovascular disease
    - Transient ischemic attacks
    - Prior cerebrovascular accident
- Severe uncomplicated hypertension (severe hypertension without hypertensive neuroretinopathy or end-organ dysfunction)

tachycardia. With the severe acute elevation of blood pressure a number of complications can occur, including hypertensive encephalopathy, intracerebral hemorrhage, and pulmonary edema due to acute left ventricular diastolic dysfunction. Thus, catecholamine-related hypertensive crises require prompt recognition and control of blood pressure to avert disaster.

Preeclampsia is a hypertensive disorder unique to pregnancy that usually presents after the 20th week of gestation with proteinuria, edema, and hypertension. Eclamptic seizures may ensue and without treatment may result in death. It is believed that eclampsia may be a subtype of hypertensive encephalopathy (8). Hypertensive disorders of pregnancy and their management are discussed in Chapter 53.

Poorly controlled hypertension in a patient requiring emergency surgery is a hypertensive crisis because of the increased cardiovascular risk that accompanies inadequate preoperative blood pressure control. Surgical manipulation of the carotid arteries or open-heart surgery (especially coronary artery bypass) is occasionally followed by severe hypertension in the immediate postoperative period. *Severe postoperative hypertension* represents a crisis requiring immediate blood pressure control because it can cause hypertensive encephalopathy or intracerebral hemorrhage, or jeopardize the integrity of vascular suture lines and thereby lead to postoperative hemorrhage.

In patients with progressive systemic sclerosis, *scleroderma renal crisis* can occur with sudden onset of hypertension that may enter the malignant phase. There is a rapid progression to ESRD within days to weeks unless the vicious cycle of hypertension, renal ischemia, and activation of the renin-angiotensin–aldosterone axis is interrupted.

Severe acute hypertension can also occur in patients with extensive burns or children receiving high-dose cyclosporine for allogeneic bone marrow transplantation. In quadriplegic patients, hypertensive crises may develop due to autonomic hyperreflexia resulting from stimulation of nerves below the level of the spinal cord injury. Hypertensive crises due to autonomic hyperreflexia can also develop in Guillain-Barré syndrome. Hypertensive crises may also complicate acute rejection or transplant renal artery stenosis in patients with renal allografts. In each of these conditions, a sudden increase in blood pressure may cause acute pulmonary edema, hypertensive encephalopathy, cerebrovascular accident, and/or death.

On the other hand, severe hypertension or the presence of hypertensive complications does not always imply the existence of a hypertensive crisis requiring immediate control of the blood pressure. Patients with benign hypertension (no hypertensive neuroretinopathy) and chronic stable end-organ dysfunction do not require emergent reduction of blood pressure, although a long-term lack of adequate blood pressure control often results in further deterioration of end-organ function. The term *benign hypertension with chronic stable complications* includes hypertension occurring in the setting of primary renal parenchymal disease with chronic kidney disease, chronic congestive heart failure, atherosclerotic coronary vascular disease (stable angina pectoris or prior myocardial infarction), or chronic cerebral vascular disease (prior transient ischemic attacks or cerebrovascular accident).

It is important to emphasize that the finding of severe hypertension does not always imply that a hypertensive crisis is present. In patients with severe hypertension that is not accompanied by acute end-organ dysfunction or evidence of malignant hypertension (hypertensive neuroretinopathy) eventual complications due to stroke, myocardial infarction, or congestive heart failure occur over a time frame of months to years rather than hours to days. Although long-term control of blood pressure can prevent these complications, a hypertensive crisis cannot be diagnosed, as there is no evidence that acute reduction of blood pressure results in any improvement in short-term or long-term prognosis. *Severe uncomplicated hypertension* is defined by a diastolic blood pressure higher than 115 mm Hg without evidence of malignant hypertension (no hypertensive neuroretinopathy) or signs of acute end-organ dysfunction. Although this is not a true hypertensive crisis as defined earlier, it is the most common presentation of severe hypertension. Severe uncomplicated hypertension is usually found in patients

with chronic essential hypertension who are undiagnosed, undertreated, or not adherant with medical therapy. It is most often discovered incidentally in an otherwise asymptomatic patient. There is no evidence of hypertensive encephalopathy or other acute end-organ dysfunction. The fundi do not show striate hemorrhages, cotton-wool spots, or papilledema. Since the potential complications of severe uncomplicated hypertension develop with a time frame of months to years, the once common practice of abrupt reduction of blood pressure with oral antihypertensive agents prior to discharge from the acute care setting is no longer accepted as the standard of care (9–11). Instead, the goal of treatment should be the gradual reduction of blood pressure to normotensive levels over a few days in conjunction with frequent outpatient follow-up visits to modify the antihypertensive regimen and reinforce the importance of lifelong adherence with medical therapy. In the past this entity has been termed *urgent hypertension*. Use of the more descriptive term severe uncomplicated hypertension is preferable because there is no need for urgent reduction of blood pressure as would be required in patients with true hypertensive crises.

MALIGNANT HYPERTENSION

Historical Perspective

In 1914, Volhard and Fahr (12) introduced the descriptive terms *benign* and *malignant,* aimed at separating renal arteriosclerosis into two distinct types. The type without renal failure was called *benign nephrosclerosis* and was characterized pathologically by arteriosclerosis of the kidney. The type with renal failure was called *malignant nephrosclerosis* and was characterized by necrotizing arteriolitis with inflammatory changes in the glomeruli in addition to the arteriosclerosis seen in the benign form (13). Volhard later abandoned the concept of inflammatory changes and substituted prolonged ischemia secondary to vascular spasm as the cause of the renal lesion in the malignant type (14).

The prognostic importance of eyeground changes in hypertensive patients has long been recognized. *Albuminuric retinitis* was the term used by Liebreich in 1859 (15) to describe the retinal changes in some patients with advanced Bright's disease that were characterized by papilledema, ill-defined white exudates, a macular star, and linear hemorrhages. Volhard noted that the appearance of albuminuric retinitis was often the first sign heralding a transition from the benign to the malignant type of nephrosclerosis (12,14).

In 1928, Keith et al. (16) coined the term *malignant hypertension* to describe a clinical syndrome characterized by severe hypertension with arteriolopathy manifested by papilledema, hemorrhages, and exudates (grade IV retinopathy). Virtually all of their patients died within 1 year of various combinations of brain, heart, and kidney failure. Interesting to note, at the time of presentation with severe hypertension and grade IV retinopathy, the majority of these patients had normal or only mildly impaired renal function.

By 1935, the concept had emerged that the clinical syndrome of malignant hypertension was not due to a single etiology but to a variety of different etiologies. Derow and Altschule (17) pointed out that malignant hypertension occurred in association with essential hypertension as well as secondary forms of hypertension such as chronic glomeulonephritis, chronic pyelonephritis, Cushing's syndrome, pheochromocytoma, renal artery stenosis, and polyarteritis nodosa.

Over the ensuing decades it was recognized that regardless of the underlying etiology of malignant hypertension, the relentless progression of the disease could be slowed or even reversed by reduction of blood pressure with any one of a number of treatments including sympathectomy (18,19), pyrogens (20), rice diet (21), excision of a unilaterally diseased kidney, adrenal resection in Cushing's syndrome (22), excision of a pheochromocytoma (23), and a variety of drugs that lower arterial pressure (24).

Untreated Prognosis and Natural History

A review of the survival statistics in the era before effective antihypertensive drugs became available reveals the reason why this disorder was called "malignant" hypertension. Keith et al. (5,16) described 81 patients with grade III or grade IV retinopathy in whom initial renal involvement was mild. Even in the absence of significant nephropathy, the short-term prognosis was grave. Their classic mortality curve is shown in Figure 56-1. They reported 1-year death rates in hypertensive patients catagorized on the basis of initial funduscopic findings. In group I (mild arteriolar narrowing or sclerosis of retinal vessels), the 1-year death rate was 10%; in group II (moderate sclerosis with increased light reflex and arteriovenous compression or localized arteriolar narrowing), 12%; in group III (retinal hemorrhages and exudates), 35%; and in group IV (hemorrhages and

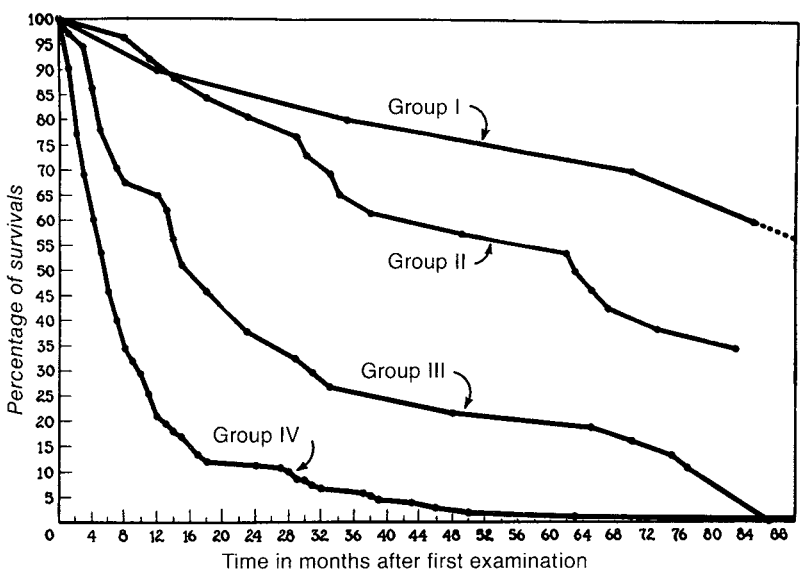

FIGURE 56-1. Survival curves based on the Keith and Wagener grade of hypertensive retinopathy. (From: Keith NM, Wagener HP, Barker NW. Some different types of essential hypertension: their course and prognosis. *Am J Med Sci* 1939;197:332, with permission.)

exudates plus papilledema), 80% 1-year mortality. Many other series of untreated hypertensive patients with severe retinopathy confirmed this dismal prognosis (25–29). The reported causes of death in untreated patients with malignant hypertension were similar in most series. Uremia was the most common cause of death, followed by congestive heart failure and cerebrovascular accident. Ellis (28) outlined four different clinical courses in patients with severe hypertension and hypertensive neuroretinopathy. In the *cerebral* type, patients died due to intracerebral hemorrhage or hypertensive encephalopathy. In the *cardiac* type, severe congestive heart failure with attacks of acute pulmonary edema dominated the clinical picture. With the *renal* type, death was the result of terminal uremia. In the combined type, patients died with manifestations of both congestive heart failure and uremia. Each type showed evidence of widespread vasculopathy and had a rapidly fatal course. In these series, the most important prognostic factor in untreated patients with malignant hypertension was the level of renal function at the time of diagnosis (25,26). Thus, the majority of untreated patients with malignant hypertension can be expected to die from uremia within 1 year. Congestive heart failure is often present. Some patients succumb earlier due to hypertensive encephalopathy or intracerebral hemorrhage at a time when renal involvement may not be pronounced.

Etiologies of Malignant Hypertension

Hypertension of virtually any etiology can enter a malignant phase (Table 56-2). Thus, malignant hypertension is not a single disease entity but rather a syndrome in which hypertension can be either primary (essential) or secondary to one of any number of different etiologies (17). Moreover, in the individual patient with malignant hypertension, on clinical grounds it is often difficult to distinguish whether the underlying hypertension is primary or secondary.

Malignant hypertension usually develops in patients with preexisting, poorly controlled, or undiagnosed hypertension. However, occasional patients have been described who experience an abrupt onset of so-called *de novo* malignant hypertension without a preceding phase of benign hypertension (27,29). The presence of de novo malignant hypertension almost always indicates an underlying secondary cause of hypertension (27).

TABLE 56-2

ETIOLOGIES OF MALIGNANT HYPERTENSION

Primary (essential) malignant hypertension[a]
Secondary malignant hypertension
Chronic kidney disease
Chronic glomerulonephritis[a]
Chronic pyelonephritis[a]
Analgesic nephropathy[a]
Immunoglobulin A nephropathy[a]
Acute glomerulonephritis
Radiation nephritis
Ask-Upmark kidney
Renovascular hypertension[a]
Oral contraceptives
Renal cholesterol embolization
Scleroderma renal crisis
Antiphospholipid (anticardiolipin) antibody syndrome
Chronic lead poisoning
Endocrine hypertension
Pheochromocytoma
Aldosterone-producing adenoma
Cushing's syndrome
Congenital adrenal hyperplasia

[a] Most common underlying etiologies.

Primary (Essential) Malignant Hypertension

In the era prior to the introduction of antihypertensive drugs, malignant hypertension evolved from underlying essential hypertension in more than 50% of patients (27,30). However, more recent series found a lower incidence of primary malignant hypertension, most likely reflecting prevention of malignant hypertension through effective control of blood pressure among patients with essential hypertension (31,32). In a series of patients collected between 1979 and 1985, primary malignant hypertension was found in only 20% (32). This observation may not apply to black patients, because among blacks, essential hypertension continues to represent the most common underlying etiology of malignant hypertension (33–35). Essential hypertension appears to be a rare cause of malignant hypertension in children. Secondary causes of hypertension such as chronic pyelonephritis, chronic glomerulonephritis, and renovascular hypertension are much more common in this younger age group (36).

Secondary Malignant Hypertension

The most common secondary cause of malignant hypertension is primary renal parenchymal disease. *Chronic glomerulonephritis* was reported to underlie the development of malignant hypertension in up to 20% of patients (30,32). Unless a history of an acute nephritic episode or long-standing hematuria or proteinuria is available, the underlying glomerulonephritis may be apparent only if a renal biopsy is performed (32). Recently, immunoglobulin A (IgA) nephropathy was reported as a frequent cause of malignant hypertension in series from Spain and Australia (32,37,38). In one series of 66 patients with IgA nephropathy, malignant hypertension developed in 10% (38).

Chronic pyelonephritis was reported as a cause of malignant hypertension in 9% to 16% of patients in recent series (31,32). In children, chronic atrophic pyelonephritis is the most frequent cause of malignant hypertension (39,40). Children with reflux nephropathy causing chronic atrophic pyelonephritis often present with either hypertensive encephalopathy or malignant hypertension (40).

In Australia, malignant hypertension complicates up to 7% of cases of *analgesic nephropathy* (41). Transient malignant hypertension responsive to volume expansion, an entity that is rare with other causes of malignant hypertension, can occur in the setting of analgesic nephropathy (41). It has been suggested that the salt-wasting state caused by tubulomedullary dysfunction contributes to the pathogenesis of malignant hypertension by causing severe volume depletion with activation of the renin-angiotensin axis (42).

Malignant hypertension is both an early and a late complication of *radiation nephritis* (25,44). In a series of patients with seminoma treated by radiotherapy to the posterior lymph nodes, acute radiation nephritis occurred in 13 patients with a latent period of 6 to 12 months. This was characterized by hypertension, anemia, albuminuria, and renal insufficiency. Hypertensive neuroretinopathy (striate hemorrhages, cotton-wool spots, and papilledema), indicating the development of malignant hypertension, occurred in five of these patients, three of whom died as a consequence of congestive heart failure, hypertensive encephalopathy, and uremia (44). In some patients, malignant hypertension developed as a late complication of radiotherapy with a latent period of 1.5 to 11.0 years, despite the absence of a history of prior acute radiation nephritis (44).

Congenital unilateral renal hypoplasia (Ask-Upmark kidney) is a rare cause of malignant hypertension in children and

adolescents. This entity is characterized by unilateral renal hypoplasia with an enlarged and deformed renal pelvis that has one or more recesses that end blindly near the surface of the kidney. There is debate as to whether this represents a distinct clinicopathologic entity (43), or is simply the result of chronic pyelonephritis in a congenitally hypoplastic kidney (45).

Renovascular hypertension due to either fibromuscular dysplasia or atherosclerotic renal artery stenosis is a well-recognized cause of malignant hypertension. Its frequency, however, varies in different series. Some series in adult patients reported only a 3% to 4% incidence of underlying renovascular hypertension (25,27). A 10% incidence of renal artery stenosis was reported among children with severe hypertension (40). In contrast to these series, others found a very high incidence of underlying renovascular disease among patients with malignant hypertension (46). In a series of 123 patients with malignant hypertension, renovascular hypertension was found in 43% of white patients and 7% of black patients. The prevalence of renovascular hypertension was the same when the group with hemorrhages and exudates without papilledema was analyzed separately from the group with papilledema (46).

Although hypertension is usually mild to moderate in patients with *polyarteritis nodosa,* malignant hypertension has been reported (47). In polyarteritis, even in normotensive individuals, lesions indistinguishable from primary malignant nephrosclerosis may be seen in the interlobular arteries (proliferative endarteritis) and afferent arterioles (fibrinoid necrosis). However, the finding of healed and active lesions in larger medium-sized muscular arteries of the kidney (arcuate and larger arteries), mesentery, lungs, pancreas, and adrenals is unusual in primary malignant hypertension and suggests the diagnosis of polyarteritis nodosa (30).

In women of childbearing age, *oral contraceptives* may cause malignant hypertension (48–49). Some of the reported patients were normotensive prior to the initiation of oral contraceptives, although several patients had gestational hypertension during a prior pregnancy. In the absence of underlying renal disease, discontinuation of oral contraceptives is associated with an excellent long-term prognosis (49). However, a more recent series of women of childbearing age with malignant hypertension suggests that oral contraceptive use was not a common underlying etiology; instead a past history of pregnancy-induced hypertension was the most important risk factor (50).

Severe hypertension, which can enter the malignant phase, is a frequent complication of *atheroembolic renal disease* (cholesterol embolization) (51–54). In patients with severe aortic atherosclerotic disease undergoing aortic angiography, cardiac catheterization, or vascular surgery, evidence of cholesterol embolization may develop immediately after the procedure, with lower extremity livedo reticularis and purple toes, abdominal pain, eosinophilia, eosinophiluria, hypocomplementemia, and acute renal failure. Severe benign or malignant hypertension may develop acutely. Alternatively, the patient may present with malignant hypertension weeks to months after the inciting procedure, at a time when clinical signs of cholesterol embolization have entirely resolved.

Scleroderma renal crisis is the most acute and life-threatening manifestation of progressive systemic sclerosis. It is characterized by severe hypertension (sometimes malignant) with rapidly progressive renal failure (55,56). In one large series, scleroderma renal crisis occurred in 7% of white patients and 21% of black patients with progressive systemic sclerosis (57). The renal histology in scleroderma renal crisis is often virtually indistinguishable from that of primary malignant nephrosclerosis (58). However, in progressive systemic sclerosis, involvement of the renal vasculature, with proliferative endarteritis involving the interlobular arteries and fibrinoid necrosis of the afferent arterioles, may be a primary event that precedes either hypertension or renal insufficiency (58). The renal ischemia that results from these lesions causes hypertension through activation of the renin–angiotensin system, leading to a vicious cycle of severe hypertension and renal ischemic injury. Scleroderma renal crisis was once a uniformly fatal complication of progressive systemic sclerosis. With the introduction of angiotensin-converting inhibitors as treatment, outcomes have improved significantly, though 39% to 50% of patients with scleroderma renal crisis continue to have poor outcomes, including permanent dialysis and death (56).

Patients with *antiphospholipid (anticardiolipin) antibody syndrome,* either primary or secondary to systemic lupus erythematosus, can develop malignant hypertension with renal insufficiency due to thrombotic microangiopathy even in the absence of overt lupus nephritis (59).

Malignant hypertension rarely complicates the course of immunoglobulin G (IgG) myeloma. Although the pathogenesis is not known, hyperviscosity has been implicated (60). Malignant hypertension may also develop in patients with immunotactoid glomerulopathy (Nolan, unpublished observation).

Patients with *chronic lead poisoning* can develop severe hypertension and the neuroretinopathy typical of malignant hypertension (17).

A number of endocrine disorders occasionally lead to secondary malignant hypertension. Malignant hypertension is a rare complication of *pheochromocytoma* (61). Although malignant hypertension secondary to *aldosterone-producing adenoma* is rare, occasional cases have been described (62). However, a diagnosis of primary hyperaldosteronism must be made with caution in patients with a history of malignant hypertension. Following successful treatment of malignant hypertension, plasma renin activity (PRA) rapidly returns to normal, whereas aldosterone secretion may remain elevated for up to a year. This observation has been attributed to persistent adrenal hyperplasia due to long-standing hyperreninemia with disordered feedback control (tertiary hyperaldosteronism) (63). During this period, hypokalemia, metabolic alkalosis, and hyperaldosteronism may persist, despite suppressed PRA, thereby mimicking primary hyperaldosteronism. These patients with tertiary hyperaldosteronism were found to have bilateral nodular adrenal hyperplasia at surgery (64,65). Although adrenalectomy alleviates hypokalemia in this setting, there is no improvement in blood pressure (64,65).

Cushing's syndrome with bilateral adrenal hyperplasia is most often associated with benign hypertension, although occasional cases of malignant hypertension have been reported (25,66). *Congenital adrenal hyperplasia* in patients with 11 β-hydroxylase deficiency (67) or 17 α-hydroxylase deficiency (68) can cause malignant hypertension.

Epidemiology of Malignant Hypertension

Incidence

Although malignant hypertension is often a complication of preexisting hypertension, the risk of its development in hypertensive patients is difficult to estimate. In early series the incidence of malignant hypertension among hypertensive patients was 1% to 7% (25,69). In the era of effective antihypertensive therapy for benign hypertension, the incidence of malignant hypertension appears to have declined to some extent. A review of death certificates in New York City between 1958 and 1974 revealed that the overall mortality due to malignant hypertension had declined by 78% from 2.25 deaths to 0.48 deaths/100,000 population/year (70). Although some of the decreased mortality was probably due to successful treatment of patients with malignant hypertension with antihypertensive drugs and dialysis, the authors speculated that the

overall incidence of malignant hypertension had declined to less than 1% due to successful treatment of benign hypertension. However, despite recent advances in the treatment of essential hypertension, malignant hypertension is clearly not a disease that has vanished. In the United States, during the period from 1983 to 1992, the number of hospital admissions with malignant hypertension or accelerated hypertension as the primary diagnosis (International Classifcation of Diseases, *ICD-9* Code 401.0) doubled from approximately 16,000 to 32,000. Moreover, the number of admissions in which one of these conditions was listed as a diagnosis tripled from approximately 23,000 to 75,000 (2). Reported experience in a multiracial population in England indicates that malignant hypertension is still common with a small proportion of hypertensive patients presenting with malignant hypertension each year (71). The incidence rate of malignant hypertension for the entire population was approximately one to two cases/100,000/year. Moreover, the incidence rate was not changed over the 24-year period from 1970 to 1993. Thus, despite the plethora of drugs available for the treatment of hypertension, failure to identify or adequately treat hypertension remains problematic and malignant hypertension undoubtedly will remain a clinical challenge for the foreseeable future (72,73).

Age

Malignant hypertension occurs more frequently in younger subjects. The mean age of patients with malignant hypertension ranges from 40 to 50 years, with 57% of patients between 30 and 50 years old (27). No difference has been found in the age at onset in men compared to women or whites compared to blacks (25–27,29,74). Pickering (45) suggests that the age dependency of malignant hypertension could be related to the increased frequency of secondary, more severe forms of hypertension in the young. Alternatively, it is possible that hypertension in patients destined to enter the malignant phase may be more rapidly progressive from the onset, so that the disease would be expected to occur predominantly in younger patients. Malignant hypertension is a rare development in patients beyond the age of 65 (27). The declining incidence of malignant hypertension in patients with essential hypertension relative to age is in marked contrast to the overall incidence of benign hypertension, which reaches a peak in the eighth decade (27). Patients over age 60 with malignant hypertension usually have underlying renovascular hypertension or primary renal parenchymal disease (75).

Gender

In most series of patients with malignant hypertension, males predominate over females by as much as 2 to 1 (25,26,29,30, 69,74).

Race

Blacks have an increased incidence of essential hypertension compared to whites. Moreover, several studies demonstrate that blacks with essential hypertension also have an increased risk of developing malignant hypertension. In a population in which 31% of all hypertensive patients were black, 46% of 200 patients with malignant hypertension were found to be black (29). In a study of 135 pairs of black and white hypertensive patients matched for age and gender, 4.4% of the black patients had retinopathy consistent with malignant hypertension, whereas only 0.74% of the white patients had these funduscopic findings (76). The increased frequency of malignant hypertension among blacks may be due to the fact that they presented later in the course of essential hypertension, that antihypertensive therapy in blacks was inadequate to prevent the development of malignant hypertension, or that essential hypertension may be a more aggressive and likely to enter the malignant phase in blacks than whites (77).

Preceding Duration of Benign Hypertension

Although there are occasional case reports in which the malignant phase appears to begin *de novo*, the majority of patients show evidence of a variable period of preceding benign hypertension before the onset of malignant hypertension. Among 77 patients with malignant hypertension, the documented duration of benign hypertension was 0 to 6 months in 4%, 6 months to 1 year in 10%, 1 to 2 years in 12%, 2 to 4 years in 23%, 4 to 6 years in 16%, 6 to 8 years in 17%, and 8 to 10 years in 4%. Only 14% had benign hypertension for more than 10 years prior to the onset of the malignant phase (25).

Smoking and Alcohol as Risk Factors

The risk of malignant hypertension is higher among hypertensive patients who smoke (78–80). In one series, 82% of the patients with malignant hypertension were smokers versus 50% of inpatients and 43% of outpatients with benign hypertension and 52% of normotensive control subjects (80). The relative risk for developing malignant hypertension was five times higher in hypertensive patients who smoked. Among patients with malignant hypertension, at initial presentation, renal insufficiency was also more common among smokers. The mean serum creatinine concentration for nonsmokers was 1.2 mg/dL, compared to 2.5 mg/dL for smokers. Moreover, of the 18 patients with a serum creatinine value over 2.8 mg/dL, 17 were smokers and one was a former smoker (80).

In contrast to the findings with regard to smoking risk, no significant difference has been found for the prevalence or quantity of alcohol consumption in groups of patients with benign or malignant hypertension (79).

Clinical Features of Malignant Hypertension

The clinical features of untreated malignant hypertension as outlined by Volhard and Fahr in 1914 (12) are still valid today: (a) elevation of diastolic blood pressure, usually fixed and severe; (b) funduscopic changes of hypertensive neuroretinopathy with striate hemorrhages, cotton-wool spots, and papilledema; (c) renal insufficiency; (d) rapid progression to a fatal outcome, usually due to uremia if inadequately treated; and (e) renal histology demonstrating malignant nephrosclerosis with fibrinoid necrosis of afferent arterioles and proliferative endarteritis of interlobular arteries.

Unless hypertensive neuroretinopathy is present, malignant hypertension cannot be diagnosed regardless of the height of the arterial blood pressure (4). However, the other clinical features need not be present initially to substantiate a diagnosis of malignant hypertension. There is no critical level of blood pressure that defines the presence of malignant hypertension. An acute increase in blood pressure in previously normotensive individuals can precipitate the malignant phase at a diastolic blood pressure as low as 100 to 110 mm Hg. Conversely, very high diastolic blood pressures may persist for many years in patients with essential hypertension without the development of malignant hypertension (81).

With untreated malignant hypertension, severe renal impairment inevitably occurs, although there may be minimal renal involvement at the time of presentation. In this regard, in patients dying early in the course of malignant hypertension due to cerebrovascular accident or congestive heart failure, histologic features of malignant neuphrosclerosis may be absent.

Some authors have distinguished *accelerated hypertension* (hemorrhages and cotton-wool spots) from *malignant hypertension* (hemorrhages, cotton-wool spots, and papilledema).

However, since the finding of striate hemorrhages and cotton-wool spots has the same prognostic significance whether or not papilledema is present (6,7), it has been recommended that accelerated hypertension and malignant hypertension be regarded as synonymous terms for a clinical syndrome in which there is widespread hypertension-induced acute arteriolar injury. The World Health Organization has recommended that the term *malignant hypertension* be used to describe this disease process (4). Use of the term *accelerated hypertension* should probably be abandoned because it is now commonly used to describe patients who have increasingly severe or resistant hypertension independent of the funduscopic findings that characterize true accelerated or malignant hypertension.

Presenting Symptoms

The most common presenting complaints in patients with malignant hypertension are headache, blurred vision, and weight loss (25–27). Less common presenting symptoms include dyspnea, fatigue, malaise, gastrointestinal complaints (nausea, vomiting, epigastric pain), polyuria, nocturia, and gross hematuria (26,27). In many series, the onset of symptoms was noted to be remarkably sudden, such that it could often be dated precisely (26,27,29). In contrast, an "asymptomatic" presentation of malignant hypertension is not uncommon, especially in young black males who deny any prior symptoms when they present in the end-stage of the hypertensive process with florid failure of the brain, heart, and kidneys.

Headache is the most frequent presenting complaint in patients with malignant hypertension. Unfortunately, headache is a nonspecific finding that also occurs frequently in patients with benign hypertension. Nonetheless, when patients with severe hypertension experience headaches of recent onset or the intensification of an existing headache pattern, malignant hypertension should be excluded (25).

In one large series, visual symptoms were present at initial diagnosis in 76% of patients and visual problems eventually developed in 90% (26). The most common complaints were blurred vision and decreased visual acuity. Sudden blindness occurred in 14% of patients. Scotoma, diplopia, and hemianopsia were also reported.

Weight loss is a very common symptom early in the course of malignant hypertension, and often occurs before the onset of anorexia or uremia (25,29,81). In many patients, at least a portion of the weight loss can be attributed to volume depletion resulting from a spontaneous natriuresis with the onset of malignant hypertension (42,82,83).

Level of Blood Pressure

There is apparently no absolute level of blood pressure above which malignant hypertension invariably occurs. In most series of patients with malignant hypertension, the average diastolic blood pressure is higher than 120 to 130 mm Hg (26,27). However, two series found considerable overlap of blood pressure levels in patients with benign and malignant hypertension (25,84) (Fig. 56-2).

Funduscopic Manifestations

Examination of the ocular fundus is of great importance in the assessment of patients with severe hypertension, especially with regard to prognosis (84–88). The description by Keith et al. (16) of the prognosis of hypertensive patients based on a grading system for hypertensive retinopathy was the landmark study in this field. They graded retinal findings in untreated hypertensive patients as follows: grade I—mild narrowing or sclerosis of arterioles; grade II—moderate sclerosis with an increased light reflex and arteriovenous compression; grade III—retinal hemorrhages and exudates; and grade IV—the findings in grade III plus papilledema. The presence of papilledema was associated with the worst prognosis and became synonymous with the term *malignant hypertension*. In subsequent years, the term *accelerated hypertension* was adopted to describe patients with grade III retinopathy (89).

The clinical utility of the Keith and Wagener classification, although widely accepted, has been questioned (6,57,87). It

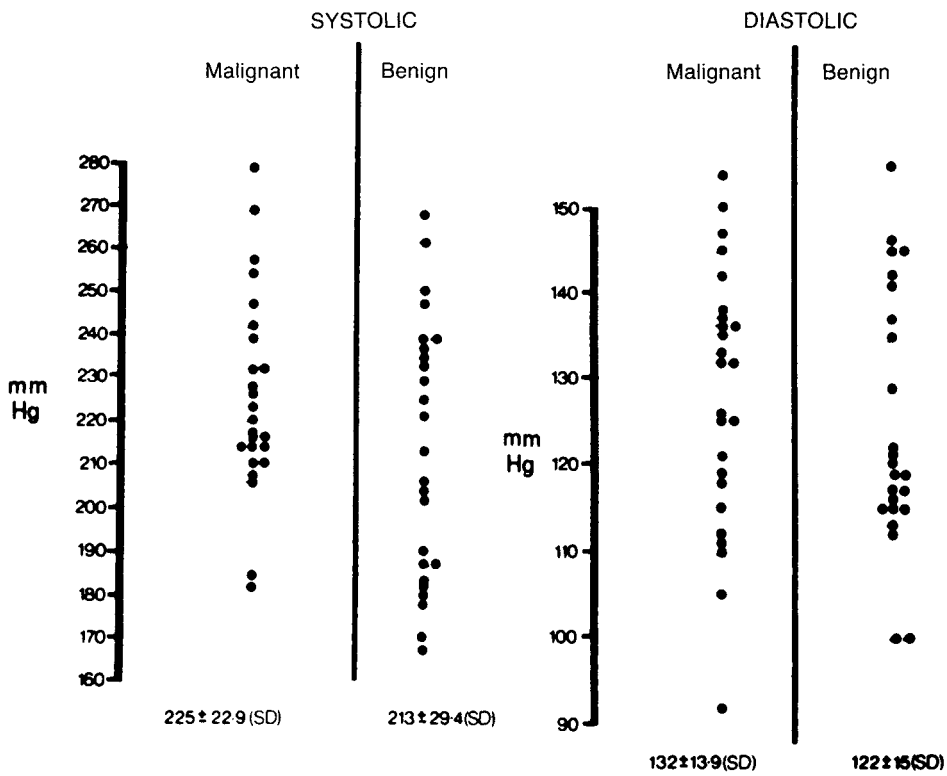

FIGURE 56-2. Systolic and diastolic blood pressures in patients with malignant hypertension compared with age- and gender-matched patients with severe benign hypertension. (From: Kincaid-Smith P. Malignant hypertension: mechanisms and management. *Pharmacol Ther* 1980;9:245, with permission.)

is extremely difficult to quantitate arteriolar narrowing (87). Moreover, there is observer bias such that patients with mild hypertension and questionable narrowing are inevitably placed in this group (87). Thus, the finding of grade I changes is of limited usefulness. There is also great interobserver variability with regard to the definition of arteriovenous crossing changes (90). Another objection to the Keith and Wagener classification is that it does not clearly distinguish between the retinal changes of benign and malignant hypertension (45). For example, the clinical significance of a large, ill-defined white exudate (cotton-wool spot) appearing in the fundus of a young man with severe hypertension is quite different from the clinical significance of a sharply defined, glistening, hard exudate in a 60-year-old patient with moderate hypertension. In the first example, the cotton-wool spot and the clinical circumstances suggest the onset of malignant hypertension. In the older patient, the retinal picture is consistent with retinal arteriosclerosis characteristic of benign hypertension (45). The therapeutic and prognostic implications of these two types of exudate are clearly different, although both would be assigned to grade III in the Keith and Wagener classification.

A number of authorities have recommended abandonment of the Keith and Wagener classification in favor of the hypertensive retinopathy classification initially proposed by Fishberg and Oppenheimer (86). This classification draws a distinction between *retinal arteriosclerosis with arteriosclerotic retinopathy,* which is characteristic of benign hypertension, and *hypertensive neuroretinopathy,* which defines the presence of malignant hypertension (Table 56-3). In essence, two different types of retinal disease occur in patients with hypertension: one that reflects changes induced by arteriolar narrowing (retinal arteriosclerosis); and one that represents acute retinal vascular injury induced by severe hypertension (hypertensive neuroretinopathy).

Retinal arteriosclerosis with or without arteriosclerotic retinopathy is seen in patients with long-standing benign hypertension from either primary or secondary causes. Retinal arteriosclerosis (arteriolosclerosis) is characterized histologically by the accumulation of hyaline material in arterioles. In the early stages, the material is deposited in the intima beneath the endothelium, while in older lesions deposits extend into the media and ultimately involve the entire vessel wall. Funduscopic changes reflecting retinal arteriosclerosis include irregularity of the lumen and focal narrowing, arteriovenous crossing changes, broadening of the light reflex, copper or silver wiring, perivasculitis (parallel white lines around blood column), and generalized arteriolar narrowing. Arteriosclerotic retinopathy, which results from this arteriosclerotic process, is manifested by the presence of hemorrhages and hard exudates. The hemorrhages are usually solitary, round or oval, and confined to the periphery of the fundus. They are caused by venous or arterial occlusion (87). Hard exudates may appear as multiple small white dots that give a powdery appearance to the retina, or they may appear as large glistening spots that are sharply defined from the adjacent retina. Arteriosclerotic retinopathy can also cause localized areas of retinal edema and hemorrhage due to occlusion of small branch veins. However, the principal findings of hypertensive neuroretinopathy, namely, striate hemorrhages, cotton-wool spots, and papilledema, are absent (Table 56-3).

TABLE 56-3

RETINAL CHANGES IN HYPERTENSION

Retinal arteriosclerosis and arteriosclerotic retinopathy
Arteriolar narrowing (diffuse)
Focal arteriolar narrowing
Arteriovenous crossing changes
Broadening of the light reflex
Copper or silver wiring
Perivasculitis
Solitary round hemorrhages
Hard exudates
Central or branch venous occlusion
Hypertensive neuroretinopathy
Generalized arteriolar narrowing
Striate (flame-shaped) hemorrhages[a]
Cotton-wool spots (soft exudates)[a]
Bilateral papilledema[a]
Macular star

[a]Features that distinguish hypertensive neuroretinopathy (characteristic of malignant hypertension) from retinal arteriosclerosis (characteristic of benign hypertension).

The finding of retinal arteriosclerosis in hypertensive patients usually does not imply a poor prognosis. Even patients with severe arteriosclerotic retinopathy may live for many years before the development of morbid events due to coronary artery disease, congestive heart failure, or cerebrovascular accident. Furthermore, the presence of retinal arteriosclerosis in patients with essential hypertension is typically not associated with significant renal impairment. This observation is in sharp contrast to patients with hypertensive neuroretinopathy in whom renal impairment, if not already present, is imminent without treatment.

Furthermore, the finding of retinal arteriosclerosis in hypertensive patients is of no prognostic significance with regard to the risks of coronary atherosclerosis and cerebrovascular disease (85,87,88). As the arteries visualized with the ophthalmoscope are technically arterioles with a diameter less than 0.1 mm (87), hyaline arteriolosclerosis of the retinal vessels is a process that is entirely different from the atherosclerotic process that can affect larger muscular arteries. Thus, the finding of retinal arteriosclerotic changes is not predictive of the presence or absence of atherosclerotic disease of the coronary or cerebral vessels or other major arterial branches of the aorta (85). The prognostic significance of retinal changes in benign hypertension has also been questioned on the basis of the observation that normotensive control subjects between the ages of 40 and 60 have a high incidence of retinal arteriosclerosis, presumably reflecting age-related vascular changes (91).

In a study designed to assess the usefulness of ophthalmoscopy in mild to moderate hypertension, 25 patients with untreated essential hypertension were evaluated with direct ophthalmoscopy, assessment of fundus photographs, ambulatory blood pressure monitoring, estimation of left ventricular mass by electrocardiography and two-dimensional echocardiography, and measurement of urinary microalbumin excretion. No statistical relation was found between either clinic or ambulatory blood pressure readings and the severity of retinal arteriosclerosis as defined by the presence of arteriolar narrowing or arteriovenous crossing changes. Moreover, there was no independent relationship between retinal changes and age, measures of left ventricular mass, creatinine clearance, or urinary microalbumin excretion. Thus, the finding of retinal arteriosclerosis has little clinical utility in the evaluation of patients with mild to moderate hypertension (90).

The lack of clinical significance of retinal arteriosclerosis in hypertensive patients contrasts markedly with the importance and prognostic significance of the finding of hypertensive neuroretinopathy. The appearance of striate hemorrhages and cotton-wool spots with or without papilledema closely parallels the development of severe arteriolar damage (fibrinoid necrosis and proliferative endarteritis) in the circulation of other organs including the brain and kidneys. Hypertensive

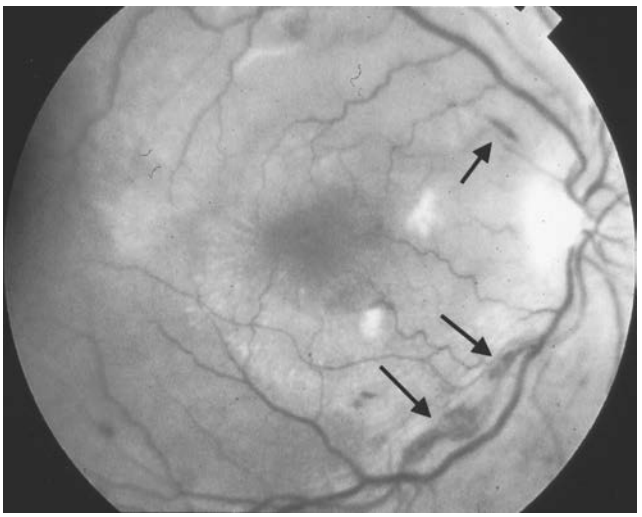

FIGURE 56-3. Striate hemorrhages (*arrows*) in the fundus of a 48-year-old white female with secondary malignant hypertension due to underlying immunoglobulin A nephropathy.

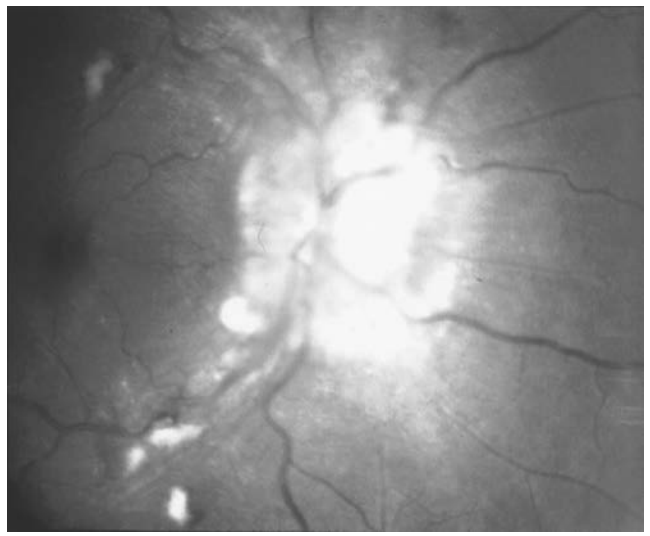

FIGURE 56-5. Papilledema in the fundus of an 18-year-old African American male with primary malignant hypertension. Cotton-wool spots are also apparent. This asymptomatic patient was incidentally noted to have severe hypertension during a routine dental examination.

neuroretinopathy is the clinical *sine qua non* of malignant hypertension and therefore signifies a far more ominous prognosis than does the finding of retinal arteriosclerosis in benign hypertension.

The appearance of small striate hemorrhages is often the first sign that malignant hypertension has developed. These hemorrhages are linear or flame-shaped and are most commonly observed in a radial arrangement around the optic disc (85,87) (Fig. 56-3). They arise from superficial capillaries in the nerve fiber bundles, which have high intravascular pressure because they are perfused directly by arterioles (85). The hemorrhages extend along nerve fibers parallel to the retinal surface. They often have a frayed distal border due to extravasation between nerve fiber bundles. Even when widespread, hemorrhages are rarely seen lateral to the macula in hypertensive neuroretinopathy. Striate hemorrhages often occur adjacent to cotton-wool spots (Fig. 56-4) and most likely arise from capillary microaneurysms at the margins of the spots. Since hemoglobin absorbs fluorescein, hemorrhages appear black with fluorescein angiography (85). Striate hemorrhages can usually be distinguished from the hemorrhages seen in retinal arteriosclerosis, which are solitary, round, and confined to the periphery of the fundus (87).

Cotton-wool spots are the most characteristic feature of malignant hypertension and are the result of ischemic infarction of nerve fiber bundles caused by arteriolar occlusion. They usually surround the optic disc and most commonly occur within three disc diameters of the optic disc (Figs. 56-4–56-6). Cotton-wool spots begin as grayish-white discoloration of the retina, but within 24 hours they become shiny white with fluffy margins. Red dots may be seen in the bed of the exudate (microaneurysms). Cotton-wool spots are not specific for

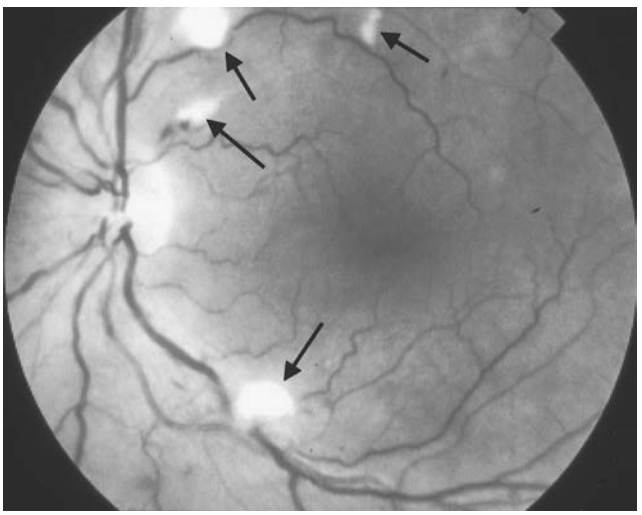

FIGURE 56-4. Cotton-wool spots (*arrows*) in the fundus of a 48-year-old white female with secondary malignant hypertension due to underlying immunoglobulin A nephropathy. Striate hemorrhages are also seen adjacent to some of the cotton-wool spots.

FIGURE 56-6. Full-blown hypertensive neuroretinopathy in fundus of a 30-year-old man with malignant hypertension demonstrating linear (striate) hemorrhages, cotton-wool spots, papilledema, and a star figure at the macula. (Photograph courtesy of Daniel J. Mayer, MD.)

hypertensive neuroretinopathy and can also be seen with diabetic retinopathy, retinal emboli, and central and branch retinal vein occlusion. However, differentiation of these disorders from malignant hypertension is usually not difficult.

Papilledema can occur in patients with hypertensive neuroretinopathy, but it is not invariably present. In malignant hypertension, papilledema is usually accompanied by striate hemorrhages and cotton-wool spots (Figs. 56-5 and 56-6). When papilledema occurs alone, the possibility of a primary intracranial process such as a tumor or cerebrovascular accident should be considered (45). However, lone bilateral papilledema has recently been described as a variant of hypertensive neuroretinopathy (92). Once intracerebral pathology has been excluded, these patients require aggressive treatment just as those with hypertensive neuroretinopathy accompanied by striate hemorrhages and cotton-wool spots.

A star figure at the macula represents hard exudates arranged in a radial fashion from the central fovea (Fig. 56-6). Although a star figure can occasionally be seen in arteriosclerotic retinopathy, it usually develops in conjunction with the florid retinal changes in malignant hypertension. In hypertensive neuroretinopathy the exudates form lines or sheets around the macula rather than the discrete dots around the macula that occur in arteriosclerotic retinopathy (45).

Hypertensive neuroretinopathy almost always precedes clinically apparent damage in other end organs but there are occasional reports of malignant nephrosclerosis appearing before the onset of hypertensive neuroretinopathy (93). It should also be noted that the findings of striate hemorrhages, cotton-wool spots, and papilledema are not specific for malignant hypertension. Funduscopic findings that are indistinguishable from those of hypertensive neuroretinopathy can occur with severe anemia, subacute bacterial endocarditis, systemic lupus erythematosus, polyarteritis, temporal arteritis, and scleroderma (45). In these disorders the retinopathy may develop even in the absence of hypertension. Central retinal vein occlusion can also mimic hypertensive neuroretinopathy but is usually unilateral, whereas hypertensive neuroretinopathy is almost always bilateral.

Severe hypertension can also affect the choroidal as well as the retinal circulation. Hypertensive choroidopathy can occur with malignant hypertension and is manifested by lesions known as acute *Elschnig's spots,* which are white areas of retinal pigment epithelial necrosis with overlying localized serous detachments of the retina (94) (Fig. 56-7). The serous retinal detachments may vary from one-third to six disc diameters. Fluorescein angiography reveals staining of the damaged pigment epithelium and leakage into the subretinal space (94). Although most patients with this hypertensive choroidopathy also have typical changes of hypertensive neuroretinopathy with striate hemorrhages and cotton-wool spots, if the elevation of blood pressure is relatively sudden, the changes of hypertensive choroidopathy may predominate (94).

As stated earlier, papilledema should not be regarded as an essential requirement for the diagnosis of malignant hypertension. By life table analysis, the 10-year survival rate for hypertensive patients was 46% in patients with hemorrhages and exudates and 48% when papilledema was also present (7). The lack of association between papilledema and the length of survival was confirmed using the Cox's proportional hazards model, which revealed associations between survival and age, smoking habit, initial serum creatinine concentration, and the level of blood pressure control achieved with therapy. No association was found with papilledema. When other covariates were controlled simultaneously, no association was found between the presence of papilledema and survival (Fig. 56-8). The failure of recent studies to find the previously reported difference in survival between grade III and grade IV retinopathy (5) may be due to the fact that the earlier study involved untreated patients. Papilledema may be associated with a worse prognosis only when the hypertension is untreated or ineffectively treated (7).

There is no evidence to indicate that the apparent severity of hypertensive neuroretinopathy is predictive of a more severe hypertensive vasculopathy or more advanced end-organ

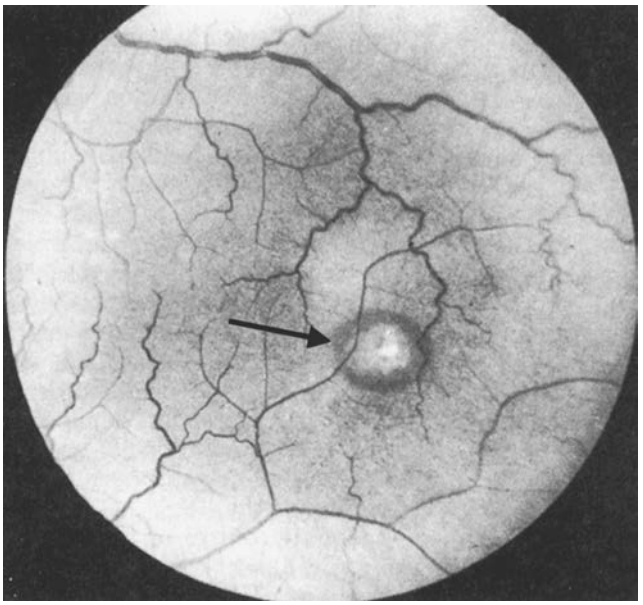

FIGURE 56-7. Hypertensive choroidopathy in malignant hypertension demonstrating focal serous detachment of the sensory retina with a whitish lesion at the level of the retinal pigment epithelium (acute Elschnig's spot). (From: de Venecia G, Jampol LM. The eye in accelerated hypertension: II. Localized serous detachments of the retina in patients. *Arch Ophthalmol* 1984;102:68, © 1984, American Medical Association, with permission.)

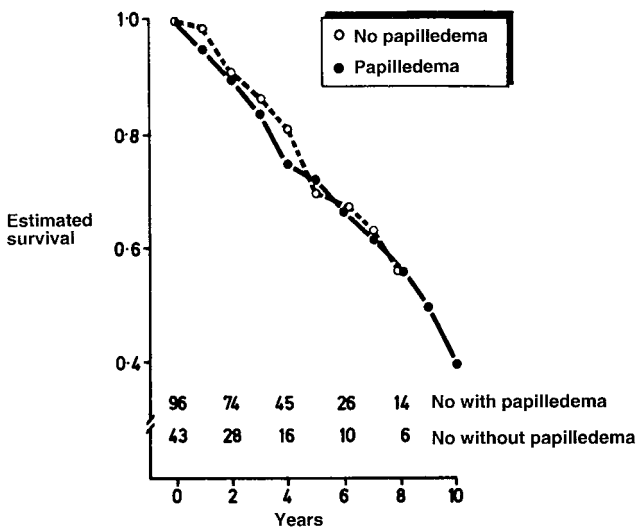

FIGURE 56-8. Relation between papilledema and survival in 139 hypertensive patients with bilateral retinal hemorrhages and exudates after controlling for age, gender, smoking habit, initial serum creatinine concentration, and initial and achieved blood pressure by multivariate analysis. Failure of papilledema to influence prognosis was confirmed by likelihood ratio test ($X = 0.89$, 1 df, $p = 0.34$). (From: McGregor E, et al. Retinal changes in malignant hypertension. *Br Med J* 1986;292:233, with permission.)

destruction. Papilledema is not always present even when there is severe malignant nephrosclerosis presenting as oliguric acute renal failure. In four series with a total of 25 patients presenting with malignant hypertension and acute renal failure, only 14 patients had papilledema. The other 11 patients had hemorrhages and cotton-wool spots but no papilledema (95–98). This lack of a difference in prognosis for patients with hypertensive neuroretinopathy whether or not it is accompanied by papilledema may be explained by the fact that cotton-wool spots and papilledema share a similar pathogenesis (*vide infra*) (99,100).

The diagnosis of malignant hypertension may be made in the setting of severe hypertension when only a single cotton-wool spot is observed. The approach to the treatment of hypertension in this setting should be just as aggressive as in patients with full-blown hypertensive neuroretinopathy with extensive striate hemorrhages, cotton-wool spots, and papilledema. Since the funduscopic findings in patients with malignant hypertension may sometimes be difficult to visualize, the evaluation of patients with severe hypertension should include a careful ophthalmologic examination after pupillary dilation with 1% tropicamide (101). Because the presence of even subtle hypertensive neuroretinopathy has important therapeutic and prognostic implications, if the retina cannot be adequately visualized, formal ophthalmologic evaluation with indirect ophthalmoscopy should be considered. Furthermore, retinal photographs provide permanent objective documentation of the presence of malignant hypertension.

Renal Manifestations

Malignant hypertension is a progressive systemic vasculopathy in which renal involvement is a secondary and relatively late development. Patients with malignant hypertension may present with a spectrum of renal involvement ranging from minimal albuminuria with normal renal function to ESRD indistinguishable from that seen in patients with primary renal parenchymal disease (25,27,74).

The first sign of renal involvement in malignant hypertension is often the abrupt appearance of proteinuria. About 20% of patients also have painless gross hematuria, while 50% have microhematuria (27). Pickering (45) regards the appearance of blood or more than a trace of protein in the urine of patients with essential hypertension to be an indication of the onset of malignant nephrosclerosis, as significant proteinuria and hematuria are rare in benign nephrosclerosis (27). However, recent reports suggest that nephrotic-range proteinuria can occasionally develop with severe benign nephrosclerosis (102,103).

Quantitation of 24-hour protein excretion in patients with malignant hypertension has revealed less than 2 g in one-third, between 2 and 4 g in one-third, and more than 4 g in one-third of patients (26). The level of protein excretion is of little value in the differentiation of primary (essential) malignant hypertension from malignant hypertension due to secondary causes (25,74).

Hematuria is a very important clinical finding in patients with essential hypertension. In the absence of primary renal parenchymal disease or a urologic source, the onset of hematuria is virtually diagnostic of malignant nephrosclerosis (45). In one series, hematuria was found in 100% of patients with malignant hypertension (74). However, the absence of hematuria does not exclude the diagnosis. Of interest is the fact that red blood cell casts were observed in patients with malignant hypertension who had no evidence of glomerulonephritis at renal biopsy (97).

Pyuria has been demonstrated in 75% of patients with malignant hypertension. However, the presence of pyuria does not differentiate between primary and secondary malignant hypertension (26).

Renal size is variable and depends on the duration of prior benign hypertension. In patients with primary (essential) malignant hypertension, the size of the kidneys may be normal to only slightly reduced. In fact, there may be little reduction in renal size even when patients develop terminal renal failure (25).

The clinical spectrum of renal involvement in malignant hypertension is variable. Four clinical renal syndromes have been described. Progressive subacute deterioration of renal function leading to ESRD occurs in some patients. In patients presenting with malignant hypertension and initially normal renal function, in the absence of adequate treatment, it is common to observe deterioration of renal function with progression to ESRD over a period of weeks to months. The second clinical renal syndrome observed in malignant hypertension is transient deterioration of renal function following the initial control of blood pressure. This well-described entity occurs in patients presenting with mild to moderate renal impairment. In the third clinical renal syndrome, patients with malignant hypertension present with established renal failure. The close similarity between the terminal stage of primary malignant nephrosclerosis and chronic nephritis with superimposed malignant hypertension has long been recognized. In this regard, it may not be possible to ascertain whether a patient presenting with severe hypertension, hypertensive neuroretinopathy, and renal failure has primary or secondary malignant hypertension (45). While a history of an acute nephritic episode or long-standing proteinuria or hematuria may suggest underlying primary renal parenchymal disease, the distinction between primary malignant nephrosclerosis and chronic nephritis often requires a renal biopsy (45,104). In the fourth clinical renal syndrome, patients with malignant hypertension present with oliguric acute renal failure. Cases of malignant hypertension have been described that were characterized by diastolic blood pressure higher than 130 mm Hg; advanced hypertensive neuroretinopathy; marked weight loss; and with an active urine sediment with proteinuria, hematuria, and red blood cell casts (97,98). Renal size was normal. There was often evidence of microangiopathic hemolytic anemia. Although the initial blood urea nitrogen (BUN) concentration was less than 60 mg/dL, in each case oliguric renal failure occurred and necessitated the initiation of dialysis within a few days of hospitalization. Despite dialytic therapy, the blood pressure was extremely difficult to control and each patient died. Renal histology revealed malignant nephrosclerosis with fibrinoid necrosis and proliferative endarteritis. The glomeruli were normal except for ischemic changes. Multifocal tubular necrosis was present and presumed to be secondary to ischemia. In most of these patients, the diagnosis of malignant hypertension was delayed because the patients were initially considered to have rapidly progressive glomerulonephritis or systemic vasculitis, which was treated with high-dose steroids. The diagnosis of malignant hypertension was not suspected until autopsy revealed malignant nephrosclerosis.

Neurologic Manifestations

Clarke and Murphy (105) detail the neurologic findings among 190 patients with malignant hypertension. Central nervous system involvement was present at some time during the course in 42% of patients. Of the 65 patients for whom a cause of death could be ascertained, 33 had a fatal neurologic event. Of the total deaths, 20% were due to a neurologic cause. Intracerebral hemorrhage occurred in 23 patients. Episodes of focal brain ischemia, presumed due to cerebral thrombosis, occurred in 35 patients. Generalized seizures occurred in 11 patients and focal seizures in 8. Bell's palsy occurred in seven patients. Primary subarachnoid hemorrhage occurred in 4 patients. The incidence of headache was comparable in patients

with and those without neurologic complications. Thus, the presence of headache did not necessarily imply central nervous system involvement. In this series, hypertensive encephalopathy was found in only 1% of patients; however, other series reported a higher incidence (106). The clinical presentation, pathophysiology, and treatment of hypertensive encephalopathy are discussed in detail later in this chapter under Hypertensive Encephalopathy.

The cerebrospinal fluid (CSF) findings in patients with malignant hypertension are variable. Even among patients with papilledema, CSF pressure was greater than 200 mm of water in only 65% (26,105). In contrast, Pickering (107) found that patients with malignant hypertension had higher CSF pressures than did patients with benign hypertension and that there was a direct correlation between the level of blood pressure and the CSF pressure. Blood-stained or xanthochromic fluid was found only in patients with intracerebral or subarachnoid hemorrhage (105). Protein concentration was higher than 60 mg/dL in 69% of patients (range, 11 to 307 mg/dL). No pleocytosis was reported. Although Clarke and Murphy (105) report no complications from lumbar puncture, others report a 12% incidence of complications including severe headache, sudden blindness, coma, and death due to cerebellar herniation (26).

Gastrointestinal Manifestations

The most common gastrointestinal manifestations of malignant hypertension are nonspecific symptoms including nausea, vomiting, and epigastric pain. However, acute pancreatitis has been reported as a rare complication. In a series of 42 patients with malignant hypertension, severe acute pancreatitis that could not be attributed to gallstones or alcohol abuse developed in seven patients (108). All of the patients were black and were on maintenance hemodialysis for renal failure caused by malignant nephrosclerosis. The blood pressure remained poorly controlled while the patients were on dialysis. In another series reporting on the frequency of pancreatitis in a dialysis population, the majority of patients were found to have hypertensive nephrosclerosis as the cause of ESRD (109). It has been proposed that acute pancreatitis occurs with increased frequency in patients with malignant hypertension because of the use of hemodialysis. Although dialysis prevents death from uremia, if the blood pressure remains poorly controlled, hypertensive vasculopathy persists in other organs such as the pancreas. In this setting, the use of heparin for dialysis might lead to this complication by causing hemorrhage in inflamed pancreatic tissue (108).

Patients with malignant hypertension can present with an acute abdomen (110). Abdominal exploration revealed necrotic bowel with involvement of the distal ileum and ascending colon. Pathologic examination revealed fibrinoid necrosis and thrombotic occlusion of the small arteries of the bowel wall. Moreover, malignant hypertension may increase the risk of subsequent development of mesenteric ischemia in patients on chronic hemodialysis (111). Gastrointestinal hemorrhage can occur in patients with malignant hypertension due to hypertension-induced necrotizing mesenteric arteriolitis (112).

Hematologic Manifestations

A variety of hematologic findings have been observed in patients with malignant hypertension. Elevation of the erythrocyte sedimentation rate has been reported (25). The hemoglobin concentration at the time of presentation may correlate with the etiology of the malignant phase. A hemoglobin concentration higher than 12.5 g/dL is more often associated with primary malignant hypertension, while a lower value is more often associated with chronic glomerulonephritis or pyelonephritis (25,26).

There are numerous reports of microangiopathic hemolytic anemia in association with malignant hypertension. In one series of 24 patients with malignant hypertension, 16 were found to have evidence of microangiopathic hemolysis (113). Other significant abnormalities reported with malignant hypertension include thrombocytopenia, increased fibrin degradation products, increased factor VIII levels, increased fibrinogen, and increased urokinase sensitivity consistent with decreased fibrinolysis (114).

Cardiac Manifestations

Congestive heart failure can be a presenting feature of malignant hypertension. Moreover, heart failure, alone or in combination with uremia, was a common cause of death prior to the advent of effective antihypertensive drugs (26,27). Heart failure in patients with malignant hypertension is predominantly left-sided with pulmonary congestion resulting in orthopnea, paroxysmal nocturnal dyspnea, cardiac asthma, and recurrent episodes of acute pulmonary edema. Peripheral venous congestion with dependent edema or hepatic congestion may be minimal or absent even when death results from congestive heart failure. The management of acute pulmonary edema in patients with malignant hypertension is discussed later in this chapter under Acute Hypertensive Heart Failure.

Angina and acute myocardial infarction, though common with long-standing benign hypertension, are uncommon with malignant hypertension (25). Aortic dissection is also rare in patients with malignant hypertension (25).

Abnormalities of the Renin-Angiotensin-Aldosterone Axis

Evidence of activation of the renin-angiotensin-aldosterone axis is present in many, but not all, patients with malignant hypertension (63,115). Among 53 patients with malignant hypertension not secondary to renal artery stenosis, 55% had increased plasma renin activity (PRA) (116). Among 25 patients with malignant hypertension secondary to renal artery stenosis, PRA was consistently elevated (116).

Aldosterone secretion rate has been studied in patients with malignant hypertension (65). There was a marked increase in secretion rate in seven of eight patients with malignant hypertension (papilledema present), and in five of eight patients with accelerated hypertension (retinal hemorrhages without papilledema). The aldosterone secretion rate in these patients was often higher than that seen in patients with aldosterone-producing adenoma. Postmortem examination of the adrenal glands in seven patients with malignant hypertension revealed bilateral areas of focal nodular hyperplasia, especially in the zona glomerulosa (65). The renin-angiotensin axis was evaluated in a series of patients with malignant hypertension; the underlying disease was essential hypertension in 33 cases and chronic glomerulonephritis in 26 cases (117). Plasma renin activity was significantly higher in the group with essential hypertension than in the group with underlying chronic glomerulonephritis and angiotensin receptor blocker therapy induced a significant reduction of blood pressure only in the former group. These finding suggest that the renin-aldosterone system (RAS) plays a significant role in elevating blood pressure in primary malignant hypertension but may be less important in the pathogenesis of secondary malignant hypertension related to glomerulonephritis.

Of interest, in patients with malignant hypertension and elevated PRA and aldosterone secretion rate, there was often a transient period during therapy in which PRA returned to normal yet aldosterone secretion rate remained elevated. This

FIGURE 56-9. Fibrinoid necrosis in a large arteriole (*arrow*). Intimal onionskin formation is also present. (Trichrome stain.) (Photograph courtesy of Steve Guggenheim, MD.)

dissociation often persisted for months. The authors postulate that, with prolonged hyperreninemia, hyperplasia of the zona glomerulosa occurs. While renin levels quickly return to normal with therapy, persistent hyperplasia of the zona glomerulosa and a delay in resetting feedback control mechanisms lead to over secretion of aldosterone despite normal PRA (tertiary hyperaldosteronism) (63).

Electrolyte Abnormalities

Hypokalemic metabolic alkalosis was found in up to 50% of patients with malignant hypertension, presumably reflecting a state of hyperreninemia and secondary hyperaldosteronism (65). After effective therapy, aldosterone hypersecretion can persist long after volume depletion is corrected and renin levels have returned to normal. Thus, the findings of hypokalemia, increased urinary potassium losses, and aldosterone hypersecretion with suppressed PRA may mimic the findings of primary hyperaldosteronism (63).

Hyponatremia is not uncommon in patients with malignant hypertension, particularly when sodium restriction is instituted. Patients with malignant hypertension due to renal artery stenosis occasionally present with the striking hyponatremic hypertensive syndrome (118–120). The characteristic features of this syndrome include severe hypertension, hypertensive neuroretinopathy, polyuria, polydipsia, weight loss, and salt craving. Biochemical changes include hyponatremia, hypokalemia, and low total exchangeable sodium and potassium, with markedly elevated PRA, angiotensin II, aldosterone, and arginine vasopressin (AVP) levels. This syndrome may result from a vicious cycle of volume depletion with further activation of the renin–angiotensin axis as a result of a pressure-induced natriuresis from the contralateral kidney (119).

Pathologic Findings

Renal Pathology

With malignant nephrosclerosis, small pinpoint petechial hemorrhages may be present on the cortical surface, giving the kidney a peculiar flea-bitten appearance. The renal size varies depending on the duration of preexisting benign hypertension or the presence of underlying primary renal parenchymal disease. When terminal renal failure occurs in patients with primary malignant hypertension, the kidneys may be normal in size (81). However, when secondary malignant hypertension is superimposed on primary renal disease, the kidneys may be small.

Fibrinoid necrosis of the afferent arterioles has traditionally been regarded as the hallmark of malignant nephrosclerosis (25,26) (Fig. 56-9). The characteristic finding is the deposition in the arteriolar wall of a granular material that appears bright pink with hematoxylin and eosin stain. On trichrome staining, this granular material is deep red. This fibrinoid material is usually found in the media, but it may also be present in the intima. Histochemical and immunofluorescent techniques have identified this material as fibrin. Within the media, muscle fibers cannot be identified and cell nuclei are lost or fragmented. Whole or fragmented erythrocytes may be extravasated into the arteriolar wall. The hemorrhages that occur may account for the petechiae observed on the cortical surface. The arteriolar lumen may be reduced in size as a result of wall thickening and intraluminal fibrin thrombi. Infrequently, polymorphonuclear leukocytes and monocytes may infiltrate the arterioles, giving the appearance of necrotizing arteriolitis.

The interlobular arteries reveal characteristic lesions variously referred to as *proliferative endarteritis, productive endarteritis, endarteritis fibrosa,* or the onionskin lesion. The typical finding is intimal thickening that causes moderate to severe luminal narrowing. In severely affected vessels, the luminal diameter may be reduced to the size of a single red blood cell. Occasionally, there is complete obliteration of the lumen by a fibrin thrombus.

Traditionally, three patterns of intimal thickening in malignant nephrosclerosis have been described (121). The *onionskin* pattern consists of pale layers of elongated, concentrically arranged, myointimal cells. Delicate connective tissue fibrils give rise to a lamellated appearance (Fig. 56-10). The media often appears as an attenuated layer stretched around the expanded intima. *Mucinous* intimal thickening consists of a scarcely cellular lesion containing a lucent, faintly basophilic-staining amorphous material (Fig. 56-11). In *fibrous* intimal thickening, there are hyaline deposits, reduplicated bands of

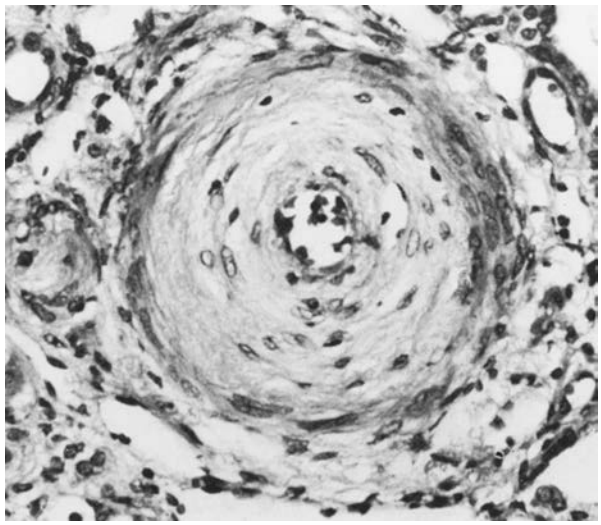

FIGURE 56-10. Onionskin lesion consisting of pale layers of elongated, concentrically arranged myointimal cells and delicate connective tissue fibrils that produce a lamellated appearance. The media is attenuated and stretched around the thickened intima. (Hematoxylin and eosin stain ×350.) (From: Sinclair RA, Antonovych TT, Mostofi FK. Renal proliferative arteriopathies and associated glomerular changes: a light and electron microscopic study. *Hum Pathol* 1976;7:565, with permission.)

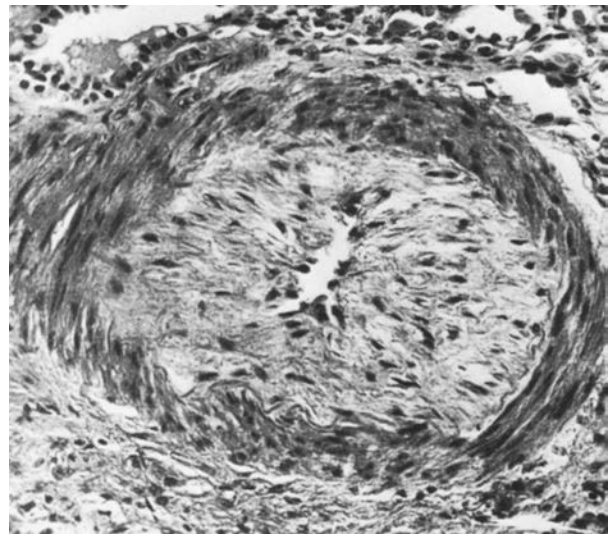

FIGURE 56-12. Fibrous intimal thickening. The lesion consists of a thick layer of connective tissue, which stains for collagen and elastin. (Hematoxylin and eosin stain ×300.) (From: Sinclair RA, Antonovych TT, Mostofi FK. Renal proliferative arteriopathies and associated glomerular changes: a light and electron microscopic study. *Hum Pathol* 1976;7:565, with permission.)

elastica, and coarse layers of pale connective tissue with the staining properties of collagen (Fig. 56-12). In rare cases, fibrinoid necrosis may also be apparent in the interlobular arteries (121).

The renal histology in blacks with malignant hypertension may be somewhat different (34,35). Although fibrinoid necrosis of the afferent arterioles is not found, there is instead a marked degree of arteriolar hyalinization. In addition, there is a prominent and characteristic finding in the larger arterioles and interlobular arteries known as *musculomucoid intimal hyperplasia* (34,35,122) (Fig. 56-13). The arterial walls are thickened due to the presence of hyperplastic smooth muscle cells. A small amount of myxoid material, which stains light blue with hematoxylin and eosin, is observed between the cells. With periodic acid-Schiff staining this material resembles basement membrane. Staining for acid mucopolysaccharide suggests the presence of chondroitin sulfate and possibly hyaluronic acid.

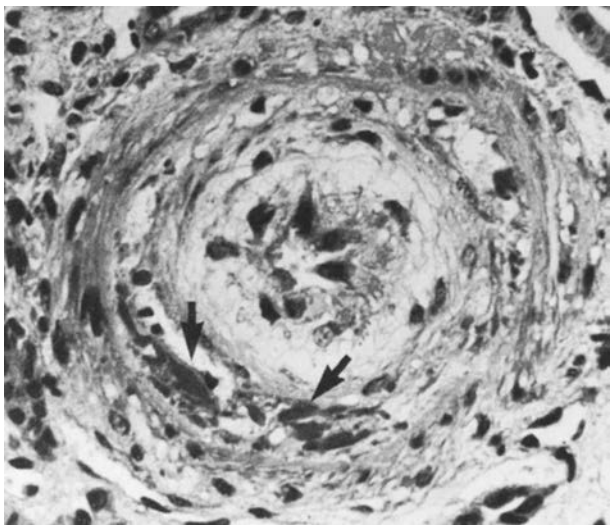

FIGURE 56-11. Mucinous intimal thickening. The lesion is sparsely cellular and consists mainly of a lucent, faintly basophilic-staining amorphous material. There are small foci of fibrinoid necrosis (*arrows*) deep within the intima. (Hematoxylin and eosin stain ×350.) (From: Sinclair RA, Antonovych TT, Mostofi FK. Renal proliferative arteriopathies and associated glomerular changes: a light and electron microscopic study. *Hum Pathol* 1976;7:565, with permission.)

By electron microscopy, in each of the above-mentioned types of intimal thickening, the most abundant cellular element is a modified smooth muscle cell called a *myointimal cell*. In these cells there are smooth musclelike ultrastructural features including cytoplasmic myofilaments and abundant rough endoplasmic reticulum (121,123). In the pure onionskin variant, the intercellular space is occupied by multiple strands of nonperiodic fibrils with the ultrastructural features of basement membrane (121). In the mucinous variant, broad electron-lucent zones with scattered finely granular material are found in the intercellular space (123). With the fibrous variant, numerous bundles of collagen, recognizable by characteristic banding, are dispersed between the myointimal cells (123).

There are no characteristic changes in the arcuate and larger renal arteries in malignant hypertension. However, fibrous thickening and elastic reduplication may be found if longstanding benign hypertension is also present.

In large autopsy series from the pretreatment era, focal and segmental fibrinoid necrosis was the typical glomerular finding (30,124,125). Glomerular lesions often occurred in continuity with a necrotic afferent arteriole. Glomerular crescent formation and segmental proliferation in areas of necrosis were also found. Rupture of these necrotic capillaries gave rise to hemorrhage into the glomerular or tubular space, accounting for some of the petechiae seen grossly. The occurrence of this necrotizing glomerulonephritis led Volhard and Fahr (12) to propose that malignant nephrosclerosis was due to arteriosclerosis with superimposed exogenous nephritis. However, even in cases of terminal uremia, the percentage of involved glomeruli was typically only 5% to 30% (30,124,125). Thus, the focal and segmental nature of the glomerular lesion in primary malignant

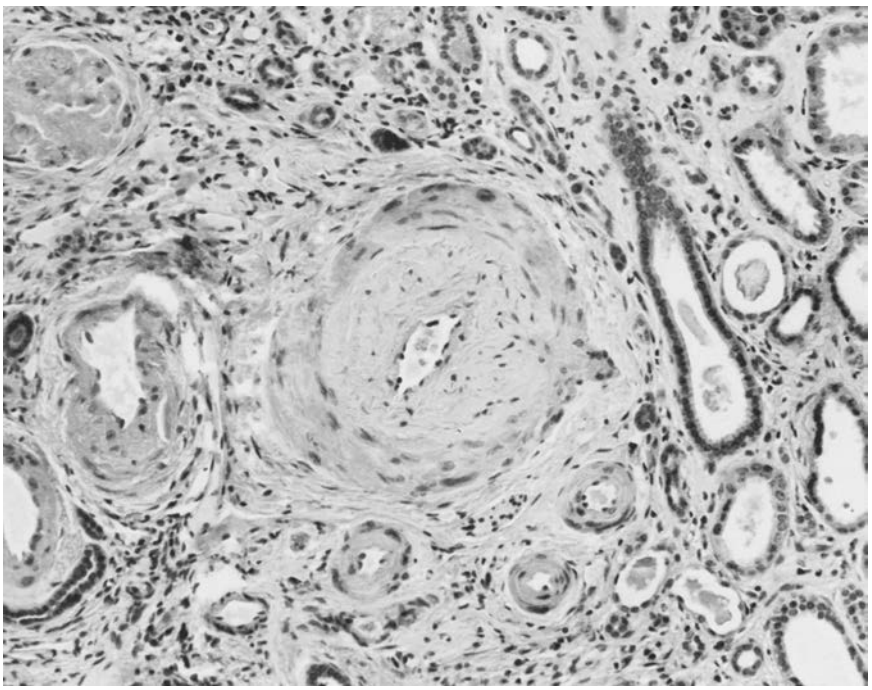

FIGURE 56-13. Musculomucoid intimal hyperplasia of an interlobular artery. The arterial walls are thickened by hyperplastic smooth muscle cells. A small amount of myxoid material is seen between the smooth muscle cells. (Hematoxylin and eosin stain ×170.) (From: Pitcock JA, et al. Malignant hypertension in blacks: malignant intrarenal arterial disease as observed by light and electron microscopy. *Hum Pathol* 1976;7:333, with permission.)

hypertension can be used to distinguish this entity from chronic glomerulonephritis with superimposed malignant nephrosclerosis, in which glomerular involvement is diffuse and global (124).

The focal and segmental necrotizing lesions that were originally described in autopsy cases of untreated malignant hypertension are now rarely seen in tissue obtained at renal biopsy in treated patients (35,126). This observation might be due to the sampling error inherent in closed renal biopsy. Alternatively, these lesions may resolve rapidly with initiation of antihypertensive therapy, and may thus not be apparent if renal biopsy is performed following adequate control of blood pressure.

In patients who have received antihypertensive therapy, as well as blacks with treated or untreated malignant hypertension, the most characteristic glomerular lesion in malignant nephrosclerosis is accelerated glomerular obsolescence secondary to the intense ischemia produced by the obliterative arterial lesions (35,126). The earliest glomerular changes consist of thickening and wrinkling of the basement membrane (35,126) (Fig. 56-14). Later, there is shrinkage of the tuft such that it does not fill Bowman's space. There is laminar reduplication of Bowman's capsule around the shrunken glomerulus (121). The end stage is the obsolescent glomerulus, which is an avascular, wrinkled glomerular tuft surrounded by a

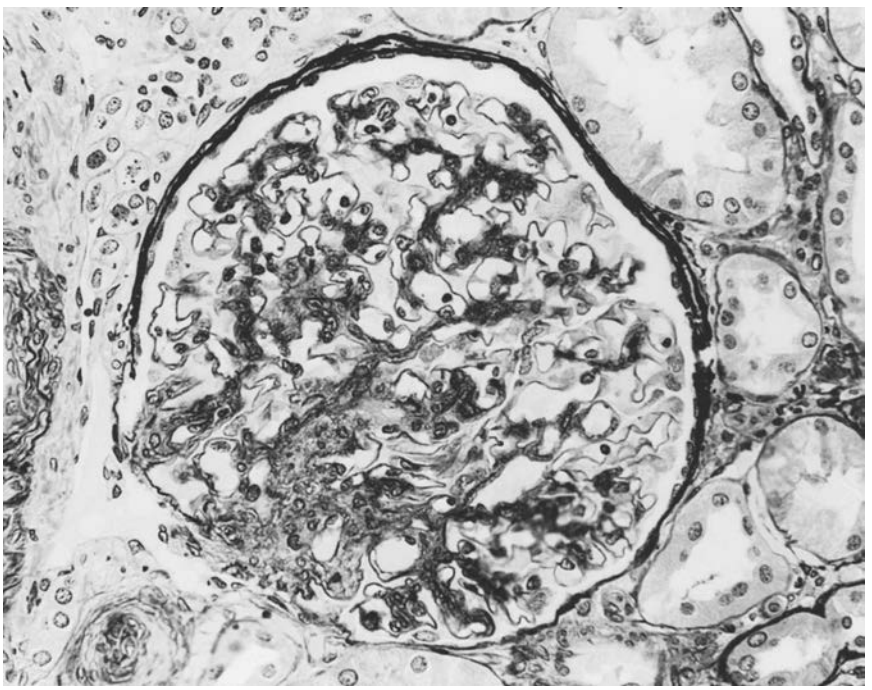

FIGURE 56-14. The earliest ischemic glomerular change in malignant hypertension consists of some basement membrane wrinkling, particularly in areas adjacent to the mesangium, with a slight increase in mesangial matrix. (Periodic acid–silver methenamine stain ×250.) (From: Pitcock JA, et al. Malignant hypertension in blacks: malignant intrarenal arterial disease as observed by light and electron microscopy. *Hum Pathol* 1976; 7:333, with permission.)

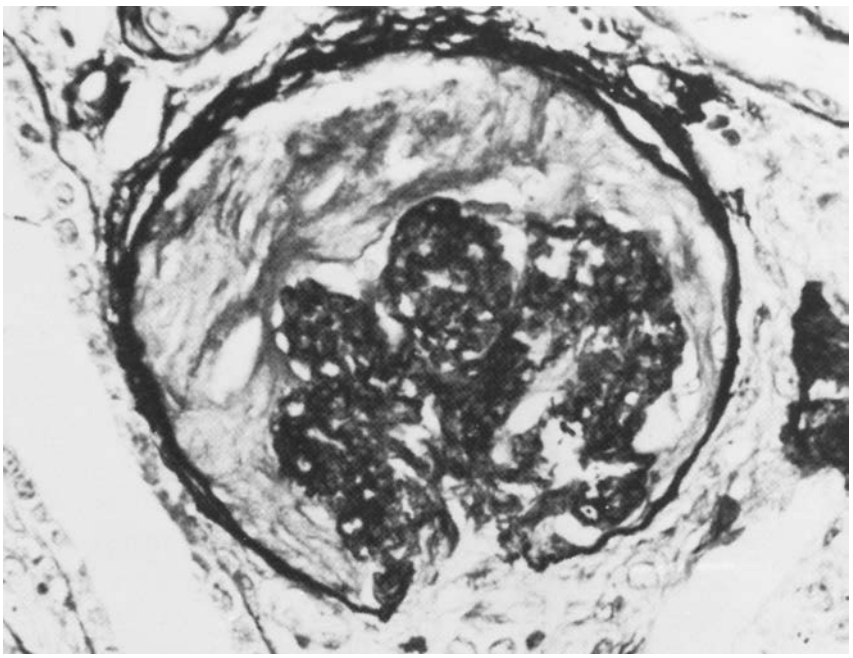

FIGURE 56-15. Glomerular obsolescence in malignant hypertension. The collapsed, avascular glomerular tuft consists predominantly of markedly convoluted basement membranes. The sclerosed tuft is partially enclosed within a collar of hyaline material filling Bowman's space. (Periodic acid–silver methenamine stain ×485.) (From: Sinclair RA, Antonovych TT, Mostofi FK. Renal proliferative arteriopathies and associated glomerular changes: a light and electron microscopic study. *Hum Pathol* 1976;7:565, with permission.)

collagenous scar that fills Bowman's space (Fig. 56-15). Focal segmental glomerulosclerosis may occur in primary malignant hypertension either as the result of glomerular hyperfiltration or fibrinoid necrosis, and may contribute to renal dysfunction. In an autopsy series of 38 black South Africans with primary malignant hypertension, mucoid intimal hyperplasia was present in all sections while fibrinoid necrosis was seen in 76%. Glomerulosclerosis was present in 38 cases, and was axially distributed in 18%, segmental in 58% and global in 24% of sections. Cases with segmental sclerosis tended to have the highest proteinuria, while those with glomerulosclerosis had the highest serum creatinine levels (127).

By electron microscopy, the lamina densa of the glomerular capillary basement membrane is thickened and wrinkled (126) (Fig. 56-16). Eventually, the entire basement membrane becomes thickened. These glomerular changes are not specific for malignant nephrosclerosis as they also can occur in scleroderma renal crisis, hemolytic–uremic syndrome, and even severe benign nephrosclerosis. However, the glomerular changes in malignant nephrosclerosis differ from the simple ischemic obsolescence observed in benign hypertension. In addition to the wrinkled basement membrane observed in benign nephrosclerosis, there is constriction of the glomerular vascular bed in malignant nephrosclerosis due to the deposition of a

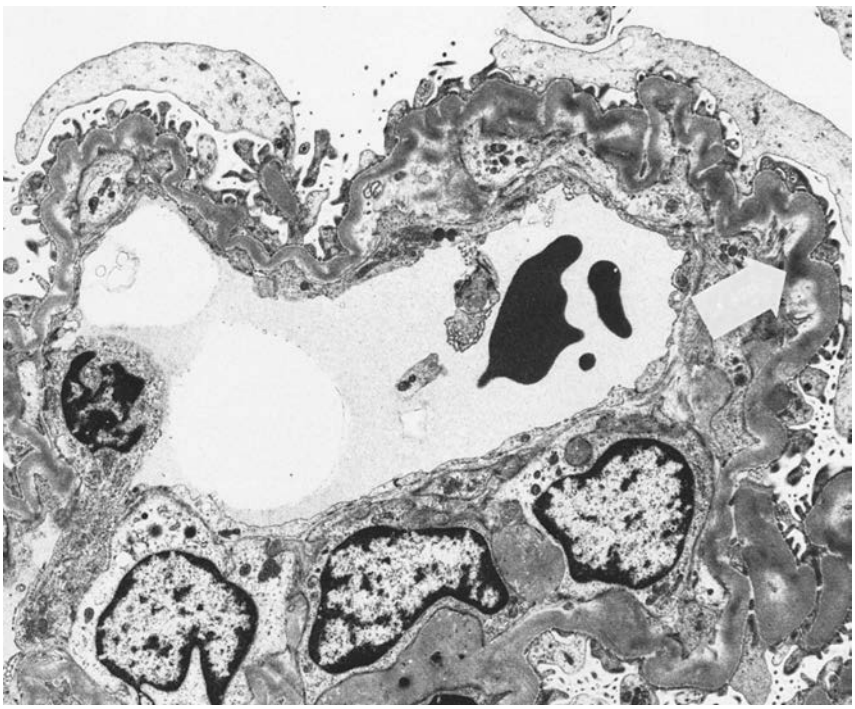

FIGURE 56-16. Accelerated glomerular obsolescence in malignant hypertension. The glomerular capillaries show striking basement membrane wrinkling (*arrow*) and some reduplication of the inner basement membrane. (Uranyl acetate and lead citrate ×4,250.) (From: Jones DB. Arterial and glomerular lesions associated with severe hypertension: light and electron microscopic studies. *Lab Invest* 1974;31:303, with permission.)

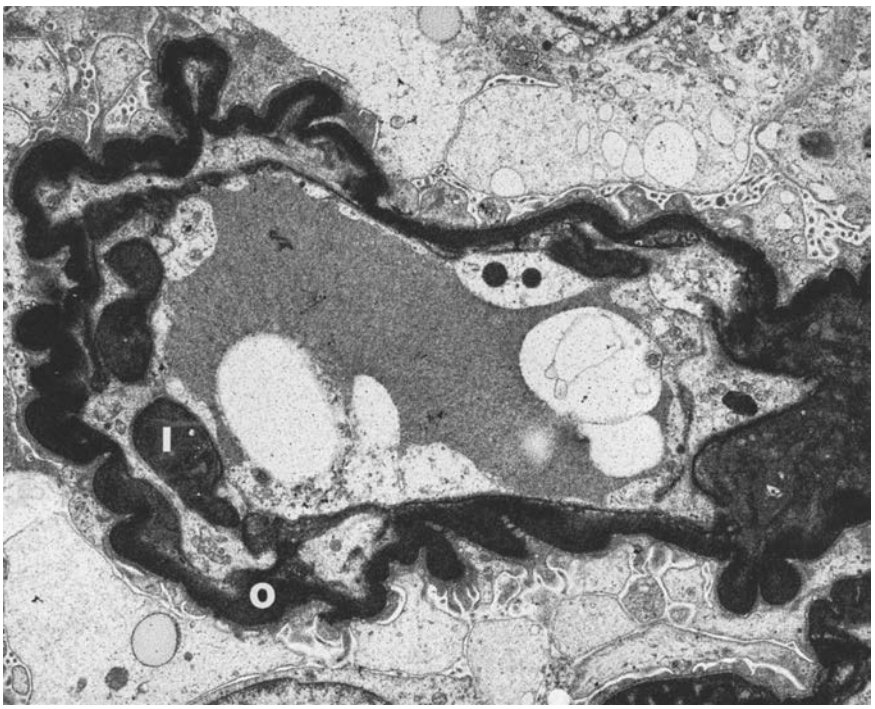

FIGURE 56-17. Accelerated glomerular obsolescence in malignant hypertension. The outer basement membrane (*O*) is thickened and wrinkled. There is a reduplicated inner basement membrane (*I*) with the capillary lumen still patent. (Uranyl acetate and lead citrate stain ×4,250.) (From: Jones DB. Arterial and glomerular lesions associated with severe hypertension: light and electron microscopic studies. *Lab Invest* 1974;31:303, with permission.)

new subendothelial layer of basement membrane material inside the original basement membrane (126) (Fig. 56-17). The new capillary lumen formed by this process is smaller, resulting in decreased blood volume in the ischemic glomerulus.

In malignant nephrosclerosis, the tubules may be atrophied and focally destroyed in areas supplied by severely narrowed arteries. Occasional tubules may be dilated and filled with eosinophilic cast material (30,125). In the interstitium in these areas, there may be a fine reticular fibrosis and chronic inflammatory cells. In malignant hypertension, as in primary renal parenchymal diseases, renal insufficiency appears to correlate best with the degree of tubular atrophy (35).

Immunofluorescence microscopy in patients with malignant nephrosclerosis has demonstrated deposition of gamma globulin, fibrinogen, albumin, and sometimes complement components in the walls of arterioles demonstrating fibrinoid necrosis by light microscopy (128). Some of the glomeruli, especially those with focal necrosis, may contain gamma globulin, albumin, and complement. Fibrinogen may be found diffusely along capillary basement membranes. Fibrinogen may also be found in the intima of interlobular arteries that by light microscopy show cellular or mucinous thickening (125).

Striking juxtaglomerular hyperplasia has been reported in patients with malignant hypertension (126,129,130). This ultrastructural finding is consistent with the hyperreninemic state often noted clinically (129).

Effective antihypertensive therapy may alter the pathology of malignant nephrosclerosis (130–132). Within days, there may be resolution of fibrinoid necrosis, which leaves behind residual hyaline deposits in the arteriolar wall. In contrast to benign nephrosclerosis in which arteriolar hyaline change is often subendothelial, in treated malignant hypertension the hyaline material may be present throughout the entire vessel wall. Fibrosis of the arterioles with collagen replacement of the arteriolar muscle and elastica may also occur. Within several weeks after initiation of therapy, the glomerular segmental fibrinoid necrosis may also resolve, leaving behind an area of hyaline deposition that can mimic focal segmental glomerulosclerosis (FSGS). Furthermore, with treatment, in the intima of the interlobular arteries there may be an evolution from cellular hyperplasia to a more fibrous form of intimal thickening. A newly formed internal elastic lamina often separates this new collagen from the narrowed lumen. Heptinstall has postulated that the cellular lesion is an early finding implying active disease, whereas the acellular fibrotic lesion is a later process often reflecting a response to treatment (125). These modifications in the interlobular arteries that occur following treatment may not be accompanied by any increase in the caliber of the lumen. Severely narrowed interlobular arteries often do not improve and the renal parenchyma distal to these arteries undergoes severe ischemic atrophy and scarring (131). However, the nephrons supplied by interlobular arteries of normal caliber may undergo substantial hypertrophy following treatment of malignant hypertension. These histologic changes may explain the improvement in renal function that sometimes occurs in some patients following institution of antihypertensive therapy with resolution of malignant hypertension.

In summary, although fibrinoid necrosis was the hallmark of malignant nephrosclerosis in untreated patients at autopsy, it is now rarely observed. In treated patients with malignant hypertension or blacks with untreated malignant hypertension, closed renal biopsy most often reveals marked intimal hyperplasia of the interlobular arteries in association with accelerated glomerular obsolescence (34,35,126).

Renal Pathology in Secondary Malignant Hypertension

A variety of primary renal parenchymal diseases can cause secondary malignant hypertension. Although it is often impossible to differentiate primary (essential) malignant hypertension from secondary malignant hypertension by clinical criteria, this distinction can usually be made by renal biopsy.

Malignant hypertension can develop during the course of chronic glomerulonephritis. When glomerulonephritis causes malignant hypertension, there is usually evidence of diffuse glomerular disease in addition to the vascular lesions seen in malignant nephrosclerosis. In contrast to the focal and segmental glomerular lesions seen in primary malignant hypertension, in secondary malignant hypertension due to chronic

glomerulonephritis, diffuse and global changes usually involve more than 90% of glomeruli (125,133).

Chronic pyelonephritis can also cause secondary malignant hypertension. The pyelonephritic process can be unilateral or bilateral. There is coarse irregular scarring of the kidney(s), as well as difference in the size and shape of the two kidneys (25). The cortex is affected in a patchy fashion with alternating areas of scarred and normal-appearing tissue. Histologically, alternating areas of sharply demarcated normal and scarred parenchyma are observed (30). In scarred areas, there are dilated, colloid-filled tubules, crowded hyalinized glomeruli, and periglomerular fibrosis. In the interstitium there is severe fibrosis with a chronic inflammatory infiltrate. Vascular lesions indistinguishable from malignant nephrosclerosis may be seen in scarred areas, even in normotensive subjects with chronic pyelonephritis. However, with superimposed malignant hypertension, vascular lesions of malignant hypertension are found in unscarred areas of the kidney (30,134). Moreover, in unilateral pyelonephritis with superimposed malignant hypertension, histologic findings of malignant nephrosclerosis occur in the contralateral kidney (30).

In the microscopic form of polyarteritis nodosa (PAN), fibrinoid necrosis of the afferent arterioles and proliferative endarteritis of the interlobular arteries can occur in the absence of malignant hypertension. However, malignant nephrosclerosis and PAN can usually be differentiated histologically. In PAN, there is usually diffuse glomerular involvement as well as an active cellular infiltrate in the necrotic vascular and glomerular lesions. In addition, there are often healed and active necrotizing lesions typical of PAN in larger medium-sized muscular arteries of the kidney, mesentery, pancreas, and adrenals. Involvement of vessels of this size in primary malignant hypertension is rarely observed (30).

In scleroderma renal crisis, changes in the renal vessels may be virtually identical to primary malignant hypertension (58). The characteristic extrarenal manifestations of progressive systemic sclerosis must be used to differentiate these two entities.

Distribution of Vascular Lesions

Malignant hypertension is a diffuse hypertension-induced vasculopathy and in its terminal stages, widespread arterial and arteriolar lesions are found in a variety of organs (25,135). These vascular changes are similar to those seen in the kidney, namely, proliferative endarteritis of small arteries and fibrinoid necrosis of arterioles. The vascular beds of the pancreas, gastrointestinal tract, and liver are most frequently involved (136). Similar vascular lesions have also been observed in the retina (137), brain (137), myocardium (136), prostate (25), and skeletal muscle (135). The pathologic changes in the various organs and tissues are secondary to ischemia caused by these obliterative vascular changes.

Ophthalmic Pathology

In patients with malignant hypertension, thickening and hyalinization of the walls of the arterioles and capillaries of the retina and choroid are invariably present (137). Fibrinoid necrosis may also be found in some patients. Fibrin thrombi may be present in arterioles. Microaneurysms at or near the occluded segments of capillaries also occur. Small ischemic infarcts of the retina (cytoid bodies), corresponding to the cotton-wool spots seen clinically, are present in most patients (137). These infarcts are located in the nerve fiber and ganglion cell layers of the retina in the vicinity of vascular lesions. The arterioles supplying the optic nerve are thickened and hyalinized to varying degrees and fibrinoid necrosis may be present (137).

Cardiac Pathology

Left ventricular hypertrophy with normal chamber size is the predominant finding in most patients with malignant hypertension (25,26). Significant coronary atherosclerosis is a rare finding in these patients (25,26).

Pulmonary Pathology

In the era before effective antihypertensive therapy, uremic edema of the lung was frequently found in fatal cases of malignant hypertension (138). Gross examination of the lungs revealed widespread gelatinous consolidation that was most intense in the perihilar region.

Adrenal Pathology

Fibrinoid necrosis of the small arterioles of the adrenal glands is observed in up to 50% of fatal cases of malignant hypertension (25). The adrenals are often enlarged with multiple macroscopic nodules. This adrenal hyperplasia reflects sustained activation of the renin-angiotensin-aldosterone axis (65).

Gastrointestinal Pathology

Significant involvement of the mesenteric arterioles is a frequent finding in patients with malignant hypertension (136). In addition, among 100 patients with malignant hypertension, 60% had proliferative endarteritis of the pancreatic arterioles (139). Pancreatic lesions including infarcts associated with arterial thrombosis, focal parenchymal necrosis, and foci of atrophy and fibrosis are frequently found.

Pathophysiology

On the basis of the clinical presentation, natural history, and renal pathology, it can be concluded that benign and malignant hypertension are distinct clinicopathologic entities rather than a spectrum of the same disease. However, the mechanisms that initiate the transition from benign to malignant hypertension are not entirely clear. The primary function of the microcirculation is to ensure that the cardiac output is distributed to a variety of tissues that vary widely in metabolic requirements. The arterioles control the blood flow to the capillary network through the process of autoregulation. Thus, it is not surprising that the arterioles are the major target of the acute vascular damage in malignant hypertension. However, the central question is whether severe hypertension alone is sufficient to cause the vascular injury characteristic of malignant hypertension, or whether additional cofactors are required.

Role of Increased Blood Pressure Per Se (the Pressure Hypothesis)

According to the pressure hypothesis, the development of fibrinoid necrosis and proliferative endarteritis is a direct consequence of the mechanical stress placed on vessel walls by severe hypertension (45,104,125,140,141). Several lines of evidence support the hypothesis that severe hypertension is the fundamental pathogenic process in malignant hypertension. In this regard, although there are numerous diverse etiologies of malignant hypertension, the process is reversible given adequate blood pressure control (22). Moreover, the transition from benign to malignant hypertension is usually accompanied by a dramatic rise in blood pressure (45). In addition, the occurrence of fibrinoid necrosis tends to correlate with the height of the arterial pressure (125).

Another finding supporting the crucial role of severe hypertension in the development of the vascular lesions of malignant hypertension is the absence of these lesions in vascular

beds protected from the elevated blood pressure. In the two-kidney, two-clip model of malignant hypertension in the dog, vascular necrosis occurs in mesenteric arterioles and other vessels exposed to the high pressure, but not in renal arterioles protected from the high perfusion pressure (142). Likewise, in rats with two-kidney, one-clip malignant hypertension, vascular necrotic lesions develop in the nonclipped kidney and the systemic circulation but not in the clipped kidney (143). One report described patients with unilateral renal artery stenosis and malignant hypertension in whom arteriolar necrosis occurred in the contralateral kidney but not in the stenotic kidney (144).

A number of experimental models of malignant hypertension lend support to the pressure hypothesis. Brief overdistention of the arteriolar system by forceful injection of saline solution into the aorta results in a sudden increase in mean arterial pressure of 80 to 90 mm Hg accompanied by the development of fibrinoid necrosis in the interlobular arteries and afferent arterioles of the kidney (145). Fibrinoid necrosis does not develop if the kidney is protected from the sudden rise in pressure.

In studies that utilize windows in the skull and abdominal wall to view the microcirculation in rats with one-kidney, one-clip malignant hypertension, cerebral and mesenteric arterioles develop focal constrictions and dilations giving rise to a string of beads or sausage-string pattern (146). Intravenous injection of trypan blue results in patchy leakage of dye in dilated regions but not in constricted regions. The dilated regions are the sites of fibrinoid necrosis (147).

The sausage-string pattern develops rapidly in mesenteric arterioles in the rat when the blood pressure is increased acutely with angiotensin or norepinephrine. When colloidal carbon particles are injected, carbon deposits in the walls of dilated segments but not constricted segments, thereby suggesting that the abnormal vascular pattern is the direct result of the elevated blood pressure and that the dilated segments represent the earliest sites of vascular damage as manifested by increased permeability to plasma proteins (148).

The extent of carbon deposition is related to the height of the arterial pressure rather than to the type of pressor substance infused (angiotensin, norepinephrine, or renal extract) (147). If the increase in blood pressure is prevented by the administration of hydralazine, the sausage-string pattern fails to develop and carbon is not deposited in the vascular walls. Thus, the abnormal permeability in dilated segments appears to be the result of hypertension-induced structural damage to the vessel wall. These studies do not support the concept of a direct vasculotoxic effect of angiotensin or norepinephrine independent of a pressor effect.

These sausage-string lesions have been evaluated using electron microscopy (148). In dilated segments, breaks in the endothelium are observed as a result of disruption of intracellular junctions or destruction of the cell body. These lesions give rise to gaps in the endothelium that are permeable to intravenously injected colloidal carbon particles. Amorphous deposits consisting of plasma, carbon particles, and fibrinlike material deposit in the media. The vascular lesions do not appear to be caused simply by a direct vasculotoxic effect of angiotensin because identical results have been reported with deoxycorticosterone acetate (DOCA)-salt hypertension, a model in which angiotensin levels are suppressed (149).

Based on these experiments, it has been concluded that the mechanical stress of severe hypertension is the principal cause of fibrinoid necrosis in malignant hypertension. Moreover, all of the vascular changes can be attributed to the indirect pressor effect of the infused substances rather than to any direct effect of these substances on vascular permeability. The following mechanism for the development of fibrinoid necrosis has been proposed (147,148,150). With mild to moderate elevations in blood pressure, the initial hemodynamic response is arterial and arteriolar vasoconstriction. This autoregulatory process maintains tissue perfusion at a relatively constant level and prevents the elevated pressure from being transmitted to the smaller, more distal vessels. With increasingly severe hypertension, however, autoregulation eventually fails such that hypertension is transmitted to more distal vessels. The resulting rise in downstream perfusion pressure damages the arterioles and capillaries. Because smooth muscle may not be uniformly distributed along arterioles, when the arterial pressure is sufficiently increased by whatever means, local areas of the arterial wall are unable to withstand the mechanical stress and become forcibly dilated (sausage-string pattern). By the law of Laplace, as the radius increases in dilated segments, wall tension increases proportionately. As a consequence, the endothelium becomes stretched, damaged, and abnormally permeable. Disruption of the vascular endothelium then allows extravasation of plasma proteins and fibrinogen into the vessel wall, compressing and destroying smooth muscle. Local fibrinogen deposition occurs, producing fibrinoid necrosis and obliteration of the vascular lumen.

The Vasculotoxic Hypothesis

The major criticism of the pressure hypothesis is that there is substantial overlap between the levels of blood pressure observed in patients with benign hypertension and those with malignant hypertension (25,84). Moreover, occasional patients tolerate severe diastolic hypertension for long periods without developing malignant hypertension (81). The abrupt change from an asymptomatic patient with severe hypertension to a critically ill patient with a multisystem disease suggests that factors in addition to blood pressure may contribute to the transition from benign to malignant hypertension (41). According to the vasculotoxic theory, severe hypertension is necessary but not sufficient to cause malignant hypertension.

The vasculotoxic theory proposes that humoral factors interact with the hypertension-induced hemodynamic stress to cause the vascular damage observed in malignant hypertension. Based on his classic dog model of renovascular hypertension, Goldblatt (142) believed that renal failure was a necessary cofactor for the development of malignant hypertension. He proposed that a vasculotoxic factor accumulated in severe renal disease. However, it has subsequently been demonstrated in both humans and experimental animals that malignant hypertension can develop in the absence of uremia and even before the vascular lesions of malignant nephrosclerosis develop in the kidney (16,28).

It has been suggested that vascular permeability factors such as renin, angiotensin, catecholamines, and vasopressin may cause vascular damage independent of their pressor effects. For example, administration of rat kidney extracts to nephrectomized rats produces a lethal syndrome characterized by large pleural effusions, ascites, edema of the pancreas, and the arteriolar fibrinoid necrosis characteristic of malignant hypertension (152). To explain these findings, the presence of a vascular permeability factor of renal origin has been postulated.

There is convincing evidence that angiotensin II does have a direct effect on the vascular endothelium to increase permeability (153). The injection of 100 pg of angiotensin II into a segment of rabbit abdominal aorta isolated between two ligatures causes a diffuse increase in permeability of the aortic endothelium as evidenced by extravasation of intravenously injected Evans blue dye. Areas of the aorta not exposed to angiotensin II do not show any increase in permeability. Injections of Ringer's solution (vehicle) produce no blue staining of the aortic endothelium. Evaluation of the areas exposed to angiotensin II by electron microscopy reveals rounding and shortening of the endothelial cells with widening of spaces between endothelial cells.

Studies of dermal capillary permeability in response to vasoactive agents have confirmed the fact that there appears to be a direct effect of angiotensin II on vascular endothelium (153). When norepinephrine or angiotensin I is injected into the dermis, there is no extravasation of Evans blue dye. However, when angiotensin II is injected in low concentrations, there is severe capillary leak. The increased permeability can be prevented by simultaneous administration of angiotensin II receptor antagonists but not by antihistamines. Angiotensin II appears to increase vascular permeability independent of any pressor effect by causing contraction of endothelial cells (153).

Role of the Renin-Angiotensin Axis

In patients with malignant hypertension there is often evidence of activation of the renin-angiotensin system (65,115). At the time of presentation, some but not all patients have increased PRA (63). Hyperplasia of the juxtaglomerular apparatus is often present. The adrenal glands frequently reveal hyperplasia and nodularity of the zona glomerulosa (65).

In patients with malignant hypertension, activation of the renin-angiotensin system may be primary or secondary. For example, activation of the renin-angiotensin system could be a critical step in the transition from benign to malignant hypertension through either direct (vasculotoxic) or indirect (hemodynamic) effects on the vasculature. Conversely, hyperreninemia may be a secondary phenomenon occurring in response to renal ischemia caused by the arteriolar lesions of malignant hypertension. These two processes are not mutually exclusive. In fact, both may occur simultaneously, resulting in a vicious cycle of hypertension-induced vascular damage causing renal ischemia, which leads to enhanced renin release that may exacerbate the hypertension.

In a two-kidney, one-clip model of renovascular hypertension in the rat, there is onset of malignant hypertension after 3 to 5 weeks (154). The sequence of events following the application of a sufficiently small clip to one renal artery has been well characterized. Initially there is evidence of activation of the renin-angiotensin system with a resulting increase in blood pressure. When the systolic blood pressure surpasses a critical level of 180 to 190 mm Hg, spontaneous natriuresis and diuresis occur. Water intake increases, but weight loss and hyponatremia ensue. Eventually, renal function deteriorates, and the histologic findings of malignant nephrosclerosis become apparent in the contralateral kidney. The rats eventually die due to renal failure, heart failure, or cerebral hemorrhage. In this model of malignant hypertension, it is apparent that a vicious cycle develops following activation of the renin-angiotensin system. Renal ischemia results in activation of the renin-angiotensin system with the development of hypertension. The sudden, severe hypertension causes natriuresis, diuresis, and volume depletion. Volume depletion further stimulates the renin-angiotensin system. As the gain on this cycle increases, hypertension-induced vascular damage or the direct vasculotoxic effect of angiotensin induces vascular damage and deterioration of renal function. Volume depletion is pivotal in the pathogenesis of malignant hypertension in this model. Rats with malignant hypertension, given a choice of deionized water or normal saline, exhibit compulsive saline drinking (155). During the first 24 hours of saline drinking, there is a marked decrease in blood pressure and an increase in body weight. Moreover, there is correction of the abnormal levels of hematocrit, serum sodium, renin, and angiotensin II. With continued saline drinking for 2 to 7 days, the blood pressure increases to the previous high levels, but signs of malignant hypertension do not recur. If saline is withdrawn at this time, within 2 days the cycle of volume depletion, activation of the renin-angiotensin system, and malignant hypertension recurs. Thus, the pathogenesis of malignant hypertension in this model appears to be critically dependent on spontaneous natriuresis, volume depletion, and activation of the renin-angiotensin system.

The unclipped kidney is clearly the source of the spontaneous natriuresis and diuresis in the two-kidney, one-clip model. In the one-kidney, one-clip rat model, there is no phase of renal salt loss (156). Likewise, in rats with two-kidney, two-clip hypertension, the phase of salt loss does not occur. However, if one clip is removed, there is an immediate onset of a salt-losing state associated with development of the syndrome of malignant hypertension with fibrinoid necrosis in the unclipped kidney.

In the stroke-prone spontaneously hypertensive rat (SHR-SP) model of malignant hypertension, life-long treatment with an antihypertensive dose of the converting enzyme inhibitor ramipril completely prevents the renal arteriolar neointimal proliferation and sclerosis, fibrinoid necrosis, glomerulopathy and tubular-interstitial fibrosis observed in placebo-treated animals (157). The nephroprotective effect of ramipril was associated with a dose-dependent inhibition of plasma and renal tissue angiotensin-converting enzyme activities. Although there was some subtle evidence of nephroprotection with a lower non-antihypertensive dose of ramipril, prevention of hypertension with high-dose ramipril was also required to completely prevent the development of malignant nephrosclerosis in the SHR-SP model.

Over the last decade, polymorphisms in the genes regulating the RAS system have been identified. These include the 287 base pair sequence deletion (D) insertion (I) polymorphism of the angiotensin-converting enzyme (ACE) gene and the methionine (M) to threonine (T) point mutation polymorphism in the angiotensinogen gene (AGT). Genotypes with respect to ACE I/D and AGT M/T loci were evaluated in a series of 42 patients with malignant hypertension, 42 patients with nonmalignant hypertension and 85 normotensive controls (158). The frequency of the DD genotype was significantly increased in patients with malignant hypertension (43%) compared with patients with nonmalignant hypertension (14%) and normotensive control subjects (18%). The frequency distribution of AGT M/T genotype did not differ between groups. These data suggest that ACE gene polymorphism is a significant risk factor for initiation of malignant hypertension.

Whereas activation of the renin-angiotensin system may be crucial in the development of the malignant phase in some experimental models and some patients with malignant hypertension, the clinical and pathologic features of malignant hypertension sometimes occurs in the absence of activation of the systemic renin-angiotensin system (63). For example, widespread necrotizing arteriolar disease occurs with hypertension caused by bilateral nephrectomy in experimental animals. On the other hand, there is emerging evidence from transgenic animals of activation of a local paracrine renin-angiotensin system in the pathogenesis of the vascular injury in malignant hypertension. Animal models provide pathological, pharmacologic, and genetic evidence supporting the hypothesis that intrarenal generation of AII and exposure of the microcirculation to elevated blood pressure cooperate in causing tissue damage in malignant hypetension (159).

Elevated levels of angiotensin II in malignant hypertension may contribute to renal injury by mediating renal inflammation independent of any blood pressure effect. In a solvent-treated two-kidney/one-clip rat model of malignant hypertension, low-dose angiotensin receptor blockade with valsartan does not prevent an increase in blood pressure but prevents the development of lethal malignant hypertension (160). In nonclipped kidneys of solvent-treated renovascular hypertensive rats, there was a prominent interstitial infiltrate of macrophages and increased expression of monocyte chemoattractant protein-1. These changes were completely prevented by low-dose

valsartan. These finding suggest that reduction of AII-induced inflammation in the kidney may contribute to the protective effects of valsartan in this model of malignant hypertension.

In summary, neither hypersecretion of renin nor the presence of kidneys appears to be required for the development of malignant hypertension. On the other hand, a high concentration of angiotensin II can be an aggravating factor or in some circumstances, can actually trigger the onset of malignant hypertension. Moreover, activation of the renin-angiotensin system by renal ischemia may contribute to the vicious cycle of severe hypertension and renal injury observed in malignant hypertension.

Role of Volume Depletion

In several experimental models, spontaneous natriuresis appears to be the initiating event in the transition from benign to malignant hypertension (154,155,161,162). In patients with malignant hypertension, an abrupt onset of weight loss early in the course of the disease has been reported (16,25,27). In the series of Kincaid-Smith et al. (25), the onset of malignant hypertension often appeared suddenly. Despite minor increases in blood pressure, the patients became suddenly ill with weakness, wasting, and profound weight loss. The rapidity of the weight loss could only be explained by natriuresis-induced volume depletion and may be the human counterpart of the rat two-kidney, one-clip model of malignant hypertension in which spontaneous natriuresis is the inciting event (154,155). In patients with analgesic nephropathy, volume depletion often accompanies malignant hypertension, whereas restoration of normal volume status leads to lowering of blood pressure and resolution of malignant hypertension (42).

In summary, it appears that volume depletion due to spontaneous natriuresis is often associated with the transition from the benign to the malignant phase of hypertension in both experimental and human malignant hypertension. The mechanism of the spontaneous salt wasting is not known. In the isolated, perfused kidney, an acute increase in arterial perfusion pressure results in increased urine flow rate and sodium excretion. Because glomerular filtration rate and renal blood flow remain unchanged, the natriuresis has been attributed to inhibition of tubular sodium resorption by elevated blood pressure.

Role of Localized Intravascular Coagulation

Evidence of microangiopathic hemolytic anemia and disorders of coagulation and fibrinolysis has been reported in experimental models of malignant hypertension (163). This anemia is characterized by red cell fragmentation and hemoglobinuria with iron deposition in the renal tubules.

Localized intravascular coagulation has been proposed to play an important role in the pathogenesis of malignant hypertension. Severe hypertension, perhaps augmented by vasculotoxic factors, injures arteriolar walls. This injury leads to increased endothelial permeability to fibrinogen and other plasma proteins. The clotting cascade is activated by tissue thromboplastin and fibrin that is deposited in the vessel wall and lumen. Platelets are deposited, and microangiopathic hemolytic anemia is produced by fragmentation of red cells as they traverse intravascular fibrin strands. The lysis of platelets and red cells produces adenine diphosphate and thromboplastin, which aggravate intravascular coagulation and produce a vicious cycle of hemolysis and fibrin deposition. Tissue ischemia is produced by a combination of intravascular fibrin deposition and arteriolar wall thickening that constrict the vessel lumen.

In this scheme, intravascular coagulation is the consequence of hypertension-induced vascular injury. It has also been postulated that the coagulation abnormalities may be the primary event that incites the development of malignant hypertension (114). This theory is based on the observation that renal vascular lesions identical to those in primary malignant nephrosclerosis can occur with idiopathic postpartum renal failure and the hemolytic–uremic syndrome. In these disorders, abnormalities of coagulation and fibrinoid necrosis often precede the development of hypertension (164).

To investigate the hypothesis that abnormalities of thrombogenesis and endothelial damage/dysfunction are greater in malignant hypertension compared with uncomplicated nonmalignant essential hypertension, Lip et al. measured markers of endothelial function (von Willebrand factor), platelet activation (soluble P-selectin) and fibrinogen in 18 consecutive patients with malignant hypertension, 50 patients with untreated essential hypertension and 34 healthy controls (165). Mean plasma fibrinogen and von Willebrand factor levels were highest in patients with malignant hypertension, intermediate in the nonmalignant hypertension group and lowest in the normotensive controls. Soluble P-selectin levels were higher in both hypertensive groups than in normotensive controls. These data suggest that endothelial damage (elevated von Willebrand factor) and platelet activation may be involved in the pathogenesis of malignant hypertenson.

Role of Prostacyclin

Abnormal prostacyclin (prostaglandin I_2 [PGI_2]) metabolism has been postulated to play a role in the pathogenesis of malignant hypertension in cigarette smokers and women taking oral contraceptives (50). Enhanced PGI_2 synthesis by vessel walls may be a protective mechanism that limits the vascular injury caused by hypertension (167). For example, PGI_2 may limit the extent of thrombus formation at sites of endothelial injury. Both oral contraceptives (166) and cigarette smoking (168) are associated with lower concentrations of 6-keto-prostaglandin $F_{1\alpha}$, a stable metabolite of PGI_2. Impaired vessel wall synthesis of PGI_2 may thus predispose to the development of malignant hypertension (50). Moreover, absence of the protective effect of PGI_2 could amplify the vascular endothelial injury caused by severe hypertension.

Role of Intracellular Calcium

Increased availability of free calcium in vascular smooth muscle may be important in the pathogenesis of hypertension. Moreover, it has been suggested that an excess of cytosolic calcium may be a crucial step in the development of malignant hypertension and that this deleterious calcium overload may be activated or inhibited independent of the arterial blood pressure (169). Dahl salt-sensitive rats on a high-salt diet develop fulminant hypertension with a necrotizing vasculitis in the kidney. However, treatment with nifedipine, which inhibits calcium influx via activated membrane calcium channels, prevents the rise in blood pressure and the occurrence of necrotizing vascular lesions. A similar protective effect has been described with nisoldipine and nitrendipine but not with captopril (169).

The stroke-prone spontaneously hypertensive rat is another experimental model of malignant hypertension that mimics the renal and vascular changes seen in primary malignant hypertension in humans (170). In salt-loaded stroke-prone spontaneously hypertensive rats, treatment with either nimodipine or parathyroidectomy dramatically reduces vascular injury and mortality despite an insignificant effect on the level of blood pressure. Since hypertension-induced vascular injury can be prevented by manipulation of calcium influx into cells, it has been postulated that vascular injury in malignant hypertension is mediated by intracellular calcium overload in vascular smooth muscle cells (169).

Role of Dietary Potassium

In Dahl salt-sensitive rats on a high-salt diet, supplementation of dietary potassium intake can prevent the hypertension-induced intimal thickening of the interlobular arteries without a concomitant reduction in blood pressure (171). In addition, in the stroke-prone spontaneously hypertensive rat model of malignant hypertension, a high-potassium diet prevented the intimal thickening of mesenteric and cerebral arteries, independent of an effect on blood pressure (172).

It has been suggested that the low-potassium diet characteristically consumed by African Americans in the southern United States may exacerbate the hypertension-induced endothelial injury in the interlobular arteries, leading to the development of pronounced musculomucoid intimal hyperplasia (malignant nephrosclerosis) (173). Low dietary potassium intake may, at least in part, explain the high frequency of ESRD due to hypertension among African Americans.

Role of the Kallikrein-Kinin System

In addition to enhanced activity of vasopressor hormones, decreased activity of vasodilator hormone systems may be involved in the pathogenesis of malignant hypertension. Kinins are potent vasodilators that exert a marked influence on renal salt and water excretion. Plasma levels of kininogen are markedly decreased in patients with malignant hypertension compared with either patients with benign hypertension or normotensive control subjects (174). Urinary kallikrein excretion is also significantly decreased in patients with malignant hypertension, particularly those with primary (essential) malignant hypertension (175). Decreased urinary kallikrein could be indicative of depressed activity of the renal kallikrein-kinin system, which may be a risk factor for development of malignant hypertension in individuals with essential hypertension.

Role of Endothelium-Derived Relaxing Factors and Endothelin

Hypertension is associated with various functional changes of the vascular endothelium including decreased formation of nitric oxide, the endothelium-derived relaxing factor (EDRF), and increased release of contracting factors such as endothelin. This dysfunction of the hypertensive endothelial organ may contribute to the elevation of peripheral resistance and the development of hypertensive complications in the cerebral, coronary, or renal circulation (176,177).

Plasma immunoreactive endothelin-1 (ET-1) levels have been measured in various rat models of hypertension (178). Endothelin-1 levels are not increased in the prehypertensive and benign hypertension phases in spontaneously hypertensive rats compared to normotensive Wistar-Kyoto rats. In contrast, treatment of spontaneously hypertensive rats (SHR) with DOCA and salt results in the development of malignant hypertension with renal insufficiency and increased plasma ET-1 levels. However, ET-1 levels do not increase in Wistar-Kyoto rats treated with DOCA-salt. In the DOCA-salt-treated SHR model of malignant hypertension, treatment with combined endothelin type A/type B receptor antagonists not only reduced blood pressure but also prevented mesangial hypercellularity, glomerular sclerotic changes, and tubulo-interstitial damage (179). Reduction of blood pressure with hydralazine alone was less effective in preventing DOCA–salt-induced renal structural injury in this model.

The ability of endothelin receptor blockade to prevent or to treat established cerebral and renal injury has recently been investigated in the salt-loaded, SHR-stroke prone model of malignant hypertension using an endothelin receptor subtype-A antagonist (180). Initiation of endothelin receptor blocker treatment at the start of salt loading prolonged survival and completely prevented the development of cerebral edema and reduced blood pressure and proteinuria in a dose-dependent fashion. However, delaying treatment until after the onset of cerebral edema failed to prolong survival. These data suggest that in the SHR-stroke prone model the endothelin A receptor participates actively in the development of increased blood pressure and initiation of target-organ damage but has a minimal role in the pathogenesis of established malignant hypertension and the progression of target-organ damage.

Role of Oxidate Stress

Oxidative stress has been implicated in the pathogenesis of hypertension. In the stroke-prone spontaneously hypertensive (SP-SHR) rat model of malignant hypertension treatment with the superoxide dismutase mimetic, tempol prevented the progression of hypertension (181). Tempol reduced the media:lumen ratio compared to controls. Moreover vascular superoxide anion levels were lower and plasma total antioxidant levels were higher in the tempol-treated group. These data suggest that oxidative stress may play an important role in the vascular damage associated with malignant hypertension in the SP-SHR.

Development of Fibrinoid Necrosis and Proliferative Endarteritis

In experimental models of malignant hypertension, vascular damage from either the mechanical stress of hypertension or vasculotoxic hormones leads to endothelial injury that is manifested by the sausage-string pattern and accompanied by seepage of plasma proteins including fibrinogen into the vessel wall. Contact of plasma constituents with smooth muscle cells activates the coagulation cascade, and fibrin is deposited in the wall. Fibrin deposits cause necrosis of smooth muscle cells and the development of fibrinoid necrosis.

Proliferative endarteritis occurs when the vascular smooth muscle cells undergo a phenotypic change from the normal contractile phenotype to the proliferative–secretory phenotype that was predominant during embryologic development. It has been proposed that the sudden severe elevation of blood pressure produces forced vasodilation of the interlobular arteries with denudation of the vascular endothelium, resulting in the attachment of platelets at the sites of endothelial injury with synthesis and release of platelet-derived growth factor (PDGF) (182,183). PDGF stimulates chemotaxis of medial smooth muscle cells to the intima, where they proliferate and secrete mucopolysaccharide and later collagen and other extracellular matrix components, resulting in the lesions characteristic of proliferative endarteritis or musculomucoid intimal hyperplasia.

Summary of Pathophysiology

Based on the foregoing discussion, it is clear that the exact pathophysiologic mechanism underlying the transition from benign to malignant hypertension is not fully understood. Undoubtedly, a marked increase in blood pressure is pivotal. Severe hypertension is the common element in malignant hypertension in humans and in each of the animal models of malignant hypertension. Moreover, reduction of the blood pressure leads to a resolution of the malignant phase regardless of the underlying etiology. Thus, a significant elevation of the blood pressure is necessary for the development and progression of malignant hypertension. The major issue is whether the mechanical stress of severe hypertension alone is *sufficient* to cause the transition from benign to malignant hypertension. Because there is considerable overlap in the levels of blood pressure seen in patients with benign and those with malignant hypertension, it is likely that severe hypertension alone is *not*

sufficient to cause the malignant hypertension in all patients and that additional factor(s) probably participate. These cofactors are not necessarily the same in every case. For example, activation of the renin-angiotensin axis may be important in some patients but not in others. In some patients, perhaps catecholamines, or activation of the clotting cascade interact with hemodynamic stress to induce malignant hypertension.

The vicious cycle of malignant hypertension is best demonstrated in the kidneys but applies equally well to the vascular beds of the pancreas, gastrointestinal tract, retina, and brain (Fig. 56-18). In this scheme, severe hypertension is central. Hypertension may be either essential or secondary to any one of a variety of disorders. The interaction between the level of blood pressure and the adaptive capacity of the vasculature may be important. Chronic hypertension results in thickening and remodeling of arterial walls, which may be an adaptive mechanism to prevent vascular damage from mechanical stress (184). However, when the blood pressure rises to a level or at a rate that is excessive, these adaptive mechanisms may be overwhelmed, resulting in vascular damage. As a result of the mechanical stress of increased transmural pressure, focal segments of the vascular system become dilated, producing the sausage-string pattern. Other hormonal factors may act synergistically with hypertension to damage the arterial vasculature. For example, spontaneous natriuresis at some critical level of blood pressure may result in volume depletion with activation of the renin-angiotensin, vasopressin, or catecholamine systems, which further elevates blood pressure. In addition, these hormones may be directly vasculotoxic. Fibrinogen and other plasma proteins may permeate the damaged vascular wall and activate the intrinsic clotting system, causing deposition of fibrin in the vessel wall and lumen, leading to fibrinoid necrosis. The onset of localized intravascular coagulation may cause a cycle in which red blood cells and platelets are disrupted on intravascular strands, with release of adenine diphosphate and thromboplastin with further activation of the clotting cascade. Platelet adherence to damaged endothelium with release of PDGF leads to myointimal proliferation in the

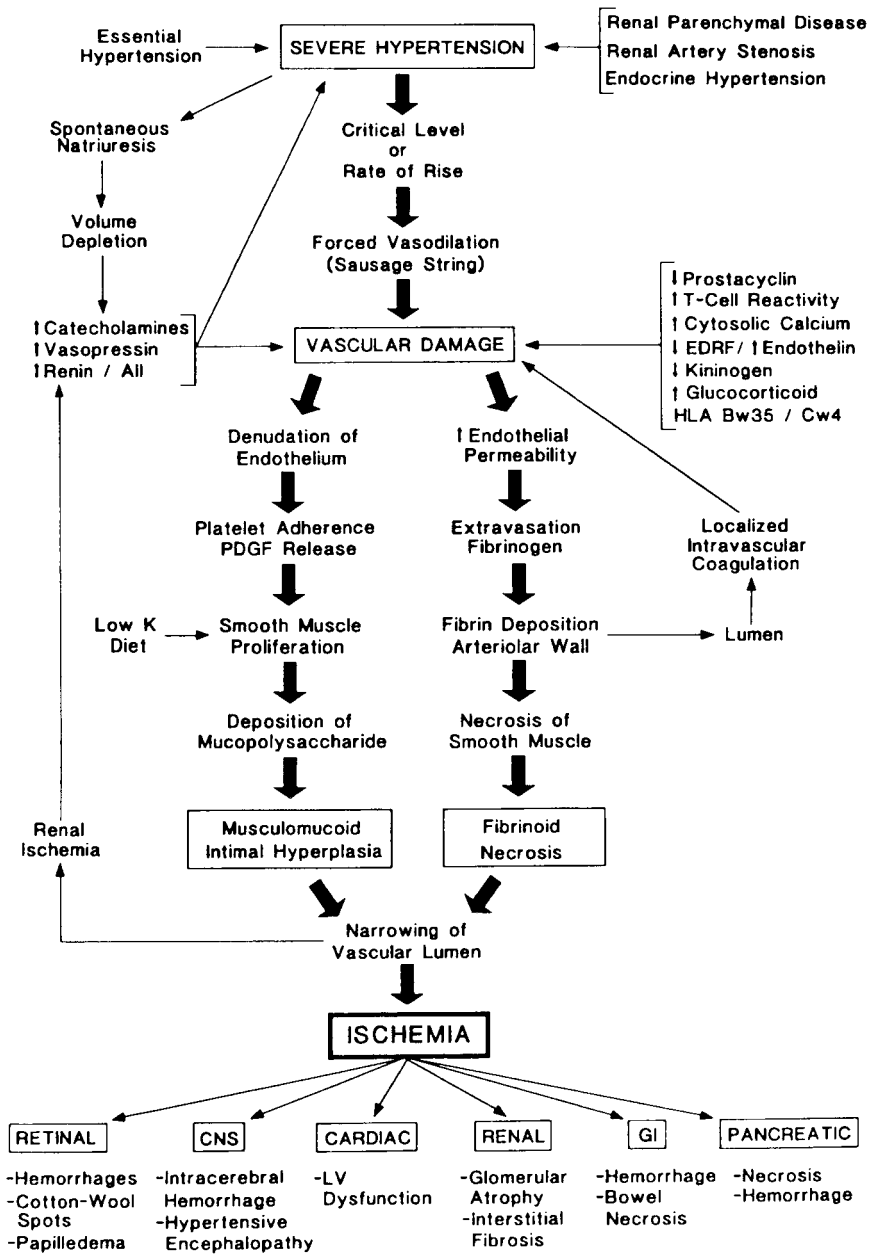

FIGURE 56-18. Pathophysiology of malignant hypertension. *AII*, angiotensin II; *EDRF*, endothelium-derived relaxing factor; *PDGF*, platelet-derived growth factor; *LV*, left ventricular.

interlobular arteries, which results in proliferative endarteritis. The wall thickening and luminal narrowing in the interlobular arteries and afferent arterioles result in glomerular ischemia, accelerated glomerular obsolescence, and renal insufficiency. Renal ischemia may lead to increased activation of the renin-angiotensin system, which may cause further increases in blood pressure, vascular damage, and pressure-induced natriuresis, such that a vicious cycle of hypertension, vascular damage, and renal insufficiency develops.

Other factors, although less certain, may also be important in the development of malignant hypertension. Low dietary potassium may predispose to hypertensive arterial injury in black patients with severe hypertension. Abnormalities in membrane sodium transport and cytosolic calcium may mediate hypertension-induced vascular injury. Cigarette smoking or oral contraceptives may decrease prostacyclin production and inhibit repair of hypertensive vascular damage. Abnormalities in the kallikrein-kinin system may also be important in some cases.

Unfortunately, the chain of pathogenetic processes leading to fibrinoid necrosis and neointimal proliferation have not yet been well studied at the molecular level to accurately assess the role of cytokines and growth factors in the development of these vascular lesions in the systemic and renal vasculature (185).

Pathophysiology of Hypertensive Neuroretinopathy

Retinal arteriolar vasculopathy in malignant hypertension leads to obliteration or rupture of vessels, resulting in striate hemorrhages, cotton-wool spots, and papilledema. Hypertensive neuroretinopathy is not simply the result of renal failure as hypertensive neuroretinopathy can clearly occur in malignant hypertension prior to the onset of clinically significant renal disease (86). It also appears that hypertensive neuroretinopathy often occurs in the absence of increased intracranial pressure (86).

The retinal circulation is under autoregulatory control and does not have a sympathetic nerve supply. As the systemic blood pressure increases, if autoregulation is intact, the retinal arterioles constrict to keep the retinal blood flow constant. The appearance of hypertensive neuroretinopathy implies that autoregulation has failed (85).

Striate hemorrhages result from bleeding from superficial capillaries in the nerve fiber bundles near the optic disc. These capillaries originate from arterioles, so that when autoregulation fails, the high systemic pressure is transmitted directly to the capillaries. This leads to breaks in the continuity of the capillary endothelium with subsequent hemorrhage (85).

Cotton-wool spots result from ischemic infarction of nerve fiber bundles due to arteriolar occlusion (85). Fluorescein angiography demonstrates that cotton-wool spots are areas of retinal nonperfusion (186). Embolization of pig retina with glass beads produces immediate intracellular edema followed by accumulation of mitochondria and other subcellular organelles in the ischemic nerve fibers (187). It has been postulated that the normal axoplasmic flow of subcellular organelles is disrupted by ischemia such that accumulation of organelles in ischemic nerve fiber bundles results in a visible white patch (100). Cotton-wool spots tend to distribute around the optic disc because the nerve fiber bundles are most dense in this region.

The pathogenesis of papilledema in malignant hypertension has been controversial. Pickering (107) maintained that papilledema results from increased intracranial pressure. However, intracranial pressure is not always increased in malignant hypertension (86). Papilledema has been produced in rhesus monkeys by occlusion of the long posterior ciliary artery, which supplies the optic disc (188). Thus papilledema, like cotton-wool spots, most likely results from ischemia of nerve fibers in the optic disc (99,189).

Treatment

Malignant hypertension must be treated expeditiously in order to prevent complications such as hypertensive encephalopathy, intracerebral hemorrhage, acute pulmonary edema, and renal failure. The hypertensive patient with hypertensive neuroretinopathy (hemorrhages, cotton-wool spots with or without papilledema) should be hospitalized for intensive medical therapy. Initiation of appropriate therapy should not be delayed pending extensive laboratory and roentgenographic examinations aimed at defining a potential underlying etiology. The workup for secondary causes should be deferred until the blood pressure has been controlled and the patient stabilized.

The traditional approach to patients with malignant hypertension has been the initiation of therapy with rapid-acting parenteral hypotensive agents such as sodium nitroprusside, trimethaphan, and diazoxide (190). In Table 56-4 are listed the settings in which the use of parenteral antihypertensive agents is recommended for the initial management of malignant hypertension. In general, parenteral therapy should be utilized in patients who have evidence of acute end-organ damage or who are unable to tolerate oral medications. The management of patients with acute hypertensive heart failure, hypertensive encephalopathy, or intracerebral hemorrhage is discussed later in separate sections on each of these topics.

The drug of choice for the management of patients with malignant hypertension requiring parenteral therapy is sodium nitroprusside (191). Preliminary studies suggest that the dopamine receptor (DA_1 selective) agonist fenoldopam may also be useful for parenteral treatment of malignant hypertension (3,192). Trimethaphan is an alternative, but a number of significant adverse effects limit its usefulness. Diazoxide, employed in mini-bolus fashion, may be advantageous in selected patients for whom monitoring in an intensive care unit is not available. Mini-bolus injections of labetalol have also been used for the treatment of malignant hypertension (191).

There are no absolute guidelines for the blood pressure goal during parenteral therapy. The theoretic risks of rapid reduction of blood pressure are discussed later in the section on the controversy over gradual versus rapid reduction of blood pressure. As a general rule, it is safe to initially reduce the mean arterial pressure by 20% or to a level of 160 to 170 mm Hg systolic and 100 to 110 mm Hg diastolic (193). During the reduction of blood pressure with parenteral antihypertensives, the patient should be monitored closely for evidence of cerebral or myocardial hypoperfusion. The use of a short-acting agent

TABLE 56-4

INDICATIONS FOR PARENTERAL THERAPY IN MALIGNANT HYPERTENSION

Patients unable to tolerate oral therapy due to intractable vomiting
Hypertensive encephalopathy
Rapidly failing vision
Intracerebral hemorrhage
Acute pulmonary edema
Acute myocardial infarction
Rapid deterioration of renal function
Acute pancreatitis
Gastrointestinal hemorrhage
Acute abdomen secondary to mesenteric vasculitis

such as sodium nitroprusside or fenoldopam has obvious advantages because the blood pressure can be stabilized quickly at a higher level if complications develop during rapid blood pressure reduction. If there is no evidence of vital organ hypoperfusion following this initial reduction of blood pressure, the diastolic blood pressure can gradually be lowered to 90 mm Hg over a period of 12 to 36 hours.

Oral antihypertensive agents should be initiated as soon as possible so that the duration of parenteral therapy can be minimized. However, a common error in the management of patients with malignant hypertension is the abrupt discontinuation of parenteral therapy immediately after oral therapy has been initiated. With this approach, severe rebound hypertension often develops before the oral antihypertensive regimen becomes effective. Ideally, oral antihypertensives should be initiated as soon as the patient has been stabilized and is able to tolerate medications by mouth. The nitroprusside infusion should be continued until the oral agents have taken effect and have been titrated to an effective dose. The nitroprusside or fenoldopam infusion can then be weaned as the oral regimen is gradually increased.

Although other agents may be effective in the long-term management of patients with malignant hypertension, the cornerstone of initial oral therapy should be an arteriolar vasodilator such as hydralazine, sustained-release nifedipine, or minoxidil. Vasodilators may reflexively activate the adrenergic system and cause tachycardia with an increase in cardiac output, which may blunt the hypotensive response. Therefore, treatment with β-adrenergic blockers is usually also required. Direct-acting vasodilators also cause renal salt and water retention, fluid overload, and the development of pseudotolerance to the hypotensive effect of the drug. Thus, although diuretics may not be required for the initial management of patients with malignant hypertension *(vide infra)*, they are usually required as a part of the long-term maintenance antihypertensive regimen. The regimen that follows has proved to be generally effective in the conversion from parenteral to oral therapy. After the blood pressure has been controlled with sodium nitroprusside and while the infusion is continued, hydralazine (50 mg) and propranolol (40 mg) are administered orally. As the oral agents become effective and the blood pressure declines, the nitroprusside infusion is tapered. Brief interruption of the infusion can be used to assess the hypotensive response to oral agents. If after 6 to 8 hours the diastolic blood pressure remains higher than 100 mm Hg, a second dose of hydralazine (100 mg) should be given. The propranolol dose is increased as needed to maintain adequate β-blockade (heart rate, 60 to 80 beats/minute). The usual dose of propranolol is 80 to 120 mg administered twice daily, but larger doses occasionally may be required. If the blood pressure is not controlled with hydralazine at a dose of 100 mg twice daily, minoxidil should be substituted for hydralazine. The starting dose of minoxidil (2.5 mg) is increased by 2.5 to 5.0 mg every 6 to 8 hours until the blood pressure is adequately controlled. The usual effective dose is 5 to 10 mg twice daily. Treatment with a beta-blocker is recommended as for hydralazine. As the blood pressure is brought under control with oral agents, the sodium nitroprusside infusion is gradually weaned. When the convalescing patient is mobilized, upright blood pressure should be carefully monitored to avoid significant orthostatic hypotension. A diuretic, usually furosemide at a starting dose of 40 mg twice daily, is added to either the hydralazine or the minoxidil regimen when it becomes evident that salt and water retention is beginning to occur.

Volume Status and the Role of Diuretics

It has traditionally been taught that patients with malignant hypertension require potent parenteral diuretic therapy during the initial phase of treatment with parenteral vasodilators (190). However, there is now evidence suggesting that routine parenteral diuretic therapy during the acute phase of treatment for malignant hypertension may actually be deleterious (41). Overdiuresis may result in deterioration of renal function due to superimposed prerenal azotemia. Moreover, volume depletion may activate the renin-angiotensin axis and other pressor hormone systems.

Even patients with malignant hypertension and pulmonary edema may not have an increase in total body salt and water content. Pulmonary congestion in this setting may result from an increase in left ventricular filling pressure due to a decrease in the compliance of the left ventricle (diastolic dysfunction) rather than an increase in left ventricular volume per se. With severe hypertension, the ventricle may become noncompliant due to the excessive workload imposed by the elevated systemic vascular resistance. As a result, left ventricular end-diastolic pressure (LVEDP) increases dramatically even though left ventricular end-diastolic volume may be near normal. With vasodilator therapy, the systemic vascular resistance decreases, left ventricular compliance improves, LVEDP decreases, and left ventricular end-diastolic volume may actually increase (194). Despite the increase in left ventricular end-diastolic volume, pulmonary congestion improves because of the reduction in pulmonary capillary pressure. Thus, even in patients with malignant hypertension complicated by pulmonary edema, afterload reduction rather than vigorous diuretic therapy should be the mainstay of initial therapy.

Some patients with malignant hypertension may actually benefit from a cautious trial of volume expansion. Intravascular volume depletion in patients with malignant hypertension should be considered in patients with exquisite sensitivity to vasodilator therapy manifest by a precipitous drop in blood pressure at relatively low infusion rates. Patients with malignant hypertension due to analgesic nephropathy are particularly prone to be severely volume-depleted at presentation due to the presence of chronic interstitial damage with a salt-wasting nephropathy (41,42).

In summary, the need for diuretic therapy during the initial phase of treatment for malignant hypertension depends on an assessment of volume status. Unless obvious fluid overload is present, diuretics should not be given initially. Although vasodilator therapy will eventually result in salt and water retention by the kidney, an increase in total-body sodium content cannot occur unless the patient is given sodium. Thus, in the initial phase of treatment, patients should be placed on a no-added salt diet with close monitoring of intravenous fluid administration. Nonetheless, during subsequent long-term treatment with oral vasodilators, the use of diuretics is usually mandatory in order to prevent fluid retention and maintain adequate blood pressure control.

Management of Malignant Hypertension Complicated by Renal Insufficiency

All patients with malignant hypertension should receive aggressive antihypertensive therapy to prevent further renal damage, regardless of the degree of renal impairment. Control of blood pressure in patients with malignant hypertension and renal insufficiency occasionally precipitates oliguric acute renal failure, especially when the initial glomerular filtration rate is less than 20 mL/minute. However, this is not a contraindication to aggressive antihypertensive therapy aimed at normalization of the blood pressure. Control of hypertension protects other vital organs such as the brain and heart whose function cannot be replaced. Moreover, with tight blood pressure control, even patients who appear to have ESRD due to malignant nephrosclerosis have recovered renal function (95,97,195–205).

In patients in whom aggressive control of hypertension precipitates the need for dialysis, dialysis is utilized to control

serum chemistry values, treat uremia, and correct fluid overload. However, since dialysis alone rarely results in adequate control of blood pressure in patients with malignant hypertension, concomitant antihypertensive drug therapy is almost always required. A regimen with minoxidil and propranolol has proved to be particularly efficacious in this setting (95,96,200,203,206,207).

Role of Nephrectomy

In the past, the use of bilateral nephrectomy to control severe hypertension in patients with malignant hypertension and azotemia was advocated (208–210). In many patients with malignant hypertension and uremia accompanied by encephalopathy, bilateral nephrectomy was lifesaving. In 1972, Lazarus and associates (209) proposed a role for urgent bilateral nephrectomy in patients with malignant hypertension who had life-threatening complications such as cerebrovascular accident, rapidly progressive congestive heart failure, or encephalopathy. The authors suggested that nephrectomy might be of value if performed early, even before the development of ESRD. Patients with serum creatinine levels as low as 7.0 mg/dL were sometimes nephrectomized.

However, following the introduction of minoxidil, the role of nephrectomy in the management of malignant hypertension with azotemia was questioned (211). Eleven patients with malignant hypertension who had been refractory to maximal doses of conventional antihypertensive agents were reported. Seven of these patients had advanced renal failure and were candidates for nephrectomy to control blood pressure. Institution of a regimen of minoxidil combined with diuretics or dialysis to control fluid retention and propranolol to control reflex tachycardia resulted in blood pressure reduction to normotensive levels in all patients with remarkably few side effects (207). Even in patients with renal failure requiring dialysis, hypertension can usually be controlled with minoxidil (202,206,207,211). Given the proven efficacy of minoxidil, bilateral nephrectomy is rarely, if ever, indicated to control refractory hypertension. Nephrectomy should clearly be a last resort as delayed recovery of renal function is possible in many cases.

In summary, the long-term management of patients with ESRD secondary to malignant hypertension should include vigorous antihypertensive therapy with the goal of sustained normotension. Dramatic recovery of renal function may occasionally occur. Even if renal function fails to recover, adequate control of blood pressure is essential to prevent other potentially fatal complications of malignant hypertension such as hypertensive encephalopathy, intracerebral hemorrhage, congestive heart failure, and hemorrhagic pancreatitis.

Initial Oral Therapy

While many patients with malignant hypertension require prompt treatment with parenteral antihypertensive agents, some patients may not yet have evidence of cerebral or cardiac complications, or rapidly deteriorating renal function and therefore do not require instantaneous control of the blood pressure (41,190,212,213). These patients may be safely managed with an intensive oral regimen designed to bring the blood pressure under control over a period of 12 to 24 hours.

In patients with malignant hypertension, a multidrug oral regimen is often required to achieve adequate blood pressure control. The most useful combinations include a diuretic, a β-adrenergic blocker, and an arteriolar vasodilator. Minoxidil appears to be particularly well suited for the initial management of malignant hypertension that requires prompt but not immediate blood pressure reduction (212,214–216). Alpert and Bauer (216) describe the use of a triple regimen of furosemide, propranolol, and minoxidil in nine patients with a diastolic blood pressure higher than 120 mm Hg. Seven of these patients had malignant hypertension. Furosemide (40 mg) and propranolol (40 mg) were given initially by mouth. Two hours later, if the diastolic pressure was higher than 120 mm Hg, a loading dose of minoxidil (20 mg) was administered. If the diastolic pressure was still over 100 mm Hg 4 hours after the loading dose, a booster dose of minoxidil was given. The amount of the booster dose was estimated based on the magnitude of the response to the loading dose. Maintenance therapy with minoxidil was begun with one-half the sum of the loading and booster doses given twice daily, with adjustment of beta-blocker and diuretic doses as necessary for control of heart rate and fluid balance. Following the booster dose of minoxidil, a sustained decrease in blood pressure was seen in all patients. No overshoot hypotension or other adverse effects were encountered. During long-term therapy, the physicians were able to substitute hydralazine for minoxidil in five patients. However, the remaining four patients required chronic minoxidil therapy for adequate blood pressure control (212,216).

Initial oral therapy with sustained-release nifedipine recently was shown to be effective in the management of malignant hypertension in black patients who did not require parenteral therapy for hypertensive encephalopathy or acute pulmonary edema (213). No precipitous decreases in blood pressure or neurologic complications were encountered. However, despite adequate control of blood pressure during the first 24 hours with sustained-released nifedipine, all patients eventually required one or more additional drugs for long-term blood pressure control. Although treatment with sublingual or oral nifedipine capsules has also been recommended for the initial management of malignant hypertension (217,218), the sustained-release nifedipine preparation is preferable because there appears to be less risk of overshoot hypotension (213).

The angiotensin-converting enzyme inhibitors have also been reported to be effective in the treatment of malignant hypertension. However, angiotensin-converting enzyme inhibitors can produce profound hypotension in volume-depleted patients, so they should be used with caution during the initial phase of treatment. Moreover, they may not always be effective in the acute management of malignant hypertension (219).

Oral loading regimens with clonidine have been advocated in severe uncomplicated (urgent) hypertension (220). However, there is limited information on the use of oral clonidine loading in the initial management of malignant hypertension. Clonidine loading can cause significant sedation, which may interfere with the assessment of potential neurologic complications during acute blood pressure reduction. Moreover, common side effects such as sedation and dry mouth can have a negative impact on compliance in patients treated with clonidine for the long term. Thus, oral clonidine loading regimens are not indicated for the initial management of malignant hypertension.

Long-Term Management

After the immediate crisis has resolved and the blood pressure has been brought under control with parenteral therapy, oral therapy, or both, lifelong surveillance of the blood pressure is essential. Close follow-up and aggressive treatment are mandatory because noncompliance or inadequate therapy may have devastating consequences. If blood pressure control becomes inadequate, malignant hypertension may recur even after years of successful antihypertensive therapy. In a study of the quality of care provided to patients with a history of malignant hypertension who subsequently died, only 27% of patients had an average treated diastolic blood pressure of less than 110 mm Hg (221). Thus, meticulous long-term treatment of hypertension is imperative in patients with a history of malignant hypertension. Triple therapy with a diuretic, a beta-blocker, and

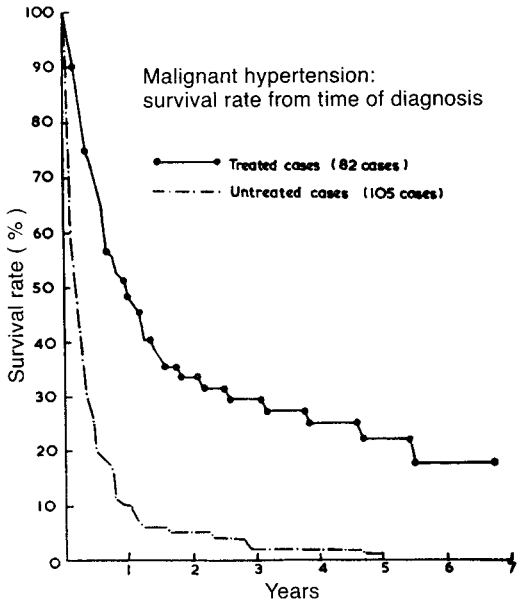

FIGURE 56-19. Survival in patients with malignant hypertension. Treated patients received ganglionic blocking agents. Untreated patients are historical controls from the pretreatment era. (From: Harington M, Kincaid-Smith P, McMichael J. Results of treatment in malignant hypertension: a seven-year experience in 94 cases. *Br Med J* 1959;2:969, with permission.)

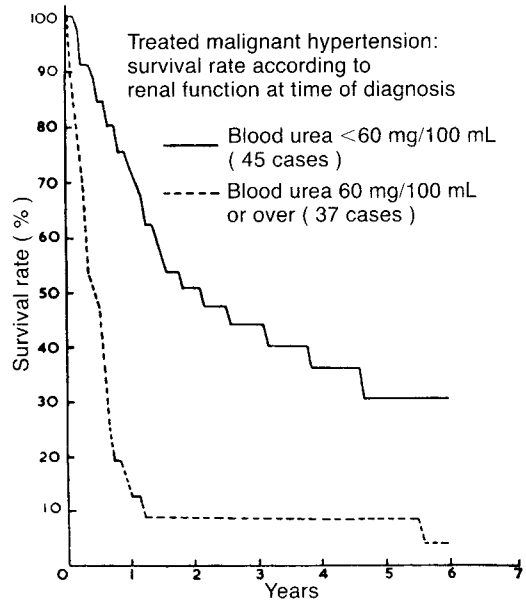

FIGURE 56-20. Survival in treated patients with malignant hypertension according to the level of renal function at the time of initial presentation. (From: Harington M, Kincaid-Smith P, McMichael J. Results of treatment in malignant hypertension: a seven-year experience in 94 cases. *Br Med J* 1959;2:969, with permission.)

a vasodilator is often required to achieve satisfactory blood pressure control.

Response to Therapy

In the absence of adequate blood pressure control, malignant hypertension has a uniformly poor prognosis. Without treatment, the 1-year mortality rate approaches 80% to 90%, and uremia is the most common cause of death (25,26). However, since the introduction of potent antihypertensive agents, studies have shown that with control of blood pressure, dialysis-free survival can be substantially prolonged (130) (Fig. 56-19).

In a more recent series of treated patients collected between 1979 and 1985, the 1-year and 5-year survival rates were 94% and 75%, respectively. The rates for renal survival, defined as patients surviving with native renal function, were 66% at 1 year and 51% at 5 years (32). In another series collected between 1980 and 1989, the 5-year survival was 74% (221). Median survival time was found to be significantly shorter in black patients compared to white or Asian patients (221). The poorer prognosis for black patients could be explained by their late presentation with severe hypertension and the higher prevalence of renal impairment at presentation. The improved patient survival in recent years has been attributed to a decrease in uremic deaths due to not only the availability of dialysis but also the prevention of ESRD because of adequate blood pressure control (28,29). Nonetheless, malignant hypertension remains a disease with a poor overall prognosis associated with a substantial risk of progression to death or chronic renal failure (221).

Prognostic Importance of Renal Function at the Time of Diagnosis

Numerous series have documented the prognostic significance of the level of renal function at the time of the initial presentation with malignant hypertension. In the early studies of the response of malignant hypertension to antihypertensive therapy, those patients with an initial blood urea level higher than 60 mg/dL (BUN greater than 30 mg/dL) had a 13% 1-year survival rate, compared with a 73% 1-year survival rate among those with an initial blood urea less than 60 mg/dL (130) (Fig. 56-20). Thus, with antihypertensive therapy, renal function tended to remain normal in patients with good renal function at presentation. In contrast, despite blood pressure control, renal function often deteriorated in patients with renal insufficiency at presentation. It was concluded that hypotensive therapy did not halt the progression of established renal insufficiency, and that a good long-term prognosis could be anticipated only in patients with nonuremic malignant hypertension.

Based on the experience between 1950 and 1965, most published series concluded that aggressive treatment of malignant hypertension in patients with renal insufficiency often resulted in worsening of renal function and sometimes even precipitated ESRD. As recently as the mid-1960s, it was routinely stated that azotemic patients with malignant hypertension should be managed conservatively, and that if the BUN was higher than 60 mg/dL, no antihypertensive therapy should be undertaken (222). Woods and Blythe were the first to report that treatment of hypertension prolonged survival in patients with malignant hypertension and severe renal insufficiency (223,224). Subsequent studies demonstrated that aggressive antihypertensive therapy could result in substantial improvement in renal function (225,226). The crucial factor in determining the risk of progression to ESRD appeared to be the adequacy of long-term blood pressure control.

There may be two distinct groups of patients with malignant hypertension and renal insufficiency (225). In one group, malignant hypertension is secondary to underlying renal parenchymal disease. Renal biopsy reveals evidence of primary glomerulonephritis with superimposed vascular changes of malignant nephrosclerosis. In this group, initial control of blood pressure may result in a temporary stabilization of renal function. However, despite adequate blood pressure control, eventually

the underlying renal disease slowly progresses to end-stage. In the other group, primary (essential) malignant hypertension is present and renal biopsy reveals only malignant nephrosclerosis with ischemic glomeruli. In these patients, intensive antihypertensive therapy may result in an improvement in renal function, especially if initial renal impairment is mild to moderate. Even in patients with severe renal impairment, recovery of renal function sometimes occurs during sustained normotension. However, many months of therapy may be required before recovery of renal function is apparent, presumably reflecting the time required for resolution of the vascular lesions (205).

Recently, the observation has been made that the combined length of the two kidneys at presentation (combined renal length) as determined by ultrasonography is predictive of the chance for recovery of renal function in patients with malignant hypertension and severe renal failure (227). The mean combined renal length was 20.2 cm in the group that recovered renal function and 14.2 cm in the group with persistent renal failure despite strict blood pressure control.

Despite the prognostic significance of the initial level of renal function in patients with malignant hypertension (32), a number of recent reports describe dramatic recovery of renal function in patients with presumed ESRD due to malignant nephrosclerosis, even after maintenance hemodialysis for months to years. Recovery of renal function in these cases has been attributed to strict control of blood pressure. These reports challenged the notion that established renal failure in malignant hypertension is irreversible (95,96,195–205). In the majority of these reports, recovery of renal function was associated with the use of the potent peripheral vasodilator minoxidil in combination with a β-adrenergic blocking drug and dialysis. Often patients who had refractory hypertension or disabling side effects while taking maximal doses of conventional antihypertensive agents eventually became normotensive with minimal side effects after the initiation of this regimen (206,207). Recovery of renal function with strict control of blood pressure has been reported up to 26 months after the initiation of maintenance hemodialysis (96). In most of the reports describing the recovery of renal function after prolonged maintenance dialysis, the initial clinical presentation was that of oliguric acute renal failure (200). In the largest reported series, 12 out of 54 patients with primary malignant hypertension requiring dialysis recovered sufficient renal function to allow withdrawal of dialysis (228). Substantially delayed recovery of renal function following initiation of dialysis for malignant nephrosclerosis is thus a distinct possibility and should be considered in such patients before long-term strategies such as renal transplantation are contemplated (205). In the modern treatment era it appears that the severity of malignant hypertension and the magnitude of renal impairment at presentation do not always predict outcome (229). Instead, the best predictor of long-term renal function seems to be the adequacy of the blood pressure control obtained during follow-up.

In patients with malignant hypertension who progressed to ESRD and were treated with long-term dialysis, the survival rates at 1, 5, and 8 years were 87%, 82%, and 50%, respectively. This was not different from age-matched controls with other causes of renal failure admitted to the same dialysis unit (230). One study found that the chance of recovery of renal function was better for patients with primary malignant hypertension treated with continuous ambulatory peritoneal dialysis compared with conventional hemodialysis (231).

Mechanism of Recovery of Renal Function

The mechanism of recovery after prolonged renal failure in malignant hypertension is uncertain. Extensive tubular damage resembling acute tubular necrosis has been reported in patients with oliguric acute renal failure (97,200). This tubular damage is thought to result from ischemia caused by the obliterative vascular lesions of malignant nephrosclerosis. In this regard, it has been postulated that the mechanism of recovery is the resolution of tubular necrosis (200). However, it is not clear why the need for dialysis often persists well beyond the usual time frame for resolution of ischemia-induced acute tubular necrosis.

Kincaid-Smith postulated that the initial loss of renal function results from glomerular ischemia due to narrowing of the interlobular arteries by proliferative endarteritis (41). Although endarteritis may be arrested by adequate blood pressure control, narrowing of the arterioles may persist. Therefore, improvement in renal function may not result from the resolution of arteriolar lesions, but rather from hyperfiltration by the remaining nephrons supplied by patent interlobular arteries. Regardless of the mechanism, it is clear that recovery of renal function is critically dependent on sustained normalization of blood pressure.

Reversal of Hypertensive Neuroretinopathy

The funduscopic changes associated with hypertensive neuroretinopathy are reversible with control of blood pressure (85,232). Striate hemorrhages cease to form as soon as the blood pressure is controlled. Clearance of existing hemorrhages takes 2 to 8 weeks. Cotton-wool spots may continue to form for several days after the blood pressure is controlled. The cellular (axonal) debris that comprises the cotton-wool spots is cleared away within 2 to 12 weeks. Hard exudates clear more slowly. A macular star may require more than a year to resolve completely. Papilledema often continues to increase during the first few days of treatment. However, in the majority of patients, it resolves slowly over several weeks. In contrast, the changes reflecting retinal arteriolosclerosis such as arteriolar narrowing, arteriovenous crossing defects, and changes in the light reflexes usually persist despite adequate blood pressure control (232).

Evaluation for Secondary Causes

The various secondary causes of malignant hypertension were discussed previously in the section on etiologies of malignant hypertension. Whereas less than 5% of patients with benign hypertension have an underlying secondary cause of hypertension, malignant hypertension may be associated with a secondary cause in up to 50% of patients. For example, among patients with benign hypertension, the incidence of renovascular hypertension was less than 0.5% (233). In contrast, there is a substantial incidence of renovascular hypertension (43% in whites, 7% in blacks) among patients with malignant hypertension (46). Thus, after malignant hypertension has been treated successfully, the possibility of underlying renovascular hypertension should be investigated. Noninvasive screening tests such as rapid sequence intravenous pyelography and radionuclide renal scans are of little value because of the high frequency of false-positive and false-negative results (233). Renal arteriography is the procedure of choice to exclude the possibility of anatomic renal artery stenosis. The diagnosis and treatment of renovascular hypertension is discussed in detail in Chapter 52.

Pheochromocytoma is a rare cause of malignant hypertension. However, given the likelihood of surgical cure or amelioration of hypertension, pheochromocytoma should be considered if symptoms consistent with catecholamine excess persist following control of blood pressure. The approach to the diagnosis of pheochromocytoma is discussed in Chapter 55.

Primary hyperaldosteronism due to an adrenal aldosterone-producing adenoma is an extremely rare cause of malignant

hypertension. However, the biochemical abnormalities in patients with treated malignant hypertension may mimic those of primary hyperaldosteronism. Long after the malignant phase has resolved, hypokalemia with inappropriate urinary potassium wasting, increased aldosterone secretion, and suppressed PRA may persist. This phenomenon may represent a form of tertiary hyperaldosteronism (63). With sustained treatment of hypertension, this hyperaldosteronism eventually resolves. Because primary hyperaldosteronism is an unusual cause of malignant hypertension, an evaluation for primary hyperaldosteronism should not be undertaken unless these abnormalities persist for more than a year after malignant hypertension has resolved.

The role of renal biopsy in the diagnosis of possible underlying primary renal parenchymal disease in patients with malignant hypertension is controversial. In patients presenting with malignant hypertension and renal failure, it may not be possible on clinical grounds to distinguish primary malignant hypertension from chronic glomerulonephritis or chronic interstitial nephritis with superimposed malignant nephrosclerosis. A renal biopsy may be required to make this distinction. When the kidneys appear small by ultrasonography, a biopsy is not indicated because it is unlikely that the results of the biopsy will alter therapy. In contrast, when the kidneys are normal in size, a renal biopsy may provide useful information. If primary malignant nephrosclerosis with ischemic but viable glomeruli is found, then intensive antihypertensive therapy may be associated with the eventual recovery of renal function, even after months of maintenance dialysis. Conversely, the finding of chronic glomerulonephritis or chronic interstitial nephritis with superimposed malignant nephrosclerosis suggests a less favorable long-term outcome.

Malignant hypertension can mimic acute glomerulonephritis or vasculitis. Patients can present with severe hypertension and oliguric acute renal failure with a nephritic urinary sediment (97). In this setting, diagnostic renal biopsy is essential since acute glomerulonephritis or vasculitis may require specific therapy in addition to antihypertensive treatment.

Since uremia and severe hypertension predispose to serious hemorrhagic complications after renal biopsy, it is prudent to manage the patient with dialysis and blood pressure control for 1 to 3 weeks prior to performance of a percutaneous renal biopsy. Unfortunately, this delay in obtaining tissue may make the diagnosis of malignant nephrosclerosis more difficult because the lesions of fibrinoid necrosis may heal rapidly with the institution of antihypertensive treatment, leaving a residual hyaline or fibrous scar (130,132). Moreover, given the sampling error inherent in closed renal biopsy, the patchy lesions of malignant nephrosclerosis might be missed. Thus, the diagnosis of malignant nephrosclerosis is often made on the basis of the findings of accelerated glomerular obsolescence and marked intimal hyperplasia of the arterioles (126).

Unilateral renal disease from atrophic pyelonephritis occasionally causes malignant hypertension in both children and adults (22,39,40). However, the experience with unilateral nephrectomy for hypertension control has been disappointing (234). In children, cure of malignant hypertension has been reported after partial nephrectomy of a scarred segment if high renin values are documented on segmental renal vein catheterization (235,236).

Benign Versus Malignant Hypertension

Since the original description by Volhard and Fahr (12), two forms of essential hypertension have been recognized, benign and malignant. It is worth emphasizing that these two forms of hypertension should be conceptualized as distinct clinical and pathologic entities. In benign hypertension there is usually a long asymptomatic phase, with death resulting from complications of cerebrovascular disease, atherosclerotic disease, or congestive heart failure, rather than renal disease (29). In benign essential hypertension (i.e., without underlying primary renal disease or superimposition of malignant hypertension), ESRD seldom occurs (81,93,125,237,238). In contrast, malignant hypertension left untreated, uniformly progresses to ESRD.

There is much controversy in the field of hypertension regarding the frequency with which benign hypertension (benign arteriolar nephrosclerosis), in the absence of occult primary renal disease or superimposed malignant hypertension, causes ESRD. Recent reviews suggest that the number of patients reaching ESRD attributable to benign nephrosclerosis might have been significantly overestimated (239,240). Goldring and Chasis (93) extensively evaluated renal function in a large group of patients with essential hypertension in the pre-antihypertensive treatment era. Most patients with long-standing essential hypertension had anatomic lesions in kidneys consistent with hyaline arteriolar nephrosclerosis. Moreover, the majority had demonstrable renal abnormalities including abnormal urinalysis with hyaline and granular casts, low-grade proteinuria (less than 1 g/day), decreased tubular maximum for para-aminohippurate, decreased renal blood flow, normal to slightly decreased glomerular filtration rate, and increased filtration fraction. However, they found that ESRD rarely occurred in patients with benign hypertension. Among 150 hypertensive patients with ESRD, only one was found to have benign nephrosclerosis as the sole underlying etiology (93). These authors concluded that in patients with benign hypertension, functional failure occurred earlier in the heart and brain than in the kidney and that death from renal failure without superimposed malignant hypertension was a rare event.

In contrast to these early reports, which were based principally on renal histologic findings at autopsy, in more recent series, "hypertensive nephrosclerosis" is listed as a common cause of ESRD, especially among African American patients. For example, blacks have a four- to eightfold elevation in the risk of hypertension-induced ESRD compared to whites (241,242). The studies suggest that much of the excess risk of ESRD among blacks can be explained by an extraordinarily high rate of renal failure from hypertensive nephrosclerosis. On a national scale, an estimated 29% of blacks with ESRD have hypertension as the primary cause (241). However, in these recent studies, classification of the causes of ESRD was based on clinical rather than histologic evidence. Furthermore, in these studies it was not clear whether the term *hypertensive nephrosclerosis* refers to benign or malignant nephrosclerosis. In the few available studies detailing the pathologic findings in blacks with ESRD due to hypertension, the characteristic findings have been those of malignant nephrosclerosis, namely, musculomucoid intimal hyperplasia of the interlobular arteries and accelerated glomerular obsolescence (35,173). Moreover, there appears to be a racial bias with regard to the diagnosis of hypertensive nephrosclerosis. In a recent survey, nephrologists were asked to review identical case histories of patients with ESRD in which only the race of the patient was randomly assigned as either black or white. It was found that black patients were twice as likely as white patients to be labeled as having ESRD secondary to hypertensive nephrosclerosis (243).

The relationship between essential hypertension and ESRD remains circumstantial despite the fact that these syndromes have long been associated in the medical literature (240). Nephrologists credit essential hypertension as the cause of ESRD in 25% of patients initiating Medicare-supported renal replacement therapy. Surprisingly, the widely held notion that benign hypertension with benign nephrosclerosis is a common cause of ESRD is difficult to support (81,239,240,244). In contrast to the large body of literature relating mild to moderate

benign hypertension to excessive cardiovascular morbidity, there is a dearth of information available regarding the corresponding risk of significant renal disease (244). In available studies, serum creatinine levels infrequently increase in patients with long-standing mild to moderate hypertension. An analysis of the data from three recent large clinical trials in patients with essential hypertension revealed that advanced renal failure developed in less than 1% of 10,000 patients during the 4 to 6 years of follow-up (89,245–247). Moreover, a very low incidence of clinically significant deterioration of renal function was also noted in the Hypertension Detection and Follow-up Program (248). Another study of untreated patients with mild to moderate essential hypertension found only minor declines in glomerular filtration rate (1.6%/year) and renal blood flow (2.1%/year), which did not differ from the renal function decline associated with aging in normotensive individuals (249). Even severe untreated hypertension (diastolic blood pressure, 120 to 150 mm Hg), in the absence of a malignant hypertension (hypertensive neuroretinopathy), caused only a minor decrement in glomerular filtration rate (1.7%/year) (249). Thus, hypertensive nephrosclerosis is commonly reported to Medicare as the cause of ESRD despite the fact that the risk of progressive renal dysfunction in clinical studies of patients with essential hypertension appears to be very low. This paradox could possibly be explained by the fact that the number of patients with essential hypertension is so large that even the small percentage at risk constitutes a relatively large number of patients who eventually develop ESRD. Long-term follow-up data from the Multiple Risk Factor Intervention Trial (MRFIT), in which over 322,000 men were screened for possible entry, support this hypothesis (250). A direct correlation was found between the initial blood pressure and the risk of development of ESRD from any cause at 16-year follow-up. Nonetheless, the age-adjusted rate of ERSD in this group was only 0.34% at 16 years.

Patients classified as having hypertensive ESRD typically present with advanced disease, making the processes that initiated the renal disease difficult to discern. It has been proposed that many patients classified as having hypertensive nephrosclerosis actually have intrinsic renal parenchymal disease (often IgA nephropathy), unrecognized renal artery stenosis with ischemic nephropathy, unrecognized episodes of malignant hypertension, or primary renal microvascular disease (239,240). At least among white patients with hypertension and renal impairment, if renal artery stenosis and malignant hypertension have been excluded, the most likely diagnosis is underlying primary renal parenchymal disease rather than benign nephrosclerosis (81,251).

In contrast to these studies, a provocative study found that mild to moderate benign hypertension did cause renal insufficiency that progressed despite adequate blood pressure control (252). However, since renal biopsies were not performed, the data do not exclude the possibility of occult primary renal parenchymal disease in patients demonstrating progressive renal insufficiency (253).

In summary, while it is clear that malignant hypertension is a frequent cause of ESRD, especially among blacks, there remains considerable controversy regarding the commonly held belief that benign hypertension *per se* commonly causes ESRD. The critical issue that has yet to be resolved is why blacks constitute a disproportionately high percentage of patients with ESRD in the United States (241). Epidemiologic studies suggest that essential hypertension occurs more frequently in blacks and is associated with more severe cardiovascular end-organ damage for any given level of blood pressure (254). In angiographic studies of patients with mild to moderate essential hypertension and normal renal function, blacks tended to have more severe angiographic evidence of nephrosclerosis than did whites (255). Tobian postulates that the low-potassium diet characteristically consumed by blacks in the United States (30 mmol/day versus 65 mmol/day in the general population) accelerates the intimal thickening of the renal vasculature that occurs due to hypertensive damage. He proposes that this might account for the increased risk of progressive renal insufficiency due to hypertension among blacks (173).

There are several other plausible explanations for the high frequency with which hypertensive nephrosclerosis is reported as a cause of ESRD in the black population. Since most of the available data are based on clinical diagnoses, there may be a tendency on the part of physicians to identify hypertension as the cause of ESRD given the known high prevalence of hypertension in blacks, even when a primary renal parenchymal disease cannot be excluded on clinical grounds (244). Another possibility is that blacks with essential hypertension tend to develop more severe benign nephrosclerosis, which, unlike benign nephrosclerosis in whites, more often results in progressive renal insufficiency and ESRD (240). Results from the African American Study of Kidney Disease (AASK) Trial indicate that benign nephrosclerosis can be accurately diagnosed in black patients with hypertension and renal insufficiency. A renal biopsy was performed in 39 nondiabetic black patients with chronic renal failure who did not have marked proteinuria (urine protein to creatinine ratio less than 2.0). Only changes compatible with benign nephrosclerosis were seen in 38 patients. The remaining patient most likely had primary focal segmental glomerulosclerosis (256). It is possible that genetic factors may increase the susceptibility of blacks to renal damage induced by benign hypertension. Animal studies in which genetically different but histocompatible kidneys were exposed to the same blood pressure in an individual host have clearly demonstrated that some kidneys are more sensitive than others to hypertension-induced renal damage (257). Finally, it is possible that recurrent bouts of unrecognized or inadequately treated malignant hypertension are an underestimated cause of the high incidence of ESRD in minority populations. In this regard, a recent study of 100 patients admitted to an inner-city hospital with a diagnosis of hypertensive emergency showed that two-thirds had malignant hypertension based on funduscopic findings (258). These patients were predominantly young, male, black, or Hispanic individuals of lower socioeconomic status. At least 93% of these patients had been previously diagnosed as hypertensive, and at least 83% were aware of their diagnosis of hypertension. At least 87% were known to have received prior pharmacologic treatment for hypertension. However, no source of regular health care could be documented in 60% of patients. More than 50% were noted to have stopped their antihypertensive medications more than 30 days prior to admission and only 24% had taken any medication on the day of admission. If the overrepresentation of young blacks with ESRD is due to undiagnosed or inadequately treated malignant hypertension, this would have tremendous public health implications given that because malignant hypertension is clearly preventable, and even significant renal dysfunction is potentially reversible with tight control of blood pressure.

HYPERTENSIVE ENCEPHALOPATHY

Most of the deleterious effects of hypertension on the brain are the result of long-standing mild to moderate elevations of blood pressure, including atherothrombotic infarction, lacunar infarction, and intracerebral hemorrhage. Occasionally, severe acute hypertension can produce dramatic and life-threatening cerebral complications. Hypertensive encephalopathy is an acute cerebral syndrome that develops in association with a sudden, sustained elevation of blood pressure (106). It can

occur with malignant hypertension or severe hypertension that is not accompanied by hypertensive neuroretinopathy. Hypertensive encephalopathy is a medical emergency that demands prompt diagnosis and rapid control of blood pressure to prevent irreversible brain damage or death. The clinical *sine qua non* of hypertensive encephalopathy is the prompt resolution of symptoms when the blood pressure is brought under control.

Clinical Presentation

The diagnosis of hypertensive encephalopathy is usually made on clinical grounds. The appearance of cerebral symptoms usually follows the sudden onset of hypertension in previously normotensive individuals or an abrupt increase in blood pressure in patients with chronic hypertension. The abrupt blood pressure elevation usually occurs 12 to 48 hours before the onset of symptoms, although this is often difficult to document. Symptoms may appear at lower levels of blood pressure in previously normotensive individuals compared to those with chronic hypertension. For example, in children with acute glomerulonephritis or pregnant women with eclampsia, hypertensive encephalopathy may occur when the blood pressure is no higher than 160/100 mm Hg (259). However, the syndrome rarely occurs in chronically hypertensive individuals at pressures less than 200/120 mm Hg and may not occur until the blood pressure is more than 250/150 mm Hg.

The initial symptom of hypertensive encephalopathy is usually a severe, generalized headache that increases steadily in severity (260). Unfortunately, headache is a nonspecific symptom, and even among patients with malignant hypertension, it does not necessarily imply central nervous system damage (105). Weakness, nausea, and vomiting (sometimes projectile) are often present. Neck stiffness is an occasional finding. Loss of vision is another common feature. Visual loss may be caused by the retinal edema and hemorrhages that accompany hypertensive neuroretinopathy or as the result of cortical (occipital) blindness (261). Denial of visual loss or loss of vision in the presence of a normal light reflex suggests cortical blindness.

Altered mental status is a prominent clinical feature of hypertensive encephalopathy. Apathy, somnolence, and confusion are the initial manifestations that usually appear several hours to days after the onset of headache. If treatment is not instituted, coma and death can occur. Recurrent seizures are common, and they can be either focal or generalized.

There are numerous reports of transient focal neurologic disturbances in patients with hypertensive encephalopathy including fleeting paresthesias and numbness in the extremities, transient paralysis, and aphasia (106,261). Thus, the presence of focal neurologic deficit in a patient with severe hypertension does not necessarily exclude the diagnosis of hypertensive encephalopathy.

Hypertensive neuroretinopathy (striate hemorrhages, cotton-wool spots, and papilledema) is present when hypertensive encephalopathy occurs in patients with malignant hypertension. However, it may be absent when hypertensive encephalopathy develops in the setting of acute glomerulonephritis, eclampsia, monoamine oxidase inhibitor–tyramine interactions, antihypertensive drug withdrawal syndromes, or pheochromocytoma (259,261,262).

Many authors have cautioned that lumbar puncture should be avoided in patients with suspected hypertensive encephalopathy because of the risk of cerebellar herniation (26,263). When performed, lumbar puncture has revealed elevated CSF pressure in most patients ranging from 230 to 560 mm of water (137,261). CSF protein concentration is usually moderately elevated (48 to 90 mg/dL) but may be normal. The cell count is usually normal (137,261), but neutrophilic pleocytosis has also been reported in hypertensive encephalopathy (264).

Computerized tomography and magnetic resonance imaging (MRI) reveal characteristic findings in hypertensive encephalopathy (260,263,265,266). Abnormalities on imaging include areas of low white-matter attenuation on computerized tomography scans and T1-weighted hypointense and T2-weighted hyperintense areas on MRI (267,268). These changes probably represent cerebral edema with increased water in the white matter. The most common location of the white-matter abnormalities on neuroimaging is the posterior regions of the cerebral hemispheres. The multifocal abnormalities include both hemispheres and tend to be symmetric (266). Commonly involved areas in descending order of frequency include the occipital lobes, the posterior parietal lobes, and the posterior temporal lobes. The pons, the thalamus, and the cerebellum are occasionally involved. The term *reversible posterior leukoencephalopathy syndrome* has been coined to describe patients with these typical radiographic findings and a reversible syndrome of headache, altered mental status, seizures, and loss of vision (266). A reversible hypertensive brainstem encephalopathy with predominant involvement of the brainstem and relative sparing of supratentorial regions has also been reported (269).

Etiologies

Although hypertensive encephalopathy can complicate malignant hypertension, not all patients with hypertensive encephalopathy have malignant hypertension. In fact, it most commonly occurs in previously normotensive individuals who experience sudden, severe hypertension (Table 56-5). The reported causes of hypertensive encephalopathy include acute glomerulonephritis (260,261,269), eclampsia (270, 271), renovascular hypertension (259), postcoronary artery bypass hypertension (272), clonidine withdrawal (273), monoamine oxidase inhibitor–tyramine interactions (274), pheochromocytoma (275), phencyclidine (PCP) poisoning (276), licorice ingestion (277), phenylpropanolamine overdose (278,279), acute renal artery occlusion (261), acute lead poisoning (261), immunosuppressive therapy with cyclosporine or tacrolimus for kidney, liver, or bone marrow transplantation (266,280,281),

TABLE 56-5

ETIOLOGIES OF HYPERTENSIVE ENCEPHALOPATHY

Malignant hypertension of any etiology
Acute glomerulonephritis
Eclampsia
Renovascular hypertension
Postcoronary artery bypass hypertension
Abrupt withdrawal of antihypertensive therapy
Monoamine oxidase inhibitor-tyramine interactions
Pheochromocytoma
Phencyclidine (PCP) poisoning
Phenylpropanolamine overdose
Recombinant erythropoietin therapy in dialysis patients
Scorpion envenomation, especially in children
Cocaine hydrochloride or alkaloidal (crack) cocaine
Acute renal artery occlusion
Acute lead poisoning in children
Cyclosporine-induced or tacrolimus-induced hypertension
Transplant renal artery stenosis or acute rejection
Femoral lengthening procedures
Acute or chronic spinal cord injuries

chemotherapy for acute leukemia in children (282), transplant renal artery stenosis or acute rejection (283,284), and femoral lengthening procedures in children (285). The preeclampsia–eclampsia syndrome has been hypothesized to reflect a subtype of hypertensive encephalopathy accompanied by impaired cerebral autoregulation and endothelial dysfunction (8,262,266,270, 271). The clinical and radiographic findings in patients with cyclosporine-induced neurotoxicity have been found to be identical to those seen in hypertensive encephalopathy (281). The only major factor found to be associated with the neurotoxic effect of cyclosporine in all patients was hypertension. Subcortical edema, affecting the posterior regions of the brain, tends to resolve following reduction in blood pressure, with or without concomitant reduction in cyclosporine dose. Hypertensive encephalopathy may also occur in patients with acute or chronic spinal cord injuries if there is autonomic hyperreflexia due to bowel or bladder distention (286,287). Acute elevation of blood pressure during recombinant human erythropoietin therapy occasionally results in hypertensive encephalopathy and seizures (288). This complication is unrelated to the extent or rate of increase in hematocrit, but is associated with a rapid increase in blood pressure and may occur in previously normotensive patients. Scorpion envenomization results in stimulation of the autonomic nervous system and adrenals and in children can lead to severe hypertension and a clinical picture consistent with hypertensive encephalopathy (289). Cocaine use can also induce a sudden increase in blood pressure accompanied by hypertensive encephalopathy (290).

Pathogenesis

The breakthrough theory of autoregulation originally proposed by Lassen and Angoli (291) is the generally accepted view of the pathogenesis of hypertensive encephalopathy (Fig. 56-21). Under normal circumstances, there is autoregulation of the cerebral microcirculation such that over a wide range of perfusion pressures, cerebral blood flow remains constant. It has been proposed that in the setting of a sudden, severe increase in blood pressure, autoregulatory vasoconstriction fails, and there is forced vasodilation. The dilation is initially segmental (sausage-string pattern), but eventually becomes diffuse. The endothelium in the dilated segments becomes abnormally permeable, and there is extravasation of plasma components with the development of cerebral edema. This theory may explain the clinical observation that hypertensive encephalopathy develops at a much lower blood pressure in previously normotensive individuals than it does in those with chronic hypertension. With long-standing hypertension, structural changes and remodeling of the cerebral arterioles may lead to a shift in the autoregulatory curve such that much higher perfusion pressures can be tolerated before forced vasodilation and breakthrough of autoregulation occur (292,293).

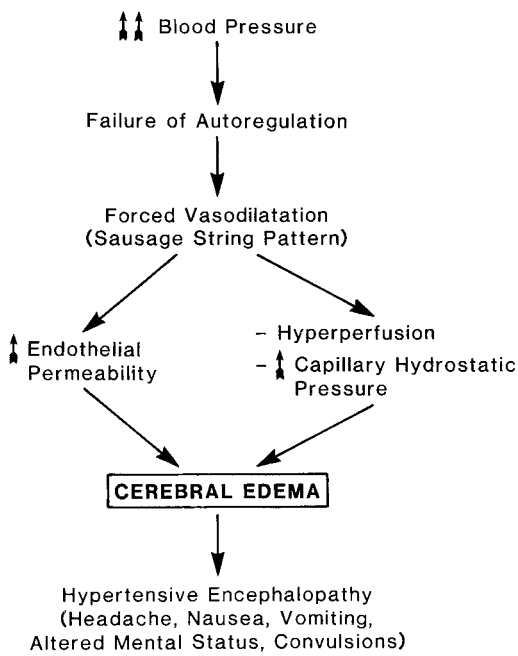

FIGURE 56-21. The breakthrough theory of hypertensive encephalopathy.

Strandgaard and co-workers (294) report studies on the regulation of cerebral blood flow in baboons with acute hypertension. Cerebral blood flow was measured using the xenon washout technique as the blood pressure was gradually increased during angiotensin II infusion. Cerebral blood flow remained constant up to a mean arterial pressure of 120 to 139 mm Hg by virtue of an increase in cerebrovascular resistance (intact autoregulation). However, at this level of mean arterial pressure, cerebrovascular resistance reached a maximum. At higher mean arterial pressures, cerebral blood flow increased significantly as cerebrovascular resistance decreased, consistent with a breakthrough of autoregulation. There was no evidence of spasm or decreased cerebral blood flow (overregulation) in response to severe hypertension.

Hypertensive-induced damage to the blood-brain barrier develops within minutes of a sudden, marked increase in blood pressure (296). The injury is most likely due to overstretching of vessels rather than from spasm and ischemia, as hypoxic injury to the blood-brain barrier would require several hours to develop (295). In a rat model of hypertensive encephalopathy due to one-kidney, one-clip renovascular hypertension, the sausage-string pattern develops in pial vessels in response to severe hypertension. Injection of colloidal carbon particles demonstrates that there is increased vascular permeability in the dilated segments (148).

Structural damage to the blood-brain barrier may not be required for the formation of cerebral edema in response to sudden hypertension. Cerebral arterioles and capillaries become abnormally permeable to protein-bound dyes within seconds after induction of severe hypertension (297). Pinocytotic vessels transport these large molecular markers through the structural components of the blood-brain barrier during periods of acute hypertension. The passage of protein molecules by pinocytosis may result in the extravascular accumulation of protein-rich fluid (cerebral edema).

Treatment

The treatment of choice for hypertensive encephalopathy is prompt reduction of blood pressure. When the diagnosis of hypertensive encephalopathy seems likely, antihypertensive therapy should be initiated prior to obtaining the results of time-consuming laboratory and radiologic examinations. The goal of therapy, especially in the previously normotensive patient with acute hypertension, should be the reduction of blood pressure to normal or near-normal levels as quickly as possible (259). Although cerebral blood flow could theoretically be jeopardized by failure of autoregulation during rapid reduction of blood pressure in patients with chronic hypertension (292,293), clinical experience has shown that the prompt reduction of blood pressure with the avoidance of frank hypotension is beneficial in patients with hypertensive encephalopathy

(259). Of the conditions in the differential diagnosis of hypertension with acute cerebral dysfunction, only cerebral infarction might be adversely affected by the abrupt reduction of blood pressure (298). Pharmacologic agents that have a rapid onset and short duration of action such as sodium nitroprusside or possibly fenoldopam should be utilized so that the blood pressure can be carefully titrated with close monitoring of the patient's neurologic status. The clinical *sine qua non* of hypertensive encephalopathy is a prompt clinical response to blood pressure reduction. Conversely, when antihypertensive therapy is associated with the development of new or progressive neurologic deficits, other diagnoses should be considered, and the blood pressure should be stabilized at a higher level.

In women with eclampsia, convulsions and other neurologic manifestations occur and are indistinguishable from those observed in nonpregnant individuals with hypertensive encephalopathy, except that in eclampsia they occur at a lower level of blood pressure (271). Eclampsia is associated with extreme risk to both the mother and the fetus. Although delivery of the fetus is the definitive cure in most cases, rapid control of the blood pressure and encephalopathic manifestations is essential before the induction of labor or performance of a cesarean section (262,270).

ACUTE HYPERTENSIVE HEART FAILURE

Both malignant hypertension and severe benign hypertension can be complicated by acute pulmonary edema. Acute fulminant pulmonary edema was a frequent cause of death among patients with malignant hypertension in the pre-antihypertensive treatment era (25,30). However, with the advent of effective antihypertensive therapy, the prognosis for hypertensive patients with left ventricular failure has improved dramatically.

Traditionally, congestive heart failure has been equated with systolic dysfunction in which there is an inability of the myofibrils to shorten against a load such that the left ventricle loses its ability to eject blood into the high-pressure aorta. The end result is a dilated, poorly contractile left ventricle with a low ejection fraction and a reduced cardiac output. However, in recent years, there has been increasing recognition that hypertension very frequently causes abnormalities in the diastolic function of the left ventricle that result in symptoms of congestive heart failure despite the presence of a normal ejection fraction and normal cardiac output (299–302). Diastolic dysfunction implies that the ventricle cannot fill normally at low filling pressures. Ventricular filling is slow, delayed, or incomplete unless the atrial pressure increases (299,301). A compensatory increase in filling pressure occurs and is sufficient to maintain normal systolic function but at the expense of pulmonary and systemic venous congestion. Thus, signs and symptoms of pulmonary or systemic venous congestion are not always the result of systolic dysfunction; instead, they may result from isolated abnormalities of the diastolic properties of the left ventricle. The treatment of hypertension-associated heart failure varies depending on whether the diastolic dysfunction manifests as chronic congestive heart failure or a hypertensive crisis with acute pulmonary edema.

Hypertensive patients with chronic congestive heart failure manifested by dyspnea and symptoms of pulmonary and systemic venous congestion are not infrequently found to have isolated diastolic dysfunction as defined by echocardiographic or radionuclide evaluation of diastolic filling (300,302,303). Thus, in patients with hypertension, left ventricular hypertrophy, and evidence of congestive heart failure, the possibility of hypertensive cardiomyopathy with chronic diastolic dysfunction should be considered. The left ventricular hypertrophy that develops in response to chronic systemic hypertension may cause abnormal myocardial relaxation and increased left ventricular chamber stiffness, which lead to the impairment in diastolic filling that characterizes diastolic dysfunction. In clinical practice the presence of dyspnea, pulmonary rales, and radiographic evidence of pulmonary venous congestion, despite a normal ejection fraction, should suggest the possibility of diastolic dysfunction (299). Traditional treatment for congestive heart failure with digitalis and arterial vasodilators may be deleterious in patients with isolated diastolic dysfunction due to hypertensive heart disease (303). This type of chronic diastolic dysfunction in hypertensive patients is best managed with beta-blockers, calcium channel blockers, or both. These drugs decrease heart rate and improve the balance between myocardial oxygen supply and demand and thus may improve myocardial relaxation and overall diastolic function. Classes of drugs that are associated with regression of left ventricular hypertrophy such as angiotensin-converting enzyme inhibitors, nondihydropyridine calcium channel blockers, beta-blockers, and centrally acting α-adrenergic agonists may result in an improvement in diastolic function as the hypertrophy regresses (304). Diuretics and salt restriction may be used to treat congestive symptoms but care should be taken to avoid excessive preload reduction that may compromise systolic function (299). Venodilation and preload reduction with nitrates may also improve symptoms of pulmonary congestion (299).

Isolated diastolic dysfunction is also the pathophysiologic process that underlies the development of acute pulmonary edema in patients with either malignant hypertension or severe benign hypertension. However, in the setting of *acute hypertensive heart failure,* the proximate cause of the left ventricular diastolic dysfunction is the markedly increased workload imposed on the heart by a pronounced increase in systemic vascular resistance (194,305). Hypertension complicated by acute pulmonary edema represents a crisis requiring immediate control of blood pressure with potent peripheral vasodilators, such as sodium nitroprusside, in order to quickly reduce the high systemic vascular resistance that underlies this disorder.

The hemodynamic derangements in acute hypertensive heart failure were characterized in a study comparing five patients with severe long-standing essential hypertension complicated by acute pulmonary edema with a control group of five patients of similar age who had long-standing hypertension of similar severity but who had no history of congestive heart failure (194,305). The subjects in both groups had electrocardiographic evidence of left ventricular hypertrophy and chest radiographic evidence of cardiomegaly with left ventricular prominence. However, pulmonary venous engorgement was evident only in the group with heart failure. The hemodynamic findings in the two groups of severely hypertensive patients are displayed in Figure 56-22. The mean arterial pressure, heart rate, cardiac index, and stroke work index were the same in both groups. The left ventricular end-diastolic volume was similarly elevated in both groups. In fact, the only hemodynamic difference between the two groups was a significant elevation of left ventricular filling pressure (pulmonary capillary wedge pressure) in the patients with acute hypertensive heart failure. Thus in this small series of patients with acute hypertensive heart failure, systolic function was normal as evidenced by the normal resting cardiac index. The finding of elevated left ventricular end-diastolic pressure (LVEDP) despite normal ejection fractions and cardiac indices implies the presence of isolated diastolic dysfunction. The increase in LVEDP was not the result of volume overload because the left ventricular end-diastolic volume was the same in both groups (Fig. 56-22). The increase in left ventricular filling pressure despite a similar end-diastolic volume can only be explained on the basis of a decrease in left ventricular compliance in the patients with acute

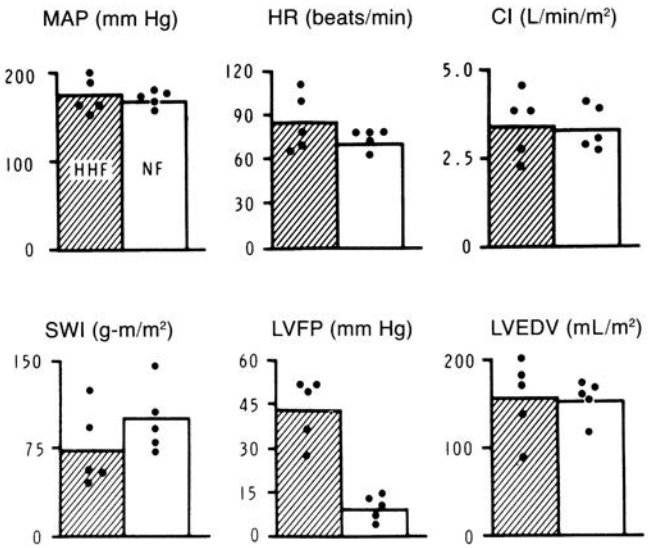

FIGURE 56-22. Pretreatment hemodynamics in patients with acute hypertensive heart failure (HHF) and those with severe hypertension but without heart failure (NF). *CI,* cardiac index; *HR,* heart rate; *LVEDV,* left ventricular end-diastolic volume; *LVFP*, left ventricular filling pressure; *MAP,* mean arterial pressure; *SWI,* stroke work index. The MAP, HR, SWI, and LVEDV were the same in both groups. The only difference between the groups was a significant elevation of LVFP in the patients with HHF. These findings suggest a decrease in left ventricular compliance in the patients with HHF, as the LVFP was significantly increased even though the LVEDV was the same as in patients without heart failure. (From: Cohn JN, Rodriguera E, Guiha NH. Hypertensive heart disease. In: Onesti O, Kim KE, Moyer JH, eds. *Hypertension: mechanisms and management*. New York: Grune & Stratton, 1973, with permission.)

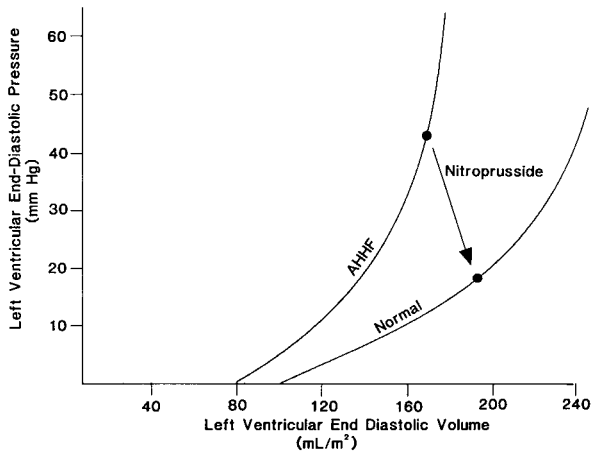

FIGURE 56-23. Schematic representation of the left ventricular end-diastolic pressure–volume relationship in a patient with acute hypertensive heart failure (AHHF) treated with sodium nitroprusside. In AHHF, the pressure–volume curve is shifted up and to the left, reflecting a decrease in left ventricular (LV) compliance. A higher than normal left ventricular end-diastolic pressure (LVEDP) is required to achieve any level of left ventricular end-diastolic volume (LVEDV). Normal LV systolic function is maintained but at the expense of a very high wedge pressure, which results in acute pulmonary edema. Treatment with sodium nitroprusside causes a reduction in the high systemic vascular resistance with a concomitant decrease in impedance to LV ejection. As a result, LV compliance improves. Pulmonary edema resolves due to a reduction in LVEDP despite the fact that LVEDV actually increases during sodium nitroprusside infusion.

hypertensive heart failure (194,305). The importance of decreased left ventricular compliance in the pathogenesis of acute hypertensive heart failure was confirmed by the hemodynamic responses to vasodilator therapy (194,305). Sodium nitroprusside infusion resulted in prompt relief of congestive symptoms in the patients with acute hypertensive heart failure with a dramatic decrease in LVEDP from a mean of 43 to 18 mm Hg. The decrease in left ventricular filling pressure was not due to venodilation and decreased venous return because the left ventricular end-diastolic volume actually increased during sodium nitroprusside infusion. Thus the benefit of sodium nitroprusside therapy was mediated through a decrease in systemic vascular resistance, which led to improvement in left ventricular compliance. The signs and symptoms of pulmonary congestion improved because there was a reduction in LVEDP. This reduction in wedge pressure occurred despite an increase in left ventricular volume because of simultaneous improvement in ventricular compliance.

A schematic representation of the changes in the left ventricular end-diastolic pressure–volume relationship in patients with acute hypertensive heart failure treated with sodium nitroprusside is displayed in Figure 56-23. The diastolic pressure–volume relationship is considered to be an index of left ventricular compliance. In acute hypertensive heart failure, the pressure–volume curve is shifted up and to the left, reflecting a decrease in compliance such that a higher LVEDP is required to achieve any given level of left ventricular end-diastolic volume. Normal systolic function is maintained but at the expense of a very high wedge pressure that results in acute pulmonary edema. Treatment with sodium nitroprusside causes a reduction in the high systemic vascular resistance. The concomitant decrease in impedance to left ventricular ejection results in an improvement in compliance such that a lower filling pressure is required to maintain systolic function. Symptoms of pulmonary edema resolve as a result of the reduction in LVEDP despite the fact that the left ventricular end-diastolic volume actually increases during sodium nitroprusside infusion.

Compliance is only one index of diastolic performance. Aortic cross-clamp experiments in a canine model have been used to study the effects of acute increases in systemic vascular resistance (afterload) on other indices of left ventricular diastolic function (306). During acute cross-clamping, the isovolumic relaxation rate and early diastolic filling rate are inversely proportional to the left ventricular systolic pressure. Thus, an acute increase in systolic load results in instantaneous changes in left ventricular diastolic function. The authors postulate that in patients with severe hypertension complicated by acute pulmonary edema, an acute increase in left ventricular systolic load due to increased systemic vascular resistance may lead to abnormal myocardial relaxation and diastolic filling, resulting in an elevation of left ventricular filling pressure and pulmonary congestion.

It is not clear why heart failure does not develop in some patients with long-standing severe hypertension, while it develops as a relatively early complication of hypertension in other patients. The rate of rise of blood pressure may be important. Sudden worsening of preexisting hypertension, as occurs in patients with malignant hypertension superimposed on chronic essential hypertension, may precipitate heart failure. Moreover, acute hypertension in previously normotensive patients, as occurs in the setting of preeclampsia or acute glomerulonephritis, may cause left ventricular failure even though the blood pressure is only modestly elevated. In contrast, more severe hypertension, which develops gradually, may be tolerated for years without cardiac decompensation.

It is possible that with longstanding hypertension, the development of left ventricular hypertrophy may be a compensatory mechanism that serves to decrease left ventricular wall

stress in the face of the increased impedance to left ventricular ejection (194). However, when the onset of hypertension is abrupt or there is a sudden worsening of chronic hypertension, compensatory mechanisms may not be fully developed. Under these circumstances, precipitous left ventricular diastolic dysfunction with pulmonary and systemic venous congestion may occur (194).

In summary, available evidence suggests that acute hypertensive heart failure results from a primary increase in systemic vascular resistance, which causes an increase in aortic impedance or resistance to left ventricular ejection. Systolic dysfunction (low cardiac output) does not occur because there is an increase in left ventricular wall tension that is sufficient to overcome the impedance to ejection. However, acute pulmonary edema develops because abnormalities of diastolic function such as delayed myocardial relaxation, decreased early diastolic filling, and reduced left ventricular compliance result in an increase in LVEDP that is transmitted to the pulmonary capillaries, resulting in transudation of fluid into alveoli (194,306).

These pathophysiologic mechanisms have important therapeutic implications in the treatment of hypertensive patients with acute pulmonary edema. Since the proximate cause of the impaired diastolic performance in acute hypertensive heart failure is the contraction load imposed on the ventricle by the increase in systemic vascular resistance, potent peripheral vasodilators are clearly the treatment of choice (194,305). However, it is important to distinguish patients in whom acute pulmonary edema is secondary to severe hypertension (acute diastolic dysfunction) from those in whom hypertension is a reflex response to acute respiratory distress during an exacerbation of heart failure due to chronic *systolic* dysfunction. A history of chronic hypertension, diastolic blood pressure over 120 to 130 mm Hg, funduscopic changes of hypertensive neuroretinopathy, and most important, failure of the hypertension to respond rapidly to the administration of oxygen, potent diuretics, and morphine are findings that should suggest that severe hypertension may be the proximate cause of acute pulmonary edema. Furthermore, even in patients with suspected reflex hypertension, if hypertension persists after institution of therapy with preload reducing agents, treatment with a parenteral antihypertensive agent is indicated.

Sodium nitroprusside is the preferred drug for the management of acute hypertensive heart failure because it reduces both preload and afterload. There is no absolute blood pressure goal. The dose of sodium nitroprusside should be increased until signs and symptoms of pulmonary congestion subside or the arterial pressure falls to hypotensive levels. However, it is rarely necessary to lower the blood pressure to hypotensive levels since a reduction to levels still within the hypertensive range is usually associated with a dramatic improvement in symptoms. Although hemodynamic monitoring is not always required, it is essential in patients in whom concomitant myocardial ischemia or compromised cardiac output is suspected. Recent evidence suggests that nitric oxide (NO) donors such as sodium nitroprusside and nitroglycerin may directly modulate diastolic relaxation in patients with a hypertrophied myocardium (307). Intracoronary infusion of nitroglycerin or sodium nitroprusside was found to cause a marked fall in LVEDP with only a slight change in left ventricular peak systolic pressure (afterload), which is consistent with a direct beneficial effect of NO donors on diastolic function.

After the acute episode of hypertension with acute pulmonary edema has resolved, oral therapy can be substituted as the sodium nitroprusside infusion is weaned. Unfortunately, guidelines for long-term antihypertensive treatment in patients with a history of acute hypertensive heart failure are not well defined. Despite the fact that direct-acting vasodilators may sustain or even promote left ventricular hypertrophy, in some patients with severe or resistant hypertension, adequate blood pressure control may require use of hydralazine or minoxidil in conjunction with beta-blockers to control reflex tachycardia and use of diuretics to prevent reflex salt and water retention. Nonetheless, as in the treatment of hypertensive patients with chronic symptoms of congestive heart failure due to diastolic dysfunction, agents such as beta-blockers and calcium channel blockers, which not only decrease blood pressure but also improve diastolic function, may represent the most logical first-line therapy. Moreover, control of blood pressure with beta-blockers, calcium channel blockers, converting enzyme inhibitors, or central α-adrenergic agonists may lead to a regression of left ventricular hypertrophy (304). However, it has not been demonstrated that regression of left ventricular hypertrophy leads to a long-term improvement in diastolic function, congestive symptoms, or prognosis. Furthermore, it is conceivable that regression of left ventricular hypertrophy might predispose to subsequent episodes of acute hypertensive heart failure if severe hypertension suddenly recurs.

HYPERTENSION COMPLICATING CEREBROVASCULAR ACCIDENT

The importance of hypertension as a risk factor for cerebrovascular accident is well established. The Framingham Study shows that regardless of gender or age, hypertension is associated with an increased incidence of ischemic and hemorrhagic stroke (308). Several prospective, randomized clinical trials demonstrate that long-term antihypertensive drug therapy results in a significant reduction in morbidity and mortality from cerebrovascular accident (309). Despite the proven benefits of blood pressure control in the prevention of stroke, the role of treatment of hypertension in the acute phase of stroke remains controversial. Whether antihypertensive therapy is indicated depends not only on the magnitude of the blood pressure elevation, but also on the type of cerebrovascular accident. It should be emphasized that the management of hypertension accompanying cerebral infarction is different from that for hypertension complicating either intracerebral hemorrhage or subarachnoid hemorrhage.

Cerebral Infarction

In the cerebral circulation, the sites of predilection for atherosclerosis are the bifurcations of the common carotid arteries, the carotid siphons, the origins of the vertebral and basilar arteries, the circle of Willis, and the proximal parts of the cerebral arteries (310). Cerebral infarction can result from partial or complete occlusion of an artery by a plaque or embolization of atherothrombotic debris from a plaque. The atherothromboembolic infarcts produced by one of these mechanisms typically involve the cerebral or cerebellar cortex or the pons (310). In contrast, hypertension-induced lipohyalinosis of the small penetrating cerebral end arteries is the principal cause of the small, deep lacunar infarcts that occur in the basal ganglia, pons, thalamus, cerebellum, and deep hemispheric white matter (310).

Hypertension is common in the setting of acute cerebral infarction. In a series of 334 consecutive patients admitted for acute stroke, the blood pressure was elevated in 84% of the patients on the day of admission. Even without specific antihypertensive treatment, the blood pressure decreased spontaneously by an average of 20 mm Hg systolic and 10 mm Hg diastolic in the 10 days following the acute event (311).

This early elevation in blood pressure most likely represents a physiologic response to brain ischemia. Decreases in blood pressure accompany recovery of brain function.

Because of the known benefits of antihypertensive therapy with regard to stroke prevention, it has been assumed that reduction in blood pressure would benefit patients with acute cerebral infarction. Unfortunately, because treatment of hypertension in this setting has never been evaluated in a prospective, randomized trial, there are no good data to guide management. Moreover, there is no evidence to suggest that rapid reduction of blood pressure is beneficial. In fact, several cases have been reported in which worsening of the patient's neurologic status was apparently precipitated by emergency treatment of hypertension (312,313). The rationale for not treating hypertension in acute ischemic strokes is based on concerns regarding impairment in autoregulation of cerebral blood flow in this setting (313–315). In normal individuals, cerebral blood flow is maintained constant at mean arterial pressures ranging between 60 and 120 mm Hg. However, in patients with chronic hypertension as well as older adult patients, the curve is shifted such that the lower limit of autoregulation occurs at a higher mean arterial pressure. Furthermore, there is evidence that local autoregulation of cerebral blood flow is disturbed in the so-called ischemic penumbra that surrounds an area of acute infarction (314). Without intact autoregulation, the regional blood flow becomes critically dependent on the perfusion pressure. Thus, to some extent, the presence of hypertension may be beneficial in the setting of acute cerebral infarction, whereas reduction of blood pressure may cause a regional decrease in blood flow with extension of the infarct.

Because there is no evidence that mild to moderate hypertension has a deleterious effect on the outcome of cerebral infarction during the acute stage, it is probably wise to allow the blood pressure to seek its own level during the first few days to weeks after the event. In most cases, the hypertension tends to resolve spontaneously over the first week without specific therapy (311). On the other hand, if hypertension persists for more than 3 weeks in a patient with a completed stroke, gradual reduction of blood pressure into the normal range can be accomplished safely (316). The goal of long-term antihypertensive treatment in hypertensive stroke survivors is the prevention of stroke recurrence. The benefits of antihypertensive therapy in secondary stroke prevention are uncertain, but large clinical trials are in progress that should provide helpful guidelines for clinical practice.

Although benign neglect of mild to moderate hypertension is prudent in the setting of acute cerebral infarction, there may be certain indications for active treatment of hypertension. When the diastolic blood pressure is sustained more than 130 mm Hg, many authorities recommend cautious reduction of the systolic blood pressure to 160 to 170 mm Hg and diastolic to 100 to 110 mm Hg with a short-acting parenteral agent such as sodium nitroprusside (293,313,317–319). Stroke accompanied by other hypertensive crises such as acute myocardial ischemia or left ventricular dysfunction with acute pulmonary edema is also an indication for cautious blood pressure reduction (313,318). Stroke due to carotid occlusion caused by aortic dissection mandates aggressive blood pressure reduction to prevent propagation of the dissection (313,318). In some patients with severe hypertension, it may be impossible to distinguish between hypertensive encephalopathy and cerebral infarction on clinical grounds. Since rapid lowering of the blood pressure may be lifesaving in the patient with hypertensive encephalopathy, a cautious diagnostic trial of blood pressure reduction with a short-acting parenteral antihypertensive agent, such as sodium nitroprusside, may be indicated (318). In patients who have suffered a stroke and require anticoagulation therapy, moderate control of severe hypertension into the 160 to 170 mm Hg systolic and 100 to 110 mm Hg diastolic range may also be prudent. In the severely hypertensive patient with progressing stroke in whom continued deterioration is believed to be secondary to concomitant cerebral edema, cautious blood pressure reduction may be warranted. Appropriate management of such patients may require continuous intracranial as well as intraarterial pressure monitoring so that cerebral perfusion pressure can be optimized (318).

In a recent study, sodium nitroprusside, given at a dose that reduced mean arterial pressure by 10 mm Hg, significantly inhibited platelet aggregation and adhesion molecule expression and improved regional cerebral blood flow in patients with acute ischemic stroke (320). These findings were attributed to beneficial effects of nitric oxide on platelet function and local vasodilation in the area of the ischemic penumbra.

In the setting of acute cerebral infarction, hypertension tends to be very labile and exquisitely sensitive to hypotensive therapy. Even modest doses of oral antihypertensive agents may cause profound and devastating overshoot hypotension (312). Antihypertensive treatment, when indicated, should be initiated with extreme caution using small doses of short-acting agents such as sodium nitroprusside. Use of oral or sublingual nifedipine may be associated with overshoot hypotension resulting in extension of the infarct and is contraindicated for the treatment of hypertension accompanying acute cerebral infarction. Oral clonidine loading is also contraindicated because it may induce overshoot hypotension or lead to sedation, which will interfere with assessment of mental status. It had been proposed that there the calcium channel blocker nimodipine, which is a cerebral vasodilator, might theoretically minimize arterial spasm and therefore improve cerebral ischemia. However, a large controlled clinical trial demonstrated no improvement in outcome in patients with thrombotic stroke treated with nimodipine when compared to placebo treatment (321).

Intracerebral Hemorrhage

Hypertension is a major risk factor for intracerebral hemorrhage. The small-diameter, penetrating cerebral end arteries are especially vulnerable to the deleterious effects of hypertension because they arise directly from the main arterial trunks (310). The most common sites of hypertension-associated hemorrhage include the basal ganglia, pons, thalamus, cerebellum, and deep hemispheric white matter (322). Lacunar infarcts arise from the same vessels and are similarly distributed.

Hypertensive hemorrhage most often occurs in patients older than 50 years of age. Intracerebral hemorrhage characteristically begins abruptly with headache and vomiting followed by steadily increasing focal neurologic deficits and alteration of consciousness (322). More than 90% of hemorrhages rupture through brain parenchyma into the ventricles, producing a bloody CSF (322). Patients presenting with acute intracerebral hemorrhage invariably have elevated blood pressure. In fact, the finding of a normal or low blood pressure makes the diagnosis of intracerebral hemorrhage unlikely (322). In contrast to cerebral infarction, the blood pressure does not tend to decrease spontaneously during the first week after the event (311). Once the hemorrhage has occurred, the patient's condition worsens steadily over a period of minutes to days until either the neurologic deficit stabilizes, or the patient dies (322). When death occurs, it is most often due to herniation caused by the expanding hematoma and surrounding edema.

Small hemorrhages, which may be clinically indistinguishable from cerebral infarction, probably require no specific therapy (310). The issue of treatment of hypertension in the setting of a large (greater than 3 cm) intracerebral hemorrhage is controversial. There is almost always a rise in intracranial

pressure accompanied by a reflex increase in systemic blood pressure (311). Because cerebral perfusion pressure is a function of the difference between systemic arterial pressure and intracranial pressure, reduction of blood pressure may compromise cerebral perfusion. Furthermore, the hematoma impairs the local autoregulatory response in the surrounding area of marginal ischemia (313). Because there is no good evidence that persistent hypertension promotes further bleeding, some authorities strongly advise against treating hypertension in patients with intracerebral hemorrhage (1,312,315). On the other hand, cerebral vasogenic edema may develop as a consequence of an abrupt, severe increase in blood pressure (310), and treatment of hypertension may be beneficial by virtue of a reduction in cerebral edema and intracranial pressure. Thus, in deciding to treat hypertension, a precarious balance must be struck between prevention of cerebral edema on the one hand, and deleterious reduction of cerebral blood flow on the other. In a study of eight patients with intracerebral hemorrhage treated with trimethaphan, cerebral blood flow measurements revealed that the cerebral autoregulation curve was intact but shifted such that the lower limit of autoregulation was at 80% of the initial level of blood pressure (323). Thus, a 20% decrease in mean arterial pressure should be considered the maximal reduction of blood pressure during the acute stage. Active treatment of the blood pressure should only be undertaken in the intensive care environment where intracranial pressure and intraarterial pressure can be closely monitored (310,324).

The drug of choice for the management of hypertension in the setting of intracerebral hemorrhage is a matter of debate. Sodium nitroprusside has traditionally been regarded as the best agent because its brief duration of action allows for rapid titration with avoidance of the catastrophic consequence of sustained overshoot hypotension (1,319). However, concern has been expressed that because sodium nitroprusside causes an increase in venous capacitance as well as cerebral arterial vasodilation, the resulting increase in cerebral blood volume may cause a further elevation of intracranial pressure (325–327). Other cerebral vasodilators such as intravenous nitroglycerin, hydralazine, or calcium channel blockers also can cause potentially deleterious elevations of intracranial pressure in patients with compromised intracranial compliance due to intracranial disease (327). Because labetalol and urapidil (a postsynaptic α-receptor blocker) may not alter intracranial pressure, they have been recommended for treatment of hypertension in patients undergoing neurosurgery (327). Unfortunately, these agents have the potential to cause overshoot hypotension, which may be difficult to quickly reverse. Thus, despite the theoretic risk of elevation of intracranial pressure, sodium nitroprusside remains the treatment of choice when severe hypertension must be treated in the patient with intracerebral hemorrhage because its brief duration of action allows for cautious, graded blood pressure reduction, which can be quickly reversed if the patient's neurologic status deteriorates or a further increase in intracranial pressure occurs. Of interest, some patients with cerebral infarction or hemorrhage have extreme elevations of catecholamine levels that may render hypertension refractory to sodium nitroprusside in the absence of concomitant beta-blocker therapy (328).

Cerebellar hemorrhage represents a neurosurgical emergency requiring prompt diagnosis and treatment (329). Typically, patients complain of the sudden onset of dizziness, nausea, vomiting, headache, and difficulty walking. Truncal ataxia, nystagmus, and ipsilateral sixth nerve paresis may be present (329). If the process continues unchecked, brainstem compression or herniation produces progressive stupor and coma. The untreated mortality is extremely high. The diagnosis can usually be confirmed by computerized tomography. Treatment consists of emergency suboccipital craniotomy with evacuation of the hematoma (329).

Subarachnoid Hemorrhage

Subarachnoid hemorrhage (SAH) accounts for less than 10% of all cerebrovascular accidents. Rupture of a congenital aneurysm is the most common cause. Rupture is heralded by the sudden onset of a profound headache and is often followed by brief syncope. If the mass of the hemorrhage is large, patients rapidly become comatose. As the hemorrhage diffuses throughout the subarachnoid space, the patient may awaken and experience headache, nausea, vomiting, and seizures. Within 24 hours, nuchal rigidity and other meningeal signs develop. Initially, neurologic findings are nonfocal. Computerized tomography can be used to confirm the diagnosis.

Recurrent hemorrhage is a potential complication associated with a high mortality. Whether treatment of hypertension after SAH reduces the risk of recurrent bleeding or improves prognosis is uncertain. In the setting of elevated intracranial pressure or cerebral arterial vasospasm, hypertension may actually be protective because it helps to maintain cerebral perfusion pressure. Thus, reduction of the blood pressure could conceivably result in aggravation of cerebral vasospasm and ischemia.

Early surgical repair of the aneurysm has reduced the incidence of rebleeding in patients with SAH. In fact, delayed cerebral ischemia due to cerebral arterial vasospasm has been found to be the most important cause of morbidity and mortality in patients who survive the initial hemorrhage (330,331). Vasospasm, which is probably caused by the irritating effects of blood in the subarachnoid space closely opposed to the large arteries, usually develops 4 to 12 days after the acute hemorrhage. Symptoms include a gradual deterioration of the level of consciousness, accompanied by focal neurologic deficits.

Surgical clipping of the aneurysm is usually undertaken as soon as possible to prevent rebleeding (331,332). There is conflicting evidence as to whether or not postoperative treatment with intravascular volume expansion, in conjunction with deliberate induction of arterial hypertension using dopamine or dobutamine, may be an effective means of reversing the ischemic neurologic deficits caused by cerebral vasospasm (333,334).

Nimodipine, a 1,4-dihydropyridine calcium channel blocker, has been approved for the prevention and treatment of delayed cerebral ischemia caused by subarachnoid hemorrhage from ruptured congenital aneurysms. Nimodipine is highly lipid-soluble and readily crosses the blood-brain barrier (335). It dilates cerebral blood vessels at concentrations lower than those required for dilation of the peripheral vasculature (335). Thus, it may dilate intracerebral vessels at doses that do not result in a significant reduction in mean arterial pressure. Furthermore, inhibition of calcium uptake by neurons may also protect against ischemic injury at the cellular level, independent of an effect on cerebral blood flow (335). Nimodipine has been shown, in randomized, placebo-controlled trials, to reduce the severity of neurologic deficits resulting from vasospasm in patients who have had a recent subarachnoid hemorrhage (336,337). The recommended dosage is 60 mg orally every 4 hours for 21 consecutive days beginning within 96 hours of the subarachnoid hemorrhage. The liquid content of the capsules can be given through a nasogastric tube in unconscious patients. The optimal timing of surgery in nimodipine-treated patients has not yet been defined.

Hypertension Complicating Severe Head Trauma

Systemic hypertension can contribute to the increase in intracranial pressure that often accompanies traumatic head

injury (338). In patients with severe head injury, the degree of intracranial hypertension correlates with mortality. If the intracranial pressure is less than 20 mm Hg, mortality is about 20%. However, if the intracranial pressure exceeds 40 mm Hg, mortality is more than 80% (339). The primary danger of intracranial hypertension is a compromise of cerebral blood flow with secondary ischemic injury. Severe, uncontrolled intracranial hypertension can result in rapid brain death due to global cerebral ischemia. The minimum cerebral perfusion pressure (mean arterial pressure minus intracranial pressure) necessary to prevent secondary cerebral ischemia is 50 mm Hg (338).

A major goal of treatment in the patient with a head injury is to maintain intracranial pressure at levels less than 30 mm Hg. However, effective treatment requires measurement of intracranial pressure with a device such as a ventricular catheter, subarachnoid bolt, or epidural transducer. Major treatments for reducing elevated intracranial pressure include hyperventilation, osmotic diuretics, removal of CSF, corticosteroids, high-dose barbiturates, and control of arterial blood pressure (338). Autoregulation of cerebral blood flow is impaired in patients with severe head injury such that changes in mean arterial pressure will cause parallel changes in intracranial pressure through alterations in cerebral blood volume. Moreover, severe hypertension may cause a breakthrough of cerebral autoregulation, leading to cerebral edema in a manner analogous to hypertensive encephalopathy. On the other hand, some increase in blood pressure may be beneficial with regard to maintenance of cerebral perfusion pressure in the patient with increased intracranial pressure. Therefore, rational treatment of hypertension in the setting of severe head trauma requires continuous monitoring of mean arterial pressure, intracranial pressure, pulmonary capillary wedge pressure, and cardiac output. Frequent neurologic examinations must be performed to assess response to therapy. The choice of antihypertensive agent is also important. Vasodilators, when used alone, tend to be relatively ineffective so the patient should also be pretreated with β-adrenergic receptor blockers. Vasodilators such as sodium nitroprusside and intravenous nitroglycerin are the treatments of choice (338). If an increase in intracranial pressure accompanied by compromise of cerebral perfusion pressure occurs with vasodilator therapy, intravenous labetalol may be a suitable alternative. Diuretics should be avoided because a decrease in intravascular volume reduces cardiac output and increases sympathetic tone.

HYPERTENSION COMPLICATING ACUTE MYOCARDIAL INFARCTION

Transient systemic hypertension is a frequent occurrence during the early stages of acute myocardial infarction, even among previously normotensive patients. This postinfarction hypertension has been attributed to a hyperadrenergic state resulting from release of catecholamines from infarcted myocardium or to an increase in sympathetic tone in response to stress, pain, or anxiety. Serial measurements of plasma epinephrine and norepinephrine in patients with acute myocardial infarction have revealed a significant direct correlation between plasma catecholamine levels and systolic blood pressure (340). A cardiogenic hypertensive chemoreflex has been described. Injection of serotonin into the left atrium or branches of the proximal left coronary artery in dogs produces an intense pressor response that is dependent on vagal afferent impulses to the central nervous system and is blocked by the α-adrenergic blocking agent phentolamine. By histologic examination, a small structure resembling a chemoreceptor has been identified. This chemoreceptor receives its blood supply from the left coronary artery (341). It has been postulated that in the setting of acute myocardial infarction, platelet deposition in stenosed vessels results in the release of serotonin with activation of this chemoreceptor. The chemoreceptor reflex results in increased sympathetic tone and systemic hypertension (340).

In most patients, hypertension is a transient finding early in the course of acute myocardial infarction that resolves without specific therapy other than pain control and sedation. The short-term changes in blood pressure in untreated patients with acute myocardial infarction have been well characterized (342). During the first hour of hospitalization the mean systolic blood pressure averages 150 $\pm$ 30.7 mm Hg, and systolic pressure of at least 160 mm Hg is present in 30% of patients. The mean diastolic blood pressure averages 92 $\pm$ 18.7, and diastolic pressure of at least 100 mm Hg is present in 42% of patients. Overall, 45% of patients have a blood pressure of at least 140/90 mm Hg and 32% have a blood pressure of a least 160/100 mm Hg during the first hour of hospitalization. However, during the subsequent 6 hours, the blood pressure spontaneously normalizes in the majority of patients. By the sixth hour of hospitalization systolic pressure falls to 130 $\pm$ 24 mm Hg, and diastolic pressure decreases to 81 $\pm$ 15.5 mm Hg. Among the patients with an initial blood pressure of at least 140/90 mm Hg, only 25% are still hypertensive by 6 hours. Patients with an initial blood pressure of at least 160/100 mm Hg demonstrate a similar decrease in blood pressure, such that it remains above this level at 6 hours in only 20%. No difference was found in the clinical course of the patients with and those without hypertension (342). Based on this study it was concluded that in early, uncomplicated acute myocardial infarction, no specific therapy of hypertension is indicated other than attention to relief of pain and adequate sedation (342).

In contrast, a number of studies indicate that hypertension in the setting of acute myocardial infarction signifies a less favorable prognosis. In a study of 143 patients with acute myocardial infarction, high systolic blood pressure on admission indicated a worse prognosis for 2-year survival (343). In another study of 106 patients with acute myocardial infarction who had systolic blood pressure of at least 170 mm Hg that persisted for at least 30 minutes, the blood pressure fell spontaneously to less than 150 mm Hg within 72 hours in all patients (344). No antihypertensive therapy was employed. The control group consisted of 106 patients with acute myocardial infarction who had a systolic pressure of 120 to 150 mm Hg and a diastolic pressure of 100 mm Hg or lower. Mean peak aspartate aminotransferase (AST) levels were significantly higher in the systolic hypertension group than in the normotensive group. The duration of systolic blood pressure of at least 170 mm Hg before return to normotension correlated with the mean peak AST level and presumably infarct size. The overall mortality, incidence of major arrhythmias, and incidence of cardiac failure were higher in the hypertensive group.

Postinfarction hypertension may be the most important risk factor for cardiac rupture (345). Although the incidence of chronic hypertension prior to acute myocardial infarction was similar in patients with and those without rupture, 40% of the patients with cardiac rupture had postinfarction hypertension (diastolic pressure $\geq$90 mm Hg) compared with 15% of patients without cardiac rupture.

A major objective of therapy in acute myocardial infarction is to minimize myocardial infarct size. The extent of ischemic damage is dependent on the balance between myocardial oxygen supply and demand. In experimental models, factors that increase myocardial oxygen demand increased infarct size. Conversely, infarct size was minimized by reducing myocardial oxygen consumption (346). Heart rate, wall tension, and myocardial contractility are the major determinants of myocardial oxygen consumption.

Treatment with β-adrenergic receptor blockers leads to a reduction in myocardial oxygen demand through a reduction in heart rate, systemic vascular resistance, and myocardial contractility. In addition, beta-blockers counter the excess production of catecholamines commonly seen in patients with acute myocardial infarction. They also have antiarrhythmic properties. When given intravenously within the first few hours after the acute event, beta-blockers can reduce both infarct size and early in-hospital mortality (347,348). Intravenous beta-blocker therapy should be considered in all patients with acute myocardial infarction unless contraindications such as severe bradycardia, heart block, systemic hypotension, severe left ventricular systolic dysfunction, and reactive airways disease are present (347). The presence of mild to moderate left ventricular systolic dysfunction should not necessarily be considered a contraindication to acute or chronic treatment with beta-blockers. Several of the larger trials did include high-risk patients with a history of compensated heart failure or with acute signs suggesting mild left ventricular dysfunction. These trials indicated that beta-blocker treatment was well tolerated by patients with left ventricular dysfunction, both in the acute phase of myocardial infarction and during long-term treatment (349). Long-term trials showed a marked (43% to 47%) reduction in the likelihood of sudden death among patients with left ventricular dysfunction treated with beta-blockers (349).

In patients with acute myocardial infarction who have relative contraindications to β-blockade such as evidence of severe left ventricular dysfunction, obstructive airways disease, and bradycardia, dose titration with intravenous esmolol may be a safe alternative (350). Moreover, the ability to tolerate esmolol infusion is a good predictor of subsequent outcome with oral beta-blocker therapy (350).

Secondary prevention trials showed that chronic beta-blocker treatment after myocardial infarction reduces both nonfatal reinfarction rate and long-term mortality (347,351). Impressive effects on morbidity and mortality have been obtained with propranolol, timolol, and metoprolol, whereas beta-blockers with intrinsic sympathomimetic activity are less effective (347). Recent studies also suggest that treatment with converting enzyme inhibitors, started within 24 hours of the onset of acute myocardial infarction, may be beneficial in patients with a history of hypertension in that they decrease the risk of severe congestive heart failure and reduce 1-year mortality rate (352).

During the first few days after an acute myocardial infarction, the systemic arterial pressure is the most important determinant of LVEDP (353). Accordingly, it has been proposed that in the setting of postinfarction hypertension, reduction of the blood pressure with arteriolar vasodilators might prevent extension of ischemia by reducing LVEDP, wall tension, and myocardial oxygen demand. Studies of vasodilator therapy with intravenous nitroglycerin, sodium nitroprusside, or trimethaphan in patients with hypertension complicating acute myocardial infarction demonstrate improved cardiac performance with decreased LVEDP and stable or increased cardiac output, findings that should be associated with a reduction in myocardial oxygen demand (354,355). Moreover, intravenous nitroglycerin has been shown to cause reversal of the restrictive left ventricular diastolic filling pattern on pulsed-wave Doppler in patients with acute anterior wall myocardial infarction (356).

In the setting of acute myocardial infarction, patients with a blood pressure higher than 160/100 mm Hg that lasts longer than 1 hour and is unresponsive to intravenous beta-blocker therapy should be considered candidates for treatment with parenteral vasodilators to decrease systemic vascular resistance, afterload, and myocardial oxygen demand. Because systemic hemodynamics can change rapidly in the setting of acute myocardial infarction, the use of agents with a short duration of action is recommended. Intravenous nitroglycerin and sodium nitroprusside are preferred in this setting. Nitroglycerin has theoretic advantages as a vasodilator in the setting of acute myocardial infarction because it dilates intercoronary collaterals and improves blood flow to the ischemic myocardium (357–359). Diazoxide and hydralazine are contraindicated because their use may result in reflex activation of the adrenergic system, resulting in increases in heart rate, cardiac output, and myocardial oxygen demand.

Acute reduction of blood pressure in patients with acute myocardial infarction necessitates careful monitoring of filling pressure and cardiac output. Definition of an arbitrary blood pressure goal is impossible. The blood pressure should be gradually reduced over a period of 10 to 15 minutes with frequent checks of systemic hemodynamics. The goal of therapy should be the reduction of system vascular resistance such that LVEDP is reduced to the range of 15 to 18 mm Hg without reflex tachycardia or compromise of cardiac output (360). The blood pressure may be reduced to normotensive levels as long as cardiac output remains stable or increases, the heart rate does not increase, and there is no evidence of increased myocardial ischemia (pain or increased ischemic changes on electrocardiogram). Vasodilator therapy can usually be weaned within 24 hours as the hypertension resolves.

Despite the fact that afterload reduction can improve myocardial performance and decrease myocardial oxygen demands, it should be undertaken with great caution. Myocardial blood flow is critically dependent on coronary perfusion pressure, and overshoot hypotension can worsen ischemia and extend the infarct. Afterload reduction should be restricted to patients with increased LVEDP (wedge pressure $\geq$15 mm Hg) (360). The use of vasodilator therapy in patients with a normal or reduced filling pressure can cause a decrease in cardiac output and reflex tachycardia, which can worsen myocardial ischemia (360).

Aortic Dissection

Acute aortic dissection is a hypertensive crisis requiring immediate antihypertensive therapy aimed at halting the progression of the dissecting hematoma. Patients with acute aortic dissection should be stabilized with intensive antihypertensive therapy to prevent life-threatening complications.

A small intimal tear usually initiates aortic dissection. In 60% to 65% of patients, the intimal tear arises in the ascending aorta within a few centimeters of the aortic valve. In 30% to 35%, it begins in the descending thoracic aorta just distal to the origin of the left subclavian artery, while in 5% to 10% the dissection originates in the transverse aortic arch (361). The most clinically useful classification of aortic dissection is based on the presence or absence of involvement of the ascending aorta regardless of the site of the original intimal tear (362,363) (Fig. 56-24). Proximal dissections include all dissections that involve the ascending aorta, including those that begin in the descending aorta and propagate retrograde into the ascending aorta. Distal dissections involve only the descending aorta. In general, the type of dissection, proximal or distal, defines whether management should be accomplished with drug therapy plus surgery or intensive medical therapy alone.

Degenerative changes in the aortic media underlie most cases of aortic dissection. This medial degeneration is believed to be the result of chronic stress on the aortic wall. Chronic hypertension is the most important risk factor for the development of aortic dissection (364). Typical patients with aortic dissection are 60- to 80-year-old men with a long history of essential hypertension. Less common factors predisposing to aortic dissection include Marfan's syndrome, Ehlers-Danlos syndrome, bicuspid aortic valve, coarctation of the aorta, and

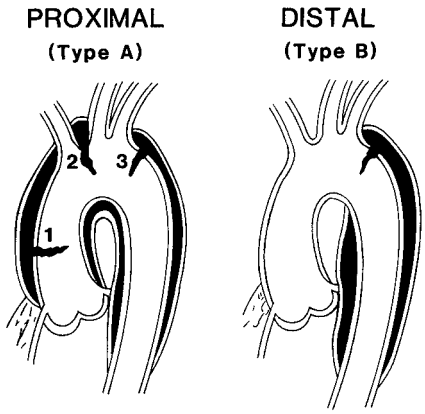

FIGURE 56-24. Classification of aortic dissection based on the presence or absence of involvement of the ascending aorta. The dissection is defined as proximal if there is involvement of the ascending aorta. The primary intimal tear in proximal dissection may arise in the ascending aorta (*1*), transverse aortic arch (*2*), or descending aorta (*3*). In distal dissections, the process is confined to the descending aorta; the ascending aorta is not involved. The primary intimal tear occurs most commonly just distal to the origin of the left subclavian artery. Proximal dissections account for approximately 67% and distal dissections 43% of all acute aortic dissections. (Adapted from: Wheat MW Jr. Acute dissecting aneurysms of the aorta: diagnosis and treatment, 1979. *Am Heart J* 1980;99:373.)

pregnancy (365). Patients with Marfan's syndrome and aortic dissection often have a family history of dissection. There may also be an association between Marfan's syndrome and dissection that occurs in the third trimester of pregnancy.

Following the initial intimal tear, a column of blood driven by the force of arterial pressure enters the aortic wall and destroys the media while stripping the intima for a variable distance along the length of the aorta. The extent of propagation of the dissecting hematoma is determined by several mechanical factors, including the systolic blood pressure, velocity of shearing forces, turbulence of blood flow, and the steepness of the pulse wave (contractility) (361). Experimental evidence suggests that the two most important factors are the blood pressure and the steepness of the pulse wave (366,367). Without treatment, acute aortic dissection is almost always fatal. In a review of survival in untreated patients, one-fourth died within 24 hours, one-half died within 1 month, and more than 90% died within 1 year. The three major complications of aortic dissection are rupture of the aorta, occlusion of major arterial branches arising from the aorta, and acute aortic insufficiency (361). The most frequent mechanism of death is through-and-through rupture of the weakened aortic adventitia (361). The most common site of rupture is the ascending aorta. Because the parietal pericardium is attached to the aorta just proximal to the origin of the innominate artery, rupture of any portion of the ascending aorta leads to hemopericardium and pericardial tamponade. Rupture of the aortic arch causes hemorrhage into the mediastinum. Rupture of the descending thoracic aorta leads to hemorrhage into the left pleural space. Retroperitoneal hemorrhage results from rupture of the abdominal aorta.

The clinical features of acute aortic dissection have been extensively reviewed (363,365,368). Men predominate over women by a ratio of 3:1. The peak incidence is in the sixth and seventh decades. The pain is usually cataclysmic in onset and maximal at its inception in contrast to the crescendo nature of pain seen with acute myocardial infarction. The pain is often described as tearing, ripping, or stabbing. Another characteristic of the pain is its tendency to migrate from the point of origin along the path followed by the dissecting hematoma. The location of the pain is suggestive of the site of origin. Pain felt maximally in the anterior thorax is more frequently due to proximal dissection, whereas pain felt maximally in the interscapular area is more common in distal dissections. Vasovagal symptoms such as diaphoresis, apprehension, nausea, and vomiting are common. Less common presenting symptoms include syncope (usually due to cardiac tamponade) and acute pulmonary edema (secondary to acute aortic insufficiency). In proximal dissections, stroke or altered consciousness can occur due to extension of the dissection into the carotid arteries with diminished carotid blood flow. Occlusion of coronary ostia can lead to acute myocardial infarction. Horner's syndrome can occur if there is compression of the superior cervical sympathetic ganglion. Vocal cord paralysis accompanies compression of the left recurrent laryngeal nerve. Involvement of the descending aorta can lead to mesenteric ischemia, renal insufficiency, lower extremity ischemia or pulse deficits, and focal neurologic abnormalities due to spinal artery occlusion with spinal cord ischemia. Occlusion of renal artery ostia may be signaled by the development of severe hypertension due to renin release from the ischemic kidney (363).

Although the majority of patients with aortic dissection have evidence of long-standing hypertension, the blood pressure can fall such that hypertension is absent at the time of presentation. In one study, at presentation, 56% of patients with distal dissections were hypertensive compared with 9% of patients with proximal dissections (364). True hypotension, which is more common with proximal dissections, is attributable to rupture of the dissected aorta with cardiac tamponade or hemorrhage into the pleural space or retroperitoneum (364). Pseudohypotension can be caused by compromise of flow through either or both subclavian arteries (364). Significant variation in blood pressure between the two arms is not uncommon.

Acute aortic insufficiency can develop with proximal dissection due to dilation of the aortic root or widening of the annulus by the dissecting hematoma so that the valve leaflets fail to oppose during diastole (363). In contrast to the finding in primary aortic valve disease, the murmur of aortic insufficiency is most commonly heard along the right sternal border. Moreover, the murmur may be quite short due to rapid ventricular filling with early equilibration of aortic and left ventricular diastolic pressures.

Although the chest roentgenogram may show widening of the mediastinum, this sign is present in only 40% to 50% of patients (361). The mediastinum bulges to the right with involvement of the ascending aorta and to the left with involvement of the descending thoracic aorta. At the aortic knob, more than 1 cm of separation of intimal calcification from the adventitial border, the so-called calcium sign, is highly suggestive but not diagnostic of aortic dissection (363). A left pleural effusion due to hemothorax can also occur.

In the past decade there has been a dramatic shift away from invasive diagnosis of aortic dissection with aortography to the use of noninvasive diagnostic modalities such as transesophageal echocardiography (TEE) or MRI (368). Imaging modalities are used to confirm the diagnosis and identify the presence or absence of involvement of the ascending aorta thereby defining the dissection as proximal or distal. Additional diagnostic information available from these studies includes the extent of the dissection and the sites of entry and reentry, presence of thrombus in the false lumen, presence of aortic insufficiency or pericardial effusion, and involvement of the coronary arteries or other arterial trunks. These data are crucial in deciding between medical and surgical therapy and for planning surgical intervention.

TEE has rapidly become the preferred imaging technique for evaluation of suspected aortic dissection (369). Although it requires esophageal intubation, TEE can easily be performed in

the emergency room to provide an accurate diagnosis within minutes. In a study designed to assess the comparative diagnostic value of TEE and retrograde aortography, TEE actually had better sensitivity (98% vs. 88%) and negative predictive value (97% vs. 85%) than did aortography (370). The superior sensitivity of TEE was due to the ability to identify noncommunicating dissection (dissection without an apparent intimal tear). However, angiography was more accurate in assessing the site of entry of the dissection (97% vs. 78%). There were no significant differences between the techniques with regard to assessing secondary tears, aortic regurgitation, coronary dissection, or extension of the dissection. Biplane and multiplane TEE are widely used since they permit visualization of the ascending aorta in multiple imaging planes (371). Thus, either TEE or aortography is adequate to diagnose aortic dissection and plan surgical intervention.

MRI also provides an accurate noninvasive technique for evaluating the thoracic aorta in patients with suspected dissection (372). The main disadvantages are that it is difficult to closely monitor the patient during prolonged scanning and that MRI is not readily available on an emergency basis at many institutions. However, MRI may be quite useful for long-term follow-up of patients with aortic dissection.

Treatment of Acute Aortic Dissection

Intensive medical therapy should be instituted immediately in patients with suspected acute dissection, preferably even before definitive diagnostic procedures are undertaken. The initial therapeutic goal is the elimination of pain (which correlates with a halting of the dissection process) and reduction of systolic blood pressure to the 100 to 120 mm Hg range or the lowest level compatible with maintenance of adequate renal, cardiac, and cerebral perfusion. Even in the absence of hypertension, therapy should be instituted. Antihypertensive therapy should be designed not only to lower blood pressure but also to decrease the steepness of the pulse wave. The most commonly used treatment regimen consists of an intravenous β-adrenergic blocking drug such as propranolol, metoprolol, or esmolol in combination with sodium nitroprusside (363,364,373). Beta-blockade should be initiated prior to nitroprusside in order to prevent an adrenergic-mediated reflex increase in cardiac contractility that could further propagate the dissection. After an initial test dose of 0.5 mg, propranolol is administered in 1-mg increments over 5 minutes until there is adequate β-blockade as evidenced by a pulse rate of approximately 60 beats/minute (365). However, the total dose should not exceed 0.15 mg/kg. Subsequent propranolol doses should be given every 4 to 6 hours to maintain β-blockade (374). Pretreatment with intravenous esmolol or metoprolol is also acceptable (374). In patients with bronchospasm, metoprolol can be administered in loading doses of 1 mg every 5 minutes followed by 5- to 15-mg maintenance doses every 4 to 6 hours as necessary. After pretreatment with beta-blocker, sodium nitroprusside is administered to lower the systolic blood pressure into the 100- to 120-mm Hg range.

Trimethaphan has been considered by some to be the preferred drug for the treatment of acute aortic dissection (361,375). In animal models, doses of propranolol much larger than those necessary to produce bradycardia are required to prevent the reflex increase in contractility associated with the use of sodium nitroprusside (375). In contrast, trimethaphan is not associated with reflex increases in heart rate or contractility because it blocks the adrenergic system. Unfortunately, the prolonged use of trimethaphan is limited by its sympathoplegic side effects as well as the rapid development of tachyphylaxis (363).

Labetalol, by virtue of its combined α_1- and β-blocking properties, may be useful in the management of acute dissection. However, its long duration of hypotensive action may not be desirable in critically ill patients with acute aortic dissection who may require urgent surgical intervention. Selective arteriolar vasodilators such as diazoxide, hydralazine, and minoxidil, which activate the adrenergic system, are contraindicated in acute aortic dissection.

After the blood pressure is controlled and the patient is pain-free, TEE or angiography should be performed. When one is deciding between medical and surgical therapy, the most important diagnostic finding is involvement of the ascending aorta. Collective results from long-term follow-up studies indicate that surgical therapy is superior to medical therapy alone in patients with proximal dissections (361,376). Operative mortality for proximal dissections at experienced centers varies from 7% to 20%, which is well below the more than 50% mortality with medical therapy alone. Conversely, in patients with distal dissections, intensive drug therapy leads to an 80% survival rate compared with only 50% in surgically treated patients (361).

Surgical therapy is advantageous in patients with proximal dissection because progression of the dissecting hematoma can result in devastating consequences including neurologic compromise, acute aortic insufficiency, and aortic rupture with cardiac tamponade. Surgical therapy involves excision of the intimal tear, obliteration of proximal entry into the false lumen, and reconstitution of the aorta with interposition of a synthetic vascular graft (377). In patients with aortic insufficiency, restoration of aortic valve competence can be accomplished by resuspension of the native aortic valve or by aortic valve replacement.

There are a number of explanations for the advantage of medical therapy over surgical therapy for acute distal dissection. These patients are generally at greater surgical risk because they are older and have a higher incidence of advanced atherosclerosis and coexistent cardiopulmonary disease (365, 377). A major complication of surgery for distal dissections is spinal cord ischemia and resultant paralysis. Moreover, the risk of life-threatening complications such as cardiac tamponade, aortic insufficiency, and cerebrovascular accident is less than that with proximal dissections. Although medical therapy is generally the treatment of choice in distal dissections, there are situations in which surgery is required. These include inability to control blood pressure, inability to control pain (which implies continued propagation), compromise or occlusion of a major branch of the aorta, or the development of a saccular aortic aneurysm during long-term medical therapy. There is also general agreement that acute distal dissection in patients with Marfan's syndrome should be managed surgically (363).

Long-Term Medical Management of Aortic Dissection

After diagnosis by TEE or aortography, patients with uncomplicated distal dissection should be continued on propranolol and nitroprusside or trimethaphan infusions. A transition to oral antihypertensive therapy should be initiated after the blood pressure has stabilized and clinical evidence of progression or complications of dissection have subsided. Survivors of surgical intervention should also receive long-term medical therapy. The preferred antihypertensive agents for the long-term management of patients with aortic dissection are those that have a negative inotropic effect such as beta-blockers without intrinsic sympathomimetic activity, verapamil, diltiazem, labetalol, methyldopa, and reserpine. Vasodilators such as prazosin, hydralazine, and minoxidil, which cause a reflex increase

in sympathetic tone, should be avoided. Nifedipine and isradipine can cause a reflex increase in heart rate and cardiac output and should probably be avoided or used only in combination with a beta-blocker (374). Converting enzyme inhibitors may also be useful for long-term medical management of aortic dissection (374). The objective of therapy should be to maintain the systolic blood pressure below 130 mm Hg. Even patients without hypertension should be given β-adrenergic blocking drugs postoperatively if at all possible (363).

Reoperation may be required for late complications including progressive aortic insufficiency, localized saccular aneurysm formation, and recurrent dissection (363). The 10-year actuarial survival rate of patients who leave the hospital is approximately 60% (376). In a long-term study of surgically treated survivors of aortic dissection, 29% of all late deaths were due to the development and eventual rupture of a localized saccular aneurysm (378). Thus, close lifelong monitoring of treated survivors of aortic dissection is required. It may be useful to perform a baseline thoracic MRI prior to discharge with follow-up examinations at 6 months and 1 year. Subsequent follow-up studies are usually performed every 1 to 2 years.

HYPERTENSIVE CRISES IN THE PATIENT REQUIRING SURGERY

Poorly Controlled Hypertension in the Patient Requiring Surgery

In the preoperative setting, the goals of blood pressure management include: (a) assessment of the perioperative risks of acute or chronic hypertension; (b) modification of the risk to minimize perioperative cardiac complications; and (c) sometimes the substitution of alternative antihypertensive agents for the patient's chronic oral antihypertensives during the perioperative period (379). Hypertension in the preoperative patient is a common problem. In a series of 1,000 patients over the age of 40 presenting for surgery, 28% were hypertensive (380). However, studies of perioperative cardiac risk have demonstrated that mild to moderate hypertension is not an independent risk factor for the development of postoperative myocardial infarction, pulmonary edema, ventricular tachycardia, or cardiac death (380,381). On the other hand, one study that evaluated intraoperative systemic hemodynamics in patients with either preoperative normotension, adequately treated hypertension, or inadequately treated hypertension demonstrated the benefits of preoperative control of blood pressure (382). During anesthesia, cardiac output decreased by 30% in all three groups. Normotensives and adequately treated hypertensives had only minor changes in systemic vascular resistance resulting in modest decreases in mean arterial pressure of 23% and 33%, respectively. In contrast, the inadequately treated hypertensives experienced, on average, a 27% decrease in systemic vascular resistance which, coupled with the declines in cardiac output, resulted in reduction of mean arterial pressure by 45% during anesthesia. Electrocardiographic changes consistent with myocardial ischemia were commonly observed in the latter group. Hypertensive patients can develop wide swings in blood pressure intraoperatively, which increase the risk of postoperative cardiac and renal complications (383). Thus, while mild to moderate hypertension with diastolic blood pressure under 110 mm Hg may not be an independent risk factor for adverse cardiac outcomes, it may predispose to the development of intraoperative hypotension or hypertension which in turn increases the risk of postoperative complications (379).

Poor control of preoperative hypertension, with a diastolic blood pressure higher than 110 mm Hg, is a relative contraindication to elective surgery. In patients with a diastolic blood pressure higher than 110 mm Hg, perioperative morbidity and mortality are increased due to a high incidence of intraoperative hypotension accompanied by myocardial ischemia and postoperative acute renal failure (384).

However, it should be noted that these data were collected in patients hospitalized for preoperative evaluation in which multiple blood pressure readings were available to document persistently poor preoperative blood pressure control. It is not clear whether these criteria should be applied to patients presenting for outpatient surgery who are found to have a diastolic blood pressure over 110 mm Hg. The finding of elevated blood pressure in this circumstance may not necessarily be reflective of long-term inadequate blood pressure control. In this setting, if there is no history of long-standing inadequate blood pressure control and if the blood pressure responds satisfactorily to sedation, sublingual nifedipine, or mini-bolus labetalol, it may be possible to proceed with elective outpatient surgery (385).

Malignant hypertension clearly represents an excessive surgical risk, and all but lifesaving emergency surgery should be deferred until the blood pressure can be controlled and organ function stabilized (385).

Some authorities believe that mild to moderate, uncomplicated, preoperative hypertension (diastolic blood pressure $\leq$110 mm Hg) does not significantly increase the risk of surgery and is therefore not a reason to postpone elective surgery (384,386,387). However, patients with mild to moderate hypertension and a preexisting complication such as ischemic heart disease, cerebrovascular disease, congestive heart failure, or chronic renal insufficiency represent a subgroup with a significantly increased perioperative risk (384). In these patients, adequate preoperative control of blood pressure is imperative (388). Even though the blood pressure in patients with severe or complicated hypertension can usually be controlled within hours using aggressive parenteral therapy, such precipitous control of hypertension carries the risk of significant complications such as hypovolemia, electrolyte abnormalities, and marked intraoperative and postoperative blood pressure lability. These risks predispose to myocardial ischemia, cerebrovascular accidents, and acute renal failure (386). In these high-risk groups, if possible, elective surgery should be postponed and blood pressure brought under control for a few weeks before surgery (386). Ideally, sustained adequate preoperative blood pressure control should be the aim in all hypertensive patients (386,388).

In patients with adequately treated hypertension, antihypertensive and antianginal medications should be continued up to and including the morning of surgery. Such treatment decreases intraoperative blood pressure lability and protects against the hypertensive response associated with endotracheal intubation and other noxious stimuli during surgery (388,389). Oral administration of blood pressure medications with a small amount of water (15 to 20 mL) a few hours before surgery does not increase the risk of gastric aspiration during anesthesia induction (386). Since hypovolemia increases the risk of intraoperative hypotension and postoperative acute renal failure, diuretics should be withheld for 1 to 2 days preoperatively except in patients with overt heart failure or fluid overload. Adequate potassium supplementation should be provided to correct hypokalemia well in advance of surgery. Drugs such as clonidine, which carry the potential for withdrawal reactions or hypertensive rebound during the postoperative period, may be electively tapered and replaced with other medications over 1 to 2 weeks preoperatively. Alternatively, the clonidine transdermal therapeutic system has been recommended for the perioperative management of patients receiving long-term centrally acting α_2-agonist therapy (390).

Theoretically, β-adrenergic blockers could cause hemodynamic instability in the setting of surgical stress, blood loss,

and the myocardial depression caused by anesthetics. However, studies have shown that elective withdrawal of beta-blockers preoperatively not only is unnecessary but also may be deleterious in patients with underlying coronary artery disease. Continuation of beta-blockers until a few hours before general anesthesia does not appear to impair hemodynamic function (391). Furthermore, patients pretreated with beta-blockers have less hypertension, tachycardia, myocardial ischemia, and dysrhythmias during endotracheal intubation than do patients who do not receive beta-blockers (392).

The use of converting enzyme inhibitors in the preoperative management of the hypertensive patient is controversial (393). The current consensus is that patients receiving chronic therapy with a converting enzyme inhibitor should continue the drug up until surgery and then restart therapy as soon as possible postoperatively. However, given the physiologic role of the renin-angiotensin system in patients subjected to a hypovolemic insult intraoperatively, concern has been raised regarding the risk of intraoperative hemodynamic instability in patients undergoing extensive surgical procedures involving large amounts of blood loss or fluid shifts (394). In contrast, there are data that suggest that converting enzyme inhibitors may be beneficial in patients undergoing coronary artery bypass surgery. Comparison of systemic hemodynamics and renal function in patients pretreated with either captopril or placebo showed that renal plasma flow, glomerular filtration rate, and urinary sodium excretion were higher in patients treated with captopril (395).

The choice of anesthetic technique in the hypertensive patient should be individualized (386,389). For peripheral procedures, regional nerve block involves minimal physiologic stress and may be the procedure of choice. Although spinal anesthesia for lower extremity and certain abdominal procedures minimizes myocardial depression and is not associated with sympathetic discharge during endotracheal intubation, the concomitant sympathetic blockade is not quickly reversible and may lead to cardiovascular collapse in high-risk hypertensive patients (386). For general anesthesia, most of the commonly used anesthetics are acceptable for use in the hypertensive patient. However, drugs such as ketamine that provoke hypertensive responses should be avoided. During anesthesia there are often wide and rapid fluctuations in blood pressure that require close monitoring, often by the direct intraarterial method (386). Continuous electrocardiographic monitoring is essential to monitor for evidence of myocardial ischemia. Accurate measurement of urine output is a helpful indirect measure of the adequacy of renal perfusion. Patients with severe hypertension undergoing upper abdominal or thoracic surgery may require central venous pressure monitoring as a guide to fluid replacement. Monitoring of cardiac output and wedge pressure should be considered in patients with a history of left ventricular failure or ischemic heart disease or those undergoing major thoracic or cardiovascular procedures (386).

The hypertensive surge during endotracheal intubation can be managed with infusion of sodium nitroprusside, esmolol, or minibolus labetalol (389,396,397). In complicated patients with a history of cardiovascular disease or congestive heart failure, intraoperative and postoperative hypertension should be managed with short-acting agents such as sodium nitroprusside or possibly fenoldopam until the preoperative oral antihypertensive regimen can be resumed (3). Given the benefits of intravenous nitroglycerin with regard to coronary vasospasm and the collateral circulation, it may be a useful agent for the management of perioperative hypertension in patients with coronary artery disease undergoing either noncardiac surgery or coronary artery bypass surgery (398). In postoperative patients, trimethaphan is contraindicated because of the risk of bowel and bladder atony.

In uncomplicated patients, intermittent intravenous labetalol injections may be useful in the management of mild to moderate postoperative hypertension (399,400). However, tachycardia and paradoxical hypertension may occur in the setting of volume depletion in patients with chronic hypertension. Therefore, physiologic tachycardia in response to volume depletion should always be excluded prior to parenteral administration of any beta-blocker. Parenteral agents such as furosemide, beta-blockers, and hydralazine, or oral antihypertensive agents given by nasogastric tube have also been recommended for the management of uncomplicated postoperative hypertension until oral therapy can be resumed. Newer agents such as nicardipine and fenoldopam may offer potential advantages over older agents in the treatment of perioperative hypertension (401). However, the cost–benefit ratio of these newer agents must also be considered. Despite the fact that perioperative hypertension is aggressively treated, there are no long-term, large-scale study data indicating that aggressive treatment affects long-term patient outcome (401).

Many patients with long-standing severe hypertension require much smaller doses of antihypertensive medications in the early postoperative course. Thus, the preoperative regimen should not be automatically restarted. Routine measurements of supine and standing blood pressure should be utilized as a guide to dosage adjustments during the postoperative recovery period. In most instances, the requirement for antihypertensive medications will gradually increase over a few days to weeks to eventually equal the preoperative regimen.

Postcoronary Bypass Hypertension

Paroxysmal hypertension in the immediate postoperative period is a frequent and serious complication of cardiac surgery. It is the most common complication of coronary artery bypass surgery, occurring in 30% to 50% of patients (401). Postbypass hypertension is mediated by increases in systemic vascular resistance. The heightened systemic vascular resistance increases cardiac work and myocardial oxygen demand. The accompanying increase in LVEDP impairs subendocardial perfusion and can cause myocardial ischemia in patients with limited coronary reserve. The acute increase in afterload can also impair cardiac performance and precipitate acute pulmonary edema. In addition, hypertension increases the incidence and severity of postoperative bleeding in recently heparinized patients. Therefore, postbypass hypertension should be diagnosed and rapidly treated.

There are numerous precipitating factors for hypertension in the postoperative setting including emergence from anesthesia, tracheal or nasopharyngeal irritation from the endotracheal tube, pain, hypothermia with shivering, ventilator asynchrony, hypoxemia, hypercarbia, myocardial ischemia, and withdrawal phenomena resulting from preoperative discontinuation of antihypertensive medications (401). Hypervolemia, though often cited as a mechanism of postoperative hypertension, is a rare cause of hypertension in this setting except in patients with renal failure (401). Marked sympathetic overreaction to hypovolemia is a common, often unrecognized, cause of severe postoperative hypertension and impaired tissue perfusion (403,404).

Hypertension after myocardial revascularization occurs as often in previously normotensive patients as in those with chronic hypertension (405). The increase in blood pressure usually occurs during the first 4 hours after surgery and tends to resolve 6 to 12 hours postoperatively. The hypertension, which results from a rise in systemic vascular resistance without a change in cardiac output, may be related to an increase in sympathetic tone due to activation of pressor reflexes from the heart, great vessels, or coronary arteries (401,405).

The initial management of postbypass hypertension should include attempts to ameliorate the reversible causes of

hypertension previously mentioned. Hypertension resulting from pain and anxiety should be managed with analgesics or sedatives. Hypothermia should be treated with warming blankets; intravenous fluids should be preheated to 37°C. Patients with paradoxical hypertension in response to volume depletion are exquisitely sensitive to vasodilator therapy and may develop precipitous hypotension even with low-dose infusions of sodium nitroprusside or nitroglycerin. Hypertension in this setting should be treated using careful volume expansion with crystalloid or transfusion as required (403). If these general measures fail to control the blood pressure, further therapy should be guided by measurement of systemic hemodynamics. Parenterally administered vasodilators are the treatment of choice for postbypass hypertension. Sodium nitroprusside or intravenous nitroglycerin can be utilized to provide controlled reduction in the systemic vascular resistance and blood pressure. Intravenous nitroglycerin is as effective for hypertension in this setting as is sodium nitroprusside (398). Nitroglycerin may be the preferred drug because it dilates intercoronary collaterals and causes less intrapulmonary shunting than does sodium nitroprusside. Moreover, sustained infusion of even low-dose sodium nitroprusside may result in cyanide toxicity, which should be considered in the differential diagnosis in postcoronary bypass graft patients who develop unexplained neurologic, cardiac, or pulmonary complications (406). Recent studies suggest that fenoldopam may be useful for the management of hypertension following bypass surgery (407,408). Postbypass hypertension is usually transient and resolves over 6 to 12 hours, after which the vasodilator can be weaned. The hypertension does not usually recur after the initial episode in the immediate postoperative period (405).

An intravenous beta-blocker is occasionally recommended for control of hypertension in patients with tachycardia prior to surgery. However, beta-blockers are generally not indicated in the setting of postbypass hypertension because the hypertension is usually secondary to increased systemic vascular resistance rather than to increased cardiac output (402,405). Moreover, the tachycardia may be a physiologic response to volume depletion. Beta-blocker therapy may be detrimental as these agents compromise cardiac output and increase systemic vascular resistance. In this regard, labetalol has been shown to cause a significant reduction in cardiac index in patients with hypertension following bypass surgery (409).

Postcarotid Endarterectomy Hypertension

Hypertension in the immediate postoperative period is extremely common after carotid endarterectomy. In one large series, 58% of patients had postcarotid endarterectomy hypertension as defined by an increase in systolic blood pressure of more than 35 mm Hg or systolic blood pressure requiring treatment with sodium nitroprusside (410). A history of hypertension, especially with poor control of hypertension preoperatively, dramatically increases the risk of postoperative hypertension (411,412). Severe postoperative hypertension following carotid endarterectomy is associated with an increased incidence of stroke with focal neurologic deficits, intracerebral hemorrhage, and increased postoperative mortality (411–414).

The mechanism of postcarotid endarterectomy hypertension is poorly understood. The incidence of hypertension is the same whether or not the carotid sinus nerves are preserved (411). A mechanism for the development of postoperative hemorrhage due to hypertension has been proposed (413). In patients with high-grade carotid artery stenosis, the distal cerebral bed has been protected from systemic hypertension by the stenosis. Following removal of the obstructing lesion, a relative increase in perfusion pressure occurs in the previously protected arteriocapillary bed. Especially in the setting of postoperative hypertension, cerebral autoregulation may fail such that there is overperfusion and rupture resulting in hemorrhagic infarction.

Because poor preoperative blood pressure control increases the risk of postoperative hypertension, strict blood pressure control is mandatory prior to elective carotid endarterectomy. Furthermore, intraarterial blood pressure should be monitored intraoperatively and in the immediate postoperative period. Ideally, the patient should be awake and extubated prior to reaching the recovery room so that serial neurologic examinations can be used to assess the development of focal deficits. When the systolic blood pressure exceeds 200 mm Hg, an intravenous infusion of sodium nitroprusside should be initiated to maintain systolic blood pressure between 160 and 200 mm Hg (411). The use of a short-acting parenteral agent is imperative to avoid overshoot hypotension and cerebral hypoperfusion.

Hypertensive Crises After Repair of Aortic Coarctation

During the first week after repair of coarctation of the aorta, severe systemic hypertension frequently develops (415). This so-called paradoxical hypertension usually resolves spontaneously if the repair has been satisfactory. There are two distinct phases to the paradoxical hypertensive response: an acute rise in systolic blood pressure on the first postoperative day and a later rise in the diastolic pressure during the second through fourth postoperative days (415,416). The immediate postoperative increase in systolic blood pressure lasts 8 to 12 hours and is similar in mechanism to the postmyocardial revascularization hypertension previously described. The second phase of hypertension, which is accompanied by abdominal pain and signs of an acute abdomen, causes considerable morbidity and mortality due to the development of mesenteric endarteritis (417).

After repair of coarctation, the decline in blood pressure in the upper body results in baroreceptor activation. The release of the sympathetic axis from tonic baroreceptor inhibition results in "vasomotor storm" with markedly increased heart rate and blood pressure. The renin-angiotensin axis may also be activated by the increase in sympathetic tone, and angiotensin-mediated vasoconstriction may account for part of the pressor response (415). The mesenteric vessels that had been exposed to the low pressure distal to the coarctation are suddenly exposed to severe hypertension. This may cause endarteritis and vascular necrosis with intestinal ischemia and infarction.

Prophylactic oral propranolol, at a dosage of 1.5 mg/kg/day in divided doses for 2 weeks before surgery, can prevent paradoxical postcoarctectomy hypertension (415). Sodium nitroprusside and parenteral beta-blockers have also been used successfully to treat postcoarctectomy hypertension. The goal of therapy should be to maintain systolic blood pressure in the range of 120 to 150 mm Hg. As soon as possible, oral propranolol should be instituted as the sodium nitroprusside infusion is weaned (418).

Hypertension Complicating Postoperative Bleeding

Hypertension in the postoperative period can result in severe and intractable bleeding from vascular suture lines. Hypertension can also aggravate bleeding in the setting of severe epistaxis, and tracheal, gastrointestinal, or urinary tract hemorrhage. Retroperitoneal hemorrhage after closed renal biopsy can be exacerbated by hypertension. In each situation, control of the blood pressure is required for normal hemostasis.

Sodium nitroprusside should be utilized for immediate and precise control of the blood pressure.

CATECHOLAMINE-RELATED HYPERTENSIVE CRISES

Hypertensive Crises with Pheochromocytoma

The diagnosis and treatment of pheochromocytoma are discussed in detail in Chapter 55. The comments here are restricted to treatment of hypertensive crises in patients with pheochromocytoma, with emphasis on the perioperative management of hypertension. In the majority of patients, pheochromocytoma causes sustained hypertension that occasionally enters the malignant phase. In roughly 30% of patients, paroxysmal hypertension is present. Paroxysms usually occur spontaneously and consist of severe hypertension, headache, profuse diaphoresis, pallor of the face, coldness of the hands and feet, palpitations, and abdominal discomfort. Marked elevation of blood pressure can lead to intracerebral hemorrhage, hypertensive encephalopathy, or acute pulmonary edema (419). Prompt reduction of blood pressure is mandatory to prevent these life-threatening complications. Although the nonselective α-adrenergic receptor blocker phentolamine is often cited as the treatment of choice for pheochromocytoma-related hypertensive crises, sodium nitroprusside is equally effective (420,421). Phentolamine is given in 5- to 10-mg intravenous boluses every 5 minutes as necessary to control blood pressure. Given its short duration of action, a continuous infusion of phentolamine can also be utilized. After the blood pressure has been controlled with sodium nitroprusside or phentolamine, intravenous β-adrenergic receptor blockers such as esmolol and propranolol can be used to control tachycardia or arrhythmias. After resolution of the hypertensive crisis, oral antihypertensive agents should be instituted as the parenteral agents are weaned.

Skillful preoperative management of blood pressure and volume status is clearly a prerequisite to successful surgical intervention (420–422). Usually, the nonselective α-blocker phenoxybenzamine is administered for 1 to 2 weeks prior to elective surgery. The initial dose of 10 mg twice daily is increased every other day until normotension, accompanied by moderate (15 mm Hg) asymptomatic orthostatic hypertension, has been attained and paroxysms are well controlled (421,422). The last dose of phenoxybenzamine is usually administered at 10 PM on the evening before surgery. After adequate α-blockade has been achieved, oral beta-blocker therapy can be initiated if needed to control tachycardia. Oral or intravenous beta-blockers should *never* be administered before adequate α-adrenergic blockade has been achieved. Administration of a beta-blocker to patients with catecholamine-secreting tumors can lead to severe hypertension with acute pulmonary edema as the result of intense α-adrenergic-mediated vasoconstriction that is no longer opposed by β-adrenergic vasodilatory stimuli. Prazosin, a selective α_1-antagonist, has been used for preoperative management of hypertension (423). However, hypertensive crises responsive to low-dose phenoxybenzamine have been observed in patients receiving apparently adequate α-blockade with prazosin (424). Labetalol has also been advocated for the preoperative management of hypertension in patients with pheochromocytoma (425). However, hypertensive crises precipitated by the use of labetalol have been reported (426). The paradoxical increase in blood pressure is due to the fact that labetalol exhibits more potent β-blockade that α-blockade.

Careful attention to volume status is imperative in the preoperative period (421,422). Alleviation of the chronic state of catecholamine-induced vasoconstriction by α-blockade results in increases in both arterial and venous capacitance. Preoperative volume expansion guided by measurements of central venous pressure or pulmonary capillary wedge pressure has been advocated to reduce the severity of intraoperative hypotension (422). However, other authors maintain that a high-salt diet or infusions of crystalloid are usually not necessary in the majority of patients during the preoperative period because treatment with α-adrenergic blockade for 1 to 2 weeks alleviates the chronic state of vasoconstriction and allows for spontaneous restoration of normal plasma volume (420). Moreover, caution has been advised if intravenous fluids are administered during the preoperative period because pulmonary edema can occur if an underlying catecholamine-induced cardiomyopathy is present (420).

Cardiac status should be evaluated carefully in the preoperative period. Approximately 25% of patients with catecholamine-secreting tumors have some degree of cardiomyopathy with biventricular dysfunction caused either by a direct toxic effect of catecholamines on the myocardium or indirectly by chronic hypertension (420). This catecholamine-induced cardiomyopathy is associated with an increased risk of sudden death from arrhythmias, as well as increased surgical risk. Thus, preoperative evaluation should include echocardiography to assess ventricular function. The cardiomyopathy is usually reversible with adequate preoperative chronic adrenergic blockade. Surgical intervention should generally be deferred until serial echocardiograms confirm that ventricular function has improved in response to treatment with adrenergic blocking drugs.

During surgery, rapid and wide fluctuation in blood pressure should be anticipated (421). Adequate premedication should be used to minimize the risk of sympathetic activation during endotracheal intubation and induction of anesthesia. Diazepam and short-acting barbiturates are the agents of choice for premedication (421). Droperidol, phenothiazines, and morphine are contraindicated because they can cause catecholamine release. Atropine should be avoided because its vagolytic effect results in tachycardia in the setting of high-circulating catecholamine levels.

Careful intraoperative monitoring of intraarterial blood pressure, cardiac output, pulmonary capillary wedge pressure, and systemic vascular resistance is required to manage rapid swings in blood pressure (421). Despite adequate preoperative α-blockade with phenoxybenzamine, severe hypertension can occur during intubation or intraoperatively due to catecholamine release during tumor manipulation. Though intermittent bolus phentolamine has been advocated in this setting, prolonged α-blockade may predispose to significant hypotension following tumor devascularization (421). Therefore, sodium nitroprusside, with its immediate onset and short duration of action, is the agent of choice for controlling acute hypertension during pheochromocytoma surgery (421). Infusions of esmolol, propranolol, or lidocaine can be used for short-term control of arrhythmias (420,421).

At the opposite end of the spectrum, severe intraoperative hypotension can occur. Hypotension or even frank shock can supervene following isolation of tumor venous drainage from the circulation, with a resultant abrupt decrease in circulating catecholamine levels. This hypotension is caused by a precipitous reduction in vascular tone, which can be aggravated further by operative blood loss, downregulation of adrenergic receptors in response to chronic increases in catecholamines, α-adrenergic blockade, or impaired heart rate response resulting from β-adrenergic blocking drugs (421). Volume expansion with crystalloid, colloid, or blood as needed is the recommended treatment for intraoperative hypotension. Volume repletion should be guided by measurements of pulmonary

capillary wedge pressure and cardiac output. Pressors should only be employed when hypotension is unresponsive to adequate volume repletion (421). The risk of hypotension due to hypovolemia extends into the postoperative period during which close monitoring of volume status is essential. In the postoperative period, required volume replacement not uncommonly exceeds measured fluid losses (421).

Hypertensive Crises Secondary to Withdrawal of Antihypertensive Therapy

Abrupt discontinuation of high doses of centrally acting antihypertensive agents such as clonidine (273,427), methyldopa (428), and guanabenz (427,429) can produce a withdrawal syndrome characterized by sympathetic overactivity (430). Symptoms consisting of headache, nausea, restlessness, agitation, insomnia, and tremor usually begin 12 to 72 hours after discontinuation of the drug. Occasionally, this withdrawal syndrome is accompanied by a rapid increase in blood pressure to above pretreatment levels (overshoot hypertension) (431). The abrupt rise in blood pressure can precipitate a hypertensive crisis with hypertensive encephalopathy or acute pulmonary edema.

The symptoms that develop following cessation of centrally acting α-receptor agonists are suggestive of sympathetic overactivity. It has been postulated that the syndrome may be related to excessive circulating catecholamine levels (430). Because the antihypertensive action of central α-agonists is due to a reduction in catecholamine release from nerve terminals, abrupt discontinuation may provoke a sudden catecholamine surge. Increased plasma and urine catecholamine levels have been found after abrupt discontinuation of high-dose clonidine (273). The renin-angiotensin system may also be involved in withdrawal phenomenon. As clonidine and methyldopa suppress plasma renin activity (PRA), it is possible that a rebound increase in PRA and angiotensin II could mediate the hypertensive overshoot following drug withdrawal (432).

In general, withdrawal symptoms or rebound hypertension occur only after cessation of large doses of drugs. Withdrawal symptoms rarely appear after discontinuation of clonidine in doses less than 1.2 mg/day (433). The average dose of guanabenz in the reported cases of withdrawal syndrome was 48 mg/day (429). However, the withdrawal syndrome can occasionally be precipitated by cessation of lower doses of drugs. This is especially apt to occur in patients with underlying renal insufficiency or renovascular hypertension (432). Patients treated with beta-blockers may be predisposed to develop severe hypertension during withdrawal of centrally acting α-agonists (434). Beta-adrenergic receptor blockade inhibits the vasodilatory effect of β_2-receptors on the peripheral vasculature, leaving vasoconstrictor α_1-receptors unopposed.

Treatment of antihypertensive drug withdrawal syndromes should be individualized. In patients with generalized symptoms of sympathetic overactivity but without excessive blood pressure elevation, reinstitution of the previously administered drug is usually all that is required (430). However, if the withdrawal syndrome is associated with severe hypertension, hypertensive encephalopathy, or acute pulmonary edema, rapid control of blood pressure with parenteral antihypertensive agents is imperative. Sodium nitroprusside or phentolamine should be used for the management of these hypertensive crises. After the blood pressure is controlled with parenteral agents, oral clonidine, guanabenz, or methyldopa should be restarted. The offending drug should then be gradually withdrawn with close monitoring for withdrawal symptoms and rebound hypertension. Another oral antihypertensive regimen, preferably without a beta-blocker, should be initiated simultaneously.

Hypertensive Crises Secondary to Monoamine Oxidase Inhibitor Interactions

Severe paroxysmal hypertension complicated by intracerebral or subarachnoid hemorrhage, hypertensive encephalopathy, or acute pulmonary edema can occur in patients receiving monoamine oxidase (MAO) inhibitors after ingestion of certain foods or drugs (274,435). The three major MAO inhibitors available in the United States are the antidepressant drugs tranylcypromine (Parnate), phenelzine (Nardil), and isocarboxazid (Marplan).

Although catechol O-methyltransferase is important in the metabolism of circulating catecholamines, MAO is required for the degradation of intracellular amines including epinephrine, norepinephrine, and dopamine (436). Since MAO normally limits intracellular amine accumulation, MAO inhibitors cause an increase in the quantity of amines within storage granules. The amino acid tyramine releases these stores of catecholamines from nerve endings, causing a profound pressor response. Certain foods contain substantial amounts of tyramine including natural or aged cheeses, Chianti wines, champagne, imported beers, pickled herring, chicken liver, yeast, soy sauce, fermented sausage, coffee, avocado, banana, chocolate, overripe or spoiled food, and aged fish or meat (salami, pepperoni, and bologna) (435,437). As a result of hepatic and intestinal MAO-inhibition, tyramine escapes oxidative degradation and causes release of norepinephrine from nerve endings. Sympathomimetic amines in nonprescription cold remedies such a phenylpropanolamine can also provoke neurotransmitter release. A hyperadrenergic state resembling pheochromocytoma then ensues.

In a large series of patients treated with MAO inhibitors, symptoms typically began within 10 minutes to 2 hours after ingestion of the offending food or drug. Symptoms include sudden onset of severe pounding headache, palpitations, throbbing vessels in the neck, flushing or pallor, profuse diaphoresis, nausea, vomiting, and extreme prostration. Abrupt onset of marked hypertension is a characteristic finding. The mean increase in blood pressure is 55 mm Hg systolic and 30 mm Hg diastolic. Complications include intracerebral hemorrhage, subarachnoid hemorrhage, hypertensive encephalopathy, and acute pulmonary edema. The duration of the attacks varies from 10 minutes to 6 hours (435).

Either sodium nitroprusside or phentolamine can be used to manage this type of hypertensive crisis. Because most patients are normotensive prior to the onset of the hypertensive crisis, the goal of treatment should be the normalization of blood pressure. Intravenous beta-blockers may also be required for control of heart rate and tachyarrhythmias. Because hypertensive crisis with MAO inhibitor–tyramine interactions is usually self-limited, the nitroprusside or phentolamine infusion can be weaned without institution of an oral antihypertensive agent.

Hypertensive Crises Due to Nonprescription Sympathomimetic Amines

Phenylpropanolamine, phenylephrine, ephedrine, and pseudoephedrine are sympathomimetic amines available in a wide variety of over-the-counter drug preparations that are marketed as nasal decongestants, appetite suppressants, or stimulants. However, toxic effects can result from overdose (278,279,438). Moreover, there may be substantial abuse

potential for use as amphetamine substitutes (279). A recent study in healthy, normotensive subjects found that 150 mg of phenylpropanolamine (the amount contained in a double dose of an over-the-counter appetite suppressant) substantially elevated blood pressure (439). Review of adverse drug effect case reports suggests that overdose of phenylpropanolamine can cause a significant increase in blood pressure that can be complicated by severe headache, hypertensive encephalopathy, intracerebral hemorrhage, seizures, and even death (278). Given the majority of serious adverse events, the FDA has recently banned the use of phenylpropanolamine in over-the-counter medications (440). In patients who present with hypertensive encephalopathy of unknown origin, the possibility of recent ingestion of over-the-counter sympathomimetic amines should be investigated. Hypertensive crises secondary to sympathomimetic amines should be treated with a rapid-acting agent such as sodium nitroprusside or phentolamine. This is generally the only treatment required because these drugs are rapidly eliminated and the duration of the toxic reaction is usually less than 6 hours (279).

AUTONOMIC HYPERREFLEXIA FOLLOWING SPINAL CORD INJURY

Autonomic hyperreflexia (or autonomic dysreflexia) is an acute medical emergency that occurs in quadriplegics and paraplegics whose spinal cord lesion lies above the greater splanchnic outflow from the thoracolumbar preganglionic sympathetic neurons (lesions at T-6 or above) (287,441–444). This potentially life-threatening syndrome results from interruption of normal feedback mechanisms in the sympathetic pathway (441). Attacks of autonomic hyperreflexia usually begin at 4 to 6 months after the injury and can recur episodically for the rest of the patient's life (444). Autonomic hyperreflexia develops in 50% to 80% of patients with spinal cord injury (287,444). Hypertensive crises, presumably due to autonomic hyperreflexia, have also been reported in patients with Guillain-Barré syndrome (445). Noxious stimuli arising below the level of the injury, most commonly due to distention of the bladder or bowel, trigger a response mediated by the sympathetic nervous system (442). Afferent impulses from nerves below the level of the cord lesion cause excess stimulation of preganglionic sympathetic neurons. The result is reflex sympathetic outflow via the splanchnic nerves with profound vasoconstriction in the visceral arteries of the splanchnic bed leading to a sudden increase in blood pressure. The elevated blood pressure stimulates baroreceptors in the carotids and aortic arch and signals are sent to the vasomotor center in the brainstem. Parasympathetic efferent impulses from the brainstem via the tenth cranial nerve cause bradycardia, which may be transient. However, in patients with spinal cord lesions above the major splanchnic sympathetic outflow, descending inhibitory feedback is blocked so that reflex vasoconstriction of the peripheral and splanchnic vasculature continues unabated. The end-result is a sudden increase in blood pressure, often reaching systolic pressures over 250 mm Hg. It is important to note that the normal resting systolic blood pressure in patients with spinal cord injury is often 80 to 90 mm Hg, and a systolic blood pressure of 130 mm Hg may be a sign of autonomic hyperreflexia. The most common symptom during a paroxysm of autonomic hyperreflexia is severe headache, but the sudden increase in blood pressure may trigger a hypertensive crisis with seizures, cortical blindness, hypertensive encephalopathy, intracerebral hemorrhage, or acute hypertensive heart failure. Additional symptoms and signs include feelings of doom, facial flushing, nasal congestion, diaphoresis and piloerection above the level of the cord lesion, and cool clammy skin below the level of injury. Bradycardia is present in only 50% of cases. Educating patients about dysreflexia is an important part of the initial rehabilitation from spinal cord injury. Most patients can recognize the occurrence of their specific pattern of signs and symptoms of dysreflexia.

Autonomic hyperreflexia can be triggered by any noxious stimulus in the dermatomes, muscles, or viscera supplied by nerves below the level of the cord injury. Stimuli related to distention of a hollow viscus are particularly effective in eliciting this response (441–444). Bladder distention and fecal impaction are the most frequent inciting stimuli (286). Urinary tract instrumentation, gynecologic instrumentation, or labor and delivery may also provoke an attack. Spinal cord injury patients undergoing abdominal or bladder surgery are also at high risk. In addition, a variety of medical problems including pressure sores, occult fractures, deep venous thrombosis, and heterotopic ossification may cause symptoms of dysreflexia. Patients at risk should be cautioned about the use of over-the-counter sympathomimetic medications, such as decongestants and appetite suppressants, which may also provoke an attack.

Autonomic hyperreflexia is best managed by prevention. If routine bowel or bladder care (bladder catheterization or bowel programs) trigger an attack, local anesthesia with Xylocaine lubricant may be used. Individuals with frequently occurring symptoms may benefit from prophylactic treatment with nifedipine or phenoxybenzamine. Drug regimens should only be implemented after a thorough search for potentially underlying correctable causes. Management of hypertensive crises due to autonomic hyperreflexia requires prompt recognition with correction of precipitating causes. Failure to recognize autonomic hyperreflexia or misdiagnosis can lead to substantial morbidity. Any spinal cord injury patient presenting with an emergency or strange symptoms should be suspected of having autonomic dysreflexia until proven otherwise (443,444). The severe hypertension must be treated expeditiously to avoid such complications as retinal hemorrhage, seizures, hypertensive encephalopathy, or intracerebral hemorrhage. During an acute episode, the patient should be brought to a sitting position with legs dangling to take advantage of the natural orthostasis that occurs with spinal cord injury. The bladder should be checked for distention by immediate catheterization. If bladder distention is not found, rectal examination should be performed to exclude fecal impaction. If these measures fail, medication to blunt the sympathetic response should be administered. Transdermal nitroglycerin may break the hypertensive reflex and this agent can be removed when the blood pressure normalizes. Oral clonidine or nifedipine may also be useful alternatives. Once the autonomic reflex is broken, long-term antihypertensive drug therapy is seldom needed. Occasionally, development of a hypertensive crisis with encephalopathy or seizures may necessitate treatment with parenteral antihypertensive agents such as diazoxide, trimethaphan, nitroprusside, or fenoldopam. Nitroprusside is particularly useful for management of intraoperative hypertension in patients with spinal cord injury.

MISCELLANEOUS HYPERTENSIVE CRISES

Hypertensive crises have been reported in a wide variety of clinical settings. Patients with extensive second- or third-degree burns may develop hypertensive crises 3 to 4 days after hospitalization (446). Hypertensive crises have been reported as idiosyncratic reactions to a number of drugs including amphotericin B (447), lithium (intoxication) (448), amitriptyline

(449), and metrizamide (450). Severe hypertension with hypertensive encephalopathy has been reported to occur in a dose-related fashion in children treated with high-dose cyclosporine for allogenic bone marrow transplantation (280,281). Hypertensive crises can also complicate the use of illicit drugs including cocaine hydrochloride or alkaloidal (crack) cocaine (451,452), and phencyclidine hydrochloride (PCP) (276).

In each of these conditions sudden elevation of blood pressure in previously normotensive individuals can cause hypertensive encephalopathy, intracerebral hemorrhage, or acute pulmonary edema. Sodium nitroprusside is the treatment of choice for the management of hypertension in these diverse settings.

Hypertensive crises have been reported in patients with insulin-induced hypoglycemia who are concomitantly treated with beta-blockers (453). It has been postulated that epinephrine release induced by hypoglycemia, in the presence of vascular β_2-receptor blockade by propranolol, causes severe hypertensive reactions due to unopposed α_1-receptor-mediated vasoconstriction.

Hypertensive crises may develop in renal allograft recipients due to acute rejection, high-dose glucocorticoid treatment, or transplant renal artery stenosis (283,284,454). Refractory hypertension complicated by hypertensive encephalopathy can also occur in patients with chronic allograft rejection who have returned to dialysis. This complication tends to develop during tapering of immunosuppressive therapy and may be caused by superimposed acute allograft rejection. Allograft nephrectomy may be indicated for the long-term control of blood pressure in this setting.

THE CONTROVERSY OVER GRADUAL VERSUS RAPID REDUCTION OF BLOOD PRESSURE

Over the last several years, some authors have cautioned against rapid lowering of blood pressure in patients with hypertensive crises and have recommended a more gradual reduction of blood pressure (319,455–457). The case for gradual reduction of blood pressure is based largely on the finding of altered autoregulation of cerebral blood flow in hypertensive patients and scattered case reports of serious neurologic sequelae resulting from overly aggressive reduction of blood pressure in patients with severe hypertension or hypertensive crises (294,458–464).

In both hypertensive and normotensive individuals, cerebral blood flow is maintained constant, at approximately 50 mL/minute/100 g of brain tissue, over a wide range of perfusion pressures, by virtue of various intrinsic and neurohumoral autoregulatory mechanisms. The lower limit of cerebral blood flow autoregulation is the blood pressure below which autoregulatory vasodilation becomes maximal and cerebral blood flow decreases. In normotensive subjects, the lower limit of autoregulation is a mean arterial pressure of 60 to 70 mm Hg. In chronically hypertensive patients, the lower limit of autoregulation is shifted so that autoregulation fails and cerebral blood flow decreases at a higher blood pressure than in normotensive individuals (292,293). This effect may be the result of hypertension-induced changes in the cerebral arterioles. In animal models, chronic hypertension causes hypertrophy of the walls of cerebral vessels with a reduction in internal diameter. Moreover, during chronic hypertension, cerebral arterioles undergo structural remodeling, which results in a smaller external diameter and encroachment on the vascular lumen (184).

On the one hand, these structural changes are protective in that the thickened cerebral arterioles are able to maintain constant cerebral blood flow at a higher perfusion pressure than would be tolerated by normotensive individuals. In this regard, in chronically hypertensive individuals, the mean arterial pressure at which autoregulatory vasoconstriction gives way to pressure-induced forced vasodilation and hyperperfusion, that is, the upper limit of cerebral blood flow autoregulation, is shifted to a higher level compared to the upper limit in normotensive individuals (see discussion of breakthrough theory in the above section on hypertensive encephalopathy) (292,293). However, as a consequence of these structural changes, the arterioles are not able to dilate fully at low mean arterial pressures, which could predispose hypertensive patients to cerebral ischemia if the blood pressure is lowered excessively.

Fortunately, with long-term control of blood pressure these changes in cerebral arterioles appear to be at least partially reversible given the observation that patients with previously severe but adequately treated hypertension have a lower limit of autoregulation, which is shifted toward the range for normotensive subjects (465) (Table 56-6).

The upward shift in the autoregulatory curve in patients with chronic hypertension is one of the major arguments put forward by those who favor gradual reduction of blood pressure in patients with hypertensive crises (319,456). However, the clinical importance and therapeutic implications of this shift in the autoregulatory curve may have been overemphasized. The demonstration of hypertensive adaptation of cerebral autoregulation should not be interpreted to mean that acute reduction of blood pressure in hypertensive crises is

TABLE 56-6

AUTOREGULATION OF CEREBRAL BLOOD FLOW DURING TRIMETHAPHAN-INDUCED HYPOTENSION[a]

	MAP (mmHg)			Percent of resting MAP %	
Group	Resting level	Autoregulation	Tolerated MAP	Autoregulation	Tolerated
Uncontrolled severe hypertensives ($N = 13$)	145 ± 17	113 ± 17[b,c]	65 ± 10[b]	79 ± 10	45 ± 6
Controlled hypertensives ($N = 9$)	116 ± 18	96 ± 17	53 ± 18	72 ± 29	46 ± 16
Normotensives ($N = 10$)	98 ± 10	73 ± 9	43 ± 8	74 ± 12	45 ± 12

MAP, mean arterial pressure.
[a]Values given as mean ± SD.
[b]$p < 0.01$ for difference between normotensives and uncontrolled hypertensives.
[c]$p < 0.01$ for difference between controlled and uncontrolled hypertensives.
(Adapted from: Gifford RW Jr. Effect of reducing elevated blood pressure on cerebral circulation. *Hypertension* 1983;5[Suppl III]:III-17, with permission.)

unwise (293). In the various hypertensive crises in which rapid reduction of blood pressure is indicated (vide supra), the proven benefits of acute reduction of blood pressure (i.e., decreased risk of intracerebral hemorrhage, hypertensive encephalopathy, or acute pulmonary edema) clearly outweigh the theoretic risk of blood pressure reduction (i.e., possible cerebral ischemia).

In practice, moderate, controlled reduction of blood pressure in hypertensive crises rarely causes cerebral ischemia (293). This clinical observation may be explained by the fact that even though the autoregulatory curve is shifted toward a higher blood pressure in chronically hypertensive patients, there is still a considerable difference between the presenting blood pressure and the lower limit of autoregulation (Table 56-6). Strandgaard (465) has studied the autoregulation of cerebral blood flow during controlled hypotension produced with trimethaphan and a 25-degree head-up tilt in 13 patients with untreated or ineffectively treated hypertension. At least eight of these patients had grade III or grade IV changes on funduscopy consistent with the diagnosis of malignant hypertension. The control groups included 9 patients who had been severely hypertensive in the past but whose blood pressure was effectively controlled at the time of the study, and 10 normotensive subjects. Baseline mean arterial pressures in the three groups were 145 ± 17, 116 ± 18, and 98 ± 10 mm Hg, respectively (Table 56-6). The lower limit of mean arterial pressure at which autoregulation of cerebral blood flow failed was 113 ± 17 mm Hg in uncontrolled hypertensives, 96 ± 17 mm Hg in controlled hypertensives, and 73 ± 9 mm Hg in normotensive individuals. Although the absolute level at which autoregulation failed differed substantially in the three groups, the percentage reduction of mean arterial pressure at which autoregulation failed was similar. The mean arterial pressure at the lower limit of autoregulation was 79% ± 10% of the resting mean arterial pressure in the uncontrolled hypertensives, 72% ± 29% in the controlled hypertensive group, and 74% ± 12% in the normotensive group. Thus, a reduction in mean arterial pressure of approximately 20% to 25% from the baseline level was required in each group to reach the lower limit of autoregulation. Therefore, even in uncontrolled hypertensive patients, there was a considerable safety margin before the limit of autoregulation was reached. Another important observation from this study was that symptoms of cerebral hypoperfusion did not occur until the blood pressure was reduced substantially below the lower limit of autoregulation (465). Studies have shown that with normal cerebral blood flow, oxygen extraction is not maximal because oxygen saturation in the jugular venous blood at rest is normally 60% to 70%. Thus, even when the mean arterial pressure is reduced below the lower limit of autoregulation, cerebral metabolism can be maintained and ischemia prevented by increasing oxygen extraction from the blood (293). The lowest tolerated blood pressure, which was defined as the level at which mild symptoms of brain hypoperfusion were encountered (yawning, nausea, and hyperventilation with hypocapnia), was 65 ± 10 mm Hg in patients with uncontrolled hypertension, 53 ± 18 mm Hg in patients with controlled hypertension, and 43 ± 8 mm Hg in normotensive subjects. These values were 45% ± 6%, 46% ± 16%, and 45% ± 12% of the resting baseline mean arterial pressures, respectively. Thus, symptoms of cerebral hypoperfusion did not occur until the mean arterial pressure was reduced by an average of 55% from the resting level (Table 56-6).

In summary, with regard to the shift in cerebral autoregulation in chronically hypertensive patients, there is a therapeutic threshold above which the blood pressure can be reduced safely in patients with hypertensive crises who require immediate control of hypertension. Strandgaard concludes that the upward shift in cerebral autoregulation should not be taken as a warning against aggressive antihypertensive therapy in hypertensive crises. It merely implies that the initial treatment should be aimed at partial reduction but not complete normalization of blood pressure (465,466).

The second argument used to support the recommendation for gradual reduction of blood pressure is based on case reports of the occurrence of acute neurologic sequelae during rapid blood pressure reduction in the treatment of severe hypertension or hypertensive crises (394,458–464).

Franklin reviews 19 reported cases of neurologic complications following aggressive antihypertensive therapy (193). The average age of the patients was 36 years. All had evidence of severe antecedent hypertension with an average mean arterial pressure of 188 ± 19 mm Hg. Malignant hypertension, based on the finding of hypertensive neuroretinopathy, was present in 79% and hypertensive encephalopathy was present in 53% of these patients. Aggressive antihypertensive treatment resulted in a reduction of mean arterial pressure to 84 ± 18 mm Hg. This represented a 56% decrease from the baseline blood pressure level, a level clearly below the predicted autoregulatory range for hypertensive patients. The time course of blood pressure reduction was within minutes in 26% and over hours in 74% of patients. However, the most critical factor in the development of neurologic sequelae was the long duration of drug-induced overshoot hypotension, which varied from a period of hours to days. Neurologic complications consisted of permanent blindness in 47%, coma in 32%, pyramidal tract signs in 32%, residual neurologic deficits after therapy in 58%, and death in three patients. The majority of these patients (80%) had received a large intravenous bolus of diazoxide. Three patients received no parenteral agents but had sustained hypotension induced with multiple oral agents. Franklin concludes that rather than the rapidity with which blood pressure was reduced, the duration of excessive hypotension was the factor that correlated best with the development of neurologic complications.

In summary, the data suggest that in the treatment of patients with hypertensive crises who require prompt control of blood pressure, potent parenteral agents can be used safely if excessive lowering of blood pressure is avoided. The studies of Strandgaard suggest that autoregulation of cerebral blood flow can be maintained in hypertensive patients as long as the mean arterial pressure is not reduced below 120 mm Hg (465,466). This value is two standard deviations above the average mean arterial pressure at which patients in the reported series developed neurologic sequelae.

In general, an initial blood pressure reduction to 160 to 170 mm Hg systolic and 100 to 110 mm Hg diastolic or to a mean arterial pressure of 120 to 130 mm Hg can be safely accomplished in patients who require immediate control of blood pressure in the setting of hypertensive crises (193). Alternatively, the initial antihypertensive therapy can be individualized based on the pretreatment level of blood pressure. In the individual patient, reduction of the mean arterial pressure by 20% should be the initial therapeutic goal. At this level, the blood pressure should still be above the predicted autoregulatory lower limit. Once this goal is obtained, the patient should be carefully evaluated for evidence of cerebral hypoperfusion. Further reduction of blood pressure can then be undertaken if necessary in a controlled fashion based on the overall status of the patient. In previously normotensive individuals in whom acute hypertensive crises develop, such as patients with acute glomerulonephritis complicated by hypertensive encephalopathy, eclampsia, and autonomic hyperreflexia, the autoregulatory curve may not yet be shifted and the initial goal of therapy will often be normalization of the blood pressure.

The use of potent parenteral agents with a rapid onset and short duration of action, such as sodium nitroprusside, has obvious advantages. If overshoot hypotension or neurologic sequelae develop, they can be quickly reversed by allowing the blood pressure to stabilize at a higher level. Agents with a long

duration of action all have an inherent disadvantage in that excessive reduction of blood pressure cannot be easily reversed. Thus, diazoxide, labetalol, minoxidil, hydralazine, converting enzyme inhibitors, calcium channel blockers, and central α-agonists should be used with extreme caution in patients requiring rapid blood pressure reduction in order to avoid prolonged overshoot hypotension.

Although in the great majority of hypertensive patients, cautious blood pressure reduction can be undertaken without a significant risk of causing cerebral hypoperfusion, it should be noted that there is one clinical setting in which there is a significant risk of causing cerebral ischemia even with moderate blood pressure reduction. In patients with acute cerebral infarction, because of failure of autoregulation in the surrounding marginally ischemic zone, even moderate blood pressure reduction can be detrimental. Therefore, in acute cerebral infarction, the aforementioned considerations regarding the general safety of acute blood pressure reduction do not apply. The management of hypertension complicating acute cerebral infarction has been outlined in the section entitled Hypertension Complicating Cerebrovascular Accident.

PHARMACOLOGY OF DRUGS USEFUL IN THE TREATMENT OF HYPERTENSIVE CRISES

Sodium Nitroprusside

In 1929, intravenous administration of the color indicator sodium nitroprusside was reported to lower blood pressure (467). Nonetheless, concern that the hypotensive action of the drug was related to the release of cyanide led to a delay in the introduction of the drug. In 1955, intravenous infusion of sodium nitroprusside was shown to be a safe and effective method for achieving short-term blood pressure control (468). However, it was not until 1974 that sodium nitroprusside (Nipride) was approved for clinical use. Over the last 25 years, it has remained the drug of choice for the management of virtually all hypertensive crises. Sodium nitroprusside is useful for the management of hypertensive crises due to malignant hypertension, pheochromocytoma, and other catecholamine-related hypertensive crises, hypertensive encephalopathy, acute pulmonary edema, intracerebral hemorrhage, aortic dissection (in combination with propranolol), and perioperative hypertension (360,469).

Mechanism of Action

Sodium nitroprusside is a potent intravenous hypotensive agent with an immediate onset and brief duration of action. The site of action is the vascular smooth muscle. It has no direct effect on the myocardium, although it may indirectly affect cardiac performance through alterations in systemic hemodynamics. In therapeutic doses it has no effect on duodenal or uterine smooth muscle (468). It has no direct central nervous system effect. Sodium nitroprusside causes vasodilation of both arteriolar resistance vessels and venous capacitance vessels. Its hypotensive action is the result of a decrease in systemic vascular resistance. Venodilation results in a decrease in venous return; hence preload is reduced. The combined decrease in preload and afterload reduces left ventricular wall tension and myocardial oxygen demand.

The net effect of sodium nitroprusside on cardiac output and heart rate depends on the intrinsic state of the myocardium (436,469). In the absence of congestive heart failure, venodilation and preload reduction can result in a small decrease in cardiac output with a reflex increase in sympathetic tone and heart rate (470–472). In contrast, in patients with left ventricular dysfunction and elevated left ventricular end-diastolic volume or pressure, sodium nitroprusside causes an increase in stroke volume and cardiac output as the result of a reduction in afterload and impedance to left ventricular ejection. There is usually a reduction in heart rate as the result of improved cardiac performance (470–472).

The cellular mechanism of action of nitroprusside has been well defined (473,474). Nitroprusside is an iron coordination complex with five cyanide moieties and a nitroso group. The action of sodium nitroprusside, as well as that of other nitrogen oxide-containing vasodilators, is mediated by a reaction with cysteine to form nitrosocysteine and other short-acting S-nitrosothiols. Nitrosocysteine, a potent activator of guanylate cyclase, causes cyclic guanosine monophosphate accumulation and relaxation of vascular smooth muscle (473,474).

Pharmacokinetics

The hypotensive effect of sodium nitroprusside appears within seconds and is immediately reversible when the infusion is stopped. It is rapidly metabolized, with a reported half-life of 3 to 4 minutes. Cyanide is formed, as a short-lived intermediate product, by direct combination of sodium nitroprusside with sulfhydryl groups in red cells and tissues (468). The cyanide groups are rapidly converted to thiocyanate by the liver in a reaction in which thiosulfate acts as a sulfur donor. Thiocyanate is excreted unchanged by the kidney with a half-life of 1 week in patients with normal renal function (469).

Dosage and Administration

The contents of a 50-mg sodium nitroprusside vial should be dissolved in 2 mL of dextrose in water. No other diluent should be used. The stock solution is diluted in 250 mL of dextrose in water to yield a concentration of 200 μg/mL. The container is immediately wrapped in aluminum foil to prevent decomposition on exposure to light. A small portion of the tubing can be left uncovered to observe the solution for color changes during administration. The freshly prepared solution has a faint brownish tint. The nitroprusside molecule reacts with a wide variety of organic and inorganic substances to yield highly colored reaction products. Therefore, the infusion fluid should not be used as a vehicle for the delivery of other drugs. If a color change occurs, the solution should be replaced. Regardless, the solution should be changed every 24 hours.

In patients who are not taking other antihypertensive agents, the average effective dose is 3.0 μg/kg/minute (range, 0.5 to 10.0 μg/kg/minute). The initial infusion rate should be 0.5 μg/kg/minute. The flow rate should be increased in increments of 1 μg/kg/minute every 2 to 3 minutes until the desired hypotensive response is obtained. The solution should be administered by infusion pump or microdrip regulator to allow for precise measurement of flow rate. The blood pressure should be monitored every 30 to 60 seconds during the initial titration and every 15 minutes thereafter. To avoid excessive accumulation of thiocyanate and the risk of cyanide toxicity, the infusion rate should not be increased above 10 μg/kg/minute. Sodium nitroprusside failures are extremely rare, and tachyphylaxis does not occur. Concomitant oral antihypertensive agents should be initiated as soon as possible and the sodium nitroprusside infusion weaned as it becomes effective.

Adequate facilities, equipment, and personnel must be available for close monitoring of blood pressure during sodium nitroprusside administration. Auscultatory or oscillometric pressure is usually adequate, so that intraarterial pressure monitoring is not routinely required (469). However, in hypertensive patients with acute myocardial infarction or acute pulmonary edema, hemodynamic monitoring may be required for

assessment of left ventricular filling pressure and cardiac output (469).

Adverse Effects

Nitroprusside is the most effective parenteral agent for the management of hypertensive crises. When properly administered in an intensive care unit setting, it is very safe and clinically significant adverse reactions are uncommon. Overshoot hypotension can result from accidental bolus infusion, faulty infusion equipment, or failure to frequently monitor the blood pressure. However, the hypotensive action is evanescent and hypotension can be reversed easily by slowing or discontinuing the infusion.

The most frequent side effects include anorexia, nausea, vomiting, abdominal cramps, diaphoresis, headache, apprehension, restlessness, and palpitations. Most of these adverse reactions result from rapid blood pressure reduction per se and they usually disappear if the infusion is slowed.

Thiocyanate accumulation and toxicity can occur when a high-dose or prolonged infusion is required, especially in the setting of renal insufficiency. When these factors are present, thiocyanate levels should be monitored and the infusion reduced or discontinued if the plasma level exceeds 10 mg/dL. Thiocyanate toxicity is rare in patients with normal renal function requiring less than 3 μg/kg/minute for less than 72 hours. Symptoms of thiocyanate toxicity include fatigue, anorexia, weakness, tinnitus, blurred vision, and disorientation, which may progress to frank organic psychosis with hallucinations. Seizures have also been reported. Treatment consists of discontinuing the infusion. Thiocyanate is also efficiently removed by both peritoneal dialysis and hemodialysis (469).

Cyanide poisoning is a very rare complication of sodium nitroprusside use. Since hepatic clearance of cyanide may be deficient in patients with severe liver disease (360) and in rare conditions such as Leber's optic atrophy or tobacco amblyopia (475), the use of sodium nitroprusside is contraindicated in these settings. Most of the reported deaths from cyanide poisoning occurred when very high doses of nitroprusside (20 μg/kg/minute) were required for the control of refractory hypertension or in normotensive patients in whom very large doses were used to induce deliberate surgical hypotension (476,477). The cyanide ion combines with cytochrome *c* and inhibits aerobic metabolism so that lactic acidosis results. Cyanide toxicity most often occurs within the first 6 to 8 hours of therapy. Cyanide toxicity should be considered if there appears to be increased tolerance to the drug. Tachyphylaxis and an increased anion gap metabolic acidosis are the most reliable early signs of cyanide toxicity. Other signs include the smell of bitter almonds on the breath, anxiety, headache, stiffness of the lower jaw, dyspnea, and widely dilated pupils. Coma, seizures, and death may follow. Occult cyanide toxicity has been reported in patients who are treated with prolonged low-dose infusion of sodium nitroprusside following cardiac surgery (406). Treatment of cyanide toxicity consists of amyl nitrite inhalation, and sodium nitrite, thiosulfate, and hydroxocobalamin infusions (360,478).

The safe use of sodium nitroprusside during pregnancy has not been established. In animals, nitroprusside readily crosses the placenta. In a study of eight normotensive gravid ewes, five required high doses of nitroprusside (mean, 25 μg/kg/minute) to reduce blood pressure by 20% for 1 hour (479). Among these five animals, a marked accumulation of maternal cyanide occurred. Fetal blood levels of cyanide were even higher and all of these fetuses died. However, in the other three ewes, hypotension was achieved with low doses of sodium nitroprusside (less than 1 μg/kg/minute). In this group, all of the fetuses survived and umbilical cord blood cyanide levels were low.

When sodium nitroprusside was used to achieve normotension for 50 minutes in ewes with norepinephrine-induced hypertension, the mean infusion rate required to control blood pressure was only 2.3 μg/kg/minute, and no fetal or maternal deaths occurred (480). Neither maternal nor fetal blood samples contained more than 50 μg/L of cyanide (toxic levels in humans, 5,000 μg/L).

There are some reports on the safe use of sodium nitroprusside for hypertensive crises in pregnant women (481–483). It has been recommended that the use of sodium nitroprusside for hypertensive crises during pregnancy be restricted to patients who are unresponsive to intravenous hydralazine or diazoxide (484). When nitroprusside is required, it should only be used briefly to manage the acute crisis, and delivery should be performed as quickly as possible.

In summary, sodium nitroprusside has several characteristics that make it nearly the ideal drug for the short-term management of hypertensive crises. These include rapid onset of action, immediate reversibility, specific effects on resistance and capacitance vessels with no direct effect on the myocardium or central nervous system, lack of tachyphylaxis, and high potency. It is also a very safe drug when used appropriately. It is the most useful and consistently effective drug available for parenteral use in the treatment of hypertensive crises.

Fenoldopam

Fenoldopam is a selective dopamine receptor (DA_1) agonist. Recent studies have shown that intravenous fenoldopam, when used in the setting of hypertensive crises or perioperative hypertension, can safely lower blood pressure while maintaining or improving renal function (3,485). Fenoldopam, a benazepine derivative of dopamine was initially developed as an oral agent for the treatment of hypertension, renal insufficiency, and congestive heart failure. However, it was eventually withdrawn from development because of poor oral bioavailability. When subsequent studies demonstrated that intravenous fenoldopam exhibited a short-half life and predictable pharmacokinetics and dose-response characteristics, it was subsequently evaluated as a potential alternative to sodium nitroprusside for parenteral treatment of hypertension. Intravenous fenoldopam mesylate (Corlopam) was approved by the Food and Drug Administration in 1997 for use in hypertension when oral therapy is not feasible or possible and for use in patients with severe hypertension, with or without target-organ damage (3).

Pharmacology and Pharmacokinetics

Fenoldopam selectively binds to DA_1 receptors and functions as a dopamine agonist. It does not bind to DA_2 receptors or β-adrenergic receptors. Fenoldopam is also an α-adrenergic receptor antagonist with greater activity at α_2 than α_1 receptors. However, this activity is observed only at higher concentrations than those required for activation of DA_1 receptors and it is unlikely that α-adrenoreceptor antagonism contributes to the hemodynamic and renal effects of therapeutic doses of fenoldopam. Peripheral DA_1 receptors are located postsynaptically in the systemic and renal vasculature, and at various sites in the nephron and gastrointestinal tract. These receptors mediate systemic, renal, and mesenteric vasodilation. Fenoldopam exerts its hypotensive effect by decreasing systemic vascular resistance. Unlike sodium nitroprusside, it also increases renal blood flow and causes a natriuresis and diuresis. It is six times as potent as dopamine in causing renal vasodilation. In patients with severe hypertension, intravenous infusion of fenoldopam significantly increases renal blood flow, and decreases renal vascular resistance with a significant increase in creatinine clearance, urine flow rate, and sodium excretion (486). Because of its selective receptor binding characteristics, fenoldopam exhibits minimal adrenergic effects. Although DA_1receptors are

present in the central nervous system (CNS), fenoldopam does not have any direct CNS effect because it does not cross the blood-brain barrier. Fenoldopam is metabolized in the liver to a variety of nontoxic methyl, sulfate, and glucuronide metabolites. There are two principal inactive metabolites, 7- and 8-methoxy-fenoldopam, that are eliminated by the kidney (80%) and in the feces (20%). Less than 1% is excreted unchanged in the urine; therefore dosage adjustment is not required in the setting of renal insufficiency. Moreover, pharmacokinetic parameters do not appear to be significantly altered in the setting of hepatic insufficiency (486). Fenoldopam is not metabolized by the cytochrome P 450 system and has no major drug-drug interactions although concomitant acetaminophen administration may increase fenoldopam levels by 30% to 70%. Following intravenous administration, the onset of action is within 10 minutes, and the half-life is 9.8 minutes. There is no evidence of rebound hypertension after stopping the infusion. The volume of distribution is 0.6 L/kg.

Dosage and Administration

Fenoldopam is available in 5-mL ampules at a concentration of 10 mg/mL. Following dilution, the solution, which is light stable, can be used for up to 24 hours. For the treatment of severe hypertension or hypertensive crises, fenoldopam is administered by continuous infusion with an initial dose of 0.1 μg/kg/minute. The infusion may be increased in increments of 0.1 μg/kg/minute every 20 minutes until the target blood pressure is achieved. The maximum recommended dosage is 1.7μg/kg/minute. The average infusion rate required is 0.25 to 0.5 μg/kg/minute. Mean plasma fenoldopam levels after a 2-hour infusion at 0.5 μg/kg/minute is between 13 to 50 ng/mL. When the desired response has been achieved, fenoldopam infusion may be discontinued gradually or abruptly, as rebound elevation of blood pressure has not been observed. Oral antihypertensive medications may be started as the fenoldopam infusion is weaned.

Adverse Effects

Adverse events attributed to fenoldopam in the treatment of hypertensive emergencies and urgencies were generally mild, occurred within the first 24 hours, and were related to the vasodilatory action of the drug (486). Headache was reported in 11% to 36% of patients, flushing in 7% to 11%, nausea in 20%, and dizziness in 10%. Asymptomatic ST-segment abnormalities occurred in 6% to 33% of patients. The etiology of these nonspecific ST- and T-wave abnormalities, which are similar to those seen with the use of other vasodilators, is unknown. They appear to be a benign phenomenon related to blood pressure lowering with alterations in myocardial repolarization rather than an indication of subclinical myocardial ischemia (486). Less frequently reported adverse events included palpitations, transient hypotension, asthenia, and sinus bradycardia. Fenoldopam, unlike sodium nitroprusside produced a reversible, dose-related increase in intraocular pressure and should be used with caution in patients with glaucoma. In comparative trials, the adverse event profiles of fenoldopam and sodium nitroprusside were generally similar, although fenoldopam may be associated with a lower incidence of transient hypotension than sodium nitroprusside.

Use for Treatment of Hypertensive Crises

Fenoldopam has been compared mostly with sodium nitroprusside in patients with acute severe hypertension (either severe uncomplicated hypertension or true hypertensive crises) (485,487,488). Treatment with fenoldopam or sodium nitroprusside reduced mean diastolic blood pressure to a similar extent and to goal levels in most patients (486). The time to achievement of goal blood pressure was similar to that with sodium nitroprusside. There was no evidence of rebound hypertension following cessation of either drug. There was no evidence of tolerance to the antihypertensive effect of either drug during maintenance infusion. In patients with hypertension following noncardiac surgery or coronary artery bypass grafting, fenoldopam and sodium nitroprusside were equally efficacious in lowering blood pressure (489,490).

The efficacy, safety and cost of sodium nitroprusside versus fenoldopam has been compared in a retrospective analysis of consecutive patients with hypertensive crises admitted to a level-1 trauma center and treated with nitroprusside ($N = 21$) or fenoldopam ($N = 22$) (488). Neither the mean pretreatment mean arterial pressure (nitroprusside 168 $\pm$ 19; fenoldopam 163 $\pm$ 9; $p = 0.45$), time to reach MAP goal (3.6, range 0.4 to 30 hours vs. 4.0, range 1 to 22 hours; $p = 0.5$), nor the duration of infusion (18, range 0.7 to 113 hours vs. 18, range 3 to 74 hours; $p = 0.45$) differed between the treatment groups. Time to imitation of oral antihypertensive therapy was similar between nitroprusside (4.5, range 0.5 to 22 hours) and fenoldopam (6.5, range 1 to 100 hours) treated patients, $p = 0.45$. Change in creatinine clearance and the incidence of tachycardia did not differ between the two groups. No symptoms of cyanide toxicity were detected in nitroprusside-treated patients. Cost of therapy was less with nitroprusside (2.66, range \$1.68 to \$3.48) than with fenoldopam (567, range \$199 to \$6,675 dollars). Thus, treatment of hypertensive crises with fenoldopam appears to result in patient outcomes equal to those with nitroprusside but at substantially higher cost.

Additional studies are needed to compare fenoldopam and sodium nitroprusside in the treatment of true hypertensive crises. Because fenoldopam preferentially dilates the renal vasculature, it has theoretical advantages in the treatment of patients with severe hypertension associated with renal impairment. Moreover, fenoldopam is not associated with the risk of toxicity from thiocyanate accumulation or cyanide. It is possible that it may also offer advantages in patients in whom cross clamping of the aorta above the level of the renal arteries is required.

Diazoxide

Diazoxide (Hyperstat) is structurally related to the thiazide diuretics, but its pharmacologic effect is a direct relaxation of smooth muscle (arteriolar, uterine, and gastrointestinal). The major role of diazoxide is in the treatment of malignant hypertension or hypertensive encephalopathy in situations in which administration of sodium nitroprusside is not feasible. It is also useful in the management of acute obstetric hypertensive emergencies refractory to hydralazine (491–494).

Diazoxide lowers blood pressure by relaxing arteriolar smooth muscle and reducing systemic vascular resistance. It has no effect on venous capacitance vessels. Although it has no direct cardiac effect, it does produce reflex sympathetic activation. Heart rate increases and the cardiac output may double (470). In patients with preexisting atherosclerotic disease, diazoxide may cause myocardial ischemia as a result of the increase in myocardial oxygen demand (495). Moreover, given the reflex increase in heart rate and contractility, use of diazoxide is contraindicated in patients with aortic dissection.

Diazoxide, like other arterial vasodilators, causes avid renal salt and water retention (496). With prolonged use, fluid retention can cause pseudotolerance. This has led to the recommendation that loop diuretics be given concomitantly with diazoxide. However, routine use of diuretics at the initiation of diazoxide therapy is not recommended. Because patients with malignant hypertension may be volume-depleted, the combined use of furosemide and diazoxide can lead to overshoot

hypotension. Unless there is obvious fluid overload, diuretic use should be avoided.

Following a bolus injection of 50 mg of diazoxide, a hypotensive response begins within 1 minute and reaches a peak within 5 minutes. The bolus dose can then be titrated to as high as 300 mg depending on the blood pressure response. Thereafter, the duration of the hypotensive effect ranges from 4 to 20 hours. This rapid onset and long duration of action may be an advantage. Continuous infusion is not required and once the desired blood pressure has been achieved, continuous blood pressure monitoring is not required. The long half-life results from extensive protein binding of the drug. Approximately 50% of the drug is eliminated unchanged by the kidney, while the other 50% undergoes hepatic metabolism. The hypotensive response to diazoxide is increased in uremia because the drug is displaced from plasma protein-binding sites. Thus, in uremic patients the dose of diazoxide should be reduced (497).

Diazoxide activates ATP-sensitive K^+ channels, which hyperpolarizes smooth muscle cells and leads to vasodilation (498). It has a generalized effect on smooth muscle, as myometrium and gastrointestinal smooth muscle are also affected.

Although administration of a single large bolus infusion has been recommended in the past, it is now known that diazoxide is effective in more than 90% of patients when it is administered as a series of small injections rather than a single large bolus injection (499,500). In the past, diazoxide was administered as a 300-mg bolus over 15 seconds, given the belief that a large bolus was necessary to saturate plasma protein-binding sites so that a sufficient quantity of free drug would be available to interact with vascular smooth muscle (496). The sustained hypotensive effect was thought to be caused by irreversible binding to vascular receptors. Unfortunately, this large bolus injection technique was often associated with a significant risk of severe, sustained overshoot hypotension (457,458,463). Since the total plasma diazoxide concentration can be directly correlated with its hypotensive action independent of the rate of administration, use of the large (300 mg) single bolus injection technique is no longer recommended (499,500).

Through the use of multiple small injections of diazoxide, the blood pressure can be more carefully titrated. In the minibolus technique, 50 to 100 mg is rapidly injected over 15 to 30 seconds every 10 to 15 minutes until the desired hypotensive response is obtained. The hypotensive action lasts 4 to 20 hours. Repeated small bolus injections of 50 to 100 mg every 4 to 6 hours can then be utilized to maintain the hypotensive response. Most patients respond after total doses of 150 to 450 mg, although some require 600 mg or more (499,500). Slow continuous infusion of diazoxide at 15 mg/minute for 20 to 30 minutes (5 mg/kg total dose) also can be safe and effective treatment for severe hypertension (491). The diazoxide solution is very alkaline (pH 11.6), and extravasation can cause severe local pain and cellulitis.

Although myocardial infarction, angina, arrhythmias, electrocardiographic abnormalities, strokes, seizures, and coma have all been reported with diazoxide use, most of these adverse events occurred in patients with precipitous hypotension induced by the rapid administration of a 300-mg bolus, especially in patients with underlying cardiovascular and cerebrovascular disease. Precipitous hypotension is more likely to occur in patients who are volume-depleted due to prior diuretic administration or who are receiving other antihypertensive agents. Diazoxide is contraindicated in patients with acute myocardial infarction or aortic dissection (501). Because diazoxide can precipitate cerebral ischemia, it is contraindicated in patients with cerebrovascular disease or intracerebral hemorrhage (190).

Diazoxide causes hyperglycemia by inhibiting insulin release from pancreatic islet cells (502). The hyperglycemia is usually mild and rarely requires therapy but the blood glucose concentration should be closely monitored in patients with renal insufficiency or type II diabetes mellitus. Failure to recognize and treat hyperglycemia can lead to diabetic ketoacidosis and nonketotic hyperosmolar coma (503).

The use of diazoxide to treat pregnancy-related hypertensive crises is controversial, but has been recommended as an alternative to hydralazine (504,505). Since diazoxide relaxes uterine smooth muscle, uterine hypotonia and cessation of labor can occur. However, this effect can be overcome with oxytocin. The drug crosses the placenta and can cause neonatal hyperglycemia and hyperbilirubinemia (506). Overshoot hypotension, which may compromise uteroplacental blood flow and result in fetal bradycardia, is usually reversible with fluid administration (491).

Trimethaphan

Trimethaphan camsylate (Arfonad) is a potent parenteral ganglionic blocking agent that is utilized infrequently now that other parenteral agents with fewer side effects have become available. However, some authors consider it to be the drug of choice for the management of acute aortic dissection (376). Moreover, it has been used for the management of hypertensive encephalopathy, subarachnoid hemorrhage, and hypertension complicated by acute pulmonary edema. It is also useful in the management of autonomic hyperreflexia in patients with spinal cord injuries.

The antihypertensive effect of trimethaphan is produced by ganglionic blockade. It blocks both sympathetic and parasympathetic autonomic ganglionic transmission by occupying postsynaptic receptor sites, thereby preventing binding of acetylcholine liberated from presynaptic terminals (436). The sympathetic blockade causes dilation of arteriolar resistance vessels. In addition, there is venodilation so that venous return and preload are reduced. Unlike nitroprusside and other peripheral vasodilators that can cause reflex sympathetic stimulation, trimethaphan blocks sympathetic reflexes so that peripheral vasodilation is not accompanied by a reflex increase in inotropy or chronotropy. Therefore, trimethaphan does not cause an increase in cardiac output in patients with normal left ventricular function, because preload is reduced and sympathetic reflexes are blocked.

The onset of action of trimethaphan occurs within minutes. After the infusion is stopped, the duration of the hypotensive effect is 5 to 15 minutes. The metabolic fate of trimethaphan is unclear. The relatively brief duration of action is believed to be due to destruction of trimethaphan by cholinesterase (507).

Tachyphylaxis often develops after 24 to 48 hours so that the dose may have to be increased to maintain the hypotensive response. In addition, pseudotolerance can be caused by renal salt and water retention. In this circumstance, responsiveness can be reestablished with parenteral diuretic administration.

Most of the side effects of trimethaphan are those expected of parasympathetic blockade. Blurred vision results from paralysis of accommodation and mydriasis. The use of trimethaphan is contraindicated in patients with glaucoma. Paralytic ileus results from decreased tone and mobility of the gastrointestinal tract. Thus, it should not be used for the postoperative management of hypertension. Urinary retention occurs with use of trimethaphan for more than 48 hours. Despite bladder distention, patients experience no urge to void. Thus, a bladder catheter is required in most patients. Ganglionic blockade results in inactivation of pupillary reflexes. This can cause confusion in the evaluation of comatose patients with hypertensive encephalopathy, head injury, or intracerebral hemorrhage. Since hypoventilation and respiratory arrests were

associated with the curare-like action at the neuromuscular junction, trimethaphan is contraindicated in patients with respiratory insufficiency (508). Use of trimethaphan during pregnancy can lead to meconium ileus in the newborn (509).

Intravenous Nitroglycerin

Intravenous nitroglycerin is particularly useful for the management of hypertension complicating acute myocardial infarction and hypertension occurring after coronary artery bypass. Nitroglycerin causes relaxation of vascular smooth muscle. The predominant effect at lower doses is venodilation. At higher doses, both venous and arterial dilation occur in a dose-dependent fashion (510). As with nitroprusside, the effects of intravenous nitroglycerin on stroke volume and cardiac output vary, depending on the presence or absence of left ventricular dysfunction. In patients without heart failure, the reduction in preload usually predominates and stroke volume falls. In contrast, in patients with left ventricular systolic dysfunction, the decrease in afterload results in a decrease in the impedance to left ventricular ejection such that stroke volume is maintained despite a reduction in preload.

For the treatment of hypertension complicating acute myocardial infarction or postcardiac bypass hypertension, nitroglycerin may have an advantage over sodium nitroprusside (358). In a study of 10 patients with acute myocardial infarction treated with nitroprusside at a rate that lowered the mean arterial pressure by 25 mm Hg, all of the patients showed an increase in ST segment elevation by precordial mapping, suggesting a worsening of regional myocardial ischemia (511). In five patients, subsequent use of sublingual nitroglycerin reduced mean arterial pressure by 14 mm Hg. However, there was a concomitant decrease in ST segment elevation, suggesting an improvement in regional ischemia.

Nitroglycerin and nitroprusside have different effects on regional myocardial blood flow (357,359). Although both drugs dilate coronary vessels, nitroglycerin has a predominant effect on large coronary conductance arteries, including intercoronary collaterals, and relatively little effect on small resistance arterioles. This phenomenon is explained by the fact that coronary resistance vessels less than 100 microns in diameter cannot convert nitrates to nitric oxide such that there is preferential dilation of the larger epicardial collateral vessels (512). In contrast, sodium nitroprusside predominantly dilates the resistance vessels and has less effect on intercoronary collaterals. In the setting of regional myocardial ischemia, resistance vessels in the ischemic region are already maximally dilated. Thus, sodium nitroprusside may dilate resistance vessels in nonischemic areas and shunt blood away from ischemic areas (coronary steal). Nitroglycerin, by predominantly dilating conductance vessels, improves blood flow to the ischemic region. Given the potentially deleterious effect of nitroprusside on regional myocardial blood flow, it has been recommended that intravenous nitroglycerin be used in preference to nitroprusside for the treatment of hypertension with left ventricular dysfunction in association with acute myocardial infarction (358).

Nitrates produce vasodilation through the formation of nitric oxide (endothelium-derived relaxing factor), which activates guanylate cyclase (513). There appears to be tight coupling between the cyclic guanosine monophosphate (cGMP) production and smooth muscle relaxation. A cGMP-dependent protein kinase is stimulated, resulting in alterations in the phosphorylation of various proteins in smooth muscle. Dephosphorylation of the light chain of myosin leads to smooth muscle relaxation (514).

Intravenous nitroglycerin has a rapid onset and brief duration of action with a half-life of 1 to 4 minutes. It is metabolized in the liver by a glutathione-dependent organic nitrate reductase. Intravenous nitroglycerin is supplied in 10-mL bottles containing 50 mg, which should be diluted in 5% dextrose in water or 0.9% sodium chloride. Usually one bottle is diluted in a 250 mL volume to yield a final concentration of 200 μg/mL. Nitroglycerin interacts with many types of plastic. Thus, the drug should be diluted only in glass parenteral solution bottles. Special infusion sets that have been developed absorb fewer nitroglycerins than standard polyvinyl chloride tubing. The initial infusion rate should not exceed 5 μg/minute. The dose is titrated in 5 μg/minute increments every 3 to 5 minutes until the desired hypotensive response is achieved. There is no standard optimal dose of nitroglycerin. There tends to be great variability in response from patient to patient. Blood pressure should be monitored every 30 seconds during the titration phase and every 15 minutes thereafter. As with nitroprusside, close monitoring in an intensive care unit setting is required. In the setting of acute myocardial infarction, monitoring of cardiac output and left ventricular filling pressure is essential.

Intravenous nitroglycerin has also been recommended for the management of the potentially dangerous posttreatment hypertensive response that inevitably follows electroconvulsive therapy (515).

Labetalol

Intravenous labetalol may be of value in a variety of hypertensive crises including malignant hypertension (192), hypertensive encephalopathy (192,516), aortic dissection (517), and hypertensive crises during pregnancy (517).

Labetalol has selective α_1- and nonselective β-blocking properties (518,519). The ratio of β- to α-blocking potency is 7:1 for intravenous labetalol. The acute antihypertensive effect after intravenous administration appears to be caused by a decrease in systemic vascular resistance without an appreciable change in cardiac output (519). However, when used in the treatment of hypertension following open heart surgery, labetalol causes a significant reduction in cardiac output (409). The β-blocking effect offsets the baroreceptor-mediated sympathetic response to hypotension. Thus, heart rate remains unchanged or decreases slightly.

After intravenous injection, the full antihypertensive effect occurs within 5 to 10 minutes, and the blood pressure gradually rises to pretreatment levels over 16 to 18 hours. The duration of action, defined as the time from the last injection until the diastolic blood pressure rises 10 mm Hg above the nadir pressure, ranges from 2.0 to 6.5 hours (192). The major route of elimination is via glycuronide conjugation in the liver. Thus, the labetalol dose must be decreased in patients with liver dysfunction but need not be modified in patients with renal failure.

Labetalol is supplied in 20-mL ampules containing 100 mg of drug. It is usually administered by repeated mini-bolus injections through an intravenous line. The initial dose is 20 mg (4 mL) injected slowly over a 2-minute period. The maximum hypotensive response usually occurs within 5 minutes of the injection. If the desired hypotensive response is not obtained after 10 minutes, a 40-mg bolus is administered over 2 minutes. Additional injections of 40 to 80 mg can be given at 10-minute intervals until the desired hypotensive response is obtained or the maximum total dose of 300 mg has been given.

Labetalol can also be given by continuous infusion. The contents of two ampules (200 mg, 40 mL) are added to 160 mL of diluent to yield a volume of 200 mL with a final concentration of 1 mg/mL. The infusion is begun at 2 mg/minute. The infusion is continued until the desired response is obtained and

then discontinued. Again, the maximum total dose of 300 mg should not be exceeded.

After the blood pressure is controlled with either the mini-bolus or the continuous infusion technique, oral therapy can be initiated with labetalol as soon as the supine diastolic pressure increases by 10 mm Hg above the minimum obtained with parenteral therapy. The initial oral dose is 200 mg. Thereafter the oral dose is titrated beginning at 200 mg twice daily and increased to 600 mg twice daily as required. The addition of a diuretic often enhances the long-term blood pressure response.

As with other parenteral antihypertensive agents, intravenous labetalol can cause precipitous hypotension, which can result in cerebral ischemia. Exaggerated hypotensive responses are usually reported when the initial injection is large (1.5 to 2.0 mg/kg); however, overshoot hypotension can also develop with either the mini-bolus or the continuous infusion technique. Chronically hypertensive patients sometimes develop paradoxical hypertension in response to volume depletion (403,404). In this setting treatment with labetalol can cause sustained overshoot hypotension. Before labetalol is used to treat a patient with hypertension and tachycardia, the possibility of physiologic tachycardia due to volume depletion should be considered.

Other side effects of labetalol are related to its nonselective β-blocking properties. It should be avoided in patients with severe sinus bradycardia, heart block greater than first degree, bronchial asthma, or congestive heart failure.

Oral labetalol has been used safely for pregnancies complicated by pregnancy-induced hypertension (520). However, intravenous labetalol should be used with caution because it has been associated with evidence of neonatal β-adrenergic blockade such as hypoglycemia, bradycardia, and hypotension (521).

Labetalol can cause a significant reduction in cardiac index when used in the setting of hypertension after open-heart surgery (409). The hypotensive action of the drug in this setting appears to result from a decrease in cardiac output rather than from a decrease in systemic vascular resistance. Thus, labetalol should be avoided after open-heart surgery, a setting in which nitroglycerin or sodium nitroprusside is preferred for management of hypertension.

Although there are reports of preoperative management of pheochromocytoma with labetalol (425,522), β-blockade can result in exacerbation of hypertension if α-blockade is incomplete. In this regard, there have been reports of paradoxical hypertension when labetalol was used to treat pheochromocytoma (426,523). Therefore, routine use of labetalol for the preoperative management of pheochromocytoma is not recommended.

Although intravenous labetalol has been recommended as an effective agent for the treatment of severe acute hypertension in patients with chronic renal failure (524), life-threatening hyperkalemia has been reported in patients with renal failure that received intravenous labetalol for the treatment of hypertensive crises (525,526). Beta-adrenergic stimulation is known to shift potassium into cells and β-agonists have been proposed as acute therapy for hyperkalemia in dialysis patients. Conversely, hyperkalemia may be caused by nonselective beta-blockers through inhibition of Na-K-ATPase with decreased cellular uptake of potassium, independent of effects on insulin or aldosterone (527). Thus, labetalol and other nonselective beta-blockers should probably be avoided for the acute management of postoperative hypertension and other hypertensive crises in patients with renal failure.

In summary, although intravenous labetalol has been used to treat a variety of hypertensive crises, its long duration of action and beta-blocking properties are potential disadvantages. For this reason, sodium nitroprusside usually represents a more logical choice for the acute management of patients with hypertensive crises requiring parenteral therapy.

Phentolamine

Phentolamine is useful in the management of catecholamine-related hypertensive crises including pheochromocytoma, MAO inhibitor–tyramine interactions, and clonidine, methyldopa, or guanabenz withdrawal reactions. It is not consistently effective in other hypertensive crises. In fact, phentolamine has largely been replaced by sodium nitroprusside in the management of catecholamine-related hypertensive crises.

Phentolamine is a nonselective α-adrenergic blocking agent that competitively inhibits the effect of norepinephrine on vascular smooth muscle α_1-receptors. It does not have β-blocking activity and therefore does not block the cardiac effects associated with β_1-receptor activation by catecholamines. Phentolamine produces dilation of both arteriolar resistance vessels and venous capacitance vessels (436,528).

The intravenous injection of 1 to 5 mg produces a hypotensive effect within 2 to 3 minutes; however, the duration of action may be only 15 to 30 minutes, so that frequent dosing is required to control blood pressure. Phentolamine is supplied in ampules containing 5 mg. The initial dose should be 1 mg. Subsequent boluses of 1 to 5 mg are administered up to a total dose of 20 to 30 mg or until the blood pressure is controlled. After the desired blood pressure is achieved, intermittent injections are given as necessary to maintain the response.

Side effects due to phentolamine are common. Tachycardia and arrhythmias can occur due to β-adrenergic cardiac stimuli that are not blocked by phentolamine. Gastrointestinal side effects include abdominal pain, nausea, vomiting, and diarrhea. Exacerbation of peptic ulcer disease can occur, so phentolamine should be used with caution in patients with a history of gastritis or peptic ulcer disease (436).

Hydralazine

In the past, parenteral hydralazine was often used for the treatment of hypertensive crises. Most obstetricians still consider hydralazine to be the drug of choice for the management of hypertensive crises during pregnancy (484). However, aside from its use during pregnancy, hydralazine has largely been replaced by other agents in the treatment of hypertensive crises.

The hypotensive response to either intramuscular or intravenous hydralazine is unpredictable. The onset of action occurs 10 to 30 minutes after a parenteral dose. The duration of action is 3 to 9 hours. The dose and frequency of administration needed to control the blood pressure are highly variable (472). Profound and sustained hypotension can occur with an intravenous dose as low as 10 mg. Hydralazine is a direct-acting arteriolar vasodilator. It causes reflex activation of the adrenergic nervous system (472). Because venous capacitance vessels are not affected, venous return is maintained. In association with activation of the adrenergic system, there are increases in heart rate and stroke volume (472). Hydralazine is contraindicated in the treatment of aortic dissection because the increase in myocardial contractility can result in propagation of the dissection. It is also contraindicated in patients with ischemic heart disease because the increased myocardial oxygen demand can precipitate angina or myocardial infarction.

Parenteral hydralazine is still used in acute hypertensive crises of pregnancy. In the majority of patients, hydralazine reduces the blood pressure to acceptable levels and is well tolerated by both mother and fetus, despite reflex activation of the adrenergic system (484). Dosing guidelines for the use of parenteral hydralazine during pregnancy are well established

(529). Because maternal hypertension helps to maintain placental perfusion, there is concern that aggressive treatment aimed at normalization of blood pressure might further compromise placental perfusion to the detriment of the fetus. Therefore, hydralazine treatment is usually instituted only if the diastolic blood pressure is more than 110 mm Hg and the goal of therapy is a diastolic pressure in the 90 to 100 mm Hg range. After an initial intravenous dose of 5 mg, additional 5- to 10-mg doses are administered every 15 to 20 minutes until the desired response is obtained. Because preeclampsia is associated with intravascular volume depletion, it is important to initiate therapy with a low dose to avoid overshoot hypotension. Intramuscular injection of hydralazine is unsatisfactory because the onset of action and magnitude of response are unpredictable.

Calcium Channel Blockers

Intravenous *nicardipine* has been reported to be effective in the acute treatment of severe hypertension in both adults and children (530–532). It may be useful in the management of postoperative hypertension in both cardiac and noncardiac patients (532). Intravenous nicardipine is also effective in preventing circulatory responses to laryngoscopy and tracheal intubation in hypertensive patients (533). Safe use of nicardipine in preeclamptic patients has also been reported (534).

Nicardipine is a dihydropyridine calcium channel blocker that inhibits the transmembrane influx of calcium into vascular smooth muscle, resulting in vasodilation with a decrease in systemic vascular resistance. The effect on heart rate is dependent on the intrinsic state of the myocardium. In patients with intact systolic function, reflex increases in heart rate may occur in response to blood pressure reduction. In patients with impaired left ventricular function, cardiac output may increase in response to afterload reduction.

Compared to other parenteral medications available for the treatment of hypertensive crises, the pharmacokinetic properties of nicardipine (as well as other calcium channel blockers) are unfavorable. The currently available dihydropyridine calcium channel blockers have very long half-lives. The β–half-life of nicardipine is 40 minutes, whereas its γ–half-life is approximately 13 hours. Because about 14% of the drug is eliminated during the γ-phase, the hypotensive effect is prolonged. Discontinuation of the infusion is followed by a 50% reduction in the hypotensive action within 30 minutes but a gradually decreasing antihypertensive effect may last for about 50 hours. Thus, nicardipine may not be the best choice for true hypertensive crises in which moment-to-moment titration of the blood pressure is the desired therapeutic goal.

In the past, *nimodipine* had been recommended for the treatment of patients undergoing cardiac valve replacement to decrease the incidence of postoperative neurologic sequelae by increasing cerebral blood flow and protecting against anoxic brain-cell damage. However, a recent placebo controlled trial of oral nimodipine following cardiac valve replacement was terminated prematurely because of a lack of evidence of benefit of nimodipine and an unexpected increase in the death rate of patients treated with nimodipine compared to placebo (535). The higher mortality rate was attributed to an increase risk of major bleeding in patients treated with nimodipine. Excess bleeding in patients treated with calcium channel blocker may be explained by the combination of vasodilation and the antiplatelet action of calcium antagonists.

The clinical use of nifedipine for severe uncomplicated hypertension and hypertensive crises has been reviewed (218,536,537). Nifedipine produces a prompt fall in systemic arterial pressure after a single oral dose. The antihypertensive effect results from arteriolar vasodilation with a decrease in systemic vascular resistance. Nifedipine produces a prompt reduction in systolic, diastolic, and mean arterial pressures of about 25% below the baseline value in most patients (537).

Nifedipine is usually administered as a 10-mg sublingual, buccal, or oral dose. The onset of action occurs 5 to 10 minutes after sublingual or buccal administration of the liquid drug, which has been squeezed or aspirated with a needle and syringe from the capsule. With oral administration of the intact capsule, the onset of action occurs at 15 to 20 minutes. A recent study showed that absorption of nifedipine from the oral mucosa is negligible and that most absorption occurs in the stomach (538). The rapid onset of action when the liquid is administered by the sublingual route is explained by the absorption of swallowed liquid from the stomach. The lag in onset of action when the intact capsule is swallowed is due to the time required for dissolution of the capsule. The most reliable method of administration of the drug may be to bite and swallow the capsule (538). The peak effect occurs in 20 to 30 minutes. The duration of action is 4 to 6 hours regardless of the route of administration (218,538).

The major acute side effects of nifedipine include a burning sensation in the face and legs, facial flushing, headache, and palpitations. Overshoot hypotension has been observed, especially in hypovolemic patients or patients pretreated with diuretics (539,540). Exaggerated hypotension can cause myocardial ischemia in patients with underlying coronary atherosclerosis (539,541).

Sublingual or oral nifedipine may be useful in the management of patients with malignant hypertension who do not have an absolute indication for parenteral antihypertensive therapy. Extended-release nifedipine may also be useful in this setting (213). However, in patients with hypertensive crises requiring careful titration of the hypotensive response, the prolonged duration of action and the potential risk of overshoot hypotension with nifedipine are major disadvantages. Sodium nitroprusside is clearly preferable for the management of true hypertensive crises. The role of nifedipine in the acute treatment of severe uncomplicated hypertension in the emergency room setting prior to discharge is discussed in the section entitled Severe Uncomplicated Hypertension.

Minoxidil

Minoxidil is a potent antihypertensive agent that is available only for oral use. In combination with a potent diuretic and a beta-blocker, it is very useful in the control of hypertension refractory to conventional antihypertensive regimens. The efficacy of a triple-drug regimen with minoxidil in the management of the patient with malignant hypertension and azotemia has already been discussed. Minoxidil is often employed for the long-term control of blood pressure in patients with malignant hypertension after initial control of the blood pressure with parenteral medications. Furthermore, in patients with malignant hypertension not requiring immediate blood pressure reduction, an oral triple-drug regimen consisting of minoxidil, a beta-blocker, and a loop diuretic can effectively control the blood pressure over a period of hours to days and thereby eliminate the need for parenteral antihypertensive therapy (see Treatment subsection under Malignant Hypertension earlier in this chapter).

Minoxidil is a direct-acting arteriolar vasodilator. Its antihypertensive effect results from a decrease in systemic vascular resistance (542). It has no effect on venous capacitance vessels. The hypotensive response to minoxidil is accompanied by a baroreceptor-mediated reflex increase in sympathetic tone, which results in an increase in heart rate, contractility, and cardiac output. Unopposed, the cardiac output may increase threefold to fourfold and attenuate the fall in blood pressure (542,543). The resulting increase in myocardial oxygen

demand may precipitate ischemia in patients with limited coronary reserve. For this reason minoxidil is usually given concomitantly with a β-adrenergic blocking drug.

As with other peripheral vasodilators, minoxidil induces profound renal salt and water retention (542). This fluid retention is probably related to the hypotensive effect of the drug. A similar antinatriuresis occurs with both hydralazine and diazoxide. Minoxidil causes more fluid retention because it is a more potent arteriolar vasodilator. Several factors enhance renal salt and water retention (543). Decreased peritubular capillary pressure is a potent stimulus for salt and water reabsorption in the proximal tubule. Increased adrenergic tone also enhances proximal tubular salt and water reabsorption. Like other vasodilators, minoxidil increases renin release, which leads to increased aldosterone production and enhanced distal tubular sodium reabsorption (543). Pseudotachyphylaxis to the original hypotensive effect of minoxidil can occur if either β-blockade or diuretic therapy is inadequate.

The serum half-life of minoxidil is 4.5 hours; however, the duration of action is longer than the half-life would predict (543). After oral administration, the antihypertensive effect begins within 30 to 60 minutes, reaches a maximum in 2 to 4 hours, and slowly abates over the next 12 to 18 hours. The prolonged hypotensive effect is probably due to persistent binding of minoxidil at the site of action in vascular smooth muscle. About 15% of the parent compound is excreted in the urine, while the remainder is metabolized in the liver by glucuronide conjugation (543).

Although the serum half-life is 4 hours, the persistent hypotensive effect allows for a twice-daily dosing schedule. Prior to the initiation of minoxidil, all other antihypertensives except diuretics and beta-blockers should be discontinued. Minoxidil is started at a dose of 2.5 mg twice daily and increased in 5-mg/day increments every 2 to 3 days until the desired response is obtained. The usual effective dose is 10 to 40 mg/day. The doses of loop diuretic and beta-blocker are titrated to maintain dry weight and prevent tachycardia, respectively.

When more rapid control of arterial pressure is required, incremental changes in minoxidil dosage can be made every 6 hours. The initial 2.5-mg dose is doubled every 6 hours up to a maximum dose of 20 mg, or until the desired response is obtained. The effective dose should then be administered every 12 hours and the dose of diuretic and beta-blocker titrated as necessary (543).

The dose of beta-blocker required to prevent reflex tachycardia in patients treated with minoxidil is often in excess of the usual β-blocking dose. This is because the sympathetic nervous system is activated by minoxidil and beta-blockers compete with catecholamines for receptor binding (543). The starting dose of beta-blocker should be propranolol at 160 mg/day or an equivalent. The dose is then titrated to maintain resting heart rate at 70 to 80 beats/minute.

In general, thiazide diuretics are not potent enough to counteract minoxidil-induced antinatriuresis, especially if renal insufficiency is present. The starting dose of furosemide is 40 mg twice daily. However, a dose of 300 to 400 mg/day may be required to prevent fluid retention and maintain dry weight.

The most common side effects of minoxidil are related to its pharmacologic properties. Fluid retention can lead to weight gain, edema, anasarca, congestive heart failure, and pericardial effusion. With inadequate β-blockade, reflex sympathetic stimulation can lead to angina or myocardial infarction in patients with underlying coronary disease. Electrocardiographic changes following the initiation of minoxidil have been reported. In more than 90% of patients flattening or inversion of T waves develops (543). Although often marked, these changes do not necessarily indicate myocardial ischemia, and they usually resolve with continued therapy (542,543).

Pericardial effusion has been reported with minoxidil treatment; however, progression to cardiac tamponade is rare. The cause of the effusion is unknown, but it occurs most commonly in patients with renal failure, collagen vascular diseases, or inadequate diuretic therapy. A hemodynamically insignificant effusion is not necessarily a reason to discontinue minoxidil, but the patient should be treated aggressively with diuretics and followed closely for signs of tamponade (542,543). Patients on dialysis should have a trial of intensive daily dialysis to achieve and maintain dry weight.

Reversible hypertrichosis of the face, back, and arms occurs in almost all patients taking minoxidil and is the most frequent reason for discontinuation of the drug, especially among female patients. Calcium thioglycolate depilatory agents and shaving are used to control this cosmetic side effect.

Triple therapy with minoxidil, a beta-blocker, and a loop diuretic is often dramatically effective in the long-term management of malignant hypertension, even when conventional antihypertensive regimens are unsuccessful or produce intolerable side effects (202,206,207,544).

Angiotensin Converting Enzyme Inhibitors

Captopril has been used successfully in the treatment of hypertensive crises. Both oral and sublingual routes of administration have been described (545,546). Angiotensin converting enzyme inhibitors are clearly the treatment of choice for scleroderma renal crisis (55).

Unfortunately, first-dose hypotension has been reported as a significant risk in the treatment of hypertensive crises with converting enzyme inhibitors. Hypotension is most likely to occur in patients with high levels of angiotensin II, underlying renovascular hypertension, or intravascular volume depletion resulting from spontaneous natriuresis in malignant hypertension or from prior diuretic treatment. In addition, the use of converting enzyme inhibitors in the initial management of patients with renal insufficiency can lead to confusion if the renal dysfunction persists or worsens. Use of converting enzyme inhibitors is contraindicated in pregnancy because they can cause acute renal failure in the neonate (547).

Although malignant hypertension is often characterized by high PRA, this is not invariably the case. Therefore, converting enzyme inhibitors may not be effective in all patients with malignant hypertension. Moreover, whereas angiotensin-converting enzyme inhibitors may be useful in the long-term management of patients with malignant hypertension, captopril has not been shown to be superior to other antihypertensive agents in preventing the recurrence of malignant hypertension (219).

Thus, although angiotensin-converting enzyme inhibitors may be useful in the long-term management of hypertension in patients with a history of malignant hypertension or other hypertensive crises, converting enzyme inhibitors are not usually recommended for the initial management of hypertensive crises except in patients with scleroderma renal crisis.

Methyldopa

In the past, parenteral methyldopa was often recommended for the treatment of hypertensive crises. However, it has several disadvantages including a delayed onset of action, unpredictable hypotensive effect, and central nervous system sedation. More rapidly acting and predictable parenteral agents such as sodium nitroprusside and diazoxide have largely replaced methyldopa.

Reserpine

Intramuscular reserpine in a dose of 1 to 5 mg was widely used in the past for the treatment of hypertension complicating acute

pulmonary edema, pheochromocytoma, toxemia of pregnancy, and aortic dissection (548). However, with the advent of more reliable agents with fewer side effects, the use of reserpine for the management of hypertensive crises can no longer be recommended (2190).

Clonidine

Oral clonidine loading has been recommended for the management of severe hypertension that is *not* accompanied by evidence of end-organ dysfunction (220). However, oral clonidine loading is *not* recommended for the management of the true hypertensive crises outlined in Table 56-1. Thus, if hypertension is accompanied by hypertensive neuroretinopathy (malignant hypertension), hypertensive encephalopathy, congestive heart failure, acute myocardial infarction, aortic dissection, or central nervous system catastrophe, oral clonidine loading is not recommended (220). In patients with hypertensive encephalopathy or another central nervous system catastrophe, clonidine can cause sedation, which would interfere with the assessment of mental status. Moreover, the relatively long duration of action represents a disadvantage in the treatment of hypertensive crises requiring moment-to-moment titration of blood pressure. The oral clonidine loading regimen was described specifically for the management of severe hypertension that is not associated with end-organ dysfunction, an entity known as *urgent hypertension* or severe uncomplicated hypertension (220). There has been an unfortunate tendency to utilize this type of regimen for the treatment of true hypertensive crises in which potent parenteral medications described earlier are clearly indicated. Use of oral clonidine loading in the outpatient setting for the management of severe uncomplicated hypertension is discussed in the next section.

SEVERE UNCOMPLICATED HYPERTENSION

The benefits of acute reduction of blood pressure in the setting of true hypertensive crises are obvious. Fortunately, hypertensive crises are relatively rare events that never affect the vast majority of hypertensive patients. Another type of presentation that is more common than true hypertensive crisis is the patient who presents with severe hypertension (diastolic blood pressure greater than 115 mm Hg) in the absence of the hypertensive neuroretinopathy or other acute end-organ damage that would signify a true crisis. This entity, which is known as *severe uncomplicated hypertension*, is very common in the emergency department setting. In a recent study of severe uncomplicated hypertension treated in an emergency room, 60% of the patients were entirely asymptomatic and had presented for prescription refills or routine blood pressure checks, or were found to have elevated blood pressure during routine examinations. The other 40% presented with nonspecific symptoms such as headache, dizziness, and weakness in the absence of evidence of acute end-organ dysfunction (549).

In the past, this entity has been referred to as *urgent hypertension,* reflecting the widely accepted notion that acute reduction of blood pressure, over a few hours prior to discharge from the emergency room, was essential to minimize the risk of short-term complications from the severe hypertension (220,550). Commonly used treatment regimens include oral clonidine loading, or sublingual nifedipine given to acutely reduce the blood pressure prior to initiation of a maintenance antihypertensive regimen (220,549,550).

In recent years, however, the urgency of treatment in patients with severe uncomplicated hypertension has been questioned (9,11,551). While it is clear that in comparison to patients with mild or moderate hypertension, patients with severe uncomplicated hypertension are at increased long-term risk of cardiovascular complications (552), they are generally not in any immediate danger of an untoward event (10). The argument supporting the acute reduction of blood pressure is based on the following assumptions: (a) It is important to reduce blood pressure immediately to avoid complications; (b) oral antihypertensive loading prior to initiation of maintenance therapy produces improved immediate and long-term blood pressure control; and (c) there are no adverse consequences of this form of treatment (9). Two studies provided some useful information regarding the need to reduce blood pressure immediately with the aim of preventing hypertensive complications. In the Veterans Administration Cooperative Study of patients with severe hypertension (552), there were 70 untreated patients who had no evidence of malignant hypertension or significant end-organ dysfunction despite the presence of diastolic blood pressures averaging 121 mm Hg. Among these patients, 27 experienced morbid events at an average of 11 $\pm$ 8 months into follow-up. The earliest morbid event occurred after 2 months. Likewise, a similar study in Baltimore showed that among 42 untreated patients with severe but uncomplicated hypertension, 19 patients experienced morbid events (congestive heart failure, onset of malignant hypertension, cerebrovascular accident, or evidence of declining renal function) at a mean of 12 $\pm$ 7 months into follow-up. The earliest morbid event occurred at 2 months (553). These data suggest that patients who have severe but uncomplicated hypertension need not be exposed to the risk of "urgent" blood pressure reduction in the emergency room setting because hypertensive complications tend to occur over a matter of months to years rather than hours to days.

Another study addressed the question of whether antihypertensive loading prior to the initiation of maintenance therapy improves or hastens blood pressure control (11). Sixty-four asymptomatic patients with severe hypertension were randomized to treatment with hourly doses of clonidine followed by clonidine and thiazide diuretic maintenance therapy, or an initial dose of clonidine followed by hourly placebo and then subsequent maintenance therapy, or initiation of maintenance therapy without prior antihypertensive loading. There was no difference between the first two groups with regard to the time required to achieve acceptable blood pressure control during loading therapy. Furthermore, there were no differences between the three groups with regard to adequacy of blood pressure control at 24 hours or 1 week. The authors conclude that sustained blood pressure control resulted solely from maintenance therapy and that the time to adequate control and eventual level of blood pressure were independent of the administration of an initial loading dose. They suggest that the common practice of acute oral antihypertensive loading to treat severe, asymptomatic hypertension should be reconsidered (11). In this regard, a recent study of 32 patients with severe uncomplicated hypertension found that a significant decrease in blood pressure frequently occurred in the emergency department even before pharmacologic intervention was initiated. The mean arterial pressure decreased by 6% without treatment within 1 hour after the initial blood pressure reading (554). The authors suggest that given a short period of observation, many patients with severe uncomplicated hypertension will experience a decrease in blood pressure to mildly or moderately hypertensive levels, which would clearly make acute blood pressure reduction with an antihypertensive loading regimen unnecessary.

Although generally safe, the oral antihypertensive loading regimens occasionally cause significant adverse effects. Sublingual nifedipine can produce severe headache and profound overshoot hypotension (540). The marked blood pressure reduction can exacerbate underlying ischemic heart disease, resulting in angina or myocardial infarction (539,541). It has even been suggested that a moratorium be placed on the use of sublingual nifedipine for the treatment of severe uncomplicated

hypertension (555). Loading doses of clonidine cause sedation in 60% of patients and some of these patients are difficult to awaken and require assistance in returning home (549). Furthermore, the recommended conversion from the oral loading dose to a twice-daily dose of clonidine (220) may represent special problems in the treatment of patients with severe uncomplicated hypertension. Clonidine produces a number of common side effects including dry mouth, drowsiness, and constipation, which may interfere with long-term compliance with medical therapy. The risk of hypertensive rebound on abrupt discontinuation of clonidine (427) should also be considered since many patients with this form of hypertension are noncompliant with medical therapy (11).

While the acute reduction of blood pressure in patients with severe uncomplicated hypertension with sublingual nifedipine or oral clonidine loading regimens has become the de facto standard of care in the acute care setting, this practice is often an emotional response on the part of the treating physician to the dramatic elevation of blood pressure (10). This aggressive approach may also be motivated by fear of medicolegal repercussions in the unlikely event that an untoward hypertensive complication occurs shortly after the emergency room visit (10). Although observing and documenting the dramatic fall in blood pressure prior to discharge is a satisfying therapeutic maneuver, there is no scientific basis for this approach and it is unclear if even the small but definite risks of acute blood pressure reduction are justified. There is, at present, no literature to support the notion of an absolute level of blood pressure above which the acute reduction of blood pressure is mandatory before the patient can be discharged from the acute care setting. For asymptomatic patients with severe uncomplicated hypertension, acute reduction of blood pressure in the emergency room is often counterproductive because it can produce untoward symptoms that render the patient less likely to comply with long-term drug therapy. Because the available data suggest that the risks to the patient are not immediate, therapeutic intervention should focus on tailoring an effective, well-tolerated maintenance antihypertensive regimen with emphasis on patient education to enhance long-term compliance (11). Therefore, oral antihypertensive loading in this setting is of little value. If the patient has simply run out of medications, reinstitution of the previous regimen should suffice. If the patient is thought to be compliant with an existing drug regimen, a sensible change in therapy such as an increase in a suboptimal dosage of an existing drug or the addition of a drug of another class is appropriate. Addition of a low dose of a thiazide diuretic as a second-step agent to existing monotherapy with converting enzyme inhibitor, calcium channel blocker, beta-blocker, or central α_2-agonist is often efficacious (390). Another essential goal of the intervention should be to arrange for suitable outpatient follow-up within a few days. Gradual reduction of blood pressure to normotensive levels over the next few days to a week should be accomplished in conjunction with frequent outpatient follow-up visits to modify drug regimens and reinforce the importance of lifelong compliance with therapy. Though less dramatic than acute reduction of blood pressure in the emergency room, this type of approach to the treatment of this chronic disease is more likely to prevent long-term hypertensive complications as well as recurrent episodes of severe uncomplicated hypertension.

Finally, an important entity that can masquerade as severe uncomplicated hypertension deserves special mention. *Pseudohypertension* is a condition in which indirect measurement of arterial pressure using a cuff sphygmomanometer is artificially high in comparison to direct intraarterial pressure measurements (556). Failure to recognize pseudohypertension can result in unwarranted and sometimes frankly dangerous treatment. Pseudohypertension can result from Mönckeberg's medial calcification, advanced atherosclerosis with widespread calcification of intimal plaques, or azotemic arteriopathy (metastatic vascular calcification in patients with ESRD) (556). In these entities, stiffening of the arterial wall may prevent its collapse by externally applied pressure, resulting in artificially high indirect blood pressure readings affecting both systolic and diastolic measurements. Pseudohypertension should be suspected in the patient with severe hypertension in the absence of significant target-organ damage. The presence of a positive *Osler's maneuver,* in which the radial or brachial artery remains clearly palpable despite being made pulseless by proximal inflation of a cuff above systolic blood pressure, is an important physical examination finding that should suggest the diagnosis (557). Roentgenograms of the extremities will often reveal calcified vessels (556). However, the diagnosis can only be made definitively by direct measurement of intraarterial pressure. If unrecognized, pseudohypertension may result in unwarranted treatment. Patients with pseudohypertension are often older adults and therefore may have critical limitation of blood flow to the brain or heart such that inappropriate blood pressure reduction may precipitate life-threatening ischemic events (556).

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SECTION IX: GLOMERULAR, INTERSTITIAL, AND VASCULAR RENAL DISEASES

CHAPTER 57 ■ MECHANISMS OF TISSUE INJURY AND REPAIR IN RENAL DISEASES

STEPHAN SEGERER, MATTHIAS KRETZLER, FRANK STRUTZ, AND DETLEF SCHLÖNDORFF

INTRODUCTION

In order to consider "the mechanisms of tissue injury and repair" for "glomerular, interstitial and vascular renal diseases," the general stage has to be set. This will involve a brief description of inflammation as well as factors contributing either to repair, with resolution and maintenance of overall function, or to persistent tissue injury with progressive fibrosis, and loss of function. The overall goal of the inflammatory process is to eliminate the original insult, to clear up the battlefield by removing cell and matrix debris, and to repair the lost tissue components. Ideally this results in a reconstitution of the original tissue architecture and function.

Complete healing requires maintanance of the tissue microarchiteture. It can only be achieved when the extracellular matrix (ECM)—predominantly the basement membranes—remain intact, as a scaffold on which the tissue can regenerate. Once this scaffold is severely damaged or lost, the original architecture can not be reestablished, and the damaged tissue will be replaced by fibrous scar tissue. This involves the interaction of infiltrating leukocytes and perhaps bone marrow–derived fibrocytes, activation of fibroblasts and epithelial-mesenchymal transition. The latter can occur when epithelial cells have lost their basement membrane, allowing them to migrate out beyond their normal environment and boundaries. Eventually it will result in a local distortion of the parenchymal architecture and a loss of function, setting the stage for a progressive process.

A GENERAL SCHEME OF TISSUE INJURY AND REPAIR

Cell Injury

Differentiated cell function depends on a specific functional and anatomical environment. Cells can adapt to stress within certain limits (1). If the adaptive capacity of the cell is exceeded, injury becomes irreversible and cell death occurs. Cell death follows two major patterns, with some overlap (i.e., necrosis and apoptosis).

Necrosis is characterized by cellular swelling, protein denaturation, organellar swelling and breakdown with release of proinflammatory products, which result in inflammation.

Apoptosis in contrast is a controlled and programmed form of cell death and removal, with intracellular organized breakdown of proteins, RNA and DNA, and rapid uptake of residual particles (apoptotic bodies) by phagocytic cells. The uptake of apoptotic particles initiates an anti-inflammatory program, since apoptosis does generally not result in inflammation (1).

In the kidney the general type of cellular stress and injury are of the same types as in other organs. However, because of the intricate anatomical and functional arrangements of the kidney, these injury responses may result in nephron segment specific responses, that may spread from the original site to involve "downstream" injury. The causes of injury involved in renal diseases are based on immunologic reactions (immune complexes [ICs] or immune cells), oxygen deprivation (local hypoxia as well as ischemia), chemical agents (ranging from drugs to endogenous substances in high concentrations, e.g., glucose), and genetic defects. The cellular responses to stress are dependent on the type, the severity and duration of injury (2). The systems that are generally most vulnerable to injury are membrane organization, adenosine triphosphate (ATP) generation, maintenance of calcium homeostasis, protein synthesis, and the integrity of the genetic apparatus (1,3,4).

Repair Involves Inflammation in the Postembryonic State

Irrespective of the initial insult to the tissue, an inflammatory response will ensue (5–8). Only in the embryo can loss of tissue be repaired without inflammation, scarring, or fibrosis (5,6). After birth repair is always associated with an inflammatory process, irrespective of the eventual outcome, such as healing, or limited or progressive fibrosis. Overall, inflammation follows a rather uniform scenario involving the local release or generation of soluble factors, an increase in local vascular permeability, activation of endothelial cells, and the emigration of leukocytes (9). The term *leukocyte* is used here in a generic way, referring to all types of circulating inflammatory cells. Inflammation is closely related to tissue repair with regeneration of parenchymal cells and filling of tissue defects with fibrous tissue, that is, scar formation. The inflammatory response therefore represents a two-edged sword: beneficial in terms of the repair process to injury; detrimental when proceeding in an uncontrolled manner, leading to progressive fibrosis with loss of function.

Soluble Mediators Are Involved in Inflammation and Tissue Repair

The major classes of soluble mediators generated at sites of renal tissue injury and involved in leukocyte migration, activation and tissue repair include the cytokines (especially the chemokines and growth factors), as well as lipid mediators, such as prostaglandins (PGs), leukotrienes (LTs), lipoxins (LXs), and platelet activating factor (PAF). The previous edition of this chapter includes an excellent review of the subject in the context of the kidney.

Cytokines

Cytokines are a large group of polypeptide molecules, predominantly generated and released by leukocytes, but also in a more restricted manner by other cells (9–11). Cytokine production requires cell stimulation and is tightly regulated. Specific cytokines in turn will activate synthesis of other cytokines by the same cell (autocrine), by adjacent cells (paracrine), or even by distant cells (endocrine). Thus they act in a network fashion. Cytokines also include growth factors such as vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), connective tissue growth factor (CTGF), tumor growth factor-β (TGF-β), and insulin-like growth factor 1 (IGF-1). The effects of the various cytokines on leukocytes, fibroblasts, endothelial, epithelial and vascular smooth muscle cells, is generally termed pleiotropic, that is, it can vary depending on the cell type and the general context. Thus depending on their state of "preconditioning" by other mediators or cytokines, by extracellular-matrix components, or by neighboring cells the response can be very different.

The major groups of cytokines (excluding general growth factors) are:

Cytokines involved in regulating growth, differentiation, and activation, for example, IL-2 for lymphocytes and macrophage colony stimulating factor (M-CSF) for monocytes

Cytokines involved in a direct response to tissue injury as part of innate immunity (e.g., TNF, IL-1)

Cytokines that activate inflammatory cells, that is, monocyte/macrophages (e.g., interferon-γ [IFN-γ], interleukin-12 [IL-12])

Cytokines that stimulate the growth and development of the various hematopoetic cell lines (e.g., M-CSF, granulocyte macrophage colony stimulating factor (GM-CSF), interleukin-3 [IL-3])

Chemotactic cytokines, the chemokines (Table 57-1)

Obviously considerable overlap exists between these groups and their function. The complexity and pleiotropic aspects of cytokine action are probably best illustrated by the TGF-β members, namely, TGF-β1, 2, and 3. They may require preactivation, release from matrix, can be pro- and antiproliferative, pro-apoptotic, cause epithelial-mesenchymal transition (EMT), act in a profibrotic manner by stimulating the formation of matrix components and inhibiting metalloproteinases, or can even act in an anti-inflammatory and antifibrotic manner (12,13). Thus the effects of TGF-β will vary depending on the specific isoform, and the local anatomical and cytokine context. Furthermore, studies of TGF-β by messenger RNA (mRNA) determination, immunohistology, or enzyme-linked immunosorbent assay (ELISA) may not at all reflect its actual activity. Therefore the common notion of TGF-β as the major profibrotic cytokine is a vast oversimplification (12–14). We will discuss the cytokines in further detail in the context of the specific renal diseases.

TABLE 57-1

CHEMOKINE RECEPTOR, CHEMOKINE LIGANDS, AND THE CORRESPONDING CHEMOKINE RECEPTOR EXPRESSING CELL TYPES

Chemokine receptor	Chemokine ligands	Chemokine receptor-positive cells
CCR1	CCL3, CCL5, CCL7, CCL8, CCL13, CCL14, CCL15, CCL23	Monocytes, dendritic cells (immature), T cells, neutrophils, mesangial cells, NK cells
CCR2	CCL2, CCL7, CCL8, CCL13	Monocytes, dendritic cells (immature), basophiles, NK cells, B and T cells
CCR3	CCL5, CCL7, CCL8, CCL11, CCL13–CCL15	T cells, NK cells, fibroblasts
CCR4	CCL17, CCL22	Eosinophiles, basophiles, T cells (TH2), dendritic cells, NK cells
CCR5	CCL3, CCL4, CCL5, CCL8, CCL11, CCL13, CCL14	Monocytes, dendritic cells (immature), T cells (TH1), basophiles, B cells
CCR6	CCL20	T cells, B cells, dendritic cells (immature)
CCR7	CCL19, CCL21	Dendritic cells (mature), T cells, B cells
CCR8	CCL1, CCL16	Dendritic cells (mature), T cells, B cells, thymocytes
CCR9	CCL25	T cells, thymocytes
CCR10	CCL27, CCL28	T cells, thymocytes
CXCR1	CXCL5, CXCL6, CXCL8	Neutrophiles, monocytes, astrocytes, endothelial cells
CXCR2	CXCL1, CXCL2, CXCL3, CXCL5, CXCL7, CXCL8	Neutrophiles, monocytes, eosinophils, endothelial cells
CXCR3	CXCL9, CXCL10, CXCL11	T cells (TH1), B cells, NK cells
CXCR4	CXCL12	T cells, dendritic cells, monocytes, B cells, neutrophils, platelets, astrocytes, thymocytes, endothelial cells
CXCR5	CXCL13	T cells, B cells, astrocytes
CXCR6	CXCL16	T cells
CX3CR1	CX3CL1	T cells, monocytes, NK cells, neurones, microglia
XCR1	XCL1, XCL2	T cells, NK cells

Chemokines

Chemokines are members of a large family of *chemo*tactic cyto*kines*, which play pivotal roles during the recruitment of subpopulations of inflammatory cells (15–19). In addition to cell positioning (e.g., during inflammation, lymphopoiesis and, immunregulation), chemokines can activate effector functions of leukocytes (including fibrotic processes), influence hematopoiesis and the release of stem-cells from the bone marrow, modulate angiogenesis, and even play roles during carcinogenesis and metastasis (18,20,21). Over 40 chemokines and 19 corresponding chemokine receptors have been described to date (15,22). Chemokines are subclassified into four groups (CC, CXC, CX3C, and C chemokines), according to a shared structural motif of conserved cysteine residues. The CC and CXC chemokines represent the vast majority of chemokines. CX3CL1/Fraktalkine combines the functions of a chemokine and an adhesion molecule. It can be directly tethered to the cell membrane via a transmembrane segment (a long mucin stalk), and it can induce chemotaxis and leukocyte arrest on endothelial cells. Furthermore it can be released by proteases and become a soluble chemokine (23). Chemokine functions are mediated through seven transmembrane spanning serpentine Gi/Go-protein coupled receptors, which are sensitive to pertussis toxin (Table 57-1). The specificities can overlap as some chemokines bind to multiple receptors, and some receptors can bind multiple chemokines.

Upon appropriate stimulation, essentially all cell types appear capable of expressing chemokines (24). The production occurs in a cell- and stimulus-specific manner. Various substances that is cytokines, ICs, growth factors, serum proteins, and even chemokines themselves are capable of inducing chemokine expression. A common denominator for the stimulation may be the generation of reactive oxygen species, and subsequent activation of transcription factors, such as NF-κB (25). In general, proinflammatory cytokines such as TNF, IL-1β, IFN-γ, and LPS, especially in combination, rapidly (within hours) induce CCL2/MCP-1, CXCL8/IL-8, and CXCL10/IP–10, whereas the induction of CCL5/RANTES is usually slower (12 to 48 hours). ICs induce upregulation of CCL2/MCP-1, CCL5/RANTES, CXCL8/IL–8, and CXCL10/IP-10 expression. Growth factors such as PDGF and bFGF can induce CCL2/MCP-1 and CCL5/RANTES expression. This may relate to the macrophage influx seen during proliferative responses in tissue repair, regeneration, and remodeling (14–21).

The role of two chemokine binding proteins DARC and D6 during inflammation is still under investigaion (26–28). Both proteins bind multiple chemokines but do not signal. It is possible that these proteins are involved in the transendothelial transport, positioning, and presentation of chemokines on vascular and lymphatic vessels and even angiogenesis. Therefore these proteins might be doorkeepers for the migration of inflammatory cells both into, as well as out of specific tissues. Furthermore, Duffy antigen/receptor for chemokines (DARC) on erythrocytes may serve as a sink or even reservoir of chemokines in the circulation.

Lipid Mediators

The lipid mediators, the eicosanoids prostaglandins (PGs), LTs, and LXs, and the phopholipid derivative PAF play considerable roles in inflammation.

Prostaglandins (PGs) belong to the oldest known class of lipid mediators of inflammation. With the molecular characterization of two forms of cyclooxygenases (i.e., COX1 and COX2) and the cloning of multiple prostaglandin receptors, with different signaling cascades and functional effects, the role of postaglandins in physiology and pathophysiology has undergone a renaissance (Fig. 57-1 [29,30]). The stimuli for the activation of phospholipases and subsequent eicosanoid production include mechanical or hypoxic cell stress, proinflammatory cytokines and growth factors and vasoactive peptides (e.g., angiotensin II, etc.). The type of prostaglandin generated depends predominantely on the cell type. While cycloxygenases will generate the intermediate endoperoxides irrespective of cell type, the subsequent conversion to the specific prostaglandins (e.g., prostacyclin, PGI2, thromboxane, PGE2, PGF2α, or PGD2) depends on the cell-specific expression of isomerases and synthetases (29,30).

The leukotrienes (LTs) are also generated from arachidonic acid. The leukocyte-restricted 5-lipoxygenase generates leukotriene A4 (LTA4) which can be converted to LTB4 by the LTA4-hydrolase or to the cysteinyl leukotrienes LTC4, LTD4, and LTE4 (30). LTs and PGs act through a variety of G-protein coupled receptors that are also expressed in a cell-specific manner. Although the chemotactic activity of LTB4 was known for a long time, new roles for LTB4 and PGD2 in T cell trafficking have been identified recently (31). This depends on the expression of the LTB4 receptor BLT and the PGD2 receptor DP-2 on specific T-cell subsets. DP-2 is present on a small fraction of CD8-positive T cells producing IL-4 and IL-13. This may be of special interest for progressive tissue injury, as IL-13 production favors fibrosis, at least in liver and lung (32,33). LTB4 converts rolling of specific T cell subsets to firm adhesion on endothelial cells, an effect mediated by BLT-1 receptor activation of integrins (30,34).

The generation of the LIPOXINS (LXs), which are also arachidonate derivatives, involves the interaction of neutrophils and platelets (35). The neutrophil 5-lipoxygenase generates LTA4, which can be converted by adjacent platelets via 12-lipoxygenase to lipoxin A4 (LXA4) and B4 (LXB4). LXA4 causes vasodilation and antagonizes the vasoconstrictor effect of the cysteinyl-leukotriene LTC4. Furthermore, it decreases chemotaxis and adhesion of neutrophils while enhancing that of monocytes. It has been suggested that the lipoxins are the natural brake for actions of leukotrienes thereby limiting the local inflammatory process (35).

Platelet activating factor (PAF) is another but less cell-type specific lipid mediator of inflammation (36). PAF received its name from the original observation that it aggregated and degranulated platelets. Chemically it is an ether-phospholipid, that is, 1-alkyl, 2-acetyl glycerophosphocholine. It is generated from membrane phosphocholine lipids with an ether bond in position one, by the action of phospholipase A2 (releasing arachidonic acid at the same time), followed by acetylation in position 2. PAF is inactivated by acetylhydrolases assuring a tightly regulated system. PAF is a very potent vasoactive agent, activator of leukocyte adhesion, including selectin activation, chemotaxis, leukocyte degranulation, and oxidative burst. PAF may also be part of local amplification loops as it can in turn stimulate the synthesis of cytokines, chemokines, and eicosanoids by the infiltrating leukocytes (36). Thus there is an intricate connection between the lipid and peptide mediators of inflammation.

Complement

The complement cascade is composed of a large number of effector and regulatory proteins (37). The activation of the complement cascade can be mediated through the classical, the alternative, and the lectin pathway. The progression down the cascade involves the proteolytic cleavage and subsequent activation of complement components (37). C5b plays a role in the activation of downstream complement events, while C5a acts as a potent chemoattractant for monocytes and neutrophils,

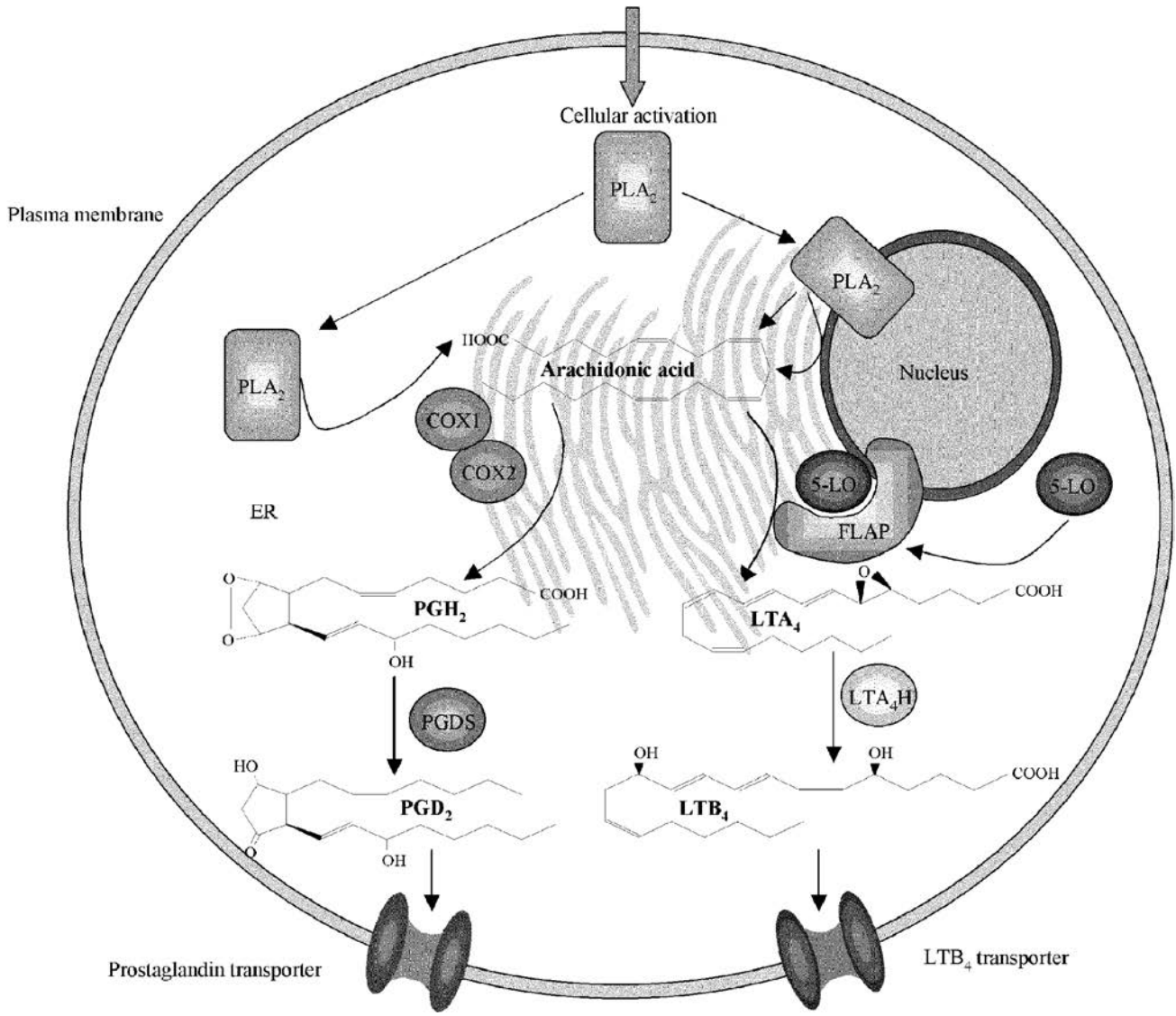

FIGURE 57-1. Schematic scheme of the synthesis of lipid mediators. Cell activation by various stimuli (e.g., trauma, cytokines, etc.) triggers the translocation of cytosolic phospholipase A2 (PLA2) to the nuclear membrane as well as to the endoplasmatic reticulum (ER), leading to the release of arachidonic acid. 5-Lipoxygenase (5-LO) leads to the production of leukotrienes (LTs, *right*, 5-LO activating protein: FLAP, LTA4 hydrolase: LTA4H). COX lead to the production of 4PGH2 which is converted to PGD2 via PGD synthase. (Modified from: Luster AD, Tager AM T-cell trafficking in asthma: lipid mediators grease the way. *Nat Rev Immunol* 2004;4:711.)

increases vascular permeability, upregulates adhesion molecules, and propagates contraction of smooth muscle cells (38). The classical pathway is initiated when immunoglobulins (e.g., immunoglobulin G 1–3 [IgG1–3] or immunoglobulin M [IgM]) bind their respective antigen leading to the formation of the C3 convertase (C4b2b). A C3 convertase can also be formed through the alternative pathway by fragments of C3 and factor B (C3bBb). Basement membrane fragments or bacterial cell wall components can activate the alternative pathway (37,39). The third pathway, the lectin pathway, is triggered by distinct carbohydrates and involves the mannose-binding lectin and the MBL-associated serine protease (37,39). All three pathways converge upon the generation of C3 convertase, which cleaves C3 into C3a and C3b. C3b promotes the first step in the activation of the terminal complement complex, as it associates with either the classical or alternative C3 convertase to generate a C5 convertase. C5 convertase cleaves C5 into C5a and C5b, which directs formation of the membrane-attack complex composed of C6, C7, C8, and C9. C5a is a ligand for a seven-transmembrane-segment G protein coupled receptor, which is abundantly expressed by neutrophils, mast cells, basophils, endothelial cells, and monocyte/macrophages (39).

NO and Vasoactive Substances

Nitric oxide (NO) is generated by three isoforms of NO synthase, the neuronal nNOS, the inducible iNOS, and the constitutive endothelial eNOS (40). The latter is predominantly present on endothelial cells and can be activated by shear stress, bradykinin, and thrombin. The iNOS is expressed on endothelial smooth muscle cells and on macrophages. It can be induced by inflammatory mediators such as IL-1, TNF, IFN-γ, and endotoxin. NO's role in inflammation includes vasodilatation, antagonizing platelet activation, reducing leukocyte recruitment, decreasing proinflammatory cytokine and chemokine

production, and acting as a microbicidal agent (40). With lack of appropriate co-factors the NO synthetase can, however, become a major source of reactive oxygen species and as such turn into a perpetuator of tissue injury (40).

Finally it should be briefly noted that the renin-angiotensin system also has an immune modulator activity that appears to be independent of its vasoactive properties (41). This "inflammatory" aspect of angiotensin may also play a role in progressive disease processes (24).

Vascular Changes Set the Stage for Leukocyte Extravasation

Acute inflammation is short in duration (hours, a few days) and is initially characterized by a predominant influx of neutrophils followed by monocytes/macrophages (42–44). The extravasation of leukocytes occurs through the specialized endothelia of postcapillary venules (Fig. 57-2). Only in the special cases of the pulmonary alveolar and renal glomerular microcirculation can leukocytes also leave the bloodstream through capillaries. The reasons for this restriction to these special microvascular anatomic sites most likely involve the high velocity of blood flow with high shear stress forces in other vascular beds, as well as differences in the expression of adhesion molecules on the various endothelia. Of note is the absolute prerequisite that both the leukocytes and the endothelial cells be activated in order for their normally brief encounters to be of consequence and result in leukocyte adhesion and extravasation (Fig. 57-2).

During acute inflammation arteriolar vasodilatation leads to an expansion of the capillary bed, which together with an increase in vascular permeability results in extravasation of fluid into the extracellular space. The blood viscosity increases and the blood flow slows, ultimately resulting in stasis. Leakiness of the microvasculature is mediated through endothelial cell contraction (by histamines, bradykinin, LTs resulting in gaps in venules), endothelial cell retraction (mediated by cytokines, e.g., TNF, IL-1 which results in reorganization of the cytoskeleton), and endothelial cell injury (45,46).

Leukocyte Extravasation

Vascular dilatation, increased permeability and slowing of flow allows the margination of leukocytes. This is followed by a cascade of events involving rolling, activation, firm adhesion and transmigration of leukocytes on the activated endothelium (Fig. 57-2 [34,42–47]). After extravasation leukocytes follow various chemotactic gradients, mostly in a haptotactic, that is, matrix bound manner, toward the site of tissue injury. Leukocyte extravasation is mediated through the interaction of adhesion molecules with their respective counter-receptors, in

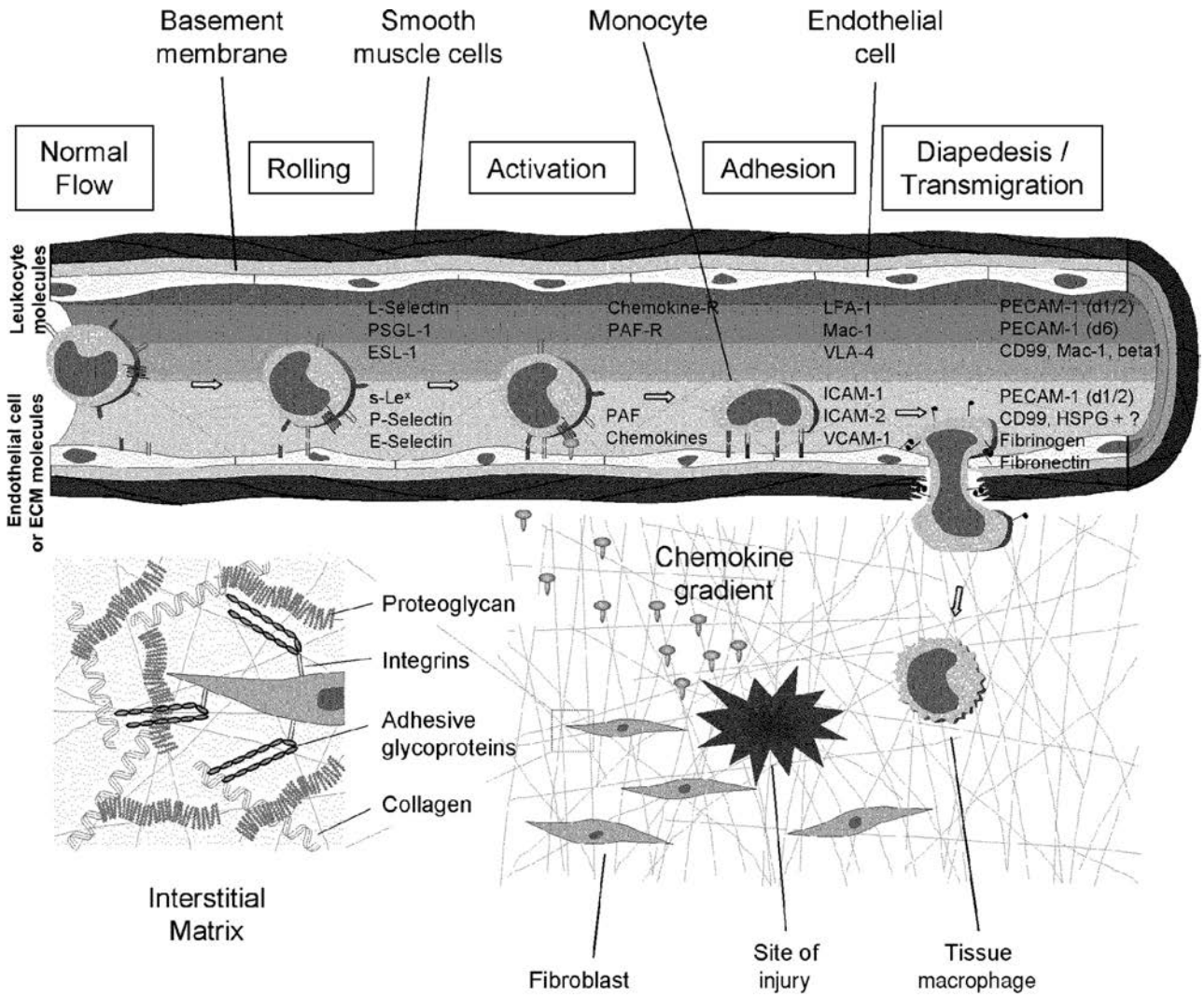

FIGURE 57-2. Extravasation cascade of inflammatory cells.

concert with activation by lipid mediators, chemokines, and cytokines, which can activate integrins and regulate the expression of adhesion molecules.

Selectins and Their Ligands Establish the Initial Contact Between Leukocytes and Endothelial Cells

The decrease in shear stress forces allows the low-affinity contact between the first class of adhesion molecules, the selectins, and their carbohydrate ligands (see Table 57-2). Selectins are expressed on leukocytes and endothelial cells (34,48–50). At present three members of the selectin family are known. Their adhesive character is determined by amino-terminal lectin domains. Following the lectin domain is an epidermal growth factor (EGF) domain, a variable number of complement regulatory domain sequences, and a single transmembrane sequence with a cytoplasmic tail. The number of complement regulatory domains varies on the different selectins. The exact function of the complement regulatory elements remains unknown, but altering them results in different binding characteristics. On leukocytes the L-selectin is concentrated on cell projections allowing the early contact with their counterparts on the endothelial surface. Leukocyte L-selectin is expressed

TABLE 57-2

ADHESION MOLECULES, THE CORRESPONDING LIGANDS AND THE MAJOR FUNCTION IN CELL RECRUITMENT

Adhesion molecule	Ligand	Function
Selectins		
L-Selectin (CD62L, on leukocytes)	MadCAM-1 (mucosal addressin cell adhesion molecule, HEVs of Peyer plaques) CD34 (endothelium) GlyCAM-1 (endothelium) Podocalyxin Mannose receptor	Rolling of neutrophils and monocytes
P Selectin (CD62P, on platelets and endothelium)	PSGL-1 Sialyl Lewis X modified proteins	Rolling of neutrophils, monocytes, and lymphocytes
E-Selectin (CD62E on endothelium)	Sialyl Lewis X modified proteins (on leukocytes)	Rolling and adhesion of neutrophils, monocytes, and T cells
Integrins		
VLA-4 ($\alpha 4\beta 1$)	VCAM-1 Fibronectin (CS-1)	Adhesion of monocytes, lymphocytes, and eosinophils
LFA-1 (CD11a/CD18)	ICAM-1 ICAM-2 ICAM-3	Adhesion and arrest of monocytes, lymphocytes, and neutrophils
MAC-1 (CD11b/CD18)	ICAM-1 C3bi Fibrinogen Factor X	
CD11c/CD18 (p150,95)	Fibrinogen ICAM-1	
CD11d/CD18	Unknown	
$\alpha 4\beta 7$	MadCAM-1 VCAM-1	
Adhesion molecules of the immunoglobulin superfamily		
ICAM-1 (on endothelium, mesangial cells, epithelial cells, and leukocytes)	CD11a/CD18, CD11b/CD18 CD11c/CD18 CD43 Fibrinogen Hyaluronic acid	
ICAM-2 (on endothelium, leukocytes)	CD11a/CD18 DC-SIGN (CD209)	
ICAM-3 (leukocytes)	DC-SIGN (CD209)	
VCAM-1 (parietal epithelial cells, endothelium, epithelial cells, mesangial cells)	VLA-4	
PECAM-1 (CD31, on leukocytes, endothelium)	PECAM-1 (CD31) $\alpha 5\beta 3$ CD38	
Others		
CD44	Hyaluronic acid	Rolling

constitutively, while E-selectin surface expression on endothelial cells requires *de novo* synthesis after prestimulation by inflammatory mediators. P-selectin is stored in platelets, and in Weibel-Palade bodies in endothelial cells, and is rapidly distributed to the surface upon stimulation. With continued stimulation P-selectin can be present constantly on the endothelium in areas of chronic inflammation.

Similarly to the expression of selectins, their counterpart glycoproteins are also regulated. The regulation involves mostly alterations in the "sticky" sugar coating. In the case of the L-selectin ligand CD34 on endothelial cells, the proper decoration of the protein backbone with the Sialyl-Lewis carbohydrates has to occur by stimulation of the respective enzymes, for example, fucosyl- and sialyl-transferases. Even the major P-selectin glycoprotein ligand-1 (PSGL-1), which is constitutively present on neutrophils and T-cells, may require some change in its carbohydrate decoration to allow for optimal interactions with P-selectin. So already the initial adhesion contact allows for a fine tuning by combining constitutive and regulated expression with rapid surface transfer of the molecules, and modification of their sugar moieties (34,48–50).

The tethering of leukocytes on the endothelium, termed rolling, by itself would be inconsequential, as the binding forces between the selectins and their ligands are too weak, and will only result in short lasting leukocyte-endothelial contact without firm adhesion. Only if the endothelial cell presents leukocyte-activating molecules (e.g., chemokines, LTB4 or PAF) on its surface for the respective receptor on the passing leukocytes, will this brief encounter be converted to firm adhesion of the leukocyte (42–44,46).

Integrins and Their Counterparts of the Immunoglobulin Superfamily Mediate Firm Leukocyte-Endothelial Adhesion

Before the next crucial step, that is, firm adhesion can occur, the involved adhesion molecules, the integrins on the leukocytes need to be activated to a status of high affinity. Integrins are a family of cell surface molecules that are involved in attachment of cells to their extracellular matrix and also in cell–cell binding (9,46). They are heterodimetric receptors consisting of paired α- and β-chains (Table 57-2). The β-chain determines the type of integrin. Different types of leukocytes can express essentially two types of β2-integrins. The β2 (CD18) can be paired with either αL (CD11a) or αM (CD11b), resulting in CD11a/CD18 (or LFA-1) or CD11b/CD18 (or Mac-1), respectively. In addition, many leukocyte types express the β1 integrin $\alpha 4\beta 1$ or very late antigen 4 (VLA-4). Under basal conditions the integrins on leukocytes are in a low-affinity binding state. Upon activation by inflammatory mediators the strength of their interaction with endothelial ligands is enhanced by conformational changes and by increasing their density on the binding surface, via clustering at the site of leukocyte-endothelial contact. The activation of the different types of leukocytes occurs predominantly by soluble mediators of inflammation, which may, however, be presented in a membrane-bound form by the endothelial cell surface. Most of the respective receptors on leukocytes belong to the serpentine, seven transmembrane spanning, G protein-coupled receptors (9,46). The predominant class of mediators include the chemokines, LTB4, PAF, complement C5a, and formylated peptides of bacterial origin (e.g., FMLP). The endogenous mediators such as chemokines, LTB4, and PAF are locally produced by the cells of the injured tissue—be they parenchymal or endothelial—and reach the vascular endothelial luminal surface either by transcytosis or by diffusion (9,46). On the endothelial surface they present themselves to the rolling leukocytes bound to proteoglycans, which serve as lampposts for presentation to the specific leukocyte receptors. Different types of leukocytes are activated by different types of chemokines and also use different molecules for rolling and firm adhesion. Not only are the integrins regulated, but also the endothelial integrin counterparts, members of the immunoglobulin family ICAM-1, VCAM-1, PECAM can be modified by cytokines (9,46).

Leukocyte-Endothelial Transmigration

Firm adhesion is followed by diapedesis of leukocytes at the specialized endothelial-endothelial junctions, a step involving yet another group of adhesion molecules (9,46). During the transmigration of leukocytes the adherens junctions of adjacent endothelial cells have to disorganize temporarily to allow the squeezing through of the leukocytes (42–45,47,51,52). Within minutes, however, the junctions are reformed and during the whole process a tight seal seems to form between the endothelial cells and the transmigrating leukocytes as demonstrated by the maintenance of electrical resistance during the entire process. In contrast to the heterophilic interactions between integrins and VCAM, ICAM, and PECAM during leukocyte adhesion to the endothelium, the molecules involved during transmigration engage in homophilic interactions. Thus PECAM-1/CD31 and CD99 on endothelial cells engage PECAM-1 and CD99, respectively on leukocytes. Blocking PECAM-1 *in vitro* or *in vivo* will inhibit diapedesis. CD99, a highly glycosylated protein seems to be involved in the homophilic interactions required for the transmigration process at a step distal to PECAM-1. Blockage of CD99 will also decrease leukocyte transmigration and blocking both PECAM-1 and CD99 results in additive inhibition (52). Furthermore, components of the endothelial-endothelial junctions, such as junctional adhesion molecules (JAMs) and cadherins are involved in the leukocyte transmigration (45,51). While it is generally accepted that the predominant site of leukocyte emigration is between endothelial cells, there is also some evidence that under certain conditions leukocytes can actually migrate through endothelial cell bodies. In the case of fenestrated endothelia, as present in the glomerulus and in vasa recta in the kidney, the possibility of leukocytes migrating across these fenestrae exists, though we are not aware of any evidence in favor or against this possibility.

Leukocyte Migration Through the Extracellular Matrix (ECM)

Once the leukocyte has transmigrated the endothelial layer, which is a matter of seconds or minutes, it will get arrested on the underlying basement membrane. The molecules and mechanisms involved in the crossing of basement membranes by leukocytes are much less understood, but may also involve interaction of adhesion molecules such as integrins on leukocytes with ligands consisting of matrix components (53,54). In addition, local activation of matrix metalloproteinases (MMPs) may allow the leukocyte to "eat" its way across the basement membrane. Similar considerations apply to the subsequent migration of leukocytes through the ECM toward the site of tissue injury. Subgroups of inflammatory cells use different (but at times overlapping) combinations of mediators that may be generated at different times (53–55). The type of recruited cell therefore not only depends on the type and the site of injury, but also on the duration of the process. Neutrophils predominate early (within the first 24 hours) and are replaced by monocytes/macrophages which may be explained by the pattern of chemotactic factors and adhesion molecules generated with

a different time points. The short-lived neutrophils undergo apoptosis within 2 days after exiting the bloodstream, whereas monocytes persist for long periods.

Having left the bloodstream, leukocytes follow gradients of chemotactic substances toward the site of tissue injury. During the migration through the extracellular space the chemokines may be matrix bound (a process termed haptotaxis) or exist as a diffusible gradient (chemotaxis) (15–19). Having reached the site of injury, leukocytes frequently engage in an amplification loop, that is, they release further mediators such as cytokines, chemokines, reactive oxygen species (ROS), NO, and lipid mediators (53–55).

Leukocyte Activation at the Site of Tissue Injury

Once at the site of tissue injury the leukocytes will start the repair process by ingesting, and processing the inciting agents, be they infectious, immune complexes (ICs), or remnants of cells and matrix. Phagocytosis and release of degradative enzymes are early events after recruitment of leukocytes to the site of injury (53–55) (Fig. 57-3). Phagocytosis involves recognition and attachment (facilitated by opsonins), engulfement, and degradation of the ingested material. The most important opsonins are IgG, complement split products (C3b), and carbohydrate binding lectins (collectins). Activation of leukocytes further involves receptors for chemokines and cytokines, receptors for pathogen-associated molecular patterns (i.e., toll-like receptors), and scavenger receptors (53–55). Activated leukocytes will generate an oxidative burst, degranulate and discharge lysosomal enzymes, release and activate various enzymes such as phospholipases and MMPs. The released enzymes include elastase, collagenases, cathepsin, and other proteases, which can degrade ECM components as well as generate vasoactive or pro-inflammarory peptides from precursors (e.g. components of the complement, kinin, or coagulation systems). The action of the proteases on the matrix will help in removing damaged matrix and thus clear the way for the repair process. If, however, this process becomes extensive, it could also remove the extracellular scaffold itself, for example, the basement membrane that is required to reestablish the original tissue architecture. This could create gaps allowing epithelial cells to migrate out of their normal cellular organisation and open up the possibility for epithelial-mesenchymal transition (EMT), which could contribute to fibrosis and loss of functional units (56).

In this context the special roles of infiltrating macrophages has to be considered. The activated macrophage can exist in multiple forms, depending on the surrounding milieu, interacting cells, and on its mode of activation (7,54,57,58). It can develop into a major source of growth factors (e.g., PDGF, FGF, TGF-β) that are required for tissue regeneration. Some of these, for example, VEGF, PDGF are angiogenic and favor neovascularization, others may favor matrix deposition. Upon exposure to IL-4 and especially IL-13 macrophages can develop into a profibrotic phenotype, that, at least in lung and liver, may be TGF-β independent (32,33). Alternatively, the macrophage may evolve to generate a cytokine profile favoring a T helper type 2 (TH2) immune response (leading to a humoral response) or develop into dendritic cells (7,54,57). Specific circulating monocyte-like precursor cells, also termed fibrocytes, may not only become a source of profibrotic cytokines such as TGF-β, but may actually become themselves a major source of matrix components for example collagen I (59–61).

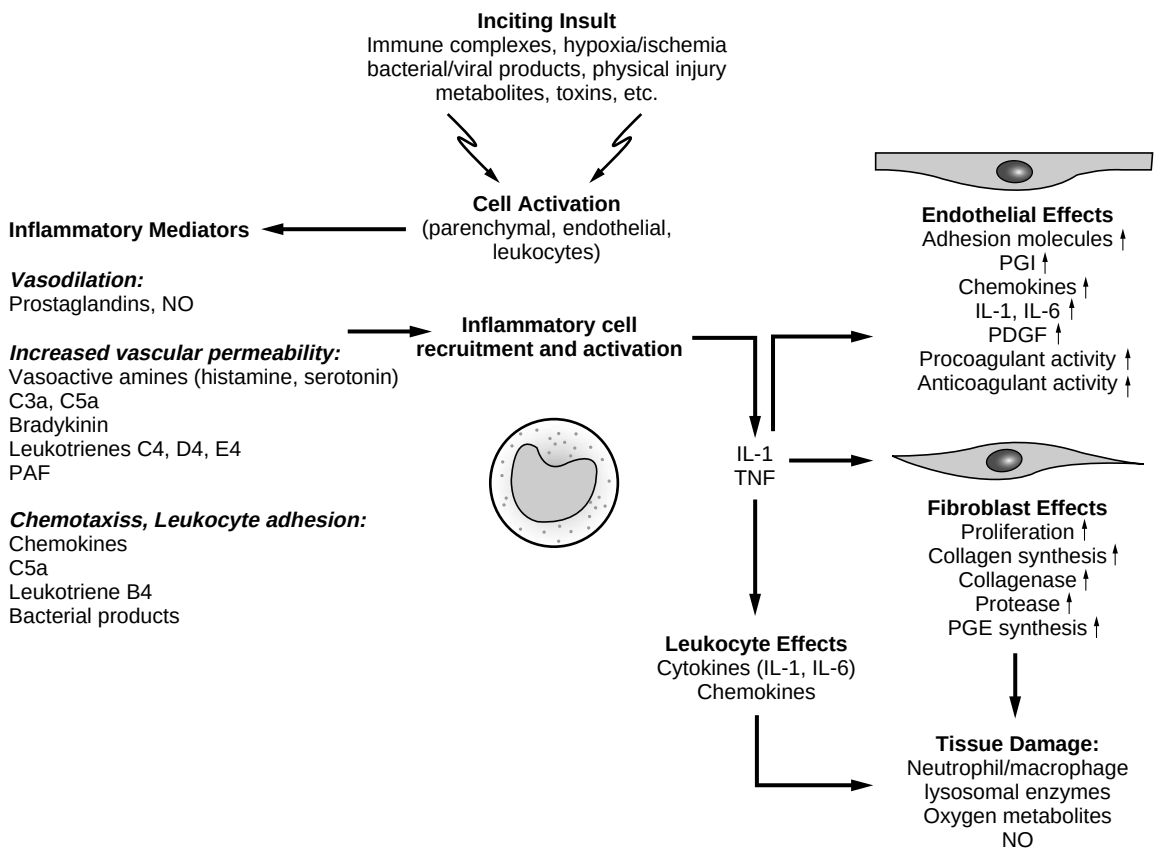

FIGURE 57-3. Scematic scheme of cellular activation, resulting in tissue damage.

In general, the myofibroblast has been considered as the major collagen-producing cell in fibrosis. In this scheme the myofibroblasts arise from activation (predominantly by TGF-β of macrophage or parenchymal origin) of interstitial fibroblasts or by transition of parenchymal epithelia to cells with mesenchymal characteristics (56). This epithelial-mesenchymal transition could result in the emigration from the organized epithelial layer into the interstitial space, provided the basement membrane loses its integrity. There they become myofibroblasts and a major source of excess collagen and hence fibrosis. Lately evidence for a different origin of excess collagen formation has been generated in models of pulmonary and liver fibrosis (59–61). Here the collagen-producing cells were of bone marrow origin and probably represent so-called "fibrocytes." These fibrocytes had been identified earlier as a unique cell population, that circulates in low number in the peripheral blood (61). They express the chemokine receptors CXCR4 and CCR7, and migrate in response to the corresponding chemokines CXCL12/SDF-1 and CCL21/SLC (59). Potentially the latter chemokines, generated at the site of tissue injury by the infiltrating macrophages, could attract the fibrocytes and involve them in the fibrotic process. Whether this mechanism is universal or specific to some forms of lung and liver injury remains to be evaluated. Reevaluation of the potential source of myofibroblasts, that is, local parenchymal versus bone marrow, in progressive fibrosis obviously might also have considerable therapeutic implications.

TGF-β1 or -2, either released and activated by the macrophage themselves, or released from its binding to ECM by proteases, can cause epithelial-mesenchymal transition; fibroblast activation; generation of matrix components and inhibition of their breakdown by MMPs (11–14); local immunsuppression; apoptosis, etc. TGF-β3 may have opposite effects, but in the adult tissue is only present in low amounts (5). The uptake of apoptotic bodies is a major function of macrophages in the repair of tissue injury (62–64). It represents a very important function, since nonremoval of apoptotic bodies will result in the generation of proinflammatory components and thus exacerbate inflammation. By contrast, the normal uptake and removal of apoptotic cells by macrophages alters their phenotype to an immuno-inhibitory, antiinflammatory one, which will produce less CXCL8/IL-8 and other chemokines, less TNF and IL-1β, and secrete more NO, TGF-β, and PGE2. The latter will further inhibit local chemokine and cytokine generation in an auto- and paracrine manner. Furthermore, upon uptake of apoptotic cells macrophages will suppress local T-cell proliferation, interrupting a vicious cycle of mutual stimulation (62–64). In this context it should be pointed out that once macrophages and T cells have reached the site of tissue injury, they engage in a dialogue. Initially the macrophage activates the lymphocyte, which in turn release proinflammatory mediators (7,54,57). The uptake of the apoptotic bodies will interrupt this vicious cycle as outlined above.

Because of the functional plasticity and the multiple and dynamic phenotypes of the macrophage, it has been questioned recently if indentification of the various macrophage activation states can be achieved. Defining macrophage subsets by surface or functional phenotype during an inflammatory episode might be akin to defining chameleons by their color display at a given moment (57).

Role of Heparan Sulfate Proteoglycans (HSPG) in Inflammatory Cell Recruitment

HSPGs play multiple roles in the process of leukocyte adhesion, and in endothelial and extracellular matrix transmigration (65,66). Proteoglycans consist of a core protein of varying size (molecular weight [MW] = 11,000 to 220,000) to which one or more (up to 100) sulfated carbohydrate glycosaminoglycans (GAGs) are attached. Proteoglycans are named according to the principal repeating disaccharides (e.g., heparan sulfate, chondroitin sulfate), which are heavily negatively charged and bind to other matrix and cell membrane components. The HSPGs are extremely versatile and diverse as their side chains can be enzymatically modified by *N*-deacetylases, *N*- and *O*-sulfotransferases, and epimerases. This occurs in a cell-specific manner and can be modified by inflammatory mediators. Proteins that can bind to HSPGs include chemokines, growth factors, adhesion molecules such as selectins, integrins, PECAM, enzymes, enzyme inhibitors and ECM proteins (fibronectin, collagens, laminin, fibrillin, thrombospondin, vitronectin). The functional consequences of the binding of the ligand to the HSPGs are: (a) immobilization resulting in a high local concentration of the ligand; (b) conformational changes of both the HSPG and the ligand with functional consequences; (c) oligomerization of the ligands, which, in the case of cytokines and chemokines, allows dimerization and thus receptor signaling; and (d) storage and protection from degradation of the ligands.

During inflammation the surface binding of for example chemokines to HSPGs on endothelial cells and ECM allows for the establishment of a gradient for the leukocytes to follow. Endothelial and leukocyte surface HSPGs are directly involved in cell–cell interaction via for example selectins and β2 integrins. Activated endothelial cells and leukocytes exert heparanase activity, which facilitates leukocyte transmigration by degrading HSPGs (e.g., the ECM). Furthermore, the haparanase may release bound chemokines and growth factors (e.g., TGF-β, EGF, FGF, PDGF, VEGF).

The HSPGs syndecan-1, -3, and -4 and glypican are differentially expressed on different endothelial cells. In the kidney only syndecan-1 and 4 have been identified (66). Beta glycans contain heparan sulfate and chondroitin sulfate chains. They bind TGF-β and are present in the interstitial space and on micro-vascular endothelium. CD44, the cell-surface receptor for hyaluronic acid, is expressed on restricted endothelial cells and on mesangial cells. Hyaluronic acid (HA) is a polysaccharide of the GAG family and is found in the ECM of many tissues. HA exists in a glycosylated and nonglycosylated form, and in numerous isoforms. Both CD44 and HA play roles in inflammation, including cell proliferation.

Outcome of Tissue Injury, Inflammation, and Repair

As long as the inflammatory response is short-lived and the injury locally limited with minimal disruption of the underlying tissue architecture, the repair process will restore almost normal histology and function. Obviously this requires that no new insults occur, that production of pro-inflammatory mediators ceases, or that their action becomes neutralized, and that leukocytes accumulated at the site of tissue injury disappear by either undergoing apoptosis locally or by leaving the site via blood or lymphatic vessels (reverse migration) (42–44,55,62). Furthermore, the extracellular matrix scaffold has to be remodeled to its original form and the parenchymal cells have to replace the lost ones in the original pattern. Unfortunately this scenario is the exception rather than the rule. Under most conditions there is enough disruption of the original intricate tissue structure, so that repair can only occur in an incomplete manner, that is with scar-fibrosis formation. As the tissue structure based on the ECM scaffold and the specific cell-cell organization is lost, it will be replaced by an amorphous fibrous tissue. The fibrotic process involves a similar set of players as the initial

inflammation, that is, leukocytes, mediators of inflammation including cytokines, chemokines, etc. However, these now fulfill different functions, for example, the leukocytes inducing EMT and fibroblast formation with deposition of excess extracellular fibrous matrix. As capillary blood supply may also be lost due to tissue injury, the area of repair may become chronically hypoxic and thus susceptible to progressive inflammation, unless adequate neovascularization occurs. Thus, in tissues with high oxygen demands such as the kidney, scar formation and fibrosis may predispose to smoldering hypoxic-ischemic damage resulting in chronic inflammation and progressive fibrosis with loss of function (67). In this process the persistence of macrophages and lymphocytes play a major role through the production of proinflammatory, profibrotic cytokines (see above). Under many circumstances, including progressive renal diseases, it remains unclear what causes the local retention, proliferation, and activation of macrophages and lymphocytes. Factors that could contribute are cytokines and other mediators released by "stressed" parenchymal-epithelial or even endothelial cells. The stress may include chronic hypoxia (see above [68]), "overwork" by the remaining parenchymal cells with generation of metabolic mediators, including ROS. During these processes hidden antigens could be "unmasked" or neo-antigens generated resulting in de novo local autoimmune processes with progressive inflammation, fibrosis, and loss of function.

Tissue Injury and Repair in the Kidney

Anatomical and Functional Specificities

The Glomerular and Peritubular Vasculature is Arranged in Sequence. Essentially the renal microvasculature is organized in two capillary beds in sequence, that is, the glomerular capillary convolute and the peritubular capillary network. This sequential organization allows for an exquisite balance of the glomerular filtration with the tubular re-absorption, that is, the maintenance of glomerular tubular balance. The downside of this physiologically extremely dynamic adaptive and fine-tuned system is its vulnerability to upstream interference with blood flow, that is, a decrease in preglomerular or glomerular blood flow will inevitably jeopardize the downstream peritubular blood flow. This is especially pertinent as there is essentially no collateral blood supply for the postglomerular microcirculation. Thus any acute or chronic glomerular injury, with a decrease in glomerular blood flow, will be associated with an obligatory reduction in peritubular blood flow which, depending on the degree of hypoxia, will entail tubulointerstitial injury and tissue remodeling.

Another consequence of these sequential capillary beds is that there are two potential sites for leukocyte extravasation during inflammatory renal disease, the glomerulus or the tubulointerstitium. However, as the peritubular circulation is downstream of the glomerular circulation, inflammation, or at least some mediators involved, may spill over during glomerular inflammation from the glomerular vascular convolute into the peritubular microcirculation and thus contribute to the frequently observed intersititial inflammatory reaction in glomerulonephritis.

Glomerular and Tubular Functions Are Organized in Sequence. The downstream position of the tubulus in respect to the the glomerulus does not only apply to the blood flow but also to the ultrafiltrate. While this is part of the elegant homeostatic system of kidney function in health, in disease it may become another avenue for spreading a primarily glomerular disease to the tubulointerstitial compartment. Due to the increased glomerular capillary permeablility and loss of permselectivity in glomerular diseases, the tubular epithelial cells will be exposed to an abnormal ultrafiltrate, and thereby to a variety of potentially noxious substances, which may in turn set off tubular epithelial injury with secondary peritubular inflammation. Thus the dual downstream position of the tubulointerstitium, that is, in terms of blood and utlrafiltration flow, which in health ensures an exquisite functional tuning of the glomerular-tubular balance, may become the Achilles tendon in disease with secondary injury to the tubulointerstitium in primary glomerular processes.

Obviously the reverse would not be true, as primary insults to the tubulointerstitium would not reach the glomerulus. Indeed in most forms of primary tubulointerstitial renal diseases the glomerular architecture is preserved until far advanced interstitial fibrosis occurs. Thus the concept of the nephron as a functional unit may not only apply to renal physiology, but also to the pathophysiology of renal disease. The glomerulus itself should also be regarded as a functional unit with each individual constituent, that is, endothothelial, mesangial, and epithelial cells and their extracellular matrix representing an integral part of normal function. Damage to one will influence the other in part through direct cell–cell connections (e.g., gap junctions), soluble mediators such as chemokines, cytokines, growth factors, changes in matrix and basement membrane composition.

The Glomerulus as the Site of the Primary Insult

Glomerular Injury in Human Kidney Diseases. From a pathogenetic standpoint human glomerular diseases can be broadly divided into those

1. Without glomerular inflammation or deposition of immunoglobulins (e.g., minimal change disease, idiopathic focal, and segmental glomerulosclerosis [FSGS]).
2. With deposition of immunoglobulins, but without relevant glomerular inflammation, most likely due to the localization of the immunoglobulins (e.g., membranous nephropathy)
3. With deposition of immunoglobulins leading to increased cellularity (proliferative glomerulonephritities, e.g., lupus nephritis, IgA nephropathy, anti-GBM, postinfectious GN)
4. With severe glomerular injury and inflammation, but without deposition of immunoglobulins (e.g., pauci immune glomerulonephritis).

This is of course an oversimplification, as some glomerular lesions are not included in this system (e.g. diabetes or amyloidosis), but it helps to separate broad glomerular disease entities (69). The podocyte seems to be the major target in the first and second group, which therefore will be described below (69). This will be followed by diseases of the third group, in which ICs lead to activation of intrinsic renal cells (via Fc receptors and complement products) resulting in inflammatory cell recruitment. For space reasons we will not describe the injury process in pauci-immune crescentic GN, diabetic nephropathy, and renal involvement in paraproteinemia. For these entities please refer to Chapters 60, 73, and 75 in this book.

Podocytes in Injury. Glomerular podocytes are highly specialized cells covering the outer aspect of the GBM with interdigitating foot processes. Foot processes intervene with slit diaphragms, a crucial element for the selective glomerular permeability. Injury to podocytes leads to proteinuria, a hallmark of most glomerular diseases. Recent studies implicate a loss of podocytes as a driving force of progressive glomerular disease (70). Podocytes can be lost via apoptosis or detachment from the GBM, and can be recovered in urine of mouse and human (70,71).

Damage to the Glomerular Filtration Barrier Results in Proteinuria. Podocytes maintain the large filtration surface through the slit membranes and are responsible for 40% of the hydraulic resistance of the glomerular filtration barrier. During proteinuric diseases, the ultrafiltration coefficient (*K*f) and thus the GFR are often lowered (for a detailed review see Deen et al. [72]). Proteinuria may be caused by defects of the podocytes, the GBM, the endothelial cells, and/or by alterations of the negatively charged proteins present on all three components of the glomerular filtration barrier. However, it is still a matter of debate whether the size selectivity of the glomerular filtration barrier is disturbed in proteinuria and whether the glomerular filtration barrier interacts with proteins by virtue of charge characteristics or its gel filtration properties (73).

Our understanding of the function and failure of the filtration barrier has been greatly improved by studies showing the essential role of slit diaphragm molecules, including ZO-1 (74), nephrin (75), CD2AP (76), FAT (77), Neph1 (77), Densin (78), and P-cadherin (79) in human or murine hereditary glomerular diseases. This was first shown for nephrin in the congenital nephrotic syndrome of the Finnish type (75), followed by the identification of mutations in the gene for podocin in autosomal-recessive (80) and alpha-actinin-4 in a autosomal-dominant FSGS (81). Several studies could also demonstrate the regulation of these molecules in acquired glomerular diseases (82). The molecular interaction and intrinsic signaling properties of the various molecules involved have been reviewed recently (83).

Glomerular Basement Membrane Alteration in Proteinuria. During glomerulogenesis, the GBM is generated by glomerular endothelial and epithelial cells as two separate layers and fused together to produce the mature GBM. The podocyte continues to add and assemble matrix molecules to the GBM, maintaining a hydrated meshwork consisting of collagen IV, laminin, entactin, agrin, and perlecan (84). The flexibility and dynamic of mature GBM requires a constant turnover. To achieve this, podocytes not only produce GBM components, but also secrete matrix-modifying enzymes (85,86).

The relevance of the GBM composition for podocyte architecture is exemplified by foot process fusion and proteinuria in the laminin beta 2-chain–deficient mouse (87). Alpha 5-, beta 2-, and gamma 1-laminin chains are assembled to form the GBM-specific heterotrimeric laminin 11 (88). The lack of a single laminin monomer is sufficient to cause severe disruption in the filtration barrier, leading to a nephrotic phenotype.

Specific matrix receptors anchor the podocyte foot processes by binding to their ligands in the GBM (88,89). Initial studies concentrated on a specific integrin heterodimer, consisting of alpha3-/beta 1-integrins, found at the "sole" of podocyte foot processes facing the GBM (90,91). Alpha3-/beta 1-integrins have been described to bind to collagen IV alpha 3-, 4-, and 5-chains, fibronectin, laminin, and entactin/nidogen, in the GBM (92). A series of experimental and genetic studies indicate an involvement of integrin-mediated podocyte matrix interaction in failure of the filtration barrier (89,90,93). Genetic evidence for the requirement of alpha 3-integrins for an intact filtration barrier was generated with the alpha 3-integrin knockout mouse, exhibiting an immature GBM and foot process effacement at birth (94). These findings led to the hypothesis that changes in podocyte integrins could result in proteinuria by interfering with the dynamic of foot process anchoring in the GBM. Integrin signaling via focal adhesion kinase (FAK) und integrin linked kinase (ILK) has also been shown to be activated in podocyte damage, *in vitro* and *in vivo* (95–97).

The expression of a second matrix receptor in podocytes, the dystroglycan complex, is negatively correlated with proteinuria in animal models (98) and in minimal-change disease in humans (99). Both integrins and dystroglycans are coupled via adapter molecules to the podocyte cytoskeleton. A series of studies have shown an association of altered GBM, loss of slit membranes, and proteinuria with rearranged cytoskeletal proteins. Systematic ultrastructural and immunohistochemical analysis detected a rearrangement of cytoskeletal proteins (F-actin and alpha-actinin) in response to foot process effacement in anti-GBM antibody-induced Masugi nephritis (100) and in puromycin nephrosis (PAN) in rats (101).

Cytoskeletal Alterations in Podocyte Damage. The integrity of the foot process is crucial for establishing stability between the cell–cell and the cell–matrix contact of podocytes. Foot process effacement, also referred to as process simplification, retraction, or fusion, is initiated at the cytoskeleton of the podocyte and results in the alteration of the cell–cell contacts at the slit diaphragm and in a mobilization of the cell matrix contacts (102,103). It is accompanied by an increase in microfilament density forming a mat of intercrossing stress fibers at the sole of the foot process (104,105). Podocyte damage models, by polycations such as protamine sulfate or the cytotoxic antibiotic puromycin, cause foot process effacement and proteinuria in rats *in vivo* (103,106) and allow the study of cytoskeletal responses in podocyte damage *in vitro* (79). Kerjaschki et al. blocked actin dynamics via calcium depletion, low temperature, or cytochalasin B and reduced proteinuria by 50% in the protamine sulfate perfused kidney (105). In contrast, interference with microtubular function with colchicine or vinblastine did not alter foot process dynamics (105).

Alpha-actinin-4 is widely expressed in podocyte foot processes, colocalizing with actin stress fibers (107) and is increased in podocyte damage *in vivo* (100,108). Mutations of ACTN4, encoding alpha-actinin-4, were found to cause a late-onset autosomal-dominant focal segmental glomerulosclerosis in humans (81). Initial functional experiments are consistent with an increased F-actin bundling by mutant alpha-actinin-4. Smoyer et al. evaluated the regulation of hsp27, a chaperone protecting cytoskeletal integrity in oxidative stress in podocyte failure (109). After puromycin-induced nephrosis, an increase of both total hsp27 and phosphorylated hsp27 was found (110). Hsp27 was shown to regulate the morphologic and actin cytoskeletal response of cultured podocytes to puromycin-induced injury (111).

Cytokines and Chemokines in Podocyte Damage. Podocytes may be the target of systemically produced cytokines and chemokines or might be the source of inflammatory mediators. As the development of nephrotic syndrome is associated with allergic reactions in some cases, TH2-inflammatory mediators may play a central role in podocyte failure (112). Podocytes from patients with minimal change nephropathy and undifferentiated rat podocytes express the TH-2 cytokine receptors for IL4 (IL-4R) and IL-13 (IL-13R2) (113). The respective ligands (IL-4 and IL-13) decrease transepithelial electrical resistance *in vitro* (113,114). Both cytokines also activate basolateral proton secretion, secretion of the lysosomal proteinase procathepsin L and induce the redistribution of the small GTPases, Rab5b and Rab7, *in vitro*. IL-4 decreases podocyte survival, changes the pattern of the tight junction protein ZO-1 (115), and inhibits the release of the glomerular endothelial survival factor VEGF (116,117). However, conclusive *in vivo* evidence of a pathogenic role of this pathway is still lacking.

In a subpopulation of patients with primary FSGS massive proteinuria can already reappear hours after renal transplantation. A soluble factor seems to mediate the filtration barrier failure, as plasmapheresis can effectively lower proteinuria and plasma from these patients is able to induce proteinuria in rats. Using an *in vitro* assay of glomerular permeability, studies are under way to uncover the molecular identity of the so far elusive "proteinuria factor" (117).

The expression of functional active chemokine receptors CCR4, CCR8, CCR9, CCR10, CXCR1, CXCR3, CXCR4, and CXCR5 has been described in human differentiated podocytes *in vitro*. Ligands of these chemokine receptors increased intracellular calcium and stimulated the generation of superoxide anion in podocytes, making podocytes a target for chemokine induced inflammatory response (118). The significance of these findings obtained in cell culture for the *in vivo* situation remains to be established, as so far there is only scanty evidence for the expression of these chemokine receptors on podocytes *in situ* in the kidney. Concerning the chemokine ligands, expression of CXCL8/IL-8, a ligand for the CXCR1/CXCR2 receptor, might represent an autocrine activation of podocytes (118). CXCL8/IL-8 has been incriminated in minimal change nephropathy and complement-mediated glomerular injury (119). A neutralizing antibody against CXCL8/IL-8 prevented podocyte foot process retraction and proteinuria in an acute immune complex-mediated glomerulonephritis, supporting a role for CXCL8/IL-8 for the development of proteinuria (120).

A chemokine-based mesangium-podocyte cross-talk has been detected with CCR7 expression on mesangial cells and CCL21/SLC production by podocytes (121). Activation of the mesangial receptor resulted in migration, proliferation, and inhibition of Fas/CD95-mediated apoptosis of mesangial cells. Besides CXCL8/IL-8 and CCL21/SLC, undifferentiated rat podocytes have been shown to release CCL2/MCP-1, further supporting the notion of the inflammatory potential of activated podocytes (122).

Generation of Reactive Oxygen Species as a Key Mechanism for Podocyte Injury. Reactive oxygen species (ROS) appear to mediate damage to podocytes induced by toxins, antibodies, complement factors, and mechanical stress. The reactive oxygen product hydrogen peroxide (H_2O_2) acts either directly or serves as a source of hypochloride. It is formed in the presence of chloride and myeloperoxidase, an enzyme secreted by polymorphonuclear leukocytes (123). The glomerular toxicity of H_2O_2 has been directly demonstrated in studies of intraarterial infusion of H_2O_2, which caused derangements in glomerular permselectivity without structural or hemodynamic changes of the glomerulus. H_2O_2-induced proteinuria was inhibited by pretreatment with catalase or deferroxamine, suggesting that iron-dependent metabolites of hydrogen peroxide mediate the effects of H_2O_2 (124). In more physiologic experimental setings overproduction of ROS has been detected in several glomerular diseases, including puromycin nephrosis (125), Heyman nephritis, a model of membranous nephropathy (126), and the Mpv 17 (–/–) mouse, a model for steroid-resistant focal segmental sclerosis (127). In these glomerular diseases, pretreatment of animals with ROS scavengers prevented foot process effacement and proteinuria (125,127). In puromycin nephrosis, phosphatidylcholine bound superoxide dismutase decreased proteinuria to the control level, improved podocyte density, and stabilized alpha 3-integrin expression (128). In biopsies of patients with membranous nephropathy, Grone et al. demonstrated oxidatively modified proteins in podocytes, mesangial cells, and basal membranes (129). In addition, an increase in glomerular xanthine oxidase activity due to a conversion of xanthine dehydrogenase to the oxidase form has been shown to be responsible for ROS production (130). Podocytes seem to be not only the target but also to be the source of ROS. In Heymann nephritis in rats sublytic complement C5b-9 attack complex on podocytes can cause upregulation and membrane translocation of the NADPH oxidoreductase enzyme complex. ROS are produced locally, reach the GBM matrix, initiate lipid peroxidation, and result in subsequent degradation of GBM collagen IV, leading to proteinuria (131).

Podocyte depletion is a crucial hallmark of glomerulosclerosis, considered a central problem in the progression of renal diseases (132). Experimentally sequential administration of PAN reduces glomerular podocyte numbers, most likely via apoptosis. The segment of the glomerulus lacking podocytes then develops glomerulosclerosis (70).

Recently, the molecular pathogenesis of membranous nephropathy could be defined for a group of patients with perinatal membranous GN (MGN). This study for the first time elegantly demonstrated, that the principle of the Heyman nephritis model can indeed be applied to human membranous nephritis. In Heyman's nephritis injection of antibodies directed against megalin, which is found on the sole of the rat podocyte foot process, causes the typical histologic picture of MGN (133). In humans, however, megalin is not expressed in podocytes and the antigen of MGN remained elusive. A case of perinatal MGN allowed the identification of neutral endopeptidase (NEP) as the podocyte antigen for immuncomplex formation. In a series of studies, the generation of anti-NEP antibodies was demonstrated in NEP deficient mothers, after immunization against the placental expressed fetal antigen. The transplacental transmission of maternal anti-NEP1 antibodies caused the typical histological and clinical picture of MGN, with subepithelial deposition along the GBM and nephrotic range proteinuria in the newborn. With the disappearance of the maternal antibodies after birth, all patients identified underwent spontaneous remission of proteinuria (134,135). These findings are a proof of principle, that MGN can be initiated by antibodies against renal antigens in humans. However, in adult-onset MGN no NEP-1 antibodies could be detected in these studies, so that antigens responsible for most cases of human MGN still remain to be determined.

From Podocyte Damage to Progressive Nephron Loss

Classic Focal-Segmental Glomerulosclerosis (FSGS). A sequence of glomerular alterations was established in models with glomerular hypertension and hyperfiltration, from which a working hypothesis for podocyte damage and loss was derived. Alterations of the filtration barrier consist of podocyte cell hypertrophy, foot process effacement, cell body attenuation, pseudocyst formation, cytoplasmic overload with reabsorption droplets, and, finally, detachment from the GBM (136,137). Cell-body attenuation and pseudocyst formation may result directly from mechanical overextension (138). After podocyte loss from a capillary loop, compensatory hypertrophy of the remaining cells results in further acceleration of podocyte damage. If podocyte loss per glomerulus reaches a threshold, progression to segmental or global glomerulosclerosis follows (70).

Collapsing FSGS. In HIV-infected patients, a distinctly different kind of glomerulopathy is found with proliferation of dedifferentiated podocytes and collapse of the glomerular tuft, termed collapsing glomerulosclerosis (139). The dedifferentiated podocytes have lost the foot process architecture including most components of the cytoskeleton, the expression of several podocyte-specific proteins including WT-1, and synaptopodin (140,141). Podocyte proliferation can obstruct the entire Bowman's space and this is paralleled by a collapse of the glomerular tuft, resulting in a "collapsing" glomerulosclerosis.

The glomerular damage can progress to loss of the respective nephron by damage to the glomerulus itself and the downstream tubular system (see below).

In summary, podocytes are, as a consequence of their highly specialized function, the most vulnerable element of the filtration barrier. They can be injured by a wide variety of mechanisms, ranging from genetic defects to soluble mediators (some of them as yet to be determined), ICs, mechanical stretch, or

complement activation. Oxidative stress appears to be a common denominator for many types of podocyte damage. Interference with any of the three essential functional elements of the podocyte (slit diaphragm, the podocyte cytoskeleton, and the cell-matrix interaction), results in proteinuria, a key progression factor of tubulo-interstitial damage in animal models and human disease.

Glomerular Injury Due to Deposition of Immunoglobulins

The Thy 1.1 Nephropathy as a Model of Glomerular Injury and Repair. One of the most commonly studied rodent models of glomerular injury and repair is the Thy 1.1 nephropathy in rats. Only in rats is the Thy 1.1 antigen restricted to mesangial cells. Therefore the injection of an antibody against this antigen results in rats in complement mediated mesangial cell lysis. The mesangiolytic phase of anti-Thy 1.1 nephropathy is complement dependent, and therefore complement depletion prevents the injury process. Consistently, the blockade of the complement inhibitor CRRY increased the severity of glomerular injury. The subsequent repair process involves a phase with increased mesangial cell numbers ("proliferative GN"). The hypercellularity is due to proliferation of mesangial cells, the recruitment of bone marrow derived mesangial cell precursors, and inflammatory cell influx. As long as the scaffolding, that is the basement membrane, is preserved this results in complete healing, without loss of function or glomerular scarring. Increased cell numbers are cleared in part via an increased rate of apoptosis.

Platelet derived growth factors have received a lot of attention in the Thy 1.1 model. The PDGF family consists of dimers of PDGF-A chain and-B chain (forming PDGF AA, AB, and BB). PDGF-A binds to PDGFRα chain, while PDGF-B binds both PDGFRα and PDGFRβ chains (forming the receptor dimers). Two additional chains PDGF-C and PDGF-D (forming PDGF CC and PDGF DD) have recently been described. PDGF-B is essential for the development of the glomerulus as both PDGF-B and PDGFRβ deficient mice do not form glomerular tufts as they lack mesangial cells and die perinatally (142,143). PDGFRβ is constitutively expressed by mesangial cells, and activation through PDGF BB leads to cell proliferation and increased production of extracellular matrix, both *in vitro* and *in vivo* (144). Antagonizing PDGF BB in the Thy 1.1 model using various approaches reduced mesangial cell accumulation and matrix expansion. Furthermore PDGF seems to be involved in the recruitment of bone marrow progenitor cells into the glomerulus during Thy 1.1 nephropathy (145–147).

During Thy 1.1 nephropathy vascular endothelial growth factor VEGF is expressed by glomerular epithelial cells, mesangial cells, and infiltrating leukocytes. Treatment with VEGF significantly enhanced endothelial cell proliferation and capillary repair by increasing endothelial cell proliferation and the number of glomerular capillaries (148). Using morphologic methods, a role for capillary growth (nonsprouting angiogenesis) has been suggested to be important in the recovery phase (149).

Induction of various chemokines (e.g., CCL2/MCP-1) has been described, which correlates with the recruitment of macrophages during Thy 1.1 nephropathy. Treatment with chemokine blocking agents reduced the macrophage influx.

During the Thy 1.1 nephropathy a co-activation of the arachidonic acid cyclooxygenase pathway synthesis of prostaglandins (PG) and thromboxane (mainly TXA_2) and of lipoxygenase pathways toward synthesis of hydroxyeicosatetraenoic acids (HETEs) and leukotrienes (LTs) has been demonstrated. Blockade of TXA_2 lead to increased production of prostaglandins with beneficial effects, the blockade of TXB_2 prevented the fall in GFR (150).

An increased rate of apoptosis clears the increased cell number and finally results in resolution of the injury process (151).

In summary the "one-shot" Thy 1.1 injection into rats is a good model of glomerular injury with full repair. Accordingly the predictions outlined in the general part are fulfilled in this model: with complement mediated cell lysis, generation of the various mediators, activation and proliferation of mesangial cells, activation of adhesion molecules, monocyte influx, matrix remodelling and cellular repopulation (due to space limitations only some of which have been described above, for further details refer to (151–153)). Unfortunately, the Thy 1.1 model does not reflect any particular human disease, perhaps with the exception of mesangiolysis that can sometimes be seen during thrombotic microangiopathy. The healing phase of Thy 1.1 injury with mesangial expansion, though self-limited in the model, may serve to some extent as a model of mesangial proliferative diseases, such as IgA nephropathy.

General Mechanisms of Immune-Injury to Glomeruli in Humans. The etiologic agents in human glomerulonephritis are mainly unknown with a few exceptions (e.g., beta streptococci in poststreptococcal GN, or hepatitis C virus in cryoglobulinemic membranoproliferative glomerulonephritis). In the most common forms of human glomerular diseases the primary insult to glomeruli is the deposition of ICs (either formed *in situ* or deposited from the circulation). Antigens for *in situ* binding of immunoglobulins can be intrinsic glomerular proteins (e.g. anti-GBM), or antigens planted in the glomerulus. Self (e.g., DNA-nucleosome complexes in systemic lupus), and non-self antigens might become localized to glomeruli through charge affinity, passive trapping, or local precipitation (as it is likely in hepatitis C associated cryoglobulinemia).

Immune complexes (Ics) can be found in the mesangium (as in IgA nephropathy, lupus nephritis, and postinfectious GN), along the glomerular basement membrane (as in anti-GBM disease), in the subendothelium (in systemic lupus erythematodes [SLE], membranoproliferative glomerulonephritis [MPGN]), and in the subepithelial area (as in membranous nephropathy, and postinfectious GN) (154,155). The response to injury depends on the site of IC deposition, for example, mesangial deposits mainly lead to mesangial cell proliferation, and subendothelial deposits predominantly result in inflammatory cell recruitment. Only when circulating inflammatory cells can be activated through the contact with immunoglobulins, or with soluble products released by intrinsic renal cells, a strong inflammatory response can result. This is the case in subendothelial, mesangial, or membrane deposits. ICs on the outer surface of the glomerular basement membrane (as in membranous nephropathy) do not seem to provoke glomerular inflammation. It is generally thought that circulating cells do not get in contact with the site of subepithelial immunoglobulin and complement deposition. Besides the site of deposition the response depends on the properties of the deposited immunoglobulins with more severe injury caused by complement-fixing IgG subtypes (e.g., IgG1 and IgG3). Additionally, the *in situ* formation with complement activation may cause more severe injury as compared to trapped ICs.

Glomerular Injury Mediated by ICs. ICs can initiate multiple inflammatory pathways in intrinsic renal cells (e.g., the release of cytokines, growth factors, chemokines, prostaglandins etc.), leading to inflammatory cell recruitment. Disease models, which have been used most extensively for studies on immune mediated glomerular injury (IC-GN) are rodent models of nephrotoxic nephritis (NTN), repeated injections of foreign antigens (e.g., horse appoferritin injection into mice) and lupus-prone mouse strains (e.g., NZBXNZW-F1, MRL-Fas^{lpr}). These models mimic IC-GN in humans to some extent and allow detailed studies on the response to injury.

The Role of Complement in IC-GN. Complement activation is a main factor mediating tissue injury after IC deposition (37). The activation of complement can lead to activation of intrinsic glomerular cells (endothelial cells, mesangial cells and podocytes) through formation of sublytic doses of the C5-9 membrane attack complex. Additionally, it can be involved in leukocyte recruitment through the release of chemotactic C5a and C3a. Sublytic quantities of C5b-9 result in cell activation with production of oxidants, chemokines, proteases, and up-regulation of adhesion molecules (which might be more important for cell recruitment than C5a/C3a) (156).

NTN was significantly ameliorated in mice transgenic for the complement inhibitor CRRY (a rodent homolog of the human complement receptor 1) as well as in mice deficient in the complement components C3 and C4. Treatment of MRL-Fas^{lpr} mice with recombinant CRRY reduced glomerular injury and proteinuria (157). Although glomerular injury was reduced by complement inhibition, circulating IC levels were markedly higher in complement-inhibited animals, indicating a role of complement in immune complex handling. A monoclonal antibody against C5 ameliorated glomerulonephritis and prolonged survival in lupus mice (NZB/W-F1) (158). Deposition of complement C3 has long been used as a diagnostic marker in renal biopsies in human glomerular diseases (e.g., lupus nephritis, MPGN, and postinfectious GN).

It should be mentioned that it becomes increasingly apparent that the deposition of ICs or complement are not sufficient to cause injury when animals are deficient in downstream pathways. For example, deficiency in IL-12 protected from glomerular injury in a lupus nephritis model although the amount of deposited ICs was the same as in wild-type animals (159). Similar results have been obtained in mice deficient in CCL2/MCP-1, CCR2, IFN-γ, and Fc receptors (160).

The Role of Cytokines in IC-GN. In the general part some of the most important cytokines, chemokines and growth factors involved in renal inflammation have been introduced. A comprehensive review of the existing literature goes beyond the scope of this chapter, but some aspects should be discussed herein. The most widely studied cytokines are proinflammatory substances (IFN-γ, IL-1, TNF-α, IL-6), anti-inflammatory cytokines (IL-4, IL-10, IL-13) and growth factors (e.g., PDGF, see under The Thy 1.1 Nephropathy as a Model of Glomerular Injury and Repair).

In response to ICs mesangial cells in culture produce a variety of proinflammatory cytokines including IL-1, IL-6, and TNF-α. These mediators can activate glomerular cells in an autocrine (e.g., mesangial cells), as well as a paracrine way (e.g., endothelial cells produce IL-6 in response to IL-1 and TNF-α). Gene profiling by oligonucleotide microarrays in NTN demonstrated an early induction of IL-1 and IL-6, TNF-α was increased during the entire disease process (161).

Type I interferons (IFN), consisting of IFN-α and -β, have antiviral activity, whereas the Type II interferon (IFN-γ), has a multitude of immunoregulatory functions (162). The production of IFN-γ by T cells can be triggered via T cell receptor activation. IFN-γ activates macrophages, and increases macrophage cytotoxicity by increasing oxidative burst and the production of other oxidants such as nitric oxide (162). IFN-γ is a key cytokine that controls antibody production. It plays a key role in lupus models (MRL/*lpr* mice, NZB × NZW mice) which has been demonstrated by increased expression during the disease course, exacerbation through the treatment with IFN-γ, and amelioration of disease by the inactivation of IFN-γ (163,164). Studies of IFN-γ deficient mice demonstrated that this cytokine is necessary for autoantibody production in MRL/*lpr* mice. IFN-$\gamma^{+/-}$ MRL/*lpr* mice are protected from glomerulonephritis despite intact autoantibody production and IC deposition (165). By transfer of IFN-$\gamma^{+/+}$ monocyte/macrophages into IFN-$\gamma^{/-}$ mice it could be demonstrated that IFN-γ production by infiltrating macrophages is responsible for adhesion molecule up-regulation, macrophage accumulation, and renal inflammation, in the absence of autoantibody deposits (163).

In an anti-GBM model induced by the immunization with alpha3-alpha5(IV)NC1 heterodimers IFN-$\gamma^{-/-}$ mice developed a more severe form of glomerular injury as compared to the wild-type counterparts. This implies that depending on the context of the immune response IFN-γ has different effects (166).

Using the lupus model of MRL-Fas^{lpr} in mice deficient in type I IFN receptor, type II receptor, or both, demonstrated an amelioration of the disease in IFN II receptor deficient animals, but an unexpected worsening in IFN I receptor deficient animals. Double knockouts demonstrated a phenotype intermediate between wild-type and IFN RII deficient mice (167). In renal biopsies from patients with lupus nephritis IFN-γ expression correlated with the activity index (168). Therefore IFN-γ seems to play a key role in IC mediated diseases, both mediating antibody production as well as local inflammation.

Besides resident cells TNF-α is released by infiltrating monocytes/macrophages in response to ICs. TNF-α can reduce glomerular blood flow and filtration rate, and propagates formation of capillary thrombi and recruitment of inflammatory cells (169). The expression can be amplified by IL-1. Bioactivity of TNF might be regulated through soluble TNF-binding proteins (e.g., TNF receptor released by proteolytic cleavage). Administration of soluble TNF receptors to rats with NTN reduced glomerular injury and prevented crescent formation.

IL-1β is a pluripotent, proinflammatory cytokine that orchestrates inflammatory cells, augments T-cell responses, increases expression of vascular adhesion molecules, and induces proinflammatory cytokines, and chemokines (170). The resulting inflammatory reaction is dependent on the balance between IL-1 and the IL-1 receptor antagonist (171,172). Treatment with anti IL-1β antibodies decreased proteinuria in NTN. Bone marrow tranplantation from IL-1β knockout animals into wild-type mice clearly demonstrated that leukocyte- and not renal cell–derived IL-1β was involved in the induction of TNF and in glomerular injury. Absence of IL-1RI on leukocytes had no beneficial effect, whereas the absence of renal cell IL-1RI decreased glomerular TNF expression and glomerular injury (173).

It is thought that the inflammatory response after activation of intrinsic glomerular cells is switched off via the induction of antiinflammatory cytokines (e.g., IL-4, IL-10, IL-13). Treatment with IL-4 reduced albuminuria, and macrophage recruitment in NTN. The expression of IL-4 messenger RNA (mRNA) progressively increased during NTN in rats (172). Consistently, NTN induced in IL-4 deficient mice lead to increased macrophage recruitment and decreased renal function as compared to wild-type animals (174). The beneficial effect of IL-4 might be mediated in part by a downregulation of proinflammatory mediators by macrophages. Similar to the situation described for IL-4, the treatment with IL-10 was of significant benefit in NTN and genetic deficiency of IL-10 increased disease severity of NTN. IL-10 immunoreactivity was also significantly increased in renal biopsies from patients with IgA nephropathy. Treatment with FK506 of lupus mice (NZBXNZW-F1) resulted in a suppression of proinflammatory IL-2, IFN-γ, while the anti-inflammatory IL-4 and IL-10 cytokines were not changed significantly. Beneficial effects were demonstrated by increased survival, decreased IC deposits, and decreased proteinuria (175).

These are just some recent examples of how the roles of cytokines (pro vs. anti-inflammatory) and their sources (intrisic cells vs. infiltrating cells) become increasingly apparent through

the use of genetically engineered mice (for further details on cytokines in glomerular injury see (171,176–181)).

The Role of Chemokines and Chemokine Receptors in IC-GN. The role of chemokines and chemokine receptors in renal disease has extensively been reviewed (24,182–184). Immunoglobulins or ICs can stimulate chemokine production by intrinsic glomerular cells via Fc receptors. Chemokine release by intrinsic glomerular cells is an important early event in the recruitment of inflammatory cells to the glomerular tuft (24). In mouse models of lupus nephritis the induction of chemokines appears earlier in the time course than the inflammatory cell recruitment, and in reversible models the reduction of inflammatory cells follows downregulation of chemokine expression (185). A variety of chemokines (e.g., CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β, CCL5/RANTES, CCL7/MCP-3, CCL22/MDC, CXCL1/MIP-2, CXCL4/PF4, CCL21/TCA3, CX3CL1/fractalkine, CXCL10/IP-10) are upregulated in nephritic glomeruli, both in rodents (e.g., NTN, appoferritin induced IC-GN), as well as in human glomerulonephritis (e.g., lupus nephritis) (24,186). Deficiency in either CCL2/MCP-1 or the corresponding receptor CCR2 ameliorates renal injury and proteinuria in the MRL-Fas^{lpr} lupus model, although the amount of glomerular ICs is not different to wild-type mice (160). A large number of studies illustrate a potential benefit of chemokine blockade (e.g., blockade of CCL2/MCP-1, CCL22/MDC (186) or growth-related oncogene protein-alpha (GRO-α) reduced the glomerular macrophage accumulation), although some studies also demonstrated negative results (implying regulatory roles during some disease phases) (187). In human lupus nephritis the accumulation of macrophages in glomeruli is associated with CCL2/MCP-1 expression in this compartment and with an increased excretion of CCL2/MCP-1 in the urine. Furthermore, a CCL2/MCP-1 polymorphism predisposes to the development of SLE and these patients are at higher risk of developing lupus nephritis (188).

The Role of Prostaglandins in IC-GN. Proinflammatory cytokines (e.g., IL-1β, TNF-α) and even IC can induce the release of PGs (e.g., PGE2) by mesangial cells. Induction of COX-2 as well as PLA2 has been demonstrated *in vitro*. In various models (e.g., NZB/W lupus, NTN) a protective effect of PGE1 and PGI2 (probably due to the vasodilatory, and antiplatelet effects as well as suppressive effects on cyto- and chemokine generation) has been suggested. The expression of adhesion molecules is also influenced by prostaglandins. For example, intraglomerular expression of ICAM-1 was suppressed by PGI2 in NTN in rats, which resulted in decreased macrophage recruitment. Furthermore, PGI2 is involved in ECM turnover through the regulation of metalloproteinases like MMP-9 and MMP-2 (by mesangial cells). On the other hand TXA_2 with its pro-aggregatory, vasoconstrictive and chemotactic actions is thought to have a negative impact. TXA_2 was significantly increased in glomeruli isolated from NTN mice and it retards the clearance of aggregated proteins in nephritic glomeruli via a decreased uptake by mesangial cells.

Cyclooxygenase (COX) exists in two isoforms, COX-1 and COX-2, with specific functions in different segments of the nephron (189). COX-2 deficient mice develop fatal nephropathy, which implies that COX-2 plays an important role during nephrogenesis (190). An increased production of prostaglandins and thromboxane has been demonstrated in human glomerulonephritis (e.g., lupus nephritis). Upregulation of COX-2 was found in human glomeruli during active lupus nephritis, as compared to normal controls. The expression was localized mainly to glomerular macrophages and to a lesser extent to mesangial cells. In contrast the expression of COX-1 did not change during lupus nephritis.

In summary, expression of cyclooxygenase (particularly COX-2) is induced during glomerular injury and a complex interaction between the prostaglandin system and other pathways like the chemokines, angiotensin and the adhesion molecules exists during glomerular inflammation (reviewed in [24,191,192]).

The Role of Leukotrines and Lipoxins in IC-GN. Both leukotrienes (LTs) and lipoxins (LXs) are lipoxygenase products of arachidonic acid. While LTs are proinflammatory, LXs antagonize many leukotriene effects, attenuate neutrophil recruitment, and might promote resolution of inflammation. Mesangial cells proliferate and contract in response to LT C4 and LT D4 (193,194). Intrarenal infusion of LT B4 into rats with NTN lead to increased polymorphonuclear cell recruitment and decreased GFR (195). Treatment of rats with NTN with an LT B4 receptor antagonist reduced proteinuria, glomerular monocytes/macrophage accumulation, and the number of crescents (196). A 5-lipoxygenase inhibitor given to rats with NTN also resulted in a significant reduction in proteinuria (197).

The lipoxin LX A4 inhibits both PDGF and LT D4 stimulated proliferation of mesangial cells (193). LXs are produced in large amounts during NTN. A decreased production of LXs (LX A4) was found during NTN in p-selectin deficient mice, resulting in an increased recruitment of inflammatory cells. It has been proposed that lipoxins might be important during the resolution phase of glomerular inflammation.

The Role of PAF in IC-GN. PAF is produced in large amounts by cytokine activated mesangial cells. Exposure of mesangial cells to PAF resulted in the expression of TGF-β, leading to an induction of fibronectin and type IV collagen expression (198). In the lupus model of NZB/W mice urinary PAF excretion correlated with proteinuria and a PAF receptor antagonist delayed the onset of proteinuria and prolonged survival (199). In NTN in rats, treatment with PAF antagonists also reduced urinary protein excretion, but did not result in a histopathological improvement. Thus PAF may be part of the proinflammatory mediators in IC-GN.

The Role of Adhesion Molecules in IC-GN. P-selectin is present on platelets and endothelial cells. It is not expressed by normal glomerular endothelium, but is upregulated during glomerular inflammation (in animal models and humans) (200). Blockade of P-selectin during NTN in mice reduced glomerular neutrophil influx (201). Administration of a P- and L-selectin antagonist (fucoidan F7) on the other hand did not change the number of glomerular leukocytes (7). The genetic deficiency in P-selectin resulted in an increased number of glomerular neutrophils (likely due to decreased lipoxin expression) indicating the complexity of the system. E-selectin although not expressed by normal glomerular endothelium has been described to be upregulated in humans with acute GN, lupus nephritis and IgA nephropathy. Little is known about the corresponding mucin ligands. ICAM-1 is only weakly expressed in the normal glomerulus (by endothelial and mesangial cells), but is induced during NTN as well as in human IgA nephropathy and human proliferative lupus nephritis. ICAM-1 blockade (or blockade of the counterreceptor LFA-1) reduced macrophage accumulation in NTN in rats (7). It is important to note that ICAM-1 can also be expressed in the absence of a glomerular infiltrate, for example, during minimal change and membranous disease. Thus expression of the adhesion molecule alone is insufficient for glomerular leukocyte recruitment, as to be expected.

VCAM-1 expression has not been detected on normal human glomerular endothelium, but has been shown to be present in glomeruli from MRL-Fas^{lpr} mice. In rats with NTN the

blockade of VCAM-1 or the ligand VLA-4 had no beneficial effect. Further studies are clearly needed to define the role of the different combinations of adhesion molecules and soluble mediators in the different renal compartments.

HSPGs are expressed on both endothelial cells as well as in the ECM and modulate inflammatory cell behaviour. During human lupus nephritis a decreased staining of glomerular GBM heparan sulfate correlated with Ig deposits and albuminuria, indicating a role in proteinuria (e.g., via decrease in sulfate-related anionic sites). The significance of the HSPG for chemokine and cytokine binding and presentation was discussed above in the general introduction. Inflammatory cells release elastase and heparanase which might mediate the decrease in heparan sulfate staining in glomeruli with proliferative GN (66). Urinary excretion of GAGs and heparan sulfate was found to be increased in patients with lupus nephritis (202). The exploration of the GAG system in GN is currently under investigation (for details see [66]).

The Role of Inflammatory Cells in IC-GN. As described above, recruitment of inflammatory cells depends on the interplay between induction and activation of adhesion molecules by proinflammatory cytokines and chemotactic factors. Neutrophils and macrophages are the predominant intraglomerular cell types in human glomerulonephritis. Neutrophil recruitment is thought to be an early and short lived (days) event, for example, in postinfectious GN (203). The appearance of macrophages follows closely and in an overlapping manner but persists unless remission ensues. Having reached the site of IC deposition, neutrophils and macrophages ingest ICs, and undergo a respiratory burst. Furthermore, activated neutrophils release serine proteases, such as elastase and cathepsin G, which by degrading the extracellular scaffold of the glomerular tuft, may lead to irreversible injury. Consistent with this scheme elastase inhibitors suppressed glomerular injury in NTN.

Monocytes/macrophages are a prominent feature in the glomerular tuft of lupus nephritis and MPGN (particularly in association with cryoglobulins). An association between glomerular macrophages and chemokine expression (e.g., CCL2/MCP-1) has been described in mouse and human. Macrophages can be both targets as well as the source for chemokines (leading to secondary waves of cell recruitment). Using bone marrow transplantation experiments it could be demonstrated that cytokines that activate macrophages come from different sources, for example, TNF-α is mainly produced by intrinsic renal cells, whereas IFN-γ is produced by intrinsic as well as infiltrating cells. The increase in number of macrophages in glomeruli results from recruitment, and to some extent also local proliferation (also demonstrated in human biopsies). In NTN macrophages mediate glomerular injury while depletion of macrophages (e.g., by radiation, by antisera), reduces injury, and results in decreased proteinuria. Macrophage effector mechanisms involved in glomerular injury are the production of oxygen radicals, PAF, coagulation products, and nitric oxide (204). Macrophages release various cytokines which lead to mesangial cell proliferation, for example, PDGF, FGF-2, and IL-1 (205). Still little is known about the macrophage phenotype during different phases of glomerular diseases. It is important to recall that the major goal of macrophages during glomerular inflammation is the removal of injurious products such as ICs. Interference with macrophage infiltration during an early phase of disease induction may therefore also have an untoward downside.

In summary, during recent years the various pathways involved in IC-GN have been described in further detail. Many of the injury processes mentioned in the general part of this chapter have been demonstrated in models of glomerular injury. Intrinsic glomerular cells (mesangial cells, endothelial cells), and/or their adjacent basement membrane or matrix are the *primary* site of the immune-deposits (Fig. 57-4). The deposition of ICs in glomeruli results in intrinsic glomerular cell activation (e.g., via Fc receptors and complement). The cells proliferate, and produce inflammatory mediators and extracellular matrix. Additionally, ICs might contain antigens or nucleic acids (RNA, DNA) from infectious particles, which could activate intrinsic renal cells via pattern recognition receptors (e.g., the toll-like receptors, TLRs) (155). *Secondary* injury results from free radicals, soluble mediators, and proteases released by recruited inflammatory cells (mainly neutrophils and macrophages). The soluble factors, for example, cytokines, growth factors, chemokines, eicosanoids, complement components further propagate the recruitment of inflammatory cells. They are filtered into the ultrafiltrate because of loss of permselectivity and thereby reach tubular cells; they also leave the glomerulus via the bloodstream to reach the peritubular vessels. Thus the inflammatory mediators generated in the glomerulus would activate podocytes and tubular epithelial cells via the ultrafiltrate and the peritubular circulation via the bloodstream thereby propagating tubulo-interstitial injury during a primary glomerular inflammation.

The Tubulointerstitium During Primary Insult and Progressive Disease

Significance of Fibrosis. Chronic progressive renal disease is characterized by the triad of glomerulosclerosis, tubular atrophy, and tubulointerstitial fibrosis. Fibrosis is the eventual consequence of persistent inflammation. The involvement of the tubulointerstitium may be at least as important as that of the glomeruli for the progression and hence prognosis of any renal disease. This may not be too surprising in view of the interconnection between glomeruli and tubules (see Introduction) and the fact that tubules and interstitium occupy more than 90% of kidney volume (206).

The exact mechanisms whereby increasing fibrosis leads to deterioration of renal function remain to be clarified. A number of theories exist, including the proposals that loss of postglomerular capillaries (207), formation of nonfiltering atubular glomeruli (208), and tubular atrophy (209) contribute to the loss of renal function. A morphometric analysis by Bohle et al. of biopsies from patients with a variable degree of impaired renal function demonstrated a robust correlation between reduced number and area of postglomerular capillaries and the rise in the serum creatinine (210). Recently Kang et al. confirmed a loss of microvasculature in progressive renal disease (207). This loss may be due to sclerosis of glomeruli with loss of downstream circulation, a lack of nutrients and growth factors, such as VEGF, and a predominance of factors such as thrombospondin-1 that promote endothelial cell apoptosis.

A second mechanism by which fibrosis may result in decreased renal function is the formation of atubular glomeruli. In 1939, Oliver described some glomeruli without attached tubules in patients with glomerulopathies, leading him to coin the term *atubular glomeruli* (211). Since no ultrafiltration can occur in atubular glomeruli, overall filtration rate is reduced (212). Marcussen and co-workers expanded this observation to document that atubular glomeruli are characterized by a reduced number of glomerular capillaries and a smaller size than their normal counterparts (212). The number of atubular glomeruli was lowest in diabetic nephropathy (8.8%) and highest in chronic pyelonephritis (50%) and renal artery stenosis (52%) (213–215). All studies on atubular glomeruli concurred that there was a good correlation between the number of glomeruli deprived of tubular connection and the degree of interstitial fibrosis.

Atrophic tubules may be the result of decreased ultrafiltrate containing various nutrients, hypoxia, and tubular toxicity by abnormal constituents of the ultrafiltrate such as free iron

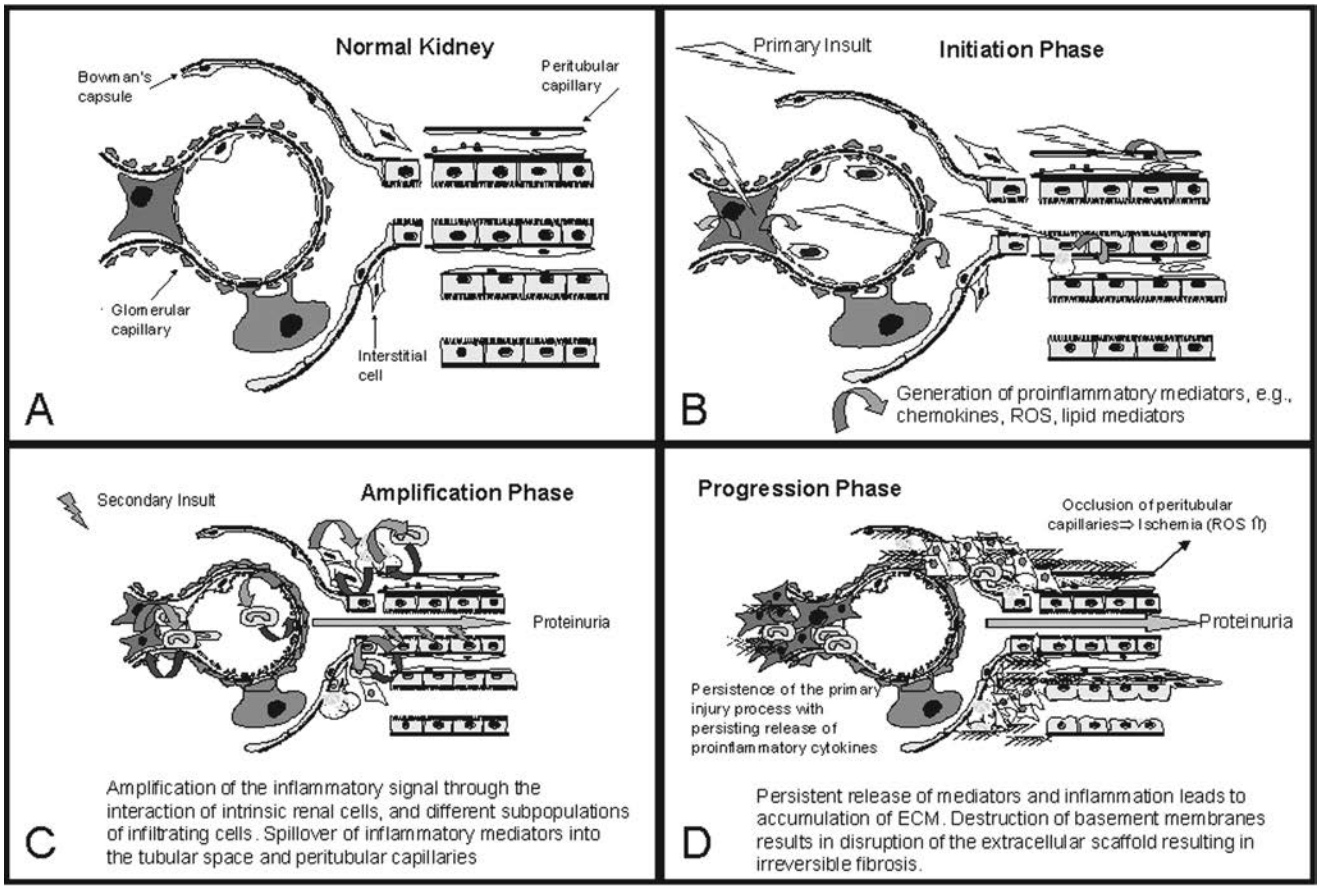

FIGURE 57-4. Hypothetical scheme of progressive renal diseases (Modified from: Segerer S, Nelson PJ, Schlondorff D. Chemokines, chemokine receptors, and renal disease: from basic science to pathophysiologic and therapeutic studies. *J Am Soc Nephrol* 2000;11:152.)

(released from transferrin–iron complexes) or even proteinuria itself (216–219). Apoptosis was shown to contribute directly to the decline in renal function in polycystic kidney disease (220), in a transgenic mouse model of interstitial fibrosis (221) as well as in a mouse model of chronic toxic nephropathy (222).

Renal Fibrogenesis. The development of tubulointerstitial fibrosis follows the general scheme outlined in the introduction to tissue injury and repair (see under A General Scheme of Tissue injury and Repair). It involves the generation of soluble mediators at the site of tissue injury, the extravasation of leukocytes across the endothelium, and subsequent secretion of various mediators by infiltrating leukocytes and tubulointerstitial cells, which results in the activation of profibrotic cells. The latter may then, through local disturbance in anatomical, hemodynamic and metabolic balances, initiate a vicious cycle of cell stress leading to generation of proinflammatory and profibrotic mediators, leukocyte infiltration and eventually fibrosis.

Induction and Development of the Inflammatory Response. Essentially all forms of interstitial fibrosis are associated with an inflammatory infiltrate. What are the factors involved in the formation of this interstitial infiltrate in tubulointerstitial and glomerular diseases? While a small number of interstitial macrophages may originate from *in situ* proliferation of resident interstitial macrophages, most of these cells migrate from the circulation through the postcapillary venules and peritubular capillaries into the interstitial space following gradients of chemoattractant and chemokinetic molecules as described above. All types of renal cells can generate such soluble mediators, and especially chemokines, when appropriately stressed (24). In tubulointerstitial fibrosis, stimuli for tubular cells include: hypoxia, ischemia, infectious agents, exogenous (e.g., antibiotics, xenobiotics) and endogenous toxins (e.g. altered lipids, high glucose, paraproteins) or genetic factors, for example, in cystic renal diseases. In glomerular diseases, the loss of permselectivity exposes tubular cells to proteins normally not filtered, such as complement components, proinflammatory cytokines, and albumin in high concentrations. All of the above have been shown to induce chemokine production by tubular epithelial cells (24). The significance of proteinuria for progressive tubulointerstitial fibrosis derives from clinical studies showing that patients with a higher degree of proteinuria have a more rapid decline in renal function (223). Table 57-3 gives an overview of the factors involved. The majority of these has been discussed in the general introduction and in more detail elsewhere (224).

Recently, Johnson et al. suggested that uric acid may also play an important role in the promotion of interstitial infiltrates and progression of disease (225). Uric acid may be proinflammatory due to stimulation of synthesis of CCL2/MCP-1 in vascular smooth muscle cells (226) and of IL-1β, IL-6, and TNF-α in mononuclear cells (227). In the remnant kidney model, rats with experimentally induced hyperuricemia had higher scores of glomerulosclerosis and interstitial fibrosis compared to the normouricemic remnant kidney controls (207).

The potential role of the tubular epithelial cell in the formation of secondary interstitial inflammation is reviewed in detail elsewhere (224). In addition to epithelial cells, involvement

TABLE 57-3

FACTORS INVOLVED IN THE FORMATION OF TUBULOINTERSTITIAL INFILTRATES

Proteinuria
Immune deposits
Chemokines
Cytokines
Calcium phosphate
Metabolic acidosis
Uric acid
Lipids
Hypoxia
Reactive oxygen species

of the endothelium has to be considered in mediating the interstitial infiltrate since all infiltrating cells have to transmigrate this barrier (see under Leukocyte-Endothelial Transmigration). However, knowledge about endothelial changes during the early phase of fibrogenesis is surprisingly limited. In acute renal failure de Greef et al. recently observed that endothelial cells of the vasa recta displayed *de novo* expression of the co-stimulatory molecule B7-1 starting 2 hours after reperfusion. Blocking this molecule by neutralizing antibodies completely prevented reperfusion injury (228). Similar observations have been made with other adhesion molecules. It remains to be established how these findings relate to renal fibrogenesis in chronic kidney disease.

The Inflammatory Infiltrate. This phase is characterized mainly by the influx of mononuclear cells. In almost all forms of primary or secondary glomerular disease, interstitial infiltrates have been described (229). Infiltrating mononuclear cells are composed of monocytes/macrophages and lymphocytes, particularly T-lymphocytes (230). The degree of interstitial infiltrate correlates with renal function in several forms of immune-mediated renal disease, including lupus nephritis and membranous glomerulonephritis (231). The number of tissue CD4-positive T cells in particular displays a close correlation with renal function (230) as does the number of CD3 T cells bearing the chemokine receptors CCR5 and CXCR3 (232,233).

While a role for macrophages in tissue damage, including renal diseases, has been established, a correlation of CD14 positive monocytes/macrophages with histological damage is less clear (234). Of interest are observations by Kondo et al. showing a good correlation between the number of infiltrating mast cells and the degree of interstitial fibrosis, pointing to the mast cell as a potentially underestimated player in progressive fibrosis (235).

Profibrotic Cytokines. The phase of inflammatory matrix synthesis is characterized by the stimulation of fibroblasts by cytokines from infiltrating inflammatory cells as well as from resident renal cells. Table 57-4 gives an overview over the most important profibrotic cytokines, which will be discussed briefly (see Cytokines).

One of the most relevant cytokines from a clinical point of view is angiotensin II. A wealth of data supports a beneficial role of blocking the renin-angiotensin system in the progression of experimental and human renal disease (summarized in [206]). Mice deficient in angiotensin II or its type I receptor develop less severe interstitial scarring compared to wild-type mice in the unilateral ureteral obstruction (UUO) model (236) and anti-GBM disease (237). Pharmacologic blockade of the angiotensin system decreased TGF-β levels in patients

TABLE 57-4

PROFIBROTIC FACTORS INVOLVED IN RENAL FIBROGENESIS

Angiotensin II
TGF-β1
CTGF
PDGF
FGF-2
EGF
ET-1
Mast cell tryptase

with chronic allograft nephropathy and diabetes mellitus, thus preserving renal function (238,239). Angiotensin II can induce TGF-β synthesis in tubular epithelial cells and fibroblasts. However, at least some of the effects of angiotensin II are independent of TGF-β1 as was recently demonstrated by Ma et al. in mice lacking integrin beta6, which is required for activation of latent TGF-β (240). It induces hypertrophy in tubular epithelial cells, which is at least in part dependent on connective tissue growth factor (CTGF) expression (241). Angiotensin II can induce tubular hypertrophy and collagen expression *in vitro* in interstitial fibroblasts (242) and tubular epithelial cells (243).

TGF-β is currently viewed as the key cytokine in renal fibrogenesis though not all effects in renal fibrogenesis can be tracked to TGF-β (see under Cytokines about the pleotrophic effects of the TGF-β). *In vitro* it mediates chemotaxis for fibroblasts, transition of fibroblasts to myofibroblasts, and the synthesis of extracellular matrix proteins such as fibronectin and collagen type I. However TGF-β also has major functions in immune regulation and induction of apoptosis. Deficiency of TGF-β by genetic manipulation leads to a severe form of autoimmune-like wasting disease. Therefore TGF-β's role has to be viewed in the special circumstances and cannot simply be considered as universally profibrotic.

Evidence for the particular role of TGF-β in renal disease comes from studies using TGF-β transgenic mice, which develop glomerulosclerosis and interstitial fibrosis (244). Three isoforms have been described in humans and at least two have profibrotic effects on cultured rat tubular cells and fibroblats (245). Increased expression of TGF-β1 is documented in numerous models of renal, pulmonary, and hepatic fibrosis (246–252). TGF-β1 is predominantly produced by infiltrating inflammatory mononuclear cells and to some extent also by resident renal cells (see under Cytokines about difficulties in evaluating TGF-β by immunohistology). *In vitro*, TGF-β1 formation can be induced by angiotensin II, ET-1, glucose, insulin-like growth factor-1 (IGF-1), atrial natriuretic factor, PAF, thromboxane, and certain drugs such as cyclosporine A (206). In addition, autoinduction of TGF-β1 may occur. Secreted TGF-β requires extracellular activation to release the active 25 kD dimer (253). This activation is mainly performed by thrombospondin-1, though other mechanisms may play a role as well (254). Thus, upregulation of thrombospondin-1 expression is required in addition to increased TGF-β1 secretion. Active TGF-β binds to the type II receptor, which subsequently activates the type I receptor. The receptor complex then activates cytoplasmic proteins of the SMAD family by phosphorylation (255). Most cells express the TGF-β receptors indicating that receptor availability is not rate-limiting. However, even the active form of TGF-β1 may be modified by binding to extracellular matrix or to individual matrix molecules including the proteoglycans decorin and biglycan. Schaefer et al. found significantly increased interstitial matrix deposition in mice deficient in decorin expression

(256). These findings were extended, when the effects of decorin (and biglycan) were shown to be mediated by induction of fibrillin-1 expression (257).

Effects of TGF-β can also be mediated indirectly by a downstream mediator, that is, CTGF (258). CTGF was originally isolated from endothelial cells. Its expression is upregulated in human interstitial fibrosis, and it may mediate TGF-β induced collagen synthesis (258,259). Furthermore, increased expression has been described in models of diabetic nephropathy and UUO (260,261). Both cytokines may have synergistic effects. In a model of skin fibrosis only combined injections of CTGF and TGF-β1 resulted in persistent scarring (262). The receptor and signaling events induced by CTGF are still unclear and further studies are necessary to define the role of CTGF in renal fibrogenesis.

Very recently, IL-13 has attracted attention as a chemotactic and growth factor for fibroblasts that also promotes collagen type I synthesis (263–265). In models of liver and lung fibrosis the effects of IL-13 were largely independent of TGFβ (33). It remains to be seen, whether this effect of IL-13 also applies to fibrosis in the kidney.

The role of EGF in renal fibrogenesis is still controversial. Whereas Chevalier and co-workers described an attenuation of renal fibrosis in the UUO model after administration of EGF (266), a study by Terzi et al. using transgenic mice expressing a dominant negative form of the EGF receptor came to the opposite conclusion—that EGF was a strong profibrotic cytokine (267). The latter assumption is also supported by some *in vitro* data (268).

Endothelin-1 (ET-1) may also have a role in renal fibrosis. Three different isoforms and two types of receptors have been described. Type A receptors are expressed mainly on vascular smooth muscle cells and type B receptors on endothelial cells. Besides its induction of TGF-β1, ET-1 may directly stimulate matrix synthesis and decrease collagenase activity. Transgenic mice overexpressing human ET-1 did not have hypertension, but displayed severe interstitial fibrosis and tubular cysts (269). Furthermore, addition of ET-1 type A receptor blockers decreased the severity of tubulointerstitial fibrosis in models of chronic transplant nephropathy (270) and lupus nephritis (271).

Fibroblast Proliferation and Activation. Once inflammatory cells have infiltrated the tubulointerstitial space, one begins to see activation and proliferation of fibroblasts. While a number of studies in human glomerular disease describe a good correlation between the number of α-smooth muscle positive interstitial cells and renal function, these studies probably do not sample the universe of the entire fibroblast participation (272). Fibroblast activation is uneven and the pool of fibroblasts engaged in fibrogenesis is heterogenous in relation to their cellular characteristics and biochemical synthetic profiles (273,274). Immunohistochemical profiles in normal human kidney suggest that a subpopulation of interstitial fibroblasts may constitutively express α-smooth muscle actin, as well as the PDGF receptor α-chain (275). For most fibroblasts, activation is required in order to express α-smooth muscle actin, which is normally confined to vascular smooth muscle cells, hence the name myofibroblasts. It is currently unclear if the increase in number of α-smooth muscle actin-positive cells is the result of proliferation of smooth muscle cells or of fibroblasts, constitutively expressing this protein; or of *de novo* expression by formerly non-expressing cells. Furthermore, for perhaps the majority of fibroblasts, activation is not associated with *de novo* expression of α-smooth muscle actin (274). The degree of change in phenotype in the transition from fibroblast to myofibroblast may be underappreciated since expression of α-smooth muscle actin is not the only biochemical change to occur (276). For example, Rodemann and Muller demonstrated that fibroblasts from fibrotic kidneys expressed a number of proteins not detectable in their counterparts derived from normal kidneys (277). Even so-called myofibroblasts may exhibit a higher degree of variability and complexity due to different levels of α-smooth muscle actin expression (278). These studies support the notion that fibroblasts from kidneys with interstitial fibrosis may differ from fibroblasts from normal kidneys. Thus the origin and even the significance of myofibroblasts for excess collagen production, and for interstitial fibrosis remain to be fully clarified.

Based on a classic paper by Cohnheim in 1867 (279) it had been thought that fibroblasts derived from migrating leukocytes. However in 1970 Ross performed a study in rats which indicated that tissue fibroblasts were probably of local origin (280). Recently, the concept of bone marrow derived cells populating the renal interstitium has returned (281). Abe et al. demonstrated that collagen-producing fibrocytes can be derived from a CD14 positive enriched cell population within the blood, confirming Cohnheim's postulate from over 130 years ago (61). A number of studies have confirmed the existence of bone marrow–derived fibrocytic cells within the interstitium (282) and the transition of bone marrow cells to tubular epithelium (283). In fibrosing models of lung disease, recent studies have emphasized the role of circulating cells of hematopoietic origin as the major source of collagen production in pulmonary fibroblasts (59,60,284). These cells were attracted to the lung in response to the chemokine CXCL12/SDF-1. At present, it is unclear if these findings apply to other organs as well.

Evidence using genetically tagged fibroblasts and tubular epithelial cells indicates that fibroblasts derived from bone marrow comprise about 12% of the resident interstitial population in normal murine kidneys (281). During interstitial disease this percentage did not change compared to an increase up to 36% of cells producing extracellular matrix that were derived from tubular epithelial cells by EMT. Though many studies demonstrated the possible contribution of EMT to renal fibrogenesis, fibroblasts may still be the predominant cells producing extracellular matrix in the diseased kidney. Fibroblasts become activated by cytokines, mostly derived from infiltrating macrophages. One of the major mitogens for fibroblasts is PDGF. It is secreted mainly by macrophages (285) and exists in at least four isoforms (286). Application of recombinant PDGF-BB to rats resulted in the influx of macrophages and the formation of myofibroblasts (287). Furthermore, increased expression of the PDGF receptor B subunit has been described in human kidney disease (288). Recently, a novel form of PDGF that is PDGF-C was found in glomerular and interstitial scars in several rat models (289). In human renal biopsies Eitner et al. described upregulated expression of PDGF-C in podocytes and interstitial cells (290). Hudkins et al. injected it into mice to further analyze the functions of PDGF-B, -C, and –D (286). Mice overexpressing PDGF-D after injection of adenovirus constructs showed a severe mesangial proliferation, but only mild changes within the tubulointerstitium and overexpression of the other PDGF isoforms resulted in even more discrete interstitial changes. Final conclusions on the role of the PDGF isoforms in renal fibrosis will require further studies.

Another important mitogen for renal fibroblasts is bFGF-2. Due to different translation start sites, five different isoforms of bFGF or FGF-2 can be distinguished of which only the 18 kD isoform gets secreted. The effects of bFGF are mediated by four different high affinity receptors. Additionally, various heparan sulfate proteoglycans serve as low-affinity receptors. The particular significance of bFGF for fibrogenesis is underlined by a seminal paper by Whitby and Ferguson who examined the differences between fetal (scarless) and neonatal (scarring) wound healing (see introduction) and found no bFGF in fetal wounds, whereas the cytokine was abundantly present in healing after birth (291). Infusion of rats with high doses of bFGF results in glomerulosclerosis and interstitial fibrosis (292).

In vitro bFGF induced proliferation of primary cortical fibroblasts and promotes the expression of α-smooth muscle actin, but had only marginal effects on the synthesis of extracellular matrix proteins (293). *In vivo* many of the above cytokines may be involved in a concerted action during tissue repair and fibrosis, so that most likely no single profibrotic "master-cytokine" exists.

Epithelial-Mesenchymal Transition (EMT). EMT was first demonstrated in a murine model of anti-tubular basement membrane disease by cloning of a specific fibroblast marker, named fibroblast specific protein-1 (FSP-1), a member of the S100 family (294). Its expression is constitutive in tissue fibroblasts under physiologic conditions (294) and its promoter contains a *cis*-acting element (FTS-1) highly specific for fibroblasts (295,296). In mouse models of chronic progressive renal disease FSP-1 expression was robustly upregulated in the tubulointerstitium. The initial staining pattern followed the distribution of collagen-producing cells in a rabbit model of anti-GBM disease (297). In addition, *de novo* expression of the fibroblast-specific protein could be detected in tubular epithelial cells suggesting their transition to a mesenchymal phenotype (298). This phenomenon had been described in a number of organs (reviewed in 299). Moreover, EMT plays a central role in embryogenesis, tumor formation, and in the diseased kidney which replicates the renal developmental programs (300). Ng and co-workers described the *de novo* expression of α-smooth muscle actin in tubular epithelial cells starting at day 21 after 5/6 nephrectomy (301). Tubular epithelial cells lost their apical-basal polarity and seemed to migrate into the interstitium, as demonstrated by electron microscopy (Fig. 57-5). Of particular interest, and consistent with the general principles of tissue repair outlined initially, loss of epithelial characteristics occurred exclusively in areas where the basement membrane was disrupted.

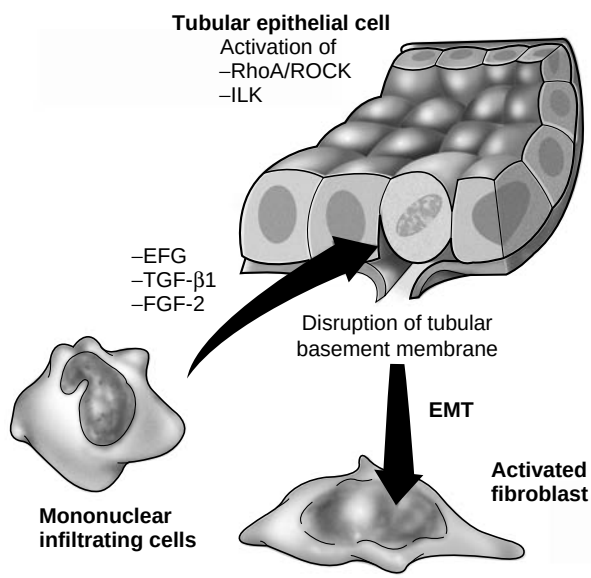

FIGURE 57-5. Hypothetical scheme of EMT in renal fibrogenesis. Stimulation of tubular epithelial cells by cytokines such as EGF, FGF-2, and TGF-β1, secreted mainly by infiltrating mononuclear cells, results in a partial loss of epithelial and de novo expression of mesenchymal proteins (transitional cell). If there is complete disruption of the tubular basement membrane, complete transition to an activated fibroblast ensues with synthesis of extracellular matrix proteins. (Modified from: Strutz F, Neilson EG. New insights into mechanisms of fibrosis in immune renal injury. *Springer Semin Immunopathol* 2003;24:459.)

What causes the plasticity of EMT? Various cytokines can induce the expression of FSP-1 in tubular epithelial cells *in vitro*. These include TGF-β and epidermal growth factor (EGF) (268,302). The group of Strutz described the effects of FGF-2 on four aspects of EMT: expression of epithelial and/or mesenchymal cell markers, cell motility, secretion of MMPs and matrix synthesis in two murine epithelial cell lines. FGF-2 had similar effects as EGF, but its main effect was the potentiation of the induction of EMT by TGF-β1 (303). Only TGF-β1 induced matrix synthesis, whereas all cytokines resulted in expression of mesenchymal markers, cell motility and MMP-2 and -9 secretion. *In vivo*, the stimulation by cytokines is probably not sufficient to induce EMT, and changes in extracellular matrix may also be required (304). For example, disrupting the tubular epithelial membrane by inhibition of collagen type IV assembly with soluble α1NC1-domains resulted in EMT conversion (304). Conversely, the tubular basement membrane did have a stabilizing function on the epithelial phenotype. These *in vitro* studies confirmed the aforementioned *in vivo* study by Ng et al. that complete transition to a mesenchymal cell can only be observed when the tubular basement membrane is disrupted (301). This concept is further corroborated by a study of Yang et al. in the UUO model of progressive fibrosis, where experimentally decreasing the activity of MMP-9 led to less degradation of type IV collagen and basement membrane, associated with less staining for α-smooth muscle actin in tubular epithelial cells, interpreted as less EMT (305). Changes in the basement membrane occur in progressive kidney disease, particularly in diabetic nephropathy (306). Such changes in the structure and the composition of the basement membrane could also promote EMT (300).

The signal transduction mechanisms of EMT point toward activation of integrin-linked kinase (307) and of the RhoA/Rho-associated coiled-coil kinase (ROCK) (308) pathways as important factors resulting eventually in the loss of the adhesion molecule E-cadherin. E-cadherin is important for the maintenance of the epithelial-epithelial adhesion and epithelial phenotype in the context of an organized tubular structure (300). Figure 57-4 illustrates the mechanisms involved in EMT schematically.

Postinflammatory Repair and Progression. The phase of postinflammatory matrix synthesis distinguishes tissue fibrogenesis from typical wound healing where resolution is expected. In this phase, the primary inflammatory process, be it in the glomerulus, the interstitium, or the vasculature, has subsided, yet interstitial inflammation may persist in a few areas. Several hypotheses about smoldering fibrosis have been entertained: First, stimulation by the remaining interstitial infiltrate is strong enough to result in continuous fibroblast activation. Second, autocrine loops in activated fibroblast may result in autonomous stimulation of these cells. Roles for IL-1, FGF-2 and TGF-β1 have been postulated in this context (293,309). A third mechanism postulates the interaction between secreted PDGF, TGF-β and tubular epithelial cells and fibroblasts (310–312). However, the existence of autocrine loops has only been described *in vitro* and their existence *in vivo* remains to be determined. Furthermore these schemes imply a persistent stimulation of one cell type or another, which in turn indicates continued cell stress and injury as the basis for the fibrosis.

In addition to persistent proteinuria another stimulus could be the aforementioned reductions in number of interstitial capillaries leading to hypoxia, which in turn may induce matrix formation (313). Hypoxia is a potent regulator of a number of genes, to a large extent mediated by the transcription factor: hypoxia induced factor 1 (HIF 1). The most prominent HIF-1 induced gene in the kidney is erythropoietin (314). In a model of progressive renal failure the expression of HIF-1α correlated

closely with the number of peritubular capillaries (315). Moreover, Manotham et al. showed that the number of capillaries was reduced 4 and 7 days after induction of the remnant kidney model (316). This reduction was subsequently followed by the development of interstitial fibrosis. Similar findings were obtained in a rat model of progressive glomerular disease (317). Synthesis and secretion of ET-1 and TGF-β1 in proximal tubular epithelial cells could be stimulated by hypoxia (318,319). Moreover, hypoxia induced the synthesis of collagen type I but not of type IV in tubular epithelial cells (319). Hypoxia may also promote apoptosis of tubular epithelial cells through an upregulation of HIF-1 and Bax (320). Finally, hypoxia may induce EMT in cultured tubular epithelial cells (321). Hypoxia may also induce the formation of proinflammatory mediators, including eicosanoids, PAF, cytokines, and chemokines via generation of free radicals by any of the cell type stressed. All of the above factors could contribute to a persistent inflammatory infiltrate, either by influx or persistence of leukocytes, their activation perpetuating the vicious cycle of inflammation, fibrosis, hypoxia, and loss of functional nephrons.

Reversibility of Fibrosis. A central question in renal fibrogenesis concerns the potential reversibility of fibrotic lesions. In order to achieve this, the imbalance between matrix synthesis and degradation would have to be corrected. Until very recently, fibrotic lesions were thought to be irreversible. However, work by Fogo et al. demonstrated potential reversibility of mild focal segmental glomerulosclerosis in an animal study by treatment with an angiotensin receptor blocker (322). Similar results were obtained by Boffa et al. in a model of nitric oxide deficient hypertensive rats (323), by Adamczak et al. in the 5/6th nephrectomy model (324) and by Koo et al. in a model of reversible UUO (325).

In recent studies the role of bone morphogenetic protein (BMP)-7 in renal fibrosis was studied. Generation of BMP-7-deficient mice demonstrated changes of bone formation, lens development, and surprisingly also severe impairment of renal development resulting in death from renal failure a few days after birth (326,327). Morphologically, kidneys were hypoplastic and displayed dilated collecting ducts separated by stromal cells and extracellular matrix (326). BMP-7 expression begins at day 11.5 of mouse development in the ureteric bud as well as in the condensing mesenchyme, where it acts as an important cell survival factor (328). BMP-7 expression, unlike other morphogens, persists postnatally, particularly in distal and medullary collecting tubules where it may function as a cellular differentiation factor (329). BMP-7 effects are mediated via Alk 3 and Alk 6 receptors. Therapeutically, BMP-7 was first tested in acute renal failure. Vukicevic and co-workers were able to demonstrate that administration of BMP-7 (formerly known as OP-1 ["osteogenic protein]) reduced the severity of acute renal failure (330). Hruska and co-workers administered BMP-7 to animals with UUO demonstrating a delay of the loss of renal function by 5 days (331). Interestingly, in that study BMP-7 was more effective than the ACE-inhibitor enalapril. Similarly, a positive effect on renal fibrogenesis by BMP-7 was demonstrated in a model of Alport's disease. However, therapy was initiated at an early time point in that study. In order to resemble the clinical situation more closely, BMP-7 application was delayed in nephrotoxic serum nephritis. This delayed treatment not only prevented progression, but even resulted in regression of fibrotic changes and tubular atrophy (332). Zeisberg et al. demonstrated that BMP-7 counteracted the effects of TGF-β1, particularly on EMT of tubular epithelial cells. *In vivo*, placebo treated animals displayed expression of FSP-1, and a simultaneous downregulation of E-cadherin in tubular epithelial cells, indicating EMT. These changes were prevented in BMP-7 treated mice. Similar results were recently obtained by Wang et al. in a model of diabetic nephropathy (329). Conversely, Ikeda et al. applied BMP-7 to rats undergoing protein overload proteinuria and found only modest effects on the disease course, possibly due to the fact that tubular atrophy and EMT are not prominent features in that model (333). At the present stage, BMP-7 represents one of the most exciting strategies to prevent progression and possibly even reverse fibrosis.

What is the situation in humans regarding reversibility of fibrotic lesions? Fioretto et al. demonstrated that regression of early diabetic sclerotic changes is possible in principle (334). Type 1 diabetics who underwent pancreas transplantation with some degree of renal involvement at that point were observed for a period of 10 years. Renal biopsies were taken at 0, 5, and 10 years after pancreas transplantation. Whereas after 5 years there was a tendency for deterioration of sclerotic changes in the glomeruli, a definite reduction of mesangial matrix score was noted 10 years after the normalization of glucose levels. Although this study was performed in only a small number of patients with early diabetic changes and the tubulointerstitial space was not evaluated, it demonstrates two things: First, early sclerotic changes are potentially reversible even in humans and second, regression of sclerotic changes may require a very long time period, at least in diabetes. Reversibility of fibrotic lesions has also been described in other organs, particularly liver (335) and heart (336). Thus, the former paradigm of irreversibility of fibrotic lesions may not be true. However, the majority of studies showing reversibility have been performed in mild fibrotic lesions, where the underlying architectural scaffolding was intact and thus full restoration still a possibility. It remains to be seen if severe and long-lasting fibrosis with distortion of basic nephron architecture is truely reversible or if there may be a point of no return. Finally, not all fibrotic processes may be equal, so that the degree of reversibility may also depend on the underlying disease.

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CHAPTER 58 ■ ACUTE POSTSTREPTOCCAL GLOMERULONEPHRITIS AND OTHER BACTERIAL INFECTION-RELATED GLOMERULONEPHRITIS

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Acute glomerulonephritis is characterized by the sudden appearance of hematuria, proteinuria, and red blood cell casts. The differential diagnosis of this syndrome is listed in Table 58-1(1). The initial diagnostic approach includes clinical evaluation and serologic determinations. Pathologic evaluation is very useful in confirming the diagnosis and defining the extent of inflammation and fibrosis. This chapter will consider glomerulonephritis associated with bacterial infections. Glomerular disease that accompanies infection with other organisms is covered in subsequent chapters. Acute poststreptococcal glomerulonephritis is the prototype, and it is distinguished from the other causes of acute glomerulonephritis by its typical serologic, histologic, and chronological features. A link between streptococci and acute glomerulonephritis can be traced to epidemics of scarlet fever in the 18th century (reviewed in [2]). During the earlier part of the 20th century, it was recognized that infection with β-hemolytic streptococci could lead to glomerulonephritis (2–5). Since this discovery, the clinical, serologic, and histologic features of the disease have been carefully documented, and there has been considerable progress in identifying the pathogenic mechanisms that participate in this disease. These features will be the focus of the initial discussion; consideration of other bacterial infections that may lead to acute glomerulonephritis will follow.

ACUTE POSTSTREPTOCOCCAL GLOMERULONEPHRITIS (APSGN)

Epidemiology and Incidence

APSGN may occur sporadically or in epidemic form. Although the sporadic form is more common, information obtained about the disease through analysis of epidemics has been particularly revealing (6–24). It affects children more than adults, with the peak age incidence from 2 to 6 years (Table 58-2). Approximately 5% of cases are found among children younger than 2 years, and 5 to 10% of patients with the disease are older than 40. Spread among family members is common, and nephritogenic streptococci have also been isolated from household pets with scabies (25). Males more often have clinical evidence of nephritis, although females are more likely to have subclinical disease (14,26). Patients with subclinical nephritis outnumber those of overt nephritis by a ratio ranging from 4:1 to 10:1 (2). This is consistent with pathologic review of biopsy specimens (nonepidemic form), where evidence of healed disease is greater than anticipated from the clinical diagnosis (27). In more temperate zones, APSGN occurs more commonly in winter months, and most often after pharyngitis, whereas in the tropics, skin infection (i.e., impetigo) during the summer is usually the initiating event (28). Cyclical outbreaks of epidemic forms have been reported by Rodriguez-Iturbe and others, although the reason for these cycles has not been fully explained (20,29,30).

APSGN follows infection with only certain groups of streptococci, and these strains are designated nephritogenic. Group A streptococci are responsible for the vast majority of cases, and among them certain types predominate (2). Occasionally non–group A streptococci have been linked to glomerulonephritis (31,32). "Nephritogenic" group A streptococci have been characterized serologically by their cell wall proteins, termed M and T proteins, and the known nephritogenic strains include the M types: 1, 2, 3, 4, 12, 18, 25, 49, 55, 57, 59, 60, and 61 (2,33–39). The risk of nephritis following infection with nephritogenic strains depends on the location of infection. For example, with type-49 streptococci, the risk of nephritis is five times greater with skin infections than with pharyngitis (28). Nephritis following pyoderma with types 47, 55, 57, and 60 is also common (2,14). Many cases have also been associated with strains that do not have an M or a T type designation. Nevertheless, the identification of nephritogenic strains suggests that factors unique to these strains are responsible for nephritis (see below). However, it should be emphasized that host factors are also important, because only a minority (approximately 10%) of patients infected with nephritogenic strains develop overt disease. ASPGN has been reported following renal transplantation, although these patients are at no greater risk for the disease (40).

Pathology

Most commonly there is diffuse glomerulonephritis, although the severity of involvement usually varies among glomeruli and within segments of individual glomeruli (40–44). On light microscopy, there is cellular infiltration and glomerular cellular proliferation (45) (Fig. 58-1). The predominant cell types depend on the timing of the biopsy. Within the first 2 weeks of disease, neutrophils, eosinophils, lymphocytes, and monocytes are present in the capillary lumen and in the mesangium, and endothelial and mesangial cell proliferation is prominent (2,35,46). CD4 T cells usually exceed CD8 cells early on, whereas later CD8 cells predominate. Periglomerular accumulation of T cells may also be observed (47). Occlusion of capillary lumen is not unusual, and mesangial expansion is typical (44). Intracapillary fibrin thrombi and deposits and/or necrosis are observed in some cases. This pattern characterizes the so-called exudative phase. During this period, intermittent thickening of capillary walls, corresponding to large

TABLE 58-1

MAJOR CAUSES OF ACUTE NEPHRITIS

Low serum complement level[a]	Normal serum complement level
Systemic diseases	**Systemic diseases**
Systemic lupus erythematosus (focal ~75%, diffuse ~90%)[a]	Polyarteritis nodosa
Cryoglobulinemia (~85%)	Wegener's granulomatosis
Subacute bacterial endocarditis (~90%)	Hypersensitivity vasculitis
"Shunt" nephritis (~90%)	Henoch-Schönlein purpura
	Goodpasture's syndrome
	Visceral abscess
Renal diseases	**Renal diseases**
Acute poststreptococcal glomerulonephritis (~90%)	IgG-IgA nephropathy
Membranoproliferative glomerulonephritis	Idiopathic RPGN (rapidly progressive glomerulonephritis)
Type I (~50%–80%)[b]	Anti-GBM disease
Type II (~80%–90%)	Pauci-immune[c] (no immune deposits)
	Immune-deposit disease

Normal serum complement levels indicate that production of complement components is keeping up with consumption; it does not exclude participation of complement in the inflammatory process. Repeat measurements useful (2–3 × 1 week apart). Consistently normal serum levels are useful in narrowing the diagnostic possibilities.
[a]Percentages indicate the approximate frequencies of depressed C3 or hemolytic complement levels during the course of disease.
[b]Most common pathologic findings associated with hepatitis C infection.
[c]Pauci-immune indicates lack of significant glomerular deposition of immunoglobulin by direct immunofluorescence. Many patients have circulating ANCA.
(Reprinted with permission from: Madaio MP, Harrington JT. The diagnosis of glomerular diseases: acute glomerulonephritis and the nephrotic syndrome. *Arch Intern Med* 2001;161.)

subepithelial immune deposits, are often observed (i.e., by trichrome staining). Focal capsular adhesions or segmental crescents are relatively common. Abundant crescent formation is unusual, although it is occasionally observed in more severe situations (2,48). Over 4 to 6 weeks, polymorphonuclear neutrophils (PMNs) are no longer present, and hypercellularity with mononuclear cells (mesangial cells and/or infiltrating monocytes) predominates. During this latter phase, capillary lumens are usually patent. Glomerular hypercellularity usually slowly resolves, although mesangial hypercellularity may persist for months. Extraglomerular abnormalities are usually not as prominent during either phase, however interstitial edema, tubular necrosis, scattered mononuclear interstitial infiltrates, and/or mild arteriolitis has been observed (49). Severe vasculitis has been reported but is unusual (50–52).

By immunofluorescence microscopy, deposits of immunoglobulin G (IgG) and C3 are distributed in a diffuse granular pattern within the mesangium and capillary walls (25,44, 53–55). C3 is invariably present, whereas the quantity of IgG depends on the timing of the biopsy, and it is not uncommon to see only C3 deposits very early or late in disease. IgM is often present early in disease but may also be observed in smaller amounts later on. Significant amounts of IgA suggest an alternative diagnosis (e.g., IgA nephropathy or Henoch-Schönlein purpura). C1q and C4 are not usually detected; however, properdin and terminal complement components (C5b-9) are often present and distributed in a granular pattern. Fibrin deposits are occasionally detected in more severe cases. Different patterns of immune deposition have been observed, however they are likely related to the timing of renal biopsy. Early in the disease (the first few weeks), the fine granular appearance of immune deposits often gives a "starry-sky-like" appearance; this pattern is associated with glomerular hypercellularity (53–55). With resolution of the disease (i.e., 4–6 weeks after onset), the immune deposits take on a more mesangial pattern, prior to disappearing. C3 may be present in the absence of detectable Ig, either very early in the disease (less than 2 weeks) or with disease resolution (i.e., with resolution of the IgG deposits). In about one-fourth of cases, the deposits are large, and they aggregate in a "rope" of "garland-like" pattern, and this pattern may be associated with persistent mesangial hypercellularity on light microscopy. When these type deposits are present, they may last for months, and they may be associated with persistence of heavy proteinuria and development glomerulosclerosis (53–56). By contrast, transition to a more mesangial pattern (IF) is usually associated with clinical and pathologic resolution. Occasionally immune deposits in small vessels occur in the setting of vasculitis.

Dome-shaped subepithelial electron-dense deposits that resemble camel "humps" are the most characteristic feature on electron microscopy (46). These humps contain Ig, are most abundant within the first month, and are frequently observed near epithelial slit pores (10,42,54,57). They have been associated with heavy proteinuria, and they usually resolve within 4 to 8 weeks. In later stages of the disease, they may be absent; however, remnant electron-lucent areas are occasionally

TABLE 58-2

GENERAL CHARACTERISTICS OF APSGN

Age:	Children > adults (5% <2 yr; 5%–10% >40 yr)
Sex:	Male > Female
Clinical manifestations: subclinical 4–10 × >overt nephritis	
Site of infection: pharynx (temperate zones), skin (tropics)	

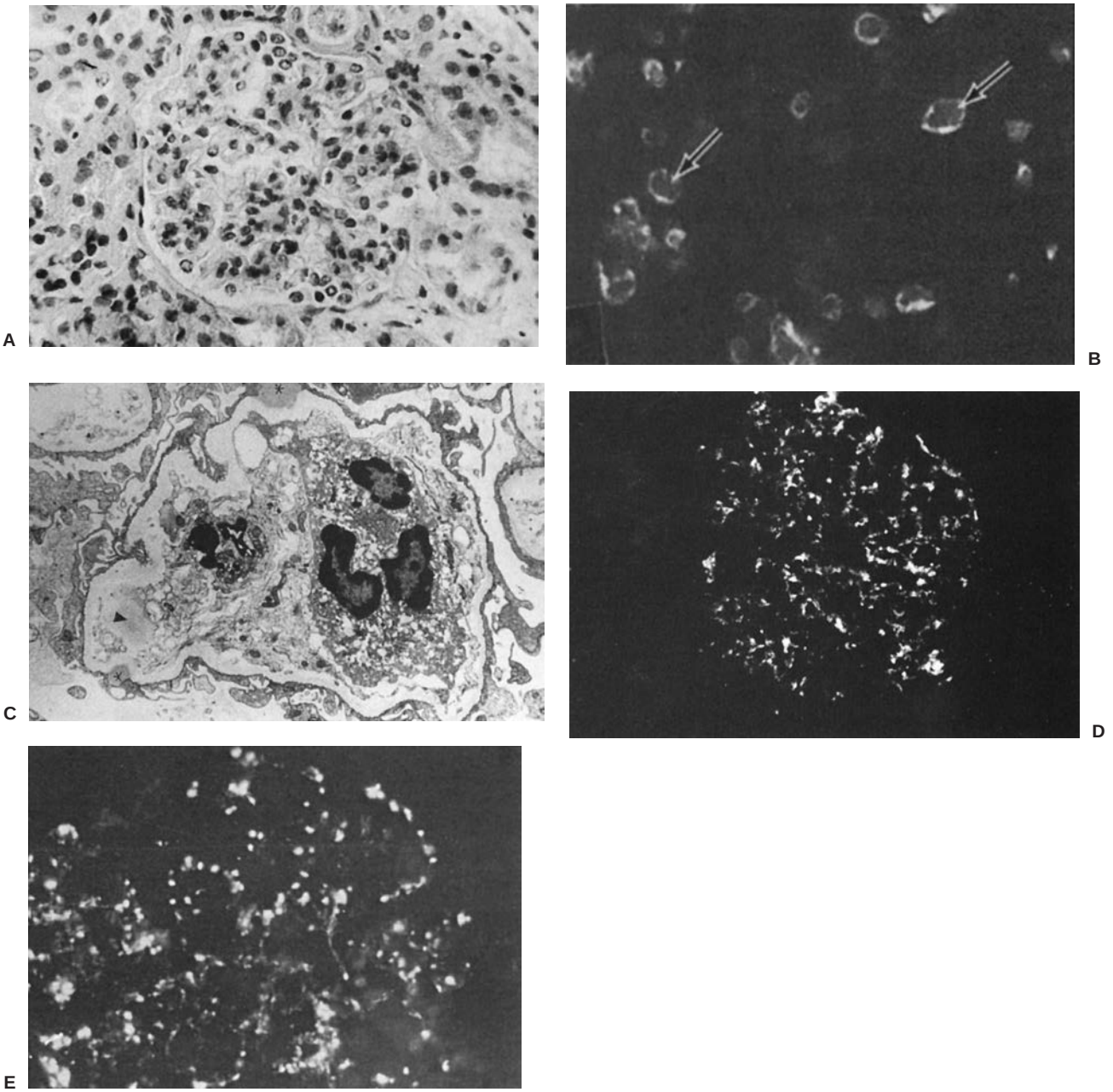

FIGURE 58-1. Pathology of poststreptococcal glomerulonephritis. **A:** Endocapillary proliferation with increased number of messangial cells and glomerular infiltration with neutrophils (PMN). Biopsy specimen taken 10 days after the beginning of symptoms. (Hematoxylin & eosin ×500.) **B:** Intraglomerular cells reactive with OKM1 monoclonal antibody (*arrows*) in a biopsy specimen obtained 14 days after the initial symptoms. Monocytes and neutrophils are recognized by the antibody, and reactivity with antihuman lactoferrin (which identifies PMN) in serial sections was use to define glomerular moncyte infiltration. **C:** Glomerular capillary loop with polymorphonuclear leukocytes in the lumen. Electron-dense deposits are present in subepithelial ("humps") (*) and subendothelial (◁) locations. (×12,000). **D:** C3 deposits (+1) in the glomerular basement membranes and mesangium. (FITC-labeled antihuman ×500.) **E:** Glomerular deposition of the membrane attack complex of complement in a biopsy specimen obtained 16 days after onset identified with monoclonal poly-C9 antibody, which recognizes a neoantingen on C9. Pattern and localization of deposits is similar to the one found for C3 and C5. (B and E reproduced with permission from: Parra G, et al. Cell populations and membrane attack complex in glomeruli of patients with poststreptococcal glomerulonephritis: identification using monoclonal antibodies by indirect immunofluorescence. *Clin Immunol Immunopathol* 1984;33:324.)

observed and provide diagnostic clues (58). Subendothelial, mesangial, and intramembranous deposits (along with smaller subepithelial deposits) are often present in variable amounts, and they usually persist after resolution of subepithelial deposits (humps) (58). Patients with large subendothelial deposits, without mesangial deposits, were found to have more proteinuria and edema (59). Large intramembranous deposits are associated with the garland-like pattern of immune deposits on immunofluorescence described previously (54). The basement membrane itself is usually of normal diameter, although thickening has occasionally been observed (49). Cellular infiltration and proliferation depends upon timing of the biopsy, as described above in the description of light microscopic changes.

Pathophysiology

The association with streptococcal infections has largely stimulated investigation of the pathogenesis of this disease. However, since only certain strains of streptococci are "nephritogenic," leading to the notion that there are unique properties of these strains. Furthermore, not all individuals infected with nephritogenic streptococci (i.e., as detected during epidemics of nephritis) develop disease, suggesting that host factors are also important for disease expression. Based on these observations, a number of theories pertaining to pathogenesis have emerged. For disease expression, the susceptible host must both be infected with a nephritogenic strain and mount a pathogenic immune response. With regard to the former, recent analysis of nephritogenic streptococci and their products has provided novel insights, although differences of opinion pertaining to the precise sequence of events leading to immune deposit formation and inflammation persist. Three major pathogenic theories have emerged, and they will be considered briefly.

A popular theory that originated in the 1960s invokes deposition of streptococcal antigens within glomeruli (60,61). Accordingly, nephritogenic streptococci produce proteins with unique antigenic determinants that, in addition to eliciting a potent antibody response, have a particular affinity for sites within the normal glomerulus. Following release into circulation, streptococcal protein fragments encounter ligands within glomeruli and lodge there, affixed to sites for which they have intrinsic affinity. Once glomerular-bound, they activate complement directly by attracting properdin, leading to stimulation of the alternate complement pathway. At the same time, the glomerular-bound streptococcal proteins serve as planted antigens for immune complex formation with circulating anti-streptococcal antibodies. Immune complex formation leads to additional complement fixation that, in turn, leads to the recruitment of additional inflammatory mediators and cells. As importantly, the glomerular bound antibodies engage Fc receptor on circulating cells, which further enhances cellular infiltration, including mononuclear cells.

In support of this hypothesis, a number of candidate proteins have been identified that meet most of these criteria. A relatively small complex of intracellular anionic proteins, termed endostreptosins, have been isolated from nephritogenic streptococci by Lange, Treser, Yoshizawa and co-workers (36,61–64). Antibodies specific for these proteins react with glomeruli of patients with nephritis (presumably containing this antigen), and elevated antibody titers are present in patients with active disease. Administration of endostreptosin to rats resulted in glomerular localization of antigen for the first four days after injection, and this was followed by deposition of IgG and complement (65). This particular antigen complex is not unique to nephritogenic streptococci, however, and elevated antibody titers have been found in patients with streptococcal infections that do not develop nephritis, raising questions about its pathogenic relevance. Nevertheless, this is not surprising since *both* a nephritigenic antigen *and* a pathogenic immune response are necessary for disease expression. Treser et al. provide further insights (66). They isolated a potentially relevant antigen from this complex, termed PA-Ag (for preabsorbing antigen), with a molecular weight of 43,000 and pI of 4.7. Antibodies to PA-Ag were present in 30 of 31 patients with APSGN, whereas they were only detected in 1 of 36 individuals with uncomplicated group A streptococcal upper respiratory infections. Particularly noteworthy, PA-Ag, and not other streptococcal components were detected in glomeruli of patients with APSGN. Furthermore, PA-Ag was capable of activating the alternate complement pathway, suggesting that it is involved in the pathogenesis of this disease. In a series of elegant studies, Oda, Yoshizawa, and co-workers recently extended these observations (67,68). They isolated an antigen termed nephritis associated plasmin receptor (NAPlr) that binds to plasmin and extends its proteolytic activity. Both plasmin and the receptor (NAPlr) were present in biopsy specimens of ASPGN patients but not in other disease samples. This lead the investigators to postulate that when released into the circulation from nephritogenic strains, NAPlr binds to glomeruli. Thereafter it engages circulating plasmin, which, in turn, initiates nephritis by activating metalloproteinases and recruiting cells, and then by serving as a planted antigen for subsequent immune complex formation.

Zabriski and co-workers have also identified disease related protein (or set of proteins) (69,70). They described an extracellular, 46 kD protein, termed nephritis strain-associated protein (NKAP), isolated from strains derived from patients with APSGN, the secreted protein was not usually produced by other strains. Anti-NKAP antibodies were identified both in sera and 18 of 21 renal biopsies of APSGN patients (71). Although NKAP was initially thought to be streptokinase, subsequent evidence has proved that this is not the case (37,71,72). Peake and co-workers isolated a protein with similar properties that was capable of both activating complement and binding to normal human glomeruli (73). Poon-King et al. identified yet another closely related protein (with similar properties as NKAP (74), initially termed nephritis plasmin binding protein (NPBP). Subsequently, sequence analysis of NPBP demonstrated sequence homology with streptococcal pyrogenic exotoxin B precursor (SPEB, previously termed zymogen). Since plasmin is capable of binding to glomerular-bound NPBP, this could account for activation of the complement cascade and initiation of inflammation, observed prior to the deposition of IgG early in disease. In support of this conclusion, sera from ASPGN patients has been observed to have anti-SPEB antibodies, and the SPEB antigen was demonstrated in glomerular lesions (75).

There has been support for other nephritogenic proteins, and there may not be a single species. Vogt et al. identified a cationic proteinase produced by nephritogenic streptococci, related to an erythrogenic toxin (76). Serum antibodies to this protein are present in many patients with APSGN but not other forms of nephritis. Bergy and Stinson described a soluble heparin-inhabitable basement membrane binding protein derived from a nephritogenic strain that also bound to tissue sections (77), and Glurich et al. identified streptococcal-derived proteins that bind to rabbit kidney *in vitro* and *in vivo* (78). Streptokinase has also been implicated by its capacity to induce nephritis in normal mice (78–80). Of potential clinical relevance, streptokinase activated complement directly within the kidney prior to antibody deposition, in a manner analogous to previous observations of glomerular complement activity patients in the early stages of APSGN. Although these and other proteins have not been as extensively studied as those described previously, it is likely that different proteins are

involved in different patients, with host factors contributing to disease expression.

Another hypothesis, initially raised by McIntosh and co-workers, invokes alteration of normal IgG by streptococcal enzymes as the initial event. This has two effects. The altered IgG is recognized as a "foreign" antigen and elicits an antibody response. It also acquires affinity for glomeruli (e.g., it becomes more cationic) and deposits therein (81). Once deposited, this altered and relatively cationic IgG serves as a fixed or "planted"' glomerular antigen for the subsequent binding of anti-IgG. In support of this hypothesis, Fc receptors are present on streptococci (82,83). Furthermore, neuraminidase-producing streptococci are capable of desialation of Ig (making it more cationic) (84,85). Therefore streptococci have the capacity to engage IgG and render it pathogenic. Furthermore, elevated levels of both serum rheumatoid factor, neuraminidase activity, and free sialic acid are often present in patients with acute poststreptococcal GN, and anti-Ig antibodies have been eluted from the kidney of a patient with this disease (84,85).

Although neuraminades may effect neutrophil activity locally (86), neuraminadase-producing streptococci are not unique to patients with acute poststreptococcal glomerulonephritis, and rheumatoid factor activity is present in many individuals with streptococcal infection who do not develop glomerulonephritis.

The possibility of antigenic mimicry, between nephritogenic streptococci and normal glomerular antigens, has also been considered (reviewed in [36]). This hypothesis invokes the production of antibodies against streptococcal membrane proteins that share structural determinants with glomerular antigens. Early reports demonstrated that soluble glycoprotein derived from nephritogenic strains of streptococci induced nephritis in animals, and disease could be transferred to normal animals using antiserum from the diseased animals (87-89). Subsequently, antibodies against streptococcal cell membranes, derived from nephritis patients, were observed to cross-react with glomeruli (36). Furthermore, homologies between M proteins derived from nephritogenic strains and basement membrane antigens are consistent with this notion (90). Antibodies produced against a tetrapeptide (Ile-Arg-Leu-Arg) derived from the M-1 protein of a nephritogenic strain were found to cross-react with normal glomerular antigens in vitro (91,92). However, although the tetrapeptide shares homology with the cytoskeletal filament protein, vimentin, the pathogenic relevance of this cross-reaction is unclear (91). Similarly, antibodies to other extracellular antigens, including laminin, heparin sulfate, and type IV collagen have been detected in serum from patients with APSGN (93–95). It is tempting to speculate that different antibody-antigen interactions are responsible for glomerular deposits at different sites within glomeruli.

Other factors may also contribute to disease susceptibility. Outbreaks among families were first noted by Wells (96). This was confirmed by Dodge et al. and later by Rodriguez-Iturbe and co-workers; they observed that the familial incidence of disease during epidemics was higher that the attack rate in the population at large (26,41). Unfortunately genetic studies have thus far failed to support linkage (as opposed to rheumatic fever where genetic linkage has been found [97]). T cells, monocytes, platelets and endogenous glomerular cells (along with cytokines and chemokines produced by these cells) have been detected within APSGN lesions. They participate in the local inflammatory response, and along with upregulation of adhesion molecules (47,54,98–100), they influence the severity of disease. Additionally, streptococcal cell wall proteins and exotoxins may serve as superantigens to engage larger populations of T and B cells in the process.. The contribution of both infectious and host factors likely influence the specific characteristics and severity of disease. The reader is referred to Chapter 57 for a more general discussion of the role of these individual participants in glomerulonephritis.

Clinical Manifestations

Although not diagnostic, the symptoms of the disease are quite characteristic. However most patients present with only a few of the features of the *acute nephritic syndrome* (101). Typical presentations include edema, gross hematuria, and back pain, although the actual incidence of symptoms varies somewhat among reports (Table 58-3) (9,10,12–14,20,23,25,41,102). Anasarca is more common among young children (2). Occasionally, patients with gross hematuria will complain of dysuria. Transient oliguria is present in approximately one-half of the patients, but anuria is rare (2). Hypertension is found in 60% to 80% of patients at initial presentation, although hypertensive encephalopathy is unusual. Nevertheless, if untreated it may be associated with seizures (2,10). In others, encephalopathy may occur in the absence of significant hypertension, and this may be due to cerebral vasculitis (103). Some patients present with signs and symptoms of congestive heart failure; however, the coexistence of rheumatic fever is rare (14). Hypertension and heart failure are mostly due to sodium retention, and the renin-angiotensin system is typically suppressed (2). Decreased glomerular filtration rate may also contribute to sodium retention (14). Rapidly progressive glomerulonephritis with acute renal failure is unusual but has been well documented. (104). Hypertension and heart failure usually resolve after diuresis. Generalized symptoms, including anorexia, malaise, nausea, and vomiting are common at initial presentation, whereas arthralgias and skin rashes are uncommon. When the latter symptoms are observed, other causes of glomerulonephritis should also be considered (i.e., lupus, vasculitis). Backache in the lumbar region has been attributed to intrarenal edema with stretching of the renal capsule.

Children are more frequently affected than adults, although the disease can occur at any age, and it may be overlooked in the elderly (2,14,20,26,30). During epidemics, most infected with nephritogenic strains develop only subclinical evidence of nephritis (i.e., abnormal urinalysis; see above) (26,28,41,105),

TABLE 58-3

CLINICAL AND LABORATORY MANIFESTATIONS OF APSGN

Clinical	
Edema	85%
Gross hematuria	30%
Back pain	5%
Oliguria (transient)	50%
Hypertension	60%–80%
Nephrotic syndrome	5%
Laboratory	
Urinalysis: proteinuria, hematuria, casts	100%
Nephrotic range proteinuria	10%
Serum creatinine ≥2 mg/dL	25%
Streptococcal antibody profile (streptozyme)	
in patients with pharyngitis	>95%
in patients with skin infections	80%
false positive rate	5%
Early Abic Rx prevents antibody response	
C3, C4, and/or CH50 depressed in	>90%
Hypergammaglobulinemia	90%
Cryoglobulinemia	75%

indicating that the disease may go unrecognized. Nephrotic syndrome occurs in 5% to 10% of children, but it is more frequently observed in adults (20%) (2). It may occur either at initial presentation or later in the course, with improvement of glomerular filtration rate (GFR). Rapidly progressive glomerulonephritis with acute renal failure occurs in fewer than 2% of children, and it is slightly more common in adults. In children, the clinical symptoms of acute glomerulonephritis usually resolve within 1 to 2 weeks; in adults, resolution may be more prolonged, and there is a higher incidence of progressive renal disease (see below).

The latent period between streptococcal infection and nephritis depends on the site of infection. Following pharyngitis, it is usually 1 to 3 weeks, whereas after skin infection, it is more prolonged at 3 to 6 weeks (14). Shorter latent periods of days (i.e., hematuria following upper respiratory infection) suggest an alternative diagnosis (i.e., IgA nephropathy). The preceding infection may be accompanied by severe symptoms (i.e., fever, pharyngitis), or be relatively asymptomatic. In many cases, it is not possible to identify an antecedent infection with certainty. Regional lymphadenopathy may be present, even after other symptoms and signs of the primary infection have resolved, and their presence should provide clues to the diagnosis. Typically the acute nephritic syndrome lasts 4 to 7 days, however it may be more prolonged in adults, especially in those presenting with rapidly progressive glomerulonephritis (RPGN) and crescentic glomerulonephritis (23). Coincident rheumatic fever is unusual (106), as is coincident arthritis (107), however glomerulonephritis may be associated with infections at other sites. Recurrent episodes are uncommon, however repeated bouts of hematuria may occur during the initial episode. Although *de novo* disease involving transplanted kidneys is usual, it may be associated with deterioration of graft function (108). Extrarenal manifestations are uncommon but include arthritis and choroiditis (109).

Laboratory Findings and Diagnosis

The urinalysis is always abnormal (see Table 58-3). Hematuria and proteinuria are invariably present, and casts are common. Red blood cell casts are frequently detected in a freshly voided specimen, and dysmorphic red blood cells indicative of glomerular hematuria are usually detected by phase-contrast microscopy. Renal tubular cells and casts, granular and/or pigmented casts, and white blood cells are also commonly found. White blood cell casts are occasionally observed in patients with severe exudative glomerulonephritis. Proteinuria is characteristic; however, nephrotic syndrome occurs in only 5% of patients at initial presentation (43,110). Occasionally in patients with severe disease at onset, there may be a transient increase in proteinuria to the nephrotic range with improvement in the glomerular filtration rate associated as the disease resolves.

The GFR is reduced and the serum creatinine is usually elevated, but the serum creatinine may remain within the upper limits of the normal laboratory range. Approximately 25% of patients have a serum creatinine greater than 2 mg/dL (2). The GFR, when measured, is almost always depressed in the initial stages of the disease, and it returns toward normal with disease resolution. Anemia may be present during the acute illness and early recovery period (111).

Throat cultures are frequently positive in affected individuals with pharyngitis. In the first 2 weeks of active nephritis the C3 and CH50 levels are significantly depressed in more than 90% of patients (2,101,112–114). The C4 and C2 levels are usually normal or only mildly decreased; marked depression suggests another diagnosis. Properdin levels are decreased in over 50% of patients and reflect activation of the alternate pathway of complement activation (115). Elevation of plasma levels of C5b–9 levels reflect the contribution of the membrane attack complex to pathogenesis (116). In most cases, the serum complement levels return to normal by 4 weeks; however, occasionally, it may take as long as 3 months (117). In patients with insidious and/or prolonged symptoms prior to visiting their physician, complement levels may have returned to normal at the time of initial presentation. The level of reduction of complement levels does not have prognostic significance. Persistent hypocomplementemia, however, is unusual and suggests another diagnosis (114). C3 nephritic factor may be present in low amounts, but marked and/or persistent elevations are more typical of membranoproliferative glomerulonephritis (101). Hypergammaglobulinemia is present in 90% of the patients, and polyclonal cryoglobulinemia (containing both IgG and IgM) is present in 75% of individuals with overt nephritis (2,118). Rheumatoid factor activity is found in about a third of patients early in the course of disease (2).

Antibodies to extracellular products of streptococci, as measured in the streptozyme test, are positive in more than 95% of patients with pharyngitis and 80% of patients with skin infections (2,14,39,119). These include anti-streptolysin (ASO), anti-hyaluronidase (AHase), anti-streptokinase (ASKase), anti-nicotinamide-adenine dinucleotidase (anti-NAD), and anti-DNAse B antibodies. The ASO, anti-DNAse B, anti-NAD, and AHase are more commonly positive after pharyngeal infections, whereas anti-DNAase B and AHase are more often positive following skin infections (120–122). Overall, these tests are relatively specific for streptococcal infections, with a 5% false-positive rate. However, since the incidence of streptococcal infections in the general population is relatively high (especially in young children), they may be elevated in patients with unrelated streptococcal infection and glomerulonephritis. Antibodies to nephritogenic streptococcal antigens may prove to be of diagnostic significance, but they require further evaluation (2,119) (123). Overall, antibody titers are generally elevated at 1 week, peak at 1 month, and fall toward their preinfection level after many months (124,125). An increasing antibody titer is indicative of recent infection. Antibodies against M proteins are type-specific and confer strain-specific immunity (38). They are detectable at 4 weeks following infection and persist for years, however the antibody levels (to M proteins) are unrelated to the severity of disease. Early treatment with antibiotic therapy often prevents the antibody response to both extracellular products and M proteins. Negative results in a patient who previously received antibiotics, therefore, do not exclude the diagnosis.

Natural History and Prognosis

In general, the overall prognosis is very good, as less than 0.5% die of the initial disease and, fewer than 2% of patients die or develop end-stage renal disease (6,8,11,15–17,20,21,24,43,126,127). Both the natural history of this particular disease and management of its complications contribute to the excellent outcome. Children have a better prognosis than adults, and patients older than 40 years with rapidly progressive renal failure and crescentic glomerulonephritis have a worse prognosis (11,20,30). Patients with RPGN associated with post-streptococcal disease have a better prognosis than other forms of RPGN; recovery after short-term dialysis dependence is not atypical, although renal function may not return completely to normal. There appears to be no difference in outcome with either epidemic or sporadic forms, although conclusions regarding prognosis are largely derived from well-documented epidemics in children. By contrast, opinion for less favorable outcomes come from smaller series of sporadic cases in adults (2). Persistent urinary and histologic abnormalities are

common in both adults and children and may persist for years (11,19,128). The persistence of proteinuria at 3 and 10 years is approximately 15% and 2%, respectively (128). In most large series of well-documented cases, these abnormalities eventually resolve, and the incidence of chronic renal failure is low.

Patients with prolonged nephrotic syndrome or persistence of heavy proteinuria have a worse prognosis, and this is often associated with an evolution to a "garland"-like pattern of immune deposits with disease progression (21,53–55). In one series of adults with APSGN, a larger percentage of patients developed persistent hypertension and/or end-stage renal disease years after the initial episode of acute of acute glomerulonephritis (43,129). It was unclear, however, whether progressive renal failure was due to nephrosclerosis associated with poorly controlled hypertension or progressive glomerular scarring. As previously discussed, most studies of patients with APSGN have not confirmed this pessimistic outlook, and it is likely that selection of patients for follow-up influenced the results in the one series (2). Nevertheless, the results are consistent with the notion that a small group of patients with residual damage may develop progressive renal insufficiency.

Treatment and Prevention

The therapy for patients with acute post-streptococcal glomerulonephritis is symptomatic and dependent on the clinical severity of the illness. The major aims of therapy of acute nephritis are control of blood pressure and treatment of volume overload. During the acute phase of the disease, salt and water should be restricted. If significant edema and/or hypertension develop, diuretics should be administered. Furosemide usually provides a prompt diuresis, with reduction of blood pressure. For hypertension uncontrolled by diuretics, vasodilators (i.e., calcium channel blockers or angiotensin converting enzyme [ACE] inhibitors) are usually effective. Intravenous agents may be required for management of rare cases of either severe or malignant hypertension. The serum potassium should be monitored and treated appropriately. Dialysis may be necessary to treat uremia. Restriction of physical activity is appropriate during the first few days of the illness but is unnecessary once the patient feels well. The most acute phase of the illness usually resolves within a week, and most patients undergo spontaneous diuresis after that interval.

Steroids, immunosuppressive agents, and/or plasmapheresis are generally not indicated. In patients with rapidly progressive renal failure, however, a renal biopsy may be indicated. If crescents are present in over 30% of glomeruli, we recommend treatment with a short course of intravenous pulse steroid therapy (500 mg to 1 g per 1.73 M^2 of intravenous Solu-Medrol [Upjohn] daily, for 3 to 5 successive days). Although there are no controlled trials of this therapy in patients with acute poststreptococcal glomerulonephritis, it has been beneficial in patients with rapidly progressive glomerulonephritis associated with other diseases (see Chapter 60). We do not recommend more prolonged treatment with steroids or other immunosuppressive therapy. Long term antihypertensive therapy in patients with hypertension and chronic renal insufficiency is essential to prevent the development of renal failure, and angiotensin converting enzyme inhibitors are the drugs of choice for this purpose.

Specific therapy for streptococcal infections is an essential part of the therapeutic regimen. This includes treatment of both the patient (if infected) and any infected family member or close personal contact. Throat cultures should be performed on all these individuals, and treatment with penicillin (oral penicillin G 250 mg four times a day [qid] for 7 to 10 days) or erythromycin (250 mg qid for 7 to 10 days) in patients allergic to penicillin, is indicated to prevent both the development of nephritis in carriers and the spread of nephritogenic strains to others. Whether or not early treatment of infected patients prevents nephritis is not known. For patients with skin infections, attention to personal hygiene is also essential. In epidemics, empirical prophylactic treatment of patients at risk, including close contacts and family members is recommended (130).

BACTERIAL ENDOCARDITIS

Epidemiologic Patterns

The precise incidence of glomerulonephritis in patients with bacterial endocarditis is unclear because prospective analysis of renal tissue in a large cohort of patients has never been conducted. Investigations to define the incidence of this complication have the typical limitations of retrospective studies or/and lacked histologic confirmation (131,132). Autopsy studies are difficult to interpret, as the presence of azotemia in patients with endocarditis may be associated with higher mortality rates, thereby overestimating the incidence of glomerulonephritis. Conversely effective antibiotic therapy may underestimate the frequency of this problem. Rates derived from renal biopsy studies often exaggerate the severity and underestimate the prevalence of glomerulonephritis, because biopsy is usually performed in patients with the most significant renal dysfunction. By contrast, studies that depend solely on urinalysis for diagnosis overestimate the prevalence of disease, because hematuria and leukocyturia may be due to either tubulointerstitial nephritis or embolic disease. Furthermore, advances in early, more effective therapies, and changes in the most common causative organisms have led to changes in the incidence of glomerulonephritis over recent years.

Despite these limitations, two trends have emerged. The overall incidence of glomerulonephritis associated with *Streptococcus viridans*–induced endocarditis has declined in the antibiotic era. Coincident with this decline has been the rise in glomerulonephritis associated with acute bacterial endocarditis, particularly involving *Staphylococcus aureus*. While *S. viridans* and *S. aureus* remain the most common causes of glomerulonephritis associated with subacute and acute endocarditis, respectively, isolated reports of other organisms such as Gram-positive and Gram-negative bacteria, *Coxiella burnetti*, and fungi have also been linked to glomerulonephritis associated with endocarditis. Several new associations between bacterial endocarditis and glomerulonephritis have been reported in recent years, including endocarditis due to *Bartonella henselae*, Brucellosis, and Actinobacillus (131,132). A heightened index of suspicion as well as better culture techniques to identify fastidious organisms may explain the emergence of these associations. The evolutionary trend regarding *S. viridans* and *S. aureus* is briefly reviewed below.

In the preantibiotic era, glomerulonephritis was documented in as many as 82% of patients dying from subacute bacterial endocarditis (SBE) (133–136). Following the availability of antibiotics, the prevalence of postmortem documentation of glomerulonephritis in patients dying from subacute bacterial endocarditis decreased precipitously to 25% (132–136). Early studies also suggested that glomerulonephritis occurred less frequently in patients with acute bacterial endocarditis; estimates of glomerulonephritis in the antibiotic era ranged from 36% of patients with fatal acute bacterial endocarditis to 82% of patients with fatal subacute bacterial endocarditis (133). It was assumed that the higher incidence of glomerulonephritis in subacute disease was due to differences in the virulence of the causative organisms. Investigators reasoned that infection with less virulent organisms led to a more indolent and prolonged course, resulting in a greater antibody response that

lead to a higher incidence of immunologically mediated events (129,132,137–139). More recent studies, however, indicated that acute endocarditis is responsible for a greater proportion of endocarditis-associated glomerulonephritis than what was originally reported, suggesting that either strain or host dependent factors were also operative. In this regard an autopsy series reported by Neugarten et al. found that *S. aureus* caused a disproportionate frequency of glomerulonephritis due to acute bacterial endocarditis (132).

Several factors may account for the changing epidemiologic pattern. *S. viridans*–associated subacute bacterial endocarditis has become much less common, due to both the declining occurrence of rheumatic fever in the United States as well as the successful implementation of prophylactic antibiotic regimens in patients with known valvular lesions. In addition, earlier recognition of endocarditis, with institution of more effective antibiotic and/or surgical therapy has led to a shorter duration of infection and associated complications (136).

Coincident with this decline, there has been an increase in acute endocarditis in intravenous drug abusers. This is often due to *S. aureus* bacteremia leading to infection of normal heart valves, and clinical evidence of glomerulonephritis has been found in 40% to 78% of patients with *S. aureus* acute bacterial endocarditis (132,140,141). Particularly noteworthy, the mean duration of clinical illness prior to the onset of glomerulonephritis is less than 10 days, and many patients are treated with antibiotics during the prodromal period. This suggests that either intrinsic properties related to the causative organism, the immune response or both, predispose these individuals to this form of glomerulonephritis.

Pathologic Features

Although, like ASPGN, many features have been reported, a few typical patterns are more common. In general, the pathology can be divided into subacute and acute forms. Glomerular changes occurring with subacute endocarditis (e.g., due to *S. viridans*) are usually less severe. The lesions are typically focal and segmental, with increased cellularity in the mesangial and endothelial portions of the tuft (133,142). Polymorphonuclear cells (PMN) and macrophage infiltration contribute to the hypercellularity, along with endogenous cell proliferation. The capillary walls are thin and single contoured. By contrast, patients with the more acute form of the disease (e.g., due to *S. aureus*) typically have more severe disease with diffuse proliferative glomerulonephritis. The hypercellularity involves mesangial and endothelial cells along with infiltration of neutrophils and monocytes (131). Double contours of capillary walls may be present to varying degrees. Epithelial and fibroepithelial crescents, both partial and circumferential, may also be seen (132,143). Rarely, features more typical of membranoproliferative glomerulonephritis have been reported (132,144,145).

The interstitium is often edematous and infiltrated by mononuclear leukocytes (131,132,136,146). These changes may be either immune-mediated or related to infection. Alternatively, interstitial disease due to antibiotics (i.e., hypersensitivity) may complicate the situation (131,147). One or all of these features may be present in an individual patient, and the precise cause(s) may be difficult to reconcile. Histologic evidence of renal embolization has been reported in 30% to 60% of patients with fatal bacterial endocarditis and should be considered in patients with unexplained renal failure, although (148) clinical manifestations of renal embolization are infrequent (132).

By direct immunofluorescence, granular mesangial and subendothelial capillary wall deposits of IgG and/or IgM, and complement (C3 predominantly, C1q less frequently) are observed in all glomeruli in patients with either focal or diffuse glomerulonephritis (131,146,149). Extraglomerular deposits are infrequent. By electron microscopy, subendothelial and mesangial deposits are usually present, although the quantity of deposits varies (146,149). By contrast, patients with acute *S. aureus* endocarditis often have subepithelial and intramembranous deposits 4 (149).

Clinical Features

The clinical manifestations depend on the duration and severity of disease. Microscopic hematuria, pyuria, and proteinuria are the most frequently detected abnormalities in patients with focal glomerulonephritis, however, these features may be absent despite histologic evidence of active disease (132,146). Less commonly, heavy proteinuria, hypertension, and renal dysfunction develop, and advanced renal failure leading to uremia may result. This latter scenario is more prevalent when bacteriologic cure is not achieved (132,146,150–152).

Microscopic hematuria and proteinuria are always present in patients with diffuse glomerulonephritis, and the nephrotic syndrome is not uncommon (i.e., ~15%) (132). Gross hematuria may be observed in patients with diffuse glomerulonephritis, although its appearance should raise suspicion of other conditions, such as either renal infarction or drug-induced interstitial nephritis (153). Pyuria occurs in approximately two-thirds of patients, and 15% to 30% of them have positive urine cultures (154,155). Unlike other forms of postinfectious glomerulonephritis, hypertension occurs infrequently (perhaps due to cardiac involvement) (132,156). Reduced GFR is variable, although it may occasionally be the presenting manifestation (148).

In the preantibiotic era, uremia contributed to the death of 5% to 10% of patients. With the advent of antibiotic therapy, mortality has fallen to less than 5% (156). With successful therapy, GFR typically is at its nadir prior to or shortly after the commencement of antibiotic therapy, and it usually improves with eradication of the infection (132). However, patients with advanced renal dysfunction at presentation, may have further deterioration of the GFR, requiring dialysis. The mortality rate is high in this population, and this is most likely related to the combination of severe infection and uremia.

Laboratory Findings

Although there are no pathognomonic findings, many serologic abnormalities are often present in patients with bacterial endocarditis. Rheumatoid factor is variable (i.e., detected in 10% to 70% of patients, depending on the series), however correlation between rheumatoid factor activity and the presence of glomerulonephritis is uncertain (157,158). Although the levels vary considerably, circulating immune complexes are found in 90% of patients with endocarditis, and high levels have been associated with a more indolent course, right sided lesions, and less virulent organisms (132,159–162). While mixed cryoglobulins are present in the serum of up to 95% of patients with endocarditis, there is no correlation between the levels of the cryoglobulins and the presence of glomerulonephritis (141,160). Recently, high titers of antineutrophil cytoplasmic antibody (ANCA) were found in a few patients with bacterial endocarditis-associated vasculitis and glomerulonephritis (163–165). Thus, endocarditis should be excluded in ANCA-positive patients, where either infection is suspected or manifestations of vasculitis are atypical.

Hypocomplementemia (depressed C3, C4 levels) is a frequent, but not invariable or specific, indicator of glomerulonephritis during the course of bacterial endocarditis (140, 150,151,156,160,161). In patients with acute bacterial endocarditis with diffuse glomerulonephritis, the serum complement levels were depressed in 68%. By contrast, hypocomplementemia has been documented more frequently in patients

with diffuse glomerulonephritis associated with subacute bacterial endocarditis (~90%), whereas 60% of those with focal glomerulonephritis had reduced levels (132). Typically C4 and C1q levels are significantly depressed, invoking activation of the classic pathway (140,161). Nevertheless, there have also been reports of primarily alternative pathway activation, particularly in patients with *S. aureus* endocarditis and glomerulonephritis (132). Based on these observations, it was suggested that *S. aureus* bacterial wall antigens may be capable of direct activation of the alternative complement pathway, as described previously for nephritogenic streptococci. In this manner direct activation of complement by *S. aureus* (localized within glomeruli) may lead to glomerulonephritis soon after the onset of clinical endocarditis, without significant glomerular IgG deposition (132,166). Normalization of complement levels usually occurs with bacteriologic cure and resolution of glomerulonephritis. By contrast, persistent hypocomplementemia suggests failure to control infection, which in turn may lead to progressive renal failure (167). Therefore, in patients who initially manifest hypocomplementemia, serial complement determinations may serve as a guide to the activity of glomerulonephritis (132,167).

Differential Diagnosis

In addition to glomerulonephritis, several other renal abnormalities can occur in patients with endocarditis. These considerations are especially important in patients with deteriorating renal function. Embolization of valvular vegetations to the kidney can result in infarction, producing the gross pathologic appearance of "flea-bitten" kidneys (168). The clinical presentation is gross hematuria, sometimes associated with flank pain. Septic emboli may lead to renal abscesses, and endocarditis should always be considered in patients with multiple renal abscesses. Alternatively, septicemia, with or without hypotension, can result in acute tubular necrosis. Thus in patients with endocarditis and acute renal failure, distinguishing glomerulonephritis from other causes may be difficult. Nevertheless, the presence of heavy proteinuria, red blood cell casts, and dysmorphic red blood cells suggests glomerulonephritis. Alternatively, temporal relationship of therapy, the presence of fever, rash and eosinophilia, or eosinophiluria in a patient with sterile pyuria should raise the possibility of hypersensitivity interstitial nephritis. Occasionally, renal biopsy is necessary to distinguish glomerulonephritis and acute hypersensitivity interstitial nephritis.

Treatment and Outcome

Eradication of infection with antibiotic and valve replacement (when appropriate) remain the mainstays of therapy. With control of infection, the urinary abnormalities and mild to moderate degrees of renal insufficiency usually resolve (167). Proteinuria and microscopic hematuria can persist for months and rarely for years after bacteriologic cure. The outcome of patients with severe renal dysfunction is variable, ranging from continued improvement over weeks to months in some, to persistent and progressive renal failure requiring dialysis in others, despite bacteriologic cure. Patients with a high proportion of glomerular crescents in renal biopsy specimens are more likely to have irreversible disease or progressive renal insufficiency (132,167).

The role of immunosuppressive therapy in patients with progressive renal insufficiency, despite optimal antibiotic and surgical treatment, remains controversial. Anecdotal case reports suggested that plasmapharesis, corticosteroids and cytotoxic agents, alone or in combination, may be useful in this situation (143,159,169–173). However, in addition to the usual adverse effects of these agents, this therapy poses the risk of exacerbating the underlying infectious process. Therefore, these agents should only be considered under very specific circumstances. In our opinion, there should be clinical and laboratory evidence of bacteriologic cure, and histologic evidence of acute glomerulonephritis with the absence of overwhelming, severe chronic changes.

Conlon et al recently evaluated the risk of developing renal failure in 204 consecutive episodes of bacterial endocarditis (174). One third developed an elevated serum creatinine (≥2 mg/dL), and there was a fivefold increase in mortality in this subgroup. Factors associated with an increased risk of acute renal failure included: increased age, hypertension, thrombocytopenia, *S. aureus* infection, and prosthetic valve involvement. Although renal biopsies were not performed in the majority of patients in this retrospective analysis (i.e., the cause of renal failure was not determined), it is likely that glomerulonephritis leading to renal failure contributed to their poor outcome.

Shunt Nephritis

Epidemiologic Patterns

Surgically implanted ventriculoatrial, ventriculojugular, and ventriculovenal caval shunts have been commonly used to treat hydrocephalus (175). Overall, infection occurs in 6% to 27% of patients with these ventriculovascular shunts (175). The most common organism isolated is *Staphylococcus epidermidis*, accounting for 75% of infections (131,176). Other organisms that have been isolated include *S. aureus*, diphtheroids, *Listeria monocytogenes*, *Peptococcus* species, *Serratia* species, *Bacillus subtilis, Corynebacterium bovis*, *Gemella morbillorum, Propionibacterium acnes*, and a variety of fungi (177–186). It has been estimated that glomerulonephritis occurs in 1% to 4% of patients with infected shunts (177). In recent years, ventriculovascular shunts have been virtually abandoned and replaced by ventriculoperitoneal shunts. The latter devices are more resistant to colonization and infection, and associated glomerulonephritis is rare (187,188). The majority of cases of shunt nephritis have been reported in children, but rare cases have been documented in adults (175,179,184).

Clinical Manifestations

Symptoms of glomerulonephritis may develop within weeks to years after the shunt placement. Fever is present in nearly all patients with nephritis (175). Arthralgias, malaise, and weight loss are common and suggest infection (142,175). Clinical examination may reveal the presence of purpura, lymphadenopathy, and hepatosplenomegaly, and anemia is often present (131,142). Hematuria is universal, with gross hematuria present in half of the reported cases, and the nephrotic syndrome develops in 30% of patients (175). At initial presentation one-half of the patients have mild to moderate renal dysfunction, one-fourth have normal renal function, while the remainder have severe renal insufficiency (131,175). Hypertension is uncommon.

Laboratory Findings

Nondiagnostic abnormalities are characteristic. Hyperglobulinemia, cryoglobulinemia (associated with elevated rheumatoid factor titers), and raised plasma immune complex levels are usually present, suggesting an immunologic basis for this disorder (175,179,182). Hypocomplementemia, with reduced levels of both C3 and C4, is usually present with shunt nephritis, whereas patients with uncomplicated shunt infection usually have normal complement levels (189). The serum complement levels normalize with successful treatment of the underlying infection and resolution of the glomerulonephritis,

and therefore serial determinations of serum complement may be a useful monitor of the activity of glomerulonephritis. Persistently depressed levels suggest either inadequate therapy or another cause of glomerulonephritis (190).

Blood and cerebrospinal fluid cultures are often positive. However, sometimes the organisms are difficult to grow, and identification of bacteria may be possible only by culture of material from the removed shunt.

Pathologic Features

The glomerular lesions are similar to membranoproliferative glomerulonephritis type I, with endocapillary proliferation, double-contoured capillary walls, and lobular accentuation. A few crescents are occasionally observed (142,149,175). Rarely, diffuse proliferative changes similar to post-infectious glomerulonephritis may be present (191). Granular IgM, IgG, and C3 deposits are usually observed by immunofluorescence, and bacterial antigens have been detected within these deposits. Electron microscopy typically demonstrates subendothelial and mesangial electron dense deposits.

Treatment and Outcome

Because antibiotic therapy alone is usually unsuccessful, treatment should include prompt removal of the infected shunt. Full recovery of renal function has been reported in two-thirds of patients after successful eradication of infection (176,178,189,192). The remaining third, however, demonstrate persistent azotemia and urinary abnormalities (131,175). Rarely, progression to end-stage renal disease has been reported (175). Immunosuppressive therapy has not been reported to be successful in these patients (181).

Visceral Sepsis-Associated Glomerulonephritis

Epidemiologic Patterns

Subacute or chronic infections including intrathoracic and intraabdominal abscesses, osteomyelitis, dental and maxillary sinus abscesses, septic abortions, and aortofemoral bypass graft infections have all been associated with glomerulonephritis (193–196). Glomerulonephritis has also been reported coincident with tuberculosis, pneumococcal pneumonia, *Campylobacter (Helicobacter) jejuni* enteritis, *Salmonella -Schistosoma mansoni* infections, and typhoid fever (193–196).

Clinical Manifestations

Many affected patients have signs and symptoms consequent to the underlying infectious process. They are often severely ill with high fever and weight loss. The interval between the onset of infection and diagnosis of glomerulonephritis varies from 2 weeks to 3 years (193). Rarely, patients present with glomerulonephritis as the initial manifestation of their illness. Common extrarenal manifestations include purpura (usually lower extremities) and arthralgias in approximately one-third of patients, and these phenomena are usually related to circulating cryoglobulins (193,197). Renal manifestations include hematuria, proteinuria, and acute renal failure. Similar to the situation described for glomerulonephritis associated with endocarditis, other causes of impaired renal dysfunction may be present in these patients. However, the presence of proteinuria and hematuria, especially dysmorphic red blood cells and red blood cell casts, usually distinguishes glomerulonephritis from other causes of acute renal failure. Approximately 50% of patients are oliguric and hypertensive (193,197).

Laboratory Findings

Blood cultures are frequently negative, and this has been attributed to the antecedent administration of antibiotics in many cases. Mixed cryoglobulins are usually present at the time of diagnosis and disappear with eradication of the underlying infection (193,197). The serum C3, C4, and CH50 levels are typically normal, unless there is an associated endovasculitis; C3 nephritic factor has occasionally been identified (1,142,198). Circulating immune complexes may be present in some patients. Rheumatoid factor is usually absent (198).

Pathologic Features

Proliferative glomerulonephritis is typical. However, a diverse group of lesions have been reported. These include mesangial proliferative glomerulonephritis, membranoproliferative glomerulonephritis, and diffuse proliferative glomerulonephritis; on occasion crescents are present (142,194,198). Immune deposits consisting primarily of C3 are the most consistent feature on immunofluorescence studies, although IgG and IgM may also be observed in mesangial, subendothelial, or subepithelial locations. While electron microscopic studies have not been commonly reported, electron-dense deposits in the mesangium as well as the occasional presence of subepithelial humps have been observed (131).

Treatment and Outcome

Complete remission of glomerulonephritis is usually achievable with early and complete eradication of the underlying infection (194,196,198). However, delayed and unsuccessful treatment may result in irreversible loss of renal function (198).

Methicillin-Resistant Staphylococcus-Associated-Glomerulonephritis

In 1995, Koyama et al. described 10 cases of glomerulonephritis following methicillin-resistant Staphylococcus aureus (MRSA) infection (199). Subsequently, several case series and reports have confirmed this association. MRSA-associated glomerulonephritis differs from staphylococcal endocarditis induced glomerulonephritis and staphylococcus associated shunt nephritis (200,201) in that serum complement levels are usually normal (albeit the lower limits of normal); there is polyclonal elevation of serum IgA and IgG levels; and IgA is often present within glomerular deposits along with IgG and C3 (202).

In a review of 26 patients with MRSA associated glomerulonephritis, Kobayashi et al. reported that the glomerulonephritis presented an average of 5.4 weeks following the onset of severe MRSA related infection. Approximately 50% of these infections were associated with pleural or abdominal abscesses. The renal presentation was usually that of either RPGN and/or the nephrotic syndrome (200). Thirty percent of patients had leukocytoclastic vasculitis and thrombocytosis was seen in 77%. Renal pathology showed a variety of types of mesangial and/or endocapillary proliferative glomerulonephritis with varying degrees of crescent formation. Immunofluorescence revealed glomerular deposits of IgA, IgG, and C3. There were electron-dense deposits in the glomerular basement membrane, mesangium and capillary walls. S. *aureus* antigens and staphylococcal enterotoxins were not demonstrable.

MRSA-associated glomerulonephritis appears to improve in the majority of cases where antibiotic therapy successfully eradicates the MRSA infection (203). However, some patients do not respond to antibiotic therapy and progress to end-stage renal disease (204). In two cases, corticosteroids were administered following apparent successful treatment of the underlying

MRSA infection (203). In these patients, relapse of the infection occurred, and they later died from sepsis. Therefore, great caution should be exercised before initiating immunosuppressive therapy. In cases refractory to antibiotic therapy, hemoperfusion with polymyxin B–immobilized fiber may be a useful therapy to reduce proteinuria (205).

Recently, isolated case reports have described MRSA infections associated with amyloid A renal amyloidosis, Henoch-Schönlein purpura, IgA nephropathy, and an IgA-dominant acute glomerulonephritis complicating diabetic nephropathy (206–208). These findings underscore the importance of renal biopsy in distinguishing the underlying cause of disease.

Syphilitic Glomerulopathy

Epidemiologic Patterns

Nephrotic syndrome is more common with congenital syphilis (up to 8% of patients) than in secondary forms (<1%) (206–208). Since the advent of mass screening and treatment campaigns, these forms of syphilis are now seen less commonly in developed countries.

Pathologic Features

The most common lesion identified on light microscopy resembles membranous nephropathy (209). In some patients, mild mesangial and endothelial cell proliferation, associated with mesangial deposits of IgG and IgM, may also be present (142). Electron microscopy usually consists of variable thickening of the basement membrane with subepithelial and occasional subendothelial dense deposits. Rarely, the histology resembles lesions associated with APSGN. Treponemal antigen and antitreponemal antibody have been identified within glomeruli of affected patients, which supports their role in the pathogenesis of nephritis (210).

Clinical Manifestations

Affected children with congenital syphilis usually present at the ages of 1 to 4 months with edema and hypertension (209,211,212). Rash and hepatosplenomegaly are common, and the typical radiologic findings associated with congenital syphilis are usually present. Adults most often present with features of the nephrotic syndrome during active secondary syphilis (212). Rarely, acute glomerulonephritis is the principal manifestation of renal involvement.

Laboratory Findings

Positive results on serologic testing for syphilis in the appropriate clinical setting, in association with renal histologic findings, support the diagnosis. Serum complement levels (C3 and C4) are depressed in congenital syphilis, but they are usually normal in adults with secondary syphilis and nephropathy (142). Circulating immune complexes are frequently present during the active stage of syphilitic nephropathy and disappear following successful treatment.

Treatment

Penicillin is the treatment of choice (142). For congenital syphilis, aqueous crystalline penicillin G, 50,000 units/kg/day, is given intravenously in divided doses for 10 days, or penicillin G procaine, 50,000 units/kg/day, is given intramuscularly for 10 days. For secondary syphilis, penicillin benzathine, 2.4 million units, is given intramuscularly (one dose) or penicillin G procaine, 600,000 units/day, is given intramuscularly for 8 days. Proteinuria subsides within 6 weeks of successful therapy in most patients.

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CHAPTER 59 ■ VIRAL GLOMERULAR DISEASES

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There are few citations regarding renal disease associated with viral illness in the classic renal texts of the 1970s. Wilson and Dixon (1) outlined renal diseases associated with hepatitis B virus (HBV) (polyarteritis nodosa [PAN], membranous nephropathy [MN], and membranoproliferative [mesangiocapillary] glomerulonephritis [MPGN]), measles, oncornavirus, Coxsackie B virus, dengue, mumps, varicella, and Epstein-Barr virus infections in humans and animals. The pathogenic link between these viral infections and renal disease, however, often had not been directly established.

An association between HBV and human renal disease was noted in the 1970s, shortly after the characterization of the Australia or hepatitis-associated antigen (HAA) in 1964 (2), and the later identification of the virus. A serum sickness-like disease, associated with HBV infection, characterized by arthritis, proteinuria and hematuria, renal insufficiency, and hypocomplementemia (3,4), was presumptively identified as an immune complex-mediated disease. Immune complex mechanisms, immunodeficiency, and the chronicity of viral infections were thought to be the most important factors in mediating the pathogenesis of renal disease (5). Lack of proper reagents to document the presence of viral antigens in renal tissue and lack of epidemiologic and pathologic screening studies in appropriate populations to link viral infections to glomerular disease were impediments to establishing direct diagnostic associations (5). These latter authors (5) posed the question of how much renal disease was intimately related to the viral infections themselves and suggested that the host response, in particular interferon (IFN) production, might exacerbate nephropathy. Since that time, IFNs have been recognized as not only a component of the host response to viral infection but also a possible treatment for viral illnesses.

In a symposium published in 1990, human renal diseases were directly linked with HBV, human immunodeficiency virus (HIV), hantavirus, yellow fever, mumps, and BK papovavirus infections (6) (Table 59-1). Measles virus and varicella were indirectly associated with the pathogenesis of human renal disease (6). Although its contribution to renal interstitial disease was recognized, the role of cytomegalovirus (CMV) infection in the pathogenesis of glomerular disease, and in particular immunoglobulin A (IgA) nephropathy, was unclear (7). Of note, the virus associated with non-A, non-B hepatitis had not been isolated; therefore, there were no reports of hepatitis C virus (HCV)–associated renal disease. By 1990, the pathogenic mechanisms putatively involved in the development of immune complex renal disease associated with viral infection had been elaborated and partially delineated. In his paper on this subject, Glassock (8) outlined several etiologic possibilities for the pathogenesis of renal diseases associated with viral illnesses, as summarized in Table 59-2.

Pathways by which viruses may cause immunologic renal disease can be broadly classified into circulating and in situ mechanisms. The former mechanisms may involve circulating viral antigens, either deposited in the kidney alone or, classically, bound to antiviral antibodies. Alternatively, circulating endogenous antigens, induced by viral changes in membrane proteins, may be shed into the circulation and induce a host antibody response. Such processes may account for the failure to detect viral antigens in renal tissue of patients with clinically diagnosed viral-associated renal diseases.

The role of *in situ* pathogenic mechanisms of immune complex-mediated nephropathy, originally outlined in models of lupus nephritis and MN (9), has been considered for renal diseases related to viral illnesses. *In situ* processes may be critical in the pathogenesis of renal diseases previously thought to be unassociated with viral infections (10–14). Mechanisms of nephropathogenesis may include viral gene expression in renal tissue or viral antigen binding to renal cells, which induces a secondary host humoral-mediated or cell-mediated immune response. These may include the synthesis of new host proteins, such as cytokines and chemokines (13,15–22) which may initiate or amplify local immune responses.

In clinical settings, it is often difficult to prove a pathogenic relationship between the renal disease and the postulated causative infectious agent in glomerulonephritides associated with viral illnesses. Clinical criteria to establish causality include the demonstration of improvement in renal disease simultaneously with clearance of the suspected antigen or the recurrence of glomerulonephritis after antigenic rechallenge or reinfection with a particular virus. Detection of specific immune complexes in the circulation supports an association, but to establish a diagnostic causal linkage with glomerulonephritis, viral antigens and host antibodies must be identified in the mesangium or glomerular capillaries in higher concentration than in the circulation (8,12,13).

Our expertise as diagnosticians has certainly advanced compared to our relatively limited capability only three decades ago. Better understanding of the etiology and pathogenesis of renal disease depends on precise identification of clinical syndromes and infective agents, improved serologic diagnosis (which requires knowledge of viral structure and immune response), epidemiologic characterization of infected populations (which is dependent on proper screening studies), and ultimately, rigorous virologic and molecular analysis of pathologic tissue (Table 59-3). Understanding these factors is critical to permit the development of rational and effective treatments. Current technology permits us to move beyond the association of viral illnesses solely with immune complex mechanisms.

The present armamentarium of tools used to address diagnostic issues is vast, and includes classic histochemical methods as well as molecular biologic approaches. Microdissection and the polymerase chain reaction (PCR) can be used to detect and amplify viral nucleic acid sequences in tissue (23–25). In situ hybridization techniques and in situ PCR technology are used to localize viral sequences in pathologic tissue (26–28), and the development of transgenic animal models has added a wealth of information to the study of renal diseases (28–31) (Table 59-4).

TABLE 59-1

VIRAL INFECTIONS MEDIATING RENAL DISEASE

Hepatitis B virus
Hepatitis C virus
Human immunodeficiency virus
Cytomegalovirus
Hantavirus
BK papovavirus
JC virus
Yellow fever
Mumps
Measles
Varicella
Others

The expression of renal disease associated with viral infection is likely to be affected by the host response (Table 59-5). Factors that may modulate expression of renal disease include the patient's age, gender, genetic background, and immunologic responsiveness (including the extent and duration of humoral as compared to cell-mediated responses). A humoral response resulting in the development of cryoprecipitable immune reactants, a characteristic of the response to viral infections (32–36), may have late sequelae that seem unrelated to the original viral illness. Socioeconomic status and access to care may also ultimately modify organ responses to viral infections. An important question to be considered is whether a virus of interest infects renal cells. The expression of viral proteins or abnormal host proteins in the kidney may result in the development of renal disease through various pathways. Viral proteins may engender cell death through necrosis or apoptosis, or may cause cell dysfunction (13,16,17,37,38–41). Alternatively, such proteins might increase the propensity to develop circulating or *in situ* immune-mediated pathogenic mechanisms or might lead to increased matrix synthesis and/or decreased matrix degradation (13,16,18,19). Selected viral or host proteins could induce the synthesis of cytokines, chemokines, growth factors, or adhesion molecules, which may result in the development or worsening of nephropathy (11,13,16–22). Finally, some renal diseases seen in patients with viral infections may be related to treatment.

TABLE 59-2

PATHOGENESIS OF IMMUNE COMPLEX NEPHROPATHY IN VIRAL DISEASES

Circulating immune complex disease involving viral antigens and host antiviral antibody
Circulating immune complex disease involving endogenous antigens released by viral-induced injury to cells and host-autoantibody
In situ immune complex disease involving viral antigen binding to glomerular structures and host antiviral antibody or cell-mediated immunity
Autoimmune reactions to host glomerular structures induced by viruses (e.g., induction of self-reactive T or B cells, release of sequestered antigen, "molecular-mimicry," or activation of idiotype–antiidiotype networks involving "peptidic self")
Virus induced activation of cytokines and/or cell adhesion molecules
Direct cytopathogenic effect of virus on glomerular cells

(From: Glassock RJ. Immune complex-induced glomerular injury in viral diseases: an overview. *Kidney Int* 1991;40(Suppl 35):S-5, with permission.)

TABLE 59-3

STEPS INVOLVED IN ELUCIDATING PATHOGENIC MECHANISMS OF VIRAL-INDUCED NEPHROPATHY

1. Identification of clinical syndromes and infective agents
2. Serologic diagnosis—dependent on knowledge of viral structure and immune response
3. Epidemiologic characterization of infected populations by proper screening studies
4. Virologic, immunologic, and molecular analysis of pathologic tissues

Cryoglobulins, which are part of the spectrum of the host response to viral infection, are immunoglobulins that precipitate in the cold (32–34,36,42–44). Cryoglobulins are classified, according to Brouet et al. (33), into three types. Type I cryoglobulinemia is characterized by a monoclonal immunoglobulin, usually IgM, and is usually associated with either myeloma or Waldenström's macroglobulinemia. Mixed cryoglobulinemia (types II and III) is defined by the presence of cryoprecipitable circulating immune complexes of at least two different immunoglobulin classes. One of the immunoglobulins must be polyclonal. Type II cryoglobulinemia usually consists of a monoclonal IgM-κ with rheumatoid factor activity, and a polyclonal IgG. Type III cryoglobulinemia consists of more than one polyclonal immunoglobulin, of which one, usually IgM, has rheumatoid factor activity. Both forms of mixed cryoglobulinemia have been associated with a variety of illnesses eliciting an immune response (e.g., viral, bacterial, parasitic, spirochetal, and fungal infections and lymphoproliferative disorders) (32). Until a little more than a decade ago, many cases were termed "essential" since no underlying cause could be identified. Since the availability of serologic testing to diagnose HCV infection, many cases are now thought to be the result of HCV infection (45–47). The syndrome of mixed cryoglobulinemia includes weakness, palpable purpura (a physical finding suggestive of vasculitis), Raynaud's phenomenon, leg ulcers, arthritis, peripheral neuropathy, and liver function test abnormalities. Renal disease associated with cryoglobulinemia has long been recognized (48) and occurs in approximately half the cases of cryoglobulinemia (49–51). MPGN is the classic expression of cryoglobulinemia-associated renal disease (52), characterized by glomerular endocapillary proliferation, intraluminal thrombi, double-contoured thickening of the glomerular basement membrane, small- and medium-sized vessel vasculitis, and glomerular and interstitial mononuclear cell infiltration.

The pathophysiology of renal disease associated with mixed cryoglobulinemia remains incompletely delineated, since no correlation has been found between the quantity of cryoglobulins in serum and the presence and severity of renal disease. It has been postulated that the renal lesions could result from

TABLE 59-4

VIRUS HUNTING IN THE KIDNEY

Microdissection and the polymerase chain reaction (PCR)
In situ hybridization/PCR techniques
Transgenic animal models

TABLE 59-5

INFLAMMATORY VIRAL RENAL DISEASE

Host factors
Age
Gender
Genetic background
Immune status
Socioeconomic status
Access to care
Immunologic factors
Classic immune complex disease
Host antiviral antibody reactive with viral antigens
Host antibody reactive with circulating endogenous antigens induced by viral changes in host membrane proteins shed into the circulation
In situ mechanisms of nephropathogenesis
Viral gene expression in renal tissue
Abnormal host protein expression in renal tissue
Viral antigen binding to renal cells with subsequent host antibody- or cell-mediated immune response
Host chemokine and cytokine expression

the interaction of antibodies either with circulating antigens deposited in the kidney or with renal structural antigens. Support for the latter hypothesis includes the observation that solubilized mixed cryoglobulins from patients with cryoglobulinemia and renal disease resulted in the development of MPGN when injected into mice. The IgM monoclonal component of these cryoglobulins was found to be deposited in glomeruli, suggesting *in situ* immune complex formation. Subsequently, a candidate antigen in kidney tissue was identified as alpha-enolase, a glycolytic enzyme present on renal tubular cell membranes that functions to catalyze the dehydration of 2-phosphoglycerate to phosphoenolpyruvate (53). Subsequent studies will be necessary to elucidate more completely the role of the interaction between cryoglobulins and structural antigens in the kidney in the pathophysiology of renal disease associated with cryoglobulinemia.

Current diagnostic and investigative technologies will allow us to use renal diseases caused by viruses as models to answer questions about and elucidate pathogenic mechanisms regarding nephropathies associated with viral infections, as well as disorders not previously linked with viral infections. Such tools will ultimately allow us to understand the complex and probably multiple reasons that some viruses are nephropathogenic while others are not.

HEPATITIS B VIRUS–ASSOCIATED GLOMERULONEPHRITIS

Glomerular disease resulting from HBV infection has been said to be the prototype of viral-induced glomerular injury (3). Much of the information known about injury associated with HCV infection and human immunodeficiency virus (HIV) infection has resulted directly from the information gleaned from the study of HBV infection.

HBV infection is one of the most common infectious disorders in the world. It has been estimated that the incidence of infection is greater than 50 million persons per year and that the prevalence may exceed 1 billion persons (54). Infection results in transient and minor illness in most persons, who become free of virus after a period of several weeks. A minority of patients develop chronic sequelae of HBV infection, including chronic hepatitis, cirrhosis, and hepatocellular carcinoma. A few patients develop complications related to the presence of circulating HBV-associated antigens in the circulation and the antibody response(s) that they evoke. The circulating immune complexes give rise to a variety of disorders reminiscent of those seen with serum sickness, including several different forms of glomerulonephritis, as well as a number of vasculitic disorders. Other patients may develop complications from the deposition of HBV-associated antigens in tissues, leading to antibody binding and injury from immune mechanisms. Either circulating or deposited antigens may evoke a wide array of responses independent of classically defined immune mechanisms. The heterogeneity of responses associated with HBV infection is not consistent with a simplistic view of the mechanism of injury. Unfortunately, investigators have focused largely on descriptive studies. There are few studies that delineate pathogenesis and even fewer that address the issue of treatment strategies for HBV-related renal disorders.

The HBV is a DNA virus of the hepadnavirus family, and infection is restricted to humans and nonhuman primates. It is depicted schematically in Figure 59-1 (55). The complete virus is known as the Dane particle, a 42-nm spherical structure consisting of an envelope of viral-encoded proteins and lipid components derived from the host. The envelope contains the hepatitis B surface antigen (HBsAg), against which neutralizing antibody (HBsAb) is directed. The inner core of the virion contains the hepatitis core antigen (HBcAg), viral DNA, and the DNA polymerase protein. The virus also produces 22-nm dimorphic spherical and filamentous particles that are composed of envelope proteins only. Since these particles do not contain the HBV genome, they are noninfectious. They are the most abundant forms of virus particles in the circulation, and are present in the circulation in concentrations several logs greater than intact viruses. The genome of HBV is semicircular, partially double-stranded DNA approximately 3,200 base pairs in length. HBV is classified into seven genotypes (A to G) based upon differences in the complete nucleotide sequence. The clinical significance of the genotypes is not yet clearly delineated (56).

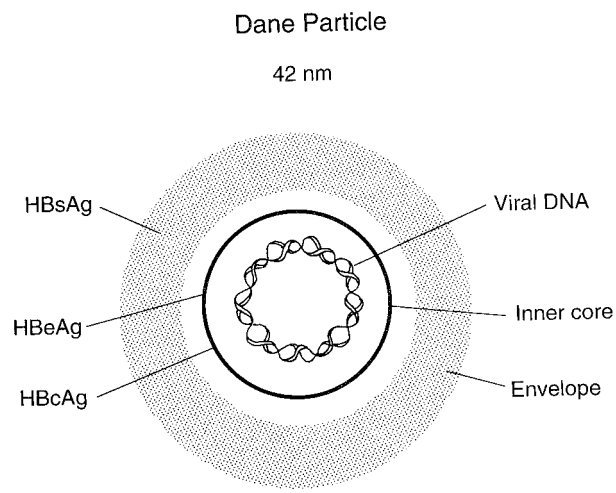

FIGURE 59-1. Schematic depiction of hepatitis B virus. The Dane particle consists of an envelope, inner core, and circular, double-stranded DNA. Several of the viral proteins may be found in the circulation of infected patients. Antibodies directed against all the depicted viral antigens may be found in the patient's circulation at different stages of the infection. *HBcAg*, hepatitis B virus core antigen; *HBeAg*, hepatitis B virus e antigen; *HBsAg*, hepatitis B virus surface antigen; *HBV*, hepatitis B virus; *viral DNA*, double-stranded HBV DNA.

Patients infected with HBV express three antigenic proteins to which antibodies may be formed: HBcA, giving rise to anti-HBcAg (HBcAb); hepatitis B e antigen (HBeAg), against which anti-HBeAg is formed (HBeAb); and HBsAg, which evokes generation of anti-HBsAg (HBsAb). If the patient is immunocompetent, generation of antigen-specific antibody results in clearance of the antigen and resolution of infection. Circulating antibody becomes a marker of previous infection. If infection is prolonged, so that antigenemia is chronic, immune complexes may form and be detected in the circulation. The pathogenicity of the immune complexes depends in part on their size and electrical charge and the physicochemical factors that result in lattice formation (57).

The molecular biology and immunology of the HBV have been extensively reviewed (56–59). There are four open reading frames that encode the envelope, core/pre-core, polymerase, and X proteins. Envelope proteins include the large (L), middle (M), and small (S) envelope proteins. The M and S envelope proteins are found in all forms of viral and subviral particles. The L envelope protein is found predominantly in complete virions. Proteins encoded by the core/pre-core reading frames include a precore polypeptide which is posttranslationally modified into a soluble protein, HBeAg. Further translation produces the core protein (HBcAg). The open reading frame encoding the polymerase overlaps with the core, envelope, and X reading frames. The polymerase protein consists of a protein primer, a spacer, a reverse transcriptase/DNA polymerase, and an RNAase H domain. The X protein, which is not essential for viral replication, is a transcriptional transactivator of many promoters including HBV and cellular oncogenes. The HBx protein has been implicated in hepatocarcinogenesis.

The HBsAg has a molecular weight of 3.5 to 4.4 million daltons, with its antigenic determinants expressed on peptides that are considerably smaller (22,000 to 49,000 daltons) (60). The inner core of HBV contains HBcAg, which consists of multiple copies of a 22-kd molecular weight protein (p22). HBcAg is not found in the circulation. HBeAg is a 15-kd protein that can be released from the core antigen by treatment with proteolytic enzymes. The HBeAg can circulate in a variety of forms: as a 30-kd dimer bound to albumin or as a hexamer bound to specific immunoglobulin G (IgG) antibodies (anti-HBeAb). HBeAg-IgG is a large complex, with a molecular weight of 240,000 to 540,000 daltons and a cationic isoelectric point. There is substantial evidence implicating the albumin-bound "small" HBeAg and IgG-associated "large" HBeAg in the pathogenesis of glomerular disease. The presence of any form of the HBeAg in the circulation correlates with the presence of the intact virus in the circulation and is indicative of infectivity. The presence of HBeAb connotes viral clearance and subsequent immunity.

The prevalence of chronic HBV infection, particularly as assessed by chronic HBsAg antigenemia, varies widely according to geographic area. It varies from 0.1% to 2.0% in low-prevalence areas (United States and Canada, Western Europe, Australia and New Zealand), to 3% to 5% in intermediate-prevalence areas (Mediterranean countries, Japan, Central Asia, Middle East, and Latin and South America), to 10% to 20% in high-prevalence areas (southeast Asia, China, and sub-Saharan Africa (54,61).

The etiology of the infection also varies according to the area of study. In adults, the transmission of infection is often horizontal and is frequently accompanied by an episode of clinically apparent infection. Since blood transfusions in developed countries are an increasingly uncommon cause of HBV infection, infection in adults often results from the communal use of needles among intravenous drug users or as a sexually transmitted disease. Conversely, HBV infection in children is more often transmitted in a vertical pattern, passing from mother to child. It has usually been thought that such children are asymptomatic and have no history of overt hepatitis (62). Bhimma et al. examined the HBV status and measured proteinuria in family members and household contacts of index children with HBV-associated MN. These investigators found that 37% of the subjects were HBV carriers, and 27% had abnormal proteinuria. The prevalence of proteinuria was much higher than in community-based control subjects, although it was not dependent upon carriage of HBV (63).

There are several defined renal clinicopathologic disorders associated with HBV infection, including a number of different forms of glomerular disease and a variety of serum sickness-like and vasculitic illnesses. Although these disorders are not uncommon, they do not occur or are not detected with a frequency commensurate with the prevalence of HBV infection in the population. Therefore, they probably do not result from HBV infection alone, but from a combination of the infection and the immunologic responses of the host.

HBV infection has been thought to result in glomerular disease through immune complex mechanisms since the early descriptions of HBV-associated renal disease. At least one of these mechanisms involves deposition of an antigen in renal tissue, followed by binding of a strategically placed antibody, which results in complement fixation and immunologic injury. Alternatively, circulating immune complexes composed of HBV antigens and antibodies may deposit in glomeruli and result in nephropathy. Current concepts regarding the pathogenesis of HBV-associated nephropathy, however, are considerably more complex. While it is clear that HBV results in renal damage through circulating immune complex mechanisms in certain instances, there are other pathogenic pathways that remain incompletely understood. In addition to immune complex deposition, other potential pathways include a direct cytopathic effect induced by the virus, viral-induced specific effector mechanisms (specific T cell or antibody) which damage the kidney, or renal injury mediated through viral-induced cytokines or other mediators (64). While we have long been aware of the association between HBV infection and renal disease, our understanding of the pathophysiology of this relationship is still inadequate.

Several glomerular diseases have been associated with HBV infection. In most reports, MN has been the most frequent pathologic lesion encountered in patients with HBV infection and renal disease. MPGN has been noted less frequently, as has IgA nephropathy. Two other disorders with renal manifestations are closely associated with HBV: PAN and cryoglobulinemia.

Hepatitis B Virus and Membranous Nephropathy, Membranoproliferative Glomerulonephritis, and Immunoglobulin A Nephropathy

MN was the first renal disorder to be associated with HBV. Combes and co-workers (65) reported a 53-year-old man who presented with nephrotic syndrome 18 months after receiving a blood transfusion. Four months after the transfusion, he developed jaundice, and circulating Australia antigen was detected. Nearly 2 years later, this man was found to have proteinuria and underwent renal biopsy. The biopsy revealed diffusely thickened glomerular capillary basement membranes and mesangial hypercellularity, with ultrastructural changes typical of MN. The authors demonstrated staining for the Australia antigen with indirect immunofluorescence using guinea pig antibody reactive against commercially obtained Australia antigen and rabbit anti-guinea pig antibody. They concluded that these findings were highly suggestive that the renal disease was caused by deposition of Australia antigen-containing

immune complexes in the kidney, although they were not able to elute such complexes from renal tissue.

Subsequently Kneiser et al. (66) reported three patients with chronic HBV infection, each of whom had a different form of glomerulopathy (i.e., MPGN, focal glomerulosclerosis, and MN). HBsAg was detected in each patient's renal tissue by indirect immunofluorescence using rabbit anti-HBsAg and guinea pig anti-rabbit globulin. The authors emphasized the similarities between the morphologic findings in their patients and those in the studies of circulating immune complex induction of glomerulonephritis performed by Oldstone and Dixon (67). That HBV MN was a circulating immune complex disease was supported by the studies of Kohler et al. (68), who reported a 42-year-old man with chronic active hepatitis and MN. HBsAg was detected by indirect immunofluorescence in glomerular capillary walls. The patient's serum contained 10 mg/dL of cryoprecipitate composed of antibody directed against HBsAg as well as the HBsAg. The demonstration of HBsAg in renal tissue, as well as the detection of circulating immune complexes containing both antigen and its antibody, was convincing evidence of the pathogenic role of circulating immune complexes in HBV MN. The concept that circulating immune complexes, consisting of the HBsAg and its antibody, could localize in glomeruli and result in disease became a well-accepted paradigm.

Subsequently, however, Couser and Salant (9) proposed that immune complexes could be formed in situ, offering a mechanism for immune complex injuries that did not require them to be in the circulation. Simultaneously, the complexity of the structure of the HBV became more apparent (9). It seemed obvious that at least some of the components of the HBV, because of their size or charge, would not be able to traverse the glomerular capillary basement membrane to localize in the subepithelial space. It was more likely, and consistent with the hypothesis of *in situ* immune complex formation, that an alternative potential mechanism of HBV-associated glomerular injury was the deposition of HBV-associated antigen in the glomerular capillary, followed by antibody deposition. A second mechanism might involve the formation of new antigens from renal tissue damaged by HBV. These alternative hypotheses for the pathogenesis of HBV-associated glomerular disease served to address two areas of concern. First, the subepithelial location of immune deposits thought to be HBV antigens and accompanying antibodies in renal biopsy material seemed improbable because the deposits were too large. Second, circulating immune complexes could not be found in the majority of specimens (69–71).

The clinical manifestations of HBV-associated MN have been reported in several large, older series (72–75). In most reports, the diagnosis of HBV-associated MN was based on one or more of the following criteria: persistent HBV-associated antigenemia, absence of evidence of other causes of glomerulonephritis, and detection of at least one HBV antigen in renal tissue. The majority of patients reported were seropositive for at least one HBV-associated antigen, usually HBsAg. Inclusion of patients without other glomerular diseases was determined on clinical grounds.

The detection of HBV-associated antigens in renal tissue was more problematic. Many cases reported as HBV-associated MN did not have any HBV-associated antigen detected in renal tissue, and few investigators reported elution of immune complexes or HBV antigen-specific antibody from renal tissue. Thus, the clinical criterion used to define HBV-associated nephropathies in many early clinical studies was, in fact, simply the occurrence of renal abnormalities in a patient with HBV infection. For example, Lai et al. (72) reported 25 patients with MN from a cohort of 74 patients with HBsAg antigenemia and a variety of different forms of glomerulonephritis, of which MN was the most common (35%). All biopsies revealed the ultrastructural changes typical of MN. The investigators attempted to detect HBsAg, HBcAg, and HBeAg using immunoperoxidase techniques with polyclonal monospecific rabbit antihuman antisera on paraffin sections of renal tissue, or with indirect immunofluorescence with the same antisera on frozen sections of renal tissue. They were only able to detect renal tissue HBcAg in three patients, HBsAg in two patients, and both renal tissue HBcAg and HBsAg in two patients. Given the inability of the investigators to detect HBV-associated antigens in renal tissue, this study could be interpreted as simply a description of patients with HBV-associated antigenemia with incidental MN. Other studies were similar. Lee and others (74) reported 87 patients with chronic HBsAg antigenemia who had glomerular disease. Twenty-nine patients had MPGN, and 18 had MN. These 47 patients were said to have HBV-associated glomerulonephritis. However, the authors could not detect HBsAg in the six biopsy samples tested and did not test for other HBV-associated antigens.

Moreover, while HBV-associated antigens were absent from the tissue of many of the HBsAg-positive patients thought to have HBV-associated renal disease, HBsAg was reported in glomerular deposits in patients seronegative for HBsAg, leading to further confusion about the importance that HBV-associated antigens might play in the pathogenesis of glomerular injury (75,76).

In virtually all reports in which HBsAg was detected in renal tissue, it was detected by indirect immunofluorescence. This technique involved raising antibody to the HBsAg or other HBV-associated antigens in rabbits and layering the immunoglobulin on the renal tissue of interest. Then, anti-rabbit antibody was used to detect whether binding of the anti-HBV antibody by renal tissue had occurred. The specificity of staining in this technique should be confirmed by using anti-rabbit antisera as a control. However, so few studies of putative HBV MN had detectable renal tissue HBV-associated antigens determined by rigorous criteria that Magiore and co-workers (77) questioned the specificity of staining for HBV-associated antigens in renal tissue. These investigators systematically examined the problem of antibody specificity and determined that endogenous immunoglobulin M (IgM) antibody in glomerular tissue had antiimmunoglobulin activity that resulted in false-positive staining for HBsAg. They demonstrated improved specificity with the use of fluoresceinated $F(ab')_2$ probes prepared from commercial rabbit antisera. They performed studies that clearly demonstrated that methods that did not use $F(ab')$ probes to detect HBV-associated antigens in renal tissue resulted in false-positive staining for these antigens. Thus, much of the clinical literature, including reports of patient material identified as HBV-associated glomerulonephritis, which had been published prior to the Magiore report, was of limited heuristic value.

Subsequently, Hiroshi et al. (78) devised a method of detecting HBV antigens in renal tissue that abrogated the problem of false-positive staining. At the same time, these authors demonstrated that the principal HBV antigen involved in HBV MN was the HBeAg, rather than the HBsAg. They immunized BALB/c mice with plasma containing HBeAg and high titers of HBsAg. Spleen cells were harvested and fused with mouse myeloma cells. Subsequently, clones yielding anti-HBe were grown in ascitic mice, and the resultant gamma globulin fraction was precipitated with acid. This monoclonal antibody was digested with pepsin to obtain $F(ab')_2$ fragments, which were then conjugated with fluorescein isothiocyanate and used to stain tissue. These monoclonal antibody fragments were shown to be quite specific by blocking and inhibition studies. Immune deposits in the glomeruli of 10 of 16 patients with MN, seropositive for HBsAg, were positive for HBeAg. None of the deposits was positive for HBsAg or HBcAg. Moreover, of the 10 patients with glomerular deposits of HBeAg, nine were

seropositive for HBeAg, and the one that was not had circulating anti-HBeAb. These studies confirmed that previous studies using conventional immunofluorescence were flawed. They also demonstrated that HBeAg, not HBsAg, was the principal antigen associated with HBV MN.

HBeAg had previously been found to be a part of the HBV capsid and to exist in both small and large forms (79). Subsequently, its participation in the pathogenesis of HBV-associated renal disease was reported by Takekoshi et al. (80). These investigators studied two children seropositive for both HBsAg and HBeAg who developed nephrotic syndrome, in both of whom renal biopsy revealed MN. The authors detected two forms of HBeAg in the serum: a small form, and a larger form bound to IgG. They also detected HBeAg in the same pattern as IgG in renal tissue, which did not stain for other HBV antigens. Through an elegant series of blocking and inhibition studies, the authors ensured the specificity of the detection of HBeAg. This reemphasized the importance of HBeAg in the pathogenesis of HBV-associated MN. These investigators also helped to delineate the relationship between the nature of the HBV-associated antigen and the propensity to develop glomerular disease. They noted that the molecular weight of HBeAg, even if bound to IgG, was substantially less than that of HBsAg and concluded that HBeAg, because of its size, was far more likely to localize in the subepithelial space than the very large HBsAg.

Subsequently, Lai and co-workers (81) studied 21 patients with adult-onset HBV MN. These patients had persistent HBsAg antigenemia and proteinuria greater than 1 g/day. The diagnosis of HBV-associated glomerulonephritis was restricted to only those patients with glomerular capillary deposition of a HBV antigen detected by F(ab) monoclonal antibody fragments. None of the patients had a history of hepatitis, although there was either historical or serologic evidence of HBV infection in some relative in the majority of their families. There were 17 males and 4 females, with a mean age of 30 years (range, 15 to 53 years). Twelve had nephrotic syndrome, 7 had asymptomatic proteinuria, and 2 had chronic renal failure. The duration of symptoms ranged from 1 to 36 months. Mean protein excretion was 4.1 g/day (range, 1.0 to 12.5 g/day). One-fourth of the patients had some impairment of renal function, while one-third were hypertensive at the time of presentation. All biopsy specimens revealed electron microscopic subepithelial electron-dense deposits, although virus-like particles were seen in only one specimen. The investigators searched for HBV-associated antigens with indirect and direct immunofluorescence using $F(ab')_2$ fragments of murine monoclonal and polyclonal antibodies and immunoperoxidase techniques. Sixteen of the patients had granular deposits of HBeAg detected in capillary walls. The other five patients had an HBV-associated antigen in biopsy tissue, although the specificity of the polyclonal antibody resulted in some crossreactivity between HBcAg and HBeAg. Polyclonal antibodies had previously been shown to be less specific than monoclonal antibodies when used to detect HBV-associated antigens in kidney tissue (82). This was the first study to describe patients with HBV-associated MN in which diagnostic criteria restricted entry to only those patients with specifically detected HBV-associated antigen(s) in the appropriate anatomic location in renal tissue.

Using these more restrictive diagnostic criteria, Lai and co-workers (83) subsequently reported a large series of patients with HBV-associated glomerular disease. They studied 100 consecutive patients who presented from 1985 to 1992 with primary glomerulonephritis who were HBsAg seropositive. Thirty-nine patients had at least one HBV-associated antigen in renal tissue as detected by monoclonal antibodies. Of these 39, HBsAg was detected in renal tissue in 21, HBcAg in 2, and HBeAg in 27. The glomerular pathologic descriptions were heterogeneous and are depicted in Table 59-6. Of the 21 patients with glomerular HBsAg, MPGN with immunofluorescent features characteristic of IgA nephropathy was the most common lesion, with MN a distant second. Several patients had combination lesions, such as MN and IgA nephropathy, or MN and MPGN. There were two patients with glomerular HBcAg: 1 with MN and 1 with IgA nephropathy. Twenty-seven patients had glomerular HBeAg, of whom 15 had MN, 1 had IgA nephropathy, and 2 had MPGN. The remainder had combinations of lesions, such as MN–IgA nephropathy and MN–MPGN.

TABLE 59-6

HEPATITIS B VIRUS-ASSOCIATED GLOMERULOPATHIES: TISSUE IMMUNOREACTANT DIAGNOSES

Tissue immunoreactant	Pathology
HBeAg	MN; mixed lesions
HBsAg	IgA nephropathy; MN; mixed lesions
HBcAb	MN; IgA nephropathy[a]

[a]Two patients only.

This study is notable for several reasons. First, it is the largest description of patients with HBV-associated glomerulonephritis in which the essential diagnostic criterion is detection of HBV-associated antigens in glomerular tissue by the use of monoclonal antibodies. Second, it clearly demonstrates that HBV-associated glomerulonephritis has diverse morphologic presentations, although three lesions appear to be most common. They include MN, MPGN, and IgA nephropathy. The previously widely accepted concept that most HBV-associated glomerulonephritis is MN has been changed because it is now clear that HBsAg is most commonly associated with mesangial proliferative lesions, while HBeAg is most commonly detected in cases of MN. Third, the study underscores the relative infrequency with which the HBcAg is associated with glomerulopathy. Finally, the study demonstrates that combination lesions, such as MN–IgA nephropathy or MN–MPGN, are not uncommon.

Lai and co-workers extended these observations using molecular biologic techniques to corroborate their findings with monoclonal antibodies. They used in situ hybridization and PCR techniques to probe for HBV DNA and RNA in renal biopsy tissue from 40 chronic HBV carriers with a variety of morphologic presentations, including MN, MPGN, and IgA nephropathy. They found HBV DNA to be located mainly in the cytoplasm of proximal tubular cells rather than glomeruli. HBcAg RNA was found in the nuclei and cytoplasm of both glomerular and tubular cells in 56%, 20%, and 36% of renal biopsies showing MN, MPGN, and IgA nephropathy, respectively. These findings, in which viral transcripts were found in renal cells, support even more strongly an etiologic role for HBV in the pathogenesis of renal disease (84). Similar data were provided by He et al. in a study of children (85).

The availability and use of monoclonal antibodies and molecular techniques to detect HBV-associated antigens in renal tissue has allowed investigators to be more confident that the glomerulonephritis seen in patients with HBV is really HBV-associated glomerulonephritis. Moreover, it has allowed investigators to define clinical populations more precisely. This led to the ability to observe not only the natural history of HBV-associated glomerulonephritis but also, in certain instances, patients' responses to therapy. Multiple studies in the era before

precise definition of HBV-associated glomerulonephritis was possible suggested that the nephrotic syndrome usually remitted in 30% to 60% of patients, while the remainder had a more indolent course. Lai and co-workers (72) had previously noted that, in a study of 69 patients with HBV-associated glomerular disease, 14% had progressive renal failure, although none reached end-stage renal disease (ESRD).

In one large clinical study with follow-up that defined patients with HBV MN more precisely, Lin (86) studied 34 children (25 boys, 9 girls) with HBV-associated MN, in whom F(ab′) fragments of monoclonal antibodies were used to detect HBeAg in the glomeruli from 30 (88%). All but 4 children had nephrotic syndrome, and all were seropositive for HBsAg; all but 2 were seropositive for HBeAg. Many patients were hypocomplementemic during the first 6 months of their renal illness, although serum complement concentrations became normal in most after several months, independent of whether the renal disease remitted. Serum levels of circulating IgM and HBsAg immune complexes were elevated initially but returned to values not different from those of seropositive controls without renal disease on remission of proteinuria. Four patients progressed morphologically, with the MN lesion having changed from stage I or II to stage III. One child had impaired renal function. Ito and others (87), in an earlier report in which serial renal biopsies of 6 children with HBeAg-associated MN were described, noted regression of the pathologic features of MN and regression of proteinuria in 2 patients who seroconverted from HBeAg-positive to HBeAb-positive. Second biopsies confirmed the absence of HBeAg in patients in whose kidney it had previously been detected, as well as resolution of deposits in glomerular basement membranes. The suggestion that seroconversion from antigen positivity could result in resolution of glomerular disease had previously been made by Knecht and Chisari (88), who reported a patient who was seropositive for HBsAg and had HBV-associated MPGN. The patient's nephrotic syndrome resolved in conjunction with loss of HBsAg seropositivity.

There are no large-scale follow-up studies in adults in whom HBV MN has been properly defined. At present it appears that HBV-associated renal disease has a varied natural history, although so few precisely defined patients have been longitudinally studied that generalizations about natural history are of limited value. Progression to ESRD does, however, appear to be uncommon, at least as is reflected in the literature. Seroconversion from HBV antigen positivity may be associated with a more favorable renal prognosis, although there are limited data to support a more definitive statement about seroconversion.

Treatment

The treatment of HBV MN has been the subject of largely anecdotal reports because there are virtually no adequately designed prospective controlled trials. In parallel with the enthusiasm afforded corticosteroids (CS) in the treatment of idiopathic MN, some patients with HBV infection have been treated with CS in attempts to induce remission of their renal disease, usually the nephrotic syndrome. In the previously mentioned study by Lin (86), 32 patients were treated with CS. One had an early response, while 11 had persistent nephrotic syndrome. Thirteen patients responded but had frequent relapses. Four patients sustained complete remission months after completion of the CS regimen, without clearing HBsAg or HBeAg from the circulation.

Following this study, Lin (89) extended his observations in this same cohort and demonstrated that treatment with CS might be harmful. He harvested macrophages, B cells, and T cells from some of these patients and performed Southern blot hybridization using EcoRI digestion of an HBV-plasmid clone, pTWL1 (90). In one patient who had not received CS, HBV DNA was found in macrophages, T cells, and B cells. Six months later, the HBV DNA was no longer detectable. In a patient who had received CS, cellular HBV DNA was detectable 3 years later. Moreover, dexamethasone was able to stimulate production of both HBsAg and HBeAg from peripheral blood mononuclear cells. Lai et al. (91) had observed a similar effect of CS therapy to enhance viral replication in a study of eight patients with HBV-associated MN. HBV DNA was determined in serum by dot blot hybridization using an oligonucleotide probe (92). Five of the six patients treated with CS and studied by this technique had an increase in serum HBV DNA, which decreased when CS therapy was discontinued. No favorable effect of CS was noted on any renal parameter when compared to control patients treated with diuretics alone.

The unsatisfactory results of treatment with CS prompted a search for more effective regimens. Scullard et al. (93) reported the disappearance of HBcAg, HBeAg, and HBsAg from the sera and livers of patients treated with IFN and the antiviral agent adenine arabinoside. This prompted a few reports of treatment of patients with HBV-associated glomerular disease with IFN. Garcia and co-workers (94) treated two patients, one adult and one child, both of whom had MN associated with seropositivity for HBsAg, with leukocyte IFN. The adult was treated for 4 weeks, with a rapid fall in DNA polymerase activity, loss of HBsAg and HBeAg, development of HBeAb, and resolution of nephrotic syndrome. However, despite the continued absence of HBV antigens, the adult patient's nephrotic syndrome recrudesced 7 months after the IFN was stopped. The child had a relapsing course, accompanied by a fall in DNA polymerase activity but no change in his HBV antigen status. After several courses of IFN, the child's nephrotic syndrome remitted, although he remained seropositive. This experience prompted Lisker-Melman and co-workers (95) to prospectively administer recombinant human IFN to five patients seropositive for HBsAg, HBeAg, HBV DNA, and DNA polymerase who had MN (four patients) or MPGN (one patient). Patients were treated with 5 million units of IFN subcutaneously daily for 4 months. Serum levels of HBV DNA and DNA polymerase activity decreased in all five patients, and four became negative for both these markers as well as for HBeAg; three of the four became seropositive for HBeAb. Urine protein levels fell from a mean of 7.9 g/day to 2.5, 2.8, and 1.0 g/day 4, 8, and 12 months after therapy, respectively. Unfortunately, side effects, although transient, were extremely common.

Jonas et al. (96) reported their experience with a 7-year-old child who they treated with IFN. Although the girl had resolution of her nephrotic syndrome, she had seroconverted from HBeAg to HBeAb a short time before receiving the IFN. Therefore, the response may have been unrelated to the treatment. Esteban and co-workers (97) treated a 9-year-old boy with HBV MN with adenine arabinoside for 6 weeks. This was associated with a prompt fall in urinary protein excretion, conversion of HBeAg to HBeAb, and disappearance of HBV DNA from serum. The patient was entirely well after 11 months of follow-up.

Lin (98) reported his experience with the treatment of HBV-associated MN in children with recombinant IFN. In this prospective, controlled, open-labeled trial, 40 children who had HBV-associated MN diagnosed by strict criteria were randomly assigned to receive either subcutaneous recombinant IFN or supportive therapy alone. All patients had undergone a 2-month trial of CS and had not responded and all had heavy proteinuria. All patients had normal serum creatinine concentrations, and none was hypertensive. Group 1 patients received 5 million units of IFN subcutaneously three times weekly for 1 year. Group 2 patients received only supportive therapy. The mean age of the patients was 6.2 and 6.8 years, and the mean protein excretion was 4.1 and 4.0 g/24 hours in groups 1 and 2,

respectively. All patients were seropositive for both HBeAg and HBsAg at the start of the trial.

Two months after IFN therapy was begun, heavy proteinuria disappeared in 16 of 20 (80%) patients in group 1, while 4 patients (20%) had only light proteinuria. By the end of the third month, all 20 patients in this treatment group were free of proteinuria. At both 1 and 2 years after the start of the trial, no patients had relapsed with proteinuria. Conversely, after 2 months of supportive therapy, 12 of 20 (60%) patients in group 2 continued to have heavy proteinuria, while 8 of 20 (40%) continued to have light proteinuria, which increased during upper respiratory infections. By the end of the third month, 50% of the patients continued with heavy proteinuria, and 50% had light proteinuria, which increased concomitant with respiratory infections. By the end of the first year of the trial, 6 of 20 (30%) patients in group 2 persisted with heavy proteinuria, 12 of 20 (60%) had light proteinuria with frequent relapses, while only 2 of 20 (10%) were free of proteinuria. By the end of the second year, 3 of 20 (15%) and 10 of 20 (50%) patients in group 2 had heavy and periodically relapsing light proteinuria, respectively. Only 7 of 20 (35%) patients were free of proteinuria.

Even more striking was the effect of treatment with IFN on the rate of HBV-associated antigen seroconversion. After 2 years, 11 of 20 patients in group 1 had HBeAg and HBsAg seroconversion. Five of 20 were HBeAb-positive, while 4 of 20 persisted with both HBsAg and HBeAg. Conversely, in the group 2 patients, all patients remained seropositive for both HBsAg and HBeAg after 2 years. No patient in either group developed renal dysfunction over the time course of the study.

The side effects of IFN were significant. All patients developed flulike symptoms and fever. Fever generally remitted after 3 weeks of therapy. There was no leukopenia. Late side effects included anxiety, depression, and suicidal ideation, the latter two symptoms affecting nearly 20% of the patients. All adverse psychiatric effects disappeared with reduction in the dose of IFN. No hematologic, infectious, or autoimmune complications were noted.

Lin (98) interpreted these results cautiously and suggested that children with HBV-associated MN with heavy proteinuria persistent for more than 2 months should be considered for therapy with IFN. Not only did such therapy appear to exert an extremely salutary effect on the duration of proteinuria, but it also was associated with a significant rate of seroconversion from HBV-associated antigenemia. It is possible that proteinuria would eventually resolve in untreated patients, as that trend was apparent in the untreated group. Nonetheless, the persistence of HBV-associated antigenemia in this group for at least 2 years may represent a marker of impaired immunoregulation and may be a risk factor for progressive renal dysfunction.

Information about treating adults with HBV infection and renal disease with IFN is more limited. Conjeevaram et al. (99) reported the results of a 4-month trial of IFN in 15 patients with HBV MN and MPGN. Eight patients had a long-term serologic response, with loss of HBeAg and HBV DNA. Seven of the eight responding patients showed gradual but marked improvement in proteinuria. The seven nonresponding patients had continued evidence of active liver and renal disease. Chung et al. (100) reported the results of therapy with IFN in eight adults with a variety of different forms of HBV-associated GN. One of the eight was only treated for 2 months because of side effects. The response of serologic markers of HBV infection to IFN was variable, while proteinuria diminished in only two patients. The results of these two relatively small cohorts of patients suggested that prolonged IFN therapy represented a promising, albeit not entirely proven, therapeutic option for patients with HBV-associated MN. Its cost, however, given the dose and length of treatment that appear to be necessary, may be prohibitive. Side effects and risks associated with such therapy may exceed its benefits.

Recently there have been reports about the use of the agent lamivudine in the treatment of HBV-associated MN. Lamivudine is a cytosine analog which, after triphosphorylation, is incorporated into the viral DNA by hepatitis B virus polymerase, resulting in DNA chain termination. Connor et al. reported on a six-year-old male with HBV-associated MN with nephritic syndrome who was treated with oral lamivudine. After two months of treatment, his nephritic syndrome had resolved, and all immunologic markers of HBV infection had disappeared. He was treated for a total of 12 months, and after nearly a year of observation, remained completely well (101). Filler et al. reported a 15-year-old Vietnamese female with HBV-associated MN who received lamivudine for a total of slightly over 13 months. After two months of treatment, the nephrotic syndrome and markers of viral infection disappeared, but ultimately she sustained a relapse of both her MN and evidence of active viral infection (102). Although such reports are preliminary, it is intriguing that the toxicity of lamivudine appears to be remarkably less than that of INF. The oral agent is relatively well tolerated, and undoubtedly will be incorporated into future clinical trials, Moreover, a number of other promising new antiviral agents, including adefovir, dipivoxil, emtricitabine (FTC), entecavir, beta-L-thymidine, and clevudine are being evaluated in clinical trials of treatment of chronic HBV infection. To our knowledge, none of these agents has yet been tested in a trial of patients with HBV-associated MN.

At present the appropriate therapy for patients with HBV MN, particularly in adults, remains unknown. CS generally should not be used because they promote viral replication. Clinical experience with the use of antiviral agents in conjunction with IFN is so limited that firm recommendations regarding the use of these agents in HBV-associated glomerular diseases are not possible. At present, there are still no data from well-designed, controlled trials regarding the use of newer antiviral or immunosuppressant therapies in patients with renal disease associated with HBV infection. There continues to be a need for properly designed, long-term, prospective trials of patients adequately stratified for clinical, immunologic, virologic, and histologic variables to determine whether antiviral agents in conjunction with immunomodulators are efficacious in these disorders.

Another issue that needs to be examined is the true incidence and prevalence of HBV-associated glomerular disease. Multiple studies have been performed in the Far East, an area in which HBV infection is endemic, with a carrier rate in the general population of nearly 15%. There are no data of which we are aware regarding the incidence and prevalence of HBV-associated renal disease in areas of the world where HBV infection is not endemic. Properly designed epidemiologic studies would permit assessment of the scope of the problem and assist in the development of well-designed natural history and intervention trials.

We must also determine whether the use of molecular biologic tools such as in situ DNA hybridization and PCR will actually improve our ability to understand the pathophysiology of HBV-associated renal disease. For example, Lin (103) studied 14 children with biopsy-proven HBV MN, diagnosed according to strict criteria, of whom eight had follow-up biopsies. He used in situ DNA hybridization and determined that seven of eight patients demonstrated HBV DNA in glomerular and tubular cells within 6 months of onset of disease. In contrast, only three of 14 patients had HBV DNA in tubular epithelia, and none in glomeruli, when samples were taken more than 6 months after the onset of the illness. Finally, he found that patients with progressive disease continued to have detectable HBV DNA in the kidney, while those with nonprogressive disease no longer had detectable HBV DNA.

PCR technology allows detection of attamole quantities of nucleic acid in renal tissue and has been used to advance hypotheses about the progression of renal disease (104). Clearly, further advances in our understanding of HBV-associated glomerular disease will require innovative thought to generate hypotheses that are testable using the techniques of molecular biology.

Finally, it may be essential to examine more carefully the role of preventive strategies that may be capable of reducing not only the rate of infection with HBV, but also the incidence of HBV-associated MN. Lee et al demonstrated that mass immunization against HBV reduced the HBV carriage rate in children from about 10% to 0.9% (105). Sun et al. (106) reported that the incidence rate of HBV-associated MN was remarkably reduced in children after mass immunization against HBV was begun in China in 1992. In their retrospective study, the investigators found only 6 cases of HBV-associated MN in 231 vaccinated children (2.6%), compared to 40 cases in 381 unvaccinated children (10.5%). Unfortunately, vaccination did not eradicate the occurrence of HBV-MN in some children, who had serologic evidence of either incomplete vaccination or vaccine failure. Finally, government health officials in Durban, South Africa reported that vaccination against HBV reduced incidence rates of HBV-associated MN in children from 0.33 per 10^5 population to 0.03 per 10^5 population within a decade (107). These data, obtained from areas endemic for HBV infection, underscore the possibility that wide spread immunization policies may represent a real opportunity to reduce the rate of HBV infection and its associated diseases.

HEPATITIS B VIRUS AND POLYARTERITIS NODOSA

Classic PAN is a form of vasculitis in which small- to medium-sized vessels are affected (50). A similar form of vasculitis can affect smaller vessels, including arterioles and capillaries, in which case the term microscopic polyangitis is used (108). PAN is a classic multisystem disease that affects a number of different organ systems. In its untreated state, PAN carries a high mortality. Fortunately, CS and cyclophosphamide have been shown to be effective, and complete remission is common in patients in whom the disorder is diagnosed early. The diagnosis of PAN is made using 10 objective criteria, one of which includes a tissue diagnosis (50,108–110). These criteria are listed in Table 59-7.

TABLE 59-7

CRITERIA FOR THE DIAGNOSIS OF POLYARTERITIS NODOSA[a]

Livedo reticularis
Testicular pain and/or tenderness
Myalgias or muscle weakness
Mono- or polyneuropathy
Diastolic blood pressure >90 mm Hg
Elevated blood urea nitrogen and/or creatinine
Hepatitis B virus in sera (antigen or antibody)
Arteriographic abnormality
Biopsy of small or medium artery with polymorphonuclear neutrophil leukocytes

[a]Three of 10 criteria should be present for diagnosis.
(Modified from: Conn DL. Polyarteritis. In: Klippel JH, Dieppe PA, eds. *Rheumatology.* St. Louis: Mosby, 1994, with permission.)

Although the pathogenesis of PAN remains unclear, a small proportion of cases appear to be causally linked to HBV infection. The mechanism that is best supported in the literature is one in which disease results from circulating immune complexes that contain an HBV-associated antigen and complement-activating immunoglobulin. These immune complexes deposit in vascular tissue and result in destructive inflammation. It is not yet clear why certain vessels appear to be affected more than others, nor why only a minority of patients with circulating immune complexes develop injury. The paradigm of the pathogenic role of HBV-associated immune complexes in PAN is that of serum sickness, in which there is sustained, usually foreign, antigenemia, which evokes host antibody formation. Subsequently, circulating immune complexes form, deposit in target tissues, and activate the complement cascade. There are substantial data to support this paradigm, both in laboratory animals (111), as well as in humans. Lawley and co-workers (112) reported their experience with 12 patients who had received prolonged courses of intravenous horse antithymocyte globulin for bone marrow failure. Eleven patients developed circulating immune complexes concomitant with the development of clinical signs and symptoms of serum sickness, although no mention was made of renal abnormalities. Both C4 and C3 levels fell precipitously as immune complexes appeared in sera. Biopsy of skin lesions in five patients revealed immune deposits in the blood vessels of three patients.

The circulating immune complex mechanism has traditionally been proposed to explain the relationship between HBV infection and PAN. The first association between hepatitis and vasculitis was noted by Paull (113), who reported four army officers who developed hepatitis and fatal vasculitis after yellow fever immunization. The link between HBV infection and PAN was first suggested by Gocke and others (114), who detected immune complexes by immunofluorescence in inflamed vessel walls in a patient with vasculitis. The immune complexes were shown to consist of the Australia antigen, IgG, and C3. This finding was taken as evidence that Australia antigen-containing immune complexes could be pathogenic.

Subsequently, Alpert et al. (115) reported 18 patients with acute viral hepatitis who had arthritis, arthralgias, or urticaria. None, however, had renal disease. Nine patients were extensively evaluated and found to have depressed concentrations of both total hemolytic complement (CH50) and C4. Serum complement concentrations were lowest when hepatitis-associated antigen titers were at a maximum level. Moreover, joint and skin symptoms correlated with depressed levels of complement. Wands et al. (116) later extended the observations of this group of investigators and found that the circulating immune complexes in cryoprecipitates from six patients with arthritis and acute viral hepatitis contained HBsAg, IgG, and IgM. The IgG subtypes were complement fixing. HBsAg and anti-HBs were concentrated several fold higher in the cryoprecipitates than in serum. The immune complexes were able to activate complement in patients with active arthritis, whereas cryoprecipitates from patients with uncomplicated acute hepatitis were unable to activate complement. These investigators concluded that the presence of complement-activating immune complexes were pathogenic in the arthritis of HBV infection.

Trepo et al. (117) investigated the circulating immune complexes found in the sera of 55 patients with histologically proven PAN, of whom 23 had active disease. Few clinical details were reported, nor was there information about renal involvement. They found HBsAg seropositivity in 30 of 55 patients (54.5%) and HBsAb in 13 of 45 (28%). Five of 45 patients (11%) had both. The Trepo group detected a variety of different particles, including the 42-nm Dane particle and 20-nm spherical and filamentous particles associated with HBsAg, in HBsAg-positive sera by electron microscopic examination. No particles were detected in sera containing HBsAb.

These investigators demonstrated the presence of coexistent HBsAg and HBsAb in the sera of 4 of 23 (17.3%) patients with active vasculitis and could not demonstrate such findings in patients with quiescent vasculitis or liver disease alone. They were concerned that they could not demonstrate HBsAg/HBsAb immune complexes in a higher proportion of cases of active vasculitis and speculated that their immune complex detection methods were not sufficiently sensitive.

The concept that immune complexes were pathogenic in PAN was supported by the work of Michalak (118), who reported an autopsy series of seven cases of PAN in HBsAg-seropositive subjects. Six of the seven had glomerulonephritis, although no clinical details were given. Michalak was able to detect HBsAg, IgG, and C1q in vascular lesions and, on elution of these complexes from vascular tissue, found that the complexes contained HBsAg and immunoglobulin fragments when they were dissociated with sodium thiocyanate and ammonium sulfate. He also noted that the amount of HBsAg immune complexes was largest in the vascular lesions with the greatest degree of acute inflammation and absent from healed vascular lesions. He interpreted these findings as supporting a role for HBsAg-containing immune complexes in the pathogenesis of PAN.

Gupta and Kohler (119) investigated the nature of circulating immune complexes in sera obtained from five patients with arteritis and three patients with venulitis, all of whom were thought to have vasculitis associated with HBV infection (119). Using a variety of techniques to dissociate the immune complexes, they determined that the immune complexes in both cohorts of patients contained several different antigen moieties of HBsAg, ranging in molecular weight from 23 to 97 kd. The antigen components in the immune complexes were identical in all patients; however, the antibody composition of the complexes differed among patients. This led the authors to conclude that while the antigenic components in different patients with HBV-associated PAN were the same, individual patients' antibody responses were different and might determine the degree of injury to vessel walls and expression of disease.

In 1976 Sergent and co-workers (120) reported nine patients with systemic vasculitis and HBsAg antigenemia. The patients ranged from 26 to 63 years of age; all were white. All were seropositive for HBsAg, including three patients who were thought to have been infected through blood transfusions and three through venereal transmission. In all cases, the vasculitis followed either exposure to the hepatitis virus or overt hepatitis. No test was performed to determine whether any patient had circulating immune complexes, although in the two patients in whom complement concentrations were measured, the complement was depressed in one. Although this report is rich in clinical detail, it is of limited use in advancing our understanding of the causal relationship, if any, between HBV infection and PAN.

Adu and others (121) reported a large series of patients with polyarteritis in the first study to provide detailed information about renal involvement in a large cohort. The majority of the 43 patients studied, however, had microscopic polyangiitis rather than classic PAN. No information about HBV status was provided.

McMahon et al. (122) provided compelling evidence of the strong relationship between HBV infection and PAN in their report of 13 patients with PAN and HBV infection. All patients were Yupik Eskimos from an area of Alaska hyperendemic for HBV infection. All patients were seropositive for both HBsAg and HBeAg at diagnosis, and in eight patients clinical and/or serologic evidence indicated that the PAN had followed recent HBV infection. In the 10 patients in whom components of the complement system were measured, 4 had depressed levels of C3 or C4, while such levels were normal in the remaining 6.

Our understanding of the pathogenesis of certain forms of systemic vasculitis, including Wegener's granulomatosis, PAN, and the form of rapidly progressive glomerulonephritis known as pauci-immune glomerulonephritis, has increased with the discovery of antineutrophil cytoplasmic antibodies (ANCA) (50,123). There are two forms of ANCA, both of which can be detected by indirect immunofluorescence of polymorphonuclear leukocytes fixed in absolute alcohol, as well as by enzyme-linked immunosorbent assay (ELISA). Cytoplasmic ANCA (c-ANCA) is directed against proteinase 3, a constituent of primary granules. Perinuclear ANCA (p-ANCA) is directed against myeloperoxidase. The spectrum of renal disorders associated with ANCA has been extensively reviewed (50,124). There was little information as to whether ANCA participated in the pathogenesis of HBV-associated PAN (125,126) until the studies of Guillevin et al. (127), who studied 28 patients with HBV-associated PAN, in whom they tested sera for the presence of ANCA. Sera from 11 men and women with biopsy-proven, active PAN, all of whom had either HBsAg or HBeAg in sera or tissue, were tested for ANCA by indirect immunofluorescence and ELISA. Three of 28 (10.7%) were positive for ANCA by immunofluorescence, while 2 of 18 (11.1%) were positive by ELISA; these latter 2 were determined to have ANCA directed against myeloperoxidase. The authors concluded that ANCA is uncommon in HBV-associated PAN.

There are very few reports of treatment of HBV-associated PAN (128,129). While CS and cyclophosphamide have long been considered the most effective form of therapy for idiopathic PAN, the use of CS in HBV-associated PAN has been considered ill-advised because of the known deleterious effect of CS on the ability to resolve HBV infection. There is an absence of well-designed and powered randomized controlled therapeutic trials.

Nonetheless, a prospective study using sequential therapy with CS, the antiviral agent vidarabine, and plasma exchange shows promising results. Guillevin (130) chose to use CS to control severe life-threatening aspects of PAN, followed by vidarabine to induce HBeAg/HBeAb seroconversion, with concomitant plasma exchange to remove immune complexes. He initially studied 33 patients with HBV-associated PAN, all of whom had histopathologic or arteriographic evidence of active vasculitis, were seropositive for HBsAg and HBeAg, had no detectable HBV-associated antibodies, and had evidence of active replication of HBV as measured by HBV DNA or DNA polymerase activity in sera. Seventeen men and 16 women with a mean age of 50.9 $\pm$ 17.5 years were studied. Two patients had glomerulonephritis, and six had renal insufficiency. Seven patients had renal artery microaneurysms, and nine had renal infarction detected angiographically. No renal biopsies were performed. Remission was defined as stabilization or improvement of clinical symptoms and normalization of laboratory abnormalities while on therapy. Control of disease was defined as improvement in the patient's overall condition, absence of new or improvement of existing symptoms, and normalization of the erythrocyte sedimentation rate. Complete recovery was considered to have occurred when the criteria defined for control of disease were met and maintained for at least 18 months after discontinuation of all treatment. Clearance of HBV antigenemia was not a treatment-dependent outcome.

On entry into the study, all patients were initially treated with 1 mg per kg per day of prednisone for 1 week; prednisone was rapidly withdrawn over the second week. Immediately thereafter, vidarabine was administered by continuous intravenous infusion at a dosage of 15 mg per kg per day for the first week and 7.5 mg/kg/day for the next 2 weeks. Plasma exchange was initiated at entry into the study and was performed three times each week for the first 2 weeks, followed by 14 exchanges over the ensuing 3 weeks, while the patients continued therapy with vidarabine. Following completion of

vidarabine therapy, plasma exchanges were performed three times weekly for 3 weeks and then twice weekly for 2 weeks. Exchanges could then be performed once or twice weekly thereafter, depending on the clinical situation. IFN, given at a dose of 3 million units thrice weekly, could be administered to those patients who did not develop HBeAb or who could not tolerate vidarabine.

No side effects of CS therapy were noted. Three patients developed transient thrombocytopenia during vidarabine therapy. IFN was given to four patients. The mean number of plasma exchanges performed was 23 (range, 6 to 34). No serious complications of therapy were noted.

Six months after entry into the study, 26 patients (78%) were controlled, while 4 (12.1%) and 3 (9.1%) were either uncontrolled or dead, respectively. After 5 years of observation, 1 patient remained in remission, while 24 (72.7%) had made a complete recovery. Eight patients (24.2%) had died. Only 1 patient died of renal causes. Seroconversion to HBsAb and HBeAb occurred in 15 (45.4%) and 6 patients (18.1%), respectively. Most patients who seroconverted did so after the first several months following the end of therapy.

Subsequently, Guillevin et al. (131) reported the results of their nonrandomized prospective trial of IFN and plasma exchange in HBV-associated PAN. Their cumulative clinical experience included 41 patients, many of whom were included in the earlier study (130). Thirty-five patients were treated with vidarabine, while six were treated with INF. All patients received plasma exchange therapy. More than half of the patients no longer expressed serologic evidence of HBV replication after therapy. Thirty-three of 41 patients (80.5%) were considered cured, while 8 patients died during the study period. None of these deaths were attributed to the treatment protocol. Only one-third of the patients had clinical renal disease, and few details are available to permit determination of the effect of treatment on renal parameters.

Case reports have also documented a salutary effect of treatment of vasculitis associated with HBV infection with lamivudine, but details regarding renal parameters are incomplete (132–136). Guillevin and his colleagues reported on the use of lamivudine combined with short-course CS and plasma exchange in the treatment of HBV-associated PAN (137). In a multicenter, prospective, observational trial, 10 patients (8 men, 2 women) with previously untreated HBV-related PAN were treated with prednisone (1 mg/kg/day) for 1 week, which was then tapered and withdrawn within 1 week. Then, lamivudine (100 mg/day) was started for a maximum of 6 months. Plasma exchanges were performed on a tapering schedule until HBeAg to HBeAb seroconversion was obtained or until 2–3 months of clinical recovery was sustained. The primary trial endpoint was clinical recovery from HBV-PAN at 6 months. The secondary end point was loss of detectable serum HBeAg and HBV DNA, and HBeAg to HBeAb seroconversion at 9 months. One death, from catheter–related sepsis, occurred. By six months, all nine survivors had achieved clinical recovery and by nine months, six of nine had seroconverted. The investigators concluded that a strategy of short-term steroid therapy followed by lamivudine and plasma exchanges not only led to recovery from HBV-PAN, but also because of its oral administration and good safety profile, lamivudine should be considered the antiviral agent of choice to treat HBV-related PAN. Unfortunately, as in previous reports, few details are available regarding kidney disease in these patients, and it is difficult to assess the effects of the treatment on kidney outcomes (137).

Nonetheless, the results of these studies are quite encouraging and offer promise that more effective and less complicated therapeutic regimens for HBV-associated PAN may be developed. The response of patients to plasma exchange certainly suggests that circulating immune complexes are involved in the pathogenesis of the disease. Often, however, whether immune complexes were measured is not reported. Moreover, the response to plasma exchange should not be used as conclusive evidence that immune complexes are the sole mechanism underlying the pathogenesis of the disease (138,139). Plasma exchange may exert salutary effects on disease through a variety of mechanisms in addition to removing complexes from sera. For example, the effects, if any, of plasma exchange on immunomodulators such as cytokines have not been extensively evaluated.

Therefore, although there is an extensive literature that relates PAN to HBV infection, our understanding of this disorder remains rather limited. The same sophisticated diagnostic maneuvers that are being used in other forms of renal disease associated with viral infections should be applied to a cohort of patients with this disorder. The field requires implementation of well-designed and powered randomized controlled therapeutic trials of antiviral therapies, immunosuppressants, and plasma exchange in patients in whom virologic and immune parameters, as well as side effects, are carefully delineated. Inclusion of patients with clinically important kidney disease in such studies may permit inferences regarding the specific effects of treatment on renal outcomes. It seems likely that safe and effective therapy will depend on the outcome of such studies.

HEPATITIS B VIRUS AND CRYOGLOBULINEMIA

Although an association between cryoglobulinemia and acute and chronic liver diseases had been noted (140), cryoglobulinemia was first associated with HBV infection by two groups in 1976 (141) and 1977 (142,143). Two-thirds of patients with essential mixed cryoglobulinemia had abnormal liver function tests, and the majority had circulating antibodies reactive with HBsAg. Almost three-fourths of patients had detectable HBsAg or anti-HBsAb in the cryoprecipitates (142). Approximately half the patients had glomerulonephritis (143). Renal histologic data and the relationship of renal disease to the antibody status and composition of the cryoprecipitate were not presented in these studies. The generalizability of these findings, furthermore, has been disputed (144,145). Speculation centered on whether differences between populations (146), consequences of hepatic dysfunction, or other etiologic agents might be confounding factors that would explain the disparate findings (147). It has even been conjectured that HBV infection in patients with essential mixed cryoglobulinemia might have been a result of transfusions received as therapy for symptoms of the cryoglobulinemia (148). Rarely has the renal disease been fully delineated in studies of patients with cryoglobulinemia in the setting of HBV infection (i.e., shown to be definitively related to the HBV infection), or the components of the cryoprecipitate shown to be reactive with HBV antigens.

Contemporary studies should focus on the incidence and prevalence of the complication in HBV-infected patients who have not been exposed to HCV. Recently, for example, Ferri et al. reported on 231 patients with mixed cryoglobulinemia. Using sophisticated serologic and virologic assessment, the investigators found that HBV infection was likely to be causative in only four patients in the entire cohort, whereas evidence of hepatitis C infection was evident in more than 92% of patients (149).

Reports of patients with cryoglobulinemia often do not distinguish between essential and secondary forms of the disease, and treatment trials of therapy in patients with hepatic disease often do not distinguish patients with cryoglobulinemia and/or renal disease for separate analysis (150,151). Various

regimens have been used to treat such patients, with therapy most often directed at the underlying viral illness. There are no adequate trials limited specifically to patients with HBV infection and cryoglobulinemia that have been prospective, well designed, and properly controlled. In addition, many patients are often infected with other viruses, potentially confounding analytic strategies. Although immunosuppressive therapies consisting of CS, cytotoxic drugs, and plasmapheresis have been employed to treat essential mixed cryoglobulinemia (150,151), recommendations for the use of such therapy in patients with HBV infection, cryoglobulinemia, and glomerulonephritis cannot be easily made. There is a need for properly designed and conducted clinical trials including surveillance of virologic outcome in addition to renal parameters, with stratification of clinical, immunologic, and histologic variables to determine which therapies may be effective in this disorder. In individual cases, the possible benefits of a specific therapy must be balanced by any conceivable risks, including exacerbation of viral replication. Plasma exchange is likely to be the least harmful therapy, but its efficacy is unknown in this specific population of patients (152).

HEPATITIS C VIRUS–ASSOCIATED GLOMERULONEPHRITIS

The identification of a syndrome of non-A, non-B hepatitis was dependent on serologic identification of hepatitis A virus (153) and HBV (154). HCV, cloned in 1989 (155), has been recognized as the major cause of non-A, non-B hepatitis (156,157). HCV is a single-stranded RNA virus (158,159) in the Flaviviridae family. It is a 50- to 65-nm particle, composed of a lipid envelope, several glycoproteins, and single-stranded RNA in the nucleus. The recombinant immunoblot assay (RIBA) is directed against the C 22 antigen. Alternatively, second or third generation immunoassays (EIAII or EIAIII) that detect a number of different capsular and core antigens are available. HCV infection is characterized by persistent, prolonged viremia in the face of an ongoing immune response, which promotes the development of immune complex disorders. Resolution of viremia is uncommon, but patients may note few or no clinical symptoms. Infection is transmitted through transfusion of blood products, intravenous drug use, and sexual and maternal–infant routes. Infection with HCV may culminate in chronic active hepatitis, cirrhosis, or hepatocellular carcinoma.

Studies using the first-generation EIA documented that a clinically significant proportion of patients with ESRD treated with hemodialysis in Europe and the United States had been exposed to HCV. Subsequent studies confirmed a 10% to 40% prevalence of HCV infection, depending on the center, and suggested the prevalence of infection may be even higher if second-generation assays or the detection of HCV RNA are used as the gold standard (160–162). The prevalence of HCV infection in patients treated for ESRD with hemodialysis outside the United States may be greater. The incidence and prevalence of HCV infection in dialysis patients is stable or declining (162,163) and the latest data suggest a prevalence of about 8-10% (163,164). Curiously, one report suggested the viral burden may be higher in males undergoing hemodialysis compared with females undergoing hemodialysis (165). The reasons for this difference are unknown but may include behavioral and risk factors that differ between the sexes or differences related to sex hormones. The mechanisms involved in transmission in dialysis units are unknown, but transfusion-related or dialysis-related processes may be involved, since a relatively lower proportion of patients on continuous ambulatory peritoneal dialysis may be infected (160,163,166). These findings are important because of uncertainty whether HCV infection in ESRD settings is solely treatment-related or whether HCV seropositivity represents an association with or is the cause of the renal disease. In a study assessing the prevalence of exposure to HCV infection in patients with chronic renal insufficiency, 7.9% of patients with renal disease in a Spanish cohort were infected with HCV, compared with a significantly lower proportion of patients (1.03%) who were healthy blood donors (167). There was also a higher prevalence of HCV seropositivity in patients with chronic glomerulonephritis compared with patients with other renal diseases. The high prevalence of HCV seropositivity in patients with renal disease, and more specifically, glomerulonephritis, suggests the infection might be a causal factor in the pathogenesis of nephropathy. Alternatively, patients with renal disease may acquire infection because of altered immune function related to renal insufficiency (168–170) or other factors, such as the use of intravenous drugs, that could be associated with both conditions (167).

There is a high prevalence of circulating, cryoprecipitable HCV RNA and/or antibodies in patients with mixed cryoglobulinemia (46,163,171–178). A study based in research centers in France demonstrated 56% of HCV-infected patients had mixed cryoglobulinemia (179). Interestingly, serologic and PCR analyses for HCV antibodies and RNA showed the PCR to be a much more sensitive test (175). In some patients, it is possible that the immune response comprises antibodies not detected by currently employed immunoassays. Misiani et al. (180), however, showed the vast majority of patients with cryoglobulinemia had HCV antibodies detected. In an autopsy study, a relatively large proportion of patients with HCV infection had various glomerular diseases (57.4%), although almost one-fourth had minimal abnormalities (181). Renal disease severely affected prognosis, increasing mortality risk. Ferri et al demonstrated that HCV RNA could be detected in the sera of 86% of patients with cryoglobulinemia (172). These findings were soon corroborated by Agnello el al. (175). A recent review by Ferri et al. (149) showed HCV infection in 92% of cases, while HBV infection was present in only 1.8% of cases with mixed cryoglobulinemia.

Beddhu et al described 17 patients with renal disease and cryoglobulinemia. Eleven of the patients were seropositive for HCV, and three additional patients had clinical abnormalities consistent with a connective tissue disorder. Only three patients had no demonstrable systemic illness. Eleven patients, some HCV negative, had MPGN on renal biopsy. Patients presented with a wide array of renal findings, including nephritic syndrome, inflammatory urinary sediments, renal failure, and hypertension (182). Differences in epidemiologic factors and viral strains in the studies may, in part, explain discrepancies in the proportion of patients with cryoglobulinemia and HCV infection detected in different investigations. Recent work has expanded our understanding of the pathogenesis and clinical consequences of the syndrome (45,183).

Several studies have also demonstrated an association of HCV infection and MPGN (180–196), independent of cryoglobulinemia. Evidence suggesting the viral infection is directly linked to glomerulonephritis comes from the work of Johnson et al. (191), who demonstrated antibody directed against HCV peptides and HCV RNA, determined by immunoblot and PCR respectively, in the cryoprecipitates of all eight patients they studied with MPGN and HCV infection.

The overwhelming majority of the patients studied by the Johnson group had microscopic hematuria, edema, renal insufficiency, hypertension, and normochromic normocytic anemia (191). Half had hepatomegaly. All patients had hypoalbuminemia, abnormally elevated serum aminotransferase concentrations, diminished total hemolytic complement levels, and increased rheumatoid factor activity. Most patients had low levels of C3 and C4. All patients had proteinuria; the majority had more than 3.5 g/day. Urinalysis revealed red blood cell

casts in over one-third of patients. Approximately two-thirds of patients had cryoglobulinemia and circulating immune complexes. Most patients had circulating IgG reactive with nonstructural HCV antigens, and all had antibodies to HCVc, the nucleocapsid core antigen. Anti-HCVc and anti-c33-c (a nonstructural protein) IgG antibodies were present in the cryoprecipitates of all three patients studied. Serum and cryoprecipitates also contained IgM antibodies reactive with HCVc antigen (191,192). Typically the renal disease is indolent (197).

Renal biopsies in all eight patients showed MPGN, with a lobular increase in glomerular cellularity, and interstitial mononuclear infiltration. Immunofluorescence demonstrated glomerular capillary IgM, IgG, and C3. IgA and C1q were present in the minority of cases. Electron microscopy demonstrated interposition of mesangial cells with subendothelial, mesangial, and rare intramembranous deposits. Immunotactoid structures were present in several patients. Of note, HCV antigens and anti-HCV immunoglobulins were not identified in renal tissue in a characteristic anatomic distribution in this study. Therefore, although the evidence linking the renal disease to the virus is circumstantially strong, direct causal association between HCV antigens and the occurrence of MPGN remains to be demonstrated (198). Further work by Johnson's group (192) demonstrated that patients with HCV infection exhibit a spectrum of clinical renal syndromes, including asymptomatic urinary abnormalities, such as hematuria and proteinuria, variable levels of renal insufficiency, and the nephrotic syndrome (199) (Table 59-8). A spectrum of histologic changes, including MPGN, MN (200–204), vasculitis, and proliferative glomerulonephritis (163,192) have been noted in HCV-infected patients with renal disease. The majority of patients were white and male. More than half of the patients had cryoglobulinemia, often with characteristic symptoms. The majority had abnormal transaminase levels and hypoalbuminemia, as well as longstanding HCV infection, but only approximately 20% had physical signs of hepatic disease. Hypocomplementemia (CH50, C4) and the presence of rheumatoid factors may be noted in most patients. Low C3 levels were noted in almost half of the patients (199). Renal disease may be present, however, in the absence of clinical signs and symptoms of the HCV infection or cryoglobulinemia. Rapidly progressive glomerulonephritis (205), IgA nephropathy (206), and thrombotic microangiopathic diseases (207) have also been reported in HCV-infected patients (208,209) but the etiologic relationships are less clear in these cases. The spectrum of disease has also been reported in Japan (210,211).

A proliferative glomerulopathy with pathologic features similar to MPGN has been noted in HCV-infected patients after liver (189) and renal (192,212,213) transplantation. The relationship of this lesion to chronic transplant allograft nephropathy remains unclear (214). Cosio et al. measured serum antibodies to HCV in patients with native kidney disease and compared the results to those found in patients with acute and chronic transplant allograft nephropathy. Native renal diseases included focal segmental sclerosis, diabetic nephropathy, MN, and MPGN. HCV serologies were negative in all patients with native renal disease without a history of intravenous drug abuse. Of the patients with acute and chronic transplant allograft nephropathy, 29% and 33%, respectively, had detectable HCV antibodies. The authors concluded that there was an association between HCV infection and certain forms of allograft nephropathy (215).

Cruzado and co-workers studied six HCV-infected renal allograft recipients with proteinuria and hematuria. All had circulating immune complexes and cryoglobulins. In all six patients, the cryoglobulins were type II IgG polyclonal–IgMk monoclonal. Renal tissue demonstrated features of a proliferative lesion, in which IgM was deposited diffusely. HCV RNA was found in higher concentrations than in serum, leading the authors to conclude that the infection might be the etiology of the glomerular lesions (216). Conversely, Morales et al. reported 15 patients with HCV infection who developed de novo allograft MN 2 years after transplantation. There was a 10-fold higher incidence in the development of MN in allografts of HCV-positive patients than in HCV-negative patients. In the majority of patients, renal function declined until dialysis had to be instituted (217).

Kendrick and others studied 197 liver transplant recipients, of whom 91 were HCV-infected. The other 106 were uninfected with HCV. Four of 10 HCV-infected patients who developed proteinuria had MPGN on renal biopsy, while none of seven HCV-uninfected patients with proteinuria had MPGN. Several patients received treatment with INF, which appeared to stabilize proteinuria, but did not reverse the renal dysfunction (218).

TABLE 59-8

GLOMERULAR SYNDROMES ASSOCIATED WITH HEPATITIS C VIRUS INFECTION

Hepatitis C virus-associated immune complex disease
Membranoproliferative glomerulonephritis
With cryoglobulinemia
Without cryoglobulinemia
Vasculitis
Proliferative glomerulonephritis
Membranous glomerulopathy
Transplant nephropathy?

Pathology

MPGN type I is the most common pathologic lesion identified in clinicopathologic studies of patients with HCV infection and renal disease (187–199,219,220). Increased glomerular cellularity and double-contoured glomerular basement membranes are hallmarks of the disease. Mesangial proliferation and sclerosis may be seen. Mesangial and glomerular capillary deposition of IgM, IgG, and C3 are often present. Subendothelial deposits are seen on electron microscopic examination in MPGN type I, but occasionally mesangial and subepithelial electron-dense deposits may also be noted. Ultrastructural features of cryoglobulins, including granular, fibrillar, and immunotactoid structures may be seen within the subendothelial space. Glomerular findings are often accompanied by tubulointerstitial inflammation and fibrosis. Small- and medium-sized renal vasculitis may be present. More rarely, subepithelial electron-dense deposits (characterizing MPGN type III) are present. Viral genome or gene products have not usually been identified in glomeruli in studies. In one study of a patient with diffuse proliferative glomerulonephritis, however, viruslike particles, similar to those seen in sera of patients with non-A, non-B hepatitis, were found in the mesangium (201). Microtubular inclusions in endothelial cells were noted as well. Since the HIV status of this patient was not reported, the significance of these ultrastructural findings is unclear.

Johnson et al. (192) and others (200–204) have also noted several patients with MN in the presence of HCV infection. A causal relationship between the viral infection and the renal disease has not been established, but the renal disease is a possible consequence of a secondary immune response (40,41,198). Such patients have typical pathologic findings, and no evidence of hypocomplementemia, rheumatoid factor activity, or

cryoglobulinemia (194,199). HCV infection has also been associated with fibrillary glomerulonephritis and immunotactoid glomerulopathy (191,192,220–222). The clinical presentation is indistinguishable from other forms of glomerulonephritis. Renal biopsy is required for diagnosis.

Pathogenesis

Glomerular deposition of circulating immune complexes likely plays a role in the pathogenesis of the renal diseases associated with HCV infection (1,8–12). Immune-mediated renal disease may be facilitated because of the persistent viremia and ongoing antibody response seen in HCV-infected patients. Glomerulonephritis associated with HCV-induced cryoglobulinemia has been linked to IgM kappa rheumatoid factors (195,223). Specific immunochemical or physicochemical characteristics may be associated with nephropathogenicity. The generation of idiotypic antibodies may be important in the development of renal disease because of physicochemical characteristics (1,9–12) or directly through immune dysregulation (1,8,12,13), or it may only reflect the immune dysregulation noted in a chronic viral illness (8,12,13). HCV proteins (224) and genetic material (201) have been demonstrated in tissue of patients with MN associated with HCV infection and cryoglobulinemic membranoproliferative glomerulonephritis (225). A study demonstrated the presence of HCV RNA in renal biopsies in patients with HCV infection, and none in the absence of HCV infection (226). Interestingly, *in situ* hybridization localized HCV RNA to tubular epithelial cells and tubulointerstitial blood vessels. Few studies have assessed molecular evidence for virologic involvement in the pathogenesis of glomerular disease (198). The small study sizes, difficulty in localization of nucleic acid in distinct or specific compartments in diseases linked to HCV infection (226), and lack of definitive evaluations in large control populations, render determination of a pathogenic link from these studies problematic.

Treatment

IFN-α has been used for the treatment of "essential" mixed cryoglobulinemia (227–229), HCV infection (230,231), and cryoglobulinemia associated with HCV infection (232–235). Treatment may result in suppression of viremia and improvement in parameters of hepatic disease, but disease markers often recur in patients after discontinuation of therapy. In uncontrolled studies, Johnson et al. (199) treated 17 patients with MPGN with and without cryoglobulinemia with IFN-α. Patients had decreased urinary protein excretion and normalization of liver function test abnormalities during treatment with IFN-α of up to 6 months' duration. There was no significant change, however, in renal function noted in this study. Loss of circulating HCV RNA during therapy may be a favorable prognostic sign. Viremia and signs of renal disease, however, often recurred after cessation of therapy. Several other studies of HCV-infected patients with MPGN (236,237) show improvement in parameters of renal disease, perhaps in association with use of higher doses of INF (236). Relapses may have been less frequent when higher doses of INF were used (163,164). In a Japanese study, glucocorticoid therapy was associated with improvement in renal function parameters, while there was no effect of therapy with INF-α (210). Steroid therapy, however, was associated with an increase in circulating titers of HCV RNA.

Side effects of therapy with IFN-α include a flulike syndrome in the majority of patients, but the occurrence of erythema multiforme and worsening neuropathy often necessitates discontinuation of treatment. It is unclear whether progressive renal insufficiency, which may occur during therapy, represents a side effect of treatment (238–243) or progression of the underlying nephropathy. Because of the unknown natural history of HCV-related renal disease, the heterogeneous long-term response to treatment, the lack of prognostic determinants, and the often transitory nature of improvement in renal and hepatic disease markers, this costly therapy must be considered on an individual basis, the risks and benefits being carefully weighed for each patient.

The sustained response of HCV infection to therapy with INF has been disappointing, leading to the development of regimens including the addition of ribavirin to INF (163) and the use of pegylated INF therapy (163). Three randomized controlled trials demonstrated the superiority of pegylated INF compared with therapy with INF (163,244–246). Trials have also used the combination of ribavirin and pegylated INF, compared with pegylated INF alone and combinations using INF in HCV-infected patients (163,247–249). Therapy with ribavirin has been associated with a dose-related occurrence of hemolysis, and the drug is cleared by the kidneys. Combination therapy may also result in infectious and autoimmune complications, as well as rarely being associated with nephrotoxicity (163). These regimens have often not been well studied in patients with coexisting renal disease, in part because of their exclusion in clinical trials (163).

Classically, therapy for cryoglobulinemic renal disease has included oral and intravenous CS, with or without plasmapheresis, and supplemental immunosuppression with cytotoxic drugs, such as cyclophosphamide (49,50,151,192,250). Nafomostat mesilate, an inhibitor of complement serine proteases, has been used to treat patients with immune complex-mediated glomerulonephritis, including essential mixed cryoglobulinemia, with a resultant decrease in proteinuria (251). INF has been used in treatment of HCV-associated renal diseases, cryoglobulinemia, and vasculitis, but it has shortcomings (163,164,235,238–243,252–258). Casato and co-workers reported a response rate of 62% in patients with cryoglobulinemia treated with INF, but the relapse rate was high. Only 10% of patients experienced a long-term remission (259). INF has also been associated with the appearance or worsening of nephropathy in treated patients (238–243,260–264). Addition of ribavirin has been considered for patients with HCV-associated cryoglobulinemia and vasculitis (44,265–269). Rossi et al. (270) treated three patients with cryoglobulinemia and glomerulonephritis in the setting of HCV infection with ribavirin 15 mg/kg/day, adjusted according to the level of hemoglobin, and added IFN-α2b after 4 weeks. Treatment with ribivarin for four weeks was not associated with clinical change in renal parameters. Level of urinary protein excretion decreased and creatinine clearance increased, and either normalized or approached normal levels in all three patients. Similar cases were described by Misiani et al. (271), Garini et al. (272), Loustad-Ratti et al. (273), and Sabry et al. (274). Rituximab has recently been suggested as an additional treatment for HCV-associated cryoglobulinemia (275–277). Rocatello et al. (277) treated six patients with HCV infection and type-II mixed cryoglobulinemia with rituximab, with improvement in levels of cryocrit and urinary protein excretion. Alric et al. (278) reported on 25 patients with nephrotic syndrome due to HCV-associated membranoproliferative glomerulonephritis and HCV infection. All patients initially received therapy with CS. They tested the effects of standard or pegylated interferon alfa and ribavirin in 18 patients compared with therapy with prednisone, furosemide and plasmapheresis alone in 7 patients with cryoglobulinemia and MPGN or mesangial proliferative glomerulonephritis associated with HCV infection in a nonrandomized study. In the group treated with

antivirals, 12 patients were responders and 6 did not respond. Urinary protein excretion decreased in "responder" patients treated with antiviral therapy, but there was no decrease in level of serum creatinine concentration in any of the groups. These patients also had a reduction in cryoglobulin levels compared with pretreatment values.

Infliximab has also been used to treat refractory vasculitis in patients with HCV infection and mixed cryoglobulinemia (279). D'Amico has suggested antiviral therapy using pegylated interferon and ribavirin for 48 weeks, adding steroids and cyclophosphamide in the face of severe symptoms, and plasmapheresis as necessary (163), but such treatment has not been validated in well-controlled randomized trials.

End-Stage Renal Disease

Relatively few studies have assessed the use of INF in ESRD patients infected with HCV (163). The sustained virologic response in HCV infected patients treated with dialysis has been about 40%, which is higher than that obtained in patients in clinical trials in the absence of renal disease (163). This response, however, has been associated with high rates of serious adverse events, dose modification, and early withdrawal from studies (163). It is possible such findings relate to the diminished clearance of INF in ESRD patients (163). The recommendation of a workshop held in 2002 was that therapy with INF in ESRD dialysis patients is controversial, and should be reserved for patients with severe liver disease, minimal comorbidity, and a reasonable prognosis, especially if renal transplantation is being considered (163), and others have endorsed similar strategies (280). Ribavirin is relatively contraindicated in ESRD patients treated with dialysis because of the occurrence of hemolytic anemia, but dosage adjustment schemes and use of erythropoietin have been attempted to facilitate treatment in patients with renal disease(163,269,281–285).

Interferon is generally contraindicated in patients who have had renal transplantation, because of the possible increased risk of graft rejection (163), but some studies have evaluated outcomes in such patients (212,286,287).

Long-term survival for HCV-infected ESRD patients is shortened compared with uninfected patients (163,288,289). Recipients of donor hepatitis C virus infected kidneys have worsened survival, although the mechanisms underlying the finding are unknown and may relate in part to selection biases (163,290). Type of immunosuppression may affect outcomes (163,291).

Conclusions

Relatively few well-designed and controlled trials have been available to guide clinical practice in treating patients with HCV infection and renal disease. The long-term effects of steroid and immunosuppressive treatment on the viral infection, which may be associated with increased viremia (199), may be a critical factor in determining the treatment options in patients with severe, acute illness. There is a need for properly designed and conducted clinical trials of combination therapy with immunosuppressants, plasmapheresis, and antiviral medications such as ribavirin (292,293) and immunologic therapies such as rituximab (275–277), including surveillance of virologic outcome in addition to renal parameters, with stratification of clinical, immunologic, and histologic variables to determine which therapies and drug doses may be effective in patients with various HCV-associated renal diseases. Carefully controlled studies of treatment of patients with HCV infection and acute and chronic kidney disease, evaluating outcomes such as viremia, viral burden, renal functional parameters, and morbidity and mortality, may clarify which patients may respond to a specific therapy with the fewest complications.

HUMAN IMMUNODEFICIENCY VIRUS–ASSOCIATED RENAL DISEASES

Since the beginning of the epidemic, a spectrum of renal diseases has been associated with acquired immunodeficiency syndrome (AIDS) and HIV infection (17,294–299). Several well-defined clinicopathologic disorders have been reported in patients with HIV infection, including a number of different forms of glomerular diseases and a variety of immune complex and vasculitic illnesses (17,294–299). These disorders do not occur or are not detected with a frequency commensurate with the prevalence of HIV infection, affecting at most 10% of the population at risk, in the era before highly active antiretroviral drug therapy (HAART) (294,298–300). Therefore, the renal diseases probably do not result from HIV infection alone, but from a combination of the infection and the immunologic responses of the host (17,295,298–301). Renal complications affect specific subsets of the HIV-infected population, primarily male African American patients in the United States (294–305), suggesting that a host response or genetic component may be associated with the incidence of the disease (294–305). Factors linked to socioeconomic status may also be related to disease pathogenesis (305).

The common renal syndromes in HIV-infected patients include asymptomatic urinary abnormalities, acute renal failure, chronic renal insufficiency, and nephrotic range proteinuria associated with focal glomerulosclerosis, the classic HIV-associated nephropathy (HIVAN) (294–300,302,303,306), and various proliferative glomerulonephritides, which may be termed HIV-associated immune complex disease (HIVICD) (294,295,297–299,302,303,307–320) (Table 59-9). Over the past decade it has become clear that there are four syndromes of chronic renal disease intimately associated with HIV infection. These are HIVAN, HIVICD, including HIV-associated IgA nephropathy, and HIV-associated thrombotic microangiopathy (17,295,298,299). Other renal diseases in HIV-infected patients, including cryoglobulinemia and vasculitis, may be related to host responses to the infection, or

TABLE 59-9

GLOMERULAR SYNDROMES ASSOCIATED WITH HUMAN IMMUNODEFICIENCY VIRUS INFECTION

HIV focal glomerulosclerosis (classic HIV-associated nephropathy)
HIV mesangial hyperplasia
HIV-associated immune complex disease
IgA nephropathy
Idiotypic
Other mechanisms
Immune complex proliferative glomerulonephritis
Postinfectious
Lupus-like
Mixed immune complex–sclerotic

infections complicating the immunodeficiency state. Additionally, treatment effects may be associated with adverse renal outcomes (298–321).

CLASSIC HUMAN IMMUNODEFICIENCY VIRUS–ASSOCIATED NEPHROPATHY

Epidemiology and Clinical Features

Classic HIVAN is the most commonly reported chronic renal disease associated with HIV infection (17,294–300,322,323). This may be due in part to its association with the nephrotic syndrome and the reluctance of performing biopsies in patients with HIV infection and mild urinary abnormalities. The disease disproportionately affects men of African heritage (294,296–300,302,305,322,323), which may also account for the relative frequency of HIVAN reported in biopsy series from Europe and the United States. Clinical highlights of the disease are nephrotic range proteinuria and renal insufficiency. In most reports, hypertension and edema are relatively uncommon. This may be a consequence of the stage of disease or the presence of malnutrition in patients. The kidneys are large and echogenic on ultrasonic examination (294,296,297,302,303,322,323).

Pathology

HIVAN has been termed a "pan nephropathy" because of characteristic involvement of the glomeruli, tubules, and interstitium (324). Glomerular capillary wall collapse of varying severity is often noted (296–299,324,325). Mesangial hyperplasia and other abnormalities may be an early stage of the nephropathy (294), or this histologic variant may differentially involve children. Glomerular visceral epithelial cells are characteristically abnormal, exhibiting hyperplasia, hypertrophy, and vacuolation. Varying degrees of segmental or diffuse and global increased mesangial matrix are seen, as well as obsolescent glomeruli (17). Tubular cells show marked degenerative changes, flattening, simplification, brush-border abnormalities, and necrosis. Microcystic tubular dilation and hypertrophy are common. The interstitium shows immune cell infiltration of mononuclear cells, primarily macrophages and T-lymphocytes (17,325,326). Interestingly, T-lymphocytes are more prevalent than B-lymphocytes (326). Interstitial fibrosis and edema of the interstitium are common. Results of immunofluorescent evaluations are variable and nondiagnostic. Electron microscopy shows glomerular epithelial cell foot process effacement, wrinkling, and abnormalities of glomerular basement membrane structure (294,325). Tubular reticular structures are common, but not pathognomonic. They are found in glomerular and peritubular capillary endothelial cells and are probably a concomitant of the action of IFN-α.

Although the individual features of HIV focal glomerulosclerosis are not specific, the concomitant findings of glomerular collapse, glomerular and tubular epithelial cell abnormalities, increased mesangial matrix, renal tubular atrophy or microcystic dilation, and interstitial immune cell infiltration, in combination with tubular reticular structures are virtually diagnostic of classic HIVAN (17,299,324,325). The clinicopathologic characteristics of HIVAN have been compared to those of the collapsing variant of focal segmental glomerulosclerosis in the absence of HIV infection and found to be similar (327). Although it can be suspected on clinical grounds, the diagnosis of HIVAN cannot be made reliably in the absence of a biopsy, since other diseases such as diabetic nephropathy and HIV-associated immune complex renal disease can have similar presentations and features.

Pathogenesis

Although early in the epidemic, HIVAN was thought by some to be an epiphenomenon, or a complication secondary to intravenous drug use in patients, several lines of evidence suggest the renal disease is intimately related to infection with HIV (17,294,295,299,322,323). The development of classic HIVAN in the infant children of mothers with HIV infection (328) and the development of renal disease reminiscent of HIVAN in transgenic murine models (29,296,329–332) and in primates infected with simian immunodeficiency virus (SIV) (333–335) underscore the pathogenic relationship between the virus and the renal disease. Expression of HIV proteins within renal tissue appears to be a prerequisite for the development of renal disease (16,17,29,295,296,299,322,323,329,330). HIV proteins may affect renal cell biologic responses in diverse ways (299). HIV peptides are toxic to many cell types (16,17). Apoptosis of renal cells secondary to exposure to HIV peptides or as a result of their expression within renal tissue is a probable cause of renal disease and a matter of current research interest (16,17,295,296,299,322,323,329,330,336,337).

Abnormal proliferation and dedifferentiation of podocytes may be related to HIV infection of renal tissue and the expression of Nef (299,338) on signal transduction pathways (338) and cyclin biology (338,339). Stat3 and MAPK1,2 phosphorylation were increased in podocytes from kidneys of patients with HIVAN, compared to those of uninfected patients with focal segmental glomerulosclerosis (FGS) and other renal diseases, as well as kidneys from HIV-infected patients in the absence of renal disease, implicating these pathways in HIVAN pathogenesis, however other mediators are involved (338). Kajiyama et al. showed, in HIV-1 transgenic mice, that Nef is not necessary for induction of renal disease, but may rather potentiate nephropathy dependent on the expression of other HIV-1 gene products (340). Dickie et al. showed, in transgenic mice with mutations in either or both nef and vpr accessory genes, that proteinuria and FGS developed only in animals carrying the vpr gene. In animals encoding Tat and Vpr, Vpr protein was localized by immunostaining in glomerular and tubular epithelium. Breeding experiments produced mice with increased severity of nephropathy without increase of Vpr expression, leading the authors to conclude multiple genes must contribute to the development of renal disease in HIV-1 transgenic mice (341).

Inhibition of cyclin-dependent kinase improved urinary and histopathologic parameters of nephropathy in HIV-1 transgenic mice (342,343). Dysregulation of podocyte biology may be an important mediator of the development of HIVAN, and abnormal expression of glomerular epithelial cell proteins is associated with the disease (16,299,322–345). Podocan expression was increased in podocytes of HIV-1 transgenic mice compared with controls (346). Increased expression of transforming growth factor-β, perhaps as a result of viral infection, is also a possible mediator of nephropathy (347–350). Interestingly, expression of transforming growth factor beta has been linked to apoptosis of podocytes (350).

We compared tissue levels of transforming growth factor-β and chemokines from biopsies of patients with HIV-associated nephropathy, HIV-associated glomerulonephritis and idiopathic focal glomerulosclerosis without HIV infection (299,351). Transforming growth factor-beta, monocyte chemoattractant protein-1 (MCP-1) and RANTES levels were

increased in renal glomerular and interstitial tissue from patients with HIV infection, regardless of the type or presence of renal disease, suggesting chemokine expression might be a nonspecific response. Renal chemokine expression could act to interfere with local HIV infection by engaging renal chemokine receptors. These data are also consistent with renal deposition of circulating chemokines. In contrast, proteins associated with antigen presentation and response to infection, such as MHC-Class II proteins, interferon-α, and interferon-γ receptor protein were specifically increased in glomerular and interstitial tissue of biopsies of patients with HIV-associated nephropathy, compared with uninfected control tissue with and without renal disease, or renal diseases in the setting of HIV infection. The findings suggest genetic susceptibility, host responses, and a immunologically activated microenvironment may be critical to disease pathogenesis. High levels of tissue interferon-α are consistent with the pathologic feature of tubuloreticular inclusions (299). The pathogenesis of HIVAN is probably a concomitant of relationships between renal cellular infection and immune cell infiltration, effects on renal and infiltrating cellular cytokine, growth factor, and chemokine responses, and host factors. In addition, clinical and animal studies suggest an important role for genetic susceptibility in association with the development of nephropathy (301,352). Socioeconomic status, including access to antiretroviral medications may also be an important determinant of outcome (298,299,353).

Many of these processes depend on the presence of productive infection of renal cells by HIV. We showed that HIV DNA was present in renal tissue of patients with and without nephropathy (23). Detection of HIV RNA in renal tissue of patients with HIVAN has recently been confirmed (322–356). Renal cells can be infected by HIV *in vivo* (16,295,296,354,355,357). Tubular and glomerular epithelial cells can be infected by HIV *in vitro* (337,358), but in some studies mesangial cells could not (359). Infection of the glomerular epithelial cell and subsequent podocyte injury may be critical to the pathogenesis of HIVAN (16,299, 322,323,330,344). The infiltration of immune cells in renal tissue may be associated with progressive nephropathogenesis (326). Preliminary evidence suggests chemokine receptors are not present on renal cells from biopsy tissue (360–362), although they may be expressed in pathologic states in inflammatory cells in renal tissue (360), and may be detected by molecular techniques *in vitro* (337). Differential expression of chemokine receptors in renal tissue in different HIV-infected populations could be associated with different patterns of susceptibility to renal disease. The role of expression of CD4 and coreceptors for HIV infection in renal cells must be further investigated.

Prognosis and Treatment

Treatment studies have sharpened our understanding of the pathogenesis of HIV-associated diseases. In the era before widespread availability of antiretroviral therapy, HIVAN was characterized as a disease with an extremely rapid progression to its end stage (294,363,364). Several anecdotal studies suggested antiretroviral therapy improved the course of HIVAN (reviewed in [16,363,364]). Four therapeutic approaches have been used in patients with HIVAN. Limited data suggested cyclosporine ameliorated the course of the renal disease (363), but few patients have been assessed with this therapy. In addition, concern has been raised regarding the risk/benefit ratio of immunosuppression in patients with HIV infection. Several of these reports are difficult to evaluate since treated patients were not always evaluated with renal biopsies. Individual case reports (17,363–367) have suggested glucocorticoids may be effective in slowing the progression of HIVAN. A case series of patients with HIV infection and renal disease, treated with steroids, showed impressive diminution in urinary protein excretion and improvement in renal function (368). These studies, unfortunately, were not performed in a randomized controlled manner. In addition, there was a relatively high frequency of side effects such as psychosis, gastrointestinal bleeding, and infections (368). Finally, since survival analyses were not conducted in this series, the long-term effects of such treatments were poorly understood. More recently, Eustace et al. (369) found a salutary effect of treatment of patients with HIVAN with CS. Almost a quarter of patients maintained sufficient renal function to remain independent of need for renal replacement therapy for up to a year, in contradistinction to previous findings. Patients treated with CS, however, had longer hospital stays. Concurrent therapy with antiretroviral drugs and drugs which inhibit the renin-angiotensin system, as a result of advances in therapeutics, may have influenced outcomes in this uncontrolled study.

The angiotensin-converting enzyme (ACE) inhibitors, captopril and fosinopril, have been used in patients with HIVAN both to decrease urinary protein excretion and to halt progression of disease. Therapy with captopril increased length of time before death or start of dialysis in a case-control study (17,363,364). Nine patients with HIVAN were treated with captopril. Their course was compared to age, race, gender, CD4 count, and serum creatinine concentration versus matched control patients untreated with ACE inhibitors. All patients in both groups had HIVAN evaluated on renal biopsy. Renal survival was longer in patients treated with captopril. In another study, HIV-infected patients with proteinuria were treated with fosinopril (370). Patients who refused fosinopril treatment had an increase in serum creatinine concentration compared with those treated with fosinopril. These studies suggest a therapeutic effect of ACE inhibitors in patients with HIVAN. Neither of these studies was randomized or placebo-controlled. Wei et al. recently reported long-term results in the patients treated with fosiniopril, suggesting excellent outcomes (371). Twenty-eight patients were treated with fosinopril, compared to 16 in an untreated comparison group, in this nonrandomized, non–time-controlled study. Mean renal survival was 479.5 days in treated patients compared with 146.5 days in the comparison group. All patients untreated with ACE inhibition went on to ESRD, compared with one patient in the fosinopril group during a more than five year observation period. The authors however could not show an independent effect of treatment with antiretroviral drugs in this small uncontrolled study. Once again, the influence of change in clinical practice, especially regarding the use of antiretroviral drugs and HAART must be considered as potential confounding factors.

These findings were corroborated in studies of Bird et al. (372), who showed administration of captopril to HIV-transgenic mice that exhibited characteristics of HIVAN was associated with improvement of several renal functional parameters, such as urinary protein excretion, azotemia, and histologic abnormalities, despite the expression of HIV genes. A likely mechanism underlying the action of these drugs is the decrease in the expression of transforming growth factor beta afforded by ACE inhibitors (347,363,364,372).

Studies of treatment with antiretroviral drugs before the HAART era were inconclusive, because of small size and lack of definitive controls (298,299), but often showed beneficial effects on the nephropathy, as well as morbidity and mortality (298,299,322,323,354,373). Recent uncontrolled studies have suggested improved outcomes in patients with HIV infection and renal disease treated with HAART (374–376). Interpretation of the findings is often difficult because biopsies are often not performed in all patients reported. In patients treated with captopril, use of antiretrovirals was independently associated with improved renal survival (364).

A case report detailed improvement in the nephrotic syndrome in a child treated with HAART (377). Two cases of patients with HIVAN complicating early stages of infection demonstrate treating patients with HIV-associated nephropathy with HAART may cause resolution of both functional and pathologic abnormalities (354,378), although productive infection of kidney tissue may continue (354). Recent work in an uncontrolled observational cohort of patients with HIV infection and renal disease who underwent renal biopsy suggests treatment with antiretroviral drugs was associated with improved outcomes in patients with HIVAN (353). Such data also bolster evidence for the causal relationship between viral infection and nephropathy.

ACE inhibitors represent a safe, possibly effective long-term therapy for patients with HIVAN. Because there is evidence that treatment with both HAART and ACE inhibitors is associated with improved outcomes in patients with HIVAN, the field requires implementation of well-designed and powered randomized controlled therapeutic trials of antiviral therapies and ACE inhibitors in patients stratified for age, gender, and histologic parameters, in whom virologic and immune parameters, as well as side effects, are carefully delineated. In some patients treated with both ACE inhibitors and HAART, in whom the disease progresses, consideration may be given to the use of CS. The role of treatment with CS, however, is unclear and awaits the performance of controlled clinical trials to determine its utility and safety. The development of renal disease in a patient with HIV infection may be an indication for HAART, regardless of stage of the viral illness, unless it is specifically contraindicated (298,299,322). Because not all renal disease in HIV infected patients is HIVAN, consideration should be given to performing a renal biopsy if the diagnosis is unclear (298,299,353).

Initial reports indicated survival of HIV infected patients with ESRD treated with dialysis was poor (299,379). While the initial growth of HIV infection in the U.S. ESRD program was quite high, recently the incidence has stabilized at 1.25% to 1.5% (298,299,380). The prevalence of HIV infected patients in the ESRD program has increased dramatically, from 0.45% in 1995 to 0.83% in 2000, in part because of the improved survival of patients with HIV infection (380–382). It is possible that trends in both incidence and prevalence of ESRD since 1996 have been affected by the availability of HAART in the US (299,380). HIV infection in the ESRD program is more common in men (380). Outcomes for patients treated with peritoneal and hemodialysis have been equivalent (383,384). In contrast, very few HIV-infected children have been treated for ESRD, and only 50% are male (385). Survival of children was better than for HIV-infected adults with ESRD (385). Girls with HIV infection and ESRD have better survival than boys (385).

Recent studies have concentrated on treatment of HIV infected patients with ESRD with renal transplantation (298,299,386,387–390). Consideration for such experimental treatment currently requires patients to be treated with HAART and have undetectable viral loads (298,299).

HUMAN IMMUNODEFICIENCY VIRUS–ASSOCIATED GLOMERULONEPHRITIS

Less common than classic HIVAN, HIVICD (13,298,299,312, 317) comprises proliferative glomerulonephritis, renal insufficiency, and proteinuria. Circulating immune complexes composed of immunoglobulins, characteristically IgG or IgA, reactive with specific HIV antigens such as p24, gp41, and gp120 are present, and identical complexes may be eluted in higher titer from renal tissue. HIV peptides may also be demonstrated intracellularly in eluted glomerular tissue by laser-enhanced microscopy (317). Three main types of clinically distinguishable HIV-associated immune complex renal disease may be delineated: HIV-associated immune glomerulonephritis, a mixed sclerotic–immune complex nephropathy (319), and HIV-associated IgA nephropathy (17,23,295,298,299,312). Mesangial hyperplasia may also be a glomerulopathy associated with HIV infection (294,328,365,391), but it is most often considered a part of the spectrum of FGS. A "lupus-like" appearance to the glomerulonephritis has also been noted in a subset of cases (319,392)

HUMAN IMMUNODEFICIENCY VIRUS IMMUNE COMPLEX–MEDIATED RENAL DISEASE

Circulating immune complexes, often identifiable as HIV-related, are common in HIV-infected patients at all stages of the disease (393–398). MPGN, diffuse proliferative glomerulonephritis, immunotactoid glomerulopathy, and MN have been reported in patients with HIV infection (202,294,297, 302,303,307–309,317–319,325,399–407). In several studies of HIV-infected patients with nephrotic range proteinuria, more than one-fourth had glomerulonephritis as opposed to HIV-associated focal glomerulosclerosis (13,23,353,408).

Epidemiology

Of 28 patients with HIV infection and nephrotic range proteinuria who underwent evaluation in our series (23), 28.6% had a form of proliferative glomerulonephritis; 87.5% of the latter were African American. In subsequent studies, 40% of patients with HIV infection and renal disease who underwent renal biopsy had findings consistent with an immune-mediated glomerular disease (13). Excluding patients with IgA nephropathy, 30% of patients with renal abnormalities and HIV infection evaluated by renal biopsy had a type of proliferative glomerulonephritis. Nochy and co-workers (319) describe a population of European, Caribbean, and African patients with HIV infection and renal disease evaluated in Paris. Approximately 37% of patients had immune complex glomerulonephritis. Twenty-one percent of black patients had immune complex renal disease. Interestingly, only one black patient in that series had immune complex glomerulonephritis alone; the remainder had mixed focal glomerulosclerosis and immune complex nephropathy. Approximately half of the white patients had immune complex glomerulonephritis, commonly with a variable tubulointerstitial nephritis. The majority of these patients were homosexual. Only one of the white patients, an intravenous drug user, had the "mixed" nephropathy. Three other European studies from the United Kingdom and Italy emphasize a high proportion of patients with nephrotic syndrome in the setting of HIV infection have glomerulonephritis (400–402). A study from Thailand of HIV infected patients with renal disease reported a diverse set of glomerulonephridites, but no cases of classic HIVAN (407). While in general, patients of African heritage are more likely to have HIVAN, European and Asian populations more often appear to have glomerulonephritis. HIVICD did occur in patients of African heritage in the European studies (319,400,401). In the large Italian study (402), although there were a variety of glomerulonephritides delineated, no patient of Italian heritage had HIVAN. In a recent U.S. multicenter study in which 89 patients with HIV infection and renal disease underwent renal biopsy, 14.6% had immune

complex glomerulonephritis, 9% had MN, 5.6% had MPGN, and 1.1% had IgA nephropathy and MCD (353).

Clinical Features

All patients we studied with HIVICD were African Americans with renal failure and proteinuria (13,317). Three had proliferative glomerulonephritis, crescentic and sclerotic changes, marked renal insufficiency, and nephrotic proteinuria. One had a clinical constellation consistent with postinfectious glomerulonephritis, with mild renal insufficiency. Most patients were homosexual, but one patient was thought to have acquired HIV infection through heterosexual transmission. Clinical stage of HIV infection was not related to the occurrence of disease (317,319). The patients with HIVICD usually had mild hypertension, in contrast to those with HIVAN. All patients had proteinuria, often in the nephrotic range. Hematuria was not an invariable finding. Red blood cell and granular casts were variably present on urinalysis. Renal insufficiency and hypocomplementemia to a variable degree were encountered. All patients in our series had circulating immune complexes composed of immunoglobulins reactive with HIV proteins, which, in most cases, were eluted from renal tissue in higher titers than in the plasma. Circulating immune complexes were isolated in all four patients, composed of IgA-p24, IgG-gp120, and IgG-p24. Identical complexes were eluted from renal tissue in the first three cases; only p24 and complement were eluted from the fourth. Eluted antibodies reacted with the HIV antigens isolated from the circulating immune complexes. We were not able to demonstrate cryoprecipitation in any case.

Renal Pathology

Nochy and co-workers (319) describe several subtypes of HIV-associated immune complex glomerulonephritis. These include a diffuse exudative endocapillary proliferative form termed "postinfectious;" a second type that resembles lupus nephritis with diffuse endocapillary proliferative changes, wire loops, hyaline thrombi in capillary lumina, and mesangial, intracapillary, and subepithelial deposits of immunoglobulin, C3, and C1q; and a "mixed" type with features of both focal glomerulosclerosis and immune complex nephropathy. Two of the patients in our series might be considered of the mixed type (317). We found a spectrum of pathologic changes, including variable degrees of mesangial expansion, segmental increase of mesangial cells and matrix, and segmental or diffuse proliferation of glomerular tufts. Increased cellularity with lobular transformation, segmental condensation or simplification of the glomerular tuft, and segmental or global proliferative and sclerosing changes were seen. Visceral epithelial cells were often prominent, and fibrocellular crescents were often present. Microcystic tubular dilation and atrophy and interstitial fibrosis or edema were often noted. Biopsies were characterized by interstitial infiltration with mononuclear cells, primarily macrophages and lymphocytes, and occasionally plasma cells, polymorphonuclear leukocytes, and eosinophils. Occasionally, infiltrating cells disrupted the tubular basement membrane. The proportion of interstitial macrophages was higher in tissue from patients with classic HIVAN compared with tissue from patients with HIVICD. On the other hand, interstitial tissue from biopsies of patients with HIVICD had a higher percentage of B cells compared with HIVAN tissue (326). Such findings are consistent with different populations of infiltrating immune cells causing different histologic types of disease or different tissue chemokine profiles in different nephropathies caused by the same virus, which attract different cell types to the site of injury.

Immunofluorescence microscopy demonstrated intramembranous and mesangial deposits of immunoglobulins and complement (317). Electron microscopy usually reveals subendothelial, intramembranous, and mesangial electron-dense deposits. Electron-dense deposits are found within mesangial cells, and glomerular capillaries occasionally exhibit both subepithelial and subendothelial deposits. Foot processes of visceral epithelial cells are approximated. Tubular reticular structures can be detected in endothelial cells.

Diagnosis

Renal biopsy is important in determining the histologic diagnosis in patients with HIV infection and renal disease, including those who present with nephrotic range proteinuria (13,23,317,353), and is the only definitive way to diagnose HIVICD. Several of the patients in our series were thought to have HIVAN before biopsy. Renal biopsy must show histologic evidence of glomerular inflammation, with immunofluorescent microscopy confirming deposition of immunoglobulin and complement and electron microscopy demonstrating mesangial or capillary deposits. More precise diagnosis may be achieved by identifying HIV protein–immunoglobulin complexes in glomerular capillaries or the mesangium in higher titer than in the circulation, although such studies are often primarily research techniques. The role of specific therapeutic interventions could be evaluated in controlled trials in HIV-infected patients with renal disease with defined clinical and histologic parameters.

An interesting subgroup is comprised of patients coinfected with HCV and HIV who have renal disease (35,186,409–411). The majority of reported cases were intravenous drug users who had glomerulonephritis, typically MPGN, rather than HIVAN. MN and immunotactoid and fibrillary glomerulonephritis have also been reported (35,202,405). HCV RNA was detected in renal tissue in half of the patients evaluated in the study by Stokes et al. (409). Etiologic relationships between the immune complexes, the inciting antigen, and the renal disease have not often been reported. Renal biopsy is necessary to make a clinical diagnosis.

Prognosis and Treatment

Prognosis depends, in part, on renal functional status and histologic findings. All patients we evaluated with findings of "mixed" disease progressed to ESRD. Another patient with mild urinary abnormalities and well-preserved renal function had relatively stable renal function for almost 2 years while the viral illness progressed. Antiretroviral therapy had been instituted in some patients with HIVICD without obvious effect. Its role in the treatment of HIV-related glomerulonephritis must be studied rigorously. One patient with advanced renal insufficiency and nephrotic range proteinuria treated with steroids had transient improvement in renal function of several months' duration but later progressed to ESRD (317). Patients in other studies have had variable outcomes, but follow-up has usually not been extensive. Patients with glomerulonephritis and HCV and HIV infection appear to have a variable, but worse outcome than patients with HCV-associated glomerulonephritis (353,409,410). In a recent U.S. multicenter observational study, patients with renal diseases other than HIVAN had a longer course until the development of ESRD (731 $\pm$ 642 compared with 254 $\pm$ 331 days) and had better overall survival (353). No differential effect of treatment

with antiretroviral drugs was noted in the group of patients without HIVAN.

HUMAN IMMUNODEFICIENCY VIRUS-ASSOCIATED IMMUNOGLOBULIN A NEPHROPATHY

IgA nephropathy has been described in several patients with HIV infection. IgA antibodies directed against HIV antigens are part of the early response to HIV infection (412–416). Circulating antigen–antibody complexes containing IgA are often present in HIV-infected patients. Rather than being the chance association of two unrelated diseases, it appears that the renal disease is intimately associated with the viral infection. In HIV-associated IgA nephropathy, we detected idiotypic IgA immunoglobulins directed against anti-HIV antigen–antibody complexes, which were identical to those found in renal tissue (312). Immunochemical analysis revealed circulating, idiotypic IgA antibodies, bound to IgG-gp41 and IgM-p24 complexes (312). Identical immune complexes were eluted from biopsied tissue, in higher concentration than the circulating immune complex. In addition, HIV gene sequences were amplified by PCR from renal tissue. These data are similar to findings in IgA nephropathy in non-HIV-infected populations (417), suggesting immune mediation and a genetic predisposition to a particular pathologic outcome.

Epidemiology and Clinical Features

Almost all patients with HIV IgA nephropathy reported are white or Hispanic (310–316). Hsieh and others reported a black patient with HIV infection and crescentic IgA nephropathy (320). Two Southeast Asian patients with HIV infection and IgA nephropathy have been reported (407). Most patients are homosexual men, although the disease has also been reported in boys. Patients have hematuria and proteinuria, sometimes in the nephrotic range. Red cell casts are usually noted. Renal insufficiency is common but is often stable and can improve. Serum IgA levels are increased, but this is a common finding in HIV-infected patients in the absence of renal disease. Occasional patients have hypertension. Katz et al. (311) evaluated three white homosexual men and a Hispanic boy with HIV infection and IgA nephropathy. All patients had circulating immune complexes composed of immunoglobulins, including IgA reactive with various HIV antigens and IgA rheumatoid factors (311). We demonstrated the presence of circulating immune complexes composed of idiotypic IgA antibodies directed against IgG or IgM antibodies reactive with HIV peptides in two patients (312). The IgA antiimmunoglobulin response in both patients was specific both for types of immunoglobulin and for immunoglobulin reactive with specific HIV antigens. The anti-HIV antigen response was also both viral peptide-specific and patient-specific. Cryoprecipitation was demonstrated in one case.

The low reported prevalence of IgA nephropathy in HIV-infected patients may be related to a true low incidence of the disease (312,418), perhaps related to host and genetic background in infected patients (312,417–419). Alternatively, the low prevalence may reflect the reluctance to perform renal biopsies in patients with urinary abnormalities and mild renal insufficiency, in the absence of perceived effective treatment, and in the presence of a disease that is thought to be more linked to survival than the nephropathy (312). A study from France, however, suggests almost 8% of postmortem cases show mesangial deposition of IgA (420). In a recent U.S. multicenter study in which 89 patients with HIV infection and renal disease underwent renal biopsy, only 1.1% had IgA nephropathy (353).

Renal Pathology

Light microscopy usually shows diffuse or segmental increase in mesangial matrix, with segmental proliferative changes. Rarely, thrombi are seen in glomerular capillaries, and areas of segmental sclerosis may be noted. Occasionally fibrocellular crescents are seen (311,312,316,320). IgA is the predominant immunoglobulin in the mesangium and in glomerular capillary walls, along with C3, IgM, and less often IgG. Electron microscopy shows increased mesangial matrix with mesangial and peripheral (intramembranous, subepithelial) electron-dense deposits. Tubular reticular structures may be noted in glomerular endothelial cells. Nuclear bodies may be seen in interstitial cells (311).

Prognosis and Treatment

Jindal et al. (316) used steroid therapy in a patient with renal insufficiency and IgA nephropathy before the HIV infection was diagnosed. The level of serum creatinine decreased, suggesting a beneficial clinical response may have been related to this treatment. Most patients, however, have had mild, nonprogressive renal insufficiency, and there are few data regarding treatment. The effects of CS and antiretroviral therapy on the natural history of the disease are unknown. A patient with HIV infection treated with didanosine, and IgA nephropathy with urinary protein excretion of 12 g/day was given captopril. Over 3.5 months, urinary protein excretion decreased to 0.5 g/day, associated with an increase of circulating levels of serum protein and albumin (421). Prospective, controlled studies are necessary to evaluate the role of inhibition of the renin-angiotensin system and the use of HAART in HIV infected patients with IgA nephropathy.

HUMAN IMMUNODEFICIENCY VIRUS–ASSOCIATED THROMBOTIC MICROANGIOPATHIES

Both thrombotic thrombocytopenic purpura (TTP) and hemolytic–uremic syndrome (HUS) have been reported in patients with HIV infection (422–431). A French study suggests the thrombotic microangiopathies are a common cause for acute renal failure in HIV-infected patients (423). Evidence suggests the disease can be caused in animal models by retroviral infection (431,432), and that the virus might mediate dysfunction of endothelial cells (431,433–437). The diseases manifest their common presentations, but because of the protean manifestations of HIV infection, may prove difficult to diagnose. Abnormalities of the peripheral blood smear remain the criterion for making the diagnosis (422). Although a variety of different therapeutic approaches have been employed (298,299,431,438), plasmapheresis remains the safest and perhaps the most effective treatment for HIV-infected patients with TTP. The potential therapeutic role of antiretroviral therapy in such cases, while attractive on theoretical grounds, has not been tested adequately in well-designed clinical studies (439,440). Few studies exist on the treatment of HUS in the setting of HIV infection. Randomized controlled trials of therapy in patients with HIV infection and the thrombotic microangiopathies have been lacking (298,299,431).

RENAL DISEASE RELATED TO TREATMENT

The host response to ongoing viremia may include continued antibody synthesis, cell-mediated immune responses, and cellular antiviral responses. Therapy with IFN-α has been associated with a variety of renal disorders. We studied an HIV-infected patient treated with IFN-α who developed MPGN. An IFN-α-immunoglobulin immune complex was identified in the circulation and in higher titer in renal eluates, demonstrating that glomerulonephritis in HIV-infected patients may be secondary to a host response to treatment (239). Distinguishing the progression of disease from iatrogenic effects may be an important clinical dilemma in patients treated with this immunomodulator who develop increased renal insufficiency. The extent of renal disease related to therapy with IFN-α in patients with HIV, HBV, or HCV infection, however, is unknown.

The newer antiretroviral drugs, especially the protease inhibitors, in particular indinavir (321,363,441,442) and to a lesser extent, ritonavir (363,443) may cause reversible nephropathy by several mechanisms (299,321,441,444–446). It is important to distinguish such effects from the progression of the underlying renal disease. Rhabdomyolysis may be an increasingly common cause of acute renal failure as patients with lipodystrophy induced by antiretroviral therapy are treated with statins (299,447–450). Adefovir, cidofovir, abacavir and tenofovir have all been associated with the development of tubular injury (299,451–455).

RENAL DISEASE INDIRECTLY ASSOCIATED WITH VIRAL INFECTION

Glomerular diseases may occur in HIV-infected patients that are unrelated to the viral infection, are related to coinfections, or reflect the immune dysregulation seen in HIV infection (13,16,17,294,297,456,457). A patient with glomerulonephritis and HIV infection exhibited characteristics of poststreptococcal glomerulonephritis (456). The biopsy, however, showed tubular reticular structures, which are a hallmark of HIV-associated renal diseases. Therefore, this patient may have had two different glomerular diseases, or the electron microscopic findings may have been nonspecific. The slow resolution of the renal disease in this case may reflect the disordered immunoregulation seen in HIV-infected patients, compared with immunologically normal hosts who develop glomerulonephritis after streptococcal infections. We evaluated a white man with nephrotic syndrome in the presence of HIV and HBV infection. The renal biopsy was suggestive of MN secondary to HBV infection, rather than an HIV-associated glomerulopathy (399). The association of these two diseases was confirmed when the proteinuria remitted concurrently with the clearance of HBeAg from the patient's circulation (458). There are a paucity of well-described cases of lupus nephritis in the setting of HIV infection (392,459). It is possible that the disordered immunoregulation associated with the viral infection mediates altered renal outcomes in such patients.

CRYOGLOBULINEMIA AND VASCULITIS

Occasionally patients with vasculitis and/or cryoglobulinemia in the presence of HIV infection have been reported (460–466), although the extent of renal involvement in such patients is often unclear. A report outlined 16 HIV-infected patients with large vessel disease (467). Another study of HCV-infected patients linked the presence of cryoglobulinemia to vasculitis and coexisting HIV infection (179). Coinfection with HBV and HCV make the pathogenesis of this disorder difficult to assess, in light of the abnormal immunoregulation present in HIV infection, in the absence of rigorous, precise identification of tissue-deposited immunoreactants (468).

Antibodies reactive with HIV p24 have occasionally been noted in patients with autoimmune diseases (469). Sera from several patients with essential cryoglobulinemia reacted with human T-cell lymphotrophic virus type I proteins, and reverse transcriptase activity was found in several patients as well (469). None of the sera reacted with HIV-1 peptides. The significance of these findings is unclear, but it is possible that these antibodies may only be cross-reactive with the retroviral proteins or be directed against endogenous proteins, coded by retroviral-like sequences in the human genome or sharing homology with retroviral proteins (469–471). Vasculitis and cryoglobulinemia, however, appear to be relatively rare compared with the prevalence of HIV infection. It is also frequently difficult, in the absence of intensive immunologic analysis, to assess whether the cryoglobulinemia is associated with HIV or other coexistent infections. Renal vasculitis has not been an important clinical finding in such patients. In a patient with HIV and HBV infection, cryoglobulinemia and proliferative glomerulonephritis were evaluated. Two circulating immune complexes were detected in this patient. One was composed of an IgG–HIV p24 complex, the other a cryoprecipitable IgA–IgG complex. Interestingly, the HIV peptide complex was not implicated in the pathogenesis of the nephropathy (13). This case illustrates the importance of a careful immunochemical evaluation in establishing a precise diagnosis of the renal disease in HIV-infected patients.

MECHANISTIC POSSIBILITIES IN THE PATHOGENESIS OF HUMAN IMMUNODEFICIENCY VIRUS-ASSOCIATED IMMUNE COMPLEX DISEASE

The pathogenesis of HIVICD is largely unknown; however, in our studies, the overwhelming majority of HIV-infected patients with renal disease show the presence of HIV genome in the kidney (13,23,317). Cohen et al. (472) also demonstrated proviral HIV DNA in tubular and glomerular epithelial cells in biopsies and autopsy tissue from HIV-infected patients with immune complex glomerulonephritis using *in vitro* DNA hybridization techniques. They also found such renal tissue markers in patients with AIDS without clinically obvious renal disease. However, they were unable to demonstrate the presence of either HIV core or envelope protein antigens in glomerular deposits in their patients with immune complex glomerulonephritis. Interestingly, we also demonstrated the presence of HIV genome in autopsy tissue of patients *without* the presence of renal lesions (13). Such findings suggest the presence of HIV genomic material in the kidney is not alone sufficient to induce nephropathy. Triggering and facilitating mechanisms seem likely, therefore, to be associated with mechanisms that cause nephropathy. Understanding the pathogenesis of such renal disease and the nature of such mechanisms might lead to preventive or ameliorative strategies.

The role of cellular incorporation of HIV genome products in the development of renal disease, contrasted with circulating immune mechanisms, is unknown. The development of glomerulonephritis may be dependent on the renal parenchymal incorporation of HIV antigens, although renal cellular HIV

infection may not be a sufficient condition for disease expression. Evidence suggests HIV may infect renal tissue, specifically glomerular epithelial cells (322,323,337,354–356,472) and mesangial cells *in vitro* (357,473). The tubular epithelium may also be a target for infection (337,358). Tubular cells can be infected by HIV *in vitro* (358).

It is possible that renal cellular viral infection itself, by the expression of viral antigens or alteration of renal cellular proteins, might lead to subsequent attachment of circulating anti-HIV antibodies or complexes to an implanted or transformed antigen. Alternatively, such findings may be the result of deposition of circulating immune complexes in renal tissue, which subsequently initiate an inflammatory response. Analysis of pathologic tissue cannot differentiate definitively between these two pathogenic mechanisms. The pathogenic significance of a positive PCR remains to be established.

Similarly, the role of genetic and host responses may be crucial in determining renal pathologic outcomes. It is of interest that a substantial proportion of the polyclonal immunoglobulin response in HIV-infected patients is composed of IgA, and that IgA-containing immune complexes are prevalent in patients with HIV infection and AIDS (412–416). These immune responses may partially explain the prevalence of IgA nephropathy seen in HIV-infected patients. It is possible that certain specific immune responses, perhaps related to specific modes of antigen presentation, or specific circulating immune complexes are more likely to provoke an ongoing renal inflammatory response. Finally, the role of concurrent or intercurrent viral infection in affecting renal responses remains to be determined.

Since HIVICD is characterized by a dense interstitial infiltrate (13,23,312,317,325,326) that aids in the identification of its HIV-associated nature, immune cell infiltration in renal tissue may be important in its pathogenesis. Because different cell populations characterize different nephropathies, the immune cell population may also have an important effect on histologic outcome. Alternatively, different pathogenic processes may elicit different infiltrating cells. Cytokines and growth factors, which are products of immune cells, may also enhance nephropathogenicity. IFNs are expressed in response to viral infections (474). Treatment with IFNs has been associated with reversible nephropathy, including interstitial nephritis and glomerulonephritis (239,240–243,475–478). Several cytokines and growth factors can modulate the growth and function of mesangial cells in culture, including platelet-derived growth factor, epidermal growth factor, interleukin-1 (IL-1), tumor necrosis factor, and transforming growth factor-β (21,479–488). A role for cytokines and growth factors in the activation of latent HIV-1 virus has also been suggested (489–495). In addition, HIV-1 proteins may have cytotoxic or other effects on renal cells that could enhance nephropathogenicity. The HIV protein gp120 is cytotoxic, causing death of astrocytes in human brain cultures *in vitro* and inducing cellular IFN-α and IFN-γ, tumor necrosis factor-α, IgG, IL-1, and IL-6 production (496–498). The HIV-1 *trans*-activator protein Tat has variable stimulatory or inhibitory effects on cell proliferation (499–502). gp120 and gp120-anti-gp120 antigen–antibody complexes are immunomodulatory (498,503,504). Therefore, HIV peptides and antibodies directed against them may modulate renal cellular function, leading to susceptibility to immune complex renal disease.

CYTOMEGALOVIRUS (CMV) INFECTION AND GLOMERULONEPHRITIS

CMV infection has been associated with transplant glomerulopathy and chronic allograft nephropathy (505–511) and acute rejection (511,512), although this notion is still a controversial area of research and vigorously disputed (513–518). The lesion may be associated with vascular rejection (512,513,515,519). A case report demonstrated the presence of immunotactoid glomerulopathy with renal failure in a renal transplant patient who simultaneously had CMV viremia (520). The glomerular lesion and renal dysfunction resolved concurrently with successful treatment of the CMV infection, circumstantially suggesting an association between the viral disease and the glomerulopathy.

Controversy also exists regarding whether CMV infection is intimately associated with glomerulonephritis in general and specifically with IgA nephropathy (417,521,522). A report implicated CMV infection in the development of recurrence of glomerulonephritis in a patient with preexisting MPGN (522) by virtue of improvement after treatment with ganciclovir. No evidence of CMV renal infection, however, was reported. Detwiler et al. reported a necrotizing, crescentic glomerulonephritis in a patient who received a renal transplant (523). Interestingly, although there was little evidence of immune-complex mediated mechanistic processes, CMV was detected in renal tissue by immunochemistry and *in situ* hybridization techniques. In this case, ganciclovir therapy also ameliorated signs of renal disease.

CMV can infect and replicate in human mesangial cells (524), and CMV infection in mice is associated with glomerulonephritis (525). In animal models, IgA nephropathy may result from various viral infections (417). Ozawa and Stewart (526) described a patient with hematuria and proteinuria, with normal renal function, who died of gastrointestinal hemorrhage while being treated with CS. Autopsy revealed focal proliferative mesangial hypercellularity. IgG, IgA, C3, and C4, as well as CMV antigens, localized by guinea pig anti-CMV antiserum, were present in a granular pattern within the mesangium (526). IgG antibody reactive with CMV was detected in renal eluates in lower titer than in the circulation. The authors interpreted these findings as consistent with a CMV immune-mediated glomerulonephritis. Gregory and co-workers (7) consistently detected CMV antigens in tissue from patients with IgA nephropathy using indirect immunofluorescence techniques, employing polyclonal antihuman CMV antibodies (7). Waldo and others (527) suggested these results may have been nonspecific, and other investigators have been unable to confirm the positive results (528,529). Ortmanns et al. described a patient with IgA nephropathy who was treated with immunosuppressive drugs who subsequently developed CMV infection (530). Renal function improved after immunosuppression was discontinued and therapy with ganciclovir initiated. After discontinuation of ganciclovir, renal function worsened. Renal function improved after reinstitution of ganciclovir. The authors interpreted these findings as evidence for an important role of CMV in the pathogenesis of IgA nephropathy, but it is also possible that two distinct renal pathogenic mechanistic processes occurred simultaneously. Lai et al. (531), using monoclonal antibodies directed against CMV antigens in indirect immunofluorescence studies, were unable to detect CMV peptides in tissue from patients with IgA nephropathy. They suggested the conflicting findings might be based on differential antibody specificity and the positive findings were artifactual.

Studies have investigated whether the CMV genome may be found in renal tissue of patients with IgA nephropathy. Okamura and co-workers (532) and Kanahara co-workers (533) were unable to detect CMV DNA in renal tissue from patients with IgA nephropathy. Smith et al. (534) were also unable to confirm the presence of CMV antigens in a large proportion of renal tissue from patients with IgA nephropathy using immunochemical techniques. Similarly, they were unable to localize CMV DNA in such tissue using in situ DNA hybridization. Bene et al. (535), using monoclonal antibodies directed

against CMV antigens, were unable to detect CMV peptides and, using PCR technology, were able to detect CMV genome in only 1 of 14 samples of renal tissue from patients with IgA nephropathy. The authors concluded that CMV infection was not associated with IgA nephropathy. Telenti and co-workers (536) failed to show CMV DNA in renal tissue from 10 patients with IgA nephropathy.

Muller et al. (537,538), however, detected CMV DNA by PCR in renal tissue from 74% of 19 biopsies of patients with IgA nephropathy. Immunohistochemical examination of tissue for CMV antigens was positive in only 2 of 17 evaluations. CMV antigens were detected in a mesangial distribution. The CMV genome, however, was also detected in 4 of 18 (22%) samples of frozen tissue from normal kidneys, although the difference between the proportions of positive detections was statistically significantly different in the two groups. CMV genome was not amplified from samples of kidneys from patients with focal glomerulosclerosis (537). In addition, detection of the CMV genome was less common (only 1 of 17, or 6%) in paraffin-embedded sections in contrast to frozen material. However, only a few specimens were assessed in tandem using the two preparations from individual patients. These results are in accordance with the notion that CMV infection is associated with the pathogenesis of IgA nephropathy but do not establish a causal relationship. Alternatively, the host immune response to CMV infection may culminate in the development of IgA nephropathy in selected patients.

Kadereit et al. (539) showed, in an early study, that CMV genome could be detected in 40% of renal biopsies of patients who had circulating antibodies directed against CMV, indicating prior exposure to the virus. However, 30% of seronegative patients also had a positive PCR evaluation in renal tissue, suggesting the technique is more sensitive than serologic assessments. A seronegative control without kidney disease also had a positive PCR determination. All negative genomic controls had negative PCR evaluations. There was no correlation of a positive PCR result with type of nephropathy. Such data suggest the finding of a positive CMV PCR may be relatively common in renal tissue of patients with and without renal disease, reflecting the high prevalence of CMV infection. Park et al. (540), however, used the PCR technique to amplify CMV DNA from renal tissue from patients with IgA nephropathy and tissue from both patients with other glomerulonephritides and normal renal tissue as controls. These investigators were able to amplify CMV gene products from 60% of paraffin-embedded renal biopsy tissue from patients with IgA nephropathy, but CMV DNA was also detected in 71% of control tissue from patients with other forms of glomerulonephritis. They were unable to demonstrate CMV antigen, however, by immunologic techniques in renal tissue from patients with IgA nephropathy. These results suggest that the presence of CMV genome in renal tissue from patients with IgA nephropathy is not specific and that renal cellular infection with CMV may be associated with glomerulonephritis. This association may not, however, be one of cause and effect.

PCR results are likely to be more sensitive (13,23,24) but also are dependent on the primers and probes used and the technique employed (541) and are subject to contamination. In addition, material may be present during PCR that may inhibit the reaction, leading to false-negative results. Therefore, in addition to scrupulous technique, it is important that positive and negative controls, from the same laboratory, be simultaneously assessed in this type of study to allow valid conclusions to be drawn from the data. Geographic variations, considering the genetic predispositions to development of IgA nephropathy and differences in rates of endemic viral infections and strain differences worldwide, should also be considered when analyzing such results. Ultimately, the demonstration of specific viral peptides and antiviral antibodies in characteristic anatomic locations will be necessary to validate a causal association between CMV infection and glomerulonephritis.

HANTAVIRUS INFECTION AND THE KIDNEY

The hantaviruses are a group of single-stranded RNA viruses of the Bunyaviridae family (542). Although hantavirus is endemic in Korea and other parts of Asia, cases have been noted in Western Europe, and exposure to hantavirus infection has been documented in inner city populations in the United States (543). Since a greater proportion of patients in Baltimore, Maryland with renal disease and hypertension had antibodies to hantavirus compared with control groups of patients seen in an emergency setting and in a sexually transmitted disease clinic, some have speculated on an association between exposure to hantavirus and the pathogenesis of chronic renal disease in the United States (543). Such preliminary results were confirmed in a study of biopsy-proved cases of acute and chronic renal disease (544). Three percent of patients with congenital renal disease had serologic evidence of exposure to hantavirus, while more than one-fourth of a group of patients with various acute and chronic renal diseases had antibodies against hantavirus. Patients with tubulointerstitial nephritis, necrotizing glomerulonephritis, and IgA nephropathy were most likely to have had antibodies directed against hantavirus. The pathogenic mechanisms underlying these associations remain to be clarified. Studies suggest chronic alterations in kidney function, including glomerular filtration, glomerular permeability and tubular function may occur after viral infection (545–548).

Hantavirus infections have been associated with acute renal failure, including nephropathia epidemica, associated with Puumala virus infection, and the more severe hemorrhagic fever with renal syndrome, in those infected with Hantaan virus. Infection with the Seoul virus has been associated with a less severe form of hemorrhagic fever with renal syndrome (549). Characteristic clinical signs of hemorrhagic fever with renal syndrome include fever, hemorrhagic manifestations, and renal failure. The disease is divided into five phases: febrile, hypotensive, oliguric, diuretic, and convalescent. In many cases, renal function returns to normal, but often over a period of months. The renal disease is often characterized by tubular, rather than glomerular disorders. Pathologic findings of hemorrhage in medullary interstitium, inflammatory cell infiltration, and glomerular epithelial cell and basement membrane abnormalities are encountered. Tubular lesions such as sloughing and necrosis of epithelial cells are common, but glomerular abnormalities are not. The pathogenic contributions of vasodilation, microcirculatory abnormalities, platelet dysfunction, and disseminated intravascular coagulation to the pathologic findings are unclear. Immune complex-mediated mechanisms have been thought to be important because they may be detected in the circulation and glomeruli (542,549). Complement activation has been noted concurrently with high levels of immune complex detection and peak vascular injury. Treatment of the renal disease consists of supportive measures and appropriate renal replacement therapy. Several reviews have considered this area (550,551), while the number of case reports and series describing specific aspects of the disease has burgeoned.

In a large series of patients from Finland with nephropathia epidemica diagnosed serologically, tubular and interstitial abnormalities were most common, although in 20 of 53 patients who had glomeruli for analysis, abnormalities such as mesangial hypercellularity were noted (552). Half these glomeruli had negative immunofluorescent evaluations. In the other half, a variety of immunoreactants, including complement, were noted in a granular mesangial distribution, with patterns ranging from

focal and segmental to diffuse and global. In one case, the diagnosis of IgA nephropathy was made. Although rare case reports of glomerulonephritis in patients with hantavirus infection exist (546,553,554), it is not clear whether the glomerulonephritis was directly related to the viral infection or represented the occurrence of two distinct diseases.

EPSTEIN-BARR VIRUS INFECTION

Rare cases of renal disease have been reported in patients with Epstein-Barr virus (EBV) infection (555–567). Several cases of immune complex renal disease with glomerular ultrastructural abnormalities have been reported in association with EBV infection (555–557,559,560,562,563) including a case of IgA nephropathy (558) and diverse types of glomerular abnormalities (555,565). Causal links cannot as yet be conclusively established between the glomerulonephritis and the viral illness, however, using strict criteria (8,10,13). The majority of cases of acute renal failure in the setting of EBV infection have been either due to rhabdomyolysis or interstitial nephritis (563–568), although a case of renal lymphoma associated with EBV infection has been reported (569). An elegant study suggests EBV infection is characteristic of renal tissue of patients with chronic interstitial nephritis (570). The role of EBV infection in modifying outcomes in renal transplantation is unclear (511,571,572).

POLYOMAVIRUS INFECTIONS

JC and BK viruses are double-stranded DNA viruses of the Polyomavirus family that have been associated with interstitial renal disease in renal transplant patients (573,574). BK virus, which has a seroprevalence of 60% to 80% in different populations, has a high degree of homology with simian virus 40 (SV40), as does JC virus. The role of BK virus in mediating renal disease in the absence of immunosuppression or preexisting nephropathy is unclear (575,576). One early study attempted to implicate BK-associated immune complexes with various immune-complex glomerular diseases (576). A recent study suggested BK virus infects glomerular epithelial cells in transplant patients (577). Renal tissue shows renal tubular abnormalities and interstitial infiltration (578). Treatment involves curtailing immunosuppression, but this may lead to rejection. Better transplant kidney function was associated with less virus detected in a study involving the use of protocol transplant biopsies (578). Quantitative PCR may allow more precise diagnosis of BK virus allograft nephropathy (579). Recently patients with BK nephropathy have been treated with cidofovir (580), although the drug can exhibit nephrotoxicity.

Recent preliminary data suggest SV40, a simian Polyomavirus, can be detected more commonly from cells of urine of patients with renal disease, compared to those of normal controls (581). A role for SV40 in the pathogenesis of focal glomerulosclerosis was suggested. Further work has tested such hypotheses (582). Such data, although associative, provide a basis for investigation of the role of SV40 in the initiation or mediation of renal disease.

PARVOVIRUS INFECTIONS

Parvovirus B19 is a single-stranded DNA virus that is a member of the Parvoviridae family. It is the only virus of this family to infect humans. Its seroprevalence ranges between 40% and 60% in adults in developed countries. It has been associated with glomerulonephritis in patients with diagnoses such as sickle cell disease (583,584) and vasculitis (585,586), as well as a variety of other glomerulonephridites (35,587–593) including Henoch-Schönlein purpura (594,595). Mesangioproliferative glomerulonephritis, mesangiocapillary proliferative glomerulonephritis, and acute endocapillary proliferative glomerulonephritis have all been reported associated with parvovirus infection. Patients may have hypocomplementemia (590,591). Histologic studies have linked the presence of viral protein (590,592) and genome (588,592,594) to the renal disease with varying assessments of control tissue, but causality has not been definitively proved.

Viral DNA was detected in a higher proportion of tissue from patients with focal glomerulosclerosis compared with tissue from patients with other glomerular diseases or normal tissue (596). Viral DNA, however, was not detected by *in situ* hybridization. Parvovirus B19 has since been linked to the presence of collapsing glomerulopathy by several investigators (35,597–600). Moudgil et al. showed parvovirus B19 DNA could be detected in renal tissue from patients with collapsing glomerulopathy with higher frequency (78.3%) than from tissue from patients with HIV-associated nephropathy or focal glomerulosclerosis (599). Viral genome was detected in glomerular epithelial cells and tubular cells, suggesting a possible pathogenic pathway for the development of the disease. The investigators speculated host factors could be critical in determining disease expression.

Finally, parvovirus infection has been associated with anemia and renal dysfunction in patients who received kidney transplants (507,598,601–603). Murer et al. reported four cases of thrombotic microangiopathy in renal allografts associated with parvovirus infection (604). The role of this virus in mediating the initiation and progression of various forms of chronic renal diseases deserves further study.

CONCLUSION

Renal disease occurs in patients with HBV, HCV, and HIV and other viral infections, and is often immune complex mediated. Viral-associated glomerulonephritis comprises a spectrum of proliferative glomerulonephritis to MN, likely reflecting differential deposition of antibody and antigen–antibody complexes, *in situ* immune mechanisms, and variation in host responses and genetic background. The role of the association of viral infection in the pathogenesis of focal segmental glomerulosclerosis is under active investigation. Infection of glomerular epithelial cells by particular viruses may contribute to the pathogenesis of focal segmental glomerulosclerosis. Differences in expression of renal disease may vary with the nephropathogenicity of specific viral peptides and the physicochemical characteristics of the associated immune complexes, the anatomic location of their deposition, and systemic and local clearance rates. Diagnosis and therefore the true prevalence of viral-related glomerulonephritis will depend on the specificity and sensitivity of the tests employed in the diagnosis. The demonstration of both viral antigens and host antibody in the mesangium and in glomerular capillaries and the isolation of identical viral immune complexes from distinct renal anatomic areas, in higher titers than the circulation, remain the gold standard for diagnostic purposes. Immunochemical and molecular biologic evaluations will prove increasingly important for our understanding the pathogenesis of viral and nonviral renal diseases in animal models and in human tissue.

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CHAPTER 60 ■ RAPIDLY PROGRESSIVE GLOMERULONEPHRITIS

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Rapidly progressive glomerulonephritis (RPGN) is one of the most calamitous of nephrologic conditions, and patients can progress from normal renal function to end-stage renal failure within weeks. RPGN is a syndrome and consists clinically of sudden and relentless deterioration in renal function associated with the pathologic finding on renal biopsy of extensive crescent formation involving most glomeruli.

Many types of glomerulonephritis can exhibit crescent formation and sometimes renal failure. These types are listed in Table 60-1 and include both primary glomerular disease and systemic disease with a glomerular component. Consequently, the definition of RPGN has been difficult, and the terminology has varied over the years, incorporating either the clinical or pathologic components, or both.

Crescents were first described by Langhans (1) over 100 years ago (Fig. 60-1). Volhard and Fahr (2) used the term *extracapillary glomerulonephritis* in 1914 because of the glomerular crescents surrounding the capillary tuft, and Lohlein (3) called this type of glomerulonephritis a *stormy course* because of the renal failure that led to death. Ellis (4) coined the phrase *rapidly progressive type 1,* but the disease has also since been referred to as *malignant* (5), *acute necrotizing* (6), *acute anuric* (7), *proliferative, with crescents* (8), and *nonstreptococcal rapidly progressive glomerulonephritis* (9). The most commonly used synonym for RPGN today is *idiopathic crescentic glomerulonephritis* (10).

Therefore, in this chapter, *RPGN* is used to describe this relatively rare syndrome, consisting clinically of an acute glomerulonephritis, with a rapid and progressive deterioration in renal function, and pathologically of extensive crescent formation involving most of the glomeruli in the absence of another type of primary glomerulonephritis or systemic disease. In spite of these restrictions, RPGN is still a heterogeneous entity and can be divided further on immunopathogenetic criteria into subgroups with different prognoses and responses to treatment. RPGN is traditionally divided into three entities: antiglomerular basement membrane disease (anti-GBM, so-called Goodpasture's syndrome), immune GN, and the previously named pauci-immune group that we now know to be varieties of vasculitis, usually antineutrophil cytoplasmic antibody (ANCA) positive.

PATHOLOGY

Crescents

The pathognomonic feature of RPGN is the presence of crescents in Bowman's space (Figs. 60-2 and 60-3). A glomerular crescent may be defined as an aggregation of cells, at least two layers deep, in Bowman's space that may encroach on and destroy the capillary tuft. There is no agreement on the extent of crescent formation required for the diagnosis of RPGN to be made. Studies vary from 20% of glomeruli involved (11) to more than 80% (12). Neild et al. (13), in an extensive review of the Guy's Hospital experience, introduced the concept of the importance of the degree of the glomerular circumferential involvement. They defined the pathologic criteria for RPGN as greater than 60% of circumferential involvement of more than 60% of glomeruli. In general, those patients with the most rapid progression and most severe renal failure have a greater number of glomerular crescents (12,14).

Crescents are composed of cells and fibrous connective tissue. During the acute phase, crescents are predominantly cellular (Fig. 60-3), whereas chronicity is typified by progressive fibrosis and eventually sclerosis of the crescent (Fig. 60-4). Early crescents are composed of large, pale-staining, elongated cells with occasional mitoses evident (Figs. 60-2 and 60-3). It was traditionally held that these cells were derived from proliferated epithelial cells, and the term *epithelial crescent* was used (9,10,15). However, studies using monoclonal antibodies as specific markers have shown that the major identifiable cell type in crescents is of macrophage origin (16). Consistent with these data, tissue culture studies of glomeruli isolated from patients with RPGN produced predominant outgrowths of macrophages (17–19) (Fig. 60-5). A cellular crescent also frequently contains erythrocytes, polymorphonuclear leukocytes, and deposits of fibrin (Fig. 60-3). Over time, progression from a cellular to a sclerosed, fibrous crescent is seen as acute infiltrating leukocytes and epithelial cells are lost through apoptosis or migration out of the crescent and are replaced by myofibroblasts and fibroblasts, which secrete matrix and contribute to fibrosis and eventual sclerosis of the crescent (see Pathogenesis, below). RPGN frequently is a nonuniform process, and various degrees and stages of crescent formation may be seen in different glomeruli from the same biopsy.

Glomerular Tuft

Most patients also have glomerular tuft involvement, which may consist of focal and segmental necrosis of glomerular capillaries, fibrin deposition, cellular infiltration, and cellular proliferation (Fig. 60-3). In cases of extensive crescent formation, the glomerular tuft can be almost completely compressed and ischemic changes may be evident. In less severely damaged glomeruli, there may be proliferation of resident kidney cells (endothelial and mesangial cells) and accumulation of leukocytes (macrophages, polymorphs, and lymphocytes). Multinucleated giant cells also have been found (20). Cells in the tuft may proliferate, become activated, release proinflammatory cytokines, and mediate thrombosis, tissue damage, and fibrosis (see Pathogenesis below) (Fig. 60-6).

The advent of monoclonal antibodies (21) enabled specific identification of individual antigens on both leukocytes and intrinsic glomerular cells (22–26), making it possible to assess

TABLE 60-1

TYPES OF GLOMERULONEPHRITIS THAT CAN BE ASSOCIATED WITH RAPID DETERIORATION IN RENAL FUNCTION AND GLOMERULAR CRESCENT FORMATION

Primary Renal Diseases
RPGN—anti-GBM without lung involvement
RPGN—immune complex deposition
RPGN—without immune deposits (>80% ANCA positive)
Membranoproliferative glomerulonephritis
Membranous nephropathy
Immunoglobulin A disease
Hereditary nephritis
Systemic Diseases
Goodpasture's syndrome
Postinfectious
Poststreptococcal (Chapter 58)
Endocarditis (Chapter 58)
Shunt nephritis (Chapter 58)
Abscess (Chapter 58)
Henoch-Schönlein disease (Chapter 61)
Lupus nephritis (Chapter 65)
Polyarteritis (Chapter 68)
Wegener's granulomatosis (Chapter 68)
Cryoglobulinemia (Chapter 69)
Scleroderma (Chapter 66)
Relapsing polychondritis
Malignancy
Malignant hypertension (Chapter 56)

ANCA, antineutrophil cytoplasmic antibody; *anti-GBM*, antiglomerular basement membrane disease; *RPGN*, rapidly progressive glomerulonephritis.

the number of individual glomerular cell types and infiltrating leukocytes *in situ*. Glomeruli from patients with RPGN have increased numbers of macrophages and polymorphonuclear leukocytes (27–34). Intraglomerular T cells have been demonstrated in glomeruli of patients with crescentic glomerulonephritis (29–34). Such T cells are functionally active, as demonstrated by their association with activated monocytes and fibrin, the other components of cell-mediated immunity (35), and their expression of receptors for interleukin-2 (IL-2R), denoting immune activation (3,32).

Interstitial Infiltrate

In most renal biopsies of patients with RPGN, there is a prominent interstitial inflammatory cell infiltrate (10). It usually is widespread throughout the interstitium but often is most marked surrounding Bowman's capsule (Figs. 60-2 through 60-4). The interstitial changes in RPGN may well relate more to the outcome of the disease than do the glomerular changes (28). It has been shown that there is a highly significant correlation between the degree of interstitial mononuclear cell infiltration and impairment of renal function, whereas there is minimal correlation between glomerular cell numbers and renal function (28). All these findings suggest that the interstitial mononuclear cell infiltrate is a very important component of the renal response to glomerular injury (36,37).

The composition of the interstitial leukocytic infiltrate in RPGN has been evaluated (28,29). Hooke et al. (28) compared the interstitial cell infiltrate in 14 patients with RPGN with 84 other types of glomerulonephritis. This study found that the interstitial leukocyte numbers were greater in the RPGN group of patients, that the T lymphocyte was the major cell involved (64%), and that the CD4+/CD8+ ratio was 1.0, which was the same as in other glomerulonephritides and in normal biopsies. The other interstitial mononuclear cells were monocytes (22%), B cells (10%), and granulocytes (2%). A typical interstitial leukocytic infiltrate stained by the monoclonal antibody PHM1, which recognizes a cell membrane antigen that is present on all human leukocytes (38), is shown in Figure 60-7. A further study demonstrated IL-2R expression by a high proportion of the interstitial mononuclear cells in RPGN, indicating an activated phenotype (3). Stachura et al. (29) published similar interstitial findings in 16 patients with RPGN. They found that the mononuclear interstitial infiltrate consisted mainly of T lymphocytes (80%) and monocytes (19%) and very small numbers of B cells and natural killer cells. Most of the T lymphocytes, both in the interstitium as well as in glomeruli, were of the helper (CD4+) phenotype in normal patients as

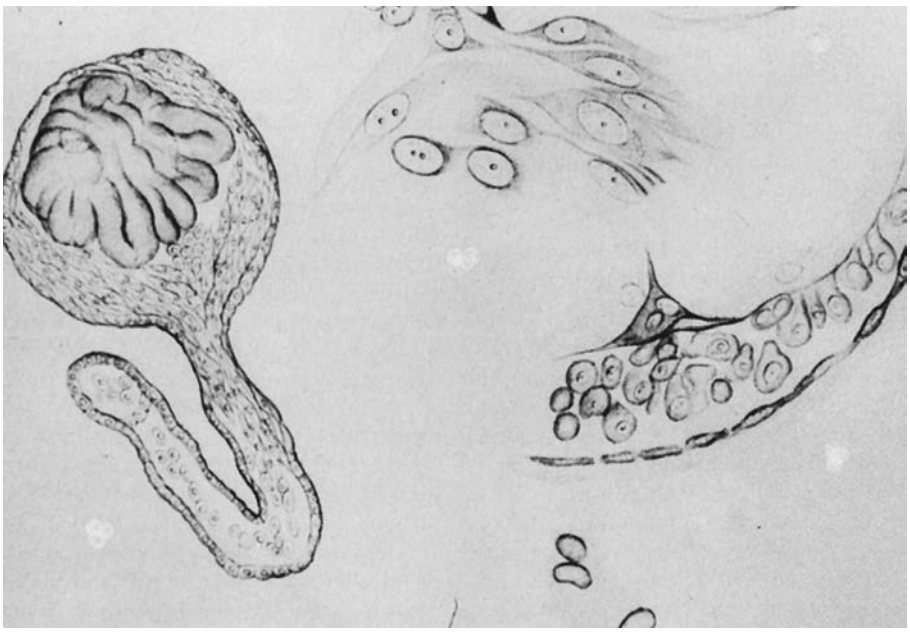

FIGURE 60-1. Reproduction of drawings from Langhans's paper, published over 100 years ago, which first described crescents in Bowman's space and inferred an epithelial cell origin. (From: Langhans,T. Uber die vernaderungen der glomeruli bei der nephritis nebst einigen Bemerkungen uber die Entshehung der Fibrinzylinder. *Arch Pathol Physiol Klin Med*1879;76:85.)

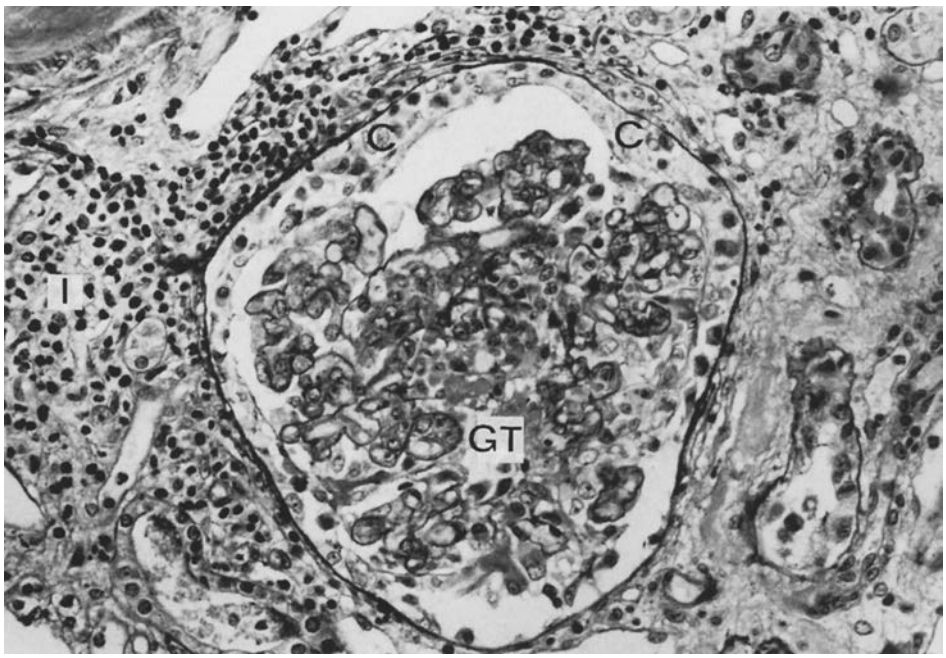

FIGURE 60-2. A small cellular crescent (C) surrounding a glomerular tuft (GT) from a 67-year-old patient with rapidly progressive glomerulonephritis. The glomerulus shows some mesangial and endothelial cell proliferation. There is a pronounced interstitial cellular infiltrate (I). (Silver-Masson trichrome, magnification ×400.)

well as in patients with RPGN. These studies clearly support a role for cellular immunity in RPGN.

Immune Deposits

The nature of the immune deposits (immunoglobulin and complement) in crescentic glomerulonephritis, as detected by immunofluorescence or an immunoperoxidase technique, depends on the underlying type of glomerulonephritis. The three patterns are (a) linear GBM deposition of immunoglobulins (anti-GBM antibody), with or without complement (C3); (Fig. 60-8A); (b) granular deposition of immunoglobulin and complement along capillary loops and mesangium, suggesting an immune complex disease (Fig. 60-8B); and (c) no or very scanty immunoglobulin or complement deposition—so-called pauci-immune RPGN (Fig. 60-8C). The proportion of cases in each individual group varies markedly from one series to another (12,13,39–45). In very general terms, however, approximately 30% of cases of RPGN account for each category of immunopathogenesis, although more recent studies indicate higher numbers of the pauci-immune group. In addition, there seems to be a great geographic variation. In the United States and Australia, anti-GBM disease is relatively common, whereas in Europe it is uncommon. In a series of 195 biopsies from patients from various disease areas and containing more than 50% crescents, 11% of the patients had anti-GBM disease, 29% had immune complex glomerulonephritis, and 60%

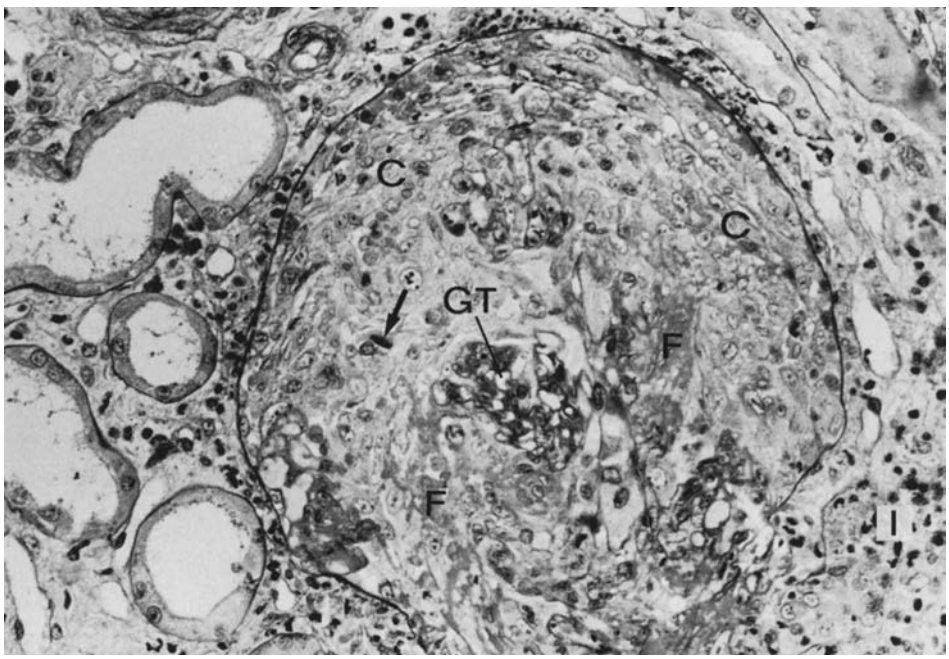

FIGURE 60-3. An extensive cellular crescent (C) from the same biopsy as that shown in Figures 60-2 and 60-7B. There is virtual obliteration of the glomerular tuft (GT). There are large areas of fibrin deposition (F) in the crescent. A mitotic figure can be seen in the crescent (*arrow*). An interstitial mononuclear cell infiltrate (I) surrounds the glomerulus. (Silver-Masson trichrome, magnification ×400.)

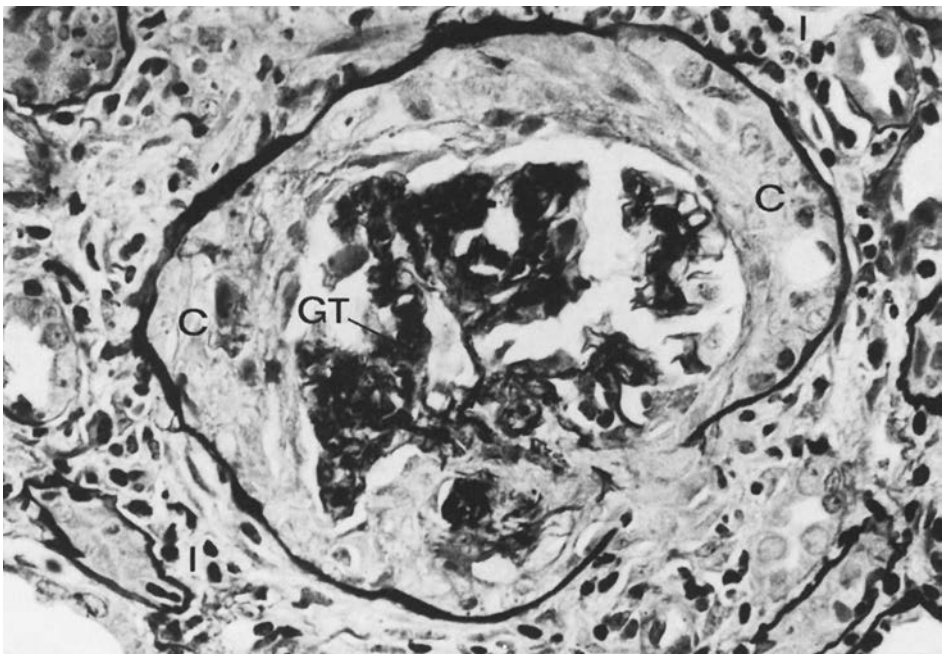

FIGURE 60-4. A glomerulus showing a fibrocellular crescent (C) surrounding the glomerular tuft (GT) from a patient with rapidly progressive glomerulonephritis, 90% crescent formation, and no immunoglobulin deposition. Interstitial infiltration with mononuclear cells is present (I). The patient is the same as in Figure 60-6. (Silver-Masson trichrome, magnification ×400.)

had pauci-immune glomerulonephritis (46). Fibrin/fibrinogen deposition usually is detected in both the crescent and the capillary tuft.

Electron microscopy confirms the presence or absence of mesangial or capillary loop immune deposits in immune complex crescentic glomerulonephritis. Collapse of the capillary wall and necrosis of glomerular cells in the tuft frequently are seen (9,15,47–49), and ruptures in the capillary loops are common and may be important in crescent formation (50). Electron microscopy also commonly demonstrates macrophages, epithelioid cells, and epithelial cells in the crescent (47,50), although this technique is not well suited to the determination of the relative proportion of each of these cell types in the crescent. Fibrin normally is seen between the cells of the crescent and, as the crescent evolves, basement membrane material and collagen fibers also may be seen. The electron micrograph shown in Figure 60-9 illustrates many of these features in a crescent from a patient with RPGN.

PATHOGENESIS

Our current understanding of the immunopathogenesis of this disease has come largely from examination of glomerular immune deposition and immune cell participation in patients and the study of experimentally induced RPGN in animals. More recent studies also have indicated that progressive tubulointerstitial injury is a prominent feature of RPGN (51,52).

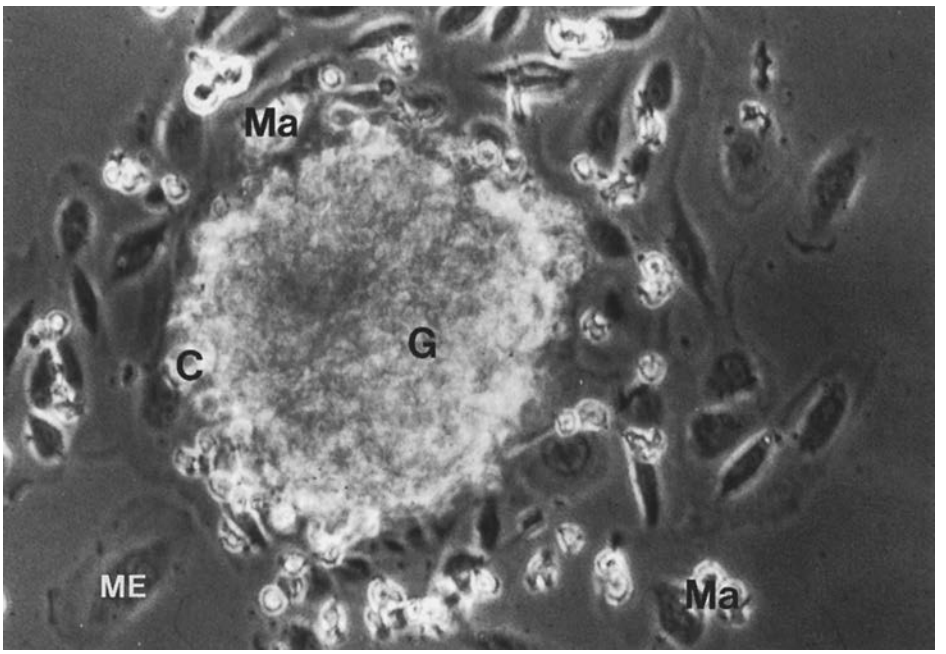

FIGURE 60-5. Phase-contrast photomicrograph of an isolated glomerulus (G) in tissue culture from a patient with rapidly progressive glomerulonephritis and 100% crescent formation. A large population of macrophages (Ma) is seen in the crescent (C) and egressing into the culture. Mesangial cells (ME) also are present in the culture outgrowth. (Magnification ×150.)

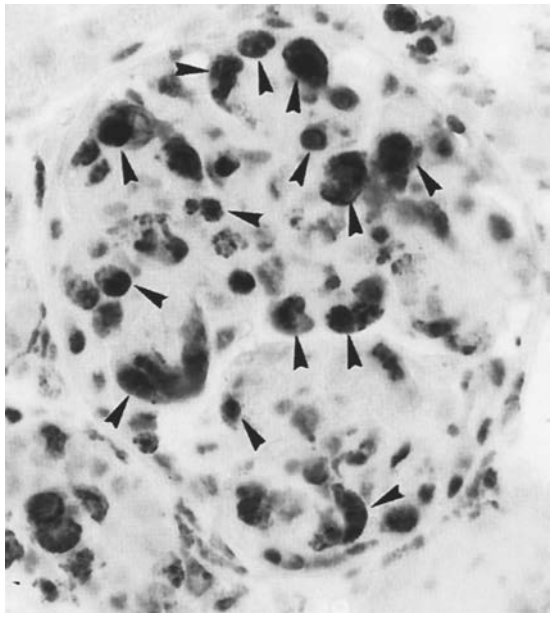

FIGURE 60-6. A rat glomerulus demonstrating hypercellularity from an animal with induced anti-glomerular basement membrane nephritis. Double immunostaining demonstrated that numerous glomerular macrophages (*arrows*; ED-1 positive [*brown*]) were proliferating (proliferating cell nuclear antigen positive [*blue/black*]) and contributing to the glomerular hypercellularity. (Periodic acid-Schiff, magnification ×400.) (See Color Plate.)

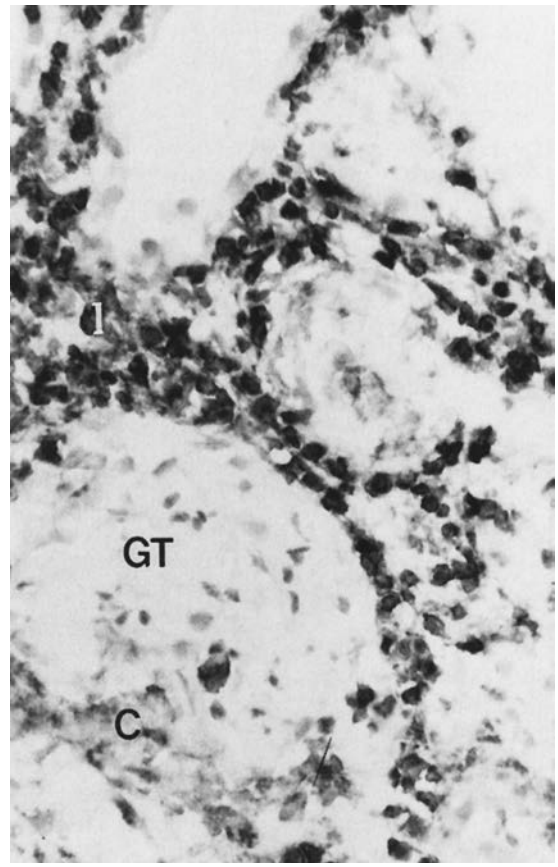

FIGURE 60-7. A cryostat section of a biopsy from the patient with rapidly progressive glomerulonephritis in Figure 60-4. Leukocytes are identified by the monoclonal antibody PHM1 (91) and a four-layer peroxidase anti-peroxidase technique giving black staining. The total infiltrating leukocyte population is identified by PHM1 as it recognizes a leukocyte common antigen. Large numbers of interstitial leukocytes (l) can be seen, particularly surrounding the glomerulus (GT). There also are mononuclear leukocytes identified in the glomerular tuft (GT). Many cells in the crescent (C) also are stained. (Magnification ×125; courtesy of Dr. D. Hooke.)

The histologic pattern and clinical course of the entity of RPGN resulting from several underlying immunopathogenetic mechanisms is discussed. However, it is likely that these mechanisms can coexist and perhaps may be variations in a spectrum of disease (40,46,53,54).

Glomerular Deposition of Anti–Glomerular Basement Membrane Antibody

The pattern of immunoglobulin deposition in the glomerulus separates this condition from the immune complex group (see above discussion). In approximately one-fifth of all patients with RPGN, linear deposition of immunoglobulin (predominantly immunoglobulin G [IgG]) along the GBM is detected. In these patients, anti-GBM antibody also usually is detected in the circulation, particularly if a sensitive and specific radioimmunoassay is used to detect the antibody (55). In some patients, this antibody also reacts with the pulmonary basement membrane and results in pulmonary hemorrhage (56), thus producing Goodpasture's syndrome. The anti-GBM antibody also can cross-react with renal tubular basement membrane, and this has been linked to a more severe interstitial component (57). The "Goodpasture antigen" has now been identified in the noncollagenous globular domain, termed NC1, of type IV collagen. More specifically, more than 90% of patients have antibodies to the $\alpha 3$ (IV) chains of the type IV collagen (58,59). The molecular organization of the autoantigen in the native alpha3alpha4alpha5(IV) collagen network of the GBM has recently been described (60).

Rapidly progressive glomerulonephritis caused by anti-GBM antibody can be induced in animals, notably in sheep, by the repeated injection of particulate heterologous (e.g., rabbit) or homologous (i.e., sheep) GBM in complete Freund's adjuvant, causing Steblay's nephritis (61). If serum from sheep with anti-GBM glomerulonephritis (induced by rabbit GBM) is injected intravenously into rabbits, then the anti-GBM antibody fixes to the rabbit GBM, inducing proteinuria (heterologous phase of nephrotoxic nephritis). With the development 5 to 6 days later of a rabbit antibody response to the sheep antibody on the rabbit GBM, a rapidly progressive crescentic glomerulonephritis develops (autologous phase of nephrotoxic nephritis) (62). This type of passive transfer of anti-GBM serum can be used to induce a rat model of accelerated anti-GBM nephritis with resultant disease that closely mimics human Goodpasture's syndrome, complete with pulmonary hemorrhage (63,64).

Evidence that human anti-GBM antibodies are pathogenic comes from studies in which anti-GBM antibodies, eluted from human kidneys, induced acute glomerulonephritis upon transfer into monkeys (65). Using mice that have been genetically modified to only produce human immunoglobulins (XenoMouse II), Meyers et al. (66) demonstrated that immunization with $\alpha 3$(IV)NC1 collagen leads to the production of human anti-GBM autoantibodies that, when transferred to a normal XenoMouse, induced proteinuria and proliferative glomerulonephritis. However, while these studies provide clear evidence that anti-GBM antibodies are pathogenic, there is also

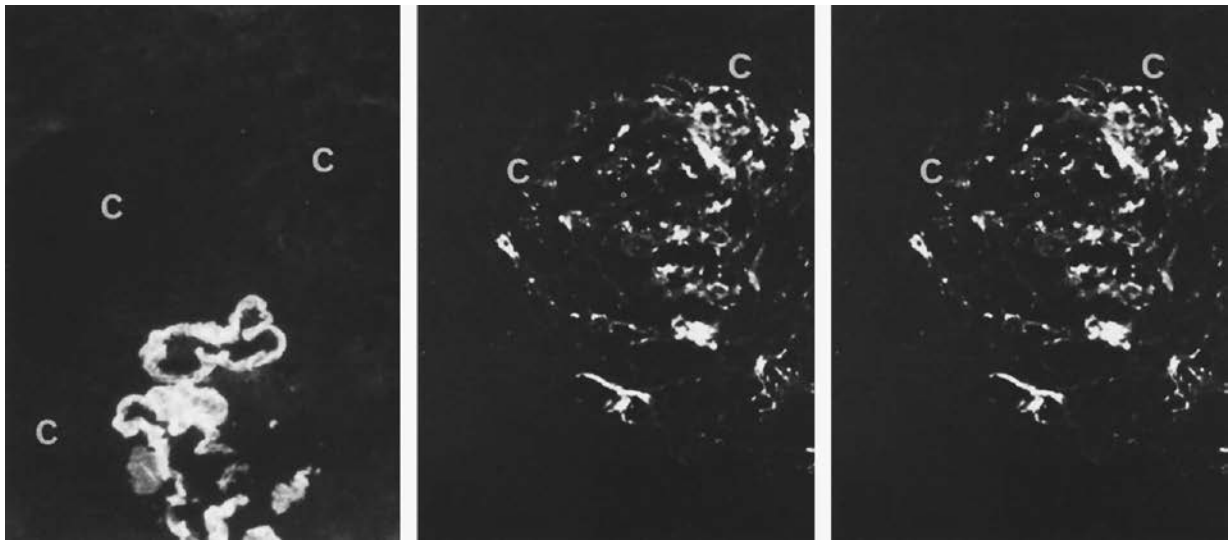

FIGURE 60-8. Immunofluorescence micrographs of renal biopsies from three different patients with rapidly progressive glomerulonephritis (RPGN) demonstrate the three underlying immunopathogenetic mechanisms. The crescents (C) are identified. **A:** Linear pattern of immunoglobulin G (IgG) deposition along the glomerular basement membrane of a patient with anti–glomerular basement membrane RPGN. **B:** Granular fluorescence of IgG along the capillary loops of the same patient with RPGN as in Figures 60-2 and 60-3, due to immune complex deposition. **C:** No definite immunoglobulin deposition is seen in the capillary tuft, but some deposits of C3 are evident on the visceral layer of Bowman's capsule in a patient with idiopathic or no-immune-deposit RPGN.

a critical role for cell-mediated immunity in disease pathogenesis (see below).

Glomerular Immune Complex Deposition

A granular deposition of immunoglobulin and complement along the capillary loops and in the mesangium is seen in approximately one-third of patients with RPGN. This pattern strongly suggests an immune complex pathogenesis, although circulating immune complexes are not commonly detected in these patients. The actual antigens involved are not known. The possibility of a viral etiology has been suggested by the demonstration of type IV nuclear bodies in the kidneys of some patients with crescentic glomerulonephritis (67). RPGN due to deposition of immune complexes can be induced in animals, especially the rabbit, by the daily intravenous injection of bovine serum albumin (BSA) in a dose according to the animal's antibody response to the BSA (chronic serum sickness). Immune complexes of BSA–anti-BSA can be detected in large amounts in the circulation and deposit in the glomerular capillary loops and mesangium (68). RPGN of this type may, in some cases,

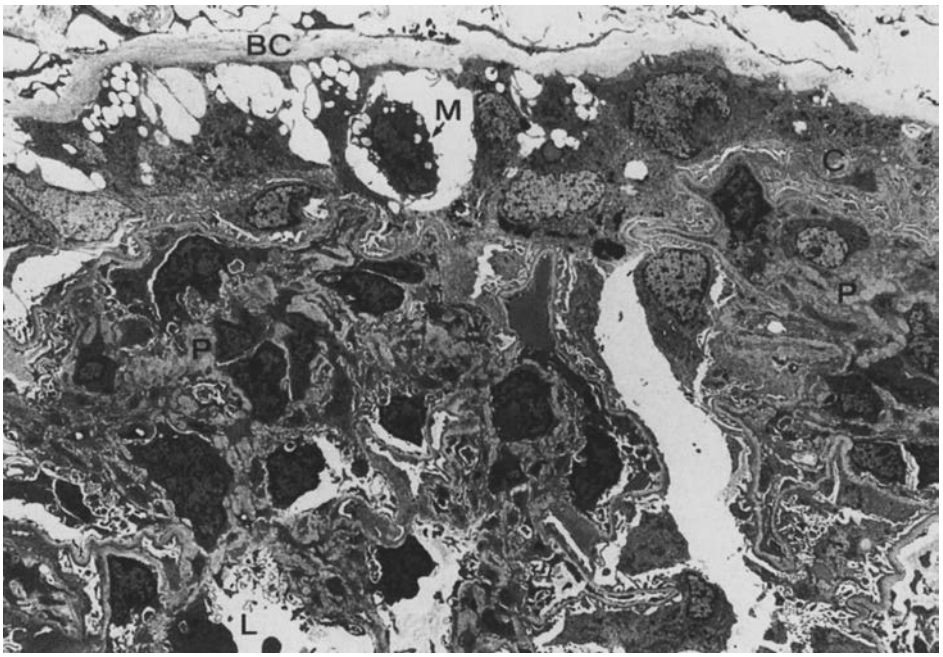

FIGURE 60-9. Transmission electron micrograph of part of a glomerulus from a renal biopsy of a 12-year-old boy with rapidly progressive glomerulonephritis, illustrating an early crescent (C) adjacent to the urinary space in which a macrophage (M) can be seen. Bowman's capsule (BC) surrounds the glomerulus. Some capillary loops (L) remain patent, but there are areas of marked endocapillary cell proliferation (P), one of which is adjacent to the early crescent. (Magnification ×3,000; courtesy of Prof. E. F. Glasgow.)

simply represent the severe end of the spectrum of common forms of glomerulonephritis (e.g., crescentic immunoglobulin A [IgA] nephropathy).

Minimal or Absent Glomerular Immune Deposits

In many patients with RPGN, significant immune deposits are not detected in the glomeruli (11). Hence, it has been difficult to incriminate humoral immune mechanisms in these patients. However, over 80% of these patients have circulating ANCA (43,46,68,69) and it appears that these patients have renal vasculitis, with or without systemic clinical manifestations (70) (see below).

Anti-neutrophil cytoplasmic antibodies (ANCA), first described by Davies et al. in 1982 (71) are a marker for "idiopathic" glomerulonephritis when it occurs with no evidence of extrarenal disease and in glomerulonephritis associated with systemic necrotizing arteritides, including microscopic polyangiitis and Wegener's granulomatosis (43,46,72–76).

Anti-neutrophil cytoplasmic antibodies have been detected in approximately 80% of patients with pauci-immune crescentic glomerulonephritis in one center (43,46,73). Jayne et al. (77) found that the sera of 28% of 889 consecutive patients with suspected RPGN were positive for ANCA. Tervaert et al. (78) reviewed 35 consecutive patients with crescentic glomerulonephritis without glomerular immunoglobulin deposition and concluded that both vasculitis-associated and idiopathic crescentic glomerulonephritis were specifically associated with ANCAs to myeloid lysosomal enzymes. Thus, it appears likely that idiopathic crescentic RPGN without glomerular immune deposits is part of the spectrum of the vasculitides. Although there are obviously ANCAs to various antigens (46,77,78), it may be that c-ANCA (cytoplasmic staining to a serine proteinase) occurs predominantly in Wegener's granulomatosis and p-ANCA (with perinuclear staining predominantly to myeloperoxidase) is found in patients with so-called microscopic polyarteritis (or polyangiitis) with crescentic glomerulonephritis but little or no systemic disease (79). Both of these conditions predominantly involve small-caliber arteries/arterioles. True polyarteritis nodosa is a disease of medium-sized arteries not commonly associated with glomerulonephritis, and is more commonly ANCA-negative.

The potential role of ANCAs in disease pathogenesis has been a controversial subject. However, recent studies in animal models have provided convincing evidence that ANCA are indeed pathogenic (80). Immunization of rats with myeloperoxidase (MPO) induces autoantibodies to rat and human MPO that aggravate renal injury induced by anti-GBM antibodies or by renal artery perfusion with a neutrophil lysosomal extract and hydrogen peroxide (81,82). Definitive experimental evidence that ANCAs are pathogenic has come from a recent study in which MPO gene deficient mice were immunized with mouse MPO. Purified anti-MPO IgG was transferred into immunodeficient (*Rag2–/–*) or normal mice, resulting in focal necrotizing and crescentic glomerulonephritis with a paucity of glomerular Ig deposition (83).

Immunoglobulin Receptors

Receptors for the Fc portion of immunoglobulin G (FcRγ) play an important role in the pathogenesis of experimental anti-GBM glomerulonephritis. There are both activating and inhibitory Fc receptors for IgG. Human and mouse share two activating Fc receptors (FcγRI, FcγRIIIa) and a single inhibitor receptor FcγRIIb (84). The two activating Fcγ receptors require a common FcRγ chain for their cell-surface assembly and signaling functions. Thus, mice deficient for FcRγ chain lack functional FcγI and FcγRIIIa receptors and these mice are completely protected from fatal, acute anti-GBM glomerulonephritis (85). Subsequent studies have confirmed that deletion of both FcRγI and III gives protection from crescentic anti-GBM glomerulonephritis, but there is debate as to the relative contribution of FcRγI versus FcRγIII (86,87). In addition to FcR promoting renal injury, it has been shown that the inhibitory FcRγIIB is protective. Deletion of FcRγIIB makes mice susceptible to the spontaneous autoimmune glomerulonephritis depending on the background strain (88). Furthermore, immunization of FcRγIIB deficient mice with collagen type IV leads to Goodpasture's syndrome with linear deposition of IgG along the glomerular and tubular basement membranes, massive pulmonary hemorrhage with neutrophil and macrophage infiltration and crescentic glomerulonephritis (89).

Cell-Mediated Immunity—T Cells

Prominent T-cell and macrophage accumulation and activation in glomeruli, crescents, and the tubulointerstitium are striking features in RPGN suggesting that cellular immunity plays a central role in the pathogenesis of these diseases. Indeed, the immunopathologic features of RPGN show marked similarities to delayed-type hypersensitivity (DTH) reactions, which include local accumulation of T cells and macrophages, multinucleated giant cell formation, and fibrin deposition – even in the absence of immunoglobulin deposition (3,11,16,20,28,29, 31,34,57,90–94).

The role of T cells in RPGN was first suggested by studies performed in 1970 (95) in which lymphocytes from patients with RPGN showed *in vitro* delayed-type hypersensitivity (DTH) responses in the presence of GBM. More recently, it has been shown that the Goodpasture antigen is expressed in normal human thymus. In Goodpasture's disease, limiting dilution analysis identified increased frequencies of CD4+ T cells reactive with alpha3(IV)NC1 in patients with acute disease, which reduced during the recovery phase. Indeed, this loss of autoreactive CD4+ T cells may explain why recurrences of this disease are infrequent (96).

Detailed time course studies in a rat model of anti-GBM glomerulonephritis that shares many features of human RPGN has shown that immune-activated T cells are exclusively localized to the areas of histologic damage, appearing to play a role in Bowman's capsular rupture, glomerular crescent fibrosis, tubulointerstitial fibrosis, and pulmonary fibrosis (63,64,97,98). Quantitative analysis showed that the number of interstitial T cells, particularly activated T cells, correlates significantly with the severity of proteinuria and renal impairment (63), suggestive of a pathogenic role for T cells in the mediation of the disease.

There is now strong evidence that T cells play a key role in the pathogenesis of crescentic glomerulonephritis. Bhan et al. (99) demonstrated that transfer of sensitized T cells into rats with planted heterologous anti-GBM globulin results in proliferative glomerulonephritis. Bolton et al. (100) induced a similar glomerular injury in chickens after transfer of T cells sensitized to GBM. Rennke et al (101) induced a granulomatous nephritis with glomerular crescent formation in the rat with sensitized T cells. These examples of glomerular injury occurred in the absence of antibody deposition.

Antibody-based depletion of either CD8+ or CD4+ T cells with monoclonal antibodies resulted in prevention of macrophage accumulation, proteinuria and crescent formation in different rat models of passive anti-GBM glomerulonephritis (102,103). While T cell depletion is a useful experimental tool, it has little clinical applicability. Therefore, efforts have

been made to block T cell activation in order to prevent or suppress the renal cellular immune response. The induction of autoantibody formation and crescentic glomerulonephritis in Wistar-Kyoto (WKY) rats immunized with rat GBM can be prevented by administration of CTLA4-Ig which blocks the CD28-CD80 and CD28-CD86 co-stimulation interactions involved in T cell activation (104,105). Furthermore, blockade of only the CD28-CD80 interaction substantially prevented T cell and macrophage infiltration, proteinuria, glomerular lesions and renal failure without affecting production and glomerular deposition of anti-GBM antibodies, demonstrating that T cell activation is essential for disease development in this model (105).

Blockade of another T cell co-stimulation interaction, CD40-CD154, can also prevent the induction of autoimmune anti-GBM glomerulonephritis in WKY rats. Delaying the anti-CD154 antibody treatment to 1 week after GBM immunization still provided moderate protection, but delaying treatment for two weeks had no significant effect upon disease development (106).

The most direct demonstration of T cell dependent RPGN comes from studies in which CD4+ antigen-specific T cell lines were derived from WKY rats after immunization with a recombinant form of the glomerular basement membrane (GBM) antigen, Col4α3NC1. Adoptive transfer of these CD4+ T cells to normal rats induced severe proteinuria with glomerular crescent formation in 5 of 11 animals in the complete absence of antibody or C3 deposition (107). Furthermore, immunization of WKY rats with a single peptide from Col4αa3NC1 has been shown to induce severe anti-GBM glomerulonephritis featuring nearly 100% of glomeruli with crescentic lesions or tuft necrosis in the complete absence of glomerular antibody deposition (108).

The mechanisms by which T cells coordinate the immune response in RPGN have been investigated. In the current paradigm of helper T-cell function, the role of a dominant type 1 helper T-cell (Th1) response in RPGN has been postulated, and the evidence for this was reviewed by Holdsworth et al. (109). While severe renal injury can be induced by passive transfer of anti-GBM antibodies to sensitized C57BL/6J (Th1-biased) and BALB/c (Th2-biased) mice, glomerular leukocyte infiltration and crescent formation was only seen in the Th1-biased strain (110). Augmentation of the Th1 response by the administration of interleukin (IL)-12, or via IL-4 gene knockout, enhanced crescent formation in anti-GBM glomerulonephritis in C57BL/6J mice (111,112), whereas antagonism of the Th1 response through IL-12 blockade (111) or IL-4 plus IL-10 administration diminished crescent formation (113). The situation is less clearly defined in larger rodents and in humans. Anti-Th1 therapy with IL-10 was studied in an accelerated model of anti-GBM disease in Sprague-Dawley rats (114). Administration of IL-10 after priming, but before disease initiation, failed to prevent crescent formation or renal injury despite inhibiting a skin DTH response to the same antigen (114). The systemic humoral immune response was enhanced by treatment, and this may have contributed to renal injury and crescent formation.

Immunohistochemical evaluation of human biopsy specimens from patients with RPGN supports a role for cell-mediated immunity, although the Th1/Th2 orientation of T cells promoting the response has been difficult to identify. Peripheral T cells specifically reactive with potentially nephritogenic GBM antigens have been identified, but not classified as either Th1 or Th2 (115,116). The immunoglobulin subclasses of potentially pathogenic antibodies produced by patients with RPGN have been studied to look for Th1 (IgG3) or Th2 (IgG4) bias, but no clear consensus has emerged (117,118). Identification of regulatory CD25+ T cells that can suppress the T cell response to the Goodpasture antigen in human anti-GBM disease raises the possibility that T cell may also play an important immunoregulatory role in RPGN (119).

Cell-Mediated Immunity—Macrophages

After the original discovery that large numbers of macrophages could be identified in glomerular cultures from patients with RPGN (120), immunohistochemistry-based studies showed the macrophage to be the major cell type found in crescentic glomeruli in biopsy tissue obtained from patients with this disease (16,17,92,121). Analysis of these biopsies also revealed marked accumulation of macrophages in areas of tubulointerstitial damage (28,122). Activation of the renal macrophage infiltrate has been demonstrated by macrophage production of cytokines such as IL-1, tumor necrosis factor-α (TNF-α) and macrophage migration inhibitory factor (MIF) (123–126), gene transcription of inducible nitric oxide (iNOS) (127) and matrix metalloproteinase-12 (MMP-12) (128), and expression of activation antigens MRP8/14 and CD86 (129,130).

Despite the fact that most studies examining the role of macrophages in RPGN have focused on mediation of renal injury, it is important to remember that macrophages are highly heterogeneous cells and can perform many different functions. Macrophages can efficiently process and present antigens to T cells and modulate the nature (Th1/Th2) of the immune response through their profile of cytokine secretion. Upon activation, macrophages have the potential to cause tissue damage by the secretion a wide array of pro-inflammatory cytokines (i.e., IL-1, TNF-α, MIF), oxidants (i.e., reactive oxygen species, nitric oxide), proteases (i.e., MMP-12, elastase), growth factors (platelet-derived growth factor [PDGF]), fibroblast growth factor-2 (FGF-2), and transforming growth factor-β1 [(TGF-β1]) and expression of procoagulant activity. Indeed, macrophages can be activated to produce these mediators via T cell-derived cytokines (i.e., IL-1, TNF-α, interferon-γ [IFN-γ]) or via T cell-independent mechanisms (i.e., immune complexes, complement, cytokines produced by intrinsic renal cells, endotoxin). The potentially beneficial aspect to renal macrophage infiltration is the removal of microorganisms via the innate immune response and promoting tissue repair through the production of antiinflammatory molecules, phagocytosis of apoptotic cells, removal of immune complexes and fibrin, matrix remodelling and enhancing vascularization.

Depletion strategies have been used to examine the pathogenic role of macrophages in experimental RPGN. Methods such as X-irradiation (131), antimacrophage serum (132), and nitrogen mustard treatment (133) have been used to induce systemic monocyte/macrophage depletion in animal models of anti-GBM glomerulonephritis, resulting in a significant reduction of kidney macrophage infiltration and abrogation of renal injury.

Direct evidence of macrophage-mediated renal injury has come from adoptive transfer studies. Early studies in a rabbit model of anti-GBM disease showed that transferred peritoneal macrophages can be recruited into the glomeruli of animals in which anti-GBM antibody is deposited, resulting in mild proteinuria in some animals (133). In recent studies, we have used adoptive transfer in the rat to demonstrate that macrophages recruited to the glomerulus directly induce moderate proteinuria and mesangial cell proliferation in acute anti-GBM disease (134). Activation of bone marrow-derived macrophages with IFN-γ prior to transfer augmented macrophage-mediated renal injury in rat anti-GBM disease, whereas treatment with glucocorticoids prevented macrophage activation and inhibited renal injury (135). Furthermore, macrophage-mediated renal injury in this model was shown to be dependent upon signaling through the c-Jun amino terminal kinase (JNK) pathway, with *in vitro* studies identifying a role for JNK signaling in

macrophage production of TNF-α, monocyte chemoattractant protein-1 (MCP-1), and nitric oxide (136).

An alternative to systemic monocyte/macrophage depletion is to prevent circulating monocytes from entering the kidney. Two chemotactic factors which are relatively specific for monocytes, MCP-1 and osteopontin (OPN), have been extensively studied in RPGN. Increased renal MCP-1 production is associated with glomerular and interstitial macrophage accumulation in human RPGN (137,138). Similarly, the induction of tubular OPN expression is tightly associated with interstitial macrophage accumulation (139,140). Antibody-based neutralization of MCP-1 inhibits glomerular macrophage accumulation and the induction of proteinuria and glomerular lesions in anti-GBM glomerulonephritis (141–143). In contrast, Tesch et al. (144) found that glomerular macrophage accumulation was unaffected in anti-GBM glomerulonephritis in MCP-1 gene deficient mice, but that interstitial macrophage accumulation and tubular damage were attenuated. Antibody-based blockade of OPN has also been shown to prevent glomerular and interstitial macrophage accumulation in rat anti-GBM glomerulonephritis with a consequent reduction in proteinuria and histologic damage (145). A role for macrophages in mediating progressive renal injury in RPGN has come from studies in which administration of neutralizing antibodies to MCP-1 or OPN in established anti-GBM disease were shown to suppress disease progression (143,145).

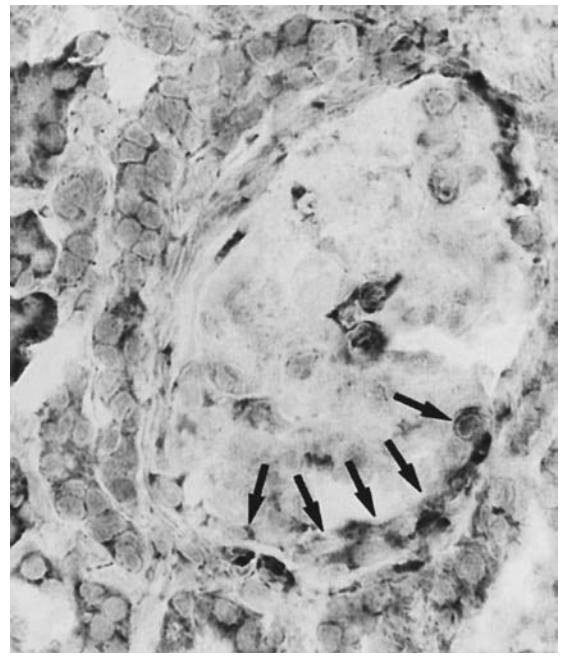

FIGURE 60-10. *In situ* hybridization demonstrating intercellular adhesion molecule-1 (ICAM-1) messenger RNA (mRNA) expression in a crescent from a rat with anti–glomerular basement membrane nephritis (*arrows*). The ICAM-1 mRNA has been depicted using *in situ* hybridization histochemistry. (Magnification ×400.) (See Color Plate.)

Adhesion Molecules

Adhesion molecules play a critical role in facilitating leukocyte attachment to the endothelium and their subsequent migration into sites of inflammation (146). Studies of human RPGN have identified upregulation of intracellular adhesion molecule-1 (ICAM-1; CD54) and vascular adhesion molecule-1 (VCAM-1; CD106) in endothelium, mesangial cells and tubules which is associated with T cell and macrophage accumulation (147–149).

Upregulation of selectin expression in the glomerular endothelium is a very early event following the deposition of anti-GBM antibodies in rat RPGN. Antibody-based blocking studies have shown a pathogenic role for P-selectin in the early, transient glomerular neutrophil accumulation and induction of proteinuria following administration of anti-GBM antibodies to experimental animals (150). Endothelial ICAM-1 expression is also upregulated rapidly following administration of anti-GBM antisera in rats, with co-localization of leukocyte function associated antigen one (LFA-1) and ICAM-1 at sites of monocyte adhesion to activated endothelium in the glomerulus and interstitium identified using immuno-electron microscopy (151,152). Up-regulation of tubular ICAM-1 expression during development of this disease model is associated with macrophage accumulation and tubular necrosis (152) (see Fig. 60-10). The pathological role of the ICAM-1/LFA-1 interaction was demonstrated by the ability of neutralizing anti-ICAM-1 plus anti-LFA-1 antibodies to abrogate leukocyte infiltration, proteinuria and histologic damage in rat anti-GBM glomerulonephritis (153,154). Consistent with these findings, ICAM-1 gene deficient mice are largely protected from the induction of anti-GBM glomerulonephritis, with reduced macrophage accumulation, proteinuria, and histological damage (155). In contrast, anti-VCAM-1 antibody treatment had no effect upon the development of anti-GBM glomerulonephritis in WKY rats. However, blockade of the VCAM-1 ligand, very late activation antigen-4 (VLA-4; CD29/49d) in this model protects against proteinuria and crescent formation without affecting glomerular macrophage recruitment (156). Furthermore, commencing anti-VLA-4 antibody treatment in established disease was shown to attenuate disease progression (157).

Cytokines

Interleukin-1 is a classic proinflammatory cytokine. Renal production of IL-1 activity was first described in cultured glomeruli from patients with RPGN (123). Subsequent studies have identified IL-1 gene and protein expression in infiltrating macrophages as well as intrinsic renal cells in human and experimental RPGN (124,158,159). Blockade of IL-1 activity in the acute, neutrophil-mediated phase of rat anti-GBM glomerulonephritis has produced variable results (160–164). However, longer periods of IL-1 blockade have been shown to suppress disease. Treatment with the IL-1 receptor antagonist (IL-1ra) during the first 14 days of rat anti-GBM glomerulonephritis suppressed glomerular macrophage accumulation and proteinuria, and prevented glomerular crescent formation and a loss of renal function (160). This effect was attributed to preventing upregulation of leukocyte adhesion molecules ICAM-1, OPN, and CD44 (165–167). Furthermore, delaying IL-1ra treatment until crescentic disease was already established was shown to halt disease progression and return renal function to normal (168), demonstrating the therapeutic potential for IL-1 blockade in RPGN.

Tumour necrosis factor-α (TNF-α) is another classic proinflammatory cytokine that plays an important role in RPGN. Upregulation of TNF-α has been described in human RPGN (124,125,158), and administration of a neutralizing anti-TNF-α antibody was shown to reduce renal injury in acute anti-GBM disease in the rat (161,163,164,169). In longer-term studies, administration of a soluble TNF receptor in rat anti-GBM glomerulonephritis produced a 55% to 70% inhibition in macrophage accumulation, 34% reduction in proteinuria, prevented a loss of renal function and abrogated glomerular crescent formation (170). Furthermore, delayed administration

of a soluble TNF-α receptor in established anti-GBM disease in WKY rats significantly reduced albuminuria and glomerular inflammation, including the prevalence of crescent formation. (171). The success of these studies in experimental RPGN has led to clinical trials in vasculitis (see Experimental Approaches to Treatment, below).

One of the earliest described cytokines is macrophage migration inhibitory factor (MIF) which plays a key role in the DTH reaction (172). MIF has proinflammatory actions as well as being a counter-regulatory of glucocorticoids (172). Blood lymphocytes from patients with RPGN produce MIF activity *in vitro* in the presence of GBM (95). More recently, an *in situ* hybridization and immunohistochemistry study identified increased MIF expression in human RPGN. The prominent T cell and macrophage infiltrates in human crescentic glomerulonephritis were largely restricted to areas of MIF upregulation and contributed to glomerular hypercellularity, glomerular focal and segmental lesions, crescent formation, tubulitis and granulomatous lesions (126). In addition, increased urinary MIF excretion has been identified in patients with crescentic glomerulonephritis and was found to correlate with renal dysfunction, histological damage and leukocytic infiltration (173).

Production of MIF-like bioactivity has been described in rat anti-GBM disease (174). More recently, increased MIF messenger RNA (mRNA) and protein expression has been quantified and localized in rat crescentic anti-GBM glomerulonephritis (175). Anti-MIF antibody treatment in this model attenuated leukocyte infiltration, reduced proteinuria, prevented renal dysfunction, reduced proteinuria, and markedly reduced histological damage, including glomerular crescent formation (176). Furthermore, delayed anti-MIF treatment was able to partially reverse established crescentic disease (177), demonstrating the therapeutic potential of this strategy. Using a different approach to demonstrate the pathologic effects of MIF, Sasaki et al. (178) induced transgenic overexpression of MIF in mouse podocytes that resulted in glomerulosclerosis and renal failure.

Signaling Pathways

An alternative approach to blocking individual cytokines or growth factors that mediate renal inflammation or fibrosis is to target common intracellular signalling pathways by which these factors exert their actions. Engagement of a wide range of cell surface receptors (i.e., IL-1 and TNF-α receptors, Toll-like receptors [TLR], T cell receptor, CD40) can lead to activation of the nuclear factor-kappaB (NF-κB) signaling pathway, resulting in inflammation, immune regulation, survival, and cell proliferation responses (179). Activation of the NF-κB pathway has been demonstrated in human and experimental RPGN (180–182). Efforts to block NF-κB signaling have suffered from a lack of specific reagents. However, gene therapy of rat anti-GBM disease using a specific NF-κB decoy, substantially inhibited proteinuria and histologic damage in association with a significant reduction in leukocytic infiltration, renal expression of cytokines, and leukocyte adhesion molecules (183). Further evidence that NF-κB signaling may promote renal injury comes from a study in which administration of unmethylated CpG oligonucleotides (common in bacteria and viruses but not in mammalian cells), that bind to and activate TLR9, caused increased macrophage accumulation, worse proteinuria, and more severe histologic lesions in a mouse model of immune-complex glomerulonephritis (184).

Cellular stresses (i.e., reactive oxidative species, nitric oxide, ultraviolet [UV] light, and osmotic stress) and cytokines and growth factors (i.e., IL-1, TNF-α, angiotensin II, TGF-β1) all induce signaling through the p38 and c-Jun NH2-terminal kinase (JNK) mitogen-activated protein kinase (MAPK) pathways (185). A marked increase in p38 MAPK activity has been described in human RPGN, although the individual cell types involved in p38 MAPK signaling is controversial (186,187). Stambe et al. (190,191) identified p38 MAPK activation in infiltrating macrophages as well as many intrinsic renal cells including podocytes and tubular epithelial cells, and found a significant correlation between the number of cells with phosphorylation (activation) of p38 MAPK and the degree of renal dysfunction and histologic damage. Administration of small molecule inhibitors of p38 MAPK have been shown to inhibit the development of renal injury in rat models of anti-GBM glomerulonephritis (189,190). Specifically, upregulation of P-selectin gene expression and the transient glomerular neutrophil influx that follows the deposition of anti-GBM antibodies was shown to be dependent upon p38 MAPK signaling (190).

Increased signaling through the JNK pathway has also been described in rat crescentic anti-GBM glomerulonephritis (181,191). *In vitro* studies suggest that blocking JNK signaling may be beneficial in RPGN, but no specific blocking studies in disease models have yet been reported.

Glucocorticoids are a mainstay treatment of RPGN. One of the beneficial effects of glucocorticoids may derive from their ability to inhibit signaling through the NF-κB and JNK signaling pathways (181,192,193). Furthermore, JNK can inhibit glucocorticoid receptor-mediated transcriptional activation by directly phosphorylating the glucocorticoid receptor (194). Thus, development of specific pharmacologic inhibitors of NF-κB and JNK signaling pathways may provide more effective treatment with fewer side effects than with current glucocorticoid therapy.

Bone morphogenic protein-7 (BMP7), a member of the TGF-β1 superfamily, can block TGF-β1-induced fibrosis (195). Administration of recombinant human BMP7 protein in a mouse model of anti-GBM glomerulonephritis beginning one week after disease induction prevented disease progression and delaying BMP7 treatment until week 3, after disease induction was still able to substantially protect the animals from end-stage renal disease and reverse disease pathology (196). Thus, antagonism of TGF-β1-induced cell signaling is another approach with therapeutic potential in RPGN.

THE MECHANISM OF CRESCENT FORMATION

The composition of cellular crescents and the mechanisms by which these arise has been an issue of debate for many years. Despite this, however, there is general agreement that cellular crescents can undergo fibrous organization by progressing through a fibrocellular stage to become a fibrous crescent (197). Cellular crescents can resolve as seen in cases of poststreptococcal crescentic glomerulonephritis and flares of crescentic IgA nephropathy. The key issue as to whether cellular crescents can resolve or progress may center on whether Bowman's capsule remains intact or becomes ruptured.

Epithelial Cells in Crescent Formation

The initial histologic studies describing crescent formation were done over 100 years ago by Langhans (1), and his sketches are reproduced in Figure 60-1. Since then, the cellular crescent traditionally has been considered to be composed of proliferated epithelial cells. This belief has been based primarily on light and ultrastructural studies of cells of the crescents

(8,9,15,198). Analysis of crescents by immunohistochemistry has identified monocyte/macrophages and epithelial cells as the predominant cell types involved, with fibroblasts present with the development of fibrous organization. However, the relative proportion of macrophages to epithelial cells varies widely in the different studies reported (16,50,199,200). Although this variation may simply reflect the highly heterogenous nature of crescents within individual biopsies and the heterogenous nature of the patient groups exhibiting glomerular crescent formation, there may also be an important mechanistic reason. A study by Boucher et al. (92) found that cellular crescents contained 55% to 95% epithelial cells and 15% to 35% mononuclear leukocytes when Bowman's capsule remained intact, whereas macrophages and T cells were the dominant population in crescents when Bowman's capsule was ruptured. Thus, the integrity of Bowman's capsule may have a profound influence on crescent composition and progression to fibrous organization—a factor not examined in most human studies.

Recent studies in mouse anti-GBM glomerulonephritis have identified early cellular crescents containing epithelial cells which lack any detectable infiltration of F4/80+ macrophages, indicating an epithelial origin of the early cellular crescents (201,202). The presence of F4/80+ macrophages was only seen in crescents at a later stage when Bowman's capsule became ruptured enabling migration into Bowman's space of the prominent periglomerular F4/80+ macrophage infiltrate. In this model, podocyte bridges between the glomerular tuft and Bowman's capsule have been proposed as the first step in the process of crescent formation (202). The use of genetic markers demonstrated that many of the epithelial cells seen in these crescents were of podocyte origin that had dedifferentiated and lost their characteristic podocyte markers upon detachment from the GBM (203). While this is an appealing hypothesis, a note of caution is warranted in that glomerular macrophages are phenotypically heterogeneous and, in particular, mouse glomerular macrophages do not express the F4/80 antigen (204). Furthermore, macrophages in early cellular crescents have been identified in mouse anti-GBM glomerulonephritis using alternative markers (110,204).

Mononuclear Cells in Crescent Formation

The first direct demonstration that mononuclear phagocytes are involved in crescent formation came from studies in which large numbers of highly motile cells which phagocytosed latex and yeast particles, and had ultrastructural characteristics of macrophages, were observed in the outgrowth from cultured glomeruli that had been isolated from patients with RPGN (120). These macrophage outgrowths were predominantly from crescents, with much smaller macrophage outgrowths detected in other types of non-crescentic glomerulonephritis (18).

To identify the cell types present in glomerular crescents in patients with RPGN, we developed specific monoclonal antibodies to human epithelial cells and to macrophages and monocytes (23,38,205). To assess the cell types present in the different stages of the evolution of the crescent, 12 patients with RPGN, 9 with cellular, and 3 with sclerosed crescents, were examined (16). Cellular crescents consisted of 35% macrophages, 12% polymorphonuclear leukocytes, and 10% epithelial cells. Sclerosed crescents contained few macrophages (5%) but similar proportions of polymorphonuclear leukocytes (11%) and epithelial cells (12%). Many of the crescent cells were unlabeled, but they were probably fibroblasts because of their morphologic appearance and the expression of surface fibronectin. Figure 60-11 demonstrates the complementary staining of epithelial cells and macrophages in the glomerular tuft as well as the crescent in the renal biopsy of a patient with RPGN. The presence of large numbers of macrophages in crescents was confirmed by the unique study of Schiffer and Michael (206). Crescentic glomerulonephritis developed in two human female kidney grafts that had been transplanted into male recipients. As demonstrated by the presence of Y-body positivity, 35% of cells in the crescents were infiltrating leukocytes from the recipient.

The accumulation of macrophages within crescents is thought to be part of a DTH-like reaction. The components of the DTH reaction (T-cell infiltration and activation, macrophage accumulation, procoagulant activity and fibrin deposition), as well as local production of MIF, have all been documented in RPGN (3,16,31,32,90,95,96,120,126). The presence of humoral immune reactants, such as immunoglobulin and complement, are not necessary for crescent formation (11). The presence of DTH reactants has also been characterized in ANCA-associated RPGN (94).

Animal studies provide strong support for a pivotal role of macrophages in crescent formation. In early studies, Kondo et al. (207) performed a morphologic evaluation of a rabbit model of anti-GBM glomerulonephritis in which large numbers of clear cells in the crescent and glomerular tuft, with the features of monocytes, were described. Further morphologic support came from studies on serial renal biopsies of animals developing crescentic nephritis (208). The initial event of crescent formation was shown to be the deposition of fibrin within Bowman's space. This was followed by an influx of mononuclear cells into Bowman's space, where they phagocytosed fibrin. Epithelioid and giant cell transformation then occurred. These morphologic observations suggest that crescent formation is a result of the transformation of infiltrating macrophages (Fig. 60-12). In Figure 60-13, a scanning electron micrograph of an isolated glomerulus in tissue culture from a rabbit with nephrotoxic nephritis, with illustrated macrophages in the crescent.

In experimental RPGN induced by either anti-GBM antibodies (209) or immune complexes (68), enzyme histochemistry has demonstrated that most crescentic cells have an enzymatic profile consistent with a macrophage origin. Cattell and Arlidge (210) using irradiation to induce leukocyte depletion, also suggested that the crescents originate from circulating leukocytes. Furthermore, in an ultrastructural analysis of crescent formation in both rats and rabbits with nephrotoxic nephritis, Clarke et al. (211) concluded that the crescent cells evolve predominantly from circulating macrophages.

As discussed earlier, a variety of strategies have been used to inhibit T cell and macrophage accumulation activation and thus prevent renal injury in experimental models of RPGN (see Pathogenesis). In particular, delaying treatments that target T cell co-activation molecules (CD28-CD80/86 interaction), cytokines (IL-1, TNF-α, or MIF), adhesion molecules (VLA-4) or chemokines (OPN and MCP-1) until crescentic disease is already established has been successful in suppressing further crescent formation and disease progression (104,143, 145,157,168,171,177).

Parietal epithelial cells may be important in attracting macrophages into Bowman's space via the secretion of chemotactic molecules and expression of leukocyte adhesion molecules (Fig. 60-10). Parietal epithelial cells are induced to express, or upregulate, a variety of molecules involved in glomerular macrophage and T cell accumulation, including chemotactic molecules (MCP-1, OPN, MIF) and leukocyte adhesion molecules (ICAM-1, VCAM-1, CD44) (143,156,165,175, 212,213). A common feature of these chemotactic and adhesion molecules is that they can be induced by the cytokine IL-1. Indeed, one of the reasons for the success of IL-1 blockade in established crescentic anti-GBM disease is the suppression of glomerular expression of ICAM-1, OPN, and CD44 by intrinsic glomerular cells (165–168).

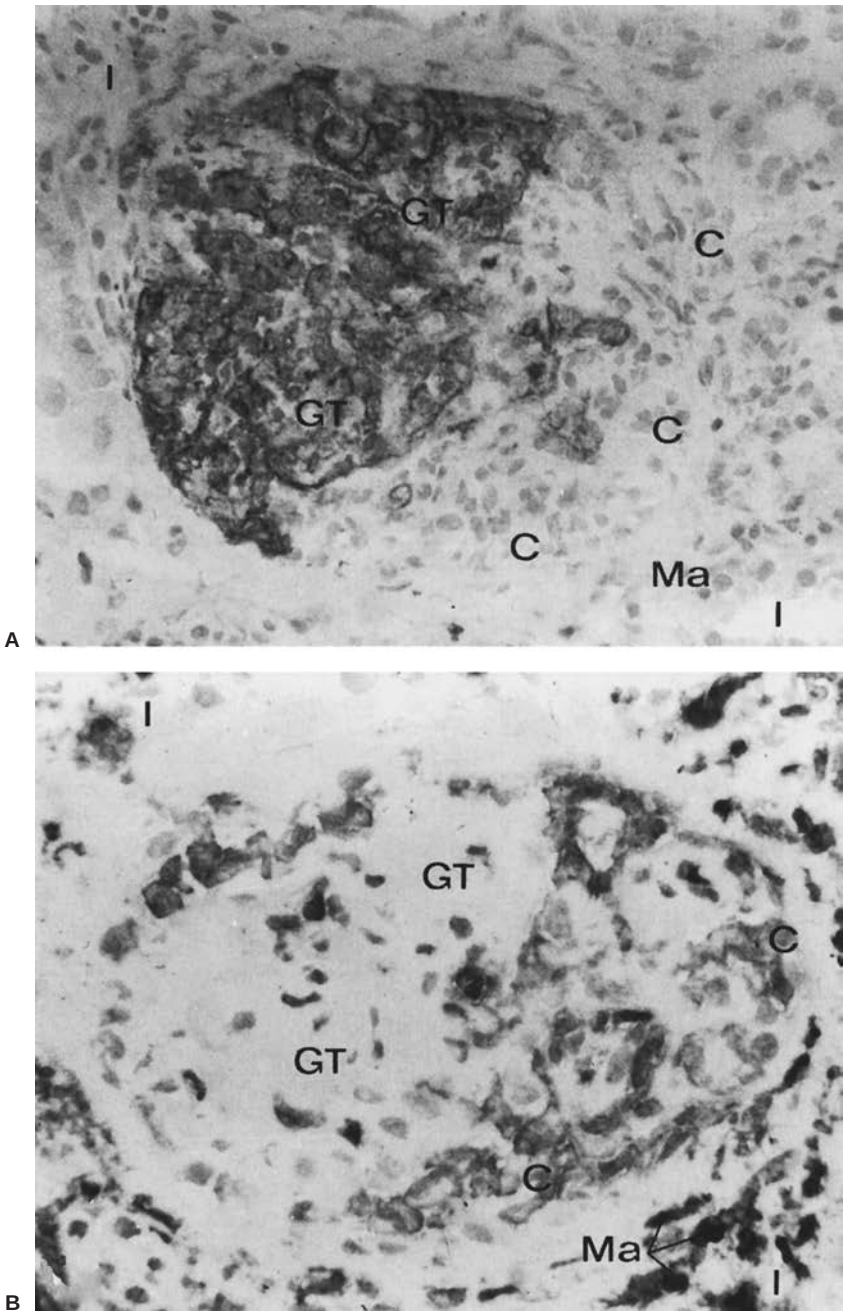

FIGURE 60-11. Serial cryostat sections from the renal biopsy of a patient with rapidly progressive glomerulonephritis, with immunohistochemical labeling of monoclonal antibodies by the four-layer peroxidase antiperoxidase technique (23). In both **A** and **B,** the glomerular tuft (GT) has been compressed into the left half of Bowman's space by the crescent (C), which occupies the right half. **A:** The epithelial cells of the glomerulus (GT) are stained using the antiglomerular epithelial cell marker PHM5. This labels all the epithelial cells in the compressed glomerular tuft (GT), but virtually none of the cells of the crescent (C). **B:** Macrophages (Ma) are stained using monoclonal antibody FMC32 recognizing monocyte/macrophages. Over 90% of the cells in the crescent are stained together with many macrophages (Ma) in the interstitium (I) surrounding the glomerulus. (Hematoxylin and eosin, magnification ×250.)

Local proliferation is another mechanism of macrophage accumulation during crescent formation within Bowman's space (Fig. 60-14). In rat anti-GBM glomerulonephritis, greater than 50% of glomeruli develop crescent formation by day 21 with macrophages accounting for 50% to 80% of total crescent cells (214). A striking finding was that over half of the macrophages within crescents were proliferating on the basis of PCNA expression, bromodeoxyuridine incorporation and the presence of macrophage mitotic figures, which was a higher level of macrophage proliferation than that present in the glomerular tuft (214). Marked macrophage proliferation within crescents has been confirmed in studies of human RPGN (93). Furthermore, expression of macrophage colony-stimulating factor (M-CSF) within crescents suggests that local M-CSF production drives the local macrophage proliferation within Bowman's space (122).

Yet another mechanism by which macrophages and T cell may enter Bowman's capsule during crescent formation is through ruptures in Bowman's capsule. Boucher et al. (92) demonstrated that epithelial cells were present in greater numbers than macrophages in cellular crescents when Bowman's capsule was intact, but that this pattern was reversed with rupture of Bowman's capsule. Periglomerular infiltration of macrophages and T cells is a prominent feature in both human (31,92,215,216) and experimental crescentic glomerulonephritis (63,97,101). Indeed, it may be case that the periglomerular infiltrate may actually mediate rupture of Bowman's capsule. In a study of rat anti-GBM glomerulonephritis, 25% to 52% of glomerular cross-sections exhibiting Bowman's capsule rupture had no evidence of crescent formation, whereas focal periglomerular accumulation of immune activated (IL-2R$^+$) CD4$^+$ T-cells and macrophages were invariably present

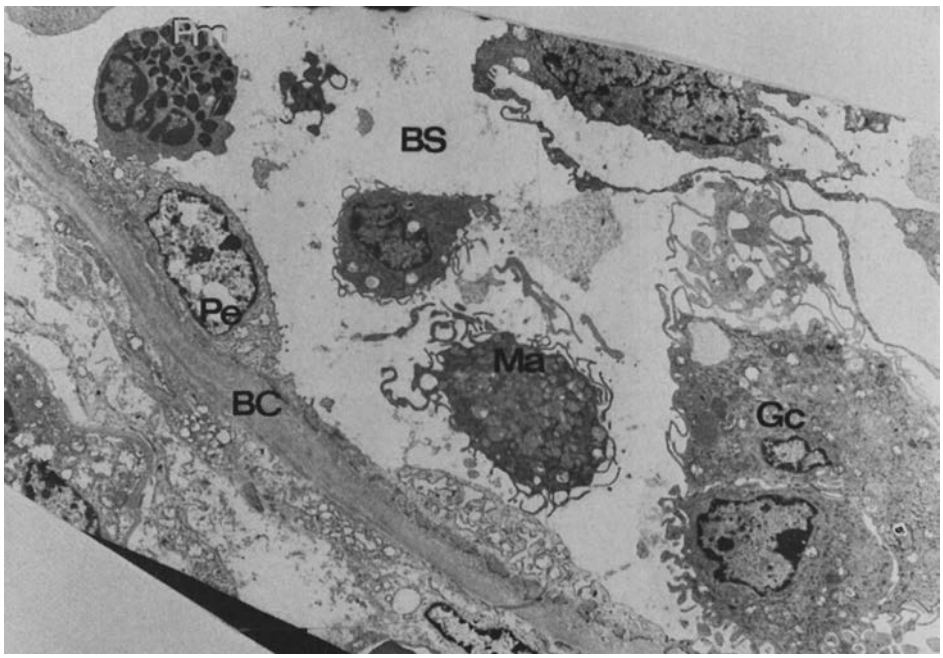

FIGURE 60-12. Transmission electron micrograph depicting a glomerulus from a rabbit with nephrotoxic nephritis at day 10 of the disease (i.e., the stage of early crescent formation). Bowman's space (BS), bounded on one side by Bowman's capsule (BC) and a parietal epithelial cell (Pe), contains a number of exudative macrophages (Ma) and a polymorphonuclear leukocyte (Pm). A macrophage that has undergone giant cell (Gc) transformation can be seen. (Magnification ×4,800; courtesy of Prof. E. F. Glasgow.)

at the site of Bowman's capsule rupture (97). Disruption of Bowman's capsule facilitated the entry of periglomerular fibroblasts, macrophages and activated (IL-2R$^+$) T-cells into Bowman's space and the development of glomerular fibrosis (97).

Deposition of Fibrin in Crescent Formation

The experiments of Vassalli and McCluskey (217) provided the first evidence that the presence of fibrin in Bowman's space is an important stimulus for crescent formation. A causal link between fibrin deposition and macrophage migration into Bowman's space was provided by studies in which defibrination of animals with ancrod prevented crescent formation in rabbit anti-GBM glomerulonephritis, despite the inflammatory reaction and macrophage accumulation in the glomerular tuft being unaltered (218). This finding is in accord with the observation in humans that fibrin is most commonly found in freshly formed crescents and tends to disappear as the crescents become sclerotic (45). In addition, we demonstrated that macrophages in the crescent in human RPGN have tissue factor expressed on their surface, as recognized by a specific monoclonal antibody, and that these macrophages are associated with fibrin deposition (90). Fibrin deposition is also present in crescents in ANCA-associated RPGN (94).

The importance of fibrin deposition in the progression of rabbit crescentic anti-GBM disease has been demonstrated by Zoja et al. (219). Recombinant plasminogen activator, which causes lysis of fibrin clots by activating plasminogen to plasmin, reduced glomerular fibrin deposition and crescent formation together with prevention of renal failure. Consistent with this

FIGURE 60-13. Scanning electron micrograph of a nephrotoxic glomerulus in tissue culture. The glomerulus itself is covered by a cellular crescent (Cr) from which macrophages (Ma) are emerging. (Courtesy of Prof. E. F. Glasgow.)

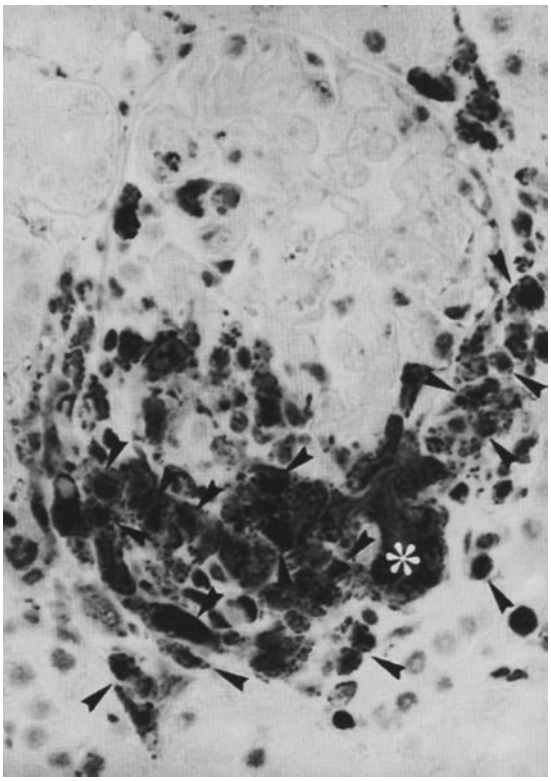

FIGURE 60-14. A rat glomerulus demonstrating an extensive crescent from an animal with anti-glomerular basement membrane nephritis. Macrophages are demonstrated using the ED-1 monoclonal antibody (*brown*), and the proliferating cell nuclear antigen antibody (*blue*) has been used to demonstrate proliferating cells. Proliferating macrophages are indicated by *arrowheads* and mononuclear giant cells by the *asterisk*. (Periodic acid-Schiff, magnification ×400.) (See Color Plate.)

finding, mice deficient for the fibrinogen gene develop a less severe anti-GBM glomerulonephritis compared to wild-type mice with a significant reduction in glomerular crescent formation (220).

Macrophages have been shown to be responsible for the glomerular fibrin deposition and express augmented procoagulant activity in rabbit anti-GBM disease (221). Furthermore, blockade of tissue factor, using either neutralizing antibodies or recombinant tissue factor pathway inhibitor, have been shown to inhibit renal injury in rabbit anti-GBM glomerulonephritis (222). In addition, an important role for thrombin in mediating fibrin deposition and consequent renal injury and glomerular crescent formation has been demonstrated in a mouse model of anti-GBM glomerulonephritis using hirudin as a selective thrombin antagonist and mice deficient in protease-activated receptor (PAR)-1—a cellular receptor for thrombin (223).

Fibroblasts in Crescent Formation

Cellular crescents can undergo a process of fibrous organization which involves a fibrocellular stage characterized by the presence of fibroblasts and collagen deposition within Bowman's space followed by a fibrous stage in which progressive collagen deposition within Bowman's space and a loss of cells leads to scar formation (197). At this stage, the glomerular tuft is often substantially compressed with capillary obliteration.

The entry of fibroblasts into Bowman's space is generally accepted as the key event in determining fibrous organization of cellular crescents. There are at least four possible origins of the fibroblasts that accumulate in fibrocellular crescents. First, the major source of these cells is probably from the periglomerular area. As discussed above (see Mononuclear Cells in Crescent Formation), rupture of Bowman's capsule is an important determinant of crescent composition. Fibroblast accumulation and upregulation of collagen mRNA levels in the periglomerular area is a very early event in experimental anti-GBM glomerulonephritis (224), and a number of studies have suggested that periglomerular fibroblasts enter Bowman's space via ruptures in Bowman's capsule to induce fibrous organization of cellular crescents (97,225,226). Second, glomerular mesangial cells can differentiate into α-smooth muscle actin (SMA)—expressing motile cells with a myofibroblast-like phenotype (227). These mesangial-derived SMA+ myofibroblasts could easily migrate into Bowman's space and promote fibrosis. Third, epithelial-mesenchymal transdifferentiation of parietal epithelial cells into SMA+ myofibroblasts has been described in crescents using immunohistochemistry and electron microscopy in rat anti-GBM glomerulonephritis (228). Fourth, a circulating fibrocyte population has been described which has been shown to migrate into sites of tissue damage and promote tissue fibrosis (229,230). Such a cell type has the potential to contribute to the development of fibrocellular crescents, but no studies to date have provided evidence to support this possibility. Irrespective of the origin of the SMA+ myofibroblast-like cells that enter cellular crescents, the high level of cell proliferation evident in this population is likely to make an important contribution to the ongoing accumulation of these cells (214,228). The production of growth factors such as PDGF, FGF-2, and TGF-β1 within crescents is likely to promote myofibroblast proliferation and matrix deposition (228,231,232).

CLINICAL FEATURES

Rapidly progressive glomerulonephritis is an uncommon disease, comprising approximately 2% of all cases of glomerulonephritis, although the reported incidence varies from 1.5% to 5% (12,42,57,233). In population terms, a German study reported a calculated annual incidence of RPGN of 0.7 per 100,000 population (234). A recent French report noted an increasing incidence of crescentic nephritis, with incidence rates for the 60 to 79 year age group increasing from 5 per million prior to 1985 to 27 per million since 1996 (235). Similarly, a Chinese report of over 10,000 renal biopsies noted an increase from 0.5% of all biopsies to 2.2% when comparing the 1980's to the 1990's (233). The disease is predominantly seen in the third to sixth decade, with a reported median age ranging from 39 to 58 years (9–13,236,237). Occurrence in childhood is very rare (235,238–240). Patients with RPGN associated with anti-GBM antibody or endocapillary cell proliferation tend to be younger than those with ANCA-positive RPGN (14). In contrast, severe crescentic glomerulonephritis of poststreptococcal etiology is most frequent in children (10,238) whereas crescentic disease in the elderly is not infrequently associated with systemic vasculitis, particularly with microscopic polyangiitis (10,241–243). Men are more commonly affected than women (2:1) (10–14) and this ratio is higher in patients with anti-GBM disease. There is a genetic predisposition to RPGN in that there is a susceptibility to anti-GBM antibody synthesis in patients who are human leukocyte antigen (HLA)-DR2 (244,245) The resultant glomerulonephritis is reported to be more severe when it is associated with HLA-B7 (244).

Despite the varying underlying immunopathogenetic mechanisms of RPGN, the clinical features are remarkably similar in all three subgroups (Table 60-2). It is not possible to reliably predict on clinical grounds which immunopathologic group the patient belongs to (anti-GBM, immune, or ANCA positive). Indeed, the signs and symptoms depend more on the stage of

TABLE 60-2

CLINICAL FEATURES OF RAPIDLY PROGRESSIVE GLOMERULONEPHRITIS

Median age: fifth and sixth decades	
Sex: M/F = 2:1	
Prodromal illness: common (upper respiratory infection, "flulike" illness, fever)	
Presenting features:	Percent of patients:
Nonspecific symptoms of malaise, lethargy	>90
Symptoms of renal failure	60
Oliguria	>60
Edema	60–70
Macroscopic hematuria	20–30
Nephrotic syndrome	10–30
Acute nephritic syndrome	10–20
Hypertension	10–20

the disease at presentation. Usually, the onset is insidious, and patients present with nonspecific symptoms for several weeks (7,9,12,79,236,237,243,246–250).

These symptoms include malaise, lethargy, weakness, anorexia, and nausea. At presentation, many have symptoms of renal failure. Most patients with advanced disease are oliguric, and some are anuric. Occasionally, the onset is very acute, with features of an acute nephritic syndrome, and advanced renal failure develops over a few days. Macroscopic hematuria is not uncommon, and microscopic hematuria is invariable. Edema is seen in most patients, and the nephrotic syndrome is seen in 10% to 45% (9,12,237,251). Hypertension may be present in up to 60% (251), but is mild when not due to sodium and water retention (14). In approximately 50% of patients, an upper respiratory or influenza-like illness precedes the other symptoms; however, only rarely has a specific viral etiology been established. Aronson and Phillips (252) found evidence of infection with coxsackie virus B5 in six patients with acute oliguric renal failure, and an outbreak of influenza A2 has been associated with RPGN with anti-GBM antibody (253). A greater than expected association with exposure to hydrocarbon solvents also has been reported, especially for anti-GBM disease (254) and other researchers have noticed an increased association with myocardial infarction (11), malignancy (11,255), and membranous glomerulonephritis.

By contrast, patients with crescentic glomerulonephritis associated with vasculitis may have prominent systemic symptoms, such as recurring fever, purpuric skin rash, arthritis/arthralgia, mucosal ulceration, and respiratory symptoms, including dyspnea and hemoptysis (79,256). Similarly, patients with crescentic glomerulonephritis associated with systemic illnesses such as scleroderma, systemic lupus erythematosus (SLE), endocarditis, cryoglobulinemia, or Schönlein-Henoch syndrome may have the systemic features of these diseases.

Frank hemoptysis suggests Goodpasture's syndrome (glomerulonephritis and pulmonary hemorrhage associated with anti-GBM antibody), but this also can be a prominent feature of systemic vasculitis (56). Goodpasture's syndrome is the combination of hematuria, hemoptysis, and (acute) renal failure, but is not specific for anti-GBM disease and is relatively common in the vasculitides, especially Wegener's granulomatosis (234). Indeed, in one study of patients with pulmonary-renal syndrome, 54% had ANCA disease, 7% had ANCA and anti-GBM antibodies, and only 6% had anti-GBM alone (257). Thus, presenting symptoms may be relatively nonspecific and variable and, apart from a rapid deterioration in renal function, provide little to alert the clinician to the seriousness of this relatively rare disease.

LABORATORY FINDINGS

The laboratory findings are summarized in Table 60-3.

Nondiscriminatory Findings

Microscopic hematuria is invariable, the red cells being dysmorphic, indicating a glomerular lesion (258). Red cell, granular, and leukocyte casts are frequent, although the urinary sediment correlates poorly with the severity of disease.

Proteinuria is always present, although in the nephrotic range in only 10% to 45% (9,12,237,251). More severe disease is less likely to be associated with heavy proteinuria, which simply reflects the low glomerular filtration rate. Fibrin degradation products may be elevated in both the serum and the urine (259–261).

Most patients have elevated serum creatinine and blood urea nitrogen levels at presentation and often are in severe renal failure, with a serum creatinine elevated to more than 5.0 mg/dL and a creatinine clearance that is less than 10% of normal. The degree of renal failure at presentation depends on the chronicity and severity of the renal lesion. However,

TABLE 60-3

LABORATORY FEATURES OF RAPIDLY PROGRESSIVE GLOMERULONEPHRITIS

Urine:	Microscopic hematuria (dysmorphic): 100% of patients
	Casts (red cell, granular, leukocyte): common
	Proteinuria: 100% (>3 g/24 hr: 10%–30%)
Renal function:	Impaired: 100%
	Creatinine clearance <20 mL/min: (30%)
Normal renal size	
Normocytic normochromic anemia	
Erythrocyte sedimentation rate: moderate rise (<100 mm first hour)	
Circulating antiglomerular basement membrane antibody (30%)	
Circulating immune complexes (10%–15%)	
Serum complement components: normal	
Anti-DNA antibody: negative	
Antistreptolysin 0 titer: no rise	
Antineutrophil cytoplasmic antibody (80% if absent glomerular immune deposits)	

TABLE 60-4

PROGNOSIS OF RAPIDLY PROGRESSIVE GLOMERULONEPHRITIS ACCORDING TO URINE OUTPUT AT PRESENTATION

	No. of patients	Percentage of patients with renal function at 12 months		
		All patients (%)	Nonoliguric (%)	Oliguric (%)
Whitworth, 1976 (12)	60	72	87	53
Morrin, 1978 (14)	29	31	59	20
Neild, 1983 (13)	19	52	100	10
Heilman, 1987 (199)	24	40	78	22
Weighted mean		54	81	34

as discussed in the following section, most untreated patients progress to end-stage renal failure within several weeks or months.

A normochromic normocytic anemia frequently is observed and often is more profound than expected from the degree of renal failure. In patients with lung hemorrhage, especially those with anti-GBM disease, anemia may be severe, and these two factors contribute to poor tissue oxygenation. Lung hemorrhage frequently is associated with an increased diffusing capacity for carbon monoxide (262). The erythrocyte sedimentation rate usually is elevated; however, if it is very high (>100 mm in the first hour), an underlying systemic disease such as vasculitis should be suspected.

Findings Specific to Rapidly Progressive Glomerulonephritis

Serum complement levels typically are not depressed (9,44, 263). Circulating immune complexes are present in 20% to 30% of patients with immune deposits in glomeruli, suggesting an immune complex etiology for their disease (264,265). However, circulating immune complexes also have been described in sera of patients with no glomerular immune deposits (3,266,267) and probably are of little clinical value. The presence of ANCA suggests the vasculitic group (43,46,69,268). In the group of patients with RPGN who have linear deposition of antibody along the GBM, circulating anti-GBM antibody usually is detected (269–271), particularly if a specific and sensitive radioimmunoassay is used (55,272,273). About a quarter of patients with anti-GBM antibodies also have detectable ANCA antibodies (274).

Findings Related to an Underlying Condition

The presence of circulating cryoglobulin has been reported in some patients (266,272,275), particularly in association with hepatitis C (276). Anti-DNA antibodies usually are not found outside of those patients with SLE. Hypocomplementemia may be seen in the crescentic glomerulonephritis of both poststreptococcal glomerulonephritis and SLE.

NATURAL HISTORY

As mentioned previously, most patients have severe renal failure at the time of presentation, reflecting extensive glomerular damage. Thus prognosis already is predetermined, at least in part, by the degree of irreversible renal damage.

In several reported series of patients with RPGN (9,44,251, 277), the authors have attempted to determine prognostic factors and the effect of treatment. These series are not strictly comparable because there are variations in the criteria for diagnosis—the underlying immunopathogenetic processes, the extent of crescent formation (>30% crescents, >50% crescents, or >80% crescents), and the inclusion or exclusion of clinically suspected microscopic vasculitis or Goodpasture's syndrome. Moreover, a valid life table analysis of persistence of renal function and survival of patients rarely has been undertaken. However, a synthesis of these reports indicates that approximately 20% of patients present with permanent end-stage renal failure. Approximately 50% progress to end-stage renal failure by 1 year, and 75% by 2 years, although these results are better in ANCA disease, with up to 90% of patients in remission at 1 year (278) (Table 60-4). A variety of patterns of deterioration or improvement in renal function are seen. Some patients show spontaneous rapid improvement in renal function soon after presentation. In a few patients, this improvement is sustained, and in others it is temporary, with the patients drifting into renal failure within months. Other patients show progressive decline in renal function after presentation, whereas others maintain stable renal function. These variable patterns of outcome probably reflect a number of factors, including the persistence or loss of the causative factors, the degree of initial renal damage, the severity of the scarring process that subsequently evolves (e.g., fibrosis of crescents and interstitial fibrosis), and the development or absence of vascular damage and hypertension. These patterns also may be altered by the various therapeutic measures used in this disease.

Urine Output at Presentation

It has become evident that the presence of oliguria at presentation of RPGN is associated with a significantly poorer outcome than is seen in patients who have maintained good urine output. Table 60-4 summarizes the results from four series of patients with RPGN (12–14,277) and the persistence of renal function at 12 months, in patients who presented with or without oliguria, is listed. Although there is a wide variation, each series shows a significant difference in survival between oliguric and nonoliguric patients. Temporary or sustained recovery of renal function was seen in only 34% of patients who were oliguric at presentation. On the other hand, 81% of nonoliguric patients were alive at 12 months with adequate renal function.

Patients who are anuric at presentation are less likely to recover renal function. The poor outcome associated with oligoanuria reflects the degree of crescent formation, as in 80% of oligoanuric patients, more than 80% of the glomeruli are involved (13,239,279).

TABLE 60-5

IMPROVEMENT IN RENAL FUNCTION FOLLOWING RAPIDLY PROGRESSIVE GLOMERULONEPHRITIS ACCORDING TO THE EXTENT OF CRESCENT FORMATION

	100%	90–99%	80–89%	70–79%	60–69%
Number of patients (total 200)	66	43	36	35	20
Number of patients with improvement in renal function	10	14	18	20	11
Percentage	15%	32%	50%	56%	55%

(Adapted from Neild GH, et al. Rapidly progressive glomerulonephritis with extensive glomerular crescent formation. *Q J Med* 1983;207:395, with permission.)

Extent of Crescent Formation

Several studies have shown a positive correlation between the percentage of glomeruli affected by crescent formation and the severity of initial and subsequent renal failure (11–14,90, 236,243,273,280).

In two studies, all patients with crescents in 100% of the glomeruli progressed rapidly to end-stage renal failure (14, 239), whereas other authors have reported the recovery of renal function in less than 10% of such patients (281,282). Habib (239,283) found a similar incidence of end-stage renal failure in patients with more than 80% crescents. Morrin et al. (14) found that 39% of patients with more than 80% crescents showed some recovery of renal function, compared with 90% of patients with less than 60% crescents. Neild et al. (13) reviewed 11 published series of RPGN, totaling 200 patients, and confirmed the findings that the more extensive the crescent formation, the less there is a chance of recovery of renal function (Table 60-5).

The development of crescents in other forms of primary glomerulonephritis (e.g., membranoproliferative glomerulonephritis) and in glomerulonephritis of a systemic disease also is associated with a poor outcome. However, in the setting of IgA nephropathy with synpharyngitic hematuria, crescents may not be indicative of a poor prognosis (284).

Changes in the Glomerular Tuft

Endocapillary cell proliferation (glomerular hypercellularity) has been reported as a good prognostic feature compared with normocellularity of the glomerular tuft (13,14,27,237). Morrin et al. (14) reported recovery of renal function in only 30% of patients with RPGN who had normal glomerular tuft cellularity, compared with 50% of patients with hypercellular glomerular tufts.

Glomerular tuft necrosis is thought to be a poor prognostic feature (14,237,285). This notion is supported by the observation that in the microscopic form of polyarteritis, where segmental or diffuse tuft necrosis is very prominent, severe renal failure may be present with minimal crescent formation. Global glomerular sclerosis and breaks in Bowman's capsule also are poor prognostic features (286).

Interstitial Fibrosis and Tubular Atrophy

It has been reported that the extent of interstitial fibrosis correlates with the severity of initial and ultimate renal dysfunction (237,240,286). Striker et al. (237) found that if interstitial fibrosis involved more than 30% of the biopsy tissue, then recovery of renal function was unusual, and the patients often were anuric at presentation.

Immunopathogenesis and Outcome

The group of patients with ANCA-positive RPGN seems to have the best prognosis (11), although this has not been the experience of all authors (12). Granular immune deposits also have been reported as indicative of a better prognosis (14). On the other hand, other authors reported a poor prognosis for patients with granular immune deposits (12). This conflict of findings may reflect the inclusion, in some series, of patients with poststreptococcal crescentic glomerulonephritis. The group with RPGN associated with anti-GBM antibody has the worst prognosis (13,14,20,234). The reason for this occurrence is not clear. The chronicity of the immune insult may explain the worse prognosis. Urine protein excretion, degree of interstitial cellular infiltration, and age show a weak correlation with outcome (277). A summary of the factors determining the outcome of RPGN is outlined in Table 60-6.

TREATMENT

Many therapeutic regimens have been used in the treatment of RPGN, and the relative benefits of these regimens are very much in dispute. Because of the rarity of RPGN, there have been very few controlled trials comparing either one treatment regimen with another or with no treatment. Many studies have simply assessed outcome compared with historical controls. This is not a valid comparison, particularly given the advances made in the minimization of progression of renal disease with less specific therapy, such as control of hypertension, use of angiotensin-converting enzyme inhibitors, diet, and the more successful management of renal failure, including temporary dialysis. In addition, many of the therapies used to treat RPGN have the potential to induce serious complications, including sepsis, hemorrhage, sterility, and malignancy.

Few controlled trials have been performed and therapy has been largely empirical. Further evaluation of the various therapies should, therefore, continue. It also must be said that several authors have noted spectacular recovery from RPGN in an appreciable number of patients given immunosuppressive and anticoagulant agents, in whom the expected recovery rate would be nil (e.g., advanced renal failure with 100% crescents). These observations coupled with our understanding of the immunopathogenesis of RPGN have been sufficient evidence for

TABLE 60-6

FACTORS DETERMINING THE OUTCOME OF RAPIDLY PROGRESSIVE GLOMERULONEPHRITIS

Factors	Poorer prognosis	Better prognosis
Urine output at presentation	Oliguric	Nonoliguric
Extent of crescent formation	>80%	50–80%
The glomerulus	Fibrinoid necrosis	Endocapillary cell proliferation
Glomerular immune deposits	Linear deposition (antiglomerular basement membrane)	Granular deposition (immune complex) or no immune reactants
Interstitium	Interstitial fibrosis and tubular atrophy	

many renal physicians routinely to use this therapy for such patients.

Corticosteroids

Because of their antiinflammatory properties, corticosteroids are frequently used in the treatment of RPGN. There are very few published data on the use of corticosteroids alone in experimental crescentic glomerulonephritis. However, several reports have demonstrated marked improvement in renal function with intravenous methylprednisolone (287–289). Holdsworth and Bellomo (290) and Tipping and Holdsworth (291) have shown that glomerular injury induced by macrophages in rabbits can be reduced substantially by corticosteroids, whereas injury induced by polymorphonuclear leukocytes is unaffected. As discussed earlier, both polymorphonuclear leukocytes and macrophages are thought to be injurious in crescentic glomerulonephritis. More recent data suggest that corticosteroids inhibit the activation of NF-κB, an important transcription protein in the immune response, resulting in inhibition of inflammatory cytokine production (292).

There is no evidence that oral steroids alone are of benefit in nonvasculitic RPGN (7,243,281,293). Their use is usually in conjunction with immunosuppressive agents. The lack of benefit from steroids alone is strikingly different from their often dramatic effect in necrotizing (ANCA positive) crescentic glomerulonephritis associated with vasculitis (263,294,295). In the latter condition, corticosteroids alone offer a remission rate similar to that obtained with other therapy (see below), but a threefold higher relapse rate (296). However, several reports indicate that high-dose intravenous "pulse" methylprednisolone therapy, given daily or on alternate days for three to six doses, can lead to dramatic and sustained improvement in renal function in nonvasculitic RPGN (11,280,296–302). Bolton et al. (297,303) found this benefit only in patients with RPGN that was not caused by anti-GBM antibody. All these investigators have commented on the low incidence of side effects of intravenous pulse steroid therapy. There is some evidence that intravenous methylprednisolone has a distinct effect of decreasing superoxide production by neutrophils from patients with ANCA-positive crescentic glomerulonephritis (304).

Cytotoxic Agents

Immunosuppression with either azathioprine or cyclophosphamide (or occasionally chlorambucil) is commonly used in the treatment of crescentic glomerulonephritis, again usually in combination with steroids and, more recently, plasma exchange.

There appears to be little or no evidence to support the use of either cyclophosphamide or azathioprine as single treatments. Several authors have reported substantial benefit from the combination of cyclophosphamide with anticoagulation, steroids, and antiplatelet drugs, whereas others have been unable to demonstrate benefit (282,285,305,306). There are very few controlled data to support the use of cyclophosphamide (or azathioprine) in RPGN, although most clinicians believe they are necessary components of the treatment. The use of intravenous cyclophosphamide in the treatment of RPGN has only rarely been reported (307,308) and has not demonstrated any advantage over daily oral dosing, unlike its use in lupus nephritis. In the vasculitic subgroup, intravenous cyclophosphamide, given as a monthly pulse dose, was as effective as a daily oral dose and had fewer side effects in one study (309), but another retrospective study of patients specifically with Wegener's granulomatosus with renal involvement suggested a worse outcome with monthly intravenous pulses of cyclophosphamide (310). Given the paucity of data, it is difficult to make recommendations regarding oral versus IV-pulse cyclophosphamide use in RPGN.

Anticoagulants

For a number of years, it has been appreciated that glomerular fibrin deposition is prominent in crescentic glomerulonephritis, in the glomerular tuft, and especially in Bowman's space and the crescent (10,242,264). The role of the coagulation process in development of the crescent in experimental glomerulonephritis has already been discussed. Several studies in experimental glomerulonephritis have shown that anticoagulation with heparin, before the onset of the glomerular injury, can reduce the formation of crescents and severity of renal failure, although enormous doses of heparin were required (311–313). Some authors have failed to demonstrate a significant benefit of this approach (314,315). Anticoagulation with warfarin also has been shown to reduce, to a smaller degree, crescent formation (217,316). It has been suggested that the pathogenetic mechanisms occurring locally in the glomerulus are capable of producing local coagulation, overriding the effects of circulating anticoagulants. This occurrence could possibly result from a failure of heparin, a highly charged molecule, to penetrate the mesangium and Bowman's space. Another explanation for the relative ineffectiveness of heparin is that the fibrin deposition results from thrombin-independent mechanisms, as discussed previously.

Anticoagulation with heparin for human RPGN was first reported by Kincaid-Smith et al. in 1968 (317). The subsequent use of heparin in this disease usually has been in combination with one or more other drugs, including steroids, immunosuppressives, and antiplatelet agents (49,285,305,318–320). It therefore has been difficult to determine the relative benefit of the anticoagulation, and even now there is still no clear-cut evidence that use of these agents is beneficial.

Antiplatelet Age

Platelets are potential mediators of glomerular injury, particularly through the release of vasoactive amines. However, little direct evidence exists that they are involved in crescentic glomerulonephritis, although a role in the coagulation process in the glomerulus has not been excluded (318,321,322). Antiplatelet agents, such as dipyridamole and sulfinpyrazone, have been used in the disease as part of a "cocktail," with no proven benefit.

Plasma Exchange

Since the demonstration of the definite benefit of plasma exchange for patients with Goodpasture's syndrome (75,323–326), the technique has been used widely in a variety of types of glomerulonephritis, including RPGN (241,267,268,279, 327–332), usually in conjunction with immunosuppression, although not always with success (333). Glockner et al. (334) reported in 1988 a randomized study of plasma exchange in 26 patients with RPGN, also given steroids and immunosuppression, and were unable to demonstrate a difference in outcome of renal function after 8 weeks. Another randomized study from the Canadian Apheresis Study Group failed to demonstrate any additional benefit of plasma exchange over prednisolone and azathioprine out to 12 months in 32 patients with idiopathic crescentic nephritis (335). In the latter study, there may have been a benefit of plasma exchange in dialysis-dependent patients, but numbers were too small. On the other hand, several prominent groups have reported on the additional benefits of plasma exchange in severe ANCA-positive crescentic nephritis (336,337). The best results appear to be in those who were dialysis dependent at presentation but still were not dramatically better than those obtained with steroids and cyclophosphamide without plasma exchange (337). A reanalysis of two French studies designed to assess the value of plasma exchange in systemic vasculitis demonstrated no additional benefit of plasma exchange in those patients with renal involvement (338). A more recent randomized study compared six treatments of either plasma exchange or immunoadsorption in a total of 44 patients with RPGN (339). All patients also received corticosteroids and cyclophosphamide. There was no difference in outcome with these two treatment modalities. However, in the study, 6 of 6 dialysis-dependent anti-GBM patients remained on dialysis, whereas 7 of 10 non–anti-GBM patients who were initially dialysis dependent improved to become dialysis independent (339). Earlier data from the Hammersmith group had suggested that unless pulmonary hemorrhage was present, treatment of patients with anti-GBM disease and a serum creatinine over 600 μmol/L (6.8 mg/dL) with plasma exchange was futile (337). This then creates a paradoxical situation: Plasma exchange is of most value in anti-GBM disease when the creatinine is less than 600 μmol/L, but in vasculitis and probably immune-RPGN, the best results with plasma exchange appear to be in those who are dialysis dependent at presentation. The reason for this difference is not clear.

Experimental Approaches to Treatment

The most commonly used alternative agent is mycophenolate mofetil. This agent is gathering favor as an alternative to cyclophosphamide, especially in the maintenance phase of management. In Class IV lupus nephritis (see Chapter 65), mycophenolate is gaining a defined role based on several trials of its use either as induction (340) or maintenance therapy (341). However, in vasculitis, there are as yet anecdotal reports only. As an example, one report detailed 14 patients with Wegener's granulomatosis who had all received induction therapy with cyclophosphamide and glucocorticoids and then received mycophenolate as maintenance therapy with excellent results (342). Another approach, particularly applied in vasculitis is the use of anti-TNF-alpha therapies. The soluble p75 TNF receptor etanercept has been used with some success in vasculitis, although there have been reports of a high relapse rate. Curiously, there are also reports of etanercept induced vasculitis, including RPGN (343). Infliximab, a humanized anti-TNF chimeric antibody, has also been used with success to treat difficult cases of ANCA positive vasculitis with a reported relapse rate of 20%. There was some concern over a high rate of infection with encapsulated organisms (344). Other agents that remain at an early phase of development include leflunomide, interleukin-1 receptor antagonist, and the antibodies Mabthera and CAMPATH II.

Renal Transplantation

Successful transplantation is possible in patients with RPGN (234,345). The disease can recur in the allograft (236,345,346), but at least for anti-GBM disease, this is unusual if transplantation is delayed until the circulating anti-GBM antibody has disappeared. The transplantation of a normal kidney into a patient with Alport's syndrome potentially incites an antibody response to the normal glomerular basement membrane. Although linear IgG may be seen in the glomeruli of as many as 30% of these transplants, crescents are not common and the course is most often benign (347). Recurrent ANCA-positive vasculitis is reported to occur in approximately 20% of cases in the setting of a positive ANCA titer at the time of transplantation (348). *De novo* RPGN occurring in a renal transplant also has been described (349).

Factors To Be Considered in the Treatment of Rapidly Progressive Glomerulonephritis

The simple diagnosis of RPGN alone is not sufficient to determine the type of treatment. Several other factors are likely to be relevant to the potential benefit, or not, of treatment.

1. The acute or chronic nature of the glomerular lesion is significant. The presence of acute cellular crescents with extensive fibrin deposition is more indicative of a likely positive response to immunosuppression than disease in which crescents are undergoing fibrosis and in which glomerulosclerosis and interstitial fibrosis are prominent.
2. The presence of segmental glomerular capillary loop necrosis should raise the possibility of an underlying vasculitis and thus a greater chance of a response to immunosuppression. Similarly, the presence of systemic symptoms other than those of renal failure should suggest the possibility of vasculitis.
3. Immunosuppression, anticoagulation, and the use of plasma exchange all have the potential for inducing severe and occasionally fatal side effects (350–352). It could be argued

that the patient particularly at risk from these side effects (e.g., the elderly, infected, or patients with peptic ulceration) is better treated by aggressive control of hypertension and renal failure rather than by immunosuppression. If immunosuppression is to be successful, early diagnosis of RPGN is essential before irreparable damage has occurred. Early diagnosis depends on urinalysis and assessment of renal function in any patient presenting to a general practitioner with persistent nonspecific symptoms (353).

The authors' approach to the treatment of RPGN is to treat all patients, unless renal biopsy shows an end-stage kidney, with a trial of immunosuppression, consisting of corticosteroids and cyclophosphamide, without anticoagulation. Pulse methylprednisolone is given intravenously (1 g) on each of 3 successive days. This is followed by oral prednisolone at a dosage of 60 mg/day for 4 to 6 weeks, which is then reduced gradually to a maintenance dose of 10 to 15 mg/day and sustained for 6 months to 1 year, depending on the clinical progress. Oral cyclophosphamide is used at 2 to 3 mg/kg of body weight, usually for 3 to 6 months. Monthly pulses of cyclophosphamide are used routinely in lupus nephritis and occasionally in ANCA-positive vasculitis. Anticoagulation with heparin is not used routinely, nor is dipyridamole or other antiplatelet agents. Plasma exchange is always used for anti-GBM disease, typically as 3- to 4-L exchanges every other day for six exchanges. Short-term plasma exchange also may be considered in other forms of severe RPGN in patients who are refractory or become dialysis dependent, especially in ANCA-positive forms of the disease.

Failure of response to these measures leads to an early rather than late cessation of therapy to minimize complications. Maintenance dialysis remains the alternative for those patients who are not treated or are not responding to treatment.

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CHAPTER 61 ■ IMMUNOGLOBULIN A NEPHROPATHY AND HENOCH-SCHÖNLEIN PURPURA

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Immunoglobulin A (IgA) nephropathy is the most common form of primary glomerulonephritis, and is a major contributor to the worldwide burden of end-stage renal failure. It is characterized pathologically by proliferation of the glomerular mesangium and mesangial deposition of IgA molecules, and clinically by wide variations in its presentation and prognosis. Its recognition as a distinct disease in its own right dates to the first description of the typical mesangial IgA immunofluorescence by Berger in 1968 (1). Despite considerable effort, elucidation of its etiology as well as satisfactory treatments have both remained largely elusive.

Henoch-Schönlein purpura (HSP) is a small vessel vasculitis that exhibits identical renal histopathologic appearances to IgA nephropathy, but with additional systemic features that readily identify it as a syndrome in its own right. It will be discussed in more detail at the end of this chapter.

IgA NEPHROPATHY

Incidence and Prognosis

The reported incidence and prevalence of IgA nephropathy varies widely, and is dependent to an important degree on variations in renal biopsy policies between different countries. Studies from Europe have estimated the incidence to be 15 to 40 new cases per million population per year. The incidence is higher in Japan, where routine screening for urinary abnormalities is performed in all school-aged children. Prevalence rates, expressed as a percentage of renal biopsy diagnoses, are reported to be 20% to 40% in Asia, Australia, Finland and Southern Europe, but as low as 2% to 10% in the United States. While the local enthusiasm for detecting asymptomatic urinary abnormalities and then biopsying those individuals undoubtedly contributes greatly to these variations, there also appear to be important differences in susceptibility across different ethnic groups (2–4). For example, it is less common in African Americans, black South Africans, and New Zealand Polynesians than in Caucasians of European origin. Subclinical disease without urinary abnormalities may also be much more common than these figures indicate. For example, a recent study of renal allografts in Japan revealed mesangial IgA deposition in 16% (82 out of 510), and 19 of those had mesangial proliferation (5). Two separate studies have found IgA mesangial deposition in 2% and 4.8% of unselected autopsy series (6,7).

Uncertainties about true incidence and different approaches to individuals with asymptomatic urinary abnormalities also affect estimations of prognosis. Centers that trouble to diagnose milder cases will naturally observe a more benign disease course. Undoubtedly the majority of sufferers of IgA nephropathy do have a benign disease that does not lead to progressive renal dysfunction, and in some all signs of disease resolve with time. However, the significant majority have persistent urinary abnormalities (microscopic hematuria +/− proteinuria), and 20% to 40% will eventually progress to end-stage renal failure (8–11). The combination of relative frequency of the disease and a high proportion with poor prognosis means that IgA nephropathy is a major contributor to the number of individuals who require end-stage renal failure replacement therapy. For example, according to the Australian and New Zealand dialysis and transplant registry, 7.0% of patients diagnosed with end-stage renal failure in 2002 had biopsy proven IgA nephropathy (12). For some, the renal demise will be relatively rapid (months to several years), because of more aggressive disease and/or late presentation, whereas others can take decades from first presentation. It will be clear in the later discussion about treatment that estimating an individual's prognosis is crucial when considering active intervention.

Clinical Presentation

The variability of presentations and subsequent course is a feature of IgA nephropathy, and a list of the wide range of initial manifestations is shown in Table 61-1. There is an approximately 3:1 male preponderance.

Macroscopic Hematuria

The most characteristic and, at least to the patient, most dramatic presentation of IgA nephropathy is with episodic macroscopic hematuria in a young adult. Approximately one-third of diagnoses of IgA nephropathy occur as a result of such a presentation. There is a highly characteristic close temporal relationship between its onset and an upper respiratory tract infection, especially pharyngitis or tonsillitis, the hematuria coinciding with or occurring within 1 to 2 days of the sore throat. This has led to wide use of the term "synpharyngitic hematuria," and the timing differentiates it from the 2 to 3 week gap between infection and macroscopic hematuria in postinfectious glomerulonephritis. Less frequently, such hematuria also accompanies infections of other mucosal surfaces (e.g., gastroenteritis and urinary tract infections). The hematuria is painless but often associated with systemic symptoms such as fever, malaise, fatigue, diffuse muscle aches, and abdominal and dull loin pain. It is usually short-lived, lasting 1 to 5 days, and its disappearance coincides with resolution of the systemic symptoms. Occasionally there is associated transient acute renal impairment.

Differentiation from other causes of macroscopic hematuria is important, because frequent and unnecessary radiographic and urologic investigations may ensue if the true cause is not recognized. Of paramount importance is examination of the centrifuged urinary sediment, which displays dysmorphic red

TABLE 61-1

PATTERNS OF CLINICAL PRESENTATION OF IgA NEPHROPATHY

Common
Synpharyngitic macroscopic hematuria +/− loin pain
Microscopic hematuria, usually with proteinuria
Hypertension
Chronic renal failure
Henoch-Schönlein purpura
Uncommon
Malignant hypertension
Acute nephritic syndrome
Acute renal failure
Nephrotic syndrome

blood cells (of glomerular origin), plus granular and red cell casts. Under these circumstances, renal biopsy is the appropriate first diagnostic procedure. The urinary abnormalities of microscopic hematuria +/− proteinuria persist in between episodes of macroscopic hematuria.

Macroscopic hematuria due to IgA nephropathy occurs far more frequently in children and young adults than in middle-aged or elderly patients, in whom such a symptom should raise the suspicion of urinary tract malignancy or stones. Almost universally the episodes stop by the age of 40 or younger, even though mucosal infections still occur and the underlying disease process continues. The reason for resolution of these episodes with age is unclear.

Asymptomatic Hematuria and Proteinuria

At least another third of diagnoses of IgA nephropathy are made following investigation of incidentally discovered microscopic hematuria, usually accompanied by proteinuria. This can occur at any age, and is typical in the older patient. Again, local attitudes to screening and investigating asymptomatic urinary abnormalities dictate the frequency of such presentations. A common source of such referrals is medical examinations performed for work or life insurance purposes.

Proteinuria and Nephrotic Syndrome

Proteinuria in the absence of hematuria is distinctly uncommon in IgA nephropathy. Nephrotic-range proteinuria is also uncommon, but can occur in the presence of either very active acute disease or advanced disease with considerable scarring. At times, both IgA nephropathy and minimal change disease occur together, which may simply be chance association of two relatively common disorders. It is important to recognize this possibility, as the nephrotic syndrome should be treated as for minimal change disease in isolation, with a similar expectation of response (13).

Hypertension and Malignant Hypertension

An important (and in our experience, increasing) proportion of patients with IgA nephropathy are detected during investigation of newly diagnosed hypertension, and it is one of the major causes of secondary hypertension in young adults. The more widespread use of blood pressure screening programs than urine screening programs may account for this increase. The more dramatic presentation of malignant hypertension is also well recognized, and the renal biopsy findings will often include evidence of severe and long-standing glomerular disease.

Acute Renal Failure

Acute renal failure is an uncommon feature of *de novo* IgA nephropathy. As described above, it may occur during episodes of macroscopic hematuria, possibly as a result of tubular obstruction by red blood cells. Such episodes resolve without specific therapy apart from occasional resort to temporary dialysis (14). Rapidly progressive renal failure can also occur as a result of acute necrotizing, crescentic glomerular injury. It is important to recognize this severe manifestation of the disease, as it is perhaps the strongest indication for aggressive therapeutic intervention.

Chronic Renal Failure

The precise proportion of patients with IgA nephropathy who have chronic, established renal failure at presentation is clouded by the fact that many who come to our attention late in the course of renal disease do not have a renal biopsy performed. Undoubtedly many of those with end or near-end-stage renal failure with small kidneys had unrecognized IgA nephropathy for years or decades. The lack of a reliable peripheral diagnostic marker of IgA nephropathy is an obvious deficit. We have on several occasions made a presumptive, retrospective diagnosis in renal transplant recipients with a failing graft where a transplant biopsy showed recurrent IgA nephropathy.

Disease Associations

The literature is replete with descriptions of associations of diseases with IgA nephropathy, although it is likely that many of these are chance associations. Some of the important and more established associations are shown in Table 61-2.

Deposition of IgA in the mesangium is relatively common and long-recognised in chronic liver disease, particularly alcoholic cirrhosis (15–17), although more overt renal disease is less common. Impaired clearance of IgA by the damaged liver cells is thought to account for this observation. In contrast, despite high levels of circulating IgA in IgA paraproteinemias, there have only been very occasional reported associations with IgA nephropathy (18,19). Likewise, there is a polyclonal increase in IgA levels in human immunodeficiency virus/acquired immune deficiency syndrome (AIDS), but no clear increase in the incidence of IgA nephropathy in association (20). These observations support the current view of the pathogenesis of IgA nephropathy, that it is alterations in the structure of the IgA molecule rather than the serum levels that lead to disease (see below).

Clinical Course

The clinical course of IgA nephropathy may be very clear at the time of diagnosis, in that those presenting late in the course of the disease may already have established renal impairment

TABLE 61-2

DISEASE ASSOCIATIONS WITH IgA NEPHROPATHY

Henoch-Schönlein purpura
Liver disease (alcoholic)
Inflammatory bowel disease
Celiac disease
Dermatitis herpetiformis

or even end-stage renal failure. Others usually follow one of several patterns of disease behavior, although precise estimates of the frequency of each pattern is not possible due to wide variations in screening and diagnosis approaches (discussed above). The patterns are: resolution of mild disease, ongoing mild disease without progressive renal failure, slowly progressive renal failure, and rapidly progressive renal failure. Hypertension is a frequent association, and is invariable and often severe in those with progressive disease. It is reasonable to assume that there is a large body of individuals with benign, asymptomatic disease with good prognosis who are never recognised. Pooling of data from a number of clinical studies has given useful information about the natural history of the disease and predicting its prognosis (8–11).

An important minority, perhaps 15% of those diagnosed, will have mild disease at presentation (i.e., normal renal function, minimal proteinuria and hematuria) and with time undergo spontaneous resolution of all signs of the problem. Associated disappearance of mesangial IgA is suggested by a study of repeated biopsies (21). Approximately 50% of patients diagnosed with IgA nephropathy have persistent but benign disease. Typical is a pattern of ongoing low-grade microscopic hematuria, low levels of or absent proteinuria, normal renal function, and normotension or mild, easily controlled hypertension. Such individuals should be monitored regularly, but longish intervals are reasonable as significant changes in their disease status will be measured in years or even decades. Both of these groups of patients do not require specific treatment for the IgA nephropathy—the challenge is predicting at the time of diagnosis those who will have benign disease.

Most of the remaining patients will have slowly progressive renal impairment, over years to decades, eventually leading to end-stage renal failure if lifespan permits. A more malignant course, measured in months, to severe renal failure is uncommon but well recognized. Frank crescentic disease on diagnostic renal biopsy predicts a rapidly progressive course. Specific and nonspecific (e.g., control of hypertension) treatment needs to target individuals with or at high risk of progressive disease.

Clinical and histologic features can be used to generate meaningful prognostic information (8–11,22,23). Hypertension, persistent proteinuria of >1g per 24 hours, and impaired renal function at time of diagnosis, are consistent and strong clinical predictors of poor renal survival. Histologicly, glomerular sclerosis, tubular atrophy, interstitial fibrosis, and hypertensive vascular changes all correlate with poor outcome. Episodic macroscopic hematuria, initially thought to be a poor prognostic sign (24), is now considered more likely to indicate a benign prognosis. The lead-time bias following early and more likely diagnosis because of the dramatic clinical presentation probably accounts for this skewing to milder disease. Individuals with persistent normal blood pressure and proteinuria of <0.2 g per 24 hours have a negligible risk of progression (10). However, presentation with isolated microscopic hematuria may not be a reliable sign of good long term outcome. A study in Hong Kong patients found that 44% of 72 patients presenting with microscopic hematuria and minimal proteinuria (<0.4g per 24 hours) developed proteinuria >1gm per 24 hours, hypertension, or renal impairment over 7 years (25). In 14% the hematuria resolved.

Diagnosis and Laboratory Features

While clinical suspicion based on presenting features (e.g., synpharyngitic hematuria) will often lead to the correct diagnosis of IgA nephropathy, a renal biopsy is an absolute requirement for confirmation. No other investigation has been proven to reliably distinguish IgA nephropathy from other renal diseases. This is frustrating because it means that a renal biopsy still is required for diagnosis—a procedure often judged unnecessary in a person with just microscopic hematuria. In the latter situation, glomerular-type hematuria usually is due either to mild IgA nephropathy or to thin basement membrane nephropathy.

Most accounts describe an elevation of serum IgA concentrations in up to 50% of cases, which has no diagnostic power (14). Neither, despite the original claims (26), has the detection of elevated plasma IgA–fibronectin complexes (27). An elevated serum IgA/C3 ratio in combination with elevated serum IgA level improves the diagnostic accuracy, but cannot supplant the histopathology (28).

Pathology

The histopathologic diagnosis of IgA nephropathy is usually clear and straightforward, with little opportunity to consider a differential diagnosis. The key is the immunohistochemical identification of IgA deposition in the glomerular mesangium. It is an excellent example of the strength of clinicopathologic correlation and of the power of the combination of light microscopy, immunohistochemistry, and electron microscopy. Histologic classifications have been proposed but are not widely used, and, apart from in advanced disease with significant scarring, have limited prognostic value (29–31). The histologic features are illustrated in Figure 61-1.

Light Microscopy

The light microscopic hallmark of IgA nephropathy is focal or diffuse expansion of mesangial regions with cells and matrix. In their mildest forms, the mesangial changes may be quite focal as well as segmental. Appreciable numbers of glomerular tufts may appear to be normal and, even in those affected, the mesangial cells and matrix changes may be quite marginal. Classically, however, the disease is diffuse, although the tuft changes may be segmental or global in distribution. As a result of mesangial proliferation, the tuft stalk expands and becomes accentuated, often markedly so, an appearance described as *arborization*.

Capillary loops usually are patent, with a normal configuration of capillary walls. However, in more florid disease, mesangial proliferative activity results in the matrix extending peripherally and circumferentially in the capillary walls, resulting in double-contouring or "tram-line" effect, usually with lumen narrowing.

In active disease, there may be tuft necrosis associated with an exudate of fibrin and infiltration of neutrophils, some of which may show karyorrhexis. This occurrence is associated with crescent formation.

In long-standing disease, areas of segmental tuft collapse and sclerosis, sometimes with overlying hyalinosis, are seen, which usually are associated with broad synechiae. In progressive disease, the end result is total glomerular obsolescence and sclerosis. The presence in one biopsy of all the lesions described is not infrequent.

Proportional to the degree of glomerular damage, there may be tubulointerstitial disease. When active glomerular disease is present, there often is interstitial edema associated with mild to moderate infiltrate of mononuclear cells and scattered neutrophils. Secondary tubular damage also may be evident. Interstitial scarring and tubular atrophy are features of advanced disease and correlate best with long-term outcome. Hypertensive changes complicate advanced cases, and related vascular lesions become evident.

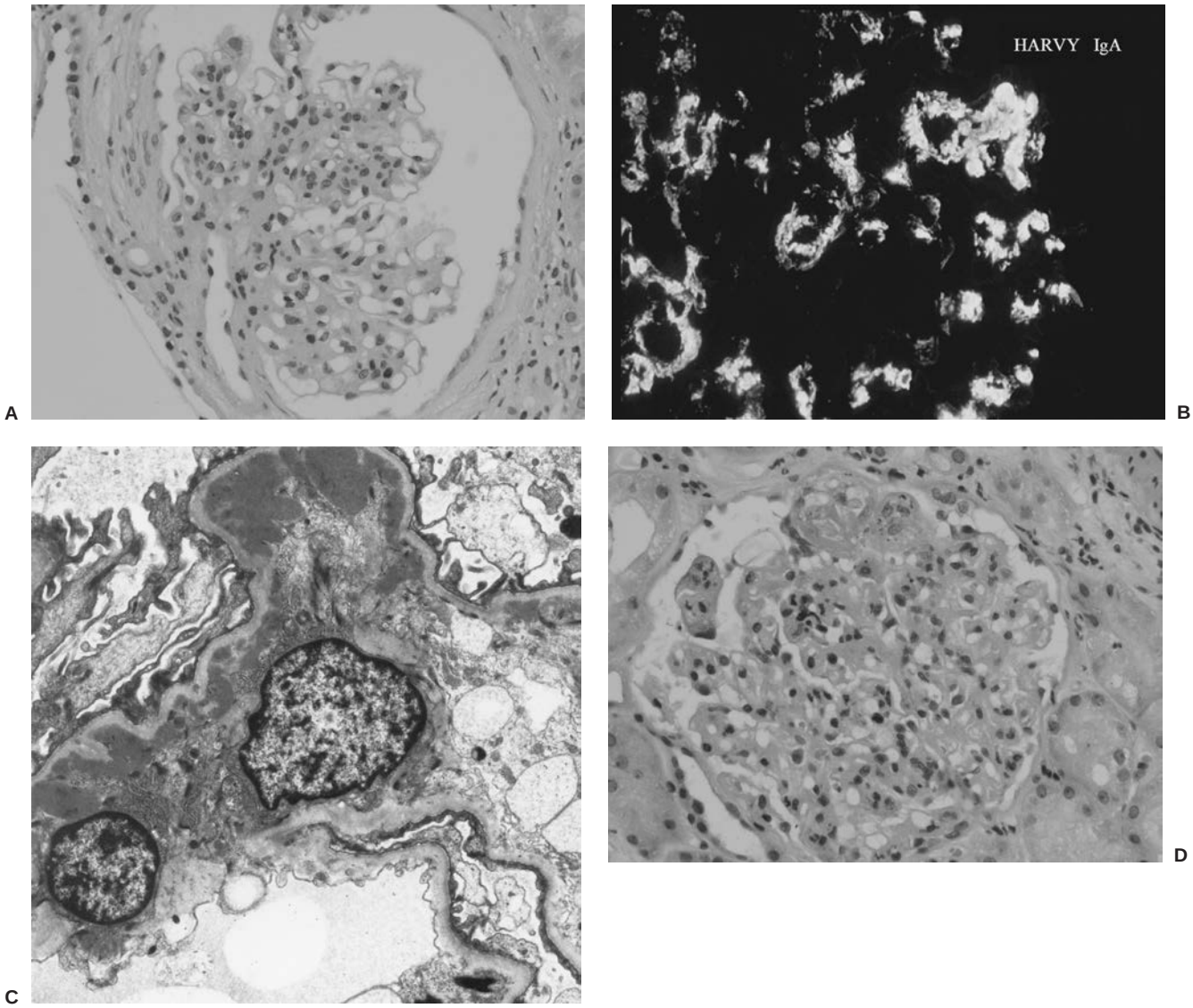


FIGURE 61-1. A: Glomerulus showing mesangial hypercellularity and increased mesangial matrix. (Hematoxylin and eosin stain, magnification, ×200.) **B:** Glomerulus, with brightly fluorescing mesangial deposits of IgA. (Fluorescinated anti-human IgA, magnification 200x). **C:** Electron micrograph of glomerular mesangium showing multiple mesangial electron dense deposits typical of IgA nephropathy. (Magnification ×3,000.) **D:** Glomerulus showing an acute lesion with segmental fibrinoid change and karryorhectic debris. (Hematoxylin and eosin, magnification ×200.) (*continued*)

Immunohistochemistry

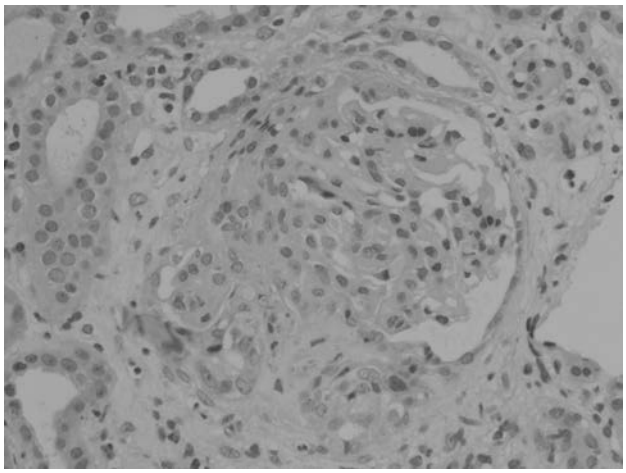
Mesangial expansion is not specific to IgA nephropathy, and can be seen in other glomerular diseases including diabetic nephropathy and focal segmental glomerulosclerosis. A definitive diagnosis can be made only with the aid of immunofluorescent or immunoperoxidase techniques applied to renal tissue. Granular deposition of IgA and usually C3 is present predominantly in the mesangium, even in apparently normal or minimally affected glomeruli. In addition, there may be variable deposits of immunoglobulin G (IgG), immunoglobulin M (IgM), or both, frequently fluorescing with lesser intensity. C1q and C4 are found rarely.

Capillary loop fluorescence for IgA is observed most commonly when active disease is present. Such cases also may show mesangial and capillary wall fibrinogen; the latter also is present in crescents. IgM also is present in areas of sclerosis. Walls of small and medium-sized blood vessels may contain abundant granular C3, particularly if the patient is hypertensive.

Electron Microscopy

There is a varying degree of expansion and proliferation of both mesangial cells and matrix, and electron-dense deposits of differing sizes and amounts are present in the matrix. Not infrequently, the deposits are particularly evident in perimesangial regions or even localized to this site. Corresponding to the segmental nature of the disease process, the distribution and amount of deposits may be quite patchy. Some mesangial sites may be distinctly free of deposits, yet others in the same glomerulus may be packed with them. The deposits usually are solid and homogeneous.

Nephrotic syndrome with the clinical and pathologic features of minimal-change disease also has been noted in patients with IgA nephropathy (13). Occasionally, glomerular basement



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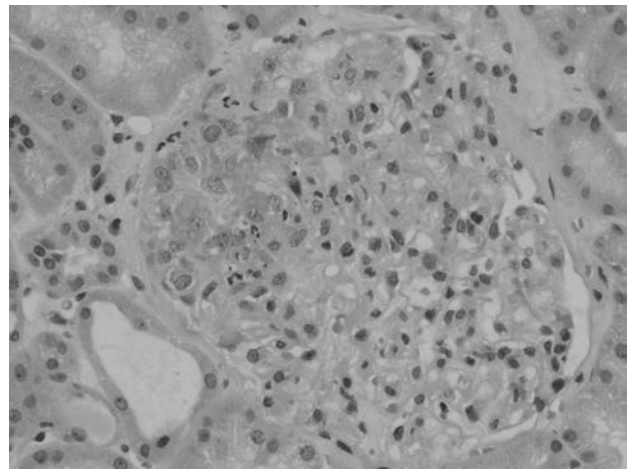

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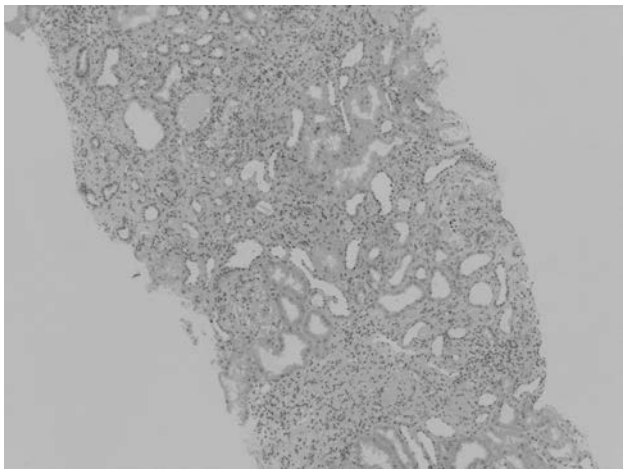

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FIGURE 61-1. (*Continued*) **E:** Glomerulus showing an acute lesion with segmental crescent. (Hematoxylin and eosin, magnification ×200.) **F:** Sclerosing glomerulus with mesangial proliferation. (Hematoxylin and eosin, magnification ×200.) **G:** Low-power view of advanced IgA nephropathy, showing tubular dropout, interstitial fibrosis and chronic inflammatory interstitial infiltrate. (Hematoxylin and eosin, magnification ×40.) All photographs courtesy of Dr. James Nolan.

membranes are uniformly thin and this observation probably signifies the presence together of two common conditions, IgA nephropathy and thin basement membrane nephropathy. However, one study of IgA nephropathy biopsies (32) describes 40% with thin glomerular basement membranes, so perhaps mesangial IgA deposits interfere in some way with normal glomerular basement membrane synthesis. This association, however, does not have any obvious prognostic implications.

Pathogenesis

The cause (or causes) of IgA nephropathy has been frustratingly elusive. Its frequent recurrence following renal transplantation (33–36), and documented resolution of IgA mesangial deposits in renal allografts (37,38), point to problems in the host's IgA immune system rather than the kidney itself. While it is accepted that the initiating event in the renal disease is the mesangial deposition of IgA, there is considerable variability in the subsequent events. These range from little or no renal disease, through continued deposition of IgA but minimal glomerular inflammation without renal impairment, to continued deposition and progressive renal failure. With the possible exception of capillary loop IgA deposition, which may indicate a poor prognosis, the extent and site of deposition bears no relationship with prognosis. This heterogeneity suggests that IgA nephropathy is more than just a simple disease caused by a single, defined abnormality.

Immunobiology of Immunoglobulin A

A working knowledge of the immunobiology of IgA is important to understand the pathogenesis of IgA nephropathy (39–41). IgA is the major immunoglobulin in mucosal secretions but is present in relatively low concentrations in serum. Its predominant function is in mucosal defense, where polymeric IgA binds and neutralizes microbes and toxins.

Two subclasses exist in humans, IgA1 and IgA2, both of which occur in monomeric (mIgA) and polymeric (pIgA) forms. IgA1 appears very late in the evolutionary chain, being present only in chimpanzees, orangutans, and humans. Plasma cells in the gastrointestinal tract and respiratory tract produce both IgA1 and IgA2 that is found in mucosal secretions at these sites. In the bone marrow, lymph nodes, and spleen, the plasma cells predominantly produce IgA1 that is expressed in the serum. The IgA that is found in the serum is 90% monomeric, whereas that found in mucosal secretions is virtually all polymeric. IgA1 accounts for 90% of serum IgA, whereas IgA2 comprises 60% of IgA in secretions (Table 61-3). Polymeric IgA (which is usually a dimer) contains a bridging or joining polypeptide—the J chain—that is linked to the heavy chain during the formation of the multimers within the plasma cell. IgM also exists as polymers linked by J chains.

IgA1 possesses an 18 amino acid hinge region situated between the CHI and CH2 domains of the heavy chain. This is absent in IgA2, and is the main structural difference between the two subclasses. The hinge region consists of an unusual repeating sequence of proline, serine, and threonine residues, and carries multiple O-linked carbohydrate side chains connected

TABLE 61-3

PROPERTIES OF NORMAL HUMAN IgA

	Serum	Mucosal
Concentration	Low	High
Size	Mostly monomeric	Mostly polymeric
Subclass	Mostly IgA1 (90%)	IgA1 (40%) and IgA2 (60%)
Source	Bone marrow	Mucosal plasma cells

to the serines and threonines. The grouping of O-linked sugars in the IgA1 molecule increases its size significantly and confers to it a highly negative charge because of sialylation of the sugars. Such O-linked sugars are uncommon on circulating proteins. The glycosylation pattern varies, so that an array of different IgA1 O-glycoforms is found in the serum at any time.

Plasma cells that produce IgA are mainly located at mucosal immune sites, and the IgA is rapidly transported across the adjacent epithelial cells into the external secretions. Very little gets into the blood. Secretion of the multimeric IgA across the mucosa depends on the acquisition of secretory component (SC), which is a portion of the polymeric immunoglobulin receptor expressed at the basolateral surface of epithelial cells. Secretory IgA (sIgA) is more stable at these sites than serum IgA, which is readily degraded. The IgA found in the serum is predominantly produced in the bone marrow. Regulation of IgA production is broadly under T lymphocyte control. It is of interest that, in contrast to IgG, interleukin 10 and transforming growth factor-β both stimulate B lymphocyte production of IgA (42).

The liver has an important role in the clearance of IgA and IgA-containing immune complexes. IgA-binding receptors present in the liver are the hepatocyte asialoglycoprotein receptor and the Fcα receptor CD89 on Kupffer cells (43–45). The asialoglycoprotein receptor recognizes the terminal galactose and galactosamine carbohydrate residues of the IgA molecule (46). Macrophages and neutrophils also express CD89 and are able to remove and catabolize IgA. Expression of CD89 by these cells is decreased in patients with IgA nephropathy, which potentially compromises this clearance pathway (47).

Animal Models of IgA Nephropathy

Many animal models of IgA nephropathy have been developed, but they have added little to the understanding of the human disease. In mice, the deposition in mesangia of immunoglobulins, and specifically IgA and IgG, occurs naturally in many breeds, so that results of experimentally induced diseases must be interpreted against this background. More important, perhaps, is the understanding that IgA1 as a protective immunoglobulin appears very late in the evolutionary chain, with only humanoids possessing this subclass of IgA. In other animals the IgA is structurally more like human IgA2 and does not have the hinge region characteristic of IgA1. It is likely, therefore, that if abnormally glycosylated IgA1 plays a significant role in the pathogenesis of the human diseases (see below), then most animal models do not represent what occurs in humans.

Production of IgA in IgA Nephropathy

Much circumstantial evidence points to the probability that there exists a defect in mucosal immunity in IgA nephropathy. The most easily recognizable and dramatic symptom of macroscopic hematuria coincides with infections of mucosal surfaces. It is likely that the site of the immune response is important, not the antigen. Increased IgA antibodies against a variety of antigens have been described, but none is ubiquitous, and the finding of such antigens in the glomerular deposits has not been established. IgA nephropathy also occurs in association with diseases with known mucosal defects such as celiac disease (48).

Approximately one-third to one-half of patients with IgA nephropathy have elevated serum levels of IgA, and this elevation is restricted to IgA1 (49,50). In patients with IgA nephropathy, there is increased production of IgA in response to viral and bacterial vaccines and tetanus toxoid, compared with control subjects without IgA nephropathy (50–52). Overproduction (overresponse) of these and recall antigens is restricted to the IgA1 subclass.

Elevated serum IgA by itself is insufficient to cause the disease (18–20). A key appears to be the increased levels of specifically polymeric IgA (51), including pIgA against mucosal antigens (52,53). This macromolecular IgA, which is normally predominantly found in mucosal secretions, is likely to be more prone to trapping within the glomerular mesangium.

It is not, however, a simple case of elevated mucosal production of polymeric IgA1 which then spills over into the systemic circulation. Both mucosal IgA plasma cells and polymeric IgA levels are normal or even decreased, whereas bone marrow IgA plasma cells numbers are increased in IgA nephropathy (54–57). This points to a defect leading to relative overproduction of polymeric IgA1 in systemic sites such as the bone marrow.

Clearance of IgA in IgA Nephropathy

The liver clearance of IgA is reduced in IgA nephropathy, although there is no clear evidence for a specific liver cell defect (58,59). It is also known that liver disease, where IgA clearance is impaired, is associated with mesangial IgA deposition, but not necessarily clinical disease (15–17). The overproduction of pathogenic polymeric IgA1 may either overwhelm the normal clearance mechanisms, or be of a form that resists the normal clearance pathways.

Mesangial Deposition of Abnormally Glycosylated IgA

The mesangial IgA found in IgA nephropathy is not necessarily fixed. It is known that it clears when disease remission occurs or when such kidneys are inadvertently transplanted into individuals without IgA nephropathy.

A consistent finding, though, is that it is IgA1 that is deposited in the mesangium in clinical disease (60–62). Several studies have found that the mesangial IgA1 in IgA nephropathy is abnormally glycosylated, and that the same abnormal IgA is increased in the serum in patients with disease (63–67). The difference between the IgA1 molecule in healthy people and patients with IgA nephropathy resides in the O-glycosylation of residues in the hinge region. A normal variant exists with terminal N-acetylgalactosamine or sialylated N-acetylgalactosamine and reduced galactosylation and sialylation, but this is rare in normal serum. It is uniquely more common in IgA nephropathy patients (68,69). The altered O-galactosylation in IgA nephropathy may result from a B-cell–restricted downregulation of $\beta 1$-3 galactosyltransferase activity (70), which limits the addition of D-galactose to GalNAc, rather than any aberration of the nucleotide sequence of the IgA_1 hinge region, which is similar in structure and sequence to the hinge region found in healthy people (71,72).

These variant IgA1 molecules are more likely to self-aggregate, and form antigen-antibody complexes with IgG antibodies directed against IgA1 hinge epitopes (68,73). IgA1 from patients with IgA nephropathy persists longer in the circulation than that from normal controls when injected into mice

(74). Liver clearance is reduced, perhaps because the polyclonal IgA1 is unable to pass through endothelial fenestrae in the liver to reach the hepatocytes (75). The net effect of this structural alteration is to increase serum levels of polyclonal IgA1 that is more prone to mesangial deposition and/or is more resistant to clearance from the mesangium.

It remains unclear whether there is a specific receptor on mesangial cells for IgA1. Indeed, the possibility exists that there is no necessity for a specific IgA receptor on mesangial cells because the altered molecule may bind nonspecifically to mesangial structures. However, it appears most likely that mesangial cells express at least one receptor that differs from the known IgA receptors (CD89, asialoglycoprotein receptor, polymeric immunoglobulin receptor (76)). Candidates include the transferring receptor (CD71), an asialoglycoprotein receptor, an Fc α/μ receptor, and a novel Fcα receptor (77–81). Irrespective of the presence or otherwise of a specific receptor, mesangial cells are capable of endocytosis and catabolism of IgA and are therefore likely to contribute actively to its clearance (79, 82).

Events After Mesangial Deposition of IgA

There is no doubt that the IgA1 deposited in the mesangium incites an inflammatory reaction, at least in those patients with clinical disease, and the classic mediators of inflammation such as complement, cytokines, leucocytes, platelets, and coagulation/fibrinolysis are involved. The mechanisms of crescentic disease are similar to those in other crescentic glomerular diseases. Acute lesions, although visually impressive, often are self-limiting. Much more important in the long term is the development of increased mesangial matrix, glomerulosclerosis, interstitial scarring, and hypertensive vascular disease.

Polymeric, but not monomeric, IgA molecules initiate an inflammatory glomerulonephritis in a rat model of acute nephritis (83,84). Interactions with IgA complexes and mesangial cells induces a proinflammatory and prosclerotic response, including mesangial cell proliferation and production of cytokines (e.g., interleukin-6, platelet-derived growth factor [PDGF], and transforming growth factor [TGF]-β) and matrix proteins (85–88). The altered glycosylation structure of the variant IgA1 molecules deposited in the mesangium may also specifically predispose to the pathogenic mesangial responses that follow (89).

The mechanisms of the subsequent inflammation and scarring in the glomerulus and interstitium in IgA nephropathy are probably not significantly different from other inflammatory glomerular diseases. Resolution or progression of the disease process may depend on the presence or otherwise of critical glomerular podocyte damage (90,91).

Genetics of IgA Nephropathy

The genetic makeup of the individual may dictate the initiation, progression, or resolution of IgA nephropathy. In support of a genetic predisposition are the apparent racial differences in prevalence of IgA nephropathy, and instances of occurrence in first-degree relatives (with many others having similar immune abnormalities *in vitro*) (92–94). On the other hand, more than 90% of cases of IgA nephropathy occur sporadically. Results of genetic studies, however, in general have been disappointing and associations reported are inconsistent. Associations have been described with DR4 (95,96) and, also on chromosome 6, with complement genes (C4A and C4B null phenotypes (93), and with the immunoglobulin "switch region" on chromosome 14 (97). A disease susceptibility gene has been localized to a region on chromosome 6 (98). Polymorphisms of genes encoding cytokines (TNF-α, interferon-γ, interleukin-4) have also been associated with development or progression of disease (99–101).

Progression of disease has also been associated with a deletion polymorphism of the angiotensin-converting enzyme (ACE) that increases serum and tissue ACE levels (94). However, further studies have not supported the proposal that this polymorphism in isolation is a significant factor in disease progression (102,103).

Summary of Pathogenesis

Current opinion about the pathogenesis of IgA nephropathy centers around the presence of excess serum levels of abnormally glycosylated immunoglobulin A molecules. These aggregate into polymeric IgA, which is less efficiently cleared by the liver and is prone to trapping in the glomerular mesangium. Evidence points to the bone marrow as the source of the abnormal IgA molecules rather than the mucosa. Ill-defined genetic influences may predispose individuals to development of this disease.

Treatment

There is no known cure for IgA nephropathy despite studies of many possible treatments. Current options mainly aim to control disease progression, and in general these do not differ significantly from recommended strategies for other progressive renal diseases. Establishing efficacy of a specific treatment for a disease such as IgA nephropathy is difficult where the majority of sufferers have a benign course, predicting those with unfavorable outcome is often difficult, and the time course of progression may be measured in decades. For the same reasons, it is difficult to establish a satisfactory risk versus benefit profile for proposed interventions such as immunosuppressive therapy. Relevant to this is the observation that IgA nephropathy frequently recurs in renal transplants despite the use of corticosteroids, azathioprine, mycophenolate mofetil, cyclosporine, and tacrolimus. For these reasons, treatments frequently have been used only in those with a known poor prognosis, that is, those patients who already have consistent proteinuria, hypertension, and impairment of renal function. A disappointingly small number of randomized controlled trials have been conducted of treatment of this common and important condition (104).

"Good prognosis" disease (i.e., minimal or no proteinuria, normal blood pressure, normal renal function, little or no scarring on renal biopsy) requires no more than regular observation. Hypertension commonly develops with time and should be managed aggressively (aim for <135/85 mm Hg).

The dramatic presenting symptom of synpharyngitic hematuria has prompted popularization of tonsillectomy as a treatment option, particularly in Japan and some parts of Europe. Results from studies have been conflicting (105–107), and prospective randomized studies are needed before it can be recommended. Supportive therapy, including analgesia as required, is all that is required for acute episodes of macroscopic hematuria, as well as any clinically indicated search for an alternative cause (e.g., urinary tract infection, renal calculi, malignancy).

An early controlled trial of phenytoin, which reduces serum IgA levels, failed to show any benefit (108). There is also no benefit in other methods to reduce antigen or antibody load, such as dietary restrictions or prophylactic antibiotics.

An array of studies, of varying quality, have examined combinations of immunosuppressive agents for treatment of IgA nephropathy. Results to this point have not been sufficiently clearcut to routinely recommend such an approach, except perhaps in the relatively uncommon cases of crescentic, rapidly progressive disease or in cases with nephrotic syndrome due to concurrent minimal change disease. In the latter the proteinuria typically responds to standard treatment for isolated minimal change disease.

In a randomized trial, corticosteroids alone (intravenous methylprednisolone 1g per day for 3 consecutive days at beginning of months 1, 3, and 5, plus oral prednisolone 0.5 mg per/kg alternate days for 6 months) in IgA nephropathy patients with significant but subnephrotic proteinuria (1.0 to 3.5 g per day) reduced proteinuria by approximately 50% and reduced the risk of a 50% increase in serum creatinine by 50% at 5 years (109). Three other small trials of corticosteroids alone showed a reduction in proteinuria and some improvement in histologic parameters, but without effect on renal function (110–112). A small trial of 2 years of corticosteroids plus azathioprine in children with early disease modestly reduced proteinuria but had no effect on renal function (113). A more recent randomized, prospective trial of prednisolone and cytotoxic agents (oral cyclophosphamide and then azathioprine) in patients with progressive IgA nephropathy and controlled hypertension showed a significant preservation of renal function compared to controls out to 5 years. Proteinuria was also reduced (114). Corticosteroids +/− cytotoxic agents may be cautiously recommended in poor prognosis disease (heavy proteinuria, early but progressive renal impairment), taking into account the risk of important side effects.

Other immunosuppressive drugs such as cyclosporine (115) and mycophenolate (116) also have been used, but the high incidence of recurrence in renal transplants casts doubt on their efficacy.

The risk/benefit equation shifts toward aggressive treatment in the setting of rapidly progressive, crescentic IgA nephropathy, where there is likely to be a rapid decline to end-stage renal failure without intervention. The treatment that is usually used (plasmapheresis, prednisolone, cyclophosphamide) is similar to that for other forms of rapidly progressive, crescentic renal disease. There is a lack of good quality, randomized trials. Small studies show an early response to treatment but a disappointing longer term outlook (117,118).

An alternative approach adopted with some enthusiasm in parts of the world has been the use of fish oil/omega-3 fatty acids for treatment, particularly of progressive IgA nephropathy (119). In the largest controlled study, it slowed progression into renal failure in poor prognosis treated patients compared with controls (120), and this effect has been long-lasting (121). A later study showed no benefit of high doses of fish oil over low doses (122). This study has been criticized because of the relatively rapid progression of disease in the control group, and other studies have not shown such benefit (104,123,124,125). There remains insufficient evidence to routinely recommend this approach.

There do not appear to be substantive differences in the mechanisms of progressive renal impairment in established IgA nephropathy compared to other glomerular diseases, and so it is not at all surprising to find that similar approaches to delaying disease progression have been studied and used extensively in this condition. It has long been established that control of hypertension is crucial to delaying progression of renal impairment in glomerular diseases. More recently, seminal studies, first in diabetic nephropathy and then in other renal conditions, have shown important and specific advantages of blood pressure control with angiotensin-converting enzyme (ACE) inhibitors (126). Comparable advantages exist with angiotensin II receptor (AIIR) blockers, and the most recent evidence suggests that there is a synergistic advantage in using both. Experimental evidence indicates that such agents reduce glomerular capillary hypertension, decrease proteinuria, and protect against hyperfiltration injury. An established aim of strategies to delay progression is now the targeting of proteinuria in addition to hypertension, and trial evidence supports the notion that incremental reductions in proteinuria are associated with improvements in renal prognosis.

Few studies to date have specifically examined the role of ACE inhibitors or AIIR antagonists in progressive IgA nephropathy, and much of the evidence favoring their use in this condition is based on studies of other proteinuric glomerular diseases (e.g., diabetic nephropathy) (127). Some studies have shown a reduction in proteinuria with ACE inhibition without improvement in renal function (128,129), although 2 more recent studies found that it delayed progression as well (130,131). AIIR blockers cause comparable proteinuria reduction in IgA nephropathy (132). The combination of both ACE inhibitor and AIIR blocker synergistically reduces proteinuria in IgA nephropathy, and there is now evidence that this confers additional benefit in delaying progression of renal impairment (133–135). Despite the relative paucity of specific data, blockade of the renin-angiotensin system is recommended where there is significant proteinuria (>1 g per 24 hours).

Transplantation for IgA Nephropathy

Although kidney transplantation should be offered to all patients with IgA nephropathy provided there is no other contraindication, they should be made aware of the possibility of disease recurrence, the risk of which is approximately 45% (33,34). Those who already have lost a graft because of recurrent disease may be at high risk for repeated graft loss, but experience is too limited for this to be an absolute contraindication to repeat transplantation (35,36). Despite the risk of recurrence, the prognosis of transplants for this group is comparable with that of patients with other causes of end-stage renal failure.

Recurrent disease has been considered to be mild and usually of little clinical significance (136,137). However, other studies challenge this concept (33,35). In one of these, 17 of 29 grafts had recurrent IgA deposits and 5 of those 17 affected had clinical and histologic evidence of recurrent IgA disease, with failing grafts (33). In our series of 81 grafts in 76 patients, the mean time from transplantation to the onset of clinical disease (microhematuria) was 4.8 years (36). In those with more severe recurrent disease causing graft loss, the mean time from transplantation to graft loss was 4.6 years. It is suggested that graft loss due to recurrent IgA nephropathy is underreported, and unless immunofluorescence studies are performed on transplant biopsies and nephrectomies, losses will be attributed to chronic rejection or transplant glomerulopathy rather than recurrent disease.

HENOCH-SCHÖNLEIN PURPURA

The almost identical renal immunopathologic features of Henoch-Schönlein purpura (HSP) and IgA nephropathy questions the separate identity of these conditions. Historically, HSP is a much older disease than IgA nephropathy, as it is a clinical syndrome readily recognized because of the overt purpuric rash, arthritis, gut manifestations, and glomerulonephritis. Whereas Schönlein (138) in 1837 associated the purpura and arthritis, and Henoch in 1874 (139) recognized the gastrointestinal and renal manifestations, the first clinical description of the disease was probably by Heberden in 1806 (140). Until the 1970s, most accounts of HSP were descriptive but, once again, the advent of immunofluorescence technology allowed exploration of possible immunopathogenetic mechanisms. As with IgA nephropathy, positive IgA immunofluorescence occurs in the glomerular mesangium, skin (especially in purpuric lesions) (141), and other organs.

Although the clinical features, incidence, and age of onset tend to vary, the many similarities of these two conditions suggest that they may represent a spectrum of the same systemic

disease (142,143). HSP is most prevalent in the first decade of life, and much more common in children than in adults. In children it is frequently a transient disease without long-term consequences. The prevalence of glomerular disease in patients with HSP, especially in children, varies from 20% to 100% (144), depending on how carefully evidence for it is sought. When glomerular disease occurs in HSP, frank nephritic or nephrotic syndrome, or acute renal failure due to crescentic disease, are more common than in IgA nephropathy. The natural history of HSP is less well defined than IgA nephropathy, and is confounded by the fact that many patients have elements of the syndrome without clinical glomerulonephritis. Complete recovery occurs in 94% and 89% of children and adults, respectively, and the renal disease may be more likely to be severe and progressive in adults than in children (145). Progression to end-stage renal failure is well described, and like IgA nephropathy, can occur relatively rapidly or over decades. The nonrenal features of the syndrome are transient although recurrent in approximately one-third of patients. The renal disease resolves in most but persists in an important minority.

The characteristic purpuric rash, joint, and gastrointestinal involvement usually make the syndrome of HSP clearly identifiable in children and adults. It commonly occurs soon after an upper respiratory tract infection. In one series, the relative frequency of the major symptoms in children was purpura (100%); arthritis (82%); abdominal pain (63%); renal disease (40%); and gastrointestinal bleeding (33%) (146). The rash is distributed symmetrically over the arms and legs. Pain in multiple large joints (typically knees and ankles) without frank arthritis or permanent damage is typical, and the abdominal pain may be severe, disabling, and accompanied by visible rectal bleeding. Although these symptoms may cause much acute morbidity, recovery is usual. Renal involvement usually is apparent within 4 weeks of diagnosis, although it can develop much later. As with IgA nephropathy, intermittent macroscopic hematuria or persisting microscopic hematuria are the most common findings. Likewise, more severe cases have significant proteinuria, nephrotic syndrome, hypertension, and renal impairment, and synpharyngitic hematuria is typical.

In patients with HSP, most clinical features are a reflection of a systemic leukocytoclastic vasculitis, characteristically affecting the skin, gastrointestinal tract, and joints. It is a disease of the small blood vessels, particularly the postcapillary venules, and is probably the result of immune complex deposition, with activation of complement and consequent leukotaxis. Neutrophils attracted to the site frequently undergo karyorrhexis. There is variable endothelial cell proliferation, mural fibrin, and, in severe cases, fibrinoid necrosis. These changes are more common in the glomerulus in HSP than in IgA nephropathy. In severe cases, there may be cutaneous infarction with ulcer formation. Deposits of C3, frequently associated with IgA and IgM, may be found in blood vessel walls. Electron-dense deposits and fibrin have been demonstrated in early lesions.

Studies of HSP are much more limited than IgA nephropathy, but similar aberrations exist in the control of IgA production. As with IgA nephropathy, the IgA in the mesangial deposits of HSP is of the IgA1 subclass, and there are circulating IgA1-containing immune complexes or immune aggregates (147–149). The circulating IgA1 immune complexes are larger in HSP than IgA nephropathy, and levels of plasma IgE are increased (142). HSP has been associated with hypersensitivity reactions (150). Like IgA nephropathy, abnormally glycosylated IgA is more abundant in individuals with HSP and glomerulonephritis (151).

Most patients with the acute syndrome neither require nor receive specific therapy. Corticosteroids may speed resolution of the arthritis and abdominal pain, but do not appear to prevent recurrent disease (146). Specific treatment of the renal disease should be considered only in patients with marked proteinuria and/or impaired renal function during the acute episode. Aggressive corticosteroid therapy may be beneficial in patients with crescentic nephritis (152). Limited data from uncontrolled studies support a role for plasmapheresis in aggressive acute disease (153). There is no specific controlled data for treatment of non-acute, progressive HSP renal disease, but the similarities to IgA nephropathy suggest that an identical approach is reasonable. Renal transplantation is a valid option should end-stage renal failure result, although recurrence of HSP nephritis in the graft is not uncommon (154). Most recurrences are subclinical, but it leads to graft loss in approximately 10%.

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CHAPTER 62 ■ MEMBRANOPROLIFERATIVE GLOMERULONEPHRITIS

GIOVANNI BARBIANO DI BELGIOJOSO AND FRANCO FERRARIO

The term *membranoproliferative glomerulonephritis* (MPGN), or *mesangiocapillary glomerulonephritis* (GN), refers to the histopathologic entity characterized by (a) intense glomerular hypercellularity, accompanied by increase of mesangial matrix (1–4); (b) thickening of the peripheral capillary walls by subendothelial deposits (type I) or dense intramembranous deposits (type II) (5); and (c) mesangial interposition into the capillary walls with apparent splitting of the glomerular basement membrane (GBM) (1,2,6–9). The designation MPGN is used by most authors throughout the world, but *mesangiocapillary glomerulonephritis* is an equally acceptable term (10) that emphasizes the importance of peripheral capillary wall thickening and interposition of mesangial cellular and fibrillary material. Other descriptive names have been used over the years: hypocomplementemic chronic glomerulonephritis (6,11–13), chronic lobular glomerulonephritis (7,14), and mixed membranous and proliferative glomerulonephritis (15). As already mentioned, on the basis of capillary wall lesions, all authors have subtyped MPGN into types I, and II; some also include a type III. Type II, or dense intramembranous deposit disease, historically included in the MPGN group, is considered by several authors to be a separate disease entity because of its peculiar ultrastructural aspect and pathogenetic mechanisms (16–21).

Membranoproliferative glomerulonephritis designates a morphologic pattern that occurs, in most cases, in the absence of underlying diseases (idiopathic MPGN). It can be observed in association with a variety of systemic, neoplastic and infectious disorders; the secondary forms of MPGN are listed in Table 62-1. Therefore, MPGN is a term that indicates a morphologic pattern that must be integrated into an etiologic context whenever possible, and underlying systemic conditions must be excluded.

Epidemiologic data indicate that MPGN is a rare disease (4% to 5% among the histologically proven primary glomerulonephritides) and that its incidence has decreased in the developed countries of the world since the early 1980s (22–30). In fact, from epidemiologic studies of primary glomerulonephritis in France, Italy, and Spain, among adult and pediatric, rural and urban populations, the incidence of type I MPGN fell from 18% to 20% in early 1970s to approximately 12% in 1980, to 6% to 8% in 1990 and still lower since the mid-1990s. Similar reduction was noted in Japan (31). Frequency of MPGN, as reported by the Italian Registry of renal biopsy was, during the years 1996 to 2000, 6.2% for males and 8.0% for females (32). Jungers et al. found that in the Paris area, the annual incidence of type I MPGN among 1,003 cases of primary glomerulonephritis, for the 1987 to 1991 period, was 5.8% (33). The decrease of annual incidence of MPGN was confirmed by Simon et al. (34) after a 27-year follow-up in a study carried out from January 1976 to December 2002 in a region located in western France.

The relative frequency of type I MPGN as a cause of unexplained adult nephrotic syndrome in a large series of primary glomerulonephritis studied in Chicago (35) declined from the 1976 to 1979 period to the 1995 to 1997 period (6% to 2%). By contrast, the incidence of type II MPGN has remained unchanged. In still developing countries MPGN has not decreased in frequency (36,37). Other etiologic factors in MPGN such as age, sex, mode of presentation, also have not changed during the periods studied (25). The reductions in incidence in industrialized countries have multiple explanations. Improved hygienic and environmental factors in the past few decades have been postulated, with a decline of bacterial infections due to greater use of antibacterials. It has also been suggested that many cases of apparently idiopathic MPGN were due to hepatitis C virus (HCV) infections, with or without cryoglobulinemia that had been misdiagnosed. More careful screening for HCV led to a decline in HCV infection in the general population and subsequently in MPGN (38). Overall, a more precise diagnosis of underlying disease also may have contributed to the decline of "idiopathic" MPGN. The reduction in the frequency of MPGN also may depend partly on the overall increase in the age of biopsy population from the earlier to the later periods of study, because MPGN has a relative higher frequency in younger patients (35). The classical hypothesis that elevated incidence of bacterial infections in developing countries correlates with higher frequency of MPGN as well as other proliferative glomerulopathies has been recently questioned by Johnson et al. (39), following the striking finding of the lack of evidence for such active infections in cases of MPGN in Peru (36). The authors suggest that MPGN might result from an aberrant immune response to an innocuous antigen rather than a strong immune response to a virulent infective agent. Therefore an imbalance of immune system, induced by hygiene and other environmental factors, may underlie the pathogenesis of MPGN as well as other glomerular diseases. According to these authors, the type of immune response, as revealed by Th1/Th2 balance, may be more relevant in determining the variant of glomerulonephritis and in particular MPGN rather than the presence of specific antigen (39,40). This idea will be developed later in this chapter.

Membranoproliferative glomerulonephritis still presents many challenging issues for investigators, and the following items are still debated: (a) the relationships between immune complexes and mesangial interposition or complement abnormalities in the development of the lesion; (b) the precise nature of the dense deposit in type II MPGN and their connection with complement activation; (c) the nosologic definition and pathogenesis of type III MPGN; (d) the role HCV infection in noncryoglobulinemic MPGN and the involvement of HCV antigen in mediating the immune complex glomerular lesion; (e) the nature of spontaneous clinical remissions which occur

TABLE 62-1

MEMBRANOPROLIFERATIVE ASSOCIATED DISORDERS

Systemic immune disorders
Systemic lupus erythematosus
Sjögren's syndrome
Hereditary complement and immunoglobulin deficiencies
Rheumatoid arthritis
Autoimmune tyroiditis
Neoplastic diseases
Light-chain disease and plasma cell dyscrasia
Leukemia and lymphomas
Nephroblastoma
Melanoma
Infectious diseases
Bacterial
Subacute endocarditis
Visceral abscesses and infected shunts
Viral
Hepatitis C virus, with or without cryoglobulinemia
Hepatitis B virus
Human immunodeficiency virus
Cocksackie, EBV
Other
Malaria, schistosomiasis, candida
Chronic liver disease
Chronic active hepatitis
Liver cirrhosis
α1-antitrypsin deficiency
Miscellaneous
Partial lipodystrophy
Sarcoidosis
Sickle cell disease
Portosystemic shunt
Transplant glomerulopathy

in all types of MPGN independent of therapy; and (f) the lack of proven effective therapy.

PATHOLOGY

Membranoproliferative glomerulonephritis is a histologically distinct entity characterized by intense glomerular hypercellularity mainly due to mesangial proliferation involving both cells and matrix and thickening of peripheral capillary wall with "double contour" appearance (2,7,9,21,41–43). Since the early 1980s, several forms or variant of so called MPGN have been described. Two major morphologic categories have been identified referred by the majority of authors as type I and type II (2,8,9,16–18,43–54).

Type I is characterized by the presence of numerous glomerular subendothelial deposits, marked involvement of the mesangium and extension of the mesangium into the glomerular capillary walls. In type II, homogeneous dense deposits in many renal basement membranes (glomerular, tubular, and arteriolar) are typical. At the glomerular level the dense deposits are clearly intramembranous, and very rarely the mesangium extended into the capillary walls with "double contour" appearance. In the past, type II was considered a MPGN because its morphological alterations on light microscopy were extremely similar to those of type I MPGN (5,18,55–57). In spite of many morphologic and clinical similarities, in particular with regards to the presence in both diseases of hypocomplementemia, there are sufficient differences from histologic and immunologic points of view to suggest that type II form is a separate and distinct entity and should not be considered a subtype of MPGN (20,48,58,59). Further variations or types of the membranoproliferative pattern have been noted by a number of investigators. A type with many of the features of MPGN type I, but with the added presence of numerous electron-dense deposits on the subepithelial side of the glomerular capillary basement membranes, was designated "type III" by Burkholder et al. (15). In this pattern, some glomerular capillaries were indistinguishable from those type I MPGN, whereas other segments of the glomerular tuft showed changes similar to those as classic membranous glomerulonephritis. Although this terminology did not find much favor, the issue is now complicated by the use of the term "type III" to designate a form of MPGN described by two other groups of workers (15,60,61). This form is characterized by the simultaneous presence of subendothelial and subepithelial deposits associated with lamination and disruption of the lamina densa of the GBM at electron microscopy (62–65). However, many reports (47–48) and recent personal data from a multicenter study of Italian Group of Renal Immunopathology, based on retrospective clinicohistologic analysis of 368 cases of idiopathic MPGN, suggest that these features are insufficient to warrant a separate entity, because epimembranous deposits are found in 20% of both types of MPGN and their number vary in repeat biopsies (66). Moreover the complement abnormalities, immunohistologic features, and clinical presentation and course of type III MPGN are similar to those in type I, and thus these two forms should be considered variants of the same disease rather than separate entities (64,66). Finally, there is some doubt over whether MPGN in all its subtypes should be considered a homogeneous and distinct histologic entity. The list of various forms of diseases associated with a membranoproliferative pattern is constantly growing. In fact, these patterns (especially type I) can occur as an idiopathic (primary), apparently isolated disease or can be associated with or stem from a wide variety of well-defined disease states, such as bacterial endocarditis, sickle cell disease, hepatitis B or C infection, cryoglobulinemia, and systemic lupus erythematosus (SLE). Although the etiologic and pathogenetic mechanisms might not be identical in all patients, most authors believe that MPGN is likely a result of chronic antigenemia. Moreover in a primary, apparently isolated (idiopathic) MPGN, several morphologic variants have been described because of heterogeneous intensity and distribution of the main basic histologic lesions; these possibly are the expression of different morphogenetic/pathogenetic mechanisms (66–69).

Type I Membranoproliferative Glomerulonephritis (Subendothelial Deposits)

Light Microscopy

By light microscopy, the most common form is the "classic" pattern characterized by widespread and massive mesangial proliferation and mesangial matrix expansion (1,2,4,7–9,21,41,44–50,70–72) (Fig. 62-1). The hypercellularity is usually global involving all portions of each glomerular tuft to approximately the same degree. Mesangial expansion is due to cellular proliferation, matrix growth, and to the presence of immunodeposits. Sometimes the increase in cells in the mesangial regions and the amount of mesangial matrix creates a much larger mesangial or centrilobular area, with the lobules assuming a club shape (lobular pattern) (Fig. 62-2).

There is marked diffuse thickening of the glomerular capillary walls; the thickening can be more prominent in some

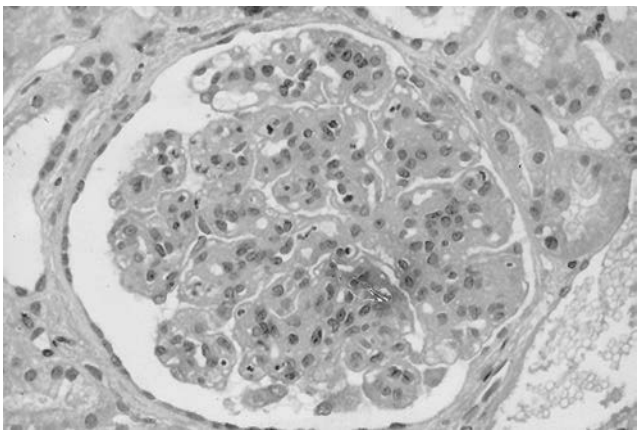

FIGURE 62-1. Type I membranoproliferative glomerulonephritis, classical pattern: widespread and massive mesangial proliferation, mesangial matrix expansion, and diffuse glomerular basement membrane thickening with "double-contour" appearance. (Trichrome, magnification, ×250.)

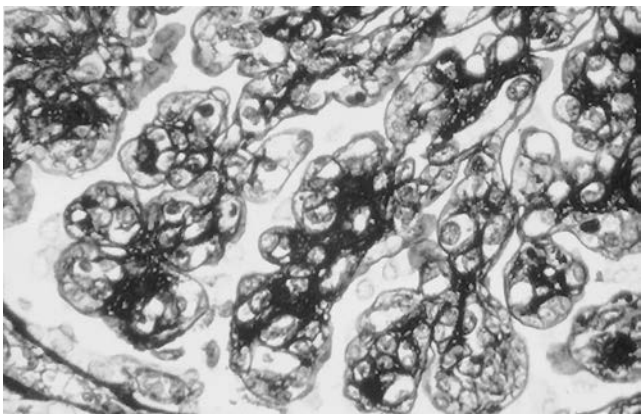

FIGURE 62-3. Type I membranoproliferative glomerulonephritis, note diffuse glomerular basement membrane thickening with clear "double-contour" appearance. (Silver stain, magnification ×400.)

glomeruli and in some capillary loops than in others. Periodic acid-Schiff (PAS) and silver methenamine stains show that the thickened glomerular capillary walls often have two basement membranes with a clear or nonargyrophilic region between them. This double contour is sometimes termed *tram-tracking, splitting,* or *duplication* of the GBM (Fig. 62-3). In some capillaries, the replication of basement membranes is very complex, resulting in multiple laminations. The double contour is created by the outward migration of mesangial cells along the inside of the capillary walls. Because mesangial cells produce basement membrane-like material, the cytoplasm of these cells is covered on the outside by the original basement membrane and on the inside by the newly formed "membrane." Both the membranes stain positively with silver and so give rise to the double-contour appearance. Capillary lumina usually are narrowed as a result of mesangial expansion, endothelial cell proliferation, and accumulation of subendothelial deposits. Using trichrome staining, large subendothelial deposits sometimes may be identified, although they are better demonstrated using immunofluorescence and electron microscopy. Mesangial deposits are rare and usually small. In type I MPGN, in addition to subendothelial deposits, many authors have described morphologic evidence of subepithelial deposits resembling to "humps" (2,3,41,43,47,48,66,69,72). In some cases mesangial matrix expansion and sclerosis is particularly evident giving a pattern of nodular glomerulosclerosis (Fig. 62-4).

Personal experience and the descriptions of other investigators (66,73–76) suggest that a true "nodular" pattern should be distinguished from lobular form of classical pattern. The lesions are mainly characterized by massive centrolobular sclerosis, always associated with microaneurysmal dilatation of glomerular capillaries, probably due to a mesangiolytic process with disruption of anchoring points at which peripheral capillary loop basement membrane attaches to mesangial stalks (Fig. 62-5). This process could be similar to that described in other nephropathies with nodular lesions as in diabetic glomerulosclerosis and light chain deposition disease (77–82). The morphogenetic mechanism of nodule formation with concentric enlargement of the mesangial matrix, strictly associated with microaneurysmal dilatation, suggests that the nodular pattern is not the late sclerotic stage of classical form but a different morphologic variant of type I MPGN, probably associated with more severe mesangial damage with consequent mesangiolysis or more severe vascular damage (66). Moreover, a morphologic transformation from classical to nodular

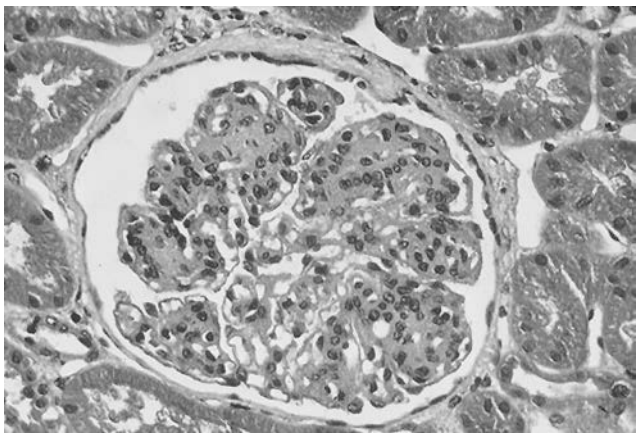

FIGURE 62-2. Type I membranoproliferative glomerulonephritis, lobular pattern: marked mesangial proliferation with mesangial matrix expansion and pronounced lobulation of the glomerular tuft. (Trichrome, magnification ×250.)

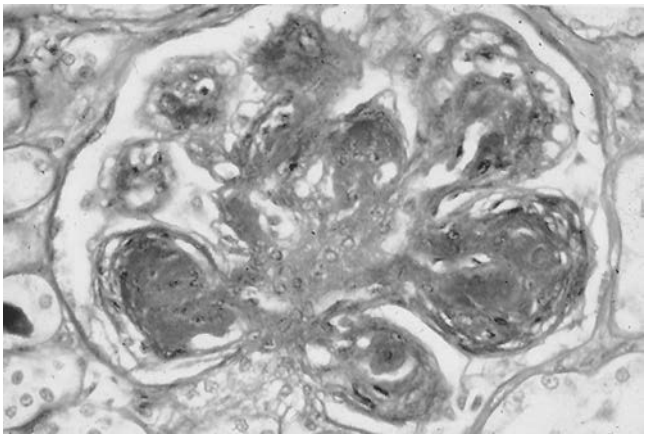

FIGURE 62-4. Type I membranoproliferative glomerulonephritis, nodular pattern: marked mesangial matrix expansion and sclerosis with large acellular nodule formation. (Trichrome, magnification ×250.)

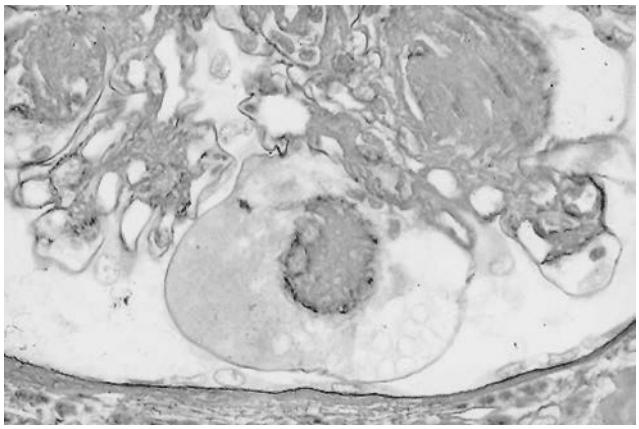

FIGURE 62-5. Type I membranoproliferative glomerulonephritis, nodular pattern: nodules of glomerulosclerosis associated with microaneurysmatic dilatation of glomerular basement membrane. (Silver stain, magnification ×400.)

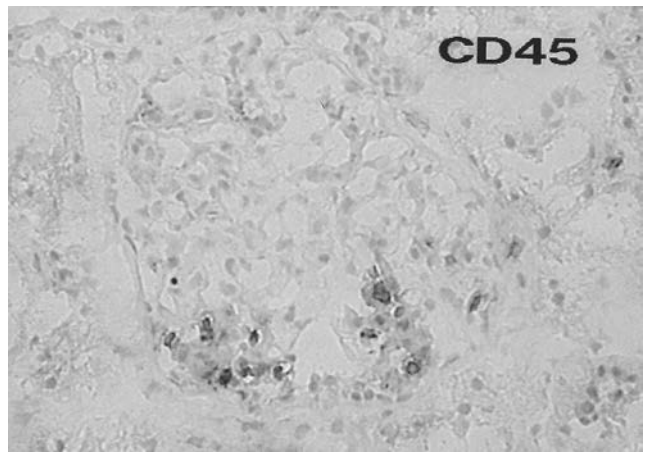

FIGURE 62-7. Type I membranoproliferative glomerulonephritis, exudative pattern: intraglomerular accumulation of monocyte-macrophages. (CD68, immunoperoxidase, magnification ×250.)

pattern has been described only rarely in studies using serial biopsies (66,68,83,84). Monga et al. suggested the possibility of double or superimposed glomerulopathies, such as describing diabetic nodular glomerulosclerosis associated with MPGN (85).

Although a moderate infiltration of neutrophils and monocytes is often present in the classical form of type I MPGN, the infiltration of inflammatory cells, mainly monocytes, is marked in approximately 20% of cases (4,86-89) (Fig. 62-6). Immunohistochemical methods, using specific antibody against monocyte-macrophages (CD68) confirm the prevalence of these cells in the glomerular tuft (Fig. 62-7). In this morphologic form, there is a marked and clearly visible "double contour" aspect of the thickened basement membrane, mainly due to the subendothelial interposition of the infiltrating inflammatory cells rather than to mesangial interposition (Fig. 62-8). This variant of "exudative" glomerulonephritis closely resembles the morphologic pattern of secondary forms of diffuse lupus nephritis, of cryoglobulinemic nephritis and some nephritis associated with other infectious diseases (88–94). Although subepithelial deposits are common in these cases the chronic clinical course, with persistent hypocomplementemia, and the immunohistologic feature of diffuse subendothelial deposition of C3, sharply differentiate this type of glomerulonephritis from the acute postinfectious nephritides, and characterize this morphologic entity as a variant of idiopathic MPGN (95,96).

Many reports of focal and segmental forms confirm the heterogeneity of morphologic lesions in type I MPGN (41,65,68,71,84,97,98)(Fig. 62-9). In these cases, only some glomeruli show lesions whereas others do not, and membranoproliferative alterations frequently are restricted to a segment of the glomerular tuft. The question of whether the focal form is a stage of less marked damage during the natural course of the disease, or is a separate entity, and thus an expression of less aggressive disease, is still open to debate. Some studies on repeat biopsies have demonstrated morphologic transformation from the focal to diffuse pattern and vice versa. Taguchi and Bohle (83) have described sequential biopsies from 33 patients with MPGN (types I and II). Twenty-four of 25 patients with diffuse forms of MPGN maintained that pattern on subsequent biopsies, whereas 4 of 6 patients with a focal MPGN pattern showed signs of a diffuse form on second biopsy. Two patients who had no histologic findings of MPGN on initial biopsy (one had focal MPGN and other had mild mesangial proliferative glomerulonephritis with small crescents) later showed evidence of a diffuse form of MPGN

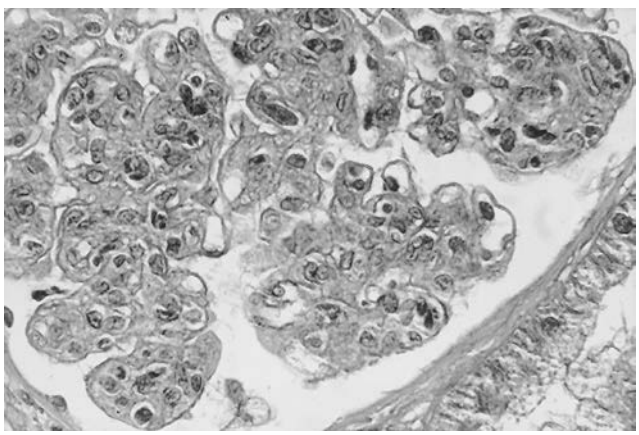

FIGURE 62-6. Type I membranoproliferative glomerulonephritis, exudative pattern: marked endocapillary hypercellularity mainly due to intracapillary infiltration of inflammatory cells. (Trichrome, magnification ×250.)

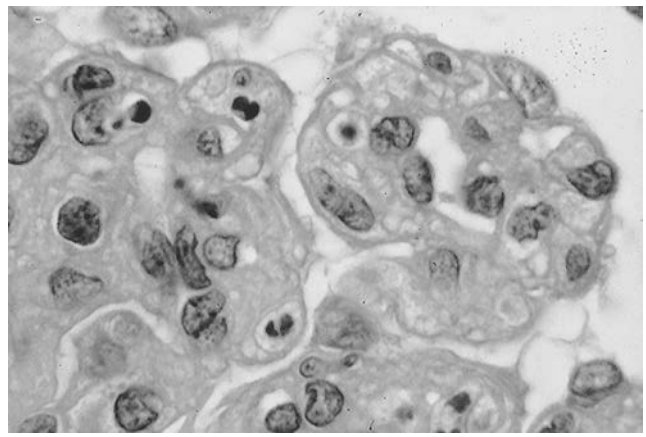

FIGURE 62-8. Type I membranoproliferative glomerulonephritis, exudative pattern: clearly visible "double contour" aspect of thickened glomerular basement membrane with subendothelial interposition of infiltrating inflammatory cells. (Trichrome, magnification ×1,000.)

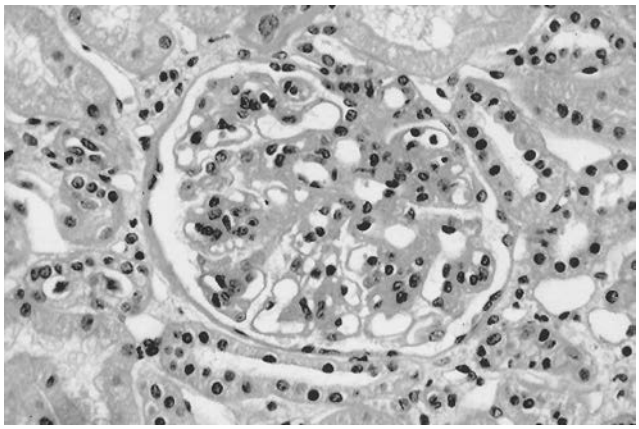

FIGURE 62-9. Type I membranoproliferative glomerulonephritis, focal segmental pattern: mesangial proliferation, mesangial matrix expansion, and glomerular basement membrane thickening are restricted to segmental part of the tuft. (Trichrome, magnification ×250.)

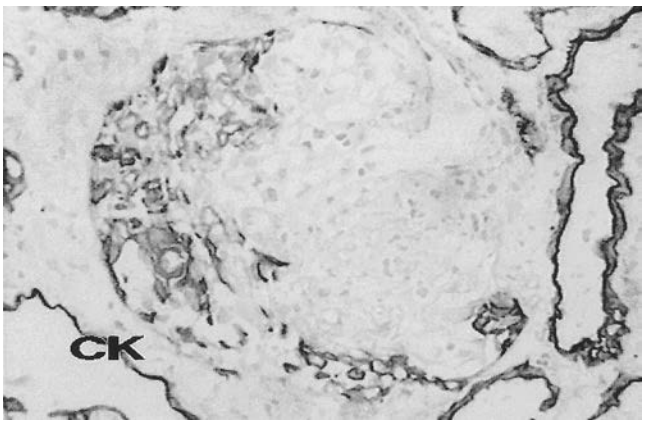

FIGURE 62-11. Type I membranoproliferative glomerulonephritis, extracapillary pattern: crescent formation strongly positive for a cytokeratins. (Cytokeratin, immunoperoxidase, magnification ×250.)

on subsequent biopsy. Our experience that the subendothelial deposits of C3 detected by immunofluorescence often are diffuse in each glomerulus supports the hypothesis that focal and segmental form could be stages of less marked damage during the natural course of the classical pattern (66–84).

Crescents also may be present in approximately 15% of patients with MPGN (8,48,99–103). These crescents may be small and segmental, or larger and circumferential, affecting most of the glomeruli (Fig. 62-10). These crescentic forms present some morphologica features that are totally different from necrotizing-extracapillary lesions typical of anti-neutrophil cytoplasmic antibody (ANCA)–associated renal vasculitis (104). The membranoproliferative crescentic patterns has minimal intraglomerular leukocyte infiltration, usually which is massive in vasculitis, and vascular cell adhesion molecule-1 is completely negative in the glomerular tuft in MPGN. Moreover, almost all cells in the crescent are positive for cytokeratins (Fig. 62-11) and their positivity for proliferating cell nuclear antigen suggests widespread proliferation of the parietal epithelial cells of Bowman's capsule (Fig. 62-12). Necrotizing glomerular lesions usually are not detected by light microscopy, whereas intraglomerular proliferation always is present. In addition, immunofluorescence demonstrates fibrinogen positivity only in the crescents. All these features suggest that membranoproliferative crescentic nephritis is based on a different pathogenetic/morphogenetic mechanism than necrotizing-crescentic forms typical of primary renal vasculitis (104), in which necrosis of the glomerular tufts induces a marked inflammatory reaction with massive accumulation of monocytes and macrophages.

Our experience in a reevaluation of 41 cases of "idiopathic" crescentic glomerulonephritis, (104) and some previous ultrastructural studies (105–107), suggest that in crescentic forms of MPGN, the pathogenetic mechanism is segmental disruption of the GBM with escape of cells and fibrin through these gaps and subsequent epithelial cell proliferation and formation of circumferential crescents in an intact Bowman's capsule, leading to compression of glomerular tuft. The poor response of these patients to the same therapeutic regimen that improves necrotizing crescentic vasculitis lends support to this hypothesis (99,102,104).

Tubulointerstitial Lesions

Only since the mid-1990s has research on renal diseases concentrated on the presence and prognostic value of tubulointerstitial lesions in primary or secondary glomerulonephritis (108). Recent studies have demonstrated that the degree of

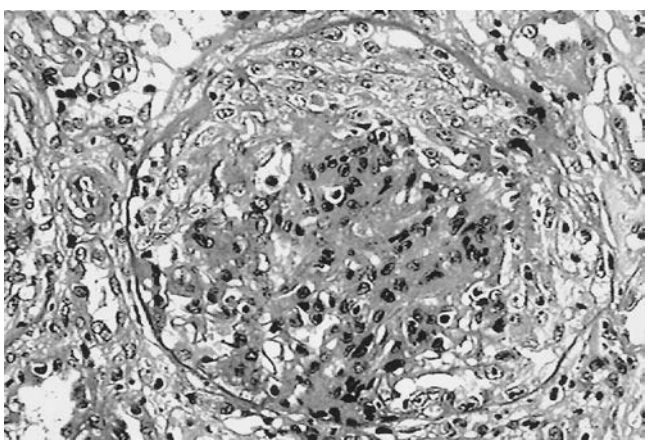

FIGURE 62-10. Type I membranoproliferative glomerulonephritis, extracapillary pattern: circumferential cellular crescent totally filling the Bowman's space, leading to compression of glomerular tuft. (Trichrome, magnification ×250.)

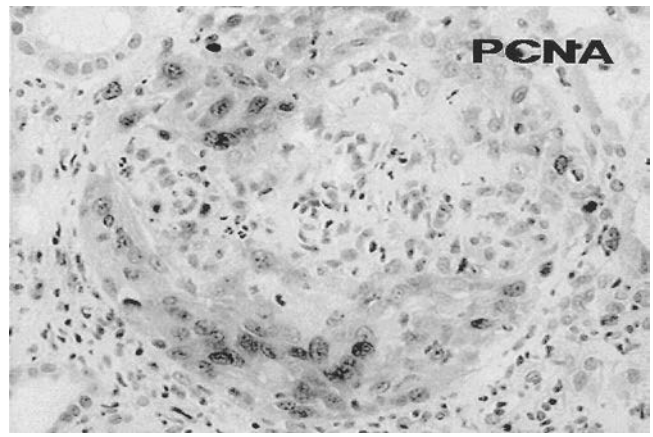

FIGURE 62-12. Type I membranoproliferative glomerulonephritis, extracapillary pattern: most crescent cells are positive for proliferative cell nuclear antigen. (Immunoperoxidase, magnification ×250.)

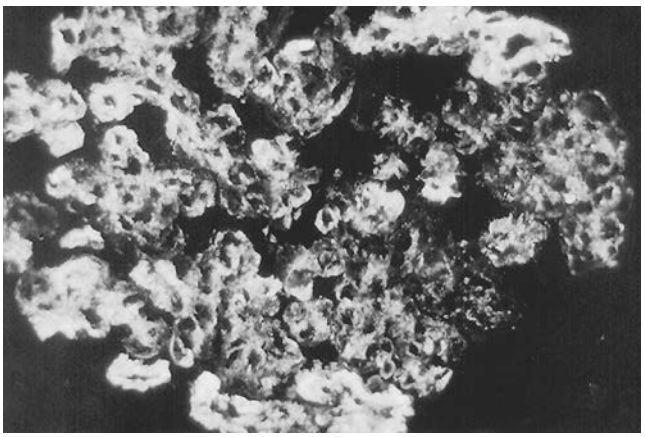

FIGURE 62-13. Type I membranoproliferative glomerulonephritis, immunofluorescence demonstrates diffuse and intense mesangial and parietal deposits. (Anti-C3, magnification ×250.)

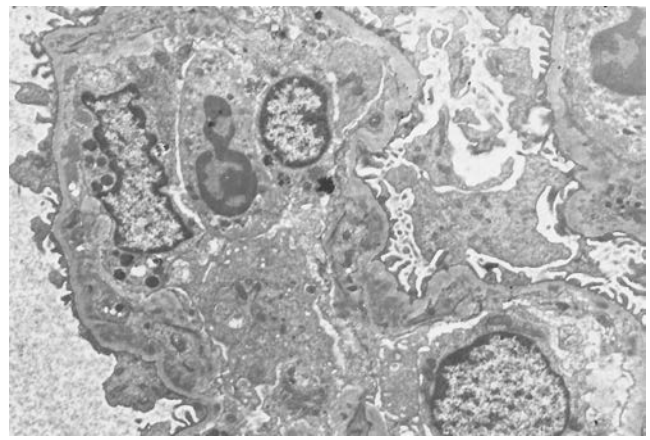

FIGURE 62-15. Type I membranoproliferative glomerulonephritis, electron micrograph of a part of a glomerulus shows subendothelial electron-dense deposits, double contour appearance of the glomerular basement membrane and some inflammatory cells. (Magnification ×3,600; courtesy of Dr. E. Schiaffino, Department of Pathology, San Carlo Hospital, Milan.)

tubulointerstitial lesions rather than glomerular changes determines renal outcome (107–112). The same correlation between the severity of interstitial changes and renal function also has been described in type I MPGN (113–117); it is becoming an accepted hypothesis that some factors produced during glomerular inflammatory process may induce reactive changes in the tubular cells, favor the passage of macromolecules into the interstitium, and finally trigger a T-cell-mediated immune reaction that can progress as an independent phenomenon. This T-lymphocytes and monocytes interstitial infiltration, which can be found early in the course of this disease, can stimulate proliferation of fibroblasts with resultant fibrosis and consequent impairment of renal function (108–112,118).

Immunofluorescence Findings

Immunofluorescence staining shows some variability in composition and distribution of deposits, confirming the heterogeneity of the pathogenetic mechanisms in patients with type I MPGN (2,41,46,71,119–124). There is almost always granular staining for C3 with two major patterns of deposition: one pattern is characterized by diffuse and regular deposits along the internal side of GBM associated with mesangial deposits (Fig. 62-13); the other pattern consists of deposits along the GBM outlining a lobulated tuft, but none in the mesangial stalk (Fig. 62-14). The latter pattern is associated mainly with lobular or nodular lesions seen by light microscopy (71,125). Early components of complement activation (C4 and C1q) are present in approximately 50% of cases, whereas properdin is almost always present (9,46,119,121,124). In two-thirds of cases, glomerular structures stain positive for immunoglobulins (Immunoglobulin G [IgG] and immunoglobulin M [IgM]) in a manner similar to that for C3, although usually with less intensity (9,46,121,123). IgA usually is absent; when present, it is extremely important to determine if it is the predominant antiserum so that an MPGN secondary to IgA nephritis (Berger's disease, liver diseases) can be diagnosed (126,127). Rarely, C3 deposits are present in Bowman's capsule and the tubular basement membrane (128).

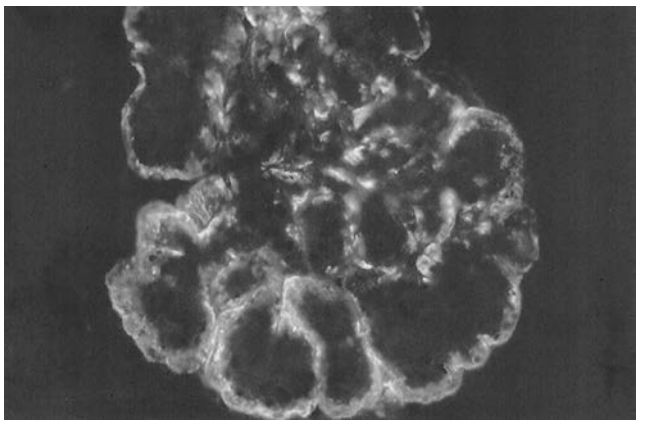

FIGURE 62-14. Type I membranoproliferative glomerulonephritis, immunofluorescence shows granular staining of the capillary wall with peripheral lobular accentuation. (Anti-C3, magnification ×250.)

Electron Microscopy Findings

Electron microscopy in type I MPGN helps clarify light microscopy observations, showing increased mesangial cells and matrix, widespread subendothelial electron-dense deposits, and thickened basement membranes with the double-contour appearance (2,9,21,41,43,45,46,129–131) (Fig. 62-15). Usually, a diffuse increase in the number of mesangial cells is accompanied by a proportional increase in the amount of mesangial matrix, but in some cases (focal forms), the degree of mesangial proliferation varies between glomeruli, being different even in individual segments of a given glomerulus. Mesangial deposits usually are scarce and rarely abundant. The double-contoured GBM lesion consists of a normal native basement membrane with a new basement membrane-like material formed beneath the endothelium. This thickened capillary wall also is composed of interposed mesangial cells and electron-dense immunodeposits. The deposits, usually described as "subendothelial," range from small and segmental to large and diffuse (Fig. 62-16).

In more exudative forms, the double contour aspect of the thickened basement membrane is due mainly to the subendothelial interposition of infiltrating inflammatory cells rather than to mesangial interposition (23–66). This variant of MPGN closely resembles the morphologic pattern of the secondary forms of cryoglobulinemic glomerulonephritis (93,132,133). To distinguish the two forms it is important to perform ultrastructural studies at high magnification to exclude the deposits that have the typical structure seen in cryoglobulinemia (93,132,133). In approximately 30% of cases, glomerular

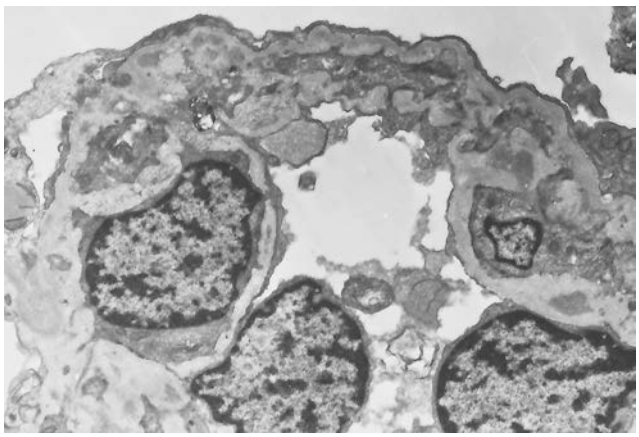

FIGURE 62-16. Type I membranoproliferative glomerulonephritis, electron micrograph of a capillary wall shows thickened glomerular basement membrane (GBM), subendothelial electron-dense deposits and double-contour appearance of GBM. (Magnification ×6,000; courtesy of Dr. E. Schiaffino, Department of Pathology, San Carlo Hospital, Milan.)

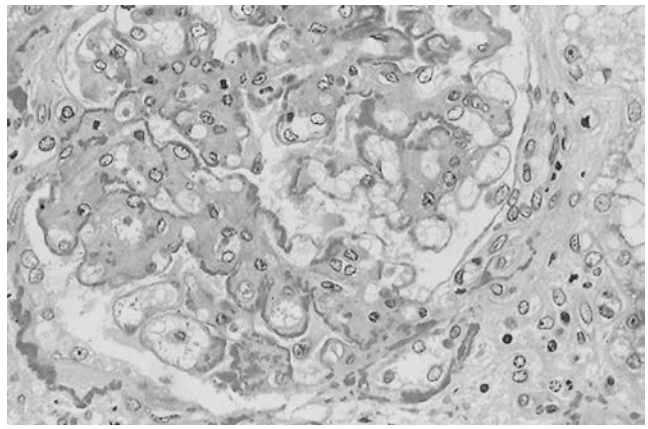

FIGURE 62-17. Type II membranoproliferative glomerulonephritis, with diffuse dense intramembranous deposits. Similar deposits are present in the basal lamina of Bowman's capsule. (Thricrome, magnification ×250.)

subepithelial deposits quite similar to the "humps" typical of acute postinfectious glomerulonephritis are present (2,71,130,131). Subepithelial deposits can be small and isolated or diffuse and abundant emphasizing that a clear separation between type I and type III of MPGN is problematic. The endothelial cells may be increased and swelling and the foot processes of visceral epithelial cells may be diffusely effaced.

Type II Membranoproliferative Glomerulonephritis (Dense-Deposit Disease)

In 1963, Berger and Galle (5) described a particular basement membrane lesion characterized by dense intramembranous deposits, mainly apparent on electron microscopy, in some patients with a membranoproliferative pattern of disease. Since the first description, this pattern of nephritis has been considered by many researches to be a variant of MPGN because in many cases it presents histologically with intense mesangial proliferation, frequent lobular accentuation of the tuft, and glomerular capillary wall thickening (16–20,44,50,56,57,59,134–139). Moreover, types I and II MPGN show clinical similarities, in particular the presence in both diseases of hypocomplementemia. However, many authors have stressed the morphologic differences on light, immunofluorescent, and electron microscopy between types I and II MPGN, suggesting that the latter form should clearly be designated as a separate disease entity (18,20,51,57,58,135,136).

Light Microscopy

The characteristic lesion of type II MPGN is thickened GBM due to irregular intramembranous deposits that are ribbon-like, brightly eosinophilic, and sometimes refractile (Figs. 62-17 and 62-18). They are intensely PAS positive and appear fuchsinophilic with trichrome stain. The glomerular intramembranous deposits vary greatly in size and number, and it is this feature that determines the extent of the glomerular capillary wall thickening. Although some authors believe that dense deposit disease can be diagnosed by light microscopy, in some cases electron microscopy may be needed to establish the presence of deposits with certainty. In some biopsies, PAS- and trichrome-positive mesangial deposits are evident, although it is often difficult to be sure that these represent bona fide mesangial deposits. Electron microscopy often reveals mesangial deposits that cannot be identified by light microscopy. Deposits also are noted in the basal lamina of Bowman's capsule and the basement membranes of both proximal and distal tubules; the extent of this extraglomerular involvement is quite variable (140–142). Discrete glomerular subepithelial electron-dense deposits similar to the subepithelial humps noted in classical acute postinfectious (poststreptococcal) glomerulonephritis may be seen in dense deposit disease (16,41,47). Rarely, spikes of GBM-like material (as seen in membranous glomerulonephritis) may be noted around the subepithelial humps. Electron dense deposits may be occasionally identified in same arterioles (16,143).

In addition to characteristic and variable capillary wall lesions, the glomeruli show, as in type I MPGN, a great range of mesangial involvement, from minimal to diffuse, proliferative lesions and a diffuse crescentic pattern (21,41,51–53,135,140). Recent International Collaborative Study Group on Dense Deposit Disease (DDD) organized by Renal Pathology Society, confirmed the variability of morphologic lesions in the largest collected series of DDD to date (unpublished data). Five patterns emerged from the review of 71 cases: classical DDD (MPGN features) 25%; focal proliferative DDD 45%;

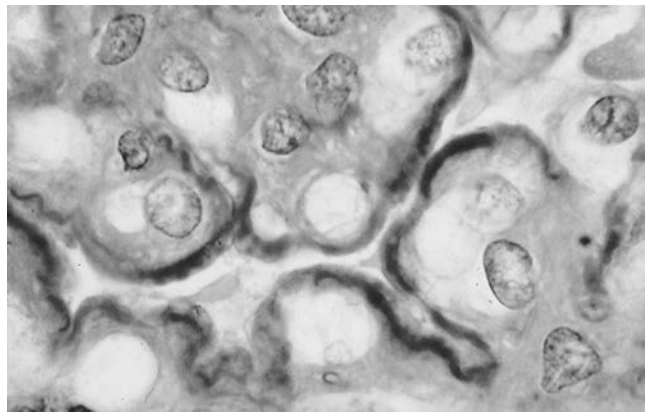

FIGURE 62-18. Type II membranoproliferative glomerulonephritis, showing overt dense intramembranous deposits. (Trichrome, magnification ×1,000.)

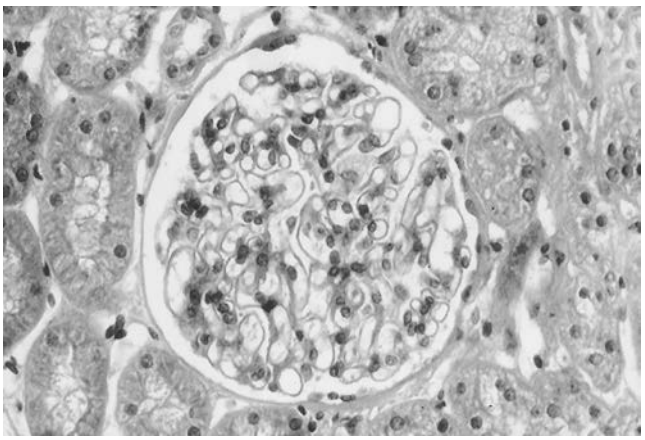

FIGURE 62-19. Type II membranoproliferative glomerulonephritis, with minimal mesangial proliferation and segmental thickening of the capillary walls. (Trichrome, magnification ×250.)

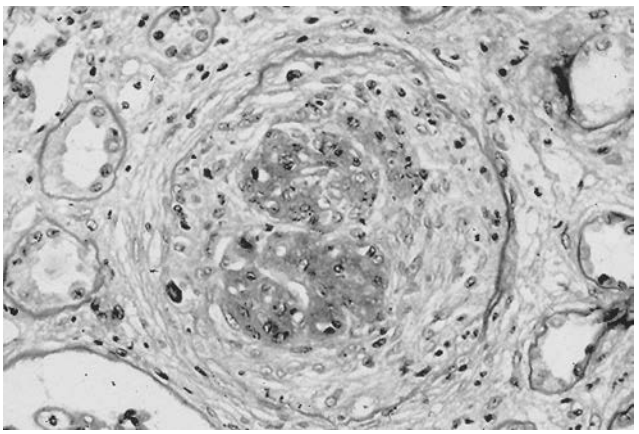

FIGURE 62-21. Type II membranoproliferative glomerulonephritis, the circumferential cellular crescent completely fills the Bowman's space. (Trichrome, magnification ×250.)

crescentic DDD 40%; acute proliferative and exudative DDD 13%; and atypical DDD (interrupted and markedly thick dense deposits) 3%. There were differences between the American and European Centers. Only one case of crescentic DDD and no cases of atypical DDD were seen in the European Centers. Crescentic DDD accounted for 35% of North American cases.

An inconsistent degree of mesangial proliferation may be seen with a picture of quite normal glomeruli but with some glomerular capillary wall thickening (Fig. 62-19). Other renal biopsies show a more pronounced glomerular tuft hypercellularity with a diffuse proliferative glomerulonephritis (Fig. 62-20). Leukocytes may be more common when the lesions are diffuse than when they are focal and segmental. Glomeruli may show areas of marked endothelial swelling that cause narrowing of the capillary lumina. The mesangium may display differing degrees of matrix proliferation (mesangial sclerosis), and, at the end of the spectrum, there are sclerotic nodules with an accentuated lobular pattern (so-called lobular glomerulonephritis). We have never seen a "true" nodular pattern in our cases of type II MPGN. Crescents may be present in a large or small numbers and with diffuse and circumferential extracapillary proliferation (51,135) (Fig. 62-21). By contrast, we have never seen focal and segmental necrotizing lesion occasionally noted by Siblay and Kim (135). There may be interstitial inflammation, mostly caused by chronic inflammatory cells. Foam cells are sometimes present, indicative of the nephrotic syndrome. Interstitial fibrosis appears as renal failure develops. As in all other forms of glomerulonephritis, the *tubulo-interstitial lesion* may play a crucial role in the progression of the disease.

Immunofluorescence Findings

The characteristic immunohistologic finding in type II MPGN is intense staining for C3 along the glomerular capillary walls and in the glomerular mesangial regions (16,17,19,21,41,44, 51,52,119,135–137,139). The immunohistologic pattern has been variously described as linear, pseudolinear, ribbonlike, granular, or nodular. Moreover, the intensity on distribution of deposits varies considerably. The most common finding is C3 along the capillary walls in a double-contour linear configuration giving a "railroad track" appearance and ring-shaped mesangial linear deposition (19,119,135) (Fig. 62-22). Granular staining, distinct from the linear deposits and the mesangial rings, are also frequently found in the mesangium. C3 also is frequently present in Bowman's capsule and along tubular basement membranes (119,141,142) (Fig. 62-22). The early components of complement (C4 and C1q) are noted rarely (144). Deposition of immunoglobulins is rare and tends to be focal,

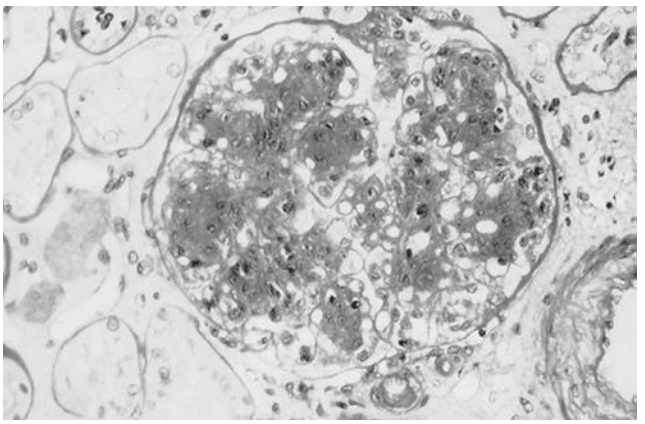

FIGURE 62-20. Type II membranoproliferative glomerulonephritis, showing pronounced glomerular tuft hypercellularity with a diffuse proliferative pattern. (Trichrome, magnification ×250.)

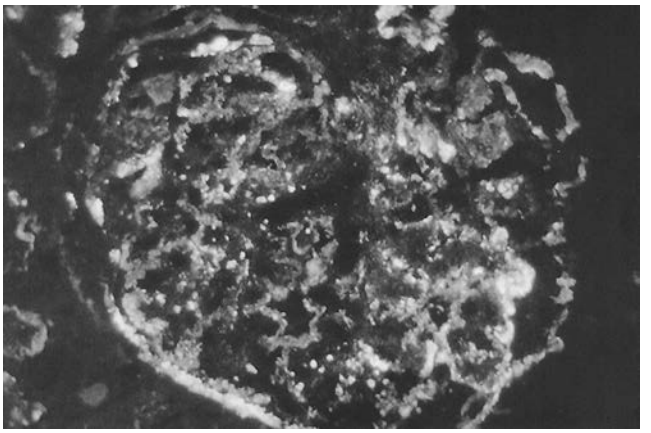

FIGURE 62-22. Type II membranoproliferative glomerulonephritis, immunofluorescence shows linear staining of the peripheral capillary wall, Bowman's capsule and tubular basement membranes. Note bright granules in mesangium. (Anti-C3, magnification ×250.)

segmental, and less intense than that of complement components (19,119).

Many studies using various staining methods showed major differences in the pattern and composition of the deposits between dense deposit disease and type I MPGN, providing valuable information for making the histologic diagnosis and understanding the morphogenesis of type II MPGN. Some investigators demonstrated that dense deposits stain brightly with thioflavine-T (18,145). Muda et al. (54) in an ultrastructural histochemical investigations, demonstrated that the dense deposits have a higher affinity for osmium than the lamina densa of normal basement membranes, and that the electron density is strictly osmium dependent, suggesting the presence of a lipid component. Nevins (146) and Galle and Mahieu (57) suggested that the dense deposit basement membrane changes are characterized by the accumulation of a glycoprotein membrane material that contains large amount of sialic acid. Further studies are warranted to determine the exact chemical composition of the dense deposit material.

Electron Microscopy Findings

There is generally good agreement that confirmation of the diagnosis of dense deposit disease requires electron microscopy examination (5,16–18,41,44,57). The characteristic lesion is a basement membrane thickening due to the presence of electron-dense material within the lamina densa (Fig. 62-23). Distribution can be segmental, discontinuous, or diffuse. The electron dense deposits do not show a particular substructure and are different from the fine granular deposits that characterize the various forms of immunocomplex glomerulonephritis, and therefore they can be clearly differentiated from the deposits seen in type I MPGN.

Similar electron-dense material usually is present in the mesangial area. These deposits may be well circumscribed and correspond to the mesangial rings made on immunofluorescence. Electron-dense deposits usually are noted along the basal lamina of Bowman's capsule, along the tubular basement membranes and in capillaries, arterioles and small arteries (16,136). More frequently than in type I MPGN, humplike subepithelial deposits are noted (16,44,136,143). Other changes seen on electron microscopy include mesangial proliferation and mesangial matrix expansion, with great variability in intensity and diffusion of these lesions.

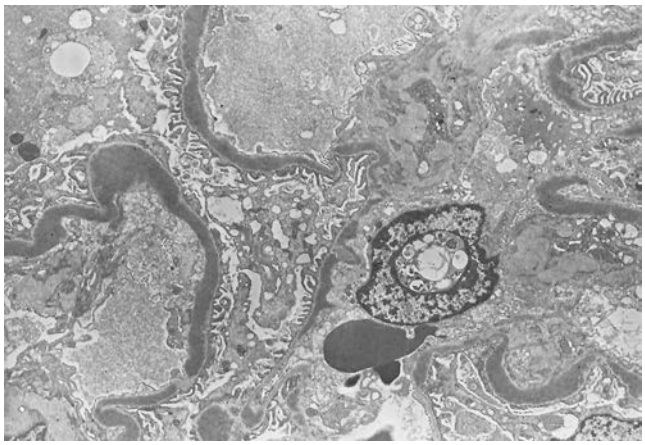

FIGURE 62-23. Type II membranoproliferative glomerulonephritis, electron micrograph of a part of a glomerulus shows the thickened capillary basement membranes; a homogeneous electron-dense material replaces the lamina densa with a diffuse distribution. (Magnification ×2,800; courtesy of Dr. E. Schiaffino, Department of Pathology, San Carlo Hospital, Milan).

The presence of dense-deposits in other organ system (e.g., brain, heart, lung, liver, adrenal glands, or pancreas) has not been reported. Eye fundus lesions, described by Duvall-Young et al. (147) and others (148), are described later in this chapter.

Type III Membranoproliferative Glomerulonephritis

Burkholder et al. (15) discussed a type of MPGN they termed *mixed membranous and proliferative glomerulonephritis*. This pattern had some of the features of typical type I MPGN, such as thickening of the glomerular capillary walls, double contours, mesangial interposition, and subendothelial deposits. In addition, however, there were silver-positive spikes along the GBM and trichrome-positive subepithelial humps such as seen in stage II membranous glomerulonephritis. Ultrastructural studies confirmed the presence of glomerular electron-dense deposits, and immunofluorescent staining showed a heavy granular pattern along the glomerular capillary walls and mesangium for C3 and sometimes for IgG and IgM. The clinical features were similar to those of type I MPGN and DDD; the serum C3 level was depressed in two of five patients.

Anders et al. (62) and Strife et al. (63), using mainly electron microscopy, characterized another variant that they also designated type III MPGN. The lamina densa was disrupted often with thickening and expansion of the basement membrane and layering by a silver-negative basement membrane material. There were glomerular subendothelial and subepithelial deposits that were contiguous or connect through the lamina densa of GBM (3,62,63,129). The controversy over whether type III should be recognized as an established and distinct clinico-pathologic entity or a simple variant of type I MPGN remains open. Jackson et al. (64) concluded that "despite some differences, type I and III should be considered variants of the same disease, rather than separate diseases."

ASSOCIATION WITH OTHER DISORDERS: DIFFERENTIAL DIAGNOSIS

Morphologic lesions referred to as MPGN have been described in numerous forms with recognizable etiologic agents or as occurring in association with other diseases. Usually the clinical features and specific laboratory findings allow the clinician and pathologist to propose a precise differential diagnosis. Moreover, many forms of MPGN associated with systemic diseases show peculiar histologic, immunohistologic and ultrastructural features that allow a clear morphologic differentiation from the "idiopathic" form.

Mixed Cryoglobulinemia (MC)

Cryoglobulinemia is a pathologic condition in which the blood contains immunoglobulins that precipitate reversibly at cold temperatures. According to the widely used classification, there are three types of cryoglobulinemia (93,149). In type I cryoglobulinemia, the cryoprecipitable immunoglobulin is a single monoclonal immunoglobulin, usually a myeloma protein or a macroglobulin (Waldenström's macroglobulinemia). In type II MC, a monoclonal immunoglobulin (usually an IgM) is bound to a polyclonal IgG and acts as an anti-IgG rheumatoid factor, whereas in type III, the anti-IgG rheumatoid factor is a polyclonal immunoglobulin. Both types of MC are frequently

associated with a single monoclonal immunoglobulin, usually a myelomatous protein. Although the glomerular lesions are variable and nonspecific in the few cases of type III MC, a particularly well-characterized pattern of glomerular involvement called "cryoglobulinemia glomerulonephritis" has been described in type II MC, in which the monoclonal rheumatoid factor usually is an IgM-k (93,94,150– 154).

The glomerular pattern of cryoglobulinemic glomerulonephritis is characterized by massive endocapillary hypercellularity due to an infiltration of leukocytes, mainly monocytes (4,104,154,155). Immunohistochemical analysis with monoclonal antibodies confirms this massive accumulation of monocytes, which is more impressive than in idiopathic MPGN (88).

Another typical morphologic feature is the frequent and sometimes massive presence of amorphous, eosinophilic, PAS-positive deposits totally filling the capillary lumina with aspect of "intraluminal thrombi" (93,94,152,153,156,157). Thickening of GBM, with a double-contoured appearance, is more diffuse and evident than in SLE and idiopathic MPGN, and is due mainly to peripheral interposition of monocytes (93,94,155). In spite of the intense intracapillary proliferation and exudation, extracapillary proliferation is an uncommon finding. In at least one-third of patients with MC, vasculitis of small and medium-sized arteries is found (94,151,152,158).

Immunohistologic features follow a predictable pattern, and the pattern of deposition or the identity of the prevailing immunoreactants is particularly important in the differential diagnosis (152,158–160). The pattern is characterized by large deposits in capillary lumina as intraluminal thrombi and diffuse granular staining of peripheral capillary walls (subendothelial deposits). IgM and IgG are the prevailing immunoreactants, suggesting that the deposits are locally trapped or precipitated cryoglobulins.

Electron-dense deposits can be found in the capillary walls and lumina (thrombi). Sometimes they are amorphous, immune complex–like deposits, but often they show a typical and peculiar fibrillar or crystalloid structure, identical to that seen in the *in vitro* cryoprecipitate of the same patients (93,152,155,156,158,161–164). Ultrastructural examination confirms the massive infiltration of monocytes in the capillary lumina and in the double contour of thickened GBM (4,154,164).

Systemic Lupus Erythematosus (SLE)

Renal morphologic features similar to those of idiopathic MPGN, with marked and homogeneous mesangial proliferation and diffuse double-contoured appearance of thickened capillary walls, are uncommon in SLE. According to new SLE nephritis International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification, the differential diagnosis should be posed with class IV global diffuse lupus nephritis (90). Class IV-G is characterized by diffuse and global endocapillary, mesangial, and frequent extracapillary proliferation (165).

Many active lesions, usually absent in type I MPGN, may be present in class IV-G: widespread wire-loops, capillary loop necrosis, karyorrhexis, and hematoxylin bodies (167–169).

The immunohistologic pattern in class IV-G lupus glomerulonephritis is characterized by massive parietal deposits mimicking the wire-loop seen at light microscopy (170). IgG is found in all patients and IgM in approximately 60% of cases, whereas IgA is rare. (170,171). Of the complement components, C3 is the most frequent, followed by C1q which usually stains very intensively. The intense positivity for C1q in SLE nephritis is an important feature in the differential diagnosis with regard to other forms of MPGN (172). Finally, ultrastructural evidence of deposits simultaneously located in the mesangial, subendothelial, intramembranous, and subepithelial sites is considered suggestive for SLE-associated glomerulonephritis (89,166,173,174).

Light-Chain Disease

The appearance of glomerular lesions occurring in light-chain disease may be variable but the most common feature is characterized by massive mesangial nodules similar to those seen in the nodular form of type I MPGN (80, 81,175–178). The staining characteristics of the glomerular nodules, however, are different in the two diseases: those in light-chain disease are more PAS positive, and negative on silver stain. Moreover, the constant presence in light-chain disease of tubular basement membrane, vascular, and interstitial deposits frequently is sufficient to trigger the suspicion that the underlying cause of the nephropathy is systemic deposition of immunoglobulin fragments. Immunofluorescence allows a further differentiation, with a strong staining for K light chains in glomeruli, tubular basement membrane, vessels and interstitium (175,176,179,180). Finally, electron microscopy shows typical fine granular deposits of extremely electron-dense material distributed through mesangial area and along GBM (181–183).

Light-chain nephropathy occasionally may resemble type II MPGN, although the electron-dense deposits of light chain nephropathy tend to be more coarsely granular and subendothelial in location, and do not stain for C3 by immunofluorescence.

Liver Disease

A pattern of membranoproliferative glomerulonephritis in liver disease has been described by many authors (127,184). The morphologic picture often is indistinguishable from the glomerular lesions of classical type I MPGN. The prevalent and intense positivity for IgA in the mesangium and capillary walls is the most important characteristic finding.

CLINICAL MANIFESTATIONS OF IDIOPATHIC MEMBRANOPROLIFERATIVE GLOMERULONEPHRITIS

Since the mid-1960s, the clinical and laboratory features of MPGN have been described in a number of clinical studies and reviews (1,2,6–8,10,11,15,16,44,67,71,185). Idiopathic MPGN affects children and adolescents more frequently than adults, and rarely occurs before 5 years of age and after the sixth decade (17,186). Type II MPGN is more frequent at younger ages. Sex predominance varies in single reports, but in the cumulative experience, both sexes are equally affected. The disease is more common among whites than among black and Asian populations. Familial cases of both type I and type II have been reported, which argues in favor of genetic factors being involved in their origin (187,188). Tissue typing studies showed that the extended haplotype on chromosome 6q, human leukocyte antigen (HLA)-B8, DR3, SC01, GL02, is found in 13% of patients with MPGN types I and III and in 1% of control haplotypes (189). However, this haplotype is not specific for MPGN and also is associated with SLE, gluten-sensitive enteropathy, and type I diabetes mellitus. Type II MPGN is much less common than type I. In series with consecutive patients

of both types, type II usually represents 10% to 20% of total cases and type I most of the rest; approximately 5% of cases have type III.

In type I MPGN, the clinical presentation is nephrotic syndrome for 60% or more of cases, usually accompanied by microscopic hematuria. Acute nephritic syndrome may be the presenting feature in 15% to 20% of cases, recurrent macroscopic hematuria occasionally is seen. The remainder present with asymptomatic microscopic hematuria or proteinuria. These clinical types and symptoms often overlap. Two children with few urinary abnormalities and hypocomplentemia were described with typical type I MPGN at renal biopsy; the clinical picture remained unchanged for many years (190). The onset of disease can be preceded, especially in children, by an infectious episode in the upper respiratory tract, more commonly in type II. Hypertension increases in frequency during the course of disease, even before the onset of a reduction in glomerular filtration rate (GFR). On initial diagnosis, hypertension is present in 30% of cases, and renal insufficiency in 20%. Renal symptoms at presentation may be accompanied in approximately 25% of cases by fatigue, weight loss, pallor, lack of appetite. A moderate degree of anemia (hemoglobin between 8 and 10 g/dL), which is out of proportion of the degree of renal function impairment, is present in 25% to 40% of cases. It may be caused by increased hemolysis, related to complement activation on red blood cells surface.

The presenting syndrome in type II MPGN is similar to that in type I, but acute nephritic syndrome or recurrent macroscopic hematuria are more common. Some authors (50) report a reversal of acute nephritic episodes in type II MPGN, most often occurring during the onset of disease, but this observation has not been confirmed by others (9). More recently, ocular alterations have been demonstrated in type II MPGN. Autopsy studies have shown dense deposit material using PAS staining of the wall of choroidal blood vessels and Bruch's membrane (147). These patients had been diagnosed with dense deposit disease by renal biopsy 2 years earlier. These deposits were confirmed by electron microscopy, and were similar to those seen in renal tissue, they were localized in the basement membrane of the ciliary epithelium, the capillaries and along the inner collagenous layer of Bruch's membrane. The high frequency of eye abnormalities has been confirmed by several clinical studies with fundal examination by ophthalmoscopy and fluorescein angiography. The ophthalmoscopically examined lesion resembles basal laminar drusen and worsens in extension and number with the duration of disease (191, 192). Michielsen et al. (193) found diffuse retinal pigment alterations in 11 out of 12 patients with type II MPGN and 4 of these patients also had disciform macular detachment and choroidal neovascularization. Subretinal neovascularization is therefore a complication of dense deposit disease (194). Type I MPGN is not associated with fundus lesions.

The clinical presentation and course of type III MPGN are similar to those of the other types, except that the patients tend to be older, the disease has more insidious onset, complement abnormalities are different from type I (62,195), the outcome may be more serious, and clinical and laboratory manifestations less responsive to steroids.

LABORATORY FINDINGS

Membranoproliferative glomerulonephritis exhibits a distinctive laboratory feature that is unique among the primary glomerular diseases. This is the frequent finding of an abnormal serum complement profile. The abnormality in type I consists of a reduction of serum C3 in 60% to 70% of cases, and, more rarely, of C4 at time of diagnosis; hypocomplementemia is more pronounced during follow-up, but normalizes with time in some patients. Nevertheless, normal values throughout an entire course of observation can be seen. C3 is more frequently (60% to 70% of cases) and severely reduced in type II than type I, but the differences are less pronounced than formerly believed. Adults and children have similar complement profiles. Most patients with type III MPGN exhibit a depression of C3, but C4 is almost always normal (64). The C3 nephritic factor of amplification loop (NFa), an IgG autoantibody to a C3 convertase (C3b,Bb), isolated from sera of patients with type II disease, is found in 80% of cases in this form and was initially believed to be characteristic of type II MPGN. However, it can also be found in few patients with type I disease. This factor is a conformational autoantibody that binds to C3b,Bb, the C3 convertase of alternate pathway that normally cleaves C3 into C3a and C3b. Binding of this autoantibody protects C3b,Bb from enzymatic inactivation and stabilizes the convertase, thereby allowing continued C3 breakdown and increasing C3 consumption.

It therefore is responsible for the persistent depression of C3. Thus, membrane-bound C3/C5 convertase does not form, and activation of the terminal pathway does not occur. C3NFa is more common in children with type II MPGN than in adults, is a fast activator, with a molecular weight of 240 kd. Another autoantibody against the terminal complement components in patients with type I MPGN has been observed (196–199). This terminal nephritic factor (NFt), an autoantibody against C3b,Bb,P, is a slow activator of the terminal components C5b,6,7,8, providing a binding site for C3b. It is found in type I (approximately 20% of cases), rarely in type II, and in approximately 70% of type III (199). NFt is properdin dependent and has a molecular weight of 980 kd and its presence in sera is associated with reduced concentrations of C3 and terminal complex C5b,6,7,8,9. Finally, a nephritogenic factor (NFc), which causes C4 activation, is found in sera of 15% of patients with type I MPGN. This autoantibody affects classical pathway C3 convertase, stabilizes C4b,2a and increases the classical pathway activation in the presence of circulating immune complexes (200). In about half of patients with type I MPGN the terminal C5b-9 complement complex (TCC) levels were found to accumulate in plasma and urine. TCC was also found in glomerular deposits. Increased TCC was associated with acute phase hypocomplementemia, with a lower response to steroids and poor long-term prognosis (201). Vitronectin and clusterin, which are both regulatory proteins for TCC, are also involved (202) and play a role in reducing the formation of TCC in glomerular tissue.

Circulating immune complexes are found in both types I and II MPGN, more frequently in type I (60% to 70% of cases) than type II (30% to 40%) (203). Serial measurements of circulating immune complexes have shown that the complexes are found intermittently, transiently, with wide variations over time (204).

In routine laboratory investigations, patients with MPGN show the aforementioned anemia, which is of the normochromic normocytic type. When present, all laboratory features of nephrotic syndrome, (e.g., hypoalbuminemia, lipid abnormalities) are detectable. Obviously, the urinalysis reveals various degrees of glomerular nonselective proteinuria on electrophoretic examination and dysmorphic erythrocytes and red cell casts in the urinary sediment.

Other laboratory investigations in all patients with a histological diagnosis of MPGN must be geared toward a complete search for the underlying disease. This procedure is mandatory before a patient is categorized as affected by a primary or idiopathic MPGN because of the frequent association of MPGN with systemic disease, including a chronic bacterial or viral infection. These investigations include testing for antinuclear and anti-DNA antibodies, cryoglobulins, serum and urine protein immunoelectrophoresis or immunofixation for paraproteins,

ANCA, serology for hepatitis B and C, hepatic enzymes, and screening studies for malignancies, especially in older patients. Moreover, blood cultures for a possible bacterial infection should be performed. Screening for HCV antibody or HCV RNA by polymerase chain reaction is important. If an MC related to HCV accompanies a MPGN, C4 will be severely depressed, unlike the primary form of MPGN, in which C3 is often decreased to a greater extent than C4. The relation between HCV and MPGN is discussed later in this chapter.

PATHOGENESIS

The pathogenesis of all types of MPGN remains incompletely understood. Type I MPGN has many features that suggest an immune complex-mediated disorder. In favor of this hypothesis is the frequent detection of circulating immune complexes (203,204), the subendothelial presence of immunoglobulins and complement components in glomeruli by direct immunofluorescence (2,9,16,17,44,48,119), the evidence in serum of activation of the classical complement pathway (6,205–207), the analogy with experimental models induced by immune complexes, and the similarity between MPGN and human diseases caused by immune deposits such as chronic bacteremic or viremic states and SLE, whose antigen is well known (208,209). In type I MPGN, unlike type III or II, the constant presence of subendothelial deposits during hypocomplementemic accords with the hypothesis of nephritis arising from immune complexes deposition. Deposition of circulating immune complexes is the predominant nephritogenic agent in type I MPGN, with evidence for classical pathway activation, even though other nephritic factors may be present in some patients. In a few severely hypocomplementemia patients with type I disease, paramesangial deposits were observed that correlated with the hypocomplementemia, which is in agreement with the presence in serum of native convertase in excess and of circulating nephritic factors (210,211).

However, because many patients with circulating immune complexes do not acquire MPGN, additional factors, such as type of antigen, type and charge on the antibody, size of the complexes, their ability to dissociate and reassociate, and local glomerular factors must play a role. In addition, mechanisms of immune complexes clearance can be defective. Other glomerular complexes are formed *in situ*, depending on the antigenic stimulus and antibody response.

Moreover, in type I MPGN, besides the classical pathway of complement activation (low C4, C2, C1q, B, C3), terminal (low C3, C5, C8, C9) and alternate pathway activation (low C3) also sometimes are observed. Therefore three patterns of complement activation are seen in type I MPGN. In fact, in type I MPGN, besides the classical immune complex mechanism, both NFa and NFt are involved. In some cases, the C1 bypass, which reacts with C1, properdin and factor B, also is found.

Complement abnormalities may be relevant to disease's pathogenesis, because frequent defects of complement components in type I MPGN are observed. These primary defects include inherited partial deficiencies of C3, C3 factor B, C6, and C7, and structural abnormalities in C3 and C7 (212–216). A membranoproliferative glomerulonephritis was described in a 10-year-old female patient with an inherited C4 deficiency. Focal MPGN was observed at renal biopsy with electron-dense deposits in paramesangium and subepithelial regions and isolated C3 deposits along GBM and mesangial areas (217). Such deficiencies, related to a defect in a structural or regulatory gene, could play a permissive role by interfering with the clearance and solubilization of antigen–antibody complexes after a viral or bacterial infection, leading to increased glomerular deposition and development of MPGN. For example, in Finnish Landrace lambs (218), which are C3 deficient, type I MPGN develops, with a predominant C3 glomerular deposition as an early lesion and subsequent deposition of immunoglobulins and development of a more generalized immune complex disease. A mixture of different types of MPGN can be observed in the same family. Berry et al. (219) described two siblings, a brother who had type III disease and a sister with type I; in a second family, two brothers had type I disease. In conclusion, even though the complement profile in type I MPGN may sometimes indicate the presence of a nephritic factor, circulating immune complexes appear to be basic to pathogenesis (210).

The mechanisms of capillary wall injury can be divided into two phases. A first "active" phase, the more proximal layers of the glomerular capillary walls are involved directly, by complement cytolytic activity and by accumulation and infiltration of inflammatory cells, in particular neutrophils and monocytes (220). Such cells in MPGN show an increased expression of some surface adhesion molecules, which interfere with intercellular adhesion molecule-1, which is expressed in endothelial and mesangial cells. The second injury phase, results in expansion of mesangial matrix and duplication of basement membrane. During the second phase tissue repair with thickening of the peripheral capillary wall, the characteristic double-contoured basement membrane and expansion of the mesangium are the prevailing features (220).

Type II MPGN is a unique lesion with no experimental analog and many factors differentiate the disease from the other histologic variants of MPGN (26,51), including the presence of peculiar and specific intramembranous deposits, the absence of immunoglobulins in glomerular deposits, the more frequent and persistent reduction of C3, the evidence of NFa in serum, the occasional association of partial lipodystrophy, and the recurrence of the disease in a transplanted kidney (16,17,44,56,137,221,222). Type II MPGN is considered to be associated with the alternative pathway of complement activation, with the amplification loop mechanism being operative.

Factor H deficiency and dysfunction have been described in type II MPGN (210). Factor H, a β1-globulin located on the gene cluster on chromosome 1, is a regulatory protein of complement activation. It inhibits C3/C5 convertase activation of C3 by cleaving Bb from C3,Bb. Factor H dysfunction leads to accumulation of alternative pathway convertase C3b,Bb and C3 consumption. Factor H also serves as a cofactor for the C3b-cleaving enzyme, factor I. Homozygous factor H deficiency was associated with type II or type I MPGN (223). In the same series other patients with homozygous or heterozygous factor H deficiency presented with typical or atypical hemolytic-uremic syndrome (223). This report confirms that glomerular lesions in MPGN are secondary to uncontrolled C3 alternative pathway activation.

Partial lipodystrophy is a rare disease, characterized by a symmetric loss of facial fat and of the disappearance, in some cases, of fat from the arms and upper part of trunk. These patients have hypocomplementemia, circulating serum Nfa and activation of the alternative pathway of complement, and a type II MPGN develops in some of them. In this disease, hypocomplementemia precedes the onset of nephritis. This suggests that additional factors also may be involved, such as circulating immune complexes. NFa also is involved in damage to adipocytes; adipocytes produce a protein (adipsin) that is identical to factor D of alternative pathway of complement activation. The pattern of damage is related to the amount of factor D produced by the adipocyte (186). More rarely, types I and III are associated with partial lipodystrophy.

Whether type II MPGN has the same genetic predisposition as type I is uncertain. Power et al (188) described the incidence of C3 nephritic factor, partial lipodystrophy and type II MPGN in members of two generations of a single family. This suggests that the pathogeneses of these conditions may be linked and that genetically determined factors may, in some circumstances, contribute to disease susceptibility.

Biochemical analysis of membranes containing dense deposits in type II MPGN showed an accumulation of a biochemically modified glycoprotein membrane substance that had increased sialic acid and decreased cysteine (57). GBM containing dense material lose its endothelial antigens (57), and has decreased antigenic binding sites. It has been reported (146) that dense deposits had a selective reactivity for certain lectins, indicating the presence of an appreciable amount of internally linked *N*-acetylglucosamine. The Cincinnati group (224) identified the mesangial deposits in type II MPGN as paramesangial (granules at the base of the capillary tuft) and, more importantly, demonstrated that their presence correlates closely with hypocomplementemia. Such deposits more likely result from circulating convertase stabilized by the NFa. More recently the same investigators (225) observed that paramesangial deposits, present only during hypocomplementemia, in type II MPGN are composed only of C3c, which can be formed from a circulating complement breakdown product, induced by NF. The C3c deposits are devoid of properdin, while C5 is present in only small amounts. These observations suggest that NF has a role in the pathogenesis of type II MPGN, the C3 level having a possible prognostic significance.

The pathogenesis of type III MPGN is uncertain. The general absence of IgG and C4 from the glomerular deposits and the aforementioned changes in the complement profile, together with the lack of classical pathway of activation, tends to rule out an origin from circulating immune complexes. Type III MPGN is a consequence of the formation of the NFt autoantibody, a slow acting, properdin-dependent antibody at the terminal pathway. Late terminal components are activated to a greater extent than in other types (197,198). Hypocomplementemia is therefore due to circulating nephritic factor which stabilizes the convertase, C3b, Bb, P. Hypocomplementemia was found to be correlated with the presence of subendothelial and subepithelial deposits, but if the patient becomes normocomplementemic during the course of disease, the deposits slowly disappear. The first deposits to disappear are subendothelial, followed by paramesangial deposits. Much later, the "type III lesions" (i.e., association of subendothelial deposits and interruptions of lamina densa) also disappear (211). In type III subendothelial deposits are present only in half of the cases in hypocomplementemic patients and are absent in normocomplementemic patients. This could depend on a less complete rearrangement of the deposited material than in type I, because of the absence of IgG (62). Paramesangial subepithelial deposits are a hallmark of type III MPGN, they are correlated with elevated circulating levels of the convertase C3b. These deposits, in type III MPGN, differ from those in type II in that they can persist for up to a year after NFt has disappeared and hypocomplementemia resolved (210,211). Remission of type III MPGN, eventually induced by steroids, is coincident with normocomplementemia and absence of type III lesion and deposits. On the other hand type III lesion can be absent at the onset of disease, when severe hypocomplementemia is observed; the histologic lesions can develop later in the course of disease.

In conclusion, abnormal circulating levels of the convertase, stabilized by the nephritic factors, are closely associated with glomerular deposits and therefore are nephritogenic (211). The elimination of nephritic factors, and consequent normalization of serum C3 level, is the primary goal of the therapy of types II and III MPGN.

Hepatitis C Virus and Membranoproliferative Glomerulonephritis

Various studies have demonstrated an association between HCV infection and MPGN. Occult chronic infection with HCV in apparent primary MPGN is thought to be more common than previously detected (38,226–228). Two-thirds of HCV-related MPGN cases had cryoglobulinemia and circulating immune complexes. The MPGN was type I and type III. The real prevalence of noncryoglobulinemic MPGN, compared with the more frequent cryo-mediated GNMP, is still debated. Patients with MC and MPGN have HCV antibodies of the IgG type that stimulate the production of cryoprecipitable IgM-k anti IgG. The production of glomerular lesions is related to cryoglobulin formation. The hypothesis that HCV antigen-containing immune complexes without IgM-k also can be responsible for noncryoglobulinemic MPGN is controversial. Some patients apparently without systemic manifestations exhibit symptoms of cryoglobulinemia later in the course of disease.

The explanation for the finding of noncryoglobulinemic MPGN associated with HCV infection, at least in some geographic areas such as the United States and northern Japan, is that the production of cryoglobulins might not reach a level detectable by standard laboratory technique. In addition, patients could produce antibodies of the "rheumatoid factor" type but that could be without the ability to induce immune complexes precipitable by cold. Finally, these patients can have an immune complex–mediated MPGN without detectable circulating cryoglobulins (220,229) although the cryoglobulins may be detected after a few months of disease.

Nevertheless, the prevalence of HCV antibody in patients with apparent "idiopathic" MPGN in many series is low, and comparable to the prevalence in the general population (230–238). In conclusion, HCV infection induces glomerular damage mainly through endogenous production of cryoprecipitable IgM-k anti-IgG antibody. Nevertheless, the induction of an immune complex mediated GN without IgM-k is a possible event (238–240).

Cellular Immune Function

Cellular immune function in patients with idiopathic MPGN has been investigated with inconclusive results (241,242). Prominent cell-mediated immunity and related immune response have been recently studied in single glomerular diseases. Human immune response leading to different patterns of injury can be directed by two specific subsets of T helper cells, which involve cell-mediated effector mechanisms and promote antibody production and antibody mediated effector mechanisms. Th1 or Th2 predominance of nephritogenic immune response is relevant for the single histopathologic patterns and outcomes of glomerular diseases. Evidence that Th1 predominant immune response is associated with MPGN has been reported a few years ago (243). Th1 or Th2 predominance can also be evaluated by IgG subtypes involvement or cytokine production (40). Elevated serum IgG3 values in patients with MPGN, in parallel with IgG3 glomerular deposits, are in agreement with a prevalent Th1 pattern in MPGN (244). Moreover, in the same patients, high levels of serum cytokines IL-2 and IL2-R also suggest that Th1 pattern has an important role in the pathogenetic mechanism of MPGN (244). Accordingly, the principal function of CD4+ cells expressing Th1 phenotype is the complement-fixing antibody, while IgG3 subclasses are strong initiators of complement activation. As already mentioned, prevailing Th1/Th2 balance directs the nephritogenic immune response and may be driven by hygiene and other environmental

factors (hygiene hypothesis) (39). This could explain the divergent prevalence of MPGN in different geographic areas worldwide and changes in epidemiology according to improved socioeconomic status.

Role of Platelets

Evidence of platelet involvement in the pathogenesis of MPGN has been found in experimental and clinical studies (245). Platelets may participate in the glomerular damage in models of experimental MPGN, together with other mediators (246). Selective platelet consumption and reduced survival have been detected in most patients with MPGN. Platelets contain a potent mitogenic factor, platelet-derived growth factor (PDGF), that recruits and mitogenically stimulates mesenchymal cells (247). Platelet-derived growth factor and the platelet-specific protein factor-4 have chemotactic activity for neutrophils and monocytes (248). Activated platelets also release transforming growth factor-β, which plays a predominant role in the evolution of glomerular lesions to sclerosis by stimulating matrix protein deposition and inhibiting their reabsorption. George et al. (249) found selective platelet consumption in patients with different types of glomerulonephritis, including MPGN. Donadio et al (250) provided evidence for decreased platelet survival in 70% of patients with MPGN, with a favorable effect of antiplatelet agents (dipyridamole and aspirin). Whether platelet activation and consumption can cause direct endothelial damage through tissue deposition or release of mitogenic proteins is unknown. The true role and importance of platelet activation in initiation and progression of injury remain unknown.

NATURAL HISTORY AND PROGNOSIS

The clinical course of MPGN appears to be comparable for all types of MPGN, and variations in the clinical severity of the disease can occur with prolonged remissions, either spontaneous or after various forms of treatment (186,251,252). Clinical remissions also were correlated with a regression of the histologic lesions (248,253,254). Episodes of acute deterioration of renal function or rapid variations of proteinuria can occur without any obvious triggering events. Moreover, the disease can remain stable for years, despite persistent proteinuria. Clinical remissions have been reported in 7% to 10% of patients in older series and persistent proteinuria in 30% to 40% of cases. Chronic renal insufficiency in 10% to 15%, and uremia with need for replacement therapy is observed in 40% to 45%. The mean renal survival rate is approximately 50% at 10 years (47,131,251,255). Droz et al. (251) reported a survival rate at 10 years of 60% for "pure" MPGN, and of 36% for the lobular form. Pediatric series have a better prognosis (256,257).

Type II MPGN does not show a different clinical course, even though clinical remissions are uncommon (16,48) and loss of renal function is somewhat faster than for type I. The mortality rate is 50% at 10 years from diagnosis for children (16) and at 8 years for a series of children and adults (48). More prolonged courses are reported by others (50). Children with type II MPGN had a slightly better prognosis in some studies, but longer follow-up series did not show any difference in outcome from that of the adult population (48,258). In four long-term follow-up studies, similar patient survival or renal failure rates were found for all the morphologic groups of MPGN (2,44,47,50). A comparison between clinical course in type I and type III MPGN was performed recently by the Cincinnati group (195). Twenty-one children with types I and 25 with type III MPGN, with similar clinical characteristics at onset and both treated with alternate day steroids, were followed for a period of at least 5 years. Patients with type I disease had a lower initial mean GFR. The frequency of complications of therapy did not differ between the two groups. Ninety percent of patients with type I and less than 50% of those with type III disease normalized their serum C3 levels after 1 year; this difference was statistically significant. After 3 years, 95% of patients with type I and 67% of those with type III had normalized their complement level. A disease relapse at 3 years occurred in six patients of type III and in none of the patients with type I disease. At last follow-up, a slight improvement in mean GFR was observed for the patients with type I disease, whereas a 25% decrease in mean GFR was seen in patients with type III. Residual urinary abnormalities were significantly more frequent in patients with type III than type I MPGN.

Two large, multicenter series of type I MPGN, 220 patients in a German study (113) and in an Italian study (66,259), reported similar data for clinical courses after a mean follow-up of 83 months from the apparent onset: 30% to 40% of patients maintained normal renal function with persistent urinary abnormalities, 25% to 30% had chronic renal insufficiency, and 25% had end-stage renal disease with replacement therapy. In the German study, there was a high mortality rate (23%). The calculated actuarial renal survival rates at 10 years from renal biopsy were 64% for the German and 60% for the Italian population. The Italian study also showed no difference in renal survival compared with 28 patients with type II MPGN. In both series, there was a mixed cohort of treated and untreated patients.

Clinicopathologic Correlations

Many clinical and histological studies have tried to identify clinical or histologic parameters that were correlated with a worse prognosis (2,47,48,50,255,256). Factors at onset of disease that adversely influenced the progression to end-stage renal disease were hypertension, early impaired renal function, and the nephrotic syndrome; among the histologic parameters, epithelial crescents and interstitial damage indicated a poor prognosis (113). Other reports did not indicate persistent nephrotic syndrome as a poor prognostic sign (113,251). There is more agreement about renal impairment at presentation, with most patients with early azotemia progressing to advanced renal failure (2,9,49,113,255,256). Hypertension is also a commonly reported bad prognostic index (9,113,252), and many investigators have found that epithelial crescents in more than 40% of glomeruli was associated with an adverse outcome (47,48,260,261). Macroscopic hematuria was found by some authors to have a bad prognosis (2,262), but this was not confirmed by others (255). Serum complement levels, presence of C3 nephritic factor, or detection of circulating immune complexes had no influence on prognosis (2,56,204–207,262–265). In the Italian study multivariate analysis showed that reduced GFR, severe proteinuria, and hypertension at presentation were significant independent risk factors. The German study did not report nephrotic syndrome as an adverse factor but gave similar results for the other clinical parameters (113). Among the histologic data, surprisingly, glomerular sclerosis was not significantly correlated with prognosis in either study, whereas interstitial infiltration and fibrosis were indicators of adverse outcome in both series. Interstitial damage also is related to a worse prognosis in other glomerular diseases, such as focal glomerulosclerosis (266), membranous nephropathy (267), and IgA nephropathy (268,269). This observation points to the role of a cell-mediated mechanism of interstitial damage, possibly responsible for the progression of disease (118,270). Whether this interstitial damage is

dependent on concomitant glomerular involvement is controversial (66, 117). Arteriolar hyalinosis correlated with a worse prognosis in the Italian study, but was not evaluated in the German report. Moreover, in the two studies, renal survival was compared in subgroups with glomerular changes of different severity. In both series, the subgroup with epithelial crescents had poor survival rates; in the Italian study, the nodular variant had a significantly worse prognosis. Although the nodular pattern is considered a distinct entity by some (271), it was not considered as a separate subgroup in the German study (113).

Pregnancy

During pregnancy, proteinuria can increase because of the hemodynamic features of pregnancy. When the patient is in renal failure, spontaneous abortion in early pregnancy is more frequent (272). Rapid deterioration of renal function has been described in patients with MPGN and renal insufficiency who became pregnant. The renal function that can be adversely influenced by pregnancy has been estimated at approximately 50 mL/minute GFR or less (273).The morphologic counterparts of such deterioration is fibrinoid thrombi in the capillary lumina and crescent formation, with subsequent focal sclerosis (274). Fairley (275) observed the deterioration of renal function in a patient with MPGN whose repeat biopsy had shown crescents. Preexisting hypertension is a factor that predisposes to a higher risk of preeclampsia. The outcome for the fetus in preexisting hypertension is worse, with a fetal loss rate of 25% (276). Overall, pregnant women with MPGN have a higher risk of development of nephrotic syndrome and a higher incidence of preterm and small-for-gestational-age infants, especially if they are hypertensive (277).

Membranoproliferative Glomerulonephritis in Renal Allografts

The reported incidence of recurrent MPGN in renal allografts varies in different series (222,278,279). It depends on the renal transplant biopsy policy and on accurate morphologic diagnosis of the primary renal disease. Recurrence of type I MPGN occurs with a frequency, that varies 18% to 30% (222,280) and the rate of graft loss due to recurrence is higher than 50%. The true frequency of type I recurrence may be difficult to establish because of its similarity of its clinical and histologic features to those of chronic allograft glomerulopathy (281). A primary cytomegalovirus infection can induce an apparent recurrence of type I MPGN (282). If patients with type I MPGN have had a rapid deterioration in native kidney function, a similar pattern is more likely to occur in their transplant allograft. Type II MPGN recurs in the renal allograft with a frequency of 80% to 90% (48,278,279). Dense deposits are easy to recognize and are the earliest histologic marker. Other morphologic changes, such as mesangial hypercellularity and matrix interposition come later (16, 283, 284). In some patients, recurrence is limited to the presence of deposits in the mesangium without GBM abnormalities, whereas in one-quarter the presence of diffuse crescents causes loss of graft (285). Serum complement levels or presence of C3 nephritic factor are not reported to be predictors or markers of dense deposit disease recurrence (286), but some authors do not agree with this observation (287). Renal failure in transplanted patients is much more often the consequence of rejection, rather than secondary to recurrence (283).

Late in the course of successful transplantation, the appearance of heavy proteinuria, decline of renal function, hypertension, and abnormal urinary sediment can be attributed to a distinct glomerular disease, called *glomerular transplant disease or allograft glomerulopathy*, rather than to the recurrence of primary MPGN (288,289). This condition has many histologic features similar to those of idiopathic MPGN, but mesangial changes are less conspicuous and consist of rarefaction followed by sclerosis, with little cell proliferation (208).

De novo type I MPGN has been observed only rarely in transplanted patients, and type II has never been reported (290,291). Elimination of MPGN as the primary disease obviously is necessary to establish the diagnosis of *de novo* MPGN.

TREATMENT

The efficacy of therapeutic regimens in MPGN is difficult to assess because of the small number of patients in short-term, controlled clinical trials reported in the literature; the larger trials have been uncontrolled. Moreover, spontaneous fluctuations of disease activity in natural history of patients with MPGN and the morphologic heterogeneity of the disease make the analysis of therapeutic results more difficult. There is no proven successful treatment for MPGN but examination of single drugs or groups of drugs represents a simple way to assess their efficacy in therapeutic approach to MPGN.

Corticosteroids

Long-term, uncontrolled studies of corticosteroids therapy were performed in children with types I, II, and III diseases by the Cincinnati group (263), using an alternate-day regimen (2.0 to 2.5 mg/kg). The dose was slowly reduced to 1.5 mg/kg at 2 years and to 0.8 to 1.0 mg/kg at 4 years, always administered every other day. The authors' latest results (258) indicate an improvement in renal function and glomerular morphology with prolonged renal survival and reduced severity of proteinuria. In the absence of a control untreated group, survival was compared with previously reported series of untreated children (2). The beneficial effect of alternate-day steroids was more evident after 3 years of treatment and only if prednisone was started within 12 months from clinical onset. A prospective, multicenter, randomized trial was carried out by the International Study of Kidney Disease in Children (ISKDC) (292). Patients were children affected with type I MPGN, with a GFR of a least 70 mL/minute/1.73 m^2 and a persistent proteinuria over 40 mg/hour/m^2. Eighty children were randomized to receive prednisone 40 mg/m^2 every other day or placebo for a mean of 41 months, with termination of treatment for occurrence of renal failure. All three types of MPGN were included. Thirty-three percent of the treated patients had a treatment failure (30% increase in serum creatinine) and the same event occurred in 58% in the placebo group. At 130 months, 61% of patients under steroids had a stable renal function, whereas the rate of preserved renal function among those taking placebo was 12%. Nevertheless, in the treated group the interval between apparent onset of disease and study entry was only 8.9 $\pm$ 1.3 months, as opposed to 18.1 $\pm$ 3.9 months in the control group. The researchers concluded that long-term treatment with prednisone appears to improve the outcome of children with MPGN (292,293). Ford et al. (294) conducted another uncontrolled study in 19 children with type I MPGN, tailoring the dose of steroids based on renal function and proteinuria of single subjects. Treatment was usually started within 1 year of diagnosis. The highest dose was intravenous pulses methylprednisolone, 30 mg/kg for three doses, followed by oral prednisone 2 mg/kg/day. The lowest dosage was prednisone 20 mg every other day. The mean follow-up was 6.5 years, and the total duration of treatment was 38 $\pm$ 3 months. Repeat renal

biopsies, performed after approximately 3 years, showed a reduced histologic activity in most patients. Forty-two percent of the treated patients had a complete remission, with protein-free urines. No patient had uremia. The mean creatinine clearance rose from 78 to 126 mL/minute during the course of treatment. This study suggests that in children with type I MPGN, aggressive and early steroid treatment, with a dosage tailored to severity of disease, may be of some benefit. Nevertheless, no control group was included and patients' outcomes had been favored by a better control of hypertension and the use of angiotensin-converting enzyme inhibitors (ACEI). A recent comparison of the long-term effects of an alternate-day prednisone regimen in types I and III MPGN in children, gave clear evidence of a significant difference with respect to changes in urinalysis, serum creatinine, and C3 levels (195). The outcome of patients with type I MPGN patients treated with prednisone was in general good, whereas similarly treated patients with type III showed reduced renal function, slower rise of serum C3 levels, and more persistent urinary abnormalities. They also experienced more disease relapses. These data confirm the clinical differences between types I and III MPGN.

In adults, steroids trials are very limited. Results have been reviewed by Donadio and Offord (295), who concluded that available information does not support a favorable effect of glucocorticoids. Nevertheless, in many studies treatment was started too late in the course of disease. The early use of methylprednisolone pulse therapy in patients with nephrotic syndrome or severe proliferative lesions, followed by oral alternate-day prednisone, can improve the prognosis and presumably exert an "antiinflammatory" effect over glomerular lesions (296).

Alkylating Agents

The use of alkylating drugs had been poorly investigated until comparatively recently in MPGN because of unfavorable results obtained by these agents in combination with others such as warfarin, heparin, or dipyridamole (252,297). More recently, uncontrolled trials were conducted using oral cyclophosphamide in combination with methylprednisolone in pulses, followed by oral prednisone. Faedda et al. (298) treated 19 patients between 9 and 65 years of age with various types of MPGN with this aggressive regimen, and obtained a complete remission in 15 of 19 patients and a partial remission in 3 of 19 patients. Four patients progressed to uremia and some of the responders had a relapse. The survival rate, after a mean follow-up of 7.4 years, was 79%. The rate of remission was higher than that reported for untreated "historical" series, but the overall survival rate did not differ greatly from that observed in children treated with steroids alone.

Anticoagulants and Antithrombotics

Available data involve patients with type I MPGN. Dipyridamole and warfarin were used in one study, in a randomized, crossover, prospective trial that enrolled 22 patients; only 13 of whom completed both a control and a treatment year (299). The treated group showed a better preserved renal function, but the rate of complications was high, with significant hemorrhagic problems in 37% of treated patients. A combination of aspirin 975 mg/day and dipyridamole 325 mg/day was studied in 40 patients who were treated for 1 year in a well-designed randomized trial. In the treated group, GFR, measured by the iothalamate method, was better preserved and platelet survival time was normalized. After an average extended follow-up period of 4 years, treated patients had a lower percentage of advanced renal failure than the placebo group. There was no change in proteinuria, hematuria, or complement profile; 15% of patients had to discontinue the trial because of bleeding complications. Dipyridamole combined with steroids proved to be useful in children with type I MPGN (300).

More recently (301), a nonblinded multicentric prospective controlled trial randomly assigned 18 adult patients either to aspirin (500 mg/day) plus dipyridamole (75 mg/day) or to placebo for a period of 36 months. Fifteen patients had type I and 3 had type II MPGN. All patients had impaired renal function and nephrotic proteinuria. Treated patients had a greater drop in proteinuria, and a partial remission was observed in 7 of 10 patients in the aspirin-dipyridamole group and in 2 of 8 in the placebo group. The treatment time and follow-up period were too short to observe the postrandomization effect on renal function. Donadio and Offord (295) stressed that these studies (aspirin plus dipyridamole) followed the course of disease from introduction of therapy and not from the diagnosis. The follow-up period was too brief to offer any conclusion on the long-term effect of this therapy on the natural history of MPGN.

Other Therapeutic Approaches

A few uncontrolled trials have been reported the use of cyclosporine in various types of primary glomerular disease (302,303). Cyclosporine affects proteinuria, whose reduction is related to a reversible lowering of GFR. It may act through its suppression of T-cell activity. This drug, used at the dosage of 4 mg/kg/day for 3 to 6 months, because of its potential nephrotoxicity and aggravation of hypertension, should be carefully employed in MPGN.

Levin presented successful treatment of a patient with MPGN and severe nephrotic syndrome using mycophenolate mofetil (304). A beneficial effect on proteinuria was reported for other anecdotal cases (305,306).

Jones et al. (307) recently treated five adult MPGN patients with mycophenolate plus oral steroids and compared outcome measures with six control untreated patients. A significant reduction in proteinuria was observed during an 18-month follow-up. No significant difference between the two groups was found for creatinine clearance, but a decrease was present in the control group, though without reaching a statistical significance.

In conclusion, preliminary data indicated that mycophenolate, a well-tolerated but expensive drug, in cases of MPGN has a favorable effect in reducing proteinuria and in stabilizing renal function.

Angiotensin-converting enzyme inhibitors could be used for their effect in reducing proteinuria and in preserving renal function (308). Experimental and clinical data indicate that angiotensin II receptor antagonists (AIIRA) added to ACEI have an additional protective effect (309,310). As in other glomerular diseases, combined therapy have an additive efficacy in reducing proteinuria and slowing the progression of renal decline (311). A low-protein diet can also play a role in lowering proteinuria and possibly slowing the rate of progression of renal disease.

Conclusions

In conclusion, from available data we can recommend three main lines of treatment in idiopathic MPGN, depending on clinical presentation:

1. Children with preserved renal function and nephrotic proteinuria should be given a long-term, alternate-day corticosteroid treatment with careful monitoring of blood pressure.

A period of 6 to 12 months of such therapy has been suggested (312), rather than the longer time of treatment recommended previously.

2. Adults with proteinuria and reduced GFR, could be treated with a combination of aspirin and dipyridamole, with attention to potential hemorrhagic complications. Patients with low proteinuria and normal renal function do not need any treatment, except diet and ACEI and/or AIIRA.
3. In patients with rapidly progressive renal failure or a recent sharp reduction in renal function, aggressive treatment with methylprednisolone pulse therapy followed by oral prednisone plus oral cyclophosphamide is an appropriate therapeutic approach, especially if crescents are present on histologic investigation, indicating more severe disease.

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CHAPTER 63 ■ MEMBRANOUS NEPHROPATHY

MITCHELL ROSNER AND WARREN KLINE BOLTON

Membranous nephropathy (MN), also know as membranous glomerulonephritis, represents the most common cause of the nephrotic syndrome in elderly adults, representing up to 30% of all cases in patients over age 50 (1–4). It is recognized by its characteristic pathological findings of diffuse subepithelial immune deposits without glomerular hypercellularity (5–7). It occurs most commonly in isolation (idiopathic MN, 80%), but may be a feature of an underlying disease (secondary MN, 20%), most often either autoimmune, infectious or malignant (8–10). The pathogenesis of MN is likely autoimmune in nature based upon animal models where the disease can be reproduced with antibodies that react to antigens expressed on the glomerular epithelial cell (GEC) (11–15). Interestingly, a recent case of congenital MN in humans has also been determined to be secondary to a passively transmitted maternal antibody that reacted to an endogenous antigen on the GEC (16). In animal models, immune complex deposition leads to complement activation and subsequent injury to the GEC and the glomerular basement membrane with the resulting expression of proteinuria and depressed glomerular filtration rate (17–21). Its course is variable with spontaneous remissions and relapses and leads to end-stage renal disease (ESRD) in up to 30% to 40% of patients over an extended period of time (22–24). Despite this good prognosis, the high incidence of MN makes it the second or third most common primary glomerular disease to progress to ESRD (25). Additional morbidity and mortality from the nephrotic syndrome accrues from accelerated vascular disease, hyperlipidemia, and the occurrence of thromboembolic events (26). Despite numerous clinical trials, therapeutic approaches remain controversial and the subject of much debate.

EPIDEMIOLOGY

Idiopathic (or primary) MN is the cause of nephrotic syndrome in approximately 25% of adults (1,27). It is a disease that affects patients most commonly around the fourth and fifth decade (median age approximately 40) but can present at any age (28,29). In patients older than age 60, MN can be seen in up to 35% of biopsy specimens (4). Secondary MN (either related to autoimmune disease, infections, malignancy, or drugs) is seen more commonly at the extremes of young and old age. In younger patients, especially in Asia, hepatitis B infection is a much more common etiology (30,31). In older patients, related malignancies are more commonly seen and may be responsible for up to 20% to 30% of all cases in patients older than age 60 years of age (32,33). MN is more common in men than in women (2 or 3:1) (34).

Determining the true incidence and prevalence of MN is difficult. In Victoria, Australia over a 2-year period, the incidence of MN identified by renal biopsy was 13.27 cases per million person-years (35). A larger study from the United Kingdom gave an annual incidence of 11 cases per million population per year (36). While the majority of patients (80%) present with signs and symptoms of the nephrotic syndrome, up to 20% to 25% of patients may be detected as having asymptomatic proteinuria from urinalysis as part of a medical examination (37). Since most physicians do not routinely screen for proteinuria and the onset of the disease is insidious, these numbers are likely an underestimate. Furthermore, many of these patients never develop the nephrotic syndrome and would go unbiopsied and undetected.

The United States Renal Data Service records MN as the cause of 0.6% of all of the ESRD population (25). This number has remained constant over the past decade.

Geographic variations in the prevalence of MN have been reported and may, in part, be related to differences in the rate of secondary MN due to infectious diseases such as hepatitis B and malaria (38).

SECONDARY MEMBRANOUS NEPHROPATHY

In over 75% of MN cases, no etiological agents can be determined. However, certain underlying conditions have been reported to be associated with the development of MN and account for up to 25% of cases (Table 63-1) (37). In many of these cases, the causal link is tenuous and consists only of isolated case reports making causation speculative. In other cases, the condition has been described so frequently as being associated with MN that causation is more definitive. The best evidence comes from conditions in which treatment of the underlying process (infection, malignancy, autoimmune disease) or removal of an offending agent or drug is associated with resolution of the nephropathy. Furthermore, in some cases (such as hepatitis B infection and malignancy) an associated antigen can be localized to the glomerular site of injury further substantiating causality (30,39–41).

In most cases, the lesion of secondary MN appears pathologically identical to the idiopathic form. The notable exception to this is MN associated with underlying systemic lupus erythematosus in which subendothelial and mesangial immune deposits as well as mesangial hypercellularity are seen (42,43).

Autoimmune Conditions

Various rheumatologic disorders have been described in association with MN (Table 63-1). Systemic lupus erythematosus (SLE) is the most common rheumatologic disease associated with MN (43–48). About 15% to 25% of patients with lupus nephritis are classified with a class V (membranous) World Health Organization (WHO) lesion with predominately subepithelial deposits (49,50). Clinically, the presentation is that of the nephrotic syndrome and is indistinguishable from idiopathic MN. The majority of these patients are young females and in a substantial number the onset of the nephrotic

TABLE 63-1

AUTOIMMUNE DISEASES ASSOCIATED WITH MEMBRANOUS NEPHROPATHY

Systemic lupus erythematosus (43–56)
Rheumatoid arthritis (57,58)
Mixed connective tissue disease (101)
Dermatomyositis (102)
Ankylosing spondylitis (103,104)
Systemic sclerosis (105)
Myasthenia gravis (79)
Bullous pemphigoid (106)
Autoimmune thyroid disease (75,76)
Sjögren's syndrome (107,108)
Temporal arteritis (109)
Crohn's disease (77)

(Adapted from: Short C, Mallick NP. Membranous nephropathy. In: Schrier RW, ed. *Diseases of the kidney*. 7th ed. Philadelphia: Lippincott Williams and Wilkins; 2001:1743.)

TABLE 63-2

INFECTIOUS DISEASES ASSOCIATED WITH MEMBRANOUS NEPHROPATHY

Hepatitis B (81–84)
Hepatitis C (96–98)
Human immunodeficiency virus (114)
Malaria (115)
Schistosomiasis (116)
Filariasis (117)
Syphilis (110–113)
Enterococcal endocarditis (118)
Hydatid disease (119,120)
Leprosy (121)

(Adapted from: Short C, Mallick NP. Membranous nephropathy. In: Schrier RW, ed. *Diseases of the kidney*. 7th ed. Philadelphia: Lippincott Williams and Wilkins; 2001:1743.)

syndrome predates the development of other signs of SLE (51,52). Thus, there should be a high degree of suspicion for SLE in any young female who presents with the nephrotic syndrome and MN on renal biopsy. Up to 25% to 50% of patients have negative anti-nuclear antibody levels, and often the antibody, if present, is of low avidity (51,52). Complement levels are usually normal. Morphologically, the lesion in class V lupus nephritis can look identical to that of idiopathic MN, but more often there are distinguishing features on renal biopsy (43). Light microscopy can reveal mesangial hypercellularity and expansion. Immunofluorescence reveals a more varied immunoglobulin response with the presence of immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM) as well as C1q deposition. Electron microscopy can reveal typical tubuloreticular inclusions in glomerular endothelial cells. Otherwise, the clinical course of lupus MN mirrors that of the idiopathic form with an excellent long-term prognosis in excess of 85% renal survival at 10 years (53–56).

When patients with rheumatoid arthritis develop MN, it is usually in the setting of treatment with agents such as gold, penicillamine, bucillamine, or nonsteroidal antiinflammatory agents (57–68). Recently, cyclo-oxygenase-2 (COX-2) inhibitors have also been associated with a lesion of MN in conjunction with acute interstitial nephritis (69). In these cases, proteinuria develops soon after exposure to the drug and resolves slowly over a period of months after withdrawal of the offending agent. The pathologic lesion is identical to idiopathic MN.

The association of MN with other autoimmune disorders is less definitive. The immune response that leads to immune complex formation in MN has been linked to the human leukocyte antigen (HLA) class II antigen DR3 (70–71). There is a threefold or greater increase in the relative risk for MN is patients with HLA-DR3 (70–72). HLA-DR3 has also been associated with several other autoimmune conditions such as Sjögren's syndrome, dermatitis herpetiformis, and Grave's disease (36). Interestingly, many of the patients with rheumatoid arthritis who develop MN in the setting of drug therapy are also HLA-DR3 positive (60–62). Thus, the association of MN with these disorders may simply reflect this common HLA association.

Many other miscellaneous conditions that have an autoimmune basis have been associated with MN. These include diverse diseases such as Grave's disease, sarcoidosis, Crohn's disease, myasthenia gravis and multiple sclerosis (73–80).

Infectious Diseases

Numerous infectious diseases have been associated with the development of MN (Table 63-2). In all cases, these represent chronic infections with long-standing and persistent antigenemia. The etiological role of these infectious diseases is strengthened when the nephrotic syndrome resolves with treatment of the infection.

The association of MN with hepatitis B viral (HBV) chronic infection is particularly strong having been first described by Combes et al. in 1971 (81). HBV infection may account for up to 30%–40% of cases on MN in Asia (but less than 1% of MN in the United States) and is particularly prevalent in childhood cases of MN throughout the world (accounting for 80% of cases in Asia, and 20% of cases in the United States) (30,31,38,82). Patients present with the nephrotic syndrome and have a history of viral hepatitis with laboratory evidence of chronic active or persistent hepatitis. Serologically, these patients are usually positive for HB surface antigen (HBsAg) and in 60% to 80% of patients, HBeAg as well (30,83–84). In contrast to idiopathic MN, C3 and C4 levels are mildly depressed and immune deposits can be found in the mesangium and subendothelial regions of the glomeruli (30). The pathogenesis of HBV-associated MN is unknown. However, HBsAg, HBcAg and HBeAg have been localized by immunofluorescence to the glomerular capillary wall in patients with HBV-associated MN supporting a possible role for these antigens in leading to immune complex formation in the glomerulus (39–41). HBeAg seems to have a particularly strong predilection to localize to the capillary wall (41). It is also possible that autoantibodies could be induced secondary to chronic liver disease and that the findings of viral antigens in capillary walls may reflect passive trapping in damaged glomerular tissue (85). Adults with HBV associated MN generally have a good prognosis (30). However, up to 10% of untreated patients may require dialysis. Children have a better prognosis with the majority undergoing spontaneous remission within 5 years (30). Remission is often associated with clearance of HBeAg (30). Treatment of HBV infection with either alpha-interferon or lamivudine has been associated with remission of the nephrotic syndrome in a number of cases (86–91).

Hepatitis C viral (HCV) infection has been associated with mixed cryoglobulinemia and the development of membranoproliferative glomerulonephritis (MPGN) (92–95). However, HCV has also been associated with MN (96–98). These patients are anti-HCV IgG and HCV RNA positive. Core antigen, which is strongly cationic, has been localized to the

glomerular capillary wall in two patients with MN associated with HCV (99). Unlike HCV-associated MPGN, cryoglobulin is undetectable and complement levels are normal. Combination alpha-interferon and ribavirin therapy has led to significant reduction in proteinuria and improved renal function in several patients (100).

Syphilis has rarely been associated with the development of MN (110–113). This association is strengthened both by the demonstration of treponemal antigens in the glomeruli of patients with MN and the resolution of the nephrotic syndrome with treatment of the infection (110–112). Human immunodeficiency viral (HIV) infection has also been rarely described in association with MN (114).

Malignancy

In patients older than age 60, up to 20% of patients with MN may have an occult or diagnosed malignancy (Table 63-3) (85). The majority of these cancers are carcinomas of the gastrointestinal tract, lung or breast. The incidence of carcinoma in patients diagnosed with MN is 10-fold higher than that expected for an age-matched normal population (32,33,122–125). In up to 70% of patients, the malignancy may be occult at the time of diagnosis of the nephrotic syndrome and thus careful follow-up of elderly patients with MN is warranted (125). Anecdotal evidence supports the view that the majority of these cancers manifest within 2 years after the diagnosis of MN (33,125). There are rare reports of resolution of the nephrotic syndrome after tumor resection or chemotherapy (126–128). Whether these represent evidence of true causation or simply spontaneous remission of MN is unknown. Supporting a direct role in the causation of MN is the finding of tumor-related antigens, including carcinoembryonic antigen, in glomerular deposits in several patients (129–132). Whether this may simply represent passive trapping of antigen to a damaged glomerulus rather than a direct role in pathogenesis is not known. A recent case of MN in association with an adrenal ganglioneuroma demonstrated the presence of an antibody from the patient's serum that reacted against the tumor as well as the glomerular basement membrane (127). Resection of the tumor was associated with disappearance of this antibody from the serum along with clinical remission of the nephrotic syndrome. The pathological lesion was identical to that of idiopathic MN. There are no data on the response of malignancy associated MN to steroids or immunosuppressive therapy (33). Unfortunately, malignant disease is often advanced in patients with MN with a 75% mortality 12 months after the diagnosis of MN (33,122,133).

TABLE 63-3

MALIGNANCIES ASSOCIATED WITH MEMBRANOUS NEPHROPATHY

Carcinomas	Noncarcinomas
Lung (135,136)	Hodgkin's lymphoma (143)
Esophageal (137)	Non-Hodgkin's lymphoma (144)
Colon (129,130)	Leukemia (145)
Breast (138)	Mesothelioma (146)
Stomach (131,134)	Melanoma (147,148)
Renal (139)	Wilm's tumor (149)
Ovary (140)	Hepatic adenoma (150)
Prostate (141)	Angiolymphatic hyperplasia (151)
Oropharynx (142)	Schwannoma (152)
	Neuroblastoma (153)
	Adrenal ganglioneuroma (127)
	Associated with graft-versus-host disease after stem cell transplant (154)

(Adapted from: Short C, Mallick NP. Membranous nephropathy. In: Schrier RW, ed. *Diseases of the kidney,* 7th ed. Philadelphia: Lippincott Williams & Wilkins; 2001:1743.)

TABLE 63-4

DRUGS/TOXINS ASSOCIATED WITH MEMBRANOUS NEPHROPATHY

Gold (59,60,156)
Penicillamine (157–159)
Bucillamine (61)
Mercury compounds (160)
Captopril (161–163)
Probenicid (164)
Trimethadione (165)
Non-steroidal anti-inflammatory drugs (65–68)
Cyclooxygenase-2 inhibitors (69)
Clopidogrel (166)
Lithium (167)
Formaldehyde (168)
Hydrocarbons (169)

(Adapted from: Short C, Mallick NP. Membranous nephropathy. In: Schrier RW, ed. *Diseases of the kidney,* 7th ed. Philadelphia: Lippincott Williams & Wilkins; 2001:1743.)

Drugs

Drug-associated MN can develop at any age and typically develops soon after exposure to the offending agent (Table 63-4) (155). The most commonly implicated drugs are gold, penicillamine, and NSAIDs (68,156–159). Mercury compounds have also been implicated (160). In these cases, removal of the offending drug usually leads to resolution of the nephrotic syndrome over a prolonged period of months to even years. Once again, caution should be exercised in attributing causation based upon isolated case reports alone.

Miscellaneous Conditions

MN has also been associated with diabetes mellitus, with or without associated diabetic nephropathy (170,171). Other diverse diseases that have been described in association with MN are listed in Table 63-5. Whether these are merely fortuitous associations or true causation is a matter of speculation, especially given the rarity of these reports.

Renal Allograft Membranous Nephropathy

While MN can recur in the renal allograft, a more common occurrence is the appearance of *de novo* membranous nephropathy (185–191). Prevalence rates for *de novo* MN in renal transplant recipients vary from 0.4% to 9.2% (185–191). In renal transplant patients who develop the nephrotic syndrome, *de novo* MN is the diagnosis in up to 30% and is the second most common cause of the nephrotic syndrome in renal transplant recipients, behind chronic allograft nephropathy (185–191). In the majority of cases, no secondary cause has been identified, although hepatitis B, C, and HIV infection have been identified in some patients (190,192). The pathogenesis is unknown but may relate to chronic production of low-avidity

TABLE 63-5

MISCELLANEOUS CONDITIONS ASSOCIATED WITH MEMBRANOUS NEPHROPATHY

Diabetes mellitus (170,171)
Sarcoidosis (73,74)
Sickle cell disease (172)
Polycystic kidney disease (173)
Alpha1-antitrypsin deficiency (174)
Weber-Christian disease (175)
Primary biliary cirrhosis (176,177)
Systemic mastocytosis (178)
Guillain-Barré syndrome (179,180)
Urticarial vasculitis (181)
Hemolytic-uremic syndrome (182)
Dermatitis herpetiformis (183)
Myelodysplasia (184)
(Adapted from: Short C, Mallick NP. Membranous nephropathy. In: Schrier RW, ed. *Diseases of the kidney,* 7th ed. Philadelphia: Lippincott Williams & Wilkins; 2001:1743.)

antibodies to histocompatibility antigens. While recurrent MN usually develops in the allograft within the first 6–12 months post-transplant, *de novo* disease usually occurs 2 or more years after transplantation (193–195). The majority of patients develop nephrotic-range proteinuria. Graft outcome in *de novo* disease is variable, with some patients progressing to graft failure (usually in the setting of heavy proteinuria and preexisting graft dysfunction), and others maintaining stable graft function (196,197). Overall, the bulk of data reveals no increased risk in graft failure in patients with *de novo* MN. Alteration of immunosuppression or additional cytotoxic therapy has not demonstrated any benefit and treatment should focus on reduction of proteinuria, control of blood pressure, and management of complications such as hyperlipidemia and venous thrombosis (198–200).

PATHOLOGY

It is essential that all histologic techniques be utilized in examining the renal tissue of patients with the nephrotic syndrome. While MN derives its name by the characteristic global thickening of the basement membrane on light microscopy, early in the course of the disease glomeruli may appear normal and the diagnosis can only be made with electron microscopy and/or immunofluorescence (201–203). Conceptually, it is useful to think of membranous nephropathy as beginning with the deposition of immune complexes in the subepithelial region of the glomerular basement membrane followed by the response of the basement membrane and glomerular epithelial cell to this deposition. Subsequent histologic changes are due to this response.

Light Microscopy

Light microscopy, with either hematoxylin and eosin (H&E) or periodic acid-Schiff (PAS) staining, reveals diffuse and uniform thickening of the glomerular basement membrane (Fig. 63-1). The basement membrane takes on the appearance of a thick eosinophilic loop (Fig. 63-2). These stains, however, do not distinguish the etiology of the basement membrane thickening (i.e., thickening due to deposition of immune complexes or increased basement membrane matrix production). These changes are best seen with silver methenamine, which binds to basement membrane matrix but not to immune complexes (Fig. 63-3). With this staining technique, characteristic ultrastructural changes in the basement membrane have been described by Ehrenreich and Churg (Table 63-6) (5). These stages represent a morphological but not necessarily a pathophysiological continuum (204). The initial stage is the formation of subepithelial immune complexes which is followed by the synthesis of new basement membrane that ultimately surrounds the complexes and is followed by the eventual digestion and disappearance of the immune complexes. On light microscopy with silver staining these changes can appear as a spectrum ranging from projections of the basement membrane ("spikes") (Fig. 63-3) corresponding to new basement membrane matrix synthesized by the GEC, to a chainlike appearance where the basement membrane completely incorporates the immune complexes (5). Later stages result in areas of lucency within the basement membrane that represent resolving immune complexes. These morphologic stages have not been correlated with

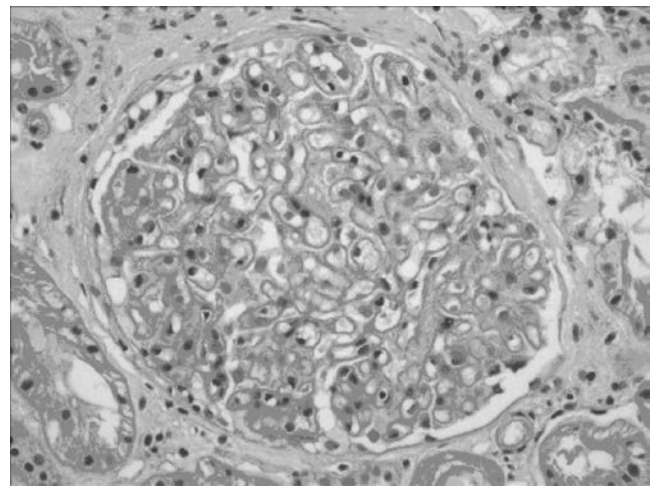

FIGURE 63-1. Hematoxylin and eosin stain (×250) of a glomerulus from a patient with idiopathic MN. There is diffuse thickening of the basement membrane without associated hypercellularity of the glomerular tuft. Inflammatory infiltrates are not seen and the capillary loops are widely patent. (Courtesy of Dr. Helen Cathro.)

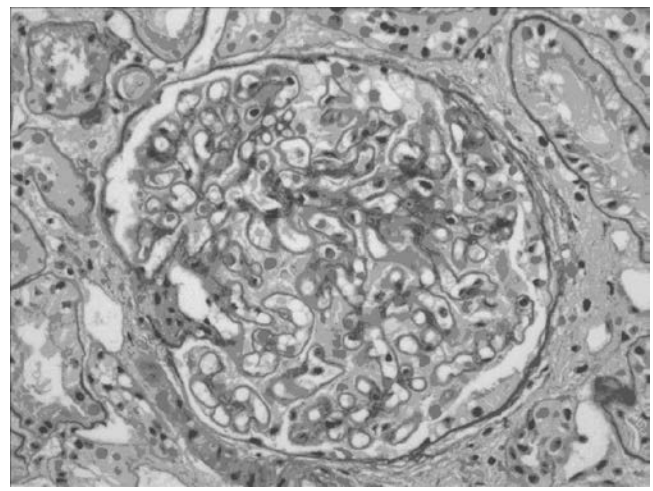

FIGURE 63-2. Periodic acid-Schiff stain of a glomerulus from a patient with idiopathic MN (×250). The basement membrane surrounding the capillary loops is diffusely thickened giving the appearance of "wire loops." (Courtesy of Dr. Helen Cathro.)

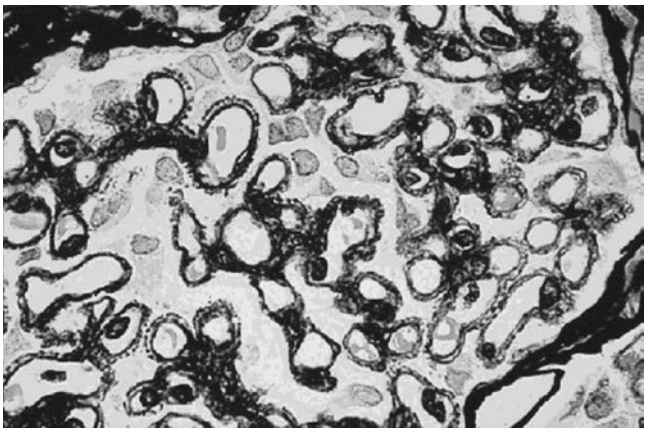

FIGURE 63-3. Jones silver stain (×250) of a glomerulus from a patient with idiopathic MN demonstrating "spikes" corresponding to newly synthesized basement membrane surrounding immune complexes. (Courtesy of Dr. Edward Klatt.)

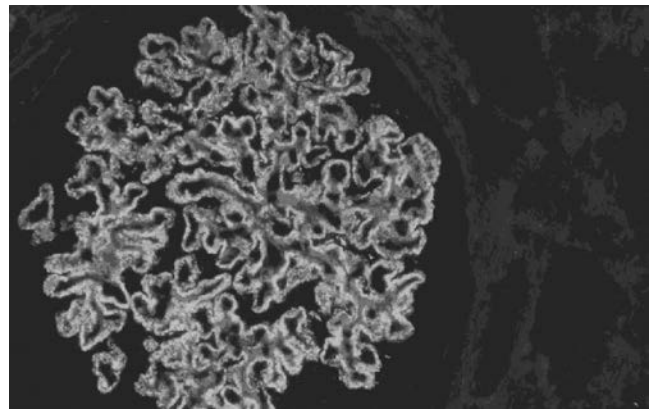

FIGURE 63-4. Immunofluorescence staining (anti-IgG) of a glomerulus from a patient with idiopathic MN (×250). Diffuse granular staining along the basement membrane is evident and corresponds to the deposition of immune complexes. Mesangial areas are free of immune deposits.

clinical findings and do not predict the degree of proteinuria, changes in renal function, responses to therapy or prognosis (204).

Other compartments of the glomerulus usually appear normal. There is usually no evidence of mesangial cell proliferation or expansion, except in the setting of SLE (205). There is also no evidence of inflammatory cell infiltrates which may relate to the fact that subepithelial immune complexes are unable to transmit chemotactic signals to effector cells in the circulation (as opposed to subendothelial deposits). In some cases, concurrent focal and segmental glomerulosclerosis can be seen and if present portends a worse prognosis (206,207).

Over time, the interstitial compartment develops fibrotic changes along with tubular atrophy. It is thought that these changes may reflect chronic, toxic effects of proteinuria on tubule cells (208–210). These tubulointerstitial changes correlate best with changes in renal function and offer important prognostic information (208–212).

Immunofluorescence Microscopy

Immunofluorescence (IF) microscopy reveals fine granular deposits of IgG and often complement components that follow the glomerular basement membrane and spares the mesangium (Fig. 63-4) (213). The predominant immunoglobulin is always IgG (often IgG4 subclass) with minimal staining for IgA and IgM (214,215). The presence of strong staining for other immunoglobulins and C1q is suggestive of MN due to SLE or other secondary causes (216). In approximately 50% of cases, staining for C3 is seen and tends to be a marker for active and more severe disease (217,218). If performed, staining for the membrane attack complex (C5b-9) is usually positive and likely reflects the important role of this complex in mediating injury to the GEC (217–219).

TABLE 63-6

PATHOLOGICAL STAGING OF MEMBRANOUS NEPHROPATHY

Stage	Electron microscopy
I	Subepithelial electron-dense deposits
II	Subepithelial electron-dense deposits with intervening basement membrane ("spikes")
III	Incorporation of subepithelial electron-dense deposits into the basement membrane
IV	Reabsorption of deposits with loss of electron-dense deposits and development of lucent area in the basement membrane
	Remodeling of basement membrane and loss of electron-dense deposits

(From: Ehreneich T, Churg J. Pathology of membranous nephropathy. *Pathol Annu* 1968;3:145.)

Electron Microscopy

As noted above, Ehrenreich and Churg described a morphologic continuum of evolutionary stages of MN by electron microscopy (5). Four stages (I through IV) are described ranging from early changes (stage I) to advanced and late changes (stage IV) (Table 63-6). These stages serve as a useful descriptive tool but have not been correlated with clinical features of MN (211).

The hallmark of MN is the finding of electron-dense deposits corresponding to immune complexes (Fig. 63-5). These deposits are located along the glomerular basement membrane in a subepithelial location adjacent to the GEC (220). Electron-dense deposits are not found in the mesangium except in the case of SLE (221). In stage I lesions, the deposits tend to be small and finely granular and there is no response of the basement membrane. However, there is evidence of GEC injury as foot cell processes are effaced. Progressive changes to the glomerular basement membrane then ensue. The basement membrane begins to project between the immune deposits (stage II) forming the characteristic spikes seen on silver staining. Eventually these outgrowths of new basement membrane matrix material begin to coalesce and completely surround the immune deposits (stage III). Over time, the immune deposits undergo dissolution leaving electron lucent areas in the basement membrane (stage IV). At this stage, the glomerular basement membrane is thickened and considerably disrupted. While it is generally believed that these stages develop in a continuum, there are no studies of follow-up biopsies in single patients over time.

The changes to the glomerular basement membrane seen on light or electron microscopy do not relate to the degree of proteinuria or to renal function (211). While it is believed that

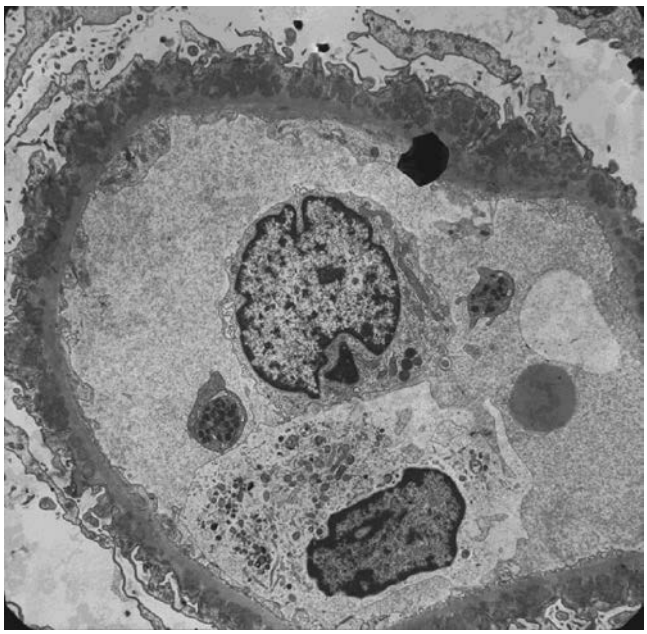

FIGURE 63-5. Electron micrograph of a glomerulus from a patient with idiopathic MN revealing characteristic electron-dense subepithelial deposits (×5000). In this micrograph, basement membrane can be seen to encircle the deposits forming the spikes seen on Jones silver staining (stages II and III). (Courtesy of Dr. Helen Cathro.)

patients with earlier stage lesions may have a better prognosis, it has been demonstrated that patients can go into clinical remission at any morphological stage (204). Renal function and prognosis is more dependent on the extent of interstitial damage.

PATHOGENESIS

Various lines of evidence support the idea that MN represents an autoimmune disorder. There is an association with other autoimmune diseases and a strong association with HLA-DR3 (70-72). There is characteristic deposition of immune complexes in the glomerular basement membrane and a strikingly similarity of the human lesion to the rat lesion of Heymann nephritis mediated by antibodies to the GEC (11–15). The recent finding of a human case of antenatal MN induced by maternally transmitted antibodies also supports an autoimmune etiology (16).

The majority of experimental work on the pathogenesis of MN has been performed in rats utilizing the Heymann nephritis model (11–15). In this model, a crude preparation of tubular brush-border extract (Fx1α) is injected into allogeneic animals. The injected animals then develop pathogenic antibody-antigen complexes that are localized to the subepithelial space and a lesion identical to MN develops (11–15). How immune complexes localize to their unique position in the subepithelial region has been the object of much speculation (222–226). First, intact circulating immune complexes could become trapped as a preformed entity in the subepithelial space. Alternatively, these immune complexes could dissociate and then reform after traversing the basement membrane. Finally, immune complexes could form *in situ* in the subepithelial space with circulating antibodies reacting to intrinsic glomerular antigens or to extrinsic antigens (either endogenous or exogenous) that become planted in the subepithelial space. Further work on the Heymann nephritis model supports the model that circulating antibodies react with either intrinsic glomerular epithelial cell antigens or to planted extrinsic antigens (227). An accelerated form of this nephritis can be induced by the injection of preformed antibody into rats (passive Heymann's nephritis), suggesting that subepithelial immune deposits can be formed without the formation of circulating immune complexes (228–230). The antigen responsible for the development of the immune complex in the rat model has been determined to be a large glycoprotein (megalin or gp330) complexed to a smaller receptor associated protein (RAP) which is expressed in the clathrin-coated pits of the glomerular epithelial cell foot processes (231–235). Once antibody binds this antigen complex, the complex is patched in the clathrin-coated pits, capped and extruded from the cell membrane (227,236). The complexes then lie in the subepithelial region where they persist as the electron-dense deposits seen on electron microscopy. Although megalin has been found in human proximal tubules, it has not been detected in human glomeruli or in immune deposits in patients with MN, thus limiting the applicability of the Heymann nephritis model (237). However, a recent case of antenatal MN in humans has been described in which maternal antibodies to neutral endopeptidase (NEP) were passively transferred to the infant (16). These antibodies reacted against NEP antigen on the glomerular epithelial cell with the formation of subepithelial immune complexes and the development of the nephrotic syndrome. The mother had no apparent renal abnormalities since she was deficient in NEP and had previously been sensitized to this antigen from a prior miscarriage (238). Thus, one mechanism of subepithelial immune complex formation in MN is the reaction of circulating antibody with antigens expressed on the GEC. Whether exogenous antigens (especially small cationic proteins) can localize to the subepithelial region and also elicit immune complex formation is still unknown.

In the rat model, once immune complexes form in the subepithelial space, complement activation occurs and is necessary to produce proteinuria (239–243). Complement activation leads to the cleavage of C5 with the formation of C5a and C5b. Given the absence of inflammatory cells in MN, it seems plausible that the chemotactic factor C5a is filtered in the urine and does not move backwards across the basement membrane to attract inflammatory cells. C5b then combines with other complement components to form the membrane attack complex (C5b-C9) which inserts into the membrane of the GEC (244–248). The complex is endocytosed into the GEC and then extruded into the urinary space where it can be identified in the urine (249–254). The GEC is resistant to the lytic effects of the C5b-C9 complex but instead undergoes metabolic activation with the resulting production of proteases (matrix metalloprotease 9), prostaglandin metabolites, oxidants, growth factors, and cytokines (255–258). A protein that is upregulated in response to GEC activation is cytochrome b_{558}, an integral component of the NAPDH oxidoreductase complex that leads to the formation of toxic reactiveoxygen species that damage the basement membrane (259). Transforming growth factors are also produced by the GEC and lead to formation of new basement membrane matrix components such as type I and IV collagen (260). Finally, the podocyte slit diaphragm, an important barrier to proteinuria, is altered by the activation of complement (261). Nephrin, a major and essential component of the slit diaphragm, is linked to the actin cytoskeleton of the GEC via adapter molecules that include CD2AP and podocin (262–264). In passive Heymann nephritis, activation of complement led to alteration in the distribution of nephrin and in its association with actin, thus disrupting the slit diaphragms (261). This dissociation of nephrin from actin was not seen if complement was depleted. Thus the consequences of injury induced by C5b-9 are multifaceted and ultimately lead to the clinical expression of proteinuria and altered renal function.

Antigens on the surface of the GEC may have important enzymatic roles and antibodies to these proteins may lead to

important alterations in function that may have pathogenic significance (238). For instance, NEP is a metallopeptidase that is responsible for the cleavage and inactivation of vasoactive mediators such as bradykinin and endothelin (265). Neutralizing antibodies to this antigen would result in higher local concentrations of these vasoactive mediators that could alter glomerular hemodynamics and capillary wall permeability. Whether these mechanisms are operative in the pathogenesis of MN remains speculative.

CLINICAL FEATURES

The onset of clinical disease is usually insidious with the majority of patients (70% to 80%) presenting with peripheral edema and the nephrotic syndrome (266). In 20% of cases, non-nephrotic range proteinuria is found and usually these patients are identified through an abnormal urinalysis that is performed for an unrelated reason such as an insurance examination (267). Secondary causes of MN may present with a more abrupt onset (268). The proteinuria tends to be nonselective and averages 5 to 10 g/day (although this can vary widely depending upon salt and protein intake and medications that the patient may be taking). Microscopic hematuria occurs in up to 50%, but macroscopic hematuria is rare. Hypertension may be seen in up to 30% of patients but is usually mild. Renal function at the time of presentation is usually well preserved and when impaired often reflects prerenal factors, such as over zealous diuretic use, more than intrinsic renal disease. Proteinuria is usually present for an extended period of time (months to years) before renal impairment develops (269–271).

Thromboembolic complications, such as deep vein thrombosis, pulmonary embolism, and especially renal vein thrombosis, can be the presenting feature in some patients (272, 273). These complications are more common in patients with heavy, persistent proteinuria and serum albumin concentrations below 2.5 g/dL (274,275). These patients tend to have hyperfibrinogenemia with increases in procoagulant factors (factors V and VIII), along with decreases in anticoagulant factors (such as antithrombin III) (276,277). High levels of lipoprotein (a) in MN patients may serve as an inhibitor of plasminogen and thus inhibit thrombolysis (278). Renal vein thrombosis (RVT) is especially common in MN, developing in anywhere from 5 to 50% of cases (279–281). MN is the most common associated condition seen in patients with RVT. Patients may be asymptomatic or present with flank pain, hematuria, or deterioration in renal function (279–281). Alternatively, patients may present with embolic complications such as pulmonary emboli (279–281). The diagnosis can be made with noninvasive imaging studies such as Doppler ultrasonography, spiral computerized tomography, or magnetic resonance angiography. Venous angiography is the gold standard and is reserved for diagnostic dilemmas.

Uncommonly, patients with MN may present with or develop a superimposed rapidly progressive glomerulonephritis usually in the presence of anti–glomerular basement membrane antibodies (282–286). Renal biopsy will demonstrate typical crescents as well as subepithelial immune deposits.

LABORATORY FINDINGS

Laboratory findings in patients with MN generally reflect the ongoing proteinuria and nephrotic syndrome. Thus, hypoalbuminemia, hyperlipidemia and lipiduria are common findings (287). A typical lipid profile will reveal increased levels of total cholesterol, low-density lipoproteins (LDL), and very-low-density lipoproteins (VLDL) (288). High-density lipoprotein levels are either normal or low (288). The elevation in LDL is thought to be secondary to hepatic overproduction and is stimulated by the low serum albumin level and reduced oncotic pressure (289). Lipoprotein (a) levels are also elevated (290,291).

Studies should be performed to exclude secondary causes of MN and include: anti-nuclear antibody, C3 and C4 levels, hepatitis B and C profiles, and age-appropriate cancer screening (mammography, prostate-specific antigen, chest radiography, colonoscopy, and careful thyroid examination). In those patients over age 60, an underlying malignancy should always be suspected. Syphilis serologies may also be useful in selected patients. Since the differential diagnosis of MN includes other causes of the nephrotic syndrome such as minimal change disease, focal glomerulosclerosis, membranoproliferative glomerulonephritis, diabetic glomerulosclerosis, monoclonal immunoglobulin deposition diseases, and amyloid; other useful laboratory tests include assessment of HIV status and serum and urine protein electrophoresis. Renal biopsy is essential for the correct diagnosis and biopsy material must be analyzed by all histologic techniques. Unlike the diagnosis of other renal diseases, sample size is not a problem in making the pathologic diagnosis of MN. The diagnosis can be made on a single glomerulus with typical subepithelial immune deposits.

Given the importance of the membrane attack complex (C5b-C9) in the pathogenesis of MN, there has been much interest in measuring the levels of this complex in the urine of patients with MN (250–254,292,293). In experimental animals, the appearance of C5b-9 in the urine closely parallels the level of disease activity and the formation of new immune deposits (250). In one human study, C5b-9 levels in the urine were elevated in 12 of 17 patients with progressive disease and in only 2 of 18 patients with stable disease (254). This assay is not widely available and further studies are still needed before this assay can be routinely used in the diagnosis and treatment of MN.

NATURAL HISTORY AND PROGNOSIS

The clinical course of MN is quite varied and complex (37). Various natural history studies have been performed and describe several possible outcomes (9,10,23,24,124,125,294–297). These include: (a) complete and lasting remission of the nephrotic syndrome with stable renal function; (b) complete remission of the nephrotic syndrome punctuated by one or more relapses but with stable renal function; (c) a partial remission of the nephrotic syndrome (generally defined as greater than a 50% reduction in proteinuria and proteinuria $\leq$3.5 g/day) with either stable or slowly deteriorating renal function; (d) persistence of nonnephrotic range proteinuria with or without progressive deterioration in renal function; (e) persistence of nephrotic range proteinuria with slowly progressive deterioration of renal function; or (f) persistence of nephrotic range proteinuria with development of superimposed acute or rapidly progressive renal failure (37). The approximate prevalence of these outcomes in untreated patients has been determined from several natural history studies and from placebo arms of intervention studies (9,10,23,24,124,125,294–297). Spontaneous complete remissions are seen in approximately 25% to 30% of patients within 3 to 5 years after presentation. Another 20% to 25% of patients will have partial remissions with persistent proteinuria $\leq$3.5 grams per day and stable renal function. However, of these patients who undergo remissions, nephrotic-range proteinuria may recur in 15% to 30% of patients. Thus, nearly 50% of patients will have persistent nephrotic syndrome. These latter patients are at much higher risk of developing progressive renal dysfunction (298).

In fact, 25% to 40% of these patients will develop end-stage renal disease (ESRD) or death within 5 years. This number rises to 50% or more after 10 years of persistent proteinuria. Progression to ESRD is uncommon (<1%) in patients who experience at least one complete remission and in those with persistent non-nephrotic-range proteinuria (299). One caveat to these natural history studies is that many of these patients did not receive aggressive anti-proteinuric therapies such as angiotensin-converting enzyme inhibitors (ACE-inhibitors) or angiotensin receptor blockers (ARBs). Furthermore, lipid abnormalities were not aggressively treated with statin drugs. Whether the natural history would be altered by these therapies is not known.

Several prognostic factors have been identified that when present are associated with an unfavorable course (9,10,23,24,124,125,294–303). These include: advanced age, male sex, reduced renal function at presentation or within 5 years of presentation, nephrotic range proteinuria (especially if persistent), excretion of increased quantities of low-molecular-weight proteins (signifying tubular abnormalities), hypertension, persistent hyperlipidemia, persistently elevated urinary C5b-C9 excretion, chronic tubulointerstitial fibrosis or focal sclerosis on renal biopsy, and the presence of HLA-B18 DRw3, or BfF1 DR5. The factors that seem to be most important in predicting both a spontaneous remission and its durability are persistent, low-grade (subnephrotic) proteinuria and female gender (9,10,23,24,124,125,294–302).

The natural history of MN makes treatment decisions difficult. Since only a subset of patients will progress to renal failure and this progression occurs over an extended period of time, therapy (especially with potentially toxic immunosuppressants) must be tailored to those patients at greatest risk for a poor outcome. Thus, efforts to identify these patients are critically important. In this regard, several of the prognostic features listed above have greater predictive ability than others. The most important prognostic variables have proven to be: age, male sex, level of renal function, and degree of proteinuria (9,10,23,24,124,125,294–303). The likelihood of progression to renal failure in those patients with an elevated serum creatinine level may be as high as 70% as compared to less than 20% in patients with normal renal function (298,304). Proteinuria is excess of 3.5 grams per day is associated with a two- to three-fold increased risk of progressive disease and has often been used as criteria to initiate therapy. If the proteinuria is massive (>8 g/day) and persistent (lasting >6 months) the risk for progression is especially high (84%) (298,304). Cattran et al. have developed a predictive model using data from 184 patients with MN from the Metro Toronto Glomerulonephritis Registry (305,306). This model had 85% accuracy in predicting which patients will subsequently develop renal insufficiency. The model has also been validated in a total of 179 patients from Italy and Finland (306). The model calculates a probability of progression from knowledge of the highest level of persistent (≥6 months) proteinuria, the creatinine clearance at the beginning of this period and the slope of the creatinine clearance during the observation period. Based upon calculations from this model, if immunosuppressive agents were restricted to only those patients at high risk for progressive disease, then of the 363 patients analyzed, only 100 patients would receive potentially toxic therapy (305). This model has not been applied prospectively and thus cannot be widely recommended at this time.

THERAPY

The goal of therapy for patients with MN is the prevention of renal failure as well as the reduction of proteinuria and minimization of the complications associated with MN. These aims must be weighed against the risk of therapy, especially given the variable natural history of the disease. Multiple controlled trials have investigated numerous therapeutic approaches to patients with MN. Despite the wealth of trials, therapy for MN remains controversial with proponents advocating a spectrum of therapy ranging from aggressive immunosuppressive therapy to no disease-specific therapy. In part, these divergent opinions are due to difficulty in interpreting the trial data. Many trials enroll small numbers of patients with limited periods of follow-up. Given a slowly progressive disease with spontaneous remissions and relapses, long-term studies with comparable control groups are needed to adequately evaluate interventions. In many studies, many of the variables that determine prognosis are not controlled. Thus, the risk of progression in the control and intervention groups may be quite different. Finally, in many older studies, non-disease-specific therapies such as antihypertensive therapy with ACE-inhibitors or ARBs, lipid-lowering therapy, and dietary manipulations are not incorporated into the treatment regimen. Despite these limitations, several options for the treatment of MN exist.

Non-Disease-Specific Therapy

These nonimmunosuppressive therapies should be implemented in all patients with MN and include strategies to lower proteinuria, decrease the rate of disease progression, and prevent complications of the nephrotic syndrome, especially hyperlipidemia (307). Numerous studies have documented the role of ACE-inhibitors and ARBs in reducing proteinuria and the rate of renal disease progression in both diabetic and non-diabetic nephropathy (308–312). These drugs reduce intraglomerular pressure, improve glomerular barrier size selectivity, and have antiinflammatory properties (309,310,313). The benefit of ACE-inhibitors seems to be greatest in those patients with the greatest degree of proteinuria (308–312). Few studies specifically comment on the use of ACE-inhibitors in patients with MN. In one small study of 14 patients with MN, enalapril decreased proteinuria from 7.1 to 5.0 g/day (314). This was accompanied by an improvement in glomerular membrane size-selectivity as determined by dextran sieving coefficients. Given that the goal of therapy is to reduce proteinuria to non-nephrotic ranges or ideally to less than 500 mg per day, it is unlikely that ACE-inhibitors alone will be able to achieve this goal in nephrotic patients. Furthermore, the goal blood pressure in proteinuric patients should be 125/75 mm Hg, a difficult goal on a single antihypertensive medication. Recent evidence supports that combination therapy with both ACE-inhibitors and ARBs may affect more complete blockade of the renin–angiotensin–aldosterone system (315). Several trials have shown a synergistic reduction of proteinuria using this combination. In one trial, including 28 patients with MN, this translated into slower progression of nephropathy (316). One small study also investigated the addition of the aldosterone antagonist spironolactone to ACE-inhibitor therapy in 11 patients with MN. In this trial, blood pressure fell with the addition of spironolactone but urine protein excretion remained unchanged (317). In all cases, a low-sodium diet (100 mEq/day) should be instituted to maximize the antiproteinuric effects of these medications.

Nonsteroidal antiinflammatory agents also have been shown to have antiproteinuric effects (318–320). Their role in the treatment of MN is controversial. In one study where indomethacin was added to ramipril therapy, there was no additional reduction in proteinuria (321). However, there was a higher risk of side effects including hyperkalemia and a reversible rise in serum creatinine. These agents are not routinely recommended.

Patients with MN and the nephrotic syndrome typically have elevated cholesterol levels, with 66% of patients having

total cholesterol levels greater than 300 mg/dL, and 70% of patients with low-density lipoprotein (LDL) levels greater than 160 mg/dL (288,322). This translates into an increased risk for cardiovascular events (288,322). In a prospective cohort study of 142 nephrotic patients the relative risk for myocardial infarction versus controls was 5.5 and for cardiac death it was 2.8 (323). Furthermore, in patients reaching ESRD, the leading cause of mortality is cardiovascular disease (25). Finally, hyperlipidemia has also been associated with an increased risk for declining GFR, and a meta-analysis has concluded that lipid reduction may have a role in reducing the rate of progression of renal disease (324–328). Treatment of hyperlipidemia rests on treatment of the underlying nephrotic syndrome, dietary modification, and antihyperlipidemic medications (329–332). 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors can lower LDL and total cholesterol levels by up to 50% in nephrotic patients and are the cornerstone of antihyperlipidemic therapy (329–332). Other agents include: ezetimibe, fibric acid derivatives, and bile acid sequestrants (329–334). While target goals for LDL have not been set for patients with the nephrotic syndrome, it seems reasonable to place these patients in the group at high risk for cardiovascular events with a goal LDL of less than 100 mg/dL (335).

Patients with MN are clearly at higher risk for venous thromboembolism (279–281). Whether to initiate prophylactic anticoagulation with warfarin at time of diagnosis of MN or to postpone treatment until a thrombotic event occurs remains controversial. Some clinicians recommend prophylactic anticoagulation in patients with serum albumin levels less than 2 g/dL and nephrotic range proteinuria, believing that the risk of thromboembolism in this group is greater than any risk of bleeding events (336). However, no prospective trial has examined this question. In those patients who do develop a thrombotic event, anticoagulation with warfarin to a goal international normalized ratio (INR) of 2 to 3 should be initiated (279–281). There are no data on the use of antiplatelet agents in this setting.

Corticosteroids

Two early controlled trials in the United Kingdom reported a small but statistically insignificant benefit in patients with MN treated with corticosteroids (336,337). However, these study were small (15 patients in one study and 19 in the other), used low doses of corticosteroid (20 to 30 mg/day) and had short-term follow-up. In 1979, the Collaborative Study of the Adult Idiopathic Nephrotic Syndrome was performed (338). In this prospective, randomized, placebo-controlled trial, 72 patients with the nephrotic syndrome due to MN were randomized to either an 8- week course of high-dose alternate-day prednisone (125 mg) followed by a tapering dose or a placebo. In the prednisone-treated group there were an increased number of remissions of the nephrotic syndrome. Over time, there were also an increased number of relapses in this group, so that at the end of 2 years, the prevalence of both complete and partial remissions were identical in the two groups. The major difference between the groups was in the level of renal function. After 6 months of therapy, the serum creatinine had doubled in only 2 of 34 steroid-treated patients, as compared to 11 of the 38 patients who received placebo. This study has been widely criticized since the risk and rate of progression of the control group was much higher than previous studies had reported for an untreated cohort of MN patients. Thus, the risk for disease progression was likely different in the two groups.

Subsequently, two other trials have examined the role of corticosteroids in the treatment of MN. In 1990, the MRC study evaluated 103 patients with MN using a similar protocol to the U.S. Collaborative Study (339). Patients were followed for a mean of 4 years and during this time there were no significant differences in either urinary protein excretion or rate of renal loss between the treatment and control groups. The Toronto Glomerulonephritis Study Group examined 158 patients with MN (340). Patients received either a 6-month course of prednisone (45 mg /m^2 of body-surface area) or placebo and were followed for a mean of 48 months. The proportion of patients with complete remission of proteinuria was similar in the two groups, as was the annual change in creatinine clearance. Progression to ESRD was identical in the two groups. A multivariate analysis adjusting for factors that may be related to the risk of disease progression failed to show any effect of prednisone on the outcome. One important difference between the U.S. Collaborative Study and the Toronto Study was that patients in the Toronto study had a longer median duration of disease prior to treatment (15 to 17 months vs. 6 months in the U.S. Collaborative Study) and more advanced glomerular changes (stage III to stage IV) on biopsy. Whether corticosteroid therapy is beneficial in patients with early lesions and a short duration of disease is not known. A meta-analysis of trials using corticosteroids for MN could find no evidence of benefit (341–342). Overall, the balance of evidence does not support the utility of corticosteroid therapy alone in either leading to remission of the nephrotic syndrome or in improving renal survival.

Cytotoxic Therapy

Initial trials of cytotoxic agents with or without corticosteroids generally involved small numbers of patients with short follow-up. In 1971, the MRC investigated the role of prednisone plus azathioprine in 14 patients with MN with no statistically significant effect of therapy on the rate of disease progression, although there was a trend toward lower serum creatinine in the treated group (337). A study from the Canadian Medical Association also found no benefit from azathioprine therapy (343). A more promising approach has been the use of either cyclophosphamide or chlorambucil. In 1975, Lagrue et al. studied the effects of a 1-year course of azathioprine (11 patients), chlorambucil (16 patients), or no treatment (14 patients) (344). There was no effect of azathioprine with 25% of patients in both the azathioprine-treated and control groups achieving a remission. However, 81% of patients treated with chlorambucil achieved either a partial or complete remission. Several subsequent studies utilized cyclophosphamide (often in addition to warfarin and dipyridamole) and found that active treatment tended to diminish proteinuria while having no effect on renal function (346–349). The interpretation of these studies is hampered by the small numbers of patients studied, the short duration of follow-up and lack of appropriate control groups.

The most effective regimen utilizing cytotoxic agents has been reported by Ponticelli et al. (350). This complex protocol involves three doses of intravenous methylprednisolone (1.0 gram daily), followed by oral prednisone (0.4 to 0.5 mg/kg/day) for a month. This regimen is then alternated monthly with oral chlorambucil (0.2 mg/kg/day) for a month. The entire treatment protocol lasts 6 months. The first report of this protocol was published in 1984 (349). Sixty-seven patients were randomized to either treatment with methylprednisolone plus chlorambucil or to placebo. After a mean follow-up of 31.4 months, 23 of 32 treated patients were in complete (12 patients) or partial remission as compared with 9 of 30 control patients. Renal function remained stable in the treated patients whereas there was progressive deterioration in the control group. Five years later, a larger cohort of patients that had received this protocol for an average of 5 years was reported (350). Plasma creatinine increased by 50% or more in 19 (49%) control patients but in only 4 (10%) treated patients. In the control group, only 9 patients (23%) achieved remission

of the nephrotic syndrome versus 67% of the treated patients. Follow-up of this protocol has now been extended out to 10 years with 40% of the control patients reaching an endpoint of either death or dialysis as compared with 8% of the treated patients (351). When patients with MN were treated with either methylprednisolone versus methylprednisolone plus chlorambucil it was clear that the beneficial effects of this regimen were related to the alkylating agent (352).

There have been concerns over the use of chlorambucil given its potential toxicity (leukopenia and risk of infectious complications). Furthermore, most clinicians in the United States have had little experience with this drug. Ponticelli et al compared oral chlorambucil and cyclophosphamide in a head–to-head trial utilizing the same 6-month protocol as prior studies (353). Cyclophosphamide was dosed orally at a daily dose of 2.5 mg/kg. In this study, 36 of 44 (82%) patients on chlorambucil achieved a complete or partial remission versus 40 of 43 (93%) patients assigned to cyclophosphamide. Relapses occurred in 30.5% of the chlorambucil-treated patients and in 25% of cyclophosphamide-treated patients over 6 to 30 months of follow-up. Renal function remained stable in both groups. Importantly, herpes zoster infections were more common (9%) in patients treated with chlorambucil. A similar study from Williams et al. also compared chlorambucil and cyclophosphamide as given by the Ponticelli protocol (354). After a mean follow-up of 32 months, the cyclophosphamide group had better renal function, with ESRD occurring in only 1 of 17 patients as compared to 5 of 15 patients treated with chlorambucil. Infectious complications were less common in the cyclophosphamide group (354). Several other studies have confirmed the benefit of oral cyclophosphamide in protocols similar to that of Ponticelli et al. (355,356). Interestingly, two controlled studies have not shown a benefit of monthly intravenous pulse cyclophosphamide therapy (357,358).

A regimen of corticosteroids and cytotoxic agents has been studied in patients with progressive renal insufficiency. Bruns et al. studied in 11 patients with MN and rising serum creatinines and showed a decline in serum creatinine as well as proteinuria with a regimen of one year of cyclophosphamide 100 mg orally a day along with daily prednisone (359). Branten et al. treated 39 patients with a 1-year course of oral cyclophosphamide with corticosteroids and demonstrated a 38% median reduction of proteinuria (360). Jindal et al. treated 9 patients with MN with a mean baseline creatinine of 2.6 mg/dL with oral cyclophosphamide and corticosteroids (355). At the end of the treatment period there was a fall in mean creatinine to 2.2 mg/dL and proteinuria fell from 11.1 to 2.2 g/day. However, after treatment there was a progressive rise in creatinine levels to 2.9 mg/dL after 83 months.

In those patients who do suffer a relapse after a course of cytotoxic therapy, a second course of cytotoxic agents has been shown to be beneficial in reduction of proteinuria and in extending dialysis-free survival (361–363). However, these studies are small, uncontrolled, and subject patients to the risks of cumulative cytotoxic therapy.

Two meta-analyses of cytotoxic therapy for MN have shown that cytotoxic therapy leads to a more frequent remission of the nephrotic syndrome (342,364). However, a conclusion regarding the effects of therapy on renal function could not be drawn given the lack of large, prospective trials with long-term follow-up.

Mycophenolate Mofetil

Mycophenolate mofetil (MMF), an inhibitor of purine biosynthesis in activated lymphocytes, has also been studied in the treatment of resistant MN. Many of the published reports describing the efficacy of this agent are uncontrolled case reports subject to reporting bias. Choi et al. studied 17 patients with MN that were either resistant or intolerant to therapy with corticosteroids or cytotoxic agents. There was a significant reduction in proteinuria with two patients achieving a complete remission and 8 achieving a partial remission (365). Renal function remained stable in the treated patients. Miller et al. studied 16 patients with MN at high risk of progression with baseline proteinuria over 9 g/day (366). All patients had failed corticosteroid therapy, six patients had failed cytotoxic therapy, and five patients had failed cyclosporine. Patients were treated for a mean of 8 months with MMF with a target dose of 2 g/day. There was no change in mean proteinuria during the study and only six patients had a partial response. There was no change in serum creatinine during the study. At this time, the experience with MMF is too limited to draw any firm conclusions.

Cyclosporine

Cyclosporine has been studied in several randomized prospective clinical trials in patients with MN (367–373). Generally, the patients who have been studied are those that have moderate or high degrees of risk for progressive disease and had often failed other therapies such as cytotoxic agents.

Patients that had failed an 8-week course of corticosteroid therapy and had persistent nephrotic-range proteinuria were randomized to either cyclosporine (CSA) (28 patients) or placebo (23 patients) (371). Cyclosporine trough levels were maintained between 125 and 225 ng/mL (average dose 3.7 mg/kg/day) for a total of 6 months of therapy. Patients also received prednisone therapy, up to a maximum of 15 mg/day. Therapy was well tolerated with no patients discontinuing therapy. At the end of 6 months of therapy, 75% of the CSA-treated patients versus 22% of the control group had a remission in proteinuria. After 1 year, remission rates fell to 48% in the CSA-treated group and 13% of the placebo group. The overall rate of the change in renal function between the two groups was identical. Another trial from China randomized 30 patients to either CSA (initial dose of 5 mg/kg/day which was tapered after 3 months to 2 mg/kg/day and continued for a total of 15 months) or captopril (373). At the end of 15 months, 11 of 15 patients in the CSA-treated group were in either complete or partial remission as compared to 2 of 15 patients treated with captopril alone. In both trials there was a high rate of relapse (up to 50%) with longer follow-up after CSA withdrawal. Despite this, there remained a larger fraction of patients in remission after CSA treatment than with placebo.

Rostoker et al. performed an open-label study in patients with MN at high risk for progression (369). These patients had shown a progressive rise in serum creatinine before the initiation of therapy. Fifteen patients were treated with CSA to maintain a 12-hour trough level between 100 and 250 ng/mL. Four of the 15 patients showed no response to therapy and the drug was discontinued after 4 months. In the remaining 11 patients, CSA was continued for 6 months, followed by tapering over 6 months. In this group, four patients had a complete remission and seven had a partial remission. Nine patients discontinued CSA, and 33% had a relapse of their proteinuria that responded to reintroduction of the drug.

Another open-label study in patients at high-risk for progressive disease studied 41 patients with a mean urine protein excretion of 10.9 g/day (375). Patients received a mean CSA dose of 3.3 mg/kg/day for 1 year. At the end of therapy, 14 patients had entered a complete remission with a mean decrease in proteinuria of 6 g/day. In those patients who entered a complete remission, the mean treatment time before a response was seen was 225 days. There was only a small rise in serum creatinine (0.3 mg/dL) during the trial.

Only one randomized controlled trial has been performed in patients at high risk for progressive renal disease (372). Entry criteria for this trial included an absolute fall in creatinine

clearance of 8 mL/minute or greater over a 4- to 12-month observation period. Furthermore, patients had persistent nephrotic-range proteinuria. Seventeen patients were selected for randomization to either CSA alone (trough level of 110 to 170 ng/mL) or placebo. Proteinuria fell in the treatment group by an average of 4.5 g/day as compared to a rise in proteinuria of 0.7 g/day in the placebo group. Most importantly, CSA therapy improved the average change in the slope of creatinine clearance versus time (from –2.4 mL/min/month prior to therapy to –0.7 mL/min/month with therapy). There was no change in the rate of decline in renal function in the placebo group. Importantly, the effects of CSA on improved renal function were maintained for up to 2 years after the medication was stopped.

In these trials, CSA therapy has been associated with transient increases in serum creatinine, hypertension, and gingival hyperplasia (368-375). Infectious complications were not seen and overall the medication has been well tolerated. The major drawback has been a relapse rate as high as 50% within 2 years of drug withdrawal (368–376). This may be minimized by extended therapy but further trials are needed. The mechanism by which CSA may decrease proteinuria and preserve renal function is unclear. The drug may act to decrease T cell–derived cytokine production as well as directly altering glomerular permeability to proteins through an unknown mechanism (376).

Alternative Therapies

Several alternative therapies have been studied in patients with MN. These are generally small, uncontrolled trials with short follow-up but have provided interesting results and point the direction for future therapies.

Intravenous immunoglobulin has been given in repeated doses to patients with severe MN (377,378). In these cases, both proteinuria and renal function has shown improvement. There are no reports of the long-term outcome of these patients.

Pentoxifylline, a phosphodiesterase inhibitor, was given to 10 patients with MN for 6 months and significantly reduced proteinuria from 11 to 1.8 g/day (379). Renal function remained stable in these patients. Pentoxifylline is an inhibitor of tumor necrosis factor-alpha (TNF-α) and plasma and urinary levels of TNF-α significantly fell in the treated patients (380). Whether pentoxifylline may serve as a useful adjunct to cytotoxic or CSA therapy has not been studied.

Based upon the experimental data demonstrating the importance of B cell activation and immunoglobulin production in the pathogenesis of MN, a monoclonal antibody that targets the B cell surface antigen CD20 has been employed in the treatment of patients with MN (381–383). Eight patients with MN and persistent nephrotic-range proteinuria were treated with four weekly infusions of the anti-CD20 monoclonal antibody rituximab. Two patients entered complete remission and three patients had a partial remission. In all patients, there was a substantial fall in urinary protein excretion with the mean protein excretion falling from 8.6 to 3.0 g/day at 1 year of follow-up. Renal function remained stable in all patients. The drug was well tolerated with no major adverse events during the trial. This therapy, which may specifically target autoreactive B cell clones, holds great promise for a disease-specific therapy that may have minimal long-term side effects. Obviously, larger randomized controlled trials with extended periods of follow-up are needed.

Future therapies are being developed that target the immune system. Eculizumab is a humanized C5 complement inhibitor that prevents the cleavage of C5 into its proinflammatory components (385). It has undergone phase II testing in patients with membranous nephropathy and 12 months of therapy with the agent resulted in an increased remission rate (386). Future clinical trials with this agent are anticipated.

Other targets for therapy include the induction of immune tolerance, modulation of inflammatory mediators (such as anti-TNF-α therapy with infliximab), blockade of co-stimulation (with anti-CD40L or CTLA4-Ig) or blockade of mechanism that lead to progressive glomerular scarring and tubulointerstitial injury (384).

Treatment Recommendations

In recent years the treatment of autoimmune diseases has undergone a revolution and new therapies that specifically target the molecular pathways involved in inflammation and immunologic regulation have become available (385). In MN, where immune complex localization to the subepithelial region of the glomerulus leads to complement activation and subsequent damage to the glomerular basement membrane and epithelial cell, therapies that intervene in the various steps in the process could be envisioned. Such therapies are being developed but have not been subject to human trials. Thus, current treatment of MN relies on nonspecific antiproteinuric strategies as well as global immunosuppression. Most clinicians reserve treatment with cytotoxic agents to those patients at greatest risk for progressive renal disease. Thus, patients with persistent high-grade proteinuria and/or worsening renal function should receive steroids and cytotoxic therapy. In those patients who do not respond to therapy or who are intolerant these drugs, cyclosporine and MMF are additional options. In the future, therapies that directly target the pathogenic mechanisms involved in glomerular injury will hopefully be available.

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CHAPTER 64 ■ NEPHROTIC SYNDROME: MINIMAL CHANGE NEPHROPATHY, FOCAL SEGMENTAL GLOMERULOSCLEROSIS, AND COLLAPSING GLOMERULOPATHY

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The term *nephrotic syndrome* refers to a classic tetrad of proteinuria, hypoproteinemia, edema, and hyperlipidemia. Although a relationship among these findings was recognized as early as the 15th century, the term *nephrosis* first achieved widespread acceptance in the early part of the 20th century, when Volhard and Fahr employed it as one of the major divisions of bilateral kidney disease (1). Later developments, notably the advent of percutaneous renal biopsy, facilitated further delineation of the many forms of kidney disease that result in the nephrotic syndrome (2,3). We have divided these diseases into three general categories, as shown in Table 64-1: (a) primary nephrotic syndrome, in which the process that initiates proteinuria is not immediately apparent from histopathologic evaluation; (b) inflammatory glomerular lesions; and (c) glomerulopathy secondary to other diseases that affect the kidney. Regardless of the underlying cause, all of these diseases share a common denominator in that each involves proteinuria of sufficient severity to produce hypoproteinemia. Typically, when the serum albumin concentration falls below a critical level of approximately 2 g/dL, the other clinical features of the nephrotic syndrome appear.

An analysis of the diseases underlying nephrosis is complicated because studies have used varied definitions; different methods of acquiring patient populations; and groupings that reflect clinical, functional, or histologic criteria. Clearly, the relative frequency of different causes of nephrosis varies with age and has changed over time. The data in Table 64-1 were developed more than 25 years ago and indicated that approximately 80% of children with renal disease had primary nephrotic syndrome, as opposed to only 25% of adults. Chronic glomerulonephritis was responsible for about half of the cases of nephrotic syndrome in adults but only 10% to 15% of childhood cases. These glomerulonephritides may result from a systemic disease, such as systemic lupus erythematosus, or they may be idiopathic, such as in membranous nephropathy. The remaining cases of nephrotic syndrome were associated with diseases such as diabetes mellitus and amyloidosis. They accounted for up to 20% of adult cases but a very small percentage of childhood cases. This general pattern of causes for nephrotic syndrome is still observed in most industrial countries; additional causes are more likely to be seen in developing nations (4–8).

This chapter focuses on the group of diseases subsumed under the category of *primary nephrotic syndrome*. We employ this term to describe the clinical picture of nephrotic syndrome that occurs in the absence of evidence for glomerulonephritis or systemic disease that would be sufficient to account for massive proteinuria. Primary nephrotic syndrome includes patients who have been described as having *minimal change nephropathy* (MCN), also called lipoid nephrosis, "nil" disease, idiopathic nephrotic syndrome of childhood, minimal change nephrotic syndrome (MCNS), or steroid-sensitive or steroid-responsive nephrotic syndrome. We have chosen to use MCN as the preferred term for this entity. We will reserve the alternate term, MCNS, for the clinical presentation in which MCN has caused the nephrotic syndrome. Nephrosis is not always present, particularly in adult patients (9,10). Although most pathologists require at least the presence of nephrotic-range proteinuria prior to treatment to make the diagnosis of MCN, occasional patients may lack edema, hypoalbuminemia, or hypercholesterolemia but have renal histology and ultrastructure that are otherwise typical. Furthermore, describing the lesion as a nephropathy offers a descriptor that is in parallel with the other forms of primary nephrotic syndrome, in that it is based upon the defined histopathology rather than a potentially variable clinical picture. Some patients with primary nephrotic syndrome have only a small amount of immunoglobulin M (IgM) deposited in the glomeruli, which usually is believed to be insignificant; some biopsy specimens reveal mild mesangial hypercellularity. These patients are considered to represent variants of MCN. A larger group of patients have significant extracellular matrix accumulation or glomerular capillary collapse. These patients are defined as having *focal segmental glomerulosclerosis* (FSGS), also called focal sclerosis and hyalinosis; and *collapsing glomerulopathy*, respectively. A spectrum of pathologic findings may be observed. Moreover, some patients, regardless of the underlying pathology, respond to treatment with corticosteroids; others with an apparently identical histologic lesion are resistant to steroid therapy.

Examination of the incidence of these diseases illustrates both the age-dependence of diagnoses and the changing nature of the underlying lesion. A large series of adults in Chicago is depicted in Table 64-2, in which was evaluated the renal biopsies of 1,000 consecutive patients who presented with the nephrotic syndrome. At this referral center, the relative incidence of different causes of nephrosis is changing, with significantly more FSGS and less MCN (10). Although this study could reflect some degree of referral bias, it is likely that these numbers approximate the general distribution of histologic diagnoses in nephrotic patients. The data for children shown in this table describe all children 0.5 to 19 years of age in the province of Ontario who were diagnosed as having nephrotic syndrome. In this group, the incidence of FSGS also is increasing. Consistent with the data shown in Table 64-1, the overall proportion of patients with primary nephrotic syndrome in children is greater than 90% (11) compared with 50% in the adult study. The increasing incidence of FSGS (more than doubled in both children and adults) is considered further in the section on Focal Segmental Glomerulosclerosis and Collapsing Glomerulopathy, later in this chapter.

TABLE 64-1

TYPES OF KIDNEY DISEASE CAUSING THE NEPHROTIC SYNDROME IN PEDIATRIC AND ADULT PATIENTS

	Relative incidence	
	Children	Adults
Primary nephrotic syndrome	79	24
Nephrotic syndrome associated with a glomerulopathy		
Chronic glomerulonephritis and systemic inflammatory disease	13	52
Secondary glomerulopathy	8	24

(Data derived from: International Study of Kidney Disease in Children. A controlled therapeutic trial of cyclophosphamide plus prednisone vs. prednisone alone in children with focal segmental glomerulonephritis. *Pediatr Res* 1980;14:1006; and Glassock RJ. The nephrotic syndrome. *Hosp Pract* 1979;14:105.)

In addition to the clinical observation that inflammation does not underlie the proteinuria of primary nephrotic syndrome, advances in our understanding of these disorders suggest that the origin of all these diseases resides in the specialized visceral epithelial cell of the glomerular filter—the podocyte. This cell contributes to the final barrier that determines the nature of the glomerular filtrate, and podocyte lesions appear to play a critical role in progressive forms of primary nephrotic syndrome. In Table 64-3 is shown a taxonomy of the primary podocyte diseases, grouped according to histologic characteristics. An analysis of the pathology, physiology, and genetics of these diseases, and insight derived from examining acquired causes of each lesion, was utilized in this categorization. Patients who show few or no glomerular abnormalities by use of light microscopy include those with classical MCN and its histologic variants, and patients with Finnish-type congenital nephrotic syndrome. A second group has diffuse mesangial sclerosis and represents mostly young children with congenital forms of nephrosis, frequently resulting from a single gene mutation. The third category, FSGS, represents an entity the incidence of which is rising rapidly throughout the world. We have chosen to define a fourth group: patients with collapsing glomerulopathy. Although this diagnosis previously has been thought to be a part of FSGS, the histologic appearance and clinical course strongly suggest that it represents a distinct lesion. Further details regarding the definition of the categories in this table will be provided in this chapter.

It is important to note that the nephrotic syndrome has many physiologic consequences that are not limited to the classic tetrad of proteinuria, hypoalbuminemia, edema, and hyperlipidemia. These include abnormalities of electrolyte balance, coagulation, hormonal function, and immunity. This chapter reviews the mechanisms underlying these manifestations of nephrosis, our understanding of the pathogenesis of different forms of primary nephrotic syndrome, and the clinical features and management of patients with this entity.

PATHOPHYSIOLOGY OF THE NEPHROTIC SYNDROME

Virtually every abnormality observed in primary nephrotic syndrome can be traced directly or indirectly to the urinary loss of protein. Thus, the mechanisms responsible for this proteinuria have systemic consequences that are manifested in the clinical signs and symptoms of nephrosis.

TABLE 64-2

CHANGING ETIOLOGIES OF NEPHROTIC SYNDROME

	Percentage of total sample			
	Children[a]		Adults[b]	
Diagnosis	1985–1993	1993–2002	1976–1979	1995–1997
Minimal change nephropathy	81[c]	65[c]	23	15
Diffuse mesangial hypercellularity	3	7	—	—
Focal segmental glomerulosclerosis	11	25	15	35
Membranous nephropathy	1	2	36	33
Membranoproliferative glomerulonephritis	1	0	6	2
Chronic glomerulonephritis	—	—	5	<1
IgA Nephropathy	—	—	3	9
Amyloid Nephropathy	0	0	7	4
Other	3	1	7	2

[a]Data from: Ontario (Filler G, Young E, Geier P, et al. Is there really an increase in nonminimal change nephrotic syndrome in children? *Am J Kidney Dis* 2003;42:1107).
[b]Data from: Chicago (Haas M, Meehan SM, Karrison TG, et al. Changing etiologies of unexplained adult nephrotic syndrome: a comparison of renal biopsy findings from 1976–1979 and 1995–1997. *Am J Kidney Dis* 1997;30:621).
[c]Includes children with steroid-sensitive nephrotic syndrome and presumed minimal change nephropathy.

TABLE 64-3

CLASSIFICATION OF PRIMARY PODOCYTE DISEASES

Pathology	Genetic (Mendelian inheritance)	Acquired	Medication-induced
Minimal change histology	• Congenital NS, Finnish type *NPHS1* *NPHS1* + *NPHS2*	• MCN • MCN variants Mesangial hypercellularity IgM nephropathy Glomerular tip lesion C1q nephropathy • MCN, association Hodgkin disease Infection (see Table 64-5)	• Nonsteroidal anti-inflammatory agents • Gold • Penicillamine • Lithium • Interferon-α and -β
Diffuse mesangial sclerosis (DMS) histology	• Congenital presentation *LAMB2* (Pierson syndrome) • Childhood presentation *WT1* (Denys-Drash syndrome, isolated DMS)	• Isolated DMS	
Focal segmental glomerulosclerosis (FSGS)	• Congenital presentation *ITGB4* • Infancy/childhood presentation *NPHS2* *NPHS1* + *NPHS2* *WT1* (Denys-Drash syndrome, Frasier syndrome) *PAX2* (renal-coloboma syndrome with oligomeganephronia) mtDNA (MELAS syndrome) *COQ2* • Adult presentation *ACTN4* *CD2AP* *TRPC6* mtDNA (MELAS syndrome)	• Idiopathic FSGS Columbia classification 1. Not otherwise specified 2. Perihilar variant 3. Cellular variant 4. Tip lesion variant 5. Collapsing FSGS • C1q nephropathy • Postadaptive FSGS Follows an adaptive response consisting of glomerular hyperperfusion and hypertrophy (a) Reduced nephron mass: renal dysplasia, oligomeganephronia, surgical renal mass reduction, reflux nephropathy, chronic interstitial nephritis (b) Initially normal nephron mass: obesity, increased muscle mass, sickle cell anemia, cyanotic congenital heart disease, hypertension*	• Cyclosporine, tacrolimus • Interferon-α • Lithium • Pamidronate
Collapsing glomerulopathy	• Action myoclonus-renal failure syndrome	• Idiopathic collapsing glomerulopathy • C1q nephropathy • Collapsing glomerulopathy associated with infection HIV-1 parvovirus B19* *Loa loa* filariasis Visceral leishmaniasis • Collapsing glomerulopathy, other associations Adult Still disease* Allograft vascular diseases* Multiple myeloma*	• Interferon-α • Pamidronate

ACTN4, α-actinin-4; *CD2AP*, CD2 associated protein; *COQ2*, coenzyme Q synthetase 2; *FSGS*, focal segmental glomerulosclerosis; *ITGB4*, integrin β4; *LAMB2*, laminin β2; *NPHS1*, nephrin; *NPHS2*, podocin; *MCN*, minimal change nephropathy; *mtDNA*, mitochondrial DNA; *TRPC6*, transient receptor patontial cation channel 6; *WT1*, Wilms tumor 1.

A classification scheme for the MCN/FSGS/collapsing glomerulopathy spectrum is presented. The forms of FSGS that are assigned to the acquired and medication-induced categories may have genetic risk components that have not been well-defined at present. The Columbia classification divides idiopathic FSGS into five variants; in the present classification system the fifth variant, collapsing FSGS, has been termed idiopathic collapsing glomerulopathy. In recognition that there may be distinct forms of glomerular tip lesion with divergent prognoses, this entity has been divided into two forms, glomerular tip lesion MCN variant and glomerular tip lesion FSGS variant (these forms may have distinct clinical outcomes but similar pathologic appearance). The diagnosis of postadaptive FSGS requires the exclusion of specific glomerular disease, for example, immune-mediated glomerulonephritis and diabetic nephropathy (which may manifest focal and segmental scarring but are not considered FSGS). Possible associations of idiopathic FSGS and collapsing glomerulopathy with other disease states have been treated somewhat conservatively, so that associations based on isolated case reports and controversial associations are excluded or designated with an asterisk. C1q nephropathy can present as MCN, FSGS, and collapsing glomerulopathy, as well as other forms of glomerulopathy.

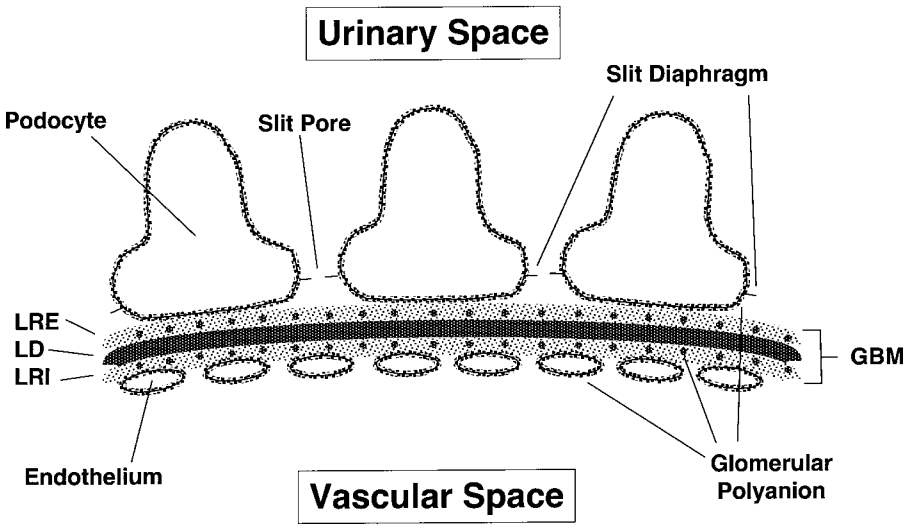

FIGURE 64-1. The glomerular filtration barrier. The distribution of glomerular polyanion in the glomerular basement membrane and on the endothelial and epithelial cell layers is shown. *LRE*, lamina rara externa; *LD*, lamina densa; *LRI*, lamina rara interna; *GBM*, glomerular basement membrane.

Mechanisms for Proteinuria

The renal factors contributing to albumin homeostasis include both glomerular filtration and tubular reabsorption. In its simplest form, the glomerulus functions as a means to promote fluid and solute flux from the blood vessel to the urinary space, from where most constituents of the filtrate are then reabsorbed. This model acquires significant complexity as solute particles approach the size limits that are characteristic of the glomerular filter. Further, recent progress in understanding tubular handling of protein has demonstrated that the reabsorptive component also has a significant impact on albumin homeostasis.

Renal Handling of Macromolecules

The glomerular barrier to filtration consists of three layers: fenestrated endothelial cells, the trilaminar glomerular basement membrane (GBM), and the epithelial cell layer (Fig. 64-1). The epithelium does not constitute a continuous layer; rather, the interdigitating extensions from adjacent epithelial cells or podocytes are separated by spaces readily apparent on electron microscopy. The GBM has been considered a major barrier to filtration (12). Experimental evidence supports a hypothetical construct in which the GBM is a thixotropic gel (one containing spicules that retard the passage of macromolecules through it) (13). Diffusion through this gel plays a significant role in restricting protein passage (14). Thus, the filtration of protein is restricted in the same manner that regulates protein movement during gel electrophoresis, where small molecules most easily penetrate (15). At the same time, other studies support a model in which macromolecules encounter a porous structure that limits the passage of larger molecules by steric hindrance (16). Glomerular filtration is possible because the interdigitations of the podocytes are separated by a small portion of the urinary space. These spaces are partly occluded by the epithelial slit diaphragm (Fig. 64-1), which has rectangular pores (17) that likely constitute the limiting barrier structure causing steric hindrance (18). The barrier itself is composed primarily of nephrin, a cell–cell adhesion molecule that interdigitates between adjacent cell processes (19), and is supported by accessory molecules including CD2-associated protein (CD2AP), FAT, Neph1, and P-cadherin (20). The result is a latticework of proteins with openings of approximately 4 × 14 nm (21). Therefore, both the GBM and the slit diaphragm contribute to steric influences on macromolecular filtration.

The porous component is demonstrated by permselectivity curves that plot the renal clearance of macromolecules, relative to the glomerular filtration rate (GFR), against molecular radius, describing a sigmoid shape (Fig. 64-2) between approximately 2 and 5 nm (20 and 50Å) (22). Therefore, some restriction in filtration of dextrans occurs with molecules of about

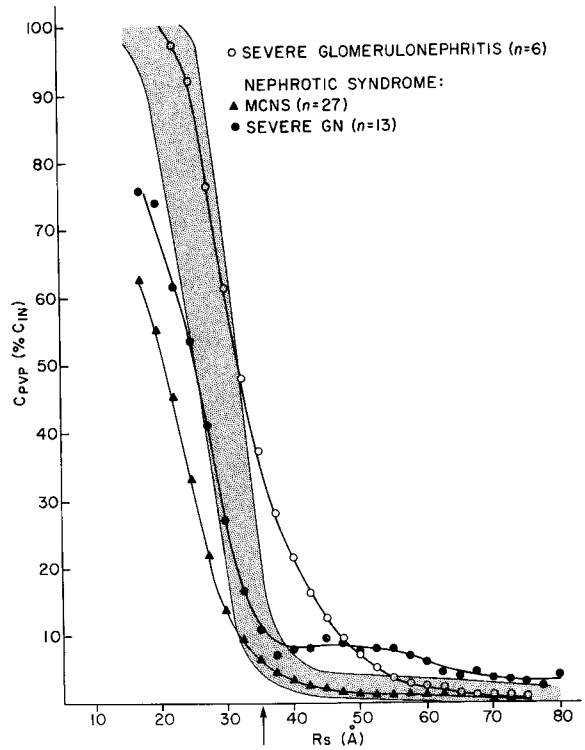

FIGURE 64-2. Permselectivity curves for patients with severe proliferative glomerulonephritis and for those with nephrotic syndrome secondary either to the minimal change nephropathy (MCN) or to glomerulonephritis. Normal values are depicted by the *shaded area*. The *arrow* indicates the molecular size of albumin. The fractional clearance of larger macromolecules is increased in severe glomerulonephritis. In minimal change nephrotic syndrome, the fractional clearance of smaller molecules is decreased. Patients with nephrotic syndrome secondary to glomerulonephritis show a hybrid curve. (Data modified from: Robson AM, Cole BR. Pathologic and functional correlations in the glomerulopathies. In: Cummings NB, Michael AF, Wilson CB, eds. *Immune Mechanisms in Renal Disease*. New York: Plenum; 1982:109, with permission.)

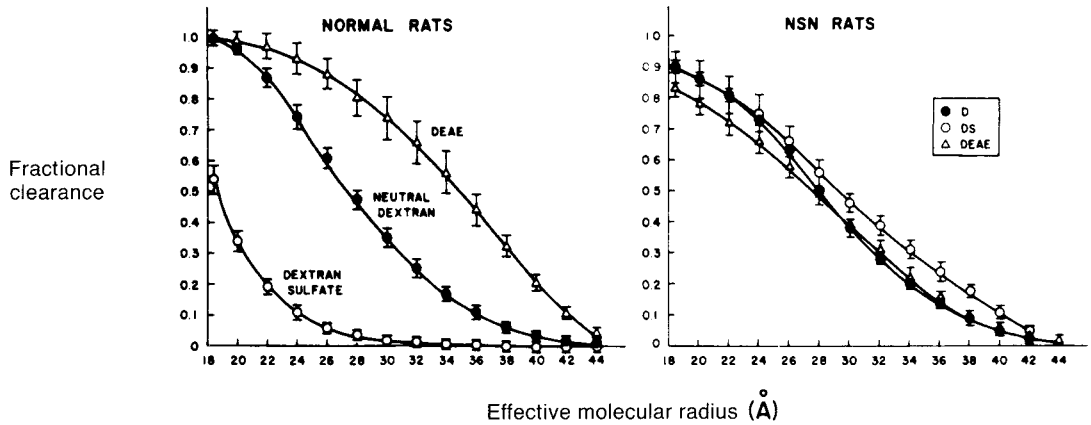

FIGURE 64-3. Clearance of neutral dextran (*D*), negatively charged dextran sulfate (*DS*), and positively charged diethylaminoethyl (*DEAE*) dextran of varying molecular size in normal rats and in those made albuminuric by treatment with nephrotoxic serum (NSN). In normal animals, clearance of negatively charged dextrans is retarded, and that of cationic dextrans is enhanced, demonstrating charge selectivity by the glomerular filter. In NSN, charge discrimination is lost. (Reprinted from: Bohrer MP, Baylis C, Humes HD, et al. Permselectivity of the glomerular capillary wall: facilitated filtration of circulating polycations. *J Clin Invest* 1978;61:72; by copyright permission of the American Society for Clinical Investigation.)

a 2-nm radius; restriction increases with increasing molecular size and approaches 100% for molecules of a radius of 5 nm (23). In addition to size, the ability of macromolecules to cross the glomerular barrier is affected by molecular configuration, shape, deformability, and flexibility (24). Permselectivity also is modified by glomerular hemodynamic factors, although the mechanism for this effect remains a subject of some controversy (15).

Initially, it was believed that macromolecule handling could be accounted for by an isoporous model for glomerular filtration, one in which steric hindrance of glomerular passage of macromolecules results from the presence of uniform pores in the barrier, each with a radius of approximately 5 nm. The size of these pores may be increased in models of increased permeability of the GBM (25). However, it has become apparent that a heteroporous model may be more appropriate (26). In this model, there are two pathways: one subject to classic steric hindrance, and a "shunt" pathway unaffected by size selectivity. As demonstrated by clearance of very large dextrans, glomerular filtration of macromolecules through this second pathway is enhanced in most forms of nephrosis and exacerbated by colloid volume expansion (27), and is ameliorated in humans by antihypertensive therapy (28), pressor doses of angiotensin II (in contrast to the effect in rats) (29), or indomethacin (30). Therefore, there appears to be a hemodynamic component to activation of this mechanism for proteinuria. The impact of this shunt is most noticeable for large molecules (greater than 6 nm); its effect on albumin clearance remains to be determined.

Steric hindrance is not sufficient to account for all aspects of permselectivity. Although proteins are handled in a manner similar to that for inert macromolecules (31), protein clearances tend to be less than those of dextrans of comparable size (24). Part of this difference is explained by the relatively rigid structure of the proteins. However, albumin, which has an effective molecular radius of 3.6 nm, is cleared by the normal kidney considerably less than are the equivalent-sized dextran molecules. Albumin carries a negative electrostatic charge, and its clearance is only slightly less than that of similarly sized dextran carrying a negative charge (32). This apparent charge selectivity has been attributed to negatively charged sialoglycoproteins in the glomerular filter (33), which are present at regularly spaced intervals in the laminae rarae of the basement membrane (34), at the endothelial fenestrae (35), and lining the epithelial podocytes (36). Collectively, these constitute the glomerular polyanion (Fig. 64-1). The presence of such negative-charge sites was proposed to be responsible for both the facilitated transport of polycations (37) and the restricted transport of polyanions (38) relative to that of neutral molecules of comparable size (Fig. 64-3). These effects are most apparent in the size range that is affected by some degree of steric hindrance. Thus the determinants of glomerular permeability for a given particle are steric hindrance, glomerular hemodynamics, and electrostatic charge.

Tubular Handling of Protein. Renal protein metabolism also is affected significantly by tubular function. The glomerular filtrate normally contains a small amount of protein. A proximal tubular system has sufficient capacity that, under physiologic conditions, little intact protein from the filtrate is present in the urine. For example, filtered albumin is subject to lysosomal degradation in the proximal tubule, with fragments appearing in both the plasma and the urine (39). However, studies of rat kidneys, isolated but perfused *in situ* with radiolabeled albumin, indicate that some albumin is reabsorbed intact (40). One mechanism of tubular protein reabsorption is demonstrated by its absence in Dent disease, a defect in chloride transport resulting from a mutation in the gene for a renal-specific, voltage-gated chloride channel, CLC-5, leading to hypercalciuric nephrolithiasis (41). Proteinuria in this disease results from disruption of both receptor-mediated and fluid-phase endocytosis (42). Patients with Dent disease have characteristic urinary losses of retinol-binding protein (RBP) and albumin (43). The failure of protein reabsorption in this lesion has permitted an estimate that the glomerular filtrate contains 22 to 32 mg/L of albumin, or roughly 3 to 6 g per day in the normal human adult, virtually all of which is reabsorbed under normal conditions. This represents greater than 4% of the total plasma albumin (44).

This saturable mechanism for albumin reabsorption is mediated by two proteins that are associated with clathrin-coated pits in the proximal tubular cell (45). Megalin is a 600-kDa, transmembrane protein and a member of the low-density lipoprotein-receptor family. It co-localizes in cultured opossum kidney (OK) cells with exogenous albumin and with cubilin, a 460-kDa protein that does not have a transmembrane domain. Ligands for cubilin in the glomerular filtrate include not only albumin but also immunoglobulin light chain and apoA-I. Megalin binds to the vitamin-binding proteins, RBP and vitamin

D-binding protein, hormones, enzymes and β_2- and α_1-microglobulin, as well as albumin (46,47). As will be discussed in the section to follow on Consequences of Proteinuria, the loss of many of these proteins has clinical significance in nephrotic syndrome. Another albumin "rescue" pathway that appears to facilitate reabsorption of intact albumin has been attributed to the FcRn immunoglobulin receptor (48).

Altered Permselectivity in Nephrosis

Permselectivity patterns obtained in patients with MCNS (49,50) (Fig. 64-2) or animal models of selective albuminuria (Fig. 64-3) (38) show a relative decrease in macromolecular clearance even in the presence of marked proteinuria. In contrast, patients with glomerulonephritis show increased macromolecular clearances (Fig. 64-2), presumably due to structural damage to the GBM, which may be visible in renal biopsy material from patients in these disease states; this concept is supported by work in animals (51). Thus the mechanisms for proteinuria in both MCN and FSGS appear to be distinct from those in glomerulonephritis. In the former, proteinuria is relatively selective for albumin and occurs even though clearance of macromolecules comparable in size to albumin is decreased. In the latter, permselectivity of macromolecules that are 2.5 nm (25 Å) or larger is increased, resulting in poorly selective proteinuria. Patients with glomerulonephritis and proteinuria that is sufficiently severe to cause the nephrotic syndrome may show a pattern of permselectivity (Fig. 64-2) that is a hybrid between those found in MCNS and those found in uncomplicated glomerulonephritis (49). In these patients, as in MCNS, clearance of smaller molecules is relatively decreased. However, in contrast to the situation in MCNS, the relative clearance of larger molecules is increased. Similar hybrid curves have been described in diabetic glomerulosclerosis (52).

Therefore, nephrotic proteinuria does not result from a simple defect in glomerular filter steric hindrance. Several theories have been advanced to account for albumin loss. A prominent one is a decrease in glomerular electrostatic charge selectivity. Renal biopsy material from patients with nephrotic syndrome shows decreased staining for glomerular polyanion (53–56). Indeed, studies in MCNS patients suggested that albuminuria results from a reduction of fixed negative charge by approximately 50% (57). Rats with nephrotic syndrome induced by puromycin aminonucleoside (PAN), which causes predominant albuminuria, show decreased staining by cationic dyes (58) and decreased sialic acid content (59). Animals with puromycin nucleoside (PAN)-induced nephrotic syndrome (60) as well as those with acute heterologous nephrotoxic serum nephritis (32) show increased clearance of negatively charged dextrans, with permselectivity curves approximating those of neutral dextrans. Further, intravenous infusion of various polycations into animals results in loss of staining for glomerular polyanion, increased porosity of the glomerular filter, and heavy proteinuria (61–63). Unilateral renal artery infusion of the polycation protamine sulfate causes ipsilateral albuminuria and depletion of glomerular polyanion (63). Finally, studies in patients suggest that neutralization of vascular anionic charges may be systemic in nature (64,65) rather than confined to the kidney. This could result from effects of a protease present in the circulation such as hemopexin (66,67). However, sieving curves generated in rats by glomerular localization of neutral or negatively charged polysaccharides were unable to demonstrate charge selectivity of the glomerular filter (68), suggesting that technical factors or differences in experimental approach could significantly affect the validity of experiments demonstrating charge selectivity. In these studies, bovine serum albumin (BSA) "uptake" was extremely high relative to other markers. Another study found a role for negative charge in modulating renal protein handling in rats infused with neutral or anionic horseradish peroxidase. These results suggested that proteins may be more affected than polysaccharides by charge, but in this model charge selectivity was lost after inhibiting tubular protein uptake with lysine or ammonium chloride, suggesting the conclusion that charge selectivity does not reside in the glomerulus (69).

In mice, the plasma elimination rate of albumin (effective molecular radius of 36 Å) was comparable to that of much larger Ficoll molecules (≥65 Å). When the animals were treated with PAN, albumin clearance increased through an unknown renal mechanism (70). Although charge selectivity could explain these findings, any mechanism that is involved could have affected either the glomerulus or the tubule. A study in analbuminemic rats treated with PAN showed no effect on renal size selectivity with treatment. There also was no change in the characteristics of urinary protein excretion (71). Previously, it has been assumed that greatly increased delivery of albumin to the proximal tubule saturated transport mechanisms or that the other proteins lost in the urine were bound to albumin, in either case causing loss of the nonalbumin proteins. However, the pattern of proteinuria observed in this study was similar to the findings in the CLC-5-null mouse, where low-molecular-weight proteinuria occurs, and suggests that at least some protein losses in nephrosis result from specific tubular mechanisms. These data support a significant role for derangement of tubular protein handling in nephrotic syndrome.

Several investigators have suggested that impairment of the rescue pathway and other tubular mechanisms are a significant cause of nephrotic proteinuria (15,72). However, several lines of evidence suggest that this is not the case. Patients with Dent disease, and the CLC-5-null mouse, have "nephrotic-range" proteinuria but do not have nephrosis. The salvage of some of the 4% of the plasma albumin that is filtered per day (44) is likely to contribute positively to homeostasis, but a significant portion of albumin rescued by the tubule is degraded (39). Assuming that half of the albumin is reclaimed intact, it is unlikely that losing 2% of plasma albumin per day will have a major effect on plasma albumin concentration. Finally, Deen and Lazzara (73) modeled the sieving coefficient for albumin. They performed a mass-transfer analysis to determine whether the sieving coefficient could be similar to, rather than greatly less than, that for neutral Ficoll of the same size. The higher value, which would have been required in the models supported by adherents of a causal role for tubular proteinuria, would generate tubular albumin concentrations located 1 mm distal to the glomerulus that are 20-fold higher than has been measured by rat micropuncture studies. The authors concluded that the glomerulus was the primary restricting site for albuminuria (73). Although the possibility of charge selectivity was considered, it could not be tested by this analysis. Therefore, the mechanism of nephrotic proteinuria remains uncertain.

Even if glomerular charge neutralization does not cause proteinuria, it could account for other aspects of nephrosis. Electron micrographs of renal tissue obtained after protamine sulfate infusion sufficient to deplete glomerular polyanion show podocyte alterations identical to those seen in nephrotic syndrome (74). Perfusion of the kidney with heparin (which is negatively charged) after infusion of polycation results in reestablishment of the normal podocyte structure (74). Furthermore, chemical removal of the sialic acid coating also causes foot-process fusion/effacement (75). These findings support the notion that repelling electrostatic effects of the negatively charged cellular coating may contribute to the normal separation between adjacent podocyte foot processes. An alternative view holds that albuminuria itself may cause foot-process fusion (76).

Alteration of podocyte architecture also may account for the generally decreased fractional clearance of smaller macromolecules in nephrotic syndrome cited previously. It has been suggested that simplification of the foot process makes the glomerular pore less complex, thereby allowing for increased clearance of some long, narrow, rigid molecules. However, most plasma proteins are prolate ellipsoids (stubby cigar-shaped) and show decreased clearance (77). The effective pore radius was reported to be decreased in both MCNS and FSGS (78). In this study, the ratio of total pore area to pore length was reduced by more than 50%. Further support for decreased pore area is found in studies indicating decreased filtration slit frequency (likely secondary to foot-process fusion) (79) or decreased pore number (80). These findings would account for decreased macromolecular clearance but not enhanced albumin clearance.

An alternative to charge neutralization as an explanation of proteinuria is suggested by the data indicating that permselectivity patterns show enhanced clearance of larger neutral dextrans in FSGS (80). Studies by Yoshioka and colleagues (81) suggest that there is enhanced clearance of albumin by less-affected glomeruli, implicating hemodynamic factors related to hyperfiltration in remnant nephrons (82). This could be accounted for by the heteroporous model, in which a different class of pores greater than 60Å in radius is increasingly utilized. This shunt pathway is active in angiotensin II-stimulated proteinuria (83) and Heymann nephritis (84). Further, the hemodynamic implications of proposing a role for the shunt pathway are supported by the salient effect of angiotensin converting enzyme inhibition on glomerular size selectivity in disease (85). However, since patients with MCN do not necessarily have increased utilization of the shunt pathway (80), it is not clear that shunting represents a mechanism of proteinuria common to all causes of the nephrotic syndrome, or is the major cause of nephrotic albuminuria.

A third hypothesis regarding the stimulus for proteinuria could account for changes in both charge and steric hindrance in the glomerular filtration barrier. Small anions such as Cl^- are freely filtered by the glomerulus, whereas negatively charged molecules such as albumin that are large enough to interact with the filtration barrier (but pass through) are affected by electrostatic hindrance. Modest increases in slit diaphragm pore size, perhaps mediated by alterations in cytoskeletal function (86), may be sufficient to both decrease steric hindrance of, and reduce electrostatic interference with, albumin transit, even before considering the amplifying effects of barrier charge neutralization or increased shunt pathway utilization.

Consequences of Proteinuria

It is generally accepted that the central feature of the nephrotic syndrome, irrespective of its underlying renal cause, is hypoalbuminemia resulting from urinary loss. There is increased fractional catabolism of albumin in nephrotic syndrome (87), mostly within the renal tubule after increased filtration of plasma proteins (88). Rates of hepatic synthesis of albumin are increased (89), but this increase is inadequate to compensate for urinary losses (90). Although gastrointestinal losses are possible through transudation of albumin across the bowel wall in nephrosis, these are not likely to contribute significantly to decreased plasma albumin concentrations.

Nonetheless, urinary losses cannot be considered an isolated phenomenon. It is apparent that a special relationship exists among protein synthetic capability, urinary loss of protein, and plasma protein concentrations. For example, patients undergoing chronic peritoneal dialysis lose "nephrotic range" amounts of protein, yet they usually have close to normal serum albumin concentrations (91). In nephrosis, the rate of hepatic albumin synthesis is related to dietary protein intake. However, increasing protein intake leads to glomerular hyperfiltration (92,93) and enhanced loss of protein in the urine, resulting in lower serum albumin concentrations in patients on high-protein diets (94). The increase in dietary intake appears to stimulate selective hepatic expression of messenger ribonucleic acid (mRNA) for albumin, indicating that the stimulus is specific for albumin production and not generalized to other proteins as well (95). The dietary stimulus can be dissociated from potential effects of alterations in plasma oncotic pressure (96). Although specific plasma amino acid content is unchanged, nitrogen balance is rendered more positive by angiotensin-converting enzyme (ACE) inhibition (97), which decreases hyperfiltration and thus the amount of protein lost in the urine. Indeed, enalapril decreases $U_{Albumin}V$ (absolute albumin excretion) and fractional catabolism of albumin in normal or nephrotic rats on high-protein diets (98,99).

Edema Formation

One of the major consequences of hypoalbuminemia is edema formation. The major forces that maintain vascular volume are believed to be those described by Starling (100), namely, the algebraic sum of hydrostatic and oncotic pressures acting at the level of the peripheral capillary beds (Fig. 64-4). Hydrostatic pressure is the dominant force at the arteriolar end of the capillary, where it is generated by arterial blood pressure. Pressure

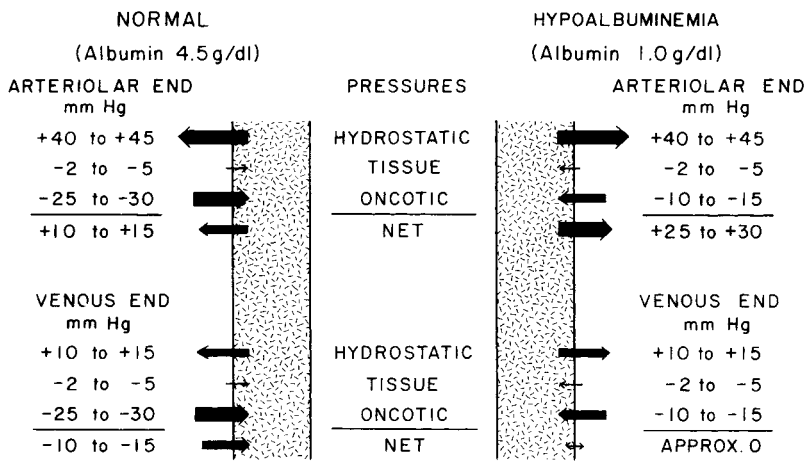

FIGURE 64-4. The forces that govern the movement of fluid across the peripheral capillary wall in healthy persons and in patients with primary nephrotic syndrome. The *shaded area* represents the lumina of the capillaries. The size and direction of the *arrows* are in proportion to the magnitude and direction of the force described by that arrow. In MCNS, hypoalbuminemia causes a marked reduction in oncotic pressure. This increases the driving force for fluid out of the arteriolar end of the capillary and decreases the forces available for return of fluid at the venous end. The result is the development of increased amounts of fluid in the interstitial space and the beginning of edema formation. See text for more details. (From: Robson AM. Edema and edema forming states. In: Klahr S, ed. *The kidney and body fluids in health and disease*. New York: Plenum; 1984:119, with permission.)

is lower in the capillaries (40 to 45 mm Hg) than in the arterial system, but it is markedly higher than tissue pressure, which ranges from 2 to 5 mm Hg. Hydrostatic pressure is opposed by plasma oncotic pressure (the osmotic pressure generated by colloidal solute), which is 25 to 30 mm Hg in healthy individuals. The resulting net force (10 to 15 mm Hg) drives an ultrafiltrate of blood from the capillaries into the interstitial fluid space. By the venous end of the capillary, hydrostatic pressure has been further dissipated (Fig. 64-4) and is exceeded by oncotic pressure, so that there is a net force for return of fluid into the capillaries. In health, the loss of fluid at the arteriolar end of the capillaries slightly exceeds the amount resorbed at the venous end. The difference is returned to the circulation through the lymphatic system (101).

Albumin, because of its abundance and its relatively small molecular size, is the plasma protein primarily responsible for the generation of oncotic pressure (102). A decrease in plasma albumin concentration thus results in a decrease in oncotic pressure, so that the net driving force for loss of fluid at the arteriolar end of the capillary bed is increased and that for return of fluid at the venous end is reduced. Consequently, fluid accumulates in the interstitial space, initiating edema formation. This accumulation occurs first where tissue pressure is lowest, for example, in the eyelids or in the scrotum; it also appears in the most dependent parts of the body because venous hydrostatic pressure is highest at these sites and is transmitted to the venous end of the capillaries.

In this traditional model of nephrotic edema formation, often referred to as "underfilling" (103), the translocation of fluid from the vascular to the interstitial fluid space as edema forms should decrease blood volume. The physiologic responses precipitated by such a reduction would then be important factors in producing the massive amounts of edema often seen in nephrotic syndrome. These changes include the release of antidiuretic hormone (ADH), the release of renin with increased production of angiotensin II, and decreases in renal blood flow and GFR (104–106). All these changes favor renal retention and positive balances of both sodium and water unless intakes are decreased. Indeed, patients may exhibit increased thirst, which is probably stimulated both by angiotensin II (107) and by the decrease in blood volume monitored through baroreceptors and volume receptors. Retained sodium and water do not remain in the vascular space. Because of the hypoalbuminemia, they add to the edema.

In practice, the pathophysiology of edema formation in nephrotic syndrome is more complex than this traditional concept. Animal studies have documented that hypoproteinemia alone does not result in edema (108). Humans with congenital analbuminemia do not develop nephrosis-like edema and have a normal plasma volume even in the virtual absence of serum albumin (109). Furthermore, if the traditional theory is correct, patients in relapse of nephrotic syndrome should have decreased blood volumes and values should return to normal during remission from the disease. Although reduced values for blood volume have been reported (110), normal or even increased levels have been documented, too (111,112). A survey of the literature (113) found that only 38% of patients with nephrosis had measurements indicating blood volumes reduced by 10% or more from normal; 48% had normal values and 14% had increased values. Furthermore, patients with carefully documented MCNS studied during relapse and again during remission did not show a consistent increase in blood volumes with remission; indeed, in most, the values did not change (113,114). Therefore, in contrast to the "underfilling" model, others have proposed an "overflow" hypothesis, in which the vascular tree is filled to excess, with increased hydrostatic pressure leading to fluid extravasation. Remarkably, nail-bed micropuncture measurements in nephrotic patients did not support the notion that capillary overfilling occurs, but capillary leak appeared more important than underfilling in differentiating nephrotic from normal subjects (115).

There are several possible explanations for these conflicting models, each of which appears valid in some cases. One is that the reported patients had varying underlying causes of their nephrotic syndrome, in some cases involving significantly decreased renal function. Nephrotic syndrome secondary to glomerulonephritis usually is associated with a normal or expanded blood volume (116). A second potential confounding factor is that some patients were receiving treatment when studied. In addition to specific treatments, albumin infusion might enhance volume, and diuretic therapy may reduce both blood and interstitial fluid volume in nephrotic subjects (117). A third issue is that measurements of blood volume are difficult to interpret because of methodologic problems. Labeled red cells may not circulate ideally in volume-depleted states, so that peripheral hematocrit may not reflect total body hematocrit; labeled albumin may have an increased volume of distribution in nephrotic syndrome, especially if vascular integrity to albumin is decreased (118). Thus both methods could be subject to errors (116). Indeed, if the suggestion that nephrotic syndrome results from a generalized loss of negative-charge sites (65) is correct, loss of such charge sites in capillary beds could cause increased losses of albumin into edema fluid (119). This occurrence not only would alter the apparent volume of distribution for albumin, but also might increase net extravascular oncotic pressure at the level of the capillaries. Support for this hypothesis is found in the observation that large changes in extracellular fluid volume cause little change in plasma volume in nephrotic patients (120).

An attractive explanation for variations in reported blood volume is that the patients were studied in different phases of their disease process. Blood volume could be reduced during the pathogenesis of the nephrotic state, particularly in MCNS, but return to normal as anasarca develops. Therefore, the decrease in plasma volume after experimental depletion of serum proteins can be prevented by massive expansion of the extracellular fluid with saline solution (121). Nephrotic subjects progress through a sodium-retaining phase but eventually enter into a new steady state in which they no longer accumulate edema and once again demonstrate the ability to excrete a sodium load (106). With this new steady state, sodium and water retention may be so marked and edema accumulation so massive that tissue hydrostatic pressure is increased and blood volume is returned to normal. This may explain reports in which nephrotic subjects could be separated into those with high and those with low urine sodium concentrations (114). The high-volume state, whether from massive fluid intake or decreased renal function, represents the overflow pathogenesis of nephrotic edema. It is likely that both underfilling and overflow occur, perhaps at different times in the same patient.

Hormonal mechanisms. Regardless of whether underfilling or overflow is paramount, a third model suggests that hormonal mechanisms are of primary importance. Although we have emphasized a primary role for hypoalbuminemia in oliguria and sodium retention, patients with MCNS often undergo a marked, remission-induced diuresis beginning as soon as urinary albumin concentrations start to decrease and before normalization of serum albumin. Initial studies of the pathophysiology of nephrosis suggested that fluid redistribution results in aldosterone-mediated sodium retention designed to replenish vascular volume (122,123). Accordingly, aldosterone activity was thought to be more important in the genesis of fluid retention than either serum albumin or colloid osmotic pressure (114). Consistent with this notion, patients with nephrotic syndrome show an increase in distal renal tubular sodium reabsorption (106,124). Increased tubular sensitivity to aldosterone may further enhance edema formation (125).

Renin–angiotensin–aldosterone system. Inconsistencies in reported plasma renin activity (PRA) results could be due to clinical factors similar to those that confound interpretation of blood volume measurement. These include different stages of both disease process and sodium balance as well as variations in therapeutic regimen. For example, immunofluorescence staining of renin-producing cells in renal biopsy material from nephrotic patients revealed increased numbers of these cells in hypoalbuminemic states. However, the increase correlated with a number of variables, most notably the presence of vascular disease (126). In an attempt to standardize some of these variables, renin–sodium profiles were performed on patients with nephrotic syndrome. Two groups of patients were identified. In keeping with traditional concepts, the classic form was typically seen in patients with MCNS, in whom high levels of PRA and aldosterone activity were associated with vasoconstriction and hypoalbuminemia; values were further stimulated rather than suppressed by salt loading and decreased spontaneously before the occurrence of steroid-induced diuresis. In the hypervolemic, overfilling, form, seen typically with chronic glomerulonephritis and renal insufficiency, low renin activity was associated with sodium retention and increased normally with sodium depletion (116). Other studies correlated PRA with plasma volume, serum albumin concentration (127), or the state of sodium balance (113). Natriuresis in MCNS was associated with an increase in PRA and presumably a decrease in plasma volume (113), whereas that induced by water immersion, presumably mediated by an increase in blood volume, was associated with a measured decrease in PRA (128). Therefore, PRA appears to correlate better with plasma volume than with rate of urinary sodium excretion.

Difficulties in confirming a definitive role for the renin–angiotensin system in the genesis of nephrotic edema are similar to those in explaining edema formation in cirrhosis (129). A multiplicity of interacting factors may be responsible in both of these disease states. Therefore, plasma renin levels could be controlled tightly by a variety of feedback mechanisms so that subtle changes, too small to be detected by current laboratory methods, are all that occur to maintain the altered homeostasis.

Other Hormonal Regulators of Fluid and Electrolyte Balance. In addition to renin, other factors affecting volume status may include abnormal vascular tone (130) and altered levels of catecholamines (131).

Antidiuretic hormone secretion. Nephrotic patients with MCN may show decreased solute-free water excretion, although the capacity to generate solute-free water remains intact (105). Increased ADH secretion may reflect a physiological response to decreased intravascular volume (110). In contrast, maximal urine osmolarity may be decreased in experimental rat nephrosis due to decreased renal tubular expression of aquaporin (132,133).

Prostaglandin metabolism. Elevated levels of prostaglandin E_2 (PGE_2) were found in the serum of patients with nephrotic syndrome, the majority of whom had MCN (134). The highest values were observed when the patients had clinically apparent edema. Urinary PGE_2 levels were increased in patients with idiopathic nephrotic syndrome who had a low urine sodium concentration as well as elevated plasma renin–aldosterone activity (135). The observation that the administration of indomethacin to nephrotic patients results in an increase in body weight and decrease in GFR suggests that prostaglandins may play a role in either maintenance of GFR or amelioration of edema in nephrotic syndrome. Indomethacin also decreased proteinuria and PRA (135). Response to indomethacin is dependent on concurrent sodium intake. When the agent was given to nephrotic patients on sodium-restricted diets, it resulted in a decrease in GFR; a similar drug regimen for patients with more liberal sodium intake did not affect renal hemodynamics (136).

Atrial natriuretic peptide. Because atrial natriuretic peptide (ANP) causes renal vasodilation, an increase in GFR, and increased sodium excretion (137), it has been suggested that abnormal metabolism of this hormone could mediate sodium retention in nephrosis. The acute increase of plasma volume following albumin infusion in nephrotic children is accompanied by a fivefold increase in ANP levels (138). However, this may simply reflect a change from low plasma volume status before the infusion is begun in patients who likely have MCNS. Plasma concentrations of ANP were determined to be low in nephrotic patients compared to patients who had acute glomerulonephritis, and ANP levels correlated well with the degree of edema in nephritis but not in nephrosis (139). Therefore, regulation of ANP appeared to be appropriate for presumed volume status. In rats with adriamycin-induced nephrotic syndrome, changes in GFR after infusion of ANP were similar to those in control animals, indicating that nephrosis does not alter glomerular filtration by changing ANP sensitivity (140). In a similar model, no change was detected in ANP receptor density in nephrotic kidneys (141). Nephrotic patients respond physiologically to ANP infusion (142), although the mechanism by which this occurs may be different from that in normal subjects (143). It has been proposed that ANP mediates the diuretic response to head-out immersion in nephrosis (144), but the effect of ANP infusion, unlike that of immersion, is blocked by enalapril (145).

Physical and Anatomic Factors Affecting Glomerular Filtration Rate. Taken together, these findings suggest that, although secretion of ANF may in part mediate diuresis, physical factors are of greatest importance in the fluid retention of nephrosis, with abnormalities of ANF representing appropriate responses for the patient's physiology (146). These physical factors may include a significant intrarenal component. Children with MCNS have decreases in both GFR and filtration fraction (104,147,148). Decreased GFR could be due to a decrease in the ultrafiltration coefficient (K_f), causing a reduction in single-nephron GFR (146), and has been suggested to result from effacement of the glomerular epithelial cell foot processes (149). Alternatively, the decreased GFR could be a consequence of raised intratubular hydrostatic pressure in the proximal tubule secondary to the presence of filtered albumin, an increase in resistance to tubular flow (150), or decreased proximal reabsorption of tubular fluid as a result of a reduction in peritubular capillary oncotic pressure (146). There also may be a local role for the renin–angiotensin system, as saralasin infusion in experimental unilateral PAN-induced nephrotic syndrome resulted in an increase in single-nephron GFR in the experimental, but not the control, kidney (146). In another animal model of nephrotic syndrome, that of nephrotoxic serum nephritis, the K_f was reduced, but compensatory mechanisms maintained renal blood flow and whole-kidney and single-nephron GFR. These responses appeared to be intrarenal in origin and caused an increase in glomerular capillary pressure (151).

Another factor affecting GFR is plasma albumin concentration. Hypoalbuminemia has been postulated to decrease glomerular plasma flow, thereby decreasing GFR. However, lower albumin also decreases plasma oncotic pressure, which should increase GFR. Löwenborg and Berg report that GFR and filtration fraction vary directly with serum albumin but inversely with mean arterial blood pressure in children with MCNS (152). This finding supports a role for altered K_f in relapse, consistent with foot-process effacement. K_f is determined by the total filtration slit length, as shown by mathematical

modeling of experimental data (153). Some studies suggest that there is a weak correlation between the amount of proteinuria and the extent of foot-process effacement, but these studies included patients in relapse and in remission with MCN (154) and with multiple nephrotic diseases (155). A recent study of 23 MCN patients in relapse showed no correlation between proteinuria and foot-process effacement ($r = 0.25$, $p = 0.25$) (156). The authors make the important point that assessment of the quantitative relationship between podocyte foot-process effacement and proteinuria should exclude patients in remission, as these patients have normal podocyte morphology (and therefore do not address the hypothesis that the degree of effacement correlates with proteinuria). Foot-process effacement reverses when patients undergo spontaneous remission or glucocorticoid-induced remission.

Other Physiologic Changes in Fluid and Electrolyte Metabolism

A curious phenomenon in primary nephrotic syndrome, perhaps related to decreased filtration fraction, is the occurrence of reversible or permanent renal failure unexplained by the underlying disease process. This has been reported in association with both MCN (157–159) and FSGS (130). In some patients, renal failure was associated with the use of nonsteroidal antiinflammatory drug (NSAID) therapy (160,161). These episodes occur in the absence of renal vein thrombosis (vide infra) or other systemic symptoms. Because fractional excretion of sodium is low in these patients (162), it is likely that the marked decrease in GFR occurs for hemodynamic reasons (163) rather than because of acute tubular necrosis or vasomotor nephropathy. In a study of 15 patients with MCNS and renal failure, GFR measured by inulin clearance was decreased out of proportion to clearance of *para*-aminohippurate (PAH), with filtration fraction reduced to between 3% and 9% (164). Improvement of renal function occurred in association with diuretic therapy either with or without albumin infusion. In patients who improved with pharmacologic diuresis, the serum creatinine level again rose on return to an edematous state. The authors postulate that glomerular hemodynamics was altered by the presence of intrarenal edema, which occurred when peripheral edema developed.

Other circulatory abnormalities have been observed in patients with nephrotic syndrome. The occurrence of hypovolemic shock and hypotension has been related to a variety of medical procedures (165). However, hypotension may occur spontaneously. These episodes usually are seen in patients during relapse who have an intercurrent illness causing fluid loss, such as emesis or diarrhea. The patients usually show marked responsiveness to small amounts of intravenous saline that are insufficient to replenish all fluid losses, suggesting a failure in maintenance of vascular tone. Recovery usually occurs if this complication is identified early and treated promptly. Sequelae may include acute tubular necrosis, renal vein thrombosis (RVT), or death.

Hyperlipidemia

Lipemic serum has long been recognized as a cardinal feature of the nephrotic syndrome (166). Abnormalities in postprandial lipid metabolism were described more than 40 years ago (167). Biochemical evaluation has shown that all lipid components of the plasma are increased, with cholesterol increasing more rapidly than phospholipid. Thus, as acute severity of the disease worsens, the ratio of cholesterol to phospholipid increases (168). Triglycerides are relatively normal at the initiation of relapse but increase as the disease continues (169); lactescence occurs when the plasma triglyceride content exceeds 400 mg/dL. Hyperlipidemia may persist well into remission (170), suggesting a residual effect of nephrosis on lipoprotein transport (171).

Depending on the classification employed, the most common patterns of hyperlipoproteinemia seen in nephrosis are types II and IV (172) or types IIa, IIb, and V (173). Low-density lipoproteins (LDLs) and very low-density lipoproteins (VLDLs) show the greatest increase in concentration. Values for high-density lipoprotein (HDL) cholesterol have been reported to be elevated (174,175), normal (176,177), or decreased (169,178,179). This variation may relate to the age of the patients studied, the underlying cause of the nephrotic syndrome, patient treatment, and whether renal insufficiency is present. Studies of lipoprotein cholesterol have produced conflicting results (169,174–179). The ratio of cholesterol to phospholipid or to triglyceride in various lipoproteins is altered, indicating abnormalities in quality as well as quantity of lipoproteins.

Several events may contribute to these abnormalities. Lipid metabolism is normally accomplished through a series of complex steps (Fig. 64-5). Through the action of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, mevalonate is produced from acetate in the liver. This in turn is used to make cholesterol, which is incorporated into lipoproteins. The greater the triglyceride content of the lipoprotein, the less dense it is. Dietary fat absorbed from the intestine is formed into chylomicrons by being surrounded with a coat of apolipoprotein that is critical for transport of the hydrophobic lipid. The triglyceride content of the chylomicron is reduced in the periphery (mainly by the action of lipoprotein lipase [LPL]), and the resulting particle containing apolipoprotein (apo) B-48

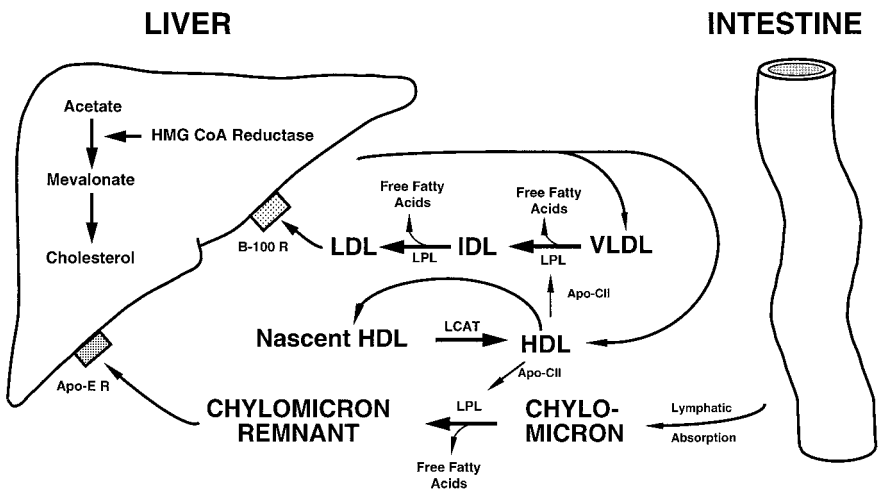

FIGURE 64-5. Normal pathways of lipid metabolism. *apo-CII*, Apolipoprotein C-II; *Apo-E R*, chylomicron remnant (apo E) receptor; *B-100 R*, apolipoprotein B-100 (LDL) receptor; *HDL*, high-density lipoprotein; *IDL*, intermediate-density lipoprotein; *HMG CoA reductase*, 3-hydroxy-3-methylglutaryl coenzyme A reductase; *LCAT*, lecithin-cholesterol acyltransferase; *LDL*, low-density lipoprotein; *LPL*, lipoprotein lipase; *VLDL*, very-low-density lipoprotein. Figure composed with the assistance of Nader Rifai.

and apo E binds to the hepatocyte via a chylomicron remnant receptor. VLDL is synthesized in the liver and metabolized in the periphery through the action of LPL to intermediate-density lipoprotein (IDL), and then to LDL. LDL is bound to apo B-100, which is then taken up by the hepatocyte LDL receptor (180,181). This brings additional cholesterol back to the liver, suppressing HMG CoA reductase activity and decreasing new cholesterol synthesis. The liver also produces HDL, which participates as a transport protein in catabolism of lower-density moieties, being regenerated by lecithin-cholesterol acyltransferase (LCAT). HDL also carries apo C-II, which activates LPL. Abnormalities at any step of metabolism from lipid uptake to the enterohepatic secretion of bile could result in the hyperlipidemia of nephrosis. Likely contributing factors include increased hepatic synthesis of lipoprotein; abnormal transport of lipid through the metabolic pathway; and abnormal catabolism secondary to decreased enzyme activity.

Lipoprotein Synthesis. It is clear that hepatic synthesis of lipoproteins is increased in nephrotic patients (168,182–185). The signal for this event appears to be related to hypoalbuminemia because daily infusion of albumin into nephrotic patients, sufficient to raise serum levels, also decreases serum lipid, triglyceride, and cholesterol levels (186). Increasing the plasma oncotic pressure in nephrotic patients or animals, by infusion of dextrans, decreases hepatic lipoprotein synthesis (167,187). Additional laboratory studies suggest that the regulatory signal could be viscosity rather than oncotic pressure (186,188,189). Cholesterol biosynthesis also has been investigated (190,191). These studies show increased incorporation of ^{14}C from labeled mevalonate into cholesterol by the liver in experimental nephrosis. Although this result is consistent with the interpretation that rates of hepatic cholesterol synthesis are increased in nephrosis, artifactual changes due to the addition of exogenous substrate (mevalonate) could not be ruled out in these experiments.

Lipid Transport. Several aspects of lipid transport may be impaired in nephrosis. The major cholesterol-transporting protein associated with the LDLs in the plasma is apo B-100 (192). This also has been implicated as a significant apolipoprotein in atherogenesis. A recent study of nephrotic patients found that elevated serum concentrations of cholesterol, triglycerides, and phospholipids resulted mostly from changes in apo B-100-containing lipoproteins. The size of the apo B-100 pool in patients was two to three times that found in healthy subjects or in patients in remission. Fractional catabolism was decreased only slightly, suggesting that the major problem was overproduction rather than decreased breakdown (193). Hepatic uptake of LDLs may be decreased (194) if the structural composition of LDLs in the circulating pool is abnormal, or if systemic neutralization of membrane negative charge leads to less efficient uptake of the largely cationic liposomes (195); this would exacerbate hypercholesterolemia by decreasing negative feedback affecting hepatic synthesis. Alternatively, decreased hepatic uptake of LDLs could result from, rather than cause, hepatic overproduction of cholesterol (192).

Metabolism of Lipids. At least one report indicates that while LDL synthesis may be increased in nephrosis, VLDL catabolism is decreased (185). Another study demonstrates that apolipoprotein E-rich IDL from nephrotic patients, but not from normal controls, inhibits sterol synthesis and cholesterol esterification (196). This finding suggests that cellular apo E metabolism may be deranged in nephrosis. Consistent with this finding, genetic variations in the expression of *apo E* alleles may influence the degree of lipid abnormality in nephrotic patients (197).

Interest regarding catabolism of lipids in nephrosis has focused on two enzymes: LPL, which facilitates the breakdown of ester bonds in glycerides, and LCAT, which catalyzes the reaction of lecithin and cholesterol to form lysolecithin and cholesterol ester (198). In nephrotic children, elevated serum lipid levels correlate with decreased postheparin LPL activity (199). In another study of nephrotic patients, most of whom had MCNS, hepatic LPL activity was normal, but serum and adipose tissue LPL activities were decreased in association with elevated plasma triglycerides (174). Decreased hepatic (200) and adipose tissue (199) LPL activity in experimental rat models of nephrosis may contribute to altered lipoprotein levels in these animals. LCAT activity also is decreased in experimental nephrosis (201), with levels appearing to correlate with serum albumin concentration (202).

Activity of these enzymes may be affected both directly and indirectly by urinary protein loss. Albumin binds to free fatty acids (FFAs); decreases in serum albumin concentration lead to FFA accumulation, thereby inhibiting LPL activity (203). LPL activity also may be inhibited by cholesterol (202). LCAT activity is inhibited by the accumulation of triglyceride and cholesterol esters (204), suggesting that abnormal LCAT activity could be a result, rather than a cause, of nephrotic hyperlipidemia. However, lysolecithin, a reaction product that binds to albumin, inhibits LCAT activity *in vitro*; this feedback mechanism is blocked by addition of physiologic levels of albumin (204,205). Therefore, urinary loss of albumin may lead to inhibition of lipolytic enzyme function.

Albumin loss does not account entirely, however, for the elevated lipid levels. Although infusion of albumin decreased serum lipid levels in an acute animal model of nephrotic syndrome, normalization occurred only after simultaneous infusion of heparin. This suggests the need for an additional factor that aids in clearing lipid from the plasma (206). In further experiments with this model, nephrectomy resulted in greater improvement of the hyperlipidemia than did albumin infusions alone (207), indicating that the factor may be lost in the urine. Further support for loss of a specific regulatory molecule in the urine is provided by the observation that alteration of dietary protein intake markedly modulates the hepatic albumin synthetic rate but does not alter the hepatic synthesis of lipoproteins (208). In this study, lipoprotein synthesis correlated directly with the urinary clearance of albumin, suggesting that albumin, or another substance lost in parallel with albumin, was needed to suppress lipoprotein synthesis. Experiments with analbuminemic rats indicate that albumin itself is not likely to be the critical molecule (209). It has been suggested that the lost factor is LCAT (168). HDL, which plays an essential role in catabolism of VLDL, also may be lost in the nephrotic urine (210,211). Conversely, other studies indicate that HDL excretion is low (187), especially in MCNS (212). Apo C-II also may be lost in the urine (192,213).

Clinical Significance. Regardless of the cause, the clinical significance of the lipid abnormalities in nephrosis must be considered. Hyperlipidemia has been associated with cardiovascular disease in otherwise healthy young adults, but studies evaluating such a correlation in nephrotic patients produced conflicting results. Premature coronary atherosclerosis (214) and a high incidence of myocardial infarction and other cardiovascular diseases (215,216) have been documented in nephrotic subjects, as well as a higher incidence of hypertension in nephrotic men than in control subjects (217). In this respect, the observation that macrophage morphology and function are altered by the hyperlipidemia of nephrosis (218) could relate to the development of atheromatous plaques. In addition, plasma levels of lipoprotein(a), a strong risk factor in cardiovascular disease, are increased (219). In contrast, other studies have not confirmed a predisposition to atherosclerosis in patients with

nephrosis (220,221). These discrepant results may reflect limitations of population base or selection bias (174,212). In the studies that did not demonstrate an increased risk, it is unclear whether stratification of the patients into cohorts according to the degree of lipid abnormality would have shown an increased risk in patients with the highest consistent elevations in lipoprotein levels. Age, underlying diagnosis, disease course, and incidence of other complicating factors such as hypertension also may be important. Another significant consideration is the possible ameliorating effect of HDL on hyperlipidemia (220–223). In several studies (174,212), HDL levels were normal or increased in MCNS. This could have a protective effect and decrease the likelihood of cardiovascular complications. Further study of larger groups of nephrotic patients would allow differentiation among patients with other cardiac risk factors in addition to the potential hazard of elevated serum lipid levels.

A second risk involves the role of lipids in causing or enhancing the progression of the renal disease itself. Rats with PAN nephrosis fed high-cholesterol diets develop mesangial foam cells and mesangial proliferative changes (224). The relationship of systemic hypertension and hyperlipidemia to atherosclerosis parallels the relationship of intraglomerular hypertension and high lipid levels to focal sclerosis (224). Effective therapy of hyperlipidemia ameliorates single-nephron hyperfiltration (225) and retards progression of renal failure in obese Zucker rats (226) and in nephrotic rats with reduced renal mass (227). In obese Zucker rats, a relative decrease in polyunsaturated fatty acids (PUFAs), rather than high cholesterol levels, may be the most important lipid-related factor in progression of renal disease, since dietary supplementation with n-6 PUFA (sunflower oil) or n-3 PUFA (fish oil) slowed progression of renal disease but only fish oil decreased serum cholesterol levels (225).

In view of these considerations, and the fact that treatments for nephrosis such as steroids and diuretics may exacerbate hyperlipidemia, clinicians have invested increasing effort in controlling the lipid abnormalities of nephrosis (228). Traditional dietary therapy is of marginal value, and may actually worsen the hyperlipidemia (229). Cholestyramine may, by increasing secretion of cholesterol into the bile, predispose toward the development of cholesterol gallstones (192). Nicotinic acid has significant side effects and has not been studied extensively. Probucol may cause concomitant loss of HDLs (229). However, it has been shown experimentally to reverse lipid-mediated vasoconstriction (230), and to be effective in treating patients who were 5 to 20 years old (231). Two other classes of drugs found to be effective in treating nephrotic hyperlipidemia are fibric acids and HMG CoA reductase inhibitors. Gemfibrozil, a fibric acid, caused a 51% reduction in serum triglyceride levels but only a 15% decrease in cholesterol when given at a dose of 600 mg twice a day to adult nephrotic patients; a 26% reduction in apo B was achieved (232). Lovastatin, an inhibitor of HMG CoA reductase, caused a 27% to 29% reduction in total cholesterol, LDL cholesterol, and apo B at a dose of 20 mg twice daily in adult patients with nephrosis due to MCNS or other diseases (193). In patients with nephrotic-range proteinuria, doses up to 40 mg twice daily caused similar decreases regardless of whether the patients were on corticosteroid therapy. A slight increase was noted in serum HDL concentrations (233). Kinetic studies showed that lovastatin enhances the catabolism of VLDL triglycerides and lowers LDL cholesterol by decreasing input rates for LDLs (234), most likely through inhibition of LDL–apo B synthesis from VLDL (235). Atorvastatin also is effective in nephrotic hyperlipidemia (236). In children, HMG CoA reductase inhibitors may be effective, but some clinicians have urged caution regarding their use in the very young child, raising the possibility that inhibiting cholesterol synthesis might impair neural myelination. This concern needs to be weighed against the more immediate issues of cardiovascular complications and renal disease progression.

Disorders of Hemostasis

The association between nephrotic syndrome and intravascular coagulation has been known for more than a century, but it was not until 1948 that the concept of a thrombotic diathesis in nephrotic patients was proposed (237). In a review of 3,377 children with nephrotic syndrome, the incidence of thromboembolic complications was 1.8% (238). The prevalence of such complications in adult nephrotic subjects is much higher and averaged 26% in eight series of patients (239). Thrombosis may occur at any stage during the course of the nephrotic syndrome, but it is most frequent in the early months.

Extent of Clinical Involvement. Deep vein thrombosis of the leg is the most common thrombotic complication in the nephrotic adult and was responsible for one third of the thromboembolic complications in the largest published series of nephrotic children (238). Other reported sites of venous thromboses include the subclavian, axillary, external jugular, portal, splenic, hepatic, and mesenteric veins as well as the superficial cerebral cortical sinus, where thrombosis has been observed in both children and adults and may be fatal (240,241). Arterial thrombosis occurs less frequently and is seen primarily in children. Thrombosis of the aorta and of the mesenteric, axillary, femoral, ophthalmic, carotid, cerebral, renal, pulmonary, and coronary arteries has been reported, as has intracardiac thrombosis (240,241). The pulmonary (242) and femoral arteries are particularly susceptible, the former potentially resulting in infarction (243) and the latter usually as a complication of attempted blood sampling from the femoral vein. Although recanalization of the artery does occur, a relatively high proportion of patients with arterial thrombi die (242).

The lesion that has attracted the most attention, however, is RVT or renal vein thrombosis. It is most often seen with membranous glomerulopathy (244), to the extent that at one time there was some controversy about whether RVT was the cause, rather than a complication, of the glomerular lesion. The reported frequency of RVT in patients with membranous disease has ranged from 4% to 51%, depending on the methods used to establish the diagnosis and to select the patient population for study (245). The mean prevalence is 12%. There is a high incidence of RVT in mesangiocapillary glomerulonephritis and the nephritis of systemic lupus erythematosus, and renal vein thrombosis can complicate numerous other renal diseases (240). It is relatively uncommon in nephrotic children except in those with congenital nephrotic syndrome of the Finnish type (246).

The thrombosis may involve only the renal venous system or it may extend into the inferior vena cava. Therefore it is not surprising that pulmonary emboli develop in about 40% of adult patients with RVT, although pulmonary emboli occur rarely in children. Death from pulmonary emboli is uncommon (238,240).

Diagnosis of acute RVT is suggested by flank pain, costovertebral angle tenderness, gross hematuria, increased proteinuria, and acute reduction in renal function; intravenous pyelography may show ureteral notching or pelvocaliceal irregularities (245). Ultrasonography may demonstrate only a large kidney or may visualize the thrombus if it extends into the renal vein or inferior vena cava. However, Doppler ultrasound analysis often shows decreased venous blood flow. A more chronic form of RVT may be asymptomatic and may be identifiable only by venography (245,247). The mode of presentation of other thromboses depends on their site. Diagnosis can be difficult and the existence of arterial thrombosis may not be realized until

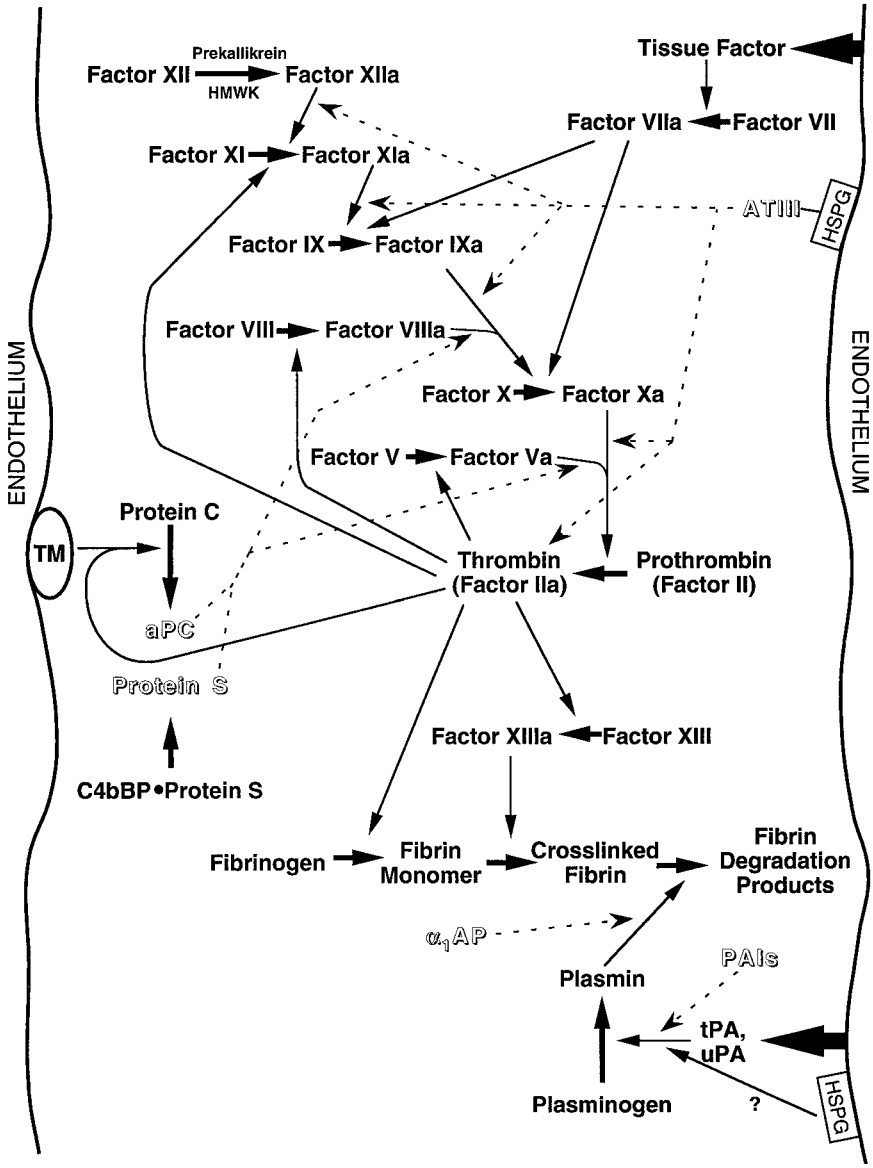

FIGURE 64-6. Interactions among circulating participants in the coagulation cascade. The intrinsic pathway begins in the upper left; the contact system of factor XII, prekallikrein, and high-molecular-weight kininogen (HMWK) that initiates the intrinsic cascade is shown in condensed form. The extrinsic pathway begins in the upper right. The action of factor VIIa on the activation of factor IX has blurred the distinction between these two limbs of the cascade. The common pathway begins with activation of factor X and results in thrombin activity and fibrin crosslinking. Coagulation is counteracted by the fibrinolytic pathway, including plasminogen activators and plasminogen/plasmin. In addition, there are several anticoagulant pathways, most notably inhibition of factors Va and VIIIa by the inhibitory cofactors protein S and activated protein C (aPC). Alpha-2-macroglobulin, which binds to and inhibits most of the enzymes in this system, is not shown here for the purpose of simplicity. The relationship between heparan sulfate proteoglycans (HSPGs) and plasminogen activator activity shown in the lower right should be regarded as hypothetical for human pathophysiology. *Thick solid lines with arrows* indicate reactions; *thin solid lines with triangular arrows* denote catalytic effects; *joining lines* show cofactors in catalysis. *Broken lines with fork-tailed arrows* represent inhibitory actions. α_1 *AP*, α_1-antiplasmin; *ATIII*, antithrombin III; *C4bBP*, complement factor 4b-binding protein; *PAIs*, plasminogen activator inhibitors; *TM*, thrombomodulin; *tPA*, tissue-type plasminogen activator; *uPA*, urokinase plasminogen activator. PAI-1 and PAI-3 bind to aPC; this interaction may cause mutual inhibition of the action of these proteins. (Figure composed with the assistance of G.A. Soff.)

autopsy. Ultrasonography and angiography are the preferred studies.

Regulators of Coagulation. The blood coagulation pathway represents a complex series of events that regulate the dynamic balance between the ability of the blood to remain fluid and its tendency to assume a gelled state in the presence of altered flow conditions or exposure to nonendothelial surfaces. Contributing to hemostatic balance are several opposing systems that contribute to a cascade through which a series of enzymes regulates fibrin polymerization (Fig. 64-6). Coagulation is initiated by activation of prekallikrein to kallikrein (intrinsic pathway), or by exposure to nonendothelial tissues (extrinsic pathway). These pathways meet in the activation of factor IX, initiating a common pathway in which a central role is played by thrombin (factor IIa). This enzyme stimulates activation of fibrinogen to fibrin and aggregation of platelets. It also activates factors V, VIII, and XIII. Factor XIIIa triggers crosslinking of fibrin monomer into a stable polymer. Two systems oppose clot formation and stability. Protein C is processed to activated protein C (aPC) by thrombin complexed with thrombomodulin. With free protein S as a cofactor, aPC inactivates factors Va and VIIIa. The other system that opposes coagulation is the fibrinolytic pathway, in which plasminogen activators convert plasminogen to plasmin, which degrades fibrin polymer. Several proteins inhibit these pathways: Antithrombin III and α_2-macroglobulin inhibit thrombin; α_1-antiplasmin and α_2-macroglobulin inhibit plasmin; and the plasminogen activator inhibitors (PAIs) inhibit plasminogen activators and aPC. Activated protein C, in turn, opposes PAI effects. Many of the components of these pathways are altered in nephrosis. In addition, physical conditions of the nephrotic syndrome, such as venous stasis, hemoconcentration, increased blood viscosity, and possibly the administration of steroids, may also contribute to enhanced blood clotting. These nephrotic effects on coagulation pathways, which are listed in Table 64-4 and discussed in detail elsewhere (240,247,248), are considered here briefly.

Platelet Aggregation. Platelets may play a role in the genesis of the coagulopathy of nephrotic syndrome. Thrombocytosis is commonly found, especially early in the disease course (249), and platelets show markers of activation (250). Platelet aggregability is increased and platelet degranulation has been described (251). In addition, plasma levels of the platelet release

TABLE 64-4

COAGULATION SYSTEM ABNORMALITIES IN THE NEPHROTIC SYNDROME

Increased platelet aggregation
Thrombocytosis
β-Thromboglobulin
Platelet factor 4
Increased procoagulant activity
Physical factors
Hemoconcentration
Hyperviscosity
Increased factor production
Intrinsic pathway—factors VII and IX (variably)
Extrinsic pathway—factor VII (variably)
Common pathway
Fibrinogen
Factors V and VIII
Factors X and II (variably)
Urinary loss of anticoagulants
Antithrombin III
Free protein S
Increased inhibitors of anticoagulation
α_2-Macroglobulin
C4b-binding protein
Increased plasminogen

substance β-thromboglobulin are increased (249,252). Levels of platelet factor 4 are normal (253) or increased (249). In contrast, platelet calcium ion release and ATP secretion have been found to be decreased in nephrosis (254). The authors of that report suggest that platelets may become desensitized to platelet activating factor (PAF) because of exposure to consistently high ambient concentrations.

Platelet hyperaggregability correlates with the degree of proteinuria and with plasma cholesterol levels. It can be reversed by the addition of urine protein (253). These findings suggest that the urinary loss of albumin (255) or of some factor that normally inhibits platelet aggregation is responsible for the changes seen in nephrotic syndrome. Alternatively, hyperlipidemia could result in the changes, as platelet aggregation is increased in patients with type II hyperlipoproteinemia to a degree that is comparable to that seen in nephrotic syndrome (256). Altered platelet function could be a response to hypoalbuminemia, because the conversion of arachidonic acid into metabolites that aggregate platelets is known to be regulated by albumin (257,258). Thus platelets show greater production of thromboxane B_2 and malondialdehyde in nephrotic plasma than in normal plasma when challenged with arachidonic acid. Addition of albumin to the nephrotic plasma corrects this abnormality (259). Finally, it is possible that alterations in platelet membranes could be responsible for increased platelet activity. Platelet membranes contain a sialoglycoprotein with a pK of 1.8 to 2.2 (260). This may be important in preventing spontaneous platelet aggregation or platelet interaction with the vessel wall (261). Because systemic negative-charge sites may be reduced during relapses of nephrotic syndrome (65), the same mechanism responsible for the reduction of negative-charge sites in the GBM could enhance platelet aggregation.

Coagulation Factors. Evidence that various functions of blood coagulation are activated in nephrosis is provided by increased concentration of the D-dimer of fibrinogen (262). Elevated levels of this breakdown product of crosslinked fibrin indicate that both the coagulation and fibrinolytic pathways are concurrently activated. Plasma fibrinogen is consistently elevated in nephrotic syndrome due to increased hepatic synthesis. Chromatography demonstrates both increased polymerization and increased proteolytic derivatives of fibrinogen or fibrin. These changes reverse as patients with nephrotic syndrome enter remission (263). This evidence for increased intravascular fibrin formation is supported by the finding of increased plasma levels of fibrinopeptide A, at least in FSGS (263).

The concentration in nephrotic patients of coagulation factors that initiate fibrin formation likely reflects the balance between the increased hepatic synthesis of these proteins, triggered as part of a nonspecific response to hypoproteinemia as described previously for lipoproteins, and urinary losses. Therefore, lower molecular-size proteins (approximately less than 70 kDa) may be lost in the urine, whereas higher molecular-size proteins (greater than 300 kDa) are likely to be increased in the plasma. For example, most studies agree that levels of factors V and VIII are increased in the plasma, whereas those of factors IX, XI, and XII are decreased (264) despite the possibility that production may still be increased. The magnitude of the increase in concentration of factors V and VIII, for example, correlates with the degree of reduction in serum albumin and the likely resulting increased hepatic synthesis of these factors, stimulated by hypoalbuminemia (265). Plasma levels of factors II, VII, X, and XIII are often found to be increased (266–268). There is no direct evidence that any of these changes are responsible for the hypercoagulable state. Indeed, the alterations in blood levels of these factors are often inconsistent and of minor degree. Therefore, these abnormalities may be of more biochemical than clinical interest. Most of the changes in concentration of these zymogen factors reverse with clinical remission of the nephrotic syndrome.

Inhibitors of Coagulation. The most well-studied biologic antagonist of coagulation, antithrombin III, is decreased in the plasma of nephrotic patients (269–273). This is presumed to be due to urinary loss of antithrombin III, which has a relatively low molecular weight. Indeed, plasma antithrombin III levels in nephrotic syndrome correlate well with those of serum albumin and inversely with the renal clearance of antithrombin III. Because hereditary antithrombin III deficiency is associated with frequent thrombosis, it was hypothesized that the low plasma antithrombin III levels were insufficient to inactivate procoagulant factors and were the major cause for the hypercoagulable state and the development of thrombosis in nephrotic syndrome (271). However, only patients with plasma albumin levels below 2 g/dL show significant reductions in plasma antithrombin III levels (274), whereas hypercoagulability may be present in patients with albumin levels exceeding this value. Further, normal plasma levels of antithrombin III were found in nephrotic subjects who had loss of antithrombin III in the urine and who had thromboembolic complications (275). Indeed, decreases in antithrombin III levels may be compensated for by increased plasma levels of α_2-macroglobulin (268), leading to increased total antithrombin activity (263).

A complex effect of nephrosis has been noted on the anticoagulation pathway by which protein C is activated by thrombin and thrombomodulin to aPC. Activated protein C and protein S combine to inhibit factor VIIIa (decreasing activation of factor X) and factor Va (decreasing activation of factor XI). Protein S exists in circulation in two forms: free and bound to C4b binding protein. Only the free protein S can serve as a cofactor with protein C to inactivate factors Va and VIIIa (276). In nephrotic syndrome, although small amounts of protein C are lost in the urine, serum concentrations are normal (277,278), indicating that hepatic synthesis is able to compensate. In contrast, although plasma levels of C4b binding protein–protein S

complex are usually normal to elevated, free levels are markedly decreased (279). This finding is consistent with the molecular sizes of the complex (640 kDa) and the free protein S (69 kDa). Acquired dysfunction of this system is a common cause of thrombotic diathesis (276,280). Indeed, intractable deep vein thrombosis in an unusual nephrotic child with decreases in both protein S and C4b-binding protein concentrations (281) supports the notion that abnormalities of this system are clinically significant.

Fibrinolysis. Alterations in the concentrations of several of the components of the fibrinolytic system have been documented (282). Decreased fibrinolytic activity has been associated with hypertriglyceridemia (283). Of the individual components of the fibrinolytic system, decreased concentrations of plasminogen have been found (270,284); levels of tissue-type plasminogen activator (262) and PAI-1 (285) are elevated. Varying levels of α_2-antiplasmin have been reported (262,263), possibly depending on whether thrombosis has occurred. Of the serine protease inhibitors that modulate both the fibrinolytic and thrombin systems, levels of α_2-macroglobulin are increased and those of α_1-antitrypsin are decreased (268). Again, this probably reflects the effect of urinary loss on plasma concentrations. It also is possible that local vascular conditions affect fibrinolysis. Infusion of a variety of polyanions causes an immediate local increase in release of plasminogen activator and PAI activity in the sow ear. The PAI activity immediately returns to normal but the increase in plasminogen activator activity is sustained (286), suggesting that negative charges in the vascular tree are important for inducing the plasminogen activator pathway. If nephrosis is associated with a generalized reduction of fixed negative charge sites in the vascular space (65), it is likely that decreased negative charges could have an impact on induction of fibrinolysis. Coupled with increased α_2-antiplasmin concentrations, decreased tissue-type plasminogen activator activity would significantly impair fibrin degradation. However, the increase in circulating D-dimer cited previously indicates that at least some fibrinolysis occurs in nephrosis. It also is possible that fibrin polymerization is impaired in nephrotic patients. In one study of a broad spectrum of adults with nephrosis, most had prolonged thrombin times. Half of the patients showed decreased ability to polymerize fibrin monomer. There was no correlation of this finding with prothrombin time, partial thromboplastin time, fibrin degradation products, antithrombin III concentration, or platelet count (287).

Environmental Factors Affecting Coagulation. Physical factors such as increased blood viscosity also may contribute to the generation of thromboembolic complications (288). Both children and adults with MCNS and well-preserved renal function may have marked hemoconcentration with elevated hematocrit and hemoglobin concentrations. Such changes are associated with disproportionate increases in viscosity and could be aggravated by the therapeutic use of diuretics, especially if these cause further hemoconcentration. In addition, when plasma fibrinogen levels increase, especially to values as high as 1 g/dL as can be seen in nephrotic syndrome, they cause increased erythrocyte aggregation and marked increases in plasma viscosity (289). A role for physical factors is supported by the high incidence of renal vein thrombosis, as hemoconcentration of the blood and the effect of urinary inhibitor loss will be most pronounced in the radicles of the renal vein (290).

Steroid administration increases the concentrations of several clotting factors and modifies coagulation mechanisms (240). Moreover, a high incidence of thromboses was recorded after these drugs were first used to treat nephrotic syndrome (291). Both arterial and venous thromboses, however, have been found in nephrotic subjects not receiving steroids. Furthermore, a hypercoagulable state is present in untreated MCNS patients, and levels of the coagulation factors do not change after steroid treatment is implemented (263).

Except for the protein S data, the potential relationship between various biochemical findings and the clinical importance of thrombus formation remains largely theoretical. For example, the biochemical abnormalities in children may be more severe than those in adults with the nephrotic syndrome, whereas the incidence of thromboembolic phenomena is worse in adults (292). This may reflect the fact that MCN is more common in children, whereas membranous nephropathy (see Chapter 63) is more common in adults. Therefore, the underlying nature of the disease may be important in determining the occurrence of intravascular coagulation. Finally, it is important to consider the physiologic conditions within the circulatory tree. For example, one study suggested that although platelet aggregation is increased in nephrosis, fibrin conversion inhibits platelet interaction with the vessel wall extracellular matrix, actually decreasing the likelihood of platelet participation in thrombus formation. In this system, the data suggest that increased fibrin formation, but not increased platelet aggregation, contributes to the hypercoagulability of the nephrotic syndrome (293). In support of a primary role for the coagulation cascade, nephrotic patients show biochemical evidence for endothelial injury (294). The effects of negative-charge sites must also be considered. Like plasminogen activator activity, antithrombin III is active in association with vascular wall heparan sulfate proteoglycans. If MCNS involves a generalized decrease in negative-charge sites, antithrombin III activity may be impaired.

Infections

It has long been known that patients with MCNS have increased susceptibility to infection. This increase may be related to the prolonged presence of gross edema or ascites, which is composed of fluids that represent ideal culture media for bacterial growth. Infection risk may be potentiated by therapy with steroids or immunosuppressive drugs, although the high incidence of infections was noted in the era before these drugs were available. Humoral responses to bacteria may be defective. Plasma concentrations of IgG are markedly reduced during relapse (295), and the ability of MCN patients to generate specific antibodies is impaired (296) between as well as during relapses. Although the role of these abnormalities in predisposing nephrotic subjects to infections remains to be elucidated, it may be significant that boys with MCN respond poorly to hepatitis B vaccine (297). This same population has a higher incidence of chronic hepatitis B surface antigenemia than that found in a control population (298). Another factor that could contribute to a high rate of infections in patients with nephrosis is a decreased serum level of alternative complement pathway factor B. Absence of this factor has been linked to defective opsonization of *Escherichia coli* in nephrotic patients (299) and to defective neutrophil function (300). Serum levels of hemolytic factor D also are decreased in patients during relapse and return to normal with remission (301). Levels of both of these factors correlate strongly with serum albumin concentration, suggesting that decreased serum levels result from urinary loss. These concerns have led to recommendations that both pediatric and adult patients with nephrosis should receive pneumococcal vaccine (302,303). In addition, the use of penicillin prophylaxis may be required in children younger than 2 years of age, or in older patients who have low antibody titers or recurrent pneumococcal infection (304). Immune system abnormalities more specifically associated with MCN are unlikely to result entirely from albuminuria, since they are specific for that

disease; these will be considered in the section on MCN later in this chapter.

Consequences of Loss of Other Proteins

Numerous proteins in addition to albumin are lost in the urine. In most instances, these proteins are of a similar or smaller size than albumin. Such losses could alter function in the endocrine system or in metabolic pathways. Therefore, loss of insulin-like growth factors could contribute to poor growth in some nephrotic children (305). The urinary loss of thyroxin-binding globulin (TBG) correlates well with total urinary protein excretion (306). In addition to TBG, losses of thyroxine (T_4) and triiodothyronine (T_3) in nephrotic urine are associated with decreased serum levels of T_3 and TBG. Most of the patients studied were clinically euthyroid and their serum levels of free T_4 and thyroid-stimulating hormone (TSH) did not differ from those in normal control subjects (307). In addition, their values for T_3 uptake were normal. Another study documented urinary losses, but normal serum concentrations of TBG (308). The patients had low or low-normal T_4 levels. Such differences in findings could relate to the underlying cause for nephrotic syndrome, whether it is associated with selective or nonselective proteinuria and whether it is accompanied by uremia. Children with MCNS have serum T_4 or free T_4 levels that are marginally low (309). They have been interpreted as having mild thyroid failure based on increased baseline TSH levels and their response to thyrotropin-releasing hormone (310). In patients who have congenital nephrotic syndrome, nephrectomy to eliminate proteinuria is associated with normalization of thyroid status, indicating that these abnormalities result from massive proteinuria rather than an intrinsic glandular defect (311).

Total serum calcium is markedly reduced, primarily because of hypoalbuminemia and the consequent decrease in protein-bound calcium. Serum ionized calcium levels may be reduced as well (312,313), even in nephrotic subjects with normal renal function; this may result in symptomatic hypocalcemia. At least some of the reduction in ionized calcium is the consequence of loss of 25-hydroxyvitamin D (25-[OH]-D) in nephrotic urine (314,315); other metabolites of vitamin D may be lost, as well (316). Low plasma levels of 25-(OH)-D, 1,25$(OH)_2$-D, and 24,25-$(OH)_2$-D have been reported in patients with nephrotic syndrome (314), and intestinal absorption of calcium is reduced (312); serum parathyroid hormone levels are increased (313). From these observations, it has been postulated (317) that urinary loss of vitamin D complex results in decreased absorption of intestinal calcium, skeletal resistance to parathyroid hormone, and reduced serum calcium levels. In turn, these changes cause increased parathyroid hormone (PTH) production and could result in defective bone mineralization. Although it has not been proved that the changes described cause significant bone disease (318), ongoing steroid therapy may increase the likelihood of clinically significant problems (319,320). In 60 children and adolescents with relapsing nephrotic syndrome, bone mineral density (BMD) was decreased but whole-body bone mineral content was higher because of an increase in the body-metabolic index (321). In patients with unremitting proteinuria and low 25-OH-D levels, supplementation with oral calcium and 25-OH-D should be considered (322).

Carbohydrate metabolism also may be deranged. Of 38 adult nephrotic patients who had not received any drugs, including glucocorticoids, for at least 2 months, 14 had oral glucose tolerance test results that were similar to those found in diabetic patients (323). Affected patients had increased insulin secretion that was thought to be secondary to increased growth hormone levels. The initiating event for these changes was not determined. There was no correlation of these findings with either serum albumin levels or renal histopathology. This observation raises the question of whether nephrotic hyperlipidemia could contribute to the development of noninsulin-dependent (type 2) diabetes mellitus.

Alterations in trace metal metabolism may be due to urinary losses of either the metals or their carrier proteins. Decreased serum levels of both iron and copper, associated with a low serum iron-binding capacity and low erythrocyte copper content, have been documented in nephrotic syndrome (324). Serum levels of copper, but not iron, were improved by oral administration of the metal. Urinary iron and copper concentrations correlated with protein excretion. The intravenous infusion of albumin led to increased albuminuria and increased metal excretion. In each case, the abnormalities appeared to be related to urinary protein loss. Nephrotic children may develop anemia secondary to urinary loss of transferrin and iron (325,326). Serum zinc levels are low in nephrotic syndrome, but urinary excretion of zinc is not elevated. Zinc binds to albumin so that serum zinc levels change with alterations in albumin levels, regardless of the etiology of the nephrotic syndrome. However, decreased zinc content in the hair of these patients suggests that other aspects of zinc metabolism also may be deranged (327).

Many drugs are protein-bound in the plasma. Hypoalbuminemia decreases the number of drug-binding sites and could result in increased toxicity of drugs that normally are bound to protein. For example, digoxin is 25% bound to proteins in the plasma, digitoxin is 90% bound, hydrochlorothiazide is 60% bound, and furosemide is 96% bound; hydralazine, prazosin, and diazoxide are all approximately 90% bound, whereas the binding of barbiturates varies from 5% to 80% depending on molecular structure (328).

General Approach to the Treatment of Nephrotic Syndrome

Based on the consequences of proteinuria described in the preceding text, symptomatic treatment regimens have been developed for the care of all nephrotic patients, even beyond those classified as having a form of primary nephrotic syndrome. These treatments, which are aimed at the physiology of nephrosis rather than the etiology of the disease, have implications beyond the reduction of nephrotic proteinuria and edema.

Diuretics and Fluid Management

Many patients with nephrotic syndrome respond to the acute use of diuretics with increased urinary losses of sodium and water. Although diuretics may be effective, there are limited indications for their use in the acute treatment of nephrotic subjects, especially children. The degree of diuresis and natriuresis they induce is small compared to that observed when the patient responds to treatment directed at the underlying cause. Furthermore, it is possible that diuretic use, by depleting intravascular as well as interstitial fluid volume, may contribute to the development of shock seen in some patients with MCNS (165).

Nonetheless, oral or parenteral diuretics are effective and often are indicated in the management of persistent edema. Parenteral furosemide is more effective than orally administered drug. Treatment may be initiated at 1 mg/kg (up to 40 mg) with judicious increases up to three to four times the usual dose (329) in an effort to elicit a response. Diuretic therapy may be less effective in patients with primary nephrotic syndrome than in most other patients, in part because of a combination of factors resulting in a physiologically decreased ability to excrete sodium. This is especially true of the loop

diuretics such as furosemide, which also may be inhibited by its binding to albumin present in the tubular lumen (330). Although spironolactone interferes with distal nephron sodium reabsorption and thus is a theoretically useful diuretic, in practice its delayed onset of action and relatively weak potency limit its usefulness to being a potentiating agent with loop diuretics. Metolazone, a thiazide-like drug that impedes both proximal and late distal nephron sodium absorption (331), is a singularly effective oral diuretic in patients with sodium retention secondary to nephrotic syndrome. There is a possibility that diuretic therapy will deplete the intravascular volume without significantly reducing the tissue edema. Therefore, diuretics should be administered with care and withheld in patients for whom a rapid response to steroids is anticipated.

If the patient has anasarca, if respiratory embarrassment results from ascites or pleural effusion, if scrotal or vulval edema is sufficiently severe to threaten tissue breakdown (332), or if peritonitis is present, then more aggressive therapy is warranted to decrease the amount of edema. A useful regimen consists of oral spironolactone, 1 mg/kg per day, and daily intravenous infusions of albumin, 0.5 g/kg initially and increasing, if well tolerated, to 1 g/kg per day. The albumin infusion should be preceded by the intravenous infusion of furosemide, 0.5 mg/kg. A repeated dose of diuretic is given toward the end of the albumin infusion. Blood pressure should be monitored throughout the albumin infusion to help avoid complications from rapid mobilization of edema fluid into the circulation, although the regimen usually is free from significant side effects when used in children and young adults. Recent observations suggest that the administration of albumin may result in more severe glomerular epithelial changes, raise the oncotic pressure of the tissue space, delay the response to corticosteroid therapy, and induce more frequent relapses after remission (333). In view of the potential for complications of albumin infusion (334), this treatment should be reserved for the specific indications of respiratory embarrassment, tissue breakdown, or the need to elicit urine output to confirm the diagnosis of nephrosis.

Management of the acute phase of nephrotic syndrome should include dietary sodium restriction. During relapse, dietary sodium intake optimally should be reduced to about 0.5 g per/day, which is approximately equivalent to a 1-g salt diet or about 20 mEq of sodium per day. Such severe dietary restriction is difficult to accomplish even in a carefully controlled hospital setting. It is important to emphasize that severe restriction of sodium intake will not result in weight loss when nephrotic patients are in the sodium-retaining phase of their disease. In such patients, the normal extrarenal losses of sodium may amount to less than 10 mEq per/day. Therefore, severe dietary sodium restriction is intended to prevent further accumulation of edema. Use of a salt substitute may facilitate compliance with the sodium-restricted diet, but in patients with renal insufficiency it must be limited because these preparations consist of potassium and ammonium salts.

At home, most patients can rarely manage dietary restriction below that of a no-added-salt diet. This provides a sodium intake of 40 to 60 mEq per/day depending on the patient's size. Even in remission, it should be employed not only to lessen the risk of edema formation if the patient has a relapse, but also to reduce side effects from steroid administration.

Although there is some debate regarding fluid management, we believe that fluid intake also should be restricted, at least initially. If intake equals insensible fluid losses plus urine output, the patient's weight will remain stable without further accumulation of edema. To accomplish loss of weight, fluid intake must be reduced below this level. Some nephrotic patients experience intense thirst. If sodium intake is limited and fluid intake is great, the patient can become hyponatremic and will remain edematous.

Anecdotal experience suggests that bed rest may potentiate a diuresis, perhaps by redistributing fluid from the peripheral tissues to the vascular space, thereby increasing renal blood flow. Bed rest also may accelerate a response to steroids and, when practical, should be advised for patients with anasarca. Other therapies that may facilitate a diuresis in some patients by mobilizing tissue fluid include local pressure using surgical elastic stockings or immersion up to the neck in warm water (128). After remission is induced, a high-protein diet may increase the rate at which plasma protein concentration returns to normal (335).

Dietary Treatment

As already indicated, dietary sodium and water restriction is important in the management of the acute phase of nephrotic syndrome. Long-term reduction of dietary sodium intake in combination with diuretics can be most effective in controlling edema in patients resistant to steroids and other pharmacologic agents.

Other dietary manipulations have received attention. High-protein diets can be beneficial in special groups of patients such as those with congenital nephrotic syndrome (336). The concept of increasing dietary protein in all nephrotic subjects has not proved to be beneficial. Although albumin synthesis increases with such diets, urinary protein excretion increases too, possibly due to an angiotensin II-induced increase in glomerular permeability. Therefore, it has been proposed that inhibitors of ACE should be used in conjunction with the high protein intake (94).

Conversely, low-protein diets will decrease albuminuria and increase albumin mass (337). This reflects conservation of essential amino acids in response to proteinuria (338). The use of soy-based, low-protein, low-fat diets rich in polyunsaturated fats and supplemented with essential amino acids or keto analogs results in decreases in urinary protein excretion and in serum total and LDL cholesterol levels (339–341). Supplementing diets with fish oils containing omega ω-3 fatty acids has not proved beneficial (342). Concerns about such dietary manipulations include their cost, the lack of patient acceptance, and whether strict adherence might result in specific nutritional deficiencies (337,343). Reducing dietary fat intake in hyperlipidemic but otherwise healthy children to the levels recommended by the National Cholesterol Education Program is safe and does not affect the children's growth or development (344). The safety of more stringent restrictions in children with renal disease remains to be determined.

Lowering dietary fat intake has a limited effect on reducing serum lipids in the nephrotic patient. Therefore, attention has focused on whether there is a role for lipid-lowering drugs in managing hyperlipidemia in these patients (see following paragraph). The efficacy of oligoantigenic diets or those that eliminate specific antigens has been tested in nephrotic subjects (see section on Atopy and Minimal Change Nephropathy). This approach is based on the possibility that some cases of nephrotic syndrome may be the consequence of food allergies, especially to milk and dairy products.

Lipid-Lowering Drugs

Long-term administration of HMG CoA reductase inhibitors in nephrotic patients will induce reductions of serum triglycerides as well as serum total and LDL cholesterol levels; HDL cholesterol values are maintained (192,236,345,346). Long-term benefits from this therapy have yet to be proved, although anecdotal experience suggests that it may help to reduce proteinuria and maintain GFR (347), as observed in experimental animals (see section on Hyperlipidemia).

Other Medical Therapy

Anticoagulation has been employed as indicated in patients with intravascular thromboses. Some clinicians utilize small doses of aspirin in an effort to prevent repeated thromboembolic episodes (348), but there are no studies confirming the efficacy of this approach.

Although hypocalcemia is common in relapses of nephrotic syndrome, it is likely that most patients in relapse of brief duration do not require routine treatment with calcium or with vitamin D or one of its metabolites (349). Supplementation with oral calcium (1 g per day) and 25-OH-vitamin D (25 μg per day) maintains normal bone status in steroid-dependent nephrotic patients (322). Vitamin D treatment may be more routinely necessary in patients who are steroid-resistant (350) (see section on Consequences of Loss of Other Proteins previously in this chapter). Similarly, patients who develop other deficiencies secondary to renal losses, such as iron-deficiency anemia, will require appropriate treatment.

Medical Treatment to Reduce Proteinuria. In patients who prove to be unresponsive to therapy directed at the underlying cause of proteinuria, efforts have been employed to decrease protein loss by employing drugs that appear to be directed against the physiology of glomerular proteinuria. The intent behind this treatment is to facilitate general medical management by decreasing proteinuria, increasing serum albumin, and thereby lessening edema formation. Thus, indomethacin is effective in ameliorating intractable nephrosis (351). Because of the association of nonsteroidal agents with renal failure in some nephrotic patients as described in the section on Complications, more recently clinicians have emphasized the use of ACE inhibition (97,352). Each of these treatments appears to reduce glomerular capillary hydraulic pressure, by different mechanisms. ACE inhibitors act by decreasing post-glomerular arteriolar resistance, whereas nonsteroidal drugs enhance preglomerular capillary resistance (353). Consistent with these mechanisms, Garini and colleagues (354) compared the effects of indomethacin, captopril and the calcium-channel blocker, nifedipine, on the changes in renal hemodynamics and proteinuria induced by a high-protein mean. The increases in GFR and renal blood flow were not blocked by captopril or nifedipine, but were blocked by indomethacin. The increase in protein excretion was blocked by indomethacin and captopril but not by nifedipine (354). Indeed, dihydropyridine calcium channel blockers do not decrease proteinuria (355) and may even increase it. Therefore, the effects on hemodynamics were different and the effect on proteinuria could be distinguished from both systemic effects on blood pressure and effects on GFR and RBF. In patients with membranous nephropathy, ACE inhibition may have a size-selective effect (356), decreasing the fractional clearance of larger (>60 Å) molecules, suggesting an effect on the shunt pathway of macromolecular clearance. Such studies have supported efforts by clinicians to utilize ACE inhibitors to decrease proteinuria not only for symptomatic management but also in an effort to delay or prevent the progression of chronic kidney disease. The efficacy of this treatment will be considered further in the section on FSGS.

It is possible that at least a part of the antiproteinuric effect of ACE inhibition is not mediated by the glomerulus. Angiotensin blockade enhances megalin expression and albumin reabsorption (357).

Nephrectomy. Unilateral nephrectomy has proved beneficial in some infants presenting with nephrotic syndrome in the first year of life (358), and bilateral nephrectomy may be a useful part of the aggressive approach required in patients with congenital nephrotic syndrome if they are to survive to an age when transplantation is feasible (359,360) (see section on Nephrotic Syndrome in the First Year of Life in the Clinical Variants of FSGS, later in this chapter). Bilateral embolization of renal arteries can be an important therapeutic option in carefully selected patients with nephrotic syndrome who appear destined to progress to end-stage renal failure (361).

MINIMAL CHANGE NEPHROPATHY

Although MCN is often thought of as a pediatric disease, it is one of the most common causes of nephrotic syndrome in adults as well. It is now the third most common cause of nephrosis in adults (10) and remains the most common cause of nephrotic syndrome in children younger than 16 years of age. Indeed, it is the second most common primary renal parenchymal disease in that age group. Two to seven new cases occur annually per 100,000 children (362), and the prevalence is about 15 cases per 100,000 children. Although most children with MCN achieve permanent remission of symptoms by the time they reach puberty, some cases persist into adulthood (363). Furthermore, new cases have been reported in the eighth decade of life (364). However, the relative incidence of MCN as the etiology of nephrotic syndrome decreases with age in both children and adults (10,364–366). Although it is not clear that adult-onset disease represents the same entity as that found in childhood, or that all patients with the clinical picture of MCNS have an identical disease, the clinical course and outcome of pediatric and adult cases appear to be sufficiently similar (364) to consider all cases together.

Minimal change disease can appear in the first year of life, but it is more common later, with a peak incidence at 2 years of age. Most pediatric surveys report that it occurs twice as often in boys as in girls, whereas it has an equal sex incidence in adults (367). Although no precipitating cause may be apparent in many children, it is not unusual for the development of edema and proteinuria to be preceded by an upper respiratory tract infection, an allergic reaction to an insect sting or other immunogenic stimuli, or the use of certain drugs (367–394) (Table 64-5). In both adult and pediatric patients, malignancies, especially Hodgkin disease, have been associated with the development of MCNS (see section on Nephrotic Syndrome and Malignancies).

Clinical Findings

Edema formation may begin within a few days of the inciting event. Facial edema usually is noted first, with few other indications of an ongoing disease process. This can be confused with allergic symptoms, especially if associated with an upper respiratory tract infection. Edema usually increases gradually. It becomes detectable in the adult only when several liters of fluid have accumulated; by the time medical advice is sought, the patient typically has pitting edema involving the sacrum and the lower extremities. When anasarca is present, periorbital edema can be so severe that the eyelids are swollen shut, scrotal or vulval edema may be marked, and there may be significant abdominal distension. Respiratory embarrassment may occur from accumulation of either pleural or ascitic fluid, although the infrequency of dyspnea or orthopnea in the setting of massive fluid retention is striking. This reflects the absence of increased pulmonary capillary wedge pressure needed to generate pulmonary edema. Headaches and irritability are common accompanying complaints of edema. The patient may note vague symptoms such as malaise, easy fatigability, irritability, and depression. Rarely,

TABLE 64-5

DRUGS AND OTHER FACTORS REPORTED TO HAVE PRECIPITATED MINIMAL CHANGE NEPHROPATHY (MCN)

Drugs
Gold (368)
Penicillamine (369)
Ampicillin (370)
Mercury-containing compounds (371)
Nonsteroidal antiinflammatory agents (372–375)
Trimethadione, paramethadione (376)
Atopy
Pollen (377)
Food allergy (378,379)
House dust (380)
Contact dermatitis (poison ivy and oak) (381)
Bee (382) or wasp (395) stings
Tumors
Lymphoma (384,385)
Others (383)
Infections
Various viral infections (386)
Schistosomiasis (387)
Ehrlichiosis (388)
Stimuli-associated with immune activation or inflammation
Guillain-Barré syndrome (389)
Still disease (390)
Immunizations (391,396)
Dermatitis herpetiformis (393)
Epidermolysis bullosa (394)
Thyroiditis (397,398)
Sclerosing cholangitis (399)

the development of cellulitis, peritonitis, or pneumonia may be the first indication of an underlying nephrotic syndrome. The pallor resulting from edema can be misinterpreted as indicating anemia.

On physical examination, dependent edema is the most prominent finding. The retina has a characteristic "wet" appearance. Subungual edema may reverse the usual color pattern on the fingernails—the normally white lunulae may be pink and the rest of the nail bed white. Horizontal white lines that may be seen on both the fingernails and the toenails are referred to as *Muehrcke bands*. Inguinal and umbilical hernias may be present, especially if the patient has had severe ascites for a prolonged period. The elasticity of the cartilage in the ear appears to be decreased.

Blood pressure in patients with MCN usually is normal, but elevated systolic pressure has been recorded in 21% and elevated diastolic pressure in 14% of the children evaluated by the International Study of Kidney Disease in Children (ISKDC) (400). Hypertension is seen more commonly in adult patients with MCN (401).

Growth failure occasionally may be found in children, most often in those who have had multiple relapses of MCNS requiring frequent courses of steroids (321). Evidence for infection, especially peritonitis, cellulitis, or pneumonia, should be sought as part of the physical examination. These infections may be associated with septicemia and shock.

In MCNS, the chest radiograph usually shows a small or normal-sized heart; pleural effusions may be present, as may pneumonic infiltrates. The presence of an increased heart size and congestive changes in the hilar regions suggests that the nephrotic syndrome is secondary to glomerulonephritis.

Laboratory Findings

Urinalysis. Clinicians who first characterized the nephrotic syndrome noted that the urine often foams excessively when voided and that it coagulates when heated. These findings result from marked proteinuria, now indicated by a dipstick reading of 3+. Other edema-forming, hypoalbuminemic states, such as malnutrition, milk protein sensitivity, and protein-losing enteropathy, can mimic nephrotic syndrome but do not manifest significant proteinuria. The amount of protein in the urine of nephrotic patients can range from less than 1 to more than 25 g per/day. The value for adult patients usually is between 3.5 and 16 g per/day; that for children typically is lower than this amount, even when allowances are made for body size (402), and averages about 50 mg/kg of body weight per/day (403). Because urine protein is a function of plasma, and thus of filtrate, protein concentration (404), children with MCNS, who may have serum albumin levels of 1 g/dL or less, may occasionally have amounts of urinary protein as low as 100 to 200 mg per/day. This finding in patients with low plasma albumin concentrations also reflects removal of much of the protein from the glomerular filtrate as it traverses the proximal convoluted tubule (405) (see previous section on Tubular Handling of Protein in Mechanisms for Proteinuria). As a consequence of proteinuria, the urine specific gravity in nephrosis usually is high, often exceeding 1.035. Exceptions include patients who are not in an edema-accumulating state (see section on Consequences of Proteinuria, earlier in this chapter) and the patient with nephrotic syndrome and renal failure or tubular dysfunction, in whom lower (but not isosthenuric) values of urine specific gravity are found. Physiologic responses of the kidney to the nephrotic state may cause further urine concentration.

The spectrum of excreted proteins depends on the renal disease responsible for the nephrotic syndrome. In primary nephrotic syndrome, most of the urine protein is albumin; in other diseases, such as glomerulonephritis, both albumin and globulins are lost in increased amounts. This occurrence has led to determination of "protein selectivity" being proposed as a noninvasive method to separate MCNS from other causes of nephrotic syndrome (406). By comparing clearance of albumin to that of larger molecules such as IgG or transferrin, a curve can be generated, indicating whether the protein loss is *selective* and restricted to small molecules, or *nonselective*, consisting of both large and small molecules. Patients with MCNS tend to show more selective proteinuria, whereas those with nephrotic syndrome from other causes have nonselective proteinuria. Unfortunately, this generalization is limited by considerable overlap in results from patients in different diagnostic categories, so that clinical determination of protein selectivity has limited value for individual patients. This limitation may reflect factors other than molecular size that also affect entry of proteins into the glomerular filtrate, and differences in tubular function, which modify reabsorption of filtered protein (407). A refined electrophoretic technique has been used to indicate protein selectivity and to predict outcome. Patients with primarily albumin and transferrin in urine as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) proved to be steroid-sensitive, whereas patients who were steroid-resistant also excreted considerable amounts of IgG, lysozyme, and other larger molecules (408). Some of these larger molecules could be derived from tubular cells, reflecting tubulointerstitial injury rather than glomerular filtration, or from activation of the shunt pathway for filtration of macromolecules discussed earlier in this chapter. In analbuminemic rats with PAN nephrosis, fractional clearances of

various macromolecules are similar to those in normoalbuminemic rats. The absence of competition for albumin implied by this finding suggests that urinary loss of these proteins in nephrotic patients does not result from "overload" of tubular reabsorption by filtered albumin (71).

The urine sediment from nephrotic subjects often contains oval fat bodies. Lipiduria is better diagnosed, however, using a microscope equipped with polarized light to demonstrate doubly refractile fat bodies ("Maltese crosses") in degenerative fatty vacuoles in the cytoplasm of desquamated renal epithelial cells or free in the urine as neutral fat droplets. Frequently, urine with large amounts of protein also contains hyaline casts.

Other urinary findings vary with the cause of the disease. Up to one third of patients with MCN may have microscopic hematuria. Gross hematuria may be seen in patients with uncomplicated MCN but is extremely rare (409). By contrast, it is more common in patients with prominent mesangial proliferation. Hematuria is more likely to be seen with FSGS than MCN (410). In patients with nephrotic syndrome secondary to glomerulonephritis, the urine shows more abnormalities, with cellular elements and granular, cellular, and mixed hyaline casts being present. However, patients with MCN cannot always be differentiated from those with glomerulonephritis on the basis of urine sediment abnormalities alone.

Blood Studies. Hypoproteinemia is common to all nephrotic patients and is caused, primarily, by hypoalbuminemia. Total serum protein is characteristically reduced to between 4.5 and 5.5 g/dL; serum albumin concentrations usually fall to below 2 g/dL, and, in children, may be less than 1 g/dL (363). Although serum albumin concentrations are usually decreased, those of total globulins are remarkably well preserved in MCN despite massive proteinuria. Typically, serum α_1-globulin concentrations are normal or slightly decreased, whereas levels of serum α_2- and β-globulins are increased. Although the concentration of γ-globulin determined by electrophoresis is normal or reduced, the levels of individual components vary. In MCN (295,386), serum IgG levels average approximately 20% of normal, whereas IgA levels are less severely reduced; IgM and possibly IgE levels are increased. The changes in serum IgG and IgA concentrations are less pronounced in patients with nephrotic syndrome of other causes; IgM and IgE are typically normal in these subjects (295,386).

Hyperlipidemia is one of the findings that define the nephrotic syndrome. Serum total cholesterol level is usually elevated, especially when the serum albumin level has fallen to 2 g/dL or less (167,295,386). Values average 400 mg/dL, but levels in excess of 1,000 mg/dL have been recorded. Other changes in plasma lipids are summarized in the section on Consequences of Proteinuria.

Most often, serum electrolyte concentrations are within the normal range even when anasarca is present, indicating proportionate retention of sodium and water. Factitiously low serum sodium concentrations (~130 mEq/L) may be measured with marked hyperlipidemia. This pseudohyponatremia results from the nonaqueous, nonsodium-containing component of the serum or plasma (lipid) being increased. It does not require treatment, because the sodium concentration in the aqueous phase of blood is normal, as is plasma osmolality. This artifact is not observed when sodium levels are determined by techniques that measure sodium activity with ion-specific electrodes or after sample ultracentrifugation. Low serum sodium may be an accurate finding in the case of excess free water ingestion relative to dietary sodium intake (411), compounded by potential effects of elevated plasma vasopressin (412). This problem may be exacerbated by diuretic therapy. A decreased anion gap is associated with decreased total serum protein or albumin levels. This finding is common to all hypoalbuminemic states and does not directly reflect either renal dysfunction or altered serum lipid levels (413). Serum calcium may be low, mainly as a result of the hypoalbuminemia. Normally, 40% of total serum calcium is bound to protein. A decrease in serum albumin concentration of 1 g/dL results in a decrease in total serum calcium of 0.8 mg/dL. In contrast, 1 g of globulin binds only 0.16 mg of calcium. In some cases, the hypocalcemia may be out of proportion to the hypoalbuminemia and is caused by a reduction of ionized calcium levels (414) by as much as 5% to 20%, possibly because of urinary loss of 25-OH-D (see section on Consequences of Proteinuria). Acute symptoms of hypocalcemia rarely occur. Total and ionized calcium levels return to normal with remission. Serum phosphorus is normal unless the nephrotic syndrome is associated with renal insufficiency.

Blood urea nitrogen (BUN) and serum creatinine values are usually close to normal in MCN, but may be mildly elevated if decreased intravascular volume from nephrosis causes prerenal azotemia. The BUN may be elevated because of either increased intrarenal urea circulation or increased protein catabolism if the patient has received steroids. Glomerular filtration rate measured by inulin clearance is reduced to an average of 80% of normal (147); occasionally values are reduced to 20% to 30% of normal. This may represent decreased renal perfusion secondary to hypovolemia. Reduced GFR at the onset of MCN is reversible and does not imply an unfavorable outcome (104). The presence or absence of azotemia therefore cannot be used as a reliable indicator in the differential diagnosis of the nephrotic syndrome.

Hemoglobin levels and hematocrit values may be normal or even increased if there is hemoconcentration secondary to loss of fluid into the peripheral tissues. This factor may help to differentiate azotemic patients with MCN from those who have severe renal insufficiency from parenchymal disease, in whom anemia is more typical. However, as noted previously, iron deficiency may cause nephrotic patients with normal renal function to become anemic.

Measured concentrations of serum complement and its components are generally considered to be normal in MCN. Although urinary losses cause decreases in low-molecular-weight complement components, serum concentrations of the components measured to detect activation of the complement cascade are unchanged (415). Thus, reduced levels of the third component of complement (β-1-C globulin; C3) or C4 indicate that a glomerulonephritis underlies the nephrotic syndrome; conversely, such changes do not occur invariably with glomerulonephritis. Circulating immune complexes may be elevated in MCN or in FSGS (416,417). Plasma renin activity may be increased in some patients who manifest physiologic changes consistent with decreased intravascular volume.

Histopathology

Minimal Change Nephropathy

The morphologic classification of nephrotic syndrome in childhood derives from classic papers by Churg and colleagues (418) and White and colleagues (419). The term *minimal change nephrotic syndrome* was used to describe the pathologic appearance on light microscopy of biopsies from nephrotic patients in which there are no definitive changes from normal in glomeruli (Fig. 64-7). Here, we have chosen to use the term *minimal change nephropathy* because some adult patients have been reported with proteinuria but no nephrosis (9,10) and in order to describe a histologic and functional entity in parallel to FSGS. The degree of change from normal histology that is considered significant remains the subject of some controversy. The spectrum of these changes is classified in Table 64-3 at the beginning of this chapter. Other terms that have

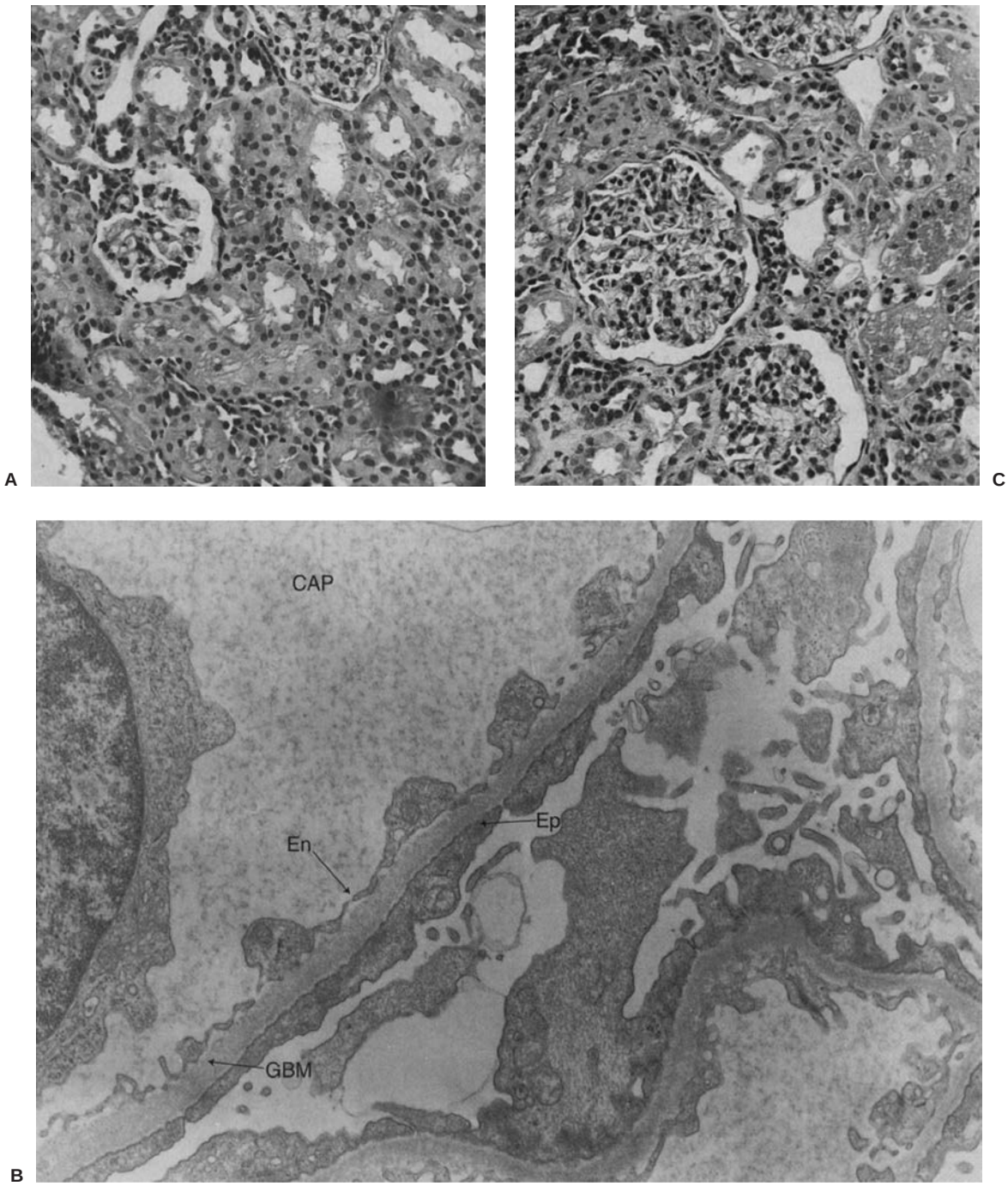

FIGURE 64-7. Findings on renal biopsies from three children with the clinical features of minimal change nephropathy. **A:** Light microscopy of a patient with MCN. Portions of two glomeruli are shown. Cellularity is normal and the capillary loops are patent. Tubular and interstitial structures are normal in appearance. (Magnification for all light microscopy ×350.) **B:** Electron microscopy from the same patient. The endothelial cells (En) lining the capillary loop show a normal fenestrated structure; the glomerular basement membrane (GBM) is uniform in thickness and structure. The epithelial cell (Ep) layer shows characteristic fusion of the epithelial foot processes, with the podocytes being in continuous contact with the GBM. Proteinaceous material and a nucleated cell are present in the capillary lumen (CAP). **C:** Light microscopy in a patient with mesangial hypercellularity. The tubular and glomerular capillary structures are normal, but an increased number of nuclei are present in the mesangial areas of the glomeruli. Immunofluorescent microscopy was negative for immunoglobulins and C3. The patient behaved clinically as one with MCNS. (Histology courtesy of Dr. John M. Kissane.)

been used to describe this general entity include *nil disease* and *steroid-sensitive nephrotic syndrome*. Changes in the proximal tubule cells reflect increased reabsorption of protein. Tubular cells may contain apparent vacuoles that are doubly refractile and are similar to the fine lipid droplets seen in oval fat bodies in the urine. This pathologic abnormality generated the term *lipoid nephrosis*, in which there is no tubular atrophy and the renal interstitium is normal. Older patients may show some globally fibrosed glomeruli with associated nephron loss. This finding is rare in children and should not involve more than 5% to 10% of glomeruli, even in elderly patients (3). Staining for glomerular polyanion with Alcian blue, colloidal iron, or ruthenium red may be reduced in the glomerular tufts. No immunoglobulin or complement deposition is observed by immunofluorescence. Electron microscopy (Fig. 64-7) reveals only glomerular epithelial cell foot-process effacement (see next paragraph) (420). Recent advances may permit this finding to be visualized by high-resolution light microscopy (421). The diffuse effacement of the podocytes that often contain protein-reabsorptive droplets typically results in the appearance of an almost continuous layer of cytoplasm on the urinary side of the GBM. Epithelial cells may appear detached in segments, producing denuded areas of the GBM. The GBM itself, however, appears normal. There are no electron-dense deposits adjacent to the GBM (422). Historically, 65% to 85% of children with primary nephrotic syndrome have this lesion (423), compared to a prevalence of about 30% of primary nephrotic syndrome and 15% of all nephrosis in adults (10).

Podocyte Foot Process Effacement

Podocyte foot process effacement is due to retraction, widening, and shortening of foot processes and is not due to fusion, as reviewed recently (424). With complete effacement, the GBM is covered by thin, sheetlike processes of podocyte cytoplasm, with gaps between the processes of adjacent cells (where protein filtration presumably occurs). Foot-process effacement is associated with a reduction in GFR, which is caused mainly by a reduction in the ultrafiltration coefficient K_f (see section on Physiologic and Anatomic Factors Affecting GFR in Consequences of Proteinuria, earlier in this chapter).

Podocyte effacement is accompanied by striking morphologic changes in the cellular cytoskeleton. The continuous layer of podocyte cytoplasm that overlies the GBM shows an increase in microfilaments and the appearance of a dense cytoskeletal band located within the basal portion of the podocyte, adjacent to the GBM. The cytoskeletal band has regions of high density at regular intervals. *En face* views demonstrate that the filaments are distributed radially from these central densities, suggesting that they may function to distribute mechanical strain and thereby prevent glomerular capillary expansion (425). Endlich et al. exposed cultured podocytes to biaxial cyclic stress, in order to model stress that might be experienced by podocytes in glomerulomegaly (426). Transverse stress fibers disappeared and were replaced by radial stress fibers connected to a single actin-rich center. The stress fibers were composed of myosin II, α-actinin, and synaptopodin. These findings differ in important ways from podocytes *in vivo*, where the actin-rich center is absent and actin is confined to the foot processes. Nevertheless, the results do suggest that podocyte response to stress consistently involves formation of radial cytoskeletal structures.

It appears likely that podocyte foot process effacement arises by different mechanisms in different settings. Certain podocyte injury models (protamine infusion, reactive oxygen species infusion, PAN administration) are associated with redistribution of α-dystroglycan from the basal surface of the podocyte, a process that occurs within as little as 15 minutes (427). MCN but not FSGS is associated with loss of podocyte dystroglycan expression (428). Kojima and Kerjaschki (429) have suggested that polycations compete with GBM laminin for binding to its receptor, dystroglycan; free dystroglycan is then internalized into podocyte endosomes. This process is dependent upon cellular ATP and participation of the actin cytoskeleton.

Related but distinct mechanisms may explain foot process effacement in FSGS, particularly postadaptive FSGS. Shirato (424) and Kriz et al. (430) have proposed that the podocyte supports the essential but contradictory functions of structural stability and leakiness (hydraulic conductivity). Cytoskeletal hypertrophy represents cellular adaptation to increased stress. The source of stress might be glomerular enlargement, increased glomerular P_{GC} (associated with the overload state) or increased GBM distensibility (due to GBM damage). Johnson has pointed out that the transmembrane oncotic pressure gradient influences the net filtration pressure (P_{UF}) and thereby might also contribute to net hydraulic stress (431). P_{UF} is the difference between transmembrane hydraulic pressure and the transmembrane oncotic pressure gradient. In the setting of nephrotic-range proteinuria, plasma albumin is reduced, which reduces the transmembrane oncotic pressure gradient across the capillary wall and thereby increases P_{UF}. In the face of mechanical or hydraulic stress, the capillary wall has three defenses: the mesangial cell, the podocyte, and the GBM. The mesangial cell may undergo proliferation and hypertrophy. Little is known about how the GBM responds to stress. The podocyte cannot proliferate and adapts by elaborating a more complex cytoskeleton to defend the structural integrity of the capillary wall. This occurs, however, at the cost of a reduction in total slit diaphragm width, podocyte effacement, and reduced K_f.

Variations in Histopathology

Immunoglobulin Deposition. Some patients with all the clinical features of MCN may have minor morphologic differences from those already described. A common variation is the presence of IgM in the glomerular mesangium. An early report (432) suggested that this variant represents a separate entity, which was termed mesangial IgM nephropathy. All of the patients, whose ages ranged from 1.5 to 59 years, showed a slight increase in mesangial matrix, and in addition to the IgM deposits, some had C3 and rare IgA deposition. Dense mesangial deposits were noted by electron microscopy in 9 of the 12 subjects. Subsequent observations did not support this as being a separate entity. In one study, 40% of 149 consecutive patients with the clinical picture of primary nephrotic syndrome were found on biopsy to have mesangial IgM deposits. Of these, 20 had mostly or entirely IgM without complement. They could not be differentiated clinically from other MCN patients (433). Because the presence of mesangial IgM deposit in patients with clinical MCN does not appear to affect either the patient's response to treatment or the disease outcome (434), it is now believed that this finding has little significance.

Mesangial IgM deposits in apparent MCN may, in some cases, be associated with immune complexes (435). Because deposits are often found in patients who undergo biopsy after receiving a trial of corticosteroid therapy, it is of interest that experimental models of immune complex metabolism suggest that steroid administration may prolong the systemic half-life of larger complexes and increase and sustain their appearance in the mesangium (436). The presence in the glomeruli of immunoglobulins in addition to IgM usually indicates a diagnosis of a disease other than MCN (437). One group of patients with a clinical diagnosis of MCN showed some glomerular proliferation associated with immunoglobulin deposits, primarily IgG, in the glomeruli. This lesion was more often observed in African American children (119), whereas a racial predilection may not be present in adults (438). Although the patients described in

this report responded to treatment with steroids initially, their subsequent course was one of frequent relapses or the development of resistance to treatment.

Mesangial Proliferation. Some patients with otherwise typical MCN have increased numbers of mesangial cells in the glomeruli. One study that correlated glomerular morphometry with the patient's clinical course found increased numbers of mesangial nuclei and smaller nuclear size in patients who had frequent relapses. The authors proposed that this indicated mesangial cell activation. They cautioned, however, that disease duration could play a role in the development of this finding, because the frequently relapsing patients had a 4-year course compared to 1.4 years in the population with infrequent relapses (439). Mesangial hypercellularity may be associated with a decreased response to steroid therapy (134,440,441), frequent relapse (441), steroid dependency (442), or a poorer prognosis (442–444). The ISKDC found that approximately 2.5% of children with nephrotic syndrome had mesangial hypercellularity (445).

Mechanisms of mesangial proliferation. Based on the finding of identical immunohistochemistry in patients with or without mesangial proliferation, it has been argued that mesangial IgM deposition does not appear to play a role in the induction of the mesangial cell response (440). Intrinsic kidney cells and cells migrating into the kidney as part of the inflammatory response, release factors that regulate mesangial cell proliferation. Mesangial cells produce platelet-derived growth factor (PDGF), a stimulant of mesangial and endothelial cell growth and wound healing (446); prostacyclin and thromboxane, produced by a variety of cells, are stimulatory cofactors for mesangial cell proliferation (447). In addition, two autocrine growth mechanisms have been defined. The first involves interleukin (IL)-1. Mesangial cells in culture secrete a mesangial cell growth factor with characteristics identical to those of IL-1 (448). Indeed, these cells express messenger RNA (mRNA) for IL-1 *in vivo* (449). The second autocrine system involves IL-6. Mesangial cell-derived IL-6 stimulates mesangial cell growth *in vitro* (450). Moreover, mice transgenic for the human IL-6 gene show marked mesangial proliferation (451). In human disease, urinary excretion of IL-6 and mesangial staining of biopsy material for IL-6 were associated with mesangial proliferation by some (452), but not all (453) authors. Because mesangial proliferative changes are associated with steroid resistance, it is noteworthy that IL-6-induced cell activation is not inhibited by steroids (454). Finally, negative regulation of mesangial cell growth may be provided by the GBM itself, as proliferation of mesangial cells is decreased by heparan sulfate (455).

Tip Lesion. A group of patients has been described as having steroid-responsive nephrotic syndrome with intercapillary foam cells adherent to Bowman's capsule in a tuft near the tubular origin (the glomerular tip lesion). Although this adhesion is irreversible, the patients appear to have a good prognosis closer to that of MCN than that of steroid-resistant nephrotic syndrome (9,456). Tip lesion is considered more extensively in the section on Selected Clinical Variants of FSGS.

C1q Nephropathy. Biopsies from some nephrotic patients show mesangial deposits of the complement component, C1q, despite the presence of normal serum complement levels and the absence of complement-activating diseases such as systemic lupus erythematosus. Accompanying pathologic findings can be protean (457), including FSGS (458), crescentic glomerulopathy (459), or minimal changes (460). Patients may present with congenital nephrotic syndrome (461), steroid-sensitive disease (460), or steroid resistance (462). It is not certain whether C1q deposits represent a distinct entity or an incidental finding.

Disease Processes and Other Findings Associated with MCN

Several clinical findings have been associated with MCN, including specific malignancies, atopy, various HLA haplotypes, and abnormalities in immune function. These associations, which could elucidate issues of both causality and treatment, will be considered here.

Malignancy. Several glomerulopathies, notably membranous glomerulopathy, have been associated with neoplasia. A significant number of patients with cancer-related nephrotic syndrome, however, have MCN (Table 64-6). The relationship between MCN and lymphomatous disorders, particularly Hodgkin disease, is especially striking (383). In a survey of the literature, 33 of 134 patients with cancer-related nephrotic syndrome had MCN, as determined by biopsy (385). Of the patients with MCN, 26 had Hodgkin disease and an additional two had non-Hodgkin lymphoma. In another review (384), 36 of 44 patients who had Hodgkin disease and the nephrotic syndrome had MCN and only two had membranous glomerulonephritis. There was a much higher incidence of nephritic diseases in patients with other types of neoplasia. Nonlymphomatous tumors that have been associated rarely with MCN include renal oncocytoma (463), embryonal cell tumors (385), pancreatic carcinoma (464), nephroblastoma (465), Waldenström's macroglobulinemia (466), bronchogenic carcinoma (467), and cecal adenocarcinoma (468).

TABLE 64-6

NEPHROTIC SYNDROME IN PATIENTS WITH MALIGNANCY

	Renal histology[a]		
Type of malignancy	Minimal change disease	Membranous nephropathy	Membranoproliferative glomerulonephritis
Hodgkin lymphoma	26	4	0
Non-Hodgkin lymphoma	2	2	1
Leukemia	1	4	1
Multiple myeloma	0	0	1
Carcinoma	3	33	4
Benign or embryonal tumors	1	1	0

[a] Only cases with defined renal pathology and without amyloidosis are shown.
(Data from: Eagen JW, Lewis EJ. Glomerulopathies of neoplasia. *Kidney Int* 1977;11:297.)

Evidence suggests that in these cases the tumor may be directly involved in the pathogenesis of the MCN. MCN can be the initial presenting sign of a lymphomatous disorder (469) and may precede clinical evidence of lymphoma by several years (470). With appropriate and successful antineoplastic therapy, either medical or surgical, the proteinuria in tumor-related MCN resolves, renal function remains normal, and the nephrotic syndrome remits (385,463,467,470). The relationship between relapse of the tumor and of the nephrotic syndrome (471,472) also strongly suggests an etiologic role for the tumor in the pathogenesis of MCN in these patients. These observations indicate the importance of considering a malignancy as an underlying cause in any adult (471–474) and, rarely, in pediatric (475) patients who present with apparent primary nephrotic syndrome. If Hodgkin lymphoma is the cause, it is essential to treat the neoplasm rather than the renal disease.

Atopy and MCN. A relationship between allergy and MCN has long been suspected. Anecdotal reports suggest that exposure to allergens may precipitate the nephrotic syndrome (377,379,475,476); rhinorrhea and allergic skin reactivity frequently precede relapses (477), and a high prevalence of allergic symptoms has been observed in nephrotic patients. Highly allergic patients who had a pathologic diagnosis of MCN based on renal biopsy experienced a decrease in urinary protein excretion when placed on an elemental diet and did not require treatment with corticosteroids. Challenge with milk led to a decrease in serum C3 and increased protein excretion, strongly suggesting that hypersensitivity was causally linked to proteinuria (378). A human basophil degranulation test was positive in 16 of 28 adults with MCN and 14 of 18 with FSGS; in contrast, only 5 of 29 patients with glomerulonephritis and 1 of 11 healthy donors showed a positive response (478). In addition, atopy and MCN were associated with increased frequency of expression of human leukocyte antigen (HLA)-B12 and -DRw7 (478–480).

Although values ranged widely, mean serum IgE levels were significantly elevated in patients with MCN compared to those with other renal problems (481); elevated levels also are associated with frequent relapse in children (482). In one study, the majority of adult MCN patients had serum IgE levels more than two standard deviations above the normal mean; of these, more than 70% had associated allergic symptoms (483). Other investigators have made similar observations, but sought to draw a distinction between primary allergic disease and the elevated IgE levels found in nephrotic syndrome. They suggest that because IgE deposition in the glomerulus is rare (484), elevated serum IgE levels could represent not the causal factor in MCN but rather evidence of more generalized derangement in the immune system (485). This concept is supported by a finding that increased serum IgE may persist even with remission. If this view is correct, it could explain why attempts to treat MCN in atopic patients with inhaled disodium cromoglycate (486) or an orally administered analog (477) were unsuccessful.

These apparently conflicting observations may be resolved by study of specific antigens. Therefore, a majority of adult nephrotic patients studied by Meadow et al., and Lagrue,Laurent, et al. had detectable elevations of specific IgE titers, with the most common sensitizing agent being house dust or dust mites. After remission induced by institution of specific desensitization and sodium cromolyn, several of these patients had a relapse on re-exposure to the allergen (477,487,488).

Immunogenetics. There may be a familial incidence of MCN. A survey from Europe, which excluded patients with the congenital nephrotic syndrome (489), found that 63 of 1,877 nephrotic children had affected family members. This prevalence of 3.35% was higher than that predicted from the frequency with which MCN occurs in the general population. Siblings were most often affected. The similarity of pathologic findings and clinical course for affected members within a family was striking (490), although in at least one report siblings showed differences in these features (491). Familial nephrotic syndrome in children has been divided into two broad categories: (a) patients with an infantile onset and a poor prognosis regardless of renal morphology and (b) patients with a juvenile onset and a generally good response to conventional therapy, provided MCN is found by renal biopsy.

An indication of a possible genetic predisposition for development of MCN is the reported association of MCN, and in some cases atopy (480,508), with certain histocompatibility-complex antigens. The most commonly cited are HLA-B8 (480 493,495,508), and -DRw7 (494,496). Not all studies have confirmed these associations. Indeed, a variety of HLA antigens have been associated with MCN (479,480,492–500,505,507) (Table 64-7) and negative associations have been reported, too. HLA-B8 was found frequently in families with more than one member having childhood nephrosis (509). In one study the combined occurrence of B8 with DR3 and DR7 produced a relative risk of 21.5 (500). In another study DR7 was linked to steroid-sensitive disease and DR3 to steroid-resistant disease (498). In French and German patients, specific DQB1 alleles have been associated with MCNS, most strongly in steroid-dependent or frequently relapsing patients (501). German studies also suggested that patients with HLA-DR7/-DR3 together are less steroid-sensitive (504) and those with HLA-DR7 are less likely to respond permanently to alkylating agent treatment of frequent relapse (506).

MCN was associated with HLA-DQB1*0601, -DRB1*01 and -DRB1*07011 in Egyptian children (507,510,511) and -DQA*0201 in German children (505,507,510). Studies from Japan linked steroid-sensitive MCN in adult patients to HLA-DRw8 and -DQw3 (497) and to specific DQB1 alleles (502,503). Different results obtained in Singaporean (512) or Bengali (513) children are likely due in part to racial or geographic differences. The observation that two extended HLA haplotypes (HLA-A1, -B8, -DR3, -DRw52, SCO1; and HLA-B44, -DR7, -DRw53, FC31) occurred with higher than expected frequency in children with steroid-sensitive, frequently relapsing MCN provides the strongest evidence to date for an immunogenetic predisposition to the disease (499).

The association between HLA type and MCN has been made primarily in children, with some studies being unable to make similar correlations in adult patients. This finding suggests that MCN in adult and pediatric patients could represent different diseases that share common pathologic and clinical features. For example, HLA-DR7, which has been linked to MCN (Table 64-7), was observed in only 18% of adult European MCN patients, a frequency not different from that of control subjects. If the data were analyzed according to age at onset of the nephrotic syndrome, 45% of patients in whom onset was before the age of 15 years were HLA-DR7, whereas the equivalent incidence in adult-onset patients was only 7% (514). It remains unclear whether these associations represent linkage disequilibrium with another gene or reflect potential underlying immune influences on the development and response of MCN.

Disordered Immunity in MCN. It has long been recognized that immunogenic stimuli may precipitate presentation or relapse of MCN. In addition to the frequent association with atopy discussed previously, episodes may follow upper respiratory tract infections, bee stings, or diseases linked with abnormal immune responses. The relationship of immunogenic events to onset of disease and the finding of disordered immune responses in these patients led Shalhoub (515) to propose a unifying hypothesis relating MCN to the immune

TABLE 64-7

RELATIONSHIP BETWEEN MINIMAL CHANGE NEPHROPATHY AND HUMAN LEUKOCYTE ANTIGEN (HLA) TYPE

Author (ref.)	Year	Country of origin	No. of patients	HLA association	Relative risk	Children	Comments
Thomson, et al. (480)	1976	England	71	B12	6.3	Yes	Relative risk with B12 and atopy—13
Trompeter, et al. (492)	1980	England	116	B12	3.1	Yes	Could not confirm atopy data
O'Regan, et al. (493)	1980	Ireland	54	B8	3.5	Yes	—
				B18	3.16		
Alfiler, et al. (494)	1980	Australia	42	DRw7	5.9	Yes	MCN confirmed by biopsy; decreased DR2
Noss, et al. (495)	1981	Germany	45	B8	2.81	Yes	
deMouzon-Cambon, et al. (479)	1981	France	54	DRw7	4.4	Yes	Related to atopy; decreased DR2
Nunez-Roldan, et al. (496)	1982	Spain	50	DRw7	4.5	Yes	Decreased DR2
Kobayashi, et al. (497)	1985	Japan	40	DRw8	3.74	No	Studied adult subjects
				DQw3	1.11		
Cambon-Thomsen, et al. (498)	1986	France	72	DR7	6.9	Yes	Steroid-sensitive
			27	DR3	3.0	Yes	Steroid-resistant
Lagueruela, et al. (499)	1990	United States	32	A1, B8, DR3, DRw52, SCO1, and/or B44, DR7, DRw53, FC31	—	Yes	Steroid-sensitive, frequently relapsing
Ruder, et al. (500)	1990	Germany	91	B8, DR3, DR7	21.5	Yes	Steroid-sensitive
Konrad (501)	1994	France, Germany	161	DQR1*07 DQB1*0201 DQA*0201, *0301, *0302	6.2 7.8 4.1	Yes	RR = 16.5 with both DRB1*0301 and DRB1*07
Abe (502)	1995	Japan	24	DQB1*0302	6.2	Yes	DQA1*0103 decreased
Kobayashi (503)	1995	Japan	30	DQB1*0301, *0601	8.6	Yes	—
Bouissou (504)	1995	Germany France	152 199	DR3/DR7	5.6 7.7	Yes	Less steroid-sensitive; Decreased association with DR2. DR6, DQ1
Haeffner, et al. (505)	1997	Germany	167	DQB1*0601	38.9	Yes	DQA1*0102 and DQB1*0602 decreased
				DQA1*0201 DQB1*0201	3.8 8.5		
Konrad (506)	1997	Germany	54	DR7	—	—	Poor response to alkylating agents
Bakr and El-Chenawy (507)	1998	Egypt	121	DRB1*01	23.4	Yes	—

MCN, minimal change nephropathy.

system. He cited four points: (a) evidence for abnormal humoral immune responsiveness; (b) marked sensitivity of the disease process to treatment with corticosteroids or immunosuppressive agents; (c) remission of MCN upon infection with measles, an inhibitor of cell-mediated immunity; and (d) the association of MCN with Hodgkin disease. In view of the lack of significant morphologic evidence of renal damage, Shalhoub suggested that MCN represents the renal manifestation of a systemic immunologic abnormality, perhaps a T-lymphocyte disorder, rather than a primary renal parenchymal disorder. Although subsequent investigation has not yet demonstrated a causal immunologic event, numerous abnormalities in both humoral and cellular immune responses (516) have been noted in nephrotic patients (295,296,299–301,416,417,517–520) (Table 64-8). These may, in time, provide a pathogenetic mechanism.

TABLE 64-8

IMMUNOLOGIC ABNORMALITIES IN MINIMAL CHANGE NEPHROPATHY

Defective opsonization
Decreased factor B (299,300)
Decreased factor D (301)
Decreased neutrophil chemiluminescence (300)
Abnormal reticuloendothelial function (518)
Circulating immune complexes (416,417,519,520)
Abnormal immunoglobulin production
Altered serum immunoglobulin concentrations (295,521–524)
Decreased specific antibody reactivity (296,525,526)
Decreased synthesis stimulated *in vitro* (527,528)
Increased spontaneous *in vitro* synthesis (521,528)
Alterations in cell-surface markers (527,529–532)
Altered cellular immunity
Cytotoxicity to renal tubular epithelium (533)
Proliferation in response to glomerular basement membrane (534)
Decreased delayed-type hypersensitivity (535–537)
Decreased experimental local graft-versus-host disease (538)
Decreased induced lymphocyte blast transformation (517,539)
Increased inducible suppressor cell activity (540)
Humoral immune abnormalities
Serum toxicity to lymphocytes (541)
Inhibition of rosette formation by serum (518,542,543)
Altered antibody-dependent cellular cytotoxicity (544)
Increased interleukin (IL) production (545)
Decreased IL production (546,547)
Suppressor lymphokines (548–550)
Monocyte migration inhibitory factor (534,551)
Vascular permeability factor (552,553)
Soluble immune-response suppressor (554,555)
Tumor necrosis factor (556)
Lymphocyte activation
Increased secretion of β_2-microglobulin (557)
Soluble IL-2 receptor production (558–561)
Increased production of IL-2, IL-4, and IFN-γ (562) IL-13 (563)

IFN, interferon; *IL*, interleukin.

Immunoglobulin synthesis. Clinical and *in vitro* assays show impaired immunoglobulin synthesis in MCN. Serum IgG levels are decreased significantly in children with MCNS, whereas IgM levels are markedly increased (295,522–524). These values return toward normal with remission, although the IgM levels may remain elevated. Not all studies have found an equal tendency toward normalization with remission, nor is this abnormality confined to MCN in all cases (564,565). Although nephrotic proteinuria may be associated with urinary loss of IgG (566), such losses are insufficient to explain the very low serum IgG levels often found in MCN (521). This pattern of increased IgM and decreased IgG levels in the serum also is associated with some other immune-deficient states, most notably X-linked immunodeficiency disease (521).

Depression of specific antibody titers, such as those to the common streptococcal antigens endostreptosin, streptolysin O, and streptozyme, was observed in children and adults with idiopathic nephrotic syndrome (526). Levels were low during active disease, remained low for up to 20 years afterward, and were not changed by steroid therapy. Patients who were nephrotic from chronic glomerulonephritis, systemic lupus erythematosus, membranous nephropathy, diabetes mellitus, or amyloidosis did not have depressed titers. These data suggest a chronic, specific impairment of response in patients with MCN. Inability to generate (296) or to maintain (525) specific titers against pneumococcal polysaccharide has been described in MCN, but not all studies confirmed this observation (567). Thus depression of specific antibody titers may be restricted to certain patients or certain antigens.

Several groups evaluated the *in vitro* secretion of immunoglobulins by lymphocytes activated with lectins. Consistent with the decrease in serum IgG levels, pokeweed mitogen-stimulated synthesis of IgG by patient lymphocytes was decreased in MCN patients during the active stage of disease, returning toward normal with remission (521,523,527). Unstimulated secretion of immunoglobulin may be increased (521,528), suggesting spontaneous activation of lymphocytes in MCN. Studies of IgA and IgM synthesis by lymphocytes obtained from patients with nephrotic syndrome of other causes produced more variable results (568). Decreased immunoglobulin production *in vitro* or *in vivo* could result from either abnormalities of lymphocytes or the presence of inhibitory agents systemically or on the cell surface. Evidence suggests that both mechanisms may be present in MCN.

Studies of lymphocyte surface marker expression. These studies were employed to determine whether the immunoglobulin abnormalities in MCN reflect some form of immune cell dysfunction. Cells infiltrating the renal interstitium are predominantly T lymphocytes (569). The ratio of helper cells to suppressor cells in the glomerulus can be similar to that found in lymphocytes in the peripheral circulation (569) but may vary from one patient to another (570). Circulating lymphocyte subsets in MCN were initially reported to show no significant alterations in helper–suppressor cell ratios (571). Studies of B-cell and T-cell subpopulations also produced conflicting data, regardless of whether patients with MCN or FSGS were studied (527,529,532,535,572). A potential increase in the number of cells co-expressing B-cell and T-cell surface markers has been reported, comparable to findings in X-linked immunodeficiency (531). However, in most studies of lymphocyte subpopulations in primary nephrotic syndrome, there are few significant changes. The meaning of the differences that were found remains to be determined. In general, studies showing alterations in lymphocyte subpopulations may be useful in suggesting the possible presence of immune derangement, but inferences of a potential role for these changes in disease pathogenesis should be made with caution unless corroborated by accompanying functional analysis. Some progress in this direction is provided by reports of two-color flow cytometry indicating that the counts for circulating activated total T cells and suppressor or suppressor/inducer T cells are increased, whereas those for activated helper T cells are decreased, during relapse (530,573). Another study found that populations of activated suppressor-inducer cells and suppressor-effector cells are increased in patients whose nephrosis was sensitive to steroid treatment, accompanied by decreased memory T cells and decreased lymphocyte proliferation in response to tetanus toxoid. Lymphocytes of steroid-resistant patients also had increased suppressor-inducer cells, but decreased suppressor-effector cells and increased memory T cells, with increased responses to tetanus toxoid (574).

Cellular immunity. In studies of delayed-type hypersensitivity to common antigens, MCN patients in relapse had decreased skin reactivity to purified protein derivative of tuberculin (PPD), *Candida*, live varicella vaccine, streptokinase–streptodornase, and topical dinitrochlorobenzene (535–537). Reactivity returns when the patient enters remission (537). In addition, the lymphocytes of patients with MCN manifest decreased local graft-versus-host activity when injected into rats,

a finding that can be normalized by preincubation of the cells with thymic humoral factor (575). These observations may not be restricted to MCN (576).

In vitro studies also showed abnormal cellular responses in nephrotic patients. Lymphocytes from MCN patients, but not from normal control subjects or patients who were nephrotic secondary to proliferative glomerulonephritis, were toxic to cultured renal tubular epithelial cells (533). Lymphocytes from some patients also proliferated on exposure to GBM (534). It is not clear from these reports whether the findings represent a primary process or the result of immunologic sensitization after renal damage. Blast transformation of patient lymphocytes was decreased in the presence of control or nephrotic serum (539), returning to normal after entry of the patient into remission (535). Other results showed that MCN is associated with increased concanavalin A-activated suppressor cell activity. This finding demonstrates at least the potential for exaggerated suppressor lymphocyte responses and is not consistently found in other renal diseases (540,577).

Evidence for abnormal lymphokine activity. Serum from adult nephrotic patients inhibits leukocyte migration in the presence of renal antigens (551), and serum monocyte migration inhibitory factor activity present during relapse of MCN disappears with remission of the disease (534). Furthermore, serum from most patients with MCN as well as some with diffuse proliferative glomerulonephritis is lymphocytotoxic, whereas serum from patients with acute tubular necrosis or urologic disease is not (541). These findings suggest that an immune inhibitory agent, or a series of such agents, is present in the circulation of patients with primary nephrotic syndrome. Sera from nephrotic patients may inhibit the ability of cells to form rosettes (542,543), although this is not specific for MCN, and patient sera do not support *in vitro* antibody-dependent cell-mediated cytotoxicity (ADCC) assays (544). Decreased splenic uptake of radiolabeled complexes was correlated with deficient Fc receptor function in nephrotic patients and could be due to the presence of an inhibitory protein that attaches to cell surfaces (518). Finally, multiple studies demonstrate a suppressive effect of patient sera on blastogenesis by normal lymphocytes (548,578–580). The specificity of this phenomenon for MCN varies from one study to another. Efforts to attribute suppressive activity in nephrotic sera to a lymphokine should attempt to exclude the possibility that the observed effects are caused by nonspecific toxicity to immune responses, resulting from the biochemical abnormalities that occur in nephrotic syndrome. For example, the suppressive activity in plasma from nephrotic children segregates in the lipid-rich fraction (581). It could thus be derived from constituents of LDLs and VLDLs that are present in increased concentrations in nephrotic plasma and suppress *in vitro* cellular immune responses (582,583). However, this finding also is consistent with migration of an immunosuppressive agent with the lipid-rich fraction. Alternatively, oxidized LDL could itself affect lymphocyte function. In either case, it is noteworthy that a patient with refractory nephrotic syndrome entered remission after LDL apheresis (584).

Several studies that partially characterized the suppressive activity suggested the production of a suppressor lymphokine. A heat-stable substance, present in the serum of nephrotic patients, inhibits lymphocyte proliferation. It binds to lymphocytes in the assay system and is not removed by washing (549). One study found a heat-stable inhibitory substance in the plasma of 51 (76%) of 67 children with MCN and six of nine children with FSGS (550). Only one sample from seven patients with membranous glomerulonephritis or 31 healthy adults and children showed similar activity. The factor was toxic to normal lymphocytes and was between 100 and 300 kDa. The presence of tumor necrosis factor (556), IL-4 (563,585), and IL-13 (565) in the serum of some patients may be related to suppressive activity.

Urine and serum samples from children and adults with steroid-sensitive nephrotic syndrome, but not other causes for proteinuria, contain the lymphokine soluble immune response suppressor (SIRS) (554). This factor, which inhibits antibody production (586) and delayed-type hypersensitivity responses (587), is secreted by patient lymphocytes without a requirement for exogenous stimulating agents. SIRS production thus could account for the suppression of immune responses seen in nephrotic patients. Suppressive activity disappears from the urine after initiation of corticosteroid therapy but before urinary protein loss decreases significantly. Patient serum activates normal lymphocytes to produce SIRS by a steroid-sensitive process (555), and a regulatory mechanism has been proposed by which CD4+ T lymphocytes from patients in relapse secrete a protein that activates CD8+ T cells to produce SIRS (588). Although the parallel between the sensitivity of SIRS production to steroids and steroid responsiveness of nephrotic proteinuria in patients who produce SIRS is striking, there is no evidence to indicate that SIRS itself causes nephrotic proteinuria. It is, however, a clear marker for steroid-sensitive mechanisms of proteinuria. The means by which SIRS acts on immune responses is not known. Although this issue remains intriguing, little recent progress has been made in this area.

Circulating immune complexes. A variety of glomerular diseases have been associated with soluble circulating immune complexes. The circulating complexes reflect immunoglobulins found in the kidney; patients with MCN had little or no IgG or IgA complexes but did have marked variation with regard to circulating IgM complexes (589). Although circulating immune complexes have been documented in some patients with MCN or FSGS (520), not all studies have confirmed this observation (519) and the relationship of this finding to mesangial immune complexes remains uncertain. A possible reason for varied results is the use of different assay systems (416). In screening studies that employed liquid-phase and solid-phase C1q binding and Raji cell assays, at least one assay was positive in serum from 11 of 14 adults with MCN, 13 of 27 patients with FSGS, and 26 of 55 patients with membranous nephropathy. Prednisone treatment did not affect the prevalence of circulating immune complexes in this study (590). In another report, 17 of 18 MCN patients had IgG immune complexes that did not bind to Clq; in seven of nine patients assessed longitudinally, immune complexes disappeared within 6 weeks of entry into remission (417). This temporal relationship and the absence of glomerular IgG in patients with MCN suggest that circulating immune complexes could be a result rather than a cause of the disease. Although they may not cause the disease, some circulating immune complexes could account for the apparently nonspecific presence of IgM in the mesangium of some patients. In support of this is the finding that large, neutral, or anionic complexes show focal to diffuse mesangial localization (591,592). Complexes containing IgM tend to be large. In contrast, low-avidity, polycationic, and small immune complexes tend to deposit in capillary or mesangiocapillary distribution.

Other findings related to immunity. Further evidence of a potential role for the immune system in MCN is the possible relationship between this disease and allergic phenomena, already discussed, as well as the unique association between MCN and tumors of immune cell origin. Impaired lymphocyte blast transformation was found in the presence of plasma from patients with MCN and Hodgkin's disease (473,593); *in vitro* responses improved significantly after antitumor therapy (594). The strong association of lymphoid tumor, abnormal cellular immune responses, and MCN supports a role for deranged immunity. The nature of this derangement is unclear. Despite the

clinical evidence of suppressed immune responses, the underlying abnormality paradoxically may be general immune system activation. In support of such an event, production of a number of specific cytokines and their regulators may be elevated. Increased IL-2 mRNA expression in patient lymphocytes (595), increased IL-8 production in steroid-resistant patients (596), and increased occurrence of one polymorphism of IL-1 receptor antagonist (597) have been reported. Circulating soluble IL-2 receptor concentrations are increased in MCN (558–561). Production of gamma interferon (IFN-γ) may also be increased in nephrotic children. Further, although various responses of stimulated patient lymphocytes are decreased, unstimulated cells from patients show, for example, increased immunoglobulin production relative to that of unstimulated control cells. Taken together, these findings suggest that in MCN, the immune system is generally activated, whereas the induction of responses to specific stimuli is impaired (516).

Relationship of the immunologic abnormalities to disease pathogenesis. Despite all of these studies, Shalhoub's hypothesis has not yet been proved. There is strong support, however, for the concept that cellular immunity may be a mediator of proteinuria. Monocytes or macrophages are important in the pathogenesis of some forms of glomerulonephritis (598) and in the genesis of proteinuria (599,600). These studies imply a role for mononuclear cells but do not explain how they may act. One possibility is through release of the lymphokine, vascular permeability factor (VPF), which is produced by activated lymphocytes from some nephrotic patients and which, when injected intradermally, causes increased permeability of vessels to macromolecules (601). This protein, usually referred to by its function as an endothelial cell stimulant called vascular endothelial cell growth factor (VEGF) (553), was detected in supernatants of unstimulated cultures of patients' cells but not those of normal controls (602). A VEGF-like serine protease in patient serum decreased staining for polyanion when used to treat histologic sections of normal glomerular tissue (603). However, the effect of VEGF does not appear to be specific for permeability of albumin, making it an unlikely cause of selective proteinuria. VEGF activates endothelial cells in numerous ways; by causing the cells to "round up" it disrupts tight cell–cell adhesions, promoting permeability of an endothelial monolayer (604). It also promotes the formation of endothelial fenestrae (605). However, since the glomerular capillary endothelium is not the final barrier to glomerular permeability, it is not clear how increased fenestration would cause albuminuria in primary nephrotic syndrome. Furthermore, a similar substance was described in IgA nephropathy, even in the absence of nephrotic syndrome (606), indicating that VEGF activity could be secondary to renal disease rather than a cause of proteinuria. VEGF production could be enhanced by IL-12 (607) or IL-15 (608). Substances produced by T-cell hybridomas derived from the lymphocytes of nephrotic patients (609) or found in culture media conditioned by mononuclear cells from nephrotic children (610), may prove to represent one or more selective permeability factors. One such factor could be IL-8, which is produced by lymphocytes from patients with steroid-responsive nephrosis (611) and increases renal clearance of protein in an *ex vivo* model (612). However, varied circulating levels have been reported in disease (596,613). IL-2 (562) and TNFα (614) production by patient lymphocytes normalized after the patients entered remission. A single case report describes remission of steroid-resistant disease after infliximab anti-TNFα therapy (615).

Despite the absence of proof for Shalhoub's hypothesis, the indirect evidence of an immunogenic basis for many cases of MCN remains compelling. Onset is often preceded by an immunogenic stimulus. Measles, which induces remission, inhibits lymphokine production but not proliferation by lymphocytes (616). Studies of MCN induced by nonsteroidal anti-inflammatory drugs (617) or cimetidine (618) indicated that these cases of disease may be associated with abnormal T-cell function. The relationship of disease to altered immunity is particularly striking with regard to suppressor cell activity. A good therapeutic response to cyclophosphamide was associated with decreased suppressor cell activity after treatment (619), although others were unable to confirm this finding (620). Furthermore, as described previously, cellular and humoral immune responses are suppressed in MCN. Thus it is intriguing that recombinant leukocyte interferon A, an agent that induces production of SIRS, caused nephrotic syndrome with minimal glomerular changes in a patient with T-lymphocyte malignancy (621) and that the antihelminthic agent levamisole, which inhibits SIRS activity (586), has been used successfully to treat MCN (622,623). Despite evidence suggesting that cytokines may be involved in promoting vascular permeability (624), the data regarding lymphokines do not address their role as a pathogenic agent in nephrosis and are equally consistent with the interpretation that production of these substances is an epiphenomenon of the derangement that causes albuminuria. In addition, the existence of differences between studies or even within a patient group in a given study suggest that multiple etiologies may exist for MCN, only one (or several) of which may be immunologic. Furthermore, the pathogenesis of proteinuria remains unclear. Given the importance of the epithelial slit diaphragm in the integrity of the glomerular filter, the podocyte is likely the target cell. However, the way in which the podocyte is affected is unknown. Considerable work remains to be done in this area.

Treatment of Minimal Change Nephropathy

Indications for Renal Biopsy

Although most older children and adults will undergo renal biopsy prior to starting treatment, the indications for and benefits of this procedure in patients with primary nephrotic syndrome remain somewhat controversial (625). Children 1 to 9 years old with all the features of MCNS and no atypical features Table 64-9 may be given a therapeutic trial of steroids without prior histologic confirmation of the diagnosis. Induction of a complete remission by steroids in such patients is considered to be adequate confirmation of the diagnosis of MCN (423), although other benign conditions may occasionally appear to respond to steroid therapy (626). A histologic diagnosis by renal biopsy is recommended in all patients who present with nephrotic syndrome in the first year of life or after the age of 9 years. Risk–benefit analysis at one point suggested that biopsy may not be diagnostically superior to a therapeutic trial of steroids, even in adults (627), but the increasing incidence of FSGS in nephrotic adults has led most clinicians to perform a biopsy at the time of presentation. All patients who are steroid-resistant (see Table 64-10 for some commonly used descriptors of nephrotic patients and their responses) should undergo biopsy, although the timing of this decision remains uncertain. Although steroid resistance in children usually is defined by 8 weeks of nonresponse, many clinicians recognize that most children who respond do so by 4 weeks. Therefore, it may be appropriate to perform a biopsy after 4 weeks or less, since those who have not responded by then are more likely to have a different disease that might require an alternative treatment. Other pediatric patients who may require a biopsy include those who have frequent relapses, or who are candidates for therapy with immunosuppressive drugs. Some experts have suggested that any steroid-dependent pediatric patients who continue to be steroid-sensitive are, statistically, so likely to have MCN (400,445) that biopsy is not indicated.

TABLE 64-9

FEATURES SUGGESTING THAT NEPHROTIC SYNDROME IS CAUSED BY A DISEASE OTHER THAN MINIMAL CHANGE NEPHROPATHY (MCN)

History
- Onset: before 1 year or after 9 years of age[a]
- History: skin rashes, joint pains, "nephritis," or hematuria[a]
- Family history: family member with Alport's syndrome or development of end-stage renal disease[a]

Physical examination
- General: clinical features of collagen vascular disease
- Blood pressure: marked elevation or evidence of vascular changes in fundi[a]
- Skin: purpura[a]
- Subcutaneous tissue: lipodystrophy

Laboratory findings
- Urine: dilute urine sediment containing more than 10 red blood cells per high-power field; cylindruria[a]; nonselective proteinuria[a]
- Renal function: markedly decreased[a]

Chest X-ray: enlarged heart, vascular engorgement, pulmonary edema

Serology: decreased C3 or C4, antinuclear antibody or anti-DNA positive

Serum cholesterol: only mild increase[a]

Plasma protein: only mild decrease in albumin[a] markedly decreased or increased globulin

[a] Although these features are unusual for MCN, their presence does not exclude the lesion.

TABLE 64-10

SOME DEFINITIONS USED TO DESCRIBE PATIENTS WITH PRIMARY NEPHROTIC SYNDROME

Nephrotic-range proteinuria
- Pediatric: Urine protein: creatinine ratio >2
- Adult: 24-hour protein excretion >3.5 g

Relapse
- Proteinuria (>1+ on dipstick or >4 mg/hr/m^2 surface area) for at least 1 week

Complete remission (CR)
- Pediatric: Protein-free urine (<1$^+$ on dipstick or <4 mg/hr/m^2 surface area) for at least 3 days
- Or, Urine protein: creatinine ratio <0.2
- Adult: 24-hour protein excretion <0.3 g

Partial remission (PR)
- Pediatric: ≥50% fall from baseline and/or <2.0 (or <0.3) and/or loss of edema and/or serum albumin >2.5 mg/dL
- Adult: PR 50%: 50% fall from baseline and <3.5 g/day
- Or, PR2: ≤2.0 g/1.73m^2

Limited response (LR)
- Pediatric and Adult: Fall to ≤50% baseline but not PR

Steroid-sensitive nephrotic syndrome (SSNS)
- Response to prednisone (60 mg/m^2 surface area/day) within 8 weeks of starting treatment[a]

Steroid-resistant nephrotic syndrome (SRNS)
- Pediatric: No response to prednisone (60 mg/m^2 surface area/day) within 8 weeks of starting treatment (ISKDC)[a]
- Adult: No change or increased proteinuria compared to baseline

Frequent relapsing nephrotic syndrome
- Initially responsive to prednisone: At least two relapses in a subsequent 6-month period or five relapses in 18 months

Steroid-dependent nephrotic syndrome
- Initially responsive to prednisone
- Either two consecutive relapses during period of steroid taper, or two consecutive relapses, each occurring within 2 weeks of ending a course of corticosteroid therapy

ISKDC, International Study of Kidney Disease in Children.
[a] Some studies define steroid-sensitive patients as those responding within 4 weeks of starting treatment. Those responding between 5 and 8 weeks are referred to as late responders.

Alternatively, response to steroids may itself be a more important determinant of disease process and outcome than is histopathology (628).

A renal biopsy may be technically difficult to perform in the patient with anasarca; surrounding fluid allows the kidney to be balloted by the biopsy needle. In such subjects, it is preferable to delay the attempted biopsy until after a spontaneous or drug-induced diuresis has occurred and most of the ascites has resolved.

Treatment of Minimal Change Disease

The optimal treatment for MCN is based on corticosteroids but continues to evolve. It is noteworthy that in the era before current drugs were available, 25% of children with idiopathic nephrotic syndrome underwent a spontaneous remission (629). Even today, a careful history will often suggest that prior to the presenting episode, the nephrotic patient has had one or more periods of edema that resolved without treatment. The association between MCN and both allergy and malignancy was reviewed earlier in this chapter. An occasional patient with frequently relapsing MCN may enter long-term remission only when a dietary allergen is withdrawn (379) or an underlying malignancy is identified and treated (472,475). Therefore, the possibility that either of these conditions is responsible for the patient's symptoms should be considered before any drug therapy is instituted.

Prior to 1940, the mortality rate for nephrotic syndrome was 40%; the major cause of death was infection (630). After the introduction of antibiotics, mortality was reduced by more than 50%. The development of corticosteroids has further reduced mortality to between 3% and 7% (409,419,630). The major benefits of steroids, however, may well be the faster induction of a remission and reduction of morbidity, although this has never been subjected to a controlled trial. In the older adult nephrotic patient, the risk of undesirable steroid side effects could outweigh the benefits of these drugs (631). Despite these caveats, adrenocorticosteroids remain the therapeutic agent of choice for MCN in patients of all ages.

Corticosteroid Therapy. As stated in the preceding text, cessation of urinary protein excretion in response to the oral administration of prednisone is virtually diagnostic of this entity in children (632). The standard dosage regimen for inducing a remission is 2 mg/kg of body weight per day or 60 mg/m^2 of body surface area per day (maximum of 80 mg per day) (633). Although the initial ISKDC studies used the same daily dose but divided it into three equal parts, we prefer the alternative approach of giving the entire daily amount in a single dose. The Cochrane group has reported that the single daily dose is as effective as a more complex dosing schedule (634). Furthermore, short-term side effects such as hunger and hypomania

are more manageable with use of the single daily dose. In addition, a single dose is more convenient to administer, with a greater likelihood of compliance. The daily dose is rounded up to the nearest multiple of 20 mg. Because nephrotic patients may be anergic, a chest radiograph should be obtained to rule out the possibility of subclinical pulmonary tuberculosis before prednisone therapy is begun. Typically, patients respond with a diuresis (114) followed by resolution of proteinuria. In most instances, response can be considered to have occurred if the patient has had protein-free urine for at least 3 days (Table 64-10). There are as yet no universally agreed-upon definitions for complete remission (CR) or partial remission (PR). Nevertheless, those presented in Table 64-10 represent a starting point for discussion. Among children, CR is commonly defined as the absence of proteinuria on dipstick or alternatively of random (or first-void) urine protein/creatinine ratio of <0.2. Among adults, where 24-hour urine collections remain the standard for definition, CR is commonly defined as <0.3 g per/day (although the upper limits of normal protein excretion at various clinical laboratories may be 150–250 mg per/day). The definitions of PR are much more problematic. Cattran et al. have argued for a 50% decrease in proteinuria from baseline and becoming subnephrotic (634a).

This definition has the merit of capturing the benefit of a substantial reduction in proteinuria, for example from 10 to 3 g per/day, and has been recently shown to correlate with long-term preservation of kidney function. Others have used a more restrictive definition of PR, for example, proteinuria <2g per/day. There may be some utility to capturing lesser degrees of benefit (e.g., limited response (LR),defined as a 50% fall in proteinuria that does not reach subnephrotic levels) but such a benefit has not been tested in longitudinal studies. In children, CR may occur within 1 week of starting treatment, and is seen within 2 weeks of treatment in 75% of responders and by 4 weeks in more than 90% (Fig. 64-8).

According to the definitions derived by the ISKDC and others (Table 64-10), patients who do not respond after 4 weeks of glucocorticoid therapy considered to be steroid-resistant. However, response may be delayed for up to 8 weeks or more in a small percentage of patients, so that approximately 95% of patients with MCNS eventually will prove to be steroid-sensitive. Serum albumin levels may not return to normal for up to 3 months (114), and serum lipid abnormalities may persist for protracted periods (170).

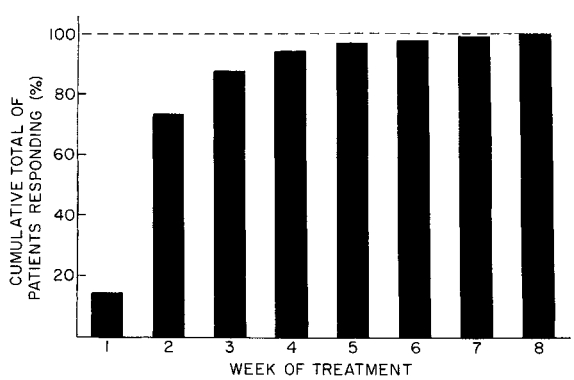

FIGURE 64-8. The cumulative rate of response to treatment with prednisone of children with primary nephrotic syndrome. Only steroid-sensitive patients are included in this analysis. The majority of patients respond in the second or third week of treatment. A small percentage, however, may take 7 or 8 weeks to respond. (Data derived from: International Study of Kidney Disease in Children. The primary nephrotic syndrome in children: identification of patients with minimal change nephrotic syndrome from initial response to prednisone. *J Pediatr* 1981;98:561, with permission.)

The persistence of some proteinuria does not necessarily indicate steroid resistance. Postural proteinuria may persist in a minority of patients and, in others, contraction of blood volume consequent to a brisk diuresis may result in renin-mediated proteinuria (635). In either case, the degree of proteinuria is modest, typically registering 1+ to 2+ on dipstick and being <1 g per/day. This should not be misinterpreted as partial responsiveness and should not be used to justify continuing steroid therapy in high doses. Alternatively, failure to respond to steroids may indicate the presence of an occult infection (633).

Studies in children have demonstrated that, *for the initial episode only*, a prolonged period of treatment with a full dose of daily corticosteroids decreases the incidence or frequency of subsequent relapses. After 6 weeks of daily treatment (rather than the standard 4 weeks of daily treatment) with prednisone, 60 mg/m^2, the patient is switched to 40 mg/m^2 on alternate days (636). Although some controlled studies may disagree (637), meta-analysis by the Cochrane Group has determined that initial treatment with high-dose daily and then prolonged alternate-day oral prednisone decreases the likelihood of relapse over the first 12 to 24 months, and suggests that long-term continuation of alternate-day steroids has increasing benefit for up to 7 months (634). Most groups continue prednisone (total of daily and alternate day) for about 3 months and then discontinue the drug. For subsequent relapses, the steroids are tapered beginning with a switch to alternate-day therapy only a few days after the response to treatment. The dose is then reduced in stepwise fashion during the next 4 to 8 weeks. Longer tapering periods may decrease the likelihood of subsequent relapses (636,638,639). For example, in a relapsed patient treated initially with prednisone, 60 mg per day, the dose is reduced to 40 mg daily after response (3 days of protein-free urine) for 2 weeks, then to 40 mg on alternate days. At 2-week intervals the doses are reduced by 50%, with the final dose of 5 mg on alternate days being given for 3 weeks. It should be noted that this regimen is a matter of preference. The only regimen that has been established by a controlled trial as efficacious is the prolonged course for children of daily and then alternate-day steroids in the initial episode. It is of interest that although prolonged treatment of the first episode seems to consolidate remission in children, those children who respond most rapidly to the initial course of corticosteroids tend to have longer remissions (640). This relationship was not observed in adults (641).

The presence of IgM deposits does not have an effect on response to steroids (642). Although initial observations suggested that patients with mesangial hypercellularity respond to treatment in a manner similar to that seen with MCN (643), subsequent studies indicated that a considerable proportion of these patients may be steroid-resistant (441,644). Conversely, evaluation of large groups of children indicated that persistence of proteinuria is only weakly predictive of mesangial hypercellularity on biopsy (168,445).

Because bacterial peritonitis is a relatively common occurrence in patients with MCN (see Complications of MCN later in this chapter), many clinicians support immunization with pneumococcal polysaccharide vaccines. Concern regarding possible poor response to immunogens in nephrotic patients, and the potential for an immunogenic stimulus to trigger a relapse, has made the issue of childhood immunization in nephrotic patients somewhat controversial. A survey of North American pediatric nephrologists indicated that most modified their approach to immunization but that there was little evidence upon which to base a standard approach (645). However, a recent British study suggests that administration of meningococcal C conjugate vaccine may increase the likelihood of relapse (396). Current recommendations on vaccination in children and adults and use of immunoglobulins (646) support the

use of polyvalent pneumococcal vaccine (302,303) and (in the case of children) *Haemophilus influenzae* type B vaccine. Live virus vaccines should not be given to immunocompromised patients.

Frequent Relapse. Although earlier reports suggested that as high as 50% of steroid-sensitive children do not have a relapse after the initial episode of nephrotic syndrome, subsequent observations indicate that this figure approximates only 25% (362). The difference could be due to an altering pattern of disease but also may reflect the types of patients being referred to reporting centers. Another 25% to 30% of children have infrequent relapses and usually respond well to further courses of steroids, as already described. The remaining patients have either frequently relapsing or steroid-dependent nephrotic syndrome (Table 64-10). Several attempts have been made to correlate pathology with response to steroids or with the pattern of relapses (647). Frequency of relapse showed no correlation with histopathology in a study by the ISKDC (648), but early relapse after the initial episode was predictive of more frequent relapses subsequently.

Attempts to control frequent relapses with steroids may result in their protracted use and possible steroid toxicity. In an effort to minimize these side effects, alternative regimens have been proposed using smaller doses of steroids to treat relapses. Unfortunately the lower response and higher relapse rates associated with most of these approaches usually result in higher cumulative doses of steroids and may not be justified (638). One study suggested that for some frequently relapsing patients, maintenance on daily rather than alternate-day steroids during the tapering regimen can result in a less frequent relapse rate and an equal or lesser cumulative steroid dose (649).

Corticotropin (adrenocorticotropic hormone, ACTH) stimulation tests suggest that prolonged postprednisone adrenocortical suppression may predispose to frequent relapses. A normal response to ACTH predicted a remission for 6 months or longer; a subnormal response was associated with remissions of less than 6 months (650). Early relapse in children with poor adrenal function can be prevented with low-dose maintenance hydrocortisone administration (651).

Nitrogen mustard was found to prolong the duration of remission in patients with MCN (652), but less toxic alternatives such as cyclophosphamide and related drugs are now used in frequently relapsing and steroid-dependent nephrotic patients, especially in those with marked steroid side effects (653). Ideally, cyclophosphamide should not be used until a patient has been followed for 2 to 3 years to document a frequently relapsing course. Although some centers prefer histologic confirmation of MCN before the drug is used, steroid responsiveness may eliminate the need to obtain a biopsy specimen in children, since the disease remains overwhelmingly likely to be MCN (654).

The original cyclophosphamide regimen used doses of 5 mg/kg per day, but it was associated with a high incidence of leukopenia, hair loss, and cystitis (655). Most centers obtain satisfactory results with a dosage of 2 mg per kg per day for children (1.5 mg/kg/d for adults) given for no more than 90 consecutive days; others reported equally good results with 2.5 mg per kg per day for 8 weeks (655–658). At 2 mg/kg per day, a course of cyclophosphamide limited to 8 weeks is followed by a higher relapse rate (659). Most centers utilize cyclophosphamide in conjunction with steroids (657). The induction of a remission with steroids before therapy with cyclophosphamide is instituted, permits a liberal fluid intake to induce a high urine flow rate during treatment with the immunosuppressive agent, thereby reducing the risk of cystitis. The steroid taper is accomplished more rapidly, over a 6-week period.

Approximately 65% of frequently relapsing patients remain in remission for at least 5 years after a course of cyclophosphamide (657,660,661). Response to cyclophosphamide may be predictable from the patterns of relapse after steroids. Of patients who have a relapse immediately after tapering of steroids, two-thirds also have a relapse quickly after cyclophosphamide treatment. Conversely, frequently relapsing patients who can maintain remission for more than 14 days after steroid therapy is discontinued have longer remissions and fewer relapses after cyclophosphamide treatment (659).

It is rare to see acute side effects with the current cyclophosphamide regimens, although white blood cell counts should be monitored at least weekly, especially early in the course of therapy. The frequency of cystitis, alopecia, and leukopenia has been reduced markedly by institution of smaller daily and cumulative doses of the drug, and is further minimized by encouraging fluid intake and frequent voiding for 4 hours after administration. The gonadal toxicity of cyclophosphamide also appears to be cumulative-dose related; testicular and ovarian toxicity is uncommon if the total dose is less than 200 mg/kg (639,662,663). However, such doses do decrease sperm counts (664), and some men experience gonadal toxicity even at low doses (665). Higher doses lead to more significant testicular dysfunction (666). In children, the drug is usually given well before puberty to minimize the risk of gonadal toxicity, although it has been suggested that prepubertal males are more sensitive than pubertal males (667). Injection of slowly absorbed testosterone may protect against iatrogenic azoospermia (668). Female patients treated with a mean total dose of 439 mg/kg at an average age of 8.7 years were followed for a mean of 12.3 years. All had normal pubertal development and regular menstrual patterns. Hormonal studies did not show obvious ovarian or pituitary–gonadal dysfunction, and two patients gave birth to normal children (666). There are no data available to convincingly demonstrate a protective effect of ovarian suppression with oral contraceptives during alkylating agent therapy (669). Concern has been expressed about the potential for cyclophosphamide to induce malignancies. It remains uncertain whether this is a major risk of therapeutic regimens currently used in nephrotic syndrome, although leukemia occurred in at least one child treated for nephrotic syndrome with prednisone and cyclophosphamide (670). Furthermore, the use of cyclophosphamide may be associated with findings of increased sister chromatid exchange (671) and long-lasting immunosuppressive effects (517).

Anecdotal experience in patients who are refractory to conventional therapies suggests that adding six monthly intravenous injections of cyclophosphamide, 0.5 g/m^2 of body surface area per injection, to the oral steroid regimen may result in prolonged remission of the nephrotic syndrome even after discontinuation of the steroids (672). This bolus therapy has potential advantages related to facilitated compliance and the availability of newer agents to counter drug toxicity.

An alternative alkylating agent, chlorambucil, shares many of the side effects seen with cyclophosphamide but does not induce cystitis. Unlike cyclophosphamide, however, it may produce seizures in some patients (673) and induces electroencephalographic changes in the absence of seizures in others (674). Malignancies have developed in at least three nephrotic children treated with this drug (650,675,676). However, the overall incidence of serious complications with chlorambucil is low, especially when it is used in a dosage of 0.1 to 0.2 mg/kg per day for an 8-week course. It may produce a more stable remission than cyclophosphamide does (677,678) and be effective in some steroid-dependent and cyclophosphamide-resistant children with nephrotic syndrome (679). A meta-analysis found that both cyclophosphamide and chlorambucil are effective in frequently relapsing patients and was unable to discern a difference between the two treatments (680).

Cyclosporine and other calcineurin inhibitors can be beneficial in the management of patients with frequent relapses,

especially those who do not achieve a long-term remission with an alkylating agent. The usual dose of cyclosporine, 150 mg/m^2 per day (7 mg/kg per day), is effective in inducing and sustaining remissions in these patients. An analysis of 129 children from nine studies showed complete remission in 84.5% (681). With cessation of the cyclosporine, however, there is a high rate of relapse, the subsequent relapses possibly being more difficult to control than those prior to cyclosporine treatment. Although some reports have indicated that cyclosporine is a safe, steroid-sparing agent for treating steroid-dependent nephrotic syndrome (682), others suggest that prolonged use can be associated with nephrotoxicity (683,684). An alternative and successful approach to management is the long-term use of low-dose cyclosporine combined with low-dose, alternate-day prednisone. Because of the potential for toxicity, serum creatinine should be monitored periodically in patients receiving cyclosporine.

Levamisole, an antihelminthic drug and immunopotentiating agent, can be used to treat children with MCN, especially those who have frequent relapses (623,685). The usual dose is 2.5 mg/kg of body weight given by mouth on alternate days. The drug may be valuable as a steroid-sparing agent. In a controlled trial, 14 of 31 patients taking levamisole were able to discontinue steroids and remain in remission for the 112 days of the trial, compared to only four of the 30 control subjects taking placebo (622). When the drug was added to the regimen of patients after a steroid-induced remission, the relapse rate fell from 5.2 episodes to less than 0.7 episodes per year during a 2-year period of treatment. The beneficial effect appeared to last beyond completion of the course of levamisole (686). Side effects of levamisole include a decreased neutrophil count or transient granulocytopenia in two-thirds of patients. More severe complications such as skin rash, a flu-like illness, vomiting, thrombopenia, and neurologic symptoms (insomnia, hyperactivity, and seizures) have been reported (687).

Mycophenolate mofetil has been reported to successfully treat frequently relapsing minimal change disease in adults (688,689) and children (690,691). No results of extensive controlled trials are yet available.

The use of intravenous immunoglobulin as an adjunct to steroids has not resulted in any clinically important extension of the period of remission in patients with frequent relapses (692).

Steroid-Dependent Patients. The management of these patients is similar in many respects to that of frequently relapsing patients. A minority of these individuals can be controlled with long-term, low-dose oral prednisone. Unfortunately the dose of prednisone required to maintain a remission is usually sufficiently high to result in unacceptable side effects. Therefore, alternative approaches to management are required.

Methylprednisolone pulses have not been shown to be beneficial in this population of patients (693). Some patients respond to cyclophosphamide and, as is the case with frequent relapses, the likelihood of a prolonged remission is increased if the course of drug is extended to 12 weeks (694).

The results of using cyclosporine in steroid-dependent patients are similar to those in the frequently relapsing group. Some clinicians advocate a course of alkylating agents before resorting to cyclosporine (695). Remissions can be maintained for 2 years or longer, especially if the cyclosporine is given continuously. However, 40% of these patients also require low-dose, alternate-day steroids to stay in remission. Unfortunately, long-term remission after discontinuing the cyclosporine is relatively rare (696). Treated patients show an average 20% decline in GFR after 3 months of therapy, but GFR stabilizes during subsequent therapy and has the potential to return toward normal with discontinuation of the drug (697). Nonetheless, toxicity may be chronic (698). A controlled multicenter study evaluating both pediatric and adult patients with either frequently relapsing or steroid-dependent nephrotic syndrome documented that cyclosporine and cyclophosphamide have a similar degree of efficacy but that more patients given cyclophosphamide have stable remissions (699). One study has suggested that cyclosporine given concomitantly with cytotoxic agents may diminish the efficacy of the latter treatment (700).

For most clinicians, when a steroid-dependent or frequently relapsing patient shows signs of steroid toxicity, the next drug to be used is cyclophosphamide, given for 12 weeks. If frequent relapses recur, cyclosporine in the lowest possible dose would be the next therapy utilized (701). Levamisole is reserved for patients who continue to have relapses. Mycophenolate also has been effective in steroid-dependent disease (690,691).

A significant number of patients who are steroid-dependent are resistant to all alternative forms of treatment and must be controlled symptomatically on a chronic basis with dietary sodium restriction and diuretics. Oral furosemide or metolazone, used alone or in combination, or in conjunction with spironolactone, is usually effective. Most patients learn to individualize their dosage. Unless there is evidence for progressive loss of renal function, this regimen probably is preferable to attempting control of the nephrotic syndrome with longer-term steroid therapy because of the significant complication rate of this latter approach. However, progressive interstitial nephritis has occurred in nephrotic patients chronically treated with diuretics, especially furosemide (702).

Treatment of Adult Patients with MCN. Most of the recommendations made for pediatric patients are equally applicable to their adult counterparts because there are remarkable similarities of MCN in the different age groups. In general, the response rates to steroids in adult and pediatric patients with MCN are comparable (657,703). However, the prevalence of relapses has not been documented systematically in adults. Differences between pediatric and adult patients with MCN may include a less rapid response to corticosteroids in adults (704) and a more effective response in adults to cyclophosphamide used alone (705,706); however, these differences may reflect technical or experimental design issues. For example, adults generally receive a lower dose of steroids on a per-weight basis.

Adult patients may be somewhat more prone to steroid complications (707), particularly in the skeleton. This has resulted in a philosophical debate about how aggressive one should be in the use of these drugs as well as immunosuppressants when treating nephrotic syndrome. However, given (a) the possibility of irreversible glomerular lesions from prolonged heavy proteinuria, (b) undesirable and potentially harmful effects of hyperlipidemia and protein deficiencies, and (c) the potential reduction of side effects from glucocorticoids and cytotoxic alkylating agents if the course of drugs is brief and the dosage not excessive (708), most internists employ aggressive therapy in treating patients with MCN.

Treatment of Steroid-Resistant MCN

Approximately 5% of children with MCN are unresponsive to a standard steroid regimen. Before considering a patient to be steroid-resistant, infection or occult malignancy must be ruled out. A small but significant percentage of those who initially are steroid-responsive will become steroid-resistant after one or more subsequent relapses. This development is an indication for renal biopsy and often denotes the presence of FSGS.

Alternative steroid regimens have been attempted to improve response to therapy. High-dose boluses of intravenous methylprednisolone induced remission in five of eight

corticosteroid-resistant children (693). In another experience, methylprednisolone pulses reduced proteinuria but did not result in remission (709). One major problem in this group of patients is the development of severe side effects from protracted use of steroids. Therefore, regimens combining drugs in an attempt to reduce the amount of steroid administered, or alternative drugs to steroids, have been tried.

Occasionally a patient with biopsy-confirmed MCN who does not respond to oral steroid therapy will respond to an equivalent dose of the drug given as methylprednisolone by intramuscular injection. In several patients we have found that a tapered course of parenteral, sustained-release, methylprednisolone in combination with prolonged chlorambucil (3 months at a dose sufficient to decrease the peripheral white blood count to $<5{,}000/mm^3$) either induced remission or decreased proteinuria sufficiently that the patient was edema-free with little or no additional diuretic therapy. The effects appear to be long-lasting. This treatment must be employed cautiously after full consideration of its potential long-term side effects such as risk of neoplasm. It is not clear whether this improved response to injected steroids relates to the patient's compliance, to poor absorption of oral drugs, or to the metabolism of steroids by the liver. The routine measurement of prednisolone kinetics (710), however, does not appear to help in the management of children with MCN.

Cyclosporine therapy in steroid-resistant MCN has had disappointing results. In a summary of nine studies, only 12 of 60 (20%) children had complete remission and many had a relapse with cessation of therapy. Cyclosporine in association with prednisone is more effective in inducing remission than is cyclosporine alone (653), and some steroid-resistant patients became steroid-sensitive after a course of cyclosporine. Potential nephrotoxicity is of concern in any patient receiving cyclosporine for protracted periods. Tacrolimus has been reported anecdotally to be effective (711).

Therapy with nonsteroidal antiinflammatory drugs also has produced variable results in patients with MCN (712). Some patients with frequently relapsing or steroid-resistant MCNS who were treated with one of these drugs demonstrated a reduction in proteinuria, but all remained nephrotic and a high percentage had no response (713). Thus, drugs such as indomethacin and meclofenamate may at best represent a useful adjunct in selected patients who are receiving symptomatic treatment only. One potential explanation for the disappointing results is that patients selected for this therapy typically are unresponsive to all standard forms of treatment and may represent a recalcitrant population (713). NSAID should be used cautiously because of association with renal failure in nephrotic patients (see section on Consequences of Proteinuria).

Steroid-resistant patients who do not respond to alternative therapies often have to be maintained on a regimen of sodium and fluid restriction combined with judicious use of diuretics. Balancing the desire to minimize tissue edema against the importance of avoiding intravascular volume depletion is a rigorous challenge for the clinician (see section on Symptomatic Treatment of Nephrotic Syndrome).

Outcomes

Although late relapses of MCN have been reported (714,715), the majority of children with MCN enter permanent remission either before or at puberty. Their long-term prognosis is good, with at least 70% entering adult life without renal or urinary abnormalities. This finding contrasts with the much less favorable outcome if the nephrotic syndrome is associated with glomerulonephritis (716). A minority of pediatric patients who are initially steroid-responsive eventually progress to renal failure. Most are found to have FSGS (443,717), although this lesion is not always present (718).

There have been many attempts to predict the long-term course either from renal histology or from patterns of response to treatment with steroids. For example, in one study of children, the presence of mesangial hypercellularity and immune complexes in the glomeruli was associated with an increased relapse rate (441). In contrast, the ISKDC (648) was unable to correlate frequency of relapse with (a) the histopathologic subgroups of MCN, (b) clinical or laboratory characteristics present at the time of diagnosis, (c) the timing of initial response, or (d) the interval between the initial response and the first relapse. Frequent relapses in the first 6 months, however, were highly predictive of frequent relapses subsequently. In contrast, children who present with minimal edema and are steroid-sensitive follow an extremely favorable clinical course (719).

Adult patients with MCN also have a good prognosis; in one series, more than 90% survived for 10 years or more without development of end-stage renal disease (ESRD) (720,721).

Complications

The most common complications observed in patients with nephrotic syndrome are secondary to therapy. Steroid-induced side effects are well known and include the typical changes in facies, obesity, hirsutism, striae, and pseudotumor cerebri. Acutely, patients receiving large doses of corticosteroids may complain of difficulty sleeping or abdominal distress from high gastric acidity. Hypertension can occur but is seen less often in patients adhering to a sodium-restricted diet. Although growth retardation may be seen in children, especially if they receive high doses of steroids for protracted periods (722), the incidence of significantly decreased height in prepubertal children is relatively low, even with repeated courses of corticosteroid therapy (723), particularly when the prednisone is given on alternate days (724). Catch-up growth often occurs when steroid therapy is discontinued (725). Patients with steroid-responsive nephrotic syndrome who had received repeated courses of high-dose steroids during childhood and who had completed growth had a mean height equivalent to the 40th percentile (726); total corticosteroid dose, however, correlated only weakly with the height scores. In another study, patients were slightly but significantly shorter than their peers. More importantly, they had a higher BMI than controls (321). Corticosteroid-induced cataracts were found in a high percentage of children (727), although visual acuity was not impaired. The complications of cytotoxic drugs were discussed previously.

Peritonitis is a particularly important complication of nephrotic syndrome in children (728). Patients who have one such episode are at increased risk for subsequent episodes. Peritonitis typically occurs during relapses of the disease associated with gross edema and ascites. Clinical evidence of peritoneal irritation usually is present even in patients receiving steroids. The most common infecting agent remains *Streptococcus pneumoniae*, which is found in approximately half of the affected patients (728); *Escherichia coli* is cultured in an additional 25%; a variety of other organisms, including *Haemophilus influenzae*, may be found in a small percentage of patients, and the peritoneal fluid may be culture-negative in some patients. Interestingly, this complication is far less common in adults; the basis for this disparity is not known.

Infections were responsible for the majority of deaths in nephrotic patients in the preantibiotic era. Although infections occur with much less frequency now, they continue to have serious implications. For example, in a report on long-term

outcomes of treatment from the ISKDC in which 389 children with MCNS were followed for 5 to 15 years, 6 of the 10 deaths were due to infections. Other causes of death included one episode of dural sinus thrombosis and one incident of cardiorespiratory failure following infusion of salt-poor albumin. One child died in chronic renal failure after development of FSGS not seen in initial biopsy specimens, and one death was from uncertain causes. Five initially nonresponsive patients and four patients manifesting early relapse died. The number of fatalities among nonresponders was particularly striking; 20% of all initial nonresponders in the study died (729). Nonrenal causes of death not mentioned in this study but that may be encountered include other thromboembolic phenomena, hemorrhagic pancreatitis (403), and hypovolemic shock.

Nonfatal complications of MCNS include azotemia, hypovolemic shock, thrombosis, anemia, and effects of decreased vitamin D levels. These were discussed in the section on Consequences of Proteinuria.

FSGS AND COLLAPSING GLOMERULOPATHY

Focal segmental glomerulosclerosis (FSGS) is a common cause of nephrotic syndrome in children and adults. Synonyms for this lesion include *focal and segmental glomerulosclerosis with hyalinosis*, *focal sclerosing glomerulonephritis*, and *focal sclerosing glomerulopathy*. Some patients, particularly adults, may present with subnephrotic proteinuria or may lack other features of nephrotic syndrome. This is particularly true of postadaptive FSGS, as compared with idiopathic FSGS.

FSGS should be distinguished from the finding of occasional globally sclerosed (obsolescent) glomeruli, a benign pattern that is seen in the United States in up to 1% to 3% of glomeruli until age 40 to 55 years and then rises steadily, reaching 30% in individuals 80 years of age (730,731). Howie makes the point that FSGS "cannot be defined in a sensible, useful, unambiguous way" (456). The problem is that there are at least three meanings to the term and that definitions must include both pathologic description and clinical information: (a) idiopathic nephrotic syndrome with segmental glomerulosclerosis (which in this chapter will be termed *idiopathic FSGS*), (b) FSGS arising as a consequence of structural and function adaptation to various conditions, all of which are characterized by glomerular hypertrophy and hyperfiltration (which in this chapter will be termed *postadaptive FSGS*), and (c) segmental glomerulosclerosis arising in the setting of other glomerular diseases, such as proliferative glomerulonephritis, membranous nephropathy, and diabetic nephropathy (which are not dealt with in this chapter, out of the belief that they should not be considered part of the spectrum of primary podocyte diseases).

A related histologic entity has been termed *collapsing focal segmental glomerulosclerosis* or *collapsing glomerulopathy* (as suggested by Detwiler et al (732). We have chosen the latter term because we believe that the histology, biology, and etiology of collapsing glomerulopathy are sufficiently distinct to merit a separate classification. Moreover, the glomerular lesions may be global rather than segmental and the defining lesions are podocyte hyperplasia and capillary collapse rather than sclerosis, all of which make the use of the term FSGS inappropriate. Nonetheless, the nephrology and pathology literature continues to most commonly include collapsing glomerulopathy within FSGS, and, therefore, in this chapter will follow that convention when necessary.

Another disorder is *diffuse mesangial sclerosis* (DMS), an uncommon syndrome generally restricted to the pediatric population. Many cases of DMS are associated with genetic mutations in podocyte genes, placing this syndrome within the spectrum of podocyte diseases.

Presentation and Epidemiology

The clinical presentation of FSGS is highly variable. Nephrotic range proteinuria is seen in approximately 90% of children and 70% of adults (733), although this fraction of non-nephroptic patients in a particular practice will clearly depend upon the inclination of the nephrologist to perform a renal biopsy in the setting of subnephrotic proteinuria. Common associated features include microscopic hematuria (55% children, 45% adults) and renal insufficiency (20% children, 30% adults) (733).

FSGS is now the leading cause of primary nephrotic syndrome among adults, as shown by review of renal biopsy archives from Chicago (10), Springfield, Massachusetts (734), and New York (365). Therefore, it has replaced membranous nephropathy as the leading cause of adult nephrotic syndrome in the United States, with a relative incidence of 30% to 40% among patients undergoing renal biopsy.

A similar pattern has been seen among children. In Houston, FSGS was present in 35% of renal biopsies in children with primary nephrotic syndrome prior to 1990, rising to 47% (and the leading diagnosis) among biopsies performed after 1990 (735). In India, the prevalence of FSGS on kidney biopsy rose from 20% to 47% during the 1990s (736). In South Africa, over the period from 1970 to 1995, FSGS as a cause of nephrotic syndrome rose from 2% to 20% among Indian children and from 5% to 28% among black children (737). Among children in Ontario (with a largely Caucasian population), who represent a closed population evaluated by single pathology center, FSGS now represents 18% of the renal biopsies performed for nephrotic syndrome. This is a 2.5-fold increase in incidence, from 0.37 cases/100,000 during 1985 to 1993 to 0.94 from 1993 to 2002 (11). Furthermore, FSGS remains a leading cause of ESRD in children ages 0 to 19 (738). Over the period from 1999 to 2002, 4,859 children progressed to ESRD; in 1,262 children the cause was glomerulonephritis, of which 592 cases were FSGS (representing 47% of glomerulonephritis ESRD and 12% of all ESRD in children). By comparison, other major ESRD categories included "other cause" (including chiefly congenital renal and urologic abnormalities), 1,262 children and "missing or unknown cause," 670 children.

To date, there has not been a population-based study of FSGS incidence in the United States and there is no national registry for glomerular disease. Therefore, the increased relative incidence of FSGS could represent a decline in other diagnostic entities or an absolute increase in FSGS incidence (other possibilities, such as a change in biopsy practice or diagnostic classification, seem unlikely to account for more than a small fraction of the changes). However, as a proxy for incident FSGS trends, Kitiyakara and colleagues examined the incidence of FSGS ESRD (excluding AIDS nephropathy) and found a steady increase over the last two decades (739,740). This was not unique to FSGS (e.g., there was a similar increase in the category, "other glomerular disease") but it contrasts with a more modest increase in membranous nephropathy ESRD and a drop in ESRD due to glomerular disease, "histologically not examined." Thus, it appears to be likely that there has been a true increase in incident FSGS cases in recent years. The reasons behind these trends are not understood.

Worldwide, there is considerable heterogeneity in the relative incidence of FSGS compared to other causes of adult nephrotic syndrome, ranging from 10% to 45% (Fig. 64-9). Factors contributing to this variability likely include population genetic differences, renal biopsy practices, and environmental factors including HIV-1 infection. There are also

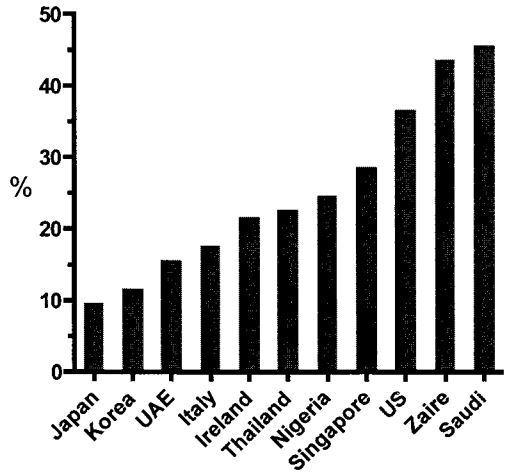

FIGURE 64-9. Relative incidence of FSGS as a cause of adult nephrotic syndrome. The fraction of adult nephrotic syndrome attributed to FSGS (which probably includes collapsing glomerulopathy in most or all series) is presented in select countries.

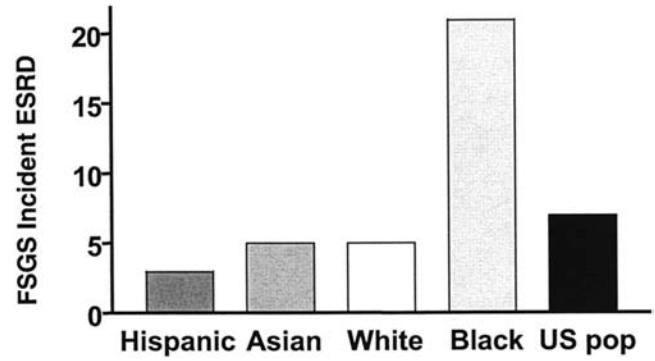

FIGURE 64-11. Incidence of FSGS ESRD by age and race in the United States. The incidence of ESRD (cases/million population/year) attributed to FSGS is presented by age and race; data from the USRDS. (Reprinted from: Kitiyakara C, Kopp JB, Eggers P, et al. Trends in the epidemiology of focal segmental glomerulosclerosis. *Semin Nephrol* 2003;23:172, with permission.)

striking racial differences in the incidence of FSGS ESRD. In the United States, blacks are at approximately fourfold increased risk for FSGS ESRD compared to whites, Hispanics (who may be of any race), and Native Americans (Fig. 64-10). Presumably genetic factors contribute to this risk. Progress is being made in identifying loci that contribute to the increased risk for ESRD among African Americans (741) and in FSGS in particular (discussed in subsequent text). Differences in FSGS ESRD can be resolved, at least in theory, into differences in FSGS incidence and differences in the progression of FSGS to ESRD. The FSGS incidence data, relative to other nephrotic diseases, that were cited previously from various U.S. cities suggest that blacks are at a higher risk for FSGS than are other racial groups. With regard to progression to ESRD, studies of adults (742) and children (743,744) have suggested that blacks are more likely to progress to ESRD than are those of other races, by a factor of approximately two- to fourfold. The reasons for these ethnic disparities remain to be elucidated; genetic differences likely contribute but environmental factors such as access to medical care, differences in the types of FSGS, and the presence of comorbid conditions could play a role. Interestingly, there also is a racial difference in the age of onset of FSGS ESRD (Fig. 64-11). In blacks, the incidence peaks in the early 50s, whereas in whites incidence peaks two decades later (when there is a second peak among blacks).

Histopathology

Fahr, writing in 1925, first noted that patients with lipoid nephrosis who progress to renal failure showed focal glomerular damage (745). In 1957, Rich examined autopsy tissues from 20 children with nephrotic syndrome and otherwise typical lipoid nephrosis and described progressive sclerosis of glomeruli, affecting first the juxtamedullary glomeruli. Heptinstall, in the 1966 edition of his renal pathology textbook, confirmed that some patients with lipoid nephrosis had hyalinization of the glomerular tuft, particularly affecting the juxtamedullary glomeruli (746). McGovern (747) and Hayslett et al. (748) showed that in some patients whose initial renal biopsy was consistent with MCN, a later renal biopsy showed FSGS. In 1970, two reports demonstrated that FSGS was the second most common cause of nephrotic syndrome in children (418,419). Thus, by 1970, it was clear that FSGS was a distinct and common glomerular disease.

Classically, the pathologic abnormalities affect only some glomeruli (focal), with only part of the glomerular tuft involved (segmental). There may be accumulation of acellular hyaline subendothelial deposits within glomerular capillaries; these represent the insudation of plasma protein below an injured endothelium. Tubular atrophy and interstitial inflammatory infiltrates are common (418). The first glomeruli affected are those located near the medulla. As a consequence, an early or superficial renal biopsy may miss the lesion. Furthermore, evaluation of sclerosis on the basis of one section or a few

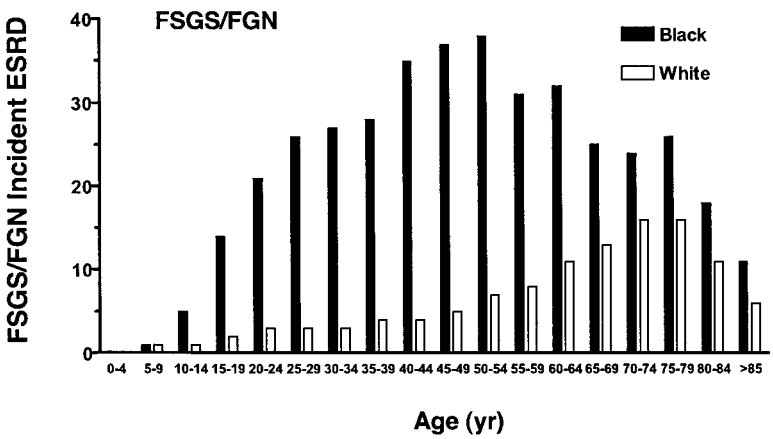

FIGURE 64-10. Racial differences (cases/million population/year) in FSGS ESRD in the United States. The annual incidence of ESRD attributed to FSGS for the years 1995 to 2000 are shown.

sections, as is typical of routine pathologic analysis, will significantly underestimate the fraction of glomeruli that are affected (749,750).

Immunofluorescent microscopy may demonstrate IgM and C3 deposits in a granular pattern, particularly within affected sclerotic segments (751). Unaffected glomerular areas also may reveal IgM and C3 in a mesangial distribution. Electron microscopy shows epithelial cell foot-process effacement that in patients with heavy proteinuria is diffuse and involves areas of the glomeruli that do not demonstrate sclerosis. Mesangial hypercellularity is present variably. In a patient with otherwise normal glomeruli, the presence of glomerular hypertrophy, focal interstitial fibrosis or tubular atrophy may reflect FSGS that is not present in the glomeruli that are sampled in the renal biopsy (752).

The incidence of FSGS in a subsequent biopsy when the original biopsy shows MCN has varied from less than 10% to more than 40% in individual studies (702), which may reflect whether each report presents a primary or referral population.

Histopathologic Variants: FSGS and Collapsing Glomerulopathy

It is now clear that the spectrum of primary podocyte diseases (MCN, DMS, FSGS, and collapsing glomerulopathy) represent several histopathologic patterns, each including multiple disease entities. The relationships among these entities remain enigmatic; we present one schema (Table 64-3). We have excluded other disorders that might otherwise be placed in the MCN/FSGS spectrum, such as idiopathic nodular sclerosis, which has been proposed to have a vascular etiology (753). Others have presented parallel but distinct approaches (754–756).

The existing classification schemes for the primary podocyte diseases, including the one presented here, rely upon a mixture of morphologic, immunologic, genetic, and historical criteria in ways that are not ideally integrated. Importantly, the presence of a risk factor for a diagnostic entity in a particular case under investigation will not always mean that that particular factor has been responsible. Therefore, although obese patients are at increased risk to develop postadaptive FSGS, they remain at some risk for other FSGS variants. Likewise many patients with FSGS have hypertension, and distinguishing hypertension-associated FSGS from other variants can be problematic. As a practical matter, information about genetic history and the presence of relevant comorbid conditions may not be available to the pathologist at the time of diagnosis. All of these comments point out the limitations of relying upon clinical factors in determining pathologic diagnosis or, conversely, determining disease based solely on histopathology. This issue may be successfully addressed by new molecular diagnostic techniques, which may improve the accuracy of clinical diagnosis and may generate more useful classification schemes in the future. Immunostaining to evaluate protein expression is one approach. For example, dystroglycan, a podocyte membrane protein contributing to cellular adhesion to the GBM, is reduced in MCN but not in FSGS (428). Another approach is quantitative analysis of RNA extracted from microdissected glomeruli. Kretzler and colleagues showed that the ratio of podocin/synaptopodin mRNA distinguished MCN from FSGS and predicted steroid-responsiveness in cases where the distinction of MCN from FSGS was uncertain (757).

Columbia Classification

D'Agati and colleagues have proposed a working classification (the Columbia classification) for idiopathic FSGS, which comprises five categories: collapsing variant, tip lesion, cellular lesion, perihilar variant, plus a final category for those cases that do not have the diagnostic criteria for the other categories, not otherwise specified (NOS), corresponding to classic FSGS. (Table 64-11, Fig. 64-12) (754).

Evaluation of the renal biopsy requires immunologic and ultrastructural studies to exclude other disease entities. The Columbia classification of FSGS variants is based solely on semiquantitative analysis of morphology by light microscopy. There is no minimum glomerular number required to make a diagnosis, but obviously diagnostic accuracy will increase with larger sample size. A single glomerulus with the defining characteristic findings is sufficient, as long as the other criteria are met. This classification scheme was not specifically designed to serve clinical or research purposes, but rather to address both needs.

The collapsing variant of idiopathic FSGS is defined by the presence of at least one glomerulus with segmental or global collapse *and* with podocyte hypertrophy or hyperplasia. Thus podocyte changes alone are insufficient to make the diagnosis (such findings would indicate cellular FSGS). This entity is termed idiopathic collapsing glomerulopathy in Table 64-3. HIV-associated collapsing glomerulopathy has an identical histologic appearance, so that the appearance of classic or other forms of FSGS in a patient with HIV-1 infection is sufficiently unusual to raise the issue of coincidental idiopathic FSGS. Both idiopathic collapsing FSGS and HIV-associated FSGS are frequently associated with tubulointerstitial injury and fibrosis that is out of proportion to the extent of glomerular involvement.

The glomerular tip variant of idiopathic FSGS is defined by the presence of at least one glomerulus with segmental scar involving the glomerular tuft adjacent to the proximal tubule. The collapsing variant must be excluded (it would take precedence over the diagnosis of the tip variant). Most tip variants have increased cellularity as well as the required sclerosis. Mesangial hypercellularity may be present. Perihilar sclerosis cannot be present but other peripheral (non-tip, non-hilar) lesions containing IgM and C3 may be present within the tip lesion. Heavy proteinuria and extensive foot-process effacement is typically present.

The cellular variant of idiopathic FSGS has had a complicated history. Grishman and Churg described the ultrastructure of 16 patients with FSGS and found that 5 patients had podocyte abnormalities, including cellular degeneration and detachment, together with glomerular capillary collapse; 3 of these patients exhibited rapid clinical deterioration (758). Schwartz and Lewis coined the term *cellular lesion of FSGS*. The original cellular definition included segmental or global hypercellularity (representing proliferation and/or infiltration, or hypercellularity within Bowman's space overlying segmental scar, or capillary collapse) (759). Thus, many of these cases meet the Columbia criteria for collapsing variant. In the Columbia framework, diagnosis of cellular variant requires the presence of endocapillary proliferation (segmental or global) in at least one glomerulus; these cells include endothelial cells, macrophages, and foam cells. Podocyte hypertrophy and hyperplasia may be present. Mesangial hypercellularity may also be present but is uncommon. Consequently, the focus of the Columbia definition of cellular variant has shifted away from podocytes to endocapillary cells, although abnormalities of both cellular compartments may be present. A tubulopathy is present that may be disproportionate to the extent of glomerular involvement. This may include a focal microcystic dilation and tubular epithelial cells manifesting acute injury (including focal acute tubular necrosis), regeneration, and chronic injury (cellular atrophy and thickening of the tubular basement membrane). Tip variant and collapsing variant must be excluded (these diagnoses would take precedence over cellular variant).

The Columbia classification makes the perihilar variant a formal diagnostic entity. This variant is diagnosed when

TABLE 64-11

COLUMBIA CLASSIFICATION OF THE MORPHOLOGIC VARIANTS OF FSGS

Variant	Positive criteria	Negative criteria
FSGS, Not otherwise specified	• At least one glomerulus with segmental increase in matrix obliterating the capillary lumina • There may be segmental GBM collapse without podocyte hyperplasia	Exclude other defined variants below
Perihilar variant	• At least one glomerulus with perihilar hyalinosis, with or without hyalinosis • Perihilar sclerosis and hyalinosis involving >50% of segmentally sclerotic glomeruli	Exclude cellular, tip, and collapsing variants
Cellular variant	• At least one glomerulus with segmental endocapillary hypercellularity occluding lumina, with or without foam cells and karyorrhexis	Exclude tip and collapsing variants
Tip variant	• At least one segmental lesion involving the tip domain (outer 25% of the tuft next to the origin of the proximal tubule) • The tubular pole must be identified in the defining lesion • The lesion must have either an adhesion or confluence of podocytes with parietal or tubular cells at the tubular lumen or neck • The tip lesion may be sclerosing or cellular	Exclude collapsing variant Exclude if any glomeruli show perihilar sclerosis
Collapsing variant	• At least one glomerulus with segmental or global collapse and podocyte hypertrophy or hyperplasia	No exclusions

FSGS, focal segmental glomerulosclerosis; *GBM*, glomerular basement membrane.
(Adapted from: D'Agati V. Pathologic classification of focal segmental glomerulosclerosis. *Semin Nephrol* 2003;23:117; and D'Agati V, et al. Pathologic classification of focal segmental glomerulosclerosis: a working proposal. *Am J Kidney Dis* 2004;43:368.) The flow of diagnostic decision-making begins at the bottom, as the pathologist diagnoses or excludes collapsing variant, then tip variant, then cellular variant, then perihilar variant, and, finally, FSGS, not otherwise specified (NOS).

perihilar hyalinosis is present in one or more glomeruli and perihilar sclerosis and/or hyalinosis is present in at least 50% of segmentally sclerotic glomeruli. Hyalinosis represents the accumulation of glassy, homogeneous, eosinophilic material within the capillary wall and is believed to consist of plasma proteins. In the remnant rat model, hyalinosis appears first as the accumulation of PAS-positive and electron-dense material beneath damaged glomerular capillary endothelial cells, with later expansion and encroachment on the capillary lumen (760). Glomerulomegaly is common. The perihilar variant is commonly associated with what we describe as *postadaptive* FSGS (defined in subsequent text). Cellular variant, tip variant, and collapsing variants must be excluded (these diagnoses would take precedence over perihilar variant).

The most common variant of FSGS (NOS) may include features of any of the prior variants, but lacks sufficient criteria to make a more specific diagnosis. The term *classic FSGS* would include FSGS NOS.

The Columbia classification represents a very important step forward in the process of developing a robust, consistent, and clinically useful diagnostic classification system for FSGS. By laying out consensus diagnostic criteria for FSGS for the first time, D'Agati and colleagues have framed the issues that the field will address in the coming years. As the authors recognize, this is a working proposal that will almost certainly undergo revision. The next iteration might usefully address some of the following issues, and may well make use of use molecular markers to refine the classification system.

First, the relationship between the cellular variant and collapsing FSGS (collapsing glomerulopathy) remains controversial. Schwartz and colleagues note that in their terminology, the cellular lesion includes collapsing FSGS, whereas the Columbia classification includes cellular lesion and collapsing FSGS as distinct entities. (761). No patient series has yet been published that describes the clinical characteristics and outcome of patients with the cellular lesion, as defined by the Columbia classification, and so this syndrome is not yet well-characterized.

Second, and related to the previous issue, the importance of different forms of podocyte injury has become apparent as we have developed new understanding of the biologic processes underlying FSGS (podocyte depletion) and collapsing glomerulopathy (podocyte proliferation). Morphologically, podocyte proliferation is characteristic of the collapsing variant, but must be coupled with capillary loop collapse for the diagnosis to be made. When capillary loop collapse is absent and podocyte changes are coupled with endothelial cell proliferation, the diagnosis of cellular lesion is made. Thus, when podocyte hyperplasia is present, the diagnosis depends upon whether capillary collapse or endocapillary proliferation is present (762). The biologic rationale for this distinction is not immediately clear, as we do not understand the mechanisms responsible for glomerular collapse. If further studies indicate that the podocyte phenotype is similar in collapsing FSGS and those cases of cellular variant FSGS with podocyte hyperplasia, and that response to therapy and prognosis are also similar, it is probably more logical to combine these categories.

Third, the stability of classification when serial biopsies are performed needs to be defined. The few studies available suggest that patients may change diagnostic categories on

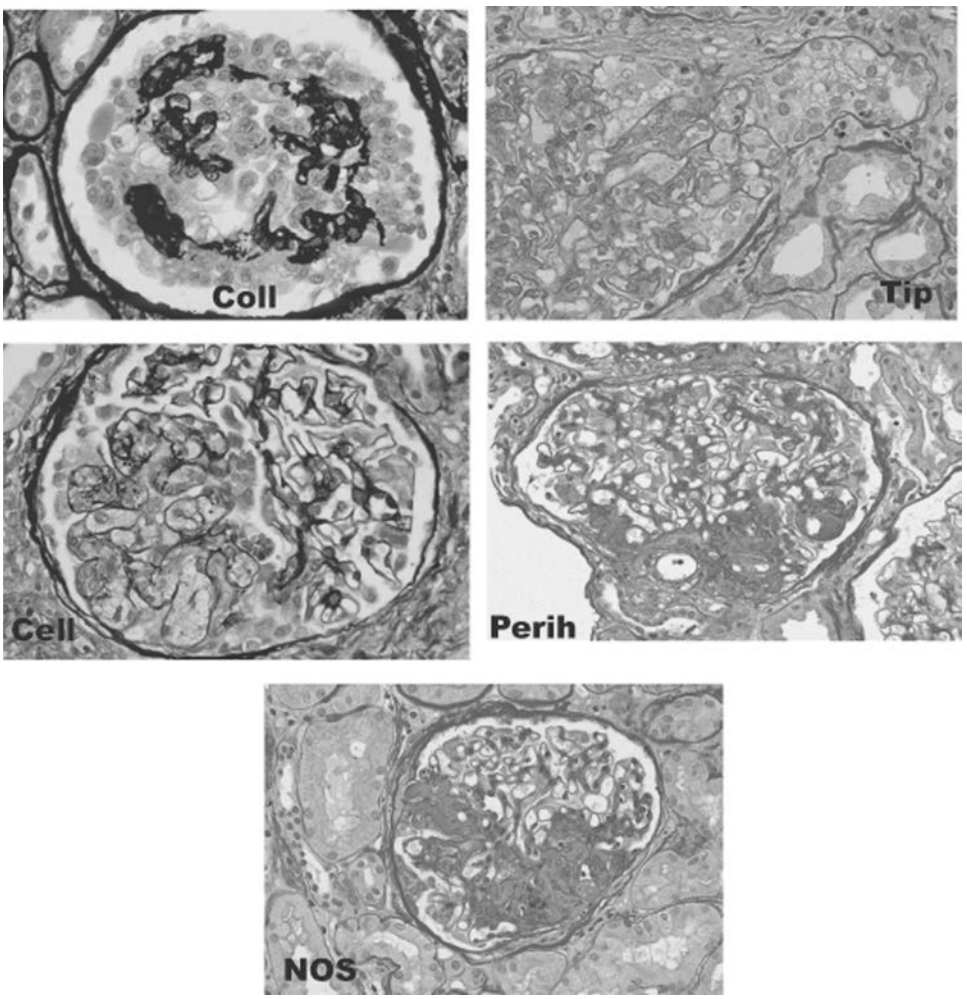

FIGURE 64-12. Variants of FSGS: the Columbia classification. This classification was developed for diagnostic purposes and involves the stepwise consideration of collapsing variant (coll), tip lesion (tip), cellular variant (cell), perihilar variant (perih) and FSGS not otherwise specified (NOS). (Figure reproduced from: D'Agati V, Fogo AB, Bruijn JA, et al. Pathologic classification of focal segmental glomerulosclerosis: a working proposal, *Am J Kidney Dis* 2004;368:43, with permission.)

subsequent biopsies (discussed in subsequent text). Future studies involving serial renal biopsies of FSGS patients would be an essential starting point. Careful consideration must be given as to whether these events represent disease evolution (category change) or disease progression (progressive scarring), and what the implication for the classification system might be.

Fourth, inter-rater reliability needs to be rigorously evaluated by practicing pathologists to ensure that the existing diagnostic framework yields reproducible results. Finally, the ability of diagnostic categories to make predictions as to etiology and prognosis, based solely on morphology and independent of clinical history, must be tested both retrospectively and prospectively on patient cohorts drawn from diverse ethnic and racial populations. Despite these considerations, the Columbia classification remains an excellent starting point, providing a common set of diagnostic criteria with which the field can advance.

Postadaptive FSGS

We propose the term *postadaptive FSGS* to include those forms of FSGS that are believed to arise as a consequence of structural adaptation (glomerular hypertrophy) and functional adaptation (glomerular hyperperfusion and hyperfiltration) to either reduced nephron mass or particular disease states (obesity, sickle cell anemia, cyanotic congenital heart disease, and others). The term *secondary FSGS* has also been used to describe this form of FSGS, although the term sometimes has been extended to HIV-associated nephropathy, to drug-associated FSGS, and to segmental scarring arising in proliferative glomerulonephritis; these varied uses have diminished the utility of the term.

The diagnosis of postadaptive FSGS remains a challenge. Ideally, the diagnosis would be made on pathologic grounds alone. The problem with relying upon clinical factors in reaching a diagnosis is that patients with obesity, hypertension, and other disorders might present with either idiopathic FSGS or postadaptive FSGS. As these entities may have different responses to therapy, particularly immunosuppressive therapy, the distinction becomes important. Three approaches are available at present.

First, postadaptive FSGS may manifest less foot-process effacement than idiopathic FSGS. Chiang et al. studied renal biopsies obtained from 30 children and found that the extent of foot process effacement was similar in MCN (63% ± 21%), FSGS (70% ± 25%), and MCN that subsequently evolved into FSGS (56% ± 23%) (763). The large standard deviation suggests that many patients in all three disease categories had <50% effacement. Furthermore, the extent of foot-process effacement did not correlate with proteinuria, although only 15 patients were included in the analysis. D'Agati and colleagues defined obesity-associated glomerulopathy as glomerulomegaly in the setting of BMI >30 kg/m^2, with or without FSGS (764). They found that obesity-associated glomerulomegaly cases had a mean foot-process effacement 40% (range 10% to 100%), whereas idiopathic FSGS patients had mean

foot-process effacement 75%, range 30% to 100%). Importantly, although there were significant differences in the group means for the extent of podocyte foot-process effacement in idiopathic FSGS versus postadaptive FSGS, there was much overlap, so the utility of particular diagnostic criteria (such as podocyte foot process effacement >50% in the former and <50% in the latter) is limited (765).

Second, the initial lesion in postadaptive FSGS is preferentially localized to the hilum. Rat models of FSGS that are characterized by increased transcapillary hydraulic pressure are associated with predominant or exclusive perihilar sclerosis. This is probably explained by the observation that the first capillary branches of the afferent arteriole are the largest, and thus by Laplace's law these branches would have the highest wall tension and be the most susceptible to podocyte injury (766,767). The problems in clinical use are that correctly identifying the location of the sclerosis, even with multiple sections, and the number of glomeruli available for study is limited. (It remains to be determined whether the perihilar variant of idiopathic FSGS also arises as a consequence of glomerular overload from an unrecognized risk factor or biologic process. In this context, it would be interesting to see a detailed analysis of the clinical and physiological characteristics of these patients.)

Third, glomerulomegaly can be demonstrated by direct measurement of glomerular size. D'Agati has proposed defining glomerulomegaly as 1.5-fold increase in glomerular diameter over control (comparable to a 2.5-fold increase in glomerular area and 3.4-fold increase in glomerular volume, assuming that the glomerulus is a sphere) (765). D'Agati and colleagues have also defined glomerulomegaly as average glomerular diameter >180 μm, based on study of four biopsy levels and the measurement limited to glomeruli in which hilus was identified (764). In normal controls, glomerular diameter averaged 168 μm (range 138 to 186 um), and in obese subjects with glomerulomegaly, glomerular diameter averaged 226 μm (range 172 to 300 μm).

Therefore, presently available methods to identify postadaptive FSGS include determining the fraction of glomeruli with perihilar sclerosis and measuring glomerular size. Needed are prospective studies evaluating each of these approaches, and defining the receiver-operating characteristic (ROC) curves for the diagnostic thresholds. Even with these data, a major limitation remains the requirement for sufficient numbers of intact glomeruli. An important research goal will be to identify molecular markers that reflect the glomerular adaptation; these might include proteins that are upregulated by podocytes or mesangial cells in response to mechanical stress.

Diffuse Mesangial Sclerosis and Related Disorders

Diffuse mesangial sclerosis represents a small fraction of pediatric renal biopsies (0.9% in India [768]) and all-age renal biopsies (0.45% in the United States [753]). In the pediatric population, many cases are associated with *WT1* and *LAMB2* mutations, as discussed elsewhere in this chapter. Some cases seen in children and adults are idiopathic. Unidentified genetic mutations may contribute. Two adult cases have been reported in association with multiple myeloma (769). One note of diagnostic caution is that it may be difficult to exclude advanced FSGS in renal biopsies lacking glomeruli with segmental sclerosis.

Disease Mechanisms of FSGS and Collapsing Glomerulopathy

Animal Models

A number of animal models have been used to delineate the mechanisms of FSGS and collapsing glomerulopathy, as has been reviewed recently (770). These models have been crucial for elucidating mechanisms, particularly for postadaptive FSGS, HIV-associated collapsing glomerulopathy and, most recently, for genetic causes of FSGS. In particular, they have led the field to highlight the central role of podocytes in FSGS and collapsing glomerulopathy. A significant gap is the absence of generally accepted animal models for MCN, idiopathic classic FSGS, or recurrent FSGS after renal transplantation.

Available animal models include experimentally induced disease (Table 64-12) (771–783), spontaneous genetic models

TABLE 64-12

ANIMAL MODELS OF FOCAL SEGMENTAL GLOMERULOSCLEROSIS (FSGS): INDUCED

Model	Species	Comment	Reference
Remnant nephron, excision	Rat	Hyperfiltration	(771)
Remnant nephron, infarction	Rat	Hyperfiltration	(773)
Remnant nephron, ligation and cautery	Mouse	Hyperfiltration	(774)
Aging plus uninephrectomy	Rat		(775)
Aging plus uninephrectomy	Mouse		(776)
Doxorubicin (Adriamycin)	Rat		(777)
Doxorubicin (Adriamycin)	Mouse	Less consistent than rat	(778)
Bromethylamine-induced papillary necrosis	Rat	Probable hyperfiltration associated with nephron loss	(779)
Cyclosporine (chronic)	Rat	Probable hyperfiltration associated with nephron loss	(780)
FGF-2 (chronic)	Rat		(781)
Puromycin aminonucleoside, single dose	Rat		(782)
Puromycin aminonucleoside, multiple dose	Rat	Glomerular hypertrophy	(783)

TABLE 64-13

ANIMAL MODELS OF FOCAL SEGMENTED GLOMERULOSCLEROSIS (FSGS): SPONTANEOUS GENETIC MODELS

Model	Comment	Reference
Spontaneously hypertensive rat (SHR)	Hypertensive	(824)
Dahl salt-sensitive rat	Hypertensive	(784)
Fawn-hooded hypertensive rat	Hypertensive, perihilar scar	(766)
Sabra hypertensive rat	Hypertensive	(785)
Munich-Wistar-Frömter rat	Normotensive	(786)
Spontaneously hypercholesterolemic rat (SHC)	Hyperlipidemic	(787)
Imai rat	Hyperlipidemic	(788)
Obese, fa/fa Zucker rat	Hyperlipidemic, nonhypertensive	(789)
Buffalo MNA rat	Normotensive, recurs after renal transplant	(790,791)
FSGS/Kist mouse or Nga mouse		(792)
APA strain, Syrian hamsters		(793)

(Table 64-13) (766,784–793), null mutation animals (Table 64-14) (794–804), and transgenic animals (Table 64-15) (798,805–823). Each model has particular strengths and limitations and reflects a certain portion of the spectrum of human podocyte disease. The interested reader is referred to the provided references; here we will consider the pathogenesis of FSGS and collapsing glomerulopathy by considering the structural and functional processes involved, rather than a detailed review of particular molecular pathways.

A critical aspect of these models is disordered podocyte function. Direct injury to the podocyte underlies many of the animal models of glomerulosclerosis, including toxic injury (adriamycin, PAN), proliferation (FGF-2 administration), viral gene expression (HIV-1 accessory genes, SV40 T antigen) and gene (*NPHS2*, *CD2AP*, *ACTN4*) deletion or modification. Podocyte stress likely contributes in models of postadaptive FSGS associated with nephron loss (remnant nephron, aging plus uninephrectomy, bromo-ethylamine-induced papillary necrosis). The genetic rat models demonstrate the importance of systemic and glomerular hypertension and hyperlipidemia, although the restriction of these traits to particular strains suggests that particular genetic loci are required for the full interaction of the hypertension and lipids with renal injury.

Glomerular Adaptation: Glomerular Overload and Glomerulomegaly

Reduction in nephron mass is a well-established model of postadaptive FSGS in experimental animal models (Table 64-12). In 1952, Platt and colleagues noted in rats subjected to 5/6 nephrectomy, creatinine clearance fell to a lesser degree than the reduction in renal mass would predict (825). Subsequently, Morrison and Howard reported similar findings using inulin clearance and went on to suggest that hyperfiltration might play a role in glomerulomegaly, podocyte hypertrophy, and the subsequent appearance of glomerulosclerosis (771,826). In 1981, in a highly influential study, Hostetter

TABLE 64-14

ANIMAL MODELS OF FOCAL SEGMENTAL GLOMERULOSCLEROSIS (FSGS): NULL MUTATIONS AND KNOCK-IN MUTATIONS

Gene class	Gene	Histology	Reference
Podocyte slit diaphragm and cytoskeletal genes	*CD2AP*	Diffuse global glomerulosclerosis (homozygotes) FGGS (heterozygotes)	(794,795)
	NPHS2	Mesangial sclerosis	(796)
	ACTN4	Podocyte injury, foot process effacement	(797,798)
	WT1 mutant knock-in	Mesangial sclerosis	(799)
	WT1 mutant knock-in (R394W)	Mesangial sclerosis	(800)
Other	MPV17	Mesangiolysis, focal global glomerulosclerosis	(801)
	BCL2	Oligomeganephronia	(802)
	LCAT	Mesangial proliferation and sclerosis	(803)
	Rrm2b	Collapsing glomerulopathy	(804)

TABLE 64-15

ANIMAL MODELS OF FOCAL SEGMENTAL GLOMERULOSCLEROSIS (FSGS): TRANSGENIC MICE AND RATS

Protein class	Species	Promoter	Transgene	Histology	Reference
Podocyte protein	Mouse	Nephrin	Mutant *ACTN4*	FSGS; proteinuria	(798,805)
Ligands and receptors	Rat	Renin-2	Murine renin-2		(806,807)
	Rat	Renin and angiotensin	Human renin and angiotensin	Nephrosclerosis	(808)
	Mouse	Albumin	*TGFB1*	Diffuse global glomerulosclerosis	(809)
	Mouse	PEPCK	*TGFB1*	GS	(810)
	Mouse	Metallothionein-1	Hepatocyte growth factor	FSGS	(811)
	Mouse	Endothelin-1	Endothelin-1	GS	(812)
	Mouse	Metallothionein-1	Growth hormone	GS	(813)
	Mouse	Metallothionein-1	Growth hormone releasing factor	GS	(813)
	Mouse	CMV/actin/globin	Macrophage migration inhibition factor	Mesangial expansion	(814)
	Rat	Nephrin	Angiotensin receptor type 1	FSGS	(815)
Other proteins	Mouse	MMTV	p53	FSGS	(816)
	Mouse	CMV	Cux-1	Mesangial expansion	(817)
	Mouse	Hemoglobin	Hemoglobin SAD (triple mutant)	FSGS	(818)
	Mouse	Thy1.1	Thy1.1	FSGS	(819)
Viral proteins	Mouse	SV40 early region	SV40 T Ag	FSGS	(820)
	Mouse	HIV-1 LTR	HIV-1 *env* + 6 regulatory genes	Collapsing glomerulopathy	(821)
	Rat	HIV-1 LTR	HIV-1 *env* + 6 regulatory genes	Collapsing glomerulopathy	(822)
	Mouse	HIV-1 LTR	HIV-1 *tat/vpr*	Collapsing glomerulopathy	(823)

FSGS, focal segmental glomerulosclerosis; *DMS*, diffuse mesangial sclerosis; *GS*, glomerulosclerosis is not otherwise described; *CMV*, cytomegalovirus; *LTR*, long terminal repeat; *MMTV*, mouse mammary tumor virus; *PEPCK*, phosphoenolpyruvate carboxylase.
Histology is as described in the original or subsequent publications. Models of diabetic glomerulosclerosis have been excluded.

et al. demonstrated that the remnant nephron model is characterized by increased single nephron SNGFR, due to increased transcapillary hydraulic pressure (772). The glomerular overload (or glomerular hyperfiltration) hypothesis states that some feature of adaptation to reduced renal mass, including possibly hyperperfusion, hyperfiltration, glomerulomegaly, and/or podocyte mechanical stretch, underlies some forms of FSGS and accounts for the progressive nature of chronic kidney disease in general.

In human subjects, removal of one kidney (e.g., with donor nephrectomy) is not associated with increased risk of renal disease after long-term follow-up. There is a poorly defined boundary of minimal renal mass, below which patients are at risk for progressive renal disease. It is quite possible that the boundary differs among patients, based on various factors including nephron endowment. Novick et al. studied 14 patients who had a solitary kidney and then underwent partial nephrectomy for cancer, with 25% to 75% of the solitary kidney having been removed (827). Five patients subsequently developed proteinuria, including three with biopsy-proven FSGS (828).

The Barker hypothesis proposes that prenatal programming influences fetal development in ways that influence adult susceptibility to diseases, including hypertension, coronary heart disease, and type 2 diabetes (829). The Brenner corollary of this hypothesis proposes that a reduced number of nephrons at birth predisposes to hypertension and progressive renal disease during adult life (830). Low birth weight may be a predictor of low nephron number, although studies in various human populations have come to discrepant findings (831,832). Studies support a relationship between low birth weight and essential hypertension (833), and between low birth weight and end-stage renal disease (odds ratio 1.4 for birth weight <2.5 kg compared to birth weight 3 to 3.5 kg) (834), but no data have been published that relate to birth weight to FSGS risk.

Glomerulomegaly. Human glomeruli increase in size during childhood, with mean diameters increasing from 112 μm at birth to 167 μm at age 15, as assessed in an autopsy study where maximal glomerular diameter was measured (835). In normal kidney donors ages 24 to 53, mean glomerular area was not correlated with age or sex (836). When assessed by microdissection, mean glomerular diameter remains stable until about age 40 to 50, after which there is a modest decline (837). The mechanism of age-associated decline in glomerular diameter is unknown, but could be due to an increasing fraction of obsolescent glomeruli. By contrast, children with FSGS have increased glomerular size and there is no correlation with age (838).

Glomerulomegaly is a hallmark of adaptation to reduced nephron mass. Glomerular enlargement may arise as a consequence of widening of the glomerular capillaries, increasing glomerular capillary length, or some combination of both. Experimental rat models suggest that both mechanisms occur with adaptation to reduced renal mass, at different ages. In *young rats* there are large increases in capillary length and only small increases in capillary diameter (839,840). In *older rats*, the major change is increased capillary diameter (841,842). In *children* undergoing uninephrectomy for postadaptive FSGS in the setting of reflux nephropathy, the nephrectomized kidney shows glomerulomegaly without increased capillary diameter (843). In *adults* with FSGS, glomerulomegaly is associated primarily with an increase in capillary diameter (844). Kidneys showing greater numbers of sclerotic glomeruli (more advanced disease), however, showed greater capillary lengthening, whereas there was no relationship between extent of sclerosis and capillary diameter. In conclusion, (a) the results in rats and humans show similar age-dependent patterns, with capillary lengthening predominantly in the young individual and capillary lumen increase predominating in the mature individual and (b) the results in adults with FSGS suggests that the increase in capillary lumen diameter may occur early (as a consequence of unknown mechanisms) and capillary lengthening may later occur in response to reduced functional nephron mass.

Glomerulomegaly is also present in experimental and clinical settings characterized by glomerular overload (defined as an increase in glomerular blood flow or glomerular filtration or both). These settings include obesity, sickle cell anemia, and cyanotic congenital heart disease. Thus in obese patients with glomerular disease and proteinuria, mean glomerular diameter has been reported as 226 μm (range 172 to 300 μm), compared to a mean of 168 μm (range 138 to 186 μm) in age-matched normal subjects (764), and 256 μm (range 192 to 280 μm) (845).

Glomerular Overload: Hyperperfusion and Hyperfiltration. Glomerular hyperperfusion and hyperfiltration are present at the *single nephron level* in animal models of reduced nephron mass. Under normal circumstances in the rat, the pressure gradient across the glomerular capillary wall (P_{GC}) is approximately 50 mm Hg and SNGFR is approximately 30 nl/min; in the remnant nephron model P_{GC} exceeds 60 mm Hg and SNGFR exceeds 60 nl/min (772). Furthermore, glomerular hyperfiltration is present at the *whole kidney level* early in the disease course in experimental models of postadaptive FSGS and most, if not all, human forms of postadaptive FSGS.

Conversely, there are several experimental settings where glomerular hyperperfusion, glomerular hyperfiltration, glomerulomegaly, and FSGS are less closely correlated. Yoshida et al. compared two rat models, both with 1/3 left nephrectomy: in one group the right kidney was removed and in the other group the right ureter was diverted into the peritoneum (846). SNGFR and P_{GC} increased to an equivalent degree in both models, but glomerulomegaly and glomerulosclerosis were blunted (although present) in the urinary diversion group. Thus, remnant nephrons in the left kidney acted differently in the presence of a filtering but nonexcreting right kidney. It remains unclear by what mechanism the remnant nephrons apparently sense total body functioning nephron mass; perhaps a circulating molecular product of glomerular cells contributes. In any case, although this experiment suggests caution in assuming that hyperfiltration will necessarily lead to glomerulomegaly and FSGS, the urinary diversion model has uncertain relevance to clinical situations involving glomerular adaptation.

Furthermore, glomerular hyperperfusion and hyperfiltration are clearly not essential for the development of all forms of FSGS. In rats with PAN nephrosis and rats with adriamycin nephrosis, average P_{GC} values remain normal despite the subsequent appearance of sclerosis (847). In the former model, increased single nephron GFR was uniformly absent and in the latter model, although some glomeruli exhibit increased GFR, these glomeruli did not have elevated P_{GC} and did not subsequently develop sclerosis, at least within the time-frame of the study. Consequently, hyperperfusion and hyperfiltration appear to characterize most if not all postadaptive FSGS models, but are absent from FSGS models that involve direct podocyte injury (presumably models of idiopathic FSGS).

In obese human subjects, absolute GFR (without correction for body size) has been reported to be increased by 25% to 60% (848–850), and this is partially returned to normal values by weight loss (850). Obese subjects have both increased GFR and increased filtration fraction, whereas even in nonobese subjects there is a positive correlation between BMI and filtration fraction (but not GFR) (851). Others have not found an increase in absolute GFR, despite similar levels of BMI, although the reasons for the discrepant findings are not apparent (852). The increased GFR is more striking in central compared to peripheral

obesity (853). The mechanisms of obesity-related glomerular hyperfiltration are unknown but may include protein intake, various features of the metabolic syndrome (discussed in subsequent text) and possibly particular adipokines.

Most but not all studies suggest that early in the course of children with sickle cell anemia, GFR and renal plasma flow are increased (854–856). Limited data available in children with cyanotic congenital heart disease suggest that glomerular filtration may be slightly impaired (857) or normal (858). In the latter study, GFR was normal and renal plasma flow was decreased, indicating an increased filtration fraction. The authors proposed that increased blood viscosity associated with polycythemia increases renal vascular resistance and intraglomerular blood pressure.

There is at least one clinical situation in which the link between glomerulomegaly and glomerular overload is absent. Fogo et al. studied 42 pediatric MCN patients, of whom 10 subsequently experienced renal functional decline and a repeat renal biopsy showed FSGS (859). In those patients who later developed FSGS, glomerular area was on average 76% larger (which would correspond to a 1.3-fold increased diameter, assuming the glomerulus was spherical). Although the data on creatinine clearances were not provided, there is no reason to suspect hyperfiltration, and these patients had no obvious clinical features that would suggest reduced renal mass. It remains uncertain what processes might account for glomerulomegaly in this setting.

Podocyte Depletion Hypothesis

The podocyte depletion hypothesis proposes that an absolute or relative reduction in podocyte number is a critical process in FSGS of all types. Furthermore, as podocytes undergo dedifferentiation and loss of matrix adhesiveness in collapsing glomerulopathy, a loss of functional podocytes may contribute. Finally, it now appears that podocyte depletion also is present in other glomerular diseases, including diabetic nephropathy (860) and primary glomerulonephritis (861), and may promote sclerosis in these settings.

Podocytes are postmitotic cells in normal human kidney. In a study of 164 kidneys, podocyte mitoses were found in one kidney (the diagnosis was given as FSGS) and binucleate cells were found in four kidneys (FSGS, lupus nephritis, and IgA nephropathy) (862). In a seminal study, Fries et al. noted that in rats subjected to 3/4 nephrectomy plus adriamycin, the remnant nephrons undergo compensatory hypertrophy, more than doubling tuft volume (863). As part of this process, endothelial cells and mesangial cells proliferate but podocytes do not. As podocyte volume density declines, each podocyte must cover a larger capillary surface area. Areas of podocyte detachment were present, particularly in areas of segmental sclerosis. Extending these studies to human renal biopsies, Bhathena found that the unit distance of GBM covered by podocytes increased in oligomeganephronia, unilateral renal agenesis with FSGS (but not unilateral renal agenesis without FSGS), uninephrectomy with FSGS (but not uninephrectomy without FSGS), and renal transplantation with late (nonrecurrent) FSGS (but not without FSGS) (864). Thus, podocyte hypertrophy characterizes human forms of postadaptive FSGS. Podocyte depletion is also characteristic of idiopathic FSGS. Reduced podocyte numbers are present in children with FSGS compared to those with MCN (838).

Evolution of Segmental Sclerosis: The Misdirected Filtration Hypothesis

Kriz and Lemley, and Kriz et al. have proposed an intriguing model of misdirected glomerular filtration to explain the propensity for segmental sclerotic scars of FSGS to expand, to progress to global sclerosis, and to be associated with tubular damage and interstitial inflammation (767,865–867). This model was developed from intensive study of rat models of FSGS, including the hypertensive Fawn hooded rat and the normotensive Milan rat, with more limited studies of human FSGS.

The process begins when one or more podocytes undergo apoptosis or necrosis, or loses adhesion and is released into Bowman's space. Naked GBM tends to lie in apposition to the parietal cells lining Bowman's capsule, perhaps due to the ballooning of the glomerular capillary in response to the loss of mechanical restraint consequent to podocyte loss or perhaps due to a response on the part of the parietal cells to contact the GBM. This manifests as tuft adhesion (synechia). Tuft adhesion involves a loosening of contacts between parietal cells, allowing glomerular ultrafiltrate to penetrate between parietal cells. A loop of glomerular capillary also penetrates between parietal cells, leading to an expanding paraglomerular space located beneath the parietal cells or extending between layers of Bowman's capsule. These capillary loops remain perfused, at least for a time, and this allows continued delivery of glomerular filtrate into the paraglomerular space. This filtrate, having passed through an abnormal capillary wall lacking podocytes, is likely enriched in growth factors, chemokines, and other inflammatory mediators. These mediators promote the recruitment and activation of fibroblasts and leukocytes, both within the paraglomerular space and in the surrounding interstitium.

Once the paraglomerular space has formed, there are several possible outcomes. First, the process may stabilize and a segmental scar may remain. Second, the enlarging paraglomerular space may engulf additional capillary loops, thereby compromising podocyte integrity in those loops and eventually leading to global sclerosis. Third, and overlapping with the preceding outcomes, the paraglomerular space may expand and encircle the proximal tubule. This has the potential of separating the tubule from peritubular capillary, leading to tubular atrophy and the development of an atubular glomerulus. This model has considerable power to explain the pathology of FSGS. It remains to be determined which forms of human idiopathic FSGS, postadaptive FSGS, genetic FSGS, and medication-associated FSGS follow this model.

Cellular Injury and Response

A consensus has emerged that podocyte injury plays a central role in the pathogenesis of FSGS. This notion is supported by animal models and genetic mutations in human FSGS (all mutations identified to date are in genes that are exclusively expressed in the podocyte or that play critical roles in the podocyte). Pavenstadt et al. (868) provide a superb comprehensive review of podocyte cell biology.

In vivo, podocyte abnormalities are most prominent in cellular FSGS and collapsing glomerulopathy, where podocyte dedifferentiation and proliferation are characteristic, but podocyte abnormalities are seen in FSGS as well (428,869–883) (Table 64-16).

Podocyte proliferation and associated dedifferentiation are likely the critical biologic process in the pathogenesis of collapsing glomerulopathy. This striking distinction between podocyte depletion without proliferation in FSGS and podocyte hyperplasia in collapsing glomerulopathy provides the rationale for separating these diagnostic categories.

Contribution of Other Glomerular and Nonresident Cells. Mesangial cell injury and response is an important component of progressive glomerular injury in FSGS. There is extensive literature on the role of mesangial cells in elaborating extracellular matrix. It remains unclear by what pathways

TABLE 64-16

EXPRESSION OF PODOCYTE DIFFERENTIATION, INJURY, AND PROLIFERATION MARKERS IN HUMAN DISEASE

	Normal	MCN	Idiopathic FSGS	Collapsing glomerulopathy and/or cellular FSGS	Reference
Nephrin	Present	Unchanged or irregular (LM) ↓ per μm (EM)[a]	Unchanged; lost in sclerotic areas	ND	(869,870)
Podocin	Present	Unchanged or ↓	↓ In all glomeruli, absent from sclerotic areas	ND	(871,872)
Synaptopodin	Present	↓	↓ Or absent from all glomeruli	Absent in abnormal podocytes; ↓ in some podocytes in unaffected glomeruli	(872–877)
Podocalyxin	Present	ND	Absent	Absent in abnormal podocytes	(873)
GLEPP 1	Present	ND		Absent in abnormal podocytes	(873)
$\alpha\beta$ Dystroglycan	Present	Reduced	Normal	ND	(428)
α5 Integrin	Present	ND	↓, Lost with progressive sclerosis	ND	(878)
WT-1	Present	Unchanged	Absent in sclerotic areas	Absent in abnormal podocytes, ↓ in some podocytes in unaffected glomeruli	(873,877)
Pax-2	Absent	Absent	Absent	Present in abnormal podocytes	(877)
Vimentin	Present	Present	Variably ↓ in sclerotic areas	↓	(877)
Cytokeratin	Absent	ND	ND	Present in abnormal podocytes	(874,879)
Ki67	Absent	Absent	ND	Present in abnormal podocytes	(873)
Cyclin D1	Present	ND	ND	↓ in abnormal podocytes, ↓ in some unaffected glomeruli	(875,880)
Cyclin E	Present	ND	ND	↑ in abnormal podocytes	(880)
p21	Absent	Unchanged	ND	↑ In abnormal podocytes	(882)
$p27^{kip1}$, $p57^{kip1}$	Present	Unchanged	ND	↓ In normal and abnormal glomeruli, absent in proliferating podocytes	(875,879,882)
$p57^{kip2}$	Present	Unchanged	ND	↓ in normal podocytes, absent in proliferating podocytes, ↓ in occasional podocytes, normal glomeruli	(879,882,883)

Podocyte proteins were localized by immunostaining or by ultrastructural labeling in human tissue. Diagnoses are shown as identified in the references; in some references the type of FSGS was not specified. For simplicity, details of altered subcellular distribution of proteins have been omitted. In some cases, discrepant findings were reported. *ND*, not determined; *LM*, light microscopy; *EM*, electron microscopy.
[a]Absent in congenital nephrotic syndrome due to nephrin mutations.

podocyte injury stimulates mesangial cell activation. One possibility, relevant to postadaptive FSGS, is that glomerulomegaly may lead to mechanical stretch being imposed on mesangial cells (reviewed in [884]). Cultured mesangial cells respond to stretch with an increase in expression of collagens, fibronectin, laminin, transforming growth factor (TGF)-β1, connective tissue growth factor (CTGF), macrophage chemotactic protein (MCP)-1, and intercellular adhesion molecule (ICAM)-1, the latter two capable of promoting leukocyte immigration and attachment (885–890). The role of mesangial cell proliferation in progressive scarring is less certain; it could simply be an index of mesangial cell activation. The mechanisms stimulating mesangial proliferation are discussed in the section on the histopathology of MCN.

Endothelial cell injury and response are also important components of progressive glomerular injury. In the remnant nephron model, the glomerular capillary endothelial cell manifests the first structural changes (760) and the first upregulation in mRNAs for extracellular matrix genes (fibronectin, laminin-β1) and TGF-β1, occurring 24 days after ablation (891). In this model, there is an early and rapid increase in endothelial cell number and total capillary surface per glomerulus in parallel with glomerular hypertrophy; enalapril therapy partially reverses these established changes (892).

Lymphocytes, particularly CD8+ T cells, and monocyte/macrophages may be present in human glomeruli affected by FSGS (893,894). The latter may be recognized within capillary loops as lipid-laden foam cells. In the rat remnant nephron model, bone marrow suppression via X-irradiation is associated with a transient reduction in glomerular macrophage numbers and mesangial matrix scores, suggesting that macrophages might contribute to glomerular matrix expansion (895). Conversely, blockade of chemokine (C-C motif) receptor (CCR)-1 (the major receptor for macrophage inflammatory protein [MIP]-1 alpha and Regulated on Activation, Normal T cell Expressed and Secreted [RANTES]) in murine adriamycin nephropathy reduced interstitial fibrosis but had no effect on proteinuria or glomerulosclerosis (896). Therefore, the pathophysiologic role of infiltrating glomerular leukocytes in FSGS remains to be established. The potential role of infiltrating tubulointerstitial leukocytes in the progression of chronic renal disease is considered elsewhere in this text.

Cytokines and Other Mediators

Although many mediators have been implicated in glomerulosclerosis and progressive kidney disease, there is a paucity of data directly defining a role for these mediators on FSGS. Experimental data indicate that these mediators could play a role in regulating the balance between *extracellular matrix (ECM) synthesis and degradation* in the glomerulus. The most prominent of these mediators is TGF-β (897), which both directly (898) and indirectly, through induction of the cytokine CTGF (899) and generation of reactive oxygen species (900), stimulates mesangial and additional cell types to produce collagens, laminin and other ECM proteins. There are two parallel systems regulating ECM degradation (901). The balance between the matrix metalloproteinases and the tissue inhibitors of matalloproteinases (TIMPs), has been implicated in animal models of progressive kidney disease (902,903). The plasmin system enhances glomerular ECM turnover (904), and is inhibited by the plasminogen activator inhibitor, PAI-1 (905). Biologic roles for these molecules in regulating cellular functions beyond matrix turnover also have been proposed.

Cytokine mediators play a role in *proliferative changes* in the glomerulus, although the data have been limited to effects on mesangial cells. Interestingly, despite the significance of podocyte proliferation in collapsing glomerulopathy, no data are available to convincingly link a particular mediator with this proliferation. Basic fibroblast growth factor (bFGF, FGF-2) has been linked with multiple podocyte abnormalities, including proliferation, in experimental membranous nephropathy (906). Another important aspect of glomerulosclerosis is the possible loss of a continuous podocyte support structure for the glomerular capillaries. Cytokines have equally important and complex roles in podocyte *apoptosis and phenotypic changes*, as shown for TGF-β1 (907) and endothelin-1 (ET-1) (908).

Other systems play a significant part in glomerulosclerosis. An important one is the multiple potential roles of the renin-angiotensin-aldosterone system (RAAS). Spironolactone decreases fibrosis in some animal models. Aldosterone stimulates expression of TGF-β and PAI-1, and fibrotic changes mediated through the generation of reactive oxygen species (909). A role for the RAAS is supported by the strong data demonstrating that angiotensin converting enzyme inhibition and angiotensin receptor blockade ameliorate progressive glomerulosclerosis and renal fibrosis in multiple diseases. These data support a role for the RAAS in altering glomerular cell phenotypes (e.g., angiotensin II stimulates mesangial cell TGF-β production [910]), as well as in hemodynamic mechanisms that relate to glomerular perfusion and filtration. Further, it is not clear whether the RAAS stimulates glomerular hypertrophy through its effects on hemodynamics or via direct, growth factorlike effects on resident cells. Other systems regulating hemodynamics that also may have direct effects upon cellular fibrogenic activity include those representing the balance between nitric oxide and ET-1 (911), or those involving arachidonic acid metabolites (912). Leptin also has multiple effects relevant to glomerulosclerosis (913).

Recent studies demonstrated an interaction between putatively hemodynamic and sclerogenic mediators (Fig. 64-13).

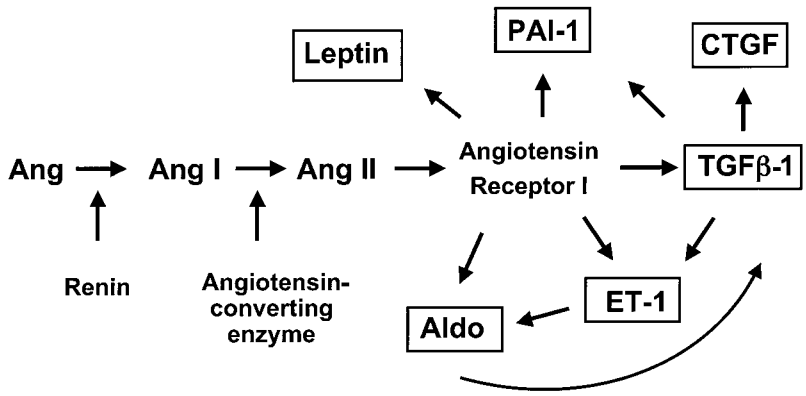

FIGURE 64-13. The renin-angiotensin system and interaction with profibrotic cytokines. In addition to the role of renin-angiotensin-aldosterone system in regulating extracellular fluid volume and potassium homeostasis, it has been become clear that this system also influences vascular remodeling and tissue fibrosis. These vascular and profibrotic effects are mediated by at least five effector molecules (*shown within boxes*), which are present in the plasma and also produced locally in tissues. *Aldo*, aldosterone; *Ang*, angiotensinogen; *ET-1*, endothelin-1; *TGF-β1*, transforming growth factor-β1; *CTGF*, connective tissue growth factor; *PAI-1*, plasminogen activator inhibitor-1.

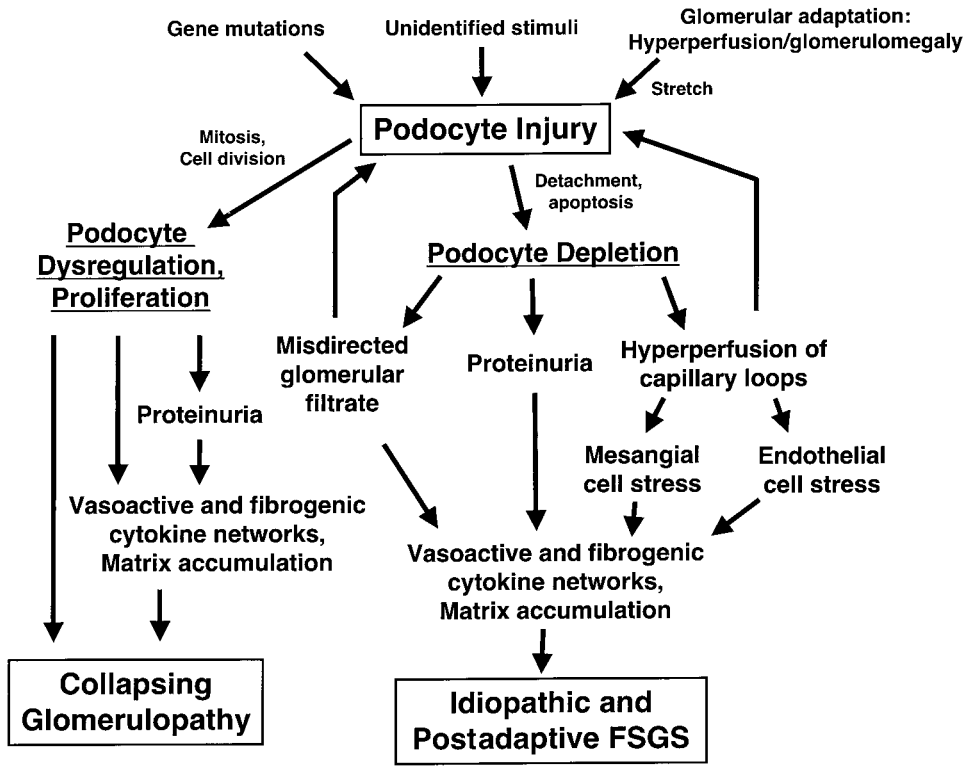

FIGURE 64-14. Proposed pathogenetic schema for FSGS and collapsing glomerulopathy. Both lesions begin with podocyte injury, resulting from a mutated gene, glomerular hyperperfusion (postadaptive) or an unidentified stimulus. Depending upon the nature of the injury, this may lead to podocyte dysregulation and proliferation, causing collapsing glomerulopathy; or relative podocyte depletion in the case of FSGS (note that podocyte depletion can be absolute, resulting from either decreased numbers of podocytes, or relative, when there are a fixed number of podocytes but a larger glomerular capillary surface area). In either FSGS or collapsing glomerulopathy, the result is a decrease in the integrity of the glomerular filtration barrier and proteinuria. Misdirected glomerular filtrate, exposure of the podocyte to excess protein, and cellular stretch/stress lead to glomerular cellular activation, changes in glomerular perfusion, and extracellular matrix accumulation. Thus, podocyte injury evolves into distinct glomerular diseases by various mechanisms, some of which are pathway-specific and others are common.

Angiotensin activation leads to the activation of the type I angiotensin receptor, which leads directly or indirectly to the production of aldosterone, ET-1, PAI-1, and TGF-β, with subsequent production of CTGF and reactive oxygen species, and extracellular matrix accumulation. This one system can thus contribute to glomerular hypertension/hyperfiltration, sodium retention, glomerular hypertrophy, podocyte dysfunction/apoptosis, and fibrosis—all of the manifestations of FSGS.

Based on the data presented in this section, a pathogenetic schema for FSGS and collapsing glomerulopathy can be proposed (Fig. 64-14). Both lesions begin with podocyte injury, resulting from a mutated gene, adaptive glomerular hyperperfusion, or an unidentified stimulus. Depending upon the nature of the injury, this may lead to podocyte dysregulation and proliferation, causing collapsing glomerulopathy, or podocyte depletion in the case of FSGS. Podocyte depletion may be absolute or relative, in the latter case when glomerulomegaly increases capillary surface area. It is noteworthy that, despite significant differences in disease etiology and course for FSGS and collapsing glomerulopathy, both manifest decreased integrity of the glomerular filtration barrier and proteinuria. Misdirected glomerular filtrate, exposure of the podocyte to excess protein, and cellular stretch/stress lead to glomerular cellular activation, changes in perfusion, and extracellular matrix accumulation. Therefore, distinct pathways may utilize similar mechanisms that result in distinct diseases, with the critical difference being the presence or absence of podocyte proliferation.

Selected Clinical Variants of FSGS and Collapsing Glomerulopathy

Glomerular Tip Lesion: A Diagnostic Cluster

The glomerular tip lesion has been a controversial subject since its description by Howie in 1986 (914). In the intervening years, as Howie has recently pointed out, the term *glomerular tip lesion* has been used in three senses, which are given distinct names here (915,916). Estimates of the incidence of glomerular tip lesion vary enormously by definition and population: 13% of FSGS patients in Chicago (761) and 66% of patients with segmental lesions (cellular or sclerosis) in Britain (915).

First, the *glomerular tip lesion MCN variant* (term introduced here but with a definition that follows Howie's original description) is situated at the portion of the glomerular tuft located adjacent to the origin of the proximal convoluted tubule and consists of a localized collection of vacuolated podocytes and intracapillary foam cells. In some cases the podocytes rest in apposition to tubular epithelial cells. The lesion may consist partly or predominantly of sclerosis. Importantly, other

glomerular lesions must be absent, including mesangial hypercellularity and sclerosis located elsewhere in the glomerulus. IgM and C3 may be present within the tip lesion. Both affected and unaffected glomeruli typically show diffuse foot-process effacement.

Second, a *glomerular tip lesion FSGS variant* has been recognized and made part of the Columbia classification. There are several differences from the preceding entity: mesangial hypercellularity may be present and peripheral scars (non-tip, non-hilar) can be present in other glomeruli. D'Agati and colleagues identified 49 cases (0.46% of their biopsy archive). There were 45 adults and 2 children. Cellular lesions (81% average per biopsy) were more typical than scarring lesions (19%). Twelve cases had glomerular tip lesion only, 18 had peripheral lesions, and 17 had associated indeterminate lesions (i.e., in some glomeruli the tip and the hilum could not be identified.) Focal mild mesangial hypercellularity was present in 45%; no cases exhibited diffuse mesangial hyperplasia. At presentation, the clinical features of glomerular tip lesion were more like those of MCN than other idiopathic FSGS: Caucasian race 77% and 59% versus 52% (p = NS), mean age 48 and 48 versus 33 ($p < 0.001$ overall) and nephrotic syndrome in 89% and 97% versus 54% ($p < 0.001$ overall). Among those with the glomerular tip lesion, 59% of patients entered CR with steroids, in some cases supplemented with other therapies, and 14% of patients entered PR. Therefore, the response to therapy is much better than classic FSGS and worse than MCN. Patients with glomerular tip lesion alone versus those with peripheral and/or indeterminate lesions had similar likelihood of CR versus PR versus nonresponse ($p = 0.88$), but this may be due to small numbers in this two-by-three group analysis. Importantly, those with other glomerular lesions had higher initial serum creatinine and higher likelihood of nonresponse ($p < 0.02$). The authors conclude that the glomerular tip lesion occupies an intermediate location along the MCN/FSGS spectrum and that the presence of sclerosis in a non-tip location confers a worse prognosis. These data can be interpreted to argue for distinguishing between a glomerular tip lesion MCN variant (lacking non-tip sclerosis) and a glomerular tip lesion FSGS variant (allowing peripheral and indeterminant scars but not perihilar scars), as these forms appear to have distinct outcomes.

Another study of the outcome of glomerular tip lesion published by Howie and colleagues came to generally similar conclusions (915). Two biopsy series of adult nephrotic syndrome comprised 108 patients, with biopsies showing segmental lesions and lacking a non-FSGS diagnosis (e.g., classic MCN, collapsing glomerulopathy, or other glomerular disease). Segmental cellular or scarring lesions at first or only biopsy were divided into two categories: tip lesions (confined to the tubular pole) and multiple lesions (at least one non-tip lesion, a lesion extending from the tubular pole to the hilum, and lesions at various sites). After 10 years, renal survival was 84% in those with tip lesions and 45% in those with multiple lesions ($p < 0.001$ by Cox proportional hazards analysis). The statistical significance of the beneficial effect of tip lesion was lost in multivariable analysis, when other variables (number of segmental lesions, extent of global sclerosis, and chronic tubular damage) were included in the model. No other factor showed an independent association with outcome; instead, all these variables showed correlation with each other. Therefore, the presence of a tip lesion conferred a favorable diagnosis when it was narrowly defined (no other segmental lesions). Forty patients underwent repeat biopsies, generally for declining renal function, but also in some cases when proteinuria recurred in an allograft. Tip lesions were frequently (perhaps always, given the uncertainty of localizing all lesions) the initial segmental lesions. With progressive loss of renal function, lesions at other glomerular locations appeared. These data are important, as they suggest that in some cases tip lesion can evolve into classic FSGS. Like the preceding paper, these data can be read as supporting a glomerular tip lesion MCN variant and glomerular tip FSGS variant, although it is unclear whether the two can be reliably distinguished at initial biopsy.

These findings support a third category, various nondiagnostic *glomerular tip changes* (Howie's suggested term), which have been described in other proteinuric conditions, including membranous nephropathy (present in 64% of cases) (917), IgM variant of MCN (918), postinfectious glomerulonephritis (919), and diabetic nephropathy (920). Experimentally, glomerular tip lesions develop in rats with crescentic glomerulonephritis; Howie showed that the initial change involves prolapse of injured podocytes into the tubule, followed by localization of macrophages to the adjacent capillary tuft and adhesion of the tuft to Bowman's capsule (921). The predisposition to injury of the podocytes at the glomerular tip remains unexplained. Haas has argued that the tip lesion is a response to prolonged heavy proteinuria and does represent a specific disease entity. In autopsy cases of severe, untreated MCN from patients who died before 1950, Haas and colleagues identified tip lesions in 5 of 8 cases (922). Among those with tip lesion, the average number of affected glomeruli was 1.8% (range 0.3% to 4.4%); there was no predilection for juxtamedullary glomeruli (unlike FSGS lesions). Thus there is a consensus that proteinuric states are commonly associated with tip changes.

In conclusion, a consensus probably exists that there is a glomerular tip lesion MCN variant and a glomerular tip lesion FSGS variant, with the former having a distinctly better prognosis but both exhibiting some degree of steroid-sensitivity. Some glomerular tip MCN variants progress over time to glomerular tip FSGS variants or classic FSGS. Important questions, however, remain to be resolved. Is it clinically useful to identify a glomerular tip lesion MCN variant rather than identify this morphology as a consequence of heavy proteinuria; in other words, is the prognosis affected, independent of the degree of proteinuria? This question remains open, as there is no published case series that compares the outcome of typical MCN patients with the glomerular tip lesion to MCN patients lacking this feature. Can we distinguish glomerular tip FSGS variants that will progress to classic FSGS? Finally, what molecular diagnostic markers might help us to differentiate between forms of tip lesion, to improve our understanding, classification, and prognostication of these disorders?

FSGS, Cellular Variant

As noted in the preceding text, the cellular variant of FSGS was first described by Schwartz and colleagues to include segmental or global endocapillary proliferation, hypercellularity in Bowman's space, or podocytes manifesting a reactive phenotype. Clinically, these patients were more likely than were those with only segmental scars (90% versus 49%) to have nephrotic range proteinuria. Compared to patients with classic FSGS, patients with cellular lesion FSGS were more likely to be black (70% versus 49%), to have a higher likelihood of proteinuria >10 g per/day (44% versus 11%), and to have a higher likelihood of progression to ESRD (40% versus 18%) (761,923). Steroid sensitivity (remission defined as CR or PR) was similar in cellular FSGS and classic FSGS (53% versus 52%). The fraction of glomeruli involved was an important predictor: >20% glomerular involvement involved defined a group that was almost exclusively black (94%), had heavier proteinuria (67% with >10 g per/day), and were less responsive to treatment (23% remission), compared to those with a lesser degree of glomerular involvement.

It appears that most or all of these patients would be classified as collapsing glomerulopathy (collapsing FSGS in the

Columbia classification). There are as yet no published data on the clinical characteristics or outcomes of patients with the cellular lesion as defined in the Columbia classification.

Postadaptive FSGS

As detailed earlier in this chapter, data as disparate as the remnant kidney model of glomerulosclerosis and epidemiologic evidence for a relationship among low nephron number, hypertension, and progressive renal scarring support the notion that increased workload per nephron can lead to glomerulosclerosis. Some clinical criteria have been developed for identifying postadaptive FSGS (Table 64-17). Here, we consider two clinical circumstances in which this can occur: reduction of nephron number or increased workload with a fixed number of nephrons.

Postadaptive FSGS with Reduced Nephron Mass. There are characteristic features of FSGS associated with reduced nephron mass. First, glomerular enlargement is common in this setting. Bhathena studied patients with FSGS appearing more than 2 years after renal transplantation, in association with chronic allograft nephropathy and interstitial nephritis (925). These patients had larger glomeruli compared to those without FSGS or those with recurrent FSGS, diagnosed within 2 years of transplantation. Subsequently Bhathena showed that glomeruli are larger in unilateral renal agenesis and following uninephrectomy when FSGS is present compared to patients with the same disorders when FSGS is lacking (926). Second, the segmental scars are preferentially located adjacent to the vascular hilum. In the rat nephrectomy model, the sclerosis preferentially affects the vascular pole (927). When colloidal carbon is injected immediately after nephrectomy, it is found preferentially within perihilar sclerotic lesions 4 months later (928). These findings suggest that some feature of this glomerular zone, possibly related to hemodynamic stress, leads to increased uptake (presumably by mesangial cells) long before the appearance of sclerotic lesions. Third, podocyte effacement is modest, typically involving <50% of the interface between podocyte and GBM, as discussed previously. There is considerable variability, however, so that this characteristic may be of limited value in clinical diagnosis. Fourth, hypoalbuminemia and edema are uncommon despite nephrotic range proteinuria (924).

Postadaptive FSGS Associated with Obesity. Obesity is associated with increased renal blood flow, glomerular filtration rate, and microalbuminuria, features that it shares with diabetes mellitus. The mechanisms for these renal abnormalities are not well understood (recently reviewed [929]). Contributing factors may include increased renal venous pressure, hyperlipidemia, and increased production of vasoactive and fibrogenic substances by adipocytes, including angiotensin II, insulin, leptin, and TGF-β1. Dogs fed a high-fat diet to produce a 60% increase in body weight manifested an increased blood pressure, plasma renin activity, and GFR, together with increased mesangial matrix and TGF-β expression (glomerular size was unchanged) (930). Obese, pre-diabetic monkeys manifest hyperinsulinemia and glomerulomegaly, suggesting that some feature of the insulin-resistant state may contribute to glomerular hypertrophy (931). Leptin is of particular interest, as it acts on cultured glomerular endothelial cells to stimulate proliferation and production of TGF-β1 and collagen I, and it stimulates expression of TGF-β type II receptor and collagen I by mesangial cells (913). Furthermore, leptin also stimulates production of angiotensin II. Therefore, leptin may initiate a pro-fibrogenic circuit within the glomerulus.

D'Agati and colleagues reviewed their experience with obesity-related glomerular disease, including 14 patients with glomerulomegaly alone and 57 patients with glomerulomegaly plus FSGS (764). The combined prevalence of these disorders

TABLE 64-17

CLINICAL RECOGNITION OF POSTADAPTIVE FOCAL SEGMENTAL GLOMERULOSCLEROSIS (FSGS)

Feature	Utility	Limitations
High-risk clinical setting: (a) Reduced nephron mass: renal dysplasia, ureteral reflux (b) Initially normal renal mass: sickle cell anemia, cyanotic congenital heart disease	++	The life-time incidence of postadaptive FSGS in these uncommon or rare conditions is probably relatively high, although precise incidence values are not available
Moderate-or low-risk clinical setting: obesity, hypertension	+	These are common clinical conditions, where the lifetime incidence of postadaptive FSGS is probably low; idiopathic FSGS may also occur in these individuals
Nephrotic proteinuria without edema or hypoalbuminemia	+	This pattern is more common in postadaptive FSGS but also occurs in idiopathic FSGS (924)
Perihilar FSGS variant: At least one glomerular showing perihilar hyalinosis and perihilar hyalinosis and/or sclerosis involving >50% glomeruli	++	Sensitivity and specificity remain to be determined
Glomerulomegaly (average glomerular diameter >185 μm)	+++	Requires a sufficient number of glomeruli (probably 5–10), multiple levels (possibly 4), and measurement of glomeruli cut at or near hilus (764)
Podocyte foot-process effacement	+	There are significant group differences between idiopathic FSGS and postadaptive FSGS, but there is considerable overlap between the groups, so this has limited utility in a particular case (764)

When confronted with a renal biopsy that shows FSGS, no history suggesting a genetic cause of FSGS (onset in childhood, family history of FSGS, extrarenal manifestations) and no use of FSGS-associated medication, the pathologist and clinician must weigh the likelihood of idiopathic FSGS versus postadaptive FSGS. Multiple features must be considered in making this distinction.

rose from 0.2% of all renal biopsies during the period from 1986 to 1990 to 2% during the period from 1996 to 2000; 53% had BMI >40 kg/m^2 (morbid obesity) and 47% had BMI 30 to 40 kg/m^2. Therefore morbid obesity is not required for the diagnosis. Compared to patients with idiopathic FSGS, patients with obesity-associated glomerulopathy had lower levels of proteinuria (nephrotic-range proteinuria in 48% compared to 66%), less foot-process effacement, and a more indolent course (progression to ESRD 4% versus 42%). By contrast, Praga et al. found that the outcome in obesity-related disease was almost as poor as that of idiopathic FSGS (845). Over an 82-month observation period, 8 of 15 patients maintained stable renal function and 7 of 15 patients demonstrated progressive loss of GFR, with five reaching ESRD. Proteinuria was in the nephrotic range at presentation or on follow-up in 80% of patients. The level of proteinuria correlated with BMI ($R = 0.45$, $p < 0.05$). Despite heavy proteinuria, >10 g per day in three patients, edema and hypoalbuminemia were uniformly absent. With the epidemic of obesity spreading to adolescents in the United States, particularly in minority populations, obesity-associated FSGS is appearing in that age range also (932).

Recently, glomerular hypertrophy and FSGS also have been reported in patients with increased BMI associated with increased muscle mass but without increased body fat, suggesting that adiposity is not required (933). Important questions remain. We do not know whether glomerulomegaly is a precursor lesion to FSGS, although that appears likely; we do not know whether there is a threshold BMI for the appearance of glomerulomegaly and FSGS; and we do not understand the molecular signals by which increased fat mass or BMI lead to glomerular hyperfiltration.

Hypertension and FSGS

Hypertension is the attributed cause of approximately 15% of incident ESRD cases in Europe and approximately 30% of incident ESRD cases in the United States (discussed in depth in Chapter 51). Few of these patients, however, undergo renal biopsy and there remains doubt about the nature of the role of hypertension in initiating renal injury. It is undisputed that malignant hypertension can lead to ESRD; it is more controversial whether benign nephrosclerosis leads to ESRD. This controversy has been the subject of several thoughtful reviews in recent years (934–936). Hypertensive nephrosclerosis (benign nephrosclerosis) is defined by the presence of arteriolar changes consisting of hyaline arteriosclerosis and myointimal hypertrophy that particularly affect the afferent arteriole and small arterioles lacking an internal elastic lamina. The initial glomerular morphologic changes include thickening and wrinkling of the GBM, following by contraction of the capillary tuft toward the vascular pole. As the tuft contracts, fibrotic material accumulates within Bowman's space. The glomerulus ultimately shrinks to a hyalinized mass at the vascular pole. In contrast to FSGS, glomerular tufts showing global glomerulosclerosis in hypertensive nephrosclerosis are not enlarged, but rather are shrunken (937).

In recent biopsy series a substantial fraction of patients with a diagnosis of hypertensive nephrosclerosis manifested segmentally sclerosed glomeruli. In a study from Brazil of 90 hypertensive patients with renal insufficiency and subnephrotic proteinuria, 19% were found to have segmental sclerosis, most of whom also had vascular features of hypertensive injury (938). In a study from Japan, 33% of patients with hypertensive nephrosclerosis had segmental sclerotic lesions; these patients had significantly higher serum creatinine values (939). Patients who experienced progressive loss of renal function during follow-up had larger glomeruli, but the authors did not state whether those with segmental sclerosis had larger glomeruli. In a study from Tennessee, Fogo and colleagues found segmental sclerosis in 34% of patients with hypertensive nephrosclerosis (940). The authors excluded idiopathic FSGS on the following grounds: lack of nephrotic range proteinuria, lack of extensive foot-process effacement, and glomerulosclerosis in proportion to vascular lesions. Segmental sclerosis was associated with global sclerosis and was more common in blacks than in whites. Importantly, the authors found that the extent of vascular injury did not correlate with the extent of global and segmental glomerulosclerosis, and statistical modeling failed to identify a link between blood pressure and the severity of the glomerular lesions. The authors concluded that a simple causal pathway, whereby hypertension causes vascular sclerosis which in turn causes glomerulosclerosis, is not supported by their data.

Kincaid-Smith noted that the original description of hypertensive nephrosclerosis, made 50 years ago, did not include segmental sclerosis. She makes the provocative proposal that many cases that are clinically diagnosed as hypertensive nephrosclerosis without renal biopsy, or pathologically diagnosed as hypertension-associated FSGS, may in fact be postadaptive FSGS due to obesity and associated conditions, including insulin resistance associated with the metabolic syndrome and hyperuricemia (936). This hypothesis is testable, with closer attention to clinical factors (obesity, proteinuria, uric acid levels, and levels of insulin and other endocrine factors) and pathologic variables (glomerular size, podocyte number, and extent of foot-process effacement) in patients with FSGS arising in the setting of hypertension. In conclusion, it remains uncertain whether hypertension causes a form of postadaptive FSGS, or whether hypertensive vascular changes coexist with segmental sclerosis lesions, which arise as a consequence of obesity and other clinical factors associated with hypertension.

C1q Nephropathy

C1q nephropathy was first described by Jennette and Hipp at Chapel Hill as the presence of mesangial C1q staining, either dominant or co-dominant with IgG, IgM, and/or C3 and the lack of serologic or clinical findings of lupus (462). The immune deposits were predominantly mesangial, although occasionally the deposits extended into glomerular capillary loops. In this series of 15 patients (2% of renal biopsies, age range 14–27 years), the glomerular histology ranged from normal to mesangial proliferation to focal or diffuse proliferative glomerulonephritis.

Two subsequent series have suggested that the disease probably fits best in the MCN/FSGS/collapsing glomerulopathy spectrum. Iskandar et al. at Winston-Salem reported on 15 children with prominent mesangial C1q staining and histology consistent with MCN (8 cases) and FSGS (7 cases) (941). Markowitz et al. in New York described 19 patients (9 children, 10 adults), representing 0.2% of all renal biopsies (458). Histologies ran the gamut of podocyte diseases, including MCN (2 patients), FSGS (2 patients with the cellular variant, 9 patients with the NOS variant), and collapsing glomerulopathy (6 patients). Mesangial hypercellularity was seen in one MCN case and 10 of the other cases.

In the series from Chapel Hill, North Carolina; Winston-Salem, North Carolina; and New York, blacks were generally over-represented (60%, 27%, and 74%, respectively) compared to their presence in the local populations. Nephrotic-range proteinuria was common (71%, 80%, and 79%, respectively), although many patients (particularly adults) did not have nephrotic syndrome. Response to therapy is variable but has improved in the most recent series, with CR or PR seen in 0% (0/9 patients treated) at Chapel Hill, 44% (4/9 patients treated) at Winston-Salem, and 57% in New York (7/13 with available data; 2 additional patients progressed to ESRD during follow-up), respectively. In the New York series, CR or PR occurred in 2/2 patients with MCN, 4/11 with FSGS, and

1/6 with collapsing glomerulopathy, mirroring the steroid responses typical of other forms of these diseases. Other reports have also suggested that some patients with C1q nephropathy remit spontaneously or with therapy, but some of these patients had atypical features, such as prominent subendothelial or subepithelial deposits (457,942).

C1q and other members of the newly recognized C1q/TNF superfamily share a structurally similar globular domain (943). C1q, a portion of the tripartite C1 molecule, functions as major link between innate and acquired immunity, as it binds a range of ligands via the globular domain and promotes apoptosis and phagocytosis of cells and cellular debris (944–946). Human genetic C1q deficiency is associated with lupus, presumably by impairing apoptotic cell clearance and thereby exposing the immune system to nuclear and cytoplasmic antigens. Interestingly, the classical swine fever virus may provide an animal model of C1q nephropathy. Pigs experimentally infected with classical swine fever virus develop immune complex glomerulonephritis characterized by viral infection of glomerular endothelial cells and podocytes, macrophage infiltration, and deposition of IgG, IgM, and C1q in mesangial, subendothelial, and subepithelial locations (947).

Therefore, by histology and clinical course, C1q nephropathy would appear to fit into the spectrum of MCN/FSGS/collapsing glomerulopathy, as D'Agati and colleagues have proposed (458). The pathogenesis remains poorly understood, and specifically it is unclear how to link C1q mesangial deposits with podocyte injury.

Idiopathic Collapsing Glomerulopathy

HIV-associated nephropathy was the first recognized form of collapsing glomerulopathy, and this topic is covered in Chapter 59. Idiopathic collapsing glomerulopathy was first described in Ohio (948), with subsequent reports from elsewhere within the United States and Europe (732,949–952). A retrospective review of the renal biopsy archive at Columbia University found the first case to have been seen in 1979, with a rapid increase to causing approximately 25% of idiopathic FSGS by the early 1990s (949). This epidemiologic pattern strongly suggests a new environmental agent, possibly a virus or toxin, although little progress has been reported in identifying what that agent might be. *De novo* collapsing glomerulopathy also has been described following renal transplantation (953,954). Recent reviews are available (955,956).

Idiopathic collapsing glomerulopathy presents clinically as the sudden onset of heavy proteinuria, although some patients have subnephrotic proteinuria. Progression to ESRD may be rapid but this in not invariably the case. Some patients report a viral-like prodrome occurring prior to presentation, with symptoms that may include fever, cough, and diarrhea.

Collapsing glomerulopathy occurs in several distinct clinical settings. It has been associated with systemic diseases, particularly adult Still's disease (957,958). Parvovirus B19 infection has the been linked with collapsing glomerulopathy (959), although controversy remains about the strength and specificity of the association (960). Most recently SV40 infection has been linked to both collapsing glomerulopathy and other forms of FSGS; these data await replication (961).

The mechanism of podocyte proliferation remains largely unexplained. Hattori et al. studied patients with cellular lesion, although it is likely that many or all of these had collapsing glomerulopathy (962). They noted that glomerular expression of smooth muscle α-actin (a marker for activated mesangial cells, exhibiting a myofibroblastic phenotype) was much greater in cellular FSGS compared to those with classic FSGS. Expression of collagen III was similar. Therefore, intense mesangial activation is part of the process and may account for the propensity to rapid progression.

Medication-associated FSGS and Collapsing Glomerulopathy

A number of medications have been associated with FSGS and collapsing glomerulopathy, as shown in Table 64-3. Cyclosporine is associated with FSGS in renal transplant patients and nonrenal organ transplant patients. In renal transplant recipients, cyclosporine contributes to the pathogenesis of chronic allograft nephropathy, but the role of other factors makes the specific role of these agents difficult to determine. The appearance of FSGS in nonrenal organ transplant recipients and its link to cyclosporine (963–965) makes the relationship more compelling. Tacrolimus likely has a very similar risk but fewer data are available. Cyclosporine induces FSGS following chronic administration to rats (966). The mechanism of glomerular toxicity may include increased matrix synthesis by glomerular cells (966) and glomerular ischemia. The 5-year risk for chronic renal failure in nonrenal organ transplantation ranges from 7% to 21% (967). The use of a calcineurin inhibitor was significantly associated with increased risk, but the degree of relative risk was modest (1.25), probably due to the study design (only use on initial hospitalization was available) and the fact that 98% of patients used these agents.

Lithium has been associated with FSGS (968–970). Chronic exposure to lithium causes tubulointerstitial injury in rats (971), which would be evidence in favor of a postadaptive mechanism. Conversely, some patients have extensive foot-process effacement (969), and nephrotic syndrome may resolve with cessation of therapy (972). These findings suggest the alternate possibility of a direct toxicity to glomerular cells.

Interferon-α has been associated with both FSGS (973–978) and collapsing glomerulopathy (979). Pamidronate has been associated with collapsing glomerulopathy (956,980). Very little is known about mechanism of renal injury with these medications.

Medications associated with idiopathic collapsing glomerulopathy include pamidronate (981) and interferon-α (979). The mechanism is unknown.

Nephrotic Syndrome in the First Year of Life

Nephrotic syndrome may occur in the first year of life and can result from MCN. Much more often, however, it is due to congenital nephrotic syndrome, especially that associated with infantile microcystic disease (982–984). This lesion is most commonly found in Finland and in people of Finnish ancestry, but it has been documented as well in many other ethnic groups throughout Europe and North America. The disease is inherited as an autosomal-recessive trait. Affected infants typically have a large placenta (983), with the placental–fetal weight ratio ranging from 0.25 to 0.43, compared to a normal ratio of 0.14. They often are born prematurely, may show signs of perinatal asphyxia, and have a high perinatal mortality rate. Indeed, the incidence of early death from congenital nephrotic syndrome may be higher than that reported, as edema and failure to thrive may not become apparent for several weeks or months, even though proteinuria is present from birth. The natural course in infancy is usually one of progressive deterioration, with hypoalbuminemia, edema, and wasting dominating the clinical picture and being more significant than decreasing renal function. In the past, half of the patients died before the age of 6 months and few survived past 2 years. However, aggressive management has dramatically improved the outlook (336).

The majority of patients with this disease have abnormal expression of the gene for nephrin, a putative cell–cell adhesion molecule that is expressed only by the glomerular podocytes (985). The gene is located on chromosome 19q13.1 (986). Histologic changes of congenital nephrotic syndrome can be subtle

in the newborn. They may include dilated cortical tubules (the origin of the term *microcystic*), mesangial hypercellularity, and glomerulosclerosis, none of which are pathognomonic for congenital nephrotic syndrome of the Finnish type. A significant reduction in the number of negative-charge sites in the GBM has been reported (56). Prenatal diagnosis has been obtained by finding increased α-fetoprotein levels in the amniotic fluid. However, levels may be high in amniotic fluid from pregnancies with heterozygous (carrier) phenotype (987). Studies of patients and their families suggest that direct analysis of the fetal nephrin gene can be used for prenatal diagnosis in many patients (988).

Other idiopathic types of congenital nephrotic syndrome have been described. These are less well defined and probably represent a heterogeneous group of diseases, most of which do not appear to be inherited (985). They are distinguished from the Finnish type by the combination of clinical characteristics and histologic picture. Most have a poor prognosis, but the clinical course is often more protracted. Attempts have been made to classify these lesions according to the dominant pathologic findings. According to this approach, the presence of microcystic disease has been used to diagnose the Finnish type of congenital nephrotic syndrome. In the absence of microcystic tubules, the disease has been classified on the basis of the most prominent glomerular lesion, for example, mesangial proliferation, DMS, FSGS, or global glomerular sclerosis. Without such changes, the patient is diagnosed as having MCNS. A study that summarized the problems of this classification system emphasized that microcystic tubules are neither specific nor diagnostic for the Finnish lesion and may be acquired as a consequence of severe proteinuria in the immature kidney (989).

In many cases DMS is the dominant pathologic picture. Prognosis is poor, irrespective of whether the onset of disease is congenital or infantile. Congenital glomerulosclerosis characterized by hyalinized glomeruli in otherwise normal kidneys also has been found in infants developing nephrotic syndrome and renal failure early in life. Although such lesions could be the consequence of intrauterine infections (990), the development of DMS more frequently reflects a genetically determined metabolic or structural abnormality. It is found in a variety of syndromes associated with the gene for the WT-1 transcription factor (see discussion of *WT1* and Denys-Drash and Frasier syndromes in the next section). Other entities that have been associated with the nephrotic syndrome in the first year of life (246,465,989,991–999) are summarized in Table 64-18.

Age at presentation is an important factor in outcome. In one study, 97% of 177 patients died or subsequently required care for ESRD if they presented with nephrotic syndrome in the first 3 months of life (1000). In contrast, death or end-stage disease occurred in only 31% of patients with onset of nephrotic syndrome between the ages of 3 and 12 months. In an effort to identify children with the most severe, progressive disease, the ratio of urinary heparan sulfate to chondroitin sulfate was measured in 37 patients and 17 healthy controls. Patients with Finnish-type disease, DMS, and focal sclerosis had elevated ratios, whereas children with MCNS had results similar to those of normal subjects (1001).

However, it is advisable to determine the nature of the lesion by tissue diagnosis as early as possible after the initial presentation, since early intervention is optimal. Congenital nephrotic syndrome is associated with profound malnutrition, dehydration, hypercoagulability, and hypothyroidism. All of these problems are amenable to medical therapy. Patients may be stabilized with daily or even more frequent albumin infusions, enteral hyperalimentation, anticoagulants, and thyroid replacement. In some cases, uninephrectomy or even bilateral nephrectomy is required to overcome the effects of severe protein loss. It is our practice to begin with albumin infusions and progress to uninephrectomy in the hope that the child will be permitted to grow for as much as a year before entering into ESRD care.

TABLE 64-18

CAUSES OF THE NEPHROTIC SYNDROME IN THE FIRST YEAR OF LIFE

Relatively common
Congenital nephrotic syndrome of the Finnish (inherited) type
Other congenital nephrotic syndromes
Less common
Diffuse mesangial sclerosis
Focal segmental glomerulosclerosis, congenital glomerulosclerosis, and hyalinosis
Minimal change nephrotic syndrome (MCNS)
Acquired immunodeficiency syndrome
Rare
Cytomegalic inclusion disease (277)
Hemolytic–uremic syndrome (278)
Mercury intoxication (279)
Nail–patella syndrome (280)
Pseudohermaphroditism (281,282)
Renal vein thrombosis (283)
Syphilis (284,285)
Toxoplasmosis (286)
Wilms tumor (50,287)
XY Gonadal dysgenesis (287)

Kidney transplantation for children with congenital nephrotic syndrome usually is effective with the nephrotic syndrome rarely recurring in the recipient. The major exception is the child with Finnish-type congenital nephrotic syndrome. Because such children are born without full-length nephrin, they may recognize intact nephrin in the transplanted kidney as foreign, developing anti-nephrin antibodies. This problem is treated most effectively with a combination of plasmapheresis and alkylating agents (987).

Genetics of FSGS and Collapsing Glomerulopathy

A major advance in the last decade has been the recognition of loci responsible for FSGS with Mendelian inheritance (Table 64-19). Molecular diagnosis of mutations, particularly podocin (*NPHS2*) and *WT1*, has now become an important part of the evaluation of pediatric FSGS. Genotyping for *NPHS1* and *NPHS2* is commercially available and is rapidly becoming a routine part of clinical practice.

As shown in Tables 64-3 and 64-19, the age at presentation and renal histology help identify the most likely genetic causes:

- Congenital nephrotic syndrome: *NPHS1* homozygous mutations or *NPHS1* + *NPHS2* compound heterozygous mutations
- DMS, congenital presentation: *LAMB2*
- DMS, congenital presentation: *WT1*
- FSGS, congenital presentation: *ITGB4*
- FSGS, infancy/childhood presentation: *NPHS2, NPHS1* + *NPHS2, WT1, PAX2,* mtDNA, COQ2
- FSGS, adult presentation: *ACTN4, CD2AP, TRPC6,* mtDNA

Collapsing glomerulopathy rarely presents with familial inheritance (1002–1004). A single genetic syndrome has been reported, action-myoclonus-renal failure syndrome, in which

TABLE 64-19

GENETIC CAUSES OF HUMAN PODOCYTE DISEASES

Gene	Gene product	Function	Inheritance (chromosome)	Renal syndrome	Prevalence	Extrarenal findings
NPHS1	Nephrin	Slit diaphragm complex	AR 19q13.1	Congenital nephrotic syndrome; infantile onset FSGS	Insufficient data	None
NPHS2	Podocin	Slit diaphragm complex	AR 1q25-31	FSGS onset <20 year	Up to 20% of familial or sporadic pediatric FSGS	None
CD2AP	CD2 associated protein	Slit diaphragm complex	AD 6	FSGS onset >20 year	Two reported cases, one with family history	None
ACTN4	α-Actinin-4	Cytoskeleton	AD 19q13	FSGS onset >20 year	Five reported families	None
TRPC6	Transient receptor, cationic, 6	Channel	AD 11q21-q22		Six reported families	None
WT1	Wilms tumor–1	Transcription factor	AD 11p13	DMS or less commonly FSGS (Denys-Drash syndrome); FSGS (Frasier syndrome)	Uncommon	Wilms tumor, gonadal abnormalities
ITGB4	β-1 integrin	Matrix protein receptor	AR 17q11-qter	Congenital FSGS	Uncommon	Epidermolysis bullosa, pyloric atresia
LAMB2	Laminin β2	Basement membrane component	AR 3p21	Congenital mesangial sclerosis	Five reported cases	Ophthalmic defects (Pierson syndrome)
PAX2	Pax2 homeobox protein	Transcription factor	AR 10q24.3-25.1	Renal dysplasia, oligomeganephronia		Ophthalmic defects (coloboma)
mtDNA	Mitochondrial tRNA	Protein synthesis	Maternal	FSGS	>40 Reported cases	MELAS syndrome, diabetes mellitus, cardiomyopathy
COQ2	COQ2 Synthetase	Enzyme	AR 11	FSGS, infantile or early childhood		Myopathy, central nervous system disorders
Galloway-Mowat syndrome	Unknown	Unknown	AR	DMS or FSGS, congenital, infancy, or early childhood		Microcephaly, hypotonia
Action myoclonus-renal failure syndrome	Unknown	Unknown	AR	Collapsing glomerulopathy, onset 9–30 years	Nine families	Myoclonus, seizures

AD, autosomal dominant; *AR*, autosomal recessive; *DMS*, diffuse mesangial sclerosis; *MELAS* syndrome, myopathy, encephalopathy, lactic acidosis, stroke.

renal failure occurred in 12 patients from 8 families of diverse European ethnic background; the gene responsible has not been identified (1005).

NPHS2

In 2000, Antignac and colleagues identified *NPHS2*, encoding podocin, by positional cloning in the evaluation of 14 of 16 families with autosomal recessive steroid-resistant nephrotic syndrome (1006). Subsequently, NPHS2 mutations have been identified in steroid-resistant nephrotic syndrome and FSGS from patients from a wide variety of racial and ethnic backgrounds (1007–1021). Homozygous or compound heterozygous mutations have been found in 35% of familial autosomal recessive FSGS and 8% of sporadic FSGS (1022,1023). To date, all patients have presented by age 25 and most patients present before age 10.

NPHS2 is a highly polymorphic gene. In Figure 64-15 is shown the location of 58 *NPHS2* sequence variants within the exon structure, including mutations (defined as variants associated with renal disease either in homozygosity or in compound heterozygosity, which involves two distinct *NPHS2* mutations on different alleles) and sequence variants (nucleotide variants, which are present in healthy individuals and do not meet the criteria for mutations). Podocin is a transmembrane protein, restricted to the podocyte that contributes to the maintenance of a normal slit diaphragm. Podocin is located within lipid rafts and interacts with other molecules that constitute the slit diaphragm complex, including nephrin and CD2AP (1024,1025). Further, podocin facilitates the signaling of nephrin and CD2AP via phosphatidylinositol and AKT (1026,1027). *NPHS2* mutants are either retained within the endoplasmic reticulum or delivered to the plasma membrane but fail to associate with lipid rafts (1028). *NPHS2* mutants that are retained within the endoplasmic reticulum are associated with earlier onset of FSGS compared to those that are targeted to the plasma membrane (1029).

In patients with *NPHS2* mutations, FSGS can recur following renal transplantation, but the risk appears to be lower than in patients with idiopathic FSGS. Hildebrandt reported recurrence in 7 of 20 (35%) patients without *NPHS2* mutations and 2 of 24 (8%) patients with *NPHS2* mutations (1021). In an update of an earlier publication that reported a surprisingly high rate of recurrence (1030), Ghiggeri and colleagues reported that 5 of 65 (8%) of subjects with homozygous or compound heterozygous mutations had recurrence (1022).

The *NPHS2 R229Q* variant is a common polymorphism, occurring in approximately 7% of European-derived populations and approximately 3% of African-derived populations (1014,1031). The pathologic significance of this variant remains unclear. FSGS has been associated with homozygous *R229Q* mutation in only one individual (1021); if this common polymorphism were sufficient to induce FSGS, one would expect more cases to have been identified. Tsukaguchi et al. (1014) found that 11 of 91 African Americans with idiopathic FSGS were R229Q heterozygotes, of whom two were compound heterozygotes with A248V. The prevalence of simple R229Q heterozygous was not statistically different from the control population. Conversely, two infants with congenital FSGS were found to have tri-allelic mutations (compound *NPHS1* heterozygotes with the *NPHS2* R229Q mutation), suggesting that the R229Q variant may modify the typical *NPHS1* presentation (1013). This finding of gene interaction between *NPHS1* and *NPHS2* has recently been challenged (1032). Finally, the R229Q variant may be associated with microalbuminuria in the Brazilian population (relative risk 2.8-fold, $p < 0.01$) (1031). Therefore, it may be that the R229Q variant acts in concert with other genetic and environmental factors to compromise glomerular function but the hypothesis will require further testing.

WT1

Mutations in *WT1*, the gene encoding the Wilms tumor suppressor gene 1, cause four syndromes: WAGR (Wilms tumor, aniridia, genitourinary malformations, retardation), Beckwith-Wiedemann syndrome, Denys-Drash syndrome, and Frasier syndrome (1033). The rates of Wilms tumor among these syndromes are widely divergent, ranging from <5% in Beckwith-Wiedemann syndrome to >90% in Denys-Drash syndrome. Patients have a single mutant *WT1* allele; in XX female patients with heterozygous *WT1* mutations, Wilms tumors frequently have mutations in both alleles, suggesting that loss of heterozygosity has occurred. The WT1 protein is a transcriptional regulator of genes important in renal development, including amphiregulin (1034) and *PAX2*, and genes important

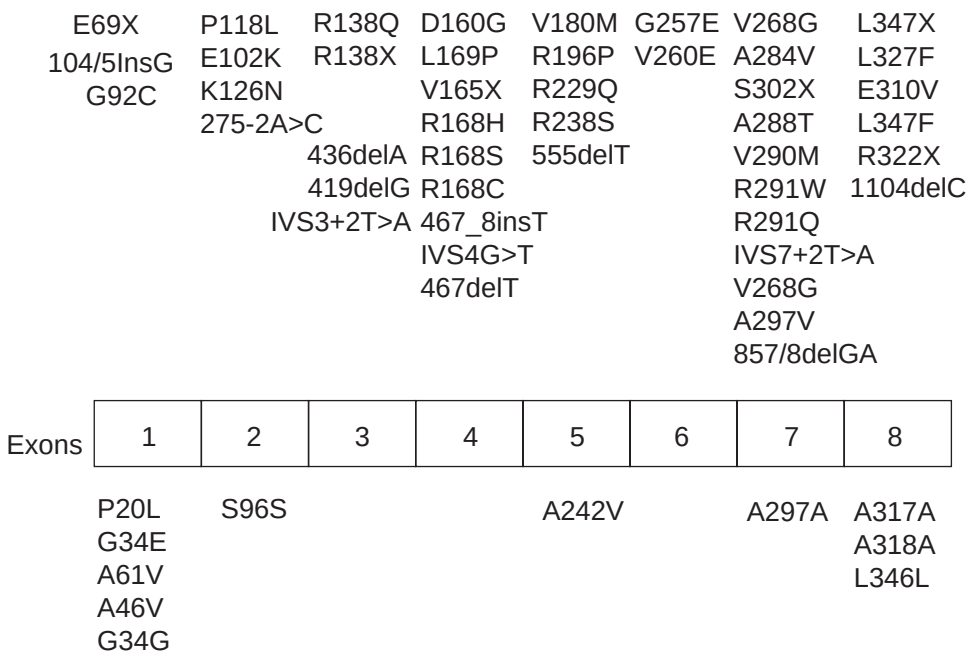

FIGURE 64-15. *NPHS2* (podocin) gene structure and variants. The eight exons of podocin are shown schematically, without portraying relative size. Mutations within the coding region (defined as variants which are associated with FSGS, when present in homozygosity or compound heterozygosity) are shown above the structure. Variants not associated with FSGS are shown below. Missense mutations are shown by the change in amino acid at the given position, with X denoting a premature stop codon. Insertions, deletions and splice site variants are also shown.

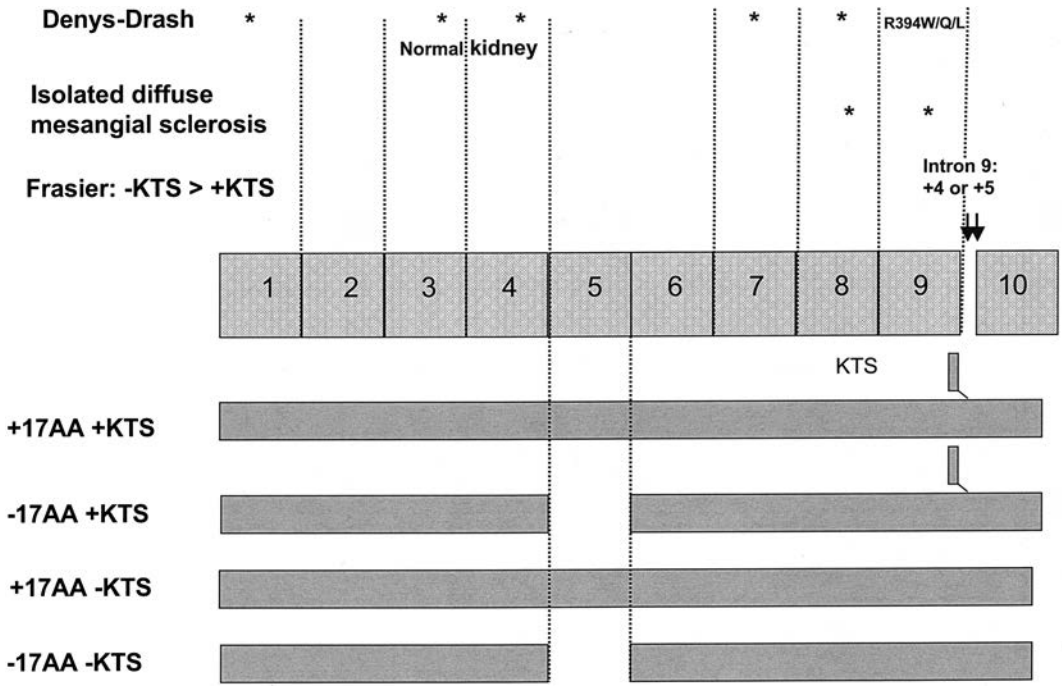

FIGURE 64-16. *WT1* gene structure, mRNA structure, and mutations associated with kidney disease. The *WT1* gene contains 10 exons (*shaded boxes*). At least 24 distinct mRNAs are generated, of which 4 predominate (*bottom*): these either do or do not contain the 17 amino acids encoded by exon 5, based on alternate splicing, and do or do not contain the three amino acids lysine-threonine-serine (KTS), based on the use of alternate splice acceptor sites located within intron 9. mRNAs lacking KTS generate more potent transcriptional activators. The Denys-Drash syndrome is associated with mutations in exons 3 and 4 (associated with normal kidneys) and in exons 7, 8, and 9 (especially the hotspot *R394W/Q/L* mutation; all are associated with DMS). Isolated diffuse mesangial sclerosis is also associated with mutations in exons 8 and 9. Frasier syndrome is associated with mutations in intron 9 at position +4 or +5, which disrupt the second splice acceptor site and lead to an excess of mRNAs that do not encode KTS (KTS-).

in maintaining the differentiated podocyte phenotype, including $p21^{Cip1}$, nephrin (*NPHS1*), and podocalyxin (1035). As shown in Figure 64-16, the *WT1* gene is composed of 10 exons, with the last 5 exons encoding the 4 C_2H_2 fingers comprising the zinc finger domain responsible for transcriptional regulation.

As depicted in Figure 64-16, the *WT1* gene undergoes a complex pattern of alternate splicing, producing at least 24 distinct mRNAs. Four mRNAs predominate: these do or do not contain the 17 amino acids encoded by exon 5, based on alternate splicing, and do or do not contain the three amino acids lysine-threonine-serine (KTS) located between zinc fingers 3 and 4, based on the use of alternate splice acceptor sites located within intron 9. mRNAs that do not encode KTS (KTS-) generate more potent transcriptional activators (1036). In cells from normal individuals, the +KTS/-KTS ratio ranges from 1.5 to 2; in cells from Frasier syndrome patients with intron 9 splice-site mutations, the ratio falls below 1, which would increase transcriptional activity (1037). By contrast, Denys-Drash syndrome is associated with point mutations and premature stop codon mutations, particularly involving zinc fingers 2 and 3. Many of the involved mutations disrupt the zinc finger structures, suggesting that the mutant protein might act in a dominant negative fashion to suppress or alter activation of target genes (although this has not been formally demonstrated) (1038).

The Denys-Drash syndrome is a triad composed of nephropathy (DMS or FSGS), male pseudohermaphroditism, and Wilms tumor, although many patients lack the full triad and some may have isolated DMS. Most patients have a 46, XY karyotype but a 46, XX karyotype can occur. Patients present commonly with ambiguous genitalia at birth, although up to 40% are phenotypically female and may present with primary amenorrhea. Rare patients are phenotypically male. Nephropathy typically presents in infancy or early childhood, occasionally at birth. The renal biopsy shows focal or DMS, or less commonly, FSGS. Patients typically progress rapidly to ESRD, often within months and almost always by age 10. Mutations in *WT1* are found in 90% of patients with Denys-Drash syndrome (1039–1043). The locations of the mutations are either in the N-terminal domain (exons 1-3, often in patients lacking nephropathy) or the exons 7-9, disrupting the zinc finger domains (especially at R394). Podocyte proliferation has also been reported, with the development of pseudocrescents and shrunken glomeruli (881). The authors did not specifically describe capillary collapse, but these findings suggest that the Denys-Drash phenotype may occasionally extend to collapsing glomerulopathy. Slowly progressive FSGS has been reported with a *F392L* mutation (1044).

The Frasier syndrome is a triad composed of nephropathy, male pseudohermaphroditism, and gonadoblastoma, although many patients lack the full triad. Patients are most often phenotypically female. Wilms tumors are not seen. Mutations in the intron 9 splice donor site are uniformly present (1037,1039,1045,1046). The mutations are usually *de novo*, but in one case, maternal inheritance has been described (1047). Onset of proteinuria is typically between 2 and 18 years of age, with ESRD reached between 8 and 20 years. FSGS is most common and occasionally DMS is present. In one case, a patient with Wilms tumor was shown to have MCN with mild segmental hypercellularity (1048). FSGS was not demonstrated in a sample that included more than 100 glomeruli; nevertheless the patient progressed to ESRD.

WT1 mutations have not been identified in sporadic FSGS or collapsing glomerulopathy, but this genomic region may harbor susceptibility loci. Denamur et al. found only 1 of 37 children with steroid-resistant nephrotic syndrome (SRNS) or FSGS to have a *WT1* intron 9 mutation, and this child had other manifestations including genitourinary abnormalities (1049). Orloff et al. studied 218 African American FSGS patients, mostly with adult-onset kidney disease, and found no pathogenic mutations (1050). They studied two candidate genes *WT1* and *WIT1*,which lie in close proximity on chromosome 11, by genotyping subjects for seven SNPs in *WT1* and one SNP in *WIT1*. Three SNPs were associated with idiopathic FSGS or collapsing glomerulopathy (but not HIV-associated collapsing glomerulopathy): two SNPs in the *WT1* gene (OR 1.7) and OR 1.9) and one SNP in *WT1* (OR 1.8). One SNP within a *WT1* intron was associated with HIV-associated collapsing glomerulopathy (OR 4.3). Extending these analyses to haplotypes (particular allelic combinations at multiple loci along a chromosomal segment), they observed that a haplotype comprising one *WIT1* SNP and four *WT1* SNPs was associated with HIV-associated collapsing glomerulopathy, but not idiopathic FSGS or collapsing glomerulopathy. These data suggest that genetic variation within *WT1*, *WIT1*, or nearby genes, may contribute to susceptibility to both idiopathic and HIV-associated glomerular diseases in African Americans, but that the contributions of particular genetic variants are complex.

ACTN4

Pollak and colleagues identified *ACTN4* mutations in three families with FSGS showing autosomal dominant inheritance (1051). The mutations (K228E, T232I, R235P, recently renumbered as K256E, T260E, S263P) were in exon 8 (out of 21 exons). Affected individuals showed a range of phenotypes, from microalbuminuria and subnephrotic proteinuria (including elderly individuals) to ESRD. Penetrance was incomplete, as some patients with mutations lack proteinuria. Importantly, in the cases identified to date, proteinuria does not develop until adulthood. The α-actinins are actin-binding and cross-linking proteins; α-actinin-4 is expressed in many tissues. Podocytes express α-actinin-4 in abundant amounts and the mutant α-actinin-4 variants were initially shown to bind actin more tightly than does the wild-type protein. Mutant proteins also have an increased propensity to form aggregates, which Pollak and colleagues suggest could explain the apparent increase in actin binding, and the mutant molecules have a greatly decreased half-life (798). Two models are proposed: the mutant protein is unavailable for cytoskeletal interactions or alternatively aggregates are cytotoxic.

ACTN4 null mice develop glomerular disease, consisting of foot-process effacement and GBM duplication, but in contrast to humans, two abnormal genes are required (797). Interestingly, heterozygous transgenic mice expressing the mouse homolog of the human K256E *ACTN4* mutation develop proteinuria and FSGS (805). Nephrin mRNA and protein expression, but not synaptopodin protein expression, were reduced in mouse podocytes *in vivo*, suggesting that cytoskeletal alterations affect the podocyte transcriptional program. By contrast, homozygous knock-in mice bearing ACTN4 K228E manifested proteinuria and podocyte abnormalities but did not develop FSGS (798). These discrepancies between the two mouse models may indicate the limitations of this model system, perhaps due to the short life of the rodents, to model a disease that takes decades to develop in humans.

CD2AP

CD2-associated protein (CD2AP) was first identified as an adaptor protein that interacts with the cytoplasmic tail of CD2. Surprisingly, homozygous *CD2AP* null mutation mice develop diffuse global glomerulosclerosis (794) and heterozygotes develop FSGS (795). Subsequently it was demonstrated that CD2AP is expressed in podocytes, co-localized with F-actin in lamellipodia of cultured podocytes (1052) and to the slit diaphragm *in vivo* (1053). CD2AP interacts with F-actin (1054) and nephrin (1027,1055). CD2AP interacts with phosphatidylinositol 3-kinase, stimulating the AKT signaling pathway and suppressing apoptosis (1056). In a study of 45 African Americans with FSGS and collapsing glomerulopathy, 6 *CD2AP* variants were identified. One variant, affecting the splice effector site in exon 7, changes amino acid coding (P243S) and generates a truncated protein (795). Two unrelated FSGS patients had the *P234S* variant; one patient lacked a family history of kidney disease and the other patient had two family member with kidney disease of uncertain cause. Family studies of these individuals have not been performed, and so it remains unclear whether the variant is truly pathogenic.

TRPC6

Winn et al. recently reported that a missense mutation (*P112G*) in *TRCP6* (a member of the transient receptor potential channel protein family) was responsible for autosomal dominant FSGS in a New Zealand family of British origin (1057). Affected individuals presented in the third or fourth decades of life with heavy proteinuria and 60% progressed to ESRD. TRPC proteins are nonselective cation channels, which increase calcium flux in response to diacylglycerol. TRCP6 is expressed in the glomerular cells, probably including the podocyte, and the P112G mutant protein exhibits increased activity (1057). TRCP6 participates in VEGF receptor signaling (1058). Thus, it is possible that increased signaling via the VEGF or other receptors induces podocyte injury.

Mitochondrial Proteins: Mutations in Mitochondrial and Nuclear Genomes

The mitochondrial genome consists of a circular DNA molecule, approximately 16 kb in length. Mitochondrial DNA (mtDNA) encodes 13 essential subunits of the respiratory chain, 22 transfer RNA (tRNA) molecules, and 2 ribosomal RNA molecules. Cellular DNA replication is accompanied by mtDNA replication. mtDNA has a high rate of mutation when compared to nuclear DNA. Somatic cells possess 10 to 1,000 copies of mtDNA, whereas the oocyte has ~100,000 copies. When an mtDNA mutant arises or is inherited from a precursor cell, it exists in pool of mtDNA that includes wild type and mutant; this variety is termed *heteroplasmy*, and at least theoretically can range from near 0% to near 100%.

The MELAS syndrome involves children who present with mitochondrial encephalopathy, lactic acidosis, and strokelike episodes. In 1990, Goto et al. identified an mtDNA mutation in 26 of 31 MELAS patients; this mutation is located at position 3243 of mtDNA (A3243G) and involves mitochondrial $tRNA^{Leu}$ (1059). MELAS has been associated with FSGS in both children and adults, with at least 14 reports describing patients with FSGS (1060–1074). Of a total of 30 patients with FSGS described in these reports, 17 children had typical MELAS syndrome and FSGS and 13 pediatric and adult patients presented in an atypical fashion, either with isolated FSGS or with deafness or type 2 diabetes or both. Importantly, the extrarenal manifestations may become apparent only after renal disease has appeared, with a lag time in some cases of many years. The diagnosis requires evaluation of mtDNA by re-sequencing or by other molecular techniques. Due to heteroplasmy, leukocyte DNA may or may not show the mutation, and it may be important to test urinary cells or kidney

tissue. Of the 30 patients with FSGS, all but 2 patients have had the *A3243G* mutation; single patients have been described with other tRNA mutations: $tRNA^{Ile}$ A269G (1063) and $tRNA^{Tyr}$ A5843G (1064).

The mechanism of glomerular injury is not well understood, but giant (and presumably abnormal) mitochondria are present in podocytes and tubular epithelial cells (1072). Some patients have isolated tubulointerstitial nephritis, raising the possibility that in some cases FSGS could arise as a postadaptive response to nephron loss.

Recently, a mutation in the nuclear gene encoding the enzyme coenzyme Q synethase 2 (COQ2) has been indentified as cause of at least some cases of autosomal recessive infantile encephalopathy and nephropathy syndrome, where the renal histology typically shows FSGS (1074a). COQ2 is an essential enzyme in the synthesis of COQ10, also known as ubiquinone. COQ10 plays an important role in electron transport within the mitochondrion, and also serves as an antioxidant. Children with the syndrome present as infants or in some cases as young children; they may also manifest with cerebellar ataxia, sensorineural deafness, and myopathy involving skeletal muscle and heart. Other mutations in COQ10 synthetic enzymes may well exist and will likely present as phenocopies, with similar syndromic features.

Oligomeganephronia

Oligomeganephronia is characterized by bilateral renal hypoplasia without dysplasia or urinary tract abnormalities. Pathologic findings include diminished nephron number and glomerulomegaly, leading ultimately to postadaptive FSGS. Renal function is typically impaired from birth, rises as an absolute value during development, reaches a plateau in early childhood, and begins to decrease in middle or late childhood. By contrast, children and adults who present with a single kidney most commonly have renal aplasia rather than renal agenesis; the distinction lies in the fact that a small kidney is present at birth and subsequently undergoes regression (1075). This is a common finding, affecting 1:1,300 newborns, and does not appear to be associated with oligomeganephronia or FSGS.

Oligomeganephronia occurs in the setting of several congenital syndromes. The *renal-coloboma syndrome* is due to mutations in *PAX2*, one of a family of homeobox genes, encoding paired-box-containing transcription factors (1076). These children may present with syndromic oligomeganephronia, accompanied by ocular defects, or isolated oligomeganephronia (1077,1078). The *acro-renal ocular syndrome* may include various urinary tract abnormalities including renal hypoplasia (1079); some cases overlap with syndromes associated with *SALL4* mutations (1080). Patients with *branchio-oto-renal syndrome* present with mixed hearing loss, bilateral branchial cleft fistulas, and bilateral renal dysplasia; the gene responsible is unknown. The *bilateral renal agenesis or dysplasia syndrome* may be familial, with dominant inheritance (1081). The locus has not been identified.

FSGS Susceptibility Genes

The mutations listed in Table 64-19 have been associated with FSGS that is inherited in typical Mendelian fashion, and additional genes are likely to be identified in the years ahead. As noted in the preceding text, there are striking racial discrepancies in the incidence of FSGS that may be due to genetic variation, but the loci responsible are not well understood. It is likely that there is a subtle interplay between genetic susceptibility factors (genes, nephron number), environmental factors (viruses, toxins), and risk factors (obesity, hypertension, dietary protein) that determines risk for both idiopathic FSGS and postadaptive FSGS. Therefore, FSGS likely fits the pattern of a complex genetic disease, like hypertension, type 2 diabetes, and asthma, in which multiple genes are believed to interact with each other and with environmental factors. The search for susceptibility genes is challenging and requires convincing evidence of association. The probability of false positive identification is high, given the many genes and the many polymorphisms that can be tested. A convincing case that a polymorphism (or better, a haplotype covering most or all of a gene) is relevant, requires significant associations that are reproduced in multiple studies, ideally in geographically (and possibly racially) diverse populations.

The *ACE* gene is highly polymorphic, but most published renal disease association studies have focused on a single polymorphism: the intron 16 *Alu* element insertion/deletion polymorphism. This variant has not been shown to alter gene expression, but could conceivably be in linkage disequilibrium with pathogenic mutations. In Table 64-20 are presented the results of eight studies including a total of 324 patients (1082,1083) and other references. Although the results are not consistent in all populations, some evidence suggests that the D allele is weakly associated with FSGS in Asian populations and stronger evidence indicates that the D allele (or perhaps the DD genotype) may increase the risk for progressive loss of renal function.

TABLE 64-20

ASSOCIATION STUDIES OF *ACE* INSERTION/DELETION GENOTYPES AND FOCAL SEGMENTAL GLOMERULOSCLEROSIS (FSGS) AND FSGS PROGRESSION RISK

Country, year	FSGS cases (*N*)	Disease association	Progression association	Reference
Germany, 1997	17	No association	No association	(1084)
Korea, 1997	30	DD	DD	(1083)
Israel, 1998	47	No association	ID, DD	(1085)
Croatia, 2000	39	No association	DD	(1086)
USA, 2002	47	No association	DD	(744)
Japan, 2001	43	DD, ID	No association	(1082)
Germany, 2003	71	No association	ID, DD	(1087)
Turkey, 2004	30	No association	DD	(1088)

Eight published studies were identified that correlated the prevalence of the insertion/deletion *ACE* genotypes (II, DD, or ID) in FSGS cases and controls or progression risk (variously assessed as glomerular filtration rate [GFR] decline slope or end stage renal disease [ESRD] incidence).

Treatment of FSGS and Collapsing Glomerulopathy

The goal of treatment of all podocyte diseases is to reduce proteinuria to normal (ideally) or alternatively to the lowest level possible. There is increasing evidence that the level of proteinuria predicts long term outcome. The definitions of response are presented in Table 64-10.

Treatment of Children

Approaches to pediatric FSGS show considerable heterogeneity. In 1997, Vehaskari surveyed 181 members of the American Society of Pediatric Nephrology to learn their therapeutic practices (1089). Daily glucocorticoid therapy lasting more than 3 months was used often or sometimes by 50% of respondents (which the author found surprising, as there are no trials of this approach in children). Intravenous glucocorticoids were used often or sometimes by 52% of respondents. Oral cytotoxic therapy (cyclophosphamide or chlorambucil) were used often or sometimes by 85% of respondents. The combination of intravenous glucocorticoids plus oral cytotoxic therapy (the Tune-Mendoza protocol and variants) was used often or sometimes by 44% of respondents. Cyclosporine was used often or sometimes by 74% of respondents. ACE inhibitors were used often or sometimes by 92% of respondents.

Glucocorticoids. Because younger children undergo a therapeutic trial of corticosteroid prior to biopsy, they may be considered by the standard ISKDC nomenclature to be steroid resistant by the time they are diagnosed as having FSGS. However, most pediatricians do not wait for 8 to 12 weeks of nonresponse before performing a biopsy, and the ISKDC standard is sufficiently narrow that it does not preclude the possibility that patients will respond to some form of glucocorticoid therapy. Of those patients who are found to have FSGS, about 15% will respond to conventional oral steroid therapy and have a high likelihood of retaining kidney function long term. The remaining 85% of patients are steroid-resistant and are at substantial risk to progress to ESRD (1090).

More commonly, however, a more aggressive regimen is necessary for steroids to be effective. In the Tune-Mendoza treatment regimen (Table 64-21), patients receive high-dose, intravenous boluses of methylprednisolone (1093) with or without an accompanying alkylating agent (1091). On this regimen, more than half of patients developed stable remission and the majority had normal renal function several years later (1094,1095). However, treatment is accompanied by significant steroid toxicity, including neoplasm (1095) and serious infection (1096). The frequent administration of high-dose, intravenous medications also is labor-intensive. Nonetheless, this protocol continues to be used by a significant number of pediatric nephrologists (1097).

Calcineurin Inhibitors. As an alternative to prolonged glucocorticoid therapy, cyclosporine has been the subject of several randomized trials in pediatric SRNS. The New York-New Jersey Pediatric Nephrology study group randomized 25 patients to cyclosporine (3 mg/kg, to achieve a trough level of 300 to 500 ng/mL) or placebo treatment for 6 months (1098). Proteinuria decreased by 71% in the cyclosporine group and was unchanged in the placebo group, although the CR and

TABLE 64-21

COMPARISON OF STEROID DOSES FOR STEROID-RESISTANT NEPHROTIC SYNDROME OR FSGS

Protocol	Ref.	Age group	Duration	Child, (28 kg, 128 cm = 1 m^2) Total prednisone equivalent (g)	Adult (80 kg, 180 cm = 2 m^2) Total prednisone equivalent (g)
Tune-Mendoza MP 30 mg/kg IV three times weekly W1–2 MP 30 mg/kg IV weekly W3–10 MP 30 mg/kg IV every 2W W11–18 MP 30 mg/kg IV every 4W W19–50 MP 30 mg/kg IV every 8W W51–82	(1091)	Children	82 weeks	43.9	NA
Waldo MP 20 mg/kg IV three times weekly W1–2 MP 20 mg/kg IV weekly W3–10 MP 20 mg/kg IV every 2W W11–26 MP 20 mg/kg IV every 4W W27–78	(1092)	Children	78 weeks	24.0	NA
Commonly-used adult regimen Prednisone 1 mg/kg orally, W1–16 Prednisone 0.5 mg/kg orally, W17–24 Prednisone taper off, W26–32	(1148)	Adults	32 weeks	NA	12.6
NIH FSGS Dexamethasone 25 mg/m2, 4 pulses orally every 4 W	Unpub.	Adults	32 weeks	(5.0) No pediatric patients treated	10.0

MP, methylprednisolone; *W*, weeks. In the Tune-Mendoza and Waldo protocols, MP doses are capped at 1 g/dose. The conversion to prednisone-equivalent doses assumes 100% bioavailability and that the equipotent antiinflammatory doses are as follows: hydrocortisone 1, prednisone 4, methylprednisolone 5, and dexamethasone 25. Importantly, the Tune-Mendoza protocol also involves intermittent cyclophosphamide therapy.

PR responses are not provided. In one uncontrolled pediatric study, very high-dose cyclosporine was used to counteract the hyperlipidemia of unresponsive nephrotic patients (1099). In a smaller, double-blind controlled study, significant reduction in urinary protein was accomplished in patients receiving cyclosporine treatment (1100). Large, prospective studies have shown that cyclosporine may be at least as effective as high-dose methylprednisolone in treating FSGS (1101,1102). Tacrolimus has been used in a small number of steroid-resistant nephrotic patients including some with FSGS. The drug helped to control the level of urinary protein loss in some of these patients (1103). Given the positive results with cyclosporine and the potential benefits of tacrolimus data, calcineurin inhibitors are used frequently by the clinician treating steroid-resistant FSGS in children.

Cytotoxic Therapy. The response of children with FSGS to treatment with cytotoxic drugs has been extensively studied. A review of nine series involving children (702) revealed that 23% of 247 children with FSGS were steroid-responsive; 70 of the patients were treated with cytotoxic drugs, and 21 (30%) of the 70 responded; at the time of their last examination, 19.5% of the 247 children were in remission. It was not possible to determine how many of these children had FSGS documented when they presented with nephrotic syndrome and how many were found to have this lesion only when they became steroid-resistant after having previous episodes of steroid-responsive nephrotic syndrome. Although chlorambucil (1104) and intravenous boluses of cyclophosphamide (12) have been reported to be effective, these agents have not been evaluated in a larger, prospective controlled trial.

Treatment of Adults

There is a wide range of therapeutic approaches to adults with FSGS and collapsing glomerulopathy. (For simplicity, the following discussion will use the term FSGS for both conditions, as most references have not distinguished between the two disorders.)

Glucorticoids. Glucocorticoids are widely accepted as initial therapy for adult FSGS and collapsing glomerulopathy, although there is considerable controversy as to dose and duration of therapy and no randomized controlled trials have been carried out. The mechanism of action is unclear. Possible mechanisms include immunosuppression, activation of podocyte anti-oxidant enzymes (1105), and stabilization of the podocyte cytoskeleton (1106).

In Table 64-22 are summarized nine studies of glucocorticoid therapy for adult FSGS that have been reported in the last 20 years. Two studies included patients who received additional immunosuppressive therapy: 10% of patients in the Chicago study and 41% of patients in the Toronto study received such therapy; studies with more than 50% of patients treated with nonglucocorticoid therapy were excluded from this summary. Eight reports provide attained CR and PR rates, with relapse rates provided in four studies; the North Carolina study provided only sustained CR rates at follow-up. Two studies were prospective, uncontrolled trials and seven studies were retrospective analyses. Entry criteria included nephrotic-range proteinuria in five studies. Exclusion criteria included collapsing glomerulopathy in the North Carolina study, postadaptive FSGS, variously defined, in all but the Toronto study, and impaired GFR in the Italian study (patients with serum creatinine levels of >3 mg/dL). Although most patients in these studies likely had idiopathic FSGS, diagnostic standards for postadaptive FSGS have differed, so it is quite possible that some patients had postadaptive FSGS. The mean or median duration of high-dose daily glucocorticoid therapy (generally prednisolone or prednisone 1 mg/kg/day) was 2 to 4 months, and the duration of total therapy was a mean or median 3 to 9 months. Two studies used pulse therapy, either as initiation or as sole treatment.

Does glucocorticoid therapy increase the likelihood of remission? Spontaneous remission is rare, occurring with conservative treatment in 4% (two patient with CR) (1090) and 6% (two patients with PR) (1108). As shown in Table 64-22, with treatment, CR rates ranged from 0% to 44% and combined remission rates (CR plus PR) ranged from 33% to 59% (with data on PR lacking from two studies). None of the studies were randomized, but three studies described the outcome in a comparison group that did not receive immunosuppressive treatment. Rydel et al. reported that remission rates were higher with treatment (CR 33%, PR 17%) than with conservative treatment (CR 0%, PR 7%) (1108). Conversely, Stiles et al. noted that remission rates were similar in patients receiving either prednisone (0% CR, 42% PR) or conservative therapy including ACE inhibitors (0%, 60% PR) (1111). Franceschini et al. found that CR rates at 18 months of observation were similar with prednisone (11%) and conservative therapy (11%), but did not provide PR rates; it appears that CR is uncommon with ACE inhibitor and angiotensin receptor blocker (ARB) therapy (1110). One consideration is that aggressive use of ACE inhibitors for proteinuric patients is a relatively recent phenomenon, becoming more common after the publication of the landmark captopril trial in diabetic nephropathy in 1993 (1112). This might explain the lower response rate in the conservative treatment group observed by Rydel and colleagues (patients diagnosed 1975 to 1993), compared to Stiles and colleagues (patients diagnosed from 1992 to 1999).

Does a longer total duration of glucocorticoid therapy improve the chance of remission (combined CR plus PR) or CR? Two intrastudy comparisons argue for this hypothesis and two argue against this hypothesis. In the Italian study, patients treated longer than 16 weeks were also more likely to reach CR than those treated less than 16 weeks (61% versus 15%, relative risk 4.0, $p < 0.01$). Multivariate analysis indicated that only duration of glucocorticoid therapy (and not other clinical or histologic variables) predicted CR. These authors found that most responders entered remission after 6 months of therapy, although this observation derives from all treated patients (adults and children, glucocorticoids and other immunosuppressive therapy). In the Lucknow study, patients treated for more than 16 weeks were more likely to remit than those treated for less than 16 weeks ($p < 0.01$); the relative risk of remission was similar in nephrotic and non-nephrotic patients (1.6 and 1.5, respectively), although treatment duration was not significantly different between remitters and nonremitters in either group studied alone. Conversely, in the Chicago study, duration of daily prednisone therapy was similar in remitting patients and in nonremitting patients (5.7 versus 5.5 months). In the Toronto study, the median duration of daily prednisone was similar for those patients in CR at follow-up and those not in CR (5 months compared to 6 months). Furthermore, the median time to CR was 4 months, with a range of 0.5 to 6 months, which the authors interpreted to mean that therapy beyond 6 months is unlikely to add to the response rate.

Does a longer duration of high-dose daily therapy improve the chance of remission? Only one (retrospective) study addresses this hypothesis. In the Chicago study, duration of daily prednisone at a dose of ≥60 mg per day was greater in remitting patients than in nonremitting patients (mean 2.7 months versus mean 1.5 months, $p < 0.01$, and median 3 months versus median 1 month).

How do the high-response rate studies differ from the low-response rate studies? The studies can be arbitrarily dichotomized into four high-response studies (CR ≥25%) and

TABLE 64-22

CLINICAL TRIALS OF GLUCORTICOID THERAPY FOR ADULTS WITH FOCAL SEGMENTAL GLOMERULOSCLEROSIS (FSGS): REGIMEN, REMISSION, RELAPSE, AND LONG-TERM OUTCOME

First author location, year	Design	N	Black subjects	Nephrotic proteinuria	Glucocorticoid therapy	Total prednisone dose equivalent	Other immuno-supressive therapy	CR definition	CR	PR definition	PR	CR+ PR	Relapse	Final status (duration observation)	Reference
Agarwal, New Dehli, 1993	Prospective	65 (23 lost)	0%	100%	High dose 8–12 w Taper to 6 m	ND	None	<0.3 g/d	18%	2 g/d	15%	33%	NS	ESRD 5% (34 m)	(1107)
Rydel, Chicago, 1995	Retrospective	30	63%	100%	High dose ≥8 w in 67%, total therapy mean 5 m	ND	Cytotoxic therapy (10%)	<0.25 g/d	33%	<2.5 g/d	17%	50%	From CR or PR: 67%	CR 30%, PR 16% ESRD 20% (79 m) (additional therapy used)	(1108)
Cattran, Toronto, 1998	Retrospective	17	0%	100%	Total therapy median 5 m range 2–50 m	6.4 g	Cyclophos-phamide (41%)	<0.2 g/d	47%	ND	ND	ND	From CR: 38%	CR: 47% ESRD 29% (155 m)	(1090)
Ponticelli, Italy, 1999	Retrospective	53	0%	100%	A) High dose for 8 w, total therapy mean of 6 m B) High dose for 8 w, total therapy mean of 5 m	A) 6.4 g B) ND	None	<0.2 g/d	40%	<2 g/d	19%	58%	From CR or PR: 55%	ND	(1109)
Chitalia, New Zealand, 1999	Retrospective	28	0%	ND	High dose 6–8 w, maximum duration 12 w	ND	None	<0.3 g/d	21%	ND	ND	ND	ND	ND	(1166)
Stiles Washington DC, 2001	Retrospective	12	83%	100%	High dose prednisone mean 4 m (alternate day in 17%)	8.4 g	None	<0.2 g/d	0%	<3 g/d	42%	42%	ND	ND	(1111)
Pokhariyal, Lucknow, India, 2003	Retrospective	83 (12 lost)	0%	59%	High dose prednisolone mean 3 m	5.6 g	None	<0.3 g/d	25%	<2 g/d or 50% decrease if sub-nephrotic	25%	50%	ND	ND	(1114)
Franceschini, North Carolina, 2003	Retrospective	36	31%	ND	High dose daily or alternate day for mean of 9 m	10.6 g	Cyclophos-phamide 3, cyclosporine 3 (17%)	<0.6 g/d	ND	ND	ND	ND	ND	CR 11% ESRD 18% (27 m)	(1110)
Smith, Bethesda, 2003	Prospective	14	50%	100%	Pulse oral dexmaethsone for 8 m	8.6 g	None	<0.3 g/d	7%	<2 g/d	29%	36%	From CR or PR: 60%	CR 7%, PR: 14% ESRD 0% (20 m)	(1117)

The results of nine studies, describing 338 adult FSGS patients with nephrotic range proteinuria who were treated primarily with glucocorticoids, are presented in chronological order. The number (N) of patients with nephrotic range proteinuria who were treated with glucocorticoids is shown. Nephrotic range proteinuria was variously defined as >3 g/d, >3.5 g/d or >3 g/d/1.73 m^2. High dose prednisone is defined as prednisone or prednisolone at a dose of ~1 mg/kg/d. In the Italian study, patients received either regimen A) prednisone 1 mg/kg/d for 8 weeks, followed by a taper to a mean of 24 weeks, or regimen B) 3 doses of intravenous methylprednisolne, followed by prednisolone 0.5 mg/kg/d for 8 weeks, and then a taper to a mean of 19 weeks. In the Bethesda study, patients received oral dexamethasone at a dose of 100 mg/m^2 monthly. For total mean prednisone dose, the value was provided by the reference or was calculated from the mean duration and assumes a mean weight of 70 kg if the reference described the total dose in mg/kg or a mean body size of 1.73 m^2 if the dosing was by body surface area. The definitions of complete remission (CR) and partial remission (PR) are shown. The two studies from India lacked complete follow-up for end-point determination; for the present analysis, calculation of outcome made here on the conservative intention to treat basis with the assumption that patients lost to follow-up were non-responders. The mean duration of follow-up is shown and percent of patients who achieved remission are shown. *CKD*, chronic kidney disease; *ESRD*, end-stage renal disease; *ND*, no data provided.

four low-response studies (CR <25%). The North Carolina study is excluded because it did not provide attained response rates and provided sustained response rates at follow-up. The high-response studies (Chicago, Toronto, Italy, and Lucknow) showed CR rates of 25% to 44% and combined response rates of 50% to 59% (PR data were not provided in the Toronto study). The low-response studies (New Delhi, New Zealand, Washington DC, and Bethesda) showed attained CR rates of 0% to 21% and combined response rates of 33% to 42%. (The Lucknow study would have been classified as a high-response study (31% CR) if the analysis excluded lost patients, as the authors proposed). A qualitative interstudy comparison of the high-response studies with the low-response studies follows:

- Similar prevalence of African Americans in high-response studies (0, 0, 0, 63%) and low response studies (0, 0, 50, 83%)
- Similar *mean duration of high dose* daily glucocorticoid therapy (approximately 1 mg/kg) in high-response studies (8, 8, 12, 12 weeks) compared to low-response studies (7, 10, 16 weeks; not applicable in the Bethesda study)
- Similar *minimum duration of high dose* daily glucocorticoid therapy (approximately 1 mg/kg), specified in only three studies: high-response studies (8 weeks in the Chicago study) versus low-response studies (6 weeks in the New Zealand study, 8 weeks in the New Delhi study)
- Similar use of *daily* glucocorticoid therapy (as opposed to alternate day or intermittent pulse therapy) in high response studies (3 of 3 studies, excluding the Italian study which used both) and low response studies (3 of 4 studies; the Washington DC study used alternate day therapy in 17% and the Bethesda study using pulse therapy in 100%)
- Similar *mean/median total duration* of glucocorticoid therapy in high-response studies (3, 5, 5.5, 5 months) compared to low-response studies (4, 6 months; not applicable in the Bethesda study and no data from the New Zealand study)
- Similar total prednisone equivalent doses in high-response studies, when data were provided (5.6, 6.4, 6.4 g) compared to low-response studies (8.4, 8.6 g)
- More use of other immunosuppressive therapy in high response studies (2 of 4 studies) than in the low-response studies (0 of 4 studies)

Although these interstudy comparisons are qualitative in nature, because of the limitations of varying study design and study reporting, they do not suggest any compelling hypotheses to explain the difference between high-response and low-response studies.

Relapses were common, occurring in 38% to 67% of remitting patients. Often, patients who experienced relapse were brought back into remission with additional glucocorticoid therapy or other treatment. Surprisingly, remission status at last follow-up was generally higher with greater length of observation (duration from presentation or from initiation of therapy), ranging from 11% (CR only, mean 18 months observation) through 21% (CR plus PR, mean 20 months observation) to 46% (CR plus PR, mean 79 months observation) and 44% (CR only, 155 months).

Do children respond better than adults to therapy, including with glucocorticoids? In the only study that compared children and adults, Cattran et al. suggested that CR rates were similar in children (47%) and adults (44%) (1090).

Can clinicians identify patients who are most likely to respond to glucocorticoid therapy? Shiiki et al. studied the likelihood of response to any immunosuppressive therapy in 35 adults with idiopathic FSGS, of whom 66% experienced a CR or PR, and found the following were significant predictors of nonresponse on multivariate logistic regression analysis: larger mean glomerular diameter (OR 2.93, CI 1.07–7.92, $p = 0.04$) and more severe tubulointerstitial changes (OR 8.86, CI 1.06–43.9, $p = 0.04$) (1113). By contrast, other investigators studying 30 adult FSGS patients (1108) and 81 adult FSGS patients (1114) could not identify any clinical or histologic features that predicted response to therapy on multivariate analysis.

What is the toxicity of a prolonged course of glucocorticoid therapy in adults? There are few published data on the toxicity of daily prednisone when used for FSGS. The Toronto study, with mean observation time of 155 months, reported four deaths (two patients who received only glucocorticoids, one patient who had received glucocorticoids and cyclophosphamide, and one patient who received neither). The Washington, DC study, with a mean observation time of 18 months, reported that 41% of patients experienced severe adverse events (diabetes mellitus, hypertension exacerbation, cellulitis, and severe myopathy). In the Lucknow study, 16% of patients experienced significant adverse events (diabetes mellitus, infections, Cushingoid facies, and dyspepsia).

Is alternative day glucocorticoid therapy an effective option, with the prospect of less toxicity? This regimen is widely used, particularly for patients who are at increased risk of adverse events, but the published data supporting this regimen are sparse. In 1977, Bolton et al. reported on the treatment of 81 adult patients with nephrotic syndrome, including 10 patients with FSGS, using prednisone 60 to 120 mg on alternate days for up to 10 years (1115). In the FSGS group, CR or PR (defined as a >50% decrease and <3 g/day) occurred in 3 (30%). In the entire patient group, the regimen was well tolerated, being associated with one adverse event per 12 patient-years of therapy. These adverse events included Cushingoid facies (10%), cataracts (6%), bone disease (5%), psychosis (1%), cytopenia (7%), infection (2%), and stroke (2%). Nagai et al. reported a case series of 17 patients older than age 60 with FSGS; 82% were nephrotic. Nine patients received 100 mg prednisone on alternate days, with two patients also receiving oral cyclophosphamide (1116). Five patients entered remission (4 CR, 1 PR), all of whom received prednisone only. A control group of 8 patients did not receive treatment and experienced only 1 PR. Two patients died during follow-up (of pancreatic cancer and stroke); other adverse events were not described. These limited data do support further studies of alternate-day therapy. Some patients in the North Carolina study received alternate-day prednisone, but there no data were presented comparing daily and alternate day therapy (1110).

If the high response-rate studies suggest that total therapy duration in excess of 4 months is desirable, does intermittent pulse therapy for an extended duration offer similar efficacy with an acceptable safety profile? The Bethesda study was a small open-label study of oral pulse dexamethasone (25 mg/m^2, given on each of the first 4 days of each 4 week cycle), administered over 32 weeks to 14 patients (1117). A distinguishing feature of the inclusion criteria was nephrotic proteinuria despite aggressive ACE inhibitor or ARB therapy, which might be expected to reduce the PR rate compared to studies that added these agents as part of the regimen. The efficacy outcomes would place this study in the low-response category: CR response rate (7%) and combined CR and PR response rates (36%). A low rate of adverse events was observed, limited to bone loss exceeding 3% in one patient (7%) and no hypertension, diabetes mellitus, avascular necrosis, glaucoma, cataract, or adrenal suppression.

Calcineurin Inhibitors and Cytotoxic Therapy. The literature on glucocorticoids is extensive due to the number of studies available and the controversies involved. By contrast, calcineurin inhibitors, and cyclosporine in particular, are generally accepted as standard therapy, although these agents do have significant limitations. Cyclosporine has been used to treat FSGS for approximately 20 years, as reviewed recently (1118).

Six studies have suggested combined CR and PR rates of 30% to 80%, CR rates of 0% to 25%, and relapse rates of 43% to 100% (699,1119–1123). Two of these trials were prospective trials. Ponticelli et al. studied 28 adult patients and 19 pediatric patients with FSGS; of those receiving cyclosporine 57% experienced CR or PR and 40% of these remained in remission after 2 years (1124). In the highest quality study, Cattran et al. studied 49 adult FSGS patients with steroid resistance, defined as nephrotic proteinuria despite ≥8 weeks of glucocorticoid therapy (1123). Patients were randomized to cyclosporine or placebo, together with low-dose prednisone (0.15 mg/kg/day). At the end of the study, CR occurred in 12% of the treatment arm and 0% of the control arm, whereas PR (defined as a 50% decrease in proteinuria, becoming subnephrotic, and a preserved GFR) occurred in 57% of the treatment arm and 4% of the control arm. At 78 weeks and again at 104 weeks of follow-up, 50% of those with CR and PR had experienced relapse. Cyclosporine also significantly reduced the chance of doubling serum creatinine over a 2-year period. Taken together, these six studies show a consistent effect of cyclosporine to reduce proteinuria in patients with FSGS (and possibly collapsing glomerulopathy, although this diagnosis was not standard when these trials were carried out).

Current recommendations are typically to initiate cyclosporine therapy at a dose of 3.5 mg/kg per day in a divided dose, with dose adjustment to achieve trough levels of 125 to 225 ng/mL (some nephrologists might aim for the slightly lower level, perhaps 100 to 150 ng/mL) (1125). Prednisone is not generally added, unless a patient is already receiving it. Given the high relapse rate, many nephrologists opt to extend therapy to 1 or 2 years, or in some cases for a longer duration. A note of caution about pursuing prolonged cyclosporine therapy was raised by Meyrier and colleagues, who noted that histologic injury progressed in some patients, including some patients who remained in CR (1126). Risk factors for histologic progression included cyclosporine dose >5.5 mg per day, renal insufficiency prior to initiating cyclosporine therapy, and a high percentage of glomeruli with FSGS on initial renal biopsy. It seems prudent to taper cyclosporine over several months when a patient has been in CR or PR, rather than abrupt cessation of therapy, but there are no published data that support this recommendation.

There are limited data addressing the use of tacrolimus in FSGS. Three studies involving a total 29 patients have been published: one study in children (711) and two studies in adults (1103,1127). There is no reason to predict that tacrolimus would be superior to cyclosporine in terms of nephrotoxicity, and the response rates appear to be similar to those seen with cyclosporine. The toxicity profile differs between cyclosporine and tacrolimus and this may influence the selection medication for a particular patient.

The mechanisms by which cyclosporine and tacrolimus reduce proteinuria, in primary podocyte diseases and in other glomerular diseases, are unknown. The immunosuppressive action of these agents may contribute, but there is no evidence that this is the case. Sharma et al. demonstrated that cyclosporine antagonizes the permeability enhancing properties of FSGS permeability factor on isolated rat glomeruli, suggesting that cyclosporine may have direct effects on glomerular cells (1128,1129).

Cytotoxic therapy, including cyclophosphamide and chlorambucil, plays a very limited role in adults with steroid-resistant FSGS and collapsing glomerulopathy. Korbet et al. reviewed the literature and found that among 105 steroid-resistant patients, cytotoxic therapy was associated with CR in 11% and PR in 11% (1130). By contrast, the responses were better among 33 steroid-responsive patients (CR 52%, PR 24%), suggesting that in the patient with steroid-dependent or frequently relapsing FSGS, these agents may be a reasonable choice.

Mycophenolate Mofetil. Cyclosporine has demonstrated but limited efficacy in treatment of FSGS, and has the drawbacks of exacerbating hypertension and potential nephrotoxicity with prolonged therapy. Mycophenolate mofetil (MMF) is an appealing alternative therapy. Choi et al. in Baltimore reported results with MMF used in clinical practice in 18 adults with FSGS (at least one of these patients had collapsing glomerulopathy) (1131). CR was defined as a urine protein/creatinine ratio of <0.3; PR was defined as a ≥50% fall in the urine protein/creatinine ratio. MMF was administered for a mean of 8 months (range 4 to 22 months) to 9 nephrotic patients, 7 of whom also received glucocorticoids. At the end of treatment, 1 patient was in CR, 3 in PR, and 5 did not respond (response rate 44%). At follow-up, 2 patients were in CR (MMF continued and 8 months after MMF withdrawal, respectively) and 1 patient was in PR (12 months after MMF withdrawal), for a sustained response rate of 33%.

Cattran et al. reported the results of a prospective, multicenter uncontrolled study of patients with glucocorticoid-resistant FSGS in Toronto, Baltimore, and New York (1132); 18 patients participated, of whom only 22% were African American. Therapy consisted of 6 months of MMF (dosage 1.5 g/day, actual duration 3 to 18 months, as two patients were noncompliant) combined with prednisone (0.25 mg/kg/day, tapered to a maximum of 10 mg/day over 6 weeks). Inclusion criteria included nephrotic range proteinuria and creatinine clearance ≥30 mL/minute/1.73 m^2. Outcomes included 0 CR; 6 PR (defined as 50% fall, becoming subnephrotic, and stable GFR), 33%; 2 patients with proteinuria response (defined as 50% fall in proteinuria), 11%; and 10 patients with no response, 56%. Among the 6 PR, further follow-up included 1 CR on MMF plus cyclosporine, 1 continued PR on MMF, 2 sustained PR off MMF (duration of observation off therapy approximately 10 months) and 2 relapses off MMF. Therefore, the sustained PR off-therapy was 12% (2/16 patients, with 2 patients continuing therapy). These results are less favorable than those from the initial single-center report. Further studies are needed to examine efficacy, in particular, sustained remission rates, in additional populations.

Effect of Immunosuppressive Therapy on Renal Survival. Does immunosuppressive therapy (glucocorticoids alone or glucocorticoids combined with other therapy) increase the likelihood of renal survival? There are no randomized controlled studies that address this question, and, therefore, the possibility of treatment selection bias limits the utility of the four nonrandomized studies that are available. Alexopoulos et al., in a study from Greece, found improved survival in treated nephrotic patients compared with untreated nephrotic patients, with 5-year renal survival of 86% versus 65% (p <0.03 by Kaplan Meier survival analysis (1133). Four retrospective studies were identified that provided outcome analysis for immunosuppressive treatment and control groups, with separate identification of nephrotic and non-nephrotic patients (Table 64-23). A pooled analysis of these studies shows that immunosuppressive therapy is associated with relative risk of ESRD of 0.52 (CI 0.32–0.84, $p = 0.02$) in nephrotic patients and 0.45 (CI 0.11–1.84, p = NS) in non-nephrotic patients. Therefore, the treatment-effect size is similar in nephrotic and non-nephrotic patients, but only in nephrotic patients is the treatment effect statistically significant. The Greek study is an outlier in two respects: use of other immunosuppressive therapy (70% of patients, compared with 10% in the other three studies combined) and the effect size (no ESRD observed with treatment of nephrotic patients). If the Greek study is omitted from analysis, the relative risk of ESRD with treatment in the remaining three studies is 0.59 (CI 0.36–0.97), which remains statistically significant ($p = 0.02$) and clinically significant.

TABLE 64-23

EFFECT OF IMMUNOSUPPRESSIVE TREATMENT ON LONG-TERM RENAL SURVIVAL IN FSGS

Location, year	Population	Immunosuppressive treatment	Duration of observation	Nephrotic, treated	Nephrotic, untreated	Relative risk of ESRD in treatment group (CI)	Nonnephrotic, treated	Nonnephrotic, untreated	Relative risk of ESRD in treatment group (CI)	Reference
Washington, DC, 2001	Adults	Prednisone	~20 F/U	ESRD 2 No ESRD 10	ESRD 3 No ESRD 7	0.55 (0.11–2.70)	ND	ND		(1111)
Greece, 2000	Adults	Prednisolone; CSA, CTX in some patients	57 mo	ESRD 0 No ESRD 11	ESRD 3 No ESRD 3	0.43 (0.12–1.5)	ESRD 2 No ESRD 5	ESRD 6 No ESRD 3	0.43 (0.12–1.51)	(1133)
Chicago, 1995	Adults	Prednisone cytotoxic therapy in some patients	79 mo	ESRD 6 No ESRD 24	ESRD 10 No ESRD 20	0.80 (0.33–2.0)	ND	ND		(1108)
Ontario, 1998	Children, adults	Prednisone; cyclophosphamide in some patients	155 mo	ESRD 16 No ESRD 33	ESRD 15 No ESRD 15	0.65 (0.38–1.11)	ESRD 1 No ESRD 2	ESRD 4 No ESRD 7	0.92 (0.15–5.4)	(1090)
Total				ESRD 24 No ESRD 78	ESRD 31 No ESRD 45	**0.58 (0.37–0.90)** **p = 0.02**	ESRD 3 No ESRD 7	ESRD 10 No ESRD 10	**0.60 (0.27–1.70)** **p = 0.44**	

Four retrospective studies are presented in order of increasing mean duration of observation (months). All treatment regimens included glucocorticoids; other immunosuppressive agents were used in selected patients as shown. The relative risk of end-stage renal disease (ESRD) and the 95% confidence interval (CI) for the outcome data from each study are shown; none of the individual studies showed a significant treatment effect when analyzed by Fisher exact test (not shown). Although each study individually is underpowered, there is a consistent pattern favoring treatment in both nephrotic and non-nephrotic patients. Pooled analyses suggest a clinically and statistically significant treatment effect in nephrotic patients, with relative risk for ESRD of 0.58. The treatment effect was of the same magnitude in non-nephrotic patients, although the group size is smaller and the results were not statistically significant.

Without randomized trials, the problem of treatment selection bias is a major concern and greatly limits the impact of these findings. In this regard, the authors of the North Carolina study carried out proportional hazards analysis and found that when other controlling for relevant clinical factors (age, sex, race, smoking status, use of ACE inhibitors or ARB, entry serum creatinine level, and entry proteinuria), immunosuppressive therapy had no effect on renal survival (1110).

Other Therapies. Several other therapeutic approaches have been tried for therapy-resistant FSGS patients. In an uncontrolled pilot study, the nonsteroidal anti-inflammatory agent meclofenamate reduced urinary protein loss by 40% without decreasing GFR in more than half of the 30 steroid-resistant nephrotic adults studied, 16 of whom had FSGS (1134). The antioxidant vitamin E, 200 IU twice daily, was studied in an open-label study involving 11 therapy-resistant pediatric FSGS patients and was associated with a fall in the mean protein/creatinine ratio from 9.7 to 4.1 ($p < 0.005$) (1135). The antifibrotic agent pirfenidone was also studied in an open-label trial involving 20 adult FSGS patients and was found to slow progressive loss of GFR by 35% ($p < 0.03$) without affecting proteinuria (1136).

Although plasmapheresis has been used to treat recurrent FSGS in patients undergoing transplantation (see Transplantation section, later in this chapter), there is no evidence to support routine use of this treatment in FSGS occurring in native kidneys (1137).

Conservative Therapy. All patients, whether they are receiving immunosuppressive therapy or not, should adhere to a conservative regimen if they have proteinuria (best evidence for this recommendation if proteinuria >1 g/day) or impaired GFR. Blood pressure should be controlled to <130/80 mm Hg as recommended by the Joint National Committee VII (1138). ACE inhibitors and ARB reduce proteinuria and slow progression in both diabetic and nondiabetic nephropathies, and the COOPERATE study demonstrated that the combination was superior to either agent alone in slowing progression a combined endpoint of creatinine doubling or ESRD (1139).

Although certain ACE inhibitor trials have included FSGS among multiple diagnoses (1140), few studies have provided data on substantial numbers of idiopathic FSGS patients (reviewed in [1141]). Stiles et al. retrospectively compared the results in 22 nephrotic adults with idiopathic FSGS, of whom 10 received prednisone at a dose of approximately 1 mg/kg per day for a mean of 4 months (range 1 to 6 months) plus ACE inhibitors and 10 received ACE inhibitors alone (1111). Outcomes were similar, with no CR, and PR occurring in 5 patients and 6 patients, respectively. Proteinuria fell by 33% with glucocorticoids and 65% with ACE inhibitors. The patient population at the Walter Reed Army Medical Center was predominantly young and male and disproportionately (86%) African American. The authors concluded that while the study was too small for definitive conclusions, conservative therapy was not inferior to glucocorticoids in this population. In a small controlled trial, losartan reduced proteinuria from 3.6 to 1.9 g per day in 13 FSGS patients, whereas a control group experienced an increase in proteinuria (1142). Conversely, a retrospective study of 42 African American patients treated with ACE inhibitors, glucocorticoids, both, or neither, was unable to demonstrate a protective effect of ACE inhibitors in delaying progression to ESRD compared to 6 untreated patients (1143).

ACE inhibitors and ARB have a major role to play in postadaptive FSGS. Praga et al. showed that captopril therapy over 12 months reduced proteinuria to greater extent in postadaptive FSGS (associated with reduced nephron mass and reflux nephropathy) compared to idiopathic FSGS (1144). Next Praga and colleagues studied 17 patients with obesity-associated proteinuria (>1 g/day); renal histology obtained from 5 patients documented FSGS in only 2 patients (1145). Captopril therapy in 8 patients lowered proteinuria by 79% (mean 3.4 g/day falling to 0.7 g/day); interestingly, weight loss in 9 patients (mean BMI falling from 37 kg/m^2 to 33 kg/m^2) was associated with a similar fall in proteinuria, by 86% (mean 2.9 g/day falling to 0.4 g/day). Unfortunately, Praga and colleagues subsequently reported that the proteinuria reduction induced by ACE inhibitors is lost after 12 months (845). Still, early treatment with ACE inhibitors appeared to preserve renal function in obesity-associated FSGS, although appropriate controls are lacking.

Current Therapeutic Recommendations. Some experts propose that nephrotic adults with idiopathic FSGS who have preserved renal function and lack prominent contraindications, receive 4 to 6 months of daily therapy with glucocorticoids beginning with prednisone at a dose of 1 mg/kg per day (maximum 80 mg) (1141,1146,1147). Many experts would recommend a dose reduction after 3 to 4 months, particularly for those who have not shown a significant reduction in urine protein. Those who fail 4 to 6 months of daily glucocorticoid therapy are then labeled steroid-resistant and progress to other therapies.

The Nephrology and Hypertension Medical Knowledge Self-Assessment Program expresses reluctance to endorse this aggressive approach in the absence of a controlled trial demonstrating efficacy and safety (1148). Instead, this text recommends, for adults with FSGS and serum creatinine <2.5 mg/dL and <20% interstitial fibrosis area on renal biopsy, a trial of daily prednisone at a dose of 1 mg/kg of ideal body weight (to a maximum dose of 80 mg/day), until CR or *8 weeks* have been reached. In the case of a CR, the recommendation is to prescribe an ~20-week tapering schedule of prednisone on alternate days, tapering at a rate of 20 mg every month. If there is no CR, the recommendation is to reduce the dose to 0.5 mg/kg until a CR or PR or *another 8 weeks* have been reached. If there is a CR or PR, the recommendation is to prescribe an ~20-week tapering schedule of alternate day prednisone, which would require tapering at a rate of 10 mg every other month. If there is no response, the recommendation, is a slow taper involving *~40 weeks* of alternative-day prednisone while adjunctive therapy (ACE inhibitors or ARB are initiated). Therefore, patients with steroid-resistance would receive 16 weeks of daily prednisone and up to 40 weeks of alternate daily prednisone, for a total dose in an 80-kg person of 7.7 g. Further options during prolonged taper would include cyclosporine for 1 year or oral cyclophosphamide or chlorambucil for 8 to 12 weeks. A newer option that was not included would be mycophenolate mofetil.

Hildebrandt and colleagues noted that that pediatric patients with homozygous or compound heterozygous *NPHS2* mutations appear refractory to glucocorticoids (defined as 6 weeks of therapy) and have only limited responsiveness to cyclosporine (1021). By contrast, *NPHS2* homozygous and compound heterozygous mutations were absent from 124 children with steroid-sensitive nephrotic syndrome. Although these data are not representative of all racial and ethnic groups, it would appear at the present time that immunosuppressive treatment is of limited benefit in children with *NPHS2* homozygous and compound heterozygous mutations.

We have few data about the adult patients with *ACTN4*, *CD2AP*, *TRPC6*, and mtDNA mutations, but it would seem prudent to withhold or limit the duration of immunosuppressive therapy, particularly glucocorticoids. Similarly, FSGS or collapsing glomerulopathy associated with medication is probably best managed by withdrawing the implicated medication and instituting conservative therapy.

It should be noted that there are significant parallels but some differences between the approach to pediatric and adult

patients. In particular, the majority of nephrotic children (those age 1 to 9 years) will have received an empirical trial of corticosteroids for 4 to 8 weeks before biopsy, and already been defined as being steroid resistant, albeit by ISKDC criteria rather than by response to the longer course used in adults. This may be appropriate given the relatively aggressive course of classical FSGS often observed in young children. There are many areas of uncertainty where there are no published data to determine the balance between the risks and benefits of immunosuppressive therapy, including the following patient populations:

- Patients with subnephrotic proteinuria at presentation, especially those with proteinuria <2 g per/day; most experts would not use immunosuppressive therapy
- Patients who become subnephrotic with therapy with ACE inhibitors, ARB, or the combination, coupled with sodium restriction and blood pressure control to <130/80 mm Hg, particularly those proteinuria falls to <2 g per/day
- Patients with postadaptive FSGS, with the diagnosis established by compatible history and glomerulomegaly on renal biopsy
- Patients with idiopathic FSGS who are at significantly increased risk of toxicity associated with glucocorticoid therapy, including with those with obesity (particularly BMI >35 kg/m^2), diabetes mellitus or a pre-diabetic state, severe osteoporosis, prominent peptic ulcer diathesis, or advanced age

Novel Therapies. New approaches to therapy may supplement existing medication in one of several ways: (a) agents that induce a durable CR with less toxicity than glucocorticoids and cyclosporine, (b) agents that reduce proteinuria to a greater extent than ACE inhibitors and ARBs, and (c) agents that slow progressive renal scarring in patients who have proven refractory to remittive therapy. In Table 64-24 is provided a nonexhaustive list of 21 agents, including small molecules, peptides, and other biologics, which have shown promise in animal models of glomerulosclerosis, including but not exclusively FSGS, either reducing proteinuria or retarding progression of fibrosis or both (1149–1167). For some agents, further animal testing is required to define efficacy. For other agents, human trials in one or more forms of podocyte disease are probably warranted.

Clinical Course and Outcome

FSGS and collapsing glomerulopathy have the worst prognosis of the common primary nephrotic diseases. Ten-year event rates for reaching a combined endpoint of ESRD or death are as follows: for children, USA study, 21% (410) and Toronto study, 32% (1090), and for adults, Toronto study, ~28% (1090), New Zealand study, 38% (1166), Hong Kong study, 40% (1167), and Chicago study, 43% (ESRD only) for nephrotic patients (compared with 8% for nonnephrotic patients) (1108) (Table 64-25).

It has been known for some time that FSGS patients who enter a CR, even if they experience a relapse, have an excellent chance of renal survival. Recently, Cattran et al. showed that patients who experience a PR (defined as a 50% fall in proteinuria and a fall to <3.5 g/day) have a significantly improved renal survival (Fig. 64-17). These data provide justification for considering the combined response rate (CR plus PR) as well as CR alone in weighing the relative costs and benefits of particular therapeutic approaches.

What clinical factors predict long-term prognosis for renal survival? The conflicting data on the impact of treatment on outcome have been reviewed in the preceding text. In

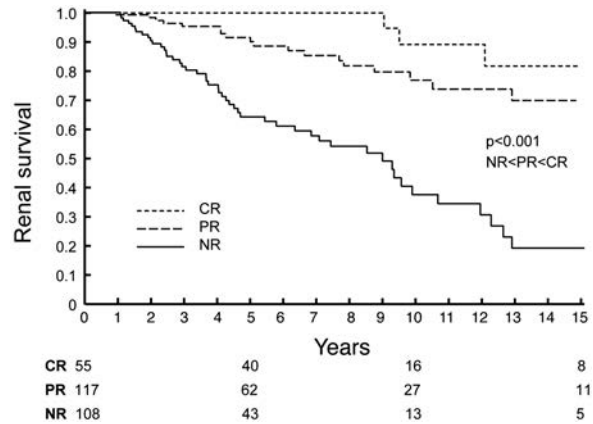

FIGURE 64-17. Long-term renal survival in FSGS by remission status. The fraction of patients with renal function is shown in nephrotic FSGS patients who enter CR (defined as <0.3 g/day) or PR50% (defined as a 50% fall in proteinuria and falling to <3.5 g/day), and those who never reach either remission (NR). (Adapted from: Troyanov S, Wall CA, Miller JA, et al. Focal and segmental glomerulosclerosis: definition and relevance of a partial remission. *J Am Soc Nephrol* 2005;16:1061, with permission.)

Table 64-26 are summarized the findings of 10 studies that have sought clinical and histologic variables that predict long-term renal function. The variables that consistently appear as significant predictors include baseline serum creatinine, baseline proteinuria, and tubulointerstitial damage/fibrosis score. The role of black race has been controversial. Ingulli and Tejani found that among children, progression to ESRD occurred in 78% of blacks and 33% of whites, despite similar baseline serum creatinine and proteinuria values (743). Conversely, Korbet and colleagues have found that in adults the progression rates are similar among blacks (64%) and whites (55%) (1108). They did find that blacks were more likely to be nephrotic at presentation (88% versus 55%). Earlier in the chapter, data were reviewed from nonrandomized controlled studies that treatment may improve renal survival. Thus, patients who enter CR have an excellent outcome, with few developing ESRD on follow-up (1090,1102).

Recurrent FSGS After Renal Transplantation

FSGS recurs following renal transplantation with a frequency that ranges from 15% to 50% depending upon the population, with the average figure cited typically being approximately 25%. When a prior allograft has manifested recurrent FSGS, the risk of recurrence rises to 70% to 80%. Recurrence is heralded by the sudden appearance of heavy proteinuria, often within the first week after transplantation. Renal biopsy obtained within the first few weeks of recurrence uniformly shows extensive foot process effacement without other changes. Subsequent biopsies commonly demonstrate FSGS. Most recurrent FSGS appears within the first 6 months following renal transplantation, as shown in the graphic depiction of data abstracted from 13 reports (1173–1186), and unpublished data kindly provided by Dr. Alok Kalia (Fig. 64-18). FSGS that appears more than 12 months after renal transplantation is typically not considered recurrent FSGS; alternative diagnoses include *de novo* FSGS and FSGS appearing as a manifestation of progressive chronic allograft nephropathy.

In patients with low-level proteinuria originating from their native kidneys, screening for recurrent FSGS may be done via urine dipstick testing performed by the patient (or parents). For those with significant proteinuria from their native kidneys, it is advisable to obtain monthly urine protein/creatinine

TABLE 64-24

NOVEL THERAPIES FOR PROGRESSIVE GLOMERULOSCLEROSIS

Class	Agent	Molecular target or pathway	Animal model	Ref.	Proteinuria	Matrix	Human use
Small molecules	Bosentan	ET_A, ET_B receptor antagonist	Ren-2 transgenic rats	(1149)	Reduced	Reduced	Approved
	Darusentan	ET_A receptor antagonist	Puromycin (rat)	(908)	Reduced	Reduced	IND
	Thiazolidine-diones	PPAR-gamma agonist	Remnant nephron (rat)	(1150)			Approved
	Eplerenone	Mineralocorticoid receptor	Uninephrectomy plus NaCl (rat)	(1151)	Reduced	ND	Approved
	Isotretinoin	Retinoid acid receptor	Thy-1 nephritis (rat)	(1152)	Reduced	Reduced	Approved
	Gefitinib	EGF-receptor tyrosine kinase inhibitor	L-NAME induced hypertension (rat)	(1153)		Reduced	Approved
	Pirfenidone	TGF-β expression? TNF signaling?	Remnant nephron, KIST mouse	(1154)	Reduced	Reduced	IND
	Tranilast	TGF-β antagonist	Remnant nephron	(1155)	Reduced	Reduced	IND
	AVE 7688	ACE and NEP inhibitor	Remnant nephron	(1156)	Reduced	Reduced	Pre-clinical
	CDK inhibitors	Cell cycle	HIV-transgenic mouse	(1157)			Pre-clinical
	Fasudil	Rho-kinase inhibitor	Dahl rat	(1158)	Reduced	Reduced	Pre-clinical
	Prolyl hydroxylase inhibitors	Collagen cross-linking	Chronic allograft nephropathy (mouse)	(1159)	ND	Reduced	Pre-clinical
	BX471	CCR1 antagonist	Adriamycin	(896)	No effect	Reduced	Pre-clinical
Peptides	IFN-γ	Fibroblasts	Remnant nephron	(1160)	Reduced	Reduced	Approved
	Bone morphogenetic protein 7	Possible TGF-β antagonist	COL4A3 null mutation mouse	(1161)	Reduced	Reduced	IND
	Hepatocyte growth factor	Possible TGF-β antagonist	ICGN mouse	(1162)	ND	Reduced	Pre-clinical
	Relaxin	Not defined	Remnant nephron (rat)	(1163)	ND	Reduced	Pre-clinical
	Decorin (proteoglycan)	Binds TGF-β	Thy-1 nephritis	(1164)	ND	ND	Pre-clinical
Monoclonal Ab and others	Etanercept, Infliximab	Binds TNF-α	ND	ND	ND	ND	Approved
	Anti-TGF-β	Binds TGF-β	Puromycin	(1165)	ND	Reduced	IND
	Sulodexide (GAG)	Unknown	ND	ND	ND	ND	IND

Approved denotes approved by the FDA for a nonrenal indication. *IND* denotes under study for any indication under the Investigational New Drug process of the FDA. *CCR1*, chemokine receptor; *CDK*, cyclin-dependent kinase; *ET*, endothelin; *GAG*, glycosaminoglycan; L-*NAME*, NG-nitro-L-arginine methyl-ester; *ND*, no data.

ratios during the first 12 months, and particularly for the first 6 months following renal transplantation. In either case, a significant worsening of proteinuria is an indication for immediate renal biopsy to establish the diagnosis. Response to therapy appears substantially greater if initiated within 2 to 4 weeks of clinical recurrence.

Pretransplantation risk factors for recurrent FSGS include the following: rapid progression of initial disease, typically defined as <3 years from diagnosis to ESRD; age of onset of FSGS between 6 and 15 years; mesangial hyperplasia; white race and recurrent FSGS in a prior renal transplant (1187). The risk of recurrence for individuals with FSGS associated with podocin mutations is controversial. Although an initial report suggested a higher rate of recurrence (1188), there is now a consensus that the risk of recurrent FSGS in individuals with *NPHS2* mutations is lower than in other patients with FSGS, ranging from 0% (1006) to 8% (1015,1021).

Older studies suggested that living donor transplants may be associated with a higher rate of FSGS recurrence, but recent data suggest that living donor transplants still retain an overall advantage in outcomes for the FSGS patient with ESRD. A review of data from the U.S. Renal Data System (USRDS) found a higher rate of allograft loss due to FSGS recurrence with living donor allografts (19% of graft loss due to recurrence) compared to cadaveric allografts (8% of graft loss due to recurrence), but overall allograft survival remained higher

TABLE 64-25

LONG-TERM OUTCOMES IN FOCAL SEGMENTAL GLOMERULOSCLEROSIS (FSGS)

Location, year	Population	Average observation (yr)	Complete remission	Persistent renal abnormalities	CKD	ESRD	Non-renal death	Lost to follow-up	Reference
North Carolina, 1976	16 children 17 adults	7 (children) 3.8 (adults)	56% 0%	24% 0%	19% 76%	0% 24%	(6%) 0%	0%	(1168)
London, 1978	12 children 28 adults	9.5	10%	18%	20%	50%	2%	0%	(720)
Montreal 1981	25 children	7.4	24%	40%	4%	20%	12%	0%	(1169)
USA, 1985	75 children	4.8	11%	37%	23%	21%	0%	8%	(410)
Hong Kong, 1991	2 children 30 adults	6.8	9%	ND	ND	16%	ND	ND	(1167)
Chicago, 1995	81 adults	5.1	ND	ND	ND	17%	ND	ND	(1108)
Toronto, 1998	38 children 55 adults	11	42% 22%	13% 24%	11% 13%	34% 42%	0% (4%)	0%	(1090)
Christchurch, New Zealand, 2000	165 adults	6.9	29%	8%	16%	44%			(1166)

All reports that included at least 30 patients and provided group outcomes after average duration of >4 years are presented in chronologic order. Most patients appear to have had idiopathic FSGS but some reports included a limited number of post-adaptive FSGS or focal global glomerulosclerosis. Children are variously defined as individuals <15–18 yr at the time of initial presentation, depending upon reference. When data are presented for children and adults separately, the data for children are presented first. Chronic kidney disease (CKD) is defined as impaired GFR. The percentage of patients experiencing non-renal deaths is shown in parentheses when these patients are also included in another category. Empty cells indicate that no data were provided from the report.

for living donor allografts (1189). Recipient factors that contribute to increased FSGS recurrence risk include prior recurrence, younger age and white race (1189). Also, there was an increased FSGS recurrence risk with black donor kidney and white recipient.

Posttransplantation risk factors for recurrent FSGS remain controversial; the studies are difficult to interpret in that none are controlled and many rely upon historical controls. Some observers have suggested that recurrence has increased in the recent years and this has been linked to particular lymphocyte depletional therapies. Raafat et al. found that recurrence was greater in patients who receive antilymphocyte serum (53% compared to those who did not (11%) (1178). The Miami group suggests that recurrence rose from 38% to 83% after the introduction of daclizumab (1190).

FSGS Permeability Factor

The rapid recurrence of FSGS following renal transplantation appears to be due to a circulating molecule. Molecular identification has been elusive and the activity has been referred to by various names; we will use the term FSGS permeability factor (FPF). A critical advance was the development by Savin and colleagues of an *in vitro* assay, which relies on the albumin permeability of isolated rat glomeruli as reflected by volume changes induced by an oncotic gradient (1191). There is a correlation between the level of FPF and the likelihood of recurrent FSGS following renal transplantation (1184,1192). FPF is unique to FSGS, being absent from patients with membranous nephropathy and with ESRD due to other causes. Dantal and colleagues developed a variation on Savin's assay. In contrast to the findings of Savin and colleagues, they reported that although FPF levels were higher in FSGS patients with ESRD compared to patients with other causes of ESRD, FPF levels did not predict recurrence of FSGS (1193). The reasons for the discrepant findings might include subtle differences in assay conditions and differences in patient populations. Resolving these discrepancies may not be possible until the molecular identity of FPF is established. In summary the FPF assay has important limitations: it is laborious; it lacks high specificity, sensitivity, and precision; and it has not been validated by all centers that work with it. Therefore, the assay must be viewed as a research tool, with limited clinical utility to supplement clinical indicators of risk for recurrence FSGS.

FPF is a protein, as shown by sensitivity to proteolytic enzymes and heat (1194). Immunoadsorption of patient plasma using a protein A column reduces proteinuria in recurrent FSGS, which is a characteristic of immunoglobulin, but plasma fractionation to remove immunoglobulin does not remove FPF

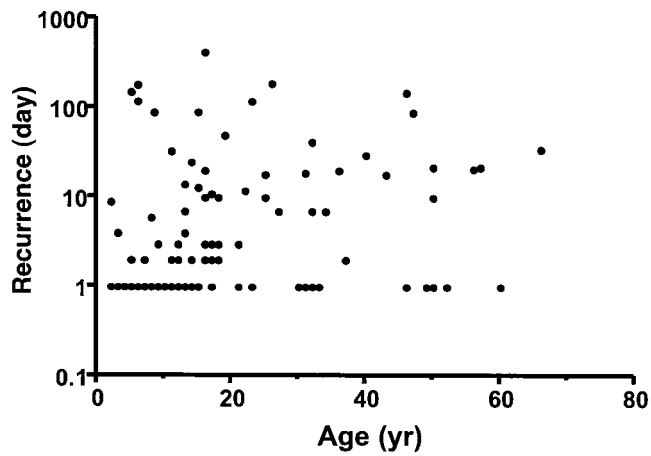

FIGURE 64-18. Timing of FSGS recurrence following renal transplant, correlated with age. Data on the timing of recurrent FSGS and patient age at renal transplantation were available for 101 patients, including 55 children <18 years of age and 46 adults. There was no relationship between age and time to FSGS recurrence (R = 0.01). In 63 cases (62%) recurrent FSGS was diagnosed within one week of renal transplantation.

TABLE 64-26

CLINICAL AND HISTOLOGIC PREDICTORS OF RENAL SURVIVAL IN FOCAL SEGMENTAL GLOMERULOSCLEROSIS (FSGS)

Location, year	*N*	Population	Analytic approach	Baseline clinical variables	Histologic variables	Reference
London, 1978	40	Children, adults	Univariate analysis	• Proteinuria	None tested	(720)
Rochester, Minnesota, 1983	64	Children, adults	Univariate analysis	• Proteinuria • Serum creatinine	• Tubulointerstitial damage (only severe)	(1170)
Tübingen, Germany, 1990	250	Adults	Multivariate analysis	• Proteinuria	• Tubulointerstitial damage (fibrosis, atrophy)	(1171)
New York, 1991	57	Children	Multivariate analysis	• Serum creatinine • Serum cholesterol	None tested	(743)
Hong Kong, 1991	32	Children, adults	Multivariate analysis	• Serum creatinine	None tested	(1167)
Chicago, 1995	81	Adults	Multivariate analysis	• Serum creatinine • Proteinuria (only when those entering remission were excluded)	None predictive	(1108)
Columbus, Ohio, 1996	49	Adults	Multivariate analysis	• Serum C3 (high normal levels are protective)	• Combined glomerular, tubular, interstitial, and vascular score	(1172)
Christchurch, New Zealand, 1999	111	Children, adults	Multivariate analysis	• Serum creatinine	• Interstitial fibrosis	(1166)
Thessaloniki, Greece, 2000	33	Adult	Multivariate analysis	• Serum creatinine (only nephrotic patients) • Age	• Mesangial sclerosis (only non-nephrotic patients) • Interstitial infiltrates (only non-nephrotic patients)	(1133)
Jackson, Mississippi, 2000	42	Adults	Multivariate analysis	• Serum creatinine	• Interstitial fibrosis • Global glomerulosclerosis	(1143)

Each study shown tested either clinical variables alone or both clinical and histologic variables, using either univariate analysis or multivariate analysis (in the latter case only variables that contributed to the model are shown).

activity (1195). Savin and colleagues have used sequential column chromatography to generate plasma fractions that have increased activity (10,000-fold purification) but these fractions remain complex, with multiple proteins and glycoproteins of <30 kDa (1196,1197). Some of these glycoproteins are absent from normal plasma and from sera following therapeutic plasma exchange. Ghiggheri and colleagues have taken a similar approach and identified a number of proteins (albumin isoforms, vitronectin, fibrinogen gamma chain, fibulin, and mannan-binding lectin-associated serine protease), but have not demonstrated activity *in vivo* (1198). Partially purified fractions are able to induce proteinuria in rats (1199,1200).

There also may be plasma factors that antagonize FPF activity, and it remains possible that recurrent FSGS is due to a deficiency of such a molecular inhibitor. Thus normal serum blocks the activity in the rat glomerulus assay (1201). Ghiggerhi and colleagues confirmed these findings, and reported that inhibitory proteins include the apo allelic variants E2 and E4 and apo J (1202). Nephrotic urine but not normal urine contains inhibitory activity (1203). Pharmacologic inhibitors of FPF activity *in vitro* include cyclosporine, indomethacin, cAMP analogs, 12-HETE, and serine protease inhibitors (1129,1204–1206).

Therapy of Recurrent FSGS

The recurrence of nephrotic syndrome after renal transplantation is particularly refractory to treatment, and none of the available therapies can be recommend without serious qualifications. Plasma exchange (using either plasma or albumin as replacement solution) or immunoabsorption (using columns bearing ligands such as protein A, which bind immunoglobulins) have been used with limited success. A single course of plasma exchange or immunoabsorption is associated with response rates (CR and PR) of approximately 58% (Table 64-27) (1173,1175–1177,1186,1207,1208). The number of plasma exchange treatments has varied greatly among different centers, and has not been subjected to careful studies. Davenport reviewed the literature and identified 44 recurrent FSGS cases, using a (liberal) definition of response as a 50% fall in proteinuria in 32 patients (73%) (1209). He concluded that optimal apheresis dose was nine treatments, which he proposed

TABLE 64-27

PLASMA EXCHANGE AND IMMUNOADSORPTION THERAPY FOR RECURRENT FOCAL SEGMENTAL GLOMERULOSCLEROSIS (FSGS)

Report	Location	Modality	*N*	Age (y)	Complete response	Partial response	Prolonged response
Dantal, 1991 (1173)	France	PE	9	A	4	1	1 PR
Artero, 1994 (1186)	Italy	PE	9	P, A	3	3	3CR/2PR
Dantal, 1994 (1195)	France	Adsorption	8	A	2	4	1 CR
Dantal, 1998 (1174)	France	Adsorption	4	A	2	2	0
Andresdottir, 1999 (1175)	The Netherlands	PE	7	A	5	0	3 CR
Greenstein, 2000 (1176)	New York	PE	6	P	5	0	ND
Matalon, 2001 (1177)	New York	PE	13	A	1	1	ND
Shariatmadar, 2002 (1207)	Miami	PE	11	A	5	2	ND
Total			**67**		**27 (40%)**	**13 (19%)**	**10 CR (27%)** **3 PR (8%)**

This table summarizes the studies that used plasma exchange or plasma absorption to treat recurrent FSGS, in studies that treated more than three patients. CR is defined as proteinuria <0.5 g/day and PR is defined as a fall in proteinuria to <2 g/day. *PE*, plasma exchange. Long-term outcome was at ≥1 year following recurrence. Chronic therapy includes that for patients who had subsequent repeat course of PE or who required intermittent, maintenance PE.

be delivered as three daily treatments followed by six treatments delivered on alternate days. Unfortunately, most patients relapse. In some patients, particularly children, a maintenance schedule of plasma modulating therapy may sustain a remission.

The limitations of this approach have led to the empiric use of cyclophosphamide, with or without plasma exchange, in four trials of pediatric patients (Table 64-28) (1181,1183, 1184,1210). The long-term outcome appears to be markedly better than those studies using only plasma exchange or immunoadsorption. This approach has not been reported in adults. Clearly randomized trials are needed before firm conclusions can be reached about either approach.

High-dose cyclosporine has been used in two pediatric trials. Salomon et al. administered intravenous cyclosporine, dose adjusted to achieve trough levels of 250 to 350 ng/mL, and achieved CR in 14 of 17 (82%) of consecutive patients with recurrent FSGS (1211). Plasma exchange was also used in 4 patients. Sustained remission was noted in 11 of 17 (65%) of patients. Raafat et al. administered oral cyclosporine, with the dose increased until proteinuria resolved or nephrotoxicity (rising serum creatinine) was noted (1212). They found that 13 of 16 (81%) of patients entered remission, either CR (11 patients) or PR (2 patients). Cyclosporine doses ranged from 6 to 25 mg/kg per day and cyclosporine trough levels ranged from 200 to 1000 ng/mL. Remission was sustained in all patients with CR, with conversion to tacrolimus in some patients. Toxicity included hirsutism and gingival hypertrophy in all patients. Although these are intriguing data, more experience is needed, especially in view of the potential toxicity of such high cyclosporine doses; whether adults would tolerate this approach without unacceptable adverse events is unclear.

Prophylactic Therapy to Prevent Recurrent FSGS

Toma and colleagues assigned FSGS ESRD patients to undergo plasma exchange prior to or immediately after renal transplantation (1213). Recurrent FSGS was seen in 4 of 19 treated patients and 9 of 19 in untreated patients. Ohta et al. reported data from 21 patients and compared patients undergoing prophylactic plasma exchange (transplantations from 1991 to publication) with historical controls (transplantation prior to 1991) (1214). Recurrence was seen in 5 of 15 versus 4 of 6 patients. Neither study had sufficient power for a definitive result, and a prospective, adequately powered trial remains to be done. If prophylactic plasma exchange is effective, the mechanism of benefit remains somewhat puzzling. Evidence suggests that the permeability factor returns following plasma exchange; therefore, any benefit from transient reduction would

TABLE 64-28

CYCLOPHOSPHAMIDE, WITH OR WITHOUT PLASMA EXCHANGE, FOR RECURRENT FOCAL SEGMENTAL GLOMERULOSCLEROSIS (FSGS)

Report	Population	Treatment	*N*	Complete response	Partial response	No response	Long term response	Graft preservation
Cochat, 1993 (1181)	Children	PE + CTX 3 mo	3	3	0	0	3 CR (1 retreatment)	3
Kershaw, 1994 (1183)	Children	CTX 2–3 mo	3	3	0	0	3 CR (1 relapse)	3
Dall'Amico, 1999 (1184)	Children	PE + CTX 2 mo	11	9	0	2	7 CR	7
Cheong, 2000 (1210)	Children	PE + CTX 3 mo	6	3	3	0	2 CR	5
Total			**23**	**18 (78%)**	**3 (13%)**	**2 (9%)**	**15 CR (60%)**	**18 (78%)**

suggest that the factor is particularly injurious in the immediate peritransplantation period.

CONCLUSIONS

From the discussion in this chapter, several conclusions can be drawn regarding the group of diseases under consideration. First, the nephrotic syndrome represents a complex of symptoms resulting from urinary protein loss rather than a disease entity characterized by specific pathology. The abnormalities found include not only the hypoalbuminemia, edema, and hyperlipidemia classically associated with nephrotic syndrome, but also derangements of hemostasis, metabolism, and endocrine function. All of these findings can be attributed to the characteristics and effects of nephrotic proteinuria. Second, we have defined a group of diseases in which the common attribute is that the lesion appears to initiate with the podocyte. In MCN, the restrictive component of the glomerular filtration barrier fails, yet podocyte effacement decreases the area of the GBM that is open to the urinary space, decreasing effective filtration surface area. In FSGS genetic, morphologic and functional data all suggest that podocyte abnormality initiates the disease. Like MCN, there is massive proteinuria, but in contrast to the more benign condition, there is disruption of podocyte architecture. FSGS is accompanied by podocyte injury and depletion, whereas collapsing glomerulopathy manifests podocyte proliferation and capillary collapse. In both cases, these changes are accompanied by the extracellular matrix accumulation that is a hallmark of this disease. Multiple cell types may serve as effector cells for fibrosis, but the common denominator of podocyte involvement suggests that a more appropriate appellation for these diseases might be *podocytopathies* (1215).

A third conclusion relates to the observation that these conditions are characterized by heterogeneity of therapeutic response and prognosis. Although certain subgroups may be associated more often with specific patterns of response to treatment and outcome, such categorizations are not absolute. Most of the patients with MCN and some of those with FSGS are steroid-sensitive. The remaining patients with MCN and a larger proportion of those with FSGS are steroid-resistant. Of particular distinction is the group of patients who have collapsing glomerulopathy. Few of these patients respond to treatment, and the nature of their lesion is so distinctive that we have chosen to consider them as belonging to an entirely separate subgroup of primary nephrotic syndrome. It should be clear that all of the patients having a poor prognosis with regard to long-term renal function are steroid resistant. Moreover, a few patients with MCN, and more with FSGS, particularly those with postadaptive FSGS, may not have nephrotic syndrome. These findings suggest heterogeneity of pathogenetic mechanisms, even within a given histopathologic subgroup.

In view of the differences among MCN, FSGS, and collapsing glomerulopathy, the assumption previously held by many clinicians that patients may move from one disorder to another should be revisited. Collapsing glomerulopathy, the rapidly progressing lesion, appears quite distinct. There are many features that also differentiate MCN from FSGS. These include (a) differences in permselectivity curves, where patients with FSGS demonstrate greater utilization of the shunt pathway (78); (b) association of FSGS with podocyte gene mutations, whereas mutations have not yet been defined for classical MCN (as opposed to congenital nephrotic syndrome); and (c) more clearly defined and more likely irreversible podocyte abnormalities in FSGS. In particular, as technical capability for detecting subtle mutations or polymorphisms in podocyte-specific genes improves, it is likely that mutations will be associated with FSGS with increasing frequency (1019). Therefore, although both MCN and FSGS initiate with a podocyte lesion, the abnormalities in FSGS appear more profound, either leading to or reflecting the pathogenetic mechanism(s) of FSGS. Given these differences, it is likely that many, perhaps a great majority, of observations of progression from MCN to FSGS have represented sampling error or early lesion on biopsy.

An unanswered question is the basis for the presently surging incidence of FSGS. Clearly, there is a still-unknown risk factor for progressive renal disease. Hypertension, increasing obesity and perhaps environmental factors may contribute. This interpretation suggests a model in which FSGS is, finally, an idiopathic form of progressive renal disease.

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CHAPTER 65 ■ RENAL INVOLVEMENT IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Systemic lupus erythematosus (SLE) is an autoimmune disease of unknown etiology, characterized by the involvement of multiple organ systems. Glomerulonephritis is a frequent and important complication of SLE, and the presence and extent of kidney involvement greatly influence the long-term outcome of this disease. Systemic lupus erythematosus is caused by the production of autoantibodies, and a hallmark of the disease is the presence of serum antibodies directed to nuclear constituents (i.e., antinuclear antibodies, ANAs). The clinical presentation and course of SLE are extremely variable. For example, some patients have spontaneous remissions, others may have mild skin and joint involvement that respond favorably to conservative measures, and a few die from progressive multisystem disease unresponsive to high-dose corticosteroids and cytotoxic drugs. Variability is also a feature of renal involvement in SLE, and the evaluation, treatment, and prognosis for each patient need to be individualized. In this chapter, we review current ideas regarding the immunopathogenesis and pathology of glomerulonephritis in SLE. We also describe lupus nephritis in terms of clinical and laboratory findings and current approaches to therapy.

Criteria for the classification of SLE (Table 65-1) have been established by the American College of Rheumatology (ACR) to facilitate the uniform reporting of SLE cases (1). A patient is said to have SLE if four of the 11 criteria shown in Table 65-1 are satisfied. Although not designed for diagnosis, these criteria have helped physicians recognize and diagnose this disease. It is emphasized, however, that these criteria occasionally lack sensitivity for diagnosing early or mild cases of SLE, and occasional patients may fulfill four criteria and still not have SLE.

The preceding criteria emphasize that patients with lupus nephritis usually demonstrate nonrenal manifestations of SLE. The physician must be aware of these other nonrenal problems because they can be serious and can greatly complicate the evaluation and management of the disease. The reader is referred to several general reviews of the clinical manifestations of SLE (2–4).

Women of childbearing age are primarily affected, although SLE can occur at nearly any age. In cases that begin between ages 15 and 40 years, more than 90% of the patients are female (5). The female-to-male ratio is closer to 2:1 for disease that develops during childhood and after the age of 65. Epidemiologic studies have shown that the incidence of disease increases in women of childbearing age and then decreases after menopause, whereas the incidence for men is more evenly distributed and may continue to rise through the seventh decade. Together, these data strongly suggest that sex hormones influence the probability of developing or expressing SLE, and studies in animal models of lupus have supported a potential role for estrogens enhancing and androgens protecting against expression of disease (6). It should be emphasized that although men develop SLE less frequently than do women, their illness, including extent of renal involvement, may be severe (7).

The overall prevalence rate of SLE in the continental United States has been reported to range between 15 and 50 cases per 100,000 persons, and overall incidence has been estimated to be 1.8 to 7.6 cases per 100,000 persons per year (5). These rates can vary greatly depending on the risk of the population being studied. The chance that a white woman in the United States will develop SLE in her lifetime is approximately one in 700. The incidence is three to four times greater for black women, who are also more likely to demonstrate severe renal involvement and have higher disease-related mortality rates (5). Certain Asian populations, North American Indian tribes, and Hispanic individuals also may have a greater predisposition toward SLE compared to individuals of mixed-European descent. These racial differences are consistent with the known importance of genetic factors in disease development (discussed in the following section), but also partly may result from environmental influences.

PATHOGENESIS

Overview

Similar to other autoimmune diseases, the development of SLE is a complicated and multistep process (8). Major events believed to be important in the immunopathogenesis of SLE are shown in Figure 65-1. Considerable evidence indicates that the development and progression of SLE have a strong genetic basis and that these genetic contributions may operate at several different levels. The T-cell driving force in SLE involves $CD4^+$ T cells, which interact with autoreactive B cells to cause immunoglobulin G (IgG) autoantibody production and various disease manifestations. IgG autoantibody production is the central immunologic problem in SLE. These autoantibodies may mediate pathology, for example, by binding directly to their cell-surface target or by forming immune complexes, although the mechanism by which many autoantibodies are associated with particular disease manifestations in SLE is unknown. The heterogeneity in clinical manifestations among SLE patients is likely related to differences in the types and amounts of autoantibodies produced.

Studies using several murine models have contributed greatly to the elucidation of SLE pathogenesis (6,9–11). The most well-characterized lupus-prone models are New Zealand hybrid, MRL, and BXSB mice. The New Zealand murine model was first described more than 40 years ago, and usually involves crosses or recombinant strains of New Zealand black (NZB) and New Zealand white (NZW) mice. MRL-*lpr/lpr* (currently referred to as MRL-Fas^{lpr}) mice, which are homozygous for the *lymphoproliferation (lpr)* mutation in the gene encoding Fas, demonstrate an accelerated form of disease compared to non-*lpr* litter mates (10). BXSB mice most frequently studied carry the *Yaa* accelerating gene encoded on the Y chromosome

TABLE 65-1

THE 1982 REVISED CRITERIA FOR CLASSIFICATION OF SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)[a]

Criterion	Definition
Malar rash	Fixed erythema, flat or raised over the malar eminences, tending to spare the nasolabial folds
Discoid rash	Erythematous raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring may occur in older lesions
Photosensitivity	Skin rash a result of unusual reaction to sunlight, by patient history or physician observation
Oral ulcers	Oral or nasopharyngeal ulceration, usually painless, observed at physical examination
Arthritis	Nonerosive arthritis, involving two or more peripheral joints, characterized by tenderness, swelling, or effusion
Serositis	Pleuritis: convincing history of pleuritic pain or rub heard by a physician or evidence of pleural effusion
	Pericarditis documented by electrocardiography or rub or evidence of pericardial effusion
Renal disorder	Persistent proteinuria greater than 0.5 g/day or >3+ if quantitation not performed
	OR
	Cellular casts (red blood cell, hemoglobin, granular, tubular or mixed)
Neurologic disorder	Seizures in the absence of offending drugs or known metabolic derangements (e.g., uremia, ketoacidosis, or electrolyte imbalance)
	OR
	Psychosis in the absence of offending drugs or known metabolic derangements (e.g., uremia, ketoacidosis, or electrolyte imbalance)
Hematologic disorder	Hemolytic anemia with reticulocytosis
	OR
	Leukopenia: WBCs, <4,000/μL total on two or more occasions
	OR
	Lymphopenia: <1,500/μL on two or more occasions
	OR
	Thrombocytopenia: <100,000/μL in the absence of offending drugs
Immunologic disorder	Positive LE cell preparation[b]
	Anti-DNA: antibody to DNA in abnormal titer
	OR
	Anti-Sm: presence of antibody to Sm nuclear antigen
	OR
	False-positive serologic test for syphilis known to be positive for at least 6 months and confirmed by TPI or FTA-ABS
Antinuclear antibody	An abnormal titer of ANA by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs known to be associated with "drug-induced lupus" syndrome

LE, lupus erythematosis; *TPI*, *Treponema pallidum* immobilization test; *FTA-ABS,* fluorescent treponemal antibody absorption test; *ANA,* antinuclear antibody.

[a]For the purpose of identifying patients in clinical studies, a person shall be said to have SLE if any four or more of the 11 criteria are present, serially or simultaneously, during any interval of observation. Taken from Tan et al. (1).

[b] The Diagnostic and Therapeutic Criteria Committee of the American College of Rheumatology (ACR) has suggested that this criterion be replaced with a positive test for antiphospholipid antibodies (elevated serum level of anticardiolipin antibodies or positive test for lupus anticoagulant using a standard method).

(From: Tan EM, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982;25:1271, with permission; and Rus V, Hochberg MC. The epidemiology of systemic lupus erythematosus. In: Wallace DJ, Hahn BH, eds. *Dubois' Lupus Erythematosus,* 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2002.)

(11). All of these murine lupus models develop a lupus-like immune-complex-mediated glomerulonephritis associated with high levels of IgG autoantibodies to nuclear antigens, including double stranded DNA (dsDNA).

The animal models of lupus have provided systems for dissecting genetic contributions to disease, understanding the immunopathogenesis of autoimmunity, and characterizing the pathogenic events in lupus nephritis. The ability to study many related offspring with similar disease manifestations after directed breeding of autoimmune and nonautoimmune strains is a great advantage compared to genetic studies of the human disease, in which many multiplex families with heterogeneous disease manifestations must be studied (12). Studies of these animal models, in contrast to patients, also offer the opportunity to control environmental exposures. In addition, spontaneous animal models permit the characterization of immunologic changes before, during, and after disease development. Therefore, one can attempt to distinguish the early immunologic abnormalities that are central to disease development from later defects that are secondary to disease. Studies involving these animal models that emphasize certain principles are discussed in the following sections.

Genetic Contributions

Evidence for a strong genetic predisposition in SLE has come from genetic epidemiology studies that determine: (a) disease prevalence within families in which there is an affected individual, when compared with the prevalence in the general population; and (b) disease prevalence in identical versus nonidentical twins (12,13). The increased risk for a sibling of an SLE patient

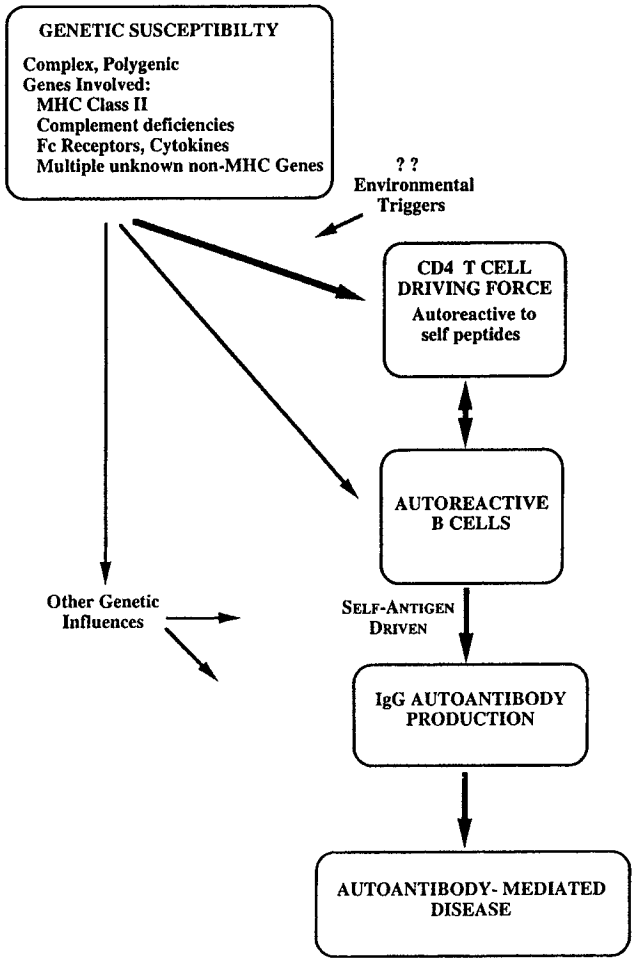


FIGURE 65-1. Genetic and immunologic events in the pathogenesis of systemic lupus erythematosus. *shape MHC,* major histocompatibility complex. (From: Kotzin BL. Systemic lupus erythematosus. *Cell* 1996;85:303, with permission).

to develop the same disease is estimated to be 15- to 20-fold greater than for the general population (12–14). Recent studies also have suggested concordance rates for identical twins of ~24% compared with ~2% for dizygotic twins (15). These results strongly support the importance of genetic contributions to disease development, but the twin data also suggest a role for other factors and/or stochastic processes in disease expression. Serologic studies indicate that identical twins discordant for disease expression may still demonstrate similar autoantibody profiles, supporting the hypothesis that certain genetic contributions operate at the level of autoantibody production.

Systemic lupus erythematosus, like other autoimmune diseases, is a complex genetic trait, which by definition is not inherited in a simple Mendelian way (12–14). Multiple genes, including major histocompatibility complex (MHC) and non-MHC genes, determine susceptibility to SLE, and no particular gene is necessary or sufficient for disease expression. The low penetrance of each contributing gene, that is, the small increase in probability of disease expression given a particular disease allele, is the main reason why diseases such as SLE are so complex genetically. Furthermore, as exemplified by the concordance rates in identical twins, overt autoimmune disease frequently does not occur even in the presence of a full complement of susceptibility alleles. Genetic complexity in SLE is also determined by genetic heterogeneity, in which the same phenotype (e.g., anti-dsDNA autoantibody production or lupus nephritis) is the result of a different set of genes in different individuals.

Many genetic studies in human SLE have focused on genes that affect immune responses, particularly those encoded within the MHC. Associations of disease with particular MHC class II alleles have been demonstrated in studies of total populations of SLE patients (i.e., inclusion of patients is based solely by meeting classification criteria) (14,16,17). However, these associations generally have been weak and consistent only within a given ethnic group. For example, in some populations, associations with HLA-DR2 and/or HLA-DR3 have been observed, but increased relative risks for disease development generally have been less than five (16,17). Furthermore, it has been suggested that the association with DR3 may be related to a null allele of complement C4A (C4A*QO), which is inherited on some extended MHC haplotypes in linkage disequilibrium with HLA-DR3 (14) or a particular allele of the gene encoding tumor necrosis factor-α (TNF-α) frequently linked with DR2 (14,18). In contrast to these weak associations with disease susceptibility in general, MHC class II genes exert a decisive influence on the production of specific types of autoantibodies in SLE (16,17). The antibody response to several autoantigens, including dsDNA, in SLE has been associated with particular MHC class II (HLA-DQ) alleles or combinations of these alleles. Overall, the data suggest that the contribution of MHC class II molecules in SLE is predominantly at the level of specific autoantibody production, perhaps reflecting the presentation of specific autoantigens.

Inherited deficiencies of classic pathway complement components, some of which are encoded within the MHC, have a significant effect on susceptibility to SLE and lupuslike disease (14,19). For example, most individuals with genetically determined complete deficiencies of complement C1q, C1r/C1s, or C4 (both C4A and C4B) develop a syndrome resembling SLE. C2 deficiency has been associated with a more than 40-fold increased risk of developing SLE. These complete deficiencies of complement components are, however, rare and account for only a very small percentage of SLE patients. In contrast, C4A deficiency may be present in as many as 13% to 15% of SLE patients (versus 1% of the general population) and studies have suggested a 15- to 20-fold increased risk of developing SLE. Furthermore, heterozygous C4A deficiency (one C4A*QO allele) is present in 35% to 60% of SLE patients compared to 13% to 20% of controls (relative risk for developing SLE is approximately two to three), although the interpretation of these data is more difficult because of the linkage disequilibrium of C4A with other possible predisposing MHC genes.

The association of complement deficiency states and SLE is likely to be etiologic, but the mechanism for this influence is unknown. Some investigators have postulated defects in the clearance of infectious particles, immune complexes, or apoptotic cells as leading to enhanced autoantibody production (20). Complement deficiencies also could affect B-cell function and the tolerance process. Alternatively, complement gene defects may exemplify genetic defects that operate distal to autoantibody production by allowing enhanced formation or deposition of immune complexes in various organs. It is emphasized that, although complement deficiencies can predispose to the development of SLE, complement activation is important for the pathogenic effects of autoantibodies and immune complexes in this disease. In SLE patients with certain types of complement deficiencies, clinical manifestations may be altered. In addition, inhibition of complement activation has been shown to suppress lupus nephritis in animal models (21), and therapeutic trials with blocking monoclonal antibodies to C5 are in progress in human SLE.

Patients with lupus nephritis have autoantibodies to the complement classical pathway protein C1q. It is not known whether these anti-C1q antibodies can cause lupus nephritis. Trouw et al. (22) developed a murine mAb, JL-1, that recognizes the tail domain of C1q. JL-1 administered alone in normal

control mice resulted in glomerular deposition of C1qand anti-C1q antibodies without inducing glomerular damage. Combination of JL-1 with a subnephritogenic dose of C1q-fixing anti-glomerular basement membrane (anti-GBM) antibodies caused renal damage manifested by decreased renal function and increased albuminuria. The glomerular injury was not seen when a non-C1q-fixing anti-GBM preparation was used. Therefore, anti-C1q autoantibodies deposit in glomeruli together with C1q but induce overt renal disease only in the context of glomerular immune complex disease. This study demonstrates, for the first time, a causal link between anti-C1q antibodies and lupus nephritis (22).

Associations also have been demonstrated between SLE and alleles of the genes encoding cell-surface receptors for IgG (i.e., Fcγ receptors) (23,24). For example, investigators have demonstrated an association between SLE and alleles of the gene encoding Fcγ RIIA (CD32), expressed on monocytes–macrophages and neutrophils (23). Alleles of this gene differ substantially in the ability to bind human IgG2, and studies found a decrease in the prevalence of the high affinity binding allele in SLE, particularly in patients with lupus nephritis. Thus, possibly similar to complement deficiencies, the genetic contribution of this Fcγ receptor deficiency may reflect a relatively late event in the pathophysiologic scheme of SLE-related immune-complex damage in lupus nephritis. Consistent with this hypothesis, other studies have noted the under representation of a high-binding Fcγ RIIIA allele (expressed on NK cells and monocytes) in patients with SLE, especially with lupus nephritis (24). The Fcγ receptor molecules and related pathways appear to be extremely important in immune-complex-mediated glomerulonephritis and the generation of lupuslike disease. In studies of the (NZB $\times$ NZW)F_1 murine model, knockout of Fcγ RIII function had no apparent effect on the deposition of immune complexes in glomeruli but nearly completely prevented the development of severe glomerular damage (25). In contrast, knockout of the gene encoding Fcγ RIIb, which negatively regulates B-cell activation, results in lupuslike renal disease in normal mice (26).

Association studies have also focused on other immunologically relevant genes in the pathogenesis of SLE. In addition to classical complement component deficiencies, alleles of mannan binding protein have been associated with susceptibility to SLE and infection in the setting of SLE (27). Studies of genetic polymorphisms that affect expression of certain cytokines such as TNF-α, IL-10, and IL-6 have also been suggested to be associated with disease (28–30), although further confirmation of these effects are needed. In contrast, most mouse and human studies have not found a major role for polymorphisms in either the T-cell receptor (TCR) gene complex or immunoglobulin (Ig) gene complex. This is consistent with a large amount of evidence that abnormalities in these lymphocyte receptors are not required for expression of disease (8). A number of different Ig genes can be utilized to encode pathogenic autoantibodies in lupus, and multiple different TCR variable regions appear to be sufficient for disease development.

The development of techniques to map the position of disease susceptibility loci in genome-wide screens without regard to candidate genes has been a significant advance in genetic analysis in the last few years (12,13). Genetic mapping studies, including genome-wide scans, have been reported in SLE as well as murine models of these diseases (14,31–33). Unfortunately, the susceptibility genes underlying most mapped loci have not been identified to date. Except for the *lpr* mutation of the gene encoding Fas and *gld* mutation in the Fas ligand gene, none of the approximately 15 to 20 non-MHC susceptibility loci mapped in murine models of lupus have been identified with any certainty to date. In addition, although multiple loci have been suggested in mapping studies in human SLE, no non-MHC genes have yet been identified. In a recent follow-up study of a directed linkage study in SLE (34), transmission disequilibrium testing was used to implicate the gene encoding poly-ADP-ribose polymerase (PARP or *ADPRT*) on distal human chromosome 1 (35), but other investigator groups have not been able to confirm this observation (36).

A possible step forward in understanding genetic contributions in lupus relates to the identification of *lpr* and *gld* in murine studies as mutations in the genes encoding Fas and Fas ligand, respectively. These genes are involved in programmed cell death (apoptosis); the traits resulting from these mutations have been described extensively (10). Homozygosity for these mutations results in the acceleration of lupuslike autoimmunity as well as a massive accumulation of CD4– CD8– (double-negative) T cells. Although the mechanism by which mutations in Fas lead to accelerated autoimmunity is unknown, the strongest hypothesis is that self-reactive T and B cells arise when they fail to undergo apoptosis normally (8). Studies have shown that both T and B cells must carry the *lpr* mutation for maximal autoantibody production to occur (37,38). The evidence indicates that Fas is not critically important in central (intrathymic) tolerance during T-cell development (8,39,40). Instead, studies support the contention that peripheral T-cell tolerance mechanisms are primarily affected by the *lpr* mutation (40). In a similar manner, studies also suggest that central B-cell tolerance may be relatively independent of Fas (41,42), and surface expression of Fas on B cells may be most important in preventing inappropriate $CD4^+$ T-cell-dependent expansion of autoreactive B cells in the periphery (43). *Fas* mutations have been identified in a few children with lymphoproliferative syndromes and evidence of autoimmunity (44). However, it is important to emphasize that there is really no counterpart to the *lpr* or *gld* phenotype in human SLE, and recent studies have not found defects of these genes in SLE patients. Still, there is a belief that other genes involved in apoptosis or related cell-signaling pathways may be involved in the genetic susceptibility and pathogenesis of the human disease.

In animal models, many single gene knockout and transgenic manipulations have resulted in systemic autoimmune syndromes with lupuslike autoantibody production and immune-complex renal disease (14,20,26,31). Targeted perturbations of genes involved in apoptosis, T- or B-cell signaling, B-cell activation, cell cycle processes, as well as genes encoding complement components, Fc receptors, and cytokine or cytokine receptor expression have been reported to cause the development of lupuslike disease. In many studies, the background of the strain in which the knockout is placed can greatly alter the expression of disease (20,26). Although single gene models of lupus have pointed to particular genes and mechanistic pathways for study in human disease, as discussed, genetic contributions to human SLE are generally complex and determined by the combination of multiple susceptibility genes (14,31,32).

Overall, there is great optimism that a number of non-MHC alleles that contribute to SLE will be defined in the next few years. Multiple abnormalities of both B- and T-cell phenotype and function have been described in SLE and murine models of lupus. However, many of these defects probably reflect secondary events in the disease process, and it is unclear which of these lymphocyte abnormalities are primary checkpoints in the pathogenesis of disease. The genes that predispose to autoimmunity are likely related to primary events in pathogenesis; therefore, their identification will almost certainly provide important insight into the development of autoimmunity and the cause of autoimmune disease.

Environmental Triggers and Influences

In the proposed model of pathogenesis (Fig. 65-1), environmental factors interact with susceptibility genes to result in

autoimmune disease; however, the existence and importance of any particular environmental trigger in SLE has yet to be defined. Geographic clustering has not been documented in SLE, and a number of postulated chemical exposures or dietary effects have not been confirmed to be associated with SLE when careful epidemiologic studies were performed. The possible viral etiology of SLE has been vigorously investigated. One recent study has shown that children with SLE have a higher prevalence of Epstein-Barr virus (EBV) infection compared to control groups (45). Although the studies are harder to perform in adults (because of the higher frequency of EBV in the general adult population), preliminary studies suggest a similar conclusion. The basis for these interesting findings in terms of which condition is predisposing to the other has yet to be clarified.

Environmental influences on the expression of disease manifestations are clearly seen in SLE. These include the exacerbation of skin rash (or even systemic symptoms) after sun exposure, exacerbations of disease after viral or bacterial infections, and changes in disease activity after administration of exogenous hormones. Several important pieces of evidence also support the contention that estrogens increase the risk of developing SLE and/or exacerbate disease. Epidemiologic studies have documented an increased frequency of disease in postmenopausal women taking estrogen supplementation (46) and in women taking birth control pills (47,48). These studies are consistent with the known increased expression of disease in women of childbearing age (5) and studies in certain animal models showing that estrogens enhance disease expression (6). There is also evidence from animal models that androgens may have a suppressive effect on disease course. Perhaps related, treatment of patients with dehydroepiandrosterone (DHEA), an abundant adrenal steroid with slight androgenic activity, has been shown in at least one controlled study to be mildly effective in patients with mild to moderate disease activity (49).

A large number of drugs have been associated with the production of ANA and development of a lupuslike syndrome (50) (see discussion later in this chapter). Drug-induced lupus, however, is not the induction of lupus in individuals predisposed to develop SLE. Compared to SLE, drug-induced lupus occurs in older patients (related to the age of patients using the offending medication) and the sex ratio is close to unity. Drug-induced lupus in general also differs from idiopathic SLE in that it tends to cause predominantly joint and pleural–pericardial involvement, and the development of lupus nephritis is very unusual. The disease remits with time when the offending drug is discontinued. The mechanism for how drugs, such as procainamide and hydralazine, induce lupuslike disease remains poorly understood.

T Cells and T-Cell Cytokines

Studies showing the association of autoantibody production with particular class II MHC genes, the requirement for class II MHC molecules in murine models of lupus, as well as the characteristics of IgG autoantibodies in SLE strongly suggest that $CD4^+$ T cells are critically involved in the pathogenesis of this SLE (8). Moreover, treatment of murine models with monoclonal anti-CD4 antibodies can ameliorate the disease, documenting an important role for $CD4^+$ T cells in the disease process (8,51–53). The specificities of the $CD4^+$ T cells involved in SLE induction have not been fully defined. The nature of T-cell help in lupus may differ from conventional responses to foreign antigens because of the unusual structure of the target autoantigens, and the T-cell specificities may not necessarily match the antigens that are stimulating and driving B cells. There is no evidence, for example, that T cells specific for DNA are involved in the anti-DNA antibody response in spontaneous murine lupus. Most lupus antigens exist as a component of large complexes (such as nucleosomes or spliceosomes), containing multiple protein as well as nucleic acid molecules. These complexes may be more capable of cross-linking Ig receptors on B cells, making the specific B cells more susceptible to T-cell help. Proteins within these complexes may also be internalized, processed, and presented for T-cell recognition. In recent studies, pathogenic T cells recognizing peptides derived from histones (the major protein component of chromatin) have been identified in both murine and human lupus (54,55), and these T cells were capable of helping B cells produce anti-DNA antibodies.

Current theories to explain the development of autoreactive T cells in SLE are based on the mechanisms of T-cell tolerance that operate in normal individuals. For example, it is conceivable that there is a generalized failure of negative selection in the thymus. However, studies in lupus mice have repeatedly shown that high-affinity responses to self-antigens are tolerized normally in the thymus (8,39,40,56,57). Similar to other autoimmune diseases, lupus more likely involves a failure of peripheral tolerance mechanisms, including a failure to maintain T-cell ignorance, a failure to induce anergy after exposure to self-antigens in the periphery, or a failure of normal regulatory (suppressor T-cell) mechanisms. To date, there is little information to pinpoint a particular defect, and research on these questions remains an extremely active area of investigation.

Any self-antigen needs to be processed and presented with MHC molecules to allow for negative selection in the thymus. Cryptic determinants are not presented adequately, and cells capable of recognizing these peptides escape central tolerance and are part of the normal peripheral T-cell repertoire (58). Immunization of normal mice with cryptic peptides of nuclear antigens has been shown to result in lupuslike autoantibody production that can spread to other determinants on the same molecule and spread to other nuclear antigens (intramolecular and intermolecular determinant spreading) (59–62). The ability to stimulate T cells that are reactive with cryptic epitopes through molecular mimicry has been suggested as a possible initiating event in the pathogenesis of SLE.

Numerous quantitative, phenotypic, and functional abnormalities of T cells in SLE and murine models of lupus have been documented (63). Studies have suggested that T-cell signaling through the TCR and CD3 is defective, associated with deficient TCR ζ-chain expression, protein kinase A activity, and downstream activation steps (64,65). Other work has documented abnormally increased expression and release of costimulatory molecules, such as CD40 ligand (CD40L; CD156), in SLE patients versus controls, which could allow for increased T-cell activation and T-cell dependent activation of B cells (66–68). Additional T-cell–related abnormalities reported in SLE include T-cell lymphopenia, alterations in $CD4^+$ T-cell numbers and certain $CD4^+$ T-cell subsets, increased numbers of activated $CD4^+$ T cells, increased numbers of CD4– CD8– (double-negative T cells) and aberrant T-cell help from this subset, defective T helper cell activity, aberrant helper activity from $CD8^+$ T cells, defective T-cell suppressor activity, deficient *in vitro* IL-2 production and response to IL-2, and abnormal *in vitro* production of other cytokines (63). It remains unclear which of these defects are important events in disease pathogenesis and which are just secondary abnormalities induced by the markedly abnormal immune state of lupus.

Although more sharply defined in mice compared to humans, it is clear that T cells after activation may evolve into two major subsets of T helper cells, distinguished by the cytokines that they produce. Type 1 helper T (Th1) cells mainly synthesize interleukin-2 (IL-2) and interferon-γ (IFN-γ), as well as other proinflammatory cytokines such as lymphotoxin and tumor necrosis factor (TNF). Type 2 helper T (Th2) cells are distinguished primarily by their secretion of IL-4, IL-5, IL-10, and

IL-13. Th1 cells appear to be critically important in autoimmune diseases such as multiple sclerosis and type 1 diabetes (69). Th2-type responses may be relatively more important in SLE compared to these organ-specific diseases. However, in spontaneous murine models of lupus, the IgG isotypes involved in pathogenicity suggest the additional influence of Th1-type cytokines and especially the importance of interferon-γ (IFN-γ) (70,71). It is important to note the critical involvement of IFN-γ in the pathogenesis of disease in murine models of lupus (71), and evidence supports a role for this cytokine in human SLE (72) as well as its potential as a therapeutic target (71). The mechanism for the influence of IFN-γ in SLE has not been defined.

T-Cell Co-stimulation and T-Cell–B-Cell Interactions

Autoreactive T cells, like T cells specific for foreign antigens, must receive several signals to be activated. One signal is antigen-specific and provided by engagement of the T-cell receptor for antigen (TCR). Costimulatory molecules and their interactions provide additional signals. Two of the most important costimulatory interactions involve CD28 and CD40 ligand on the T cell, which bind to B7 (B7-1 CD80 and B7-2 CD86) and CD40, respectively, on antigen-presenting cells and B cells. The interaction of CD40 ligand with CD40 is clearly bidirectional in that it provides signals important for both T- and B-cell activation (73). Studies in human SLE have suggested that cell-surface CD40 ligand expression may be aberrantly increased (66,67) and that soluble CD40 ligand levels may be increased with resultant excessive B-cell activation (68). In murine models of lupus, blocking of the CD40 ligand–CD40 interaction or the CD28–B7 interaction suppresses disease activity (73,74), and currently these therapies are being developed to treat human SLE patients. Blocking both these costimulatory interactions in combination is one of the most potent treatment strategies in murine lupus at present (75).

B Cells

Generalized polyclonal B-cell hyperactivity and activation are frequently present in SLE (76), and some patients demonstrate markedly increased serum immunoglobulin levels, probably reflecting this process. However, the majority of available data also indicate that pathogenic autoantibody production in SLE is selective for only certain self-antigens and driven by self-antigen at the B-cell level. In SLE and lupus mice, for example, a subset of B cells producing IgG anti-DNA antibodies are clonally expanded and their Ig genes are modified by somatic mutation (77–79). This IgG autoantibody production appears to mimic a normal T-cell–dependent response to foreign antigen and involves similar mechanisms of somatic mutation, affinity maturation, and IgM to IgG class switching. Studies by several groups have indicated that anti-DNA autoantibodies in lupus preferentially utilize a subset of immunoglobulin heavy- and light-chain variable region gene segments (77–79). In addition, the generation of high affinity anti-DNA antibodies through immunoglobulin gene rearrangement and somatic mutation involves the accumulation of basic arginine residues in the immunoglobulin regions that bind antigen (complementarity determining regions), suggesting selection by anionic antigens, perhaps DNA itself.

B cells capable of secreting pathogenic autoantibodies characteristic of SLE are present and can develop from the normal B-cell repertoire (8). The transfer of alloreactive $CD4^+$ T cells and generation of chronic graft-versus-host disease causes pathogenic lupuslike autoantibody production in normal recipient animals (80,81). Other studies have shown that immunization of normal animals to certain peptides derived from nuclear antigens can result in spreading autoantibody responses to other determinants on the same molecule and to other molecules in the same nuclear complex (e.g., anti-Sm to anti-U1RNP and anti-Ro/SS-A to anti-La/SS-B) and can lead to lupuslike disease (59–62). This epitope spreading shows the presence of self-reactive B cells in these normal mice. Therefore, defects in central B-cell tolerance do not appear to be necessary to allow for pathogenic autoantibody production (8). Similar to regulation of autoreactive T cells, studies suggest that regulation of B cells in peripheral lymphoid tissues may be most important for the prevention of B-cell autoimmunity. Evidence also suggests that one of the genetically determined immune defects in SLE may cause generalized B-cell dysfunction and disrupt the peripheral B-cell tolerance process (26,38,76).

B cells are more important in the development of SLE than just as a source of autoantibodies (82). Studies in murine lupus have indicated that disease-related $CD4^+$ T-cell activation is dependent on the presence of B cells (82). Studies also have demonstrated an important role for B cells in epitope spreading (59), probably related to the effectiveness of antigen-specific B cells to present additional determinants after binding and internalizing antigenic complexes.

Autoantibodies

Among autoantibodies produced in SLE, those directed against components of the cell nucleus are the most characteristic and are found in more than 95% of patients (2,83–85). These antinuclear antibodies can be directed to DNA, RNA, as well as multiple different proteins contained in protein–nucleic acid complexes (Table 65-2). Cytoplasmic proteins associated with RNA also can be targets of autoantibodies in SLE (86). In general, these nuclear and cytoplasmic molecules are involved in critically important cell functions, including storage of genetic material, cell division, regulation of gene expression, RNA transcription, and RNA processing. The third major group of autoantigens in SLE is cell surface molecules (e.g., those on the surface of red blood cells, platelets, or lymphocytes). In general, the only well-studied autoantibody specificities relevant to renal involvement are antibodies to double-stranded DNA (dsDNA). In a subset of SLE patients with renal involvement, antiphospholipid antibodies may contribute to renal pathology by causing thrombosis and a thrombotic microangiopathy (discussed in more detail later in this chapter).

Antibodies to Double-Stranded DNA and Their Role in the Pathogenesis of Lupus Glomerulonephritis

Anti-dsDNA antibodies (87–89), especially when present in high levels in serum, are found essentially only in SLE patients. The titer of these antibodies fluctuate with disease activity, including systemic disease activity, but appear to correlate best with expression of glomerulonephritis in SLE. In some patients, serial measurements are useful in predicting disease flares, and quantitation of anti-dsDNA antibody activity in serum may be useful in the management of individual patients with SLE. It is clear, however, that some patients with active nephritis lack detectable serum anti-dsDNA activity, whereas other patients demonstrate persistently high levels without clinical evidence of nephritis. Some of these discrepancies may reflect problems with the assays used to measure anti-dsDNA activity, and studies have demonstrated that anti-dsDNA antibodies vary greatly in their pathogenic potential. In contrast to anti-dsDNA antibodies, antibodies to single-stranded DNA (ssDNA) have little

TABLE 65-2

EXAMPLES OF AUTOANTIBODIES IN SYSTEMIC LYPUS ERYTHEMATOSUS (SLE) AND THEIR CLINICAL ASSOCIATIONS

Target	Clinical associations
sDNA	High diagnostic specificity for SLE Correlation with disease activity (especially nephritis)
sDNA	Low diagnostic specificity
Histones	SLE and drug-induced lupus
Sm (snRNP proteins B, B′, D, E)	High diagnostic specificity for SLE No correlation with disease activity
U1 RNP Sm (snRNP proteins A, C, 70 kDa)	MCTD or other overlap syndrome (when not accompanied by anti-Sm)
SS-A/Ro (60-kDa and 50-kDa proteins)	Neonatal lupus (with anti-SS-B/La) Photosensitivity and subacute cutaneous LE
SS-B/La (48-kDa protein)	Associated with Sjögren's syndrome Neonatal lupus (with anti-SS-A/Ro)
Ku	High diagnostic specificity for SLE
PCNA/cyclin	High diagnostic specificity for SLE
Ribosomal P	High diagnostic specificity for SLE Cytoplasmic staining Psychiatric disease
Phospholipids (β2-glycoprotein 1)	Lupus anticoagulant Thrombosis Recurrent abortions/fetal wasting Focal neurologic deficits Thrombocytopenia Livido reticularis
Cell surface antigens	
Platelets	Thrombocytopenia
Red blood cells	Hemolytic anemia
Lymphocytes	Lymphopenia
Neuronal cells	Diffuse neurologic deficits

SnRNP, small nuclear ribonucleoprotein; *MCTD,* mixed connective tissue disease.
(From: Kotzin BL, O'Dell JR. Systemic lupus erythematosus. In: Frank MM, et al., eds. *Samter's Immunologic Diseases,* 5th ed. Boston: Little, Brown; 1994, with permission.)

diagnostic specificity and correlate poorly with renal disease activity.

IgG antibodies directed to dsDNA appear to play a prominent role in lupus nephritis (2,87–89). The evidence for this includes: (a) detection of anti-dsDNA antibodies in the glomeruli of patients and animals with active disease; (b) studies showing enrichment of IgG anti-dsDNA antibodies in glomerular tissues relative to serum and other organs; (c) longitudinal studies in a subset of SLE patients demonstrating that high levels of circulating anti-dsDNA antibodies frequently precede, or coincide with, active glomerulonephritis; and (d) demonstration in animals that injection of certain monoclonal IgG anti-dsDNA antibodies or expression of genes that encode pathogenic IgG anti-dsDNA activity, can lead to glomerular pathology.

Anti-DNA antibodies do not appear to mediate renal damage in SLE through the deposition of circulating immune complexes. Even in patients with active glomerulonephritis or animal models with actively increasing amounts of anti-DNA antibodies in the glomerulus, DNA–anti-DNA complexes have been difficult to demonstrate in the circulation (87,90). Two alternative theories have been proposed to explain the pathogenic mechanisms of these antibodies. In the first, anti-DNA–DNA complexes are proposed to form in the glomerulus (*in situ* complex formation) rather than being deposited from the blood (91). Evidence supports a model in which DNA first binds to the glomerulus and is then recognized and bound by anti-DNA antibodies (92–94). Recent studies suggest that the negatively charged DNA may be binding to the glomerular basement membrane through positively charged histones, and that the target of autoantibodies is a DNA–histone–Type IV collagen complex within the glomerular basement membrane (94–97). It is of interest that increased amounts of circulating DNA have been detected in the blood of patients with SLE, which appears to be in the form of nucleosomes and therefore mostly composed of DNA and histones (98,99). The circulating nuclear material thus could become the planted renal target for a subset of pathogenic anti-DNA antibodies. This model also opens the possibility that selected antihistone, antichromatin, or other antinuclear specificities also could be pathogenic with mechanisms of damage similar to anti-DNA antibodies. There is evidence also that qualitative differences in anti-dsDNA antibodies are critically important in determining potential pathogenicity, and these differences may operate at the level of *in situ* complex formation. Other properties that have been proposed to affect pathogenicity include charge (cationic antibodies appear to be more pathogenic), avidity for DNA, idiotypes carried by the autoantibody, and the epitopes on DNA being recognized (87,88). Finally, in the planted antigen model, the release of antigen (i.e., DNA or nucleosomes) may be an additional variable in determining pathogenicity in lupus nephritis. This variable may explain studies in mice showing that "pathogenic" anti-dsDNA antibodies (either administered or induced) are not sufficient for the induction of severe renal disease (100,101). Analogous situations in human lupus nephritis also seem likely.

In an alternative model, the subset of pathogenic anti-DNA antibodies has been hypothesized to cross-react with glomerular antigens that are not DNA in origin. Thus, the potential to cross-react would be the determinant of pathogenicity. This model is supported by data showing that anti-DNA antibodies do contain other specificities and can bind to different glomerular structures (87,88,102–104). The model is also supported by data from other disease systems in which antibodies to intracellular antigens have been associated with glomerulonephritis, through cross-reactivity with glomerular antigens.

It should be emphasized that the correlation of anti-dsDNA autoantibody levels with activity of lupus nephritis is a general one. For example, numerous studies have shown that glomerulonephritis in SLE can occur in the absence of detectable anti-DNA antibodies (87,88). Although these cases may represent a failure of the sensitivity of currently available techniques to measure anti-DNA antibodies, it also suggests that autoantibodies directed to non-DNA antigens may participate in lupus renal damage in some cases. In most spontaneous murine models of lupus nephritis, antibodies to a self-retroviral envelope glycoprotein (gp70), and gp70-anti-gp70 immune complexes, are important in the development of severe nephritis (105). As discussed in the preceding paragraphs, there is reason to believe that a subset of antibodies directed to non-DNA antigens of chromatin also may be pathogenic. The relative contribution of autoantibody systems in addition to anti-DNA antibodies in lupus nephritis needs to be clarified.

Complement Activation and Fcγ Receptors in Renal Damage

The activation of complement components through the classical pathway appears to be critically involved in the

pathogenesis of immune complex-mediated glomerular damage in lupus. Direct damage as well as recruitment of inflammatory cells are likely to be involved. Thus, IgG anti-DNA antibodies that are complement-fixing are most likely to be pathogenic (88). Studies in murine lupus nephritis have shown dramatic suppression of renal disease after injection of blocking monoclonal antibodies specific for complement factor C5 (21). Inhibition of complement activation in a similar fashion has been proposed as a possible therapy for patients with lupus nephritis, and studies are in progress to test this idea. In addition to the role of complement, murine studies have shown that effector cells bearing Fcγ receptors for IgG (Fcγ receptors), particularly FcγRIII, are necessary for immune complexes to mediate the full extent of damage in lupus nephritis (25).

CLINICAL–PATHOLOGIC CORRELATION IN LUPUS NEPHRITIS

Almost all patients with SLE demonstrate abnormalities on renal biopsy, especially if immunofluorescence and electron microscopy analyses are performed. Approximately half of all patients have clinically significant renal involvement at time of diagnosis. Importantly, lupus nephritis demonstrates remarkable variability in its pathologic appearance, clinical presentation, and course. Morphologic differences can be apparent among biopsies from different patients as well as among glomeruli in the same biopsy.

In order to confidently classify lupus glomerulonephritis, the renal biopsy should contain a minimum of 10 glomeruli for light microscopic analysis (106). Immunofluorescence is necessary for complete analysis and should include staining for IgG, IgA, and IgM isotypes, kappa and lambda light chains, and complement components C3 and C1q. Glomerular immune deposits in lupus nephritis should always contain dominant polyclonal IgG as well as C3 and usually C1q with lesser amounts of IgM and IgA (i.e., full house pattern). Immunofluorescence patterns not containing IgG should suggest an alternative diagnosis.

Each of the histologic types of lupus nephritis represents a nonspecific response to immune complex deposition. The one pathologic finding that is relatively specific for lupus is the presence of tubuloreticular structures in the glomerular endothelial cells seen on electron microscopy. These structures are composed of ribonucleoprotein and membrane and their synthesis is stimulated by alpha interferon (107).

World Health Organization Pathologic Classification

The World Health Organization (WHO) pathologic classification system for lupus nephritis was formulated in 1974 and modified in 1982 and 1995 (Table 65–3) (108,109). This histologic scheme addresses only glomerular lesions and in general correlates with clinical severity and prognosis in patients with lupus nephritis (110–115). However, it has been emphasized that knowledge of the WHO histologic type of renal disease may add little clinically useful information over and above that already known from clinical laboratory studies (urinalysis, protein excretion, and especially renal function studies) (113–117). When interpreting histologic findings, one must keep in mind that the renal biopsy is only a reflection of what is going on in the kidney at the time it is done and that changes from one pathologic stage to another over time are well documented in patients with lupus nephritis (110–112,118–121). In patients who undergo biopsy a second time, up to 40% may have undergone a change to another histologic class. This consideration is particularly true if patients are biopsied early in the course of their lupus and before therapy.

TABLE 65-3

MODIFIED WORLD HEALTH ORGANIZATION (WHO) MORPHOLOGIC CLASSIFICATION OF LUPUS NEPHRITIS[a]

CLASS I	Normal glomeruli a. Nil (by all techniques) b. Normal by light microscopy, but deposits by electron or immunofluorescence microscopy
CLASS II (12%–25%)	Pure mesangial alterations (mesangiopathy) a. Mesangial widening and/or mild hypercellularity (+) b. Moderate hypercellularity (++)
CLASS III (16%–22%)	Focal segmental glomerulonephritis (associated with mild or moderate mesangial alterations) a. With "active" necrotizing lesions b. With "active" and sclerosing lesions c. With sclerosing lesions
CLASS IV (37%–46%)	Diffuse glomerulonephritis (severe mesangial, endocapillary or mesangiocapillary proliferation and/or extensive subendothelial deposits) a. Without segmental lesions b. With "active" necrotizing lesions c. With "active" and sclerosing lesions d. With sclerosing lesions
CLASS V (11%–21%)	Diffuse membranous glomerulonephritis a. Pure membranous glomerulonephritis b. Associated with lesions of class II c. Associated with lesions of class III d. Associated with lesions of class IV
CLASS VI (3%–4%)	Advanced sclerosing glomerulonephritis

[a]Percentages under each WHO class represent frequency of biopsy results in various genes of systemic lupus erythematosus (SLE) patients in which biopsies were systematically performed (105–111,114–117).

Renal biopsies showing normal glomeruli by light microscopy are designated WHO Class I. These are subdivided into completely normal by all techniques and those that have deposits demonstrated by immunofluorescence and/or electron microscopy. Mesangial nephritis (WHO Class II) (Figs. 65-2 and 65-3) is characterized by immune deposits in the mesangium that are best seen by immunofluorescence and electron microscopy. Light microscopy reveals mesangial hypercellularity and/or increased/expanded matrix. Deposits in the capillary loops are not apparent or minimal in this class of disease. Patients with mesangial nephritis usually demonstrate little clinical evidence of renal involvement, with normal or near normal urinalysis and renal function, and rarely require any treatment for their renal disease. It should be emphasized that mesangial changes represent a common denominator in lupus nephritis, and other lesions described subsequently are superimposed on these abnormalities.

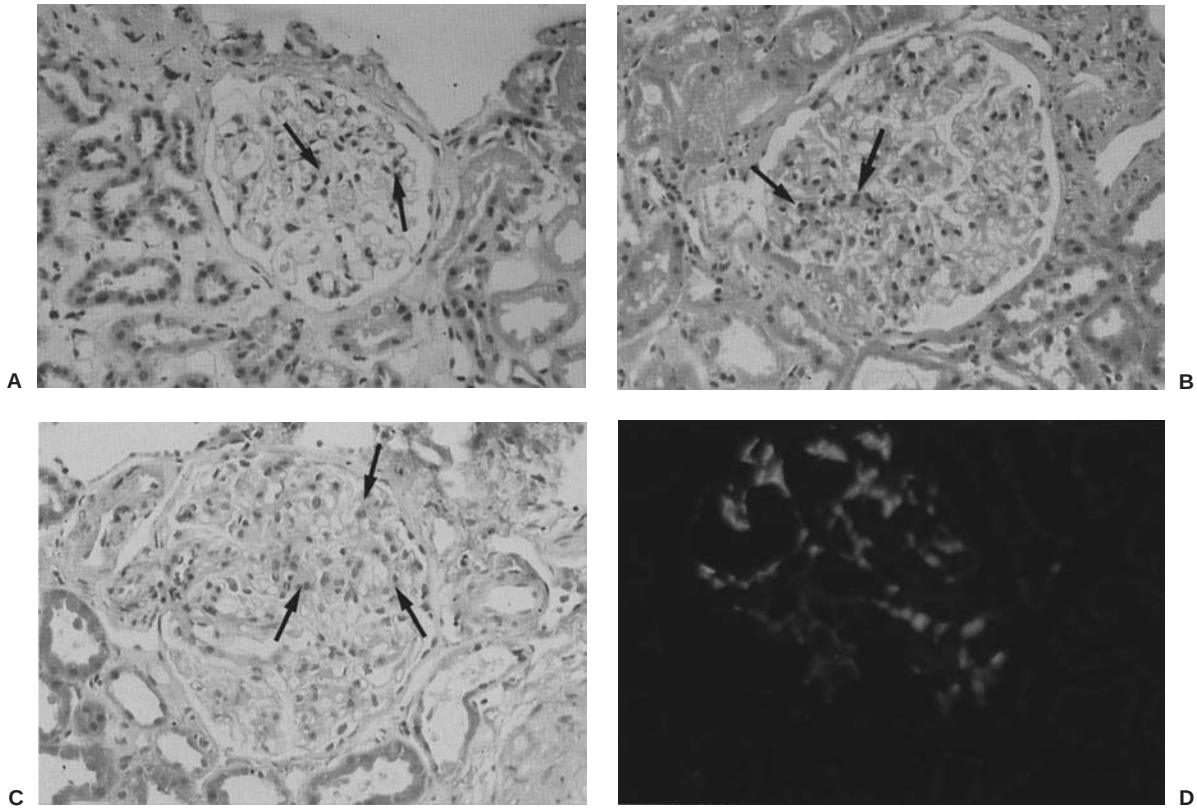


FIGURE 65-2. Histologic characteristics of mesangial glomerulonephropathy (World Health Organization Class II) in a patient with systemic lupus erythematosus. **A:** A normal glomerulus for comparison. Note the number and distribution of mesangial cells (*arrows*). (Hematoxylin and eosin, magnification ×200.) **B:** An early mesangial nephropathy displaying a subtle increase of mesangial cells (*arrows*). Compare with the normal glomerulus in **A**. (Hematoxylin and eosin, magnification ×200.) **C:** Another glomerulus from the same patient as in **B** but with a segmental accentuation of mesangial cells (*arrows*). This would be classified as a World Health Organization Class IIb lesion. (Hematoxylin and eosin, magnification ×200.) **D:** The mesangial matrix is accentuated by immunofluorescent deposits of IgG. This is an immunofluorescence analysis of the biopsy material shown in **B**. (Anti-IgG, magnification ×200.) (See Color Plate.)

Focal segmental (proliferative) glomerulonephritis (WHO Class III) (Fig. 65-4) is characterized histologically by hypercellularity owing to increases in mesangial, endocapillary, and/or infiltrating cells. These changes result in encroachment of the glomerular capillary space. Active inflammatory lesions are present. Lesions involve less than 50% of the glomeruli and are usually apparent in a segmental pattern (i.e., involve less than 50% of the glomerular tuft). Some investigators consider segmental glomerular necrosis to be the defining feature of WHO Class II lesions. Consequently, this class of lupus nephritis is further subdivided into those with active necrotizing lesions, combined active and sclerosing lesions, and primarily sclerosing lesions. Patients with this pattern frequently demonstrate proteinuria and hematuria, but severe (nephrotic range) proteinuria or progressive loss of renal function is less common. However, focal proliferative nephritis should be viewed as on a continuum with diffuse proliferative glomerulonephritis because the lesions are qualitatively similar (Figs. 65-5 and 65-6) and may progress to the more severe category with time.

Diffuse proliferative glomerulonephritis (DPGN) (WHO Class IV) is seen in about 35% to 45% of lupus patients with clinical renal disease and in most SLE patients who progress to renal failure (108–113,119–121). Diffuse proliferative glomerulonephritis is characterized by involvement of more than 50% of the glomeruli, with generalized hypercellularity of mesangial and endothelial cells. Inflammation with inflammatory cellular infiltrates, cellular destruction with nuclear fragmentation, and areas of fibrinoid necrosis are common in severe disease (Fig. 65-5). These changes ultimately may lead to obliteration of the capillary loops and sclerosis. Subendothelial deposits within the capillary loops and the resultant thickening of the membrane contour can result in "wire loop" lesions (Figs. 65-5 and 65-6A). Hyaline thrombi from massive aggregates of intraluminal immune complex deposits also may be present. Regions of basement membrane thickening are usually present. Crescents also may be seen in a subset of patients with progressive glomerulonephritis (Chapter 60). A subset of patients may demonstrate histologic abnormalities characteristic of true membranoproliferative (mesangiocapillary) glomerulonephritis (Chapter 62). Similar to Class III, WHO Class IV is further subdivided based on the presence of active, sclerosing, or mixed types of glomerular injury. Immunofluorescence microscopy demonstrates extensive deposition of immunoglobulin and complement in the mesangium and capillary loops, and electron microscopy frequently shows immune complex deposits in both subendothelial and subepithelial distributions (Figs. 65-5 and 65-6). Clinically, patients almost always have proteinuria and hematuria and not infrequently have decreases in renal function. Hypertension is common. Rarely, patients with no clinical evidence of renal disease have been biopsied and found to have focal or even diffuse proliferative glomerulonephritis (122), but progression does not occur without the development of urinalysis abnormalities.

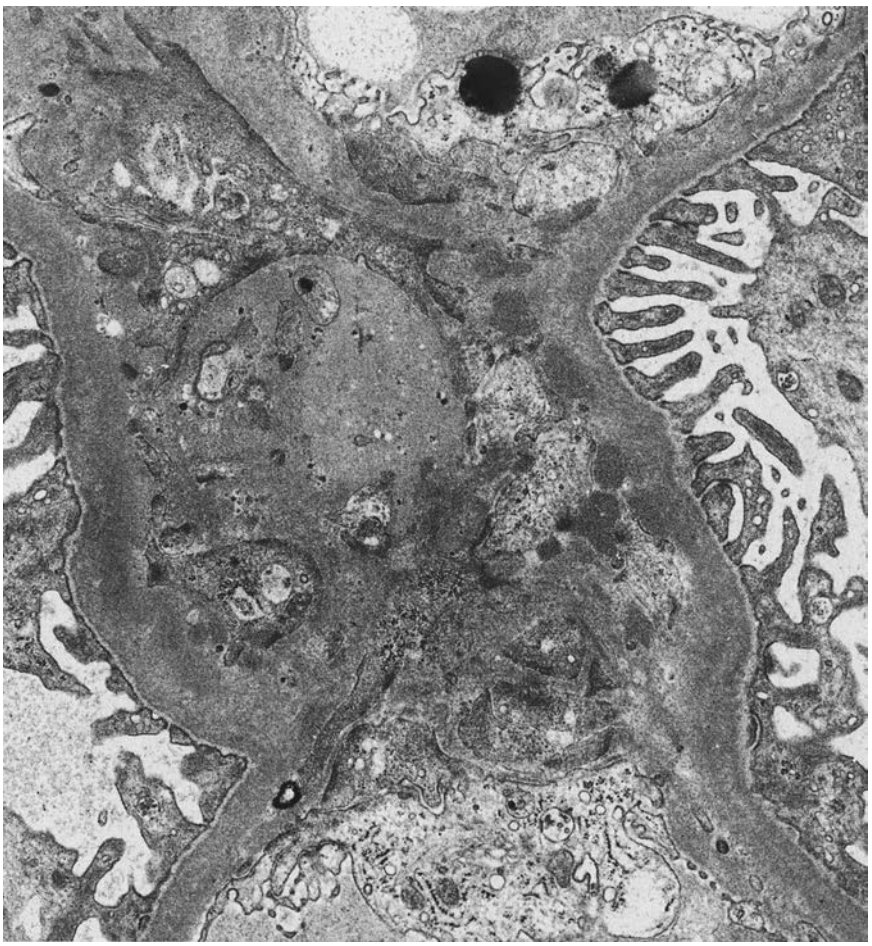


FIGURE 65-3. Electron microscopic analysis of mesangial glomerulonephropathy in a patient with systemic lupus erythematosus. Multiple electron-dense deposits are evident in the mesangium of a WHO Class IIb lesion. (Magnification ×9,000.) Light microscopic and immunofluorescence analyses of mesangial glomerulonephropathy are shown in Figure 65-2.

Membranous nephropathy (WHO Class V) (Figs. 65-7 and 65-8) is characterized histologically by diffuse thickening of the basement membrane (108–112,123–125) (Chapter 63). Glomeruli usually have normal cellularity. Lesions are further subdivided based on the presence of mesangial hypercellularity (Class II) and overlaps with focal proliferative (Class III) and diffuse proliferative (Class IV) lupus nephritis. Subepithelial deposits containing immunoglobulin and complement are apparent along the basement membrane on electron microscopy and immunofluorescence microscopy (Figs. 65-7 and 65-8). Immune deposits are also seen in the mesangium helping to separate it from idiopathic membranous nephropathy.

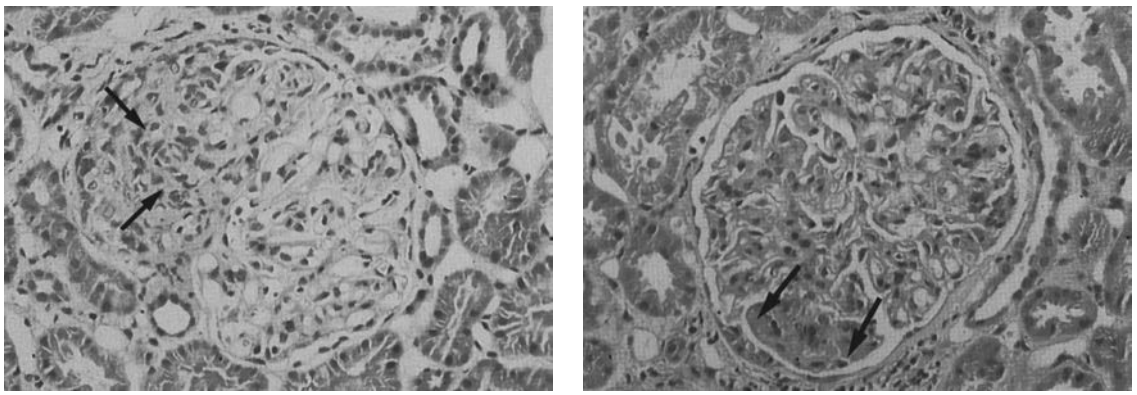


FIGURE 65-4. Histologic characteristics of focal proliferative glomerulonephritis (World Health Organization Class III) in a patient with systemic lupus erythematosus. **A:** Approximately one-half of this glomerular tuft is involved by mesangial proliferation (*arrows*), which at medium power is discernible as a segmental lesion. (Hematoxylin and eosin, magnification ×200.) **B:** Another glomerulus from the same patient. Here there is a mild global increase of mesangial cells and a segmental lesion with a suggestion of early tuft necrosis (*arrows*). (Hematoxylin and eosin, magnification ×200.) Less than half of the glomeruli in this biopsy demonstrated segmental lesions. (See Color Plate.)

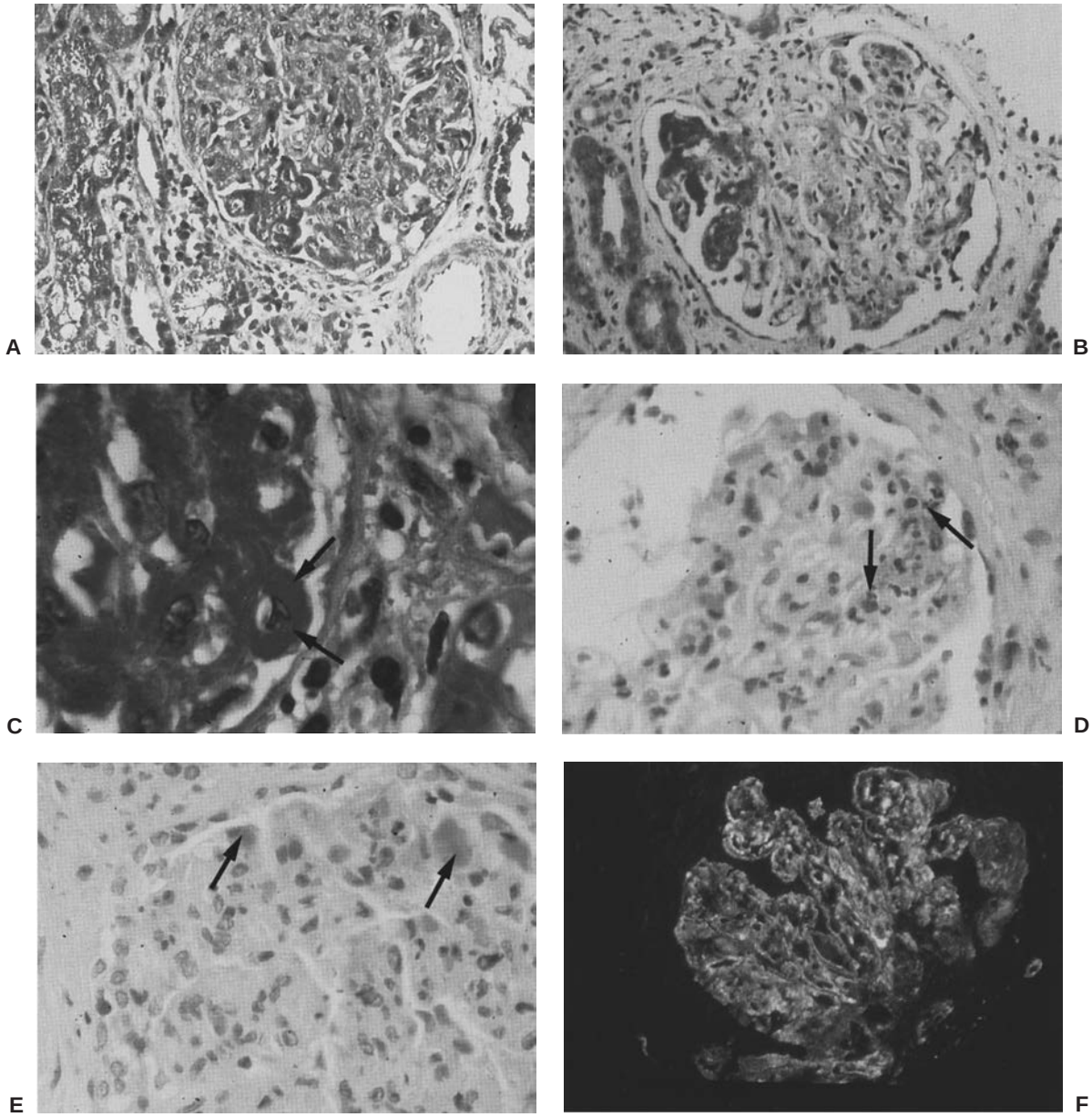
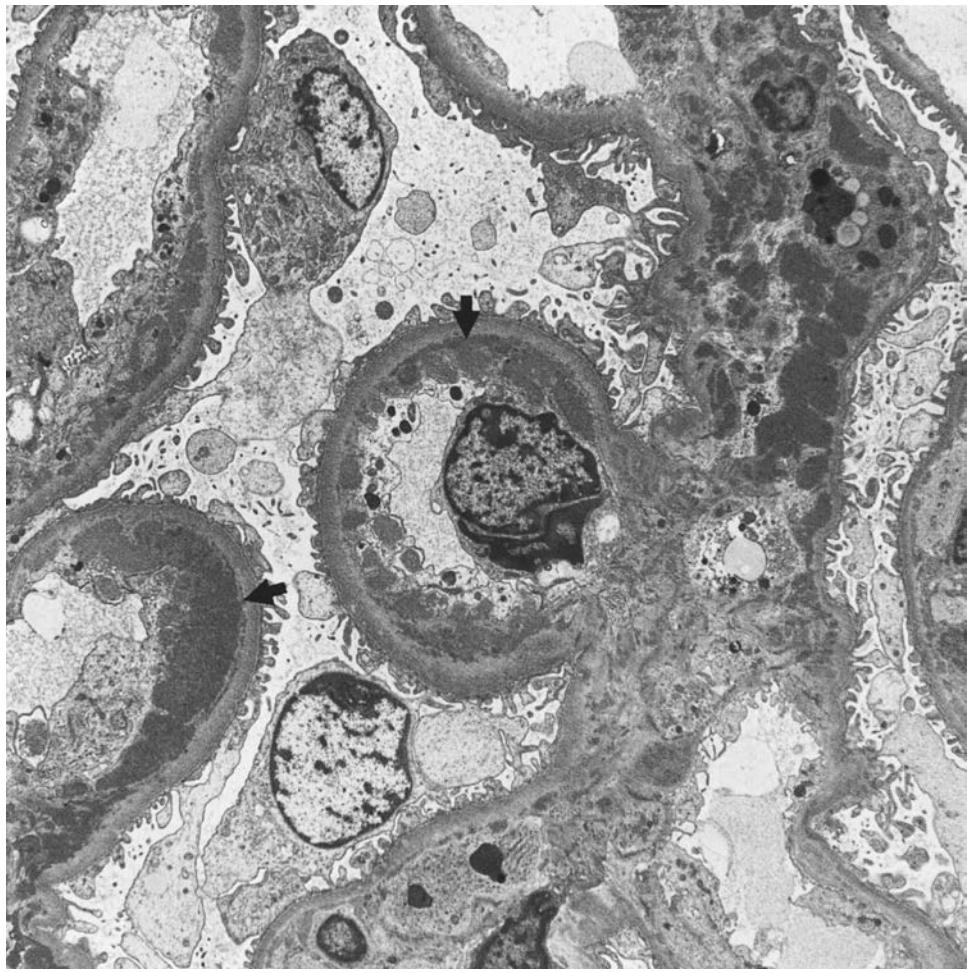


FIGURE 65-5. Histologic characteristics of diffuse proliferative glomerulonephritis (World Health Organization Class IV) in a patient with systemic lupus erythematosus. This plate also shows examples of the abnormalities used in the scoring of the activity index (Table 65-4). **A:** The majority of glomeruli in this case were involved by a global proliferation of mesangial cells and varying indicators of acute activity. (Trichrome, magnification ×200.) **B:** This trichrome stain accentuates the intracapillary thrombi present in the same biopsy as **A**. **C:** Subendothelial deposits are seen within the capillary loop (*arrows*). The resultant thickening of the membrane contour is the source of the descriptive term "wire loop." (Trichrome, magnification ×800.) **D:** Multiple neutrophils are apparent within the mesangium and capillary lumens (*arrows*). (Hematoxylin and eosin, magnification ×400.) **E:** Hematoxylin bodies (*arrows*) are not often seen in biopsy material. They are the result of altered nuclear DNA and are considered pathognomonic of lupus nephritis. Notice also the neutrophilic infiltrates and cellular proliferation, both features of an active lesion. (Hematoxylin and eosin, magnification ×400.) **F:** Extensive glomerular deposition of immunoglobulin demonstrated by immunofluorescent staining for IgG. Compare the distribution and intensity of staining with the immunofluorescence analysis of mesangial nephropathy shown in Figure 65-2D. (From: Kotzin BL, O'Dell JR. Systemic lupus erythematosus. In: Frank MM, et al, eds. *Samter's Immunologic Diseases,* 5th ed. Boston: Little, Brown; 1994, with permission.) (See Color Plate.)

Clinically, patients who have pure membranous disease frequently have extensive proteinuria but only minimal hematuria or functional renal abnormalities. Complement levels and titers of anti-dsDNA antibodies are frequently normal and do not correlate with the activity of this type of nephropathy. A subset of patients, estimated at 10% to 30%, slowly progresses to chronic renal failure within a 10-year period. The natural history of this form of membranous disease is somewhat unclear because nearly all patients with severe clinical manifestations are treated. Membranous disease also can be observed as a transition stage after treatment for proliferative glomerulonephritis (121,126).



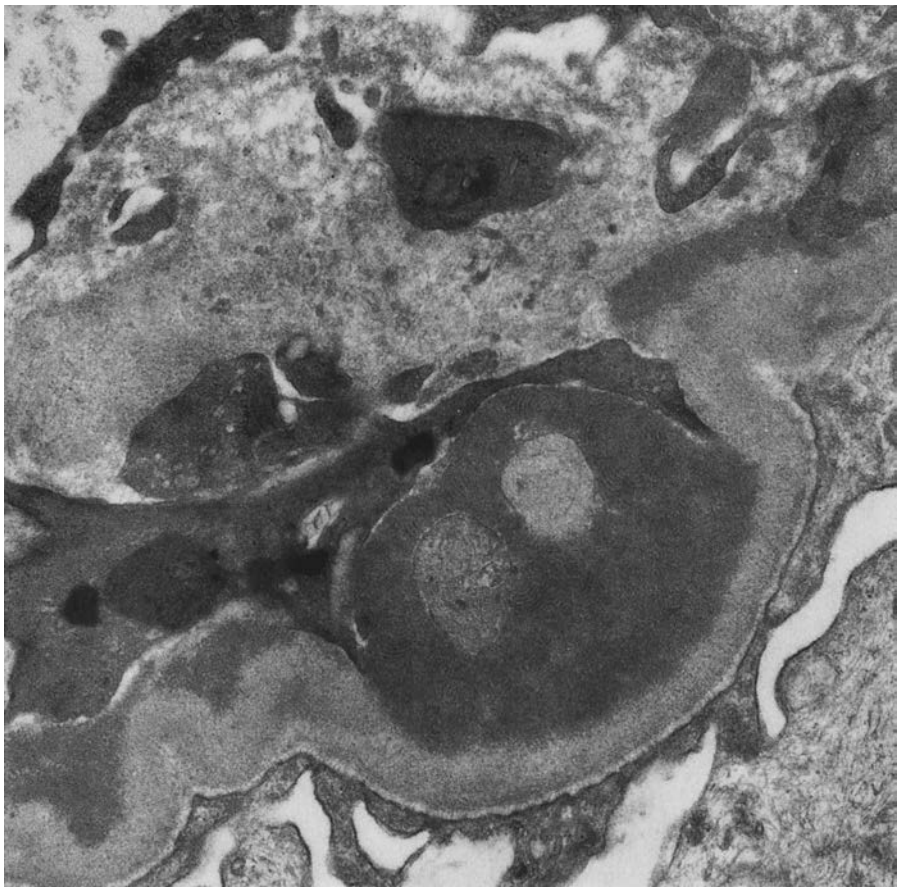
A

FIGURE 65-6. Electron microscopic analysis of diffuse proliferative glomerulonephritis (World Health Organization Class IV) in a patient with systemic lupus erythematosus. **A:** In this electron microscopic view of a wire-loop lesion, prominent electron-dense deposits (*arrows*) partially fill the subendothelial space. Dense deposits are also seen throughout the mesangium, which is characteristic of lupus nephritis. (Magnification ×9,000.) (*continued*)

Advanced sclerosing glomerulonephritis (WHO Class VI) was introduced when the WHO classification was first modified. This lesion is the result of progressive Class III, IV, or V glomerulonephritis. The use of numerous subdivisions has made the modified WHO classification system cumbersome and not widely accepted by all pathologists.

In 2003, an international group of renal pathologists, nephrologists, and rheumatologists developed an expanded classification system shown in Table 65-4 (127). This revised classification preserves the six WHO classes of lupus nephritis but introduces and defines important differences between Class III and IV lesions that are clinically relevant. In addition, activity and chronicity lesions are further described. This classification builds on the widely accepted WHO classification, attempts to eliminate the ambiguity about the definitions of classes III and IV lupus nephritis and includes segmental glomerulonephritis with 50% or greater glomerular involvement. A new subdivision of class IV as segmental or global has been included based on evidence that global involvement may have a different prognosis compared to segmental. This classification system is based on glomerular pathology, but the group strongly recommended separate documentation on the extent and severity of tubulointerstitial and vascular lesions, which can clearly affect renal prognosis. Whether this system is accepted by pathologists, improves uniformity of reporting between centers, and reflects clinical outcomes better than the previous classifications is under investigation.

Lupus can also involve the renal tubules, kidney interstitium, and blood vessels (108–112,128–130). Areas of lymphocytic interstitial infiltration may be apparent, and immunofluorescence may demonstrate antibodies bound to renal tubular cells (128,129). However, this almost always occurs in conjunction with glomerular involvement, and, in general, renal outcome is determined by the severity of the glomerular lesion. Interstitial features of the chronicity index (described subsequently) appear to be closely related to glomerular damage and glomerular dropout rather than secondary to chronic interstitial inflammation; these features of irreversible damage predict a poor outcome. The role of mononuclear cell infiltrates in the pathogenesis of lupus nephritis remains poorly understood. Vascular involvement in renal biopsies from patients with SLE can vary considerably, being related to hypertension, hyperlipidemia, a small-vessel vasculopathy or small-vessel thrombosis associated with antiphospholipid antibodies, or rarely vasculitis (130,131).



B

C

FIGURE 65-6. (*Continued*) **B:** Subendothelial deposits in a moderately active Class IV lesion, which show the concentric and swirling laminations dubbed "fingerprinting." Although not pathognomonic, they are commonly associated with lupus. (Magnification ×39,000.) **C:** Tubuloreticular structures within subendothelial cells (*arrows*). (Magnification ×62,000.) (Courtesy of Dr. William Hammond, Denver, CO.) Light microscopic and immunofluorescence analyses of diffuse proliferative glomerulonephritis are shown in Figure 65-5.

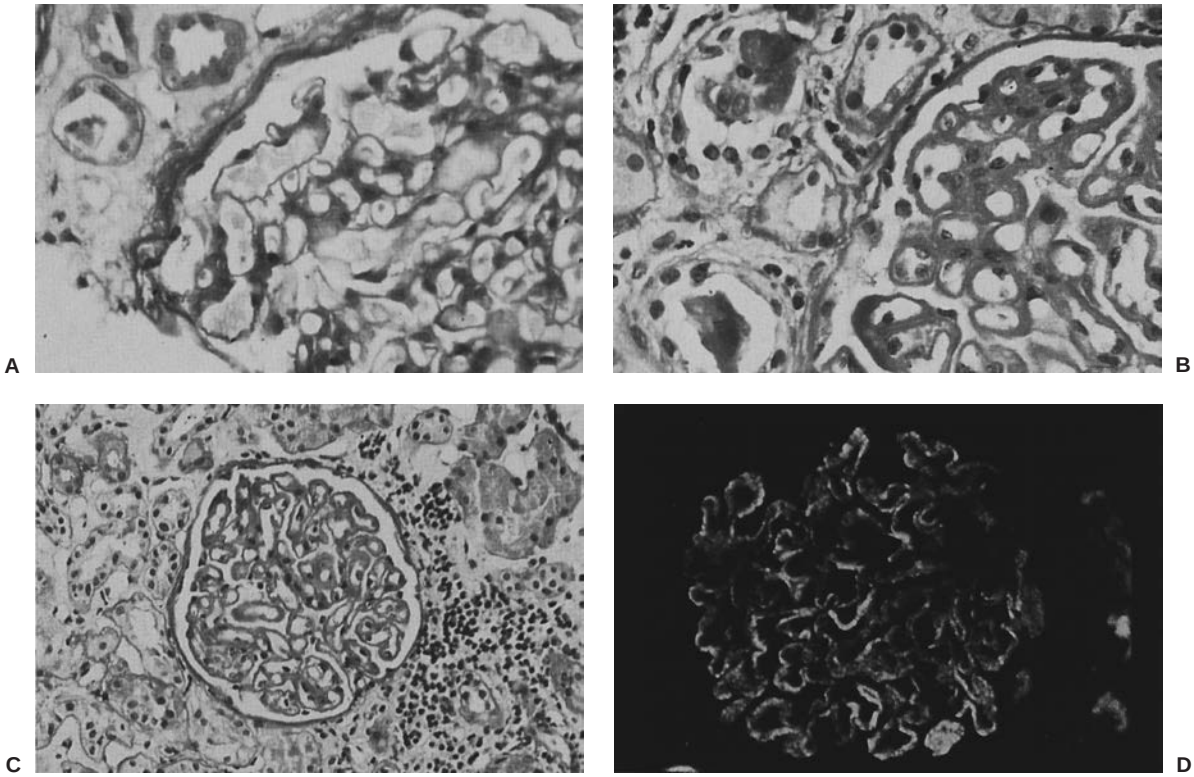


FIGURE 65-7. Histologic characteristics of membranous nephropathy (World Health Organization Class V) in a patient with systemic lupus erythematosus. **A:** Normal glomerulus for comparison. Notice the thin, delicate profile of the basement membranes. (Periodic acid–Schiff, magnification ×400.) **B:** Membranous nephropathy with thickened basement membranes. (Periodic acid–Schiff, magnification ×400.) Compare with the normal glomerulus in **A**. **C:** The thickened, rigid-appearing basement membranes of advanced membranous nephropathy are obvious even at medium power. (Periodic acid–Schiff, magnification ×200.) Lymphocytes are seen in the interstitium, and there is a hint of tubular dropout, an indicator that this is a chronic lesion. **D:** Immunofluorescence study of the same patient illustrated in **C**. The membranes are highlighted by fluorescent deposits of IgG. (Anti-IgG, magnification ×200.) (See Color Plate.)

Pathologic Indices of Activity and Chronicity

A semiquantitative histologic scoring system for lupus nephritis has been developed in an effort to more accurately predict renal outcome and to help determine which patients are most likely to benefit from aggressive therapy (7,119,132,133). In this system, a *chronicity index* measures four histologic components of chronic irreversible renal damage, and an *activity index* measures six histologic components of activity of lupus nephritis (Table 65-5, Figs. 65-5,65-6,65-9). In the last several years, these systems have come into major use, and a number of studies have reported on their predictive associations and usefulness. Most, but not all, studies have found that a higher chronicity index is associated with greater risk for progression to renal failure (7,113,114,117,132–144). For example, Austin and coworkers (133) showed that none of 29 patients with lupus nephritis and a low chronicity index (defined as scores of 0 to 1) progressed to renal failure over a 5-year period. In contrast, 40% of the patients with scores greater than 3 progressed to renal failure. Based on its apparent validity as a prognostic indicator as well as the fact that the presence of chronic damage identifies disease with destructive potential, several investigators have recommended the use of the chronicity index as a guide to the aggressiveness of therapy. In particular, treatment with agents such as cyclophosphamide was predicted to be most beneficial for patients with intermediate chronicity index scores (7,133). In contrast to the chronicity index, the validity of the activity index as a predictor of renal outcome is less clear; approximately half of these studies have shown that a higher score correlates with increased risk of renal failure (7,117,132–144). This may relate to the caveat that active lesions are amenable to therapy, whereas chronic lesions represent irreversible destruction. It seems likely that increased activity index scores identify patients most likely to respond to aggressive treatment.

Some studies have not been able to confirm the usefulness of the chronicity index for prognosis and management of patients with lupus nephritis (140–143). Most of the preceding studies validating this scoring system were performed in specialized academic centers with pathologists educated and skilled in the use of this histologic grading system. There is limited information on treatment and prognosis of lupus nephritis in primary care facilities. At least one study of renal pathologists from a nonreferral setting has suggested that problems with interreader reliability could explain some of the conflicting reports regarding the usefulness of the chronicity index scoring system (145). Importantly, small differences in chronicity index scoring could result in major differences in risk group assignment, and such differences in scoring could prevent the chronicity index from being a guide to therapy.

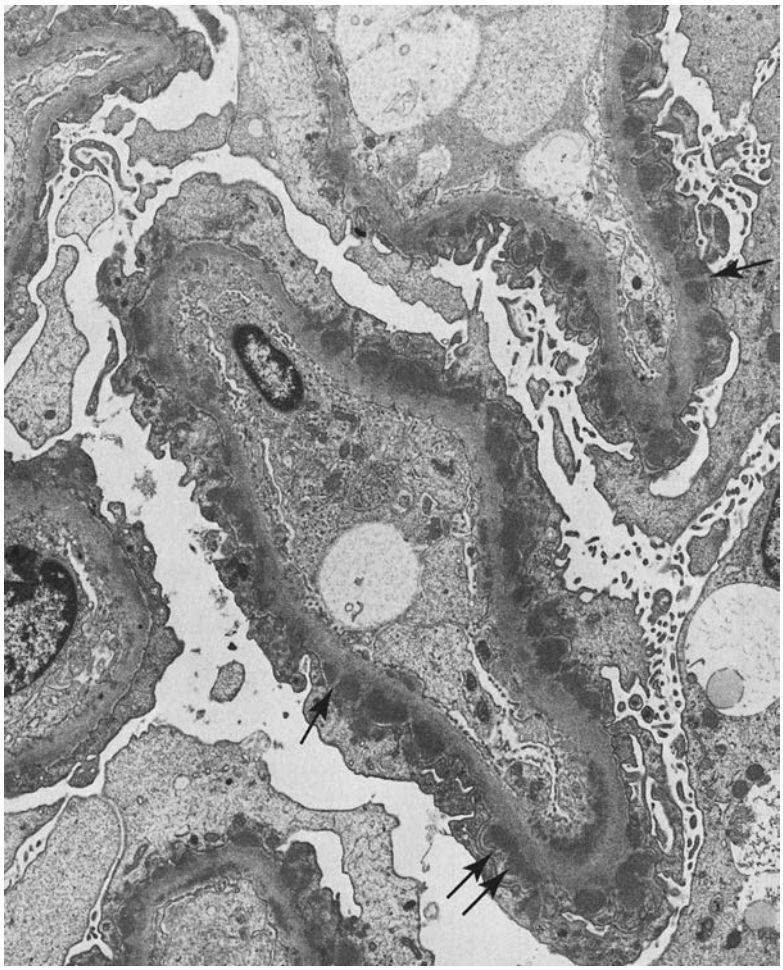


FIGURE 65-8. Ultrastructure of membranous nephropathy (World Health Organization Class V) in a patient with systemic lupus erythematosus, displaying numerous dark deposits along the outer aspect of the glomerular basement membrane. Basement membrane material has been formed and is insinuating itself between the deposits, forming so-called spikes (*single arrows*). The spikes will coalesce and envelop the deposits (*double arrows*), eventually incorporating the deposits into the membrane itself, where it will then be absorbed. (Magnification ×9,000.) Light microscopic and immunofluorescence analyses of membranous nephropathy are shown in Figure 65-7.

CLINICAL AND LABORATORY ABNORMALITIES IN LUPUS NEPHRITIS

Clinical Features

The development of lupus nephritis greatly influences long-term outcome in patients with SLE and is the most frequent reason for patients to be treated with high-dose corticosteroids and cytotoxic medications. At time of diagnosis, 50% of patients have an abnormal urinalysis with or without an elevated serum creatinine. Over the course of the disease, nearly 75% of all SLE patients demonstrate evidence of renal involvement, with urinalysis abnormalities (proteinuria (80%), hematuria (40%), or casts) or functional impairment with decreased glomerular filtration rate (GFR), as measured by creatinine clearance. Although clinically significant nephritis can occur at any time in the course of SLE, it usually occurs in the first few years (<3 years) of disease, rarely develops after 5 years, and can be the presenting feature of disease in 5% of patients (110,146–150). However, urinalysis screening at regular intervals is mandatory, since the onset of nephritis is frequently asymptomatic. Examination of an early morning, second voided urine is recommended to ensure a concentrated acidic urine specimen, which is the best condition to find urinary casts. Patients can also present with foamy urine (proteinuria), nocturia (tubular dysfunction), nephrotic syndrome, or nephritic syndrome with hypertension, depending on the extent of immune deposits and inflammation within the glomerulus. Rarely patients can present with macroscopic hematuria, rapidly progressive glomerulonephritis (doubling of creatinine within 3 months), or acute renal failure. Renal tubular acidosis is unusual and usually does not require therapy.

Individual studies have indicated that renal disease is more frequent and severe in childhood onset than adult onset SLE patients (149). In adults, young age at onset (<33 years old), male sex and nonwhite race or socioeconomic status are risk factors for developing early and more severe nephritis (5,7,144,147,150). Alternatively, patients with onset after age 50 usually have milder disease, including nephritis, although some patients can develop renal damage (151). Except for hypertension, no nonrenal clinical manifestation of SLE is reasonably reliable in predicting the likelihood of developing lupus nephritis or predicting the severity of renal involvement and renal outcome (152). As discussed in the preceding section, variability and unpredictability are major features of the course of SLE.

Hypertension is common in patients with lupus nephritis and may be important in the progression of renal damage in patients with severe disease (153). Several studies have suggested that it predicts a poor renal outcome (143,154,155). Hypertension also plays a significant role in the increased incidence of coronary artery disease seen in the SLE population. Control of hypertension is critically important for a successful outcome in lupus nephritis.

TABLE 65-4

INTERNATIONAL SOCIETY OF NEPHROLOGY/RENAL PATHOLOGY CLASSIFICATION OF LUPUS NEPHRITIS (2003)

CLASS I	**MINIMAL MESANGIAL LUPUS NEPHRITIS** Normal glomeruli by light microscopy, but mesangial immune deposits by immunofluorescence
CLASS II	**MESANGIAL PROLIFERATIVE LUPUS NEPHRITIS** Purely mesangial hypercellularity of any degree or mesangial matrix expansion by light microscopy, with mesangial immune deposits A few isolated subepithelial or subendothelial deposits may be visible by immunofluorescence or electron microscopy, but not by light microscopy
CLASS III	**FOCAL LUPUS NEPHRITIS**[a] Active or inactive focal, segmental or global endo- or extracapillary glomerulonephritis involving <50% of all glomeruli, typically with focal subendothelial immune deposits, with or without mesangial alterations
Class III (A)	Active lesions: focal proliferative lupus nephritis
Class III (A/C)	Active and chronic lesions: focal proliferative and sclerosing lupus nephritis
Class III (C)	Chronic inactive lesions with glomerular scars: focal sclerosing lupus nephritis
CLASS IV	**DIFFUSE LUPUS NEPHRITIS**[b] Active or inactive diffuse, segmental or global endo- or extracapillary glomerulonephritis involving ≥50% of all glomeruli, typically with diffuse subendothelial immune deposits, with or without mesangial alterations. This class is divided into diffuse segmental (IV-S) lupus nephritis when ≥50% of the involved glomeruli have segmental lesions, and diffuse global (IV-G) lupus nephritis when ≥50% of the involved glomeruli have global lesions. Segmental is defined as a glomerular lesion that involves less than half of the glomerular tuft. This class includes cases with diffuse wire loop deposits but with little or no glomerular proliferation.
Class IV-S (A)	Active lesions: diffuse segmental proliferative lupus nephritis
Class IV-G (A)	Active lesions: diffuse global proliferative lupus nephritis
Class IV-S (A/C)	Active and chronic lesions: diffuse segmental proliferative and sclerosing lupus nephritis
Class IV-G (A/C)	Active and chronic lesions: diffuse global proliferative and sclerosing lupus nephritis
Class IV-S (C)	Chronic inactive lesions with scars: diffuse segmental sclerosing lupus nephritis
Class IV-G (C)	Chronic inactive lesions with scars: diffuse global sclerosing lupus nephritis
CLASS V	**MEMBRANOUS LUPUS NEPHRITIS** Global or segmental subepithelial immune deposits or their morphologic sequelae by light microscopy and by immunofluorescence or electron microscopy, with or without mesangial alterations Lupus nephritis may occur in combination with class III or IV in which case both will be diagnosed Class V lupus nephritis may show advanced sclerosis
CLASS VI	**ADVANCED SCLEROTIC LUPUS NEPHRITIS** ≥ 90% of glomeruli globally sclerosed without residual activity

Indicate and grade (mild, moderate, severe) tubular atrophy, interstitial inflammation and fibrosis, severity of arteriosclerosis or other vascular lesions.
[a]Indicate the proportion of glomeruli that are active and with sclerotic lesions.
[b]Indicate the proportion of glomeruli with fibrinoid necrosis and/or cellular crescents.

Pregnancy is not an infrequent occurrence in SLE patients and can be a difficult management problem (156,157). Pregnancy in the setting of active disease, especially active or progressive renal disease, can result in severe problems and a bad outcome for both the mother and baby. Among women with lupus nephritis, the incidence of preeclampsia can be as high as 66%. It may be difficult to distinguish preeclampsia from an exacerbation of lupus nephritis, although the presence of an active urinary sediment, elevated anti-dsDNA antibody levels, and hypocomplementemia favor lupus nephritis (158). Patients with active lupus nephritis have up to a 52% risk of fetal loss during pregnancy (159). Furthermore, disease flares and exacerbations of glomerulonephritis may occur, particularly in the third trimester and immediate postpartum period, even in patients who have been clinically quiescent (160). Notably, these flares can also occur following elective termination of pregnancy. Therefore, women with lupus nephritis should be encouraged to delay pregnancy until their disease is treated and inactive for at least 6 months (156).

Autoantibodies

As reviewed in the preceding text, antinuclear antibodies (ANAs) are the hallmark of autoantibody production in SLE, and more than 98% of SLE patients with nephritis demonstrate a positive ANA test (83–85). Antinuclear antibodies are detected in most laboratories by an indirect immunofluorescence assay with human tumor cells (usually HEP-2 cells) as the substrate fixed to the slide. These cells allow the greatest sensitivity and best definition of binding patterns. A variety of immunofluorescent staining patterns (e.g., peripheral or rim, diffuse, speckled, or nucleolar) can be observed on ANA testing. However, because of the development of tests that measure

TABLE 65-5

PATHOLOGIC INDICES OF ACTIVITY AND CHRONICITY IN LUPUS NEPHRITIS

Chronicity index[a]	Activity index[b]
Glomerular sclerosis	Cellular proliferation
Fibrous crescents	Fibrinoid necrosis, karyorrhexis
Tubular atrophy	Cellular crescents
Interstitial fibrosis	Hyaline thrombi, wire loop lesions
	Leukocyte infiltration in glomerulus
	Mononuclear-cell infiltration in interstitium

[a]To obtain a chronicity score, each parameter is graded 0 to 3 depending on severity of involvement, and the grades are added. Glomerular sclerosis and fibrous crescents are graded as follows: 0, absent; 1+, <25% of glomeruli involved; 2+, 25% to 50% of glomeruli involved; 3+, >75% of glomeruli involved. Tubular atrophy and interstitial fibrosis are graded as follows: 0, absent; 1+, mild; 2+, moderate; 3+, severe. The maximum chronicity score is 12.
[b]To obtain an activity score, each parameter is graded 0 to 3 depending on severity of involvement, and the individual grades are added. Fibrinoid necrosis and cellular crescents have been given a "weighting" factor of 2. The maximum activity score is 24.

specific ANA specificities, these patterns have less clinical significance today. Although elevated serum levels of ANA are very sensitive for the diagnosis of SLE, it is emphasized that this test is not at all specific for SLE or other connective tissue diseases. In a study of 15 international laboratories, nearly one-third of healthy individuals were found to have a positive ANA test at a serum dilution of 1:40 (161). The frequency of a positive ANA in healthy individuals was 5% at a serum dilution of 1:160 and 3% at 1:320. The sensitivity for the diagnosis of SLE at a 1:40 serum dilution was 97% compared to 87% at 1:320 (161). The specificity for SLE was only 68% at a titer of 1:40 but was 95% at 1:320. These results indicate that a low dilution (1:40) is most valuable for excluding the diagnosis of SLE, whereas titers higher than 1:160 have the best positive-likelihood ratio for the diagnosis of SLE.

If the screening ANA test is determined to be positive, many clinical laboratories will offer a panel to detect various specific ANAs, including antibodies to dsDNA, Sm, U1 RNP, SS-A/Ro, and SS-B/La (Table 65-2). A number of different techniques are used to detect and quantitate anti-DNA antibodies, including immunoprecipitation, radioimmunoassay (RIA), enzyme-linked immunoabsorbent assay (ELISA), and immunofluorescence staining. Using the hemoflagellate *Crithidia luciliae,* which has a kinetoplast containing only dsDNA, an indirect immunofluorescence assay has also been developed to specifically detect antibodies to dsDNA. Although ELISA and *Crithidia* techniques can be made specific and sensitive for antibodies that bind dsDNA, these assays may not best measure the subset of potentially pathogenic anti-dsDNA antibodies that correlate with lupus nephritis (88); for example, these assays may be capable of detecting lower avidity anti-dsDNA antibodies compared to other detection techniques (e.g., a modified Farr assay) that rely on precipitation of antibodies bound to DNA. Some studies have suggested that anti-DNA antibodies with high avidity have greater pathogenic potential (88).

Antiribosomal P is a highly specific lupus autoantibody that reacts with the 60S ribosomal subunit located in the cell's cytoplasm. Antiribosomal P antibodies are detected in about 15% of lupus patients and correlate with disease activity, particularly lupus cerebritis, and, more recently, nephritis. Reichlin

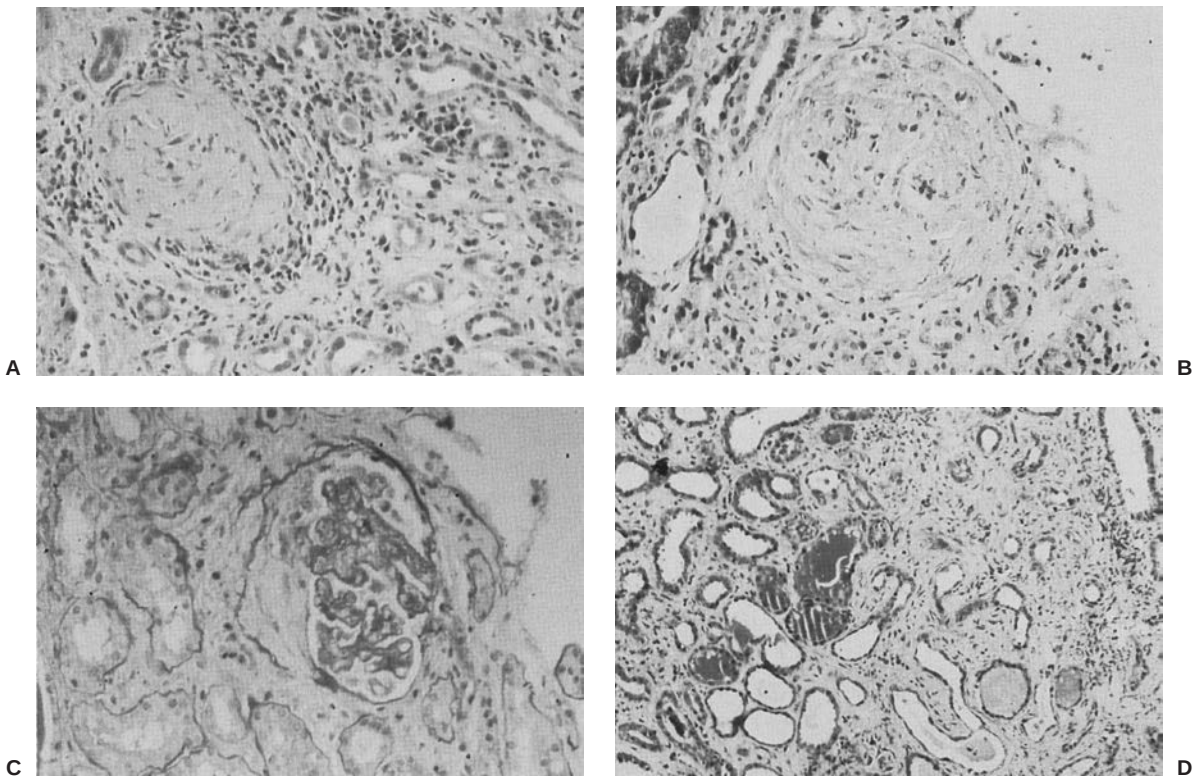


FIGURE 65-9. Examples of histologic features utilized in the chronicity index. **A:** An obsolescent glomerulus is surrounded by small strophic tubules. (Hematoxylin and eosin, magnification ×200.) **B:** The residuum of a glomerulus is barely visible in this example of a fibrous crescent. (Trichrome, magnification ×200.) **C:** A shrunken glomerular tuft is displaced by a fibrous crescent. **D:** Simplified, dilated tubules in a fibrotic stroma. (Hematoxylin and eosin, magnification ×200.) (See Color Plate.)

and Wolfson-Reichlin reported that the occurrence of both antiribosomal P and anti-ds DNA antibodies together were more associated with lupus nephritis than was the presence of either one alone (162). Interestingly, antiribosomal P antibodies can cross-react with DNA and are capable of penetrating live cells resulting in suppression of protein synthesis (163).

Serial monitoring of specific ANA in SLE should probably be limited to anti-dsDNA as there is no evidence that changes in the levels of other ANAs correlate with renal disease activity. Monitoring of anti-DNA antibody levels appears to be of most value in a subset of patients with lupus nephritis. They occur more frequently and in higher titers in patients with Class III or IV proliferative nephritis than with Class V (membranous) glomerulonephritis. Changes, particularly an increase in titer of anti-dsDNA antibodies, are more valuable than the absolute height of the antibody level (164.) Importantly, some patients with high titers of anti-dsDNA antibodies never develop nephritis, whereas severe nephritis can occasionally occur in patients with low titers of these antibodies. However, in general, patients with rising titers should be followed more closely for disease exacerbation.

The value of antichromatin/nucleosome and anti-C1q antibodies for assessing lupus nephritis activity and predicting flares is being actively investigated. Chromatin is the native histone-DNA complex organized into a repeating series of nucleosome in the nucleus of eukaryotic cells. Nucleosomes (chromatin) from apoptotic cells are autoantigens in SLE patients. Immune complexes composed of antichromatin antibodies and chromatin can bind to the glomerular basement membrane. Antichromatin antibodies have been reported to be associated with lupus nephritis with a sensitivity of up to 81% (165). Patients with antichromatin antibodies have a twofold higher prevalence of lupus nephritis and higher disease activity scores than patients without these antibodies. Similarly, anti-C1q antibodies are present in 30% to 45% of patients with SLE. These antibodies are strongly associated with lupus nephritis, and rising titers appear to predict renal flares (166). In addition, these antibodies appear to predispose patients to more severe forms of proliferative lupus nephritis. The binding of anti-C1q to C1q on immune complexes may facilitate immune complex deposition and/or amplify pathogenic complement activation once the immune complex has deposited or formed in the glomerulus (167).

Complement

Complement is consumed primarily via the classical pathway during active immune complex deposition disease; therefore, patients with active SLE characteristically have decreased levels of C3, C4, and total hemolytic complement. Low complement levels are associated with active disease, especially when active lupus nephritis is present. However, hypocomplementemia may be present in SLE patients with active cutaneous disease and other types of clinical activity and occasional patients who do not have clinical activity. Therefore, the presence of hypocomplementemia should alert the clinician to the possibility of active organ involvement but should not by itself dictate therapy. As discussed, a subset of SLE patients demonstrates hereditary deficiencies of complement components. The presence of normal levels of C3 and undetectable hemolytic complement activity (e.g., CH50) levels should alert the clinician to the possibility of a hereditary complete complement component deficiency. In these situations, assays for the individual complement components should be performed. Studies have suggested distinguishing clinical and laboratory characteristics, including less renal disease, in SLE patients with complete complement deficiencies. Serial monitoring of complement levels is discussed in the section on management.

SLE is a disease characterized by complement activation when active. Consequently, measurement of complement split products, complement receptors (CR1 on erythrocytes), and the complement membrane attack complex (C5b-9) is presently being studied for monitoring disease activity. Of these, measurement of C4d on erythrocytes (E-C4d) holds the most promise. The complement split product, C4d, has the capacity to attach covalently to cell surface components and persist longer than soluble split products in plasma. Recent studies have demonstrated that abnormally high E-C4d levels have a diagnostic sensitivity and specificity of 70% to 80% (168). In addition, the levels fluctuate over time and correlate with disease activity. Due to the short half-life of reticulocytes (2 days) compared to erythrocytes (120 days), measurement of reticulocyte-bound C4d may be an even better measure of current and ongoing disease activity. The value of measuring membrane-bound C4d levels to monitor lupus nephritis is currently being evaluated.

Tests Monitoring Renal Involvement

Routine laboratory tests used to diagnose and monitor the extent of glomerulonephritis in patients with SLE are similar to those used for other renal diseases (Chapters 10 and 11). Frequent urinalyses with microscopic examination, serum creatinines, and 24-hour creatinine clearance and urinary protein excretion studies usually are necessary in SLE patients with renal involvement. Scrupulous monitoring for changes in kidney function is critical in the management of patients with lupus nephritis. Similar to other types of glomerular disease, advanced lupus nephritis may also be associated with excessive tubular creatinine secretion, owing to tubular dysfunction (169,170); therefore, both the creatinine clearance and serum level of creatinine may overestimate true GFR in these patients. The insidious and persistent loss of GFR in lupus patients is an important marker of poor outcome and is closely related to the presence of chronic destructive lesions seen histologically (7,114,117,134,135,143,144,154). In contrast, acute decreases in kidney function often indicate the presence of newly active and potentially treatable disease.

As discussed, severe glomerulonephritis in SLE is unusual in the absence of proteinuria, and nephrotic-range proteinuria may occur in up to 25% of patients (147,148). Reduction in proteinuria is an important measure of favorable response to treatment in lupus nephritis. Studies have shown that initially severe proteinuria and persistent proteinuria (>1 g/24 hours) after treatment place patients at high risk for the development of progressive disease (143,171). Decreases in kidney function in the absence of proteinuria should alert the clinician to drug-related effects, especially when nonsteroidal antiinflammatory drugs (NSAIDs) or ACE inhibitors are being taken. Patients with SLE are at higher risk for developing decreasing renal function with these medications (Chapter 44).

RENAL INVOLVEMENT IN CLINICAL SYNDROMES CLOSELY RELATED TO SYSTEMIC LUPUS ERYTHEMATOSUS

Overlap Syndromes and Mixed Connective Tissue Disease

Patients with features of SLE or even those classified as SLE by the ACR criteria may demonstrate manifestations of other

rheumatic diseases, including systemic sclerosis, polymyositis–dermatomyositis, rheumatoid arthritis, and Sjögren's syndrome. One type of overlap syndrome, referred to as mixed connective tissue disease (MCTD), has distinguishing clinical and serologic characteristics (172). This disease is partially defined by the presence of specific autoantibodies to U1 RNP in the absence of antibodies to Sm and the relative absence of other types of ANA. Features of SLE, systemic sclerosis, polymyositis/dermatomyositis, and, less commonly, a destructive arthropathy resembling rheumatoid arthritis characterize these patients clinically. Especially early in the disease process, patients tend to resemble each other with common features that include Raynaud's phenomena, puffy or edematous hands, arthritis involving the small joints of the hands, and serositis. Sclerodactyly and esophageal dysfunction also may be present. As the syndrome evolves, one type of disease process may predominate. In up to one-third of cases, patients develop a disease in which features of systemic sclerosis predominate. Severe lupuslike glomerulonephritis is much less common in MCTD as compared with SLE.

Drug-Induced Lupus

A large number of drugs have been associated with the production of ANA and development of a lupuslike syndrome (50). Most of the cases have been associated with hydralazine or procainamide. Drug-induced lupus in general differs from idiopathic lupus in that it tends to cause predominantly joint and pleural–pericardial involvement. Lupus nephritis and central nervous system manifestations are very unusual in drug-induced lupus. The disease remits when the offending drug is discontinued, but the time to remission may be prolonged (months), and manifestations may require a period of treatment with NSAIDs or steroids. Drug-induced disease is associated with positive ANA tests, and antihistone antibodies are present in almost all patients (50). Other autoantibodies frequently seen in SLE (e.g., anti-dsDNA and anti-Sm) usually are absent. Recently, minocycline has been added to the list of drugs causing a lupuslike syndrome. In addition to arthritis and a positive ANA, minocycline-induced lupus is frequently associated with hepatitis, a positive pANCA without myeloperoxidase specificity, and negative antihistone antibodies.

Antiphospholipid Antibodies and the Antiphospholipid Antibody Syndrome

Antiphospholipid antibodies are associated with an increase in thrombotic events resulting in a variety of clinical problems, including recurrent venous and arterial thrombosis, repeated spontaneous miscarriages, and focal neurologic findings secondary to cerebrovascular occlusion (173,174). Less common features associated with these autoantibodies are livedo reticularis, thrombocytopenia, verrucous thickening of cardiac valves, and Coombs-positive hemolysis. Antiphospholipid autoantibodies are present in more than 30% of patients with SLE, and complications are seen in a significant subset of these patients. Kidney involvement related to the presence of these antibodies may contribute to progressive renal damage in patients with lupus nephritis (130,131,174). The antiphospholipid antibody syndrome (APS) with complications can occur in patients without SLE or a related autoimmune disease (e.g. primary APS), and these autoantibodies are an important health problem in the general population. Prospective studies have found lupus anticoagulants in 8.5% to 14% of patients who presented with venous thromboembolism for the first time (up to 35,000 of the 250,000 cases per year) (175).

Kidney involvement related to the presence of antiphospholipid antibodies can occur in 25% of patients with primary APS (176,177). This renal involvement is characterized by noninflammatory occlusion of renal blood vessels that can range from the main renal artery and vein to a thrombotic microangiopathy affecting the glomerular capillaries (178). Histopathologic evaluation shows the vessels to have a reactive intimal mucoid thickening, medial hyperplasia, and thrombosis. The clinical presentation depends on the size of blood vessel involved.

Patients with SLE and antiphospholipid antibodies can develop any of the renal complications seen in primary APS (179). An isolated thrombotic microangiopathy causing significant renal dysfunction occurs in up to 10% of SLE patients with these antibodies. In addition, up to one-third of biopsies in lupus patients with renal abnormalities due to lupus nephritis have glomerular capillary thrombi. Lupus nephritis patients with these microthrombi were significantly more likely to have antiphospholipid antibodies (particularly lupus anticoagulant) and to have a history of previous thrombotic episodes. It is important to note that patients with lupus nephritis, glomerular vessel thrombi, and antiphosholipid antibodies were more likely to have an elevated serum creatinine, hypertension, interstitial fibrosis on renal biopsy, and the eventual development of chronic renal failure (180).

Antiphospholipid antibodies are usually detected as antibodies to cardiolipin in ELISA or as the "lupus anticoagulant" (173,174,181). Lupus anticoagulants interfere with phospholipid-dependent coagulation tests without inhibiting the activity of specific coagulation factors. The name is misleading in that it is not at all specific for SLE, and, paradoxically, it is clinically associated with venous and arterial thrombosis rather than bleeding problems. These antibodies are usually detected initially by finding an elevated partial thromboplastin test (PTT), and the abnormal PTT is not corrected by mixing the patient's plasma with normal plasma, implicating a circulating inhibitor. Antiphospholipid antibodies inhibit *in vitro* coagulation tests at the level of activation of prothrombin to thrombin (i.e., inhibit the prothrombin activator complex), dependent on the amount of phospholipid in the assay. Dilution of the phospholipid (which increases the inhibiting activity of these antibodies) or adding excess phospholipid (which saturates the antibodies) are methods used to specifically screen for this type of lupus anticoagulant. One example is the Russel viper venom test. It is emphasized that inhibition of coagulation by these antibodies is an *in vitro* phenomenon, because thrombosis and not bleeding represent their clinical consequences.

The mechanism for the recurrent thrombotic events observed in these patients is not well understood but likely involves autoantibody-mediated alterations in anticoagulant activity (181). Studies have shown that the antiphospholipid antibodies detected in the various assays are mostly directed to β 2-glycoprotein I (usually with bound phospholipid), and newer assays that specifically detect autoantibodies to β 2-glycoprotein I may increase the accuracy of predicting which patients will suffer complications from these antibodies (i.e., thrombosis) (181). Several studies have suggested that these antibodies may promote thrombosis by inhibiting activation of the protein C/protein S system, which is involved in inactivation of clotting factors Va and VIIIa. Alternatively, it has been suggested that antiphospholipid antibodies promote thrombosis by interacting with platelet phospholipid and inducing platelet aggregation. Finally, complement activation by these antibodies may contribute to the propensity to clot.

Prophylaxis in patients who demonstrate antiphospholipid antibodies but no prior clotting episodes may include antiplatelet agents. Treatment and prevention of recurrent thrombosis usually requires full anticoagulation with heparin (and subsequent warfarin), antiplatelet agents, or both, depending

on the severity of the episode. Although high-dose corticosteroids can be shown to decrease antiphospholipid antibodies, the effect is frequently lost as the drug is tapered. Therefore, corticosteroid treatment is usually not a reasonable long-term therapeutic approach to prevent or treat thrombotic complications in these patients. In patients with lupus nephritis and glomerular capillary microthrombi due to these antibodies, anticoagulation may be an important addition to standard immunosuppressive therapy.

MANAGEMENT

Overview

The management of lupus nephritis is complicated by extreme variability in clinical presentation, course of disease, and response to therapy. Treatment must be tailored to the individual patient. The goal of therapy is to suppress disease while minimizing the cumulative toxicity of treatment, especially that resulting from high-dose corticosteroids. In addition, appropriate treatment of complicating problems, such as hypertension, hyperlipidemia, osteopenia, as well as pneumococcal prophylaxis, is crucial for a successful outcome in the treatment of SLE and lupus nephritis.

Outcome in SLE has been steadily improving over the last 30 years. In patients with lupus nephritis, 5- and 10-year renal survival rates have been reported as high as ~90% and ~80%, respectively (154). Substantially worse renal outcomes have been reported in centers with a high proportion of black patients (150). Some studies have emphasized the bimodal pattern of mortality in SLE and lupus nephritis, with those dying early in their disease from active lupus, whereas coronary artery disease is the major cause of death after years of disease and treatment (5,182,183). A large fraction of the deaths now occur secondary to large-vessel atherosclerotic disease or infection. Factors playing roles in the coronary disease include corticosteroid therapy, hypertension, hyperlipidemia, coagulation abnormalities, obesity, and possible vasculopathy from inflammation and immune injury (5,184). Aggressive treatment, especially with corticosteroids, has clearly contributed to the increased number of infectious deaths. Physicians also have become aware that infection can mimic manifestations of the disease as well as result in disease flares. The possibility of infection should always be considered in the management of SLE, and infection must be excluded prior to institution of corticosteroids or cytotoxic drugs.

Many aspects of the management of lupus nephritis are controversial, including the clinical value of renal biopsy, the use of certain serologic correlates of renal disease activity, and choice of therapeutic agents.

Use of the Renal Biopsy to Guide Management

Studies have suggested that the WHO histologic classification of lupus nephritis (Table 65-3) generally correlates with the clinical severity of renal disease and prognosis (108–114). However, knowledge of the histologic subtype appears to add little to the treatment plan beyond the information already known from the urinalysis, 24-hour protein excretion values, and studies of renal function (113–117). Furthermore, use of the histologic classification alone cannot reliably predict progression to renal failure and thus identify patients who should be treated more aggressively. In contrast, as discussed, the extent of chronic damage on renal biopsy (Table 65-5) may provide important prognostic information and help in treatment considerations (7,113,114,117,132–139,144). This incremental information from a biopsy may be most valuable for patients who do not demonstrate a persistent decrease in renal function (7,114). It has been suggested that patients with no evidence of chronic damage may do well with corticosteroid therapy alone. In contrast, those who demonstrate a limited degree of sclerosis and fibrosis may benefit most from cytotoxic drugs in addition to corticosteroids to reduce the risk of renal failure. Additional studies are required to verify these predictions.

We believe that initial treatment can be started without the need of a renal biopsy in many patients with renal disease. Immediate histologic information may be helpful for those patients with factors associated with an adverse prognosis, such as decreased renal function, hypertension, or black race. Histologic information may be most useful in those patients with evidence of continuing renal disease activity despite treatment with high doses of corticosteroids. At such a time of therapeutic decision making, a renal biopsy may be particularly valuable in identifying patients with nephritis of destructive potential (increased chronicity index), who have an increased chance of progression to renal failure and may most benefit from the addition of cytotoxic drugs. The renal biopsy also is useful to determine the extent of irreversible versus active disease, particularly in those patients who have lost renal function slowly (over months to years) or for whom the duration of loss is unknown. Renal biopsy may be useful for patients with SLE who have another possible cause of renal disease and for patients with glomerulonephritis of unknown etiology in whom the diagnosis of SLE is being considered.

Monitoring Serum Levels of Anti-dsDNA Antibodies and Complement

As discussed, most patients with active proliferative glomerulonephritis have elevated serum levels of antibodies to dsDNA and decreased serum complement levels. At the time therapy is initiated, however, the levels of these markers do not have prognostic significance (114,117). These tests may aid in predicting disease flares in some patients in clinical remission (185,186). Clearly, the chance of exacerbation is less in patients who normalize anti-dsDNA and complement levels with therapy, and progression of renal disease is more likely to occur in patients with persistent serologic abnormalities, especially decreased complement levels (144,187,188). The clinical usefulness of this information is, however, limited because: (a) these serologic results are frequently redundant to what is known clinically, and (b) a significant percentage of patients with abnormal tests do not have an exacerbation of lupus nephritis during extended follow-up periods. There is no evidence, at present, to suggest that therapy for lupus nephritis should be directed at normalizing the anti-dsDNA or complement levels. If there is a lack of correlation between serum anti-dsDNA or complement levels and clinical or routine laboratory evidence of nephritis, we recommend that parameters of glomerular filtration and permeability (i.e., serum creatinine, creatinine clearance, urinary sediment, and 24-hour levels of proteinuria) be used as the most important indicators of response. Increasing anti-dsDNA antibody titers or decreasing complement levels in an otherwise stable patient, however, should prompt closer follow-up.

Drug Therapy

Not all patients with lupus nephritis need to be treated aggressively with corticosteroids and/or cytotoxic drugs. Initial therapy should be tailored to the clinical severity of glomerular

involvement. For example, patients with low levels of proteinuria (<1 g/24 hours), minimal urinary sediment abnormalities, and normal stable renal function may not need aggressive therapy directed at the kidney involvement. Close monitoring is critical, especially early in the disease course. Not infrequently, treatment in these patients may need to be directed at extrarenal manifestations. At the other end of the spectrum are patients with late-stage lupus nephropathy who have gradually progressed to chronic renal insufficiency (serum creatinine >3 to 4 mg/dL) with renal biopsy showing extensive irreversible chronic damage and little activity. These patients should not be candidates for immunosuppressive therapy, as these drugs have little effect on the progression to end-stage renal failure but do increase the likelihood of infection. Systemic lupus erythematosus patients who have slowly progressed to end-stage renal failure frequently demonstrate a marked decrease in serologic abnormalities, and clinical evidence of extrarenal disease is usually minimal. Dialysis and renal transplantation for these patients are discussed elsewhere in this chapter.

The first line of therapy for previously untreated patients with active lupus nephritis and severe clinical manifestations (i.e., decreasing renal function and/or high-grade proteinuria) is high doses of corticosteroids. An attempt should be made to control disease activity quickly. The initial dosage of the most commonly used drug, prednisone, should be approximately 1 mg/kg per day (~60 to 80 mg/day) in three divided doses. It may take several weeks to achieve control of active nephritis. The rate at which the steroid dose can be decreased is dependent on the response to therapy. An initial taper should include changing a divided dose to a one-dose-per-day (AM) schedule. After the serum creatinine has returned to normal and proteinuria has decreased to low levels, initial reductions can be relatively large (e.g., ~10 mg every 2 weeks) to a dosage of 40 mg per/day. As the daily dose decreases, reductions should be at progressively smaller increments. The goal is to achieve the lowest daily dose that maintains adequate control of renal disease activity. Occasional patients can be tapered to an alternate-day schedule as a maintenance regimen. In those instances in which a 6- to 8-week course of high-dose prednisone has not restored serum creatinine levels to normal or the proteinuria continues at greater than 1 g per/day, a renal biopsy can be performed to determine whether glomerular sclerosis, fibrous crescents, and irreversible tubulointerstitial changes are present. If these poor prognostic indicators are observed, especially with evidence of continued activity, the addition of cytotoxic drugs or other immunosuppressive modalities should be considered.

The toxicity of continuous high-dose corticosteroid therapy is cumulative and severe (189). Perhaps most serious in SLE patients is the associated immune suppression and increased susceptibility to severe infections. Endocrine and metabolic problems are numerous, and include truncal obesity, moon facies, acne, hirsutism, menstrual irregularities, and impotence. Patients may also demonstrate a catabolic state with negative nitrogen balance, hyperglycemia, and hyperlipidemia, which may contribute to the increased mortality from coronary artery disease in long-term treated SLE patients. Steroid-induced osteopenia can result in an increased incidence of bone fractures, and high-risk patients should be evaluated for the need for early prophylaxis. Fluid and electrolyte abnormalities secondary to corticosteroids include sodium and water retention and rarely hypokalemia. These alterations can result in or exacerbate hypertension. Corticosteroid therapy, especially high doses, has been associated with psychological problems ranging from anxiety and insomnia to frank psychosis. Patients are also at risk for the development of a proximal myopathy, avascular necrosis, and ophthalmologic complications such as glaucoma and cataracts. Less common complications include pancreatitis, peptic ulcer disease, and pseudotumor cerebri.

Although shown to be associated with less toxicity than daily administration, alternate-day steroid therapy is usually not successful in suppressing severe disease activity. Some patients, however, can be tapered to alternate-day therapy as a maintenance regimen. It was originally believed that pulse intravenous corticosteroid therapy (e.g., 1 g of methylprednisolone per day for 3 days) might obviate the need for continuous high-dose daily treatment. This does not appear to be true in the majority of cases. Furthermore, the addition of pulse steroids may add significantly to certain steroid toxicities such as increased susceptibility to infection and avascular bone necrosis. Complications such as sudden death rarely have been associated with these large boluses of drug. Although pulse corticosteroid therapy may improve efficacy when added to maintenance oral prednisone in a small subgroup of patients, its long-term benefit in most patients appears to be minimal. At this time, despite its relatively widespread use, alternate-day steroid therapy cannot be recommended as a routine therapy in patients with lupus nephritis. Controlled trials have demonstrated that monthly pulse methylprednisolone is not as effective as intermittent cyclophosphamide in preserving renal function (190,191).

The use of cytotoxic drugs in the treatment of lupus nephritis should be reserved for the subgroup of patients with severe, refractory disease. These include: (a) patients with evidence of active and severe glomerulonephritis (see preceding discussion) despite treatment with high-dose prednisone, (b) patients who have responded to corticosteroids but who require an unacceptably high dose to maintain a response, and (c) patients with unacceptable side effects from corticosteroids. In addition, as discussed, evidence of chronic damage on the renal biopsy or other indicators of a poor prognosis may suggest the need for early introduction of cytotoxic drug therapy. The most commonly studied cytotoxic drugs have been oral azathioprine, oral cyclophosphamide, and intermittent intravenous cyclophosphamide. These drugs are given in association with a dose of prednisone (usually 0.5 mg/kg/day) required to control extrarenal manifestations. Cytotoxic drugs, especially cyclophosphamide in combination with prednisone, have been shown to prevent progression to renal failure more effectively than prednisone alone (7,190–199). However, because of potentially severe toxicity, proportional improvements in mortality have been more difficult to demonstrate (196). It is recommended that intravenous cyclophosphamide be given under the supervision of a physician experienced in its use.

Azathioprine is rapidly converted to 6-mercaptopurine after absorption. It should be initiated at 1.0 to 1.25 mg/kg/day. Maximal clinical benefit may not be observed for weeks to months. If the clinical response is unsatisfactory, the dosage can be slowly increased to a maximal level of 3.0 mg/kg/day. The most serious toxicity during therapy is bone marrow suppression, primarily leukopenia; therefore, complete blood counts must be followed regularly during treatment. Gastrointestinal intolerance is common, but giving the dose at bedtime can decrease this. Some patients develop elevated liver enzymes early in therapy, which usually resolves during continued treatment. The major long-term risk of azathioprine use is the potential for developing a malignancy, especially lymphoma. This risk appears to be extremely low, and lower than that with cyclophosphamide. The risk also appears to be less in patients with SLE than in patients who have undergone renal transplantation. Azathioprine appears to be less toxic than cyclophosphamide. It has not been associated with hemorrhagic cystitis and has a much lower incidence of premature ovarian failure compared with cyclophosphamide. At this time, azathioprine might be recommended for a patient who wishes to preserve ovarian

function; however, available data also suggest that cyclophosphamide is more potent in suppressing lupus renal disease activity compared with azathioprine (195). Cyclophosphamide, therefore, should be preferentially considered for patients with continued deterioration of renal function and those at high risk for progression to renal failure.

Recent studies have indicated that when given correctly, intermittent intravenous cyclophosphamide has a lower incidence of hemorrhagic cystitis compared with daily oral cyclophosphamide (195,196). Furthermore, recent experience indicates that improvement may be more rapid after intravenous boluses compared with daily oral administration. There is little evidence for additional toxicities with intravenous cyclophosphamide not encountered during oral administration. Therefore, the intravenous regimen is recommended in patients for whom cyclophosphamide is being considered. In current protocols, the initial dosage of cyclophosphamide is 0.5 to 0.75 g/m^2 of body surface area given intravenously over approximately 60 minutes. The lower dose should be employed in patients whose creatinine clearance is less than 33 mL/minute. The dose can be gradually increased to 1.0 g/m^2, but the leukocyte nadir should be no lower than 2,000 cells/mm^3 (>1,500 neutrophils/mm^3). Following the initial pulse, five additional doses are given at monthly intervals, and additional infusions usually are given at 3-month intervals (190,191). Each cyclophosphamide infusion must be accompanied by vigorous hydration (3 L/m^2 with half normal saline in 5% dextrose) over the subsequent 24-hour period to prevent hemorrhagic cystitis. The cyclophosphamide metabolite acrolein appears to be the major irritant responsible for damage to the bladder wall. Studies have suggested that the concomitant use of mesna (2-mercaptoethane sulfonate) likely reduces the risk of bladder complications of cyclophosphamide therapy, and in many centers, this drug is given during the cyclophosphamide infusion and early postinfusion period. Prophylaxis for nausea and vomiting frequently include dexamethasone and serotonin receptor antagonists, such as granisetron or ondansetron.

The most serious toxicity related to cyclophosphamide therapy is leukopenia, which can further predispose patients to serious infections. Even in the absence of leukopenia, an increased incidence of bacterial infections and especially herpes zoster has been reported (191,195,196). Ovarian or testicular damage is also a relatively common problem with long-term therapy; this problem correlates with age and cumulative dose (200). A long-term risk of cyclophosphamide may relate to the induction of malignancy, including acute leukemia and non-Hodgkin lymphoma, but the risk in SLE patients appears to be low.

It is currently unclear how long to continue any regimen of cytotoxic drugs in the treatment of lupus nephritis. Short courses of cyclophosphamide (<6 months) have been associated with frequent relapses and the need for repeat induction therapy (190,191). Some protocols have recommended continuing treatment for 1 year of remission and no longer than 4 years of total treatment duration (190). The cytotoxic drugs then should be slowly tapered. Replacement of maintenance therapy with less toxic regimens such as azathioprine or mycophenolate mofetil has been considered because of the potential toxicity of prolonged cyclophosphamide therapy. It remains unclear whether these approaches to reduce toxicity will be successful at maintaining remission and preventing long-term progression.

Limitations of the intravenous high-dose cyclophosphamide regimens include (192): (a) no effect on survival rates; (b) no differences in outcome compared to oral cytotoxics; (c) risk of severe infections; (d) no high relapse rate. Thus the Euro-Lupus Nephritis Trial has studied low-dose cyclophosphamide followed by azathioprine. Specifically, intravenous pulses of 500 mg cyclophosphamide weekly or fortnightly for a few months followed by oral azathioprine maintenance therapy was compared to high-dose intravenous cyclophosphamide. In the Euro-Lupus Nephritis Trial, after a median follow-up of 73 months, there was no significant greater cumulative probability of end-stage renal disease (ESRD) or doubling of serum creatinine in the low-dose versus high-dose regimens (193).

High-dose corticosteroids are the mainstay of drug therapy in a pregnant patient who demonstrates severe exacerbations of lupus nephritis. Azathioprine also appears to be an acceptable adjunct, if necessary, based on the experience in female patients receiving renal transplants. Cyclophosphamide and other cytotoxic drugs should not be used in pregnant patients.

Newer Therapies

A variety of experimental therapies have been utilized in SLE, especially in patients with severe lupus nephritis. Studies have shown that plasmapheresis does not have long-term benefit in the treatment of lupus nephritis (201), and it cannot be recommended as a routine adjunct to drug treatment. Therapies utilized in the setting of transplantation, such as cyclosporin A, tacrolimus (FK506), and total lymphoid irradiation, have been reported in the treatment of lupus nephritis as alternatives to cyclophosphamide therapy with at least anecdotal efficacy (202–209); however, none has been shown to be an acceptable alternative to the therapies described. Animal studies and anecdotal case series initially suggested that mycophenolate mofetil might be efficacious in lupus nephritis (205,210). A follow-up controlled study in patients with relatively mild DPGN showed that initial remission rates with this drug and prednisolone were comparable to responses with oral cyclophosphamide and prednisolone (followed by azathioprine and prednisolone) (206). The 1-year follow-up reported, which is early in the course of treated lupus nephritis, and study design does not allow conclusions regarding whether mycophenolate mofetil will be able to prevent progression to renal failure similar to cyclophosphamide, especially in patients with more severe disease.

Mycophenolate mofetil has recently been studied as maintenance therapy. Fifty-nine patients with lupus nephritis (12 in World Health Organization class III, 46 in class IV, and 1 in class Vb) received induction therapy consisting of four-to-seven monthly boluses of high-dose intravenous cyclophosphamide (0.5 to 1.0 g/m^2 body surface area) plus corticosteroids. The patients were then randomly assigned to one of three maintenance therapies: quarterly intravenous injections of cyclophosphamide, oral azathioprine (1 to 3 mg/kg body weight/day) or oral mycophenolate mofetil (500 to 3,000 mg/day) for 1 to 3 years. Five patients died (four in the cyclophosphamide group and one in the mycophenolate mofetil group) and chronic renal failure developed in five (three in the cyclophosphamide group and one each in the azathioprine and mycophenolate mofetil groups). The 72-month event-free survival rate for the composite end point of death or chronic renal failure was higher in the mycophenolate mofetil and azathioprine groups than in the cyclophosphamide group ($p = 0.05$ and $p = 0.009$, respectively). The rate of relapse-free survival was higher in the mycophenolate mofetil group than in the cyclophosphamide group ($p = 0.02$). The incidence of hospitalization, amenorrhea, infections, nausea, and vomiting was significantly lower in the mycophenolate mofetil and azathioprine groups than in the cyclophosphamide group. No statistically significant differences were found between the mycophenolate mofetil and azathioprine groups (207). A study comparing mycophenolate mofetil and azathioprine for maintenance therapy is underway (192).

Mycophenolate mofetil has recently been studied as induction and maintenance therapy (208). Sixty-four Chinese patients with biopsy proven diffuse proliferative lupus nephritis were studied with a median follow-up of 63 months. Thirty-three patients were randomized to receive mycophenolate mofetil and 31 were randomized to receive high-dose oral Cytoxan (2.5 mg/kg/day) followed by azathioprine. Both regimens were equally effective in induction-maintenance therapy. The mycophenolate mofetil group had fewer infections.

A 24-week randomized, open-label, noninferiority trial comparing oral mycophenolate mofetil (initial dose, 1000 mg/day, increased to 3000 mg/day) with monthly intravenous cyclophosphamide (0.5 g per square meter of body-surface area, increased to 1.0 g per square meter) as induction therapy for active lupus nephritis, has recently been completed (211). The primary end point was complete remission at 24 weeks (normalization of abnormal renal measurements and maintenance of baseline normal measurements). A secondary end point was partial remission at 24 weeks. Of 140 patients recruited, 71 were randomly assigned to receive mycophenolate mofetil and 69 were randomly assigned to receive cyclophosphamide. In this 24-week trial, mycophenolate mofetil was more effective than intravenous cyclophosphamide in inducing remission of lupus nephritis and had a more favorable safety profile.

Recent work has suggested that very-high-dose (immunoablative) cyclophosphamide, with or without bone marrow transplantation, may provide long-term benefit for patients with very severe disease, and trials are in progress to test this idea (212). Trayner et al. reported 15 patients with persistently active SLE after intravenous cyclophosphamide (CYC) therapy who underwent immunoablation and autologous hematopoietic stem cell transplantation (HSCT) (213). Seven of the patients were critically ill. There were no deaths. After a median follow-up of 36 months, all patients had a gradual, but marked, improvement. The SLE Disease Activity Index decreased to less than 6 in 12 patients. Serology normalized and marked improvements in end-organ function occurred in all subjects. Ten patients were able to discontinue immunosuppressive medications after 1 year.

Immunoablative doses of cyclophosphamide without HSCT have been studied in lupus nephritis (214). Fourteen patients with refractory SLE were treated. There were no deaths or fungal infections. In nine patients with lupus nephritis there was marked improvement in proteinuria.

There is also great excitement about several new biologic inhibitors being developed for therapy of patients with SLE. For example, inhibitors of the CD40-CD40 ligand interaction with monoclonal antibodies to CD40 ligand (73) and the CD28-B7 interaction with CTLA4-Ig (74), especially in combination (75), have demonstrated potent suppression of lupus nephritis in murine models (see previous discussion of pathogenesis). In a clinical study, a short course of BG9588 (anti-CD40 ligand antibody) improved serology in patients with proliferative lupus nephritis. However, the study was discontinued prematurely because of thromboembolic events (215). Based on studies in murine lupus, clinical trials are also being started with biologic agents that inhibit the production or signaling of interferon-γ (71) and the activation of complement C5 (21).

PROGNOSIS

Clinical Remission and Renal Flares

Clinical remission is defined by some investigators as an inactive urine sediment, a creatinine level of less than or equal to$\leq$ 1.4 mg/dL, and protein excretion of less than 330 mg per/day. Patients who achieved a remission had a greater than 90% renal survival at 5 and 10 years compared to 46% and 31% of patients who did not achieve remission (216). In addition, overall patient survival was much improved at 10 years in those in renal remission (95% versus 60%).

Relapse of lupus nephritis after initial immunosuppression occurs in 27% to 66% of patients (217–219). In patients receiving maintenance immunosuppression the rate of relapse is decreased to 20% to 25%. Renal flares have been classified as nephritic or proteinuric (nephrotic). Nephritic flares are subgrouped into mild, moderate, or severe. A mild/moderate nephritic flare is characterized by active urinary sediment (>10 RBCs/HPF or cellular casts), an increase in proteinuria (<2 g/day for mild, >2 g/day for moderate, and stable serum creatinine level). Severe nephritic flares are accompanied by active urine sediment, increased proteinuria, and a creatinine level of greater than 30% over baseline. Most nephritic flares are associated with low C3 and elevated anti-dsDNA antibodies. Nephrotic (or proteinuric) flares are characterized by only an increase in proteinuria (>2g/day) (220).

Risk factors for patients likely to have flares of lupus nephritis have been identified (217–219). Demographically, young age, male sex, and nonwhite race are significant risk factors for relapse. Disease activity factors include severe SLE, a high activity score, rising anti-dsDNA antibodies, and arterial hypertension. A delay (>5 months) in initiating treatment and difficulty or failure to achieve a complete remission of lupus nephritis are also significant risk factors for future renal relapses.

Patients who develop a flare of their lupus nephritis need to be treated aggressively to preserve renal function (221). Patients who relapse frequently take up to three times longer to remit compared to their initial episode of nephritis. Up to 35% fail to remit or progress to end-stage renal disease in spite of reinstitution of immunosuppressive therapy. Nephritic flares with a rapid increase in creatinine (doubling or >2 mg/dL) associated with a high activity score and/or chronicity index at baseline biopsy are especially likely to progress to ESRD (218).

Rituximab is an anti-CD20 monoclonal antibody that depletes B cells. It is used to treat Hodgkin lymphoma. In a prospective observational study of 8 patients with idiopathic membranous nephropathy, rituximab treatment resulted in a significant decrease in proteinuria. (222). In an open-label trial, 10 patients with active proliferative lupus nephritis were treated with rituximab (223). Rituximab was well tolerated and resulted in B-cell depletion for 1 to 7 months. Five patients had a complete remission (normal serum creatinine level, inactive urine sediment, 24-hour urine protein <500 mg). Three patients had a partial remission (50% improvement in renal parameters). Therefore, there is preliminary evidence supporting the safety and efficacy of rituximab as monotherapy in proliferative lupus nephritis. Randomized controlled studies are needed comparing rituximab with cyclophosphamide and mycophenolate mofetil for induction and maintenance therapy.

LJP 394 selectively eliminates pathogenic autoantibody-producing B cells, sparing the non-autoimmune B-cell compartment. LJP 394 selectively decreases anti-dsDNA antibodies. In a randomized, double-blind placebo-controlled study, 230 patients were studied (224). In the intent-to-treat population, the time to institution of high-dose steroid and/or cyclophosphamide therapy was significantly prolonged and the number of treatments was significantly lower than placebo. In 189 patients with high-affinity antibodies to LJP 394, the time to renal flare was significantly longer compared to placebo, and the number of renal flares and treatments with high-dose steroid and/or cyclophosphamide therapy was significantly lower compared to placebo. A multinational, prospective, randomized

study is ongoing in SLE patients with a history of renal disease and high-affinity antibodies at baseline.

End-Stage Renal Disease and Transplantation in Lupus Nephritis

Approximately 20% to 30% of patients with severe lupus nephritis progress to ESRD over a 10-year follow-up period (7,113,114,117,132–144,146,150,154,155,190,194–196,198,225). Lupus nephritis accounts for up to 3% of cases of end-stage renal failure requiring dialysis or transplantation. In a subset of patients, active lupus early in the dialysis period may require treatment with corticosteroids and/or other immunosuppressive medications. The use of these medications is associated with increased morbidity, especially infectious complications. For unclear reasons, SLE patients with progressive renal failure and those on dialysis frequently demonstrate a decrease in nonrenal clinical manifestations of active SLE as well as a decrease in serologic markers of active disease (226–231), although some patients fail to improve (232). In SLE patients with absent or minimal disease activity, clinical course and survival on dialysis (hemodialysis or continuous ambulatory peritoneal dialysis [CAPD]) compare favorably to other patient groups (230). However, SLE patients with antiphospholipid antibodies have an increased incidence of recurrent thrombosis in arteriovenous grafts, which may be lessened with warfarin therapy, and patients receiving CAPD have increased infectious complications if on immunosuppressive medications (233,234).

Although controversial, it has been recommended that patients with SLE should wait 6 to 12 months while on dialysis prior to transplantation. With time, SLE patients appear to be excellent candidates for transplantation. Recurrence of active lupus nephritis in the transplant occurs in 10% to 30% of patients (226–236). It is important to note that recurrence contributed to graft loss in less than half of these patients. However, in contrast to earlier studies (226–232), more recent follow-up studies have suggested that renal allograft survival in SLE patients is lower compared to most other patient groups (237,238). This is particularly true in patients with antiphospholipid antibodies who may lose their allograft due to renal thrombosis if not adequately anticoagulated (239–241).

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CHAPTER 66 ■ RENAL DISORDERS ASSOCIATED WITH SYSTEMIC SCLEROSIS, RHEUMATOID ARTHRITIS, SJÖGREN'S SYNDROME, AND POLYMYOSITIS-DERMATOMYOSITIS

ROBERT W. JANSON AND WILLIAM P. AREND

SYSTEMIC SCLEROSIS

Systemic sclerosis (SSc) is a relatively rare disorder of connective tissue, the clinical hallmark of which is fibrotic skin induration (scleroderma). This manifestation in some patients is no less dramatic than that described by Osler (1), who wrote that "to be 'beaten down and marred and wasted' until one is literally a mummy, encased in an ever-shrinking, slowly contracting skin of steel, is a fate not pictured in any tragedy, ancient or modern." Although such skin involvement is a cruel form of morbidity, visceral involvement of this systemic disease equally influences morbidity and frequently determines mortality. Organ systems other than the skin—including the gastrointestinal tract, kidneys, lung, and heart—may be affected by characteristic and widespread fibrotic, inflammatory, and vascular changes. The etiology of this pathologic process remains elusive (2).

Renal involvement remains one of the most feared complications in SSc. Scleroderma renal crisis (SRC), once a uniformly fatal process and the leading cause of death in SSc, is now a form of renal failure that can be treated successfully in a substantial proportion of patients in whom it develops. However, prevention of permanent renal failure rests on early recognition of SRC and prompt, aggressive treatment with angiotensin-converting enzyme (ACE) inhibitors. It is beyond the scope of this chapter to give a detailed account of all the manifestations of SSc or an in-depth examination of its pathogenesis; therefore, the following is an overview with focus on renal involvement. Readers are encouraged to refer to recent texts and reviews for a broader discourse on this disorder (2–4).

Classification

Scleroderma occurs in localized and systemic forms. In the former, involvement is isolated to the skin and subcutaneous tissue and can be classified as *morphea* or *linear scleroderma*. In the systemic form *(systemic sclerosis)*, visceral involvement occurs in addition to skin induration. Further classification of systemic sclerosis divides this entity into two relatively distinct clinical variants: systemic sclerosis with *limited* scleroderma (lSSc) and systemic sclerosis with *diffuse* scleroderma (dSSc), based on extent of skin involvement (5). Although significant overlap exists, type and frequency of various clinical manifestations differ between these variants to the extent that they behave as two distinct clinical entities. Therefore, the pattern of extradermal involvement, in addition to characteristic serologic findings, can be of diagnostic utility to help distinguish lSSc from dSSc. An overview of these characteristics is outlined in Table 66-1.

Limited systemic sclerosis has replaced the acronymic term CREST syndrome, which remains a useful reminder of this variant's typical features of subcutaneous *c*alcinosis, *R*aynaud's phenomenon, *e*sophageal hypomotility, *s*clerodactyly (skin induration limited to the digits), and *t*elangiectasias. Raynaud's phenomenon and telangiectasias often precede by years the skin involvement, which is limited to the distal extremities and face. Visceral involvement occurs late in the course of lSSc and differs from dSSc by the rarity of renal and myocardial involvement. A leading cause of late mortality in lSSc is pulmonary hypertension occurring in the relative absence of interstitial lung disease. Such isolated pulmonary hypertension, which develops rarely in dSSc, affects 8% to 16% of patients with lSSc (6,7). The immunologic marker for lSSc is the anticentromere antibody, which is detected in 20% to 40% of affected individuals (8).

In *diffuse systemic sclerosis,* more widespread skin induration develops within months to a few years of disease onset, by definition involving the trunk and/or extremities proximal to the elbows and knees (5). Typically, Raynaud's phenomenon develops concomitantly with distal skin signs of edema and early induration. A more notable contrast to lSSc is early visceral involvement of the gastrointestinal tract, kidneys, heart, and lungs in dSSc. Anti-Scl-70 antibodies are relatively specific for dSSc and are detectable in 20% to 40% of those with this variant (8,9). The antigen to which this antibody is directed has been identified as topoisomerase I, a DNA gyrase (10). More recently, anti-RNA polymerase antibodies, particularly anti-RNA polymerase III, have been found to be relatively specific and somewhat more sensitive serologic markers for dSSc, and are detectable in approximately 20% of individuals with dSSc (11). Regarding prognosis, it has been generally accepted that dSSc is associated with a higher mortality rate than limited disease, with 10-year survival rates on the order of 55% and 70% in dSSc and lSSc, respectively (12,13). The major causes of death in dSSc are pulmonary, cardiac, and renal involvement, whereas pulmonary hypertension is the leading cause of death in lSSc. Recent studies have suggested that the two variants may now have nearly equal survival rates, which likely reflects decreasing mortality of SRC in dSSc as a result of earlier and more effective treatments (14–16). Poor prognosis is often associated with diffuse skin involvement, late age of onset of disease, African American descent, lung vital capacity of less than

TABLE 66-1

SYSTEMIC SCLEROSIS (SSC) SUBSETS

Organ system/manifestation	dSSc	lSSc
Skin	Trunk and/or proximal extremity induration	Distal extremity induration (may include face) Calcinosis Telangiectasias
Raynaud's phenomenon	Onset within 1 year of skin involvement	Frequently precedes skin involvement by years
Pulmonary	Early development of pulmonary fibrosis (>50%)	Late development of isolated pulmonary hypertension (10% to 20%); pulmonary fibrosis (<40%)
Renal	Scleroderma renal crisis (10% to 20%)	Rare involvement
Gastrointestinal	Involvement of esophagus, small intestine, colon	Involvement usually limited to the esophagus
Cardiac	Primary involvement of the myocardium/microvasculature	Secondary involvement owing to pulmonary hypertension
Musculoskeletal	Joint contractures; arthralgias/arthritis; tendon friction rubs; myositis	Acral joint contractures; arthralgias/arthritis less common; myositis
Nail-fold capillaries	Dilation and dropout	Dilation without dropout
Serologic studies		
Antinuclear antibody	>90%	>90%
Anti-topoisomerase	20% to 40%	<15%
Anticentromere	<5%	20% to 40%
Anti-RNA polymerase III	20%	6%
10-Year survival	55%	70%

55% predicted, and significant renal, cardiac, gastrointestinal, or pulmonary disease (8).

Discussion of classification must not exclude those patients with normal skin who have typical internal organ changes, and vascular, and serologic features of SSc. *Sine scleroderma* is the term used to refer to this uncommon presentation (3). It should also be noted that systemic sclerosis may occasionally occur in *overlap* with features of other autoimmune rheumatic disorders such as rheumatoid arthritis, systemic lupus erythematosus, polymyositis-dermatomyositis, and Sjögren's syndrome.

Epidemiology

Systemic sclerosis is a rare disorder that has a worldwide distribution affecting all racial groups. Epidemiologic studies of SSc have likely underestimated its true incidence and prevalence because many such studies have been based on referral center data. The published incidences range from 1 to 19 cases per million, with an estimated prevalence of about 250 cases per million (16). Peak age of onset occurs in the fourth and fifth decades, with women being affected more commonly than men at a ratio of approximately 4:1. Although SSc affects all races, certain racial groups, such as African Americans, appear to have a higher incidence compared to whites (16). Disease occurs without identifiable environmental exposures in the majority of patients. However, certain environmental and occupational factors are associated with development of SSc and SSc-like disorders. These include exposure to silica dust and various organic compounds such as vinyl chloride, epoxy resins, and organic solvents (8). It should be noted that there has yet to be shown a convincing link between SSc and silicone breast implants (17–19).

Genetic predispositions and familial clustering have been well described in other rheumatic diseases such as rheumatoid arthritis and systemic lupus erythematosus; however, genetic associations have been weak and familial clustering rare in SSc. Relatively weak human leukocyte antigen (HLA) associations have been demonstrated in SSc, and findings have varied among ethnic groups, geographic regions, and investigating centers. Reported associations have included certain HLA-DR2, HLA-DR3, and HLA-DR5 haplotypes in addition to the C4 null alleles C4AQ0 and C4BQ0 (20,21). These HLA associations may be more related to the presence of particular autoantigens than to the disease (20). Environmental factors may play a more prominent role in the pathogenesis of this disease because there are only scarce reports of SSc occurring in familial clusters (22) and concordance in monozygotic twins is an infrequent observation (16).

Pathogenesis

One of the striking observations in SSc is the similarity of pathologic lesions in the various affected organs, that is the ubiquitous presence of vasculopathy and fibrosis. Although the inciting event triggering such systemic injury is unknown, a working hypothesis has been proposed that attempts to integrate the pathogenic abnormalities affecting the immune system, microvasculature, and connective tissue (2,23).

Immune System

Because of the observation that 95% of patients with SSc have detectable antinuclear antibodies (ANAs) using the widely used Hep-2 cell line as the detection substrate (24), SSc has long been viewed as an autoimmune disorder. Supporting this view is the observation that SSc occurs in overlap with other ANA-associated autoimmune disorders such as polymyositis, systemic lupus erythematosus, and Sjögren's syndrome. As discussed, relatively specific autoantibodies (anticentromere, anti-Scl-70, and anti-RNA polymerase III) are present in SSc, more strongly suggesting a role for the immune system in pathogenesis. However, these autoantibodies have not been shown

to be pathogenic per se, at least not in the usual sense of immune complex-mediated tissue injury (25). Although circulating immune complexes have been detected in SSc (26), and immunofluorescence studies have demonstrated vascular deposition of immunoglobulins and complement components, the characteristic inflammatory sequelae expected as the result of immune-complex deposition are absent by histologic examination. Thus, convincing evidence is lacking for immune complex-mediated vascular injury in SSc.

The potential role of cellular immunity in the pathogenesis of SSc was first suggested by the observation of perivascular dermal mononuclear cell infiltrates in the early edematous phase of skin involvement (27). The extent of these dermal infiltrates has been shown to correlate with the severity of subsequent skin induration. Phenotypic examinations have shown activated CD4-positive T cells to be the predominant cell type (23,28). Elevated levels of interleukin-2 (IL-2) and its soluble receptor (sIL-2R) have been demonstrated in SSc sera and correlate with disease severity and patient survival, supporting the concept of a T-cell contribution to tissue injury in SSc (29). Perhaps the strongest evidence that cell-mediated immune injury is of pathogenic importance in SSc comes from reports of SSc-like manifestations occurring in chronic graft-versus-host disease (GVHD). Features of this disease include Raynaud's phenomenon, dermal sclerosis (particularly in the digits), positive ANA test, and vascular changes similar to those seen in SSc (30,31). A similar syndrome in mice with chronic GVHD is dependent on the presence of immunocompetent donor T cells reactive to incompatible recipient H-2 locus antigens (32). The finding that a T-cell response to cellular antigens can induce a syndrome similar to SSc, combined with the knowledge that major histocompatability complex (MHC) antigens can be expressed on activated endothelial cells, has obvious implications regarding the potential role for cell-mediated immunity in the pathogenesis of SSc. Furthermore, recent research suggests that microchimerism, the persistence of circulating fetal immune cells in previously pregnant women, could possibly, when activated by a second event, induce a GVHD-like response contributing to the development of SSc (2,23,33).

Endothelial Cell and Vasculature

The nearly universal manifestation of Raynaud's phenomenon in SSc and the finding of abnormal nail-fold capillaries in greater than 85% of patients, both usually developing early in the course of disease and often before the onset of dermal sclerosis, emphasize the importance of vasculopathy in SSc (34). The three most apparent manifestations of widespread vascular abnormalities in SSc are: (a) vasomotor instability leading to transient ischemia not only of the digits but also of the viscera, (b) structural abnormalities of small vessels characterized by endothelial cell damage and obliterative intimal proliferation, and (c) intravascular abnormalities such as enhanced coagulability and platelet activation. Development of this systemic vasculopathy, with its resultant tissue ischemia, may be the most important pathogenic mechanism of visceral organ damage in SSc, with scleroderma renal crisis being one of its more dramatic consequences.

Vasoreactivity is enhanced in SSc. Easily provoked by cold exposure, vasospasm of structurally abnormal vessels results in transient tissue hypoperfusion, with Raynaud's phenomenon being the most familiar clinical consequence. The enhanced vasoreactivity is likely multifactorial in nature, as suggested by the variety of vasopressors that have been proposed as mediators of this phenomenon, including serotonin (35), catecholamines (36), prostaglandins (37), thromboxane A_2 (38), and angiotensin (39). In addition, serum levels of endothelin, an endothelium-derived peptide and the most potent vasoconstrictor yet discovered, have been shown to be elevated in individuals with primary Raynaud's phenomenon and SSc (40,41). Kahaleh (41) demonstrated that endothelin is mitogenic for fibroblasts and stimulates collagen synthesis, thereby invoking a potential link between vascular and connective tissue abnormalities in SSc.

Morphologic studies in SSc have revealed evidence of endothelial cell (EC) injury and altered EC function. Additional observations include EC swelling and retraction with loss of intercellular junctions, ECs in various stages of cell death or apoptosis, and basement membrane abnormalities (34). The first *in vivo* evidence to support EC injury in SSc was the finding of elevated plasma levels of EC-specific von Willebrand's factor (42), a phenomenon seen in other disorders associated with underlying EC damage such as vasculitis (43). Interest in EC "activation" has led to the finding of increased expression of intercellular adhesion molecule-1 (ICAM-1), among other integrins, in the skin of individuals with recent onset SSc (44), suggesting a homing mechanism for pathogenic lymphocytes. Other investigators have demonstrated that serum levels of soluble ICAM-1 (s-ICAM-1), E-selectin, and P-selectin are increased in patients with SSc compared to controls and correlate both with their *in situ* activity and with clinical disease activity (45,46). Therefore, s-ICAM-1 and other selectins may be useful markers of EC activation.

Damaged and activated endothelial cells are potentially capable of stimulating intimal proliferation directly by elaboration of EC-derived growth factors and indirectly by triggering platelet activation and degranulation (34). This process results in the characteristic obliterative intimal lesion of SSc. Compounding the effect of the vascular structural changes is that of intravascular coagulation abnormalities. Histologically, this is indicated by the formation of luminal thrombi in affected vessels. Clinically, this is manifested by the development of microangiopathic hemolytic anemia and supported by the findings of increased fibrinogen turnover (47), increased circulating platelet aggregates, and elevated levels of ß-thromboglobulin (48).

Although EC injury and activation appear to be of primary importance in the development of vascular abnormalities in SSc, the mechanisms by which such injury occurs are not clear. As discussed previously, immune complex injury seems unlikely. Antibodies directed against EC have been detected by several investigators but have not been shown to be directly cytotoxic (49). However, a potential role for antibody-mediated damage has been implied by the observation that SSc sera are capable of mediating antibody-dependent cellular cytotoxicity against EC, although sera from only about 20% of patients exhibit this property (49,50). Cytokine-mediated EC injury may be a contributing factor based on the findings that transforming growth factor-β (TGF-β) and tumor necrosis factor-α (TNF-α) can inhibit endothelial cell growth *in vitro* (2). Nonimmune mechanisms of EC damage have also been described. Over the last decade, contradictory reports have appeared regarding evidence for a nonimmunoglobulin serum factor in SSc that is cytotoxic to EC (49). One group of investigators partially purified a low-molecular-weight ($\leq$5 kDa) cytotoxic factor from SSc sera, which led to elevated plasma levels of von Willebrand's factor, capillary dilation, and arterial/arteriolar intimal hyperplasia when serially injected into rabbits (51).

Fibroblast

Perhaps the most characteristic feature of SSc is that of widespread fibrosis, the result of faulty regulation of collagen synthesis leading to excessive accumulation of types I and III collagen and other extracellular matrix components in the skin and visceral organs (52). LeRoy was the first of several

investigators to show that cultured dermal fibroblasts from SSc patients synthesized two to fourfold more collagen than control fibroblasts, and that this ability persisted for several cell passages before declining toward control levels (53). Evidence has accumulated to indicate that this increased collagen production by SSc fibroblasts occurs as a result of enhanced messenger RNA (mRNA) transcription (54), the probable result of cytokine stimulation. The potential ability for mast cell–elaborated products, such as heparin, to stimulate fibroblast collagen production in SSc has been reviewed (55). Of potential fibroblast stimulatory cytokines, TGF-β has been found to be the most potent inducer of collagen synthesis, likely because of its ability to upregulate the transcription of collagen mRNA (56). Recently, it has been shown that cultured scleroderma fibroblasts possess increased levels of membrane TGF-β receptors (57). Unfortunately, the finding of elevated TGF-β receptor levels in patients with SSc has not been universally reproduced (58). Transforming growth factor-β can also induce endothelial cell production of endothelin, the most potent vasoconstrictor known (59). Other soluble mediators—including the interleukins 1, 2, 4 (a profibrotic cytokine), 6, 8, and 17 (enhances the proliferation of fibroblasts), TNF-α, platelet-derived growth factor (PDGF), and fibronectin—may play a role in the pathogenesis of SSc (2,34).

A working hypothesis has been proposed in an attempt to unify the pathogenic abnormalities in SSc discussed in the preceding paragraphs (2,23). An unidentified inciting event may trigger both EC injury and immune system activation, with the latter potentiating and/or perpetuating EC damage. A cytokine cascade, the result of immune cell activation and platelet degranulation triggered by EC damage, then provokes systemic fibroblast activation and proliferation, the end result being fibrosis. Whether the associated autoantibodies are pathogenic or merely an epiphenomenon remains unclear.

Renal Involvement

Historical Perspective

Descriptions of cutaneous manifestations compatible with those of systemic sclerosis were noted in writings as early as those of Hippocrates (460–370 BC) and Galen (131–201 AD) (60). The earliest convincing description of a patient with SSc appeared in a 1753 monograph written by Carlo Curzio (61). In 1847, Gintrac (62) coined the descriptive term *sclérodermie,* which soon thereafter became the widely accepted term of reference. The earliest description of renal involvement in SSc is attributed to Auspitz (63), who in 1863 described a 29-year-old locksmith with tightness and hyperpigmentation of the skin who succumbed to "Bright's disease." Later in that century, in the first edition of his renowned medical text, Osler (64) mentioned that patients with scleroderma "are apt to succumb to pulmonary complaints or to nephritis." Although other authors subsequently acknowledged the visceral manifestations of SSc, it was not until well into this century that more detailed attention was given to renal disease in SSc. Moore and Sheehan (65) provided the first detailed clinical and pathologic description of renal involvement in three patients with SSc who developed acute hypertension and died 6 to 8 weeks later of renal failure. The concept that a direct relationship exists between SSc and renal disease became widely accepted subsequent to this convincing report.

Frequency and Type of Renal Involvement

Review of the literature makes it clear that clinically significant renal disease develops much less commonly in SSc than do the renal pathologic abnormalities. Multiple autopsy studies in SSc have demonstrated the frequency of typical renal histopathologic lesions to be in the range of 50% to 80%; these studies have been previously reviewed (66,67). In an attempt to better estimate this frequency and understand the natural history of scleroderma renal disease, Kovalchik and colleagues (68) performed renal biopsies in nine normotensive SSc patients in whom there was no evidence for renal disease. Six of the nine biopsies demonstrated presence of vascular changes, and mild nonspecific glomerular lesions were seen in all nine. Despite the frequency of these findings, only one of these patients eventually developed SRC.

In SSc, clinical renal disease ranges from less severe renal abnormalities including hypertension, proteinuria, and azotemia (serum creatinine >1.2 mg/dL) unrelated to SRC, to overt life-threatening SRC. Cannon et al. (69) found that 45% to 60% of 210 patients with SSc had hypertension, proteinuria, or azotemia unrelated to SRC. Common causes of these abnormalities in patients with SSc include prerenal effects from congestive heart failure, diarrhea or dehydration from scleroderma gastrointestinal involvement, volume depletion from infection, and drug reactions resulting from the use of diuretics, D-penicillamine, cyclosporine, or nonsteroidal antiinflammatory drugs (NSAIDs) (70). Despite classic renal pathologic changes occurring in a large number of patients, chronic progressive renal insufficiency is rare outside of the setting of SRC. Because hypertension, proteinuria, and azotemia can all result from causes other than SSc, their use as specific indicators likely overestimates true SSc-related renal disease. However, the diagnosis of SRC always should be considered in the setting of these renal abnormalities, particularly if they are abrupt in onset.

The frequency of SRC in a University of Pittsburgh cohort of 1,118 SSc patients followed between 1972 and 1990 was 10%; the frequency in dSSc was 10-fold higher than in lSSc (19% and 2%, respectively) (71). There is much less risk of SRC in lSSc, particularly if the patient is anticentromere antibody positive. Langevitz et al. (72) reported SRC developing in only 7 (3%) of 243 SSc patients (5% frequency in dSSc) followed prospectively between 1979 and 1989, leading to the suggestion that the incidence of SRC may be declining. This observation may be due to earlier diagnosis and treatment with ACE inhibitors.

Several cases of pauci-immune crescentic glomerulonephritis associated with myeloperoxidase-specific anti-neutrophil cytoplasmic antibodies (MPO-ANCA) have also been reported in patients with SSc (73–76). Although this entity was originally thought to be a complication of treatment with D-penicillamine, occurrence in the absence of D-penicillamine has been reported (75,76). In contrast to SRC, patients with this disorder are typically normotensive with nephrotic range proteinuria and renal insufficiency, and do not have microangiopathic hemolytic anemia or thrombocytopenia. Treatment regimens for microscopic polyangiitis with corticosteroids and immunosuppressive agents may be beneficial.

Scleroderma Renal Crisis

Clinical Features. Renal crisis in SSc is a distinct clinical syndrome of abrupt onset representing a medical emergency, which inexorably results in renal failure and death if left untreated. The best predictor of the development of SRC is rapid progression of cutaneous involvement in patients with dSSc (77). The highest risk occurs early in the course of disease, with greater than 70% of cases developing within 4 years of disease onset (70,77). Investigation of multiple other clinical parameters has identified as other apparent risk factors diffuse skin involvement, anemia, pericardial effusions, congestive heart failure, the presence of anti-RNA polymerase III antibodies, and use of high-dose corticosteroids (prednisone, ≥30 mg/day) especially

TABLE 66-2

CLINICAL FACTORS AND SCLERODERMA RENAL CRISIS (SRC)

Factors predictive of SRC	Factors not predictive of SRC
Diffuse cutaneous Involvement	Baseline renal function
Early disease: within 4 years of disease onset	Urinalysis abnormalities
Rapidly progressive skin induration	Baseline blood pressure
Cardiac involvement	Plasma renin activity
Congestive heart failure	Renal pathologic abnormalities
Pericardial effusion	Anti-topoisomerase antibodies (in diffuse systemic sclerosis)
New anemia	
Possibly high-dose corticosteroids (normotensive SRC)	
Anti-RNA polymerase III antibodies	

in normotensive patients (70,77–79) (Table 66-2). Case reports of rapidly progressive SSc with SRC associated with a malignancy (breast, ovarian, lung, and renal cell carcinoma) suggest that screening for occult malignancy should be considered in this population and that SSc patients with malignancies should be monitored closely for renal, cardiac, and pulmonary complications (80).

The syndrome of SRC is classically characterized by the precipitous onset of severe hypertension, rapidly progressive renal failure, microangiopathic hemolytic anemia, consumptive thrombocytopenia (rarely <50,000 platelets/mm^3), and hyperreninemia. Although symptoms may be absent at the onset of SRC, most patients experience fatigue, headache, visual disturbances, encephalopathy, or seizures, all of which may be secondary to marked hypertension. Hypertensive funduscopic changes are frequently noted and may include hemorrhages, exudates, and papilledema. Signs and symptoms of left ventricular failure are not infrequent at presentation or shortly after onset of SRC. Pulmonary hemorrhage, which is not usually seen in SSc, can be a rare life-threatening complication (78,81).

The diagnosis of SRC is easily made when typical manifestations develop in a patient with known or obvious SSc. Diagnosis of normotensive SRC, which accounts for approximately 10% of presentations (78), depends on identification of other clinical features of SRC such as new-onset azotemia and microangiopathic hemolytic anemia. Renal crisis may occasionally develop in the setting of minimal signs of SSc or in the absence of skin involvement, termed sine scleroderma. In such cases, making an early and correct diagnosis hinges on recognition of noncutaneous manifestations of SSc such as the presence of Raynaud's phenomenon, tendon friction rubs, carpal tunnel syndrome, nail-fold capillary abnormalities, and supporting serologic findings such as antitopoisomerase (anti-Scl-70) and anti-RNA polymerase III antibodies (70,82).

Hypertension in the absence of SRC has a frequency in SSc similar to the prevalence of essential hypertension in the general adult population, in the range of 15% to 20% (69,77). Hypertension may or may not be present before SRC onset, and its presence does not appear to predict development of SRC or overall survival (77,83). A number of cases of SRC occurring in the absence of concomitant hypertension have been reported, beginning with two of the original three patients described by Moore and Sheehan (65). Helfrich et al. (78) at the University of Pittsburgh reported that of 131 patients with SRC, 15 (11%) had normal blood pressure during the complication. Compared with hypertensive SRC patients, these patients demonstrated a higher frequency of microangiopathic hemolytic anemia, thrombocytopenia, and previous corticosteroid exposure along with a poorer 12-month survival rate.

Laboratory Features. Azotemia and the hematologic manifestations of SRC have been mentioned previously. The serum creatinine concentration tends to rise rapidly and may continue to rise for several days, even after effective control of the hypertension. Proteinuria occurs frequently in SRC, but is not usually greater than 2.5 g per/24 hours. The finding of proteinuria on urinalysis is definitely not specific for SRC; mild degrees of proteinuria may be present in 20% to 36% of SSc patients in whom SRC is absent (69,71). Proteinuria has not been found to be a helpful predictor of SRC development, although its presence has been suggested to be a marker for higher mortality (77). Urinalysis may also reveal the presence of microscopic hematuria or granular casts; RBC casts are an unusual finding and would suggest a scleroderma-overlap syndrome. Abnormalities of the renin–angiotensin system are described in the following.

Course and Prognosis. As mentioned, untreated SRC is a uniformly fatal disorder. Uremia, hypertensive complications, and congestive heart failure can lead to death within 7 to 10 days. Prior to the last decade and antecedent to use of ACE inhibitors, the average survival after SRC onset was 1 to 3 months (84), despite availability of antihypertensive agents and hemodialysis. The first indication that disrupting the renin–angiotensin system was of therapeutic importance in SRC came when it was shown that bilateral nephrectomy improved mortality (84,85). LeRoy and Fleischman (85) showed that survival beyond 6 months was only 10% in those without nephrectomy, compared to 43% in those who had undergone the procedure.

A revolution in treatment of SRC began with the first report of captopril therapy reversing SRC (86). Subsequently, it was confirmed that ACE inhibition was the most effective therapy for SRC. Steen and colleagues (87) reviewed outcomes in 108 patients with SSc before and after availability of ACE inhibitors. The cumulative 1-year survival rate in 55 patients receiving ACE inhibition therapy was 76% compared to 15% in 53 patients not treated. Moreover, of 20 ACE inhibitor-treated patients requiring dialysis and surviving for 3 or more months, 11 (55%) were eventually able to discontinue dialysis; none of 15 patients who were not treated with an ACE inhibitor were able to do so. Subsequently, Steen and Medsger (88) reported the outcomes of 145 patients with SRC treated with ACE inhibitors: 61% of patients with SRC had good outcomes (55 patients received no dialysis, and 34 patients received temporary dialysis) and 39% of patients had bad outcomes (permanent dialysis or early death). The 34 patients who received temporary dialysis continued taking ACE inhibitors, and dialysis was discontinued after 2 to 18 months. The 5-year cumulative survival rate was 90% in the no dialysis and temporary dialysis groups, similar to their cohort of patients with SSc without renal crisis. Patients who had SRC requiring permanent dialysis had a 5-year cumulative survival rate of only 40%.

It seems clear that treatment with ACE inhibitors has rendered SRC a much less ominous manifestation of SSc. The use of ACE inhibitors, combined with the observation that SRC may be declining in frequency, appears to account for the recent observation that renal disease has been superseded by cardiopulmonary complications as the leading cause of death in SSc (14,72,89).

Pathophysiology of Renal Involvement

Renal Circulation

Both functional and structural vascular abnormalities contribute to impaired renal cortical perfusion in SSc, setting the stage for the development of clinical renal disease. Urai et al. (90) were the first to demonstrate reduced renal plasma flow in SSc. Their study of 25 SSc patients without evidence of renal disease revealed reduced *p*-aminohippurate clearances in 23 (92%). In a supporting study, Cannon et al. (69) demonstrated normal to slightly diminished renal cortical perfusion using a xenon-133 washout technique in patients with markers of renal involvement in the absence of renal failure. In contrast, extreme to absent renal cortical perfusion was noted in those patients with SRC. This dramatic observation was corroborated by Woolfson et al. (91), who showed almost complete loss of renal perfusion and parenchymal uptake in three SRC patients studied by dynamic renal scintigraphy. Unfortunately, these techniques have not been successful in predicting the development of SRC.

The angiographic appearance of the scleroderma kidney demonstrates well the underlying structural abnormalities of the renal vasculature. Lester and Koehler (92) first reported the striking angiographic findings of irregular narrowing of the interlobular and arcuate arteries with prolonged contrast transit time. Winograd et al. (93) described abnormal cortical lucencies of the nephrogram in two SSc patients having undergone arteriography. This "spotted nephrogram" is suggestive of focal cortical necrosis and appears to be relatively specific for SSc, particularly when seen in the absence of large vessel abnormalities (69,94).

Vasospasm may contribute to renal hypoperfusion in SSc. In a study of renal perfusion, Cannon et al. (69) found that induction of Raynaud's phenomenon by cold exposure resulted in an acute reduction in cortical perfusion. In addition, they showed that intrarenal infusion of a vasodilator resulted in increased blood flow. Vasospasm leading to decreased renal perfusion could result in the release of renin. These and other investigators have observed a higher frequency of SRC during the cold months of the year, suggesting that vasospasm may play an initiating role in SRC (84); others have not found as strong an association (77).

Renin–Angiotensin System

Plasma renin activity (PRA) acutely and rapidly rises to values 2 to 100 times that of normal during SRC (84). However, in the absence of renal involvement, PRA is frequently normal, and levels do not appear to predict development of SRC. Traub et al. (84) reported that baseline PRA determinations were normal in all 13 SRC patients who happened to have had PRA measured from 1 week to 1 year prior to SRC onset. Other investigators have confirmed this observation (95). Supporting the concept of a Raynaud's-like renal vasoconstriction, Kovalchik et al. (68) observed significant PRA elevations with cold pressor testing in four of their patients with SSc, with evidence of vasculopathy on renal biopsy, but not in those without such histologic changes. As discussed, all of the subjects in this study lacked clinical evidence of renal disease.

Considering the preceding observations, it is reasonable to conclude that in the setting of SRC, both structural and functional renal vascular abnormalities lead to hypoperfusion of the juxtaglomerular apparatus, stimulating renin secretion. With marked elevation of PRA, excessive angiotensin II is formed, exacerbating systemic and renal vasoconstriction. A self-perpetuating cycle is initiated with the resultant systemic hypertension and worsened renal cortical ischemia. The beneficial therapeutic effects of ACE inhibition support the importance of this mechanism in the pathophysiology of SRC.

Histopathology

Gross Appearance

The gross appearance of the scleroderma kidney varies depending on the relative chronicity and severity of disease. In patients with acute SRC, the kidneys are generally normal in size with a smooth to finely granular surface. In florid cases of SRC with associated renal failure, the renal cortex may display areas of hemorrhagic or yellow-gray infarction. The kidneys become shrunken in size and may exhibit coarsely granular surfaces in patients who have survived SRC but have been left with chronic renal disease. These gross changes are not specific for the scleroderma kidney because they also are seen in other forms of chronic renal failure, such as malignant nephrosclerosis (66).

Microscopic Appearance

Arteries. The small interlobular and arcuate arteries, measuring 150 to 500 μm in outer diameter, exhibit the characteristic lesions of scleroderma renal involvement (69). Intimal edema is the earliest abnormality seen. This is followed by cellular intimal proliferation with associated deposition of mucoid ground substance composed primarily of mucopolysaccharide and, to a lesser extent, glycoproteins. The cellular component of this process arises from proliferation of myointimal cells, which ultrastructurally can resemble either smooth muscle cells or fibroblasts (66). The myointimal cells often arrange themselves in a concentrically whorled or "onionskin" configuration (Fig. 66-1). As a consequence, the vascular lumen becomes markedly narrowed. Endothelial cells lining the lumen are typically swollen, and in some areas may have sloughed. The internal elastic lamina is intact. The media becomes thinned, often to one or two cell layers. Typically, no significant inflammatory cell infiltrates (lymphocytes or other mononuclear cells) are present. Mild adventitial and periadventitial fibrosis may be seen, a finding reported to be relatively specific for SSc and rarely noted in malignant hypertension not associated with SSc. The nonfibrotic arterial abnormalities described are much less specific because they are difficult to distinguish from those seen

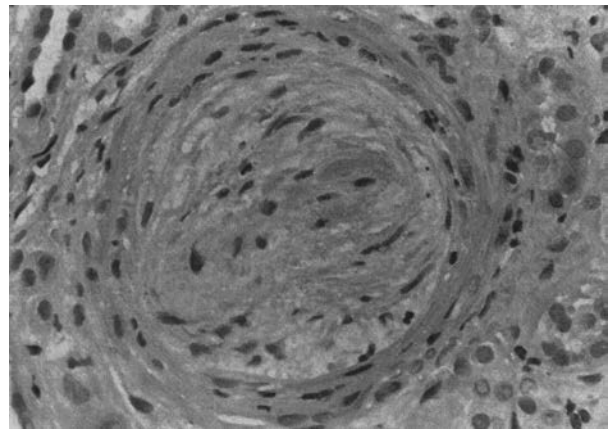

FIGURE 66-1. Photomicrograph of the kidney from a patient with systemic sclerosis with diffuse scleroderma and scleroderma renal crisis. The small muscular artery demonstrates intimal edema and concentric proliferation of myointimal cells with complete occlusion of the lumen (hematoxylin and eosin, magnification ×300). (Courtesy of William S. Hammond.)

in association with essential hypertension. In contrast to essential hypertension, where development of these renal vascular changes appears to be dependent on the level of blood pressure elevation, the lesions in SSc appear to precede or occur in the complete absence of hypertension (68,96).

With more chronic renal involvement, mucoid edematous intimal proliferation of the arteries is replaced by a dense, concentric, intimal fibroelastosis that continues to narrow the lumen. Moderate concentric reduplication of the internal elastic lamina usually can be demonstrated. In contrast to acute lesions, the media in chronic SSc renal disease lacks a stretched appearance and may appear mildly sclerotic. The adventitia is unremarkable and no inflammatory infiltration is present in the vessel wall (66).

Arterioles. Arterioles (external diameter of 50 to 150 μm) rarely display mucoid intimal proliferation. Rather, subintimal and, to a lesser degree, intramural fibrinoid necrosis affects vessels of this size, but vasculitis is rarely seen. This process, frequently extending to the hilus of the glomerulus, severely narrows the arteriolar lumen, which also may be occluded by intraluminal fibrin thrombi. These changes are indistinguishable from those found in malignant hypertension (66) (Fig. 66-2).

Glomeruli and Interstitium. Acutely the histologic characteristics of glomeruli in SSc renal disease range from normal to showing areas of infarction and necrosis. Juxtaglomerular apparatus hyperplasia is seen, nonspecific to SSc, and consistent with the hyperreninemia found in SRC. Chronically the glomeruli exhibit progressive glomerulosclerosis, a finding in common with malignant hypertension and hemolytic–uremic syndrome. Basement membrane wrinkling and thickening are noted, which in the past have improperly been referred to as "wire looping," a term now reserved for the glomerular capillary wall thickening caused by subendothelial deposits in active lupus nephritis (66). The renal tubular epithelial cells may be flattened and may exhibit hyaline droplet degeneration. The latter finding preferentially affects certain nephrons and not others, suggesting a greater degree of protein leakage from the more severely affected glomeruli. The interstitium may show varying degrees of fibrosis and mild chronic inflammatory infiltrates consisting primarily of lymphocytes and plasma cells.

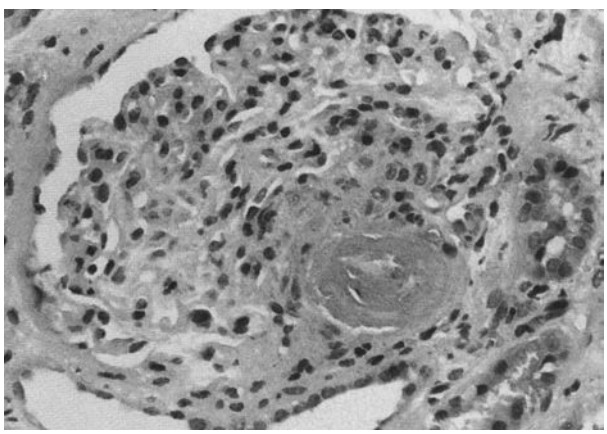

FIGURE 66-2. Photomicrograph of the kidney from the same patient as in Figure 66-1. The glomerular afferent arteriole demonstrates fibrinoid necrosis (hematoxylin and eosin, magnification ×300). (Courtesy of William S. Hammond.)

Immunofluorescence and Elution Studies. Studies utilizing immunofluorescence microscopy in SSc have described nonspecific focal deposits of immunoglobulins (primarily IgM), complement components (C1q, C3, and C4), and fibrinogen in the intima and lumina of small renal arteries and arterioles (66). Glomerular staining is more inconsistent and less intense for these components compared to vascular staining. Elution of immunoglobulin (primarily IgG) and antinuclear antibodies from kidneys of SSc patients has been reported (97–99). Although these findings have been interpreted as providing evidence for immune complex injury in the pathogenesis of SSc renal vasculopathy, this interpretation is now viewed with less certainty. Electron-dense deposits suggesting immune-complex deposition have not been demonstrated on electron microscopic examination of vessels and glomeruli in SSc (66). In addition, immunofluorescence staining in the early stages of malignant nephrosclerosis is quite similar to that seen in SSc, suggesting that this finding is nonspecific and the likely result of disruption of vascular integrity with increased vascular permeability (66,96).

Management of Renal Crisis

For reasons previously discussed, ACE inhibition is the most important intervention in the management of SRC treatment. Short-acting ACE inhibitors that can be easily titrated, such as captopril, should be started immediately on SRC diagnosis. Later, longer-acting agents may be substituted without loss of efficacy. The dose of the ACE inhibitor should be increased every 6 to 12 hours as needed to reduce the systolic blood pressure by about 10 to 20 mm Hg per day to achieve a normal blood pressure (about 120/70 mm Hg) within 48 to 72 hours (70). Although this control may be achieved with maximum ACE inhibition alone, other antihypertensives (calcium channel blockers, hydralazine, minoxidil) may be necessary while continuing the ACE inhibitor if this treatment does not normalize the blood pressure within 48 hours. Nitroprusside and lobetolol are not usually required as they may lead to arterial vasodilation with arterial underfilling resulting in further renal hypoperfusion (100). Excessive falls in blood pressure should be avoided and volume status should be monitored to maximize renal perfusion. For this reason, diuretic therapy is contraindicated. In the case of normotensive SRC, it is advised that the highest possible dose of ACE inhibitor be used that avoids hypotension. Other treatments such as corticosteroids, immunosuppressives, anticoagulation, and plasmapheresis have no role in the therapy of SRC. The use of angiotensin II receptor blockers (ARBs), such as losartan, or the combination of an ACE inhibitor with an ARB has not been extensively studied in SRC. Caskey et al. (101) reported the failure of losartan to control blood pressure in one patient with SRC. Although both ACE inhibitors and ARBs diminish the effects of angiotensin II, only ACE inhibitors increase levels of the potent renal vasodilator, bradykinin (102). Despite normalization of the blood pressure with ACE inhibition, the serum creatinine concentration may continue to rise for 7 to 10 days prior to returning toward normal. This rise in creatinine level usually is not attributable to ACE inhibitor therapy, which should be continued (70,72).

Factors associated with early death or dialysis in patients with SRC treated with ACE inhibitors include male sex, older age, congestive heart failure, inability to control blood pressure within 72 hours, and a pretreatment serum creatinine level of greater than 3 mg/dL (70). Renal replacement therapy, when needed, may take the form of either hemodialysis or continuous ambulatory peritoneal dialysis (CAPD). Although these interventions are successful in many patients with SSc, complications may occur more frequently than in end-stage renal failure patients without SSc. With hemodialysis, there may be

difficulty fashioning and maintaining adequate vascular access and problems with hemodynamic instability. With CAPD, altered peritoneal permeability, caused by sclerosis, and diminished peritoneal blood flow, caused by underlying vasculopathy and vasospasm, may decrease effectiveness of this intervention (103). It should be reiterated that continuing ACE inhibition to avoid any hyperreninemia during renal replacement therapy appears to be of therapeutic importance. Steen and coworkers (87) described that by continuing ACE inhibitor therapy, patients surviving more than 3 months have a 55% chance of becoming independent of dialysis.

Renal transplantation should be considered in those patients with SRC who remain dependent on dialysis for greater than 18 to 24 months and in whom there are no disease-related contraindications such as significant pulmonary, cardiac, or intestinal involvement. Richardson (104) reported the first successful case of renal transplantation for SRC-induced renal failure in 1973. Subsequently, small numbers of other cases have been reported (105–107). Tsakiris et al. (106) reported graft survival greater than 50% at 1 year and 44% at 3 years in 28 patients with SSc who underwent renal transplantation between 1977 and 1993. Recently, Chang and Spiera (107) reported on 86 SSc renal transplant recipients who were monitored by the United Network for Organ Sharing (UNOS) Scientific Registry from 1987 through 1997. This study showed graft survival rates of 62%, 57%, and 47% at 1, 3, and 5 years, respectively. Unfortunately, data regarding characteristics of the patient population, length of time to graft failure, the cause of graft failure, and the immunosuppressive therapy regimen were lacking from this registry. In comparison, graft survival rates in patients with systemic lupus erythematosus (SLE) from the UNOS registry were 82%, 73%, and 62% at 1, 3, and 5 years, respectively (107). Similarly, Bleyer et al. (108), using the UNOS registry from 1987 through 1996, showed decreased patient and graft survival (84% and 72%, respectively) at 3 years in scleroderma patients in comparison to a reference condition, immunoglobulin A (IgA) nephropathy. The survival rate of patients with SSc on hemodialysis is approximately 35% to 40% at 3 to 5 years (88,106).

There have been several reports of a possible recurrence of SSc in renal allografts; however, the histologic changes suggesting recurrence are not easily differentiated from those found with chronic graft rejection or malignant hypertension in renal disease (103,105,107). In addition, the glomerular and vascular histologic abnormalities seen in cyclosporine-induced nephrotoxicity can appear similar to those seen in the scleroderma kidney (109). This observation and the known cyclosporine-related adverse effects of vasoconstriction and endothelial injury notwithstanding, it remains unclear whether cyclosporine increases the risk of recurrence of SSc in the allograft and whether SSc renal transplant recipients are more vulnerable to cyclosporine toxicity (110).

Conclusion

Systemic sclerosis is a disorder of unclear etiology that can vary widely in its severity. To date, no therapy, when tested in a prospective controlled fashion, has been shown to halt or reverse the vasculopathy or fibrotic process. The long-term hope is that further understanding of the pathophysiology of SSc will lead to a therapy that will arrest these processes. Current treatment is limited to those agents that may alleviate specific clinical manifestations (e.g., calcium channel blockers for Raynaud's phenomenon and potent antacids or proton-pump inhibitors for gastroesophageal reflux symptoms). Of such interventions, only the use of ACE inhibition for SRC has made a significant difference in the long-term outcome of this disorder. Although no study has been performed to address whether "prophylactic" use of ACE inhibitors prevents SRC, it is recommended that even mild hypertension in a patient with SSc be treated with an ACE inhibitor, particularly if SRC risk factors are present. Diuretics should be avoided in the routine management of hypertension in patients with SSc because hypovolemia could potentially precipitate the onset of SRC.

RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic inflammatory disease of unknown etiology, characterized primarily by symmetric polyarthritis. Rheumatoid arthritis is also a systemic disease with extraarticular manifestations in up to 40% of patients, including constitutional symptoms, serositis, vasculitis, ocular involvement, rheumatoid nodules, pulmonary lesions, and cardiac disease (111). Rheumatoid arthritis is the most common of the inflammatory rheumatic diseases, with a prevalence of 1% and more than 3 million cases in the United States alone. This disease is more common in women by a ratio of 2.5:1 and can occur at any age, although the peak onset is in the fourth and fifth decades (112).

Renal involvement is generally considered to be quite rare, although RA is often characterized by the presence of rheumatoid factors and circulating immune complexes (113). Clinically, renal disease attributable to RA itself usually is mild and not a major problem in clinical management. However, studies showed that measurement of serum creatinine concentrations might underestimate renal impairment in patients with RA, possibly because of the decreased muscle mass of these patients (114,115). Furthermore, population-based studies have shown that patients with RA have an increased mortality relative to age-matched controls and that a significant proportion of this increased mortality can be attributed to renal disease (116,117). These studies suggest that a combination of insults to the kidneys over time in rheumatoid patients may contribute to renal compromise and make the kidneys more susceptible to subsequent diseases such as infection, hypertension, or atherosclerosis (116–119).

Two broad categories of renal disorders can be distinguished in RA: lesions caused by the disease itself and lesions occurring as a result of side effects of therapeutic agents. In Table 66-3 are listed the types of renal lesions that have been described in RA. It is often difficult to distinguish between disease-associated and drug-associated renal lesions because patients with RA are frequently treated with multiple drugs for many years. Many studies in the literature suffer from a failure to make this distinction. In addition, many studies from the 1940s and 1950s included a significant number of patients with other connective tissue diseases such as SLE in their series of patients with RA, thereby further confusing the definition of RA-associated renal

TABLE 66-3

RENAL INVOLVEMENT IN RHEUMATOID ARTHRITIS (RA)

Renal disorders related to RA
Amyloidosis
Glomerular lesions
Vascular lesions
Tubulointerstitial lesions
Renal disorders related to drug therapy
Nonsteroidal antiinflammatory drugs
Analgesics
Gold/penicillamine
Cyclosporine

disease. Nevertheless, substantial literature exists describing renal lesions in RA.

Renal Disorders Related to Rheumatoid Arthritis

Amyloidosis

Amyloidosis is a syndrome characterized by deposition of an insoluble proteinaceous material in the extracellular matrix of multiple organs. These deposits have a unique fibrillar ultrastructure characterized by a rigid, nonbranching filament of about 100 Å in diameter. Amyloidosis is generally classified according to the type of fibril deposited in tissue, being either secondary amyloidosis (AA amyloidosis) or primary amyloidosis associated with plasma cell dyscrasias (AL amyloidosis). Rheumatoid arthritis is one of a number of chronic inflammatory or neoplastic diseases that may eventually lead to secondary amyloidosis (AA amyloidosis). The initial manifestation of renal involvement in amyloidosis is proteinuria; it progresses at a variable rate and most patients eventually develop the nephrotic syndrome and/or uremia (Chapter 75).

The frequency with which amyloidosis is found at postmortem examination of patients with RA varies widely from 10% to 60%, although most authors report a prevalence of 10% to 20% (117,120–124). The reported frequency of amyloidosis is considerably lower in living patients. Using a Congo red stain on liver biopsies, Unger et al. (124) found amyloidosis in six of 56 patients with RA, all of whom had proteinuria. Fearnley and Lackner (122) surveyed 183 patients with RA, of whom 24 had persistent proteinuria. Eight of these patients were found to have amyloidosis, giving an overall prevalence of eight of 183, or 4%. Arapakis and Tribe (120) performed rectal biopsies on 115 patients with RA in search of amyloidosis; six patients were positive, only three of these patients had proteinuria. A recent study from Finland reported the prevalence of amyloidosis to be 5.8% in 1,666 patients with RA who died during 1989 (125). In this study, more than 90% of the cases of amyloidosis were diagnosed during life. Thus, although more frequent at autopsy, clinically significant amyloid renal disease may occur in only 3% to 6% of living patients with RA.

Amyloidosis is seen with increasing frequency in patients with long-standing RA (>10 years' duration) and is also more common in patients who are positive for rheumatoid factor (RF) and who have significant joint destruction (126). This observation implies that if arthritis is kept under good control, the frequency of secondary amyloidosis can be reduced. Parkins and Bywaters (127) described two patients in whom proteinuria and clinical renal disease improved when their arthritis went into remission, but renal disease recurred when the RA relapsed. Recently, Elkayam et al. (128) reported resolution of proteinuria and stabilization of amyloid deposits by serial ^{123}I-labeled serum amyloid P scintigraphy in a woman with rheumatoid arthritis of 10 years' duration and proteinuria (900 mg per 24 hours) treated with infliximab. Wright and Calkins (129), in a series of patients (1967 to 1979) in which management of RA was presumably more aggressive and more successful, reported a lower frequency of amyloidosis at autopsy (3.6%) than in earlier studies. These data are consistent with the hypothesis that aggressive management of active joint inflammation may decrease the frequency and severity of AA amyloidosis in patients with RA.

The clinical course of AA amyloidosis in patients with RA is usually one of progressive renal failure, as there are no satisfactory treatments aside from aggressive treatment of the underlying disorder. Cardiac involvement and serum creatinine level greater than 2.0 mg/dL at presentation appear to be poor prognostic factors (130). Patients with amyloidosis generally do poorly on dialysis, and recurrence of amyloid deposits has been reported in transplanted kidneys (131). However, because there are other causes of proteinuria in RA, the diagnosis of amyloidosis as a cause of clinical renal disease should be made only with biopsy confirmation and after other conditions have been excluded.

Glomerular Lesions

The existence of a glomerular lesion specific for RA has been debated for a number of years. Early studies reported nonspecific glomerulitis on kidney biopsy or autopsy and decreased renal function in 20% to 60% of patients with rheumatoid arthritis (132–139). However, a number of these patients may have had SLE, casting doubt on the conclusions of these studies. Subsequent studies have demonstrated either membranous or mesangial nephritis in some patients with RA (140–150). Membranous glomerulonephritis is almost always secondary to drug therapy with gold or penicillamine. A few cases of membranous glomerulonephritis occurring independent of drugs have been reported that present with mild proteinuria (140–143). Mesangial glomerulonephritis is common on renal biopsies from patients with RA (142–150). This lesion is characterized by increased mesangial matrix and/or hypercellularity and mesangial deposits of IgM, IgA, and C3 (146–150). Korpela et al. (147,150) have shown that 5% to 8% of patients with RA have histologic features of IgA nephropathy. However, whether this prevalence of IgA nephropathy is greater than that in the normal population is not clear.

Despite the findings of glomerulitis on renal biopsy, the clinical consequences of these lesions are minor. The most frequent manifestations of glomerulitis in RA are microscopic hematuria and low-grade proteinuria. Follow-up of these patients has shown a benign course, with progression to renal insufficiency and/or increasing of proteinuria occurring only rarely (145,146,148,149).

Proliferative glomerulonephritis is a rare cause of renal disease in RA. Davis et al. (151) reviewed the records of 5,232 patients with the diagnosis of RA who were hospitalized over a 22-year period. Proliferative glomerulonephritis was diagnosed in only four patients, most of whom met the criteria for SLE or mixed connective tissue disease. However, a well-documented case of proliferative glomerulonephritis in RA in the absence of any other disorder was reported (151). Although this patient was on gold therapy at the time of the renal biopsy, proteinuria and hematuria both preceded initiation of gold therapy. The renal biopsy showed mesangial hypercellularity along with proliferative changes in the glomeruli, and electron microscopy revealed subendothelial deposits. The authors raised the question as to why a proliferative glomerulonephritis is not seen more often in RA, because circulating immune complexes frequently are present in this disease. Weisman and Zvaifler (152) speculated that reduced complement-fixing ability of the immune complexes in RA might explain the paucity of renal lesions. However, the reason why immune complex nephritis is not seen more often in RA remains unclear (153).

Necrotizing glomerulonephritis complicating RA appears to be an uncommon entity. There are several reported cases of necrotizing glomerulonephritis occurring in RA associated with concurrent systemic vasculitis (154–156) and additional cases occurring in the absence of systemic vasculitis (156–159). Of interest, the majority of the renal biopsies from these cases revealed a pauci-immune necrotizing glomerulonephritis. Furthermore, the patients in the more recent case reports were perinuclear-ANCA (p-ANCA) positive (156,159). Although rare, p-ANCA positive pauci-immune necrotizing glomerulonephritis should be considered in all patients with RA and

acute renal insufficiency with proteinuria and/or microscopic hematuria.

In summary, early studies describing a rheumatoid glomerulopathy are clouded by the inclusion of patients with diseases other than RA and by the inclusion of patients taking a variety of potentially nephrotoxic drugs. It appears that proliferative and necrotizing glomerulonephritis are extremely rare in RA, occurring only in isolated cases. A mild form of mesangial or membranous glomerulopathy does appear to occur in RA independent of drug therapy. Functionally, this disease is usually mild, presents with microscopic hematuria and low-grade proteinuria, and follows a benign course.

Vascular Lesions

Systemic vasculitis is the most feared of all the complications of RA and is associated with an appreciable morbidity and mortality. Schmid et al. (160) described 34 patients with RA and arteritis in whom extraarticular disease was present. The most common clinical symptoms were peripheral neuritis in 16 patients, episcleritis and pericarditis in five patients, and hypertension in four patients. Eight patients in their series were found to have proteinuria (24%), and this occurred in 43% of patients with extraarticular manifestations of RA summarized from the literature. No mention was made, however, of impaired renal function in any patient. Scott et al. (161) reviewed 50 cases of systemic rheumatoid vasculitis; 12 (24%) of their cases had clinical features of renal disease. Four patients had amyloidosis, two had chronic renal failure of undetermined cause, and six developed proteinuria or hematuria when typical lesions of cutaneous vasculitis appeared. Renal function was not impaired in these six patients.

There are, however, a few reports in which patients with RA developed a polyarteritis-like clinical syndrome with renal vessel involvement. A case report described a patient with rheumatoid vasculitis who presented with abdominal pain and had intrarenal aneurysms on arteriography (162). Ball (163) reported five cases of rheumatoid arthritis with diffuse vasculitis. Only one of the five patients had clinical renal involvement during life, but all had microscopic evidence of renal artery vasculitis.

Tubulointerstitial Lesions

Tubulointerstitial disease of the kidneys is common in RA. However, the primary cause of these changes is Sjögren's syndrome, which is seen as a secondary manifestation in up to 35% of RA patients. The renal lesions of Sjögren's syndrome are discussed elsewhere in this chapter. Another major cause of tubulointerstitial disease in RA is the use of NSAIDs. Although interstitial nephritis may occur with any of these agents, it appears to be most frequently associated with fenoprofen. Investigators examining renal changes in biopsy or autopsy specimens described some degree of interstitial fibrosis in 20% to 46% of rheumatoid patients (164,165). However, none of these studies excluded patients taking NSAIDs or patients with Sjögren's syndrome. It is, therefore, unclear as to whether RA itself causes a tubulointerstitial lesion distinct from that associated with medications or Sjögren's syndrome.

Renal Disorders Related to Drug Therapy

Nonsteroidal Antiinflammatory Drugs and Salicylates

Nonsteroidal antiinflammatory drugs (NSAIDs) are widely used in the treatment of RA. The antiinflammatory properties of these drugs result, in part, from their ability to inhibit cyclooxygenase, one of the major enzymes involved in the synthesis of prostaglandins. Inhibition of renal prostaglandin synthesis by NSAIDs may result in reduced glomerular blood flow and elevation of serum creatinine levels (166,167) (discussed in more detail in Chapter 44). In addition to this reversible rise in serum creatinine, NSAIDs also can cause an interstitial nephritis. Interstitial nephritis has also been shown to be reversible when the drugs are discontinued (Chapter 44). Although all NSAIDs and salicylates (168) can induce a rise in serum creatinine and/or an interstitial nephritis, there is some evidence that sulindac may result in a less severe decrease in renal blood flow than may occur with other NSAIDs (169–171). However, this point is controversial and may depend on the duration of therapy and severity of any underlying renal or liver disease (172). All NSAIDs should be regarded as potential inducers of renal dysfunction. The selective cyclooxygenase-2 inhibitors have the same risk for adverse effects on renal function, as do other NSAIDs (167). As mentioned, the use of NSAIDs has made analysis of renal lesions in RA more difficult to interpret. Practically, when a patient with RA presents with evidence of decreased renal function or interstitial changes, discontinuation of NSAIDs should be one of the first steps before further diagnostic or therapeutic maneuvers are undertaken.

Analgesic Nephropathy

Analgesic nephropathy is characterized by a combination of papillary necrosis and interstitial nephritis (Chapter 44). This type of injury is most widely attributed to phenacetin but may also be seen with NSAIDs (173,174). Rare cases of papillary necrosis have been reported in association with ibuprofen, phenylbutazone, fenoprofen, naproxen, and mefenamic acid, as well as with several combinations of NSAIDs. The risk of rheumatoid patients developing analgesic nephropathy appears to be lower than would be expected on the basis of their regular consumption of NSAIDs (116). This observation may be due to less frequent use of analgesic combinations or to closer physician monitoring. However, the frequency of subclinical renal disorders in patients with RA should raise the possibility that chronic NSAID therapy may cause some additional degree of renal damage.

Renal Lesions Associated with Gold Therapy

Gold salts have been advocated for the treatment of many diseases including pulmonary tuberculosis. In the belief that tuberculosis and RA had a common infectious etiology, Forestier in 1935 first described the use of gold salts in the treatment of RA (175). He noted improvement in 70% to 80% of more than 500 cases, a finding that has been confirmed in multiple studies since that time. With the availability of newer effective therapies, gold treatment is used less often in selected patients with RA, psoriatic arthritis, or juvenile chronic arthritis. It is referred to as a "disease-modifying antirheumatic drug," or DMARD, because some studies have shown healing of bone erosions after its use (176). The clinical effects of weekly gold injections do not develop until after 3 to 6 months of therapy, and the patient must receive maintenance monthly injections for years. Recent studies have shown that relatively few patients are able to remain on gold therapy for the long term, however, owing to the cumulative incidence of side effects, including nephropathy, bone marrow suppression, rash, and stomatitis. An oral form of gold (auranofin) introduced in the early 1980s has a lower incidence of serious toxicity than parenteral gold but is less effective (177,178).

Clinically, the hallmark of renal toxicity from gold is proteinuria, occurring in 3% to 10% of RA patients receiving gold injections (177,179–183). Although microscopic hematuria can accompany proteinuria, isolated hematuria or the appearance of cellular casts is rare and should prompt an investigation into other causes of renal disease. Nephrotic syndrome develops in up to one-third of patients with gold-induced

proteinuria (116). The onset of proteinuria is most common 4 to 6 months after the initiation of gold therapy (total dose 1,000 to 1,500 mg at 50 mg/week), although it may occur at any time during therapy. Furthermore, the severity of proteinuria is not proportional to the dose of gold received. The frequency of proteinuria in patients receiving oral gold (auranofin) appears to be very similar to the frequency in those patients receiving injectable gold (177).

Hall et al. have extensively studied the natural history of gold nephropathy (184–187); the proteinuria resolves gradually with discontinuation of gold. Proteinuria resolved completely by 21 months in all 21 patients studied, with the mean time to resolution of proteinuria being 11 months (185). In those patients who developed the nephrotic syndrome, treatment with a high-protein diet and diuretics was sufficient to control symptoms; steroids were not required. No changes in creatinine clearance were observed in any patient during or after the resolution of proteinuria. Despite these reports, high-dose glucocorticoid therapy (prednisone 60 mg daily) may be beneficial in gold-induced nephrotic syndrome. Hall and Tighe (187) examined the outcome of nephropathy when gold was continued after the onset of proteinuria. Follow-up of these patients indicated an identical pattern of resolution of renal disease even when discontinuation of gold was delayed for up to 11 months after the onset of proteinuria. Indeed, a number of reports suggest that the continuation of gold after proteinuria develops does not necessarily lead to progressive proteinuria and renal deterioration. Klinkhoff and Teufel (188) successfully reinstituted parenteral gold therapy at a lower dose in five of eight patients with gold-induced proteinuria once their proteinuria had resolved (4 to 12 months). However, because nephrotic syndrome and renal failure can occur, it is recommended that gold therapy be discontinued if progressive proteinuria develops because a number of alternative DMARDs that are not nephrotoxic are now available for the treatment of RA.

The renal histology associated with gold-induced proteinuria and the nephrotic syndrome is membranous glomerulopathy. Light microscopy shows basement membrane thickening, and electron microscopy shows subepithelial deposits (189–191). When proteinuria is of short duration, the only changes noted are subepithelial deposits on electron microscopy. Basement membrane thickening can be detected on light microscopy with long-standing disease. Light and electron microscopy revealed no correlation between the site or degree of deposition of gold particles in the kidney and the presence of proteinuria. These observations suggest that gold nephropathy is an immune complex disease, although the inciting antigen remains unknown.

Wooley et al. (192) have offered a potential explanation for the finding of proteinuria in only some patients with RA receiving gold. They found that 14 of 15 episodes of gold-induced proteinuria occurred in patients who possessed the HLA antigens B8 and DR3. The relative risk of proteinuria during gold therapy was increased 32 times in patients with the DR3 antigen. An increased frequency of an extended haplotype containing a specific *DQA1* allele (as well as DR3 and DR5) has been found in patients with gold nephropathy (193).

In summary, it appears that gold-induced glomerulopathy is mediated by immune complexes and is more prevalent in those patients with a specific genetic background that predisposes them to the development of autoantibodies. The association of gold-induced nephropathy with the B8/DR3 haplotype may also help to explain the predilection of patients with gold nephropathy to develop renal disease when treated subsequently with penicillamine because penicillamine nephrotoxicity is associated with the same haplotype (see the following section). However, the risk of developing gold nephropathy in patients with the B8/DR3 or DQA extended haplotype does not merit HLA typing in rheumatoid patients before instituting gold therapy.

Renal Lesions Associated with Methotrexate and Other DMARD Therapy

Methotrexate is a folic acid antagonist that has become the major DMARD used in the treatment of RA over the last two decades. Although high-dose methotrexate used in the treatment of neoplasia has been associated with renal failure, renal failure associated with the low doses of methotrexate (5 to 25 mg/week) used to treat RA is extremely rare. It was shown, however, in 13 patients with RA that doses of 15 mg/week of methotrexate reduced glomerular filtration rate (GFR) by an average of 10% (194). Renal impairment can increase the risk of side effects of low-dose weekly methotrexate therapy, particularly respiratory and hematologic toxicities (195). Bressolle et al. (196,197) have shown in patients with RA that methotrexate clearance decreases with decreasing GFR. These authors suggest a 50% dose reduction of methotrexate in patients with a GFR of less than 45 mL/minute (197). Methotrexate therapy should be avoided in patients with severe renal dysfunction. Renal function should be monitored periodically in patients taking the drug, particularly in those patients with preexisting renal insufficiency, because methotrexate is primarily excreted unchanged in the urine. Other commonly used DMARDs such as antimalarials, azathioprine, leflunomide, sulfasalazine, and the newer biologic agents etanercept and infliximab appear to have little or no renal toxicity (198).

Renal Lesions Associated with Penicillamine Therapy

Penicillamine was introduced into clinical medicine in the 1950s for the treatment of Wilson's disease (199). Within a few years it had been tried in RA, and it became accepted as a disease-modifying drug in the 1970s. However, the use of penicillamine has declined because of the recent addition of a number of other less toxic DMARDs to treat RA. It is a slow-acting drug with responses generally not observed until after 3 to 6 months of therapy (200–202).

Clinically and histologically, penicillamine-induced nephropathy is similar to gold nephropathy. The initial clinical manifestation is hematuria or proteinuria, occurring in 5% to 30% of patients receiving penicillamine. Nephrotic syndrome occurs in one-third to two-thirds of patients who develop proteinuria (186,200–204). The onset of proteinuria is usually within the first 12 months of therapy (average, 8 months), but it may occur at any time. Initial studies using high doses of penicillamine (>1,000 mg/day) indicated a rapid onset of proteinuria with a higher frequency of this complication (200,201,203). With newer treatment regimens starting therapy at lower doses, toxicity has decreased but proteinuria has been reported at doses as low as 125 mg per day. Penicillamine-induced proteinuria resolves spontaneously after the discontinuation of penicillamine, with a mean time to resolution being 8 months (range, 1 to 21 months) (184,186). No patients demonstrated changes in creatinine clearance owing to penicillamine, and no patients required steroid therapy. Continuation of penicillamine after the onset of proteinuria has been shown to have a benign outcome, similar to gold (187). Several investigators have advocated that penicillamine can be continued in patients with proteinuria if renal function is closely monitored. However, as with gold therapy, this usage rarely seems justified in light of other agents presently available for the treatment of RA.

Histologically, penicillamine-induced renal lesions appear similar to those of gold-induced nephropathy. In the studies by Hall et al. (184,185), 29 of 33 patients with proteinuria secondary to penicillamine had membranous glomerulonephritis,

2 of 33 had minimal change nephropathy, and 2 of 33 patients had only mesangial changes. Studies by Dische et al. (205) showed glomerular capillary wall thickening and increased mesangial matrix, with electron microscopy revealing subepithelial deposits, similar to gold-induced nephropathy or idiopathic membranous glomerulonephritis. The possible genetic predisposition to membranous glomerulopathy seen with gold therapy is also present with penicillamine (192). Penicillamine-induced rapidly progressive MPO-ANCA-positive crescentic glomerulonephritis that requires aggressive immunosuppressive therapy is a rare occurrence (74,206).

Cyclosporine

Cyclosporine is an immunosuppressive agent that primarily affects T cells and has been used for a number of years in transplantation medicine to prevent allograft rejection (207). A number of studies have shown that cyclosporine may be beneficial in refractory RA (208–211). It has also been used to treat other rheumatic diseases such as refractory psoriatic arthritis and polymyositis-dermatomyositis. However, cyclosporine should be reserved for those patients who have not responded to more conventional therapy owing to its potential renal and hypertensive toxicities. The major factor preventing more widespread trials of cyclosporine in RA has been nephrotoxicity, which has been reported to occur in 30% to 70% of patients receiving this drug (207,212–214). The mechanism of cyclosporine nephrotoxicity is not clear but appears to be primarily vascular, resulting in decreased GFR, decreased creatinine clearance, increased serum creatinine, hypertension, and hyperkalemia (207,215). The severity and frequency with which nephrotoxicity occurs are directly related to the dose of cyclosporine administered. Gordon et al. (216) reported that in renal transplantation patients, nephrotoxicity correlated best with trough serum levels of cyclosporine. Patients displaying cyclosporine nephrotoxicity showed trough serum levels of greater than 200 ng/mL; 96% of patients who developed impaired renal function owing to cyclosporine had trough serum levels greater than or equal to 250 ng/mL.

Treatment regimens employing cyclosporine at 2.5 to 10.0 mg/kg per day have been investigated in RA. Berg et al. (212) showed that patients receiving 5 mg/kg per day had a mean increase in serum creatinine of 19.7% and a decrease in creatinine clearance of 22%. Patients receiving 10 mg/kg per day had a mean increase in serum creatinine of 59.5%. Of note is that patients taking NSAIDs with cyclosporine demonstrated increased serum creatinine levels of only 16.6%. Renal dysfunction usually occurred within 2 to 3 weeks after the initiation of cyclosporine therapy and remained stable during the full course of treatment. After discontinuation of therapy, serum creatinine levels improved but remained slightly higher than baseline for up to 12 weeks. Boers et al. (217) demonstrated that renal function remained stable for up to 2 years after discontinuation of the drug; no improvement was noted in those patients who had developed renal impairment, but no further deterioration occurred. In a study by the International Kidney Biopsy Registry, cyclosporine nephropathy was rare in patients who did not take a daily dose in excess of 5 mg/kg per day (218,219). Renal biopsies of RA patients on cyclosporine have shown only minimal changes (tubular atrophy and interstitial fibrosis) (219,220).

In summary, cyclosporine is still an experimental drug in refractory RA. If prescribed, it should be used at a low initial dosage (2.5 to 3.5 mg/kg), and the maximum dose should not exceed 5 mg/kg per day (218,221–223). The maximum dose of the oral cyclosporine preparation Neoral should not exceed 4 mg/kg per day (223). Plasma creatinine levels should be closely monitored throughout the course of therapy. Cyclosporine dosage should be decreased if serum creatinine levels increase by greater than 30% above baseline. Concurrent use of NSAIDs should be discouraged because this may exacerbate nephrotoxicity. Cyclosporine should be used with extreme caution in older patients with RA or in patients with preexisting hypertension or renal disease. Adjustment of cyclosporine dosage based on serum creatinine levels is mandatory to minimize renal toxicity. The need to monitor trough plasma cyclosporine levels at these doses is controversial.

Renal Disease in Juvenile Chronic Arthritis

Juvenile chronic arthritis (JCA), also called juvenile rheumatoid arthritis (JRA), is defined as an inflammatory arthropathy occurring in individuals less than 16 years old. JCA can be divided into three types: (a) polyarticular (five or more joints), which resembles adult RA; (b) oligoarticular (four or fewer joints), which primarily affects young females and is associated with antinuclear antibodies and chronic iritis; and (c) systemic onset disease (Still's disease), characterized by arthritis, fever, rheumatoid rash, leukocytosis, splenomegaly, and serositis. Clinically significant renal disease in children with any of the forms of JCA is rare, although as in adults, proteinuria, hematuria, and amyloidosis have been reported. Studies in children have the same problems as studies in adults with RA; frequently the effects of drug therapy cannot be separated from the spontaneous variations of the disease itself.

Anttila (224) studied 165 children with JCA treated in Finland between 1967 and 1970. Proteinuria was observed in 42.5% of the children on at least one occasion during the 3-year period. Persistent proteinuria, however, occurred in only 2.4% of the total patient population. None of the children developed the nephrotic syndrome, although on renal biopsy two patients were found to have amyloidosis as a cause of proteinuria. Hematuria was found in 38 patients with JCA (23%). Seven of these children had two or more episodes of hematuria, whereas 31 had only one episode. Of the seven patients with JCA with more than one episode of hematuria, amyloidosis was present in two patients and coagulopathy in one, with four patients receiving treatment with gold. Repeated episodes of pyuria occurred in 10 children, or 6% of the patients with JCA. Creatinine clearance was diminished in 5.6% of the group who exhibited only joint symptoms and in 18.5% of the group who demonstrated extraarticular manifestations of JCA. Creatinine clearance was more often depressed in patients receiving salicylate therapy.

Renal biopsies were performed on 57 of the 165 patients with JCA, and autopsy specimens were obtained from three patients (224). Amyloidosis was observed in one biopsy and one autopsy specimen. Except for proteinuria, hematuria, and pyuria, amyloidosis had not caused major impairment of renal function. Glomerular changes were observed in 28% of the cases. In 13 cases it was described as a "local glomerulitis," which would now be described as mesangial changes. Twelve of these 13 patients with JCA had proteinuria and/or hematuria. Other renal biopsy changes included mild basement membrane thickening in 4 patients and interstitial changes in 8 patients. No vascular abnormalities were noted in any biopsy. Overall, approximately 40% of JCA patients in this study had an abnormal kidney biopsy, although renal function was not significantly compromised in any patient.

Amyloidosis in JCA is the subject of many reports (225–231). Of 1,272 patients with JCA, Schnitzer and Ansell (226) observed 51 who had developed amyloidosis, a cumulative incidence of 4%. A large amount of geographic variation in the incidence of amyloidosis in JCA has been reported. In North America, the frequency is estimated to be as low as 0.1%, compared to 0.25% in France, 3% in Germany, and up to 10% in Poland (225,227). Because of the poor prognosis of

amyloidosis associated with JCA, Schnitzer and Ansell initiated therapy with chlorambucil in selected patients in 1967. An 8-year survival rate of 80% was reported for those patients given chlorambucil, as compared to 55% to 60% for those patients receiving intermittent or no chlorambucil therapy. Several studies since that time have indicated that chlorambucil can improve survival and decrease renal disease in a significant percentage of these patients (225,229–231). In a study by Deschénes et al. (225), 8 patients with JCA and systemic amyloidosis were treated with chlorambucil. After a mean 10-year follow-up period, one patient had died from renal disease, two had persistent nephrotic syndrome, and five were free of proteinuria or renal disease. It is of interest that the three patients who did poorly all had persistent activity of their arthritis, suggesting, as has been the experience in adult RA, that control of joint inflammation in JCA can improve renal amyloidosis. A retrospective study of 79 JCA patients with amyloidosis revealed a 10-year survival rate of 80% in those patients treated with chlorambucil compared to 23.5% in patients not treated with chlorambucil (229). In this study, the cause of death was renal failure in 82% and infection in 12% of patients. Serious side effects of chlorambucil therapy include leukopenia, thrombocytopenia, acute leukemia, and infections.

In 1985, Stapleton et al. (232) described 2 patients with JCA who had hematuria, hypercalciuria, and urolithiasis. They subsequently studied 38 children with JCA and found increased urinary calcium-creatinine ratios in 12 (32%). Six of the 12 patients with hypercalciuria had hematuria, compared to three of the 26-normocalciuric patients. Detailed studies of calcium metabolism in four of the hypercalciuric patients showed normal serum calcium levels, normal parathyroid function, and no evidence of acidosis or vitamin D abnormalities. The authors speculated that the hypercalciuria in JCA might result from immobilization and bone demineralization rather than renal calcium wasting. In an earlier paper, Lorenz and Schneider (233) reported kidney stone formation in six of 89 German children with JCA. Taken together, these reports suggest that hypercalciuria should be considered in the evaluation of children with JCA and that such children may be at risk for developing urolithiasis.

In conclusion, clinically significant renal disease in JCA is uncommon. Reports of frequent proteinuria, hematuria, and mesangial nephropathy are clouded by the inclusion of patients taking a variety of potentially nephrotoxic drugs. Although a few cases of crescentic glomerulonephritis in patients with JCA have been reported, a direct association has not been established (234,235). Renal amyloidosis can occur in JCA in frequencies ranging from as low as 0.1% in North America to 3% to 10% in Europe. There is some evidence that aggressive control of the inflammatory arthritis in patients with amyloidosis can improve outcome. Finally, children with JCA may become hypercalciuric and be at risk for the development of renal stones.

SJÖGREN'S SYNDROME

Sjögren's syndrome is a chronic systemic autoimmune disease characterized by the infiltration of lymphocytes into multiple organs, primarily the exocrine glands (236,237). The salivary and lacrimal glands are most frequently involved, resulting in the clinical symptoms of dry eyes (keratoconjunctivitis sicca) and dry mouth (xerostomia). The presence of these two symptoms together is known as the sicca complex. Although dry eyes and dry mouth are the major features of Sjögren's syndrome, in up to one-fourth of patients other organs also may be affected by the systemic autoimmune process including the lungs, gastrointestinal tract, pancreas, liver, central nervous system, and kidneys. Histologically, the salivary glands are infiltrated with CD4+ T cells and B cells with the salivary epithelial cells expressing high levels of HLA-DR. This and other findings have led to the hypothesis that Sjögren's syndrome may be triggered by an immune response to foreign antigens or altered self-antigens in the exocrine glands (237). Rarely, the lymphoid infiltrates may distort the normal architecture of the organ or of lymph nodes, resulting in a syndrome known as pseudolymphoma (238). When this occurs, the distinction between benign and malignant tissue lesions can be difficult. Patients with Sjögren's syndrome have a 44-fold higher risk for the development of non-Hodgkin B-cell lymphomas in comparison to age- and sex-matched control subjects.

In addition to the local cellular immune response, Sjögren's syndrome is characterized by the presence of autoantibodies to tissue-specific antigens (salivary duct antibodies) and to non–tissue-specific antigens, represented by ANA and RF. Antibodies to two nuclear antigens, SS-A and SS-B, are particularly characteristic of this disease, although they also may be found in other autoimmune diseases, including SLE. Patients with Sjögren's syndrome frequently develop hypergammaglobulinemia and mild anemia indicative of a generalized inflammatory state (237).

Sjögren's syndrome can be broadly divided into two categories. Primary Sjögren's syndrome is diagnosed when the symptom complex exists in the absence of another autoimmune disease. Secondary Sjögren's syndrome can occur in primary biliary cirrhosis or in conjunction with another autoimmune disease such as RA, SLE, or scleroderma. Sjögren's syndrome has been reported to occur in up to 35% of patients with RA and is frequently seen in patients with SLE, mixed connective tissue disease, or SSc. In these diseases, the presence of Sjögren's syndrome is considered to be an additional feature of the underlying autoimmune disease process.

Renal Abnormalities in Sjögren's Syndrome

The renal abnormalities in Sjögren's syndrome arise as a result of either lymphocytic infiltration into the renal parenchyma or immune-complex deposition in the glomeruli (Table 66-4). The renal abnormalities owing to secondary Sjögren's syndrome are often minor in comparison to the renal abnormalities caused by the primary rheumatic disease such as SLE or SSc. For this reason, the following discussion will focus on the renal abnormalities seen in primary Sjögren's syndrome. There is no evidence, however, that the renal abnormalities caused by primary Sjögren's syndrome differ in any way from those of secondary Sjögren's syndrome. It must also be noted that many patients with Sjögren's syndrome are taking medications such as NSAIDs that may further contribute to renal pathology.

Tubular Dysfunction

Histologic studies of the kidneys in patients with Sjögren's syndrome have demonstrated mild to moderate interstitial infiltration of lymphocytes, with varying degrees of tubular

TABLE 66-4

RENAL ABNORMALITIES IN SJÖGREN'S SYNDROME

Tubulointerstitial disease
Hyposthenuria
Renal tubular acidosis
Fanconi's syndrome
Interstitial nephritis
Glomerulonephritis

atrophy and interstitial fibrosis (239). Immunofluorescence studies of the infiltrating cells have indicated that they are CD4+ T and B cells, a pattern identical to that seen in the exocrine glands (240,241). This type of cellular infiltration is different from that seen in many other forms of interstitial nephritis in which cytotoxic T cells are the predominant cell type (240). This finding has led several authors to postulate that the pathogenesis of renal disease in Sjögren's syndrome is similar to that seen in the salivary glands and arises as a direct result of lymphocytic infiltration into the renal interstitium. The frequency with which lymphocytic infiltrates occur in the kidney in Sjögren's syndrome has not been extensively examined. Tu et al. (239) studied nine patients with Sjögren's syndrome and found interstitial changes in six patients. There are numerous other small series of patients with Sjögren's syndrome and abnormal findings on renal biopsy. Although the presence of interstitial infiltrates appears to be common, the exact frequency cannot be determined from the present literature.

Clinically, renal dysfunction in Sjögren's syndrome is usually mild and subclinical. The most frequently reported functional abnormalities are hyposthenuria, renal tubular acidosis (RTA), Fanconi's syndrome, and interstitial nephritis.

Hyposthenuria. In 1962, a patient with Sjögren's syndrome who had nephrogenic diabetes insipidus was reported. Detailed studies on eight additional patients demonstrated that four demonstrated a persistently impaired ability to concentrate urine with water deprivation (242). This defect was not associated with proteinuria or other clinically apparent renal abnormalities. In 1965, Shearn and Tu (243) reported a case of a young woman whose presenting manifestation of Sjögren's syndrome was vasopressin-resistant nephrogenic diabetes insipidus. Her polydipsia and polyuria antedated the symptoms of dryness of the eyes and mouth by 10 years. Although hyposthenuria may exist as an isolated abnormality in Sjögren's syndrome, in many of the reported cases it has been associated with other tubular abnormalities including RTA and aminoaciduria.

Renal Tubular Acidosis. Distal RTA (type I) has been reported to occur in up to 20% to 25% of patients with Sjögren's syndrome (244), and proximal RTA (type II) can also occur (although less frequently). Shearn and Tu (245) used an acid-loading test to detect latent RTA in 10 patients with Sjögren's syndrome who lacked overt abnormalities of acid–base balance. They found impairment of acid excretion in three of the 10 patients studied. Many of these patients also demonstrated a defect in renal concentrating ability. Urinary acidification was impaired in six of 12 patients with Sjögren's syndrome studied by Talal et al. (246). The RTA was clinically evident in three patients, subclinical in two, and associated with glomerulonephritis in one patient. Creatinine clearance was not altered in those patients with RTA. Distal tubular acidosis can be associated with hypocitraturia, renal stones, nephrocalcinosis, and rare cases of osteomalacia. Hypokalemic paralysis and respiratory arrest owing to distal RTA in patients with Sjögren's syndrome have been reported (247–249).

Two studies have examined Sjögren's syndrome patients for the presence of renal tubular acidification defects by ammonium chloride loading (250,251). Shiozawa et al. (250) showed that six of 17 patients with Sjögren's syndrome had abnormal urine acidification, and a higher incidence of abnormal concentrating ability, although this defect was not clinically evident. In this study, the presence of RTA and abnormal concentrating ability did not necessarily correlate with one another, suggesting that Sjögren's syndrome may affect tubular function at multiple functional and anatomic sites.

Hypergammaglobulinemia, which is common in Sjögren's syndrome, has also been associated with RTA (252–255). It is unlikely, however, that the development of RTA in Sjögren's syndrome is simply due to the associated hypergammaglobulinemia. Tubular dysfunction can occur in the absence of hypergammaglobulinemia, and tubular dysfunction is frequently not present despite significant degrees of hypergammaglobulinemia (254,256).

Other Tubular Defects. Fanconi's syndrome (aminoaciduria, glycosuria, and phosphaturia) has been reported in several patients with Sjögren's syndrome (257), although it appears to be uncommon. In Shiozawa's study (250), six of 17 patients with Sjögren's syndrome showed decreased tubular reabsorption of phosphate.

Interstitial Nephritis. Although infiltration of the interstitium with lymphocytes is common in Sjögren's syndrome, severe interstitial nephritis resulting in impaired creatinine clearance is relatively rare. Gentric et al. (258) reported five patients with primary Sjögren's syndrome who developed severe interstitial nephritis with decreased creatinine clearance, proteinuria, and hematuria. Two patients eventually required chronic hemodialysis, but three patients improved after taking corticosteroids. Other cases of this unusual complication of Sjögren's syndrome have recently been summarized (259,260).

Glomerulonephritis

Glomerulonephritis is rare in patients with primary Sjögren's syndrome, with only scattered cases reported in the literature (261–264). The histologic types of glomerulonephritis are usually membranoproliferative, mesangial, or membranous. These patients may present with heavy proteinuria, active urinary sediment, or renal insufficiency. Renal biopsy specimens in patients with Sjögren's-associated glomerulonephritis have shown glomerular deposits of immunoglobulins and C3 on immunofluorescence studies (262,263). It is hypothesized that the pathogenesis is most likely due to deposition of immune complexes formed by cryoprecipitable monoclonal IgM RF along with polyclonal IgG and IgA (263). Cryoglobulinemia, associated with hypocomplementemia, is often found in these patients. There may be a correlation between glomerulonephritis and the development of lymphoma (263). Treatment of the glomerulonephritis with corticosteroids and cytotoxic drugs has been reported to be successful in some patients (261,263). Rare cases of ANCA-positive crescentic glomerulonephritis in patients with primary Sjögren's syndrome have been reported (265).

In summary, some form of renal involvement can occur in 30% to 50% of patients with Sjögren's syndrome. Inability to concentrate the urine, RTA, and other tubular dysfunctions can be demonstrated frequently with stress testing. However, clinically significant tubular dysfunction appears to be unusual in Sjögren's syndrome and severe interstitial nephritis resulting in renal insufficiency is rare. Glomerulonephritis is distinctly unusual. Drug-induced renal abnormalities always should be considered in these patients (see section on Rheumatoid Arthritis), because many patients with Sjögren's syndrome are treated with NSAIDs and other drugs.

POLYMYOSITIS-DERMATOMYOSITIS

The inflammatory myopathies are a heterogeneous group of diseases in which the muscles are diffusely damaged by an endomysial and occasional perivascular infiltration of inflammatory cells, which are predominantly lymphocytes (266). The

clinical syndrome associated with these pathologic changes is termed *polymyositis*. Although this syndrome is frequently pleomorphic in presentation, the cardinal feature in essentially all patients (98%) is muscle weakness. When the syndrome occurs in association with characteristic skin lesions it is termed *dermatomyositis*.

Five clinical or laboratory abnormalities are present in most patients with polymyositis and dermatomyositis and thus are used to define or diagnose the disease (267). The first criterion is symmetric proximal muscle weakness, which is the initial manifestation in up to 69% of patients and is present overall in 98% at some time during the course of the disease. The second criterion is abnormal myopathic electromyographic findings, present in 90% of patients. The third criterion is elevated serum muscle enzymes (creatine kinase, aldolase), found in 95% of patients with myositis. The fourth criterion is the muscle biopsy, which should show evidence of necrosis of type 1 and 2 muscle fibers, areas of degeneration and regeneration of fibers, phagocytosis, and an interstitial or perivascular inflammatory process. The fifth criterion consists of the characteristic skin changes seen in dermatomyositis. These skin lesions include: an erythematous, scaly rash over the face, neck, and shawl area, as well as the extensor surfaces of the knees, elbows, and medial malleoli; a heliotrope rash (a periorbital violaceous discoloration, often with associated edema); and Gottron's papules (scaly erythematous flat plaques over the dorsum of the metacarpophalangeal and proximal interphalangeal joints).

Although a number of different classification schemes of polymyositis-dermatomyositis have been suggested, the scheme proposed by Bohan and Peter and Bohan et al. (267,268) is the most widely used (Table 66-5).

Organ systems other than skin and muscle may be involved in patients with polymyositis or dermatomyositis (266). This is true even when those patients with overlap syndrome (Group V) are excluded. The more common organ systems involved include the joints, with up to 35% of patients experiencing arthralgias. Synovitis can occur but is uncommon. Gastrointestinal manifestations are also common, with involvement of both the proximal and distal esophagus in adults and abdominal crisis secondary to vasculitis sometimes seen in children (266–268). Pulmonary involvement may be found in up to 50% of patients with polymyositis or dermatomyositis, with interstitial lung disease being the most common abnormality (269). Rare cardiac manifestations include electrocardiographic abnormalities and cardiomyopathy.

Renal Disease

Involvement of the kidneys in polymyositis-dermatomyositis is distinctly uncommon and in fact is not mentioned in some reviews or chapters on polymyositis-dermatomyositis in standard textbooks. Early references to renal disease with dermatomyositis have included patients who would now be classified as having mixed connective tissue disease, including SLE or scleroderma in association with myositis (270,271). Dyck et al. (272) identified five patients with primary idiopathic polymyositis who developed proteinuria associated with urinary sediment abnormalities. Renal biopsies from these patients showed a focal mesangial proliferative glomerulonephritis with deposits of both immunoglobulin and complement. After treatment of the polymyositis with corticosteroids, the proteinuria and urinary sediment changes disappeared as the muscle disease improved. These authors speculated that immune complexes might be implicated in the etiology of this renal disease, although results of serum complement studies and serum cryoglobulins were normal in all patients except one. Other rare cases of mesangial and membranous glomerulonephritis have been reported in patients with polymyositis and dermatomyositis (273–276).

Membranous glomerulopathy is reported in up to 10% of all malignancies (277). A case report by Rose (278) describes a 58-year-old woman who presented with the nephrotic syndrome with renal biopsy features of membranous glomerulopathy who subsequently developed dermatomyositis and shortly thereafter an oat cell tumor in her lung. The relationship between these three conditions is unclear, although some interesting speculations are possible. Membranous nephropathy is thought to be an immune complex disease, which in this case may have resulted from the deposition of immune complexes induced by the tumor. Similarly, dermatomyositis is known to exhibit both humoral and cell-mediated abnormalities. Therefore, membranous glomerulonephritis may be seen in conjunction with polymyositis-dermatomyositis because both disorders may be associated with an underlying neoplasm (Group III).

Myoglobinemia and myoglobinuria may be present in up to 50% of patients with dermatomyositis and polymyositis (279). The degree of myoglobinuria is usually mild except in acute, severe disease. Myoglobinuric acute renal failure has been reported in a few cases of polymyositis (280–283).

Childhood dermatomyositis (Group IV) is frequently associated with vasculitis involving the muscles and gastrointestinal tract. Bitnum et al. (284) studied 13 children with dermatomyositis and performed percutaneous renal biopsy in six of these children because of proteinuria. All six of the biopsies were abnormal, showing mild to moderate cellular proliferation of the glomerular tuft. The glomerular basement membrane also was thickened in two patients. The authors were unsure of the implications of their findings but did note that none of the patients developed signs of clinical renal failure. Others investigators have also described diffuse glomerular changes in patients with juvenile dermatomyositis (285). As in the study by Bitnum et al. (284), these authors also found that renal function was maintained, although proteinuria was present in the children.

In summary, renal involvement in polymyositis-dermatomyositis is distinctly uncommon. It may occur in the setting of another connective tissue disease (Group V) in which the renal lesion is found to be that of either SSc or SLE. Membranous glomerulonephritis may occur in association with malignancy (Group III). In acute fulminant myositis, tubular dysfunction or acute renal failure may be associated with myoglobinemia and myoglobinuria. This usually responds to treatment of the myositis and progression to chronic renal failure is rare.

TABLE 66-5

CLASSIFICATION OF POLYMYOSITIS/DERMATOMYOSITIS

Group I	Primary idiopathic polymyositis
Group II	Primary idiopathic dermatomyositis
Group III	Dermatomyositis or polymyositis associated with malignancy
Group IV	Childhood dermatomyositis or polymyositis often associated with vasculitis
Group V	Dermatomyositis or polymyositis associated with another connective tissue disease

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CHAPTER 67 ■ THROMBOTIC THROMBOCYTOPENIC PURPURA, HEMOLYTIC-UREMIC SYNDROME, AND ACUTE CORTICAL NECROSIS

PIERO RUGGENENTI, MICHELE CERUTI, AND GIUSEPPE REMUZZI

HISTORICAL PERSPECTIVE AND DEFINITIONS

Thrombotic thrombocytopenia purpura (TTP) and hemolytic-uremic syndrome (HUS) have been considered to be indistinguishable or separate entities. No pathologic feature is specific to one of the two entities, but they actually have different clinical manifestations. Several research studies have been performed in attempt to discover new parameters or methodological approaches for the distinction of the two entities. Hemolytic-uremic syndrome has been defined as the consequence of some infections due to an *Escherichia coli* strain. Conditions in which cerebral symptoms predominated were thought to be more specific for TTP, but HUS has been described with the same pathologic features. Familial forms of both entities have been discovered and studied. Thrombotic thrombocytopenia purpura or HUS has been found in nonhereditary subjects as adverse effects of some drugs. Posttransplantation forms have been found in subjects with different underlying pathologies.

Recently, a new paradigm of distinction has been proposed according to the plasma activity of a von Willebrand factor protease. The decrease of this parameter may be associated with the diagnosis of TTP, but a significant number of patients with clinical features of HUS has been found with the same laboratory finding.

Thus, there are forms that, regardless of the underlying etiology, cannot be definitively assigned to TTP or to HUS. Consequently, it may be better to use the broader term of thrombotic microangiopathy (TMA). This form defines lesions, common to both TTP and HUS, characterized by vessel wall thickening (mainly arterioles or capillaries), with endothelial cell swelling and/or detachment from the basement membrane with accumulation of fluffy material in subendothelial space, intraluminal platelet thrombosis, and partial or complete obstruction of the vessel lumina. Thrombotic microangiopathy was introduced in 1952 by Summers to replace thrombotic thrombopenic purpura and revived in 1979 by E. Rossi. It provides a natural link between the two syndromes and is still the best term to encompass all of the lesions just described.

A classification into subgroups may be performed according to laboratory and genetic findings with no aim to overlap old classifications that distinguished TTP and HUS according to clinical features. The new classification attempts to distinguish TMA subgroups according to genomics and molecular biologic findings.

The term thrombotic thrombocytopenic purpura (TTP) was first introduced in 1925 by Moschcowitz (1), who described a 16-year-old female patient with a fulminant febrile attack, hemolytic anemia, bleeding, renal failure, and neurologic involvement. Pathologic changes were characterized by widespread hyaline thrombosis of small vessels. In Moschcowitz's view, thrombi were constituted by agglutinated hyalinized erythrocytes. The disease was believed to be caused by a powerful "toxin that has both agglutinative and hemolytic properties" (1). Since then and up to 1980 almost 500 cases of TTP have been reported in the literature, and several criteria have been proposed for the diagnosis (2–4). The term TTP should be used when two of the three major criteria (thrombocytopenia, microangiopathic anemia, and neurologic involvement) are associated with two minor criteria (fever, renal changes, and presence of thrombi in the circulation) (5). The term hemolytic-uremic syndrome (HUS) is most commonly used when renal changes result in severe renal insufficiency. Hemolytic-uremic syndrome is more frequent in children and TTP in adults, but this is not a general rule (1,3,6–8). The same patients may be classified as having TTP or HUS during different episodes of disease exacerbation (9–11). There are also cases with severe renal failure that have been classified as TTP, and cases of HUS often have neurologic symptoms (12,13).

Idiopathic TTP has been separated from secondary TTP (5). TTP may occur in pregnancy or may complicate the postpartum period (14,15). Cases of TTP have been discovered in association with neoplastic disorders such as lymphoma (16), Sjögren's syndrome (17), rheumatoid arthritis (18), polyarteritis (19), and systemic lupus erythematosus (SLE) (20,21). Thrombotic thrombocytopenic purpura has been recognized in patients with endocarditis (22) or after treatment with sulfonamide (23,24), iodine (25), oral contraceptives (26), and some poisons (27).

The disease is more common in women, with a female to male ratio of 3:2 (3). Although the peak incidence occurs in the third decade of life, cases of TTP have been described in patients ranging from 1 to 90 years (2,28). Recurrent episodes of TTP are not exceptions, but relapses cannot be predicted by any clinical or hematologic feature (29–31). Based on cases of TTP occurring in siblings, a genetic predisposition has been suggested, and some reports indicate that there is a possibility that an autosomal recessive trait underlies TTP (32–34).

However, there are forms that, regardless of the underlying etiology, cannot be surely assigned to TTP or to HUS. In such cases, it may be better to use the broader term of TMA. As already noted, TMA defines histopathologic lesions common to both TTP and HUS. These histopathologic changes are almost invariably accompanied by thrombocytopenia and hemolytic anemia. Thrombocytopenia derives from platelet consumption in the microcirculation. The reason of hemolytic anemia is not as clear, but it may be a consequence of the mechanical fragmentation of erythrocytes during flow through partially

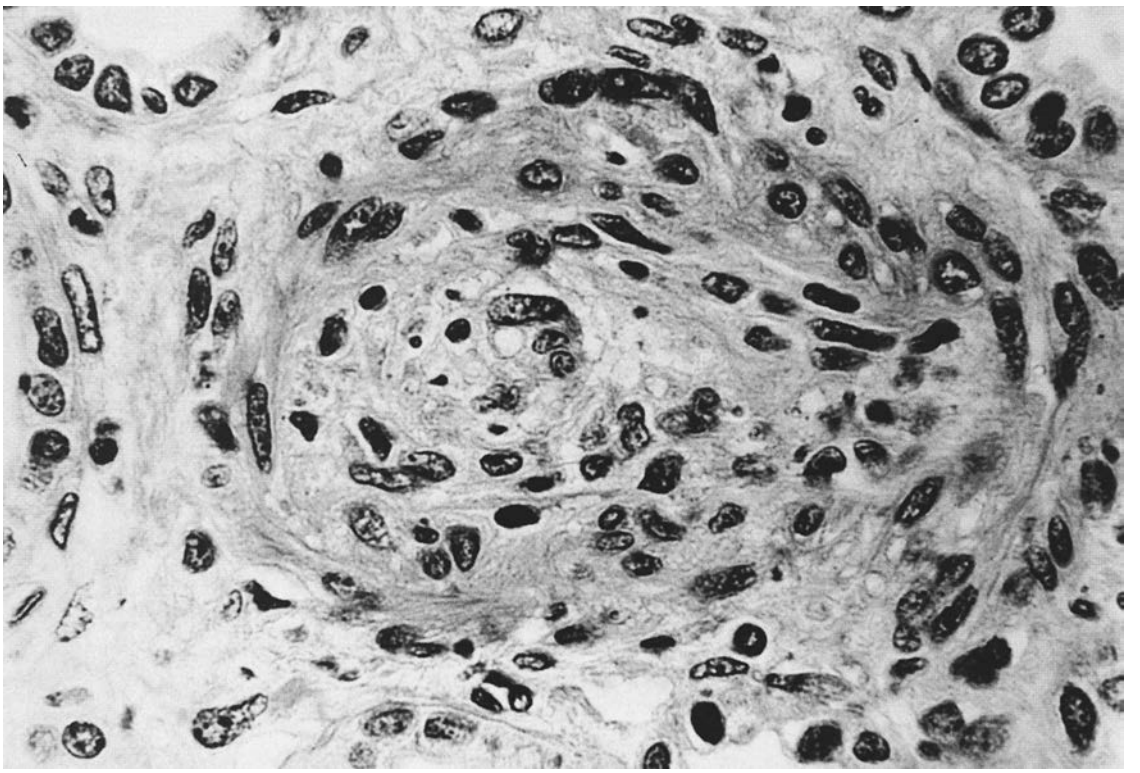

FIGURE 67-1. Marked endothelial and myointimal cell proliferation with occlusion of the lumen of an interlobular artery in a case of thrombotic thrombocytopenic purpura (trichrome, ×375).

occluded microvessels. Peripheral smear shows fragmented red blood cells with the typical aspect of burr or helmet cells. Elevations in lactate dehydrogenase (LDH) and low or undetectable haptoglobin levels are markers of hemolysis (2). Reticulocyte count is almost invariably elevated. Coomb's test is negative. Prothrombin time, partial thromboplastin time (PTT), factor V, factor VIII, and fibrinogen are normal in most cases. In some patients, high levels of fibrin degradation products and prolonged thrombin time have been observed

PATHOLOGY

Thrombotic Thrombocytopenic Purpura

The typical pathologic changes of TTP are the thrombi that occlude capillaries and arterioles in many organs and tissues (35,36). These thrombi are constituted by fibrin and platelets, and their distribution is widespread. They are most commonly detected in kidneys, pancreas, heart, adrenals, and brain (35,37,38). Compared to HUS, pathologic changes of TTP are more extensively distributed, probably reflecting the more systemic nature of the disease.

Other types of lesions have been described in TTP; the subendothelial accumulation of hyaline material has led to the term prethrombotic lesions (35), and frequently microaneurysms (36) or glomeruloid structures (10) are found. Altogether thrombi, prethrombotic changes, aneurysmal dilation of vessels, and glomeruloid structures have been considered typical of TTP, but recent studies cast doubt on the specificity of these lesions (38,39). Recently, it has been observed that gingival biopsies and bone marrow clot sections have the highest percentage of positivity for microthrombi (39,40).

Fibrin has been detected in the thrombi by immunofluorescence (41–43). Although the presence of platelets in the thrombi has been shown by electron microscopy (41), staining with antiplatelet sera is often negative (41,42,44). C3, C1q, and immunoglobulin deposits have been occasionally detected (42,45). In the kidney, fibrin–platelet thrombi are more frequent in arterioles than in glomerular capillaries (46). Reflecting the more chronic and relapsing character of the disease, proliferation of endothelial and myointimal cells (Fig. 67-1) is more pronounced in TTP than in HUS (47).

Hemolytic Uremic Syndrome

The kidneys are the main target organs involved in HUS. Pathologic changes originally described by Gasser et al. (48) in children with HUS were consistent with a picture of cortical necrosis. Since then, lesions of HUS have been described in more detail (49–53), and a term that successfully defines the characteristic pathologic changes of HUS is thrombotic microangiopathy (54). The classic lesion is characterized by thickening of the capillary walls secondary to the presence of a fluffy material in the subendothelial space (53,55,56). These changes, plus occasional thrombi, lead to occlusion of the glomerular capillary lumen. In the vessels from the tissue section, fragmented red blood cells or cells at different degrees of hemolysis may be detected (Fig.67-2). In the acute phase, arteriolar and capillary thrombi are more common, whereas the subendothelial fluff is the dominant lesion seen days or weeks after the acute attack. A widening and fibrillar appearance of the mesangium coexists with the glomerular capillary wall alterations (54,57). The vascular lesions may be differently distributed (55,58,59), and the percentage of affected glomeruli may influence the prognosis (59).

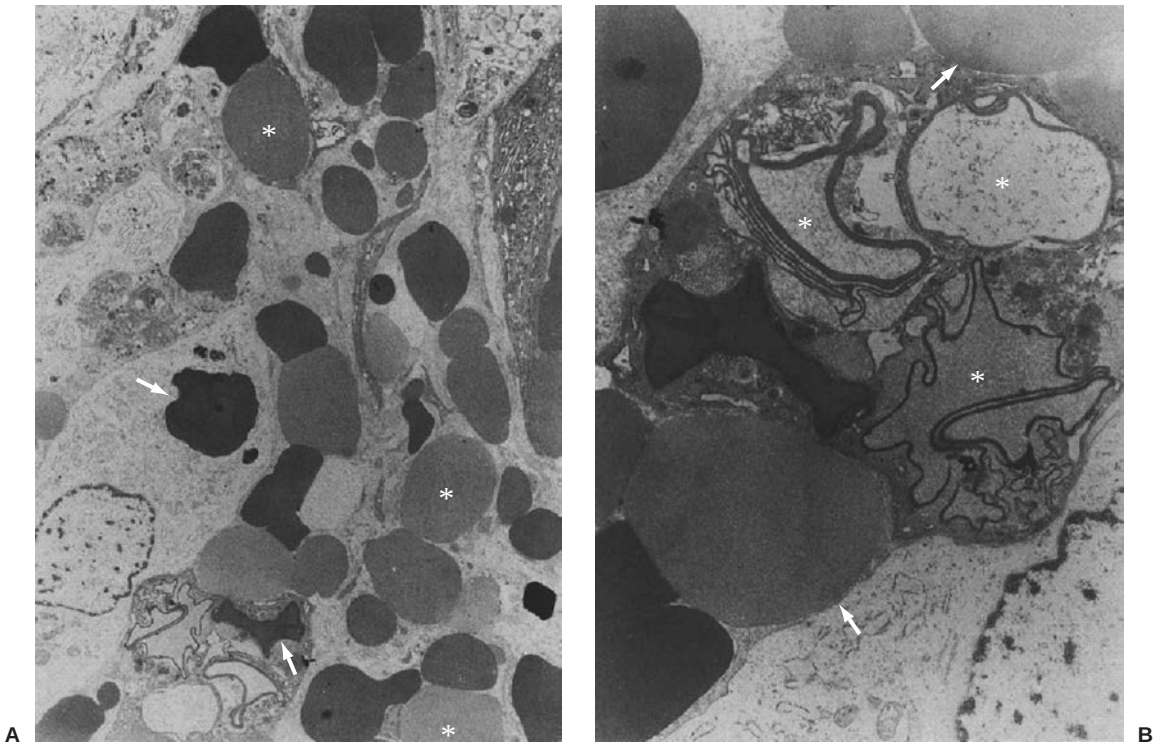

FIGURE 67-2. A: Electron micrograph showing fragmented red blood cells (*arrows*) and red cells at different stages of hemolysis (*asterisks*) in a small renal vein from a patient with hemolytic uremic syndrome (×5,000). **B:** High magnification of inset of Figure 67-2A showing red blood cells at different stages of hemolysis (*arrow*) and the presence of "ghosts" of red blood cells (*asterisks*) in a small renal vein from a patient with hemolytic-uremic syndrome (×12,000). (Both micrographs courtesy of Dr. C. L. Pirani and Dr. V. D'Agati.)

The finding of pure thrombotic microangiopathic lesions derives mainly from studies in children, especially children younger than the age of 2 years. However, in older children and adults, the histologic pattern is usually more complex and is characterized by a prevalence of arterial involvement with necrosis and thrombosis of interlobular arteries associated with ischemic changes in glomeruli.

Three patterns of renal lesions can be found in HUS—one with prevalent glomerular lesions of the microangiopathic type, one with predominant arterial involvement, and a third with simultaneous glomerular and arterial involvement. Other tentative classifications for HUS have been proposed (59). Recently, Thoenes and John (60) proposed the term endotheliotropic hemolytic nephroangiopathy. In the opinion of the authors, this term, in addition to including the main clinical features of the disease, also emphasizes the decisive pathogenetic role of endothelial damage. To emphasize the pathogenetic role of vascular changes in HUS, Bohle et al. (61) used the definition primary malignant nephrosclerosis.

A detailed analysis of renal changes in younger children includes swelling of glomerular endothelial cells leading to narrowing or complete occlusion of glomerular capillary lumens (Fig.67-3). Glomerular capillary walls are thickened, and often the endothelial cells are separated from the glomerular basement membrane (GBM). A fluffy material accumulates in the subendothelial space, giving a double-contoured appearance to the glomerular capillary walls (Fig.67-4). Together with the swelling of the endothelial cells, the widening of the subendothelial space contributes to the occlusion of the glomerular lumen. Thrombi having the characteristic staining for fibrin are occasionally seen. Mesangial matrix has a fibrillar appearance without any sign of mesangial hypercellularity. Occasionally red blood cells and platelets are seen in the glomerular capillary lumens. Not all glomeruli are equally affected. In some cases damage is more severe, whereas in others less-pronounced changes (see Fig 67-4) are found. Arterial involvement is extremely variable, but some widening of the subendothelial space containing fluffy material is observed.

In older children and in adults, the major changes are restricted to the arteries (59,62). Interlobular arteries are often occluded by thrombosis, necrosis, or intimal edema or proliferation (62) (Figs. 67-5 and 67-6). In the more chronic phases, fibroplasia of small arteries and arteriosclerotic changes are seen. In some cases glomeruloid structures of the small arteries are observed, probably as a result of endothelial and myointimal proliferation of small arteries adjacent to the glomeruli (47). Glomerular lesions are ischemic, as shown by the thickening and wrinkling of the glomerular capillary walls, by atrophy of the glomerular tufts, and by thickening of Bowman's capsule (Fig.67-7). In a small number of patients a histologic pattern of cortical or tubular necrosis is detected (59). Not infrequently, however, the pattern of glomerular involvement coexists in the same patient with significant arterial damage (58,59).

Immunofluorescence shows fibrinogen almost invariably present in the glomeruli, along the glomerular capillary walls, and in the thrombi and vessel lumina (63). Granular deposits of C3 and immunoglobulin M (IgM) are also found in glomeruli and vessels (64,65). Ultrastructural injury to glomerular endothelial cells is pronounced. Endothelial cells are swollen and are often detached from the underlying GBM (Fig. 67-8). Beneath, the endothelium is an electron-lucent fluffy material that may be associated with a thin, newly formed GBM (Fig. 67-9). The composition of this fluffy material is not known, but it has been suggested that it might consist of fibrinogen–fibrin

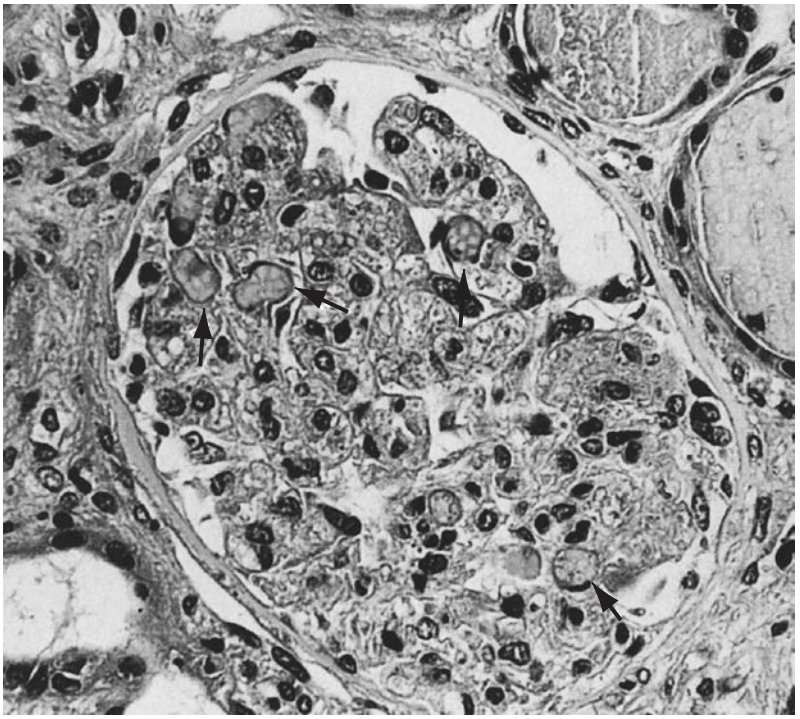

FIGURE 67-3. Swelling of glomerular endothelial cells and occlusion of almost all capillary lumens packed with red blood cells (*arrows*) in a case of hemolytic-uremic syndrome. (Trichrome, ×250.)

breakdown products as well as fibronectin (47). Glomerular capillary lumens are markedly narrowed by the combination of endothelial and subendothelial changes. Foam cells can be detected within the glomeruli, generally in the late stages of the disease, and contain lipid droplets and myelin figures. Glomerular mesangium may be the site of fibrin deposits, but the most common lesion is edema of the mesangial matrix, leading to marked reticulation and even complete dissolution, called "mesangiolysis" (66). Endothelial damage predominates in arteries and arterioles, which demonstrate detachment of endothelium, swelling of endothelial cells, and subendothelial fluffy deposits.

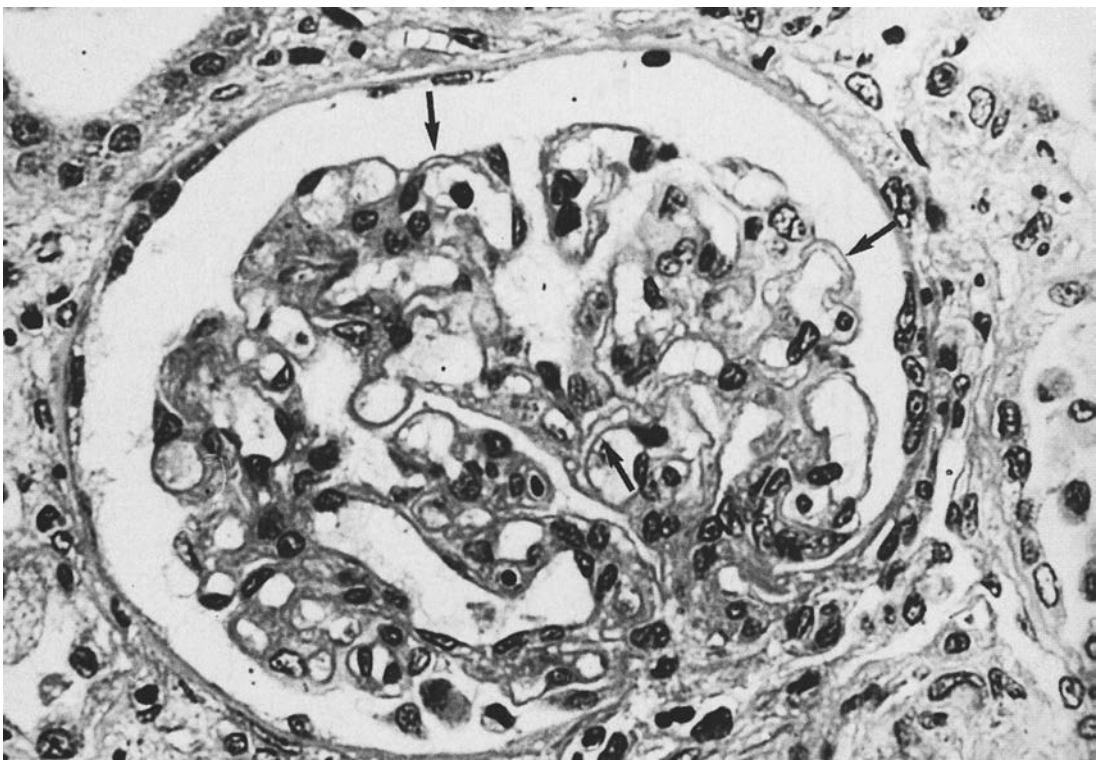

FIGURE 67-4. Double tracks (*arrows*) in some areas of glomerular capillary walls of a patient with hemolytic-uremic syndrome. (Trichrome, ×375.)

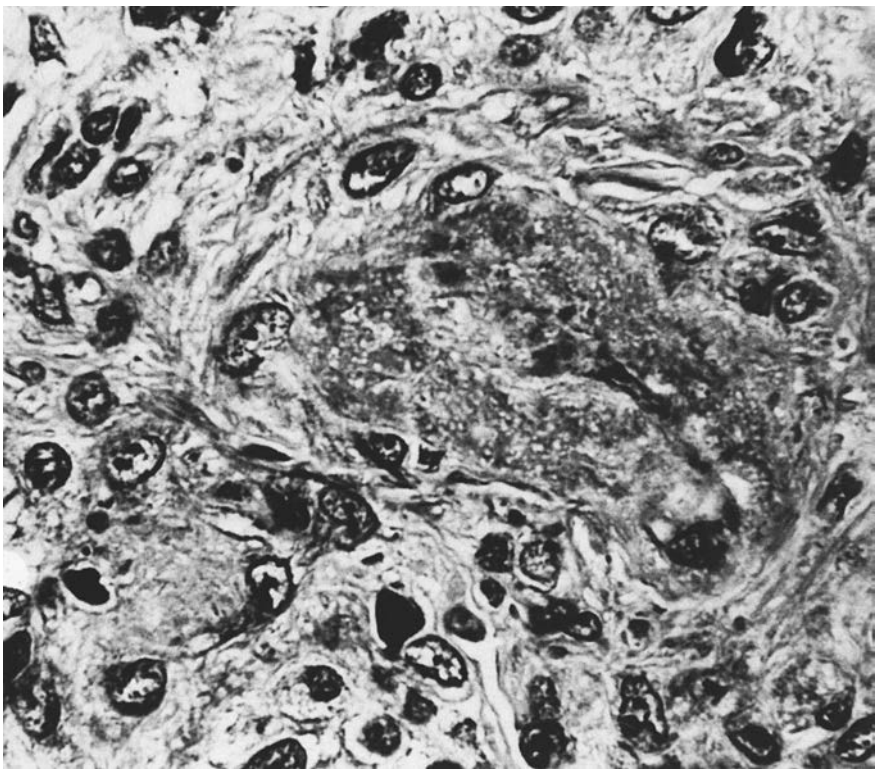

FIGURE 67-5. Thrombotic and necrotic changes in a small artery from an adult patient with hemolytic-uremic syndrome. (Trichrome, ×375.)

PATHOGENETIC MECHANISMS

The Central Role of Endothelial Damage

Toxins, immune reactants, and drugs may cause endothelial damage, which, in turn, may result in decreased prostacyclin bioavailability, increased shear stress, and oxidative stress that contribute to amplify the damage in a vicious circle resulting in microvascular thrombosis.

Toxins

Shigatoxin. In 1978, Koster et al. (67) found an association between Shigella dysenteriae type 1, severe colitis, and children with HUS, which is now reported quite frequently in India (68,69) where HUS is epidemic). Further studies (70) found that *E. coli* can produce exotoxins related to the 70-kDa protein encoded in *S. dysenteriae* DNA. In 1983, Riley et al. (71) reported the association of *E. coli* (serotype O157:H7) infection and two outbreaks of hemorrhagic colitis, and a paper in *The Lancet* described verotoxin-producing *E. coli* (VTEC)

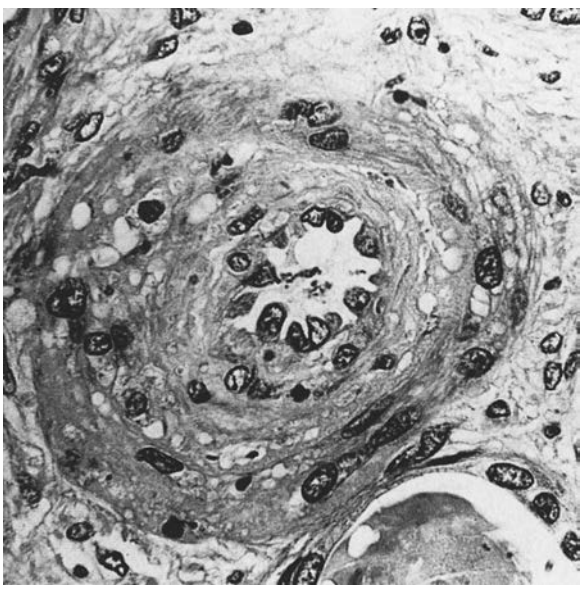

FIGURE 67-6. Occlusion of an interlobular artery with intimal swelling and myointimal proliferation in a case of adult hemolytic-uremic syndrome. (Trichrome, ×375.)

FIGURE 67-7. Ischemic glomerular lesions characterized by thickening and wrinkling of glomerular capillary walls and atrophy of glomerular tuft in a case of adult hemolytic-uremic syndrome. (Silver, ×250.)

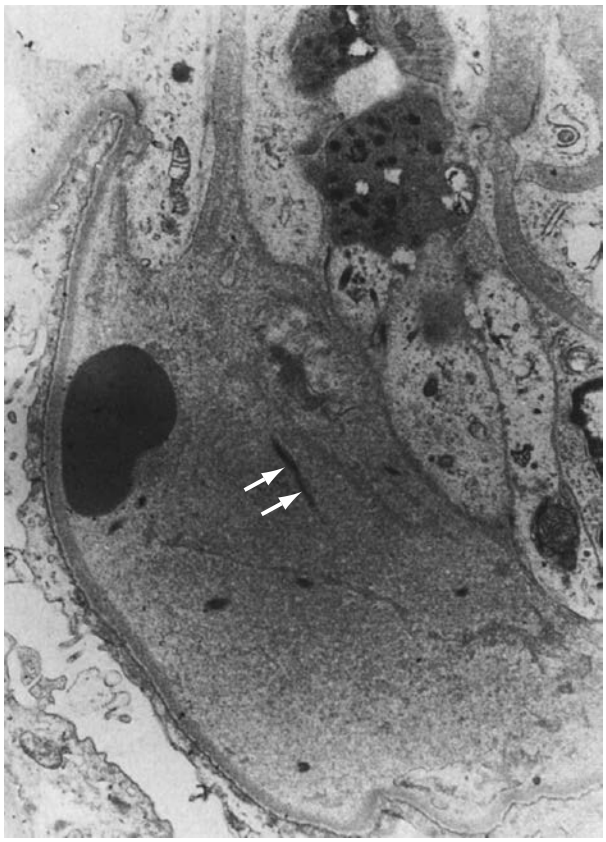

FIGURE 67-8. Detachment of endothelial cell from the underlying glomerular basement membrane in a case of hemolytic-uremic syndrome. A red blood cell is in close contact with the glomerular basement membrane. Electron-lucent "fluffy" material and a few strands of fibrin (*arrows*) are present in subendothelial space (×7,000). (Courtesy of Dr. C. L. Pirani and Dr. V. D'Agati.)

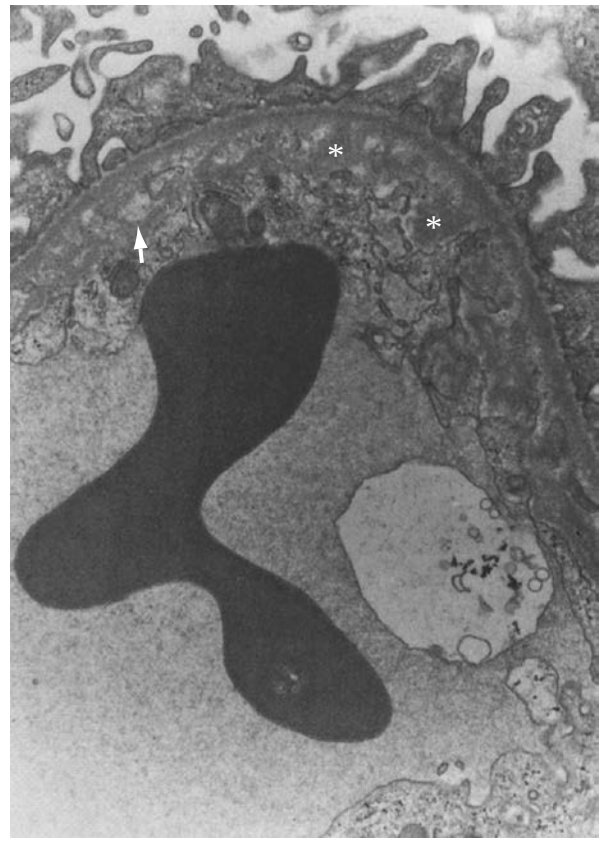

FIGURE 67-9. Electron-lucent "fluffy" material (*arrow*) with some electron-dense deposits (*asterisks*) is located between the cytoplasm of an endothelial cell and the glomerular basement membrane in a segment of glomerular capillary from a patient with hemolytic-uremic syndrome (×12,000). (Courtesy of Dr. C. L. Pirani and Dr. V. D'Agati.)

infection (72) in 11 of 15 cases of sporadic HUS. From now on, only the term Shigatoxin (or Stx) should be used to name exotoxins produced both *S. dysenteriae* and by *E. coli*, and the other terms such as Shiga-like toxin, verotoxin, or VTEC should be abandoned (73).

Humans may be infected from contaminated milk, meat, and fecally contaminated water, or from contact with infected animals, humans, or the excreta of either (74–76). Rowe et al. (77) found that undercooked meat and foods are not necessarily implicated in the transmission of the disease, which is more likely to occur in patients who have been exposed to a family member with diarrhea in the 7 days before the onset of symptoms than in healthy controls. This suggested a person-to-person transmission of Stx-producing *E. coli* that is also a plausible explanation for most cases of sporadic HUS (78–81). It can be estimated that in North America and western Europe about 90% of children with HUS have some evidence of Stx infection, with the serotype O157:H7 in 70% of cases (82–85). O157:H7 serotype is not common in Argentinian cases of Stx-induced diarrhea (but without hemorrhagic colitis) complicated or not by HUS (86). This would suggest that Stx of serotypes other than O157:H7 is mostly associated with nonbloody diarrhea.

Following ingestion, *E. coli* and *S. dysenteriae* localize on the colonic mucosa, invade epithelial cells, multiply, and cause cell death. (The action of such organisms normally gives diarrhea), but only strains that produce Stx's damage the vasculature of the mucosa so as to cause hemorrhagic colitis (87). Shigatoxins are picked up by polarized gastrointestinal cells via transcellular pathways and translocate into the circulation, probably facilitated by the transmigration of polymorphonuclear cells (PMNs), which increase paracellular permeability (88,89).

Although it has been shown *in vitro* that Stx can bind to human erythrocytes, platelets, and activated monocytes, recent studies have underpinned a role for PMN in Stx carriage in blood, since Stx rapidly and completely binds to PMN when incubated with human blood (90). Consistently, circulating PMN-bound Stx has been detected in blood of Stx-HUS patients (91). Moreover, PMN Stx receptor has a 100-fold lower affinity to the ligand than the high-affinity receptor expressed on glomerular endothelial cells, thereby facilitating the intercellular transition. Actually, *in vitro* and in co-cultures, PMN loaded with Stx transfer the ligand to glomerular endothelial cells so that at the end of the incubation Stx molecules were found on glomerular endothelial cells but no longer on PMN (90).

The human disease is caused by two distinct exotoxins—Stx-1 and Stx-2—almost identical to the toxin produced by *S. dysenteriae* type 1 (92). Stx DNA is incorporated into the genoma of a restricted number of some *E. coli* O:H serotypes, which can produce Stx-1 alone or Stx-2, or both. Both Stx-1 and Stx-2 are 70-kDa AB5 holotoxins comprising a single A subunit of 32 kDa and a five 7.7-kDa A subunit. Interestingly, a new AB5 toxin comprising a single 35-kDa A subunit and a pentamer of 13-kDa B subunits have been recently isolated from a highly virulent *E. coli* strain (O113:H21) responsible for an outbreak of HUS, which may represent the prototype of a

new class of toxins, accounting for HUS associated with strains of *E. coli* that do not produce Stx's (93). Despite their similar sequences, Stx-1 and Stx-2 cause different degrees and types of tissue damage as documented by the higher pathogenicity of strains of *E. coli* that produce only Stx-2 than of those that produce Stx-2 alone (94–96). In a recent study in children who became infected by Stx-*E. coli*, *E. coli* strains producing Stx-2 were most commonly associated with HUS, whereas most strains isolated from children with diarrhea alone or remaining asymptomatic only produced Stx-1 (97). This is also true in mice and baboons (98).

Stx-1 and Stx-2 bind to different epitopes on the Gb3 molecules and they also differ in binding affinity and kinetics (99). Surface plasmon resonance analysis showed that Stx-1 easily binds to and detaches from Gb3, in contrast to Stx-2, which binds slowly but also dissociates very slowly, thus leaving time enough for the cell's incorporation (99).

After binding to endothelial cell receptors, the toxin is internalized in the cytosol by endocytosis (100) within 2 hours (101) and inhibits protein synthesis within 30 minutes (Fig. 67-10). The number of high-affinity receptors is a major determinant of susceptibility of cells to Stx (102). Therefore, cell viability and protein synthesis of endothelial cells of the kidney were reduced by 50% upon exposure to 1 pM Stx, unlike endothelial cells of umbilical vein that were viable up to greater than 1 nM exposure to the toxin. These findings are consistent with basal levels of Stx receptors 50 times higher in renal endothelium than in the umbilical cord endothelium (103). During internalization, the alpha subunit of the toxin dissociates from the beta subunits. Approximately 10% of the alpha subunit protein is removed in a trypsinlike process, resulting in a maximally active 27-kDa subunit enzyme. It is well established that this fragment is a direct inhibitor of protein synthesis and is responsible for the cytotoxic action of the toxin. Stx selectively inactivates 60S ribosomal subunits by removing one nucleotide in the 28S ribosomal RNA in a nucleotide-specific manner (104).

For many years it was assumed that the only relevant biologic activity of Stx was the block of protein synthesis and destruction of endothelial cells. Recently, however, it has been shown that treatment of endothelial cells with sublethal doses of Stx, exerting minimal influence on protein synthesis, leads to increased mRNA levels and protein expression of chemokines, such as interleukin (IL)-8 and monocyte chemoattractant protein-1 (MCP-1) and cell adhesion molecules, a process preceded by NF-kB activation (105). Analysis of genome wide expression pattern of human endothelial cells stimulated with sublethal doses of Stx evidenced 25 and 24 genes upregulated by Stx-1 and Stx-2, respectively, mostly encoding for chemokines and cytokines, cell adhesion molecules, including P-selectin and intercellular adhesion molecule 1 (ICAM-1), and transcription factors (EGR-1, NFkB2, and NFkBIA) (106).

Adhesion molecules seem to play a critical role in mediating binding of inflammatory cells to endothelium. This is supported by adhesion experiments under flow showing that Stx-2 treatment enhanced the number of leukocytes adhering and migrating across a monolayer of human endothelial cells (107). Moreover, preventing IL-8 and MCP-1 overexpression by adenovirus-mediated NF-kB blocking, inhibited adhesion and transmigration of leukocytes (105).

Therefore, it can be inferred that Stx, by altering endothelial cell adhesion properties and metabolism, favor leukocyte-dependent inflammation and induce loss of thromboresistance

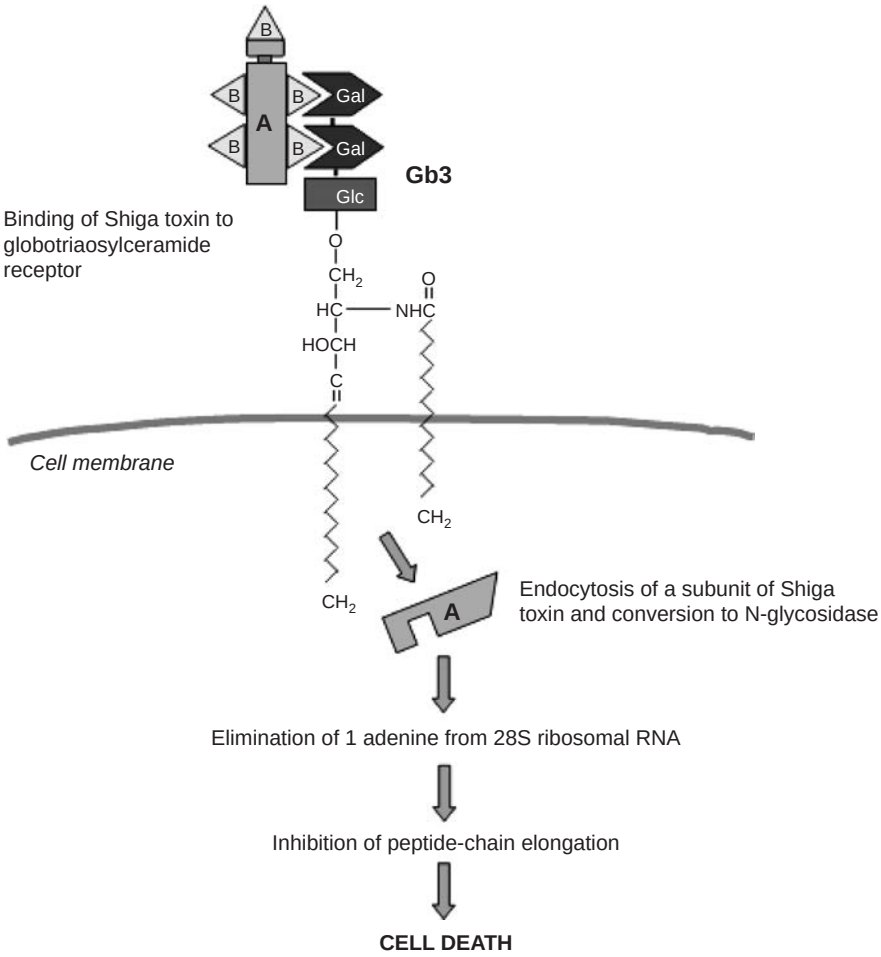

FIGURE 67-10. Binding and mechanism of action of Shiga-like toxin. The B subunits of Shiga toxin molecules attach to galactose (gal) disaccharides of globotriaosylceramide (Gb3) receptors on the membrane of monocytes, polymorphonuclear cells, platelets, glomerular endothelial cells, and tubular epithelial cells. The toxin is internalized via retrograde transport through the Golgi complex. Then the A and B subunits dissociate, and the A subunit is translocated to the cytosol. The A subunit blocks peptide chain elongation by eliminating one adenine from the 28S ribosomal RNA.

in endothelial cells, leading to microvascular thrombosis. Evidence for such sequence of events has been obtained in experiments of whole blood flowing on human microvascular endothelial cells, pre-exposed to Stx-1, at high shear stress (108). In these circumstances early platelet activation and adhesion occurs, followed by formation of organized endothelial P-selectin and platelet-endothelial cell adhesion molecule (PECAM)-1-dependent thrombi. This offers a likely pathophysiologic pathway for microvascular thrombosis in HUS. This report may also be taken as a demonstration of a link between bacteria and their products and arterial thrombosis, as suggested by the evidence of *in vivo* coagulation disturbances, that is, increase of prothrombin fragment 1+2, found in children who developed HUS upon infection with *E. coli* O157:H7 (109–111). Although early studies suggested that fibrinolysis is augmented in Stx-HUS, more recent work revealed the presence of higher than normal levels of plasminogen-activator inhibitor type 1, indicating that fibrinolysis is substantially inhibited (111).

Endotoxins. Endotoxemia is often associated with HUS (112), which would suggest that endothelial cell damage is the result of the toxic effect of Stx, added to that of endotoxin (113). Lipopolysaccharide (LPS, endotoxin) may combine with Stx to amplify its cytotoxic potential. Such a synergism was dose-dependent on umbilical vein and renal endothelial cells in culture and was maximal after preincubation of cells with endotoxin (114). A similar synergism has also been demonstrated between Stx and tumor necrosis factor-α (TNF-α) (113). This increase occurs mainly via TNF receptor 55 upon protein kinase C activation (115). Of interest to understanding the pathogenesis of renal lesions in HUS are findings that Stx induces TNF-α biosynthesis in the kidney, but not in other organs or tissues (116).

TNF-α, IL-1 and IL-6 have been demonstrated to upregulate the expression of globotriaosylceramide on renal endothelial cells and increase the binding of Stx-1 (108,117). According to recent studies, Stx-1 may induce the endothelial cells to secrete unusually large multimers of von Willebrand factor and enhance the expression of vitronectin ($\alpha v\beta 3$ integrin) receptors, P-selectin, and PECAM-1 on cell surfaces (108). Moreover, in another study (118) Stx-1 was shown to bind to and activate platelets through globotriaosylceramide or globotriaosylceramide-like receptors.

Yamada et al. (119) documented that endotoxin requires the presence of granulocytes to damage endothelial cells in culture. Because the damage could be reduced by suppressing granulocyte adhesiveness or by oxygen free-radical scavengers, the authors concluded that cytotoxic activity of endotoxin could be due in part to its capability of promoting granulocyte adhesion to endothelium and stimulating granulocyte production of toxic oxygen radicals. Moreover, neutrophils from children with diarrhea-associated HUS have an enhanced capacity to adhere to endothelial cells and cause injury (120).

Meyrick (121), using both *in vitro* and *in vivo* models, showed that endotoxin can cause a direct injury to pulmonary endothelium and that this damage is enhanced by activated complement and granulocytes by their interaction with the altered endothelium. In more recent years data became available indicating that similar to cytokines such as TNF and IL-1, endotoxin induces a dramatic increase in endothelial cell adhesiveness for leukocytes by stimulating the expression of endothelial cell adhesion molecules. Specifically, endotoxin acts directly upon endothelium *in vitro* to induce the expression of E-selectin, ICAM-1, and P-selectin (122–124). Munro et al. (125) showed that endotoxin injection into baboon skin resulted in the *de novo* endothelial expression of E-selectin, which was associated with concurrent extensive adhesion and extravasation of neutrophils. In another model in which endotoxin was administered by intravenous injection to rats, Coughlan et al. (124) documented a rapid onset of neutropenia in association with induction of P-selectin expression on microvascular endothelial cells in kidney, liver, and lung. Treatment with an antibody to P-selectin blocked neutropenia and neutrophil accumulation in tissues.

Neuraminidase

Neuraminidase, produced by some bacteria and viruses, also can damage vascular endothelium. Klein et al. found that neuraminidase, by removing sialic acids from the cell membranes exposes the Thomsen-Friedenreich receptor present on glomeruli, red cells, and platelets (127). The formation of an anti-Thomsen-Friedenreich IgM antibody would promote agglutination of red cells and platelets.

Immune Reactants

Antibodies (128–132) and immune complexes (133–135) can induce endothelial injury and trigger massive sequestration of platelets and polymorphonuclear leukocytes in the microvasculature (131), as in acute allograft rejection in humans (132). It is likely that circulating antibodies or immune complexes or both play a pathogenetic role in the development of thrombotic microangiopathies in the course of connective tissue disorders, such as SLE (20,21), Sjögren's syndrome (17), rheumatoid arthritis (18), polyarteritis (19), the most frequent association being with SLE, although the percentage incidence varies in the different reports (136,137).

Occasional patients with TTP have cytotoxic antibody to cultured endothelial cells *in vitro* (138,139). This cytotoxicity is complement dependent and related to the IgG fraction of immunoglobulins. That autoimmune-mediated endothelial damage may be the cause of microvascular injury is supported by the findings of Leung et al. (140), who described the consistent presence of cytotoxic anti-endothelial cell antibodies in the serum of patients in the acute phase of TMA. The same authors found that sera from 13 of 14 children with acute HUS contained complement-fixing IgG and IgM antibodies that specifically injured cultured human umbilical vein endothelial cells. The endothelial cell antigen was lost after treatment of the cells with gamma interferon. In contrast, only 3 of 5 adult patients with acute, nonrelapsing TTP had lytic anti-endothelial antibodies, and only one of these recognized an antigen lost upon treatment with gamma interferon. None of 32 control sera contained lytic anti-endothelial cells antibodies. These data suggest that, at least in some children, HUS may involve a disorder of immunoregulation so that a unique class of anti-endothelial cell antibodies is detectable in such patients. The possibility of an antibody mediated injury to endothelial cells is supported by the findings of Platt et al. (141) who recently showed that exposing cultured porcine endothelium to human serum as a source of natural antibodies and complement caused cleavage and release of endothelial cell proteoglycans that preceded irreversible cell injury. These *in vitro* findings might be interpreted to indicate that the loss of endothelial cell proteoglycan is critical to the pathogenesis of vascular damage and that stimuli other than endotoxins can also induce endothelial injury.

Experimental endothelial damage can also be induced by immune complexes. A model of endothelial damage probably due to immune-complex deposition has been described in rabbits treated with BSA, which classically induces acute serum sickness (133,134). In this model, endothelial damage is probably mediated by basophil and platelet activation. These cells release vasoactive amines responsible for increasing vascular permeability and facilitating the further localization of immune complexes at the level of endothelial injury. In these animals there is extensive endothelial destruction and exfoliation with

fibrin deposits in the glomerular capillaries and mesangium (133).

Hyperacute renal allograft rejection and acute serum sickness are examples of immunologically mediated endothelial damage. Hyperacute rejection of the kidney is mediated by secondary injury following antigen–cytotoxic antibody reaction at the interface of the recipient's blood and the donor's endothelium (129,130). The earliest event after fixation of antibodies and complement to the endothelium is believed to be massive sequestration of platelets and, to a lesser extent, of polymorphonuclear leukocytes in the microvasculature of the graft (131,132). Widespread microthrombosis with concomitant vasoconstriction is a crucial event leading to vascular occlusion and ischemia (142). The endothelial injury has been observed in several experimental models, such as hyperacute cardiac allograft rejection and skin allograft rejection; information obtained from rejection of these organs has greatly helped in our understanding of the mechanisms and targets of renal rejection. Dvorak et al. (143), in a model of skin allograft rejection, found diffuse microvascular damage preceding infarction of skin allograft. Vascular damage associated with hypertrophy, necrosis, and sloughing of endothelial cells was the major cause of skin graft rejection. Forbes et al. (144), studying cardiac allograft rejection in the rat, observed extensive endothelial injury that was complement dependent and associated with platelet aggregation.

Drugs

About 200 cases of TMA have been described in the course of neoplastic diseases and appear to depend on the use of some anticancer drugs, such as mitomycin, vinblastine, cisplatin, bleomycin, cytosine arabinoside, and daunorubicin (145–151). Cancer-associated TMA has been reported in 4% to 15% of patients given mitomycin. Most patients develop TMA after cumulative doses that exceed 60 mg (151). Mytomycin added to cultured endothelial cells of human umbilical cord vein inhibits prostacyclin production and cell proliferation in a dose-dependent manner (152). Perfusion of the renal artery *in vivo* also induces structural abnormalities reminiscent of HUS, which include endothelial injury followed by platelet deposition, subendothelial expansion, and obliteration of arterioles and capillaries (153). Immunotherapeutic drugs, namely cyclosporine, tacrolimus, OKT3, interferon, and quinidine, have been frequently reported to induce non–Stx-HUS (126).

Thrombotic microangiopathy has been described in women using an estrogen-containing birth control pill (154,155,156,157), but the incidence, prevalence, and strength of this association are not known. Possible predisposing factors other than birth control pills, such as "flu-like illness" (154,158), pregnancy (157), or severe hypertension (159–161), were recognized in most reports. Hence, some cases defined as "birth control pill–associated HUS" were actually forms of familial (162–164) or recurrent (165) HUS. In turn, evidence that the prevalence of women who use the birth control pill and developed HUS or TTP is comparable with the prevalence of age-matched women who use the birth control pill in the general population (166) strongly argues against a direct cause and effect relationship between birth control pill and TMA.

The platelet anti-aggregating agent ticlopidine has been associated to the development of TTP with an estimated incidence of 1 case per 1,600 to 5,000 (167–170). The first occurrence of TTP in association with ticlopidine was clearly recognized only 7 years after the approval of the drug (167–170). Thrombocytopenic purpura developed within 2 weeks after the initiation of treatment in 15% of reported cases and within one month in 80% of cases. The overall mortality rate for ticlopidine-associated cases of TTP has been estimated as 33%. Fifty-seven percent of the reported cases were treated with plasma exchange (167,170). Although a comparison is faulted by the use of a retrospective analysis, it should be noted that among the patients who received plasmapheresis the mortality rate was 18%, whereas it was 58% among those who did not.

A new antiplatelet agent, clopidogrel, whose mechanism of action and indications are similar to those of ticlopidine, was very recently associated with 11 cases of TTP (171); 10 of them developed TTP within 2 weeks of the beginning of treatment with the drug. All were treated with plasma exchange; one died and all the other recovered, although for two cases 20 exchanges or more were required before recovery.

Quinine was recently described to induce TMA characterized by predominant renal impairment. Despite the dramatic presenting symptoms and severe renal failure, the outcome is usually good if cessation of quinidine and institution of plasma exchange are provided early enough (73).

Recently, the use of antimotility agents has been described to be associated with an increased risk of HUS following E. coli infection (74,172). A very recent report described a case of TTP associated with treatment with zoledronic acid in which an immune-mediated reaction was suspected (173). The clinical course of a patient who presented a TTP pattern after being started on simvastatin, a HMG-CoA inhibitor, is described (174).

The Role of Platelet Activation

Damaged endothelial cells may favor platelet activation and aggregation at the site of endothelial injury. Therefore, "exhausted platelets" from patients with acute HUS contain less βbeta-thromboglobulin and serotonin and poorly aggregate in vitro (175–177). Elevated plasma levels of platelet derived proteins, beta-thromboglobulin, and platelet factor 4, and low platelet–plasma serotonin ratios in children with acute HUS (176,178), further indicate that intravascular platelet activation is a prominent pathophysiologic feature that may contribute to the genesis of the thrombocytopenia.

Platelet activating factor (PAF), a lipid mediator of inflammation released from injured endothelial cells, platelets, and leukocytes that *in vitro* induces platelet and neutrophil adhesion on the endothelial cell surface (179), is excreted in increased amounts in the urine of children during the acute phase of HUS (180). However, although PAF promotes platelet activation and impairs renal function, whether PAF release from renal vascular endothelial cells has a role in the pathogenesis of the disease or is rather a specific marker of endothelial injury has not been established to date.

Specific platelet activators have also been identified in TTP. Lian et al. (181) found that plasmas from 3 patients with acute TTP induce *in vitro* clumping of platelets from both normal subjects or from the same patients during remission. The platelet clumping substance was not adenosine diphosphate (ADP) or collagen and its activity was not inhibited by antiplatelet agents. The same authors identified a 37-kDa protein (p37) with platelet agglutinating activity that was independent from ADP release, energy metabolism, and the cyclooxygenase pathway. In turn, p37 was completely inhibited by IgG from healthy human adults or from TTP patients after recovery, likely through the formation of a p37-IgG complex (182). p37 activity is likely mediated by a specific and concentration-dependent binding to a 97-kDa platelet membrane protein. Because this protein was identified by anti-glycoprotein IV (GPIV) antibodies on either platelet or endothelial cell membranes, Lian hypothesized that p37-GPIV binding on endothelial cells may cause endothelial injury and mediate platelet–endothelial cell interaction. Also a of 59-kDa protein purified from plasma of some patients with acute TTP was found to cause the aggregation of platelets from healthy subjects. Its activity was

calcium dependent, required energy metabolism and fibrinogen, and was inhibited by prostacyclin, normal plasma, and monoclonal antibodies against the GPIIb/IIa complex, but not by heparin and aspirin. Further studies are needed to better characterize these proteins and their role in the pathogenesis of TTP.

The Role of Deficient ADAMTS13 Activity

The relationship between unusually large (UL) von Willebrand factor (vWF) multimers and thrombotic microangiopathies remained elusive until the recent demonstration of the presence in human plasma of a vWF-cleaving protease (183,184), which was first partially characterized in 1996, and then identified in 2001 and shown to be a member of the so-called ADAMTS (a disintegrin and metalloprotease with thrombospondin type 1 domains family of protease) (184). This metalloprotease was shown to cleave vWF physiologically at the peptide bond between amino acid residues 842Thr and 843Met in the A2 domain of the subunit (183). ADAMTS13 is expressed by the homonymous gene restricted on chromosome 9q34. Mutations in this gene lead to an inactive enzyme as it was demonstrated in available data (185).

Two primary mechanisms for deficiency of the ADAMTS13 activity have been identified, namely a constitutive deficiency and the presence of a circulating acquired inhibitory antibody. Whereas patients affected by Upshaw-Schulman syndrome (186), which is synonymous with hereditary TTP (117), and by recurrent and familial HUS (183) have a severe deficiency of ADAMTS13 caused by heterozygous or homozygous ADAMTS13 gene defect, the role of antibody-mediated ADAMTS13 deficiency in the pathogenesis of sporadic TTP continues to be a disputed topic of discussion (184). IgG antibodies inhibiting the metalloprotease has been found in 48% to 80% of patients with acquired idiopathic TTP (117,187); these data are controversial. Moreover, a decrease in ADAMTS13 antibody titers has not been demonstrated consistently during plasma exchange procedures, which are the most effective therapeutic techniques (117,183,184).

The discovery of deficient ADAMTS13 activity in TTP has been rapidly integrated into the prevailing model of the pathophysiology of vWF-mediated thrombotic microangiopathies. According to this model, congenital or autoimmune dysfunction of ADAMTS13 prevents the normal proteolysis of large vWF multimers as they are secreted from injured endothelial cells. This ultimately causes the development of circulating UlvWFs multimers. Such material does not normally circulate but is stored in endothelial Weibel-Palade bodies, and its presence in the peripheral blood reflects endothelial cell injury. In conditions of high shear stress, ULvWF multimers are capable of supporting platelet aggregation more efficiently than normal multimers, by means of glycoprotein Ib receptors on platelet membrane (117). This condition would predispose patients to microvascular thrombosis at sites of endothelial cell injury. However, direct proof of this has never been provided.

Results of analysis of the relationship between vWF multimeric pattern and ADAMTS13 activity in TTP and HUS patients may offer alternative opportunities. On one hand, UL multimers in 2 patients with recurrent TTP have been found with normal ADAMTS13 activity. On the other hand, many patients with either TTP or HUS, with complete or severe deficiency of the ADAMTS13 activity, did not have UL multimers in their circulation. In addition, patients with deficiency of ADAMTS13 activity showed increased vWF fragmentation during the acute phase, as indirectly documented by a lower high molecular weight–low molecular weight (HMW–LMW) multimer ratio, rather than in remission. The same results were reported by Veyradier et al., who found no correlation between ADAMTS13 activity levels and ULvWF multimers in their large series. It was also found that the normal 189-, 176-, and 140-kDa (187) fragments were present in the blood of all patients with deficient ADAMTS13 activity, and the percentage of the native intact vWF subunit was even lower than in healthy subjects, at least in the acute phase. These results indicate that besides ADAMTS13, other proteases are present in the blood of patients with TMA that cleave vWF to the normal fragments and support the possibility that deficiency of the ADAMTS13 activity is not the only determinant of vWF abnormalities in these diseases. Consistent with the present findings, many studies have documented that in sporadic (190), recurrent (189–191), and familial (189) TMA, and in the acute phase of HUS resistant to plasma therapy (192), rather than the presence of ULvWF multimers, there is a loss of such multimers and an increase of LMW forms that indicate enhanced proteolytic fragmentation of the molecule.

In two large clinical studies (189,193), deficiency of ADAMTS13 activity was found in patients with diagnosis of TTP but not in those with HUS. These data seemed to indicate that the presence or absence of this activity could be used to classify patients as having TTP or HUS (183). Nevertheless, severe deficiency of ADAMTS13 activity was found in patients with disorders of thrombocytopenia other than TTP and HUS, including idiopathic thrombocytopenia (one case), SLE (two cases), and disseminated intravascular coagulation (three cases) (188,194). It is important however to recall that microvascular platelet thrombi in disseminated intravascular coagulation are vWF negative and fibrinogen positive, the opposite of thrombi in TTP patients (195). In addition, deficient ADAMTS13 activity is not the only determinant of the presence of ultralarge ULvWF multimers in some phases of the clinical disease of these patients (183).

It may be concluded that low values of ADAMTS13 activity may not be specific for TTP, at least in recurrent and familial forms. However, there is evidence of a link between ADAMTS13 and vWF, and this may be studied using the broader term of TMA (thrombotic microangiopathies), classifying patients in ADAMTS13-associated TMA, as well as in Stx-TMA or HF-dependent TMA (see the following text).

No ADAMTS13 inhibitor was found in patients with familial TTP, despite a complete deficiency of activity of the protease. However, the method used in this study would not detect the presence of antibodies that do not inhibit ADAMTS13 activity but do enhance the clearance of the protease *in vivo*, so that the prevalence of antibodies might have been underestimated in this study. However, in the available asymptomatic parents of the patients described in the preceding text, levels of ADAMTS13 activity were consistent with carriership (approximately 50% activity), indicating that the ADAMTS13 defect was inherited. These results support the view that the deficiency of ADAMTS13 activity in thrombotic microangiopathies may be acquired as a result of an autoimmune mechanism or may be inherited (183).

The Role of Uncontrolled Complement Activation

Genetically determined defects in some components of the complement system such as the factor H 1 (HF1) and the membrane cofactor protein (MCP) may result in uncontrolled complement activation, secondary endothelial damage, and eventual microvascular thrombosis.

Defective Factor H Bioavailability

HF1 consists of 20 homologous units, named short consensus repeats (SCRs). Human glomerular endothelial cells and

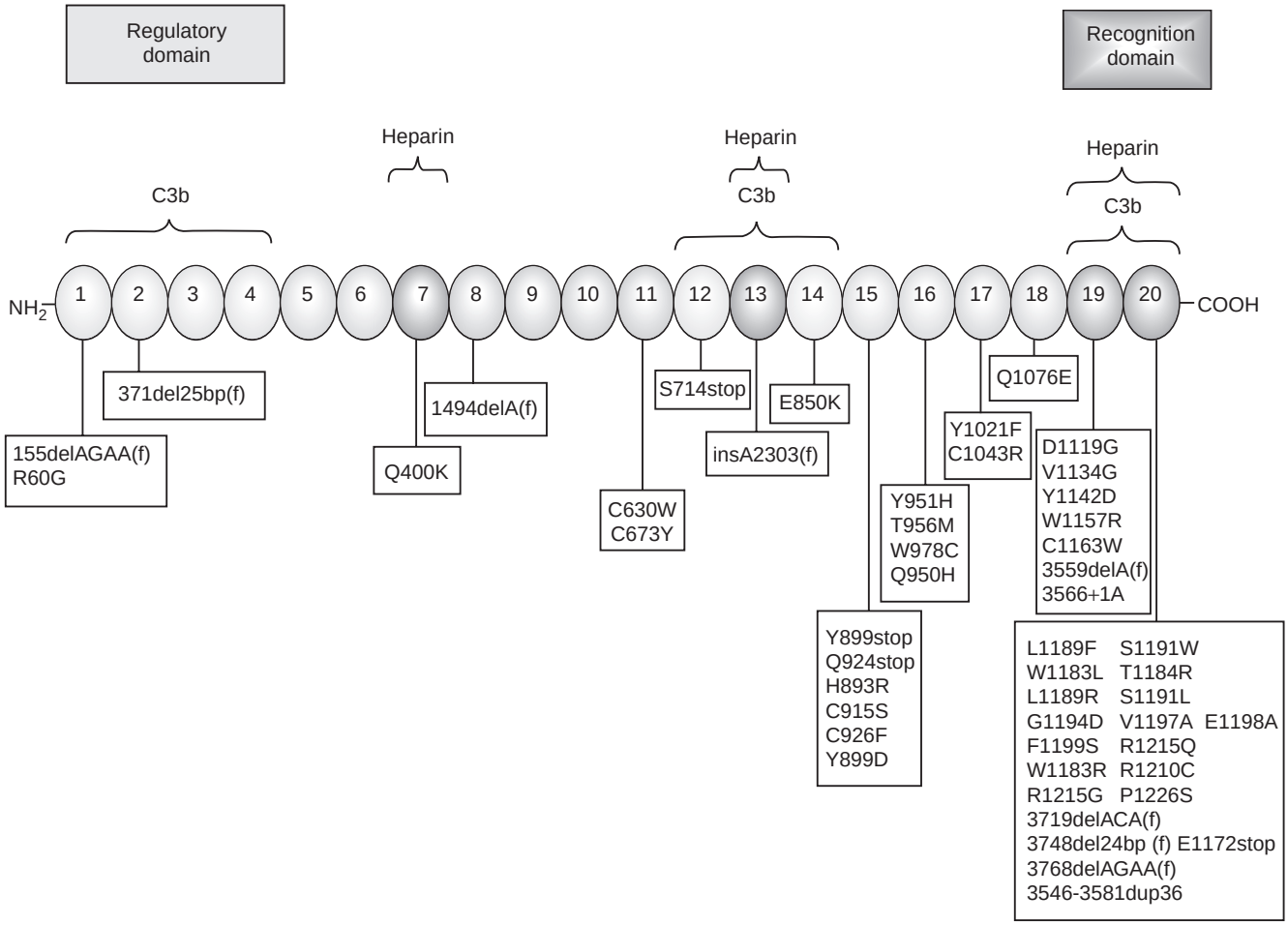

FIGURE 67-11. Factor H mutations associated with HUS. The figure shows the structure of human factor H with the 20 short consensus repeats. The locations of the N-terminal regulatory domain responsible for cofactor activity and the binding sites for C3b and polyanions (heparin) are indicated. The majority of the mutations found in patients with HUS clusters in the C-terminus of factor H that is important for binding to polyanions and to surface bound C3b and for the control of C3b deposition on cell membranes and extracellular matrix.

kidney glomerular basement membrane are rich in polyanionic molecules so that HF1 deposited on their surface would provide an efficient shield against complement attack (196,197). Fifty different HF1 mutations have been found to date in 80 patients who had familial (36 patients) and sporadic (44 patients) forms of non-Stx-HUS (198–201) (Fig. 67-11). Alterations in other genes encoding for complement regulatory proteins could theoretically be involved in determining predisposition to sporadic non-Stx-HUS. Alternatively, these forms could be caused by an acquired autoimmune HF1 defect similar to what has been observed in some patients with TTP in whom the acute episode is triggered by antibodies against ADAMTS13 (202). HF1 mutations are heterozygous in most HUS patients, who are commonly presenting normal HF1 plasma levels, in opposition to patients with type II membranoproliferative glomerulonephritis who carry homozygous HF1 mutations causing severely reduced HF1 levels (200). In a recent study (203), it has been proposed that HF1-related complement regulatory defects could be detected in serum with an *ex vivo* hemolytic assay, which may point out a more severe lysis of sheep erythrocytes with serum of patients with HF1 mutation than with serum from patients with normal HF1 activity. This, if confirmed, could represent a useful tool to select HUS patients who deserve studies of HF1 and other complement regulatory proteins.

Membrane Cofactor Protein Abnormalities

A heterozygous mutation has been recently found in MCP, a widely expressed transmembrane glycoprotein that regulates complement activation, in two patients with familial history of HUS. The mutation determines a 3-amino acid change at position 233–235 followed by a premature stop-codon that causes loss of MCP transmembrane domain and results in severely reduced MCP cell-surface expression (204). Membrane cofactor protein could be the second putative candidate gene for TMA associated with genetic defects; it is highly expressed in the kidney and plays a main role in regulating glomerular C3 activation. Reduced expression of MCP upon complement-activating stimuli may fail to restrict complement deposition on glomerular endothelial cells, leading to microvascular cell damage and tissue injury (204).

Pathophysiology of the Damaged Endothelium

Decreased Prostacyclin Bioavailability

In both TTP and HUS, damaged vessels form less than normal prostacyclin (PGI2) and serum binding capacity for

PGI2 is reduced (205–209). Although the former are indirect measurements, data of reduced urinary excretion of the renal metabolite of PGI2, 6-Keto PGF1-alpha (that normalizes in remission) (210) strongly support that, in vivo, children with acute HUS form less PGI2 than normal. PGI2, an inhibitor of platelet aggregation (211), is the major product of arachidonic acid formed by vascular endothelium in physiologic states, which inhibits platelet–platelet interaction, thereby limiting thrombus formation at the site of endothelial injury.

Shear Stress

A major factor to explain perpetuation of endothelial cell dysfunction in HUS is fluid shear stress. Shear stress changes, the force produced by flowing blood upon endothelial cell surface, have a profound influence on the pathophysiology of vascular endothelium and act as a determinant of vWF handling by enhancing the susceptibility of vWF to proteolytic cleavage (212). Specifically, a recent study found a shear-rate dependent loss of the largest multimers when normal plasma was perfused through long capillary tubings achieving shear rates normally encountered in the circulation. As the large multimers decreased, an increase in the smaller multimers was detected, including 200 and 350-kDa bands. These bands are the dimers of the 140-kDa and of the 176-kDa fragments (212). One can speculate that increased shear forces in damaged microvessels account for abnormal vWF fragmentation observed during the acute phase of TMA and serve to amplify and propagate microvascular lesions.

Oxidative Stress

There are recent data that increased flow rate *in vitro* caused a consistent release of nitric oxide (NO), a potent vasoactive derivative of L-arginine, from vascular endothelial cells (213) and upregulated NO synthase mRNA (214). Since, as documented by animal experiments, NO is a potent mediator of vascular damage (215), it derives that shear-stress mediated overproduction of endothelial NO can act as a major toxic factor for microvascular endothelium. Furthermore, convincing evidence is available that most of the toxic effects of neutrophil-derived oxygen radicals on vascular endothelium are mediated by the interaction of NO with O_2^- to form peroxynitrite, which is further transformed into HO•, the most potent and toxic oxygen-derived product radical so far known (216). It is conceivable that in HUS, NO released by endothelial cells upon the initial insult, serves to amplify endothelial damage via its direct cytotoxic potential as well as by interacting with leukocyte-derived oxygen radicals.

THE KEY SYNDROMES

TMA may be considered an idiopathic genetic or disimmune disease, or a secondary microangiopathic complication of systemic infectious or neoplastic diseases, according to the pathogenic mechanisms that have been discussed in the preceding text. In an attempt to sort out these topics, TMA may be divided according to key syndromes: TMA associated with infectious diseases or exogen infection-related toxins; TMA associated with or secondary to genetic defects; TMA associated with autoantibodies, suggesting an autoimmune disorder; TMA associated with pregnancy; TMA associated with transplant; and forms of TMA secondary to systemic diseases, such as cancer, malignant hypertension, HIV and dysimmune systemic diseases, such as scleroderma, SLE, and antiphospholipid syndrome; lastly, if no clear triggering condition could be found, then idiopathic TMA. Post-HUS chronic nephropathy is the common sequela of any form of TMA resulting in chronic kidney damage after resolution of the acute microangiopathic episode (Table 67-1).

TABLE 67-1

A SUGGESTED CLASSIFICATION OF THROMBOTIC MICROANGIOPATHIES (TMA) BASED ON THE UNDERLYING PATHOGENETIC MECHANISMS

TMA associated with infectious diseases
Stx-associated HUS
Neuraminidase-associated HUS
HIV infection-associated TMA
TMA associated with ADAMTS 13 abnormalities
TMA associated with genetically determined ADAMTS 13 abnormalities
TMA associated with immune-mediated ADAMTS 13 abnormalities
TMA associated with abnormalities of the complement system
HUS associated with genetically determined factor H deficiency
HUS associated with immune-mediated factor H deficiency
HUS associated with membrane cofactor protein (MCP) abnormalities
TMA associated with pregnancy
Pregnancy associated TTP
The Hemolysis, Elevated Liver enzymes and Low Platelet count (HELLP) syndrome
Postpartum HUS
TMA associated with transplanation
De novo HUS
Recurrent HUS
TMA associated with metastatic cancer, chemotherapy, or radiotherapy
TMA associated with systemic diseases (scleroderma, systemic lupus erythematosus, vasculitis, malignant hypertension)
Idiopathic TMA
Post-TMA chronic nephropathy

TMA Associated with Infectious Diseases

Associations between TMA and Coxsackie A (217), Coxsackie B (218), other unspecified viruses (219), microtatobiotes (9), *Mycoplasma pneumoniae* (220), *Legionella pneumophila* (221), and with recent vaccinations (222,223) have been described, and fatal simultaneous occurrence of TTP in a husband and wife (224) and in siblings (225) argue further for an infectious etiology in some cases. Neame hypothesized that viruses may cause the syndrome by producing platelet aggregation, endothelial cell damage, or the production of immune complexes (14). Circulating immune complexes are probably involved in the pathogenesis of TTP associated with bacterial endocarditis (226). TMA may complicate human immunodeficiency virus (HIV) type 1 infection as well as acquired immunodeficiency syndrome (AIDS) related complex (227–229). Elevated platelet-associated levels of IgG and IgM and of the third and fourth complements component suggest an immune-mediated pathogenesis of the syndrome (229). Among the different forms associated with infectious diseases, Stx-associated

HUS and neuraminidase-associated HUS deserve particular attention.

Stx-associated HUS

Epidemiology and Clinical Features. Typical (epidemic) HUS is also referred as D+HUS, since it is associated with prodromal diarrhea. D+HUS is mainly a disease of childhood (but no age group is exempt), with a slight prevalence in females among older children. Caucasians are more commonly affected with peak incidence in Britain, America, and India. It is now clear that Stx is associated with illnesses that include asymptomatic infection, diarrhea, hemorrhagic colitis, and HUS. Stx infection from O26, O111, O113, O121, O145, and O157 serotypes was recognized in 30 of 40 patients from Ontario and Quebec with classical HUS (230).

Gastrointestinal symptoms usually precede full manifestation of the disease and may be followed by a symptom-free period. Hemorrhagic colitis presents with abdominal cramps and watery diarrhea followed by a discharge resembling lower gastrointestinal bleeding. Barium enema reveals filling defects (pseudotumors) that are also features of HUS with bloody diarrhea. In some instances Stx-associated colitis may mimic surgical emergencies including appendicitis (231), acute ulcerative colitis with megacolon (232), and intestinal intussusception (233), which may occasionally call for unnecessary laparotomy that increases mortality. However, some individuals may well require a hemicolectomy for bowel infarction or perforation (234). These cases carry the worst prognosis. Shigellosis-associated HUS also has a high mortality rate (about 30%) and may be associated with systemic intravascular coagulation, acute cortical necrosis, and renal death (235,236). Gastrointestinal losses may cause hypovolemia, and, in combination with hemoglobinemia, hyperbilirubinemia, and hyperuricemia, may favor acute renal failure. Renal symptoms include macro- or micronematuria, proteinuria (occasionally nephrotic), oligoanuria, or polyuria. Oligoanuria, fluid retention, and hypertension may precipitate congestive heart failure. Prolonged oligoanuria and/or persistent hypertension are markers of more severe disease that often lead to residual renal impairment. Some patients with D+HUS have leukocyte counts of greater than 20,000 units and this is considered a predictor of poor outcome, especially in male patients (237). The yearly incidence of the disease (according to an epidemiologic survey in the United Kingdom) was of about 1/100,000, with a peak in children of 1 to 2 years of age (3.3/100.000/year) and between April and October (238). Diarrhea was bloody in 71.6% of cases. Stx infection was demonstrated in 62% of cases; 62% of children needed dialysis and 5% died during the acute phase of the disease. Hyponatremia was associated with an increased risk of seizures. At 1 year no patient was on chronic dialysis. All the available data indicate that patients with D+HUS tends to recover within 2 or 3 weeks without sequelae. Mortality today approaches about 6% of cases (239). However, at 10-year follow-up only 60% of patients have a normal glomerular filtration rate (GFR), whereas the rest have a GFR less than 80 mL/minute per 1.73 sqm (240). In a French series, 15 to 25 years after an episode of classical childhood HUS, only 10 of 25 patients had no renal abnormalities. Of the remaining 15 patients, four had reached end-stage renal failure, four had chronic renal failure, and eight had residual proteinuria or hypertension. All of these patients with residual renal disease had either patched cortical necrosis (11) or TMA involving at least 50% of the glomeruli at the time of disease presentation (241). Somnolence, confusion, and occasionally seizures and coma may be the consequence of uremia or hyponatremia and, in some cases, of TMA of the brain, occasionally with infarctions or hemorrhages (242). The neurologic symptoms usually subside with the remission of the disease, but sequelae including developmental retardation, learning and behavioral problems, focal motor deficits, and convulsions may persist for years (243).

Prognosis and Treatment. A recent meta-analysis of 49 published studies (3,476 patients, mean follow-up of 4.4 years) describing long-term prognosis of patients who survived an episode of Stx-HUS, reported death or permanent end-stage renal disease (ESRD) in 12% of patients and GFR of less than 80mL/minute per 1.73 m^2 in 25% (244). Correction of fluid and electrolyte abnormalities and dialysis played a major role in better short-term outcomes of children with HUS.

There is no clear consensus on whether antibiotics should be administered to treat Stx-E. coli infection. There are data indicating that exposure of *E. coli* O157:H7 to sulfamethoxazole/trimethoprim *in vitro* increases toxin production, whereas ciprofloxacin may reduce it (245). In a prospective cohort study of 71 children who had *E. coli* O157:H7 associated diarrhea, Wong et al. showed that antibiotic treatment was significantly associated with an increased incidence of HUS (246). The cohort comprised children infected with *E. coli* O157:H7, 9 of whom were treated with antibiotics. HUS developed in 10, 5 of 9 (56%) of those were treated with antibiotics and 5 of 62 (8%) of those were not treated. It was postulated that antibiotic-induced injury to the bacterial membrane might favor the acute release of large amounts of toxins. Conversely, a recent meta-analysis on 26 reports failed to show a higher risk of HUS associated with antibiotic treatment (247). Of note, in the study by Wong et al., no patient had bacteremia. Although bacteremia is very common in Stx-HUS precipitated by Shigella dysenteriae type 1, in which patients eventually progress to death if antibiotics are not started early enough, it is only rarely found in Stx-HUS caused by *E. coli* O157:H7 infection. A recent report of an adult patient with *E. coli* O157:H7-induced HUS with bacteremia and urinary tract infection showed that early antibiotic therapy rapidly resolved hematologic and renal abnormalities (248). Consequently, it may be suggested that in patients with Stx-*E. coli* gastrointestinal infection antibiotics should be avoided unless the patient presents with severe systemic bacteremia.

In occasional cases, enterohemorrhagic colitis progresses to severe ischemic bowel disease and perforation, which may require colectomy. Specific therapies, including corticosteroids, heparin, streptokinase, antiplatelet agents, and prostacyclin, were found of no benefit in cases of typical HUS of childhood (249,250). Only three prospective studies are available on the use of heparin. In one study (251) heparin was used in 10 of 30 severe cases of childhood HUS and appeared to worsen the outcome of the disease. Loirat et al. (252) failed to find differences in duration of hemolysis, thrombocytopenia, and oliguria, as well as differences in renal pathology and long-term renal function in 15 children with HUS given heparin and urokinase as compared to 18 controls who only had symptomatic treatment. Of note, in four patients antithrombotic therapy was associated with severe hemorrhages and was discontinued. Similarly, Van Damme-Lombaerts (253) did not find differences in duration of disease, outcome, and renal pathology in 58 children with HUS randomly given heparin plus dipyridamole or supportive therapy alone. Streptokinase and urokinase have definitely been associated with more hemorrhages and should no longer be used. Infusion of vitamin E (alpha-tocopherol, which prevents lipid peroxidation) was encouraging in uncontrolled studies. In 16 children with HUS, 14 of whom were oliguric and 11 required dialysis, oral vitamin E at the dose of 1g/m^2 per day for at least 1 week was associated with a good clinical course (254). All patients survived, with normal renal function in all but one 3 months later. These results appear better than those

of a comparable group of children given heparin, streptokinase, or antiplatelet agents, but, in the absence of larger controlled studies, the role of vitamin E remains elusive. A double-blind controlled study by Sheth et al. (255) found that intravenous infusion of gamma-globulins had no long-term benefits in 20 children with HUS. Therefore, the risk of anaphylaxis and infection (256) provides support against their use as first choice therapy for HUS. A study with SYNSORB-Pk, a synthetic material composed of particles of silicon linked to globotriaosylceramide, given orally failed to find any effect of SYNSORB over placebo (257).

Since 1980 some patients were given plasma infusion or plasma exchange with fresh frozen plasma (258,259). Published literature on pediatric cases with classic HUS given plasma manipulation is sparse. Sheth et al. (260) found that duration of anemia and thrombocytopenia and incidence of residual renal or neurologic abnormalities were comparable in 12 children given fresh-frozen plasma and in 31 historical controls. Ogborn et al. (261) compared retrospectively the outcomes of 18 children with typical HUS treated with hemodialysis and plasma infusion to that of 18 children treated with hemodialysis alone. Severity of the disease, length of hospital stay, duration of renal dysfunction, and disease-related complications were comparable. At discharge the prevalence of hypertension was higher in the plasma group than in the control. In a controlled study of plasma infusion in 32 children with severe HUS (262) the study group was given 30 mL/kg of plasma at admission and then 10 mL/kg per day until the platelet count exceeded 150,000. The average number of infusion days was 9.7. The control group had supportive therapy alone. No significant benefit of plasma could be demonstrated in the acute phase at 24 months follow-up. Renal biopsies taken one month after the onset of the disease showed comparable lesions at light microscopy. Ultrastructural studies of renal tissue, however, revealed arteriolar damage in 5 of 7 control patients, whereas none of the plasma-treated patients had such lesions. The French study by Loirat et al. (263) showed comparable blood pressure, serum creatinine, and proteinuria at 1 year follow-up in 39 children treated with plasma infusion (10 mL/kg/day for 7 days) and in 40 controls. However, renal biopsy studies revealed extensive cortical necrosis in 7 of 27 control patients, whereas no cases of cortical necrosis were found in the 27 patients who were given plasma. Therefore, despite plasma infusion in these preliminary studies appearing to ameliorate renal sequelae in children with HUS, none of the studies performed so far could demonstrate significant differences in major clinical end points, and none had the appropriate sample size to demonstrate differences, if one existed. Side effects of plasma treatment in children can be relevant and include risk of infection and fluid overload. The latter can be avoided by plasma exchange. Due to the benign course of typical HUS, risks and costs of specific treatments outweigh the potential benefits. Until new strategies become available for clinical practice, it appears that for the moment careful supportive management is still the most appropriate form of treatment.

Neuraminidase-Associated HUS

This is a rare but potentially fatal complication of pneumonia or, less frequently, meningitis caused by *Streptococcus pneumoniae*. The clinical picture is usually severe with respiratory distress, anuria, neurologic involvement, and coma (264). The outcome is strongly dependent on the effectiveness of antibiotic therapy. In theory, plasma infusion or exchange is contraindicated, since adult plasma contains antibodies against the Thomsen-Friedenreich antigen that may accelerate agglutination and hemolysis (265). Therefore, patients should be treated only with antibiotics and washed red cells. In some cases, however, plasma therapy, occasionally in combination with steroids, has been associated with recovery.

TMA Associated with Genetic or Immune-Mediated ADAMTS 13 Abnormalities

Differential diagnosis between TMA associated with genetic or immune-mediated ADAMTS 13 abnormalities is important to predict disease outcome and to guide specific treatments. Disease related to genetic defects may affect different members of the same family and tend to recur more times in the same individual (familial and recurrent TMA). Immune-mediated disease does not cluster in families, may unmask an underlying autoimmune disease or follow the exposure to certain drugs and may have a chronic, relentless course that parallels the continuous production of the anti-ADAMTS 13 autoantibody. The rationale of treatment is also different, replacement of the defective activity being the key component of treatment of genetic disease and inhibition of the autoantibody production being the main target of treatment in immune-mediated disease. Monitoring the severity of ADAMTS 13 deficiency and the levels of the autoantibodies is also important in following the patient response to treatment.

TMA Associated with Genetically Determined ADAMTS 13 Abnormalities

A recent large series of patients showed a 91% specificity of the ADAMTS13 defect in distinguishing between TTP and HUS (187), leading Veyradier et al. to conclude that "vWF-cleaving protease deficiency specifically concerns a subgroup of TMA corresponding to the TTP entity" and to formulate the paradigm that "a single laboratory test may enable physicians to distinguish TTP from HUS," according to ADAMTS13 activity. However, a few recent studies have challenged this paradigm, showing that patients with a diagnosis of HUS may have complete ADAMTS13 deficiency (183), albeit less frequently (187). Actually, in a recent study, most patients with a clinical diagnosis of recurrent or familial TTP had complete or severe deficiency of the ADAMTS13 activity during the acute phase of the disease, and in a subgroup the defect persisted at remission. Complete deficiency of ADAMTS13 activity was also found in plasma samples from 5 of 9 patients with recurrent and familial HUS during the acute phase and in 5 patients during remission. These results have been confirmed by evaluating the cleavage of recombinant vWF A1-A2-A3 domains, which overcomes possible artefacts of the collagen-binding assay due to the presence of endogenous undegraded vWF in test samples (202,274,275). In the series of the same authors each of two studied families had one patient with TTP and one with HUS who completely lacked ADAMTS13 activity (183). Because the patients within each family inherited the same genetic defect, these data can be taken to indicate that deficiency of ADAMTS13 may lead to either the TTP or the HUS phenotype. At variance with the series of Veyradier et al. showing 91% specificity of the ADAMTS13 defect in distinguishing between TTP and HUS, in the study previously described, a 44% specificity was found, thus challenging the quoted paradigm put forward by Veyradier and indicating that the protease defect cannot be narrowed to specific subtypes of TMAs. Differences in patient population, including only familial and recurrent TTP and HUS in one series (183), and mostly sporadic cases in the other one (187), may account for discrepancies in results.

TMA Associated with Immune-Mediated ADAMTS13 Abnormalities

IgG autoantibodies have been shown to react with different antigenic regions of the ADAMTS13 molecule in patients with acute TTP, suggesting an autoimmune pathogenesis for acute TTP (184,187,193,202). Further evidence of the pathogenetic role of this autoantibody is that it usually disappears from the circulation when remission is achieved by effective treatment and this occurs in parallel with the normalization of plasma vWF-cleaving protease activity. Of note, autoantibodies against ADAMTS13 have also been observed in patients developing TTP during treatment with antiplatelet drugs such as ticlopidine and clopidogrel (276).

Clinical Features

The syndrome manifests in most cases with the features of TTP. The clinical presentation is dominated in most patients by hemorrhages and neurologic symptoms (2,3). In more than 90% of cases purpura is the initial manifestation, which may or may not be associated with retinal hemorrhage, epistaxis, gingival bleeding, hematuria, gastrointestinal hemorrhage, menorrhagia, and hemoptysis (2,3,45,277). More rare symptoms are malaise, fatigue, pallor, abdominal pain, arthralgia, myalgia, and jaundice (2,3,278). Although fever is not frequently seen at onset, it is almost always present during the illness (2,3,40,45,277). Anemia is severe, with average values between 7 and 9 g/dL of hemoglobin (2,4,279). Transient and fluctuating neurologic manifestations are present in almost all patients (8). These include confusion, headache, paresis, aphasia, dysarthria, visual problems, and coma. Angiographic and electroencephalographic studies have not been performed extensively but do not appear to offer major diagnostic contributions. Ocular involvement is frequent, retinal and choroid hemorrhages being the most common manifestations (280).

Laboratory findings show microangiopathic hemolytic anemia in almost all patients (Table 67-1). A detailed analysis (5) of all the reported series of patients with TTP revealed that hemoglobin levels are less than 10 g/dL in 99% of patients and less than 6.5 g/dL in 38%. Reticulocyte counts are elevated and in 35% of patients are greater than 20% of circulating red blood cells. Serum levels of lactate dehydrogenase (LDH) are frequently correlated with the course of the disease (2,281). Leukocytosis is relatively frequent, but the white blood cell counts rarely exceed 20,000/mm^3 (3). The platelet count is generally low (2,4,40,45,277) and only rarely exceeds 60,000/mm^3. Platelet survival studies have shown reduced platelet survival, suggesting peripheral destruction or consumption (218,283). The complement system has not been studied extensively, but in most instances appears to be normal (14,284,285). Positive lupus erythematosus (LE) cell preparations and antinuclear antibody factors have been reported in a few cases (2,3,286).

Renal involvement is common, with proteinuria and microhematuria the most constant findings (2,40,198,279). Renal function is depressed in 40% to 80% of patients, although severe renal insufficiency is rare (2,4,40,277). Heart involvement is infrequent, although congestive heart failure and conduction disturbances have occasionally been reported (287,288). In rare instances, lungs may contain some alveolar and interstitial infiltrates (289). Abdominal pain has been reported in 10% to 30% of cases (2,3,286) and has been interpreted as secondary to the involvement of small vessels of the gastrointestinal tract (290,291). Pancreatitis has also been described (292).

Prognosis and Treatment

Historical Perspective. Amorosi and Ultmann (3) reported only 10% survival in 1964, but Ridolfi and Bell (277) documented a 46% survival between 1964 and 1980, that increased up to 90% in two large series published in 1991 (293,294). Factors that have improved survival in TTP include intensive care units and recent technical advances in the care of critically ill patients, but it seems unlikely that an eightfold increase in survival is merely the result of improved supportive care or statistical artefact.

Some authorities believe that antiplatelet agents have improved the prognosis of TTP. Dextran 70 was apparently used successfully (295), and subsequent reports described remissions following aspirin—alone or in combination with dipyridamole, sulfinpyrazone, and ticlopidine (296). However, cases in which the disease progressed while the patients were on antiplatelet agents as well as the increasing number of patients experiencing HUS onset with ticlopidine and clopidogrel (126) clearly challenge the significance of the previously described observations (296). Moreover, the 108 patients reported by Bell et al. (294) who never received antiplatelet agents had the same survival rate (90%) as the 102 patients of Rock et al. (293), all of whom had aspirin (325 mg per day) and dipyridamole (400 mg per day) plus steroids and plasma manipulation. All these observations seriously question the value of antiplatelet agents in the treatment of TTP.

Findings of reduced PGI2 activity have stimulated research on the possible use of PGI2 infusion as a substitutive therapy (297). PGI2 (usually as epoprostenol) was given to several patients with TTP with conflicting results (298–302). Because only a limited number of patients have been treated, there is not enough information to draw conclusions about the possible effectiveness of PGI2.

The Role of Plasma Therapy. The most effective form of treatment is plasma exchange or infusion. Exchange transfusion was first attempted in 1956 by Wile and Sturgeon in a 10-month-old infant but attracted greater attention only in 1976 when Bukowski et al. reported a 50% survival in 16 patients treated with this procedure (286). Thereafter many reports described favorable results with exchange transfusion and plasma exchange. The overall response rate to plasma exchange procedures that was 76% in 1981 (303), is 90% in the most recent series with fresh-frozen plasma as replacement therapy (293). A recent randomized trial of plasma exchange versus plasma infusion documented an apparent superiority of exchange over infusion (293). However, the total volume of plasma infused was three times more during exchange than during infusion alone (21.5 $\pm$ 7.8 L versus 6.7 $\pm$ 3.3 L), which suggested that exchange appeared more effective because more plasma was infused. Alternatively, plasma exchange may be more effective because toxic substances are removed from the circulation. The case of a patient with chronic relapsing TTP (304) who received many courses of plasma exchange or infusion during different relapsing episodes documented that infusion rather than removal of plasma is the effective maneuver (305).

Patients who properly respond to plasma therapy usually have a good prognosis if the patient survives the acute episode. Recent data have indicated that infusion of cryosupernatant of plasma (i.e., plasma devoid of vWF multimers, fibrinogen, and fibronectin, which remain in the cryoprecipitate) induced prompt remission in a small series of patients who either did not respond or did not respond adequately to repeated plasma exchanges with fresh-frozen plasma (306). Patients who do not respond to plasma therapy usually have a very poor prognosis, but occasional responses to splenectomy (4,220,295,307) or vincristine (308) have been reported. A 79% response has been reported in 19 patients who had a relapse after withdrawal of plasma therapy (309). However, the results of delayed splenectomy are possibly biased by patient selection and normally evade the early mortality of TTP. Bell et al. (294) reported a pronounced deterioration in clinical status

TABLE 67-2

A SUGGESTED ALGORITHM TO GUIDE TREATMENT OF TMA ASSOCIATED WITH DEFECTIVE ADAMTS 13 ACTIVITY

Form	Treatment[a]	Comment
Genetically determined	Plasma exchange or infusion	To replace defective activity, the exchange procedure allowing more plasma supply without risk of fluid overload
Immune mediated	Plasma exchange	To remove the autoantibody and replace the inactivated protease
	Steroids/immunosuppressive agents (?)	To aspecifically inhibit the production of the autoantibody
	Rituximab (?)	To specifically inhibit CD20 lymphocyte clones possibly involved in the production of the autoantibody
	Splenectomy (?)	Rescue therapy of unproven benefit for refractory and life-threatening forms

[a]Regardless of the underlying etiology, plasma exchange is first therapy in any severe case with neurologic signs or acute renal failure
(?) Unproven efficacy.

accompanied by a decrease in hematocrit and platelet count and an increase in serum LDH levels in six patients undergoing splenectomy because of refractoriness to corticosteroids and plasma exchange; four patients became comatose and one died suddenly. The five patients who survived progressively recovered after the reinstitution of plasma exchange. Splenectomy was not further considered by these authors to treat TTP refractory to plasma therapy. A recent study showed a higher mortality rate and longer disease duration in 13 patients undergoing splenectomy to treat their first episode of TTP as compared to 39 patients continuing on plasma therapy (310). Despite that this study is far from conclusive due to its retrospective design, the role of splenectomy remains most debated until new data becomes available.

A New Rationale for Established Treatments. Better understanding of the pathogenetic mechanisms may help explain the heterogeneous responses to treatment of apparently similar diseases. Patients with genetically determined ADAMTS 13 deficiency, such as the case with chronic relapsing TTP described in the preceding text (304), may benefit from both plasma infusion or exchange, since both procedures may replace the defective activity, the exchange procedure offering the possibility of supplying larger amounts of plasma without the risk of fluid overload (Table 67-2). Those with immune-mediated deficiency definitely benefit the most from the exchange procedure that, in addition to supplying an extra amount of protease that may saturate the autoantibody activity, may also remove the autoantibody from the circulation. Therefore, future trials of plasma therapy should consider separately genetic from immune-mediated forms. The same considerations apply to the use of steroids (3,294), vincristine (311–313), immunoglobulins (314–317), immunosuppressants, and splenectomy (4,220,294,295,307,310). In particular steroids have been extensively used in the past to cure patients with the so called idiopathic TTP, with inconsistent results. Indeed, in immune forms these treatments may aim at inhibiting the production of the autoantibody and, combined with plasma exchange, may result in an effective clearance of the autoantibody from the circulation. However, they are not effective for treatment for genetic forms. Trials considering the two forms together invariably diluted the potential benefits of steroids or immunosuppressive therapy in subjects with immune-mediated disease. This may explain the inconclusive results of previous studies in TTP. Novel studies should likely focus on the role of steroids as first-line therapy, and on vincristine, high-dose immunoglobulins, or other immunosuppressants as second-line therapy, with splenectomy considered as rescue therapy for those patients with refractory disease and life-threatening thrombocytopenia or neurologic involvement (Table 67-2).

TMA Associated with Genetic or Immune-Mediated Abnormalities of the Complement System

TMA associated with abnormalities of the complement system usually manifests with the clinical features of HUS.

HUS Associated with Genetically Determined Factor H Deficiency

In 35 cases of familial TMA belonging to 10 families, identified through the Italian Registry of Recurrent and Familial TMA, decreased serum level of the third component of complement (C3) was associated with an increased risk of the disease (266). This was initially attributed to an inherited defect in C3 synthesis (267), but much more convincing data are now available that low C3 in HUS derives from either lack (268–270) or altered function (271) of factor H, a regulatory component of the alternative pathway of the complement system (272,273), which protects host tissues (126). Compelling molecular evidence is available that genetic alterations in factor H are involved in both autosomal dominant and recessive HUS.

Familial forms have been reported to occur in children and adults (33,163,318,319–325). Both are uncommon and share a poor prognosis. A hereditary autosomal recessive form, which may present as the relapsing variant, is most common in children (163,319). In adults the inheritance of HUS appears of autosomal dominant model (321) and male predominance has been found in a few kindreds (320). Malignant hypertension is a frequent complication of the syndrome, and the prognosis is extremely poor. Irreversible renal failure has been reported in five members of two families with autosomal dominant HUS (324). The syndrome was triggered by a virus-like disease and was complicated, despite good control of arterial blood pressure, by severe retinopathy with exudates and hemorrhages. In two cases, severe lung involvement with hemoptysis,

respiratory failure, and x-ray findings of diffuse shadows were reported and improved following bilateral nephrectomy (324).

Plasma infusion or exchange has been used in patients with HUS and factor H_1 (HF_1) mutations, with the rationale to provide normal plasma HF1 activity to correct the genetic deficiency (126). In one study patients died or developed ESRD (326). In very recent studies, patients remained chronically ill or required infusion of plasma at weekly intervals in order to raise protein HF1 plasma levels enough to maintain remission (327). Only one case of total sustained remission in a patient with acute HUS and HF_1 mutation after plasma exchange has been described by Stratton and Warwicker (328). After 3 months of weekly plasma exchange in conjunction with intravenous immunoglobulins, the patient had normal renal function and hemodialysis was not needed. At one year after stopping plasma therapy, the patient did not have any relapse and remained dialysis independent.

Graft failure occurs in 30% to 100% of kidney transplants, according to different surveys (126). Given the fact that HF1 is a plasma protein mainly of liver origin, indirect evidence is available that a kidney transplantation does not correct the HF1 genetic defect (204). Simultaneous kidney and liver transplantation has been recently performed in two young children with non-Stx HUS and HF_1 mutations, with the objective of correcting the genetic defect and preventing disease recurrences. However, for reasons that possibly involve an increased liver susceptibility to immune or ischemic injury related to uncontrolled complement activation, both cases (329,330) treated with this procedure were complicated by premature irreversible liver failure. In the first case (329), the child developed hepatic encephalopathy and coma that recovered with a second, uneventful, liver transplantation. The second case (330) was complicated by a fatal, primary nonfunction of the liver graft. Graft hypoperfusion, due to a sudden drop of arterial blood pressure soon after reperfusion, triggered severe ischemia/reperfusion damage and complement deposition in the liver, conceivably as the result of defective HF1 complement regulatory potential. Thus, combined kidney and liver transplantation should not be performed unless the patient with HF1-dependent HUS is at imminent risk of life-threatening complications (126).

HUS Associated with Immune-Mediated Factor H Deficiency

An autoimmune mechanism involving regulatory proteins of the complement alternative pathway, that is, factor H (HF) and membrane co-factor protein (CD46), was first studied in familial and recurrent HUS (see subsequent text), and then it was surveyed in sporadic forms in which it was demonstrated in several studies (126,331). In a recent French study, Dragon-Durey et al. investigated the presence of HF autoantibodies by enzyme-linked immunosorbent assay (ELISA) using coated purified human HF in a series of 48 children who presented with atypical HUS (331). IgG antibodies reacting with HF were detected in the plasma of three children who presented with recurrent HUS. The HF plasma activity was found to be decreased, whereas plasma HF antigenic levels and HF gene analysis were normal (332). This report supports the possible occurrence of sporadic HUS as an autoimmune disease through the development of anti-HF antibodies.

HUS Associated with Membrane Cofactor Protein (MCP) Abnormalities

HUS may also occur in patients with a genetic abnormality of the membrane cofactor protein (MCP). The disease manifests with fever, hemolytic anemia, and thrombocytopenia (333), and the outcome is usually poor (126). Plasma infusion or exchange still remains a valid therapy that may have some effect. Unlike cases associated with congenital abnormalities of HF1 in which the microangiopathic process invariably recurs after kidney transplantation, it is tempting to speculate that forms associated with MCP abnormalities can be corrected by transplanting a normal kidney. Four successful kidney transplantations have been performed to date in such patients with no disease recurrence (334). Because MCP is a membrane-bound protein highly expressed in the kidney, a kidney graft would reasonably correct local MCP dysfunction. The graft, which is physiologically bearing wild-type MCP, highly expressed in the kidney, should conceivably be protected from the recurrence of a microangiopathic process (204,333).

TMA Associated with Pregnancy

Pregnancy-associated TTP may be distinguished from preeclampsia, the hemolysis elevated liver enzymes and low platelet (HELLP) syndrome (usually near term), and postpartum HUS (usually within 3 months after delivery).

Pregnancy-associated TTP

TTP develops during the antepartum period in 89% of cases, usually within 24 weeks. Measurement of plasma antithrombin III (AT III) activity has been suggested as a useful tool to differentiate TTP and preeclampsia. Before gestational week 28 and when AT III plasma activity is normal, TTP is most likely (73). After week 34 of gestation, preeclampsia is most likely. In pregnant patients with TTP, despite limited experience, available series show that the maternal mortality rate has fallen from 68% to almost zero with the institution of plasma therapy (335). Plasma therapy could be continued until term and/or complete remission of the disease. Delivery can be considered as "rescue" after failure of plasma therapy (73).

The HELLP Syndrome

The HELLP syndrome (an acronym for hemolysis, elevate liver enzymes and low platelet count) is simply a form of severe preeclampsia in which in addition to hypertension and renal dysfunction, there is evidence of microangiopathic hemolysis and liver involvement. It arises in the antepartum period in 70% of cases. Symptoms can arise within 24 to 48 hours postpartum, occasionally after an uncomplicated pregnancy (335). Diagnosis is based on: (a) hemolysis (defined as fragmented erythrocytes in the circulation and lactic dehydrogenase $\geq$600 U/L), (b) elevated liver enzymes (serum glutamicoxaloacetic transaminase >70 U/L), and (c) low platelets (platelet count $<10^3/mm^3$) (191). Overt disseminated intravascular coagulation (DIC) is reported in 25% of cases. Intrahepatic hemorrhage, subcapsular liver hematoma, and liver rupture are rare, life-threatening complications. The maternal and perinatal mortality rates range from 0% to 24% and from 7.7% to 60%, respectively (73). In the HELLP syndrome, termination of pregnancy is the only definitive therapy, but by no means the treatment of choice. Hydralazine or dihydralazine are the first choice drugs to control pregnancy-induced hypertension, and magnesium sulfate is the first choice to prevent and treat convulsions. Both peritoneal dialysis and hemodialysis have been used to treat acute renal failure. Platelet transfusions are needed for clinical bleeding or severe thrombocytopenia (platelet count $<20.000/\mu L$). In approximately 5% of patients with HELLP syndrome, symptoms and laboratory abnormalities do not improve after delivery. There are cases with central nervous system abnormalities, associated with renal and cardiopulmonary dysfunction and activation of coagulation. Uncontrolled studies suggest that plasma exchange may help recovery in patients with persistent evidence of disease 72 hours or more after

delivery. However, plasma therapy is ineffective during pregnancy and may increase fetal and maternal risk when used to delay delivery. Preliminary evidence suggests that, postpartum, corticosteroids may speed up disease recovery, and, antepartum, may postpone delivery of pre-viable fetuses and reduce the mother's need for blood products (73).

Between 28 and 34 weeks of gestation, because the differential diagnosis between the HELLP syndrome and TTP is ambiguous, the optimal treatment is controversial. If there is no evidence of fetal distress and plasma AT III activity is normal, a course of plasma therapy can be reasonably attempted before inducing delivery (336).

Postpartum HUS

Postpartum HUS is a rare event that manifests within 3 months after delivery. When it ensues early postpartum it can be considered a late manifestation of severe preeclampsia or the HELLP syndrome and may recover spontaneously or with plasma therapy. On the contrary, the relationship with pregnancy is uncertain for cases that ensue in the later postpartum period. In most of these cases renal failure and neurologic symptoms are severe and are associated with severe hypertension (318). The mortality rate has ranged from 50% to 60% in the various reports (337,338). Patients who survive the acute phase of the disease have residual renal dysfunction and hypertension (337,339,340). The best management of patients with postpartum HUS is a supportive treatment (i.e., dialysis if necessary and careful fluid management). A 53% survival in heparin-treated patients versus 17% survival in untreated patients was found in a retrospective analysis of uncontrolled studies in post-partum HUS (341). However, because of severe hypertension and thrombocytopenia, heparin and antiplatelet agents enhance the risk of intracranial hemorrhage in these patients. Isolated reports of successful therapy of postpartum HUS with plasma infusion or exchange are available, but data are not as convincing as for TTP.

TMA Associated with Transplantation

Transplantation-associated HUS usually ensues with the clinical features of HUS. The disease may occur *de novo* or may recur in the transplanted kidney in subjects who progressed to ESRD because of HUS. The underlying etiology and the outcome may differ substantially. *De novo* HUS may complicate a toxic or immune-mediated damage to the kidney (such as in subjects on immunosuppressive therapy with cyclosporin A or tacrolimus, or with vascular rejection of the kidney graft) and may recover with the resolution of the underlying disease. Recurrent HUS almost invariably affects subjects with a genetic predisposition and results in irreversible graft failure regardless of treatment.

De novo HUS

De novo HUS may occur in patients receiving renal transplants and other organs, as a consequence of the use of calcineurin inhibitors (CsA and tacrolimus) or due to humoral (C4b positive) rejection (126,342). Since the first description of thrombocytopenia, hemolytic anemia, and renal failure in a patient treated with CsA for preventing graft-versus-host disease after allogenic bone marrow transplantation (343), several cases of HUS have been reported in patients given CsA to prevent bone marrow (343,344), liver, heart, and kidney graft rejection (345). It is known that cyclosporine at pharmacologic doses induces a potent vasoconstrictory effect that may result in kidney hypoperfusion and ischemia. Enhanced release of endothelin and other mediators might then amplify endothelial damage and sustain the microangiopathic process (346). In addition, HUS has been described to occur in approximately 1% of renal transplantation patients receiving tacrolimus (342). Recently, Hochstetler et al. (347) reported the onset of thrombotic microangiopathy in 4.1% of 512 patients receiving a cadaveric renal transplant, usually in the first posttransplantation month. There were 5 deaths (24%) and 7 transplant nephrectomies (33%), resulting in an overall graft loss of 57%. These values exceeded the 6-month posttransplantation mortality rate (4%) and graft loss (12%) experienced by renal allograft recipients without TMA. Evidence of cytomegalovirus (CMV) infection during their acute TMA was found in 42% of the patients and, in combination with CsA therapy, might have favored the onset of the disease.

Removal or treatment of the underlying cause of renal damage is the key component of treatment. In most cases associated with calcineurin inhibitor therapy, early withdrawal of cyclosporine or tacrolimus may result in full recovery. Plasma therapy is usually recommended, in particular in those cases in which there is not prompt recovery with treatment withdrawal. Replacing calcineurin inhibitors with other inhibitors of IL-2 activity that are free of direct nephrotoxicity such as rapamycin and specific monoclonal antibodies against IL-2 receptors (346), may help disease recovery without increasing the risk of graft rejection. Noteworthy, two cases successfully treated with intravenous IgG infusion and without CsA discontinuation, have been recently reported (347). The outcome of *de novo* HUS associated with vascular rejection is most often severe and closely reflects the outcome of the underlying disease. Lymphocytolytic therapy and plasma exchange may help limiting the disease, but graft failure is the most common outcome.

Recurrent HUS

HUS may recur in 0% to 25% of renal grafts (348). However, the risk of recurrence, which is minimal in cadaveric kidney grafts (349,350), may approximate 50% in living related kidney transplants (351). Comparable rates of recurrence are reported among patients with or without CsA therapy (351). Miller et al. (348) found HUS to recur in 12 of 18 patients with non-Stx-HUS and only in 1 of 6 with Stx-HUS, usually within the first 2 months posttransplantation. Ten of the grafts had severe vascular lesions and 9 were irreversibly lost. Life-time analysis showed significantly poorer graft survival for HUS patients compared to overall renal transplantation population, but surprisingly this was not associated with an increased risk of graft loss due to disease recurrence. These data were confirmed by Gagnadoux et al. (350) who found that 1- and 5-year graft survival was 66% and 37% in 25 HUS children versus 80% and 69%, respectively, in all other pediatric recipients. These authors concluded that although recurrence of disease is a relatively uncommon cause of graft loss in HUS patients, actually only the minority with atypical non-Stx HUS would be at risk (348), as compared to chronic vascular rejection. For unknown reasons, it is remarkably more frequent in HUS patients as compared to the general transplantation population. One should also consider that some of the graft losses attributed to HUS in the literature are likely the consequence of allograft rejection. Actually, difficulties in differentiating recurrent HUS from antibody-mediated acute vascular rejection may account for the different rates of HUS recurrences reported in the various series.

A genetic predisposition to develop HUS after exposure to Stx has been proven in a small proportion of patients with Stx-HUS. There is concern whether this predisposition may favor recurrence of disease in such patients after kidney

transplantation. However, because patients who received a kidney transplant because of ESRD secondary to Stx-associated HUS are relatively few, numbers of series are too small to definitely establish the role of genetic predisposition in posttransplantation recurrence (346). Neurologic and other extrarenal manifestations are relatively uncommon in posttransplantation HUS. However, central nervous system involvement has been described in both adults and children and has presented with spontaneous cerebral hemorrhage (352,353).

Some of the therapies effective in other forms of HUS have been empirically tried in recurrent posttransplantation HUS, but only a minority of patients recovered renal function, whereas most of them lost their graft suddenly.

Plasma infusion or exchange is the first used treatment approach in this setting but there is still no definite evidence of effectiveness. Adding plasma infusion/exchange might limit the microangiopathic process in cases sustained by plasma abnormalities (such as defective factor H activity) that can be corrected by normal plasma (346). Whether this may apply to other familial/recurrent forms is reasonable, but not proven.

Other treatments, such as immunoperfusion, that is, perfusion of autologous plasma over filters containing staphylococcal protein A, have been attempted, but there is no reason to believe that this procedure may offer any advantage as compared to plasma exchange. Finally, high-dose intravenous immunoglobulin infusion has been reported to be effective in some cases associated with CMV infection. It is possible, but not proven, that this treatment was effective by limiting viral replication (346). Combined liver and kidney transplantations have been performed in children with factor H associated HUS with the rationale to replace the defective activity with the factor H produced by the transplanted liver (354,355).

However, this procedure is not recommended due to the unacceptably high risk of premature and irreversible failure of the liver graft (see TMA associated with genetic or immune-mediated defects of the complement system).

TMA Associated with Cancer

Cancer-associated HUS complicates almost 6% of cases of metastatic carcinoma (356). Gastric cancer alone accounts for about half such cases. These patients have an extremely poor prognosis and most of them die within weeks. A form of TMA resembling HUS has also been described in cancer patients given the anticancer drug mitomycin. Platinum- and bleomycin-containing chemotherapeutic combinations may also trigger HUS. The risk of developing HUS following mitomycin-C therapy is 2% to 10% (357). Manifestations of the disease are dose related, so that cumulative doses higher than 60 mg are almost invariably associated with the disease. The case fatality rate of mitomycin HUS is 60% to 70%, and the median time to death is about 4 weeks. Deaths occurring within the first few months is usually related to complications of the syndrome, including renal failure and pulmonary edema, or sepsis. Patients surviving the acute phase of the syndrome, may have remission of the microangiopathic process but remain on chronic dialysis.

Specific therapy is minimally effective. Blood transfusions often exacerbate the disease and may trigger pulmonary edema (358). Perfusion of autologous plasma over filters containing staphylococcal protein A, which binds the Fc portion of immunoglobulin G, has been attempted to remove circulating immune complexes with encouraging results (359). HUS may also be a complication of radiation nephritis. It usually occurs late after radiotherapy and is invariably associated with irreversible kidney failure.

TMA Associated with Systemic Diseases, Including HIV Infection

TMA may complicate SLE, scleroderma (sclerodermic crisis), malignant hypertension or vasculitis. Clinical features and outcomes strongly depend on the underlying disease. Seventy percent of patients may require dialysis during the acute phase of the disease (360). The mortality rate from neurologic complications is high during the acute phase of the disease, and residual kidney dysfunction is frequent, with death or ESRD an almost invariably outcome of scleroderma or malignant hypertension. Older age is also associated with an increased mortality.

HIV infection-associated HUS was firstly reported in 1984 in a homosexual man (227). Since then, TMA diagnosed as either TTP or HUS is part of the complications of AIDS and accounts for up to 30% of hospitalized cases of TMA at least in some institutions (229). Stx infection (361) and cocaine abuse (362) are possible triggers. The clinical course is poor and depends heavily on the severity of the underlying disease. Management is the same as recommended for non-AIDS cases. However, steroids, vincristine, and immunoglobulins may increase the risk of opportunistic infection in already immunosuppressed patients and should be given with caution. Plasma exchange or infusion is likely the only feasible therapy for AIDS-associated TMA. In one series of seven patients with HIV-associated TMA the short-term response rate to plasma exchange (86%) was comparable to the response rate (80%) reported in the pooled literature of non-HIV cases (363). However, none of these patients survived more than 2 years after remission was achieved. Due to sporadic reports of remission of the syndrome after treatment with the antiretroviral agent zidovudine (364) and of recurrence of the disease after its withdrawal (365), it has been suggested that zidovudine may play a role in the management of HIV-related TMA. However, cases of TMA ensuing during zidovudine therapy make this possibility very unlikely (366).

Idiopathic TMA

Idiopathic forms of TMA encompass all thrombotic microangiopathies with an unclear etiologic pattern. It mainly consists of "non-Stx-HUS" forms in which no clear link was found with any genetic or immune-based mechanism. In opposition to genetic and familial forms, sporadic TMA appears in patients with no history of TMA in their family. Sporadic TMA manifests at all ages, but is more frequent in adults. According to a recent U.S. study, the incidence of sporadic TMA in children is approximately two cases/year/1,000,000 total population (204).

The disease manifests without prodromal diarrhea in children of all ages and in adults, and accounts for approximately 5% to 10% of all cases (238). Typically there is no evidence of Stx infection and the mortality rate may be high (260). Plasma therapy is the treatment of choice, but whether it effectively helps renal function recovery is unknown.

Post-HUS Chronic Nephropathy

Chronic sequelae may complicate any form of TMA, including Stx-associated HUS. Hypertension, proteinuria, and worsening renal function are the typical features and relentless progression to ESRD may occur over years or decades. Careful blood pressure control and renin–angiotensin system blockade may be particularly beneficial in the long term for those patients with chronic renal disease after an acute episode of HUS. A

recent study (367) in 45 children with renal sequelae of HUS followed for 9 to 11 years documented that early restriction of proteins and use of ACE inhibitors may have a beneficial effect on long-term renal outcome, as documented by a positive slope of 1/Serum Cr values over time in treated patients. In another study, 8- 15-year treatment with ACE-inhibitors after severe Stx-HUS normalized blood pressure, reduced proteinuria and improved GFR (368). Finally, kidney transplantation should be considered as an effective and safe treatment for those children with Stx-HUS who progress to ESRD. The recurrence rates actually range from 0% to 10% and graft survival at 10 years is even better than in control transplanted children with other disease (369–371).

In occasional patients, severe vascular changes in the kidney may sustain refractory hypertension and microangiopathic hemolysis even after recovery from the acute phase of the disease. In those patients with extensive microvascular thrombosis at renal biopsy, refractory hypertension, and signs of hypertensive encephalopathy when conventional therapies including plasma manipulation are not enough to control the disease (i.e., persistent severe thrombocytopenia and hemolytic anemia), bilateral nephrectomy has been performed with excellent follow-up in some patients (192). When the genetic studies exclude a congenital predisposition to the disease, these patients may eventually benefit with a kidney transplant.

ACUTE CORTICAL NECROSIS

Acute cortical necrosis represents a rare cause of acute renal failure (ARF), occurring in about 2% of patients (372). The term acute cortical necrosis defines a condition of destruction of the renal cortex except for a thin tissue rim under the capsule and usually a thicker layer under the corticomedullary junction. This phenomenon reflects a disturbed blood flow to interlobular and afferent arterioles, whereas the arcuate arteries, which supply blood to the juxtamedullary nephrons, are usually spared. The lack of necrosis in subcapsular nephrons is due to the presence of anastomoses with extrarenal vessels that allow minimal perfusion to superficial nephrons, just enough to prevent necrotic changes. In 50% to 70% of the series considered, acute cortical necrosis is a complication of pregnancy (especially in multiparous women older than 30 years of age). Abruptio placentae is the most common prior complication of the ARF (372,373). Preexisting toxemia seems to be an important predisposing factor (66,374,375), but there is no general agreement on this issue (372). Intrauterine death, hemorrhage from placenta previa, septic abortion, postpartum hemorrhage, and, occasionally hyperemesis, are other conditions that may be complicated by acute cortical necrosis (372, 376–379).

Recently, Donohoe (380) followed pregnant women throughout the two decades from 1961 to 1980. Comparing the incidence of acute cortical necrosis during these periods, he found that in the second group (1971–1980) abruptio placentae occurred almost three times less often than in the first group. Acute renal failure and acute cortical necrosis also decreased further in these 10 years (1971–1980), proving that not only a better management of pregnancy but also a prompter and more appropriate approach may prevent the occurrence of necrotic changes in the cortex. In contrast, a recent experience (337) has indicated that cases of cortical necrosis associated with pregnancy are increasing despite an overall reduction in the number of patients suffering from ARF after pregnancy.

Bacterial and postoperative shock, pancreatitis, dissecting aneurysms, gastrointestinal hemorrhage, trauma, burns, phosphorus and diethylene glycol poisoning, snake venom bites, and sometimes TTP and HUS are other conditions that can be complicated by acute cortical necrosis (373,381–392). In children, cases of cortical necrosis have been reported, most frequently after protracted vomiting and diarrhea with marked dehydration. Moreover, as in adults, the disease has been seen concomitantly with infections such as peritonitis, septicemia, pharyngitis, transfusion reactions, and phosphorus poisoning (391,393–397).

The generalized Shwartzman reaction (GSR) is an experimental model of bilateral cortical necrosis of the kidneys induced in rabbits by two intravenous doses, spaced 18 to 24 hours apart, of endotoxin from gram-negative bacteria (398).

The earliest histologic lesion in the GSR is the deposition of a homogeneous, eosinophilic material with the staining properties of fibrinoid within the lumen of the glomerular capillaries of the kidneys (399). Similar material is deposited in the vessels in other visceral organs in association with necrotizing and hemorrhagic lesions (398,399).

There is experimental evidence that in pregnant animals (rabbits and rats) (400,401) or in animals pretreated with corticosteroids (398), sympathomimetics (alpha-agonists) (402), or synthetic acid polymers (403), the GSR can be triggered by a single dose of endotoxin, whereas two spaced endotoxin injections are required in nonpregnant or untreated animals. Although recent studies have emphasized the role of endothelial injury that follows the exposure of endothelium to endotoxin, direct and definitive evidence of this mechanism is still lacking. Raij et al. (404), in a model of unilateral cortical necrosis in rabbits, showed that after an *in situ* perfusion of one kidney with endotoxin followed by a systemic injection of endotoxin 24 hours later, the GSR was confined to that kidney. Therefore, it appears that endotoxin might have a specific local effect on the vascular endothelium that is sufficient as an initial event that predisposes to cortical necrosis. In agreement with this view is the finding of Arhelger et al. (405) that in rats after an injection of nephrotoxic serum, which is known to induce immediate endothelial injury, a single dose of endotoxin was sufficient to elicit the GSR. Hoyer et al. (406) extended this experiment by infusing nephrotoxic serum into the left renal artery of pregnant rats and obtained a Shwartzman reaction restricted to the left kidney. More recently, Campos et al. (407) demonstrated that vascular prostacyclin (PGI_2) infusion prior to endotoxin significantly inhibited the GSR in pregnant rats. Although the lesions of the GSR were not completely abolished, the extent and severity of the histologic changes were significantly less than in PGI_2-untreated animals.

PGI_2 might exert its protective effect against the GSR by inhibiting platelet aggregation, regulating polymorphonuclear leukocyte adherence to endothelial cells, and influencing the participation of leukocytes in the GSR. The relevance of PGI_2 in antagonizing the occurrence of the GSR is strengthened by the observation that bradykinin, which increases the synthesis of renal prostaglandins (408), prevents the GSR elicited by a single injection of endotoxin in pregnant rats and cortisone-sensitized rabbits (409). The protective effect of bradykinin on the GSR seems to be mediated by prostaglandins, since it is abolished after administration of aspirin.

Pathogenesis

The pathogenesis of cortical necrosis still remains a mystery. The two major theories proposed to date only partially explain the disease. According to Sheehan and Moore (373), who studied specimens from patients who died after abruptio placentae, vasospasm is the primary event causing cortical necrosis.

Alternatively, it has been suggested that acute vascular injury followed by activation of coagulation and thrombosis plays a key role in the development of cortical necrosis (403,410). Both these theories do not convincingly account for the fact that after the initial "trigger" a cascade of events takes place leading to a necrotic process localized at the renal cortex.

Sheehan and Davis (411) observed that prolonged experimental clamping of the renal pedicle can produce cortical necrosis. The same lesion was obtained in experimental animals by the infusion of a large amount of vasoactive substances such as epinephrine and oxytocin (412–414). However, no convincing evidence is available so far to support the idea that vasospasm is the key event in acute cortical necrosis in humans (380).

Experimental Shwartzman reaction produced in pregnant rabbits after a single injection of bacterial toxin supports the theory that vascular injury is the major etiologic event in cortical necrosis (415). The Shwartzman reaction produces DIC. The crucial role of the latter in the development of cortical necrosis is documented by the fact that heparin reduces the incidence of cortical necrosis in the model of GSR of nonpregnant rabbits (416). However, the main difference between experimental Shwartzman reaction and cortical necrosis in humans is that in animals the necrotic process involves also the renal medulla and organs other than the kidney. Probably in humans selective damage of the cortical vasculature predisposes to the subsequent development of localized damage as soon as a "trigger event," for example, abruptio placentae, occurs. In this context, recent experimental data are particularly relevant. Unilateral Shwartzman phenomenon confined to a single kidney has been produced by local perfusion of low-dose endotoxin before systemic injection of endotoxin (404). Cortical necrosis in experimental animals has also been obtained using liquid or diethylene glycol (383,417). The mechanisms by which these toxic agents lead to cortical necrosis are far from being understood, but these agents are known to cause endothelial damage.

Another intriguing issue is the significance of glomerular fibrin thrombi. Early reports have focused on the possible crucial role of glomerular thrombosis in the pathogenesis of the disease (376,418). However, a detailed analysis of the most representative series reported in the literature revealed that glomerular fibrin thrombi are relatively rare in cortical necrosis. Only occasionally have extensive intraglomerular thrombi been documented (372,378). Altogether, the available data do not support the idea that cortical necrosis is the consequence of a mechanical blockage of glomeruli by fibrin thrombi. Moreover, in the two largest series (378,419) reported so far of patients affected by DIC with glomerular fibrin thrombi, the majority of which had bacterial sepsis, cortical necrosis was found in only four of the 63 cases studied.

Finally, it has been suggested that cortical necrosis might be a consequence of immunologic mechanisms. Most evidence in support of this hypothesis comes from the observation that pathologic findings similar to cortical necrosis have been found in hyperacute rejection of renal allografts (420). Cortical necrosis in these cases presumably results from direct immunologic injury to transplanted cortical vessels by preformed antibodies. This event occurs in completely denervated allografts, and, therefore, vascular endothelial damage rather than vasospasm may be the primary event in the overall pathogenesis of at least some forms of this dramatic disease.

Clinical Features

The most typical clinical sign of acute cortical necrosis is sudden oliguria, the amount of urine ranging from 0 to 100 mL per day (421). Sometimes this is preceded by gross hematuria. Lumbar pain, if present, constitutes a rather nonspecific symptom and may be associated with fever and leukocytosis. Urine contains protein, red blood cells, white blood cells, epithelial cells, and various types of casts. Hypertension may occur, but generally the blood pressure is only slightly elevated. A picture of acute renal failure with hyperazotemia, metabolic acidosis, and hyperkalemia emerges from laboratory data. LDH and glutamic oxaloacetic transaminases in serum are elevated during the first days of the disease (417). DIC is frequent, especially in obstetric patients. Fibrinogen and platelet counts fall very low, prothrombin time is prolonged, and fibrinogen degradation products (FDPs) in serum are often elevated (380).

Acute cortical necrosis must be suspected when oliguria or anuria tends to persist for a long period. Renal biopsy provides the definitive diagnosis. However, the patient's clinical condition may not always permit the performance of a biopsy, and in some cases the diagnosis may be missed because, especially in the incomplete form of disease, the specimen does not allow for detection of the typical changes. Radiologic techniques are very useful in the evaluation of the diagnosis of acute cortical necrosis (372,422,423). Renal echography may exclude obstruction. Selective arteriography may provide information about the extent of lesions, permitting a distinction between the complete and incomplete forms. The most typical radiologic sign of acute cortical necrosis is the renal cortical calcification, which, however, is uncommon and does not occur in the early phases of the disease (417).

Hemodynamic studies with krypton or xenon washout techniques indicate reduced renal blood flow; however, these techniques, except in cases of complete acute cortical necrosis, may not differentiate between patients with incomplete acute cortical necrosis and those with acute tubular necrosis (372).

Pathology

In 1953 Sheehan and Moore (373) described in detail the pathologic changes characteristic of cortical necrosis. In massive or complete cortical necrosis, almost the whole cortex is affected by necrosis except the corticomedullary junction and a thin rim of cortical tissue under the capsule. On gross examination, the kidneys are enlarged and weigh 200 to 300 g. The cortex has a yellowish-white appearance, but congested areas are detected in the periphery. Moreover, the columns of Bertin are necrosed. The main renal arteries—the lobar and the arciform—are generally spared. Microscopic examination shows pathologic changes appearing 48 to 72 hours after the initial injury. Glomeruli and tubules show extensive necrotic changes, whereas the afferent arterioles are occluded by thrombi extending to the interlobular arteries. At the periphery of the necrosis, large-scale infiltration of polymorphonuclear leukocytes fully develops after 3 to 4 days.

In addition to the pattern of complete cortical necrosis, Sheehan and Moore (373) described other forms of acute cortical necrosis characterized by more limited necrotic changes, the so-called incomplete acute cortical necrosis. The latter includes the focal form, in which the necrotic lesion can reach a diameter ranging from 3.0 to 0.5 mm, and the patchy form, with much larger necrotic areas. The authors described an additional variant of acute cortical necrosis called confluent focal cortical necrosis, which differs from the forms previously described in the following aspects: (a) The focal lesions are so numerous that they merge with one another, (b) the typical changes are present in tubules and glomeruli but not in the arterioles and arteries and appear at different stages in the course of the disease, and (c) the pattern is not associated with abruptio placentae.

Histologically, the lesions of incomplete cortical necrosis are essentially the same as those in the complete form. The edge of the necrotic area forms a sharp border with normal renal tissue. In the late phases of the disease, kidneys are reduced in size, interstitial fibrosis occurs in the injured areas, and sclerotic substitutions occur in glomeruli and in vessels. Calcium deposits detected by Kossa stain can be found in glomeruli or in arteries.

Obviously the organ most often affected by acute cortical necrosis is the kidney; however, though to a lesser extent and more mildly, other organs are sometimes injured too, such as the adrenals, spleen, liver, large intestine, and particularly the pituitary gland sinusoids (372,424–426).

Prognosis and Treatment

The course of acute cortical necrosis is characterized by death during the first days of the disease unless dialysis treatment is undertaken. Death was almost unavoidable in the past before dialysis and better management of acute renal failure (372,386). In one of the largest series, reported by Kleinknecht et al. (372), 21 of 38 patients died. However, almost all these patients were seen before dialysis was available for the treatment of acute renal failure. The most typical clinical course, especially in the obstetric forms, is characterized by prolonged oliguria requiring dialysis. After a period of 1 to 3 months, renal function may partially recover, so that patients become dialysis independent. Urine output progressively increases, and renal function may improve over a period of 1 to 2 years, to a final plateau of 20 to 25 mL/minute (380,417). Hypertrophy of the juxtamedullary nephrons has been suggested as a factor contributing to the partial restoration of renal function (380).

A large percentage of patients require chronic dialysis, and these patients are probably those with the most severe and diffuse form of acute cortical necrosis. In this context two opposite possibilities must be mentioned: (a) the late return to dialysis a number of years after the occurrence of cortical necrosis; and (b) the late resumption of a degree of renal function sufficient to maintain patients off dialysis (380). Many patients have received renal transplants, and the prognosis of such patients has greatly improved during the last few years (417).

No specific therapeutic approaches are available for acute cortical necrosis. All the supportive maneuvers commonly employed in ARF are performed. Substitutive treatment must be started as early as possible, and daily dialytic therapy may be necessary considering the high catabolic rate often present in these patients. In experimental models of cortical necrosis, many measures favorably affect pathologic changes. These include employment of anticoagulants, general anesthesia, β-blocker agents, mannitol-induced diuresis, and the use of nitrogen mustards to induce leukopenia (416,427–430). However, none of these measures has been employed with unequivocal beneficial effects in humans.

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CHAPTER 68 ■ VASCULITIC DISEASES OF THE KIDNEY

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The last decade has witnessed an explosion of knowledge in the field of vasculitis. The advent of antineutrophil cytoplasmic antibodies (ANCA) has led to new approaches in the clinical diagnosis of the vasculitides. There has been a reclassification of vasculitis, and modern tools of molecular immunology are shedding light on the pathogenesis of these disorders. Treatment strategies are slowly changing, no longer aimed only at improving patient and renal survival, but also at decreasing the short- and long-term toxicity of current therapies. This chapter focuses on the many facts of small-, medium-, and large-vessel vasculitis.

DIAGNOSTIC CLASSIFICATION AND PATHOLOGY OF VASCULITIDES

Vasculitis can affect any vessels in the body and thus can cause various clinical signs and symptoms. Most of these manifestations are indicative of vessel involvement in a particular organ rather than a specific pathologic category of disease. Therefore, vasculitis cannot be accurately diagnosed on the basis of clinical features alone. Serologic and other laboratory data can be very helpful in narrowing the differential diagnosis or providing additional support to a presumptive diagnosis, but data are rarely definitive. As with all tissues, vessels have a limited number of nonspecific patterns of response to injury. For example, many different inflammatory stimuli cause histologically indistinguishable acute and chronic inflammation with and without necrotizing or granulomatous features. To further complicate pathologic evaluation, vasculitic lesions evolve through various stages of active (Fig. 68-1) and sclerosing injury (Fig. 68-2). Specific categories of vasculitis have a particular predilection for involvement of certain types of vessels, although there is so much overlap that type of vessel involvement alone does not provide adequate categorization (Table 68-1, Fig. 68-3). Therefore, vasculitis cannot be diagnosed accurately on the basis of pathologic features alone, especially if these are evaluated only by routine light microscopy. The best current approach to a specific diagnosis is to combine clinical, laboratory, and histologic data to identify distinctive clinicopathologic categories of vasculitis. Vasculitis categorization schemes will certainly be improved in the future; however, current systems provide valuable guidance for prognostication and for determining the most effective management strategy.

There are a number of approaches to the diagnostic categorization of vasculitis. The system that we use in this chapter is the so-called Chapel Hill Nomenclature System, which was agreed on by an international group of clinicians and pathologists with a special interest in vasculitis (Table 68-2) (1).

Knowledge of the historical evolution of vasculitis categorization is helpful to understand the current diagnostic criteria for the classification of vasculitides. The following discussion of diagnostic classification includes a brief review of the historical events in the recognition of each category. The discussion is divided into sections dealing with large-vessel vasculitis, medium-sized vessel vasculitis, and small-vessel vasculitis (Tables 68-1, 68-2, Fig. 68-3). Large-vessel vasculitides were first recognized because of the reduced pulses and ischemic manifestations caused by chronic narrowing of major arteries. Medium-sized vessel vasculitides were first recognized because of the pseudoaneurysms caused by necrotizing lesions of medium-sized arteries, and small-vessel vasculitides were first recognized because of the glomerulonephritis and purpura caused by involvement of glomerular capillaries and dermal venules, respectively. Most of the discussion focuses on small-vessel vasculitides because these cause a higher frequency of renal disease.

Large-Vessel Vasculitis: Takayasu's Arteritis and Giant Cell Arteritis

Large-vessel vasculitis affects the aorta and its major branches, such as the arteries to the extremities and to the head and neck (1,2). During the acute phase of disease, large-vessel vasculitis is characterized pathologically by chronic granulomatous inflammation that often contains giant cells in the inflammatory infiltrates during the active phase of disease. The chronic phase is characterized by extensive vascular sclerosis with little or no active inflammation. Inflammatory and sclerotic thickening of the aorta and the arteries causes narrowing of lumina, which in turn causes ischemia and the resultant clinical manifestations. Involvement of the renal artery may cause renovascular hypertension. The two major categories of large-vessel vasculitis are Takayasu's arteritis and giant cell arteritis.

In 1856, William Savory described patients with diminished peripheral pulses who probably had Takayasu's arteritis involving the major arteries to the extremities (3). However, this category of vasculitis is named for Mikito Takayasu, a Japanese ophthalmologist who reported the ocular ischemic effects of this chronic granulomatous arteritis in 1908 (4). Takayasu's arteritis, which also includes "aortic arch syndrome" and "pulseless disease," most often involves the aorta and its major branches, although the pulmonary arteries may be affected (2,5–7). Takayasu's arteritis is most common in Asia, although it occurs worldwide. It rarely occurs in patients older than 40 years and is usually diagnosed during the second decade of life. Clinically, it often presents with reduced pulses, vascular bruits, claudication, and renovascular hypertension.

Giant cell arteritis rarely occurs in patients younger than 50 years and is most common in patients of northern European ethnicity (8,9). Like Takayasu's arteritis, giant cell arteritis affects the aorta and its major branches; however, it has a much greater predilection for the extracranial branches of the carotid artery. Frequent clinical manifestations include headache, jaw claudication, blindness, deafness, tongue dysfunction, extremity claudication, and reduced peripheral pulses. Pathologic

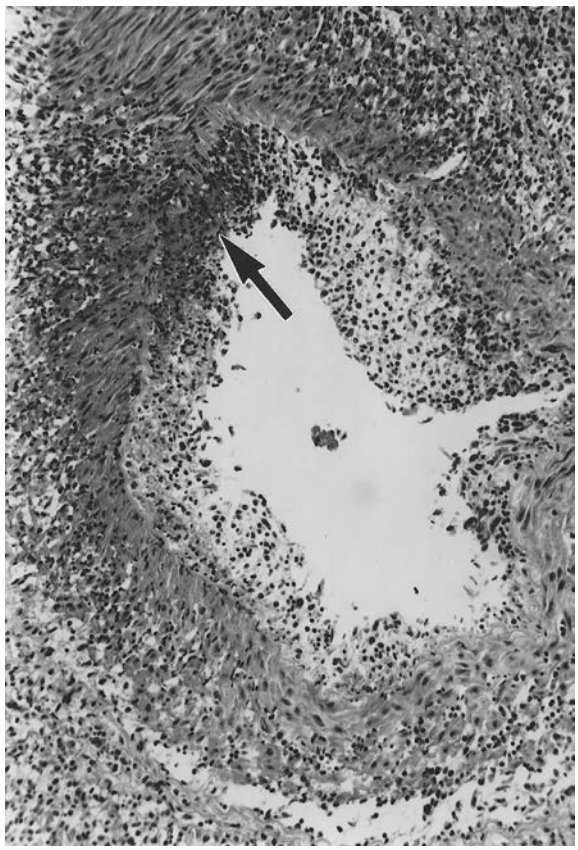

FIGURE 68-1. Acute necrotizing arteritis affecting a renal interlobar artery in a patient with Kawasaki's disease. There is (*arrow*) transmural inflammation and necrosis. (Hematoxylin and eosin stain, magnification ×125.)

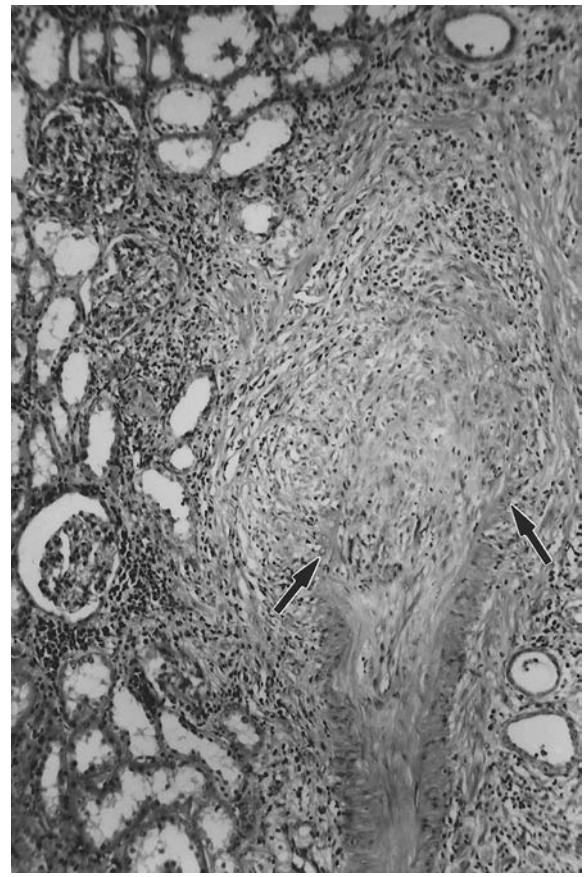

FIGURE 68-2. Chronic scarring in an arcuate artery from a patient with polyarteritis nodosa. The muscularis is completely destroyed (*arrows*), indicating that the sclerosis is secondary to a necrotizing arteritis rather than severe arteriosclerosis. (Hematoxylin and eosin stain, magnification ×75.)

involvement of the renal artery is common in giant cell arteritis, but symptomatic renovascular hypertension is rare. This is in contradistinction to Takayasu's arteritis, which often causes renovascular hypertension.

Giant cell arteritis has also been called "temporal arteritis," partly because one of the earliest descriptions of this type of vasculitis in 1890 by Hutchinson emphasized temporal artery involvement (10). However, the term "giant cell arteritis" is more appropriate than "temporal arteritis" because a) not all patients with giant cell arteritis have temporal artery involvement and b) vasculitides other than giant cell arteritis can cause temporal artery inflammation, such as polyarteritis nodosa, Wegener's granulomatosis, and microscopic polyangiitis (MPA) (1). If a patient with clinical manifestations of temporal artery inflammation is found by temporal artery biopsy to have a necrotizing rather than a granulomatous arteritis, the differential diagnosis should include polyarteritis nodosa, MPA, Wegener's granulomatosis, and other forms of necrotizing vasculitis. The frequent association of polymyalgia rheumatica with giant cell arteritis is a useful diagnostic aid, although not all patients with giant cell arteritis have polymyalgia rheumatica and not all patients with polymyalgia rheumatica have giant cell arteritis.

Takayasu's arteritis and giant cell arteritis cannot be accurately distinguished on the basis of pathologic evaluation of involved arteries. Polymyalgia rheumatica and involvement of branches of the carotid artery are more in favor of giant cell arteritis, and preferential involvement of the aorta and arteries to the extremities is slightly in favor of Takayasu's arteritis. However, the best diagnostic discriminator is age. If a patient with clinical or pathologic features of chronic granulomatous arteritis is older than 50 years, a diagnosis of giant cell arteritis is warranted, whereas a diagnosis of Takayasu's arteritis is warranted if the patient is younger than 50 years (1). The presence of renovascular hypertension in a child or a young adult is suggestive of Takayasu's arteritis. In a patient older than 50 years, renal artery involvement by a chronic sclerosing process is more likely secondary to atherosclerosis than to giant cell arteritis, and Takayasu's arteritis is essentially ruled out by the age of the patient.

Medium-Sized Vessel Vasculitis: Polyarteritis Nodosa and Kawasaki's Disease

The medium-sized vessel vasculitides are necrotizing arteritides that have a predilection for arteries that lead to major viscera and their initial branches. In the kidneys, the major targets are the interlobar and arcuate arteries, with less frequent involvement of the main renal artery and interlobular arteries (Fig. 68-3). The two major categories of medium-sized vessel vasculitis are polyarteritis nodosa and Kawasaki's disease. Pathologically, both are characterized in the acute phase by necrotizing arteritis with transmural inflammation that initially includes neutrophils and foci of fibrinoid necrosis (Fig. 68-1). The acute necrotizing inflammation often erodes completely through the artery wall and into the adjacent perivascular tissue, thereby forming a pseudoaneurysm (Fig. 68-4). Secondary

TABLE 68-1

MAJOR DIAGNOSTIC CATEGORIES OF VASCULITIS

Large-vessel vasculitis (chronic granulomatous arteritis)
Giant cell arteritis
Takayasu's arteritis
Medium-sized vessel vasculitis (necrotizing arteritis)
Polyarteritis nodosa
Kawasaki disease
Small-vessel vasculitis (necrotizing polyangiitis)
Pauciimmune small-vessel vasculitis (usually antineutrophil cytoplasmic antibody (ANCA) positive)
Microscopic polyangiitis
Wegener's granulomatosis
Churg-Strauss syndrome
Drug-induced ANCA vasculitis
Immune complex small-vessel vasculitis
Henoch-Schönlein purpura
Cryoglobulinemic vasculitis
Lupus vasculitis
Rheumatoid vasculitis
Goodpasture's syndrome
Serum sickness vasculitis
Hypocomplementemic urticarial vasculitis
Drug-induced immune complex vasculitis
Infection-induced immune complex vasculitis
Behçet's disease
Paraneoplastic small-vessel vasculitis
Lymphoproliferative neoplasm-induced vasculitis
Carcinoma-induced vasculitis
Myeloproliferative neoplasm-induced vasculitis
Inflammatory bowel disease vasculitis

complications of the arteritis include thrombosis, infarction, and hemorrhage. In only a few days, the lesions evolve from an acute neutrophil-rich inflammation to a "chronic" inflammation with predominantly mononuclear leukocytes. Sites of thrombosis and necrosis develop progressive scarring (Fig. 68-2). By definition, medium-sized vessel vasculitides do not cause glomerulonephritis, although they can cause hematuria, proteinuria (usually less than 2 g per 24 hours), and renal insufficiency as a result of renal infarction. Pseudoaneurysms near the renal surface may rupture and cause severe, even fatal, retroperitoneal and intraperitoneal hemorrhage.

The meaning of the diagnostic term "polyarteritis nodosa" has evolved over the past century, and substantial confusion continues over how best to use it (11). The problem and the solution that we propose is best understood in historical context. Systemic necrotizing arteritis was first clearly described by Kussmaul and Maier in the mid-1800s (12). They reported a patient with widespread visceral nodules caused by acute inflammation of arteries and called the process "periarteritis nodosa." Ferrari introduced the term "polyarteritis nodosa" (13), which is more appropriate because the inflammation is transmural rather than perivascular. For approximately 50 years, essentially all patients with any pattern of necrotizing arteritis were included in the polyarteritis nodosa category. During the early to mid-1900's, astute investigators recognized that many patients with necrotizing arteritis had distinctive distributions of vascular inflammation or characteristic pathologic processes that warranted their separation from patients with arteritis alone. For example, Kawasaki's disease, Wegener's granulomatosis, Churg-Strauss syndrome, and MPA were initially included in the category of polyarteritis nodosa but now are recognized as distinct entities (1). The removal of these vasculitides from the polyarteritis nodosa category is justified not only on the basis of different patterns and distributions of vessel involvement, but also because they have different natural histories, prognoses, and treatment requirements.

The reduction of polyarteritis nodosa to a more homogeneous and clinically useful category of vasculitis began when Arnaout, among others, recognized that some patients with necrotizing arteritis had lesions that could be seen only by microscopic examination (14). In about 1950, Zeek et al. (15,16) and Godman and Churg (17) carried out careful evaluations of patients with arteritis and concluded that polyarteritis nodosa should be separated from the "microscopic" form of vasculitis that was characterized by involvement of not only small arteries but also venules and capillaries. As discussed later in the section on small-vessel vasculitis, Godman and Churg also concluded that polyarteritis nodosa was distinct not only from MPA but also from Wegener's granulomatosis and Churg-Strauss syndrome, and that MPA, Wegener's granulomatosis, and Churg-Strauss syndrome were related to one another.

The diagnostic approach that we advocate defines polyarteritis nodosa as necrotizing inflammation of medium-sized or small arteries without glomerulonephritis or vasculitis in arterioles, capillaries, or venules (Table 68-2.) (1). This allows the separation of polyarteritis nodosa from other types of vasculitis, such as Wegener's granulomatosis, MPA, and Churg-Strauss syndrome, which have necrotizing arteritis as a component of a systemic polyangiitis that affects capillaries, venules, and arteries. By using this approach, the presence of glomerulonephritis rules out a diagnosis of polyarteritis nodosa and indicates the presence of some type of small-vessel vasculitis. In Table 68-3 are compared some of the features of polyarteritis nodosa and MPA. Note that glomerular capillaritis (glomerulonephritis) or pulmonary alveolar capillaritis with pulmonary hemorrhage rule out a diagnosis of polyarteritis nodosa and raise the possibility of MPA. Peripheral neuropathy is not a discriminator, because involvement of small epineural arteries in peripheral nerves may occur with polyarteritis nodosa or MPA. As we discuss in more detail later, testing for ANCA is useful for distinguishing between polyarteritis nodosa and the ANCA-associated small-vessel vasculitides (11,18–21). In a patient with necrotizing arteritis, a positive ANCA result decreases the likelihood of polyarteritis nodosa and increases the likelihood of MPA, Wegener's granulomatosis, or Churg-Strauss syndrome, i.e.; increases the likelihood that the patient has or will develop necrotizing inflammation of vessels other than arteries, such as pulmonary alveolar capillaritis or glomerular capillaritis (glomerulonephritis).

Necrotizing arteritis that is pathologically indistinguishable from the necrotizing arteritis of polyarteritis nodosa can occur in patients with Kawasaki's disease (Figs. 68-1, 68-3). Kawasaki's disease is an acute febrile illness of childhood that is characterized by the mucocutaneous lymph node syndrome, which includes nonsuppurative lymphadenopathy, polymorphous erythematous rash, erythema of the oropharyngeal mucosa, erythema of the palms and soles, conjunctivitis, and indurative edema and desquamation of the extremities (22–25). A major cause for morbidity and mortality in patients with Kawasaki's disease is the development of a necrotizing arteritis (24–27). This arteritis has a predilection for coronary arteries but can occur anywhere, including the kidney (Figs. 68-1, 68-3). Symptomatic renal involvement is rare in Kawasaki's disease.

The treatment of Kawasaki's disease is different from the treatment of polyarteritis nodosa. Kawasaki's disease usually is treated with aspirin and intravenous γ-globulin therapy (28–31), whereas polyarteritis nodosa usually is treated with high-dose corticosteroids and cyclophosphamide. Therefore, differentiation between the arteritis of Kawasaki's disease and that of polyarteritis nodosa is very important. The presence or absence of the mucocutaneous lymph node syndrome is an effective diagnostic discriminator between Kawasaki's disease and polyarteritis nodosa.

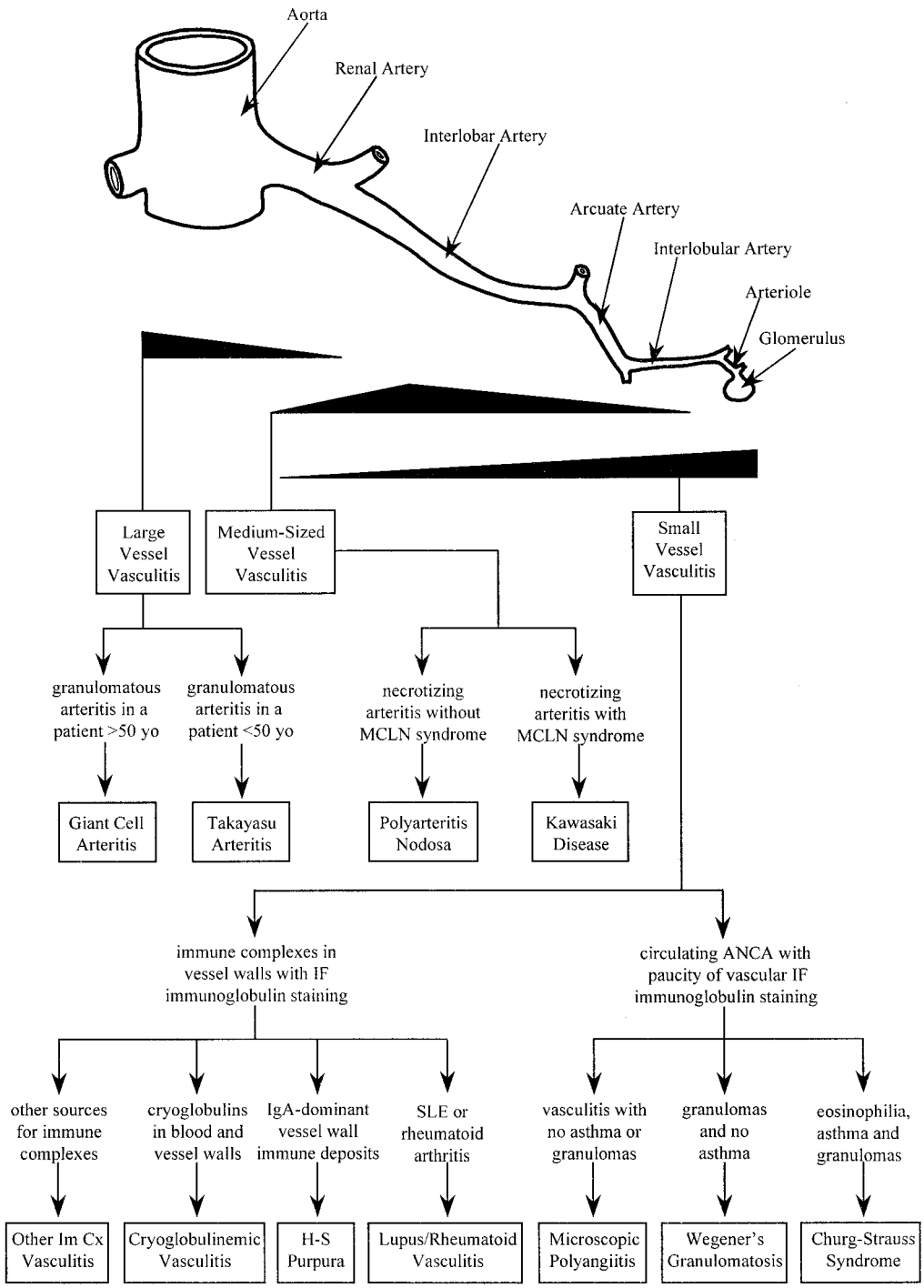

FIGURE 68-3. Predominant vascular involvement by large-vessel vasculitides, medium-sized vessel vasculitides, and small-vessel vasculitides as indicated by the positions and heights of the solid triangles. The algorithm suggests clinical and pathologic features that discriminate among different diagnostic categories of vasculitis. *yo*, years old; *MCLN*, mucocutaneous lymph node syndrome; *IF*, immunofluorescence microscopy; *ANCA*, antineutrophil cytoplasmic autoantibodies; *Im Cx*, immune complex; *SLE*, systemic lupus erythematosus; *H-S*, Henoch-Schönlein. (From: Jennette JC, Falk RJ. Renal involvement in systemic vasculitis. In: Greenberg A, Cheung AK, Coffman TM, et al, eds. *National Kidney Foundation Nephrology Primer.* 2nd ed. San Diego, CA: Academic Press; 1998:200, with permission.)

Small-Vessel Vasculitis: Microscopic Polyangiitis, Wegener's Granulomatosis, and Churg-Strauss Syndrome

Small-vessel vasculitides are characterized by necrotizing inflammation of multiple types of vessels. Arteries, veins, arterioles, venules, and capillaries may be affected; however, venules and capillaries are the most frequent targets (11). An understanding of the small-vessel vasculitides is important for nephrologists because these diseases often involve the kidneys and frequently cause glomerulonephritis (11,32). All of the small-vessel vasculitides listed in Table 68-1 can involve the kidneys. As mentioned earlier in the discussion about the

TABLE 68-2

NAMES AND DEFINITIONS OF VASCULITIS ADOPTED BY THE CHAPEL HILL CONSENSUS CONFERENCE ON THE NOMENCLATURE OF SYSTEMIC VASCULITIS

Large-vessel vasculitis[a]	
Giant cell arteritis	Granulomatous arteritis of the aorta and its major branches, with a predilection for the extracranial branches of the carotid artery. Often involves the *temporal artery. Usually* occurs in patients older than 50 years and often is associated with *polymyalgia rheumatica*.
Takayasu's arteritis	Granulomatous inflammation of the aorta and its major branches. Usually occurs in patients younger than 50 years.
Medium-sized vessel vasculitis[a]	
Polyarteritis nodosa	Necrotizing inflammation of medium-sized or small arteries without glomerulonephritis or vasculitis in arterioles, capillaries, or venules.
Kawasaki's disease	Arteritis involving large, medium-sized, and small arteries, and associated with mucocutaneous lymph node syndrome. *Coronary arteries are often involved.* Aorta and veins may be involved. Usually occurs in children.
Small-vessel vasculitis[a]	
Wegener's granulomatosis[b]	Granulomatous inflammation involving the respiratory tract, and necrotizing vasculitis affecting small-to medium-sized vessels, for example, capillaries, venules, arterioles, and arteries. Necrotizing glomerulonephritis is common.
Churg-Strauss syndrome[b]	Eosinophil-rich and granulomatous inflammation involving respiratory tract and necrotizing vasculitis affecting small-to medium-sized vessels, and associated with asthma and blood eosinophilia.
Microscopic polyangiitis[b]	Necrotizing vasculitis with few or no immune deposits affecting small vessels, for example, capillaries, venules, or arterioles. Necrotizing arteritis involving small- and medium-sized arteries may be present. Necrotizing glomerulonephritis is very common. Pulmonary capillarities often occurs.
Henoch-Schönlein purpura	Vasculitis with IgA-dominant immune deposits affecting small vessels, for example, capillaries, venules, or arterioles. Typically involves skin, gut, and glomeruli and is associated *with arthralgias or arthritis.*
Cryoglobulinemic vasculitis	Vasculitis with cryoglobulin immune deposits affecting small vessels, for example, capillaries, venules, or arterioles, and associated with cryoglobulins in serum. *Skin and glomeruli are often involved.*
Cutaneous leukocytoclastic angiitis	Isolated cutaneous leukocytoclastic angiitis without systemic vasculitis or glomerulonephritis.

[a] "Large artery" refers to the aorta and the largest branches directed toward major body regions (e.g., to the extremities and the head and neck); "medium-sized artery" refers to the main visceral arteries (e.g., renal, hepatic, coronary, and mesenteric arteries); and "small artery" refers to the distal arterial radicals that connect with arterioles. Note large and medium-sized vessel vasculitides do not involve vessels other than arteries.
[b] Strongly associated with ANCA.
(From: Jennette JC, Falk RJ, Andrassy K, et al. Nomenclature of systemic vasculitides. Proposal of an international consensus conference. *Arthritis Rheum* 1994;37:187, with permission.)

evolution of the definition of polyarteritis nodosa, small-vessel vasculitides with arterial involvement once were subsumed in the polyarteritis nodosa category. Zeek et al. (15,16) and Godman and Churg (17) were among the first to recognize that vasculitides that involve capillaries and venules in addition to arteries have clinical and pathologic features that are clearly distinct from those of polyarteritis nodosa.

The two major categories of small-vessel vasculitis include the "pauciimmune small vessel vasculitides" and the "immune complex–mediated small vessel vasculitides" (Table 68-1) (11). Immune complex–mediated vasculitides, such as Henoch-Schönlein purpura (HSP), cryoglobulinemic vasculitis, lupus vasculitis, and antiglomerular basement membrane (anti-GBM) vasculitis, have extensive localization of immunoglobulin and complement in vessel walls as a consequence of deposition of circulating immune complexes or *in situ* immune-complex formation between circulating antibodies and planted or constitutive antigens. The pauciimmune small-vessel vasculitides have little or no vascular wall localization of immunoglobulins (11,33–35). The pauciimmune small-vessel vasculitides often have necrotizing and crescentic glomerulonephritis as a component of the systemic necrotizing vasculitis. A pathologically identical pauciimmune necrotizing and crescentic glomerulonephritis also occurs as a renal limited process, which has sometimes been referred to as "idiopathic crescentic glomerulonephritis" or "renal vasculitis" (11,34–38). Pauciimmune crescentic glomerulonephritis, which usually is a component of systemic pauciimmune small-vessel vasculitis, is the most common type of crescentic glomerulonephritis (Table 68-4).

The three major categories of systemic pauciimmune small-vessel vasculitis are MPA, Wegener's granulomatosis, and Churg-Strauss syndrome (CSS) (17,34,39). Table 68-2 and Figure 68-3 provide an approach for differentiating these three vasculitides and for distinguishing them from other types of vasculitis (11). MPA, Wegener's granulomatosis, and Churg-Strauss syndrome share a histologically identical necrotizing vasculitis that can affect arteries (Fig. 68-5), arterioles (Fig. 68-6), venules (Fig. 68-7), and capillaries, especially glomerular capillaries (Fig. 68-8). At all of these sites, the acute lesion is characterized by segmental necrosis with mural and perivascular fibrinoid material, sometimes accompanied by thrombosis in the vascular lumen. The initial inflammatory infiltrate has conspicuous neutrophils, often undergoing leukocytoclasia (Fig. 68-7), but this usually transforms into a predominantly mononuclear leukocyte infiltrate within a few days. The

FIGURE 68-4. Acute necrotizing arteritis affecting a renal interlobar artery in a patient with Kawasaki's disease. The necrotizing inflammation (*arrows*) has eroded into the perivascular tissue to produce a pseudoaneurysm. (Hematoxylin and eosin stain, magnification ×350.)

TABLE 68-3

CLINICAL DIFFERENCES BETWEEN POLYARTERITIS NODOSA AND MICROSCOPIC POLYANGIITIS

Clinical feature	Polyarteritis nodosa	Microscopic polyangiitis
Rapidly progressive nephritis	No	Very common
Pulmonary hemorrhage	No	Yes
Peripheral neuropathy	Yes	Yes
Microaneurysms by angiography	Yes	Rare
Renovascular hypertension	Occasional	No
Positive hepatitis B serology	Uncommon	No
Positive antineutrophil cytoplasmic antibodies serology results	Rare	Frequent
Relapses	Rare	Frequent

(From: Guillevin L, Lhote F, Amouroux J, et al. Antineutrophil cytoplasmic antibodies, abnormal angiograms and pathological findings in polyarteritis nodosa and Churg-Strauss syndrome: Indications for the classification of vasculitides of the polyarteritis nodosa group. *Br J Rheumatol* 1996;35:958, with permission.)

glomerular lesion of pauciimmune ANCA-associated glomerulonephritis begins with segmental fibrinoid necrosis (Fig. 68-8) that quickly leads to crescent formation (Fig. 68-9). At the time of renal biopsy, approximately 90% of patients with pauciimmune crescentic glomerulonephritis have some degree of glomerular necrosis and crescent formation, although this may involve fewer than 50% of glomeruli (34,40). Arteritis, arteriolitis, and medullary angiitis are seen in less than 20% of renal biopsy specimens. Medullary angiitis can be severe enough to cause focal papillary necrosis.

In addition to the renal vasculitic lesions illustrated in Figures 68-5–68-9, patients with all three systemic pauciimmune small-vessel vasculitides share histologically identical inflammatory vascular lesions in other tissues, such as pulmonary

TABLE 68-4

FREQUENCY OF IMMUNOPATHOLOGIC CATEGORIES OF CRESCENTIC GLOMERULONEPHRITIS IN MORE THAN 3,000 CONSECUTIVE NONTRANSPLANT RENAL BIOPSIES EVALUATED BY IMMUNOFLUORESCENCE MICROSCOPY IN THE UNIVERSITY OF NORTH CAROLINA NEPHROPATHOLOGY LABORATORY

	Any crescents ($N = 540$)	>50% crescents ($N = 195$)	Arteritis in biopsy ($N = 37$)
Immunohistology			
Pauciimune (<2 positive immunoglobulin)	51% (277/540)	61% (118/195)[a]	84% (31/37)
Immune complex (≤2 positive immunoglobulin)	44% (238/540)	29% (56/195)	14% (5/37)[c]
Antiglomerular basement membrane	5% (25/540)[b]	11% (21/195)	3% (1/37)[d]

[a] 70 of 77 patients tested for antineutrophilic cytoplasmic antibodies (ANCA. were positive (91%) (44 p-ANCA and 26 c-ANCA).
[b] 3 of 19 patients tested for ANCA were positive (16%) (2 p-ANCA and 1 c-ANCA).
[c] 4 patients had lupus and 1 poststreptococcal glomerulonephritis.
[d] This patient also had a p-ANCA (myeloperoxidase-ANCA).
(From: Jennette JC, Falk RJ. The pathology of vasculitis involving the kidney. *Am J Kidney Dis* 1991;24:130, with permission.)

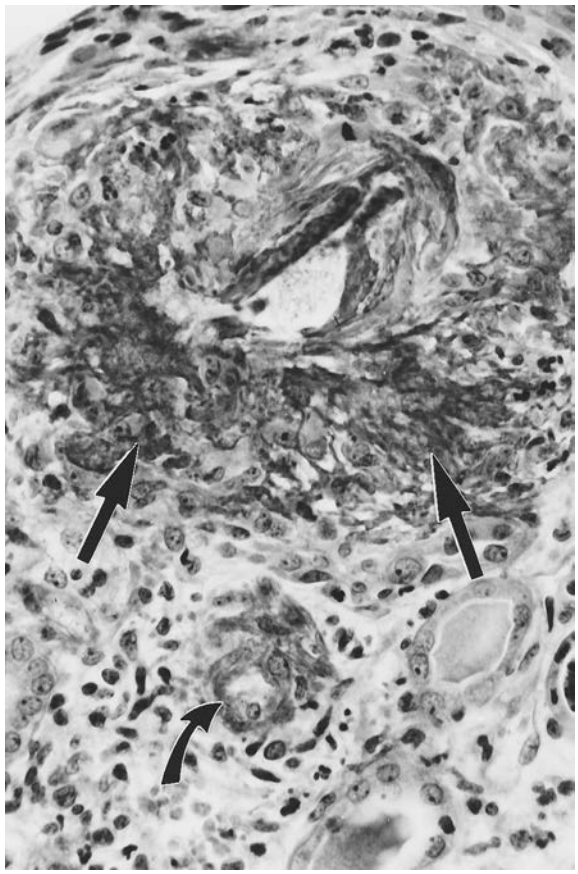

FIGURE 68-5. Necrotizing arteritis affecting an interlobular artery in a patient with microscopic polyangiitis. Note the extension of fibrinoid material into perivascular interstitium (*straight arrows*). Also note the necrotizing arteriolitis (*curved arrow*). (Masson trichrome, magnification ×350.)

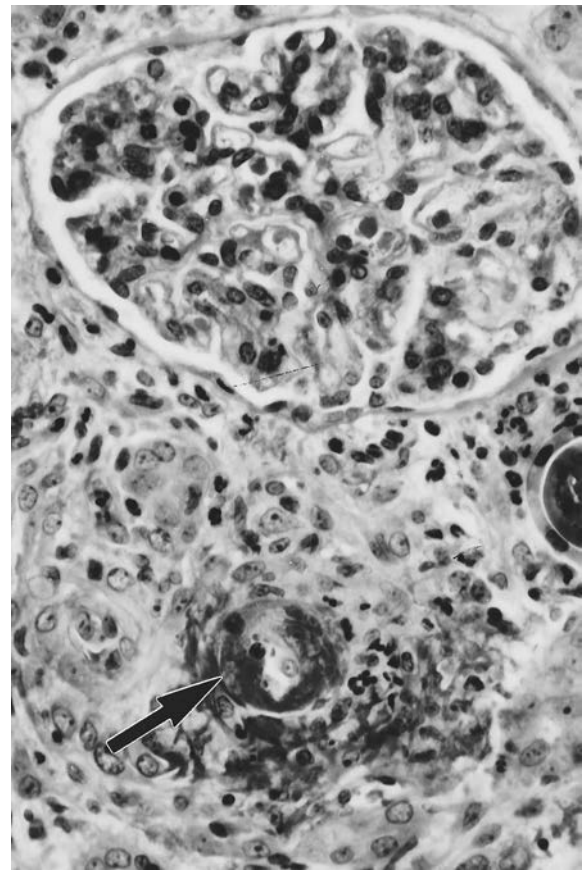

FIGURE 68-6. Necrotizing arteriolitis (*arrow*) affecting an arteriole in the renal cortex of a patient with microscopic polyangiitis. Note the fibrinoid material in the vessel wall and adjacent interstitium, and the focal perivascular leukocytoclasia. (Masson trichrome stain, magnification ×350.)

hemorrhagic alveolar capillaritis, dermal leukocytoclastic venulitis, and necrotizing inflammation of arteries in many tissues, including but not limited to peripheral nerves, skeletal muscle, gut, liver, pancreas, and skin. The diagnostic distinctions among the three diseases are not based on the pathologic or clinical features of vasculitis *per se*, but on the presence or absence of accompanying features, specifically granulomatous inflammation, asthma, and blood eosinophilia (1;11). As detailed in Table 68-2 and diagrammed in Figure 68-3, a diagnosis of MPA is warranted if a patient has systemic pauciimmune small-vessel vasculitis with no evidence for necrotizing granulomatous inflammation or asthma. A diagnosis of Wegener's granulomatosis is warranted if a patient has systemic pauciimmune small-vessel vasculitis with necrotizing granulomatous inflammation, usually in the upper or lower respiratory tract, and no asthma. A diagnosis of CSS is warranted if a patient has systemic pauciimmune small-vessel vasculitis with asthma and blood eosinophilia. Reaching these diagnostic conclusions does not necessarily require pathologic documentation of the lesions if reasonable clinical surrogates are identified. For example, in a patient with a renal biopsy that demonstrates pauciimmune necrotizing and crescentic glomerulonephritis, radiographic demonstration of cavitary lung nodules (in the absence of infection) or lytic bone lesions in the nasal septum are reasonable evidence for necrotizing granulomatous inflammation and thus warrant a diagnosis of Wegener's granulomatosis.

Serologic testing for ANCA is useful for making the diagnosis of pauciimmune small-vessel vasculitis or renal-limited pauciimmune crescentic glomerulonephritis. As discussed in more detail elsewhere in this chapter, the major types of ANCA are those that have specificity for proteinase 3 (PR3-ANCA) and for myeloperoxidase (MPO-ANCA) (18–21,41). In an indirect immunofluorescence microscopy assay, PR3-ANCA usually causes cytoplasmic staining of neutrophils (c-ANCA. and MPO-ANCA usually causes perinuclear staining (p-ANCA).

The clinical differential diagnosis of ANCA-associated pauciimmune small-vessel vasculitis also includes immune-complex small-vessel vasculitis. In Table 68-5 is demonstrated a great deal of overlap in organ system involvement among different types of pauciimmune small-vessel vasculitis and immune-complex small-vessel vasculitis. Upper or lower respiratory tract involvement has the greatest discriminatory value because respiratory tract involvement is common with pauciimmune small-vessel vasculitis and rare with immune-complex small-vessel vasculitis. In Table 68-6 and Figure 68-3 are detailed a number of observations that can be used to conclusively differentiate among MPA, Wegener's granulomatosis, CSS, cryoglobulinemic vasculitis, and HSP (11). Direct immunofluorescence microscopy of vessels in biopsy specimens, such as glomerular capillaries or dermal venules, is useful because this demonstrates immunoglobulin A (IgA)-dominant vascular immunoglobulin deposits in HSP, IgG and IgM deposits in cryoglobulinemic vasculitis, and little or no

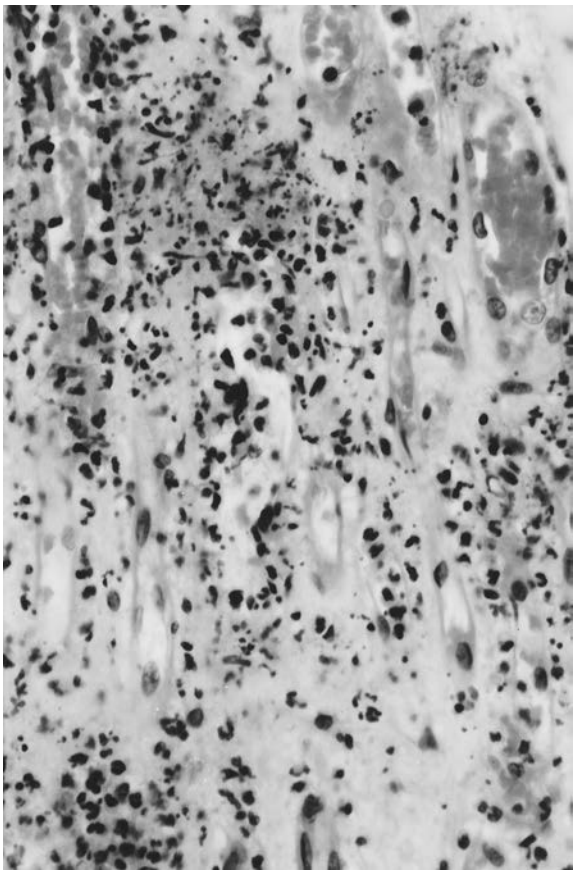

FIGURE 68-7. Leukocytoclastic medullary angiitis affecting the peritubular vasa recta in a patient with Wegener's granulomatosis. (Hematoxylin and eosin stain, magnification ×350.)

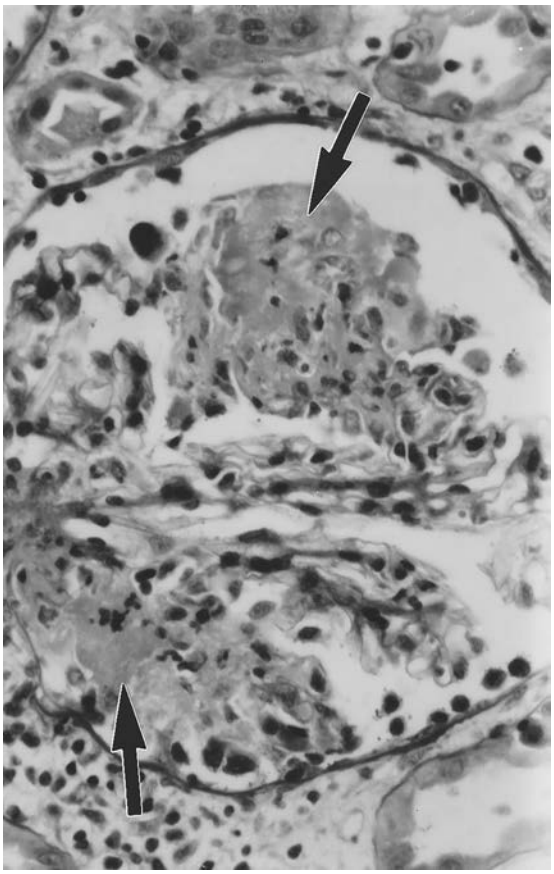

FIGURE 68-8. Segmental fibrinoid necrosis (*arrows*) in a glomerulus from a patient with microscopic polyangiitis. (Hematoxylin and eosin stain, magnification ×350.)

immunoglobulin in pauciimmune small-vessel vasculitis. Serologic testing also helps to focus the differential diagnosis, for example, testing for ANCA, anti-GBM, cryoglobulins, hepatitis C or B, antinuclear antibodies (ANA), and complement component levels.

In summary, the precise and accurate diagnosis of different categories of vasculitis, including small-vessel vasculitis, requires the knowledgeable integration of clinical, laboratory, and pathologic data.

EPIDEMIOLOGY OF SYSTEMIC VASCULITIS

In the past, the incidence of systemic vasculitis was difficult to determine, largely because of an inconsistent classification strategy. The advent of the Chapel Hill Nomenclature System allowed for a more precise estimation of incidence and prevalence. The incidence of giant cell arteritis varies from 15 to 30 per 100,000 in individuals older than 50 years. There is an increased incidence with age and a female-to-male ratio of 2:1 (42). Giant cell arteritis is more common in Caucasians and is uncommon in African Americans. Takayasu's arteritis has been described worldwide, but the disease is much more prevalent in Japan, where there are approximately 150 new cases per year. In Olmstead County, Minnesota, the incidence is 2.6 cases per million per year.

The incidence of the small-vessel vasculitides associated with ANCA (Wegener's granulomatosis, MPA, and CSS) appears to be on the order of 20 cases per million (42,43). In contrast, polyarteritis nodosa has become a rare disease. It is possible that the perceived increase in the incidence of MPA is a consequence of the development and widespread use of ANCA testing. Nonetheless, the incidence of MPA appeared to be more common in the 1990's than in the 1980's (19.8 versus 7 cases per million). Two interesting studies reported a much higher incidence of ANCA-associated vasculitis. One study reported a much higher incidence in Alaskan Indians, in which all cases were associated with hepatitis B (43). The other report from Kuwait after the Gulf War found an increased incidence of 16 cases per million of polyarteritis nodosa and 24 cases per million of MPA (44). The incidence of Wegener's granulomatosis was 0.7 per million per year from 1980 to 1986, increasing to 2.8 million per year from 1987 to 1989. In the 1990s, the prevalence of Wegener's granulomatosis was closer to 10.6 cases per million in the United Kingdom. The annual incidence of (CSS) is on the order of 4 cases per million (45). A recent study estimated the prevalence of polyarteritis nodosa (PAN), MPA, Wegener's granulomatosis and CSS in a large multiethnic suburb of Paris based on a three-source capture-recapture method. During the calendar year 2000, the prevalences per 1,000,000 adults were estimated to be 30 for PAN, 25 for MPA, 24 for Wegener's granulomatosis, and 11 for CSS. The overall prevalence was 2.0 times higher for subjects of European ancestry than for non-Europeans ($P = 0.01$) (46).

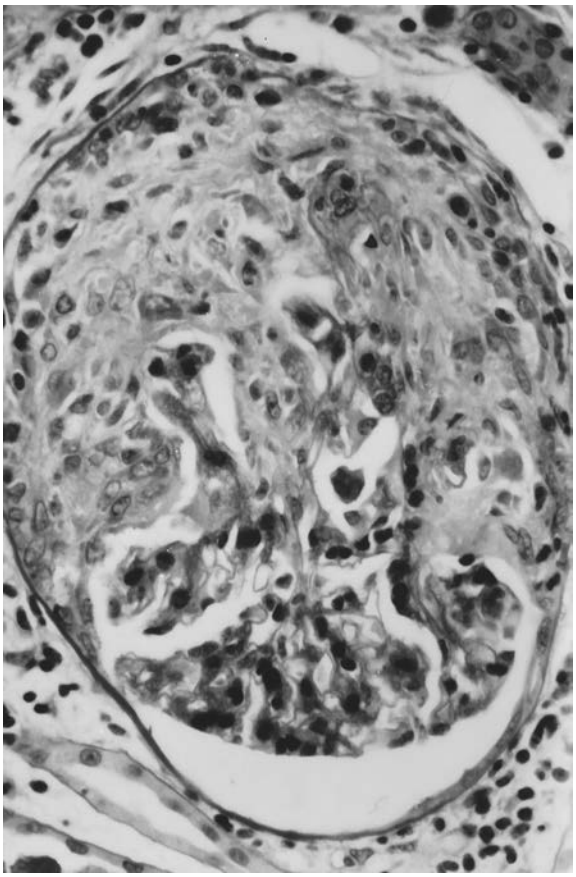

FIGURE 68-9. Cellular crescent in a glomerulus from a patient with microscopic polyangiitis. (Hematoxylin and eosin stain, magnification ×350.)

SMALL-VESSEL VASCULITIDES ASSOCIATED WITH ANCA

Demographics

ANCA-associated small-vessel vasculitis affects men and women equally. All ages are susceptible to disease, although the mean age of our population is 55 years. Almost 90% of our population is Caucasian, and 10% is African American. There appears to be a seasonal variation of the onset of disease, most commonly occurring in the late fall and early spring (47). It is possible but not proven that in northern latitudes, Wegener's granulomatosis predominates, whereas MPA is more common in southern climes.

Clinical Features

Renal Disease

The renal manifestations of small-vessel vasculitis are several. Many, if not most, patients present with rapidly progressive glomerulonephritis with hematuria, proteinuria, and a rising serum creatinine over the course of days to weeks. This clinical presentation is associated with the pathologic findings of glomerular necrosis and crescent formation. Invariably, interstitial inflammation results in interstitial fibrosis. At the other end of the spectrum are patients who present with much milder disease marked by isolated hematuria and low-grade proteinuria. On biopsy, these patients have focal areas of necrosis that result in areas of focal glomerulosclerosis.

Persistent microscopic hematuria can be the harbinger of different renal outcomes. In some patients, persistent hematuria correlates with focal inflammation in the kidney that with additional inflammatory stimuli (e.g., infection and environmental exposure. transforms into an aggressive acute nephritis. The acute nephritic presentation is usually associated with renal insufficiency, hypertension, and, on biopsy, with diffuse glomerular necrosis and crescent formation. In contrast, some patients with persistent microscopic hematuria without proteinuria have a clinically indolent disease that in the absence of a renal biopsy is ascribed to IgA nephropathy or thin basement membrane disease. Frequently, a renal biopsy is delayed until azotemia or significant proteinuria develops. By then, the biopsy reveals a picture of chronic glomerulonephritis with widespread glomerular sclerosis and only focal necrosis. Unfortunately, in such cases, the persistent microscopic hematuria was a reflection of unrecognized, unchecked glomerular inflammation. The treatment of such patients must be evaluated on a case-by-case basis depending on the degree of scarring and renal insufficiency, as the risks of aggressive antiinflammatory and immunosuppressive treatment may outweigh the potential benefits.

Proteinuria in ANCA glomerulonephritis is usually due to glomerular sclerosis or severe necrotizing and crescentic glomerulonephritis. In most cases of acute nephritis or rapidly progressive glomerulonephritis, the amount of proteinuria is on

TABLE 68-5

COMPARISON OF APPROXIMATE FREQUENCY OF MANIFESTATIONS OF MICROSCOPIC POLYANGIITIS WITH SEVERAL OTHER FORMS OF SMALL VESSEL VASCULITIS

	Microscopic polyangiitis	Wegener's granulomatosis	Churg-Strauss syndrome	Henoch-Schönlein	Cryoglobulin vasculitis
Cutaneous	40%	40%	60%	90%	90%
Renal	90%	80%	45%	50%	55%
Pulmonary	50%	90%	70%	<5%	<5%
Ear, nose, and throat	35%	90%	50%	<5%	<5%
Musculoskeletal	60%	60%	50%	75%	70%
Neurologic	30%	50%	70%	10%	40%
Gastrointestinal	50%	50%	50%	60%	30%

(From: Jennette JC, Falk RJ. Small vessel vasculitis. *N Engl J Med* 1997;337:1512, with permission.)

TABLE 68-6

FEATURES THAT ALLOW DIFFERENTIATION OF MICROSCOPIC POLYANGIITIS FROM SEVERAL OTHER FORMS OF SMALL-VESSEL VASCULITIS

	Henoch-Schönlein purpura	Cryoglobuline vasculitis	Microscopic polyangiitis	Wegener's granulomatosis	Churg-Strauss syndrome
Small-vessel vasculitis signs and symptoms[a]	+	+	+	+	+
IgA-dominant immune deposits	+	0	0	0	0
Cryoglobulins in blood and vessels	0	+	0	0	0
ANCA in blood	0	0	+	+	+
Necrotizing granulomas	0	0	0	+	+
Asthma and eosinophilia	0	0	0	0	+

[a] All of these small-vessel vasculitides can manifest any or all of the shared features of small-vessel vasculitides, such as purpura, nephritis, abdominal pain, peripheral neuropathy, myalgias and arthralgias. Each is distinguished by the presence and just as importantly the absence of certain specific features.
(From: Jennette JC, Falk R. Small vessel vasculitis. *N Engl J Med* 1997;337:1512, with permission.)

the order of 500 to 3,000 mg per 24 hours. The mean 24-hour urine protein excretion of our entire population at presentation is only 800 mg per 24 hours. Certainly there are cases of nephrotic-range proteinuria typically associated with diffuse glomerulosclerosis.

Acute interstitial nephritis is an unusual expression of small-vessel vasculitis. These patients generally present with pyuria and white blood cell casts without evidence of hematuria or proteinuria. In these cases, the glomeruli are completely spared, and inflamed vasa rectae are accountable for the clinical findings.

There are many examples of patients in whom ANCA-associated small-vessel vasculitis coexists with other forms of glomerular injury, the most common of these being anti-GBM disease. Typically these patients with both ANCA and anti-GBM have vasculitis affecting vascular beds other than the kidney and the lung. Their clinical course is more consistent with an ANCA glomerulonephritis than with anti-GBM disease. However, a recent retrospective analysis of patients with both ANCA and anti-GBM suggests that these patients have more severe renal disease and a poorer prognosis than patients with ANCA alone (48). It is unclear whether patients with both antibodies and severe renal failure share the poor renal prognosis of dialysis-dependent patients with anti-GBM disease alone. Indeed, some patients may respond to treatment with a dialysis-free interval of months to years.

Similarly, there are patients with ANCA-associated small-vessel vasculitis and immune complex forms of glomerulonephritis (e.g., membranous glomerulopathy or IgA nephropathy). Typically, the renal biopsy reveals areas of crescent transformation or glomerular necrosis. The clinical finding of a sudden decrease in renal function and worsening of the hematuria herald this pathologic event. ANCA glomerulonephritis may occur in patients with scleroderma, in whom the renal dysfunction is not attributable to a thrombotic microangiopathy, but to ANCA-induced necrosis and crescent formation.

As noted previously in this chapter, vessels larger than capillaries and venules are targets of inflammation. Small arteries, including the renal artery, can be injured. The most common clinical consequence of this process is renal infarction resulting in flank pain and renal insufficiency. Persistent disease of the renal artery causes stenosis and poststenotic dilation, with the clinical presentation of renovascular hypertension. Renal arteriography is necessary to delineate the degree of renal artery disease. Angioplasty or surgical correction of the stenotic vessel is frequently curative.

Skin Disease

Because the most commonly affected vessels of ANCA-associated small-vessel vasculitis are capillaries and postcapillary venules, the typical skin lesion is palpable purpura. Lesions tend to occur in "crops," primarily on the lower extremities. With time, the lesions flatten and either disappear or leave small hyperpigmented areas. In addition to this classic dermal presentation, there are several other cutaneous lesions, including petechiae, ecchymosis, ulceration, nodules, and plaquelike lesions, livido reticularis, and urticaria. Several cases of urticarial vasculitis have been observed in the absence of hypocomplementemia and immune-complex deposition. In unusual circumstances, vasculitic lesions give rise to erythema nodosum or pyoderma gangrenosum–like lesions. Biopsy of the affected area reveals a leukocytoclastic angiitis. However, in the case of nodular lesions, a simple punch biopsy may not provide sufficiently deep material to sample an involved vessel of larger caliber. A deep "excisional" biopsy is necessary.

The differential diagnosis of renal dermal vasculitic syndromes is important. In addition to the ANCA-associated small-vessel vasculitides, SLE, cryoglobulinemia, and HSP cause cutaneous vasculitides and renal disease. Each of these conditions is associated with a different natural history and treatment approach. For instance, many patients with HSP require only supportive care. If the renal dermal vasculitic syndrome is a consequence of ANCA-associated small-vessel vasculitis or lupus, immunosuppressive therapy is warranted. In addition, cutaneous vasculitis is frequently the consequence of a drug reaction. There are numerous classes of drugs that cause leukocytoclastic lesions, the most common being propylthiouracil, minocycline, phenytoin, and penicillamine.

Pulmonary Disease

The pulmonary consequences of ANCA-associated small vessel vasculitis are numerous and involve not only the lung parenchyma, but also the respiratory tract from the subglottis to the alveolar sacs. Several clinical presentations are common. Patients with MPA typically present with pulmonary infiltrates

that are frequently initially ascribed to an infectious process. Generally, these patients describe hemoptysis, although many patients have no overt evidence of pulmonary bleeding. In some cases, the infiltrates wax and wane spontaneously. Infiltrates may coalesce, resulting in dyspnea and hypoxemia. The most alarming consequence of pulmonary capillaritis is the development of massive pulmonary hemorrhage. In our series, the occurrence of pulmonary hemorrhage was the most powerful predictor of death (49). As is discussed later, the prompt institution of plasmapheresis substantially decreased the mortality rate when compared with conventional immunosuppressive treatments.

Necrotizing granulomatous inflammation is a hallmark of Wegener's granulomatosis. Focal necrotizing lesions progress to confluent areas of necrosis that when surrounded by palisading histocytes, are called "geographic necrosis" (50). Nodular lesions are of varying size, from those that can only be seen by spiral computed tomography (CT) to those that occupy a complete lobe of the lung. In general, the larger lesions tend to cavitate. The differential diagnosis must include aspergillus or tuberculous infection, as well as other opportunistic infections. Determining whether the nodular and cavitary lesions are due to granulomatous infection or are a consequence of an opportunistic infection can present a difficult diagnostic dilemma. These two processes may coexist at times.

Similarly, recurrent alveolar hemorrhage in immunosuppressed patients may be attributable to infection. Alveolar lavage is useful in determining an infectious cause of alveolar hemorrhage rather than as a consequence of small-vessel vasculitis alone. Bronchioalveolar lavage should be performed, carefully considering the possible deterioration in oxygenation immediately after the procedure. Careful attention to the protection of airway patency for modern ventilatory support is mandatory. Transbronchial biopsy of the lung in patients with these diseases often results in non-diagnostic results. Open lung biopsy or procedures play a role in the diagnosis of these diseases.

In addition to the characteristic pulmonary nodules of Wegener's granulomatosis, endobronchial lesions, similar to those found in the subglottic region and trachea, can cause airway obstruction and may result in areas of collapsed lung. The lesions are usually quite sensitive to systemic glucocorticosteroid treatment (51).

CSS is characterized by the presence of asthma and eosinophilia in the circulation and within tissues. The pulmonary infiltrates tend to result in diffuse alveolar involvement but nodules and cavitations also occur. Eosinophilic pneumonia of other causes can be indistinguishable from the pulmonary presentation of CSS. Therefore, it is important to verify that a small-vessel vasculitis exists before determining that the patient has CSS (52).

Patients with subglottic masses or stenosis present with stridor or a sense of breathlessness. Results of flow–volume loop study results are abnormal. These lesions necessitate emergent attention to avoid life-threatening critical airway narrowing. Direct laryngoscopy with fiberoptic instrumentation allows visualization of the lesion. Glucocorticoid treatment is usually effective, but surgical intervention may be necessary. Areas of tracheal and bronchial granulomatous lesions can occur throughout the respiratory tree and result in bronchial obstruction.

The long-term consequences of intermittent pulmonary capillaritis result in pulmonary fibrosis. In some individuals, the diagnosis of idiopathic pulmonary fibrosis prompts consideration of ANCA-associated small-vessel vasculitis. Similarly, some patients with bronchiolitis obliterans with organizing pneumonia have had an underlying small-vessel vasculitis.

Upper Respiratory Tract Disease

Vasculitis frequently affects the areas of the ear, nose, and throat. By far the most common localization occurs in the nose, especially in patients with Wegener's granulomatosis. Other areas of involvement include the nasopharynx, the paranasal sinuses, and within the larynx. With respect to the nose, persistent or repetitive episodes of rhinosinusitis are one of the first symptoms. Small ulcerations lead to a nasal discharge that becomes hemorrhagic. Once these lesions become inflamed, a thick purulent material oozes from bloody crusts covering much larger areas of ulceration and granulation tissue. Histologic evaluation of these tissues most commonly reveals nonspecific acute and chronic inflammation. With a good sample, one may find areas of fibrinoid necrosis or granulomatous inflammation. Focal areas of ischemia and infarction occur. Repetitive bouts of inflammation eventually lead to septal perforations, the loss of turbinates, and may result in a loss of support of the nasal bridge (saddle nose deformity of Wegener's granulomatosis). Even with treatment, the nasal mucosa becomes atrophic and crusty. The crusts cause epistaxis when patients sneeze or blow their nose. *Staphylococcus aureus* superinfections may be the root cause of these ulcerations and an important factor in their development. Topical treatment with antibacterial ointments or systemic treatment with antibiotics decreases nasal symptoms and limits the number of relapses (53).

Sinusitis typically occurs with bloody nasal or postnasal discharge. Computed tomography may reveal bony erosions caused by granulomatous lesions typical of Wegener's granulomatosis. Necrotizing capillaritis associated with MPA or CSS frequently causes necrotizing lesions in the sinuses as well but do not lead to bony erosions. Granulomatous inflammation that blocks the eustachian tubes leads to serous otitis media. Bacterial superinfections lead to infectious otitis media. Ventilating tubes placed in the tympanic membrane may lessen the problem. Facial nerve paralysis may occur as a result of entrapment of the nerve by granulomatous inflammation anywhere along the course of the nerve. Large granulomatous pseudotumors invade the orbit and may lead to loss of an eye.

Gastrointestinal Tract Disease

The gastrointestinal tract represents one of the least well-studied areas of involvement of small-vessel vasculitis. In our experience, at least one-third of patients with active necrotizing glomerulonephritis have abdominal complaints, either on presentation or at some point during the course of disease. One of the more common areas of vasculitic involvement is the gastric mucosa, causing non-healing gastric or peptic ulcers. It is sometimes difficult to determine whether these ulcers are the consequence of vascular inflammation or glucocorticoid treatment. Endoscopic biopsy can provide a diagnosis, yet a presumptive diagnosis is made by a favorable response to glucocorticoid therapy. Similarly, pancreatitis, small bowel infarction, and ulcers throughout the gastrointestinal tract lead to abdominal pain. The most catastrophic of all abdominal vasculitic disease is transmural infarction of the bowel, leading to viscus perforation and polymicrobial sepsis. Prompt diagnosis and treatment is mandatory.

Autoimmune hepatitis and sclerosing cholangitis occur with p-ANCA that is not specific for MPO. The liver is usually not involved with necrotizing vasculitis.

Patients with small-vessel vasculitis may have medium-sized artery involvement as well. Aneurysmal dilation and fibrinoid necrosis of mesenteric or renal arteries results in infarction of the affected organ. If a mesenteric artery is involved, infarctions cause substantial abdominal pain or an ischemic colitis. These

patients require mesenteric arteriography to identify the areas of arteritic involvement.

Neurologic Disease

Mononeuritis multiplex, or a pattern of multiple mononeuropathies, is caused by nerve impairment in anatomically separate regions. Most commonly, these areas of peripheral nerve ischemia are found in areas in the midthigh or mid upper arm, in watershed zones of poor vascular perfusion. Lesions of peripheral neuropathy occur abruptly and are very painful. The pain is described as a deep ache that is difficult to localize. Symptoms of the cutaneous distribution of the nerve occur several days after the onset of weakness and are described as a burning pain. Nerve biopsy should be performed in individuals with neuropathy as the major manifestation of vasculitis. A negative sural nerve biopsy result does not rule out the diagnosis. Repetitive biopsy of the nerve is almost useless. In a series of 200 patients with vasculitis and a neuropathy, only 27% had a vasculitis demonstrated in a muscular specimen only, 35% in a nerve only, and 27% in the nerve and the muscle (54). Many patients develop distal peripheral sensory neuropathies. Whether these symptoms are a consequence of vasculitis, pharmaceutical treatment, or malnutrition is not clear.

The central nervous system is an uncommon locus for vasculitis disease. Vasculitis involving the central nervous system usually results in a headache (55), without which the diagnosis is unlikely. Rarely, seizures are the presenting manifestation of central nervous system vasculitis. In Wegener's granulomatosis, meningeal disease can occur. Magnetic resonance imaging (MRI. with gadolinium infusion may reveal enhancing lesions in many separate foci. Most commonly, however, small-vessel vasculitis affects vessels that are too small to produce a positive magnetic resonance imaging (MRI) scan. Cerebral angiography may reveal abnormalities in less than half of patients. If the patient has systemic hypertension, cerebrovascular disease from atherosclerosis, or renal insufficiency resulting in uremia, it may not be possible to ascertain the precise cause of central nervous system symptoms. Treatment with antihypertensive agents and dialysis can exclude or decrease the possibility that hypertension and uremia are the cause of the symptoms. Many patients with ANCA-associated small-vessel vasculitis are older adults, and vasculitis may be impossible to differentiate from atherosclerosis in that population.

Other Organ System Diseases

Any organ system or capillary bed may be inflamed by small vessel vasculitis, resulting in numerous other clinical manifestations. Ocular manifestations of disease include iritis, uveitis, and peripheral keratitis. These lesions result in a red eye and are observed using a slit lamp by a qualified ophthalmologist.

Cardiac vasculitic disease results in subendocardial ischemia. The lesions can be difficult to see by using coronary arteriogram. Whether patients have coronary vascular disease as a consequence of atherosclerotic disease or of vasculitis is difficult to determine. In our population, 5% of patients had myocardial infarction at the time of their generalized disease process. Pericarditis is much less common in patients with ANCA-associated small-vessel vasculitis than in those with lupus vasculitis. A pericardial friction rub should raise the specter of a separate disease process.

In more than 90% of our patients, constitutional features are hallmarks of disease. Fatigue represents a ubiquitous finding that persists even after all of the other specific manifestations of vasculitis appear to be in remission. In addition, fever, myalgias, and arthralgias are common. Arthralgias are frequently migratory in which joint pain occurs in one joint, only to resolve and appear in another joint at another time. Frank arthritis with synovial thickening occurs in at least 10% of patients.

LABORATORY FINDINGS

Abnormal laboratory findings in patients with small vessel vasculitis include normochromic and normocytic anemia, mild to marked leukocytosis, and mild thrombocytosis. Eosinophilia is uncommon in patients with Wegener's granulomatosis and MPA but is required for the diagnosis of CSS. Several markers of inflammation such as the C-reactive protein and the erythrocyte sedimentation rate are elevated, especially at times of disease exacerbation. Rheumatoid factor levels are positive in some individuals.

In the differential diagnosis of ANCA-associated small-vessel vasculitis, a number of other vasculitic syndromes can be excluded by serologic tests. These include tests for lupus, including ANA, anti–double-stranded DNA (dsDNA. antibodies, serum complement levels, and cryoglobulins. Rarely, a patient may have an overlap syndrome of SLE and ANCA-associated small-vessel vasculitis, with positive ANA, anti-dsDNA, and usually anti-MPO antibodies. Unlike with polyarteritis nodosa, screening for infectious diseases usually yields negative results, including assays for hepatitis B and hepatitis C. Anti-GBM antibodies should be measured at least once in the differential diagnosis of crescentic glomerulonephritis. Tests for circulating immune complexes are not reliable.

The laboratory findings in patients with CSS include eosinophilia in all patients. The degree of eosinophilia may reach 50% of the total leukocyte count. Elevated serum IgE levels and IgA containing immune complexes are found in some patients (56).

ANCA Serologic Studies

Since Richard Davies reported eight patients with antibodies to neutrophils associated with necrotizing glomerulonephritis (57), substantial advances have been made in the serologic analysis of ANCA. ANCA reacts not only to neutrophils, but also to monocytes. Effective ANCA testing must use both indirect immunofluorescent microscopy in conjunction with antigen-specific tests using highly purified MPO and PR3 antigens (18–21,41,58). Indirect immunofluorescent microscopy has elucidated two different ANCA patterns. On ethanol-fixed human neutrophils, cytoplasmic ANCA (c-ANCA) result in diffuse immunofluorescent staining of the cytoplasm. In contrast, perinuclear ANCA (p-ANCA) stain the periphery of the nucleus using ethanol-fixed cells but have a cytoplasmic pattern when using formalin-fixed leukocytes. Most c-ANCA react with PR3, a serine proteinase found within the primary granule of neutrophils and monocytes. This serine proteinase has substantial homology with elastase and cathepsin G. Some c-ANCA (less than 10%) react with bacterial/permeability increasing protein. This pattern of reactivity is found mainly in patients with cystic fibrosis and inflammatory bowel disease (59).

p-ANCA react with MPO in more than 90% of cases. The MPO is a member of a multichain peroxidase family that also includes thyroperoxidase, eosinophil peroxidase, and lactoperoxidase. Many if not most of the p-ANCA found in diseases other than pauciimmune necrotizing glomerulonephritis and vasculitis do not react with MPO, such as in ulcerative colitis, primary sclerosing cholangitis, and Felty's syndrome. The most confusing situation occurs in patients with lupus erythematosus. In these patients, p-ANCA are usually attributable to ANA, although in some rare cases, antilactoferrin and antielastase

TABLE 68-7

APPROXIMATE FREQUENCY OF ANTINEUTROPHIL CYTOPLASMIC ANTIBODIES (ANCAS) WITH SPECIFICITY FOR PROTEINASE 3 (PR3-ANCA, C-ANCA) OR MYELOPEROXIDASE (MPO-ANCA, P-ANCA) IN PATIENTS WITH ACTIVE UNTREATED MICROSCOPIC POLYANGIITIS, WEGENER'S GRANULOMATOSIS, AND CHURG-STRAUSS SYNDROME

	Microscopic polyangiitis	Wegener's granulomatosis	Churg-Strauss syndrome	Renal-limited vasculitis[a]
PR3-ANCA c-ANCA	40%	75%	10%	20%
MPO-ANCA p-ANCA	50%	20%	60%	70%
Negative ANCA	10%	5%	30%	10%

[a]Renal-limited vasculitis refers to pauciimmune necrotizing and crescentic glomerulonephritis with no apparent extrarenal vasculitis.

antibodies are found. Patients with lupus may have a false-positive anti-MPO test result by enzyme-linked immunosorbent assay (ELISA) (60).

The antigen specificity of circulating ANCA is not diagnostic for a particular clinicopathologic variant of ANCA-associated small vessel vasculitis, although there are differences in the relative frequency of PR3-ANCA and MPO-ANCA in different types of pauciimmune small-vessel vasculitis (Table 68-7). For example, most patients with Wegener's granulomatosis have PR3-ANCA (c-ANCA), whereas most patients with renal-limited pauciimmune crescentic glomerulonephritis have MPO-ANCA (p-ANCA).

A positive ANCA result in a patient with strong clinical evidence for crescentic glomerulonephritis or another manifestation of small-vessel vasculitis, such as purpura or pulmonary hemorrhage, has a high positive predictive value. A positive ANCA result in a patient with weak evidence for crescentic glomerulonephritis or small-vessel vasculitis, such as isolated hematuria and proteinuria with normal renal function, has a much lower positive predictive value (Table 68-8). However, in a patient with weak clinical evidence for crescentic glomerulonephritis or small-vessel vasculitis, a positive result increases the likelihood to a level that requires expeditious additional diagnostic evaluation, possibly including a renal biopsy, to confirm or refute the presence of a pauciimmune small-vessel vasculitis or crescentic glomerulonephritis. A negative ANCA result is more effective at ruling out pauciimmune crescentic glomerulonephritis in a patient with weak clinical evidence of small-vessel vasculitis than in a patient with strong clinical evidence for small-vessel vasculitis (Table 68-8).

The ANCA result is typically negative in patients with polyarteritis nodosa, Takayasu's arteritis, or giant cell arteritis. There are individuals who have an overlapping disease with vasculitic involvement not only of large vessels, but also of small arteries. These patients have either PR3- or MPO-ANCA.

PATHOGENESIS

There has been an explosion of knowledge pertaining to the pathogenesis of ANCA small-vessel vasculitis and pauciimmune necrotizing glomerulonephritis, but much still remains to be discovered. While Wegener's granulomatosis, microscopic polyangiitis, and CSS share the hallmark of pauciimmune necrotizing small-vessel vasculitis, each presents phenotypic differences. It is still unclear what causes the granulomatous lesions of Wegener's granulomatosis or stimulates the eosinophilia and asthma in CSS. Furthermore, the severity of disease varies from one patient to another. To date, the host factors that produce minimal disease in some patients and severe disease in others remain unknown. More intriguing is the observation that while vasculitis affects many capillary beds (e.g., kidney, skin, or lung) in some patients, it is limited to one organ in others. In many instances, the disease processes are focal. For example, only a segment of a capillary bed is affected,

TABLE 68-8

PREDICTIVE VALUE OF COMBINED INDIRECT FLUORESCENT ANTIBODY (IFA) AND ENZYME IMMUNOASSAY (EFA) ANTINEUTROPHIL CYTOPLASMIC ANTIBODY TESTING FOR PAUCIIMMUNE CRESCENTIC GLOMERULONEPHRITIS

Adult with	Prevalence pretest likelihood	Positive predictive value post-test likelihood	Negative predictive value post-test unlikelihood
RPGN	47%	95%	85%
Hematuria, Proteinuria, (creatinine >3 mg/dL)	21%	84%	95%
Hematuria, Proteinuria, (creatinine 1.5–3 mg/dL)	7%	60%	99%
Hematuria, Proteinuria, (creatinine <1.5 mg/dL)	2%	29%	100%

Data derived from an analysis of 2,315 patients, with ANCA assay sensitivity 81% and specificity 96%.
(From: Lim LC, Taylor JG III, Schmitz JL, et al. Diagnostic usefulness of antineutrophil cytoplasmic autoantibody serology. Comparative evaluation of commercial indirect fluorescent antibody (IFA) kits and enzyme immunoassay (EIA) kits. *Am J Clin Pathol* 1999;111(3):363, with permission.)

leaving an adjacent segment spared. This is exemplified by the observation that a segmental necrotizing lesion in a glomerulus may sit adjacent to an ostensibly normal glomerular segment.

A number of clinical and basic science observations suggest that ANCA play a pivotal role in the pathogenesis of pauciimmune vasculitis and glomerulonephritis (11,61–63). ANCA are found in 85% to 90% of patients with pauciimmune glomerulonephritis and small-vessel vasculitis. There is an absence of evidence for more clearly delineated pathogenetic mechanisms, such as immune-complex disease or direct antibody attack–mediated disease. ANCA titers tend to correlate with disease activity in most but not all patients, yet the most convincing human data come from patients who develop a drug-induced ANCA-vasculitis. In some cases, cessation of the offending agent is associated with remission of small-vessel vasculitis and diminution of ANCA titers. Finally, there is a clear description of trans-placental transfer of MPO-ANCA from a mother with active MPA during pregnancy resulting in a pulmonary-renal vasculitic syndrome in the newborn infant (64,65).

ANCA more than likely participate in the pathogenesis of vasculitis by activation of primed leukocytes and monocytes in the circulation and increasing their adherence or leukocytes adherent to the vascular endothelium. A number of laboratories have confirmed that ANCA induce neutrophil and monocyte activation using various methods. These include analysis of respiratory burst and degranulation and measurement of several chemokines and cytokines (66–69). How these antibodies bind to cognate antigens is likely a consequence of a number of factors. It is possible that there is a genetic increase in the expression of PR3 on the surface of neutrophils and monocytes (70,71). Another possibility stems from the observations that in patients with ANCA vasculitis, neutrophils and monocytes have substantially increased expression of genes for MPO and PR3. In fact, the expression of RNA from these cells was correlated with disease activity. Interestingly, while the MPO and PR3 genes are on different chromosomes, message from both of these genes was coordinately increased, suggesting a similar, but unrecognized, transcription factor that may be regulating the production of many granular constituents (72).

Once antigen is available for antibody binding, neutrophils and monocytes are then activated by two coordinated and separate signal transduction pathways. In the past, controversy abounded as to whether ANCA activation of neutrophils and monocytes occurred by the Fc receptor alone or whether there was F(ab′)2 stimulation (73). Human neutrophils constitutively express receptors for IgG, including FcRIIa and FcRIIIb. The former is a widely expressed receptor, whereas the latter is a low-affinity receptor with expression that is restricted to neutrophils and eosinophils (74). Engagement of the Fc receptor results in a number of neutrophil-activation events, including respiratory burst, degranulation, phagocytosis, cytokine production, and upregulation of adhesion molecules. Fc receptors are thus likewise engaged in the activation of neutrophils and monocytes by ANCA (75,76). Polymorphisms of the Fc receptors could play an important role in the development of ANCA vasculitis. Whereas the FcRIIa single nucleotide polymorphisms do not appear to be important (77,78), some evidence suggests that the FcRIIIb polymorphism may influence disease severity (79,80). In addition to Fc receptor–induced activation, there are substantial data that the $F(ab')_2$ portion of the antibody molecule also plays a role in leukocyte activation. ANCA $F(ab')_2$ not only induce oxygen radical production (73), but also induce the transcription of cytokine genes in normal human neutrophils and monocytes. Whereas some genes are upregulated by both whole ANCA immunoglobulin and ANCA $F(ab')_2$, other genes are upregulated by only one or the other (81). It is most likely that $F(ab')_2$ portions of ANCA are capable of low-level neutrophil and monocyte activation (73). The Fc portion of the molecule almost certainly causes leukocyte activation once the $F(ab')_2$ portion of the immunoglobulin has interacted with the antigen, either on the cell surface or in the microenvironment (75). Recently, the signal transduction pathways of F(ab′)2 and Fc receptor activation have been nicely elucidated. Both of these possible activation pathways appear to activate a specific p21ras (Kristen-ras) through separate but coordinated pathways (82). This is an important observation in that it may provide a focus for a therapeutic target.

As described in several studies, once neutrophils and monocytes are activated by ANCA, they interact with endothelial cells causing damage to the endothelium (83–86). It has been thought that it is possible that endothelial cells expressed ANCA antigens, especially PR3, but this observation has been excluded by a number of investigators. What has emerged, however, is the possibility that, in addition to direct damage of the endothelium by the respiratory burst properties of leukocytes, ANCA antigens released from neutrophils and monocytes enter endothelial cells and cause endothelial cell damage. For example, PR3 released into the microenvironment enters endothelium, resulting in IL-8 production (87) and chemoattractant protein-1. PR3 is also capable of inducing apoptotic events from both proteolytic and non-proteolytic mechanisms (88,89). The internalization of PR3 into endothelial cells is receptor mediated (90–92).

Similarly, MPO has been shown to be internalized into endothelial cells by an energy-dependent process (93), and to transcytose intact endothelium to localize within the extracellular matrix. There, in the presence of the substrates H_2O_2 and NO_2-, MPO catalyzes nitration of tyrosine residues on extracellular matrix proteins (94), resulting in the fragmentation of extracellular matrix protein (95).

There have been a number of *in vitro* models of ANCA neutrophil and endothelial cell interaction using flow models and intravital microscopy. With treatment using tumor necrosis factor (TNF), endothelial cells capture neutrophils from the circulation. With the addition of ANCA over these rolling neutrophils, a more substantial adhesion of leukocytes to endothelial cells and transmigration of leukocytes occur. This occurs through a conformational change on CD11b that reveals an activation epitope (86).

The Role of T Cells

It has long been known that there are circulating T cells in patients with ANCA small-vessel vasculitis that have a Th1-type cytokine profile (96–101). Within tissues, Th-1 cytokine profiles have also been reported, especially in T cells from granulomatous tissue of patients with Wegener's granulomatosis (102). Very little is known, however, about T cells and ANCA vasculitis since it has been difficult to demonstrate that circulating T cells derived from affected patients are activated by the ANCA target antigens. There is mounting evidence implicating T cells in the pathogenesis of ANCA glomerulonephritis. T cells and monocytes are the predominant cell types in inflammatory vascular and perivascular lymphoid infiltrates in ANCA glomerulonephritis (103). The predominance of IgG1 and IgG4 subclasses of ANCA denotes the effects of T-cell help and IL-4 on isotype switching (104). Furthermore, in patients with ANCA glomerulonephritis, the concentrations of soluble IL-2 receptor, which is a marker of T-cell activation, correlate well with disease activity (105).

In vitro analyses of peripheral blood T-cell proliferation in response to MPO and PR3 yielded conflicting results. Recent studies have shown little or no difference in T-cell reactivity to PR3 between patients with ANCA-disease and controls (106,107). In the largest study looking at T-cell proliferative responses in 45 patients at various stages of disease (with and without treatment), PR3 responses were seen at all stages of disease activity, and to a lesser degree in healthy controls (108).

Interestingly, T cells from only two patients with PR3-ANCA, and none of the controls, proliferated in response to MPO.

Analysis of the profile of cytokine secretion by T cells derived from tissue with granulomatous inflammation (nasal mucosa or bronchiolar lavage fluid. as well as from peripheral blood T cells revealed a T-helper 1 (T_H1) pattern of cytokines (99,101). This is corroborated by the finding that T cells from patients with Wegener's granulomatosis have a decreased expression of CD28 as compared to healthy controls (102). CD28 costimulation promotes the production of T_H2 cytokines (109). Conversely, a recent study looking at the cytokine profile of T cells from patients with ANCA disease in complete remission and receiving *no* immunosuppressants revealed a TH2 cytokine profile with elevated production of IL-6 and IL-10 and low production of interferon gamma (IFN-γ) (110). These differences in the detected cytokine profiles could be due to the state of disease activity.

Antiendothelial Cell Antibodies

Antiendothelial cell antibodies (AECA) have been recognized for some time in several autoimmune diseases. AECA are more common in vasculitides associated with SLE, rheumatoid arthritis, systemic sclerosis, and antiphospholipid-induced vasculopathy (111,112). These antibodies recognize endothelial cell antigens expressed on large arterial vessel endothelial cells (Takayasu's arteritis) and on microvascular cells from both the arterial and the venous system. The precise nature of the target antigen has still not been elucidated. However, it is reasonable to presume that planted antigens complex with AECA resulting in *in situ* immune complex formation. Such antigens include DNA or DNA-histone complexes in SLE (113). Once AECA react with the surface of the endothelium, they likely induce a pathogenetic event by fixing complement.

When endothelial cells are cultured from a variety of tissues, including those from the human kidney, liver, lung, and nasal carriage, there is increased binding of AECA to unstimulated cells and cytokine-treated lung endothelial cells when compared to human umbilical vein endothelial cells or liver sinusoidal cells (114). These observations provide a newer concept: that AECA are specific for certain endothelial cells in target organ and may recruit leukocytes to their site of engagement.

Role of *Staphylococcus aureus* and Superantigens

Clinical studies reveal that 60% to 70% of patients with Wegener's granulomatosis have a chronic nasal carriage of *S. aureus* (53) that is associated with an eightfold increased rate of relapse (115). Importantly, in a placebo-controlled randomized trial, patients with nasal carriage of *S. aureus* treated with trimethoprim-sulfamethoxazole had a significantly lower rate of relapse of the nasal or upper respiratory tract disease. *S. aureus* superantigens are most likely implicated in disease activity. For instance, patients with superantigen-positive *S. aureus* strains are more likely to have a relapse of disease than carriers of superantigen-negative strains (116). The staphylococcal acid phosphatase appears to bind to the endothelium as a consequence of its cationic nature and is recognized by the sera of patients with Wegener's granulomatosis (116). In addition, Brown-Norway rats immunized with staphylococcal acid phosphatase and then perfused with this same protein developed severe crescentic glomerulonephritis (117).

Environmental Factors (Including Infections)

The first report of ANCA was an association in eight patients with necrotizing glomerulonephritis and arbovirus infection with the Ross River virus (55). Several animal models suggest an association of arteritis and infection. For instance, parvovirus B19 is associated with a vasculopathy not only in humans, but also in the Aleutian mink (118). These animals develop a chronic parvovirus illness that results in immune complex–mediated vasculitis. There are several animal models of infection-mediated vasculitis in which there may be direct invasion of the vascular wall.

Environmental factors, particularly exposure to silica dust and other silica-containing compounds, may increase the risk of developing a number of different autoimmune diseases including scleroderma, rheumatoid arthritis, systemic sclerosis, SLE, and vasculitis (119). The early data were derived from studies that primarily evaluated cohorts of workers in occupations with high exposure to silica dust. This type of study is not ideal for assessing rare outcomes, such as autoimmune diseases. Case-control studies of specific autoimmune disorders have offered more insight into diseases potentially associated with silica dust exposure. Previous case-control studies have shown an association between ANCA-associated diseases and exposure to silica dust or other silica-containing compounds (120–122) with odds ratios ranging from 4.4 to 14.0. More recently, a case-control study reported an association between primary systemic vasculitis and farming activities and exposure to occupational solvents (the association with exposure to silica could not be ascertained (123). In a study involving 129 patients with ANCA-vasculitis with glomerulonephritis and 109 matched controls, only prolonged silica exposure (>23 years) was statistically significantly more common among patients than controls. There was no difference between the two groups in exposure to silica of shorter duration (124). In contrast, exposure to silica dust was not associated with the development of lupus nephritis, which is in contrast to reports from occupational cohorts (122,125,126).

Many drugs can induce vasculitis. In fact, 10% to 20% of cutaneous reactions to drug exposure are vasculitic in nature. A list of the most frequently implicated drugs includes anticonvulsants, antibiotics, penicillamine, hydralazine, nonsteroidal antiinflammatory drugs, and propylthiouracil (127). One of the drugs most commonly related to the development of anti-MPO–induced disease is propylthiouracil (127).

Animal Models

There are now excellent animal models of ANCA-induced small-vessel vasculitis. The most direct evidence of the pathogenic role of anti-MPO antibodies stems from a model of transfer of either splenocytes or anti-MPO antibodies into Rag 2−/− mice (128). In this model, MPO knockout mice (MPO−/−) were immunized with purified mouse MPO, and developed mouse anti-mouse -MPO antibodies. When splenocytes from these mice were transferred into Rag2−/− mice, which lack functioning T and B cells, these animals developed a systemic necrotizing vasculitis and severe necrotizing and crescentic glomerulonephritis. When anti-MPO antibodies derived from immunized MPO knockout mice were transferred into Rag 2-/- mice, a pauciimmune necrotizing and crescentic glomerulonephritis was induced. This disease process occurred without antigen-driven T cells. Several follow-up studies have now been performed using this mouse model. In particular, the disease process was aggravated by the administration of lipopolysaccharide (LPS) into recipient mice by increasing the percentage of glomeruli involved with necrotizing and

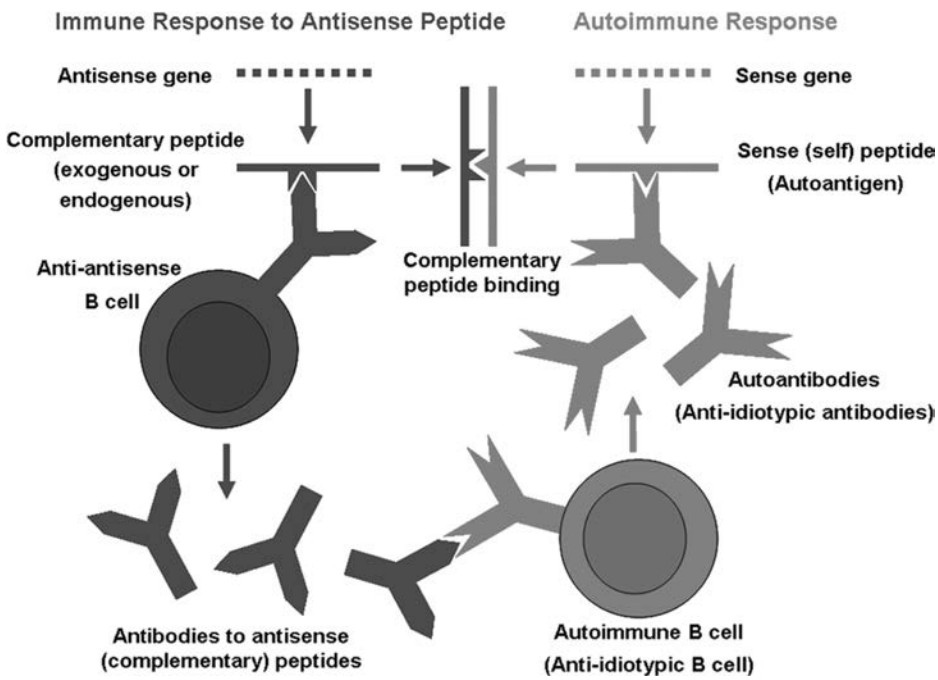

FIGURE 68-10. Schematic of a mechanism for the development of autoimmunity (theory of autoantigen complementarity) as a consequence of an immune response to a protein whose amino acid sequence is complementary to a self protein.

crescentic glomerulonephritis when anti-MPO antibodies were transferred into these mice (129). The role of neutrophils in this response was highlighted by the abrogation of disease when the neutrophils of anti-MPO recipient mice were depleted by a selective anti-neutrophil monoclonal antibody (NIMP-R14) (130).

The pathogenic role of anti-MPO antibodies is also documented in a second animal model (131). In this model, rats immunized with human MPO developed anti-rat-MPO antibodies. These animals then developed a necrotizing and crescentic glomerulonephritis, as well as pulmonary capillaritis. Microscopy of superior mesenteric vessels demonstrated that when a chemokine was applied to the vessel, the anti-MPO antibodies induced adherence and margination of leukocytes to the vessel wall. These two animal models document that anti-MPO antibodies are capable of causing a necrotizing and crescentic glomerulonephritis and a widespread systemic vasculitis, and also demonstrate that cytokines and chemokines exacerbate the injury in a manner that mimics the *in vitro* studies of ANCA-induced leukocyte activation. A model of anti-PR3-induced vascular injury was developed in which a perivascular infiltrate was observed around cutaneous vessels in the setting of anti-PR3 antibodies and cytokine exposure (62,132). In summary, these animal studies document that both anti-MPO and proteinase-3 antibodies are capable of causing disease.

Theory of Autoantigen Complementarity

For all autoimmune diseases, the critical question is, is the most proximate cause of the formation of the autoantibody or the abnormal T cell clone. There have been a number of theories that may provide a basis for the alteration of self antigens, the most plausible of which is known as the theory of molecular mimicry (133–135). This theory suggests that there is an immune response directed against a microbial antigen that mimics the amino acid sequence or structure of a self protein. To date, it has been difficult to demonstrate this theory in human autoimmune disease. A serendipitous finding in ANCA vasculitis has spawned a theory of autoantigen complementarity. While the details of this theory and the proof that it may pertain to ANCA vasculitis is beyond the scope of this chapter, a brief description of this observation is germane for the understanding of ANCA vasculitis (136,137) (Fig. 68-10).

It has been known for decades that proteins transcribed and translated from the sense strand of DNA bind to proteins that are transcribed and translated from the anti-sense strand of DNA. Some patients with PR3 ANCA harbored antibodies to an antigen complementary to the middle portion of PR3. These anti-complementary PR3 antibodies formed an anti-idiotypic pair with PR3-ANCA. Moreover, cloned complementary PR3 proteins bind to PR3 and function as a serine proteinase inhibitor. What is the source of the complementary PR3 antigen? Preliminary data suggest that these proteins are found on a variety of microbes, some of which have been associated with ANCA vasculitis and also found in the genome of some patients with both PR3- and MPO-ANCA (137). These studies need to be confirmed and expanded to understand what the source of the complementary PR3 antigen is in any given person, and just as importantly, whether these complementary proteins are capable of inducing disease. If these observations remain true, they may provide a promising avenue for the detection of the proximate cause of the autoimmune response in any given person.

TREATMENT

The treatment of ANCA-associated small-vessel vasculitis and glomerulonephritis rests primarily on the use of induction methylprednisolone, high-dose corticosteroids, and cyclophosphamide. Considering the importance of the serum creatinine concentration at the time treatment is initiated as a determinant of long-term renal outcome (49), pulse methylprednisolone (7 mg/kg per day for 3 days) is used to curb the active inflammation as soon as possible. This is followed by instituting prednisone at a daily dosage of 1 mg/kg per day (not to exceed 80 mg per day for the first month of therapy) Corticosteroids are then tapered over the second month to an alternate-day dosing schedule and subsequently decreased every week by

10 to 20 mg per day until they are eventually discontinued by the end of the fourth to fifth month. The rate of decrease in corticosteroid dosing should be tailored based on an assessment of each patient's disease activity.

The beneficial role of cyclophosphamide in the treatment of acute ANCA-associated small-vessel vasculitis is evidenced by the substantial improvement in the rate of remission (56% to 85%) and a threefold decrease in the risk of relapse is associated with the use of this drug (138). Cyclophosphamide may be administered as a daily oral regimen or as monthly intravenous pulses. When the intravenous route is used, it is usually started at a dose of 0.5 g/m^2 of body surface area, which is subsequently increased to a maximal dosage of 1 g/m^2. This dose is adjusted to maintain the 2-week leukocyte nadir at more than 3,000/mm^3. When the daily oral regimen is used, cyclophosphamide is given at a daily dosage of 1.5 to 2 mg/kg (139). To prevent severe leukopenia, careful attention to the leukocyte count must be maintained throughout this therapy. Cyclophosphamide is usually continued for a total of 6 to 12 months, depending on the patient's response to treatment. Whether one form of cyclophosphamide therapy is superior to the other is a subject of continued investigation. In general, the intravenous regimen allows for a 2 to 3 times smaller total dose of cyclophosphamide than the oral regimen. In prospective and retrospective analyses, intravenous therapy was associated with a significant decrease in the rate of clinically significant neutropenia and other complications. A regimen of daily oral cyclophosphamide may be associated with a decreased risk of relapse (140). However, in a meta-analysis of three randomized controlled trials comparing pulse versus oral continuous cyclophosphamide, pulse cyclophosphamide attained a statistically higher rate of remission, and lower rates of leucopenia and infections. Pulse cyclophosphamide was associated with a higher rate of relapse which was not statistically significant (141). The final outcomes of patients (death or end stage renal disease [ESRD]) were no different in the two groups despite the lower rate of relapse in the oral cyclophosphamide group (141).

Patients presenting with pulmonary hemorrhage also benefit from the institution of plasmapheresis in a regimen similar to that used for patients with Goodpasture's disease. Although no controlled data are available, early and aggressive institution of plasmapheresis has in our experience substantially diminished the mortality rate associated with massive pulmonary hemorrhage (142). Plasmapheresis is typically performed daily until the pulmonary hemorrhage ceases and then every other day for a total of seven to ten treatments. Plasma is replaced with a solution of 5% albumin, but two units of fresh-frozen plasma are administered at the end of the treatment to replace clotting factors and minimize the risk of persistent or renewed bleeding. On the basis of several relatively small studies, plasmapheresis does not seem to be of added benefit over the use of corticosteroids and cyclophosphamide in patients without pulmonary hemorrhage (143–145) or severe renal involvement. In a randomized trial of plasma exchange versus methylprednisolone as additional therapy in patients with severe ANCA glomerulonephritis (creatinine >500 μmol/L or dialysis), the of use plasma exchange was associated with a significant improvement in the recovery of renal function and dialysis-free survival (146).

Once remission is attained, an alternative maintenance regimen consists of switching cyclophosphamide to oral azathioprine at the end of 3 months. Azathioprine is then continued for 12 to 24 months. This regimen offers the advantage of a limited use of cyclophosphamide and results in similar rates of remission and relapse as the cyclophosphamide-only–based therapies (147).

Patients suffering primarily from mild ANCA-associated small-vessel vasculitis without renal involvement may benefit from the use of methotrexate in lieu of cyclophosphamide. In an uncontrolled study, methotrexate afforded rates of remission comparable to those published for cyclophosphamide (148). An open-label study suggests that methotrexate could be used for maintenance therapy after the induction of remission with cyclophosphamide and corticosteroids (149), but may be associated with a relatively high rate of relapse. In a randomized controlled trial of induction therapy among patients with "early" ANCA-associated vasculitis comparing weekly methotrexate (15 mg/week escalating to a maximum of 20 to 25 mg/week by 12 weeks) to daily oral cyclophosphamide (2 mg/kg/day to a maximum of 150 mg/day), the rate of remission at 6 months was comparable among the two treatment groups (89.8% for methotrexate vs. 93.5% for cyclophosphamide, $p = 0.041$). However, the onset of remission in methotrexate-treated patients with relatively extensive disease or pulmonary involvement was delayed. Methotrexate was associated with a significantly higher rate of relapse than cyclophosphamide (69.5% vs. 46.5%), and 45% of relapses occurred while patients were receiving methotrexate (150). Importantly, patients enrolled in this trial did not have organ- or life-threatening manifestations, or significant renal involvement. The dose of methotrexate must be reduced in patients whose creatinine clearance is less than 80 ml/min, and its use is contraindicated when creatinine clearances are less than 10 ml/min. Most experience of methotrexate in Wegener's granulomatosis has involved patients with no renal involvement or with glomerulonephritis and near-normal renal function.

With the use of an alkylating agent, the rate of remission is on the order of 70% to 85%. Patients who require dialysis at the time of diagnosis have a decreased probability of recovering sufficient renal function to discontinue dialysis (about 50%). Patients that do recover sufficient renal function, do so within the first 3 months of treatment. For that reason, and in the absence of active extrarenal vasculitis, immunosuppression may be stopped after 3 months if no signs of renal recovery have occurred.

Relapse of ANCA-associated small-vessel vasculitis occurs in about 30% of patients (138). In our experience, 80% of relapses occur in the first 18 months after immunosuppressive therapy is discontinued. Other authors did not detect such a clustering of relapses in the early months after discontinuing therapy (151). Recurrent disease may resemble clinically the initial presentation but is sometimes associated with new organ involvement.

The risk of relapse is not uniform among patients with ANCA-associated vasculitis. Multivariate analysis of 258 patients with the disease who were treated and attained remission showed that presence of PR3-ANCA antibody and involvement of the lungs and upper respiratory tract were independent risk factors for relapse. Of the patients who presented none of these risk factors, 26% relapsed in a median of 62 months (median among those who relapsed was 20 months). In contrast, 47% of the patients who presented with a single risk factor experienced a risk for relapse (95% CI 1.1-3.9, $p = 0.038$). Of patients presenting with all three risk factors, 73% relapsed in a median of 17 months (median among those who relapsed was 15 months), corresponding to a 3.7-times increased risk of relapse (95% CI 1.4-9.7, $p = 0.007$) compared to those with no risk factors (152).

Whether ANCA titers are predictive of a relapse is a matter of controversy. To determine the occurrence of a relapse, serial measurements of ANCA titers should be interpreted only in the context of the clinical history and physical and laboratory examination of the patient. Recurrent glomerulonephritis is usually indicated by the recurrence or worsening of hematuria with an increase in serum creatinine. An increase in proteinuria

alone or the gradual increase in serum creatinine without hematuria may be the result of progressive chronic scarring, rather than recurrent active inflammation. Repeated renal biopsy is sometimes indicated to best differentiate between recurrent disease and progressive scarring and to avoid unnecessary immunosuppression in the latter case.

Whether ANCA titers are clinically useful in ascertaining the occurrence of relapse or of predicting its occurrence in the future remains a subject of investigation. Although ANCA titers correlate with disease activity when a group of patients are considered, the ANCA titer may not correlate in an individual patient. Some patients maintain a high titer level despite clinical remission, whereas others exhibit clinical evidence of active vasculitis in the absence of a rise in titer. ANCA titers are best used in serial measurements and interpreted in consideration with each patient's pattern.

Several studies have addressed whether ANCA could reliably predict the future occurrence of a relapse (153). Although a rise in ANCA titer tends to predict recurrent disease, the relapse may not occur for several months. In a study by Cohen Tervaert et al., one-third of patients with a rise in titer did not experience clinical signs of relapse even after 18 months (154). In this context, and considering the toxicities of immunosuppression, the prophylactic use of high-dose corticosteroids or cyclophosphamide to prevent relapse would needlessly expose many patients to their toxic side effects. If alternative, less-toxic therapies are shown to be effective in the treatment of ANCA vasculitis, the risk–benefit ratio may make such a preemptive or prophylactic approach more appealing. To date, the evidence does not support the use of preemptive immunosuppressive therapy to prevent a relapse in patients with an increase in ANCA titer.

Relapsing ANCA-associated vasculitis responds to immunosuppression with corticosteroids and cytotoxic agents with a similar response rate as the initial disease. The decision regarding the repeated use of pulse methylprednisolone can be based on the total amount of corticosteroid that has been administered to the patient over the course of the disease, as well as the severity of the relapse. Patients with a history of relapsing disease pose a particular challenge because they are particularly subject to the cumulative toxic effects of cytotoxic agents and corticosteroids. Some may require the use of long-term "maintenance" immunosuppressive therapy with either low-dose prednisone or azathioprine. Although the use of trimethoprim-sulfamethoxazole or cotrimoxazole is beneficial in the prevention of relapses involving the nose and upper respiratory tract, no benefit is seen in disease affecting the kidneys or other organ systems (52). The concomitant use of trimethoprim-sulfamethoxazole and methotrexate is contraindicated because it may result in severe bone marrow toxicity.

In an effort to limit the exposure to cytotoxic agents, a number of immunomodulatory drugs and antibodies are being evaluated for the treatment of patients with recurrent vasculitis. The various studies can conceptually be divided into two categories: studies aimed at treating patients who are resistant to conventional treatment with cyclophosphamide and corticosteroids, and studies aimed at the prevention of relapse. Table 68-9 summarizes various "novel" therapies that have been or are being evaluated. The efficacy of any such agents is currently

TABLE 68-9

A SUMMARY OF VARIOUS "NOVEL" THERAPIES THAT HAVE BEEN OR ARE BEING EVALUATED IN THE TREATMENT OF ANCA-ASSOCIATED SMALL-VESSEL VASCULITIS AND GLOMERULONEPHRITIS

Therapy	Possible role	References
Plasmapheresis	Adjunctive therapy in patients with pulmonary hemorrhage or advanced renal disease	Gaskin 2001(266); Frasca 1992, 1993(267,268); Pusey 1991(145); Zauner 2002(269); Klemmer 2003(142); Cole 1992(144)
Intravenous Immunoglobulin	Adjunctive therapy in patients with persistent disease on standard therapy	Jayne et al. (155)
Methotrexate	Alternative to cyclophosphamide in patients with 'early disease' and without significant renal or pulmonary disease.	Sneller 1995(148); Langford 2000(270); DeGroot 2005(150)
	Prevention of relapse?	Isaacs 1996(161), Booth 2002(167), Stegeman 1997(271)
Azathioprine	Prevention of relapse?	Jayne 2003(147)
Mycophenolate mofetil	Prevention of relapse?	Nowack 1999(170); Langford 2004(272)
Leflunomide	Prevention of relapse?	Metzler 2004(273).
Rituximab	Adjunctive therapy for cyclophosphamide-resistant or relapsing patients?	Specks 2001(274), Keogh 2005(156); Eriksson 2005(157)
Infliximab	Adjunctive therapy for cyclophosphamide-resistant or relapsing patients?	Lamprecht 2002(164); Bartolucci 2002(165); Booth 2004(166); D'Haens 1999(275)
Alemtuzumab	Adjunctive therapy for cyclophosphamide-resistant or relapsing patients?	Kirk 2003(160)
Etanercept	Shown NOT to be efficacious in the prevention of relapse	WGET(276)
Trimethoprim-sulfamethoxazole	Prevention of relapses that affect the upper respiratory tract.	DeRemee 1985(277), Reinhold-Keller 1996(278); Stegeman 1996(53)

not established, and they should not be considered as first-line therapies.

Potential Adjunctive Treatment for Patients with Resistant Disease or Contraindications to Conventional Therapy

Several agents have been evaluated as adjunctive therapy for patients with resistant forms of ANCA vasculitis. Adjunctive therapy with intravenous immunoglobulin (IVIg) (single course of a total of 2 gm/kg) was evaluated in a randomized controlled trial in patients with persistently active ANCA vasculitis despite conventional therapy. Patients treated with IVIg experienced a more rapid decline in disease activity (as measured by a 50% reduction in Birmingham Vasculitis Activity Score [BVAS]) and C-reactive protein at 1 and 3 months, but there was no significant difference between the two groups after 3 months with respect to disease activity or frequency of relapse (155).

Rituximab, a chimeric monoclonal antibody directed against the CD20 antigen, effectively depletes B lymphocytes, but not plasma cells. Preliminary evidence indicates that rituximab may have a role in the management of ANCA-associated vasculitis that is resistant to or relapsing after standard therapy based on small, uncontrolled case series (156–158). In these studies, the use of rituximab (375mg/m^2 IV weekly × 4 or 500 mg IV weekly × 4 fixed doses) in conjunction with corticosteroids, resulted in remission in the majority of patients and was generally well tolerated. In contrast, in a fourth open label study of 8 patients with severe, refractory Wegener's granulomatosis, the addition of rituximab (375mg/m2 every four weeks × 4 doses) to cyclophosphamide, mycophenolate mofetil or methotrexate was associated with limited benefit (159). The use of rituximab as induction therapy is currently compared to cyclophosphamide in an ongoing multicenter, randomized controlled trial.

Alemtuzumab (Campath-1H) is a humanized monoclonal IgG1 antibody directed against the CD52 antigen expressed on the surface of peripheral blood lymphocytes, monocytes and macrophages (160). Treatment with alemtuzumab results in complement-mediated lysis, antibody-dependent cellular cytotoxicity and induction of apoptosis of target cells and results in depletion of T cells and B cells (161). Alemtuzumab has been used to treat a select group of patients with refractory or relapsing autoimmune diseases, including 70 patients with ANCA-associated vasculitis (162). These patients received at least one course of 135 mg intravenous alemtuzumab over 5 days. Remission was achieved by 83% of surviving patients. Unfortunately, this treatment regimen was associated with high rates of serious infections and death (18% at 1 year) and a 43% rate of relapse.

Similarly, the chimeric monoclonal antibody directed against TNF-α (163) Infliximab was been evaluated in four open-label uncontrolled trials of small numbers of patients (164–167). In these studies, the treatment regimen included infliximab plus corticosteroids, and either cyclophosphamide or other immunosuppressive agents. In the largest of these trials, which included 32 patients with acute or resistant disease, infliximab was associated with a remission rate of 88% and a relapse rate of 20% (166). These promising results are mitigated, however, by an elevated rate of serious infectious complications.

The immunosuppressant, 15-Deoxyspergualin, used in Japan for the treatment of steroid-resistant renal transplant rejection, was also evaluated for the treatment of refractory Wegener's granulomatosis in a small uncontrolled trial (168).

Potential Agents for the Prevention of Relapse

Preliminary small-scale pilot studies suggest that mycophenolate mofetil may have a role in the treatment of mild or moderate ANCA-associated small-vessel vasculitis (169) or as "maintenance" therapy to prevent relapse (170). Currently there are not sufficient data to support its use in the treatment of life- or organ-threatening disease or as a first-line drug. The efficacy and safety of the TNF receptor–Fc fusion protein etanercept (171) in the treatment of patients with ANCA-associated vasculitis was compared in a controlled trial to that of the traditional regimen of daily oral cyclophosphamide or methotrexate and corticosteroids. Although the rate of remission was comparable in the two treatment groups, patients treated with etanercept had a higher rate of solid tumors (172).

RENAL TRANSPLANTATION

Renal transplantation has been recognized as an option of renal replacement therapy in patients with ANCA-associated Wegener's granulomatosis, MPA, or necrotizing crescentic glomerulonephritis. Successful renal transplantation in patients with ANCA-associated small-vessel vasculitis has been reported in patients who were in full remission and with negative ANCA test results, in patients with positive ANCA test results (173–175), and even in patients with evidence of active vasculitis at the time of transplantation (176). Recurrent disease after transplantation has also been described as occurring as early as a few days posttransplantation (177) and as late as several years posttransplantation (178). Just as with the initial ANCA-associated small-vessel vasculitis, reported recurrences after transplantation involve a spectrum of various organs and are not limited to the transplanted kidney (179).

Based on a pooled analysis (180), ANCA-associated small-vessel vasculitis recurs in about 19% of all patients, with an average time from transplantation to relapse of 31 months. The presence of ANCA at transplantation does not appear to increase the rate of relapse posttransplantation. Therapy with cyclosporine does not appear to have a significant protective effect on recurrent ANCA-associated small-vessel vasculitis over that afforded by other immunosuppressant regimens (corticosteroids and azathioprine). The evaluation of the role of mycophenolate mofetil in the treatment of ANCA-associated vasculitis is in its infancy, and it remains to be seen whether this drug will reduce the incidence of recurrent disease posttransplantation. Patients with Wegener's granulomatosis had a relative risk of relapse of 2.75 when compared with patients with MPA or necrotizing crescentic glomerulonephritis alone. Conversely, ANCA pattern (c-ANCA or p-ANCA) or antigen specificity (PR3 or MPO) was not associated with differences in relapse rate posttransplantation.

A review of the reports of recurrent ANCA-associated small-vessel vasculitis posttransplantation reveal a good response to cyclophosphamide in the treatment of relapsing disease, although recurrent disease can lead to graft loss and even patient death (181).

In summary, renal transplantation is a beneficial option in the management of patients with ANCA-associated small-vessel vasculitis and end-stage renal disease. Although the presence of circulating ANCA is not a sufficient contraindication to transplantation, it is current practice not to perform transplantation in patients with active vasculitis, but to delay surgery until the disease is in remission. No data are currently available about the need to wait a certain period of time after remission is attained and before proceeding to transplantation.

POLYARTERITIS NODOSA

Clinical Features

Patients with polyarteritis nodosa typically have constitutional symptoms, including fever and weight loss. The presence of mononeuritis multiplex, myalgias, and arthralgias, as well as nodular skin lesions in half of patients, characterize the disease. Vasculitis of the coronary arteries may lead to cardiac symptoms. The renal disease seen in polyarteritis nodosa is primarily related to vasculitis of the renal arteries resulting in renovascular hypertension and/or renal parenchymal infarction. Patients with polyarteritis nodosa do not have evidence of small-vessel vasculitis, glomerulonephritis, or pulmonary capillaritis. In fact, the lung is rarely injured in polyarteritis nodosa, in contrast to the frequency of pulmonary disease in patients with Wegener's syndrome, MPA, or CSS (182). Although not distinguishing features, gastrointestinal complaints and peripheral neuropathy are more common among patients with polyarteritis nodosa than patients with MPA (182). The prognosis of polyarteritis is really a reflection of the involvement of kidneys, heart, central nervous system, or gastrointestinal tract (183,184).

Pathogenesis

The etiology of polyarteritis nodosa remains unclear, and most cases of polyarteritis nodosa are probably idiopathic. An association between polyarteritis nodosa and hepatitis B infection is evident by the fact that patients with hepatitis B antigenemia are at greater risk of developing polyarteritis nodosa. However, the incidence of polyarteritis nodosa among these patients varies in different populations worldwide (185–187). A role for hepatitis B antigenemia in the pathogenesis of polyarteritis nodosa is further suggested by reports of vasculitis after hepatitis B vaccination (188). Furthermore, treatment with antiviral agents and plasma exchange has led to resolution of the vasculitis in patients whose serology converts from being positive for the HBe or HBs antigens to the corresponding antibodies (189,190).

Polyarteritis nodosa has been associated with a number of cancers, especially hairy cell leukemia (191–194). Treatment of hairy cell leukemia with interferon-alpha (INF-α) may be associated with resolution of polyarteritis nodosa (195). As opposed to patients with small-vessel vasculitis, patients with polyarteritis nodosa are ANCA-negative (182).

Renal Manifestations

Both polyarteritis nodosa and Kawasaki's disease are medium-sized vessel vasculitides that affect the kidney. These arteritides result in necrotizing lesions in the major renal arteries and aneurysm formation with thrombosis and renal infarction. The aneurysms are not true aneurysms but pseudoaneurysms. The arteritis of Kawasaki's disease most commonly involves the coronary arteries, but in at least 25% of patients the lesions also involve the kidney. Kawasaki's disease is distinguished from polyarteritis nodosa by the pathognomonic *sine qua non* feature of mucocutaneous lymph node syndrome. Renal arterial involvement by polyarteritis nodosa, including interlobar and arcuate arteries, results in renal ischemia, infarction, and hemorrhage. One of the most painful and catastrophic consequence of this disease is rupture of an arterial pseudoaneurysm that causes retroperitoneal and sometimes intraperitoneal hemorrhage.

Treatment

The treatment of classic polyarteritis nodosa has been based on the use of high-dose corticosteroids with the addition, in moderate to severe or organ-threatening cases, of cyclophosphamide. Unfortunately, most studies pertinent to the treatment of polyarteritis nodosa antedate the 1994 Chapel Hill consensus conference, which classified MPA among the small-vessel vasculitides separate from classic polyarteritis nodosa. Consequently, most of the older studies and reports include a substantial number of patients who would now be diagnosed with MPA and not classic polyarteritis nodosa. As a result of the consensus conference, the incidence of classic polyarteritis nodosa involving medium-sized vessels alone and without evidence for glomerulonephritis is very low and is not readily amenable to large-scale evaluation of various therapies. The problem is compounded by the relatively recent recognition of the association of polyarteritis nodosa with hepatitis B virus (HBV) infection in a subset of patients with polyarteritis nodosa that varies from 10% to 50%, depending on the population studied.

In the absence of HBV infection, the mainstay of treatment of classic polyarteritis nodosa continues to rest on the use of high doses of corticosteroids. Typically, prednisone is initiated at a dosage of 1 mg/kg per day for the first month. Over the course of the second month, the dosage is tapered to an alternate-day regimen so that the patient is receiving 1 mg/kg every other day by the end of the second month. It is subsequently tapered slowly by 5 mg per day weekly as tolerated. The addition of an alkylating agent such as cyclophosphamide in the treatment of polyarteritis nodosa is not as well established as in the treatment of MPA or Wegener's granulomatosis, although some studies report improved patient survival when immunosuppressive drugs were added (196). In another study, the addition of cyclophosphamide to corticosteroids and plasma exchange led to decreased relapse in the cyclophosphamide-treated group, but no improvement in the 10-year survival rate (197). The beneficial effects of cyclophosphamide were also demonstrated when this drug was added to the corticosteroid regimen of patients who failed to respond to corticosteroids alone (198). No randomized study exists to critically assess the value of a daily oral regimen of cyclophosphamide compared with pulse cyclophosphamide in the outcome of patients with classic polyarteritis nodosa. Plasma exchange does not seem to improve the outcome, decrease relapse, or improve long-term survival of patients with polyarteritis nodosa not associated with HBV (199).

In current practice, cyclophosphamide should be reserved for patients with severe or organ-threatening disease, patients with disease that fails to respond to treatment with corticosteroids alone, patients who require unacceptably high doses of corticosteroids, or patients who are intolerant to corticosteroid side effects (200). Although azathioprine has been used in the treatment of classic polyarteritis nodosa, this agent is better reserved for maintenance therapy or as a steroid-sparing agent. The successful use of infliximab in PAN resistant to "conventional therapy" has been reported in a small number of cases (201,202).

In the setting of HBV-associated polyarteritis nodosa, treatment with immunosuppression consisting of corticosteroids with or without cyclophosphamide is thought to be deleterious because it facilitates viral replication, delays the development of protective anti–hepatitis B antibodies, and may lead to an aggravation of hepatic involvement (203). For this reason, it has been advocated that only a short course of corticosteroids (1 mg/kg per day) be used for 1 week, followed by a rapid taper over the following week. The prompt institution of antiviral therapy may ameliorate the vascular inflammation.

Treatment with plasma exchange has been advocated to clear circulating immune complexes thought to be important in the pathogenesis of this disease (204), although no controlled trial has critically assessed the need for plasmapheresis. The antiviral agents used have included vidarabine, INF-α-2b, or more recently combination therapy of INF-α-2b in addition to lamivudine (205) or famciclovir (190). No large-scale trials of antiviral therapy are available to critically assess the efficacy of these combinations in patients with HBV-related polyarteritis nodosa. Current work in the treatment of chronic HBV is focusing on the use of INF-α-2b, modified purine analogs such as famciclovir or L-stereoisomers of pyrimidine derivatives such as lamivudine (206). In an uncontrolled study, the combination of a short course of corticosteroids followed by a 6-month course of lamivudine and scheduled plasma exchange (until hepatitis B antigen or anti-HBe antibody seroconversion) resulted in a clinical remission of the vasculitis (207). In a recent retrospective analysis of 115 patients with HBV-associated polyarteritis nodosa (according to the Chapel Hill nomenclature) followed by the French Vasculitis Study Group between 1972 and 2002, the overall remission rate was 80.9% with a subsequent overall 9.7% relapse rate (208). The rates of relapse or death were not significantly different among patients treated with antiviral agents (vidarabine, INF-α, or lamivudine) (n = 80) when compared to patients treated with corticosteroids alone, or with cyclophosphamide, or plasma exchanges (n = 35) (5% vs. 14.3% relapse; and 30% vs. 48.6% death, respectively). However, the use of antiviral agents has led to a significantly higher rate of seroconversions from HBeAg to anti-HBeAb (49.4% vs. 14.7%; $p < 0.001$). Such seroconversion was associated with a clinical remission and absence of relapse.

Outcome

Most studies examining patient outcome and predictors of patient survival in polyarteritis nodosa antedate the Chapel Hill consensus conference of 1994. These studies are based on cohorts of patients that include those with MPA, CSS, and hepatitis B–associated classic polyarteritis nodosa. Earlier studies report a 5-year survival rate of approximately 55% in patients primarily treated with corticosteroids alone (209). The addition of cyclophosphamide or immunosuppressive therapy to glucocorticoids seems to have improved the 5-year survival rate to about 80%. Patients with bowel infarction, serious gastrointestinal bleeding, or renal insufficiency had particularly poor prognosis. In a more recent prospective study including 342 patients, of whom 119 had classic polyarteritis nodosa without HBV (89 patients with HBV, 52 patients with MPA, 82 patients with CSS) (183), proteinuria of 10 g per day, renal insufficiency, and gastrointestinal tract involvement were the major prognostic markers for a worse outcome.

BEHÇET'S DISEASE

Behçet's disease is a systemic vasculitic syndrome classically characterized by a triad of recurrent oral ulcerations, genital ulcerations, and ocular lesions usually consisting of uveitis, iritis, or retinal vasculitis. Behçet's disease can present with protean manifestations with multiple organ involvement, either concomitantly or consecutively. Other organ system involvement includes the skin, musculoskeletal system with arthralgias and myalgias, central nervous system, and lungs, and gastrointestinal, cardiac, and genitourinary systems. Vascular involvement may affect large blood vessels, capillaries, venules, and veins. The diagnosis of Behçet's disease is based on an established set of criteria (210). The criteria include the presence of oral ulcerations, and two or more of the following: recurrent genital ulcerations, eye lesions, skin lesions, and positive pathergy test results. The latter test represents a nonspecific skin hyperreactivity induced by intradermal needle prick (211).

Epidemiology

Although Behçet's disease has been reported worldwide, the highest incidence of disease appears to be in Japan, the Middle East, and around the Mediterranean basin. The incidence ranges from 1 to 2 per 10,000 in Japan and Saudi Arabia to as low as 0.3 per 100,000 in Northern Europe. The peak age of onset is within the third decade, and there is a male preponderance in most published case series. Men are also reported to have more severe disease than women.

Pathogenesis

The etiology of Behçet's disease remains unknown. Associations with infectious agents such as herpes simplex virus I, *Streptococcus sanguis,* parvovirus B19, and *Mycobacterium tuberculosis* have been hypothesized and evaluated to various degrees. However, no direct link has been convincingly established. Human leukocyte antigen (HLA) typing reveals a close association between Behçet's disease and HLA-B51 (especially the allelic variants HLA-B*5101) (212) and HLA-B*5108 and HLA-B*57 among Caucasians (213). Other studies point to an association with a microsatellite located between the HLA-B locus and the TNF gene rather than an association with the HLA-B*51 gene itself (214). Therefore, the TNF promoter allele TNF-1031 was found to be independently associated with susceptibility to Behçet's disease among Caucasians (213). The presence of antibodies to a number of autoantigens such as alpha-tropomyosin (215) has been described. The role of such autoantibodies in the pathogenesis of the disease is unclear. Similarly, T cells are likely involved in the pathogenesis of Behçet's disease as evidenced by an increase in $\gamma\delta$T cells (216), a predominance of Th1 cell phenotype (217) and autoreactive T cells (218).

Renal Involvement

Renal involvement in Behçet's disease appears to be more frequent than previously recognized. The spectrum of involvement ranges from subtle urinary abnormalities to end-stage renal disease and can conceptually be divided into five categories: (i) glomerulonephritis, (ii) amyloidosis, (iii) renal vascular involvement, (iv) interstitial nephritis, and (v) other problems, such as complications of drug therapy or genitourinary system abnormalities (219). The nephrotic syndrome and renal failure occurring in the setting of Behçet's disease can be associated with the presence of AA amyloidosis more typically found in patients with long-standing disease (220). Based on an extensive review of the published case reports (totaling 159 patients), amyloidosis was the most commonly reported lesion (43% of cases), whereas glomerulonephritis accounted for 32% of cases (219). Although an early study reported the presence of hematuria, proteinuria, or both in about one-third of patients (221), a recent extensive retrospective review of more than 4200 cases identified such urinary abnormalities in about 11% of patients tested and documented glomerulonephritis in only 7 (0.16%) patients (222). The pathologic lesions associated with Behçet's disease include focal and diffuse proliferative glomerulonephritis, membranoproliferative glomerulonephritis, focal segmental necrotizing glomerulonephritis with crescents, and

minimal change disease. In Benekli's review, predominant IgA deposits are reported in 11 of 40 cases of glomerulonephritis associated with Behçet's disease. The report of several cases of focal segmental necrotizing and crescentic glomerulonephritis (223,224) in the absence of immune complex deposition raises the question of an association with ANCA. The presence of such autoantibodies has been reported in rare cases (225), but not in systematic screening of patients with Behçet's disease (226).

Treatment

A number of immunomodulatory and immunosuppressant agents are used in the treatment of Behçet's disease. These include corticosteroids, calcineurin inhibitor, azathioprine, and interferon-alpha (227,228). More recently the use of agents that block the TNF pathway has also been reported (229). Because of the rarity of glomerular involvement in Behçet's disease, no definitive data for treatment are available. The use of corticosteroids has been reported with variable outcomes. In cases of Behçet's disease with severe vasculitic disease or glomerulonephritis, the use of corticosteroids and immunosuppressive therapy with azathioprine or cyclophosphamide may be justified.

TAKAYASU'S ARTERITIS AND TEMPORAL (GIANT CELL) ARTERITIS

Aortitis is a common feature of Takayasu's and giant cell arteritis but is also associated with other vasculitides such as syphilis, tuberculosis, mycosis, Behçet's disease, and Kawasaki's disease. The most commonly involved vessels are the subclavian arteries in more than 90% of patients. Diagnostic differentiation between Takayasu's and giant cell arteritis is largely based on age, with patients younger than 40 years having Takayasu's arteritis and those older than 50 having giant cell arteritis. Aortic aneurysm rupture represents a morbid complication of giant cell arteritis. Aortitis may result in ischemic symptoms or infarction of the area supplied by the involved vessel. Asymptomatic aortitis may be a more common phenomenon than previously thought (230).

Laboratory Findings

Laboratory findings in large-vessel vasculitis include a mild anemia, elevated levels of C-reactive protein, elevated erythrocyte sedimentation rate, and a generalized elevation in γ-globulin levels. Other serologic study results, including those from tests for lupus and infections, are typically negative. Patients typically present with only mild proteinuria and hematuria. The most common presentation is associated with hypertension and renal insufficiency, whereas renal failure is uncommon.

Pathogenesis

The pathogenesis of giant cell and Takayasu's arteritis is not known. However, there are several tantalizing clues that infectious agents may play a role in these diseases. In animals, there is evidence that gamma herpes virus 68 causes arteritis in mice lacking the interferon-γ receptor. In humans, an association exists between giant cell arteritis and parvovirus B19 infection (231). However a recent study using polymerase chain reaction (PCR) and immunohistochemistry techniques on 147 temporal artery biopsies found no evidence of parvovirus B19 DNA in the arteries of patients with giant cell arteritis (232). A cyclic occurrence of disease, with a peak incidence occurring every 5 to 7 years, suggests an infectious cycle. Certain genetic factors are associated with the development of giant cell arteritis. This form of vasculitis is more common in individuals of Northern European descent living in Europe or the United States (233), and there is clustering of cases among families (234). The development of giant cell arteritis also correlates with the expression of HLA-DR4, which is also found in high frequency among patients with polymyalgia rheumatica (235).

Giant cell arteritis may be a consequence of either or both the humoral and cellular immune responses. The clinical and experimental findings suggest that a cell-mediated process is most likely (236). Most inflammatory cells that invade the vessel walls are CD4-positive T cells. Elevated levels of IL-6 correlate with the severity of the disease and decrease quite rapidly when glucocorticoids are administered (237). Levels of several other cytokines and chemokines are similarly elevated. It is hypothesized that activated monocytes infiltrate the adventitia of large vessel walls via the vasa vasorum and become macrophages that then produce interferon-γ and recruit additional leukocytes, including macrophages. Unfortunately, the antigen responsible for these interactions has yet to be elucidated.

Renal Involvement

Renal involvement in Takayasu's arteritis and giant cell arteritis is usually a consequence of inflammation and scarring of the aorta adjacent to the orifice of the renal artery, leading to stenosis of the renal artery and ischemic renal failure. One of the most common clinical presentations of this phenomenon is renovascular hypertension affecting more than 50% of patients with Takayasu's arteritis (238). In Japan, Takayasu's arteritis is an important cause of hypertension in adolescents.

Glomerular lesions and necrosis occurs in patients with large-vessel vasculitis, but this may be an overlap of a small-vessel vasculitis. Several cases of glomerulonephritis in the setting of Takayasu's arteritis have been reported in the literature. The renal pathology in these cases varies from case to case, including focal segmental sclerosis (239), mesangial proliferation (240), membranoproliferative lesions (241), and crescentic lesions (242).

Treatment

The cornerstone of treatment of giant cell arteritis is based on the use of high-dose corticosteroids. Several guidelines or recommendations have been published. Typically, prednisone is started at 1 to 1.5 mg/kg per day, until the erythrocyte sedimentation rate is normal and the patient is asymptomatic. Initial treatment on an alternate-day basis is ineffective in the treatment of giant cell arteritis. When compared with patients receiving daily corticosteroids, only 30% (versus 85%) of patients treated with an alternate-day dosing enter an early remission, and 75% (versus 15%) experience a flare of disease activity (243). Intravenous pulses of methylprednisolone (1 g per day for 3 to 5 days) is recommended for patients with severe visual loss, because this treatment seems to prevent additional visual loss or fellow-eye involvement after initiation of corticosteroids (244). The initiation of corticosteroids within 2 weeks before a temporal artery biopsy does not change the characteristic pathologic findings (245,246). A delay in treatment to obtain a temporal artery biopsy is therefore not warranted.

Once a clinical remission is attained, a subsequent slow taper of corticosteroids is undertaken by decreasing the dose by 10% every 2 weeks to a dose of 10 mg per day, then by 1 mg per day. Other similar tapering protocols have been suggested, but no critical assessment of these recommendations is available. A switch to an alternate-day regimen may be similarly efficacious and perhaps less toxic (247). Symptoms usually resolve within 2 to 3 days and the erythrocyte sedimentation rate usually normalizes within 4 to 6 weeks. Most patients with giant cell arteritis require 2 years of corticosteroids, and a few remain on a low-dose regimen indefinitely. Patients who continue to require "maintenance" dosages of more than 15 mg per day may be considered "steroid resistant."

The high rate of complications attributable to the prolonged duration of corticosteroid therapy and the age distribution of patients with giant cell arteritis has led to an interest in identifying steroid-sparing alternative drugs. Dapsone, azathioprine, cyclosporine, antimalarials, cyclophosphamide, or gold have not been found to reduce corticosteroid toxicity and still maintain therapeutic effectiveness (248). The reported beneficial effect on the rate of relapse of adding methotrexate to corticosteroids (249), was not confirmed by a multicenter, placebo-controlled trial (250). A retrospective study suggests that the concomitant use of low-dose aspirin decreases the rate of visual loss and cerebrovascular events in patients with giant cell arteritis (251). Information on the use of TNF-alpha-blocking agents in the treatment of giant cell arteritis is currently limited to very small case series (252–254).

The treatment of Takayasu's arteritis is similarly based on high-dose corticosteroids. In a National Institutes of Health study, treatment was initiated at 1 mg/kg (up to 60 mg per day) for 1 to 3 months, followed by a slow taper to an alternate-day regimen over the following 4 to 8 weeks, and a subsequent slow taper over the following 6 to 12 months. This regimen is associated with a remission rate of 60% and an estimated median time to remission of 22 months (238).

Unfortunately, relapses occur in as much as 45% of patients, leading to multiple or prolonged courses of corticosteroids. Up to 40% of patients require the addition of cytotoxic drugs such as cyclophosphamide or methotrexate. In an open-label study of 18 patients with persistent or refractory Takayasu's arteritis despite treatment with corticosteroids alone, the use of methotrexate was associated with an 81% remission rate (255). Fifty percent of patients achieved a corticosteroid-free remission on methotrexate, and half of these patients remained in remission after methotrexate was withdrawn. About 20% of patients did not attain remission despite corticosteroids and methotrexate. The successful use of infliximab has been reported in several case reports of patients with active Takayasu arteritis despite conventional therapy with corticosteroids and cyclophosphamide (256) or methotrexate (257). The use of anti-TNF therapy was assessed in a pilot, open-label trial involving a total of 15 patients with active, relapsing Takayasu's arteritis. Seven patients were initially treated with etanercept and eight with infliximab. The use of anti-TNF agents led to remission in 10 of the 15 patients that was sustained for 1 to 3.3 years without glucocorticoid therapy. Four patients achieved partial remission, with a >50% reduction in the glucocorticoid requirement. Two relapses occurred during periods when etanercept was interrupted, but remission was reestablished upon reinstitution of therapy (258).

The optimal treatment of patients with Takayasu's arteritis is further complicated by the results of biopsies of affected vessels obtained at the time of bypass surgery. These data revealed evidence of persistent vascular inflammation even in the absence of clinical signs or symptoms of active disease and in the setting of a normal erythrocyte sedimentation rate (238,259,260).

The diagnosis and treatment of hypertension represents a very important aspect of the care of patients with Takayasu's arteritis because congestive heart failure, ischemic or hemorrhagic stroke, and renal failure account for most of the deaths from this disease (261). The diagnosis of hypertension may be missed if it is based on measurement of blood pressure in the upper extremities alone, because of the high incidence of subclavian artery stenoses, which may be bilateral. In some cases, lesions in the thoracic or abdominal aorta or the iliac or femoral arteries may give misleading normal blood pressures in the lower extremities as well. It is thus recommended that arteriographic studies be performed with pressure transducers so that aortic pressures can be compared with extremity pressures, and to identify the extremity where blood pressure monitoring is most reliable and reflective of true blood pressure (261).

THERAPEUTIC CONSIDERATIONS COMMON TO ALL VASCULITIC SYNDROMES

As the mainstay of therapy of severe vasculitis remains based on corticosteroids and alkylating agents, it is associated with short- and long-term complications. The most prominent side effects of this form of therapy are infection, ovarian failure (especially with a prolonged course of cyclophosphamide), bone disease, and cataract formation. In addition, the prolonged use of cyclophosphamide is associated with a 15% risk of developing a transitional cell carcinoma of the bladder over the course of 5 to 10 years (214). Whether the use of monthly pulse intravenous cyclophosphamide (which is associated with a smaller incidence cumulative dose and a lower incidence of hemorrhagic cystitis) can reduce the rate of bladder cancer is not yet ascertained.

The institution of attentive supportive care is crucial in minimizing the short- and long-term complications. Compulsive attention must be paid to the early detection and aggressive treatment of infections, because they remain an important cause of morbidity and death (262). Whenever possible, the use of trimethoprim-sulfamethoxazole for the prevention of *Pneumocystis carinii* pneumonia should be considered.

Whenever corticosteroids are used, measures must be taken to minimize the development of osteoporosis (263). Specific recommendations include calcium (1.2 g per day) and vitamin D supplementation, and, in selected patients with established osteoporosis, calcitonin nasal spray or alendronate for patients in whom the drug is not contraindicated (e.g., azotemia or esophagitis). Rigorous control of blood pressure with sodium restriction and antihypertensive therapy is essential to minimize the additive effect of hypertension in loss of renal function after active nephritis. Current research directions include the preservation of gonadal function by hormonal manipulation during cytotoxic therapy. In a small study, the use of testosterone during cyclophosphamide treatment appeared to prevent azoospermia (264). The gonadotropin-releasing hormone agonist leuprolide appears to be effective in the prevention of cyclophosphamide-induced ovarian failure based on a small prospective uncontrolled trial of patients with lupus nephritis (265).

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CHAPTER 69 ■ MIXED CRYOGLOBULINEMIA

GIUSEPPE D'AMICO

The term "cryoglobulinemia" describes a group of proteins with the common property of precipitating from cooled serum (1–5). On the basis of immunochemical studies, Brouet et al. (6) identified three types of cryoglobulins.

In type I, the cryoprecipitated immunoglobulin is a single monoclonal immunoglobulin. Types II and III cryoglobulinemias are both mixed types with at least two immunoglobulins; in both types, polyclonal immunoglobulin G (IgG) is bound to another immunoglobulin, which is an antiglobulin, acting as an anti-IgG rheumatoid factor (RF). The important difference between these two types of mixed cryoglobulins (MCs) is that in type II the antiglobulin component, which usually is of the IgM class, is monoclonal, whereas in type III it is polyclonal. Both components of MCs, IgG and IgM RF, are necessary for precipitation in the cold, whereas the individual components do not have this property.

The majority of MCs, defined as "secondary mixed cryoglobulins," have been detected in patients with connective tissue disorders, lymphoproliferative disorders, noninfectious hepatobiliary diseases, or immunologically mediated glomerular diseases.

Until the end of the 1980s, the cause of approximately 30% of both type II and type III mixed cryoglobulinemias was not clear; this subset of cryoglobulinemias was called "essential" (7). Over the past 10 years, anti-hepatitis C virus (HCV) antibodies and HCV RNA (a marker of active viremia) have been detected in the great majority (up to 90%) of patients with "essential" mixed cryoglobulinemia of both types (8–17). Positivity for HCV RNA was even greater in the cryoprecipitate (11,13,16).

The clinical syndrome of mixed cryoglobulinemia (MC) was first described by Meltzer et al. in 1966 (7). It was characterized by purpura, weakness, arthralgias, and, in several patients, proliferative glomerulonephritis (GN). Many subsequent reports further defined this syndrome, confirming that renal involvement is particularly frequent in MC (18–37). Kidney involvement ranges from 8% to 58% in large series of patients and is more frequent in women and in type II MC. The incidence of MC nephritis varies in different geographic areas, probably reflecting a different distribution of hepatitis C. The disease seems to be more frequent in some Mediterranean countries such as France, Spain, and, particularly, Italy, whereas it is very rare in northern Europe and in North America. Although the glomerular lesions are variable and nonspecific in the few cases of type III MC with renal involvement, in type II MC in which IgMk is the monoclonal component, a particularly well-characterized pattern of glomerular disease has been described (24,26,27,31,38,39), that Mazzucco et al. (39) termed "cryoglobulinemic glomerulonephritis."

RENAL HISTOLOGY

Light Microscopy Findings

Glomeruli

Cryoglobulinemic glomerulonephritis is a membranoproliferative, exudative GN. It is different from the idiopathic type of membranoproliferative GN as well as from the diffuse proliferative GN of systemic lupus by having the following features:

1. Endocapillary hypercellularity mainly due to a frequently massive infiltration of monocytes and sometimes T lymphocytes (38–44) (Fig 69-1). Some of the infiltrating monocytes express anti-tissue factor procoagulant activity and are therefore activated cells (44).
2. Large, amorphous, eosinophilic, periodic acid-Schiff (PAS)–positive, Congo red-negative deposits lying against the inner side of glomerular capillary wall and filling the capillary lumen, the so-called *intraluminal thrombi*, seen in about one-third of all patients and especially in those with more acute renal disease and more massive proliferation and exudation (26,27,31,43,45,46) (Fig. 69-2).
3. Thickening of the glomerular basement membrane, with a "double contour" appearance, which is more diffuse and evident than in lupus nephritis and idiopathic membranoproliferative GN (Fig. 69-3). As seen by electron microscopy, the double contour is mainly due to the peripheral interposition of monocytes, whereas mesangial matrix and cell interposition are less evident than in lupus nephritis and in idiopathic membranoproliferative GN (37,39,43,47,48).

Not all patients with type II MC have these characteristic glomerular lesions. In about 10% of patients only a mild segmental mesangial proliferation, without significant monocytes infiltration, is found. In another 10% of patients the glomerular pattern is that of a lobular GN, with mesangial expansion and centrolobular sclerosis. With the exception of this small group of patients, glomerular segmental and global sclerosis is rather mild and inconstant even many years after the onset of renal disease (26,31,43).

Interstitium and Vessels

Monocytes and T lymphocytes accumulate in the interstitium in the acute stages (44). Interstitial fibrosis, however, is not a prominent finding, even in late biopsy specimens.

An acute vasculitis of small and medium-sized arteries is present in at least one-third of patients who are biopsied

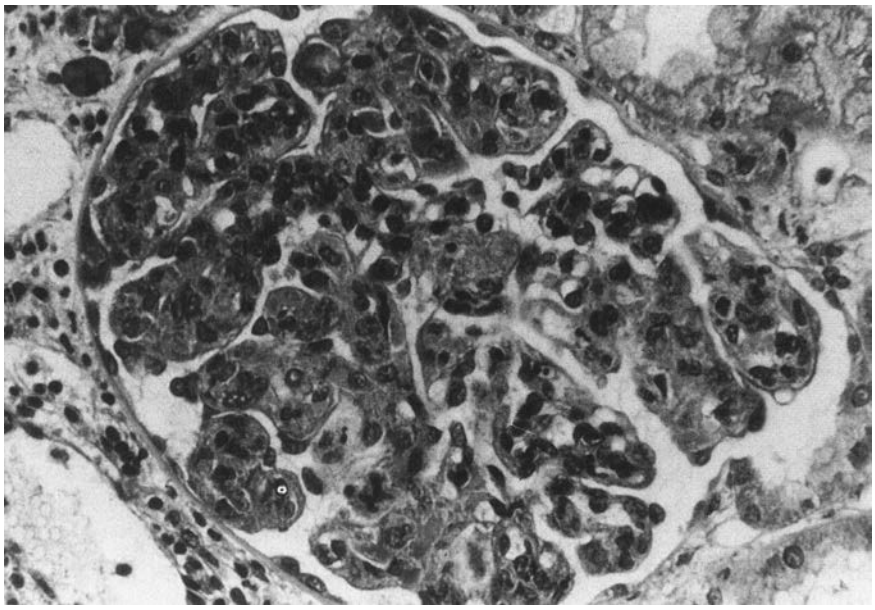

FIGURE 69-1. Cryoglobulinemic glomerulonephritis. Note the marked infiltration of the glomerular tuft with mononuclear cells. Light microscopy. (Masson trichrome, magnification ×320.)

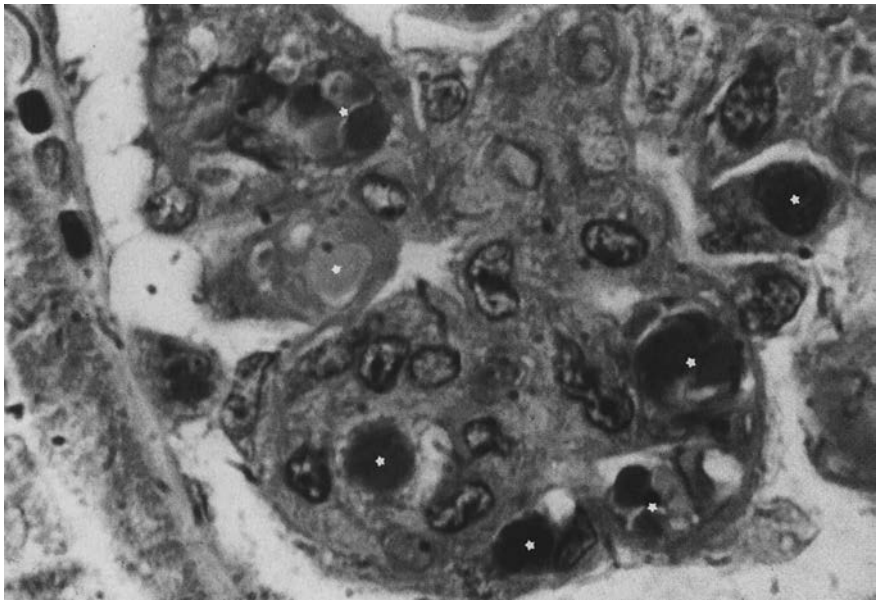

FIGURE 69-2. Numerous hyaline thrombi (*) occluding many capillary lumina. Note their different tinctorial affinity. Light microscopy. (Masson trichrome, magnification ×750.)

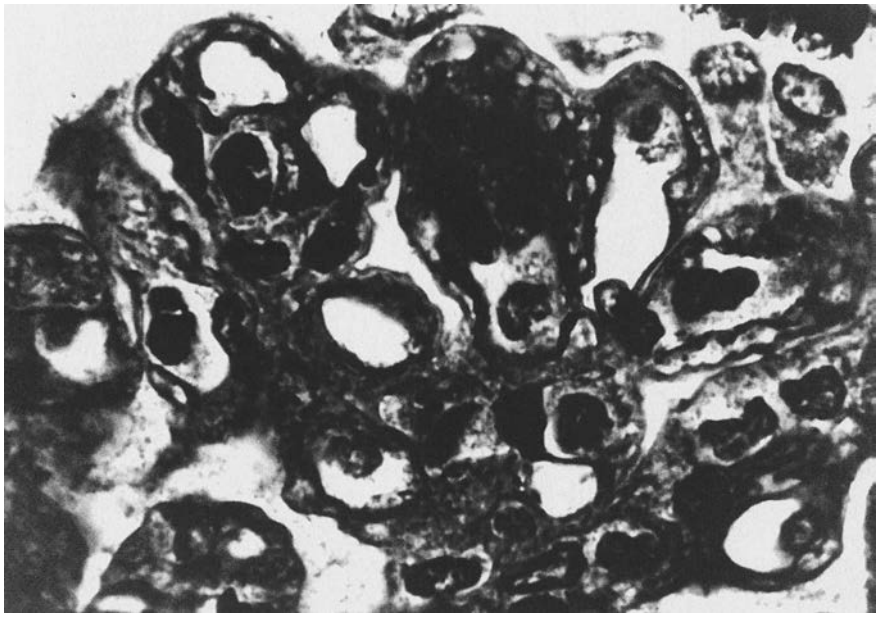

FIGURE 69-3. Glomerular capillary walls with "double-contour" appearance. Light microscopy. (Jones silver methenamine, magnification ×750.)

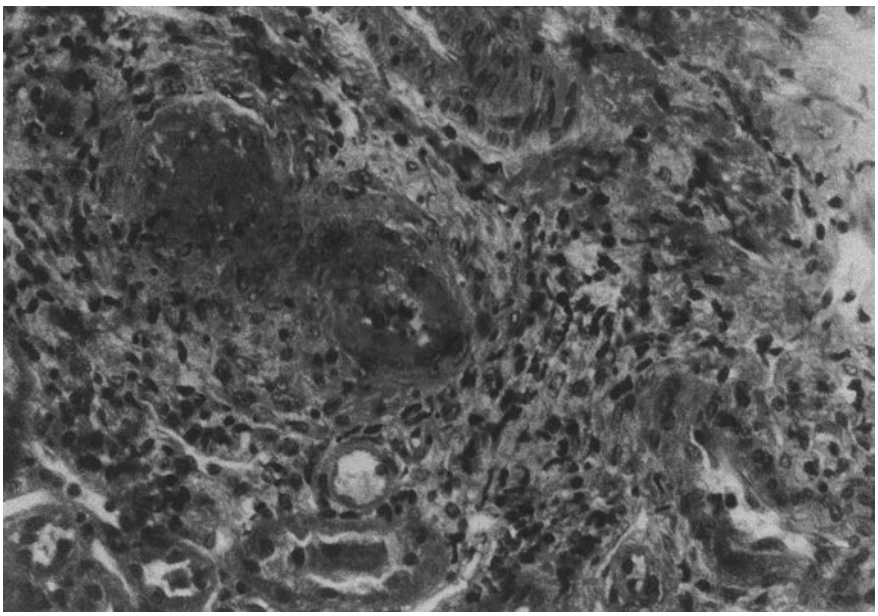

FIGURE 69-4. Severe necrotizing arteritis involving an interlobular artery. Light microscopy. (Masson trichrome, magnification ×320.)

during the acute stage of the renal disease, and in a larger percentage of postmortem specimens (31,47). It can be found even in the absence of obvious glomerular damage. The acute vasculitis is characterized by fibrinoid necrosis of the arteriolar wall, with infiltration of monocytes around the wall (Fig. 69-4). Even when the fibrinoid necrosis of the arterial wall is severe, the lesions of segmental necrosis of the capillary loops, with crescentic extracapillary proliferation (now considered the equivalent of vasculitic damage of the glomerular capillaries), is absent, suggesting that the vasculitic damage is limited to arterial vessels of larger size.

Electron Microscopic Findings

Electron-dense deposits can be found in the capillary lumen, usually in a subendothelial position but sometimes also filling the capillary lumen. They are sometimes amorphous immune complex-like deposits, but often they have a peculiar fibrillar or crystalloid structure (24,26,27,31,49,50), which is identical to that seen in the *in vitro* cryoprecipitate in the same patients, consisting of cylinders that are 100 or 1000 nm long and having a hollow axis (Fig. 69-5). In cross-sections they look like annular bodies with a light center, a dense ring, and lighter peripheral protein coat. The crystalloid deposits, when present, are often surrounded by areas of amorphous, weakly osmiophilic and translucent material, attributed to degradation of the deposits. Both types of deposits are infrequently seen in mesangial area, and subepithelial deposits are quite unusual. Circulating aggregates or structured material can sometimes be found in peritubular capillaries and arterioles. Ultrastructural examination confirms that monocytes in the capillary lumina are responsible mainly for the intracapillary proliferation, and increase in mesangial matrix can also be found. Monocytes very often appear to be in close contact with subendothelial and intraluminal amorphous or crystalloid deposits. In fact, these cells appear to be filled with large amorphous protein droplets with different osmiophilia, which are probably products of phagocytosis (phagolysosomes) (38,40,50). These intracellular protein droplets do not have a crystalloid structure, suggesting that the cryoglobulins lose their structure during phagocytosis. When a large number of these monocytes with giant protein droplets accumulate in the capillary lumen with the amorphous or crystalloid deposits, they contribute to the complete occlusion of the lumen. Monocytes can frequently be found interspersed with the subendothelial deposits, between the glomerular basement membrane and the endothelial cells or the newly formed basement membrane-like material in those areas in which capillary walls are double-contoured.

Immunohistologic Features

Three patterns of glomerular deposition can be seen by immunofluorescence (30,31,45,51,52).

1. Intensive, massive staining of huge deposits that fill the capillary lumen (intraluminal thrombi), usually associated with faint, irregular, segmental parietal staining of some peripheral loops, in a subendothelial position (Fig. 69-6).
2. The same pattern of faint, irregular, segmental parietal staining of some peripheral loops, in a subendothelial position, but without any intraluminal staining.
3. Intense granular diffuse staining of peripheral loops, with a subendothelial pattern (Fig. 69-7).

IgM and IgG are the prevailing immunoreactants in type II MC, suggesting that they are locally trapped or precipitated cryoglobulins. This identity was confirmed by the demonstration (53) that the immune deposits have antiglobulin activity similar to that of the serum cryo-IgM. More recently, using monoclonal antibodies against cross-reactive idiotypes present on rheumatoid factors, we identified the same idiotype of the circulating monoclonal rheumatoid factors in renal biopsy specimens from patients with MC-GN (54). C3 is present very frequently in the parietal deposits, with a distribution similar to that of immunoglobulins, although with a lower intensity. Intraluminal thrombi only occasionally show positive fluorescence for complement components. Deposits of earlier complement components (C1 and C4) and fibrinogen are also found in the parietal deposits in about 30% of cases. Vascular deposits of IgM, IgG, C3, and fibrinogen are detected in about one-third of cases.

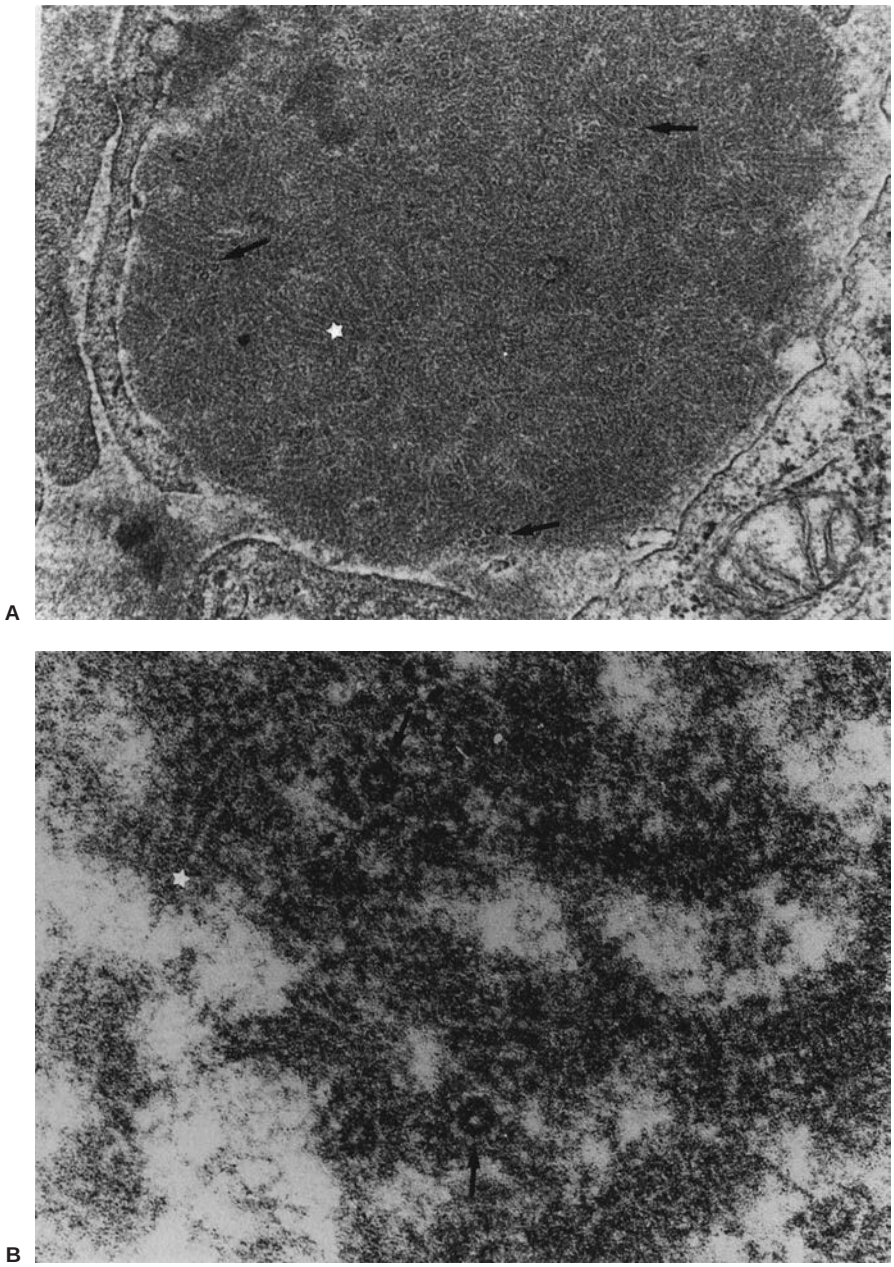

FIGURE 69-5. A: Crystalloid structure of deposits in the glomerulus. **B:** Identical structure in the *in vitro* cryoprecipitate of the same patient. The deposits consist of straight or slightly curved paired cylinders (✯) and annular structures (→).

CLINICAL MANIFESTATION

Clinical Features at Presentation

In most patients, type II MC is diagnosed between the fifth and the sixth decades of life, but in many patients some symptoms of the disease may first appear some 10 to 20 years before a correct diagnosis is made. Renal involvement usually manifests many years after the first symptoms develop. In some cases, however, renal and extrarenal manifestations appear concomitantly. More rarely, renal disease may be the first presenting manifestation.

The most frequent presenting renal syndrome is isolated proteinuria with microscopic hematuria, sometimes associated with signs of moderate chronic renal insufficiency. An acute nephritic syndrome characterized by severe proteinuria, microscopic or macroscopic hematuria, arterial hypertension, and a sudden rise in serum creatinine is the first renal manifestation in about 20% to 30% of patients and may be complicated by oliguria in some 5% of cases. In another 20% of patients, renal involvement presents with a nephrotic syndrome (26,27,30,34,37).

Arterial hypertension is observed in more than 80% of patients at the time of onset of renal disease (31,34,37). Extrarenal symptoms and signs are usually observed at presentation (Table 69-1).

Natural History and Prognosis

The severity of the disease may be remarkably variable. Extrarenal symptoms may show a fluctuating course with alternations of quiescence and exacerbation. Most patients have

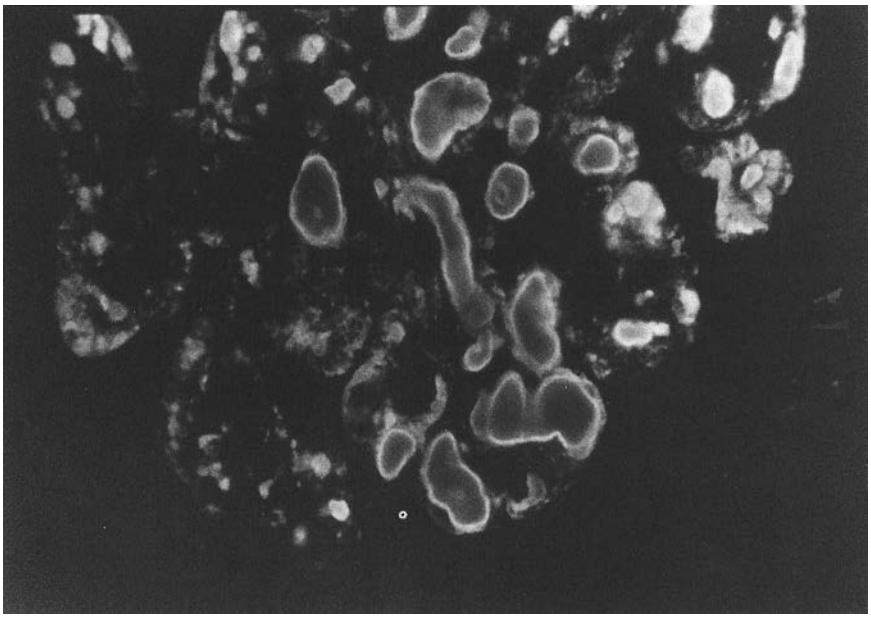

FIGURE 69-6. Large thrombi in many capillary lumina and scanty deposits along glomerular basement membranes. The antiserum stains only the periphery of the thrombi. Immunofluorescence study. (Anti-IgM serum, magnification ×450.)

recurrent, nonpruritic, palpable purpura or petechiae, usually affecting the lower extremities. Joints involved in MC include the hands, knees, ankles, and elbows, often in a migratory fashion. Fever is a relatively frequent manifestation of MC. About one-fourth of patients in whom infection can be excluded may present with prolonged episodes of fever. Liver involvement is frequent and can have serious consequences, but very often, despite the concomitant HCV infection, liver function abnormalities are mild and rarely symptomatic. Abnormal liver function tests were detected in about 25% of cases in two large series (27,34) of patients with renal involvement, in higher percentages of patients in other series of nonselected patients (25,35,36). Eighty to 100% of patients tested with antibodies or polymerase chain reaction have been found to be positive for HCV (8,9,11–15). An association with hepatitis B surface antigen has been reported in the past by some investigators (55,56), but this association was not confirmed. It is possible that some HBV-positive patients were also HCV positive. Histology shows a chronic hepatitis with a striking mononuclear cell infiltrate (56). In rare cases patients with MC may develop severe intestinal vasculitis with consequent ischemia, which can pose problems of differential diagnosis. Lung involvement is frequent in MC, probably as a consequence of immune complex deposition. Tests that are considered to be indicative of small airways disease frequently demonstrate abnormalities in MC (35). Several patients can show attack of spasmodic asthma, pleurisy, and hemoptysis with roentgenographic or histologic abnormalities (35,57). An acute respiratory distress syndrome requiring mechanical ventilation may rarely occur (58). Peripheral neuropathy was considered to be a rare complication of MC in the past, but when electromyography was routinely made, an abnormal peripheral nerve conductivity was found in 68% to 82% of patients (59,60). Lymphadenopathy, thrombocytopenia, Sjögren syndrome, Raynaud phenomenon, retinal vasculitis, and central nervous system involvement are rarely observed (32,34,61,62).

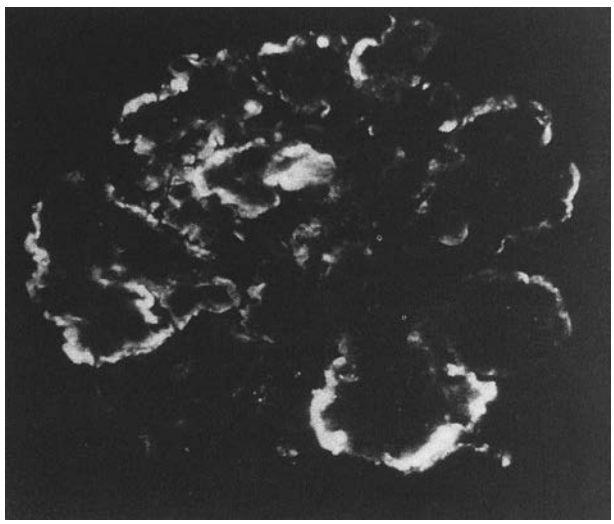

FIGURE 69-7. Diffuse granular deposits irregularly distributed on peripheral capillary loops. Immunofluorescence study. (Anti-IgM serum, magnification ×320.)

Kidney involvement in MC often heralds an ominous prognosis, although renal disease may have a variable course. Some 10% to 15% of patients may attain complete or partial remission of renal symptoms even though some of them presented with an acute nephritic syndrome (23,29). In another 30% of patients the renal disease follows an indolent course and does not progress to end-stage renal failure despite the persistence of urinary abnormalities and even some degree of renal dysfunction. In about 20% of patients, several episodes of an acute nephritic syndrome can occur, sometimes associated with flare-ups of the systemic signs of the disease. These episodes may be treated with high-dose corticosteroids and/or plasmapheresis but sometimes may spontaneously reverse and reappear; a moderate degree of renal insufficiency is rather frequent, usually long after the onset of the disease. However, the development of terminal renal failure is relatively rare. In the large series of patients with MC nephritis studied in Milan, Tarantino et al (29) reported that after a mean follow-up of 131 months only 15 of 105 patients required regular dialysis. However, in the meantime, 42 patients died because of extrarenal complications. Cardiovascular complications, infections, liver failure, and neoplasia were the most important causes of death (Table 69-2). Cardiovascular and cerebrovascular accidents were particularly frequent in patients with

TABLE 69-1

EXTRARENAL SIGNS AND SYMPTOMS IN 116 PATIENTS WITH ESSENTIAL MIXED CRYOGLOBULINEMIA NEPHRITIS

Purpura	88%	Raynaud's phenomenon	35%
Hepatomegaly	88%	Nervous system involvement	31%
Arthralgias	78%	Abdominal pain	27%
Weakness	67%	Thrombocytopenia	26%
Fever	56%	Increase in liver enzymes	25%
Anemia	53%	Weight loss	23%
Splenomegaly	50%	Pleurisy	23%

(From: Tarantino A, et al. Prognostic factors in essential mixed cryoglobulinemia nephropathy. In Ponticelli C, Minetti L, D'Amico G, eds. *Antiglobulins, Cryoglobulins and Glomerulonephritis*. Dordrecht: M. Nijhoff; 1986:219.)

arterial hypertension, which occurs early in MC and is often severe and difficult to control. In that study, the 10-year probability of being alive without dialysis was 49%. Older patients, those with purpura, splenomegaly, high serum cryocrit, low serum C3 levels, and high serum creatinine were more likely to die or to reach end-stage renal failure. An even higher mortality rate in patients with MC nephritis has been reported by others. Death was reported in 18 of 22 patients by Gorevic et al. (20) in 12 of 15 patients by Cordonnier et al. (22), and in 8 of 12 patients by Frankel et al. (27).

In summary, two groups of patients with MC nephritis can be identified: (a) those with multisystemic involvement including renal disease who have a tumultuous course and may die before end-stage renal failure develops, and (b) those with fewer signs of systemic vasculitis who generally have a benign course without developing renal failure. Whether these differences are correlated to the nature of immunoglobulins involved in the phenomenon of cryoprecipitation or to the physicochemical characteristics and other properties of cryoglobulins remains to be ascertained.

TABLE 69-2

CAUSES OF DEATH IN 42 PATIENTS WITH MIXED CRYOGLOBULINEMIA NEPHRITIS

Cause of death	No. of patients
Cardiovascular accidents	12
Cerebral hemorrhage	4
Heart failure	3
Intestinal infarction	2
Myocardial infarction	2
Pulmonary embolism	1
Infections	9
Sepsis	5
Tuberculosis	1
Meningitis	1
Pneumonia	1
Peritonitis	1
Liver failure	8
Neoplasia	4
Chronic lymphatic	1
Leukemia	1
Non-Hodgkin's lymphoma	1
Laryngeal cancer	1
Acute respiratory distress	1
Unknown	8

(Data from: Tarantino A, et al. Long-term predictors of survival in essential mixed cryoglobulinemic nephritis. *Kidney Int* 1995;47:618.)

Laboratory Data

As already discussed, cryoglobulins in type II MC are composed of two immunoglobulins, one of which is monoclonal and acts as an antiglobulin against polyclonal IgG. The monoclonal immunoglobulin is usually an IgM but can also be an IgG or IgA (18,63–66). Characterization of the cryoglobulins in patients with renal disease shows the exclusive monoclonal nature of antiglobulin IgM, which is nearly always an IgM-k (26,27,31). On the contrary, in rheumatologic surveys, patients with polyclonal IgM (type III cryoglobulinemia) outnumber those with monoclonal IgM. Serum titers of IgM rheumatoid factor (RF) activity are usually elevated and there are high serum levels of immune complex material as assessed by the C1q-binding assay (69–71).

The cryocrit value may vary considerably from 2% to 80% in different patients and can fluctuate over time in single patients. Also characteristic, although not invariable, is the pattern of complement activation in serum, with selective depletion of early components of the classic pathway and normal factor B levels (70). The serum pattern of complement is rather specific for MC. C1q, C4, and CH50 are usually low; C3 may be slightly low or normal; and the late components (C5-C9) C3PA and C1NH tend to be higher than in normal controls. The C3 breakdown product C3d is sometimes increased (26,70–72). These complement component abnormalities have been attributed to complement hyposynthesis (70,73), but it is also possible that the consumption of the early serum complement components with the characteristic sparing of C3 might be attributed to the C4-binding protein that controls the mechanism of the classic pathway C3 convertase (71,74). A retarded clearance of IgG coated autologous erythrocytes, indicative of abnormal Fc receptor function, is specific for patients with MC and renal involvement or motor neuropathy (75).

A peculiar hematologic finding of all types of cryoglobulinemias is represented by pseudoleukocytosis and/or pseudothrombocytosis, with an abnormal histogram as determined by a Coulter Counter (76,77). These abnormalities, which disappear if the blood sample is warmed at 37°C, can alert the clinician to the possible diagnosis of MC. Therefore, particular attention should be paid to the leukocytes and platelet counts in patients with MC (78).

No correlation has been found between the degree of activity of MC and the values for cryocrit, IgM rheumatoid factor, or early complement components (21,25,29,70). However, patients who have died had significantly higher cryocrit values and lower levels of IgG than did those who have survived, thereby suggesting a possible prognostic value of these parameters (79).

PATHOGENESIS

The Role of Hepatitis C Virus (HCV) Infection in Inducing Mixed Cryoglobulinemia

The polyclonal anti-IgG immunoglobulins involved in type III MC are derived from perturbation and magnification of the physiologic mechanism of production of antigloblulins concerned with immunoregulation and are antigen-driven, whereas the monoclonal anti-IgG immunoglobulins of type II MC may be derived from abnormal proliferation of a special clone of B lymphocytes, probably as a consequence of lymphoproliferative disorder (even though only a small number of patients with type II MC develop lymphoid malignancy) (37,80). Obviously, the same chronic antigen stimulation that induces the production of polyclonal RFs by B lymphocytes might in some cases favor the growth and amplification of a specific clone of B cells that produces a monoclonal RF. With a panel of mouse monoclonal anti-RF idiotypes, an increased percentage of idiotype-positive (μ +) B cells has been found in patients with type II MC (81). Peripheral B lymphocytes from MC patients produce larger quantities of IgM-RF than normal B lymphocytes, both spontaneously and after *in vitro* stimulation with pokeweed mitogen (82). Moreover, Agnello's group in the United States found the same cross-idiotype (WA) in the monoclonal IgM of type II essential MC and of type II MC secondary to malignancies of the immune system (83). It has been suspected that some antigen, possibly the same antigen that triggers the production of RFs, is bound to the polyclonal IgG of the MC of type II or III, so that the IgM RFs acts as anti-immune complex antibody. The recent demonstration of a very high rate of positivity of anti-hepatitis C virus (HCV) antibodies and of HCV RNA in the sera of patients with all type of mixed cryoglobulinemia suggests a direct role of such chronic infection in their pathogenesis.

Because it has been found recently that peripheral blood mononuclear leukocytes, and, especially B lymphocytes, can be the site for extrahepatic viral replication of HCV (84–86), particularly when there is chronic infection (84), we have postulated (32,80,87) that in a subgroup of patients with chronic infection direct active viral replication in B lymphocytes induces type III MC by triggering the activation of clones of B cells to hyperproduce polyclonal IgM RF. We have further postulated,that some additional, as yet uncharacterized, events might induce, in a small subgroup of this population of patients with polyclonal B cell activation due to viral infection, the shift to the abnormal proliferation of a clone of B cells producing a monoclonal IgM rheumatoid factor (type II MC).

The Role of HCV Infection for the Pathogenesis of Cryoglobulinemic Glomerulonephritis (and Other Types of Membranoproliferative Glomerulonephritis?)

It is now accepted that the large majority of patients with type II mixed cryoglobulinemia and membranoproliferative glomerulonephritis (MPGN) have an active HCV infection; however, in almost 10% of our large group of patients with cryoglobulinemic GN, we found constant and protracted negativity of all signs of HCV infection (80).

The role of the virus in inducing renal lesions is still a matter of controversy. It is still debated whether HCV antigens are directly involved in mediating the immune complex glomerular lesions, independent of the concomitant existence of a cryoglobulinemia (as HBV antigens do in chronic hepatitis B infection), or whether the renal lesion is mediated by the virus through induction of the lymphoproliferative disorder, which activates the B-cell clones that produce monoclonal IgM, and is present only when a type II mixed cryoglobulinemia is also present. Data from the United States (88,89) and from Japan (90) show that, although type II MC and MPGN usually coexist in patients with HCV infection, the same type of glomerulonephritis can be found even in the absence of cryoglobulins. On the contrary, no cases of idiopathic MPGN concomitant with HCV infection, in the absence of type II MC, were found in another report from the United States (91), in Italy (32), in France (92), in Spain (93), in Turkey (94), and in Hong Kong (95).

In an experimental mouse model recently developed by Fornasieri et al. (96), an MPGN very similar to human cryoglobulinemic GN could be induced by intravenous injection of 37°C solubilized cryoglobulins from patients with this renal disease and HCV infection; IgMk isolated from such cryoglobulins, and certainly not containing any viral antigenic component, when injected separately could deposit in the glomerulus, suggesting a specific affinity of IgMk RF components of the cryoglobulins for some glomerular structure, as has been demonstrated for light chain disease (97). These investigators demonstrated also that the same purified IgMk binds to cellular fibronectin, a known constituent of mesangial matrix (98).

On the basis of this preliminary evidence, we hypothesize that cryoglobulinemic GN might be initiated by the binding, either *in situ* or in circulation, of IgG antibodies-HCV complexes to IgMk RF, the nephrotoxicity being due to the particular affinity of the IgMk RF for the glomerular matrix.

Treatment

Antiviral Therapy of Hepatitis C Viral Infection with Associated Mixed Cryoglobulinemia. It is probable that eradication of the viral infection can affect production of new cryoglobulins, and thus also systemic and renal damage.

In 1987, before the viral cause of essential MC had been proved, Bonomo et al. (99) had already reported a remarkable improvement of clinical signs in seven cryoglobulinemic patients treated with interferon alfa (IFNα), used for its potential antiproliferative and immunoregulatory effects.

After the viral etiopathogenesis of MC was demonstrated, the same antiviral drug, interferon-α, was used extensively. However, only three controlled trials in patients with HCV infection and associated MC have been reported in the literature (100–102). All of them showed a significant improvement of systemic signs, mainly purpura, with a reduction of cryoglobulins and transaminases. However, although eradication of viremia was obtained in only a fraction of patients, a recurrence of viremia and a rebound of the clinical signs, and of cryocrit, was reported by all these investigators. With the exception of the study of Misiani et al. (101), no clear renal involvement was present in the treated patients.

More extensive information (103–124) has been accumulated in these last years on the effect of interferon-α, administered alone or in association with another antiviral agent, ribavirin, in reducing the viremia and the liver damage in patients with HCV infection, and in controlling the systemic symptoms of MC in the subgroup of patients with associated cryoglobulinemia.

The most effective standard treatment is the combination of pegylated IFNα and ribavirin (125). In the pegylated form of

interferon, the interferon-a molecule is linked to a polyethylene glycol molecule to ensure sustained interferon concentrations after single weekly injection (126–133).

Pegylated interferon-α potently inhibits the replication of HCV and has immunomodulatory properties that probably accelerate the clearance of infected cells. Ribavirin exerts a weak and transient early antiviral effect and acts primarily by preventing relapses during and after therapy in patients who have had an initial response. Ribavirin's mechanisms of action are unknown.

The main end-point of therapy is a "sustained virologic response," defined by an undetectable HCV RNA level in the patient's blood 24 weeks after the end of treatment. In most patients in whom a sustained virologic response is achieved, HCV infection appears to be definitively cured. Sustained virologic response, that did not exceed 40% to 45% of patients when the association nonpegylated IF-α-ribavirin was used, has been increased to 55% to 60% of them. In patients with no sustained virologic response with standard therapy, the lack of efficaciousness can be predicted already after 3 months of treatment. It has been found that in patients who do not have a reduction in HCV RNA of at least 2 $\log_{10}$ international units or an undetectable HCV RNA level by 12th week of treatment, there is virtually no chance of achieving a sustained virologic response. These patients can stop therapy at week 12. However, not all patients who have a reduction or disappearance of the virus at week 12 will subsequently have a sustained virologic response (125). Two pegylated interferon molecules have been commercialized. The recommended dose of peginterferon-α 2a is of 180 μg/week, that of peginterferon-α 2b is 1.5 μg/kg/week. The recommended dose of ribavirin is 1,000 to 1,200 mg/daily, based on body weight.

It has been found that both race and viral genotype influence the response to antiviral therapy. Blacks have been reported to have significantly lower response rates than the white and Asian populations. Treatment failure is more frequent among patients infected with HCV genotype 1.

As for the control of the extrarenal clinical manifestations of cryoglobulinemia, one study (133) suggested that improvement in cryoglobulinemia-related symptoms can be achieved, even without complete biochemical or virologic response.

Treatment of the Renal Complications of Mixed Cryoglobulinemia Associated with Hepatitis C Virus Infection

Although a sustained virologic response to the standard viral therapy described in the preceding text can interrupt the progression of the hepatic damage, and frequently improves the extrarenal signs of cryoglobulinemia, the results on the control of the clinical signs of the renal complication and on its progression are still controversial. Improvement, sometimes even protracted, of the renal disease has been reported in some anecdotal cases (134–137). In a more recent study in 25 consecutive patients with mixed cryoglobulinemia and proteinuria in the nephrotic range due to MPGN bioptically documented (138), a significant reduction of proteinuria and cryocrit after the standard combined antiviral therapy, without modifications of the serum creatinine, was found in the subgroup with sustained virologic response. The reduction in cryoglobulin level was sometimes persistent. Other clinical manifestations of cryoglobulinemia, such as arthralgia and polyneuropathy, were not clearly influenced by antiviral treatment.

There is now clear evidence that, when acute exacerbations of the glomerular disease occur, especially if they are associated with signs of acute systemic vasculitis, the antiviral therapy is not sufficient to control the disease (17,133,138–140), and may even exacerbate it (141–143).

According to our experience, steroids, and, in most severe cases, also plasmapheresis and cyclophosphamide, extensively used by us and by many others to treat "essential" MC in the 1980s, are still necessary to control flare-ups of cryoglobulinemic GN, despite the potential detrimental effect of the immunosuppressive drugs in increasing viral titers and aggravating hepatic damage.

Let us consider each of them separately.

Steroids. After the initial report by De Vecchi et al. (144), the subsequent experience of treatment of acute flare-ups of cryoglobulinemic GN and vasculitis has demonstrated that intravenous high-dose methylprednisolone (usually 1g per/day given every 24 hours for 3 consecutive days), followed by oral prednisone (0.5 mg/kg/day, rapidly tapered to 0.1 to 0.2 mg/kg/day) for at least 6 months, allows rapid improvement of the renal damage (serum creatinine and proteinuria) together with dramatic amelioration of the extrarenal symptoms.

Cyclophosphamide. This cytotoxic drug is usually in addition to steroid treatment, at the dose of 2 to 3 mg/kg per/day, for a minimum of 4 months, in the most severe cases of renal involvement, and in particular when renal and/or systemic vasculitis coexist. It has been accepted as a valid remedy despite the lack of controlled trials.

Plasmapheresis. The removal of significant amounts of cryoproteins from the blood, thereby preventing local cryoprecipitation in small renal vessels and restoring reticuloendothelial system functions that have been saturated by the chronic overload of circulating cryoglobulins, has been accepted as a useful therapy for the last 20 years. Many uncontrolled studies have reported good results, with improvements in serum creatinine, proteinuria, and cryocrit (7,30,145–151), and a very transient reduction of HCV viremia (152). Although in the majority of cases intensive plasma exchange (exchanges of 3 L of plasma per week for 2 to 3 weeks) has been performed in association with steroids and cyclophosphamide to block the production of new cryoglobulins

TABLE 69-3

THERAPY OF THE ACUTE FLARE-UPS OF CRYOGLOBULINEMIC GLOMERULONEPHRITIS AND VASCULITIS ASSOCIATED WITH HEPATITIS C VIRUS (HCV) INFECTION

INTERFERON-α
3 million units 3 times a week or peginterferon-α 2a 180 μg/week for 1 year (drug can be discontinued after 6 months if HCV-RNA does not disappear from the blood), and can be associated from the beginning to *ribavirin* (0.8–1.0 g/day)
STEROIDS
0.75–1.0 g/day of *methylprednisolone,* intravenously, for 3 consecutive days, followed by oral *prednisone* for 6 months (0.5 mg/kg of body weight daily, tapered over a few weeks until small maintenance doses are achieved)
CYCLOPHOSPHAMIDE
2 mg/kg of body weight, for 3 to 4 months (especially when signs of severe renal and systemic vasculitis are present)
PLASMAPHERESIS
Exchanges of 3 L of plasma 3 times/week, for 2 to 3 weeks (only most severe cases of acute renal involvement)

by the immunocomponent cells and the possible rebound, one group of investigators from Pisa, ltaly (147,153) has used plasmapheresis alone, repeated every few weeks for 2 to 24 months, to prevent clinical relapses.

Our current policy, derived from an extensive experience accumulated also with the treatment of "essential" MC in the 1980s, is to use antiviral agents (possibly a combination of IFNα and ribavirin), associated with small doses of oral steroids when necessary to control the systemic signs of MC. When an acute flare-up of the renal disease is present, with rapid deterioration of renal function and/or proteinuria in the nephrotic range, the schema indicated in Table 69-3 is used.

There was no evidence of any worsening of the liver involvement, as indicated by the level of hepatic enzymes in more than 50 patients treated with such a therapy, even though the potential increase of HCV replication in peripheral blood mononuclear cells, both *in vitro* and *in vivo*, after corticosteroid boluses has been recently reported (154).

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CHAPTER 70 ■ RENAL ARTERY THROMBOSIS, THROMBOEMBOLISM, ANEURYSMS, ATHEROEMBOLI, AND RENAL VEIN THROMBOSIS

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This chapter focuses on vascular complications of the main renal arteries and veins. The complex variety of vascular complications include (a) acute thrombosis of the renal artery, most often arising from trauma; (b) thromboembolism to the renal arterial vasculature; (c) acute dissection of the renal artery; (d) atheroembolic disease of the kidneys; (e) rupture of renal arterial aneurysms; and (f) acute and chronic renal vein thrombosis. The latter condition occurs primarily in patients with the nephrotic syndrome. Therefore, special attention is paid to the hypercoagulability of the nephrotic syndrome. In addition, other thromboembolic problems associated with this condition are examined. Furthermore, certain conditions, such as fibromuscular dysplasia and atherosclerotic disease of the renal vessels, which generally cause "renovascular" hypertension, are not considered in this chapter except as they relate to the differential diagnosis of the occlusive disorders just noted.

ACUTE THROMBOSIS OF THE RENAL ARTERY FROM TRAUMA

Acute thrombosis of the renal artery can be classified as traumatic and nontraumatic in origin. Blunt abdominal trauma of the acceleration or deceleration type is the most common cause of renal artery thrombosis (1). This occurs more often in young people, in the setting of motor vehicle accidents and falls. Thrombosis, lacerations with hemorrhage, and contusions may involve the main renal artery, branch vessels, and renal veins (2). Thrombosis is more commonly unilateral than bilateral, and is more likely to be left-sided (3), presumably because the left renal artery is shorter and more acutely angled to the aorta than the right renal artery. Thrombosis of the renal artery often occurs in conjunction with injury to other intraabdominal organs, which often obscures the diagnosis. Thrombosis may occur in 1% to 3% of severe blunt abdominal trauma (4–6). In a review of 250 patients who underwent surgery for traumatic renal artery injury, thrombosis (52%) was the most common finding, followed by avulsion (12%), and branch injury and lacerations (3%) (7). Renal lesions were bilateral in 22%, and injuries to other organs were present in 45% (7).

The rapid development of renal infarction with irreversible loss of renal function is the major concern of acute thrombosis. The maximal warm ischemic time tolerated by the human kidney before the development of infarction is uncertain, but it may be as short as 1 to 2 hours in complete occlusions (8). Incomplete occlusions or occlusions to kidneys with previous stenosis that have developed collateral circulation are viable for longer periods. Early experimental animal studies of temporary arterial occlusion shed light on this issue. Dogs that had a unilateral renal artery clamped to induce complete occlusion for variable hours had split renal function evaluated 4 days after the occlusion (9,10). When the ischemic clamp time was 1 hour, 60% of those kidneys had good function (more than two-thirds the glomerular filtration rate (GFR) of the contralateral kidney) 4 days later; in the group with 2 hours of clamping, this dropped to 38% of the kidneys, and after 3 hours of ischemia, none were functioning. Some of the acute renal failure may have been ischemic acute tubular necrosis and not infarction. Other dogs underwent partial occlusion of the suprarenal aorta, which allowed a low mean arterial pressure downstream, 17 to 30 mm Hg, to perfuse the kidneys. The kidneys tolerated 1 and 2 hours of this partial occlusion very well. Four days after the occlusion, GFR was similar to pre-ischemia control values in all the dogs. This suggests that in the dog, very low perfusion pressures that are inadequate to maintain glomerular filtration, can maintain renal viability for at least 2 hours.

There are no specific clinical or laboratory findings specific for renal artery thrombosis. Anuria or oliguria following abdominal trauma suggests the presence of bilateral thrombosis, but these can be seen in unilateral occlusion. Abdominal and flank pain are usually present. Hematuria may be noted, either gross or microscopic, but may be absent in 25% of patients (1). Elevations of creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and alkaline phosphatase may be present.

An early diagnosis is of paramount importance if surgical revascularization is contemplated. Computed tomography with contrast is the preferred study in severe abdominal trauma (11). The main computed tomographic (CT) findings in renal artery thrombosis are the lack of renal parenchymal enhancement and the absence of contrast excretion (Fig. 70-1). Occasionally, enhancement of the peripheral renal cortex, the "cortical rim sign," may be noted, which is attributed to capsular or collateral perfusion of the cortex (12). Other renal findings can be subcapsular, perinephric and intrarenal hematomas, renal lacerations, and extravasation of contrast media (Fig. 70-2). Depending on the severity of the lesion, it may be advisable to proceed to early angiography, which can provide the definitive diagnosis. Angiography, the gold standard for occlusive renal artery disease, can identify the severity of the lesion and the presence or absence of collateral circulation (13). With digital subtraction technology, angiography can be performed using very little iodinated contrast or none at all, if carbon dioxide and/or gadolinium or one of its derivatives is used instead (14). With these methods, concerns of contrast-induced renal failure are no longer a major concern, even in patients considered to be high risk for this complication. In the critical patient

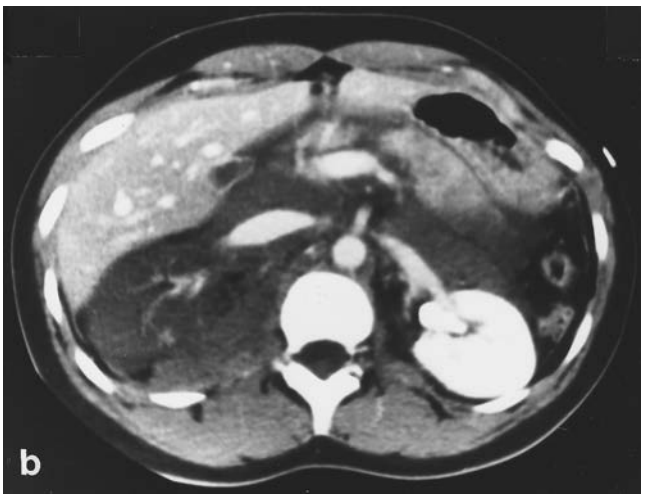

FIGURE 70-1. Arterial phase of computed tomographic (CT) image with contrast. Note the absence of enhancement of the right kidney because of renal artery thrombosis, and avulsion because of trauma. There is reflux of blood to the right kidney from the inferior vena cava because of decreased pressure in the right renal veins. (From: Lang, EK. *Radiology of the Upper Urinary Tract*. Heidelberg: Springer; 1991, with permission.)

who is too unstable to undergo these preoperative diagnostic studies, a bolus injection of contrast material followed by a "one-shot" excretory urogram may be extremely valuable to the surgeon preoperatively. A normal urogram of the kidney in question would exclude the presence of major trauma to that kidney. Alternatively, if the surgeon has to perform an emergent nephrectomy to control hemorrhage, a normal urogram of the contralateral kidney would suggest that it has good function (15).

Early surgical revascularization has been attempted to avoid renal infarction and irreversible loss of renal function, but the results for renal salvage have been mixed and often disappointing (15–18). The warm ischemic time and the size of the thrombosed artery (e.g., main renal artery or a branch), and the extent of occlusion (complete or partial) are major factors (15,17–23). Most successful revascularizations were performed within 12 hours of the onset of thrombosis. Maggio and Brosman reported 80% success of renal salvage when revascularization was performed within 12 hours of trauma, 57% success for repairs performed between 12 and 18 hours, and 0% for later attempts (23). Clark et al. described a dismal 17% success rate for renal salvage even though revascularization attempts were performed early, between 3 and 18 hours after the injury (7). Hass et al. reported surgical outcomes in 20 cases of bilateral occlusion and 34 cases of unilateral renal arterial occlusion (24,25). These cases were a mixture of their own cases and data from reports in the literature. Surgical revascularization was successful in 56% of the bilateral cases and in only 26% of the unilateral cases.

Early revascularization is indicated in cases of bilateral renal artery thromboses and of thrombosis to solitary functioning kidneys in order to avoid severe irreversible renal failure. However, in patients with unilateral thrombosis and a functioning contralateral kidney, surgical outcomes may not be better than medical management alone, so the indication for surgery in this setting is not clear. Despite the concerns regarding ischemic time, there are scattered reports of restored renal function when revascularization for traumatic renal artery thrombosis was performed long after the period of injury (21,22,23,27). In these cases renal function may have been preserved by collateral circulation if preexisting stenosis was present or the occlusion was incomplete.

Surgical revascularization should not consist of a thrombectomy alone (25,26). The occluded segment of the renal artery should be resected, with reinsertion in the aorta, or, alternatively, with interposition of an aorto-renal graft, with either PTFE or great saphenous vein (28). Nephrectomy may be required in emergent situations to control hemorrhage.

NONTRAUMATIC ACUTE OCCLUSIVE DISEASES OF THE RENAL ARTERIES

Acute occlusion of the renal artery or its branches may be due to thrombosis from trauma or other causes, from acute embolic events usually arising from the heart, or from dissecting aneurysms. The major consequence of acute arterial occlusion is the rapid development of renal infarction and irreversible loss of renal function.

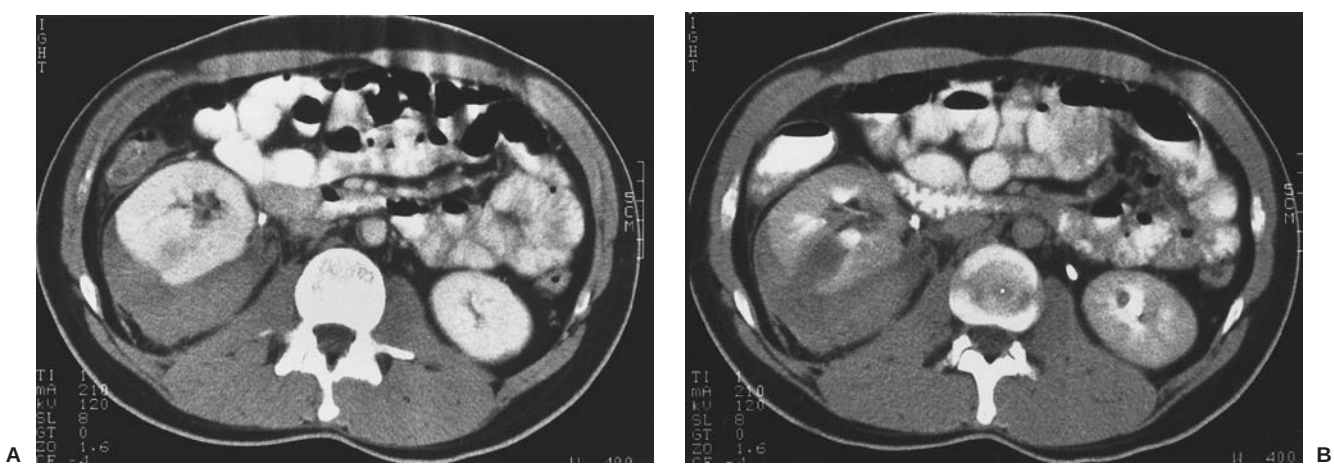

FIGURE 70-2. Computed tomography with contrast. Arterial phase (**A**) and late phase (**B**) demonstrate a wedge-shaped traumatic infarct localized to the posterolateral midpole of the right kidney. A large right perirenal hematoma is present as well. (From: Lang, EK, *Radiology of the Upper Urinary Tract*. Heidelberg: Springer; 1991, with permission.)

Nontraumatic Renal Artery Thrombosis—Causes

In Table 70-1 are listed the nontraumatic causes of renal artery thrombosis. Damage or disruption of the arterial endothelial surface initiates platelet adhesion and aggregation, and leads to thrombus formation. The most common setting for this is atherosclerotic renovascular disease. Other settings include fibromuscular dysplasia (28,29), inflammation, infection, renal artery aneurysms, and dissecting aneurysms. Thrombosis of the main renal arteries or branches has been described in the various vasculitides, including polyarteritis nodosum (30,31), Takayasu arteritis (32,33) Kawasaki's disease, and thromboangiitis obliterans. Other causes include Behçet's disease (34–37), syphilis (38), inflammatory diseases such as phycomycosis (39,40), cocaine use (41–43), neurofibromatosis (44), and infiltrating urothelial carcinoma of the renal pelvis (45). Thrombosis has been attributed to strenuous aerobic exercise (46) and to trauma from car seat belts (47). Renal infarction has been observed with sickle cell anemia (48).

Hypercoagulable states usually lead to venous thrombosis, not to arterial thrombosis. Exceptions to this include the antiphospholipid syndrome (APS) and heparin-induced thrombocytopenia. Severe cases of catastrophic diffuse arterial and venous thromboses can occur in both diseases. Thrombosis in the APS may occur in any location within the renal vasculature, that is, from the renal artery trunk to the renal veins. Renal artery thrombosis has been noted in both primary and secondary (lupus-related) APS (49–59). In a recent report of abdominal CT scan findings of abdominal thrombosis and ischemic events in patients with APS, renal infarctions of varying sizes were found in 22 of the 42 patients (60). Factor V Leiden mutation, which is associated with a short partial thromboplastin time, has been implicated as the cause of renal artery thrombosis in native kidneys and in renal allografts (61). Arterial thrombosis has also been observed rarely in patients with the nephrotic syndrome (62–64).

TABLE 70-1

NONTRAUMATIC CAUSES OF ACUTE OCCLUSIVE RENOVASCULAR DISEASE

1. Renal artery thrombosis
   - (A) following damage/disruption of endothelial surface
     - atherosclerotic disease
     - fibromuscular dysplasia
     - renal artery aneurysms and dissecting aneurysms
     - polyarteritis nodosum
     - Takayasu's arteritis
     - Kawasaki's disease
     - thromboangiitis obliterans
     - Behçet's disease
     - syphilis
     - cocaine
   - (B) specific hypercoaguable states
     - antiphospholipid antibody syndrome
     - heparin-induced thrombocytopenia
     - factor V Leiden mutation
     - nephrotic syndrome (rare)
2. Renal artery thromboembolism
   - (A) cardiac origin
     - atrial fibrillation
     - endocarditis
     - myocardial infarction
     - congestive cardiomyopathy
     - paradoxical emboli through patent septal defect
   - (B) aortic or renal artery source
     - severe atherosclerosis/thrombosis

Thromboembolism of the Renal Arteries—Causes

Emboli to the renal vasculature most commonly originate in the heart. These emboli, depending on their size, will travel downstream and obstruct arteries depending on their size. Cardiac disease and dysrhythmias, in particular atrial fibrillation, are the most common settings as an embolic source (65–67). Mural atrial and ventricular thrombi may develop in the settings of myocardial infarction or in congestive cardiomyopathy. Thrombi or vegetations may form on prosthetic or diseased heart valves and be the source of renal emboli. Before the use of antibiotics, renal emboli often occurred as a consequence of subacute bacterial endocarditis. Rarely, paradoxical renal emboli may result from venous clots passing through patent interatrial or interventricular septal defects (68). In addition to a cardiac source, peripheral emboli may originate from clots that develop along diseased endothelium or in aneurysms of the suprarenal aorta or renal artery.

Iatrogenic causes of renal artery thrombosis and thromboemboli have increased in recent years following endovascular aortic and renovascular procedures, and with stent placements by interventional radiologists and vascular surgeons. The risk of major complications associated with renal artery procedures is 7% to 10% according to a recent review. These include thrombosis, dissection, rupture, and atheroembolic showering (69,70). Morris et al. reported renal artery rupture or occlusion from acute thrombosis in 4.2% of 308 procedures of percutaneous transluminal angioplasty or stent dilatation of the renal artery (71) Two German centers reported renal complications following endovascular repair of thoracic and abdominal aortic aneurysms using a variety of endografts in 775 patients (72,73). New renal infarctions were noted in 10% of the cases when pre-procedure CT images were compared to postprocedure studies. New infarcts were considered to be the consequence of thromboembolic complications of the endovascular repair. Most of these infarctions were asymptomatic, and were small wedge-shaped findings on CT; however, one of the studies reported a 2.6% incidence of total unilateral renal artery occlusion following the procedure (72,73).

The downstream complications from renal angioplasty and stenting may be diminished with the use of distal filter protection devices. Holden and Hill reported their early experience with distal devices in 46 procedures of renal artery stent revascularization for atherosclerotic renal artery stenosis (74). Sixty-five percent of the distal protection baskets contained embolic material, including fresh and chronic thrombi and atheroemboli. More experience with distal filters is needed to determine their merit.

Pathology of Renal Infarction

Renal infarction is a fairly common autopsy finding, but an infrequent clinical diagnosis. In a review of 14,411 autopsies, renal infarctions of varying sizes were found in 1.4% of the cases, and most of them were postmortem diagnoses (75). Renal infarctions may occur in venous thrombosis, but they are much more common with arterial occlusions. Depending on the size of the occluded artery, the infarction could be a small wedge-like lesion or encompass the entire kidney. The gross appearance of the infarct depends on the size of the occluded

artery, the age of the infarct, and whether infection is present. Early on the infarct is red and pyramidal, and within hours it becomes gray with a narrow red rim of congested parenchyma. The necrotic area is eventually replaced by collagenous tissue. The area shrinks, leaving behind a V-shaped scar. Infarctions involve only the renal cortex; the medulla is usually spared (76).

On microscopic examination, sterile infarcts have the classic picture of coagulative necrosis (76). The initial findings of marked congestion are followed by cytoplasmic and nuclear degenerative changes, with gradual loss of viable cytologic structure. The cytoplasm becomes homogeneous and eosinophilic, and the nuclei undergo condensation and karyorrhexis. Surrounding this necrotic area is a transitional zone of sublethal injury with findings similar to acute tubular necrosis. This peripheral area becomes infiltrated with polymorphonuclear leukocytes. Eventually the central necrotic area becomes smaller, with eventual collapse, and is replaced by a collagenous scar (76).

Clinical Features and Diagnosis of Acute Occlusive Renal Arterial Disease

There are no specific signs or symptoms specific for arterial occlusion (Table 70-2). Most patients with acute thrombosis or thromboembolism to the kidney seeking medical care present with flank and/or abdominal pain. The pain is not specific, and may vary in intensity from a mild dull ache to excruciating pain. Nausea and vomiting, and fever and chills, may also be present, but fever may not develop until the second or third day after the onset of pain. The patient may have noted anuria or oliguria, and gross hematuria. Anuria or severe oliguria raises the suspicion of bilateral renal artery occlusion. However, oliguria may be seen in unilateral occlusion with a normal contralateral kidney, and it may last for several days. The decreased renal function and oliguria have been attributed to arteriolar spasm of the contralateral kidney. On physical examination, blood pressure may be mildly to markedly elevated. The abdomen and/or flank are usually tender. Bowel sounds may be absent, and guarding and peritoneal signs may be elicited. Conversely, small infarcts may be painless and asymptomatic in many cases. Gorich et al. found 12 new small renal infarcts on CT imaging following endovascular procedures that were considered iatrogenic thromboemboli; all 12 patients were asymptomatic (73). Obviously, the size of the occluding artery and the extent of renal ischemia play a critical role in the patients' symptoms. Early diagnosis of acute arterial occlusion is usually missed because the initial diagnostic considerations are typically directed toward other more common diseases such as gastroenteritis, pancreatitis, cholecystitis, nephrolithiasis, and pyelonephritis.

Laboratory abnormalities include an elevated LDH level, which may rise to 2,000 IU/L in cases of large infarctions. Serum AST and alanine aminotransferase (ALT) levels may be increased, but usually not to the same extent as LDH. Moderate elevations of alkaline phosphatase occur in 30% to 50% of patients, and may persist for up to 10 days. The course of changes of various serum enzymes in a patient with thromboembolic disease is shown in Figure 70-3. Most patients have microscopic hematuria (77), and mild proteinuria and leukocytosis are frequent. A low urinary sodium concentration, suggestive of renal hypoperfusion, is occasionally noted (Fig. 70-4) (78).

Imaging with CT with contrast, magnetic resonance angiography (MRA), or angiography, are the best means of diagnosis (Figs. 70-5 and 70-6). Angiography has the added advantage in that thrombolytic agents can be infused directly to treat an occluded artery if it is found. Noncontrast studies will miss the underperfused areas of the kidney. Isotopic renal flow scans can show absent or markedly reduced perfusion defects (Fig. 70-7). Their accuracy may be limited if acute renal failure is present. Ultrasonographic evaluation of the renal arteries using color and power-Doppler techniques is of limited value because the imaging of the entire renal artery is technically difficult, the procedure takes a long time, and the quality of the studies is operator-dependent.

TABLE 70-2

CLINICAL AND LABORATORY FEATURES OF THROMBOEMBOLIC DISEASES OF THE KIDNEY

Feature	Approximate incidence[a]
History and physical findings	
Pain and tenderness (flank, abdominal, chest, or back)	75%
Nausea and vomiting	50%
Gross hematuria	20%
Cardiac disease (myocardial infarction, atrial fibrillation, rheumatic valvular disease)	90%
Laboratory features	
Leukocytosis (11,000–32,000/μL)	95%
Microscopic hematuria (>15 erythrocytes per high-power field)	90%
Pyuria (>10 leukocytes per high-power field)	80%
Proteinuria (1+ to 4+)	95%
Increased enzymes (LDH, SGOT, SGPT, alkaline phosphatase)	95%–100%
Special diagnostic procedures	**Finding**
Intravenous urogram	Decr. or absent function; delayed appearance
Renal ultrasonography	No obstruction; rarely, wedge-shaped mass
Isotope renal flow scan	Decreased flow to all or part of kidney
Computed tomography (with contrast material)	Area of decreased accentuation; cortical rim of accentuation

[a]Refer to text.
LDH, lactic dehydrogenase; *SGOT*, glutamic-oxaloacetic transaminase; *SGPT*, glutamic-pyruvic transaminase; *Decr.*, decreased.

Therapy for Nontraumatic Acute Occlusive Renal Arterial Disease

The overwhelming concerns of renal arterial occlusion are renal ischemia and imminent infarction with irreversible loss of renal function. A rapid diagnosis and initiation of therapy, usually intraarterial thrombolysis, are critical to avert infarction and preserve renal function. The duration of warm ischemia, the size and extent of the vascular occlusion (e.g., bilateral versus unilateral; main, branch, or peripheral artery; complete versus partial occlusions) and the presence or absence of collateral

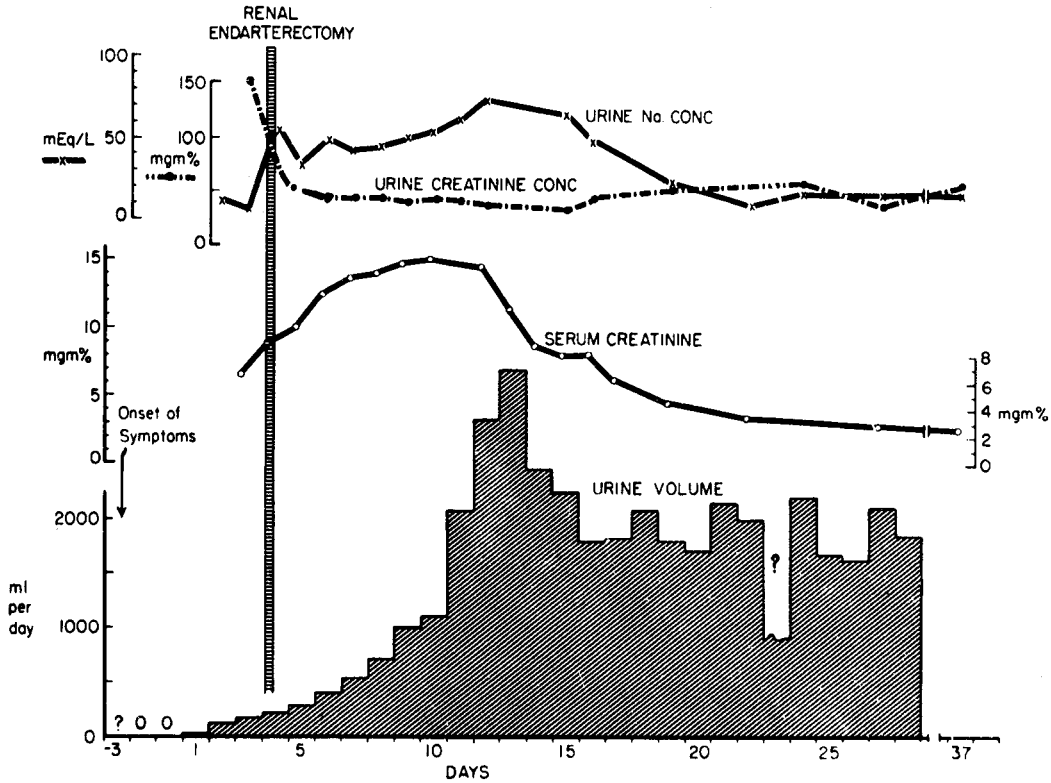

FIGURE 70-3. Course of a 56-year-old woman with abrupt onset of anuria and right lower quadrant and low back pain; she had atrial fibrillation and mitral stenosis owing to rheumatic heart disease. One year earlier she had undergone a left nephrectomy for malignant hypertension. (Earlier, she had had a stroke and blindness of the left eye owing to an embolism.) A diagnosis of thromboembolism to the renal artery was confirmed by aortography (Fig. 70-6), and surgery was perfumed with removal of the embolus and endarterectomy. The fractional excretion of sodium was 0.36% before surgery and increased to 7.3% after removal of the thrombus. There was ultimate recovery from acute tubular necrosis, as indicated. (From: Lessman, RK, et al. Renal artery embolism: clinical features and long-term follow-up in 17 cases. *Ann Intern Med* 1978;89:477, with permission.)

circulation are important factors in determining whether renal function can be preserved. The general rule is the shorter the duration of renal ischemia, the greater the chance of preserving renal function.

Blum et al. described 14 patients with acute embolic renal artery occlusion who were treated with thrombolytic therapy: intraarterial streptokinase, urokinase, or tissue-type plasminogen activator (79). The estimated ischemic time from onset of symptoms to therapy varied from 12 hours to 8 days. Recanalization and adequate renal perfusion were achieved in 13 of the 14 patients. Despite these results, renal function did not improve in any patient with complete occlusion, but did stabilize in those patients with partial occlusions. In their review of the literature, the duration and degree of renal artery occlusion were crucial; in 50 patients with complete occlusion of the main renal artery and ischemic time greater than 3 hours who underwent a successful reperfusion procedure by either thrombolysis or surgery, evidence of irreversible renal damage as shown by

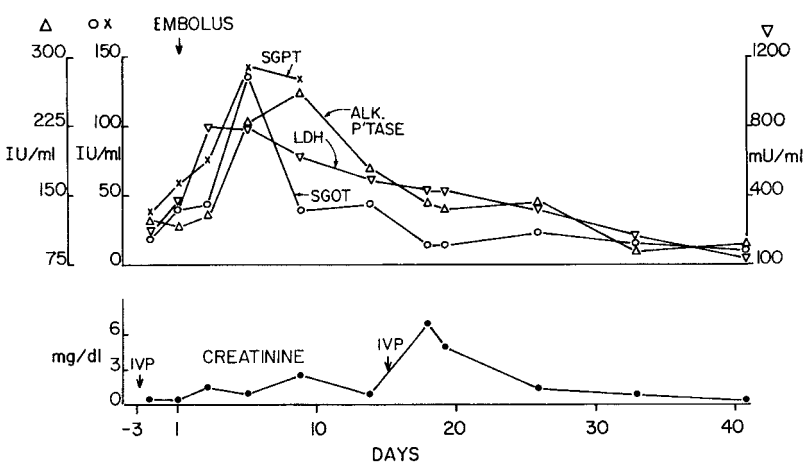

FIGURE 70-4. Serial serum levels of glutamic-oxaloacetic transaminase (SGOT, ○) glutamic-pyruvic transaminase (SGPT, X), lactic dehydrogenase (LDH, ▽), alkaline phosphatase (ALK P TASE, △) and creatinine (●) in a 62-year-old man with thromboembolism to a single kidney 10 days after a myocardial infarction. Right flank and chest pain and lower abdominal tenderness appeared on day 1. The patient also exhibited an increase in serum creatinine level following the second intravenous pyelogram (IVP). (From Lessman, RK, et al. Renal artery embolism: clinical features and long-term follow-up in 17 cases. *Ann Intern Med* 1978;89:477, with permission.)

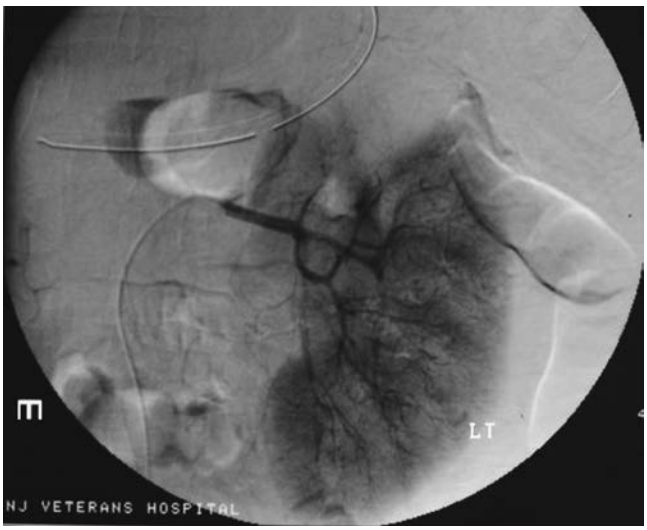

FIGURE 70-5. Left renal angiogram shows a wedge-shaped perfusion defect involving the upper pole of the kidney due to a thromboembolism.

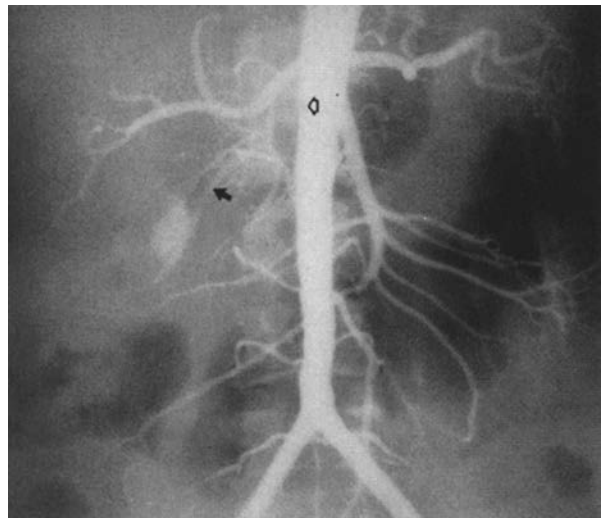

FIGURE 70-7. Aortogram of a patient with an embolism to a solitary right kidney. There is a faint lucency projecting into the lumen of the aorta (*open arrow*) at the site of an embolus to the right renal artery. The distal renal artery filled faintly (*solid arrow*), presumably via collaterals. (From: Lessman, RK, et. al. Renal artery embolism: clinical features and long-term follow-up in 17 cases. *Ann Intern Med* 1978;89:477, with permission.)

repeat scintigraphic perfusion defects was noted in all. In the small number of patients with complete occlusion of the main artery and short ischemic time, less than 3 hours, renal function was preserved. Patients with either incomplete occlusion of the main renal artery or with branch artery occlusions fared better.

Recent convincing case reports refute this "critical ischemic period" (80–83). A patient with bilateral occlusion and another with occlusion of a solitary functioning kidney with relatively prolonged ischemia (20 and 36 hours, respectively), underwent successful thrombolytic therapy and correction of severe renal failure to normal function. Another report describes an elderly patient with new-onset flank pain, anuria, severe acute renal failure requiring dialysis, and bilateral occlusion by angiography with a complete lack of the left nephrogram noted 4 days after the onset of pain. Following angioplasty, renal function returned to near-normal values over time, and repeated imaging studies showed normal perfusion to the whole left kidney.

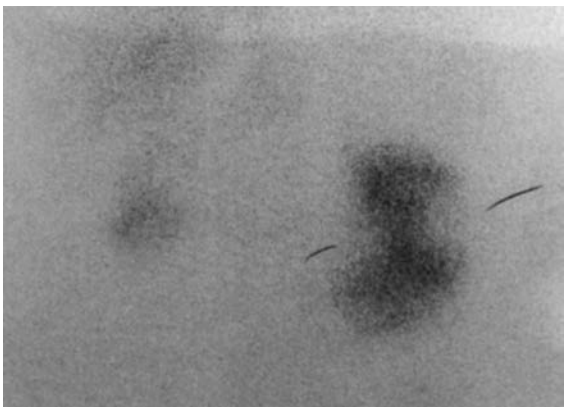

FIGURE 70-6. Renal scan with technetium 99m-labeled dimercaptosuccinic acid (DMSA) showing evidence of segmental renal infracts of the left kidney of a 74-year-old man who was hospitalized with abrupt appearance of a supraventricular tachycardia. There was a progressive rise in serum creatinine from a baseline value of 2.2 to 4.4 mg/dL over 5 successive days. He was never oliguric; urinalysis showed 2+ proteinuria. His white blood cell count rose from 10,200/μL on admission to 15,400/μL after 4 days; there was no eosinophilia. The serum alanine aminotransferase level rose from 33 to 42 U/L and lactate dehydrogenase, from 49 to 173 U/L. A technetium-labeled "flow" scan disclosed markedly reduced flow bilaterally that was more marked on the left than the right. There was a history of emboli to his feet, with the serum creatinine level increasing from 1.2 to 2.2 mg/dL in association with an acute myocardial infarction several years earlier. On the basis of DMSA scan, a diagnosis of thromboembolism to the kidney was made; the patient received anticoagulants and his serum creatinine level gradually fell to 2.4 mg/dL.

Surgical outcomes for preserving renal function in acute nontraumatic obstructions are mixed. Lacombe showed very good surgical results (84). Twenty patients with acute obstruction of the main renal arteries (25 kidneys at risk), 5 from acute embolism, and 15 from acute thrombosis underwent revascularization 18 hours to 68 days after the onset of occlusion. The kidney salvage rate was 64%, but the postoperative mortality rate was 15%. It is unclear whether some of these cases may have been caused by thrombosis associated with atherosclerosis with collateral circulation, which may have a better prognosis for renal salvage than an acute occlusion without collateral circulation. Other studies do not show such optimistic outcomes. In another study, renal artery embolectomy failed to restore renal function in 13 patients with acute renal artery embolism (85).

In summary, an aggressive approach at revascularization should be attempted in bilateral occlusions or occlusions to solitary functioning kidneys, since the threat to major renal function is great. For unilateral occlusions with normal contralateral kidneys, clinical judgment is required to determine whether to intervene with one of these. Either therapy is probably futile if prolonged complete occlusion of the renal artery without collateral circulation persists for more than a day or two. Conversely, intervention may be successful for shorter ischemic durations, and the shorter the period the better. If the obstruction is incomplete or if collaterals are present, the kidney may remain viable much longer. For unilateral occlusions, thrombolysis may be preferable because of the lower risks compared to surgery. If a unilateral segmental embolus is detected, anticoagulation may be all that is necessary.

Many reports describe the rapid relief of pain, which may be very severe, during thrombolytic intervention. Severe pain, which suggests ongoing ischemia, is probably a good indication for thrombolytic intervention. In patients with embolic disease, particular attention should be paid to the source of embolization. Some patients, for example, those with atrial fibrillation, will require long-term anticoagulation to avoid future recurrences of embolization to the kidneys or other vital organs.

RENAL ARTERY ANEURYSMS

Renal artery aneurysms (RAAs) are uncommon in the general population, and are usually diagnosed serendipitously on imaging studies. From large autopsy studies, the incidence of RAAs in the general population is about 0.01% (86). In patients who have undergone renal arteriography for any reason, not uncommonly to evaluate renovascular hypertension, RAAs were found in 0.3% to 1% (87,88). In one study, RAAs were diagnosed in 83 of 8,525 patients (0.97%) who underwent renal arteriography (87). Sixty-one percent of the aneurysms were right-sided, and 7% were bilateral.

Renal artery aneurysms are classified as saccular, fusiform, dissecting, and intrarenal (88,89). Saccular aneurysms, the most common type, comprise about 80% of RAAs. They may be located anywhere along the vascular tree. About 90% are extrarenal, and most are located at the first-order bifurcation of the main renal artery. Less than 10% are within the renal parenchyma.

Fusiform aneurysms, less common, (90–94), usually follow areas of stenosis, and give the image of poststenotic dilation. In fibromuscular dysplasia there may be several series of stenoses followed by dilations, giving a "string of beads" appearance on arteriography. Fusiform aneurysms are most commonly found in young women who undergo renal angiography for the evaluation of renovascular hypertension.

Intrarenal aneurysms comprise 10% to 15% of RAAs, and are frequently multiple. They may be congenital, posttraumatic (e.g., following renal biopsies), or associated with polyarteritis nodosa (91,95) (Fig. 70-8).

The histologic findings of the RAA resemble medial fibromuscular dysplasia. In the arterial wall, degeneration of the internal elastic lamina with fragmentation, increased collagen, and a lack of elastic tissue are observed. Atherosclerotic lesions may be the cause of the aneurysm, or more likely, may be a secondary factor (96). Calcification of the arterial walls may occur.

FIGURE 70-8. Renal arteriogram shows a traumatic intrarenal aneurysm located in the lower pole of the right kidney. (From: Lang, EK. *Radiology of the Upper Urinary Tract*. Heidelberg: Springer; 1991, with permission.)

Table 70-3 shows demographic and clinical data of 277 patients who underwent surgical correction of renal aneurysms in three recently published studies (96–98). The mean age of the patients was around 50 years, and ranged from 13 to 78 years in one study. Women outnumbered men by 65% to 35%. Hypertension was common, present in 73% to 89%. In other series, hypertension was found in 35% to 100% of the patients (100). Medial fibromuscular dysplasia, present in 34% to 54%, was considered the major cause, and was the

TABLE 70-3

DATA FROM THREE LARGE STUDIES REPORTING THEIR SURGICAL OUTCOMES OF RENAL ARTERY ANEURYSMS IN 277 PATIENTS

Studies-Authors (ref.)	English (96)	Pfeiffer (98)	Henke (97)
No. patients	62	94	121
No. RAAs repaired	72	107	168
Time-period of surgery (yr)	1987–2003	1980–2002	1965–2000
Mean patient age (yr)	46	51	51
Male/female (%)	70/30	61/39	60/40
Hypertension (%)	89	80	73
Pathogenesis			
Fibromuscular dysplasia (%)	54	51	34
Atherosclerosis (%)	35	30	25
Outcomes			
Perioperative mortality	1 (1.6%)	0	0
Perioperative morbidity (%)	12	17	?
Hypertension improved (%)	54	22	?
Hypertension cured (%)	21	25	?
Long-term artery patency (%)	91	81	98

probable reason for the female gender preference. Atherosclerosis was present in 25% to 35%. A small number of cases were due to arteritis, including polyarteritis nodosum and giant cell arteritis, Marfan's syndrome, dissection, mycotic aneurysms, and trauma (96–100).

Most patients with RAAs are asymptomatic. Approximately 20% may complain of nonspecific flank or abdominal pain, and there may be hematuria and an abdominal bruit. Complications include rupture, renovascular hypertension, thrombosis with acute arterial occlusion and infarction, distal embolization, and erosion into adjacent veins with the formation of arteriovenous fistulae. The latter may lead to the development of high-output heart failure (89,90).

Rupture of a renal artery aneurysm, a potentially catastrophic event, is the most serious complication. This can present or quickly lead to hemorrhagic shock, irreversible loss of that kidney's function, and death. In cases of rupture, severe flank pain is usually present and flank ecchymoses may develop later.

It is generally accepted, though not proven in prospective studies, that the larger the RAA, the greater the risk of rupture. The risk of rupture of small RAAs, less than 1.5 to 2.0 cm in diameter, appears to be low based on data from several studies that followed the natural history of RAAs. However, RAAs of any size in the setting of pregnancy, and large aneurysms, especially those greater than 4.0 cm in diameter, have a greater tendency to rupture. The older literature suggested that aneurysms without calcified walls may also have a greater tendency to rupture, but this is questionable. Six studies described the natural history of small aneurysms in more than 200 patients with small aneurysms, who were followed conservatively without surgery for up to 17 years (86,97,101–104). Most of these aneurysms were less than 2.0 cm. During follow-up, none of the aneurysms ruptured, and very few of them caused symptoms or had a considerable increase in size. Henriksson et al. described 34 patients with RAAs who underwent repeat angiographic studies (102). Twenty-eight of the 34 patients exhibited no change in size of the RAA on follow-up, and 5 had slight enlargement, thrombosis, or calcification. One patient had a worrisome dilation of the RAA and underwent surgical repair. These studies suggest that small aneurysms, less than 2.0 cm in diameter, are unlikely to rupture, enlarge, or cause symptoms. It appears safe to follow them conservatively with periodic imaging studies.

Pregnant women make up a disproportionate number of cases of RAA rupture. In a review of 43 cases of rupture, 18 (42%) occurred in pregnant women (103). Most occurred during the last trimester of pregnancy, but case reports describe rupture and hemorrhage occurring earlier in pregnancy and during the postpartum period (105–108). Renal artery rupture during pregnancy has also been described in a renal transplant recipient (109). Many of the pregnant women who had RAA rupture did not have hypertension before or during their pregnancy (106,110,111). The reason for increased rupture in pregnancy is not certain. Pathogenic considerations include increased renal blood flow, particularly during the third trimester, steroid hormone effects on the vasculature, and increased intraabdominal pressure (110). The role of pressure on the pelvic vasculature caused by the enlarged uterus, particularly in certain positions, may be a factor (106, 111,112). Emergent nephrectomy is usually required in this setting to control the hemorrhage. In recent years, maternal mortality has decreased to 6% and fetal mortality to 25% if the pregnancy reached the third trimester (113,114). If rupture occurred before the third trimester, fetal mortality approaches 100%.

Renal angiography and MRA will diagnose renal artery aneurysms. Computed tomography and radionuclide scanning may be useful screening techniques.

Three surgical centers specializing in renovascular surgery recently published very good surgical outcomes in a combined number of 277 patients who underwent surgical RAA repair (96–98). The mean size of the aneurysms was reported from 1.5 cm to 2.6 cm. Primary surgical success was around 97%, and long-term arterial patency free of stenosis was 81% to 91%. In one center, unplanned nephrectomy had to be performed in 8 of 121 surgeries (6.6%), a risk for this surgery. Perioperative death rate was 0% to 1.6%, and morbidity was 12% to 17%. Hypertension was considered cured in 21% to 25%, and improved in another 22% to 54%. The specific surgical procedures are detailed in the studies (96–98,115–120). An experienced surgical team is essential for successful outcomes.

The following points should be considered in deciding whether or not to surgically correct RAA: the increased risk of rupture of large aneurysms, and the low risk of small ones, less than 1.5 cm; the increased risk of rupture in pregnancy; the benefits of curing or improving renovascular hypertension, especially in a young person; the risk of major surgery; the risk of a failed procedure, which frequently results in renal infarction and irreversible loss of renal function. There are no set criteria for surgery, but recent recommendations for surgery are the following (91,96–98):

1. RAA diameter greater than 1.5 to 2.0 cm in a healthy, normotensive person;
2. RAA greater than 1.0 cm in women of child-bearing age, since rupture during pregnancy is increased;
3. RAAs with associated renal artery stenosis or evidence of distal embolization.
4. RAA showing significant expansion during follow-up imaging studies.

If medical management is chosen, blood pressure should be well controlled, and periodic CT monitoring of the aneurysm should be undertaken.

Endovascular approaches have been used to treat RAAs as an alternative to surgery. Radiologic transcatheter placement of stent grafts, and exclusion of the aneurysm through the placement of microcoils and with Onyx, a radiopaque nonadhesive liquid embolic agent, have been described in limited cases (121–124). The long-term outcome of these procedures is not known. Superselective end-branch embolic therapy may be used to occlude intraparenchymal RAAs.

DISSECTING ANEURYSMS OF THE RENAL ARTERY

Tears of the arterial intima and medial necrosis lead to dissection of the arterial wall. Atherosclerosis and fibromuscular dysplasia are common predisposing factors that lead to intimal tears. Iatrogenic dissection due to angiographic procedures may be induced by trauma from guide wires, catheters, or angioplasty balloons (125). Rarely, dissections occur after blunt trauma of the abdomen or flank (126,127). Occasionally, renal artery dissections are found as incidental findings at autopsy, without any apparent clinical manifestations during life.

Dissecting aneurysms can cause acute or chronic occlusions of the renal artery, with consequent renal ischemia and infarction and renovascular hypertension (128). They occasionally can rupture and cause a retroperitoneal hemorrhage. The discussions in the preceding text regarding acute renal artery occlusion and renal infarction apply also to dissecting aneurysms. Chronic renal artery dissection most commonly manifests itself as renovascular hypertension (129).

In acute dissections, new-onset hypertension or worsening hypertension and flank pain are common. Occasionally,

patients may experience malignant hypertension. Headache may occur, probably as a result of hypertension. Some patients, especially those who develop the lesion iatrogenically from an angiographic procedure, may show no symptoms or signs other than hypertension.

Dissections are about three times more common in males than females (128,129). They occur most commonly between 40 and 60 years of age, but young adults can be affected, particularly if fibromuscular dysplasia and hypertension are present. Dissecting aneurysms are more commonly right-sided, and are bilateral in 20% to 30% of cases (128,129).

Proteinuria may be detected, and hematuria is present in 20% to 35% of patients. Impaired renal function with serum creatinine level greater than 1.5 mg/dL was present in 9% and 33% of patients in two series (127,128).

Renal angiography or MRA may diagnose dissection. Selective arteriography typically reveals an abrupt narrowing of the arterial lumen (Fig. 70-9). Less often, both the true and false lumina fill with contrast, giving the appearance of a double lumen separated by the intimal flap. The dissection may extend from the main renal artery, distally to the first bifurcation and into the branch arteries. Follow-up arteriography may show persistent dissection, but others have described some degree of reversibility, with gradual improvement of renal function and improvement of hypertension (127,130). Renal function generally is relatively well maintained. Approximately 50% of patients in one study had lateralization of renal vein renin levels, and the isotope renogram showed unilateral abnormalities in a similar fraction (128).

The appropriate therapy depends on the severity of the hypertension and its response to medical management. Some patients have persistent severe renovascular hypertension resistant to antihypertensive therapy. They may benefit from surgical revascularization, or nephrectomy if renal infarction is present, with improvement or complete resolution of hypertension following these procedures (131,132). In other patients, hypertension can be controlled with medications, and these patients may become normotensive again with time. Edwards and colleagues noted an adequate response of the hypertension to medical therapy in the majority of patients (129).

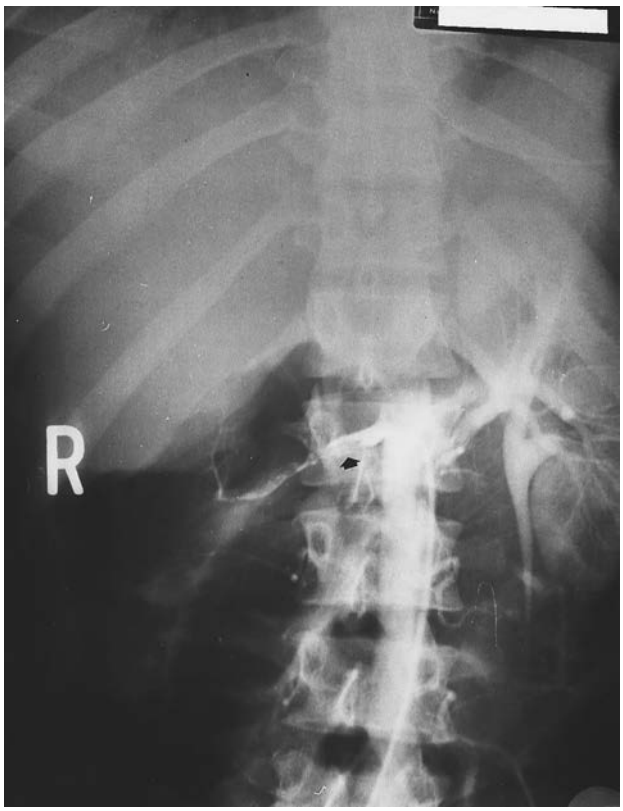

FIGURE 70-9. Aortogram demonstrates an intimal flap and dissection with thrombosis of the right renal kidney. Note the absence of enhancement of the right kidney. (From: Lang, EK. *Radiology of the Upper Urinary Tract*. Heidelberg: Springer; 1991, with permission.)

ATHEROEMBOLIC RENAL DISEASE

The clinical presentation of atheroembolic disease (AED), also known as cholesterol embolic disease, differs from acute renal ischemia and infarction produced by the larger thromboemboli to the renal artery and medium-sized arteries. In atheroembolic disease, tiny cholesterol-containing plaques with fibrin slough off the aorta and large arteries and travel downstream, eventually occluding many small arteries and arterioles with diameters of 150 to 200 μm (133). Local ischemia and inflammatory reactions follow. This leads to the acute or insidious onset of renal impairment if the renal vasculature is showered, as well as a wide range of clinical manifestations depending on which other organs are showered. The presentation lacks the clinical features of a large renal infarct due to thromboemboli or acute thrombosis described in the previous sections.

Atheroembolic disease is a disease of the elderly, who typically have evidence of diffuse atherosclerosis, and especially smokers. Precipitating factors are invasive procedures with aortic or arterial catheters, vascular surgery, and therapy with thrombolytic agents or anticoagulants; less commonly, AED occurs spontaneously without a precipitating event. Clinical manifestations can be mild or severe depending on the degree of atheroemboli. Almost any organ can be involved, but the most common clinical manifestations involve the skin, kidneys, gastrointestinal tract, and central nervous system. No specific therapy is known to limit or halt the cholesterol showering and the clinical sequelae.

Pathology

Autopsy studies show that cholesterol-containing microemboli in the renal vasculature are fairly common. Large autopsy studies describe renal cholesterol emboli in 5% of men and 3% of women older than 50 years of age. (134). This is far higher than the clinically apparent disease, and it suggests that only the more severe cases of AED have clinical manifestations, whereas mild cases are asymptomatic. The incidence of symptomatic cholesterol emboli involving the skin or kidneys following cardiac catheterization was found to be 1.4% in a large prospective Japanese study (135). In autopsy studies of patients with AED, the kidney is the most commonly involved internal organ. Renal involvement was found in 75% of 173 autopsy cases of AED (136). Cholesterol emboli were widespread, and each autopsy averaged 3.4 organs with microemboli. In addition to renal involvement, other common organ sites for atheroemboli were the spleen, pancreas, and gastrointestinal tract (137).

Fragments of atheromatous plaques become embedded in small arteries, arterioles, and sometimes in the glomerular capillaries. The arcuate, interlobular, and terminal arterioles are involved (138). Pathologic examination of the occluded vessels reveals characteristic biconvex, needle-shaped clefts that remain after the lipid is dissolved during histologic processing (Fig. 70-10). Complete obstruction of the small arteries or arterioles leads to distal areas of small infarction and necrosis;

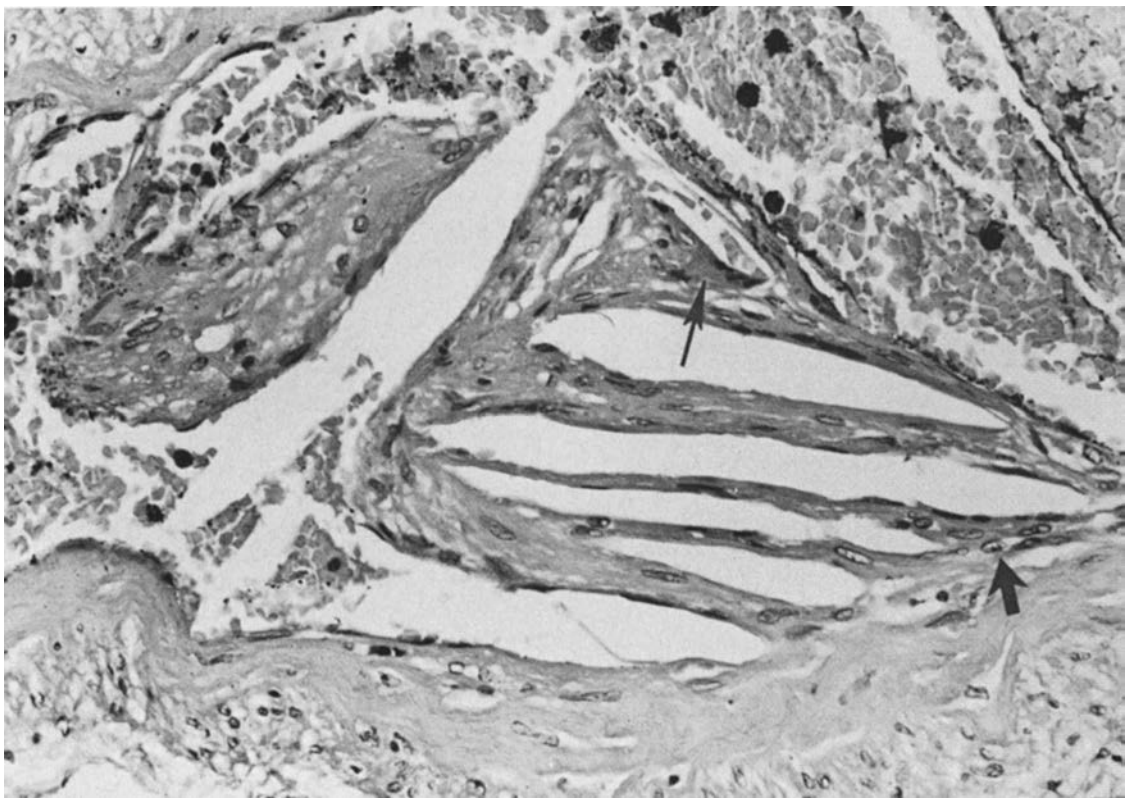

FIGURE 70-10. Light microscopy illustrates the needle-shaped clefts of atheroemboli in a renal arteriole. Foreign-body giant cells (*arrows*) surround the cholesterol clefts.

in incomplete obstruction, distal areas exhibit ischemic atrophy.

During the early period after atheroembolization, cholesterol crystals may be surrounded by eosinophilic material, with little inflammatory reaction in the surrounding interstitium. Subsequently, there is intimal thickening, and the appearance of macrophages, lymphocytes, and multinucleated giant cells of the foreign body type (138). Later there may be marked intimal thickening with concentric fibrosis. Necrosis of the arterial wall is not present. The ischemic glomeruli may become hyalinized, and the tubules become atrophic (139). Grossly, the kidney may shrink and have a rough granular surface with wedge-shaped scars. The lesions produced in experimental animals closely parallel the pathologic features observed in humans (140,141).

Renal impairment arises from recurrent and multiple episodes of cholesterol microembolization. The debris and cholesterol crystals found at the site of occlusion are identical to the material found in the severe atheromatous lesions observed in the aorta of these patients (142,143). Indeed, the incidence of AED and the severity of aortic atherosclerosis have been correlated (143,144). The irregular and ulcerating internal surface of the aorta is commonly covered with fibrin and platelet thrombi. These pliable and soft thrombi can be dislodged easily. The intima may be completely eroded, revealing direct communications between an atheroma and the lumen of the aorta.

A secondary form of focal glomerulosclerosis (FGS) has been described in cases of AED. In a report of 24 patients with renal biopsy evidence of AED, FGS was found in the majority of patients (145). In the patients with nephrotic-range proteinuria, the collapsing variant of FGS was common. The authors speculated that glomerulosclerosis and collapse were the consequences of chronic ischemia and progressive damage of the remaining glomeruli to hyperfiltration. In addition, there are several reports of necrotizing glomerulonephritis including antineutrophil cytoplasmic antibody positive pauci-immune extracapillary glomerulonephritis associated with atheroembolic disease (146–148).

Clinical Features

Atheroembolic renal disease is a disease of the elderly, and is rare in patients younger than 50 years of age. The disorder is more common in males than females, and in a U.S. series, it is described predominantly in caucasians (137,138,149,150). Several Japanese series have been published recently (151,148). Evidence of aortic atherosclerosis is almost invariably present, and symptomatic peripheral or cerebral vascular disease, coronary artery disease, hypertension, and mild renal insufficiency are common. Most patients are smokers. In three clinical studies and one large review of AED, which jointly included 350 patients with AED, the mean age was 66 years age, more than three-fourths were male, and more than 90% were caucasian (137,138,149,152,153). Cigarette smoking was present in 79% to 92% of the patients. Hypertension was present in 61% to 100%, coronary artery disease in 44% to 73%, peripheral vascular disease in 59% to 75%, and cerebrovascular disease in 32% to 62%. Abdominal aortic aneurysms were noted in 25% to 67%, and baseline renal insufficiency was common, noted by a baseline serum creatinine of 1.8 mg/dL (137,149,153). The clinical characteristics of AED are summarized in Table 70-4.

Invasive angiographic procedures, vascular surgery, and therapy with anticoagulants or thrombolytic agents have been implicated as precipitating factors for cholesterol atheroemboli. Coronary, carotid, and aortic angiograms as well as cardiovascular, aortic, and renal artery surgery have been

TABLE 70-4

CLINICAL CHARACTERISTICS OF ATHEROEMBOLIC DISEASE AND THEIR APPROXIMATE INCIDENCE[a]

Age (yr)	60 and higher (rare lower than 50)
Male (%)	75%
Caucasian race	90%
Cigarette smoking history	70%–90%
Medical and vascular disease	
Hypertension	60%–90%
Peripheral vascular disease	60%–75%
Coronary artery disease	40%–75%
Abdominal aortic aneurysm	20%–45%
Diabetes mellitus	10%–35%
Hypercholesterolemia	20%–50%

[a]Refer to text.

complicated by AED (154–157). Thrombolysis and anticoagulation with heparin and Coumadin were considered sole or contributing factors in 14% to 37% of cases (158–165). Less commonly, blunt abdominal trauma, intraaortic balloon placement, cardiopulmonary resuscitation, and percutaneous renal angioplasty have been implicated as causing AED. Occasionally, AED occurs spontaneously without precipitating events. Only 13% of 95 cases were spontaneous in an Italian study (152,165).

The time from the precipitating event to clinically apparent AED is variable, with a range of 1 day to a month or two. In a study of 52 patients with AED and renal failure, clinical manifestations, most often skin manifestations, were seen within 1 day of the precipitating event in 50% of the patients (149). Skin lesions are the most common clinical finding in AED, and are described in 35% to 50% of patients (149,167). Skin findings include purpura, petechiae, violaceous mottling of the toes and feet, the so-called "purple toe syndrome," livedo reticularis, gangrene, and ulcers. The lower extremities are most commonly involved and they are frequently bilateral, although other parts of the body may be involved. Severe scrotal and penile necrosis due to AED has been described (166–168).

Renal involvement is frequently severe and often is the most critical problem. The presentation of renal failure may be acute and fulminant, or it may follow a more insidious, sometimes stepwise course. The onset of renal failure may follow the precipitating event closely, within 1 or 2 days, or it may develop weeks or months later. From three reports of 91 patients who developed acute renal failure from AED, 17% to 44% had severe failure that required dialysis (137,138,149). About 20% of these patients recovered sufficient function to no longer require dialysis, after 42 to 122 days (149). Predictors of end-stage renal disease (ESRD) among 95 patients with AED were preexisting chronic kidney disease and longstanding hypertension; use of statins was independently associated with a decreased risk of ESRD (152). Atheroemboli to transplanted renal allografts have been described (169,170).

The gastrointestinal (GI) tract is showered with microemboli in 18% and 48% of cases. (171–173). Abdominal pain and hemorrhage develop from mucosal ulcerations and small infarcts. Diarrhea and postprandial pain may also be present. Pancreatitis (174), and, less commonly, small bowel perforation, bowel obstruction, cholecystitis, and splenic infarcts have been described (172).

The central nervous system may be involved, with findings of confusion, obtundation, and focal neurologic deficits. Visual deficits and the funduscopic finding of Hollenhorst plaques,

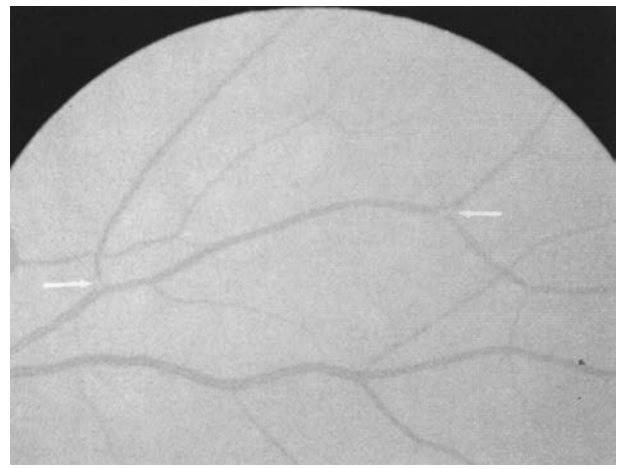

FIGURE 70-11. Retinal image shows two bright yellow cholesterol plaques (Hollenhorst plaques) at two bifurcations of retinal arterioles (*arrows*). This was noted in a 68-year-old man with progressive renal insufficiency owing to atheroembolic disease following cardiac catherization. (From: Goehagen M. Ophthalmology Service, Department of Veterans Affairs Medical Center, West Los Angeles, Wadsworth Division, with permission.)

bright copper-yellow plaques usually lodged at the bifurcations of the retinal arterioles, may be noted (175,176) (Fig. 70-11). Several cases of pulmonary involvement with hemoptysis and a pulmonary-renal presentation have been described (177–179). Patients may have nonspecific findings of weight loss, fever, and muscle aches.

Laboratory results are often abnormal but not specific. Occasionally, mild leukocytosis and eosinophilia may be present. Reports on the incidence of eosinophilia in AED have been varied. Eosinophilia was seen in only 14% of 37 patients in one report, but was found transiently in 71% of 80 patients and in 77% in another study (180–182). The erythrocyte sedimentation rate is commonly elevated, and hypocomplementemia has been observed (183). Mild proteinuria is generally present, but nephrotic-range proteinuria has also been described (145,184,185).

Microscopic hematuria occasionally occurs, and hyaline and granular casts may be found on urinalysis. Rarely, dysmorphic red blood cells and red blood cell casts, findings of a nephritic sediment, may be seen. Eosinophiluria detected by the Hansel stain was found in 8 of 9 patients with AED, but noted in only 2 of 37 patients in a report that used the Wright stain (149,186). Depending upon the site of the atheroembolic showering, increases of amylase, lipase, creatine kinase, and liver enzymes may be present.

A high index of suspicion for AED should be present when elderly patients, especially those with diffuse atherosclerotic disease, develop renal failure following a vascular procedure. On the other hand, the diagnosis of spontaneously occurring AED may be perplexing. In these latter cases, a tissue diagnosis is often necessary. The safest and easiest tissue to biopsy is the skin, which is diagnostic for AED in 90% of biopsies (137,167,187). Renal biopsies are often diagnostic, but they may miss the typical lesions because of the patchy distribution of atheroemboli. Other organs that showed cholesterol emboli were the muscle, bone marrow, prostate, stomach, and lung (177,178,188).

At present, there is no satisfactory therapy for patients with atheroembolic renal disease. General medical supportive measures and the avoidance of anticoagulants may be the best current approach, since there is the concern that these agents could worsen the atheroembolic showering (189–195).

Corticosteroids, antiplatelet agents, and vasodilators have unproven effects. If patients require dialysis, it may be safest to perform heparin-free dialysis.(152,191,195,196).

The vascular surgical literature describes improvement of renal function with revascularization of the kidney when AED is the consequence of severe atherosclerosis of the renal artery and when renal artery stenosis is present (137,197,198). In these cases, renal function was allowed to stabilize for a few months following the initial episode of atheroemboli before intervention, either surgical bypass or intra-arterial stent placement, was performed. Surgical benefit has also been described in AED, mainly for limb salvage (197–199). In a study of 100 patients with AED, surgery was attempted to remove the source of the atheroemboli. Surgical outcome and long-term prognosis, especially limb salvage, were good in the patients in whom the infrarenal aorta or iliac artery were the source of the atheroemboli. However, clinical outcomes were poor and mortality rates were high in patients who underwent suprarenal aortic surgery (197). In another study, 42 patients with AED underwent bypass surgery or endarterectomy, most of them at the level of the infrarenal aorta or more distally. They reported good limb salvage, low operative mortality, and excellent long-term relief of embolization (198). Surgical candidates among this elderly population, many with comorbid conditions, must be chosen carefully.

RENAL VEIN THROMBOSIS

Thrombosis of the renal vein was originally thought to be a relatively uncommon vascular complication of the kidney. Although the first description of thrombosis of the renal vein was made by J. Hunter (200), Rayer in 1840 was the first to make an association between renal vein thrombosis (RVT) and the nephrotic syndrome (201). Much later, Abeshouse extensively reviewed the medical literature in regard to thrombotic disease of the renal vein (202). Among the most important causes cited were infectious suppuration, malignancy, and trauma. It is important to note that in all of these patients reported to have RVT, the diagnosis was made postmortem. Later, with the development of more advanced radiographic techniques and selective catheterization, antemortem diagnosis of RVT was made possible, and the number of patients in the adult population diagnosed with RVT increased. Although RVT may be caused by trauma or tumor, it occurs most commonly in the nephrotic patient.

Because of the early descriptions, emphasis was placed on the presence of lumbar pain with flank tenderness, edema, and the appearance of a lumbar mass. As a consequence, the early reports following this description focused attention on these symptoms, and it was generally assumed that RVT always presented suddenly with florid symptomatology. However, Harrison et al. in 1956 described two groups of patients with RVT (203). The first, with complete acute thrombosis of the renal vein, was characterized by severe lumbar or abdominal pain, enlargement of the affected kidney, proteinuria, edema, and deterioration of renal function. A second group had only the nephrotic syndrome and absence of acute symptomatology.

Etiology

The incidence of RVT in the adult population is difficult to establish. A review of 29,280 necropsy studies performed in the Mayo Clinic from 1920 to 1961 revealed 17 adults with bilateral RVT, an incidence of approximately 0.6% per 1,000 necropsies (204). Among the 17 patients, only 2 had the nephrotic syndrome; however, during the last decade, prospective studies evaluated the incidence of RVT in the nephrotic patient and found it to be significant, although variable. In Table 70-5 are displayed data from various prospective studies evaluating the incidence of RVT in patients with the nephrotic syndrome and membranous nephropathy (210–216). These prospective studies evaluated patients undergoing routine renal venograms regardless of the presence or absence of symptoms suggestive of RVT. It can be appreciated that the overall incidence of RVT in both the nephrotic syndrome and membranous nephropathy is significant; however, there are marked differences, ranging from 5% to 62%. The reason for such differences is not clear. One possibility, in light of current immunologic advances, is that membranous nephropathy may include different immunologic entities and some patients may be more prone to develop RVT than others. Another possibility is that the duration of the nephrotic syndrome and the persistence and magnitude of the hypoalbuminemia may have varied in these studies. It is generally agreed that the most common underlying nephropathy associated with RVT is membranous nephropathy. A review of all our patients with

TABLE 70-5

PROSPECTIVE STUDIES EVALUATING THE INCIDENCE OF RENAL VEIN THROMBOSIS (RVT) IN PATIENTS WITH NEPHROTIC SYNDROME (NS) AND MEMBRANOUS GLOMERULOPATHY (MGN)

	Patients with RVT in NS	Incidence (%)	Patients with RVT in MGN	Incidence (%)
Bennett (4)	—	—	5/10	50
Noel et al. (154)	—	—	5/16	31
Wagoner et al. (158)	—	—	14/27	52
Cameron et al. (76)	—	—	2/15	13
Pohl et al. (155)	1/54	2	1/20	5
Llach et al. (152)	33/151	22	20/69	29
Monteon et al. (153)	15/53	28	15/24	62
Vosnides et al. (157)	7/44	16	5/30	17
Velasquez-Forero and Garcia-Prugue (156)	8/19	42	3/5	60

(From: Llach, F. The hypercoagulability and thrombotic complications of nephrotic syndrome. *Kidney Int* 1985;28:4259. Editorial review, with permission.)

TABLE 70-6

ETIOLOGY OF THE NEPHROTIC SYNDROME IN 151 PATIENTS

Renal diagnosis	Patients with renal vein thrombosis (no.)	Patients without renal vein thrombosis (no.)	Total
Membranous nephropathy	20	49	69
Membranoproliferative glomerulonephritis	6	21	27
Lipoid nephrosis	2	8	10
Rapidly progressive glomerulonephritis	1	1	2
Amyloidosis	1	5	6
Focal sclerosis	1	3	4
Renal sarcoidosis	1	0	1
Lupus nephritis	1	10	11
Diabetic nephropathy	0	15	15
Focal glomerulonephritis	0	3	3
Acute poststreptococcal glomerulonephritis	0	2	2
End-stage renal disease	0	1	1
Total	33	118	151

(From: Llach F, Koffler A, Massry SG. Renal vein thrombosis and the nephrotic syndrome. *Nephron* 1977;19:65, with permission.)

nephrotic syndrome is shown in Table 70-6. Of 151 patients with the nephrotic syndrome, 33 had RVT, and of these, 20 had membranous nephropathy (205). However, other causes such as membranoproliferative glomerulonephritis, minimal change nephrosis, and amyloidosis may be associated with RVT. Of our 33 patients with RVT, only 4 had an acute mode of presentation.

The concept of the relationship between RVT and the nephrotic syndrome has changed in the last two decades. For many years, it was thought that RVT was the cause of the nephrotic syndrome, a belief that is no longer held. Several lines of evidence seriously questioned this hypothesis. First, experimentally induced RVT causes only mild proteinuria, and renal histology by immunofluorescent findings in these cases does not resemble that of membranous nephropathy (206,207). Second, according to the surgical literature, RVT can occur in the absence of the nephrotic syndrome (208,209). Moreover, in autopsy studies of patients with RVT, nephrotic syndrome was present antemortem in only a few patients. Third, most patients with RVT and nephrotic syndrome who have been subjected to renal morphologic study exhibit an identifiable glomerulopathy, most of the time membranous nephropathy, which is responsible for the nephrotic syndrome (210–216). Finally, as shown recently, RVT occurs after the onset of the nephrotic syndrome (216). Therefore, the general view today is that the nephrotic syndrome provides a favorable milieu for the development of RVT.

Pathophysiology

An important factor in the causation of RVT in patients with the nephrotic syndrome is the presence of a hypercoagulable state. Various investigators (217–231) observed profound clotting factor abnormalities in patients with the nephrotic syndrome. There are five major functional classes of coagulation components: (a) zymogens (factors II, V, IX, XI, and XII), which are activated by enzymes and cofactors (factors V and VIII) the major role of which is to accelerate the role of enzymes; (b) fibrinogen and products from the conversion of fibrinogen to fibrin; (c) the fibrinolytic system; (d) clotting inhibitors; and (e) components of the platelet reaction and thrombogenesis. Alterations in all of these coagulation components have been observed in the nephrotic patient.

Alterations in zymogens and cofactors include a decrease in the levels of factors IX, XI, and XII. The low levels of these proteins are due most likely to urinary loss secondary to their small molecular size rather than to impaired protein synthesis. An increase in the levels of factor II and combined factors VII and X has also been described (222–227). In general, most of these zymogen abnormalities tend to normalize with clinical remission of the nephrotic syndrome. Most consistently, increased levels of cofactors (factors V and VIII) have been noted in the nephrotic syndrome (231–233). A number of studies showed a correlation between increases in factors V and VIII and a fall in serum albumin level (232,233). These alterations in cofactors result from increased synthesis of these proteins by the liver; the mitochondria of the liver cells are the final sites of production of most of these proteins, and a decrease in plasma oncotic pressure or a decrease in serum albumin concentration or both may be sensed by these liver cells, which in turn respond with an increased production of different proteins (234).

During the earlier description of the hypercoagulable state of the nephrotic syndrome, the hypothesis was advanced that an increase in cofactors may lead to hypercoagulability and may explain the high incidence of thrombosis in these patients. However, two important points must be made regarding this hypothesis. First, all of these factors are normally present in great excess in the circulation, with only a small amount of any given factor being activated during thrombus formation. Therefore, it seems unlikely that high levels of any of these zymogens would lead to thrombosis or that reduced levels of some coagulation factors would be a sensitive marker of the presence of thrombosis. Second, there is no evidence suggesting that the increased level of cofactors may lead to thromboembolic phenomena. High levels of these factors are usually present during acute inflammatory responses because they are acute-phase reactant proteins. There is no current evidence that these conditions are associated with an increased risk of thrombosis.

An elevation of plasma ^{131}I-labeled fibrinogen levels is a consistent and significant abnormality observed in nephrotic patients (235–238). As demonstrated by the use of ^{131}I-labeled fibrinogen, the rate of fibrinogen catabolism is normal in these patients, and the observed increase in plasma fibrinogen is due to an increased liver synthesis that is proportional to the urinary protein loss (237). In addition, there is a significant correlation between fibrinogen and cholesterol levels, and both are related inversely to the levels of serum albumin. The level of fibrinogen in nephrotic patients may be as high as 1 g/dL and can alter plasma viscosity considerably (232). Therefore, increased plasma fibrinogen concentration reflects its increased hepatic synthesis; contracted intravascular distribution may be present, and there is a normal degradation rate of fibrinogen in the nephrotic patient. It is possible that the high fibrinogen levels, by significantly increasing blood viscosity, are important in the hypercoagulable state of nephrotic syndrome.

Various tests for the determination of the products of fibrinogen to fibrin conversion have been developed for diagnosis of a prethrombotic state. An increase in the plasma concentration of fibrinogen degradation products (FDPs) is not commonly observed in nephrotic patients but has been observed in the urine of patients with glomerulonephritis (238–241). Some nephrotic patients have increased urinary levels of FDPs (238). However, these findings should not be taken as definite evidence for increased fibrinolysis in the systemic or renal vasculature, because in patients with nonselective proteinuria, fibrinogen is filtered at the glomerulus and may undergo proteolytic degradation by protease. In this regard, gel chromatography shows clearly that material that in the past was interpreted as FDP is actually filtered fibrinogen that has been degraded in the tubules (238).

Alterations in the fibrinolytic system have also been observed in nephrotic patients (233). The basic reaction of the fibrinolytic system is the conversion by plasminogen activators of β-globulin, plasminogen, to an active serum protease, plasmin. This system is modulated by inhibitors of both plasminogen and plasmin. A number of clinical studies reported an association between defective fibrinolysis and thrombosis; the association included oral contraceptive ingestion (242), pregnancy (243), postoperative states (244), malignant disease (245), obesity (246), and the nephrotic syndrome (247). The data in regard to the fibrinolytic abnormalities in nephrotic patients have shown in general a decrease in plasma plasminogen concentration (248–251) that is correlated with a low serum albumin concentration and the magnitude of the proteinuria (251). The clinical significance of this abnormality is not known, and a cause–effect relationship of these abnormalities and RVT has not been made. However, Du et al. (252) identified a plasmin inhibitor that is identical to α_2-antiplasmin. They evaluated 14 nephrotic patients with RVT together with 30 nephrotic patients without RVT. In both groups the level of total fibrinolytic activity was normal and that of the plasma inhibitor (α_2-antiplasmin) of plasminogen activation was elevated. However, the plasmin inhibitor was elevated in 13 of 14 patients with RVT and in only 12 of 30 patients without RVT. The authors suggested that the increased level of α_2-antiplasmin may be a factor in determining susceptibility to the development and persistence of RVT in nephrotic patients. Further studies are needed to confirm the importance of these observations.

Alterations in coagulation inhibitors have been observed in nephrotic patients. The components of the coagulation system exist in the circulation as zymogens, and they are cleaved to form proteolytic enzymes. Activated clotting factors are inhibited by naturally occurring coagulation inhibitors (253–255). The most important physiologically of these inhibitors is antithrombin III (AT III), an α_2-globulin that is the main inhibitor of thrombin and also inhibits activated factors XII, IX, X, and XI and plasmin (256–258). The rate of inhibition of these enzymes by AT III is markedly increased in the presence of heparin. In patients from families with an inherited deficiency of AT III, an increased incidence of thromboembolic complications is generally observed when AT III levels are less than 75% of normal (256).

Kauffman et al. studied AT III levels in 48 patients with proteinuria and their relationship with the occurrence of thromboembolic phenomena (259,260). Nine patients had evidence of thrombosis, including 4 with RVT. In 8 of these 9 patients, the serum AT III levels were less than 70% of normal. There was a significant correlation between AT III concentration and urinary protein excretion. Only 6 of the 32 patients who excreted less than 10 g per 24 hours showed depressed AT III levels of less than 85%, whereas 13 of 16 patients with a urinary protein loss higher than 10 g per 24 hours showed depressed AT III levels. Because the molecular weight of AT III is relatively low, excretion in the urine would be expected in patients with proteinuria. Because of similar molecular weights, the renal clearance of AT III and albumin was compared. A significant correlation was noted between the plasma concentrations of these two proteins. In addition, there was a significant correlation between the renal clearance of AT III and the degree of AT III deficiency. It was concluded that thrombosis in nephrotic patients may be associated with deficiency of AT III due to increased urinary loss and that low levels of AT III may be insufficient to inactivate procoagulant factors, thus resulting in the development of thrombosis. These results are in apparent conflict with those of studies showing normal or increased antithrombin activity in nephrotic children (261,262). This finding may have been due to the nonspecific *in vitro* inhibition of thrombin by α_2-globulin and α_2-antitrypsin (two other clotting inhibitors), and, therefore, the apparent increment in AT III activity may not have reflected a true increase in AT III levels. In addition, AT III deficiency was generally associated with a serum albumin concentration of less than 2 g/dL by other investigators (259–260), whereas in the earlier studies only a few patients had severe hypoalbuminemia. Panicucci et al. reported normal levels of AT III despite high urinary AT III levels (263). They suggested that increased AT III synthesis compensated for its renal loss. Later, Vaziri et al. observed a significant decrease in AT III plasma concentration and activity in 20 nephrotic patients compared with normal subjects (264). In addition, substantial urinary losses of AT III were demonstrated in the nephrotic patients. Therefore, although the rate of synthesis and degradation of AT III and its distribution have not been determined, it is likely that renal losses of AT III in nephrotic patients contribute to AT III deficiency. It is possible that the danger of thromboembolic phenomena may arise with sudden changes in the activity of the renal disease, resulting in abrupt renal losses of AT III while hepatic synthesis of AT III has not yet increased. An interesting observation is the increase in AT III levels in nephrotic children after steroid therapy (263). Furthermore, of interest is the recent observation in a healthy non-nephrotic 13-year-old girl presenting with acute flank pain and anuria due to RVT (265). She was noted to have a marked familial deficiency of AT III levels. Urgent surgical thrombectomy and anticoagulation resulted in recovery of renal function. Consequently, an early diagnosis of this familial condition may lead to the use of preventive measures or acute specific therapeutic intervention at the onset of the acute thrombosis.

Important data about the role of coagulation inhibitors in the development of thrombosis have been reported. Protein C and protein S have been identified as potent anticoagulants. Protein C is a vitamin K–dependent serum protease zymogen that is homologous with other known vitamin K–dependent serum proteases (266–269). This protein is an anticoagulant because it prolongs the clotting time of plasma in various

clotting assays (270). The clinical role of protein C as an important antithrombotic regulatory molecule was demonstrated by identifying a familial thrombotic disease that is associated with an inherited partial deficiency of protein C (271). Consequently, families with a deficiency in plasma protein C have recurrent thrombosis. It appears that protein C levels less than 50% result in thrombosis. Although AT III appears to be a major regulatory protein in limiting the activity of procoagulant plasma enzymes, activated protein C may represent a major regulatory protein limiting the activity of activated procoagulant factors (factors V and VIII). In this respect, the anticoagulant properties of activated protein C and AT III are complementary.

The rate of inactivation of factor V by activated protein C is stimulated by another vitamin K–dependent protein, protein S (272). Protein S has no effect on factor V activity in the absence of activated protein C, indicating that it is not a protease. In protein S–deficient plasma, the anticoagulant activity of protein C is restored. It appears that the complex between protein S and activated protein C is formed only in the presence of phospholipids (266,267). Because protein S is required for the expression of the anticoagulant activity of activated protein C, it is not surprising that a deficiency of protein S predisposes to recurrent thrombosis (272). Comp and Esmon (271) identified six unrelated persons with severe, recurrent venous thrombosis who were deficient in protein S, with levels between 15% and 37%. Early high protein C and S levels were observed in the nephrotic patients (273).

Other investigators noted normal protein C levels in nephrotic patients (271). However, it was observed that the functional levels of protein S did not correlate with the immunologic levels. Further, protein S was noted in two forms in plasma, as free and functionally active protein S and complexed to C4b-binding protein. When compared with control subjects, nephrotic patients had reduced functional levels of protein S, despite having elevated levels of total protein S antigen (274). Decreased total protein S activity was caused by significant reductions in free (active) protein S levels due to selective urinary loss of free protein S and elevation of C4b-binding protein levels that favors complex formation. These authors also observed that the specific activity of protein C (activity-antigen ratio) was lower in nephrotic patients than in controls. The authors concluded that acquired protein S deficiency occurs in nephrotic syndrome and may be a risk factor for the development of the thromboembolic complications.

Platelet abnormalities have also been observed in nephrotic patients. Therefore, thrombocytosis is often present (274–276), and an increased platelet aggregation with adenosine diphosphate (ADP) and collagen but not with epinephrine has been observed (277,278). Remuzzi et al. observed that the degree of platelet function abnormalities correlates with the degree of hypoalbuminemia and the severity of the proteinuria (276). The levels of β-thromboglobulin, a specific protein released by platelets on aggregation, are significantly elevated in nephrotic patients and return to normal with clinical remission (277). This suggests that nephrotic patients have an increase in platelet aggregation. However, normal β-thromboglobulin levels have also been reported in nephrotic patients (278). One study of nephrotic patients demonstrated that thrombotic complications occur only in patients with increased platelet aggregation and elevated β-thromboglobulin levels (279). In addition, these complications occurred primarily in patients with a serum albumin level of less than 2 g/dL.

In summary, the hypercoagulable state of the nephrotic syndrome is characterized by low zymogen factors, a marked increase in cofactors, an increase in plasma fibrinogen levels, a decrease in the levels of AT III and antiplasmin activity, thrombocytosis, increased plasma aggregation, and increased levels of β-thromboglobulin. The high levels of fibrinogen leading to increased plasma viscosity may be an important factor in the hypercoagulable state. A convincing relationship has been found between low AT III and thrombosis, and it is likely that increased platelet aggregation may also be an important factor in hypercoagulability; the increased levels of β-thromboglobulin may be a reliable marker of platelet aggregation. The severity of the hypoalbuminemia, by increasing hepatic synthesis of fibrinogen and platelet aggregation, may play a pivotal role in the generation and maintenance of these abnormalities.

In regard to the role of a protein S deficiency in the hypercoagulable state, additional studies in nephrotic patients with thrombosis are necessary to reach any conclusion. However, because protein S deficiency is an acquired problem, it should be determined in patients with thromboembolic complications.

In addition to hypercoagulability, other factors may be important in the pathogenesis of RVT. Therefore, a persistent reduction in plasma volume, an important feature in some patients with the nephrotic syndrome, especially those with membranous nephropathy and normal renal function, may provide a milieu favorable to RVT. Theoretically, a sustained reduction in blood volume could lead to decreased renal venous flow, thereby favoring the development of RVT. In this regard, we have been impressed by the marked decrease in washout time in renal venograms in patients with membranous nephropathy who do not have RVT (235). Diuretics may enhance volume depletion and thereby contribute to the thromboembolic phenomena of the nephrotic syndrome. Recent preliminary data presented by Cheng et al. (280) in evaluating 97 nephrotic patients, strongly suggest that intensive diuretic therapy is associated with a high incidence of RVT. Third, the nature of the immunologic injury may also be important. One study investigating the relationship between membranous nephropathy and RVT separated a subpopulation of patients with membranous nephropathy and RVT (281). It is attractive to speculate that such complexes may be the triggering factor in the coagulation process. In one study, the presence of factor XII and prekallikrein in subepithelial deposits was observed in 29 patients with membranous nephropathy (282). It is tempting to relate the high incidence of RVT in membranous nephropathy to activation of factor XII, a factor that is at the crossroads of important proteolytic pathways. Clinically, patients with nephrotic syndrome and membranous nephropathy have a high incidence of other thromboembolic phenomena in addition to RVT (235,236). Furthermore, greater disturbances of hypercoagulability were noted in patients with membranous nephropathy than in those nephrotic patients with minimal change disease (235). An underestimated factor predisposing to acute peripheral vascular thrombosis and even RVT may be arterial diagnostic puncture as well as placement of central catheters (283). Finally, the role of steroids in the pathogenesis of RVT has not yet been defined. Steroids can aggravate the hypercoagulable state (284), and historically the advent of steroid therapy coincided with an increase in thromboembolic complications (285,286). The previously mentioned study by Cheng et al. (280) indicated that steroid therapy was associated with a high incidence of RVT. Therefore, these agents should be used cautiously in the treatment of nephrotic syndrome.

In summary, the pathogenesis of RVT in patients with nephrotic syndrome may be multifactorial. A general integrated scheme of the pathogenetic factors leading to RVT and other thromboembolic complications is displayed in Figure 70-12.

Clinical Manifestations: Acute Versus Chronic

The pattern and mode of clinical presentation of the nephrotic syndrome and RVT may differ from those mentioned in the early literature. Rayer's description of RVT included lumbar pain with tenderness, swelling, and the appearance of a lumbar

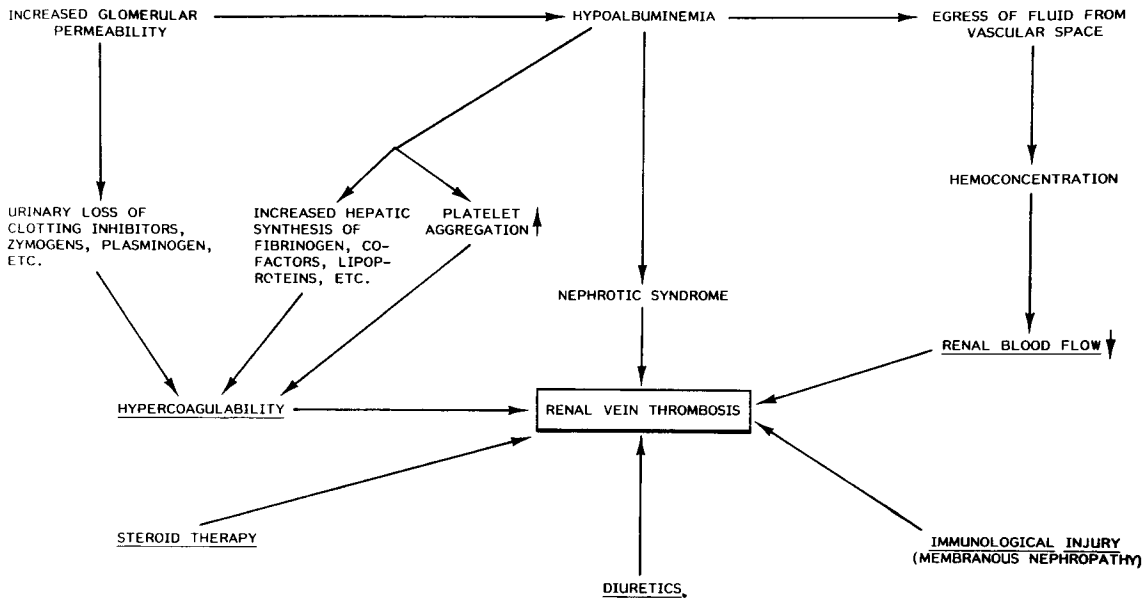

FIGURE 70-12. Schematic representation of pathogenetic factors leading to renal vein thrombosis in nephrotic syndrome.

mass (201). Subsequent reports stressed the presence of flank pain and macroscopic hematuria in the clinical presentation of RVT (287). However, descriptions of more cases revealed that many patients with RVT did not have any local symptoms and signs. The clinical spectrum of RVT varies from patient to patient. (216) The rapidity of venous occlusion and the development of venous collateral circulation determine the clinical presentation and subsequent renal function; however, in general, patients with RVT usually have two modes of clinical presentation: acute and chronic. The chronic presentation of RVT is observed most frequently and in general is asymptomatic. Acute RVT is characterized by sudden onset and usually occurs in younger patients complaining of persistent acute flank pain, which may be colicky at times; marked costovertebral angle tenderness and macroscopic hematuria are usually present. The following case is a representative example of acute RVT:

A 25-year-old white man came to the hospital with a year-long history of proteinuria and ankle swelling. Physical examination revealed no acute distress with normal blood pressure. The only physical finding was ankle edema. Urinary sediment demonstrated 3+ proteinuria. Biochemical data were consistent with the presence of nephrotic syndrome and normal renal function. Renal biopsy revealed early membranous nephropathy. Lung scan and intravenous pyelogram (IVP) were within normal limits. An inferior vena cavogram as well as a left renal venogram were within normal limits. At the time the left renal venogram was performed, the patient experienced acute left flank pain that lasted 4 to 5 hours and was accompanied by macroscopic hematuria and marked tenderness in the left costovertebral angle. The procedure was terminated and the patient was returned to the ward. A second IVP showed marked enlargement of the left kidney with poor visualization of pelvocaliceal system. A selective left renal venogram obtained 1 week later revealed complete obstruction of the main renal vein. The creatinine clearance rate was reduced, and the patient was given anticoagulant therapy. In 2 days, the flank pain resolved completely, and the creatinine clearance rate, after 6 months of anticoagulant therapy, increased from 40 to 90 mL/minute.

On occasion, acute RVT may be bilateral, resulting in marked oliguric acute renal failure and flank pain. The severity of the symptomatology and oliguria and the magnitude of renal function deterioration depend on various factors. Therefore, previously compromised renal function, absence of collateral circulation, and a large thrombus involving both renal veins may result in a florid clinical presentation as well as rapid deterioration in renal function.

Other causes of acute RVT with or without the nephrotic syndrome are trauma, ingestion of oral contraceptive agents, dehydration (mostly in infants), and steroid administration.

RVT secondary to trauma is usually accompanied by renal artery thrombosis. The history of the trauma, the severe acute flank pain, and a palpable mass are usually suggestive of this condition.

The use of oral contraceptives is implicated occasionally as a cause of RVT. In a young female on oral contraceptives without the nephrotic syndrome or any traumatic event, acute RVT developed with florid clinical manifestations (288). Whether there was a cause–effect relationship between the contraceptive agents and the RVT was not clear.

Dehydration is clearly associated with RVT in infants (289). In this condition thrombosis develops in the small renal veins, predominantly the arcuate or intralobular vein. The majority of these infants do not have nephrotic syndrome or even significant proteinuria. This syndrome develops initially in the clinical setting of diarrhea, vomiting, and often shock. Oliguria and hematuria rapidly ensue. Contributing factors are a history of maternal diabetes, congenital heart disease, and performance of angiocardiography. An enlarged palpable kidney may be found in 60% of affected infants. The common clinical presentation is a hyperosmolar hypovolemic syndrome. The mortality of RVT in infants is high. Fortunately, the frequency of this syndrome has diminished due to earlier therapy and control of volume-depleting events.

Steroid administration is clearly associated with acute and chronic RVT as well as other thrombotic complications (284,285); however, the causative role of these agents in RVT remains to be established. The role of steroids cannot be appraised until other factors influencing thrombus formation are understood. As mentioned earlier, Cheng et al. (280) presented preliminary data from 97 nephrotic patients who were prospectively evaluated. Of these, 44 patients had RVT as shown by

routine renal venography. Multivariate regression analysis revealed that steroid therapy was associated with a high tendency to develop RVT ($p < 0.01$).

Recently, acute RVT was noted with increasing frequency in the transplanted kidney (290–292), which, unlike the native kidney, has a single drainage system. In this setting RVT usually leads to permanent damage of the graft within hours. Predisposing factors are the use of OKT3 and cyclosporine therapy (293). The available evidence suggests that cyclosporine may predispose to vascular thrombosis by exacerbating the hypercoagulability (294). In fact, rupture of renal allografts, an uncommon complication, usually is an early manifestation of acute RVT that often is attributable to cyclosporine therapy (293).

Finally, abdominal tumors, especially hypernephromas, are a common cause of RVT; however, in the majority of these patients chronic rather than acute RVT is the common clinical presentation.

The radiologic manifestations of acute RVT are well defined and characteristic in contrast to those of chronic RVT, which often are minimal. Experimentally, with complete occlusion of the renal vein, the kidney increases rapidly in size within the first 24 hours, reaching a peak within 1 week of renal vein occlusion (295). Thereafter, there is a progressive decrease in renal size over the next 2 months, resulting in a small, atrophic kidney. A progressive decrease in the caliber and length of the renal artery follows the occlusion.

Clinically, in a patient with acute RVT, the plain film of the abdomen and IVP initially reveal an enlarged kidney (see Fig. 70-13). In the IVP, if the obstruction is sudden and complete, there may not be any visualization of the collecting system. However, in most patients, because of the presence of some collateral circulation, there is renal enlargement and opacification of varying degree with some visualization of the kidney. Often the renal pelvis can be visualized and is usually stretched, distorted, and blurred (see Fig. 70-13). This may be the result of severe interstitial edema and swelling of the pelvocaliceal system. At this stage the radiographic appearance has been compared to that observed in polycystic kidney disease, and on occasion it has led to this mistaken diagnosis. The acute symptomatology of RVT in association with this radiologic appearance establishes the diagnosis. Ureteral edema may progress to the point at which the collecting system is completely obliterated; there have been cases of complete ureteral obstruction in which during retrograde pyelography the catheter could not be advanced into the pelvis. A characteristic radiographic finding of RVT is notching of the ureter, which usually occurs when collateral veins in close relation to the ureters become tortuous as they dilate to form an alternative drainage route. Originally the notching of the ureters was interpreted as representative of mucosal edema; however, more detailed radiographic studies have shown indentation of the ureters by the collateral venous circulation (296,297). Notching of the ureter is a very infrequent finding in nephrotic patients with RVT and usually occurs only in a minority of patients with chronic rather than acute RVT (296).

Retrograde pyelography may be useful in the patient with complete RVT who does not excrete the contrast material. In such instances, retrograde pyelography may demonstrate a rectangular, linear mucosal pattern with irregular renal pelvic outlines similar to those described with the IVP.

Inferior vena cavography with selective catheterization of the renal vein establishes the diagnosis of RVT. If the inferior vena cava is patent and free of filling defects, and if a good stream of unopacified renal blood is demonstrated to wash out contrast material from the vena cava, a diagnosis of RVT is unlikely. The Valsalva maneuver is useful during vena cavography; by increasing the intraabdominal pressure the transit of contrast agent and blood from the inferior vena cava is slowed, the proximal part of the main renal vein may be opacified, and the patency of the lumen or even the outline of the thrombus may be demonstrated. On occasion, a lack of washout in the area of the renal vein may be suggestive of RVT; sometimes partial defects in the area of the renal vein may be demonstrated, characteristic of renal venous thrombus extending into the inferior vena cava. In the presence of complete inferior vena cava obstruction below the renal vein it is desirable to demonstrate the proximal extent of the thrombus, and this can be accomplished readily by transbrachial catheterization with passage of the catheter into the inferior vena cava via the subclavian vein.

Often the inferior vena cavogram is not diagnostic and selective catheterization of the renal vein must be performed. A normal renal venogram demonstrates the entire intralobular venous system to the level of the arcuate vein. In general, use of epinephrine for better visualization of the smaller vessels is not necessary. However, in the presence of normal renal blood flow all contrast material is washed out of the renal vein within 3 seconds or less, and occasionally only the main renal vein and major branches are visualized. Therefore, in this situation there may be uncertainty about thrombi in major or smaller branches. The use of intrarenal arterial epinephrine by decreasing blood flow enhances retrograde venous filling and allows later visualization of the smaller intrarenal veins. An abnormal

FIGURE 70-13. Intravenous pyelogram of a patient with acute renal vein thrombosis. Note blurring and irregularities of the left pelvocaliceal system as well as marked enlargement of the left kidney. (From: Llach, F. Renal vein thrombosis and the nephrotic syndrome. In: Llach, F. *Renal Vein Thrombosis*. Mount Kisco, NY: Futura; 1993: 155, with permission.)

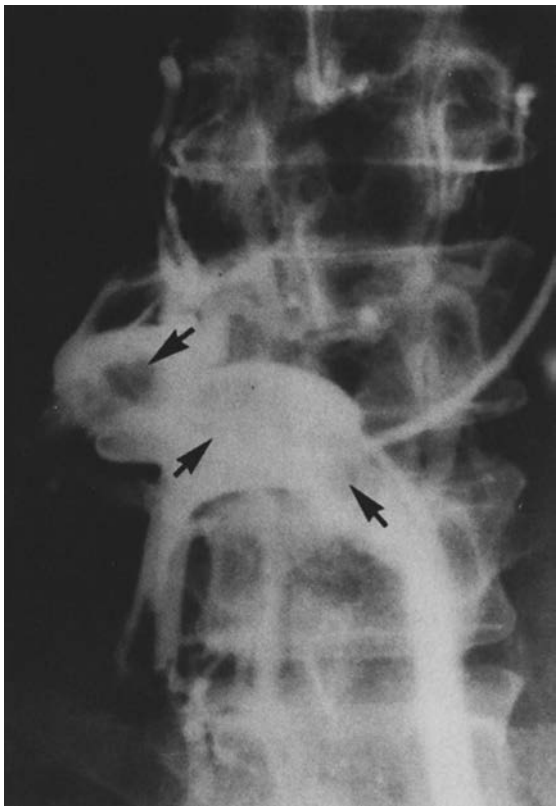

FIGURE 70-14. Left renal venography from a patient with renal vein thrombosis. Note the arrows indicating filling defects, which reflect accumulation of thrombotic material, surrounded by contrast material. There is complete obstruction of the main renal vein as well as of collateral circulation. (From: Llach, F, Papper, S, Massry, SG. The clinical spectrum of renal vein thrombosis: acute and chronic. *Am J Med* 1980;69:819, with permission.)

renal venogram usually demonstrates a thrombus within the lumen as a filling defect surrounded by contrast material (Fig. 70-14). In the presence of partial thrombosis, extensive collateral circulation can be demonstrated. Thus, the presence of such collaterals usually reflects the chronicity of the RVT and may explain the lack of renal functional deterioration.

Many investigators originally considered renal arteriography preferable to renal venography for the diagnosis of RVT. With renal arteriography important information is obtained about the status of the renal parenchyma. In addition, the theoretical danger of dislodging the clot material from the renal vein during renal venography and precipitating a pulmonary embolism is avoided. However, this complication did not occur following renal venography in several extensive radiographic series of patients with RVT (297,298). Renal arteriography may be useful in patients being evaluated for RVT associated with renal trauma or tumor because of the common involvement of the renal artery phase. Deviation and stretching of the interlobular arteries are usually observed in these conditions. In the nephrographic phase, the medullary pyramids are more densely opacified than the cortex, and instead of presenting the usual triangular appearance, they appear to bulge and sometimes are even ovoid in appearance.

Renal ultrasonography may be a useful potential diagnostic procedure for the diagnosis of RVT (297). The sonographic diagnosis of RVT is based on direct visualization of thrombi within the renal vein and inferior vena cava, demonstration of renal vein dilation proximal to the point of occlusion, loss of normal renal structure, and an increase in renal size during the acute phase. These ultrasonography findings, however, usually have to be confirmed with other diagnostic procedures.

Preliminary information on the use of combined ultrasonic scanning and Doppler ultrasonography for the diagnosis of RVT has been advanced. This is a noninvasive technique that essentially measures the renal venous flow velocity. Although the results of this approach are not definite in RVT, some studies suggest that Doppler ultrasonography may be helpful in the diagnosis of RVT in the transplanted kidney (297).

Computed tomography is the procedure of choice for the noninvasive evaluation of acute RVT. Lower attenuation density shows a thrombus and may be identified within the renal vein and inferior vena cava, or the venous diameter may be enlarged owing to obstruction. In general, intravenous infusion of contrast material, together with computed tomography, may help to visualize the thrombus. In the near future it is likely that noninvasive diagnosis of RVT may be possible using either ultrasonography or computed tomography, or both, together with clinical findings. The radiographic findings include enlargement and distention of the affected renal vein, with visualization of the clots within the vein and sometimes extension into the inferior vena cava (297). Persistent parenchymal opacification with kidney enlargement is usually observed. Capsular venous collaterals, thickening of Gerota's fascia, and pericapsular "whiskering" are often observed.

Preliminary observations of isolated cases suggest that magnetic resonance imaging (MRI) may be, in the future, the diagnostic procedure of choice in the noninvasive diagnosis of RVT. Because MRI produces high-contrast images between flowing blood, vascular walls, and surrounding tissues, vascular patency may be best determined by this technique. A major potential advantage in using this method is the avoidance of contrast material.

Clinical Course and Treatment

The experience to date on the course, prognosis, and treatment of RVT in nephrotic patients is limited. In 1963 Kowal et al. reviewed 65 patients with RVT (296). Only 14 of these patients were alive after 2 years of follow-up. Unfortunately, clinical data and literature references were given only for the 14 surviving patients. Ten of these patients had acute RVT. Recurrent thromboembolic phenomena were cited as the most common cause of death. Later, Rosenmann et al. followed 11 of 15 nephrotic patients with RVT over a period of 24 to 115 months (287). Only 4 of these patients had symptoms suggestive of acute RVT. The incidence of thromboembolic phenomena was high and the phenomena were fatal in 7 patients. One or more episodes of pulmonary embolism occurred in 7 patients, and 4 had evidence of repeated episodes of thrombosis involving the renal venous system. Three patients received anticoagulant therapy and had no new episodes of pulmonary emboli. These data suggest that the prognosis for nephrotic patients with RVT may be poor and is determined by the presence or absence of recurrent thrombotic complications.

In 1970 Richet and Meyrier reviewed 112 cases of RVT reported in the literature. Of the 112 patients, 72 had died, constituting a 64% mortality rate (299). Earlier, McCarthy et al. estimated the average survival period after the onset of RVT to be 9 months (204). These data, however, may not be representative of the present prognosis of this entity. First, most of the earlier data about the course and prognosis of RVT were obtained from autopsy studies. Therefore, the mortality may have been overestimated. Second, renal insufficiency secondary to RVT was a common cause of death in these patients. Dialysis therapy has, of course, reduced the rate of uremic death considerably. Third, the new diagnostic techniques and better understanding of this entity together with better use of

TABLE 70-7

FOLLOW-UP DATE ON NEPHROTIC PATIENTS WITH ACUTE AND CHRONIC RENAL VEIN THROMBOSIS (RVT)

Patients	Urine protein (g/24 hr)	Creatinine clearance (mL/min)	Serum albumin (g/dL)	Serum cholesterol (g/dL)	No. of patients undergoing dialysis
29 with chronic RVT	5.9 ± 3.9[a] (4.8 ± 2.3)	71 ± 25[a] (65 ± 29)	2.4 ± 0.7[a] (2.9 ± 0.7)	370 ± 110[a] (360 ± 88)	4
4 with acute RVT	5.2 ± 1.2[a] (5.0 ± 1.2)	76 ± 19[a] (98 ± 8)[b]	2.1 ± 0.2[a] (2.3 ± 0.2)	347 ± 19[a] (332 ± 26)	0

[a]Initial laboratory data (mean ± SD). Data obtained after anticoagulant therapy are shown in parentheses.
[b]$p < 0.05$.

anticoagulant therapy have contributed to better management of these patients. Nevertheless, a serious complication in patients with RVT is still thromboembolic phenomena, most often pulmonary embolism.

Lavelle et al. recently reevaluated 27 nephrotic patients with RVT, 10 of whom had acute lumbar pain and acute renal failure (300). Eleven patients died within the first 6 months. Survivors were followed for 6 months to 19 years. Nephrotic syndrome improved or even disappeared in 12 patients, and renal function did not worsen throughout the follow-up period. The main prognostic factors were initial renal function and type of nephropathy; that is, patients with membranous nephropathy had significantly better renal function and lower mortality than did patients with other nephropathies. Initial renal insufficiency was significantly associated with a poor prognosis. In fact, 6 of the 8 patients with acute renal failure died; hemorrhagic complications were the major cause of death in these patients.

Renal function may dramatically improve in patients with acute RVT treated with anticoagulant therapy. In Table 70-7 are shown the follow-up data on patients with acute RVT who had significant improvement in renal function with anticoagulant therapy during the follow-up period, and it is recommended that these patients be maintained on long-term anticoagulant therapy (205,210,216,217,220,221,239,251). Convincing evidence has been gathered suggesting that anticoagulant therapy reduces the incidence of new thromboembolic episodes and often reverses the deterioration of renal function that occurs with acute RVT (220,301). Patients treated with anticoagulant therapy may have recanalization of the renal vein, and in some instances a total dissolution of the clot may occur (301). Heparin is the initial therapy of choice. In patients with a large RVT and pulmonary embolism, the clearance rate of heparin is increased, and these patients may need higher doses of heparin in the early stages of therapy. Although the dosage of heparin varies from patient to patient, the aim is to maintain the clotting time of two to two and one-half times normal. In general, because of fewer complications, continued infusion of heparin is preferable to intermittent intravenous administration. Warfarin therapy is instituted once the patient has been treated with heparin for 5 to 7 days and the partial thromboplastin time is within the desired range. Warfarin is started orally with small loading doses. Common clinical problems with warfarin therapy are the drug interactions related to the kinetics of warfarin such as drug absorption, protein binding, metabolism, and excretion. These drug interactions are very common and may play an important role in the enhancement of or decrease in the anticoagulant effect of heparin. It should also be remembered that drugs such as aspirin and indomethacin can increase the risk of bleeding in these patients owing to the effect on the gastrointestinal mucosa and platelet aggregation. From all these observations it is obvious that individualization of warfarin therapy is essential, and the clinical status, patient sensitivity, metabolism, and possible drug interactions must all be taken into consideration. Once oral anticoagulant therapy is established, the prothrombin time should be kept at about one and one-half to two times normal. The recommended duration of anticoagulant therapy in these patients is difficult to establish. The severity of the hypoalbuminuria is a good indicator of the magnitude of the hypercoagulability. Thus, the nephrotic patient should probably be treated with anticoagulants as long as the serum albumin level is below 2.5 g/L. Relapses with new episodes of acute RVT have been observed after cessation of anticoagulant therapy (251). It is our belief that in general these patients should be on anticoagulation therapy as long as they have the nephrotic syndrome with significant hypoalbuminemia.

Early resolution of the acute RVT has been reported using either streptokinase or urokinase, both systemically or with selective infusion (302–304). However, it appears that selective infusion of these agents is also successful in RVT of the transplanted kidney (305,306).

Surgical treatment for RVT is rarely used today because the role of thrombectomy in the treatment of RVT has not been established as beneficial. Although marked improvement in renal function is occasionally observed after thrombectomy, the majority of these patients do not improve with surgery. This modality of therapy may be theoretically useful in patients with acute bilateral RVT who are not otherwise expected to survive the acute episode, especially when recurrent pulmonary emboli occur despite anticoagulation therapy.

Thromboembolic Complications Other than Renal Vein Thrombosis

The high cumulative risks of thromboembolic complications in the nephrotic patient have been recognized in the last three decades (217,220,251,305). These complications have been observed previously in the pulmonary arteries (307); axillary and subclavian veins (308); and femoral, coronary, and mesenteric arteries (309–310). However, the most common observation may be deep vein thrombosis of the extremities. A summary of thromboembolic complications studies is shown in Table 70-8. Therefore, Andrassy et al. studied 84 nephrotic patients and observed 37 episodes of thromboembolic complications in 30 patients during a period of 3 years (310). There were 23 episodes of deep vein thrombosis, an incidence of 44%, among the highest number encountered in medical patients. Kanfer et al. observed arterial and venous thrombosis in 5 of 8 children and in 10 of 29 adult nephrotic patients during a period of 7 years (231). Although lower (27%), this is still a significant incidence. Four other investigators observed a 17%

TABLE 70-8

SUMMARY OF PUBLISHED STUDIES EVALUATION THROMBOEMBOLIC COMPLICATIONS IN THE NEPHROTIC SYNDROME

	No. of patients	No. with thrombosis[a]	Venous			Arterial			Total no of other episodes[a]
			Renal	Pulmonary	DVT	Heart	Brain	Peripheral	
Andrassy et al. (256)	84	30 (38)	6	7	23	3	1	3	37 (44)
Kauffman et al. (206)	48	9 (19)	4	4	3	—	—	1	8 (17)
Kanfer et al. (178)	45	13 (29)	3	3	6	—	2	1	12 (27)
Pohl et al. (155)	59	5(10)	1	2	3	—	—	—	5 (8.5)
Kuhlmann (226)	17	4 (23)	1	2	1	—	—	—	3 (17)
Velasquez-Forero et al. (156)	19	8 (42)	8	1	2	—	—	—	3 (16)
Llach et al. (159)	151	41 (26)	33	18	3	2	2	1	26 (17)
Kendall et al. (179)	35	4 (23)	1	3	—	1	1	1	6 (17)

[a]Numbers in parentheses represent percentages.
DVT, deep vein thrombosis.

incidence of mostly deep venous thrombosis (210,213,214). In our prospective studies (216), 26 episodes of thromboembolic complications other than RVT were noted in 151 nephrotic patients (17%). Noteworthy is the finding that Pohl et al. reported only 1 patient with RVT of 54, but they observed 5 episodes of thrombosis, an 8.5% incidence, which is still high (212); as shown in Table 70-6 the incidence of thromboembolic complications ranges from 8.5% to 44%. Peripheral venous thrombosis and pulmonary embolism were the most frequent complications, but arterial thrombosis also occurred.

In our prospective study, ventilation-perfusion lung scanning was performed in 94 nephrotic patients, 24 with and 70 without RVT (220). Asymptomatic perfusion defects in the presence of normal-appearing chest X-ray films were observed in 12 patients, 5 with and 7 without RVT. Because pulmonary angiography was not done in these patients, a definitive interpretation of this defect is not possible. However, these data suggest a significant incidence of pulmonary embolism in the nephrotic patients. Similar observations noted (19%) of 37 nephrotic patients with abnormal ventilation-perfusion lung scans and only 1 with RVT (304).

Clearly, there is a significant incidence of thromboembolic phenomena other than RVT in nephrotic patients, but the morbidity and mortality of these complications are not well defined and prospective longitudinal studies must be done to elucidate both the magnitude of this problem and the use of appropriate prophylaxis and therapy in the patient with a high risk of thrombosis.

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CHAPTER 71 ■ THE LONG-TERM OUTCOME OF GLOMERULAR DISEASES

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At first sight, the task of describing the outcome of a particular disease seems to be a simple one; in fact, nothing could be further from the truth. First, we have the problem of definition, that is, what the "disease" under consideration may be. Without getting into a deep philosophical discussion of what the word *disease* signifies, at a practical level, a number of difficulties arise. Next, the very idea of a disease in its own right is an abstraction: As many clinicians emphasize, there are no "diseases"—only diseased patients—so in essence, we are describing the behavior and fate of groups of patients. But how are they to be defined for inclusion in a study?

Immediately new problems arise because there are no hard boundaries for the disease we are dealing with in the glomerulus. Various clinical presentations can be defined but in real life they have fluid boundaries; there are histologic appearances both on optical and immunofluorescence microscopy, between which there is no one-to-one correspondence; and finally, there are proved or (more usually) probable precipitating events, organisms, or multisystem diseases that affect other organs.

To cite an example, when we consider the outcome of acute glomerulonephritis, we meet accounts in the literature of patients with the clinical presentation of acute glomerulonephritis, many or all of whom did not have a renal biopsy; we have other accounts of patients with a similar presentation, but in whom a streptococcal etiology was established by cultures or antibody titers, with or without biopsies; and we have accounts of patients with streptococcal glomerulonephritis, some of whom had mesangioproliferative glomerulonephritis (MPGN) as shown by biopsy, or severe crescents.

Inevitably, there will be discrepancies in the conclusions drawn about the outcomes from these similar, but not identical, groups. A further difficulty arises because almost certainly some of the histologic appearances of the diseased glomerulus—for example, membranous nephropathy—are no more single diseases than the broad clinical features they present. We must think of these as appearances—patterns of glomerular injury—rather than diseases.

There may be several different pathogenic routes into a single histologic appearance, and each of these may have a different outcome. If we cannot distinguish or are ignorant of these subgroups, then a different mix of the subgroups inevitably leads to different descriptions of outcome. The second major problem is more obvious but equally neglected when we look at follow-up data from diverse sources: This is the question of patient selection (Fig. 71-1). The only unassailable data are those that derive from a population survey in which techniques that will select all the patients with a particular condition are applied, and all those patients are followed without fallout for a given period. Such studies do not exist in the study of glomerular diseases. Many of the conditions we discuss in this chapter such as IgA-associated glomerulopathy, minimal change disease, and membranous nephropathy exist in the population without ever being detected. The more diligently the population is examined, and the more frequently the urine is tested, the more such "patients" (who are, in fact, symptomless individuals) will be detected. The second stage of selection is when these patients are discovered; just how much are they studied or referred for investigation? Many patients with proteinuria or hematuria discovered on routine examination for insurance purposes may not be followed.

Specialist centers also will attract (because of their skills in management) patients who present with considerable problems, and it is often from such centers that descriptions of outcomes are published. It is usual for these first studies to present an overly pessimistic outlook for the prognosis of disease, and subsequent recognition of milder, more indolent, forms may restore the balance, with an apparent decrease in gravity in the view of the condition. Thus, the best outlook probably approximates more closely the true description of outcome for the general worldwide population.

Even if a patient is referred, a crucial question is whether to perform a renal biopsy. Because the criteria for many of the conditions discussed in this chapter depend on the availability of renal biopsy data, this point is crucial. It is often impossible to know exactly what the criteria for renal biopsy in a health care facility at a particular time may have been, because the authors do not tell us. Usually most adult patients with a nephrotic syndrome, or profuse proteinuria, undergo renal biopsy. The policy for biopsy in patients having isolated minor (0.5 to 1.5 g per 24 hours) proteinuria, isolated hematuria, or hematuria with minor proteinuria is particularly variable, among health care facilities and in different countries, sometimes depending on non-medical factors such as the local availability of skills and funding structures for medical investigations. The presence or absence of routine screening of populations, for example, military inductees, will depend on other factors, such as whether military service is obligatory.

Even when a population of patients, however gathered, is available for study, fallout begins as patients are lost to follow-up. Diligent work will often lead, even 10 to 40 years later, to more than 90% of "lost" patients being recovered, but very often the data available may be limited to those who are entirely healthy or who are dead. Built into any system of follow-up, however, is the inevitable fact that some patients enter the study early and some later, so the length of follow-up may vary from 1 to 2 years or to several decades. How do we best handle these incomplete data in order to extract the best information?

First, it is clear that in the end all diseases result in one of two states: Either the patient is dead or cured (Fig. 71-2). The proportion of patients who follow each of these two courses can vary from 1:99 to 99:1. Equally, the time required for the progression of this disease may vary; in practice, most glomerular diseases have run their course within 15 to 20 years of apparent onset, with the possible exception of IgA-associated nephropathies, which appear to have a slower evolution. This statement brings up yet another difficulty: When did the disease really start? What we have on record is the earliest point at which it reached clinical attention or, in retrospect, when the

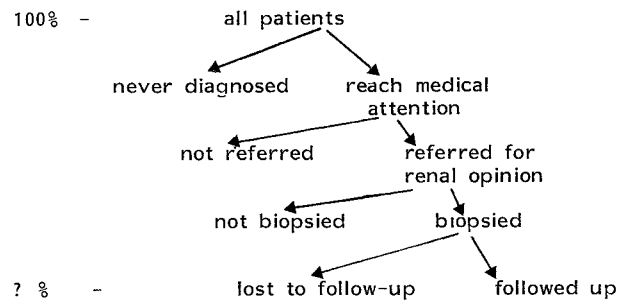


FIGURE 71-1. The various levels of patient selection that confuse descriptions of diseases and their outcome.

patient had symptoms (e.g., frothy urine or edema). In the case of symptomless hematuria and proteinuria, this point may be years or even decades from the true onset.

The next question is to describe outcome. A technique that is much used in this area and that appears throughout this chapter is that of the *actuarial life table,* usually presented in graphic form as a survival curve. It is worth discussing here the strengths and limitations of this technique, which is described in detail by Peto et al. (1). The table was developed by actuaries to provide estimates of survival of populations, for example, at different ages, mainly for insurance purposes. It was first applied in medicine to studies of cancer data and was used in renal disease in the mid-1960s to analyze transplant survival of kidney graft. Finally, in the early 1970s it was introduced to the study of glomerular and other chronic diseases, and it has become popular as its advantages have been recognized.

Life tables were originally designed to be applied to large populations—thousands or tens of thousands—whereas in medical practice, they are rarely used for the analysis of more than a few hundred persons, especially for relatively rare diseases, such as those affecting the glomeruli; many series involve less than 100 individuals. The importance of this is that as with any statistical estimate, the potential error of the estimate achieved will increase sharply when small numbers are under consideration. In general terms, all data on glomerular diseases deal only with small (or very small) numbers of patients.

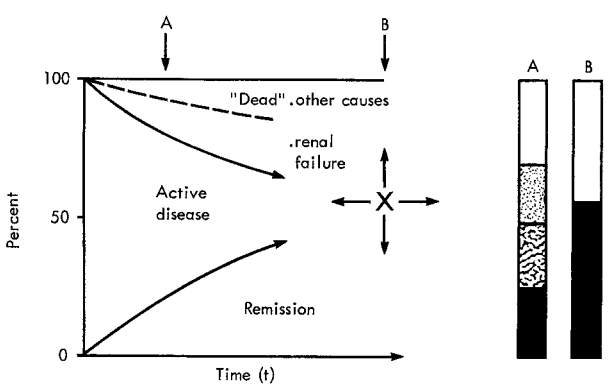


FIGURE 71-2. A generalized diagram to describe the outcome of a glomerular disease. Vertically, the proportion of patients in a given state is shown: dead (either incidentally or as a result of the glomerular disease), with continued proteinuria, or in remission. The time *(t)* at which the final state of all patients being in remission or dead will be reached will depend on the aggression of the disease; and the proportion dead or in remission, on its severity. If a cross-section of patients is studied at time A or B, a proportion of patients will still have active disease, with or without impairment of renal function, as represented in the bar graphs (**right**). Diagrams such as these can be used to summarize the outcome of glomerular diseases, the curves being calculated by the best estimate obtained from life table analysis of the necessarily incomplete data (see text).

The life table technique produces an estimate often referred to as the Kaplan-Meier (2) estimate of what the behavior of the population at risk might be if all patients had been followed for the full period under analysis. As already noted, this is almost never the case, except for very short follow-up periods. The lines in a figure may be drawn in a bold fashion, but it is the possible error—usually expressed as the standard error for each point on the life table—that is crucial. For example, an estimated population survival of 60% at 10 years may have 95% confidence limits of 20% to 90%, so the estimate is almost useless for purposes of comparison; in fact, the only valid method of comparison between two life tables is to compare the whole curve with the contrasted one—that is, assuming that the two populations are comparable (1), which is almost never the case except in carefully randomized prospective controlled studies.

With all these reservations in mind, we can examine how a life table is constructed. There are several ways to do this in detail, and a number of computer programs are available. For any period under study, first the number of patients "at risk" of having the event in question is calculated. The event can be any event of interest—loss of proteinuria, absence of hypertension, entry into end-stage renal disease (ESRD), or death, for example. The number of patients at risk throughout the interval in question clearly is related to the number being followed at the start of the follow-up period but will be less than this latter number because some patients will be lost to follow-up during the interval.

The calculation of a standard error of any point on the curve is possible using the following formula:

$$\text{Cumulative survival rate}\sqrt{\frac{1 - \text{Cumulative survival rate}}{\text{Number at risk}}}$$

And two life table curves can be compared for the statistical difference between them by statistics discussed originally by Kaplan and Meier (2) and more recently by Peto et al. (1) and Breslow (3). In essence, this procedure is a χ^2 test, weighted by the distribution of the data in the life table.

Obviously it is possible, by drawing up a life table for terminal renal failure and another for loss of proteinuria, to generate a diagram of the type shown in Figure 71-2 for any given glomerular condition or group of patients. Programs are available to construct life tables on microprocessors from raw data of dates of entry and the date of event or most recent follow-up—whatever the reason for exiting from the follow-up may be. If one wishes purely to examine the rate of entry into uremia, rather than true survival, then death from unrelated causes can be treated as "lost to follow-up" for the purposes of analysis. This may seem bizarre, but a similar problem arises in the analysis of transplantation data when immunologic graft loss is being examined and a patient dies of another cause with a functioning graft.

This raises the difficult problem of deaths attributed to causes other than renal failure in any study. Clearly in the end, all the patients in the study will be dead, including those who are in remission. In addition, the numbers of those dying within a decade or two of causes other than uremia will increase sharply if the mean age of the population under study is older than 60 years. Ideally, any life table analysis should include the expected life table for the population at risk, assuming no renal diseases were present. In Western countries, at the age of 35 years, at least 95% of patients will survive 15 years; therefore, in the case of most glomerular diseases that affect predominantly a young population, the effect of neglecting expected mortality is trivial. However, only 85% of 60-year-olds will survive a decade, the average survival time being 18 years for women and 20 years for men; at 65 years old,

the corresponding values are 12 and 16 years. Consequently, for some diseases, such as membranous nephropathy and vasculitis, some account of age of presentation must be taken.

It is not always clear what should be considered "other" causes of death and what role secondary hypertension may play, both as a direct cause of death and as a cause of accelerated fall-off in renal function. Similarly, vascular disease would be considered by some as a possible complication of a persisting nephrotic syndrome. How should deaths from complications of therapy be treated for statistical purposes? Clearly, they are the result of the disease, but this statistical treatment may not be appropriate to some observers.

Therefore, the technique of life tables allows us to make a description of the general average outcome of a glomerular disease or its appearances, but of course this is not what the patient wants to know. He or she wishes to know what his or her individual outcome will be, and from the data on population, the physician can make only the most general statements of risk. The possible evolution of the disease in individual patients can be made more precise by attempting to assess the predictive value of various individual characteristics. At a clinical level, such observations as the presence or absence of hematuria in a patient with proteinuria, the degree and persistence of proteinuria, and the presence or absence of hypertension or reduced renal function can be examined; and on histology, various glomerular and tubulointerstitial or vascular features can be assessed for their effect on prognosis. The crude way to do this is simply to examine the outcomes of patients with a certain feature and compare that with outcomes of patients without the same feature (univariate analysis), but this process ignores possible interactions, either purely statistical or causative, which may relate to certain features. This evaluation requires simultaneous multivariate analysis, for which a number of methods are available.

Perhaps the best technique for this type of analysis is the Cox method of proportional hazards (4). This technique has been applied surprisingly little to the study of glomerular disease (5–9), perhaps because the number of patients under study is so small that the technique may not be appropriate, but perhaps also because of ignorance of the technique. Analysis by the Cox method can be calculated only by the use of a computer program and is included in the widely available statistical software packages on the internet. A discussion on the use of the Cox model and related statistics in renal data can be found in the article by Beukhof et al. (5). It is often forgotten that when applying this type of technique, predictive variables are analyzed sequentially, so events that may be useful or convenient in the clinical sphere become nonsignificant statistically after the analysis shows they are secondarily dependent on some stronger variable. Therefore, in many analyses, only proteinuria and interstitial fibrosis emerge as independent determinants of outcome.

Obviously, the monitoring of proteinuria and indices of renal function, such as plasma creatinine and the glomerular filtration rate (GFR), followed sequentially, is among some of the most powerful tools available that allow prediction of disease course in individual patients. For example, it has become apparent that functional progression rarely, if ever, occurs in proteinuric renal disease if the excretion of protein falls to within normal limits even for a short while (10), although hypertensive damage is possible subsequently. Furthermore, the persistence of profuse proteinuria within or near the nephrotic range in patients with structurally disordered glomeruli (e.g., other than minimal change) is a clear indicator of a poorer prognosis in most patients with this phenomenon (Fig. 71-3), and in membranous nephropathy is a continuous variable—the greater the protein loss, the poorer the prognosis (see later discussion).

The behavior of the plasma creatinine concentration (P_{creat}) also has been the subject of much study. Mitch et al. reminded us 15 years ago (11) that GFR varies as $1/P_{creat}$ and several articles (11,12) have emphasized that the plot of $1/P_{creat}$ against time may be relatively linear, the slope being characteristic for each patient. The linearity of such plots has been greatly exaggerated, and the *r* value for correlation plots, even with the best fit, rarely if ever exceeds 0.70 (13).

Study of glomerular diseases confirms this point, and although membranous nephropathy shows relatively linear plots (14), study of data from the Medical Research Council (MRC) trial in the United Kingdom (15) showed that only 18 of 41 nephrotic membranous patients with a decline in renal function showed substantially linear fall-off in reciprocal creatinine plots. Similarly, Fellin et al. (16) found few patients with IgA nephropathy, whose decline in renal function could be described in a single slope of $1/P_{creat}$. MPGN often shows a markedly nonlinear pattern (17) with sudden falloffs in renal function that are not associated with hypertension, even after periods of relatively stable function lasting several years. In addition, the predictive value of the P_{creat} for the GFR has been overemphasized; changes occur with changes in diet and muscle mass, and, conversely, a constant plasma creatinine may conceal a fall-off in GFR. The use and misuse of reciprocal and other functions of P_{creat} have been reviewed by Walser (18), Hunsicker (19), and Levey (20).

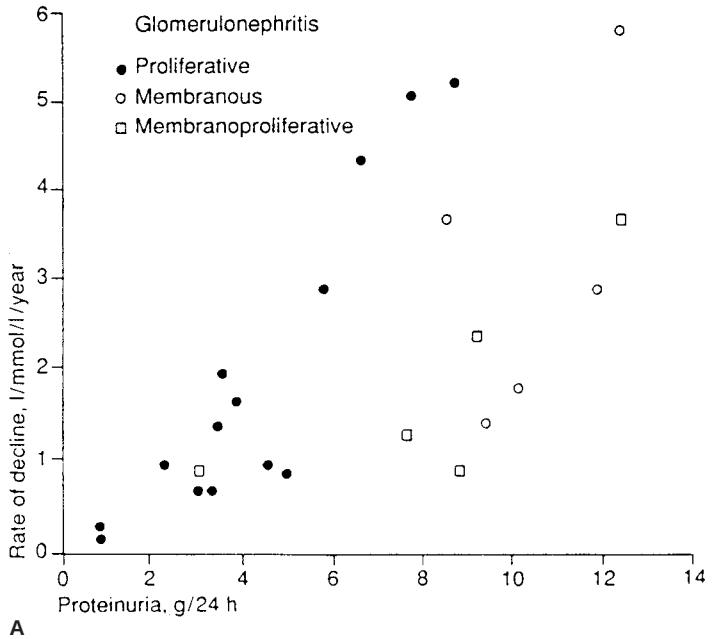
One interesting observation (14,15) is that for the main groups of progressive, proteinuric glomerular diseases (membranous nephropathy, focal segmental glomerular sclerosis [FSGS], and MPGN), progressive disease usually reveals itself by a detectable fall-off in GFR and a rise in plasma creatinine concentration within only 3 or so years of onset. Together with the observations on the presence and persistence of proteinuria mentioned previously, this information allows a better individual prognosis to be given quite early in the course of the disease. However, this is not the case for IgA nephropathy, which generally evolves much more slowly.

In this chapter, for reasons of brevity, we deal mainly with recently published observations on the outcomes of glomerular disease, published in 1980 or later. For a more detailed consideration of the literature before 1980, the reader is referred to an earlier treatment of the subject (21). Given that an etiologic classification is still beyond our grasp, this chapter is organized in a conventional fashion around the usual histologic classification of glomerular disease (22), with the many limitations of this approach acknowledged herein.

THE OVERALL PROGNOSIS OF THE NEPHROTIC SYNDROME

Unlike symptomless patients with hematuria or proteinuria, patients who have the nephrotic syndrome are likely to be referred for medical attention and very likely to have a renal biopsy as part of the investigation of their condition. It is still common to perform a biopsy on all adult patients with onset of a nephrotic syndrome, and older data exist for unselected series of children with nephrotic syndrome who had a biopsy; however, it is now no longer common practice to perform a biopsy on all nephrotic children, especially those between the ages of 1 and 5 years.

The outcome of nephrotic syndrome generally depends on four sets of circumstances: complications of the nephrotic syndrome, the effects of proteinuria itself, the influence of underlying histopathology, and the response to treatment (23). Furthermore, there is expanding interest in identifying urinary biomarkers, which may predict progression of nephrotic syndrome. Some, including fractional excretion of immunoglobulin G (IgG), urinary N-acetyl-beta-glucosaminidase, urinary



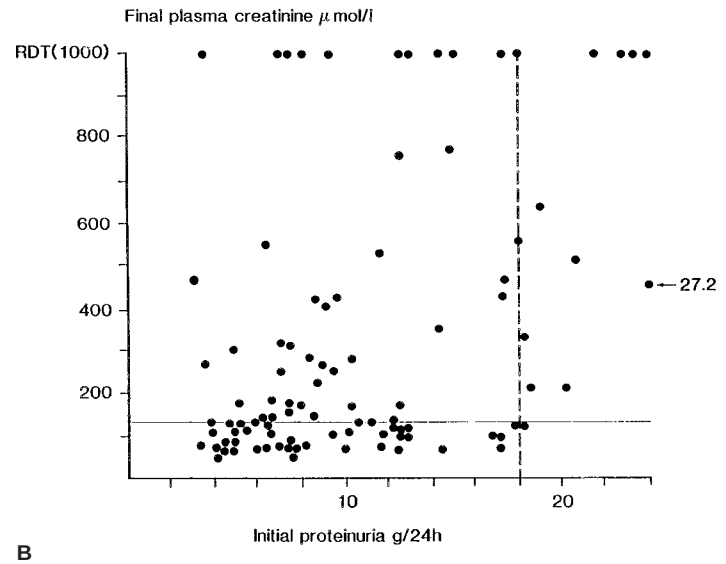


FIGURE 71-3. The important relationship between the outcome and the quantity of proteinuria is further examined by correlating plasma creatinine concentration during follow-up with the quantity of proteinuria at presentation. In various forms of glomerular diseases, the relationship between initial proteinuria and rate of change in the $1/P_{creat}$ holds. (From: Williams PS, Fass G, Bone JM. Renal pathology and proteinuria determine progression in untreated mild/moderate chronic renal failure. *Q J Med* 1988;67:343, with permission.)

beta-2 microglobulin, and urinary retinol-binding protein are promising as prognostic indicators (24–27).

Complications of the Nephrotic State

Now only few nephrotic children or adults die as a result of complications of the nephrotic state (28,29). In the study of Wass et al. (30), only one-third of adults with nephrotic syndrome remained nephrotic 4 years later. The remainder had either progressed to renal failure with diminution of proteinuria and disappearance of edema or had gone into remission either spontaneously or as a result of treatment. Today an even smaller proportion of children with nephrotic syndrome have a persistent course. Most children have minimal change lesions, and these children will either have a remission within 3 years spontaneously (two-thirds) or have earlier remissions secondary to treatment with corticosteroids or cytotoxic agents (95%) (see later discussion). According to the data reviewed by Trompeter et al. (31), after 15 to 20 years, only 3% of an unselected cohort of children with biopsy-proven minimal change nephrotic syndrome still had active disease.

Similarly, in a study of 89 adults with onset of minimal change nephrotic syndrome after the age of 15 years (32), at follow-up after 2 to 24 years, 59 (80%) of 74 surviving patients were in complete remission, and only 5 had persisting nephrotic syndrome. Therefore, in contrast to past experience, neither of these groups is exposed to possible risks of complications for prolonged periods. However, the few adults and children who have severe and persistent proteinuria are at high risk for complications. Many of these individuals, especially nephrotic children and young adults, have lesions of FSGS that are resistant to treatment with corticosteroids and cytotoxic drugs; others have membranous nephropathy or MPGN. In these unfortunate individuals, a full nephrotic syndrome may progress to chronic kidney disease (CKD) or renal replacement therapy. Even nephrectomy or renal infarction has been occasionally required (33).

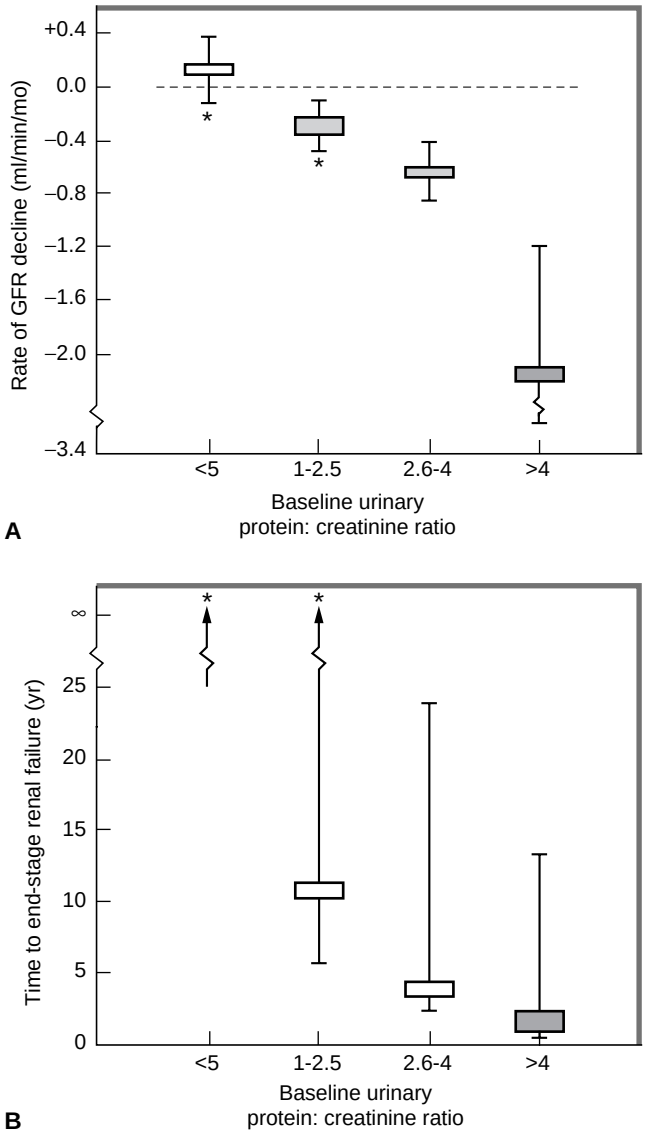


FIGURE 71-4. In chronic nondiabetic, proteinuric nephropathies, decline of glomerular filtration rate (GFR) and predicted time to end-stage renal disease (ESRD) are highly dependent on baseline urine protein/creatinine ratio. Data are from 98 patients with various glomerular diseases. Mean (±SE) Rate of Decline in the Glomerular Filtration per Patient per Month (**Panel A**) and Mean Predicted Time to End-Stage Renal Disease (Panel B). Asterisks indicate a significant difference ($p < 0.05$) from a baseline urinary protein/creatinine ratio above 4. The I bars in **Panel B** are ranges. (From: Remuzzi G, Bertani T. Pathophysiology of progressive nephropathies. *N Engl J Med* 1998;339:1448–1456, with permission.)

Infections

Although their importance is still evident, in the past, infections (34) played an even more important role in determining the outcome for the nephrotic patient. For example, the data of Arneil (35) from the Sick Children's Hospital in Glasgow, Scotland, suggest that during the preantibiotic era, more than half of nephrotic children were dead by 5 years after onset, most infections occurring within the first 2 years. There are no data from this period from a single source dealing with adult-onset nephrotic patients, but perusal of accounts in the literature of the 1920s and 1930s (36) suggests that the clinical picture was similar, although (as today) primary pneumococcal peritonitis is mostly confined to children and adolescents. This is because they have yet to develop specific antibody against pneumococcal polysaccharides and are dependent on nonspecific immunity resting on the activity of the alternative pathway of complement, which is much reduced in nephrotic patients because of protein losses into the urine (29).

Hyperlipidemia

The ubiquitous hyperlipidemia of the nephrotic syndrome (34,37,38) is usually manifest with elevated triglycerides, low-density lipoproteins (LDLs), and total cholesterol, often with low high-density lipoprotein (HDL) values (39). Any attempt to understand the clinical consequences of hyperlipidemia in relation to accelerated vascular disease must take into account the various other cardiovascular risk factors present in nephrotic patients. For example, most patients with persistent nephrotic syndrome also have hypertension, which may be a stronger risk factor for myocardial and other vascular diseases than hyperlipidemia (40). Analysis of 100 nephrotic adults demonstrated that most had one or more risks for cardiovascular disease, including hypertension, left ventricular hypertrophy (LVH), smoking, and others (41). Furthermore, nephrotic diseases with circulating immune complexes may carry their own specific risks, as shown experimentally (42,43) and in humans with systemic lupus who demonstrate premature atherosclerosis (44–46). Although studies have varied in their conclusions (30), a commonly cited study by Ordonez et al. found an elevated risk for myocardial infarction and cardiovascular death in nephrotic adults (47).

Given the information on the deleterious effects of hyperlipidemia in the general population, an exhaustive multivariate analysis implicating the hyperlipidemia of nephrotic syndrome as an *independent* risk factor for cardiovascular events is unlikely to be performed. On balance, it seems reasonable to assume that the hyperlipidemia of persistent nephrotic syndromes probably leads to an increased risk of atherosclerosis.

Thrombosis

Thrombosis is the third major risk besides infection and vascular disease (30,34,48,49). This prothrombotic state probably arises from platelet hyperaggregability, itself the result of platelet–von Willebrand factor interactions and possibly increased availability of arachidonic acid to form aggregant prostaglandins. High fibrinogen levels are also important (49). Major alterations in fluid-phase coagulation proteins are almost confined to severe nephrotic syndromes, with a serum albumin concentration of less than 2 g/dL. Unlike the risk of vascular disease, thrombosis may strike within only a few days or weeks of onset and although rare may attack most tragically those with the most reversible of nephrotic syndromes, because their urinary protein loss is great and their hypoalbuminemia and alterations in other proteins such as von Willebrand factor profound.

Prevention of Complications of the Nephrotic State

To what extent any of these risks can be minimized is controversial. Prophylactic treatment of children with a severe active nephrotic syndrome using penicillin seems sensible, and prompt treatment of cellulitis or septicemia is almost always successful. With the introduction of HMG- CoA reductase inhibitors (statins), which are effective in reversing nephrotic hyperlipidemia (50,51), therapy seems reasonable in those with persistent nephrotic syndromes or in those who are clinically likely to remain nephrotic despite treatment

(i.e., MPGN). The question of prophylaxis against thrombosis with anticoagulants in patients with active severe nephrotic syndromes remains contentious (48). Especially for idiopathic membranous nephropathy, expert opinion and decision analyses have supported the use of prophylactic warfarin in high-risk patients with serum albumin level less than 2 g/dL (52). However, controlled studies are not available for warfarin, or for antiplatelet agents, which may have less toxicity but similarly unestablished efficacy.

Proteinuria as a Factor in Progression

The second major area of risk in the nephrotic syndrome is the proteinuria itself (53–55). It is well known that progression into renal failure in the absence of proteinuria almost never occurs in glomerular disease (56), although exceptions occur (57–59). One such exception is when secondary hypertension appears after apparent "healing" of the original disease. Conversely, progression is almost always accompanied by urinary protein more than 2 g per 24 hours or elevated urine protein/creatinine ratio, and usually within the nephrotic range (Figs. 71-3, 71-4). The prognosis for patients with varying glomerular lesions is worse when a nephrotic syndrome is present, as demonstrated in Figure 71-3 and confirmed by Williams et al. (60) and Hunt et al. (61). In FSGS (62,63), membranous nephropathy (64–66), and MPGN (67), the prognosis is markedly worse for nephrotic patients compared with patients who were never nephrotic. Furthermore, if one plots proteinuria against rate of progression of renal disease (60,66), one finds that the greater the proteinuria, the more rapid the decline in renal function.

Two interpretations of these data are possible (53,68): Either profuse continuing proteinuria is a marker for more severe and eventually more serious disease, or profuse persisting proteinuria is damaging and leads to acceleration of the original disease process or to secondary renal damage (69). It has been established that proteinuria leads directly to the production of chemotactic factors (70) and a damaging interstitial infiltrate (68). Transit of protein across the glomerulus may be damaging as well. The accumulating experimental evidence for a direct role of proteinuria in initiating tubulointerstitial injury and progressive nephropathy is reviewed elsewhere (71).

There is much current interest in these hypotheses, given that it has proved possible to reduce the protein loss in patients with glomerular diseases using various agents—angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARBs), nonsteroidal antiinflammatory drugs (NSAIDs), cyclosporine, and dipyridamole—and following low-protein diets (53,72). For ACE inhibitors and ARBs, evidence in treatment of type 1 and type 2 diabetes with nephropathy suggests that reduction in proteinuria slows the decline in glomerular filtration rate (GFR) independent of blood-pressure lowering effects (73–76). This is perhaps the strongest evidence that proteinuria in and of itself may be injurious. An exception to the general rule that proteinuria dominates prognosis may be lupus nephritis (77–79), in which the outcome of this disease is more affected by treatment than are all the previously mentioned progressive glomerular diseases.

Renal Histopathology

The third factor that determines prognosis for the nephrotic patient is the histologic appearance of renal biopsy specimens, which despite many reservations remains the best guide to the nature and prognosis of the syndrome, although this has been challenged (68,80,81). The general prognosis association with the various aspects of the histopathologic appearances was reviewed in detail elsewhere (82). However, a number of general points can be made.

The first point is to examine the differing underlying histologic appearances, especially because they are different at various ages and in different parts of the world. In Figure 71-5 data are shown from a series of more than 600 adult-onset nephrotic patients seen at Guy's Hospital in London between 1965 and 1984. The data for the childhood-onset cases are in Figures 71-5 and 71-6 and are from the International Study of Kidney Disease in Childhood (ISKDC) on more than 500 unselected nephrotic children and 200 childhood-onset patients seen at Guy's Hospital before 1970, during which time all

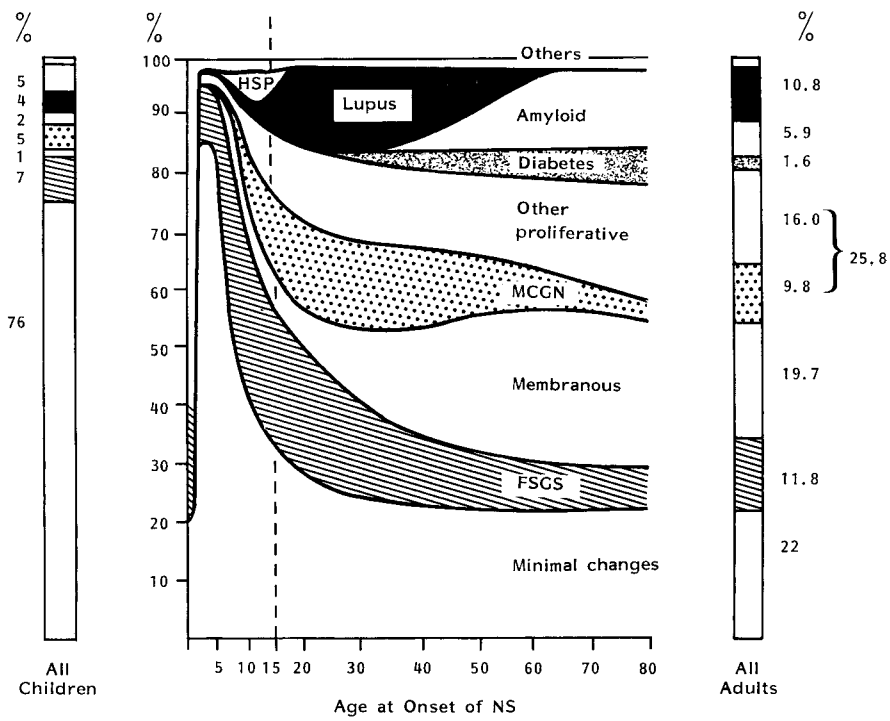


FIGURE 71-5. The underlying histopathologic appearances from a series of 607 nephrotic patients with onset after the age of 15 years, studied at Guy's Hospital from 1963 to 1984. The childhood data are derived from 521 patients studied by the International Study of Kidney Disease in Childhood and 200 patients studied at Guy's Hospital before 1970, during which time all childhood nephrotic patients underwent biopsy. The curves have been smoothed to simplify the diagram, but the adult data are shown in Table 71-2, and the childhood data are shown in more detail in Figure 71-6. (From: Cameron JS, Glassock RJ. The natural history and outcome of the nephrotic syndrome. In: Cameron JS, Glassock RJ, eds. *The Nephrotic Syndrome*. New York: Marcel Dekker; 1987, with permission.)

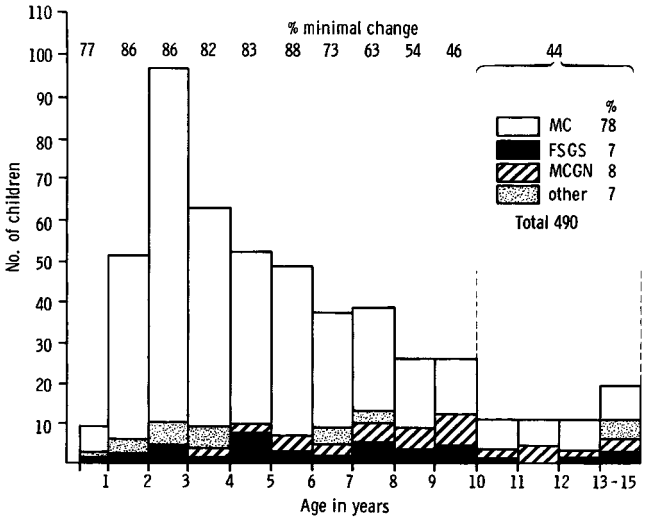


FIGURE 71-6. The underlying histopathology in more than 500 unselected nephrotic children studied by the International Study of Kidney Disease in Childhood, 1965–1976. *MC*, minimal changes; *FSGS*, focal segmental glomerulosclerosis; *MPGN*, mesangioproliferative glomerulonephritis. (From: Barnett HL, et al. Minimal change nephrotic syndrome. In: Edelmann CM, ed. *Pediatric Kidney Disease*. Boston: Little, Brown and Company; 1978:195, with permission.)

children who presented with a nephrotic syndrome underwent biopsy. The main categories shown account for almost 99% of all nephrotic patients, and a few others are discussed in subsequent text. The increase in the proportion of patients with membranous nephropathy with increasing age at onset is obvious. Although amyloidosis is rare before the age of 40, primary amyloid is a relatively common cause of a nephrotic syndrome in older adults. Lupus has the usual age distribution (ages 15 to 50 years) with or without a nephrotic syndrome. Focal segmental sclerosis is present at all ages, from neonates until old age, although the proportion of patients with minimal change lesions declines steadily from the age of 3 to the age of 20 years, but thereafter remains rather constant.

These classic descriptions on the occurrence of various histopathologic syndromes have been challenged in recent years by a growing literature suggesting that FSGS is more prevalent clinically and in large biopsy series. Although initially confined to reports in U.S. adults (83,84), similar data have emerged for nephrotic children in Canada, the United States, and India, and for adults in Peru, Korea, and other regions (85–90). Thus, the current literature strongly suggests a worldwide increase in the frequency of FSGS in both adults and children.

A number of factors distort the incidence of various diseases. First, there is a tendency not to refer older patients for biopsy. Second, the proportion of diabetic patients seen with a nephrotic syndrome depends on the referral and biopsy policy in diabetic patients with proteinuria, with or without edema. In general, many centers have avoided biopsy in diabetic nephrotic patients with retinopathy and have performed a biopsy only when there is some indication that the condition is unlikely to be diabetic nephropathy. For example, the presence of significant hematuria, or the absence of retinopathy, may be an indication for a biopsy. However, the proportion of all nephrotic patients with diabetic nephropathy, even where all nephrotic patients have undergone biopsy, does not exceed 10% in any published series. The coincidence or association of diabetes and membranous nephropathy (91) or other forms of glomerular pathology needs emphasis, and many centers are performing biopsies more often in diabetic nephrotic patients.

Although these data apply to developed Western communities, there are many variations within the much higher incidence of nephrotic syndrome found in the Tropics (92). Although poorly documented for developing countries, the admission data analyzed by Kibukamusoke (93) suggest that nephrotic syndrome may represent a much more common admission diagnosis than in Western nations. In the absence of firm data on incidence, it is difficult to judge whether minimal change disease is actually rare in the Tropics or merely submerged in a much larger number of other forms of histopathology. For example, in Malaysia (94), there may be a higher incidence of minimal change disease or very minor proliferative histology in adults. These patients are sensitive to corticosteroids in terms of decreasing urinary protein excretion and represent 40% to 50% of all nephrotic adults. Minor variations in incidence almost certainly occur throughout the Western world. For example, higher proportions of nephrotic adult patients from the United States have membranous nephropathy compared with counterparts from Europe. In the Interhospitals Study (95) of the nephrotic syndrome, 72 of 154 nephrotic patients had membranous nephropathy, whereas in U.K. data (Fig. 71-5), the proportion of adult-onset cases with this appearance remained constant at about 20% to 25%. Membranous nephropathy, unrelated to carriage of hepatitis B virus, also seems to be common in Greece (96) and the Balkans. MPGN is relatively uncommon in Japan, but in Iran, it accounts for 64% of nephrotic patients (97). Thus, it is difficult to make general assumptions regarding incidence of various diseases, given regional variations and changing frequencies of primary diseases such as FSGS.

When one is considering the histopathology of diseases that underlie a nephrotic syndrome, the general prognosis of the group of patients with any given histopathologic pattern may be estimated, but it remains the individual prognosis that is of primary concern to physician and patient. Within general statements, such as that 90% of adult type I MPGN patients with a nephrotic syndrome will be in renal failure before 10 years, or that up to 60% of adult-onset nephrotic patients with membranous nephropathy will have renal failure eventually, the prognosis and rate of progression of the individual are submerged.

Effects of Treatment

The fourth and final influence on prognosis is again an obvious one: the effect of specific treatment in resolving the nephrotic syndrome or at least diminishing the proteinuria to the point at which edema no longer forms. The treatment of nephrotic children and adults has been discussed extensively elsewhere in these volumes, but a few additional points are worth making here. Clearly, the response of the various histologic types of glomerulonephritis underlying the clinical presentations of glomerular disease differs. For example, 90% of nephrotic patients with minimal change lesions, and 45% to 65% of those with FSGS (98,99) will respond to treatment with corticosteroids, cytotoxic agents, or cyclosporine by complete or partial remission of proteinuria. As noted previously, progression into renal insufficiency is almost unknown for any patient whose proteinuria remits completely (56), and thus these patients have almost universally a good prognosis for long-term renal function. Therefore, response to early treatment is a powerful indicator of good prognosis. For example, it has been known for many years that the outlook for adult nephrotic patients who respond to corticosteroids is much superior to those who do not respond to corticosteroids (100), irrespective of renal biopsy appearances (Fig. 71-7). By the 1970s, this distinction was so clear to pediatric nephrologists that since then, it has been almost universal practice to treat all but a few

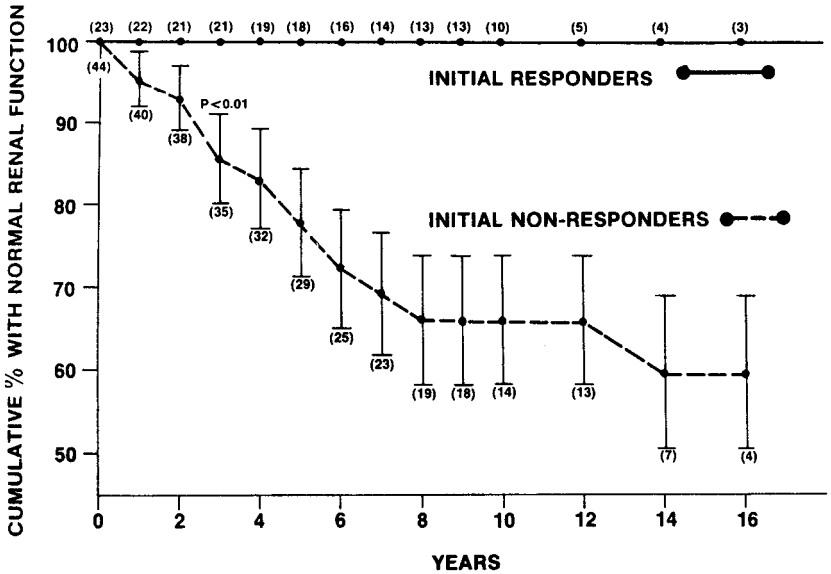


FIGURE 71-7. The long-term outcome of adult patients with a nephrotic syndrome who never underwent a biopsy and who were treated with prednisone. Those who responded to this treatment with loss of proteinuria during the initial treatment fared much better than those who did not, with respect to the outlook for renal function. (From: Idelson BA, et al. Prognosis in steroid-treated idiopathic nephrotic syndrome in adults. *Arch Intern Med* 1977;137:891, with permission.)

childhood-onset nephrotic syndrome patients with corticosteroids and to perform a biopsy at about 4 weeks in those whose proteinuria fails to remit by this time.

It has been argued (79–81) that such a policy has merit also for patients with adult-onset nephrotic syndrome, even though the proportion of adult nephrotic patients who respond to a brief course of steroids is much lower in adults than in children. Such a suggestion involves balancing the relative risks of a continuing nephrotic syndrome against the risks of renal biopsy itself and of the corticosteroid treatment to be given. Given such data as the lack of effectiveness of steroids in membranous nephropathy (101), the often protracted duration of steroid therapy required for many patients with FSGS (102), and the general safety of real-time renal biopsies using automated needles (103), it seems increasingly difficult to rationalize an empiric course of steroids in nephrotic adults. Furthermore, Nolasco (32) and Korbet et al. (104) analyzed the rates of response to corticosteroids in patients with adult-onset minimal change nephrotic syndrome treated with corticosteroids (Fig. 71-10). The response rate for adults in these series was slower and less frequently complete than that for children in the ISKDC study. Therefore, a policy of performing a renal biopsy in all adult-onset nephrotic syndrome patients without obvious diabetic nephropathy is preferred.

PROGNOSIS OF INDIVIDUAL HISTOLOGIC GROUPS

Minimal Change Lesions

Most patients who undergo biopsy and show minimal change lesions have a full nephrotic syndrome, but there are many symptomless patients with isolated proteinuria—especially adolescents and young adults (105,106), who may show appearances consistent with a diagnosis of minimal change lesions in their renal biopsy specimens (Chapter 64). However, these patients may not undergo biopsy unless the urinary protein loss is more than 2 g per 24 hours or edema appears, because of the excellent prognosis for this group (105,106) and because the changes in their renal biopsy specimens are usually benign. Therefore, this discussion is confined to patients with a minimal change lesion in the setting of a nephrotic syndrome. The outcome of minimal change disease as observed today is very much modified from its "natural" history, both by control of infections (discussed already) and by the successful use of immunosuppressive agents to eliminate proteinuria.

Childhood-Onset Minimal Change Nephrotic Syndrome

History Without Treatment: Causes of Death. As Figure 71-6 demonstrates, the minimal change lesion is most commonly found in childhood-onset nephrotic syndrome patients but also accounts for 20% to 25% of adult nephrotic patients of all ages. As discussed already (34,82), until the era of diuretics and antibiotics, death from sepsis and occasionally from thrombosis was common in patients with nephrotic syndrome. Now death from complications is rare, although Trompeter (31) and the ISKDC (107) demonstrated that a low rate of mortality from complications persists. In Trompeter's study of 183 unselected children with a nephrotic syndrome and biopsy-proven minimal change lesions, there were 11 deaths during the subsequent 15 to 22 years, 7 of which were related to the nephrotic syndrome or its treatment (e.g., infections, septicemia, and hypovolemia). The only death from 1982 to 1990 in this group was of one boy, who had brain infarction from sagittal sinus thrombosis during a relapse. Of 10 children who died of the 389 children studied by the ISKDC (107) for 7 to 15 years, 6 died of infection, 1 died of dural sinus thrombosis, 1 died of circulatory overload precipitated by intravenous albumin infusion, and 1 died in chronic renal failure; the cause of death in the final child was undetermined. Notably, all the deaths occurred in the early part of the study (i.e., before 1972). Krensky et al. (108) and Gorensek et al. (109) confirmed the continued importance of peritonitis as a complication of childhood nephrotic syndrome. In these two series, three children died of their sepsis.

Much attention has been directed to the relapsing pattern of patients with minimal change nephrotic syndrome. It has sometimes been said that this pattern is the result of "forcing" patients into remission with corticosteroids, whose disease would otherwise have persisted. This pattern of behavior was recorded, however, well before the introduction of corticosteroids; indeed, one of the first accounts of a patient with childhood nephrotic syndrome, in Paris (1833 to 1834), described two children whose urine became completely free of albumin between relapses (110). The relapses themselves

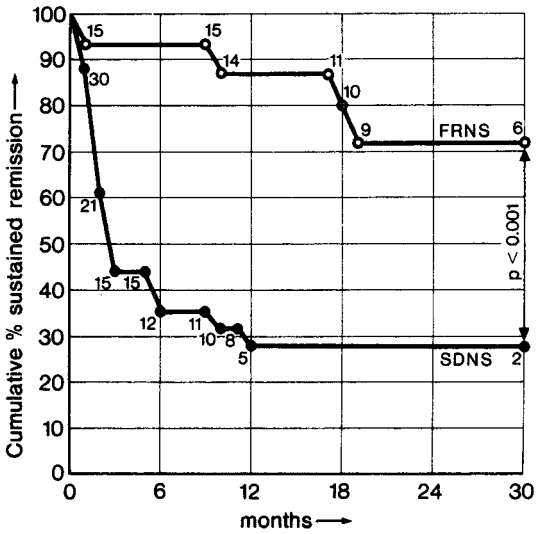


FIGURE 71-8. The stability of response of nephrotic children who have multiple relapses (Frequent Relapsing Nephrotic Syndrome *[FRNS]*) or have become steroid-dependent (Steroid Dependent Nephrotic Syndrome *[SDNS]*) (see text for definitions) treated with mustardlike agents (cyclophosphamide or chlorambucil). Those who have multiple relapses have a much superior response in the long term, compared with those who were steroid-dependent before treatment with cytotoxics. (From: Arbeitsgemeinschaft für pödiatrische nephrologie. Effect of cytotoxic drugs in frequently relapsing nephrotic syndrome with and without steroid dependence. *N Engl J Med* 1982;306:451, with permission.)

also may remit spontaneously (111). There is little doubt from the data of Arneil (35) and Schwartz (112) that the natural evolution of childhood nephrotic syndrome progresses slowly toward remission; from Arneil's data, one can calculate a spontaneous remission rate of about 65% at 3 years from onset (35).

History with Treatment. Today, this long-term natural evolution is modified by early treatment with corticosteroids. From the data of the ISKDC (113), 93% of nephrotic children will go into remission within 6 to 8 weeks on a dosage of prednisone of 60 mg/m^2 per 24 hours (Fig. 71-8). Thirty-six percent will never experience a relapse, 18% will have infrequent relapses, but 39% will experience multiple relapses, either with a period without treatment and free of proteinuria between relapses ("frequent relapser") or relapsing immediately when prednisone is withdrawn or even during modest doses of the drug (corticosteroid-dependent).

What the exact influence of the initial treatment on subsequent outcome may be has been debated. Early claims were made that more prolonged initial treatment resulted in fewer subsequent relapses (114). The German Arbeitsgemeinschaft für Pödiatrische Nephrologie (APN) (115) examined the ISKDC regimen of treatment for 8 weeks contrasted with treatment for a shorter period, the period of treatment being determined by how rapidly complete remission appeared. Sustained remission was significantly less frequent (19%), with the shorter course over the following 14 months of follow-up, compared with 39% for the standard protocol; when an even longer course (12 weeks of treatment) was given (116), the results were even more impressive: 64% had sustained remission. Choonara et al. (117) compared stability of remission in a group of relapsing children treated for similar lengths of time but with lower dosages (30 mg per 24 hours) with those given the "standard" ISKDC dosage of 60 mg/m^2; duration of remission was similar in the two groups. The French Club for Pediatric Nephrology reexamined the suggestion of Lange et al. (114) but found that stability of remission was no more after 1 year of alternate-day treatment than after 16 weeks (118).

Multiple Relapsing Nephrotic Syndrome. Much attention has been paid to the optimum management of frequently relapsing or corticosteroid-dependent patients (Chapter 64). Although cytotoxic agents such as *cyclophosphamide* and *chlorambucil* may induce a remission in such children (119–121), recent reports in small numbers of patients cast some doubt on the uniform effectiveness of cyclophosphamide in steroid-dependent nephrotic syndrome (122,123). Other corticosteroid-based therapy includes carefully titrated alternate-day prednisone (124) or lower dose prednisone (125) on an intermittent or continuous basis for a longer duration. This tendency has been strengthened by the observations of Garin et al. (126) and the German APN (127); the patients who need the relief from steroid dosage the most, that is, the steroid-dependent patients, achieve the least benefit from cytotoxics in terms of duration of remission (Fig. 71-8). The optimum duration of the course of cyclophosphamide has not been determined precisely. Initial data suggested that a course of only 2 weeks was without effect, and that 6 or 8 weeks at 3 mg/kg per 24 hours would achieve as much as longer courses (119). This view was challenged by the APN (115), who showed more stable remission when 12 weeks of treatment was used, but yet again a Japanese study found no difference (128). Schulman et al. (129) noted no difference in the proportion of prolonged remissions in those with or without IgM deposits or global sclerosis. Of note, a recent report found that children with a history of intrauterine growth retardation (IUGR) and minimal change disease were far more likely to become steroid-dependent or frequent relapsers, perhaps indicating a subgroup eligible for heightened monitoring and a low threshold for conversion to cytotoxic agents (130).

In the last two decades, *cyclosporine* has been used extensively in children with minimal change nephrotic syndrome (131), on the reasonable assumption that the inhibition of activated T cells achieved by this drug could be useful. Accumulated data (121,131–134) have produced a consensus that the drug has a useful place in the management of relapsing patients but makes only a small difference in the longer term evolution of the condition. Remission can usually be induced in steroid-dependent or frequently relapsing patients, but relapse is the norm when the dosage of the drug is stopped or tapered during the first year of treatment. However, cyclosporine may be useful in permitting a reduction in dosage or cessation of corticosteroids, although its use may substitute the side effects of cyclosporine and cyclosporine dependency for corticosteroid dependency and side effects. This is particularly important in permitting normal stature. There is, however, some evidence that longer courses of cyclosporine therapy may induce prolonged remission (135), as cytotoxic drugs may achieve, but at a much lower level of frequency; in most series, even after long-term (more than 1 year) treatment, withdrawal leads to relapse (136). Chronic cyclosporine therapy confers a significant risk of nephrotoxicity. As seen from one study, progressive nephrotoxicity in children on long-term cyclosporine can be minimized if patients are kept volume expanded and low doses are used with close monitoring of levels (137).

As in adults, the antiproliferative agent *mycophenolate mofetil* (MMF) has garnered interest as a treatment for children with steroid-dependent minimal change disease. Although numbers of patients are small, this agent has shown the ability to substantially reduce relapses (138). Another agent, the antihelminthic agent levamisole, is known to have immunomodulatory effects. Very few randomized or controlled studies are available in which levamisole has been used alone or in combination treatment in minimal change disease in children (139–141). It has been used mainly to prevent relapses

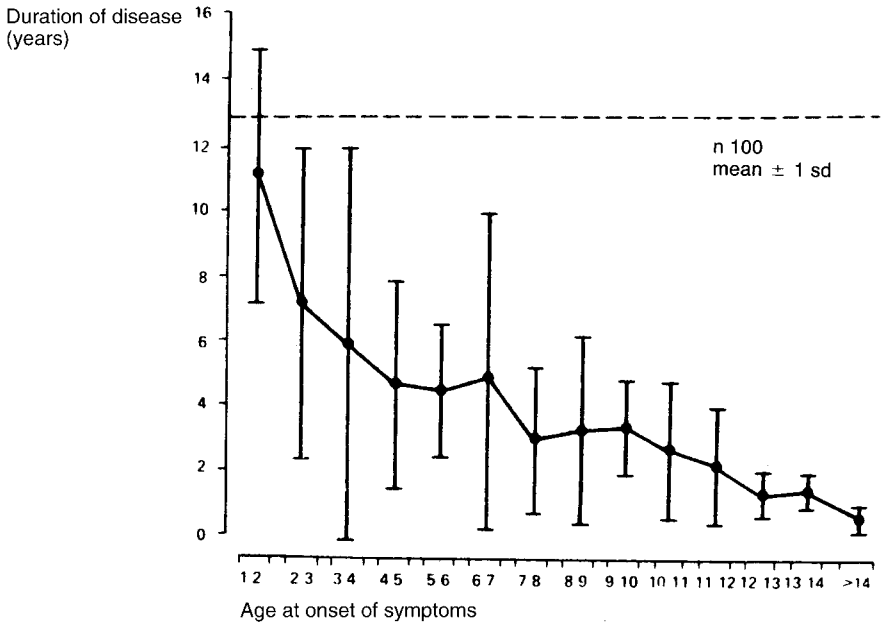


FIGURE 71-9. The duration of relapsing course in relation to age at onset in minimal change nephrotic children initially treated with corticosteroids. (From: Trompeter RS, et al. Long-term outcome for children with minimal change nephrotic syndrome: a long term follow-up. *Kidney Int* 1978;29:1215, with permission.)

after remission has been obtained with the use of prednisone. But the main problem once again is occurrence of relapse once the drug has been stopped. In a recent study from India (140), alternate-day levamisole therapy in steroid-dependent nephrotic syndrome reduced the relapse rate from a mean of 3 per year to 0.9 per year. Because of unavailability controlled or new large-scale studies, the exact place of levamisole and MMF in therapy of minimal change disease is unclear.

Relapses in the Longer Term. Although the tendency to relapse is prolonged, in most patients with minimal change nephrotic syndromes, it is not indefinite, and it is striking how few childhood patients are referred to adult clinics because they are still relapsing. Siegel et al. (142,143) studied 60 nephrotic children responsive to corticosteroids for a mean of 14.5 years, with particular emphasis on the subset of 20 relapsers who received cyclophosphamide treatment. Those so treated had all gone into remission, whereas just more than half (52%) of those treated with corticosteroids alone were still having relapses.

In Trompeter's long-term study of 183 children (31), only 10 children with a duration of disease from 15.0 to 20.5 years were still having relapses. By this time, they were 17 to 27 years old. Lewis et al. (144), from Manchester, England, followed 63 children with steroid-sensitive nephrotic syndromes for 10 to 21 years and again noted a tendency for relapses to become less frequent 4 years after diagnosis, and the percentage of patients relapsing fell steadily with time (Fig. 71-9). Only two children died: one from sagittal sinus thrombosis and one from septicemia. Niali (145) reported a patient who had relapses repeatedly between the ages of 4 and 53 years, at which point he died of tuberculosis.

Another observation of practical value is how long remission must elapse before a further relapse becomes unlikely. These data are influenced by the use of cytotoxic agents, but from other published data (145), it appears that it is rare for a relapse to occur after more than 2 years in remission and almost unknown after 7 years of remission. Nevertheless, reports of relapses occurring 10 to 25 years later have been published (146–149). These very late relapses are almost always responsive to treatment with corticosteroids, but an acquired resistance to treatment has been described (149).

It would be useful to be able to predict at the outset which children will have relapses frequently, but no features of the initial nephrotic syndrome appear to distinguish frequent relapsers with any certainty from those who will have no relapses or infrequent relapses. However, in the study of Trompeter et al. (31), there was a striking relation between persistent relapsing disease and the patient's age (Fig. 71-9). All children with persisting disease had onset before the age of 6 years, and the younger the child at onset, the more likely he or she was to have a prolonged relapsing course. Data from the ISKDC show that the pattern of frequent relapses is usually established soon after an attack, with two relapses within 6 months or three within 1 year being the pattern in most patients; these data are useful in deciding which therapy to use before long-term corticosteroids are used. In a recent retrospective study of nearly 400 children, long-term outlook was excellent if there was response in the first 8 weeks and remission was maintained for the next 6 months. These patients had very few relapses. A relapse in the first 6 months predicted further relapses in the next 3 years. Of the children who failed to achieve remission, 21% progressed to renal failure. Therefore, aggressive therapy may be required in slow responders (150).

Stature and Bone Characteristics in Children with Minimal Change Nephrotic Syndrome. The terminal height attained by children with prolonged periods of relapse and consequent prolonged treatment with corticosteroids is of particular interest. Trompeter's data (31) show that the height of 10 children who had relapses for 20 to 25 years was within 2 standard deviations (SDs) of expected, although most values were on the lower side of the mean. Similarly, Manchester data (151) showed a mean height in the 40th percentile in 80 patients, most of whom were relapsers, after 5 to 24 years; the height in only six patients was more than 2 SDs below the mean. In both studies, hypertension was absent and all patients had normal renal function. The results of the study of Berns et al. (143), from Yale, were not so encouraging; the mean height of those treated with corticosteroids alone was –0.93 ± 0.3 SD, and those who had received cyclophosphamide, –0.84 ± 0.4 SD, after a mean follow-up of 14.5 years. Padilla and Brem (152) studied growth velocity before and after treatment with cyclophosphamide. They found a doubling of growth velocity from 4.3 ± 1.3 to 8.7 ± 2.5 cm per year. The negative effects of corticosteroids on stature and growth velocity have been partially ameliorated

in a one-year pilot study using growth hormone in steroid-dependent children (153).

Focus has shifted in recent years to bone densitometry and markers of bone turnover. Mixed reports on bone-mineral density exist (154,155), but a recent, carefully controlled (and somewhat surprising) study suggests that bone mineral density is not affected by long-term glucocorticoids when controlling for factors such as age, sex, Tanner stage, and body mass index (BMI) (156). To date, there is no clear evidence that markers of bone turnover, such as urinary deoxypyridinoline and N-telopeptide, are clearly affected by corticosteroids in childhood nephrotic syndrome (155,157).

Evolution to Chronic Renal Failure in Corticosteroid-Sensitive Minimal Change Nephrotic Syndrome

It is very rare for a nephrotic patient with a minimal change lesion in biopsy tissue and demonstrated responsiveness to prednisone, with complete loss of proteinuria, to go into renal failure subsequently. Only a handful of such patients has been recorded in the literature (158–163). These exceptional patients are of great interest in influencing our ideas on the relationship between minimal change disease and focal segmental sclerosis (see the following discussion).

However, the experience of Tejani (161) is at variance with this general conclusion. Thirty-three of 48 children who had multiple relapses showed segmental sclerosis (164), IgM (165), or both in specimens taken at a second biopsy; in the remaining 9 children, minimal changes were still present, and third biopsies showed similar changes in 6. The reasons for this markedly poorer prognosis for frequently relapsing children, so much at variance with all other reports, is not clear, although the ethnic origins of this group of patients is different from the origins reported in most other published series (predominantly American Hispanic and black), and black adults frequently show progressive focal segmental lesions (166) (see later discussion).

Still rare but slightly more frequent is the patient who appears to have minimal changes on an initial biopsy sample but fails to respond to corticosteroids or cyclophosphamide (121). Often, these patients have a rather prominent mesangium in their biopsy specimens and sometimes mesangial deposits of IgM (see the following discussion). Material obtained at subsequent biopsies may show obvious lesions of focal and segmental glomerulosclerosis, and the question arises as to whether these lesions may have already been present in the kidney but not in the plane of the biopsy section or the entire biopsy specimen on the first occasion. Either way, the initial resistance to treatment with both agents is an important indicator of an adverse prognosis for renal function, and it exceeds in value the details of the histologic appearances in renal biopsy material.

Minimal Change Nephrotic Syndrome in Adults

Natural History Unmodified by Treatment. As with the childhood data, the bulk of information available represents the modified history of the condition after treatment including corticosteroids, cytotoxic agents, and cyclosporine. Data from untreated adult patients with minimal change syndrome are available from the preantibiotic era but are dominated by deaths from infections (34); data are also from the control cases from two controlled trials, that of the MRC in Great Britain (167) and that of the Interhospitals Study (168). Both series suggest that after 3 years, about two-thirds of patients with adult-onset disease will have gone into spontaneous remission, whereas one-third will still have proteinuria; there were no deaths among 28 untreated patients in the two trials.

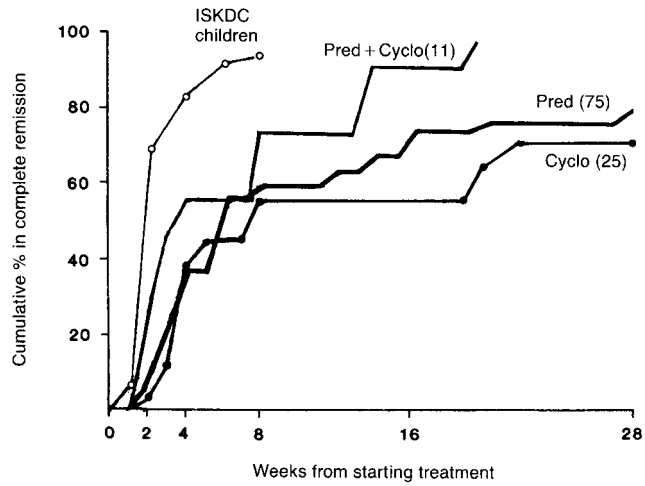


FIGURE 71-10. Rate of response (judged by loss of proteinuria) of patients with adult-onset minimal change nephrotic syndrome treated with corticosteroids, cyclophosphamide, or both, compared with data in children taken from the International Study of Kidney Disease in Childhood. Data from 89 adults in the Guy's Hospital series (32) are shown.

History Modified by Treatment. It is often forgotten that at all ages, minimal change disease accounts for one-fourth of adult nephrotic syndromes, but far fewer data are available from adults than from children with minimal change disease (32,104,121,168–170). The literature up to 1980 was reviewed by Coggins (168) and Broyer et al. (121), and Nolasco reviewed long-term follow-up of 89 adults with minimal change nephrotic syndrome (32). These cases, together with those summarized by Coggins (168), 40 cases of Korbet (104), 33 from Fujimoto et al. (169), 51 from Mak et al. (170), and 46 from Huang (171) form the bulk of the data.

Patients with adult-onset minimal change nephrotic syndrome responded more slowly and more incompletely than children to corticosteroids in all series (32,104,169,171) (Fig. 71-10). Therapy as long as 15 weeks may be required to achieve remission. This might reflect the lower dosage of prednisone normally in use on a body weight or surface area basis, when compared with pediatric use. However, the same effect was seen with cyclophosphamide, and here the dosage was identical (3 mg/kg per 24 hours for 8 weeks). In addition, a lower percentage of adult patients achieved a complete response after corticosteroid treatment. Fourteen (24%) of Nolasco's 58 patients whose proteinuria resolved completely never had a relapse, 32 (56%) had relapse on a single occasion or infrequently, and only 12 (21%) were frequent relapsers or steroid-dependent—a much lower proportion than that in children. The same gradient of a less frequent relapsing course with age was seen in patients with adult-onset minimal change syndrome, so after the age of 60, relapses were rare. Some authors also have seen this (32), in contrast to Zech et al. (172), who observed no relapses in this age group. As in children, a first relapse was uncommon after 2 years of remission and never occurred more than 8 years later.

In Nolasco's study, stability of remission after treatment with cyclophosphamide (36 patients) was superior to that found in children, with 60% of patients still in remission at 10 to 15 years. Only patients older than 55 years died, and only 5 of 15 deaths noted were related to the nephrotic syndrome, most in the period before 1975. At last follow-up, which was 1 to 24 years after onset, only 13 of 89 patients still had active disease and only 6 were still manifesting nephrotic syndrome, as were only 3 of 51 of Mak's series (170). As in children, cyclosporine has been used in frequently relapsing

and steroid-dependent adults (132,135,173), and has also been used successfully in combination with steroids as primary therapy (174).

Primary Resistance to Treatment, Histologic Changes, and Acquired Resistance to Treatment in Minimal Change Nephrotic Syndrome

As mentioned already, resistance to treatment with prednisone and cyclophosphamide is usually an ominous sign in patients with minimal change disease demonstrated by biopsy. It is easy to forget that the diagnosis of minimal change disease is a diagnosis of exclusion made with varying certainty according to whether the juxtamedullary glomeruli (most frequently and earliest affected by FSGS) are included in the biopsy material, and whether immunofluorescence or electron microscopy is available to distinguish a very early membranous pattern. Therefore, some of these patients have histologic changes, other than minimal changes, which have not been detected as such. However, within the scope of what is usually regarded as minimal changes are focal global sclerosis of glomeruli, sometimes accompanied by tubular atrophy and varying mild degrees of mesangial matrix expansion and hypercellularity. The former appears to have no effect on prognosis when present in children with a nephrotic syndrome (175). In adults, with increasing age, more and more global obsolescence of glomeruli is seen (176), together with tubulointerstitial damage and vessel changes that are probably the origin of these appearances. The interpretation of "minimal changes" in adults must take into account these alterations that occur with age.

Some initially resistant patients, however, fail to respond to corticosteroids in conventional doses or have only a diminution of continuing proteinuria but then respond promptly to cyclophosphamide. They probably represent a rather resistant group of patients who require more treatment, but are at one end of the responsive spectrum and have a good prognosis according to the ISKDC data (175); 23 of 31 initial nonresponders to prednisone (no loss of abnormal proteinuria by 8 weeks) lost their abnormal proteinuria eventually. In data from the Hopital des Enfants Malades (121,177), 46 (56%) of 83 children with nonresponsive nephrotic syndrome eventually lost their abnormal proteinuria.

The presence of mesangial expansion, proliferation, or both is a more controversial topic. In childhood nephrotic syndrome, there seems to be little doubt that as the mesangial volume increases (as judged by its area in fixed material in histologic preparations), a higher proportion of patients with hematuria, hypertension, initial resistance to corticosteroid treatment, and ultimate entry into renal insufficiency is seen (159,175,178–182). The presence or absence of IgM or minor mesangial deposits on electron microscopy does not, however, seem to alter the prognosis (178–181). Because fixation techniques affect the mesangial area, and there are no absolute criteria for normality, different observers may classify the same biopsy sample in different ways. However, a remarkable unanimity on the significance of mesangial expansion for prognosis has emerged, as outlined already. What is much less clear is what pathogenic significance the mesangial expansion, proliferation, or deposits may have. Some patients with only mesangial expansion in initial biopsy specimens, and no segmental lesions, will show segmental sclerosis on subsequent specimens. This type of observation leads many observers (113,121,159,182) to treat this group of patients as a variant of the minimal change–FSGS group, and the whole as a spectrum of presentations of a single disease. The older German American name, "lipoid nephrosis," is perhaps preferable for this group, because the name used by the Parisians (113,121,145) (idiopathic nephrotic syndrome) could equally apply to other forms of primary glomerular disease.

Late unresponsiveness to corticosteroid treatment in relapsing patients is another less common pattern of behavior in patients with minimal changes. In the ISKDC data (175), 43 of 311 initial responders acquired some degree of resistance to corticosteroid treatment during follow-up. Because only these troublesome patients usually have late biopsies, and, in many, focal segmental sclerosing lesions were found (180), it was supposed at first that this evolution was confined to resistant patients. Since then, it has become apparent that the biopsy material may remain normal in appearance in resistant patients (179) and show sclerosing lesions in responding relapsers.

The value of the presence of IgM in predicting outcome is controversial, mainly because different authors have asked different questions. There seems little doubt that within the group of patients with minimal changes on optical microscopy, the presence of IgM makes no difference to short- or long-term outlook (121,183,184). Mesangial proliferative glomerulonephritis with mesangial IgM deposition is dealt with in the following discussion.

Focal Segmental Glomerulosclerosis (FSGS)

The appearances of focal and segmental sclerosis are nonspecific (185–188), just as global glomerular sclerosis is nonspecific. Particularly in adults, this type of lesion can be found in a number of clinical and pathologic settings, such as in the presence of a reduced number of nephrons, hypertension and aging, human immunodeficiency virus (HIV) positivity, and/or intravenous drug abuse, as well as superimposed on almost any form of progressive glomerular disease. It has been suggested that the study of the details of the lesion may allow a consistent distinction between these different types of FSGS (189). Here, we exclusively discuss "primary" FSGS found as an isolated phenomenon in patients with proteinuria, usually of considerable dimensions and frequently accompanied by a nephrotic syndrome.

The relationship of this lesion to minimal changes has been much debated, and the controversy is reviewed elsewhere in detail (82,185) (Chapter 64). In short, it is not likely that we will be able to decide whether FSGS and minimal changes represent different aspects of what is pathogenically the same lesion or two distinct diseases unless and until we understand the pathogenesis of either; at the moment, despite much speculation and experimentation, this remains unknown, although a humoral factor that is possibly identical in the two appearances seems to be involved in the induction of proteinuria in both. However, data from patients with recurrent disease who underwent transplantation (190), the occasional entry of patients with a completely responsive minimal change lesion into renal failure already mentioned, the more frequent appearance of focal segmental lesions in patients with mesangial expansion, and the occasional response of even the most severe forms of FSGS to intense immunosuppression all support the thesis that FSGS and minimal changes represent aspects of the same or similar disease (121,159,185,187).

It has became obvious that the FSGS lesion is much more common in black nephrotic patients than in white patients in the United States, accounting for up to one-half of black adult nephrotic patients (166) and one-third of children (191), compared with 25% of adult whites and 10% of white children. In many of these patients, the FSGS is of what has been called the "collapsing" variety (186,187,192,193). Although it has become usual to include collapsing glomerulopathy as a variety of FSGS, it is in fact a diffuse disease in almost all instances and perhaps deserves its own category.

TABLE 71-1

INITIAL RESPONSIVENESS OF NEPHROTIC CHILDREN WITH FOCAL SEGMENTAL GLOMERULOSCLEROSIS TO CORTICOSTEROID TREATMENT

Author	Year	Reference no.	Number treated	No. responded with loss of proteinuria	Percentage
Siegel et al.	1974	(180)	22	16	73
Newman et al.	1976	(596)	15	9	60
Gubler et al.	1978	(236)	85	21	25
Tarshish et al.	1983	(150)	16	5	31
Mongeau et al.	1981	(218)	31	6	19
Arbus et al.	1982	(195)	51	19	37
Schärer et al.	1982	(627)	51	10	20
South West Pediatric Nephrology Study Group	1985	(203)	56	16	16
Trompeter (Guy's)	1987	(194)	62	10	16
Total			389	112	29

Clinical Picture

Short-Term Behavior with Treatment. Complicating the understanding of corticosteroid responsiveness in children is the reality that steroid-sensitive patients with FSGS may never receive a biopsy. Therefore, case series of FSGS in children may represent a relatively steroid-resistant group. A proportion of children with FSGS with a full nephrotic syndrome respond to treatment with the conventional 6 to 8 weeks of prednisolone, similar to those with minimal change (Table 71-1). In the published literature on 332 children, 96 (29%) responded with complete loss of proteinuria (194); many of these patients subsequently had a relapsing course, just as in the minimal change nephrotic syndrome, but some became corticosteroid-resistant later (195). In more recent series, which tend to include either higher doses or longer courses of steroids, the rate of complete remission has been higher (196), with reported completer remission rates as high as 65% (197). However, a substantial price of toxicity (growth retardation, cataracts) may be paid (197).

Whether adults can achieve high rates of sustained remission is debated in the literature (198). For example, of 39 nephrotic patients reported by Beaufils (62), 6 went into remission, but 2.5 to 10.0 years later, and only 3 of 26 treated with corticosteroids alone and 1 each treated with indomethacin and chlorambucil went into remission. In the series of Cameron (63), only 2 patients responded with loss of abnormal proteinuria, of 18 adults treated with corticosteroids alone. In an analysis of the literature (199), 17% of adult nephrotic patients with FSGS were found to have lost abnormal proteinuria completely. However, with evolution in steroid dose and duration of therapy, these pessimistic outcomes may need to be revised. Even in groups with high proportions of African Americans and nephrotic-range proteinuria, complete response rates exceeded 30%, and between 50% and 70% of patients achieved complete or partial response (196,200,201).

Almost all studies on nephrotic patients with FSGS support the idea that the prognosis of patients with complete resolution of proteinuria in response to corticosteroids is much better than the prognosis for those who do not show this initial response (Table 71-2). In contrast, however, Tejani et al. (202) and the South West Pediatric Nephrology Study Group (203) reported that the outcome in terms of renal failure was no different in those who responded initially and those who did not, so the prognosis even for those who respond must remain guarded. Both of these latter series contained, in contrast to the rest of the literature, a high proportion of black and Hispanic children. Ingulli and Tejani (191) demonstrated that as in black adults (166), FSGS is more common in black or Hispanic children with a nephrotic syndrome (57 of 177), and that 78% of this group evolved to renal failure compared with only 33% (4 of 12) of white children. However, only 30% of white children respond to initial treatment with corticosteroids, so the difference may not be as large as these authors suggest.

Therefore, the response to an initial treatment with *corticosteroids* may be a valuable indicator of likely prognosis. It also follows that the overall survival of a group of nephrotic patients with FSGS may depend on the proportion of steroid-responsive patients within the population under study. Finally, the data support the advisability of giving *all* nephrotic patients with FSGS a trial of prednisone for a minimum of 3 months; patients should not be considered steroid-resistant until a 6-month trial has been completed (200). Ponticelli et al. (201) pointed out that it may take much longer for proteinuria to resolve than with patients with minimal change disease, which highlights the difficulty of defining "corticosteroid resistance."

It is less clear whether it is worth giving *cyclophosphamide* thereafter to those who do not respond. The ISKDC, performed

TABLE 71-2

LONG-TERM OUTCOME IN NEPHROTIC PATIENTS WITH FSGS IN RELATION TO INITIAL RESPONSIVENESS TO CORTICOSTEROIDS[a]

	Responders[b]	Nonresponders	Total
All	49 (20%)	196 (80%)	245
In CRF	3 (10%)	49 (53%)	52 (45%)
"Death"[c]	2	55	57

FSGS, focal segmental glomerulosclerosis; *CRF*, chronic renal failure.
[a]Summary of literature on adults and children 1969–1978 and personal cases.
[b]Response equals complete loss of proteinuria, usually within 8 weeks.
[c]Death equals dead, dialyzed, or transplanted; mean follow-up, 6.4 years.
(Reprinted from: Cameron JS. The problem of focal segmental glomerulosclerosis. In: Kincaid-Smith P, D'Apice AJF, Atkins RC, eds. *Progress in Glomerulonephritis*. New York: Wiley; 1979:209 with permission.)

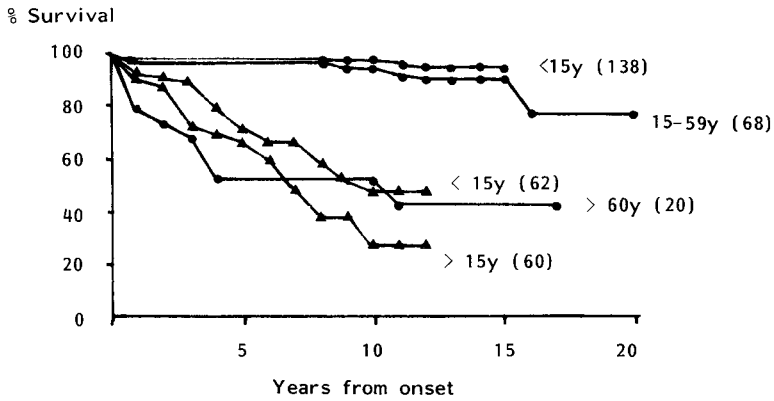


FIGURE 71-11. Survival of nephrotic children or adults with either minimal change (●) or focal segmental glomerular sclerosis (▲). (From: Cameron JS, Glassock RJ. The natural history and outcome of the nephrotic syndrome. In: Cameron JS, Glassock RJ, eds. *The Nephrotic Syndrome.* New York: Marcel Dekker Inc; 1987; unpublished data are from the Guy's Hospital series [32], with permission.)

in the 1970s, reported in 1996 that addition of cyclophosphamide to alternate-day prednisone in children resistant to 8 weeks corticosteroid therapy provided no additional benefit (204). Tune et al. used alkylating agents in 78% of children with FSGS and achieved a remission rate of 70% (197). Others have described high remission rate followed by a disappointingly high relapse rate (205), as well as low initial response rate (206). Conclusions from these scattered series are difficult to draw in children. For adult-onset patients, there is a lack of any randomized trials or even long-term prospective studies using cytotoxic agents (207). Ponticelli et al. provide some information (201). In 65 patients receiving cytotoxic agents as first-line therapy, 40% achieved a response (the majority partial remissions), and 6 of 11 patients with steroid-resistance achieved partial or complete remission. As for chlorambucil, Mendoza et al. (208) reported favorable results in white children treated with an intensive regimen of *methylprednisolone* and *chlorambucil,* but this was not confirmed in a mainly black population (205). Therefore, for both children and adults with FSGS, there is marginal evidence for benefit of cytotoxic agents after a 12- to 16-week course of steroids (207).

Cyclosporine has been used extensively in children (121,209,210) and adults (132,209) with FSGS, usually in association with corticosteroids. A higher response rate than with either corticosteroids alone or cyclophosphamide has been reported, although a reduction in urinary protein loss (see previous discussion) has often been used as a criterion for success, and this may be a purely hemodynamic event. In an analysis of the literature, 29 (27%) of 109 corticosteroid-resistant adults and 11 (25%) of 44 corticosteroid-resistant children showed complete remission (132). This benefit was confirmed by Ponticelli et al. (209): 13 of 22 treated patients attained remission of the nephrotic syndrome after 1 year of cyclosporine treatments, compared with 3 of 19 control subjects. A recent randomized controlled trial in adults supports these conclusions (211). Despite high relapse rates, a substantial lowering of the risk of active disease remained at 4 years. The French Society of Pediatric Nephrology noted remission in 42% of 65 steroid-resistant children treated with cyclosporine (210), whereas Lieberman and Tejani noted a remission rate of 66% (212). The addition of chlorambucil to a cyclosporine-containing regimen has not appeared helpful (213). In summary, there is a case for cyclosporine rather than cyclophosphamide as second-line therapy in corticosteroid-resistant patients with FSGS.

The possibility that mycophenolate mofetil (MMF) may have a future in the treatment of FSGS deserves mention. In a study of 46 patients with primary glomerular disease treated with MMF, 18 patients with FSGS experienced a decline in urinary protein excretion without a change in renal function (214). Smaller studies have also demonstrated activity against the disease (215).

Long-Term Outcome with Treatment. In adults, as with other histologic appearances, the prognosis is better for patients with FSGS if urinary protein excretion remains below the nephrotic range (62,216). Few if any patients with subnephrotic proteinuria progress to renal failure within a decade. There are only scant data on children with FSGS who are not nephrotic, perhaps because this is a rather rare finding. In the series of Yoshikawa (217), there was no difference in outcome with degree of proteinuria.

The actuarially calculated 10-year survival rates for renal function in all patients with FSGS have been reported as 38% ± 12% for adults (62) and as 45% ± 21% by Trompeter (*unpublished data,* 1978) for children. In Figure 71-11 is shown the survival curves for nephrotic adults and children with either minimal change disease or FSGS in Guy Hospital. The two curves of 60 adults and 62 children with FSGS are not statistically different. Mongeau et al. (218,219) also emphasized the better prognosis of the children in their series with FSGS, with an overall renal survival rate of 56% at 20 years, suggesting the difference resides with the type of patient referred to some centers. Much higher rates of ultimate remission have been reported in intensively treated adults by Ponticelli et al. (201), but it must be remembered that in this series, only patients with normal or near-normal renal function were included. Recently, Chun et al. reported a 10-year renal survival of 67% in a cohort of patients mostly treated with corticosteroids; ESRD was uncommon in those who achieved either a compete or partial remission (98).

"Malignant" Focal Segmental Glomerulosclerosis. Brown et al. (220) and Saint-Hillier et al. (216) drew attention to a group of patients whose condition has been called *malignant FSGS.* These patients demonstrated complete resistance to corticosteroids and conventional cytotoxic therapy. Furthermore, they usually had torrential urinary protein loss, with frequent hypovolemic episodes, and were almost always younger than 25 years. These patients had a relentless course to renal failure within only 1 to 4 years from onset of the nephrotic syndrome, maintaining the proteinuria into terminal renal failure and even on to dialysis, so bilateral nephrectomy or renal infarction may be needed (33). Recurrence of equally torrential urinary protein loss in patients with transplanted kidneys may be immediate, with later appearance of typical lesions of FSGS in the allograft and graft failure (163,190). The term *malignant FSGS* has been used also to describe the severe form of FSGS associated in black patients with glomerular "collapse" and very rapid entry into renal failure (186,192,193). When compared with age-matched controls with classic FSGS, patients with the collapsing variant exhibited more rapid progression with a time-course of only 13 months to end-stage renal failure compared with 62.5 months (221).

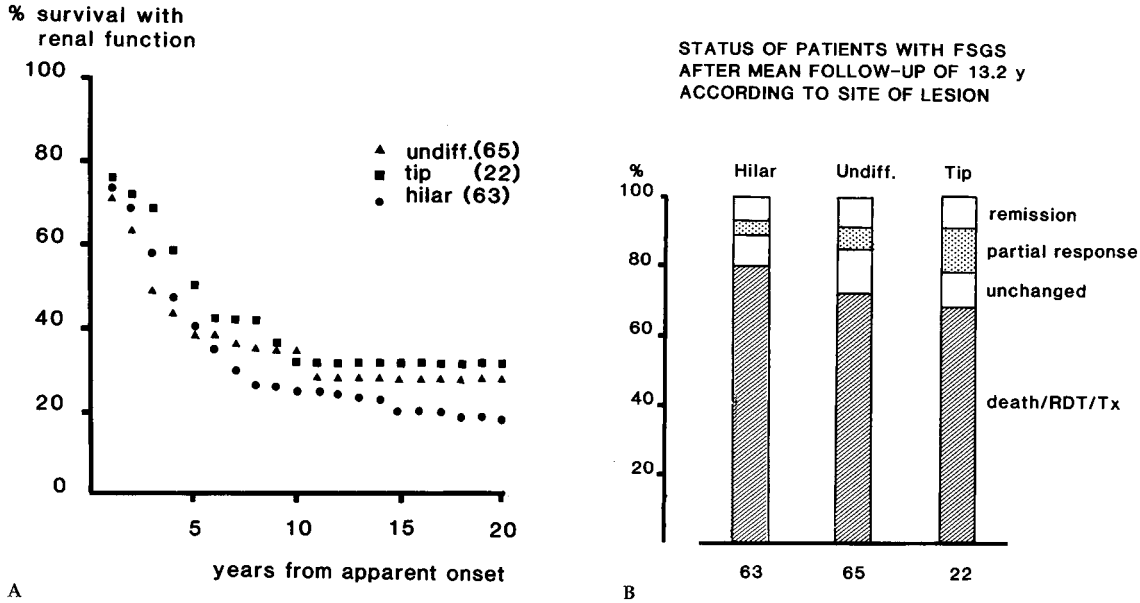


FIGURE 71-12. Data on nephrotic children and adults showing focal segmental glomerular sclerosis in their renal biopsy specimen, seen at Guy's Hospital during a 20-year period. Survival curves (**A**) and status at most recent follow-up (**B**) are shown for 22 patients in whom the site of the lesion could be localized to the glomerular "tip," 63 patients in whom the lesion was perihilar, and 65 patients in whom on the sections available no statement as to localization could be made. Patients with both tip and perihilar lesions were counted as perihilar. There is no difference in outcome between the groups. (From: Chaudhury N, Cameron JS, *unpublished data,* with permission.)

There is also a problem of recurrent FSGS in patients who have received a renal allograft (222–224). This has been attributed to the presence of a circulating factor that was identified using an *in vitro* assay measuring the permeability of rat glomeruli. It has been suggested that prophylactic use of plasmapheresis prior to transplantation may reduce early recurrence by 50% (225). Treatment of established recurrent FSGS with plasmapheresis may also be beneficial; predictors of success may include early therapy (226).

Histologic Pattern and Pathologic Markers in Relation to Outcome

Almost all biopsy tissue from patients with FSGS shows some degree of tubular atrophy, so there is not much use in determining prognosis within the group. Severe tubular atrophy is usually not a good prognostic sign. However, as in almost all other forms of progressive glomerulopathy, interstitial fibrosis emerges from multistep analysis as the major histologic determinant of outcome (227). The clinical correlate of this severe tubular disease is the finding of glycosuria, aminoaciduria (228), and excessive losses of β_2-microglobulin in the urine (229), which may be a clinical indicator of the presence of FSGS. Another important new marker of poor prognosis and poor response to therapy is an elevated fractional excretion of IgG, which is thought to represent a more severe alteration in permselectivity of the glomerular filtration barrier (230).

The lesions of FSGS have a characteristic distribution, often affecting either the perihilar area, known as *classic FSGS*, or the pole of the glomerulus opposite the hilus—named the *glomerular tip lesion* (231–235). Ito et al. (232) found that almost all those children with perihilar lesions progressed to renal failure, whereas those with "tip" lesions adjacent to the exit of the tubule from Bowman's capsule did well. These interesting observations were not confirmed by others (233). Guy's Hospital data show no difference in the rather gloomy long-term outcome of pediatric and adult patients, whatever the site of the lesion (Fig. 71-12). This area of study remains controversial, as described by Chun et al. (98), who presented a series of 87 adults with nephrotic syndrome and biopsy-proven FSGS. Patients with classic, cellular/collapsing, and tip lesions had similar prognosis and response to steroid therapy regardless of the lesion. Even those with collapsing lesions responded to steroids in 64% of cases; 80% of the collapsing patients who responded were free of ESRD at 10 years. It has also been suggested, with more general agreement, that mesangial prominence is an adverse finding (182,236,237), but again, there are data that do not agree with this conclusion (220). Finally, precocious and prominent afferent arteriolar hyalinosis has been noted in patients with a poor outcome for renal function (220), an observation that has been confirmed (238).

Membranous Nephropathy

Membranous nephropathy is a well-documented histologic appearance, presenting in general with proteinuria and affecting principally middle-aged and older adult men (Chapter 63). The short-term (less than 5-year) outcome of membranous nephropathy was extensively documented in adults during the late 1960s and 1970s; the data are reviewed elsewhere (185). In its idiopathic form, membranous nephropathy is a relatively indolent disease in most patients, with few patients entering remission or progressing to renal failure within 5 years of apparent onset; although in contrast to this statement, several observers reported 40% to 50% of patients dead or in renal failure within 5 to 6 years. Others noted a relatively benign outcome within the first 5 years, with the results in other series falling somewhere between the two extremes.

All of these older data have several handicaps. First, patients with idiopathic membranous nephropathy often were not separated from those with identifiable precipitating factors, some of which (e.g., drugs) might improve the overall prognosis

and some (e.g., malignancies) might make it worse. Second, a variable proportion had been treated for varying times with a number of agents believed to affect the outcome of the condition. Third, patients with only mild proteinuria were often mixed with those showing a full nephrotic syndrome. Fourth, the numbers in most of these series were small. Finally, the effects of late referral of patients with progressive disease were not considered.

Outlook in Untreated Patients

Over the past two decades, data that avoid some of these criticisms have become available on groups of patients. Noel et al. (239) described the outcome of a group of patients with idiopathic membranous nephropathy, none of whom had received treatment that was believed to be specific. The actuarial survival rate at 10 years was estimated at 75%. Forty-eight percent of the patients were women (now known to do better, as discussed later) and only 78% had a nephrotic syndrome, which again is an indicator of poor prognosis. Donadio et al. (240) followed 140 patients (47 women, 83% nephrotic) for up to 15 years, 89 of whom had received no treatment. They made the valuable comparison between the expected survival of this middle-aged and older adult population of patients with that expected for the population as a whole: There was no difference for the whole population of patients (75% at 10 years) and no difference whether treatment was given or not. The Bergamo group (241) reviewed another large untreated group of 100 patients, of whom 63 were nephrotic, 32 were women, and none had been treated. The 8-year survival rate for renal function was 73% ± 7%, and 42% were in remission and only 30% were still nephrotic. The relatively good outlook reported in these articles contrasted with the control, untreated group in the Interhospitals Study of the nephrotic syndrome reported from the United States in 1979 (95), in which 11 (29%) of 38 untreated patients reached a renal failure endpoint (plasma creatinine concentration of more than 4 mg/dL) within 48 months of entry.

Davison et al. performed a three-center retrospective analysis of 62 untreated patients with idiopathic adult-onset membranous nephropathy, all of whom were nephrotic (242). Of 62 untreated idiopathic nephrotic patients followed for a mean of 4.5 years, only 32 (50%) showed a deterioration in renal function. The rate of change of plasma creatinine concentration was only approximately linear for each patient, but the slopes of the data vary greatly from patient to patient. Three patients went into renal failure within 2 years of onset of a nephrotic syndrome, as previously described by Coggins (168) in untreated patients, and by the end of 5 years, only 9 (14%) had started dialysis. Data on untreated patients are available from four further prospective controlled trials in nephrotic patients, from Italy (243,244), Canada (245), and the United Kingdom (164). A total of 190 control patients were entered into these trials using roughly the same criteria (data from the Canadian trial are included here only for those patients with a nephrotic syndrome). After a follow-up averaging just less than 5 years, 18 patients (11%) had actually started dialysis, and at least 9 more patients (6%) had plasma creatinine concentrations of more than 400 μmol/L (data not available in the report by Ponticelli et al. (243).

Other series of untreated patients have also been studied and reported from individual units: Donadio (240), 89 patients; Honkanen et al. (246), 19 patients; Kida et al. (247), 45 patients; Murphy et al. (248), 79 patients; and a large collected series (249). Although not all the patients in these studies had a nephrotic syndrome, these series in general confirmed the apparently better prognosis—at least during the first decade—found in recent studies of untreated membranous nephropathy.

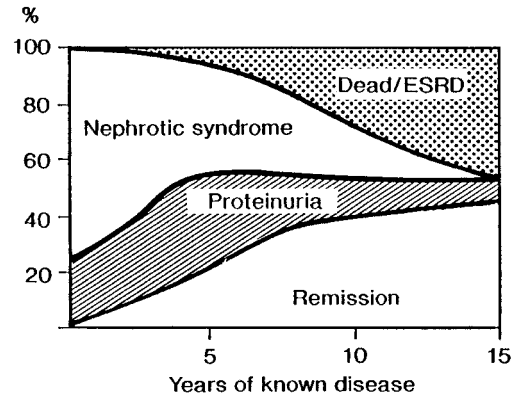


FIGURE 71-13. The course of membranous nephropathy, summarized from J.S. Cameron's data and the literature up to 1978. The evolution is relatively slow, and remissions and entry to uremia may occur more than 15 years after onset, although some patients evolve much more rapidly. *ESRD*, end-stage renal disease.

Estimated 10-year renal survival rates were 77% (249), 81% (248), 64% (240), 82% (246), and 92% (247). Figure 71-13 has thus been modified from that in previous editions of this book, in light of these more recent data, to show that more than 40% of patients with adult-onset membranous nephropathy will go into remission and only about 10% enter into renal failure or die within 10 years. Although some 10- to 15-year data are available (250–252) from long-term studies of patients studied earlier, which suggest a 50% renal failure/mortality rate at this time, little 20-year outcome is available. An exception is a recent national report on Japanese patients with idiopathic membranous nephropathy (253); the 20-year survival was 60.5%, despite excellent renal survival at 10 years (90%) and 15 years (81%). It is thus likely that only 40% of all patients with membranous nephropathy eventually experience renal failure, even if a nephrotic syndrome is present to begin with.

Relapses

Another change in the evaluation of the outcome in membranous nephropathy has been the realization that relapses and a relapsing course are not that rare. For example, of the 141 patients who went into remission in the controlled trials just cited (95,164,243,245)—either spontaneously or in apparent response to treatment—46 subsequently had relapse; of these, only 8 were among 39 untreated patients, perhaps suggesting a higher relapse rate in patients with corticosteroid-induced remissions. Passerini et al. (56) examined the prognostic implications of complete remission of proteinuria after treatment, even if it returns. In 33 patients followed for a median of 8 years, none showed an abnormal plasma creatinine concentration, although half had a relapse, 9 with a full nephrotic syndrome. Using Kaplan-Meier statistics, they estimated that two-thirds of those who go into remission will have a further relapse of proteinuria before 10 years have gone by.

Problems for Trials of Treatment of Membranous Nephropathy

The realization that the prognosis for membranous nephropathy with regard to renal function is better than formerly supposed presents formidable problems in the design and conduct of therapeutic trials. First, the rather good survival and spontaneous tendency toward remission make it difficult to show improvement in terms of renal or patient survival, at least within the usual length of trials in practice. Therefore, trial endpoints

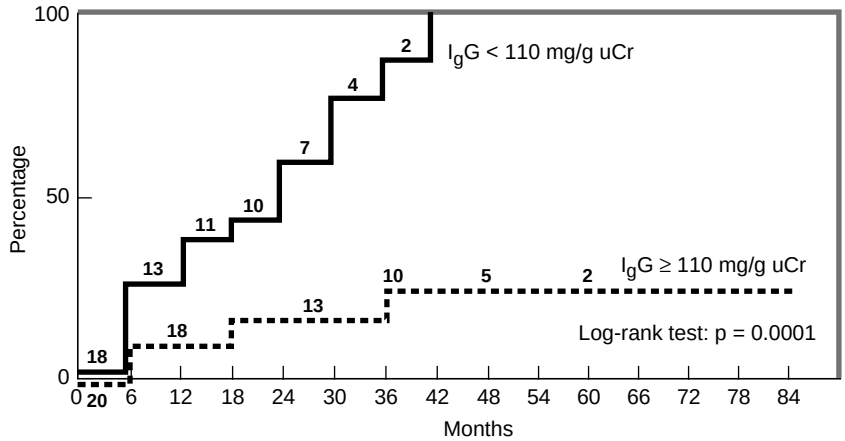


FIGURE 71-14. In a study of 38 patients with membranous nephropathy, nephrotic syndrome, and normal renal function (sCr, 0.99 ± 0.20 mg/dL), probability of complete or partial remission was highly dependent on urinary IgG excretion values less than 110 mg/g uCr versus 110 mg/g uCr or greater. (From: Bazzi C et al. Urinary excretion of IgG and alpha(1)-microglobulin predicts clinical course better than extent of proteinuria in membranous nephropathy. *Am J Kidney Dis* 2001;38:240, with permission.)

must depend on levels of renal function and urinary protein excretion. Second, any trial that randomizes patients at apparent onset will result in the control group including three or four of every 10 patients who will have a remission spontaneously within 5 years and thus can only have side effects without benefit from the treatment. Thus, it may be preferable to treat patients with nonspecific therapy (ACE-inhibition, hyperlipidemic therapies, blood pressure control) for 6 months prior to cytotoxic therapy or enrollment in a clinical trial.

Predictors of Poor Prognosis

Possible interventions and trials in membranous nephropathy are discussed in detail in Chapter 63. In this discussion, we consider which criteria might allow us to predict entry to renal failure at or near onset in idiopathic membranous nephropathy (252).

Clinically, several features seem to carry a poorer prognosis in univariate analysis: male sex (242,254–256), increasing age (242), urinary protein loss in excess of the nephrotic range (66,239,242,255), reduced renal function (239,242,249, 256,257), and raised blood pressure (240,249,255). Of these, persistent proteinuria is probably the strongest predictor of renal failure in multivariate analysis; initial creatinine clearance and decline in creatinine clearance have also been demonstrated as independent predictors (66). Kobayashi et al. (257) considered in detail the prognosis of non-nephrotic patients with membranous nephropathy. Of 123 patients with membranous nephropathy and subnephrotic proteinuria, including 18 of their own patients, only 13 (10%) had gone into renal failure over a mean follow-up of 4 years, even though 40 had later developed a full nephrotic syndrome. Twenty-five (22%) were in complete remission. In a recent update comparing Canadian data to those of other populations in Italy and Finland, a predictive model has been outlined and validated (258). The most important factor determining long-term outcome in patients with membranous nephropathy was found to be the highest level of proteinuria sustained over 6 months. The model was able to predict patients at high risk of progression with an accuracy of more than 85%, despite differences at baseline.

Genetically, in white populations a strong association between membranous nephropathy and the whole major histocompatibility complex haplotype HLA-A1, B8, DR3, and each of its component genes has been described, whereas in Japanese populations, the association is with HLA-DR2 (Chapter 63). Despite some earlier data that suggested correlations between outcome and major histocompatibility complex type among white populations (259), the largest study to date, that of the British MRC trial (164), failed to reveal any associations between either HLA or Bf allotypes and outcome in more than 100 patients with nephrotic syndrome and idiopathic membranous nephropathy.

Urinary markers have also been explored as predictors of eventual renal failure in idiopathic membranous nephropathy. In a prospective study, urinary excretion of beta-2 microglobulin and IgG were found to have sensitivities of 88% and specificities of 88% and 91%, respectively, for the development of eventual renal failure in a group of patients with nephrotic syndrome and serum creatinine (SCr) less than 1.5 mg/dL. When the two tests were combined, specificity reached 97% (260). Similarly, excretion of N-acetyl-beta-glucosaminidase was also found to be superior to total protein excretion in predicting prognosis and response to treatment, as was IgG and beta-2 microglobulin (24, 25, 261). These studies bring optimism to the idea that prognostic information as well as selection of patients for treatment may be possible with noninvasive urinary studies (Fig. 71-14).

Patients with milder (class I and II) glomerular disease (239,249,251) as determined by histopathologic evaluation have been suggested to do better than those with class III and IV lesions, but several other groups failed to find any correlation (242,250,256). Bohle et al. (262) and others (250) noted that interstitial lesions best predicted a poor outcome, and more recently Wehrmann et al. (249) performed a multivariate analysis confirming that tubulointerstitial lesions predict a poor outcome better than any other criterion; Ponticelli et al. (244) came to the same conclusion. In addition, if phenotype of the interstitial infiltrate is determined, the numbers of both monocytes and helper T cells in the interstitium predict the GFR after 5 years of follow-up (263).

Some patients show focal sclerosing lesions (264), which may have some value in predicting outcome. Vascular lesions have also predicted outcome in a univariate analysis with long-term follow-up (250). Finally a few patients with membranous nephropathy develop crescentic nephritis (265–267), with or without circulating antiglomerular basement membrane (anti-GBM) antibody. A poorer prognosis is experienced by these unfortunate patients, but luckily they are rare.

As in most glomerular diseases, in membranous nephropathy, once renal function begins to fall, it usually continues to decline at a varying rate over the following years until ESRD is reached (242). However, one or two circumstances may lead to acute reversible declines in renal function; these include renal venous thrombosis, the appearance of a spontaneous or drug-induced acute interstitial nephritis, and the formation of new crescents, as just discussed. In the course of the tri-hospital study (242), it became apparent that some patients with

membranous nephropathy might show a reversible decline in function without any of these circumstances being present; in the MRC trial (164), six patients showed remarkable slowing in renal function loss and one showed reversal of decline without any identifiable event or intervention such as control of hypertension, treatment with immunosuppressive agents, or use of low-protein diets. This obviously needs to be considered when evaluating the data of serial renal function studies in relation to late treatment interventions.

Treatment History

The treatment of membranous nephropathy is discussed in detail in Chapter 63; therefore, only salient aspects are discussed here. Whether major differences in the outcome of adult nephrotic patients with idiopathic membranous nephropathy emerge from treatment with cytotoxic agents and corticosteroids remains contentious (101,268–272).

Membranous Nephropathy in Children. In white children, membranous nephropathy is rare, accounting for only 1% of nephrotic syndromes in childhood in the unselected data of the ISKDC. However, in some areas of the world, particularly in Asia and Poland, membranous nephropathy in association with hepatitis B virus carriage accounts for 5% to 15% of nephrotic syndromes in childhood (92,271,273).

Therefore, 40% of children have some associated disease, principally infections (273), contrasting with only about 20% to 25% of adults (274,275). Because patients with such forms of membranous nephropathy usually do well, it is not surprising that the overall prognosis for children with membranous nephropathy is good; however, even in children with the apparently idiopathic form, the outlook is much better than it is in adults, with less than 10% going into renal failure. Such an evolution is almost unknown with onset before the age of 10 and rare with onset before the age of 20 (274). One interesting observation is that children with membranous nephropathy never, and adolescents very rarely, develop renal venous thrombosis.

Membranous Nephropathy in Adults. About one-third of adult patients with membranous nephropathy reach end-stage renal failure. What can be done to alter this outcome? Based on a critical review of the literature, high-dose oral steroids should not be used as sole therapy because they are ineffective in either producing a sustained remission of the nephrotic syndrome or preserving renal function (101,272). The use of azathioprine is not associated with any significant benefits and is therefore not justified. The alkylating agents cyclophosphamide and chlorambucil are efficacious in the management of membranous nephropathy (272,276,277). Because of their long-term toxicities, use of these drugs should be reserved for patients with a moderate-to-high likelihood of progression to ESRD (severe proteinuria and renal insufficiency). Alkylating agents should always be used with steroids. Cyclosporine has a growing role in the treatment of membranous nephropathy; some have suggested that it may be more effective than cytotoxic agents in patients at highest risk of progression, that is, those with renal failure and/or greater than 8 g of protein per/day (207,278). Other agents, such as MMF and rituximab, will be discussed in Chapter 63; long-term results for these agents are not yet available (279–282). Based on the decision model by Sarasin and Schifferli (52), prophylactic anticoagulation is suggested to be beneficial in membranous nephropathy patients with persistent nephrotic syndrome and albumin levels of less than 2g/dL. However, no randomized controlled trial has been done to study the effect of anticoagulation in these patients.

PROGNOSIS OF DISEASES ASSOCIATED WITH GLOMERULONEPHRITIS

IgA-Associated Nephropathy (Berger's Disease)

Despite almost 40 years of research, we still do not know whether the IgA present in the mesangium plays any role in glomerular injury or what the significance of the raised serum IgA concentrations, the IgA-containing immune complexes, or IgA autoantibodies in the serum may be (283). In addition, it must be noted that classifying patients with glomerular disease by the presence or absence of predominant IgA on immunohistology cuts across both clinical presentation and conventional histologic classifications, as outlined already here and elsewhere in this book. There is great variability in disease progression for patients who are given the diagnosis of IgA nephropathy. On the one hand, this variability of outcomes raises doubts regarding the current definition and diagnosis of IgA nephropathy. On the other hand, such diverse outcomes also emphasize the need to identify those patients with a worse prognosis whenever possible.

Because IgA-associated nephropathy commonly presents without symptoms through abnormal findings on urine tests, there is considerable possibility for variation in any population of patients under study, for example, depending on whether systematic urine testing of schoolchildren has been performed, as in some Japanese reports, or testing of the urine in all army recruits, as in Singapore. Furthermore, the local criteria for performing a renal biopsy becomes critical; if it is policy simply to watch patients with isolated hematuria, then a group with a generally favorable prognosis will be excluded. If in contrast all such patients undergo a biopsy, then the group of patients with isolated symptomless hematuria and isolated mesangial IgA will be included and "improve" the prognosis of that particular series.

The prognosis of IgA nephropathy has been examined in considerable detail, perhaps because of the large number of cases available worldwide (72). A series of conferences (held in Milan in 1983) was devoted to the subject of IgA nephropathy (284), and other useful reviews have been published (72,285–289) including two meta-analyses (287,289). The reader is also referred to Chapter 61 of this book.

IgA Nephropathy in Adults

All reports describing IgA-associated disease suggest a condition of indolent evolution toward healing or renal damage, although an aggressive course associated with extensive crescents may be seen in a few patients (290,291). In general, the entry into renal dysfunction and finally failure is slower in IgA nephropathy than in any of the other diseases discussed in this chapter (289,292), occurring over a time scale of 30 years or more (Table 71-3), in contrast to the 5 to 15 years that is more usual in other glomerulopathies. This may reflect earlier diagnosis in patients with macroscopic hematuria, but many patients with the slowest evolution into renal failure present with symptomless urinary abnormalities on casual urine testing.

The long-term outcome of several large series of adult patients with IgA nephropathy has been described (286,293–297) and the literature summarized (287,289). Schena (289) highlighted the differing proportions of patients with hypertension, already elevated plasma creatinine concentrations, macroscopic hematuria, and profuse proteinuria noted in differing

TABLE 71-3

ACTUARIAL RENAL SURVIVAL AT 10 YEARS AND CLINICAL FEATURES AT PRESENTATION IN LARGE POPULATIONS OF ADULT PATIENTS WITH IgA NEPHROPATHY ACCORDING TO THE MOST ACCURATE STUDIES IN THE LITERATURE

		Clinical features at presentation							
Reference	Country	No. of patients	Mean age at presentation (y)	High serum creatinine (%)	High blood pressure (%)	Proteinuria >3 g/24 hr (%)	History of macroscopic hematuria (%)	Mean duration of follow-up (mo)	Actuarial renal survival at 10 years (%)
Europe									
D'amico et al. (1986) (298)	Italy	365	29	24	36	7	55	79	85[a]
Beukof et al. (1986) (303)	The Netherlands	75	24	—	37	—	46	92	84[a]
Droz et al. (1984) (297), Noël et al. (1987) (628)	France	280	—	—	6	10	37	>60	85[a]
Velo et al. (1987) (629)	Spain	153	22	—	—	1	78	>60	81[a]
Bogenschütz et al. (1990) (630)	Germany	239	—	34	19	—	26	59	81[b]
Rekola et al. (1989,1990) (631,632)	Finland	209	25	16	11	1	64	76	83[c]
Alamartine et al. (1991) (304)	France	282	28	2	9	3	27	96	94[a]
Johnston et al. (1992) (296)	UK	220	30	28	26	32	—	65	83[a]
Australia									
Nicholls et al. (1984) (305)		244	32	36	43	6	39	60	87[c]
Ibels et al. (1994) (633)		121	39	36	31	16	40	107	93[a]
Asia									
Woo et al. (1986) (294)	Singapore	151	27	6	33	4	24	65	82[a]
Kusumoto et al. (1987) (320)	Japan	87	27	—	31	15	—	114	80[a]
Katafuchi et al. (1994) (308)	Japan	225	32	36	22	16	20	48	74[c]
Yagame et al. (1996) (287)	Japan	206	30	—	—	—	—	110	87[c]
Koyama et al. (1997) (634)	Japan	448	>10 in 95%	19	29	3	24	142	85[a]
United States									
Wyatt et al. (1984) (635)		58	27					>60	78[a]
Radford et al. (1997) (636)		148	39	59	47	30	—	45	67[c]
Haas et al. (1997) (637)		109	~40	Mean = 2.2 ± 1.9 mg/dL	49	33	35	>18	57[a]

[a] After the first manifestation.
[b] Not specified.
[c] After the biopsy.
(Reprinted from: D'Amico G. Natural history of idiopathic IgA nephropathy: role of clinical and histological prognostic factors. *Am J Kid Dis* 2000;36(2):227, with permission.)

parts of the world such as Europe, North America, East Asia, and Australia, which are bound to influence final data on follow-up (see later discussion). He also drew attention to the fact that in the reports from North America, follow-up was notably shorter than that elsewhere in the world, which again will affect reported outcomes. Various studies have reported renal survival rates between 57% to 94% after 10 years (287). Studies from the United States have reported worse renal outcomes than those in Europe and Asia, perhaps due to the inclusion of a more severely ill patient population (287). At least 25%—and maybe 50%—of adult patients with IgA nephropathy will end up in renal failure; the proportion of the remainder that may develop renal insufficiency as well in their third, fourth, or fifth decades is unknown.

IgA nephropathy has been examined in great detail for factors that may predict prognosis for groups of patients (294,298–302) and for individuals (303) using univariate and multivariate (72,303,304) techniques. Clinically, proteinuria (especially if profuse), hypertension, and a raised plasma creatinine concentration were determinants of a poorer prognosis in most studies. Male sex is a risk factor for a poor prognosis in several studies in which it has been examined (287). Macroscopic hematuria has given discordant results: Bennett and Kincaid-Smith (292) pointed to a poorer prognosis in patients with macroscopic hematuria, a conclusion reinforced in a later paper from this group (305), whereas all other studies, including those of D'Amico (286) and Beukhof (303), showed that macroscopic hematuria is a favorable sign. In multivariate analyses (298,303), proteinuria was the single most powerful clinical predictor of a poor outcome and the only independent clinical variable.

Pathologically, interstitial scarring is the most powerful predictor (298,303), along with number of crescents (306), vascular hyaline lesions (307), the number of obsolescent glomeruli and the degree of glomerular segmental sclerosis (308), and proliferation. Several groups (309–312) counted and determined the phenotype of the cells infiltrating the interstitium in IgA nephropathy and related these findings to prognosis. It has also been observed that only the rather small numbers of interstitial B cells correlated with rapid entry to renal failure (309), an interesting finding in view of the general B-cell overactivity in this condition. This area deserves further study.

Studies suggest that a polymorphism in the *ACE* gene may have an impact on the progression of IgA nephropathy. The polymorphism consists of insertion (I) or deletion (D) of a 287-bp DNA fragment, which may be the silencer portion of the *ACE* gene (313). The *DD* genotype of the *ACE* gene is found more frequently in patients with progressive IgA nephropathy than is the *ID* or *II* genotype. This was supported by a study done by Pei et al., in which polymorphisms in the *ACE* gene were important markers for predicting progression to chronic renal failure in 168 white patients with IgA nephropathy (314). In another study and meta-analysis by Schena et al. (315), however, the *ACE* gene polymorphisms did not correlate with disease progression. No relationship of the disease has been found with the angiotensin II receptor gene (313,316,317).

There are surprisingly few data on entry into *complete remission* for adults with IgA nephropathy. Nicholls et al. (305) noted that only 6% of their patients had entered remission after 5 years of follow-up, and Kitajima et al. (318), in a collaborative study of 1,394 adults, found only 6.5% after an average of 36 months; Schena (289) did not address the question in his otherwise admirable review, perhaps because so few authors comment on this aspect. Consequently, we simply do not know what proportion of adult patients will be in complete remission with normal urine after, say, 20 years. One is left with the impression that even in those with persisting normal renal function, at least for a decade or two, urinary abnormalities persist in the great majority.

IgA nephropathy is notably uncommon in blacks, although it is frequent in whites and Asians. One study of long-term outcome in black patients with IgA nephropathy found no difference from that of white patients (319).

IgA Nephropathy in Children

Although IgA nephropathy is a common form of glomerulopathy in children, documentation of the longer term outlook has not been extensive until recently. A relatively benign outlook for children presenting with hematuria, macroscopic or microscopic, was noted in the past (290,292) but did not include histologic data or information on the presence or absence of IgA. Levy et al. (290) described one of the larger follow-up studies on 91 children with IgA nephropathy, and their data are summarized in Table 71-4. Only eight children developed renal failure (six terminal, two more modest) within the follow-up period (mean, 13.5 years); the 10-year survival rate was estimated to be 92% ± 2%. Most patients in this series had macroscopic hematuria at some point in their course, 66 at presentation, and only 7 had microscopic hematuria associated with proteinuria. Levy et al. (290) noted 800 children previously described in the literature, with only 8 patients entering renal failure 5 to 44 years after onset and another 11 showing some degree of renal insufficiency 3 to 17 years after onset.

Data are available also from Japanese children, in whom IgA-associated nephropathy is particularly common. The study of Kitajima et al. (318), included in Levy's analysis of the literature, summarized data collected from 491 Japanese children;

TABLE 71-4

PROGNOSIS OF IgA-ASSOCIATED GLOMERULONEPHRITIS IN CHILDREN

		Subsequent behavior 1–16 (mean, 5.6) years			
Appearance on optical microscopy	*N*	No. with recurrent macroscopic hematuria	Remission	Nephrotic syndrome	Renal failure
Minimal changes	26	25	16	0	0
Mesangial proliferation only	3	3	1	0	0
Focal segmental proliferative glomerulonephritis + mesangial proliferation	41	28	14	0	0
Proliferative glomerulonephritis + crescents	21	13	2	0	8
	91	69	33	0	8

(Reprinted from: Levy M, et al. Berger's disease in children: natural history and outcome. *Medicine (Baltimore)* 1985;64:151, with permission.)

only 9 developed chronic renal failure or died. Kusumoto et al. (320) reported on 98 children with IgA nephropathy, with renal failure occurring in only 9 and an estimated 10-year survival rate of 94%. Most of these children presented with proteinuria and hematuria, and only two with isolated hematuria. Yoshikawa et al. (321,322) reported on follow-up of 200 Japanese children with IgA-associated nephropathy. Only 2 of 83 children younger than 8 years at apparent onset went into renal failure, but follow-up was relatively short (5.5 ± 2.8 years). A much higher proportion than in an adult series (93 of 200) had normal urine by this time. Of 117 children aged 9 to 15 years at onset, 8 went into renal failure after a similar brief period. In Kusumoto's study (320), the outlook for renal function was significantly poorer in 86 patients with adult-onset disease studied in parallel, so a gradient of poorer prognosis with age at onset seems to be present.

Therefore, the overall prognosis in the medium term seems to be generally good for children with IgA-associated nephropathy. However, the very slow evolution of the disease must not be forgotten. After 10 years in Levy's study, only one-third of patients were in complete remission with normal urine, and the longer term fate (20 or 30 years) of such patients has not been established. Other authors have emphasized the poorer prognosis of their pediatric patients with IgA nephropathy (323,324), probably because of referral and biopsy policies determining that only patients with more severe disease reach tertiary referral centers. In children, the same prognostic indicators as those in adults, although less extensively studied (323), have emerged as indicators of a poorer early prognosis; on histology, extensive crescent formation, interstitial fibrosis, and sclerosing glomerular lesions carry a poorer outlook. Clinically, proteinuria, hypertension, and older age at onset seem the most important variables.

Unusual Patterns of Evolution in IgA Nephropathy

A particular feature of IgA-associated nephropathy is that although generally proteinuria has been an adverse factor in determining prognosis, a small but widespread number of patients have been described in whom a full nephrotic syndrome, normal renal function, and prompt response—and often a relapsing course—followed corticosteroid therapy (325). These patients, more often children than adults, almost always show only minimal changes on optical microscopy, although some show mesangial expansion or even FSGS. However, in the few patients examined by electron microscopy, characteristic electron-dense aggregates have been absent. Whether these patients represent a subset of IgA-associated nephropathy or the coincidence of minimal change nephropathy with symptomless IgA deposition within the mesangium, as may occur in the healthy population (e.g., organ donors), is not known. Many of the patients described in the literature, even from non-Asian countries, are ethnic Chinese.

Another unusual pattern in IgA nephropathy is recurrent attacks of acute renal failure (326), first described by Talwalkar et al. (327) in 1978. It has been proposed that the acute hematuria is toxic to renal tubules (292,301), and red blood cells ingested by the tubules have been demonstrated, which is supported by a report of a hematuric patient who did not have IgA nephropathy (328). These patients must be distinguished from those going into acute renal insufficiency because of crescentic glomerulonephritis (290,292), and thus a repeated renal biopsy is usually necessary.

Treatment of IgA Nephropathy

The reader is referred to Chapter 61 for the details of treatment. The effect of treatment on the outcomes reported in various series is difficult to determine, but recent evidence suggests that treatment does affect the long-term outcome. Although D'Amico noted that there have been similar results in those studies reported before and after the widespread use of *ACE* inhibitors (287), several studies have now shown a benefit for patients treated with *ACE* inhibitors (329–332), and all proteinuric patients should probably be treated with these agents. Previously it was felt that patients with proteinuria of more than 3 g per day and preserved renal function should be treated with steroids to reduce proteinuria and stabilize renal function (333,334). Pozzi et al. also recently reported the 10-year follow-up data for a cohort of patients with mild initial renal disease (plasma creatinine ≤1.5 mg/dL and urinary protein excretion of 1 to 3.5 g per day) who were randomized to treatment with steroids for 6 months or supportive treatment. In this study, treatment with steroids significantly improved renal survival and led to a statistically significant reduction in the percentage of patients who doubled their plasma creatinine (335). In patients with progressive disease, fish oil may be tried because there is some benefit seen in some studies (336,337). In a 6-year follow-up study of the earlier trial (338), Donadio et al. concluded that early and prolonged treatment with fish oil slows renal progression for high-risk patients with IgA nephropathy. Cyclosporine has not been shown to be of benefit.

Membranoproliferative Glomerulonephritis

Since the early 1970s it has been the practice to divide the light microscopic appearances of membranoproliferative glomerulonephritis (MPGN, also mesangiocapillary glomerulonephritis) (Chapter 62) into two categories: the more common variety with subendothelial immune aggregates (type I), and a variety with extensive replacement of the lamina densa of the capillary basement membranes and those of the tubules and Bowman's capsule, with a birefringent, rather continuous material that takes stains avidly (type II). On light microscopy, not all type I patients with MPGN show a true mesangiocapillary pattern—that is, expansion of the mesangium with a complex thickening of the glomerular capillary walls by mesangial cell cytoplasm and new material. Some authors (339–342) distinguish a "type III" MPGN in which the immune aggregates are found by electron microscopy not only at a subendothelial site, but also throughout the basement membrane with subepithelial aggregates, so that in isolation the appearances resemble those of an advanced membranous nephropathy. Some authors regard this as a later stage of type I MPGN and it is so treated in this chapter.

One striking feature of data from Europe has been the decline in the number of new patients presenting with either type of MPGN (343,344), suggesting that some precipitating environmental agent or agents, most probably infectious, has declined in frequency. MPGN has always been unusual in adults and children in Japan, although it is very common in other parts of the world: In Iran, MPGN is the underlying histologic appearance in two-thirds of adult-onset nephrotic syndromes (345). MPGN may be seen complicating various infections and as part of systemic disorders. Infection-related MPGN needs to be separated from the apparently idiopathic disorder, because the outlook for infection-related MPGN, whether from endocarditis (346), infected juguloatrial shunts (347), or deep sepsis (347), is generally good, provided that the infection can be controlled. The prognosis of MPGN in a setting of lupus or Henoch-Schönlein purpura (HSP) is considered in later sections of this chapter.

Long-Term Outcome

The survival of patients with idiopathic type I or type II MPGN was documented in a number of series during the 1970s and a

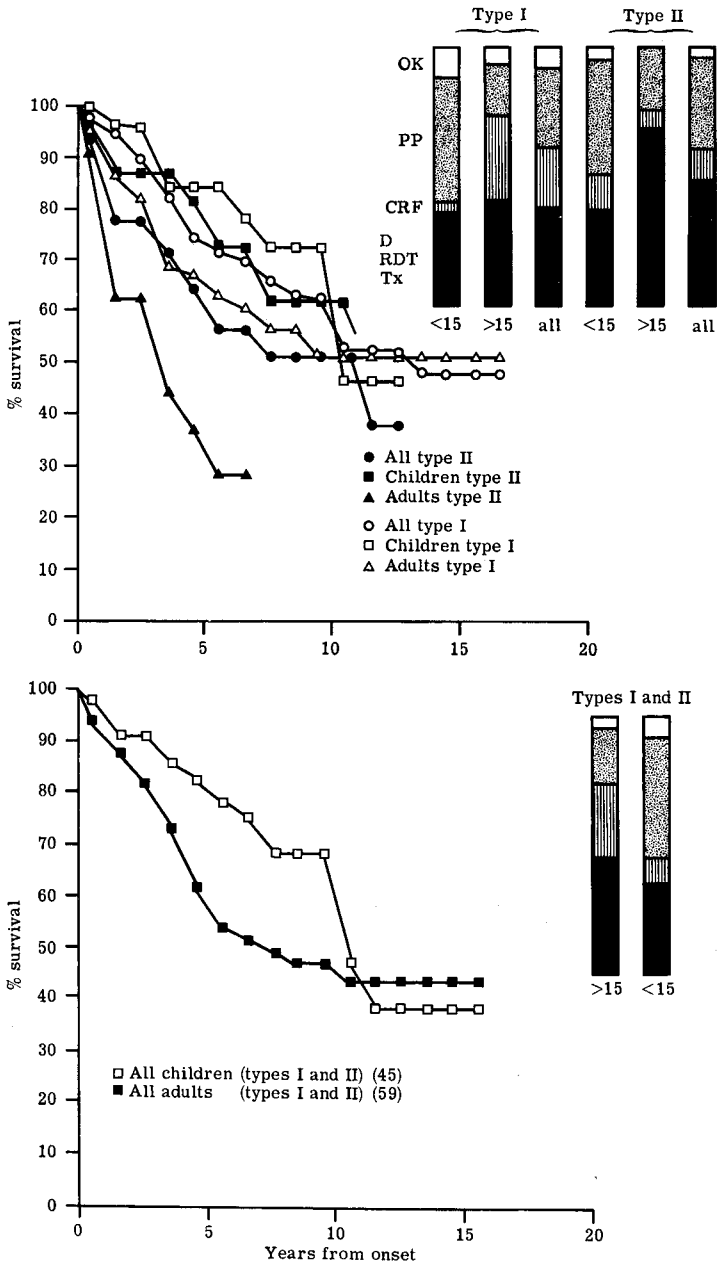


FIGURE 71-15. (**Top**) Survival and status at last follow-up evaluation in patients with mesangioproliferative glomerulonephritis (MPGN). The survival curves were calculated by the actuarial method. None of the curves differ from each other when tested by the log-rank method (Peto et al. [1]), although the adult patients with type II MPGN approach significance when compared with similar children ($p = 0.08$). The most recent status is shown at top right: OK, well, normal urine, normal renal function; PP, persistent proteinuria with or without a nephrotic syndrome but with normal renal function; CRF, glomerular filtration rate of less than 80 mL per minute/1.73 m^2 and persistent proteinuria with or without a nephrotic syndrome; D, RDT, Tx, dead, regular dialysis treatment, or transplantation. Data are presented for each of type I and II MPGN for patients older and younger than 15 years at apparent onset. In type I MPGN, children have a significantly better prognosis than adults ($p < 0.01$) compared in this fashion. Similarly, data for types I and II together show a significant difference ($p = 0.003$). In the case of patients with type II alone, the differences between children ($N = 22$) and adults ($N = 13$) were not significant ($p = 0.12$). (**Bottom**) Survival in children and adults with MPGN. There is no significant difference between the two curves, although there is an excess of early deaths among the adult patients. When the data for most recent status are examined, there is a significantly worse prognosis for adults than children ($\kappa^2 = 7.74$; $p = 0.003$). (From: Cameron JS, et al. Idiopathic mesangiocapillary glomerulonephritis: comparison of types I and II in children and adults and long-term prognosis. *Am J Med* 1983;74:175, with permission.)

few more recent sets of data. These show that in general MPGN of either type is usually a progressive disorder in white and Chinese (67) populations, with the estimated survival rate for renal function at 10 years being about 60% and the 15-year survival rate being no more than 50%. In agreement with this, a large study from Italy (348) found an estimated 10-year survival rate of 60% to 65%. Because the patients in general are children or young adults (67,129), the impact of nonrenal deaths is minor compared with that of membranous nephropathy, for example, and near 100% survival for at least 10 years can be expected for the control population. Data from MPGN type I are rather concordant, whereas those for type II show much more varied outcomes, perhaps because of the small number of patients studied in individual series. In general, no form of treatment appears to affect the outcome of either form of MPGN, with the exception of a single series discussed later in this chapter, so untreated and treated patients are not separated in the data presented here.

Follow-up data have been reported for 104 patients (349), both children and adults (69 type I and 35 type II) who had idiopathic disease. These data (Fig. 71-15) are in general agreement with other published data, with only 7 patients showing complete remission. The group of the Hospital des Enfants Malades in Paris presented its data from children in the same year (350). Complete remission was equally rare; this occurred in only 1 patient with type II and 4 patients with type I MPGN of 44 and 84 patients with idiopathic MPGN, respectively. Of the 44 patients with type II MPGN, 18 were either dead or in ESRD, 1 was uremic, 11 had a persisting nephrotic syndrome, and 11 had persisting proteinuria and normal renal function. Of the 84 patients with type I MPGN, 21 were dead or in ESRD treatment, 9 had chronic renal failure, 18 had a persisting nephrotic syndrome, and 32 had persisting proteinuria with normal renal function.

In type II MPGN, only two groups noted a better prognosis. In children Klein et al. (351) found that 5 of 18 patients were

in remission after 11 ± 1 year of follow-up. Similarly, Strife et al. (342) reported 17 children with type II MPGN, with only 4 showing progressive disease and 3 being nephrotic, whereas no child presenting with subnephrotic proteinuria showed progression.

Finally, controlled trials in the United States (352,353) gave some data on serial GFRs in adult patients with type I MPGN. In the former trial (352), over 18 months, the control group lost 9 mL/minute and the treatment group did marginally worse, at 14 mL/minute. In the Mayo Clinic trial (353), the control group lost a mean of 19 mL/minute over 1 year, whereas those treated with aspirin and dipyridamole lost only 5 mL/minute. However, long-term follow-up results were identical in the two groups despite continued treatment (353). The rate of fall-off in renal function was varied, as in membranous nephropathy; Chan et al. (67) analyzed rates of change in $1/P_{creat}$ and noted similarly wide variations in rates of decline in renal function.

Indicators of Prognosis

Several groups studying type I MPGN (67,349,354) were able to identify *clinical* indicators of a poor prognosis using univariate analysis (Table 71-5). As in most forms of progressive glomerular disease, and in most other MPGN series, a nephrotic syndrome—particularly if persistent—was a major clinical indicator of a poor outcome, but Schmitt et al. (354) did not confirm this observation. Hypertension was modestly predictive of a poor outcome in both sets of data (67,349), but there was (in sharp contrast to membranous nephropathy) no difference in outcomes between adults and children. In one series, the presence, absence, or degree of hypocomplementemia had no relation to outcomes in either type of MPGN, suggesting that the prominent *in vivo* complement breakdown is an epiphenomenon of the disease, although Swainson et al. (355) and Klein et al. (351) reported the opposite in smaller series. Magil et al. (57) found that men did worse than women, as in other forms of glomerular disease, but this trend did not reach significance.

Hardly surprising, reduced GFR or elevated plasma creatinine concentration (354) at presentation had an adverse effect on outcome. Donadio et al. (352) found that onset with an acute nephritic syndrome and macroscopic hematuria (common in children) was an adverse feature, and Bennett et al. (356) noted a higher incidence of macroscopic hematuria in patients with type II MPGN who did poorly. The presence of hypertension is controversial; Schmitt et al. found it to be a strong predictor of adverse outcome and a major direct cause of death (354), whereas others (67) found no effect on outlook.

On *histologic* evaluation, crescents have been shown to indicate a worse prognosis (67,344,350). Schmitt et al. (354) performed a more elaborate analysis of the glomerular histology, with five grades, the last of which included crescent formation; patients with this last grade did worst of all. Although Barbiano di Belgiojoso et al. (344) noted a poorer prognosis for those patients with more lobular forms of glomerulonephritis, Schmitt's data did not support this suggestion. Schmitt et al. (354) also assessed interstitial changes carefully and found, as in other glomerular diseases, that the degree of tubulointerstitial changes was strongly predictive of outcome (Fig. 71-16).

Possible Effects of Treatment

Treatment in adults and children with idiopathic MPGN is reserved for patients with nephrotic-range proteinuria, interstitial disease on biopsy, or impaired renal function—all markers for poor prognosis.

Children. One group (357–359) has consistently stated that their treatment results—using alternate-day, high-dose oral prednisolone—have been significantly better than those in children with type I MPGN managed in other centers, with a 10-year survival rate for renal function of 82% but falling to 56% after 20 years. Another group supported the idea that this may represent an improvement in prognosis induced by the high-dose, alternate-day corticosteroid regimen used (360). Likewise, the ISKDC (361), which was a prospective randomized controlled trial using steroids, showed that 61% of the treatment group and 12% of the placebo group had stable renal function at the end of the study.

Adults. A study using aspirin and dipyridamole demonstrated a clinically significant effect of reduction in proteinuria in the treatment group but no change in GFR (362). The general

TABLE 71-5

EFFECT OF FEATURES AT ONSET ON PROGNOSIS FOR RENAL FUNCTION IN TYPE I AND TYPE II MESANGIOCAPILLARY GLOMERULONEPHRITIS

	Type I		Type II		All	
	"Dead"[a] vs. alive	"Uremia"[b] vs. persistent proteinuria and remission	"Dead" vs. alive	"Uremia" vs. persistent proteinuria and remission	"Dead" vs. alive	"Uremia" vs. persistent proteinuria and remission
Child or adult (< or >15 yr)	NS[c]	<0.001	(0.07)	NS	NS	0.003
Hematuria at onset	NS	NS	NS	NS	0.003	NS
Nephrotic syndrome at onset	0.01	NS	NS	NS	0.02	NS
Nephrotic syndrome at any time	0.001	0.06	0.04	NS	0.0001	0.003
Blood pressure elevated at onset	0.03	NS	NS	NS	NS	NS
Glomerular filtration rate decreased at onset	(0.07)	0.002	NS	NS	NS	NS

[a] "Dead," dead, dialysis, or transplantation.
[b] "Uremia," "dead" or persistent disease with glomerular filtration rate <80 mL/minute/1.73 m^2.
[c] NS = $p > 0.05$ (χ^2 test).
(Reprinted from: Cameron JS, et al. Idiopathic mesangiocapillary glomerulonephritis: comparison of types I and II in children and adults and long-term prognosis. *Am J Med* 1983;74:175, with permission.)

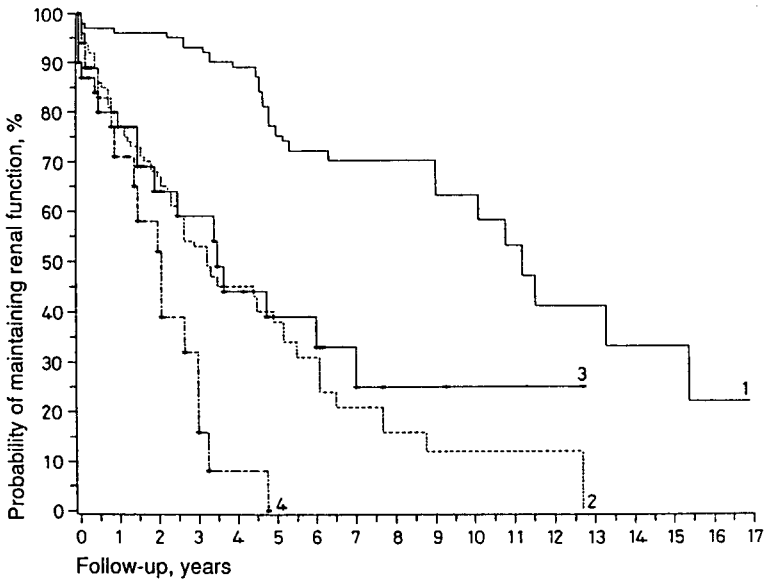


FIGURE 71-16. Probability of entering renal failure in patients with membranoproliferative glomerulonephritis type I, according to the degree of interstitial changes. Curve 1 is derived from 92 patients with a normal interstitium in the initial biopsy; curve 2, from 77 patients with interstitial fibrosis only; curve 3, from 32 patients with tubular damage only; and curve 4, from 19 patients with both tubular damage and interstitial fibrosis present. (From: Schmitt H, et al. Long-term prognosis of membranoproliferative glomerulonephritis type I. Significance of clinical and morphological parameters: an investigation of 220 cases. *Nephron* 1990;55:242, with permission.)

opinion is that no treatment reliably affects the long-term outlook of MPGN in adults.

Postinfectious Glomerulonephritis

In patients with postinfectious glomerulonephritis, there are particular problems with definition, as mentioned in the first section in this chapter (see also Chapter 58). Different observers may be writing about different types of patients, and it is essential to be absolutely clear about which are and perhaps even more important which are not the subject of study. Three main sets of observations help to define this group of patients: the clinical presentation (an acute nephritic syndrome), a precipitating event (e.g., documented antecedent streptococcal infection), and the histopathology involving exclusively, or at least predominantly, the endocapillary wall, and with infiltrates of leukocytes in the early stages.

Apart from "core" patients who satisfy all three criteria there will be other patients who variably do not. In temperate climates, most patients with an acute nephritic syndrome do not seem to have a renal disorder that has arisen from streptococcal infections, which are now rare given better hygiene and the absence of insect vectors. Patients with an acute nephritic syndrome and streptococcal infection may also present with mesangiocapillary patterns of nephritis or extensive crescent formation. Obviously, different mixes of patients within these groups will lead to different impressions of outcomes. Because this chapter is organized predominantly according to appearances viewed by light microscopy, this discussion is limited only to those patients with an exudative endocapillary pattern, because the prognosis of those patients with streptococcal infection with acute nephritis who show either MPGN or extensive crescents on biopsy specimens resembles closely that of patients without streptococcal infection with a similar histology. Even within the "core" group, there are two separate clusters to consider: those patients associated with epidemics of disease and those in whom disease appears to be sporadic. The prognosis of the latter may well be not as good as it is for those observed in an epidemic setting.

There has been some controversy regarding the frequency of occult or evident streptococcal glomerulonephritis as a cause of chronic renal failure, and, conversely, the frequency with which persistent renal disease may be expected in patients with postinfectious glomerulonephritis (363,364). Part of the problem arises from the fact that the mix of patients observed in epidemics, mostly not in a Western urban environment, and the sporadic disease patients seen in city center referral specialist units are not the same.

Few cases of acute postinfectious nephritis are reported in industrialized countries, and most of those are not the sequel of streptococcal infection (365,366). Conversely, an unknown number of patients may have subclinical attacks of poststreptococcal nephritis; these numbers at least equal the number of obvious cases in the epidemic situation and may exceed them severalfold (367–370). The urine may show little or no abnormality (371) and hypertension may be the principal feature. Unless these atypical patients are investigated very thoroughly with serology, serial complement estimations, and renal biopsy with electron microscopy, it may be difficult to exclude other forms of glomerulonephritis. Occasional patients with a typical insidious nephrotic syndrome may show typical active diffuse endothelial nephritis in renal biopsy specimens. Some otherwise typical acute nephritic patients may have rather profuse proteinuria and show, rather briefly, a full nephrotic syndrome; this certainly can occur with the biopsy appearances discussed here, but it raises the suspicion of MPGN. An occasional patient with only acute endothelial proliferative glomerulonephritis presents with anuria and acute renal failure (372).

Childhood

Many early statements of the prognosis of acute nephritis in children are difficult to evaluate, because in almost all cases, histology was not available and the assumption was made (often without good evidence) that acute glomerulonephritis must necessarily be poststreptococcal. These early reports are summarized by Perlman et al. (373). One report is worthy of summary here, however, because it represents an early attempt to assess the very long-term prognosis of acute nephritic children (374). Twenty-seven patients who had acute nephritis in childhood severe enough to result in hospital admission were studied 19 to 32 years later. These patients represented only 25% of the 130 patients the author set out to trace. None of the 27 showed evidence of disease, and all urine test results and blood pressures were normal. Only one patient had died, and that was from tuberculosis.

Epidemic Cases. The prognosis of epidemic-proven acute streptococcal glomerulonephritis appears to be equally benign. Perlman et al. (373) studied the children who had acute glomerulonephritis 10 years previously in the streptococcal epidemic in the Red Lake Indian reservation. All children were studied initially and were shown to have streptococcal infection. After 10 years, renal function was normal in all, and the degree of proteinuria and hematuria did not differ from that in control subjects. Renal biopsies were performed in 16 of the 61 children; 12 specimens were entirely normal and 4 showed minor mesangial changes. Sanjad et al. (375) reported on 153 cases of acute glomerulonephritis from Florida. In this study, 103 patients were followed and none showed any clinical evidence of progressive disease up to 10 years later. However, ten children showed minor urinary abnormalities and all six who underwent biopsy had some glomerular abnormality.

Potter et al. (376–378), in an important study, described the 2- to 6-, 7- to 12-, and 12- to 17-year follow-up of a group of patients, mostly children, with sporadic or epidemic poststreptococcal nephritis, collected in Trinidad in 1965 and the following years. This study involved more than 700 persons, almost all of whom were still available in 1977 (378). Only 2 patients had died in renal failure, 19 had proteinuria, 3 had hematuria, and 3 had both. The incidence of hypertension (16 patients) was no higher than what is expected for the Trinidad population as a whole. The 1981 survey (377) was less complete. Only 534 patients with urinary abnormalities were identified, and all had normal plasma creatinine concentrations. It is worth noting that in the histories of the three patients who died, it was not clear whether they died of renal failure or if so, whether this was attributable to their nephritis.

An interesting observation of Nissenson et al. (378) was of 13 persons who had abnormal urine samples, but no symptoms during the attack (i.e., subclinical nephritis); none showed abnormalities 7 to 12 years later. The other study of this type of patient is that of Smith et al. (379), who performed biopsies and followed symptomless patients with acute poststreptococcal nephritis and minor urinary abnormalities. After 1 to 4 years, 8 of 28 patients showed persisting urinary abnormalities, and 2 had hypertension. Rodriguez-Iturbe et al. (369,380,381) summarized their experience of epidemic poststreptococcal glomerulonephritis in Maracaibo; only 1 of 71 patients followed for 11 to 12 years went into renal failure, but 15 (21%) still showed urinary abnormalities.

Sporadic Cases. Evidence is also available from patients with sporadic poststreptococcal glomerulonephritis in childhood. In an extensive study from Galveston (367,382), 60 patients were followed for at least 3 years, an important aspect in a condition with a relatively benign prognosis. Only 1 of 60 patients died after 2 years, and his initial biopsy specimen showed an unstated number of epithelial crescents; another child died of causes unrelated to renal disease at 18 months. At 3 years, only 8 of 45 children had urinary abnormalities (367), and at 5 years, only 2 of 32 still under observation showed proteinuria. Similarly, in the study from Lewy et al. (383) of 46 children with sporadic poststreptococcal glomerulonephritis, only 4 patients died, all showing extensive crescent formation in their biopsy samples. Two children still showed proteinuria after 5 years of follow-up. One of these two children had crescents in the initial biopsy specimen. Only three patients were lost to follow-up. Hinglais et al. (384) presented evidence from 29 children with acute nephritic syndromes; 16 of their 65 patients had raised antistreptolysin levels, but it is impossible to deduce which of these 16 patients were children. Of the 29 children, 25 had various forms of endocapillary glomerulonephritis, including exudative patterns with "humps" in 16. No patient with only endocapillary glomerulonephritis did poorly, and at the assessment point 2 years later, all those patients with endocapillary glomerulonephritis with humps had normal urine samples. Popovic-Rolovic et al. (385) followed 88 patients with sporadic disease in Serbia for 10 to 17 years. Only hypertension (3.4%) and proteinuria (2.3%) and microscopic hematuria were observed. The creatinine clearance was normal in all patients.

Evidence of persistent disease or progression was confined to those patients showing lesions other than endocapillary lesions, as in the series of Mota-Hernandez et al. (386). Baldwin et al. (387) included 37 children in their mainly adult study. One child required dialysis after 9 weeks, and her biopsy specimen showed extensive epithelial crescents. In six children, GFRs were measured 3 to 10 years from onset; all were more than 80 mL/minute except in one patient with a GFR of 77 mL/minute (uncorrected). In another series (388), 38 children with acute poststreptococcal glomerulonephritis were first studied between 1962 and 1970. Two patients died during the acute illness with crescentic nephritis and 33 of the remaining 36 were studied a mean of 9.4 years later (range, 6 to 13 years) (389). Two of the other three patients were known to be alive and in good health and only one was lost to follow-up. One patient showed persistent hypocomplementemia, and a second biopsy in this child revealed MPGN (390). All others showed normal blood urea and plasma creatinine concentrations, and in 21, creatinine clearance rates were normal (mean, 113 mL/minute; range, 80 to 70 mL/minute per 1.73 m^2). However, nine patients showed persisting urinary abnormalities: hematuria plus proteinuria in three, and isolated urinary protein loss in six, ranging from 0.2 to 0.5 g per 24 hours. Blood pressure was within the normal range in all patients. All of these patients were recalled for restudy a mean of 19.4 years (range, 14.6 to 22 years) later (349). Only one was hypertensive, three patients had microscopic hematuria, and the patient with MPGN had proteinuria. All showed normal plasma creatinine concentrations.

Although GFR appears to be conserved, renal reserve may be impaired. Cleper et al. (391) reported on 36 patients who had recovered from an episode of poststreptococcal glomerulonephritis 1 to 16 years earlier without apparent sequelae. Basal creatinine clearance was no different from that in control patients. However, the renal reserve in response to protein loading was significantly decreased. The magnitude of the impairment in renal reserve appeared to increase with more time since the initial episode of poststreptococcal glomerulonephritis. A similar note of caution regarding the degree of recovery was sounded by Herthelius and Berg who reported that although the GFR is normal, the filtration fraction remains high, suggesting possible glomerular hyperperfusion (392).

In summary, the accumulated evidence suggests that in epidemic and sporadic poststreptococcal glomerulonephritis and other acute nephritic syndromes in childhood, a similar pattern emerges. If the biopsy reveals endocapillary changes only, whether or not "humps" are present, then healing within 5 years is the rule. The few patients who do badly almost always show some other histologic pattern and are usually associated with other adverse features such as the appearance of a full nephrotic syndrome.

Adults

The evidence that progression can occur in acute poststreptococcal glomerulonephritis is somewhat stronger in adults, even in those with only an endocapillary glomerulonephritis demonstrated by renal biopsy. In addition, other patterns of histology such as the presence of extracapillary crescents are more common. In Jennings and Earle's classic paper of 1961 (393), the only patient of their 36 who died, still anuric, had extensive crescent formation in his biopsy specimen. Thirteen other patients had clinical evidence of continuing disease at follow-up

5 months to 4 years after onset. McCluskey and Baldwin (394) reported on a mixed group of adults showing an acute nephritic onset; 5 patients died and 14 showed evidence of progressive disease. Some of their patients showed either diffuse crescentic glomerulonephritis or MPGN. More recently, Baldwin et al. and Baldwin and Schacht (364,387,395) reported their findings in 89 adults with proven poststreptococcal nephritis, drawing the widely quoted conclusion that "up to one-half" of patients with this condition show evidence of progressive disease. This conclusion deserves careful examination.

The ten patients older than 15 years at onset who died or required dialysis had extensive crescent formation, and four of these patients had a rapidly progressive course over 3 months into renal failure. The other patient with crescentic glomerulonephritis became uremic 6 months after a second attack of glomerulonephritis. Of the other five who died, only two died later, at 2 and 6 years of follow-up. Six of these ten fatal patients showed a full nephrotic syndrome at presentation. These patients, therefore, although part of the spectrum of poststreptococcal nephritis, are not typical of this condition and were seen in a tertiary referral center.

In the remainder of the patients in this study, the evidence for persisting disease must be considered against the large fallout of patients. Unlike the childhood series discussed previously, only 56 of 126 patients completed even 1 year of follow-up, only 29 were still in contact after 5 years, and only 5 at 10 years or later. The fact that 50% of patients showed a raised blood pressure after 8 to 10 years is made less impressive if one remembers that the 10 patients studied at this point represent only 7% of the whole group; the assumption cannot be made, as discussed at the beginning of this chapter, that those patients lost to follow-up had the same characteristics as those retained. In most studies of relatively benign disease, as noted already, patients with the more severe disease progressively concentrate in the follow-up group.

However, 8 of 16 adults whose GFR was measured 3 to 15 years after their attack of glomerulonephritis showed values of less than 80 mL/minute. An inulin clearance rate of 100 mL/minute was used as the lower limit of normality. These data are not corrected for sex, surface area, or age, and other published figures suggest that this would not represent the mean minus 2 SDs, and the blood pressures were not compared with normal data for sex and age. Twenty patients still had proteinuria at 5 years, and four at 10 years, although the description of proteinuria as evidence of irreversible disease is not justified. Hypertension, judged as a diastolic blood pressure of more than 90 mm Hg, was equally common. Baldwin and Schacht (395) presented further data on this group of patients; 125 of 176 patients in the study had a renal biopsy, and 105 were followed for 2 years or longer. By 1975, nine patients entered terminal renal failure within a maximum of 6 months from onset, whereas six others progressed to uremia from 1 to 12 years after onset; all the patients in the latter group showed typical acute endocapillary glomerulonephritis in their initial biopsy specimen.

What have other authors reported? Hinglais et al. (384), in their study of 36 adults with sporadic acute nephritis, found 25 patients with only endocapillary lesions in the renal biopsy specimen. Seven of these showed persistent proteinuria when restudied at 2 years. In contrast, 10 of the other 11 patients with changes other than endocapillary glomerulonephritis (mesangiocapillary, extracapillary) had continuing disease. However, GFRs were not measured in this study. Lien et al. (396) also studied 57 patients, 52 of which were adults who had sporadic poststreptococcal glomerulonephritis, for a mean range of 7 ± 4 years. All had an initial biopsy and 33 had second biopsies. Two patients died with abnormal renal function, and 11 showed evidence of some renal damage and four additional patients had only hypertension. Vogl et al. (397) described 2- to 13-year follow-up for 72 of 101 patients with adult-onset, sporadic acute poststreptococcal nephritis in Germany, Luxemburg, and Austria. Only three patients entered ESRD, one in only 5 months after transformation into a diffuse crescentic nephritis as shown by a second biopsy, as others have described (389,398). Only two other patients had raised plasma creatinine concentrations and continued proteinuria. Four other patients died, however: one from accelerated hypertension after only 2 months, another of sepsis, and two others of unknown causes. Vogl et al. (397) confirmed the suggestion of Hinglais et al. (384) and others that onset with a nephrotic syndrome indicates a poorer prognosis. Similarly, Rodriguez-Iturbe reported that of adult patients presenting with nephrotic-range proteinuria, as many as 77% will develop ESRD (399).

In a more recent study (400), the long-term outcomes were reported for patients diagnosed with infection-associated glomerulonephritis between 1979 and 1999. In this study, the prognosis was significantly worse for patients who also had a severe underlying disease. After a mean follow-up of more than 7 years, 64% of those without underlying disease were in complete remission, whereas only 14% of those with severe underlying disease (such as cirrhosis or malignancy) achieved complete remission.

Baldwin's group has also emphasized that widespread vascular sclerosis of arterioles may be a marker of progression (401). On the clinical side, profuse proteinuria, anuria, the persistence of low complement levels, and hypertension beyond about 4 weeks after onset are valuable indicators of a poor outlook, and many such patients show MPGN in their biopsy specimens. Patients may begin with a biopsy specimen that shows a pure endocapillary disease, which then develops into MPGN (389) or into severe crescentic nephritis (389,397,402). However, it must be emphasized again that pure endocapillary glomerulonephritis can occur with anuria and that anuria does not always indicate crescentic nephritis (403).

One practical problem in uncomplicated cases is to know just how long one has to wait before the urine can be expected to become free of blood, trace protein, or both, and whether late renal biopsy should be performed. There are surprisingly few data on this important point, but it has been known for many years that it could take up to several years for complete clinical "healing" to occur. Joseph and Polani (404) provided evidence from a trial of bedrest (which showed no effect) that more than 90% of children with sporadic poststreptococcal nephritis will be free of hematuria by 6 months.

In summary, the prognosis of acute glomerulonephritis, both epidemic and sporadic, postinfectious or poststreptococcal, probably becomes worse with age. Recovery is almost invariable in the preschool child, there is some suggestion of chronicity in older children, and the disease becomes chronic in a significant minority of adults. With increasing age, an increasing proportion of patients show diffuse extracapillary proliferation or MPGN, which accounts for an increased incidence of precocious renal failure. It is difficult to generalize from these controversial figures, but of 100 acute nephritic children, 1% or less will show crescentic glomerulonephritis and go on to early uremia; 75% will be free of proteinuria in 2 to 3 years, and a further 20% by 5 years. Only 1% or 2%, usually older children, will develop uremia later. Some of these will have shown MPGN or crescentic glomerulonephritis at onset. In adults, perhaps 5% will show persisting proteinuria, and some of these patients will have reduced renal function and hypertension. Their fate is not known, but some may progress to uremia within a decade or two. Thus, most of the difficulties and apparent inconsistencies in the various reports of the outcome of acute nephritic patients resolve themselves if the differences in age, etiology, epidemiology, and type of histology seen in renal biopsy specimens are taken into account.

TABLE 71-6

VARIOUS GLOMERULAR CONDITIONS THAT MAY BE ASSOCIATED WITH EXTENSIVE CRESCENT FORMATION

Primary crescentic glomerulonephritis
Common
Idiopathic[a] crescentic glomerulitis, usually necrotizing segmental
ANCA-associated vasculitis
Antiglomerular basement membrane nephritis
Membranoproliferative glomerulonephritis (I or II)
Uncommon
IgA nephropathy
Membranous nephropathy
Secondary crescentic glomerulonephritis
Common
Henoch-Schönlein purpura
Microscopic polyarteritis
Uncommon
Systemic lupus erythematosus
Behçet's syndrome
Mixed cryoglobulinemia
Shunt nephritis
Subacute bacterial endocarditis
Neoplasia

[a] Considered by some always to represent microscopic polyarteritis (see text).

Crescentic Glomerulonephritis

The group of patients with crescentic glomerulonephritis is neither pathologically nor clinically homogeneous (Table 71-6). Only the thesis that extensive crescent formation is a dominant determinant of progression, overriding that of the underlying glomerulopathy, justifies considering them together (405–407) (Chapter 60). In practice, the outlook for patients with extensive crescent formation, whatever the underlying disease, is so much poorer than that noted for other forms of glomerulopathy (17), that this approach seems worthwhile, at least for purposes of clinical management.

Types

Three main groups of patients may be included under the heading of "crescentic glomerulonephritis." The proportion of each varies with the ages of the patients and geography. For example, crescents complicating proliferative glomerulonephritis form a greater proportion of cases in children (408–411) and in the developing world (411–414). Some degree of extracapillary proliferation is relatively common in patients with primary proliferative glomerulonephritis. This is true of postinfectious endocapillary nephritis, MPGN, and less commonly, IgA nephropathy. The changes are usually minor and often segmental. In some patients, however, a significant number of glomeruli show multilayered crescents affecting a large proportion of the glomerular circumference. In the minority of patients who are of concern, most or all glomeruli show such circumferential extracapillary proliferation.

Interpretation of the distorted and compressed glomerular tuft engulfed by a crescent is always difficult and may be impossible, but on immunohistologic examination, granular IgG and C3 are seen in various distributions in other patients with endocapillary or mesangiocapillary nephritis, or lupus, and IgA is seen in patients with crescentic IgA nephropathy (415–417) or HSP (405,408–411).

Anti-GBM disease and *vasculitis* of various types are the other two main varieties of crescentic nephritis and form most cases in adults in developed countries (405–407). Extracapillary crescent formation is present in most patients, together with necrotizing lesions within the glomerulus. On immunohistology, fibrin is seen within the crescents; this is particularly common in crescentic nephritis associated with vasculitis (see later discussion). Linear deposits of IgG and complement, and occasionally IgA, are seen in anti-GBM disease along the capillary walls, whereas in vasculitic patients (with the exception of those with HSP), no glomerular immune aggregates are visible.

Apart from these three main groups, the so-called "idiopathic crescentic glomerulonephritis" remains. In patients with this form, there is usually a segmental necrotizing glomerulitis and collapsed but otherwise normal glomerular tufts (418–420). Many of these patients have antineutrophil cytoplasmic antibodies (ANCA) in their plasma, directed against myeloperoxidase (MPO) (see later discussion). However, very few patients do not fit this description, do not have circulating ANCA, and if untreated, do not develop vasculitis. Some authors have never seen such cases (421,422), and certainly they are very uncommon (423,424).

How Many Crescents Make Crescentic Nephritis?

It is impossible to draw a firm line at any point to distinguish what should be called "crescentic glomerulonephritis." For purposes of discussion, criteria of 50%, 60%, 70%, and 80% of glomeruli affected by crescents have been used by different authors to define crescentic glomerulonephritis. Because the prognosis, at least of the untreated disease, may depend on the proportion of affected glomeruli, this is not an academic point. Expressing the percentage involvement from a total number of glomeruli of less than, say, 10 may not seem to be a useful exercise, and reservations about the sampling error are certainly present. However, because the extent of the involvement in an individual glomerulus varies with the proportion affected (412,425), the conclusions may be more secure than at first appears. The important point made earlier is that although the presence of extensive crescent formation may dominate the histologic and clinical picture, justifying the separation of the group, the glomeruli themselves may show various changes and the patients various clinical pictures.

Clinical Presentation

Despite this heterogeneity, most patients with extensive crescent formation have a characteristic presentation and clinical course. The most common presentation is with sudden onset of rapidly progressive renal failure, sometimes with anuria. Other patients have a course over several months, which is still quick enough to justify the description of "rapidly progressive glomerulonephritis" (RPGN). Of course, the apparent onset of the disease may not be anywhere near its true beginnings. RPGN may also supervene in a patient with an indolent course of nephritis, for example, with membranous nephropathy, as discussed previously. Overall, the prognosis for patients with crescentic nephritis is poorer than that for any other form of nephropathy and relates in broad fashion in the untreated patient to the extent and severity of the crescent formation. Successful treatment of the two major groups (vasculitis and anti-GBM disease) has removed this correlation. Despite this generally poor outlook in the absence of treatment, occasionally patients make remarkable recoveries, sometimes without any specific intervention; however, few patients with crescentic nephritis escape treatment. A search of the literature from 1964 to 1980 shows 115 patients with crescentic nephritis who

TABLE 71-7

PROGNOSIS OF UNTREATED AND TREATED PATIENTS WITH CRESCENTIC-GLOMERULONEPHRITIS

Total no. of patients	No. not treated	No. judged to have "improved"	Total no. treated	No. judged to have improved
328	115	13 (11.3%)	213	90 (42%)

Summary of treated and untreated patients with crescentic glomerulonephritis (>50% of glomeruli with crescents).
World literature 1964–1980, excluding patients with (i) anti-glomerular basement membrane disease, (ii) known or suspected vasculitis, (iii) systemic lupus erythematosus, and (iv) Henoch-Schönlein purpura.

received no specific treatment, of which thirteen (11.3%) made a spontaneous recovery (Table 71-7).

Prognostic Features

Most authors (422,426–429) reached the conclusion that within this severely affected group, the following clinical and histologic features indicate a (relatively) favorable prognosis: (a) history of prior infection, (b) presence of a urine output, (c) lesser extent of crescent formation, (d) absent or mild tubular atrophy and interstitial fibrosis, (e) presence of glomerular immune aggregates shown by immunohistology or electron microscopy, (f) presence of endocapillary proliferation, (g) intact Bowman's capsule, and (h) ANCA titers.

On *histology,* the correlation with the proportion of glomeruli affected by extensive crescents has been mentioned previously (Table 71-8). In view of the difficulty in defining "crescents," the change in number and extent of crescents with time, and the small sample available, it is not surprising that the correlation is only approximate: Even when 100% of glomeruli are so affected, a proportion of patients may yet recover function. As usual, the extent and severity of tubulointerstitial disease correlates well with outcome, as reported for almost all other forms of glomerulonephritis. In one series (418) using a scoring system for severity of tubular atrophy and interstitial fibrosis, of 26 patients with scores of less than 3 (of 8), 14 patients showed improvement in renal function, which was sustained in 12. In contrast, of 13 patients with scores of 3 or higher, only 5 showed improvement and this was sustained in only a single patient. Therefore, tubulointerstitial changes together with the extent of crescent formation can give a very good idea of which patients may respond to treatment and point to those whose disease has evolved to a point at which recovery is likely.

TABLE 71-8

FREQUENCY OF AN OLIGOANURIC PRESENTATION IN CRESCENTIC GLOMERULONEPHRITIS

Crescents (%)	Patients with oligoanuria (%)
60–70	0
70–80	40
80–90	44
90–100	72
100	92

Excluding anti-glomerular basement membrane, polyarteritis, systemic lupus erythematosus, and Henoch-Schönlein purpura.
(Data from: Rees AJ, Cameron JS. Crescentic nephritis. In: Cameron JS, et al., eds. *Oxford Textbook of Clinical Nephrology.* London: Oxford; 1992:418, with permission.)

Persistently high or rising titers of ANCA are often associated with disease relapses (430,431). However, this association may not occur in 10% to 30% or more of those with such ANCA profiles during one or more years of follow-up, but the use of an elevation in ANCA titer as the sole parameter to justify immunosuppressive therapy cannot be endorsed. In a prospective study of 100 ANCA-positive patients observed over a 2-year period, relapse did not occur in 43% and 29% of those with a rise in ANCA titers by immunofluorescence and in PR3-ANCA titers by enzyme-linked immunosorbent assay (ELISA), respectively (430,431). Thus, treating all patients who have increases in ANCA titers would have resulted in unnecessary risks of toxicity in a substantial percentage of patients. Therapy should be instituted only on the basis of unequivocal evidence of clinical relapse, but patients with rising titers should be followed closely for signs of clinical activity. Conversely, if a patient was ANCA-positive during a period of active disease, a persistently ANCA-negative status is consistent with, but not absolutely proof of, remission. This was illustrated in a report in which 37 of 100 patients with ANCA-associated vasculitis suffered flares during the period of observation (430,431). Of these, 13 were ANCA negative at the time of relapse.

Clinical Course

Postinfectious nephritis, even when crescents are present, has a reputation of being more benign than comparable idiopathic disease; the apparently good prognosis in poststreptococcal nephritis is, however, the result—at least in part—of the inclusion in published data of patients with relatively minor degrees of crescent formation. In the analysis of Heaf et al. (432), only 51% of patients in the infection-related group had more than 80% of the glomeruli affected by crescents, compared with 69% of the anti-GBM group and 65% of the "idiopathic" group. The prognosis for recovery diminishes only when the number of glomeruli affected by occluding crescents reaches or exceeds 60%; the literature on poststreptococcal crescentic nephritis is particularly poor in its definitions of "extensive" crescent formation, as we have discussed elsewhere (17). Consequently, the prognosis for postinfectious nephritis with extensive crescent formation may not be as benign as supposed in the past.

Second, oligoanuria is a factor indicating a poor prognosis. In Table 71-8, the frequency of oligoanuria in relation to the extent of crescent formation is shown in data taken from five articles in the literature (406). Of 100 patients presenting with oliguria of less than 500 mL per 24 hours, a GFR of less than 3 mL/minute, only 20 showed any recovery of renal function, with or without treatment, which varied from series to series. Bolton and Couser (433) reached an even more pessimistic conclusion from their analysis of the literature: Only 7% of oligoanuric patients recovered function. Age, surprisingly, does not play a major part in determining recovery (432).

TABLE 71-9

PROGNOSIS OF CRESCENTIC GLOMERULONEPHRITIS IN RELATION TO ASSOCIATED GLOMERULAR DISEASE

Histologic diagnosis	No. of patients	"Improved"	Death or dialysis
Postinfectious	47	23 (49%)	24 (51%)
No immune deposits/immune deposits	70	13 (19%)	57 (81%)
Antiglomerular basement membrane disease	81	9 (11%)	72 (89%)
Vasculitis	69	4 (6%)	65 (94%)
All patients	521	124 (24%)	297 (76%)

(Reprinted from: Glassock RJ. A clinical and immunopathologic dissection of rapidly progressive glomerulonephritis. *Nephron* 1978;22:253, with permission.)

The outlook for different groups of crescentic nephritis varies, as might be expected. In Glassock's analysis (427) (Table 71-9) dating from the early and mid-1970s, the prognosis for patients with vasculitis and those with "idiopathic" immune deposit–negative crescentic glomerulonephritis—many of whom had vasculitis—was particularly poor. As shown in the next section, this "natural" history of vasculitic crescentic nephritis has been much modified for the better. Crescentic nephritis complicating proliferative glomerulonephritis, other than poststreptococcal nephritis, is rare in adults in developed countries, and few cases of crescentic MPGN (90) or IgA nephropathy (417) have been described. In general, the prognosis is worse than it is in patients not affected by crescents (see previous discussion in section on MPGN and IgA nephropathy) but the data are not extensive.

Apart from HSP, crescentic nephritis is rare in children. At least four extensive series have been published (408–411), for a total of 164 cases including 82 children with primary glomerulonephritis (postinfectious, MPGN). Only nine (5%) had anti-GBM nephritis, emphasizing the rarity of this form of nephritis in childhood. Fourteen children had crescentic nephritis without deposits, many (17%) had occult vasculitis, and the remainder had various forms of glomerulonephritis, including seven (4%) with crescentic IgA nephropathy; thus, this type of crescentic nephritis, secondary to identifiable glomerulopathy, appears relatively more common in children than in adults. Twenty-two children (13%) had postinfectious nephritis and 17 had MPGN (six had type II MPGN). One child had a juguloatrial shunt, and in 15, the crescentic nephritis could not be further characterized. Twenty-five, the largest single group, had HSP.

Clinicopathologic correlations were similar to those found in adults. A total of 84 of 158 children with follow-up entered end-stage renal failure, more than half within 1 month of onset and the remainder, from several months to 5 years later. No patient in the South West Pediatric Group series (409) entered into complete remission, but seven in the series from Paris (408) and three in London (410) did. The remainder retained proteinuria and hematuria with varying degrees of renal dysfunction. Therefore, the outlook for children with crescentic nephritis does not differ greatly from that for adults, and even patients with postinfectious glomerulonephritis may go into renal failure.

At several points in this section, the effects of *treatment* on the "natural" history of crescentic nephritis are mentioned (432–434). Of course, dialysis alone without specific treatment may allow time for spontaneous healing, when otherwise an early death from uremia could be expected. Treatment of both ANCA-related vasculitis and anti-GBM nephritis (discussed later) has had dramatic effects. What evidence is there that treatment affects the outcome of other forms of crescentic nephritis? The earliest data comes from Heaf et al. (432) who undertook an extensive analysis of the effects of treatment in all forms of crescentic nephritis, but their analysis included patients with both anti-GBM and vasculitic nephritis (Table 71-10). The "best" results are obtained with the use of intravenous methylprednisolone, which is relatively inexpensive, safe, and simple to administer, or with plasma exchange, which is none of these things. Bolton and Sturgill (435) and Couser (436) reviewed the use and results of intravenous methylprednisolone and concluded that about three-fourths of treated patients with idiopathic crescentic nephritis maintain or recover renal function (44 of 59 in Couser's (436) analysis). Because these results are as good as or better than those of more complicated regimens including prolonged anticoagulation or plasma exchange, this form of treatment seems preferable for all forms of crescentic nephritis, with the exception of anti-GBM nephritis, in which intravenous methylprednisolone appears to have little or no effect (435) (see later discussion).

The longer term outcome of patients with crescentic nephritis has been less studied (437–439), perhaps because until recently, survival of patients with severe disease was unusual. Long-term follow-up of responders has revealed that more than one-half have a sustained improvement in renal function, whereas some patients slowly progress to ESRD. The series of Bruns et al. (437) included eight patients with crescentic nephritis without immune deposits (i.e., probable vasculitis) and two patients with anti-GBM disease. However, in the remaining 13 patients, the disease was immune complex–related and its evolution is of interest here. Seven patients entered chronic renal failure after temporary recovery, two within a few months, but five from 1 to 2 years later, and four remained in various degrees of renal failure (P_{creat} of 2 to 4 mg/dL) for 2 to 11 years. Despite the initial clinical improvement, repeated renal biopsy usually shows a marked increase in glomerulosclerosis, presumably reflecting healing of injury induced during the active stage of the disease (440). Therefore, early therapy is essential in this disorder to minimize the inflammatory damage that leads to late glomerular scarring.

Anti-GBM Nephritis

The prognosis of anti-GBM nephritis, with or without pulmonary hemorrhage (Goodpasture's syndrome), is now very different from that described two or three decades ago (441) (Chapter 60). First, many of the older descriptions were unknowingly contaminated by patients who had pulmonary hemorrhage as part of vasculitic syndromes (442). Second, as anti-GBM antibody assays have become widely available, a group of less severely affected patients have been described with more

TABLE 71-10

APPARENT INFLUENCE OF TREATMENT ON PROGNOSIS IN CRESCENTIC GLOMERULONEPHRITIS (ALL TYPES)

		Results						
Treatment	No. of patients	Alive, normal renal function (%)	Alive, abnormal renal function (%)	In uremia (%)	In dialysis (%)	Death (%)		Uremia + dialysis + death
Symptomatic	82	3	7	3	42	45	=	90
Immunosuppression	144	11	15	4	22	47	=	71
Anticoagulation[a]	94	13	9	4	27	47	=	78
Plasma exchange	117	3	30	25	29	13	=	65
Methylprednisolone	39	20	36	7	24	12	=	43
Plasma exchange and methylprednisolone	7	0	42	0	42	14	=	56

[a]Regarding these data, it must be remembered that comparable patients were not allocated to all the treatment groups and that some data are predominantly recent (e.g., plasma exchange) and others less recent (e.g., immunosuppression alone).
Often combined with immunosuppression.
(Reprinted from: Heaf JG, Jorgensen F, Nielsen NP. Treatment and prognosis of extracapillary glomerulonephritis. *Nephrons* 1983;35:217, with permission.)

indolent disease arising from anti-GBM antibody who do not have extensive crescent formation (443). Finally, treatment has modified the "natural" history of the condition in the last 15 years.

We realize now that anti-GBM disease may present first with overt or occult pulmonary hemorrhage and with anemia, and only later will renal manifestations supervene. Some patients who show linear deposits of anti-GBM antibody along the GBM do not have clinical manifestations of renal disease. Some patients have no lung hemorrhage, some have occult lung hemorrhage detectable by the presence of severe anemia or a raised K_{co} (pulmonary carbon monoxide transfer), whereas in others the pulmonary hemorrhage is the dominant feature of the disease and may be life-threatening.

At first, the prognosis for anti-GBM nephritis was worse than that for almost any other form of nephritis, at least with regard to renal function (Table 71-9). Some additional patients died from pulmonary hemorrhage with relatively good renal function. Therefore, only 18 of 136 patients reported in the 1960s and early 1970s survived. Even as recently as 1977, Beirne et al. (426) found only 3 of 17 patients who retained independent renal function and survived. Although occasional dramatic recoveries have been described, even in those requiring dialysis (444), they usually are related to patients without very extensive crescent formation (3 of 13 glomeruli in the case just cited) (444).

Plasma exchange to remove the circulating antibody, together with cyclophosphamide to prevent its resynthesis, has changed the outlook for anti-GBM disease dramatically (Chapter 60), although no controlled studies are available. It has become evident that plasma exchange is normally effective in protecting renal function only if the patient is treated early enough, before oligoanuria and a requirement for dialysis has become established (Table 71-11). Among patients already requiring dialysis (445,446) very few recover renal function. Walker et al. (447), however, reported more encouraging results: 5 (45%) of 11 oligoanuric patients in their series of 22 patients recovered renal function. Savage et al. (448), as well as reviewing their extensive personal data from the Hammersmith Hospital (Table 71-11), reviewed collected data from other units on 59 patients presenting between 1980 and 1984. Of a total of 58 patients of 108 local and collected patients who were oligoanuric and who received treatment with plasma exchange and cyclophosphamide, 18 died and 51 continued on dialysis; only 7 patients (13%) requiring dialysis recovered function with aggressive treatment. In the series from Herody et al. (449) in Paris, only 16 of 29 patients required dialysis, illustrating the changed presentation of anti-GBM disease. The more uremic patients did worse. Twenty-five of the 35 patients of Merkel et al. (450) developed ESRD. In both series, the combination of extensive crescents and oligoanuria predicted a poor outcome. More severe disease and a poorer outlook are associated also with the HLA type; those with HLA-DR2 along with HLA-B7 doing poorly (451).

There are few longer term data on the survival of patients with anti-GBM disease; renal survival is obviously much poorer than patient survival, even though patients are at risk from pulmonary hemorrhage and the effects of intensive immunosuppression. Turner and Rees (441) noted eight deaths (28%) during the first year among 29 patients seen at the Hammersmith Hospital between 1976 and 1988. All occurred in patients with extensive crescents, an initial plasma creatinine concentration of more than 600 μmol/L, and pulmonary hemorrhage. Similarly, 8 (36%) of 22 patients in Walker's series (447) died within 12 months. Savage et al. (448) noted 15 deaths (25%) within 8 weeks in their collected series of 59 British patients seen during 1980–1984, half of which were the result of lung hemorrhage and 14 in patients requiring dialysis. In the patients seen at the Hammersmith Hospital, 8 (16%) of 49 patients died, all but 1 among those requiring dialysis, and most dying from lung hemorrhage. The later mortality is not given for either group, so 1-year figures are not available. However, Merkel et al. (450) and Herody et al. (449) noted only four deaths in the 64 patients in their two series, with 37 remaining alive on dialysis and 23 with recovery or retention of some renal function. Only eight patients had a GFR within the normal range, including one patient without clinical renal involvement.

Therefore, an important determinant of the response to therapy is early diagnosis. There is a direct correlation between the initial plasma creatinine concentration and the percentage of glomeruli with crescents; in particular, crescents are present in more than 75% of glomeruli when the plasma creatinine concentration is greater than 5 mg/dL (442 μmol/L). Avoidance of maintenance dialysis is rare in patients who require dialysis within 72 hours of presentation, particularly in those who have crescents involving all glomeruli. In comparison, prevention of ESRD can usually be achieved in less severe

TABLE 71-11

IMMEDIATE OUTCOME IN PATIENTS WITH ANTIGLOMERULAR BASEMENT MEMBRANE NEPHRITIS

Presentation P_{creat} (μmol/L)	N	Received full treatment[a]	Outcome after 8 wk: Improved	Requiring dialysis	Death
Patients from British Renal units 1980–1984 (N = 59)					
Normal	3	2	3	—	—
<600	5	5	2	2	1
>600	7	7	—	4	3
Dialysis-dependent	44	33	—	33	11
Patients seen at the Royal Postgraduate Medical School 1974–1984 (N = 49)					
Normal	4	4	3	—	—
<600	15	15	13	2	—
>600	5	5	1	4	—
Dialysis-dependent	25	25	—	18	7

Treatment	5-yr survival (%)	Author	Year
Supportive only	13	Rose and Spencer	1957
	13	Frohnert and Sheps	1964
	12	Leib et al.	1979
Corticosteroids	62	MRC (U.K.)	1960
	48	Frohnert and Sheps	1967
	57	Sack et al.	1975
	61	Cohen et al.	1980
Corticosteroids plus cytotoxics	80	Leib et al.	1979
	55	Cohen et al.	1980
Corticosteroids plus cytotoxics methylprednisolone IV and plasma exchange in severe cases			
N			
53	38 Both	Serra et al.	1984
34	65 Micropolyarteritis	Savage et al.	1985
36	64 Both	Coward et al.	1986
43	62 Micropolyarteritis	Adu et al.	1987
26	80 Both	Fuiano et al.	1988
54	56 Wegener's	Gaskin and Pusey	1992
49	64 Micropolyarteritis	Gaskin and Pusey	1992
17	74 Wegener's	Cameron	1991
35	56 Micropolyarteritis	Cameron	1991

[a]Full treatment, at least 8 weeks of cyclophosphamide + plasma exchange for 2 weeks.
(Reprinted from: Savage COS, et al. Antiglomerular basement membrane antibody–mediated disease in the British Isles 1980–4. *Br Med J* 1986;292:301, with permission.)
In the earlier series (pre-1980, e.g., Leib et al.), many patients had either no renal disease or only minor renal disease. In addition, most of these early series included some patients with large-vessel vasculitis (polyarteritis nodosa). Thus, the data are not strictly comparable with those of more recent series consisting almost exclusively of patients with either Wegener's granuloma or microscopic polyarteritis, with severe (usually crescentic) glomerulonephritis.

cases, although some do progress. From all the presented data in the preceding text one can predict that about one-fourth of patients with anti-GBM nephritis can be expected to recover or maintain renal function, but only 10% of this 25% will have a normal GFR. The other 15% of the total will remain dialysis-dependent, and of these about 10% will die within 12 months, most within the first 3 months, often from pulmonary hemorrhage. Among those who recover or maintain renal function, a proportion subsequently develop uremia (447,452) and require dialysis or transplantation later. This "late" deterioration presumably arises through whatever mechanisms operate in remnant kidneys, rather than through specific immunologic attack. Return of active disease during follow-up is exceptionally rare, although it has been described (453), which agrees with the hypothesis that anti-GBM disease results from a single time-limited episode of autoimmunity, the duration and severity of which can be inhibited by removal and prevention of resynthesis of the causative autoantibody.

ANCA-mediated Vasculitis

Wegener's Granuloma, Microscopic Polyarteritis, and the Churg-Strauss Syndrome. Wegener's granulomatosis is a systemic vasculitis of the medium and small arteries, as well as the venules, arterioles, and occasionally large arteries. "Classic" Wegener's granulomatosis primarily involves the upper and lower respiratory tracts and the kidneys. A "limited" form, with clinical findings isolated to the upper respiratory tract or the lungs, occurs in approximately one-fourth of cases. Microscopic polyangiitis is a vasculitis that primarily affects capillaries, venules, or arterioles. Involvement of small and

medium-sized arteries may also be present. This disorder is thought by some investigators to represent part of a clinical spectrum that includes Wegener's granulomatosis, since both are associated with the presence of ANCA, with similar histologic changes outside the respiratory tract (e.g., a focal necrotizing, pauci-immune glomerulonephritis), and have similar outcomes. Although the similarities between these diseases are generally accepted, histopathology in microscopic polyangiitis does not include granuloma formation, and the ear, nose, and throat are generally spared.

Until recently, there were few data on the long-term survival of such patients because untreated survival was so miserable (454–460). The 2-year survival rate of untreated vasculitic patients with severe nephritis was essentially zero, and there is overwhelming evidence—albeit uncontrolled—that aggressive treatment has modified the outlook dramatically over the last two decades. Although Fauci et al. (457,458) were the first to report the transformation of a 20% 2-year survival rate to a 93% survival rate at the same point in patients with vasculitis, scrutiny of their reports shows that most of their patients had no or only trivial renal disease.

Modern aggressive treatment of vasculitic nephritis is centered on the use of cytotoxic agents (usually cyclophosphamide) and corticosteroids (454,461–466). There is evidence that this more aggressive regime improves results, decreasing the number of patients who die of the vasculitis in the acute phase (463). For patients with advancing or severe renal failure, *many* units employ intravenous methylprednisolone, plasma exchange, or both (461–466). Taken together, the results of several small controlled trials (467–470) indicate that although the addition of plasmapheresis is of no benefit in the predialysis period, it may improve long-term survival in dialysis-dependent patients. In contrast, there is no evidence to suggest that intravenous administration of cyclophosphamide during the acute phase has any advantage (471), although intermittent intravenous cyclophosphamide may have benefits over long-term azathioprine in the longer term. No trial comparing the two has been performed, however.

Nachman et al. have recently published their experience with treatment of 97 patients with ANCA-associated microscopic vasculitis or ANCA-associated necrotizing crescentic nephritis (472). Patients received prednisone alone or prednisone with cyclophosphamide for about a year after the pulse therapy with methylprednisone. Because this was not a randomized controlled trial, multivariate analysis was performed to assess treatment response and relapses. Overall, 75 of the 97 patients (77%) went into remission. Of the 75 responders, 32 patients (43%) remained in long-term remission. Twenty-nine percent of the initial responders suffered a relapse that generally occurred within 18 months from the end of therapy. There was a significant difference in the remission rate between corticosteroid-treated patients and cyclophosphamide-treated patients (56% versus 89%; $p = 0.003$). The cyclophosphamide-treated patients had three times less risk of experiencing a relapse than did corticosteroid-treated patients.

Prognostic markers for patient and renal survival were analyzed in the same cohort of patients (473). The important clinical markers that predicted patient death were presentation with pulmonary hemorrhage, presence of c-ANCA (compared with p-ANCA), and treatment with steroids alone. Conversely, serum creatinine on presentation, race (African Americans worse than whites) and presence of arteriosclerosis on renal biopsy predicted renal survival.

Unlike in patients with anti-GBM nephritis, recovery of renal function in patients with vasculitis and crescentic nephritis who are already dialysis-dependent and oligoanuric is common, almost the rule (462–466,474). Death still occurs from other complications of the vasculitis, but now the main cause of early death is sepsis from the intense immunosuppression in patients who are frequently older adults and often frail. In earlier cases, reversal of mounting uremia is also usual. About three-fourths or more of patients can be expected to survive the early phases of the disease (462,464,474–476).

The rate for longer term patient survival using these forms of treatment is between 50% and 80% at 5 years (463–466,475,476), with similar figures reported for the few patients studied for as long as 10 years. The redefinition of this group of patients using ANCA has not altered prognosis (477,478). Unlike patients with anti-GBM disease, rather few patients survive requiring dialysis after the acute attack. For example, of 60 patients with Wegener's granuloma, Gaskin and Pusey (479) noted severe renal disease in 54. Eleven patients (20%) died during the first 2 weeks, seven among the 24 requiring dialysis, mostly the result of pulmonary complications (hemorrhage and infection); only 3 of the 24 continued on dialysis. There were three additional later deaths in those who recovered function, all the result of infection. The actuarial survival rate was estimated to be 70% at 1 year and 56% at 5 years. Similarly, of their 49 patients with microscopic polyarteritis (479), 14 initially required dialysis, 4 died without recovering function, 8 recovered function, 2 died shortly thereafter, and only 2 continued on dialysis. The patient survival rate was 78% (38 of 49) at 8 week, and renal survival was 73% (36 of 49). When Wegener's granuloma and microscopic polyarteritis are compared, even when allowing for the extent of renal damage, usually the patients with Wegener's granuloma appear to do a little better (465,479), but the difference is not striking. The patients who die later (460,479) usually still have active but suppressed so-called smoldering disease (479), but the mortality rate has recently been reduced. An additional problem with Wegener's granuloma is that many patients with this condition have little or no renal involvement (480,481), and not surprisingly, the presence of renal disease is an indicator of poorer prognosis (480,481). In some patients renal vasculitis reactivates after prolonged remission (480), even after the patient has received a transplant (482,483). After the dramatic improvement in prognosis during the 1970s and 1980s for vasculitic patients, it appears that prognosis has now stabilized and further improvement was not seen during the last decade (484).

Factors that predict outcome are now obscured by the relative success of treatment. Clinically, as already mentioned, oligoanuria does not predict failure of treatment, although as a group, oligoanuric patients do rather worse. Histologically, the extent of crescent formation also has ceased to be a predictor of longer term outcome in this data (465), although it correlates with renal function at presentation (Fig. 71-17).

Patients with vasculitis who survive the initial illness may have a relapsing course, in which the frequency depends very much on the intensity of the immunosuppression. Therefore, in the series of 52 vasculitic patients seen during the 1980s, almost all of whom were maintained on prednisolone and azathioprine for 5 years or more, relapses were rare (465). ANCA titers may be of help in diagnosing the possibility of relapse early (485,486) and in modulating immunosuppression, because relapse is unlikely when the ANCA titer is normal. Persistently high or rising titers of ANCA are often associated with disease relapses (430,431). However, this association may not occur in 10% to 30% or more of those with such ANCA profiles during one or more years of follow-up. In a prospective study of 100 ANCA-positive patients observed over a 2-year period, relapse did not occur in 43% and 29% of those with a rise in ANCA titers by immunofluorescence and in PR3-ANCA titers by ELISA, respectively (430,431). Patients with rising titers should be followed closely for signs of clinical activity. Conversely, if a patient was ANCA-positive during a period of

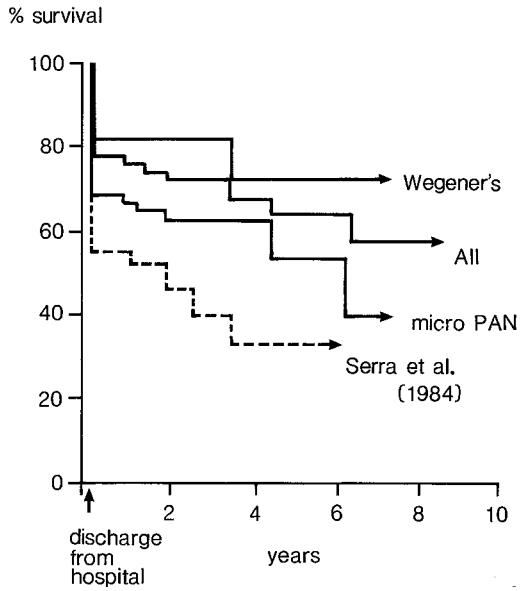


FIGURE 71-17. Probability of survival of patients presenting with renal vasculitis, 1981–1990. Guy's Hospital data are from 1980 to 1989 (429). Wegener's, 17 patients; microscopic polyarteritis (micro PAN), 34 patients; total, 51; mean age, 65 years; 19 requiring dialysis. Also shown are the data of Serra et al. (419) published in 1984 on the outcome of 53 patients presenting during the decade preceding the Guy's Hospital study. Overall survival has improved ($p<0.05$) in association with more aggressive treatment (see text), and the major cause of death is no longer the vasculitis itself but side effects of the more intense immunosuppression. Early mortality during the acute illness, although reduced, remains the main problem.

active disease, a persistently ANCA-negative status is consistent with, but not absolutely proof of, remission.

Vasculitis is rare in childhood (487), with the sole exceptions of the infantile Kawasaki disease, which usually spares the kidney, and HSP, which is addressed in the next section. Habib (488) reviewed a relatively large experience of 16 children with Wegener's granuloma or polyarteritis, whose clinical picture did not differ from that in similar adults.

There are few data on the outcome of renal disease in the Churg-Strauss syndrome, although it is now recognized that renal involvement is more common and more severe than what was thought in the past. Clutterbuck et al. (489) described the outcome of 19 patients whose renal disease resembled histologically that of Wegener's granuloma or microscopic polyarteritis. However, the prognosis was better, with only one patient requiring transplantation, five having varying degrees of chronic renal failure, and the remainder maintaining renal function for up to a decade. Only one patient died, of a restrictive cardiomyopathy.

Henoch-Schönlein Purpura Nephritis

HSP occurs more often in children than in adults, and many cases follow an upper respiratory tract infection, suggesting that the precipitating antigen may be infectious. In a review from the United Kingdom, the annual incidence was about 20 per 100,000 children (peak 70 per 100,000 between the ages of 4 and 6 years) and was little influenced by skin color or ethnicity (490). The prognosis of HSP without clinical nephritis is excellent (491,492) (Chapter 61), and nephritis forms the major cause of mortality and morbidity, apart from occasional problems with intussusception and other gastrointestinal tract complications (Chapter 61). Nephritis may appear with the initial attack but is particularly associated with recurrent attacks of purpura (493) that may continue in exceptional patients for months or even years. Even so, compared with that for most other forms of glomerulonephritis in children or adults, the prognosis even with nephritis remains good (494). Overall, after 2 years, half the children with HSP nephritis will be in complete remission, whereas one-third show persisting urinary abnormalities with normal renal function.

Few children (perhaps 3%) progress to renal failure immediately, and their renal biopsy specimens usually show extensive crescent formation (409,410). No more than 19% show continued urinary abnormalities with reduced renal function. By the time 10 years has passed (495), most of the children who had urinary abnormalities but normal renal function at 2 years will have gone into remission, but a proportion of those with decreased renal function will have progressed to renal failure.

It is difficult to judge how many will eventually do so, but Habib and Cameron (494) reported that 32 (17%) of 188 patients went into renal failure, and the total would probably be about 20%. These data come from two highly specialized referral centers, and the proportion of children with more severe disease is inevitably exaggerated in these figures (see the first section of this chapter). The prognosis for unselected children with HSP nephritis is undoubtedly better than this, but by how much is impossible to say exactly. In the series from Kobayashi et al. (496), only 3 (2.7%) of 123 children with nephritis died, and 106 (86%) were in complete remission. In the study by Koskimies et al. (491), only 1 child of 39 still showed signs of evident nephritis and another 2 had chronic disease. Kobayashi et al. (496) calculated a final remission rate of 91%, with a 9% renal failure–death rate. However, although the benign nature of the disease is usually emphasized, HSP nephritis accounts for about 3% of children going into end-stage renal failure (497).

It would be useful to know at the outset which children would be most likely to have a severe course, so treatment could be concentrated on the minority who might require it. There is some correlation between clinical onset and outcome (Table 71-12). In general, patients with only symptomless urinary abnormalities do well, whereas those with a nephrotic syndrome, especially when combined with renal insufficiency, do poorly (494,495,498–500). However, there are exceptions in both directions. Another adverse feature is age at onset (Table 71-13); there was, however, no relation with serum IgA concentrations (497,499).

The best guide to outcome is a combination of clinical data (Table 71-12) and biopsy data (494,501) (Table 71-14). Almost all of those patients without diffuse glomerular proliferation and with less than 50% of glomeruli affected by segmental lesions or crescents did well. However, those with more extensive crescent formation tended to do poorly. Of children with diffuse proliferative glomerulonephritis, only half of those who had less than 50% of glomeruli affected by segmental lesions healed clinically; the remainder showed persisting disease. In those with more than 50% of glomeruli affected by segmental lesions, the evolution was favorable much less often, and in those with more than 80% of glomeruli affected by crescents, evolution was usually into renal failure (12 of 19 patients). It is in this group that aggressive treatment with intravenous methylprednisolone and plasma exchange is usually concentrated (492). Two children in this group, however, recovered completely without aggressive intervention. Overall, the greater the number of glomeruli affected by crescents, the poorer the prognosis (409,410).

There are few data on the very-long-term outcome of children with HSP nephritis, but recently 20-year data about (502) patients studied in the past (493,501) was published. One important finding was that seven children who had apparently recovered completely according to the last analysis in 1976 had deteriorated. Schaerer et al. (503) have published data on 64 children followed for two decades. Renal survival at

TABLE 71-12

OUTCOME IN RELATION TO CLINICAL ONSET (10 YEARS FROM ONSET) IN HENOCH-SCHÖNLEIN NEPHRITIS

	Outcome (no. of patients)				
	Recovery	Minor urinary abnormalities	Moderate nephropathy	Renal failure	Total
Hematuria only	4	0	1	0	5
Proteinuria + hematuria	31	3	3	3	40
Proteinuria + hypertension	7	0	0	1	8
NS + hematuria	8	0	0	1	9
"Nephritic-nephrotic" syndrome	11	3	5	7	26
Total	61	6	9	12	88

NS, nephrotic syndrome.
(Reprinted from: Counahan R, et al. Prognosis of Henoch-Schönlein nephritis in children. *Br Med J* 1977;2:11, with permission.)

10 years was 73%, with initial renal insufficiency being the most powerful predictor of an adverse outcome.

Discussion of HSP nephritis in adults is made difficult by several circumstances. First, a number of older articles that purported to deal with the topic do not distinguish between HSP, other forms of vasculitis, and essential cryoglobulinemia, even with the technology available when the data were collected (504). Second, some articles dealt with adults and children and it is impossible to separate the data. Third, in some reports, patients without renal involvement cannot be separated from those with nephritis. And finally, because of the relative rarity of HSP nephritis in adult life, no single series is large enough to form conclusions or do statistical analyses.

However, based on the published literature (505–518) and data from a national survey of 47 patients with HSP nephritis in the United Kingdom seen from 1976 to 1986 (519), the clinical manifestations do not differ much from those in children, although a history of prior drug ingestion and severe arthralgia (80%) are more common, and abdominal signs and symptoms less common (35%). The purpura more frequently ulcerates in adults with HSP, particularly older adults, than in children. The condition may be seen even in the ninth decade of life; there is some suggestion that in younger adults, older individuals with the childhood disease are present in decreasing numbers with increasing age; then there is a "bulge" of cases in the 50- to 70-year-old age-group, corresponding to that for other forms of adult vasculitis. Coppo et al. (520) examined prognosis in a collaborative study of adults and children with Henoch-Schönlein purpura, all of whom had nephritis. In general, outcomes were similar, although slightly more adults (15.8% of 97) than children (7% of 57) entered end-stage renal failure. Two further series compared unselected adults and children with Henoch-Schönlein purpura, both with and without renal disease (521,522), noting a higher incidence of renal disease in older patients and an association of renal disease with upper body purpura. Again, most adults (89%) and children (94%) recovered completely.

However, the long-term prognosis may not be benign in all patients who appear to recover from the acute nephritic episode (523–525). A long-term retrospective study of a cohort of 250 adults with HSP and renal involvement of sufficient severity to require biopsy also reported that the renal outcome of some patients is relatively poor (523). At a median follow-up of nearly 15 years, 11% and 13% had become dialysis-dependent or had severe renal failure (creatinine clearance of less than 30 mL/minute), respectively. Sclerosis and fibrosis on initial renal biopsy correlated closely with poor outcomes.

The renal histology in adults is similar to the findings in children, and most interest has centered on whether the prognosis in the adult differs from that in children (Table 71-15). There is a suggestion that adults do a little worse than the selected children seen in referral centers, but again the adults are even more highly selected. Overall, 35 (12%) of 282 selected patients had gone into ESRD or died of renal failure, although the duration of follow-up differed greatly in the various studies included in Table 71-15. Death, as might be expected in this older population, sometimes resulted from unrelated causes (21 of 282, 7.4%). In the United Kingdom study of 43 patients (519), after 1 to 7 years of follow-up, 15 were in complete remission, 17 had urinary abnormalities (12 with normal renal function), 4 were receiving treatment for ESRD, and 7 had died (2 with normal renal function). Some patients with HSP nephritis may apparently heal but present with severe hypertension 10 to 20 years later (526), so blood pressure should be checked at least once a year, indefinitely.

TABLE 71-13

OUTCOME IN RELATION TO AGE AT ONSET IN HENOCH-SCHÖNLEIN PURPURA NEPHRITIS

	Age at onset (yr)		
	<6	6–11	<12
Recovery	19	38	3
Minor urinary abnormalities	2	4	0
Moderate nephropathy	2 (12.5%)	6 (26%)	1 (50%)
Renal failure	1	9	2
Total	24	57	6

(Reprinted from: Counahan R, Cameron JS. Henoch-Schönlein nephritis. *Contrib Nephrol* 1977;7:143, with permission.)

Systemic Lupus Erythematosus

The outlook for patients with clinically evident nephritis in a setting of systemic lupus erythematosus is difficult to summarize for several reasons (Chapter 65): First, being a multisystem disease, extrarenal organ involvement may have a major effect on the mortality and morbidity of patients with lupus, even those in whom the main involvement is in the kidney (527–530). Second, even in its "natural" untreated state, the disease may manifest over a very wide range of activity, from a limited indolent condition to a fulminant aggressive disorder affecting

TABLE 71-14

OUTCOME IN RELATION TO INITIAL BIOPSY APPEARANCE IN PATIENTS WITH HENOCH-SCHÖNLEIN NEPHRITIS FOLLOWED FOR MORE THAN 1 YEAR

	Recovery	Minimal urinary abnormalities	Persisting disease	Renal failure
Nonproliferative glomerulonephritis				
No crescents	1	0	0	0
Crescents <50%	17	9	1	2
Crescents >50%	0	0	1	3
Proliferative glomerulonephritis				
No crescents	1	1	1	0
Crescents <50%	4	4	6	1
Crescents 50–80%	3	2	3	2
Crescents >80%	2	4	1	12
Total	28	20	13	20

(Reprinted from: Levy M, et al. Anaphylactoid purpura nephritis in childhood: natural history and immunopathology. *Adv Nephrol* 1976;6:182, with permission; and from: Habib R, Broyer M, Levy M. Schönlein-Henoch purpura glomerulonephritis in children. In: Strauss J, ed. *Pediatric Nephrology 4*. New York: Garland; 1977:155, with permission.)

almost all major organ systems. Third, almost any pattern of glomerulopathy may be seen in renal biopsy specimens (Chapter 65). Fourth, the appearances may transform in sequential biopsy specimens, both spontaneously and in response to treatment (531–534).

The overall 10-year survival for patients with lupus is greater than 90% (535). Patients with nephritis still have worse survival rates than those without nephritis (88% versus 94%, $p < 0.05$) (535). During the last 20 to 30 years, however, the major differences in outlook for those with different WHO classes of nephritis (which had been evident in earlier studies [536,537]) have disappeared (Table 71-16). This is likely because treatment has been more aggressive in those with clinically or histologically more severe forms of nephritis (538,539). Those with severe proliferative lupus nephritis (WHO class IV) appear to have benefited from improved therapies (540,541), and renal failure is no longer the principal cause of mortality in this population (538,542,543). Awareness of lupus as a diagnosis is greater now than it was 30 years ago and relatively specific tests for the condition are now widely available. Thus, it is possible that some of the "improvement" in the survival of all patients with lupus results from the inclusion of milder forms in recent series, or due to earlier diagnosis of the disease (544).

TABLE 71-15

FOLLOW-UP IN PATIENTS WITH ADULT-ONSET HENOCH-SCHÖNLEIN PURPURA NEPHRITIS

							Outcome in those with f/u[a]			
Ref.	Author	Country	Period	*N*	Age (yr)	Male: female ratio	f/u (yr)	Remitted	Persisting disease (reduced function)	ESRD/dead[b]
Series in which all patients had renal involvement										
(507)	Ballard	U.S.A	1954–58	14	>20	14:0	Not stated	8	1 (1)	5/0
(512)	Fillastre	France	1964–71	28	>20	22:6	?–13	8	7 (3)	2/1
(514)	Kalowski	Australia	1959–71	18	>16	13:5	Mean 3.7	5	9 (2)	1/3
(517)	Roth	U.S.A.	1968–83	9	>16	9:0	Not stated	7	2 (1)	0
(515)	Lee	Korea	1979–82	17	>16	9:8	Mean 3.2	10	7 (0)	0
(510)	Esteve	Spain	Not stated	11	>16	5:6	Not stated	3	5 (0)	3
(511)	Faull	Australia	1975–85	27[c]	>12	16:11	0.16–9.0	6	16 (6)	2/3
(519)	Knight	U.K.	1976–86	47	>20	34:13	1–7	15	17 (5)	4/7
(513)	Fogazzi	Italy	1967–88	16	>15	11:5	1–20	2	11 (9)	3/0
				187	—	133:54	—	64	75 (27)	20/14
Series in which only some patients had renal involvement										
(509)	Debray	France	?–1971	22 (10)	>15	12:10	4	5	2 (0)	0
(508)	Bar-On	Israel	1959–69	21 (12)	>15	12:9	0.5–6.0	4	2	2/0
			Totals		209	—	—	73	79 (27)	22/14

f/u, follow-up; *ESRD*, end-stage renal disease.
[a]Normal urine plus hypertension counted as continuing disease in f/u.
[b]Dead, dead from causes other than renal failure.
[c]24 Patients older than 20 years.

TABLE 71-16

FIVE-YEAR ACTUARIAL SURVIVAL RATES FOR LUPUS, LUPUS NEPHRITIS, AND WORLD HEALTH ORGANIZATION CLASS IV NEPHRITIS OVER THE LAST 40 YEARS

	% 5-year survival (weighted mean of published series)		
Period	All lupus (no. of papers)	Lupus nephritis (no. of papers)	Class IV nephritis (no. of papers)
1953–1969	49% (4)	44% (3)	17% (2)
1970–1979	82% (6)	67% (13)	55% (9)
1980–1989	86% (5)	82% (6)	80% (3)
1990–1995	92% (3)	82% (5)	82% (4)

A number of new treatment regimens offer the prospect of improved outcomes, decreased toxicity, and possibly both (545). Because of the small number of patients now dying or going into ESRD, however, many of the recent studies are too short or too small to adequately compare these newer regimens. Clinicians now face the difficult decision of treating patients with less well-established therapy in order to minimize the side effects of therapy. Risk stratification and close clinical follow-up are, therefore, as important as ever.

Causes of Death

Because it is a multisystem disease, the causes of death in lupus are much more varied than those in other forms of primary glomerulonephritis that are seen in young adults and that are confined to the kidney. Sepsis, not surprisingly, emerges as one of the major causes of death (527,530,546–548) promoted or potentiated by immunosuppressive treatment. However, other manifestations of extrarenal lupus may prove fatal as well (527,530,546–548). Rather surprisingly, however, recent survival curves for renal function and for the patient are almost identical in most series (538,548,549). A number of observers have commented that in lupus patients, entry into chronic renal failure may be preceded by long periods of clinical quiescence (550,551), suggesting that the mechanisms other than continued activity of the nephritis determine the fate of the kidneys. A further cause of late death, even in young women with lupus, is severe atheromatous myocardial and other vascular diseases (44,46,527,547,548). The pathogenesis of these late vascular lesions is not clear, but corticosteroid treatment, hyperlipidemia, and immune complexes may all play a part. Interactions between immune complexes and hyperlipidemia have been shown in experimental animals to accelerate atheroma formation (42,43,552).

Predictors of Prognosis

The response to therapy and maintenance of remission are of prognostic importance, highlighting the necessity of adequate treatment. Korbet et al. (553) reported on the long-term experience of the Lupus Nephritis Collaborative Study Group. Defining remission as the combination of serum creatinine level of less than or equal to 1.4 mg/dL and proteinuria of less than or equal to 330 mg per day, these authors found a 10-year renal survival of 94% in patients achieving remission in contrast to only 31% in those failing to achieve remission. The importance of remaining in remission has been underscored by the study of Moroni et al. (554), who described the effect of renal disease "flares" (significant increase in proteinuria or serum creatinine level) on long-term renal function. In a group of 70 patients followed for a median of more than 10 years, the likelihood of adverse renal outcome (defined as doubling of serum creatinine level) was increased nearly sevenfold in patients with a flare of renal disease compared with those with no renal disease flares. In patients with flares characterized by a rapid rise in serum creatinine level, the risk of poor renal outcome increased 27-fold. In a study of Chinese patients with WHO class IV disease who were treated with cyclophosphamide, 38% of the patients had renal flares with a mean follow-up of 8 years after the last dose of cyclophosphamide (555). Significant independent risk factors for renal flares were chronicity score, persistently positive anti-dsDNA, and the absence of maintenance therapy (azathioprine in this study). Furthermore, nephritic flares were an independent risk factor for doubling of the serum creatinine in this study. It appears clear, therefore, that the goal of therapy must be the achievement and maintenance of remission with stable renal function and minimal proteinuria.

Race is an obvious target for analysis, because the incidence and prevalence of lupus are so much higher in blacks and Asians than in whites (556–559). Although some studies comparing black and white subjects have found no difference in outcome (528,560–564), several large studies have shown a poorer outcome for black subjects (556,565). Tejani et al. (566) suggested that black children with an onset before puberty form a particularly high-risk group. Gordon et al. (567), examining national mortality from lupus in the United States, again suggested a higher mortality among black women. Similar results have been obtained from the Glomerular Disease Collaborative Network at the University of North Carolina as reported by Dooley et al. (568). In contrast to whites, in whom 95% retained renal function 5 years after the diagnosis of lupus nephritis, African Americans had a significantly worse course, with only 58% retaining renal function at 5 years. The difference was not attributable to age, duration of lupus, hypertension, or biopsy indices of disease activity or chronicity. The difference in outcomes in black Americans compared to white Americans may, in part, be confounded by socioeconomic status (561,565). Reveille et al. (569) reported black race as a risk factor for renal disease and poorer survival and systemic lupus erythematosus. This risk was independent of socioeconomic status. Barr et al. (570), however, found that race was no longer a significant predictor of progression when adjusted for socioeconomic factors. This study also concluded that socioeconomic status, itself, is an important risk factor for disease progression. The potential role of biologic or genetic factors in the observed racial heterogeneity of outcomes remains to be defined, although some genetic polymorphisms have been suggested (571).

Clinical severity of nephritis may be a predictor of outcome. Although many patients with initially severe disease may have a favorable outcome, several studies have found that a raised plasma creatinine concentration at the onset of disease is strongly associated with a poorer outcome (548,553, 556,564,572–574). The magnitude of proteinuria or the

presence of nephrotic syndrome is more controversial. Several reports (78,549,562) have not noted a difference in outcome in those with nephrotic range proteinuria compared to those with lesser degrees of proteinuria in univariate analysis. However, Esdaile et al. (564) and Appel et al. (575) found poorer survival in their nephrotic patients. In the data by Appel et al. (575) this was particularly true in those whose nephrotic syndrome persisted. In multivariate analysis, the degree of proteinuria has also been shown to be a predictor of remission (553).

Indices of clinical activity of the lupus itself have been examined in a number of studies (563,564), many of which either did not deal with lupus nephritis or did not specify how many of the group studied had nephritis. However, there are clear correlations between immunologic findings such as complement concentrations and levels of circulating anti-dsDNA antibody, and the severity of the clinical picture, judged in various ways (576–579). In the analysis of Esdaile et al. (564) of a group exclusively consisting of patients with nephritis, thrombocytopenia, hypocomplementemia, and an elevated DNA binding at onset, all predicted poor outcome. Najafi et al. reported that the presence of anti-Ro antibodies was a predictor of the development of ESRD (574). Austin et al. (556) also found hypocomplementemia to be predictive of a poor renal prognosis, together with anemia, which was also a risk factor in the analysis by Donadio et al. (548) and in the report by Moroni et al. (554). Using the National Institutes of Health (le Riche) index, Goulet et al. (580) found good correlation between overall disease activity and renal outcomes. This was particularly important in patients without severe renal disease at presentation in whom higher disease activity index nevertheless portended an increased risk for subsequent renal insufficiency.

Age may also be a prognostic factor for patients with lupus. The outcome of lupus in childhood has been the subject of some controversy (581), with some series reporting poorer survival rates in children than adults. Wallace et al. (78) reported the outcome in 50 patients with onset before the age of 16 years. The survival was 73% at 5 years and 58% at 10 years. Only one-third of these patients had nephritis, however, and unlike in most North American series the children were predominantly White. Other series have shown better survival rates for childhood lupus. In a series of 80 patients who had the onset of lupus nephritis before the age of 20 years, the 5- and 10-year estimates of survival were 85% and 82%, respectively (581). In data from Minneapolis (533) on 70 childhood-onset cases, the 10- and 15-year survival rates were 85% and 77%. Esdaile et al. (564) performed a Cox analysis and noted no difference in renal failure or renal death in those older and those younger than 24 years at onset in a series of 87 patients. Austin et al. (582), however, found that age less than 24 years was a predictor of the development of renal failure. There seems to be little difference in the survival of adults and children or adolescents with lupus nephritis when other factors are also considered (78,563–565,582), although some authors (583) have interpreted the existing data as demonstrating that young age confers a poorer prognosis. Overall it seems that the difference in survival between children and adults, if there is one, is not a major one.

Hypertension, at least severe hypertension, is not as commonly present in lupus nephritis at apparent onset as in some other forms of glomerulopathy (e.g., 10 of 87 in Esdaile's study]). Hypertension was not examined as a prognostic factor in either of two of the largest studies cited previously (78, 565), and it was not mentioned in most follow-up studies. Esdaile et al. (564) did not find any association between outcome and high blood pressure itself, only with comorbid conditions. Similarly, the Glomerular Disease Collaborative Network (568) did not find hypertension to be an independent marker for poor renal outcome. In contrast, the Gruppo Italiano (549), Ward and Studenski (584), and Ginzler et al. (585) showed a markedly poorer survival in their hypertensive subgroups. In almost all patients, if hypertension is present at the outset or appears later, it is usually treated vigorously. Nevertheless, unlike in IgA nephropathy, for example, high blood pressure does not seem to be a powerful predictor of outcome in lupus nephritis.

The number of *disease criteria* a patient demonstrates may predict a worse outcome. Esdaile et al. (564) and Ginzler et al. (565) have found that the total number of American College of Rheumatology (ACR) criteria for the diagnosis of lupus present at onset was a useful index of a poorer outcome. Swaak et al. (586) noted a poorer prognosis in those with an increasing number of clinical relapses, although this was in a population predominantly not under heavy immunosuppression. The antiphospholipid antibody syndrome (APS) has been associated with renal artery stenosis, thrombotic microangiopathy, and APS nephropathy, and the presence antiphospholipid antibodies was associated with worse renal outcomes during long-term follow-up (587).

Several authors have studied *gender* as a prognostic factor. Male patients, of course, form only a small minority of patients with lupus, which makes comparison difficult except in the largest series. In the series of Wallace et al. (78), in which only 39% of patients had evident nephritis, there was a significantly worse outcome for the 63 men compared with the 546 women, and the data of Austin et al. (582) are in agreement with this conclusion. In the study by Swaak et al. (586), 5 of 16 men and only 9 of 94 women died. The Cox analysis by Esdaile et al. (564) suggested a higher rate of renal failure in men, but no difference in nonrenal deaths. However, Ginzler et al. (565), using a stepwise linear regression model, found no difference in their large collected series. Tejani et al. (566) and other pediatricians (588) suggested a poorer outcome for male children, especially those with a prepubertal onset. In contrast, Levy et al. (589) reported a higher frequency of death or end-stage renal failure in girls. It must be recognized, however, that the numbers are small and other confusing factors such as race (see earlier discussion) are difficult to correct for.

Renal Biopsy Data

Pattern of Glomerular Disease

At a histologic level, a considerable body of data is available. The WHO classification, which includes a category of sclerosing glomerulopathy (537,590), has been studied the most. Although the overall prognosis of lupus nephritis has improved, it is clear that the outcome varies for different classes of disease (Fig. 71-18). Appel et al. (575) noted a better survival in those with class II (mesangial) nephritis compared with the other histologic classes. Similar results have been reported in a review by Berden (591). In contrast, despite recent improvement, Austin et al. (592) reported a poorer survival rate in those with class IV nephritis, as did Nossent et al. (573) and Berden (591).

A particular problem exists with regard to patients classified as having the WHO class III appearance—focal proliferative glomerulonephritis. A diverse outcome has been reported for such individuals (58,593,594), which illustrates the great variation in severity within this group. The survival of patients with class IIIa disease (by the original WHO classification system) resembles that for milder forms of lupus nephritis (595–597), whereas survival of patients with more severe focal disease is more akin to that of patients with class IV disease. In fact, in the 10-year follow-up data from the Lupus Collaborative Study (574), patients with class III disease were significantly less likely to enter remission and had significantly worse renal survival rates than patients with class IV disease. In the revised WHO classification, the activity and chronicity of class III lesions is also described (598).

Patients showing the WHO class V pattern (i.e., membranous nephropathy) almost always have a more favorable course

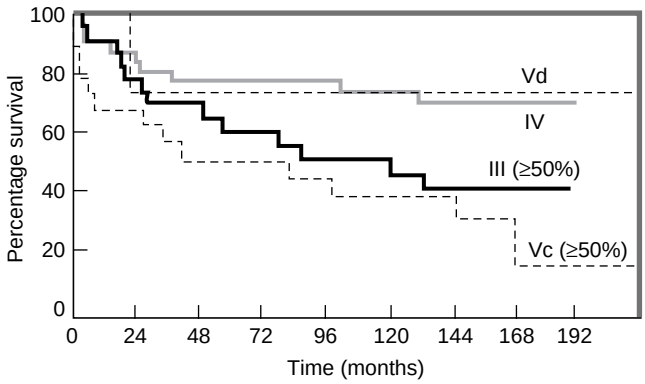


FIGURE 71-18. Renal survival based on histologic categorization and subcategorization (censoring for nonrenal death). Renal survival is shown for patients with World Health Organization (WHO) class III (≥50%; or active and/or necrotizing lesions in 50% or more of glomeruli), IV (in which there was inflammation in ≥80% of non-hyalinized glomeruli), Vc (membranous glomerulonephritis superimposed upon class III), or Vd (membranous glomerulonephritis superimposed upon class IV). Survival for class III ($N = 24$) was significantly worse than for class IV ($N = 35$) disease ($P = 0.05$, IV vs. III (≥50%). Class Vc ($N = 20$) disease had a significantly worse renal survival than class IV ($p = 0.002$). (From: Najafi CC, et al. Significance of histologic patterns of glomerular injury upon long-term prognosis in severe lupus glomerulonephritis. *Kidney Int* 2001;59:2156, with permission.)

(175,537,599–601), although renal failure occurs in a minority (601,602). Persistent nephrotic syndrome has been reported to be associated with a worse prognosis (564,572,575). It has recently become clear that the long-term prognosis of lupus membranous nephropathy varies depending on whether the patient exhibits pure membranous (Va) or just mild mesangial proliferation (Vb) versus those with coexisting proliferative disease, either focal (Vc) or diffuse (Vd) (601,603). The 10-year survival rate fell from 72% in patients with Va or Vb disease to 48% in those with Vc lesions and only 20% in patients with class Vd disease (603). As a consequence, in a recent revision of the WHO classification system, the grades Vc and Vd have been dropped (598). In this revised system, both pathologic grades should be reported for patients with a combination of class V disease with either class III or class IV so as to more clearly communicate the lesion.

Individual Elements of Histology

Analyses have been performed also to assess the predictive value of individual elements of the histologic picture. Two observations appear to show some reliability in predicting a poor outcome. The first of these is extensive subendothelial deposits, especially if they persist in repeated biopsy specimens (604–607). The value of this observation may now be reduced in most patients by aggressive treatment of such a histologic appearance. Still of value, however, are tubulointerstitial changes, which were noted by Muehrcke et al. (608) as long ago as 1957. These changes pointed to a poor prognosis in a number of studies (564,592,609). Magil et al. (572) found that the number of nonspecific esterase-positive cells (macrophages) correlated with outcome, and another study (610) showed, using monoclonal antibodies to determine the phenotype of the interstitial cells, correlations between both the numbers of infiltrating monocytes and T cells and subsequent GFRs. The presence of crescents has also been related to a poorer prognosis (611), as in other forms of nephritis, but very extensive crescentic disease is relatively uncommon in lupus. Vascular lesions within the biopsy material (612,613) and intraglomerular capillary thrombi (614) have been associated with unfavorable outcomes also, although the latter observation has been contested (595,615). In another study, the presence of intraglomerular leukocyte infiltration was found to be the strongest correlate with progression to renal insufficiency (616).

Activity/Chronicity Indices. Because of dissatisfaction with the data obtained by considering the morphologic aspects of nephritis (which is in any case variable between and within glomeruli in lupus nephritis, and also varies in sequential biopsy samples (531–534)), an alternative approach has gained support: that of assessing the histologic activity of the nephritis on the one hand, and its apparent chronicity on the other, as judged by the extent of sclerosis. This approach was pioneered by Pirani and Salinas-Madrigal (617), Morel-Maroger (590), and others in the 1960s and 1970s, but became popular after Austin et al. (592) described the calculation of activity and chronicity indices using data from glomeruli and interstitium (Table 71-17). This approach allowed these workers (592) to identify clear groups at high and low risk for a poor outcome and permitted therapeutic stratification, specifically, when and when not to use aggressive treatment.

These data were supported by Magil et al. (572), Esdaile et al. (564), and Nossent et al. (573), who all found a high chronicity index to be the best predictor of subsequent renal failure. However, several other authors found a poor or absent association of outcome with either index, or combinations of them (575,595,618), although the follow-up period in Schwartz's study (618) was only 2 years. Nevertheless, it is clear that chronic scarring is associated with poor prognosis.

Additional Value of Renal Biopsy Data. Several authors (564,572,582,606,607) using different multivariate models and different sets of data examined the additional value for predicting outcome of the information from renal biopsy specimens over that available from clinical studies. Whiting-O'Keefe et al. (606,607) concluded that only the degree of sclerosis and the presence or absence of subendothelial deposits seemed of any great value. Austin et al. (592) and Magil et al. (572) suggested that the WHO classification of biopsy data adds useful information, and that the chronicity index is an even better predictor of outcome. Esdaile et al. (564), however, found no biopsy information that added to the clinical predictions. The

TABLE 71-17

RENAL PATHOLOGY SCORING SYSTEM IN LUPUS NEPHRITIS

Activity index	Chronicity index
Glomerular abnormalities	
Cellular proliferation	Glomerular sclerosis
Fibrinoid necrosis[a]	Fibrous crescents
Karyorrhexis	
Cellular crescents[a]	
Hyaline thrombi wire loops	
Leukocyte infiltration	
Tubulointerstitial abnormalities	
Mononuclear cell infiltration	Interstitial fibrosis
	Tubular atrophy

[a]Fibrinoid necrosis and cellular crescents are weighted by a factor of 2. Maximum score of activity index is 24 and chronicity index is 12. (Reprinted from: Austin KA III, et al. Prognostic factors in lupus nephritis: contribution of renal histologic data. *Am J Med* 1983;75:382, with permission.)

TABLE 71-18

RISK FACTORS FOR PROGRESSION OF LUPUS NEPHRITIS

Strong evidence
- Response to treatment (553)
- Maintenance of remission (553–555)
- Race (556,565,566,568)
- Plasma creatinine at onset of disease (548,553,556,564, 572–574)
- Disease activity (overall disease activity [580], anemia [548,554,556], hypocomplementemia [556,564], anti-Ro antibodies [574])
- WHO histologic classification (573,574,591,592)
- Chronicity index (564,572,573,592)

Some evidence
- Socioeconomic status (570)

Conflicting evidence
- Proteinuria (549,553,562,564,575)
- Hypertension (549,564,568,584,585)
- Age (78,563–565,582,583)

value of the biopsy in directing more aggressive—and possibly more successful—therapeutic strategies is almost impossible to evaluate, but as most analysis of prognosis and response to therapy in lupus nephritis is based upon the underlying histology, evaluation of the biopsy is an essential part of risk stratification and therapeutic decision making

Inapparent Renal Disease in Lupus. A number of authors noted that patients without clinical manifestations of nephritis have changes in their glomeruli amounting to glomerular disease. There have been few follow-up studies on such patients, but the study of Leehey et al. (619) showed that most remain without clinical nephritis for some years, although even severe diffuse proliferative patterns may be observed in some patients. Similar findings have been reported by Gonzalez-Crespo et al. (620). However, it is equally obvious that all patients with clinically evident nephritis must go through a period of absent or occult disease before the disease becomes evident; it is not known how many of these patients run a subclinical course for a prolonged period.

Long-Term Outlook for Lupus Nephritis

There are some data relating to 10-year survival or longer for patients with lupus nephritis (543,548,549,621–623). The differences between the various forms of nephritis have decreased and the survival is now better than 80% at 10 years (562,595). However, recent data (543,562,595) show no further improvement in outlook during the 1990s, with a persisting minority of patients who either die or go into end-stage renal failure within a decade. There is little information on 20-year survival, although a cohort of 60 patients treated between 1976 and 1986 (543) demonstrated about 75% survival, similar to data found in the paper of Moroni et al. (623).

There are surprisingly few data also on the longer term status of patients with lupus nephritis in relation to treatment: How common is stable long-term remission after discontinuing all therapy? Is there a difference between patients treated with cyclophosphamide or azathioprine in this respect? Given the recent evidence that mycophenolate is effective for induction or maintenance therapy (624,625), the long-term outcome for patients treated with this agent are also important to know. As noted previously, in an analysis of patients treated before 1986 (543) who all had a potential follow-up of more than 15 years (actual median follow-up, 15.5 years; range, 2 to 31 years) prognosis improved in the cohort studied from 1976 compared with those who entered in 1975 or earlier, but the subsequent decade saw no further improvement (10-year survival of 57%, 81%, and 84%, respectively). After 20 years, more than three-quarters of patients can expect to be alive with renal function. Of living patients with renal function, 38% had no proteinuria, but only 11% were receiving no specific treatment. The report by Moroni and colleagues (623) on a smaller group of patients is more encouraging. Of 34 patients who had actually completed 10 years of follow-up, only 6 (17.5%) had died, 3 were receiving end-stage treatment, 6 had elevated plasma creatinine levels (3 with proteinuria), 6 had minor proteinuria with normal function, and 15 were in complete remission; 11 patients (32%) had been off all treatment for a mean of 7.5 years. None of the patients in these two series had received intravenous cyclophosphamide, and the medium-term (2 to 10 years) results are similar to those treated with this type of regimen in other hands. In a more recent report that included patients with proliferative disease who had been treated with cyclophosphamide (574), the overall 15 year renal survival was 50% and the 15-year patient survival was 70%. Unfortunately, greater detail regarding the long-term outlook for patients after receiving cyclophosphamide has not been reported. In particular, data regarding the number of patients free of treatment long term is unknown.

CONCLUSIONS

This chapter presents data suggesting that most patients with progressive forms of glomerulonephritis reach renal failure within 5 to 15 years after apparent clinical onset, and similarly, those who enter remission will usually have done so by the end of this time. Few patients show much more rapid evolution or remission, and only a few will have persisting urinary abnormalities with normal renal function.

These patients show "grumbling" nephritis, with proteinuria, hematuria, or both, persisting at various levels of apparently stable renal function for 20 years or more. This is particularly common in membranous nephropathy and in IgA-associated disease. It can also be seen in treated patients with lupus nephritis.

One major task, along with the introduction of effective treatments to slow or eliminate the deterioration in renal function, is to identify as early as possible those patients who have a progressive course so treatments can be focused on this high-risk group. Several features consistently emerge from multivariate analyses as predictors of a poor outcome in a broad range of glomerular diseases: (a) the magnitude and duration of proteinuria—although this must be interpreted in light of the renal biopsy findings, because patients with minimal change nephrotic syndrome may show the highest figures; (b) a raised plasma creatinine concentration that does not return to normal on control of the nephrotic syndrome, because reversible changes in K_f may depress the GFR temporarily; (c) tubulointerstitial damage in the renal biopsy specimen—in general the severity of glomerular changes is a much poorer indicator of outlook, with the sole exception of extensive crescent formation; and (d) a persistently raised blood pressure—again, the transient hypertension of some patients with minimal change nephrotic syndrome or reversible acute nephritis makes a period of observation necessary, and the first figures obtained on admission to hospital relatively useless.

A number of other features (e.g., male sex and increasing age) are less consistently predictive of a poor outcome. Some authors have suggested that the amount of urinary blood excreted by patients with hematuric illnesses predicts poor

outcome, but others found just the opposite. Therefore, the archetypal patient with glomerulopathy and a poor outlook is a middle-aged or older adult man with sustained hypertension, an elevated plasma creatinine concentration, persistent nephrotic-range proteinuria, tubulointerstitial damage in renal biopsy specimens, and crescents if a proliferative glomerulonephritis is present. On the contrary, a young normotensive woman whose proteinuria remits or diminishes, who has normal renal function, and whose biopsy reveals only intraglomerular changes is almost certain to do well.

Glomerulonephritis, although no longer the most common single cause of end-stage renal failure, remains the most common cause in young adults. Now we need 15-, 20-, and 30-year follow-up data on patients with glomerular diseases to answer the many questions raised in this chapter about the fate of individual groups. Collecting such data is tedious, without glamor, and difficult to fund; changes in staff over the years result in many studies never being completed. Without such data, however, we will never be able to present even a 20-year picture of what happens to patients with glomerular disease.

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CHAPTER 72 ■ CHRONIC TUBULOINTERSTITIAL NEPHROPATHIES

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Diseases of the kidney primarily affect the glomeruli, the vasculature, or the remainder of the renal parenchyma, that is, the tubules and interstitium. The chronic forms of the latter category of diseases of the kidney are considered in this chapter. Initially referred to as *interstitial nephritis* (1,2), the more descriptive terms *tubulointerstitial diseases* (3), *nephritis,* or *nephropathies* (4) are now used to classify this heterogeneous group of disorders that in its primary form affects the renal tubules and interstitium and only secondarily involves the other structural components of the kidney.

The preferential use of the qualifier tubulointerstitial was first proposed because structurally it is the tubules, not the interstitium, that comprise the bulk of the normal kidney parenchyma and sustain the brunt of the injury; and clinically, disorders of tubular function constitute the most notable component of the disordered pathophysiology of these diseases and thereby differentiate them from other forms of kidney disease resulting from glomerular or vascular lesions (3–5). In addition, increasing evidence now indicates that the tubular epithelial cells are an integral component of the pathogenesis of these diseases, in both the processing and the presentation of foreign antigens and in the progressive interstitial changes that ensue (6–9). This is not to imply that glomerular lesions do not occur or contribute to the loss of kidney function in these diseases. Although the primary lesions originate in the tubules and interstitium, many of them eventually will develop structural and functional abnormalities of the glomeruli (3,5), whose structural alterations are characterized by glomerulosclerosis, whereas the functional changes are evidenced by a progressive reduction in the glomerular filtration rate (GFR), the development of glomerular proteinuria, and ultimately, the onset of volume-dependent hypertension (4,10,11). Early in the course of tubulointerstitial diseases of the kidney, with their characteristic insidious onset and indolent course, tubular dysfunction is almost always out of proportion to any coexistent glomerular dysfunction (3,4,11). In the later phases of tubulointerstitial diseases of the kidney in which the glomerular lesions are quite advanced, it may be difficult to differentiate them from the end-stage kidney disease of any etiology. Although diseases of the glomeruli and vasculature are excluded, by definition, from primary chronic tubulointerstitial nephropathies, the secondary forms of tubulointerstitial nephritis are an important component of diseases of the glomeruli and vasculature, and the extent and severity of concurrent tubulointerstitial changes contribute significantly to their clinical course (12). It is the development of the secondary form of tubulointerstitial nephritis that attends almost all instances of progressive glomerular and vascular diseases that determines, in large part, their outcome (12–18).

The frequency with which primary tubulointerstitial diseases affect the kidney is difficult to determine. Their importance, however, can be appreciated because an estimated 20% to 40% of all patients who develop kidney failure and become dialysis-dependent have primary tubulointerstitial disease as the cause of their kidney failure (19–21). Taken together with the demonstrated role of secondary tubulointerstitial lesions in the deterioration of kidney function in those with primary glomerular or vascular disease (8,21,22), it becomes evident why it is important to evaluate and appreciate lesions of the interstitium and tubules in chronic kidney disease of any etiology.

The preferential use of the term *tubulointerstitial* should in no way minimize the role of the interstitium, because this is where the pathogenesis and evolution of the disease is perpetuated (7,8,18), and the interstitial lesions may well account for many of the disorders of tubular function that are characteristic of these diseases. The renal interstitium as an important component of kidney function has long been appreciated. The interstitial solute concentration in the cortex and in the medulla is an integral component of the regulatory mechanisms for proximal reabsorption and for concentration and dilution of urine, respectively (23,24). Furthermore, the endocrine functions of the interstitial cells, in the release of renin and erythropoietin in the cortex and of prostaglandins in the medulla, are essential modulators in the role of the kidney in maintaining homeostasis (14,23–25). Conceptually then, interstitial events could account for the pathogenesis, progression, and clinical manifestations of chronic kidney diseases in general and of the tubulointerstitial nephropathies in particular (Fig. 72-1). Still, the tubular changes bear the brunt of the insult and, because they are functionally more important, account for the clinical and functional manifestations of any interstitial nephritis. Evaluation of tubular function is extremely important because of its predictive value of the extent of tubulointerstitial nephritis, and as such would provide a readily available, noninvasive, clinical assessment of the severity and prognosis of chronic kidney diseases (26).

STRUCTURAL FEATURES OF THE INTERSTITIUM

The interstitium of the kidney consists of peritubular and perivascular spaces. The relative contribution of each of these spaces to interstitial volume varies, reflecting in part the arbitrary boundaries used in assessing them. The portion of renal tissue occupied by interstitium increases from the cortex to the papilla. In the cortex, there is little interstitium, because the peritubular capillaries fill most of the space between the tubules and the cortical interstitial cells, which are scattered between the capillaries and tubules of the cortical labyrinth and are relatively inconspicuous. In the medulla, however, there is a noticeable increase in interstitial space, and the interstitial cells have characteristic structural features and an organized arrangement. The ground substance of the interstitium contains different types of fibrils and a basementlike material embedded in a glycosaminoglycan-rich substance (27–30).

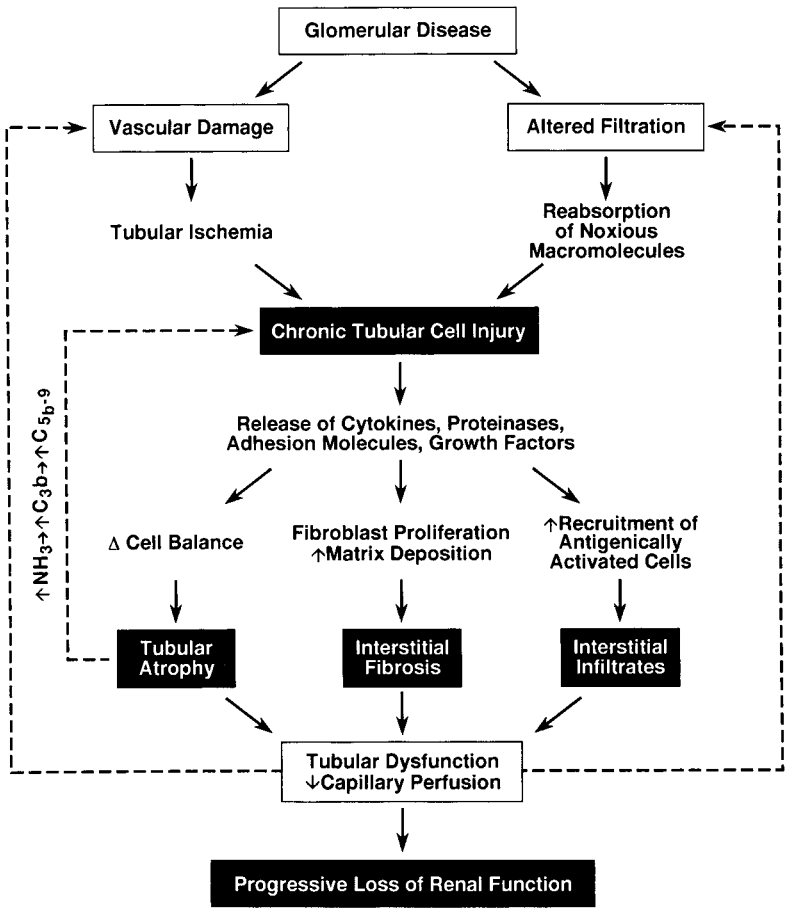

FIGURE 72-1. Schematic overview of the potential pathways in the pathogenesis of chronic tubulointerstitial nephritis due to primary tubular injury or secondary to glomerular disease.

The relative volume of the interstitium of the cortex is approximately 7%, consisting of about 3% interstitial cells and 4% extracellular space (23,31,32). The vasculature occupies another 6%; the remainder (i.e., 85% or more) is occupied by the tubules. The cortical interstitial space is distributed unevenly and has been divided into "narrow" and "wide" structural components (30,31). The tubules and peritubular capillaries are closely apposed at several points, sometimes to the point of sharing a common basement membrane, or are separated by a very narrow space, the so-called *narrow interstitium,* which occupies an estimated 0.6% of cortical volume in the rat. The narrow interstitium occupies about one-half to two-thirds of the cortical peritubular capillary surface area. The remainder of the cortical interstitium consists of irregularly shaped, clearly discernible larger areas, the so-called *wide interstitium,* which occupies an estimated 3.4% of cortical volume in the rat (30,31). Most of the cortical interstitial cells are located in the wide interstitium. The capillary wall facing the narrow interstitium is significantly more fenestrated than that facing the wide interstitium (23,30). Evidence for a possible functional heterogeneity of these interstitial spaces has been advanced on the basis of studies of the interstitial albumin pool (28). The actual significance of these findings, however, remains to be clarified.

In the medulla, the size of the interstitium increases gradually from the outer medullary stripe to the tip of the papilla (23,30), because of the increase in the relative volume contribution of both the interstitial cells and the extracellular space. Actually, in the outer stripe of the outer medulla, the relative volume of the interstitium is slightly less than that in the cortex and has been estimated to be approximately 5% in the rat. It is in the inner stripe of the outer medulla that the interstitium begins to increase significantly in volume in increments that gradually becomes greater toward the papillary tip (23,30–34). The inner stripe of the outer medulla consists of the vascular bundles and the interbundle regions, which are occupied principally by tubules. Within the vascular bundles, the interstitial spaces are meager, whereas in the interbundle region, the interstitial spaces occupy 10% to 20% of the volume. In the inner medulla, the differentiation into vascular bundles and interbundle regions becomes gradually less obvious until the two regions merge. This is coupled by a gradual increase in the relative volume of the interstitial space from the base of the inner medulla to the tip of the papilla. In the rat, the increment in interstitial space is from 10% to 15% at the base to about 30% at the tip, and, in the rabbit, from 20% to 25% at the base to more than 40% at the tip (35).

The cortex contains two types of interstitial cells. The more abundant variety of interstitial cells are the type I or the fibroblastic cells. These have a prominent rough endoplasmic reticulum and elongated mitochondria. They appear to produce the extracellular interstitial matrix and contribute to fibrosis in response to chronic irritation as they evolve into myofibroblasts. The less common type II interstitial cells are mononuclear cells or macrophages, which have an abundant chromatin and no endoplasmic reticulum with demonstrable phagocytic ability, and through their autocrine function contribute to the fibrogenesis of tubulointerstitial nephropathy (23,30). An interstitial infiltrate of recruited mononuclear cells is a prominent feature of chronic interstitial nephritis, but some of the infiltrating cells are derived from these local precursors (6,7,19).

In the outer zone of the outer medulla, the interstitial cells are similar to those in the cortex. In the inner zone of the outer medulla and the inner medulla, the cellular content is

significantly altered. A unique type of cell that is present in increasing numbers toward the tip of the papilla is the lipid-laden interstitial cell. The number of cytoplasmic lipid droplets of these cells varies from one cell to the other, and there are differences in the number of droplets among species and among individuals of the same species. A change in the number of droplets has been suggested, but not conclusively demonstrated, to occur in relation to the intake of salt and water and the level of blood pressure (36). These cells are also the source of the interstitial glycosaminoglycans in the inner medulla (30). Another characteristic feature of these medullary cells is their connection to each other in a characteristic arrangement, similar to the rungs of a ladder, with a distinct close and regular transverse apposition to their surrounding structures, specifically the limbs of the loop of Henle and capillaries, but not to the collecting duct cells (23,27). The other types of medullary cells are the mononuclear cells or macrophages and the pericytes. The latter are prominent in the descending vasa recta in the outer medulla but become rare as the vascular bundles break up in the inner medulla. The number of mononuclear cells and macrophages increases in the inner stripe of the outer medulla and is highest in the inner medulla (29). Proliferation and upregulation of resident macrophages and pericytes, in response to injury, are an essential determinant of the extent of fibrosis that characterizes tubulointerstitial nephritis.

The extracellular loose matrix is a hydrated gelatinous substance that consists of glycoproteins and glycosaminoglycans (hyaluronic acid, heparan sulfate, dermatan sulfate, and chondroitin sulfate) that are embedded within a fibrillar reticulum, which consists of collagen fibers (types I, III, and VI) and unbanded microfilaments. Collagen types IV and V are the principal components of the basement membrane lining the vessels and tubules. Glycoprotein (fibronectin, laminin) components of the basement membrane connect it to the interstitial cell membranes and to the fibrillar structures of the interstitial matrix (37). The relative increase in interstitial matrix of the medulla may be important for providing support to the delicate tubular and vascular structures in this region, and its inflammation-mediated compositional alterations might well account for the susceptibility of the structures in this region to toxic reactive metabolites (38).

FUNCTIONAL SIGNIFICANCE OF THE INTERSTITIUM

Exchange processes between the different segments of the renal tubular and vascular components are at the core of the homeostatic functions of the kidney. In most physiologic discussions, the transport of ions, water, and proteins is described as though it occurred directly from the tubule to the capillary, and the emphasis is on the permeability of the membranes concerned. In fact, the reabsorbed or secreted tubular fluid has to traverse a true interstitial space between the tubules and peritubular capillaries. The structure, composition, and permeability characteristic of the interstitial space must, of necessity, exert an effect on any such exchange. Several studies suggest that pressure and volume conditions of the interstitial space modulate renal tubule function in response to fluid-balance disturbances. The normal interstitial hydrostatic pressure of about 3 mm Hg and protein concentration of about 2 g% changes with extracellular fluid volume expansion to 5 to 10 mm Hg and 0.5 g%, respectively (39). Similarly, rapid transient changes in the hydrostatic pressure of the interstitial spaces occur after the intraaortic injection of albumin or saline solutions (39). There are also measurable changes in the volume of the medullary interstitial space during water diuresis and vasopressin-induced antidiuresis (40). Homeostasis is also controlled by paracrine mediators according to the cytoarchitectural features between the tubules and arterioles. The site where such a structure–function mechanism has been best elucidated is the conglomerate of cells at the connection between the macula densa and the glomerular vasculature that has been considered to form an "apparatus," as reflected in its accepted name: the juxtaglomerular apparatus (41).

Although the normal structural and functional correlates of the interstitial space are poorly defined, changes in the interstitial composition and structure are bound to reflect themselves in changes of tubular function. Structural changes of the interstitium, by altering equilibration or exchange, and the release of vasoactive cytokines by the infiltrating cells, could well result in a significant alteration in the normal functions of the renal tubule. Correlation of the structural changes of the interstitium to those of kidney function may be better envisioned when the rich but delicate capillary network of the renal microcirculation is taken into consideration. The periarterial loose connective tissue that envelops the intrarenal microvasculature in the cortex is continuous with that of the sparse peritubular interstitium (23,30,31). It is possible that changes in the supporting interstitium could affect the blood flow to the adjacent tubule and thereby cause tubular dysfunction (Fig. 72-1). Angiographic studies of the renal microcirculation reveal that the number and volume of peritubular capillaries are markedly compromised in the areas of interstitial inflammation and fibrosis (42–44). Interstitial alterations also may be reflected in changes in glomerular capillary pressure and filtration rate through a tubuloglomerular feedback mechanism; specifically, as the function of the affected tubular segments becomes compromised secondary to interstitial disease, solute delivery to the macula densa may be altered (24,39,45,46). On the basis of experimental models and studies correlating morphometric analysis of human kidney biopsy specimens to kidney function, it has been suggested that obliteration of postglomerular capillaries secondary to interstitial injury and fibrosis may account for changes in kidney function (44,47), and by extension to the pathogenesis of progression of kidney disease to kidney failure (48,49).

It is of special relevance, in this regard, that attempts to correlate kidney function with histologic abnormalities of the glomeruli have been conflicting. Whereas some studies found a relationship (50,51), others were unable to document any correlation (46). Conversely, several studies of abnormalities of the tubulointerstitial tissue show a close correlation with abnormalities of kidney function in an assortment of chronic kidney diseases (13,22,52–56). A careful study of 70 patients with various renal diseases, most of which were glomerular in origin, evaluated by semiquantitative morphometric analysis of the biopsy material the changes in glomerular, tubular, vascular, and interstitial components (57). Studies of kidney function, performed within 2 days of the biopsy, included measurements of GFR, effective renal plasma flow, acidifying ability, and maximal concentrating ability. The GFR showed a higher correlation with interstitial and tubular damage, regardless of the disease process (Fig. 72-2). Interstitial fibrosis and cellular infiltration were also well correlated with GFR, whereas edema was not. In contrast to these findings, there was only a modest correlation between GFR and the glomerular injury score. As might be expected, effective renal plasma flow showed a high correlation with the degree of vascular disease (Fig. 72-3); it was also highly correlated with tubular and interstitial damage but not to glomerular lesions. The urinary concentrating ability was found to be a very sensitive indicator of the presence or absence of tubular and interstitial abnormalities (Fig. 72-4). It was the severity of tubulointerstitial damage and not the primary kidney disease that seemed to be the determinant of impairment in concentrating ability. All patients whose maximal urinary osmolality was less

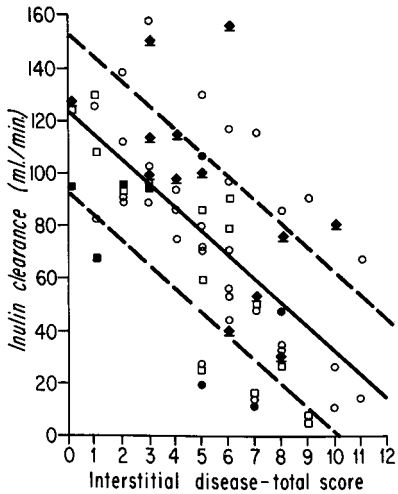

FIGURE 72-2. Relationship of inulin clearance to the severity of tubulointerstitial lesions. The regression line is $y = 122 - 8.8x$. Symbols represent categories of kidney diseases studied: ♦, acute glomerulonephritis; ○, chronic glomerulonephritis; ●, tubulointerstitial nephropathy; ■, nephrosclerosis; □, miscellaneous. (From: Schainuck LL, et al. Structural-functional correlations in renal disease. Part II: the correlations. *Hum Pathol* 1970;1:631, with permission.)

than 600 mOsmol/kg of water had moderate-to-severe tubulointerstitial lesions, whereas those able to concentrate their urine to more than 800 mOsmol/kg of water had only minimal tubulointerstitial lesions. There was only a modest, albeit significant, relationship between urinary concentrating ability and the severity of the glomerular score. Finally, the impairment in the ability to excrete titratable acid and ammonium

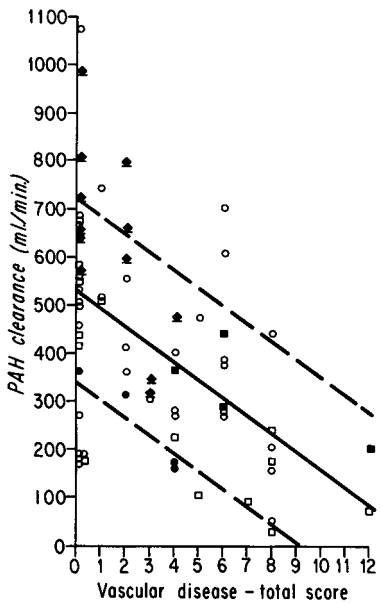

FIGURE 72-3. Relationship of effective renal plasma flow as measured by the clearance of p-aminohippurate *(PAH)* to the severity of vascular disease. The regression line is $y = 553 - 38x$. Symbols represent categories of kidney diseases studied: ♦, acute glomerulonephritis; ○, chronic glomerulonephritis; ●, tubulointerstitial nephropathy; ■, nephrosclerosis; □, miscellaneous. (From: Schainuck LL, et al. Structural-functional correlations in renal disease. Part II: the correlations. *Hum Pathol* 1970;1:631, with permission.)

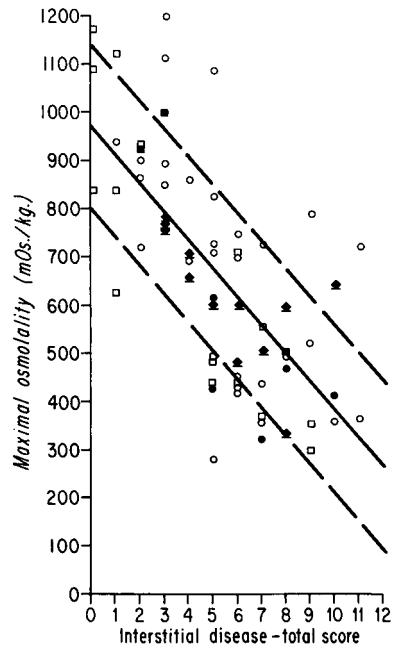

FIGURE 72-4. Relationship of the maximum ability to concentrate the urine (maximal osmolality) to the severity of tubulointerstitial lesions. The regression line is $y = 975 - 59x$. Symbols represent categories of kidney diseases studied: ♦, acute glomerulonephritis; ○, chronic glomerulonephritis; ●, tubulointerstitial nephropathy; ■, nephrosclerosis; □, miscellaneous. (From: Schainuck LL, et al. Structural-functional correlations in renal disease. Part II: the correlations. *Hum Pathol* 1970;1:631, with permission.)

in response to an acid load was highly correlated with the degree of interstitial and tubular lesions and to a much lesser degree with glomerular changes (Fig. 72-5). With increased awareness of the role of the interstitial lesion, these studies were expanded (12,14,58–60), and the presence of interstitial fibrosis has been documented to show a significant correlation with the progression of kidney diseases of glomerular origin (Fig. 72-6). Therefore, the presence of secondary tubulointerstitial lesions in primary glomerular diseases not only correlates with the severity of altered kidney function at the time of biopsy but also provides a prognostic index of the rate of progression to end-stage kidney failure (14,15,61–64).

Glomerular Influence on Tubulointerstitial Disease

Altered glomerular permeability with consequent proteinuria has emerged as an important contributory factor to the development of tubulointerstitial lesions in primary glomerular diseases (62,64,65) (Fig. 72-1). Glomerular diseases associated with proteinuria elicit tubulointerstitial inflammation, which when persistent will progress to interstitial fibrosis (18,66). Different potential pathways can account for this association, but an abnormal glomerular filtrate appears to mediate tubular epithelial cell injury, and their consequent antigenic activation is an important step in initiating the sequence of events that leads to fibrosis and scarring of the interstitium. Activated epithelial cells release chemokines, such as monocyte chemotactic peptide-1 (MCP-1) and osteopontin, which are capable of attracting leukocytes (67,68) and vasoactive peptides, such as endothelin-1, that alter vascular permeability (67–69). The activated epithelial cells also express major histocompatibility complex (MHC)-II molecules and act as antigen-presenting

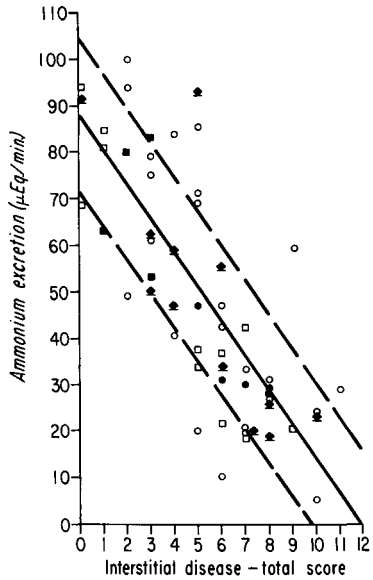

FIGURE 72-5. Relationship between ammonium excretion in response to an acute acid load and the severity of tubulointerstitial lesions. The regression line is $y = 90 - 7.6x$. Symbols represent categories of kidney diseases studied: ♦, acute glomerulonephritis; ○, chronic glomerulonephritis; ●, tubulointerstitial nephropathy; ■, nephrosclerosis; □, miscellaneous. (From: Schainuck LL, et al. Structural-functional correlations in renal disease. Part II: the correlations. *Hum Pathol* 1970;1:631, with permission.)

cells to the infiltrating interstitial T cells (70). Interstitial nephritis has been induced by protein overload in uni-nephrectomized rats (71). Significant cellular infiltration develops in the interstitium of experimental animals within 1 week of exposure to a protein overload consisting of 1 g of bovine serum albumin (BSA) injected in the peritoneum daily. The initial infiltrates consisting predominantly of macrophages are soon replaced by T lymphocytes, and by the fourth week, tubular atrophy and interstitial fibrosis is present. This has been attributed to the proximal tubular epithelial cell catabolism of the filtered albumin, which results in the generation of a nonpolar lipid that is chemotactic for macrophages (72). Apart from its effect on tubular epithelial cells, the "inflammatory filtrate" of the injured glomerulus also can diffuse into the interstitial space and elicit an inflammatory response in the periglomerular interstitium (18,66).

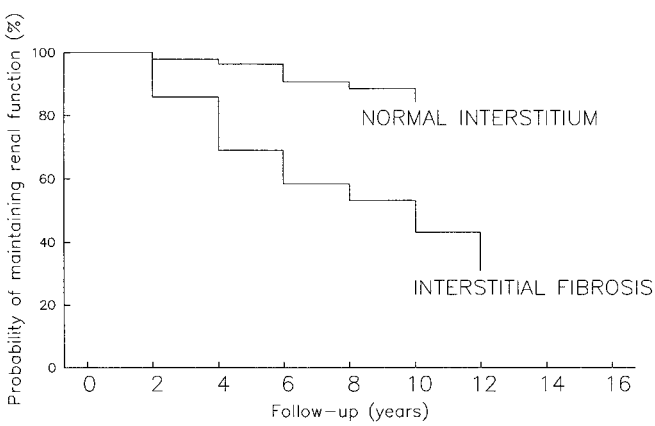

FIGURE 72-6. Effect of the presence of cortical interstitial fibrosis noted at the time of the initial diagnostic kidney biopsy on the long-term prognosis of patients with mesangioproliferative glomerulonephritis ($N = 455$), membranous nephropathy ($N = 334$), and membranoproliferative glomerulonephritis ($N = 220$). (From: Bohle A, et al. The consequences of tubulointerstitial changes for renal function in glomerulopathies: a morphometric and cytological analysis. *Pathol Res Pract* 1990;186:135, with permission.)

In addition to the effect of altered glomerular permeability on interstitial fibrosis, glomerular inflammation and scarring can compromise postglomerular blood flow and initiate ischemic tubular injury. Injury to the peritubular capillaries and rarefaction of the peritubular vasculature are components of several models of experimental tubulointerstitial nephritis (73). An early angiogenic response followed by progressive endothelial cell apoptosis and regression of peritubular capillaries has been implicated as a potential contributory factor to tubulointerstitial fibrosis (73,74). In experimental models, hypoxic tubular epithelial cells increase expression of several proinflammatory molecules that promote fibrosis, such as interleukin-6 (IL-6) and tumor growth factor-β (TGF-β) (75–77). Hypoxia also leads to the increased local production of angiotensin and the transdifferentiation of tubular epithelial cells into migrating fibroblastlike cells (78). As such, tubular ischemia triggers a localized inflammation and leukocyte recruitment and promotes the deposition of extracellular matrix (67–70). The resultant tubulointerstitial nephritis correlates with reduced renal blood flow, fall in glomerular filtration rate, and altered nitric oxide (NO) activity (79).

Iron loss in the tubular fluid also has been implicated in the pathogenesis of the tubulointerstitial injury associated with primary glomerular disease. In an experimental model of glomerulonephritis in rats, the induction of iron deficiency reduced the urinary protein excretion rate and attenuated the development of tubulointerstitial lesions and attendant deterioration of kidney function (80,81). Conceivably, iron, dissociated from filtered transferrin at the more acid pH of the tubule fluid or during its absorption in the proximal tubule, contributes to the formation of free hydroxyl radicals, which account for tubular epithelial cell injury and consequent tubulointerstitial lesions. In type II diabetic patients, transferrinuria correlates with interstitial injury, perhaps through a similar free-radical mediated mechanism (82) or possibly by stimulating platelet-derived growth factor (PDGF) (83).

These experimental studies notwithstanding, severe tubulointerstitial lesions do not invariably accompany proteinuric glomerular diseases, and the search for additional factors contributory to the development of interstitial lesions deserves continuous scrutiny.

In summary, the available evidence would indicate that the normal interstitium plays an important role in kidney function and that changes in the integrity of interstitial components show a significant correlation to the severity and extent of loss of function in various diseases of the kidney. As such, tubulointerstitial nephritis can be considered the final common pathway of all forms of chronic kidney diseases associated with progressive loss of kidney function independent of the original site of injury—glomeruli, vasculature, or tubules. The importance of the interstitium in the normal and diseased kidney is now well established. The mechanisms involved in the initiation and progression of interstitial fibrosis remain to be fully elucidated.

PATHOLOGIC FEATURES OF TUBULOINTERSTITIAL NEPHROPATHIES

Independent of its initiating process, the principal morphologic feature of chronic tubulointerstitial nephropathy is an increase

in the interstitial volume, mainly because of interstitial fibrosis and of varying degrees of infiltration with chronic inflammatory cells (Fig. 72-1). Tubular atrophy and degeneration are also common, if not invariable, findings, particularly in the late phases of the disease (84). As interstitial widening by fibrosis progresses, the tubular basement membrane (TBM) thickness increases as a result of deposition of type IV collagen and laminin (85). Depending on the duration, cause, and severity of the initiating disease process, the distribution of the lesions will vary from focal to diffuse but is generally patchy. This interstitial inflammatory reaction is a secondary feature of several progressive kidney diseases, including those of glomerular or vascular origin (62,86–90). There are, however, other diseases of the kidney in which primary tubulointerstitial lesions are the principal, and early in the course of the disease, usually the only detectable lesion on morphologic examination of kidney tissue (91). Changes of glomerular sclerosis, periglomerular fibrosis, and atrophy, if not present at the onset, will ultimately occur in primary tubulointerstitial diseases of the kidney (92). The final picture that emerges in the end-stage form of the disease may be indistinguishable from that of end-stage kidney disease of any etiology. As such, progressive tubulointerstitial lesions are a major contributory cause of progressive loss of kidney function and of all end-stage renal diseases, whether of primary or secondary etiology.

Immunohistologic examination, using monoclonal antibodies, coupled with conventional and electron microscopy indicates that most of the mononuclear cells making up renal interstitial infiltrates are T lymphocytes (93–97). Chronic inflammatory cell loci may be characterized by Ia antigen-positive T cells, considered to be markers of activated lymphocytes (86,98). The interstitial infiltrates contain varying numbers of activated lymphocytes in the absence of tubulointerstitial immune deposits (62), even in classic examples of immune complex–mediated diseases such as systemic lupus erythematosus (59). The extent of interstitial infiltrates shows a direct correlation between the severity of tubular atrophy and interstitial fibrosis determined semiquantitatively (86). The profile of immunocompetent cells suggests a major role for cell-mediated immunity in the tubulointerstitial lesions that are mediated by autoimmune mechanisms (99,100). Experimental studies have shown the sequential accumulation of T cells and monocytes after the initial immune insult, which would implicate an important role for T cells, both as inflammatory cells and in the progression of subsequent injury (7,94,96,97). The infiltrating lymphocytes may be of the CD4+ helper/inducer subset (78) or the CD8+ cytotoxic/suppressor subset (76), although there generally seems to be a selective prevalence of the former variety (94). A more limited number of natural killer cells are a component of the infiltrates. Lymphocytes that are peritubular and are seen invading the tubular epithelial cells, so-called *tubulitis,* are generally of the cytotoxic (CD8+) variety (100), with a contributory role of the natural killer cells. Nephritogenic CD8+ T-cell lines of distinct functional phenotypes, which are specifically cytotoxic to tubular epithelial cells, have been isolated and identified (101). The further characterization of T-cell clones from these lesions can better define the role of the infiltrating cells in the pathogenesis of tubulointerstitial diseases (Fig. 72-1). Fine-needle aspiration of the kidney can be useful in the characterization of the cellular infiltrates (102) and their sequential evolution (103). The interstitial accumulation of monocytes involves a host of chemo-attractant cytokines such as osteopontin (uropontin), a secreted cell-attachment glycoprotein whose messenger RNA expression is upregulated and levels are increased at sites of tubular injury in proportion to the severity of tubular damage (104,105). The expression of other cell adhesion molecules (intercellular adhesion molecule 1 [ICAM-1], vascular cell adhesion molecule 1 [VCAM-1], E selectin) also is increased at sites of tubular injury and may contribute to the recruitment of mononuclear cells and increase the susceptibility of renal cells to cell-mediated injury (66–70,106,107).

In addition to the infiltrating nephritogenic lymphocytes, macrophages and mast cells are recruited and participate in the proteolytic and fibrotic injury of the normal parenchyma (97,108). The resident fibroblastic (or type I) interstitial cells, which normally produce and maintain the extracellular matrix, proliferate and increase their well-developed rough endoplasmic reticulum in response to injury (20,109) (Fig. 72-1). The number of fibroblasts derived in cell culture from kidney biopsy material obtained from patients with interstitial fibrosis is increased by a factor of five to ten, compared with that from cultures derived from normal kidney biopsy samples (19). Growth kinetic studies of these cells show a significant increase in proliferating capacity and generation time, indicating hyperproliferative growth (110).

The type of infiltrate can be characteristic and occasionally diagnostic of the kidney disease. The presence of eosinophils can be diagnostic of drug-induced acute allergic interstitial nephritis (Chapter 48). Another cell type, the presence of which may be of diagnostic importance, is the foam cell, with its characteristic vacuolated appearance. Studies using monoclonal antibodies showed these cells to be derived from macrophages (111), which have phagocytosed lipids. Macrophage-derived foam cells can perpetuate an inflammatory reaction by release of cytokines that promote vascular smooth muscle cell proliferation and matrix deposition (112). Isolated foam cells are frequently encountered in patients with heavy proteinuria of any etiology (113). Their presence in large numbers in the glomeruli and interstitium is indicative of Alport's nephritis (114). Masses of interstitial foam cells, interspersed with inflammatory cells, occur in chronic forms of pyelonephritis and, when prominent, form the characteristic lesion of xanthogranulomatous interstitial nephritis (115). A closely related condition is the appearance of large mononuclear cells with abundant cytoplasm, containing large and laminated phagolysosomes that show progressive mineralization and form the characteristic Michaelis-Gutmann bodies seen in malakoplakia (115,116). Although malakoplakia is commonly a lesion of the urinary bladder, it occasionally involves the renal interstitium and usually occurs in the context of chronic urinary tract infection (116,117). These peculiar cells have also been observed in hemolytic-uremic syndrome (118) and in transplanted kidneys (119).

The interstitial lesions of one group of tubulointerstitial diseases can be distinguished by the presence of a characteristic noncaseating granulomatous pattern, which, when present, is highly suggestive of sarcoidosis (120,121). Usually coexistent with extrarenal granulomata there are rare reports of sarcoid granulomatous lesions localized to the kidney only, in the absence of extrarenal lesions (122,123). Interstitial granulomatous reactions also occur in response to oxalate deposition (124) and to infection of the kidney by mycobacteria (125), fungus (126), or bacteria (127). Granulomatous reactions to drugs, such as methicillin (121), sulfonamides (128), and narcotics (129), have been observed in patients with acute forms of tubulointerstitial disease. In experimental models of anti–basement membrane nephritis, granulomatous inflammation develops around the immunologically altered TBM. In the course of the granulomatous reaction, tissue monocytes evolve from recruited circulating monocytes and pursue one of two independent pathways of differentiation: (a) macrophages that evolve distant from the tubules or (b) epithelioid cells that are formed adjacent to the tubules. The latter form through cell fusion and can develop into the multinucleated giant cells of Langhans (130–133). Multinucleated giant cells also are a feature of renal involvement in multiple myeloma (134,135).

Intracellular crystals can be diagnostic of some forms of tubulointerstitial diseases. Rhomboid-shaped crystals within

the cell lysosomes are characteristic of cystinosis (136). Tophaceous deposits and birefringent deposits of sodium biurate are seen in hyperuricemia and gout (137). Birefringence on dark-field microscopy is observed in oxalosis (106,119,120). Gold therapy for rheumatoid arthritis can result in characteristic lysosomal crystalloid aggregates that can be seen by electron microscopy (140).

The interstitial spaces may be invaded by proliferative malignant cells in leukemias and lymphomas (141), typically causing an increase in kidney size. Interstitial infiltration by malignant cells can lead to increased interstitial pressure with resultant tubular obstruction and disruption of peritubular vasculature. Commonly found at postmortem in patients with clinically evident malignant disease, massive infiltration of the kidney with renal failure may be an initial presenting feature in some patients (141,142).

PATHOGENESIS AND MECHANISMS OF INJURY IN TUBULOINTERSTITIAL NEPHRITIS

Tubulointerstitial nephropathy is an inflammatory reaction that is perpetuated by continuous exposure to injury or a failure to respond to the usual controlling mechanisms in the feedback loop of the normal defensive response to injury. The demonstration that the infiltrating cells are antigenetically active implicates a principal role of cell-mediated immune reaction in this inappropriate response, which evolves into one progressive parenchymal injury (96,97). The gradual increase in interstitial spaces by infiltrating cells and fibrous tissue implicates a relentless fibrogenesis in replacing the regressive tubular atrophy and degeneration caused by the parenchymal injury (143).

The immune response to the initiating injury includes recognition of the insult, an integration of the response, and an effector phase, which in the kidney entails a complex integrated operation between the tubular epithelial cells, resident interstitial cells, and the recruited inflammatory cells (Fig. 72-7). This interactive process is mediated by a host of cytokines and autocoids released by the involved cells at various stages of the response and of the upregulated expression of specific receptors to them by the targeted cells. More of these stimulatory or responsive signals have been identified, with different, often overlapping, functions that modulate and amplify the inflammatory reaction that accounts for the tubulointerstitial lesions. Some of these are cell surface markers that have antigenic (MHC; human leukocyte antigen class II; osteonectin; and secreted protein acidic and rich in cysteine) or adhesive (ICAM-1, VCAM-1; integrins; osteopontin; thrombospondin; selectins) properties. Others are cytokines that are chemoattractant (monocyte chemoattractant protein-1; regulated on activation, normal T-cell expressed and secreted; osteopontin; eotaxin; fibronectin; macrophage inflammatory protein-1), *proinflammatory* (IL-6, IL-8, platelet-derived growth factor β [PDGFβ]; granulocyte monocyte colony-stimulating factor; tumor necrosis factor α [TNFα]; Tamm-Horsfall glycoprotein [TH]; transforming growth factor-α [TGF-α]), *vasoactive* (nitrous oxide [NO]; endothelin 1; angiotensin II [AII]; thromboxanes; prostaglandins; adenosine), *cytotoxic* (matrix metalloproteinases-1 and -2 [MMP-1 and MMP-2]; tissue inhibitor of metalloproteinase-1; TNFα; reactive oxygen species [ROS]; ferric ion), fibrogenetic (fibroblast growth factor-2; AII; aldosterone; TGF-β; PDGF; IL-1; IL-6; TNF; and plasminogen activator inhibitor [PAI]), *growth promoting* (AII, PDGF; TGF-β; endothelin 1), or *apoptotic* (clusterin; Fas; bax; osteopontin) (8,9,144–150).

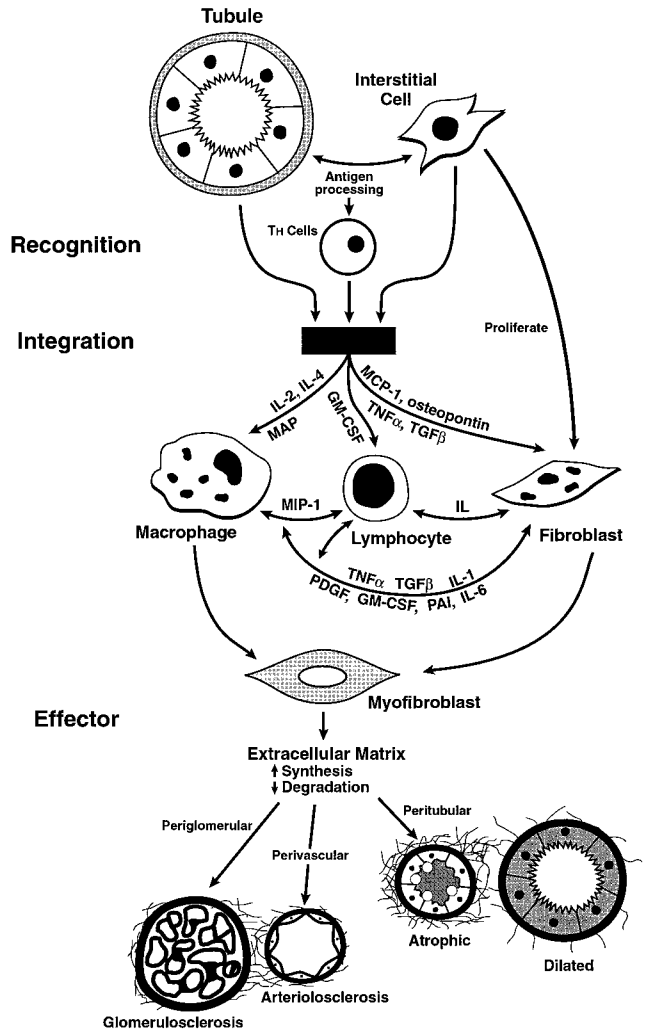

FIGURE 72-7. Pathogenesis and mechanisms of injury in tubulointerstitial nephritis. T_H, T helper cells.

The initiating insult may be a drug, an infection, a filtered noxious protein, mechanical obstruction, ischemia, or a specific antigen (41,66–70). The reactive process begins by antigenic recognition, uptake, processing, and presentation by antigen-presenting cells followed by the recruitment of T helper cells (T_H cells), which are activated and initiate the subsequent recruitment of the effector mononuclear B and T lymphocytes. In rare conditions in which the injury is induced by a specific antigen, the B cells differentiate into antibody-producing plasma cells, which result in the deposition of immunoglobulins and immune complexes along the TBM. In more common conditions, the effector mechanism is nonspecific and consists principally of inflammatory CD4+ and cytotoxic CD8+ lymphocytes, and depending on the injury, fewer and variable numbers of B cells, natural killer cells, and macrophages (96,97,145). In acute tubulointerstitial nephritis, feedback mechanisms restore this initial coordinated response to injury to its baseline steady state. In chronic tubulointerstitial nephritis, either persistent injury or perpetuation of the effector phase results in variable rates of progressive and permanent tubulointerstitial damage.

The deposition of fibrous tissue is the first indicator of progression. An integral component of this progressive stage is activation of interstitial fibroblasts, which undergo upregulation and increased expression of new or constitutive receptors for the fibrogenetic cytokines (TGF-β1, PDGF, TNF-α, IL-6,

PAI), released by the tubular epithelial cells and infiltrating cells (8,9,145–148). Infiltrating macrophages produce direct injury (proteases, ROS) and account for triggering of several fibrogenetic cytokines, among which TGF-β and PDGF appear to play a dominant role (151–153).

The subsequent evolution of the fibroblasts (derived from resident fibroblasts or the transdifferentiation into fibroblasts of epithelial cells, vascular pericytes, and recruited macrophages) into myofibroblasts, as they undergo phenotypic changes including acquisition of smooth muscle (actin), and mesenchymal cell markers (vimentin, desmin), parallels the increased deposition of extracellular matrix (66–70, 154–157). Fibronectin has been proposed as the first extracellular matricial protein to appear, and that its chemoattractant and adhesive properties then provide for the recruitment of fibroblasts and deposition of the other extracellular proteins (158). Experimental maneuvers that diminish early mediators of fibrosis (macrophages, fibronectin, myofibroblast phenotype) have been shown to attenuate interstitial fibrosis in different models of kidney disease (153,154). The deposited extracellular matrix consists principally of proteoglycans (decorin, biglycan) and collagen types I, III, and IV (143,145,155,156). In addition to the stimulated fibrogenesis, atrophy and collapse of injured tubules and contraction of the myofibroblasts have been proposed for the apparent disproportionate amount of fibrous tissue seen in the affected kidney (143).

Experimental evidence indicates that tubular epithelial cells can differentiate into interstitial fibroblasts, which contribute to the interstitial fibrosis (70,160). Hypoxia and TGF-β initiate their transdifferentiation (78,161). Inhibition of TGF-β pathways interrupts progressive fibrosis in experimental models of obstructive nephropathy (161) and glomerulonephritis (162) induced tubulointerstitial nephritis. Consequently, TGF-β has emerged as a potential target in limiting the progression of fibrosis and scarring. From a therapeutic standpoint, targeting epithelial to fibroblast differentiation and profibrotic pathways is a promising avenue of investigation. Bone morphogenic protein-7 (BMP-7), a factor expressed in tubular epithelial cells, has been shown in experimental models to inhibit this pathogenic transdifferentiation (163–165) and TGF-β synthesis (166).

In the affected kidney, myofibroblasts aggregate around the injured tubules and the arterioles. Microvascular obliteration (fibrosis) and constriction (myofibroblasts, vasoactive agents) induce ischemia, alter glomerular hemodynamics, and increase production of AII, which magnifies the fibrogenesis (144) and perpetuates (48) the injury. Although circulating levels of AII are not elevated, the concentration of AII within the kidney is several-fold higher than in plasma (147) and contributes to the proliferation of fibroblasts and their conversion into myofibroblasts (144,167,168), and to fibrosis by the activation of PDGF and TGF-β1 (169,170). In a rat experimental model, AII infusion has been shown to shift T-helper cell balance to a T_h1 subtype, an effect that could be blocked with angiotensin receptor blockers (144). Thus, the renoprotective effects of angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers is not limited to their hemodynamic effect on glomerular intracapillary pressure, but perhaps more importantly to their demonstrated ability to favorably alter the lesions of tubulointerstitial nephritis through an immunomodulatory as well as vascular effect. Part of the effect of AII may be related to its action in stimulating aldosterone, which is an independent autacoid promulgator of the injury process (170,171). Aldosterone has been shown to increase TGF-β expression in the kidney, an effect that could be blocked with spironolactone (167,172). Whether the cardioprotective effect of low-dose spironolactone also can protect the kidney in humans, as suggested from experimental evidence (149,151,168), remains to be established.

Increased ammonia generation, due to proteinuria or the remaining intact tubules, has also been implicated as a cause of progressive injury (173,174). Ammonia possesses the capability to form amidated C3, which acts on C3/C5 convertase and activates through the alternate complement pathway the membrane attack complex C5b-9 (173–176). Administration of bicarbonate to correct the acidosis reduces tubular ammonia generation and has been shown to ameliorate the tubulointerstitial lesions in the remnant model of kidney disease.

The evidence for all of these pathogenetic mechanisms has been marshaled from experimental laboratory models of tubulointerstitial disease. Several of them have been confirmed to be operative in humans from *in situ* hybridization and histochemical studies of kidney biopsies. The implicated pathogenetic mechanisms provide a basis for the therapy of tubulointerstitial nephritis. The initial inflammatory cell infiltration is amenable to steroid therapy. Evidence also supports the role of other forms of cell-directed therapy with cyclosporine or cyclophosphamide. The renoprotective effects of ACE inhibitors and AII blockers make them a logical agent of choice in management, even in the absence of hypertension. Correction of acidosis to limit activation of the alternate complement pathway initiated injury by the membrane attack complex deserves attention. Finally, accrued evidence for determinants of extracellular matrix biosynthesis and degradation provides a basis for the identification of potentially effective agents in controlling interstitial fibrosis.

FUNCTIONAL MANIFESTATIONS OF TUBULOINTERSTITIAL NEPHROPATHIES

The principal manifestations of interstitial lesions are those of tubular dysfunction, which is one reason why the term *tubulointerstitial disease* is preferred to *tubulointerstitial nephritis*. Convincing evidence has been presented that there is a correlation between the structural changes in the tubulointerstitium and tubular function, and that the magnitude of tubular dysfunction tends to relate to the extent of tubulointerstitial involvement (12,13,26,57). Because of the focal nature of the lesions that occur and the segmental nature of normal tubular function, the pattern of tubular dysfunction that results will vary, depending on the major site of injury, whereas the extent of damage will determine the severity of tubular dysfunction. The hallmarks of glomerular disease, such as salt retention, edema, hypertension, proteinuria, and hematuria, are characteristically absent in the early phases of primary chronic tubulointerstitial nephropathies (4,10,11,177). However, in keeping with the intact nephron hypothesis, glomerular damage ultimately will appear. As a rule, the magnitude of glomerular function reduction seems to correlate with the magnitude of interstitial fibrosis, whereas the enlargement of the interstitium by cellular infiltrate appears to have less of a bearing on the GFR in the early stages of the disease (57,178–180).

Basically, the tubulointerstitial lesions are localized either to the cortex or to the medulla. Cortical lesions will affect, in the main, the proximal tubule or the distal tubule. Medullary lesions will affect the loop of Henle and the collecting duct. The change in the normal function of each of these affected segments then determines the manifestations of tubular dysfunction (Table 72-1). Essentially, the proximal nephron segment reabsorbs the bulk of bicarbonate, glucose, amino acids, phosphate, and uric acid. Changes in proximal tubular function, therefore, result in bicarbonaturia (proximal renal tubular acidosis), β_2-microglobinuria, glucosuria (renal glucosuria), aminoaciduria, magnesemia, phosphaturia, and uricosuria (48,181,182). The latter two can be valuable in suspecting

TABLE 72-1

PRINCIPAL SITES OF INJURY AND PATTERNS OF TUBULAR DYSFUNCTION IN CHRONIC TUBULOINTERSTITIAL NEPHROPATHIES

Site of injury	Cause	Tubular dysfunction
Cortex		
Proximal tubule	Heavy metals Multiple myeloma Immunologic diseases Cystinosis	↓ Reabsorption of: Bicarbonate Glucose Uric acid Phosphate Amino acids
Distal tubule	Immunologic diseases Granulomatous diseases Hereditary diseases Hypercalcemia Urinary tract obstruction Sickle cell hemoglobinopathy Amyloidosis	↓ Secretion of: Hydrogen ion Potassium ↓ Reabsorption of: Sodium
Medulla	Analgesic nephropathy Sickle hemoglobinopathy Uric acid disorders Hypercalcemia Infection Hereditary disorders Granulomatous diseases	Impaired ability to concentrate urine ↓ Reabsorption of: Sodium
Papilla	Analgesic nephropathy Diabetes mellitus Infection Urinary tract obstruction Sickle hemoglobinopathy Transplanted kidney	Impaired ability to concentrate urine ↓ Reabsorption of: Sodium

tubulointerstitial disease when the serum phosphate and urate concentrations are noted to be lower than expected, particularly in individuals with reduced GFR and azotemia (178). Measurements of the fractional clearance of uric acid from a spot urine sample can also be helpful. The normal fractional clearance of urate is about 10%; values significantly higher than normal would suggest a defect in urate handling by the proximal tubule. In addition, there is a linear correlation between the fractional excretion of magnesium and the extent of tubulointerstitial fibrosis, and an increase in the fractional excretion of magnesium has been proposed as the most sensitive index to detect an early abnormality of tubular structure and function (26). The clinical conditions in which this has best been characterized are patients receiving cisplatin or gentamicin (183,184). The distal nephron segment secretes hydrogen and potassium and regulates the final amount of sodium chloride excreted. Lesions affecting primarily this segment, therefore, result in the distal form of renal tubular acidosis, hyperkalemia, and salt wasting (185–188). Lesions that primarily involve the medulla and papilla disproportionately affect the loops of Henle, the collecting ducts, and the other medullary structures essential to attaining and maintaining medullary hypertonicity. Disruption of these structures, therefore, results in different degrees of nephrogenic diabetes insipidus and clinically manifests as polyuria and nocturia (189,190).

Although this general framework is useful in localizing the site of injury, considerable overlap may be encountered clinically, with different degrees of proximal, distal, and medullary dysfunction present. In addition, the ultimate development of kidney failure complicates the issue further because of the added effect of urea-induced osmotic diuresis on tubular function in the remaining nephrons. In this later stage of tubulointerstitial diseases, the absence of glomerular proteinuria and the more common occurrence of hypertension in glomerular diseases can be helpful in the differential diagnosis of primary from secondary forms of tubulointerstitial nephritis (4,13,178). Under any circumstance though, the diagnosis and severity of tubulointerstitial disease can be established only by morphologic examination of kidney tissue.

The type of insult determines the segmental location of injury (Table 72-1). For example, agents secreted by the organic pathway in the pars recta (cephalosporins) or reabsorbed in the proximal tubule (aminoglycoside and light-chain proteins) cause predominantly proximal tubular lesions. Conditions or agents that cause hypersensitivity reactions (methicillin) or depositional disorders (amyloid and hypergammaglobulinemic states) cause predominantly distal tubular lesions. Finally, insulting agents that are affected by the urine concentrating mechanism (analgesics and uric acid) or medullary tonicity (sickle cell) cause medullary injury (11).

The clinical course of tubulointerstitial diseases depends to a great extent on the primary cause and the magnitude and persistence of the insult. In general, acute exposure to a massive insult results in the rapid deterioration of kidney function (191), whereas sporadic exposure to small amounts of the same insult results in a more indolent course, with a gradual but progressive loss of kidney function (192). Tubulointerstitial diseases are divided into acute or chronic forms on the basis of the clinical course and accompanying pathologic lesions (Table 72-2). The *acute* form is characterized by prominent interstitial edema and focal areas of varied cellular infiltrates. If the injury is severe, tubular damage and dilation are also present, and azotemia and renal insufficiency are early presenting features (Chapter 48). In the chronic form, interstitial fibrosis and tubular atrophy are

TABLE 72-2

MORPHOLOGIC FEATURES OF TUBULOINTERSTITIAL NEPHRITIS

Feature	Acute	Chronic
Interstitium[a]		
Cellular infiltrate	+ → + + + +	+ → + +
Edema	+ → + + + +	± → + +
Fibrosis	±	+ + → + + + +
Tubules		
Epithelium	Injury → necrosis	Atrophy
Basement membrane	Injury → disruption	Thickened
Shape	Preserved	Atrophy Dilation
Glomerulus		
Structure	None → MCD Sclerosis	Periglomerular fibrosis
Vascular		
Structure	Minimal Reversible	Variable Sclerosis

MCD, minimal change disease.
[a]The severity of the changes is given as an estimate with + for minimal to + + + + as severe.

present, and the cellular infiltrate is uniformly mononuclear. In this more indolent form, loss of glomerular filtration is slow to develop and the early manifestations of the disease are those of tubular dysfunction that may go undetected unless specifically elicited in the history, noted in the laboratory results, or documented by specific testing (177). Ultimately, though, kidney failure will occur. The onset of heavy proteinuria is an ominous sign and is indicative of the development of glomerulosclerosis (91,193). In contrast to glomerular disease, hypertension is a late development and occurs after significant decrements in filtration rate have occurred (10). In a study of 48 patients with biopsy-proven tubulointerstitial nephritis, hypertension was present in only half the cases (178). In the other half, the blood pressure was normal even in the presence of kidney failure. Of the 15 patients with a creatinine clearance rate of less than 15 mL per minute, 7 had normal blood pressure.

ETIOLOGIC FACTORS

Arbitrarily grouped together because the predominant morphologic involvement is in the tubules and interstitium, primary tubulointerstitial nephropathies are caused by a motley group of diseases of varied and diverse etiologies (Table 72-3). The different initiating mechanisms that have been implicated include immunologic diseases, infection, toxic and metabolic perturbations, mechanical obstruction to urine flow, neoplasia, and hereditary factors.

Immunologic Diseases

Evidence exists for three immunologic mechanisms of tubulointerstitial injury: immune complex deposition, antibodies directed against the TBM, and altered cell-mediated immunity (194–196). Although the preponderance of the evidence in human tubulointerstitial nephropathy implicates a cell-mediated immune process, the best-studied experimental model is that of

TABLE 72-3

CONDITIONS ASSOCIATED WITH CHRONIC TUBULOINTERSTITIAL NEPHROPATHY

- **Immunologic diseases**
  - Systemic lupus erythematosus
  - Sjögren's syndrome
  - Transplanted kidney
  - Cryoglobulinemia
  - Goodpasture's syndrome
  - IgA nephropathy
  - Amyloidosis
  - Pyelonephritis
- **Infection**
  - Systemic
  - Renal
    - Bacterial
    - Viral
    - Fungal
    - Mycobacterial
- **Urinary tract obstructions**
  - Vesicoureteral reflux
  - Mechanical
- **Drugs**
  - Analgesics
  - Cyclosporine
  - Nitrosourea
  - Cisplatinum
  - Lithium
  - Miscellaneous
- **Hematopoietic diseases**
  - Sickle hemoglobinopathies
  - Multiple myeloma
  - Lymphoproliferative disorders
  - Aplastic anemia
- **Heavy metals**
  - Lead
  - Cadmium
  - Miscellaneous
- **Vascular diseases**
  - Nephrosclerosis
  - Atheroembolic disease
  - Radiation nephritis
  - Diabetes mellitus
  - Sickle hemoglobinopathies
  - Vasculitis
- **Metabolic disorders**
  - Hyperuricemia/hyperuricosuria
  - Hypercalcemia/hypercalcuria
  - Hyperoxaluria
  - Potassium depletion
  - Cystinosis
- **Hereditary diseases**
  - Medullary cystic disease
  - Hereditary nephritis
  - Medullary sponge kidney
  - Polycystic kidney disease
  - Familial juvenile nephronophthisis
- **Granulomatous diseases**
  - Sarcoidosis
  - Tuberculosis
  - Wegener's granulomatosis
- **Endemic diseases**
  - Balkan nephropathy
  - Nephropathia epidemica
- **Idiopathic diseases**

anti-TBM nephritis (197,198). However, anti-TBM antibodies are a rare cause of tubulointerstitial nephritis in humans, except in association with antiglomerular basement membrane (anti-GBM) disease (195), as is that of immune complex deposits (197,199).

Immune Complex Deposition

The repeated injection of homologous kidney tissue in Freund's adjuvant into rabbits (200) and rats (201) results in tubulointerstitial immune complex disease characterized by tubule cell injury, interstitial fibrosis, and mononuclear cell infiltration, with only minimal changes noted in the glomeruli. Immunofluorescent staining reveals granular deposits of immunoglobulins and complement along the TBM. Electron microscopy confirms the presence of focal electron-dense deposits along the tubule. The postulated mechanism for this model of tubulointerstitial disease is that the circulating antibodies, which are formed in response to the injection of homologous kidney tissue, diffuse across the peritubular capillaries and combine in the interstitium with a tubular cell antigen that diffuses out of the epithelial cells, thereby forming the peritubular interstitial immune complexes seen on microscopy. A similar lesion can be produced in rats immunized with Tamm-Horsfall protein (THP), a surface membrane glycoprotein of the epithelial cells of the ascending thick limb of the loop of Henle and distal tubules. These animals develop antibodies to THP and a tubulointerstitial nephritis that selectively involves the thick ascending limb (201–203). In this model, the earliest electron-dense deposits are detected in the extracellular space between the basal infoldings of the cell membranes and the TBM. Later, they are noted within the TBM. Selective mononuclear cell infiltrates occur around the tubules at the site of the deposits. Tubulointerstitial immune complex nephritis also has been produced by passive immunization of rats with antisera to rat THP (204). In these studies, the antibodies were noted to combine with THP at the base of the cells lining the thick ascending limb of the loop of Henle and to form granular immune complexes *in situ* in the spaces between the basal cell surface membrane and the TBMs. The deposits were maximal in the juxtamedullary region and least prominent in the first portion of the ascending limb in the inner zone of the outer medulla. Immune complexes also were selectively formed, at the same sites, during perfusion of the isolated kidneys with antisera to THP. The deposits of immune complexes were maximal during the first week after injection, at a time when the circulating antibody titers were highest. As the antibody titers subsequently fell to undetectable levels, tubular immune complexes cleared rapidly and were virtually absent by about 4 weeks after the injection of the anti-THP antibodies. This is in contrast to the prolonged persistence of glomerular immune complex deposits that occurs after the experimental injection of appropriate antiglomerular antisera and may account for the rapid reversibility of immune injury in tubulointerstitial nephritis when exposure to the antibody is limited (204). In addition, although immunoglobulin deposition in the glomeruli correlates with glomerular damage, a similar association is not found between the extent of tubulointerstitial deposits and interstitial inflammation and tubular injury (194,195,205,206).

A role for THP-mediated immune complex disease has been implicated in certain forms of human chronic kidney diseases. Antibodies to THP are found in sera of patients with vesicoureteral reflux (207) and pyelonephritis (208) and are demonstrated in the interstitium of patients with medullary cystic disease (209), hydronephrosis (210), diabetes (211), and hereditary nephritis (212), as well as in the glomeruli of patients with obstructive uropathy (213). However, immunoglobulins are not regularly associated with THP deposits noted in the interstitium of human kidneys (213). In addition, the frequent occurrence of mononuclear cell infiltrates surrounding the immune complex deposits may reflect a cell-mediated immune response that accounts for the progressive nature of the kidney injury in some of these clinical conditions (Fig. 72-1). In experimental tubulointerstitial nephritis induced in guinea pigs by immunization with homologous THP, the transfer of lymphocytes and spleen cells to unimmunized animals does induce a tubulointerstitial nephritis in the recipients (214), indicating a causative role of cell-mediated immunity. Finally, it is not possible to attribute a primary role to antibodies, to THP, or to that of any other cellular antigenic component, because the immune deposits could have followed primary tubular injury by another mechanism, with the subsequent release of THP or other cellular antigenic components into the interstitium and the secondary formation of autologous antibodies. Evidence has been advanced that the rise of intrarenal ammonia concentration, engendered by the adaptation to chronic kidney disease, could determine attendant immune complex deposits (215). Because ammonia attacks the reactive thioester bond of C3 (173), the direct reaction of ammonia with C3 forms amidated C3, which is sufficient to trigger the alternative complement pathway that activates the membrane attack complex of complement and thereby propagates the injury and peritubular deposition of C3 and C5b-9 (174–176), as well as the generation of other mediators of tubulointerstitial injury (216) (Fig. 72-1). As noted previously, in a study of rats with surgically reduced kidney mass, dietary supplementation with sodium bicarbonate resulted in lower tissue levels of ammonia, less deposition of complement components, and a diminution of tubulointerstitial damage (217). It is possible, therefore, that it is the local activation of the complement system, rather than THP protein, which contributes to immune complex deposition and the propagation of interstitial injury by an immune-mediated mechanism independent of the initial etiology of the kidney disease (218).

Primary tubulointerstitial nephritis mediated by immune complex deposition is rare in humans (195). The condition in which tubulointerstitial deposits are most commonly found clinically is lupus nephritis, in which interstitial infiltrates are detected by light microscopy in more than half of biopsy specimens (219–222). The deposits may be focal or diffuse. They are located either along or within the TBM, around the peritubular capillaries, or in the interstitium. They consist principally of immunoglobulin G (IgG) and C3 and often of IgM or IgA. Evidence for immune complex–mediated injury is adduced from the demonstration of DNA in the deposits. In general, the tubular and interstitial changes correlate with the magnitude of the deposits, although interstitial mononuclear cell infiltrates can be present in the kidneys of patients who have few, if any, tubulointerstitial immune complex deposits (220–222). Moreover, even in the presence of tubulointerstitial deposits, there is no correlation between the prevalence of immune complex deposits and the severity of interstitial reaction, indicating a more important role of a cell-mediated injury (59). Nevertheless, the severity of the interstitial changes, independent of the pathogenesis, correlates quite well with the degree of reduced kidney function in lupus nephritis (59,180,223), and appraisal of the disease activity may be possible by renal imaging with gallium-67 (224). Prominent tubulointerstitial nephritis also can occur in patients with systemic lupus erythematosus with only mild glomerular involvement, and generally pursues a rapid fulminant course (225).

Tubulointerstitial immune complex deposits have been also described in kidney allografts (226), Sjögren's syndrome (100, 227–229), mixed cryoglobulinemia (221), crescentic glomerulonephritis (220), IgA nephropathy (52), Wegener's granulomatosis (230), and idiopathic tubulointerstitial nephropathy (231). In addition, the sera from some patients with idiopathic tubular dysfunction contain antibodies that react with distal

tubular epithelium (232–234). However, aside from systemic lupus erythematosus, in which antigen (DNA) has been demonstrated in the deposits, and mixed cryoglobulinemia, in which anti-IgG antibodies and IgG have been identified, there is no evidence to support a specific antigen responsible for the immune complex deposits. Therefore, direct proof of immune complex–mediated injury is lacking in most of the rare clinical instances in which immune complex deposits are noted on examination of the kidney tissue.

Antibodies Against Tubular Basement Membrane

Evidence for anti-TBM antibody disease was first obtained when guinea pigs were noted to develop a progressive, fatal tubulointerstitial kidney disease after they were injected with crude rabbit cortical tissue in Freund's adjuvant (235). Morphologic examination of the kidneys from these animals revealed tubular cell injury, peritubular multinucleated giant cells, interstitial fibrosis, and mononuclear cell infiltration. The infiltrating cells consisted of monocytes, macrophages, and T lymphocytes. Linear deposits of IgG were noted along the TBM on immunofluorescent study (235,236). Similar lesions were produced in guinea pigs immunized with bovine TBM (237) and in the Brown Norway strain of rats, but not Sprague-Dawley or Lewis rats immunized with homologous or heterologous TBM (197,238). In both species, the lesion could be reproduced in normal recipients by injecting them with serum from the immunized animals (168,170). Independent of the initiating role of anti-TBM antibodies, cell infiltrates are a prominent feature in all models studied, implicating a role for cell-mediated immunity in the pathogenesis and modulation of the interstitial lesions (197–199). Although the disease cannot be transmitted by cells in all species, it can be transferred by intravenous injection of lymph node and splenic cells from sensitized animals in other species (197,198), suggesting again the central role of cell-mediated immunity.

Evidence for anti-TBM antibodies causing tubulointerstitial disease in humans has been advanced from patients with Goodpasture's syndrome associated with anti-GBM antibodies (220) and patients with rapidly progressive glomerulonephritis who have associated anti-TBM antibodies (20,230). In both forms, absorption of eluates with solubilized GBM removed the anti-TBM activity (196). A target antigen in human anti-TBM disease has been identified as a 58-kDa glycoprotein (239–241), which in experimental models synthesizes as a high-molecular-weight glycoprotein that is processed to smaller forms that are secreted by proximal tubular cells into the extracellular matrix, where it attaches to the TBM (199,242). This noncollagenous glycoprotein constitutes about 9% of the TBM and accounts for 90% of the tubulointerstitial antigen to which anti-TBM antibodies are directed. Its overall composition is similar to that of laminin and nidogen (243,244). Target antigens or tubulointerstitial nephritis antigens (TIN-Ag) identified in human anti-TBM disease have been termed TIN1 and TIN2 and shown to promote cell adhesion and to interact with type IV collagen (245). Anti-TBM antibodies also have been demonstrated in some cases of glomerulonephritis without associated anti-GBM antibodies (246–249) , such as kidney allografts (215,226,250), and lupus nephritis (251,252), and detected in the serum of patients with biopsy proven tubulointerstitial nephritis (253). The role of these implicated antigens in the pathophysiology of chronic tubulointerstitial nephritis remains to be elucidated.

Cell-Mediated Immunity

Although the presence of anti-TBM antibodies or immune complex deposits is a variable feature of tubulointerstitial disease, interstitial cell infiltration is an almost invariable essential component of the lesion. Most of the mononuclear cells making up the renal interstitium are T cells and are generally characterized by markers of activated lymphocytes (62,86,98). Hence, the role of cell-mediated immunologic mechanisms involved in tubulointerstitial diseases can be considered on a reasonably sound basis (199,254). Because both immune complex–mediated and anti-TBM nephritis can be transferred by lymph node and spleen cells, a cell-mediated response appears to be an essential component of the immunopathology of all tubulointerstitial nephropathies (197,199). It is evident then that even in antibody-dependent forms of the disease, recruited interstitial cells play a major role in anti-TBM and immune complex–mediated interstitial lesions. Some rats injected with homologous kidney tissue develop interstitial mononuclear cell infiltrates before there is evidence of autoantibody production (194,195). This was also noted in the early studies of nonimmunosuppressed canine experimental kidney transplants (3). In a study of guinea pigs sensitized to bovine γ-globulin, the direct injection of the heterologous globulin into the renal cortex resulted in a mononuclear interstitial infiltrate with focal tubular destruction (255). This reactivity could then be transferred to unsensitized animals with lymph node cells, but not with serum, providing direct evidence for cell-mediated tubulointerstitial injury in this experimental model.

The tubular epithelial cells appear to contribute to the recruitment of the mononuclear cells (65,110,256). Cytokines and TNFs released by the activated infiltrating cells and injured tubular epithelial cells have been implicated in the pathogenesis and progression of tubulointerstitial lesions (7,8,16,257–259). Moreover, the epithelial cells of the proximal tubule are one of the few epithelial cells in the body shown to constitutively express the class II MHC molecules required to present antigens to CD4+ T cells (6) and likely are central to the initiation and perpetuation of the sequence of events that results in chronic tubulointerstitial nephropathies (Figs. 72-1, 72-7).

Infection

Many organisms have been associated with acute tubulointerstitial nephritis, either due to their direct invasion of the kidney or due to a reactive response of the kidney to a systemic infection (Chapters 34 and 48). By contrast, the bulk of the available evidence implicates direct bacterial infection of the kidney, rather than a reactive response to systemic infections, as a cause of chronic tubulointerstitial disease.

Cellular reactivity to bacterial antigens has been implicated in the pathogenesis of interstitial lesions of pyelonephritis (260). By using an indirect fluorescent method for the detection of a common enterobacterial antigen in infected tissues, it was shown that bacterial antigen persisted in renal scars after the healing of experimental pyelonephritis (261,262). In one clinical study, bacterial antigen was found in six of seven kidneys of patients with "abacterial" pyelonephritis who had not had clinical or bacteriologic evidence of infection for as long as 19 years before the study (263). This finding, however, could not be substantiated in other studies in which the antigen could be demonstrated in kidneys from patients with acute pyelonephritis caused by enteric organisms, but not from those with chronic interstitial nephritis or nonspecific renal scars or patients with pyelonephritis who were bacteriologically free of infecting organisms (260). Experimental studies have revealed that rats with acute enteric streptococcal pyelonephritis develop cellular reactivity to bacteria, as indicated by lymphocyte stimulation *in vitro*. In these animals, bacterial antigens can be shown by immunofluorescent studies within both the infiltrating leukocytes and the interstitium. However, after eradication of the infection, bacterial antigens were not demonstrable in the interstitium, and, when present, were found only within macrophages (260–262). It would seem unlikely, therefore, that

delayed reactivity against bacterial antigens could provide a mechanism for the perpetuation of the tubulointerstitial lesions of pyelonephritis beyond the period when viable organisms are present in the kidney (260–264).

Whereas the role of bacterial infection as the major determinant for the development of acute pyelonephritis is unequivocal, the role of recurrent infection as a cause of kidney damage remains doubtful. Progression from acute infection to chronic "atrophic" pyelonephritis does not appear to be a result of persistent or recurrent infection itself but may be due to chronic tubular cell injury (Figs. 72-1, 72-7), with consequent altered cellular immune mechanisms (151), and usually will develop in those with a coexistent mechanical outflow obstruction (207,264–266), thereby implicating an added role for obstruction. Several studies of many women and men with significant bacteriuria, who were followed over a decade, showed that urinary tract infection does not lead to kidney damage, provided the condition is not associated with obstruction and there are no coexistent diseases, such as diabetes and hypertension (267,268). In children, however, bacteriuria does not necessarily pursue the same relatively benign course as that seen in adults (269,270). Chronic atrophic pyelonephritis, with classic tubulointerstitial lesions, is a disease of childhood that manifests itself clinically in adult life (269). Children younger than 5 to 6 years in whom urinary tract infection coexists with vesicoureteral reflux and in whom pyelotubular backflow develops appear to be the individuals who are at increased risk of developing interstitial scarring, and, ultimately, tubular atrophy. Reflux or obstruction without complicating infection can initiate the injury to the tubules and interstitium (207,264). However, it is mainly children in whom infection and vesicoureteral reflux coexist and persist despite treatment who develop renal scars and tubulointerstitial disease (270). One study (271) with a 2-year follow-up revealed the formation of new scars in 3 of 34 children whose infection was untreated, as compared with only 1 of 26 children whose bacteriuria was treated. Therefore, the role of infection is not to be discounted. Whether urinary tract infection uncomplicated by obstruction causes sufficient interstitial injury to result in irreversible changes and progressive kidney disease is questionable (269,272–278). However, obstruction or vesicoureteral reflux can produce renal injury by causing either recurrent infection, by backpressure damage in the absence of infection, or both (207). Moreover, pyelonephritogenic strains of *Escherichia coli* that are more cytotoxic to the renal tubular epithelium because of stronger bacterial attachment and hemolysin delivery have been identified and characterized (90,279,280), as has a dysfunctional neutrophil response in pyelonephritis-prone cases (281).

Experimentally, the unmanipulated urinary tract is remarkably resistant to retrograde challenge with an infectious inoculum, whereas direct intrarenal injection of organisms causes bacterial proliferation and elicits a local cellular reaction (273). The initial inflammatory response to infection of the renal parenchyma is that of a classic acute tubulointerstitial nephritis characterized by edema, cellular infiltration, and tubular damage. To determine whether this initial response results in progressive immune-mediated injury after sterilization of the urinary tract, efforts were made to characterize the local response and the immunologic activity to the lymphoid cells isolated from the experimentally infected kidney (274–276). Within the first few days after infection, polymorphonuclear leukocytes predominate in the interstitium and tubular lumen, and the renal epithelial cells express class II MHC antigens (277) and the TNF-α gene (278), indicating their ability to act as antigen-presenting cells and to initiate the inflammatory sequence of events implicated in the pathogenesis of tubulointerstitial nephritis, such as increased excretion of TGF-β1 (282) and IL-6 and IL-8 (283). The local release of chemoattractants to recruit polymorphonuclear cells has been demonstrated to contribute to the tubular damage that results (284), as has that of free radical mediated tissue injury by the recruited leukocytes (285). Within the subsequent days, the neutrophilic infiltrate dissipates while a dense mononuclear lymphocytic infiltrate develops, indicating an active cellular response evident by the presence of B-lymphocytes and a depression of helper T-lymphocyte activity, whereas suppressor T-cell activity is increased (275). There is also evidence for the local activation and deposition of the complement system (286) and for an increased local production of IgG, IgM, and IgA by the infected kidney (287,288). During an acute infection, an increase in the level of circulating antibodies to THP has been reported (208,289). The level of antibodies decreases once the active infection is eradicated, and clearance studies showed the rapid clearance of antibodies from local deposits (204). Whether these antibodies or the local cellular infiltrates play a role in the perpetuation of chronic kidney injury and scarring is a possibility that has been proposed by some (263) but questioned by others (260). The potential for ammonia production by urease-producing organisms has been implicated in the propensity of these organisms to produce renal injury (173,218).

Preexisting kidney disease or an immunocompromised host seems to predispose to the detrimental effect of infection (290,291). The latter is best exemplified by the unusual infections causing interstitial injury encountered in kidney allografts (291,292), the kidneys of patients with acquired immunodeficiency syndrome (AIDS) (293), and following bone marrow transplantation (294). Of the various infections that have been implicated to cause tubulointerstitial lesions in the transplanted kidney the incidence of that due to polyomavirus infection appears to have increased after the introduction of mycophenolate mofetil–based antirejection therapy (295–298), and to subside after a decrease in the dose of mycophenalate based immunosuppression or after switching to cyclosporine-based antirejection therapy (295,297). Actually, recipients of kidney allograft and patients with AIDS are prone to tubulointerstitial nephritis even in the absence of superimposed infection (299–302).

Obstruction and Reflux

Interstitial changes are a prominent feature of the kidneys in urinary tract obstruction of any etiology. The initial structural changes that follow are those of tubular dilation and cortical infiltration with lymphocytes. Later, a network of fibrosis develops, extending from the capsule through the medulla of the affected pyramids. Fibrosis gradually increases and causes contraction and scarring (264). Proliferation of interstitial cells also occurs after ureteral obstruction (109,146,303). Many quantifiable pathophysiologic events occur during the first week of unilateral ureteral obstruction, which make this an important model for the study of the mechanisms of tubulointerstitial nephritis (304,305). The whole process can occur in the absence of infection. If infection is deliberately induced, or develops spontaneously, a polymorphonuclear reaction occurs and tubular casts are noted. More importantly, in the coexistence of infection, the fibrosis is more extensive.

In vesicoureteral reflux, kidney damage depends on the degree of incompetence of the ureterovesical valve, the magnitude of pressure that develops, and the length of time that the vesicoureteral reflux persists before correction. In persons with neurogenic bladder disorders, in which a high-pressure condition develops, vesicoureteral reflux occurs as a secondary event. In one study, more than 90% of children and 50% of adults with pyelonephritic scars had vesicoureteral reflux demonstrated on voiding cystourethrograms. In 89% of adults with scars, cystoscopic examination revealed abnormal ureteral orifices, suggesting ureteral reflux (306).

The importance of reflux of infected urine in the causation of tubulointerstitial lesions and atrophic kidney is now well established (264,309). Reflux, present in about 1% of newborns, is due to a number of congenital abnormalities such as defects at the vesicoureteral junction or impaired peristalsis of the urinary outflow tract (307,308). The role of sterile urine reflux, in doing so, although supported by experimental studies in pigs, remains to be documented in humans. A role for extravasation of a potentially powerful autoantigen, THP, in causing immune-mediated injury in the absence of infection has been postulated to occur in patients with vesicoureteral reflux, urine outflow obstruction, or inadequately draining transplanted kidneys (264). However, a number of studies have failed to confirm an immediate relationship between THP deposition and subsequent inflammatory response (309).

A proinflammatory potential of THP has been proposed on the basis of its demonstrated ability to bind neutrophils *in vitro* (310). Experimental support has been advanced for the notion that THP contributes to the pathogenesis of tubulointerstitial nephritis when it is increased in the interstitium after cell injury, where it initiates an autoantibody reaction and contributes to the infiltration of polymorphonuclear leukocytes (311). The oxidative burst of the now-activated adherent leukocytes then releases proteases and ROS, which contribute to the further loss of epithelial cell integrity. The tubular rupture responsible for extravasation of THP is related to the increased pressure in the tubules when the intrapelvic pressure rises in the presence of urine flow obstruction (312). Studies in 23 human neonatal kidneys obtained at autopsy showed that the range of pressure needed to cause pyelotubular backflow, with extravasation into the interstitial tissue, was 22 to 87 mm Hg, with a mean of 47 mm Hg (313). Pyelovenous reflux occurs in about 50% of cases when intrapelvic pressures of 50 to 70 mm Hg are induced experimentally and in 100% when pressures of more than 70 mm Hg are attained (313). In humans, intrapelvic pressures of more than 70 mm Hg are attained in patients with hydronephrosis in whom focal pyelotubular backflow can be demonstrated radiologically (314). Vesicoureteral reflux of a minor degree probably causes no kidney injury, but vesicoureteral reflux in a high-pressure situation or in the presence of infection does appear to be associated with tubulointerstitial injury. Thus, whereas renal scarring as a result of intrarenal reflux does occur, secondary to urinary outflow obstruction of any cause, no comprehensive uniform explanation has been advanced for the renal scarring and atrophy that develop in some but not in others.

Most of the experimental work attempting to explore the pathogenesis of scarring due to reflux has been done on rats, in which the unilobular pelvic anatomy is different from that of human kidney, which is multilobular and has 8 to 12 calices (313,315). The pig kidney used in some studies is multilobular and therefore is considered more analogous to human anatomy (207). About 75% of human kidneys have compound papillae, which fuse to drain several papillae generally in a polar location. The ducts of Bellini in some of the composite renal papillae have wide-open orifices, as opposed to the narrow slit-like orifices of the simple single papilla (316). Consequently, if the pressure of the refluxing urine is sufficient to overcome the pressure within the nephrons of the composite renal papillae, intrarenal reflux can occur (317,318). This accounts for the experimental and clinical observations that reflux can be demonstrated in only some segments of the kidney and that these are usually at a polar location. However, if the tubular flow is high and there is a large diuresis, injury from intrarenal reflux may be prevented (319).

Inflammatory pathways also play a role in the tubulointerstitial injury of urinary tract obstruction. Of interest are the changes of parenchymal cell infiltration that accompany increased intrapelvic pressure. Mononuclear cell infiltration is one of the earliest responses of the kidney to ureteral obstruction. Fairly early following acute urinary tract obstruction, cortical tubule epithelial cells express MCP-1 and RANTES, providing an impetus for the recruitment and accumulation of inflammatory cells (304,320). The infiltrating cells are macrophages and suppressor and cytotoxic lymphocytes (321,322). In experimental models of urinary tract obstruction, an increased expression of MCP-1 and RANTES is observed simultaneously with leukocyte and macrophage infiltration, with the subsequent onset of fibrosis (305). The release of various proinflammatory and vasoactive cytokines, by the infiltrating cells of the hydronephrotic kidney, appears to exert a significant modulating role in the tubulointerstitial injury and hemodynamic changes seen early in the course of obstruction (321–328). With persistent obstruction, changes of chronic tubulointerstitial nephritis occur within weeks (329), and gradually fibrosis becomes prominent (304,330,331). Cultures of the fibroblastic type of cortical interstitial cells from explants of unilateral hydronephrotic rabbit kidneys, compared with those from the contralateral normal kidney, grow significantly faster and have increased prostaglandin E_2 production in response to bradykinin (332).

Also of note in reflux nephropathy are the glomerular changes that develop in the later phases of the disease in some patients. As a rule, the glomerular changes commonly encountered are ischemic in nature, consisting of sclerosis, periglomerular fibrosis, and obsolescence, and are associated with modest proteinuria. In a few, focal and segmental glomerulosclerosis and hyalinosis develop and are manifested clinically by massive proteinuria and rapidly progressive kidney failure. The affected glomeruli commonly contain IgM and C3 as shown by immunofluorescent microscopy, suggesting an immunologic mechanism (193,333–335). The nature of the immune reaction remains unclear. Autologous (THP, brush-border antigen) or bacterial antigen derivatives have been implicated, as have hemodynamic changes that develop in the glomeruli of remaining intact nephrons of the hydronephrotic kidney, adapting to a reduction in renal mass by hyperfiltration (333).

Drugs

Drugs that are the major cause of acute tubulointerstitial disease also produce the chronic forms of tubulointerstitial nephropathy. Patients who develop the acute form of drug-induced tubulointerstitial nephritis generally recover fully. A few, however, do not recover and progress to chronic tubulointerstitial disease, as best exemplified by cisplatin (336,337). Based on a retrospective review of kidney biopsies, it has been estimated that about a third of patients with acute tubulointerstitial nephritis develop either only partially reversible or irreversible loss of kidney function (338). Most patients who develop drug-induced chronic tubulointerstitial nephritis do so insidiously, during or after the chronic use of one or more drugs. The latter (in which exposure to a mixture of more than one agent accounts for the kidney lesions) is best exemplified by analgesic abuse nephropathy (339,340), whereas cases of chronic tubulointerstitial nephritis due to chronic use of a single drug are exemplified by prolonged exposure to cyclosporine (340,341), cisplatin (342), nitrosourea (343), herbal medications (344–347), lithium (348,349), methotrexate (350), mesalazine (351,352), 5-aminosalicylic acid (353), and nonsteroidal antiinflammatory drugs (354–356). The risk of chronic tubulointerstitial nephropathy with these therapeutic agents is quite small and often preventable, and the risk–benefit ratio associated with these agents is much too small to limit their beneficial clinical use (356). Conversely, cases

occurring due to herbal medicines, which are of questionable therapeutic merit, deserve closer scrutiny (357). First reported in 1993 from Belgium, as a unique rapidly progressive, hypocellular, fibrosing interstitial nephritis in young women using a slimming regimen of Chinese herbs (356), the entity is now recognized with increasing frequency worldwide and in association with various herbal medicines (356–362). The lesions have been attributed to the content of phytotoxins—specifically aristolochic acid—which are not only nephrotoxic but also carcinogenic, in that nearly half of the patients with nephropathy have been subsequently diagnosed with transitional cell carcinomas of the urinary tract (346,358,361,362). However, aristolochic acid was not present in all herbal preparations associated with tubulointerstitial nephritis (344,346). Especially alarming is the rapid progression to end-stage kidney failure despite the discontinuation of herbal medications (363,364). The exact mechanism of aristolochic acid nephropathy is unknown. However, specific DNA adducts have been detected in the kidney of patients with aristolochic acid nephropathy that are not seen in other forms of chronic tubulointerstitial nephropathy (346). Aristolochic acid has been shown to inhibit filtered albumin reuptake by proximal tubule epithelial cells, even after cessation of its ingestion (359). Considered together with the fact that the nephropathy progresses even after cessation of aristolochic acid exposure and that uroepithelial tumors are common in these patients, it is reasonable to assume that DNA injury and mutagenesis may be an important cause of aristolochic acid nephropathy. Interestingly, the interstitial fibrosis due to aristolochic acid is not inhibited by ACE inhibition or angiotensin blockade, suggesting an injury pathway independent of the renin–angiotensin system (360).

Progressive loss of kidney function associated with a characteristic tubulointerstitial nephritis with cystic tubular dilatation and global glomerulosclerosis has been described in patients receiving chronic lithium therapy for bipolar disorders (349,365,366) and demonstrated experimentally (367). Initially, this was considered controversial because the kidney biopsies were performed in individuals whose kidney function was reduced, and similar lesions were described in psychiatric patients with reduced kidney function who had not been treated with lithium (368,369). However, confirmatory reports of loss of kidney function (365) that progresses to kidney failure and is dialysis-dependent (366,370–372) leave little doubt that lithium is nephrotoxic, particularly in those with recurrent lithium intoxication (365). The prevalence of the interstitial lesions, however, remains to be determined. Of special interest in these cases are the characteristic cystic dilations, which have been shown to be localized to the distal and collecting tubules (370). This is particularly relevant because of the commonly encountered nephrogenic diabetes insipidus that affects as many as 20% to 70% of lithium-treated patients (369), and the experimentally demonstrated lithium-induced downregulation of the vasopressin-regulated water aquaporin-2 water-channel expression in the apical plasma cells of the collecting tubules (373,374). Whereas the progression of kidney disease is dependent on the duration of therapy and cumulative dose exposure (372), the prevalence of interstitial lesions during lithium therapy remains to be determined.

The lesions of analgesic abuse deserve special consideration. To a great extent, it is the appreciation of their role in the causation of chronic interstitial nephritis that had the greatest impact in questioning the wisdom of attributing all chronic tubulointerstitial lesions to pyelonephritis. In the initial reports from Switzerland, the lesion was described as chronic interstitial nephritis (chronische interstitielle nephritis), with a high incidence of papillary necrosis (375,376). Subsequent reports established papillary necrosis as a prominent lesion of analgesic-associated nephropathy (AAN) and documented the significance and occurrence of this problem worldwide (377–382). From the outset, it was evident that there was considerable variation in the incidence of AAN in different parts of the world (from Australia to New Zealand to Scandinavia to England to Canada to the United States), with wide regional differences within each country (18,339,383–389).

Because of the over use of analgesic compounds, a common ingredient of which was phenacetin, the lesions of AAN were initially attributed to phenacetin (379–390). Subsequent clinical studies, however, questioned the role of any single agent as the cause of kidney disease (391), and despite the withdrawal of phenacetin, AAN remained one of the main causes of end-stage renal disease in Australia, Switzerland, Belgium, and Germany (384–386). The preponderance of experimental studies indicates that phenacetin, paracetamol, and aspirin given alone are only moderately nephrotoxic, even in massive doses. Renal lesions can be much more readily induced when a mixture of aspirin and paracetamol or phenacetin is used, particularly when they are combined with water deprivation (339,392). In all experimental studies, the extent of renal injury was dose-dependent, and when examined, water diuresis protected from analgesic-induced renal injury (339,393). Actually, phenacetin itself is not toxic; it is rapidly metabolized by the liver to paracetamol (*N*-acetyl-*p*-aminophenol), which is then excreted by the kidneys either as the free compound or as its conjugate. The bulk of the latter consists of the glucuronide and a smaller portion of the sulfate. Both paracetamol and its conjugates attain significant (fourfold to fivefold) concentrations in the medulla and papilla, depending on the state of hydration of the experiment animals studied. The toxic effect of these substances is apparently related to their intrarenal oxidation to reactive intermediates, which in the absence of reducing substances such as glutathione become cytotoxic by virtue of their capacity to induce oxidation (38,393,394) and induce tubular cell apoptosis (395). Salicylates are also significantly (6-fold to 13-fold) concentrated in the medulla and papilla, where they attain a sufficient level to uncouple oxidative phosphorylation and reduce the ability of cells to generate reducing substances (393–395). Thus, both agents attain sufficient medullary concentration in the kidney to individually exert a detrimental and injurious effect on cell function. Their simultaneous presence results in an additive effect because of the salicylate-induced decrease in reducing capability at a time when the concentrations of the reactive oxidative byproducts of phenacetin are increased locally. Considered in this context, it is evident why water diuresis, by reducing the medullary tonicity and therefore the medullary concentration of drug attained, protects from analgesic-induced cell injury (339,393,396). A direct role of analgesic-induced injury can be adduced from the arrest of the progressive loss and generally improvement of kidney function that are noted after cessation of analgesic abuse (397,398). That it is the use of analgesics in combination that accounts for kidney injury is evidenced from the decrease in AAN as a cause of end-stage kidney failure in Australia and European countries since legal restrictions were imposed on the sale of over-the-counter analgesic mixtures (356,399), and the epidemiologic demonstration of a direct relationship of the prevalence of AAN to the proportion of analgesic mixtures available for sale over the counter (400).

The intrarenal distribution of analgesics provides an explanation for the medullary location of the pathologic lesions of analgesic abuse (Fig. 72-8). The initial lesions are patchy and consist of necrosis of the interstitial cells, thin limbs of the loops of Henle, and vasa rectae of the papilla. The collecting ducts are spared. The quantities of tubular and vascular basement membrane and interstitial ground substance are increased. At this stage, the kidneys are normal in size and there are no abnormalities in the renal cortex. With persistent drug exposure, the changes extend to the outer medulla. Again, the lesions are initially patchy, involving the interstitial cells, the loops of

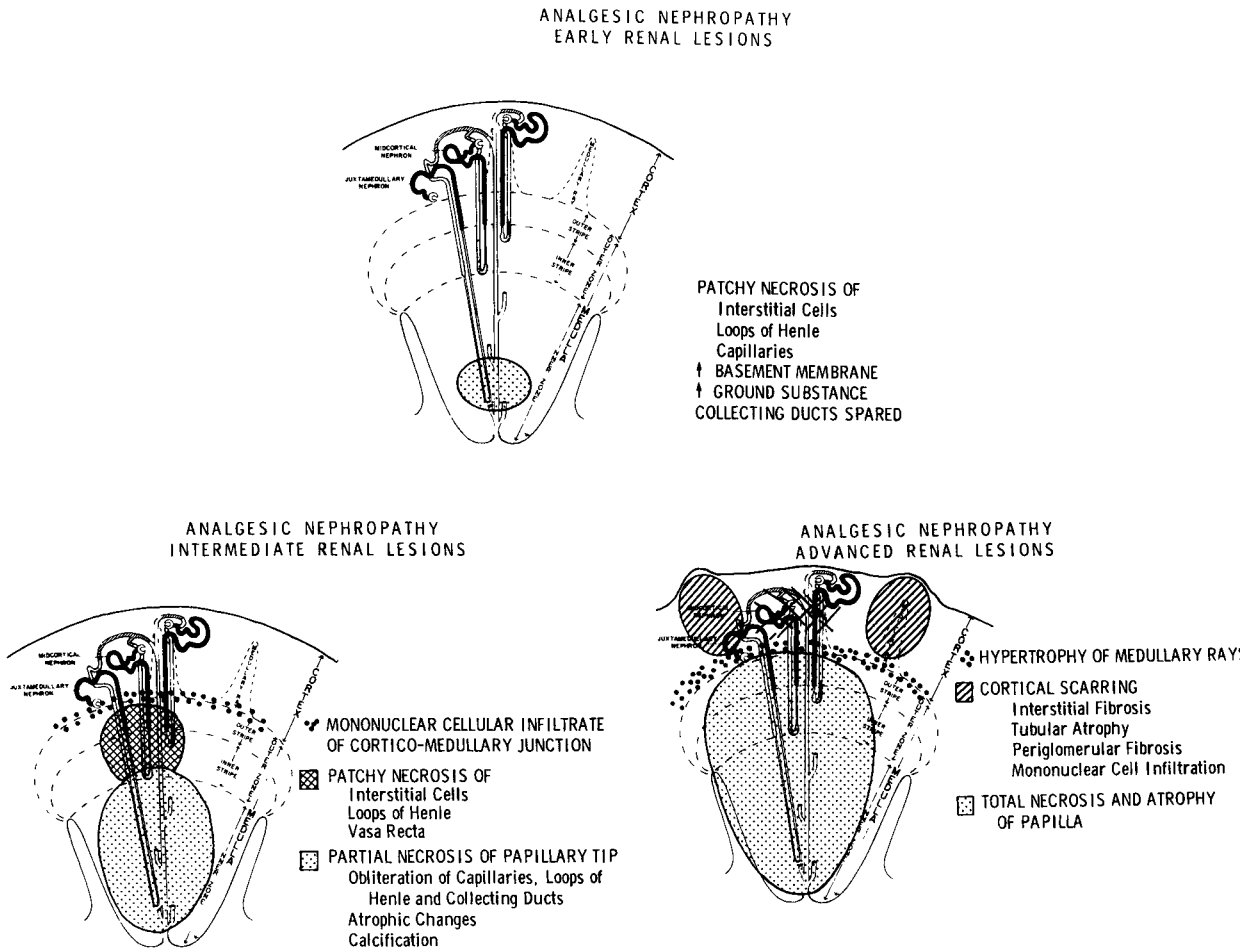

FIGURE 72-8. The course and stages of analgesic nephropathy. (From: Eknoyan G. Analgesic nephrotoxicity and renal papillary necrosis. *Semin Nephrol* 1984;4:65, with permission.)

Henle, and vascular bundles. With continued drug abuse, the severity of the inner medullary lesions increases with sclerosis and obliteration of the capillaries, atrophy and degeneration of the loops of Henle and collecting ducts, and the beginning of calcification of the necrotic foci. Ultimately, the papillae become entirely necrotic, with sequestration and demarcation of the necrotic tissue. The necrotic papillae may then slough and are excreted into the urine or remain *in situ,* where they atrophy further and become calcified. Cortical scarring, characterized by interstitial fibrosis, tubular atrophy, and periglomerular fibrosis, develops over the necrotic medullary segments. The medullary rays traversing the cortex are usually spared and become hypertrophic, thereby imparting a characteristic cortical nodularity to the now shrunken kidneys. The visualization of these configurational changes can be extremely useful in the diagnosis of AAN. A comparison of 60 analgesic abusers with 188 control subjects, using renal ultrasound and computed tomography, showed that a decrease in kidney size combined with bumpy contours of both kidneys provided a diagnostic sensitivity of 90% and a specificity of 95%. The additional finding of evidence of renal papillary necrosis resulted in an overall sensitivity of 72% and specificity of 97%, giving a positive predictive value of 92% (400). Examination of kidney tissue at this late stage of AAN reveals the classic features of cortical tubulointerstitial nephropathy (377,391,401–403).

As might be expected from the slow progressive nature of the lesion, the deterioration of kidney function is insidious. Kidney injury can be detected by appropriate testing for sterile pyuria, reduced concentrating ability, and a distal acidifying defect. These may be evident at levels of mild functional insufficiency and become more pronounced and prevalent as kidney function deteriorates. Proximal tubular function is preserved in those with mild loss of function but can be abnormal with more advanced kidney disease (356). In all cases, there is a relationship between kidney function and the duration, intensity, and quantity of analgesic consumed (404,405). The magnitude of injury is related to the quantity of analgesic ingested chronically over the years. In persons with significant impairment of kidney function, the average dose ingested has been estimated at about 10 kg over a mean period of 13 years (406). The minimum amount of drug consumption that results in significant kidney damage is unknown. It has been estimated that a cumulative dose of 3 kg of the index compound, or a daily ingestion of 1 g per day over 3 years or more, is a minimum that can result in detectable renal impairment (18). Only a minority of persons who regularly take analgesic mixtures develop kidney injury (405). There is also considerable variation in the severity of the renal lesions in those who do develop kidney injury. One reason for this variable response may be adduced from the experimental evidence for the medullary concentration of analgesics and the prevention of renal injury by hydration (393).

It is possible that those who develop analgesic nephropathy ingest lower quantities of fluid or are subjected to dehydration due to environmental conditions. This issue has not been investigated clinically, although a role for dehydration due to the climatic conditions has been proposed as one reason for the geographic variability in the incidence of analgesic nephropathy (339). Other factors that might contribute to dehydration,

such as laxative and diuretic use, have also been implicated (407,408). Differences in the content of the mixtures also could account for the geographic variation in the incidence of AAN (407,409). Finally, coexistent diseases, such as diabetes, sickle cell disease, and infection, which themselves cause tubulointerstitial nephritis, may predispose analgesic abusers to more severe kidney injury (339). An association between the ingestion of analgesic drugs and end-stage renal disease has been proposed on the basis of population-based case-control studies (385,386,410). The noted risk was greatest in heavy users of acetaminophen and nonsteroidal antiinflammatory drugs. An associated risk has also been reported with use of aspirin and salicylates (411,412). The validity of these epidemiologic observations remains to be established from prospective studies.

Consumption of phenacetin-containing analgesics has been associated with a significant increase (10% of abusers) of urothelial tumors of the renal pelvis, ureters, and bladder (37,413). The tumors occur after a latent period of decades, are typically multiple, are poorly differentiated, and spread rapidly. Epidemiologic and retrospective case-control studies of the role of paracetamol association with urothelial tumors have yielded negative results (414,415). However, paracetamol may not be free of all such side effects (413). A recent population-based case-control study showed that the risk of renal pelvic cancer was increased by phenacetin-aspirin mixtures to a greater extent than by paracetamol alone (relative risk, 12.2 versus 1.3), but the risk of renal cell carcinoma appeared to be increased to a similar degree by phenacetin-aspirin mixtures (relative risk, 1.4) and paracetamol (relative risk, 1.5) taken in any form (416). The presence of renal papillary necrosis in phenacetin abusers significantly increased the relative risk for urothelial cancer (417).

Tubulointerstitial nephritis has emerged as the most serious side effect of *cyclosporine* (418). Cyclosporine-mediated vasoconstriction of the cortical microvasculature has been implicated in the development of an occlusive arteriolopathy and tubular epithelial cell injury (419–422). Although these early lesions tend to be reversible with cessation of therapy, an irreversible interstitial fibrosis and cellular infiltrate develop with prolonged use of the drug, especially with higher doses (340,341,421–425). Cyclosporine increases the expression of renal TGF-β, which likely promotes interstitial fibrosis (426–428). The fibrosis has been shown to be inhibited by ACE inhibition (428) and by blocking the TGF-β pathway (427). The irreversible nature of this tubulointerstitial nephritis and its attendant loss of kidney function have raised serious concerns regarding the long-term use of this otherwise-efficient immunosuppressive agent (429,430), the use of which has been proposed in the treatment of tubulointerstitial nephritis (199). Chronic tubulointerstitial nephritis also occurs with tacrolimus, although kidney function is reported to be better preserved than with cyclosporine (431–433). Both agents exert a stimulatory effect on endothelin-1 (ET-1) synthesis and secretion in the kidney (434). The vasoconstrictor effect of ET-1 may account for the ischemic effect of these agents in initiating kidney injury.

Hematopoietic Disorders

The renal lesion that develops in disorders of the hematopoietic system is generally that of a tubulointerstitial nephropathy. This is by far most common in persons with a sickle cell hemoglobinopathy. Although more frequent in those with sickle cell disease, tubulointerstitial lesions also are common in those with sickle cell trait, sickle cell hemoglobin C disease, or sickle cell thalassemia (435,436). The predisposing factors that lead to the propensity of kidney involvement are the physicochemical properties of hemoglobin S that predispose its polymerization in an environment of low oxygen tension, hypertonicity, and low pH level (437). These conditions are characteristic to the renal medulla and therefore are conducive to the intraerythrocytic polymerization of hemoglobin S and consequent erythrocyte sickling that accounts for the development of typical vascular occlusive lesions (438–442). Although some of these lesions occur in the cortex, the lesions start and are predominantly located in the inner medulla, where they are at the core of the focal scarring and interstitial fibrosis (436,443,444), and account for the common occurrence of papillary necrosis in these persons (339). The incidence of radiographically demonstrable papillary necrosis is from 33% to 65% (436), and of increased renal echogenecity on ultrasonography is 17.6% (437). Tubular function abnormalities such as impaired concentrating ability, depressed distal potassium and hydrogen secretion, tubular proteinuria, and decreased proximal reabsorption of phosphate and increased secretion of creatinine are common and detectable early in the course of the disease (445,446).

Disorders of plasma cell function also produce tubulointerstitial disease. Actually, the pathogenesis of kidney involvement is of varied etiologies (186), and the renal complications of multiple myeloma are a major contributing factor in the morbidity and mortality of this neoplastic disorder (447–449). As might be expected, it has been shown that in those who present with kidney failure, ultimate survival depends to a great extent on the hematologic response to chemotherapy and much less on the coexistent kidney failure that is amenable to dialytic therapy (450,451). Kidney involvement depends on the tumor burden. Less than 5% of myeloma cases with a low tumorous mass have kidney involvement, whereas more than 40% of those with a high tumor mass will have reduced kidney function (452). The lesions that are directly the result of excessive production of light chains are those caused by the precipitation of the light-chain dimers in the distal tubules and result in what has been termed *myeloma cast nephropathy.* The affected tubules are surrounded by chronic inflammatory cells, interstitial fibrosis, and multinucleated giant cells (134,135,447). Adjoining tubules show varying degrees of atrophy. The propensity of light chains to lead to myeloma cast nephropathy appears to be related to their concentration in the tubular fluid, the tubular fluid pH level, and their structural configuration, rather than their intrinsic physicochemical properties, as was initially postulated (452–456). This accounts for the observation that increasing the flow rate of urine or its alkalinization will prevent or reverse the casts in their early stages of formation. Direct tubular toxicity of light chains also may contribute to the tubular injury (134). λ Light chains appear to be slightly more injurious than κ light chains (453,457), whereas the larger and heavier IgG myeloma protein is least likely to to be injurious (458). In fact, light-chain proteinuria is a highly significant risk factor for the kidney dysfunction, especially when it is more than 2 g per day (457,459). Binding of human κ and λ light chains to human and rat proximal tubule epithelial cell brush-border membrane has been demonstrated (460). Analysis of the binding data reveals a single class of low-affinity, high-capacity binding sites that are specific to light chains. Epithelial cell injury associated with their absorption has been implicated in the pathogenesis of tubulointerstitial nephritis (Fig. 72-1). Another mechanism whereby plasma cell dyscrasias cause tubulointerstitial lesions is related to the interstitial and perivascular deposition of paraproteins, either as amyloid fibrils that are derived from λ chains or as fragments of light chains that are derived from κ chains, and produce the so-called light-chain deposition disease (134,461). Of these various lesions, myeloma cast nephropathy appears to be the most common, being observed in one-third of the autopsied cases, followed by amyloid deposition, present in 10% of cases, whereas intramembranous light-chain deposition is relatively rare and present in less than 5% of cases (449,462,463).

The kidney is one of the most common extranodal sites of metastatic lymphomas (464), and it is not uncommon to diagnose occult lymphomas by kidney biopsy that clinically mimic chronic kidney disease (465). Infiltration of the kidney is frequent in patients with acute lymphoblastic leukemia and non-Hodgkin's lymphomas but less common in those with Hodgkin's disease (134). Renal infiltrates are generally clinically silent and only result in kidney failure either from vascular or ureteral compression or from extensive parenchymal infiltration (142).

Tubulointerstitial lesions are common in the acute stage of the hemolytic syndrome and are an integral feature of those who develop persistent kidney damage necessitating renal replacement therapy (398,466).

Heavy Metals

Environmental exposure to *cadmium* results in its accumulation in the body, where it is preferentially concentrated in the kidney, principally in the proximal tubule, in the form of a cadmium-metallothionein complex that has a biologic half-life of approximately 10 years (462). Because of its chronic local toxic effect, an insidious form of chronic tubulointerstitial nephropathy results, the principal manifestation of which is proximal tubule dysfunction (468–470), a distinctive form of low-molecular-weight proteinuria (471) and osteomalacia due to calciuria, which causes nephrolithiasis in 40% of such cases (472).

Exposure to *lead,* as an occupational hazard, environmental exposure, or its ingestion in homemade brew, results in its insidious accumulation in the body (139,473,474). This subclinical accumulation of lead has been implicated in the causation of hyperuricemia, hypertension, and kidney failure (5,475,476). Episodes of acute gouty attacks precede the onset of reduced kidney function, and hypertension develops early in the course of kidney disease at a time when the serum creatinine level is only slightly above the normal range. Focal tubulointerstitial lesions are present in as many as half of such patients. Actually, tubulointerstitial lesions of such cases once attributed to gouty nephropathy are now considered the consequence of lead overload (476). The infusion of calcium disodium ethylenediaminetetraacetic acid (EDTA) to mobilize body stores of lead is a useful diagnostic tool for the detection and treatment of this disorder (139,476–478). Environmental exposure appears to be associated with worsening of preexisting chronic kidney disease. In a randomized study of nondiabetic Taiwanese patients with chronic kidney disease and an elevated body lead burden, repeated EDTA chelation resulted in improved kidney function and a slower rate of progression over 24 months compared to a placebo-treated group (479).

Rare cases of tubulointerstitial nephropathy have been caused by exposure to *silicon, copper, bismuth, barium, uranium,* and *arsenic* (480,481), and possibly *organic solvents* (482). Early detection is important in all cases of heavy metal exposure because of the potential of reversibility. Unfortunately, the onset of kidney disease is insidious and often goes undetected. Urinary enzymatic markers of tubular injury and selective proteinuria (THP, retinal binding protein, alkaline phosphatase) can be useful in early detection but are not readily available (483,484).

Vascular Diseases

Tubular degeneration, interstitial fibrosis, and mononuclear cell infiltration are part of the degenerative process that affects the kidneys in vascular diseases that involve the intrarenal vasculature with any degree of severity as to result in ischemia (67,485–488) (Figs. 72-1, 72-7). Rarely, if the insult is sudden and massive, the lesions are those of infarction and acute deterioration of kidney function. More commonly, the vascular lesions develop gradually and go undetected until renal insufficiency supervenes (49). It is this chronic form that accounts for the tubulointerstitial lesions of arteriolar nephrosclerosis and hypertension (10,49,89,489) that accounts for the relatively high prevalence of end-stage renal disease in selected ethnic populations and contributes to the tubulointerstitial lesions of patients with diabetes (486), sickle cell hemoglobinopathy (436), vasculitis (487,488), and cyclosporine nephrotoxicity (421,422).

The kidney injury caused by radiation, which is characteristically one of tubulointerstitial nephropathy, is also ischemic in origin because of the radiation-induced injury of the renal vasculature (490,491). The rash of cases that were reported from the 1940s through the 1960s resulted from the failure to recognize the sensitivity of renal vessels to irradiation (492) and the then-prevailing misconception that the kidney was resistant to radiation injury (493). Recognition that the total dose and frequency of the administered radiation are important determinants of nephrotoxicity, and institution of shielding of the kidneys during radiation exposure has resulted in a dramatic decrease in the frequency with which radiation-induced tubulointerstitial lesions are currently encountered.

Metabolic Disorders

Hyperuricemia/Hyperuricosuria

The kidneys are the major organs for the excretion of uric acid and a primary target organ affected in disorders of urate metabolism. The renal lesions result from crystallization of uric acid, either in the urine outflow tract or in the kidney parenchyma (494–496). The determinants of uric acid solubility are its concentration and the pH of the medium in which it is dissolved (497). Consequently, the supersaturation of the tubular fluid, as the excreted uric acid becomes concentrated in the medulla, and the acidification of the urine in the distal tubule will be conducive to the precipitation of uric acid. Not unexpectedly then, the major sites of urate deposition are the renal medulla, the collecting tubules, and the urinary tract. The pH of uric acid is 5.4, and at the acid pH of the distal tubule fluid, the bulk of the filtered urate will be present in its unionized form as uric acid, whereas at the more alkaline pH of the blood and interstitium, it is in its ionized form as urate salts. If deposition occurs in an acid medium, as in the tubular fluid, birefringent uric acid crystals are formed, whereas in an alkaline medium, as in the interstitium, amorphous urate salts are deposited (498,499). Depending on the load of uric acid presented to the kidneys, one of three disorders results: acute uric acid nephropathy, uric acid nephrolithiasis, or chronic urate nephropathy. Whereas either one of the former two forms can produce tubulointerstitial lesions, only the latter is considered here, because uric acid nephropathy is an acute lesion and uric acid nephrolithiasis produces its effect by obstruction.

The principal lesion of chronic hyperuricemia is the deposition of microtophi of amorphous urate crystals in the interstitium, with a surrounding giant-cell reaction (133,500,501). The earlier change, however, is probably due to the precipitation of birefringent uric acid crystals in the collecting tubules, with consequent tubular obstruction, dilation, atrophy, and interstitial fibrosis (498,502). Experimental evidence in support of a role of tubular precipitation of uric acid has been advanced from studies on pigs fed a high purine diet (503). Additional support derives from the reported high incidence of uric acid crystals in the kidneys of patients with gout and those with Lesch-Nyhan syndrome (495,504). The proposal has,

therefore, been made that most patients whose hyperuricemia and gout are due to decreased uric acid excretion, rather than overproduction, would be spared from renal injury. However, this is not the case because kidney lesions are encountered in these "underexcretors." The kidney injury of these individuals has been attributed to hyperacidity of the urine because of an inherent abnormality in their ability to produce ammonia (505,506). The acidity of urine is important because uric acid is 17 times less soluble than urate, and, therefore, it facilitates precipitation in the distal nephron of those who are not overproducers but have an acid urine.

The earlier notion that chronic kidney disease was common in patients with hyperuricemia (504) has been questioned in light of prolonged follow-up studies of kidney function in persons with hyperuricemia (505–507). Reduced kidney function could be documented only when the serum urate concentration was more than 10 mg/dL in women and more than 13 mg/dL in men for prolonged periods. The deterioration of kidney function in those with hyperuricemia of a lower magnitude has been attributed to the higher-than-expected occurrence of concurrent hypertension, diabetes mellitus, abnormal lipid metabolism, and nephrosclerosis (508,509). Recent experimental evidence indicates that milder hyperuricemia is associated with endothelial dysfunction, hypertension, and chronic tubulointerstitial nephritis (510–514). The development of tubulointerstitial nephritis in mild hyperuricemia is crystal-dependent and is likely secondary to activation of the renin–angiotensin system and altered production of nitric oxide (NO), since the hypertension and kidney lesions were attenuated with administration of allopurinol, ACE inhibition and the NO-substrate, l-arginine (511,512). Clinically, hyperuricemia is independently associated with hypertension and cardiovascular disease, whether its treatment affects kidney function remains to be determined (511,513). An association of kidney disease and hyperuricemia exists in persons with a history of exposure to lead and consequent subclinical lead toxicity (475,476). A series of studies from New Jersey showed that patients with coexistent hyperuricemia and reduced kidney function had lead overload, whereas those with hyperuricemia and normal kidney function had normal body lead stores. The mechanism whereby lead aggravates hyperuricemia is not clear (515).

Hyperoxaluria

The increased production or intestinal absorption of oxalate, with its consequent increased renal excretion, almost invariably results in its precipitation as calcium oxalate in the urine outflow tract (138,516). Microcrystallization first occurs in the proximal tubules where oxalate secretion occurs (516–518), but the lesions that develop are more severe in the renal medulla, where the increasing concentration of the tubular fluid and its acidification promote calcium oxalate precipitation (138,139,519), and adhesion to distal nephron epithelial cells (520), particularly in the presence of Tamm-Horsfall protein deficiency (521). The exposure of tubule epithelial cells to calcium oxalate crystals results in the increased synthesis of osteopontin, MCP-1, prostaglandin E_2, and AII receptors, which are known to activate the interstitial inflammatory process and matrix deposition (522,523). This results in atrophy of the epithelial cells lining the affected tubules, interstitial edema, and inflammatory cell infiltration (138,139,523).

Hyperoxaluria may be primary or acquired (138). The primary form is a rare inherited disorder due to an enzymatic abnormality in the metabolism of glyoxylic acid. The acquired forms of hyperoxaluria are more common and result either from the ingestion of oxalate precursors, such as ethylene glycol (524) and ascorbic acid (138), and exposure to methoxyflurane anesthesia (525) or from its increased absorption from the intestinal tract of persons with inflammatory bowel disease or who have undergone small bowel resection (526–528). When the hyperoxaluria is sudden and massive, such as after ethylene glycol ingestion, acute renal failure develops. Otherwise, as with most cases of hyperoxaluria, the overload is insidious and chronic; as a result, interstitial fibrosis, tubular atrophy, and dilation result in a chronic tubulointerstitial nephritis, with progressive renal failure. The propensity to recurrent calcium oxalate nephrolithiasis and consequent obstructive uropathy contributes to the tubulointerstitial lesions.

Hypercalcemia/Hypercalciuria

Given the many and vital roles of calcium in normal cell function, it is evident why changes in calcium concentration, either in the blood or in the urine, produce immediate reversible changes in kidney function, which are followed by irreversible structural changes if calcium derangement goes undetected or remains untreated (529). In most instances, the severity and potential reversibility of the changes that occur are related to the degree and duration of the hypercalcemia (529,530). Several experimental studies noted the development of acute changes in tubular function and renal hemodynamics (531–533). A defect in the urinary concentration is the most notable tubular dysfunction (529,530,533). It is multifactorial in origin but results mainly from a direct inhibitory effect of calcium on the reabsorption of sodium in the tubule in general, but in the loop of Henle in particular (529,530,533), and from an altered responsiveness of the collecting duct to antidiuretic hormone due to downregulation of aquaporin-2 water channels (534–538). The hemodynamic changes result from an effect of calcium on the systemic and the renal vasculature, either directly or indirectly through its effect on cardiac function (530,532,539) and renal prostaglandin and renin–angiotensin production (540).

Focal degeneration and necrosis of the tubular epithelium, primarily in the medulla where calcium is concentrated, occur shortly after persistent hypercalcemia and have been attributed to increased intracellular and mitochondrial calcium content (539–542). The subsequent calcification and destruction of the TBM result in proliferative and infiltrative changes of the adjacent interstitium, whereas the sloughing of necrotic cells results in tubular atrophy and obstruction with consequent dilation and pressure injury to the proximal segments of the tubule (539,540). Early changes in the proximal tubule also have been noted (451,453). Deposition of calcium in the necrotic and injured areas results in the characteristic nephrocalcinosis seen on radiography or noted at autopsy (529,530,542,543). The deposition of calcium in the glomerular capillaries and vasculature probably contributes to the further progression of the injury. The final lesion of focal scarified areas of tubular atrophy, increased interstitial fibrosis, and mononuclear cell infiltration is a classic example of chronic tubulointerstitial disease. The propensity to kidney stone formation and therefore to obstructive nephropathy also can contribute to the tubulointerstitial lesions.

Potassium Depletion

Various well-documented abnormalities of kidney function accompany the development of potassium depletion (544,545), the hallmark of which is a vasopressin-resistant impairment of the ability to concentrate the urine (546–548), increased ammonia genesis (549), and a modest reduction of the GFR (544,546). The characteristic structural change that accompanies potassium depletion in humans is that of vacuolation and hypertrophy of the tubule epithelial cells due to the dilated cisternae of the endoplasmic reticulum and the basilar foldings of the cells (550). The lesions are generally limited to the proximal tubule segments, with only focal changes in the distal segment. With potassium repletion, the functional and

structural changes appear to be reversible (544,545). Whether they can progress to a chronic tubulointerstitial nephropathy and result in persistent kidney dysfunction is uncertain but seems likely depending on the duration and extent of potassium depletion (544,545,551). A familial form of hypokalemic tubulointerstitial nephritis with progressive kidney disease has been described (552,553). Experimental potassium depletion in rats resulted in persistent interstitial fibrosis and scarring several months after potassium repletion (554). In rats, the lesions of acute potassium depletion are more extensive than those in humans, with vacuolation and tubular hyperplasia affecting both the proximal and the distal tubule segments (555,556). These changes have been attributed to the susceptibility of potassium-depleted rats to chronic pyelonephritis (551,556), although the evidence that potassium depletion predisposes to pyelonephritis is not well established and is controversial at best (557). A principal cause of kidney injury may well be the augmented ammonia genesis associated with hypokalemia (Fig. 72-1). Ammonia directly activates the alternative complement pathway and thereby sets off an immune-mediated process of progressive tubulointerstitial injury (218). In hypokalemic rats, suppression of ammoniagenesis with bicarbonate supplementation ameliorated the tubulointerstitial lesions (558).

Potassium depletion has also been shown to induce the production in the vasculature of the kidney of cytokines implicated in interstitial fibrosis such as TGF-β, insulin-like growth factor 1, and renin (559–561). Another contributory role to fibrosis may be that of aldosterone in cases in which potassium losses are due to either primary or secondary hyperaldosteronism. Increasing evidence for aldosterone-induced fibrogenesis and its prevention by low-dose spironolactone has been presented recently (562,563) in various experimental models of kidney disease including streptozotocin-induced diabetes (564) and obstructive nephropathy (565).

Cystinosis

Cystinosis is a rare autosomal inherited disorder of amino acid metabolism that is characterized by the deposition of cystine crystals throughout the body (136). The early lesions of the kidney in these persons is a "swan neck" deformity or atrophy of the proximal tubule segments that are adjacent to cystine-containing interstitial cells. The structural changes coincide with the development of proximal tubular dysfunction characteristic of Fanconi's syndrome (566), which is one of the eponyms used in describing this entity (136). Progressive interstitial fibrosis, tubular atrophy, and interstitial inflammatory reaction result in progression to end-stage renal failure (567,568). The characteristic rhomboid-shaped crystals of cystine that are generally seen in the interstitium also have been noted in the glomerular and tubular cells (569).

Hereditary Diseases of the Kidney

Tubulointerstitial lesions are a prominent component of the structural changes of various hereditary diseases of the kidney, such as the medullary cystic disease, familial juvenile nephronophthisis, medullary sponge kidney, and polycystic kidney disease (570–574). The primary disorder of these conditions is a tubular defect that in some patients results in the cystic dilation of the affected segment. Several of the identified genes disrupted in polycystic kidney disease and nephronophthisis encode proteins expressed in the primary cilia of epithelial cells that project into the lumen, and cilium-generated signaling has been implicated in the pathogenesis of cyst formation (572,573). Altered epithelial cell proliferation and associated TBM changes account for cyst formation (574–578). It is the continuous growth of cysts and their progressive dilation that cause pressure-induced ischemic injury of the adjacent renal parenchyma (578–580). A defect in urinary concentrating capacity is an early functional manifestation of experimental and clinical cystic diseases of the kidney (581–583). Hypertension is common in these diseases, and the vascular lesions of nephrosclerosis may contribute further to the course of renal deterioration and development of tubulointerstitial lesions (584–589).

Tubulointerstitial lesions are also a salient feature of inherited diseases of the glomerular basement membrane (590). Notable among them are those of hereditary nephritis or Alport's syndrome, in which a mutation in the encoding gene localized to the X chromosome results in a defect in the α_5 chain of type IV collagen (591–593). Of interest are the lesions that occur in the transplanted kidney of these patients who develop antitubular and antiglomerular antibodies resulting in progressive loss of kidney function (594).

Granulomatous Diseases

Interstitial granulomatous reactions are a rare but characteristic hallmark of certain forms of tubulointerstitial disease (121). The two best-known forms are sarcoidosis and tuberculosis. Granulomatous infiltration of the renal interstitium may be present in as many as 40% of the patients with sarcoidosis, but it is rarely sufficiently extensive to cause detectable kidney dysfunction (5,120,122,595,596). The granulomatous reactions may develop after subsidence of pulmonary lesions (597). The lesions are usually responsive to steroid therapy (598,599). However, the regression of the active granulomatous reaction can result in interstitial fibrosis and progressive loss of kidney function (596,600). Interstitial nephritis also has been noted in the absence of granulomatous lesions in some patients with sarcoidosis (596); it may be due to the hypercalcemia, glomerular disease, and arteritis that occur in sarcoidosis (601–604).

A similar pathogenetic sequence of events occurs in tuberculosis of the kidney. The granulomatous reaction to the mycobacterial infection responds to antituberculous treatment, but the residual fibrosis can result in tubulointerstitial disease (125,605,606). A potential role for steroid treatment in conjunction with antituberculous therapy for attenuation of the interstitial lesions has been suggested (607). A contributory factor to the interstitial lesions of renal tuberculosis is the obstructive nephropathy that follows ureteral and caliceal scarring caused by tuberculous involvement of the genitourinary tract (270).

Interstitial granulomatous reactions also have been noted in xanthogranulomatous pyelonephritis (127), renal malakoplakia (116), Wegener's granulomatosis (608), renal candidiasis (126), heroin abuse (609), and hyperoxaluria after jejunoileal bypass surgery (610), and in association with anterior uveitis (611).

Endemic Diseases

Two endemic diseases in which tubulointerstitial lesions are a predominant component are Balkan nephropathy and nephropathia epidemica.

Endemic Balkan nephropathy is a progressive chronic tubulointerstitial nephritis, the occurrence of which is limited to a geographic area bordering the Danube River as it traverses Romania, Bulgaria, and the former Yugoslavia (612–614). The etiology of the disease remains unknown but has been attributed to genetic factors, heavy metals, trace elements, and infectious agents (612,615,616). Environmental industrial contaminants and aromatic hydrocarbons have also been implicated, and the frequent association of the nephropathy with

urothelial tumors attributed to them (617). The regions where the disease occurs have a high ambient humidity and heavy rainfall, and, hence, a propensity to fungal infection of food (612,618,619). Ochratoxin A, a mycotoxin found in food, is known to be nephrotoxic and cancerogenic (620). Foods with a high content of ochratoxin A are shown to cluster in the same areas where Balkan nephropathy is prominent and this mycotoxin has been implicated as its causative agent (621). Ochratoxin A has been shown to induce tubule epithelial cell injury (622,623), to modulate a number of epithelial cellular proinflammatory signals, to enhance collagen deposition (624) and to cause vasoconstriction through an AII-dependent mechanism (623). Ochratoxin A has also been implicated in chronic tubulointerstitial nephritis of unknown etiology (620,625). The disease evolves in emigrants from endemic regions, suggesting a role for inheritance or the perpetuation of injury sustained before emigration (626). It is now evident that the disease is clustered but may not be confined to the Balkans (615).

Nephropathia epidemica, initially thought to be restricted to the Scandinavian countries and termed *Scandinavian acute hemorrhagic interstitial nephritis,* has a more universal occurrence (627,628) and has therefore been more appropriately termed by the World Health Organization (WHO) as hemorrhagic fever with renal syndrome (629,630). As a rule, the disease presents as a reversible acute tubulointerstitial nephritis after an initial febrile episode but can progress to a chronic form (631,632). It is caused by a rodent-transmitted virus of the *Hantavirus* genus of the Bunyaviridae family (633). Humans appear to be infected by respiratory aerosols contaminated by rodent excreta. Antibodies to the virus are detected in the serum of affected individuals (628), and in that of patients with varied forms of chronic kidney disease and IgA nephropathy (634). Tubulointerstitial nephropathy due to other viral infections has also been reported with polyomavirus (298,635), cytomegalovirus (636,637), herpes simplex virus (291), human immunodeficiency virus (HIV) infection, and infectious mononucleosis (638).

IDIOPATHIC

Despite extensive evaluation, in several cases of primary chronic tubulointerstitial nephritis it is not possible to determine the cause of the lesions. A potential role of Epstein-Barr virus (EBV) infection of proximal tubule cells has been implicated as a contributory factor to the irritation and/or progression of the tubulointerstitial lesion of such cases. Using *in situ* hybridization and the polymerase chain reaction, the EBV genome could be detected exclusively in kidney tissue of patients with idiopathic chronic tubulointerstitial nephropathy but not in that of cases due to glomerular disease, infection, or drug-induced tubulointerstitial nephritis (639).

Mild proteinuria and microhematuria occur in 16% of patients with clinical and serologic evidence of infectious mononucleosis (640). Except in rare cases of acute renal failure (641), kidney function is well preserved in these cases. In the rare cases that have been biopsied, a monocytic interstitial cellular infiltrate is common and can result in tubulointerstitial lesions (638), but glomerular changes have been reported also (642). Whether active EBV infection or activation of latently infected proximal tubule cells may evoke the cellular immune response that results in the tubulointerstitial lesions of some of these cases remains to be established.

PAPILLARY NECROSIS

Renal papillary necrosis develops in various diseases that cause chronic tubulointerstitial nephropathy in which the lesion is more severe in the inner medulla (189). The basic lesion is one of impairment of the vasculature and consequent focal or diffuse ischemic necrosis of the distal segments of one or more of the renal pyramids (402,643–645). In the affected papilla, the sharp demarcation of the lesion and the coagulative necrosis seen in the early stages of the disease closely resemble those of infarction (646). The cellular infiltrates that characterize tubulointerstitial lesions of the cortex are sparse in the medullary lesions (84). The fact that the necrosis is anatomically limited to the papillary tips can be attributed to various features that are unique to this site, especially those of the vasculature. The renal papilla receives its blood supply from two sources: the vasa recta and branches of the arteries in the adventitia of the minor calices. The importance of the latter is evidenced by the finding that the terminal portions of the papilla may be viable at a time when the remainder of it is necrotic, presumably because the blood supply from the minor caliceal arteries remains intact when the vascular involvement is limited to the vasa recta (647,648), and by the report of a case of renal papillary necrosis due to caliceal arteritis (649). The vascular bundles formed by the vasa recta in the outer medulla and the interbundle region form distinct compartments; in the inner medulla, a subdivision into compartments is not obvious. In addition, the vascular bundles, which are widest in the outer medulla, gradually decrease in size, and at the papillary tip only single or a few communicating vessels remain (648,650). The tapering away of the bundles is due to the gradual reduction of the vessels entering and leaving at different levels of the medullary capillary plexus. Measurements of medullary blood flow notwithstanding, it should be noted that much of the blood flow in the vasa recta serves the countercurrent exchange mechanism. Nutrient blood supply is provided by small capillary vessels that originate in each given region. The net effect is that the nutrient blood supply to the papillary tip is less than that to the rest of the medulla, and hence its predisposition to ischemic necrosis caused by diseases of the vasculature or induced by hemodynamic changes such as shock and nonsteroidal anti-inflammatory drugs (651–653).

Infection is usually, but not invariably, a concomitant finding of most cases of renal papillary necrosis (189,654–658). With few exceptions, most patients with renal papillary necrosis ultimately develop a urinary tract infection. Some authors considered the infection to be the cause of the renal papillary necrosis and the suggestion has been made that renal papillary necrosis is a form of acute pyelonephritis that is more devastating than usual because its natural course is altered by associated disease states, specifically diabetes mellitus and obstruction of the urinary tract (655,659,660). However, angiographic studies revealed that vascular deficiency contributes even to the renal papillary necrosis associated with chronic pyelonephritis (661), and the necrosis of acute pyelonephritis has been attributed to compression of the thin-walled medullary vasculature by the inflammatory reaction of the interstitium (655). In addition, in considering infection as a cause of renal papillary necrosis, one must consider that infection itself represents a complication of papillary necrosis (189); that is, the infection develops after the primary underlying disease has initiated local injury to the renal medulla, with foci of impaired blood flow and poor tubular drainage. In any case, infection, if not the cause, is a frequent and important finding in most patients with renal papillary necrosis (189). It contributes significantly to the symptomatology of renal papillary necrosis because fever and chills are the presenting symptoms in two-thirds of patients and a positive urine culture is obtained in 70% (189). However, to consider renal papillary necrosis as an extension of severe pyelonephritis is inaccurate and simplistic. In most patients with florid acute pyelonephritis, renal papillary necrosis does not occur. To classify renal papillary necrosis as a form of necrotizing pyelonephritis is archaic and

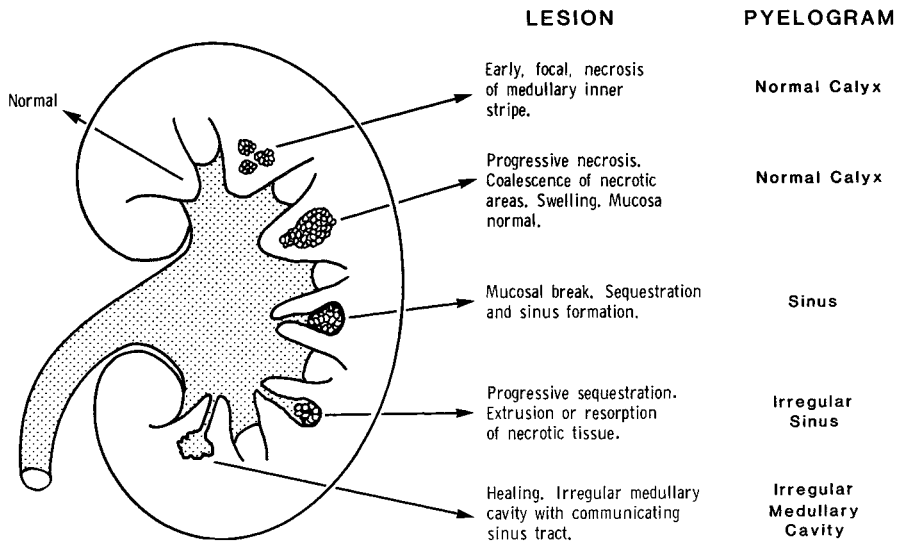

FIGURE 72-9. Pathogenesis and radiologic changes in the medullary form of renal papillary necrosis. (From: Eknoyan G, et al. Renal papillary necrosis: an update. *Medicine* 1982;61:55, with permission.)

has detracted from the recognition of renal papillary necrosis as a distinct clinicopathologic entity that can develop in the absence of pyelonephritis and urinary tract infection (189).

The necrotic lesion occurs in one of two forms. In the medullary form (Fig. 72-9), also termed *partial papillary necrosis,* the inner medulla is affected but the papillary tip and fornices remain intact; in the papillary form, also termed *total papillary necrosis,* the caliceal fornices and entire papillary tip are necrotic (662–664) (Fig. 72-10). In the latter form, renal papillary necrosis is characterized by necrosis, demarcation, and sequestration of the papilla, which ultimately sloughs into the pelvis and may be recovered in the urine. In most of these cases, however, the necrotic papilla is not sloughed but is either resorbed or remains *in situ,* where it becomes calcified or forms the nidus of a calculus (654,665). In these patients, excretory radiologic examination and computed tomography are diagnostic. Unfortunately, these changes may not be evident until the late stages of renal papillary necrosis, when the papillae are shrunken and sequestered (Fig. 72-8). Actually, even when the papillae are sloughed out, excretory radiography results can be negative (666,667). The passage of sloughed papillae is associated with lumbar pain, which is indistinguishable from ureteral colic of any cause and is present in about half of the patients (189). Oliguria occurs in less than 10% of patients. Because a definitive diagnosis of renal papillary necrosis can be made by finding portions of necrotic papillae in the urine, a deliberate search should be made for papillary fragments in urine collected during or after attacks of colicky pain of all suspected cases, by straining the urine through filter paper or a piece of gauze (668,669). The separation and passage of papillary tissue may be associated with hematuria, which is microscopic in about 40% to 45% of the patients and gross in 20% (161). The hematuria can be massive, and occasional instances of exsanguinating hemorrhage requiring nephrectomy have been reported (670,671).

The necrotic lesions of renal papillary necrosis may be limited to only a few of the papillae or involve several of them in either one or both of the kidneys. In most patients, the lesions are bilateral (189,654,655,659,665,672). In patients in whom one kidney is involved at the time of initial presentation, renal papillary necrosis develops in the other kidney within the ensuing 4 years (663). This is not unexpected, considering

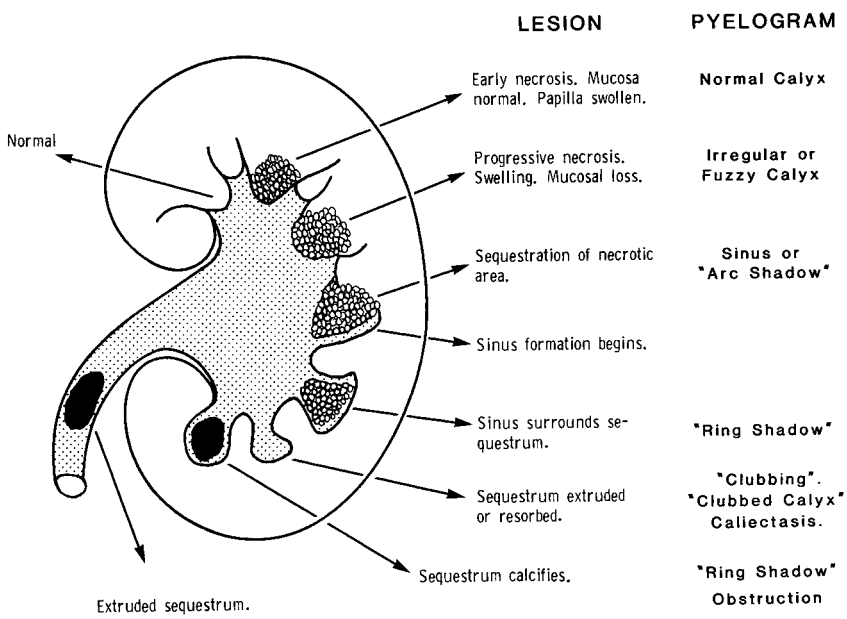

FIGURE 72-10. Pathogenesis and radiologic changes in the papillary form of renal papillary necrosis. (From: Eknoyan G, et al. Renal papillary necrosis: an update. *Medicine* 1982;61:55, with permission.)

the systemic nature of the diseases that are associated with renal papillary necrosis. Renal papillary necrosis may be unilateral in patients in whom predisposing factors, such as the infection and obstruction, are limited to one kidney. Azotemia may be absent even in bilateral papillary necrosis, because it is the total number of papillae involved that ultimately determines the level of renal insufficiency that develops (189). Each human kidney has an average of eight pyramids, such that even with bilateral renal papillary necrosis affecting one or two papillae in each kidney, sufficient unaffected renal lobules remain to maintain an adequate level of renal function. Moreover, in instances in which several papillae in each kidney are necrotic, the localization of the lesion to the inner medulla results in the loss of the juxtamedullary nephrons only, whereas the cortical nephrons, which terminate in the outer medulla, are spared, such that even in the affected pyramids, there remain several intact nephrons that are capable of contributing to the maintenance of normal homeostasis (645,665,673). Inability to concentrate the urine maximally is a more common development than kidney failure, as might be expected from the involvement of the inner medulla (665,673). Hence, a longstanding history of polyuria and nocturia can be elicited from many of these patients. With the loss of several papillae and involvement of the cortex, however, kidney failure will ultimately supervene.

The clinical course of renal papillary necrosis is variable. In its rare acute form it may occur as a rapidly progressive devastating illness that results in the death of the patient because of septicemia and kidney failure (659,660). In its more common chronic form, it will pursue a protracted course of months or years. These patients may remain totally asymptomatic, with the diagnosis made incidentally, either at autopsy or by excretory urography. Alternatively, it may be symptomatic, manifesting itself principally as episodes of nephrolithiasis or pyelonephritis (189,654,655,663,674,675). In these conditions, fever and chills or renal colic are the presenting symptoms. Of those diagnosed while living, survival is lowest and the risk of progression to end-stage kidney failure is greatest among diabetic patients (676).

Diabetes mellitus is the most common condition associated with renal papillary necrosis, accounting for some 50% to 60% of the cases reported in the major series. The occurrence of renal papillary necrosis in patients with diabetes may be more common than is generally appreciated. In an intravenous urographic study of 76 patients with longstanding insulin-dependent diabetes mellitus, renal papillary necrosis was observed in almost 25% of the patients (677). *Obstructive uropathy* has been reported as the cause of renal papillary necrosis in 15% to 40% of the major reviews. *Pyelonephritis* can result in papillary necrosis, and infection is present in most patients with renal papillary necrosis, but its exact prevalence as the cause of renal papillary necrosis is difficult to determine because infection may develop secondary to obstruction or diabetes, which remain the primary cause of renal papillary necrosis in the United States (189). Analgesic abuse accounts for about 15% to 20% of the cases of renal papillary necrosis in the United States but for as many as 70% in countries in which analgesic abuse is common (189,384,678,679), including in children exposed to analgesics and nonsteroidal anti-inflammatory drugs (680,681). Radiologic evidence for renal papillary necrosis has been reported in more than half of patients with *sickle cell hemoglobinopathy* (436,682). The transplanted kidney appears to be vulnerable to developing renal papillary necrosis, either because of allograft rejection or secondary to the usual causes of renal papillary necrosis, which may have caused the end-stage kidney disease in the first place (250,683–685). In addition, the vasculitis of allograft rejection may lead to obliteration of the vessels supplying the papilla and may result in ischemic necrosis of the papilla. Alternatively, the renal papillary necrosis may be due to the primary disease that caused end-stage kidney disease, such as analgesic abuse, sickle cell disease, or diabetes mellitus.

As a rule, renal papillary necrosis is a disease of the older age-group, with the average age of patients being 53 years. Nearly half the cases occur in persons older than 60 years, and more than 90% of cases in those older than 40 years (189). Renal papillary necrosis is rare in persons younger than 40 years, except for those cases due to sickle hemoglobinopathy (436). Renal papillary necrosis is much less common in children, in whom the chronic conditions associated with renal papillary necrosis are rare, but it has been reported in association with hypoxia and hypotension associated with dehydration and septicemia (680,686–692), particularly in children but also in adults (693,694) and in recurrent dialysis-associated hypotensive episodes (695).

Other conditions in which renal papillary necrosis has been reported are renal vein thrombosis (674,675), cryoglobulinemia (676,696), renal candidiasis (684), idiopathic acute granulomatous interstitial nephritis (697), contrast dye–induced nephropathy (698,699), Wegener's granulomatosis (608), necrotizing angiitis (700), hypotensive shock (701), pancreatitis (657), chronic alcoholism (702,703), and aplastic anemia (189). Most of these have been observed in individual patients, and at least in some of them, their association with renal papillary necrosis may have been coincidental. Actually, even in conditions commonly associated with renal papillary necrosis, more than one of the causative factors of renal papillary necrosis has been noted to be present in more than half of the patients with renal papillary necrosis (189). Therefore, in most cases of renal papillary necrosis, the lesion is multifactorial in origin and the pathogenesis of renal papillary necrosis may be considered the result of an overlap phenomenon, in which a combination of detrimental factors are operating in concert to cause renal papillary necrosis (Fig. 72-11). As such, although each of the conditions alone can cause renal papillary necrosis, the coexistence of more than one predisposing factor in any one person significantly increases the risk for renal papillary necrosis. The contribution of any one of these factors to renal papillary necrosis would be expected to differ among individuals

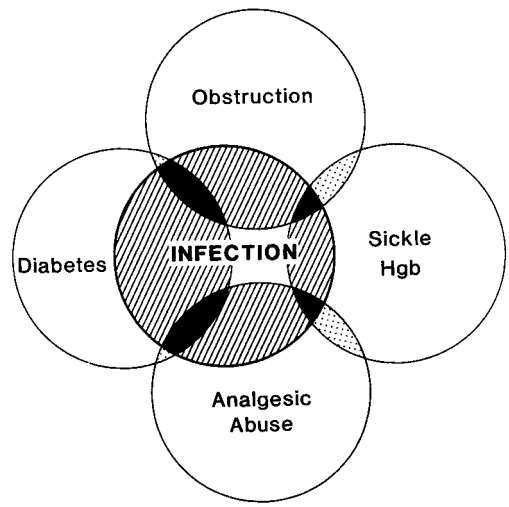

FIGURE 72-11. Spectrum of diseases that are the major causes of renal papillary necrosis, illustrating the multifactorial pathogenesis of renal papillary necrosis. Whereas each condition can, by itself, cause renal papillary necrosis, the coexistence of two (*striped areas*) or three (*black areas*) predisposing conditions increases the risk for renal papillary necrosis. (From: Eknoyan G, et al. Renal papillary necrosis: an update. *Medicine* 1982;61:55, with permission.)

and at various periods during the course of the disease. To the extent that the natural course of renal papillary necrosis itself predisposes to the development of infection of necrotic foci and obstruction by sloughed papillae, it may be difficult to assign a primary role for any of these processes in an individual patient (189). Furthermore, the occurrence of any of these factors—necrosis, obstruction, or infection—may itself initiate a vicious cycle that can lead to the other and culminate in renal papillary necrosis. Evidence that this may well be the case can be adduced not only from clinical studies (189) but also from experimental models of renal papillary necrosis. For example, in the Gunn rat with congenital hyperbilirubinemia, renal papillary necrosis occurs spontaneously secondary to the deposition of unconjugated bilirubin in the renal papillae (590). The administration of analgesics to these animals results in an acceleration of the papillary necrotic process and can be induced at doses of analgesics considerably less than those necessary to induce papillary lesions in other strains of rats (704).

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SECTION X

SYSTEMIC DISEASES OF THE KIDNEY

CHAPTER 73 ■ CLINICAL ASPECTS OF DIABETIC NEPHROPATHY

YALEM WOREDEKAL AND ELI A. FRIEDMAN

Diabetic nephropathy (DN) is one of the most common disabling complications of diabetes mellitus. Characteristically, DN is associated with increased proteinuria, hypertension, and progressive loss of renal function. DN can occur in individuals with either type 1 diabetes or type 2 diabetes (1,2), and if left untreated frequently leads to progressive renal structural damage and end-stage renal disease (ESRD). Convincing evidence indicates that the rate of progression of both early and late nephropathy in patients with type 1 diabetes, and to a lesser extent in type 2 diabetes, can be attenuated by multiple interventions (Table 73-1).

Despite the potential for changing the natural history of diabetic nephropathy with current available therapies, diabetes has become the main cause of ESRD in Western countries. Although the stages of diabetic nephropathy are well studied in type 1 diabetic patients, recent studies have shown a similar course of diabetic nephropathy in type 2 diabetic patients as well (3,4,5).

STAGES OF DIABETIC NEPHROPATHY

Diabetic nephropathy has been divided into three stages (Fig. 73-1): Microalbuminuria (incipient nephropathy), proteinuria with decreasing glomerular filtration rate (GFR), (overt nephropathy), and ESRD. Two additional stages have been described with type 1diabetes. These stages are glomerular hyperfiltration with enlarged kidneys and early glomerular lesions such as mesangial expansion and basement membrane thickening with normal urinary albumin excretion. Both these stages are clinically silent, since estimation of GFR and kidney biopsy is not routinely undertaken. Fragmentary reports suggest that the early course of type 2 diabetes also includes hyperfiltration. At onset of type 1 diabetes, increased GFRs up to 140% of normal values and enlarged kidneys are present in the large majority of individuals (6). Intensive insulin therapy normalizes hyperglycemia and corrects glomerular hyperfiltration (7). A substantial subset of 25% to 40% of individuals with type 1 diabetes who achieve renal levels of plasma glucose during typical insulin therapy still manifest a persistently elevated GFR (8,9), and less than half of all individuals with type 1 diabetes ever manifest clinical nephropathy (proteinuria or azotemia). Chaiken et al. have also found hyperfiltration in (36%) 15 of the 42 patients whose duration of diagnosed type 2 diabetes is less than a year; hyperfiltration persists for up to 10 years in 14% to 20% of these patients without progressing to diabetic nephropathy (3).

Stage 1: Microalbuminuric stage (Incipient Nephropathy)

By definition, microalbuminuria is increased urinary albumin excretion (30 to 200 mg/24 hours or 20 to 200 μg/minute) not detected by dipstick testing, which is commonly employed to detect proteinuria. Several methods for quantifying low concentration of urinary albumin are available, including radioimmunoassay, nephelometric immunoassay, and enzyme-linked immunosorbent assay (ELISA); a semiquantitative dipstick test has also been introduced (Micro-Bumintest, Miles Laboratories, Elkhart, IN).

In the early 1980s, studies in patients with type 1 diabetes linked the presence of microalbuminuria to subsequent development of overt diabetic nephropathy in a reproducible sequence (6,8,10).

The presence of microalbuminuria in patients with type 2 diabetes who are young and non-caucasian is more likely to be associated with the development of renal disease (11–13), whereas in elderly caucasians it is more closely associated to cardiovascular disease (14,15).

The American Diabetes Association in concert with the National Kidney Foundation recommends screening for microalbuminuria starting at the time of diagnosis of diabetes in all individuals with type 2 diabetes and in all persons with type 1 diabetes for longer than 5 years (16). Confounding interpretation of microalbuminuria is the fact that microalbuminuria may be produced by urinary tract infection, strenuous exercise, high protein intake, acute febrile illness, and congestive heart failure (17). (Table 73-2). Therefore, accurate assessment of microalbuminuria requires that testing be postponed if these factors are present. Considering the range and frequency of variables that influence microalbuminuria, it is not surprising that reproducibility of surveys of microalbuminuria is poor, especially when urine collection periods are shorter than 24 hours. In fact, fluctuations in daily urinary albumin excretion yield a coefficient of variation greater than 45% (18–20). For consistency, at least three of urinary albumin tests should be measured over the course of several months.

Approximately 10% to 20% of individuals with type 1 diabetes have microalbuminuria after 5 to 15 years. Estimates of incidence rates of microalbuminuria in type 1 diabetes range from 2.5% to 5.0% per year (21). In the Diabetes Control and Complication Trial, conducted over 9 years in newly diagnosed patients with type 1 diabetes who were younger than 35 years, the incidence of microalbuminuria varied from 4.5% per year in the control group given conventional therapy to 2.9% per year in the intensive glycemic therapy group (22). Similarly, the United Kingdom Prospective Diabetes Study (UKPDS 64) in 5,097 type 2 diabetic patients followed for 10 years demonstrated the incidence of microalbuminuria was 2% per year from time of diagnosis of diabetes (23).

Recent studies have observed a 30% to 45% risk of progression from microalbuminuria to proteinuria over 10 years (24), whereas about 30% of type 1 diabetic patients with microalbuminuria become normoalbuminuric as microalbuminuria persists in the remainder of patients (25). Adding to the complexity of interpreting the significance of microalbuminuria, some normoalbuminuric, longstanding type 1 diabetic patients have well-established lesions of diabetic nephropathy

TABLE 73-1

MEASURES INFLUENCING PROGRESSION OF DIABETIC NEPHROPATHY

Glycemic Control
Tight Blood Pressure Control
Low Protein Diet
Lipid Lowering Agent
Cessation of Cigarette Smoking

TABLE 73-2

COMMON CAUSES OF TRANSIENT INCREASE IN PROTEINURIA

Exercise
Pregnancy
Congestive heart failure
Urinary Tract Infection

on biopsy. Approximately 40% of all type 1 diabetic patients destined to progress to proteinuria are normoalbuminuric at initial screening (26).

Without specific intervention as detailed later, once microalbuminuria becomes constant, a progressive downhill course toward clinical nephropathy is usual (10,27). Typically, albumin excretion increases by about 25 μg/minute per/year, whereas the GFR remains normal or elevated. The GFR begins to decline, at a variable although individually constant rate, once the amount of microalbuminuria exceeds 70 μg/minute. Blood pressure elevation is higher in type 1 diabetic patients with microalbuminuria than in nonalbuminuric patients, although not necessarily higher than 140/90 mm Hg. Persistent hypertension (>140 to 160/90 mm/Hg) and microalbuminuria are present in up to 40% of populations with type 1 diabetes. These two combinations herald near-term deterioration to clinical nephropathy (>200 mg albumin per/24 hours) (28).

Stage 2: Clinical Nephropathy: Proteinuria and Decreasing Glomerular Filtration Rate

Following a variable interval, usually after several years of microalbuminuria, GFR declines below normal and proteinuria is detectable by dipstick. Proteinuria, defined as albumin excretion of greater than 200 mg/day, is universally noted in diabetic nephropathy (29). In the subset of diabetic patients who develop nephropathy, proteinuria is duration-related, increasing in time to a florid nephrotic syndrome with urinary protein excretion exceeding 3 g/day. In about 30% to 40% of patients with type 1 diabetes, periorbital and ankle edema, followed by anasarca, occurs as proteinuria rises above 500 to 3,000 mg/day. Hypertension is present in the majority of patients who enter this stage of clinical nephropathy. A full nephrotic syndrome (hypoproteinemia, hyperlipidemia, massive proteinuria, and anasarca) is seen when proteinuria rises to 4 to 40 g/day. The proportion of proteinuric patients with type 2 diabetes who develop a nephrotic syndrome is not well defined but is probably about the same as in type 1 diabetes. Anasarca develops in diabetic nephrotic patients at a higher serum albumin concentration level than in nondiabetic patients, an observation probably explained by the fact that the glycation of albumin leads to enhanced transcapillary permeability of glycated albumin compared with normal albumin (30).

The diagnosis of diabetic glomerulosclerosis can be inferred from a typical clinical course in which a diabetic patient manifests a transition from microalbuminuria to nephrotic range proteinuria that is followed by a progressive decrease in GFR. It should be kept in mind that because diabetic patients are at equal risk for nondiabetic renal disorders, other renal diagnosis should be pursued, with resorting to renal biopsy, whenever the course does not fit the usual pattern of diabetic nephropathy (Table 73-3).

Once overt nephropathy develops there is a progressive decline in GFR that can be assessed as an absolute decline in mL/minute per year. In the absence of intensive glycemic control and blood pressure control, albumin excretion rate increases at 20% to 40% per year and GFR declines at a rate of 11 mL/minute per year (31,32).

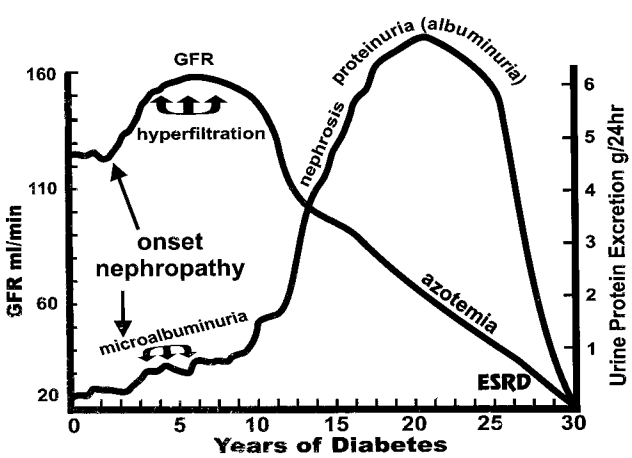

FIGURE 73-1. The natural history of kidney disease in diabetes begins with the pathophysiologic perturbations of increased glomerular filtration rate (GFR), termed hyperfiltration, and the excretion of small amounts of albumin, termed microalbuminuria. Thereafter, proteinuria, nephrosis, azotemia, and end-stage renal disease follow in sequence. These stages are similar in type 1 and type 2 diabetes.

Stage 3: End-Stage Renal Disease

In a study from the Joslin Clinic published in 1985, 10 years after the onset of proteinuria, 50% of type 1 diabetic patients progressed to ESRD (33). In contrast, several population studies found substantial racial and ethnic differences in the incidence rate of ESRD attributed to type 2 diabetes (34–37). The highest incidence of ESRD secondary to diabetes has been reported in Native Americans followed by Hispanics and African Americans. In Native Americans of the Pima tribe in Arizona, the cumulative incidence of ESRD after onset of clinically detectable proteinuria is 40% at 10 years and 61% at 15 years (37). By contrast, ESRD develops in only 11% of caucasians after 10 years of proteinuria and in 17% after 15 years (34). The incidence of diabetic ESRD is 3.6 times higher in African

TABLE 73-3

CONSIDER DISEASES OTHER THAN DIABETIC NEPHROPATHY IN DIABETIC PATIENTS

Absence of albuminuria
Rapid increase in serum creatinine
Presence of active urinary sediments

Americans (35), and 6 times higher in Hispanics when compared to caucasians (38). These racial and ethnic differences in the incidence of diabetic nephropathy reflect a complex and poorly understood interplay between genetic and environmental factors.

Therapeutic Measures in Diabetic Nephropathy

Glycemic Control

Clinical trials of enhanced metabolic regulation of hyperglycemia in type 1 and type 2 diabetes have removed any doubt about the indictment of hyperglycemia as a contributor of diabetic nephropathy (22,23). In the Diabetes Control and Complication Trial in type 1 diabetes, a reduction in HbA1c to a mean of 7.2% reduced the risk of developing microalbuminuria by 34%, as compared to convention therapy which yielded a mean HbA1c of 9%. This benefit was maintained for up to 8 years, even once the difference in glycemic control between the two groups disappeared (39). Similarly, the UK Prospective Diabetes Study in type 2 diabetes reported a 34% reduction in the risk of developing proteinuria in an intensively treated group with a mean HbA1c of 7% as compared with a conventionally treated group with a mean HbA1c of 7.9% (23). It has been shown that both hyperfiltration and enlarged kidneys were reversed to normal after intensive insulin treatment in both type 1 and type 2 diabetes (40,41). With normalization of glycemic control seen in solitary pancreas transplantation, Fioretto et al. found regression of mesangial expansion and glomerular basement thickening after 10 years, but not after 5 years, following pancreas transplantation in adolescent whites with type 1 diabetes and normal renal function (42).

Once overt proteinuria appears, it is uncertain whether glycemic control affects the progression of diabetic nephropathy. Studies have shown conflicting results as to whether improved glycemic control induces a reduction in urinary albumin excretion.

In two combined analysis of the large Steno study, better glycemic control was associated with a fall in urinary albumin excretion (UAE) rate and slow change in GFR (43). Similarly, Nyberg et al. prospectively followed 18 patients with type 1 diabetes and diabetic nephropathy for 21 months, noting a direct relationship between fall in GFR and HbA1c, with the greatest fall in GFR occurring in those with the highest HbA1c (44). In contrast, other studies have failed to demonstrate an association between glycemic control and decrease in GFR (45,46). In a cohort study, Viberti et al. followed 24 diabetic patients for an average of 43 months discovering no relationship between HbA1c and decrease in creatinine clearance (45). Similarly, in a prospective study of 115 patients (50 with type 1 and 65 with type 2 diabetes) no relationship between HbA1c and GFR was noted over 7 years (46).

From the studies described in the preceding text, it is clear that the effect of glycemic control on the progression of diabetic nephropathy is more significant during the earliest stage of diabetes. However, one should be cautious about inducing severe hypoglycemia when intensive glycemic control is attempted in patients with greatly impaired renal function and/or ESRD (47). Modification of some antidiabetic drug doses is required when GFR is subnormal. For example, metformin is contraindicated in advanced renal insufficiency (serum creatinine level >2.5 mg/dL) because of the risk of fatal lactic acidosis. In mild and stable decreased renal function, the dose of metformin should be lowered to approximately one-third that given to patients with normal GFRs (47). In patients treated with insulin, progressive reduction of insulin dose may be required both after the abrupt onset of acute renal failure and chronic renal insufficiency because of impaired degradation of exogenous and endogenous insulin (48,49). Several studies suggest a benefit of glycemic control in patients with ESRD (50,51). Morioka et al. have reported in a prospective study of 150 ESRD patients with diabetes that cumulative survival was higher in those with HbA1c <7.5% than those with HbA1c >7.5% before the start of regular hemodialysis (51).

Blood Pressure Control

Diabetes and hypertension are both common conditions associated with high morbidity and mortality. Diabetic nephropathy and hypertension occur together in 50% of diabetic patients, and result in a 7.2-fold increase in overall mortality (52). Multiple trials show that induction of normotension in type 1 and type 2 diabetic patients with persistent microalbuminuria decreases albumin excretion, whereas clinically evident nephropathy is postponed and may even be prevented.

In type 1 diabetes, microalbuminuria usually precedes a gradual but consistent rise in blood pressure by 3 to 5 years. In contrast in type 2 diabetes, there is no consistent pattern to the course of elevation of blood pressure as nephropathy progresses. Hypertension is present in 25% to 30% of type 2 diabetic patients at the onset of diabetes.

As reviewed by Parving, in those with overt diabetic nephropathy, blood pressure reduction, whether with angiotensin-converting enzyme (ACE) inhibitors or non-ACE inhibitors reduces albuminuria,delays progression of nephropathy, and improves survival in both type 1 and type 2 diabetic patients (53).

Long-term follow-up studies of initially normotensive diabetic patients with microalbuminuria showed a blood pressure–dependent decline in GFR even when the blood pressure level remained normal (54–57).

Parving et al. in a pioneer study of six hypertensive adults with nephrotic-range proteinuria due to type 1 diabetes, observed that treatment with metoprolol, hydralazine, and furosemide for 28 to 36 months reduced mean blood pressure from 162/103 to 144/95 mm Hg, resulting in a 60% reduction in the rate of fall of GFR from 1.23 to 0.4 mL/minute/month and also a reduction in albumin excretion by 5% to 10% per year (58). Subsequently, using a similar antihypertensive regimen, blood pressure was reduced from 143/96 to 129/84 mm Hg in ten patients with type 1 diabetes who were followed for 32 to 90 months, evincing a 75% decrease in the rate of decline in GFR from 0.89 to 0.22 mL/minute/month (59). Schrier et al. also showed that intensive treatment of normotensive, type 2 diabetic patients to blood pressure of approximately 125/75 mm Hg with nisoldipine (calcium channel blocker) or enalapril (ACE-I), retarded progression to incipient and overt nephropathy, decreased the progression of retinopathy, and reduced the incidence of stroke (60).

The sixth report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure first published the recommendation for the target level of blood pressure in diabetes to at or below 130/85 mm Hg (61). The American Diabetes Association adapted the recommendation in 1999 (62). However, a consensus report of the National Kidney Foundation suggests that the goal should be a blood pressure of less than 130/80 mm Hg for nonproteinuric and 125/70 mm Hg for those with proteinuria (63).

To achieve this degree of blood pressure reduction almost always necessitates more than one antihypertensive drug and a willing patient. The ideal drug combination is far from clear, although a combination of calcium channel blocker and ACE inhibition or angiotensin receptor blocker is usually satisfactory.

DO ANGIOTENSIN-CONVERTING ENZYME INHIBITORS AND ANGIOTENSIN II RECEPTOR BLOCKERS HAVE ADDITIONAL BENEFIT FOR DIABETIC NEPHROPATHY?

Although retardation of the decline in GFR was also shown with other antihypertensive medication, the effect on proteinuria was most prominent with ACE inhibitors. In the early 1990s ACE-inhibitors were found to have what have been termed renoprotective properties (64–66). Lewis et al. in a randomized-controlled trial comparing captopril to placebo in 207 type 1 diabetic patients with nephropathy found that captopril-treated patients showed a significant decline in the primary endpoints (doubling serum creatinine and the development of ESRD) and a 50% reduction in risk of combined endpoints (64). A similar effect of ACE inhibitors was also shown by Ravid et al. in a small trial in normotensive type 2 diabetic patients with microalbuminuria (66). Subsequently, many studies documented the value of ACE inhibitors in hypertensive diabetic patients with incipient nephropathy (IP). The MICRE-HOPE study comprised 1,140 patients with type 2 diabetes and microalbuminuria randomized to ramipril 10 mg/day or placebo (67). Blood pressure in all patients was kept at normal values by including addition of other medications. At 4.5 years, ramipril-treated patients had reduced myocardial infarction by 22%, stroke by 33%, cardiovascular death by 37%, and total mortality by 24%. There were slower rises in urinary albumin excretion in ramipril-treated patients. A recent multicenter double-blind, randomized study was designed to assess whether an ACE inhibitor and dihydropyridine calcium-channel blockers, alone or in combination, would prevent microalbuminuria in patients with hypertension, type 2 diabetes, and normal urinary albumin excretion. A total of 1,204 patients were randomly assigned to receive at least 3 years of treatment with trandolapril (at least 2 mg daily) plus verapamil (180 mg sustained release per day), trandolapril alone (2 mg per day), verapamil alone (sustained-release 240 mg per day), or placebo. Trandolapril plus verapamil and trandolapril alone delayed the onset of microalbuminuria by factors of 2.6 and 2.1, respectively. The effect of verapamil alone was similar to that of placebo (68). In perspective, because of their organ protective effect, efficacy, and relative mild side effects, ACE inhibitors rank as first-line treatment in both type 1 and type 2 diabetes.

Angiotensin II receptor blockers (ARBs) are a relatively new class of antihypertensive agents that inhibit the renin–angiotensin system by selectively blocking the AT_1 subtype of angiotensin II receptors. The ARBs are effective antihypertensive agents. Compared to ACE inhibitors, a similar reduction in blood pressure is obtained. The ARBs have an advantage over ACE inhibitors in terms of the side-effect profile.

Several small studies had shown a decrease in albuminuria in ARB-treated patients with diabetic nephropathy similar to the effect obtained with ACE inhibitors (69,70). A compelling renoprotective effect of ARBs was underscored when three large international prospective controlled studies were published in the same issue of *The New England Journal of Medicine* (71–73). The Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan (RENAAL) consisted of 1,519 type 2 diabetic patients with hypertension and proteinuria, compared losartan (50 to 100 mg once a day) with placebo, and was based on a composite endpoints (i.e., doubling of the baseline serum creatinine, onset of ESRD, or death). Losartan-treated patients showed reduction in risk of doubling the serum creatinine by 25%, and the risk of ESRD by 28%. The study showed that losartan-treated patients achieved 16% reduction in any primary endpoint. The authors estimated that benefits of losartan translated to a 2-year delay in the need for dialysis or transplantation (71). The other study, irbesartan diabetic nephropathy trial (IDNT), consisted of 1,715 hypertensive patients with nephropathy due to type 2 diabetes randomly assigned to treatment with irbesartan (300 mg daily), amlodipine (10 mg daily), or placebo. The target blood pressure was 135/85 mm Hg or less to all groups. Treatment with irbesartan was associated with a risk of the primary composite endpoint (doubling of serum creatinine, the development of ESRD, or death from any cause) that was 20% ($p = 0.02$) lower than that in the placebo group and 23% lower than that in the amlodipine group. Proteinuria was reduced on average by 33% in the irbesartan group as compared with 6% in amlodipine group and 10% in the placebo group (72). Parving et al. in 590 hypertensive type 2 diabetic patients with microalbuminuria compared irbesartan in two different doses (150 mg and 300 mg daily) to placebo while blood pressure was maintained in the same range (141 to 144/83 mm Hg). Both doses of irbesartan were renoprotective when compared to placebo. The higher dose had a greater effect (73). In all three studies the renoprotective effect of ARBs was considered to be independent of the effect on blood pressure. These studies (MICRO-HOPE, INDT, and RENAAL) provide a strong rationale for use ACE inhibitors or ARBs in patients with diabetic nephropathy. Indeed current standards of care for proteinuric diabetic patients are based on inclusion of an ACE inhibitor or ARB.

A few studies detected a superior effect of dual blockade of the renin–angiotensin system with ACE inhibitors and ARBs with regard to lowering albuminuria and blood pressure in diabetic patients when compared to maximal dose of an ACE inhibitor alone (74–77). A larger and longer prospective, randomized controlled trial is needed before suggesting the combination of ACE inhibitors and ARBs as the standard antihypertensive medications for diabetic patients.

Dietary Protein Restriction

It has been known that protein restriction is effective in alleviating the symptoms of uremia and may delay the need for dialysis (78). Beyond the symptomatic relief, reduced protein intake is advocated to slow or/and prevent decline in renal function (79–84). The mechanism of halting of such progression is suggested to be a reduction in the hyperfiltration that occurs in the remaining nephrons after renal injury is established (83). The value of dietary protein restriction in type 1 diabetes was suggested by Zeller et al. in a small prospective, randomized controlled study of 35 patients with type 1 diabetes mellitus and clinical nephropathy. The low-protein diet contained 0.6 g/kg/day and patients were followed for a mean period of 34.7months. There was a fourfold decrease in the rate of decrease of GFR in the low-protein diet group compared with that in 15 controls after 3 month. Mean protein excretion fell by 24% in the study group and rose by 22% in controls. At the end of the study, reduction in proteinuria in the study population was only 6%, whereas controls had a 24% increase (79).

Further to the point, Jibani et al. found that albumin excretion rates fell when patients with microalbuminuria were given a predominantly vegetarian diet in the absence of significant change in either blood glucose control or arterial blood pressure (85).

The study in type 2 diabetes by Pijls et al. (86) failed to discover favorable effect of a low protein diet, mainly because subjects did not comply with the diet. Pecis et al. (87) compared three diets—the usual diet with 1.4 g/kg/day protein, a low

protein diet (0.6 g/kg/day), and a test diet in which chicken and fish replaced red meat. They found that the chicken and fish diet had effects on GFR similar to those of the low protein diet. However, this was a short-term study with only 3 weeks on each diet.

Available evidence suggests that protein restriction can slow progression in early nephropathy, although the effect is fairly modest. At present, we advocate prescribing a diet that avoids protein in excess of 0.8 g/kg/day in both type 1 and type 2 diabetes once proteinuria reaches 0.5 g/day or more or a decreasing GFR is noted, provided that overall nutritional status is satisfactory.

Lipid-Lowering Drugs

Hyperlipidemia is recognized as a risk factor for the development of vascular disease, including nephropathy in both the animal models of diabetes and human type 1 and type 2 diabetes (88–90). There are few prospective studies that have tried to show the association between hyperlipidemia and the decline of renal function in diabetic nephropathy (91–93). Krolewski et al. (91) examined the determinants of progression to chronic renal failure in 439 diabetic patients with retinopathy and nephropathy in a *post hoc* analysis of the prospective Diabetic Retinopathy Study. One-third of patients had a rapid loss of renal function. Only elevated serum cholesterol level and elevated diastolic blood pressure at entry were significantly associated with a rapid loss of renal function. These correlations remained significant in a multiple logistic regression analysis. Similarly, Parving et al. found a significant positive correlation in a univariate analysis between serum cholesterol level and rate of decline in GFR in a 10-year prospective study of ACE inhibitors in type 1 diabetic patients with nephropathy (92).

In a prospective study, Ravid et al. followed 94 normotensive type 2 diabetic patients with microalbuminuria and normal renal function. Patients were randomized to receive enalapril or placebo and were followed for 5 years. A significant correlation between baseline and mean study values of serum total cholesterol and the subsequent decline of renal function was observed. This association persisted after stratification for blood pressure in both groups. Initial and mean plasma total cholesterol and mean blood pressure were also significant predictors of the subsequent increase in albuminuria (93).

A recent randomized, double-blind placebo-controlled study by Fried et al. compared the effect of simvastatin and diet versus diet alone on albuminuria in 39 type 1 diabetic patients with nephropathy. Although, not significant the rise in albuminuria was slower in the simvastatin-treated group compared to placebo over 2 years (94). Recently a meta-analysis on the effect of lipid-lowering therapy on the progression of renal disease assessed 13 prospective controlled trials, seven were exclusively in diabetic patients, to examine the effects of antilipid agents on proteinuria and renal function (95). Lipid lowering was associated with a slower rate of the decline in renal function compared to controls ($p = 0.008$) to an extent equivalent to the beneficial effect of an ACE inhibitor on preservation of renal function. The effect on GFR did not correlate with the type of lipid-lowering agent or the cause of renal disease. Although not obtaining significance, there was a tendency for a favorable effect of anti-lipidemic treatment on proteinuria.

In summary, to date, studies attempting to document on association between dyslipidemia and progression of diabetic nephropathy are inconclusive. Large clinical studies with longer follow-up are needed to validate lipid-lowering agents as an additional renoprotective measure. However, as cardiovascular disease is the number one cause of death in diabetic patients with nephropathy, optimizing lipid control is now a standard of care.

Cessation of Cigarette Smoking

Cigarette smoking is a risk factor for progression of diabetic nephropathy in both type 1 and type 2 diabetes (96–100). Chase et al. (96) looked at the effects of cigarette smoking on diabetic renal and retinal complication in 359 type 1 diabetic patients. The prevalence of increased albumin excretion rate was 2.8 times higher in smokers than nonsmokers. Even after corrections for glycohemoglobin level and duration of diabetes, smoking was a significant factor in a logistic regression model for albuminuria. Significant improvement was also seen when subjects ceased smoking (96). Chuahirun et al. also showed that type 2 diabetic patients who smoked had a faster decline of renal function and increased urinary albumin excretion (98) than non-smokers, despite blood pressure control and use of ACE-inhibitors. They also found that cessation of smoking ameliorates the progressive renal injury caused by continued smoking (99). This is in addition to the well-established effect of smoking on cardiovascular and pulmonary disease. It is thus important that every patient be made aware of the importance of quitting this habit.

Management of Comorbid Conditions

As diabetic kidney disease progresses, treating a diabetic patient becomes increasingly difficult and involves multiple healthcare professionals (Fig. 73-2). Diabetic patients with impaired renal function have a higher mortality rate than nondiabetic patients with equivalent renal insufficiency, usually due to cardiac disease, infection, and cerebrovascular diseases. Biesenbach and Zazgornik followed 20 type 2 diabetic patients with overt proteinuria and normal GFR until the beginning of dialysis or the time of death. Twelve patients required dialysis after a mean time of 74 months. Eight patients died before starting dialysis treatment. From the start of the study to the time of starting dialysis or to the time of death, the prevalence of cerebrovascular disease increased from 30% to 75%, cardiovascular disease increased from 45% to 91%, and peripheral vascular disease from 15% to 50% (101).

Listed in Table 73-4 are the most significant comorbid concerns in the management of diabetic patient with impaired renal function.

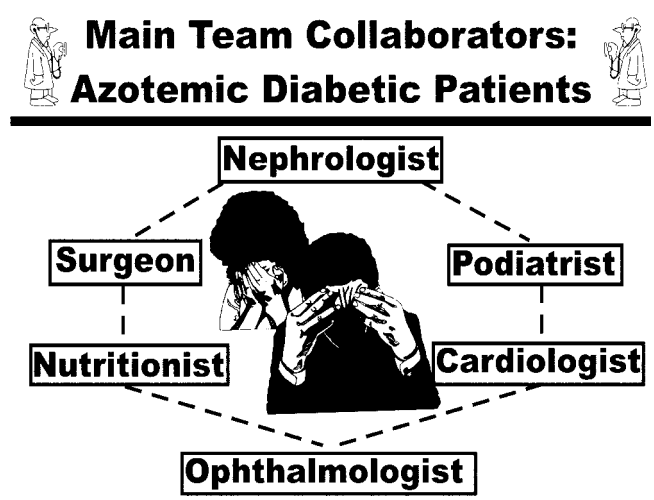

FIGURE 73-2. Comprehensive management of comorbidity in a patient with diabetic nephropathy depends on the skills of a competent team. Cardiac evaluation is especially important to detect the treat of serious coronary artery disease.

TABLE 73-4

CO-MORBID CONDITIONS: DIABETIC AZOTEMIC PATIENTS

Retinopathy
Cardiovascular disease
Cerebrovascular disease
Peripheral vascular disease
Autonomic neuropathy
Cystopathy
Gastroparesis
Peripheral polyneuropathy

Diabetic Retinopathy

Diabetic retinopathy is the leading cause of new cases of blindness in person ages 20 to 75 years in the United States (102). Type 1 diabetic patients with nephropathy nearly always present with retinopathy. However, only 35% of patients with retinopathy develop signs of nephropathy. Epidemiologic studies such as the Diabetes Control and Complication Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS) have shown a strong relationship between hyperglycemia and development and progression of retinopathy (22,23). In the DCCT treatment group, the reduction in the risk of progressive retinopathy and nephropathy resulting from intense insulin therapy in type 1 diabetic patients persisted for at least 4 years, despite increasing hyperglycemia. To examine the 14-year incidence and progression of diabetic retinopathy, Klein et al. conducted a study on 634 type 1 diabetic patients with diabetes diagnosed before age 30 years. The 14-year rate of progression of retinopathy was 86%, regression of retinopathy was 17%, progression to proliferative retinopathy was 37%, and incidence of macular edema was 26%. Increased risk of proliferative retinopathy and macular edema was associated with more severe baseline retinopathy, higher glycosylated hemoglobin, and elevated diastolic blood pressure (103).

Data from the EURODIAB Controlled Trial of Lisinopril in Insulin-Dependent Diabetes Mellitus (EUCLID) showed that lisinopril decreased the progression of retinopathy by two or three grades, and the progression to proliferative retinopathy (104). Retinopathy progressed by 13.2% in 150 patients receiving lisinopril compared to 23.4% in those receiving placebo. This reduction remained constant even when adjusted for glycemic control. These findings suggest that ACE inhibitors have not only renoprotective properties, but also might have a role in preventing progression of retinopathy.

We advise that as a component of the initial evaluation of diabetic patients with nephropathy, direct funduscopy, retinal photography, and fluorescein angiography be performed to provide a baseline facilitating interpretation of subsequent examinations.

Cardiovascular Disease

Diabetic patients have a high prevalence of morbidity and mortality secondary to cardiac causes, with up to 75% of mortality being from cardiovascular disease (105,106). Previous studies have shown that age-adjusted relative risk of death due to cardiovascular event in diabetic patients is threefold higher than in general population (105).

The Multiple Risk Factor Intervention Trial (MRFIT) (107) occurrence of cardiovascular death at 12-year follow-up was about three times greater in diabetic men than in their nondiabetic controls, regardless of systolic blood pressure, age, cholesterol, or smoking status. Systolic hypertension, elevated cholesterol, and cigarette smoking were also independent predictors of mortality, and the presence of at least one of these risk factors had a greater impact on increasing cardiovascular mortality in patients with diabetes than in those without diabetes, suggesting a key role for the management of cardiovascular risk factors in these patients.

Microalbuminuria is an independent marker for increased cardiovascular mortality in both diabetic and nondiabetic subjects (108–112). The degree and rate of progression of albuminuria also correlates with increased cardiovascular event (113). A recent analysis of the UKPDS demonstrated that among 5,097 type 2 diabetic subjects followed for more than 10 years, the risk of death was greater than the risk of ESRD in those with type 2 diabetes (114). In this study, annual rates of progression from normal to microalbuminuria, microalbuminuria to macroalbuminuria, and macroalbuminuria to elevated plasma creatinine or renal replacement therapy were all between 2% and 3% per year. The annual cardiovascular death rates for these progressive stages of chronic kidney disease were 0.7% for subjects with no nephropathy, 2% for those with microalbuminuria, 3.5% for those with macroalbuminuria, and 12.1% for those with elevated plasma creatinine or on renal replacement therapy.

Clearly, the preceding studies show that cardiovascular disease starts at the earliest stage of nephropathy in diabetic patients, hence great emphasis should be placed upon its primary prevention. Use of aspirin, ACE inhibitors or ARBs, and antilipid agents, as well as regular cardiac evaluation, should help to reduce the mortality and morbidity caused by cardiovascular disease in diabetic patients. Lastly, for patients with early nephropathy and established cardiovascular disease such as coronary artery disease or heart failure, the additional use of beta-adrenergic blocking agents favorably impacts the natural history of these conditions (115). Beta-blockers improve left ventricular ejection fraction and reduce rates of hospitalization, sudden death, and all-cause mortality (116).

Peripheral Vascular Disease

Foot ulceration and limb amputation are the leading causes of morbidity and mortality in diabetic patients. The incidence of diabetic foot ulcers is up to 5% per year, with a prevalence of up to 10% (117). The cumulative incidence of amputation in type 1 and type 2 diabetic patients is approximately 15% (118). In diabetic patients with ESRD, the incidence of foot complications is higher, reaching 25% (119) and remaining a problem after kidney or kidney and pancreas transplantation (120). Wanner et al. reported that 16% of type 2 diabetic patients undergoing hemodialysis had a prior history of amputation, whereas 44% were found to have evidence of peripheral vascular disease (121). Pugh et al. reported a diagnosis of peripheral neuropathy in 84% of patients with type 1 diabetes and 60% of those with type 2 diabetes at the start of renal replacement therapy (122). In more than 70% of limb loss, amputation is preceded by an ulcer due to ill-fitting shoes, causing repeated episodes of unnoticed trauma to the foot precipitated by loss of protective sensation as a consequence of peripheral neuropathy.

Although the amputation rate in diabetic persons is up to five times higher than in nondiabetic persons, recent studies comparing patency rates after percutaneous transluminal angioplasty or bypass grafting have not shown a significant difference between the two groups. This suggests that part of the reason for the higher amputation rate in diabetic patients might be due to delay in initiating treatment (123,124).

Initiating a program to educate patients and family members about potential limb disease, with frequent examination of the feet; wearing of comfortable, nonconstricting shoes; and frequent visits to a podiatrist for nail and callus care is a highly effective means of preventing limb loss.

Autonomic neuropathy

Autonomic neuropathy is a highly prevalent, yet frequently overlooked complication of diabetes. Although involvement of the autonomic nervous system is generally diffuse, symptoms may be confined to a single-target organ or organ system. Complications of diabetic autonomic neuropathy contribute greatly to the morbidity, mortality, and reduced quality of life. The clinical manifestation of autonomic neuropathy includes gastroparesis, postprandial hypotension, sexual dysfunction, and unawareness of hypoglycemia.

Diabetic cystopathy (impaired bladder sensation and, increased bladder capacity, sometimes accompanied by voiding difficulties) often develops insidiously and asymptomatically. Although common, it is frequently unrecognized and confused with worsening of diabetic nephropathy. The prevalence of diabetic cystopathy is 25% in type 2 diabetics and 48% to 87% in type 1 diabetic patients (125,126).

Cardiac autonomic neuropathy occurs in diabetic patients and its prevalence and severity are correlated with advancing nephropathy (127,128). Heart rate variability is useful in assessing dysfunction of the autonomic nervous system in diabetic patients (127). Meinhold et al. assessed cardiac autonomic neuropathy in 66 diabetic patients without nephropathy ($n = 24$), incipient nephropathy ($n = 26$), and overt nephropathy ($n = 16$), and found cardiac autonomic neuropathy was significantly higher in those with overt nephropathy when compared to those without nephropathy and incipient nephropathy (129). QTc interval and QTc dispersion increased significantly with increasing nephropathy. Heart rate variability analysis can detect early subclinical alterations of the autonomic nervous system in symptomatic diabetic patients (126). Absent heart rate variability secondary to diabetic cardiac autonomic neuropathy is a predictor of left ventricular failure and increased mortality due to sudden cardiac death (130). Patients with diabetes are at an increased risk of sudden cardiac death due to the increased prevalence of silent myocardial ischemia and cardiac autonomic neuropathy predisposing patients to ventricular tachyarrhythmias (131).

Impaired gastric emptying (gastroparesis) is a disorder of delayed gastric emptying that is often chronic. Up to 50% of all diabetic subjects have symptoms of gastroparesis (132,133), which include nausea, vomiting, and early satiety. Gastroparesis may frustrate efforts to synchronize insulin effect with food ingestion due to irregular gastric emptying. Diagnosis of gastroparesis requires identifying the proper symptom complex, while excluding other entities (peptic ulcer disease, rheumatological disease, and medication effect). The diagnosis can be confirmed by demonstrating gastric emptying delay during a 4-hour scintigraphic study. Treatment options are limited and rely on dietary modifications, use of available pharmacologic agents, and occasionally surgical interventions. The pharmacologic agents for treating gastroparesis include metoclopramide, erythromycin, and cisapride (only available via a company-sponsored program). All of these drugs act as promotility agents that increase the number or intensity of gastric contractions. Cisapride has been removed from the open market as a result of more than 200 reported cases of cardiac toxicity attributed to its use. Gastric pacing (134,135) and pyloric injection of botulinum toxin (136,137) have shown promise for patients with medically refractory gastroparesis, but awaits further investigation. Other manifestations of autonomic neuropathy of the gastrointestinal tract are obstipation and explosive night time diarrhea-often coexisting with gastroparesis (138). Obstipation responds to a daily dose of cascara, whereas diarrhea is treated with psyllium seed dietary supplements one to three times a day plus loperamide in repetitive 2-mg doses to a total dose of 16 mg daily.

Unawareness of hypoglycemia is a severe complication for diabetic patients and alters the quality of life because it markedly increases the anxiety of both the patients and his/her family. It has been attributed to diabetic autonomic neuropathy, probably due to inability to secrete counterregulatory hormones (glucagons, epinephrine) in response to hypoglycemia (139). In a cross-sectional survey of 178 type 1 diabetic patients over a period of 14 years, the prevalence of severe hypoglycemia increased by more than 50% despite more frequent use of multiple injection therapy and self-measurement of blood glucose (140). An important negative consequence of the struggle to attain metabolic control is the frightening increase in hypoglycemic episodes, some of which are detected late at the point of loss of consciousness; this may be the price paid by patients who are dutifully compliant. Hypoglycemic coma related to an absolute or relative excess of insulin should, in most cases, be treated at home by oral administration of carbohydrate with high-glycemic index if consciousness allows it or, if not, the injection of glucagons. In contrast, a hypoglycemic coma due to a too-high dose of sulphonylurea requires hospitalization to supervise the patient and to provide a prolonged intravenous infusion of glucose. It is mandatory that family members of any diabetic patient at risk of developing severe hypoglycemia receive specific education in order to promptly apply the appropriate treatment capable of a rapid and safe recovery from hypoglycemia.

Impairing quality of life, and present in at least one-half of diabetic men with renal insufficiency, is erectile dysfunction (141). Phosphodiesterase enzyme (PDE) inhibitors have revolutionized the management of erectile dysfunction and oral therapy (sildenafil, vardenafil, or tadalafil) is currently first-line therapy (142,143). Injectable prostaglandin or surgical insertion of a mechanical prosthesis can also be used for those in whom sildenafil is not effective or contraindicated (144).

ENDING CONSERVATIVE MANAGEMENT

Although normalizing blood pressure, tightly controlling glucose, and adhering to a low-protein diet may retard development and progression of diabetic nephropathy, many patients still progress to ESRD, resulting in diabetes being the preeminent cause of ESRD in industrialized nations.

Continuing explanation to the patient of transpiring events as renal function deteriorates builds confidence and minimizes panic, despair, and frantic behavior when the need for ESRD therapy becomes pressing. As the creatinine clearance falls to approximately 20 to 25 mL/minute, available options for ESRD therapy should be discussed and the patient's preference determined. Patient support groups, especially the American Association of Kidney Patients (AAKP), can play a vital role in guiding the patient with new ESRD in making appropriate choices regarding the type of therapy and location of facilities where treatment can be obtained. Membership in the AAKP should be encouraged as a key step in overcoming the shock, fear, bewilderment, and sometimes panic of first learning that one has ESRD. The AAKP's National Office telephone number is 1-800-749-2257.

Depending on the choice of treatment, there are specific preparatory steps that should be initiated. For example, for those opting for hemodialysis, construction of a vascular access site, preferably an internal arteriovenous fistula, is of cardinal importance, thereby avoiding the pain and discomfort of percutaneous femoral or jugular venous cannulation until a suitable vascular access is available. Predialysis management includes preserving forearm cutaneous veins by avoiding venous punctures and intravenous catheters. During the pre-ESRD interval, nutrition should be maintained and erythrocyte mass sustained at greater than a hematocrit of 33%, as necessary, by administration of erythropoietin and supplemental iron.

Metabolic bone disease due to secondary hyperparathyroidism can be minimized by use of intestinal phosphate binders along with synthetic vitamin D. Periodic (monthly) measurements of serum calcium and phosphate levels to exclude hypercalcemia against vitamin D intoxication. The exciting introduction of calcimimetic agents may greatly improve bone preservation in renal failure (145).

Dialytic therapy or kidney transplantation should be considered before the patient loses muscle mass because of the catabolic state induced by uremia.

Although not proven by prospective alternative-case trials, a consensus suggests that renal replacement therapy should begin at a higher creatinine clearance (10 to 15 mL/minute) for diabetic patients rather than nondiabetic patients, because most diabetic patients become symptomatic at a higher creatinine clearance than do those with ESRD of other etiologies. Anorexia, nausea, vomiting, and a reversed diurnal sleep pattern (insomnia coupled with daytime somnolence) are hallmark symptoms as uremia worsens. Indications for urgent dialysis include pericardial friction rub, unremitting gastric or colonic bleeding, seizures, and advancing uremic motor neuropathy (foot drop, paraplegia, quadriplegia, respiratory paralysis).

Preemptive kidney or kidney-pancreas transplantation is advocated at higher levels of residual renal function (creatinine clearance 15 to 20 mL/minute) based on the belief that by avoiding the ravages of severe uremia, rehabilitation is more likely to be successful. Even though evidence from a randomized, controlled trial showing a clear difference between early and late initiation of dialysis is sketchy and debated, an early start at a higher creatinine clearance level does ease management of blood pressure and nutritional status, while probably retarding the progression of uremic motor neuropathy. Previously restricted to patients with type 1 diabetes, the value of pancreas transplantation in individuals whose ESRD is due to type 2 diabetes is under active inquiry (146,147).

Diabetic nephropathy is the leading cause of ESRD in the United States, Japan, and industrialized Europe. The number of new diabetic patients accepted for renal replacement therapy has been increasing steadily for the last two decades. The United States Renal Data System (USRDS) in 2004 reported that 154,197 of 424,802 U.S. patients receiving either dialysis treatment or a kidney transplant in 2002, had diabetes (148). Furthermore, during 2002, of 98,313 new cases of ESRD, 44,139 (44.7%) were listed as having diabetes (Fig. 73-3).

Survival and medical rehabilitation of diabetic patients receiving renal replacement therapy is significantly inferior to that of other patients with other causes of ESRD (149,150). The highly prevalent comorbid conditions affecting diabetic patients when renal replacement therapy is initiated account for the greater risk of death and limited rehabilitation potential in these patients. With recognition and efforts to correct the impact of hypertension and dysmetabolism, survival of diabetic patients with ESRD has improved yearly since the mid-1980s (Fig. 73-4). A comprehensive team effort by both medical professionals and their patients, stressing the importance of preventive care to reduce hypertension, normalize blood glucose level, and correct hyperlipidemia, is rewarded by improved patient survival and rehabilitation.

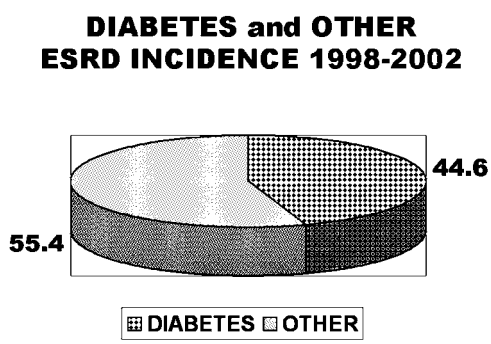

FIGURE 73-3. The number of diabetic patients accepted for renal replacement therapy has been increasing steadily for the last two decades. The United States Renal Data System (USRDS) in 2004 reported that in 2002, of 98,313 new cases of ESRD, 44,139 (44.6%) were listed to have diabetes.

OPTIONS FOR RENAL REPLACEMENT THERAPY

Diabetic patients with ESRD have similar options as nondiabetic patients for renal replacement therapy. Apart from detection and treatment of risk factors for progression of renal disease and monitoring the course of comorbid conditions, it is important to explore various modalities of renal replacement therapy to determine which option best suits the patient. The different types of renal replacement therapy available for diabetic patients with ESRD include hemodialysis, peritoneal dialysis, kidney transplantation alone, and a combined pancreas and kidney transplantation, which is unique to diabetic patients (Table 73-5). The treatment that is best for a particular patient is determined by considering the patient's age, level of education, severity of comorbid conditions, social and family support, and geographic location. For example, a patient with severe cardiomyopathy may be restricted to continuous ambulatory peritoneal dialysis (CAPD) because the stress of extracorporeal blood circulation in hemodialysis might precipitate cardiac decompensation, whereas a young, motivated, and independent patient can choose from a menu of available modalities of treatment preferable selecting kidney transplantation.

MAINTENANCE HEMODIALYSIS

According to the United States Renal Data System (USRDS) 2004 registry (Fig. 73-5), 75% of all diabetic patients with ESRD are treated with hemodialysis (center or home), 7.4% with peritoneal dialysis (CAPD) or continuous cycling peritoneal dialysis (CCPD), and 17% received a functioning kidney

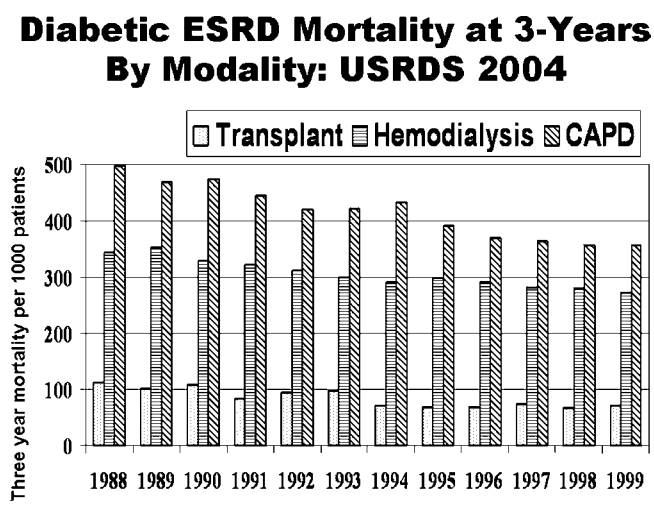

FIGURE 73-4. Three-year mortality rates of diabetic patients with end-stage renal disease (ESRD). Reflecting incremental improvement in management, especially better attention to blood pressure regulation and metabolic control, mortality rates of diabetic patients with ESRD treated with hemodialysis, peritoneal dialysis, or a cadaveric donor renal transplant have shown progressive improvement.

TABLE 73-5

OPTIONS FOR RENAL REPLACEMENT THERAPY FOR DIABETIC PATIENTS WITH ESRD

Hemodialysis
In-center Hemodialysis
Home Hemodialysis
Daily (nocturnal) Hemodialysis
Peritoneal Dialysis:
CAPD
CCPD
Organ Transplantation:
Kidney Transplantation
Simultaneous Pancreas and Kidney Transplantation
Pancreas after Kidney Transplantation

transplant (148). Given that effective hemodialysis is contingent on a continuous blood flow of up to 500 mL/minute, the need to establish a vascular access is an early concern in diabetic patients. Peripheral vascular calcification in middle-sized arteries plus atherosclerosis of small vessels and increased risk of wound infection, may pose a challenge for the vascular surgeon attempting to construct a vascular access in an azotemic diabetic patient. Although the preferable vascular access is an arteriovenous fistula (AV), preexisting vascular disease limits its utility in diabetic patients, who have a primary failure rate of 30% to 40%. A less desirable, but necessary, alternative vascular access can be constructed using a polytetrafluoroethylene graft with a half-life in excess of 1 year. Once it is established and functioning well enough to permit adequate hemodialysis, the mean 3-year survival rate of an AV in diabetic patients is approximately 80%, whereas arteriovenous grafts remain viable in only 47% after 3 years (151).

Current Dialysis Outcome Quality Initiative (DOQI) guidelines encourage placement of AVs in more hemodialysis patients. Konner et al. were able to increase the proportion of AV fistulae in diabetic patients by careful preoperative investigation for selection of adequate location for initial placement of the fistula; preference to large-diameter arteries and veins, frequently requiring use of the elbow region; variable surgical approaches including perforated vein fistula (PVF) and avoidance of initial polytetrafluoroethylene graft (152). Using the previously described principal the authors were able to create primary arteriovenous access for a total of 181 diabetic and 567 nondiabetic patients during 1993 to 1998 (153). Continuous surveillance by nephrologists and dialysis staff permitting elective revision and avoidance of thrombosis will also improve viability of primary fistulae. Complication of vascular access is the leading cause of hospitalization in diabetic patients with ESRD undergoing hemodialysis. Hemodialysis treatment for diabetic patients is similar to that in nondiabetic patients, except for the interruption of dialysis because of intradialytic hypotension or painful, intractable muscle cramps. An ideal hemodialysis regimen consists of thrice-weekly dialysis, each lasting 3.5 to 4.5 hours, during which extracorporeal blood flow must be maintained at 300 to 500 mL/minute. When dialysis sessions are timed precisely, it is noted that diabetic patients on hemodialysis actually receive a lower dialysis dose than nondiabetic patients because of interruption of dialysis due to dialysis-induced hypotension or inadequate blood flow through the access (154). Intradialytic hypotension in diabetes is thought to be due to a depressed counterregulatory reflex in response to hypotension (155). Previously hypertensive diabetics may later develop sustained hypotension, as autonomic neuropathy worsens and cardiac performance deteriorates. Limitation of intradialytic weight gain, use of sodium modeling to promote vascular refilling, use of midodrine (alpha agonist), and cooling of dialysate can reduce dialysis-related hypotension (156).

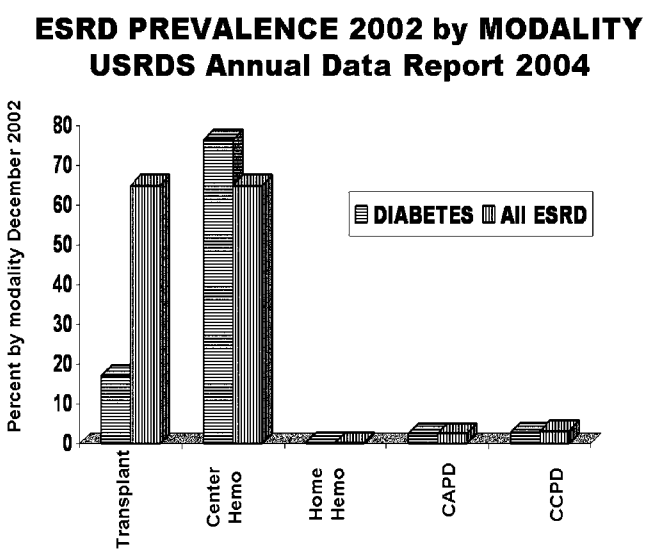

FIGURE 73-5. ESRD treatment modality, diabetic versus total ESRD population. Compared with the entire group, as shown in these data extracted from the United States Renal Data System 2004 Report, diabetic patient with ESRD are more likely to be treated with maintenance hemodialysis even though their survival is best ensured by kidney transplantation.

Glycemic control in a diabetic patient on a dialysis regimen is difficult. Insulin dosing is more complex because of unrecognized gastroparesis that disconnects absorption of ingested food from timed insulin administration, as well as reduced renal insulin catabolism that results in prolonged action of exogenous insulin (157). This combination causes erratic glucose regulation complicated by frequent episodic hypoglycemia, a potentially serious complication. Glycemic control should remain a priority in the dialyzed diabetic patient, as it may retard complications of microvascular disease. Survival during the long-term management of ESRD in diabetes has been linked to the quality of glycemic control achieved.

Defective bone matrix formation and mineralization without osteoid thickness characterize aplastic bone disease, a form of osteodystrophy affecting approximately one-third of uremic patients. Factors for this mineralization defect have not been determined. Diabetic patients on dialysis are predisposed to low bone turnover, which is unrelated to aluminium exposure (158). A prospective study of predialysis patients with a creatinine clearance of less than 10 mL/minute, none of whom had received any form of vitamin D, showed that diabetic patients had low parathyroid hormone values and fewer bone lesions of advanced hyperparathyroidism (16% versus 45%), whereas low bone turnover disease was note in 56% of diabetic patients versus 41% of nondiabetic patients (159).

Although survival of diabetic patients on dialysis has been improving over the last decade, morbidity and mortality remain significantly higher than in those without diabetes. This grim reality is mostly attributed to progression of comorbid conditions. Cardiovascular disease, infections, and withdrawal from dialysis are the leading cause of mortality in ESRD due to diabetes (148).

PERITONEAL DIALYSIS

Peritoneal dialysis is the ESRD treatment modality selected for approximately 7% of diabetic patients in the United States.

CAPD offers the advantage of freedom from a machine, performance at home, reduced cardiovascular stress, better preservation of renal function, and avoidance of heparin when compared to hemodialysis. Another postulated advantage of peritoneal dialysis over hemodialysis is the avoidance of cytokine-mediated toxic response provoked by contact of blood with biomaterials in the extracorporeal circuit and membrane used in hemodialysis (160). Disadvantages include risk of peritonitis, high rates of technical failure, and less adequate dialysis when residual renal function is lost.

As is true for hemodialysis, preparation of the patient for CAPD necessitates education, repetitive explanation, and facilitating surgery to insert an intraperitoneal permanent catheter. CAPD can be learned as a home regimen by motivated patients, even those with impaired vision, in as little as 10 to 15 days, although the typical patient requires about 4 weeks. An alternative to manual cycling of dialysate is the use of a mechanical cycling device in a regimen termed CCPD, which can be performed during sleep.

During the course of both CAPD and CCPD, there is a constant risk of peritonitis as well as a gradual decrease in peritoneal surface area which may ultimately prove to be insufficient for adequate dialysis. Diabetic patients with ESRD on CAPD experience twice as many hospitalization days as nondiabetic patients; peritonitis accounts for 30% to 50% of these hospital days (161). Surprisingly, despite the longer duration required for treatment of each episode of peritonitis, the overall risk of contracting peritonitis is no greater in diabetic patients (162).

CAPD or CCPD are specifically indicated when vascular access sites have been exhausted for hemodialysis or in patients with severe hemodialysis-associated hypotension or angina related to atherosclerotic heart disease. An advantage of peritoneal dialysis for patients with poorly compensated congestive heart failure is its relatively slower ultrafiltration rate, coupled with less rapid removal of solutes resulting in smaller shifts in serum osmolality, which account for the absence of significant hypotension in CAPD.

To achieve euglycemia when patients are continuously exposed to a dialysate glucose concentration of 1500 mg/dL, regular insulin can be added in the dialysate bags. To determine the initial intraperitoneal insulin dose for CAPD, three times the subcutaneous dose in regular units should be divided equally in four bags; the dose for the overnight dwell should be reduced by 50% to avoid nocturnal hypoglycemia (163). For cycling peritoneal dialysis, comparable glycemic control can be achieved with less insulin (approximately 85%) used in CAPD. This is because of reduced glucose absorption during the shorter cycles. Alternatively, patients usually can be treated with twice-daily subcutaneous insulin dosing. During episodes of peritonitis, insulin requirements will vary widely.

The prevalence of malnutrition in patients on CAPD is greater than on hemodialysis, and the problem is worsened in diabetic patients (164,165). Diabetic patients on CAPD become malnourished because of large amount of protein (8 to 10 g/day) losses in the dialysate fluid, and reduced appetite caused by glucose infusion from dialysate, or early satiety due to increased intraabdominal pressure and delayed gastric emptying (166). With increasing duration of CAPD-CCPD, protein loss in the dialysate fluid rises in diabetic patients, probably due to inflammation of peritoneum involving small peritoneal vessels (167). Prevention of malnutrition in these patients requires dietary counseling, maintenance of an adequate dose of dialysis, avoidance of acidemia, and aggressive treatment of superimposed catabolic illness. For those patients who have persistently inadequate nutritional intake, specific treatment such as food supplement, enteral tube feeding, intradialytic parenteral nutrition, and total parental nutrition can be used.

Of the two major options in dialytic therapy for ESRD in diabetes, the USRDS consistently reports superior survival in those treated by hemodialysis, compared with peritoneal dialysis, whether sorted by age, race, or sex (148). In contrast Vonesh et al. (168) analyzed incident data from 398,940 U.S. medicare patients who initiated dialysis between 1995 and 2000 to compare survival outcome between hemodialysis and peritoneal dialysis patients; 11.6% used peritoneal dialysis as initial therapy, 45% had diabetes, 52% were 65 years or older, and 55% had at least one comorbid condition. Among diabetic patients with no comorbidity, hemodialysis was associated with a higher risk of death among younger patients (age 18 to 44 years: relative risk (RR) 95%) confidence interval (CI) 1.24 (1.07,1.44) and a lower risk of death among older patients (>45 years: RR = 0.92 (0.85–1.00). For nondiabetic patients, adjusted mortality rate was significantly higher on hemodialysis than on peritoneal dialysis in both young and older patients (168). Similarly, when a direct comparison of equivalent-dosed CAPD and hemodialysis was made, Keshaviah et al. had found that the 2-year survival estimates were identical on hemodialysis and on CAPD in older diabetic patients (169). In a prospective cohort study of 680 incident CAPD patients in North America, dialysis in the United States compared with Canada was associated with a relative risk of death of 1.93 (95% confidence interval [CI], 1.14–3.28). The 2-year survival probability was 79% in Canada and 63% in the United States (169). This difference was not explained by race, age, gender, diabetes, history of cardiovascular disease, or dose of dialysis. The relative risk of a nonfatal cardiovascular event in the United States compared with Canada was 1.80 (95% CI, 1.21–2.67). The authors suggest that the higher acceptance rate for dialysis in the United States may explain, in part, the greater cardiovascular morbidity and the decreased survival observed.

The causes of mortality in CAPD patients are similar to those on hemodialysis. Cardiovascular event and infection are the two most common causes of death.

KIDNEY TRANSPLANTATION

In the 1970s, many transplant programs excluded diabetic patients with renal failure from consideration for renal transplantation. Repeated reports recounted the poor prognosis of diabetic patients on dialysis while their rehabilitation was preempted by multiple comorbid conditions, leading to the conclusion that diabetic patients should not be considered for renal transplantation (170–172). In centers that did perform renal transplantation in diabetic patients during this period, their survival exceeded that attained by dialysis. Confirmation of this finding coupled with better management of diabetes and uremic complications, established kidney transplantation as routine in diabetic ESRD. In our view, renal transplantation is the treatment of choice for all diabetic patients able to withstand the stress of surgery. Not only is greater patient survival achieved by renal transplantation, but there is a remarkable superior level of rehabilitation over that attained by dialytic therapy.

Since 1985, the results of renal transplantation in diabetic patients have approached parity, at least in the first 2 years, with those achieved in nondiabetic patients (148). A fall-off in survival of diabetic renal transplant recipients after 5 or more years is the result of coronary, cerebral, and other arterial diseases. The most recent report of patient survival in diabetic patients indicated 93.7% 1-year and 85.5% 3-year survival for recipients of cadaveric donor grafts, and 95.4% 1-year and 91.3% 3-year survival for those receiving living donor transplants. The annual death rates of transplant recipients are approximately one-third that of diabetic patients remaining on dialysis (173), a finding suggesting the possibility of a selection bias for healthier dialysis patients for kidney

transplantation, leaving a residual pool for dialysis of sicker patients more likely to die. Nevertheless, when diabetic patients who received kidney transplants were compared to those remaining on the transplant waiting list, patient survival was significantly better in transplant recipients (173,174). The mortality was 38% lower among transplant recipients than among patients on the waiting list.

Forty percent of deaths in renal transplant recipients are secondary to cardiovascular event such as myocardial infarction and stroke, and diabetes is the major risk factor for these events (175). Koch et al. examined 105 consecutive diabetic patients (77 type 1 and 28 type 2 diabetics) between 1989 and 1993 in the first 6 months of dialysis treatment for subsequent renal transplantation. Coronary angiography was performed in all patients regardless of symptoms of coronary artery disease (CAD). In 38 patients (36%) CAD was documented (single-vessel disease 17 patients; double-vessel disease 6 patients; triple-vessel disease 15 patients). Angina pectoris was present in 10 of 38 patients (24%) (176). Therefore, it is warranted to evaluate diabetic kidney transplant candidates for preexisting vascular disease before transplantation. Responding to the real risk of cardiac events in the pretransplantation period, we established a pretransplantation screening program starting with a thorough history and physical examination plus an electrocardiogram, echocardiogram, dobutamine stress test, and, if indicated, by equivocal results, coronary angiography. A meta-analysis of noninvasive methods of assessing coronary artery disease comparing 10 reports on dipyridamole-thallium-201 myocardial perfusion (1,994 patients) to five reports on dobutamine stress echocardiography (446 patients) found the results equivalent (177). Reliance on dobutamine stress echocardiography to exclude significant CAD in ESRD patients permits kidney transplantation with a 97% probability of being free of cardiac complication or cardiac death posttransplantation (178).

Apart from assessing the extent of cardiovascular disease present prior to kidney transplantation, periodic, at least annual, reassessment with noninvasive studies in asymptomatic high-risk patients should be performed. The majority of transplant centers ask for noninvasive testing on an annual basis in the high-risk patients on the waiting list.

Posttransplantation Management

Immediate posttransplantation management of the diabetic renal transplant recipient's metabolic control of plasma glucose concentration is best achieved by frequent hourly glucose measurement and an intravenous infusion of insulin. Protracted gastric atony from gastroparesis present in about one-third of diabetic recipients may delay resumption of oral feeding. An oral dose of a liquid suspension of metoclopramide before meals usually enhances gastric motility and improves gastric emptying. Constipation, sometimes obstipation, is common following kidney transplantation; spontaneous defecation is encouraged by early ambulation, stool softening agents, and suspension of cascara.

Urinary retention, a manifestation of diabetic autonomic neuropathy as a functional outflow obstruction, is a frequent posttransplantation complication. A regimen of hourly voiding when awake, self-application of manual external pressure above the pubic symphysis (Crede's maneuver), and administration of oral bethanechol usually permit resumption of spontaneous voiding. Rarely, repeated self-catheterization is required for an unresponsive atonic bladder. After the initial postsurgical period, most posttransplantation hospitalizations are for either graft rejection or perturbations in plasma glucose levels due to changing doses of corticosteroids. Wide swings in glucose concentration, alternating hypoglycemia and hyperglycemia, are seen, particularly during administration of high-dose steroids for allograft rejection treatment. Otherwise, care of the diabetic renal allograft recipient is not substantially different from that of the nondiabetic recipient, with the key exceptions of the need to manage evident diabetic complications as well as to protect the transplanted kidney from recurrent diabetic glomerulopathy. Recurrent diabetic glomerulopathy is first manifested as glomerular basement membrane thickening and mesangial expansion in as short a time as 2 years for recipients with type 1 diabetes (179). In type 1 diabetes, characteristic nodular intercapillary glomerulosclerosis is regularly noted after 4 or more years in kidneys from nondiabetic donors (180). By 5 years after renal transplantation, we observed a recurrent nephrotic syndrome followed by progressive azotemia, and, finally, ESRD in patients who failed to maintain acceptable levels of glucose control (181).

PANCREAS TRANSPLANTATION

Over the last decade, highly successful results have been reported for combined pancreas and kidney transplantation in diabetic patients (182–184). Combined kidney-pancreas transplantation (KP) is recommended because this option is perceived to carry no additional risk. However, most transplant centers have restricted combined KP transplant to patient with few diabetic complications and no coronary artery disease. Using the United Network for Organ Sharing database, Reddy et al. analyzed long-term survival in 18,546 patients with type 1 diabetes and renal failure who received kidney transplants between 1987 and 1996. Simultaneous kidney and pancreas transplant (SKPT) and living donor kidney recipients had a significant survival advantage over cadaveric kidney transplant recipients (8-year survival rates 72% SKPT recipients, 72% living donor kidney recipients, and 55% for cadaveric kidney recipients) (182). The SKPT recipients had a higher mortality risk relative to living donor kidney recipients through 18 months posttransplantation (hazard ratio, 2.2; $p < 0.001$), but had lower relative risk (hazard ratio, 0.86; $p < 0.02$) thereafter.

Solitary pancreas transplants, either pancreas transplant alone (PTA) or pancreas after kidney transplants (PAKs) constitute a small proportion of the total pancreas transplants in the United States. The University of Minnesota recently reported their large experience in solitary pancreas transplantation (>400 PAK and >200 PTA patients). They showed excellent patient survival and survival of the pancreas graft approaching that of PK transplant using tacrolimus-based immunosuppression (185). Survival of the kidney graft did not appear to be jeopardized by subsequent pancreas transplantation.

Repetitive hospitalizations of pancreas transplant recipients during the first year are caused by bladder pain, hemorrhage, and intraabdominal infection. Patients accept the tradeoff of freedom from insulin injections and enhanced quality of life afforded by a functioning pancreas transplant. There is evidence that pancreas transplantation improves defective glucose counterregulation resulting from prior episodes of hypoglycemia. The responses of epinephrine, glucagons, and growth hormone have been shown to improve in response to hypoglycemia as well as recognition of hypoglycemia (186,187). Pancreas transplantation also prevented progression of mild diabetic nephropathy in patients who have received a prior renal transplant (188). In addition, reversal of established lesions of diabetic nephropathy has been shown in the native kidneys of nonuremic type 1 diabetic patients 10 years after receiving a PTA (42).

Hope that a pancreas transplantation would end the siege of diabetic complications was gleaned from the first observations of the course of diabetic neuropathy following combined pancreas and kidney transplantation in which some patients had stabilization and improvement in diabetic motor neuropathy (189–190). Unfortunately, when pancreas transplantation was performed in patients with extensive extrarenal disease, there was neither cessation of further injury nor reversal of established diabetic retinopathy, diabetic cardiomyopathy, or peripheral vascular disease (191).

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CHAPTER 74 ■ THE NORMAL AND DISEASED KIDNEY IN PREGNANCY

MARSHALL D. LINDHEIMER, KIRK P. CONRAD, AND JASON GARI UMANS

Kidney disease, even when mild, was once considered so major an impediment to successful pregnancy and so dangerous to the mother's wellbeing, that all patients with chronic renal disorders were counseled to avoid conception or to terminate if pregnant (1). We now know this is not true, as there are many instances in which pregnancy in women with kidney disease carries little risk. This chapter, designed to aid physicians consulted when such patients contemplate conceiving or are already pregnant, focuses on pathophysiology, risk assessment, and management of renal disease in pregnancy, whereas hypertension during gestation is addressed in Chapter 53. First, we review the normal anatomic and physiologic changes in the urinary system during gestation, as such knowledge permits detection of abnormalities at an early stage.

PREGNANCY-INDUCED CHANGES IN ANATOMY AND FUNCTION

Anatomy

Kidney

Kidney weight, size, and volume increase in normal human gestation (2–6). Autopsy data are obviously limited, although data from one very unique series published more than 30 years ago suggested the combined weight of "normal" kidneys of 97 women dying during or shortly after pregnancy were greater than in nonpregnant women (4). Kidney length estimated by either pyelography or nephrotomography increases by approximately 1 cm, returning to nonpregnant values by the sixth postpartum month (5,6).

Increments in kidney weight *may* also occur in rodent pregnancy: one group of investigators claim that proximal tubule length and renal dry weight increase, in a manner reminiscent of events during compensatory hypertrophy after uninephrectomy (7). Davison and Lindheimer (8), however, failed to detect biochemical evidence of new kidney growth in pregnant rats. They, as others (9), measured similar renal dry weights in gravid and nonpregnant rats, concluding that the increased total weight was due entirely to the increment in water content. In essence, the changes in kidney weight and length during either human or animal gestation appear to be a consequence of the increase in renal extracellular volume that accompanies normal pregnancy (see later discussion).

Information on the microscopic anatomy of kidneys during human or animal gestations was previously limited to the light microscopic appearance in renal biopsies, usually obtained near term during cesarean delivery, described as similar to that in nonpregnant subjects. However, data from 27 autopsy cases (4) and more recently from 12 third-trimester biopsies (10) suggest that glomerular diameter is substantially greater than that measured in nonpregnant subjects. Of historical interest, the autopsies described by Sheehan and Lynch (4) were performed within 2 hours of death, between 1935 and 1946, at the Glasgow Royal Maternity Hospital by the celebrated pathologist H.L. Sheehan. We recommend his monograph (4), which focuses on the histology of the kidneys, liver, and brains of women dying with preeclampsia or eclampsia, for its methodical detail as well as for the approximately 1,700 references, virtually all which are not available in current PubMed computer searches. The authors also counted cells within the glomerulus, concluding that increments in glomerular size were due to cellular hypertrophy rather than hyperplasia.

Collecting System

The most prominent alterations in the urinary tract are in the collecting system, calices, renal pelves, and ureters, all dilated, accompanied by hypertrophy of ureteral smooth muscle and hyperplasia of its connective tissue (2,3,11–15) (Fig. 74-1). The dilation, often more prominent on the right, may be observed in the initial trimester and is present in more than 90% of gravidas at term (2,3,12,14). "Normal" limits of caliceal dilation have always been difficult to define; the reader is referred to published norms by Faúndes et al. (16), which are helpful but require confirmation.

Views as to why the urinary tract dilates differ, some pointing to hormonal causes and others to obstructive origins (2,3,15). Arguments favoring the former come from animal studies and include observations that in humans, the ureters may dilate before the uterus has enlarged sufficiently to become an obstructive factor, and that prolonged ureteral catheterization fails to reverse the dilation (2,3). The best evidence for the obstructive theory comes from studies in which intraureteral pressure was monitored in third-trimester gravidas subjected to several postural maneuvers (17). Pressure, which was greatest when the gravidas were supine or standing, decreased markedly when they assumed a lateral decubitus or knee-to-chest position or immediately after cesarian delivery of the fetus, implying that the gravid uterus can obstruct the ureters. In addition, the increased pressure was present only above the pelvic brim, which is consistent with an elegant study performed by Dure-Smith (11), who combined *in vivo* and postmortem observations, observing that dilation of the collecting system terminates at the level of the true bony pelvic brim. This is where the ureters and the iliac arteries cross (Fig. 74-1B) and where the weight of the enlarged uterus produces a pyelographic filling defect termed the "iliac sign" (Fig. 74-1A). One should note, however, that failure to observe dilation below the pelvic brim is not conclusive evidence of obstruction. This is because Waldeyer's sheath, the connective tissue that envelops the ureters as they enter the true pelvis, hypertrophies during pregnancy, which could prevent hormonal dilation below the pelvic brim.

Finally, there is another theory that ascribes ureteral obstruction to dilation of the ovarian and uterine veins

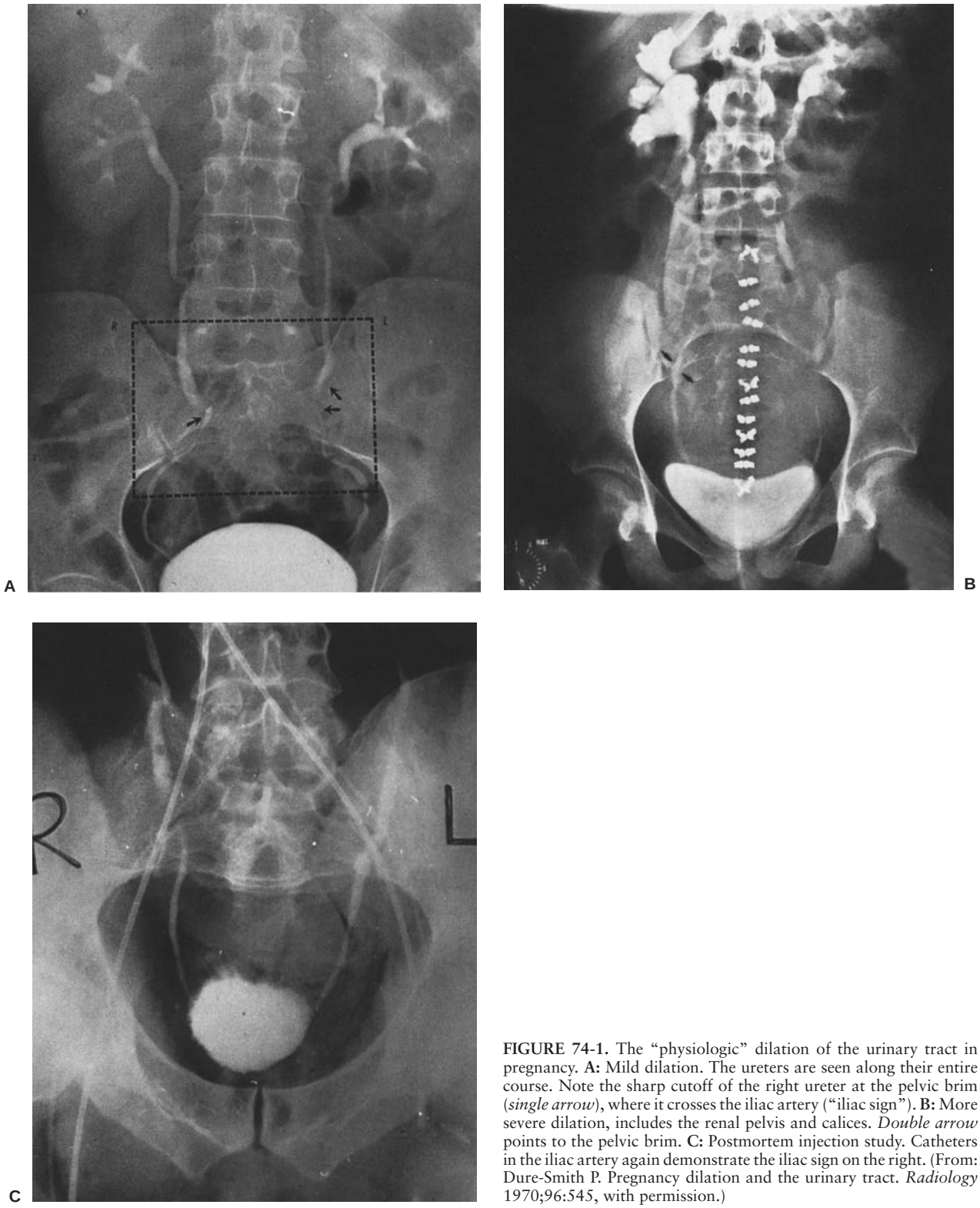

FIGURE 74-1. The "physiologic" dilation of the urinary tract in pregnancy. **A:** Mild dilation. The ureters are seen along their entire course. Note the sharp cutoff of the right ureter at the pelvic brim (*single arrow*), where it crosses the iliac artery ("iliac sign"). **B:** More severe dilation, includes the renal pelvis and calices. *Double arrow* points to the pelvic brim. **C:** Postmortem injection study. Catheters in the iliac artery again demonstrate the iliac sign on the right. (From: Dure-Smith P. Pregnancy dilation and the urinary tract. *Radiology* 1970;96:545, with permission.)

(particularly on the right) during pregnancy, and of interest an "ovarian vein syndrome" characterized by ureteral colic due to the obstruction of the ureter by an enlarged ovarian vein has been described in patients ingesting oral contraceptives (2,18). In a sense, this postulate combines the hormonal and obstructive theories, but we find the concept of venous dilation as a cause of ureteral obstruction unconvincing.

Clinical Relevance

The anatomic changes during pregnancy have considerable clinical significance (Table 74-1). Acceptable norms of kidney size should be increased by 1 cm, and, therefore, postpartum reductions in renal length from values estimated during pregnancy should not be attributed to pathologic decrements in

TABLE 74-1

RENAL CHANGES IN NORMAL PREGNANCY

Alteration	Manifestation	Clinical relevance
Increased renal size	Renal length ≈1 cm greater on radiographs	Postpartum decreases in size should not be mistaken for parenchymal loss
Dilation of pelves, calices, and ureters	Resembles hydronephrosis on ultrasound or IVP (more marked on right)	Not to be mistaken for obstructive uropathy; retained urine leads to collection errors; upper urinary tract infections are more virulent; may be responsible for "over-distention syndrome"; elective pyelography should be deferred to ≥12 wk postpartum
Increased renal hemodynamics	Glomerular filtration rate and renal plasma flow increase 35%–50%	Serum creatinine and blood urea nitrogen values decrease; >0.8 mg/dL (>72 μmol/L) creatinine already suspect; protein, amino acid, and glucose excretion all increase
Changes in acid–base metabolism	Renal bicarbonate threshold decreases; progesterone stimulates respiratory center	Serum bicarbonate and P_{CO_2} are 4–5 mmol/L and 10 mm Hg lower, respectively; a P_{CO_2} of 40 mm Hg already represents substantial CO_2 retention
Renal water handling	Osmoregulation altered (osmotic thresholds for AVP release and thirst decrease; hormonal disposal rates increase)	Serum osmolality decreases ≈10 mOsmol/L (serum Na ≈5 mEq/L); increased metabolism of AVP may cause transient diabetes insipidus in pregnancy

IVP, intravenous pyelography; *AVP*, vasopressin; P_{CO_2}, carbon dioxide tension.
(Reprinted From: Lindheimer MD, Grünfeld JP, Davison JM. Renal disorders. In: Barron WM, Lindheimer MD, eds. *Medical Disorders During Pregnancy.* 3rd ed. St. Louis: Mosby–Year Book; 2000:39, with permission.)

renal mass. The dilated urinary tract often contains substantial volumes of urine, which may lead to errors in timed urine volumes, but these can be avoided if the patient is hydrated and assumes a lateral decubitus position 45 to 60 minutes before starting and again before completing the collection. These maneuvers minimize dead space errors by producing a diuresis and ensuring that any residual urine within the urinary tract or bladder is dilute and of recent origin.

Factors that may be responsible for these anatomic changes have also been linked to several pathologic problems that arise in pregnancy. There is an "overdistention" syndrome (Fig. 74-2) characterized by abdominal pain, marked hydronephrosis, variable increases in serum creatinine levels, and even hypertension (19–24). Conservative therapy, including assuming a knee-to-chest or lateral recumbent position may ameliorate symptoms. However, some patients have required placement of ureteral stents, after which renal function improved and blood pressure normalized; the stents were then removed postpartum without recurrence of the syndrome. One should note that frank urinary obstruction may be difficult to diagnose during pregnancy, in which case the "iliac sign" and the norms suggested by Faúndes et al. (16) may prove helpful. Finally, two rare but serious complications that occur in late pregnancy are acute urinary retention and nontraumatic rupture of the ureters (19,23). Whether these complications represent extreme instances of "physiologic" dilation or reflect other underlying pathology is less clear.

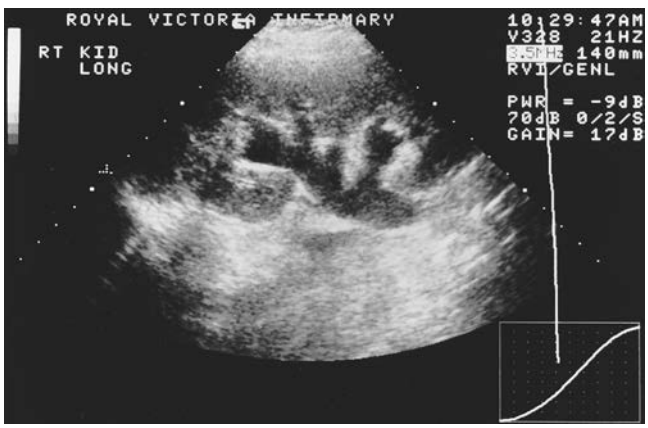

FIGURE 74-2. Overdistention syndrome. This ultrasonogram demonstrates marked dilation of the pelvic caliceal system. The patient complained of right-sided loin pain that worsened despite treatment of presumed pyelonephritis. The symptoms resolved immediately after stent placement and did not recur when the stent was removed 1 month after delivery. (From: J. M. Davison, with permission.)

Function

Renal Hemodynamics

Glomerular filtration rate (GFR) and renal plasma flow (RPF) increase markedly during gestation (Fig. 74-3). Detailed reviews of more than 50 reports can be found elsewhere (2,3,18,25–27), including discussions of important methodologic considerations that explain discrepancies in the older literature. The best studies are those in which inulin clearance (C_{inulin}) and p-aminohippurate clearance (C_{PAH}) were measured serially and simultaneously, preferably with the subjects positioned in lateral recumbency during the test. The importance of the latter is that supine or upright posture may transiently decrease GFR and RPF, especially in late pregnancy, when assuming a supine position results in the enlarged uterus encroaching on the aorta and vena cava and obstructing their flow (2,25,26). These reviews and the more recent literature permit the following synthesis: An increase in GFR and RPF is already present in the luteal phase of the menstrual cycle (26–28), and both are significantly increased by the sixth week of gestation (29).

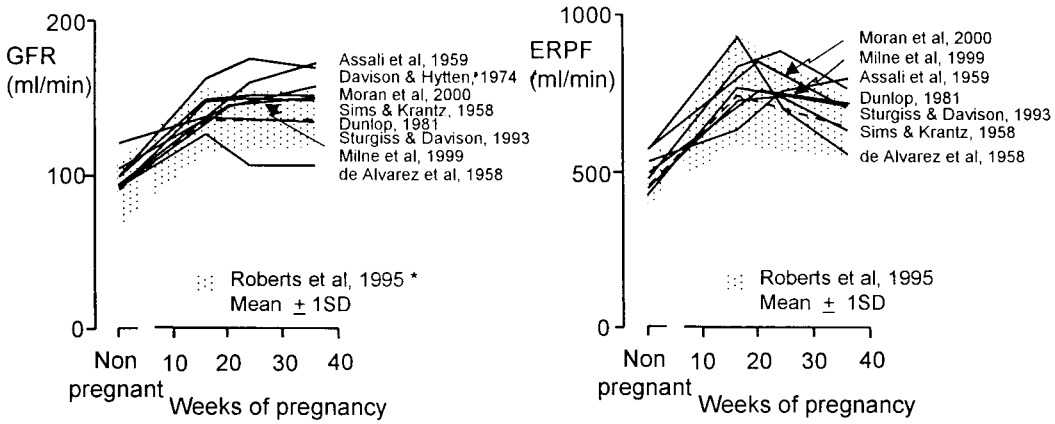

FIGURE 74-3. Serial changes in glomerular filtration rate (**left**) and effective renal plasma flow (**right**) in pregnancy. Changes reported by several workers are superimposed on the mean (*dashed line*) ± 1 standard deviation (*shaded area*) from the study by Roberts et al. (29). (From: J. M. Davison, with permission.)

The increments in GFR peak approximately 50% greater than nonpregnant values during the second trimester, and these high levels are maintained through gestational week 36, after which a decrease of 15% to 20% may occur. There are disputes about whether this decrease actually occurs, but in some reports, 24-hour endogenous creatinine clearance does return to nonpregnant levels at term (18,26,27,30).

The increase in RPF during the first two trimesters (50% to 80%) surpasses that of GFR, so in many reports, filtration fraction falls slightly. Near term, however, C_{PAH} may decline about 25%, and filtration fraction returns to that observed in nonpregnant populations, even though RPF is still considerably elevated (3,18,26,30–32).

The mechanisms responsible for the changes in renal hemodynamics during human pregnancy are unclear. There are increases in cardiac output and plasma volume that appear to parallel those of GFR and RPF (29), but vascular resistance and blood pressure fall at the same time. A number of endocrine changes occur, including circulating levels of progesterone, prolactin, and chorionic gonadotropin and salt retaining hormones, that could conceivably augment renal hemodynamics, but none alone appears to have effects as large as those seen in pregnant women. Plasma albumin concentration and oncotic pressure decrease early in pregnancy, and if similar decrements are present within the glomerular capillary, they could contribute to a rise in GFR (18,26,27,33).

Renal hemodynamics also increase during pregnancy in several animal species (2,3,18,26,27,34). Results from micropuncture protocols designed to span the entire gestation of the rat suggest that increases in single nephron glomerular filtration rate (GFR) are due exclusively to increments in glomerular plasma flow (34). Furthermore, the tubuloglomerular feedback mechanism appears to be operating normally in this species but is reset to "sense" the increased nephron filtration rate as normal (35). Gestational vasodilation does not appear to alter the intrinsic renal autoregulatory ability in rats or rabbits (36,37). Renal vasodilation during rat pregnancy involves an even reduction of the afferent and efferent arteriolar resistances so glomerular capillary pressure is kept constant (Fig. 74-4). In such a scenario, hyperfiltration should have no adverse effects on long-term renal morphology and function, a postulate elegantly shown to be true in rats after six repetitive pregnancies (38)

Direct micropuncture studies of the determinants of ultrafiltration are obviously proscribed in humans, but insight into these parameters can be obtained by measurements of the fractional clearance of neutral dextrans ($C_{dextran}/C_{inulin}$). Such data are subjected to mathematical modeling that explores the mechanism of solute transport across heteroporous membranes, and when combined with measured GFR, RPF, and plasma oncotic pressure predict the glomerular ultrafiltration coefficient (K_f) and glomerular membrane porosity. Permselectivity to neutral dextrans has been found to be altered in human pregnancy (Fig. 74-5), and theoretical analysis of these curves with either the "isoporous plus shunt" or lognormal models (32,33,39–41) suggests that hyperfiltration during human gestation is primarily due to increases in RPF with a minor contribution due to decreased oncotic pressure. There is a small increment in K_f, and membrane porosity appears to be altered. There is no evidence of increased glomerular capillary pressure in pregnant women (Fig. 74-6), similar to findings in the pregnant rat. However, one must underscore the theoretical nature of this approach in pregnant women.

Studies in animal models before 1995 involving primarily rodent gestation and designed to gain insight into the factors responsible for the striking changes in renal hemodynamics are detailed and cited elsewhere (26,34,42). Some have observed increased GFR and RPF in pseudopregnant rats, suggesting that the stimulus is maternal in origin (reviewed in 27,34). However, a host of studies designed to mimic the maternal hormonal milieu in pregnancy in both animal models and nonpregnant women have been negative or contradictory. A partial exception is progesterone, the administration of which

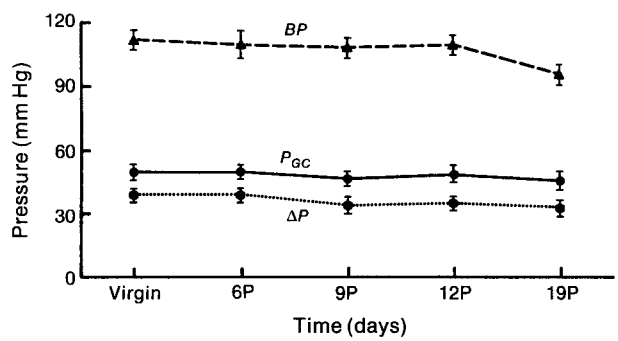

FIGURE 74-4. Glomerular capillary pressure (P_{GC}), transglomerular hydrostatic pressure difference (ΔP), and arterial blood pressure (BP) measured in virgin, and 6-, 9-, 12-, and 19-day pregnant euvolemic Munich-Wistar rats. Data are mean ± 1 standard error; only the BP on gestational day 19 is significantly lower than values in virgin animals. (From: Baylis C. Glomerular filtration and volume regulation in gravid animal models. *Clin Obstet Gynaecol (Bailliére)* 1994;8:235, with permission.)

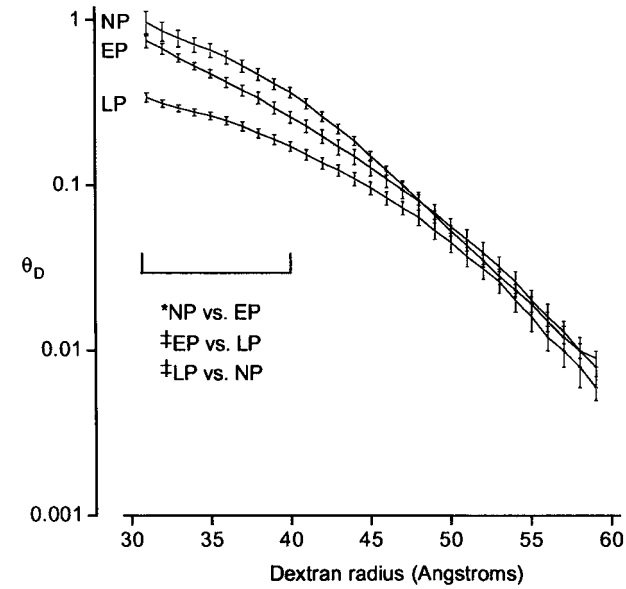

FIGURE 74-5. Serial changes in fractional dextran clearance relative to water clearance (as C_{inulin}) (ΘD) in early pregnancy (*EP*, 16 weeks gestation), late pregnancy (*LP*, 36 weeks gestation), and nonpregnant (*NP*, 16 weeks postpartum) women ($N = 11$). (*$p < 0.02$; $p < 0.0001$.) (From: Roberts M, Lindheimer MD, Davison JM. Altered glomerular permselectivity to neutral dextrans and heteroporous membrane modeling in human pregnancy. *Am J Physiol* 1996;270:F338, with permission.)

to both women and animals partially reproduces the renal functional changes of pregnancy, but in a smaller scale (27,34). Similarly, acute cyclooxygenase inhibition failed to reverse the increased GFR and RPF in pregnant animals. More promising areas are series of sequential observations by Conrad et al., (27,43). These studies were designed according to the knowledge that the preglomerular and postglomerular arterioles, as well as the contractile elements interposed between them (i.e., the mesangium), are sensitive to various vasoactive hormones and autocoids including angiotensin II, endothelins (ETs), atrial natriuretic peptides, and endothelium-derived relaxing factors (EDRFs), as well as to the alterations in their receptors. These hormones, autocoids, or receptors may be altered during pregnancy, thereby affecting hemodynamics.

Briefly, Danielson and Conrad (44) measured GFR and RPF in conscious, chronically instrumented rats at midpregnancy, when the gestation increase in renal hemodynamics peaks in this species (45). Acute inhibition of nitric oxide synthases (NOSs) decreased GFR and RPF and increased renal vascular resistance to a greater extent in gravid than in virgin rats, eliminating the differences in renal hemodynamics between groups.

Consistent with these clearance results are *in vitro* data demonstrating that endothelial removal or NOS inhibition restored the reduced myogenic reactivity of small renal arteries obtained from midterm pregnant rats to that of virgin controls (46). However, their message became more complex as pregnant rats undergoing chronic NOS inhibition (47) still manifested midterm increases in GFR and RPF (although such an "escape" has not been observed by all [48,49]), suggesting that when NO production is suppressed, compensatory mechanisms take over. These appear to be mediated by vasodilatory prostaglandins, because now the gestational rise can be abolished with meclofenamate (although prostaglandin inhibitors alone have no influence on the increased renal hemodynamics of rat gestation [26,27]). Conrad and colleagues (50) further suggested a role for ET and its receptors in the renal adaptation to pregnancy. ET is normally thought of as a potent vasoconstrictor, but using pharmacologic probes and rats genetically deficient in the ET_Breceptor (51,52), the authors suggested that ET plays an important role in maintaining low vascular tone in pregnant rodents (as well as the reduced *in vitro* myogenic reactivity of their small renal arteries) via stimulation of the endothelial ET_B receptor subtype, resulting in tonic stimulation NO release.

The same group has demonstrated that relaxin, a hormone that in rodents and women originates in the corpus luteum and whose production is markedly stimulated by human chorionic gonadotropin (hCG), increases GFR and RPF, and decreases both *in vitro* myogenic activity of small renal arteries and plasma osmolality (P_{osm}) when administered to nongravid rodents (53,54). Moreover, administering neutralizing antibodies or eliminating production of the hormone by ovariectomy (but maintaining the pregnancy with exogenous sex steroids) prevented all of the normal renal and osmoregulatory changes of rat pregnancy (54). Finally, pregnancy (relaxin)-induced increases in vascular gelatinase activity has recently been implicated as the pivotal step that drives the ET/NO vasodilatory pathway (by increasing the availability of ligand for the endothelial ET_B receptor through the conversion of big ET to $ET_{1-32)}$ (51,55).

The previously described observations led to an appealing hypothesis (Fig. 74-7), as we now have a hormone whose levels rise in the luteal phase of the menstrual cycle and even more markedly in gestation and whose actions can simultaneously explain both the enhanced renal hemodynamics and the changes in osmoregulation that occur in human gestation. Relaxin may also initiate other pregnancy adaptations, given that chronic administration to conscious nonpregnant rats increases cardiac output and global arterial compliance, and reduces systemic vascular resistance, simulating other changes of normal human pregnancy (56).

Because GFR and RPF are already markedly increased, the question has arisen whether renal vasodilation is already maximal during pregnancy or whether gravidas possess a "functional reserve" akin to that demonstrated during protein

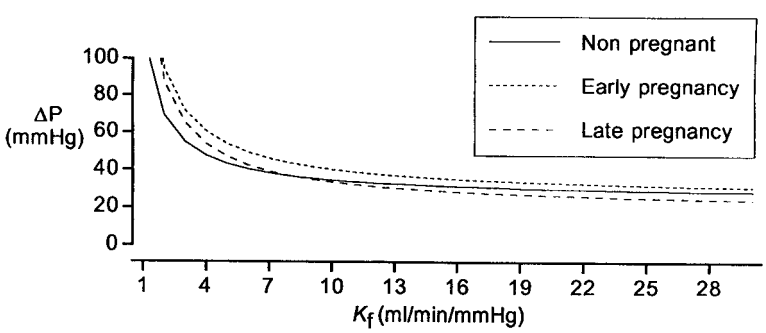

FIGURE 74-6. The theoretical relationship between the ultrafiltration coefficient (K_f) and the transglomerular hydrostatic pressure difference (ΔP) in normal pregnancy. Note that over a large range of K_f chosen to include those values we would expect in humans, there is little difference in the ΔP when results during early gestation, late pregnancy, and the nonpregnant state are compared with one another. (From: Roberts M, Lindheimer MD, Davison JM. Altered glomerular permselectivity to neutral dextrans and heteroporous membrane modeling in human pregnancy. *Am J Physiol* 1996;270:F338, with permission.)

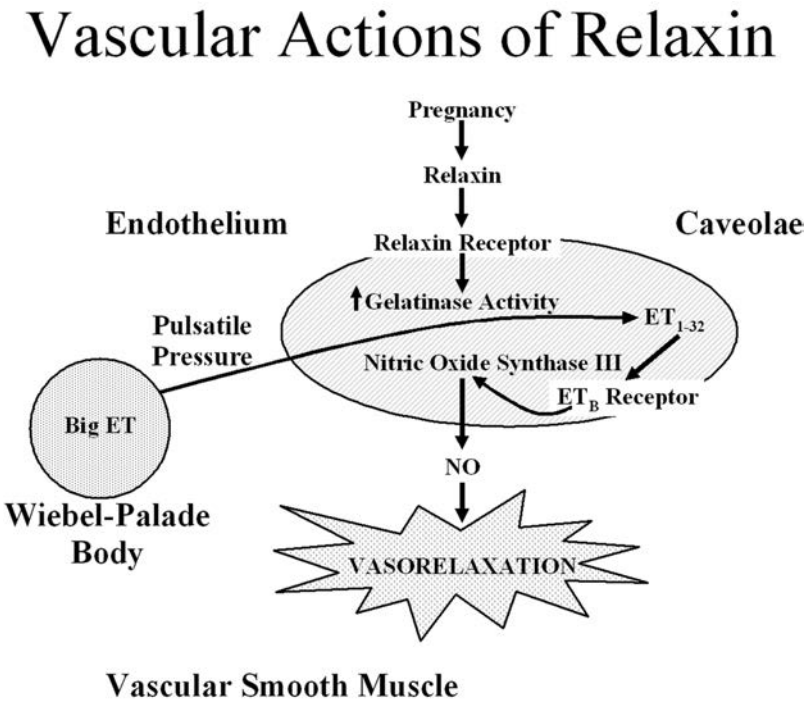

FIGURE 74-7. Working model of cellular mechanisms underlying pregnancy and relaxin-induced renal vasodilation and hyperfiltration and reduced myogenic reactivity of small renal arteries. Big ET is released from Weibel-Palade bodies in response to pulsatile pressure *in vivo* or increases of intraluminal pressure *in vitro*. MMP-2, eNOS and the ET_B receptor are localized in caveolae of endothelial cells. Whether the vascular relaxin receptor resides in the caveolae of the endothelium as depicted here, or in the vascular smooth muscle, and regulates MMP-2 expression through intermediary molecules, have not been determined. See text for further details. (Modified from: Conrad KP, Novak J. Emerging role of relaxin in cardiovascular function. *Am J Physiol* 2004;287:R250 with permission.)

loading or amino acid infusions in nonpregnant populations. Several investigators have demonstrated further increases in renal hemodynamics during the infusion of amino acids into pregnant rats (57) and women (31,39,40). Effects of oral protein loading, however, have been equivocal (58–62).

In summary, evidence from both pregnant rats and pregnant women suggests that hyperfiltration in pregnancy is primarily due to increased RPF, the latter being due to decreases in renal vascular resistance. There has been considerable progress toward understanding factors responsible for the increased renal hemodynamics during gestation, much made since the last edition of this text. This includes evidence implicating relaxin, vascular gelatinase activity, ET, and NO, primarily in the gravid rat model, although evidence is accruing that relaxin has a role in the changes in renal hemodynamics of human pregnancy, as well (63). Whatever the putative mechanism, it must explain why the pregnancy-induced rise in GFR is so powerful (being far greater than that evoked by protein loading or amino acid infusions in nonpregnant individuals), and why it occurs in subjects with kidneys already hypertrophied after uninephrectomy as well as in transplant recipients (64,65). Finally, data from micropuncture in rats and modeling of fractional dextran clearances in pregnant humans suggest that despite hyperfiltration, glomerular capillary pressure is unaltered, indicating that the sustained gestational increments in GFR should not be harmful to the kidney.

Significance of the Increased GFR and RPF

Because there is, at most, a small increase in the production and excretion of creatinine during normal pregnancy (2,66), the large increments in creatinine clearance (C_{cr}) lead to reduction in its plasma level. The concentration of serum urea also decreases, partly because of its augmented clearance, but also because there is enhanced protein synthesis. Therefore, levels of serum creatinine and urea nitrogen that average 0.7 mg/dL and 12 mg/dL (60 μmol/L and 4.3 mmol/L), respectively, in the nonpregnant state decrease to means of 0.5 mg/dL and 9 mg/dL (44 μmol/L and 3.2 mmol/L) during gestation (2,30). *Thus, values considered normal in nonpregnant women may reflect abnormal function in pregnancy.* For example, concentrations of serum creatinine and urea nitrogen exceeding 0.8 mg/dL and 13 mg/dL (80 μmol/L and 5 mmol/L), respectively, should alert the clinician to further evaluate renal function. Finally, the filtered loads of many solutes increase, explaining in part why glucosuria, aminoaciduria, and enhanced urinary excretion of water-soluble vitamins often occur during normal pregnancy (2,30). These increments in urinary nutrients may enhance the susceptibility of gravidas to symptomatic urinary tract infections.

Tubular Function

Glucose and Amino Acids. Glucose excretion normally increases during pregnancy, exceeding the upper limits of normal in nonpregnant subjects (about 100 mg per 24 hours) 85% of the time and often reaching 1 g or more per day (2,30,42, 67–69); urinary glucose then returns to nonpregnant levels during the first postpartum week (69). Gestational glucosuria is due mainly to increments in filtered load, but the tubular handling of glucose is also altered in pregnancy. Welsh and Sims (69) suggested that women manifesting overt glucosuria in pregnancy had lower maximal tubular reabsorption capacities (T_m) than those who did not, whereas, Davison and Hytten (70) demonstrated that both the T_m and fractional reabsorption of glucose (T/F glucose) decrease in all subjects during pregnancy. Of interest, those women most severely glucosuric during gestation had the lowest T/F glucose rates both when pregnant and postpartum, but were not glucosuric when not pregnant, because filtered glucose had decreased substantially. Therefore, even though the splay of the titration curve increases or the apparent T_m decreases, the striking increment in GFR remains the major cause of glucosuria in pregnancy. Glucosuria also occurs during pregnancy in the rat, where limited micropuncture studies reveal that proximal tubular reabsorption of glucose is enhanced commensurate with the increase in filtered load, and therefore the increment in urinary excretion reflects decreased distal reabsorption, the authors further implying that this may reflect a change in epithelial permeability resulting from an increased backleak in the loop of Henle (71).

The excretion of many amino acids also increases in gestation and may reach 2 g per 24 hours (2,30,42,72). The few studies designed to determine its mechanism were inconclusive,

but it appears that alterations in both GFR and tubular reabsorption would be needed to account for the magnitude of the excretion rates of some of the amino acids (e.g., glycine and histidine) (2,30,42).

Uric Acid. Urate excretion (and presumably production) increases in pregnancy, and its clearance, which is typically 6 to 12 mL per minute in nonpregnant populations, rises to 12 to 20 mL per minute during gestation (2,26,42,73,74). The absolute quantities of urate filtered and reabsorbed also increase, but there are conflicting data on whether the urate fractional clearance (C_{urate}/C_{inulin}) rises as well (25,26,42,74,75). Whatever the case might be, the net result is a fall in serum urate levels of at least 25%, so values range from 2.5 to 5.0 mg/dL (149 to 298 μmol/L), with 5.0 to 5.5 mg/dL (298 to 327 μmol/L) being the upper limit of normal at most centers (2,26,42,76). There may be racial variations of the normal range, as well as diurnal variations in urate levels during pregnancy, with the highest values noted in the morning and lowest in the evening (77–79). Circulating levels tend to be higher in the presence of multiple fetuses (79,80).

The changes in clearance and plasma levels are greatest in early pregnancy, whereas C_{urate} may decline and plasma levels rise near term (74–76,80,81). In some women, this may represent subclinical preeclampsia, because reductions in C_{urate}/C_{inulin} or increments in P_{urate} may precede overt manifestation of this disorder sometimes by many weeks (2,25,26,42,82,83), but nonrenal causes for the changes in urate levels near term have also been suggested (84). Finally, data are emerging suggesting that women with the highest plasma urate levels within the normal range in early gestation are an at-risk population for preeclampsia and other cardiovascular complications (85).

Potassium Secretion. There is uncertainty about the fate of body potassium stores during pregnancy. Some suggest that total body stores decrease early in gestation and then rise, and by gestational week 36 are ~100 mEq greater than prepregnancy levels (86). The more traditional view, however, is that cumulative potassium retention is approximately 350 mEq, most of which is stored in the fetal space and organs of reproduction (87). That potassium retention occurs is surprising, considering that gravidas, who have high circulating levels of aldosterone and other potent mineralocorticoids, ingest diets of normal sodium content, most of which is excreted into the urine each day. Furthermore, gravidas manifest bicarbonaturia at lower plasma levels than do nonpregnant women (see later discussion), which should also favor renal potassium loss. In contrast to nonpregnant subjects, gravidas have been shown to be quite resistant to the kaliuretic action of exogenous mineralocorticoids, even those women consuming diets high in sodium content (88) (Fig. 74-8). This resistance to mineralocorticoids has been ascribed to the fact that gravidas also manifest high

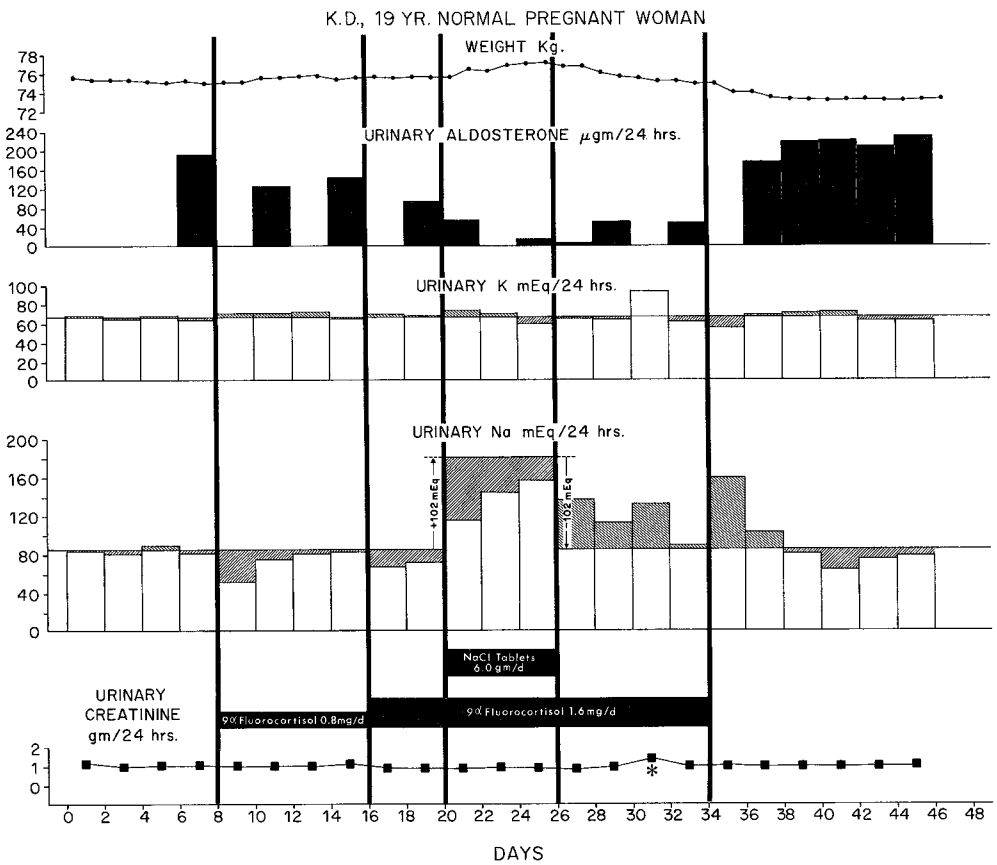

FIGURE 74-8. Effect of a mineralocorticoid (Florinef acetate) in a normal pregnant woman. Ingestion of the potent mineralocorticoid led to sodium retention, but no kaliuresis was observed even when dietary sodium was substantially increased (days 20 to 26). The high baseline aldosterone excretion is normal for pregnancy. The different treatment periods (*heavy lines*) are shown, as are the arithmetic means of urinary sodium and potassium excretions (*horizontal lines*) during the pretreatment (control) days. A small kaliuresis was observed on day 31 only, but there was also increased creatinine excretion that day, suggesting a collection or measurement error. (From: Ehrlich EN, Lindheimer MD. Effect of administered mineralocorticoids or ACTH in pregnant women. Attenuation of the kaliuretic influence of mineralocorticoids during pregnancy. *J Clin Invest* 1972;51:1301, with permission.)

circulating levels of progesterone, a view supported by studies in both humans (42,88) and animals (42,89), but not accepted by all (90).

Resistance to the kaliuretic effects of mineralocorticoids may be advantageous and problematic during gestation. The hypokalemia due to excessive renal loss of potassium in both primary aldosteronism and Bartter syndrome may remit during gestation (87,91). Such observations, however, are variable, because instances of these diseases with substantial potassium depletion do occur in gravid women. On the other hand, patients with diseases that compromise potassium secretion (e.g., sickle cell anemia) or women receiving potassium-sparing diuretics may become hyperkalemic when GFR is seemingly normal or only modestly compromised (87).

Finally, there is a very rare disorder, characterized by genetic alteration of the renal mineralocorticoid receptor in a manner in which hormones that normally antagonize the salt-retaining actions of aldosterone now act as agonists (92). When female heterozygotes from these families conceive, their 100-fold increase in progesterone results in marked salt retention, and hypertension in early gestation accompanied by renal potassium wasting, and hypokalemia, although plasma aldosterone levels remain undetectable.

Proton Excretion. There are alterations in acid–base metabolism in pregnancy that are both respiratory and renal in origin. Blood levels of hydrogen ion decrease by 2 to 4 mmol/L early in gestation, a decrement sustained throughout pregnancy that results in an arterial (or arterialized capillary) blood pH level of 7.42 to 7.44, significantly higher than the 7.38 to 7.40 measured in nonpregnant subjects (18,93). The acid–base and osmolar changes in pregnancy, combined with a small decrement in $P_{albumin}$ levels, also reduce the anion gap and "strong anion difference" modestly (94,95). Most of these changes appear to be due to the effects of increased progesterone on the respiratory center, which cause modest hyperventilation accompanied by a fall of P_{CO_2} from a mean of 39 mm Hg in the nonpregnant state to 30 mm Hg during gestation (18,93). These P_{CO_2} changes are accompanied by a decline in plasma bicarbonate of approximately 4 mmol/L, so normal values during pregnancy range from 18 to 22 mmol/L (18,93).

Studies of renal bicarbonate handling have revealed the following: raising plasma bicarbonate levels to as high as 31 mmol/L results in concomitant increments in tubular reabsorption, and, despite the presence of both hypocapnia and increased intravascular volume, there is no evidence of an increase in either the apparent tubular maximum or the "splay" of the titration curve depicting HCO_3^- reabsorption as millimolar per liter of GFR (Fig. 74-9A) (93). On the other hand, there is a small but persistent bicarbonate "leak" at lower plasma bicarbonate levels during pregnancy (even at levels of 15 to 16 mmol/L) compared with those of the same patient postpartum (Fig. 74-9B).

Urinary acidification has also been evaluated in pregnant women, and it appears that bicarbonate regeneration is comparable to or better than that in similarly tested nonpregnant women (18,93). During acute acidification induced by ammonium chloride ingestion, urinary pH level decreases and titratable acid and NH_4 excretion increase when blood pH, although reduced from 7.44 to 7.40, is still in the normal nonpregnant range. In essence, substantial bicarbonate regeneration occurs when blood pH would still be considered alkaline in nongravid women. Finally, distal proton secretory capacity appears unaltered in gestation (93), and the decrease of blood pH during exercise is similar in pregnant and nonpregnant women (96).

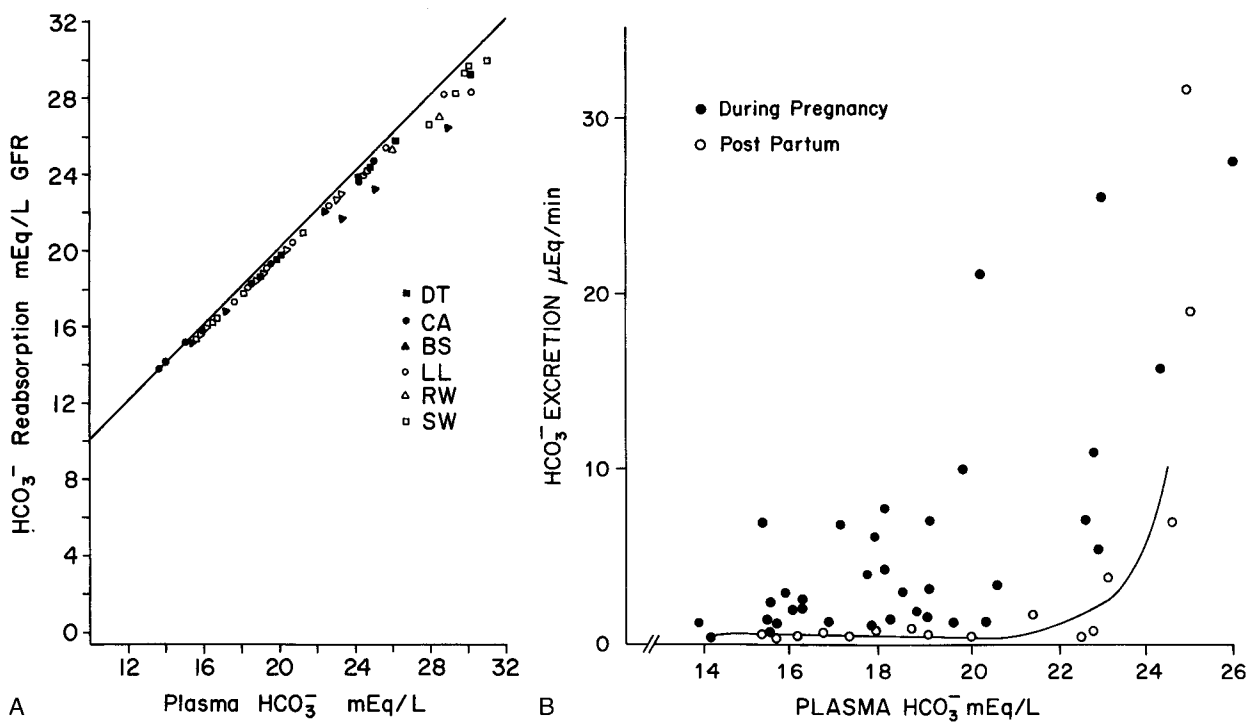

FIGURE 74-9. Bicarbonate titration curves (**A**) measured in six third-trimester women during the slow infusion of a 5% $NaHCO_3$ solution. The infusion inadvertently ran faster for 30 minutes in subject *BS*, the only volunteer to display a wide splay. Bicarbonate excretion (**B**) as a function of plasma bicarbonate levels during pregnancy (*filled circles*) and in two of the subjects studied postpartum (*open circles*). The bicarbonate threshold is lower in pregnant women (mean, 18 mmol/L), but even when surpassed during the infusion, the bicarbonaturia is still trivial. (From: Lim VS, Katz AI, Lindheimer MD. Acid–base regulation in pregnancy. *Am J Physiol* 1976;231:1764, with permission.)

Renal Water Handling and Osmoregulation. Pregnant women experience a decline in body tonicity, as reflected in a decrement in P_{osm}. The decrease starts during the luteal phase of the missed menstrual cycle (28), reaches a nadir of ~10 mOsmol/kg below nonpregnant levels early in pregnancy, after which a new steady state is maintained until term (97–99). This decrement is a true decrease in effective P_{osm}, because most of it is due to a decline in the concentration of sodium and its attendant anion. Analysis of data from both human and rodent gestation led to the following formulation of how these changes occur (98–104). First, the osmotic thresholds for thirst and antidiuretic hormone release decrease in parallel (Fig. 74-10). Lowering the threshold to drink stimulates increased water intake and dilution of body fluids. Because inhibition of arginine vasopressin (AVP) release also occurs at a lower level of body tonicity, the hormone continues to circulate and ingested water is retained. The P_{osm} thus declines until it is below the osmotic thirst threshold and a new steady state with little change in water turnover is established.

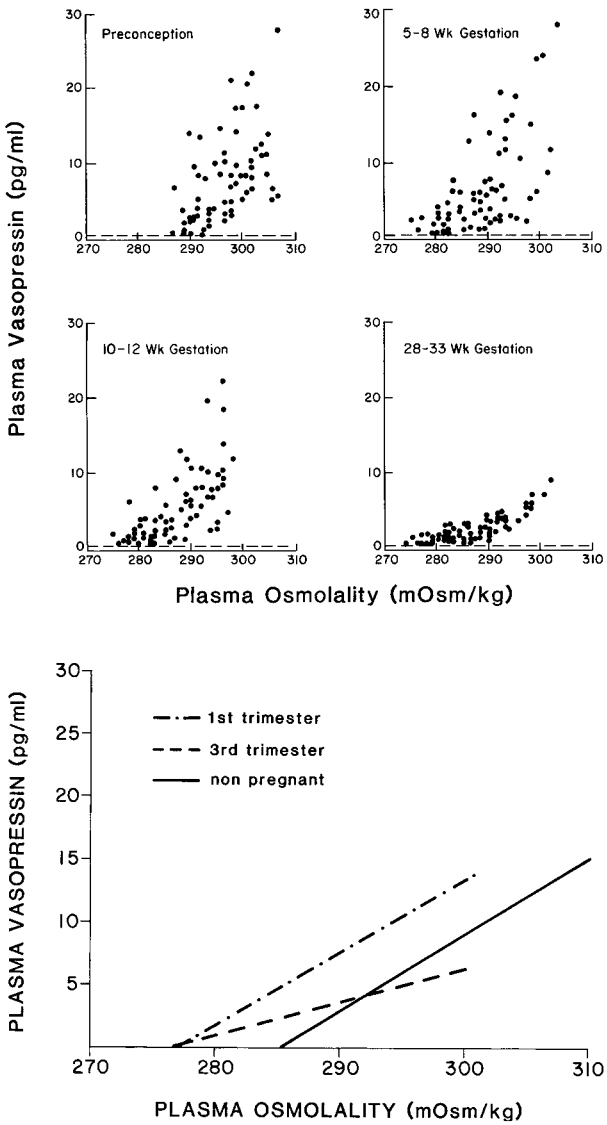

FIGURE 74-10. Relationship of plasma arginine vasopressin (AVP) to plasma osmolality in eight healthy women who underwent hypertonic saline infusions starting before conception, then during gestation, and finally postpartum. Each point represents a value from a single blood sample; data from the postpartum test, similar to those taken preconception, are not shown. The cartoon (**lower panel**) depicts the highly significant regression lines from tests performed during pregnancy and in the nonpregnant state, which demonstrate a marked decrease in the abscissal intercept (the apparent threshold for AVP release) during gestation. Note the substantial decrease in the slope during the third trimester, which is discussed further in the text. (From: Davison JM, et al. Serial evaluation of vasopressin release and thirst in human pregnancy: role for chorionic gonadotropin on the osmoregulatory changes of gestation. *J Clin Invest* 1988;81:798, with permission.)

Pregnancy also affects the disposal of AVP, its metabolic clearance rate (MCR) rising fourfold between early and midgestation (105). This increment seems to parallel both the increase in trophoblastic mass and the striking concomitant rise in the levels of circulating cystine aminopeptidase (vasopressinase). This relation has led to suggestions that vasopressinase is responsible for the rise in the MCR of vasopressin, which is also supported by studies demonstrating that the disposal rate of l-desamino-8-D-argininevasopressin (dDAVP; desmopressin), an AVP analog resistant to inactivation by vasopressinase, is hardly altered by pregnancy (106) (Fig. 74-11).

Mechanisms responsible for the altered osmoregulation in pregnancy are obscure, although hCG (104,107), the constitutive NOS (108), and relaxin (53,109) have all been implicated in these changes. Relaxin, as noted previously (53,55), is a very interesting candidate because it will explain the effects of hCG on decreasing P_{osm} and osmotic thresholds in premenopausal women (104,107) but not men (104), the osmoregulatory changes in the menstrual cycle (28), and both the increases in renal hemodynamics and alterations in osmoregulation during rodent and human gestation. Of further interest, the decrease in P_{osm} is blunted in women with primary ovarian failure who successfully carry donated ova (63).

Some authors believe that despite measurable increments in absolute intravascular volume, gestation is a state of decreased "effective arterial volume," secondary to arterial vasodilation, and they have suggested that such nonosmotic influences are important effectors of the osmoregulatory changes in pregnancy (110–112). Studies describing serial hemodynamic and humoral alterations in pregnant baboons (113) and increased vascular sensitivity of gravidas to angiotensin-converting enzyme (ACE) inhibition (114) support the concept of a decreased "effective arterial volume" in pregnancy. Most interesting are studies by Ohara et al. (115), who suggest that borderline or undetectable elevations in plasma vasopressin concentrators (P_{AVP}) are present in gravid rats who also manifest upregulation of aquaporin-2 mRNA and its water channel protein in apical membranes of the collecting ducts. Others, based on both rodent and human studies, believe that the volume-sensing AVP-release mechanisms appear to adjust as pregnancy progresses so each new volume status is "sensed" as normal (34,98,99,107,116–118). Also the observations of Ohara et al. (115) appear paradoxical, because any increments in AVP levels, even if undetectable (especially when these increased levels are attributed to decreased "effective arterial volume"), should cause increments in the density of apical water channels and blunt the animals' ability to excrete water loads. However, gravid rats excrete water loads more efficiently than virgin controls (100). It must be acknowledged, however, that the gestation rise in GFR leads to 50% more filtered water.

Significance of the Altered Osmoregulation and AVP Metabolism

The osmoregulatory changes observed in humans do not occur in all species. For example, rats demonstrate decreases in P_{Na} and P_{osm} only at midgestation, whereas P_{osm} is unaltered in pregnant sheep (98,101). The latter species, interestingly, fails to produce a functioning relaxin hormone, the AVP disposal rate is unaltered, and its placenta fails to produce vasopressinase (27,98). Therefore, the clinical relevance of these changes

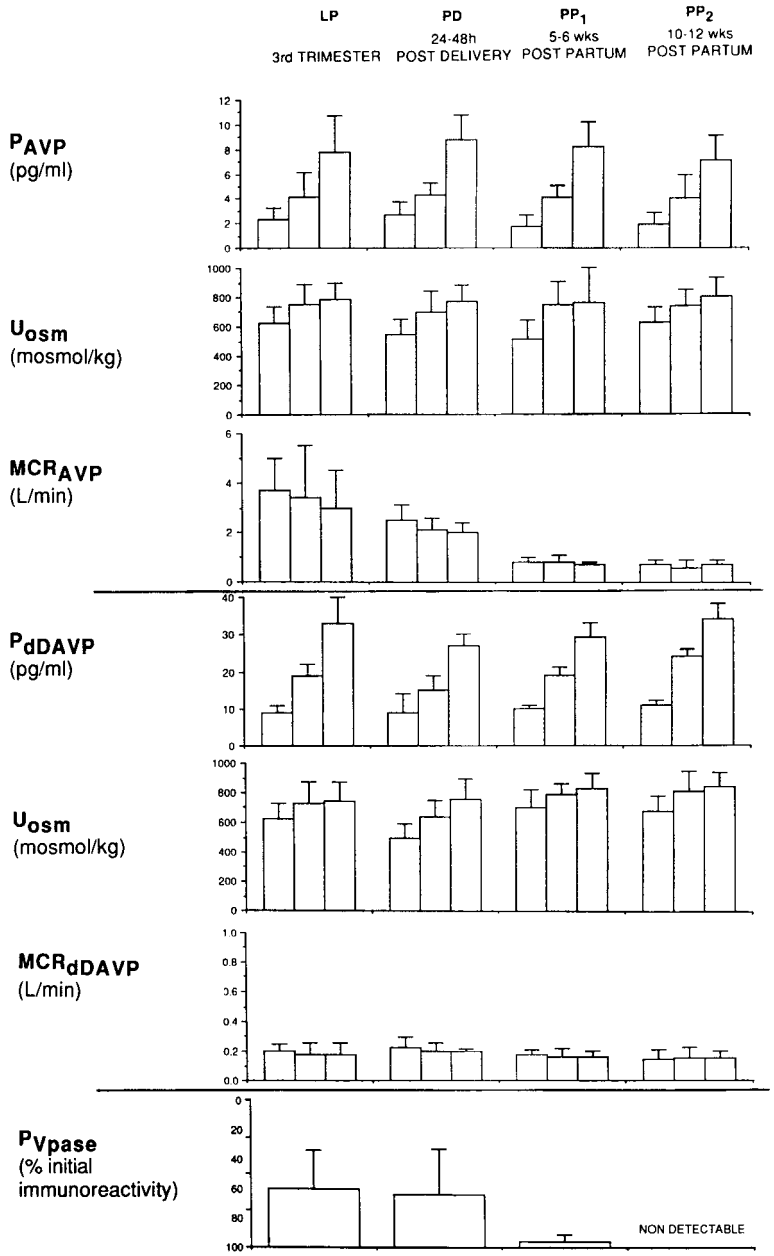

FIGURE 74-11. Plasma levels and metabolic clearance rates (MCRs) of arginine vasopressin and l-desamino-8-D-arginine-vasopressin measured in the third trimester, 24 to 48 hours after delivery, as well as 5-to-6 and 10-to-12 weeks postpartum in six women. P_{vpase}, plasma vasopressinase. Above each bar is one standard deviation. (From: Davison JM, et al. Metabolic clearance of vasopressin and an analogue resistant to vasopressinase in human pregnancy. *Am J Physiol* 1993;264:F348, with permission.)

is still unclear. One answer may relate to the physiologic increase in extracellular volumes that accompanies normal gestation. In this respect, some believe that the increase in intravascular volume optimizes fetal development, and hypoosmolality would facilitate such expansion because the ingestion and retention of less solute is required per liter of extracellular water retained. In this case, the downward resetting of the osmotic threshold for AVP release would be advantageous, especially when sodium is scarce. More striking, however, are the clinical implications of the fourfold rise in the MCR of vasopressin, first with regard to the management of patients with vasopressin-deficient states and second as an explanation of a syndrome termed *transient diabetes insipidus (DI) of pregnancy.*

Disorders of Water Metabolism in Pregnancy

Diabetes Insipidus in Pregnancy. The causes of DI antedating pregnancy are discussed elsewhere (119–122; Chapter 87). Patients are rarely treated with AVP (e.g., pitressin) anymore, but if they are, the rise in hormonal disposal rates would obviously require an increase in dosage. Currently, however, women with known central DI are managed with desmopressin (dDAVP), the MCR of which, as noted, is not altered by pregnancy (98,99). Still, there are reports of increased dDAVP requirements in gestation, in which symptoms associated with the altered threshold for thirst seem to have been misinterpreted as dDAVP "escape." In most instances, however, the preconception replacement dose has proved sufficient throughout gestation (98,119–121). Also, there is minimal, if any, transfer of dDAVP into breast milk (122).

Women who develop pituitary necrosis due to blood loss at delivery (Sheehan's syndrome) may manifest transient polyuria in the immediate puerperium. They also develop partial central DI, which is usually asymptomatic and detected only by special testing such as the plasma AVP (PAVP) response during dehydration and/or hypertonic saline loading (123–125).

Nephrogenic DI in pregnancy is rare, although carriers of the X-linked variety may become polyuric in pregnancy (D. Bichet and G. Robertson, *personal communications*). Diuretics—the mainstay of therapy in this disorder—work

by depleting intravascular volume, which results in increased proximal reabsorption of sodium and the delivery of less fluid to the diluting site. The maintenance of hypovolemia may not be optimal for pregnancy, but when the polyuria is massive, such agents may have to be used.

Transient Diabetes Insipidus of Pregnancy. On occasion a gravida develops polyuria and polydipsia late in gestation, which disappear postpartum. This syndrome, described in the older literature, was characterized further in 1984, when Barron et al. (126) studied three women whose polyuria was resistant to the administration of synthetic vasopressin during the puerperium. Their disease, however, remitted soon afterward, and they regained the ability to concentrate their urine normally with overnight dehydration or after the administration of pitressin. Plasma AVP, measured in one of the women, was 7.3 pg/mL, a level that should have evoked maximum urine concentration. The authors, however, labeled the disorder "transient vasopressin-resistant DI of pregnancy," and avoided the term "nephrogenic," aware that the AVP measured in the patient's plasma might have been but fragments recognized by the antibody used in the radioimmunoassay but devoid of biologic activity. In other words, something was destroying the hormone. This was the first indication that in certain women with transient DI of pregnancy, the disorder might reflect massive *in vivo* destruction of AVP by extremely elevated levels or exaggerated effects of vasopressinase. Figure 74-12 depicts the signal study by Dürr et al. (127) of a patient who became massively polyuric and hypernatremic and who failed to concentrate her urine after pharmacologic doses of AVP, but whose U_{osmol} increased to 800 mOsmol/kg when dDAVP was administered. The authors used two methods to demonstrate that the P_{AVP}, surprisingly only 240 pg/mL after the highest dose of AVP (25 μg) was not intact hormone but represented inactive fragments. In essence, this large dose of pitressin had been inactivated in less than 25 minutes. Dürr et al. also noted high levels of circulating vasopressinase in this patient and suggested that this is the cause of the disorder; however, alterations in the enzyme's kinetics are another possibility.

Many other examples of this transient disorder have now been cited (98,99,120–122). Many of these gravidas also have preeclampsia or display coagulation and liver function abnormalities (128). The liver is a major site of vasopressinase inactivation.

There is a second form of transient DI of pregnancy in which some women with partial central DI, asymptomatic when nonpregnant, manifest polyuria after midpregnancy coincident with the normal fourfold increase in the MCR of AVP. This disorder also remits postpartum (129–132). In addition, patients with transient DI resistant to both AVP and dDAVP have been described, the authors noting that both P_{AVP} and urinary prostaglandin E_2 were extremely elevated (133,134).

Finally, although these unusual syndromes are quite easy to diagnose (histories of thirst, and polyuria, plus a urine hypotonic to a relatively hypertonic plasma), and treat (DDAVP), they can be life-threatening if unrecognized, especially when patients are water-restricted or placed under anesthesia in the delivery suite. Unfortunately, such cases continue to be reported (135–137) in which the disorder was recognized only when neurologic signs and symptoms of extreme hypernatremia occur. This can be avoided with thorough history taking when patients present for labor, and recalling that a sodium of $\geq$140 mEq/L, is already increased in most gravidas.

Transient Inappropriate Antidiuretic Hormone Secretion in Pregnancy. Gestational hyponatremia secondary to "inappropriate" secretion of AVP has been described. This disorder is extremely rare and may be associated with preeclampsia, both when the latter is characterized by marked proteinuria and hypoalbuminemia and when such evidence of marked intravascular volume depletion is less evident (138–140).

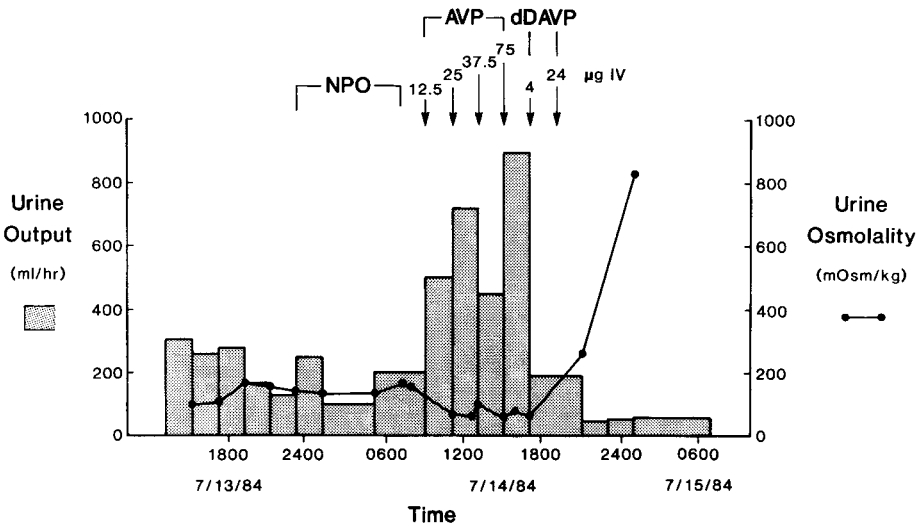

FIGURE 74-12. Study during postpartum day 6 in a patient who developed diabetes insipidus late in gestation and remained polyuric in the puerperium. She was unable to concentrate her urine during fluid restriction (nothing per os), and plasma arginine vasopressin (AVP) was undetectable despite a P_{Na} level of 153 mEq/L. Her urine remained dilute despite the stepwise parenteral administration of large doses of AVP, but urinary osmolality increased to 800 mOsmol/kg after receiving l-desamino-8-D-arginine-vasopressin. Plasma AVP measured after the last dose of AVP was 240 ng/L (when values in the thousands were anticipated), but the material identified by radioimmunoassay was not bioactive and probably represented degraded fragments of AVP. Plasma vasopressinase measured on day 12 postpartum was tenfold higher than that in women at term. (From: Dürr JA, et al. Diabetes insipidus in pregnancy associated with abnormally high circulating vasopressinase. *N Engl J Med* 1987;316:1070, with permission.)

VOLUME REGULATION

There are alterations in volume homeostasis and in the control of blood pressure in pregnancy, both of which are incompletely understood. These topics, detailed in Chapter 53 and elsewhere (42,141,142), are summarized briefly here, with a primary focus on human studies.

Weight Gain

Healthy women gain approximately 12.5 kg in their first pregnancy, and 1 kg less in subsequent gestations. However, these means have large standard deviations, and gravidas can gain twice such amounts or hardly any weight, at all, and still have normal pregnancies (2,42). Analysis of the distribution of this added weight reveals that not all the increase can be accounted for by the products of conception, by the tissues directly concerned with reproduction, or by the gain in total body water, but that 3 to 6 kg of the increment is maternal fat stores (42,86,143). Furthermore, these averages were compiled from subjects with no or only mild ankle edema during pregnancy, whereas about 15% of healthy gravidas develop generalized edema, characterized by swelling of the fingers and face as well. Weight gain in this latter group averages 14.5 Kg, of which about 5 Kg represents fluid accumulation within the extracellular space (42).

Fluid Volumes

Most of the weight added in pregnancy is in the form of fluid. The gain in total body water, measured by deuterium, by the stable isotope of oxygen (^{18}O), or by bioelectric impedance is between 6 and 9 L (2,18,42,86,141–144). Estimates of the gain in extracellular volume range from 4 to 7 L, indicating that intracellular water also increases (0.5 to 1.0 L) during gestation (18,42,142). This area, however, requires further investigation, considering the wide variations reported by most authors and the incomplete information on validation of their methods for use in pregnant women. Intravascular volume also increases in pregnancy, due to an estimated 40% increment in plasma volume and a small increase in red blood cell mass (42,96,141,142,145). Plasma volume increases most rapidly in the midtrimester, and the accumulation of interstitial volume is greatest in late pregnancy.

Renal Sodium Handling

There is a gradual cumulative retention of about 900 mEq of sodium during pregnancy, distributed between the products of conception and the increased maternal extracellular spaces described previously (2,18,42,141,142). However, late in pregnancy, when the accumulation is greatest, renal sodium retention is but a few milliequivalents per day and is difficult to detect in conventional balance studies. The reasons for these changes are obscure, with reports in the literature focusing on the hormonal changes in pregnancy that may affect renal salt handling or the effects of acute saline loading in pregnant women.

The concentration of several antinatriuretic hormones, mostly mineralocorticoids such as aldosterone and desoxycorticosterone, increases substantially during gestation (2,18,42, 142,146). The renin–angiotensin–aldosterone (RAA) system has been studied extensively in human pregnancy (2,18,141, 142,147). In Figure 74-13 are depicted the increments in renin substrate, plasma renin activity, and plasma and urinary aldosterone that accompany normal pregnancy (148). Note that urinary sodium excretion, and presumably intake, are similar or only slightly increased throughout gestation compared with values measured postpartum. Therefore, inadequate sodium intake does not seem to account for these rises in the RAA system. Other studies have demonstrated that despite apparently high circulating levels, the renin–aldosterone system responds appropriately when gravidas undergo acute or chronic expansion with saline infusions or high salt diets or when they are sodium restricted or receive diuretics (2,18,42). Furthermore, inhibition of aldosterone biosynthesis results in an inappropriate diuresis and subtle signs of volume depletion (149). In essence, the activated renin–aldosterone system in pregnancy does not appear to function autonomously, as some have postulated, because the very high levels of aldosterone (which often exceed those in patients with primary aldosteronism) are appropriate and respond to homeostatic demands.

There are also increments in the concentration of several potentially natriuretic hormones during gestation (e.g., oxytocin, vasodilating prostaglandins, and melanocyte stimulating hormone) (18,42,141,142). Progesterone levels increase manyfold over that measured during the luteal phase of the menstrual cycle. This hormone binds *in vitro* with higher affinity than aldosterone to the mineralocorticoid receptor, prompting some to question how aldosterone can function efficiently during gestation when faced with such high circulating levels of progesterone (150). Part of the answer might be upregulation, during pregnancy, of enzyme systems (e.g., in the kidney), that convert progesterone to inactive metabolites (151). Ironically, however, progesterone may actually contribute to antinatriuresis, because it is a major source of the increase in desoxycorticosterone production. The current view is that most of the maternal desoxycorticosterone arises by extra adrenal 21-hydroxylation of circulating progesterone (152,153). Activity of the enzyme responsible for this conversion may be enhanced when estrogen levels are increased, as they are in pregnancy, and renal steroid 21-hydroxylase activity may be particularly high in pregnancy, so a considerable portion of maternal desoxycorticosterone may be produced near the renal mineralocorticoid receptor (153,154). Finally, and as noted in the section on renal potassium handling, a mutation of the mineralocorticoid receptor has been described (92) that recognizes traditional aldosterone antagonists as agonists. In these rare cases, therefore, the high progesterone levels paradoxically cause marked sodium retention and early pregnancy hypertension.

The role of natriuretic factors in pregnant women is poorly understood. The various circulating peptides and membrane pump inhibitors are usually described as elevated (18,141, 142,155), but these increases may be modest when related to the magnitude of the increase in intravascular and interstitial volumes that accompanies normal gestation.

Perhaps the major factor to consider when evaluating renal salt handling in pregnancy is the striking increase in GFR. Each day more than 20,000 mEq of sodium is filtered, which must be reabsorbed by the renal tubule, a quantity far greater than the expected salt-retaining effects of high circulating levels of aldosterone, desoxycorticosterone, or estrogens. Furthermore, filtration fraction is decreased throughout most of gestation, as is plasma albumin, additional factors that should enhance salt excretion.

Effects of Acute or Chronic Sodium Loads

Studies of the effect of acute saline infusions on urinary sodium excretion have produced conflicting results (18,25,141,142) in that a decreased, increased, and unchanged ability to excrete the load have all been described. Of note are studies by

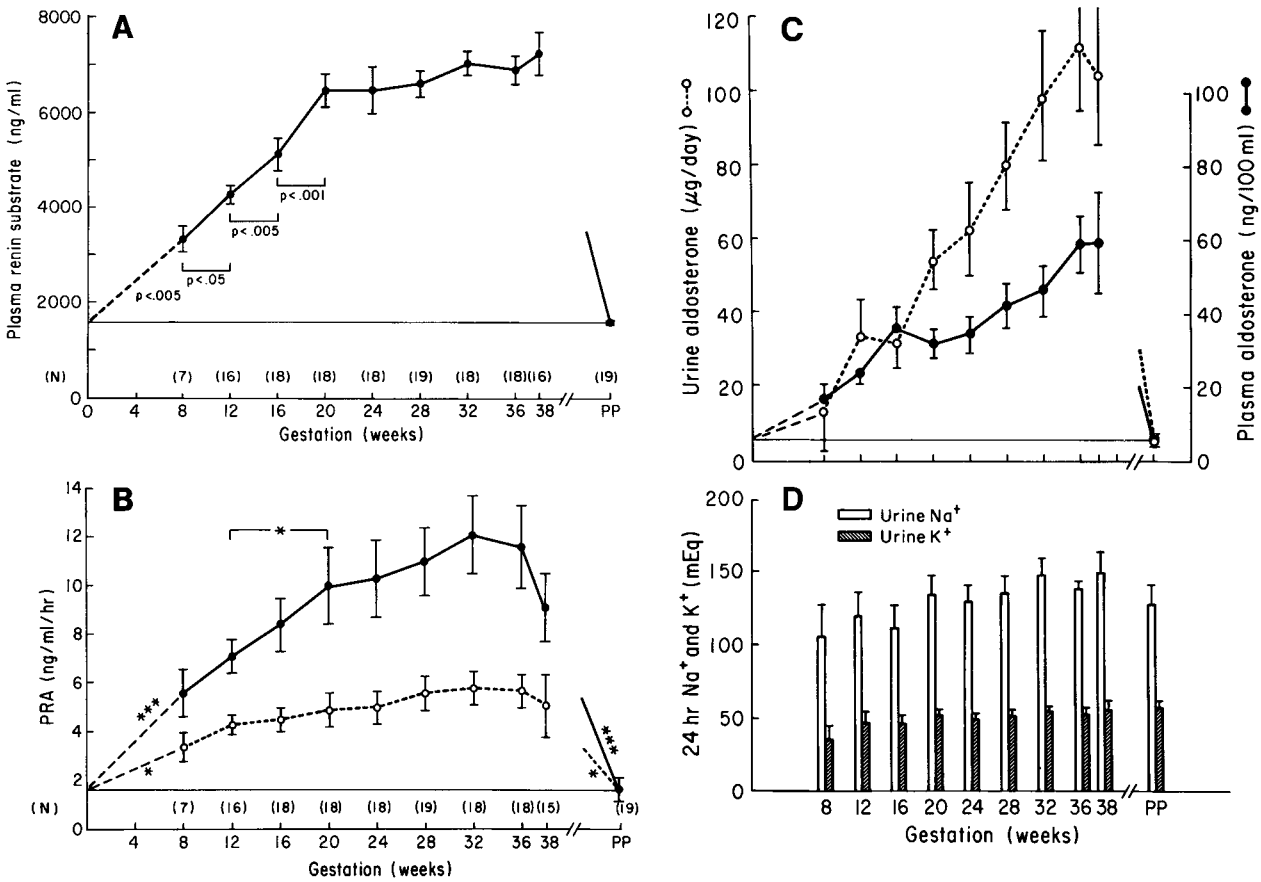

FIGURE 74-13. Changes in plasma renin substrate (**A**), its activity (**B**), and plasma (**C**) and urinary (**D**) aldosterone, during normal gestation and their relationship to urinary sodium and potassium excretion (**right lower panel**). *Dashed line* in (**B**) represents values normalized for postpartum substrate concentrations. (From: Wilson M, et al. Blood pressure, the renin-angiotensin system, and sex steroids throughout normal pregnancy. *Am J Med* 1980;68:97, with permission.)

Brown (156), Graves (66), and their respective colleagues in which salt intake before the infusion was controlled. The former found no differences in the sodium excretory capacity of normal gravidas compared with that of women in the nonpregnant state, whereas the latter suggested that both second- and third-trimester subjects respond with an enhanced natriuresis. Bay and Ferris (157) have reported that gravidas subjected to diets with a sodium content as low as 10 mEq per day achieve urinary sodium balance as quickly as nonpregnant controls. The women studied by Bay and Ferris lost weight, and, therefore, their data could be interpreted as suggesting that with extreme salt restriction, gravidas manifest subtle salt wasting (18,141,142). This is because sodium accumulation in late pregnancy should be in the range of 6 to 8 mEq per day, and restricting intake to 10 mEq should reveal differences between the pregnant and nonpregnant state.

Significance of the Volume Changes in Pregnancy

The reason maternal total body water, as well as intravascular and interstitial volumes, increase during normal gestation is unclear, which is one reason interpretation of the meaning of these changes is disputed (18,99,142). Some authorities consider the physiologic hypervolemia of human gestation a suboptimal response to the general arterial vasodilation that accompanies pregnancy (the "underfill" theory), whereas others propose that it may be an epiphenomenon secondary to factors such as the marked increase in circulating mineralocorticoids (the "overfill" theory). Still others, including ourselves, postulate that the volume-sensing mechanisms are continually reset as gestation progresses so that the increments in absolute volume are "sensed" as normal ("normal fill"). These discordant hypotheses were mentioned briefly in the section on osmoregulation and AVP release and are clarified further here.

Views supporting "underfill" include activation of the renin–angiotensin system, manifested not only by an increase in all its circulating components, but also by observations that gravidas manifest a greater increase in aldosterone release than nonpregnant subjects in response to small quantities of angiotensin II (158) and display exaggerated decreases in blood pressure when treated with ACE inhibitors (114). Those that rationalize the existence of increased absolute volume with "underfill" postulate further that marked peripheral vascular dilation precedes the rise in intravascular volume, the latter never completely compensating for this change (the "primary arterial vasodilatation" theory) (29,112,159). It is further suggested that this may be due to early upregulation of constitutive endothelial NOS in the vasculature (108). Studies of women and baboons in early pregnancy appear consistent with both the primary arterial vasodilation and "underfill" hypotheses (29,113,159). As well, adrenalectomy or sodium restriction is tolerated more poorly by pregnant rats than by nonpregnant controls (160).

Support for the "overfill" theory includes absolute increments in extracellular volumes, high levels of circulating natriuretic factors or inhibitors of the membrane pump (18,142, 155), increases in renal hemodynamics, and observations by two groups of investigators (156,161) of increased sodium excretory capacity in response to saline infusions. Women with cardiac or renal disorders appear more susceptible to volume overload complications, whereas healthy gravidas seem to tolerate blood loss better than nonpregnant women. Finally, in most studies of gravid animals, the mean circulatory filling pressure appears slightly increased (99,162).

As noted, the "normal-fill" hypothesis is supported by animal experiments that probe relationships between intravascular volume depletion and AVP release (98,99,107,116,117). In addition, studies in rats and humans suggest that sodium and water reabsorption in the proximal nephron (determined by indices such as fractional lithium or solute-free water clearances) is unaltered, and both species dilute their urine normally when water loaded during gestation (60,97,99,117). These latter observations are important because failure to dilute the urine maximally and a blunted excretory response to water loading are major pathophysiologic features of the "underfill" status in hyponatremic patients with cirrhosis or cardiac failure, considered the prototypes of diseases in which absolute extracellular volumes are increased and "effective arterial volume" is low. Finally, the previously described data are in accord with studies demonstrating a similar sodium excretory response to saline infusions in the pregnant and nonpregnant states (18,25,141,142).

Controversies about the various "fill" hypotheses are yet to be settled. It may be that all are correct at some time during pregnancy. Hormone-induced vasodilation may create temporary "underfill" in early gestation, followed quickly by compensation to "normal-fill" as pregnancy progresses, whereas during the last trimester, antinatriuretic factors predominate, at least in some gravidas (29,99,107,159). The importance of settling this dispute, however, is not trivial, because a better understanding of how the pregnant woman "senses" her volume changes will have an impact on our understanding and management of pregnant women with hypertensive complications and cardiac disorders. For the interested reader, we recommend discussions by Schrier et al. (111,112,163) and particularly Dürr and Lindheimer (99), who discuss these hypotheses; the latter includes a historical review in the form of an appendix entitled "On what is 'effective' in effective blood volume."

EVALUATION OF RENAL FUNCTION IN PREGNANCY

Examination of the Urine

Most, but not all (164), studies suggest that urinary protein excretion increases during normal gestation (18,64,99,165). Accepting the relative imprecision and variability of testing methods used by hospital laboratories, one should consider 300 mg per day (or twice the institution's cutoff for nonpregnant populations) the upper limit of normal protein excretion in pregnancy. It is unclear, however, whether urinary albumin excretion increases in normal pregnancy (26,33). We observed no difference in a small group of women studied serially when values obtained during gestation were compared with those measured 3 months postpartum (32), but follow-up collections made at 6 months or later suggest that nonpregnant levels may in fact have been slightly lower (41). In essence, if a difference can be demonstrated, it will be small, and, thus, use of the upper limits of normal for nonpregnant populations is probably permissible. More research is needed here, because the detection of microabuminuria in pregnant women may have predictive value in relation to pregnancy outcome in women with diabetes and with regard to preeclampsia (18,99,166). Finally, there is sparse information (99,167) concerning the excretion of small-molecular-weight or tubule proteins, as well as enzymuria, in both normal and abnormal gestation, and such tests have little current value in pregnancy.

There have been few attempts to quantitate the urine sediment of pregnant women. The excretion of red blood cells may rise (in pregnancy 1 to 2 red blood cells per high power field is acceptable), but whether increased leukocyturia occurs is unclear (2,5,168,169). One should be aware that microscopic and even gross hematuria may complicate otherwise uneventful gestations (170). The differential diagnosis includes all causes of hematuria in nongravid populations, but often no cause can be found and the bleeding subsides postpartum. Some of these episodes may be due to rupture of small veins around the dilated renal pelvis (170), and the hematuria may recur in subsequent pregnancies. Of importance, complete investigation of the hematuria can often be deferred until the postpartum period, and, noninvasive techniques, including ultrasonography and magnetic resonance imaging, are helpful in arriving at such decisions.

Renal Function Tests

The endogenous creatinine clearance, the primary tool to assess GFR in nonpregnant subjects, is equally useful for evaluating function in gravidas (Table 74-1). The lower limit of normal for clearances measured during gestation should be ~30% greater than that of nongravid women, the latter usually quoted as averaging 90 to 110 mL per minute. As noted by some, the 24-hour endogenous creatinine clearance may fall toward nonpregnant values during the last gestational month (18), and, therefore, a rise in serum creatinine to a level of 1 mg/dL or less (88 μmol/L) near term need not alarm the physician. Urinary creatinine results from tubular secretion and from glomerular filtration, but, unfortunately, there is little information on whether the ratio of secreted-to-filtered creatinine is altered by gestation. However, it is fair to assume that when renal dysfunction is present (serum creatinine level, $\geq$1.5 mg/dL or 133 μmol/L), a substantial proportion of the clearance may be due to secretion, resulting in overestimation of the GFR. In such circumstances, the measurement of endogenous creatinine clearance can be performed during gestation during cimetidine (Food and Drug Administration [FDA] pregnancy class C) administration, and this might aid in assessing prognosis and managing the clinical course of the patient.

Recently, other endogenous proteins easily measured in clinical laboratories have been suggested as better markers of GFR. Cystatin-C, considered superior to creatinine in nonpregnant populations, has been recommended as an alternative and possibly superior marker in pregnant women. However more recent data seriously question its use in pregnant populations (171).

Urinary concentration and dilution are similar in gravid and nonpregnant women. Supine posture can interfere with the ability of the gravida to produce maximally dilute urine, whereas lateral recumbency interferes with optimal urine concentration (97). Such tests are, therefore, best performed with the patient seated quietly. Ammonium chloride loading to assess renal acidification (rarely indicated in gestation) gives results similar to those in nonpregnant women (93). Finally, when timed urine collections prove unreliable or are difficult to obtain, random urine protein–to–creatinine ratio and the Cockcroft-Gault formula (using preconception body weight) can be used to estimate protein excretion and creatinine clearances in pregnant women, respectively (172).

Renal Biopsy

Pregnancy was once considered a relative contraindication for the performance of renal biopsy because of earlier reports that stressed several complications, particularly excessive bleeding (2). This view may have stemmed from the fact that many of these biopsies were performed in hypertensive patients, some of whom may have rapidly developed thrombocytopenia and other coagulation abnormalities associated with preeclampsia. It is now evident that if the biopsy is performed in women with well-controlled blood pressure and normal clotting indices, its morbidity is similar to that in nonpregnant patients (173).

In the past some (173) recommended liberal use of renal biopsy during gestation, but we find few indications for this procedure in pregnancy and suggest the following guidelines (174). First, renal biopsy may be considered when there is sudden deterioration of renal function with no apparent cause, because certain forms of rapidly progressive glomerulonephritis, when diagnosed early, may respond to aggressive treatments such as high-dose steroid pulses or plasma exchange. Second, renal biopsy is helpful in symptomatic nephrotic syndrome (i.e. marked edema or striking biochemical abnormalities), if present before the thirty-second gestational week. Although some may consider a therapeutic trial of steroids in such cases, we prefer to determine first whether the lesion is likely to respond to this regimen, thereby avoiding the potential complications of corticoids, such as fetal anomalies, hyperglycemia, propensity to infection, and poor wound healing. Nephrotic range proteinuria alone in a normotensive woman with preserved renal function, who does not have severe hypoalbuminuria, marked hyperlipidemia, or intolerable edema, would lead us to examine the patient at frequent intervals and defer the renal biopsy to the postpartum period. This is because most investigators believe that prognosis is determined primarily by the level of renal function and the presence or absence of hypertension, rather than by the type of renal lesion (see later discussion). A similar approach is used for patients with asymptomatic hematuria and stable preserved function. Indications for kidney biopsy between gestational week 31 and delivery are extremely rare, for at this stage, the decision to terminate the gestation is usually made independently of biopsy results, and neonatal survival rates are excellent, making an invasive procedure unnecessary.

THE CLINICAL SPECTRUM AND MANAGEMENT OF RENAL DISORDERS IN GESTATION

Acute Renal Failure

Acute renal failure (ARF) severe enough to require dialytic treatment is quite rare in pregnancy, its incidence being 1: 20,000 or less of all gestations in industrialized nations (18,175,176). Such statistics are a considerable improvement over conditions that existed ~50 years ago, when sudden and severe renal failure complicated 1 of every 8,000 gestations (2,18,175,176). This progress is due, most likely, to liberalization of abortion laws, more aggressive and effective antibiotic therapy, as well as improved prenatal care (Fig. 74-14). Moreover, maternal mortality associated with ARF, once ~18%, is now unusual (175–177). Unfortunately, these reassuring statistics do not apply to developing nations, where pregnancy-related ARF may comprise up to 25% of referrals to dialysis centers and is associated with substantial maternal and fetal mortality (175,176,179,180).

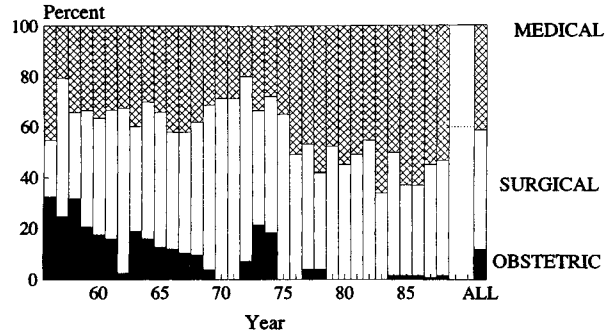

FIGURE 74-14. The relative frequency of medical, surgical, and obstetric causes of acute renal failure at the General Infirmary at Leeds, England. Note the virtual disappearance of obstetric-related causes after 1980. (From: Turney JH, Ellis CM, Parson FM. Obstetric acute renal failure. *Br J Obstet Gynaecol* 1989;96:679, with permission.)

Septic Abortion

Before the legalization of abortion in the United States, there were at least 200,000 (some estimate one million) illegal, usually nonsterile, pregnancy terminations annually, and ~5% of these women became critically ill (176). A common presentation was septic shock with renal failure, and if the infecting organism was *Clostridium,* mortality was high. Because most of these events occurred during the initial trimester, ARF in pregnancy had a bimodal distribution, the first peak occurring early in pregnancy and comprising most of the cases of septic abortion, and a second peak between gestational week 35 and the immediate puerperium, due mainly to placental abruption, bleeding, or preeclampsia (2,175,176). Fortunately, this initial peak has virtually disappeared because sterile terminations are readily available.

The clinical presentation of a gravida infected with clostridia is usually dramatic, the initial manifestations occurring several hours to 1 to 2 days after the attempted abortion. The patient is quite febrile and often manifests vomiting and diarrhea. Muscle pain, most intense in the upper limbs, thorax, and especially abdomen, may lead to erroneous diagnoses of an intra-abdominal inflammatory process, particularly when a history of attempted abortion is denied or not sought. In addition, there may be scant or no vaginal bleeding, and clostridia are difficult to culture or detect on the smear and are also normally present in the female genital tract. In essence, a high degree of suspicion is required because once symptoms develop, progression to shock and death is rapid.

Patients with clostridial sepsis are often jaundiced (due to hemolysis), and that in combination with cutaneous vasodilatation and pallor produces peculiar bronze skin coloration. Other features are marked anemia, a striking leukocytosis ($\geq$25,000/mm^3), and thrombocytopenia associated with evidence of disseminated intravascular coagulation. *Imaging* may reveal air in the uterus and/or abdomen due to gas-forming organisms or perforation. Death occurs rapidly in some patients, but most respond rapidly to the antibiotic regimen (primarily high-dose penicillin and volume resuscitation), and survival depends largely on the management of the renal failure (181). Review of the roles of hyperbaric oxygen, antitoxins, or exchange transfusions, and controversies concerning the conservative approach versus early surgical removal of the infected uterus are beyond the scope of this chapter and are discussed in many obstetric texts.

Other Causes of Pregnancy-Associated Renal Failure

The etiology of ARF in pregnant women is often similar to that in nonpregnant populations, including volume depletion (with

hyperemesis gravidarum) and bleeding (175,176). Antepartum uterine hemorrhage may be underestimated or difficult to diagnose when blood is trapped behind the placenta ("concealed hemorrhage"). Sudden renal failure during pregnancy has been reported after drug ingestion, incompatible blood transfusions, various nephritides, collagen disorders, or rarely in sarcoidosis or lymphoma (175,176,182,183). There also seems to be a greater propensity for gravidas to develop ARF in association with acute infectious pyelonephritis (175,176,184). Of interest are reports of ARF due to obstructive nephropathy, often in women with single kidneys (23,176,185,186). Causes are again similar to those in nongravid women, including stone disease and tumors, as well as the "distention syndrome," which was described previously (21–24).

A number of renal failure syndromes are peculiar to pregnancy, which include those associated with preeclampsia, acute fatty liver, and idiopathic postpartum renal failure. Preeclampsia, characterized by generalized vasoconstriction, is the major cause of renal dysfunction in gestation, but fortunately function loss is mild to moderate at most (187) and renal replacement therapy is rarely required. However, when preeclampsia is superimposed on chronic renal disease or essential hypertension, or is accompanied by marked coagulopathy (see "HELLP syndrome," Chapter 53), preeclampsia may progress to acute tubular and even cortical necrosis (176,179,180,188–190).

The incidence of ARF in women with acute fatty liver of pregnancy, once estimated at 60%, is considerably less today, perhaps due to earlier recognition of the disorder followed by rapid intervention to terminate the pregnancy (18,175,176). Both the etiology of the disease and its propensity to cause renal failure are obscure, although of interest are observations that some pregnant women developing either acute fatty liver of pregnancy or the microangiopathic *h*emolytic anemia, *e*levated *l*iver enzymes, and *l*ow *p*latelets (HELLP) syndrome harbor a long-chain 3-hydroxy acyl-CoA dehydrogenase deficiency or carry mutations of the enzyme's gene resulting in deficiencies in the fetus (191,192). Thus, ARF could be related to the coagulopathy that may accompany acute fatty liver of pregnancy or to hemodynamic factors akin to those observed in the "hepatorenal syndrome." Acute fatty liver of pregnancy was once associated with considerable maternal and fetal mortality, exceeding 70%, but maternal survival is now markedly improved (193,194). Such improved statistics may be due to aggressive intervention but also reflect earlier recognition leading to the inclusion in reported series of patients with milder disease (176,193,194). However, irreversible cases still occur, and in these instances, orthotopic liver transplantation may be lifesaving (195).

Idiopathic postpartum renal failure is characterized by the onset of renal failure in the puerperium after an uneventful gestation. This disorder, first recognized in the 1960s (196–198), is fortunately quite rare and goes by various names, including postpartum hemolytic-uremic syndrome, accelerated nephrosclerosis, postpartum malignant nephrosclerosis, and irreversible postpartum intravascular coagulation with ARF (175,176).

Idiopathic postpartum renal failure may present 24 hours to several weeks after delivery, the patient manifesting oliguria and at times anuria, rapidly progressive azotemia, and often evidence of microangiopathic hemolytic anemia or consumption coagulopathy. Blood pressure varies from normal or minimally elevated to severe hypertension. The cause of the syndrome is obscure, and suggestions have included an antecedent viral illness, retained placental fragments, and drugs, including oxytocic agents, ergotamine compounds, and oral contraceptives prescribed shortly after delivery (176). Hypocomplementemia noted in some cases has led to speculation about immunologic causes. Deficiencies in prostaglandin production or antithrombin III levels similar to decreases observed in the hemolytic-uremic syndrome have also been ascribed to idiopathic postpartum renal failure (176). In the past there were suggestions that preeclampsia and idiopathic postpartum renal failure had a common etiology (199). This concept has been extended with suggestions that those disorders, the "HELLP" syndrome, and acute fatty liver of pregnancy have a common pathogenesis, similar to that of thrombotic thrombocytopenic purpura and hemolytic-uremic syndrome (200). It has also been postulated that all these diseases are manifestations of a thrombotic microangiopathy induced by endothelial dysfunction. Although this hypothesis is intriguing, we note that there are many differences in both the manifestations and the prognosis of the various disorders listed above.

The renal pathology findings in idiopathic postpartum renal failure are detailed elsewhere (176) and fall into two general categories. In one, changes in the glomerular capillaries resemble those seen in hemolytic-uremic syndrome, and in the other, there are arteriolar lesions reminiscent of malignant nephrosclerosis or scleroderma. It may be that the lesions resembling thrombotic microangiopathy are from biopsies obtained early in the course of the disease, whereas those suggesting accelerated nephrosclerosis represent a more chronic stage. There is also an increased incidence of bleeding associated with the performance of renal biopsy in these cases (175).

The prognosis of idiopathic postpartum renal failure is poor. Some patients succumb, and most require chronic dialysis, or survive with severely reduced function (175,176). Treatment is aimed at reduction of blood pressure and general supportive measures (see subsequent text). There are anecdotal claims that early anticoagulant therapy with heparin or fibrinolytic agents may reverse the renal failure, but the data are not convincing and these drugs are not harmless. Similarly, antiplatelet therapy, infusion of blood products such as antithrombin III concentrates, and plasma exchange remain unproven treatment modalities (although the latter may be useful in a variant of acute postpartum renal failure linked to circulating lupus anticoagulant (201) or to circulating antiphospholipid antibodies (202,203). Because of the possible contributing role of retained placental fragments, dilation and curettage should be considered when the syndrome occurs very close to delivery.

Renal Cortical Necrosis

Cortical necrosis, the more ominous form of ARF, is fortunately rare, but in the past, it was more apt to be associated with pregnancy (4,175,176,204). The reason for this was obscure, but many of the women appeared to be older multiparas who perhaps had preexisting nephrosclerosis, suggesting that their kidneys were more vulnerable to inciting factors such as ischemia or coagulopathy. In this respect, cortical necrosis was most common late in gestation and occurred more frequently after placental abruption, especially when concealed hemorrhage was present (175,176). Another proposed reason was related to observations that the Sanarelli-Shwartzman reaction can be more easily produced in pregnant animals than in nongravid controls (2,176,205,206). Consistent with this possibility were cases that followed prolonged retention of a dead fetus. The incidence of cortical necrosis related to gestation has now declined markedly in industrialized nations and is presently less than 1 in 80,000 births (204).

Although cortical necrosis may involve the entire renal cortex, resulting in irreversible renal failure, it is the incomplete ("patchy") variety that is most often associated with pregnancy (175,176,207). The latter condition is characterized by severe oliguria or even anuria, which lasts longer than uncomplicated acute tubular necrosis, followed by a variable return of renal function and a stable period of moderate renal insufficiency that in some patients progresses years later to end-stage renal disease.

Management of Acute Renal Failure in Pregnancy

Management of ARF during pregnancy is generally similar to that in nonpregnant subjects (Chapter 41), but several points pertinent to treatment of antepartum patients deserve emphasis. Both peritoneal dialysis and hemodialysis may be used (176,208–210). The former may be preferable because it causes more gradual changes in fluid and solute levels and is thus less likely to be associated with precipitous hypotension or premature uterine contractions. But those higher clearances that seem to be associated with improved pregnancy outcomes are difficult to achieve with peritoneal dialysis. If hemodialysis is the modality chosen, the problems listed previously can be minimized by daily treatments for shorter periods and avoidance of excessive ultrafiltration. Because urea, creatinine, and presumably many other metabolites (or toxins) that accumulate in the blood associated with uremia cross the placenta, dialysis should be undertaken early with the aim of maintaining blood urea nitrogen levels below 50 mg/dL (18 mmol/L), if possible, which may require substantial increments in the daily prescription in patients treated with peritoneal dialysis (211). Further details concerning the hemodialysis prescription are given in the later section devoted to the management of women with end-stage renal disease treated with renal replacement therapy who conceive.

As noted, maternal prognosis has improved considerably compared with the dismal statistics published three decades ago. Survival for obstetric ARF is now markedly better than in patients whose disease is due to surgical or medical diseases. Prognosis for the fetus was once considerably worse than the mother but here, too, the outlook is improving dramatically.

Finally, although this section stresses ARF requiring dialysis, we note that sudden renal dysfunction occurs more frequently, often in association with oliguria during labor or in the immediate puerperium (187,212). These events are transient, and the clinician should refrain from vigorous attempts at fluid resuscitation, because the latter is more apt to result in volume overload than to prevent any renal disaster.

Urinary Tract Infection

Infections of the urinary tract are the most frequent renal complications encountered during an otherwise normal pregnancy. In most instances, the problem is asymptomatic bacteriuria uncovered by routine screening. However, cystitis, overt pyelonephritis, and rarely perinephric abscess may also develop. The management of these conditions may be shared by obstetricians and nephrologists, especially in the latter two disorders, which may become life-threatening.

Asymptomatic Bacteriuria

The prevalence of covert urinary tract infection in gravidas is similar to that in nonpregnant women of reproductive age, ranging from 2% to 10%, with higher rates recorded in gravidas with sickle cell disease or diabetes (2,18,213–217). However, the natural history of asymptomatic bacteriuria is different during pregnancy than in the nonpregnant state. Outside of pregnancy, the situation is quite benign, but during gestation, covert bacteriuria progresses to acute pyelonephritis in 20% to 30% of patients. This may be particularly true in women with previous evidence of renal scarring, with or without reflux (218,219). In addition, there are claims that gravidas with asymptomatic bacteriuria have a higher incidence of anemia, hypertension, intrauterine growth retardation, or "preterm/low-birth-weight" infants (2,18,25, 214,215), but data supporting these assertions are few and equivocal (25, 218–222). Most investigators currently recommend screening all gravidas and treating those with confirmed positive cultures (217). Screening is best performed during gestational week 16, because the onset of covert bacteriuria appears to be greatest between weeks 8 and 17 (223).

The practice of screening and treating asymptomatic bacteriuria is based on the as-yet unproven assertion that such an approach prevents up to two-thirds of potential antepartum pyelonephritis (214,224). Even a more modest preventive effect would avoid substantial morbidity and result in significantly less hospitalization during pregnancy (216,217,225,226). Still, there are some who question the sensitivity and cost-effectiveness of routine screening (gravidas often generate contaminated and false-positive cultures, which require repeated testing and even catheterization) and suggest that testing be limited to those women with histories of recurrent infections, reflux, or nephrolithiasis (226–228). Until this issue is resolved, we continue to recommend universal screening. When considering screening, one should note that the dipstick method has a sensitivity of $\leq$50% (229,230) compared with classic or semiquantitative culture techniques, although some have suggested the former may still be cost-effective (217).

The incidence of bacteriuria increases immediately after delivery when it can be as high as 17% (231–233). This no doubt relates to the birth process and is not unexpected, because transient bacteriuria follows sexual intercourse and can be induced by massaging the urethra during gynecologic operations (42,234). Moreover, trauma occurring during labor impairs the resistance of the bladder to infection and facilitates colonization by pathogenic organisms. Still, in the absence of symptoms, "prophylactic" antibiotics are unwarranted, because in most instances the bacteriuria will clear spontaneously, falling to about 4% by the third postpartum day (231,233).

Symptomatic Infections

Cystitis or pyelonephritis complicates about 3% of all pregnancies (214,217). The incidence of acute cystitis (about 1% to 2%) does not seem to have been influenced by universal screening. Fortunately, however, the incidence of infectious pyelonephritis, the more ominous complication, has decreased from nearly 3% to 1% or less, a decline ascribed to aggressive screening for and prompt treatment of covert bacteriuria (214).

Cystitis. Acute cystitis in gestation is considered an entity unrelated to asymptomatic bacteriuria (214,217) for the following reasons. Cystitis occurs most frequently in the second trimester, in patients with previously negative screening cultures. The peak incidence of asymptomatic bacteriuria is much earlier, and its source is the upper urinary tract in about 50% of the women. Cystitis usually responds rapidly to treatment with a recurrence rate of about 17%, whereas that of asymptomatic bacteriuria may be as high as 35%. Finally, unlike covert bacteriuria, cystitis is not associated with a substantial risk of developing acute pyelonephritis (214).

Acute Pyelonephritis. Most (about 70%) patients who develop acute pyelonephritis during pregnancy have preexisting covert bacteriuria, and in a number of cases, the offending organism seems to have been newly acquired (213,214,217). Thus, although screening seems to have reduced the incidence of acute pyelonephritis by almost two-thirds, it has not and will not eradicate this disease (214,217). Ten percent to 20% of the cases occur in early pregnancy, and approximately half in the second trimester (235–237). Of interest is a report that women developing pyelonephritis antepartum manifest greater degrees of pelvicaliceal dilation during gestation that may not regress after delivery, underscoring the need for postpartum investigation of this population (236).

The bacteriology of the various organisms that cause acute pyelonephritis are discussed in detail elsewhere (214,217).

More than 70% of the uropathogens are aerobic gram-negative rods, most of which are *Escherichia coli*. Of interest, results from a recent series (440 cases) suggest that the bacteriology of pyelonephritis in pregnancy has changed, with fewer infections from the klebsiella-enterobacter group, and more than 10% due to gram-positive organisms, mainly group B streptococcus (236). Pregnancy seems to decrease tolerance to these infections, which may be related in part to observations that during gestation, there is immunosuppression of the cytokine and specific antibody responses to gram-negative bacteria that invade the urinary tract (238). In addition, the increased invasiveness may reflect upregulation of Dr adhesins that govern bacterial adhesion to CD 55, itself upregulated during pregnancy (239). As a result, acute pyelonephritis in pregnancy can be often devastating to the health of both mother and fetus. This disorder was a cause of maternal mortality in the preantibiotic era, and currently about 20% of pregnant patients who present with pyelonephritis manifest bacteremia (214,217,235). Furthermore, there is substantial literature suggesting that upper urinary tract infections in gravidas are associated with the exaggerated effects of endotoxemia, including respiratory distress syndrome and various hematologic, hepatic, and renal functional abnormalities (2,42,214,236,240). Concerning the latter, it is noteworthy that although upper urinary tract infections seem to have little effect on the renal hemodynamics of nonpregnant patients, acute pyelonephritis in pregnant women may result in transient and marked decrements in GFR (184). As for the fetus, acute pyelonephritis is associated with premature delivery, growth retardation, and intrauterine death (214,222,241). This disease should therefore be preferably treated in a hospital setting.

Perinephric or Cortical Abscess. These are very unusual events in pregnancy but should be considered in a setting of occult or resistant infections. This is especially true during the immediate puerperium, when physicians are apt to focus on uterine and abdominal sources of infection or on pelvic thrombophlebitis, and to overlook the renal problems noted previously.

Management of Urinary Tract Infections in Pregnancy

In Table 74-2 are listed the common antibiotics used to treat asymptomatic bacteriuria and cystitis during pregnancy (214,217,242). In the past, given the tendency of asymptomatic bacteriuria to recur, many authorities suggested prolonged treatment, often for 6 or more weeks. Currently, however, most workers recommend a 7-day course, and even shorter periods are being evaluated (214,217,224). As of 2005 there was a growing literature (217,243) describing success with single-dose therapy, and a large World Health Organization (WHO) sponsored randomized prospective trial testing utility of a dipstick method to diagnose significant bacteriuria and the efficacy of single-dose therapy with nitrofurantoin to eradicate the infection was in progress and scheduled to be completed in 2006.

Some have suggested that failure of short treatment regimens identifies patients at increased risk for recurrent asymptomatic bacteriuria who may benefit from suppressive regimens (217,243). Until these issues are resolved, we suggest the standard 7-day course (Table 74-2), which appears to treat successfully 90% of the women with cystitis and will eradicate bacteriuria for the remainder of pregnancy in more than 70% of the women with asymptomatic infections. Urine cultures should be repeated 1 or 2 weeks after completion of the initial antibiotic course, and then at 4- to 6-week intervals until delivery. Relapsing infections (reappearance of the same organism), which usually occur within the first 2 weeks and suggest a urinary tract abnormality (including nephrolithiasis) or renal involvement, should then be treated with a prolonged course (2 to 3 weeks), followed by suppressive therapy until delivery (Table 74-2). Reinfections (caused by a different strain or species) normally appear more than 3 weeks after treatment and are usually confined to the bladder. They may be treated with repeated short courses of antibiotics each time they occur, or with long-term suppression. Alternatively, one may prescribe only post–coital prophylaxis (a single dose of either 250 mg of cephalexin or 50 mg of nitrofurantoin [245]). Trimethoprim-sulfamethoxazole combinations, frequently prescribed to nonpregnant women, are best avoided during gestation (246). Trimethoprim is teratogenic in animals and may also precipitate megaloblastic anemia during pregnancy, whereas sulfamethoxazole has been associated with kernicterus when prescribed near term (217).

TABLE 74-2

RECOMMENDED THERAPY FOR ASYMPTOMATIC BACTERIURIA AND CYSTITIS IN PREGNANCY

Agent[a]	Dosage
Standard courses of treatment (duration 7 days)	
Ampicillin	500 mg q.i.d.
Amoxicillin	250 mg t.i.d.
Nitrofurantoin[b]	50 mg q.i.d. or 100 mg b.i.d.
Cephalexin	250 mg q.i.d. or 500 mg b.i.d.
Amoxicillin/clavulanic acid	250/125 mg t.i.d.
Long-term prophylaxis	
Nitrofurantoin[b]	50 mg at night
Cephalexin	250 mg at night
Amoxicillin	250 mg at night

[a]All antibiotics in this table are in the FDA pregnancy risk category B.
[b]Nitrofurantoin macrocrystals may reduce the incidence of gastrointestinal tract side effects.
(From: Pedler SJ, Orr KE. Bacterial, fungal, and parasitic infections. In: Barrom WM, Lindheimer MD, eds. *Medical Disorders During Pregnancy.* 3rd ed. St. Louis: Mosby; 2000, with permission.)

Pregnant women with acute pyelonephritis are currently treated in a hospital setting because they often require considerable supportive care and treatment with intravenous antibiotics (247). As of the year 2006 there were scattered reports (217,247,248) of successful management of gravidas with acute pyelonephritis in an outpatient setting, but we believe such data to be preliminary and await better-designed studies. A recent report that routine pretreatment urine and blood cultures are of limited use because only 6% of the patients require a change in therapy after the start of empiric treatment is of interest but should be confirmed (249). Because of an increase in the number of community-acquired pathogens resistant to ampicillin (250), many consultants recommend the addition of an aminoglycoside (usually gentamicin) and clindamycin initially, adjusting therapy when culture results become available. The "extended-spectrum" penicillins and third-generation cephalosporins have also been used successfully in these women. The readers are referred elsewhere (214,217,242) for further discussion of individual antimicrobials (the latter citation lists the current pregnancy risk classification according to definitions suggested by the FDA).

Most patients respond quickly, with the temperature returning to normal within 72 hours. Continued fever or evidence of sepsis should alert the physician to seek an obstructive cause using ultrasonography or a plain abdominal x-ray film, because urinary calculi are the most common cause of obstruction in these patients, and about 90% of the stones are radiopaque.

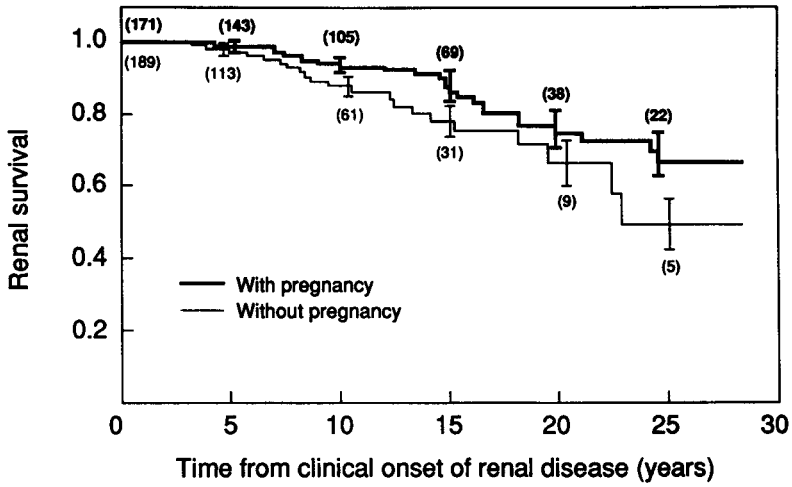

FIGURE 74-15. Renal survival in women with preexisting renal disease who were pregnant at least once (*heavy line*) compared with those who never conceived. The long-term renal prognosis was similar in the two groups. (From: Dr. P. Jungers, with permission.)

When clinical improvement is apparent, oral antibiotics may be substituted for parenteral medications, but there are limited data on treatment duration. A standard 2-week course seems appropriate, and many prescribe suppressive therapy through term thereafter, although urinary surveillance (see previous discussion of asymptomatic bacteriuria) has been claimed to be as effective as long-term suppression (251).

In summary, urinary tract infections are the most common renal complication of pregnancy, and most patients present with asymptomatic bacteriuria. Universal screening followed by treatment in culture-positive cases is debated but seems to have decreased the incidence of pyelonephritis, which in pregnant women can be a virulent disease. There is no consensus yet on the optimal treatment regimens for women with asymptomatic bacteriuria, although it is hoped that a large randomized trial to be completed in 2006 will shed light on this dilemma. Similarly, the ideal treatment of acute pyelonephritis, including a place for outpatient treatment, remains unclear. We still suggest screening all gravidas and treating those with covert bacteriuria for 7 to 10 days, and initially hospitalizing all patients with pyelonephritis, followed by 2 weeks of the appropriate antibiotic, but underscore the need for further research in this area.

Preexisting Renal Disease

Perspectives

As noted, older views that underlying chronic renal disease of any severity precluded a successful pregnancy have been radically altered, because in many instances prognosis for these patients is quite favorable (1). This more optimistic current approach is based on a series of studies dating from 1980, reported by authors who have reviewed the renal prognosis and gestational outcome in more than 1,000 patients, most with biopsy verification of their underlying kidney disorder (2,18,252–262). Results of these largely retrospective studies permit the following guidelines in which prognosis is mainly based on two functional parameters: the presence or absence of hypertension and the degree of renal insufficiency at conception. Women are arbitrarily classified in three categories: those with *mild, moderate,* or *severe* dysfunction.

Normotensive women with intact or only mildly decreased and stable renal function (serum creatinine, ≤1.4 mg/dL or ≤125 μmol/L) generally do well, experiencing more than 95% live births, about 75% of which are adequate for gestational age (252). These excellent fetal survival estimates are weighted to the more recent literature and reflect the marked advances in both perinatal and neonatal care. Gestation does not appear to alter the natural course of the renal disease (Fig. 74-15), but there is an increased incidence of superimposed preeclampsia or late pregnancy hypertension, as well as increased proteinuria that can exceed 3 g per day (18,252,258–260). Such increments rarely indicate functional deterioration; most patients with chronic renal disease and only mild dysfunction surprisingly experience a gestational-induced increase in GFR (252).

There are, however, exceptions to the optimistic outlook already described. Several nephropathies appear more sensitive to intercurrent pregnancy, including lupus nephropathy and perhaps membranoproliferative glomerulonephritis. In addition, women with scleroderma and periarteritis nodosa do poorly (especially when there is renal involvement and associated severe hypertension) and thus should be emphatically counseled to avoid pregnancy. Furthermore, authorities disagree on whether gestation influences adversely the natural history of IgA nephropathy, focal segmental glomerulosclerosis, and reflux nephropathy (18,253,258–260,263–271). Our view is that prognosis in the last three groups is similar to that of women with renal disease in general, being best when, before pregnancy, function is preserved, and high blood pressure absent. Although hypertension before conception increases the incidence of both maternal and fetal complications (253,272), the outlook of such patients has also improved dramatically, especially when the blood pressure is well controlled (273).

Prognosis is poorer when there are greater degrees of renal dysfunction. With moderate impairment (serum creatinine level, ≥1.5 to 3 mg/dL or ≥133 to 275 μmol/L), live births still approach 90%, but the incidence of fetal growth retardation or preterm deliveries exceeds 50% (18,274–277). In addition, one-third or more of such patients experience renal functional deterioration or the appearance of hypertension after midpregnancy. The latter may be severe and hard to control, and some women may experience an accelerated progression of their disease after delivery. Accordingly, pregnancy is not advisable in such patients. Note, however, that one group of investigators (262) suggests that maternal and fetal outcomes remain relatively favorable until serum creatinine levels approach 2 mg/L (or 177 μmol) and are more liberal in their recommendations.

When renal dysfunction before conception is severe (serum creatinine level, ≥3.0 mg/dL or ≥275 μmol/L, but some authorities consider the disease "severe" at creatinine levels of 2.5 mg/dL or 210 μmol/L), the risk of maternal complications is far greater and the pregnancy success rate is poor (208–210,276,277). However, such patients are often infertile, especially when they have end-stage disease but there are

exceptions (210,278–280). For instance, women receiving renal replacement therapy occasionally conceive, and the incidence may be favorably influenced by erythropoietin therapy, which is associated with a return of ovulatory cycles and improvements in libido (280,281). Fetal survival in women undergoing dialysis, once barely more than 50% a decade is slowly improving (see later discussion). However, there are still serious maternal risks, including the occurrence of intraperitoneal hemorrhage and accelerated hypertension (208–210,281–283), and we do not recommend planning or continuation of a pregnancy in women with preexisting severe renal dysfunction.

In summary, most pregancies associated with renal dysfunction appear to succeed. We must emphasize, however, that these conclusions reflect literature that is primarily retrospective and that most of the patients described had but mild functional impairment. Published series dealing with greater degrees of dysfunction comprise fewer than 300 patients in aggregate. Thus, confirmation of our guidelines and a more definitive perspective require large prospective observational studies.

Specific Diseases

In Table 74-3 are listed specific diseases associated with pregnancy.

Diabetes. Diabetic nephropathy is the most common chronic renal disorder encountered during gestation. Most studies have focused on gravidas with overt disease (defined by the presence of abnormal proteinuria before conception or early in gestation), and there is very little information on the course of pregnancy in women with microalbuminuria only (262,284–299). The maternal long-term renal prognosis in patients with preserved function does not seem to be adversely affected by gestation, which is surprising because nephrotic range proteinuria and hypertension complicate two-thirds of these pregnancies. However, there are occasional observations of patients whose increased proteinuria does not remit or whose function deteriorates after delivery. Less surprising is limited evidence of rapid functional deterioration in diabetic women with moderate or greater renal dysfunction before pregnancy (284,293,294,297,300).

Fetal outcome, once considered poor is now quite good, with the success rates exceeding 95%. However, there is a high incidence of preterm deliveries as the rise in urinary protein excretion and blood pressure noted previously often occurs early in the third trimester, is difficult to differentiate from superimposed preeclampsia, and thus frequently leads to early termination of the pregnancy (284,286,289,293,301,302). Of interest is a report of the appearance *de novo* of hypothyroidism during gestation in nephrotic diabetic patients (303).

Chronic Glomerulonephritis. Women with chronic glomerulonephritis, a general term that encompasses many morphologically and presumably pathogenetically distinct entities, seem to do well when renal function is preserved and hypertension is absent (2,252–256,259–262,270). There is evidence of

TABLE 74-3

PREEXISTING RENAL DISEASE AND PREGNANCY

Renal disease	Effects
Diabetic nephropathy	Most frequent nephropathy encountered. Pregnancy does not appear to accelerate functional loss; increased frequency of covert bacteriuria; high incidence of heavy proteinuria and hypertension after midgestation.
Chronic glomerulonephritis focal glomerulosclerosis (FGS)	There may be an increased incidence of high blood pressure late in gestation, but usually no adverse effect if renal function is preserved and hypertension absent before gestation; some disagree, believing that coagulation changes in pregnancy exacerbate these diseases, especially IgA nephropathy, FGS, and membranoproliferative glomerulonephritis.
Systemic lupus erythematosus	Expect more problems than in most glomerular diseases, but prognosis is more favorable if disease is in remission ≥6 months before conception. Poorest maternal and fetal outcomes associated with antiphospholipid antibodies or with lupus anticoagulant.
Periarteritis nodosa and scleroderma	Fetal prognosis is poor; associated with maternal deaths; reactivation of quiescent scleroderma can occur during pregnancy or postpartum; therapeutic abortion should be considered.
Chronic pyelonephritis (infectious tubulointerstitial disease)	Bacteriuria in pregnancy may lead to acute exacerbations but otherwise is well tolerated. Frequent urine cultures are recommended and chronic suppressive antibiotic therapy may be necessary.
Reflux nephropathy	Some controversy but most now agree that these patients do well when function is preserved. They require frequent urine cultures.
Polycystic kidney disease	Patients with preserved function do well, but there is an increased tendency for late pregnancy hypertension and/or preeclampsia.
Urolithiasis	The "physiologic" ureteral dilation and stasis of pregnancy do not seem to affect natural history, but infections can be more frequent. Stents have been successfully placed during gestation.
Previous urologic surgery	Other urogenital tract malformations may be present; urinary infections increase, and functional decrements have been observed; cesarean section may be necessary to avoid disruption of the continence mechanism if artificial sphincters or neourethras are present.

(From: Lindheimer MD, Grünfeld JP, Davison JM. Renal disorders. In: Barron WM, Lindheimer MD, eds. *Medical Disorders During Pregnancy.* 3rd ed. St. Louis: Mosby, 2000; with permission.)

transplacental transfer of nephritic factor(s) in association with membranoproliferative glomerulonephritis (304). This is also one of the entities in which pregnancy is said to have adverse effects on the course of the disease, such as an accelerated decline in renal function and an increased propensity to develop gestational hypertension or preeclampsia (the latter mainly ascribed to the type I renal lesion) (2,42,305). However, a review of the literature comprising 98 patients with membranoproliferative glomerulonephritis (260) suggests a maternal and fetal prognosis in this disease that is quite similar to that of gravidas with most other forms of chronic glomerulonephritis. Obviously more data are needed before firm conclusions can be reached.

IgA nephropathy is another disorder whose course in pregnancy is disputed. One group (263,266,267) described substantial declines in renal function that were only occasionally reversible, also stressing the frequent appearance of *de novo* hypertension that did not always normalize in the puerperium. In contrast are the views of two groups (253,258, 259,262,268,270) who reviewed more than 200 gestations in women with IgA nephropathy, one (268) also comparing long-term renal function in 84 such patients who had experienced pregnancy with that of 80 who had never conceived. They concluded that the natural history of this disorder and pregnancy outcome follow the generalization that the prognoses are excellent in normotensive women with minimal renal dysfunction. Lastly, we shall comment on acute poststreptococcal glomerulonephritis in this section, although it is obviously neither a preexisting nor a chronic renal disorder. Its occurrence in pregnant women is unusual, perhaps due to the suppressed immune responsiveness characteristic of normal gestation (2,25,42,306–308). When it does occur, the recovery of renal function is usually complete and gestation is successful. Pregnancy in women with a remote history of acute glomerulonephritis is also uneventful (2,42).

Focal Segmental Glomerular Sclerosis. This is yet another disease whose course during gestation is disputed. Here, however, the reason may relate to the fact that focal segmental glomerular sclerosis (FSGS) has multiple causes. One group of investigators (257,263,265) noted hypertension, frequently severe, in 75% of their pregnant patients, functional deterioration in 45% of gestations, and a fetal loss of about 45%. The high blood pressure persisted postpartum in 20% of the women. Others (253,255,262) dispute these data, noting few problems when function before or in early pregnancy is preserved and hypertension is absent. However, here too, the total published experience is sparse (100 or fewer patients described) and substantially more data are required to resolve this issue. Finally, there is an interesting report of women with FSGS experiencing two successful term deliveries, each followed by transient proteinuria in the neonate lasting several weeks (309). This is of interest in relation to the description in some patients with FSGS of a circulating factor(s) that increases glomerular permeability to albumin (310), suggesting the ability of such factor(s) to cross the placenta and affect the newborn.

Connective Tissue Disorders. The most common connective tissue disorder complicating pregnancy is, not surprisingly, systemic lupus erythematosus, a disease that primarily affects women during the childbearing years (311). The outlook for gravidas with lupus nephropathy and preserved kidney function is clearly more complex than that of a pregnant woman with the preexisting renal disorders discussed previously, and is further complicated by the fact that the natural history of systemic lupus erythematosus is unpredictable regardless of gestation. However, case series and reviews appearing after 1980 that describe the course of more than 500 gravidas have permitted a clearer understanding of the natural history and fetal outcome of gravidas with lupus nephropathy (312–315). The best outcomes occur when the disease is in remission for 6 months before conception and when renal function is preserved (312–316). Prognosis is more guarded if the disease is active within 6 months of the pregnancy, when the lupus anticoagulant or an antiphospholipid antibody is present and, of course, if there is preexisting hypertension or greater degrees of renal insufficiency (201,311,312). Of note, lupus nephritis exacerbating or presenting *de novo* during late gestation may be difficult to differentiate from preeclampsia (2,311) (Table 74-4).

There is an increased incidence of first-trimester abortions, intrauterine growth restriction, and fetal death in women with systemic lupus erythematosus (311,317). These are also more apt to occur when circulating anticoagulants or antiphospholipid antibodies are present but may also be due to transplacental passage of the many other IgG autoantibodies that circulate in the blood of these patients. The latter are responsible for the occurrence of congenital heart block and the "neonatal lupus syndrome," including life-threatening thrombocytopenia, which are described further elsewhere (42,311). Still, it appears from most series that 85% to 90% of the fetuses survived (excluding first-trimester spontaneous abortions) (312). Increases in proteinuria and "flares" appear quite common in pregnancy, but data from controlled studies show no evidence of more increases in the activity of lupus nephritis in pregnancy

TABLE 74-4

COMPARISON BETWEEN PREECLAMPSIA AND LUPUS NEPHRITIS

Clinical measure	Preeclampsia	Lupus nephritis
C3, C4, CH_{50}	May be decreased but unusual	Commonly low
Urinalysis	Red blood cell (RBC) casts are rare	RBC casts frequent
Onset of proteinuria	Commonly abrupt	Gradual or abrupt
Hepatic aminotransferases	May be increased	Rarely abnormal
Quantity of proteinuria	Will not differentiate	
Thrombocytopenia	Will not differentiate	
Hyperuricemia	Will not differentiate	
Hypertension	Will not differentiate	

(From: Lockshin MD, Sammaratano LR. Rheumatic disease. In: Barron WM, Lindheimer MD, eds. *Medical Disorders During Pregnancy.* 3rd ed. St. Louis: Mosby; 2000, with permission.)

(314,316). Management of "flares" in the mother, including pulse steroids and chemotherapy, the treatment of the antiphospholipid syndrome, and the detection and management of congenital heart block are issues beyond the scope of this chapter and are detailed elsewhere (311).

In contrast to systemic lupus erythematosus, in which the disorder is usually sufficiently benign to allow conception in women who so desire, the outcome of pregnancy in patients with renal involvement with periarteritis nodosa or scleroderma is very poor, often because of the associated hypertension, which can be malignant in nature. In fact, many of the cases described in the older literature, albeit selective and anecdotal, were of maternal deaths, and fetal survival was dismal (2,42,311). The more recent literature, although still anecdotal, contains descriptions of successful pregnancies in women with scleroderma, but these were treated with ACE inhibitors, a drug category that should be avoided during gestation (311,318). We therefore continue to counsel against conception in women with these two entities and suggest pregnancy termination when the disease is present in the first trimester. Finally, there are reports that link pregnancy, via the passage to and persistence of fetal cells or DNA in the mother, to the pathogenesis of scleroderma, albeit years later (319,320). Experience with other vasculitides, including Wegener's granulomatosis, is also sparse and anecdotal (311,321,322). Antenatal and postpartum exacerbations have been described, as well as successful pregnancies, in women treated with azathioprine and cyclophosphamide. It should be noted, however, both are pregnancy risk class D agents (see *Physicians' Desk Reference*), although there is considerable experience with the former agent, primarily in gravid allograft recipients (see later discussion).

Miscellaneous Glomerular Disorders. The literature regarding minimal change nephropathy and pregnancy is favorable but is restricted to fewer than 200 gestations (252,255,262). Serum albumin levels usually decrease approximately 1g in normal gestation, which should theoretically make gravidas more vulnerable to the complications attendant to massive urinary protein loss. Of interest, therefore, is a case description of a pregnant woman with steroid-responsive nephrotic syndrome who developed in the first trimester renal insufficiency that responded rapidly to high-dose prednisone therapy (323). There is limited experience with membranous nephropathy as well in pregnancy, but for the most part, patients with preserved renal function do well (252,255,258,262). Their proteinuria may increase markedly, however, and at least one group has commented that severe nephrotic syndrome of any origin during the first trimester portends a poorer fetal prognosis (255,262).

Some Rarer Disorders

A retrospective report of 29 pregnancies in 17 women with renal amyloidosis secondary to familial Mediterranean fever is of concern; the authors suggested that gestation had an adverse effect on the natural history of the disease, even when function was only minimally decreased (324). There is only anecdotal information on the course of Schönlein-Henoch disease and Goodpasture's syndrome in pregnancy (262,325–328). Concerning the latter are data from one report in which the authors postulated that the placenta ameliorated the disease during gestation by providing a large absorptive surface for the autoantibody (326). This would be consistent with an older report by Steblay (329) that sheep immunized to human placentas develop antibodies that react with both human placenta and glomerular basement membrane. He noted similar results when the sheep were immunized with glomeruli, suggesting that similar or identical antigens are present in the placenta and kidney.

Tubulointerstitial Disease and Reflux Nephropathy. There is sparse information on gestation in women with chronic interstitial nephritis and those with tuberculosis, but it would appear that here, too, outcome reflects functional status before conception (2,252). Of interest is the paucity of data relating to pregnancy in women with analgesic (primarily phenacetin) abuse, given the incidence of this disorder worldwide. One group of investigators (330) has suggested that women with interstitial nephritis were more prone than those with glomerular disorders to hypertension during pregnancy. This is contrary to our experience, in which pregnancy outcome was better than in those with primary glomerular diseases (252).

Reflux nephropathy is a disease that begins in childhood and affects a substantial number of women of reproductive age (331) (Chapter 24). Its presence may even remain undetected, and one should always consider this disorder in young women with frequent infections, especially if associated with unexplained hypertension. Some workers (263) have stressed that pregnancy affects adversely the course of this disease, but on careful review of their own data, these authors now note that gravidas who do poorly are mainly those who had hypertension and moderate renal dysfunction before conception (269). An analysis of 335 gestations in 135 women by Jungers' group (271) further revealed that when renal function was preserved, 92% of the gestations succeeded, although 17% of the women delivered prematurely. Functional deterioration was noted only in patients with moderately or severely decreased GFR before conception or early in pregnancy.

Because of the nature of the disease, women with reflux nephropathy require frequent urine cultures and aggressive treatment when signs of urinary tract infection occur (331). Also, because the disease may be inherited, children who are born to parents with vesicoureteric reflux or reflux nephropathy or who have affected siblings should be screened in infancy (332). Finally, women who have undergone urinary tract reconstructive surgery, especially diversion, may develop reflux when pregnant (333). They also have a high incidence of asymptomatic bacteriuria. Still, and despite the risk of developing urinary tract obstruction as the uterus enlarges, their pregnancies are usually successful and fairly benign (333).

Inherited Renal Disorders. The most common genetic renal disorder is autosomal-dominant polycystic kidney disease (ADPKD), which affects 1 of 400 to 1,000 persons (334) (Chapter 18). The other conditions encountered in women of childbearing age are renal amyloidosis associated with familial Mediterranean fever (an autosomal-recessive disorder clustering in Armenians and Sephardic Jews, discussed previously) and hereditary nephritis (335) (Chapter 19). Most of the rarer disorders manifest during childhood and have usually progressed to end-stage renal disease before or by adolescence (e.g., nephronophthisis and cystinosis). Still, such patients may conceive while receiving renal replacement therapy or after transplantation (336). Women with these disorders should be referred for genetic counseling, or in early pregnancy where antenatal predictive tests may be available (291).

Polycystic kidney disease. This disorder may remain undetected during gestation, but careful questioning for a family history of renal problems (10% or less of the cases are due to spontaneous mutations) and the judicious use of ultrasonography permit early detection (334,337). Women with ADPKD may have a greater propensity to develop late gestational hypertension or preeclampsia, but pregnancy outcomes are favorable when kidney function is preserved and hypertension is absent (337,338). There is an increased risk for upper urinary tract infections that are at times severe and require treatment with lipophilic antibiotics such as ciprofloxacin or trimethoprim (both FDA pregnancy risk category class C) (334).

Although pregnancy does not appear to affect the natural history of the renal disorder, it may influence the effect of ADPKD on other organs. Liver cysts are more prevalent and larger in women, especially in those who have been pregnant, and enlargement of cysts (increasing the chance of rupture) may occur during gestation (Chapter 18). One group of investigators (339,340) suggests that multiple pregnancies (defined as three or more) lead to an earlier onset of renal insufficiency. However, we believe that this may only reflect the effects of pregnancy once moderate dysfunction is present, and that gestation does not influence the long-term renal prognosis of ADPKD. Finally, routine preconception screening of the intracerebral vasculature is not necessary and should be reserved for patients with a family history that reveals clustering of intracranial aneurysms or subarachnoid hemorrhage (341).

Hereditary nephritis. Most patients with hereditary nephritis (85%) are men, having inherited the disease as an X-linked dominant disorder. In these families, most of carrier women manifest only mild or moderate degrees of urinary abnormalities (as dictated by the Lyon hypothesis) (335). The other 15% of patients with hereditary nephritis are examples of autosomal-dominant and autosomal-recessive inheritance. Thus, although hereditary nephritis is clinically silent in most women, there are exceptions, and the disease may manifest itself for the first time during a pregnancy. Because GFR is usually well preserved, most of these gestations succeed, but there is an interesting report describing two sisters with the disorder who developed rapidly progressive crescentic glomerulonephritis associated with pregnancy (342). Also, one should be aware of a variant of hereditary nephritis associated with macrothrombocytopenia and thrombocytopathy (2,335). Gestation in these women may be complicated by bleeding problems that appear to occur only at delivery or during surgical procedures.

Tuberous sclerosis. This autosomal-dominant disorder is characterized by skin lesions, central nervous system involvement, and visceral lesions, including renal angiomyolipomas (343). There may be renal aneurysms in these angiomyolipomas and in adjacent large branches of the renal arteries. One should attempt to detect and excise these aneurysms before a planned pregnancy, to prevent their rupture during gestation.

Von Hippel-Lindau disease. This is also an autosomal-dominant disorder in which renal involvement includes cysts and bilateral multifocal renal carcinoma. Twenty-five percent of these patients also have pheochromocytoma, often bilateral, which may also cluster in families, and is sometimes the only manifestation of the disorder (344). Because pheochromocytomas are associated with fatal outcome and are especially prone to exacerbate during gestation, all patients with von Hippel-Lindau disease should be screened for this disorder. The management of pheochromocytoma in pregnancy is discussed in Chapter 55. Details concerning genetic diagnosis and counseling are beyond the scope of this chapter and can be found elsewhere (345).

Urolithiasis. The incidence of stone disease in pregnant women varies by geographic location and is estimated to occur in 1 of every 1,500 pregnancies (346). The actual incidence, however, may be higher, because patients are often not identified until their disease is symptomatic and may even be overlooked if the symptoms are subtle (e.g., repeated urinary tract infections). There does not appear to be a marked increase in either the appearance or the recurrence rate of nephrolithiasis in pregnant women (347) (even in the face of marked calcium supplementation [348]). This is surprising because gravidas are normally hypercalciuric, and their urinary supersaturation ratios for calcium oxalate and brushite are substantially increased (349) (Fig. 74-16). Urinary citrate and magnesium excretion also rise during pregnancy, but these increments are not enough to counter the marked increases in the supersaturation ratios (349). The answer, therefore, as to why there are no striking increases in stone disease during gestation may lie in the increased production and excretion of certain glycoproteins and of the Tamm-Horsfall protein, which decrease crystal aggregation and growth, thus inhibiting stone formation (346,350).

Presentation of the disease resembles that in nongravid subjects, the most common symptom being flank pain (about 89%), and more than 90% display either microscopic or macroscopic hematuria (351). Most stones passed during pregnancy contain calcium salts, others are infectious in origin ("struvite"), and very few are of the uric acid or cystine variety (42,346). The older literature anecdotally stressed the dramatic complications associated with obstruction or infection, whereas more recent reports describe more benign courses, except for increases in urinary tract infection and perhaps spontaneous abortion (42,346,347). Gestation does not appear to affect the course of urolithiasis, and there is even the impression that mobilized stones pass more easily because the ureters are dilated.

It should be stressed that the previous summary relates primarily to women with noninfectious calcium oxalate stones, whose course is also relatively benign in nonpregnant populations. Little is known, however, of the natural history of the more serious struvite stones during pregnancy. Experience with

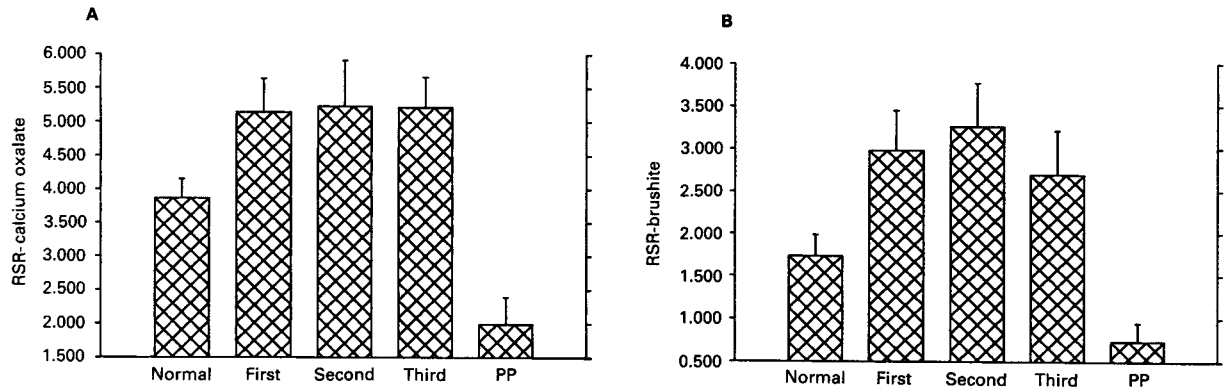

FIGURE 74-16. Relative saturation ratios for calcium oxalate (**A**) and brushite (**B**) during each trimester of pregnancy and postpartum (*PP*), as well as in a population of healthy women (normal). $p < 0.01$ for both comparisons. (From: Maikrantz P, et al. Gestational hypercalciuria causes pathological urine calcium oxalate supersaturation. *Kidney Int* 1989;36:108, with permission.)

women who have cystinuria and conceive is also limited, although the results of one small series appear reassuring (352). In the latter disease, it is important to ensure hydration early in pregnancy when women are frequently nauseous, and thus far no adverse fetal effects have been ascribed to penicillamine (FDA pregnancy safety category D). There is virtually no information relating to uric acid stones, although acute renal dysfunction secondary to hyperuricemia has been described in pregnancy (353).

When stone disease is suspected, pregnancy should not be a deterrent to x-ray examination. A routine pyelogram involves about 0.4 rad, and exposure can be minimized further by restricting the number of films or using spiral computed tomography (354). The diagnostic sensitivity of ultrasonography may be markedly improved when combining abdominal and vaginal technology (the latter to identify ureteral "jets") and determining resistance (355). If the stone obstructs the ureter, surgery may be necessary, and in this respect, about 30% of the patients described in the literature have undergone an operative procedure such as open lithotomy or percutaneous nephrostomy (42,346,356,357). Rigid ureteroscopy performed under general anesthesia has also been used in pregnancy (346,358). Currently, however, the placement of ureteral stents under ultrasonic guidance, a less invasive technique, has made it possible to manage gravidas through gestation and to defer more definitive surgical procedures until after delivery (24,42,346,359). Because there are very few data on lithotripsy during gestation, the procedure is best avoided. Finally, as noted previously, urinary tract infection in the presence of nephrolithiasis requires prolonged treatment (about 3 weeks), followed by suppressive therapy through the immediate puerperium, because the calculus represents a nidus of infection resistant to sterilization.

Guidelines on Managing Women with Preexisting Renal Disorders

Counseling

When counseling women on the advisability of conception or whether to continue a gestation already in progress, neither answers nor decisions come simply. These patients have high expectations, and the desire for motherhood is so strong as to tempt the sympathetic physician to take unwarranted risks. In addition, patients conceive against advice and refuse termination for religious, moral, or emotional reasons, in which case, both nephrologist and obstetrician (preferably a maternal-fetal-medicine subspecialist) must be ready to manage a difficult gestation and be prepared for serious complications.

We advise against attempting a pregnancy when serum creatinine levels exceed 1.4 mg/dL (124 μmol/L), but some permit gestation with preconception levels of up to 2 mg/dL (177 μmol/L), particularly in women with a single kidney, in those normotensive without treatment, and in those whose disorders are primarily interstitial in nature. As for blood pressure control, diastolic levels (Korotkoff V sound) should be 90 mm Hg or less.

In addition to the standard laboratory tests that permit the early detection of renal deterioration, the *database* should include serum uric acid and albumin levels, oxaloacetic and pyruvate transaminases, lactic acid dehydrogenase, and a platelet count, tests that will later aid in the differential diagnosis of an exacerbation of the renal disorder versus superimposed preeclampsia (Chapter 53). We suggest biweekly visits, primarily to the obstetrician's clinic or office until gestational week 32, after which the patients are examined like all gravidas on a weekly basis. Renal parameters (primarily serum creatinine levels or its clearance) should be assessed every 4 to 6 weeks, unless more frequent evaluations become necessary. Fetal surveillance with electronic monitoring should be instituted after the thirty-second week, and even as early as the twenty-eighth to thirtieth week in frankly nephrotic patients.

Nephrotic gravidas are, in general, not prescribed diuretics because they may be oligemic, and further intravascular volume depletion may compromise uteroplacental perfusion. Furthermore, both serum albumin concentrations and blood pressure normally decrease during gestation, in which case diuretics may be more prone to precipitate renal failure, circulatory collapse, or thromboembolic episodes. This is a relative recommendation, however, because there are nephrotic patients whose kidneys retain salt avidly and thus develop volume-induced hypertension. This is especially true in diabetic nephropathy, in which judicious use of diuretics may prevent or treat the presentation or aggravation of hypertension after midpregnancy and help avoid the early termination.

The treatment of hypertension in pregnancy is discussed elsewhere (360–362, Chapter 53). We use methyldopa, the preferred drug in the National High Blood Pressure Education Program's (NHBPEP) "consensus" report (360,362), because its safety during gestation is attested to by more than 25 years of postmarketing surveillance and several controlled trials; it also remains the only blood pressure medication for which there is a 7.5-year follow-up of the neonate. Currently, many hypertensive and diabetic patients who conceive are receiving either ACE inhibitors or angiotensin receptor blockers (ARBs). These drugs may cause fetopathies (sometimes irreversible), neonatal oliguric or anuric ARF, and possibly fetal anomalies (360,363). The major adverse events seem to only be associated with fetal exposure after midpregnancy, while anomolies, principally of the CNS or cardiovascular system, were rare and noted in but a single report whose authors linked prescriptions and outcome in a large data base. If discontinuing the drug prior to a planned conception may compromise management, particularly of hypertensive diabeties and some patients with proteinuric renal disease, we recommend continuing ACE inhibitor or ARB therapy until conception is confirmed and then immediately discontinuing their use. This is because major adverse events are associated with fetal exposure after midpregnancy while anomaly risks are small compared to those associated with poorly controlled blood pressure and proteinuria prior to conception. The patient however, should be informed of the various options. These medications however, are contraindicated, particularly after mid-pregnancy. Of note, although the NHBPEP report suggested that pregnant patients with chronic hypertension need not be treated until their diastolic levels reach 100 mm Hg or more (Korotkoff V sound), women with underlying renal disorders should receive therapy when these values are 90 mm Hg or more.

Many nephrologists have treated patients with renal disease, including nephrotic patients, with protein-restricted diets (Chapter 101), but we caution against such a policy in pregnant patients, because little is known regarding fetal outcome in protein-restricted women. This is because of reports (whose citation and discussion is beyond the scope of this chapter) suggesting that protein restriction of gravid animals retards the development of the fetal kidney, impairs microvascular function, adversely effects insulin signaling, and is associated with hypertension in the offspring.

In the final analysis, GFR and blood pressure remain the two maternal parameters that determine the course of the gestation. Renal functional deterioration and the appearance or rapid acceleration of hypertension are best evaluated in a hospital setting, preferably one with an obstetrical tertiary care facility. It is important to remember that a decrement in creatinine clearance of 15% to 20% may occur normally near term, and increased proteinuria in the absence of hypertension need not cause alarm. In such circumstances, hospitalization is

not necessary. When functional deterioration occurs earlier in pregnancy, there are no definite guidelines that permit prediction of postpartum reversibility, and the decision to terminate is always difficult. This is especially true when the gestation is approaching its twenty-fourth week, and 2 to 4 more weeks could improve substantially the chance for fetal survival. We usually recommend termination when serum creatinine levels have increased by 1 mg/dL, unless the values are still less than 2 mg/dL. Clearly this is an area in which more information is required.

Hemodialysis and Peritoneal Dialysis

Guidelines for the initiation and management of dialysis during pregnancy are detailed in several reviews (208–210,283,364). These patients are often rigidly protein restricted when not pregnant, but after conception, their protein intake should be liberalized (to at least 1.5 g/kg), especially in patients undergoing peritoneal dialysis (365,366). The concept of initiating early dialysis or increasing its frequency is based on the unproven but plausible hypothesis that fetal outcome is poorer in the face of marked azotemia even when uremic symptoms are not evident, and that fetal survival will improve in a less azotemic intrauterine environment. Thus, we recommend that renal replacement therapy be initiated when serum creatinine levels reach 5 to 6 mg/dL or if blood urea nitrogen levels are 60 mg/dL or more (208–210,283). Such a policy appears to facilitate volume and blood pressure control and permits a more liberal diet. Therapeutic goals include maintenance of predialysis blood urea nitrogen level at 50 mg/dL or less (17.9 mmol/L) while avoiding both hypotension and hypertension and rapid volume changes and maintaining pH and electrolyte levels near pregnancy norms (Table 74-1). For example, dialysate bicarbonate levels have to be reduced to avoid alkalosis, and after midpregnancy, the average 0.5 kg per week weight gain should be taken into account when considering dry weight. These goals usually require daily dialysis.

Some of the problems encountered in these women include the following. Dialysis may induce uterine contractions, and it is prudent to initiate intradialytic uterine monitoring with dialysis after the twenty-fifth gestational week; the placenta hydroxylates vitamin D and increasing the frequency of dialysis may lead to hypophosphatemia. Thus, the vitamin D analog and phosphate binder dosing, as well as the dialysate Ca concentration, may all need to be reduced to avoid hypercalcemia. Pregnancy is a hypercoagulable state, and intradialysis heparin requirements may be difficult to adjust. Because pregnant women tolerate infection poorly, one should be extremely vigilant with patients receiving peritoneal dialysis. Some of the advantages of increasing the weekly dialysis prescription may be offset by increased loss of potassium, minerals, and water-soluble vitamins, often-requiring nutritional supplementation and increases in dialysate potassium levels. Erythropoietin requirements increase markedly, and such needs may be the first indication that a patient on dialysis is pregnant (367). This drug seems to be well tolerated during gestation, (although data are limited), and may be even indicated in women with moderate renal insufficiency and severe anemia but not requiring dialysis (208–210,282,283).

Finally, as noted, pregnancy outcome in these women is poor and there are considerable maternal risks such as accelerated hypertension, cardiac decompensation, peritoneal bleeding (including venous erosion by the peritoneal catheter), and death (210,281,368). However, there are recent indications that prognosis may be improving, especially when the above-mentioned guidelines are adhered to (281–283). In this respect, a dialysis in pregnancy registry is maintained by Dr Hou at the Division of Nephrology, Loyola Stritch School of Medicine, Chicago, IL, who can be contacted for up-to-date information.

Renal Transplantation

Transplantation reverses the abnormal reproduction associated with advanced renal disease. Ovulation and menses resume, and it has been estimated that as many as 1 of every 50 women of childbearing age with a functional kidney transplant become pregnant (369). Thus, it is not surprising that after a 1963 report of a successful pregnancy in a recipient of a renal allograft (370), thousands more followed. In many of these instances, the patient was unaware that she was fertile, suggesting a failure on the part of physicians to counsel these women properly.

In one extensive review of the literature through 1993, Davison (371) documented 3,382 gestations in 2,409 recipients of a renal allograft (it is our experience that many more are never reported). Most of these patients received azathioprine and prednisone, although experience with cyclosporine was then quite limited and newer immunosuppressive drugs (e.g., tacrolimus, mycophenolate mofetil) were yet to be used for the management of transplant recipients. About 35% of the pregnancies did not go beyond the first trimester, about 15% due to spontaneous miscarriages (an incidence similar to that of normal pregnant populations), and the remainder to selective inductions, often for nonmedical reasons. There was a 0.4% incidence of ectopic pregnancy. More encouraging, 93% of the gestations that continued beyond the first trimester succeeded, kidney prognosis and gestational outcome appearing to parallel those of pregnancy in women with preexisting renal disease in that the better the renal function before conception, the better the results. As expected, prognosis was best when the transplanted kidney came from a living related donor.

Many women with renal transplants experience increases in GFR during pregnancy, despite the presence of a single functioning kidney that often has undergone some degree of chronic rejection (64,65). The increments, however, are smaller than those observed in normal gestations. On the other hand, functional declines, usually in late gestation, are reported in about 15% of the patients, most frequently in the patients with the poorest prepregnancy creatinine levels. Fortunately, these are transient in most instances (42,371). Proteinuria also increases during these pregnancies, often to abnormal levels (63,371,372), but in most instances, these increments are functional in nature and unrelated to the health of the allograft.

This optimistic depiction must be tempered somewhat, because despite a high success rate, there are numerous maternal and fetal problems (18,371,373–375). These include steroid-induced hyperglycemia, leukopenia, serious infections including sepsis, uterine rupture, and even death in the mother. Hypertension complicates 30% of the gestations, and in such situations, plasma uric acid levels and protein excretion are not useful markers for diagnosing the presence of superimposed preeclampsia. This is because in allograft recipients, both indices could be above the norm at any stage of the gestation in otherwise uncomplicated pregnancies. There is also evidence that diabetic gravidas with renal allografts fare much worse than their nondiabetic counterparts, but the outlook may be better in patients who received combined pancreas and kidney transplants (371,376–378).

There are additional problems with the fetus and neonate as well (371,373,374). The incidence of prematurity, growth restriction, and low-birth-weight infants is increased, and congenital anomalies, serious infections, thrombocytopenia, hypoadrenalism, and hepatic insufficiency have been reported. In addition, there are few, if any, long-term follow-up studies of the neonates, as periodic reports of chromosomal damage, as well as subtle immunologic abnormalities (secondary to immunosuppression?) have appeared in the literature (371,379).

Finally, as mentioned, the surveys on which our summary is based primarily reflect results in patients managed with

azathioprine and steroids alone, whereas virtually all allograft recipients now receive cyclosporine, tacrolimus, or mycophenolate, and newer agents are introduced all the time (380–382). In this respect, reports are generated periodically by a National Transplantation Pregnancy Registry, directed by Dr V. Armenti at Temple University, Philadelphia, PA (373,377,383), and a similar registry exists in the United Kingdom (384). Reports from the former registry that include several hundred new patients, suggest outcomes similar to those in the review by Davison, although it appears that patients receiving cyclosporine and tacrolimus are at greater risk for hypertensive complications, growth restriction, and prematurity. For patients receiving cyclosporine this may reflect the fact that they tend to have more chronic hypertension and higher creatinine levels when they conceive.

Effect of Pregnancy on Allograft Survival

There is a continuing controversy regarding the effect of pregnancy on the natural history of the renal allograft. Concerns that gestation might jeopardize the long-term health of the graft was revived in 1993 by a limited but well-designed case-control study by Salmela et al. (385). They described 29 posttransplantation pregnancies in 22 women, each matched according to original disease, type of donor, immunosuppression, serum creatinine level at conception, and interval from transplantation to pregnancy. Despite evidence of well-preserved renal function (serum creatinine averaging 1.1 mg/dL or 109 μmol/L before first gestation), the women who conceived had a lower 10-year graft survival rate (69% versus 100%; $p < 0.005$) compared with women who never did. This report rang alarm bells in transplant centers throughout the globe, even though we noted the atypical character of this study (386), in which the different results were primarily due to a highly unusual outcome in the controls, who in 10 years of follow-up had no graft failures! This is certainly contrary to the experience of most centers, in many of which statistics resemble (and may even be poorer than) those of the transplanted group who had conceived. Furthermore, the findings of Salmela et al. are at odds with results from the registry of the European Dialysis and Transplant Association (387) and other more recent reports (64,388,389). Most interesting among the latter is a survey by Sturgiss and Davison (388,390) of 18 women who had 34 pregnancies after transplantation, and that contained a considerable number of patients in the poorer prognostic category (e.g., moderate renal insufficiency, longstanding diabetes, and hypertension) (390). These women, too, were matched in case-control fashion to an equal number of women who had never conceived. There were no significant differences in GFR (measured by infusion techniques) or in the prevalence of hypertension between those who had conceived and those who had not, with the posttransplantation follow-up in each group averaging 12 years (Fig. 74-17). In summary, as of 2006, we believe there is no need to alter our generally optimistic counseling practices, although we do apprise the patients of the contrary findings of Salmela et al. (385). This is another area in which prospective data are needed.

Management Guidelines

Pregnancy in allograft recipients has been reported since the 1960s, but only recently did the American Transplant Associations convene a consensus group whose recommendations appeared in 2005 (369,391). Our guidelines are as follows:

1. Patients with transplants should wait 1 year before attempting conception. The reason for this are that outcomes appear improved when renal function is stable and well preserved without a recent rejection episode, or the need for frequent adjustment of the immunosuppressive prescription, and the women are remote from the risk of a cytomegalovirus infection (that may require teratogenic medications); all these criteria are usually achieved by 1 year posttransplantation.
2. Serum creatinine levels should be no more than 2 mg/dL (177 μmol/L), and preferably less than 1.5 mg/dL (133 μmol/L), without evidence of rejection, pelvocaliceal dilation, or infection. Proteinuria should be absent or minimal. The acceptance of higher initial serum creatinine levels by some is based on the assumption that in patients receiving cyclosporine, part of the functional loss is due to renal vasoconstriction.
3. Hypertension should be absent or easily managed.
4. Prednisone dosage should be less than or equal to 15 mg per day, and azathioprine 2 mg/kg per day or less. The azathioprine dose is based on limited data suggesting that fetal anomalies do not occur until more than 2.2 mg/kg per day is prescribed (392). We strive to maintain cyclosporine and tacrolimus levels of 100 to 159 and 6 to 10 ng/mL, respectively, and avoid use of sirolimus or mycophenolate (based on both the very limited experience and/or anecdotal reports concerning use of these latter two agents in pregnant women).

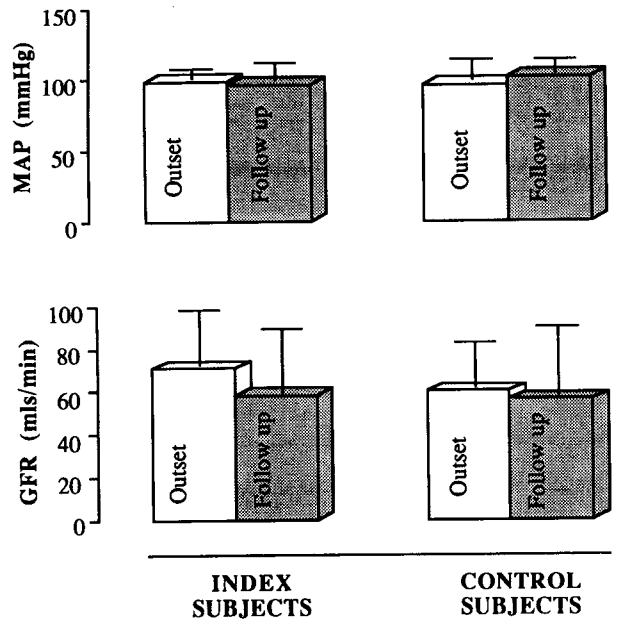

FIGURE 74-17. Mean arterial pressure (MAP) (**upper panel**) and glomerular filtration rate (GFR) measured as the clearance of inulin (**lower panel**) in renal transplant recipients who underwent pregnancies (**left**), compared with a control group who had never conceived. Open bars are at outset and closed bars are at follow-up studies. (From: Dr. J. M. Davison, with permission.)

Antenatal management is in general similar to that outlined for women with preexisting renal disease. Tests of liver function, calcium and phosphate levels, and red and white blood cell counts should be done at about 6-week intervals, because the liver of gravidas may be more sensitive to the hepatotoxic effect of azathioprine, and decreasing white blood cell counts may be predictive of neutropenia and thrombocytopenia in the newborn (both problems managed by decreasing the dose) (371). Monitoring calcium and phosphate levels is important because some women have had a subtotal parathyroidectomy, although others may manifest tertiary hyperparathyroidism. The hemogram is primarily monitored to detect marrow suppression secondary to the immunosuppressive therapy, but there are also data suggesting that the erythropoietin response to anemia may be suboptimal in these gestations (393). Because most

patients receive steroids, they should also be screened for glucose intolerance during each trimester. Screening for toxoplasmosis and cytomegalovirus during each trimester, as well as for herpes simplex virus near term, is also advisable, although outcome studies to determine whether these policies are effective are sparse or lacking. Any evidence of graft dysfunction should be investigated aggressively; renal biopsy is often necessary, given the difficulties in distinguishing among calcineurin inhibitor toxicity, acute rejection, or the appearance of preeclampsia by clinical and laboratory test criteria alone.

Gestations in transplant recipients are obviously "high risk" and require close prenatal and perinatal care. All pelvic examinations should be performed with strict aseptic techniques. The transplanted kidney is in the false pelvis and is not apt to obstruct the birth canal. Routine cesarean sections are therefore unnecessary, and the procedure is reserved for traditional indications. In this respect, many transplant patients have or have had pelvic osteodystrophy related to their previous renal failure or prolonged steroid therapy, which is of a severity that mandates an operative delivery (371). Regarding breast-feeding, steroids are secreted into breast milk but in the usual therapeutic doses are not sufficient to affect the infant. Metabolites of both azathioprine and cyclosporine also appear in breast milk (371). Their levels, too, are minimal, but in the absence of definitive data, we tend to discourage breast-feeding.

There are few guidelines concerning contraception in allograft recipients, a question frequently raised at counseling sessions or at the postpartum visit. The efficacy of intrauterine contraceptive devices (few are available in the United States) may be reduced because of the immunosuppressive and antiinflammatory agents these patients ingest (371). These devices may also aggravate menstrual problems and obscure signs and symptoms in early pregnancy, and their insertion and replacement is associated with transient bacteremia, which is an additional concern in immunosuppressed patients. Oral contraceptives may cause or aggravate hypertension or thromboembolism and may produce subtle changes in the immune system. Use of preparations with low estrogen content seems to have reduced these problems, but these drugs are yet to be systematically tested in transplant populations. Still, some prescribe them to allograft recipients, in preference to the less efficacious barrier method (371).

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Space limitations dictate restriction of the reference list; we have therefore focused on citations during the past 25 years. More extensive literature surveys exceeding 2,000 citations can be found in texts and chapters by Chesley (25), Lindheimer and Katz (2,18), Lindheimer et al. (394), and Sheehan and Lynch (4).

CHAPTER 75 ■ MONOCLONAL GAMMOPATHIES: MULTIPLE MYELOMA, AMYLOIDOSIS, AND RELATED DISORDERS

PIERRE M. RONCO, PIERRE AUCOUTURIER, AND BÉATRICE MOUGENOT

Monoclonal proliferations of the B-cell lineage, often referred to as plasma cell dyscrasias, are characterized by abnormal and uncontrolled expansion of a single clone of B cells at different maturation stages, with a variable degree of differentiation to immunoglobulin (Ig)-secreting plasma cells. Therefore, they are usually associated with the production and secretion in blood of a monoclonal immunoglobulin (Ig) and/or a fragment thereof. A decreased production of normal polyclonal Ig may occur, thereby favoring infections. An ominous consequence of secretion of monoclonal Ig products is their deposition in tissues. These proteinaceous deposits can take the form of casts (in myeloma cast nephropathy [CN]), crystals (in myeloma-associated Fanconi's syndrome [FS]), fibrils (in light-chain [LC] and exceptional heavy-chain [HC] amyloidosis), or granular precipitates (in monoclonal Ig deposition disease [MIDD], (Table 75-1). They may disrupt organ structure and function, inducing life-threatening complications. In a large proportion of patients with crystals, fibrils, or granular deposits of Ig products, major clinical manifestations and mortality are related to visceral Ig deposition rather than to expansion of the B-cell clone. Indeed, except for myeloma CN, which is generally associated with a large tumor mass malignancy, Ig precipitation or deposition diseases often occur in the course of a benign B-cell proliferation or of a smoldering or low-mass myeloma.

The presence of abnormal urine components in a patient with severe bone pain and edema was first recognized in the 1840s by Henry Bence Jones (1) and William MacIntyre (2), who described unusual thermal solubility properties of urinary proteins, far later attributed to Ig LCs (3). To perpetuate this discovery, monoclonal LC proteinuria is often referred to as *Bence Jones proteinuria*. This term is not appropriate because less than 50% of LCs do show thermal solubility. Renal damage characterized by large protein casts surrounded by multinucleated giant cells within distal tubules was identified in the early 1900s and termed *myeloma kidney*. This term must, however, be abandoned because CN with acute renal failure may occasionally occur in conditions other than myeloma and because other patterns of renal injury were subsequently found in patients with myeloma. The first of these was amyloidosis, wherein tissue deposits are characterized by Congo red binding and fibrillar ultrastructure. In 1971, Glenner et al. (4) showed that the amino acid sequence of amyloid fibrils extracted from tissue was identical to the variable region of a circulating Ig LC, thereby providing the first demonstration that an Ig component could be responsible for tissue deposition. The spectrum of renal diseases due to monoclonal Ig deposition has expanded dramatically with the advent of routine staining of renal biopsy specimens with specific anti-κ and anti-λ LC antibodies, and of electron and immunoelectron microscopy (Table 75-1). These morphologic techniques associated with more sensitive and sophisticated analyses of blood and urine monoclonal components have led to the description of new entities, including nonamyloid monoclonal LC deposition disease (LCDD) (5,6), HC (or AH) amyloidosis (7), nonamyloid HC deposition disease (HCDD) (8,9), chronic lymphocytic leukemia (CLL)–associated glomerulopathies with organized deposits (10,11), and proliferative glomerulonephritis with monoclonal IgG deposits (12). All of these pathologic entities principally involve the kidney, which appears as the main target for deposition of monoclonal Ig components. This is not only explained by the high levels of renal plasma flow and glomerular filtration rate (GFR), but also by the sieving properties of the glomerular capillary wall and by the prominent role of the renal tubule in LC handling and catabolism (13).

Polymorphism of renal lesions may be due to specific properties of Ig components influencing their precipitation, their interaction with renal tissue, or their processing after deposition. Alternatively, the type of renal lesions may be driven by the local response to Ig deposits, which may vary from one patient to another. That intrinsic properties of Ig components are responsible for the observed renal alterations was first suggested by *in vitro* biosynthesis of abnormal Ig by bone marrow cells from patients with lymphoplasmacytic disorders and visceral LC deposition (14) and by recurrence of nephropathy in renal grafts (15,16). A further demonstration of the specificity of Ig component pathogenicity was provided by Solomon et al. (17). They showed that the pattern of human renal lesions associated with the production of monoclonal LC, that is, myeloma CN, LCDD, and LC (or AL) amyloidosis, could be reproduced in mice injected intraperitoneally with large amounts of LCs from patients. The good correlation between experimental findings and human lesions observed at biopsy or autopsy led to the conclusion that physicochemical or structural properties of LCs might be responsible for the specificity of renal lesions.

A normal Ig is composed of two LCs and two HCs. LCs and HCs are themselves made up of so-called constant (C) and variable (V) globular domains. Whereas a limited number of genes encode the constant region, multiple gene segments are rearranged to produce a variable domain unique to each chain. Diversity is further amplified by mutations and variations of the linking peptide segment. Although LCs (and HCs) have many structural similarities, they also possess a unique sequence that may be responsible for physicochemical peculiarities, hence their deposition in tissue or interaction with tissue constituents. A number of structural and physicochemical abnormalities of Ig have already been described (reviewed in 18,19). They include deletions of C_H domains in HCDD (8,9) and HC amyloidosis (7), shortened or lengthened LCs and abnormal LC glycosylation in LCDD (14,20), and resistance to proteolysis of the V_L fragment in FS (21). Moreover, overrepresentation of certain V_L gene subgroups was also reported in amyloidosis (22,23) and LCDD (24). The mechanisms generating Ig diversity may randomly create HCs or LCs with peculiar

TABLE 75-1

PATHOLOGIC CLASSIFICATION OF DISEASES FEATURING TISSUE DEPOSITION OR PRECIPITATION OF MONOCLONAL IMMUNOGLOBULIN-RELATED MATERIAL

Organized			Nonorganized	
Crystals	Fibrillar	Microtubular	MIDD ("randall type")	Other
Myeloma cast nephropathy[a] Fanconi's syndrome Other (extrarenal)	Amyloidosis (AL, AH) Nonamyloid	Cryoglobulinemia kidney Immunotactoid	LCDD LHCDD HCDD	Proliferative GN with monoclonal IgG Crescentic GN (IgA or IgM)

AH, heavy-chain amyloidosis; *AL*, light-chain amyloidosis; *GN*, glomerulonephritis; *HCDD*, *LCDD*, *LHCDD*, *MIDD*, heavy-chain, light-chain, light- and heavy-chain, monoclonal immunoglobulin deposition disease.
[a] Crystals are predominantly localized within casts in the lumen of distal tubules and collecting ducts, but may also occasionally be found in the cytoplasm of proximal tubule epithelial cells.
(Adapted from: Preud'homme JL, Aucouturier P, Touchard G, et al. Monoclonal immunoglobulin deposition disease (Randall type): relationship with structural abnormalities of immunoglobulin chains. *Kidney Int* 1994;46:965, with permission.)

properties such as proneness to deposition, whereas mistakes in the rearrangement or hypermutation processes may result in altered genes encoding truncated Ig. It must be stressed, however, that some abnormal Ig chains produced in immunoproliferative disorders are not associated with any special clinical features. Conversely, structural abnormalities of LCs are not a constant feature of diseases associated with LC deposition. These observations suggest the need to increase the number of nephritogenic Ig components to be analyzed at the complementary DNA (cDNA) and protein levels.

Myeloma- and AL amyloidosis-induced renal failure accounts for less than 2% of the patients admitted to a chronic dialysis program each year. This is due in part to the relative rarity of these immunoproliferative diseases, but also to a deteriorated clinical condition of patients at the time of end-stage renal disease. A dramatic effort of prevention must therefore be carried out, relying in part on a better understanding of the structural and physicochemical properties of Ig components leading to deposition or precipitation in tissues. Any progress in this field may also enlighten the pathogenesis of immunologically mediated renal diseases, especially glomerulonephritides, because properties of monoclonal Ig components favoring their deposition may apply as well to polyclonal Ig involved in the formation of immune complexes. This is exemplified by type II mixed cryoglobulins that both contain a monoclonal Ig and form circulating immune complexes.

Because of the correlations already established between pathologic entities and physicochemical or binding properties of Ig components, and because of the lack of specificity of clinical manifestations such as acute renal failure or the nephrotic syndrome, we have classified the various forms of renal involvement in monoclonal gammopathies according to the lesions observed in renal biopsy specimens. We indeed consider that elucidation of the pathophysiologic mechanisms responsible for each type of lesion should result in the identification of patients at risk for this lesion and in the design of new therapeutic strategies.

The majority of patients (63% in a series of 87) with serum and/or urine monoclonal gammopathy who undergo renal biopsy have disease unrelated to monoclonal gammopathy deposition (25). Therefore, the diagnosis of virtually all of the entities to be discussed is critically dependent on the inclusion of κ and λ in the standard of immunofluorescence stains. In some of the rarer entities, a more refined and precise diagnosis can be made with immunofluorescence staining for the subclasses of IgG. Collectively these stains may demonstrate light chain isotype restriction and γ-heavy chain subclass restriction, which strongly favors, but does not definitely prove, the presence of a monoclonal Ig. Demonstration of monoclonality requires serum and urine studies by immunoelectrophoresis or immunofixation.

MYELOMA-ASSOCIATED TUBULOPATHIES

The prevalence of tubular lesions or dysfunction in patients with myeloma is difficult to assess because most patients do not undergo a renal biopsy, but it is most likely high and.can be approached histologically and functionally. In Ivanyi's necropsy study including immunofluorescence,18 of 57 patients (32%) had CN, whereas 6 (11%) had renal amyloidosis and 3 (5%) had κ-LCDD. The higher prevalence of CN (30%) was confirmed in the more recent autopsy series of Herrera (26). Tubular alterations are also demonstrated by increased urinary concentrations of the low-molecular-weight proteins normally reabsorbed by the proximal tubule (27), increased urinary elimination of the tubular lysosomal enzyme β-acetyl-D-glucosaminidase (27), and frequent abnormalities in renal tubular acidifying and concentrating ability (28) in patients with LC proteinuria. However, myeloma-associated FS remains an exception; it was not detected in the 42 myeloma patients whose tubular function was systematically studied by Coward et al. (29).

CN is not only the most frequent lesion in myeloma patients, it is also the major cause of renal failure, which is observed in about 25% of patients with multiple myeloma (30). In nephrology departments that usually receive only myeloma patients with severe renal abnormalities, the prevalence of CN assessed histologically varies from 63% to 87% (31–34) among the myeloma patients with renal failure. This prevalence is most likely underestimated because patients with presumed CN do not systematically undergo a renal biopsy, whereas those exhibiting significant albuminuria or *a fortiori* the nephrotic syndrome do, especially in the absence of amyloidotic deposits in nonrenal biopsy specimens. In myeloma patients with an albumin urinary output of less than 1 g per/day, there is a good correlation between the diagnosis of CN and renal failure.

Of note, CN may occur in other immunoproliferative disorders featuring urinary LC excretion including Waldenström's macroglobulinemia (35) and μ-HC disease (36). In a case of μ-HC disease, the urinary secretion of large amounts of free κ-chain was responsible for acute renal failure with a typical histologic presentation of "myeloma kidney" (36). In a case of Waldenström's macroglobulinemia, acute renal failure was

related to cast nephropathy due to (urinary) secretion of a free λ LC (35). CN was also reported in renal allograft recipients treated with tacrolimus plus rapamycin (37).

Myeloma Cast Nephropathy

Pathophysiology of Myeloma Cast Nephropathy

CN occurs mainly in patients with myeloma with a high LC secretion rate (although the pathogenic character of LC rate is much variable between individuals). That LCs are the main culprits is supported also by the following clinical, pathologic, and experimental data:

1. Renal lesions may recur on grafted kidneys (38).
2. Similar crystals may occasionally be seen within casts, proximal tubule cells, and plasma cells (39). Their usual lack of staining with anti-LC antibody is most likely due to degradation or masking of the relevant epitopes.
3. Mice injected with LC purified from patients with CN developed extensive cast formation in the distal renal tubules (17,40).

However, a number of patients produce large amounts of LCs and yet fail to present significant signs of renal involvement throughout the course of the disease. This may be related to the absence of enhancing factors such as hypercalcemia, low urinary output and high urinary solute concentration, low urinary pH, and injection of contrast medium, but this also suggests that some LCs may be particularly prone to induce renal lesions, especially cast formation.

Because lesions observed in CN associate cast formation with proximal tubule cell lesions, it is usually believed that LCs are directly toxic to epithelial cells, resulting in decreased proximal reabsorption of the LCs and increased delivery to the distal tubule in which they coprecipitate with Tamm-Horsfall protein (THP). Tubular obstruction by large and numerous casts may also contribute to the development of tubular lesions. For clarity, we will analyze separately the pathogenesis of proximal tubule lesions that result from renal metabolism of LCs, the mechanisms of cast formation, and the respective role of tubular obstruction and tubular lesions in the genesis of renal failure (Fig. 75-1). This mode of presentation is also warranted by the observation that certain LCs damage the proximal tubule, whereas others precipitate in the distal tubule, obstructing the nephron, when perfused in rat nephrons *in vivo* (41).

Renal Metabolism of Light Chains and Pathogenesis of Proximal Tubule Lesions. Normal as well as malignant plasma cells can secrete free LC, in addition to complete Ig molecules; the amount of free LC secretion is highly variable, depending on the variable (V_L) domain structure (42). The LCs are normally filtered by the glomerulus and then reabsorbed by the proximal tubule. Lambda and to a lesser extent, κ-LCs circulate mainly as covalently linked dimers that have a mass-restricted glomerular filtration. In normal individuals, several hundred milligrams per day of circulating free polyclonal LCs are filtered by glomeruli and more than 90% of these are reabsorbed and catabolized by proximal tubular cells (13,43,44). LCs bind to a single class of low-affinity, high-capacity noncooperative binding sites described by Batuman et al. on both rat and human kidney brush-border membranes (45). These sites exhibit relative selectivity for LCs compared with albumin and β-lactoglobulin. It has been shown that LCs could bind to cubilin (46), a multi-ligand receptor belonging to the large family of low-density lipoprotein receptors and located in the intermicrovillar areas of the brush border. After binding to the luminal domain of proximal tubular epithelial cells, LCs are incorporated in endosomes that fuse with primary lysosomes where proteases, mainly cathepsin B, degrade the proteins into amino acids, which are returned to the circulation by the basolateral route. Decreased LC reabsorption can be induced by infusion of cationic amino acids that compete with the LC-binding sites (47) and by impairment of proximal tubule function.

When the concentration of filtered LCs is increased as in myeloma patients, profound functional and morphologic alterations of proximal tubule epithelial cells may occur. The functional disturbances include low-molecular-weight proteinuria (27,29) and inhibition of sodium-dependent uptake of amino acids and glucose by brush-border preparations (48). Furthermore, in human proximal tubule cells, endocytosis of LCs was shown to induce cytokines including interleukin (IL)-6, IL-8 and monocyte chemoattractant protein-1 (MCP-1) through activation of NF-κB (49). Increased cytokine production may be a major mechanism mediating tubulointerstitial injury and progressive kidney disease in some patients with myeloma. Morphologically, some of the LCs infused in mice (40) or rats (50) or perfused in rat nephron *in vivo* (41,51) accumulated in endosomes and lysosomes of the proximal convoluted tubule and sometimes of the distal convoluted tubule (40). In a rat model of myeloma, crystalloid formations were frequently seen within phagolysosomes in proximal tubule cells, and lysosomes were markedly enlarged and distorted (52). Activation of the endosome/lysosome system was associated with mitochondrial

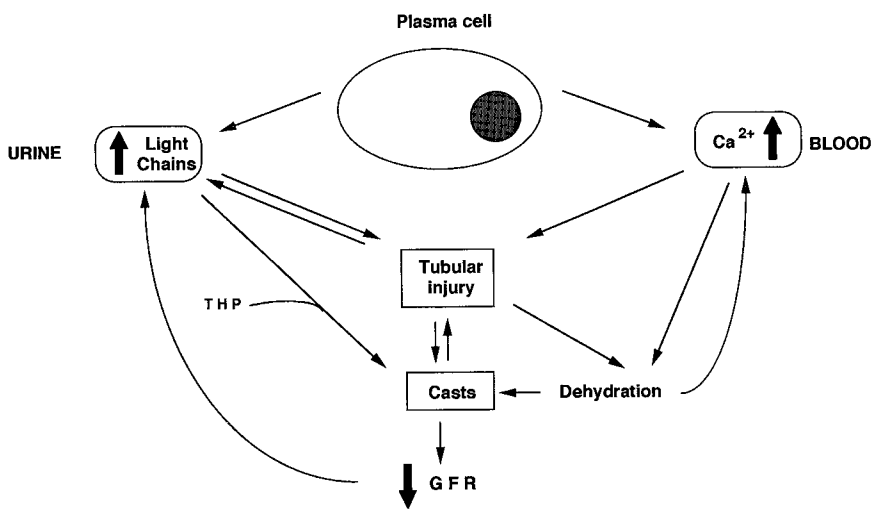

FIGURE 75-1. Schematic representation of the pathogenesis of myeloma cast nephropathy. *GFR*, glomerular filtration rate; *THP*, Tamm-Horsfall protein. (Adapted from: Winearls CG. Nephrology forum: acute myeloma kidney. *Kidney Int* 1995;48:1347.)

alterations (50), prominent cytoplasmic vacuolation, focal loss of the microvillus border, and epithelial cell exfoliation (51).

These experiments thus establish that the proximal tubule epithelium is a main target of LC toxicity, but that not all LCs are toxic to this epithelium. However, these results must be interpreted with caution. In most cases, large amounts of LCs were injected or microperfused. Moreover, clinical characteristics and renal lesions of the patients from whom LCs were obtained were defined poorly or not all. It might well be that some of the LCs, especially those prone to form crystals, were produced by patients with FS. Conversely, these experiments suggested that the proximal tubule endocytotic and lysosomal system might be overwhelmed when large quantities of LCs were filtered, thereby allowing LCs to proceed to the distal nephron.

Pathogenesis of Cast Formation. Because myeloma casts are composed principally of the monoclonal LC and THP, it has long been hypothesized that interaction of these two proteins was a key event in cast formation. THP is a highly glycosylated and acidic protein (isoelectric point [pI] = 3.2) synthesized exclusively by the cells of the ascending limb of the loop of Henle (53). It is the major protein constituent of normal urine, and an almost universal component of casts. This 80-kDa protein is also remarkable for its ability to form reversibly high-molecular-size aggregates of about 7×10^6 daltons at high but physiologic concentrations of sodium and calcium, and at low urinary pH. The role of THP in cast formation has prompted a wealth of studies on its interactions with LCs. These studies were performed with the aim of defining a population of myeloma patients at risk of developing renal damage. The role of LC pI has long been suggested. As early as 1945, Oliver (54) proposed that the occurrence of casts in the distal tubules was the result of coagulation of globulins with a low pI in an acid urine. In 1979, Clyne et al. (55) suggested that LC with a high pI (greater than 5.6) and THP could bear opposite charges in the normal urine pH range, and undergo polar interaction between charged groups and precipitation. Clyne's hypothesis was supported by Coward et al. (56), who reported a significant negative correlation between pI and creatinine clearance. However, the nephritogenic potential of LCs with a high pI was not confirmed in further experimental and clinical studies (31,52,57,58).

In an elegant series of studies, Sanders et al. implicated THP as a major pathogenetic factor in a rat model. Development of casts and injury to proximal tubule cells in renal tubules microperfused with human nephritogenic LC were not correlated with LC pI, molecular form, or isotype (41). Intranephronal obstruction was aggravated by decreasing extracellular fluid volume or adding furosemide. In perfused loop segments, cast-forming LCs reduced chloride absorption directly, thereby increasing tubule fluid [Cl^-] and promoting their own aggregation with THP (59). Pretreatment of rats with colchicine, which prevents addition of sialic acid to the protein, completely prevented obstruction and cast formation in perfused nephrons (60), and THP from those rats did not aggregate with LCs *in vitro,* contrary to THP purified from control rats. *In vitro* studies suggest that THP can undergo both self (homotypic) aggregation and heterotypic aggregation with LCs (61). Homotypic aggregation is enhanced by calcium, furosemide, and low pH, and is dependent on THP sialic-acid content. Heterotypic aggregation requires previous binding of LC to the THP protein backbone (62). A 9-residue sequence of the THP was identified as a binding site of LCs, including a histidine at position 226, which explains, at least partially, the pH dependence of molecular interactions (63). LCs bind to THP through their third complementary determining region (CDR3) (64). The sugar moiety is also essential for coaggregation of LC and THP, perhaps by facilitating homotypic aggregation of THP. THP from normal volunteers treated with colchicine had a lower sialic acid content and a decreased aggregation potential in the presence of pathogenic LCs (61).

These findings suggest that colchicine may be useful in the treatment of cast nephropathy and that it is conceivable to design peptides or analogs that would inhibit interactions of LCs with THP and theoretically prevent the clinical manifestations of myeloma CN. Conversely, an increase in dietary salt and the loop diuretic, furosemide, may be harmful because these substances not only enhance THP homotypic aggregation, but also increase expression of this protein in the rat (65).

Cast formation may not rely only on interactions between LC and THP. First, five of 12 LCs purified from the urine of patients with CN failed to react with THP by enzyme-linked immunosorbent assay (ELISA) (21). Second, myeloma casts occasionally do not stain for THP in human biopsies (66), and casts induced in mice by LC injection do not seem to contain THP during the first 24 hours (40), indicating that some LCs may undergo aggregation or precipitation in the absence of THP. This hypothesis is supported by studies showing that the deposition of certain LCs *in vivo* may be related to their capability to aggregate *in vitro* (67). Resistance of LCs to renal and macrophage-released proteases may also contribute to cast formation and persistence (21).

Role of Tubular Obstruction by Casts in the Genesis of Renal Failure. The role of casts as plugs obstructing the tubules has been clearly shown in micropuncture studies (68). In myeloma patients, the correlation between severity of renal insufficiency and the number of casts remains controversial. Hill et al. (69) found a good correlation between the extent of cast formation and degree of renal failure, whereas others (28,31,70) have failed to demonstrate this correlation. This may be explained partly by the prominent medullary localization of casts, the count of which is underestimated in superficial kidney cortex biopsy specimens. The first indication that antibodies to THP could serve as probes of tubular obstruction was provided by Cohen and Border (71), who identified the protein in glomerular urinary spaces of two myeloma patients. This finding is indicative of intratubular urinary backflow (72). We detected THP in glomerular urinary spaces in 16 of 18 biopsies of patients with myeloma CN (Ronco and Mougenot, personal data) (Fig. 75-2). The proportion of obstructed tubules is too small to account by itself for renal failure. Renal failure induced by

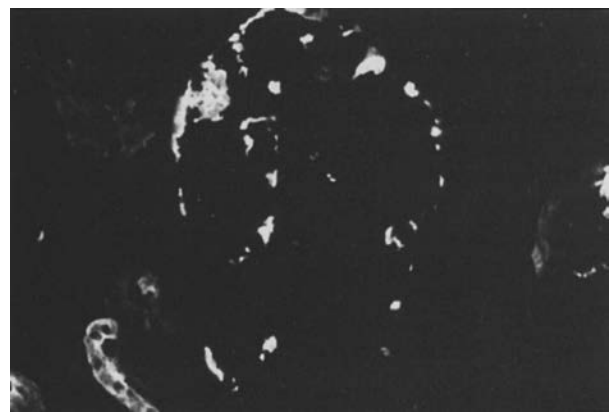

FIGURE 75-2. Myeloma cast nephropathy. Immunofluorescence stain with anti–Tamm-Horsfall protein (THP) monoclonal antibody. Glomerular deposits in Bowman's space delineate the inner aspect of Bowman's capsule and penetrate between lobules of the capillary tuft. Identification of THP in the urinary spaces of glomeruli supports the obstructive role of casts with reflux of tubular urine. (Magnification, ×312.)

CN is multifactorial, implicating also tubular epithelial cell and interstitial lesions. From a clinical point of view, tubule obstruction by casts may explain the slow recovery of renal function noted in many patients (31).

Interstitial deposits of THP were also found in 8 (44%) of the 18 biopsies examined with specific antibodies (Ronco and Mougenot, personal data). They probably result from a leakage of the protein through gaps in the tubular basement membrane favored by tubular obstruction. Clinical and experimental models have implicated the protein in the pathogenesis of tubulointerstitial nephritis. Thomas et al. (73,74) identified a single class of sialic acid–specific cell surface receptors for THP on polymorphonuclear leukocytes, and further showed that *in vitro* activation of human mononuclear phagocytes by particulate THP led to the release of gelatinase and reactive oxygen metabolites, both probably contributing to tissue damage.

In conclusion, there is considerable evidence that some LCs are intrinsically prone to induce tubular damage but the type of the predominant renal lesion (e.g., casts, crystals, or tubular epithelial cell alterations) varies from one LC to another. In addition, extrinsic factors including dehydration, hypercalcemia, low urine pH, and use of contrast medium or xenobiotics toxic to epithelial cells, most likely enhance LC toxicity. Tubular obstruction plays an important, but not exclusive, role in the genesis of renal failure. Both tubular obstruction and acute tubular lesions are theoretically reversible.

Clinical Presentation

Changing Presentation of Patients with Myeloma-Induced Renal Failure. When DeFronzo et al. reported the first series of 14 myeloma patients with acute renal failure in 1975 (75), it was established that renal failure occurred at some time during the illness in approximately half of the patients, but that the mode of presentation was usually chronic with a slow progression over a period of several months to years.

The mode of presentation of renal failure in myeloma has changed dramatically over the years. In their review of 141 patients treated in Nottingham between 1975 and 1988, Rayner et al. (76) showed that the absence of severe renal impairment at presentation predicted a low probability of developing renal failure subsequently. In only 5 of 34 patients of our own renal series (31) did the diagnosis of myeloma antedate the discovery of renal failure by more than 1 month. In 3 patients the presence of a monoclonal Ig was known for 10 to 18 years, but it only showed criteria of malignancy for less than 9 months. In 34 of the 53 cases (64%) collected in the Oxford series over the period from 1989 to 1994 (77), renal failure was discovered within a month of the diagnosis of myeloma, and in more than half of the cases it antedated the diagnosis of myeloma. Other series (32,33) also emphasize the simultaneous diagnoses of myeloma and renal failure. Such a change in epidemiology of renal failure may be interpreted in two ways. It is possible, although unlikely, that patients with established myeloma who subsequently develop renal failure are not referred for dialysis. Alternatively, more aggressive treatment of myeloma and higher awareness of the conditions that induce CN in the last two decades may have prevented LC precipitation within the tubule lumen, and hence the development of CN (77).

Demographic and Hematological Characteristics of Patients with Cast Nephropathy-Related Renal Failure. In Table 75-2 are summarized the clinical and pathologic data in four large series of myeloma patients with acute renal failure in which a renal biopsy was performed in at least 40% of the patients. A diagnosis of myeloma CN was established histologically in 81 of 99 (82%) renal biopsies, and lesions compatible with this diagnosis were found in 10 further biopsy specimens (10%). In comparison with the Mayo Clinic series of 869 unselected myeloma cases (79) in which the mean age was 62 years and the male–female ratio was 1.55, patients with acute renal failure did not show any demographic particularity. As shown in Tables 75-2 to 75-4, myeloma patients with renal failure are characterized by high tumor mass and virtually constant urinary LC loss, often of high output.

More than 72% of patients in the renal series have a high tumor burden (Table 75-2). This is confirmed by the Alexanian series, which included 494 consecutive patients referred to an oncology center (80) (Table 75-3). Only 3% of patients with myeloma of low tumor mass had renal failure, whereas 40% of those with high tumor burden had a serum creatinine greater than 180 μmol/L. These data contrast with the hematologic characteristics of patients with other renal complications of dysproteinemia including FS, amyloidosis, and MIDD, in

TABLE 75-2

CLINICAL AND PATHOLOGIC CHARACTERISTICS OF PATIENTS WITH MYELOMA-INDUCED RENAL FAILURE OF PRESUMED OR ESTABLISHED TUBULOINTERSTITIAL ORIGIN

Series (ref. no.)	No. of patients	Age (yr)	Male–female ratio	Tumor mass IIB	Tumor mass IIIB	Serum creatinine (μmol/L)	Urinary light chain >2 g/day	Renal lesions in biopsy specimen
Rota et al., 1987 (31)	34	66 (33–90)	0.88	15%	73%	975 (164–2000)	53%	26 MCN 2 ATN 2 CIN
Pozzi et al., 1987 (32)	50	63 (47–75)	1.38	12%	82%	798 (273–1518)	41%[a]	16 "Myeloma kidney"[b] 8 other
Pasquali et al., 1990 (33)	25	60 (48–74)	2.12	24%	72%	891 (455–1391)	72%	25 MCN
Irish et al., 1997 (78)	56	67 (42–82)	1.33	22%	78%	811 (302–2600)	NA	16 MCN, 5 AIN[c]

AIN, acute interstitial nephritis; *ATN*, acute tubular necrosis; *CIN*, chronic interstitial nephritis; *MCN*, myeloma cast nephropathy; *NA*, not available; *IIB*, intermediate tumor mass; *IIIB*, high tumor mass.
[a]Total proteinuria, including light chains.
[b]Presumably myeloma cast nephropathy.
[c]"Compatible with myeloma."

TABLE 75-3

RELATION BETWEEN TUMOR MASS AND RENAL FUNCTION

Tumor mass	No. of patients[a]	% of patients with serum creatinine (μmol/L) <180	180–270	>270
Low	151	97	1	2
Intermediate	183	89	5	6
High	160	60	17	23

[a]This series included 494 consecutive, previously untreated patients with multiple myeloma.
(From: Alexanian R, Barlogie B, Dixon D. Renal failure in multiple myeloma: pathogenesis and prognostic implications. *Arch Intern Med* 1990;150:1693, with permission.)

whom the monoclonal B-lymphocyte or plasma cell proliferation is either malignant but of low magnitude, or often benign from a hematologic point of view.

Another salient feature of myeloma associated with renal failure is the high prevalence of pure LC myelomas. Although they represent only about 20% of all myelomas referred to hematology or cancer centers, they are found in between 37% and 64% of patients with renal failure of presumed or established tubulointerstitial origin referred to nephrology centers. Development of CN in two studies in which this diagnosis was established histologically (31,33) was associated with urinary excretion of LCs exceeding 2 g per/day in 53% and 72% of the patients (Table 75-2). IgD myeloma has the greatest potential for causing renal disease. To give a balanced view on the prevalence of pure LC myeloma and on the output of urinary LCs in myeloma-associated renal failure, it is also necessary to analyze nonrenal series (80). When specific disease features implicated in the pathogenesis of renal failure are examined, LC protein excretion emerges as a highly significant independent factor on multivariate analysis (Table 75-4). The risk of developing renal failure is twice as high in patients with pure LC myeloma, and five to six times greater in patients with LC proteinuria greater than 2.0 g per/day compared to those with proteinuria less than 0.05 g per/day. This indicates that in patients producing complete immunoglobulin molecules, CN essentially occurs in those synthetizing an excess of LCs. The frequency of renal failure is identical in patients excreting κ or λLCs, as it is in most renal series. In the Alexanian series (80), hypercalcemia also was a prominent independent pathogenetic factor on multivariate analysis, with a risk of renal failure five times greater in those patients with corrected calcium greater than 2.87.

TABLE 75-4

FEATURES ASSOCIATED WITH RENAL FAILURE IN MYELOMA

	No. of patients[a]	% with renal failure	*P*
All patients	494	18	
Urinary LC (g/day)			
>2.0	123	39	0.00001
0.05–2.00	149	17	
<0.05	222	7	
Myeloma protein type			
Only LC protein	93	31	0.0003
Other	401	15	
Serum calcium (mmol/L)[b]			
>2.87	104	49	0.00001
≤2.87	390	10	

LC, light chain
[a]Same series of patients as in Table 75-3.
[b]Corrected calcium (mmol/L).
(From: Alexanian R, Barlogie B, Dixon D. Renal failure in multiple myeloma: pathogenesis and prognostic implications. *Arch Intern Med* 1990;150:1693, with permission.)

The Clinical and Urinary Syndrome of Myeloma Cast Nephropathy. CN-induced renal failure is remarkably silent. Clinical accompanying signs are due to myeloma (or to hypercalcemia), including weakness, weight loss, bone pain, and infection. Because of their nonspecificity and their frequency in older patients, they often do not lead patients to take medical advice or physicians to prescribe serum and urinary electrophoreses, which are the key laboratory investigations for the diagnosis of myeloma. Peaks visible on serum or urine electrophoresis are then identified by immunoelectrophoresis or immunofixation. A preserved corrected calcium at presentation in patients with unexplained renal failure should alert clinicians to the possibility of myeloma.

The main urinary feature is the excretion of a monoclonal LC, which accounts for 70% or more of total proteinuria in 80% of patients (31). LC proteinuria is usually not detected by urinary dipsticks, but only by techniques measuring total proteinuria. Certain LCs fail to react or react weakly in some widely used precipitation assays, such as the sulfosalicylic acid method, leading to falsely negative or underestimated results. The remaining proteins are composed of albumin and low-molecular-weight globulins that have failed to be reabsorbed by proximal tubule cells. In the rare patients with albuminuria greater than 1 g/day, CN is usually associated with glomerular lesions due to amyloidosis or MIDD. There is no hematuria in pure CN.

Precipitants of Cast Nephropathy. These are of paramount importance because of measures to prevent precipitation (Table 75-5). It is often difficult to identify a particular event responsible for precipitating renal failure, as these patients experience many of the complications of the disease at once, a common thread of which seems to be an effect on renal perfusion.

Hypercalcemia is an important precipitant found in 19% to 44% of the renal series (Table 75-5), and in 57% of the patients with renal failure in Alexanian nonrenal series (80). Presumably, hypercalcemia acts by inducing dehydration as a result of emesis and a nephrogenic diabetes insipidus. It may also enhance LC toxicity (81) and cause nephrocalcinosis. Calcium was shown to enhance the aggregation of LCs with THP (61).

Dehydration, with or without hypercalcemia, and infection are other major risk factors for acute renal failure. Rota et al. (31) found a high rate of urinary infections (10/34, 29%), which were associated in three cases with an increased proportion of polymorphonuclear leukocytes in the renal biopsy, suggesting an etiologic link between infection and deterioration of renal function. Infection also operates by causing dehydration and prompting the use of nephrotoxic antibiotics.

Contrast media have hitherto been considered an important precipitant of acute renal failure. It was hypothesized that the contrast medium bound to intratubular proteins, especially the LC and THP, causing them to precipitate and obstruct tubular flow. Contrast media also have vasoconstrictive effects, decreasing glomerular filtration rate (GFR) and urinary output.

TABLE 75-5

PRECIPITANTS OF ACUTE RENAL FAILURE IN MYELOMA

Series (ref. no.)	No. of patients	Dehydration	Infection	Hypercalcemia	Contrast medium	NSAIDs	None
Rota et al., 1987 (31)	34[a]	65%	44%	44% (>2.60 mmol/L)	0%	24%	—
Pozzi et al., 1987 (32)	50[a]	24%	10%	34% (≥2.75 mmol/L)	4%	0%	44%
Ganeval et al., 1992 (34)	80[b]	10%	9%	30%	11%	—	35%
Irish et al., 1997 (78)	56[a]	4%	4%	23%	0%	11%	57%

NSAIDs, nonsteroidal antiinflammatory drugs.
[a]Renal lesions are described in Table 75-2.
[b]Includes 19 patients with myeloma cast nephropathy, two with amyloidosis, and eight with LCDD (light and heavy chain deposition disease).

McCarthy and Becker (82) reviewed seven retrospective studies of myeloma patients receiving contrast media, involving 476 patients who had undergone a total of 568 examinations. The prevalence of acute renal failure (which was not defined) was 0.6% to 1.25%, compared to 0.15% in the general population. This is a low risk and contradicts the dogma that contrast media should not be used in myeloma patients. This change may reflect awareness of the risk and care taken to hydrate patients actively with alkaline solutes before and during the administration of contrast media. No clinical data currently support the preferential use of non-ionic agents in myeloma patients to decrease the risk of acute renal failure.

A number of drugs are noxious in myeloma patients. They include antibiotics, particularly aminoglycosides, and nonsteroidal antiinflammatory drugs (NSAIDs) (31,77,83–85). The mechanisms whereby NSAIDs precipitate renal failure in myeloma patients are most likely twofold (86). First, they reduce the production of vasodilatory prostaglandins that help to maintain an appropriate GFR in patients with renal hemodynamics compromised by dehydration. Second, inhibition of the diuretic effects of these prostaglandins presumably increases the tonicity of distal tubular fluid that would favor THP–LC co-precipitation and cast formation. Angiotensin-converting enzyme (ACE) inhibitors can also precipitate renal failure in myeloma patients because they reduce GFR dramatically in dehydrated patients (87). Their use as that of angiotensin type-1 receptor antagonists should be avoided in myeloma patients as long as a risk of decreased renal perfusion persists.

Recently introduced therapies including biphosphonates may also induce toxic tubular injury. Renal failure secondary to acute tubular necrosis was reported with zoledronate, a potent biphosphonate that is in widespread use for the treatment of hypercalcemia of malignancy (88), and with short-term, high-dose pamidronate (89).

Renal Pathology and the Value of Renal Biopsy

A renal biopsy should not be systematically performed in patients with a presumed diagnosis of myeloma CN. However, it is useful in three circumstances:

1. to establish the cause of renal failure in anuric patients with clinically silent myeloma without evidence of serum monoclonal component on electrophoresis (90);
2. to analyze tubulointerstitial lesions and predict the reversibility of renal failure in patients with presumed CN but multiple precipitating factors;
3. to identify glomerular lesions in patients with urinary albumin greater than 1 g per day and no evidence of amyloid deposits in "peripheral" biopsies (accessory salivary glands, rectum, abdominal fat).

Myeloma Casts. Myeloma CN (also inappropriately referred to as *myeloma kidney)*, is characterized by the presence of specific casts associated with severe alterations of the tubule epithelium. Myeloma casts are large and usually numerous. Their prevailing localization is the distal tubule and the collecting duct, but they may also be found in the proximal tubule and even in the glomerular urinary space. They often have a "hard" and "fractured" appearance, and show polychromatism upon staining with Masson's trichrome (Fig. 75-3). Casts may also have a stratified or laminated appearance. They may stain with Congo red, but only exceptionally do they show the typical yellow-green dichroism of amyloid under polarized light.

An important diagnostic feature of myeloma casts is the presence of crystals, which may be suspected by light microscopy (91). Such casts are often angular or heterogeneous because they contain multiple rhomboid or needle-shaped crystals surrounded by amorphous material and cell debris.

Casts are frequently surrounded by mononuclear cells, exfoliated tubular cells, and, more characteristically, by multinucleated giant cells whose macrophagic origin has been established by specific antibodies. These cells are often seen engulfing the casts and at times actually phagocytizing fragments. In some cases, the cellular reaction is made of polymorphonuclear leukocytes in the absence of urinary tract infection. Typical myeloma casts with a giant, multinucleated cell reaction (Fig. 75-3) can be very occasionally detected in other hemopathies including μ-HC disease (36) and Waldenstöm's macroglobulinemia (35). In myeloma CN, there is a great variability in the respective percentage of typical myeloma casts and of

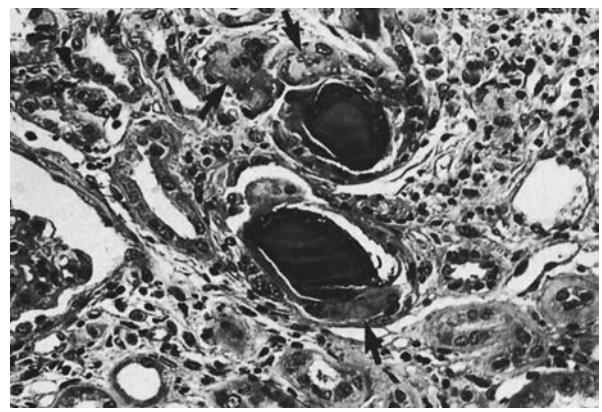

FIGURE 75-3. Myeloma cast nephropathy. Typical myeloma casts with fractured appearance are surrounded by multinucleated macrophagic cells (*arrows*) in a patient with λ–light-chain myeloma. (Masson's trichrome, ×312.)

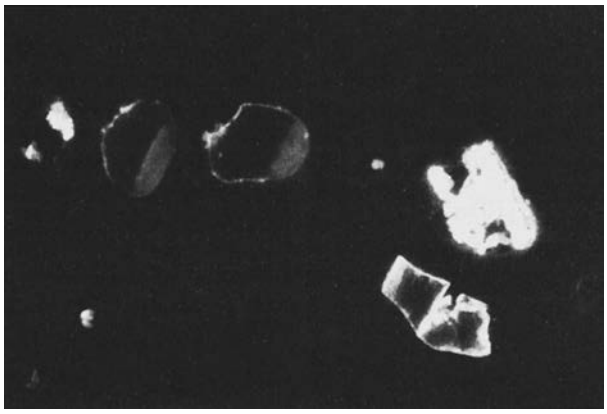

FIGURE 75-4. Myeloma cast nephropathy. Several tubules contain large casts, one of which has an angular and fractured aspect. The stain with anti-κ antibody is more intense at the periphery of most casts. (Immunofluorescence, ×312.)

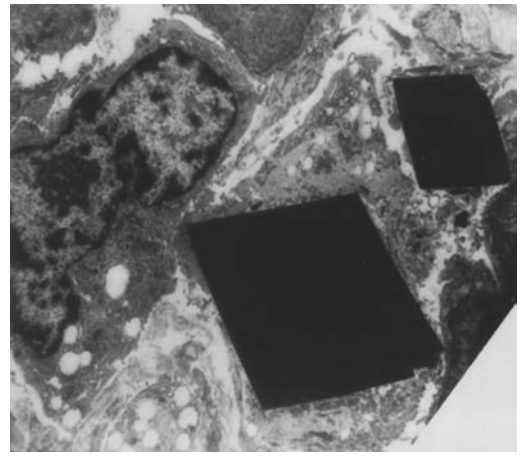

FIGURE 75-5. Myeloma cast nephropathy. Rectangular crystals presumably composed of λ-light chains in tubular cells. (Electron micrograph, uranyl acetate, and lead citrate, ×7,000.)

nonspecific hyaline casts. In some instances, most casts have nonspecific characteristics by light microscopy, even if by immunofluorescence the vast majority consists predominantly of one of the two LC types. The search for typical casts has to be conducted on all available sections if necessary.

By immunofluorescence, myeloma casts are essentially composed of the monoclonal LCs excreted by the patient, together with THP. In most cases, casts are stained exclusively or predominantly with either the anti-κ or the anti-λ antibody. However, in about 25% of myeloma biopsies, casts stain for both antibodies because they contain polyclonal LCs, together with albumin and fibrinogen (91). Staining of "angular" casts is often irregular, and more intense at the periphery (Fig. 75-4). In heterogeneous casts, the crystals themselves fail to stain, while the matrix of the cast and the surrounding cellular debris and amorphous material often stain positively for one of the LCs.

Cast ultrastructure has been studied by electron microscopy in 24 biopsies of myeloma CN by Pirani et al. (91). Crystals were detected in 14 biopsy specimens and suspected in another four. The authors have identified four major categories of casts, according to their content and ultrastructural appearance. One category characterized by large rectangular crystals, or fragments thereof, with a pentagonal or hexagonal cross-section, is found only in myeloma CN. It seems to be closely linked to the development of a giant-cell reaction around the cast. A second category also frequently contains crystals, but they are small, electron-dense, and needle-shaped, and seemingly not associated with a cellular reaction. Similar large rectangular and small, needle-shaped crystals can be found within plasma cells. They are also seen occasionally within the cytoplasm of either proximal or distal tubular cells (Fig. 75-5), surrounded by a single smooth membrane, which suggests that they are located within lysosomes.

Tubules and Interstitium. Considerable tubular damage is almost always present in myeloma CN. Epithelial tubular lesions are not only seen in the distal tubules where casts are principally located, but also in proximal convoluted tubules, where the epithelium undergoes atrophy and degenerative changes. Frank tubular necrosis may also be seen, with or without typical myeloma CN (31). By immunofluorescence, a variable number of tubule sections contain numerous "protein reabsorption droplets" staining for the monoclonal LC (71).

Interstitial lesions are often associated with the tubular damage. They may be mild and consist of inflammatory infiltrates and fibroedema, but fibrosis and its correlate, tubular atrophy, may also be fairly extensive. In severe cases with epithelial denudation and gaps in the continuity of the tubular basement membrane, often in close contact with myeloma casts, granulomatouslike formations containing macrophages and histiocytes develop around the ruptured tubules (71) (Fig. 75-6).

Glomeruli and Vessels. The glomeruli are usually normal, except for small clusters of globally sclerotic glomeruli and a mild thickening of the mesangial matrix. When mesangial thickening is more prominent, the possibility of an associated MIDD should be considered. Rarely, amorphous deposits reminiscent of myeloma casts can be seen in capillary loops or in the glomerular urinary space. In younger patients, severe chronic vascular lesions are sometimes observed, which may contribute to progression of sclerosis.

Outcome and Prognosis of Myeloma Cast Nephropathy

Until the 1980s, myeloma-induced renal failure was associated with a very poor prognosis. In the first series reported by DeFronzo and colleagues (75), only five of 14 patients survived the early period of acutely impaired renal function, and four of these died within only 2 months. Both renal prognosis and survival have significantly improved in the last 20 years, however. Most studies have provided information on the renal and vital

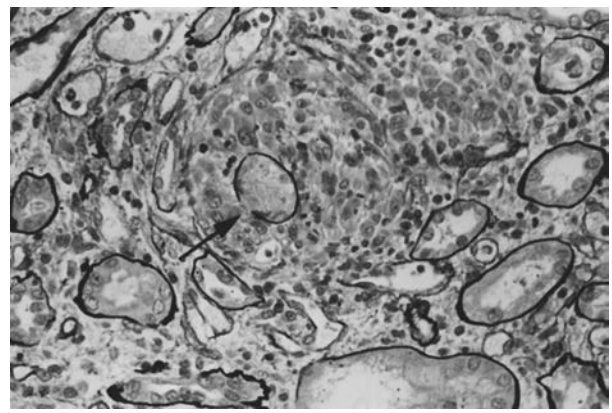

FIGURE 75-6. Myeloma cast nephropathy. Interstitial granulomatouslike formations with macrophages surrounding disrupted tubular basement membrane (*arrow*) were numerous in this λ-chain cast nephropathy. (Silver stain, ×312.)

TABLE 75-6

RENAL RECOVERY, MEDIAN SURVIVAL, AND PROGNOSTIC INDICATORS IN 604 PATIENTS WITH SEVERE RENAL FAILURE AND MYELOMA TREATED WITH CONVENTIONAL CHEMOTHERAPY (1987–2000)

Series (ref. no.)	No. of patients[a]	Renal recovery (%)	Median survival (mo.)	Pejorative renal predictors	Pejorative survival predictors
Rota et al., 1987 (31)	34	47	19	Female gender Interstitial lesions, tubular atrophy	No renal recovery
Pozzi et al., 1987 (32)	50	46	11	High serum creatinine, severity of renal lesions, number of casts	No renal recovery
Misiani et al., 1987 (92)	23	83	9	NA	Poor response to chemotherapy
Pasquali et al., 1990 (33)	37	43	17	NA	No renal recovery Hypercalcemia, early infection, interstitial fibrosis, tubular atrophy No plasma exchange
Johnson et al., 1990 (93)	21	57	22	Number of casts Poor response to chemotherapy	NA
Ganeval et al., 1992 (34)[b]	78	62	20	High serum creatinine at presentation	Poor response to chemotherapy Advanced disease stage Poor renal function after 1 mo
Irish et al., 1997 (78)	56	15	8	NA	Low plasma albumin Low platelet count
Blade et al., 1998 (94)	94	26	10	Serum creatinine >354 μmol/L Serum calcium <2.88 mmol/L Proteinuria >1g/24 hours	Poor response to chemotherapy Severity of renal failure No renal recovery
Knudsen et al., 2000 (95)	225	58	—	High serum creatinine Normal calcemia High LC excretion	Stage III disease High age Hypercalcemia Severity of renal failure No renal recovery

NA, not available.
[a]Includes early deaths.
[b]Includes 19 patients with myeloma cast nephropathy, 2 with amyloidosis, and 8 with light-chain deposition disease.

outcomes in patients treated with conventional chemotherapy (Table 75-6). As yet, there are very few series of patients treated with high-dose myeloablative therapy and autotransplantation (96,97) because severe renal failure was long considered a contraindication to high-dose chemotherapy.

Renal Outcome and Prognostic Factors. About half of patients have complete or partial reversal of renal failure after weeks to months (Table 75-6), and some come off dialysis (98). Ganeval et al. (34) noticed that patients with improved renal function showed a phase of rapid decrease in serum creatinine level within the first month, then a second phase of much slower improvement. The first phase of improvement is mostly independent of chemotherapy, whereas the second phase is likely to be the result of both chemotherapy (in responders) and continuation of measures undertaken to suppress the toxic renal effect of remnant light chains.

Elevated plasma creatinine concentration at presentation has been quoted as a marker of poor prognosis in four studies (32,34,94,95), implying that renal functional impairment of any degree should be treated as a medical emergency. In the study by Rota et al. (31), main prognostic indicators were provided by renal histology. Return-to-normal renal function was seen only in patients with typical cast nephropathy and/or tubular necrosis in the absence of interstitial damage. Global tubular atrophy and interstitial fibrosis were associated with partially or totally irreversible renal failure, whereas the number of casts has a controversial predictive value (31,32,93).

Recent data indicate that outcome of severe renal failure may be substantially improved by high-dose melphalan and autologous transplantation. Of 54 dialysis-dependent myeloma patients, 13 (24%) became dialysis independent at a median of 4 months after treatment (97). Dialysis duration of ≤6 months and pretransplantation creatinine clearance of >10 mL/minute were significant for renal function recovery.

Survival and Predictors. Myeloma patients with renal failure have a shorter survival than those with normal renal function. In the presence of renal failure mortality in the first 3 months is about 30% (32,34,94), and median survival ranges from 9 to 22 months (Table 75-6). However, Rota et al. (31) found that survival time was about 2 years in those patients whose serum creatinine level returned to normal, compared to only 5 months in patients with irreversible renal failure. The effect of recovering renal function on survival was emphasized in other series (32–34,94,95) (Table 75-6). Response to chemotherapy is another important predictor (Table 75-6).

In Alexanian's series of 494 consecutive myeloma patients (80), a large tumor mass was the only significant variable adversely affecting survival (p <0.001) on multivariate analysis. For comparable patients with high tumor mass, the frequency of response was less and the remission and survival times were

shorter with more severe renal failure, but the differences were not significant. Reversal of renal failure did not confer a survival advantage, contrary to response to chemotherapy.

These data indicate that myeloma patients with renal failure should be treated with chemotherapy just as those without renal failure, and that any measures that could contribute to improved renal function should be undertaken from the day of diagnosis.

Treatment

The treatment of patients with CN-induced renal failure has two main objectives: (a) to limit further cast precipitation by reducing precipitability of the urinary LC and LC production rate, and (b) to avoid complications of uremia by dialysis and sometimes by transplantation. Even more importantly, preventive measures are essential to reduce the incidence of renal failure.

Decreasing Precipitability of the Urinary LC by Immediate Symptomatic Measures. Because co-precipitation in renal tubules of free LC and THP is the main nephritogenic event, measures to reduce concentration and precipitability of both partners are essential and urgent. These include rehydration, correction of hypercalcemia, stopping administration of NSAIDs and ACE inhibitors, and treatment of infections with non-nephrotoxic antibiotics. Despite controversy about the role of LC pI in cast formation, alkalinization of urine remains recommended because solubility of THP is reduced at low pH (61). Therefore, a daily urine output greater than 3 L and a urine pH greater than 7.0 should be reached in all patients whose cardiac and renal function can tolerate a deliberate expansion of the extracellular fluid volume. These measures alone are sufficient to improve renal function in the majority of patients with renal impairment at presentation, especially in those with hypercalcemia (80,99). However, they must be completed by therapeutic means aimed at decreasing the amount of urinary LCs filtered by glomeruli.

Reducing the Production Rate (and Concentration) of the Monoclonal Light Chains. Because reversal of renal insufficiency results in survival outcomes similar to those of patients who have normal renal function at diagnosis, patients who are diagnosed with renal insufficiency should be aggressively treated. However, the presence of renal impairment could add significantly to the morbidity of these patients and make it difficult to tolerate aggressive treatment regimens. Therefore, the treatment approach for this group of patients should include a thorough understanding of the feasibility and outcomes of the various available treatments, including newer options such as thalidomide, as well as careful monitoring of monoclonal LC production by the serum-free LC assay (100,101).

Oral melphalan, an alkylating agent, was first used to treat myeloma nearly 50 years ago, and in combination with high-dose corticosteroids has become the standard treatment. Melphalan and prednisone with the dose of melphalan adjusted for renal failure is still a common choice. However, this choice is challenged by the following considerations. First, although the response rate is 40% to 60% (30,102), no patients go into complete remission (as currently defined by absence of monoclonal paraprotein in serum and urine by immunofixation maintained for a minimum of 6 weeks, less than 5% plasma cells in bone-marrow aspirate and trephine biopsy, no increase in number or size of lytic bone lesions, and disappearance of soft tissue plasmacytomas). Median survival is only 24 to 30 months. Second, repeated cures of melphalan are cytotoxic for stem cells, which is a problem for future autologous peripheral blood stem cell (PBSC)–supported high-dose melphalan, which is now considered standard therapy for myeloma (with or without renal failure), at least for younger patients (103,104). Third, another drawback of melphalan-prednisone combination is slow antitumor action. Regimens including vincristine, doxorubicin and dexamethasone ("VAD") induce earlier response, which is an advantage in patients with hypercalcemia and renal failure, but their long-term efficacy in terms of median survival and duration of "remission" is not better than that of melphalan-prednisone. Cytoreduction can also be achieved with other noncytotoxic regimens such as high-dose dexamethasone alone or in combination with thalidomide. However, these regimens have not radically changed the global prognosis of multiple myeloma.

In the past 10 years, the concept of high-dose chemotherapy regimens with hematopoietic stem cell support (to reduce the duration of the drug-induced myelosuppression with its high risks of morbidity and mortality) has modified the treatment of young patients with multiple myeloma (105). Transplantation from an allogeneic donor may have the advantage over autotransplantation of a potential "graft versus myeloma" effect, but the procedure still has a high level of related mortality. Many more patients are candidates for autologous transplantation that is now usually performed using peripheral blood stem cells. In 1996, a randomized controlled trial first demonstrated the benefits of high-dose therapy over conventional combination therapy in terms of complete remission rate, event-free survival, and overall survival in patients with normal renal function (103). High-dose therapy with autotransplantation can lead to a median overall survival exceeding 5 years (106).

However, until recently, high-dose therapy with stem cell transplantation had not been considered in patients with significant renal impairment. Series have appeared in the literature showing that this procedure is possible in these patients (96,97). It seems that transplant-related mortality is equivalent to that in similar patients with normal renal function, provided that the dose of melphalan be reduced.

The following recommendations are widely accepted (104, 107):

1. Patients who are younger that 65 years should receive high-dose chemotherapy (melphalan) and a single autologous stem cell transplant, provided that they are free of severe comorbid conditions.
2. Dose reduction (140 to 100 mg/m^2 melphalan) should be considered in order to decrease melphalan toxicity.
3. Dose-adjusted autotransplantation should be applied once maximum cytoreduction has been achieved, preferably with noncytotoxic regimens such as VAD or pulse high-dose dexamethasone-containing regimens.
4. Response to chemotherapy should be evaluated by the serum-free LC assay (100,101).

The use of plasmapheresis has been advocated by many to reduce LC concentration rapidly, but its efficacy has not been established convincingly, except in patients with hyperviscosity syndrome (92,93,108–112a). Three randomized controlled trials have been published to date (93,111,112b), but further trials enrolling large number of patients are required.

Supportive Therapy. Blood transfusion, analgesia, erythropoietin, and bisphosphonates are important adjuncts to therapy. Beyond delaying the onset of skeletal events, the new generation of biphosphonates, pamidronate and zoledronate, also exert antimyeloma effects indirectly by inducing osteoclast apoptosis, thereby reducing a major source of the antiapoptotic IL-6 molecule, or directly by inducing myeloma cell apoptosis. Biphosphonates were shown to reduce skeletal events in myeloma patients with bone disease (113), but their use in the absence of bone disease needs to be further evaluated in the context of potential nephrotoxicity. Cases of acute renal failure

(88,89) and nephrotic proteinuria with focal segmental glomerulosclerosis and its collapsing variant, were indeed recently reported in patients receiving zoledronate and pamidronate (114–117). Therefore, pamidronate (90 mg intravenously over at least 2 hours monthly) and zoledronate (4 mg intravenously over at least 15 minutes monthly) should be given at the appropriate doses, and careful monitoring of renal function and albuminuria should be undertaken.

Dialysis and Renal Transplantation. Dialysis is clearly indicated for the treatment of acute renal failure and end-stage renal disease, except in patients with refractory myeloma (118). It should be started early to avoid the added complications of uremia and to compensate for the hypercatabolic state induced by the use of high doses of corticosteroids. If peritoneal dialysis is chosen, the early placement of a permanent indwelling dialysis catheter is recommended to avoid infectious peritonitis, the risk of which is increased by chemotherapy-induced leukopenia (77). Residual renal function must be carefully monitored because of possible improvement after several months of dialysis (97,98). Two reports from Great Britain (119) and the United States (120) suggested that chronic dialysis could be a worthwhile treatment in patients with myeloma and renal failure. Survival at 1 year was 45% in the British study (23 patients) and 54% in the American study (731 patients). At 30 months, survival declined to 25% compared with 66% in nondiabetic end-stage renal disease patients without myeloma (120). These data have been confirmed in further studies that showed a 1-year survival rate of 63% and a median survival of 12 to 20 months in patients with myeloma undergoing hemodialysis (review in reference 121). Myeloma patients requiring long-term dialysis live as long as those with less severe renal failure (118), with little difference between hemodialysis and peritoneal dialysis (78,119,122), although most authors insist on the serious risk of infection in continuous ambulatory peritoneal dialysis (CAPD) patients (119). However, median survival was less in patients on hemodialysis with myeloma CN (12 months) than in those with AL amyloidosis (24 months) or with LCDD (48 months) (123).

The experience with renal transplantation in myeloma is extremely limited (124–126). In one patient (38), lesions recurred early in the graft but normal renal function was sustained. Transplantation should be limited to carefully selected patients with an inactive hematologic disease.

Finally, if most cases of severe renal failure cannot be prevented because they occur simultaneously with the finding of myeloma, it is necessary to avoid or correct all precipitating factors of renal failure in patients with established myeloma. It is particularly important to reduce the use of NSAIDs as analgesic drugs, to detect and control hypercalcemia as soon as possible, and to correct dehydration.

Fanconi's Syndrome

Fanconi's syndrome (FS) is characterized by renal glycosuria, generalized aminoaciduria, hypophosphatemia, and, frequently, by chronic acidosis, hypouricemia, and hypokalemia. It often includes osteomalacia, with pseudofractures. These manifestations result from functional impairment of the renal proximal tubule. The first association of FS with myeloma was reported by Sirota and Hamerman (127), although these authors considered FS and myeloma as two separate diseases. Engle and Wallis (128) identified crystal-like inclusions in both tumor cells and renal tubule epithelial cells, and suggested that FS and myeloma could be related. Costanza and Smoller (129) described the cytoplasmic inclusions as round or rodlike electron–opaque structures with longitudinally oriented fibrils. Lee et al. (130) established clearly that myeloma was a cause of adult FS. Maldonado et al. (131) reported 17 cases of FS associated with plasma cell dyscrasia, and two recent studies described the clinicopathologic features of the disease in two series of 11 and 32 patients (132,133). The disease is most likely underdiagnosed. The rarity of FS in patients with myeloma contrasts, however, with the high prevalence of tubule alterations in myeloma autopsy series. This suggests that unusual specific properties of LCs, mostly κ, are involved in the pathophysiology of FS.

Pathophysiology of Plasma Cell Dyscrasia-Associated Fanconi's Syndrome

The rare occurrence of FS in multiple myeloma, the peculiar proneness of LCs to form crystals, and their unique ability to alter proximal tubular transports, strongly suggest that LCs have unusual physicochemical properties. The peculiar propensity of certain LCs to form crystals *in vivo* is attested by experimental studies in mice (17) and rats (41,51,134). It is remarkable that the κ LCs that induced crystallization *in vivo*, also significantly reduced the glucose, chloride, and volume fluxes (51,134). The clinical characteristics of the patients from whom the LCs were isolated, especially the presence of FS, were unfortunately not specified.

Crystal composition was analyzed in a patient with myeloma-associated FS and hexagonal crystals in kidney proximal tubular cells, bone marrow plasma cells, and phagocytes (135). N-terminal sequencing and mass spectrometry studies showed that a 107-amino acid fragment corresponding to the variable domain of the κ-LC (Vκ) was the essential component of crystals forming spontaneously from the patient's urine (Fig. 75-7A). Vκ was also crystallized alone using the hanging drop technique (Fig. 75-7B). Crystals were hexagonal bipyramids and had the same 6.0-nm periodicity on electron micrographs as those found in the cells. The V domain (12-kDa) resisted proteolysis by trypsin, pepsin, and cathepsin B, self-reacted, and formed crystals *in vitro*, which may explain its accumulation in plasma cells and proximal tubular cells. The resistance of LC V domains to proteolytic enzymes including cathepsin B was confirmed in further studies (21,132), except in two patients with a high-mass myeloma and FS. At variance with the observations made in patients with CN (21, 62), LCs from patients with FS did not bind THP, except in one case where both syndromes were associated.

The unusual physicochemical behavior of FS κ-chains was tentatively correlated with their structure in a number of cases (136). Sequence analyses showed that 9 of 10 (90%) LCs belonged to the VκI variability subgroup, while this subgroup only accounts for 56% of all monoclonal κ-LCs (132,137). The VκI appeared to originate from only two germline genes, *LCO2/O12* in five cases and *LCO8/O18* in four. Analyses of the DNA sequence suggested that all structure peculiarities arose from somatic mutations in the proliferating clone (138). In the 10 available sequences, residues had never or rarely been reported among VκI subgroup LCs. The unusual presence of nonpolar or hydrophobic amino acids in the complementary determining region (CDR)-L1 loop at position 30, together with a nonpolar amino acid at position 50, seems to be specific for FS LCs derived from gene *LCO2/O12*. These hydrophobic residues are exposed to the LC surface (136) and may be involved in the pathophysiology of FS, as is suggested by site-directed mutagenesis in an experimental model for FS (139).

In summary, unusual properties of LCs associated with the FS might explain crystal formation and/or accumulation in lysosomes. After endocytosis, LCs are processed in the endosomal and lysosomal compartment where "normal" LCs are degraded. In FS, accumulation of the protease-resistant V domain fragment generated by lysosomal enzymes may induce

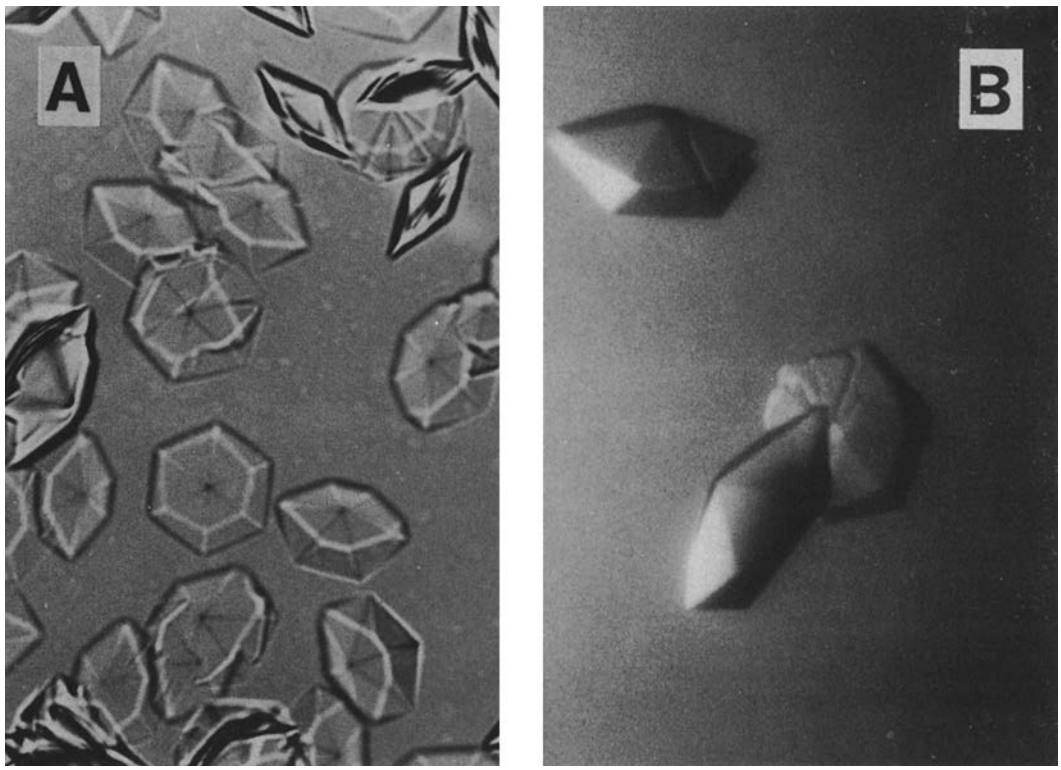

FIGURE 75-7. Plasma cell dyscrasia-associated Fanconi's syndrome. Crystals spontaneously obtained *in vitro* from a Sephadex G100 fraction of the patient's urinary proteins (**A**) and by the hanging drop technique from purified Vκ fragment (**B**). (**A**, magnification, ×400; **B**, size of these crystals, 0.25 mm.) (From: Aucouturier P, Bauwens M, Khamlichi AA, et al. Monoclonal Ig L chain and L chain V domain fragment crystallization in myeloma-associated Fanconi's syndrome. *J Immunol* 1993;150:3561, with permission.)

crystal formation. Clogging of the endolysosomal system may subsequently alter apical membrane recycling and/or adenosine triphosphate (ATP) production (hence, Na^+–K^+–ATPase functioning) as suggested by mitochondrial injury (130), and lead to progressive impairment of sodium-dependent apical transporters. However, we do not know why FS does not occur in patients with apparently the same degree of distortion of the lysosomal compartment as can be seen in certain myeloma patients with or without CN. The molecular mechanisms responsible for glycosuria, phosphaturia, generalized aminoaciduria, and uric acid loss remain poorly understood.

Clinical Presentation

The clinical features are summarized in Table 75-7 (132). The median age at diagnosis is 57 years. Most common initial manifestations are bone pain and weakness, principally due to osteomalacia. The major cause of this osteomalacia is hypophosphatemia, which results from increased urinary clearance of phosphate. Chronic acidosis and abnormal renal vitamin D metabolism further contribute to the development of bone lesions. Bone pain may also be the consequence of lytic lesions in patients with a high-mass myeloma. Other revealing signs are essentially due to the proximal tubule impairment, including hypokalemia. Renal failure occurs more frequently than one would expect in a disease of the proximal tubule.

Criteria for the diagnosis of FS (e.g., "orthoglycemic" glycosuria, increased phosphate and uric acid clearances, and generalized aminoaciduria) may not all be present together, especially in patients with renal failure (137). The diagnosis of FS is often unrecognized for several years in patients presenting with proteinuria, bone pain, or renal failure. The mean time from onset to diagnosis of FS is about 3 years (132). Typically, the diagnosis of FS precedes that of the plasma cell dyscrasia, most often a κ-LC-excreting multiple myeloma, because the hematologic disease has a low tumor burden and a slow progression. In 35 of 98 (36%) published cases (132,133), even criteria for the diagnosis of myeloma are lacking, and patients were classified initially as having a benign monoclonal gammopathy of undetermined significance (MGUS). In some patients, the diagnosis of the plasma cell dyscrasia remained undetermined between myeloma and MGUS because it may be difficult to recognize the cytologic characteristics of myeloma cells when their cytoplasm is stuffed with crystals. Two patients of 98 had Waldenström's macroglobulinemia (132,133).

Conversely, the metabolic disorders of FS may be overseen in the context of myeloma, and FS-related bone lesions should not be interpreted as the consequence of high-mass myeloma.

Pathologic Data

Typically there are prominent crystals in enlarged proximal tubular cells and degenerative changes of proximal tubules (132). Proximal tubular cells are stuffed with microcrystals that stain red or green with Masson's trichrome and are periodic acid-Schiff negative. In the most severely affected tubules, crystal-containing exfoliated cells are seen in the tubular lumen, whereas intracytoplasmic crystals are still present in atrophic tubules. In other cases, crystals can only be suspected by the presence of a finely granular material of glassy appearance in an enlarged proximal tubular epithelium (Fig. 75-8A). Their presence is more easily demonstrated by toluidine-blue staining of semi-thin sections and by hematoxylin and eosin staining of cryostat sections. In the same tubule sections, all the cells are not equally affected; cells with a normal aspect coexist with those stuffed with crystals.

TABLE 75-7

CLINICAL CHARACTERISTICS OF PATIENTS WITH PLASMA CELL DYSCRASIA-ASSOCIATED FANCONI'S SYNDROME[a]

Total no. of patients	Age mean/ extremes	Gender	Initial manifestations	Bone lesions	Renal failure[c]	Plasma cell dyscrasia	Light-chain isotype
68	57 22–81	30 males 38 females	Bone pain (25)[b] Weakness, fatigue (16) Weight loss (7) Polyuria–polydipsia (7) Hypokalemia-related signs (4) Proteinuria (18) Renal failure (16) Renal glycosuria (13)	Osteomalacia (25) High-mass myeloma (12) Plasmacytoma (1)	54	Myeloma (36)[d] MGUS (21)[e] MGUS/myeloma (4)[f] Lymphoma/CLL (4)[g] "Atypical" plasma cell dyscrasia (1)	49κ 7λ

[a]Figures in parentheses indicate number of patients.
[b]Related to osteomalacia.
[c]Serum creatinine >130 μmol/L, or creatinine clearance <80 mL/mn.
[d]Including 12 patients with a high-mass myeloma.
[e]Monoclonal gammopathy of undetermined significance (*MGUS*).
[f]Undetermined diagnosis, mostly due to cytoplasmic inclusions in plasma cells making interpretation of cytology difficult.
[g]Chronic lymphocytic leukemia (*CLL*).
(From: Messiaen, Deret S, Mougenot B, et al. Adult Fanconis syndrome secondary to light-chain gammopathy: clinicopathologic heterogeneity and unusual features in 11 patients. *Medicine* (*Baltimore*) 2000;79:135, with permission.)

A universal feature is the additional presence of severe lesions of the proximal tubule epithelium devoid of crystals. These lesions include vacuolization, loss of the luminal brush border, and focal cell sloughing, with cell fragments in the lumen of the tubules. Interstitial cellular infiltrate, including plasma cells, may contain crystalline inclusion bodies. Patchy tubular atrophy and focal interstitial fibrosis, together with a variable number of obsolescent glomeruli, are often observed.

In several cases, attempts to characterize the crystal proteins with anti-Ig conjugates, including anti-LC antibodies, have failed (140). When immunohistochemical studies are positive, crystals stain only (or predominantly) for the monoclonal LC, most often κ (Fig. 75-8B).

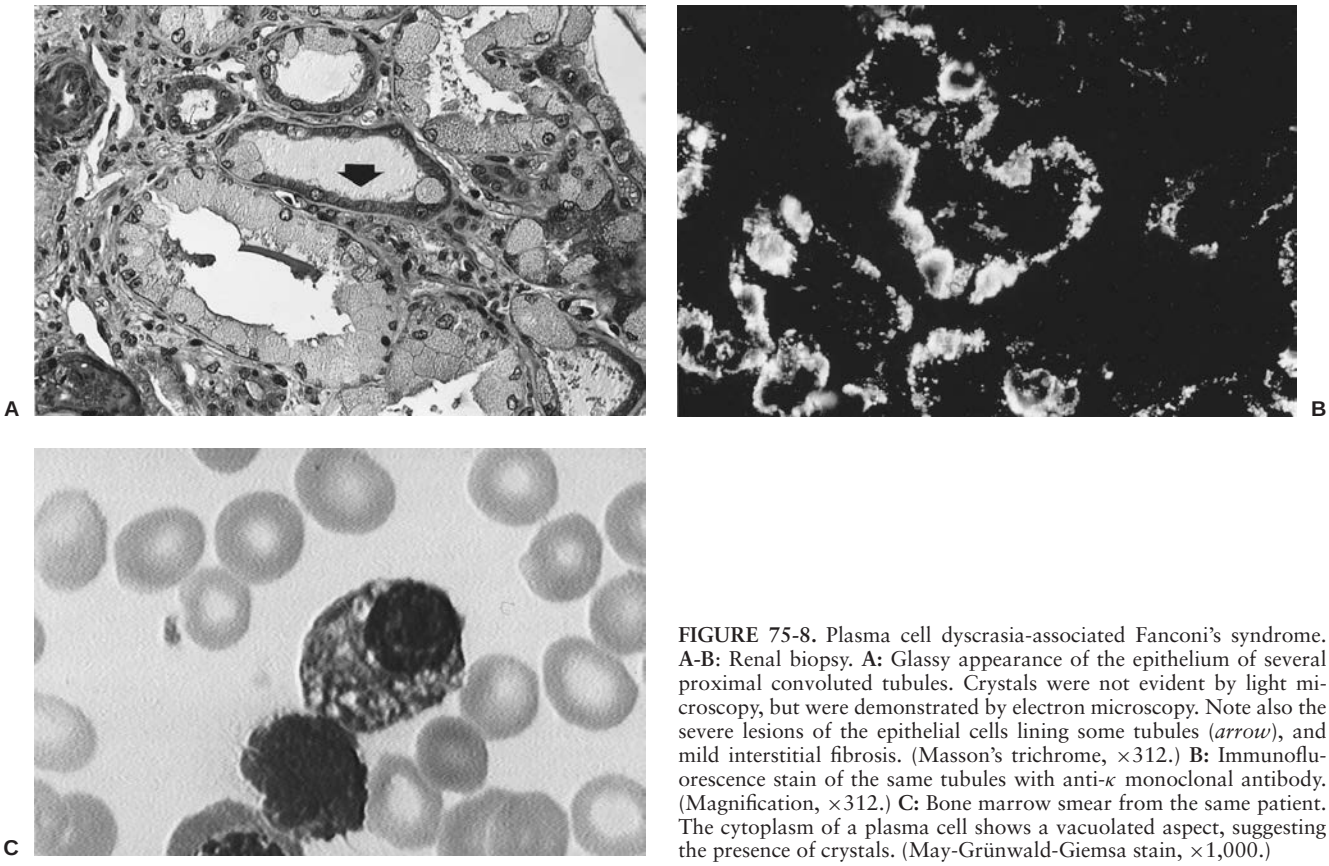

FIGURE 75-8. Plasma cell dyscrasia-associated Fanconi's syndrome. **A-B**: Renal biopsy. **A:** Glassy appearance of the epithelium of several proximal convoluted tubules. Crystals were not evident by light microscopy, but were demonstrated by electron microscopy. Note also the severe lesions of the epithelial cells lining some tubules (*arrow*), and mild interstitial fibrosis. (Masson's trichrome, ×312.) **B:** Immunofluorescence stain of the same tubules with anti-κ monoclonal antibody. (Magnification, ×312.) **C:** Bone marrow smear from the same patient. The cytoplasm of a plasma cell shows a vacuolated aspect, suggesting the presence of crystals. (May-Grünwald-Giemsa stain, ×1,000.)

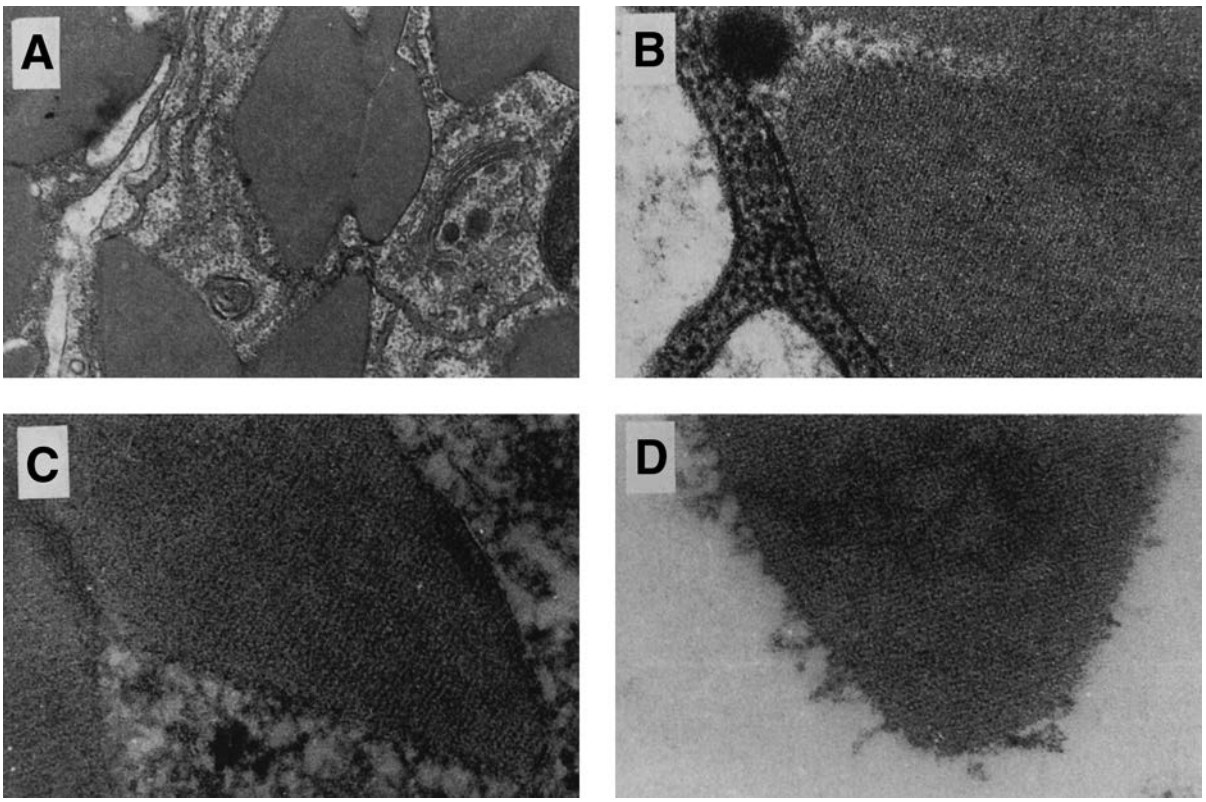

FIGURE 75-9. Plasma cell dyscrasia-associated Fanconi's syndrome. Same patient as in Figure 75-7. Electron microscopic study of intracellular (*A, B,* and *C*) and *in vitro*-formed crystals in the same patient. **A:** Bone marrow plasma cell (and a macrophage on the left). (Magnification, ×8,000.) **B:** Bone marrow macrophage. (Magnification, ×50,000.) **C:** Proximal convoluted tubular epithelial cell. (Magnification, ×50,000.) **D:** Crystal obtained *in vitro* from Sephadex G100 fraction C from the patient's urine. (From: Aucouturier P, Bauwens M, Khamlichi AA, et al. Monoclonal Ig L chain and L chain V domain fragment crystallization in myeloma-associated Fanconi's syndrome. *J Immunol* 1993;150:3561, with permission.)

By electron microscopy, crystals of various size and shape (rectangular, rhomboid, round, or needle-shaped) are detected within the cytoplasm of proximal tubule cells (Fig. 75-9C). Intracytoplasmic crystals are surrounded by a single smooth membrane, likely of lysosomal origin (91,135,141). In rare cases, crystals are also seen in distal tubule cells (141,142). In other cases, crystals are not found by light microscopy, but electron microscopy shows enlarged vesicular bodies containing dense tubular and rod-like structures (129–131,140,143,144) or fibrils and needle-shaped deposits very close to crystalline structures (142).

Crystal formation in plasma cell dyscrasia–associated FS is not limited to renal tubule epithelium but also occurs in bone marrow and tissue-infiltrating plasma cells, and in macrophages (128–131,135,143,145,146) (Fig. 75-8C and 75-9). The presence of crystals in macrophages close to the tumoral plasma cells might result from endocytosis of either freshly secreted LCs or lysed plasma cells. In plasma cells, crystals are localized not only in lysosomes, but they are also frequently found inside the granular endoplasmic reticulum (147). Crystal formation in these organelles therefore suggests incomplete proteolysis of LCs. The slow progression of myeloma disease in typical FS associated with crystal formation may be explained by the deleterious effects on cell growth of the accumulation of crystalline inclusions in the tumor plasma cells.

Although crystals are a salient feature of the plasma cell dyscrasia–associated FS, they are neither specific nor absolutely constant. Crystals were found in 16 of 28 (57%) patients in the two largest series published so far (132,133). They may also be found, albeit in low amounts, in proximal tubule epithelial cells of patients with CN (21,91), and occasionally in myeloma patients with isolated tubular lesions, that is, in the absence of distal nephron myeloma casts (148,149). In addition, recent pathologic studies of 11 patients suggest that the FS due to LC nephropathy is more heterogeneous than expected (132). FS may indeed occur in patients with a high-mass myeloma, with or without crystals in tubule cells, with or without myeloma casts in tubule lumen.

Outcome and Treatment

As expected, patients with multiple myeloma have shorter survival time than those with MGUS. In the Mayo Clinic's series, only one of the 14 patients with MGUS developed multiple myeloma, and at the end of follow-up, only five of 32 patients had evolved to end-stage renal disease (133).

In patients with osteomalacia, considerable improvement can be obtained with 1α-hydroxyvitamin D, calcium, and phosphorus supplementation. The effect of chemotherapy on the proximal tubule impairment is much more debated. Ichida et al. (144) and Orfila et al. (142) reported that the treatment of underlying myeloma improves urinary signs and tubular transport abnormalities. However, no significant change in renal function was observed in the two largest series (132,133). It has been suggested (150) that the presence of crystals within plasma cells should be added to the list of criteria defined by Kyle and Greipp (151) against chemotherapy in myeloma. Because chemotherapy, especially with alkylating agents, carries

a significant risk of complications but without much benefit for kidney function, and because patients who do not have an overt malignancy show a relatively benign course, the risks and benefits of chemotherapy should be weighed carefully.

AMYLOIDOSIS

Amyloidosis has been known to be associated with or to cause renal disease for more than 100 years. Amyloid was originally identified as a waxy substance by Rokitansky in 1842 (152), but the term *amyloid* was coined by Virchow in 1854 (153) because the substance stained with iodine in a way that was similar to starch and cellulose. Although the protein content of amyloid was recognized subsequently, the term *amyloid* persisted. The diversity of amyloidotic disease was rapidly suspected on clinical grounds, but chemical studies in the late 1960s actually provided the basis of the present classification of amyloid (Table 75-8). In 1968, Pras et al. (154) isolated and purified amyloid fibrils, which opened the way to further chemical analyses. In 1971, Glenner et al. (4) found that the amyloid fibril proteins from two patients had an N-terminal sequence identical to Ig LCs, which was the first demonstration of a relation between amyloidosis and Ig. They also generated "amyloidlike" fibrils by proteolytic digestion of some human LCs, thereby demonstrating their propensity for forming amyloid (155).

AL amyloidosis is certainly among the most severe complications of plasma cell proliferative disorders. From a practical point of view, it must be considered as such, since the only efficient therapeutic tools to date are chemotherapeutic drugs against B-cell proliferations. However, ultrastructural as well as pathophysiologic considerations that may open new therapeutic avenues are derived from the fact that AL amyloidosis belongs to the large entity of amyloidoses, covering conditions as different as familial polyneuropathies, Alzheimer's disease, type II diabetes, transmissible spongiform encephalopathies, hemodialysis-related amyloidosis, Iceland-type hereditary amyloidangiopathy, and secondary (AA) systemic amyloidosis, among others (Table 75-8). Amyloidogenesis may result from common mechanisms, as suggested for instance by studies of Alzheimer's and the prion diseases (156,157).

General Characteristics of Amyloidosis

A Common Ultrastructural Molecular Organization Defining a Morphologic Entity

Amyloidosis is the general term for a morphologic entity, defined by visceral, extracellular deposition of protein material with unique tinctorial properties and ultrastructural characteristics. After Congo red staining, amyloid deposits exhibit

TABLE 75-8

CLASSIFICATION OF AMYLOIDOSES

Amyloid protein	Precursor	Distribution	Type	Syndrome or involved tissues
AA	**Serum amyloid A**	**Systemic**	**Acquired**	**Secondary amyloidosis, reactive to chronic infection or inflammation including hereditary periodic fever (FMF, TRAPS, HIDS, FCU, and MWS)**
AApoAI	**Apolipoprotein A-I**	**Systemic**	**Hereditary**	**Liver, kidney, heart**
AApoAII	**Apolipoprotein A-II**	**Systemic**	**Hereditary**	**Kidney, heart**
Aβ	Aβ protein precursor	Localized	Acquired	Sporadic Alzheimer's disease, aging
		Localized	Hereditary	Prototypical hereditary cerebral amyloid angiopathy, Dutch type
Aβ2M	β_2-microglobulin	Systemic	Acquired	Chronic hemodialysis
ABri	Abri protein precursor	Localized or systemic?	Hereditary	British familial dementia
ACys	Cystatin C	Systemic	Hereditary	Icelandic hereditary cerebral amyloid angiopathy
AFib	**Fibrinogen Aα chain**	**Systemic**	**Hereditary**	**Kidney**
AGel	**Gelsolin**	**Systemic**	**Hereditary**	**Finnish hereditary amyloidosis**
AH	**Immunoglobulin heavy chain**	**Systemic or localized**	**Acquired**	**Primary amyloidosis, myeloma-associated**
AL	**Immunoglobulin light chain**	**Systemic or localized**	**Acquired**	**Primary amyloidosis, myeloma-associated**
ALys	**Lysozyme**	**Systemic**	**Hereditary**	**Kidney, liver, spleen**
APrP	Prion protein	Localized	Acquired	Sporadic (iatrogenic CJD, new variant CJD (alimentary?)
		Localized	Hereditary	Familial CJD, GSSD, FFI
ATTR	**Transthyretin**	**Systemic**	**Hereditary**	**Prototypical FAP**
			Acquired	**Senile heart, vessels**

The following proteins may also cause amyloidosis: calcitonin, islet-amyloid polypeptides, atrial natriuretic factor, prolactin, insulin, lactadherin, keratoepithelin, and Danish amyloid protein (which comes from the same gene as ABri and has an identical *N*-terminal sequence). *CJD*, denotes Creutzfeldt-Jakob disease; *FAP*, familial amyloidotic polyneuropathy; *FCU*, familial cold urticaria; *FFI*, fatal familial insomnia; *FMF*, familial Mediterranean fever; *GSSD*, Gerstmann-Strüassler-Scheinker disease; *HIDS*, hyper-IgD syndrome; *MWS*, Muckle-Welles syndrome; *TRAPS*, tumor necrosis factor receptor-associated periodic syndrome. **Lines in bold characters indicate amyloid types with kidney involvement.**
(Adapted from: Westermark G, Benson MD, Buxbaum JN, et al. Amyloid fibril protein nomenclature—2002. *Amyloid* 2002;9:97; Merlini G, Bellotti V. Molecular mechanisms of amyloidosis. *N Engl J Med* 2003;349:583.)

birefringence under polarized light, which indicates the presence of highly ordered structures. These deposits have been extensively studied at the ultrastructural level by electron microscopy, infrared spectroscopy, and x-ray diffraction. In an important review published in 1980, Glenner (158) clustered all amyloidoses under the more convenient denomination of β-fibrilloses on the basis of the highly similar organization of the amyloid deposits. These are "typically composed of a felt-like array of 7.5- to 10-nm wide rigid, linear, non-branching, aggregated fibrils of indefinite length." One amyloid fibril is made of two twisted 3–nm-wide filaments, each having a regular antiparallel β-pleated sheet configuration; the β-sheets are perpendicular to the filament axis. A regular packing of peptides or proteins with a β-sheet conformation results in the elongation of amyloid fibrils. The numerous hydrogen bonds between virtually all amide functions of the peptide backbones make such a structure highly stable. Other components, described in subsequent text, are supposed to stabilize the fibrils.

Amyloid Protein Precursors and Classification of Amyloidoses

Amyloid protein precursors share the property of either a native β-pleated conformation or a high propensity to form β-sheets. All are globular structures, clearly distinct from fibrillar proteins such as collagen, which are proline-rich polymers with a longitudinal arrangement. The term *fibrils* is thus confusing, and one must keep in mind that in the case of amyloid, it actually refers to a quaternary arrangement of nonfibrillar proteins.

The International Committee for Amyloidosis recommended a nomenclature essentially based on the nature of amyloid proteins (159); the abbreviated name of each amyloid protein is preceded by the letter A. The list provided in Table 75-8 is not exhaustive. More than 20 different amyloid protein precursors have been identified to date. This classification, favoring pathogenetic aspects, has the advantage of being less empirical than those based on clinical and pathologic presentations. However, it is worth noting that hereditary and secondary forms of a same disease exist and should be distinguished; for instance, systemic AA amyloidosis occurs in contexts of both chronic inflammatory or infectious diseases (secondary form), and hereditary periodic fever. Normal transthyretin is responsible for senile systemic amyloidosis, whereas certain mutations are the cause of familial amyloidotic polyneuropathy (Fig. 75-10A). Thus, multiple different factors, either intrinsic (structural) or external (concentration of the precursor proteins, tissue factors, etc.), may influence the pathogenicity of a variety of potentially amyloidogenic proteins.

Other Constituents of Amyloid

In addition to the unique "pseudocrystalline" stacking of β-sheets just described, a few structural features are shared by all types of amyloid, and might help the understanding of some

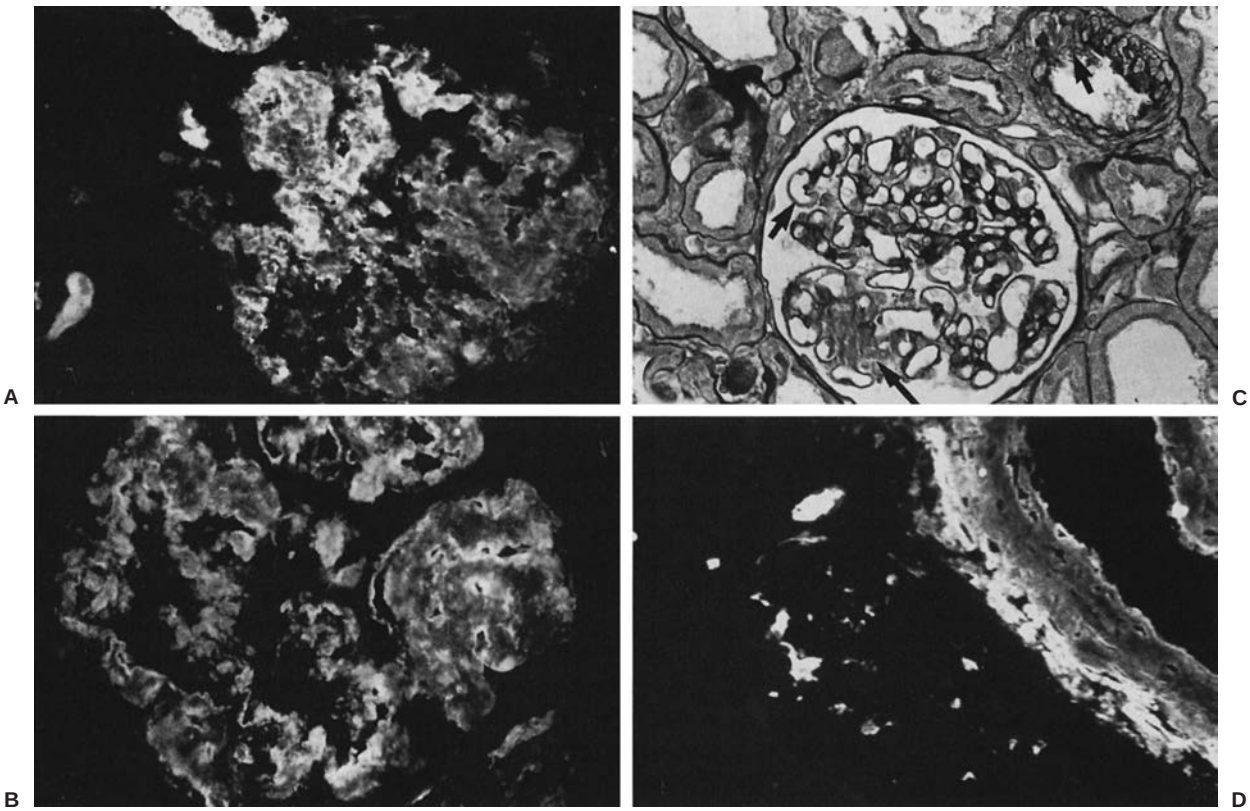

FIGURE 75-10. Amyloidosis. **A:** Glomerular and vascular heavy amyloid deposits stained with anti-transthyretin antibody in a patient with Portuguese-type hereditary amyloidosis. (Immunofluorescence, ×312.) **B:** Co-deposition of amyloid P (AP) component in a glomerulus from the same patient as in (**A**). (Immunofluorescence stain with anti-AP component antibody, ×312.) **C:** Glomerulus with early amyloid deposits in mesangium, capillary walls, and arteriolar wall (*arrows*) from a patient with AA amyloidosis. (Light microscopy, periodic acid–Schiff, ×312.) **D:** Glomerulus from a patient with AL amyloidosis. Scanty glomerular deposits contrast with almost complete replacement of arterial walls by amyloid. (Immunofluorescence stain with anti-κ antibody, ×312.)

aspects of the pathophysiology. Glycosaminoglycans (GAGs) have been found tightly associated with all isolated amyloid fibrils (160,161). GAGs are polysaccharide chains made of repeating uronic acid–hexosamine units of several types and normally linked to a protein core, thus constituting proteoglycans, which are important constituents of extracellular matrices. The invariable presence of GAGs in amyloid fibrils raises two suggestions:

1. Proteoglycans might interact with amyloidogenic precursors during the nucleation steps of amyloidogenesis (see subsequent text); indeed, most GAGs associated with fibrils are of the heparan sulfate type (162,163), and heparan sulfate proteoglycans are essential components of the basement membranes, which are preferential sites of amyloid deposition.
2. Sulfated GAGs might be important for inducing and stabilizing the β-pleated structure of the amyloid fibrils (164).

Another constituent of all amyloid deposits is a protein of the pentraxin family, the serum amyloid P component (SAP) (Fig. 75-10B). SAP is a plasma glycoprotein made up of two noncovalently linked pentamers of identical subunits. The β-pleated structure of SAP (165) is strongly homologous to that of legume lectins such as concanavalin A. It shows no allelic polymorphism and displays striking interspecies homology. Furthermore, no occurrence of SAP deficiency has yet been described, which suggests that it has essential physiologic functions. SAP is a calcium-dependent lectin, with binding affinities toward DNA (166), C4-binding protein and the collagenlike region of C1q (167), and several constituents of extracellular matrices such as fibronectin and proteoglycans. SAP was shown to bind apoptotic cells and nuclear debris, and mice with targeted deletion of the SAP genes spontaneously develop anti-DNA antibody and a syndrome resembling human systemic lupus erythematosus (168). Two calcium sites are involved in carbohydrate binding. In the presence of calcium, SAP is remarkably resistant to proteolytic digestion, suggesting a physiologic role in maintaining extracellular matrix structures. Coating of amyloid fibrils with unaltered SAP is a constant feature that could result in their protection from catabolism. It is probable that SAP binding to amyloid deposits is mediated by GAGs through the formation of multicomponent complexes (169). The high affinity of SAP toward all types of amyloid was exploited for diagnosing, locating, and monitoring the extent of systemic amyloidosis using scintigraphy with ^{123}I-labeled SAP (170,171). SAP binding to all ligands is inhibited by specific sugars such as β-D-galactose cyclic pyruvate acetal. Moreover, the knowledge of SAP structure offers the opportunity of designing competitive inhibitors as potential drugs for the treatment of amyloidoses. However, an important physiologic role of SAP in protecting from autoimmunization (168) could make this approach hardly feasible.

General Mechanisms of Fibrillogenesis

The amyloidoses are diseases of protein conformation in which a particular soluble innocuous protein transforms and aggregates into an insoluble fibrillar structure that deposits in extracellular spaces of certain tissues. Fibrillogenesis may be the consequence of several mechanisms of processing the amyloid precursor, including partial proteolysis and conformational modifications. In systemic secondary (AA) amyloidosis, removing of the C-terminal part of an apolipoprotein acute-phase reactant, SAA, yields a 5- to 10-kDa fibril-forming fragment. Phagocytic cells, in particular macrophages, supposedly play a central role in this disease by providing the intralysosomal processing of the precursor (172). In Alzheimer's disease, the metabolism of an integral membrane protein, β-amyloid precursor protein (APP), generates a hydrophobic 40- to 42-amino-acid fragment with amyloidogenic properties, the amyloid β-peptide (Aβ) (173). In other forms of amyloidosis, such as those involving transthyretin and Ig LCs, partial proteolysis has been demonstrated but may as well occur after fibrillogenesis. For instance, in AA amyloidosis, cleavage of the precursor protein also occurs after fibril formation (174). The demonstration of small fragments from the LC constant domain in deposited fibrils also argues in favor of a postfibrillogenic proteolysis in AL amyloidosis (175).

In certain types of hereditary amyloidoses due to mutations in the genes coding for the precursor protein (review in 176), amyloid formation seems to occur via a conformational change leading to a soluble partially folded intermediate. The property shared by these amyloidogenic variants is a native conformation that is thermodynamically less stable than that of the normal counterpart. A reduction in the stability of the variant was shown to favor the formation of partially folded conformers (alternative spatial arrangements of the same polypeptide) that have a strong propensity to self-aggregate and assemble into fibrils. However, whether conclusions from structural studies of transthyretin or lysozyme mutants may be extended to other amyloidoses, including AL and AA amyloidoses, remains questionable.

Amyloidogenesis seems to be a nucleation-dependent polymerization process (157). Unlike other protein deposition diseases, in which amorphous aggregates are the consequence of insolubility of the pathogenic protein in the tissues, amyloid may result from a "one-dimensional crystallization." In the latter process, formation of an ordered nucleus is the initial and limiting step, followed by a thermodynamically favorable addition of monomers leading to elongation of the fibrils. As shown in Alzheimer's and prion diseases, the nucleation step can be overrun by adding a preformed nucleus to a supersaturated solution of the amyloidogenic protein. A similar "seeding" phenomenon may explain the "amyloid-enhancing factor" activity of extracts from amyloid-containing tissues in AA amyloidosis animal models (177). The lag phase can also be shortened by injection of synthetic peptides corresponding to known amyloid fibril proteins (178).

Distribution of Amyloid: Localized Versus Systemic Amyloidosis

Tissue localization of the deposits is characteristic of many amyloidoses (Table 75-8). Single-organ involvement may reflect either local secretion or particular tropism of the amyloid precursor; for instance, pancreatic amyloidosis in patients with insulinoma or type 2 diabetes mellitus is related to cosecretion with insulin of islet amyloid polypeptide by β-cells (179). In some cases of Alzheimer's disease, molecules such as proteoglycans or lipids normally present in certain areas of the brain (amygdala, hippocampus, and neocortex) could behave as heterogeneous seeds for the polymerization of amyloid β-peptide (157). Systemic amyloidoses are derived from circulating precursors, which either display unusual structural features or are present at abnormally high plasma levels, or both. Although most cases of LC amyloidosis are due to systemic organ deposition of LCs produced by bone marrow plasma cells, localized forms of LC amyloidosis have also been reported mostly in the orbit, the nasopharynx, and the genitourinary tract (reviewed in 180). A local infiltration of plasma cells is then usually found in proximity to the amyloid deposits, and may be responsible for the secretion of an amyloidogenic LC (181).

Pathologic Data with Special Emphasis on Renal Involvement

Despite the diversity of amyloidogenic proteins, they all deposit in tissue as fibrils constituted by the stacking of β-pleated

sheets as identified by x-ray crystallography and diffraction studies (182). This unique protein conformation is responsible for the tinctorial and optical properties revealed by Congo red staining of tissue sections, and for the relative resistance of the fibrils to solution in physiologic solvents and to normal proteolytic digestion, which leads to their implacable accumulation in tissues (158).

By light microscopy, the deposits are extracellular, eosinophilic, and metachromatic. After Congo red staining, they appear faintly red and show the characteristic apple-green birefringence under polarized light. This light microscopic method is the most reliable to detect amyloid because it yields virtually no false-positive findings. Sections thicker than those usually recommended for renal pathologic examination may be necessary to produce sufficient color density. Metachromasia is also observed with crystal violet, which stains the deposits in red. The use of other stains such as thioflavine T has been proposed, but the results lack specificity. The permanganate method of Wright et al. (183) may help to discriminate AA from AL fibrils if the sections are treated with permanganate before the Congo red procedure. AL amyloid is resistant, whereas AA amyloid is sensitive to permanganate oxidation. However, this method has been supplanted by immunohistochemical analysis of the deposits.

In the kidney, the earliest lesions are located in the mesangium, along the glomerular basement membrane, and in the blood vessels (184) (Fig. 75-10C). Within the mesangium, deposits are associated primarily with the mesangial matrix, and subsequently irregularly increase by spreading from lobule to lobule and then invading the whole mesangial area. Amyloid deposits may also infiltrate the capillary basement membrane or be localized on both sides of it. When subepithelial deposits predominate, spikes recalling those seen in membranous glomerulopathy may be observed. Dikman et al. (185) showed that the severity of proteinuria correlated with the presence of spicules and podocyte destruction rather than with the amount of amyloid in the glomerulus. The spicules were associated with morphologic and clinical evidence of rapid amyloid deposition and a fulminant clinical course (185). Glomerular cell proliferation is infrequent. Advanced amyloid typically produces a nonproliferative, noninflammatory glomerulopathy, responsible for a marked enlargement of the kidney. The amyloid deposits replace normal glomerular architecture with loss of cellularity and usually without eliciting inflammation. When glomeruli become massively sclerotic, the deposits may be difficult to demonstrate by Congo red staining, and electron microscopy may then be helpful. The latter is required at very early stages, which may not be detected by light microscopy examination in patients presenting with the nephrotic syndrome (186,187). Except in fibrinogen A α-chain amyloidosis, which characteristically does not affect renal vessels, the media of the blood vessels is prominently involved at early stages. Vascular involvement may predominate, and occasionally occur alone, particularly in AL amyloidosis (Fig. 75-10D). Deposits may also affect the tubules and the interstitium, leading to atrophy and disappearance of the tubular structures and to interstitial fibrosis. In apolipoprotein AI amyloidosis, deposits markedly predominate in the interstitium, whereas glomeruli are not or are occasionally involved.

Because of the heterogeneity of amyloidotic diseases, which results in specific diagnostic and therapeutic strategies adapted to the type of protein deposited within tissues, immunofluorescence examination of snap-frozen biopsy specimens with specific antisera should be routinely performed (188–191). In the first series published by Gallo et al. (189), immunohistochemical classification of amyloid type was possible for 44 (88%) of 50 patients using anti-LC and anti-AA antisera. However, Noel et al. (188) pointed out that immunofluorescence with sera directed against HCs and LCs of Ig might be more difficult to interpret than with anti-AA antiserum. This is likely due to the frequent loss of LC constant domains in fibrils, accounting for the absence of epitopes normally recognized by antibodies. It is also possible that the pseudocrystalline structure of the fibrils makes these epitopes poorly accessible to antibodies. In a recent series, 12 of 34 patients (35.3%) with proven AL-amyloidosis had negative immunofluorescence staining for κ and λ light chains, which confirms the relatively low sensitivity of immunofluorescence microscopy in the detection of AL amyloidosis in the kidney and underscores the need to pursue additional diagnostic studies to identify the plasma cell dyscrasia (192). A genetic cause should be sought in all patients with amyloidosis that is not the reactive systemic amyloid AA type and in whom confirmation of the AL type cannot be obtained. Indeed in 350 patients with systemic amyloidosis, in whom a diagnosis of the AL type of the disorder had been suggested by clinical and laboratory findings and by the absence of a family history, amyloid mutations were present in 34 cases, most often in the genes encoding fibrinogen A α-chain (18 patients) and transthyretin (13 patients) (191). A low-grade monoclonal gammopathy was detected in 8 of the 34 patients (24%), but none of these patients had free LC identified in the urine.

By electron microscopy, amyloid deposits are characterized by randomly oriented, nonbranching fibrils with an 8- to 10-nm diameter (Fig. 75-11). Early deposits can be found in close connection with mesangial cells that undergo important changes, and in the capillary walls distant from the mesangium on both sides of the basement membrane (193). As mentioned previously, SAP has been found in all chemical types of amyloid thus far examined. In studies using double-label immunogold staining of AL and AA amyloid deposits, Yang and Gallo (194) showed that SAP represented 1.5% and 6.5% of the total gold label in AL and AA, respectively. SAP occurred as widely separated single units while the major fibril protein was labeled in single rows, similar to beads on a string.

Pathophysiologic Considerations of AL Amyloidosis

Studies on the mechanisms of AL amyloidogenesis are made particularly difficult by the unique degree of structural heterogeneity of the precursor. Each monoclonal LC is different from all others. An Ig LC typically includes two globular domains of 105 to 110 amino acids, strongly homologous to each other, that exhibit the classic conformation of all domains belonging to the "Ig superfamily" of proteins. The COOH-terminal domain (constant domain, C) is encoded by a single gene segment with very little allelic polymorphism in κ-chains and by no more than four different gene segments in λ-chains. Conversely, the NH_2-terminal domain structure (variable domain, V) results from complex somatic rearrangement and mutation events occurring in the course of B-cell differentiation, and leads to a high degree of diversity. The antiparallel β-pleated ("β-barrel") structure of Ig domains seems particularly adapted to amyloid formation. It is worth noting that another protein of the Ig superfamily, β_2-microglobulin, may form amyloid fibrils.

The implication of Ig HC in amyloidosis is exceptional. In the first case of AH amyloidosis almost entirely documented at the molecular level, the pathogenic IgG HC had an internal deletion of half the molecule, so that the V domain was directly joined to the COOH-terminal C domain C_H3, thus strikingly resembling an LC (7). Three other AH-amyloidosis cases related to deposition of IgA-related α-heavy chain (195) or of V domains only (196,197). A case of γ-HC amyloidosis was

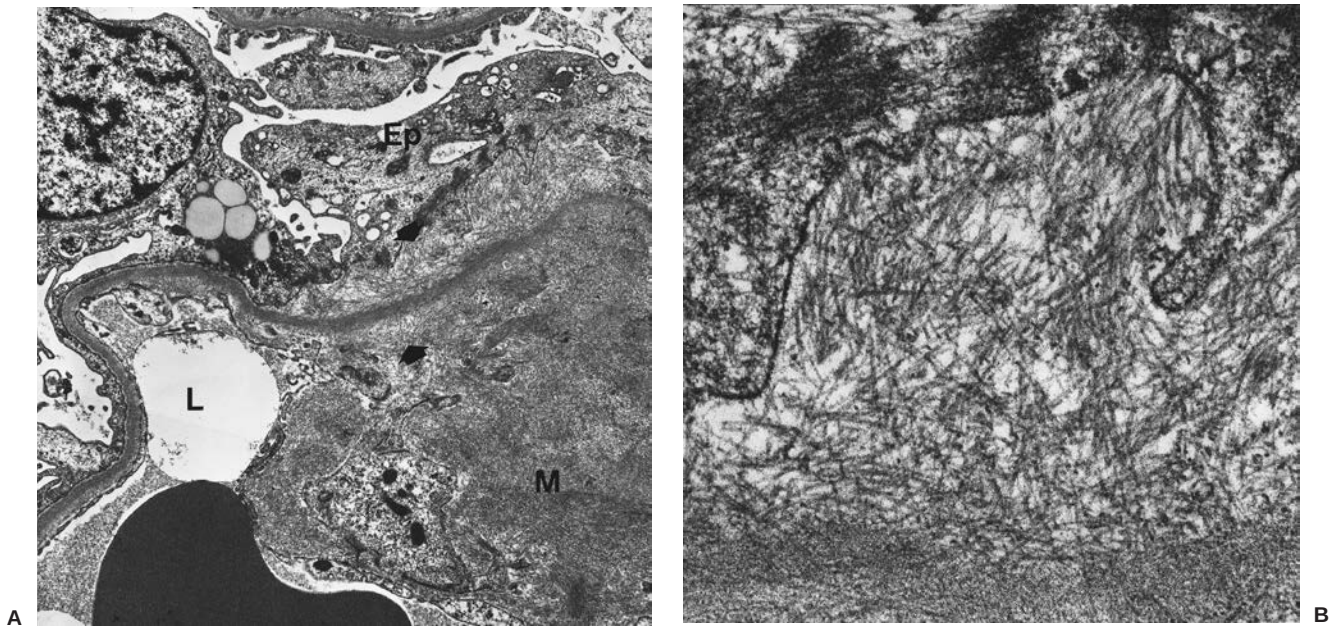

FIGURE 75-11. Amyloidosis. **A:** Electron micrograph of glomerular deposits of amyloid. Fibrils are seen in the basement membrane on both sides of the lamina densa (*arrows*). The lamina densa is attenuated. *Ep*, epithelium; *L*, capillary lumen; *M*, mesangium. (Magnification, ×5,000.) **B:** High magnification view of the randomly oriented fibrils on the epithelial aspect of the basement membrane. (Magnification, ×30,000.)

also reported in a patient whose nephrotic syndrome recurred 6 years after autologous peripheral blood stem cell transplantation for a λ-LCDD (198).

Not all Light Chains are Amyloidogenic

Despite predisposing conformation, a majority of Ig chains are not amyloidogenic, even at long-lasting high secretion rates. Several LC characteristics are considered amyloidogenic, particularly the LC isotype. First, the λ–κ ratio is between 2:1 and 4:1, depending on the series. Second, a homology family of LC V region, the $V_{\lambda VI}$ variability subgroup, was shown to be overrepresented in AL amyloidosis (22). This rare subgroup is expressed exclusively in amyloid-associated monoclonal Ig and represents 41% (17/41) of amyloidogenic λ-chains (23). A study of 55 consecutive unselected cases of primary amyloidosis showed a very skewed repertoire, as only two germline genes belonging to the λIII and λVI families, namely 3r (22% of cases, λIII) and 6a (20%, λVI), contributed equally to encode 42% of amyloid V_{λ} regions (199). Furthermore, there was a significant correlation between the use of the $V_{\lambda VI}$ germline donor, 6a, and renal involvement (200,201) and that of the $V_{\lambda III}$ gene, 3r, with soft-tissue AL (201). The use of a biased V_L gene repertoire also correlated with clinical outcome; the use of $V_{\lambda II}$ germline genes was associated with cardiac amyloidosis and affected survival adversely (201). In contrast, no significant imbalance of the variability subgroups of κ-chains was found (202), but patients with κLCs were more likely to have dominant hepatic involvement (200).

Empirical studies also show that amyloidogenicity is often associated with some physicochemical features such as the presence of low-molecular-mass LC fragments in the urine, and low pI; together with LC isotypy, these parameters allow the prediction of the amyloidogenic/nonamyloidogenic character of a monoclonal LC, with a correct allocation in 81% of tested cases (203).

The ability of two amyloidogenic LCs contrasting with the failure of one nonamyloidogenic LC to reproduce typical fibrillar deposits in mice definitely demonstrates that essential pathogenic factors relate to intrinsic properties of the LC precursors (204). This experimental model required huge amounts (up to 5.3 g/mouse) of purified monoclonal LCs to be injected intraperitoneally. Nevertheless, several interesting findings were noted, such as the presence of mouse SAP in the deposits, confirming its crucial role in either the constitution or, more probably, the maintenance of fibrils *in vivo*. Water deprivation dramatically increased the amyloidogenic potential of the LCs.

Comparison of Structures of Amyloid Light-Chain Precursors and Deposited Light Chains: A Role for Proteolysis?

Considerable works have been devoted to the study of structures of circulating monoclonal LCs or extracted fibrils. After the demonstration by Glenner et al. (205) that an LC was the predominant constituent of amyloid fibrils, they showed that NH_2-terminal sequences (up to 36 residues) were identical in the deposited and urinary monoclonal LCs from three patients (4,206). However, the possibility that a mutant form or a molecular variant of the soluble LC could be the amyloid precursor was not excluded, and only 20 years later the complete sequence identity between a circulating and a deposited LC was established (207). Association of AL amyloidosis with other deposition diseases such as LCDD (208) will raise the same question of uncertain identity between LCs forming different kinds of deposits. Mutant subclones may be encountered in myeloma, especially after treatment with alkylating agents, and have also been described in AL amyloidosis (209).

Analyses of LC precursor primary structures were performed either at the protein (210–213) or at the cDNA levels (214). All cases had an overall normal structure, including a normal C domain sequence.

The essential role of the V domain in fibrillogenesis was further supported by analyses of fibrils extracted using

adaptations of the method of Pras et al. (154), which showed it to always be the main amyloid constituent. The C domain is often, but not always (206,207), partially or totally absent from the fibrils after extraction (205,209,215–229). In many cases, heterogeneity in the length of the COOH-terminal end was noted (216,217,223,224). In a few cases, intact LC was present together with fragments (223); in most others, such heterogeneity might have been present but unrecognized because the analyses were focused on a purified predominant fragment. These results raise the hypothesis of a possible role of proteolysis, as already demonstrated in other forms of amyloidosis. Bellotti et al. (230) showed the disappearance of a conformational LC idiotope in the course of fibril formation, and suggested that polymerization results from the loss of the dimer conformation, possibly due to proteolysis of the C domain.

The question of a role of LC proteolysis in the amyloidogenic process has been addressed in different ways, but has not yet received a fully satisfactory answer. Abnormally low-molecular-mass LCs were secreted in *in vitro* biosynthesis experiments on bone marrow cells from AL amyloidosis subjects, but they might result from either abnormal synthesis or proteolytic processing, or both (231,232). *In vitro* digestion experiments using pepsin, trypsin, and kidney lysosomes yielded fibrils resembling amyloid by electron microscopy and displaying typical Congo red binding and green polarization birefringence (233–235). However, most tested LCs were from patients without amyloidosis, and the *in vitro* fibrils generally contained smaller fragments than those found *in vivo*. Therefore, instead of reproducing "normal" amyloidogenesis, these experiments merely showed the ability of peptides generated by cleavage of β-pleated structures to form amyloidlike fibrils. Another intriguing matter of all *in vitro* fibrillogenesis experiments is the absence of GAGs and SAP, which are invariable constituents *in vivo*. Although these studies contribute to the general understanding of the molecular mechanisms of fibril formation, their validity as models is questionable. In bone marrow cell culture from an AL amyloidosis patient, Durie et al. (236) found amyloidlike material immediately adjacent to macrophages, and concluded that a processing of the LCs by these cells, similar to that observed in AA amyloidosis, might lead to amyloid formation. Conversely, several observations point to the amyloidogenic potential of intact LCs; specifically, experimental mouse amyloid fibrils are made essentially of the entire injected LC (204); *in vitro* fibrils with characteristic tinctorial and ultrastructural properties of amyloid can be generated after simple reduction of the interchain disulfide bond of an intact LC dimer (237). Considering the sensitivity of C domains to proteases (238), it is conceivable that they are digested after constitution of the fibrils and tissue deposition, or during the purification process. The demonstration of C domain fragments as amyloid component argues in this sense (175).

Amyloidogenic Light Chains: Light Chains with Peculiar Sequence or with Affinity for Extracellular (Matrix) Constituents?

The search for primary sequence peculiarities of the LC V domains first led to disappointing conclusions. Several unusual features such as N-glycosylations (209,214,221–224), insertion of acidic residues (217,219), and changes of charged to hydrophobic residues (209,213) have been noted. Infrequent amino acids at certain positions have been considered to affect the secondary structure of the framework regions (219) or the LC dimerization (225). This led Stevens et al. (239) to determine amyloid-associated LC residues from the comparison of 52 pathogenic sequences with a bank of 128 other LCs. In a more recent report that collected 100 LCs of the VκI variability subgroup, including 37 amyloidosis cases, Stevens defined a limited number of structural risk factors, based on three sites of amino-acid substitution and the occurrence of N-glycosylation (240).

A study with LC mutants bearing certain amino acids found in amyloidogenic precursors suggested that an unfolding step facilitated by these substitutions could be required for fibril formation (241); however, as stressed by the authors, the *in vitro* conditions were clearly nonphysiologic, and such models with single replacements in a structural context that is different from the original pathogenic LCs have an essentially theoretical interest.

Environmental factors may also play some role. For example, the kidney contains high concentrations of urea that were shown to enhance fibril formation by reducing the nucleation lag time (242). The same LC can assemble into fibrils or form granular aggregates upon exposure to various environmental conditions (243).

One AL amyloidosis-associated LC, protein Mcg, has been extensively studied at the three-dimensional level by x-ray crystallography (244), although the fundamental results have rarely been discussed with respect to pathology. Despite all speculations on possible abnormal conformation of amyloidogenic LC V domains, the dimer Mcg is strikingly "normal" and similar to other known mouse and human LCs and antigen-binding (Fab) fragments. An interesting observation is that the combination of hypervariable regions (complementary-determining regions [CDRs]) from both monomers mimics a normal antigen-binding site with affinities toward haptenlike compounds such as dinitrophenyl (DNP)-lysine and opioid peptides (245,246). The number of contact residues and consequent binding affinity are decreased after reduction of the disulfide bond between COOH-terminal cysteinyls of the C domains (247). Because DNP-lysine can bind specifically amyloidogenic dimers (248), it is possible that covalent binding between LCs influences their pathogenicity. In other cases, specific antibodylike binding of isolated Ig LCs was shown to be dependent on the dimerization status (249,250). The hypothesis that specific recognition by amyloidogenic LCs plays a pathogenic role was enforced by the demonstration of their higher dimerization constants (251), and by the finding of high rates of somatic mutations clustered in the CDRs, suggesting antigen-driven selection (252,253).

Specific affinity of an LC toward an extracellular structure might create a nucleus that could lead to elongation of a fibril, in accordance with proposed mechanisms of other forms of amyloidogenesis. Indeed, four tested amyloidogenic LCs seemed to react more strongly than others with endothelial cell basement membranes *in vitro* (P. Aucouturier and D. Cines, unpublished results). This would also explain the specific tissue localization of amyloid and its close association with extracellular matrix components such as GAGs. Indeed, amylogenic LCs seem to display a special affinity to heparin and chondroitin sulfate B and C (254).

Mechanisms of Tissue Injury

Tissue injury is mostly the consequence of extensive deposition of amyloid. However, at least in some tissues such as the heart, the infiltration alone did not correlate well with the degree of heart failure or survival. Infusion of LCs from patients with cardiac amyloidosis caused diastolic dysfunction in isolated mouse hearts (255). Amyloid LC proteins isolated from patients with amyloid cardiomyopathy, specifically alter cellular redox state in isolated cardiomyocytes, and increase intracellular reactive oxygen species and the redox-sensitive protein, heme oxygenase-1 (256). Amyloid LCs may thus contribute directly to the pathogenesis of amyloid cardiomyopathy, independent of extracellular fibril deposition. Along the same line, LCs from AL amyloid patients incubated with cultured human mesangial cells induced a macrophage-like phenotype, whereas

those from LCDD patients induced a myofibroblastic phenotypic transformation (257).

Epidemiology and Clinical Features of AL Amyloidosis

Epidemiology

Ig LC-related (AL) amyloidosis was the first characterized at the biochemical level (205). This may be explained by the abundance of deposits, especially in certain organs (kidneys, liver, heart, spleen), and the often rapid fatal evolution of the disease, which prompted development of extraction methods from necropsy pieces (154). The incidence of primary AL amyloidosis in the United States is 8 per million per year and has remained stable during the last four decades (258). Among systemic amyloidoses with predominant renal involvement, AL amyloidosis has become more common than AA amyloidosis since the widespread use of effective antimicrobial agents. The male-to-female ratio varies from 1 to 2 according to series (259,260). The median age at diagnosis is between 60 and 65 years. About two-thirds of patients are between 50 and 70 years of age at diagnosis, and only 1% to 4% is younger than age 40 years (260,261).

Amyloid deposits are found in approximately 10% of myeloma cases (262), and this incidence reaches 20% in patients with pure LC myeloma (263). The high frequency of amyloidosis associated with myeloma (56%) in Alexanian's series (264) was attributed to the referral of more myeloma patients for chemotherapy to the authors' institution. Conversely, a minority (probably less than one of four) of patients with AL amyloidosis are considered to bear a patent immunoproliferative disease, which usually is a multiple myeloma, although other forms such as Waldenström's macroglobulinemia (265) and non-Hodgkin's lymphoma (266) may occur. In fact, the true incidence of myeloma depends on the criteria used for its diagnosis. AL amyloidosis without overt immunoproliferative disease is usually referred to as *primary amyloidosis*. If multiple myeloma is not present at the diagnosis of AL amyloidosis, it is unlikely to develop.

Clinical and Laboratory Features of AL Amyloidosis

The largest series published thus far on AL amyloidosis is that of the Mayo Clinic (261). It included 474 patients with biopsy-proved amyloidosis. Ninety-nine percent of the patients were 40 years of age or older; 69% were men and 31% were women. Seventy-one patients (15%) had a myeloma according to the authors' criteria. Two hundred and nineteen (56%) of 391 patients had an increased number (≥6%) of plasma cells in the bone marrow.

The clinical and laboratory features at presentation and at diagnosis are summarized in Tables 75-9 and 75-10 (261,267). The main clinical symptoms at presentation are weakness and weight loss. Except for bone pain, there is no difference in the incidence of initial symptoms in patients with and without myeloma. Nephrotic syndrome, orthostatic hypotension, and peripheral neuropathy are, however, more common at diagnosis in patients with AL amyloidosis without myeloma than in those with associated myeloma (Table 75-10).

Proteinuria mainly composed of albumin is noted in 55% of the patients, indicating that glomerular involvement is a common feature of AL amyloidosis. There is a poor correlation between the extent of amyloid deposits seen on a kidney biopsy specimen and the extent of proteinuria. Even small amyloid deposits have been associated with severe nephrotic syndrome (268). Microscopic hematuria is an exception, and, therefore, should prompt the search of a bleeding lesion of the urinary tract. Renal manifestations may also include renal tubular acidosis (mostly as a part of Fanconi's syndrome) and polyuria-polydipsia (resulting from urinary concentration defect), when amyloid deposits occur around proximal tubules and Henle's loops (or collecting ducts), respectively. High urinary protein excretion and high serum creatinine at diagnosis are pejorative renal predictors. Renal insufficiency occurs usually in the presence of marked kidney enlargement and is usually not associated with hypertension.

TABLE 75-9

CLINICAL AND LABORATORY FEATURES AT PRESENTATION IN 474 PATIENTS WITH PROVEN AL AMYLOIDOSIS

Initial symptoms	
Fatigue	62%
Weight loss	52%
Pain	5%
Purpura	15%
Gross bleeding	3%
Physical findings[a]	
Palpable liver	24%
Palpable spleen	5%
Lymphadenopathy	3%
Macroglossia	9%
Laboratory findings	
Increased plasma cells (bone marrow ≥6%)	56%
Anemia (hemoglobin <10 g/dL)	11%
Elevated serum creatinine (≥1.3 mg/dL)	45%
Elevated alkaline phosphatase	26%
Hypercalcemia (>11 mg/dL)	2%
Proteinuria (≥1.0 g/24 hours)	55%
Urine light chain	73%[b]
κ chain	23%
λ chain	50%

[a]A comparison of the prevalence of clinical syndromes according to the presence or the absence of myeloma is given in Table 75-10.
[b]Of 429 patients. All other figures refer to all 474 cases.
(Data from: Kyle RA, Gertz MA. Primary systemic amyloidosis: clinical and laboratory features in 474 cases. *Semin Hematol* 1995;32:45.)

TABLE 75-10

SYNDROMES AT DIAGNOSIS IN 229 PATIENTS WITH PROVEN AL AMYLOIDOSIS

Syndromes	Without myeloma (182 patients)	With myeloma (47 patients)
Nephrotic syndrome	37%	13%
Carpal tunnel syndrome	21%	38%
Congestive heart failure	23%	23%
Peripheral neuropathy	20%	6%
Orthostatic hypotension	16%	4%

(Data from: Kyle RA, Greipp PR. Amyloidosis (AL): clinical and laboratory features in 229 cases. *Mayo Clin Proc* 1983;58:665.)

Restrictive cardiomyopathy is found at presentation in up to one-third of patients and causes death in about half. Characteristic features of amyloid on ECG include low voltages and a pattern suggestive of myocardial infarction without evidence of ischemic damage on echocardiography. Infiltration of the ventricular walls and the septum may be recognized by echocardiography. The ejection fraction is frequently normal or even increased. Doppler flow studies are required to identify diastolic dysfunction frequently missed in routine studies. Amyloid may also induce dysrhythmias and the sick sinus syndrome. Amyloid deposits in the coronary arteries may result in angina pectoris and myocardial infarction. Recently, blood levels of cardiac troponins and N-terminal pro-brain natriuretic peptide (NT-proBNP) were shown to be sensitive markers of myocardial dysfunction and powerful predictors of overall survival in patients with AL amyloidosis (269–272).

Involvement of the gastrointestinal tract is also common and can cause motility disturbances, malabsorption, hemorrhage, or obstruction. Macroglossia occurs in about one-fifth of these patients. It may interfere with eating and obstruct airways. Hepatomegaly occurs initially in one-third of the patients, but abnormalities of hepatic function remain generally mild. Hyposplenism, usually associated with splenomegaly, is occasionally found. Peripheral neuropathy occurs in one-fifth of cases and is usually responsible for a painful sensory polyneuropathy followed later by motor deficits. Autonomic neuropathy causing orthostatic hypotension, lack of sweating, gastrointestinal disturbances, bladder dysfunction, and impotence may occur alone or together with peripheral neuropathy. Orthostatic hypotension is one of the major hampering complications of AL amyloidosis, causing some patients to be bedridden. Skin involvement may take the form of purpura, characteristically around the eyes, as well as ecchymoses, papules, nodules, and plaques, usually on the face and upper trunk. AL amyloidosis may also infiltrate articular structures and mimic rheumatoid or an asymmetric seronegative synovitis. Infiltration of the shoulders may produce severe pain and swelling ("shoulder-pad" sign). A rare but potentially serious manifestation of AL amyloidosis is an acquired bleeding diathesis that may be associated with deficiency of factor X and sometimes also factor IX, or with increased fibrinolysis. It should be systematically sought before any biopsy of a deep organ. It does not occur in AA amyloidosis, although widespread vascular deposits may also be responsible for bleeding. Actually, AL amyloidosis may infiltrate almost any organ other than the brain and thus be responsible for a wide variety of clinical manifestations.

On average, monoclonal LCs can be detected by immunoelectrophoresis in 73% of the urine samples, and the λ-isotype is twice as common as the κ-isotype, contrasting with the 1:2 λ-to-κ ratio observed in patients with multiple myeloma alone. With the use of more sensitive immunochemical techniques, a monoclonal Ig is found in nearly 90% of patients in the serum and/or the urine. This value could further increase with the routine use of recently developed method for serum free LC measurement (273–275). It is, however, worth noting that, even under such conditions, there is no detectable monoclonal Ig in the serum and urine of some patients.

"Primary" Amyloidosis: A True Plasma Cell Dyscrasia

It is now well established that "primary" amyloidosis (i.e., AL amyloidosis without myeloma) is a true plasma cell dyscrasia. This concept introduced by Osserman et al. (276) was elegantly confirmed by immunofluorescence and biosynthetic studies of bone marrow cells. Preud'homme et al. (231) identified, by immunofluorescence, monoclonal plasma cell populations in 12 of 14 patients with "primary" amyloidosis, even in those without detectable serum and urine monoclonal Ig and with a normal percentage of bone marrow plasma cells. Moreover, the synthesis and excretion of large amounts of free monoclonal LCs by plasma cells were demonstrated in every patient studied by Buxbaum (232) and Preud'homme et al. (231), together with the presence of LC fragments in almost all patients. This contrasted with nonmyelomatous secondary amyloidosis, which was characterized by normal distribution of bone marrow plasma cells by immunofluorescence and by synthesis of normal-sized Ig, without free LC secretion and fragments (231).

A definition of "primary" AL amyloidosis is certainly useful for inclusion of patients in prospective therapeutic trials. However, from a pathophysiologic point of view, myeloma-associated and "primary" AL amyloidoses represent two ends of a single entity. The intrinsic pathogenicity of the precursor free LC is probably highly variable from one patient to another, so that expression of the disease occurs in the context of very different tumor masses. Whatever the hematologic status, the amyloid disease is responsible for the predominant symptoms in most patients. AL amyloidosis is thus typical of these forms of plasma cell dyscrasias in which malignancy is conferred by the pathogenic LC rather than the underlying hematologic disease.

Diagnostic Procedures in AL Amyloidosis

AL amyloidosis should be considered in any patient who presents with nephrotic range proteinuria with or without renal insufficiency, nondilated cardiomyopathy, peripheral neuropathy, hepatomegaly, or autosomic neuropathy, whether or not a paraprotein can be detected in the serum or urine. Particular vigilance should be maintained in patients with multiple myeloma or MGUS, especially of the λ isotype. Initial investigation should confirm the diagnosis of amyloidosis on tissue biopsy and this should be followed by investigations to establish the type of amyloid present and the extent of organ involvement.

All patients require immunofixation of serum and urine in an attempt to demonstrate the presence of a monoclonal LC. A bone marrow specimen is necessary because 10% of patients will not have a demonstrable monoclonal LC by immunofixation, and a clone of plasma cells detected in the bone marrow by immunofluorescence or immunohistochemistry is strong supportive evidence of AL. Immunonephelometric quantitation of free LCs is a useful complement to immunofixation, because it shows remarkable specificity and sensitivity. The assay gives a positive result (raised level of either κ or λ together with an altered ratio of free κ to free λ LC) in 98% of patients with systemic AL amyloidosis (277).

Histologic diagnosis may be achieved by biopsies of various tissues. Biopsy of an affected organ is usually diagnostic, but less invasive alternatives should be preferred first. As indicated in Table 75-11, the less invasive procedures yield positive results in up to 90% of cases (261,278,279). Rectal biopsy is diagnostic in greater than 80% of cases, provided that the biopsy specimen contains submucosal vessels in which early deposits are located. Bone marrow biopsy should also be stained with Congo red for the presence of amyloid, and involvement of the bone marrow is strongly suggestive of the AL type. Evaluation of adequate specimens in experienced laboratories is necessary to maintain high diagnostic sensitivity and specificity. Both false-positive and false-negative interpretations are not uncommon.

It is not always easy to be certain that amyloidosis is of the AL type because immunohistochemical staining for Ig LCs is not fully reliable, and the presence of a monoclonal component is strong but not conclusive evidence of AL. Extreme caution is required when patients have an intact monoclonal Ig in the serum without evidence of circulating free LCs in the serum or in the urine. In those cases, hereditary forms of

TABLE 75-11

DIAGNOSTIC YIELD OF BIOPSIES IN 100 PATIENTS WITH AL AMYLOIDOSIS ACCORDING TO THE SITE OF BIOPSY

Site of biopsy	No. tested	Sensitivity of the diagnostic procedure
Bone marrow	44	52.3
Gingiva	6	83.3
Rectal mucosa	21	85.7
Small bowel	8	87.5
Subcutaneous abdominal fat	97	88.7
Kidney[a]	21	100
Heart[a]	14	100
Liver[a]	11	100
Skin[a]	3	100
Nerve[a]	2	100
All other	22	

[a]Organ biopsy was performed because of clinical manifestations.
(Data from: Skinner M, Anderson J, Simms R, et al. Treatment of 100 patients with primary amyloidosis: a randomized trial of melphalan, prednisone, and colchicine versus colchicine only. *Am J Med* 1996; 100:290.)

amyloidosis should be considered because they may produce clinical syndromes indistinguishable from AL (280) and coexist with MGUS (191). In cases of doubt, DNA analysis and/or amyloid fibril sequencing by mass spectrometry may be necessary. Imaging using SAP scanning may be helpful because demonstration of bone marrow involvement is strongly correlated with amyloidosis of AL type.

There are no universally agreed or validated criteria for defining organ involvement. Most groups use familiar but not identical criteria, and only two have reported these in detail (281,282). A consensus panel has recently established criteria that centers can now use to define organ involvement, organ response, and organ disease progression (Table 75-12) (283). Abnormalities in strain echocardiography (284), elevation of NT-proBNP (270), and elevation of cardiac troponins (269) are promising markers of myocardial dysfunction in AL amyloidosis, but they need to be evaluated sufficiently against echocardiography for these variables to be used as criteria of cardiac involvement.

Outcome and Treatment of AL Amyloidosis

"Natural" History

AL amyloidosis is among the most severe complications of plasma cell proliferative disorders. The median survival was 13 months in the series of 474 patients from the Mayo Clinic reported in 1995 (261). Despite the use of melphalan and prednisone, the median survival is still only 17 to 18 months (285). The natural history varies with the extent and nature of organ involvement, but fewer than 5% survive 10 or more years from the time of diagnosis (286). In those long-term survivors who all received alkylating-agent therapy, the monoclonal protein disappeared from the serum or urine. Few studies have specifically addressed prognostic variables (Table 75-13). A recent study found an association between certain V_L germline genes and clinical presentation and outcome (201).

TABLE 75-12

CRITERIA FOR ORGAN INVOLVEMENT, ORGAN RESPONSE AND ORGAN DISEASE PROGRESSION IN PATIENTS WITH AL AMYLOIDOSIS

	Organ involvement	Organ response	Organ disease progression
Kidney	24-Hour urine protein >0.5 g, predominantly albumin	50% Decrease (at least 0.5 g/day) of 24-hour urine protein (urine protein must be >0.5 g/day pretreatment)	50% Increase (at least 1 g/day) of urine protein to greater than 1 g/day
Heart	Echo: mean wall thickness >12 mm, no other cardiac cause	Mean interventricular septal thickness decreased by 2 mm, 20% improvement in ejection fraction, improvement by 2 NYHA[a] classes without an increase in diuretic use, or improvement by 1 NYHA class associated with a 50% reduction in diuretic requirements and no increase in wall thickness	Interventricular septal thickness increased by 2 mm, increase in NYHA class by 1 grade with a decreasing ejection fraction of ≥10%
Liver	Total liver span >15 cm in the absence of heart failure or alkaline phosphatase >1.5 times upper limit of normal	50% Decrease in abnormal alkaline phosphatase, decrease in liver size radiographically at least 2 cm	50% Increase of alkaline phosphatase above the lowest value
Nerve	P[b] (clinical): symmetric lower extremity sensorimotor neuropathy A[c]: gastric-emptying disorder, pseudoobstruction, voiding dysfunction	Improvement in electromyogram nerve conduction velocity (rare)	Progressive neuropathy by electromyography or nerve conduction velocity

[a]NYHA: New York Heart Association.
[b]P: peripheral.
[c]A: autonomic.
[d]Not related to direct organ infiltration.
(Data from: Gertz M, Comenzo R, Falk RH, et al. Definition of organ involvement and treatment response in primary systemic amyloidosis AL. *Am J Hematol* 2005;79:319.)

TABLE 75-13

PROGNOSTIC FACTORS IN PATIENTS WITH AL AMYLOIDOSIS

Pejorative prognostic indicators:
- Symptomatic or substantial echocardiographic evidence of cardiac amyloid (median survival of about 6 months) (287)
- Autonomic neuropathy (288)
- Liver involvement with hyperbilirubinemia (289)
- Associated multiple myeloma (201)
- A large whole-body amyloid load on SAP scintigraphy and evidence of accumulation of amyloid on serial SAP scans when available (277)

Better prognostic indicators:
- Proteinuria or peripheral neuropathy (without autonomic neuropathy) as the dominant clinical feature (261)
- Substantial suppression of underlying clonal disease by chemotherapy
- Regression of amyloid deposits on serial SAP scintigraphy when available

(Adapted from: guidelines Working Group of UK Myeloma Forum; British Committee for Standards in Haematology, British Society for Haematology. Guidelines on the diagnosis and management of AL amyloidosis. *Br J Haematol* 2004;125:681).

Monitoring AL amyloidosis and Assessing Response

Because amyloid fibrils in AL amyloidosis consist of monoclonal immunoglobulin LCs produced by a clone of plasma cells, treatment with alkylating agents that are effective against plasma cell dyscrasias is warranted. However, the results of chemotherapy in amyloidosis are difficult to document because there is no easy way to measure the amount of amyloid in a patient. Investigators have long recognized that resolution of the nephrotic syndrome does not necessarily reflect that of amyloid deposits, and that the progressive deposition of amyloid can occur in the presence of improved clinical and laboratory findings. Scintigraphy after the injection of ^{123}I-labeled SAP component may be helpful for monitoring the extent of systemic amyloidosis (171,277), but this technique is not readily available. The definition of a response in amyloidosis should be hematologic and organ-based (283). A complete hematologic response is defined by negativity of serum and urine for a monoclonal protein by immunofixation, normal free LC ratio, and less than 5% plasma cells in the bone marrow. Criteria for organ response and organ disease progression are depicted in Table 75-12.

Principles of Treatment

Therapy is aimed at annihilating the plasma cell clone. All patients with AL amyloidosis deserve a trial of chemotherapy because of the improved survival of responders (290). In the latter, gradual regression of AL amyloidosis deposits is possible (291,292). However, the degree by which the clonal disease needs to be suppressed to produce clinical benefit varies substantially between patients and depends on many factors, notably the turnover rate of the amyloid deposits. Supportive measures to preserve organ function are essential adjuncts to therapy.

To date, physicians treating patients with AL amyloidosis are facing a dilemma because clinical benefit from conventional chemotherapy does not occur for many months after the underlying plasma cell clone has been suppressed (285). Therefore, patients who receive slow-acting conventional chemotherapy regimens (e.g., low-dose melphalan and prednisone) often do not live long enough to derive benefit. Conversely, more intensive chemotherapy (e.g., high-dose melphalan with peripheral blood stem cell transplantation [PBSCT]) is associated with much greater treatment-related toxicity than seen in

TABLE 75-14

OUTCOME IN PREVIOUSLY UNTREATED AL AMYLOIDOSIS

Regimen	Series (ref. no.)	No. of patients	Response (% all patients)[a]	TRM[b]	Overall survival (median)	Comments
MP	Kyle et al., 1997 (285)	77	28	Not reported	18 months	Risk of MDS
	Gertz et al., 1999 (293)	52	27	Not reported	29 months	
VAD	Lachmann et al., 2002 (294)	98	54 (63% of evaluable)	7%	50 months (projected)	Selected patients
IDM	Lachmann et al., 2002 (294)	33	46	18%	Not reached	Poor risk group
M-HDD	Palladini et al., 2004 (295)	46	67	None	Not reached	Patients ineligible for PBSCT
HDD-IFN	Dhodapkar et al., 2004 (296)	93	53 (of evaluable hematologic) 45 (of evaluable organ)	7%	31 months	
PBSCT	Comenzo and Gertz, 2002 (297)	148	39 (62% of evaluable)	21%–39%	60%–70% at 1 year	Selected patients
	Skinner et al., 2004 (298)	312	40	13%	54 months	Selected patients

HDD-IFN, high-dose pulsed dexamethasone with maintenance dexamethasone/alpha interferon; *IDM*, intermediate dose melphalan; *MDS*, myelodysplastic syndrome; *M-HDD*, melphalan and high-dose pulsed dexamethasone; *MP*, melphalan and prednisone; *PBSCT*, peripheral blood stem cell transplantation; *VAD*, vincristine, Adriamycin, dexamethasone.
[a]Response criteria have varied but generally include response of either plasma cell dyscrasia and/or organ dysfunction.
[b]Treatment-related mortality (TRM) is defined as death during treatment or within 100 days from completing treatment. Note that the reported TRM in PBSCT studies did not include deaths during mobilization and reinfusion of peripheral blood progenitor cells.
[c]21% average of four single center studies; 39% average of two multicenter studies.
(Adapted from: guidelines Working Group of UK Myeloma Forum; British Committee for Standards in Haematology, British Society for Haematology. Guidelines on the diagnosis and management of AL amyloidosis. *Br J Haematol* 2004;125:681.)

patients with myeloma because of multiple organ involvement that may be clinically latent (260). A summary of available data is shown in Table 75-14. The role of PBSCT in treatment of AL amyloidosis is far from settled in the absence of data from well-designed, randomized controlled trials, which would compare risk–benefit ratios of high-dose versus an effective conventional therapy like M-Dex (299,300) chemotherapy with stem cell support.

Chemotherapy and Other Agents Without Stem Cell Support

MP (melphalan and prednisone) is the only chemotherapy regimen that has been evaluated in randomized controlled clinical trials; MP was superior to colchicine alone in terms of response and survival (285). Response, assessed by organ function and paraprotein levels, was seen in 28% of patients on MP and only 3% of those on colchicine. Survival in patients receiving MP was 18 months compared with 8.5 months for colchicine alone. Patients who respond to MP survive significantly longer than nonresponders (89 months vs. 14 months), (285). However, the median time to response is about 12 months. The adverse effects of melphalan include myelotoxicity, and there is a 20% risk of myelodysplasia, often leading to secondary leukemia in patients who survive longer than 3.5 years (301).

The VAD (vincristine, Adriamycin, dexamethasone) regimen has theoretical advantages over MP in treating AL amyloidosis because a rapid reduction in tumor burden is observed in patients with myeloma. Furthermore, unlike melphalan, VAD does not deplete stem cell reserve, which is an important advantage for subsequent PBSCT. However, its use is limited in patients which AL amyloidosis because of cardiotoxicity of Adriamycin and of exacerbation of autonomic neuropathy by vincristine. The largest series included 98 patients who received a median of four cycles of VAD (or CVAMP: cyclophosphamide, vincristine, Adriamycin, methylprednisolone) as first-line therapy (294) (Table 75-14). Patients with symptomatic heart failure, autonomic neuropathy, or severe peripheral neuropathy were excluded. Response as defined by a fall in the amyloidogenic class of serum free LC by more than 50%, was observed in 53 patients (representing 63% of 84 evaluable patients). The function of organs predominantly affected by amyloid improved in half of all patients. After a median follow-up of 21 months, the projected median overall survival was 50 months.

The intermediate dose melphalan (IDM) regimen was used as first-line therapy in 33 patients who were selected on the basis that they were not fit enough to receive VAD (294) (Table 75-14). The response rate appears similar to that seen with VAD, and toxicity is acceptable in this poor risk group.

The combination of high-dose dexamethasone (HDD) and melphalan (M-HDD) has been used to obtain a rapid response in 46 patients ineligible for PBSCT (295) (Table 75-14). Thirty-one (67%) achieved a hematologic response (median, 4.5 months) and 15 (33%) a complete remission. In 22 (48%) of the responsive patients, functional improvement of the organs involved was observed. Despite the fact that patients with advanced disease were selected, there was no treatment-related mortality. Clinical efficacy of HDD with maintenance dexamethasone/alpha interferon has also recently been examined in a multicenter study of 93 patients (296) (Table 75-14). Exclusion criteria related to AL amyloidosis were only NYHA class-IV congestive heart failure. Hematologic response occurred in 53% of 55 patients evaluable for hematologic response (median, 103 days) with complete remission in 24% (median, 173 days). Organ response in at least one involved organ was seen in 33 (45%) of 73 patients evaluable for organ response. Treatment-related mortality was observed in 6 (7%) patients. The tolerance to this regimen was lower than that of M-HDD because HDD was given three times per cycle (instead of one time per month). The use of HDD, alone or in combination, appears as a promising first-line treatment in AL amyloidosis because of high rate of response, short median time to response, and relatively low treatment-related toxicity, even in patients ineligible for PBSCT. The association of thalidomide with dexamethasone is rapidly effective (median time to response, 3.6 months) but because of frequent treatment-related toxicity (symptomatic bradycardia in 26% of 31 patients), it should be used as a second-line treatment (302).

High-Dose Chemotherapy with Stem Cell Support

The use of high-dose melphalan (HDM) therapy and PBSCT in patients with AL amyloidosis was first reported in 1996, and a series of 25 patients was reported shortly thereafter (297). Since then encouraging results have been reported in several series of patients in various centers (297,298,303,304). Therapy with HDM and PBSCT can result in reversal of the clinical manifestations of AL amyloidosis in up to approximately 60% of patients who survive the procedure. A case-match-control study recently showed a superior overall survival in the transplantation group (305). However, the efficacy of PBSCT in AL amyloidosis has not been investigated in any controlled comparative study. In a French ongoing randomized trial, M-HDD is being compared to HDM and PBSCT in a series of 100 patients (299).

A major concern is that procedure-related mortality has been consistently and substantially higher among patients with AL amyloid than among those with multiple myeloma. The 100-day treatment-related mortality comprises between 13% and 39% (297,298,303,304,306). This reflects compromised function of multiple organ systems by amyloid, and, therefore, refinement of patient selection and improvement of peritransplantation management are priorities. There is also a significant risk, including death, associated with stem cell mobilization in patients with AL amyloidosis, even when granulocyte colony stimulating factor (G-CSF) is used alone (297).

HDM and PBSCT are not recommended in patients with any of the following: age older than 70 years; symptomatic cardiac amyloid; symptomatic autonomic neuropathy; history of gastrointestinal bleeding due to amyloid; dialysis-dependent renal failure; and more than two organ systems involved (260). When using these exclusion criteria, the benefits of HDM and PBSCT over other treatments without PBSCT remain to be established. Dispenzieri et al. (282) examined data from patients with AL amyloid treated at the Mayo Clinic from 1983 to 1997, and identified 229 patients who would now have been eligible for PBSCT based on age younger than 70 years and well-preserved cardiac, renal, and hepatic function. At a median follow-up of 52 months, their median survival was 42 months and 5- and 10-year survival rates were 36% and 15%, respectively.

Data from nonrandomized studies (277) have suggested that patients who achieve a 50% or greater decrease in free LC concentration after treatment with VAD or IDM have a similar survival to patients achieving the same response with HDM and PBSCT. It may therefore be appropriate to offer patients VAD or an HDD-containing regimen initially and reserve HDM, with its high associated treatment-related mortality, for those patients who do not respond adequately to initial therapy (as assessed by quantitation of free LCs).

Renal Supportive Care and Kidney Transplantation

Organ function in amyloid is precarious, and renal or cardiac failure is easily precipitated, even in individuals with apparently normal organ function by factors such as intravascular fluid depletion or intercurrent infection.

Most studies of the clinical course and outcome of patients on dialysis include both AL and AA amyloidoses. The patients' survival rate is low, but it compares favorably with that of

patients not requiring dialysis: From 66% to 72% at 1 year, it falls to 30% to 44% at 5 to 6 years (307–309). No difference in survival was observed at any time between the patients with AL and AA amyloidoses (308). The survival rate of patients treated with CAPD is similar to that of patients on hemodialysis.

Cardiac amyloid is the most important predictor of poor survival in patients with AL amyloidosis undergoing dialysis, and cardiac deaths represent the main cause of mortality in such patients (309). Congestive heart failure, atrioventricular or intraventricular conduction defects, and dysrhythmias due to amyloid myocardial involvement often occur. The management of patients with AL amyloid on hemodialysis is also often complicated by permanent hypotension, gastrointestinal hemorrhage, chronic diarrhea, and difficulties in the creation and maintenance of vascular accesses. It has, therefore, been suggested that CAPD could have several advantages over hemodialysis in the management of end-stage renal amyloidosis, including avoiding vascular access and deleterious effect on blood pressure (310); however, CAPD may induce protein loss in the dialysates and thereby enhance malnutrition.

Renal transplantation is limited by the severity of heart involvement and the recurrence of deposits in the transplanted kidney. There are a few case reports and small series reported in the literature of renal transplantation in AL amyloidosis (311). Renal transplantation may be considered in patients whose underlying clonal plasma cell disease has remitted following chemotherapy.

Epidemiology and Specific Features of AA Amyloidosis

Although AA amyloidosis does not involve deposition of Ig fragments and thus should not be classified within the group of monoclonal gammopathies, it shares with AL amyloidosis pathogenetic pathways, high prevalence of renal involvement, and some therapeutic aspects that deserve further consideration.

AA amyloidosis develops in 5% of patients with sustained elevation of serum amyloid A protein (SAA). Patients at risk are those with long duration of chronic inflammatory disease (median, about 10 years), high magnitude of acute phase SAA response, homozygosity for SAA1 isotype, Familial Mediterranean Fever (FMF) trait (heterozygosity for variant pyrin), and family history of AA amyloidosis (50% risk).

An important epidemiologic aspect of AA amyloidosis is the changing spectrum of underlying diseases (Table 75-15). Pyogenic and granulomatous infections, especially tuberculosis, account for far fewer cases than in the older series. This is because of the efficacy of antibiotic treatments for bacteria, which shows that amyloidosis can be efficiently prevented when its cause is suppressed. In contrast, the prevalence (about 70%) of amyloid linked to autoimmune inflammatory diseases, such as rheumatoid arthritis and juvenile chronic arthritis, has increased dramatically. AA amyloidosis in patients with Hodgkin's disease has virtually disappeared with more efficient treatment of the hematologic disease. In contrast, hereditary AA amyloidoses associated with familial recurrent fever syndromes are claiming an increasing portion of about 10% of cases in recent series.

There are a number of clinical manifestations of AA amyloidosis (314,315) (Table 75-16). The main target organ by far is the kidney affected in almost all patients with AA amyloidosis. Renal dysfunction may be acute with nephrotic syndrome, or very insidious. Proteinuria is absent in about 5% of cases. Gastrointestinal disturbances (including diarrhea, constipation, and malabsorption) and hepatosplenomegaly are the most common after kidney manifestations. In contrast with AL amyloidosis, congestive heart failure, peripheral neuropathy, macroglossia, and carpal tunnel syndrome occur infrequently. The reason for the differential distribution of AA and AL tissue deposits is not understood, but should stimulate research because this clinical observation deals with the deposition process per se.

The optimal method for diagnosing AA amyloidosis remains controversial. Although kidney biopsy is positive in about 100% of symptomatic patients, less invasive biopsy procedures should be preferred first (Table 75-16). Biopsies of accessory salivary glands, abdominal fat, and rectal mucosa yield positive results in 50% to 80% of patients. Immunohistochemical staining using antibodies to SAA is required to confirm that Congo red positive amyloid deposits are of AA type. SAP scintigraphy, when available, shows early accumulation of amyloid in spleen, whereas bones are not affected (contrary to AL amyloid).

Survival time of patients with AA amyloidosis is usually longer than in AL amyloidosis (Table 75-16). As in AL amyloidosis, an elevated serum creatinine and a low serum albumin are strong adverse prognostic indicators. Main causes of death are infections and dialysis complications, but not cardiac complications. Estimated survival was about 40% at 3 years, and median survival time was approximately 2 years, in older series (314,315).

Recently, it has been clearly established that amyloid load and clinical outcome in AA amyloidosis are dependent on circulating concentrations of SAA (317). Amyloid regressed in 25 of 42 patients whose median SAA values were within the reference range (<10 mg/L) throughout follow-up, and amyloidotic

TABLE 75-15

CHANGING SPECTRUM OF UNDERLYING DISEASES IN SECONDARY AA AMYLOIDOSIS[a]

	Dahlin 1949 (312) (N = 30)	Brownstein et al. 1971 (313) (N = 100)	Browning et al. 1985 (314) (N = 75)	Gertz and Kyle 1991 (315) (N = 64)	Joss et al. 2000 (316) (N = 43)	Gillmore et al. 2001 (317) (N = 80)
Rheumatic disease	2 (7)	15 (15)	55 (73)	42 (66)	30 (70)	60 (75)
Granulomatous infection (tuberculosis, fungus, leprosy)	9 (30)	28 (28)	8 (11)	0	6 (14)	0
Pyogenic infection	10 (33)	35 (35)	5 (7)	11 (17)	0	7 (9)
Inflammatory bowel disease	2 (7)	4 (4)	3 (4)	6 (9)	1 (2)	2 (3)
Malignancy/Castleman	7 (23)	18 (18)	3 (4)	2 (3)	1 (2)	2 (3)
Other[b]	—	—	2 (3)	3 (5)	5 (12)	9 (11)

[a]Data are number of patients (percentage).
[b]Including hereditary recurrent fevers and unknown.

TABLE 75-16

CHARACTERISTICS OF PATIENTS WITH SECONDARY AA AMYLOIDOSIS

	No. of patients	Age (yrs)	Male–female ratio	Presenting clinical syndrome—no. (%)	Source of tissue for diagnosis—no. (%)	Causes of death—no./totals (%)	Survival
Browning, et al., 1985 (314)	75	57 (18–81)	0.8	Proteinuria/renal failure—49 (65) Gastrointestinal disturbance—4 (5) Hepatosplenomegaly—3 (4)	Rectum—45 (60) Kidney—11 (15)	Renal failure—18/37 (49) Bronchopneumonia—7/37 (19) Cardiac—4/37 (11)	~40% at 3 years
Gertz and Kyle, 1991 (315)	64	56 (14–80)	1.5	Proteinuria/renal failure—58 (91) Gastrointestinal disturbance—14 (22) Goiter—6 (9) Neuropathy/carpal tunnel—2 (3)	Rectum—32 (50) Kidney—24 (38) Stomach/small bowel—15 (23) Marrow—12 (19)	Uremia/dialysis complications—32/47 (68) Sepsis—4/47 (9) Cardiac—4/47 (9)	~40% at 3 years Median = 24.5 mos

organ function stabilized or improved in 39 of these cases. Outcomes varied substantially among patients whose median SAA concentration exceeded 10 mg/L, but amyloid load increased and organ function deteriorated in most of those whose SAA was persistently greater than 50 mg/L. Estimated survival at 10 years was 90% in patients whose median SAA was less than 10 mg/L, and 40% among those whose median SAA exceeded this value ($p = 0.0009$). These data emphasize the fact that underlying inflammatory diseases responsible for amyloid must be treated as vigorously as possible and SAA (preferentially to C reactive protein [CRP]) levels must be monitored monthly and maintained at target value of less than 5 to 10 mg/L. In patients with inflammatory arthritis, the new anti-tumor necrosis factor-α therapy may help to achieve this goal (318).

Three large series of renal transplantation in patients with amyloidosis have been published (311,319,320). They all come from Scandinavia and essentially include patients with rheumatic diseases. In the last series of 62 renal transplantations including 29 grafts from related living donors (311), the 1-year actuarial patient survival rate was 79%, decreasing to 65% after 5 years. Amyloid deposits recur in about 10% of the grafts (311,319). There is a high risk of infection that is the main cause of early deaths. As with dialysis, cardiac involvement is a major threat for patients receiving renal transplants.

Familial Mediterranean Fever and Other Hereditary Recurrent Fever Syndromes

Familial Mediterranean fever (FMF) represents both a particular type of AA amyloidosis and is the most common cause of familial amyloidosis. Interestingly, colchicine has proved to be efficient both in the prevention and treatment of this type of amyloidosis (321,322). FMF is usually transmitted as an autosomal-recessive disorder and occurs most commonly in Sephardic Jews and Armenians (323,324). Mutations of the gene for proteins called pyrin or marenostrin have been demonstrated (325,326). Clinically, there are two independent phenotypes. In the first, brief episodic febrile attacks of peritonitis, pleuritis, or synovitis occur in childhood or adolescence and precede the renal manifestations. In the second, renal symptoms precede and may be the only manifestation of the disease for a long time. The attacks are accompanied by dramatic elevations of acute-phase reactants, including SAA. Amyloid deposits of the AA type are responsible for severe renal lesions with prominent glomerular involvement leading to end-stage renal disease at a young age, and early death. Zemer et al. (322) showed that in a cohort of 1,070 patients with FMF, colchicine, an agent effective in preventing attacks (327), could prevent the appearance of proteinuria and deterioration of renal function (in patients with amyloidosis who had proteinuria but not the nephrotic syndrome or renal insufficiency). Life-table analysis showed that the cumulative rate of proteinuria was 1.7% after 11 years in the compliant patients and 48.9% after 9 years in the noncompliant patients ($p < 0.0001$). In 1992, the authors corrected their statement that colchicine did not improve amyloidotic kidney disease once it had reached the nephrotic stage, because colchicine reversed the nephrotic syndrome in three patients (328). They insisted on the importance of a dosage adapted to the clinical situation. The minimum daily dose of colchicine for prevention of amyloidosis is 1 mg even if attacks are suppressed by a smaller dose. Patients with clinical evidence of amyloidotic kidney disease and kidney transplant recipients should receive daily doses of between 1.5 and 2.0 mg. Most interestingly, the serum levels of SAA during attacks in patients receiving colchicine were only slightly less elevated than those in untreated patients (329), suggesting that prevention of amyloidosis by colchicine might be due, at least in part, to a peripheral effect of the drug on fibril formation.

Next to FMF, there is a growing family of hereditary autoinflammatory disorders associated with renal amyloidosis (Table 75-17). A first report of systemic AA amyloidosis in patient with the hyperimmunoglobulinemia D with periodic fever syndrome (HIDS) was recently published (330).

In summary, clinical experience of FMF and inflammation-related AA amyloidosis demonstrates that it is possible to prevent and treat AA amyloidosis efficiently. The potential for amyloid to regress and for the function of amyloidotic organs to recover, support therapeutic strategies to decrease the supply of amyloid fibril precursor proteins in amyloidosis generally.

Therapeutic Prospects

Although the existence of amyloid has been known for about 150 years, therapeutic results have been modest so far, except in FMF and infection-related AA amyloidosis. This is partly due to the fact that the specific amyloid proteins have only recently been identified and the pathogenetic pathways remain obscure. New therapeutic strategies should be directed at the three steps of the amyloidogenic process: Synthesis of the precursor,

TABLE 75-17

AA RENAL AMYLOIDOSIS ASSOCIATED WITH FAMILIAL RECURRENT FEVER SYNDROMES

	FMF	TRAPS	MWS	FCAS
Ethnic geographic background	Sephardic Jews Armenians Turkish	Europe	Europe	USA
Inheritance	Autosomal recessive	Autosomal dominant	Autosomal dominant	Autosomal dominant
Gene	MEFV (16p13)	TNFRSF1A (12p13)	CIAS1 (1q44)	CIAS1 (1q44)
Protein	Pyrin/marenostrin	55 kDa TNF receptor	Cryopyrin	Cryopyrin
Age at onset	Childhood	Variable	Variable	Childhood
Duration of fever episodes (days)	3–4	7–21	1–2	1–2 (cold-induced)
Abdominal pain	++	++	+	+
Arthritis	++	+	+	+
Skin	+/− rash	++ erysipeloid	++ urticaria	++ urticaria
Deafness	−	−	+	−

FMF, familial Mediterranean fever; *TRAPS*, TNF receptor-associated periodic syndrome; *MWS*, Muckle-Wells syndrome; *FCAS*, familial cold autoinflammatory syndrome.
(Adapted from: Pirson Y. From renal amyloid deposits to the identification of the culprit genes. *J Nephrol* 2003;16:427.)

deposition of amyloid fibrils, and removal or dissolution of amyloid fibrils.

Prevention of Amyloid Precursor Synthesis

Curing the underlying disease has proved to be extremely effective, as shown in cases of AA amyloid. This objective must be envisioned, at least theoretically, in all forms of amyloidosis. The possibility of immunotargeting against precursor synthesis is also worth exploring.

Prevention of Amyloid Fibril Deposition

As previously discussed, the target of colchicine is debated. Because colchicine affects a number of cell functions including assembly of cytoskeleton fibers, the drug might alter fibril deposition or turnover. Its efficacy seems, however, to be limited to FMF. Enhancing the proteolysis of the amyloid precursor may be a rewarding approach, as serine proteases expressed on monocyte surfaces, in supernatants from monocyte cultures and in serum are capable of degrading SAA (331,332). Another promising therapeutic strategy is blockade of RAGE (receptor for advanced glycation end-products), a multiligand receptor of the immunoglobulin superfamily that also is a receptor for the amyloidogenic form of serum amyloid A (333). Antagonizing RAGE with a soluble form of the receptor or with blocking antibodies inhibits amyloid deposition in the spleen of mice injected with amyloid-enhancing factor and silver nitrate (333). Interestingly, interaction of RAGE with AA activates genes involved in cell stress. Whether these findings can apply to other types of amyloidosis remains to be determined.

Conversely, rapidly growing data on the nucleation process might lead to the synthesis of nucleation inhibitors for all types of amyloid. For example, low-molecular-weight (135 to 1,000) anionic sulfate or sulfate compounds were shown to interfere with heparan sulfate-stimulated β-peptide fibril aggregation *in vitro* (334). When administered orally, these compounds substantially reduced murine splenic AA amyloid progression. One of these compounds, Fibrillex, is currently been used in clinical trials. Novel therapies, such as Fibrillex, will have a major impact on the remaining affected population of patients with AA amyloidosis who do not respond to therapy for the underlying disorder or who have a rare inflammatory disease. They may also apply to other types of amyloid.

Removal or Dissolution of Amyloid Fibrils

The regression of amyloid deposits under effective treatment of the underlying disease as well as the finding that amyloid deposits may undergo redistribution (335) strongly suggest that amyloid fibrils can be catabolized and removed from tissues. This process is most likely restrained by proteinase inhibitors that have been detected in amyloid deposits of various types (336,337), and by fibril coating with the calcium-dependent lectin SAP (165). A potential approach, therefore, would be to block these inhibitors, to target proteolytic enzymes at the amyloid deposition site, or to dissociate SAP with competitive ligands.

A competitive inhibitor of SAP binding to amyloid fibrils has been developed by Pepys et al. (338). This palindromic compound also crosslinks and dimerizes SAP molecules, leading to their very rapid clearance by the liver, and thus produces a marked depletion of circulating human SAP. This mechanism of drug action potently removes SAP from human amyloid deposits in the tissues and may provide a new therapeutic approach to both systemic amyloidosis and diseases associated with local amyloid including Alzheimer's disease. The drug is currently being tested in animal models and clinical trials.

At present, most attempts to use dimethylsulfoxide (DMSO) as an amyloid solvent have been disappointing because of lack of efficacy and bad odor from the patient's breath. A novel iodinated anthracycline, (4′-iodo-4′-deoxydoxorubicin) binds specifically and with high affinity to all the natural amyloid fibrils, and promotes the disaggregation of fibrils both *in vitro* and *in vivo* (339). However, its clinical efficacy remains to be determined (340,341).

Immunotherapeutic Perspectives

The immune system might be manipulated in order to recognize amyloid fibrils as harmful foreign entities. Indeed, therapeutic vaccinations were performed in mouse models of Alzheimer disease with remarkable success (342,343). In prion diseases, also featuring amyloid-like accumulations of a misfolded self-protein, induction of specific immune effectors is also an important field of research (344). In AL-amyloidosis, Hrncic et al. demonstrated that a monoclonal antibody specific for a conformational determinant could accelerate the clearing of amyloid (345). Although we are still far from applicable human

immunotherapy, such experimental approaches might lead to future developments.

MONOCLONAL IMMUNOGLOBULIN DEPOSITION DISEASE

History and Nomenclature

It has been known since the late 1950s that nonamyloidotic forms of glomerular disease can occur in multiple myeloma. Kobernick and Whiteside (346) and Sanchez and Domz (347) first described glomerular nodules "resembling the lesion of diabetic glomerulosclerosis," lacking the staining features and fibrillar organization of amyloid. The monoclonal LC content of these lesions was recognized in 1973 by Antonovych et al. (5) and confirmed by Randall et al. (6), who published the first description of LCDD in 1976.

Monoclonal HCs were found together with LCs in the tissue deposits from certain patients (348), and the terms monoclonal Ig deposition disease (MIDD) (348,349) and LC- and HC-deposition disease (LHCDD) (350,351) were proposed. More recently, deposits containing monoclonal HCs only, that is, in the absence of detectable LCs, were first observed in 1993 in patients affected with otherwise typical Randall's disease (HC deposition disease [HCDD]) (8), and reports of two series of similar patients were published later (9,352). More than 20 cases of HCDD have been reported so far, but this disease is most likely underdiagnosed. In two further cases, termed "pseudo-γ HCDD," predominant γ4 chain reposits were demonstrated with pathologic aspects similar to MIDD; the authors suggested that misfolding or denaturation of the LC was responsible for its nonreactivity with specific antibodies (353).

In clinical and pathologic terms, LCDD, LHCDD, and HCDD are essentially similar and therefore will be described together. They differ from amyloidosis by the lack of affinity for Congo red and lack of fibrillar organization. The distinction also relates to different pathophysiology of amyloid, which implicates one-dimensional elongation of a pseudocrystalline structure, and of MIDD, which would rather involve a one-step precipitation of Ig chains.

Pathophysiology

The pathogenetic mechanisms leading to MIDD remain entirely hypothetical because circulating or urinary monoclonal immunoglobulin chain precursors are frequently absent, or present at very low levels, making their purification and analysis particularly difficult, and data on the precise nature of the visceral deposits remains speculative. MIDD is due to monoclonal κ LCs in most cases, with a probable overrepresentation of the rare $V\kappa_{IV}$ variability subgroup (24); however, contrary to the $V\lambda_{VI}$ subgroup, which was found exclusively on monoclonal LCs from AL amyloidosis patients (23), $V\kappa_{IV}$ LCs may be encountered in myeloma without renal involvement.

That immunoglobulin chain deposition involves unusual immunoglobulin chain properties is supported by the absence of detectable monoclonal component in the serum and urine in 10% to 20% of patients with MIDD, the recurrence of the disease in the transplanted kidney, and the biosynthesis of abnormal LCs by bone marrow plasma cells (14,351).

Abnormal Glycosylation and Structure of MIDD LCs. Structural abnormalities of immunoglobulins in MIDD have long been suggested by empirical studies of *in vitro* bone marrow cell biosynthesis products (14,348,349,351). In a study of immunoglobulin biosynthesis by bone marrow plasma cells in eight consecutive patients (349), LCs were of normal size in two cases, and short or apparently large in the other six patients. These short or large LCs showed a striking ability to polymerize when secreted *in vitro*. Abnormal glycosylation may be responsible for an increase in apparent molecular weight of the corresponding LC.

Undetectable circulating LCs were found to be N-glycosylated in all cases where they could be studied, either by *in vitro* inhibition of glycosylation with tunicamycin, analysis of the carbohydrate content of purified LCs (349), or by treatment with endoglycosidase-F (24). Therefore, LC glycosylation might increase the propensity of LCs to precipitate in tissues and displace the equilibrium from soluble toward deposited forms so that they are no more detectable in the body fluids.

Sequence Analysis. The first complete primary structure of an LC in LCDD was determined by Cogné et al. in 1991 (20). The 30-kDa κ chain found in the kidney was presumably identical to that secreted by the malignant plasma cells, since they shared the same apparent molecular mass and 13–amino acid N-terminal sequence. It was encoded by a normal-sized κ mRNA and was N-glycosylated. The C region was entirely normal and the V region belonged to the $V\kappa_{IV}$ subgroup. Eight mutations were observed, including replacement of Pro95 (considered as essential for the conformation of the third hypervariable region). Replacement of Asp70 by Asn determined a N-glycosylation site.

The primary structures of a few further LCDD precursors were analysed at the complementary DNA (354,355) and protein levels (356). Most peculiarities are clustered in peptide loops corresponding to the complementarity-determining regions (CDRs), that is, parts of the molecules normally implicated in antigen binding, suggesting that a first step of the pathogenesis could be an LC tropism for extracellular components behaving as antigenlike structures. The most remarkable observations were unusual hydrophobic residues at positions where they could either be exposed to the solvent or strongly modify the conformation, potentially leading to LC aggregation or interaction with other hydrophobic molecules (357).

However, as in AL amyloidosis, extrinsic conditions may also contribute to aggregation of the LC. The same LC can form granular aggregates or amyloid fibrils depending on the environment, and different partially folded intermediates of this protein may be responsible for amorphous or fibrillar aggregation pathways (358).

Heavy-Chain Deposition Disease: A Disease Featured by Heavy-Chain Deletions. A deletion of the first constant domain C_H1 was found in the deposited or circulating HC in the 11 patients with γ-HC deposition disease where this deletion was searched for (8,9,352,359,360) (Table 75-18). It also was suggested in a patient with α HCDD (361). A larger deletion also including the C_H1 domain, the hinge, and the C_H2 domain was found in one case (8). In the blood, the deleted HC was associated with LCs, mostly of the λ isotype, or circulated in small amounts as a free unassembled subunit (9). It is likely that the C_H1 deletion facilitates the secretion of free HCs that are rapidly cleared from the circulation by organ deposition (362). Deletion of the C_H1 is also found in HC disease, a lymphoproliferative disorder with free HC secretion without corresponding renal tissue deposition, and in AH-amyloidosis in which deposits have a fibrillar organization. In heavy chain disease, however, the variable domain also is partially or completely deleted, which suggests that the V_H domain is required for tissue precipitation. Sequence analysis of two HCDD proteins did

TABLE 75-18

IMMUNOLOGIC CHARACTERISTICS OF PATIENTS WITH HEAVY-CHAIN DEPOSITION DISEASE (HCDD) ACCORDING TO Ig SUBTYPE[a]

	No. of patients	C_H1 deletion	Complement deposition	Complement activation
$\gamma 1$	8	6/6[b]	5/8	7/8
$\gamma 2$	1	NS	1 (weak)	0/1
$\gamma 3$	5	3/3	4/4	4/4
$\gamma 4$	3	2/2	1 (weak)	NS
γ (isotype not specified)	4	NS	1/1	0/1
α	2	NS	1/2	0/2
μ	1	NS	1/1	0/1

NS, not specified.
[a]Cases are from references 8,9,352,359,360.
[b]In one patient, C_H1 and C_H2 were deleted (8).

show unusual amino acid substitutions in the V_H, which might change their physicochemical properties, including charge and hydrophobicity (363).

Deposition Does Not Mean Pathogenicity. The finding by Solomon et al. (17) of unexpectedly frequent (14 of 40) deposition of human monoclonal LCs along basement membranes in a mouse experimental model, raises the question of the relationship between tissue precipitation and pathogenic effects. Although approximately 80% of MIDDs are caused by κ chains, human LCs, which were found deposited along basement membranes in mice, were predominantly of the λ type (9 of 14). In addition, LC deposition similar in aspect to LCDD by immunofluorescence but with only scanty granular electron-dense deposits in the tubular basement membrane, may occur in the absence of glomerular lesions and tubular basement membrane thickening (352,364). Whether the diagnosis of MIDD should be restricted to the patients with extracellular matrix accumulation, remains debated. However follow-up data indicate that the subgroup of patients with evidence of LC deposition by immunofluorescence and negative electron microscopy is not characterized by a milder course (365).

Pathophysiology of Extracellular Matrix Accumulation. A striking feature of MIDD is the dramatic associated accumulation of extracellular matrix. A role for transforming growth factor-β (TGF-β) is supported by its strong expression in glomeruli of MIDD patients, and by *in vitro* experiments using cultured mesangial cells (366). Incubation of mesangial cells with LCs from patients with MIDD induces cell changes, activation of PDGF-β and its receptor, production of MCP-1, as well as increased expression of Ki-67, a proliferation marker, whereas tubulopathic LCs chain have no effect (367). Because of the similarities between MIDD- and diabetes-induced nodular glomerulosclerosis, including the strong reactivity of lesions with the periodic acid–Schiff (PAS) reagent, it has been suggested that immunoglobulin chains might stimulate mesangial cells in a similar manner to advanced glycation-end products (AGEs). Recent experiments performed by Teng et al. (368) revealed that monoclonal LCs responsible for LCDD or AL-amyloidosis are endocytosed by cultured mesangial cells through a yet unidentified caveolae-associated receptor. In both conditions, contact with the pathogenic LCs increases expression of this receptor, and different resulting pathologic actions would result from distinct cellular trafficking.

Pathologic Features

Light Microscopy

The morphologic features of these disorders are well established, and, in retrospect, cases of undiagnosed "paraamyloid," or unidentified glomerulonephritis, or even "diabetic" glomerulosclerosis in patients with normal glycemic control were presumably MIDD. MIDD should not be considered a pure glomerular disease. In fact, tubular lesions may be more conspicuous than the glomerular damage.

Tubular lesions are characterized by the deposition of a refractile, eosinophilic, PAS-positive, ribbonlike material along the outer part of the tubular basement membrane in virtually all patients with MIDD. The deposits predominate around the distal tubules, Henle's loops, and in some instances the collecting ducts, the epithelium of which is flattened and atrophied. Typical myeloma casts are only occasionally seen in pure forms of MIDD. In advanced stages, a marked interstitial fibrosis including refractile deposits is frequently associated with tubular lesions.

Glomerular lesions are much more heterogeneous (362). Nodular glomerulosclerosis is the most characteristic (186) (Fig. 75-12A), being found in 30% to 100% of patients with LCDD (352,362). Kappa-LC deposition is more likely than λ-LC deposition to be associated with nodular glomerulosclerosis and granular electron-dense deposits (see subsequent text) (365). Expansion of the mesangial matrix was observed in all cases of HCDD, with nodular glomerulosclerosis in almost all of them (9,352). Mesangial nodules are composed of PAS-positive membranelike material and are often accompanied by mild mesangial hypercellularity. The capillary loops stretch at the periphery of florid nodules and may undergo aneurysmal dilation. The Bowman's capsule may contain a material identical to that present in the center of the nodules. These lesions resemble nodular diabetic glomerulosclerosis, but some characteristics are distinctive: The distribution of the nodules is fairly regular in a given glomerulus, the nodules are often poorly argyrophilic, and exudative lesions as "fibrin caps" and extensive hyalinosis of the efferent arterioles are not observed. In occasional cases with prominent endocapillary cellularity and mesangial interposition, the glomerular features mimic a lobular glomerulonephritis.

Milder forms simply show an increase in mesangial matrix and sometimes in mesangial cells, and a modest thickening of the basement membranes appearing abnormally bright and rigid. Glomerular lesions may not be detected by light

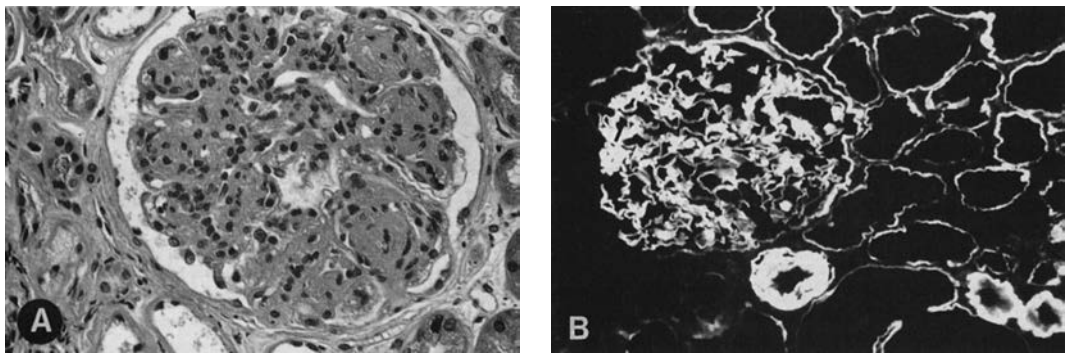

FIGURE 75-12. Monoclonal immunoglobulin deposition disease. **A:** Typical nodular glomerulosclerosis. Note membranelike material in the center of the nodules and nuclei at the periphery. Some glomerular capillaries show double contours (*arrow*). Note also thickening of the basement membrane of atrophic tubules. (Light microscopy, Masson's trichrome, ×312.) **B:** Bright staining of tubular and glomerular basement membranes, and of mesangium and arteriolar wall with anti-κ antibody in a patient with κ–light-chain deposition disease without nodular glomerular lesions. (Immunofluorescence, ×312.)

microscopy but require ultrastructural examination. These lesions may represent early stages of glomerular disease or be induced by LCs with a weak pathogenic potential. Their diagnosis would be unrecognized without the immunostaining results.

Arteries, arterioles, and peritubular capillaries all may contain LC deposits in close contact with their basement membranes.

Immunofluorescence

A key step in the diagnosis of the various forms of MIDD is immunofluorescence examination of the kidney. All biopsy specimens show evidence of monotypic LC and/or HC fixation along tubular basement membranes (Fig. 75-12B). This criterion is requested for the diagnosis of MIDD. In contrast with AL amyloidosis, the κ-isotype is markedly predominant.

The tubular deposits stain strongly and predominate along the loops of Henle and the distal tubules, but they are also often detected along the proximal tubules. In contrast, the pattern of glomerular immunofluorescence displays marked heterogeneity. In patients with nodular glomerulosclerosis, deposits of monotypic Ig chains are usually found along the peripheral glomerular basement membranes, and, to a lesser extent, in the nodules themselves. The staining in glomeruli is typically weaker than that observed along the tubular basement membranes. This may not be a function of the actual amount of deposited material, since several cases have been reported in which glomerular immunofluorescence was negative despite the presence of large amounts of granular glomerular deposits by electron microscopy (91). Local modifications of deposited LCs might thus change their antigenicity (349). In patients without nodular lesions, glomerular staining occurs along the basement membrane, but it may involve the mesangium in some cases (Fig. 75-12B). Linear Ig-chain staining is usually present along Bowman's capsule basement membrane. Deposits of Ig chains are constantly found in vascular walls (Fig. 75-12B).

In patients with HCDD, immunofluorescence with anti-LC antibodies is negative, despite typical nodular glomerulosclerosis. Monotypic deposits of γ-, α-, or μ-HC may be identified. Any γ-subclass may be observed. Analysis of the kidney biopsies with monoclonal antibodies directed to the various constant domains of the γ-HC showed that C_H1 domain determinants were undetectable in all tested cases (9,352,362) (Table 75-18) (Fig. 75-13). In addition, monoclonal antibodies to the $\gamma 1$ C_H2 domain also failed to react with the renal

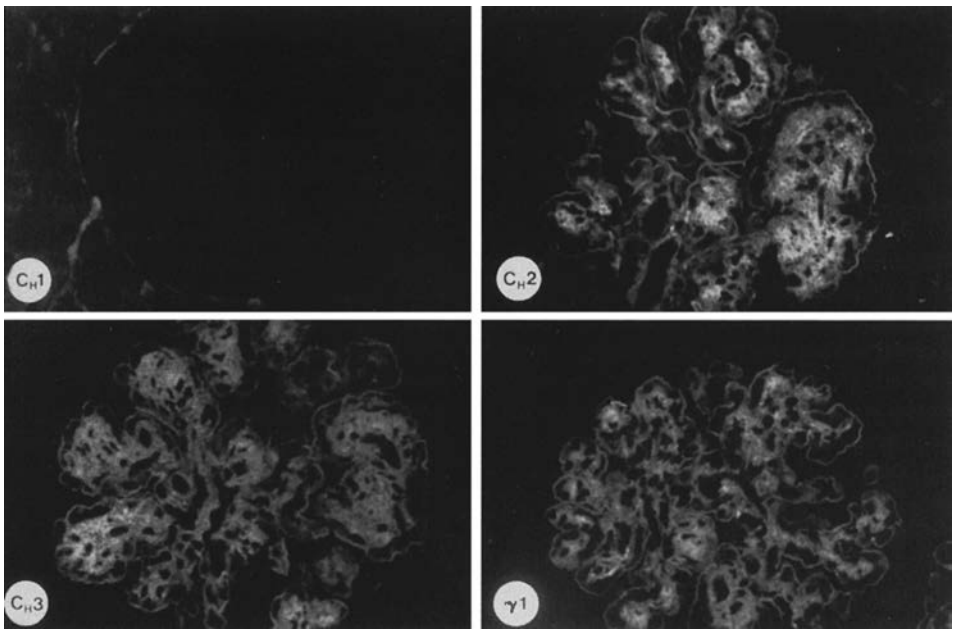

FIGURE 75-13. Heavy-chain deposition disease in a patient presenting with nodular glomerulosclerosis. Mesangial and parietal deposits stain with a monoclonal antibody specific for the $\gamma 1$-isotype in the absence of detectable light chain (**bottom right**). Immunofluorescence with a panel of monoclonal antibodies directed to the various constant domains of the γ-heavy chain shows that the glomerular deposits are stained with anti-C_H2 and -C_H3, but not with anti-C_H1 antibodies. (Magnification, ×312.) (From: Moulin B, Deret S, Mariette X. et al. Nodular glomerulosclerosis with deposition of monoclonal immunoglobulin heavy chains lacking C_H1. *J Am Soc Nephrol* 1999;10:519, with permission.)

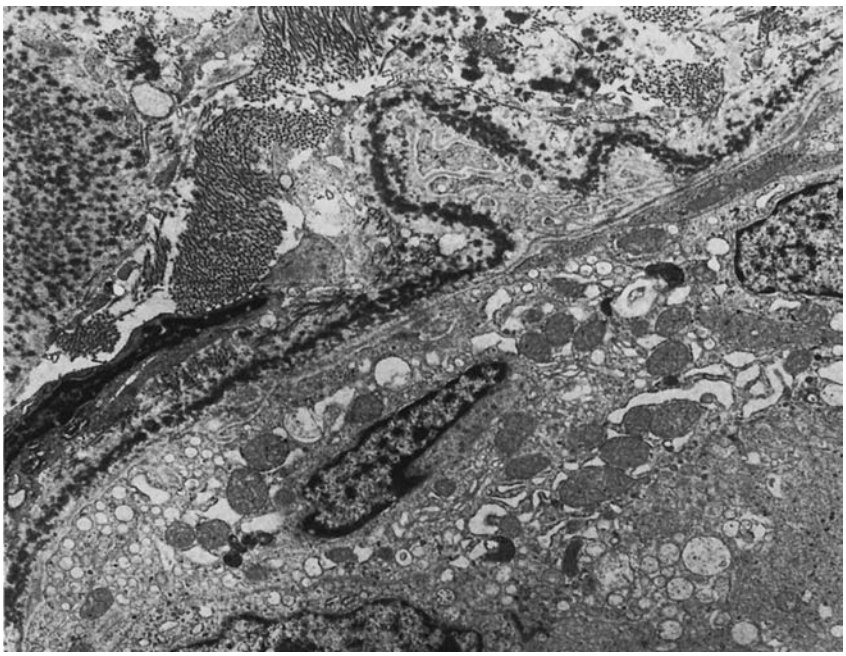

FIGURE 75-14. Light-chain deposition disease. Coarsely granular dense deposits lining the outer aspect of the tubular basement membrane. (Electron microscopy, uranyl acetate and lead citrate, ×6,000.) (From: Ganeval D, et al. Visceral deposition of monoclonal light chains and immunoglobulins: a study of renal and immunopathologic abnormalities. *Adv Nephrol Necker Hosp* 1982;11:25, with permission.)

deposits of one patient, due to a combined deletion of C_H1 and C_H2 domains (8). In most cases of HCDD, especially when a $\gamma 1$ or $\gamma 3$ chain was involved, complement components could be demonstrated in a granular or pseudolinear pattern (Table 75-18). Complement deposits were often associated with signs of complement activation in serum (9,352,360).

Electron Microscopy

The most characteristic ultrastructural feature is the presence of finely or coarsely granular electron-dense deposits that delineate the outer aspect of the tubular basement membranes (Fig. 75-14). They appear to be in contact with a well-preserved basal lamina. The deposits are usually quite large and may protrude into the adjacent part of the interstitium.

Ultrastructural glomerular lesions are characterized by the deposition of a nonfibrillar, electron-dense material in the mesangial nodules and along the glomerular basement membrane. The mesangial material is usually finely granular with a membranoid appearance (Fig. 75-15), but in some cases, it may contain strongly electron-dense granules identical to the peritubular deposits (369,370). The deposits along the glomerular basement membrane appear as a prominent, but thin, continuous band delineating the endothelial aspect of the basement membrane. The limits between the deposits and the basement membrane may be difficult to distinguish. In rare cases the deposits invade the lamina densa. Glomerular endothelial cells are separated from this material by areas of electron-lucent fluffy material. Deposits can also be found in Bowman's capsules and in the wall of small arteries between the myocytes (362).

A common feature shared by most patients with MIDD is the dramatic accumulation of extracellular matrix. Nodules are made of normal constituents (371) and stain weakly for the small proteoglycans, decorin and biglycan (372). In a series of 36 patients with LC-related renal diseases including AL amyloidosis, CN, fibrillary glomerulopathy, and LCDD, transforming growth factor-β (TGF-β) was detected only in glomeruli of the three patients with LCDD and nodular glomerular lesions (373). In the control series, TGF-β was essentially found in nodular diabetic glomerulosclerosis, which may suggest that distinct initial insults to the glomerular mesangium may trigger similar fibrogenetic pathways. A similar accumulation of matrix proteins was found in the perisinusoidal space of the liver of patients with LCDD (374).

Clinical Presentation

In Tables 75-19 and 75-20 are summarized the main data from five large series (351,352,365,375,376). They show an unexpectedly wide range of affected ages (28 to 94 years), with a male preponderance. MIDD is a systemic disease with Ig-chain deposition in a variety of organs leading to various clinical manifestations (362), but visceral Ig-chain deposits may be totally asymptomatic and found only at autopsy.

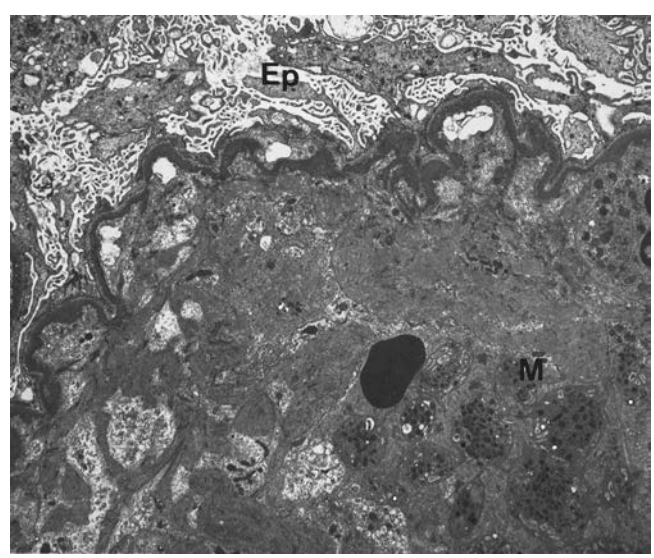

FIGURE 75-15. Light-chain deposition disease. A heavy layer of dense granular deposit lies along the inner part of the basement membrane lining a large mesangial nodule. *Ep*, epithelium; *M*, mesangium. (Electron microscopy, uranyl acetate and lead citrate, ×2,500.)

TABLE 75-19

RENAL MANIFESTATIONS AT PRESENTATION IN PATIENTS WITH LIGHT-CHAIN DEPOSITION DISEASE (LCDD) AND LIGHT-AND HEAVY-CHAIN DEPOSITION DISEASE (LHCDD)

Series (ref. no.)	Age (yrs)	Male–female ratio	Proteinuria >1 g/d	Nephrotic syndrome	Hematuria	Renal failure	Hypertension
Ganeval et al., 1982 (375) ($N = 17$)	57 (38–73)	11/6	13 (76%)	5 (29%)	5 (29%)	15 (88%)	3 (23%)[c]
Buxbaum et al., 1990 (351) ($N = 13$)	NS (35–71)	9/4	10 (77%)	3 (23%)	NS	12 (92%)	8 (61%)
Heilman et al., 1992 (376) ($N = 19$)	51 (37–77)	12/7	NS	10 (53%)	11 (58%)	17 (89%)	12 (63%)
Lin et al., 2001 (352)[a] ($N = 17$)	57 (NS)	9/8	NS	3 (18%)	8 (47%)	16 (94%)	12 (71%)
Pozzi et al., 2003 (365)[b] ($N = 63$)	58(28–94)	40/23	53 (84%)	25 (40%)	NS	60 (96%)	NS
Total ($N = 129$)	57	1.7/1	82%	36%	45%	93%	53%

NS, not specified.
[a]Cases of LCDD with MCN (N = 11) are not included.
[b]Including 10 cases with MCN that could not be distinguished from those without MCN.
[c]Plus 3 patients with past history of hypertension.

Renal Features

Renal involvement is a constant feature of MIDD, and renal symptoms, mostly proteinuria and renal failure, often dominate the clinical presentation (Table 75-19). In 23% to 53% of the patients, albuminuria is associated with the nephrotic syndrome. However, in about a one-fourth it is less than 1 g per day, and these patients exhibit mainly a tubulointerstitial syndrome. Albuminuria is not correlated with the existence of nodular glomerulosclerosis, at least initially, and may occur in the absence of significant glomerular lesions by light microscopy. Hematuria is more frequent than one would expect for a nephropathy in which cell proliferation is usually modest, with a few exceptions.

The high prevalence, early appearance, and severity of renal failure are other salient features of MIDD (6,352, 362,365,377). In most cases, renal function declines rapidly,

TABLE 75-20

HEMATOLOGIC FEATURES, EXTRARENAL MANIFESTATIONS, AND OUTCOMES OF PATIENTS WITH LIGHT-CHAIN DEPOSITION DISEASE (LCDD) AND LIGHT- AND HEAVY-CHAIN DEPOSITION DISEASE (LHCDD)

Series (ref. no)	Plasma cell dyscrasia	Monoclonal component in blood or urine	Extrarenal manifestations	Survival
Ganeval et al., 1982 (375) $N = 17$	Myeloma—9 Waldenström—1	12/17 (71%)(8κ)	Liver—10 Heart—4 Lung —2 Spleen—3 Nervous system—4	1–46 mos 8/16 deceased
Buxbaum et al., 1990 (351) $N = 13$	Myeloma—4	10/12 (83 %) (6κ)	Heart—6 Liver—3	1 mo to 10 yrs 9/13 deceased
Heilman et al., 1992 (376) $N = 19$	Myeloma—6 Malignant LPD—1	16/19 (84%) (15κ)	Heart—4 Nervous system—2	89% : 1 yr 70% : 5 yrs
Lin et al., 2001 (352) $N = 17$	Myeloma—8	15/17 (88%)[a]	NS	LCDD, 69 mo[b]s LHCDD, 13 mo[b]s 9/17 deceased
Pozzi et al., 2003 (365) $N = 63$	Myeloma—41 LPD—2	59/63 (94%) (43κ)	Heart—13 Liver—12 Spleen—5 Nervous system—5	66% : 1 yr 31% : 8 yrs 37/63 deceased
Total $N = 129$	Myeloma—53%	88% (κ65%)	Heart—24% Liver—22%	63/109 (58%) deceased

LPD, lymphoproliferative disease.
[a]14 κ chains in 17 kidney biopsies.
[b]Mean patient survival time.

TABLE 75-21

COMPARISON OF CLINICAL MANIFESTATIONS, RENAL LESIONS, AND HEMATOLOGIC FEATURES IN PATIENTS WITH MONOCLONAL IMMUNOGLOBULIN DEPOSITION DISEASE (MIDD)

Characteristics	LCDD/HCDD[a] $N = 129$	HCDD[b] $N = 24$
Male–female ratio	1.7	0.8
Age (y)	57 (28–94)	57 (26–79)
Hypertension (%)	53	90
Renal failure (serum creatinine ≥130 μmol/L) (%)	93	83
Nephrotic syndrome[c] (%)	36	46
Hematuria (%)	45	89
Nodular glomerulosclerosis (%)	31–100	96
Multiple myeloma (%)	53	24
M component (blood or urine) (%)	88	58[d]

[a]Patients are from the series of Tables 75-19 and 75-20.
[b]Cases are from references 8,9,352,359,360,361,378–385.
[c]Proteinuria ≥3 g/day.
[d]Including two cases with only free κ chain.

which is a main reason for referral. It occurs with comparable frequency in patients with either low or heavy proteinuria (349), and thus presents in the form of a subacute tubulointerstitial nephritis or a rapidly progressive glomerulonephritis, respectively. The prevalence of hypertension is variable, but must be interpreted according to associated medical history.

Renal features of patients with HCDD are basically similar to those seen in LCDD and LHCDD (Table 75-21).

Extrarenal Manifestations

Liver and cardiac manifestations occurred in about one-fourth of patients (375) (Table 75-20). Liver deposits were constant in patients whose liver was examined. They were either discrete, confined to sinusoids and basement membranes of biliary ductules without associated parenchymal lesions, or massive with marked dilation and multiple ruptures of sinusoids resembling peliosis. Hepatomegaly with mild alterations of liver function tests was the most usual symptom, but several patients developed hepatic insufficiency and portal hypertension, and some of them died because of hepatic failure (349).

Cardiac involvement is also frequent and may be responsible for cardiomegaly and severe renal failure. Dysrhythmias, conduction disturbances, and congestive heart failure are seen. Echocardiography and catheterization may reveal diastolic dysfunction and reduction in myocardial compliance similar to that seen in cardiac amyloid. As in the kidney and liver, immunofluorescence showed monotypic LC deposits in the vascular walls and perivascular areas of the heart, in all autopsy cases (349).

Deposits may also occur along the nerve fibers and in the choroid plexus, as well as in the lymph nodes, bone marrow, spleen, pancreas, thyroid gland, submandibular glands, adrenal glands, gastrointestinal tract, abdominal vessels, lungs, and skin (362). They may be responsible for peripheral neuropathy (20% of the reported cases), gastrointestinal disturbances, pulmonary nodules, amyloidlike arthropathy, and sicca syndrome. In some patients, nonamyloidotic, localized nodules, termed "aggregomas," developed in the lung or as cervical mass without systemic LCDD (386,387). It is not certain whether they are truly localized or they represent an initial expression of a silent, systemic LCDD.

Extrarenal deposits are less common in patients with HCDD. They have been reported in the heart (359), synovial tissue (359,388), skin (382), striated muscles (382), pancreas (8), around the thyroid follicles (8), and in Disse's spaces in the liver.

Hematologic Findings

The most common underlying disease in MIDD is myeloma, which accounts for about 50% of pure MIDD (Table 75-20) and greater than 90% of LC deposits associated with myeloma CN. MIDD was found at postmortem examination in 5% of myeloma cases (262). MIDD, like AL amyloidosis, often is the presenting disease that leads to the discovery of myeloma at an early stage. In some patients who first presented with common myeloma and with normal-sized monoclonal Ig without kidney disease, LCDD occurred when the disease relapsed after chemotherapy, together with Ig structural abnormalities (348,349). Because melphalan was shown to induce Ig gene mutations (389), the disease in these patients might result from the emergence of a variant clone induced by the alkylating agent. Apart from myeloma, MIDD may complicate Waldenström's macroglobulinemia, chronic lymphocytic leukemia, and nodal marginal-zone lymphoma (348,390). It often occurs in the absence of detectable malignant process, even after prolonged (more than 10 years) follow-up (Tables 75-20 and 75-21). In such "primary" forms, a monoclonal bone marrow plasma cell population can be documented easily by immunofluorescence examination.

Diagnostic Procedures in MIDD

The diagnosis of MIDD must be suspected in any patient with the nephrotic syndrome or rapidly progressive tubulointerstitial nephritis, or with echocardiographic findings indicating diastolic dysfunction, and the presence of a monoclonal Ig component in the serum and/or the urine. The same combination is also seen in AL-amyloidosis, but the latter is more often associated with the λ LC isotype. Because sensitive techniques including immunofixation fail to identify a monoclonal Ig component in 10% to 20% of patients with LCDD/HCDD and about 40% of patients with HCDD (Table 75-21), renal biopsy plays an essential role in the diagnosis of MIDD and of the associated dysproteinemia.

The definitive diagnosis is made according to the immunohistologic analysis of tissue from an affected organ, in most cases the kidney, using a panel of Ig chain-specific antibodies, including anti-κ and anti-λ LC antibodies to stain the non-Congophilic deposits. When the biopsy stains for a single heavy chain isotype and does not stain for light chain isotypes, the diagnosis of HCDD should be suspected.

The diagnosis of the plasma cell dyscrasia relies on bone marrow aspiration and bone marrow biopsy with cell morphologic evaluation and, if necessary, immunophenotyping with anti-κ and anti-λ antisera to demonstrate monoclonality. Diagnostic criteria for a multiple myeloma are present in no more than 60% of the patients with LCDD, and in about one-fourth of patients with HCDD.

Outcome and Treatment

The outcome of MIDD remains uncertain, mainly because extrarenal deposits of LCs can be totally asymptomatic or cause severe organ damage leading to death. Survival from onset of symptoms varies from 1 month to 10 years (Table 75-20). In the largest series as yet reported of patients with LCDD (365), 36 of the 63 (57%) patients reached uremia, 37 of those patients (59%) died during follow-up (mean, 27.5 months), and patient survival was only 66% at 1 year and 31% at 8 years, although 54 patients (86%) were treated by chemotherapy. Multivariate analysis showed that the only variables independently associated with renal survival were age and degree of renal insufficiency at presentation (365) or the time of renal biopsy (352). Those independently associated with a worse patient survival were age, associated multiple myeloma, and extrarenal LC deposition (365), or initial serum creatinine (352). The survival of the uremic patients treated with dialysis was not different from that of the patients not reaching uremia (365). Renal and patient survivals were significantly better in patients with pure MIDD (mean, 22 and 54 months, respectively), compared with those who presented with myeloma CN (mean, 4 and 22 months) (352).

As in AL amyloidosis, treatment should be aimed at reducing Ig production. Whether appropriate treatment can result in sustained remission has long remained unclear. Clearance of the LC deposits has been unequivocally demonstrated in some patients after intensive chemotherapy with syngeneic bone marrow transplantation or blood stem cell autografting (391,392). Disappearance of nodular mesangial lesions and LC deposits was also reported after long-term chemotherapy (393). These observations are of paramount importance. They demonstrate that fibrotic nodular glomerular lesions are reversible, and they argue for intensive chemotherapy in patients with severe visceral involvement.

In a retrospective study of 11 young (<65 years) patients with LHCDD treated by high-dose therapy with the support of autologous blood stem cell transplantation, no treatment-related death occurred (394). A decrease in the monoclonal Ig level was observed in eight patients, with complete disappearance from serum and urine in six cases. Improvement in manifestations related to deposits was observed in six patients, and histologic regression was documented in cardiac, hepatic, and skin biopsies. No manifestation related to deposits occurred or recurred in any patient. Reversal of dialysis dependency and sustained improvement in renal function was also noted in one patient with LCDD by Firkin et al. (395).

As in AL amyloidosis, monitoring of LC production should rely on free LC assay, particularly in the patients without a blood and urine monoclonal component.

Kidney transplantation has been performed in a few patients with MIDD. Recurrence of the disease is usually observed. Therefore, kidney transplantation should not be an option for LCDD patients unless measures have been taken to reduce LC production.

COMBINED GLOMERULAR AND TUBULAR LESIONS

Tubular Lesions Associated with Glomerular and Tubular Light-Chain Deposits

The association of monoclonal LC deposits, mostly along renal tubular basement membranes, with typical myeloma CN is more frequent than reported initially. It was found in 11 of 34 (32%) patients with MIDD (352). Nodular glomerulosclerosis is, however, infrequent (<10%), and some ribbon-like tubular basement membranes are seen in less than one-half of the patients. In addition, one-third of the patients do not have granular-dense deposits by electron microscopy. The lack of matrix accumulation in most of these patients who present with acute renal failure in the setting of a true myeloma may relate to insufficient time for the development of fibrosis or to a weaker sclerogenic effect of the LC, if any (362). As discussed in the preceding text, the presence of LC deposits along the tubular basement membrane is not sufficient to make a diagnosis of MIDD. Conversely, in patients with glomerular lesions associated with LCs, damage to proximal tubule epithelium frequently occurs and can become prominent in some patients (149), although typical myeloma casts are rare. In addition, the pattern of renal lesions may change with time under chemotherapy. In three patients with typical myeloma cast nephropathy on initial biopsy, casts were replaced by massive tissue deposits of LCs (κ chains in two, amyloid in one) (69), suggesting chemotherapy-induced mutation of the LCs, which might cause the LCs to be deposited in tissues rather than giving rise to myeloma casts (348,389).

More exceptional is the association of a Fanconi syndrome with AL amyloidosis. In three cases (131,143,396), a Fanconi syndrome was not caused by amyloid infiltration of the kidney. Amyloid was diagnosed shortly before death or at autopsy. Finkel et al. (143) noticed that nodular amyloid deposits were surrounded by atypical lymphoid cells containing numerous needle-shaped crystals, and first suggested that "a product" from these cells "may have been involved with both crystal formation and amyloid production." Since the nucleation processes initiating amyloid and crystal formation may share similarities, it is tempting to speculate that the responsible LC bore unusual physicochemical properties, inducing both pathologic conditions.

Combined AL or AH Amyloidosis and Monoclonal Immunoglobulin Deposition Disease

Since the description of MIDD, it was expected that the two types of deposits might coexist at different sites in a single patient. The first observations were reported by Jacquot et al. (208). A review by Gallo et al. (350) indicated that in approximately 7% of 135 cases of light-chain deposition disease, amyloid was found in one or more organs. Because amyloid deposits were focal, the true incidence of the association may be markedly underestimated. In patients with both types of deposits, amyloid P component was found in the fibrillar, but not the nonfibrillar, LC deposits by immunohistochemical methods (397). The pathophysiologic significance of this association remains controversial. Conversely, some light chains may possess intrinsic properties, which make them prone to form both fibrillar and nonfibrillar deposits, depending on the tissue microenvironment (358), and, in the absence of structural analysis of the deposited LCs, one cannot exclude that they

are generated by different variant clones. In a patient with IgD myeloma, MIDD and amyloidosis were associated with cast nephropathy (398).

Late development of systemic λ-LC amyloidosis was recently reported in a patient with γ-HCDD during long-term follow-up (399). Copeland et al. (198) reported the metachronous development of nonamyloidogenic λ-LCDD and γ-HC amyloidosis in the same patient. Given the length of time between the development of the two diseases (6 years) and the apparent success of stem cell transplantation in treating the first, it is most likely that the patient produced two different plasma cell clones.

OTHER DYSPROTEINEMIA-ASSOCIATED GLOMERULAR LESIONS

Glomerulonephritis with Intracapillary Thrombi of Immunoglobulin M

This glomerulonephritis is almost specific of Waldenström's macroglobulinemia (400). It is considered as the most common renal lesion with amyloidosis in this condition, but like amyloidosis, it has become a rare entity, probably because of the increased efficacy of chemotherapy. In the series of 16 autopsy and biopsy cases published by Morel-Maroger et al. (400), this lesion was found in six cases, and was associated with a variable degree of proteinuria and normal or slightly altered renal function. It was characterized by PAS-positive, non-Congophilic endomembranous deposits in a variable number of capillary loops. Deposits were sometimes so voluminous as to occlude the capillary lumens partially or completely, thereby forming thrombi. By immunofluorescence, thrombi and deposits were stained with anti-IgM (three cases studied) and with anti-κ (one case studied) with antibodies. Two of the six patients had cryoglobulinemia and slight glomerular cell proliferation. In the remaining four, the amount of circulating IgM was higher than in the other patients of the series with amyloidosis or no detectable renal lesion, which suggested that hyperviscosity could favor IgM deposition in glomerular capillaries where ultrafiltration further increases the protein concentration. This pathophysiologic hypothesis is also supported by the study of Argani and Kipkie (401) in which formation of diffuse intracapillary thrombi was likely precipitated by severe dehydration.

Because renal biopsy may be hazardous in patients with Waldenström's macroglobulinemia with frequently increased bleeding time, it is wise to search for amyloid deposits first by a less-invasive tissue biopsy. This is of utmost importance because amyloidosis is a major cause of morbidity associated with a much shorter survival time (265).

Glomerulonephritis with Nonamyloid Organized Monotypic Deposits

These entities are characterized by fibrillar or microtubular deposits in mesangium and glomerular capillary loops that are readily distinguishable from amyloid because fibrils are thicker and are not stained by Congo red. They were termed "fibrillary glomerulonephritis" (FGN) by Alpers et al. (402) and immunotactoid glomerulopathy (IT) by Korbet et al. (403,404). There has been considerable debate about the relationship of FGN to IT, and most authors suggested that the two denominations might cover partly different morphologic entities as defined by the size and aspect of organized structures. For Alpers (405), the distinguishing morphologic features of IT are the presence of organized deposits of large, thick-walled microtubules, usually greater than 30 nm in diameter, which are often hollow and arranged in parallel or stacked arrays, whereas FGN is characterized by more amyloidlike deposits with smaller fibrils (12 to 22 nm).

Although these criteria remain controversial (404,406–408), distinguishing IT from FGN may be of great clinical and pathophysiologic interest because the former seemed to be more often associated with monotypic Ig deposits. However, until 2002, it was difficult to assess precisely from the literature, the respective prevalence in each entity of monotypic deposits and of circulating monoclonal Ig because studies of biopsies with anti-LC antibodies were often incomplete, urine and blood data uncertain, and, even more, patients with dysproteinemias were excluded *a priori* from several series (403,404). This issue has been settled in two recent studies (408,409), which confirmed that IT has a significant association with underlying dysproteinemia. Therefore, differentiation of IT from FGN appears justified on immunopathologic and clinical grounds, and this has important therapeutic consequences.

Epidemiology and Clinical Manifestations

The incidence of glomerulopathies with nonamyloid deposition of fibrillary or microtubular material in a nontransplant adult biopsy population is estimated to be about 1% (equivalent to that of antiglomerular basement membrane [anti-GBM] disease). Despite a growing number of case reports, this is most likely underestimated because of the insufficient attention given to atypical reactions with histochemical stains for amyloid and the lack of immunohistochemical and ultrastructure studies of most biopsy specimens.

The characteristics of fibrillary and immunotactoid glomerulopathies are described in Table 75-22 by comparison with AL amyloid. Patients with IT and FGN have a mean age of 55 to 60 years (extreme: 19 to 86 years) with a male-to-female ratio that varies from a series to another (11,408,409). They usually present with the nephrotic syndrome, microscopic hematuria, and mild-to-severe renal failure. In both most recent series (408,409), there was no significant difference between IT and FGN patients in serum creatinine level, incidence of nephrotic syndrome, microscopic hematuria, hypertension, and renal failure.

Pathologic Features

In IT, renal biopsy shows either membranous glomerulonephritis (often associated with segmental mesangial proliferation) or lobular membranoproliferative glomerulonephritis (408). By immunofluorescence, coarse granular deposits of IgG and C3 are observed along capillary basement membranes and in mesangial areas. In a recent series of 23 patients based on ultrastructural appearance of the deposits, IgG deposits were monotypic in 13 of 14 patients with IT (κ, seven cases; λ, six cases), and in only one of nine patients with FGN (408). However, a circulating monoclonal Ig was detected by immunoelectrophoresis or immunoblotting in only six of the 14 patients with IT (408). Among the five cases of IT available for IgG subtype analysis reported by Rosenstock and associates (409), four cases featured monotypic deposits of IgG1 subclass, whereas deposits composed of a single gamma subtype (two IgG1 and two IgG4) but with equivalent staining for the κ and λ LCs were found in four of 19 FGN cases.

By electron microscopy, the distinguishing morphologic features of IT are the presence of organized deposits of large, thick-walled microtubules, usually greater than 30 nm in diameter, at times arranged in parallel arrays (Fig. 75-16). However, the mean diameter of the substructures did not differ

TABLE 75-22

IMMUNOLOGIC AND CLINICAL CHARACTERISTICS OF FIBRILLARY AND IMMUNOTACTOID GLOMERULOPATHIES

Characteristics	Amyloidosis (AL-type)	Fibrillary glomerulonephritis (FGN)	Immunotactoid glomerulopathy (IT)
Congo red staining	Yes	No	No
Composition	Fibrils	Fibrils	Microtubules
Fibril or microtubule size	8–15 nm	12–22 nm	>30 nm[a]
Organization in tissues	Random (β-pleated sheet)	Random	Parallel arrays
Immunoglobulin deposition	Monoclonal LC (mostly λ)	Usually polyclonal (mostly IgG4), occasionally monoclonal (IgG1, IgG4)	Usually monoclonal (IgGκ or IgGλ)
Glomerular lesions	Deposits spreading from the mesangium	MPGN, CGN, MP	Atypical MN, MPGN
Renal presentation	Severe NS, absence of hypertension and hematuria	NS with hematuria, hypertension; RPGN	NS with microhematuria and hypertension
Extrarenal manifestations (fibrillar deposits)	Systemic deposition disease	Pulmonary hemorrhage	Microtubular inclusions in leukemic lymphocytes
Association with LPD	Yes (myeloma)	Uncommon	Common (CLL, NHL, MGUS)
Treatment	Melphalan + prednisone; intensive therapy with blood stem cell autograft	Corticosteroids ± cyclophosphamide (crescentic GN)	Treatment of the associated LPD

CGN, crescentic glomerulonephritis; *CLL*, chronic lymphocytic leukemia; *GN*, glomerulonephritis; *LC*, light chain; *LPD*, lymphoproliferative disorder; *MN*, membranous nephropathy; *MP*, mesangial proliferation; *MPGN*, membranoproliferative glomerulonephritis; *NHL*, non-Hodgkin's lymphoma; *NS*, nephrotic syndrome; *RPGN*, rapidly progressive glomerulonephritis.
[a]Mean diameter of the substructures did not differ between fibrillary glomerulonephritis (15.8 ± 3.5 nm) and immunotactoid glomerulopathy (15.2 ± 7.3 nm) in Bridoux's series (408).

with their ultrastructural fibrillar or microtubular appearance in Bridoux's series (408), with the mean external diameter of the microtubules ranging from 9 to 45 nm in patients with IT.

Of the 14 patients with IT reported by Bridoux et al. (408), six had a chronic lymphocytic leukemia, one a small lymphocytic B-cell lymphoma, and three a MGUS. Intracytoplasmic crystal-like Ig inclusions were found in four patients with chronic lymphocytic leukemia and in the lymphoma patient (408). They showed the same microtubular organization and contained the same IgG subclass and LC isotype as renal deposits. Whether crystallization in lymphocytes and the glomerulus results from unusual intrinsic physicochemical properties of the monoclonal Ig, or from reactivity with a shared epitope remains to be established. These properties may also account for rapid disappearance of the Ig from the blood and its recurrence on renal graft noted in several patients (410–413).

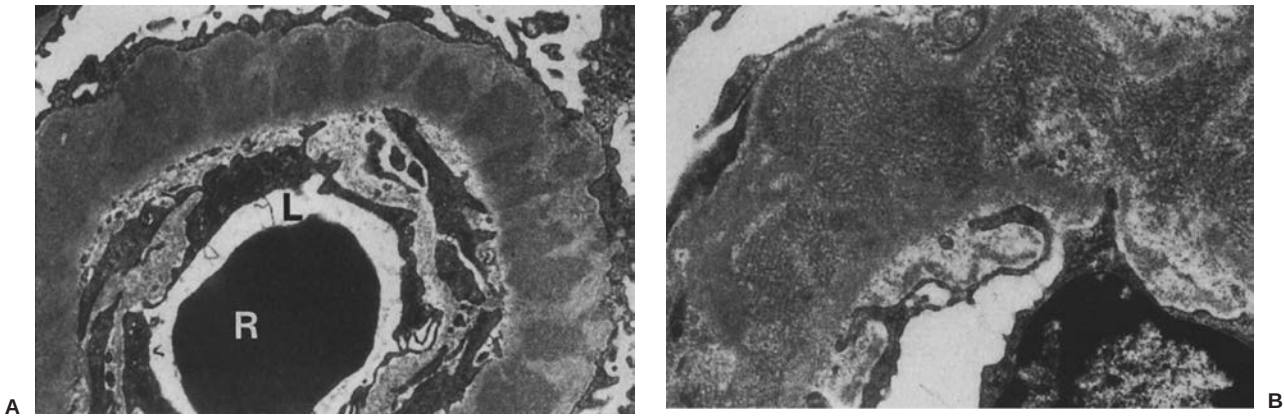

FIGURE 75-16. Immunotactoid glomerulopathy. Atypical membranous glomerulonephritis showing exclusive staining of the deposits with anti-immunoglobulin G and anti-κ–light-chain antibodies, in a patient with chronic lymphocytic leukemia. **A:** Electron microscopy of a glomerular capillary showing subepithelial deposits with effacement of the foot-processes and mesangial interposition. *L*, capillary lumen; *R*, red blood cell. (Uranyl acetate and lead citrate, ×4,400.) **B:** Higher magnification of the capillary wall showing microtubular structure of the deposits. (Magnification, ×12,000.) (From: Moulin B, Ronco PM, Mougenot B, et al. Glomerulonephritis in chronic lymphocytic leukemia and related B-cell lymphomas. *Kidney Int* 1992;42:127, with permission.)

Mesangial proliferation and aspects of membranoproliferative glomerulonephritis are predominantly reported in series of FGN. Glomerular crescents are present in approximately 30% of the biopsies (409). Immunofluorescence studies mainly show IgG deposits of the γ4-isotype (408,414) with a predominant mesangial localization. Monotypic deposits containing mostly IgGκ, are detected in no more than 15% of patients. By electron microscopy, fibrils are randomly arranged and their diameter varies between 12 and 22 nm. Of note, the fibril size alone is not sufficient to distinguish nonamyloidotic fibrillary glomerulonephritis from amyloid.

Although fibril deposition is almost always confined to the kidney, similar fibrillary deposits have been reported in the alveolar capillary membrane in patients presenting with a pulmonary–renal syndrome and in the skin of a patient with a leukocytoclastic vasculitis. In a patient with IT who suffered from severe mononeuritis multiplex of the lower limbs, peripheral nerve biopsy showed a similar ultrastructural microtubular organization to the glomerular deposits (408).

Pathogenesis

The cause of FGN is not known. The exclusive or prevailing presence of IgG4 in the immune deposits of patients with FGN is of great interest. Although not monoclonal, this isotype-restricted homogeneous material made of highly anionic Ig may facilitate fibril formation. Amyloid P component has also been found in the fibrils. The description of fibrillar cryoprecipitates consisting of Ig-fibronectin complexes in the serum of patients with FGN without evidence of systemic disease indicates that serum precursors can lead to the formation of fibrillary deposits (415).

The mechanisms of Ig deposition in lymphocytes and kidney of patients with IT are also poorly understood. Analysis of monoclonal Ig both at the protein and mRNA levels has not disclosed size abnormalities in two patients (416).

Treatment and Outcome

Patients with FGN usually respond poorly to corticosteroids and cytotoxic drugs, with an incidence of endstage renal disease of 50% (403,406,414,417). By contrast, in those with IT, corticosteroid and/or chemotherapy were associated with partial or complete remission of the nephrotic syndrome in most cases, with a parallel improvement of the hematologic parameters (406,408).

After a mean follow-up period of 54 months for the IT group and 56 months for the FGN group, patient survival (71.4% vs. 88.8%, respectively) was found to be similar for the two groups (408). The incidence of chronic renal failure (IT: 8/14, 57.1%; FGN: 8/9, 88.8%) and endstage renal failure (IT: 2/14, 14.3%; FGN: 4/9, 55.8%) tended to be lower in the IT group, but the difference was not statistically significant. Renal transplantation has been performed in only a few patients, and recurrent disease occurred in several (410–413).

A better understanding of these unusual glomerulopathies, which most likely make up a new form of monoclonal Ig-related deposition disease with organization of the deposits will require: (a) detailed ultrastructural and immunomorphologic characterization of the renal deposits; (b) careful analysis of circulating Ig with sensitive techniques, including repeated search of cryoglobulins; (c) studies on bone marrow and circulating lymphocytes (ultrastructure, *in vitro* Ig biosynthesis); as well as (d) prolonged follow-up of patients to assess eventual occurrence of extrarenal manifestations and efficacy of treatment.

IT (microtubular) glomerulopathies must now be added to the heterogeneous list of glomerulopathies caused by B-cell chronic lymphocytic leukemia and related lymphomas, including AL amyloidosis and the larger cohort of cryoglobulinemia-associated membranoproliferative glomerulonephritis (Table 75-23).

TABLE 75-23

RENAL LESIONS OBSERVED IN B-CELL PROLIFERATIONS

Renal lesions	Multiple myeloma	Waldenström's macroglobulinemia	Chronic lymphocytic leukemia and related lymphomas
Tubular lesions			
Cast nephropathy	+ + +	–	–
(Proximal) tubule lesions[a]	+	–	–
Fanconi's syndrome	+ (smoldering)	–	–
Glomerular lesions[b]			
AL amyloidosis	+ +	+	+
MIDD (nodular, membranoproliferative, minimal change)	+ +	+	–
Nonamyloid organized deposits[c]	–	–	+
Type I and type II cryoglobulinemia	+	+ +	+ +
IgM capillary thrombi	–	+	–
Other (crescentic, minimal change, etc.)	+	+	+
Interstitial lesions			
B-Cell infiltrate	+[d]	+ +	+ +
Nephrocalcinosis	+	–	–
Pyelonephritis (infections)	+	–	–

–, not or exceptionally observed; + to + + +, semiquantitative rating of the prevalence of renal lesions; *MIDD*, monoclonal immunoglobulin deposition disease

[a]Without detectable myeloma casts, sometimes acute tubular necrosis.

[b]Glomerular involvement is usually but not always preponderant.

[c]Usually atypical membranous (or membranoproliferative) glomerulonephritis.

[d]Exceptionally, plasmacytoma.

Other Types of Glomerulonephritis

Additional histologic forms of glomerulonephritis have been described in monoclonal gammopathies. In type I cryoglobulinemia, a membranoproliferative glomerulonephritis (MPGN) with macrophage infiltration is the most characteristic histologic pattern and the deposits are typically, but not invariably, organized into fibrillary or microtubular structures at the ultrastructural level (418). Type II cryoglobulinemias are much more common and are described in Chapter 69.

Recently, it has been shown in 10 patients that in the absence of detectable cryoglobulin, glomerular deposition of monoclonal IgG could produce a proliferative glomerulonephritis that mimics immune-complex glomerulonephritis by light and electron microscopy (12). Proper recognition of this entity requires confirmation of monoclonality by staining for the γ-heavy chain subclasses and the LC isotypes. Tissue fixation of complement was observed in 90% of cases, and 40% of patients had hypocomplementemia. Clinical presentation included renal insufficiency in 80%, proteinuria in 100%, nephrotic syndrome in 44%, and microhematuria in 60%. A monoclonal serum protein with the same heavy and light chain isotype as that of the glomerular deposits was identified in 50% of cases (including three IgGκ and two IgGλ). No patient had overt myeloma or lymphoma at presentation or over the course of follow-up.

Renal manifestations may also occur in POEMS syndrome (Polyneuropathy, Organomegaly, Endocrinopathy, M protein, and Skin changes), although they are not related to deposition of the monoclonal immunoglobulin (419).

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CHAPTER 76 ■ HYPERURICEMIA, GOUT, AND THE KIDNEY

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INTRODUCTION

Uric acid is a weak acid trioxypurine with a molecular weight of 168 that is composed of a pyrimidine and imidazole substructure with oxygen molecules. It is produced during the metabolism of purines, and specifically, is generated by the degradation of xanthine by the enzyme xanthine oxidase or its isoform, xanthine dehydrogenase. In most mammals uric acid is further oxidized to allantoin by the hepatic enzyme, urate oxidase (uricase). However, during early hominoid evolution (12 to 20 million years ago) a series of mutations occurred, first affecting the promoter region and then the actual gene, eventually rendering uricase nonfunctional (1,2). As a consequence, serum uric acid levels in humans are higher (3 to 15 mg/dL, 180 to 900 μM) and less regulatable than in most mammals (1 to 3 mgdL, 60 to 180 μM) (3). Great Apes (such as the chimpanzee, gorilla, and gibbon) as well as some New World Monkeys also have mutations in uricase, but tend to have lower serum uric acid levels (2 to 4 mg/dL, 120 to 240 μM), primarily due to diets low in purines and fructose. The best known consequence of an elevated uric acid in humans is the disease gout, due to the deposition of urate crystals in synovial joints, occasionally with tophi formation. However, there are also a number of well-known renal manifestations associated with elevated uric acid, including the formation of uric acid kidney stones (urate nephrolithiasis), acute urate nephropathy (due to intratubular crystal formation with obstruction), and chronic urate ('gouty') nephropathy. The latter has been historically viewed as occurring as a consequence of interstitial urate crystal deposition with local inflammation, but we will review new data that suggests this entity may occur through a crystal-independent manner. There is also the entity of familial juvenile hyperuricemic nephropathy, for which the gene responsible has recently been identified. Finally, new studies also suggest that uric acid may have a role in other renal diseases and may also have a direct role in mediating intrarenal vascular disease, hypertension, and even the metabolic syndrome. These are all discussed in subsequent text of this chapter.

URATE METABOLISM AND HOMEOSTASIS

Generation of Uric Acid

Uric acid is produced from metabolic conversion of either dietary or endogenous purines, primarily in the liver, muscle, and intestine (Fig. 76-1) (reviewed in 4). The immediate precursor of uric acid is xanthine, which is degraded to uric acid by either xanthine oxidase, which generates superoxide anion in the process, or by its isoform, xanthine dehydrogenase, which generates the reduced form of nicotinamide-adenine dinucleotide. Both exogenous purines (such as is present in fatty meat, organ meats, and seafood) and endogenous purines are major sources of uric acid in humans.

Excretion of Uric Acid

The primary site of excretion of uric acid is the kidney, with normal urinary urate excretion in the range of 250 to 750 mg per/day. Although urate (the form of uric acid at blood pH of 7.4) is freely filtered in the glomerulus, there is evidence that there is both reabsorption and secretion in the proximal tubule, and as a consequence the fractional urate excretion is only 8% to 10% in the normal adult. Some adaptation occurs with renal disease, in which the fractional excretion will increase to the 10% to 20% range. In addition, some excretion of uric acid occurs through the gut, where uric acid is degraded by uricolytic bacteria, and this may account for one-third of the elimination of uric acid in the setting of renal failure. The classic paradigm of uric acid excretion consists of a four-step model with glomerular filtration, followed by reabsorption, secretion, and postsecretory reabsorption, the latter three processes all occurring in the proximal convoluted tubule (5,6). However, ideas of the handling of uric acid by the kidney have changed greatly during the last decades, with characterization and isolation of transporters and channels mainly or exclusively restricted to urate transport (7,8). Membrane vesicle studies have suggested the existence of two major mechanisms modulating urate reabsorption and secretion, consisting of a voltage-sensitive pathway and a urate/organic anion exchanger. Recently several of these transporters/channels have been identified. Endou's group identified uric acid transporter-1(URAT-1), a member of the organic anion transporter (OAT) family, as the major organic anion exchanger for uric acid on the apical (luminal brush border) side of the proximal tubular cell (7). In the human kidney, urate is transported via URAT-1 across the apical membrane of proximal tubular cells, in exchange for anions being transported back into the tubular lumen to maintain electrical balance. Urate then moves across the basolateral membrane into the blood by other organic anion transporters, most likely OAT-1 and OAT-3 (9,10). URAT-1 has a high affinity for urate together with lactate, ketones, α-ketoglutarate, and related compounds. Pyrazinamide, probenecid, losartan, and benzbromarone all inhibit urate uptake in exchange for chloride at the luminal side of the cell by competition with the urate exchanger. In humans, urate secretion is relatively minimal and URAT-1 is thought to be the major mechanism for regulating blood level of uric acid.

Urate secretion appears to be mediated principally by a voltage-sensitive urate transporter, which is expressed ubiquitously and localizes to the apical side of the proximal tubule in the kidney. The primary candidate is urate transporter (UAT), which is in the galactin family (8). Recently, a novel human

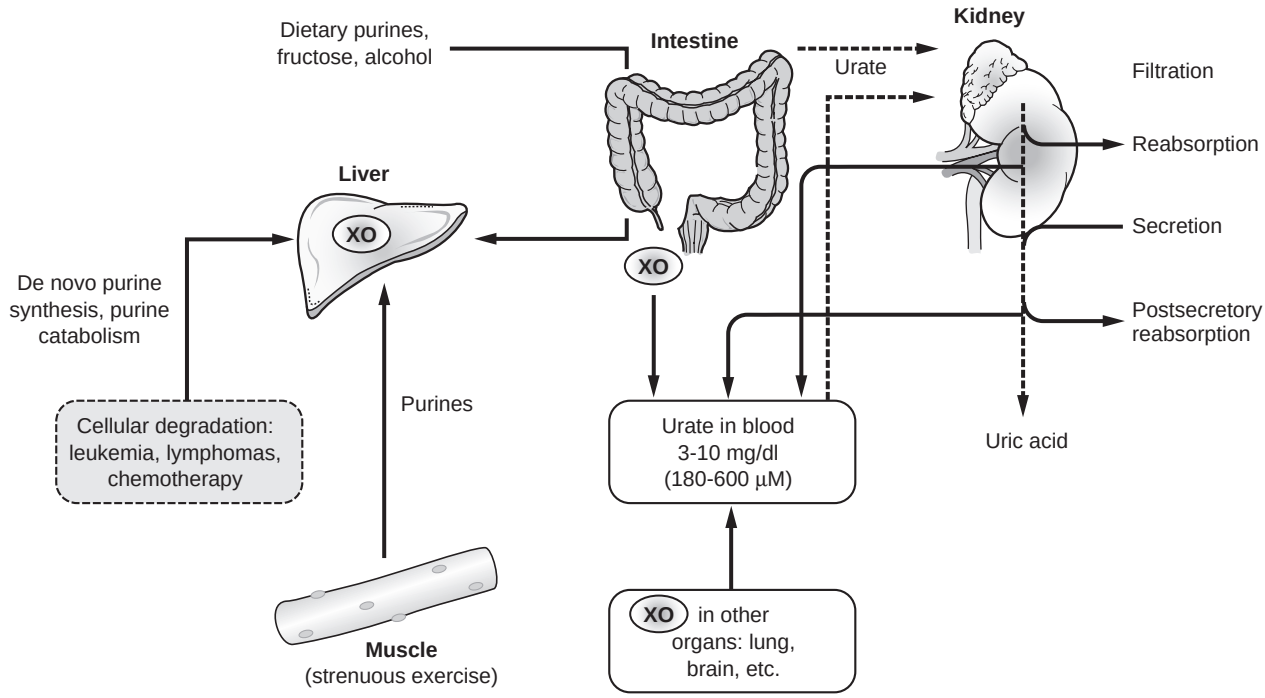

FIGURE 76-1. Urate metabolism.

renal apical organic anion efflux transporter, called MRP4, has been identified (11). MRP4 is a member of the ATP-binding cassette transporter family. It is proposed to mediate secretion of urate and other organic anions such as cAMP, cGMP, and methotrexate across the apical membrane of human renal proximal tubular cells. Human MRP4 is an ATP-dependent unidirectional efflux pump for urate with multiple allosteric substrate binding sites (12).

Another gene involved in renal transport of urate is Tamm-Horsfall protein (THP), also known as uromodulin. THP is exclusively expressed and secreted by epithelial cells of the thick ascending limb, where it has been shown to have antibacterial effects. THP also co-localizes with the Na,K,2Cl transporter in lipid rafts in the apical cell membrane, suggesting a functional interaction (13). Mutations in the human uromodulin gene have been identified in subjects with medullary cystic kidney disease type 2 and in patients with familial juvenile hyperuricemic nephropathy (see subsequent text) (14,15). It is not yet known how the THP mutation leads to hyperuricemia, as most evidence suggests that uric acid handling is restricted to the proximal tubule. However, there is some evidence that some urate secretion in the rat can occur distal to the proximal tubule (16). Furthermore, there is also some evidence that the THP mutation may lead to sodium and water wasting, possibly resulting in stimulating urate reabsorption proximally (see section following section on Familial Juvenile Hyperuricemic Nephropathy).

Causes of Hyper- and Hypouricemia

Hyperuricemia has been arbitrarily defined as >7.0 mg/dL in men and >6.5 mg/dL in women. "Normal" serum uric acid levels in the population appear to be rising throughout the last century, likely as a consequence of changes in diet (see subsequent text), and mean levels in men in the United States are now in the 6.0 to 6.5 mg/dL range (3). Uric acid levels tend to be higher in certain populations (e.g., African American and Pacific Islanders), with certain phenotypes (obesity, metabolic syndrome) and with special diets (meat eaters vs. vegetarians) (3). Uric acid also has a circadian variation, with the highest levels in the early morning (17). The serum urate concentration reflects the balance between urate production and elimination. Hyperuricemia may occur from excessive production of urate (overproduction) or decreased elimination (underexcretion), and frequently a combination of both processes occur in the same patient. Furthermore, uric acid levels may vary in the same individual by as much as 1 to 2 mg/dL during the course of a day, due to the effects of diet and exercise.

Genetic mechanisms mediating hyperuricemia include overproduction due to mutations of two enzymes: hypoxanthine–guanine phosphoribosyltransferase (HGPRT) and phosphoribosyl pyrophosphate synthetase (PRPPS) (Table 76-1). Subjects with Lesch-Nyhan syndrome (due to a mutation of HGPRT on the X chromosome) present in childhood with neurologic manifestations (mental retardation, choreoathetosis, and dystonia) and have an increased risk for nephrolithiasis, renal failure, and gout. A partial deficiency of HGPRT may manifest later in life as recurrent gout and/or nephrolithiasis (partial HPRT deficiency (Kelley-Seegmiller syndrome) (18). Other genetic mechanisms include subjects with the uromodulin mutation, who develop hyperuricemia (due to underexcretion) with early and progressive renal disease (see subsequent text). Certain populations such as indigenous peoples living in Oceania may also have higher uric acid levels than white populations. Finally, African Americans also have higher uric acid levels and a twofold higher incidence of gout compared to white or Asian populations (19); however, this likely reflects the consequence of diets high in purines and fructose (see subsequent text) rather than genetic mechanisms.

Hyperuricemia may also result from diets high in purines, from ethanol, and from fructose. Foods high in purine include anchovies, herring, trout, organic meats (liver, heart, kidney), meat gravies, broths, asparagus, and mushrooms. The effect of alcohol is in part related to increased urate synthesis, which is due to enhanced turnover of ATP during the conversion of

TABLE 76-1

MAJOR CAUSES OF HYPERURICEMIA

Genetic causes
Familial hyperuricemic nephropathy (mutation of uromodulin)
Lesch-Nyan syndrome (HGPRT mutation)
Phosphoribosyl pyrophosphate synthetase (PRPPS) mutation
Dietary causes
Diet high in purines (organ meats, shellfish, fatty meats)
Diet high in fructose (high fructose corn syrup, table sugar, honey)
Ethanol
Low salt diet
Drugs
Thiazides
Loop diuretics
Calcineurin inhibitors (cyclosporine > tacrolimus)
Pyrizinamide
Low-dose aspirin
Volume depletion
Hypoxia (systemic or tissue)
Increased cell turnover (myeloproliferative disorders, polycythemia vera)
Conditions associated with higher uric acid levels
Renal failure
Obesity/metabolic syndrome
Untreated hypertension
African American race
Preeclampsia
Vigorous exercise

acetate to acetyl-CoA as part of metabolism of ethanol (20). In addition, acute alcohol consumption causes lactate production, and because lactate is an antiuricosuric agent, it will reduce renal urate excretion and exacerbation of hyperuricemia (21). Fructose (a simple sugar present in sucrose, table sugar, high fructose corn syrup, honey, and fruits) can also induce a rapid rise in serum uric acid, due in part to its rapid phosphorylation in hepatocytes with the stimulation of AMP deaminase and ATP consumption (22). It has recently been proposed that the marked increase in fructose intake may have a role in the rising levels of serum uric acid and obesity worldwide (23).

Uric acid may also be affected by exercise, with moderate exercise reducing urate levels (probably by increasing renal blood flow) and severe exercise causing a rise in uric acid (probably due to ATP consumption with adenosine and xanthine formation). Urate levels vary among gender, in that premenopausal women have lower uric acid, a fact attributed to the uricosuric effect of estrogen (24). The mechanism may relate to gender effects on URAT-1 expression, as recent studies suggest that male mice have higher URAT-1 expression in their proximal tubules compared to female mice (25). Uric acid also tends to increase in the setting of low blood volume and/or low salt diet (due to increased proximal reabsorption), and following the administration of catecholamines or angiotensin II (due to renal vasoconstriction resulting in increased reabsorption). Urate production also relates to body size and weight, so that larger persons produce more urate than those who are smaller. Hyperuricemia is particularly common in the obesity metabolic syndrome (thought to be secondary to the effect of insulin to stimulate uric acid reabsorption) (26) and in untreated hypertension (thought to be due to reduced renal blood flow) (27). Thiazides also increase uric acid reabsorption by decreasing blood volume and via direct interaction with the organic anion exchanger.

Other drugs (cyclosporine, pyrazinamide, low dose aspirin) also increase uric acid, primarily by interfering with renal excretion. In addition, the generation of organic anions such as lactate, β-hydroxybutyrate, and others may interfere with urate secretion in the proximal tubule and cause a rise in serum uric acid. Chronic low level lead ingestion can also cause hyperuricemia by reducing urate excretion, whereas high concentrations tend to cause proximal tubular injury with no rise in uric acid.

Uric acid is also increased in the setting of tissue hypoxia (28) or with cell turnover (29). With tissue hypoxia, ATP is consumed and the isoform, xanthine oxidase, is induced, resulting in increased local uric acid concentrations. Uric acid levels are thus high in subjects with congestive heart failure, high altitude hypoxia, congenital cyanotic heart disease, and with obstructive sleep apnea. Uric acid levels are commonly elevated with certain malignancies, especially leukemias and lymphomas, and levels may sharply rise following chemotherapy (see acute urate nephropathy in the following text) (30). Finally, uric acid has a tendency to be elevated in polycythemia vera and other myeloproliferative disorders (31). In the setting of reduced renal function, the fractional excretion of urate increases but is inadequate to fully compensate for the reduction in GFR, and as a consequence serum uric acid levels rise. Conversely, uric acid excretion via the gastrointestinal tract is also enhanced (32), and therefore serum uric acid levels tend to be only mildly elevated in patients with chronic renal disease, and gout is relatively rare.

Low uric acid levels (levels <2.0 mg/dL) can occur via a variety of mechanisms, including with liver disease (due to decreased production), Fanconi syndrome (due to impaired proximal tubular function), and with diabetic glycosuria (due to proximal tubular dysfunction) (Table 76-2). Drugs such as probenecid, high-dose salicylates, sulfinpyrazone, benziodarone, benzbromarone, and losartan are all uricosuric, whereas allopurinol, febuxostat, and oxypurinol lower uric acid by blocking xanthine oxidase. Statins also lower uric acid (33), and recombinant uricase (rasburicase) can markedly reduce serum uric acid and is approved for use in children with tumor lysis syndrome (in which marked hyperuricemia may develop) (34). There is also a hereditary hypouricemia syndrome that has been observed, and is particularly common in Japan, which has been shown to be due to a mutation in the URAT-1 gene (35). These patients are particularly prone to develop acute renal failure following vigorous exercise, in which it is postulated to be due to massive uricosuria following ATP consumption in the muscle.

TABLE 76-2

MAJOR CAUSES OF HYPOURICEMIA

Liver disease
Fanconi's syndrome
Diabetes (with glycosuria)
Inappropriate secretion of vasopressin
Familial hypouricemia (due to URAT-1 mutation)
Total parenteral hyperalimentation
Medications with uricosuric property including aspirin (>2.0 g/day), x-ray contrast materials, ascorbic acid, calcitonin, outdated tetracycline and glyceryl guaiacolate

URIC ACID AND RENAL DISEASE

In the following section, we discuss the major associations of uric acid with renal disease. For a discussion of urate nephrolithiasis, please see Chapter 26.

Acute Uric Acid Nephropathy

Acute urate nephropathy is a form of acute renal failure that may occur when serum uric acid rapidly rises, such as in patients with malignancies following chemotherapy ("tumor lysis" syndrome) (30). Typically the patient has a hematologic malignancy in which rapid tumor lysis occurs, resulting in the release of DNA and RNA and their rapid metabolism to uric acid by the liver and other tissues. Serum uric acid levels may increase to greater than 14 mg/dL (>840 μM), resulting in a marked increase in urinary urate excretion that exceeds its solubility. Uric acid crystals form within the tubules, leading to obstruction and sometimes rupturing into the interstitium. Monocytes and T cells are attracted to the site, and form giant cell reactions with tubular proliferation and extracellular matrix deposition (36). Diagnosis is facilitated by the characteristic clinical syndrome and with a urinary uric acid/urinary creatinine ratio of >1 mg/mg (or >0.66 mM/mM) (37), and by the presence of urate crystals in the urinary sediment. Historically, treatment consisted of forced alkaline diuresis (to facilitate solubilizing the urate) and large doses of xanthine oxidase inhibitors (typically allopurinol 300 to 600 mg per/day). Recently, recombinant uricase (rasburicase) has become available (and is Food and Drug Administration [FDA] approved in children), which can be administered intravenously and which effectively lowers serum uric acid levels and corrects renal dysfunction more rapidly than allopurinol (34). Dialysis can also be used to acutely lower the serum uric acid levels. The natural course is one similar to that for acute renal failure of any etiology with a period of oliguria, followed by partial or complete clinical recovery. However, some degree of residual renal injury/damage is common.

"Gouty" Nephropathy: Renal Disease Secondary to Primary Gout or Hyperuricemia

In patients who have had gout for many years, renal disease is common. Natural history studies prior to the availability of uric acid–lowering drugs reported that as much as 25% of gouty subjects developed proteinuria, 50% developed renal insufficiency, and 10% to 25% developed end-stage renal disease (37,38). Histologic changes are particularly common, and have been observed in autopsy studies of 75% to 99% of patients with gout. The primary histologic findings consist of arteriolosclerosis, glomerulosclerosis, and tubulointerstitial fibrosis, similar to the findings one observes in patients with hypertensive renal disease (nephrosclerosis) or with aging (38,39). However, in addition, subjects with chronic gout often have focal deposition of monosodium urate in interstitial areas, particularly in the outer medulla. This characteristic histologic pattern has historically been termed "gouty nephropathy" and was thought to be a major etiology of chronic renal disease in the past (39). Studies performed primarily in the 1970s and 1980s challenged the concept that gouty nephropathy was a true entity. First, the focal deposition of crystals did not appear to adequately explain the diffuse disease commonly present, and crystal deposition could be found in some autopsies of other etiologies in which no renal disease was present (40). Second, studies led by Yü and Berger (41–43) argued that, although gout was often associated with renal disease, that this was likely due to mechanisms not related to uric acid. Specifically, they pointed out that most of the gouty subjects who had or developed renal disease had concomitant hypertension, or were old, both conditions associated with the development of microvascular disease, glomerulosclerosis, and tubulointerstitial fibrosis (41–43). When they excluded gouty subjects with hypertension or who were older, they found little evidence that gout could cause renal disease (41–43). Third, although some studies suggested that lowering uric acid could improve the renal disease in gout (44,45), other studies could not demonstrate any significant improvement (46). In some studies in which an improvement was observed, it was often unclear if this was due to the fact that better uric acid control would lead to less use of nonsteroidal agents, which are known to be nephrotoxic (47). Other studies have found no benefit, although most were either nonrandomized or short-term (46). Finally, it became evident that some subjects with high uric acid and renal disease had other entities, such as lead nephropathy (48), or hereditary familial hyperuricemic nephropathy (49). Although some studies suggested that with markedly elevated uric acid levels might cause chronic renal disease (such as levels >13 mg/dL in men or >10 mg/dL in women) (50), most authorities concluded that uric acid by itself was unlikely to cause a chronic nephropathy. Therefore, many authorities considered the term "gouty nephropathy" a misnomer, and concluded that uric acid had relatively little to do with the renal disease present in these subjects (51). Indeed, this led Beck to write "Requiem for gouty nephropathy," an article that summarily condemned the role of uric acid in chronic renal disease and then attempted to permanently lay it to rest (52).

New Insights on the Entity of Gouty Nephropathy

Renewed interest on the role of gout and/or asymptomatic hyperuricemia has occurred in the last several years. First, it was recognized that there were certain inherent assumptions with the types of analyses performed by the previous investigators (53). For example, it seemed inappropriate to use the presence of hypertension to explain every case of renal insufficiency in the gouty patient, since most subjects with essential hypertension have relatively preserved renal function (53). Another implicit assumption was that gouty nephropathy had to be due to crystal deposition, and that it could possibility be mediated by other effects of hyperuricemia was not considered. Furthermore, the analysis also assumed that the presence of hypertension was a separate cause of renal disease and that it had to be independent of the uric acid. This led to a proposal to reinvestigate the role of uric acid in chronic renal disease (53).

Subsequently a number of studies using classic multivariate strategies examined the relationship between serum uric acid levels and the subsequent risk for developing chronic renal disease. Interestingly, studies of the general population found that hyperuricemia was a remarkable risk factor for developing renal insufficiency, with a serum uric acid of >7 mg/dL in men and >6 mg/dL in women, conferring a 10.8-fold increased risk in women and a 3.8-fold increased risk in men compared to those with normal uric acid levels (Fig. 76-2) (54). This increased relative risk was independent of age, body mass index, systolic blood pressure, total cholesterol, serum albumin, glucose, smoking, alcohol use, exercise habits, proteinuria, and hematuria. Indeed, an elevated uric acid was more predictive for the development of renal insufficiency than proteinuria. An elevated uric acid was also independently associated with a marked increased risk of renal failure in another study of more than 49,000 male railroad workers (55). These studies have raised again the possibility that uric acid may cause renal injury.

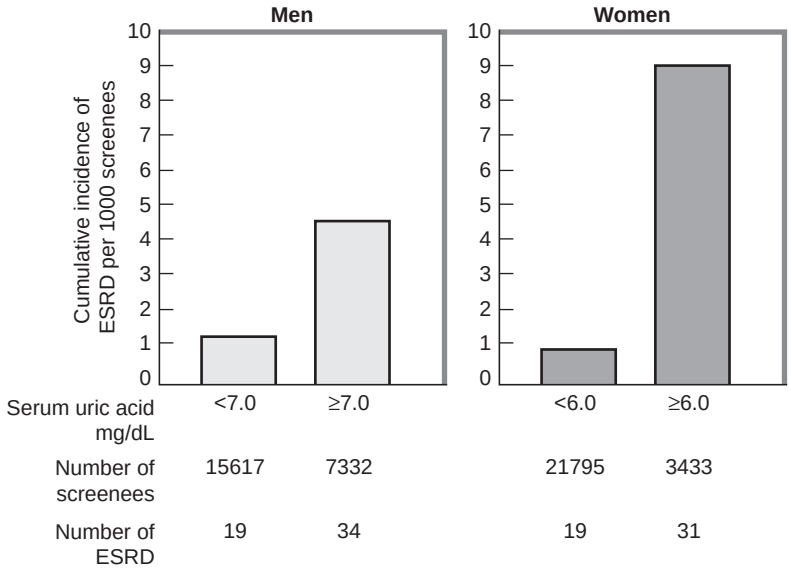

FIGURE 76-2. Cumulative incidence of end-stage renal disease (ESRD) according to baseline serum uric acid level from 1993 to 2000. (From: Iseki K, Ikemiya Y, Inoue T, et al. Significance of hyperuricemia as a risk factor for developing ESRD in a screened cohort. *Am J Kidney Dis* 2004;44:642, with permission.)

A second major breakthrough came from experimental studies in which chronic mild hyperuricemia was induced in rats (56,57). Because rats have functional uricase, the way mild hyperuricemia was induced was by administering low concentrations of the uricase inhibitor, oxonic acid, to the diet (56,57). This resulted in serum uric acid levels that were only 1.5- to 3.0-fold greater than in the normal rat, and importantly this did not result in intratubular or interstitial urate crystal deposition. Over time, however, rats developed hypertension and progressive renal disease. Early in the course the rats developed arteriolar thickening and rarely hyalinosis of the preglomerular arterioles, often accompanied by glomerular hypertrophy (Fig. 76-3) (57,58). Over time proteinuria developed, with the development of worsening vascular disease, glomerulosclerosis, and interstitial fibrosis (57). The lesion was identical to that observed with nephrosclerosis of hypertension, with aging-associated glomerulosclerosis, and with gouty nephropathy, except for the absence of crystal deposition that had been observed in the latter condition. This led the authors to suggest that chronic hyperuricemia may cause renal disease and hypertension via a crystal-independent pathway.

Further studies investigated the mechanism for the uric acid-induced renal disease. It was found that uric acid was able to induce endothelial dysfunction *in vitro*, and that it could inhibit endothelial release of nitric oxide and block endothelial cell proliferation (59). Uric acid was also shown to stimulate

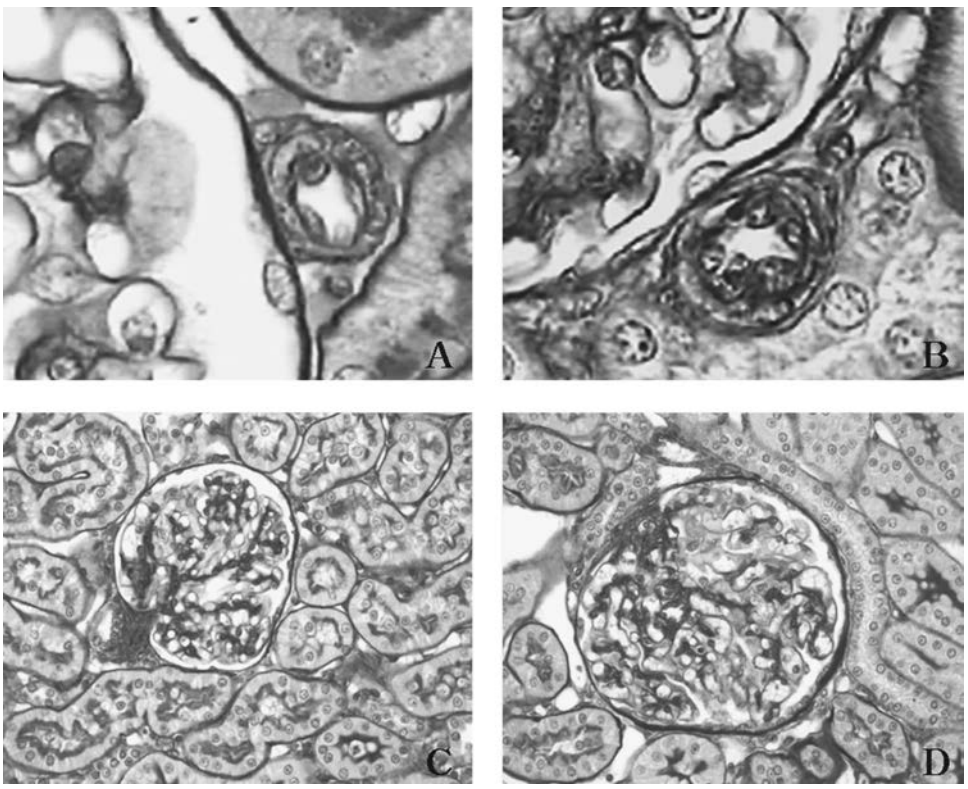

FIGURE 76-3. Afferent arteriolopathy (A, B) and glomerular hypertrophy (C, D) in rats with hyperuricemia (B, D) compared to control rats (A, C). (From: Mazzali M, Kanellis J, Han L, et al. Hyperuricemia induces a primary renal arteriolopathy in rats by a blood pressure-independent mechanism. *Am J Physiol Renal Physiol* 2002;282:F991 and Nakagawa T, Mazzali M, Kang DH, et al. Hyperuricemia causes glomerular hypertrophy in the rat. *Am J Nephrol* 2003;23:2, with permission.)

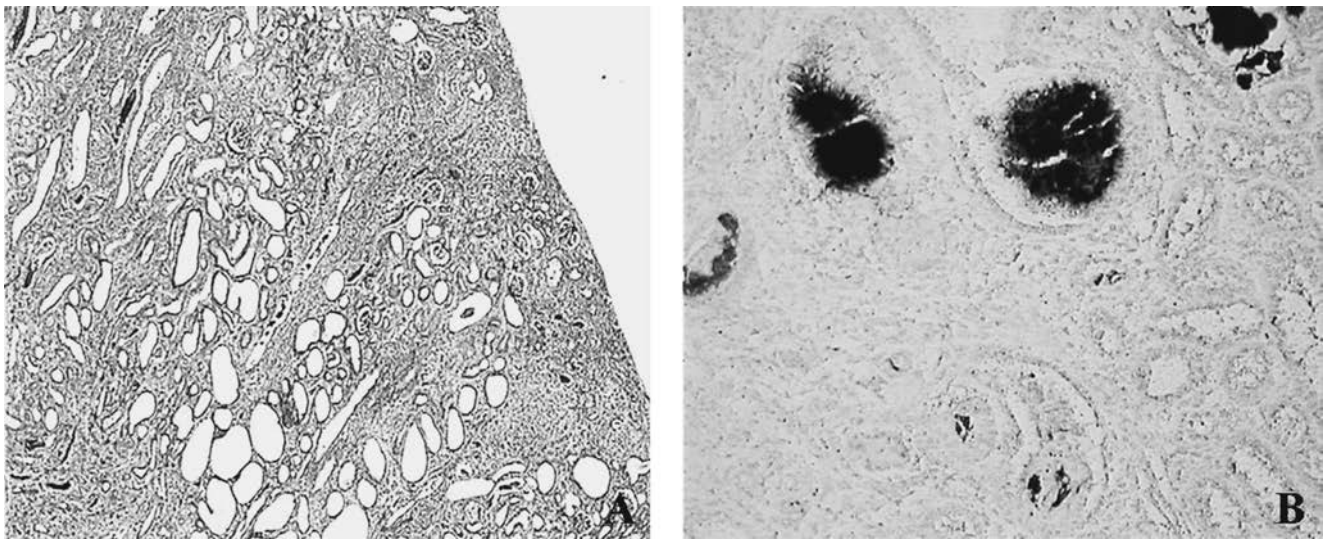

FIGURE 76-4. Acute urate nephropathy induced in rats. **A:** Shown is the renal cortex of a rat with acute urate nephropathy induced by a uricase inhibitor with uric acid supplementation. Renal cortical injury is present with tubular dilation and interstitial inflammation. (PAS, ×200.) **B:** A DeGalantha stain shows intratubular uric acid crystals (magnification, ×400).

vascular smooth muscle cell proliferation through a mechanism that involved uptake of urate into the cell with activation of MAP kinases, nuclear transcription factors (including NF-κB and AP-1), and various inflammatory mediators (including monocyte chemoattractant protein-1 and C-reactive protein) (58–62). An induction of COX-2 with thromboxane production was also shown (63). Hyperuricemic rats displayed evidence of endothelial dysfunction (with low serum nitrites reflecting low NO) and increased intrarenal renin expression (56–59). The *in vivo* renal changes could be reversed by lowering uric acid with allopurinol. In addition, micropuncture studies performed on the hyperuricemic rats demonstrated that the rats developed glomerular hypertension with a reduction in renal plasma flow, both mechanisms that could lead to renal injury (64,65). The observation that hyperuricemia is an independent risk factor for renal progression and that experimental hyperuricemia can largely reproduce the lesion of chronic gouty nephropathy has led to a reappraisal of the role of uric acid in chronic renal disease. Importantly, it is evident that new studies should be performed to determine if lowering uric acid can slow renal progression in these subjects.

Clinical Manifestations of Gouty Nephropathy

Most subjects with longstanding gout will have asymptomatic renal involvement. Although histologic disease is almost invariably present, most subjects will have either normal or only mild renal insufficiency (41–43). Renal blood flow is usually disproportionately low for the degree of renal insufficiency (41–43,66). Fractional excretion of uric acid is usually less than 10%. Proteinuria occurs in the minority of cases, and, when present, is usually in the non-nephrotic range. The urinary sediment is also usually benign. However, hypertension is frequent, occurring in 50% to 60% of subjects and increasing in prevalence as renal function worsens.

The diagnosis of gouty nephropathy classically depends on histologic diagnosis, demonstrating chronic glomerulosclerosis, tubulointerstitial fibrosis, renal microvascular disease, and focal medullary crystal deposition. Identification of the crystals is best done using ethanol fixed tissue with the DeGalantha stain, as the crystals are solubilized by normal fixation methods (Fig. 76-4). However, given recent experimental studies suggesting that crystal deposition is not necessary, the diagnosis may become problematic, for the histologic findings are similar to what is found with benign nephrosclerosis (renal injury from essential hypertension) or with aging-associated renal disease. However, a disproportionately elevated serum uric acid in relation to the degree of renal insufficiency (such as a uric acid level of >9 mg/dL for a serum creatinine of <1.5 mg/dL, a uric acid of 10 mg/dL for a serum creatinine of 1.5 to 2.0 mg/dL, and a serum uric acid of >12 mg/dL when serum creatinine is >2.0 mg/dL) should make one consider this diagnosis, as well as the diseases described in the following text.

Management of Gouty Nephropathy

Although no specific studies have addressed management, it is reasonable to treat gouty nephropathy similar to chronic renal disease of other etiologies. Therefore, one should aim for good blood pressure (BP) control (targeting a BP of <140/90 mm Hg in nonproteinuric patients and 125/75 mm Hg in proteinuric patients). Given the known benefit of blocking the renin–angiotensin system on other renal diseases, and the fact that blocking the renin–angiotensin system also prevents the renal injury induced with experimental hyperuricemia (56–58), we would recommend the use of an angiotensin receptor blocker and/or an angiotensin-converting enzyme inhibitor as part of the clinical regimen. Furthermore, losartan may be a particularly good choice as it is also uricosuric and will lower uric acid levels by affecting the urate–anion exchange in the proximal tubule through a mechanism that is not shared with other angiotensin receptor blockers (67). The use of thiazide or loop diuretics is discouraged, as these drugs have as a side effect the raising of uric acid, and recent studies suggest they provide less renal protection than other antihypertensive agents available (68). If a diuretic is required, the use of amiloride or spironolactone could be attempted provided significant hyperkalemia is not induced. However, it is recognized that some subjects will nevertheless require a thiazide to control blood pressure.

The role of lowering uric acid still remains controversial, and no definitive studies have been performed to resolve this

important issue. Based on the experimental studies, it does seem reasonable to lower serum uric acid in subjects with hyperuricemia, particularly in individuals in which it is markedly elevated (>8 mg/dL). In the setting of renal insufficiency most uricosuric agents are relatively ineffective, although some success with benziodarone has been reported in Europe (69). The most effective way to lower uric acid chronically is with the use of xanthine oxidase inhibitors, but there is concern over using allopurinol in subjects with chronic renal insufficiency due to reports of higher frequency of allergic reactions, particularly the allopurinol hypersensitivity syndrome. This is a syndrome in which subjects develop a Stevens Johnson-like syndrome often in association with fever, abnormal liver function tests, and worsening renal failure (70). In addition, the functional metabolite of allopurinol, oxypurinol, builds up in the setting of renal insufficiency. Thus, if allopurinol is to be used, one should start with 50 mg daily and then slowly increase the dose. The usual dose is 100 mg for each 30 mL/minute of GFR. A promising drug that is currently in clinical trial is febuxostat, which is a nonpurine xanthine oxidase inhibitor that appears to have minimal side-effects, to be more effective than allopurinol, and for which there is no need for dose adjustment in renal disease (71). Finally, one should consider having the subject reduce ingestion of foods that can raise uric acid, such as foods with high-purine content, ethanol, and fructose.

Chronic Lead Nephropathy

It has become recognized that some subjects with the triad of renal disease, hypertension, and hyperuricemia may have chronic lead poisoning. Studies in the 1800s linked chronic lead intoxication with the development of hypertension and renal disease, a finding that has been confirmed repeatedly in recent years. In the 1800s many of these cases were seen in England where large amounts of fortified port wine that had been contaminated with lead was consumed. More recently chronic lead-induced renal disease has been observed in individuals exposed to lead as a consequence of working in or near foundries or individuals who drink moonshine prepared with lead distilling equipment (72). Emmerson also reported a large number of subjects from Queensland who developed lead toxicity as a consequence of ingesting lead paint chips when they were children (73). Some subjects provide no history of known exposure to lead, and in certain populations (such as in Taiwan), a substantial number of subjects with chronic renal disease may have low-level lead toxicity (74).

The etiology of lead nephropathy is complex. Experimentally lead-induced hypertension is observed only with low-dose ingestion of lead acetate, and this results in hypertension and mild renal injury that can be shown to be mediated by oxidants (75). Interestingly, high doses of lead cause proximal tubular injury with intranuclear inclusions, and a Fanconi-like picture in which serum uric acid levels are low and blood pressure is normal (76). Acute lead toxicity in humans is also associated with more of a Fanconi picture due to a postulated direct tubular toxicity.

Subjects typically present with hypertension, slowly progressive renal insufficiency, and often have hyperuricemia and/or gout (termed "saturnine" gout when it is secondary to lead). The renal excretion of uric acid is impaired, and this correlates with elevated blood lead levels (77). The renal sediment is benign, similar to the findings observed with gouty nephropathy and/or arteriolosclerosis. Histologically, the renal lesion also appears like chronic hypertension, and is characterized by prominent vascular changes of arteriolosclerosis, often with variable degrees of glomerulosclerosis and tubulointerstitial fibrosis (78). The strong association of lead intoxication with renal microvascular disease led Huchard, the great French academician of the 1800s, to declare that lead intoxication was the second common cause of arteriolosclerosis (79).

Diagnosis should be suspected when one observes hyperuricemia and/or gout in the presence of chronic renal disease and hypertension. Definitive diagnosis requires the ethylene diamine tetraacetic acid (EDTA) challenge test. One gram of EDTA is administered intravenously and the total urinary lead excretion determined over the subsequent 72 hours. A urinary lead excretion of greater than 600 μg per 72 hours is considered positive (80). The differential diagnosis should include primary gouty nephropathy, familial juvenile hyperuricemic nephropathy (FJHN), and hyperuricemia accompanying other renal disorders.

Treatment consists of standard regimens for chronic renal disease of any etiology, and ideally with the avoidance of drugs that can further increase serum uric acid levels (see previous section on treatment of gouty nephropathy). The role of lowering uric acid levels in this population is unknown. More recently, double-blind and prospective studies have shown that intravenous EDTA treatment can chelate the lead and improve renal function over time (81). The dose used in the study consisted of 1 g calcium disodium EDTA in 200 mL normal (0.9%) saline given over 2 hours on a weekly basis for 3 months with reassessment of lead burden, and if the urinary excretion continued to be greater than 600 μg per 72 hours, then the course was repeated (81).

Familial Juvenile Hyperuricemic Nephropathy

A rare form of hereditary renal disease is Familial Juvenile Hyperuricemic Nephropathy (FJHN). This is an autosomal dominant disorder that has been described in Europe, Australia, and the United States. Typically children and/or young adults present with slowly progressive renal disease and marked hyperuricemia (15,82,83). Renal histology shows chronic renal injury with glomerulosclerosis, tubulointerstitial fibrosis, and arteriolosclerosis, but urate crystal deposition is rare. Gout may or may not occur in the individual. A characteristic feature is a very low fractional excretion of urate, typically <5% (82). Subjects often are normotensive initially, but hypertension is common as the renal disease progresses. Hemodynamic studies have shown that there is severe renal vasoconstriction, with marked depression in renal plasma flow relative to the glomerular filtration rate (GFR) (83). Recently the disease was shown to be due to a mutation in uromodulin, also known as the gene encoding the Tamm-Horsfall protein (14). The THP is produced only by the thick ascending limb tubular epithelial cells in the kidney, raising questions of how this mutation could result in hyperuricemia and renal failure. Interestingly, mutations in uromodulin have also been shown to be the cause of autosomal dominant medullary cystic kidney disease type 2 (15). Indeed, a recent study also suggests that this latter entity is commonly associated with severe hyperuricemia and clinically mimics the phenotype of familial hyperuricemic nephropathy (15). Indeed, for all purposes the two conditions should be viewed as the same disease.

The pathogenesis of the renal injury remains unclear. Interestingly, medullary cystic kidney disease is known to be associated with salt wasting, but it remains unclear if patients with FJHN also have a salt or water-wasting defect. However, preliminary studies in such patients suggest that they have a defect in water concentration, and this correlated inversely with the serum uric acid levels (15). Furthermore, mice with the uromodulin mutation also show a mild water and sodium wasting phenotype, and have evidence for upregulation of sodium transporters in their proximal tubules, as well as a relative defect in urinary urate excretion when factored for the sodium excretion (84, and Gersch M, et al., unpublished data). This

provides the interesting possibility that the hyperuricemia in FJHN is due to increased proximal sodium and urate reabsorption secondary to renal salt loss. Moreover, the TH mutant mouse does not develop either hyperuricemia or renal disease. Again, it is tempting to speculate that this may be due to the presence of uricase in these mice that maintains serum uric acid within normal levels.

Diagnosis is suggested by a positive family history, the early onset of renal insufficiency in the setting of elevated (often >9 mg/dL) uric acid levels, and by a fractional urinary urate excretion of <5% (82). Confirmation is now possible by having leukocyte DNA analyzed for the mutation (available commercially by Athena Diagnostics (www.AthenaDiagnostics.com)(85).

Treatment is largely supportive. Controversy exists over whether lowering uric acid level slows renal progression, but Cameron's group has suggested a benefit using this approach if treatment is started early (82).

Hyperuricemia and/or Gout: Role in Primary Renal Disease, Diabetes, and Chronic Renal Disease

Patients with established renal diseases, such as glomerulonephritis and diabetic nephropathy, may also develop hyperuricemia. As discussed earlier, there is some retention of uric acid with renal insufficiency of other causes, but this is usually mild, due to compensatory increases in the fractional excretion of uric acid and an increase in enteric excretion in the gut (32). Indeed, gouty arthritis is uncommon in progressive renal disease. This may relate to the fact that uric acid levels are often not that elevated and to the fact that uremia is known to inhibit neutrophil chemotaxis and function (86), and hence the inflammatory response may be partially subdued.

More recently interest has developed on the role of uric acid in renal progression in these diseases. Several studies have now reported that serum uric acid predicts renal progression in subjects with IgA nephropathy (87,88), including the severity of renal damage (89). Another study examined diabetes, and noted that those subjects who did not develop nephropathy often had repeated diabetic ketoacidosis and intermittent glycosuria, with low serum uric acid levels (90). Glycosuria is known to impair proximal tubular function and to cause uricosuria. In contrast, those diabetic subjects who had elevated serum uric acid levels were the ones most at risk for developing progressive nephropathy (90).

A clue to the role of uric acid in renal progression was demonstrated in an experimental model of progressive renal disease (63). Rats underwent nephrectomy with removal of two-thirds of the remaining kidney, to create the remnant kidney model. In this model glomerular hypertension develops, resulting in progressive glomerulosclerosis and tubulointerstitial fibrosis over the next 8 to 12 weeks. When rats were made hyperuricemic, a marked acceleration in renal disease was found, with worsening hypertension, proteinuria, renal function, and glomerulosclerosis. A striking finding was that hyperuricemic rats developed severe preglomerular vascular disease, with vascular smooth muscle cell proliferation in the interlobular and afferent arterioles (63). All of these changes could be reversed by allopurinol, and partially by benziodarone (a uricosuric drug used in Europe). Although experimental evidence suggests that an elevated uric acid may accelerate established renal disease, studies in humans are still lacking. Given these observations, one must be cautious in recommending lowering uric acid in such subjects at this time, particularly given reports that the risk for allergic reactions with allopurinol may be increased in the setting of reduced renal function.

Hyperuricemia in Renal Transplantation

Hyperuricemia commonly occurs following renal transplantation, and, in particular, has been associated with the use of cyclosporine, and, to a lesser extent, tacrolimus (91). Indeed, several studies suggest that hyperuricemia occurs in more than 50% of subjects and gout develops in 10% to 15% (91). The mechanism has been shown to be due to decreased renal excretion, driven in part by increased net tubular reabsorption as well as decreased glomerular filtration (92).

While the primary complication of hyperuricemia in renal transplant patients has classically been thought to be gout, again there has been recent concern that the rise in serum uric acid may contribute to the renal vasoconstriction and renal injury that occurs with chronic calcineurin inhibitors. Therefore, Mazzali et al. reported that cyclosporine-treated rats developed mild hyperuricemia with the classic lesions of arteriolar hyalinosis and tubulointerstitial fibrosis, but this lesion was markedly exacerbated if uric acid levels were further increased by administering a uricase inhibitor (93). Kobelt et al. also reported that allopurinol lowers blood pressure and improves renal blood flow in rats administered cyclosporine (94), and Assis et al. demonstrated that allopurinol improved GFR (inulin clearances) in cyclosporine-treated rats (95). Most notably, Neal et al. recently reported that allopurinol therapy lowered uric acid and improved renal function in liver transplant patients receiving cyclosporine (96).

At this time insufficient evidence has been provided to recommend uric acid–lowering therapy in renal transplant subjects. However, if a subject has repeated episodes of gout, or if serum uric acid levels are excessively elevated (>9 mg/dL), we would consider pharmacologic therapy to lower uric acid. Some care must be given with using allopurinol, since allopurinol interacts with azathioprine, and the latter should be reduced to 25% to 50% of its original dose if allopurinol is administered. In addition, we recommend initiating low doses of allopurinol (50 to 100 mg per/day) with slow increases and with a 2-to-4 week course of colchicine (0.6 mg b.i.d.) to minimize the risk for precipitating a gout attack (which is common on initiating allopurinol).

URIC ACID AND HYPERTENSION, THE METABOLIC SYNDROME AND CARDIOVASCULAR DISEASE

In addition to its controversial role in renal disease, there has also been reawakened interest in the role of uric acid in cardiovascular disease, and in particular its role in hypertension and the metabolic syndrome. Although it had been known for more than 100 years that hyperuricemic and/or gouty individuals are at increased risk for cardiovascular events and or mortality, this had historically been attributed to the fact that patients with gout frequently are obese or have other features associated with cardiovascular risk. Studies such as the Framingham analysis published in 1999 reported that this relationship of uric acid with cardiovascular disease was not independent when it was controlled for other accepted cardiovascular risk factors, such as hypertension, diuretic use, obesity, and renal disease (97). Most societies concluded that there is insufficient evidence to support uric acid as a true cardiovascular risk factor.

However, recent studies have provided provocative data that uric acid may be a true risk factor for hypertension. Thus, an elevated serum uric acid is now recognized to be an independent risk factor for the development of hypertension (reviewed in 98). This has been shown by multiple groups including a recent analysis by the Framingham group (99). Furthermore, an

elevated serum uric acid is common in subjects with new-onset hypertension. In one study of new-onset hypertension in adolescents, 89% of subjects had a serum uric acid >5.5 mg/dL, whereas this was not observed in any of 63 controls (including 22 subjects with white coat hypertension) (100). Pilot studies also found that lowering uric acid with allopurinol normalized blood pressure in four hypertensive adolescents, although no placebo group was included in the study (100). There is now an ongoing double blind randomized trial to investigate the role of uric acid in hypertension in this population.

New studies have also suggested that hyperuricemia may have a pathogenetic role in the obesity metabolic syndrome. Thus, an elevated uric acid was found to predict the development of both obesity and hyperinsulinemia in normal subjects (23,101,102), and an elevated uric acid is universally present in the metabolic syndrome (103). Furthermore, recent studies in which rats were fed fructose to induce metabolic syndrome found that lowering uric acid with allopurinol could significantly prevent the development of hypertension, hyperinsulinemia, hypertriglyceridemia, and obesity (23). Further studies are necessary to determine if lowering uric acid in humans will have similar benefit.

Although the studies described in the preceding text have been performed in subjects with normal renal function, Kang et al. observed that endothelial function assessed by the degree of endothelium-dependent vasodilation in pre-dialysis chronic renal failure patients with mean serum creatinine of 2.2 mg/dL was significantly correlated with serum uric acid levels (104). Hsu et al. also reported that a high serum uric acid level was a significant and independent predictor of cardiovascular mortality in 146 hemodialysis patients. In their study serum uric acid levels were higher in patients who died of cardiovascular disease compared to patients dying from sepsis (9.0 ± 1.0 vs. 6.7 ± 1.8 mg/dL, $p<0.05$) (105).

The mechanism by which uric acid may cause cardiovascular disease has been explored using both cell culture and animal models. It appears that uric acid must enter the endothelial and vascular smooth muscle cell via a specific organic anion exchanger, where it activates a variety of intracellular signaling molecules involved in inflammation and proliferation. In the endothelial cell there is a decrease in nitric oxide levels and an inhibition of endothelial proliferation, whereas in the vascular smooth muscle cell there is activation of proliferative and inflammatory pathways (56–62). Local activation of the renin–angiotensin system could also be shown (62).

Experimental studies have further demonstrated that the mechanism by which uric acid causes hypertension is via a decrease in endothelial-derived nitric oxide, activation of the renin–angiotensin system, and the induction of preglomerular vascular disease. The latter may promote continued salt-sensitivity, even after the serum uric acid is corrected (60). The low nitric oxide may also have a central role in the induction of insulin resistance, since insulin requires nitric oxide for its action (by stimulating blood flow to the skeletal muscle) (102).

Although these studies are primarily limited to animals, they provide provocative data to suggest that uric acid may have a role not only in the development of renal disease, but potentially in aggravating cardiovascular risk through effects on blood pressure and the metabolic syndrome.

CONCLUSION

In conclusion, there is a strong relationship of uric acid with both acute and chronic renal disease. Emerging evidence suggests that it may not only cause acute renal failure as a consequence of crystal formation within the tubular lumina, but chronic hyperuricemia may also be able to cause renal disease via crystal-independent pathways. Indeed, uric acid may not only be a primary cause of chronic renal disease (gouty nephropathy), but could be a contributory factor in lead nephropathy, primary renal diseases, diabetic nephropathy, cyclosporine nephropathy, and the progression of chronic renal disease. Uric acid may also be an unrecognized contributor to cardiovascular risk, as evidenced by recent studies linking it pathogenetically to hypertension and metabolic syndrome. The next few years should be exciting in terms of separating out the role of uric acid in acute and chronic renal injury.

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CHAPTER 77 ■ SICKLE CELL DISEASE

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In 1910, Herrick described the case of a young black student with severe anemia characterized by "peculiar elongated and sickle-shaped red blood corpuscles" (1). This was the first description of the condition later to be known as sickle cell anemia. Herrick noted a slightly increased volume of urine of low specific gravity, and thus observed the most frequent feature of sickle cell nephropathy: an inability of the kidney to concentrate urine normally.

PATHOPHYSIOLOGY

The identification of this familial autosomal-codominant disorder as an abnormality of the hemoglobin molecule was made by Pauling et al. in 1949 (2). The exact nature of the defect, the substitution of valine for glutamic acid at the sixth residue of the β chain, was established by Ingram (3), thus characterizing sickle cell anemia as a disease of molecular structure. The hemoglobin molecule is composed of two α and two β chains. The result of the point mutation in the β chain is a slight change in the three-dimensional spatial configuration of the hemoglobin molecule. Glutamic acid is a charged amino acid and is therefore very soluble in water; valine is uncharged and poorly soluble in water. The loss of charge explains the altered migration of sickle cell hemoglobin with hemoglobin separation techniques, including electrophoresis. Furthermore, the uncharged, poorly soluble valine residues allow the hemoglobin molecules to adhere to one another and to form elongated structures that distort the red cells into their characteristic sickle and other abnormal shapes (Fig. 77-1). Under conditions of hypoxia, acidosis, or hyperosmolality (the latter being present in the renal medulla), sickle hemoglobin forms a highly viscous, semisolid "gel" (4). Hemoglobin molecules form elongated double-stranded fibers that polymerize, resulting in 14 helical filaments (5,6). The process of polymerization and the formation of these structures is delayed after deoxygenation of the red blood cell, which is sufficiently long for most cells that they are returned to the lungs and reoxygenated before significant gelation and "sickling" (7). Gelation is also inhibited by the presence of normal hemoglobin A, hemoglobin F, and other hemoglobin variants (8).

The presence of sickle hemoglobin is associated with mechanical deformation of the cell due to polymerization and increased fragility with oxidative injury, altered phosphorylation and methylation of red cell membrane proteins (9). Acquired red blood cell membrane abnormalities include a loss of phospholipids, asymmetry, increased fragility and abnormal integrin–protein interactions (9). This injury, in addition to the retention of adhesion molecules, promotes sickle red blood cell adhesion to vascular endothelium (10). Because clinical disease severity has been correlated with red blood cell adhesivity, this feature of sickle cell disease likely plays a central role in the pathophysiology (11).

Intermittent vasoocclusion in the renal circulation due to adherent and/or sickled red blood cells may result in ischemia followed by reperfusion and oxidative injury, which has been investigated in the transgenic sickle cell mouse model. Nath et al. described renal enlargement, medullary engorgement, and a reduced plasma creatinine in transgenic sickle cell mice, resembling changes seen in human kidneys with sickle cell disease. Red blood cell sickling extended from the medulla into the cortical capillaries and glomeruli (12). Induction of the expression of heme oxygenase (HO)-1 was associated with increased HO levels in the mice, representing a response to oxidate stress that may arise in response to free heme release with hemolysis of sickle red blood cells and other oxidative stresses. Similar increased expression of HO-1 was seen in circulating endothelial cells from sickle cell patients, associated with increased blood levels of HO (12). Osarogioagbon et al. demonstrated increased peroxidation of polyunsaturated fatty acids after transgenic sickle cell mice were exposed to transient hypoxia (13). This oxidation was reduced by preadministration with allopurinol, suggesting a role for the xanthine oxidase (XO) system in reperfusion injury (13). Upregulation of NFκB was also noted, which might lead to apoptosis in kidney tissues. Peroxynitrite formation led to reduced phosphorylation of p38 and extracellular signal-related kinase (ERK) and increased phosphorylation of c-Jun HN(2)-terminal kinase (JNK) in renal tissue from transgenic sickle cell mice exposed to hypoxia, changes which would also be expected to lead to increased apoptosis (14). Apoptosis was observed by Bank et al. in renal tubular cells from transgenic sickle cell mice exposed to hypoxia, and nitration of tyrosine in kidney protein extracts co-located in close proximity with cellular inductible nitric oxide synthase (iNOS) by immunoreactive staining, suggesting oxidative alternation of nitric oxide metabolism (15). Upregulation of various genes, including cytochrome P450 4a14, Na^+/K^+ ATPase, and renin-1 was demonstrated by gene expression microarray of transgenic sickle cell mice kidneys in the setting of hypoxia documented by blood oxygen level-dependent magnetic resonance imaging BOLD-MRI (16). The authors suggested that in aggregate these changes may represent a hypoxia-driven cascade that increases the renal vasculature and may worsen vasoocclusion by altered arachidonic acid and nitricoxide metabolism. They also observed potentially beneficial compensatory upregulation of mitochondrial hydroxymethylglutaryl CoA synthase (mHMG-CoASyn), cytokine-mediated SH-2 containing protein (CIS), and arginase II.

In the inner renal medulla and the renal papillae in humans, where, especially during antidiuresis, osmolality is markedly increased as a result of countercurrent multiplication and countercurrent exchange, shrinkage of red blood cells is observed (17). This results in an increase of intracellular hemoglobin concentration, which may initiate the process of polymerization (18). This makes the renal medulla and papillae most vulnerable to vasoocclusion, red blood cell adhesion, oxidation/reperfusion injury and hemolysis in sickle cell disease. Patients with sickle cell disease and co-existent α-thalassemia experience potentially fewer complications, including less microalbuminuria, an early marker of sickle nephropathy (19). This apparent protective effect was not correlated with degree of

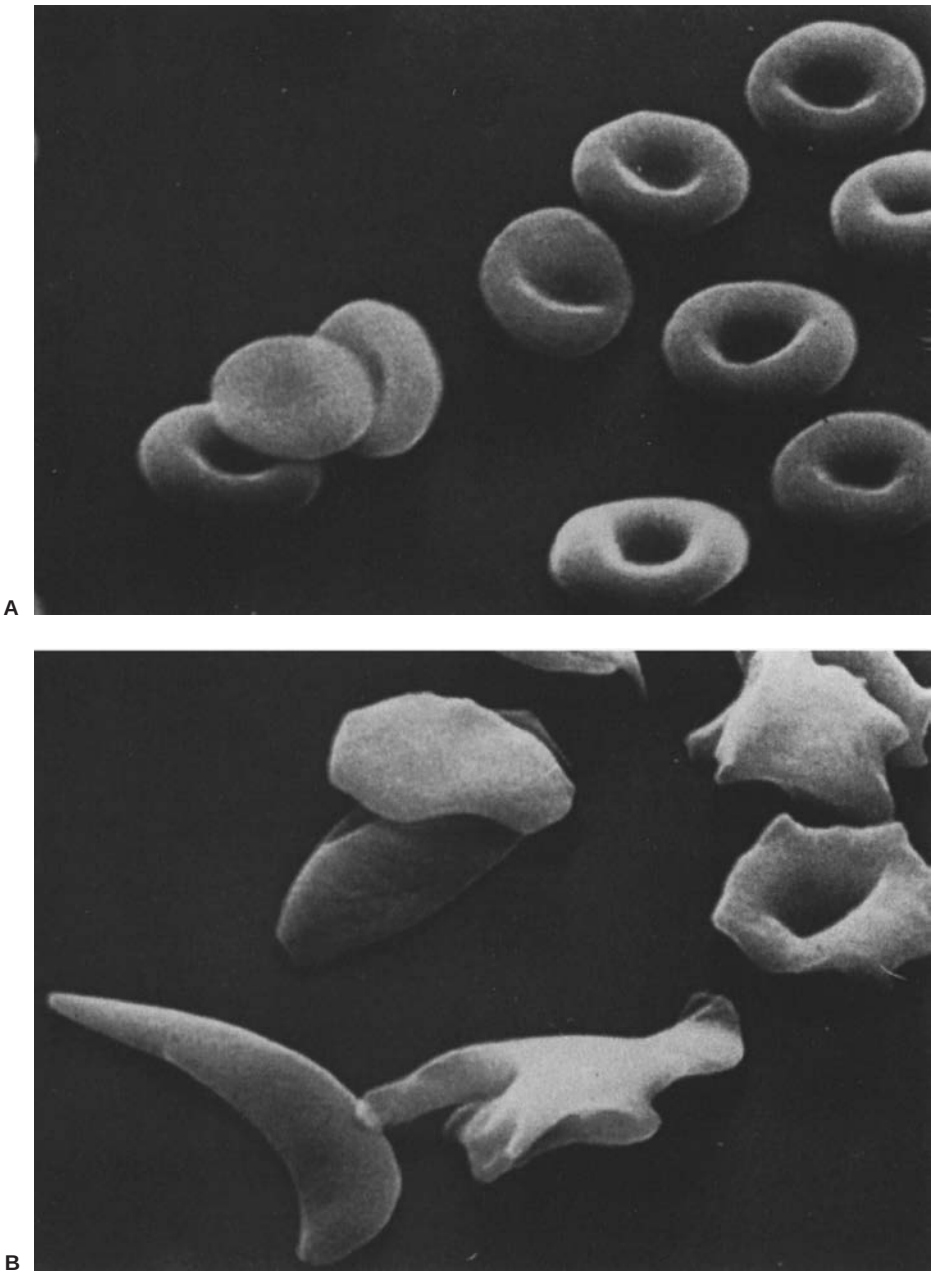

FIGURE 77-1. A: Normal red blood cells from Hb-AA subject as seen by scanning electron microscope (SEM). (Magnification, ×5,230.) **B:** Sickled red blood cells from Hb-SS subject viewed by SEM. The cells appear to be rigid and are deformed by the formation of intracellular elongated fibers formed as a result of polymerization of hemoglobin molecules. (From: Barnhart MI, et al. *Sickle Cell.* Kalamazoo, MI: Upjohn Co.; 1974, with permission from Pfizer Inc.)

anemia or severity of hemolysis. The authors suggested this may be due to the decreased mean corpuscular volume (MCV) or mean corpuscular hemoglobin concentration (MCHC) associated with co-existent α-thalassemia, which may lessen the proclivity for sickle hemoglobin polymerization.

The homozygous form of sickle cell disease, sickle cell anemia (Hb-SS), is characterized by a severe hemolytic anemia (hemoglobin 5.9 to 9.6 g/dL) with a compensatory reticulocytosis. The mean red cell survival is 30 days (normal = 120 days). These patients suffer periodic attacks (crises) of severe pain in the joints, bones, muscles, and abdomen due to vasoocclusion from sickled and/or adherent red blood cells. In the heterozygous form, sickle cell trait (Hb-AS), only 30% to 45% of hemoglobin is Hb-S; the presence of more normal hemoglobin A than hemoglobin S precludes polymerization. Except in rare circumstances, sickling of red blood cells does not occur in the systemic circulation, and this is a benign, asymptomatic condition. These patients have no hematologic abnormalities and reticulocyte counts are normal. However, renal abnormalities, such as disturbed urinary concentrating capacity, episodic hematuria, and sometimes even papillary necrosis with renal colic, may be observed due to the extreme conditions found in the renal medulla.

β Chain production is controlled by two genes, one from each parent. Sickle cell disease also occurs when Hb-S is produced by one β chain gene and another abnormal hemoglobin is produced by a mutation in the other β chain gene. These sickle cell disease patients have been called *double heterozygotes* and represent a large group of hemoglobinopathies characterized by the phenomenon of sickling. In these patients, under conditions of hypoxemia, hyperosmolality, and acidosis, intracellular hemoglobin polymerization can occur, although in variable degrees. These include hemoglobin SC (Hb-SC), Hb-SD, Hb-SO_{Arab}, and Hb-SE diseases. β-Thalassemia occurs when the mutation in the β chain gene results in the failure to produce any β globin chains (β^0thalassemia) or the underproduction

of an otherwise normal β globin chain (β^+thalassemia). When combined with Hb-S, the presence of a β-thalassemia results in either Hb-Sβ^+thalassemia or Hb-Sβ^othalassemia, sickling disorders characterized by the presence of microcytosis. In contrast to sickle cell trait (Hb-AS), the amount of hemoglobin S produced is more than the normal hemoglobin A, permitting polymerization and sickling. A renal concentration defect has been observed in patients with Hb-SC (20) and Hb-SD (21), as well as in Hb-Sβ-thalassemia patients. Hemoglobin C_{Harlem} has two substitutes on the β chain, one of which is identical to the Hb-S abnormality. Both sickling and hyposthenuria have been observed in Hb-C_{Harlem} patients (22). In any hemoglobinopathy that results in sickling of red blood cells, it might reasonably be expected that renal concentrating defects would be found.

The incidence of sickle cell anemia (Hb-SS) varies in different parts of the world. It is estimated that 8% of African Americans carry sickle cell trait (Hb-AS), and that 1 in 400 African Americans have sickle cell anemia (Hb-SS) (23). The frequency of Hb-AC is estimated at 2%. There are important geographic variations in the incidence of these important abnormal hemoglobins. One factor is the protection against infection with falciparum malaria by carriers of the sickle cell gene. This explains why the phenotypic frequencies for Hb-S vary from 10% to 40% in Africa and from 5% to 20% in Greece and Turkey, and exemplifies that sickle cell diseases occur in individuals of non-African descent. The phenotypic frequency for Hb-C is approximately 10% in West Africa (24).

This chapter describes the renal manifestations of sickle cell disease. Clinical impairment in urinary concentrating function—and to a lesser degree in urinary acidification—is observed despite normal or even supernormal glomerular filtration rate (GFR) and renal plasma flow in all patients with sickle cell disease. Chronic renal disease occurs in up to 30% of patients with increasing age, manifested first by microalbuminuria, then proteinuria, hypertension, worsening anemia, and, in some cases, focal segmental glomerulosclerosis (FSGS) and nephrotic syndrome (25). Chronic renal failure accounts for 4% to 18% of deaths in adults with sickle cell disease (25,26). Episodic hematuria, an increased tendency to pyelonephritis, papillary necrosis, and renal medullary cancer, may also occur in patients with sickle cell disease and sickle cell trait.

PATHOLOGIC FEATURES

In 1923, Sydenstricker et al. first described macroscopic and microscopic postmortem studies of kidneys of patients with sickle cell disease (27). Increasingly sophisticated techniques in the last four decades have enabled improved delineation of gross, microscopic, ultrastructural, and molecular changes and their impact on renal function in sickle cell disease.

Gross Anatomy

The kidneys of patients with sickle cell disease are usually of near-normal size in childhood, but tend to increase in length and weight with increasing age. Walker et al. observed 5 to 8 mm increases in the length of kidneys by ultrasound in patients with Hb-SC and Hb-SS disease as compared with age-matched controls (28). The increase in length was correlated with reticulocytosis in patients with Hb-SS. On cut surfaces glomeruli often stand out very prominently as red "pinheads." Kidneys removed because of severe hematuria may demonstrate submucosal hemorrhages in the pelvis, medulla, and cortex (29). Calicectasis has been observed on pyelographic examination in 10 of 17 adult (Hb-SS) patients (30). Autopsy studies on three patients with calicectasis showed lesions consistent with acute and chronic pyelonephritis. However, these lesions could also have been caused by ischemia and necrosis as the result of the sickling process itself. Radiographic changes suggesting papillary necrosis—medullary cavitations, ring shadows, and calcifications in the pyramids—have also been observed (31,32). Occasionally, minimal papillary necrosis is apparent only on microscopic examination. Renal vein thrombosis is also an occasional finding in Hb-SS disease.

Whereas renal medullary pathology is often demonstrated, cortical infarctions are less commonly reported. In a study on renal function in sickle cell anemia patients older than 40 years of age, 20% of the patients exhibited irregularity of the renal outline on intravenous urography (33).

Morgan et al. (34) made a retrospective study of hospital necropsies of 21 patients with Hb-SS who died at the age of 40 or older. Renal failure had caused or contributed to death in 10 cases. All kidneys showed papillary damage. In 13 cases, moderate-to-severe cortical irregularity, including scarring, was seen. Hypertrophic glomeruli were observed with increased cellularity and glomerulosclerosis was common. In an autopsy series of 403 sickle cell disease patients, collected at the Centralized Pathology Unit for Sickle Cell Disease at the University of South Alabama, 13% of patients had pathologic evidence of chronic renal failure and/or papillary necrosis and 23% had areas of renal infarction and/or atrophy (35).

Microradioangiographic studies have been performed on kidneys removed at autopsy from patients with normal hemoglobin, patients with Hb-SS disease, and patients with Hb-AS and Hb-SC disease (36) (Fig. 77-2). A significantly reduced number of vasa recta are seen in kidneys from Hb-SS patients. Even the vessels that are present are abnormal in that they are dilated, show spiral formation, and end bluntly. Patients with Hb-AS and Hb-SC disease show changes intermediate between those of the Hb-SS patients and normal subjects. In patients with Hb-AS, sparse bundles of vasa recta are surrounded by a chaotic pattern of dilated capillaries, with loss of the original bundle architecture. The assumption is that these changes result from occlusion of vasa recta and represent the structural basis for the development of functional changes. The loss of the highly specialized structure of parallel running loops of Henle and vasa recta makes countercurrent multiplication and exchange impossible.

Microscopic Pathology

In the first description of structural changes in sickle cell kidneys, prominent glomeruli distended with blood and necrosis and pigmentation of tubular cells were observed (27). Glomerular enlargement and congestion are more common in children beyond the age of 2 years and are most marked in juxtamedullary glomeruli (37,38). When the size of juxtamedullary glomeruli is systemically measured, there is a distinct difference between those of children with sickle cell disease and normal children. Both the afferent and efferent arterioles of the enlarged glomeruli may be dilated and engorged with sickled cells. In adult patients with Hb-SS and normal GFR, glomerular size as well as the total glomerular area per unit area of cortex have been found to be significantly greater than in patients with Hb-AS and normal control subjects. In older patients, this glomerular enlargement and congestion led to progressive ischemia and fibrosis with obliteration of glomeruli (34,39).

In the study by Falk et al. of 10 biopsied Hb-SS patients with proteinuria, glomerular enlargement was found along with peripheral focal segmental glomerulosclerosis (40). The mean

FIGURE 77-2. Injection microradioangiographs of kidneys from a subject without hemoglobinopathy (**A**), a patient with sickle cell disease (**B**), and a patient with sickle cell–hemoglobin C disease (**C**). In the normal kidney (**A**), vasa recta are visible radiating into the renal papilla; in sickle cell anemia (**B**), vasa recta are virtually absent. Those vessels that are present are abnormal, are dilated, form spirals, and end bluntly, and many appear to be obliterated. The patient with Hb-SC (**C**) shows changes intermediately between the Hb-SS patients and the normal subjects. (From: Statius van Eps LW, et al. Nature of concentrating defect in sickle cell nephropathy, microradioangiographic studies. *Lancet* 1970;1:450, through courtesy of the editors.)

($\pm$SD) glomerular area of these patients was $28.7 \pm 4.1 \times 10^3\ \mu m^2$ as compared with $15.8 \pm 4.3 \times 10^3\ \mu m^2$ in control patients without renal disease. Elfenbein et al. observed that the glomeruli of sickle cell disease patients were 74% larger than those with sickle cell trait and 87% larger than those from normal kidneys (41).

The most common structural morphologic abnormalities in the glomeruli of patients with sickle cell disease are focal segmental glomerulosclerosis (FSGS) and membranoproliferative glomerulonephritis-like (MPGN-like) disease without immune complex deposition (40). Either a collapsing or expanding pattern can be seen (41). Elfenbein et al. and Bakir et al. spec-

ulated that small fragments of red blood cells may have lodged in capillary loops and been phagocytosed by mesangial cells (41,42), which lay down subendothelial basement membrane material. This may result in the reduplicated appearance of the capillary loops.

Medullary lesions, however, are the most prominent; early changes consist of edema, focal scarring, and interstitial fibrosis. With progressive scarring there is tubular atrophy and infiltration of mononuclear cells. Iron deposition has been observed in proximal tubules and pigmented casts may be seen (31). Defective iron metabolism, suggested by decreased renal cortical spin echo with magnetic resonance imaging (MRI), may contribution to the nephrotic syndrome (43). All these changes could be the result of the observed obliteration or attenuation of the medullary circulation (36).

Ultrastructural Pathology

Young adults with Hb-SS disease (from 17 to 27 years) and limited ability to concentrate urine, but normal GFR and effective renal plasma flow (ERPF), show normal epithelial foot processes and glomerular basement membranes. Occasionally focal pedicle fusion and glomerular basement membrane thickening are observed (44). The cytoplasm of mesangial cells contains prominent electron-dense material with a homogeneous and sometimes granular or lamellated structure. The mesangial matrix may be increased in quantity with focal presence of fibrillar material. These findings cannot be considered specific for sickle cell disease, although the changes mentioned previously increase with advancing clinical renal disease in the patient with Hb-SS. Moreover, these ultrastructural changes may be present without other signs of overt renal disease and may be the earliest effects of sickle cell disease on the kidney. Systematic follow-up studies will be necessary to establish this relationship. Interstitial cells of the outer medulla sometimes contain aggregates of granular electron-dense material. The normally close relationship of the interstitial cell processes and cytoplasmic fibrils to the interstitial capillaries appear to be at least partially interrupted by the interstitial collagen.

Guasch et al. demonstrated enhanced fractional clearance of all dextrans (26 to 64 Å) as compared to healthy controls (45). Assuming a heteroporous model of filtration and analysis with hydrodynamic pore theory, the authors suggested there is a distinct alteration in capillary wall pore structure with an increase in resistant pore size.

Pathologic Changes in the Nephrotic Syndrome

In studies of patients with sickle cell anemia who have nephrotic syndrome and marked renal insufficiency, much more striking electron-microscopic changes have been reported (41,46). There is effacement of foot processes, and sheets of epithelial cytoplasm cover the external surface of the basement membrane. Mesangial matrix is increased and basement membrane duplication is seen. Another feature is the appearance of electron-dense bodies, particularly in the mesangium and occasionally in the endothelial cytoplasm. Most of these ultrastructural changes are also observed in the nephrotic syndrome resulting from a variety of causes, and in the absence of concomitant sickle cell disease; therefore, they cannot be considered specific for the sickling disorders. Bakir et al. published a survey on 240 adults with sickle cell anemia over a period of 11 years (42). Twelve had the nephrotic syndrome. In nine, the glomerular lesion, which they proposed to call *sickle glomerulopathy,* consisted of mesangial expansion and basement membrane duplication. The prognosis of this complication of sickle cell nephropathy is very poor. Of 22 patients with sickle cell glomerulopathy, 9 from the report of Bakir et al. and 13 added from the literature, 11 died within 2 years, 10 from rapidly developing renal failure. Bakir et al. concluded, based on their very large group of sickle cell anemia patients, that the nephrotic syndrome, most often caused by sickle glomerulopathy, occurs in 4% of patients, leading to renal failure in two-thirds and death within 2 years in half of the patients.

Alternatively, the nephrotic syndrome in patients with both sickle cell anemia and sickle cell trait (47–49) has been interpreted as an autologous immune-complex nephritis. Immunologic studies in seven Hb-SS patients with clinical and laboratory evidence of glomerular disease revealed immunoglobulin and complement components and renal tubular epithelial antigens in a granular pattern along the glomerular basement membrane. Tubular epithelial antigens and cryoprecipitable renal tubular antigen–antibody complexes were detected in the circulation of some patients. It was suggested that an immune-deposit normocomplementemic nephritis develops in sickle cell anemia and that the involved antigen is released from tubules damaged by the vascular alterations characteristic of this disease. However, this has been demonstrated in only 12 cases in the literature and is not representative of the preponderance of sickle cell nephropathy.

Recently, more attention has been given to the occurrence of glomerulosclerosis in sickle cell anemia. Tejani et al. found glomerulosclerosis in 8 of 13 children in whom a renal biopsy was performed for persistent proteinuria or nephrotic syndrome, whereas mesangial proliferation was found in the other 5 children (50). Children with glomerulosclerosis were older at the onset of nephropathy and presented with nephrotic syndrome more often than did those with mesangial proliferation. In addition, several case reports mentioned the occurrence of the nephrotic syndrome with focal glomerulosclerosis in sickle cell anemia (27,38,41,46). All patients with mesangial proliferation and half of the patients with focal and segmental glomerulosclerosis had supernormal renal clearances at onset of nephropathy suggesting hyperfiltration (50). The latter, both in animals and humans, may be injurious to the integrity of glomeruli and lead to mesangial proliferation and focal segmental glomerulosclerosis. It has been shown that hyperfiltration is a characteristic of the nephropathy in Hb-SS patients (51–54). This hyperfiltration may well be responsible for the glomerulosclerosis and the renal function decline in these patients (40,55). Bhathena and Sondheimer studied kidney biopsy specimens from six homozygous Hb-SS nephrotic patients (56). They observed a distinctive glomerulopathy of focal sclerosis developing in maximally hypertrophied glomeruli. The glomeruli were markedly enlarged, whether nephrotic (233.6 μm $\pm$ 25.3 [standard error, SE]) or not (control Hb-SS values 243.0 μm $\pm$ 12.5; normal control values 158.0 μm $\pm$ 12.7). Because increased hyperfiltration and glomerular capillary hypertension are injurious to remnant glomeruli that undergo hypertrophy, the observed focal segmental glomerulosclerosis is the expected pathologic lesion. Hyperfiltration-related glomerular injury that supervenes in the form of progressive focal glomerulosclerosis causes renal insufficiency and increasing proteinuria, the nephrotic syndrome, and ultimately renal failure.

Although this form of sickle cell glomerulopathy is the most typical and specific lesion in sickle cell patients to cause the nephrotic syndrome and ultimately renal failure, other causes of nephrotic syndrome can occur with Hb-SS, such as poststreptococcal acute glomerulonephritis, membranoproliferative glomerulonephritis (57), and the autologous normocomplementemic membranoproliferative glomerulonephritis (48,49).

RENAL FUNCTION

Hemodynamics

Renal hemodynamics are, as a rule, either normal or supernormal in homozygous (Hb-SS) patients younger than 30 years of age (51,54,55). In Hb-SS infants, increased values have been observed for both GFR and effective renal blood flow (ERBF), as well as for the tubular transport maximum of *para*-aminohippurate (Tm_{PAH}). ERBF was found to be normal or elevated, although less elevated than effective renal plasma flow (ERPF) because of the very low hematocrit. The extraction ratio of *para*-aminohippurate (E_{PAH}) was decreased. Filtration fraction (GFR/ERPF) has been found to be decreased (mean 14% to 18%; normal 19% to 22%) (51,54,55,58). It has been suggested that selective damage of the juxtamedullary glomeruli might result in a lower filtration fraction because these nephrons appear to have the highest filtration fractions (59,60). Microradioangiographic studies lend support to this suggestion (36).

In sickle cell trait, Hb-SC disease, Hb-CC disease, hemoglobin C trait (20), and Hb-Sβ^+thalassemia, GFR and ERPF were found to be within the range of normal. As compared to patients with Hb-SS, individuals with sickle cell trait do not appear to have significant age-related increases in GFR or microalbuminuria (61).

Multiple mechanisms may be responsible for the increase in renal hemodynamics in patients with sickle cell disease. Multiple transfusions with Hb-A blood to patients with Hb-SS result in significant, although temporary, increases in hemoglobin concentration, with gradual and almost complete replacement of Hb-S by Hb-A. Because this procedure does not reduce the supernormal GFR and ERPF (51), the cause of the supernormal renal clearances in sickle cell nephropathy cannot be explained by the anemia per se or by the presence of the abnormal hemoglobin. Also GFR and ERPF may even be slightly reduced in chronic anemia from other causes (62).

Altered nitric oxide production and renin secretion as seen in transgenic sickle cell mouse models may play a role in hyperfiltration (16,63) (See section entitled "Pathophysiology"). The ischemic damage to the medulla could also be a stimulus for increased prostaglandin synthesis, leading to hyperfiltration (55,58,64). Indeed, during prostaglandin synthesis inhibition with indomethacin, a significant fall in GFR, ERPF, creatinine clearance, and urea clearance was observed in sickle cell anemia. In control subjects none of these variables changed after indomethacin. It is therefore suggested that prostaglandins may play an important role in maintaining a normal GFR and ERPF in sickle cell anemia.

The increase in renal blood flow, whatever its cause, could secondarily cause both an increased GFR and a decreased E_{PAH} (38), although a supernormal ERPF can be accompanied by normal GFR (64). There may also be a relationship between the supernormal GFR and ERPF of sickle cell nephropathy and the observed apparent increased reabsorption by the proximal renal tubule in these patients (see section entitled "Proximal Tubular Reabsorption") (65).

A number of possible mechanisms may be responsible for the decline in renal hemodynamics with age, sometimes ending in renal failure with, at necropsy, shrunken end-stage kidneys. It could well be that, over a number of years, continued loss of medullary circulation, interstitial fibrosis, and possibly superimposed pyelonephritis may lead to a progressive decline in GFR and advanced renal insufficiency in the patient with Hb-SS disease. Support for the idea of a reduction in medullary blood flow in sickle cell disease can be found in the microradioangiographic studies of Hb-SS kidneys (36) (Fig. 77-2). There was almost no perfusion of the vasa recta by the contrast medium in the kidneys obtained at autopsy. These findings suggest an almost complete absence of vasa recta in Hb-SS. As mentioned previously, supernormal hemodynamics and hyperfiltration have been proposed as causative mechanisms of glomerulosclerosis (66), which may lead to the chronic renal failure seen in sickle cell disease. Increased apoptosis, stimulated by ischemic and/or oxidative injury, may also contribute to progressive renal dysfunction (13–15).

Estimation of Renal Function

In sickle cell anemia, fractional creatinine excretion has been found to be increased (58); the use of inulin clearances may more accurately assess GFR. Serum creatinine is a poor marker of glomerular function in patients with sickle cell disease and did not predict the presence of subnormal GFR as assessed by Tm_{PAH} and inulin clearance or reduced ultrafiltration coefficient and abnormal glomerular selectivity seen in patients with Hb-SS (67,68). In older patients (30 to 60 years of age) with Hb-SS, GFR and ERPF diminish with age to normal or even depressed levels (69,70). As compared to younger people with sickle cell disease, patients older than 40 years of age have a higher plasma creatinine concentration and lower creatinine clearance (70), but these measures still likely underestimate the severity of renal dysfunction due to the increased fractional creatinine excretion.

Urinary Concentrating Capacity

An inability to achieve maximally concentrated urine has been the most consistent feature of sickle cell nephropathy. This inability has now been documented many times, in both the homozygous and the heterozygous states (20,51,71–74). The abnormality is unrelated to GFR and ERPF, since both as a rule are normal or even supernormal in sickle cell disease. There is, however, a definite relationship between urinary concentrating capacity and age. In Hb-SS patients older than 10 years, the maximal urinary concentration is reduced to about 400 mOsm/kg H_2O and does not decrease further with advancing age. In very young children with sickle cell anemia, a concentrating defect is present, but normal urinary concentrating ability can be restored by multiple transfusions of Hb-A erythrocytes (51,72). This capacity for improvement is progressively lost with age, and in patients older than 15 years, impaired urinary concentrating capacity is irreversible (Fig. 77-3). The renal concentrating capacity of the heterozygotes (Hb-AS) is affected too, but only later in life. The presence of co-existent α-thalassemia, which variably ameliorates manifestations of sickle cell disease in some patients, also attenuates the urinary concentrating defect seen in individuals with sickle cell trait, presumably by reducing red blood cell sickling (75).

Maximally concentrated urine is produced by extraction of water from collecting duct fluid as it passes through the hypertonic medullary interstitium in the presence of antidiuretic hormone (ADH). It does not increase further after exogenous ADH administration (73,76). Sickle cell anemia patients are not able to increase urinary osmolality despite sufficiently stimulated plasma vasopressin levels (76).

In the normal human kidney, approximately 85% of the nephrons have short loops of Henle restricted to the outer medullary zone. These nephrons may be largely responsible for achieving the interstitial osmolality of about 450 mOsm/kg H_2O that exists at the transition of the outer and inner medulla. The remaining 15% of human nephrons are juxtamedullary nephrons with long loops of Henle extending into the inner

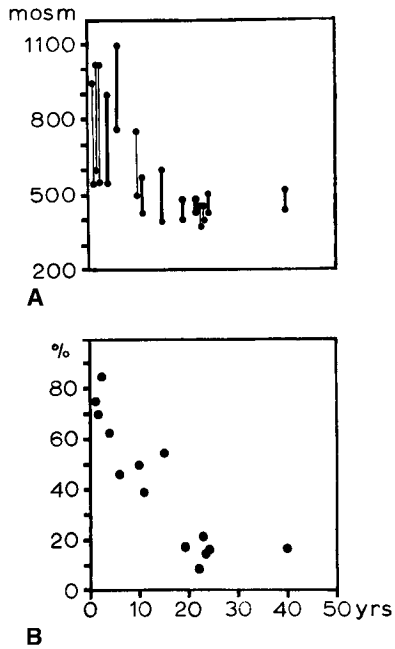

FIGURE 77-3. Relationship between age and the ability to reverse the defect in urinary concentration in patients with sickle cell disease by blood transfusions. **A:** The maximal urinary osmolality achieved prior to transfusion (**lower point of each vertical line**) and after multiple transfusions with normal blood (**upper point of each vertical line**) in 14 patients with sickle cell disease ranging from 2 to 40 years of age (8 patients, *thick lines,* from: Statius van Eps LW, et al. The influence of red blood cell transfusions on the hyposthenuria and renal hemodynamics of sickle cell anemia. *Clin Chim Acta* 1967;17:449; and 6 patients, *thin lines,* from: Keitel HG, et al. Hyposthenuria in sickle cell anemia: a reversible renal defect. *J Clin Invest* 1956;35:998.) **B:** The percentage increase in maximal urinary osmolality resulting from transfusion. Maximal urinary osmolality prior to transfusion is depressed at all ages; significant improvement after transfusion occurs only in children and adolescents. (From: Statius van Eps LW, et al. The influence of red blood cell transfusions on the hyposthenuria and renal hemodynamics of sickle cell anemia. *Clin Chim Acta* 1967;17:449, through courtesy of the editors.)

medullary zone and renal papillae. Together with the parallel hairpin vasa recta, these units are responsible for further increasing interstitial osmolality during antidiuresis in humans to about 1,200 mOsm/kg H_2O at the tip of the papillae. In experiments with rats (77), selectively removing the papillae destroys only nephrons originating in the juxtamedullary cortex. In such animal preparations, a severe loss of urinary concentrating capacity during fluid deprivation has been observed. Thus, juxtamedullary nephrons are necessary for achieving a maximal urine osmolality (78).

These pathophysiologic mechanisms help to clarify the abnormal findings in sickle cell nephropathy. On the basis of these mechanisms, the concentrating defect in sickle cell disease can be explained as a consequence of the sickling process per se and the resultant ischemic changes in the medullary microcirculation (36). Hb-SS erythrocytes form sickle erythrocytes within seconds when placed in surroundings as hyperosmotic as the renal medulla during hydropenia (17) and has been observed in the transgenic sickle cell mouse model (12). Sickling and/or adherence of red blood cells could interfere with the normal circulation through the vasa recta, preventing both the active and passive accumulation of solute in the papillae necessary to achieve maximally concentrated urine. Intermittent vasoocclusion could also produce local hypoxia, oxidative/reperfusion injury, apoptosis, and eventually infarction of the renal papillae.

The vasa recta also provide the nutrient blood supply to the inner medulla and papillae, and it is at these sites that the most conspicuous lesions of established sickle cell nephropathy occur (e.g., focal scarring and patchy interstitial fibrosis). These lesions develop over many years, and it is probable that collateral capillary circulations are formed (Fig. 77-2). These collaterals probably lack the highly specialized spatial configuration—designated *zonation* (79)—of the normal vasa recta that run parallel to the long loops of Henle in the inner renal medulla. The loss of zonation and loss of countercurrent exchange function of the vasa recta, even with adequate nutrient blood flow through the medulla, could result in defective "trapping" of solute, so that the Hb-SS kidney would be unable to maintain a normally high papillary osmolality.

Because it was demonstrated that fractional urea excretion fell markedly after indomethacin administration in sickle cell anemia patients and resulted in a rise in serum urea (65), the effect of indomethacin on renal concentrating capacity was also studied. Indomethacin normally promotes sodium reabsorption in the medulla with a consequent rise in papillary sodium and chloride concentration. Whereas indomethacin in normal patients improves renal concentrating capacity, it did not increase urinary osmolality in water-deprived Hb-SS patients (64,71) (Fig. 77-4A). This suggests a defect in the capacity to trap solute in the medulla in sickle cell anemia. Also, the ability to concentrate urine was not increased in Hb-SS children placed on a high-protein diet (80), as observed in normal subjects.

Loss of the ability to concentrate urine to more than 450 mOsm/kg H_2O is not of clinical importance under most circumstances because individuals usually drink more than the minimum amount of water necessary to excrete the average solute load of 600 to 800 mOsm/day. The obligatory urine volume to excrete this solute load in a patient with Hb-SS nephropathy amounts to about 2,000 mL/day, which is not an unusual urine volume for the normal person. Fluid deprivation or excessive fluid loss, however, will lead more rapidly to clinical dehydration in the patient with Hb-SS nephropathy than in the normal person. Thus, in hot, dry climates and in clinical syndromes attended by vomiting and diarrhea, the patient with sickle cell disease is vulnerable to more rapid dehydration than is the normal person. Enuresis occurs at a higher frequency in sickle cell disease patients than in normal children and may be associated with increased urine volumes (81). However, since some children respond to intranasal desmopressin (DDAVP) (82), other mechanisms may be implicated, and the etiology is likely multifactorial, as in children without sickle cell disease.

Generation of Negative Solute-Free Water

The capacity to generate negative solute-free water (Tc_{H2O}) was studied in patients with sickle cell disease using different protocols. After mannitol loading, conflicting results have been obtained. Although Whitten and Younes find normal Tc_{H2O} levels in Hb-SS children (83), Levitt et al. describe two patients with a Tc_{H2O} of 3.2 mL/minute/100 mL glomerular filtrate (73). Hatch et al. find a mean Tc_{H2O} of 4.2 ± 0.9 SD mL/minute/100 mL glomerular filtrate in 11 Hb-SS patients compared to a mean Tc_{H2O} of 5.7 ± 1.2 SD mL/minute/100 mL glomerular filtrate in 7 control subjects (84). These results suggest that Tc_{H2O} after mannitol loading is lower in sickle cell anemia.

After saline infusion, Hatch et al. (84) and Forrester and Alleyne (85) agree that Tc_{H2O} was impaired in Hb-SS patients.

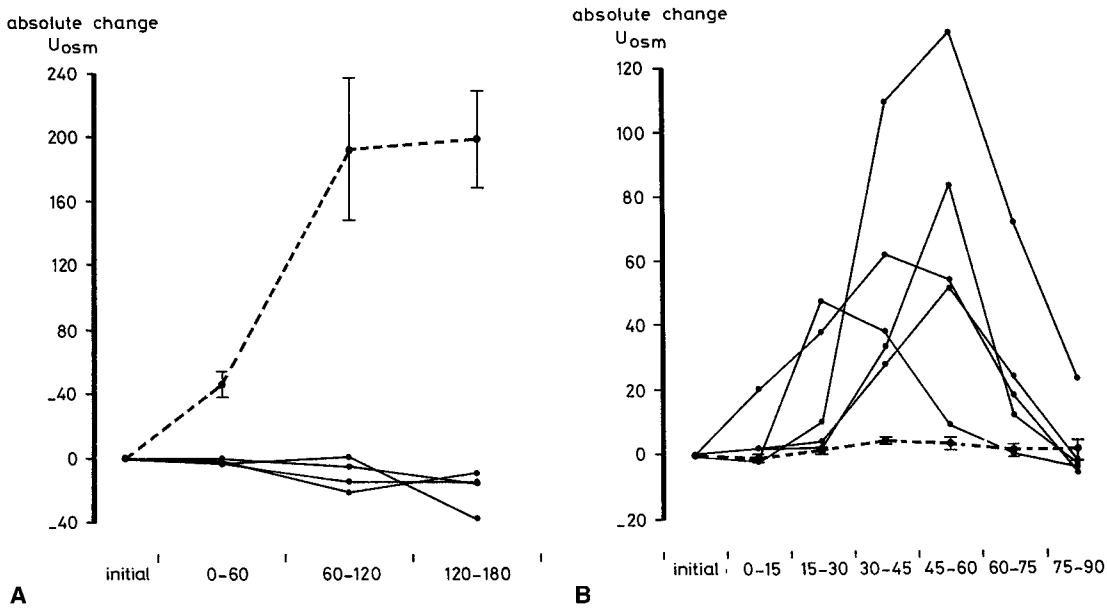

FIGURE 77-4. The effect of indomethacin (75 mg as a suppository) in the water-depleted state (**A**) and the effect of indomethacin (0.25 mg/kg body weight i.v.) in the water-loaded state (**B**). The *broken lines* represent the mean ± standard error of the mean in control subjects, and the *continuous lines* represent the individual data in patients with sickle cell anemia. (From: De Jong PE, et al. The influence of indomethacin on renal concentrating and diluting capacity in sickle cell nephropathy. *Clin Sci* 1982;63:53, through courtesy of the editors.)

From these studies it was concluded that sickle cell anemia patients have a defect in the water impermeable medullary loops of Henle that transports solute. The normal solute-free water clearance in Hb-SS patients argues against such an impairment in sodium chloride reabsorption from the ascending limb of Henle's loop. However, a normal solute-free water clearance only indicates a normal transport mechanism in the thick portion of the ascending limb in the outer medulla, whereas a normal Tc_{H2O} is depending on adequate function of the part of Henle's loop localized in the inner medulla. Sickle cell anemia patients are able to increase urinary osmolality generally to 450 mOsm/kg H_2O or higher, to the level that can be generated in the outer medulla. As shown in Figure 77-2, the capillary plexus surrounding short loops of Henle in the outer zone does not necessarily penetrate into the inner zone.

Urinary Diluting Capacity

Patients with sickle cell anemia are capable of diluting their urine normally (72,74,84). Under conditions of water diuresis, the fall in urinary osmolality has been found to be the same in control subjects and in patients with Hb-SS, and the percentage of filtered water excreted (C_{H2O}/GFR) was also identical in the two groups. Therefore, the capacity to reabsorb solute in the thick portion of the medullary ascending limb of Henle's loop apparently is intact in sickle cell anemia.

This combination of a defect in renal concentrating capacity with a normal diluting capacity is quite characteristic for sickle cell anemia. However, after indomethacin administration, the Hb-SS patient was not able to excrete water normally. There was a greater fall in C_{H2O}/GFR in sickle cell subjects compared to controls, and urinary osmolality rose from 42 to 125 mOsm/kg H_2O (69,71) (Fig. 77-4B). This result therefore suggests that renal prostaglandins are of importance in the normal diluting capacity in sickle cell anemia.

Urinary Acidification

Although systemic acidosis is generally not a feature of sickle cell disease in the absence of advanced renal failure, patients with Hb-SS or Hb-SC demonstrate an incomplete form of renal tubular acidosis (52,86–88). In response to a short-duration acid load, 79% of the patients with Hb-SS studied by Ho Ping Kong and Alleyne (86), 100% studied by Goossens et al. (87), and 29% of those studied by Oster et al. (88) were unable to decrease urine pH below 5.3, whereas normal subjects will achieve a urinary pH of 5.0 or lower. Titratable acid and total hydrogen ion excretion are lower in patients with Hb-SS or Hb-SC, but ammonia excretion is appropriate for the coexisting urine pH in most cases. The increased ammonia excretion induced by acid loading is reduced by indomethacin (52). This suggests that the assumed enhanced prostaglandin synthesis in sickle cell disease (89) may be important in maintaining a normal ammoniagenesis in sickle cell disease. When a maximal acidifying stimulus is employed, such as infusion of sodium sulfate, patients with Hb-SS may lower urine pH and increase net acid excretion to the same degree as normal subjects. Thus, the distal tubule of Hb-SS patients apparently requires a greater-than-normal stimulus to generate a normal urine-to-blood hydrogen ion gradient. In patients with sickle cell trait, renal acidification has been found to be normal (90).

The acidification defect has been classified as distal rather than proximal (87) because there is no associated wasting of bicarbonate and because it is characterized by failure to achieve a normal minimal urinary pH during acid loading. Because none of the patients studied were acidemic or hyperchloremic before acid loading and no generalized proximal tubular reabsorptive defect was observed, the acidification defect was consistent with the incomplete syndrome of distal renal tubular acidosis (91). It has been suggested that the alterations in the microcirculation of the papillae may result in an inability

of collecting ducts to maintain normally steep hydrogen ion gradients.

This very subtle defect in renal acidification generally does not cause systemic metabolic acidosis, which would increase sickling. Investigators in the West Indies (92), Nigeria (93), and the United States (88) find no evidence of metabolic acidosis in the absence of a sickle cell crisis, but find changes consistent with a mild chronic respiratory alkalosis.

Potassium Metabolism

In addition to the defect in hydrogen ion excretion, potassium excretion is also impaired in sickle cell patients (94–96). After administration of potassium chloride, sodium sulfate, or furosemide, potassium excretion was found to be subnormal in patients with sickle cell disease. Plasma renin activity and plasma aldosterone concentration in these patients were normal, both with normovolemia and after volume contraction. The defect could therefore not be explained by hypoaldosteronism. Despite the impairment in renal potassium excretion, hyperkalemia did not develop during acute potassium chloride loading. Administration of angiotensin-converting enzyme (ACE) inhibitors, potassium-sparing diuretics, and β-blockers has been associated with elevated plasma potassium concentration (97). However, significant alteration in plasma potassium concentrations has not been reported with the use of ACE inhibitor therapy for proteinuria in patients with sickle cell disease (40).

As with the defect in water and hydrogen ion excretion, the disturbance in potassium excretion could be due to an abnormality in the collecting duct secondary to the ischemic injury; potassium excretion is known to reflect primarily secretion in the distal nephron. Potassium excretion in sickle cell trait is normal (98).

A hyperkalemic, hyperchloremic metabolic acidosis in sickle cell nephropathy has been observed (94). Three patients had Hb-SS, two had Hb-AS, and one had Hb-SC; all had impaired renal potassium excretion. Five of these six patients had a moderate-to-severe decrease of GFR and all patients had spontaneous metabolic acidosis. Selective aldosterone deficiency was recognized in three patients, two with normal and one with low plasma renin activity.

Other reports describe hyporeninemic hypoaldosteronism in patients with sickle cell disease (96,99). An impaired renin-secreting apparatus may result in impaired function of the adrenal glomerulosa cells, diminished aldosterone secretion, and an impaired ability to excrete potassium loads. In these cases, the hyperkalemia responded favorably to treatment with mineralocorticosteroids.

Proximal Tubular Secretion

Although severe disturbances in medullary transport occur in patients with sickle cell anemia, proximal tubular activity, both secretory and reabsorptive, appears to be supernormal. The tubular transport maximum of *para*-aminohippurate is elevated in sickle cell anemia, particularly in children (55). Other evidence of an increased proximal tubular secretory capacity has been obtained from studies regarding uric acid excretion. Notwithstanding the increased red cell turnover with consequent uric acid overproduction, most patients with sickle cell anemia are normouricemic. Urate clearance was found to be greater in these patients (100,101). This increased urate clearance was accounted for by increased pyrazinamide-suppressible urate clearance indicating an increased secretion of urate (100,102). Hyperuricemia in Hb-SS patients also can occur with decreased urate clearance, but these patients often have decreased *para*-aminohippurate clearance (101), proteinuria (103), or decreased inulin clearance (104). Urate clearance decreases with age in these patients and the incidence of hyperuricemia increases as renal function deteriorates (101). Attacks of gout can occur (104–106) and sometimes be clinically difficult to differentiate from an acute vasoocclusive crisis or from other bone and joint manifestations of sickle cell disease (i.e., sickle cell arthropathy) (107).

As previously mentioned, the tubular secretion of creatinine has been found to be elevated, with a 20% to 29% rise in fractional creatinine excretion in Hb-SS patients compared to control subjects (58,64). However, when an intravenous load of creatinine was given to 16 adults with sickle cell disease, most without proteinuria, creatinine clearance did not increase as expected and as compared to controls; GFR remained the same in both groups (108). The authors suggested that a reduced tubular capacity for secretion of creatinine in response to a creatinine load may be an early indicator of sickle cell renal dysfunction. Abnormal tubular function may thus limit the utility of creatinine clearance measurements in sickle cell disease to estimate GFR.

Proximal Tubular Reabsorption

Maximum tubular reabsorption of phosphate per liter of glomerular filtrate (TmP/GFR) is also increased in sickle cell anemia. Consequent to this higher phosphate reabsorption, serum phosphate is elevated in some subjects (65,109). It has been concluded that the high phosphate reabsorption reflects an increased reabsorptive activity of the proximal tubule (65). In contrast, children with sickle cell anemia have lower serum phosphate level than normal children (110), and it is associated with significantly lower renal tubular resorption of phosphate. This may be due to increased parathyroid levels reported in children with sickle cell disease (111) or may simply indicate the absence of sickle cell renal injury early in life.

Because sodium reabsorption in the proximal tubule parallels phosphate reabsorption, this increased TmP/GFR suggests that increased sodium reabsorption may also occur in the proximal tubule. Indeed some studies reported an increased plasma volume in the noncrisis steady-state Hb-SS patients (112–114). Conversely, this increased proximal tubular sodium reabsorption could be a secondary mechanism to correct for defects in salt reabsorption in a more distal part of the nephron. One might expect these patients to develop a volume-depleted state as a consequence of the defects in medullary water and sodium conservation. However, such distal compensatory mechanisms would not be expected to increase plasma volume.

An increased tubular uptake of β_2-microglobulin has also been described in sickle cell anemia (115). A positive correlation between the reabsorption of phosphate and β_2-microglobulin was reported, providing further evidence of an increased activity of the proximal tubule in sickle cell anemia. Aparicio et al., however, reported a good correlation between estimates of GFR comparing ^{51}Cr-EDTA, creatinine clearance, and β_2-microglobulin determinations in sickle cell patients older than 40 years of age (116). Zinc excretion has also been found to be abnormal in sickle cell anemia. Whereas the excretion of zinc in control subjects is lower than the filtered load of zinc, in Hb-SS patients zinc excretion exceeds the filtered load (117).

Therefore, Hb-SS patients have a defect in renal medullary functions with a tendency to lose water and sodium, whereas ERPF, GFR, and proximal tubular activity are increased. In

general, the ultimate result of this compensation will be normal homeostasis of fluid and electrolytes.

RENAL HORMONES

Erythropoietin

Data on plasma erythropoietin (EPO) levels in sickle cell anemia are conflicting. Erythropoietin levels are strongly correlated with red cell mass in sickle cell disease patients (118). Increased values have been reported, both in asymptomatic patients and during infectious episodes (119). However, when comparing plasma EPO concentrations in sickle cell anemia with those in other causes of anemia, Hb-SS patients have similar or decreased (120) values. EPO titers in patients with pure red cell aplasia, for instance, were 10-fold higher than those in patients with Hb-SS.

The observations of Sherwood et al. (121) that patients with sickle cell anemia produce less immunoreactive EPO at a given hemoglobin concentration than do patients with nonsickle cell anemias agree with previous results using biologic assay (122). Pediatric patients with sickle cell anemia have significantly higher EPO levels than do adults.

Morgan and Serjeant (33), in a study of patients older than 40 years, observed a fall in the Hb concentration among these individuals. They suggest that the lower Hb concentration is due to a reduced EPO production. In sickle cell disease patients with renal failure, supplementation with erythropoietin using doses of 100 to 150 U three times a week (123–125), up to 30,000 U per week (126), result in increased reticulocyte counts, but may not increase the absolute hemoglobin value. However, renal transplantation corrected the erythropoietin-resistant anemia in 5 cases (126,127). The improvement in erythropoietin after transplantation may reflect decreased uremia or other toxins, or reduced inflammation that pre-transplantation might have suppressed erythropoiesis or enhanced hemolysis (127). Hydroxyurea therapy results in an increase in erythropoietin levels 5 to 10 days after starting therapy (128).

The potential mechanisms for the low EPO levels in sickle cell disease include interference with the renal synthesis of EPO, as a result of renal damage by the sickling process, and the displacement to the right of the oxygen equilibrium curve. Circulating red blood cell progenitors from patients with sickle cell disease have increased expression of erythropoietin receptors, which correlated with increased stimulated and autocrine erythroid colony development (129). This may be a compensatory mechanism, in the absence of renal disease, for relatively lower erythropoietin levels in sickle cell disease.

Renin–Angiotensin–Aldosterone System

Plasma renin activity and aldosterone concentration in general are found to be normal in patients with Hb-SS disease during steady-state conditions (58,95). Plasma renin and aldosterone levels in 101 patients with sickle cell disease were comparable to control patients, even though they had a lower mean arterial blood pressure (130). After volume depletion, normal or increased values were found (131), and Hatch et al. (113) showed that both supine and upright plasma renin activities for different sodium intakes always were higher in Hb-SS patients than in control subjects. Upregulation of renin-1 has been demonstrated in the kidneys of transgenic sickle cell mice exposed to hypoxia (16). As mentioned previously, some patients with hyporeninemic hypoaldosteronism and hyperkalemia have been described (96,99). Interestingly, high plasma renin activity has been described in a patient with intermittent hypertension occurring during a painful crisis. When the crisis had subsided, blood pressure normalized again (132). This is an unusual finding in sickle cell anemia, however, as hypertension seldom occurs in Hb-SS patients, even during crisis.

Renal Prostaglandins

Prostaglandin production has been documented in the interstitial medullary cells and collecting duct cells in the kidney (133) and is promoted by various vasoconstrictor stimuli (134). The interstitial cells of the medulla in patients with sickle cell anemia contain aggregates of granular electron-dense material (44), likely representative of prostaglandin production. It might, therefore, be suspected that the ischemic and/or oxidative insult to the inner medulla in sickle cell anemia induces synthesis of vasodilator prostaglandins. The role of renal prostaglandins in sickle cell anemia has been studied in several ways: by indirect methods using indomethacin as a prostaglandin synthesis inhibitor, by direct measurement of urinary prostaglandin E_2 and $F_{2\alpha}$ (PGE_2 and $PGF_{2\alpha}$) excretion, and by assessment of gene regulation in the transgenic sickle cell mouse model.

As already discussed, indomethacin administration does not change GFR and ERPF in control subjects, but a significant fall in these functions was found after indomethacin administration to Hb-SS patients (58,64). This suggests that prostaglandins are of importance in maintaining the supernormal or normal GFR and ERPF in sickle cell anemia and may be relevant with respect to the hyperfiltration and rise in GFR observed in young patients with sickle cell anemia. The rise in renal blood flow and glomerular filtration could also explain the increased proximal tubular activity in these patients.

In normal subjects, indomethacin causes sodium and water retention and a rise in body weight. In Hb-SS patients, a similar sodium retention was observed after indomethacin administration. This sodium retention, however, was not accompanied by water retention or an increase in body weight. Rather, serum osmolality increased in these patients (55).

Indomethacin administration to water-deprived normal subjects caused a rise in urinary osmolality of 836 to 1,027 mOsm/kg H_2O, whereas the defect in urinary concentration in sickle cell anemia was not improved at all after indomethacin (71) (Fig. 77-4). This result supports a diminished solute gradient in the inner medulla of patients with Hb-SS, which is not modulated by prostaglandins. During a water load, however, a rise in urinary osmolality occurred after indomethacin administration in patients with sickle cell disease, but not in control subjects. It is thus concluded that normal diluting capacity in patients with sickle cell anemia is dependent on adequate renal prostaglandin synthesis (64,71).

Both in the dehydrated and in the water-loaded state, PGE_2 excretion has been found to be normal in patients with sickle cell disease. $PGF_{2\alpha}$ excretion, however, is decreased, therefore, the $PGE_2/PGF_{2\alpha}$ ratio is higher than in normal persons (89). Because PGE_2 and $PGF_{2\alpha}$ have different and sometimes opposite effects on renal hemodynamics, renin release, and sodium and water excretion (135,136), it is possible that an abnormal balance between these two prostaglandins may contribute to some of the characteristics of sickle cell nephropathy. For example, the relative excess of the vasodilating PGE_2 could explain the rise in renal blood flow and GFR, particularly in the juxtamedullary nephrons.

In the transgenic sickle cell mouse model, cytochrome P450 4a14 is upregulated in renal tissue (16). This enzyme catalyzes arachidonic acid to 20-hydroxyicosatraaenic acid (20-HETE), which regulates renal vascular tone, tubular resorption, arterial pressure, and natriuresis (16).

Atrial Natriuretic Peptide

The renal effects of atrial natriuretic peptide (ANP) in sickle cell disease have been studied recently. In normal subjects, ANP exerts its natriuretic effects in several nephron segments dependent on prevailing ANP levels. Infusion of a low-dose ANP induced natriuresis in normal individuals but not in matched patients with sickle cell disease (137). This observation suggests that low-dose ANP inhibits sodium reabsorption in the long loops of Henle, possibly by increasing medullary blood flow and renal interstitial pressure. Natriuresis during supraphysiologic plasma levels of ANP appears to be multifactorial, by an increase in GFR and a decrease in both proximal and distal tubular sodium reabsorption. Infusion of high-dose ANP in patients with sickle cell disease and normal subjects induced a similar degree of natriuresis in both groups (137).

Hypertension and Sickle Cell Disease

The incidence of hypertension in the black population older than 18 years of age in the United States is 32%. This percentage sharply contrasts with that in sickle cell disease. Reports from both the Caribbean (138,139), the United States (113,140), and Europe (130) show that hypertension is present in only 2% to 6% of the Hb-SS patients (Fig. 77-5) and in 15% of patients older than 55 years of age. The explanation for these findings is unclear. Renal salt losing has been suggested (99), but these data are not convincing. Matustik et al. (131) and Hatch et al. (113) show that Hb-SS patients are able to conserve sodium adequately on a severely restricted sodium diet. Moreover, the demonstration of an increased, instead of a lowered, plasma volume (112–114) does not support the hypothesis that the lower blood pressures in patients with sickle cell anemia are related to a volume loss. Altered systemic vasoreactivity has been postulated, but there has been limited study. A decreased forearm vascular resistance that did not increase with cold-induced, sympathetic-mediated stimulation and an impaired pressor response to angiotensin II suggest that an altered vascular reactivity may protect these patients from hypertension (113).

Blood Coagulation in Sickle Cell Disease

Statius van Eps and Leeksma (141) have suggested the occurrence of intravascular coagulation in sickle cell disease because of decreased platelet counts, increased platelet turnover, hyporeactive "exhausted" platelets, elevated fibrinopeptide A, and fragment D-dimer levels, all observed in patients with vasoocclusive crisis. The potential role of thrombotic occlusion in sickle cell disease was summarized by Francis (142) and has been postulated to play a role in the renal circulation. Procoagulant changes have been observed associated with vasoocclusive crises in sickle cell disease (143).

Wolters et al. studied the effect of low-dose acenocoumarol in sickle cell disease (144). The enhanced thrombin formation was corrected and normalized, and the increased plasma levels of prothrombin fragments 1 and 2, reflecting enhanced endogenous thrombin generation, became normal (144). More recently, D-dimer levels were reduced with low-dose warfarin therapy (145). However oral anticoagulation has not been demonstrated to change the course of acute or chronic clinical events, and did not reduce markers of endothelial activation (146), which is thought to play a central role in the pathophysiology of sickle cell disease.

CLINICAL MANIFESTATIONS

Hematuria

In 1948, Abel and Brown were the first to discover a relationship between sickle cell disease and hematuria (147). They described a young black soldier who underwent nephrectomy because of severe and persistent unilateral hematuria. A renal neoplasm was suspected, but histopathologic examination of the excised kidney demonstrated only sickled red blood in the medullary vessels.

Hematuria is a very dramatic renal manifestation of sickle cell nephropathy. Gross hematuria occurs in patients who have sickle cell trait (Hb-AS) and,Hb-SC disease, as well as in those who have sickle cell anemia (Hb-SS) (82,148–151). The majority of cases have been reported in patients with Hb-AS and Hb-SC disease. Gross hematuria in affected patients may occur at any age, including in young children, and appears to be more common in males than in females.

The pathologic abnormalities causing hematuria have not yet been elucidated. Kimmelstiel (152) describes the changes as temporary capillary stasis after spasm. The consequence is plugging of capillaries by sickle cells, resulting in vessel wall injury and, rarely, in true capillary thrombi with ischemia and necrosis. Mostofi et al. (29) studied 21 kidneys from patients

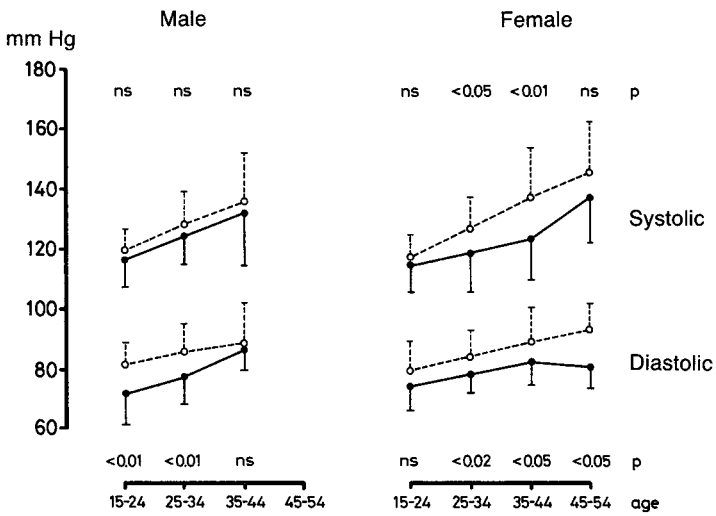

FIGURE 77-5. Mean ± standard deviation of systolic and diastolic blood pressure in control subjects (*dotted lines*) and patients with sickle cell anemia (*closed lines*) who are age and sex-matched. (Derived from: De Jong PE, et al. Blood pressure in sickle cell disease. *Arch Intern Med* 1982;142:1239; and reproduced from: De Jong PE, Statius van Eps LW. Sickle cell nephropathy. New insights into its pathophysiology. [Editorial review]. *Kidney Int* 1985;27:711, through courtesy of the editors.)

with sickle cell disease that were removed because of massive blood loss and the possibility of renal neoplasm. The absence of significant gross alterations in most of these kidneys emphasizes the fact that the lesions are inconspicuous and may be easily missed. The most striking change consisted of severe stasis in peritubular capillaries of both the cortex and the medulla; the changes were most marked in the medulla. Extravasation of blood was observed, mainly into the collecting tubules. Perillie and Epstein show that Hb-S erythrocytes transversing the hyperosmotic inner renal medulla undergo instant sickling as a result of an increase in intracellular hemoglobin concentration (17). Moreover, the renal medullary acidic and hypoxic environment promotes sickling and adherence of red blood cells in the vasa recta. Vasoocclusion and increased blood viscosity may result in formation of microthrombi, ischemia, oxidative/reperfusion injury, and/or necrosis, which could cause structural changes leading to hematuria. Interestingly, when hematuria is unilateral, which is generally the case (only about 10% of cases are bilateral), the left kidney appears to be involved four times more often than the right kidney. This may be due to the increased pressure in the left renal vein due to its length and its course between the aorta and superior mesenteric artery (82).

The presence of gross hematuria in the patient with sickle cell disease can result in misleading findings on the intravenous urography. In almost one-half of all such patients with gross hematuria, blood clots produce filling defects in the renal pelvis that may be confused with neoplasm, calculus, or hemangioma. Such findings have led to unnecessary nephrectomy. Computed tomography can generally exclude the presence of a renal neoplasm (153).

Although hematuria can be massive and life-threatening, conservative measures are the treatment of choice. These include bed rest, urinary alkalinization, and the maintenance of a high rate of urine flow. Intravenous triglycyl vasopressin was successful in two sickle cell cases with persistent hematuria (152,154).

Immergut and Stevenson showed the favorable therapeutic effect of epsilon aminocaproic acid (EACA) in the control of hematuria associated with hemoglobinopathies (155). Complete inhibition of fibrinolytic activity can normally be expected with 8 g of EACA daily. Urinary levels of EACA are 50 to 100 times those of plasma. With repeated oral dosage there is a gradual sustained renal excretion of the drug so that adequate urinary levels are maintained. The results of EACA in the treatment of hematuria of sickle cell nephropathy have been impressive, although the therapeutic regimens have varied (141,155–157). Thrombotic complications have been reported to occur in patients receiving large doses of EACA, usually in excess of 12 g per day. Control of fibrinolytic hemorrhage often can be obtained, however, with doses as low as 2 or 3 g per day (157). Therefore, it is advisable to administer EACA on a short-term basis in the lowest dosage required to inhibit urinary fibrinolytic activity.

Surgical intervention (i.e., nephrectomy) should be considered only in the presence of life-threatening hemorrhage that does not improve with conservative therapy. If the bleeding can be localized in a distinct part of the kidney, one should consider embolization.

Other causes of hematuria should be considered, including mild bleeding disorders (e.g., von Willebrand's disease), other forms of kidney disease and renal medullary carcinoma.

Autosomal-dominant polycystic kidney disease (ADPKD) in blacks has been studied and its occurrence together with sickle cell hemoglobin analyzed (158). Sickle cell hemoglobin occurred more often than expected in ADPKD. The disease was more severe with earlier onset of end-stage renal disease in blacks compared with ADPKD in whites and in blacks with sickle cell trait when compared with blacks without the trait. Both conditions can cause hematuria.

Urinary Tract Infections

In a review of 321 children with sickle cell disease, 7% have had a urinary tract infections, 60% of which had an associated febrile illness (150). The incidence of asymptomatic bacteriuria during pregnancy and the puerperium appears to be distinctively higher in women with sickle cell disease or sickle cell trait than in nonpregnant women or women without sickle cell disease or trait; a twofold increase has been observed (159). The rate of pyelonephritis is approximately 1%, similar to that report in non-sickle cell pregnant populations (160). An increased incidence of "pyelonephritis" found at autopsy in Hb-AS patients (161), though the pathologic changes due to Hb-SS nephropathy (medullary ischemia and fibrosis), however, may have been easily confused with those of pyelonephritis.

Pyelonephritis or urosepsis may precipitate a crisis, as may other infections. Most episodes of Gram-negative sepsis are secondary to urinary tract infections in sickle cell patients (162). For these reasons, sickle cell patients with a painful crisis should have a search for foci of infection, including one in the urinary tract.

Papillary Necrosis and Calicectasis

Renal papillary necrosis and calicectasis are a frequent occurrence in sickle cell disease, in both homozygotes and heterozygotes. Renal papillary necrosis as a complication of sickle cell nephropathy has an incidence ranging from 15% to 36% (31). Harrow et al. (32) were the first to stress the importance of this complication. As in sickle cell nephropathy, the distinctive abnormalities of the renal medulla and papillae are obliteration of vasa recta and medullary necrosis and fibrosis; papillary necrosis is a logical consequence of these processes.

Gross, painless hematuria (151) is a common symptom reported. Renal colic caused by the passage of blood clots or ruptured particles of necrotic papillae is less frequent.

Intravenous pyelography is the method of choice to diagnose papillary necrosis (32,151). The most common finding is a medullary type of partial papillary necrosis, appearing as cavitation within one or more of the renal papillae (SS). Multiphasic helical CT may permit early detection of medullary and papillary necrosis and visualization of the entire kidney, facilitating the identification of conditions (153).

Treatment is supportive and similar to the management of hematuria. Ureteral obstruction by thrombus or necrotic material should be relieved by stenting if required (151).

Renal papillary necrosis has not been associated with an increased risk of renal failure (25).

Microalbuminuria, Proteinuria, and the Nephrotic Syndrome

As in other disease states, microalbuminuria may be indicative of early glomerular injury and renal dysfunction. Although most often attributed to hyperfiltration, Guasch et al. suggest that there may be an early permselectivity pore defect in the glomerular capillaries (45,68). As assessed by urine albumin/creatinine ratio (U Alb/Cr), the prevalence of microalbuminuria increases with age and is uncommon before the age of 7, but may be present in up to 20% to 40% of children between the ages of 10 and 18 (163,164). The presence and

amount of albuminuria correlated with age and hemoglobin levels, but not consistently with other clinical manifestations of sickle cell disease, such as pain or other acute sickling events (164). The prevalence of microalbuminuria in adolescents and young adults with sickle cell disease ranges from 9% to 40%, depending on the type of sickle cell disease (165). Lima et al. estimated urinary albumin excretion in 72 adult patients with sickle cell disease using a spot U Alb/Cr, however, and found the results highly variable and inconsistently correlated with a 12-hour urine sample to quantitate albumin excretion (166). Furthermore, the spot U Alb/Cr did not demonstrate the reduction in albumin exertion achieved by ACE inhibitor therapy, which was detected using 12-hour collections. They suggested that the U Alb/Cr be used as a screening test, and if positive, should lead to a 12-hour collection for an accurate determination of microalbuminuria (166).

Reduction in microalbuminuria with ACE inhibitor therapy has been demonstrated. Aoki et al. treated 8 adult sickle cell patients with enalapril, which over 120 days normalized or markedly reduced albuminuria without changes in sodium, potassium, or lithium excretion or in mean arterial blood pressure (167). Two years after stopping the enalapril, 4 of 6 patients still had normal albumin excretion. A placebo-controlled randomized trial of captopril in 22 adult sickle cell patients with Hb-SS demonstrated a mean 37% reduction in albumin excretion with captopril as compared to placebo after 6 months of therapy (168). It was associated with a 5 mm Hg drop in diastolic blood pressure in the treatment group. To date, there are no results from long-term trials to demonstrate whether ACE inhibitor therapy reduces the prevalence or progression of chronic renal disease in sickle cell disease patients.

More overt proteinuria is a frequent finding in sickle cell disease, occurring in about 30% of patients when observed over a prolonged period. Nephrotic range proteinuria has been reported in children and adults with sickle cell disease (169,170), but its prevalence has not be well studied. Sklar et al. reviewed the records of 386 patients with sickle cell anemia (171). Seventy-eight patients (20.4%) had proteinuria and 17 patients (4.6%) had renal insufficiency. Both renal insufficiency and proteinuria increased with age, reaching rates of 33% and 56%, respectively, in patients 40 years of age and older. In some series, proteinuria occurs in 40% of patients with sickle nephropathy (172) and is associated with progression to chronic renal failure and early mortality (25,40,42,172).

Falk et al. investigated 381 patients with sickle cell disease for proteinuria and renal insufficiency (40). Twenty-six patients (7%) had serum creatinine concentrations greater than the normal range and 101 (26%) had proteinuria of at least 1+. Forty-four patients had a complete 24-hour urine collection. In these patients protein excretion ranged from 28 mg per/24 hours to 10.8 g per/24 hours, with a mean of 1.7 g per/24 hours and SD of ± 2.4 g. Twelve patients excreted more than 2.5 g of protein per 24 hours, associated with other features of the nephrotic syndrome. In the 10 biopsied patients with proteinuria, administration of an (ACE) inhibitor, enalapril, caused the 24-hour urinary protein excretion to decrease by 57% (range 23% to 79%) below the baseline value; it increased to 25% below baseline after discontinuation of enalapril. There was no significant change in GFR, ERPF, or filtration fraction, and there was no decrease in arterial pressure. Although there are no trials demonstrating long-term benefits of ACE-inhibitor therapy in sickle cell disease, use of this therapy is generally recommended for patients with proteinuria.

Other causes of proteinuria and nephrotic syndrome should be considered, including primary and secondary renal diseases before one assumes sickle cell nephropathy is present. Renal vein thrombosis has been diagnosed in some patients, and because sickle cell disease may predispose to thrombosis, this complication should be considered if sudden massive proteinuria develops.

Acute Renal Failure

Acute renal failure in association with sickle cell disease has rarely been described. Some reports describe a reversible acute oligoanuric renal failure in the setting of sickle cell crisis (173,174). In both of these studies rhabdomyolysis was suggested as the cause of this acute renal failure. In a study by Sklar et al. of 12 sickle cell anemia patients with acute renal failure, volume depletion in the setting of sickle cell crisis was the most common cause (175). Of the 12 patients, 10 survived and subsequently had recovery of renal function.

Hassell et al. describe a syndrome of acute multiorgan failure in 14 sickle cell patients (10 Hb-SS, 4 Hb-SC) occurring during a painful event that was unusually severe for the patients (176). There was acute failure of at least two of three organs: lung, liver, or kidney. Acute renal insufficiency developed during 13 episodes, with a rapid, reversible elevation of serum creatinine concentration greater than 2.0 mg/dL during a period of 24 to 36 hours. Acute abnormalities of liver function and acute pulmonary infiltrates were observed. All but one patient recovered after treatment with multiple blood transfusions.

Renal Replacement Therapy

Because renal function may deteriorate with age in Hb-SS patients, either as a result of the parenchymal damage due to sickling or because of primary glomerular disease, some patients with sickle cell disease will become candidates for chronic hemodialysis. Renal dysfunction is one of the most common causes of death in older patients with sickle cell anemia (35,177). Powars et al. performed a 25-year demographic and clinical cohort study to determine the incidence, clinical cause, and risk factors associated with the onset of chronic renal failure in 725 sickle cell anemia patients and 209 with sickle C disease (25). Of the sickle cell anemia group, 4.2% developed chronic renal failure compared with 2.4% of those with sickle C disease. The median ages at disease onset were 23.1 years for the sickle cell anemia group and 49.9 years for the sickle C disease group. Survival time for sickle cell anemia patients after diagnosis of chronic renal failure, despite dialysis, was 4 years, and the median age at the time of death was 27 years. Pathologic studies showed characteristic lesions of glomerular "dropout" and glomerulosclerosis. An interesting finding was that the risk for renal failure was increased in patients who had inherited the Central African Republic β gene haplotype.

Sickle cell anemia patients are presenting with increasing frequency for renal replacement therapy. Both hemodialysis (178–180) and transplantation (180–183) can be performed successfully.

Nissenson and Port (179) report the course of 77 patients with sickle cell disease and end-stage renal failure. There is a marked male predominance, and renal failure appears most commonly in the third and fourth decades. The 2-year survival among those in this group receiving renal replacement therapy was approximately 60%.

Chatterjee showed that these patients do as well as others after transplantation with respect to patient survival and graft survival (181) with short-term follow-up. Longer-term studies, however, demonstrate an increased graft loss at 3 years, as compared to other groups of patients, especially with cadaveric transplantation (180), with increased mortality between 1 and 3 years after transplantation (184). In one case report, the

recurrence of sickle cell nephropathy was suggested in a transplanted kidney 3.5 years after transplantation (185). Nonetheless, renal transplantation remains a viable option for sickle cell disease patients with chronic renal failure (180,184,186).

Renal Medullary Carcinoma

Renal medullary carcinoma is a rare malignancy that is thought to arise from the papillary epithelium (187), or, more rarely, from the proximal tubule (188). Upon review of the 55 reported cases, Dimashkieh et al. noted the majority of cases occurs in young men of African descent with sickle cell trait (187). The typical presentation included hematuria, flank or abdominal pain, weight loss, and fever. This malignancy is general refractory to various chemotherapies and immunotherapies, is rapidly progressive and almost always fatal. Gene expression of the tumor has been performed in search of potential biomarkers and therapeutic targets (189), but these remain elusive.

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CHAPTER 78 ■ TROPICAL NEPHROLOGY

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The tropical zone is that region on earth lying between the Tropics of Cancer (23.5 degrees North) and Capricorn (23.5 degrees South). There is diversity of racial and ethnic backgrounds in this region that considerably reflects on the population's susceptibility to diseases, modifies clinical patterns, and influences responses to treatment. A warm, humid atmosphere is the hallmark of tropical weather. However, the density of precipitation varies from one region to another, covering a whole spectrum from dryness in the Saharans of Africa and Arabia to the tropical rainforest climate in the equatorial zone. The tropical environment is favorable for the growth of animals, plants, and microbes, and is an important source of natural toxins. It is also a large animal reservoir serving as vectors for disease transmission.

The socioeconomic conditions and quality of governance vary widely in the tropical countries. Great variations in gross national products per capita explain the differences in standards of education, health care, and social services. In most tropical countries the income per capita is low. Poor nutrition, reflecting low socioeconomic level, is a risk factor for infection. The problem is compounded by water and air pollution, creating an unfavorable environment that favors infection. Children born from undernourished mothers can have low birth weights with lower numbers of nephrons and immunologic defects that can lead to poor health including infection, diabetes, hypertension, and renal disease at later stages of life (1). The general lack of effective disease-prevention programs and delays in diagnosis and treatment have resulted in high morbidity and mortality due to renal diseases. This however has provided opportunities to observe the natural history of some diseases that cannot possibly be studied in developed countries. Optimistically, tropical renal disease can serve as a model that may lead to better comprehension of nephrology in general. Tropical nephrology, therefore, represents interaction between the kidney and several unfavorable factors in the tropical environment. With rapid communication and globalization, renal diseases peculiar to the Tropics are global rather than local concerns.

In this chapter, an overview is given to epidemiology of common renal diseases in the Tropics that are also present in the West but with different prevalence and nephropathy due to tropical infection and toxin poisoning, which is uniquely tropical. This is followed by description of specific diseases including diseases related to chemical toxins and environment. Finally, the issue of end-stage renal disease (ESRD) and its management in different tropical regions is reviewed.

OVERVIEW

Epidemiology of Common Renal Diseases in the Tropics

Acute Renal Failure

Acute renal failure in the Tropics differs from that in Western countries with respect to etiology. Surgery and trauma account for more than 50% of cases in Western countries (2,3), whereas medical causes are the major etiologic factors in the Tropics (4–9). With socioeconomic development in the Tropics, the incidence of surgical causes is increasing, but the important causes are still medical: tropical infections, drugs, or toxins (10,11). Obstetric acute renal failure, uncommon in Western countries (2,3), is still a problem in certain areas of the Tropics, with ominous outcomes (10). There is a high incidence of bilateral cortical necrosis in India (5,12–14), probably related to late referral and a high incidence of sepsis and massive blood loss. However, the incidence of obstetric causes is decreasing with better prenatal care and fewer illegal abortions. Interestingly, when community factors are excluded, the spectrum of hospital-acquired acute renal failure as studied in northern India does not differ from that of developed countries (15).

Glomerular Diseases

The prevalence of glomerular diseases in the Tropics has been estimated to be 1 in 10,000, which is 2.5 times the rate in Western countries (16–20). The cause is not known, but could be related to the environment. In Africa, glomerulonephritis has a high incidence in areas endemic for malaria and schistosomiasis. However, the incidence of glomerular diseases has decreased remarkably following malaria eradication programs (18–21), whereas the impact of schistosomiasis control remains to be elucidated (22). Nevertheless, postinfectious glomerulonephritis remains common in the Tropics.

Among the various forms of primary glomerular diseases, minimal change disease has a lower incidence (<10%) in the blacks of South Africa (23) and Papua New Guinea (24), but is common in Indonesia and Vietnam (25). Focal segmental glomerulosclerosis is rare in India (26) and Pakistan (27). Mesangial proliferative glomerulonephritis is more frequent in Southeast Asia than in the West, especially in Myanmar and Thailand (25,28,29). The incidence of immunoglobulin M (IgM) nephropathy accounts for 50% of primary glomerular diseases in Thailand (28). In addition, IgA nephropathy has a high prevalence in the Far East and Southeast Asia (25,30,31) but is rare in Africa (32). Mesangiocapillary (membranoproliferative) glomerulonephritis is a common glomerular disease in South Africa (23) and Brunei (25), but is less common in the other Southeast Asian countries (25,28). Membranous glomerulonephritis is less common in Southeast Asia (28), South America (33), and the Caribbean (34), but is common in South Africa (35) and Pakistan (27, 36–40).

Glomerulonephritis is the major cause of chronic renal failure in most countries in the Tropics, especially in Cambodia, Indonesia, Singapore, and Vietnam (25). The incidence of diabetic nephropathy is increasing as the standard of living increases. Secondary glomerulonephritis due to infection has an impact on the development of chronic renal failure in Africa, but this is less so in Southeast Asia. Lupus nephritis has a high incidence in the Tropics, especially in Southeast Asia (28). The reason is not yet known.

Vascular Disease

Takayasu's arteritis is a common vascular disease in the Oriental tropics and South Africa. The majority of reports have come from India (41), Sri Lanka (42), Singapore (43), Thailand (44), China (45), and Japan (46). It is the most common cause of renovascular hypertension, accounting for 61% of such patients in India (41).

Interstitial Nephritis

The incidence of interstitial nephritis in the Tropics perhaps does not differ from that of Western countries, although infection may play a larger role in the pathogenesis. The role of environmental pollution remains questionable. Chronic interstitial nephritis is emerging as an important cause of chronic renal failure in certain countries (47). The use of herbal medicine and easy access to drugs could be one of the causes.

Nephropathy Due to Tropical Infection and Toxin Poisoning

Pathophysiology

As a vascularized organ, the kidney is often involved with a variety of tropical infections, and toxins (48–51). Nephropathy associated with tropical diseases is attributed either to inflammatory reaction or direct nephrotoxicity. Both infections and toxins can stimulate monocytes through their receptors to release proinflammatory cytokines (52–59). Tumor necrosis factor α (TNF-α) and interleukin 1 (IL-1) released from monocytes can induce the generation of a cascade of cytokines, chemokines, vasoactive mediators, and adhesion molecules that cause hemodynamic changes, increased vascular permeability, hemorheologic derangements, immune response, and cell injury. Norepinephrine, endothelin-1, NO, kinins, thromboxane A_2, PGI_2, PGE_2, and angiotensin II are found to be elevated both in infection and toxin models.

Hemodynamic Alterations. Hemodynamic alterations in tropical infection and toxin poisoning are similar to those observed in sepsis (60). In mild and moderate infection, systemic vascular resistance is decreased accompanied by increased cardiac output and increased renal vascular resistance. The renal blood flow and the glomerular filtration rate are decreased. In severe infection, systemic vascular resistance is either normal or slightly increased. The cardiac output is either normal or decreased. Renal vascular resistance is further increased with marked diminution of renal blood flow and glomerular filtration rate.

In uncomplicated febrile illnesses hypervolemia has been shown during fever, and returned to normal during the recovery phase (61). The rise in blood volume is believed to be attributed to vasodilation due to the effects vasodilating mediators, antidiuretic hormone (ADH), and aldosterone. Hypovolemia is noted in severe infection, owing to increased vascular permeability with fluid leakage from the intravascular compartment. This is further compounded by the increased insensible loss and sweating due to high fever. The common causes of severe hypovolemia in the Tropics are diarrheal diseases, dengue hemorrhagic fever, and intestinal anthrax.

Hemorheologic changes can be induced by increased plasma viscosity, increased erythrocyte viscosity in malaria and babesiosis, and erythrocyte swelling in snake bite (50,62). Cytoadherence, either between leukocytes and the vascular endothelium because of adhesion molecules in infection or between parasitized erythrocytes in falciparum malaria and the vascular endothelium, is a common phenomenon. Hemoglobinuria, myoglobinuria, intravascular coagulation, free radicals release, bile acids, and complement and protease activation are among nonspecific effects of infection (49,63–67). These nonspecific factors in addition to hypovolemia, hemorheologic changes, and cytoadherence further contribute to decreased renal blood flow. Ischemic renal failure is therefore common in severe infection.

Immune-Mediated Mechanisms. Humoral and cell-mediated immune mechanisms closely operated with cytokines are involved in the pathogenesis of nephropathy in tropical diseases (68). Both Th_1 and Th_2 subsets of T cells are in operation (Fig. 78-1). Th_1 cytokines, consisting of IL-2, interferon γ (IFN-γ), and TNF-α, are effective against bacteria and viruses. Th2 cytokines, comprising IL-4, IL-5 and IL-10 defend against parasitic or mucosal infection. Glomerulonephritis, usually immune-complex mediated, is the classic finding in most infectious diseases. Because IgM antibodies are produced early in infection and fix complement efficiently, granular immune-complex deposition in the glomeruli with IgM and C3 is therefore commonly observed. Immune complexes can be either circulating or in situ. Through the release of cytokines and chemokines there is recruitment of leukocytes and proliferation of interstitial cells. In certain infections, such as leptospirosis and schistosomiasis, tubulointerstitial lesion and granuloma may be observed as predominant cell-mediated injury. Persistent antigen load may result in secondary amyloidosis as seen in leprosy, leishmaniasis, schistosomiasis, and opisthorchiasis.

Direct Invasion and Direct Nephrotoxicity. Microbial invasion of the kidney, urinary tract, and lymphatics can provoke injury through the local inflammatory reaction in the form of cellular proliferation, infiltration, and cystic and granulomatous changes (50). Renal lesions in leptospirosis, echinococcosis, filariasis, schistosomiasis, and tuberculosis represent injury by this mechanism. Infections and toxins may have indirect toxicity to the kidney through high fever and a number of toxic substances including myoglobin, hemoglobin, bile acids, proteases, peroxynitrite, phospholipases, free oxygen radicals, and complement products (49,63–67) (Fig. 78-2).

In the real direct nephrotoxic model, the outer membrane of leptospires, carp raw bile, impila (Callilepis laureola), toxic mushroom, and cotton seed oil are toxic to renal tubules (49). Djenkolic acid, the main composition of the djenkol bean, obstructs renal tubules in the presence of acid and concentrated urine (49). Star fruit juice drinking causes renal failure through tubular obstruction by oxalate crystals (49). Russell's viper venom and green pit viper venom have direct toxicity to the blood vessel, glomeruli, and renal tubules (49,69).

There is a broad spectrum of renal pathologic changes. All renal structures can be involved by tropical infection and toxins (49,50,70). Various forms of glomerulonephritis can be seen. Mesangial proliferative glomerulonephritis with deposition of IgM and C3 is a common pathologic finding and is responsible for mild proteinuria and urinary sediment changes. Other forms of glomerulonephritis are also observed but are less common. Tubular necrosis is the important pathologic counterpart of acute renal failure. Cortical necrosis is common in snake bite.

Clinical Manifestations

Nonspecific urinary sediment changes consisting of few erythrocytes, leukocytes, and granular casts are often observed in febrile infectious diseases. Significant microscopic hematuria may be seen in hematotoxic snake bite. However, the findings resolve when the disease is under control.

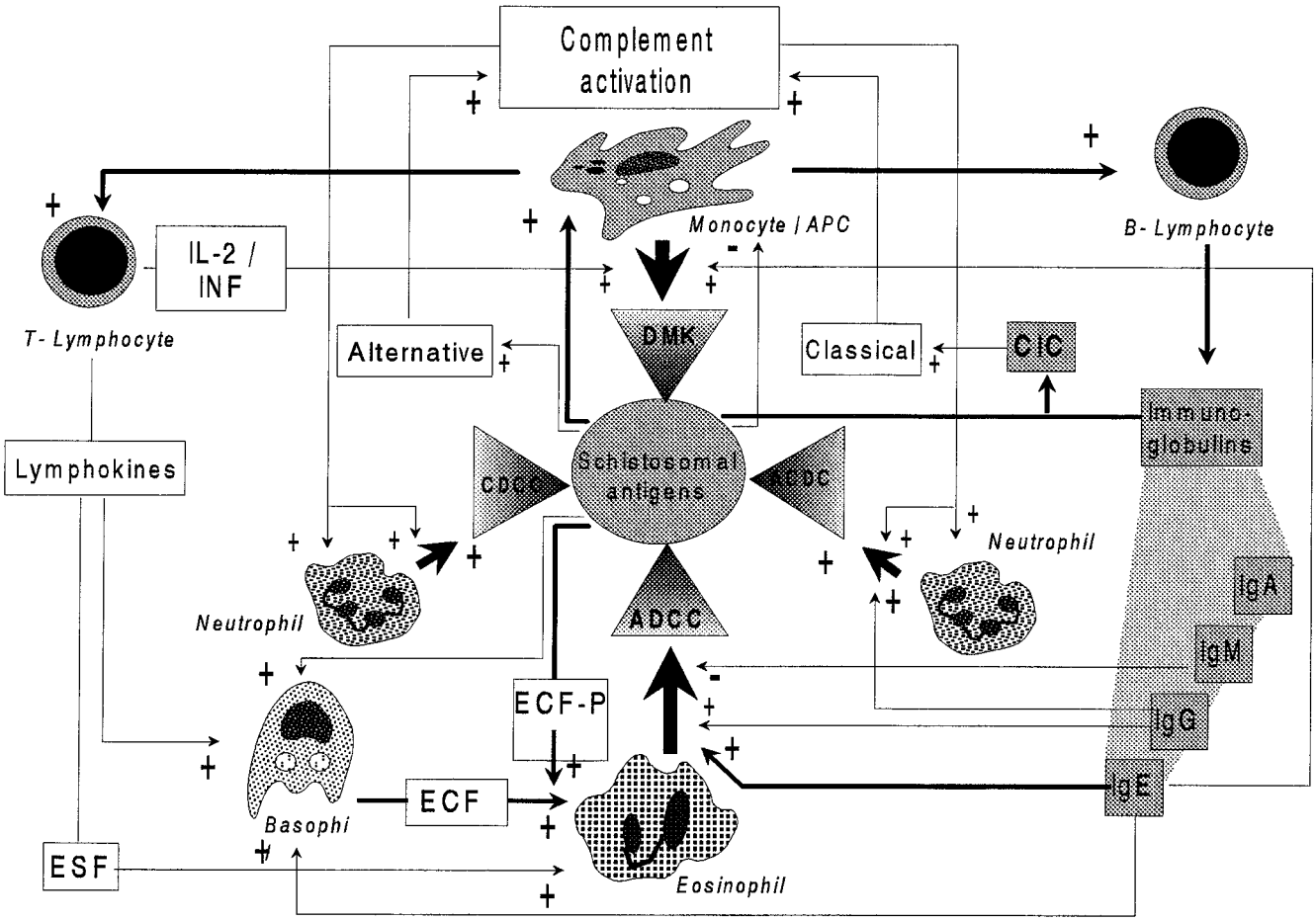

FIGURE 78-1. The immune response to schistosomiasis. The principal four killing systems are shown—direct macrophage killing (DMK), antibody-dependent cell-mediated cytotoxicity (ADCC), antibody and complement-dependent cytotoxicity (ACDC), and complement-dependent cytotoxicity (CDCC). The close association of humoral and cell-mediated immunity in activating the parasite elimination mechanisms is displayed. Note the crucial role of the eosinophil and immunoglobulin E (IgE). Also note the formation of pathogenic circulating immune complexes involving all major immunoglobulins. *IL-2*, interleukin-2; *APC*, antigen-presenting cell; *INF*, interferon; *ESF*, eosinophil-stimulating factor; *ECF*, eosinophil chemotactic factor; *ECF-P*, eosinophil chemotactic factor of parasitic origin; *CIC*, circulating immune complexes. (From: Baroum RS. Oxford Textbook of Clinical Nephrology. Oxford University Press, 2005, Oxford; New York, Toronto: 1175, with permission.)

Proteinuria is transient and often mild with total urinary protein of less than 1 g per 24 hours. Significant proteinuria, even at the nephrotic range, may be seen, but disappears when the disease resolves. Persistent proteinuria with abnormal urinary sediment has been observed in certain infectious diseases that run chronic clinical course or associated with superimposed infection or autoimmune reaction.

Hemoglobinuria is one of the common findings in venom or toxin poisoning (48). Bites by vipers and hornet or wasp stings can cause significant intravascular hemolysis (14,28–30). Glucose-6-phosphate dehydrogenase (G6PD) deficiency is common in tropical countries and may account for intravascular hemolysis in the patient with infection or with use of certain drugs. Myoglobinuria is observed in several infectious diseases with rhabdomyolysis such as leptospirosis, trichinosis, malaria, typhoid fever, viral infection, and septicemia (49). In the toxin group, sea-snake bite and insect stings can cause rhabdomyolysis and myoglobinuria.

Hyponatremia is common in the patients with febrile diseases. The causes are multiple including increased ADH, low sodium intake, sodium loss, sodium influx into the cells, and osmoreceptor resetting (49). Response to water load may be delayed. Hypokalemia attributed to respiratory alkalosis due to high fever is noted in 38% of the patients with febrile illness. Potassium loss due to diarrhea is a common cause of hypokalemia. Interestingly, cotton seed oil ingestion (49) and leptospirosis (71) can cause kaliuresis and hypokalemia. Intravascular hemolysis and rhabdomyolysis are important causes of hyperkalemia. Hypocalcemia and hypophosphatemia are seen in severe infection.

Acute renal failure, often caused by tubular necrosis either ischemic or toxic model, is frequently observed in severe infection and toxin poisoning. The magnitude of the problem varies from 0.5% to 4% of patients with tropical diseases. Renal failure is hypercatabolic in 68% of the patients, characterized by rapid rises of blood urea nitrogen and serum creatinine. Acute renal failure may be accompanied by jaundice, myoglobinuria, or hemoglobinuria. Cholestatic jaundice is a common complication, being noted in 60% of the cases. Prognosis of acute renal failure is usually good unless complicated by multiorgan

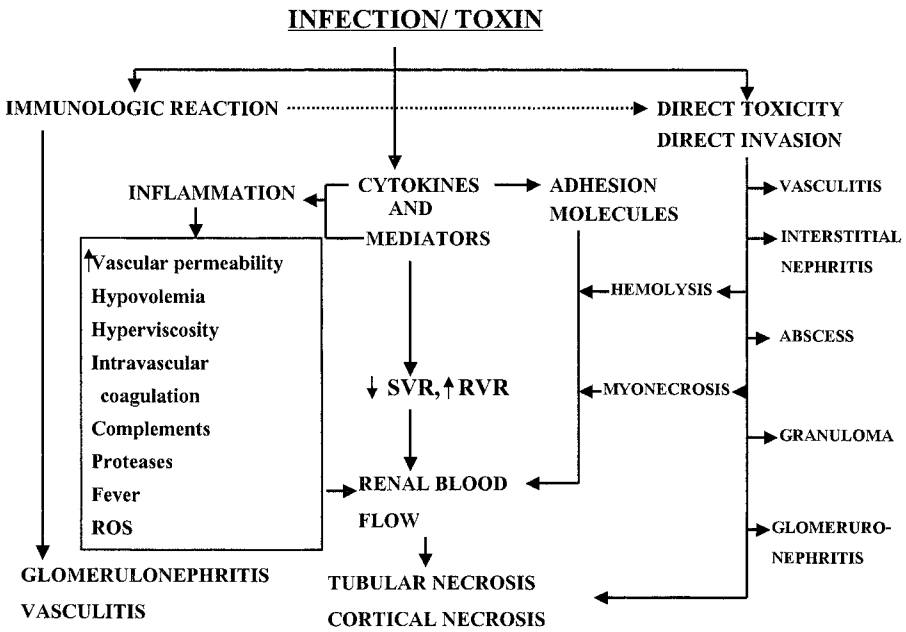

FIGURE 78-2. Pathogenesis of infection and toxin-induced nephropathy. *ROS*, reactive oxygen species; *SVR*, systemic vascular resistance; *RVR*, renal vascular resistance.

failure, cortical necrosis, or papillary necrosis. The clinical course may be prolonged in severe acute diffuse interstitial nephritis.

TROPICAL INFECTIONS

Bacterial Infections

Typhoid Fever and Salmonella Infections. Typhoid fever continues to be a common disease in tropical countries. Clinically significant renal disease with typhoid fever is uncommon, occurring in less than 6% of all patients (72–74). However, when careful and repeated urinalysis is performed, more than 50% of the patients are found to have definite findings of renal involvement (75). The spectrum of renal disease in typhoid fever includes mild-to-severe glomerular involvement (75–80) and acute tubular necrosis.

Hematuria and proteinuria are frequent during the febrile phase (75). The urinary protein loss is less than 1 g per 24 hours in the majority of patients, but can exceed this in occasional patients. The glomerular disease usually has a benign course, with complete recovery occurring in a 1- to 2-week period (75,80). Less commonly, fever, generalized edema, and hypertension mimicking acute poststreptococcal glomerulonephritis have been reported in South Africa (77). Recovery was complete with appropriate antimicrobial treatment. Renal histology, reported in several series (75,77,80), shows large glomeruli with mild-to-moderate mesangial proliferation. Immunofluorescence staining has shown variable amounts of C3, IgG, and IgM deposits (75,80). IgA deposition in the mesangial area has been described (81). The pathogenesis of typhoid glomerulonephritis is of an immunologic nature. Salmonella Vi antigen has been demonstrated in the glomerular capillary wall (80). The complement level is usually low during the acute phase in patients with renal involvement and normal in those without renal involvement (75,77,82). However, this has not been a consistent finding, especially in experimental typhoid (83,84).

Acute renal failure has occurred with typhoid fever associated with intravascular hemolysis due to G6PD deficiency, disseminated intravascular coagulation (85), severe jaundice (75,86–87), and rhabdomyolysis (88–89).

An interesting syndrome reported from Egypt is the nephrotic syndrome occurring in patients with chronic salmonellosis associated with schistosomiasis (90–93) (see subsequent text). It is amazing that, although similar combinations of salmonella and schistosomal infection occur in Brazil, the previously described pattern has not been reported (94).

Leptospirosis. Leptospirosis, an infectious disease caused by several serotypes of Leptospira interrogans, is worldwide in distribution. The disease is transmitted through contact of abraded skin or mucous membrane with blood, tissue, or urine of infected animals or through exposure to contaminated environments. It is an unusual cause of acute renal failure in Western countries (95,96); however, it plays an important role in certain tropical regions (97–99). In Southeast Asia and Sri Lanka, leptospirosis is one of the important causes of acute renal failure, accounting for 24% and 32% of all reported cases, respectively. The kidney is invariably involved in leptospirosis, and acute renal failure of variable severity occurs in the majority of the patients.

Clinically, patients present with sudden onset of chills, fever, generalized muscle pain, and variable degrees of jaundice. Urinary abnormality consists of mild proteinuria, a variable number of erythrocytes, occasional hemoglobinuria, granular casts, and bile-pigmented casts. Leptospiruria is demonstrated by dark-field illumination. Renal failure, occurring in 60% of the patients, may be mild and nonoliguric. Oliguric renal failure with hyperbilirubinemia occurs in severe cases. In severe leptospirosis hypotension is frequently observed and can lead to renal failure and pulmonary complications if not promptly treated (100). Hypokalemia secondary to kaliuresis has been observed (71). Kaliuresis in leptospirosis is due to inhibition of potassium reabsorption in the medullary thick ascending limb of the loops of Henle (101) and increased sodium–potassium exchange in the principal cells of the collecting ducts secondary to decreased sodium reabsorption in the proximal tubules (102). Renal bicarbonate wasting similar to proximal renal tubular acidosis may be observed. In severe cases (Weil's syndrome), marked renal failure with a rapid rise in serum creatinine and uric acid occurs in association with jaundice. Jaundice

is usually cholestatic. Hepatocellular jaundice may be observed when associated with shock. In rare cases, hemolytic-uremic syndrome may be seen. In patients reported from Barbados (103) and French Polynesia (104), renal failure was associated with significant thrombocytopenia; however, full-blown disseminated intravascular coagulopathy is rare. Infection with different leptospiral serotypes does not seem to explain the marked variability in renal involvement.

Although leptospirosis involves every structure of the kidney, the primary lesion is interstitial in nature, with local or diffuse mononuclear cell infiltration. Renal function can be normal. In acute renal failure, cellular degeneration of both proximal and distal tubules is seen. Glomerular changes are quite mild and limited to mild mesangial hypercellularity (105, 106). On immunofluorescent staining, nonspecific C3 and IgM uptake is seen in the mesangial area and occasionally in the afferent arterioles (98,106). On electron microscopy, occasional dense deposits are seen in mesangial, paramesangial, and intramembranous locations (98). The organism itself is rarely seen in human biopsy studies. However, in hamster models, leptospires can be seen initially in the glomeruli and then in the interstitium and renal tubules a few hours following inoculation (106).

The pathogenesis of renal changes in leptospirosis is multifactorial.

1. Hemodynamic alterations. The outer membrane of leptospires can cause a release of nitric oxide, TNF-α, and monocyte chemoattractant protein from the medullary thick ascending limb of the loops of Henle (107). Peptidoglycans and lipopolysaccharide also induce monocytes to release proinflammatory cytokines and vasoactive mediators causing hemodynamic changes. The renal blood flow is decreased through the effects of cytokines, mediators, and nonspecific inflammatory factors (Table 78-1) (108). Systemic vascular resistance is decreased along with increased cardiac output in anicteric leptospirosis, but it is increased in icteric leptospirosis with decreased cardiac output. Decreased cardiac function is attributed to myocarditis or myocardial depressant factor or jaundice. In severe leptospirosis, renal blood flow is markedly decreased in sufficient degree to cause acute renal failure.
2. Direct nephrotoxicity. Intercalation of leptospire glycolipid in the host cell membrane (109) and inhibition of membrane Na^+,K-ATPase (110) can cause cellular injury. Lipopolysaccharide, porin, and lipoprotein of the outer membrane have been shown to induce interstitial nephritis (111). The subject has been recently reviewed (112).
3. Immunologic mechanism. This mechanism plays a major role in canine leptospirosis. However, it probably does not play a significant role in human disease (106). Circulating immune complexes have been demonstrated in only one patient (98). An increased number of B lymphocytes has been observed in a patient with leptospirosis, along with a decrease in the number of CD3+ and CD4+ cells (113).

TABLE 78-1

HEMODYNAMICS IN LEPTOSPIROSIS

Severity	Anicteric	Icteric
Cardiac output	~ or ↑	~ or ↓
Blood volume	~ or ↑	~ or ↓
Systemic vascular resistance	↓	~ or ↑
Renal vascular resistance	↑	↑↑
Renal blood flow	↓	↓↓
Glomerular filtration rate	↓	↓↓

Management of renal failure in patients with leptospirosis should focus on treatment of the underlying abnormality. Penicillin, including its derivatives, is the drug of choice. Hypotension should be treated promptly with vasopressor agents. Intravenous fluid should be given cautiously because of decreased response to fluid load. A small dose of dopamine increases blood pressure and urine flow (114). Hemodialysis and peritoneal dialysis have been used successfully in patients with renal failure. Plasma exchange coupled with hemodialysis is advocated for patients with marked hyperbilirubinemia (115–117). In patients who recover from acute illness, renal function returns to normal. Prognosis is generally good. Bad prognostic indices include hyperbilirubinemia, hyperkalemia, and pulmonary complications, either pulmonary edema, adult respiratory distress syndrome (ARDS), or hemorrhage. Continuous hemofiltration, either arteriovenous or venovenous form, plasmapheresis, and blood exchange are useful in these clinical settings (112,118). In the rural area blood exchange is preferred because of simplicity and the low cost.

Leprosy. Leprosy is a common infectious disease in tropical regions including Africa, Asia, and South America. It has been estimated that of 12 million cases of leprosy in the world, 3 million are in India. Renal involvement is frequent among patients with lepromatous leprosy, although it can also be seen in those with tuberculoid or borderline types (119, 120). The prevalence of glomerulonephritis in leprosy varies from 6% to 50% in biopsy studies (121).

The clinical spectrum covers asymptomatic proteinuria and hematuria (122), nephrotic syndrome (123), nephritic syndrome, and even renal failure (124). Circulating immune complexes and cryoglobulinemia are detectable in the majority of patients. Serum complement levels may be low (125). The cell-mediated immune response is depressed in the lepromatous type. Nephrotic syndrome is common in patients with amyloidosis, but may be observed in patients with membranous and diffuse proliferative glomerulonephritis (126). Urinary concentration and acidification defects have been described (119,121). Acute renal failure may occur (124,127), and chronic renal failure often results from complicated amyloidosis. Acute renal failure is observed primarily in lepromatous leprosy with a prevalence of 63%, whereas only 2% of nonlepromatous patients have impaired renal function (127). Acute renal failure may also be a complication of multiple drug treatment of leprosy (128). Treatment should concentrate on the leprosy; management of the renal involvement is only supportive.

Pathologically, diffuse proliferative glomerulonephritis and mesangial proliferative glomerulonephritis are common (76, 119). Focal proliferative glomerulonephritis (119), membranous nephropathy (129), mesangiocapillary glomerulonephritis, crescentic glomerulonephritis (124), focal glomerulosclerosis (120), and interstitial nephritis (129) have been reported. Immunofluorescence study shows granular deposition of IgM, IgG, IgA, and C3 in the mesangial areas and along the glomerular capillary walls (130). Deposition of IgA alone has been demonstrated occasionally (123). There is electron-dense deposition in the mesangial, subendothelial, intramembranous, and subepithelial areas (119,131,132). The findings are compatible with immune-complex glomerulonephritis. The nature of the antigen has not been identified. Glomerulonephritis may be only a nonspecific reaction. The possibilities exist among mycobacterial antigens, other microbial antigens, and autoantigen. Secondary amyloidosis is noted in 2.4% to 8.4% of patients, predominantly with lepromatous and borderline lepromatous leprosy, although it may be observed occasionally in tuberculoid leprosy (121).

Melioidosis. Melioidosis is an infectious disease caused by a Gram-negative bacillus, Burkholderia pseudomallei. The disease is prevalent in the Tropics, especially in India, Thailand, Myanmar, Kampuchea (Cambodia), Laos, Vietnam, Malaysia, the Philippines, and Papua New Guinea. Melioidosis was an important health problem during the Vietnam War, when many soldiers became the victims of this deadly disease. Significant serologic titers for the organism were detected in 29% of military personnel (133). Recently, antibodies to Burkholderia pseudomallei were detected in 39.5% of the people in northeastern Thailand. There are a few reports on melioidosis of the urinary tract (134,135). The data on renal involvement are scanty, and according to a few reports the rate of renal involvement varies from 2.4% to 35.0%. Yet, acute renal failure can occur in the septicemic form of the disease. In a series of 220 patients with melioidosis in northeastern Thailand, renal failure was noted in 35% (135). The underlying causes in 56% of the patients included diabetes mellitus, renal stone, cirrhosis, and glomerulonephritis. Hypoproteinemia was present in 60% of the patients. Hyponatremia was observed in 90% of the patients. The duration of renal failure varied from 1 week to 6 weeks, averaging 3 weeks. The mortality rate was close to 90%.

Renal pathologic changes include multiple renal abscesses, tubular necrosis, and interstitial nephritis (135). The bacteria are seen in the suppurative lesions (136). In the animal model, thrombi have been demonstrated in blood vessels, and cortical necrosis has been observed (136). These have not been shown in human melioidosis. Renal changes are attributed to severe sepsis, which causes renal ischemia and inflammatory reactions to bacterial invasion.

Tetanus. Tetanus is capable of causing acute renal failure through stimulation of the sympathetic nervous system (137–139). The incidence of renal failure in tetanus is high in Brazil. In a report by Martinelli et al. (137), proteinuria was noted in 50% of the patients and impaired renal function was observed in 39% of patients in whom serum myoglobin levels were found to be elevated. There is no correlation between the serum level of myoglobin or creatine phosphokinase (CPK) and renal failure. Renal failure is usually nonoliguric and mild.

Scrub Typhus. Scrub typhus is widely distributed in the Tropics and can involve the kidney. As with the other infectious diseases, mild proteinuria with abnormal urine sediment is not uncommon. In the majority of patients, renal function is normal. Renal failure can be observed with severe infection associated with jaundice, intravascular coagulation, or hemolysis due to G6PD deficiency (140–142). The usual renal changes are those of mild mesangial proliferative glomerulonephritis. Deposition of IgM and C3 is seen in the mesangial area. In severe cases, platelet thrombi may be seen in the glomeruli, with focal thickening of the basement membrane. Tubular necrosis is observed in the presence of renal failure. Interstitial nephritis may be present (140,141,143). The infiltrate often occurs in the corticomedullary region and consists of mononuclear cells.

Perivascular infiltration and thrombophlebitis may be seen in the interlobular veins.

Diphtheria. Diphtheria can occasionally cause acute renal failure in children (144–146). In a review of 155 patients by Singh et al., renal failure was observed in only two patients (146). This could be attributed to decreased cardiac output due to myocarditis. Yet, diphtheria toxin may be nephrotoxic because it inhibits protein synthesis when it is added to the basolateral side of the renal tubular cells (147). Renal histologic changes in those with acute renal failure are consistent with tubular necrosis (144). In a report of 70 patients by Kannerstein, tubular necrosis was observed in 16% and interstitial changes in 48% (145).

Cholera. Cholera remains a global threat. In 2003, a total of 111,575 cases of cholera with 1894 deaths were reported to the World Health Organization (WHO) (148). The number of cases notified by Africa greatly exceeded the number reported from other countries. Cholera is still endemic in Asian countries. Renal failure is attributed to fluid and electrolyte loss. Acidosis in cholera is associated with an increased anion gap due to hyperproteinemia, hyperphosphatemia, and increased serum lactate levels (149). Hypokalemia can be striking. Interestingly, the ratio between blood urea nitrogen and serum creatinine may be lower than normal because of the larger loss of urea than creatinine through diarrhea and perhaps rhabdomyolysis due to hypokalemia. In addition to tubular necrosis, vacuolation of the proximal convoluted tubules due to hypokalemia may be present (150). Cortical necrosis has been described (151).

Shigellosis. Shigellosis can cause renal failure in the same fashion as cholera and other diarrheal diseases. In a series of 2018 patients reported by Bennish et al., renal failure occurred in 26% (122). By multivariate analysis, younger age, decreased serum protein, altered consciousness, and thrombocytopenia were indices of a poor prognosis (122). Because shigella toxin causes endothelial injury, hemolytic-uremic syndrome may occur (152–154). In this setting there may be cortical necrosis and diffuse fibrin deposition in the glomeruli. Srivastava et al. reported an incidence of hemolytic-uremic syndrome of 34% in patients with acute renal failure, mostly related to shigellosis; renal histology showed cortical necrosis in 40% of the patients (155). The mortality rate was 60%.

Vibrio vulnificus Infection. Vibrio vulnificus is a Gram-negative bacillus found in coastal and brackish waters. Infection due to V. vulnificus usually occurs in an immunocompromised host or chronic alcoholic hosts following consumption of contaminated sea food, or injury to the skin in a marine environment. V. vulnificus infection in humans can be expressed as primary sepsis, wound infection, or gastrointestinal manifestation. The presenting symptoms consist of fever with chills, abdominal pain, vomiting, diarrhea, and lower extremity pain. Disseminated intravascular coagulation can occur. Acute renal failure is common with tubular necrosis as a significant pathologic change (156,157). Renal failure is ischemic in origin. Yet, cellular injury can be induced by bacterial cytolysin, collagenase, protease, metalloprotease, and phospholipases (158–161). The disease may be confused with leptospirosis, scrub typhus, malaria, and other forms of sepsis.

Viral Infections

Many viral diseases can cause mild glomerular involvement. Rhabdomyolysis may be seen with viral infection and can be responsible for acute renal failure. It is, however, interesting that several viruses harbored in the kidney producing viruria without renal function changes.

Dengue. The disease is caused by dengue virus and is characterized by flu-like symptoms with headache, muscular pains, arthralgia, flushing of the face, conjunctival injection, and skin rashes. The disease is common in Southeast Asia (162,163) and is transmitted by the mosquito Aedes aegypti. Aedes albopictus, Aedes scutellaris, and Aedes polynesiensis may be important vectors in certain areas. In recent decades dengue fever and dengue hemorrhagic fever have emerged as a global public health problem. Fifty million cases of dengue infection occur

each year, with 500,000 cases of dengue hemorrhagic fever, and at least 22,000 deaths, mainly among children (164). Hypotension may occur during the second phase of the disease, a few days after the onset when fever declines. Complement activation, increased vascular permeability, and thrombocytopenia are the main pathophysiologic changes of the disease leading to hypovolemia and bleeding (165–168). Immune complexes play an important part in complement activation. Levels of anaphylatoxins C3a and C5a, cytokines, and mediators are elevated and result in plasma leakage from the intravascular space. Usual renal manifestations in dengue hemorrhagic fever include mild urinary sediment changes, mild proteinuria, and hyponatremia. Renal failure in children is often mild and usually prerenal due to hypotension. In adults, renal failure can be severe and associated with cerebral symptoms, liver dysfunction, and myoglobinuria (169–171). As in sepsis, hemodynamic alteration plays a key role in the pathogenesis. It is not clear why the disease is more severe in adults. It could be related to virus virulence, associated infection, drugs used, and late hospitalization. In addition, the disease may not be recognized earlier in adults. Jaundice is hepatocellular, with marked elevation of liver enzyme levels. The ratio between blood urea nitrogen and serum creatinine is lower than normal. Renal failure is oliguric with a prolonged clinical course.

Renal histologic changes include mesangial proliferation with IgM and C3 deposition, endothelial cell swelling, and perivascular infiltration by mononuclear cells (172). Tubules show degeneration along with interstitial edema (173). The diseases can be confused with Hantavirus infection, which can produce almost similar pathophysiology and symptoms.

Hantavirus Infection. Hantavirus is an RNA virus in the family Bunyaviridae. There are seven serotypes: Hantaan, Seoul, Puumala, Prospect Hill, Belgrade, Thottapalayam, and Sin Nombre. The disease is seen worldwide throughout Asia, Europe, North and South America, Australia, and Africa (174–177). It is considered as a rare disease in tropical Asian countries. In Korea, Hantaan and Seoul serotypes are common. Hantaan virus causes severe disease with a renal syndrome. Seoul virus causes a disease of moderate severity, and Puumala virus produces the least severe disease. The epidemiologic significance of Prospect Hill serotype is not well understood. Belgrade virus is associated with severe disease in the Balkans. Sin Nombre serotype causes the most severe disease, with a pulmonary syndrome in the United States. Thottapalayam virus is found in India. Rodents, especially rats, mice, and voles, are the important reservoirs. Infection is acquired mostly by inhalation of rodent excreta, although direct inoculation by abrasion or cuts of the skin is also possible. Increased vascular permeability is the main pathophysiology of the disease (167), owing to the effects of various mediators, cytokines, complement activation, and vascular endothelial injury, which finally result in hypovolemia, decreased renal perfusion, and acute renal failure.

The symptoms of Hantavirus infection with different serotypes vary greatly. Even in the same serotype the symptoms can vary from mild to severe. The description of Hantaan virus infection with renal syndrome is classic. Clinically, after the incubation period of 2 to 5 weeks, the disease is manifested by flu-like symptoms with fever, headache, flushed face, myalgia, abdominal pain, nausea, and vomiting. Periorbital edema, conjunctival hemorrhage, and palatal and axillary petechiae may be present. The clinical course can be divided into five phases: febrile, hypotensive, oliguric, diuretic, and convalescent (177). The febrile phase lasts for 3 to 7 days and is followed by the hypotensive phase due to hypovolemia, which develops with lysis of fever. The hypotensive phase lasts from 3 hours to 3 days and is followed by the oliguric phase, which may be prerenal or renal in origin. The duration of the oliguric phase, therefore, varies from a few days to several days. The diuretic and convalescent phases follow. In infection with other serotypes or in mild cases these five phases may not be apparent. Hypotension and oliguria may not be present. The Sin Nombre serotype is associated with mild renal involvement but a severe pulmonary syndrome.

Renal involvement is common in Hantaan virus infection. Proteinuria, hematuria, and pyuria are usually observed. Renal failure is more common in Hantaan virus infection than in infections with the other serotypes, and may be associated with pulmonary edema. Thrombocytopenia may occur in severe cases. Disseminated intravascular coagulation has been shown.

Renal pathologic changes are consistent with tubular necrosis. Medullary vessels are dilated and congested. Marked interstitial changes with edema and hemorrhage can be seen, with later infiltration by mononuclear cells. The interstitial changes are pronounced in Puumala infection. Glomerular changes are not remarkable. Mild glomerular hypercellularity may be found. IgM, IgG, and C3 deposition in the glomeruli and interstitium may be observed.

Hepatitis B. Hepatitis B virus (HBV) infection is worldwide in distribution, with a low carrier rate in Western countries. The prevalence of the hepatitis B surface antigen (HBsAg) carrier varies from 0.3% to 1.0% in North America to 1% in Western Europe; 5% in South America, Eastern Europe, Japan, and western Asia; 7% in Africa; and 10% to 20% in China, Taiwan, and Southeast Asia (178). The pathogenetic role of HBV in glomerulonephritis is a topic of great interest. The observations of a high incidence of HBsAg carriers among patients with various forms of glomerulonephritis when compared with the general population tend to support the role of HBV in the pathogenesis of glomerulonephritis. In Hong Kong, 22% of patients with glomerulonephritis are HBsAg positive, which is higher than the carrier rate in the general population. In South Africa 20% of the patients with glomerulonephritis are HBsAg positive (179). In Zimbabwe, Japan, and Taiwan, HBs antigenemia has been found in 80% to 100% of children with membranous glomerulonephritis (180–182). However, in Thailand and South Korea the prevalence does not differ from that for the general population.

Clinical presentations vary from asymptomatic proteinuria and hematuria to nephrotic syndrome and impaired renal function with hypertension. Nephrotic syndrome is common with membranous and mesangiocapillary glomerulonephritis, whereas hematuria and asymptomatic proteinuria are present with mesangial proliferative glomerulonephritis. In about 33% of patients the serum complement level is decreased. Patients with HBV infection may present with the clinical picture of essential cryoglobulinemia with purpura, arthralgia, and splenomegaly (183). Acute renal failure may occur with fulminant hepatitis (184), but may occasionally develop with uncomplicated hepatitis B (185).

The natural history of HBV-associated glomerulonephropathy is not well understood (186). Spontaneous remission occurs in 50% of membranous nephropathy patients. Seroconversion to positive antihepatitis B e antibody (anti–HBeAb) is associated with remission of proteinuria. In children the disease may run a benign course and the pathology is mainly membranous. Among patients with IgA deposition, 19% had deterioration of renal function in 40 months (186). Clearance of HBsAg from blood has been associated with remission of polyarteritis. The use of steroids as treatment for glomerulonephritis should be discouraged. Corticosteroid therapy has been associated with active virus replication and hepatic dysfunction with an appearance of viruslike particles in the glomeruli (186,187). Interferon alpha administration has been shown to suppress HBV expression with clearing of HBeAg (186), and may be tried in HBV-associated glomerulonephritis with short duration of

infection. The use of adenine arabinoside and thymic extract reduced proteinuria in 87% of membranous nephropathy patients, with a reduction of HBV DNA in T cells, B cells, and macrophages along with seroconversion from HBeAg positive to anti-HBe positive (188). The use of antiviral agents such as lamivudine can be beneficial.

Renal pathologic changes include membranous glomerulonephritis, mesangial proliferative glomerulonephritis, mesangiocapillary glomerulonephritis, and polyarteritis nodosa (178). Among these, membranous nephropathy is usually associated with deposition of HBeAg in the immune complexes, whereas mesangial proliferative glomerulonephritis is associated with HBsAg complexes (189,190). Hepatitis B core antigen (HBcAg) has been found in membranous nephropathy patients when polyclonal anti-HBcAg antiserum was used. Glomerular deposition of HBeAg and HBsAg is demonstrable in mesangiocapillary glomerulonephritis. The pathogenetic association between IgA nephropathy and HBV has attracted attention, as the geographic area with the highest endemicity of HBV infection has also the highest incidence of IgA nephropathy. Glomerular HBsAg deposition in a distribution similar to that of IgA immune staining is detected in 21% to 40% of patients (186,191). There is no HBeAg deposition.

Hepatitis C. The incidence of hepatitis C virus (HCV) carrier in the Tropics is less than 5%. Chronic HCV infection can be associated with mesangiocapillary glomerulonephritis (192–194), membranous glomerulonephritis (195), and diffuse proliferative glomerulonephritis (196). Mesangiocapillary lesion with mixed cryoglobulinemia is the common lesion. Glomerular immune complex deposition consists of HCV, anti-HCV IgG, and IgM rheumatoid factor. The cryoprecipitate containing HCV RNA and HCV antibody can be seen in the subendothelial glomerular deposits (197,198). Ohta et al. demonstrated the core antigen in the glomeruli of patients with hepatitis C in the absence of circulating cryoglobulins (199). Electron microscopy of the renal tissue shows cryoglobulinlike structures. The patients have proteinuria, hematuria, decreased renal function, and hypocomplementemia, rheumatoid factor, and circulating cryoglobulin. Nephrotic syndrome is a common presentation. Crescentic fibrillary glomerulonephritis has been described (200).

Treatment with IFN-α improves liver function and decreases urinary protein excretion with disappearance of HCV RNA (197). Exacerbation of glomerulonephritis with proteinuria and hematuria during IFN administration has been reported (201). It was suggested that renal damage was either a direct or indirect effect of interferon on the glomerular endothelial and epithelial cells. The result of treatment IFN therefore varies (201,202). The viral genotype and titer may be important determinants in response. Antiviral therapy with IFN-α with ribavirin may be beneficial (203). In comparing with the other causes of secondary glomerulonephritis in the Tropics, hepatitis C is a rather uncommon cause. However, it is an important cause of morbidity and mortality among recipients of renal transplantation and chronic dialysis patients. Pretransplant interferon may reduce the occurrence of posttransplantation HCV-related de novo glomerulonephritis (204).

Hepatitis A. Fulminant hepatitis A can produce renal failure in a fashion similar to hepatorenal syndrome. Viral hepatitis associated with G6PD deficiency may present with massive intravascular hemolysis and acute renal failure (205). Recently, there have been several reports of acute renal failure developing in patients with hepatitis A in the nonfulminant form (206–208). The mechanism of renal failure is not well understood. In some patients, there are no apparent renal histologic changes, whereas in the others tubular necrosis is seen. Perhaps various nonspecific factors in inflammation that lead to renal ischemia superimposing on hepatic dysfunction produce the renal failure. IgA-dominant immune complex glomerulonephritis with nephrotic syndrome has been reported (209).

Mycotic Infections

Mycotic infections constitute an important part of tropical nephrology. Because no particular features distinguish such infections in the Tropics from those that occur in other parts of the world, this chapter does not include detailed accounts, which can be found elsewhere (Chapters 37, 72, and 97). For easy reference, however, the important mycotic infections of clinical significance in tropical countries are highlighted.

Mycotic Nephropathies in the Immunocompetent Individual

Coccidioidomycosis. The disease is widely distributed over the globe, but endemic foci are identified in the tropical zone of the American continent, particularly Central America and Argentina. Infection is acquired through inhalation of dust containing the fungal hyphae, leading to predominantly pulmonary disease. The kidneys are involved in the rare disseminated form of the disease, particularly in non-whites (210). The lesions are interstitial, either granulomatous or suppurative, usually multiple, and often associated with lung cavities (211).

Paracoccidioidomycosis. This is a chronic granulomatous disease, also endemic in Central and South America. It is characterized by mucocutaneous manifestations, in addition to foci in different viscera including the kidneys (212).

Fungal Toxins

Ochratoxins. These fungal products often contaminate stored cereals. They can induce a form of interstitial nephritis in pigs (213) that resembles and has been incriminated in the pathogenesis of Balkan nephropathy in humans (214). It is also a potent carcinogen that induces renal adenocarcinoma in small laboratory animals, but the relevance of this observation to humans is unknown.

Chronic interstitial nephritis associated with ochratoxin A has been reported from the tropical zone, including Egypt (215) and Northwest Africa (216).

Aflatoxins. Aflatoxins B_1 and B_2, produced by Aspergillus flavus, which contaminates many foods, particularly cereals, have been associated with hepatomas in humans and in experimental animals. They can also induce adenocarcinoma and pelvic neoplasms in rats (217).

Although aflatoxins are present worldwide, they have recently attracted a lot of interest in the Tropics, where the cereal storage conditions are optimal for fungal growth. They have been incriminated in the remarkable increase in the incidence of hepatomas in most of Africa, particularly in association with persistent HBV-antigenemia (218). It is unknown whether aflatoxins are also responsible for a higher incidence of renal malignancy in the same continent.

Opportunistic Mycotic Infections in the Immunocompromised

Fungi are well-known opportunistic organisms worldwide. Fungal infections are even more prevalent in tropical countries, because of the uncontrolled use of antibiotics and immunosuppressive agents, poor general hygienic standards, and the high prevalence of acquired immunodeficiency syndrome (AIDS) in certain areas.

The principal opportunistic fungi encountered in the Tropics include the following:

1. **Candidiasis** is, by far, the most common. Urethritis, cystitis, and pyelonephritis are usually ascending infections (219). Disseminated candidiasis (220) is often a terminal event in patients with AIDS and overimmunosuppressed transplant recipients, intravascular catheters being a common source of infection (221).
2. Contrary to previous classifications, **Pneumocystis carinii** infection is currently regarded as a fungal infection on the basis of ribosomal RNA analysis. It causes serious pneumonia and occasional extrapulmonary manifestations in patients with AIDS, and less often in those receiving immunosuppressive therapy including renal transplant recipients (222).
3. Disseminated **histoplasmosis** is an important risk to renal transplant recipients in Central and South America. Both primary infection and reactivation of dormant infection seem to occur. The disease is characterized by focal necrotic lesions in different viscera, bones, joints, meninges, endocardium, skin, and oral mucosa (223).
4. Invasive **aspergillosis** is a serious infection in renal transplant recipients, particularly after bacterial infection in neutropenic patients (224) and those with active cytomegalovirus (CMV) infection (225). The disease is characterized by interstitial pneumonia or consolidation with or without cavitation, often accompanied by intracranial, gastrointestinal, hepatic, cardiac, and osseous lesions. Interstitial renal disease may also occur, presenting as proteinuria, pyuria, and hematuria, with rapid loss of function. Fungal "balls" have been observed in the urine of such patients (226).
5. The rhinocerebral, pulmonary, renal, and disseminated forms of **mucormycosis** are rare infections that may be encountered in patients with uncontrolled diabetes, particularly with ketoacidosis or renal failure (210,227). They often complicate desferrioxamine therapy in patients on dialysis and are occasionally seen in renal transplant recipients, being strongly associated with the use of azathioprine (228). Mucormycosis is being increasingly recognized as a cause of acute renal failure, even in immunocompetent patients, due to fungal interstitial nephritis (229).
6. **Cryptococcosis**, which affects as many as 10% of patients with AIDS, is not a common opportunistic infection in patients receiving immunosuppressive therapy (230).

Parasitic Infections

Parasitic infestations influence the practice of clinical nephrology in the Tropics in three ways: (i) They are the causative agents of certain renal diseases, usually referred to as parasitic nephropathies; (ii) they are among the important agents that may infect immunocompromised patients; and (iii) they often modify the classic clinical picture, prognosis, and management of renal disorders at large.

Parasitic Nephropathies

Urinary tract disease that is due to parasitic infestation was known in pharaonic medicine. Several papyri, analogous to the medical textbooks of our time, accurately describe the clinical features of a disease called "âââ" attributed to a worm, acquired by exposure to Nile water (231). That this disease is indeed what we know today as schistosomiasis is suggested by the close similarity of the clinical features and by finding calcified schistosomal ova in the bladders of ancient Egyptian mummies (232). Schistosomiasis remains one of the most common parasitic nephropathies, exerting its effect mainly through the induction of lower urinary tract pathology. That the parasite may also lead to glomerular disease was suggested only a few decades ago (233,234), and subsequently confirmed by other investigators in Latin America (235,236), Africa (237–241), and Asia (242).

Malarial nephropathy was next on the list, being referred to in the Hippocrates collection, dating back to the fourth century BC, and suspected by Atkinson late in the nineteenth century (243). It remained for Giglioli (244) to present evidence for a potential cause-and-effect relationship between malaria and the nephrotic syndrome. Other investigators subsequently confirmed this suggestion on the basis of epidemiologic, experimental, and clinical grounds (20,245–250).

Schistosomiasis and malaria remain as the most relevant parasitic nephropathies of epidemiologic concern. Renal lesions have been described with many other parasitic infections (Table 78-2), but these are generally either too mild to achieve clinical significance (e.g., trichinosis) or geographically too restricted to be of epidemiologic importance (e.g., onchocercosis). On the whole, the incidence of renal involvement seems to be relatively low when depicted in relation to the global prevalence of parasitic diseases. However, there is a place for a lot of bias in this conclusion, because of the general unawareness of such complications, the lack of the necessary facilities to detect them in endemic areas, and the known difficulties in documentation and publication.

Parasitic Infections in the Immunocompromised

The renal patient may be immunocompromised as a feature of the primary disease, due to acquired infection with agents that impair the immune response such as human immunodeficiency virus (HIV), HCV, Epstein-Barr virus (EBV), and CMV, or as a consequence of therapeutic immunosuppression. Such patients are susceptible to a number of well-defined infections (Chapter 98). In the Tropics, the list of agents known to affect the immunocompromised host is even longer and involves some 20 parasitic species. These may be (i) acquired de novo, due to impairment of immunity to primary infection (e.g., cryptosporidiosis); (ii) reactivated, having been dormant due to a balanced host–parasite concomitant immunity (e.g., Leishmaniasis); or (iii) transmitted with a transplanted organ (e.g., trypanosomiasis) (Table 78-3). Unfortunately the mode of acquisition of clinical disease remains uncertain in most posttransplantation parasitic infections. Such knowledge is important, since it will settle the debate about the need for prophylaxis in transplanted travelers (259), pre-transplantation chemotherapy (260), and specific screening of potentially infective donors (278).

Although some of these infections produce insignificant morbidity, others may be associated with severe disease that may be fatal (e.g., strongyloides hyperinfection). The main clinical presentations of important parasitic infections in the immunocompromised are summarized in Table 3.

There are several interesting considerations regarding therapy, since several antiparasitic drugs may interact with immunosuppressive agents (e.g., quinine or chloroquine with cyclosporine) (301,302), or they may acquire a specific toxicity profile in transplant recipients (e.g., antimony-induced pancreatitis) (303). Conversely, immunosuppressive agents may have antiparasitic properties (e.g., cyclosporine and strongyloidiasis) (292,293).

Impact on the Clinical Patterns and Management of Renal Disease

The clinical patterns of renal diseases in the Tropics may be modified in different ways by associated parasitic infestations. Many infestations cause, or are associated with, malnutrition which, in addition to reflecting on the severity of the infestation

TABLE 78-2

REPORTED CLINICAL SPECTRUM OF PARASITIC NEPHROPATHIES

	Acute renal failure	Asymptomatic urinary abnormalities	Acute nephritic syndrome	Nephrotic syndrome	Chronic tubulointerstitial	Ureteral obstruction	Other features	Chronic renal failure
Caused by Protozoa								
Malaria								
Quartan		++		++	+			+++
Falciparum	+++	++	+	+				
Babesiosis	++[a]							
Visceral leishmaniasis		++		+[b]	+			
Trypanosomiasis African	++							
Toxoplasmosis				+				
Congenital				+				
Acquired								
Caused by Cestoda								
Echinococcosis		+					+[c]	
Caused by Trematoda								
Schistosomiasis								
Hematobium		+	+	+[b]	+	+++		+++
Mansoni		+		+++		+		+++
Opisthorchiasis	++	+					+[b,e]	
Caused by Nematoda								
Filariasis								
Wuchereria bancrofti		+	+	+			+[d]	
Onchocerca volvulus		++		+				+
Loa loa		+		+				
Strongyloidiasis		+		+				
Trichinosis		+						

Note: +, occasionally reported; ++, infrequently reported; +++, commonly reported.
[a]Usually with asplenia.
[b]Usually with amyloidosis.
[c]Mass effect of renal cysts.
[d]Chyluria.
[e]Jaundice.

per se (malaria, schistosomiasis, strongyloidiasis), augments nephrotic edema, increases anemia and bone disease in chronic renal failure, superimposes skin and peripheral nerve complications, and increases the risk of bacterial infection (304).

The associated chronic activation of the immune system is often blamed for increasing the incidence of secondary amyloidosis (305). The same was also attributed to impairment of macrophage function in kala azar (306). Immune activation may be expressed by hyperglobulinemia, which often poses diagnostic difficulties in the interpretation of false-positive results on serologic tests (239). Having mentioned that, it is important to note that autoimmunity may, indeed, be associated with certain parasitic diseases such as schistosomiasis, malaria, and filariasis (307–309).

Most parasitic diseases are characterized by multisystem involvement, which compounds the clinical picture, prognosis, and management of the associated renal disorder. Common examples are the extensive microcirculatory disturbance in falciparum malaria associated with acute renal failure (310), the chronic hepatic and lower urinary tract pathology in schistosomiasis (311), and the chyluria of filariasis (312). Such elements in the scenario may have a considerable impact on treatment strategies, use of medications, and choice of acute (313) and chronic (314) dialysis modalities as well as on the safety and efficacy of dialysis (315). They often influence the donor selection, the recipient's immunosuppression, and the eventual outcome of renal transplantation (316).

Individual Parasitic Diseases and the Kidney

Malaria. Malaria is a parasitic disease of great epidemiologic importance in the Tropics (Fig. 78-3), largely because of the warmth and humidity that favor the multiplication of mosquitoes, of which the anopheline species is the principal vector for malarial transmission. The incidence of malaria in the world is of the order of 300 to 500 million clinical cases each year, with mortality averaging 2 million per year. The disease is caused by a protozoan, Plasmodium, of which four species are pathogenic to humans, namely, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale, and Plasmodium falciparum (317). The clinical pattern of the disease, incidence of acute and chronic complications, and, consequently, the outcome

TABLE 78-3

IMPORTANT PARASITIC INFECTIONS IN THE IMMUNOCOMPROMISED

		De novo infection	Recrudescence	Transmission with graft	Disease	Clinical presentation	References
Circulatory parasites	Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae	Natural in endemic and indigenous areas (travelers, expatriates, exposure in airports); parenteral	vivax, malariae, ? Falciparum	Kidney, liver, bone marrow, multiorgan	Malaria	Fever, anemia, thrombocytopenia, acute renal graft dysfunction, hepatosplenic $\gamma-\delta$ lymphoma	251–263
	Babessia microti	Transfusion			Babesiosis	Fever, anemia, arthralgia, hemolytic uremic syndrome	264,265
	Schistosoma hematobium, mansoni	Reinfection	S. mansoni glomerulopathy		Schistosomiasis	Hematuria, proteinuria	266,267
Tissue parasites	Leishmania donovani		Kidney, bone marrow, heart, lung	Possible, not documented	Kala-Azar	Fever, splenomegaly, pancytopenia, acute renal graft dysfunction	268–274
	Trypanosoma cruzi	Possible, not documented	Heart	Debated	Chagas disease	Fever, subcutaneous nodules, cardiomyopathy	275–279
	Toxoplasma gondii	Possible, not documented	Heart, bone marrow, liver, kidney, multiorgan	Heart, bone marrow	Toxoplasmosis	Fever, lymphadenopathy, hemophagic syndrome, encephalopathy, Guillain Barré syndrome, hepatitis, pneumonia	280–289
Alimentary parasites	Strongyloides stercoralis		Heart, kidney, stem cells	Possible, not documented	Strongyloidiasis hyperinfection	Fever, GIT symptoms, pneumonia, ARDS, Eosinophilia	290–293
	Capillaria philippinensis		Debated	Possible, not documented	Capillaria hyperinfection	Fever, eosinophilia	294
Protozoa	Crytosporidium parvum		All solid organs		Cryptosporidiosis	Diarrhoea	295
	Acanthamoeba castellani		Bone marrow, kidney, lung		Acanthamebiasis	Keratitis, encephalitis, osteomyelitis, cutaneous ulceration	296–298
	Encephalitozoon cuniculi		Kidney, bone marrow,		Microsporidiosis	Conjunctivitis, encephalitis, pneumonia	299–300

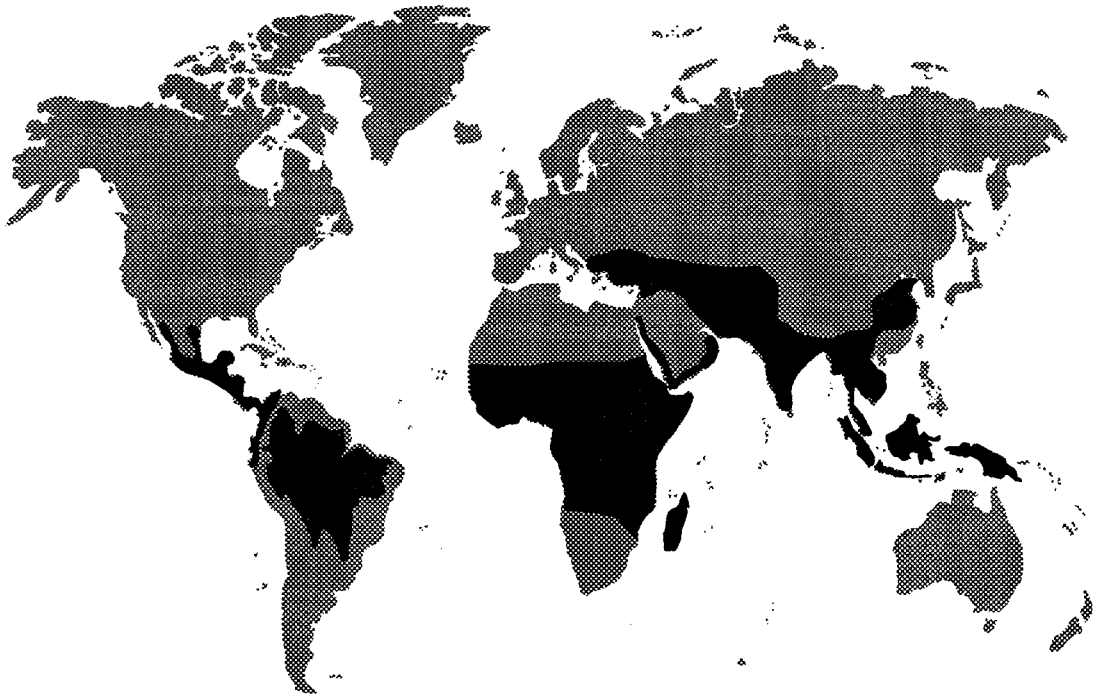

FIGURE 78-3. Global distribution of malaria.

are influenced by certain differences among the infective species. These include inherent features in their own life cycles as well as their selective adhesion to specific red blood cell and hepatocyte receptors (318). The age of infected erythrocytes is an important factor. Thus, whereas P. vivax and P. ovale infect only young red blood cells, and P. malariae infects only aging cells, P. falciparum infects erythrocytes at any age, explaining the heavy parasitemia associated with the latter.

The type and severity of clinical disease depends on the parasite's ability to modify the physical properties of parasitized red blood cells as deformability, fragility, and adhesiveness, as well as on the host's immune response to the parasite's antigens.

Clinical features. The disease is characterized by recurrent pyrexia with chills, the frequency of which varies according to the infective species. Constitutional features such as headache, malaise, and muscle and joint aches are usually encountered, more often with the first infection episode and in expatriates. Diarrhea, pulmonary edema, jaundice, coma, and circulatory failure may complicate falciparum infection, hence the term malignant malaria. The spleen is usually enlarged, particularly with relapsing disease. Anemia and neutrophil leukocytosis are prominent laboratory findings. The diagnosis is confirmed by direct visualization of the parasite in Giemsa-stained peripheral blood smears. Fluorescent staining with acridine orange enhances the diagnostic accuracy of peripheral blood examination. Routine serology was once of limited diagnostic value, particularly in endemic areas where old infection is highly prevalent. However, the recent introduction of synthetic peptides for the detection of epitopes on infected red blood cell membranes has opened a new diagnostic dimension (319). The use of polymerase chain reaction (PCR) technology for the molecular diagnosis of malaria is limited to research (320).

Renal involvement in malaria. Clinically significant renal disease may typically complicate infection with two malarial species, namely, P. malariae and P. falciparum. The former is associated with a chronic progressive syndrome, whereas infection with the latter is an acute illness that is usually reversible by adequate management. In recent years, the identity and specificity of P. malariae nephropathy has been debated, whereas P. vivax was implicated in the pathogenesis of acute renal failure (321), or the nephrotic syndrome (322). Epidemiologic studies are awaited to resolve this dispute.

Quartan malarial nephropathy. The causative link between P. malariae infection and glomerulonephritis is based on epidemiologic, experimental, and immunologic evidence (323–326). Thus, nephrotic syndrome and progressive glomerulonephritis are strikingly more prevalent in endemic areas, and their prevalence was significantly reduced with eradication of malaria (20,327–329). Glomerular disease was experimentally induced by the closely related species Plasmodium berghei (323) and Plasmodium brazilianum (330), yielding ultrastructural changes identical to those seen clinically. Even more direct evidence was obtained by finding malarial antigen in the glomeruli (331), which also suggests that glomerular injury is, indeed, immune-complex mediated (332).

Overt renal involvement is infrequent among patients with acute and chronic P. malariae infection, which suggests the interaction with other factors in the pathogenesis of the renal lesion. It is presumed that a certain immunopathologic setting may be necessary for the malarial antigen to induce clinically significant glomerular disease (333). This may be achieved by associated infection (e.g., Epstein-Barr virus) (330), autoimmunity (332), malnutrition (324), or a genetic predisposition (334).

Histopathological Features. The glomerular lesion is dominated by subendothelial deposits. These are mainly seen by light microscopy (Fig. 78-4) as thickening of the capillary walls, leading to a double-contour appearance of the basement membrane (324). By immunofluorescence, the deposits usually exhibit a coarsely granular pattern along the capillary endothelium and contain IgG (most commonly IgG3), IgM, C3 (335), as well as malarial antigens in 25% to 33% of patients, particularly

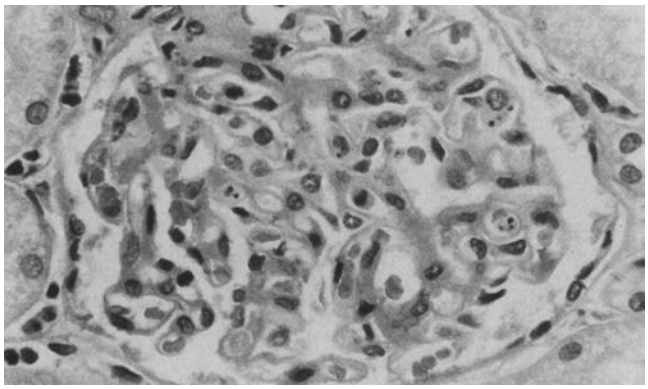

FIGURE 78-4. Malarial nephropathy. Note the pigment-laden macrophages in glomerular capillary lumina. (Hematoxylin and eosin, magnification ×200.) (From: V. Boonpucknavig, with permission.)

during early stages of disease (324,331). Less often, diffuse finely granular deposits containing IgG2 are encountered (335). Mixed patterns are not unusual. Electron microscopy shows subendothelial deposits of electron-dense or basement membrane–like material, associated with the formation of intramembranous lacunae (336).

A proliferative lesion, mainly involving the mesangium, is often encountered in adults. Crescents are rare in all age-groups. The extent of tubular involvement depends on the severity of glomerular lesions (324).

The pathology is initially focal and may indeed continue to be so, culminating with secondary focal and segmental sclerosis. In most patients, however, the pathology is diffuse and progressive, leading to ESRD.

Clinical Features. The clinical syndrome of quartan malarial nephropathy (324,329) is nonspecific, apart from its association with the features of the parasitic infection (vide supra). Most patients are children, with a mean age of 5 years. Proteinuria is encountered in a variable proportion of patients, up to 46% in the first published series (337). Microhematuria is occasionally noticed, particularly in the older age-groups. Overt nephrotic syndrome develops in a yet-undefined fraction, and hypertension is a late encounter. Serum complement levels are normal, and blood cholesterol values are usually not elevated, owing to the associated nutritional deficiency. The disease progressively leads to chronic renal failure over 3 to 5 years (324,329). Response to antimalarial treatment is poor and limited to patients in early stages (324,327).

Falciparum malarial nephropathy. Renal complications encountered in falciparum malaria include acute renal failure, glomerulonephritis, and a number of typical metabolic disturbances.

Acute Renal Failure. This is, by far, the most important malarial complication in the kidneys, being relatively frequent and potentially fatal. The reported incidence is 1% to 4% (338), but it may reach up to 60% in high-risk individuals (339). Acute renal failure usually occurs in patients with heavy parasitemia (infected erythrocytes, more than 5%), particularly in pregnant women (340), HIV-infected individuals, and foreigners visiting endemic areas. It also occurs in almost all cases of blackwater fever.

Patients developing malarial acute renal failure (MARF) have the typical ***clinical features*** of severe falciparum disease with profound disturbances of the microcirculation, multiple organ involvement, particularly the liver, and prominent metabolic disturbances including hypoglycemia, lactic acidosis, and hyponatremia.

MARF is usually oliguric and hypercatabolic, with high BUN/creatinine ratio. Hyperkalemia can be profound in patients with intravascular hemolysis. Hyperuricemia, disproportionate of the nonprotein nitrogen retention, is frequently seen. The oliguric phase usually lasts for a few days to several weeks. Mortality is usually within the range of 30%, but it may vary from 10% to 50%, depending on the severity of infection, response to antiparasitic treatment, availability of dialysis, and the predominant pathogenetic factors involved.

Therapy usually poses challenging problems, because of the complexity of the syndrome. Quinine remains the drug of choice. Artemisinin derivatives have been used with promising results in decreasing parasitemia, particularly in quinine-resistant patients. Early dialysis is often needed to treat the hypercatabolic state. Although peritoneal dialysis is less effective because of the supervening circulatory disturbances, it becomes more effective as the flow in microcirculation is improved by treatment. It is often the only available dialysis modality available in underdeveloped communities. In some cases, continuous peritoneal dialysis may be indicated. Exchange transfusion is helpful in patients with heavy parasitemia and those with severe jaundice.

Pathogenesis of MARF. MARF is basically an ischemic acute tubular necrosis (ATN), attributed to shock, intrarenal vascular abnormalities, endothelial activation, and capillary occlusion by red blood cell and platelet sludging. The ***pathogenesis*** is multifactorial, involving a complex interaction of rheologic and immunologic components (341) (Fig. 78-5).

1. *Disturbances in Blood Rheology.* The parasitized red blood cell is the cornerstone of all events. Characteristic of P. falciparum infection are particular red blood cell membrane abnormalities (342,343) leading to the formation of adhesive knobs (344,345) (Fig. 78-6), composed of abnormal proteins encoded by the parasite's genome. The major family of adhesive proteins is called plasmodium falciparum erythrocyte membrane proteins (PfEMPs) (346), which includes several members identified by sequential numbers. The PfEMP-1 appears to be the major determinant of erythrocyte adhesiveness and subsequent malarial morbidity. A striking feature of this protein is variability, being structurally and antigenically different in consecutive generations of parasitized red blood cells. This is attributed to switching in-between alleles in the "Major Var Gene" in successive merozoite generations. Switching is largely a spontaneous process (347) but is also influenced by host factors, as shown by the effect of splenectomy in experimental models (348,349). This "cross-talk" between the parasite and the host keeps the balance that permits both to survive.

   Other adhesive protein families have been identified in the erythrocyte knobs including the histidine-rich proteins (345,350), rifins (351), rosettins (352), and others. Most of these are also variable proteins of importance in the host–parasite relationship, but their relative pathogenetic significance seems to be less prominent than that of the PfEMP-1.

   The main red blood cell receptors for PfEMP-1 are the erythrocyte complement receptor (CR1) and glycosaminoglycans (GAGs) (353,354). There are many platelet and endothelial receptors. Although some are constitutively expressed, as CD36, PECAM-1/CD31, thrombomodulin and chondroitin-4-sulfate, many are induced by the host's immune response as a part of widespread endothelial activation (355) including E-selectin, P-selectin, ICAM-1, and VCAM-1 (356–361).

   The physiologic deformability of parasitized red blood cells is impaired (362) and their mechanical fragility

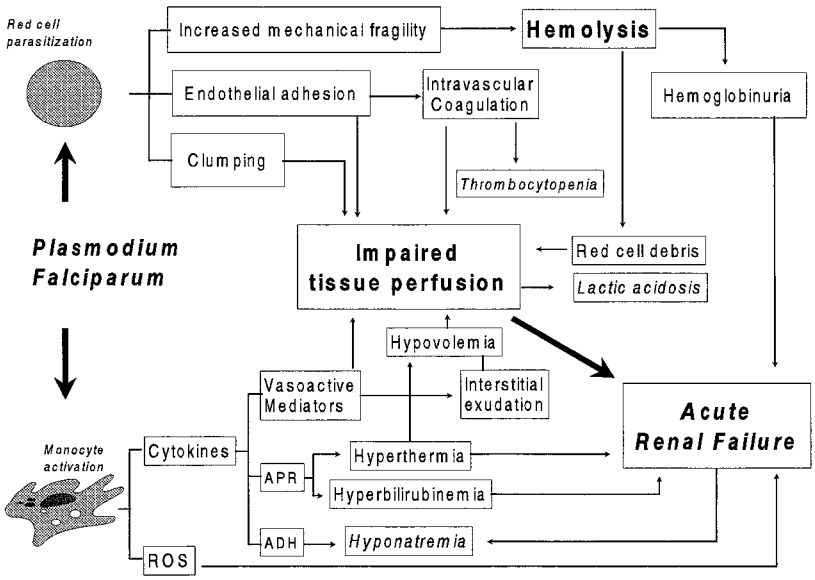

FIGURE 78-5. The pathogenesis of acute renal failure in malaria. *ADH*, antidiuretic hormone; *APR*, acute-phase reactants; *ROS*, reactive oxygen species.

increased (363), which leads to hemolysis. Although this is usually extravascular, intravascular hemolysis may also occur, particularly in patients with G6PD deficiency, who are receiving quinidine or pyrimethamine therapy. In extreme forms, massive intravascular hemolysis leads to the frank hemoglobinuria characteristic of blackwater fever. Red cell debris and their free products exert their hemodynamic and toxic ill effects, which include vasoconstriction, renal tubular toxicity, and activation of intravascular coagulation (364,365). Activation of coagulation factors, thrombocytopenia, and increased plasma fibrin degradation products (366) have been well documented. However, glomerular capillary thrombosis is uncommon, and fibrin deposits have not been consistently seen by immunofluorescence.

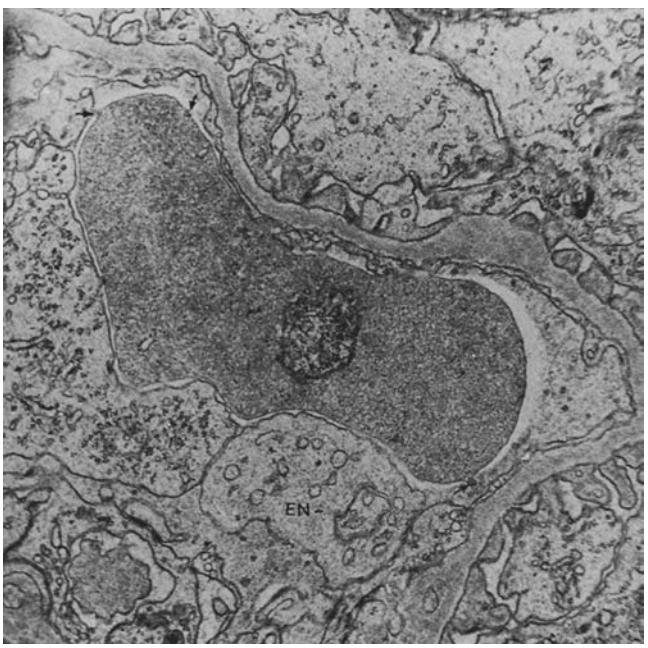

FIGURE 78-6. Adherence knobs on the parasitized erythrocyte membrane (arrows) to the glomerular endothelium (EN). (From: Sitprija V. Nephrology forum: nephropathy in falciparum malaria. *Kidney Int* 1988;34:866, with permission.)

2. *Immunologic Perturbation.* Antigenic proteins are expressed on the cell membranes of the parasite as well as the parasitized host cells. However, owing to the intracellular residence of the sporozoan during most of its life cycle in humans, it is the antigen expression on the parasitized red blood cells that constitutes the major part of the antigen load. In addition to the adhesive proteins described earlier, other antigenic proteins have been identified on parasitized red blood cells, monocytes, and hepatocytes. These include "var" gene products other than the PfEMP-1 (367), the ring-infected erythrocyte surface antigen (RESA) (368), Pf332 (369), 70-kDa and 78-kDa heat shock proteins (370), and others.

There is evidence that different antigens may provoke different immune responses. For example, glycosylphosphatidylinositol moieties covalently linked to the surface antigens of falciparum malarial parasites appear to act like endotoxin (371) that interacts with upregulated monocyte CD14 receptors (372), thereby provoking a Th_1 pro-inflammatory response. Conversely, the Pf332 antigen appears to interact with a different monocyte receptor that favors Th2 proliferation (369), which is associated with immunity to reinfection.

The hallmark of pro-inflammatory monocyte activation is the release of TNF-α, which plays a pivotal role in the pathogenesis of acute malarial morbidity (373). Of equal significance is the release of IL-6, which is the main proliferative signal for Th_1 cells (306,374). The latter secrete IFN-α; which amplifies the monocyte response by a positive feedback mechanism (375). The observed increase in neopterin serum levels in malignant malaria (376) is the consequence of this augmented release of IGF-I.

Th_1 activation also leads to B-lymphocyte proliferation, with IL-2-induced switching to IgG2 synthesis. This often leads to autoantibody formation that is usually associated with P. falciparum and P. vivax infections. Anticardiolipin (377), antiphospholipid (378), and anti-neutrophil cytoplasmic antibody (ANCA) (379) have been suggested to have a role in the pathogenesis of microvascular complications. Antibodies to triosephosphate isomerase (380) have been associated with prolonged complement-dependent hemolytic anemia following acute malaria.

Th_2 activation has an immune modulatory function and is associated with immunity to reinfection. The release of IL-4 induces B-lymphocyte proliferation, favoring IgE and

IgG4 synthesis (381). Together with IL-10 (382), IL-4 downregulates the monocytes and inhibits the release of IL-8 (383). However, Th_2 lymphocytes have been recently shown to behave as pro-inflammatory cells. This is particularly manifest in cerebral malaria, where IgE antibody levels are considerably elevated (384). Malarial antigen–IgE antibody complexes are identified by CD23 monocyte receptors, leading to increased TNF-α generation through an NO-transduction mechanism (385). The interaction of TNF-α and IL-5 leads to eosinophil activation, which seems to be crucial in cerebral malaria (386).

Peripheral gamma-delta T-lymphocytes are strongly upregulated in malaria (387). They seem to have a mandatory role in the regulation of the early immune response and the elimination of chronic infection. However, their exact role in this respect is not yet understood.

3. *The Integrated Scenario*. As a consequence of early increase in pro-inflammatory cytokines, the peripheral blood capillaries, mainly in the skeletal muscles, are dilated, leading to decreased peripheral resistance. Blood volume is consequently increased (388,389), being associated with increased cardiac output. In severe infection, blood tends to pool in the peripheral capillaries, with fluid transudation into the interstitial tissues, owing to increased permeability (389,390). The slow circulation is further impeded by the previously mentioned rheologic abnormalities, leading to impaired cardiac filling. Together with the frequently associated drug-induced left ventricular dysfunction (391), or, rarely, parasite-induced pericardial tamponade (392), this leads to a critically low cardiac output, and impaired renal perfusion. The latter is augmented by the supervening nonspecific humoral imbalance (see subsequent text), endothelial cell activation, and sludging of red blood cells and platelets. Free hemoglobin and myoglobin add their independent ill effects to the renal hemodynamics and tubular cell integrity, leading to ATN.

Glomerulonephritis. Glomerular lesions may be associated with falciparum malaria at any age, although children remain the main population at risk (338,339,393). It is impossible to estimate the true incidence because the disease is essentially mild, transient, and overshadowed by other complications. Glomerular lesions were detected in 18% of patients with falciparum malaria who underwent autopsy (394). Mild proteinuria, microhematuria, and casts are found in 20% to 50% of patients (339). Nephrotic (395) and acute nephritic (395,396) syndromes are occasionally seen, but hypertension is unusual. Serum C3 and C4 levels may be reduced during the acute phase. The disease is very rarely progressive. In contrast to quartan malarial nephropathy, falciparum glomerulopathy is reversible within 2 to 6 weeks after eradication of the infection.

The glomerular lesion is characterized by prominent mesangial proliferation with many transit cells. Mesangial matrix expansion is modest, and basement membrane changes are unusual. Deposition of an eosinophilic granular material has been noticed along the capillary walls, within the mesangium, and in Bowman's capsule (397). The glomerular capillaries are often empty, but they may contain a few parasitized red cells or giant nuclear masses in patients with intravascular coagulation (398). Immunofluorescent study shows finely granular IgM and C3 deposits along the capillary walls and in the mesangium. Malarial antigens are occasionally seen, analogous with an animal model (331), along the glomerular endothelium and the medullary capillaries (Fig. 78-7). ICAM-1 is expressed in the glomerular mesangium, vascular endothelium, and proximal tubular cells. The tubules and the vascular endothelium show expression of TNFα IL-1, IL-6, and granulocyte–macrophage colony-stimulating factor (399,400). Electron microscopy shows subendothelial and mesangial electron-dense deposits along with granular, fibrillar, and amorphous material (398).

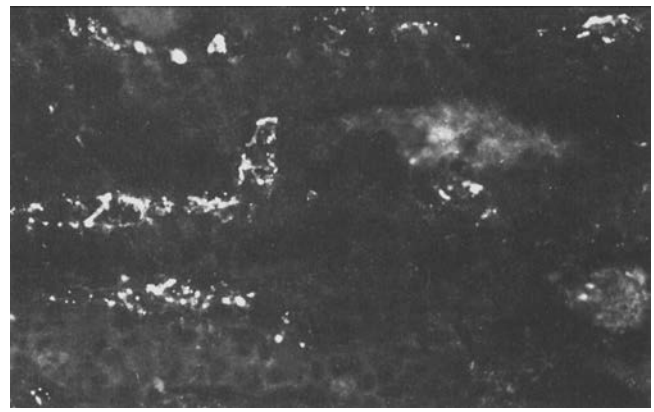

FIGURE 78-7. Deposition of granular malarial antigen along the capillaries in the renal medulla. (Anti–Plasmodium falciparum, magnification ×200.) (From: V. Boonpucknavig, with permission.)

There is general agreement that the glomerular lesion in falciparum malaria, like that associated with quartan malaria, is immune-complex mediated. Yet, there is an ill-defined proportion of cases in which the glomerular lesions are attributed to direct activation of the alternative complement pathway (366), thereby explaining the paucity of immune-complex deposits.

Metabolic Abnormalities. The metabolic abnormalities associated with, and often contributing to acute malarial nephropathies are the consequence of: (i) perturbation of the host's cell membranes, (ii) the parasite's consumption of nutrients and cofactors, (iii) repercussions of the described hemodynamic and immunologic disturbances, and (iv) side effects of treatment (401).

1. *Cell Membrane Abnormalities*. In addition to the de novo synthesis of antigenic and adhesive polypeptides encoded by the parasite's DNA, changes in the constitutive parasitized cell membrane structure may lead to important functional abnormalities. Most notorious in falciparum malaria is the inhibition of the erythrocyte magnesium-activated ATPase (343). This impairs the sodium pump in a "sick-cell syndrome" fashion, leading to internal dilutional hyponatremia (402). Secondary calcium influx alters the calmodulin-dependent erythrocyte kinetics (403), reduces hemoglobin–cell wall interaction and curtails red blood cell deformability (362). This further augments the peripheral hemodynamic derangement and increases the erythrocyte mechanical fragility. Shortened red blood cell survival is, therefore, almost invariable in malaria (404).

   Even nonparasitized red blood cells undergo membrane changes that lead to rosette formation (405). Many factors have been implicated in this phenomenon including parasite-derived rosettins (352), immunoglobulins, and other plasma protein abnormalities. As mentioned previously, rosette formation is an important contributor to the disturbed peripheral blood rheology in malaria.

   It is not clear whether the same or other factors, or the abnormal cytokine profile supervening in malaria, are responsible for the hepatocyte membrane abnormality that leads to cholestatic jaundice in the majority of cases (406). The same question applies to leaky muscle membranes associated with malarial rhabdomyolysis (407).
2. *Parasite's Energy Consumption* Plasmodia consume large quantities of glucose for their metabolism. This seems to overwhelm the host's compensatory mechanisms, often

leading to clinically overt hypoglycemia. A recent study has also shown an increase of the serum transketolase activity in patients with falciparum malaria. This indicates thiamine depletion, which is attributed to the increased demand for the parasite's glycolytic pathway (408). Because thiamine is an essential cofactor for coenzyme A, its depletion may be an additional factor in depressing the host's aerobic glycolysis, causing increased anaerobic glycolysis (Pasteur effect) and lactic acid accumulation. However, none of the clinical manifestations of severe malaria, particularly the neurologic aspects, could be statistically correlated to measurable biochemical parameters of thiamine deficiency.

Other factors contributing to hypoglycemia in malaria include hyperinsulinemia associated with quinine (409) or sulfadoxine-pyrimethamine (410) therapy and possibly the impaired glucogenesis in patients with significant hepatic complications.

3. *Consequences of the Hemodynamic and Immunologic Perturbation.* The disturbed peripheral blood rheology, the release of local cytokines, and the immune-mediated systemic inflammatory response integrate in the pathogenesis of peripheral pooling leading to impaired tissue oxygenation.

The metabolic consequences of tissue hypoxia and potential impairment of glucose availability include increased lactic acid production with increased lactate:pyruvate ratio (411), depressed mitochondrial respiration (412), and increased generation of reactive oxygen molecules (413). Inducible NO generation and abnormal lipid peroxidation are documented consequences of the increased oxidative stress in falciparum malaria (414).

A lot of secondary humoral changes are seen in falciparum malaria. These include hypercatecholaminemia (415); increased levels of circulating plasma renin activity, kinins, and prostaglandins (338); ADH secretion (416–418); and hyperinsulinemia in quinine-treated patients (409). Most of these effects are nonspecific, being attributed to the severe acute infection. However, they seem to have a significant impact on the final target of all pathogenetic factors in malignant malaria, namely, the microcirculation.

Fluid and electrolyte changes in malaria are of clinical and physiologic interest. Hyponatremia, usually asymptomatic, is observed in 67% of patients (416). Hyponatremic patients have a higher parasitemia than those with normal serum sodium levels (418). There are multiple causes including increased nonosmotic ADH release, low sodium intake, sodium loss, cellular sodium influx, and resetting of osmoreceptors. Hypernatremia, usually associated with hypothalamic injury, is rare. Hypokalemia, attributed to augmented tubular loss, is common (419). Hyperkalemia is seen in patients with intravascular hemolysis, rhabdomyolysis, or renal failure. As in sepsis, hypocalcemia and hypophosphatemia are seen in severe malaria (371,420). Hypocalcemia without renal failure may be caused by unexplained hypoparathyroidism (421). Phosphate shift into the cells induced by respiratory alkalosis accounts for hypophosphatemia (422). Both hypocalcemia and hypophosphatemia are transient and resolve with clinical improvement. Of clinical importance is the decreased response to water load, which can be observed in moderate and severe malaria. In this clinical setting, hemodynamic changes include hypervolemia, decreased peripheral vascular resistance, increased cardiac output, elevated plasma renin activity, and increased plasma norepinephrine levels, ADH, and renal vascular resistance (418). Hyponatremia is an important clinical marker in these patients. A decreased response to water load occurs in 20% of hyponatremic patients. Fluid administration to the patient with moderate or severe malaria should be cautiously done to avoid fluid overload. Furosemide should be given when there is decreased response to fluid load.

Babesiosis. This rare febrile disease (423) is closely related to falciparum malaria. It is not strictly tropical, as most cases have been described in Eastern Europe and the United States. However, it is often confused with falciparum malaria in patients returning from the Tropics. Either Babesia microti or Babesia divergens is responsible for infection. The disease manifests with fever, chills, and malaise associated with nausea and arthralgias. It is particularly severe in asplenic patients, who are more likely to develop acute renal failure as a result of hypotension, intravascular hemolysis, and disseminated intravascular coagulation. Mesangial proliferative glomerulonephritis with deposition of IgG and C3 is observed (424,425). Babesiosis has been reported in a renal transplant recipient due to transfusion with contaminated blood (265). Diagnosis is made by finding the intraerythrocytic parasite in a blood smear or by a serologic test. Therapy with quinine sulfate and clindamycin is usually effective in eradicating the parasite. Pentamidine is effective in resistant cases.

Visceral Leishmaniasis. This disease, also known as kala azar (or black sickness) (306), is one of three forms of human leishmaniasis with worldwide distribution, being highly prevalent in East and North Africa, northeastern China, Iran, the Mediterranean region, and Brazil. It is caused by Leishmania donovani, a protozoan that infects humans through a sandfly bite. The parasite lives and multiplies in the monocytes, being protected by a peculiar host–parasite interaction that involves depression of cell-mediated immunity.

Most patients remain asymptomatic, often with spontaneous cure. Leishmaniasis can occur in the immunocompromised individual. Overt disease ushers in with pyrexia, chills, sweating, asthenia, weight loss, and hepatosplenomegaly. Lymphadenopathy is often noticed but is seldom striking. Skin nodules containing the parasite may be seen, particularly at the inoculation site. If untreated, the disease may end fatally, usually due to intercurrent infections. Response to treatment with pentavalent antimony is slow and often incomplete. Primary resistance is encountered in 10% of patients.

In the kidney, the parasite induces a chronic inflammatory interstitial lesion (Fig. 78-8) that rarely progresses to any appreciable degree of fibrosis (426). The protozoan may be seen within the infiltrating monocytes, with little lymphocytic infiltration that reflects the characteristic depression of macrophage function (306). To the same mechanism is ascribed the frequent development of secondary amyloidosis (427). Immune complex–mediated glomerular lesions have also been described in kala azar (428–430), mesangioproliferative glomerulonephritis being the most common pattern (Fig. 78-9). IgM and C3 deposits are often found in the capillary walls and mesangium. Leishmania membrane antigen is detected (431).

Renal involvement in kala azar is usually subclinical. An occasional patient with secondary amyloidosis may present with gross proteinuria or even the nephrotic syndrome, but this is usually encountered as a terminal event in the patient's illness.

Trypanosomiasis. African trypanosomiasis (432), caused by Trypanosoma brucei and transmitted by the tsetse fly, is the cause of sleeping sickness. The Rhodesian version is an acute febrile illness with neurologic and cardiac manifestations, anemia, and disseminated intravascular coagulation that often ends fatally within a few weeks. Acute renal failure may be encountered during the terminal phases as a component of multiorgan failure.

The Gambian type is more chronic, initially presenting with pyrexia, lymphadenopathy, and a skin eruption, followed several months or even years later by progressive meningoencephalitic manifestations.

Experimental infection in monkeys by T. brucei leads to proliferative glomerulonephritis, with IgM and C3 deposits in the

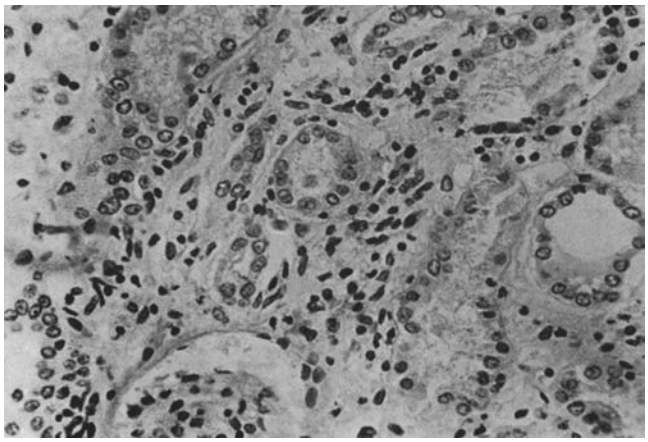

FIGURE 78-8. Renal involvement in visceral leishmaniasis: interstitial edema, interstitial mononuclear infiltration, and degeneration of some tubular cells. (Hematoxylin and eosin, magnification ×250.)

mesangium and along the capillary walls. Serologic studies in this model showed high levels of circulating immune complexes and a reduction of serum C3 levels with normal C4 levels, suggestive of direct alternative complement activation by the parasitic antigens. Similar observations were made in a murine model, in which electron-dense deposits were also found in the mesangium and subendothelial space (433). Whether similar lesions also occur in humans is unknown. Clinically relevant renal complications have not been reported.

South American trypanosomiasis, known as Chagas disease, has attracted considerable recent attention in view of its potential role in organ transplantation (vide supra).

Toxoplasmosis. Toxoplasma gondii infection (434) is common worldwide, with a current estimate of 500 million infected humans. It is acquired through contact with cats and certain birds, by ingestion of cysts or oocysts. It can also occur through transplacental transmission (congenital toxoplasmosis). In most instances, the infection is dormant, with a few cysts lying in the lymph nodes, muscle, heart, or brain and little or no inflammatory response.

Overt clinical disease is rare in the immunocompetent individual. It usually manifests by cervical lymphadenopathy associated with pyrexia and malaise. The disease is self-limiting in most patients. Progressive chorioretinitis is the notorious expression of congenital toxoplasmosis in the immunocompetent individual.

In the immunocompromised, the disease is much more disseminated, presenting with pyrexia, hepatosplenomegaly, pneumonitis, maculopapular rash, myositis, myocarditis, meningoencephalitis, or central nervous system mass lesions. The course is rapidly fatal.

Renal involvement in the immunocompetent and the immunocompromised host has been rarely described. The lesion is mainly glomerular, with a mesangioproliferative pattern. Mesangial IgM and Toxoplasma antigen deposits have been detected by immunofluorescent study. Renal infection is usually subclinical, although the nephrotic syndrome has been described in association with chorioretinitis in congenital toxoplasmosis (435).

Cryptosporidiosis. The causative protozoan, Cryptosporidium, belongs to the same family as T. gondii. The disease is also acquired through contact with infected domestic animals. It has recently emerged as an important opportunistic infection in immunocompromised patients in the Tropics and in the West, causing severe watery diarrhea, abdominal cramps, and occasional infection of the biliary system, leading to ascending cholangitis and rarely gangrene of the gallbladder. It is not surprising that renal involvement can occur through nonspecific inflammatory factors resulting in proteinuria and urinary sediment changes. There is currently no effective therapy for cryptosporidiosis (436).

Echinococcosis. Humans act as an intermediate host for Echinococcus granulosus, a parasite of worldwide distribution, mainly encountered among sheep-herding populations and those in close contact with dogs. Infection is acquired by ingestion of eggs contaminating the environment through the feces of infected dogs. The ova hatch in the gut, and larvae migrate into the liver, lungs, brain, kidneys, muscles, and other tissues where they eventually yield cysts. The cysts contain and reproduce protoscoleces, which have the potential of completing the life cycle if ingested by a dog.

Echinococcosis (hydatid disease) in humans is characterized by multiorgan infection. Involvement of the kidneys may be silent, being discovered only during routine imaging. Hilar cysts have been described to cause caliceal back pressure and to stretch the arteries, leading to renovascular hypertension (437).

Immune-mediated glomerular injury has also been described. The disease is usually mild or subclinical. It affects young adults and presents with mild-to-moderate proteinuria and an increase in the urinary cellular elements. Renal

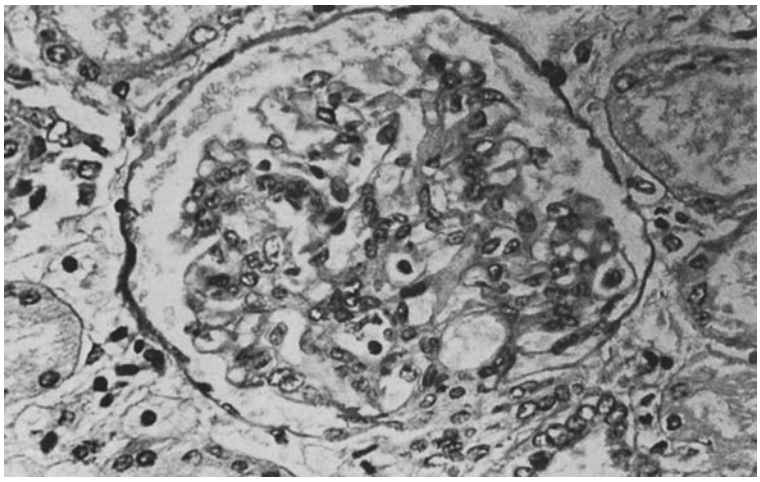

FIGURE 78-9. Kidney involvement in visceral leishmaniasis: Focal proliferative glomerulonephritis and mesangial expansion and presence of amorphous material in Bowman's space. (Periodic acid–Schiff stain, magnification ×250.)

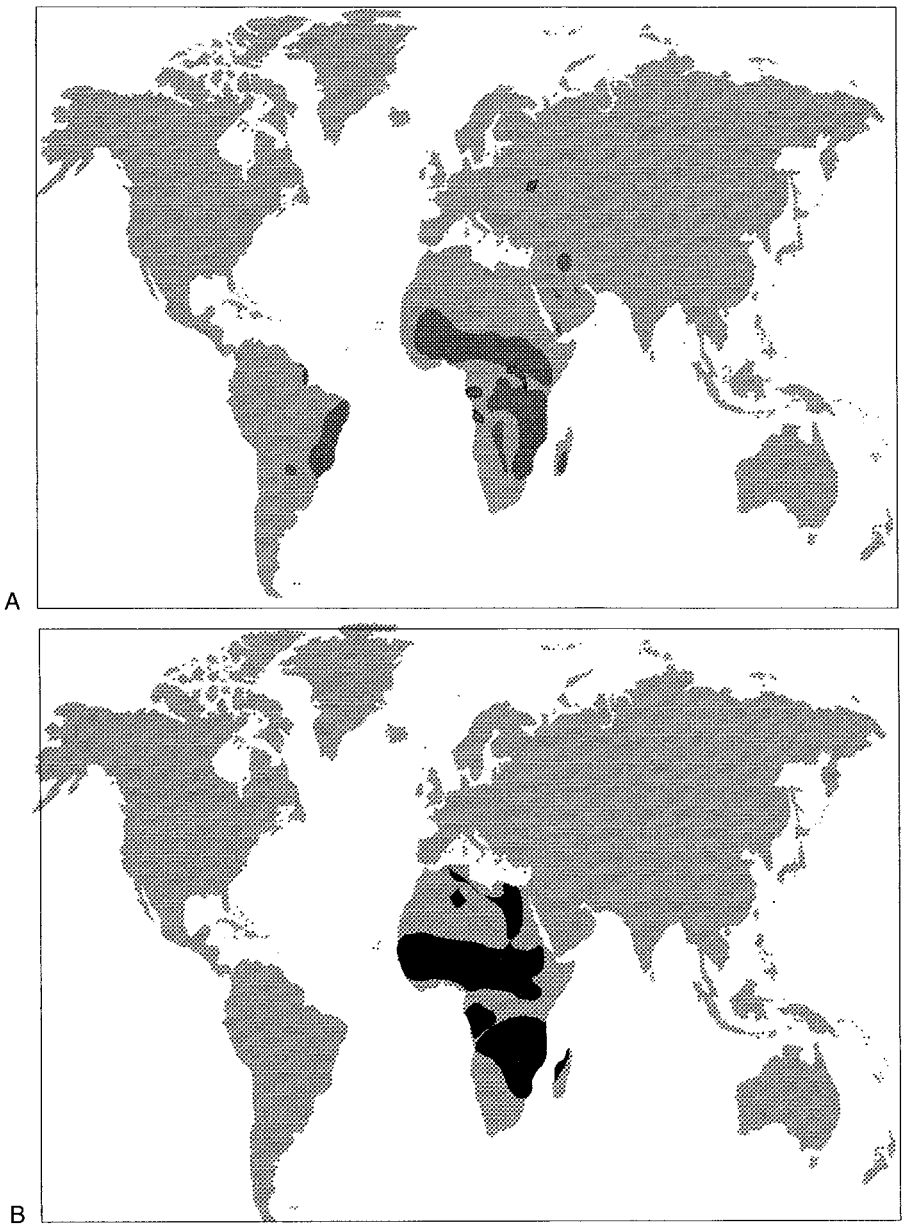

FIGURE 78-10. Global distribution of relevant species of schistosomiasis. **A:** Schistosoma mansoni. **B:** Schistosoma haematobium.

biopsy results in several patients revealed a mesangioproliferative glomerulonephritis with IgM and C3 deposits (438). Parasitic antigens have not pursued in the glomeruli, but they are readily detected in circulating immune complexes, particularly after mebendazole treatment or surgical intervention (437).

Schistosomiasis. Schistosomiasis is among the most widely spread of parasitic diseases, with an estimated 500 to 600 million people at risk, 200 million actually infested, 20 million with serious morbidity and 20 thousand annual deaths attributed directly to the disease (439) (Fig. 78-10). There are many strains of schistosomes, of which only five infect humans: Schistosoma haematobium, mainly in Africa; Schistosoma mansoni in Africa and South America; Schistosoma japonicum in China, Southeast Asia, and Japan; Schistosoma intercalatum, related to S. mansoni and found mainly in central Africa; and Schistosoma mekongi, related to S. japonicum and found in a few foci in Southeast Asia.

Infection is acquired through contact with fresh water harboring the specific snails, which act as the intermediate hosts and define the prevalence and density of infestation in different geographic areas. The infective agent is the cercaria, a fishlike organism with a bifid tail, 400 to 600 μm in length; the organism penetrates the skin or mucous membranes, loses its tail, and becomes a schistosomula. The latter gains access to the bloodstream through lymphatics and circulates in different capillary beds until it randomly reaches the portal or perivesical venous plexus. These sites contain trophic factors that promote the rapid growth of the schistosomula, which soon matures into an adult worm in the downstream veins. The adult worm is bisexual, the male being stronger and larger. The female stays in almost continuous copulation with the male in a special groove called the gynecophoral canal. It only leaves to travel against the bloodstream to lay eggs in the submucosa of the urinary bladder (S. haematobium), colon, or rectum (S. mansoni and S. japonicum). During its active sexual life, the female S. haematobium or S. mansoni lays about 300 ova per day, whereas S. japonicum may lay up to 3,000 ova per day. Most ova find their way to the exterior by virtue of spines in their shells, aided by the muscle contractions of their habitat.

Contact with fresh water leads to swelling and rupture of the shells, and release of miracidia, the infective stage to the snail, which completes the cycle (440).

Ova that fail to be exteriorized are the principal cause of pathogenicity. They cause a delayed hypersensitive reaction and granuloma formation that heals with fibrosis (441). The resulting functional consequences depend on the strategic anatomic sites of such fibrosis. Ova may be driven back along the bloodstream to deposit in different organs, where they also form granulomas. Most notorious are the hepatic granulomas, leading to periportal fibrosis and portal hypertension, and the pulmonary granulomas, which result in arteriolar occlusion and pulmonary hypertension. Other metastatic lesions include cerebral granulomas, often seen with S. japonicum infestations, subcutaneous nodules, and ocular granulomas, which are occasionally mistaken for other clinically similar conditions (442).

Renal disorders associated with schistosomiasis

S. Haematobium. The deposited ova form small tubercles that superficially resemble those of tuberculosis, hence, the name pseudotubercles. These coalesce to form nodules, which often get secondarily infected. With the evolution of the lesions, the underlying bladder wall becomes fibrotic and the trapped ova become calcified. A thin mucosal layer may cover these lesions, which appear as pale granular areas called sandy patches. Other late lesions include mucosal cysts and fibrotic nodules, cystitis cystica, and cystitis glandularis. Bladder malignancy often supervenes, a slowly growing squamous cell carcinoma being the predominant histopathologic pattern (443).

Functional consequences often complicate urinary schistosomiasis, depending on the site and extent of fibrosis. At the lower ends of the ureters, this leads to partial obstruction; at the bladder neck, it results in chronic outflow obstruction; and in the detrusor, it impairs contractility, resulting in an atonic viscus, which is often associated with vesicoureteric reflux. Upstream repercussions are attributed to ureteric obstruction, reflux, and chronic infection (311) (Chapters 24, 25, 34, and 36).

The clinical features of schistosomiasis haematobium are encountered soon after the establishment of infection. Painful terminal hematuria is the time-honored presentation. It often affects children, more frequently men, reflecting the variance in exposure imposed by social constraints. The diagnosis is confirmed by finding the characteristic ova, with terminal spines, seen with routine urine microscopy. Pyuria and bacilluria are also detected in the presence of secondary infection. If untreated, hematuria persists for many years, although its intensity declines as the process of bladder fibrosis progresses. Eventually the patient is left with the symptoms of chronic cystitis, and upstream complications may develop.

Renal morbidity (Fig. 78-11) is reported in different endemic areas to vary from 2% to 52% of patients with lower urinary tract schistosomiasis haematobium, depending on the infective strain, intensity of infestation, availability of therapy, and probably genetic predisposition (444).

Because the disease is usually localized in the submucosa of the lower ureteric sites, the proximal healthy part often undergoes significant hypertrophy, which allows adequate urodynamic compensation without any back-pressure sequelae. However, hydronephrosis develops in 9.7% to 48.0% of patients who fail to maintain this compensatory mechanism (311,444). Ascending infection is common, usually after instrumentation performed for various reasons. It is particularly common in patients with vesicoureteric reflux, again frequently induced by surgical or instrumental interventions. In addition to the regular features of acute and chronic pyelonephritis, infection often leads to the formation of stones. The proportion of patients with schistosomiasis haematobium who ultimately progress to end-stage is unknown; a broad estimate in Egypt is 1 per 1,000 (445). With the high prevalence of infestation, even this small proportion amounts to 40 patients per million of the total population.

FIGURE 78-11. Postmortem material showing the spectrum of urinary tract lesions in schistosomiasis haematobia. Note (**a**) the thickening of the bladder wall, dirty mucosa, and villous growth (squamous cell carcinoma); (**b**) the dilated ureters with cystic lesions (bilharzial cystitis cystica); (**c**) the dilated right pelvicaliceal system (due to obstruction/vesicoureteric reflux); and (**d**) the scarred left kidney of chronic pyelonephritis. (From: Barsoum RS, Francis MR, Sitprija V, et al, In: Schrier RW, ed. *Atlas of Diseases of the Kidney*. Philadelphia: Current Medicine; 1999;6:10, with permission.)

Early haematobium infestation is easy to cure. Several drugs have been used through the years, starting with the old-fashioned antimony preparations and ending up with the more modern, highly effective, and safe compounds niridazole and praziquantel (446). Surgical treatment is needed occasionally for the relief of obstruction or repair of reflux. ESRD management is classic, although often modified because of the problems imposed by active chronic pyelonephritis and bladder fibrosis (316).

S. Mansoni. This organism is principally an inhabitant of the portal venous tributaries, with colorectal disease and periportal

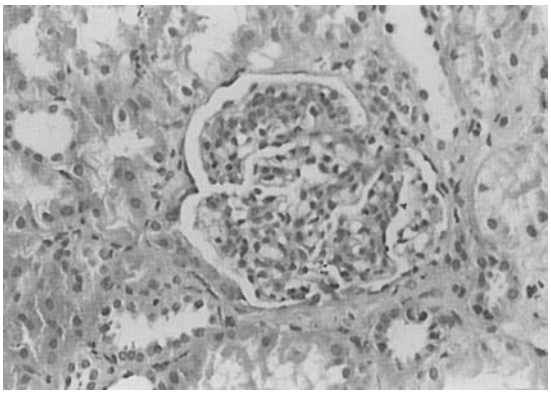

FIGURE 78-12. Axial mesangial proliferation in early schistosomal glomerulopathy. (Hematoxylin and eosin, magnification ×200.) (From: Barsoum RS. Schistosomal glomerulopathies. *Kidney Int* 1993;44:1, with permission.) (See Color Plate.)

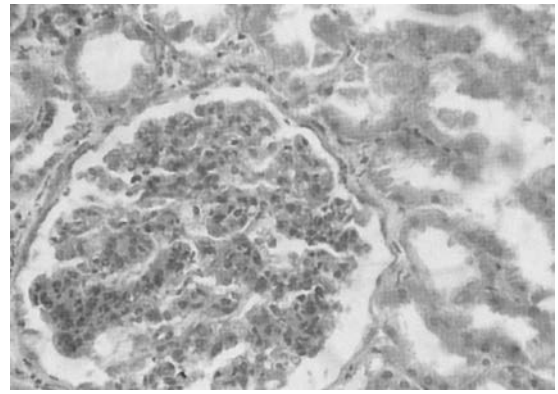

FIGURE 78-14. Exudative glomerulonephritis in the nephrotic syndrome associated with combined Schistosoma-Salmonella infection. Note the abundance of neutrophils and monocytes in the glomerular capillaries and mesangium. (Hematoxylin and eosin, magnification ×200.) (From: Barsoum RS. Schistosomal glomerulopathies. *Kidney Int* 1993;44:1, with permission.) (See Color Plate.)

hepatic fibrosis being the major consequences of infestation (434). Over the last three decades, experimental (447) and clinical (236,397) studies established that an immune-mediated glomerular injury may complicate this disease. Of the more than 100 antigens identified in the parasite, those of the adult worm's gut were incriminated in the pathogenesis of renal lesions. Particularly interesting are a proteoglycan and a glycoprotein, often referred to as cathodal and anodal antigens, respectively, found by immunofluorescent study in the early glomerular lesions associated with schistosomiasis (448,449).

The initial glomerular response in humans (450) and in experimental animals (449,451,452) is essentially mesangial, with focal or diffuse axial cellular proliferation and no matrix expansion (Fig. 78-12). IgM and C3 deposits are detected by immunofluorescent study (451,453,454), often along with parasitic antigens (Fig. 78-13) (449,454). This lesion is not specific for S. mansoni, being also observed in humans with S. haematobium infection (238,455) and in experimental animals infected with S. japonicum (447). Apart from the nature of the antigen deposits (456), this condition is morphologically similar to that seen with many other parasitic glomerulopathies.

Only mild clinical manifestations, usually limited to subnephrotic proteinuria and increased cellular excretion in urine, are associated with this lesion. There are considerable doubts about whether the lesion progresses in the absence of other factors (see subsequent text). The reported response to treatment with antiparasitic agents, steroids, and immunosuppressive agents has not been confirmed (457–461).

The initial glomerular lesions may subsequently progress into three distinct clinicopathologic syndromes.

1. *Exudative glomerulonephritis* (Fig. 78-14), which often complicates concomitant Salmonella infection, a frequent association in endemic areas (462,463), which leads to an acute, reversible nephrotic syndrome (91–93).
2. Progression into either *mesangiocapillary glomerulonephritis* (Fig. 78-15) or *focal segmental sclerosis* (Fig. 78-16), which seems to occur mainly in the presence of significant hepatic disease. The crucial role of the liver has been documented in experimental models (452,464) and clinical (465) and postmortem (466) studies. When associated with significant impairment of macrophage function (467), liver disease seems to permit a high worm antigen load to escape from the portal into the systemic circulation, thereby contributing to the formation of pathogenic immune complexes. Hepatic fibrosis has also been blamed for the defective clearance of IgA, originating in the gut in response to the parasite's antigenic products (468). Superimposed on the initial

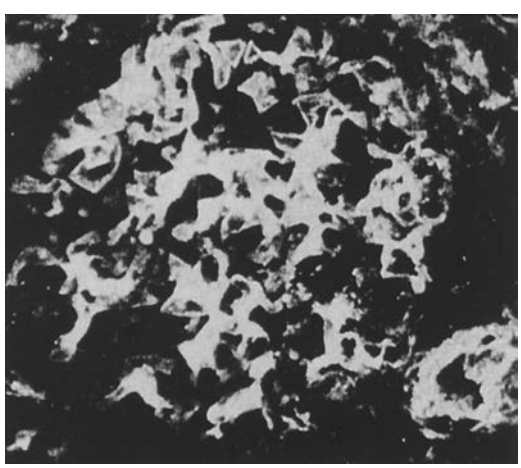

FIGURE 78-13. Mesangial deposits of schistosomal Gut antigens in early schistosomal glomerulopathy. (See Color Plate.)

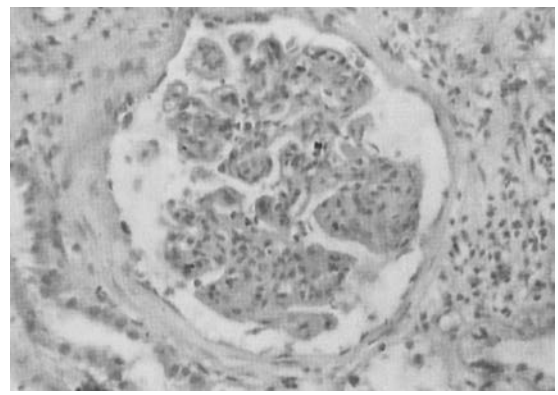

FIGURE 78-15. Mesangiocapillary glomerulonephritis in advanced schistosomal glomerulopathy. Note the mesangial matrix expansion and the thickening of the glomerular basement membrane. (Hematoxylin and eosin, magnification ×200.) (From Barsoum RS. Schistosomal glomerulopathies. *Kidney Int* 1993;44:1, with permission.) (See Color Plate.)

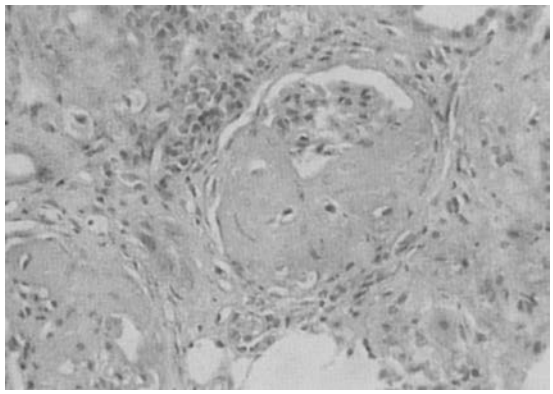

FIGURE 78-16. Focal segmental glomerulosclerosis in advanced schistosomal glomerulopathy. (Masson trichrome stain, magnification ×200.) (See Color Plate.)

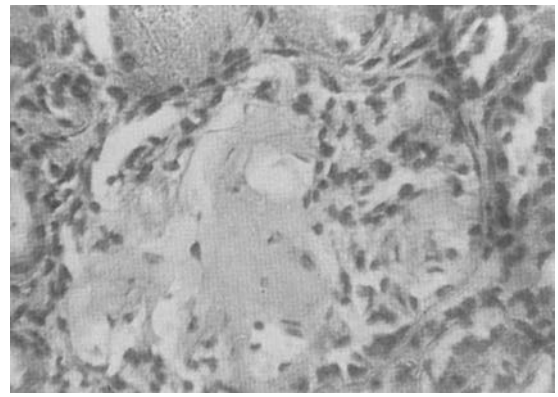

FIGURE 78-17. Glomerular amyloid deposition in mixed Schistosoma haematobium and Schistosoma mansoni infection. (Hematoxylin and eosin, magnification ×200.) (From: Barsoum RS, et al. Renal amyloidosis and schistosomiasis. *Trans R Soc Trop Med Hyg* 1979;73:367, with permission.) (See Color Plate.)

glomerular injury, IgA may be crucial in the progression of glomerular lesions into an overt nephropathy, and further on into ESRD. Autoimmunity may also have a potential role in disease progression (308).

The classic clinical presentation is the development of nephrotic edema and hypertension (50%) in a patient with typical hepatosplenic schistosomiasis. The diagnosis is supported by finding living schistosomal ova in the stools or rectal snips or by acquiring serologic evidence of active infestation. Liver biopsy results reveal the characteristic periportal fibrosis mostly sparing the hepatocytes, and may also reveal deposited ova or worm pigments. This histopathologic pattern is reflected on conventional liver function tests, which are usually nearly normal unless HBV or HCV infection is associated, which is fairly common. Endoscopic examination usually shows lower esophageal or fundal varices.

Examination of renal biopsy specimens usually reveals either of the two described patterns of glomerular injury, probably depending on genetic factors (469). Immunofluorescent study shows IgG and IgA deposits, and occasionally IgM and C3 deposits. Worm antigens are seldom seen at this stage. Variable degrees of nonspecific tubulointerstitial lesions have been observed, particularly with concomitant S. haematobium infection.

Both glomerular lesions are progressive (470,471). They do not respond to treatment. ESRD ultimately develops in those who survive the risks of ruptured esophageal varices or hepatocellular failure when the disease is compounded by viral hepatitis.

The epidemiologic importance of established schistosomal glomerulopathy as a cause of ESRD is not accurately quantitated; certain estimates suggest the figures of 10 per million of the general population (445) and 15% of those with schistosomal hepatic fibrosis (466).

3. *Renal amyloidosis* (Fig. 78-17) is the third potential outcome of schistosomal glomerulopathy, based on experimental findings in small laboratory animals (472,473) and epidemiologic evidence in endemic areas (474,475).

It is suggested that the chronic parasitic antigen load of either S. mansoni or S. haematobium may lead to increased synthesis of Amyloid-A (AA protein) and that local glomerular changes enhance deposition of this protein in the characteristic fibrillar structure (475). The clinical presentation of Schistosoma-associated amyloidosis resembles that of mesangiocapillary glomerulonephritis and focal segmental sclerosis, yet hepatosplenic disease may be less prominent and hypertension is less frequent. However, the disease progresses more rapidly to ESRD. Earlier reports of a favorable response to treatment (474) have not been substantiated.

With the increasing encounter of HCV-associated schistoma-mansoni hepatic fibrosis, a peculiar renal syndrome was recently identified (476). This consists of a mixture of glomerular amyloidosis, IgM mesangial deposits, and macroglobulinemic vasculitis and tubular casts. It is attributed to the ambiguous activation of the classical and the alternative activation of the antigen presenting cells by the virus and the parasite respectively. In addition, the T-lymphocyte response may be modified by the direct invasion of HCV via the recently described CD80 ligands.

S. Japonicum. This infestation is similar to that with S. mansoni in causing hepatic fibrosis, portal hypertension, and splenomegaly. Metastatic lesions, particularly those in the central nervous system, are more common and more serious. Although most of the experimental background on schistosomal glomerulopathy is based on S. japonicum models (447), the impact of this infection is extremely limited in clinical practice. Early mesangioproliferative lesions have been described, but overt disease is seldom reported and even denied (477). The reasons for this discrepancy are unclear; the nature of parasitic antigens, host genetic factors, limited hepatic involvement, and other factors may be involved. The high prevalence of IgA nephropathy in areas where S. japonicum infection is endemic (478) may mask the identity of S. japonicum glomerulopathy as a distinct disease.

Filariasis. Filariasis is highly prevalent in Africa, Asia, and South America (479) (Fig. 78-18). Of the eight filarial strains that infect humans, Wuchereria bancrofti, Brugia malayi, Onchocerca volvulus, and Loa loa are most frequently encountered in clinical practice. All species are transmitted by insect bites. The infective larvae migrate into the lymphatic vessels and slowly mature into adult worms over 3 to 18 months.

Adult W. bancrofti and B. malayi reside in the lymph nodes or afferent lymphatic vessels for decades. They mate and deliver the microfilariae, which either circulate in the bloodstream or migrate by way of the lymphatic channels to the dermis, awaiting their vector to complete the life cycle. W. bancrofti and B. malayi infections may be entirely dormant (asymptomatic microfilaremia), being usually identified by the accidental discovery of eosinophilia. Infected subjects with a pronounced immunologic response may present with hypereosinophilia and pulmonary involvement. The classic presentation, however, is recurrent pyrexia with chills and lymphangitis caused by the

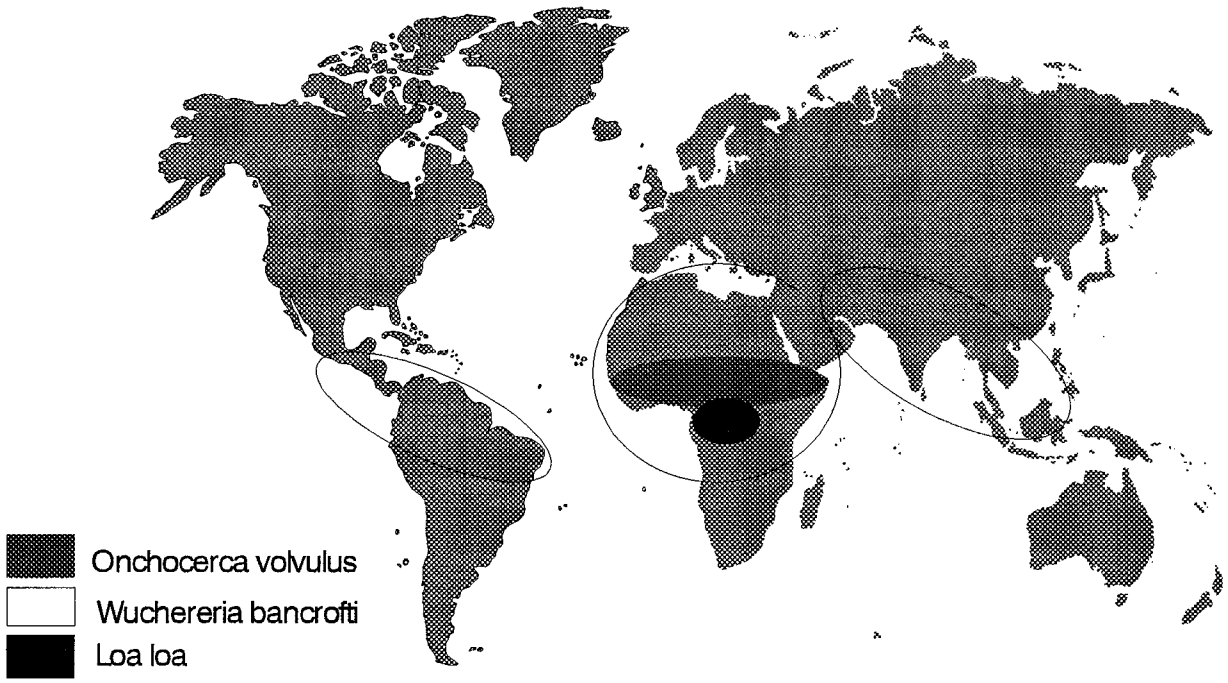

FIGURE 78-18. Global distribution of filariasis.

local immune-mediated granulomatous response against the adult worms. In B. malayi infection, this reaction often leads to the formation of local abscesses that rupture and subsequently heal with characteristic scars. With W. bancrofti, the local reaction usually leads to lymphatic obstruction, gross upstream dilation, and distortion. Elephantiasis of the extremities and genitalia is the usual consequence. Rupture of the dilated lymphatic vessels may lead to chylous effusions, usually in the form of hydrocele or ascites. Rupture of the dilated retroperitoneal lymphatics into the renal pelvis leads to chyluria (Chapter 10).

Adult O. volvulus coil into spherical bundles in subcutaneous tissues and deep fascia, leading to characteristic nodules called onchocercomas. Regional lymph nodes may be enlarged. The worms deliver millions of microfilariae, which migrate into the skin and ocular tissues. In the latter tissues, microfilariae may cause keratitis, anterior uveitis, and less often chorioretinitis. Blindness (river blindness) supervenes in 1% to 4% of patients.

Adult L. loa worms live wandering in the subcutaneous and subconjunctival tissues. They may induce a peculiar hypersensitivity skin reaction, the Calabar swellings, which are localized erythematous and angioedematous lesions. Generalized angioedema may be encountered in expatriates and undernourished native individuals.

Renal involvement in filariasis. The best-documented renal lesion in filariasis is that associated with O. volvulus. In a large epidemiologic study in Cameroon, where this strain is hyperendemic, proteinuria was significantly more frequent among infected subjects (480). Minimal change, mesangioproliferative, mesangiocapillary, and chronic sclerosing glomerulonephritis lesions are most often encountered. Subendothelial and mesangial immune complexes containing IgM, IgG, C3 (312), and onchocercal antigens (480), were detected by immunofluorescence and mesangial electron-dense deposits (481), by electron microscopy. Onchocercal glomerulonephritis is known to recur in transplanted kidneys.

Similar lesions have also been described in bancroftiasis (482,483) and loiasis (484), although specific antigens were not looked for. In addition, an exudative glomerulonephritis, referred to as acute eosinophilic glomerulonephritis, has been documented in patients with W. bancrofti infection (483), and membranous nephropathy in those with L. loa (480) infections. Microfilariae may be seen in the glomerular capillaries.

The clinical spectrum of filarial glomerulonephritis ranges from asymptomatic proteinuria to ESRD. Nephrotic syndrome is often ascribed to such infections in endemic areas. Acute nephritic syndrome in patients with bancroftiasis has been reported. Treatment with diethylcarbamazine may help to resolve early glomerular lesions, but it usually fails when the nephrotic syndrome is manifested (482). Proteinuria may even be aggravated by such treatment in some patients; this is often attributed to further release of filarial antigens (485).

Trichinosis. Trichinosis is a nematodal infection of worldwide distribution, being particularly prevalent in communities where raw, smoked, or undercooked meat, especially pork, is eaten. It is fairly common in Southeast Asia and Latin America, but for obvious reasons, is rare in countries with large Muslim and Jewish populations.

Infection is acquired by ingestion of infective larvae encysted in striated muscles. Excystation occurs by acid-pepsin digestion in the stomach; parasites mature in the upper small intestine. The adult female produces larvae, which penetrate the blood and lymphatic vessels and migrate into different host organs. Those reaching the muscles become encysted and remain viable for many years.

Although trichinosis is often associated with nonspecific gastrointestinal symptoms, its main clinical impact is related to the eosinophilic granulomatous reaction that the encysted larvae provoke in different organs, including skeletal and cardiac muscles, lungs, and the central nervous system. Proptosis and periorbital edema associated with pyrexia and myalgia are the usual clinical clues to the diagnosis.

Renal involvement in trichinosis was vaguely reported as early as 1916 (486). It became more firmly established in recent years as more elaborate diagnostic criteria became established. The lesions are mainly glomerular, in the form of

mesangial proliferation associated with immunoglobulin, C3, and occasionally fibrin deposits. Trichinella antigen has not been searched for in the glomeruli (487).

Renal involvement is usually subclinical, with mild proteinuria and microhematuria. Blood pressure is not elevated, and renal function is usually preserved or only mildly impaired (488). Hypoalbuminemia is occasionally noted, leading to contraction of the blood volume with a mild reduction of p-aminohippurate and creatinine clearances. Circulating immune complexes associated with a decrease in serum C3 level have been described. All clinical abnormalities are reversible by thiabendazole treatment (50 mg/kg of body weight per day for 2 days) (487).

Opisthorchiasis. The disease is caused by Opisthorchis felineus and Opisthorchis viverrini through the ingestion of raw or undercooked fish. O. felineus is common in the Philippines, Vietnam, and India, whereas O. viverrini is prevalent in Thailand, Laos, and Kampuchea. The liver is the target organ where the parasites lodge in the biliary tract. The incidence of cholangiocarcinoma is high in opisthorchiasis. Renal failure can occur in the patients with opisthorchiasis (489). Acute renal failure is seen in 49% of the patients with cholangiocarcinoma and severe jaundice (55). Hyponatremia and hypokalemia secondary to natriuresis and kaliuresis are frequently observed (65). The causes of renal failure are multiple and include hypovolemia, endotoxemia, cardiac dysfunction, effects of vasoactive mediators, hypotension, hyperbilirubinemia, and hyperuricosuria.

Renal pathologic changes include tubular degeneration with bile staining and vacuolation of proximal tubules in the potassium-depleted patients. IgA deposition in the mesangium may be observed. In Syrian golden hamsters infected with O. viverrini, immune complex glomerulonephritis with deposition of IgG antibody specific to the integumental membrane of the adult worm has been shown (490). Renal amyloidosis with AA protein deposition in the glomeruli and interstitium can occur.

Strongyloidiasis. Infestation by Strongyloides stercoralis is endemic worldwide (491), particularly in the warm climates of the Tropics. Infection is acquired by skin contact with soil containing the free filariform larvae. The larvae migrate into the pulmonary capillaries where they break into the alveolar spaces, ascend through the airways to the pharynx, are then swallowed, and eventually mature in the duodenum into adult worms. In most infested subjects, only female worms are found in the small intestine. They reproduce by parthenogenesis, forming eggs that hatch in the gut and release rhabditiform larvae, which eventually transform into the infective filariform larvae in the soil. This transformation can also occur in the gut of an immunocompromised host.

Strongyloidiasis is a mild disease in the immunocompetent individual, being either asymptomatic or associated with vague abdominal symptoms. In the immunocompromised, however, reinfection with filariform larvae can occur through the intestinal walls or the perianal skin. As this process is repeated, hyperinfection occurs, leading to a potentially fatal disseminated disease. As the infective larvae migrate through the lungs, they can induce pulmonary hemorrhage and acute respiratory complications that mimic the adult respiratory distress syndrome. The larvae can also disseminate into various organs including the brain, eyes, pancreas, peritoneum, kidneys, and skin where they form creeping eruptions (492). Mesangial proliferative glomerulonephritis with nephrotic syndrome has been observed in association with strongyloidiasis. The nephrotic syndrome resolved after treatment of the disease (493). The response to thiabendazole treatment is remarkable, with parasitologic cure in more than 90% of patients.

Other Parasitic Diseases. Renal lesions rarely occur in visceral larva migrans caused by the migration of larval stages of Toxocara canis or Toxocara cati (494). Granulomatous changes consisting of eosinophils and histiocytes with giant cells may be observed.

Hematuria and eosinophiluria may be noted. Immune complex–mediated glomerulonephritis has been reported in amebiasis (495). However, ameba antigen was not demonstrated in the immune deposits.

TOXIC NEPHROPATHIES IN THE TROPICS

Animal Toxins

Animal toxin poisoning is a common cause of acute renal failure and nephropathy in the Tropics.

Snake Bites. Snakebites are a worldwide problem. It is estimated that 2,000 species of snakes exist, and 400 of them are poisonous. Snakebites are blamed for 40,000 deaths per year in the world; the vast majority occur in the tropical countries of Africa, South and Southeast Asia, and Latin America. In India alone, 15,000 patients die of snakebites per year, whereas in the United States, only 20 deaths per year are reported (496).

Renal involvement in snakebites has been reviewed extensively by Chugh (497). Renal failure complicates 5.5% to 26.0% of all poisonings from snakes (498,499). Most cases are due to the Viperidae family of snakes, which includes pit viper, rattlesnake, Russell's viper, saw scale viper, Bothrops jararaca, and puff adder (498,500). Other snakes such as the boomslang, mulga snake, dugite, gwardar, Agkistrodon hypnale, and Cryptophis nigrescens and sea snakes can also cause acute renal failure.

Clinical presentation is due to either local symptoms with pain, swelling, local bleeding, or infection at the site of bite, or systemic symptoms with generalized bleeding, hypotension, or oliguria. Oliguria usually occurs 24 to 72 hours following the bite. Patients can be hypotensive at presentation, but often are normotensive. With viper bites, renal failure is usually associated with intravascular hemolysis and disseminated intravascular coagulation. Those patients with renal failure have higher urinary fibrin degradative products (FDPs) than those with normal renal function, suggesting the role of intravascular coagulation in the pathogenesis of renal failure (501). With sea snake bites, renal failure is associated with rhabdomyolysis. Sepsis can be a complicating factor at presentation (498,502). Nephrotic syndrome has been reported in a rare patient following snakebite (503). However, transient heavy proteinuria can occur in patients bitten by Russell's viper.

Renal histology has been studied by several groups (504–509). The majority of the patients show histologic findings compatible with acute tubular necrosis. Acute cortical necrosis is seen in a significant minority of the patients (497). Electron microscopic study in Russell's viper poisoning complicated by acute renal failure has shown glomerular mesangial hypercellularity, vascular endothelial swelling, tubular epithelial necrosis, and interstitial infiltrates (510). Interstitial nephritis (511), proliferative glomerulonephritis (512–515), and arteritis of interlobular vessels (507) have been reported. Granular deposition of IgM and C3 in the mesangium is often observed. There is evidence of deposition of immune complex in situ (69).

With sea snake poisoning (516,517), rhabdomyolysis is the primary event leading to myoglobinuric renal failure. Renal histology shows findings compatible with tubular necrosis (505,517). Muscle histology shows diffuse hyaline lesions involving muscle fiber (516).

Pathogenesis. Snake venom contains many proteolytic enzymes and phospholipases capable of triggering hemolysis, disseminated intravascular coagulopathy, fibrinolysis, complement activation, rhabdomyolysis, and tissue necrosis (500,502,518–521). Hypotension can result from blood loss, anaphylaxis, myocardial depression, and generalized vasodilation due to bradykinin release. Systemic and renal hemodynamics in snake envenomation bear resemblance to that observed in infection and, in fact, share the same cytokines and mediators (522–525). Given the protein nature of snake venom, acute renal failure is rarely due to one factor but rather multiple factors. Chugh et al. (500) showed that a lethal dose of Russell's viper venom did not consistently cause renal failure, and histologically no renal lesion was observed. However, when a sublethal dose was used, renal failure occurred consistently, and histologically both tubulointerstitial and glomerular fibrin deposition was seen. In an isolated perfused kidney, Russell's viper venom decreased the renal blood flow and glomerular filtration rate and increased the fractional excretion of sodium (526). The membrane potential of the proximal tubular cell is decreased in a dose-dependent fashion (70). Russell's viper venom decreases renal cortical mitochondrial oxygen consumption and increases the phosphorus and oxygen ratio (525). Toxicity to glomeruli and tubular epithelial cells has been shown (527,528). Immune mechanism plays a role in the pathogenesis of glomerulonephritis. Therefore, there is evidence of hemodynamic changes, direct nephrotoxicity and immunologic mechanism in snakebite nephropathy.

Treatment and prognosis. Treatment of renal failure caused by snake poisoning is complicated by other systemic complications of the venom. Standard treatment consists of local care, use of antivenom, generalized support, and, if needed, dialysis (529). Dialysis in patients with a sea snake bite can improve muscular symptoms (517). Early administration of sodium bicarbonate to alkalinize urine prevents the development of acute renal failure (530,531). The prognosis for patients with renal failure is very good and most recover with appropriate treatment (532). The prognosis in the minority with cortical necrosis is poor (506,509,533,534).

Insect Stings. Proteinuria, nephrotic syndrome, and acute renal failure have been reported in association with bee, wasp, and hornet stings. In a study by Elming and Solling of 20 healthy subjects stung by bees or wasps, pathologic albuminuria was found in three persons. The urine albumin excretion normalized in 2 months in two persons (535).

Nephrotic syndrome. An association between bee stings and nephrotic syndrome has long been described (536). A relapse of nephrotic syndrome following a bee sting has been reported (537). From the pooled data including our own experience, the onset of nephrotic syndrome varies from 2 to 14 days after the sting (537–542). Serum complement levels and renal function are usually normal. In most patients the serum immunoglobulin profile is normal. One report described a decrease in serum IgA, IgM, and IgG levels (541). The response to steroid treatment is favorable in 50% of the patients.

Renal pathologic changes vary. Minimal change lesions, mesangial proliferative glomerulonephritis, membranous glomerulonephritis, and glomerulosclerosis are among the changes described (537,543). Deposition of C3, IgM, and IgG is demonstrable. However, no report has demonstrated the presence of bee venom antigens in the glomeruli. Although such a finding would indicate a role for an immunologic mechanism in the pathogenesis of glomerulonephritis, the cause-and-effect relationship between the bee venom and glomerulonephritis has not been substantiated. The association between the bee sting and nephrotic syndrome with minimal lesions, erythema of the skin, and eosinophilia described in one report (541) could be related to basophil sensitization (544).

The mechanism responsible for the development of glomerulonephritis is therefore not understood. The role of the bee venom (Apis mellifera) in inducing the alteration of T-cell function in mice, which might link with glomerulonephritis, deserves further study (545).

Acute Renal Failure. Renal failure has occurred in patients stung by wasps (536,546–549), hornets (543,546,550,551), and bees (542,552–554). Stings are usually multiple. A single sting does not cause renal failure but can cause anaphylactic shock in the previously sensitized patient. Renal failure associated with rhabdomyolysis or intravascular hemolysis develops within 24 hours. Thrombocytopenia may be present with or without disseminated intravascular coagulation. Hepatocellular jaundice may be observed. Laboratory findings include hyperkalemia, hyperuricemia, hyperphosphatemia, hypocalcemia, hemoglobinuria, myoglobinuria, and elevation of muscle enzyme levels. Nonoliguric renal failure is not uncommon. Oligoanuria is often observed in the elderly. The duration of renal failure varies from 1 week to several weeks. The course of renal failure is prolonged in the elderly. Recovery of renal function is usual; however, residual renal damage can occur. The elderly and children are at high risk for a fatal outcome.

Renal pathologic changes include tubular necrosis and interstitial lesions with edema and mononuclear infiltration. The proximal, distal, and collecting tubules are affected. Renal failure is attributed to intravascular hemolysis, rhabdomyolysis, and hypotension. Acute interstitial nephritis with renal failure presumably due to hypersensitivity has been reported (555). Increased calcium uptake in the proximal tubule cells by melittin may enhance renal tubular injury (556).

Raw Carp Bile. Ingestion of raw carp bile, traditionally believed to improve visual acuity, stop coughing, decrease body temperature, and lower blood pressure, can result in renal failure. The problem is well known in Southeast Asia, Taiwan, China, and Korea. The raw bile of carp, belonging to the order of Cypriniformes, including Ctenopharyngodon idellus, Cyprinus carpio, Hypophthalmichthys molitrix, Mylopharyngodon piceus, and Aristichthys nobilis, is nephrotoxic (557). Clinical manifestations, which start from gastrointestinal symptoms, consist of abdominal pain, nausea, vomiting, and diarrhea, occurring 10 minutes to 12 hours after the ingestion of raw bile (557,558). Seizure and bradycardia may be observed (559). The amount of bile ingested varies from 15 to 30 mL. The gastrointestinal symptoms are followed by hepatitis and acute renal failure. The onset of oliguria varies from 2 to 48 hours after ingestion. Oliguria renal failure is noted in 54% of patients. Hematuria occurs in 77% and jaundice in 62%. The duration of renal failure varies from 2 to 3 weeks.

Renal pathologic changes are those of tubular necrosis. Glomeruli show no remarkable changes. The pathogenesis of renal failure is not well understood and perhaps is due to multiple factors. Nonspecific factors including diarrhea and jaundice leading to renal ischemia cannot be excluded. In rats, nephrotoxicity developed after the ingestion of carp raw bile but not hog raw bile. Therefore, mammalian bile is relatively nontoxic. Cyprinol, a bile alcohol, has been suggested to be nephrotoxic (560,561). Lin et al. showed that the toxic compound exists in the ethanol-soluble fraction of bile, which has bile acids (561).

Jellyfish Stings. The tentacles of jellyfish (Physalia physalis) have the capsules or hematocytes that contain venom consisting of 5-hydroxytryptamine, histamine, and a glycoprotein with hemolytic properties (562). Severe poisoning can cause nausea, diarrhea, hypotension, intravascular hemolysis, and

convulsions. Renal failure secondary to intravascular hemolysis has been reported (563).

Scorpion Stings. Scorpion venom can cause the release of acetylcholine, catecholamines, and various mediators, with toxic effects to the neuromuscular system. Among the clinical features following severe reaction to a scorpion sting are symptoms of autonomic nervous system stimulation, disseminated intravascular coagulation, myocarditis, cardiac failure, pancreatitis, pulmonary edema, convulsions, and hypotension (564,565). Renal failure has been described in association with disseminated intravascular coagulation and massive hemorrhage in various organs (566). Recently hemolytic-uremic syndrome has been reported (567).

Spider Bites. In most cases the symptoms of spider bite are mild. Yet, severe reaction can occur from the bite of certain spiders. Latrodectus venom can cause severe pain at the site of the bite, as well as nausea, vomiting, salivation, sweating, headache, muscular twitching, hypertension, and respiratory paralysis. Sicarius venom can cause disseminated intravascular coagulation with renal failure (568). Brown spider bites can cause local dermonecrosis and hemorrhage at the site of bite. Systemic manifestations including intravascular hemolysis, disseminated intravascular coagulation, thrombocytopenia, and renal failure have been reported (569,570). The venom has metalloproteases that cause degradation of extracellular matrix such as fibronectin, fibrinogen, entactin, heparan sulfate proteoglycan, and basement membrane (570).

Centipede Bites. A centipede bite usually causes local reactions. Severe systemic symptoms can occur following the bite of the giant desert centipede, Scolopendra heros, which is found in the southern part of the United States and Mexico. The venom contains phospholipase A_2, serotonin, and cardiotoxic protein (571). Nausea, vomiting, headache, rhabdomyolysis, and acute renal failure have been reported (572).

Lonomia Caterpillar Contact. The hemolymph and hair extract of caterpillar of moths in the genus Lonomia have strong fibrinolytic and enzymatic activities similar to tissue plasminogen activator, kallikrein, factor Xa, and urokinase, which cause both fibrinolysis and disseminated intravascular coagulation (573,574). Severe acute renal failure with a hemorrhagic syndrome resembling disseminated intravascular coagulation following contact with Lonomia caterpillars has been described (575,576).

Plant Toxins

Several kinds of plants exert pharmacologic effects on the body. Certain effects are harmful and may directly or indirectly cause nephropathy. The subject has recently been reviewed (577).

Djenkol Bean. Djenkol beans (Pithecolobium lobatum, Pithecolobium jiringa) are consumed by people in Indonesia, Malaysia, and southern Thailand (578–583). The beans are eaten as food: raw, fried, or roasted. Toxicity follows ingestion of the raw bean in large amounts (>5 beans) although there are different susceptibilities among individuals. Poisoning may be caused by a single bean in some individuals, whereas it may take 20 beans to cause toxicity in others (581). Ingestion of the boiled beans does not cause toxicity because the toxic substance, djenkolic acid, is removed from the bean. The amount of djenkolic acid may vary among beans from various sources. Poisoning is characterized by abdominal discomfort, loin pains, severe colic, nausea, vomiting, dysuria, gross hematuria, and oliguria, occurring 2 to 6 hours after the beans were ingested (582). The patient may be anuric. Hypertension may be present. In a recent report of 22 patients with djenkol bean poisoning, dysuria was noted in 17 (77%), hematuria in 15 (68%), proteinuria in 10 (45%), hypertension in 8 (36%), and renal failure in 12 (55%) patients (580).

Urine analysis reveals erythrocytes, epithelial cells, protein, and the needle-like crystals of djenkolic acid. The symptoms are caused by mechanical irritation of the renal tubules and urinary tract by the djenkolic acid crystals. Precipitation of djenkolic acid causing tubular obstruction occurs in acid and concentrated urine. Urolithiasis has been reported, with djenkolic acid as the nucleus (579). The majority of patients recover within a few days.

Diagnosis can easily be made by the history of bean ingestion and occasional sulfurous fetor in the breath. Treatment requires hydration to increase urine flow and alkalinization of urine by sodium bicarbonate (583). When the urine pH is increased from 5.0 to 7.4, the solubility of djenkolic acid is increased by 43%, and at a pH of 8.1 the solubility increases to 92% (578).

Callilepis Laureola. Callilepis laureola (impila) is a perennial herb with a tuberous rootstock found widely in South Africa, Zambia, Zaire, Zimbabwe, and the neighboring countries. The plant is used in the form of infusion for coughs, constipation, intestinal worms, and many other illnesses. An alkaloid, atractyloside, found in the tuber of the plant, is believed to have nephrotoxic and hypoglycemic effects. It has an inhibitory effect on oxidative phosphorylation (584). After medication, toxic symptoms usually occur in less than 24 hours in 40% of the patients and within a few days in 72% (585–587). Generally patients have abdominal pain, vomiting, and diarrhea; hypoglycemia is observed in 81% of patients. Convulsions and coma are common. Hepatocellular jaundice is present, and renal failure ensues in the majority of patients. Treatment is supportive. The mortality rate is greater than 50%.

The kidney shows tubular necrosis involving the proximal tubules and the ascending loops of Henle. Interstitial edema and cellular infiltration are present.

Semecarpus Anacardium. Semecarpus anacardium is the name for the marking nut tree in India and tropical forests. The bark and the pericarp of the fruit have a black caustic juice that is irritating to the skin, causing eruptions and blisters. Prolonged exposure to the sap can cause abdominal pain, vomiting, fever, and renal failure (588). Cortical necrosis has been reported. Nephrotoxicity is attributed to the phenolic substance in the sap.

Star Fruit. Star fruit (Averrhoa carambola) is a popular fruit among Asians. The fruit has high oxalate content. Ingestion of large quantities of pure fresh juice can cause within hours nausea, vomiting, abdominal pain, and backache followed by acute oliguric renal failure, due to acute oxalate nephropathy (589,590). Pure fresh star fruit juice should not be consumed in large amount, especially on an empty stomach or in a state of dehydration.

Mushrooms. Toxic mushroom poisoning leads to a variety of clinical presentations ranging from gastrointestinal symptoms to fulminant liver failure and renal failure. Amanita, Cortinarius, and Galerina mushrooms are nephrotoxic. Amatoxin, phallotoxin, and orellanine are toxic ingredients (591). Amatoxin inhibits DNA-dependent RNA polymerase II. Phallotoxin binds with F actin and polymerizes G actin. Orallanine is toxic to proximal tubular cells. Toxic symptoms occur within 10 to 14 hours after ingestion and consist of abdominal pain, nausea, vomiting, and diarrhea, followed by jaundice, renal

failure, convulsions, and coma (592). Renal failure is severe, with a mortality rate of greater than 50%. In an animal model of Cortinarius orellanus mushroom poisoning, renal dysfunction occurred within 48 hours (593). The pattern of renal impairment included decreased glomerular filtration rate, proteinuria, glycosuria, and decreased tubular reabsorption of sodium, potassium, and water. Renal pathologic changes are those of tubular necrosis, with prominent necrosis of the proximal tubules and interstitial edema (594). There are no glomerular changes. Given early in the course of disease penicillin G, silymarin, and silibinin can be useful. Mushroom poisoning with liver and renal failure is associated with a high mortality despite optimal medical therapy including plasmapheresis, charcoal hemoperfusion, and hemodiafiltration. Use of molecular absorbent regenerating system (MARS), which removes albumin-bound toxins, has been reported to favor recovery (595,596).

Cotton Seed Oil. The principal ingredient of the cotton seed oil is gossypol. Gossypol can cause kaliuresis and hypokalemia. The mechanism is not well understood. In central and southern China, where the dietary potassium is low, the incidence of gossypol induced hypokalemia is between 4% and 5% (597). It has also been reported to cause distal renal tubular acidosis in Chinese people who consume cotton seed oil (598).

Other Plant Toxins. Several plants including Securidaca longipedunculata, Euphorbia matabelensis, and Crotalaria laburnifolia have been listed as being nephrotoxic (599,600). Yet, there is no supporting scientific evidence. These plants are usually used as traditional medicine for the treatment of many illnesses and may cause renal failure through their side effects. For example, infusion of the leaves, bark, and root of S. longipedunculata can cause severe gastroenteritis with diarrhea and vomiting, which can result in acute renal failure through volume depletion without evidence of direct nephrotoxicity. Thiloa glaucocarpa is tubulotoxic due to toxic tannins. Solanum malacoxylon can cause hypercalcemia due to 1,25-dihydroxycholecalciferol (601).

An association between the use of Chinese herbal medicine containing aristolochic acid and chronic renal disease has been described (602). Renal pathologic changes include tubular atrophy, interstitial fibrosis, glomerulosclerosis, and thickening of the wall of the interlobular artery and glomerular afferent arteriole. Urothelial malignancy has been observed. Multifocal atypia of the medullary collecting ducts, the pelvis, and the ureter with overexpression of P53 has been shown (603). On the positive side, traditional Chinese herbal medicine (Saireito) with the active principal saikosaponin-d has been shown to prevent glomerulosclerosis in uninephrectomized rats with anti-thy-1 antibody injection (604).

Other Chemicals

Accidental exposure to certain chemical toxins is well documented as a cause of acute renal failure in the Tropics, as it is in the West. This subject is addressed in Chapter 47.

Chemical intoxication may also be associated with certain occupational hazards, involving exposure to industrial poisons such as lead, mercury, cadmium, uranium, and asbestos. This is highly prevalent in tropical countries, owing to the inadequacy of environmental protection measures in industrial plants. The toxicologic aspects of such exposure are detailed in Chapter 47.

Certain chemical hazards of particular importance in the Tropics deserve mentioning, namely, paraquat, copper sulfate, and diethylene glycol.

Paraquat Poisoning. This is mainly reported in Southeast Asia (605). Paraquat is an herbicide, widely used in agriculture, to which humans are exposed by ingestion (including suicidal intake), inhalation of sprays, or contact with skin abrasions (606). Pulmonary, hepatic, and renal manifestations occur with paraquat poisoning, and are attributed to the massive generation of reactive oxygen radicals. Nephrotoxicity is expressed as acute tubular necrosis (607), which leads to accumulation of the poison and increases its systemic toxicity. Plasma paraquat levels higher than 2 mg/L at 24 hours and 1 mg/L at 48 hours are of grave prognostic significance (608).

Like many other chemical intoxications, the first line of treatment is to reduce absorption by gastric lavage and the administration of adsorbing substances such as Fuller's earth. If renal function permits, forced diuresis should be attempted (609). Hemodialysis and charcoal hemoperfusion (610) are effective, whereas peritoneal dialysis is inefficient. Attempts to reduce the oxygen radical load by suppressing leukocytic function with corticosteroids and even cyclophosphamide (611) are controversial.

Copper Sulfate Poisoning. This hazard is of considerable epidemiologic significance in India (612). Copper sulfate is used in the leather industry. Exposure occurs through accidental or intentional ingestion. Acute toxicity ushers in with prominent gastrointestinal manifestations, intravascular hemolysis, hepatic injury, and renal failure.

Renal injury is the result of massive intravascular hemolysis, induced by the action of the poison on intracellular enzyme systems including G6PD, glutathione reductase, and catalase (613,614). The associated hypovolemia, due to gastrointestinal fluid losses, further aggravates the renal insult. Acute tubular necrosis with rupture of the tubular basement membrane, copper deposits in the renal tubule cells, and hemoglobin casts in the lumens, are characteristic histopathologic features.

In addition to the conventional methods for reducing the absorption of the poison, fluid replacement and induction of diuresis, dimercaprol (100 to 150 mg intramuscularly every 4 hours) is effectively used for chelation in copper sulfate poisoning. Dialysis is often necessary during the critical hypercatabolic phase, and also to assist in eliminating the toxin (615).

Diethylene Glycol Poisoning. Diethylene glycol is a known nephrotoxic agent. Poisoning with diethylene glycol in the Tropics has frequently been associated with contamination of ingestible pharmaceutical products. Outbreaks of acute renal failure by diethylene glycol–contaminated acetaminophen have been reported in Nigeria (616) and Bangladesh (617). Recently there was a large outbreak of acute renal failure with deaths in children consuming diethylene glycol–contaminated acetaminophen syrup in Haiti (618). Renal failure was severe and associated with hepatitis, pancreatitis, and central nervous system involvement with high mortality. It has been postulated that acetaminophen could provide an additive or potentiating effects for diethylene glycol toxicity. Hypovolemia due to vomiting could be another contributing factor.

Diverse Environmental Problems

In addition to the high prevalence of infections and intoxications, other features of tropical ecology may have a significant influence on the epidemiology of renal disease in certain geographic locations. These include the effects of climate, soil composition, and environmental pollution. Some of the available information on this issue is briefly reviewed.

Renal Stones. Renal stone disease is one of the common problems in tropical Asia. It accounts for 40% of renal problems in Pakistan, which is a country in the "stone belt" (619). The renal stones usually consist of mixed calcium, oxalate, and phosphate. The hot and dry climate and dietary factors play important roles. In the Arabian peninsula, the dietary ratio of oxalate to calcium and the dietary animal protein intake are higher than those in any other country (620). In Israel the high prevalence of renal stones is related to dehydration and solar hypervitaminosis D with absorptive hypercalciuria (621). In India and Pakistan the cause of renal stones is not apparent but could be related to dehydration. The prevalence of upper urinary tract stones in the northeastern Thai population has been estimated to be 3.76 per 1,000 individuals, with a male-to-female ratio of 2:1 (622). The stones, like those in other Asian populations, are composed of calcium, oxalate, and phosphate. Urinary excretion rates for calcium, magnesium, oxalate, and urate are normal. Urinary phosphate excretion is slightly decreased. The low urinary citrate concentration could significantly contribute to stone formation (622,623).

Endemic Hypokalemia and Related Problems. Hypokalemia in northeastern Thailand is endemic and reflects the effects of the environment. Northeastern Thailand is a plateau in the arid area of the country, where the weather is dry and hot. Except for the areas close to the river basin, the land consists largely of sandstone. The soil, therefore, has low levels of potassium and is infertile. The people in the villages are of low socioeconomic status and consume the products of the land. Therefore, 38% of them have hypokalemia and are potassium depleted. The erythrocyte membranes of the villagers are found to have decreased Na^+,K^-ATPase activity. The urine has a low citrate concentration. Both potassium depletion and decreased Na^+,K^-ATPase activity lead to low urine citrate through intracellular acidosis, which enhances citrate reabsorption. Intracellular sodium concentration is increased, while intracellular potassium concentration is decreased. The basic abnormalities of these northeastern Thai villagers are, therefore, potassium depletion, decreased Na^+,K^-ATPase activity, and low urinary citrate levels (623–625). These abnormalities resolved when the villagers migrated to the capital city (624). In addition to renal stones, sudden unexplained death and distal renal tubular acidosis are also important health problems of the region.

Sudden unexplained nocturnal death has received much attention in recent years. The incidence is 38 per 100,000 men between 20 and 49 years old (626). The victims are usually muscular young men of low socioeconomic class who die in their sleep without apparent organic lesion. Ventricular fibrillation has been observed and hypokalemia has been reported in some patients. These individuals have decreased Na^+,K^-ATPase activity of the erythrocyte membrane (627). It has been postulated that hypokalemia, the possible decrease in myocardial Na^+,K^-ATPase, and sympathetic stimulation due to stress could lead to cardiac arrhythmias and death (628). The causes are likely multiple and could involve genetic variations. Recent evidence indicates mutation of the sodium channel (SCN5A) in the cardiac muscle resembling Brugada syndrome (629).

The prevalence of distal renal tubular acidosis in northeastern Thailand is approximately 3.6%, with a female preponderance (630). The patients are usually admitted to the hospital during the mid-summer months because of muscular weakness or paralysis; nephrocalcinosis or renal stones, or both, are noted in 27% of the patients. Interestingly, the patients have low urinary potassium levels, a finding different from Western reports. In addition, gastric acidity is low (631), suggestive of decreased H-K-ATPase (HKα1) activity or decreased chloride bicarbonate exchange (AE2). Because of hypokalemia, aldosterone secretion is likely suppressed, and this would in turn decrease H-ATPase activity. Distal renal tubular acidosis is heterogeneous and requires further study on chloride bicarbonate exchange (AE2), H-ATPase and H-K-ATPase (HKα2) activities of the collecting ducts (632). In southeast Asian ovalocytosis with distal renal tubular acidosis, the AE1 gene mutation has been reported (633). Recently two novel compound heterozygous SLC4A1 mutations have been identified in Thai patients with autosomal recessive distal renal tubular acidosis (634).

TROPICAL ENVIRONMENTAL POLLUTION

A wide spectrum of chronic renal diseases is attributed to environmental pollution, which has been implicated in the pathogenesis of glomerulonephritis (e.g., heavy metals, hydrocarbons), tubulointerstitial disease (e.g., lead), and renal and urothelial malignancies (cigarette smoking, cadmium, and aflatoxins). This subject is reviewed in Chapter 47.

The epidemiologic significance of environmental pollution in the Tropics is unknown, but environmental pollution may very well account for the high prevalence of renal disease in general, and particularly for the progressive increase in tubulointerstitial disease in the recently industrialized tropical countries. It is noteworthy that the item "unknown" accounts for 7.4% to 54.5% of ESRD on the tropical list of causes (635–639). Although this outcome may be attributed to a lack of adequate diagnostic facilities or to late patient presentation, it may also be accounted for by environmental pollution.

Experimental Background

Most of the available information in this field is derived from animal experiments. Nephrotoxicity can be induced by various chemicals in a number of animal models using rats, mice, hamsters, guinea pigs, chickens, ferrets, dogs, pigs, monkeys, and others. Three principal patterns are often encountered:

1. "Toxic" tubulointerstitial disease, in which the renal tubules, particularly the proximal ones, are the main target. Cell injury is mediated by various mechanisms including cell membrane disruption, disturbance of intracellular organelles, enzyme systems, and metabolic pathways. Cell necrosis and subsequent regenerative hyperplasia, luminal dilation, interstitial edema, cellular infiltration, and, ultimately, fibrosis are the histopathologic hallmarks. Proteinuria, cylindruria, leukocyturia, and azotemia are the usual functional consequences. This pattern is attributed to a direct toxic effect on cell membranes or intracellular enzyme systems.
2. Acceleration of "spontaneous nephropathy" in adult male rats (640), with nephrotoxicity being expressed as a statistically significant increase in the incidence of nephropathy or the development of neoplastic lesions. The P2 segment of the proximal tubule is the target. The lesions are ushered in by the appearance of intracellular hyaline droplets, which coalesce and eventually lead to cell necrosis. The resulting debris forms intraluminal granular casts that tend to block the tubules at the corticomedullary junction, leading to pressure necrosis of the surrounding cells and upstream tubular dilation. Spontaneously initiated clones expand, starting the process of regeneration. Adenomas, and, rarely, adenocarcinomas may develop. Interstitial edema, cellular infiltration, and subsequent fibrosis and nephrocalcinosis cause progressive nephropathy in these animals.

   The α_{2u}-globulin was recently brought into focus as an important sex-linked mediator of the renal lesions in this

model (641). This protein is normally generated in the liver, filtered through the glomeruli, and taken up by the proximal tubule cells, where it is metabolized in the lysosomes. Binding with certain chemicals slows degradation of this globulin, leading to its intracellular accumulation and subsequent crystallization in the form of hyaline droplets. The subsequent mechanisms of cellular injury in droplet nephropathy are unknown, but they may involve lysosomal disruption, release of reactive oxygen species, or others.

3. Carcinogenesis, in which expansion of particular cell clones leads to neoplastic growth. This may occur at the tubular level, leading to adenomas and adenocarcinomas, or at an urothelial cell level, leading to papillomas or transitional cell carcinomas of the pelvis, ureter, or bladder.

Recent emphasis has been on the levels of kidney lipid peroxidation after the administration of chemicals with carcinogenic potential. High levels were associated with more nephrotoxicity, the appearance of eosinophilic bodies in renal tubular cells, and the development of neoplasms. Cysteine or glutathione pretreatment was protective, whereas diethyl maleate resulted in exacerbation of the effects of lipid peroxidation (642).

Effects on Humans

It is difficult to substantiate the effect of environmental pollution on humans because of the complex interaction of a large number of factors, including the nature and magnitude of pollution, duration and continuity of exposure, individual susceptibilities, associated diseases, and drug intake. Inherent differences in body size, normal life span, metabolic pathways, defense mechanisms, and other factors make it impossible to extrapolate experimental observations to humans. Accordingly, the whole concept of renal disease due to environmental pollution remains disputable, although not necessarily unimportant, particularly in tropical countries where the international standards of air and water pollution and the acceptable qualities and quantities of food additives and contaminants are not stringently respected.

Specific Issues in Tropical Environmental Pollution

Smoke, Dust, and Fumes. Tobacco smoking is now recognized as a major risk factor in cardiovascular morbidity and mortality in patients with chronic kidney disease (643), progression of renal disease (644) particularly among diabetic patients (645), and in the pathogenesis of bladder cancer (646,647) (Chapter 29). This problem is particularly relevant in tropical countries because of the lack of adequate control measures (648) and public education.

Compared with the rest of the world, the major cities in tropical countries have the highest levels of pollution from car, motorcycle, and other engine exhausts. There is unequivocal evidence that the combustion products of diesel fuel and unleaded gasoline (649), like other petroleum-derived jet fuels (650), are nephrotoxic to adult male rats, being mediated by α_{2u}-globulin. Other hydrocarbons such as paint, mineral spirit, and aromatic solvents (651) have the same nephrotoxic potential.

It is uncertain whether human immunity, conferred by the low α_{2u}-globulin profile, against hydrocarbon-induced renal injury is absolute, even in the face of continuous exposure over many decades. It is noteworthy that lead nephropathy is an independent risk in most tropical countries where commonly used gasoline is not unleaded.

Exposure to lead is also carcinogenic, as suggested by a rat model (652) and by observations of an exposed human cohort (653).

Chronic exposure to cadmium (654) and asbestos (655), which in addition to industrial intoxication are often ingested with contaminated water or inhaled with dust, also carries a high risk for renal cell carcinoma.

Food Additives. Many artificial flavors, colors, thickening substances, solvents, and even wrapping material to which humans are extensively exposed have documented nephrotoxic effects in experimental animals.

Toxic tubulointerstitial lesions have been associated with the administration of carotenes, used as artificial colors (656), and β-cyclodextrin, used as a flavor, color, and vitamin carrier and stabilizer and also in the decaffeination of coffee and tea and in reducing the cholesterol content of eggs (657,658). These lesions have also been reported with exposure to extraction solvents such as 1,2-dichloroethane (659), dichloromethane (660), α-methyl-benzyl alcohol (661), and diethylene glycol monoethyl ether (662).

Although no clinical nephrotoxicity has been attributed to most of these agents, acute renal failure was reported to complicate the accidental ingestion of large quantities of 1,2-dichloroethane (659), or inhalation of dichloromethane (663). Mild azotemia complicated acute intoxication with diethylene glycol monoethyl ether (664).

Protein droplet nephropathy has been associated with exposure of adult male rats to the flavoring agent limonene, used in beverages, chewing gum, candy, ice cream, gelatins, and puddings (665). Humans seem to be resistant to this type of nephrotoxicity because of the very small amounts of α_{2u}-globulin that they can produce (666).

Renal adenomas and adenocarcinomas have been induced in rats (667) and hamsters (668) by long-term ingestion of potassium bromide, which is often used as a flour-treatment agent. Accidental or suicidal administration in humans may be associated with acute renal failure, tubulointerstitial nephritis, interstitial fibrosis, and glomerulosclerosis (669).

Urothelial malignancy in the renal pelvis and urinary bladder has also been documented in experimental models. Of particular interest are the artificial sweeteners, most notoriously saccharin, which at high dietary concentrations of 5% or more induced bladder tumors in male rats (670). The relation between saccharin and bladder cancer in humans rests on soft evidence (671). Recent data, including a large postmortem study based on the examination of 6,503 sections obtained from 282 deceased patients (672) and a meta-analysis of 15 case-control studies (673), do not substantiate this relation in humans.

Food Contaminants. Certain substances produced during food preparation or preservation have a nephrotoxic effect on experimental animals. Typical lesions were induced in rats by chloropropanols, which are produced during the preservation of vegetables (674–676). No human disease has been attributed to these contaminants.

Exogenous contaminants are more relevant to the question of human nephrotoxicity in the Tropics, where agriculture remains the predominant occupation. These include mycotoxins, pesticides, and veterinary drug residues.

Mycotoxins are discussed elsewhere in this and in other chapters, where reference is made to ochratoxin-associated interstitial renal disease and aflatoxin-associated neoplasias.

Pesticides are ingested with drinking water, plants, fish, poultry, and meat. Adequate control is beyond the capacity of most tropical countries, because of problems with analytical methodology, standardization, and so on. Large amounts,

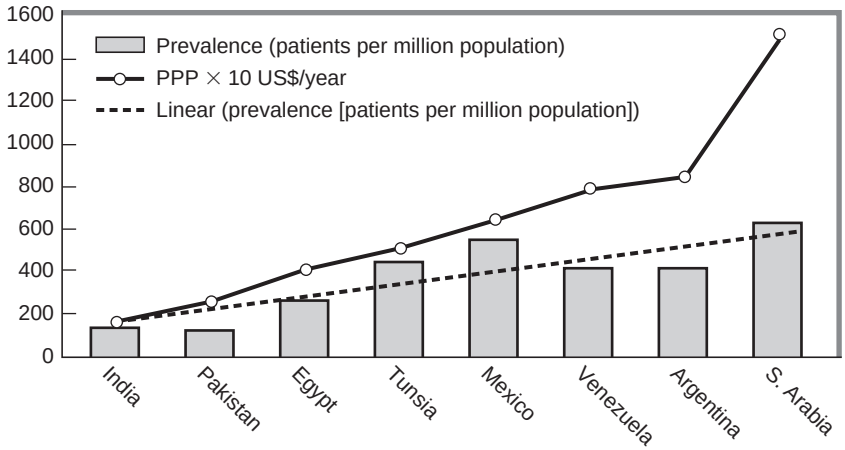

FIGURE 78-19. Impact of wealth (expressed as the Purchasing Power Parity) on the prevalence of end-stage renal disease (ESRD) in six developing countries. Note the selective parallel relationship in between PPP and the prevalence rather than the incidence polynomial trend lines.

therefore, may be inevitably ingested by tropical inhabitants for lifelong periods.

Most pesticides are organophosphates; their main toxicity is on the red blood cells, thyroid gland, and nervous system. Weight loss, anemia, hepatic adenomas, esophageal and other gastrointestinal tract tumors, skin allergy, and teratogenicity are the principal toxic manifestations. There is some experimental evidence of toxicity to the liver and kidney (677), but no clinically significant renal disease has been ascribed to pesticides.

Veterinary drug residues constitute an important source of pollution in an agricultural environment where close contact between humans and animals is inevitable. These residues are ingested with vegetables, fruits, and meat. Some residues, including certain anthelmintics, antimicrobial agents, and production aids, are nephrotoxic to experimental animals.

The broad-spectrum anthelmintic tiabendazole induces tubular degeneration and hyperplasia in the short term in mice (678). However, nephrotoxicity was not observed in a 24-week study in humans (679).

Spiramycin, a macrolide antibiotic used for the treatment of bacterial and mycoplasmal infections in animals, causes "considerable kidney damage," particularly in the loops of Henle, and necrotic changes in several areas of the renal parenchyma in dogs (680). However, administration of this drug to human volunteers caused only mild gastrointestinal disturbances (681).

Ractopamine is a phenolethanolamine α-adrenergic agonist that increases weight, leanness, and feed efficiency in pigs. It causes a transient elevation of systolic blood pressure in short-term studies in humans (682). Long-term follow-up studies are awaited.

END-STAGE RENAL DISEASE IN TROPICAL COUNTRIES

Epidemiology

Prevalence

With the progressive improvement of renal replacement therapy (RRT) worldwide, prevalence curves continue to rise in most countries. The reported prevalence is currently highest in Japan, being close to 2000 patients per million population (pmp), which is attributed to the strikingly high survival on dialysis. The respective figures are about 1500 pmp in the United States and 800 pmp in the European Union (683,684).

In the developing world, prevalence of ESRD is proportionate to national economy (Fig. 78-19) (684,685). Because there are no major differences in incidence between individual developing countries, the major determinants of prevalence are the capacity and competence of RRT programs, both of which are financially demanding.

Incidence

The incidence of new cases of ESRD in the Tropics is generally within the range of 100 to 150 pmp (685). There is no point in focusing on minor differences between individual countries or regions, since the available information is only approximate, being based on questionnaires to leaders, small samples, official governmental reports and, very occasionally, registries with doubtful credibility. These numbers are far lower than those reported in the West, where the incidence is three- to sixfold higher (683). Although underreporting may be responsible for this discrepancy, it has been estimated that the lower incidence of diabetes (686,687) in the Tropics can account for the difference (Fig. 78-20). This is quite alarming given that the incidence of diabetes is expected to boom in tropical countries during the coming two decades (686), which will undoubtedly reflect on the incidence of ESRD.

Etiology of Chronic Kidney Disease

Chronic glomerulonephritis and interstitial nephritis constitute the principal causes of tropical chronic kidney disease (CKD) (685). This reflects the high prevalence of infections and intoxication as described previously in this chapter. Of the principal bacterial infections, tuberculosis ranks quite high in India and the Arabian Gulf, being associated with ureteric strictures, back pressure, and chronic interstitial nephritis. Streptococcal infections of the throat and skin (complicating scabies) are responsible for chronic glomerular disease in a large number of African children. Of the viral infections, hepatitis C is currently the most important cause of progressive mesangiocapillary (membranoproliferative) glomerulonephritis in many countries, particularly Egypt. Several parasitic infections cause ESRD through ureteric obstruction (e.g., schistosomiasis in most of Africa), interstitial nephritis (e.g., Kala-azar in many African and Asian countries), and glomerulonephritis (e.g., malaria in West Africa, schistosomiasis in Africa and Latin America, filariasis in Nigeria).

There is a considerable variation in the histologic types of glomerulonephritis encountered in the Tropics, mirroring the predominant infectious agents as well as genetic predisposition (688). Generally speaking, IgA nephropathy is the predominant

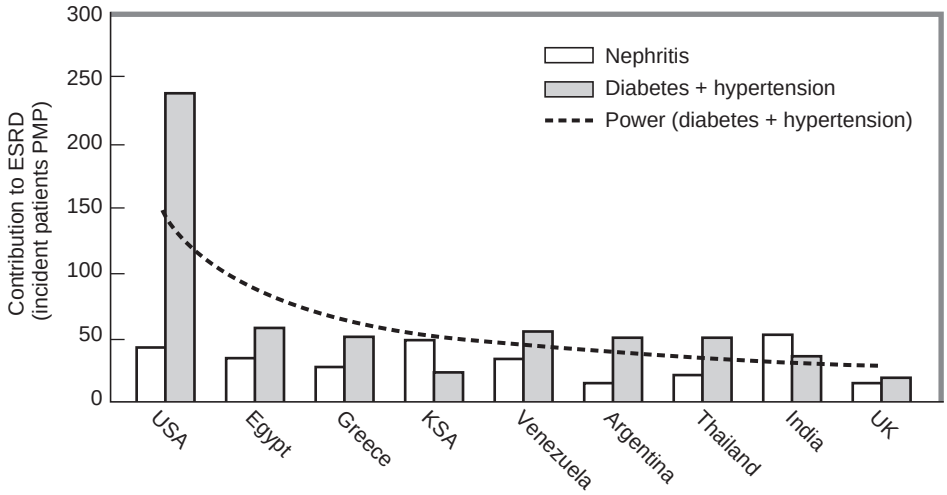

FIGURE 78-20. Effect of diabetes and hypertension on the incidence of ESRD in six developing countries compared to the USA. Note the reflection of the polynomial trend line for diabetes and hypertension as opposed to the incident rates of glomerulonephritis.

type in South East Asia and the Pacific region (35% to 45%), while being quite rare in Africa (3% to 4%). In contrast, focal and segmental glomerulosclerosis supervenes among the black populations in Africa (25% to 25%), Arabia (40%), India (up to 46%) and South America (up to 43%), probably as a consequence of the low nephron mass. Proliferative forms of glomerulonephritis are more homogeneously spread all over the developing world, being responsible for 25% to 35% of reported cases (688).

The contribution of diabetes varies from 9.1% to 29.9% in different reports. More than 80% of cases are type 2 insulin-resistant. End-stage diabetic nephropathy exhibits a constantly rising trend, attributed to increasing incident cases as well as improved survival on RRT. In a single center experience in Egypt, diabetic patients constituted 8.0% of patients on chronic dialysis in 1980, 13.3% in 1990, 18.7% in 2000, and 24.3% in 2003 (Barsoum, unpublished data).

The lack of general agreement on the role of primary hypertension in causing ESRD reflects on the statistical reports from most tropical countries. Upon standardization of the definition of this disease entity, the prevalence of hypertensive nephrosclerosis among ESRD patients in the Tropics was reported between 13% and 21% (685).

Other important causes of ESRD in the Tropics include urolithiasis with subsequent obstruction and infection, chronic drug abuse, and possibly environmental pollution. The epidemiologic significance of the latter remains questionable, being documented only in occupational exposure to lead, cadmium, and mercury (see preceding text).

Clinical Patterns

The usual clinical syndrome of ESRD is modified in the Tropics by three factors: (i) associated manifestations of the primary disease, (ii) manifestations of concomitant disorders, and (iii) late diagnosis and poor management.

Manifestations of the Primary Disease

As described previously in this chapter, renal disease in the Tropics is often secondary to an endemic infection, is attributed to environmental pollution, or is caused by a drug or traditional medication. The associated features of the primary etiology frequently modify the clinical picture of ESRD. Examples include the concomitant hepatic disease in schistosomal glomerulopathy; severe hypertension, arthropathy, and polyneuropathy with chronic exposure to lead; and persistent hyperkalemia with certain herbal intoxications.

Concomitant Disorders

Many patients have a concomitant illness, the features of which may overlap with or modify those of conventional ESRD. Such disorders reflect the general health status of the community, often dominated by endemic infections and infestations, malnutrition, and certain malignancies.

Endemic Infections and Infestations

Acute and chronic bacterial infections often complicate renal disease in the Tropics. In addition to those causing renal disease (see preceding text) and those acquired during dialysis (see subsequent text), certain infectious agents may have an independent impact. One of the most outstanding infections is tuberculosis, which is encountered in 4.1% to 11.5% of the Saudi Arabian population on hemodialysis (689), Gulf countries (690), India (691,692), Bangladesh (637), and Indonesia (693). Tuberculous peritonitis has been reported in patients on chronic ambulatory peritoneal dialysis, wherein it poses notorious diagnostic difficulties (694). Evidence of previous Salmonella infection was detected in 42% of patients on regular hemodialysis in Egypt (695). Recent Salmonella infection–induced acute graft rejections has been reported in renal transplant recipients (696). The list includes many other infections, the most common being scabies, HBV, HCV, HIV infection (see the following), and intestinal parasitic infections (218). In addition to imposing their specific symptomatology, these infections generally tend to induce a catabolic state that augments such uremic manifestations as asthenia, anemia, and bone disease.

Malnutrition

Protein-energy malnutrition is often a major problem in dealing with renal disease in the Tropics. This may be attributed to starvation, catabolic disorders, or physicians' instructions. It is unfortunate that general practitioners are still advising their patients to restrict protein intake to exceedingly low levels. Even nephrologists maintain this trend in patients on dialysis, as shown in a meta-analysis of recent European Dialysis and Transplant Association (EDTA) data (697), where 73.5% of uremic patients in developing countries were instructed to receive a daily protein intake of less than 0.6 g/kg, compared with 31.3% in Western Europe.

Specific nutritional deficiencies of iron, calcium, vitamin D, other vitamins, and trace elements are also frequently

encountered. The effects of these on anemia, bone, and peripheral nerve disease are obvious.

Malignancies

Infection-related malignancies are notoriously common in the Tropics. Examples include Burkitt's lymphoma and Kaposi's sarcoma (698). These often become a significant epidemiologic risk in immunocompromised patients.

Late Diagnosis and Inadequate Management

Owing to the prevailing standards of medical care, renal disease may not be suspected, may be neglected, or may be poorly managed for a long time before adequate nephrologic care is provided. Advanced uremic manifestations, of almost historic interest according to present-day Western standards, remain fairly common in the tropical glossary. It is not unusual to see a patient with extensive uremic frost on his or her cheeks or finger creases, or with multiple soft tissue swellings of metastatic calcification, or another crippled with bone deformities or motor polyneuropathy. Many patients are first seen in coma, in convulsions, or with a hematocrit level of 8% to 10%. The clinical picture is often further complicated by the use of medications without any dose adjustment in consideration of residual renal function.

Dialysis in the Tropics

Acute Dialysis

Hemodialysis is available for the management of acute renal failure in most of the major hospitals in the Tropics, even in those countries with the lowest Gross National Products (GNPs) (685). The efficiency of such units is widely variable, ultimately depending on socioeconomic development. For obvious reasons, acute peritoneal dialysis is more widely used. It is often implemented where limited or no specialized nephrologic care is available, at least as a first-line therapy. Unfortunately, the prevailing hygienic conditions in many tropical hospitals yield a high incidence of peritonitis.

Chronic Dialysis

The number of patients treated by chronic dialysis in the Tropics cannot be precisely defined because of the scarcity of reliable registries. Data generated from sporadic publications (which are, unfortunately, mostly found in the nonindexed local literature), national registries (by personal communication with colleagues in charge), or supranational registries (African, Arabian, Latin American, and Asian Pacific registries) suggest that the chronic dialysis activity in the Tropics is fairly extensive. The chronic dialysis pools vary from less than 10 pmp in most sub-Saharan African countries, India, and Bangladesh, to the 100 pmp range in Pakistan, Thailand, South Africa, 300 to 400 pmp in South America and Northern Africa, and greater than 500 pmp in Singapore, Saudi Arabia, and Mexico (685).

The Dialysis Environment

The patient. Young adults in their 20s or early 30s constitute 70% to 80% of the dialysis population in the Tropics, which is attributed to the nature of the prevailing primary diseases. Children constitute less than 10%. There is a male preponderance, which is partly explained by cultural factors and possibly the increased exposure to noxious environmental factors (vide supra). Most patients are poor, uneducated, and suffering from chronic endemic diseases, malnutrition, and advanced uremic state, which has a significantly negative impact on the outcome of dialysis treatment (vide supra). They do not adequately comply with the regularity of dialysis, diet, intake of medications, or rehabilitation. Although this is partly attributed to improper patient information, the lack of motivation and financial shortcomings certainly have a considerable influence.

Medical staff. Shortage of adequately trained medical and paramedical local staff is the rule in most tropical countries. The majority of countries do not have a structured program for training physicians, nurses, and technicians. Yet recent years have witnessed an increasing regional and international collaboration for such training. Training guidelines have been developed by the International Society of Nephrology and several supranational societies of nephrology; and many have launched mechanisms for bilateral collaboration between well developed dialysis units in the West and developing units in the Tropics.

Dialysis centers. The reported number of dialysis centers varies, in different tropical countries, from less than 1 to 12 units pmp (685). When detailed figures are compared with the respective numbers of patients actually receiving regular dialysis, it can be appreciated that the units are not kept sufficiently busy. The reasons for nonfunctional equipment are many and include lack of personnel, shortage of funds, poor maintenance, and inefficient organization.

Most units are located in hospitals, with a few satellite units in the better-developed countries. State-sponsored, insurance-sponsored, and private units are available in most countries, in proportions that differ according to the political systems and GNPs. Excellent state-sponsored units comprise most dialysis units in the economically better-off countries. However, the standard of service in state-sponsored units in most tropical countries with low GNPs is modest, which invites active participation of the private sector. Political system permitting, as in India, Egypt, Latin America, and most of Southeast Asia, private units tend to take the technical lead. Governments generally provide partial reimbursement for private dialysis in these countries. Insurance-sponsored units are also growing where health insurance systems are strong enough to support the cost. Unique models of charity-sponsored programs have emerged in recent years, notably in Singapore and Pakistan.

Standard equipment is the rule, including nonvolumetric hemodialysis machines, acetate-based dialysate, cuprophane membrane hollow fibers, and so on. Peritoneal dialysis fluid is often locally manufactured, although at a very high cost (vide infra). A few units accept children and older adults, but almost none is specialized in pediatric or geriatric dialysis.

Chronic dialysis modalities. With a few exceptions, hemodialysis is the predominant dialysis modality in most tropical countries, constituting 80% to 90% of the population on dialysis. In countries with the lowest GNPs, however, intermittent peritoneal dialysis (IPD) is the inevitable alternative. The implementation of continuous ambulatory peritoneal dialysis (CAPD) is widely variable, from sporadic experiences to a national policy (Mexico, 75% (699); Hong Kong, 70% (700); South Africa, 40% (685)). Most other countries refrain from using CAPD because of the high cost (vide infra), high incidence of infection, and poor patient compliance. Accordingly, CAPD is offered only as a second choice to selected patients of the higher social classes.

IPD is still used for high-risk patients such as those with severe ischemic heart disease or complicated diabetes. On the whole, it accounts for at least 60% of all chronic peritoneal dialysis treatment in the Tropics. Because adequate urea kinetics can only be achieved by an impossible number of dialysis hours, IPD is associated with an extremely poor median survival time, 1 year, and modest quality of life. Although IPD

is usually carried out in hospitals, the incidence of infection remains high.

The Cost of Regular Dialysis. Calculating dialysis costs is a difficult and complex procedure. In addition to the direct costs including the prices of consumable items, overhead expenses, salaries, and fees, one must include the costs of interdialytic therapy, transfusions, hospital admissions, transportation, and so on. In even broader terms, one should also consider the days missed from work and the reduced productivity of some patients.

The items of typical annual direct dialysis cost per capita in a tropical country are essentially the same, with the differences being mainly influenced by the proportion of imported versus locally manufactured materials and by the prevailing standards of salaries and professional fees, which vary from 10% to 35% of the total cost (685).

Peritoneal dialysis is generally the most expensive modality of ESRD therapy in most tropical countries, even when locally manufactured solutions are used. This is attributed to the extremely high overhead expenses entailed in small-scale production lines. Conversely, dialyzer reuse in many centers is often responsible for effectively reducing the comparative cost of hemodialysis.

Results and Limitations of Chronic Dialysis Treatment. The reported annual mortality rate after the first year for all dialysis modalities, for all ages, and irrespective of the primary renal disease in different tropical countries varies from 10% to 30%. The first-year mortality is about 25% to 45% higher, reaching greater than 80% in the overall experience in India (685). When considered separately, the mortality for IPD is much higher, whereas CAPD has yielded impressive results in certain series (701,702). There is a remarkable "center effect," in favor of economically privileged units that can afford recruiting better equipment and staff and that tend to attract those in the higher socioeconomic category of patients (315).

Attention to the quality of life is proportionate to an individual nation's GNPs. In the poorer countries, which constitute the majority in the tropical zone, this issue is not even raised. Patients are often content just to be alive, even though they are disabled. The incidence of complications is quite high, mainly because of inadequate dialysis, poor water quality, undernutrition, dialysis-acquired infections, and deficient interdialytic care.

Dialysis-acquired infections are extremely common, mainly including the hepatitis viruses, HIV, and CMV. HBV infection, with a prevalence approaching 90% among dialysis patients in certain countries a couple of decades ago (703), has now considerably regressed to a rate of less than 20% in most tropical countries and even to less than 6% in wealthier countries. This decline is at least partly attributed to vaccination of the populations at risk. Unfortunately, HBV infection has been largely replaced by infection with HCV, antibodies to which have been reported in 35% to 65% of populations from different tropical countries. The outcome of HCV-positive patients on dialysis is poor, with a 5-year survival of less than 20% in several series (704).

With the recognition of methicillin-resistant staphylococcal infection as an important threat in dialysis units, an increasing number of reports has confirmed the major negative impact of this infection in the Tropics. The precise incidence of this infection is unknown, but it has been certainly responsible for major outbreaks such as that reported from China in 2004 (696).

As a rule, interdialytic medical care is extremely modest in tropical countries. Even such basic goals as blood pressure control, correction of anemia, and maintenance of the calcium and acid–base balance are often overlooked. Access to active vitamin D and erythropoietin (EPO) is a matter of affordability. In the majority of tropical countries, 10% to 20% of dialysis patients receive regular EPO therapy. Exceptions to this are reported from Saudi Arabia and Argentina (60% and 64%, respectively (685). The subsequent need for multiple transfusions obviously facilitates the spread of dialysis-associated viral infections.

Renal Transplantation in the Tropics

Availability. Renal transplantation is widely available in many tropical countries. The precise data about different countries obtained from local, supranational, or different international registries are controversial. The number of transplantations per million population ranges from less than 1 (in sub-Saharan Africa) to more than 100 patients (in South America).

The Transplantation Environment. Donors for renal transplantation are either deceased, living related, or living unrelated. The major factor that determines individual country profiles is cultural, mainly dependent on whether prevailing religions and heritage accept the concepts of brain death, and removal of organs from the dead and transplanting them into strangers.

Deceased donor transplantation. The most active tropical countries in this respect are South Africa (82.5%), Venezuela (63%), and Thailand (46%). Despite earlier difficulties in harvesting and transporting donor kidneys, most of these programs are prosperous, expanding, and progressively limiting the growth of the dialysis pools. Recipients are generally selected according to standard rules and put on waiting lists with waiting times varying between 1 and 6 months.

Living donor transplantations. Living related donors constitute 15% to 85% of the overall transplant activity in different tropical countries, depending on the availability of a national deceased donor program, and the popularity of unrelated donor transplantation. The latter is particularly flourishing in Pakistan, Egypt, India, and Mexico, accounting for 50% to 80% of all transplants. It is difficult to understand the moral aspects of this practice in countries where patients with ESRD have no alternatives, and donors are left in extreme need without any kind of social support. Nevertheless, most countries do not permit officially unrelated donor transplantation; some require a formal permit of an independent ethics committee, and one country, Iran, has legalized and organized the procedure of unrelated donor transplantation through a central agency (705).

Transplantation centers. Most transplantations are performed in general hospitals. There are very few independently standing specialized transplantation centers. As with the dialysis activity and according to the same rules (vide supra), private transplantation teams share in providing the service in living donor programs.

The hygienic standards are variable. In some areas, they are far below average and are responsible for high postoperative morbidity and mortality rates, as well as for the transmission of infections such as AIDS, hepatitis, malaria, and others (706).

The Cost of Transplantation. The ultimate burden of renal transplantation on a national economy is less than that of dialysis. Despite the high initial expenses and the cost of expensive medications such as cyclosporine and monoclonal antibodies, the ultimate analysis shows that transplantation is both less

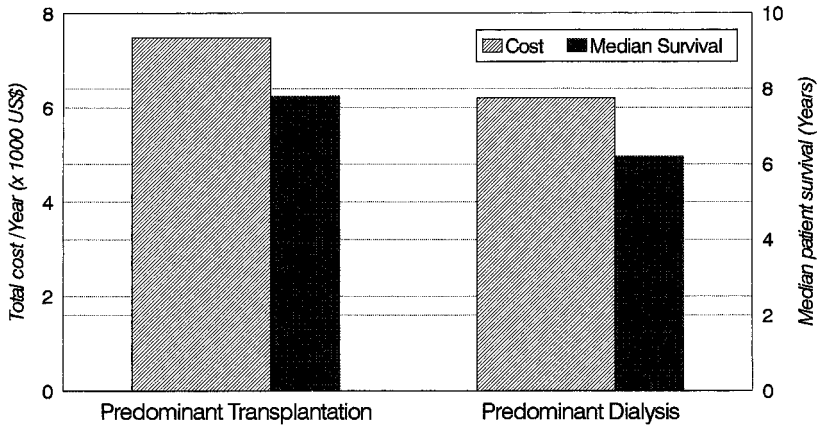

FIGURE 78-21. Estimated average annual cost of treatment and respective median survival times in two hypothetical models of end-stage renal disease treatment strategies, predominant dialysis versus predominant transplantation in a developing country. (See text for hypothesis and explanation.)

expensive and more cost effective. A local study in which cumulative survival rates were taken into consideration estimated that the annual steady-state budget for accepting 100 new patients for dialysis would be approximately $7.5 million U.S. dollars, compared with $6.2 million U.S. dollars for transplantation. The median survival times would be 2.0 and 7.1 years, respectively (707) (Fig. 78-21).

Results and Limitations of Renal Transplantation. Data obtained from national registries suggest that the outcome of renal transplantation in tropical countries is in accordance with international standards. However, it is presumable that most transplantations are not registered at all, because, for example, some countries do not have registries. It is therefore impossible to know the precise outcomes of those thousands of grafts transplanted in patients in tropical countries.

However, the published sporadic data suggest that the recipient morbidity and mortality rates are relatively high, mainly due to postoperative wound and systemic infections. The latter include activation of dormant disease as well as de novo infections. Notorious for the Tropics are such infections as tuberculosis, salmonellosis, certain parasitic infections (vide supra), and others. As outlined previously, the high incidence of certain malignancies such as Kaposi's sarcoma and Burkitt's lymphoma also accounts for the increased recipient mortality rate.

Socioeconomic Impact

ESRD is imposing a distinct socioeconomic strain on the economy, social integrity, and even morals of tropical communities at large. The financial burden in certain countries seems impossible to meet, whether by individuals or by the state. It is important to know that the cost of keeping one patient alive but partially rehabilitated on dialysis may literally exceed the average GNP per capita generated by 10 citizens (707). Yet, it is also important to realize the facts about the expenditures made by the same countries on such issues as political security, cigarette imports, and the purchase of weapons.

A few tropical countries have established independent national kidney foundations that take care of the organization and funding of ESRD therapy, promote research and development, and support staff training. This positive trend has gained success in some tropical countries.

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CHAPTER 79 ■ KIDNEY DISEASE IN CHILDREN

MELISSA A. CADNAPAPHORNCHAI, GODFREY CLARK, AND CYRIL CHANTLER

Although many renal conditions can affect both children and adults, certain disorders may be associated with different manifestations, treatment, or prognosis in these populations. The purpose of this chapter is to describe renal physiology and diseases that are unique to the pediatric population. Chapters dealing with renal conditions that affect both age groups should also be consulted for details.

KIDNEY DEVELOPMENT

Renal Embryogenesis

The anatomic stages of kidney organogenesis have been well characterized in several animal species including humans. Over the last decade, it has become apparent that normal kidney embryogenesis relies on a complex spatial and temporal interaction between stimulatory and inhibitory factors. The development of the kidney results from the phylogenetic recapitulation of three embryonic kidneys—the pronephros, the mesonephros, and the metanephros—which are derived from the intermediate mesoderm. Although the pronephros and mesonephros are transitory organs, disruption of their development can result in renal anomalies or agenesis. The metanephros is the definitive kidney in mammals and results from reciprocal interaction between the ureteric bud and the metanephric mesenchyme (Fig. 79–1) [reviewed in (1)]. Around the fifth week of gestation in humans, the ureteric bud invades the metanephric mesenchyme, resulting in branching of the ureter and transformation of mesenchymal cells into epithelial cells of the nephron (nephrogenesis). Specifically, the mesenchymal cells aggregate around the tip of each ureteric branch to form a condensate. This is followed by formation of a vesicle, which invaginates twice to form a comma-shaped body and then an S-shaped body. Mesangial and endothelial cell precursors presumably invade the lower cleft of the S-shaped body and differentiate into mesangial and vascular glomerular cells. The mesenchyme differentiates into proximal tubules, glomeruli, and vessels, whereas the ureteric bud generates the collecting ducts, the renal pelvis, and the ureteric epithelium. In humans, nephrogenesis is completed *in utero* by 34 to 35 weeks of gestation.

Disruption of branching morphogenesis is known to result in maldevelopment of the kidneys. Genetic studies in humans and animals have demonstrated critical roles for numerous transcription factors, growth factors, and cell-surface signaling molecules in the regulation of branching morphogenesis. A comprehensive list has been developed by Davies at The Kidney Development Database website [http://golgi.ana.ed.ac.uk/kidhome.html]. For example, mutations in *Eya1* (2), *Sall1* (3), and *Pax2* (4) have been shown to cause maldevelopment ranging from renal agenesis to ureteral dilation (Table 79-1).

An intact renin–angiotensin system is also critical to normal renal development. The ontogeny of this system during renal development has been described extensively (5–7). Pharmacologic inhibition of the renin–angiotensin system during nephrogenesis has been shown to cause an arrest in the branching of the kidney arterioles, abnormal thickening of the wall of intrarenal arterioles, papillary and tubular atrophy, and dilation of the renal pelvis (8). Similar findings have been observed in angiotensinogen (9), angiotensin-converting enzyme (ACE) (10), and AT1 receptor (11) knockout mice as well as the human fetus exposed to inhibitors of the renin–angiotensin system (12). Inhibition of the renin–angiotensin system during active nephrogenesis has been shown to be associated with a reduction of at least 30% in the number of nephrons (13). It is important to note that the reduction in nephron number has been proposed to result in arterial hypertension in adult life (14).

Morphologic Maturation of the Nephron

The nephrons are fully formed by 34 to 35 weeks of gestation, but those in the superficial cortex are not functionally or morphologically mature at birth. The newborn kidney contains approximately 20% of its adult complement of cells (commensurate with its corrected glomerular filtration rate [GFR]), and cell division continues until 6 months of age (15). The glomerulus is lined by cuboidal epithelium at birth and is less permeable than in the older child. The high renal vascular resistance before birth is associated with low renal blood flow, and renal function *in utero* is of primary importance in relation to the volume and composition of the amniotic fluid, which determines proper lung development. Oligohydramnios is an important sign of an infant with renal agenesis or severely dysplastic kidneys, and such infants can be born with compression deformities and an easily recognized facial appearance (Fig. 79-2). Neonatal mortality associated with these conditions is often due to lung hypoplasia.

The renal blood supply develops its interrelationship with nephron structures under the influence of angiotensin II. Cortical blood flow is restricted in the newborn kidney with a 1:1 ratio between cortical and juxtamedullary nephrons. The rapid increase in renal blood flow after birth correlates with an increase in GFR from approximately 20 to 60 mL/minute per 1.73 m^2 by 6 months of age. The GFR continues to improve over the first 2 years of life to reach adult values (Table 79-2). Because it is clinically cumbersome to assess GFR in the newborn infant due to the need for an indwelling urinary catheter, serum creatinine is generally used to monitor renal function in the newborn. Serum creatinine level is elevated at birth, reflecting maternal renal function, but rapidly decreases to a stable level of approximately 0.4 mg/dL by 1 to 2 weeks of age in the term infant. In the otherwise normal very premature infant, there is a transient increase in serum creatinine with a peak at

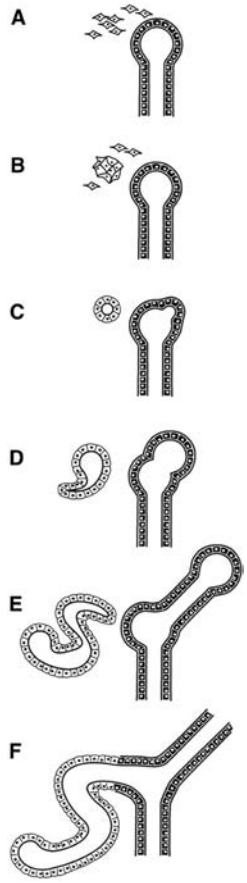

FIGURE 79-1. Morphogenic stages in early nephrogenesis. The nephrogenic pathway is initiated by inductive signaling between ureteric bud and adjacent mesenchyme. **A:** Induced condensing mesenchymal cells adhere and begin cell remodeling to epithelial phenotype. **B:** Globular aggregates close to tip of ureteric bud express basement membrane proteins and cell adhesion molecules. **C:** Renal vesicle. Lumen of sphere suggests secreted fluid and solutes. **D:** Comma-shaped body representing reorganized sphere. **E:** S-shaped body suggesting expression of an as yet unknown patterning program. **F:** Junction of upper domain of mesenchyme-derived S-shaped body with most peripheral branch of ureteric bud. (From: Horster et al. Embryonic renal epithelia: Induction, Nephrogenesis, and Cell. *Physiol Rev* 1999;79:1157, Figure 3, with permission.)

around day 4 of life, followed by a progressive decline to normal neonatal values within 3 to 4 weeks of birth (16). This peak has been attributed to tubular reabsorption of creatinine (17). Therefore, the newborn kidney's primary physiologic limitation is diminished GFR. This is of particular importance in the setting of stressors such as hypoxia, sepsis, or administration of nephrotoxic drugs.

The proximal convoluted tubule is only approximately 10% of its adult length at birth, whereas glomerular diameter is approximately one-third of its adult size. Several tubular functions are thus affected by postnatal maturation. However, tubular functions such as urinary concentrating capacity, glucose and phosphate reabsorption, and acidifying ability are normal if allowance is made for the low GFR. There is, however, greater nephron heterogeneity so that wide variation is evident in glucose and bicarbonate titration curves. The renal bicarbonate threshold is low in term infants (19 to 23 mEq/L) as compared to older children and adults, and is even lower in preterm (18 to 22 mEq/L) or very-low-birth-weight (1,300 g) infants (14 to 18 mEq/L). Postnatal maturation of carbonic anhydrases (CAs) including CA IV is responsible for these alterations in acid–base homeostasis (18). Important to note is that as the CA system matures, the excretion of an endogenous or exogenous acid load is not impaired. Consequently, even very-low-birth-weight infants can thrive despite physiologic acidemia. Tubular sodium loss is characteristic of the immediate postnatal period, with fractional excretion of sodium (FENa) approximating 5% in the term newborn as compared to 1% in the adult. The initial high FENa decreases within days in the term neonate, followed by a period of renal sodium conservation in the next 2 to 3 postnatal weeks. The development of positive sodium balance is critical for adequate growth. This process is delayed in the premature infant (16). Therefore, appropriate sodium supplementation in the postnatal period is of utmost clinical importance. Salt wasting may be apparent in the sick newborn and is common in infants whose distal tubular function is disturbed by cystic dysplasia or obstructive uropathy (19,20).

FLUID AND ELECTROLYTE REQUIREMENTS IN CHILDREN

An understanding of the relationship among renal function, growth, nutritional requirements, and fluid and electrolyte balance is fundamental for the medical care of children. Infants and children have a higher metabolic rate in relation to body mass than do adults, and this is reflected by greater energy needs and higher turnover of fluid and electrolytes. Growth obviously contributes to this increased requirement for food and is most rapid in the first 2 years of life. The average child grows 25 cm during the first year and triples his birth weight; even during the prepubertal growth spurt the gain in height per year is only approximately one-third of that achieved in the first year of life. Therefore, lack of growth in the first year of life may be difficult to compensate for later in life. The main reason for this high metabolic rate is the larger proportion of body mass that consists of metabolically active organs, especially the brain. The higher energy turnover is reflected by a higher GFR in relation to body weight after the first year (Table 79-2), which obviously assists in the maintenance of body composition. This advantage is not present in the first few months because of the immature kidney, and disturbances of body composition can occur even in healthy infants fed inappropriate foods. Human milk or an infant formula is all that is required or should be given in the first 4 months of life in normal infants, for it provides an adequate amount of energy, a large amount of fluid, and an intake of other nutrients such as protein, sodium, potassium, and phosphate, which are sufficient for growth but do not require excessive renal solute excretion.

Water requirement is directly proportional to energy needs, and, because metabolic rate correlates with body surface area (BSA), it is convenient to relate fluid requirements to surface area (21). Because 1 kilocalorie requires the consumption of 1 mL of water and basal metabolic activity requires 1,000 kcal/m^2 BSA, the basal water requirement is 1 L/m^2 BSA. Normal activity increases energy needs by 50%, so that the normal water requirement is 1,500 mL/m^2 BSA, of which 400 mL/m^2 BSA is for insensible losses in the afebrile patient. The requirements should be increased by 12% for every degree celsius of body temperature greater than 37°C. Basal electrolyte requirements can also be calculated relative to body surface area, for example, sodium 30 to 50 mEq/m^2 per day, potassium 20 to 40 mEq/m^2 per day.

An alternative method of calculating fluid requirements that avoids surface area calculations is to allow 100 mL/kg per day for the first 10 kg of body weight, 50 mL/kg per day for the next 10 kg, and 20 mL/kg per day for each kilogram over 20 kg (22). This method is based on caloric expenditure. Insensible loss is one-fourth of the total. Small or premature infants need

TABLE 79-1

HUMAN GENE MUTATIONS EXHIBITING DEFECTS IN BRANCHING MORPHOGENESIS

Primary disease	Gene	Phenotype
Apert syndrome	*FGFR2*	Hydronephrosis
Beckwith-Wiedemann syndrome	*P57KIP2*	Renal medullary dysplasia
Branchio-Oto-Renal (BOR) syndrome	*EYA1*	Unilateral or bilateral renal agenesis, dysplasia/hypoplasia; collecting system anomalies
CAKUT	*AGTR2*	Renal dysplasia/hypoplasia; collecting system anomalies
Renal-Coloboma syndrome	*PAX2*	Unilateral renal agenesis; hypoplasia; vesicoureteral reflux
Simpson-Golabi Behmel syndrome	*GPC3*	Renal medullary dysplasia
Townes-Brocks syndrome	*SALL1*	Renal dysplasia/hypoplasia; vesicoureteral reflux

(Reprinted from: Hu MC, Rosenblum ND. Genetic regulation of branching morphogenesis: lessons learned from loss-of-function phenotypes. *Pediatr Res* 2003;54:434, with permission.)

even more fluid. Maintenance electrolyte requirements can then be determined based on fluid requirements, that is, sodium 3 mEq/100 mL per day, potassium 2 mEq/100 mL per day.

It is apparent from these considerations that the fluid intake and urine output of small children is much higher in relation to body weight than for adults, and the importance of a low-solute intake in relation to energy and water intake should be appreciated. A large volume of dilute urine enables adequate conservation of water by urine concentration in response to extrarenal losses, whereas a solute intake of adult dimensions in relation to energy diminishes dilution and therefore the capacity for further urine concentration.

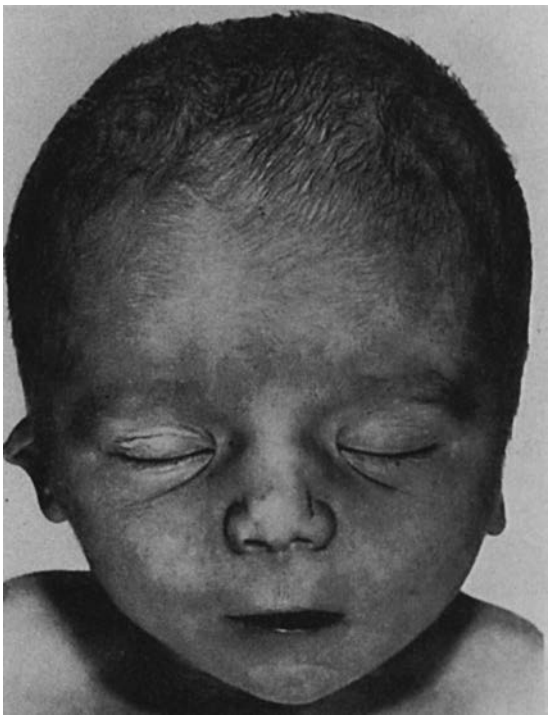

FIGURE 79-2. Facial appearance of a child with renal agenesis (Potter's syndrome). (From: Lieberman E. *Clinical Pediatric Nephrology.* Philadelphia: JB Lippincott, 1976:219; with permission.)

Relevance for Clinical Management

Close attention to the infant or child's fluid and electrolyte status is important, particularly in children with renal disease. High energy requirements may be difficult to satisfy in the sick child, and malnutrition can develop rapidly. Due to a variety of physiologic derangements, infants and young children with chronic renal insufficiency (CRI) often demonstrate growth failure. Nasogastric feeding or intravenous feeding in prolonged acute renal failure (ARF) and nasogastric or gastrostomy tube feeding in CRI are used if necessary to supply adequate energy intake. Dialysis is required earlier and more frequently to maintain body composition. Fluid balance charts are difficult to maintain with any reliability, and by far the most useful parameter in clinical management is frequent weighing combined with a clinical assessment of the state of hydration and blood volume judged by the venous pressure, blood pressure, and capillary refill.

In hyperosmolar dehydration, the normal signs of reduced skin turgor are absent because the water loss is largely intracellular; the child is irritable, the mucous membranes usually are parched, and the eyes are sunken. Extra fluid losses in vomitus and diarrhea may be considerable in proportion to body mass. Furthermore, infants lose heat rapidly and constant attention to the thermal environment is imperative.

Two considerations are important in relation to drug therapy. The volume of distribution is determined by the body weight so that the loading dose can be calculated accordingly. The maintenance dose, however, is determined by the turnover or metabolic rate that correlates with the surface area rather than body weight and is therefore higher in relation to weight than for adults. If a constant blood level is required without large peaks, the drug must be administered more frequently than for an adult.

IDENTIFICATION AND INVESTIGATION OF RENAL DISEASE IN CHILDREN

Imaging

Routine prenatal ultrasonography with views of the major organs of the fetus has led to the antenatal detection of congenital

TABLE 79-2

GLOMERULAR FILTRATION RATE IN CHILDREN IN RELATION TO BODY SIZE AND KIDNEY SIZE

Age	Kidney weight (g)	GFR mL/min	GFR/body weight (mL/minute/kg)	GFR/SA (mL/minute/1.73 m^2)	
				Mean	Range ± 2 SD
Birth	27	2.5	0.7	20	
7 day	29	4.6	1.3	38	26–60
1 mo	32	6.4	1.6	48	28–68
6 mo	51	15.5	2.0	77	41–103
1 yr	71	28	2.9	115	49–157
2 yr	93	38	3.1	127	89–165
8 yr	149	70	2.7	127	89–165
Adult	290	131	2.1	131	88–174

GFR, glomerular filtration rate; *SA,* surface area.
(Reprinted from: Oh W, Oh MA, Lind J., Renal function and blood volume in the newborn infant related to placental transfusion. Acta Paediatr Scand 1966; 56:197, with permission.)

renal anomalies in 15 per 10,000 live births (Table 79-3); upper renal tract dilatation has been reported to occur in as many as 1 per 100 pregnancies. Fetal renal ultrasonography is accurate in detecting the number, size, and position of the kidneys, upper and lower tract dilation, and liquor volume. It is reasonably good at revealing qualitative differences in renal echogenicity that may provide useful information about the presence of renal lesions, such as dysplasia and cysts (23). However, it provides little information about renal function so should not be relied on to give prognostic information to parents.

Detection of renal abnormalities antenatally allows the early proper investigation of the renal tract postnatally. In this way, obstructive uropathy can be promptly relieved, and antibiotic prophylaxis can be commenced immediately after the birth before development of serious urinary infection (24). It is hoped that early diagnosis will improve the long-term prognosis of these children. Drainage of the urinary tract can be accomplished *in utero* by inserting a suprapubic bladder catheter to drain the urine into the amniotic fluid. There is as yet no clear evidence that this modifies the outcome for renal function (25), and an accurate diagnosis is required if the fetus with a dilated but nonobstructed urinary tract is not to be placed at risk (26). Unfortunately, the hope that severe pulmonary hypoplasia, which so often is a fatal complication of severe urinary tract obstruction, could be prevented by early drainage *in utero* has not been sustained. This is not surprising because it is likely that the intervention would have to occur before the 16th week of gestation if pulmonary growth were to be enhanced (27).

TABLE 79-3

PREVALENCE OF CONGENITAL RENAL TRACT ANOMALIES

Anomaly	Rate per 10,000 births
Renal agenesis/dysplasia	4.7
Renal tract obstruction	8.0
Bladder or urethral obstruction	2.3
Exstrophy of bladder	0.3

(Reprinted from: Schulman J, Edmonds LD, McClearn AB, et al. Surveillance for and comparison of birth defect prevalences in two geographic areas–United States, 1983–1988. MMWR *Morbid Mortal Wkly Rep* 1993;42:1, with permission.)

Renal/bladder ultrasonography is often used for initial structural evaluation of the urinary tract in children. Doppler ultrasonography is used for assessment of renal artery stenosis, although this technique is not as sensitive as ^{99m}Tc-mercaptotriacetylglycine (^{99m}Tc-MAG3) nuclear renal scan or magnetic resonance angiography for this purpose. Doppler ultrasonography is also useful for evaluation of renal venous blood flow in suspected cases of renal venous thrombosis. Renal scarring may be identified by renal ultrasound scanning, particularly in the older child or when the scars are large or the kidney small and irregular, but it should not be used as the sole method to detect vesicoureteral reflux (VUR) or reflux nephropathy due to a high false-negative rate (28–30). ^{99m}Tc-Dimercaptosuccinic acid (^{99m}Tc-DMSA) is more sensitive than renal ultrasonography for detecting scars.

Micturating cystourography is valuable in the assessment of bladder abnormalities, including VUR or posterior urethral valves. The initial micturating cystogram should be performed with contrast in order to characterize urinary tract anatomy (posterior urethral valves, diverticulae, fistulae) and for diagnosis and grading of VUR. Indirect nuclide cystograms using ^{99m}Tc-MAG3 may then be used for annual follow-up of children with primary VUR. Computed tomographic scanning and magnetic resonance imaging of the abdomen provide high-resolution imaging of the kidneys and renal tract in cases of pyelonephritis, tumors, or cysts (31), as well as defining spine and spinal cord anatomy, which is useful because obstructive uropathy and bladder dysfunction can coexist.

Scintigraphy with ^{99m}Tc-MAG3 provides dynamic and functional data in suspected obstructive uropathy and exposes the child to approximately one-third less radiation than ^{99m}Tc-diethylenetriaminepentaacetic acid (^{99m}Tc- DTPA) scanning; its use in children has superceded the latter. As noted in the preceding text, ^{99m}Tc-DMSA is of particular value to assess acute parenchymal inflammation with pyelonephritis or parenchymal scarring as in reflux nephropathy.

Renal Function Tests

Assessment of GFR is the single most important test of renal function required in clinical practice. The 24-hour endogenous creatinine clearance is used most frequently in adults, but such urine collections can be difficult to obtain in infants and young children, particularly those who are outpatients. The 24-hour urine excretion of creatinine is a measure of creatinine production that is related to muscle mass and in turn correlates with

the cube of height in boys and girls from the age of 6 months to maturity (32). Because GFR correlates with BSA or the square of height, it follows that GFR corrected for BSA is related to height. Thus,

$$\mathrm{GFR} = \frac{\mathrm{UcV}}{\mathrm{Pc}} \quad [1]$$

$$\mathrm{UcV} \ \alpha \ \mathrm{height}^3 \quad [2]$$

$$\mathrm{GFR} \ \alpha \ \mathrm{height}^2 \quad [3]$$

$$\frac{\mathrm{GFR}}{\mathrm{BSA}} \ \alpha \ \frac{\mathrm{height}}{\mathrm{Pc}} \quad \text{or} \quad \frac{\mathrm{GFR}}{\mathrm{BSA}} = \frac{\mathrm{k(height)}}{\mathrm{Pc}} \quad [4]$$

where V is urine flow rate and Uc and Pc are the urine and plasma concentrations of creatinine, respectively. The value for the constant k has been empirically derived (33–35). Thus:

$$\mathrm{GFR(mL/minute/1.73m^2)} = 40 \times \mathrm{height\,(cm)/Pc\,(\mu mol/L)} \quad [5]$$

or

$$\mathrm{GFR(mL/minute/1.73m^2)} = 0.55 \times \mathrm{height\,(cm)/Pc\,(mg/dL)} \quad [6]$$

when using automated methods for the measurement of plasma creatinine. The different relation of muscle mass to height in infants younger than 1 year and in adolescent boys results in k values of 0.45 and 0.7, respectively, when plasma creatinine is measured as milligrams per deciliter (35). Due to the difficulty associated with 24-hour urine collection in young children, estimates of GFR from the height and plasma creatinine may be more reliable than the 24-hour endogenous clearance in children and obviously are more convenient. It is important to note that this method is unreliable when the normal relation between muscle mass and height is altered, as in malnutrition or muscular dystrophy, or in severe renal failure when tubular secretion of creatinine can occur.

Plasma creatinine concentration averages 0.88 mg/dL at birth, when the level is largely determined by the mother's plasma creatinine concentration. It falls to a nadir of 0.45 mg/dL at 2 years as GFR increases and then rises with the increase in muscle mass. From age 2 weeks to 5 years, a normal blood creatinine level is between 0.2 and 0.5 mg/dL. From 5 to 10 years, a normal blood creatinine level is between 0.4 and 0.7 mg/dL. Normal serum creatinine may be as high as 1.2 to 1.3 mg/dL in older teenagers or adult men. However, a serum creatinine concentration greater than 1.3 to 1.4 mg/dL should raise concern for underlying renal disease. More precision may be obtained by performing 3-hour creatinine clearance determinations under diuretic conditions or single-injection plasma clearance estimations using ^{51}Cr ethylenediaminetetraacetic acid, sodium iothalamate, or inulin.

Proteinuria

Quantitative assessment of proteinuria in children is affected by the unreliability of urine collections. However, its main purpose is to detect damage to the glomerular filter leading to increased permeability, and for this purpose the most sensitive parameter is the sieving coefficient (the relative concentration in glomerular filtrate and plasma water) of a molecule of a size that normally is just restrained by the glomerular filter. The protein–creatinine ratio provides a suitable approximation for use in clinical practice; being a ratio, it is independent of urine flow rate and can be estimated from a random urine sample (36,37). A value of less than 0.2 is considered normal and greater than 2.0 is considered nephrotic-range proteinuria in children. The use of this ratio is preferred for children rather than the commonly performed 24-hour urine excretion for which more than 200 mg or 5 mg/kg per day usually is regarded as abnormal in children. The albumin excretion rate from a timed urine collection, or microalbuminuria in diabetic children, is an important predictive marker for diabetic nephropathy (38). Rarely, dipstick albuminuria due to predominantly tubular disease is encountered.

Proximal Tubular Function

The detection of proximal tubular dysfunction rather than its quantitation is all that is usually required, and for this purpose the presence of glycosuria, aminoaciduria, hyperchloremic acidosis with bicarbonate wasting, polyuria, polydipsia, rickets, or a combination of these features, which represent Fanconi's syndrome, usually are sought. Phosphaturia can be measured and is useful for assessing both proximal tubular function and secondary hyperparathyroidism in children with CRI. The fractional excretion of phosphate ($FEPO_4$) is the proportion of filtered phosphate that is excreted. Because this is calculated as a clearance ratio, a timed urine collection is not necessary.

$$\mathrm{FEPO_4} = \frac{\mathrm{UPO_4V}}{\mathrm{PPO_4}} \times \frac{\mathrm{Pc}}{\mathrm{UcV}} \quad [7]$$

where UPO_4 is the phosphate excretion and $PPO_4 \times UcV/Pc$ is the filtered phosphate.

The fractional excretion of phosphate also can be used to calculate the theoretic phosphate threshold for renal tubular reabsorption ($TmPO_4$/GFR). For children, the use of nomograms is unnecessary and the phosphate threshold can be calculated using a random urine sample and simultaneous plasma sample from the following formula (39):

$$\mathrm{TmPO_4/GFR} = \mathrm{PPO_4} - \frac{\mathrm{UPO_4} \times \mathrm{Pc}}{\mathrm{Uc}} \quad [8]$$

The phosphate threshold provides a useful index of parathyroid hormone (PTH) activity in children (40) and is relatively insensitive to changes in phosphate intake or GFR. The $FEPO_4$ ranges between 2% and 12% in young children and rises to between 5% and 20% in adults: $TmPO_4$/GFR decreases from an average of 6 mg/dL in children to 3.4 mg/dL in adults.

Tubular proteinuria can be assessed by the clearance of retinol-binding protein, β_2-microglobulin, and α_1-microglobulin in urine relative to urinary creatinine concentration. All are low-molecular-weight proteins (LMWPs) that are present in plasma and filtered by glomerular filtration, after which 99.9% of the filtered LMWP is reabsorbed and catabolized by tubular cells. The LMWP fractional excretion is high in infants, reflecting tubular immaturity, but falls from 1.68% to 0.5% between birth and 2 years of age (41). β_2-Microglobulin is unstable in acid urine, and in clinical practice it is more convenient and accurate to use the excretion of retinol-binding protein as a measure of proximal tubular dysfunction. The normal range for the excretion of retinol-binding protein in a random sample is less than 1 to 24.5 mg per mole of creatinine (42). Tubular disease is associated with a marked increase in the excretion of LMWP (43), and this can be estimated again as a fractional excretion from a random urine and blood sample. The presence of excessive *N*-acetyl-glucosaminidase—an enzyme released from the proximal tubule cell brush border—in urine is indicative of proximal tubular damage, and has been shown to be markedly elevated in Fanconi's syndrome due to cystinosis (44) or ifosfamide treatment (45).

Distal Tubular Function

Urine Acidification

Hydrogen ion is secreted by tubular cells and neutralizes filtered bicarbonate to form carbonic acid, which is then dehydrated via carbonic anhydrase, thereby allowing carbon dioxide to diffuse into the tubular cells and then be reconstituted as bicarbonate (see Chapter 6). Distal tubular fluid is essentially free of bicarbonate. When bicarbonate is in the urine, the urine pH exceeds 6.1; for a pH of 6.1, urine bicarbonate concentration is approximately 1 mmol/L. Plasma bicarbonate level less than the normal threshold for bicarbonate reabsorption implies a defect of proximal tubular bicarbonate handling if other sources of bicarbonate loss have been excluded. The threshold may need to be defined in a child with proximal renal tubular acidosis (RTA), and this can be achieved by raising the plasma bicarbonate concentration with an infusion of bicarbonate or by stopping the bicarbonate supplements and observing the urine pH as the plasma bicarbonate falls (46). As noted in the preceding text, the threshold for bicarbonate reabsorption is lower in infants than in older children and the splay on the bicarbonate reabsorption curve is wider so that the plasma bicarbonate concentrations are lower, ranging from 19 to 23 mEq/L for infants to 20 to 26 mEq/L for children. The range of normal in premature infants extends from 14 to 22 mEq/L (47).

Urine excretion of bicarbonate, titratable acid, and ammonium are essentially determined by the urine hydrogen ion concentration, so that much can be learned from measurement of urine pH. Most children pass acid urine during the night and, if the plasma bicarbonate is normal, an early-morning urine pH of less than 5.3 excludes a defect of hydrogen ion excretion.

Renal Tubular Acidosis. Renal tubular acidosis (RTA) should be suspected in a child with a normal plasma anion gap [$Na^+ - (Cl^- + HCO_3^-) = 8$ to 16 mEq/L] and a hyperchloremic acidosis. Clinically, RTA is characterized by failure to thrive, polyuria, constipation, and in prolonged untreated RTA, rickets. Loss of HCO_3^- from the gastrointestinal tract, mild volume depletion, and the administration of hydrochloric acid or its precursors should be excluded. The urine anion gap ($Na^+ + K^+ - Cl^-$) is a useful tool to differentiate between RTA and the other conditions because it estimates urine ammonium concentration; thus, a negative gap suggests gastrointestinal loss of HCO_3^-, whereas a positive gap ($Na^+ + K^+ > Cl^-$) indicates distal RTA (48). Hyperkalemia should raise concern for type IV RTA (see Chapter 6).

The underlying defect in type 1 or distal RTA is failure of distal tubular sodium hydrogen ion exchange with associated sodium and bicarbonate wasting and urine pH greater than 5.5. Extracellular volume contraction in this condition is associated with hyperaldosteronism with potassium wasting and associated lack of renal concentrating ability (polyuria). Increased urinary calcium excretion and decreased urinary citrate excretion often result in nephrolithiasis or nephrocalcinosis in distal RTA. In fact, the urinary calcium–creatinine ratio is a useful marker for adequacy of therapy. Over the last several years, mutations of genes encoding carbonic anhydrase II, kidney anion exchanger 1, and different subunits of the hydrogen-ATPase proton pump have been shown to be responsible for inherited forms of distal RTA (reviewed in [49]). However, secondary causes of distal RTA, including obstructive uropathy, should be excluded (see Chapter 6). The diagnosis of distal RTA can be established by documenting an inability to lower urine pH below 5.3 either after ammonium chloride loading or after furosemide (50). However, such extensive testing is rarely necessary. In contrast, proximal or type 2 RTA is characterized by renal bicarbonate wasting with a resetting of the threshold for proximal tubular bicarbonate reabsorption. Because distal nephron acidification is intact, affected patients are able to generate acidic urine. Type II RTA can rarely be transmitted in an autosomal dominant or recessive manner or can occur in association with urinary tract obstruction. Generalized proximal tubular dysfunction (Fanconi's syndrome) should be excluded.

Evaluation for RTA should include a urinalysis to examine pH, to exclude glycosuria suggestive of Fanconi's syndrome, and to exclude other renal disease. Consideration should be given to obtaining a urine calcium-creatinine ratio. Serum phosphorus concentration is typically low in Fanconi's syndrome, and hypophosphatemia should prompt more extensive evaluation of proximal tubular function, including urine amino acids and tubular reabsorption of phosphate as described in the preceding text. A renal ultrasound should be obtained to exclude obstruction and nephrocalcinosis. A thorough evaluation should exclude other causes of growth failure, including inadequate caloric intake or thyroid disease. Further metabolic evaluation should be considered in children with an elevated anion gap.

Urine Concentrating Capacity

Urinary concentrating capacity is relatively impaired in younger as compared to older children. If the plasma osmolality is increased to more than 290 mOsm/kg and the urine is not concentrated, then a concentrating defect exists. Most children pass concentrated urine first thing in the morning following sleep, and if the osmolality exceeds 800 mOsm/kg, no further tests are required. If the child has frank polyuria and polydipsia, water deprivation tests should be carried out during the day with the child weighed at regular intervals and the test terminated when the weight loss exceeds 5%. A plasma osmolality can be obtained at this time to confirm that an adequate stimulus for urine concentration has been achieved and desmopressin (dDAVP) given intranasally to differentiate central from nephrogenic diabetes insipidus. Normal children respond to a standard water deprivation test by producing a urine osmolality of 1,089 ± 110 mOsm/kg (51). To perform this test, no fluid is allowed after 5 o'clock pm; the bladder is emptied at 8 o'clock pm, all the urine passed overnight is collected, and the osmolality is measured. Consideration should be given to hospitalization of infants and very young children for water deprivation testing. An alternative is to administer dDAVP (20 μg intranasally; 10 μg for infants), but water intake should then be controlled to avoid water intoxication. The magnitude of the rise in urine osmolality with water deprivation depends partly on the urea excretion and the daily protein intake. Infants on low-protein milk or human milk concentrate urine less well, although the urine concentration of nonurea solutes and the ability to conserve water are similar to those of older children (52).

Plasma and Urine Electrolytes

Sodium

Hyponatremia does not generally imply a reduction in total body sodium because plasma sodium concentration measurement must be assessed in conjunction with extracellular fluid (ECF) volume. The renal conservation of sodium is limited in premature infants, and hyponatremia is common if sodium supplements are not given. Extrarenal losses of sodium from vomiting or diarrhea leading to hypovolemia also result in water retention from increased antidiuretic hormone (ADH) secretion. Hyponatremia is common in relapse of the nephrotic syndrome when intravascular volume depletion stimulates ADH

secretion and thirst. In ARF, the continued intake of water can lead to edema and dilutional hyponatremia, particularly in neonates with unsuspected renal failure. The syndrome of inappropriate secretion of ADH often is wrongly diagnosed in the sick newborn, particularly in those with a respiratory problem who are maintained on artificial ventilation. The diagnosis requires that the secretion of ADH be inappropriate with regard both to the plasma osmolality and to the volume status of the plasma and ECF in the presence of normal thyroid function. Determination of the etiology of hyponatremia requires careful assessment of sodium and water intake, the clinical signs of ECF volume expansion or contraction, an estimation of renal function and GFR from the plasma creatinine and blood urea, and the analysis of urine sodium concentration and osmolality.

Hypernatremia is most often seen in the setting of dehydration when total body water losses exceed sodium losses (e.g., diarrhea). Hypernatremia also occurs in children with diabetes insipidus in the setting of impaired fluid intake, either due to lack of mobility or communication skills (as in infants) or with intercurrent illness (e.g., vomiting). Excessive sodium intake compared with water intake in infants with diarrhea or those fed unmodified cow's milk can cause hypernatremia; rarely, hypernatremia results from failure of normal thirst control (53) or from salt poisoning (54,55). Hypernatremia due to inappropriate fluid prescription and abnormal thirst mechanism has been reported in the hospital setting (56).

The 24-hour urine sodium excretion is simply a measure of dietary intake and provides no other useful information in a normal person or in the patient in chronic renal failure with stable body composition. In ARF, the measurement of urine sodium and urea concentration enables prerenal failure to be distinguished from intrinsic renal failure. The fractional excretion of sodium (FENa%), again calculated as a clearance ratio on a random urine and plasma sample, provides the best discrimination; in intrinsic renal failure it exceeds 5% in neonates or 2.5% in older children:

$$\text{FENa\%} = \frac{\text{UNa}}{\text{PNa}} \times \frac{\text{Pc}}{\text{Uc}} \times 100 \qquad [9]$$

where *UNa, PNa, Uc,* and *Pc* are the urine and plasma concentrations of sodium and creatinine, respectively.

Calcium and Phosphate

Approximately 50% of total plasma calcium is protein bound and, in children who are dehydrated, this can cause an increase in total plasma calcium without an increase in the physiologically important ionized fraction. Total plasma calcium measurement should be corrected in children who have apparently high values but who are dehydrated because of polyuria or for other reasons by subtracting 0.09 mg/dL (0.0225 mmol/L) for every 1 g/L by which the plasma albumin exceeds 46 g/L. Hypercalcemia can cause a renal concentrating defect, leading to an erroneous diagnosis of nephrogenic diabetes insipidus. Urine calcium excretion varies with diet, exposure to sunlight, and seasons, but the upper limit for normal is usually taken as 4 mg/kg per 24 hours or 0.25 mg of calcium per milligram of creatinine (57).

Plasma inorganic phosphate concentration is higher in children than in adults, particularly during the first year of life, and thus is associated with a higher threshold for renal tubular phosphate reabsorption (see previous text). The normal range in infants is 3.7 to 8.6 mg/dL, falling to 3.4 to 5.9 mg/dL at 1 year, 3.6 to 5.0 mg/dL at 7 years, and 2.8 to 4.8 mg/dL after puberty (58). The upper limit for the solubility product for calcium × plasma phosphorus is 60.0 (mg/dL × mg/dL) or 4.8 (mmol/L × mmol/L). It is important to monitor this parameter when managing children with CRI.

Miscellaneous

The upper limit for the urinary oxalate–creatinine ratio (mmol/mmol) falls from 0.26 before 1 year to 0.15 at 12 years and 0.083 after 12 years of age. The mean (SD) plasma oxalate–creatinine molar ratio is 0.033 (0.013) and is independent of age and renal function (59). Urinary vanillylmandelic acid excretion also is better expressed as the creatinine ratio, and the upper limit falls from 16.3 μmol vanillylmandelic acid per millimole creatinine at 6 months to 13.7 at 6 years and 7.6 in older children (60).

RENAL DISEASE IN THE FETUS AND NEONATE

Neonatal Renal Problems

More than 90% of newborns pass urine within 24 hours, and failure to do so within 48 hours is abnormal (61). Proteinuria occurs in 20% of normal newborns, and a few red blood cells occasionally are found in the urine (62). Renal pathology in this age group most often relates to congenital abnormalities of the urinary tract or to renal damage that occurs perinatally (63). With the advent of routine prenatal ultrasonography, many infants with identified renal tract anomalies can be delivered at centers with appropriate pediatric renal care. In Table 79-3 are shown the relative prevalence of various congenital renal anomalies.

Many congenital renal tract anomalies are sporadic, but inherited or syndromic abnormalities are well described (64). In addition to identifying those cases that form part of a syndrome, the pediatric nephrologist's role is to diagnose and relieve urinary tract obstruction, diagnose renal impairment and hypertension, identify conditions that lead to progressive renal failure, promote normal growth of the infant, and prevent or treat urinary tract infection (UTI) and hypertension.

Structural Abnormalities

Antenatal ultrasonographic examination of the fetus can detect many structural renal anomalies, which occur at a frequency of 15 infants per 10,000 live births. Ultrasonography can identify unilateral or bilateral renal agenesis, hypoplasia, dysplasia, cystic disease, hydronephrosis, hydroureter, and abnormal bladder, as well as abnormal renal shape (horseshoe kidney) and position (ectopic pelvic kidney). It cannot diagnose urinary tract obstruction, only dilation which may or may not be associated with obstruction. More than 300 congenital syndromes have been described that involve urinary tract anomalies, and because many are inherited, current hypotheses of renal tract maldevelopment involve genetic dysfunction in the embryo. However, the etiology of renal structural anomalies is likely multifactorial because many conditions occur only sporadically in families, and in general the risk to subsequent offspring is low. Environmental factors are known to contribute to some anomalies (e.g., renal agenesis/hypoplasia in fetal alcohol syndrome).

Bilateral renal agenesis affects 1 in 7,000 fetuses. Estimates of unilateral renal agenesis vary from 1 in 500 to 1 in 3,200 (65), and these children have a normal life expectancy if their single kidney is normal. Unilateral renal agenesis has a male preponderance; this has been attributed to earlier differentiation of the Wolffian duct, which takes place close to the time of ureteric bud formation (66). Preexisting maternal diabetes mellitus and maternal African American race have been shown to be associated with renal agenesis (65). Approximately 5%

of first-degree relatives of patients with bilateral agenesis have unilateral agenesis, which suggests that the cause is either polygenic with a threshold-determining expression or multifactorial with genetic and environmental influences.

Renal Dysplasia

Renal dysplasia is one of the most common congenital renal malformations and on ultrasonography is characterized by loss of normal corticomedullary differentiation and usually bright echogenic kidneys. The association between dysplasia and obstruction is important because the prognosis for renal function after surgical relief of the obstruction is affected by the coexistent dysplasia. Dysplasia often arises from severe obstruction to urinary flow *in utero,* such as occurs with posterior urethral valves in boys (67), prune-belly syndrome (68), or severe VUR (69), or can be associated with specific syndromes (e.g., Ivemark's syndrome [70]). It may be unilateral or bilateral, in which case it usually affects the kidneys asymmetrically and causes CRI early in childhood, often requiring renal replacement therapy. Renal histologic investigation shows developmental arrest, failure of differentiation, and the persistence of fetal mesonephric structures (71). Primitive ducts lined by columnar epithelium, nests of metaplastic cartilage, fetal glomeruli, and cysts may be found. The association of renal dysplasia and urinary obstruction and the abnormal tubular anatomy results in abnormal tubular physiology. Fractional reabsorption of sodium and bicarbonate in the proximal tubule is reduced. Distal reabsorption of sodium and hydrogen ion excretion may be impaired, causing the neonatal kidney to have an even higher fractional excretion of sodium. Chronic salt depletion compromises growth and cognitive function, and hypertension does not typically occur. Because urinary obstruction may damage the distal tubule and collecting ducts, urinary concentration is reduced, leading to variable but obligatory polyuria. These children require free access to oral fluids. Dehydration and further renal impairment occur when these children are deprived of fluid, such as occurs for hospital investigations and anesthesia, or when increased extrarenal fluid and electrolyte losses are present (e.g., vomiting, gastroenteritis). Sodium wasting is treated with salt supplements, the dose being adjusted to maintain good growth, a normal plasma sodium concentration, and normal blood pressure without clinical evidence of excessive ECF volume. The metabolic acidosis usually requires sodium bicarbonate supplements, 2 to 4 mmol/kg per day. If significant chronic renal impairment is present, attention to nutrition, which may require tube feeding to supply sufficient calories, and secondary hyperparathyroidism is needed (see subsequent text). The physician should be aware that growth may be compromised in these children with renal dysplasia, which does not depend solely on degree of renal impairment.

If urinary obstruction is present then this must be relieved. A marked postoperative increase in urine production and salt loss may occur because of the reduced proximal tubular reabsorption of sodium and poor urinary concentrating ability of the dysplastic kidney. The consequent reduction in ECF further compromises renal perfusion and GFR, so careful postoperative fluid balance should be maintained and fluid prescription increased to anticipate the increased natriuresis and diuresis. Renal adaptation with reduction in polyuria may take days or even weeks to occur.

The multicystic dysplastic kidney constitutes a form of renal dysplasia where the kidney has multiple cysts associated with an atretic ipsilateral ureter. Bilateral disease is associated with absence of renal function and is incompatible with life. Unilateral disease occurs in 1 in 4,300 live births. The unilateral multicystic dysplastic kidney typically undergoes progressive atrophy during the first 2 years of life; however, some are very large at birth and may require surgical excision. Such kidneys are only rarely associated with hypertension. Renal ultrasonography in this setting demonstrates multiple noncommunicating cysts of varying size with no identifiable renal parenchyma. A renal scan will document lack of function of the affected kidney. Up to 30% of contralateral kidneys have abnormalities including VUR, obstruction, or dysplasia, a voiding cystourethrogram should and be routinely obtained in such patients. The small risk of hypertension and equally rare incidence of malignant change contributed to routine nephrectomy of the multicystic dysplastic kidney in the past. However, more recent studies suggest that conservative management is associated with minimal risk, and it has been suggested that affected children can receive annual clinical follow-up and routine renal ultrasonography at 2, 5, and 10 years of age (72). Nephrectomy should be considered for children with significant mass effect, as can be particularly seen with preterm infants, or in the setting of hypertension, increasing cyst size, or an increasing stromal component suggestive of possible malignant transformation. Although nephrectomy obviates medical observation, the anesthetic risk is comparable to the medical complication risk (73).

Congenital Hydronephrosis

Antenatal hydronephrosis is the most common condition detected by prenatal ultrasonography, being observed in 1:100 to 1:500 studies (74). However, approximately 20% of abnormalities observed *in utero* are not present postnatally (75). Although there is consensus that follow-up renal and bladder ultrasonography should be performed on these neonates, experts debate the timing. Early scans within 48 hours (when the child may be dehydrated and have a lower GFR) may underestimate hydronephrosis. Late scans performed at 7 to 10 days increase sensitivity but risk noncompliance and loss to follow-up. Thus far, studies have shown no difference in outcome with early versus late scans (76). It is generally recommended, however, that the postnatal renal ultrasound be obtained soon after birth in children with moderate-to-severe prenatal hydronephrosis. A voiding cystourethrogram (VCUG) should be obtained on all infants with antenatal hydronephrosis to exclude vesicoureteral reflux, urethral valve position, or diverticular disease in the bladder, even if the postnatal ultrasound is normal (77). Until the VCUG confirms no reflux, prophylactic therapy should be initiated with amoxicillin (10 mg/kg per day) (74). ^{99m}Tc-MAG3 scanning should be considered to exclude obstruction to evaluate differential renal function. In general, in the absence of hydronephrosis, a solitary kidney, or suspected posterior urethral valves, the nuclear renal scan can be deferred until the first 4 to 6 weeks of life. Functional scans are best performed after 1 month of age when renal concentrating ability has matured.

Common obstructive causes of postnatal hydronephrosis include ureteropelvic junction (UPJ) obstruction (44%), ureterovesical junction obstruction (21%), multicystic dysplastic kidney, ureterocele/ureteral ectopia, duplex collecting system (12%), posterior urethral valves (9%), and, less commonly, urethral atresia, sacrococcygeal teratoma, and hydrometrocolpos (78). Nonobstructive causes include VUR (14%), physiologic dilation, prune-belly syndrome, renal cystic disease, and megacalycosis.

Indications for and timing of surgical intervention for UPJ obstruction remains somewhat controversial. It has been argued that up to 85% of infants with prenatally detected hydronephrosis do not have true urinary tract obstruction and thus will improve spontaneously (79–81). Whether early surgical intervention significantly alters renal function is also debated (82–85). It has been suggested that those children with

severe renal impairment on initial ^{99m}Tc-MAG3 scanning (<20% differential function) are unlikely to have significant recovery of function postpyeloplasty (86). Proposed criteria for pyeloplasty for UPJ narrowing include differential GFR less than 35%, severe hydronephrosis (>2.5 cm anteroposterior diameter), or deteriorating renal function of 10% or more in the affected kidney. Infants born with mild hydronephrosis (<1.5 cm anteroposterior diameter) are not likely to become obstructed. However, such children should be monitored with serial imaging studies because significant deterioration in the hydronephrosis occasionally may occur despite initial good prognostic features.

Bilateral hydronephrosis, particularly in male infants, should be more urgently investigated and treated. The presence of antenatal oligohydramnios and prematurity correlates with a poor postnatal renal outcome (87). However, antenatal renal ultrasonography is unreliable in predicting renal function postnatally (88,89). In addition to prophylactic antibiotics on day 1, plasma creatinine should be measured frequently in the first week of life to document the expected decrease in value or identify renal impairment if present. Renal ultrasonography with bladder views to measure bladder volume and wall thickness, prevoiding and postvoiding, together with micturating cystourethrography should be obtained within the first week to diagnose posterior urethral valves or VUR. Urgent investigation is indicated if renal impairment is present. If posterior urethral valves are present, continuous bladder drainage via urethral or suprapubic catheter is necessary until a pediatric urologist carries out cystoscopic resection of the valves or vesicostomy. Alternatively, if VUR is not responsible for the bilateral hydronephrosis, early ^{99m}Tc-MAG3 scanning should be performed in an attempt to document differential GFR of each kidney and any degree of urinary obstruction. In children with spina bifida, early bladder videourodynamics should be performed rather than delayed to document the degree of bladder dysfunction, because significant renal damage may occur from high-pressure VUR and infection.

Acute Renal Failure

The newborn's kidneys are at risk for development of ARF because of perinatal asphyxia, low GFR, renal vasomotor constriction, and intravascular volume depletion. Consequently, acute tubular necrosis or infarction (acute cortical necrosis) may develop in up to 8% of neonates in intensive care (90). It has been suggested that a persistent rise in plasma creatinine greater than 1.5 mg/dL (approximately 130 μmol/L), corresponding to a decrease in GFR to less than 50% of normal, be used for the definition of ARF in the newborn (90,91). Although ARF may be characterized by oliguria (<1 mL/hour/kg body weight), polyuric ARF commonly occurs particularly with congenital abnormalities of the renal tract and drug toxicity (aminoglycosides, antifungals); fractional excretion of sodium sometimes may be misleading in differentiating these two causes. The most common clinical feature of ARF in newborns is edema, owing to continued normal fluid intake in the presence of unrecognized oliguria. In contrast, edema is not generally present in polyuric ARF, which may instead be characterized by volume and sodium depletion.

As in adults, ARF in the newborn may be attributed to prerenal, intrinsic, or postrenal causes. Intrinsic ARF due to acquired renal diseases, such as glomerulonephritis or interstitial nephritis, is quite rare in the neonatal period. Therefore, in the neonate, intrinsic ARF is usually synonymous with acute tubular necrosis. Infants born with congenital heart disease also are more prone to development of ARF, caused either by heart failure or the effects of cardiac surgery (92,93). Maternal ACE inhibitor therapy can cause renal tubular dysplasia or ARF in the newborn (94,95). Predicting which infants are at risk for development of neonatal renal failure from perinatal asphyxia in the intensive care unit may be assessed in a number of ways: by measuring plasma or urine myoglobin, retinol-binding protein excretion (96), or asphyxia scores (97). Appropriate fluid restriction and salt balance can then be achieved before fluid overload and hyponatremia occur. Congenital chronic renal failure can mimic ARF in the newborn period, due to acute loss of maternal homeostatic mechanisms with delivery of the infant. Imaging studies may be helpful to differentiate these processes and to exclude renal vascular thrombosis.

Rapid correction of prerenal causes of ARF remains paramount for treating and avoiding prolonged renal dysfunction in neonates. Congenital obstruction and structural renal abnormalities such as dysplasia, hypoplasia, or agenesis should be excluded by ultrasonographic examination of the urinary tract, and a cystourethrogram usually is appropriate if hydronephrosis/hydroureter is demonstrated. Pulmonary edema should be prevented by positive-pressure ventilation, and acidosis should be corrected with intravenous sodium bicarbonate by initial half correction only of the metabolic acidosis to avoid precipitating intraventricular hemorrhage by use of excessively hyperosmolar solutions. Hyperkalemia should be treated with correction of any acidosis nebulized albuterol, intravenous 2% calcium gluconate, and glucose and insulin. The use of oral or rectal cation exchange resins may be complicated by bowel obstruction or perforation and thus are preferably not used in sick newborns already at risk for necrotizing enterocolitis. Dietary sodium intake should be about 1 mEq/kg per day in babies who are anuric or severely oliguric. In contrast, babies with polyuric ARF may have large urinary sodium losses of up to 10 mEq/kg per day and thus require significant sodium replacement. Adequate nutritional intake should be provided without causing volume overload. Supportive therapy with provision of large numbers of calories by high-concentrate dextrose intravenous infusions and parenteral nutrition and antibiotics for infection may be needed if control of the catabolic state and metabolic disturbance is to be achieved. Other factors acting on renal blood flow such as angiotensin II, endothelin I, and nitric oxide appear important in maintaining GFR in the neonatal kidney (98–100), and therapeutic intervention in these areas may become possible in the future.

Renal Artery Thrombosis

Renovascular complications and abnormalities in renal blood flow commonly occur in neonates, particularly in the clinical setting of intensive care for premature infants. Umbilical artery catheters, which are commonly used in sick newborns, may cause aortic intimal damage, mural clot formation and propagation, or embolism. If the tip of the catheter is placed above or around the orifices of the renal arteries, there is a significant risk of renal artery thrombosis or embolism. Depending on the degree of occlusion of the artery and on whether the main artery or only interlobular arteries are involved, the resulting renal ischemia can produce focal parenchymal scarring or complete infarction. Affected infants can present with hematuria, cardiovascular instability, collapse, shock or hypertension, renal impairment, anemia, or thrombocytopenia. A palpable renal mass may be present, and if the aorta also is thrombosed, the femoral pulses become impalpable and leg circulation is poor, characterized by gray, mottled skin. Urgent Doppler ultrasonography of the aorta, inferior vena cava, and renal arteries and veins is required together with arteriography to confirm the diagnosis. Intraaortic injection of a bolus of tissue plasminogen activator followed by a 24-hour infusion may dissolve the clot and salvage renal tissue and function (101–103). Although renal perfusion may recovery sufficiently to avoid long-term renal

replacement therapy, end-stage renal disease (ESRD) usually results if bilateral involvement was present initially.

Renal Venous Thrombosis

This condition usually occurs within a few days of birth in infants with perinatal asphyxia and in infants of diabetic mothers, or later in infants with severe dehydration or nephrotic syndrome. A predisposing coagulopathy may be present such as factor V (Leiden) mutation, protein S or C deficiency, or a maternal hypercoagulation state such as anticardiolipin syndrome (104,105). Such underlying diseases predisposing to thrombosis probably are more common than has been previously recognized, with new mutations in many coagulation factors being recently described.

Renal venous thrombosis commences in the small venous tributaries of the renal cortex or medulla and propagates to the main renal vein; it may affect one or both kidneys and may produce inferior vena cava obstruction. The classic diagnostic triad of flank mass, gross hematuria, and thrombocytopenia occurs in less than 15% of cases (106). Other signs and symptoms in the infant with renal venous thrombosis can include cardiovascular collapse, anemia, hyperosmolality and acidosis, oliguria, and renal impairment. Clinical management entails resuscitation of the infant's circulation, correction of acidosis that still persists once the cardiovascular volume has been restored, monitoring of coagulation parameters and fluid intake and output, and ultrasonography of the renal veins and inferior vena cava to document the extent of the thrombosis. Controlled trials of anticoagulation for unilateral or bilateral renal venous thrombosis, with or without inferior vena cava involvement, are lacking. Anticoagulation with heparin with or without tissue plasminogen activator is required for bilateral disease and significant extension of clot into the lumen of the vena cava (107,108); such agents should be used with caution in the preterm infant, who is at increased risk for intraventricular hemorrhage (108). Surgical thrombectomy is rarely required (109) but if undertaken should be followed after 10 days with oral anticoagulation using coumarin derivatives. Full anticoagulation may be required for longer periods. Dialysis may be required for bilateral renal involvement to correct the acidosis and metabolic and electrolyte disturbances. Although renal vein thrombosis in neonates is associated with low mortality, long-term follow-up shows an increased incidence of renal atrophy and hypertension (106,110,111).

RENAL DISEASE IN INFANCY AND CHILDHOOD

Genetic Renal Disease

Genetic kidney disease more commonly presents in children than in adults, although there are notable exceptions (e.g., autosomal dominant polycystic disease). Renal diseases due to genetic causes are recognized throughout childhood and have become an important area of clinical practice because increasingly more young children, even fetuses, are diagnosed with genetic disease by specialists in fetal medicine, geneticists, and pediatric nephrologists, with the latter being consulted about management of the postnatal kidney dysfunction.

Inherited Ion Transport Disorders

Although inherited disorders of ion transport are extensively reviewed elsewhere (Chapters 4 and 5), it is important to briefly review their features here, as these conditions may present in infancy or early childhood. Bartter syndrome is associated with salt-wasting, hypokalemia, and alkalosis, and is often apparent early in life as chronic volume contraction results in failure to thrive. Laboratory features resemble those observed with chronic loop diuretic use. Several genetic mutations have been identified in patients with Bartter syndrome, all of which result in direct or indirect suppression of sodium entry through the bumetanide-sensitive sodium-potassium-2 chloride (Na-K-2Cl) cotransporter (112–119). The neonatal form, characterized by polyhydramnios, premature delivery, life-threatening episodes of fever and dehydration during the early weeks of life, growth retardation, hypercalciuria, and early onset nephrocalcinosis, is now known to result from mutations in two genes, including Na-K-2Cl and the adenosine triphosphate–sensitive, inwardly rectifying potassium channel (ROMK). In contrast, older children presenting with classic Bartter syndrome with symptoms of polyuria and failure to thrive but no nephrocalcinosis have either deletions or mutations at the gene encoding a renal chloride channel (ClC-Kb). Sensorineural deafness is seen in Bartter patients with mutations in Barttin, which is an essential subunit of chloride channels in the inner ear (120). The treatment of Bartter syndrome usually includes potassium supplementation, potassium-sparing diuretics, and nonsteroidal anti-inflammatory drugs.

In contrast to Bartter syndrome, Gitelman syndrome is not typically symptomatic in infancy. Affected patients exhibit features resembling chronic thiazide use, with sodium and potassium wasting, hypomagnesemia, and hypocalciuria. Mutations in the distal nephron sodium chloride cotransporter (NCC) are responsible for this condition (121–124). Treatment includes potassium supplementation, potassium-sparing diuretics, and magnesium supplementation.

Cystinosis

Cystinosis is the most common cause of Fanconi's syndrome in children. It is an autosomal-recessive disease characterized by the abnormal accumulation of the amino acid cystine in lysosomes of all tissues due to a defective cystine transporter protein. The gene responsible for nephropathic cystinosis, *CTNS* on chromosome 17p, encodes for a 367–amino acid transmembrane protein called *cystinosin* (125). A common major deletion underlies most affected patients in Europe; other mutations have been reported (e.g., in Canadian patients of Irish ancestry and in intermediate cystinosis in adults; see Chapter 20).

Typically, cystinosis is a multisystem disease causing failure to thrive, dehydration, polyuria and polydipsia, vomiting, constipation, Fanconi's syndrome, and renal impairment in infancy. Fanconi's syndrome results in excessive urinary losses of low-molecular-weight protein, glucose, amino acids, phosphate, calcium, magnesium, sodium, potassium, bicarbonate, carnitine, and water. Affected children may be erroneously diagnosed with nephrogenic diabetes insipidus or diabetes mellitus due to polyuria and recurrent episodes of volume depletion. Chronic acidosis contributes to failure to thrive. Phosphaturia can result in hypophosphatemic rickets with metaphyseal widening, rachitic rosary, genu valgum, and elevated serum alkaline phosphatase. The hallmark of this disease is the accumulation of cystine in nearly all tissues, including kidneys. Corneal accumulation of cystine crystals leads to photophobia beginning in mid-childhood. Crystal storage in the thyroid causes hypothyroidism at an average age of 10 years (126). Feeding dysfunction including reflux, dysmotility, and swallowing abnormalities, is common (127–129), further contributing to impaired growth in this condition. Cognitive dysfunction with impaired visual and spatial abilities is common, even in heterozygous siblings who do not manifest other features of the disease (130). Serum creatinine is typically only mildly

elevated in infancy and early childhood. However, untreated, the tubulopathy progresses to chronic interstitial disease and glomerular necrosis with ESRD occurring at a median age of 9.2 years (131).

The diagnosis is made by measurement of leukocyte cystine content. Prenatal diagnosis is available through testing of amniotic fluid (132) or chorionic villus samples (133). Supportive treatment includes replacement of tubular fluid and solute losses, and affected patients require increased free water intake as well as supplementation with sodium-potassium citrate or sodium bicarbonate, sodium phosphate, 1,25-dihydrotachysterol, and possibly carnitine. Close attention to growth and nutritional needs is critical, and many centers now recommend placement of a gastrostomy tube to ensure adequate nutritional intake and compliance with the multiple required medications. Hypothyroidism should be treated appropriately. Although cystinosis is not associated with growth hormone deficiency, the administration of recombinant growth hormone to affected children has been shown to be of benefit (134).

Specific treatment involves depletion of intralysosomal cystine with cysteamine and related compounds to form mixed disulfides that are capable of being transported from the lysosome (135). Such treatment has been shown to slow the progression of renal insufficiency and thyroid disease in this condition (136–138). Specifically, chronic cysteamine therapy has shifted the point at which serum creatinine reaches 10 mg/dL from around 10 to 23 years of age (137). Corneal crystals do not respond to oral cysteamine but instead require topical treatment (139,140).

Patients with renal failure are candidates for peritoneal dialysis, hemodialysis, or renal transplantation. Cystine accumulates in renal allografts but does not cause functional abnormalities. It has been suggested that posttransplantation diabetes mellitus occurs at increased frequency in patients with cystinosis (141,142). Ongoing cysteamine treatment posttransplantation is indicated to prevent other systemic manifestations of this disease. With improved survival due to advancements in renal replacement therapy, it has become apparent that cystinosis is associated with severe neurologic sequelae, including cerebral atrophy, pyramidal signs, difficulties with speech, cerebellar ataxia, and pseudobulbar palsy (143,144). Further studies are needed to determine whether such neurologic deficits can be prevented.

Congenital Nephrotic Syndrome

Nephrotic syndrome in childhood can be attributed to a variety of primary and secondary causes, including inherited disease, infection, vasculitis, and exposure to toxic drugs or heavy metals. It is clinically useful to describe nephrotic syndrome in children by the age at presentation, with congenital nephrotic syndrome (CNS) occurring in children within the first 3 months of life, infantile nephrotic syndrome with onset between 4 and 12 months of age, and childhood nephrotic syndrome with onset after one year of age. In general, children who present with nephrotic syndrome very early in life tend to have a worse long-term prognosis than those presenting later in infancy or childhood.

Although typical causes of childhood nephrotic syndrome including minimal change disease, focal segmental glomerulosclerosis, and mesangial nephropathy can manifest in children at any age, these conditions typically have their onset later in life. Other important causes of primary CNS include CNS of the Finnish type (CNF), diffuse mesangial sclerosis (DMS), and membranous nephropathy. Causes of secondary CNS include infections (toxoplasmosis, syphilis, hepatitis B or C, HIV, malaria, cytomegalovirus, or rubella), syndromes (nail-patella syndrome, Lowe syndrome, Galloway-Mowat syndrome, etc.), systemic lupus erythematosus (SLE), or toxic mercury exposure.

It is now known that CNF is associated with mutations in both the *NPHS1* and *NPHS2* genes (145). These genes encode the proteins nephrin and podocin, respectively. These proteins are expressed in podocyte foot processes, and their interaction appears to be important in the maintenance of the glomerular filtration barrier. CNF is characterized histologically by mesangial hypercellularity and sclerosis, glomerular immaturity, and tubular cystic damage. Characteristic histologic changes may not be apparent until 3 to 8 months of age. Clinically, the condition is characterized by elevations in maternal serum and amniotic fluid α-fetoprotein concentration and increased placental weight greater than 25% of the infant's birth weight. Severe proteinuria with generalized edema is apparent immediately after birth in 25% of children, with more than 90% of affected children manifesting severe proteinuria within one week of birth (146). Hypoalbuminemia is typically severe (<1 g/dL), and albumin infusions and diuretics are needed routinely. Close nutritional follow-up is indicated. Urinary losses of immunoglobulin G result in increased risk of serious bacterial infections, particularly with encapsulated organisms such as pneumococci (147). Urinary losses of antithrombin III are associated with increased risk of thrombosis, including renal vein thrombosis (148).

Early mortality was common prior to the era of modern medicine. With supportive treatment, prolonged survival is possible in affected patients, although the majority of patients progress to ESRD by school age (146). The condition is resistant to steroids and other immunosuppressive drugs. Early bilateral nephrectomy with continuous cyclic peritoneal dialysis was commonly recommended prior to the development of ESRD in order to ameliorate complications of nephrotic syndrome (149). However, more recent reports suggest that medical management (ACE inhibition, prostaglandin synthase inhibition) and unilateral nephrectomy result in improved serum albumin concentration, decreased need for albumin infusion, maintained growth, and delay of second nephrectomy and renal replacement therapy until after 2 years of age (150). Renal transplantation has been successful in many patients. However, the main risk posttransplantation is recurrence of nephrotic syndrome, which affects up to one-fourth of patients (151) and has been attributed in several cases to the presence of anti-nephrin antibodies (148,151–154).

Diffuse mesangial sclerosis, in which glomerulosclerosis is not accompanied by mesangial hypercellularity, may occur in isolation or be associated with the Denys-Drash syndrome of male pseudohermaphroditism (XY gonadal dysgenesis) (155) and the nephrotic syndrome presenting in infancy. Mutations in the tumor suppressor gene *WT-1*, which is known to play a role in podocyte embryogenesis, have been reported in both (156–158). Diffuse mesangial sclerosis progresses to ESRD over months to a few years. Maternal or amniotic α-fetoprotein concentrations are not elevated in diffuse mesangial sclerosis, in contrast to congenital nephrotic syndrome of the Finnish type.

Other Inherited Nephrotic Syndromes

NHPS2 on chromosome 1q25-31, which causes autosomal recessive steroid-resistant nephrotic syndrome, has been mapped. Its relationship with other nephrotic syndromes, congenital or otherwise, is unknown, but *NPHS2* is expressed in the podocytes of fetal and mature glomeruli, and encodes the integral membrane protein, podocin, belonging to the stomatin protein family (159). Autosomal dominant focal segmental glomerulosclerosis has been linked to polymorphic markers on

chromosomes 1q25-31, 11q22-24, and 19q13 in three different affected families (160–162). The gene located at 19q13 encodes α-actinin-4, an actin-binding and cross-linking protein localized to podocytes (162). It has been proposed that mutations in this gene cause increased binding of α-actinin to actin filaments and thus disrupt the function of the podocyte. Clearly, the common clinical features of the nephrotic syndrome belie a heterogeneous group of disorders, many of which will have a genetic basis in children.

Hematuria

Microscopic hematuria is found in approximately 1% to 2% of school-aged children with decreasing prevalence with repeated examination (163). Diseases causing hematuria in children are listed in Table 79-4, and care should be taken not to miss conditions that mimic hematuria. Initial evaluation should include a careful history and physical examination, plasma creatinine, complete blood count, serum C3 complement, urine microscopy with consideration of culture, urine protein estimation, urine calcium-creatinine ratio (164), and renal ultrasonography. Consideration should also be given to obtaining antinuclear antibodies, antineutrophil cytoplasmic antibodies, antistreptolysin titer concentration, and sickle cell preparation in the appropriate clinical settings. In contrast to adults, cystoscopy rarely is necessary unless the history suggests a bladder or urethral problem and urine examination reveals lower tract red blood cells (see Chapter 10). The family history is important and the urine of all first-degree relatives should be tested to exclude a familial cause such as autosomal dominant thin glomerular basement membrane disease or Alport syndrome.

Most children with isolated microscopic hematuria do well, and extensive investigations, including renal biopsy, do not reveal serious disease and are not indicated. Prediction of long-term prognosis from screening of school children for hematuria is not possible and probably not worthwhile (165). Meanwhile, associated significant proteinuria is an indication for renal biopsy.

Idiopathic hypercalciuria has been recognized since 1981 as a cause of both micro- and macroscopic hematuria in childhood (166,167). This condition occurs in the setting of normal serum calcium concentrations and has been attributed to both urinary leak of calcium (renal hypercalciuria) and increased gastrointestinal absorption of calcium (absorptive hypercalciuria). There is frequently a family history of urolithiasis. It has been suggested that hematuria is the result of irritation of the urinary tract epithelium by microcalculi. Children can be asymptomatic or may complain of dysuria, renal colic, or suprapubic discomfort. Treatment involves increased fluid intake to improve urinary solubility of calcium. Thiazide diuretics should be considered for children with significant symptoms or associated urolithiasis.

TABLE 79-4

CAUSES OF HEMATURIA IN CHILDREN

Infections, including bacterial or viral cystitis or tuberculosis
Benign familial hematuria/thin glomerular basement membrane disease
Acute or chronic glomerulonephritis
Trauma
Idiopathic hypercalciuria or nephrolithiasis
Tumors
Meatal ulcers
Hematologic disorders, including sickle cell trait or disease and hemophilia
Systemic diseases (e.g., bacterial endocarditis and systemic lupus erythematosus)
Urologic malformations including ureteropelvic junction obstruction
Drugs

Glomerulonephritis

Glomerulonephritis in childhood can be associated with the acute nephritic syndrome, gross or microscopic hematuria with or without proteinuria, hematuria with nephrotic syndrome, and acute and chronic renal failure. As in adults, the clinical syndrome does not always predict the pathologic process, and the long-term prognosis is related primarily to the pathologic process (see Chapter 71). Renal biopsy is still undertaken primarily for diagnostic purposes but is not necessary in all children with glomerulonephritis, particularly in cases of postinfectious glomerulonephritis with reasonable renal function.

Postinfectious Glomerulonephritis

Postinfectious glomerulonephritis in developed countries is seen most commonly following acute pharyngitis with group A streptococci or impetigo due to staphylococci or streptococci. The typical presentation includes acute onset of gross hematuria within 7 to 21 days of streptococcal pharyngitis or 14 to 28 days of bacterial skin infection, often with associated proteinuria, hypertension, and mild azotemia and renal insufficiency. Other infectious agents can also cause this condition (Chapter 58). Serum C3 complement concentration is typically depressed, returning to normal levels within 4 to 6 weeks following acute presentation. Failure of the serum complement concentration to normalize within this timeperiod should raise concern for underlying membranoproliferative glomerulonephritis or SLE with associated nephritis. Gross hematuria and proteinuria typically resolve over several weeks although microscopic hematuria may persist for more than one year following acute presentation. Most children with postinfectious glomerulonephritis recover fully. However, particular concern should be directed toward those children who have ARF or nephrotic syndrome at presentation as well as older children.

Henoch-Schönlein Purpura Nephritis

Henoch-Schönlein purpura (HSP) was first described by Heberden. Later, Schönlein described the typical rash and the joint pains, and Henoch then recognized the association of gastrointestinal involvement and nephritis. HSP affects 13.5 per 100,000 children per year (168) and can occur at any age (see Chapter 61), but is most common in children ages 2 to 8 years; boys are affected more frequently than girls. The diagnosis is made principally from the evolution and appearance of the rash, which in its typical form is not to be confused with any other condition. The generalized vasculitis affects skin (including nonpurpuric areas), the capsules of the joints, the gastrointestinal tract, and the glomerular capillaries. The pathognomonic feature on renal biopsy is mesangial deposition of IgA-containing immune complexes. The similarity of IgA nephropathy and HSP nephritis has been noted (see Chapter 61), and identical twins have been described, one with IgA nephropathy and the other with HSP (169). Hypertension in HSP without urinary abnormalities has been reported (170).

The cause of HSP is unknown, but many cases appear to follow infections such as group A streptococcal pharyngitis or viral infections. The C4 null genotype (171) and major histocompatibility antigen (human leukocyte antigen [HLA]) alleles *DRB1*01* and *DRB1*11* have been associated with the development of HSP, with or without nephritis, whereas *DRB1*07* appears to be protective (172). Most children with HSP make a full recovery after 1 to 3 months, but during this period recurrent episodes of purpura may occur in up to one-third of patients. Significant renal involvement is seen in 20% to 60% of cases. In some patients, recurrences continue for months or years, and these patients cause particular concern because of the risk of serious renal involvement. However, less than 10% of children reach ESRD (173).

Approximately one-third of children with HSP have abnormal urinary sediment for more than 1 month after the onset of the disease (174). The prognosis usually is good, even for children with persistent urinary abnormalities, but children with combined nephritic and nephrotic syndrome, heavy proteinuria, crescentic glomerulonephritis involving more than 50% of glomeruli, or renal impairment at presentation are at greater risk for development of chronic renal failure (see Chapter 71) (168,173). Abnormal urine sediments may be found for some years before finally disappearing, and such children must be followed with every-third-month determinations of blood pressure, plasma albumin, and serum creatinine. Late deterioration may occur and is not easily predicted based on clinical features or biopsy findings for an individual.

Children with HSP who present with a combined nephrotic and nephritic syndrome tend to have more severe renal lesions on biopsy and fare less well. The presence of interstitial disease with tubular atrophy is a bad prognostic sign for long-term outcome, as is a mesangiocapillary type appearance of the glomerulonephritis. There is some evidence that intensive immunosuppression with high-dose intravenous methylprednisolone (175) followed by prednisolone and azathioprine (176) (combined with plasmapheresis if necessary [177–179]) alters the poor prognosis in children with severe HSP crescentic nephritis [179,180]. Therapy with methylprednisolone, cyclophosphamide, dipyridamole, and prednisolone for 3 months also is effective in severe HSP glomerulonephritis (181). There is no evidence to support early use of prednisolone to prevent development of nephritis in children with HSP (182).

Other Glomerulonephritides in Childhood

The clinical and histologic features of membranoproliferative glomerulonephritis in children are similar to those in adults, but treatment with prolonged moderate-dose steroids produces a better outcome in children than adults (183,184). The prognosis of membranous nephropathy is also better in children than in adults. A detailed description of glomerulonephritis can be found elsewhere in this text (Section IX).

Symptomless Proteinuria

Isolated mild, persistent proteinuria is prevalent in approximately 2% to 4% of children (185) and, in the absence of other evidence of renal disease is often benign. Orthostatic proteinuria has an excellent prognosis. Its diagnosis should be made or excluded before planning more invasive investigations. Heavy proteinuria of greater than 1 g/1.73 m^2 per 24 hours, even in the absence of other evidence of renal disease, should prompt consideration of renal biopsy because unexpected renal lesions may be discovered. Significant hematuria in the presence of proteinuria is generally an indicator for renal biopsy because children with serious forms of idiopathic glomerulonephritis such as epithelial crescentic nephritis, mesangiocapillary or membranoproliferative glomerulonephritis, and focal segmental sclerosis may present in this way.

Nephrotic Syndrome

Most children with nephrotic syndrome have minimal change disease, which nearly always responds to steroids with resolution of the proteinuria, and has an excellent prognosis even if recurrent relapses occur into adult life (186). The main indication for renal biopsy, therefore, is if steroid resistance is present at the onset or develops later. A trial of prednisone or prednisolone before biopsy at a daily dose of 60 mg/m^2 BSA or 2 mg/kg body weight for 4 weeks usually is advised except in children in whom suspicion of more serious disease is raised by factors such as age or a low C3 complement. Although some children with focal glomerulosclerosis who respond to steroids are missed by this policy, this does not present a problem because the prognosis in such children is often good (see Chapter 71). More recently, some have advocated genetic screening for mutations associated with steroid resistance at initial presentation in order to avoid unnecessary steroid exposure (187).

In the absence of such screening, the subsequent clinical course of the nephrotic syndrome can generally be predicted from the initial response and the relapses in the first 6 months of the disease. Steroid resistance with the first course of prednisolone and heavy proteinuria in the first 6 months favor progression to ESRD in 21% to 35% of patients (188). Three relapses within the first 6 months after responding to the initial steroid therapy predicts that the child will continue to relapse frequently (189). It has been suggested that the risk of subsequent relapse can be decreased by extending the initial course of steroid treatment. The prevalence of frequently relapsing disease during 2 years of observation in steroid-responsive children may be reduced from 50% to 27% by giving a weaning course of alternate-day steroids over an additional 4 months after the initial 8- to 10-week therapy (190,191).

Most steroid toxicity in children with steroid-sensitive nephrotic syndrome is caused by frequent relapses that require high-dose treatment. Once a child has been identified as a frequent relapser, several therapeutic options exist. A dose of alternate-day prednisolone may be given in the morning, which maintains remission without steroid toxicity (up to 0.5 mg/kg on alternate days), and the child maintained on treatment for a year before trying to discontinue the steroid therapy again by slowly weaning the dose (192). Levamisole 2.5 mg/kg on alternate days has been reported to be a useful adjunct in treatment of this condition, although success has been variable (193). Cyclophosphamide (194) or chlorambucil (195) at limited cumulative doses is often helpful in those children who demonstrate steroid dependence. However, the risks associated with use of such agents, including secondary malignancy and subsequent infertility, must be considered in each case. Alkylating agents allow withdrawal of all medication with a risk of relapse of 45% at 2 years; however, approximately 15% are cured (196). Long-term cyclosporine at doses of 4 to 5 mg/kg per day effectively maintains remission for 2 to 4 years and is quite safe for 5 years providing regular monitoring of plasma creatinine, GFR, and cyclosporine trough levels is carried out. Patient and parents may be offered the option of having a renal biopsy every 2 years to detect histologic changes of cyclosporine toxicity. However, after 5 years or more of cyclosporine treatment, less than 5% of patients have shown interstitial fibrosis or tubular atrophy attributed to drug toxicity, and usually this is accompanied by a decline in GFR. Seventy-five percent of steroid-responsive nephrotic children are able to discontinue steroids under cover of cyclosporine treatment. More recently, long-term tacrolimus has been found to be effective in maintaining remission in

children with steroid-dependent nephrotic syndrome and possibly inducing partial remission in children with steroid-resistant nephrotic syndrome (197–199). Although blood trough levels must be monitored when using tacrolimus, it is not associated with the undesirable side effects of cyclosporine, including hirsutism and gingival hyperplasia. Limited reports are now available suggesting that mycophenolate mofetil is effective in the treatment of idiopathic nephrotic syndrome in childhood (200–203); however, further study is necessary before its routine use can be recommended for this condition.

The main risk to a child with steroid-sensitive nephrotic syndrome is a complication of a relapse. Clinical signs of hypovolemia may be evident and may be associated with septicemia due especially to pneumococcal bacterial infection or with vascular thrombosis. The physician should have a low threshold to perform paracentesis to diagnose primary peritonitis due to pneumococcal or Gram-negative organisms, particularly if the child is taking steroids. Some pediatric nephrologists maintain children on prophylactic penicillin during relapses. Trials of immunization with a pneumococcal vaccine show that antibody responses are adequate (204) and persist for more than 5 years in more than 50% of immunized nephrotic children (205), but do not prevent all cases of *Streptococcus pneumoniae* infection (206). Nonetheless, both the 23-valent and heptavalent pneumococcal vaccinations should be provided to appropriate-age children with nephrotic syndrome. Hypovolemia should be considered in any child with a relapse, particularly if the child is unwell, and especially with vomiting or abdominal pain. The most useful clinical sign is poor peripheral perfusion with cold feet and toes, because the blood pressure may be raised from increased angiotensin production. The hematocrit rises and the urine output is low, with a low urinary sodium concentration. ARF requiring dialysis is rare, however. Elevated blood urea nitrogen (BUN) with a normal plasma creatinine is common and is due to the reduced urea clearance associated with oliguria. Treatment consists of infusing salt-poor albumin at a concentration of 25 g/dL with careful monitoring to detect vascular overload.

Children who demonstrate ongoing nephrosis that is unresponsive to treatment should receive chronic albumin infusion with diuretics as necessary. Close attention to infection is warranted as noted in the preceding text due to chronic hypogammaglobulinemia. Children with nephrotic syndrome have been shown to have acceptable antibody responses to immunization against varicella (207), influenza A (208), and hepatitis B (209), although the latter vaccination may also induce relapse (210). Periodic monitoring of iron stores and serum thyroid stimulating hormone (TSH) are indicated due to urinary losses of transferrin and thyroid-binding globulin. Low-dose aspirin or Coumadin may be considered for these children at risk for thrombosis due to urinary loss of antithrombin III and decreased intravascular volume. Plasmapheresis has been attempted for treatment of idiopathic nephrotic syndrome due to focal segmental glomerulosclerosis. However, such treatment appears to be more effective for recurrent disease following renal transplantation.

Hemolytic-Uremic Syndromes (HUS)

The hemolytic-uremic syndromes (HUSs) are broadly divided into the diarrhea-associated (D+) HUS, previously called *typical* or *epidemic form* (211), and non–diarrhea-associated (D–) HUS, previously called the *atypical* or *sporadic form* (212). Familial forms exist and are usually, but not exclusively, D(–) HUS. Underlying C3 hypocomplementemia (213) associated with genetic mutations of complement factor H (214,215) predisposes to familial D(–)HUS. The diarrheal form is associated with infection by Shiga-like toxin–producing organisms, typically *Escherichia coli* O157 (verotoxin-producing *E. coli* [VTEC]) in developed countries, *Shigella dysenteriae* type 1 in the Indian subcontinent and Africa, or occasionally *Shigella flexneri* infections. *Streptococcus pneumoniae* also may produce D(–) HUS because its neuraminidase toxin removes sialic acid residues from red blood cells, platelet membranes, and glomeruli to expose the Thomsen-Friedenreich (or "T") antigen, which reacts with circulating IgM to cause cell lysis and death (216). All forms of HUS, inherited or otherwise, have the ability to injure endothelial cells, and this seems to be a primary requisite for an agent to cause HUS (217,218). Neutrophils and complement may play important roles in this injury process, which results in thrombotic microangiopathy with hemolytic anemia (see Chapter 67). The pathogenesis of the ARF seems to be related to damage to endothelial cells with platelet activation, a rise in nitric oxide, and intracapillary thrombi.

Diarrhea-associated HUS occurs in Europe and North America with increased frequency in the summer months after an episode of diarrhea, which often is bloody and usually is caused by VTEC. Sources of VTEC associated with HUS have been found in cows, pigs, contaminated cheese and dairy products, processed meats, and stagnant water such as wells and swimming lakes, in addition to other family members. D(+)HUS is associated with microangiopathic hemolytic anemia; thrombocytopenia is almost invariable and together with uremia, these results comprise the clinical triad of HUS. Intravascular coagulation, clot fibrinolysis, and tissue-type plasminogen activator are increased, but not plasminogen activator inhibitor-1 (219). Prothrombin time and partial thromboplastin time usually are normal, and levels and activity of factors V and VIII are variable. Fibrin degradation products usually are elevated, but fibrinogen turnover is normal. Antithrombin III levels may be reduced, but protein C and S values usually are normal. Increased circulating concentrations of von Willebrand factor multimers have been described. Prostacyclin production by endothelial cells may be defective (220). It is not clear whether this failure is due to some toxic substance in the plasma of patients with HUS or the absence of a factor necessary for normal prostacyclin generation. Elevated leukocyte counts are common in postdiarrheal HUS and this correlates with circulating interleukin-8 (IL-8) concentrations, whereas normal or low counts tend to be found in D(–)HUS. When present in D(+)HUS cases, the higher the leukocyte count the worse the prognosis (221), probably signifying the greater involvement of endothelial tissue and therefore greater IL-8 release.

Hemolytic-uremic syndrome is the most common single cause of ARF in infants and young children. The presentation is usually a child with an unremarkable episode of diarrhea (except for the fact that blood may be seen in the stools), who then becomes quiet, lethargic, and pale, and is noted to be oliguric. Extrarenal involvement in HUS, although common, rarely causes severe problems; however, catastrophic central nervous system complications can occur and are a major contributor to morbidity and mortality in this condition (222). Treatment on admission to the hospital is directed toward dealing with the acute complications of renal insufficiency, anemia, and thrombocytopenia. Because these children often are extremely ill, dialysis may be required. Although the utility of plasma infusion in D(+)HUS is controversial, plasmapheresis should be considered for children with neurologic complications resembling thrombotic thrombocytopenic purpura. The prognosis in D(+)HUS is generally good with full recovery of renal function after a period of a few days to a few weeks in most cases. However, renal functional reserve may be impaired, with sequelae including chronic proteinuria, hypertension, or impaired renal function found in up to 50%

of survivors (223). Epidemiologic studies have suggested that the long-term prognosis for renal function relates to factors including age of the patient at presentation, maximum white blood cell count and duration of elevated white blood cell count, duration of anuria, and duration of need for dialysis (223,224).

Hemolytic-uremic syndrome caused by neuraminidase from *S. pneumoniae,* should be suspected in any child who is toxic with pneumonia and a positive Coombs test. The condition can also occur with other *S. pneumoniae* infections including meningitis and bacteremia (225). T-antigen activation can be detected by demonstrating red blood cell agglutination by the peanut lectin *Arachis hypogea* (226). Efficient diagnosis is critical because blood transfusion or plasma infusion may accelerate the agglutination. Such patients should be treated with penicillin or ceftriaxone to cure the infection and provided supportive therapy. Pneumococcal HUS appears to be more severe clinically than D(+)HUS, with the former group demonstrating an increased need for dialysis and platelet and red blood cell transfusions, as well as longer hospitalization time as compared to the latter group (225).

The sporadic, familial, and other D(-) forms of HUS are extremely uncommon. Familial autosomal recessive forms of HUS have been described, and these need to be distinguished from the postdiarrheal form occurring in members of the same family at the same time. The sporadic familial form without diarrhea has a poor prognosis, and these children tend to progress to ESRD or die with complications such as cerebrovascular involvement despite treatment with fresh frozen plasma or plasmapheresis (227). HUS can recur after renal transplantation, and it may be that such patients have the familial form of the disease. Autosomal dominant inheritance of HUS has been described, but these patients are usually adults and have a very poor prognosis. Hemolytic-uremic syndrome also can occur in other circumstances, as in association with drugs such as cyclosporine.

Urinary Tract Infection (UTI)

UTI can occur in children of all ages. It has been estimated that UTI is diagnosed in 3% of prepubertal girls and 1% of prepubertal boys, with an even higher incidence in pubertal girls (228). The relative risk of UTI in girls to boys ages 2 months to 2 years is 2.27 (229); this pattern is even more apparent in older children. Few children or adults who have recurrent UTI progress to serious renal disease, but chronic pyelonephritis may cause ESRD (Table 79-5). The epidemiology of chronic pyelonephritis is complicated by the virulence of the infecting organism and the specific susceptibility of the child. Investigation of children with UTI has shown that VUR is present in 25% to 33% and that the degree of VUR correlates well with the prevalence of renal scarring (230). Numerous urinary pathogens possess fimbrial or nonfimbrial (type 1 fimbriae and P-fimbriae) adhesins for mucosal attachment and colonization. P-fimbriated *E. coli* are associated with the development of renal scars, and inappropriate antibiotic therapy may favor colonization with such organisms (231). The understanding of UTI and its epidemiology with regard to congenital, genetic, and environmental aspects suggests that early diagnosis and treatment should lead to a reduction in morbidity and mortality rates.

The role of the pediatrician caring for children with UTI is to prevent recurrent UTI and new renal scar formation and consequently renal impairment by facilitating prompt diagnosis of UTI and identifying children with existing renal scars, underlying renal tract anomalies, or known complications of UTI or VUR. Indeed, without an underlying renal tract abnormality, progressive renal damage is rare in children with UTI. Population screening is not cost-effective, but early diagnosis by primary health care workers, urgent treatment of acute pyelonephritis to minimize scar formation, and prevention of infection by long-term low-dose prophylactic antibiotics (232) or the surgical correction of reflux in selected children (233) can all contribute to an improved prognosis. Successful management of UTI in childhood by routine urine culture in all febrile infants and toddlers in Sweden has reduced the prevalence of reflux nephropathy/chronic pyelonephritis causing ESRD in pediatric transplant recipients from 20% in the 1970s to less than 5% in the 1990s in that country (234).

Reflux nephropathy or 'scarring' in the presence of VUR may arise by two main independent mechanisms (235). Severe antenatal VUR causes segmental renal dysplasia or hypoplasia in the fetal kidney, whereas postnatal VUR with intrarenal reflux and urinary infection causes segmental renal pyelonephritic scars in both infants and older children (69).

TABLE 79-5

CAUSES OF CHRONIC RENAL INSUFFICIENCY IN CHILDREN. PRIMARY DIAGNOSIS BY PERCENTAGE OF 5,651 CHILDREN REGISTERED IN THE NORTH AMERICAN PEDIATRIC RENAL TRANSPLANT COOPERATIVE STUDY (NAPRTCS)

Cause	%
Obstructive uropathy	22.9
Aplasia/hypoplasia/dysplasia	17.9
Glomerulonephritis	9.0
Membranoproliferative glomerulonephritis	1.6
SLE nephritis	1.5
Familial nephritis	1.5
Chronic glomerulonephritis, unspecified	1.3
Berger's (IgA) nephritis	1.0
Idiopathic crescentic glomerulonephritis	0.7
Henoch-Schönlein nephritis	0.7
Membranous nephropathy	0.5
Diabetic glomerulonephritis	0.2
Reflux nephropathy	8.3
Focal segmental glomerulosclerosis	8.3
Polycystic kidney disease	4.1
Prune belly syndrome	3.1
Renal infarct	2.7
Hemolytic uremic syndrome	2.2
Cystinosis	1.5
Pyelointerstitial nephritis	1.4
Medullary cystic disease	1.2
Congenital nephrotic syndrome	0.8
Other systemic immunologic disease	0.4
Wilms tumor	0.4
Wegener's granulomatosis	0.3
Sickle cell nephropathy	0.2
Oxalosis	0.1
Drash syndrome	0.1
Other	12.5
Unknown	2.5

SLE, systemic lupus erythemotosus.
(Adapted from: NAPRTCS 2004 Annual Report, exhibit 13.1.)

The presence of compound renal papillae, which allow the backflow of infected urine from the calices into the collecting ducts (intrarenal reflux), is important in the generation of renal scars with VUR in animal models (236); however, the relevance of this mechanism in reflux nephropathy in children has been debated due to the rarity of such papillae in human kidneys (237). The increased frequency of UTI in infants, the presence of congenital segmental renal dysplasia, the increased risk of true infective pyelonephritic scars developing in infants, and renal impairment at presentation all demonstrate that the target population in which to detect UTI and renal "scars" is children under 18 months of age.

The clinical presentation of UTI varies with the age of the child. In young children, classic symptoms of UTI such as dysuria, frequency, urgency, and flank pain are uncommon. Instead, vague symptoms such as fever, abdominal pain, irritability, lethargy, poor feeding, or incontinence are frequent. Fever is a common symptom, and the American Academy of Pediatrics advises that UTI be excluded in any child between 2 months and 2 years of age with unexplained fever (229).

It is critical to obtain an uncontaminated urine sample for diagnosis. Bladder catheterization provides a sterile sample in the infant or incontinent child. Suprapubic aspiration, although providing definitive results in young infants, is difficult to perform in children older than 1 year of age and is becoming less popular. Bag urine specimens often are contaminated and should not be used for diagnosis of UTI. In older continent children, a clean-catch urine specimen can be obtained, but both clean-catch urine and midstream urine specimens can be difficult to acquire, particularly if parents are given the responsibility of obtaining them. A frequent mistake is to diagnose UTI from a significant growth of organisms after culture of a contaminated or improperly transported urine specimen. The specimen should be cultured within 2 hours or transported in a medium such as boric acid that prevents bacterial multiplication.

Most truly infected urines contain an excess of white blood cells and yield a pure growth of a single organism. Fresh urine without cells or visible organisms on microscopy is unlikely to be infected. Although more than 10^5 organisms per milliliter of urine is the standard criterion for UTI, in infants a moderate growth of 10,000 to 100,000 organisms per milliliter of urine may represent an infection, and further specimens should be obtained. A detailed history about fever accompanying the illness, the adequacy of the urinary stream in boys, and the presence of functional bladder problems, followed by careful palpation of the abdomen for renal masses and bladder enlargement, thorough examination of the genitalia, and assessment of perineal sensation and neurologic function in the lower limbs, should be carried out.

Appropriate investigations depend on the age of the child and the clinical assessment. Several medical committees have published guidelines on appropriate protocols to investigate UTI in children (229,238–241). However, there is significant controversy in the literature regarding the extent and timing of evaluation in children with UTI and the impact that such evaluation has on the development of reflux nephropathy. What is needed is appropriate investigation of all children with UTI if outcomes of children who present in middle or late childhood with ESRD due to reflux nephropathy are to be avoided (242). At our institution, we recommend renal ultrasonography and voiding cystourethrography in males of any age with UTI, all females under 5 years of age, and all females with pyelonephritis or recurrent UTI. A functional study such as ^{99m}Tc-DMSA renal isotope scan can be performed to detect segmental renal dysplasia or scarring in selected cases, including infants. Ultrasonography alone has been shown to be unreliable in the detection of both vesicoureteral reflux (28,29,243,244) and renal scarring (243,245–247). Voiding contrast cystourethrography is preferred to radionuclide cystography for the initial study, particularly in males, due to the former's ability to exclude structural anomalies of the urethra including posterior urethral valves. Failure to make the diagnosis, investigate thoroughly, and follow guidelines remain the major obstacles in identifying those children at risk of renal impairment or hypertension.

Management of Urinary Tract Infection

There is little evidence that older children with normal urinary tracts without renal scars who have recurrent UTI will sustain renal damage, and for such children, attention to hygiene, prevention of constipation, and an adequate fluid intake with regular bladder emptying are important. Mild or moderate reflux without upper tract dilation in young children is likely to disappear in a reasonably short time so that, if the kidneys are not scarred, medical management seems appropriate, whereas severe reflux, especially with scarred kidneys, requires longer-term follow-up, and surgery may be advisable if the quality of the surgery can be guaranteed. However, the single most important action required to reduce the morbidity and mortality of chronic pyelonephritis is the diagnosis and treatment of UTI in infants with fever.

Management of UTI involves early administration of appropriate antibiotics to treat the infection and reduce the risk of renal scarring, attention to the voiding habits of children and avoidance of constipation, encouragement of good fluid intake and good personal hygiene of the perineum and genitalia, and diagnosis of any underlying renal tract abnormality (248). Incontinence predisposes to infection, and the treatment of enuresis by bladder training is important. VUR of a significant grade may be treated medically with prophylactic antibiotics at night (232) or surgical reimplantation of the ureter. Nightly prophylactic antibiotics should also be considered for children in the absence of VUR or other renal structural anomalies when attention to fluid intake, hygiene, and other interventions, is not successful in prevention of recurrent infection. The International Reflux Study has shown that medical management of VUR is equivalent to surgery in reducing the number of new renal scars (249,250). No advantage between medical vs. surgical management has been shown after 5 years of follow-up (250,251). Prophylactic antibiotics reduce the incidence of UTI, but doubt has been cast on whether regular prophylactic antibiotics or even surgery significantly alter the rate of new scar formation—most authors agree that making an early diagnosis of UTI is most important in this regard (248). In those children who continue to reflux, cessation of prophylactic antibiotics after 8 years of age was associated with only 12% risk of another UTI (252). Antibiotic prophylaxis or surgery alone does not influence the number of children reaching ESRD—(253).

Because most infections are caused by organisms from the bowel, at least in girls, successful antibiotic prophylaxis depends on the prevention of antibiotic resistance in the bowel flora. Suitable medicines are trimethoprim, 1 mg/kg, or trimethoprim/sulfamethoxazole; nitrofurantoin, 1 to 2 mg/kg; or nalidixic acid, 15 mg/kg, all given as a single dose at night.

The complications of UTI in children include renal scarring and renal calculi. *Proteus* infections more commonly cause renal calculi than any other infection because of the organism's ability to produce alkaline urine by degrading urea. In long-term follow-up studies of up to 41 years (254), hypertension was observed in young adults in approximately 10% of cases, with people in the higher percentile range for blood pressure being more at risk than others. Approximately 10% have significant renal impairment and may reach renal failure as young

TABLE 79-6

CONDITIONS UNDER WHICH CHILDREN YOUNGER THAN 3 YEARS OF AGE SHOULD HAVE BLOOD PRESSURE ROUTINELY MEASURED

History of prematurity, very low birth weight, or other neonatal complication requiring intensive care
History of congenital heart disease
Recurrent urinary tract infections, hematuria, or proteinuria
Known renal disease or urologic malformations
Family history of congenital renal disease
Solid-organ transplant
Malignancy or bone marrow transplant
Treatment with drugs known to raise blood pressure
Other systemic illnesses associated with hypertension (neurofibromatosis, tuberous sclerosis, etc.)
Evidence of elevated intracranial pressure

(Adapted from: The National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. *Pediatrics* 2004;114:556.)

people (255). UTI, preeclampsia, fetal death, and low-birth-weight infants are more common in women with renal scarring (256,257).

HYPERTENSION

It is disappointing how frequently the measurement of blood pressure is neglected in pediatric practice. Population screening of children for UTI or urinary abnormalities is not cost-effective. This increases the responsibility of primary health care workers to identify the child with renal disease by measuring the blood pressure at routine health checks. It is now recommended in the United States that all children older than 3 years of age have their blood pressure checked at routine health care visits. Routine blood pressure measurement is recommended for children younger than 3 years of age with specific medical conditions (Table 79-6). An appropriately sized cuff has a bladder width that is 40% of the arm circumference at a point that is midway between the olecranon and the acromion and is long enough to encircle at least 80% of the arm circumference (258). The usual error is to use a cuff that is too small, which results in a falsely high reading. In the outpatient setting, blood pressure should be measured following several minutes of quiet sitting, with the arm supported at heart level. Repeated measurement is indicated for mild hypertension, as its frequency has been shown to decrease with repeated measurement. Measurement is difficult in infants because auscultation of the Korotkoff sounds often is unsatisfactory. With Doppler devices, an accurate measurement of systolic pressure can be obtained. Values for normal blood pressure in children of different ages are given in Tables 79-7A and 79-7B.

Neonates and Infants

The upper limit for mean arterial blood pressure in infants weighing more than 2.5 kg rises from 68 mm Hg at birth to 87 mm Hg at 4 weeks. Systemic hypertension, not related to coarctation of the aorta, appears to be becoming more common in newborns, both because it is now easier to measure blood pressure using Doppler ultrasound and because of thromboembolic occlusions of the renal vasculature from umbilical arterial catheters (259). It is now standard practice that the tip of the catheter lies below the origin of the renal arteries. Rarer causes of hypertension include renal emboli from clots that originate in the ductus arteriosus, congenital renal artery stenosis, renal venous thrombosis, structural renal lesions, elevated intracranial pressure, and rarely congenital adrenal hyperplasia. The most useful investigations include renal ultrasonography to evaluate structural lesions or obstruction and ^{99m}Tc-labeled MAG3 scanning to assess renal blood flow and function. Control of the hypertension is vital; propranolol up to 3 mg/kg per day and the calcium channel blocking agent nifedipine up to 3 mg/kg per day with or without a diuretic are useful. The ACE inhibitor captopril or a longer-acting analog is useful but is contraindicated when bilateral or severe renal artery stenosis is suspected or prior to the completion of nephrogenesis. More potent vasodilators such as minoxidil may be required. Acute control may be achieved with intravenous labetalol infusions at 1 to 3 mg/kg per hour, sodium nitroprusside 500 ng to 8 μg/kg per minute, or hydralazine in doses up to 3 mg/kg per day. In cases secondary to renal emboli, the prognosis often is good, with the hypertension resolving and the renal function improving with time if permanent renal ischemia is not present. With decreased mortality in preterm infants, transient systemic hypertension associated with bronchopulmonary dysplasia is becoming more frequent in older infants (260).

Causes and Investigation in Older Children

Hypertension in children is defined as an average systolic and/or diastolic blood pressure greater than the 95th percentile for healthy children of the same age, sex, and height on more than three separate occasions (261) and is usually secondary to a specific disease (Table 79-8). Although the possible causes are numerous, in practice approximately 60% to 70% of patients have parenchymal renal disease, 10% to 20% have coarctation of the aorta, 5% to 10% have renovascular problems, 3% to 5% have endocrine disease, and 1% to 5% have some other condition (262). The initial evaluation should include a careful history documenting symptoms associated with hypertension as well as any symptoms referable to the urinary tract or systemic disease; dietary habits and tobacco use; neonatal illness including placement of an umbilical artery catheter or surfactant deficiency with subsequent chronic lung disease; prior UTI; the use of drugs which contribute to hypertension, including over-the-counter decongestants and recreational drugs; and familial risk factors for cardiovascular disease and hyperlipidemia. A thorough physical examination can provide clues to the etiology of hypertension (Table 79-9). Further evaluation should include examination of the urine for blood and protein; measurement of plasma electrolytes, creatinine, and urea; renal imaging with ultrasound including Doppler studies of renal arteries; and consideration of measurement of plasma renin activity. Echocardiography should be performed when physical examination suggests aortic coarctation or when severe or chronic hypertension is present. In the appropriate clinical settings, consideration should also be given to hormonal assessment including urinary catecholamine metabolite excretion and serum aldosterone and cortisol concentrations. Hypertension secondary to endocrine disease usually is associated with suppression of plasma renin, although a secondary increase can be found with a pheochromocytoma. Supine and upright plasma epinephrine and norepinephrine levels help to identify pheochromocytomas and give an indication whether they are adrenal or extraadrenal in origin (263). Computed tomographic scans of the thorax and abdomen are more sensitive than metaiodobenzylguanidine isotope scans in locating these tumors.

TABLE 79-7A

BLOOD PRESSURE LEVELS FOR BOYS BY AGE AND HEIGHT PERCENTILE

		SBP, mm Hg							DBP, mm Hg						
		Percentile of height							Percentile of height						
Age, y	BP percentile	5th	10th	25th	50th	75th	90th	95th	5th	10th	25th	50th	75th	90th	95th
1	50th	80	81	83	85	87	88	89	34	35	36	37	38	39	39
	90th	94	95	97	99	100	102	103	49	50	51	52	53	53	54
	95th	98	99	101	103	104	106	106	54	54	55	56	57	58	58
	99th	105	106	108	110	112	113	114	61	62	63	64	65	66	66
2	50th	84	85	87	88	90	92	92	39	40	41	42	43	44	44
	90th	97	99	100	102	104	105	106	54	55	56	57	58	58	59
	95th	101	102	104	106	108	109	110	59	59	60	61	62	63	63
	99th	109	110	111	113	115	117	117	66	67	68	69	70	71	71
3	50th	86	87	89	91	93	94	95	44	44	45	46	47	48	48
	90th	100	101	103	105	107	108	109	59	59	60	61	62	63	63
	95th	104	105	107	109	110	112	113	63	63	64	65	66	67	67
	99th	111	112	114	116	118	119	120	71	71	72	73	74	75	75
4	50th	88	89	91	93	95	96	97	47	48	49	50	51	51	52
	90th	102	103	105	107	109	110	111	62	63	64	65	66	66	67
	95th	106	107	109	111	112	114	115	66	67	68	69	70	71	71
	99th	113	114	116	118	120	121	122	74	75	76	77	78	78	79
5	50th	90	91	93	95	96	98	98	50	51	52	53	54	55	55
	90th	104	105	106	108	110	111	112	65	66	67	68	69	69	70
	95th	108	109	110	112	114	115	116	69	70	71	72	73	74	74
	99th	115	116	118	120	121	123	123	77	78	79	80	81	81	82
6	50th	91	92	94	96	98	99	100	53	53	54	55	56	57	57
	90th	105	106	108	110	111	113	113	68	68	69	70	71	72	72
	95th	109	110	112	114	115	117	117	72	72	73	74	75	76	76
	99th	116	117	119	121	123	124	125	80	80	81	82	83	84	84
7	50th	92	94	95	97	99	100	101	55	55	56	57	58	59	59
	90th	106	107	109	111	113	114	115	70	70	71	72	73	74	74
	95th	110	111	113	115	117	118	119	74	74	75	76	77	78	78
	99th	117	118	120	122	124	125	126	82	82	83	84	85	86	86
8	50th	94	95	97	99	100	102	102	56	57	58	59	60	60	61
	90th	107	109	110	112	114	115	116	71	72	72	73	74	75	76
	95th	111	112	114	116	118	119	120	75	76	77	78	79	79	80
	99th	119	120	122	123	125	127	127	83	84	85	86	87	87	88
9	50th	95	96	98	100	102	103	104	57	58	59	60	61	61	62
	90th	109	110	112	114	115	117	118	72	73	74	75	76	76	77
	95th	113	114	116	118	119	121	121	76	77	78	79	80	81	81
	99th	120	121	123	125	127	128	129	84	85	86	87	88	88	89
10	50th	97	98	100	102	103	105	106	58	59	60	61	61	62	63
	90th	111	112	114	115	117	119	119	73	73	74	75	76	77	78
	95th	115	116	117	119	121	122	123	77	78	79	80	81	81	82
	99th	122	123	125	127	128	130	130	85	86	86	88	88	89	90
11	50th	99	100	102	104	105	107	107	59	59	60	61	62	63	63
	90th	113	114	115	117	119	120	121	74	74	75	76	77	78	78
	95th	117	118	119	121	123	124	125	78	78	79	80	81	82	82
	99th	124	125	127	129	130	132	132	86	86	87	88	89	90	90
12	50th	101	102	104	106	108	109	110	59	60	61	62	63	63	64
	90th	115	116	118	120	121	123	123	74	75	75	76	77	78	79
	95th	119	120	122	123	125	127	127	78	79	80	81	82	82	83
	99th	126	127	129	131	133	134	135	86	87	88	89	90	90	91
13	50th	104	105	106	108	110	111	112	60	60	61	62	63	64	64
	90th	117	118	120	122	124	125	126	75	75	76	77	78	79	79
	95th	121	122	124	126	128	129	130	79	79	80	81	82	83	83
	99th	128	130	131	133	135	136	137	87	87	88	89	90	91	91
14	50th	106	107	109	111	113	114	115	60	61	62	63	64	65	65
	90th	120	121	123	125	126	128	128	75	76	77	78	79	79	80
	95th	124	125	127	128	130	132	132	80	80	81	82	83	84	84
	99th	131	132	134	136	138	139	140	87	88	89	90	91	92	92

(*Continued*)

TABLE 79-7A

(CONTINUED)

		SBP, mm Hg							DBP, mm Hg						
		Percentile of height							Percentile of height						
Age, y	BP percentile	5th	10th	25th	50th	75th	90th	95th	5th	10th	25th	50th	75th	90th	95th
15	50th	109	110	112	113	115	117	117	61	62	63	64	65	66	66
	90th	122	124	125	127	129	130	131	76	77	78	79	80	80	81
	95th	126	127	129	131	133	134	135	81	81	82	83	84	85	85
	99th	134	135	136	138	140	142	142	88	89	90	91	92	93	93
16	50th	111	112	114	116	118	119	120	63	63	64	65	66	67	67
	90th	125	126	128	130	131	133	134	78	78	79	80	81	82	82
	95th	129	130	132	134	135	137	137	82	83	83	84	85	86	87
	99th	136	137	139	141	143	144	145	90	90	91	92	93	94	94
17	50th	114	115	116	118	120	121	122	65	66	66	67	68	69	70
	90th	127	128	130	132	134	135	136	80	80	81	82	83	84	84
	95th	131	132	134	136	138	139	140	84	85	86	87	87	88	89
	99th	139	140	141	143	145	146	147	92	93	93	94	95	96	97

The 90th percentile is 1.28 SD, the 95th percentile is 1.645 SD, and the 99th percentile is 2.326 SD over the mean.

Renal artery stenosis (RAS) may be suspected from elevated plasma renin activity and from the clinical examination, e.g. abdominal bruit but further imaging studies are usually needed to establish the diagnosis. The initial evaluation usually includes Doppler evaluation of the renal arteries; however, this technique is not diagnostic in every case, especially with distal stenosis (264). The ACE inhibitor–enhanced radionuclide renal scan appears to have improved sensitivity and specificity as compared to Doppler for diagnosis of RAS in children (265) and is relatively noninvasive. Magnetic renal angiography (MRA) is also becoming more widely used for this purpose, although young children may require sedation for the procedure. In the absence of a diagnosis and with high clinical suspicion for RAS, selective renal arteriography is indicated after the blood pressure has been controlled. The amount of contrast injected should be the minimum required and blood pressure should be monitored. Approximately 100 case reports of RAS associated with pheochromocytoma exist, so the latter diagnosis should be excluded before undertaking renal arteriography. Evidence of renal ischemia can also be obtained from the differential renal vein renin determinations. A ratio of greater than 1.5:1.0 between the affected and nonaffected kidney is a useful predictor of renal ischemia and indicates that a favorable response to surgery is likely in children (266), although successful surgery has been reported in some cases when the ratio was less than 1.5:1.0. A negative arteriogram with no significant difference between the renal vein renins and the systemic plasma renin excludes a renovascular cause for the hypertension.

Treatment

Nonpharmacologic management can be initially attempted in children with prehypertension (261) or in those with mild essential hypertension. Such treatment should include healthy eating with a no-added-salt diet; weight reduction for overweight children; aerobic exercise at least 4 times per week in children with no other contraindication to participation (267); and cessation of smoking. Should hypertension persist despite such measures, pharmacologic treatment should be considered. Drug treatment is also indicated for children who are symptomatic, those who have known secondary hypertension while the underlying etiology is being addressed, and those who have evidence of end-organ damage (e.g., left ventricular hypertrophy, hypertensive retinopathy, proteinuria) from hypertension.

Children who are symptomatic or who have severe hypertension should be admitted to the hospital for initial control of blood pressure; as in adults, malignant hypertension is a medical emergency. Controlled lowering of the blood pressure by the use of intravenous labetalol or sodium nitroprusside infusions should be achieved slowly and steadily over a period of days if severe neurologic complications and blindness are to be avoided (268). The infusion rate of these drugs can be titrated to the blood pressure. If the child is having seizures, then an initial rapid reduction of systolic blood pressure by 20 to 40 mm Hg is necessary. Patients with malignant hypertension may be volume depleted, and the blood pressure may fall precipitously when a vasodilating agent such as nifedipine, hydralazine, or diazoxide is administered. In such cases, physiologic saline should be infused rapidly to correct the volume depletion and allow an even control of blood pressure. In fact, in salt-depleted hypertensive children, 0.9% saline may be necessary to gain control of the blood pressure. The calcium channel blocking agent nifedipine, administered orally, or β-adrenergic blocking drugs are useful to control less severe rises in blood pressure. The increased risk of mortality from calcium channel blockers seen in adult studies is rarely of concern in otherwise healthy children, in whom the risk of coronary artery disease is low. ACE inhibitors should be avoided when bilateral renovascular disease, renovascular disease in the single kidney, or coarctation of the aorta are suspected.

Otherwise, oral regimens usually start with an ACE inhibitor, long-acting calcium channel blocker such as amlodipine, or both (Table 79-10). β-Blockade is useful but is contraindicated in the child with underlying bronchospastic disease. Diuretics are not routinely used for management of hypertension in children unless there is concern for fluid retention as in renal insufficiency, renin-mediated hypertension, or acute glomerulonephritis. Vasodilator drugs often lead to salt and water retention and escape from blood pressure

TABLE 79-7B

BP LEVELS FOR GIRLS BY AGE AND HEIGHT PERCENTILE

		SBP, mm Hg							DBP, mm Hg						
		Percentile of height							Percentile of height						
Age, y	BP percentile	5th	10th	25th	50th	75th	90th	95th	5th	10th	25th	50th	75th	90th	95th
1	50th	83	84	85	86	88	89	90	38	39	39	40	41	41	42
	90th	97	97	98	100	101	102	103	52	53	53	54	55	55	56
	95th	100	101	102	104	105	106	107	56	57	57	58	59	59	60
	99th	108	106	109	111	112	113	114	64	64	65	65	66	67	67
2	50th	85	85	87	88	89	91	91	43	44	44	45	46	46	47
	90th	98	99	100	101	103	104	105	57	58	58	59	60	61	61
	95th	102	103	104	105	107	108	109	61	62	62	63	64	65	65
	99th	109	110	111	112	114	115	116	69	69	70	70	71	72	72
3	50th	86	87	88	89	91	92	93	47	48	48	49	50	50	51
	90th	100	100	102	103	104	106	106	61	62	62	63	64	64	65
	95th	104	104	105	107	108	109	110	65	66	66	67	68	68	69
	99th	111	111	113	114	115	116	117	73	73	74	74	75	76	76
4	50th	88	88	90	91	92	94	94	50	50	51	52	52	53	54
	90th	101	102	103	104	106	107	108	64	64	65	66	67	67	68
	95th	105	106	107	108	110	111	112	68	68	69	70	71	71	72
	99th	112	113	114	115	117	118	119	76	76	76	77	78	79	79
5	50th	89	90	91	93	94	95	96	52	53	53	54	55	55	56
	90th	103	103	105	106	107	109	109	66	67	67	68	69	69	70
	95th	107	107	108	110	111	112	113	70	71	71	72	73	73	74
	99th	114	114	116	117	118	120	120	78	78	79	79	80	81	81
6	50th	91	92	93	94	96	97	98	54	54	55	56	56	57	58
	90th	104	105	106	108	109	110	111	68	68	69	70	70	71	72
	95th	108	109	110	111	113	114	115	72	72	73	74	74	75	76
	99th	115	116	117	119	120	121	122	80	80	80	81	82	83	83
7	50th	93	93	95	96	97	99	99	55	56	56	57	58	58	59
	90th	106	107	108	109	111	112	113	69	70	70	71	72	72	73
	95th	110	111	112	113	115	116	116	73	74	74	75	76	76	77
	99th	117	118	119	120	122	123	124	81	81	82	82	83	84	84
8	50th	95	95	96	98	99	100	101	57	57	57	58	59	60	60
	90th	108	109	110	111	113	114	114	71	71	71	72	73	74	74
	95th	112	112	114	115	116	118	118	75	75	75	76	77	78	78
	99th	119	120	121	122	123	125	125	82	82	83	83	84	85	86
9	50th	96	97	98	100	101	102	103	58	58	58	59	60	61	61
	90th	110	110	112	113	114	116	116	72	72	72	73	74	75	75
	95th	114	114	115	117	118	119	120	76	76	76	77	78	79	79
	99th	121	121	123	124	125	127	127	83	83	84	84	85	86	87
10	50th	98	99	100	102	103	104	105	59	59	59	60	61	62	62
	90th	112	112	114	115	116	118	118	73	73	73	74	75	76	76
	95th	116	116	117	119	120	121	122	77	77	77	78	79	80	80
	99th	123	123	125	126	127	129	129	84	84	85	86	86	87	88
11	50th	100	101	102	103	105	106	107	60	60	60	61	62	63	63
	90th	114	114	116	117	118	119	120	74	74	74	75	76	77	77
	95th	118	118	119	121	122	123	124	78	78	78	79	80	81	81
	99th	125	125	126	128	129	130	131	85	85	86	87	87	88	89
12	50th	102	103	104	105	107	108	109	61	61	61	62	63	64	64
	90th	116	116	117	119	120	121	122	75	75	75	76	77	78	78
	95th	119	120	121	123	124	125	126	79	79	79	80	81	82	82
	99th	127	127	128	130	131	132	133	86	86	87	88	88	89	90
13	50th	104	105	106	107	109	110	110	62	62	62	63	64	65	65
	90th	117	118	119	121	122	123	124	76	76	76	77	78	79	79
	95th	121	122	123	124	126	127	128	80	80	80	81	82	83	83
	99th	128	129	130	132	133	134	135	87	87	88	89	89	90	91
14	50th	106	106	107	109	110	111	112	63	63	63	64	65	66	66
	90th	119	120	121	122	124	125	125	77	77	77	78	79	80	80
	95th	123	123	125	126	127	129	129	81	81	81	82	83	84	84
	99th	130	131	132	133	135	136	136	88	88	89	90	90	91	92

(*Continued*)

TABLE 79-7B

(CONTINUED)

		SBP, mm Hg							DBP, mm Hg						
		Percentile of height							Percentile of height						
Age, y	BP percentile	5th	10th	25th	50th	75th	90th	95th	5th	10th	25th	50th	75th	90th	95th
15	50th	107	108	109	110	111	113	113	64	64	64	65	66	67	67
	90th	120	121	122	123	125	126	127	78	78	78	79	80	81	81
	95th	124	125	126	127	129	130	131	82	82	82	83	84	85	85
	99th	131	132	133	134	136	137	138	89	89	90	91	91	92	93
16	50th	108	106	110	111	112	114	114	64	64	65	66	66	67	68
	90th	121	122	123	124	126	127	128	78	78	79	80	8l	81	82
	95th	125	126	127	128	130	131	132	82	82	83	84	85	85	86
	99th	132	133	134	135	137	138	139	90	90	90	91	92	93	93
17	50th	108	109	110	111	113	114	115	64	65	65	66	67	67	68
	90th	122	122	123	125	126	127	128	78	79	79	80	81	61	82
	95th	125	126	127	129	130	131	132	82	83	83	84	85	85	86
	99th	133	133	134	136	137	138	139	90	90	91	91	92	93	93

*The 90th percentile is 1.28 SD, the 95th percentile is 1.645 SD, and the 99th percentile is 2.326 SD over the mean.
(Adapted from: The National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. *Pediatrics* 2004;114:556).

control if a diuretic is not administered concomitantly. In children with renal insufficiency, the thiazide drugs are less effective and furosemide usually is advocated as the diuretic. The dose of furosemide must be increased to compensate for a low GFR because the drug action depends on its intraluminal concentration. A long-acting ACE inhibitor such as lisinopril is used when renal ischemia or progressive CRI is the cause of the hypertension. This class of antihypertensive drug is especially valuable to control blood pressure in children with renin-dependent hypertension related to acute glomerulonephritis or HUS. When renal ischemia is caused by renal artery stenosis or coarctation of the aorta, captopril or related drugs may cause a precipitous fall in GFR because of efferent arteriolar dilation; therefore, caution should be exercised. Concern exists over long-term metabolic effects (hypokalemia, hyperuricemia, and hyperglycemia) with prolonged thiazide therapy in growing children and the SLE syndrome associated with hydralazine treatment.

TABLE 79-8

ETIOLOGY OF HYPERTENSION IN CHILDHOOD

Cause	<12 yr	12–18 yr
Primary	15%–30%	85%–95%
Secondary	70%–85%	5%–15%
Renal parenchymal	60%–70%	[a]
Aortic coarctation	10%–20%	
Endocrine	3%–5%	
Tumor	1%–5%	
Other	1%–5%	

[a]The frequency of the etiologies of secondary hypertension is similar in both age groups.
(Reprinted from: Flynn JT. Evaluation and management of hypertension in childhood. *Progr Pediatr Cardiol* 2001;12:177, with permission.)

Essential Hypertension

It is usual to define essential hypertension in children as a blood pressure greater than the 95th percentile for age, taking into consideration the patient's height and sex; this inevitably defines 5% of this population as hypertensive. However, screening studies have demonstrated that, with repeated measurement, only approximately 1% of a population of children has a persistently raised blood pressure. Ambulatory blood pressure monitoring discriminates between true and "white coat" hypertension. One long-term follow-up study of young adults ages 21 to 30 years showed a threefold increased risk of hypertension in the children in the highest blood pressure quintile at 9 to 14 years of age (269); these data confirm the findings of others (270). The risk of hypertension in the offspring of hypertensive parents is increased (271,272). Although population screening may not be cost-effective, blood pressure should be checked at office visits, on admission to the hospital, and in children with a family history of hypertension so that advice about weight reduction, exercise, smoking, and diet can be provided; because of familial predisposition to hypertension, this advice may well benefit other members of the family (273).

CHRONIC AND END-STAGE RENAL DISEASE IN CHILDREN

The overall prevalence of renal disease causing ESRD has remained relatively constant since the late 1970s, at 53.4 children per million population of children younger than 18 years of age. However, some racial groups have higher rates than others. For example, Indian Asians have a threefold increase in demand for ESRD treatment in the United Kingdom, in part because of congenital renal tract anomalies (274). In the United States, nearly half of children reaching ESRD are white, whereas 24% are African American and 21% are Hispanic (North American Pediatric Renal Transplant Cooperative Study [NAPRTCS] 2004 Annual Report). Whereas the overall prevalence of ESRD

TABLE 79-9

PHYSICAL FINDINGS IN HYPERTENSION

- Growth including weight and height percentiles
  - Growth failure (chronic renal insufficiency)
  - Changes in weight (thyroid disease, pheochromocytoma, cortisol excess)
  - Obesity (primary hypertension)
  - Truncal obesity (Cushing's syndrome)
- Vital signs
  - Fever (rheumatologic disease)
  - Tachycardia (hyperthyroidism, pheochromocytoma)
  - Bradycardia (hypothyroidism)
- Funduscopic exam (hypertensive retinopathy, increased intracranial pressure)
- Neurologic exam (tumor, increased intracranial pressure)
- Thyromegaly
- Cardiac
  - Murmur (coarctation of the aorta)
  - Congestive heart failure
  - Femoral pulses (coarctation of the aorta)
- Abdominal
  - Mass (renal cystic disease, neuroblastoma, Wilms tumor)
  - Bruit (renal artery stenosis)
- Genitalia
  - Ambiguous/virilization (adrenal hyperplasia)
- Extremities
  - Joint swelling (systemic lupus erythematosus, collagen vascular disease)
  - Muscle weakness (Hyperaldosteronism, Liddle syndrome)
- Skin
  - Purpura (vasculitis)
  - Café-au-lait spots (neurofibromatosis)
  - Ash-leaf spots (tuberous sclerosis)
  - Adenoma sebaceum (tuberous sclerosis)
  - Malar rash or discoid lesions (lupus)
  - Pallor, flushing, or diaphoresis (pheochromocytoma)
- Overall appearance
  - Elfin facies (Williams syndrome)
  - Broad chest, wide-spaced nipples, short neck with webbing, low hairline (Turner syndrome)
  - Moon facies, increased central adiposity, acne, striae, hirsutism (Cushing's syndrome)

has not varied significantly since the late 1970s, its etiology and the age of reaching ESRD have changed as more young children with congenital or inherited renal disease are treated. Congenital renal tract abnormalities and inherited kidney conditions now account for 41% and 72% of the need for renal replacement therapy in children in the United States and the United Kingdom, respectively.

Because CRI is rare in children compared with adults, it seems reasonable for a limited number of centers to specialize in providing treatment for children because treatment of an occasional child in adult treatment centers is likely to be unsatisfactory. A children's center should have the full range of general pediatric services, including intensive care facilities for children, a pediatric dialysis unit, pediatric nurses, social workers, dietitians, and the necessary educational and psychological staff to address the needs of chronically ill children. The aim for most children with ESRD is a successful renal transplantation, so it is advantageous if facilities for transplantation are available in the children's hospital. Obviously, such provision can be expensive but is more economical if it is established in a hospital that already has well-developed pediatric services and an active adult renal unit.

Our knowledge of the factors that affect children in CRI is sufficient to ensure a better outcome but is far from complete. With proper diagnosis and investigation of the renal tract to ensure unobstructed urine flow and adequate bladder function, nutritional support, correction of electrolyte and acidosis imbalance, treatment with recombinant growth hormone, and control of secondary hyperparathyroidism, children with CRI should grow well and develop normally. It is these areas of the clinical care of pediatric CRI that differ greatly from the experience in adult nephrologic practice.

Management of the child with CRI/ESRD is time consuming, and careful clinical and biochemical assessment is required at regular intervals. This should include accurate measurement of height and weight, skinfold thickness, and upper arm circumference. The weight-for-height index is of particular value in detecting malnutrition. Pubertal status should be documented at intervals. Radiographs of the hand and wrist should be obtained periodically for assessment of bone age and detection and supervision of renal osteodystrophy.

Growth Retardation in Children with Chronic Renal Failure

Historically, nearly half the children with CRI or ESRD were below the third percentile for height (275–277). A few decades ago, the average height for young men and women treated by dialysis and transplantation during childhood was approximately 2 SD below the mean (278), representing an average deficit of 15 cm in final height (279). This stunting of growth was a source of social embarrassment and psychological trauma.

The cause of growth failure in children with CRI is multifactorial. Children with congenital renal disease tend to be shorter than those with acquired disease because of a growth deficit during the first 2 years of life, when growth potential is maximal. Growth failure can also reflect poor nutrition (see subsequent text) (280,281) and electrolyte imbalance (282). Growth retardation is particularly marked in children with generalized tubular dysfunction (Fanconi's syndrome), in which sodium and bicarbonate wasting, polyuria and polydipsia, hypophosphatemia, and osteomalacia all contribute (283). Sodium wasting is particularly important both in these children and in infants with CRI caused by congenital urinary tract obstruction or renal dysplasia/hypoplasia. Sodium is essential for growth (19,284), and salt supplements restore growth in children with isolated sodium wasting (285). Children with syndromes, chromosomal anomalies, and some systemic conditions often demonstrate poor growth. Renal osteodystrophy, osteomalacia, and osteitis fibrosa cystica due to secondary hyperparathyroidism are all potent inhibitors of growth.

If dialysis is required, this mode of therapy provides suboptimal replacement of renal function and has many complications in infants (286,287). Improving dialysis efficiency is known to improve growth (288). With transplantation, growth usually improves but is variable because of many complex factors, with only some patients showing "catch-up" growth. Certainly younger, prepubertal children, particularly those who are most stunted during CRI or who were dialyzed, grow better initially than older children or adolescents if they receive a good functioning renal transplant. Good posttransplantation renal

TABLE 79-10

DRUG DOSES IN PEDIATRIC HYPERTENSION

Class	Medication	Dosage	Dosing interval	Maximum
ACE inhibitors	Captopril	0.15–0.5 mg/kg/dose	BID-TID	6 mg/kg/day, up to 150 mg/day
	Enalapril	0.08 mg/kg/dose	QD	0.6 mg/kg/day, up to 40 mg/day
	Lisinopril	0.07 mg/kg/dose, up to 5 mg QD	QD	0.6 mg/kg/day, up to 40 mg/day
	Benazepril	0.2 mg/kg/day, up to 5 mg QD	QD	0.6 mg/kg/day, up to 40 mg/day
ARB	Irbesartan	75–150 mg/day	QD	300 mg/day
	Losartan	0.75 mg/kg/day, up to 50 mg QD	QD-BID	1.4 mg/kg/day, up to 100 mg/day
Calcium channel blockade	Amlodipine	0.06 mg/kg/dose	QD-BID	0.6 mg/kg/day, up to 10 mg/day
	Isradipine	0.05–0.15 mg/kg/dose	TID-QID	0.8 mg/kg/day, up to 20 mg/day
β-Adrenergic blockade	Labetalol[a]	2–3 mg/kg/day	BID	10–12 mg/kg/day, up to 2.4 g/day
	Propranolol	1 mg/kg/day	BID-TID	16 mg/kg/day
	Atenolol	0.5–1 mg/kg/day	QD-BID	2 mg/kg/day, up to 100 mg/day
	Metoprolol	1–2 mg/kg/day	BID	6 mg/kg/day, up to 450 mg/day
Peripheral α_1-antagonist	Doxazosin	1 mg/day	QD	4 mg/day
	Terazosin	1 mg/day	QD	20 mg/day
Central α_2-adrenergic agonist	Clonidine	0.1 mg transdermal	Q5-7 days	0.3 mg
Vasodilator	Hydralazine	0.25 mg/kg/dose	TID-QID	7.5 mg/kg/day, up to 200 mg/day
	Minoxidil	0.1–0.2 mg/kg/dose	BID-TID	1 mg/kg/day, up to 50 mg/day
Diuretic	Furosemide	0.5–2 mg/kg/dose	BID-QID	10–15 mg/kg/day, up to 200 mg/dose
	HCTZ	1 mg/kg/day	BID	4 mg/kg/day, Up to 50 mg/day

ACE, angiotensin converting enzyme: *ARB*, angiotensin receptor blocker.
[a]Labetalol has both α and β adrenergic antagonist effects.
(Adapted from: Flynn J. Recognizing and managing the hypertensive child. *Contemp Pediatr* 2003;20:38.)

function and alternate-day steroid therapy, or, better still, no long-term maintenance steroids, produce significantly better growth than daily maintenance doses.

Nutrition in Children with CRI

Malnutrition due to the metabolic disturbances of uremia is common in adults, and in childhood it results in poor growth. Anorexia frequently accompanies CRI, and when the energy intake falls below 80% of the recommended dietary allowance, there is a correlation between the poor energy intake and poor growth (277,281). Prescribing a strict dietary regimen for the anorectic child in CRI is futile because compliance is poor. The renal dietitian is an essential part of the clinical team to offer advice on diet in children with chronic renal disease. They prescribe an amount of low-phosphate food for infants, which contains supplements of glucose polymer or lipid to increase the daily calorie intake to a minimum of 120 but ideally 150 kcal/kg per day. Infants are fed high-protein milks and protein supplements up to a total of 2.5 g/kg per day. Phosphate restriction is typically necessary for older children, with reduction of intake of dairy products along with the use of phosphorous binders. Monitoring of the average calorie intake, weight and height gain, BUN (which should be <60 mg/dL), and serum albumin concentration is sufficient to determine the dietary protein intake needed for the individual child. Protein restriction has been associated with poor growth in infants with CRI (289) and is not recommended for growing children. In clinical practice, excluding dairy products for phosphate restriction already reduces protein intake to some extent. Adequate intake of vitamins such as pyridoxine and trace minerals should be ensured. If the weight gain is poor with the child's spontaneous food intake, early use of alimentary feeding with either a nasogastric feeding tube or gastrostomy is required.

When the GFR is less than 80 mL/minute/1.73 m^2, accumulation of phosphate and secondary hyperparathyroidism occur and can be prevented by implementing dietary restriction of phosphorus with the addition of phosphate-binding drugs if needed. When the GFR reaches less than 25 mL/minute/1.73 m^2, high-dose phosphate binders [calcium carbonate or cross-linked poly-(allylamine hydrochloride) (Renagel)] together with extra vitamin D in the form of 1,25-dihydroxycholecalciferol (0.1 to 0.5 μg per day) are important components of therapy. Periodic monitoring of serum calcium, phosphorus, and intact PTH concentrations is necessary. Therapy should be adjusted to keep the PTH levels below 1.5 times the upper limit of normal. If the child is hypercalcemic, it is best to lower the plasma phosphate by diet and Renagel and then substitute calcium carbonate when control has been established.

Concern exists among pediatric and adult nephrologists regarding the long-term metabolic consequences of a diet high in carbohydrate and fat and the cardiovascular disease risks they impose. Both hypertriglyceridemia and hypercholesterolemia are common in children with CRI and ESRD, although the observed derangement varies with the degree of uremia, mode of renal replacement therapy, and drug regimens (290–292). There are no large, controlled, long-term trials of therapy for hyperlipidemia in children with progressive renal disease. Short-term studies with fish oil, 5 to 8 g per day, have shown effectiveness in reducing hypertriglyceridemia but not hypercholesterolemia (293). Both triglyceride and cholesterol are reduced in nephrotic patients with 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors.

Linear Growth in Children with CRI

The levels of the two most important growth hormones in humans, insulin and growth hormone (GH) itself, are elevated in CRI, with the balance of insulin-like growth factor-1 (IGF-1) and its binding proteins in favor of there being less biologically active IGF-1 (294–296) and therefore decreased growth. If other factors contributing to poor growth in CRI have been appropriately addressed, and the child still fails to grow and is below the third percentile for height, recombinant human GH (rhGH) therapy at a dose of 30 U/m^2 BSA per week increases the height velocity SD score (SDS) in children with CRI (297,298), those undergoing dialysis (299), and those with transplants (300). The drug is provided as a daily subcutaneous injection. It is more effective in prepubertal and younger children, when height velocity SDS may double or triple, compared with older, peripubertal children. "Catch-up" growth may occur in the first 2 or 3 years of therapy, but then height velocity reduces to more normal levels. If rhGH is stopped, "catch-down" growth is reported to occur in 75% of patients (301), whereas others report growth proceeding along the percentile lines (302). The therapy is safe, with no common deleterious effects on GFR, long-term insulin insensitivity, or, in transplanted patients, allograft rejection (303,304). Although the parents' and patient's acceptance of the treatment is good (305), compliance still may be a problem in 60% of children, and the issue should be raised, although there was little correlation with the response to therapy in one study (306).

Pubertal Development in Children with CRI

Delayed pubertal development is common in children with impaired renal function and is attributable to numerous hormonal alterations. Lower overnight gonadotropin bursts from the pituitary also are observed in peripubertal children (307); this leads to an average delay in puberty onset in CRI of 2.5 years for both sexes (308). The poor growth of CRI usually is associated with delayed skeletal maturation (309). As noted previously, GH bursts from the pituitary gland are also reduced in quantity, and in prepubertal and peripubertal boys, plasma IGF-1 levels are reduced and IGF binding protein concentrations increased, leading to less growth-promoting activity of the pituitary-GH-IGF axis (310). In late puberty, GH burst frequency increases to greater than normal but GH mass per burst remains low. The mean age of onset for menarche in girls with ESRD is 15.9 years, compared with a mean age for normal girls of 13.4 years (311). Sex steroid hormone administration may be necessary to speed puberty in older teenagers.

Anemia in Children with CRI

The management of anemia in children with CRI/ESRD has been revolutionized by the introduction of recombinant human erythropoietin. The dosage varies between 100 and 150 U/kg body weight given three times a week after dialysis. The dose should be adjusted to maintain the hemoglobin concentration in the lower half of the normal range for the child's age and sex. Erythropoietin treatment is associated with a fall in serum ferritin, and oral or intravenous iron supplementation may be required. The amelioration of anemia is associated with increased well-being and exercise tolerance, and as long as the hemoglobin level is monitored carefully, hypertension does not appear to be a significant complication. Thrombosis of vascular access is a risk if polycythemia is induced. The avoidance of blood transfusions decreases the chance of sensitization to major histocompatibility complex (MHC) antigens and is associated with a fall in antibodies in previously sensitized children.

Psychosocial Considerations

Chronic renal failure leads to severe psychological problems in some children, especially those on chronic hospital dialysis (312–315). The insecurity and painful procedures such as blood taking and fistula access may elicit aggression toward parents and staff, and the insecurity this creates in both can lead to anxiety for all concerned. The aggression may be followed by withdrawal, or refusal to cooperate (which can at times make dialysis almost impossible), and can affect food intake and survival. The further stresses of retarded growth, retarded sexual and emotional maturation, loss of schooling, abnormal appearance, and dependence on hospital and machines inhibit the normal development of independence. The whole way of life for the child and family can be affected. Although the incidences of depression and anxiety do not differ from those in normal, healthy adolescents, personal and social adjustments often are poor. The coping strategies displayed by children on dialysis are better for those on home peritoneal dialysis than for those on hemodialysis (312), and children with functioning renal transplants fare even better (313).

In one survey of adults who had been treated in childhood for an average of 11 years, most were working full or part time in a variety of different jobs that were not dissimilar to those of their peers (278). Many, particularly the girls, were married and living normal sexual lives. The mode of treatment is important in determining psychological morbidity, with transplanted children showing half the psychosocial problems of dialyzed children (316). Marriage occurred in approximately 50% of patients, with half of the couples having children of their own. Only 10% to 14% of pediatric patients with ESRD were unemployed (316,317). It was especially interesting that many families thought the experience had improved their family life rather than the reverse.

Stress in the care of the child with CRI/ESRD may be reduced if all involved staff are aware of the problems and are willing to discuss them openly to alleviate anxiety and take time to talk about the difficulties between themselves, with the families, and with the children. The environment where the children are treated is important; schooling must be available, with a liaison between the hospital and the child's own school. Social service support to alleviate at least some of the family's difficulties, financial help for the unforeseen expenses, and an opportunity for relief by providing holidays for the children away from the family are important and aid in the development of independence. Access to skilled psychiatric assistance is important. Children need to grow emotionally and socially, and this is inhibited by overprotection; thus, families should be sympathetically discouraged from overindulgence. Children mature best with the love, discipline, and support of their families, and it is no surprise that dialysis at home, whether hemodialysis or continuous cyclic peritoneal dialysis, is associated with fewer problems. A multidisciplinary team is essential if the child and family are to overcome these problems.

Intellectual performance is reduced in adults with ESRD, and some studies in infants suggested a similar problem that may be compounded by the severe disturbances in body composition that occur in these younger children (318,319). With the advent of calcium carbonate and Renagel, aluminum neurotoxicity and bone disease should not occur, but malnutrition strongly influences neurologic outcome in infants with early, severe CRI from birth (320).

Dialysis

Children require more dialysis in relation to their body weight than adults do because their metabolic rates and therefore

intakes are higher. This means that children require larger dialyzers in relation to their body weight, but if too large a dialyzer is used with a volume exceeding more than 8% of the child's circulating blood volume (80 mL/kg body weight), there is a risk of hypotension on dialysis or hypertension after "rinse back." Dialyzers should have low compliance and should be the largest that can be used while still maintaining an extracorporeal blood volume below 8% of the child's circulating blood volume. The dietary principles for the child on hemodialysis are similar to those discussed previously, and an adequate energy intake should be ensured and secondary hyperparathyroidism carefully controlled.

Chronic dialysis options for children include continuous cyclic peritoneal dialysis (CCPD), continuous ambulatory peritoneal dialysis (CAPD), and hemodialysis, which usually is hospital based. Each has its advantages and disadvantages. In general, dialysis or preemptive transplantation is required in children when the GFR is 10 mL/minute/1.73 m^2 or lower, or if hyperkalemia, fluid overload, poor metabolic control, or poor growth due to uremia occur. Ideally, no child should be accepted onto a dialysis program unless renal transplantation is intended, because this offers the most successful therapy for ESRD in children.

CCPD may be used in infants as well as in the older child or teenager. The nutritional problems of children are important, particularly in infants because of their need to achieve energy balance. They require greater dose of dialysis, and CCPD is most appropriate. Dialysis fill volumes are 40 mL/kg in the stable chronic patient, and the amount of dialysis is set by the number of cycles per night. The large variation in body size of children of different ages means that individual prescription volumes of peritoneal dialysate are required and should be reassessed at regular intervals. Peritoneal equilibration tests (PETs) or formal timed clearances may be used to adjust dialysis prescriptions to gain better general health and growth (321). In general, children were thought to have higher solute transport than adults, but more recent evidence suggests the contrary when transport rates are corrected for BSA (322). By performing CCPD overnight, children may be left with their abdomen empty during the day, which adds to their daytime mobility and comfort. Using sterile technique, peritonitis rates of approximately one episode per 14 months per patient or better are easily achievable. CAPD may be used where dialysis machinery is not available, and like adult practice, requires four exchanges to be made per 24 hours; it is not as convenient for school-aged children or their parents. Both techniques can achieve creatinine clearances of approximately 70 L/week, giving the child a sense of well-being and permitting good school attendance.

The major problems with peritoneal dialysis are infection of the exit site or catheter tunnel, peritonitis, poor catheter position or malfunction, constipation interfering with dialysate drainage, and line breaks, in common with adult practice (see Chapter 98). Peritonitis caused by pets breaking the sterile circuit, usually by gnawing on the dialysis tubing, is well described in children on home dialysis. Attention to detail with regular cleaning and dressing of the exit site and a well adherent catheter at the exit site protects against exit site and tunnel infections. Chronic colonization of the exit site often leads to peritonitis. Common pathogens that cause peritonitis in children are coagulase-negative staphylococci, *Staphylococcus aureus, Pseudomonas,* and, occasionally, *Candida*. The latter two pathogens usually require the peritoneal catheter to be removed in order to clear the infection with hemodialysis commenced. Long-term peritoneal membrane failure may lead to high transporter status or ultrafiltration failure when assessed by PET. This may lead to an increased body mass index in the child because glucose is readily absorbed through the peritoneum. Sclerosing peritonitis occurs in children as well as adults and is related to recurrent peritonitis and long-term use of peritoneal dialysis.

The hemodialysis prescription for children often is 4 hours three times per week, and kt/V or urea reduction ratio criteria have been calculated for children to assess the quality of dialysis (Chapters 98 and 99) (323–325). Younger infants may require up to six to seven hemodialysis treatments per week to maintain adequate clearance and foster growth. Hospital-based hemodialysis is becoming less popular, but it may have advantages in certain clinical settings. Nephrotic patients who have undergone recent bilateral nephrectomy are able to achieve better anabolism with hemodialysis than with peritoneal dialysis, when protein losses may further compromise a protein-malnourished child. Supervision by hospital staff also is possible in families who have difficulty coping with peritoneal dialysis, although hospital-based hemodialysis is more expensive. Long-term problems with hemodialysis arise from difficulty with vascular access. New double-lumen catheters with cuffs that are tunneled under the skin provide greatly improved access, particularly in small children and infants. Reliance on surgically performed arteriovenous fistulas or artificial arteriovenous grafts still occurs with older children and adolescents. Despite these advances, line sepsis, fistula stenosis or thrombosis, excessive interdialytic weight gains, and loss of schooling still pose significant problems for the child on hemodialysis.

Growth on dialysis remains suboptimal, as detailed previously. The mode of dialysis affects growth, with peritoneal dialysis having better growth rates than hemodialysis.

Renal Transplantation

Living related or cadaveric donor renal transplantation has been successfully performed routinely in children, from the age of 1 year, since the early 1970s. It remains the primary mode of therapy for renal failure in children and is most successful when carried out preemptively, because survival of children (and grafts) is no different from those who underwent dialysis initially (326,327). Successful transplantation gives the child with renal failure the best potential to achieve a normal life, growth, school performance, and psychosocial and emotional development. Adults who have been transplanted as children have a higher chance of being well rehabilitated, employed, and married then if they are undergoing dialysis (317).

Surgical and Perioperative Considerations in Very Young Children

Transplanting young children may present the surgeon with special problems—the discrepancy between the organ size and the recipient's abdomen may pose operative problems that the surgeon must address before surgery (328). Although infants receiving adult kidneys can have the graft placed intraabdominally with the renal vessels anastomosed to the aorta and inferior vena cava, we prefer to wait until children have reached a weight of at least 12 to 15 kg before proceeding with transplantation due to decreased allograft survival. In addition, intraabdominal renal transplants are often associated with increased third space fluid losses, postoperative ileus, temporary intolerance of feeds, and diarrhea. Age matching of donors and recipients does not improve graft survival for young children (329), although others have demonstrated in their units (330) that all recipients have less risk of graft loss if the donor age is 18 to 45 years. Graft function of adult and pediatric donors is similar within 2 years posttransplantation owing to adaptive changes dictated by the recipient's size (331). Vascular thrombosis of small allografts placed in young children is more common, as is renal artery stenosis (332). Young recipients

often have renal tract anomalies or obstructed renal tracts as the cause of their ESRD, so a thorough preoperative assessment of urine drainage through the bladder and urethra is required, including videourodynamics in many cases, to enable the site of anastomosis of the transplant ureter to be planned. In those children with a urinary diversion, when graft function is optimal and good urine flow is present, bladder augmentation or urethral operations and anastomosis of the transplant ureter to the bladder can be carried out. Corrective bladder augmentation or other urologic operations often are unsuccessful unless good urine flow is ensured (333).

After surgery, the renal transplant's vascular capacity often greatly exceeds that of the child's native kidney, so extra care must be taken to maintain intravascular volume and renal perfusion by adequate fluid replacement. Small discrepancies in fluid balance are critical to early graft function and even long-term outcome. Most important, postoperative tissue perfusion should be assessed by measurement of pulse, arterial blood pressure, and central venous pressure. The ideal postoperative cardiovascular status is to achieve an adequate central venous pressure, normal arterial blood pressure, and good urine output (>2 mL/kg/hour). Ensuring good urine production optimizes graft function and leads to low primary nonfunction rates.

Immunosuppression and Patient and Allograft Survival

The many successful immunosuppressive protocols used in children are similar to adult protocols, with adjustment of drug dosages based on BSA to reflect the discrepancy in body size and metabolic rate. Familiarity with the pharmacokinetics of immunosuppressive drugs in children is important. Most commonly, a triple regimen including tacrolimus, mycophenolate mofetil, and prednisolone/prednisone is used. Variations on this basic protocol occur with the use of cyclosporine instead of tacrolimus or azathioprine instead of mycophenolate mofetil. As expected, protocols vary from center to center. The cyclosporine era has allowed a great reduction in the dose of steroid use posttransplantation, with improvement of graft survival, side effects, and growth. Long-term maintenance, alternate-day dosing of steroids has been shown greatly to improve pubertal growth, particularly in boys, compared with daily dosing at similar doses that were used in prednisolone and azathioprine protocols (334).

The North American Pediatric Renal Transplant Cooperative Study (NAPRTCS) has reported 1- and 5-year graft survival probabilities; living related donor graft survival rates are 93% and 81%, respectively, compared with cadaveric allograft survival rates of 84% and 66% (2004 Annual Report). Chronic and acute rejection continue to be the major causes of index allograft loss, followed by graft thrombosis. Long-term survival rates in children who undergo renal transplantation before the age of 6 years are similar to those in older children and adults. Impressive patient and allograft survival rates with living related donors can be obtained in recipients younger than 2 years of age (335). This maximizes the potential for physical and neurologic development, with good catch-up growth and an increase in head circumference and developmental quotient being observed.

Loss of the renal transplant may occur from acute rejection, acute graft thrombosis, infection of the recipient that requires withdrawal of immunosuppression, or chronic allograft nephropathy, some of which is due to chronic rejection. Anti-HLA antibodies in children who have become sensitized are most troublesome when directed against MHC class II antigens and may complicate repeat transplantation. Because graft thrombosis is a common cause of graft loss in transplanted children, the pretransplantation workup of the recipient should include detailed screening for procoagulant conditions by functional and genetic testing, such as the presence of factor V (Leiden), homocystinuria, heterozygosity for factor C, S, prothrombin or antithrombin III deficiency, and others. For living related donor transplantation, these tests also should be performed on the donor.

Teenagers and Transplants

Delayed puberty and short stature are important potential problems of teenage life. Many transplanted teenagers have difficulty in meeting others socially. Teenagers with renal transplants have anxiety about rejection (often reinforced by their doctors), which may lead to depression. When this is coupled with an abnormal body image and a chaotic lifestyle, noncompliance with medication may be very common (up to 60% of patients). Concerns over body image result from drug side effects, or a dialysis fistula may dominate a teenager's attitude to his or her treatment, leading to noncompliance. If this involves immunosuppression, the renal transplant is at risk. The severe, full-face acne and central obesity induced by steroids, the hypertrichosis of the trunk and limbs from cyclosporine, and the gum hypertrophy from cyclosporine and calcium channel blocker are common concerns. Supportive counseling about their problems and a nonconfrontational approach to noncompliant teenagers is most constructive, as opposed to blaming them for their actions. The physician caring for such adolescents must show support and understanding for their concerns, adapt the drug regimen appropriately, and resist justifying the need for the drugs simply because the transplanted kidney is valuable to the patient. Education about the importance and need for immunosuppressive drugs both before and after surgery reduces posttransplantation noncompliance.

Recurrent Disease and Long-Term Complications of Pediatric Renal Transplantation

Recurrent renal disease may occur after renal transplantation in approximately 11% of transplanted children. Steroid-resistant idiopathic nephrotic syndrome, usually due to focal segmental glomerulosclerosis, recurs in 30% to 50% of patients. The recurrence often occurs very early in the transplant's clinical course and is slightly more common with living related kidneys, but is also very common in second or subsequent grafts (70% to 80% of children) if it recurred in the first transplant. Heavy and prolonged immunosuppression has been successful in treating this posttransplantation complication, and recently both immunoadsorption of plasma and multiple plasmapheresis with standard triple therapy have been used successfully (336). In contrast to these clinical findings, mesangiocapillary glomerulonephritis commonly occurs later in the posttransplantation course, particularly if it is dense deposit disease.

Oxalosis recurs in 33% of newly transplanted kidneys and long-term graft survival rates at 3 years are only 17% to 23% (337). Successful transplantation is achieved by decreasing the oxalate load by extensive preoperative dialysis and postoperative diuresis. Alternatively, liver transplantation may be curative before severe CRI occurs, or combined liver–kidney transplant may be considered.

Because of heavy immunosuppression with multiple drugs and their long-term administration with a successful allograft, and the relative lack of Epstein-Barr virus infection in young children, recipients transplanted at a young age are at increased risk for posttransplantation lymphoproliferative disorder (PTLD) and other neoplasms (see Chapter 97). PTLD is reported to affect 1% to 10% of pediatric renal transplant recipients (338). Increased general use of more potent immunosuppression in the 1990s may increase this figure in the future, a worrisome potential consequence of using potent immunosuppressive drugs in the effort to obtain better graft survival.

Transplanted children with chickenpox, which still is endemic in many countries, are at increased risk because the illness may be severe and even fatal in immunosuppressed hosts. We recommend that all children who are placed on transplant waiting lists be immunized against varicella. (It is optimal to be certain that children are fully immunized against all usual childhood illnesses prior to renal transplantation if possible.) Immunizations, other than live poliovirus vaccines, usually are not contraindicated in children with renal transplants taking maintenance immunosuppression (339).

PEDIATRIC NEPHROLOGY CLINICAL SERVICES

The successful outcome of children with renal disease depends on many factors—early identification *in utero* for congenital renal malformations, early diagnosis of renal disease or infection in susceptible target populations of children, appropriate treatment of associated hypertension, and appropriate therapy of the underlying disease. Because of the complex nature of most renal disease, which is due to the major role the kidneys play in homeostasis, it is clear that to attain the maximum potential well-being for a child with a serious renal condition, many skills are required. Moreover, for the child and his or her family, psychological and emotional, social, and financial support, together with education, usually are required.

There are no absolute contraindications to the treatment of a child with ESRD, but there are many relative ones. The purpose of treatment is to enable the child to survive and grow up to lead a full adult life. Most children can achieve this successfully only with the support of their families. Long-term dialysis and transplantation of newborns and infants with ESRD still are uncommon, but every pediatric renal unit has had some experience with these children. Treatment of the child is not always the best or kindest option for the child or the family. The final decision, given that the resources are available, must rest with the family. Obviously, as the child grows older, these contraindications to treatment lessen, but the support that is required for the successful treatment of children in ESRD and their families must never be underestimated. Only a clinical team—nephrologists, other physicians and specialists, nurses, psychologists, social workers, teachers, play specialists, and parents—can provide all the necessary skills. Such clinical teamwork, coupled with research, holds the key to a successful future for children with renal disease.

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CHAPTER 80 ■ RENAL FUNCTION AND DISEASE IN THE AGING KIDNEY

DEVASMITA CHOUDHURY AND MOSHE LEVI

INTRODUCTION

The natural process of aging affects both structure and function of the kidney. Although the aging kidney can maintain an adequate internal environment for daily function, acute intercurrent stresses from immunologic, infectious, toxic insults, or other organ dysfunction reveal the limitations of response and lead to increased failure of function. This is evident with older donor kidneys. Kidneys from donors older than 55 years of age are more likely to fail from chronic allograft dysfunction (1–5).

The current U.S. population of 35 million older than 65 years is expected to grow to 54 million by 2010 (6), with an expected proportional increase in the prevalence of moderate-to-severe renal failure in this group from present estimates of 10.8% in the nondiabetic, nonhypertensive population and 20.6% in those with preexisting diabetes and hypertension (6). Prevalence estimates of end-stage renal disease (ESRD) in the elderly are five times greater than in young adults (7,8). Renal functional loss further independently adds to risk of cardiovascular and all-cause mortality to this elderly population (6). Understanding, therefore, the anatomic, physiologic, and pathologic changes of renal aging may help in our ability to appropriately care for the elderly and prevent disastrous outcomes. The use of molecular probes has increased insight into basic processes leading to changes in the aging kidney. This chapter attempts then to review the current understanding of structural, functional, and clinical processes that impact renal function in health and disease.

MACRO AND MICRO CHANGES IN STRUCTURE

Gross kidney weight and mass both decrease as age increases. Postmortem evaluation of kidneys using intravenous urography and computed tomography note that kidneys of older individuals are smaller (9,10) and weight drops from 250 to 270 g during young adulthood to 180 to 200 g by age 90 (11). These changes in renal mass may be age-appropriate given that there is parallel decrease in body surface area with aging (12).

Ultrastructural examination of older kidneys reveals a general increase in glomerulosclerosis and tubulointerstitial fibrosis. Little cellular response is seen with hyaline depositing in residual glomeruli and Bowman's space as they become obsolescent (Fig. 80-1) (13). Much of the cortical glomeruli are ischemic, with glomerular count decreasing as much as 30% to 50% by age 70 (14), whereas medullary glomeruli appear to be relatively spared. The aged obsolescent glomeruli display an 8% to 12% increase in mesangial volume, loss of glomerular tuft lobulation, and progressive capillary collapse with obliteration (15). In aging (24-month-old) Sprague-Dawley rats there is decreased expression of proangiogenic vascular endothelial growth factor (VEGF) and increased expression of antiangiogenic factor thrombospondin-1 when compared with younger (3-month-old) rats (16). This is associated with the significant decrease in both glomerular and peritubular capillary density, hinting the possibility of impaired angiogenesis in the aging renal tissue as cause for gradual glomerular obsolescence and tubulointerstitial fibrosis.

Renal cortical arteries from tissue sections of humans from 6 to 70 years of age show progressive arterionephrosclerosis with increased intimal fibrosis and medial sclerosis and shrinkage (17), suggesting that the gradual increase in blood pressure that is seen with aging may be set according to the changes in the renal vasculature (Fig. 80-2) (18). Similarly donor biopsies from healthy elderly reveal increased arteriosclerosis of interlobular and arcuate arteries compared to younger donor kidneys (19). These changes seen in the intrarenal vasculature appear to be independent of hypertension and other renal disease. Basement membranes of both glomeruli and tubules are also wrinkled and thickened, leading to fewer more simple vascular channels (20,21). Microangiograms show redistribution of renal blood flow from the cortex to the medulla, with blood being shunted from the afferent to the efferent arterioles of the juxtamedullary glomeruli (Fig. 80-3) (22). The arteriolar vera recta of the renal medulla are intact and sustain blood flow to the medulla (21,23).

Interestingly, molecular changes in the aged renal tissue seem to parallel histologic changes. Telomere length decreases in the aging renal cortex faster than in the renal medulla (24). Acting as mitotic clocks for cell replication (25–27), critical shortening of telomere length in the renal cortex may suggest a limited survival capability for this cell population, possibly contributing to the subsequent cortical scarring seen in the aged human kidney (24,28). In addition, there is increased expression of messenger RNA (mRNA) and protein of candidate genes associated with senescence including cell cycle inhibitor p16INK4a, p53, cyclooxygenase 1 and 2 (COX-1, COX-2), transforming growth factor beta-1 (TGF-β-1) and heat shock protein A5 (HSPA5) in human renal cortical tissue that correlates with age-related histologic changes of glomerulosclerosis and interstitial fibrosis. The cell cycle inhibitor p16INK4a appears to show the most consistent correlation with both age and histological changes and correlates inversely with cell replication (29).

Glomerular senescence is accompanied by renal tubular atrophy that outpouches into distal diverticula (Fig. 80-4) (30). Histologically these tubular outpouchings may represent the beginnings of acquired renal cysts seen in elderly (30). A collection of bacteria and debris in these diverticula can then predispose to infection and pyelonephritis (31).

Tubulointerstitial fibrosis in the aging kidney may actually precede the associated focal glomerulosclerosis and tubular atrophy (32,33). Fibrosis in the aging rat tubulointerstitium is an active inflammatory process marked by fibroblast activation, accelerated surrounding cellular apoptosis, focal tubular cell proliferation, myofibroblast activation and macrophage

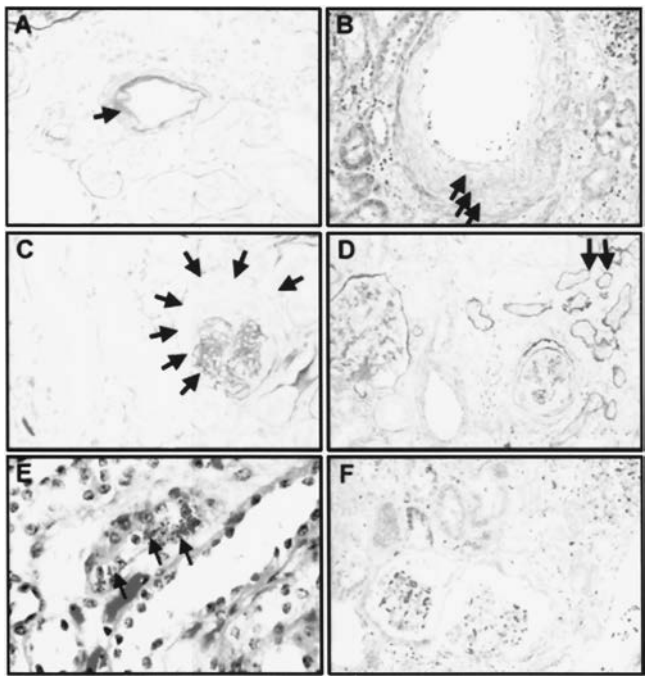

FIGURE 80-1. Histology of renal senescence: (**A**) arteriohyalinosis, (**B**) fibrous intimal thickening, (**C**) glomerulosclerosis, (**D**) tubular atrophy, (**E**) lipofuscin pigment, (**F**) interstitial fibrosis. (Reprinted from: Melk A, Halloran PF. Cell senescence and its implications for nephrology. *J Am Soc Nephrol* 2001;12:385.)(See Color Plate.)

infiltration (34). Increased immunostaining for adhesion proteins osteopontin and intercellular adhesion molecule-1 (ICAM-1) is evident in the extracellular interstitial matrix (34) (Fig. 80-5). Older rats (24-, 30-month-old) are noted to also have increased fibronectin mRNA expression as well as increased deposition of both collagen 1 and collagen IV. Diminished endothelial nitric oxide synthase (eNOS) expression resulting from ischemia and peritubular capillary injury may incite the inflammation. Collagen 1 mRNA upregulation and increased deposition in aging Sprague-Dawley rats is associated with progressive fibrosis (35). Similarly, aging human autopsy tissue also demonstrates increased collagen 1 accumulation, which correlates with the extent of interstitial fibrosis, although the same is not evident for collagen IV (36). Because mRNA levels were opposite, however, to the tissue accumulation of collagen I and collagen IV, it is possible that aging diminishes the different matrix metalloproteinases (MMPs) responsible for degradation of the different collagens, MMP-3 and MMP-9 for collagen IV, and MMP-8 and MMP-13 for collagen 1, differently, thereby leading to accumulation of collagen 1 and not collagen 4 (36).

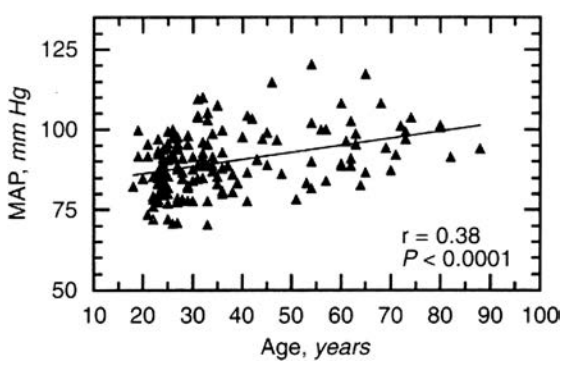

FIGURE 80-2. Mean arterial pressure (MAP) as a function of age. (Redrawn from: Hoang K, Tan JC, Derby G, et al. Determinants of glomerular hypofiltration in aging humans. *Kidney Int* 2003;64:1417.)

MEDIATORS AND MODIFIERS OF GLOMERULOSCLEROSIS AND TUBULOINTERSTITIAL FIBROSIS

It is clear that some individuals experience the changes observed with aging in the kidney faster than others. A 23-year longitudinal follow-up of healthy elderly finds two-thirds of the subjects showing a decrease in creatinine clearance, whereas one-third of the subjects did not have any absolute changes in creatinine clearance (38). Although biologic aging in the kidney results in scarring of the glomerulus and tubulointerstitium, various factors can change the rate at which sclerosis occurs including angiotensin II (AT-II), nitric oxide (NO), advanced glycosylation end products (AGEs), TGF-β-1, oxidative stress, and lipids (Fig. 80-6). The ability to modify these factors may be important in delaying the progressive age-associated changes in renal function.

Role of Angiotensin II

AT-II plays diverse roles in the kidney, from vasoactive effects to affecting proximal tubular transport of sodium and water (39,40), glomerular and tubular growth (41–45), decreasing NO synthesis (46), immunomodulation, growth factor induction, and accumulation of extracellular matrix proteins, all of which can modulate renal glomerular and tubulointerstitial scarring. Changes in intraglomerular hemodynamics with preferential efferent arteriolar vasoconstriction to maintain filtration pressure in the aging nephrons may be contributing to age-dependent glomerular damage (47). The use of angiotensin-converting enzyme (ACE) inhibitors to increase renal plasma flow (RPF) and glomerular filtration rate (GFR) indirectly implicates AT-II in supporting this mechanism of glomerulosclerosis (46). Treatment of aging rodents with ACE inhibitor decreased their intrarenal vascular resistance (RVR) and intracapillary pressure with a subsequent decrease in protein leak, and resulted in a significant reduction in the frequency of focal segmental glomerular lesions seen with aging (48,49). The increase in filtered load associated with postprandial hyperfiltration was reduced with chronic ACE inhibitor use (50). In addition, ACE inhibitor could possibly be changing glomerular capillary size selectivity or the negative glomerular barrier charge distribution (49,51). There was a significant decrease in glomerular diameter mesangial area, and total glomerulosclerosis in ACE inhibitor-treated aged mice in comparison to age- and sex-matched untreated mice (51–54) (Fig. 80-7).

With aging, the walls of the larger intrarenal vessels undergo variable sclerotic changes that are worsened with hypertension. Increased arteriosclerosis is present in the interlobular and arcuate arteries as well as other intrarenal vessels of biopsies from healthy older donors in comparison with those of the younger group (19). Renal pyelography and angiography on postmortem kidneys of normotensive subjects older than 50 years of age suggest that changes in the renal vasculature may affect loss of renal cortical tissue more than age alone (37).

Further beneficial effects of AT-II antagonism in the aging kidney are evident by the nonhemodynamic effects of AT-II in inducing profibrotic cytokines in the kidney. Via endogenous synthesis and autocrine action of TGF-β (57), AT-II stimulates collagen IV transcription (56). AT-II also stimulates mRNA and protein expression of the chemokine RANTES (Regulated

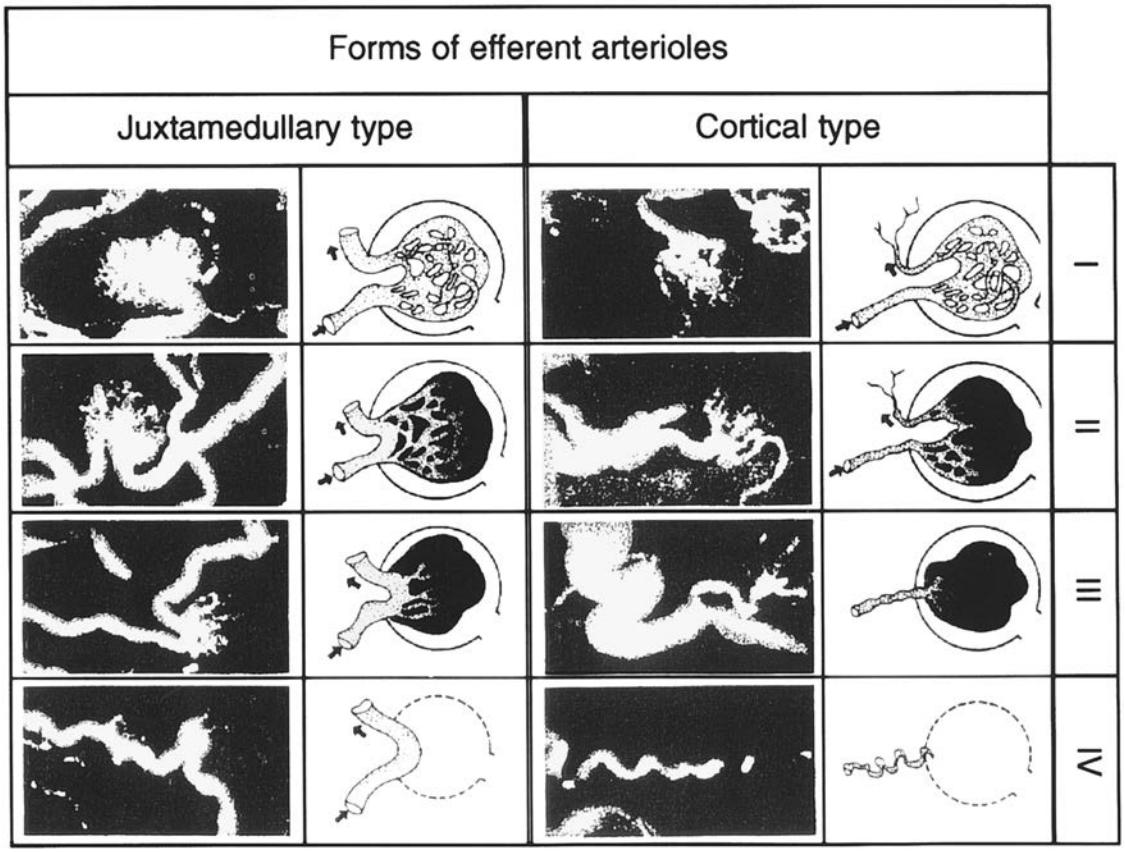

FIGURE 80-3. Progressive (Stages I to IV) vascular simplification and glomerular degeneration of the cortical and juxtamedullary arteriole-glomerular units with corresponding microangiograms. (From: Takazakura E, Sawabu N, Handa A, et al. Intrarenal vascular changes with age and disease. *Kidney Int* 1972;2:224, with permission.)

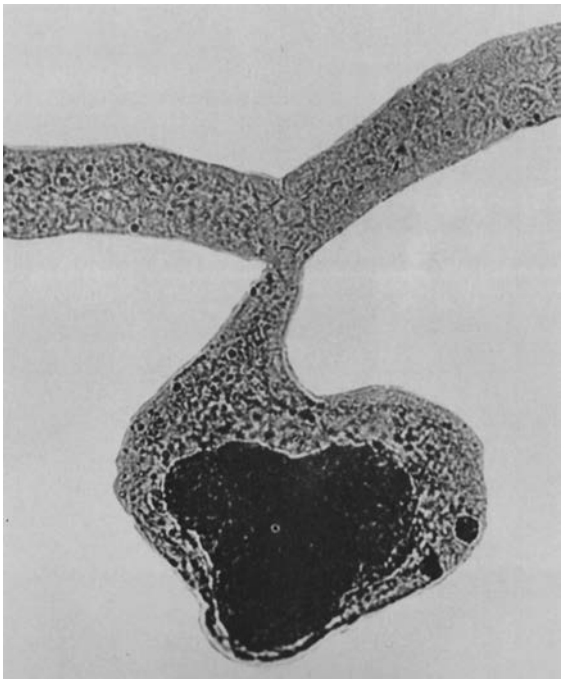

FIGURE 80-4. Microdissection of collecting tubule and associated diverticulum showing continuity between tubular lumen and diverticulum. (From: Baert L, Steg A. Is the diverticulum of the distal and collecting tubules a preliminary stage of the simple cyst in the adult? *J Urol* 1977;113:707, with permission.)

on Activation, Normal T-cell Expressed and Secreted) in endothelial cells and thus promotes monocyte–macrophage influx (58). Transcription of the proinflammatory chemokine monocyte chemoattractant protein-1 (MCP-1) is induced with AT-II inhibition of NO. Enalapril-treated aged rats showed a significant decrease in tubulointerstitial fibrosis and alpha smooth muscle cell-actin when compared with nifedipine-treated aged rats and untreated aged rats with similar blood pressure control (59). By stimulating plasminogen activator inhibitor-1 (PAI-1) from the endothelium (60), AT-II promotes matrix accumulation. Tissue plasminogen activator and urokinase plasminogen activator, involved in matrix proteolysis and fibrinolysis, are inhibited by PAI-1, resulting in matrix accumulation (61). AT-II antagonists showed regression of glomerular and vascular sclerosis and decreased collagen content in treated rats (62). Furthermore AT-II antagonists attenuated the age-associated increase in mitochondrial oxidant production and prevented age-related decrease in the capacity for mitochondrial energy production associated with cellular damage (63).

More recently, AT-II is found to downregulate *Klotho* gene expression. This gene and its protein product, primarily expressed in the kidney, are associated with the suppression of premature aging and arteriosclerosis. AT-II mediated renal morphologic injury is ameliorated in male Sprague-Dawley rats with adenovirus vector-mediated mouse *Klotho* gene transfer (64). In addition *Klotho* mRNA downregulation reversed with the use of AT-II receptor blocker losartan. This was not seen with the use of another antipressor agent, hydralazine (64). These animal experimental data highlight the importance of AT-II-mediated changes in the progression of sclerosis associated with aging, and suggest that angiotensin antagonism may

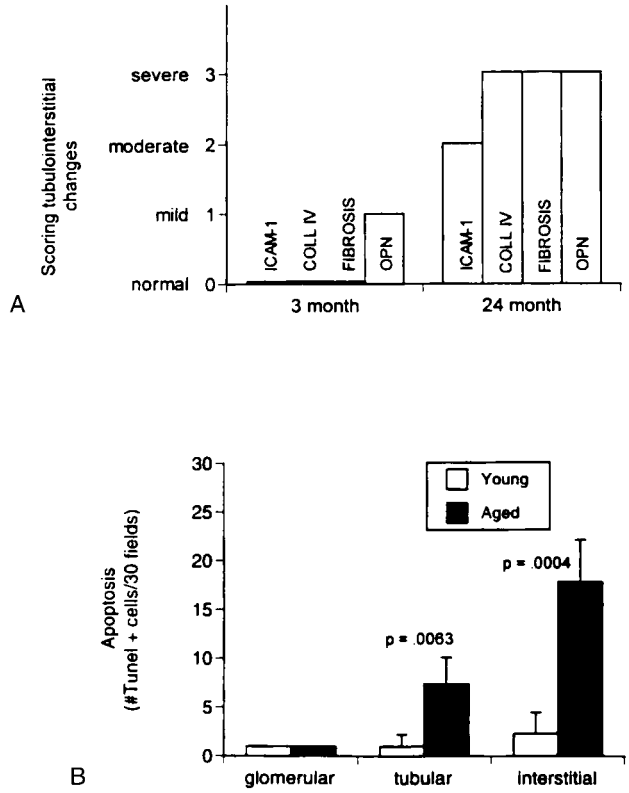

FIGURE 80-5. **A:** Tubulointerstitial fibrosis as well as immunostaining for collagen IV (Coll IV), intracellular adhesion molecule-1 (ICAM-1), and osteopontin (OPN) are increased in aged rats compared with younger rats. **B:** Apoptosis in young and aged rats was quantified by the number of terminal deoxynucleotidyl transferase (Ttd)-mediated dUTP-biotin nick end-labeling (TUNEL) cells in the glomeruli tubules and interstitium. Total number of TUNEL-positive cells/30 fields was 3.5 ± 2.5 in the 3-month-old rat versus 24.5 ± 5.3 in the 24-month-old rat ($p = 0.0001$). (Redrawn from: Thomas SE, Anderson S, Gordon KL, et al. Tubulointerstitial disease in aging: evidence for underlying peritubular capillary damage, a potential role for renal ischemia. *J Am Soc Nephrol* 1998;9:231, with permission.)

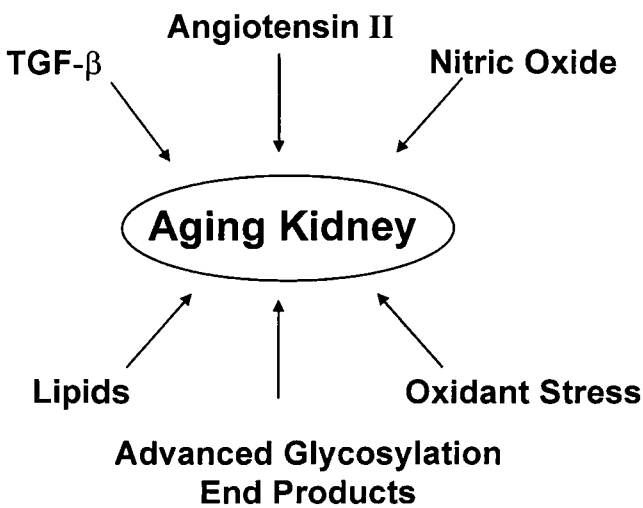

FIGURE 80-6. Factors associated with the pathogenesis of age-related glomerulosclerosis and decline in renal function.

be beneficial in the progression of injury. Specific validation in the aging human kidney is yet to occur.

Transforming Growth Factor-β

TGF-β actively modulates tissue repair, and its role in fibrosis of the aged kidney may be the result of usual or overactive wound healing. Various factors associated with aging in the kidney including AT-II, abnormal glucose metabolism, platelet-derived growth factor (PDGF), hypoxic and or oxidative stress, mesangial stretch, and increased levels of AGE stimulate TGF-β. TGF-β stimulates gene transcription and production of collagens III, IV, and I; fibronectin; osteopontin; osteonectin; thrombospondin; matrix glycosaminoglycans (65); and synthesis of MMP inhibitors that inhibit collagenase (66). The end result is glomerular and tubular scarring from tissue accumulation of extracellular matrix proteins (66–69). Aging rats have increased TGF-β mRNA expression with associated increased levels of TGF-β immunostaining in the renal interstitium (33,69) (Fig. 80-8). Modulating factors such as AT-II by long-term AT-II inhibition are associated with downregulation of TGF-β and decreased interstitial fibrosis (70). Specific studies inhibiting TGF-β expression by antisense oligonucleotides or being able to antagonize TGF-β action or neutralize its function, for instance with decorin, may be useful in defining a clearer role of TGF-β in age-associated sclerosis and subsequent prevention. Relaxin, a small peptide hormone usually produced by the pregnant ovary and prostate, has recently been found to have antifibrotic properties via direct inhibition of transforming growth factor-β (TGF-β) fibroblast stimulation, and results in decreases in collagen I and III. Aged male relaxin knockout mice have an increase in collagen I with thickened cortex, glomerulosclerosis, and a lesser extent of interstitial fibrosis. Female knockout mice for this gene appeared not to be affected. Furthermore, treatment of 12-month male knockout mice with human recombinant relaxin seems to reverse the established renal interstitial fibrosis, glomerulosclerosis, and cortical thickening with a decrease in collagen content (71). Further studies may clarify the utility of this treatment in the progressive fibrosis seen with aging.

Nitric Oxide

Nitric oxide (NO) appears to be intimately related with both sclerosis and changes in renal perfusion with aging. By its ability to inhibit NFB in the presence of reactive oxygen intermediates, NO can prevent MCP-1-associated monocyte–macrophage influx inflammatory injury (72,73). NO levels are decreased in aged rats when the stable oxidation products, nitrites and nitrates, are quantified (74,75). Endothelial NO (eNOS) expression is also diminished in aged rat peritubular capillaries. Loss of eNOS expression is found to lead to chronic tubulointerstitial ischemia and fibrosis (76). Furthermore L-arginine given to aged rats over a long period significantly improved RPF and GFR, while decreasing proteinuria and glomerulosclerosis (Fig. 80-9) (77). L-Arginine supplementation has also been shown to decrease kidney collagen and N-ϵ-(carboxymethyl)lysine accumulation (79). Recent studies in rats indicate oxidant stress mediated NO scavenging via NADPH oxidase to contribute to NO depletion in aged kidneys (78). Although the specific cause of decreased NO levels in the aging kidney is unknown, interplay with increased AT-II activity, hypoxia, and increased oxidative stress, higher AGE levels and dietary protein intake may contribute (80–83). Urinary NO excretion is found to increase and normalize with use of either AT-II antagonists or dietary protein restriction or both (75).

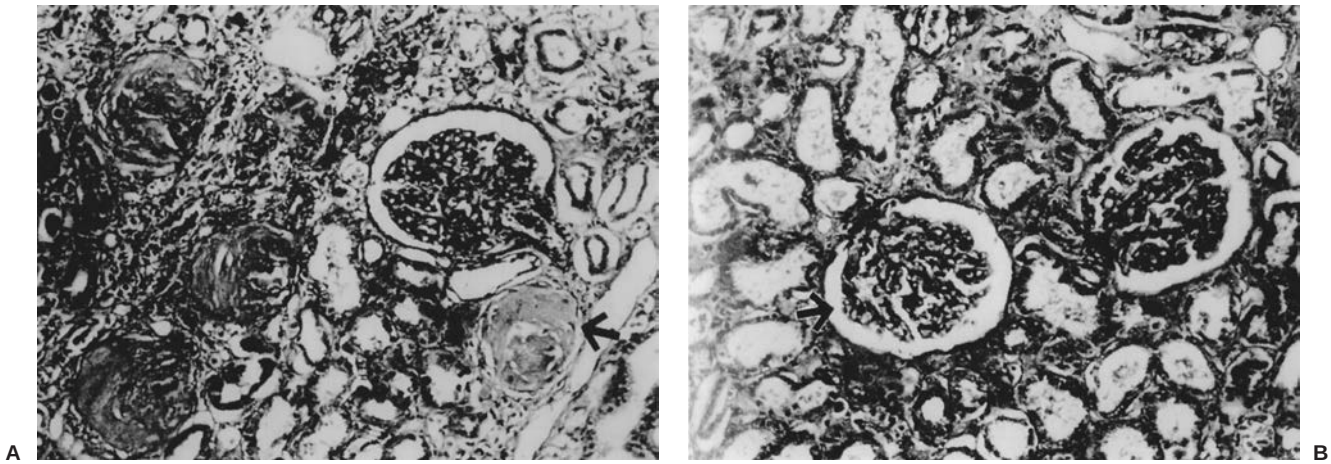

FIGURE 80-7. Angiotensin-converting enzyme inhibitor (enalapril) decreases glomerulosclerosis in aged mice. **A:** Animals treated with placebo. **B:** animals treated with enalapril. (From: Ferder L, Inserra F, Romano L, et al. Decreased glomerulosclerosis in aging by angiotensin-converting enzyme inhibitors. *J Am Soc Nephrol* 1994;5:1147, with permission.)

Advanced Glycosylation End Products

Progressive accumulation of cross-linked glycoxidated proteins, lipids, and nucleic acids during aging injure the vascular bed and renal tissue (84,85). Hyperglycemia accelerates this process (86). AGEs decrease vascular elasticity and increase monocytic chemotactic activity via AGE-receptor ligand binding, thereby promoting endothelial permeability with subsequent macrophage activation, growth factor secretion, and cytokine generation. NO-mediated vasodilation may be affected by possible chemical inactivation with accumulated AGE in the endothelium and basement membrane (87–91). AGE-related changes in the endothelium mimic those seen in diabetic patients. AGE and AGE-receptors (RAGEs) also accumulate in the mesangium and basement membrane of aged animal kidneys with associated increase in vascular permeability and induction of platelet-derived growth factor (PDGF) and TGF-β leading to glomerulosclerosis and tubulointerstitial fibrosis (86). A number of factors including an age-associated fall in GFR, increased oxidative stress leading to oxidative modification of glycated proteins and N-ϵ-(carboxymethyl)lysine, and increased insulin resistance causing abnormal glucose metabolism and protein glycation can increase both AGE and RAGE in the aged kidney. Lifelong smoking of tobacco and eating of AGE-enriched food substances may also contribute to increased AGE load and accumulation in tissues (92,93).

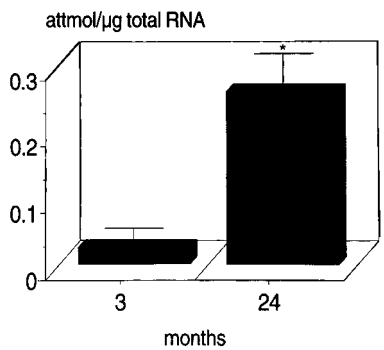

FIGURE 80-8. Quantitative analysis of the amount of transforming growth factor-1 (TGF-1) mRNA in two groups of rats, 3 months and 24 months of age. Results expressed as mean ± SEM of 10 animals, $^*p < 0.05$ versus 3-month-old-rats; attmol/μg total RNA = attomolar TGF-1 cDNA concentration per milligram of total RNA. (Redrawn from: Ruiz-Torres MP, Bosch R, O'Valle F, et al. Age-related increase in expression of TGF-β1 in the rat kidney: relationship to morphologic changes. *J Am Soc Nephrol* 1998;9:782.)

The pathologic role of AGE in age-related renal and vascular disease is strengthened by animal studies of aged rats and rabbits treated with aminoguanidine. Aminoguanidine appears to decrease proteinuria and glomerulosclerosis (94) (Fig. 80-10) as well as arterial stiffening and cardiac hypertrophy with long-term treatment in aged rats (96). Furthermore reversal of the increased vascular permeability and defective vasodilatory response to acetylcholine and nitroglycerin, as well as prevention of mononuclear cell migration can be seen in the subendothelial and periarteriolar spaces in various tissues of rats and rabbits treated with aminoguanidine (95). When a 60% calorie restriction is imposed on Brown-Norway female rats, there is decreased burden of AGE, N-ϵ-(carboxymethyl)lysine and pentosidine (97,98). There was a significant decrease in collagen AGE content in glomeruli, kidneys, and abdominal aorta of aged 30-month-old lean WAG/Rij rats on 30% calorie restriction versus 30-month-old aged counterparts on *ad libitum* diet (7 g per day). (Fig. 80-11) (99).

Preliminary animal studies indicate a possible role for aminoguanidine treatment in decreasing AGE-associated glomerulosclerosis and tubulointerstitial fibrosis. Further future studies in humans will need to clarify any true therapeutic outcomes.

Oxidative Stress

Increased free radical generation and or a deficiency of antioxidant enzymes in aging renal tissue leads to increased oxidative stress and lipid peroxidation culminating in tissue injury (100–103). Increased excretion of urinary oxidized amino acids suggests higher skeletal muscle oxidized proteins in aged rats (104). Aged kidneys also demonstrate increased levels of reactive oxygen species and thiobarbituric acid reactive substance (TBARS), markers of lipid oxidative damage (105). In a study of aged rats, increased markers of oxidative and lipid peroxidation, isoprostanes, AGE, RAGE, and heme oxygenase induction not only improved with antioxidant treatment, high-dose vitamin E, but also these rats showed significant improvement in RPF, GFR, and decreased glomerulosclerosis (106). AT-II can activate membrane-bound NADP/NADPH oxidase

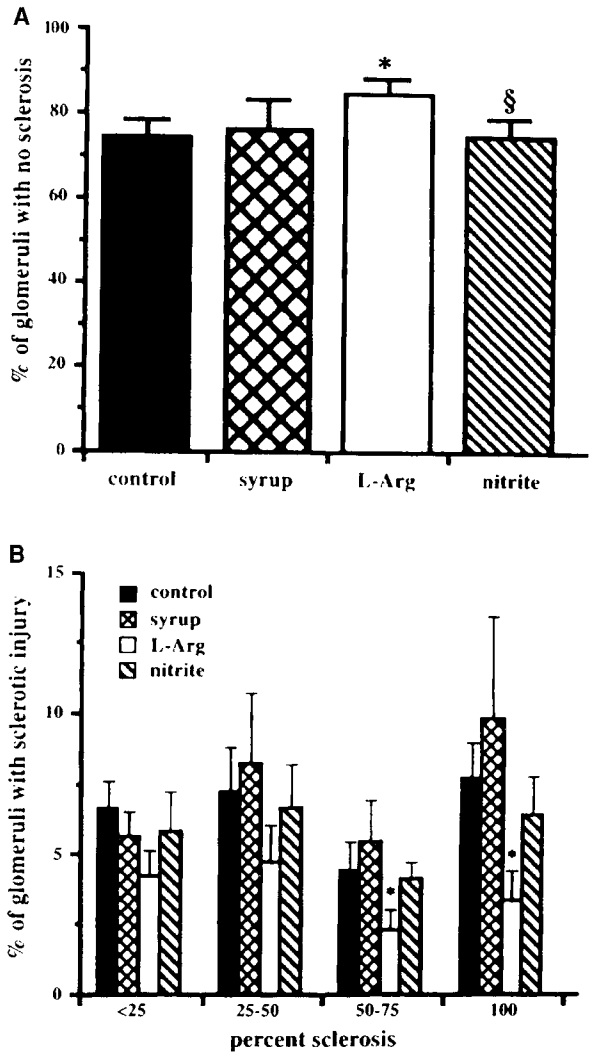

FIGURE 80-9. Morphologic examination of kidneys from aging rats. **A:** Average percentage of glomeruli per kidney with no sclerosis. $^*p < 0.05$ compared with untreated controls; $\S p < 0.05$, nitrite-treated versus L-arginine-treated groups. **B:** Average percentage of glomeruli per kidney with graded sclerotic injury. $^*p < 0.05$, L-arginine-treated rats versus other groups. (From: Reckelhoff JF, Kellum JA, Racusen LC, et al. Long-term dietary supplementation with L-arginine prevents age-related reduction in renal function. *Am J Physiol* 1997;272:R1768, Figure 3, with permission.)

to generate superoxide anions (107). Conversely, ACE inhibitor increases antioxidant enzyme activity (108). Aged Fisher 344 rats given Tempol, a superoxide radical scavenger, had restored ability to respond to AT-II receptor blocker, enalaprilat, to suppress oxygen consumption in renal cortical tissue, which is NO mediated (78) (Fig. 80-12). Furthermore, apocynin, an NADPH oxidase inhibitor, also restored the action of enalaprilat to suppress oxygen consumption in these animals (78). This suggests that decreased NO availability in the aged kidney may be secondary to destruction by superoxide produced from NADPH oxidase activity (78). TGF-β also stimulates reactive oxygen species in cultured mesangial cells, which is blocked with antioxidant taurine and ACE inhibitor (109). Oxidative stress in aging rat kidneys can also enhance mitogen-activated protein kinase (MAPK) cellular signaling pathways, including extracellular signal-related kinases (ERKs), C-Jun NH2-terminal kinases (JNKs), and p38 MAPK (110). Caloric restriction suppresses age-related oxidative stress (112) and activation of MAPK in the aging rat kidney. Also caloric restriction in aged rats appears to decrease mitochondrial lipid peroxidation and membrane damage and decrease apoptosis in the rat kidney (111). Therefore, dietary discrimination including caloric restriction, antioxidant therapy, and AT-II inhibition may be future therapeutic targets to delay age-associated renal sclerosis.

Lipids

Cholesterol is found to accumulate in a number of tissues, including the kidney, and may lead to the progression of glomerulosclerosis and proteinuria in several diseases including diabetes (113–124). Modified low density lipoproteins (LDLs), and lipoprotein (a) levels increase with higher levels of oxidative stress and AGE in aging (125–127). These lipid products can further induce oxygen free radicals, growth factors including PDGF and TGF-β, monocyte migration and adherence, mesangial and vascular cell growth, as well as inhibit NO generation. These changes can then lead to renal disease progression. In fact, high cholesterol feeding of type-2 diabetic rats enhances lipooxidative stress and induces glomerulosclerosis and tubulointerstitial fibrosis (128). Treating streptozotocin-induced diabetic rats with 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors for 12 months markedly improved glomerular volume and urinary albumin excretion compared to untreated rats (129). Both diabetic and nondiabetic patients seem to have a decrease in proteinuria and partial GFR preservation with long-term HMG-CoA reductase use and or peroxisome proliferator-activated receptor-alpha (PPAR-alpha) agonists (130–132). Investigations in aging humans are yet to be conducted.

FUNCTIONAL CHANGES IN THE AGING KIDNEY

Structural changes obviate various functional changes of the aging kidney, which may be subtle and unrecognized until the kidney is pushed to maximum functional capacity under physiologic need. Awareness of these functional changes allows for forethought in the appropriate management of elderly patients.

Renal Plasma Flow

Changes in the aging renal vasculature are associated with a decreased renal blood flow. Renal plasma flow (RPF) evaluated by para-aminohippurate (PAH) decreases by 50% from 600 mL/minute/1.73 m^2 during the third decade to 300 mL/minute/1.73 m^2 by the ninth decade (133–135) (Fig. 80-13). The rate of change is noted to be approximately 10% per decade (132–133). Healthy donor xenon washout scans indicate a preferential decrease in cortical blood flow and preservation of medullary blood flow with increasing age from 17 to 76 years of age, which parallels histologic changes (136). An age-related fall in cardiac output may explain the change in RPF (136); however, this has not borne out in the face of careful study (137,138). Changes in the anatomic and vascular responsiveness associated with renal aging may be the reason for the decrease in the renal fraction of the cardiac output (139).

Vascular reactivity changes in both aging humans and animals. When the ability to vasodilate is tested with intravenous infusion of vasorelaxants such as pyrogen (134) or atrial natriuretic peptide (140) or intraarterial acetylcholine (136) in elderly subjects, RPF increases; however, the vasodilation

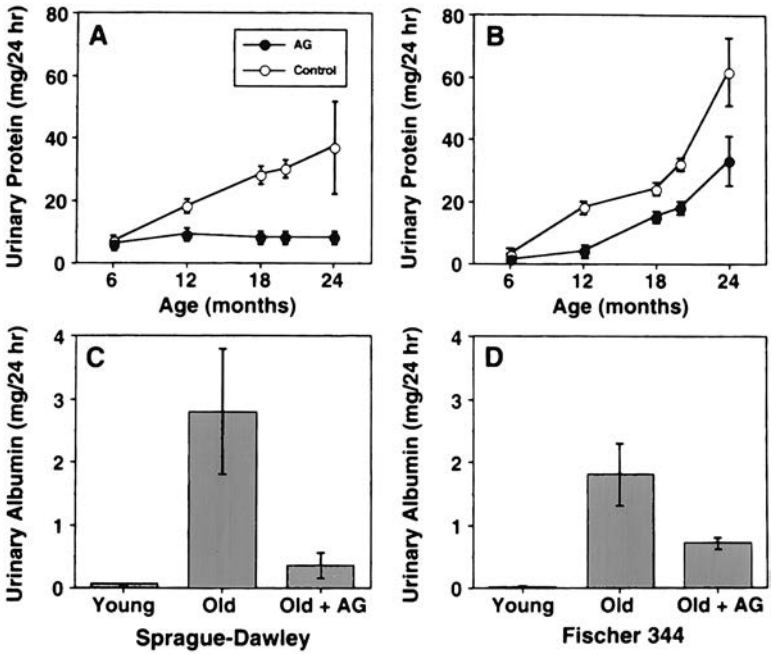

FIGURE 80-10. Age-related urinary protein (**A and B**) and albumin loss (**C and D**) in the rat is suppressed by aminoguanidine (AG). Total urinary protein concentration was determined in 24-hour urine collected every 4 months over an 18-month period from S-D (A) or F344 (B) rats treated with AG (0.1% in drinking water) (●) or untreated, age-matched controls (○). Albuminuria was determined at baseline and at the end of the study in S-D (C) or F344 (D) rats. Data are expressed as means ± SEM). Comparisons (old vs young; old vs old + AG) of all experimental groups of both strains were significant at $p < 0.05$ (N= 5 to 7 rats per group). (Donnelly T, et al. Prevention of cardiovascular and renal pathology of aging by the advanced glycation inhibitor aminoguanidine. *Proc Natl Acad Sci U S A* 1996;93:3902.)

remains blunted when compared to younger subjects. Amino acid infusion increases GFR and filtration fraction but does not affect RPF (141). With maximal relaxation of the renal vascular bed using combined dopamine and amino acid infusion to healthy elderly and young kidney donors, both RPF and GFR increase significantly, although the response is less in the elderly, and filtration fraction stays the same (141).

Changes in intracellular signaling in aged kidneys may be the reason for the impaired vasorelaxation that is seen (142). Renal sympathetic mediated vasoconstriction is exaggerated with inadequate response of cyclic adenosine monophosphate (cAMP) to β-adrenergic agonists or altered response to cyclic guanosine monophosphate (cGMP) to atrial natriuretic peptide (ANP) (143,144). Other vasomediators such as vasodilatory prostacyclin (PGI_2) are decreased in the aged rats kidneys and human vasculature when compared to the vasoconstrictive thromboxanes (145,146). Aging subjects also excrete less vasodilatory natriuretic prostaglandins such as PGE_2 (147).

The aging renal vasculature appears to exhibit a hyperresponse to AT-II mediated vasoconstriction as ACE inhibition produces an exaggerated vasodilation in the elderly (148,149). Aged rats treated with ACE inhibitor and angiotensin receptor blockers in fact have a marked increased in RPF and GFR (48). However intraarterial angiotensin produces an identical vasoconstrictive response in young and older human subjects (136). With blunted vasodilation to amino acid infusion and possible exaggerated response to ACE inhibition, but normal vasoconstriction with angiotensin infusion, the aging kidney may already be compensating for underlying sclerosis to maintain adequate renal function.

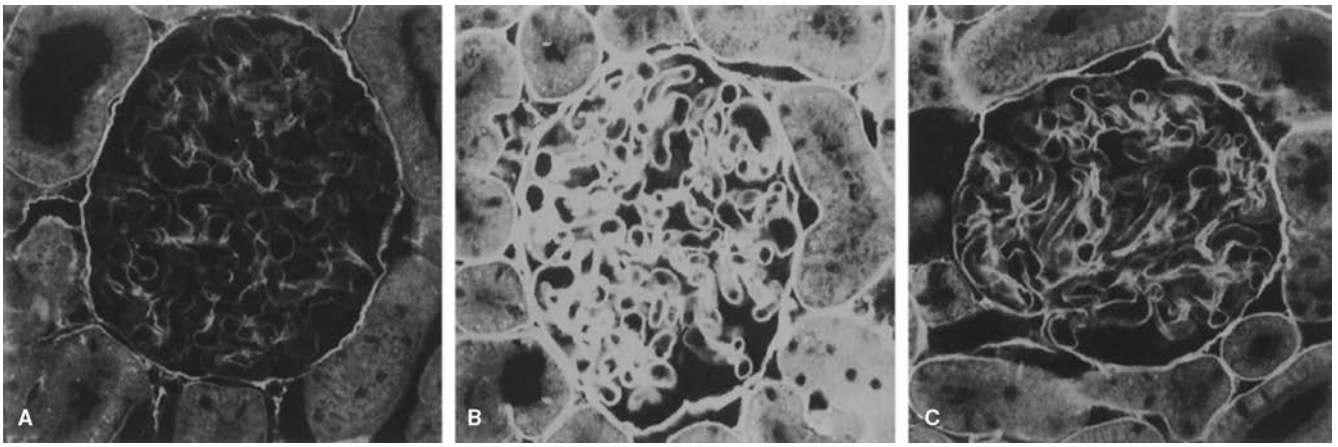

FIGURE 80-11. Immunolocalization of advanced glycosylation and products (AGE) in the renal cortex of 10-month-old (**A**) and 30-month-old (**B**) female WAG/Rij rats fed *ad libitum*, and 30-month-old animals food-restricted by 30% (**C**). AGE localized predominantly in extracellular matrix. Increased AGE accumulation was evident in tubular basement membranes, mesangial matrix, glomerular basement membranes, and Bowman's capsule between 10 and 30 months in rats fed *ad libitum*. Such accumulation was mostly prevented in food-restricted animals. (Magnification ×350.) (From: Teillet L, Verbeke P, Gouraud S, et al. Food restriction prevents advanced glycation end products accumulation and retards kidney aging in lean rats. *J Am Soc Nephrol* 2000;11:1488, with permission.)

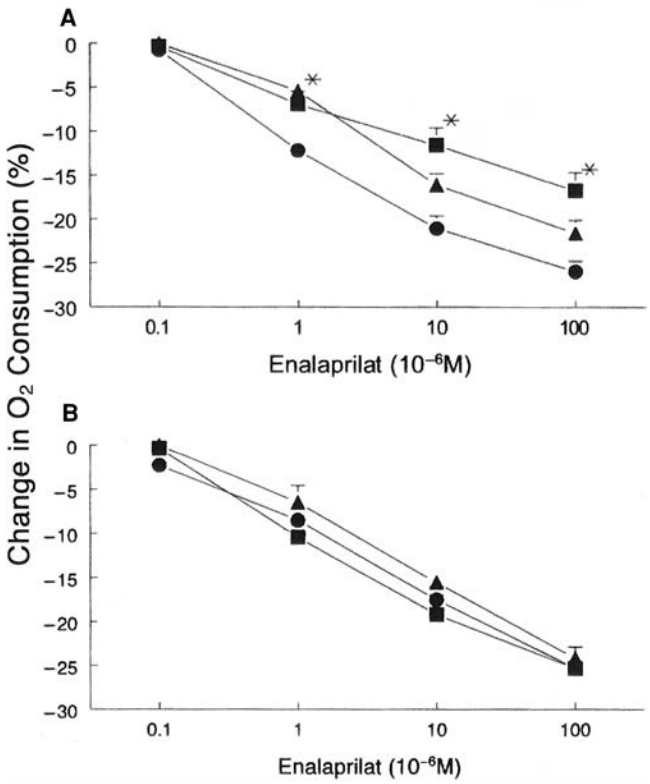

FIGURE 80-12. Effect of enalaprilat (**A**) on NO-mediated renal cortical O_2 consumption and Tempol (**B**) superoxide radical scavenger, on suppression of O_2 consumption by NO agonist enalaprilat consumption in 4-month (*circles*), 13-month (*triangles*), and 23-month-old (*squares*) Fischer 344 rats ($N = 6$ per group, $p < 0.05$). (Redrawn from: Adler S, Huang H, Wolin MS, et al. Oxidant stress leads to impaired regulation of renal cortical oxygen consumption by nitric oxide in the aging kidney. *J Am Soc Nephrology* 2004;15:52.)

Glycine infusion into aged groups of Sprague-Dawley rats exhibits a similar response in RPF as seen in humans and matches the histologic changes of progressive glomerulosclerosis (150). Renal vascular resistance (RVR increases significantly and RPF decreases in the aged Sprague-Dawley rats versus younger counterparts with L-NAME (N-nitro-L-arginine-methyl-ester), an L-arginine analog that competitively inhibits the formation of endothelium-derived relaxing factor (EDRF) (151). Micropuncture studies of young and older Sprague-Dawley rats does not show any change in the magnitude of pressor and vasoconstrictive response with AT-II infusion. Both preglomerular and efferent vessels increase arteriolar resistance with AT-II with a subsequent decrease in RPF and glomerular plasma flow, and increase in filtration fraction in both young and older rats. The glomerular capillary ultrafiltration coefficient (Kf) decreases in the older rats with a fall in GFR and single nephron GFR (SNGFR). The same is not seen in the younger rats in which there is no change in Kf, GFR, or SNGFR with A-II. Glomerular mesangial cell contraction with AT-II and a drop in filtration surface area may possibly explain these findings (152). Combining morphometric analysis with mathematical modeling of healthy renal transplant donors age 23 to 69 years to evaluate Kf in two kidneys and in individual glomeruli, Hoang et al. suggest that the drop in Kf in humans with age results from underlying structural changes lowering single nephron Kf in addition to the decrease in the number of functioning glomeruli (135) (Fig. 80-14).

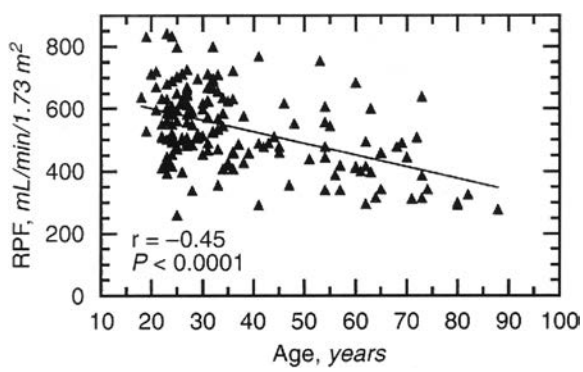

FIGURE 80-13. Renal plasma flow (RPF) as a function of age. (Redrawn from: Hoang K, Tan JC, Derby G, et al. Determinants of glomerular hypofiltration in aging humans. *Kidney Int* 2003;64:1417.)

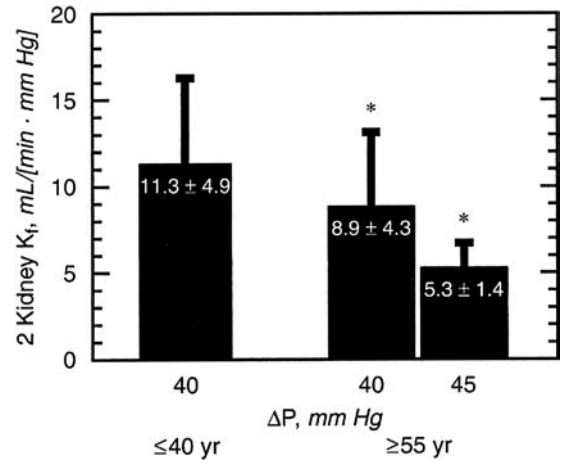

FIGURE 80-14. Two-kidney ultrafiltration coefficient (Kf) for $\Delta P = 40$ mm Hg in youthful (<40 years) and $\Delta P = 40$ or 45 mm Hg in aging subjects (≥55 years). *$p < 0.005$ vs. youthful. (Reprinted from: Hoang K, Tan JC, Derby G, et al. Determinants of glomerular hypofiltration in aging humans. *Kidney Int* 2003;64:1417.)

Although there is a linear decrease in RPF with age, filtration fraction seems to increase (135). Because juxtamedullary nephrons inherently have higher filtration fraction than cortical nephrons, preserved medullary plasma flow and decreased cortical plasma flow may help explain this finding.

Glomerular Filtration Rate

With renal senescence, there is an expected decrease in GFR; however, the rate of decline can vary in aging individuals according to various factors including measurement criteria, gender, race, genetic differences, and other underlying risks of renal dysfunction. A decrease in urea clearance with aging was noted early on in 1938 by Lewis and Alving (153), which was later confirmed by other measures of clearance including inulin, creatinine, and iothalamate (133,135,154) (Fig. 80-15). By creatinine clearance, the rate of decline in GFR is noted to be approximately 0.8 mL/minute/1.73 m^2 per year, with 140 mL/minute/1.73 m^2 at age 40 years to 97 mL/minute/1.72 m^2 at age 80 years (155). This decrease is evident even when the variability of protein intake on creatinine clearance is considered (156). Iohexol clearance suggests a rate of decline of 1.0 mL/minute/1.73 m^2 (157). Healthy normotensive elderly subjects on a 1 g/kg per day protein diet have inulin clearances of

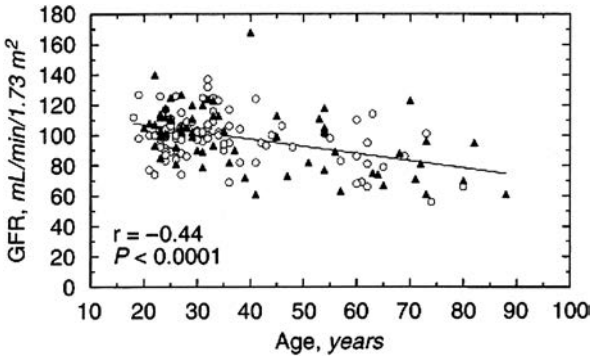

FIGURE 80-15. Glomerular filtration rate (GFR) plotted as a function of age in 164 healthy individuals. Symbols are (○) males, (▲) females. (Reprinted from: Hoang K, Tan JC, Derby G, et al. Determinants of glomerular hypofiltration in aging humans. *Kidney Int* 2003;64:1417.)

100 mL/minute/1.73 m^2, which is lower compared to similar younger subjects (158).

Because muscle mass also decreases with age, a rise in serum creatinine is not evident with increasing age despite a decrease in GFR. Therefore, the use of serum creatinine (Scr) as a surrogate to estimate GFR in the elderly often overestimates the true creatinine clearance. This becomes important in dosing medications and in the assessment of risk to the aged kidney from toxic, metabolic, and ischemic events. Formulas frequently used can also underestimate and overestimate GFR in the elderly (Table 80-1) (159–164). The Third National Health and Nutritional Examination Survey data of 15,625 individuals ages 20 and older compares a commonly used formula, Crockcroft-Gault, with previous inulin clearance in healthy men to examine this relationship (Fig 80-16) (6). Formulas derived from the Modification of Diet in Renal Disease (MDRD) study may also be useful in evaluating creatinine clearance in the elderly with underlying renal disease (equations 3 and 4 in Table 80-1) (162,163). The use of the original MDRD equation to calculate GFR in patients with chronic kidney disease has been recommended by the National Kidney Foundation and may be used to calculate GFR in many laboratories (163). More recently, a quadratic GFR equation (equation 5 in Table 80-1) was developed using serum creatinine and age and validated with iothalamate GFR clearances in both healthy individuals and in patients with chronic kidney disease (164). However, this equation will need to be validated in the elderly and African American population, groups that are underrepresented in this study. Although the use of these formulas can be useful for estimating renal function in the elderly, appropriate medication dose adjustment and drug level monitoring are recommended with medications dependent on renal excretion or metabolism. Actual measurement of creatinine clearance is best when it can be done with a 24-hour urine collection; however, variability in the urine collection from diet or other factors can occur. Newer methods to estimate GFR using serum cystatin C and serum 2-(alpha-mannopyranosyl)-L-tryptophan (MTP) may provide more accurate estimates of GFR in the elderly but are not yet widely available (165,166). Radionuclide clearance with either technetium-99m diethylenetriamine-pentaacetic acid (99mTc-DTPA), 125-iothalamate, or radiocontrast clearance with iohexol x-ray fluorescence analysis (154) may be more accurate; however, expense, radioactive exposure, and test availability limit their use as routine clearance measurement techniques.

Whether gender differences in GFR affect the rate of decline is not clear. Cross-sectional studies seem not to suggest a more gradual decline in women than men (Fig. 80-15).

However, both racial and genetic differences can affect the rate of decline of GFR in the elderly. Elderly African Americans have a faster decline in creatinine clearance in comparison to elderly caucasians (167). Similarly elderly Japanese when compared to caucasian Americans also appear to have a greater rate of decline, possibly due to an increase in nephrosclerosis (11).

The presence of underlying factors including hypertension and heart failure may also affect the rate of decline in GFR. A comparison of elderly normotensive, hypertensive, and subjects with heart failure with younger subjects finds that GFR, RPF, and RVR decrease in all elderly groups, with greater decreases among those with heart failure (158). This study did not suggest a greater decline in hypertensive subjects compared to normotensive; however, elderly hypertensives requiring medication when compared to those not requiring medical

TABLE 80-1

COMMONLY USED FORMULAS TO ESTIMATE GFR

1. Creatine clearance [mL/minute/1.73 m^2] = [1.33 − 0.64] × age[a]
2. Creatine clearance [mL/minute] = $\frac{[140 - \text{age}] \times \text{weight [kg]}}{72 \times \text{serum creatinine [mg/dL]}^a}$
3. $\text{GFR} = 170 \times [P_{cr}]^{-0.999} \times [\text{age}]^{-0.0176} \times [0.762 \text{ if patient is female}] \times [1.180 \text{ if patient is black}] \times [\text{SUN}]^{0-0.0170} \times [\text{alb}]^{+0.318}$
4. Original MDRD study equation:
   GFR [ML/minute/1.73 m^2] = $186 \times [S_{cr}]^{-1.154} \times [\text{age}]^{-0.203} \times [0.742 \text{ if female}] \times [1.210 \text{ if African American}]$
5. Quadratic GFR equation:
   $\text{GFR} = \exp[1.911 + \frac{5.249}{S_{cr}} - \frac{2.114}{[S_{cr}]^2} - 0.00686 \times \text{age} - 0.205 \text{ [if female]}$ if $S_{cr} < 0.8$ mg/dL, use 0.8 mg S_{cr}

[a] 15% less in females.
Formula 1 from: Rowe JW, Andrew R, Tobin JD, et al. Age-adjusted standards for creatinine clearance. *Ann Intern Med* 1976;84:567.
Formula 2 from: Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976;16:31.
Formula 3 from: Levey AS, Bosch J, Lewis JB, et al. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. *Ann Intern Med* 1999;130[6]:461.
Formula 4 from: Levey AS, Coresh J, Balk E, et al. National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Ann Intern Med* 2003;139:137.
Formula 5 from: Rule AD, Larson TS, Bergstralh EJ, et al. Using serum creatinine to estimate glomerular filtration rate: accuracy in good health and in chronic kidney disease. *Ann Intern Med* 2004;141:929.

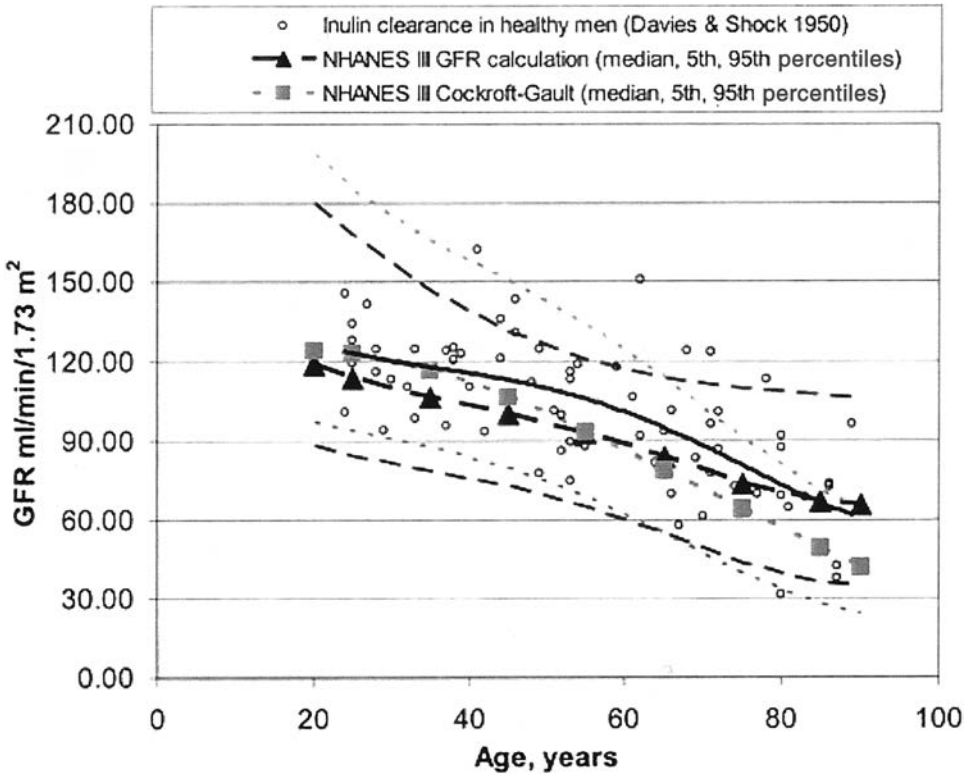

FIGURE 80-16. Percentiles of glomerular filtration rate (GFR) and Crockcroft-Gault creatinine clearance (Ccr) by age, plotted on the same graph as data by Davies and Shock on inulin clearance in healthy men. Percentiles are calculated using a fourth order polynomial weighted quantile regression. *Solid line* shows a polynomial regression to the inulin data. *Dashed lines* without symbols show the 5th and 95th percentiles for GFR estimates. (Reprinted from: Coresh J, Astor BC, Greene T, et al. Prevalence of chronic kidney disease and decreased kidney function in the US population: Third National Health and Nutrition Examination Survey. *Am J Kidney Dis* 2003;41:1.)

treatment also have lower GFRs and higher RVRs. Other studies have shown an age-associated decrease among hypertensive subjects (168,169,170,171). Intraglomerular hypertension may be hastening this decline (168,169). Aged spontaneously hypertensive rats show a functional decline in preglomerular RVR, which leads to glomerular hypertension and significant vascular or glomerular injury (171). Other systemic processes common in the elderly such as atherosclerotic vascular disease, diabetes mellitus, and abnormal lipid metabolism can add to a functional decline in GFR. A decline in GFR is seen in the elderly with impaired glucose intolerance and essential hypertension (172).

Tubular Functional Changes

Ability to Conserve Sodium

With aging, the tubules exhibit a diminished capacity to conserve sodium in the face of salt deprivation. Healthy aged subjects need twice as long to decrease urine sodium excretion with salt restriction than younger controls. With salt intake abruptly decreased to 10 mmol/day, younger 30 year-old individuals take 17.6 hours to minimize urinary sodium excretion, whereas older 60-year old individuals take 30.9 hours (173) (Fig. 80-17). Clearance studies point to a decrease in distal tubular sodium reabsorption (174). This may not be surprising given renal interstitial scarring and fewer nephrons, which can increase solute load per nephron such as that seen in chronic renal failure.

In addition, changes in the levels and response to factors such as renin, angiotensin, and aldosterone, which regulate salt excretion, are seen and may explain the changes seen in the aging kidney. Despite normal renin substrate, basal plasma renin is 30% to 50% lower in the elderly, and differences in plasma renin activity between young and old become more pronounced with stimuli known to increase renin activity such as upright

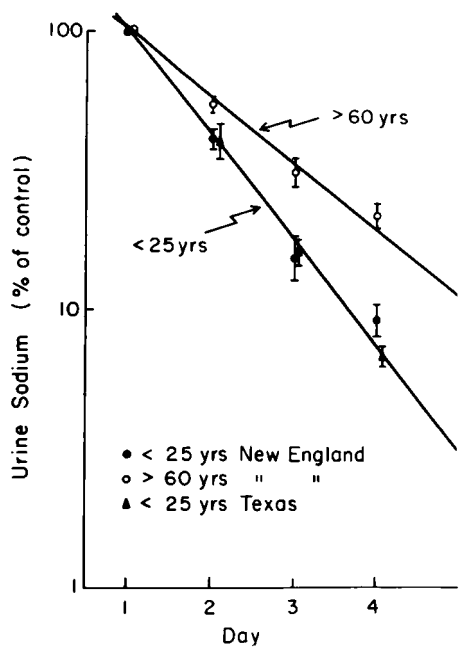

FIGURE 80-17. Response of urinary sodium excretion to restriction of sodium intake in normal humans. The mean half-time ($t_{1/2}$) for eight subjects older than 60 years of age was –30.9 ± 2.8 hours, exceeding the mean half-time of –17.6 ± 0.7 hours for subjects younger than 25 years of age (p <0.01). When the subjects younger than 25 years of age were separated according to geographic area, the mean half-time for the Texas group (>17.9 ± 0.7 hours) was similar to that of the New England group (>17.9 ± 1.4 hours; p <0.3). (Reprinted from: Epstein M, Hollenberg N. Age as a determinant of renal sodium conservation in normal man. *J Lab Clin Med* 1976;87:411, with permission.)

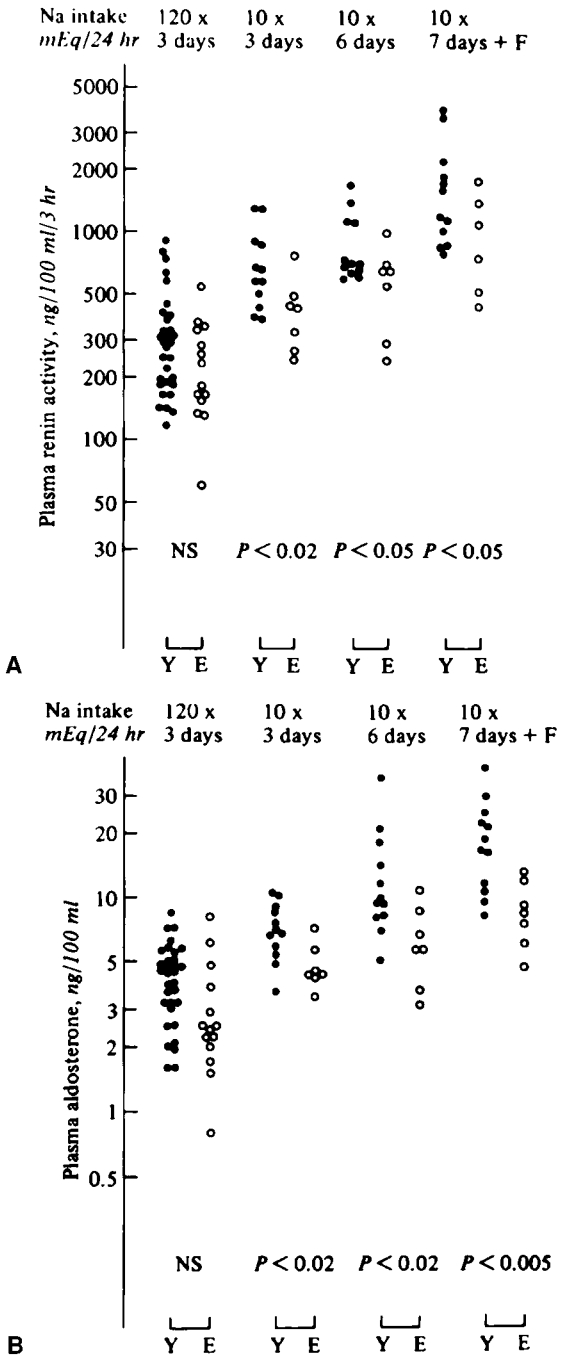

FIGURE 80-18. Distribution of individual supine plasma rennin (A) and aldosterone values (B) before and during progressive sodium depletion in young (Y) and elderly (E) healthy subjects. Values indicating statistical significance refer to differences between young and elderly subjects. Plasma renin activity values are those obtained at an incubation pH of 5.7. (Reprinted from: Weidmann P, De Chatel R, Schiffmann A, et al. Interrelations between age and plasma rennin, aldosterone, and cortisol, urinary catecholamines, and the body sodium/volume state in normal man. *Klin Wochenschr* 1977;55:725, with permission.)

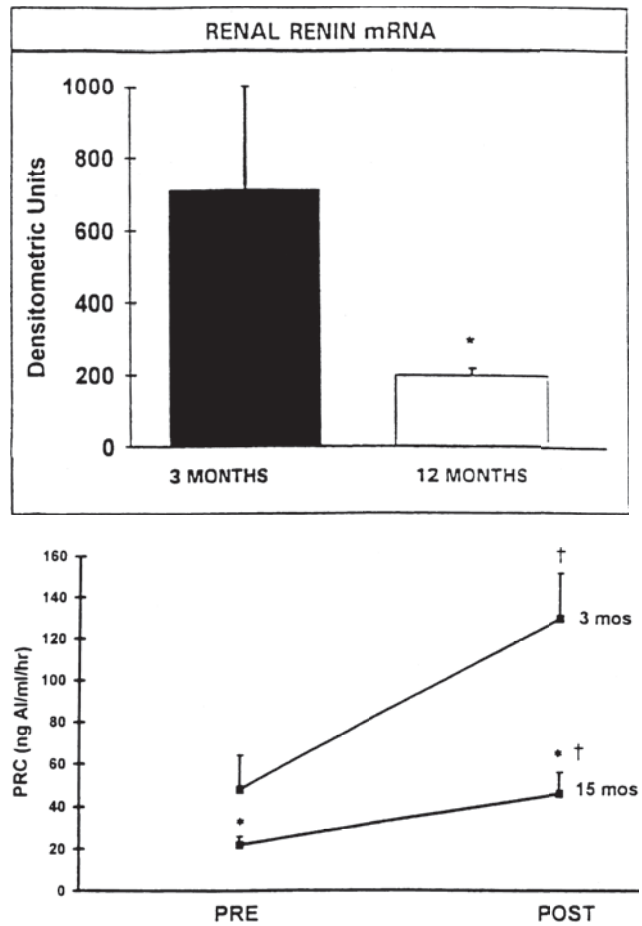

FIGURE 80-19. **Top:** Northern blot analysis of renin mRNA. Renal renin mRNA is significantly decreased in older (12 months) versus younger (3 months) rats. $^*p < 0.05$. **Bottom:** Prehemorrhage (Pre) and posthemorrhage (Post) values for plasma renin content (PRC) revealed significantly lower levels in older rats, as well as a smaller increase after hemorrhage. $^*p < 0.05$ versus young in same period; $^\dagger p < 0.05$ versus prehemorrhage. (Reprinted from: Jung FF, Kennefick TM, Ingelfinger JR, et al. Down-regulation of the intrarenal renin-angiotensin system in the aging rat. *J Am Soc Nephrol* 1995;5:1573, with permission.)

position, 10 mEq/day sodium intake, furosemide use, air-jet stress, pre- and post-hemorrhage (173–184) (Fig. 80-18). Salt-depleted aged rats with a decrease in mean arterial pressure also have a blunted rise in plasma renin activity and delayed decrease in urinary sodium excretion (187). Aging rats have a decrease in juxtamedullary nephron renin content (181), down-regulation of renin mRNA abundance, and decreased ACE levels (186), as well as a decrease of AT-II type 1 receptor mRNA expression (188). Rate of conversion of inactive to active renin is also decreased in healthy aging adults (184). Therefore, both renin synthesis and release likely contribute to decreased renin content in the elderly.

Plasma renin content pre- and post-hemorrhage in 15-month-old rats both before and after hemorrhage are significantly lower than that in 3-month-old rats (186) (Fig. 80-19). With sodium deprivation and a fall in mean arterial pressure, aged rats have a blunted increase in plasma renin activity and delayed decrease in urine sodium excretion (187). Older aging adults also seem to convert less plasma renin substrate from inactive to active renin (182). Therefore, both decreased renin synthesis and impaired renin release may in part provide an explanation for decreased renin content in the elderly.

Aldosterone levels also decrease by 30% to 50% in the supine elderly compared to younger subjects in relative to plasma renin, the difference again exaggerated with upright posture, sodium restriction, and furosemide use (185,189–191). That this is related to a decrease in renin–angiotensin deficiency rather than an intrinsic adrenal defect is demonstrated by a normal aldosterone and cortisol response to adrenocorticotropic hormone infusion in elderly subjects (185). Blocking

the renin–angiotensin–aldosterone system with ACE inhibitors reproduces the slow renal response to dietary sodium restriction (192). When aldosterone is infused into healthy elderly, there is marked improvement in sodium conservation, suggesting appropriate tubular sensitivity (193). Therefore, decreased ability for sodium conservation in the elderly may be the result of a deficiency of renin–angiotensin, or aldosterone, or both.

Ability to Excrete Sodium

The older kidney excretes a sodium load much slower than do younger kidneys (194–196). When 2 L normal saline is infused into adults older than 40 years in comparison to those younger than 40 years matched for sex, age, and race, the 24-hour urine sodium is significantly decreased (167,197) (Fig. 80-20). Circadian variation with increased age promotes a greater percentage of the salt load to be excreted at night (197). Clinically then older adults have a greater propensity for volume overload and nocturia.

Although the age-related decrease in GFR may also influence the decrease in sodium excretion seen, other factors such as changes in the renal response to ANP may also provide an explanation. ANP, an important factor in Na excretion, is released from atrial myocytes in response to atrial stretch and volume loading. It acts then via specific cell-surface receptors on renal microvasculature and tubular epithelium to induce hyperfiltration and inhibition of sodium reabsorption, and it suppresses renin release. ANP cell receptor interaction results in guanylate cyclase conversion of MgGTP to cGMP. cGMP phosphorylates intracellular proteins such that luminal membrane sodium channels are inhibited. Both serum and atrial proteases cleave proANP, the stored 126-amino acid prohormone, to biologically active 28-amino acid peptide ANP 1-28. Selective blocking of degradative enzymes and clearance receptors of ANP can prolong the half-life of ANP, which is usually rapidly degraded (198). Healthy elderly are found to have basal ANP levels three to five times those of younger healthy adults (195,199,200). Saline loading, high salt intake, or head-out body water immersion stimulates ANP to a greater extent in older than in younger subjects (195,199,201,202). Low salt intake does not produce a difference in ANP level, however, suggesting that the ANP response to salt loading is intact and that higher basal levels may be the result of decreased metabolic clearance, increased half-life, or greater volume of distribution (203–207). That decreased GFR can play a role in decreased ANP clearance is illustrated by higher ANP levels in patients with chronic renal failure in comparison to controls (207). Similarly inhibition of degradative enzymes, endopeptidases, rich in the proximal brush border, can also contribute to changes in ANP clearance (198). Rats with decreased renal mass when infused with phosphoramidon, endopeptidase inhibitor, increase ANP levels in association with higher urinary cGMP and salt excretion (208). This was also seen in humans with class II heart failure by New York Heart Association criteria. These subjects were infused with a renal neutral endopeptidase inhibitor, candoxatril, with no observed changes in renal hemodynamics. ANP half-life was also significantly prolonged in these subjects and they were noted to have decreased metabolic clearance of ANP (209). ANP infusion in the elderly versus younger adults also suggests decreased metabolic clearance (206,210). Whether aging affects the number or quality of brush-border membranes, degradative enzymes need further investigation.

It is possible that elevated ANP levels in the elderly are an adaptive phenomenon for reduced ANP sensitivity in the kidney (194,196,211). However, urinary sodium excretion is maximal at a 2 ng/kg/minute rate of ANP infusion in the elderly whereas, young subjects continue to increase sodium excretion with incremental increases in ANP infusion (Fig. 80-21), suggesting a blunted ANP response. A change in baseline cGMP, a second messenger of ANP, is not seen with age (195,202). However, a low-dose ile-ANP infusion does increase cGMP in both plasma and urine of healthy elderly subjects (206). Even with the increases in cGMP, an increase in Na excretion did not occur (206), suggesting a post-cGMP effector defect.

Concurrent measurements of plasma renin and aldosterone during ANP infusion suggest that the natriuretic function and the ability to suppress the renin–angiotensin–aldosterone system are affected differently in the elderly (194,195,206).

Urinary Concentration

The capacity of older individuals to maximally concentrate urine is impaired when compared to younger matched controls (155,212–215) (Fig. 80-22). The ability to appropriately

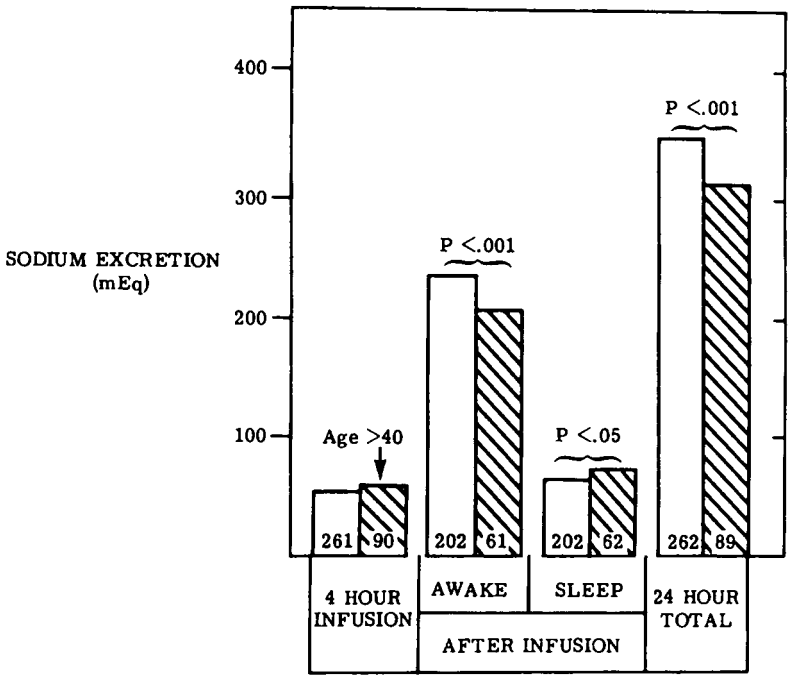

FIGURE 80-20. Comparisons of urinary sodium excretion in younger (*clear bars*) and older (*hatched bars*) subjects after 2-L intravenous normal saline. Normal subjects older than 40 years of age excrete a sodium load slower than subjects younger than 40 years of age. Numbers at the base of the bars represent the number of subjects in each group. (Reprinted from: Luft FC, Grim CE, Fineberg NS, et al. Effects of volume expansion and contraction in normotensive whites, blacks, and subjects of different ages. *Circulation* 1979;59:643, with permission.)

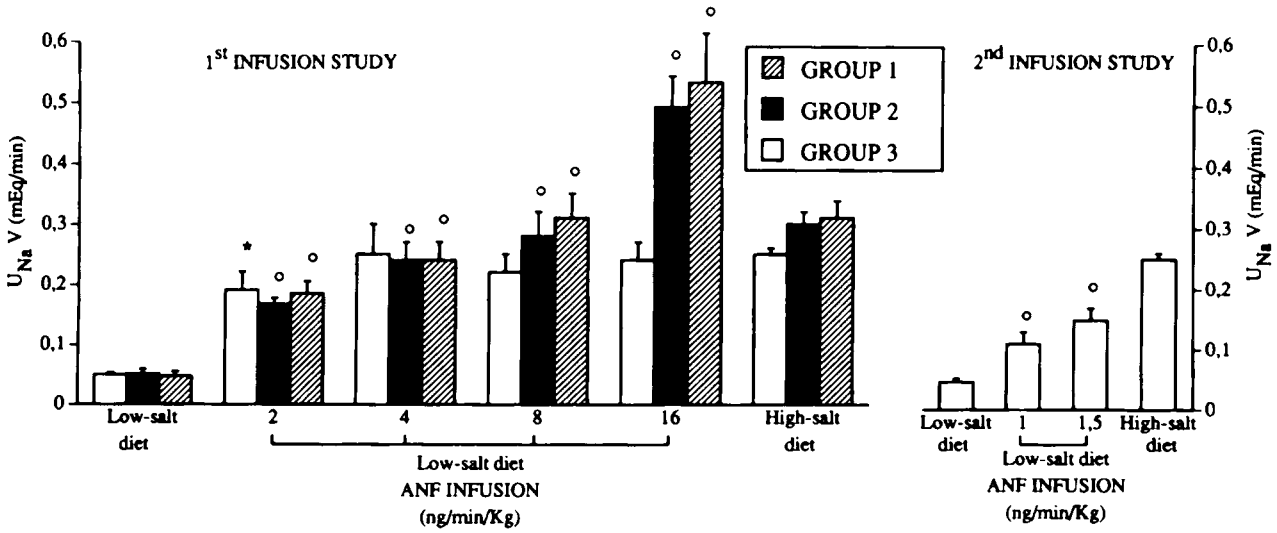

FIGURE 80-21. Urinary sodium excretion for low-salt diet (during basal conditions and after atrial natriuretic factor infusion) and at high-salt diet conditions in young (group 1), middle-aged (group 2), and elderly subjects (group 3). The columns represent means and the bars standard errors of the mean. °p <0.05 versus other steps and low-salt diet; *p <0.01 versus low-salt diet. (Reprinted from: Leasco D, Ferrara N, Landino P, et al. Effects of age on the role of atrial natriuretic factor in renal adaptation to physiologic variations of dietary salt intake. *J Am Soc Nephrol* 1996;7:1045, with permission.)

concentrate urine under conditions of water deprivation and or hyperosmolar states depends on an intact osmoreceptor and volume receptor sensitivity for arginine vasopressin (AVP) release as well as an intact tubular response to AVP in the face of maximal medullary tonicity. A combination of functional changes likely contributes to the defect in urinary concentration in the elderly.

The AVP response to both osmotic and volume pressure stimulus remains intact in the elderly, although the extent of stimulus determines the response obtained. Osmoreceptor sensitivity to AVP is enhanced as demonstrated in rats and elderly subjects. Hypertonic saline infusion to Long-Evans rats results in an increased hypothalamic-neurohypophyseal response with increased AVP release (216,217). Similarly, 3% saline infusion to elderly subjects over 2 hours shows heightened osmoreceptor sensitivity (218). Levels of basal circulating AVP are also higher in the elderly after 24 hours of water deprivation (219–222). With 9-hour water deprivation (223), plasma AVP levels are lower in elderly compared to younger counterparts, whereas 14-hour water deprivation (224) increases AVP levels to only slightly higher levels, suggesting that the greater AVP response may require maximal osmoreceptor stimulation in the elderly. Although baroreceptor-mediated AVP release from volume pressure changes such as acute upright posture decreases with age (225), plasma AVP levels increased in healthy elderly 65 to 80 years of age who underwent 60-degree head-up tilt stimulation in comparison to 20- to 34-year-old subjects (226). In addition, the oropharyngeal inhibition of AVP release in response to drinking cold liquids was noted to decrease with age (221).

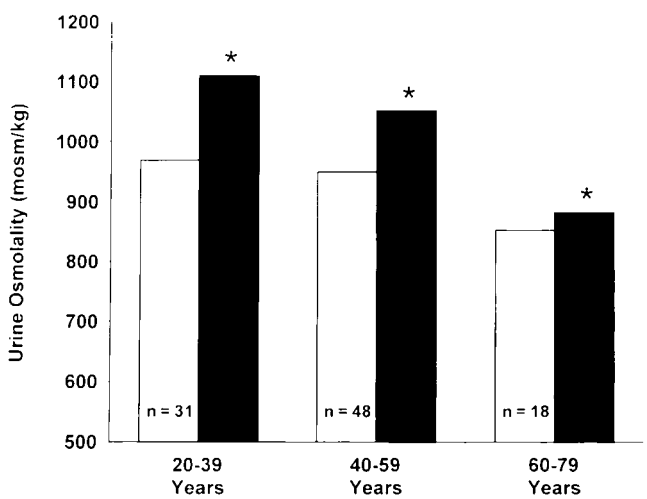

FIGURE 80-22. The effect of age on urine osmolality in response to 12 hours of water deprivation. □, Pretreatment values; ■, posttreatment values. The number of subjects in each group is noted at the base of each bar. Urine osmolality decreases significantly with age after water deprivation (*p <0.05). (Data from: Rowe J, Shock N, DeFronzo R. The influence of age on the renal response to water deprivation in man. *Nephron* 1976;17:270.)

Functional changes in the tubular process of concentrating urine may be responsible for diminished urinary concentrating capacity. Possible intrarenal resistance to AVP in the elderly is suggested as AVP infusion to healthy elderly fails to correct the underlying decrease in maximal concentrating ability (227). Medullary washout secondary to relative increased medullary blood flow in the aged kidney can also be considered. Rowe et al. tested this hypothesis in healthy community dwelling volunteers between 20 and 79 years of age who underwent 12 hours of overnight dehydration (Fig. 80-22) (215). A significant decrease in urine osmolality and an increase in solute and osmolar clearance are noted with aging, independent of creatinine clearance (215). Impaired solute transport in the ascending loop of Henle can lead to decreased medullary tonicity. This is supported by water diuresis in the elderly indicating decreased sodium chloride transport in the ascending loop of Henle (174,228). However, studies in aged rats suggest AVP resistance in the collecting tubules. Both exogenous AVP and 40 hours of dehydration failed to maximally concentrate urine in aged rats. Solute-free water formation was normal; however, solute-free water reabsorption was impaired. Inner medullary

solute content was identical in both old and young rats, thus suggesting intact ascending limb solute transport and impaired collecting tubular water transport (229).

The role of urea transporters in preserving papillary tonicity in older rats reveals a decreased expression of medullary UT-A1 and UT-B1 urea transporters and lower papillary osmolality regardless of food restriction or *ad libitum* diet (230). However chronic infusion of deamino-8-D arginine vasopressin (DDAVP) upregulated these urea transporters, increased papillary urea accumulation, and improved urine osmolality and urine flow rate in 30-month-old WAG/Rij female rats in comparison to 10-month-old rats (231) (Fig. 80-23). Dehydrated

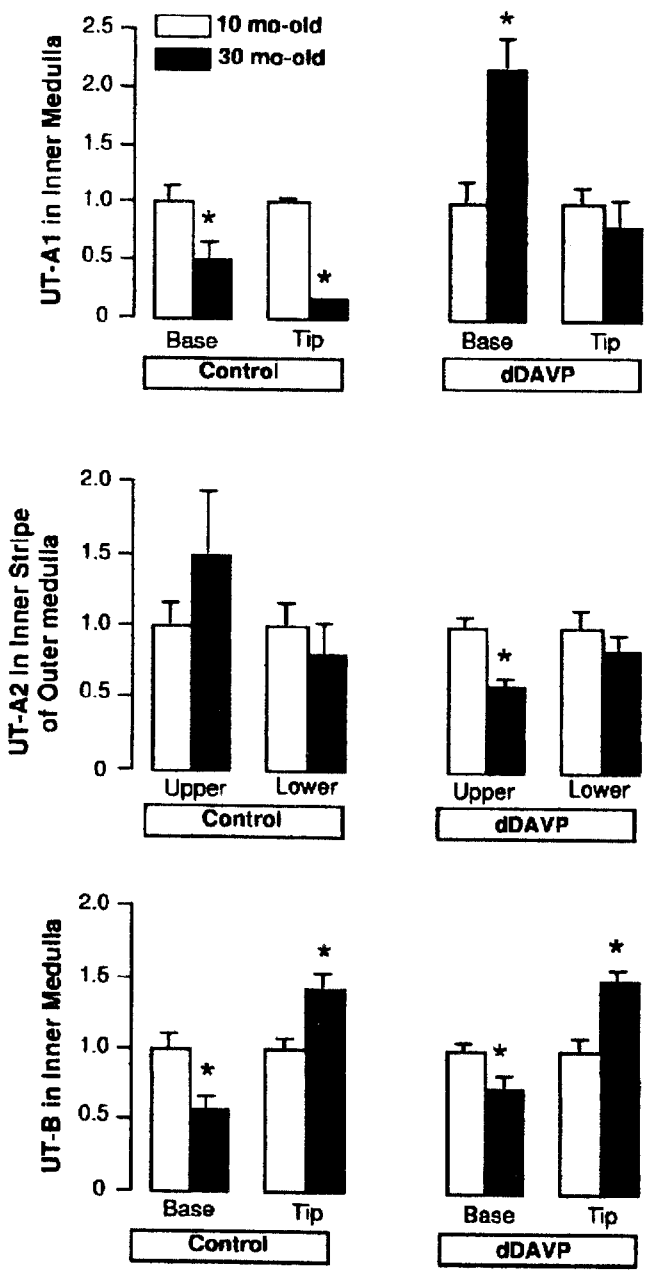

FIGURE 80-23. UT-A1, UT-A2, and UT-B abundance in medulla subzones in untreated (control) and dDAVP-treated 10- and 30-month-old rats. Values (means ± SE) are expressed relative to respective untreated or treated 10-month-old rats. $^*p < 0.05$ or less by one-way ANOVA. (Reprinted from: Combet S, Geffroy N, Berthonaud V, et al. Correction of age-related polyuria by dDAVP: molecular analysis of aquaporins. *Am J of Physiol Renal Physiol* 2003;284:F199, Figure 8.)

senescent rats responded similarly to adult rats with a sixfold increase in plasma vasopressin level and doubling papillary urea concentration, but still were not able to reach the level of urinary osmolality of younger adult rats. There was enhanced papillary urea sequestration with a great decrease in both UT-A1 and UT-A3 levels in the inner medullary tip with increased level in the inner medullary base, suggesting that lower papillary urea concentration may be a reason for the inability to concentrate urine as efficiently in older rats (232).

Inner medullary slices of both rats and mice have an age-associated decrease in AVP-induced cAMP generation (233,234). Younger, 12-month-old rats have much higher cAMP levels after AVP infusion than older 24-month-old rats. Older rats required a higher threshold dose to initiate a significant cAMP response (234). Chronically elevated plasma levels of AVP in older animals may downregulate AVP receptors and decrease cAMP response (217). However, no change in AVP receptor number or affinity has been found (235). A role for postreceptor processes can be considered. Aging kidneys express a decrease in the abundance of stimulatory guanine nucleotide-binding protein (Gs) (236), possibly from impaired adenylate cyclase activity and cAMP levels. Although *in vitro* cholera toxin- and forskolin-perfused rabbit cortical collecting tubules (CCTs) reflected the ability of Gs to stimulate adenylate cyclase in the 3-month-old CCTs (young) and 2- to 3-year-old CCTs (middle-aged), Gs stimulation of adenylate cyclase was markedly impaired in the 4- to 5-year-old rabbit CCTs (old-aged). The response of aged CCTs was only 32% of the response of the young isolated CCTs with cholera toxin infusion alone, which is known to ADP-ribosylate the G-proteins and thereby stimulate adenylate cyclase and cAMP generation. However forskolin, a potent adenylate cyclase stimulator at the level of the catalytic unit and also G-protein interaction, yielded a response of only 12% that of young isolated rabbit CCTs. These data point to a possible defect in the Gs protein with the catalytic subunit of adenylate cyclase as a mechanism for age-related changes in CCT response to AVP (237).

Northern blot analysis reveals similar degrees of downregulation of vasopressin V2 receptor mRNA and AQP-2 mRNA in dehydrated old Sprague-Dawley rats (238). Older Brown-Norway rats also have decreased AQP-2 protein abundance and have decreased V2 receptor expression, despite similar increases in AVP after moderate water restriction (239) (Fig. 80-24). Both AQP-2 and AQP-3 expression in the medullary collecting duct of 30-month-old female WAG/Rij rats are lower in comparison with that in similar 10 month-old rats. However papillary cAMP content determined by enzyme-linked immunosorbent assay (ELISA) of these normally hydrated animals was not significantly different between the two groups (240). The role of gender is not clear. The sum of these studies suggest that lower AQP-2 and AQP-3 expression in older rats occurs independent of circulating AVP and intracellular cAMP (240), and that impaired AVP response resulting from lower AQP-2 abundance and of V2 receptor expression may in part contribute to the decrease in urinary concentration capacity of aging.

Urinary Dilution

The aged kidney's ability to achieve maximum urinary diluting capacity also declines (212,241–243). Older subjects, age 77 to 88 years, achieve a minimum urine osmolality of 92 mOsm/kg H_2O during water diuresis. In contrast, younger subjects, ages 17 to 40 years, achieve a minimum of 52 mOsm/kg H_2O with solute-free water clearance decreased from 16.2 to 5.9 mL/minute in older versus younger individuals, respectively. For appropriate urinary dilution to take place, distal delivery of filtrate needs to undergo appropriate solute extraction in the ascending loop of Henle with suppression of AVP with water

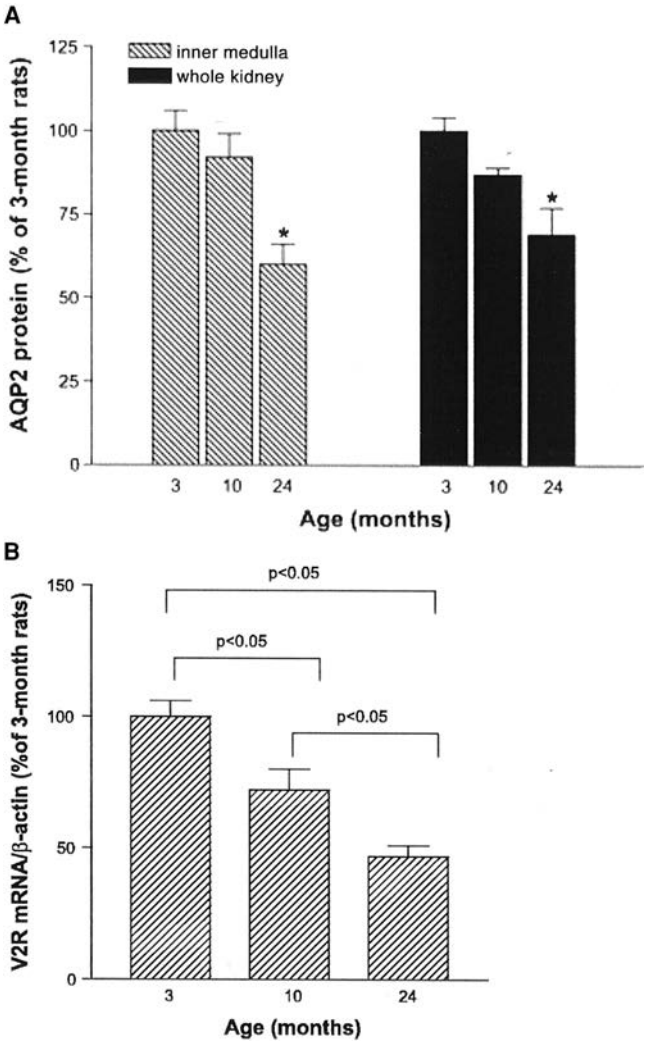

FIGURE 80-24. Age related decreases in renal aquaporin-2 (AQP2) protein abundance and in renal inner medullary V2 subtype of the AVP receptor (VR2) mRNA under baseline conditions. **A:** Group means of AQP2 proteins in whole kidney and inner medulla of 3-,10-, and 24-month-old rats under baseline conditions. **B:** V2R mRNA densitometric analyses of Northern blot bands normalized to the value of β-actin band in each lane for rat groups in A. ($N = 6$ per group, * = $p < 0.05$, one-way ANOVA for both AQP2 protein, and V2R mRNA data). (Redrawn from: Tian Y, Serino R, Verbalis JG. Downregulation of renal vasopressin V2 receptor and aquaporin-2 expression parallels age-associated defects in urine concentration. *Am J Physiol Renal Physiol* 2004;287:F797.)

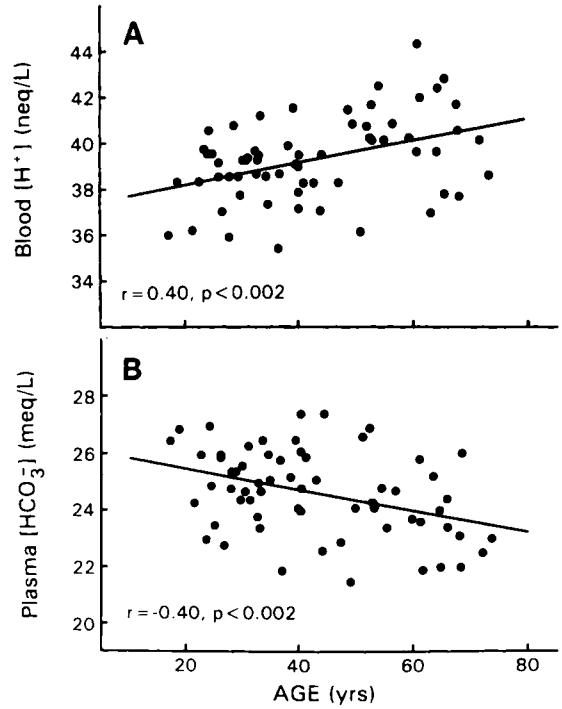

FIGURE 80-25. Relation between $[H^+]_b$ and age (**A**) and between $[HCO_3^-]_p$ and age (**B**), in normal adult humans ($N = 64$). Each data point represents the mean steady-state value in a subject eating a constant diet. Regression equations: $[H^+]_b = 0.045 \times \text{age} + 37.2$; $[HCO_3^-]_p = -0.038 \times \text{age} + 26.0$. (Reprinted from: Frassetto L, Morris RC Jr, Sebastian A. Effect of age on blood acid-base composition in adult humans: role of age-related renal functional decline. *Am J Physiol* 1996;271:F1112, Figure 2, with permission.)

loading and hypoosmolality. The decrease in GFR may contribute to the inability to maximally dilute urine in the elderly. However even with correction for GFR, solute-free water clearance is still lower in the elderly (241). The maximum suppression of plasma AVP following water loading still needs to be evaluated.

Acid–Base Balance

The aged kidney maintains homeostatic acid–base balance until acid loading occurs, and then the impaired ability to excrete the acid load becomes evident. The loss of renal mass and GFR with age is associated with this perturbation (244). A progressive low-grade metabolic acidosis develops with age in healthy subjects on a steady-state acid diet (245) (Fig. 80-25). Both the change in blood pH and plasma bicarbonate correlate with the change in GFR. The plasma chloride increases as the plasma bicarbonate decreases, similar to that seen with early renal disease and renal tubular acidosis (245). Urinary ammonia accounts for less of the total acid excretion in elderly subjects between 72 and 93 years of age taking an oral ammonia load than younger subjects (244). When glutamine is given, the rate of ammonium excretion is of equal magnitude in both young and old. Titratable acid accounts for a greater percentage of the total acid excretion, with increases in phosphate excretion. Factored for GFR, this acid excretion is related to a decrease in renal tubular mass rather than a specific tubular defect. However, another study suggests a possible tubular defect, as decreased ammonium excretion failed to reach minimal pH even after correction for GFR in ammonium-loaded elderly patients (246) (Fig, 80-26). Whether this defect results from histologic changes in the aging kidney or from changes in function such as seen with an impaired renin–angiotensin–aldosterone axis is not clear. Measurements for titratable acid did not reach statistical differences. Aged rats fed equal amounts of ammonium chloride were also noted to have a decrease in the absolute amount of ammonium excreted, accounting for a more severe acidemia as pH fell from 7.40 to 7.09, whereas in younger rats the pH decreased from 7.40 to 7.32 (247). Transport studies in renal proximal tubular apical brush-border membrane vesicles reveal similar enhancement of sodium –hydrogen exchange activity (Na/H exchange), a major regulator of proximal tubular acidification, by an acid load (247). Phosphate transport is also reduced to the same extent in adult and aged rats. Therefore, the change in ammonium excretion may mediate age-related

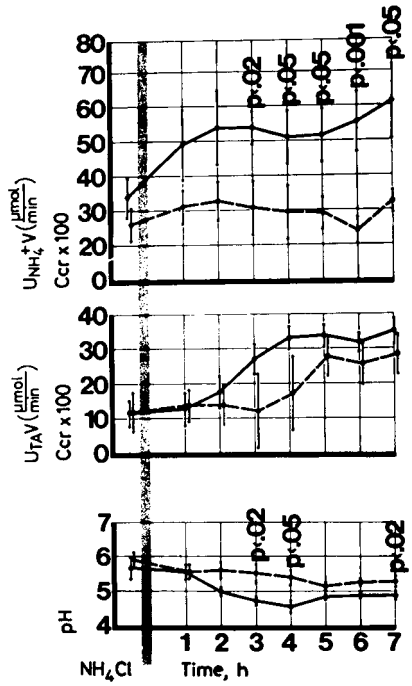

FIGURE 80-26. Acid excretion over the entire study in young and elderly subjects. Ammonium ($U_{NH4}V$) excretion corrected for GFR (Ccr $\times$100), and urinary pH were compared between young (group 1, *solid line*) and aged subjects (group 3, *dashed line*). Mean values ($\pm$SEM) along with *p* values of the differences between the two groups are shown. (Reprinted from: Agarwal BN, Cabebe FG. Renal acidification in elderly subjects. *Nephron* 1980;26:291, with permission.)

changes in renal adaptation to metabolic acidosis despite intact compensatory mechanisms (247).

Neither pH nor serum bicarbonate levels are abnormal in the elderly, despite a subtle presence of metabolic acidosis (245). However bone demineralization and muscle wasting, frequent complications of chronic metabolic acidosis, are common in the elderly. Mobilization of calcium and alkali from bone leaching in the presence of chronic acidosis inhibits calcium reabsorption and urinary calcium loss. Enhanced muscle breakdown from acidosis results from activation of an ATP-dependent pathway involving ubiquitin and proteasome (248). Even in the presence of normal serum bicarbonate, increased protein intake (frequently evident in industrialized societies) and aging may result in negative balance, osteoporosis, and greater incidence of fractures and muscle wasting (249). Potassium bicarbonate given to postmenopausal women has suggested improvement in nitrogen balance (250) and calcium balance (251). Whether bicarbonate supplementation can benefit the elderly to prevent complications of chronic acidosis needs to be evaluated.

CHANGES IN OSMOLALITY, ELECTROLYTE, AND MINERAL METABOLISM

The elderly are more prone to serum osmolar abnormalities (252–256). Tubular functional changes in solute and water conservation and excretion, in addition to various medications, infections, and emotional disability predispose the elderly to hyponatremia or hypernatremia.

Hyponatremia

Hyponatremia in the elderly is relatively common, with an incidence of 11.3% recorded over 10 months in a geriatric facility, and is noted to be as high as 22.5% in a chronic disease facility (253,256). The combination of impaired diluting capacity and enhanced osmotic AVP release predispose the elderly to a greater occurrence of hyponatremia (257). A subset of geriatric ambulatory clinic patients present with syndrome of inappropriate antidiuretic hormone (SIADH) (258). Thiazide diuretics are implicated in 20% to 30% of the hyponatremia seen in the elderly, its use exacerbating the preexisting renal diluting defect (Fig. 80-27) (253,255,256,259,260). In addition, a deficiency in prostaglandin synthesis in the elderly increases susceptibility to thiazide-induced hyponatremia (259). Many elderly are also taking numerous other medications including sulfonyl urea compounds, chlorpropamide, tolbutamide, or nonsteroidal antiinflammatory drugs (NSAIDs), which potentiate the action of AVP and can act in synergy to impair water excretion. Use of medications that stimulate the nonosmotic release of AVP or potentiate renal tubular effects of AVP act in synergy with impaired diluting ability and should be used cautiously in the elderly.

As water shifts from the extracellular to the intracellular space in the face of hyponatremia, numerous symptoms and signs including apathy, disorientation, lethargy, muscle cramps, anorexia, nausea, vomiting, agitation, depressed deep tendon reflexes, pseudobulbar palsy, and seizures can occur (261). Recognition with appropriate and timely initiation of therapy can prevent severe neurologic sequelae including central pontine myelinolysis.

Hypernatremia

Elderly are similarly susceptible to hypernatremia in the face of decreased urinary concentrating ability and impaired sodium conservation, leading to an increase in serum osmolality and volume depletion. Although thirst and volume depletion normally stimulate fluid intake, these defenses are also impaired in the elderly (221,262) (Fig. 80-28). When access to free water is limited in geriatric patients from confusion, delirium, restraint, or immobility, lethal states of hypernatremia are possible with mortality rates as high as 46% to 70% (252,254). Acute increases in serum sodium greater than 160 mEq/L are associated with 75% mortality, a result related to concurrent comorbidities.

In addition, medications that inhibit tubular AVP function such as lithium and demeclocycline, or mechanisms that can cloud the sensorium including sedatives and tranquilizers should be avoided. Careful monitoring needs to occur when using osmotic diuretics, high protein and glucose containing tube feedings, or bowel cathartics. Systemic illnesses, infections, dementia, fever, and neurologic disorders can impair AVP release and predispose the elderly to dehydration. Cellular dehydration can lead to severe neurologic sequelae including obtundation, stupor, coma, seizures, and death. As a result, cautious monitoring of debilitated elderly patients or elderly patients on medications that affect renal water conservation or the sensorium is necessary (Table 80-2).

Potassium Balance

Total body and exchangeable potassium decreases with muscle mass loss from aging, which is greater in women than in men (263,183,191). However plasma renin and aldosterone levels also decrease with age and predispose the elderly to

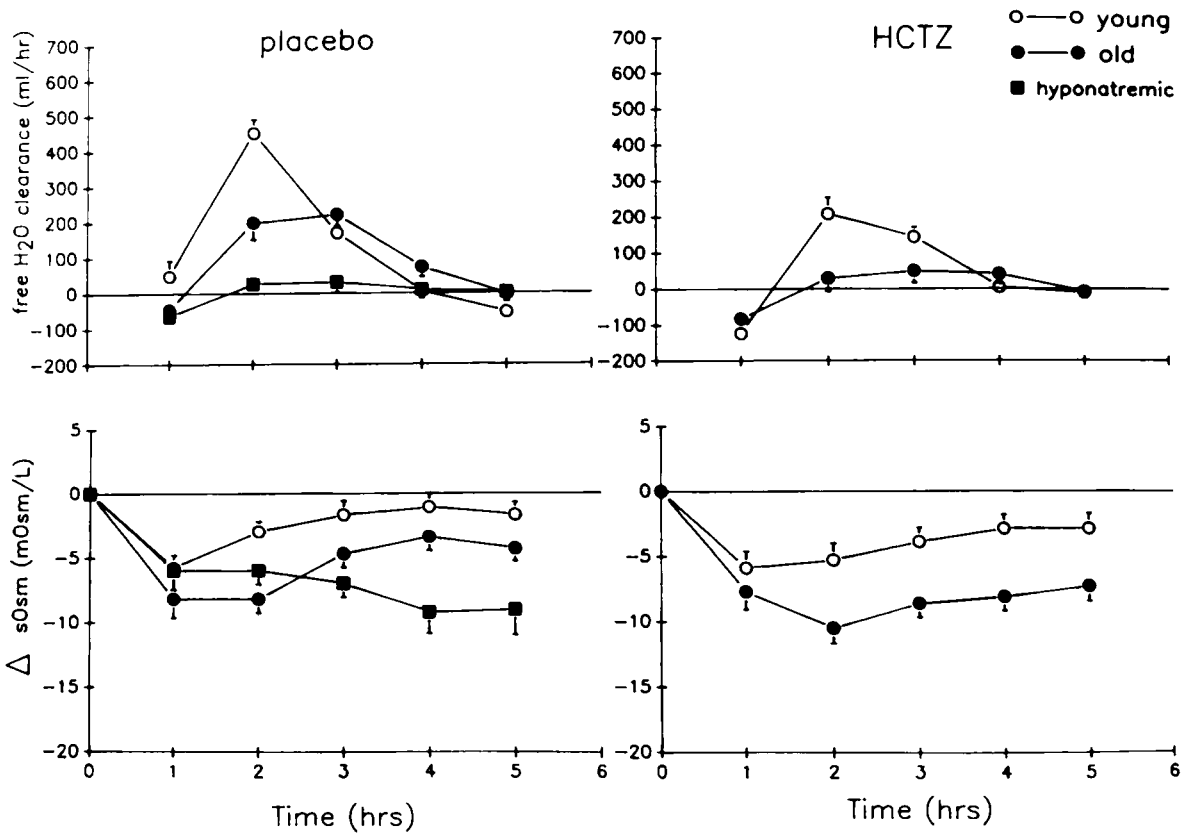

FIGURE 80-27. Free water clearance (CH_2O) and change in serum osmolality (ΔsOsm, mOsm/kg H_2O) after a water load with placebo versus hydrochlorothiazide (HCTZ) in young, old, and old with a prior history of thiazide-induced hyponatremia. CH_2O and decline in sOsm were significantly lower in the old than in the young [$p < 0.05$, analysis of variance (ANOVA)]. This difference was magnified after the use of HCTZ. Those with a history of hyponatremia had a lower CH_2O and decline in sOsm than did the healthy elderly ($p < 0.05$, ANOVA). (Reprinted from: Clark BA, Shannon RP, Rosa RM, et al. Increased susceptibility to thiazide-induced hyponatremia in the elderly. *J Am Soc Nephrol* 1994;5:1106, with permission.)

hyperkalemia. This is evident in healthy elderly subjects 65 to 85 years of age who have lower basal plasma aldosterone and inadequate aldosterone response to potassium infusion when compared to younger, 20- to 35-year-old subjects (264) (Fig. 80-29). Results of studies in aging rats suggest a defect in both renal and extrarenal potassium adaptation. With potassium chloride infusion to young and old rats receiving normal potassium diet, young and old rats have similar efficiency of potassium excretion (265). However, when on a high-potassium diet, older rats are much less efficient in excreting the potassium load, with a greater rise in plasma potassium compared to younger rats. After bilateral nephrectomy and potassium

TABLE 80-2

MECHANISM BY WHICH DRUGS CAN LEAD TO IMPAIRED WATER METABOLISM

Inhibit ADH release	Inhibit peripheral action of ADH	Potentiate ADH release
Fluphenazine	Lithium	Nicotine
Haloperidol	Colchicine	Vincristine
Promethazine	Vinblastine	Histamine
Morphine (low doses)	Demeclocycline	Morphine (high doses)
Alcohol	Glyburide	Epinephrine
Carbamazepine	Methoxyflurane	Cyclophosphamide
Norepinephrine	Acetohexamide	Angiotensin
Cisplatinum	Propoxyphene	Bradykinin
Clonidine	Loop diuretics	
Glucocorticoids		

ADH, antidiuretic hormone; *NSAIDs*, nonsteroidal antiinflammatory drugs.

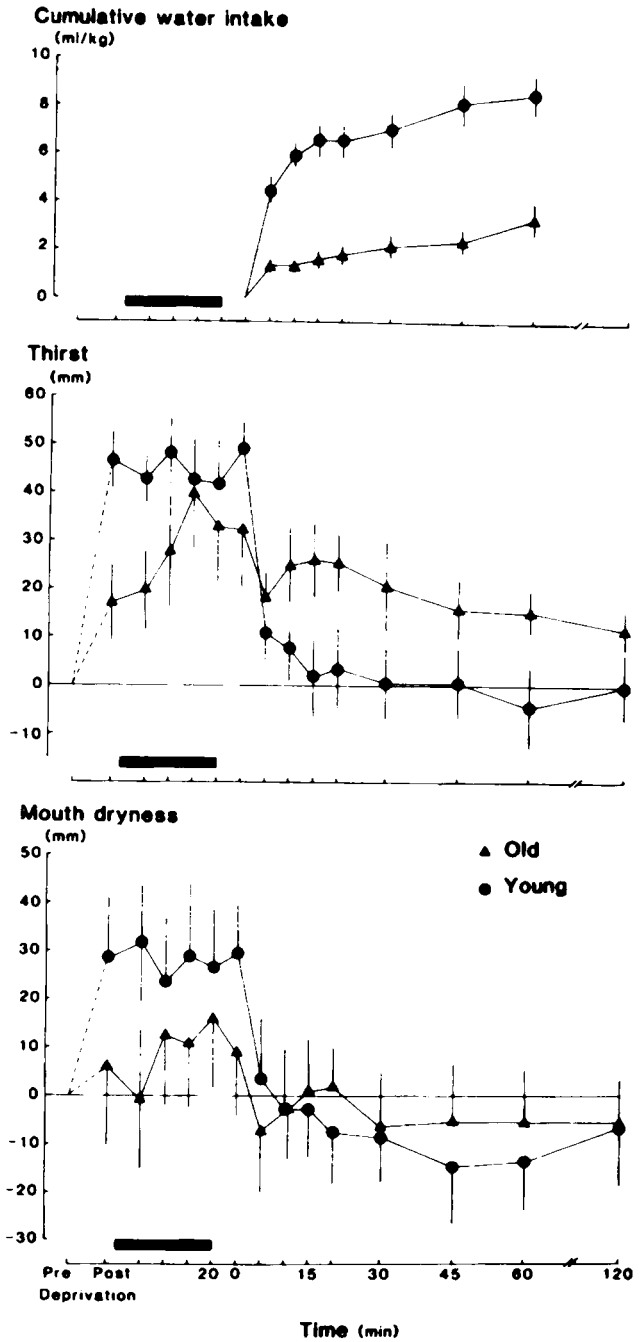

FIGURE 80-28. Cumulative water intake and changes in thirst and mouth dryness in elderly ($N = 7$) and young ($N = 7$) groups. *Symbols* represent mean values, and *bars* SEM. Changes in thirst and mouth dryness were measured on a visual analog rating scale. The *hatched rectangle* represents the single-blind sham infusion. (Reprinted from: Phillips PA, Phil D, Rolls BJ, et al. Reduced thirst after water deprivation in healthy elderly men. *N Engl J Med* 1984;311:753, with permission.)

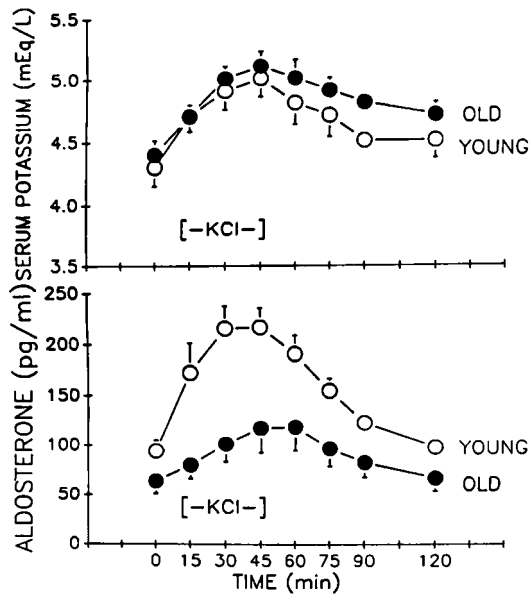

FIGURE 80-29. Serum potassium and aldosterone levels before, during, and after infusion of potassium chloride (0.5 mEq/kg body weight over 45 minutes) in six healthy young and six healthy elderly men. Changes in serum potassium levels were similar, but elderly subjects had lower aldosterone responses ($p < 0.005$, analysis of variances). (Reprinted from: Mulkerrin E, Epstein FH, Clark BA. Aldosterone responses to hyperkalemia in healthy elderly humans. *J Am Soc Nephrol* 1995;6:1459, with permission.)

chloride infusion, older rats were unable to attenuate the rise in serum potassium to levels as low as were younger rats. In addition, Na,K-ATPase activity is 38% less in older rat renal medulla versus younger rats (265). Therefore, acute adaptive mechanisms against hyperkalemia may be present in aging rats, but renal and extrarenal potassium adaptation may be attenuated (265). How this applies to older adult subjects is not known. Insulin-mediated potassium uptake remains intact in elderly humans (266). Exercise-induced plasma potassium elevation in healthy young and elderly subjects show an impaired response to β-adrenergic-induced increase in adenylate cyclase activity, with decreased activity of the Na,K-ATPase exchange in skeletal muscle (267).

Changes in renal acidification in addition to a decrease in renin–angiotensin–aldosterone system activity may help explain the increased incidence of type-4 renal tubular acidosis seen in the elderly (268). Furthermore, in the presence of a possible problem with chronic potassium adaptation in aging, medications that inhibit the renin–angiotensin–aldosterone system including ACE inhibitors, heparin, cyclosporine, tacrolimus, β-blockers, and NSAIDs, can increase the risk of hyperkalemia in the elderly. Other agents that also inhibit distal tubular potassium excretion—such as sodium channel blockers trimethoprim and pentamidine, and potassium sparing diuretics such as amiloride, triamterene, spironolactone, and eplerenone—can exacerbate defects in potassium excretion in the elderly (269–271) (Fig. 80-30).

Calcium Balance

Tubular calcium reabsorption remains intact with aging despite changes in calcium metabolism in aging. Aging rats demonstrate appropriate urinary calcium excretion and reabsorption in the face of increased or decreased dietary calcium (272). Both absolute filtered load and proximal reabsorption of calcium per nephron remain similar in both young and old rats (273).

There is decreased intestinal calcium absorption with aging, which parallels a decrease in 1-alpha-hydroxylase activity, decreased levels of 1,25-dihydroxycholecalciferol (1,25(OH)2D3), and increased basal parathyroid hormone (PTH) levels. The 1,25(OH)2D3–dependent binding protein also decreases and correlates with a decrease in calcium absorption with age (272,274). Although the initial production of

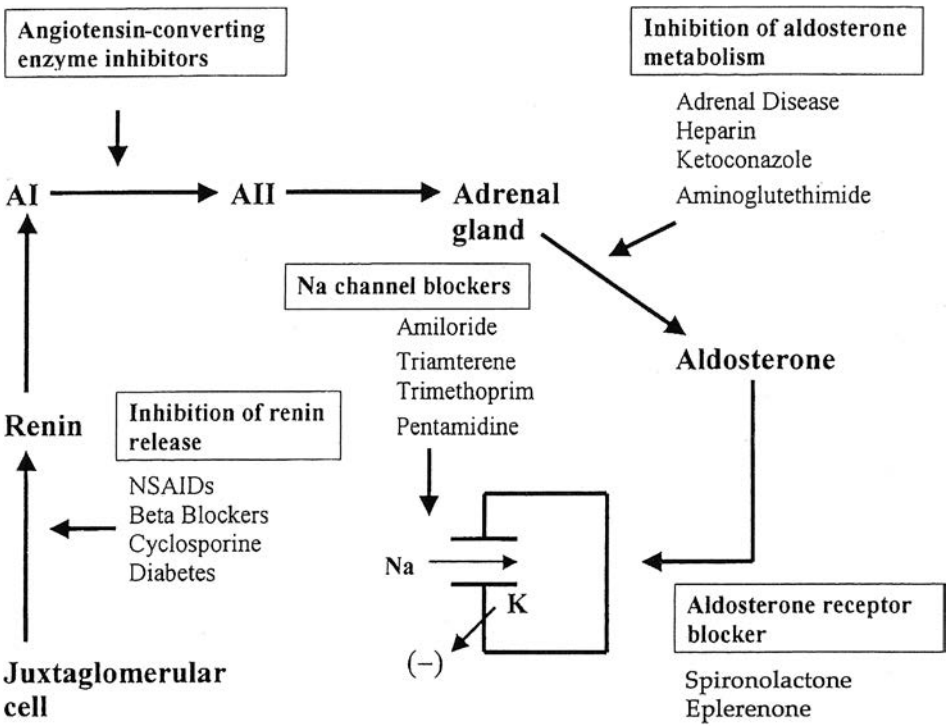

FIGURE 80-30. Site of action of various pharmacologic agents and disease states that can further impair activity of the renin–angiotensin–aldosterone axis and exacerbate hyperkalemia in aged individuals with progressive decline in renin and aldosterone levels. *A1*, angiotensin; *A11*, antiinflammatory drugs.

1,25(OH)2D3 is lower in healthy adults compared to younger subjects, the final 1,25(OH)2D3 concentration does not differ between the two groups. Even urinary cAMP and fractional phosphate excretion increase similarly in both groups, with PTH infusion suggesting an intact response (275). However, calcium regulation of PTH release may be altered in the elderly. Calcium gluconate and sodium ethylenediamine tetraacetic acid (NaEDTA) infusion in relation to PTH response suggests that the age-associated increase in serum PTH may reflect an increase in the set-point for calcium and in the number of parathyroid cells. It is not clear at this point what role the cell surface G-protein-coupled calcium-sensing receptor (CaSR), which may play in the altered set point for PTH seen in both primary and secondary hyperparathyroidism of uremia, plays in the change in set-point for PTH release seen with aging (276).

Phosphate Balance

Renal tubular phosphate reabsorption decreases with age (113,276–283) and occurs in addition to a decrease in intestinal phosphate absorption (284). Furthermore aged rats demonstrate impaired adaptation to low phosphate diet (113,277,280,284). Although PTH may be elevated, this impairment is independent of endogenous PTH activity, as parathyroidectomy in aged rats improves but does not normalize renal tubular phosphate reabsorption (277,280,281, 285,286). Primary cultures of renal tubular cells of young and old rats (287) and *in vivo* studies (277) show similar results, with a decrease in phosphate transport mediated by a decrease in maximum velocity (Vmax) of sodium gradient-dependent phosphate transport (Na/Pi cotransport) and a decrease in adaptation to low phosphate concentration of culture media. The decrease in proximal tubular Na/Pi cotransport is associated with a decrease in type IIa Na/Pi cotransporter protein and mRNA levels (288). Immunohistochemistry reveals a significant decrease in type IIa Na/Pi cotransporter protein at the level of the apical brush-border membrane (288).

An increase in membrane cholesterol content seen with aging may also affect Na/Pi cotransport activity (113,289–292). *In vitro* enrichment of old rats with cholesterol of brush-border membrane isolated from young rats demonstrates the age-related impairment in Vmax of Na/Pi cotransport (293). Similarly direct changes in cell cholesterol content of opossum kidney cells with renal proximal tubular cell characteristics modulate Na/Pi cotransport activity by changing the expression of the apical membrane type II Na/Pi cotransport protein (294). These data suggest an important role for changes in membrane cholesterol effecting age-related decrease in Na/Pi cotransport.

Whether impaired 1,25(OH)2D3 metabolism plays a role in the age-related renal and intestinal phosphate transport needs to be examined as vitamin D-deficient animals supplemented with 1,25(OH)2D3 significantly improve in both renal and intestinal phosphate transport (295–297). Interestingly, changes in phosphate transport resulting from supplementing 1,25(OH)2D3 parallel significant changes in brush-border membrane lipid composition and fluidity (298,299). Therefore, it is possible that similar lipid-modulating effects of 1,25(OH)2D3 in the aged may similarly improve renal and intestinal transport of phosphate and also calcium.

CLINICAL RENAL DISEASES IN THE ELDERLY

Acute Renal Failure

Changes in renal function with aging predispose the elderly to acute renal failure (ARF). A 9-year, prospective Spanish study of 437 patients establishes a 3.5-times greater incidence of ARF in patients older than 70 years (300). The prevalence and increased need for both medical and surgical treatment of comorbid systemic diseases including atherosclerosis, hypertension, diabetes heart failure, and malignancy in the growing elderly population further complicates common presentations of ARF such as prerenal or volume depletion, sepsis, acute interstitial nephritis, nephrotoxin-induced glomerular and tubular dysfunction, and urinary tract obstruction. Particularly common in elderly with generalized atherosclerosis is cholesterol

embolization, whether spontaneous or from invasive procedures to the arterial tree. Acute vasculitis or rapidly progressive glomerulonephritis appears less often but needs to be considered, as significant morbidity can ensue if not recognized.

Prerenal azotemia from volume loss in the elderly is relatively common, with 50% prevalence noted in two separate studies (300,301). Even mild episodes of vomiting, diarrhea, or overzealous use of diuretics can decrease GFR in the elderly who may have compromised capability to prevent continued solute loss or concentrate urine, and have decreased thirst. In addition a decrease in renal perfusion from medications, sepsis-induced vasodilation, or low cardiac output is also a common cause of prerenal azotemia in the elderly. Furthermore, decreases in baseline renal blood flow and GFR with decreased ability to autoregulate renal plasma flow and impaired renal functional reserve also renders the aging kidney susceptible to prerenal failure. Altered hemodynamics with use of NSAIDs, ACE inhibitors, AT-II receptor blockers, α-adrenergic blockers commonly used for rheumatologic, cardiovascular, and genitourinary abnormalities in the elderly can also compromise vasoregulatory mechanisms. Reversibility of prerenal azotemia in most cases is expected with appropriate intervention including careful volume repletion, discontinuation of concomitant exacerbating medications and improving cardiac output. Sixty percent of elderly patients with prerenal azotemia recovered renal function in one study (301). However evolution to acute tubular necrosis (ATN) is more common in the elderly (22%) than in younger patients (15%) (302). The use of urinary indices such as fractional excretion of sodium (FENa) to estimate volume depletion in the elderly must be interpreted cautiously. Some elderly may not have adequate tubular capacity to achieve a FENa less than 1. Therefore, a high FENa may be present despite hypoperfusion or volume depletion from preexisting tubular defect (303).

Postsurgical complications account for approximately 30% of ARF in the elderly. Common postoperative complications associated with ARF in the elderly include hypotension during or after surgery, volume redistribution to the interstitial space, volume loss from gastrointestinal fistula, postsurgical sepsis, dysrhythmias, myocardial infarction, and atheroemboli from diseased vascular manipulation.

Infection and sepsis with associated vasodilation and hypotension frequently complicate the hospital course and lead to ARF in elderly patients. Gram-negative septicemia accounts for 30% of ARF in the elderly with endotoxin-mediated renal vasoconstriction causing ATN in susceptible individuals. Associated multiorgan failure or perioperative sepsis or both result in higher catabolic rates and add to a poor prognosis in the elderly (304–306). Hemodynamic instability often with a need for nephrotoxic antibiotics, such as aminoglycosides or amphotericin, prolongs renal dysfunction. Therefore, careful dosing and monitoring and adjustment of administered antibiotics to renal clearance are crucial. GFR estimation using only Scr or formulas based on Scr alone may not take into account the decrease in muscle mass seen with aging. Furthermore, functional and biochemical changes in the aging renal tubule may predispose to increased toxicity of antimicrobials such as aminoglycosides. Age is a well-known risk factor for aminoglycoside-associated nephrotoxicity (307).

Common pathogenic organisms such as staphylococci, streptococci, legionella, cytomegalovirus, human immunodeficiency virus, and common antibiotics such as β-lactams and sulfonamides and a myriad of other medications can result in interstitial inflammation and interstitial nephritis (308). Activated macrophages in the inflammatory tubulointerstitium release degradative enzymes that can denude intact basement membranes, thereby hampering the regeneration of the tubular segment. GFR decreases secondary to the loss of functioning nephrons with failure of the remaining nephrons to compensate with hyperfiltration (308).

Although NSAIDs and AT-II antagonists can affect vasoregulating mechanisms that maintain GFR, they also can cause interstitial inflammation and acute interstitial nephritis. Presentation can be typical, with pyuria and worsening GFR, or, atypical, with nephritic-range proteinuria. Renal biopsy is notable for both minimal and membranous lesions with NSAIDs, especially propionic derivatives (309), and membranous lesions with the ACE inhibitor captopril.

Radiocontrast agents can result in acute and prolonged vasoconstriction to significantly impair renal function in the elderly (310). The osmotic load delivered to the macula densa can also trigger tubuloglomerular feedback to further reduce GFR (311).

Evident from experimental studies in aged rats is the high propensity for the aging kidney for ischemic and toxic ARF (312–316). Occlusion of the renal artery causes a greater decline and a slower recovery of renal function in older versus younger rats (317,318). Underlying glomerulosclerosis is not necessarily the main reason for this observation. When difference in glomerulosclerosis was minimized between young and old rats with low protein feeding, renal artery clamping still caused a significant decrease in GFR and RPF and an increase in RVR in older compared to younger rats (317). Despite a constant sodium intake of 240 mEq per day in euvolemic men, RVR increased with age and the response to orthostatic change in RVR was diminished (319). Magnetic resonance imaging using changes in blood oxygenation level in nine female volunteers between 59 and 79 years of age demonstrated the inability to improve medullary oxygenation with water diuresis compared to younger subjects, possibly suggesting a predisposition to hypoxic renal injury in older patients (320). Similarly, elderly cardiac surgery patients (>70 years of age) on cardiopulmonary bypass and without evidence of acute renal failure have higher levels of kidney-specific proteins N-acetyl-β-glucosaminidase, α-1-microglobulin, glutathione transferase-pi (GST-pi), glutathione-α (GST-α), and higher fractional sodium excretion postsurgery relative to presurgery in comparison to similar, younger (<60 years of age) patients (321). Ischemic acute renal failure generates oxygen-free radicals (322). With infusion of a free radical scavenger, superoxide dismutase, into ischemic aged rats, renal hemodynamics improves significantly (317).

Impaired NO production or decreased intrarenal NO levels may also predispose to ischemic ARF in aged rats (317). NO is tonically released from the vascular endothelium (323) and acts to induce vasodilation and oppose vasoconstriction in order to maintain blood pressure (74). Tonic basal NO levels play a significant role in maintaining renal perfusion in aging (74,324). Levels of NO from isolated conduit arteries are reduced in aging (325–327), and aging rats have a 40% decrease in serum nitric oxide synthase (NOS) substrate, L-arginine (327). Similar findings are noted in aging humans (328). More recently, however, results of studies in aging Sprague-Dawley rats evaluating gene-expression regulation of the renal arginine synthesis and small intestinal synthesis of citrulline (arginine precursor) show synthesis to be unchanged or upregulated in older rats compared with younger rats (329). This suggests that the decrease in NO in aging may not necessarily be the result of decreased substrate but may result from other changes in the NO biosynthetic pathway. Normotensive Japanese individuals given L-arginine infusion displayed a decrease in maximum percentage change of plasma cGMP, a marker for NO production, with aging (330). Older healthy subjects on a low-sodium diet and despite a fall in blood pressure showed an age-related decrease in urinary nitrate and nitrite and urinary cGMP, reflecting an age-associated decline in clearance (331). It is possible that older individuals maximize renal endothelial NO production to maintain stable renal function. This appears to be consistent with the blunted vasodilatory response seen in the elderly (136).

NO production has yet to be measured in the human ischemic kidney. However, older rats fed L-arginine for 7 days prior to renal artery occlusion have a marked improvement in GFR and RPF with a decrease in RVR. When L-NAME, a NOS inhibitor, was given to these rats, the renal hemodynamic response seen with L-arginine and superoxide dismutase was abolished (317). Whether feeding a normal or high protein diet would have changed these responses remains speculative, given that a high protein diet in aged rats decreases NO excretion as measured by urinary nitrite and nitrate excretion (NOx). Protein restriction maintains urinary NOx excretion similar to that of controls (75). L-Arginine supplementation in drinking water of aged animals appears to limit the structural changes seen in aged glomerular basement membranes (77,79). Future studies may clarify whether NO is in some way related to the beneficial effects of a low protein diet on age-associated glomerulosclerosis. Statin use prior to renal artery clamping in older rats seems to mitigate the pronounced decrease in GFR seen in comparison to younger rats. In older rats statin induced an increase in endothelial NO synthase (eNOS)-mRNA expression and eNOS protein, which may have played an important role in the protection against the decrease in GFR (332) (Fig. 80-31). Furthermore, there was histologic improvement in tubular ischemia of the outer stripe of the outer medulla in the

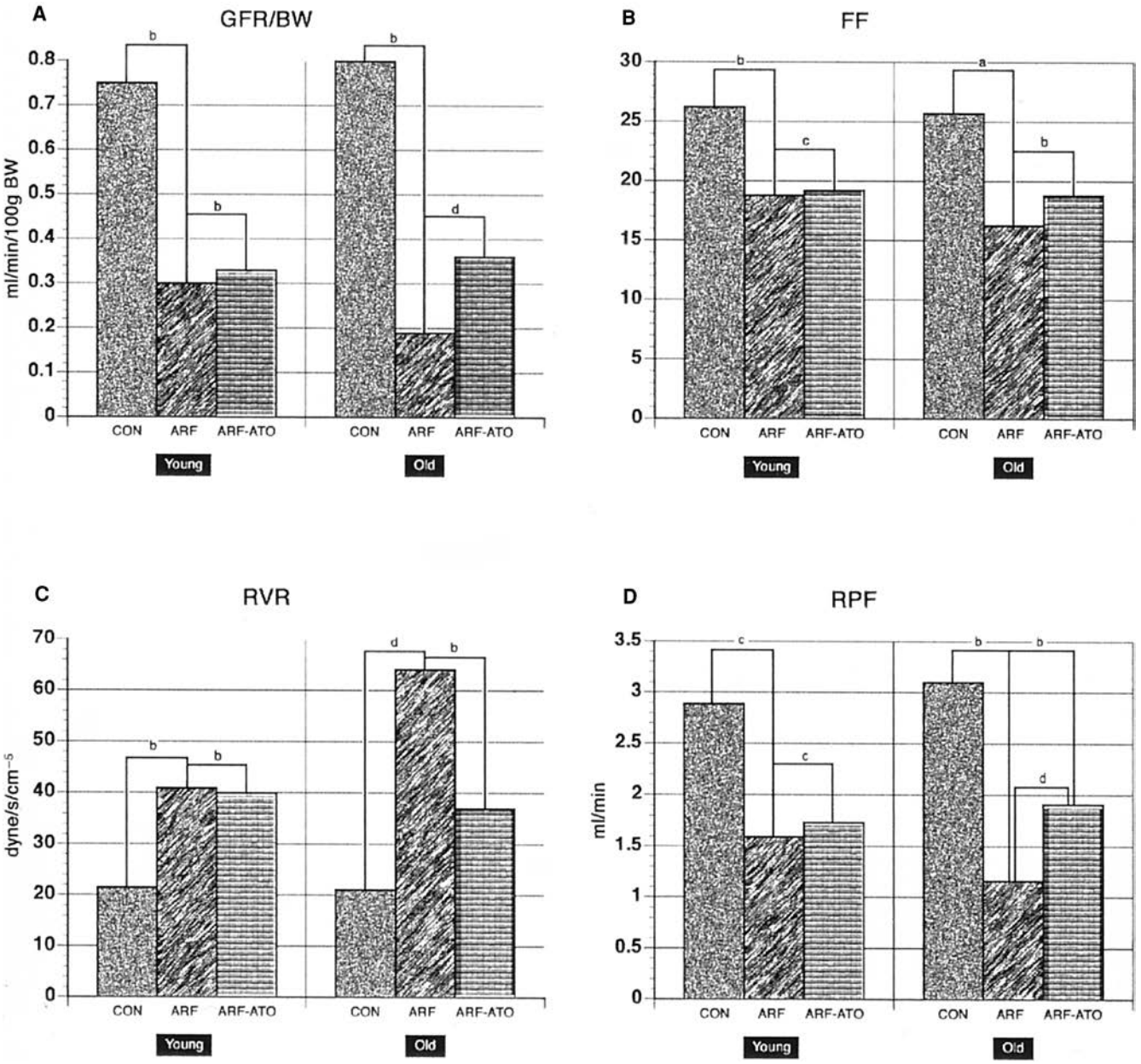

FIGURE 80-31. Renal hemodynamics (**A**) glomerular filtration factored to body weight (GFR/BW), (**B**) filtration fraction (FF), (**C**) renal vascular resistance (RVR), (**D**) renal plasma flow (RPF) in young (Y) and old (O) rats under normal conditions (CON) and 24 hours after acute renal failure (ARF), without and with ATO treatment (ATO). b = p <0.001 versus respective CON. c = p <0.05 versus respective CON, d = p <0.05 versus O-ARF group. Y-CON (n = 6), Y-ARF (n = 7), Y-ARF-ATO (n = 7), O-CON (n = 7), O-ARF (n = 8), O-ARF-ATO (n = 8). (Redrawn from: Sabbatini M, Pisani A, Uccello F, et al. Atorvastatin improves the course of ischemic acute renal failure in aging rats. *J Am Soc Nephrol* 2004;15:901.)

statin-treated older rats (332). The role of statins in acute ischemic renal failure in aging older humans needs to be evaluated.

N(G)-asymmetric dimethylarginine (ADMA), an endogenous NOS inhibitor and L-arginine analog, is noted to be significantly elevated in aged 20-month-old Sprague-Dawley rats in comparison to 6-month-old adult rats in the presence of similar levels of L-arginine (335). The ADMA levels also increase with age in humans (336). High levels of ADMA can decrease endothelial cell NO, with subsequent decreased nitrite content and NOS activity, and thereby impair endothelium-dependent vasodilation (335) and predispose the aged kidney to ischemic injury. Both higher ADMA levels and RVR in healthy elderly, and even greater levels in hypertensive elderly, are noted compared to healthy young adults in association with significantly decreased effective renal plasma flow (ERPF) (Fig. 80-32) (333). Logistic regression analysis suggests ADMA to be an independent determinant of both ERPF and RVR in these groups, suggesting that accumulation of endogenous NOS inhibitor, ADMA, may be involved in the decreased renal perfusion and increased blood pressure seen with aging (333).

Metabolic and biochemical changes in aging tubular cells can also predispose to ischemic injury (317,334). *In vitro* anoxia of renal cortical slices from younger and older rats demonstrated diminished PAH and tetraethylammonium (TEA) uptake to a greater extent in the older rats (316).

Renal atheroembolic disease with ARF is particularly common after intraarterial cannulation or surgery in elderly patients with generalized atherosclerosis. Although spontaneous cholesterol emboli also can occur (331), manipulation of the arterial tree for radiographic or surgical intervention such as carotid, coronary, renal, or abdominal angiography, aortic surgery, or percutaneous transluminal angioplasty of the coronary or renal arteries markedly increases the risk of atheroemboli. Use of anticoagulants or fibrinolytic agents in patients with diffuse atherosclerosis can also trigger cholesterol emboli (337). Renal failure is often irreversible and progressive and not always associated with other systemic manifestations such as purpura, livido reticularis of the abdominal and lumbar wall and or lower extremities, Hollenhorst plaques signifying retinal ischemia, gastrointestinal bleeding, pancreatitis, myocardial infarction, cerebral infarction, and distal ischemic necrosis of the toes (338). Associated laboratory findings may often be absent including eosinophilia, eosinophiluria, and low complement levels. There is no specific therapy for this entity except supportive care. Therefore, benefits and risk of angiographic procedures in elderly patients with widespread atherosclerotic disease need to be weighed carefully, and excessive anticoagulation should be avoided in these patients.

Elderly men presenting with symptomatic obstructive uropathy with gradual increase in BUN and serum creatinine level should be evaluated for prostatic hypertrophy (339).

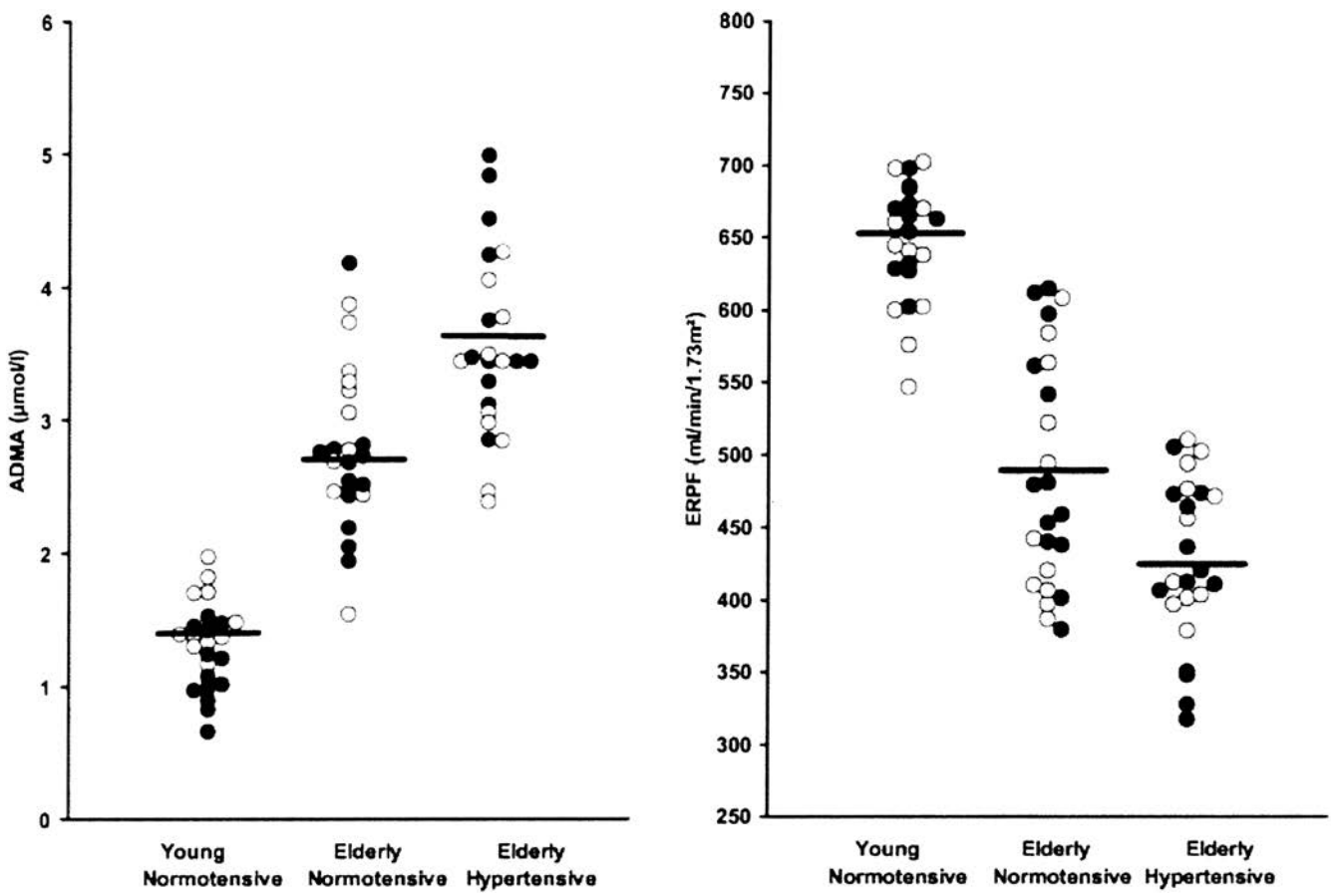

FIGURE 80-32. Individual plasma ADMA values, and ERPF values in young ($n = 24$, age = 25 ± 1 years) and old ($n = 24$, age = 69 ± 2 years) healthy subjects and elderly patients with essential hypertension ($n = 24$, age = 70 ± 2 years). • = men; ○ = women; — = mean value. (Adapted from: Kielstein JT, Bode-Boger SM, Frolich JC, et al. Asymmetric dimethylarginine, blood pressure, and renal perfusion in elderly subjects. *Circulation* 2003;107:1891.)

Pelvic tumors of the uterus or cervix in older women can encroach on the ureters to produce obstruction. Other retroperitoneal or pelvic neoplasms such as lymphoma, bladder carcinoma, and rectal tumors, can also present as ARF in the elderly. Typical obstructive symptoms of urinary frequency, voiding difficulty with starting or stopping micturition, may not always be apparent in the geriatric population. Irreversible renal failure may occur with prolonged obstruction. Careful medication review (e.g., anticholinergics) and further investigation with postvoid residual urine volume and renal ultrasonography, followed by prompt urologic intervention, can be crucial to reverse new-onset obstructive ARF. Underlying infection in the face of obstruction may worsen tubular dysfunction, renal blood flow (RBF), and GFR.

Although older individuals are at greater risk for ARF and also may take longer to recover renal function (340), age is not a critical determinant of survival in ARF (301,341) and should not be used in making therapeutic decisions concerning ARF (298,342). Many older patients respond well to dialysis support for ARF if necessary.

Renal Vascular Disease

Progressive atherosclerotic renovascular disease can be an important cause of hypertension and can lead to ischemic renal failure and ESRD in up to 15% of patients (343,344). The prevalence of renovascular disease in elderly patients age 65 and older is estimated to be 6.8% based on a cohort of study participants from the Cardiovascular Health Study (345). Patients with atheromatous involvement of the renal vasculature may present with renal artery stenosis, with complex intrarenal lesions and multiple stenosis of the renal and intrarenal vasculature, or with cholesterol embolization. Hypertension and progressive azotemia with underlying risk for generalized atherosclerosis in the elderly makes it necessary to consider of the diagnosis atherosclerotic renal disease (346–348).

Natural history of renovascular disease usually dictates progressive occlusion of the major renal arteries, particularly with luminal narrowing greater than 75% by angiography (349,350). Nearly 50% of 237 patients followed by Rimmer and Gennari showed angiographic progression of disease (351). Average decrease in GFR in 51 patients with bilateral atherosclerotic renal artery disease followed for a median of 52 months was 4 mL/minute (352). Although varying degrees of obstruction can affect one or more renal arteries, azotemic patients with high-grade renal artery stenosis in a solitary kidney or unilateral renal artery occlusion and contralateral stenosis are at greatest risk for progressive renal failure (350,353). One study notes a crude mortality rate of 45% at 60 months for subjects with bilateral renal artery stenosis (352).

Smoking is noted to be an independent risk factor and predictor for macro and microvascular renal disease in the elderly. Smokers older than 55 years of age free of other risk factors for vascular disease have decreased radionuclide measured RPF and elevated levels of endothelin-1 (ET-1) concentration compared with age- and gender-matched nonsmokers (354). Smoking can also be a predictor for renal artery stenosis (355) and increases progression to ESRD (356). With use of a multivariate "best fit" model adjusted for race, gender, weight, baseline Scr, and age for nondiabetic elderly patients older than 65 years of age in the Cardiovascular Health Study Cohort, it was determined that the number of cigarettes smoked per day was independently associated with an increase in Scr (357). Smoking also stimulates release of norepinephrine and epinephrine (358), interferes with metabolism of prostacyclin and thromboxane A2 in the endothelium (359–362), and also interferes with vascular response to acetylcholine (363), NO, and ET-1 (364,365). Renal ET-1 levels are increased in active smokers (366) and given its potent vasoconstrictor mitogenic and atherogenic activity on vascular smooth muscle, it may mediate renal arteriolar thickening (367–369). Renal ET-1 protein is found to be elevated in kidneys of healthy aging rodents (370).

Various diagnostic methods can be used to detect atherosclerotic renovascular disease. A laboratory clinical clue for either bilateral atherosclerotic renovascular disease or unilateral atherosclerotic stenosis in a solitary functional kidney is an increase in Scr with the use of AT-II antagonists (ACE inhibitor or ARB) (371). The AT-II antagonists inhibit the autoregulatory vasoconstriction of AT-II on the efferent arteriole, which preserves glomerular filtration in the context of a drop in glomerular perfusion, thereby decreasing GFR (372). By using this concept, it has been determined that renal scintigraphy with Tc-DTPA or technetium-mercaptoacetyltriglycine (99Tc-MAG3) before and after ACE inhibitor administration becomes a useful tool to investigate clinical suspicion for significant functional unilateral renal artery stenosis (373,374). Some centers have significant experience using duplex ultrasound scanning to visualize stenosis and obtain blood flow velocities in the renal arteries to determine the significance of the stenosis with high sensitivity and specificity (375). Noninvasive visualization of the renal arteries using magnetic resonance angiography is also becoming popular in many centers. Carbon dioxide angiography can also be used in the presence of decreased GFR. Contrast angiography remains the gold standard, as the renal vasculature can be visualized in full including smaller intrarenal branches. Atheromatous narrowing of distal branches of the renal arteries and microvasculature can also lead to significant hypertension and ischemic renal failure (376).

Contrast injection into diffuse atherosclerotic arteries can lead to contrast nephropathy and or cholesterol embolization. An acute reversible rise in Scr 1 to 4 days after contrast injection can be attributed to contrast dye. However, irreversible and often progressive renal failure 1 to 4 weeks after arterial contrast injection may be secondary to renal cholesterol embolization with or without systemic manifestations. Cholesterol crystals lodge in arteries 200 μm or less in diameter, including glomerular tufts (377), and appear histologically as clear biconvex clefts with surrounding inflammation because the lipid component dissolves with tissue fixation (378). Blood pressure control, avoiding further nephrotoxins or anticoagulants, and supportive care are recommended.

Procedures to preserve renal function and improve blood pressure including percutaneous transluminal angioplasty (PTCA) or surgical revascularization may be considered also if technically feasible. Many have suggested reversibility of renal failure with these procedures (380–384). Although PTCA improves renal function in some patients, almost two-thirds of patients with Scr levels of 3 mg/dL or greater have a poor outcome including failure to improve blood pressure, initiation of dialysis within months of the procedure, or death (385). Other factors associated with poor outcome of PTCA include bilateral renal artery occlusion and high-grade unilateral stenosis of a single functioning kidney (385). Easily approached lesions for angioplasty are usually non-ostial, and unilateral, although 20% of cases can recur and require repeat angioplasty (347). Intravascular stents are becoming more common (386); however, an Scr of 2 mg/dL or greater post-stent revascularization may predict poor outcome (387). Surgical revascularization is more commonly recommended for ostial, bilateral, or totally occluding lesions (347). Initial Scr of 3.2 mg/dL or greater or high-grade unilateral stenosis predicts poor outcome with surgical repair. Initial postoperative response, as reflected by unchanged or improved Scr and bilateral renal artery repair, predicts favorable long-term outcome after surgical revascularization (388). In many patients the presence of collateral circulation sometimes offers renal parenchymal

protection from ischemic injury despite the presence of significant stenosis (379,380).

Acute Glomerulonephritis

Acute glomerulonephritis in the elderly presents commonly as rapidly progressive glomerulonephritis (RPGN). Histopathologic evaluation typically demonstrates more than 50% glomeruli with crescents. In a series of 60 patients with crescentic glomerulonephritis, the average and median age was older than 60 years. Clinically the elderly patient experiences a rapid loss of renal function in the face of an active urine sediment including hematuria, pyuria, red blood cell casts, and moderate-to-severe proteinuria. Although convincing evidence for immunologic injury may be absent in many cases, immune-mediated pathogenesis is suspected. Three major types of immunohistopathology are usually seen in the kidney: type 1, the presence of antiglomerular basement membrane antibody; type 2, granular immune deposits; type 3, no immune deposits, although circulating anti-neutrophilic cytoplasmic antibody (ANCA) may be present (389). Elderly patients present more frequently with a type 2 or type 3 histologic pattern (390,391). Renal biopsy in 115 elderly patients with RPGN demonstrated the following: 19 presented with crescentic glomerulonephritis of which 9 patients had granular immunoglobulin G (IgG) deposition on immunofluorescence, 6 had no immune deposits, and 3 had antiglomerular basement membrane disease (392). In another study, 8 of 10 elderly patients with RPGN had ANCA-positive sera, although no immune deposits were evident (393). In a larger series of 259 patients 60 years and older presenting with acute renal failure pauci-immune crescentic glomerulonephritis was diagnosed in 79 (31.2%) (394).

Diffuse proliferative poststreptococcal acute glomerulonephritis in the elderly is often associated with skin and throat infection and favors a good prognosis (396,398–400). The incidence can be as high as 22.6% in patients older than 55 years of age (396,401). A study from the Indian subcontinent suggests that 82% of patients 60 years and older presenting with acute glomerulonephritis had postinfectious endocapillary glomerulonephritis (402).

In general, elderly patients presenting with RPGN have poor prognosis, although patients with poststreptococcal disease fare better. Of patients presenting with crescentic glomerulonephritis in two studies, the median age was older than 60 (395). Risk-to-benefit considerations for treatment with pulse steroids, cyclophosphamide, and or plasmapheresis for RPGN in the elderly must be individualized given significant side effects associated with the available treatment options (390,395,397).

Nephrotic Syndrome and Chronic Glomerulonephritis

Significant proteinuria, hypertension, and edema without known cause necessitate renal biopsy in the elderly. Thirty percent of elderly patients were biopsied for nephrotic syndrome as noted from the Medical Research Council Glomerulonephritis Registry from 1978 to 1990 (403). The most common histologic finding was membranous glomerulopathy (36.6%), followed by minimal change (11%) and renal amyloidosis (10.7%). Various case series from other countries including the United States, France, Israel, England, Japan, Korea, and India concur with these findings (392,402, 404–413). Cumulative data are presented in Table 80-3. Of 545 patients presenting with nephrotic syndrome, 54% are noted to have presented with membranous glomerulonephritis, 19% with minimal change, 9% with mesangial proliferative, and 9% with membranoproliferative glomerulonephritis (394,400–407,409,412,413).

Minimal change disease in the elderly can be associated with a greater risk of acute renal failure, severe proteinuria, and lower serum albumin levels than in younger patients (414). Systemic amyloidosis, commonly AL type, also is not uncommon in the elderly with nephritic syndrome. Associated paraproteinemia may be present in 40% to 68% of elderly noted to have amyloidosis (415). Therefore, elderly patients with unexplained nephrotic syndrome should have serum and urine electrophoresis done. Abnormal findings from these tests should then prompt bone marrow evaluation for multiple myeloma. Congo red stain of abdominal fat pad aspirates may be useful in the diagnosis of amyloidosis. Kidney biopsy tissue in elderly with unexplained proteinuria should be routinely stained with Congo red and evaluated with electron microscopy for amyloid fibrils. Treatment with melphalan, prednisone, and thalidomide may delay the progression of myeloma to ESRD.

Glomerulosclerosis alone can present as nephrotic syndrome in approximately 7% of renal biopsies in the elderly. This sclerosis resembles the focal and segmental

TABLE 80-3

HISTOLOGIC LESIONS IN 545 ELDERLY PATIENTS WITH PRIMARY NEPHROTIC SYNDROME

Authors	Minimal change	Membranous glomerulo-nephritis	Mesangial proliferative glomerulonephritis	Membrano-proliferative glomerulonephritis	Glomerulo-sclerosis	Chronic glomerulonephritis
Fawcett et al., 1971	6	5	—	4	16	5
Huriet et al., 1975	4	2	—	6	—	—
Moorthy and Zimmerman, 1980	9	15	7	2	1	—
Ishimuto et al., 1981	1	6	—	2	7	—
Lustig et al., 1982	2	16	—	2	3	—
Zech et al., 1982	19	31	2	4	—	3
Kingswood et al., 1984	2	16	11	3	—	—
Murphy et al., 1987	2	2	—	2	—	—
Sato et al., 1987	7	30	12	7	1	—
Johnston et al., 1992	35	116	18	—	5	—
Ozono et al., 1994	6	26	—	8	—	—
Shin et al., 2001	14	27	—	8	6	1
Total	107(19%)	292(54%)	50(9%)	48(9%)	39(7%)	9(2%)

glomerulosclerosis seen more commonly in younger patients, with juxtaglomeruli commonly affected and immunofluorescence positive for IgG and the third complement component, C3. These histologic findings are usually the end result of other glomerulopathies and or secondary advanced systemic diseases, including hypertension and diabetes (416), with hyperfiltration likely hastening the process of sclerosis (41,417). The higher filtration fraction in the juxtamedullary glomeruli make them more susceptible. Glomerular ischemia and renovascular disease can lead to adaptive glomerular enlargement and segmental sclerosis with nephrotic proteinuria in the elderly (418). These histologic features are also evident in healthy aging rats with proteinuria (419–424).

Renal biopsy is necessary for histologic diagnosis of nephrotic syndrome and subsequent therapeutic decisions in the elderly. Treatment with steroid alone is not effective in idiopathic membranous nephropathy in the elderly per retrospective review of 33 of 74 patients receiving treatment. Although the rate of change in renal function was not different compared to a younger group, the incidence of renal failure was higher (425). Decreased renal functional reserve in the elderly may contribute to a more negative outcome in the elderly with nephrotic syndrome. Partial and complete remission with protection from renal failure using cytoxic agents such as chlorambucil or Cytoxan with steroids has been accomplished in idiopathic membranous nephropathy (426). In addition, cyclosporine and mycophenolate are being used in those who have failed initial treatment with chlorambucil or Cytoxan and steroid, although successful results remain anecdotal. The use of steroids, cytotoxic agents, and immunosuppressive agents needs to be carefully evaluated and individualized in elderly patients with idiopathic membranous nephropathy. Response to treatment for membranoproliferative glomerulonephritis is quite variable. Nearly 20% of elderly can present with minimal change nephrotic syndrome and they have greater likelihood of response to steroids.

Malignancy can often precede or coexist with nephrotic syndrome (427), particularly membranous nephropathy, in the elderly and needs to be considered in the evaluation. Seven percent to 20% of patients with nephrotic syndrome present with a malignancy (411,428). The association of membranous lesions and malignancy is thought to be immune mediated, secondary to tumor-associated antigens (428). Solid tumors of lung, colon, breast, rectum, kidney, and stomach seem to be more common with membranous glomerulopathy. Therefore, a thorough history, physical examination, and basic screening for underlying malignancy need to be done in the elderly with nephrotic syndrome.

Chronic Renal Failure

Gradual progression of underlying renal disease or chronic medical illnesses affecting the kidney often leads to chronic renal failure later in life. Approximately 6.6 million people older than 60 years have GFR of less than 60 mL/minute/1.73 m_2 per NHANES III data (429). Longstanding diabetes mellitus, hypertension, chronic glomerulonephritis, ischemic renovascular disease, and obstructive uropathy lead in common diagnoses. The level of GFR independently predicts the risk of cardiovascular disease and all-cause mortality as determined by a cohort of community dwelling subjects older than 65 years in the Cardiovascular Health Study (430). The elderly may present with decompensated preexisting medical illness such as heart failure, gastrointestinal bleeding, hypertension, and dementia rather than with frank uremic symptoms *per se*. Scr may not change significantly given gradual muscle loss in the elderly, thereby masking detectable change in renal function. Renal reserve is frequently lower than that estimated by Scr.

If reversible causes of chronic progression to ESRD are excluded, then preparation for renal replacement therapy is indicated. Older patients experience uremic symptoms at lower Scr levels than do younger patients, hence often requiring earlier dialysis initiation. Subtle manifestations of uremia may present as repeated episodes of heart failure, progressive weight loss, and behavioral changes. Age alone is not exclusionary to the need for dialysis support. In the absence of other comorbid systemic illness or extrarenal organ failure, elderly adjust fairly well to dialysis. Currently more than 55% of ESRD patients on dialysis are older than 60 years of age. Longevity of older dialysis patients is less than younger patients, however (431–433). Octogenarians appear to have a median survival of 2 years on thrice-weekly hemodialysis (434). Rapid ultrafiltration from short thrice-weekly dialysis sessions may lead to more frequent hypotension and risk for silent ischemia in the elderly (435–437). Therefore, careful volume management on dialysis in the elderly is necessary.

Elderly with significant cardiovascular disease may fare better with peritoneal dialysis (432,438–441). No difference is evident in the incidence of peritonitis, type of infectious organism, or likelihood of technique failure between young and old patients (432,442). In fact, elderly patients often require fewer peritoneal catheter replacements than do younger patients (432,442).

Neither dialysis mode has been clearly proved superior to the other in the elderly (443–445). The presence of comorbid illness has produced conflicting results when considering preferential dialysis modality (432). Automated peritoneal dialysis patients older than 65 years of age in Chile fared similar to younger patients, as comorbidities resulted in decreased actuarial survival not due to peritoneal dialysis. (446). Data from ESRD elderly diabetic and nondiabetic Medicare patients suggest that hemodialysis may have a slightly better survival rate than continuous ambulatory peritoneal dialysis (CAPD) (447). Patient selection for either modality is dependent on various factors including patient preference, social support, comorbid disease (including cardiac disease), early dementia, and ability to establish a functioning vascular or peritoneal access. Patients in whom the ability to obtain patent vascular access or with significant hemodynamic instability during hemodialysis may do well on peritoneal dialysis. Similarly hemodialysis may be preferred for deconditioned patients or those unable to perform self-care dialysis. The social aspect of in-center dialysis may be preferable for those who live alone or who are depressed. Mortality of the elderly ESRD patient results from comorbid cardiovascular complications, infections, malnutrition, malignancy, and dialysis withdrawal.

The decision to pursue renal transplantation is not specifically determined by age alone. Many medically eligible elderly have undergone successful renal transplantation (448,448). Donor age appears to be more significant than recipient age in predicting delayed graft function (4,450) (Fig. 80-33). A decision-analytic model comparing cadaveric renal transplantation with hemodialysis notes that well-selected elderly patients younger than 70 years of age with fewer comorbidities have significant quality-adjusted life expectancy gains of 1.1 years with transplantation (451). When comparing patients older than 60 years of age matched demographically for comorbidities, who have undergone rigorous screening and from whom 5-year survival in anticipated in excess of 80%, those who have undergone transplantation have significant survival advantage over those who have undergone dialysis. The preoperative 1-, 3-, and 5-year survival is 98%, 95%, and 90%, compared with 92%, 62%, and 27%, respectively (452). Posttransplantation 1-year patient survival and allograft survival rates are similar for both young and old patients. The major reason for allograft loss posttransplantation in the elderly is patient demise from cardiovascular disease or infection (449,453).

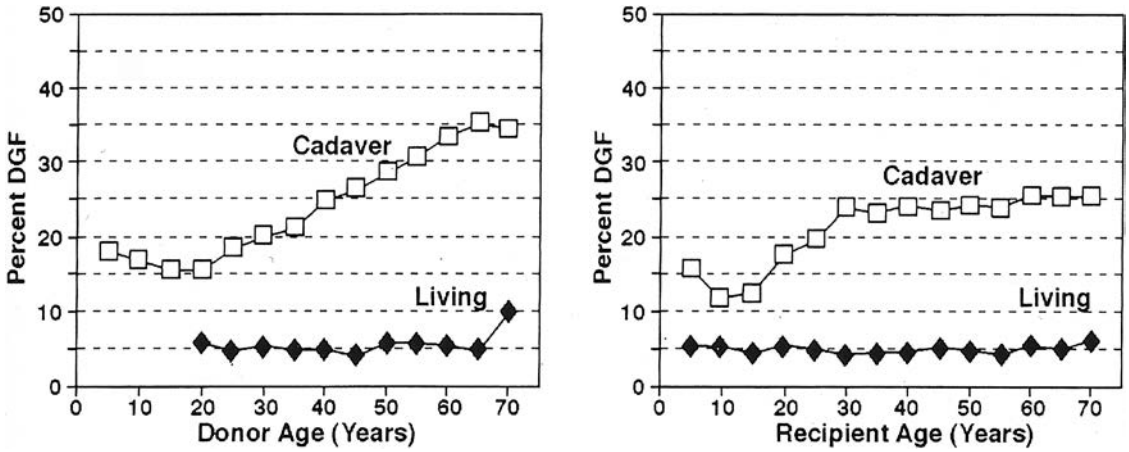

FIGURE 80-33. Effect of donor age on delayed graft function (DGF) of first transplants (1996–2000). (Reprinted from: Cecka JM. The UNOS renal transplant registry. *Clin Transplant* 2001:1, with permission.)

Review of data from the U.S. Renal Data System (USRDS) and United Network for Organ Transplant Scientific Registry from 1988 to 1997 reveals age to be an independent factor in the increased relative risk of death from infection in elderly patients who undergo transplantation (454). Older patients also seem to have a decrease in acute rejection (455). Therefore, underlying comorbid cardiovascular disease and the ability to tolerate immunosuppressive therapy in light of "biologic age" need to be carefully considered prior to renal transplantation in the elderly (455,456).

Urinary Tract Infection

The frequency of urinary tract infection may be significant in the elderly. Functional changes of the bladder, altered immune defenses, changes in pelvic musculature, prostatic enlargement, neurogenic bladder dysfunction, impaired mobility, dementia, and cerebrovascular accidents leading to poor hygiene are processes that contribute to the higher incidence of urinary tract infection in the elderly (457,458). Decreased prostatic secretions of older men and microcalculi provide the environment for harboring infection in the lower urinary tract and prostate (459). Postmenopausal women have low estrogen levels, which change the pH of the vaginal secretions, thereby allowing bacterial colonization and subsequent cystitis (460). Intravaginal estrogen use may help in the prevention of recurrent urinary tract infection in older women (461).

The prevalence of asymptomatic bacteruria in healthy older men can be as high as 20%, and increases to 25% in both men and women living in extended care facilities (462), 35% of aged in nursing homes, and 30% to 50% of older hospitalized patients (463). Creatinine clearance is decreased in elderly persons with bacteriuria in comparison to those without bacteriuria (243,464). The mechanism is not clear but it is possible that chronic inflammation in the kidney may lead to gradual tubulointerstitial fibrosis and glomerulosclerosis with loss of renal function. Predisposing factors leading to chronic bacteriuria often also account for decreased survival in these patients (465). Treatment of asymptomatic bacteriuria does not result in improved survival (466,467). Because treatment failure and relapse is high, asymptomatic bacteruria without evidence for underlying renal or urologic abnormalities need not be treated (468). Benefits of chronic suppressive therapy are still debated given possible emergence of resistant Gram-negative strains (469,470).

Renal Cysts

Simple renal cysts progressively develop in aging kidneys. With the increased frequency of diagnostic abdominal imaging studies, a greater prevalence of incidental cysts is noted. Microscopic dissection of kidneys from older adult kidneys (greater than 50 years) reveal ectasia, diverticula, and microscopic cysts not noted in 20-year-old kidneys. More than one-half of patients older than age 50 have at least one renal cyst noted on postmortem examination (471). Sonographic prevalence of acquired renal cysts is 22% in aged persons older than 70 years compared to 0% in those between 15 and 29 years of age (472). More commonly these acquired cysts are simple, painless, and asymptomatic. Some have noted abdominal or lumbar discomfort, hematuria, secondary infection, and, occasionally, renin-dependent hypertension. Simple cysts are defined as cysts with a thin smooth wall and clear, fluid-filled space void of internal echoes by sonogram. These cysts require no treatment or follow-up. Cysts that are thick walled and debris filled, with internal echoes or associated with a renal mass are designated as complicated and need to be investigated by cyst puncture, angiography, or surgery to rule out infection, bleeding, or malignancy.

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SECTION XI: DISORDERS OF ELECTROLYTE, WATER, AND ACID–BASE

CHAPTER 81 ■ MECHANISMS OF DIURETIC ACTION

DAVID H. ELLISON, MARK D. OKUSA, AND ROBERT W. SCHRIER

INTRODUCTION

The term *diuretic* derives from the Greek *diouretikos*, which means, "to promote urine." Although many substances promote urine flow, the term *diuretic* is usually taken to indicate a substance that can reduce the extracellular fluid volume by increasing urinary solute and water excretion (1). In 1553, Paracelsus recorded the first truly effective form of therapy for dropsy (edema), namely inorganic mercury (Calomel [2]). Inorganic mercury remained the mainstay of diuretic treatment until the beginning of this century. In 1919, the ability of organic mercurial antisyphilitics to effect diuresis was discovered by Vogl, then a medical student (3). This observation led to the development of effective organic mercurial diuretics that continued to be used through the 1960s. In 1937, the antimicrobial, sulfanilamide, was found to cause metabolic acidosis. Carbonic anhydrase, which had been discovered in 1932, was inhibited by sulfanilamide. Pitts demonstrated that sulfanilamide inhibited Na bicarbonate reabsorption in dogs (4) and Schwartz showed that sulfanilamide could induce diuresis when administered to patients with congestive heart failure (5). Soon, more potent sulfonamide-based carbonic anhydrase inhibitors were developed, but these drugs were associated with side effects and limited potency. Nevertheless, a group at Sharp & Dohme, Inc. was stimulated by these developments to explore the possibility that modification of sulfonamide-based drugs could lead to drugs that enhanced Na *chloride* rather than Na *bicarbonate* excretion. The result of this program was the synthesis of chlorothiazide and its marketing in 1957 (6–8). This drug ushered in the modern era of diuretic therapy and revolutionized the clinical treatment of edema.

The search for more potent classes of diuretics led to the development of ethacrynic acid and furosemide in the United States and Germany, respectively (4) The safety and efficacy of these drugs led them to replace the organic mercurials as drugs of first choice for severe and resistant edema. Spironolactone, marketed in 1961, was developed after the properties and structure of aldosterone had been discovered and steroidal analogs of aldosterone were found to have aldosterone-blocking activity (9). Triamterene was initially synthesized as a folic acid antagonist, but was found to have diuretic and potassium-sparing activity (10). The identification of the arginine vasopressin (AVP) receptor subtypes led to the more recent development of V2 AVP antagonists, which are likely to enter clinical practice during the next several years (11,12). The identification and cloning of natriuretic peptides (13,14) led to the development of drugs with similar effects (15).

The availability of safe, effective and relatively inexpensive diuretic drugs has made it possible to treat edematous disorders and hypertension effectively. Incidentally, driven by clinical drug development, specific ligands that interact with discrete Na and Cl transport proteins in the kidney were developed that permitted these transport proteins to be identified. Subsequently, these ligands were used to clone the Na and Cl transport proteins that mediate the bulk of renal Na and Cl reabsorption. The diuretic-sensitive transport proteins that have been cloned include the sodium hydrogen exchanger (NHE3; Gene symbol, *SLC9A3*) (16–18), the bumetanide-sensitive Na-K-2Cl cotransporters (NKCC2; Gene symbol, *SLC12A1*) (19,20), the thiazide-sensitive Na-Cl cotransporter (NCC; Gene symbol, *SLC12A3*) (21), and the epithelial Na channel (ENaC; Gene symbols, *SCNN1A*, *SCNN1B*, *SCNN1G*) (22–24). The information derived from molecular cloning has also permitted identification of inherited human diseases that are caused by mutations in transport proteins (25–31). The phenotypes of several of these disorders resemble the manifestations of chronic diuretic administration. Therefore, the development of clinically useful diuretics permitted identification and later cloning of specific ion-transport pathways. The molecular cloning is now helping to define mechanisms of diuretic action and diuretic side-effects. The use of animals in which diuretic-sensitive transport pathways have been "knocked out" should permit a clearer understanding of which diuretic effects result directly or secondarily from actions of the drugs on specific ion transport pathways and which effects result from actions on other pathways or other organ systems.

NORMAL RENAL NACL HANDLING

The normal human kidneys filter approximately 23 moles of NaCl in 150 L of fluid each day. Approximately 6 to 10 g of salt (i.e., 17 mEq/g of NaCl, 43 mEq/g of Na) is consumed each day by individuals who ingest a typical Western diet. To maintain balance, renal NaCl excretion must be approximately 160 mmol/day (the difference owing to nonrenal losses). Under normal circumstances, approximately 99.2% of the filtered NaCl is reabsorbed by kidney tubules, generating a normal fractional sodium excretion of less than 1%. Sodium, chloride, and water reabsorption by the nephron is driven by the metabolic energy provided by adenosine triphosphate (ATP). The ouabain-sensitive Na/K ATPase is expressed at the basolateral cell membrane of all Na-transporting epithelial cells along the nephron. This pump maintains large ion gradients across the plasma membrane, with the intracellular Na concentration maintained low and the intracellular K concentration maintained high. Because the pump is electrogenic and associates with a K channel in the same membrane, renal epithelial cells have a voltage across the plasma membrane oriented with the inside negative relative to the outside.

The combination of the low intracellular Na concentration and the plasma membrane voltage generates a large electrochemical gradient favoring Na entry from lumen or interstitium. Specific diuretic-sensitive Na-transport pathways are expressed at the apical (luminal) surface of cells along the nephron, permitting vectorial transport of Na from lumen

TABLE 81-1

EFFECTS OF DIURETICS ON ELECTROLYTE EXCRETION

	Na	Cl	K	Pi	Ca	Mg
Osmotic diuretics (45,46,297,513,514)	⇑(10%–25%)	⇑(15%–30%)	⇑(6%)	⇑(5%–10%)	⇑(10%–20%)	⇑(20%)
Carbonic anhydrase inhibitors (88,190,297)	⇑(6%)	⇑(4%)	⇑(60%)	⇑(>20%)	⇑ or ⇔ (<5%)	⇑(<5%)
Loop diuretics (187,188,190,271, 297,474)	⇑(30%)	⇑(40%)	⇑(60%–100%)	⇑(>20%)	⇑(>20%)	⇑(>20%)
DCT diuretics (188,271,297,298)	⇑(6%–11%)	⇑(10%)	⇑(200%)	⇑(20%)	⇓	⇑(5%–10%)
Na channel blockers (271,297,474)	⇑(3%–5%)	⇑(6%)	⇓(8%)	⇔	⇔	⇓
Collecting duct diuretics (297)	⇑(3%)	⇑(6%)	⇓	⇔	⇔	⇓

Values indicate approximate maximal fractional excretions of ions following acute diuretic administration in maximally effective doses. ⇑ indicates that the drug increases excretion; ⇓ indicates that the drug decreases excretion; ⇔ indicates that the drug has little of no direct effect on excretion. During chronic treatment, effects often wane (Na excretion), may increase (K excretion during DCT diuretic treatment), or may reverse as with uric acid (not shown).

to blood. Along the proximal tubule, where approximately 50% to 60% of filtered Na is reabsorbed, an isoform of the Na/H exchanger is expressed at the apical membrane (32). Along the thick ascending limb, where approximately 20% to 25% of filtered Na is reabsorbed, an isoform of the Na-K-2Cl cotransporter is expressed at the apical membrane (33,34). Along the distal convoluted tubule, where approximately 5% of filtered Na is reabsorbed, the thiazidesensitive Na-Cl cotransporter is expressed (35,36). Along the connecting tubule and cortical collecting duct, where approximately 3% of filtered Na is reabsorbed, the amiloride-sensitive epithelial Na channel is expressed (37). These apical Na-transport pathways form the targets for diuretic action.

This chapter will discuss the molecular and physiologic bases for diuretic action in the kidney. Although some aspects of clinical diuretic usage will be discussed, we have emphasized physiologic principles and mechanisms of action. Several recent texts provide detailed discussions of diuretic treatment of clinical conditions (38). Extensive discussions of diuretic pharmacokinetics are also available (39).

A rational classification of diuretic drugs is based on the primary nephron site of action. Such a scheme emphasizes, first, that more than one chemical class of drugs can affect the same ion-transport mechanism. Second, although most diuretic drugs affect transport processes of several nephron segments, most owe their clinical effects primarily to their ability to inhibit Na transport by one particular nephron segment. An exception is the osmotic diuretic. Although these diuretics were initially believed to inhibit solute and water flux primarily along the proximal tubule, subsequent studies have revealed effects in multiple segments. Other diuretics, however, will be classified according to their primary site of action.

OSMOTIC DIURETICS

Osmotic diuretics are substances that are freely filtered at the glomerulus, but are poorly reabsorbed (see Fig. 81-1). The pharmacologic activity of drugs in this group depends entirely on the osmotic pressure exerted by the drug molecules in solution. It does not depend on interaction with specific transport proteins or enzymes. Mannitol is the prototypical osmotic diuretic (40,41). Because the relationship between the magnitude of diuretic effect and concentration of osmotic diuretic in solution is linear, all osmotic diuretics are small molecules. Other agents considered in this class include urea, sorbitol, and glycerol.

Urinary Electrolyte Excretion

Although osmotic agents do not act directly on transport pathways, ion transport is affected. Following mannitol infusion, sodium, potassium, calcium, magnesium, bicarbonate, and chloride excretion rates increase (Table 81–1) (42–44). Rates of sodium and water fractional reabsorption are reduced by 27% and 12%, respectively, following the infusion of mannitol (45). Reabsorption of magnesium and calcium is also reduced in the proximal tubule and loop of Henle. In contrast, phosphate reabsorption is only inhibited slightly by mannitol in the presence of parathyroid hormone (42).

Mechanism of Action

The functional consequences that result from intravenous infusion of mannitol include an increase in cortical and medullary blood flow; a variable effect on glomerular filtration rate; an increase in sodium, water, calcium, magnesium, phosphorus, and bicarbonate excretion; and a decrease in medullary concentration gradient. The most pronounced effect observed with mannitol is a brisk diuresis and natriuresis. The mechanisms by which mannitol produces a diuresis include: (i) an increase in osmotic pressure in the lumens of the proximal tubule and loop of Henle, thereby retarding the passive reabsorption of water, and (ii) an increase in renal blood flow and washout of medullary tonicity.

Mannitol is freely filtered at the glomerulus and its presence in tubule fluid minimizes passive water reabsorption.

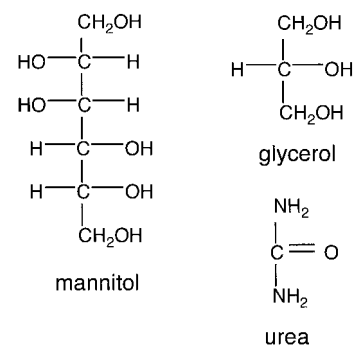


FIGURE 81-1. Structures of osmotic diuretics.

Normally, within the proximal tubule, sodium reabsorption creates an osmotic gradient for water reabsorption. When an osmotic diuretic is administered, however, the osmotic force of the nonreabsorbable solute in the lumen opposes the osmotic force produced by sodium reabsorption (46). Isosmolality of tubule fluid is preserved because molecules of mannitol replace sodium ions reabsorbed. However, sodium reabsorption eventually stops because the luminal sodium concentration is reduced to a point where a limiting gradient is reached and net transport of sodium and water ceases (42,45,47). This view was confirmed by stationary micropuncture studies (48). Quantitatively, mannitol has a greater effect on inhibiting Na and water reabsorption in the loop of Henle than in the proximal tubule. Free-flow micropuncture studies following mannitol infusion in dogs demonstrated a modest decrease in fractional reabsorption of sodium and water by the proximal tubule, but a much larger effect by the loop of Henle (42,45). Within the loop of Henle the site of action of mannitol appears to be restricted to the thin descending limb resulting in a decrease in reabsorption of water (49). In the thick ascending limb, reabsorption of Na will continue in proportion to its delivery to this segment. The sum of net transport in the thin and thick limbs will determine the net effect of mannitol in the loop of Henle. Further downstream in the collecting duct, mannitol reduces sodium and water reabsorption (50).

Renal Hemodynamics

During the administration of mannitol, its molecules diffuse from the bloodstream into the interstitial space. In the interstitial space, the increased osmotic pressure draws water from the cells to increase extracellular fluid volume. This effect increases total renal plasma flow (50). Cortical and medullary blood flow rates both increase following mannitol infusion (50,51). Conversely, single nephron glomerular filtration rate (GFR) increases in cortex and decreases in medulla (49,52); this action on the medulla washes out the medullary osmotic gradient by reducing papillary sodium and urea content (47,53,54). Experimental studies indicate that the osmotic effect of mannitol to increase water movement from the intracellular space to the extracellular space leads to a decrease in hematocrit and in blood viscosity. This fact contributes to a decrease in renal vascular resistance and increase in renal blood flow (47). In addition, secretion of vasodilatory substances is stimulated by mannitol infusion. Both prostacyclin (PGI_2) (55) and atrial natriuretic peptide (56) could mediate the effect of mannitol on renal blood flow. The vasodilatory effect of mannitol is reduced when the recipient is pretreated with indomethacin or meclofenamate, suggesting that PGI_2 is involved in the vasodilatory effect.

The effect of mannitol on GFR has been variable but most studies indicate that the overall effect is to increase GFR (40,41,45,57,58). Whereas mannitol was shown to increase both cortical and medullary blood flow, it increased cortical but decreased medullary single nephron GFR (49,52). The mechanisms by which mannitol reduces the GFR of deep nephrons are not known, but it has been postulated that mannitol reduces efferent arteriolar pressure. Micropuncture studies examining the determinants of GFR in superficial nephrons have demonstrated that the increase in single nephron GFR results from an increase in single nephron plasma flow and a decrease in oncotic pressure (58).

Alterations in renal hemodynamics contribute to the diuresis observed following administration of mannitol. An increase in medullary blood flow rate reduces medullary tonicity (54), primarily by decreasing papillary sodium and urea content (59) and increasing urine flow rate (47).

Pharmacokinetics

Mannitol is not readily absorbed from the intestine (40); therefore, it is routinely administered intravenously. Following infusion, mannitol distributes in extracellular fluid with a volume of distribution of approximately 16 L (60); its excretion is almost entirely by glomerular filtration (61). Of the filtered load, less than 10% is reabsorbed by the renal tubule, and a similar quantity is metabolized, probably in the liver. With normal glomerular filtration rate, plasma halflife is approximately 2.2 hours.

Clinical Use

Mannitol is used prophylactically or as treatment for established acute renal failure (40,62,63), although the basis for its use in established acute renal failure remains controversial (63–65). Mannitol improves renal hemodynamics in a variety of situations of impending or incipient acute renal failure. Mannitol (along with hydration and sodium bicarbonate) has been recommended by some (66,67), but not all (68), investigators for the early treatment in myoglobinuric acute renal failure and to prevent posttransplant acute renal failure (69–71). Mannitol is used perioperatively to treat patients undergoing cardiopulmonary bypass surgery. The beneficial effects may relate to its osmotic activity, thereby reducing intravenous fluid requirement (72) and its ability to act as a free radical antioxidant (73). Although some studies have shown a beneficial effect when used prophylactically to treat patients at risk for contrast nephropathy (74), most prospective controlled studies have not found mannitol beneficial in preventing acute renal failure and it is not currently recommended (64,75).

Mannitol is used for shortterm reduction of intraocular pressure (76). By increasing the osmotic pressure, mannitol reduces the volume of aqueous humor and the intraocular pressure by extracting water. Mannitol also decreases cerebral edema and the increase in intracranial pressure associated with trauma, tumors, and neurosurgical procedures (72,73,77). Mannitol and other osmotic agents have been used to treat dialysis disequilibrium (78,79). This syndrome is characterized by acute symptoms during or immediately following hemodialysis. Most significant symptoms are attributable to disorders of the central nervous system such as: headache, nausea, blurred vision, confusion, seizure, coma, and death. Rapid removal of small solutes such as urea during dialysis of patients who are markedly azotemic is associated with the development of an osmotic gradient for water movement into brain cells producing cerebral edema and neurologic dysfunction. Dialysis disequilibrium syndrome can be minimized by slow solute removal and raising plasma osmolality with saline or mannitol.

Adverse Effects

Patients who have a reduced cardiac output may develop pulmonary edema when mannitol is infused. Intravenous mannitol administration increases cardiac output and pulmonary capillary wedge pressures (60). Acute and prolonged administration of mannitol leads to different electrolyte disturbances. Acute overzealous use or the accumulation of mannitol leads to dilutional metabolic acidosis and hyponatremia (80). Accumulation of mannitol also produces hyperkalemia (81,82) as a result of an increase in plasma osmolality. An increase in plasma osmolality increases potassium movement from intracellular to extracellular fluid from bulk solute flow and increase in the electrochemical gradient for potassium secretion.

Prolonged administration of mannitol can lead to urinary losses of sodium and potassium, thereby leading to volume depletion, hypernatremia (as urinary loss of sodium is invariably less than water) and hypokalemia (83). Marked accumulation of mannitol in patients can lead to reversible acute renal failure that appears to be caused by vasoconstriction and tubular vacuolization (84–86). Mannito-linduced acute renal failure usually occurs when large cumulative doses of ~295 g are given to patients with previously compromised renal function (84).

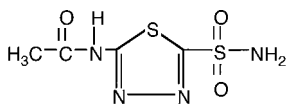
PROXIMAL TUBULE DIURETICS (CARBONIC ANHYDRASE INHIBITORS)

Through the development of carbonic anhydrase inhibitors, important compounds were discovered that have utility as therapeutic agents and as research tools. Carbonic anhydrase inhibitors have a limited therapeutic role as diuretic agents; however, because they are only weakly natriuretic. They are used primarily to reduce intraocular pressure in glaucoma and to enhance bicarbonate excretion in metabolic alkalosis. Carbonic anhydrase inhibitors have been useful in developing other diuretic agents such as thiazide and loop diuretics and have been instrumental in elucidating transport mechanisms in proximal and distal nephron segments. The structure of a prototypical carbonic anhydrase inhibitor acetazolamide, is shown in Figure 81-2.

Urinary Electrolyte Excretion

Through their effects on carbonic anhydrase in the proximal tubule, carbonic anhydrase inhibitors increase bicarbonate excretion by 25% to 30% (see Table 81-1) (87–90). The increase in sodium and chloride excretion is smaller than might be expected, because these ions are reabsorbed by more distal segments of the nephron (90). However, a residual small but variable amount of sodium is excreted along with bicarbonate. Rates of calcium and phosphate reabsorption are also blocked along the proximal tubule by carbonic anhydrase inhibitors. Because distal calcium reabsorption is stimulated, fractional calcium excretion does not increase (91). In contrast, phosphate appears to escape distal reabsorption following acetazolamide administration resulting in an increase in fractional excretion of phosphate by approximately 3% (89,91). Although proximal tubule magnesium transport is inhibited by carbonic anhydrase inhibitors, fractional excretion of magnesium is either unchanged or increased as a result of variable distal reabsorption (89).

Carbonic anhydrase inhibitors increase potassium excretion (89,90,92,93). It is likely that several indirect effects of carbonic anhydrase inhibition contribute to the observed kaliuresis. Carbonic anhydrase inhibition could block proximal tubule potassium reabsorption and increase delivery to the distal tubule, but this has not been established clearly. Whereas carbonic anhydrase inhibitors decrease proximal tubule sodium, bicarbonate and water absorption during both free flow micropuncture and microperfusion, the effects of carbonic anhydrase inhibitors on proximal tubule potassium transport have been less consistent. In free flow micropuncture studies, carbonic anhydrase inhibition did not affect proximal tubule potassium reabsorption (91), whereas carbonic anhydrase inhibition of perfused proximal tubules reduces net potassium transport (94). The effect of carbonic anhydrase inhibitors on the proximal tubule ion transport does, however, facilitate an increase in tubular fluid flow rate and sodium and bicarbonate but not chloride delivery to the distal nephron. This effect is thought to increase the concentration of nonreabsorbable anions in the distal tubule lumen, creating an increase in lumen negative voltage (95) and increase in flow rate (96), factors known to increase potassium secretion by the distal tubule. Carbonic anhydrase inhibitors can also produce a luminal composition that is low in chloride and high in nonchloride anion. This luminal fluid composition has been demonstrated to stimulate potassium secretion by the distal nephron independent of a change in lumen negative voltage (97).



acetazolamide

FIGURE 81-2. Structure of acetazolamide carbonic anhydrase inhibitor (a).

Mechanism of Action

In the kidney, carbonic anhydrase inhibitors act primarily on proximal tubule cells to inhibit bicarbonate absorption. Carbonic anhydrase, a metalloenzyme containing one zinc atom per molecule, is important in sodium bicarbonate reabsorption and hydrogen ion secretion by renal epithelial cells. The biochemical, morphologic, and functional properties of carbonic anhydrase have been reviewed (98–101). Carbonic anhydrase isoforms (CAs) can be categorized into four groups: (i) cytosolic I—II, III, VII; mitochondrial—V; membrane associated—IV, IX, XII, XIV; and secreted—VI (98). Carbonic anhydrases regulate cellular hydrogen ion secretion through catalyzing the formation of HCO_3^- from OH^- and CO_2 and by binding to transporters and directly regulating its activity (102–104). There are three major renal carbonic anhydrases. Type II carbonic anhydrase (CAII) is distributed widely comprising more than 95% of the overall activity in kidney and is sensitive to inhibition by sulfonamides (99,105). CAII is expressed in the cytoplasm and facilitates the secretion of hydrogen ions by catalyzing the formation of HCO_3^- from OH^- and CO_2 (see Equation 3, next page). In addition CAII binds to the C-terminal region of sodium proton exchanger (NHE1) and likely regulates the transport efficiency of Na/H exchange (104). CAIV, is bound to renal cortical membranes, comprising up to 5% of the remaining overall activity in kidney, and is sensitive to sulfonamides (106,107). Carbonic anhydrase activity at basolateral and luminal plasma membranes of proximal tubule cells (100,108,109), and luminal membrane of intercalated cells (110), catalyzes the dehydration of intraluminal carbonic acid generated from secreted protons (99,111). The carbonic anhydrase activity at the basolateral and luminal plasma membranes of proximal tubule cells is thought to be due in part to CAIV (112). CAIV has been shown to also bind to the extracellular loop of $NaHCO_3$ transporter 1 (NBC1) regulating its transport activity (103). Evidence for the physiologic importance of carbonic anhydrase is apparent, given that a deficiency of CAII leads to a renal acidification defect resulting in renal tubular acidosis (113). Furthermore, metabolic acidosis leads to an adaptive increase in both CAII and IV carbonic anhydrase messenger RNA (mRNA) expression in kidney (114), suggesting the importance of both carbonic anhydrase isoforms in this disorder. Recently CAXII was cloned and characterized in renal carcinoma and normal human kidney (114a). Through *in situ* hybridization and immunologicalization methods, CAXII is expressed in proximal tubules and collecting ducts and may contribute to the carbonic anhydrase activity in these segments (102,115,116).

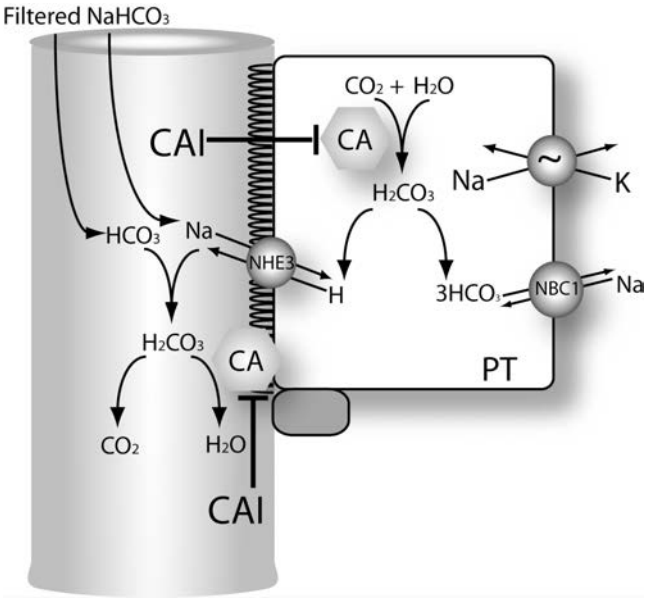


FIGURE 81-3. Mechanisms of diuretic action in the proximal tubule. The figure shows functional model of proximal tubule (PT) cells. Many transport proteins are omitted from the model, for clarity. Carbonic anhydrase (CA) catalyzes inside the cell the formation of HCO_3 from OH and CO_2. This is the result of the twostep process (please see equations 1R, 2R, and 3R, in the text, for additional details). Bicarbonate leaves the cell via the Na, HCO_3, cotransporter (280,281). A second pool of carbonic anhydrase is located in the brush border (CA). This participates in disposing of carbonic acid, formed from filtered bicarbonate and secreted H+. Both pools of carbonic anhydrase are inhibited by acetazolamide and other carbonic anhydrase inhibitors (see text for details).

Normally the proximal tubule reabsorbs 80% of the filtered load of sodium bicarbonate and 60% of the filtered load of sodium chloride. Early studies by Pitts and Alexander (117) and DuBose et al. (118) and micropuncture studies indicated that hydrogen ion secretion was responsible for bicarbonate absorption and renal acidification (118–121). The cellular mechanism by which proximal tubules reabsorb bicarbonate is depicted in Figure 81-3. The effect of carbonic anhydrase in accelerating bicarbonate is a result of the reactions that occur in both luminal fluid and in the cell. The mechanism of carbonic anhydrase action in luminal fluid (122), is shown in the following equation, where *E* represents the carbonic anhydrase enzyme:

Luminal Fluid

$$EH_2O + HCO_3^- \Leftrightarrow H_2O + CO_2 + EOH \quad [1]$$
$$EOH + H^+ \Leftrightarrow EH_2O \quad [2]$$
$$HCO_3^- + H^+ \Leftrightarrow CO_2 + H_2O \quad [3]$$

Note that the addition of reactions 1 and 2 leads to the classic reaction 3. In this scheme, the enzyme is viewed as a superhydroxylator.

Luminal carbonic anhydrase prevents H from accumulating in tubule fluid, which would eventually stop all Na/H exchange (119). Once formed, carbon dioxide rapidly diffuses from the lumen into the cell across the apical membrane.

The mechanism by which intracellular carbonic anhydrase participates in net H+ secretion is functionally the reverse of the reactions shown in the preceding equations.

Intracellular Fluid

$$EH_2O \Leftrightarrow EOH + H^+ \quad [2R]$$
$$EOH + H_2O + CO_2 \Leftrightarrow EH_2 + HCO_3^- \quad [1R]$$
$$CO_2 + H_2O \Leftrightarrow HCO_3^- + H^+ \quad [3R]$$

In this case, the enzyme splits water, thereby providing an hydroxyl ion to form bicarbonate. The bicarbonate ions then exit the basolateral membrane via $Na(HCO_3)_3$ cotransport (123–127). Therefore, in the early proximal tubule, the net effect of the process described results in the isosmotic reabsorption of $NaHCO_3$. The lumen chloride concentration increases because water continues to be reabsorbed producing a lumen positive potential (128). These axial changes provide an electrochemical gradient for transport of chloride via paracellular and transcellular pathways. The latter pathway for chloride likely involves a chloride/base exchanger operating in parallel with an Na/H proton exchanger (129,130). The dual operation of these parallel exchangers results in net NaCl absorption.

Carbonic anhydrase inhibitors act primarily on proximal tubule cells (131), where approximately 60% of the filtered load of sodium chloride is reabsorbed (132,133). Despite the magnitude of sodium chloride reabsorption in the proximal tubule segment, the natriuretic potency of carbonic anhydrase inhibitors is relatively weak. Several factors explain this observation. First, proximal sodium reabsorption is mediated by carbonic anhydrase-independent as well as carbonic anhydrase-dependent pathways. Second, the increased sodium delivery to distal nephron segments is largely reabsorbed by these distal nephron segments (88,90,91). Third, carbonic anhydrase inhibitors generate a hyperchloremic metabolic acidosis, further reducing the effects of subsequent doses of carbonic anhydrase inhibitor (134). Recent studies have demonstrated that metabolic acidosis produces resistance of bicarbonate absorption to carbonic anhydrase inhibition (135). Following the induction of metabolic acidosis, the K_i for bicarbonate absorption by membrane impermeant carbonic anhydrase inhibitors was increased by a factor of 100 to 500, suggesting that metabolic acidosis is associated with changes in the physical properties of the carbonic anhydrase protein (135). For these reasons, carbonic anhydrase inhibitors alone are rarely used as diuretic agents.

Following carbonic anhydrase inhibitor administration, proximal tubule bicarbonate reabsorption declines between 35% and 85% (87,121,136–140). Additional sites of action of carbonic anhydrase inhibitors include proximal straight tubule or loop of Henle (121,140,141), distal tubule (111), and the collecting and papillary collecting ducts (141–145). Yet, despite the effect of carbonic anhydrase inhibitors on proximal tubules as well other nephron segments, compensatory reabsorption of bicarbonate at other downstream tubular sites limits net fractional excretion of bicarbonate to approximately 25% to 30%, even during acute administration (87,88).

The relative contributions of membranebound and intracellular components of cellular carbonic anhydrase have been examined. Both species contribute to bicarbonate absorption. The role of membrane-bound carbonic anhydrase was first suggested by Rector et al. (120), who observed that carbonic anhydrase inhibitors produce an "*acid disequilibrium pH*" in the proximal tubule. The term "disequilibrium pH" refers to the difference between the pH of tubule fluid *in situ* (pH_{is}) and the pH achieved after the tubule fluid is allowed to reach chemical equilibrium at known pCO_2; an acid disequilibrium pH indicates that the tubule fluid is more acidic *in situ* than after full equilibration. When carbonic anhydrase is inhibited, the dehydration of H_2CO_3 to H_2O and CO_2 is slow, allowing H^+ to accumulate in the lumen and reducing pH. The demonstration

of an acid disequilibrium pH provided physiologic evidence in support of previous histochemical findings that a fraction of enzymatic activity was present in the tubule lumen. Although the cytoplasmic carbonic anhydrase constitutes the majority of enzyme activity in kidney, it is believed that the membrane-bound carbonic anhydrase plays a significant role in bicarbonate reabsorption by the proximal tubule. Studies addressing this question have employed carbonic anhydrase inhibitors that differ in their ability to penetrate proximal tubule cell membranes. Benzolamide is charged at normal pH and it does not penetrate cell membranes well, whereas acetazolamide enters the cell relatively easily (146). Both intravenous (120,147) and intratubular administration of benzolamide (137) lead to an acid disequilibrium pH, indicating that luminal carbonic anhydrase inhibition contributes to bicarbonate absorption. Furthermore, proximal tubular perfusion of benzolamide resulted in 90% inhibition of bicarbonate reabsorption. Despite near-equal efficacy in inhibiting proximal tubule bicarbonate reabsorption, benzolamide lowered tubular fluid pH, whereas acetazolamide increased tubular fluid pH. These results suggest that the site of action of benzolamide is the brush border, whereas the site of action of acetazolamide is largely cellular. Inhibition of luminal carbonic anhydrase causes lumen pH to decrease because of the continued secretion of hydrogen ions and its accumulation in the tubular lumen. In contrast, acetazolamide does not produce an acid disequilibrium pH (137). The conclusion that tubular fluid is in direct contact with membrane carbonic anhydrase was substantiated by the use of dextran-bound carbonic anhydrase inhibitor (148,149). In proximal tubules perfused *in vivo*, Lucci et al. (149) determined that dextran bound inhibitors, which inhibit only luminal carbonic anhydrase, decreased proximal tubule bicarbonate absorption by approximately 80% and reduced lumen pH.

Although these studies establish the importance of luminal carbonic anhydrase, they also support a role for intracellular or basolateral carbonic anhydrase. The observation that both acetazolamide and benzolamide inhibit proximal tubule bicarbonate reabsorption to a similar degree yet produce opposite effects on tubule fluid pH suggests that intracellular carbonic anhydrase contributes to proximal tubule luminal acidification.

The expression of carbonic anhydrase in the basolateral membrane of proximal tubule cells (108,109) suggests that this membranebound enzyme plays an important role in basolateral bicarbonate transport (99,150). Although it is well known that carbonic anhydrase inhibitors inhibit intracellular generation of substrate for the transporter (151,152), the direct interaction between CAIV and NBC1, the sodium/bicarbonate cotransporter (103), suggests the possibility that carbonic anhydrase inhibitors may also directly regulate anion transport activity. Functional studies using an impermeant carbonic anhydrase inhibitor, *p*-fluorobenzyl-aminobenzolamide that is 1% as permeable as acetazolamide demonstrated the importance of basolateral membrane bound carbonic anhydrase. *p*-Fluorobenzyl-aminobenzolamide reduced fluid and bicarbonate absorption when applied to the basolateral membrane of rabbit proximal tubules perfused *in vitro* (115).

In the collecting duct, carbonic anhydrase facilitates acid secretion that is mediated by a vacuolar H adenosine triphosphatase (H-ATPase) (153) and a P-type gastric H-KA-TPase (154–156). Luminal administration of acetazolamide produced an acid disequilibrium pH in the outer medullary collecting duct, suggesting the contribution of luminal carbonic anhydrase star (157). With use of a membrane impermeant carbonic anhydrase inhibitor (F-3500; aminobenzolamide coupled to a nontoxic polymer polyoxyethylene), bicarbonate absorption was reduced, thereby confirming the presence of carbonic anhydrase in the luminal membrane of the outer medullary collecting duct (135). The K_i for inhibition of bicarbonate absorption was 5 μM, consistent with the inhibition of CAIV.

Renal Hemodynamics

Inhibition of carbonic anhydrase decreases GFR acutely (94, 158–160). Systemic acetazolamide infusion decreased GFR by 30%. Single nephron glomerular filtration rate (SNGFR) was 23% lower during acetazolamide infusion, partly because increased solute delivery to the macula densa activates the tubuloglomerular feedback (TGF) mechanism, which reduces GFR. Similar results were observed following infusion of benzolamide (159,160). Nevertheless, the effects of carbonic anhydrase inhibitors to reduce GFR are not simply the result of TGF activation. Sarala[8]-angiotensin I, an angiotensin II antagonist, prevented the decrease in SGNFR, suggesting the involvement of local angiotensin II in response to benzolamide (160). Furthermore, infusion of benzolamide into targeted adenosine-1 receptor knockout mice, mice that lack a TGF response, reduced GFR by 21% (161). Taken together, these results suggest complex mechanisms by which carbonic anhydrase inhibitors reduce GFR.

Pharmacokinetics

Acetazolamide is well absorbed from the gastrointestinal (GI) tract. More than 90% of the drug is plasma protein bound. The highest concentrations are found in tissues that contain large amounts of carbonic anhydrase (e.g., renal cortex, red blood cells). Renal effects are noticeable within 30 minutes and are usually maximal at 2 hours. Acetazolamide is not metabolized but is excreted rapidly by glomerular filtration and proximal tubular secretion. The half-life is approximately 5 hours, and renal excretion is essentially complete in 24 hours (61). In comparison, methazolamide is absorbed more slowly from the GI tract, and its duration of action is long, with a half-life of approximately 14 hours.

Adverse Effects

Generally, carbonic anhydrase inhibitors are well tolerated with infrequent serious adverse effects. Side effects of carbonic anhydrase inhibitors may arise from the continued excretion of electrolytes. Significant hypokalemia and metabolic acidosis may develop (90). In elderly patients with glaucoma treated with acetazolamide (250 mg to 1000 mg/day), metabolic acidosis was a frequent finding in comparison to a control group (162). Acetazolamide is also associated with nephrocalcinosis and nephrolithiasis due to its effects on urine pH, facilitating stone formation (163). Premature infants treated with furosemide and acetazolamide are particularly susceptible to nephrocalcinosis, presumably due to the combined effect of an alkaline urine and hypercalciuria (164). Other adverse effects include drowsiness, fatigue, central nervous system depression, and paresthesias. Bone marrow suppression has been reported (165,166).

Clinical Use

The popularity of carbonic anhydrase inhibitors as diuretics has waned owing to the development of agents that are more effective with fewer toxic side effects. In general, tolerance develops rapidly and renders these drugs less effective. Daily use produces systemic acidemia from an increase in urinary excretion of bicarbonate. Nevertheless, acetazolamide can be administered for short-term therapy, usually in combination with other diuretics, to patients who are resistant or who do not respond adequately to other agents. The rationale for using a

combination of diuretic agents is based on summation of their effect at different sites along the nephron. In addition to its use as a diuretic agent, the carbonic anhydrase inhibitor is used in a number of other clinical situations.

The primary indication for the use of acetazolamide as a diuretic agent is in the treatment of patients with metabolic alkalosis that is accompanied by edematous states (100,167), or chronic obstructive lung disease (168,169). In patients with cirrhosis, congestive heart failure, or nephrotic syndrome, aggressive diuresis with loop diuretics promotes intravascular volume depletion, secondary hyperaldosteronism, and renal insufficiency, conditions that promote metabolic alkalosis. Administration of sodium chloride to correct the metabolic alkalosis may exacerbate edema. Acetazolamide can improve metabolic alkalosis by decreasing proximal tubule bicarbonate reabsorption, thereby increasing the fractional excretion of bicarbonate. An increase in urinary pH (>7.0) indicates enhanced bicarbonaturia. However, it should be noted that potassium depletion should be corrected prior to acetazolamide use because acetazolamide will increase potassium excretion. The time-course of acetazolamide effect is rapid. In critically ill patients who are on ventilators, following the correction of fluid and electrolyte disturbances, intravenous acetazolamide produced an initial effect within 2 hours and a maximum effect in 15 hours (170).

Acetazolamide is used effectively to treat chronic open-angle glaucoma. The high bicarbonate concentration in aqueous humor is carbonic anhydrase dependent, and oral carbonic anhydrase inhibition can be used to reduce aqueous humor formation. Newer topical formulations are currently in clinical trials (171,172) and could prove to be effective without the side effects of orally administered drugs.

Acute mountain sickness usually occurs in climbers within the 12 to 72 hours of ascending to high altitudes. Symptoms are characterized by a complex consisting of headache, nausea, dizziness, and breathlessness. Carbonic anhydrase inhibitors improve symptoms and arterial oxygenation (173,174).

The administration of acetazolamide has been used in the treatment of familial hypokalemic periodic paralysis (175,176), a disorder characterized by intermittent episodes of muscle weakness and flaccid paralysis. Its efficacy may be related to a decrease in influx of potassium as a result of a decrease in plasma insulin and glucose (177), or to metabolic acidosis. Carbonic anhydrase inhibitors can also be used as an adjunct treatment of epilepsy (178), pseudotumor cerebri (179), and central sleep apnea (180).

By increasing urinary pH, acetazolamide has been used effectively in certain clinical conditions. Acetazolamide is used to treat cystine and uric acid stones by increasing their solubility in urine. Acetazolamide in combination with sodium bicarbonate infusion is also used to treat salicylate toxicity. Salicylates are weak acids (pK_a 3.0); therefore, their ionic and nonionic forms exist in equilibrium. They are excreted primarily by the kidney through secretion via the organic anion transport pathway in the proximal tubule. Acetazolamide and sodium bicarbonate infusions increase urinary pH, thereby favoring formation of a nondiffusible nonionic form of salicylate and thus increasing excretion of salicylates (181).

LOOP DIURETICS

The loop diuretics inhibit sodium and chloride transport along the loop of Henle and macula densa. Although these drugs also impair ion transport by proximal and distal tubules under some conditions, these effects probably contribute little to their action clinically. The loop diuretics available in the United States include furosemide, bumetanide, torsemide, and ethacrynic acid (see Fig. 81-4).

The molecular mechanisms by which loop diuretics inhibit the Na-K2-Cl cotransporter remain unclear. Loop diuretics are organic anions. Studies that utilized radiolabeled bumetanide suggested that loop diuretics bind to one of the chloride (anion) sites on the transporter (182). According to this model, the loop diuretic would bind because of its negative charge (and its shape) and then inhibit the transport of ions because it is not transported. More recent studies utilized chimeric cloned proteins, comprising portions of different members of the cation chloride cotransporters, to investigate diuretic and ion binding by using heterologously expressed constructs. All studies have indicated that diuretic binding and ion affinities are properties of the central hydrophobic domain of the proteins (183). Isenring et al. (184,185) found that transmembrane domains 2–6 and 10–12 play roles in defining loop diuretic affinity, whereas chloride affinity is regulated by transmembrane domains 4 and 7 (185). This suggests that loop diuretics do not simply bind

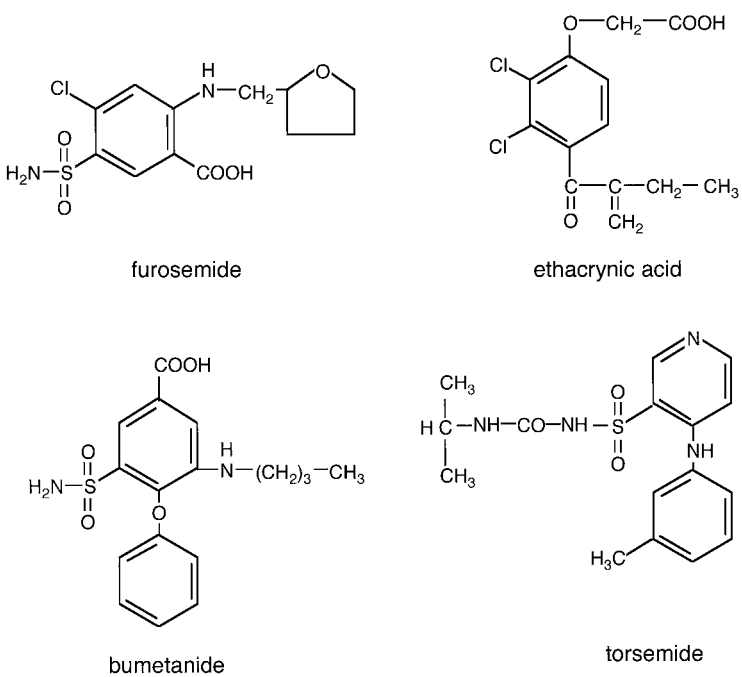


FIGURE 81-4. Structures of loop diuretics.

to one of the chloride sites on the transporter. The results of the chimeric studies, however, have been complex and it would appear that interactions between various transmembrane domains might reconcile the apparent differences in results. A full delineation of sites at which ions and diuretic drugs bind to these transport proteins awaits their crystallization.

Urinary Electrolyte and Water Excretion

Loop diuretics increase the excretion of water, sodium, potassium, chlorine, phosphate, magnesium, and calcium (see Table 81-1). The dose–response relationship between loop diuretic and urinary Na and Cl excretion is sigmoidal (see Fig. 81-5). The steep dose–response relation has led many to refer to loop diuretics as "threshold" drugs (39). Loop diuretics have the highest natriuretic and chloruretic potency of any class of diuretics; they can increase Na and Cl excretion by up to 25% of the filtered load. If loop diuretics are administered during water loading, solute-free water clearance (C_{H_2O}) decreases and osmolar clearance increases, although the urine always remains dilute (186–188). This effect contrasts with that of osmotic diuretics which increase osmolar clearance and C_{H_2O} (189). During hydropenia, loop diuretics impair the reabsorption of solute-free water ($T^C_{H_2O}$)(186–188). During maximal loop diuretic action, the urinary Na concentration is usually between 75 and 100 mM (190). Because urinary potassium concentrations during furosemide-induced natriuresis remain low, this means that the clearance of electrolyte-free water (C_{H_2O}) is increased when loop diuretics are administered during conditions of water diuresis or hydropenia (190). This effect of loop diuretics has been exploited to treat hyponatremia, when combined with normal or hypertonic saline (191,192).

Mechanisms of Action

Na and Cl Transport

The predominant effect of loop diuretic drugs is to inhibit the electroneutral Na-K2-Cl cotransporter at the apical surface of thick ascending limb cells. The loop of Henle, defined as the region between the last surface proximal segment and the first surface distal segment, reabsorbs from 20% to 50% of the filtered Na and Cl load (193); approximately 10% to 20% is reabsorbed by thick ascending limb cells. The model in Figure 81-6 shows key components of Na, K, and Cl transport pathways in a thick ascending limb cell. As in other nephron segments, the Na/K-ATPase at the basolateral cell membrane maintains the intracellular Na concentration low (approximately 10-fold lower than the interstitial concentration) and the K concentration high (approximately 20-fold higher than interstitial). Potassium channel(s) (194) in the basolateral cell membrane permit K to diffuse from the cell, rendering the cell membrane voltage oriented with the intracellular surface negative, relative to extracellular fluid. A chloride channel in the basolateral cell membrane (presumably CLCNKB (25,195) permits Cl to exit the cell (194). Together with the apical K

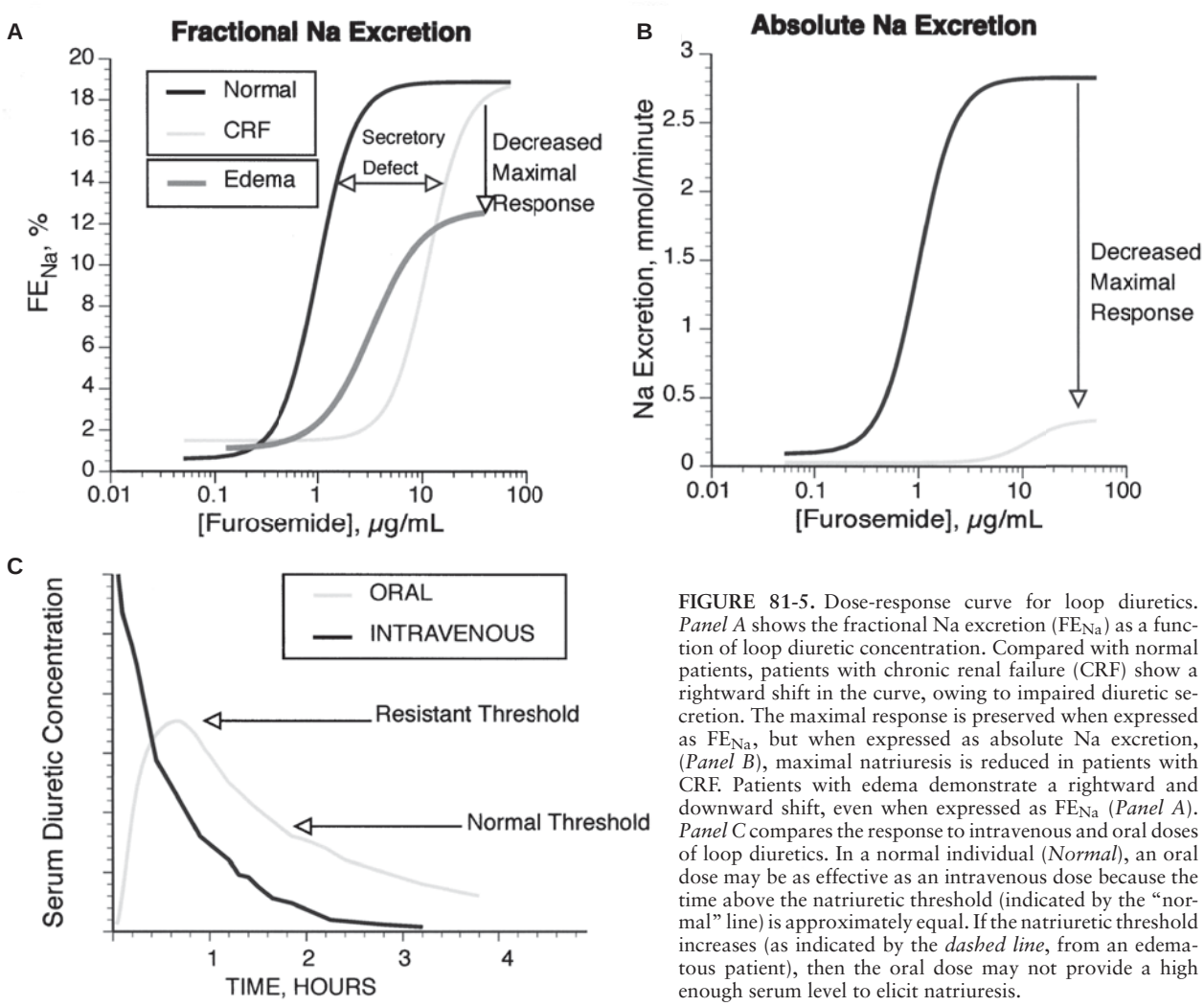


FIGURE 81-5. Dose-response curve for loop diuretics. *Panel A* shows the fractional Na excretion (FE_{Na}) as a function of loop diuretic concentration. Compared with normal patients, patients with chronic renal failure (CRF) show a rightward shift in the curve, owing to impaired diuretic secretion. The maximal response is preserved when expressed as FE_{Na}, but when expressed as absolute Na excretion, (*Panel B*), maximal natriuresis is reduced in patients with CRF. Patients with edema demonstrate a rightward and downward shift, even when expressed as FE_{Na} (*Panel A*). *Panel C* compares the response to intravenous and oral doses of loop diuretics. In a normal individual (*Normal*), an oral dose may be as effective as an intravenous dose because the time above the natriuretic threshold (indicated by the "normal" line) is approximately equal. If the natriuretic threshold increases (as indicated by the *dashed line*, from an edematous patient), then the oral dose may not provide a high enough serum level to elicit natriuresis.

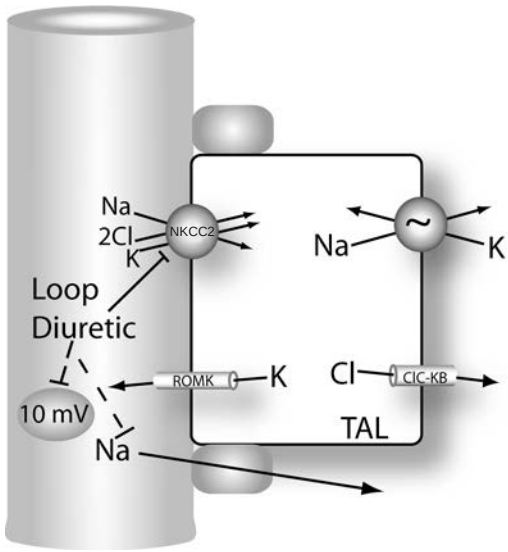


FIGURE 81-6. Mechanisms of diuretic action along the loop of Henle. Figure shows model of thick ascending limb (TAL) cells. Na and Cl are reabsorbed across the apical membrane via the loop diureticsensitive Na-K-2Cl cotransporter, NKCC2. Loop diuretics bind to and block this pathway directly. Note that the transepithelial voltage along the thick ascending limb is oriented with the lumen positive relative to blood (*circled value*, given in millivolts, mV). This transepithelial voltage drives a component of Na (and calcium and magnesium, see Fig. 81-9) reabsorption via the paracellular pathway. This component of Na absorption is also reduced by loop diuretics because they reduce the transepithelial voltage.

channel, described in the subsequent text, this chloride channel generates a transepithelial voltage, oriented in the lumenpositive direction.

The transporter inhibited by loop diuretics is a member of the cation chloride cotransporter family (183,196). This protein, referred to as the bumetanidesensitive cotransporter, first isoform (BSC-1), or as the Na-K-2Cl cotransporter, second isoform, (NKCC2), is encoded by the gene *SLC12A1*. It is believed to comprise 12 membrane-spanning domains and is expressed at the apical membrane of thick ascending limb (34) and macula densa (MD) cells (36,197). This transporter is oriented in parallel with a K channel (ROMK) permitting potassium to recycle from the cell to the lumen (198). Greger and Schlatter showed that the asymmetrical orientation of channels (apical versus basolateral) and the action of the Na/K ATPase and Na-K-2Cl cotransporter combine to create a transepithelial voltage that is oriented with the lumen positive, with respect to the interstitium (199). This lumenpositive potential drives absorption of Na, Ca, and Mg via the paracellular pathway. The paracellular component of Na reabsorption comprises 50% of the total transepithelial Na transport by thick ascending limb cells (200). It should be noted, however, that both the transcellular and the paracellular components of Na transport are inhibited by loop diuretics, the former directly and the latter indirectly. The thick ascending limb is virtually impermeable to water. The combination of solute absorption and water impermeability determines the role of the thick ascending limb as the primary diluting segment of the kidney.

Although direct inhibition of ion transport is the most important natriuretic action of loop diuretics, other actions may contribute. Thick ascending limb cells have been shown to produce prostaglandin E_2 following stimulation with furosemide (201), perhaps via inhibition of prostaglandin dehydrogenase (202,203). Blockade of cyclooxygenase (COX) reduces the effects of furosemide to inhibit loop segment chloride transport in rats (204); prostaglandin E_2, but not I_2 can restore this effect (205). Increases in renal prostaglandins may contribute to the hemodynamic effects of loop diuretics as described in subsequent text.

Although high luminal concentrations of chloride might be suspected of inhibiting diuretic efficacy, based on the relation between luminal chloride concentration and diuretic binding to the transporter, there is no clinical information to suggest that that luminal chloride inhibits diuretic efficacy *in vivo*.

Ca and Mg Transport

Loop diuretics increase the excretion of the divalent cations, calcium and magnesium. This effect to increase calcium excretion is used to advantage when furosemide is used together with saline to treat hypercalcemia (206). Although a component of magnesium and calcium absorption by thick ascending limbs may be active (especially when circulating parathyroid hormone levels are high [207]), a large component of their absorption is passive and paracellular, driven by the transepithelial voltage. As described in the preceding text, active NaCl transport by thick ascending limb cells leads to a transepithelial voltage, oriented in the lumen-positive direction. The paracellular pathway in the thick ascending limb express paracellin-1 (claudin-16), which is encoded by the gene *PCLN-1* at the tight junction that is believed to mediate both magnesium and calcium movement (208). Mutations in this gene lead to the clinical syndrome, familial hypomagnesemia and hypercalciuria (FHHNC), an autosomal recessive tubular disorder that is frequently associated with renal failure (209). The positive voltage in the lumen, relative to interstitium, drives calcium and magnesium absorption through the paracellular pathway (210). Loop diuretics, by blocking the activity of the Na-K-2Cl cotransporter at the apical membrane of thick ascending limb cells, reduce the transepithelial voltage towards or to 0 mV (211–213). This stops passive paracellular calcium and magnesium absorption.

Renin Secretion

In addition to enhancing Na and Cl excretion, effects that result directly from inhibiting Na and Cl transport, loop diuretics also stimulate renin secretion. Although a component of this effect is frequently related to contraction of the extracellular fluid volume (see subsequent text), loop diuretics also stimulate renin secretion by inhibiting Na-K-2Cl cotransport directly. Macula densa cells, which control renin secretion, sense the NaCl concentration in the lumen of the thick ascending limb (214). High luminal NaCl concentrations in the region of the macula densa lead to two distinct but related effects. First they activate the tubuloglomerular feedback (TGF) response, which suppresses glomerular filtration rate. Second, they inhibit renin secretion. The relation between these two effects is complex and has been reviewed (214), but both effects appear to result largely from NaCl movement across the apical membrane (215). Most of the ion transport pathways of macula densa cells are expressed by thick ascending limb cells. This includes the loop diureticsensitive Na-K-2Cl cotransporter (NKCC2 or BSC1) at the apical surface (36,197). Under normal conditions, an increase in luminal NaCl concentration in the thick ascending limb raises the NaCl concentration inside macula densa cells (215). Because the activity of the basolateral Na/K ATPase is lower in macula densa cells than in surrounding thick ascending limb cells (214), the cell NaCl concentration is much more dependent on luminal NaCl concentration in macula densa than in thick ascending limb cells (216). When luminal and macula densa cell NaCl concentrations decline, production rates of nitric oxide and prostaglandin E_2 are stimulated. Although the mechanisms by which Na and Cl transport regulates nitric oxide and prostaglandin production rates are not known, both mediators appear to participate importantly in effecting renin secretion.

The constitutive (neuronal) isoform of nitric oxide synthase (nNOS) is expressed at high levels by macula densa cells, but not by other cells in the kidney (217). Nitric oxide produced by macula densa cells has a paracrine effect to increase cellular cyclic AMP (cAMP) in adjacent juxtaglomerular cells. Through protein kinase A, cAMP helps to stimulate renin secretion. In juxtaglomerular cells, nitric oxide may act by increasing cellular cGMP, which inhibits phosphodiesterase 3 (218), leading to phosphodiesterase 3 inhibition and cAMP accumulation. Several laboratories reported that furosemide-induced stimulation of renin secretion is dependent on an intact nitric oxide system because nonspecific nitric oxide inhibition interferes with this phenomenon (219–221). More recent studies, however, utilized knockout models to examine the role of nitric oxide in diuretic-induced renin secretion. Using this approach, it appears that neither neuronal nor endothelial nitric oxide synthases are required for loop diuretic-induced renin secretion. Instead, nitric oxide appears to play a permissive, rather than necessary, role in facilitating diuretic-induced renin secretion (222).

Prostaglandin production also participates in regulating renin secretion. Cyclooxygenase 2 (COX-2) is expressed by macula densa cells and by interstitial cells in the kidney (223–226). This isoform is often found only after induction by inflammatory cytokines. Blockade of prostaglandin synthesis either by nonspecific COX inhibitors (227) or by specific COX-2 blockers (228,229) reduces both the natriuresis induced by loop diuretics and dramatically inhibits the renin secretory response. These results have been corroborated in humans (230).

Renal Hemodynamics

Glomerular filtration rate and renal blood flow (RPF) tend to be preserved during loop diuretic administration (231), although GFR and RPF can decline if extracellular fluid volume contraction is severe. Loop diuretics reduce renal vascular resistance and increase renal blood flow under experimental conditions (231,232). This effect is believed related to the diuretic-induced production of vasodilatory prostaglandins.

Another factor that may contribute to the tendency of loop diuretics to maintain glomerular filtration rate and renal plasma flow despite volume contraction is their effect on the TGF system. The sensing mechanism that activates the TGF system involves NaCl transport across the apical membrane of macula densa cells by the loop diuretic sensitive Na-K-2Cl cotransporter. Under normal conditions, when the luminal concentration of NaCl reaching the macula densa rises, glomerular filtration rate decreases via TGF. To a large degree, the TGF-mediated decrease in GFR is believed to be caused by afferent arteriole constriction (214). Emerging concepts regarding TGF signaling were recently reviewed by Bell et al. (233). In response to changes in NaCl transport across the apical membrane of macula densa cells, ATP is released across the basolateral membranes through a NaCl sensitive ATP-permeable large-conductance (380 pS) anion channel (234), an effect blocked by luminal furosemide (235). Once released across the basolateral membrane of macula densa cells, the role of ATP in the TGF effector response is controversial. The presence of ecto-5′-nucleotidase (236) in mesangial cells could allow for the rapid degradation of ATP to adenosine and subsequent activation of the A1 adenosine receptor (P1 purinergic receptor class) expressed on afferent arteriole. The role of A1 adenosine receptor activation in TGF is strengthened by studies in which the tubuloglomerular feedback response is absent or reduced in animals lacking adenosine A1 receptors (237) or lacking ecto-5′-nucleotidase (238). Alternatively, ATP may have direct effects on the afferent arteriole to mediate vasoconstriction. $P2X_1$ purinergic receptors, receptors for ATP have been identified on the afferent arteriole (239) and mediate vasoconstriction (240). It is likely that TGF may depend on the coordinate interaction of both P1 and P2 purinergic receptors (237,241). Loop diuretic drugs block TGF by blocking the sensing step (242). In the absence of effects on the macula densa, loop diuretics would be expected to suppress GFR and RPF by increasing distal NaCl delivery and activating the TGF system. Instead, blockade of the TGF permits GFR and RPF to be maintained.

Systemic Hemodynamics

Acute intravenous administration of loop diuretics increases venous capacitance (243). Some studies suggest that this effect results from stimulation of prostaglandin synthesis by the kidney (244,245). Other studies suggest that loop diuretics have effects in peripheral vascular beds as well (246). Pickkers et al. examined the local effects of furosemide in the human forearm. Furosemide had no effect on arterial vessels but did cause dilation of veins, an effect that was dependent on local prostaglandin production (247). More recently, loop diuretic-induced vasodilation was shown to depend on increased nitric oxide production (248). Although venodilation and improvements in cardiac hemodynamics frequently result from intravenous therapy with loop diuretics, the hemodynamic response to intravenous loop diuretics may be more complex (249). Johnston et al. reported that low-dose furosemide increased venous capacitance, but that higher doses did not (250) It was suggested that furosemide-induced renin secretion leads to angiotensin II–induced vasoconstriction. This vasoconstrictor might overwhelm the prostaglandin-mediated vasodilatory effects in some patients. In two series, 1 to 1.5 mg/kg furosemide boluses, administered to patients with chronic congestive heart failure, resulted in transient *deteriorations* in hemodynamics (during the first hour), with declines in stroke volume index, increases in left ventricular filling pressure (251), and exacerbation of congestive heart failure symptoms. These changes may be related to activation of both the sympathetic nervous system and the renin–angiotensin system by the diuretic drug. Evidence for a role of the renin–angiotensin system in the furosemide-induced deterioration in systemic hemodynamics includes the temporal association between its activation and hemodynamic deterioration (251), and the ability of angiotensin-converting enzyme (ACE) inhibitors to prevent much of the pressor effect (252). The effects of renal denervation on sympathetic responses to furosemide were studied. These results confirm that the effects are mediated both by direct renal nerve traffic, and, indirectly, by activation of the renin–angiotensin axis (253,254). Many other studies have shown that acute loop diuretic administration frequently produces a transient decline in cardiac output; whether diuretic administration increases or decrease left atrial pressure acutely may depend primarily on the state of underlying sympathetic nervous system and renin–angiotensin axis activation.

Pharmacokinetics

The three loop diuretics that are used most commonly, furosemide, bumetanide, and torsemide, are absorbed quickly after oral administration, reaching peak concentrations within 0.5 to 2 hours. Furosemide absorption is slower than its elimination in normal subjects; therefore, the time to reach peak serum level is less for furosemide than for bumetanide and torsemide. This phenomenon is called "absorption-limited kinetics" (39). The bioavailability of loop diuretics varies from 50% to 90% (see Table 81-2); furosemide bioavailability is approximately 50% (255); when furosemide dosing is switched from intravenous to oral, the dose may need to be increased to compensate for its poor bioavailability (39). The half-lives

TABLE 81-2

PHARMACOKINETICS OF LOOP DIURETICS

	Bioavailability %	Elimination half-life, hours			
		Healthy	Renal disease	Liver disease	CHF
Furosemide	10–100	1.5–2	2.8	2.5	2.7
Bumetanide	80–100	1	1.6	2.3	1.3
Torsemide	80–100	3–4	4–5	8	6

CHF, congestive heart failure.
(Data adapted from: Brater DC. Diuretic therapy *N Engl J Med* 1998;339:387.)

of the loop diuretics available in the United States vary, but all are relatively short (ranging from approximately 1 hour for bumetanide to 3 to 4 hours for torsemide). The half-lives of muzolimine, xipamide, and ozolinone, none of which is available in the United States, are longer (6 to 15 hours).

Loop diuretics are organic anions that circulate tightly bound to albumin (>95%); therefore, their volume of distribution is small except during extreme hypoproteinemia (256). Approximately 50% of an administered dose of furosemide is excreted unchanged into the urine. The remainder appears to be eliminated by glucuronidation, probably by the kidney. Torsemide and bumetanide are eliminated both by hepatic processes and through renal excretion. The differences in metabolic fate mean that the half-life of furosemide is altered by renal failure, whereas this is not true for torsemide and bumetanide.

Clinical Use

Loop diuretics are used commonly to treat the edematous conditions, congestive heart failure, cirrhosis of the liver, and nephrotic syndrome. In addition, a variety of other electrolyte, fluid, and acid–base disorders can respond to loop diuretic therapy. Details of loop diuretic use for the treatment of edematous conditions are beyond the scope of this chapter.

Adverse Effects

There are at least three types of adverse effects of loop diuretics. The first and most common side effects are those that result directly from the effects of these drugs on renal electrolyte and water excretion. The second class of side effects is toxic effects of the drugs that are dose-related and predictable. The third class includes idiosyncratic allergic drug reactions.

Loop diuretics are frequently administered to treat edematous expansion of the extracellular fluid volume. Edema usually results from a decrease in the "effective" arterial blood volume. Overzealous diuretic usage or intercurrent complicating illnesses can lead to excessive contraction of the intravascular volume, thereby leading to orthostatic hypotension, renal dysfunction, and sympathetic overactivity. Although patients with congestive heart failure usually require diuretic therapy, the combination of diuretics and ACE inhibitors or angiotensin receptor blockers (ARBs) is especially likely to cause renal dysfunction. High diuretic doses or extreme dietary NaCl restriction may predispose to renal dysfunction during therapy with diuretics and ACE inhibitors/ARBs for congestive heart failure (257,258). In such cases, it is important to attempt to continue ACE inhibitors/ARBs, in view of their effects on mortality. Functional renal failure in such patients often responds to a reduction in diuretic dose or a liberalization in dietary NaCl intake, permitting continued administration of the ACE inhibitor/ARBs.

Other patients at increased risk for renal dysfunction during diuretic therapy include the elderly (259), patients with preexisting renal insufficiency (260), patients with right-sided heart failure or pericardial disease, and patients taking nonsteroidal antiinflammatory drugs. In a case-control study of nonsteroidal antiinflammatory drug use and renal failure, diuretic users had a 2.77 relative risk of acute renal failure, compared with nonusers (261).

Disorders of Na and K concentration are among the most frequent adverse effects of loop diuretics. Hyponatremia is less common with loop diuretics than with distal convoluted tubule diuretics (see subsequent text), but it can occur. Its pathogenesis is usually multifactorial, but involves the effect of loop diuretics to impair the clearance of solute free water. Additional factors that may contribute include the non-osmotic release of arginine vasopressin (262), hypokalemia (263), and hypomagnesemia (264). Conversely, loop diuretics have been used to treat hyponatremia when combined with hypertonic saline in the setting of the syndrome of inappropriate ADH secretion (192,265). The combination of loop diuretics and angiotensin I converting enzyme inhibitors has been reported to correct hyponatremia in the setting of congestive heart failure (266). The value of adding a loop diuretic to treatment with a vasopressin V2 receptor antagonist for hyponatremic syndrome of inappropriate antidiuretic hormone (ADH) secretion has been suggested (267,268).

Hypokalemia occurs commonly during therapy with loop diuretics, although the magnitude is smaller than that induced by distal convoluted tubule diuretic (loop diuretics, 0.3 mM versus distal convoluted tubule diuretics, 0.5 to 0.9 mM (269,270). Loop diuretics increase the delivery of potassium to the distal tubule because they block potassium reabsorption via the Na-K-2Cl cotransporter. In rats, under control conditions, approximately half the excreted potassium was delivered to the "early" distal tubule. During furosemide infusion, the delivery of potassium to the "early" distal tubule rose to 28% of the filtered load (271). Therefore, it appears that a large component of the effect of loop diuretics to increase potassium excretion acutely reflects their ability to block potassium reabsorption by the thick ascending limb. Nevertheless, during chronic diuretic therapy, the degree of potassium wasting correlates best with volume contraction and serum aldosterone levels (272). These data suggest that, under chronic conditions, the predominant effect of loop diuretics to stimulate potassium excretion results from their tendency to increase mineralocorticoid hormones while simultaneously increasing distal Na and water delivery.

Metabolic alkalosis is very common during chronic treatment with loop diuretics. Loop diuretics cause metabolic alkalosis via several mechanisms. First, they increase the excretion of urine that is bicarbonate free but contains Na and Cl. This leads to contraction of the extracellular fluid around a fixed

amount of bicarbonate buffer; a phenomenon known as "contraction alkalosis." This probably contributes only slightly to the metabolic alkalosis that commonly accompanies chronic loop diuretic treatment. Loop diuretics directly inhibit transport of Na and Cl into thick ascending limb cells. In some species, these cells also express an isoform of the Na/H exchanger at the apical surface. When Na entry via the Na-K-2Cl cotransporter is blocked by a loop diuretic, the decline in intracellular Na activity will stimulate hydrogen ion secretion via the Na/H exchanger (273–275). Loop diuretics stimulate the renin-angiotensin–aldosterone pathway, both directly and indirectly, as discussed in the preceding text. Aldosterone indirectly and directly enhances proton secretion through genomic actions on the vacuolar H-ATPase. Aldosterone indirectly increases Na reabsorption by principal cells of the connecting tubule (CNT) and cortical collecting tubule (CCT), which renders the tubule lumen more negative and increases H-ATPase activity (276). Aldosterone also directly activates the vacuolar H^+-ATPase in the outer medullary collecting tubule (258). Through nongenomic actions, aldosterone, independent of its receptor, rapidly modulates vacuolar H^+-ATPase activity in the outer medullary collecting tubule (277,278). Therefore, through different mechanism, aldosterone stimulates H secretion via the electrogenic H ATPase present at the apical membrane of intercalated cells. Hypokalemia itself also contributes to metabolic alkalosis by increasing ammonium production (279), stimulating bicarbonate reabsorption by proximal tubules (280,281), and increasing the activity of the H/K ATPase in the distal nephron (155,282). Finally, contraction of the extracellular fluid volume stimulates Na/H exchange in the proximal tubule and may reduce the filtered load of bicarbonate. All of these factors may contribute to the metabolic alkalosis observed during chronic loop diuretic treatment.

Ototoxicity is the most common toxic effect of loop diuretics that is unrelated to their effects on the kidney. Deafness, which is usually temporary (but can be permanent), was reported shortly after the introduction of loop diuretics (283,284). It appears likely that all loop diuretics cause ototoxicity, because ototoxicity can occur during use of chemically dissimilar drugs such as furosemide and ethacrynic acid (283,284). The mechanism of ototoxicity remains unclear, although the stria vascularis, which is responsible for maintaining endolymphatic potential and ion balance appears to be a primary target for toxicity (285). Loop diuretics reduce the striatal voltage from +80 mV to −10 to −20 mV within minutes of application (286). A characteristic finding in loop diuretic ototoxicity is strial edema. This suggests that toxicity involves inhibition of ion fluxes (285). Ikeda and Morizono detected functional evidence for the presence of a Na-K-2Cl cotransporter in the basolateral membrane of marginal cells in the inner ear (287). According to the model proposed by these investigators, marginal cells resemble secretory cells in other organ systems, with a Na-K-2Cl cotransporter and Na/K ATPase at the basolateral cell membrane and channels for K and Cl^- at the apical surface. According to this model, loop diuretic induced shrinkage of marginal cells results from inhibition of cell Na, K, and Cl uptake across the basolateral cell membrane. This model received molecular confirmation when the secretory isoform of the Na-K-2Cl cotransporter, NKCC1, was localized in the lateral wall of the cochlea, using specific antibodies (288) and reverse transcriptase polymerase chair reaction (RT-PCR) (289). Furthermore, disruption of the ubiquitous form of the Na-K-Cl cotransporter, NKCC1, leads to deafness in mice (290–292). Loop diuretics cause loss of outer hair cells in the basal turn of the cochlea, rupture of endothelial layers, cystic formation in the stria vascularis, and marginal cell edema in the stria vascularis (293).

Ototoxicity appears to be related to the peak serum concentration of loop diuretic, and, therefore, tends to occur during rapid drug infusion of high doses. For this reason, this complication is most common in patients with uremia (294). It has been recommended that furosemide infusion be no more rapid than 4 mg/minute (295). In addition to renal failure, there may be risk for increased ototoxicity in infants, patients with cirrhosis, and patients receiving aminoglycosides or *cis*-platinum (294).

DISTAL CONVOLUTED TUBULE DIURETICS

The first orally active drug to be developed that inhibited Na and Cl transport along the distal convoluted tubule (DCT) was chlorothiazide. Chlorothiazide was developed as sulfonamide-based carbonic anhydrase inhibitors were modified in pursuit of substances that increased Cl excretion rather than bicarbonate. The identification of a substance that increased Na and Cl excretion rates was immediately recognized as clinically significant, because extracellular fluid contains predominantly NaCl rather than $NaHCO_3$ (6,8), and because acidosis limits the effectiveness of carbonic anhydrase inhibitors. Subsequent development led to a wide variety of benzothiadiazide (thiazide) diuretics (see Fig. 81-7); all are analogs of 1,2,4-benzothiadiazine-1,1-dioxide. Other structurally related diuretics include the quinazolinones (such as metolazone) and substituted benzophenone sulfonamide (such as chlorthalidone). Although the primary site at which these drugs exert their action has been a source of confusion until recently (296), molecular identification of the target ion transporter of these drugs has permitted precise delineation of the predominant site of action as the distal convoluted tubule. Although the term "thiazide diuretics" is frequently used to describe this class of drugs, a more accurate descriptor is the term *distal convoluted tubule diuretics*.

Urinary Electrolyte and Water Excretion

Acute administration of these drugs increases the excretion of Na, K, Cl, HCO_3, phosphate, and urate (see Table 81-1) (189,297,298), although the increases in HCO_3, phosphate, and urate excretion are probably related primarily to carbonic

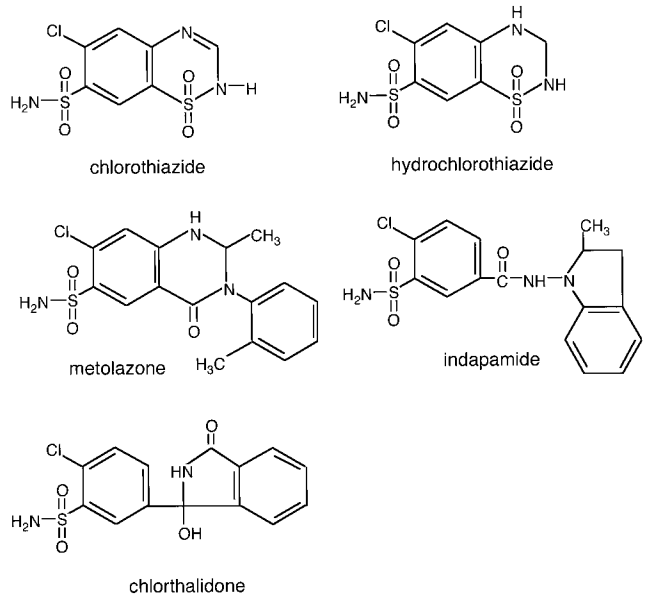


FIGURE 81-7. Structures of DCT diuretics.

anhydrase inhibition (see subsequent text). As such, the effects of DCT diuretics to increase HCO_3, phosphate, and urate excretion may vary, depending on the carbonic anhydrase inhibiting potency of particular drug. Chronically, as contraction of the extracellular fluid volume occurs, uric acid excretion declines and hyperuricemia can occur (299). Furthermore, bicarbonate excretion ceases, and continuing losses of chloride without bicarbonate coupled with extracellular fluid volume contraction may lead to metabolic alkalosis. In contrast to loop and proximally acting diuretics, DCT diuretics tend to reduce urinary calcium excretion (300,301). Although the effects on urinary calcium excretion can be variable during acute administration (297,300,302), these drugs uniformly lead to calcium retention when administered chronically.

DCT diuretics inhibit the clearance of solute free water (C_{H_2O}) when administered during water diuresis. This effect is similar to that of loop diuretics and originally led to the mistaken inference that they act along the thick ascending limb. In contrast to loop diuretics, however, DCT diuretics do not limit $T^C_{H_2O}$ during antidiuresis (188,303).

Mechanism of Action

DCT diuretics are organic anions. On the basis of studies in which [^{3}H]metolazone binding to kidney cortical membranes was studied, DCT diuretics were believed to bind to the Na-Cl cotransporter at the anion site (304,305). This conclusion derives from the observation that chloride inhibits the binding of [^{3}H]metolazone in a competitive manner. Unlike loop diuretic binding to the Na-K-2Cl cotransporter, [^{3}H]metolazone binding to the Na-Cl cotransporter does not require the presence of Na, suggesting either that chloride binds first to the transporter or that binding of ions to the transporter is not "ordered" (304,305). In contrast, Monroy et al. (306) reported that the inhibitory activity of DCT diuretics is inhibited by increasing concentration of either Cl or Na. These workers suggested a model that incorporates Na and Cl binding to the transporter in random order, with alterations in diuretic affinity occurring secondarily. Although these conclusions appear to be contradictory, it should be mentioned that in one study diuretic binding was determined, whereas in the other diuretic inhibiting affinity was determined. As is the case for the Na-K-2Cl cotransporter, described previously, firm conclusions about sites of diuretic binding await crystallization of the transport protein.

Na and Water Transport in the Proximal Tubule

As discussed in the preceding text, DCT diuretics are related chemically to carbonic anhydrase inhibitors, and most DCT diuretics retain carbonic anhydrase inhibiting activity (307). Carbonic anhydrase inhibitors interfere with the activity of the apical Na/H exchanger expressed at the luminal membrane of proximal tubule cells. Although this effect of DCT diuretics may occur when these drugs are administered acutely (as during intravenous chlorothiazide administration), it probably contributes little to the overall natriuresis during chronic use (308,309). Yet this effect may play a role in the tendency for DCT diuretics to reduce the glomerular filtration rate and activate the TGF mechanism (94). The relative carbonic anhydrase inhibiting potency (shown in parentheses) of some commonly used DCT diuretics is chlorthalidone (67) >benthiazide (50)>polythiazide (40)>chlorothiazide (14) >hydrochlorothiazide (1)>bendroflumethiazide (0.07) (310).

NaCl Absorption in the Distal Nephron

As the name indicates, the predominant site at which DCT diuretics inhibit ion transport is the DCT. Although clearance studies had identified one site of thiazide action as the cortical diluting segment (188) and a second site as the proximal tubule (311–313), micropuncture studies pinpointed the primary site of action as the superficial "distal tubule" (308). This region of the nephron, between the macula densa and the confluence with another nephron to form the cortical collecting duct, is cytologically heterogenous (314,315). It comprises a short stretch of post macula densa thick ascending limb, the DCT, the connecting tubule, and the initial portion of the cortical collecting duct. When this morphologic heterogeneity became evident, experiments were designed to determine the site of thiazide action more precisely. Microperfusion experiments in rats indicated that thiazide diuretics inhibit Na and Cl transport along the "early" portion of the distal tubule (316,317), a segment known to contain predominantly DCT cells (314,318).

Although microperfusion data from rats indicated that thiazide diuretics inhibit Na transport in the DCT, the transition between distal nephron segments in this species is gradual. For several years, therefore, it was impossible to attribute thiazide-sensitive Na-Cl transport to a specific cell type in the rat (296,319). In contrast, rabbit distal nephron segments have abrupt transitions. Therefore, it has been possible to perfuse DCTs, connecting tubules, and cortical collecting ducts separately *in vitro*. Initial work suggested that thiazidesensitive Na-Cl cotransport could be detected in connecting tubules, but not in distal convoluted tubules (320–322). In contrast, Greger and Velázquez reported evidence consistent with the presence of electroneutral Na transport in rabbit distal convoluted tubules (323), although thiazide-sensitive sodium or chloride transport was not measured directly. Pizzonia et al. immunodissected cells from mouse thick ascending limb and distal convoluted tubule (324). A clonal cell line was developed that expresses thiazide-sensitive sodium and chloride uptake, suggesting that thiazide-sensitive Na-Cl cotransport is expressed by distal convoluted tubule cells in the mouse (325). Definitive identification of cells that express the thiazidesensitive Na-Cl cotransporter, however, required molecular identification of the protein.

In 1984, Velázquez et al. reported the mutual dependence of Na and Cl transport in the superficial rat distal tubule (326). The same year, Stokes reported evidence that a directly coupled thiazidesensitive Na-Cl cotransporter is expressed in the urinary bladder of the Winter Flounder (327). Gamba et al. expression cloned a thiazidesensitive Na-Cl cotransporter from the flounder bladder (21) and Myanoshita et al. detected a similar mRNA in rat and mouse kidney (328), and then cloned a rat form of the thiazidesensitive Na-Cl cotransporter (328). Mouse, human, and rabbit forms were cloned shortly thereafter (31,329,330); the gene is *SLC12A3*. This transport protein has been variously termed the TSC (thiazide-sensitive cotransporter), the NCCT (sodium chloride cotransporter), and NCC (sodium chloride cotransporter). In rat, human, and mouse, NCC message and protein are expressed by DCT cells (35,331–334). A model of Na-Cl transport by DCT cells is shown in Figure 81-8. In human, rat, and mouse, expression of NCC extends into a transitional segment, referred to as the DCT2, which shares properties of DCT and connecting tubules (35,334). In the rabbit, the thiazidesensitive Na-Cl cotransporter was also shown to be expressed *exclusively* by DCT cells (335); connecting tubule cells do not express the transporter. Therefore, from a molecular standpoint, the NCC is expressed by distal convoluted tubule cells in all mammalian species examined to date.

Evidence for thiazide action in other nephron segments has also been obtained. *In vivo* catheterization experiments demonstrated a component of thiazide-sensitive Na transport in medullary collecting tubules of rats (336). Some (337,338) investigators have detected thiazidesensitive Na-Cl transport in rat cortical collecting ducts perfused *in vitro*. In those

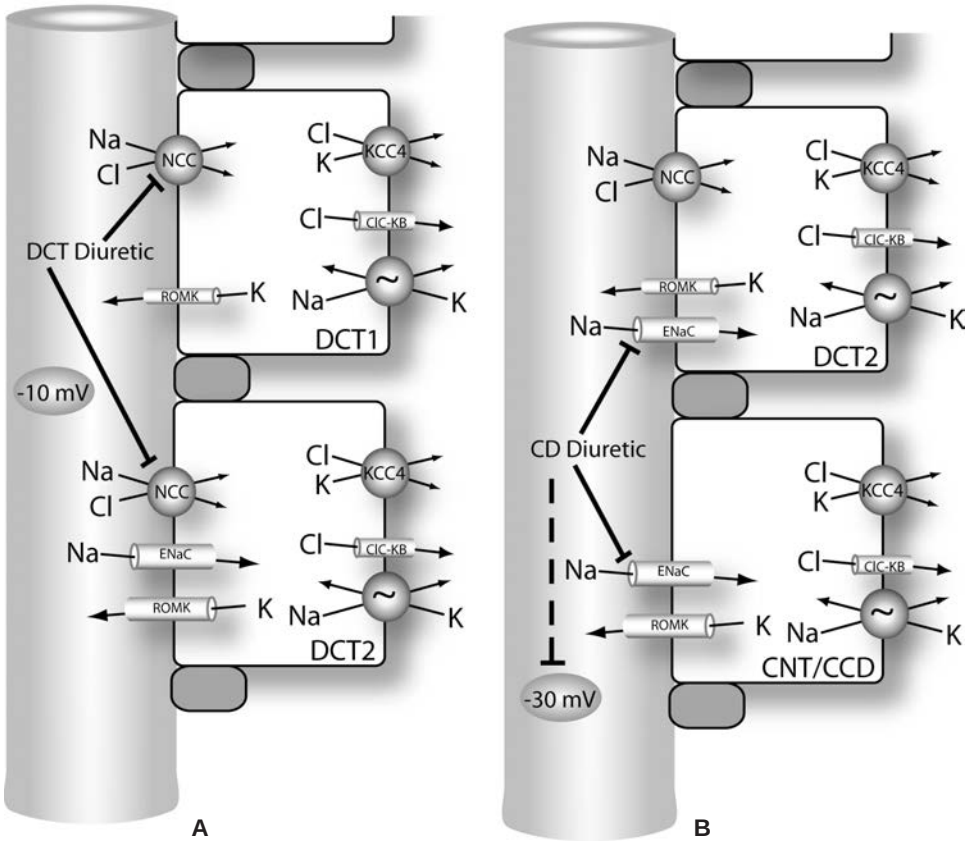


FIGURE 81-8. Mechanisms of DCT and CD diuretics. **Panel A** shows mechanism of action of DCT diuretics. In rat, mouse, and human, two types of distal convoluted tubule cells have been identified, referred to here as DCT-1 and DCT-2. Na and Cl are reabsorbed across the apical membrane of DCT-1 cells only via the thiazide-sensitive Na-Cl cotransporter. This transport protein is also expressed by DCT-2 cells, where Na can also cross through the epithelial Na channel, ENaC (35,334,443). Therefore, the transepithelial voltage along the DCT-1 is near to 0 mV, whereas it finite and lumennegative along the DCT-2. **Panel B** shows the mechanism of action of CD diuretics. The late distal convoluted tubule cells (DCT-2 cells) and connecting (CNT) or collecting duct (CD) cells are shown. Na is reabsorbed via the epithelial Na channel (ENaC), which lies in parallel with a K channel (ROMK). The transepithelial voltage is oriented with the lumen negative, relative to interstitium (shown by the *circled value*), generating a favorable gradient for transepithelial K secretion. Drugs that block the epithelial Na value reduce the voltage towards 0 mV (effect indicated by *dashed line*), thereby inhibiting K secretion.

experiments, pretreatment of animals with mineralocorticoid hormones was necessary to induce the thiazide-sensitive Na and Cl transport.

Calcium and Magnesium Transport

When administered chronically, DCT diuretics reduce calcium excretion. This effect has been utilized clinically to treat calcium nephrolithiasis (see subsequent text). Much progress in understanding mechanisms of the hypocalciuric effect of DCT diuretics has been made during the last 10 years. Acute administration of DCT diuretics has a variable effect on calcium excretion, sometimes leading to increases in calcium excretion (297,339). This probably reflects the carbonic anhydrase inhibiting capacity of these drugs, because carbonic anhydrase inhibitors increase urinary calcium excretion acutely. Calcium reabsorption by proximal tubules is functionally coupled to sodium reabsorption; drugs that inhibit proximal Na reabsorption also inhibit proximal calcium reabsorption (340). During chronic treatment, however, the filtered calcium load decreases, owing to the hemodynamic effects discussed in the following text, and the proximal calcium reabsorption increases, owing to extracellular fluid volume contraction.

The primary site where DCT diuretics increase renal calcium reabsorption, is the same segment where they inhibit Na and Cl transport. Although rat distal nephrons reabsorb both Na and Ca, Costanzo and Windhager showed (341) that thiazide diuretics dissociate the two; Na reabsorption is inhibited whereas calcium reabsorption is stimulated. Several factors are now believed to contribute to the effect of DCT diuretics to stimulate calcium reabsorption (see Fig. 81-9). As in most other cells, the intracellular calcium concentration of DCT cells is low, compared with extracellular fluid calcium (325). Calcium enters DCT cells passively, down its electrochemical gradient via specific calcium channels, primarily TRPV5 (342,343). DCT diuretics increase the intracellular calcium activity, suggesting that a primary effect is to increase apical calcium entry (325).

Brunette et al. proposed that two distinct pathways mediate apical calcium entry in DCT cells. One pathway has a high affinity for calcium and is stimulated by parathyroid hormone, whereas the other has a lower affinity for calcium and is stimulated by thiazide diuretics (344,345). Friedman (207), Gesek and Friedman (325) and Bacskai and Friedman (346) also obtained evidence for distinct mechanisms of calcium entry. They studied a calcium channel that is activated by hyperpolarization (207,325,346). When DCT cells were incubated with thiazide diuretics, the cells hyperpolarized, owing to the reduction in cellular chloride activity and the presence of a chloride channel at their basolateral membrane. The hyperpolarization was shown to activate calcium channels, leading to increased rates of calcium entry, but the molecular nature of this calcium entry pathway remains undetermined.

Bindels et al. (342) cloned a calcium channel that is expressed at the apical membranes of cells along the second part of the distal convoluted tubule (the DCT2) and the connecting tubule. This channel is regulated by vitamin D and appears to possess several characteristics suggesting that it is one of the primary apical calcium entry pathways. Lee et al. confirmed an acute effect of DCT diuretics on distal calcium uptake but found that a large portion of the chronic effects of DCT diuretic results from extracellular fluid volume depletion. They speculated that acute exposure to DCT diuretics hyperpolarizes DCT cells, as suggested by Gesek and Friedman (325), activating TRPV5 channels and also increasing their expression at the mRNA level (347). During chronic exposure, however, extracellular fluid volume contraction reduces distal Na-Cl delivery, limiting the DCT diuretic-induced hyperpolarization (347). Two groups have reported that chronic DCT diuretic treatment reduces the abundance of calcium transporting proteins (347,348).

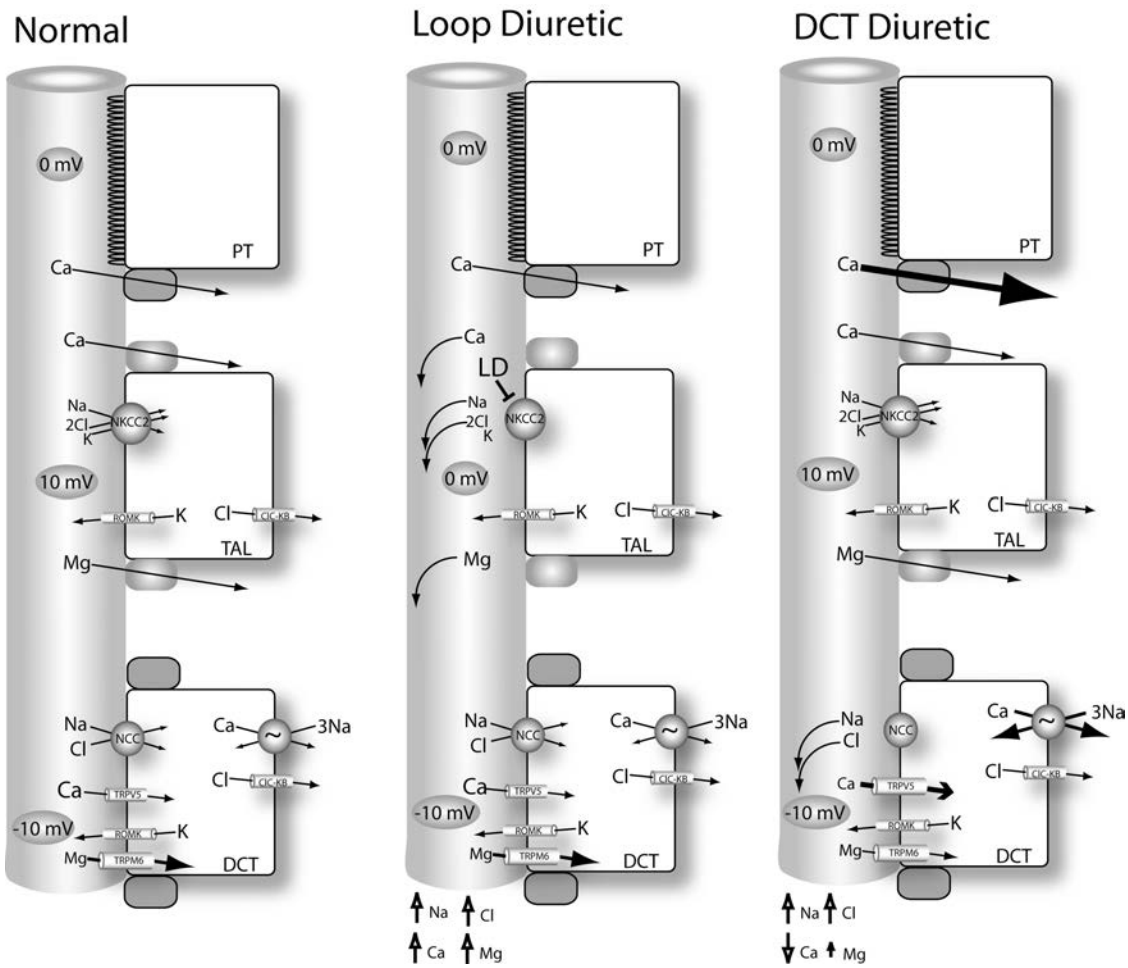


FIGURE 81-9. Possible mechanisms of diuretic effects on calcium and magnesium excretion. Typical cells from the proximal tubule (PT), thick ascending limb (TAL), and distal convoluted tubule (DCT) are shown. Calcium reabsorption occurs along the distal convoluted tubule largely via a transient receptor potential channel (TRPV5). Magnesium reabsorption occurs along the distal convoluted tubule largely via a transient receptor potential channel (TRPM6). Transepithelial voltages (representative but arbitrary values, given in millivolts, mV) are shown. Net effects on electrolyte excretion are shown at the bottom. Normal conditions are at the left. Treatment with loop diuretics (LD) is shown in the middle; treatment with distal convoluted (DCT) diuretics is shown on the right. Loop diuretics reduce the magnitude of the lumenpositive transepithelial voltage, thereby retarding passive calcium and magnesium reabsorption. Passive calcium and magnesium reabsorption appears to traverse the paracellular pathway. Chronic treatment, especially with DCT diuretics, increases proximal Na and Ca reabsorption; therefore, less calcium is delivered distally. Enhanced distal calcium absorption, driven by DCT diuretics also occurs. Effects of DCT diuretics to increase magnesium excretion remain incompletely understood.

DCT diuretics not only stimulate entry of calcium across the apical membrane but also stimulate calcium transport across the basolateral cell membrane into the interstitium. DCT cells, at least in rat, mouse, and human, express the Na/Ca exchanger (35,335,349,350). The Na/Ca exchanger is electrogenic and is believed to carry 3 Na ions into the cell in exchange for 1 calcium ion out. When DCT diuretics block the luminal entry pathway for Na and Cl, as noted in the preceding text, cells hyperpolarize and the intracellular Na activity declines. Both the hyperpolarization and the decline in cell Na concentration increase the electrochemical driving force favoring calcium movement from cell to interstitium. Although the data describing effects on apical and basolateral calcium transport were obtained in different model systems and from different species, taken together, they suggest that DCT diuretics stimulate both the apical entry pathway and the basolateral exit pathway that permit calcium reabsorption. The calcium reabsorptive pathway of the distal tubule is quite potent and the passive calcium permeability of this tubule segment is low; in stationary microperfusion experiments, the distal tubule was able to reduce the luminal calcium concentration to less than 0.1 mM (341).

The effects of DCT diuretics on magnesium excretion are incompletely characterized. Acute infusions have been reported to have little effect on magnesium excretion (297,351,352). In contrast, DCT diuretics increase urinary magnesium excretion and can cause hypomagnesemia when administered chronically (353–355). The mechanisms of magnesium transport by DCT cells and their regulation by diuretics are not completely understood, but some recent progress has been made recently, at the molecular level. Quamme et al. proposed that magnesium is transported across the apical membrane of DCT cells by a hyperpolarization-activated magnesium channel (356). They found that DCT diuretics stimulated magnesium uptake into DCT cells by reducing the intracellular activity of chloride, hyperpolarizing the membrane voltage, and activating magnesium channels (356). Amiloride was found to have similar effects on magnesium uptake (357). Konrad et al. identified

mutations in the transient receptor potential metastatin (TRPM) channel, TRPM6 (358). This protein localizes to DCT cells (and intestinal cells) at the apical membrane, and has functional characteristics suggesting it is, or is part of, the magnesium entry step along the distal nephron (359).

The mechanisms by which chronic DCT diuretic administration lead to hypomagnesemia remain controversial. Quamme proposed that DCT diuretics, by hyperpolarizing DCT cells, enhance magnesium uptake, thereby downregulating the expression of magnesium channels, leading to magnesuria (360). Ellison suggested that magnesium wasting consequent to DCT diuretic treatment resulted from the actions of aldosterone to increase the lumen-negative transepithelial voltage of the DCT (361). This would be expected to increase the electrochemical gradient favoring magnesium secretion (or inhibiting its reabsorption). Loffing et al. suggested that magnesium wasting results from the destruction of DCT cells, resulting from apoptosis induced by DCT diuretics (362,363).

Renal Hemodynamics

DCT diuretics increase renal vascular resistance and decrease the glomerular filtration rate when given acutely (94,308,316,364–366). Okusa et al. (94) showed that intravenous chlorothiazide reduced the glomerular filtration rate by 16% when measured as whole kidney clearance or by micropuncture of a superficial distal tubule. In contrast, however, when flow to the macula densa was blocked and the single nephron glomerular filtration rate was measured by micropuncture of a proximal tubule, intravenous chlorothiazide had no effect on glomerular filtration rate. These data suggest that diuretic-induced stimulation of the TGF system mediates the effect of DCT diuretics on glomerular filtration rate, yet the recent data, described in the preceding text, in which carbonic anhydrase inhibition was shown to reduce the GFR of adenosine-1 receptor deficient, TGF-deficient, animals, suggest that other mechanisms may be involved as well (161).

During chronic treatment with DCT diuretics, contraction of the extracellular fluid volume develops, thereby increasing solute and water reabsorption by the proximal tubule. This effect reduces Na delivery to the macula densa. This would be expected to return GFR toward baseline values during chronic treatment with DCT diuretics (309,367). Therefore, when used chronically, DCT diuretics lead to a state of mild extracellular fluid volume contraction, increased fractional proximal reabsorption, and relatively preserved glomerular filtration (309,367).

When administered acutely, the effect of DCT diuretics on renin secretion is variable (368). If urinary NaCl losses are replaced, these drugs tend to suppress renin secretion (369), probably by increasing NaCl delivery to the macula densa (94). In contrast, during chronic administration, renin secretion increases both because solute delivery to the macula densa declines (309) and because volume depletion activates the vascular mechanism for renin secretion.

Pharmacokinetics

DCT diuretics are organic anions that circulate in a highly protein bound state. As with loop diuretics, the amount reaching the tubule fluid by filtration across the glomerular basement membrane is small; the predominant route of entry into tubule fluid is by secretion via the organic anion secretory pathway in the proximal tubule (39). DCT diuretics are rapidly absorbed across the gut, reaching peak concentrations within 1.5 to 4 hours (39). The amount of administered drug that reaches the urine varies greatly (for a review see [39]) as does the half-life. Short-acting DCT diuretics include bendroflumethiazide, hydrochlorothiazide, tizolemide, and trichlormethiazide. Medium-acting DCT diuretics include chlorothiazide, hydroflumethiazide, indapamide, and mefruside. Long-acting DCT diuretics include chlorthalidone, metolazone, and polythiazide (39). The clinical effects of the differences in half-life are unclear, except in the incidence of hypokalemia, which is more common in patients taking the longer acting drugs such as chlorthalidone (269,370).

Clinical Use

DCT diuretics are used most commonly to treat essential hypertension. Despite a great deal of debate about the potential complications of DCT diuretics, these drugs continue to be recommended as first-line therapy for hypertension because these drugs are clearly at least as effective as more expensive agents at reducing mortality (371). DCT diuretics are also used commonly to treat edematous conditions, although they are frequently perceived as being less effective than loop diuretics. Although the maximal effect of loop diuretics to increase urinary Na, Cl, and water excretion is greater than that of DCT diuretics, Leary and Reyes have shown that the cumulative effects of DCT diuretics on urinary Na and Cl excretion are often greater than those of once daily furosemide (372). Although these studies were conducted in normal volunteers, they may extend to patients with mild cases of edema. In addition, DCT diuretics have proved useful to treat edematous patients who have become resistant to loop diuretics. In this case, the addition of a DCT diuretic to a regimen that includes a loop diuretic frequently increases urinary Na and Cl excretion dramatically (see subsequent text).

DCT diuretics have become drugs of choice to prevent the recurrence of kidney stones in patients with idiopathic hypercalciuria. In several controlled and many uncontrolled studies, the recurrence rate for calcium stones has been reduced by up to 80% (373–375). Relatively high doses of DCT diuretics are often employed for the treatment of nephrolithiasis (376). Some studies suggest that the hypocalciuric effect of DCT diuretics wanes during chronic use, in the setting of absorptive hypercalciuria (377). The observation that Gitelman's syndrome, an inherited disorder of the thiazid-esensitive Na-Cl cotransporter, may present during adulthood with hypocalciuria suggests that compensatory mechanisms may not exist for the effects of DCT diuretics on calcium transport (361). The ability of DCT diuretics to reduce urinary calcium excretion suggests that these drugs may prevent bone loss. Some (378,379), but not all (380,381), epidemiologic studies suggest that DCT diuretics reduce the risk of hip fracture and osteoporosis. A randomized controlled study confirmed that DCT diuretics reduce bone loss in women (382). A case series suggests that DCT diuretics also provide rapid recovery of bone mass in osteoporotic men (383). A recent study confirmed an effect of DCT diuretic to reduce hip fracture but noted that the protective effect wanes within 4 months of discontinuance (384). Others have indicated that DCT diuretics can be effective in patients with primary hypoparathyroidism, when combined with a low salt diet (385). Concomitant potassium bicarbonate administration may increase renal calcium retention induced by DCT diuretics (386).

DCT diuretics are also employed to treat nephrogenic diabetes insipidus, causing a paradoxical decrease in urinary volume flow rate. This action of DCT diuretics results from the combination of mild extracellular fluid volume contraction, owing to diuretic-induced natriuresis, suppression of glomerular filtration, owing largely to diuretic-induced activation of the TGF mechanism, and impaired solute reabsorption along the DCT. The DCT, like the thick ascending limb, is nearly impermeable to water (387). Solute reabsorption by the thiazide-sensitive Na-Cl cotransporter therefore contributes directly to urinary dilution. The central role of extracellular fluid volume

contraction in the efficacy of DCT diuretics in diabetes insipidus was highlighted by the observation that dietary salt restriction is necessary to reduce urinary volume effectively (367,388). DCT diuretics may also increase the antidiuretic hormone-independent water permeability of the medullary collecting tubule (389). When administered to rats lacking antidiuretic hormone (similar to patients with central diabetes insipidus), DCT diuretics did not alter the abundance of the apical water channel of the collecting duct (aquaporin-2) (390), even though they reduced urine volume. In contrast, DCT diuretic treatment increased the abundance of aquaporin-2, the thiazide-sensitive Na-Cl cotransporter (*SCL12A3*) and the alpha subunit of the epithelial Na channel (391), when administered to rats with lithium-induced nephrogenic diabetes insipidus. It was suggested that the upregulation of the abundance of the renal Na and water transporters might explain the antidiuretic effectiveness of DCT diuretics.

Adverse Effects

Electrolyte disorders, such as hypokalemia, hyponatremia, and hypomagnesemia are common side effects of DCT diuretics. A measurable decline in serum K concentration is nearly universal in patients given DCT diuretics, but most patients do not become frankly hypokalemic. In the ALLHAT trial, mean serum potassium concentrations declined from 4.3 to 4.0 and 4.1 mM at 2 and 4 years of treatment, respectively (392). The clinical significance of diuretic-induced hypokalemia continues to be debated (393–400). Unlike the loop diuretics, DCT diuretics do not influence K transport directly (401). Instead, they increase K excretion indirectly. DCT diuretics increase tubule fluid flow in the connecting tubule and collecting duct, the predominant sites of K secretion along the nephron. Increased flow stimulates K secretion along the distal nephron (96). Recent work has indicated that the flow-regulated K secretion is mediated largely by a large calciumactivated K channel (402–406). In addition, DCT diuretic-induced extracellular fluid volume contraction activates the renin–angiotensin–aldosterone system, further stimulating K secretion. Evidence for the central role of aldosterone in diuretic-induced hypokalemia includes the observation that hypokalemia is much more common during treatment with long-acting DCT diuretics, such as chlorthalidone, than with shorter-acting DCT diuretics, such as hydrochlorothiazide or with the very short-acting loop diuretics (407). Another reason that DCT diuretics may produce more potassium wasting than loop diuretics is the differences in effects on calcium transport. As discussed in the preceding text, loop diuretics inhibit calcium transport by the thick ascending limb, increasing distal calcium delivery. In contrast, DCT diuretics stimulate calcium transport, reducing calcium delivery to sites of potassium secretion. Okusa et al. (408) showed that high luminal concentrations of calcium inhibit the functional activity of ENaC in the distal nephron, thereby inhibiting potassium secretion. DCT diuretics also increase urinary magnesium excretion and can lead to hypomagnesemia, as discussed in the preceding text. Hypomagnesemia may cause or contribute to the hypokalemia observed under these conditions (409,410). Some studies suggest that maintenance magnesium therapy can prevent or attenuate the development of hypokalemia (409), but this has not been supported universally.

Diuretics have been reported to contribute to more than one-half of all hospitalizations for serious hyponatremia. Hyponatremia is especially common during treatment with DCT diuretics, compared with other classes of diuretics, and the disorder is potentially life-threatening (411). Several factors contribute to DCT diuretic-induced hyponatremia. First, as discussed in the preceding text, DCT diuretics inhibit solute transport in the terminal portion of the "diluting segment," the DCT. This impairs the ability to excrete solute-free water. Second, DCT diuretics can reduce the glomerular filtration rate, primarily by activating the TGF system. This limits solute delivery to the diluting segment and impairs solute-free water clearance. Third, DCT diuretics lead to volume contraction, which increases proximal tubule solute and water reabsorption, further restricting delivery to the "diluting segment." Fourth, hyponatremia has been correlated with the development of hypokalemia in patients receiving DCT diuretics (412). Finally, susceptible patients may be stimulated to consume water during therapy with DCT diuretics; although the mechanisms are unclear, this may contribute importantly to the sudden appearance of hyponatremia that can occur during DCT diuretic therapy. Of note, one report suggests that patients who are predisposed to develop hyponatremia during treatment with DCT diuretics will demonstrate an acute decline in serum sodium concentration in response to a single dose of the drug (413). Other studies suggest that risk factors for DCT diuretic-induced hyponatremia include older age, lower body mass, and concomitant administration of selective serotonin reuptake inhibitors (414,415).

DCT diuretics frequently cause mild metabolic alkalosis. The mechanisms are similar to those described previously for loop diuretics, except that DCT diuretics do not stimulate Na/H exchange in the thick ascending limb (TAL).

DCT diuretics cause several disturbances of endocrine glands. Glucose intolerance has been a recognized complication of DCT diuretic use since the 1950s. This complication appears to be doserelated (416,417). In the ALLHAT trial patients there was a 1.8% increase in newonset diabetes at 4 years of treatment, compared with patients treated with calcium channel blockers (418). This difference did not translate into adverse clinical outcomes in the diuretic group, but has generated a great deal of discussion. The pathogenesis of DCT diuretic-induced glucose intolerance remains unclear, but several factors have been suggested to contribute. First, diuretic-induced hypokalemia may decrease insulin secretion by the pancreas, via effects on the membrane voltage of pancreatic β cells. When hypokalemia was prevented by oral potassium supplementation, the insulin response to hyperglycemia normalized, suggesting an important role for hypokalemia (419). Hypokalemia may also interfere with insulin mediated glucose uptake by muscle, but most patients demonstrate relatively normal insulin sensitivity (299). Other factors may contribute to glucose intolerance as well. Volume depletion may stimulate catecholamine secretion, but volume depletion during therapy with DCT diuretics is usually very mild. It has also been suggested that DCT diuretics directly activate calcium-activated potassium channels that are expressed by pancreatic β cells (420). Activation of these channels is known to inhibit insulin secretion. Inhibiting the renin–angiotensin–aldosterone axis appears to reduce the development of new diabetes (421). Drugs that inhibit this pathway might attenuate the effects of diuretics to impair glucose homeostasis, but this has not been tested directly.

DCT diuretics increase levels of total cholesterol, total triglyceride, and low-density lipoprotein (LDL) cholesterol and reduce the high-density lipoprotein (HDL) concentration (299,422). Definitive information about the mechanisms by which DCT diuretics alter lipid metabolism is not available, but many of the mechanisms that affect glucose homeostasis have been suggested to contribute. Hyperlipidemia, like hyperglycemia, is a dose-related side effect, and one that wanes with chronic diuretic use. In the ALLHAT study, treatment with chlorthalidone resulted in a total cholesterol 2.2 mg/dL higher than did treatment with ACE inhibitors (418). In several large clinical studies, the effect of *low-dose* DCT diuretic treatment on serum LDL was not significantly different from placebo (423). Further, treatment of hypertension with DCT diuretics

reduces the risk of stroke, coronary heart disease, congestive heart failure, and cardiovascular mortality (418,424).

CORTICAL COLLECTING TUBULE DIURETICS

Diuretic drugs that act primarily in the cortical collecting tubule or the connecting tubule and cortical collecting duct (potassium-sparing diuretics) comprise three pharmacologically distinct groups: aldosterone antagonists (spironolactone and eplerenone), pteridines (triamterene), and pyrazinoylguanidines (amiloride, see Fig. 81-10). The site of action for all diuretics of this class is the cortical collecting duct and the connecting tubule, where they interfere with sodium reabsorption and indirectly potassium secretion. Because of the ability to minimize the normal tendency of diuretic drugs to increase potassium excretion, amiloride (425) and triamterene (426,427) are considered potassium sparing. Diuretic activity is weak partly because fractional sodium reabsorption in the collecting tubule usually does not exceed 3% of the filtered load. Another reason, however, may relate to the tendency for these drugs to produce only partial blockade of Na channels. In support of this hypothesis, knockout or disruption of sodium channel (EnaC) function leads to profound renal salt wasting (428). Because potassium-sparing drugs are relatively weak natriuretic agents, they are used most commonly in combination with thiazides or loop diuretics, often in a single preparation, to restrict potassium losses and sometimes augment diuretic action. However in certain conditions, potassium sparing diuretics are used as first-line agents (see Table 81-3). For example spironolactone is used in the treatment of edema in patients with cirrhosis (429) and amiloride or triamterene is used as a first-line treatment of Liddle's syndrome (430,431) or Bartter's syndrome (432). Spironolactone has been shown to improve mortality of patients with congestive heart failure (433). Eplerenone was shown to improve mortality in patients with left ventricular dysfunction following myocardial infarction (434).

Urinary Electrolyte Excretion

Amiloride, triamterene, and spironolactone are weak natriuretic agents when given acutely (see Table 81-1), although some studies suggest that these drugs are as effective as furosemide in some clinical settings (429). In addition, these three diuretic agents decrease hydrogen ion secretion by the late distal tubule and collecting ducts. Evidence that spironolactone decreases hydrogen ion excretion comes from the finding of metabolic acidosis associated with mineralocorticoid deficiency (435,436), and the finding that spironolactone produces metabolic acidosis in patients with cirrhosis who have mineralocorticoid excess (437). In rats, the administration of amiloride and triamterene has been shown to inhibit urinary acidification (425,427). A common mechanism is likely to be involved in mediating the effects of all three diuretic agents on hydrogen ion secretion. These drugs reduce the lumen-negative potential difference (voltage) and thus decrease the electrochemical gradient favoring hydrogen ion secretion.

Clearance studies in rats have demonstrated that amiloride decreases calcium excretion (438). In these studies, amiloride produced both a decrease in the calcium clearance/Na clearance ratio (C_{Ca}/C_{Na}), as well as a decrease in the fractional excretion of calcium. The effect of triamterene on clearance of calcium was less clear, although it did decrease the C_{Ca}/C_{Na} ratio. *In vivo* microperfusion of rat distal tubules demonstrated that the effect of chlorothiazide on calcium absorption was enhanced with amiloride, but that amiloride action was along the "late" distal tubule (probably the connecting tubule) rather than in the true DCT (316). Furthermore *in vitro* perfusion of connecting tubules has shown that amiloride stimulates calcium absorption (322). Amiloride is believed to stimulate calcium absorption through its ability to block sodium channels, thereby hyperpolarizing the apical membrane (439).

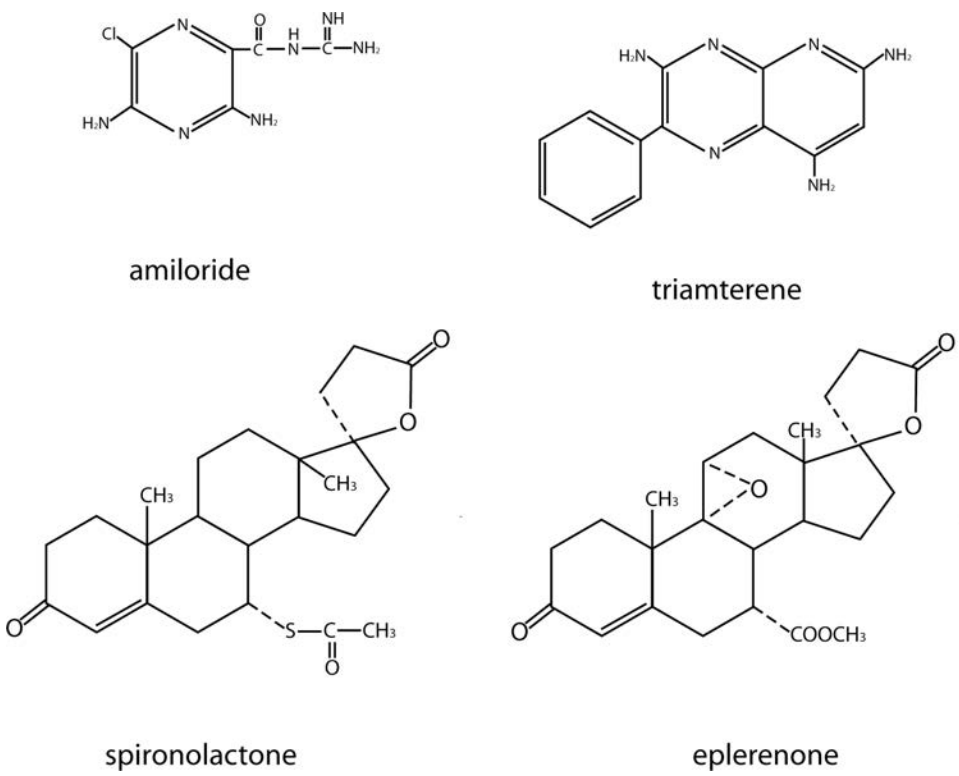


FIGURE 81-10. Structure of collecting duct diuretics. Two classes of diuretics are available, Na channel blocking drugs (triamterene and amiloride) and aldosterone antagonists (spironolactone and eplerenone).

TABLE 81-3

INDICATIONS FOR DIURETIC DRUGS

I. Indications for osmotic diuretics
  A. Acute or incipient renal failure, especially owing to heme pigment
  B. To reduce intraocular or intracranial pressure
II. Indications for carbonic anhydrase inhibitors
  A. Glaucoma
  B. Acute mountain sickness
  C. Metabolic alkalosis
  D. Cystinuria
  E. Resistant edema (used in combination with other diuretics)
III. Indications for loop diuretics
  A. Edematous conditions
    1. Congestive heart failure
    2. Cirrhotic ascites
    3. Nephrotic syndrome
  B. Hypercalcemia (with saline)
  C. Hyperkalemia
  D. Hyponatremia (with hypertonic saline)
  E. Hyperkalemic, hyperchloremic metabolic acidosis (type 4 RTA)
  F. Hypermagnesemia
  G. Intoxications
  H. Hypertension
  I. Acute renal failure
IV. Indications for DCT diuretics
  A. Hypertension
  B. Edematous conditions
    1. Congestive heart failure
    2. Cirrhotic ascites
    3. Nephrotic syndrome
  C. Nephrolithiasis
  D. Nephrogenic diabetes insipidus
  E. Osteoporosis
  F. Hypoparathyroidism
  G. Diuretic resistance (used in combination with other diuretics)
V. Indications for collecting duct diuretics
  A. Cirrhotic ascites
  B. Lithiuminduced diabetes insipidus
  C. Prevention of hypokalemia (owing to potassiumwasting diuretics)
  D. Prevention of hypomagnesemia (owing to potassium-wasting diuretics)
  E. Diuretic resistance (used in combination with other diuretics)

Hyperpolarization of the apical membrane stimulates calcium entry through hyperpolarization-activated calcium channels, as discussed in the preceding text. Amiloride has also been reported to reduce magnesium excretion (353,440) and to prevent the development of hypomagnesemia during therapy with a DCT diuretic (441).

Mechanism of Action

The site of action of potassium-sparing diuretics is the connecting tubule and collecting duct. Although a great deal of interest has centered on control of Na and K transport by the collecting duct, recent evidence has reemphasized the central role played by the connecting tubule (314,442). Recent molecular studies have indicated that sites of DCT diuretic action overlap considerably with sites of cortical collecting duct (CCD) diuretic action. Therefore, in rat, mouse and human, a transitional segment, with characteristics of both DCT and connecting tubule, is present along the distal tubule (35,334,443–446). This segment, which may comprise the bulk of the distal tubule in humans, expresses both the thiazide-sensitive Na-Cl cotransporter and the amiloride-sensitive epithelial Na channel.

Although the connecting and cortical collecting tubules reabsorb only a small percentage of the filtered Na load, two characteristics render this segment important in the physiology of diuretic action. First, this nephron segment is the primary site of action of the mineralocorticoid, aldosterone, a hormone that controls sodium reabsorption and potassium secretion. Second, virtually all of the potassium that is excreted is due to the secretion of potassium by the connecting and collecting tubules. Therefore, this segment contributes to the hypokalemia seen as a consequence of diuretic action.

The collecting tubule is composed of two cell types that have entirely separate functions. Principal cells (collecting duct cells) are responsible for the transport of sodium, potassium, and water, whereas intercalated cells are primarily responsible for the secretion of hydrogen or bicarbonate ions. The apical membrane of principal cells express separate channels that permit selective conductive transport of sodium and potassium (Fig. 81-8). Connecting tubule cells also express apical Na and K channels, permitting electrogenic Na reabsorption and K secretion. The mechanism by which sodium reabsorption occurs is through conductive sodium channels (for a review see [447]). The low intracellular sodium concentration as a result of the basolateral Na,K-ATPase generates a favorable electrochemical gradient for sodium entry through sodium channels. Because sodium channels are present only in the apical membrane of principal and connectin tubule cells, sodium conductance depolarizes the apical membrane resulting in an asymmetric voltage profile across the cell. This effect produces a lumen-negative transepithelial potential difference. The lumen-negative potential difference together with a high intracellular-to-lumen potassium concentration gradient provides the driving force for potassium secretion.

Amiloride-sensitive sodium conductance is a function of the epithelial sodium channel (ENaC). The functional channel comprises three homologous subunits, α, β, and γ ENaC (448), comprising either four (2α, 1β, 1γ) or nine subunits (449–451). A number of factors regulate this channel, including hormones such as aldosterone, vasopressin, oxytocin, intracellular signaling elements such as G-proteins and cAMP, protein kinase C, intracellular ions (sodium, hydrogen, and calcium) (448), and the cystic fibrosis transmembrane conductance regulator (452,453). Alterations in systemic acid–base balance (454) and sodium intake (455) have also been shown to regulate ENaC function. Studies that used selective subunit antisera have demonstrated specific regulation of subunit abundance or pattern of expression. In mice adapted to a high sodium diet, the α-subunit was undetectable, and the β- and γ-subunits were expressed in the cytoplasm (455). In contrast, mice on a lowsodium diet displayed subapical or apical expression of all three subunits (455). Administration of dDAVP to Brattleboro rats increased expression of all three subunits to varying degrees (456). Long-term acid-loading decreases and base loading increases β- and γ-subunits (454)

The amount of sodium and potassium present in the final urine is tightly controlled by aldosterone action on connecting and collecting duct cells. Extensive studies have demonstrated that in epithelia, aldosterone produces an early increase in sodium conductance (457) followed by a sustained increase in transepithelial sodium transport. As a result, transepithelial sodium transport is increased, an effect that depolarizes the apical membrane. An increase in the lumen-negative-potential in turn enhances potassium secretion through conductive potassium channels located in the apical membrane. The cellular

mechanisms that are responsible for these events have been extensively studied and reviewed (457). Aldosterone has been shown to have heterogeneous effects on ENaC subunits. In mammals, aldosterone has been shown to increase the abundance of the α-subunit of ENaC (457), to redistribute all three subunits to the apical region of principal cells masilamani (458) and to induce a shift in the molecular weight of the γ-subunit from 85 kDa to 70 kDa. In contrast, however, a mineralocorticoid knockout mouse demonstrated essentially normal expression of ENaC subunits in the kidney, suggesting aldosterone regulation ENaC function in a posttranscriptional manner (459). Additional nongenomic activation of ENaC function by aldosterone has been reported (460).

Mineralocorticoid Receptor Blockers. Spironolactone (Fig. 81-10) is an analog of aldosterone that is extensively metabolized (461,462), having the principal effect of blocking aldosterone action (463,464). Spironolactone is converted by deacylation to 7α-thiospironolactone or by diethioacetylation to canrenone (463). In the kidney, spironolactone and its metabolites enter target cells from the peritubular side, bind to cytosolic mineralocorticoid receptors, and act as competitive inhibitors of the endogenous hormone (465–468). In studies using radiolabeled spironolactone or aldosterone, [^{3}H]-spironolactone-receptor complexes were excluded from the nucleus. In contrast, [^{3}H]-aldosterone-receptor complexes were detected in the nucleus (466). These results are consistent with the proposal that aldosterone antagonists block the translocation of mineralocorticoid receptors to the nucleus. The mechanism by which aldosterone antagonists block nuclear localization of antagonist-receptor complexes is not known; however, it has been suggested that they destabilize mineralocorticoid receptors facilitating proteolysis (469). Mineralocorticoid receptors, like other steroid receptors, contain a steroid-binding unit associated with other cellular components including HSP90, in its inactive state. Steroid binding produces dissociation of HSP90 from the steroid binding unit uncapping the DNA-binding sites. Spironolactone facilitates the release of HSP90 and in combination with rapid dissociation of ligand could lead to degradation of the receptor (469).

Spironolactone induces a mild increase in sodium excretion (1% to 2%) and a decrease in potassium and hydrogen ion excretion (468,470). Its effect depends on the presence of aldosterone. Spironolactone is ineffective in experimental adrenalectomized animals (430) and in patients with Addison's disease (430) or humans on a high salt diet. In cortical collecting tubules perfused *in vitro*, spironolactone added to the bath solution reduced the aldosterone-induced lumen-negative transepithelial voltage (426). By blocking sodium absorption in the collecting tubule, a decrease in lumen-negative potential reduces the driving force for passive sodium and hydrogen ion secretion (426).

Spironolactone causes troubling estrogenic side effects commonly, a fact that has limited its more general use (see subsequent text). Renewed interest in the utility of aldosterone blockers, especially in the setting of congestive heart failure, more recently prompted the development of anti-aldosterone agents that are more specific inhibitors of the mineralocorticoid receptor. Eplerenone is a second competitive aldosterone antagonist, currently in clinical use. Eplerenone (see Fig. 81-10) is Pregn-4-ene-7,21-dicarboxylic acid, 9,11-epoxy-17-hydroxy-3-oxo, γ-lactone, methyl ester (7α, 11α, 17α) and was derived from spironolactone by the introduction of a 9α,11α-epoxy bridge and substitution of the 17α-thioacetyl group of spironolactone with a carbomethoxy group. *In vitro*, eplerenone exhibits 10- to 20-fold lower affinity for the mineralocorticoid receptor than spironolactone, but in humans, it appears to be 50% to 75% as potent (471,472). This change significantly enhances the relative affinity of the drug for mineralocorticoid receptors over other steroid receptors.

Amiloride and Triamterene

Amiloride and triamterene (see Fig. 81-10) are structurally different but are organic cations that use the same primary site of action (see Fig. 81-8). Triamterene is an aminopteridine chemically related to folic acid and amiloride is a pyrazinoylguanidine. Systemically administered amiloride results in an increase in sodium excretion and decrease in potassium excretion (89,271,425,473). Their actions on sodium and potassium transport, unlike spironolactone, are independent of aldosterone (473). Systemically administered amiloride produced a small increase in sodium excretion and a much larger decrease in potassium excretion (474,475). Sampling of tubule fluid from the distal tubule demonstrated an inhibition of the normal rise in the tubule fluid to plasma potassium ratio. These results indicated that amiloride decreased distal tubule potassium secretion. Experiments employing *in vivo* microperfusion of distal tubules (401,476) and *in vitro* perfusion of isolated cortical collecting tubules (477,478) demonstrated that luminally administered amiloride reduced sodium absorption and potassium secretion. Similar results were obtained following *in vivo* microperfusion with benazamil (479), a more potent amiloride analog. Amiloride decreases potassium secretion by blocking sodium conductance in the apical membrane of distal tubule and collecting tubule cells (480,481), thereby decreasing the electrochemical gradient for potassium secretion.

In high concentrations (>100 μM), amiloride interacts with different transporters, enzymes, and receptors. At concentrations of 0.05 to 0.5 μM, however, amiloride interacts specifically with sodium channels (482,483). Furthermore, aromatic substitutions on the guanidinium moiety renders the molecule even more potent (IC_{50} 10- to 20-fold lower than amiloride) (482,484). Only the parent compound, amiloride, however, is available for clinical use. The molecular mechanism by which amiloride blocks sodium channels remains incompletely defined. It is likely, however, that the positive charge on the guanidinium moiety plays an important role in occluding the sodium channel (see Fig. 81-8 [447,483]). Amiloride has been demonstrated to bind to a 150-kDa protein from the bovine renal papilla (485); however, the relationship between this protein and the putative Na-channel components, α, β and γ rENaC, is not known (483). Other approaches using mutational analysis (486,487) and anti-amiloride antibodies (488) have demonstrated contributions to amiloride binding by all 3 subunits in proximity to the channel pore. A putative amiloride binding domain, WYRFHY, of the α-subunit of ENaC has been identified (488,489).

Clearance and free-flow micropuncture studies using triamterene demonstrated results similar to studies with amiloride (271); however, the mechanism of action is not clearly defined. In earlier studies of rabbit cortical collecting tubules perfused *in vitro*, triamterene produced a gradual, reversible inhibition of the potential difference after a latent period of 10 minutes (426). More recent studies, however, suggest that triamterene binds to the epithelial sodium channel and thus has a mechanism of action similar to amiloride (490).

Pharmacokinetics

Spironolactone is poorly soluble in aqueous fluids. Bioavailability of an oral dose is approximately 90% in some but not all commercial preparations. The drug is rapidly metabolized in the liver into a number of metabolites. Canrenone is one metabolite of spironolactone (see [461,462]). This conclusion was based on fluorometric assays. Assays of spironolactone and its metabolites by the use of high-performance liquid chromatography (HPLC) demonstrated that fluorometrically measured levels of canrenone overestimated true canrenone levels (491). Using HPLC, the predominant metabolite,

7α-methylspironolactone (492), appears to be responsible for roughly 80% of the potassium-sparing effect. Spironolactone and its metabolites are extensively bound to plasma protein (98%). In normal volunteers, taking spironolactone (100 mg/day) for 15 days, the mean half-lives for spironolactone, canrenone, 7α-thiomethylspironolactone, and 6β-hydroxy-7α-thiomethylspironolactone were 1.4, 16.5, 13.8, and 15 hours, respectively. Therefore, although unmetabolized spironolactone is present in serum, it has a rapid elimination time. The onset of physiologic action is extremely slow for spironolactone, with peak response sometimes occurring 48 hours or more after the first dose; effects gradually wane over a period of 48 to 72 hours. Spironolactone is used in cirrhotic patients to induce a natriuresis. In these patients, pharmacokinetic studies indicate that the half-lives of spironolactone and its metabolites are increased. The half-lives for spironolactone, canrenone, 7α-thiomethylspironolactone, and 6β-hydroxy-7α-thiomethylspironolactone are 9, 58, 24, and 126 hours, respectively (493).

Eplerenone is rapidly absorbed, with peak serum levels at 1.5 hours (472). Its volume of distribution is 43 to 90 L, with approximately 50% protein bound. It is cleared primarily via the CYP4503A4 system to inactive metabolites with an elimination half-life of 4 to 6 hours (472). This is in contrast to spironolactone, where the half-life of the parent compound is short, but the half-life of metabolites is very long. The maximal plasma concentration and area under the curve are increased in people older than 65 years of age and with kidney failure; eplerenone is not removed by hemodialysis brown (472).

Clinical Use

The most common side effect of loop and DCT diuretics is the depletion of body potassium with or without significant lowering of serum K concentration. Hypokalemia of sufficient magnitude may produce nonspecific weakness or may be life-threatening. The more severe effects include impairment of neuromuscular function, cardiac dysrhythmia, intestinal disturbances, and partial loss of the ability to concentrate urine. Of particular concern is the potential for cardiac toxicity in patients with congestive heart failure who are maintained on cardiac glycosides. Given these potential problems, it is important to avoid potassium deficit through dietary intake of large amounts of potassium, avoidance of excessive Na-Cl intake, and monitoring of serum K concentrations. The most effective therapeutic measure is to add a potassium-sparing diuretic to the therapeutic regimen (494), but KCl supplements should be discontinued or plasma K monitored carefully if a K-sparing agent is used.

Spironolactone (or eplerenone) is most effective as a single agent in patients with primary (adrenal adenoma or bilateral adrenal hyperplasia) (495) or secondary hyperaldosteronism (congestive heart failure, cirrhosis, nephrotic syndrome) and is ineffective in patients with nonfunctional adrenal gland (496). Spironolactone is used for correction of hypokalemia. The drug is also administered alone, with thiazides, or a loop diuretic, to reduce the ECF volume without causing potassium depletion or hypokalemia. The drug is especially appropriate for the treatment of cirrhosis with ascites, a condition invariably associated with secondary hyperaldosteronism (429). In comparison to loop or thiazide diuretics, spironolactone is equivalent or more effective (497). A combination of loop diuretic in addition to spironolactone can be used to boost natriuresis when the diuretic effect of spironolactone alone is inadequate.

Interest in the use of aldosterone blockers has greatly increased during the last 10 years, sparked first by the observation that spironolactone reduces morbidity and mortality in congestive heart failure when added to a regimen of ACE inhibitors and loop diuretics (433). A subsequent study showed that eplerenone reduced morbidity and mortality of patients with left ventricular dysfunction following a myocardial infarction (498).

Spironolactone or eplerenone is the treatment of choice for hypertension owing to adrenocortical hyperplasia (499).

Triamterene or amiloride is generally used in combination with potassium-wasting diuretics (thiazide or loop diuretics), especially when maintenance of normal serum potassium concentrations is clinically important. In addition, amiloride (or triamterene) has also been used as initial therapy in potassium wasting states such as primary hyperaldosteronism (500,501), Liddle's (431), Bartter's, or Gitelman's syndrome (432). Amiloride has been used in the treatment of lithium-induced nephrogenic diabetes insipidus (502). The efficacy of amiloride in this disorder relates to the ability of amiloride to block collecting-duct sodium channels, a pathway by which lithium uses to gain entry into cells (503,504).

Adverse Effects

The most serious adverse reaction encountered during therapy with spironolactone is hyperkalemia. Serum potassium should be monitored periodically, even when the drug is administered with a potassium-wasting diuretic. Patients at highest risk are those with low glomerular filtration rates and those individuals who take potassium supplements concurrently. This problem has become more important because of the wide use of aldosterone-blocking drugs, together with ACE inhibitors, ARBs, and β-blockers in patients with congestive heart failure (505). Risk factors include kidney failure, older age, coexistent diabetes, and concomitant treatment with β-blockers (506). It is important to note that the original RALES study specifically excluded patients with several of these comorbidities. Renal failure appears to be another complication in this group (506).

In patients with cirrhosis and ascites treated with spironolactone, hyperchloremic metabolic acidosis can develop independent of changes in renal function (437). Gynecomastia may occur in men, especially as the dose is increased (507) but even at low doses (433); decreased libido and impotence have also been reported. Women may develop menstrual irregularities, hirsutism, or swelling and tenderness of the breast. Spironolactone induced agranulocytosis has also been reported (508).

Triamterene and amiloride may cause hyperkalemia. The risk of hyperkalemia is highest in patients with limited renal function (e.g., renal insufficiency, diabetes, and elderly patients). Additional complications include elevated serum blood urea nitrogen and uric acid, glucose intolerance, and gastrointestinal disturbances. Triamterene induces crystalluria or cylindruria (509), may contribute to or initiate formation of renal stones (510) and acute renal failure when combined with nonsteroidal antiinflammatory agents (511,512). The drugs are contraindicated in patients with hyperkalemia, individuals taking potassium supplements in any form, and in patients with severe renal failure with progressive oliguria.

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CHAPTER 82 ■ IDIOPATHIC EDEMA

GRAHAM A. MACGREGOR AND HUGH E. DE WARDENER

Idiopathic edema is an ill-defined syndrome characterized by intermittent edema secondary to sodium and water retention. It occurs almost exclusively in women and is much worse with prolonged standing. Typically, these patients gain excessive amounts of weight during the day compared with normal women. Although pitting edema may be a prominent symptom, particularly in the ankles and legs, the clinical findings are not always as impressive as the patient's symptoms may suggest. A concern about weight amounting to an obsession is common in some patients with a defect in body image. Abdominal bloating often occurs. Many patients spend their lives alternately dieting and binge eating.

Many claims have been made about the etiology of this syndrome, and practically all the known mechanisms that control sodium and water excretion have been suggested at one time as possible causes of idiopathic edema. The idea that idiopathic edema may result from a defect in capillary permeability has been proposed, although there is little evidence to support this hypothesis (1). All investigations in these women are difficult because they often continue to take diuretics while telling investigators that they have stopped. In the majority of women with idiopathic edema, symptoms are made worse by diuretics, and initially may have been caused either by diuretics or the intermittent fasting and binge eating to which many of these women subject themselves (2).

Thorn pointed out many years ago that many patients with idiopathic edema are emotionally labile, depression being a particularly common feature (3). Some patients find that intermittent vomiting or laxative abuse has the same effect as diuretics in that it causes a transient loss of weight and an improved sense of well being, which is then followed, as with diuretics, by a worsening of the edema.

CLINICAL FEATURES

The typical features of idiopathic edema are intermittent swelling of the legs, hands, and, rarely, the face, with abdominal bloating. In particular, there is no pattern to the weight gain, which can occur at any time and is unrelated to the menstrual cycle. Thorn arbitrarily applied a criterion of a weight gain greater than 1.4 kg per day as essential for a diagnosis of idiopathic edema, stating that normal women gain of less than 1 kg per day (3). Unfortunately, it is not clear from what studies Thorn chose these arbitrary figures and, in particular, whether the patients were taking diuretics at the time. Nevertheless, this criterion often has been used for diagnostic purposes (1). In our experience and that of others (4), this criterion is not very helpful because some normal women can gain more than 1.5 kg per day without any symptoms, whereas other women gain only small amounts of weight but have a large number of symptoms directly attributable to sodium and water retention. Indeed, one study showed that one in six women attending a gynecologic outpatient clinic would be classified as having idiopathic edema (4).

In symptomatic subjects the weight gain, particularly the edema in the legs, is made worse by standing during the day and is also aggravated by warm weather. In other patients the most prominent symptom may be abdominal bloating (5). Many patients who do not have clinical evidence of edema may often complain of fullness and tightness of the face, ankles, and hands, particularly the fingers on which there are rings. At the time the physician sees the patient, despite what may seem to the patient to be almost disabling symptoms, there is usually no clinically detectable swelling or edema.

In addition, there may be substantial psychological and emotional disturbances, which Thorn regarded as one of the criteria for diagnosis of idiopathic edema (3). Typically, the patient is emotionally labile with depressive features. Nearly all patients have an excessive concern about their weight, and invariably they are trying to lose weight. They may have a defect in the image they have of their body size, a feeling that they are overweight and appear bloated when in fact they may seem to others to be underweight. Attempts to lose weight often consist of alternately fasting for as long as possible, followed by a short period of excessive food consumption. Such a binge is accompanied by severe sodium and water retention, perhaps with edema, and certainly by all the usual symptoms (6). At this time diuretics may temporarily relieve the sodium and water retention, or, if the patient has not yet discovered diuretics, temporary relief may be obtained by the use of laxatives or surreptitious vomiting.

DIAGNOSIS

Usually the syndrome is straightforward diagnostically, but other causes of sodium and water retention must be excluded. By far the most frequent cause of intermittent water and sodium retention that must be distinguished from idiopathic edema is the menstrual cycle. In patients with idiopathic edema, the "edema" has no relation to the menstrual cycle. It is useful for diagnostic purposes to ask patients to record for a period of time their morning and evening weight and the days on which they menstruate.

A typical feature of idiopathic edema is that true gain in weight is not nearly as impressive as that claimed by the patient. There is no doubt that many patients exaggerate their symptoms, often as a result of the indifference they have encountered from doctors who have dismissed their symptoms as trivial. The patient then feels that the only way to get attention is by claiming daily weight gains that are physiologically impossible. For instance, it is not unusual for patients to claim that they gain from 6 to 8 kg in a single day. If this were owing to sodium and water retention, there would have to be a minimum fluid intake of at least 6 to 8 liters, yet most patients deny drinking this amount of fluid in a single day.

In patients in whom the diagnosis is not clear-cut, Streeten et al. have suggested that a water-loading test in the supine and upright positions might be useful (7). In this test, patients are instructed to drink 20 mL of water per kilogram of ideal body weight over 30 minutes; urine output is then followed for 4 hours. According to Streeten et al., normal subjects excrete 70% of the water load within 4 hours, in both the supine and

the upright positions, whereas patients with idiopathic edema excrete only 70% of the water load in the supine position and much less when upright. Such a test is positive in any subject who is volume depleted for any reason; therefore, if patients are furtively taking diuretics or are covertly sodium-depleting themselves in other ways, a positive test will not be diagnostic of some primary abnormality (8). There is also the risk with this test that hyponatremia may occur.

Idiopathic edema must be distinguished from other well-known causes of sodium and water retention such as heart failure and cirrhosis. Although this is not difficult in most patients, there have been occasional case reports of cardiomyopathy being diagnosed mistakenly as idiopathic edema (9). Renal disease, particularly the nephrotic syndrome, can cause edema but is relatively simple to exclude. Allergic causes of localized edema such as angioneurotic edema can usually be excluded by the history.

Pre-diabetic patients, who often experience intermittent edema before or at the time of developing diabetes, may be more difficult to distinguish from idiopathic edema (10). Patients with myxedema also may experience intermittent swelling that disappears with correction of their thyroid deficiency (11). All patients who are seen for edema, therefore, should have a fasting blood sugar measured, if not a glucose tolerance test and tests of thyroid function.

Many drugs can cause sodium and water retention, in particular, all the nonsteroidal antiinflammatory drugs. Estrogen therapy may cause sodium and water retention, and steroids with mineralocorticoid action (e.g., fludrocortisone) and drugs that inhibit 11-beta-hydroxy steroid dehydrogenase, such as carbenoxolone and liquorice also may cause edema. Chlorpromazine and similar psychotropic drugs can cause fluid retention and mimic some of the symptoms of idiopathic edema (12). Many blood pressure-lowering drugs may cause edema, particularly the postadrenergic blocking drugs (e.g., guanethidine) and the direct arteriolar vasodilators (e.g., hydralazine). Minoxidil is almost invariably associated with severe sodium and water retention with edema unless diuretics are given concomitantly. The calcium channel blockers, which are natriuretic and cause a long-term reduction in sodium balance (13), may paradoxically cause edema of the ankles and legs, particularly the dihydropyridine derivatives (e.g., nifedipine). This edema is thought to result from local arteriolar vasodilation with an imbalance in capillary filtration.

Edema of the legs also may be caused by venous or lymphatic obstruction, and the differentiation from idiopathic edema may be difficult in some patients. This entity may occur particularly with the familial lymphatic syndromes, which may initially present with intermittent pitting edema. There may also be difficulties in some patients with severe varicose veins with thromboses who develop severe dilation of the veins. They may then develop intermittent edema on standing upright, secondary to pooling of blood in the legs (a form of blood volume translocation that diminishes the "effective" arterial blood volume). Many patients may conceal that they are taking diuretics or laxatives. It is very common for patients to alternately binge and fast. This in itself causes exaggerated swings in sodium and water balance.

ETIOLOGY

It is not surprising to find considerable controversy about the potential mechanisms whereby some women develop intermittent edema because the normal mechanisms controlling sodium and water balance in humans are not fully understood. Sodium balance plays an important role in regulating the volume of extracellular fluid in mammals. During evolution, as mammals moved away from the sea, they had difficulty in obtaining enough sodium and developed powerful mechanisms to conserve sodium in urine and sweat as well as an instinctual appetite for sodium. During evolution the average sodium intake of humans has been estimated to be around 5 to 10 mmol/day. However, the more recent ability of humans to obtain salt either from mines or evaporation of seawater and the finding that salt is a good preservative of food have meant that sodium intake has now increased to around 100 to 400 mmol/day. There is no doubt that such a marked increase in salt intake has been associated with an increase in extracellular fluid volume.

Surprisingly, idiopathic edema was not described until 1955 after the introduction of oral diuretics (14); however, Streeten claimed that he saw patients with idiopathic edema as early as 1952, that is, just before the introduction of oral diuretics (15). In the earlier literature, a case report in 1922 is often cited as the first case of idiopathic edema (16); however, this case report is that of a 28-year-old man with sodium and water retention who had clinical and radiologic evidence of a pituitary tumor that had already caused visual field defects.

Nearly all the potential mechanisms that may influence sodium excretion have at one time been claimed to be the underlying cause of idiopathic edema; however, the cause of the syndrome (assuming it exists) has proved elusive. In our view, most of the abnormal findings in these women are caused by the prior use of diuretics that have been stopped a few days before, the continued use of diuretics either knowingly or covertly during the test, excessive amounts of laxatives or surreptitious vomiting, or recurrent fasting followed by binge eating. A recurring problem in the literature of idiopathic edema is that it is often not clear whether patients were studied while taking diuretics, and, if they were not, when the diuretics were stopped and what steps were taken to detect patients who continued to consume diuretics surreptitiously, take laxatives, or vomit.

Potential Etiologic Factors

Upright Posture

Whatever the cause of idiopathic edema, all investigators agree that the edema itself is made worse by prolonged periods of standing; indeed, in most patients the symptoms largely disappear if the patient lies flat during the day (1,3,7). Even in normal subjects, it is well established that adoption of the upright posture causes a reduction in sodium and water excretion. In careful studies, Streeten et al. showed that there is excessive sodium and water retention on tilting in patients with idiopathic edema compared with normal subjects (7). Indeed, they were able to classify patients with idiopathic edema into those who had what they termed "orthostatic water retention," others who had "orthostatic sodium retention," and a few in whom the edema apparently was unrelated to the upright position. This division of patients into those who retain water and/or sodium on standing upright has been criticized and seems to relate more to the severity of the patient's symptoms than to a different underlying mechanism (1). As with many other studies of patients with idiopathic edema, patients stopped the diuretic only a short time before the studies were performed, and Streeten et al. admit that some of the balance data on potassium excretion had to be discarded because of the previous effects of the diuretics (7). It is, therefore, impossible to exclude the possibility, and in our view the probability, that compared with control subjects who had never had diuretics, the long-term use of diuretics would have caused increased sodium and water retention on tilting, even though the diuretics had been stopped a few weeks before (8). One possible mechanism for this excessive sodium and water retention on standing upright even when the diuretic has been discontinued is juxtaglomerular hyperplasia secondary to prolonged

use of diuretics, which would give an exaggerated response of renin and aldosterone to the upright posture, even though the diuretic had been stopped some time previously.

Increased Renin or Aldosterone Level

Early reports of idiopathic edema claimed that patients had increased renin and aldosterone levels or an exaggerated renin and aldosterone response to standing (17–19). Most investigators now agree that these findings are likely to result from previous diuretic therapy. Our own experience and that of others is that if plasma renin activity or plasma aldosterone is raised, it is very suggestive that the patient either was taking a diuretic or was in some other way depleting herself of sodium. Assessment of the level of plasma renin activity or plasma aldosterone must be done in conjunction with a 24-hour urinary sodium excretion test, because subjects who are restricting salt intake also have elevated plasma renin activity and increased aldosterone secondary to salt restriction rather than to the use of diuretics, laxatives, or vomiting.

With the realization that plasma aldosterone concentrations are normal in patients with idiopathic edema who were not taking diuretics, it was then suggested that despite normal levels, a possible mechanism might be a failure to escape from these normal levels of aldosterone. However, studies in which large amounts of fludrocortisone were given to patients with idiopathic edema have shown that they were able to escape from the sodium-retaining effect of the steroid, albeit at a slightly slower rate than normal subjects, and that they did not develop edema, making it extremely unlikely that the "idiopathic" edema could result from a failure to escape from a normal level of aldosterone (1).

Antidiuretic Hormone

The claimed abnormal response to sodium and water excretion in the upright posture in "idiopathic edema" has prompted some authors to suggest that there may be abnormalities of antidiuretic hormone (ADH) secretion. Thibonnier et al., measuring urinary ADH, found that patients with idiopathic edema failed to show the normal suppression of ADH after water load, but only when they were in the upright posture, suggesting perhaps a posturally related defect in the feedback mechanisms controlling ADH (8). However, even if it is accepted that these results are not related to previous diuretic therapy, it is not clear how an increase in ADH secretion in the upright posture could cause edema. Excess secretion of ADH is well known to be associated with a fall in plasma sodium but not to cause sodium retention. It would seem, therefore, very unlikely that increased ADH secretion could have any direct role in the pathogenesis of idiopathic edema which necessitates both sodium and water retention (5).

Hypothalamic Disorders

A report by Young et al. claimed that there is a greater release of prolactin, luteinizing hormone, and follicle-stimulating hormone following administration of luteinizing releasing hormone and thyrotropin-releasing hormone in some patients with idiopathic edema (20). However, three of the 14 patients were studied while taking diuretics, and eight had stopped the diuretics 3 months previously. Furthermore, it is not clear from the article whether any subjects were surreptitiously taking diuretics during the study. No comment is made as to whether the three who were known to be taking diuretics during the study had the most abnormal results. This study concluded that there might be some hypothalamic abnormality that would be compatible with the well-known psychological problems that occur in many patients with idiopathic edema. Some case reports of idiopathic edema associated with raised plasma prolactin concentration and a subsequent favorable response to bromocriptine support a hypothalamic disturbance (21). However, the study by Young et al. showed no significant difference between prolactin levels in the 14 patients compared with controls (20).

Estrogen and Progesterone

The fact that idiopathic edema occurs predominantly in women has led to the suggestion that the edema might be related to an imbalance among estrogen, progesterone, or other sex hormones. However, there is little evidence of a quantitative alteration in sex hormone secretion in these patients compared with normal controls (5). Although Lagrue et al. found some evidence for reduced progesterone levels and normal estrogen levels, progesterone administration did little to relieve the symptoms (22). The reason that the condition is primarily confined to women remains elusive, although there is little doubt that women are much more conscious of their weight and appearance than are men. It is extremely unusual to see men who are addicted to diuretics or laxatives or who vomit surreptitiously to adjust their weight and appearance.

Natriuretic Hormone

Several authors have suggested that there might be an abnormality of secretion of natriuretic factors in idiopathic edema. Mach and Favre have shown that of six patients on a restricted salt diet, three had urinary natriuretic factor levels that were higher than the normal range and in the same range as normal controls taking 9α-fludrocortisone, suggesting, therefore, that in idiopathic edema there is some abnormality in the way the kidney responds to natriuretic factors (23). However, Anderson and Streeten have shown no difference in plasma atrial natriuretic peptide levels in patients with and without idiopathic edema (24).

Hypothyroidism

Many patients with hypothyroidism, and even some with subclinical hypothyroidism, may go through a phase of retaining sodium and water, developing symptoms suggesting idiopathic edema (11). It is therefore very important that thyroxine and thyroid-stimulating hormone levels are measured in all patients. The edema associated with thyroid deficiency responds well to treatment with thyroxine. Nevertheless, the majority of patients with idiopathic edema have completely normal thyroid function.

Diabetes

There is evidence that patients with diabetes or latent diabetes may go through a phase of retention of sodium and water, mimicking the symptoms of idiopathic edema (10). At the very least, all patients should have a test of fasting blood glucose levels. If there is any suspicion of an abnormality, a full glucose tolerance test should be performed. Insulin can cause sodium and water retention, and a change in insulin sensitivity could possibly play a role. The majority of patients with idiopathic edema have normal glucose metabolism. However, importantly, the condition may be aggravated or even initiated by sudden changes in carbohydrate and sodium intake.

Defective Venous Tone

If there is a severe defect of venous tone in the legs, then there will be pronounced pooling of blood on standing, which causes excessive sodium and water retention by the kidney, owing to a reduction in renal blood flow (5). This pooling of blood is usually associated with severe varicose veins or previous thrombotic disorders of the veins of the legs. For instance, we have

seen one patient who had long-standing venous problems in her legs. When she stood up, she developed severe orthostatic hypotension owing to the pooling of a considerable portion of the blood volume in her legs, and she then retained sodium and water. Eventually she developed edema if she was unable to lie down. This is a relatively rare cause of sodium and water retention and is not found in the majority of patients with idiopathic edema.

Abnormal Capillary Permeability

The idea that there may be an abnormality of capillary permeability in patients with idiopathic edema is attractive and has been accepted by some authorities without, in our view, critical appraisal of the evidence (1,15). There is, in fact, no definitive evidence to support an abnormality of capillary permeability in patients with idiopathic edema. The early case reports that have been cited seem to be describing patients who almost certainly had vasculitis associated with connective tissue disorders (25). More recently, Edwards and Bayliss have claimed that when they gave radiolabeled albumin intravenously, there was an increased disappearance of radioactive albumin from the plasma in patients with idiopathic edema compared with control subjects (1). This paper has been widely cited as evidence of abnormal capillary permeability; however, for ethical reasons, no measurements of the disappearance of radiolabeled albumin were made in control subjects. The authors therefore were in no position to decide whether the rate of loss of isotopically labeled albumin in their patients was abnormal (26,27).

The only groups that have attempted to measure directly the permeability of capillaries to albumin in patients with idiopathic edema and normal subjects are Lagrue et al. (28) and Behar et al. (29). Isotopically labeled albumin was injected intravenously, and the forearm was then monitored for radioactivity before, during, and after venous occlusion. Radioactivity rises and falls rapidly in such a maneuver, and failure of radioactivity to return to baseline levels is considered an indication of increased permeability to albumin. The results in patients with edema are difficult to interpret. The most pronounced abnormalities were found in patients with cirrhosis who were grossly edematous and in patients with edema associated with the menstrual cycle, but the abnormality was only present when the patients were edematous. Therefore, it is not clear whether the prolonged retention of albumin in the extravascular compartment of the forearm was a consequence of the edema or its cause. In other words, the possibility that an increased volume of interstitial fluid can delay the removal of radioactive albumin from the interstitial space has not been eliminated (26).

Gill et al. found that some patients with idiopathic edema had a low plasma albumin level and also claimed that there may be a low circulating albumin pool in women with idiopathic edema (31). However, this has not been confirmed. Even if such a mechanism existed in the patients of Gill et al., it is unlikely that it would be relevant to other patients with idiopathic edema because plasma albumin was in the normal range in all other reports.

If there is a defect in the capillaries, it is more likely that it relates to an attenuation of the reflex postural vasoconstriction of the precapillary sphincter on standing, as occurs in some diabetics and with treatment with nifedipine (30). This could well explain the few patients with idiopathic edema who are not in some way intermittently depleting and repleting themselves with sodium. Further studies of such patients would be very valuable.

Diuretics

Many patients find that when their symptoms are severe, the use of diuretics may temporarily alleviate both the edema and their symptoms. However, diuretics stimulate compensatory mechanisms to retain sodium and water, and these compensatory mechanisms are longer acting than the natriuretic effect of the diuretic. Therefore, whenever diuretics are stopped, there is pronounced retention of sodium and water (2,6). Many patients who regularly consume diuretics may be able to keep themselves edema free. However, one of the first patients that we studied came to us with edema while taking furosemide, 160 mg per day, which she had been taking for more than a year and a half (6). On admission to the metabolic ward, she had marked edema, and she remained edematous until the diuretic was stopped. When the furosemide was stopped, she gained 3 kg over 5 days, following which she began to lose weight. In Figure 82-1 is illustrated how her weight continued to fall until it leveled out at about 4 kg less than her weight when she was taking diuretics.

These remarkable findings led us to study 10 other patients with idiopathic edema who were taking diuretics (2). All patients were admitted to the metabolic ward and placed on a fixed sodium and potassium intake. Control observations were made while they continued to take diuretics, and the diuretics were then stopped. When the diuretics were stopped, all 10 patients retained sodium and water, as judged by a reduction in urinary sodium excretion and an increase in weight (Fig. 82-2). This retention of sodium and water and gain in body weight appeared to be directly related to the degree of stimulation of the renin–angiotensin–aldosterone system by the diuretics. During

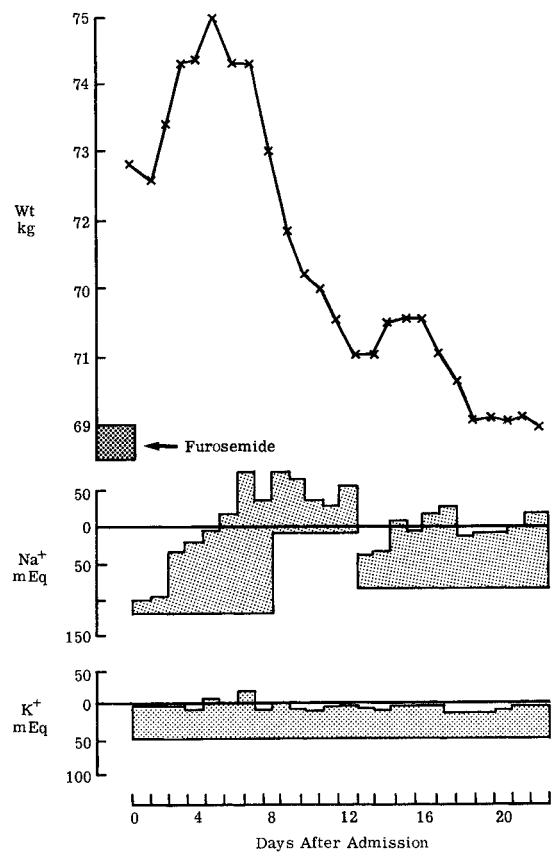

FIGURE 82-1. Changes in body weight, sodium balance, and potassium balance in a 22-year-old nurse with idiopathic edema. She was edematous while on furosemide, which was then stopped. She noted an initial 3-kg weight gain with stopping of furosemide, followed by a 6- to 7-kg weight loss. (Reprinted from: MacGregor GA, Tasker PR, de Wardener HE. Diuretic induced oedema. *Lancet* 1975;1:489, with permission.)

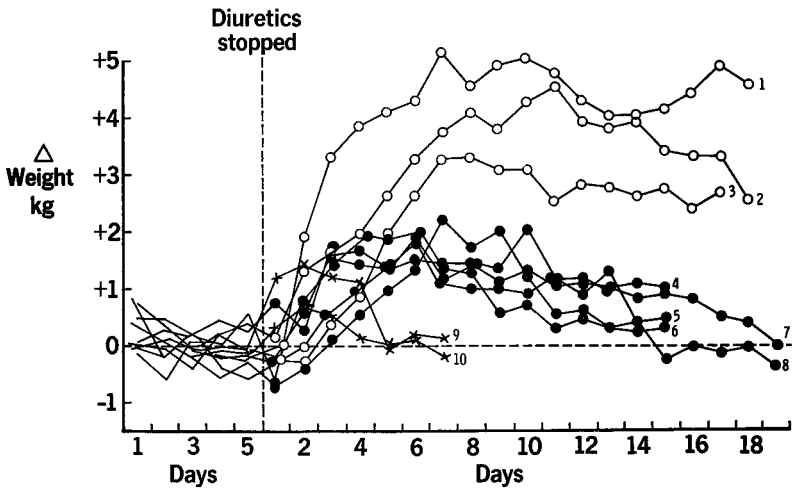

FIGURE 82-2. Changes in weight in 10 patients while taking diuretics and after stopping them. Note that weight returned to the level present while taking diuretics within 3 weeks of stopping them in patients 4 to 10, whereas weight remained elevated 3 weeks after stopping the diuretics in patients 1, 2, and 3. (Reprinted from: MacGregor GA, et al. Is "idiopathic" oedema idiopathic? *Lancet* 1979;1:397, with permission.)

the next 5 to 6 days after stopping diuretics, plasma renin activity (Fig. 82-3) and urinary aldosterone excretion (Fig. 82-4) gradually returned toward normal, urinary sodium excretion increased, and most patients then lost weight and their edema subsided. In seven patients no further treatment was necessary, and they remained well on follow-up without further episodes of edema. However, in three patients who had been taking the largest amounts of diuretics and who had the greatest stimulation of the renin–angiotensin–aldosterone system before stopping the diuretics, weight remained elevated and edema recurred subsequently during the next few months despite the absence of diuretics. In view of the recurring edema, they were placed on a moderately restricted sodium diet of approximately 50 mmol/day. In two patients the edema then disappeared, and within a year they were able to increase their sodium intake to normal levels without developing edema. The third patient was not able to control her sodium or her calorie intake, and she continued to take diuretics and to have intermittent edema.

Claims have been made that some patients who have never taken diuretics present with idiopathic edema (15). In our own practice, the reason for starting diuretics was often trivial. These patients are almost always obsessed about their weight and appearance. Many patients confess that in attempting to control their weight, they vary the amount of food they consume, both before and after they started taking diuretics. In an attempt to lose weight they may starve themselves for several days and then lose control and eat several substantial meals. Sudden changes in carbohydrate intake can cause sodium and water retention with edema (32,33). In addition, low sodium intake also stimulates the renin–angiotensin–aldosterone system, and, as with diuretics, the effect of this stimulation persists for some time after sodium intake is suddenly increased. Therefore, a sudden increase in sodium intake causes sodium retention; this is compounded by the effect of a sudden increase in carbohydrate intake, which also causes retention of sodium and water, perhaps because of an increase in sympathetic tone (34).

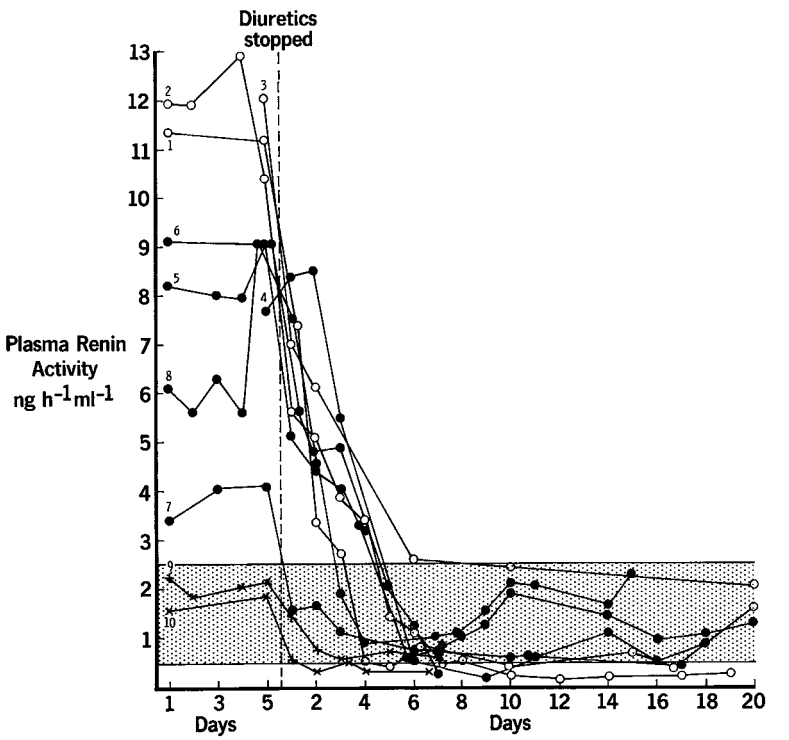

FIGURE 82-3. Changes in plasma renin activity in 10 patients before and after stopping diuretics. (Reprinted from: MacGregor GA, et al. Is "idiopathic" oedema idiopathic? *Lancet* 1979;1:397, with permission.)

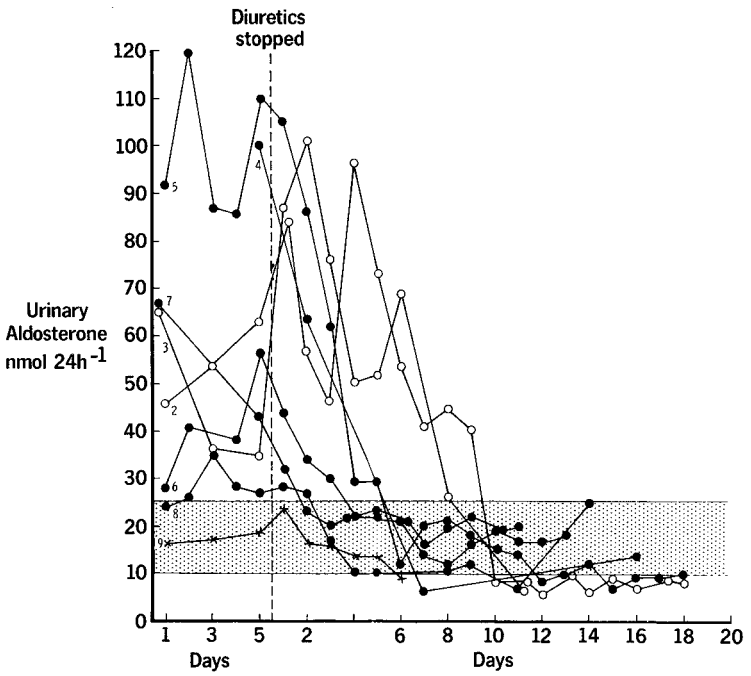

FIGURE 82-4. Changes in urinary aldosterone levels in 10 patients taking diuretics and after stopping them. (Reprinted from: MacGregor GA, et al. Is "idiopathic" oedema idiopathic? *Lancet* 1979;1:397, with permission.)

To study the effect of sudden changes in carbohydrate and salt intake in young women, we studied four normal women aged 20 to 26 years, who were first placed on a low-sodium (10 mmol/day), low-carbohydrate (80 g/day) diet and then suddenly changed to a high-sodium (350 mmol/day), high-carbohydrate (350 g/day) diet (2). This sudden, simultaneous change in sodium and carbohydrate intake caused marked retention of sodium and water with a weight gain that varied from 3 to 4 kg over 24 to 36 hours (Fig. 82-5). With this retention of sodium and water and weight gain all of these normal women complained of the typical features of idiopathic edema—that is, tightness of the ankles, hands, and face, with detectable edema in two subjects and abdominal bloating. In subsequent studies we examined the effect of alteration of carbohydrate intake alone on sodium excretion and showed that when normal women change from a similar low to a high-carbohydrate diet, there is a small degree of sodium retention and weight gain. These same women were then studied a second time when they were kept on a constant carbohydrate intake and changed from a low- (10 mmol/day) to a high-sodium (350 mmol/day) diet. This caused greater retention of sodium and weight gain, but the weight gain was less than when carbohydrate and sodium intakes were both suddenly increased. It would seem likely, therefore, that it is the combination of the change in both carbohydrate and sodium intake that causes severe sodium and water retention.

These observations in normal women suggest that the initial complaints in some patients with idiopathic edema might be owing to fluctuations in sodium and carbohydrate intake caused by the patients' concern for their weight and appearance. Sudden gains in weight with their attendant discomforts cause these women to seek medical advice. The absence of any obvious cause for the edema and the patient's persistence eventually overcome the doctor's reluctance to prescribe diuretics. Once on diuretics, the renin–angiotensin–aldosterone system is persistently stimulated both by the diuretics themselves and continued fluctuations in sodium and carbohydrate intake. Severe edema occurs if the diuretics are stopped, and both the patient and her doctor believe that the edema can be prevented only by the continuous use of diuretics.

Many of the previous findings in patients with idiopathic edema, such as reduced blood volume (1), elevated plasma renin activity, and increased aldosterone level (17,18), could be directly owing to the previous use of diuretics. Associated findings, including a tendency toward sodium and water

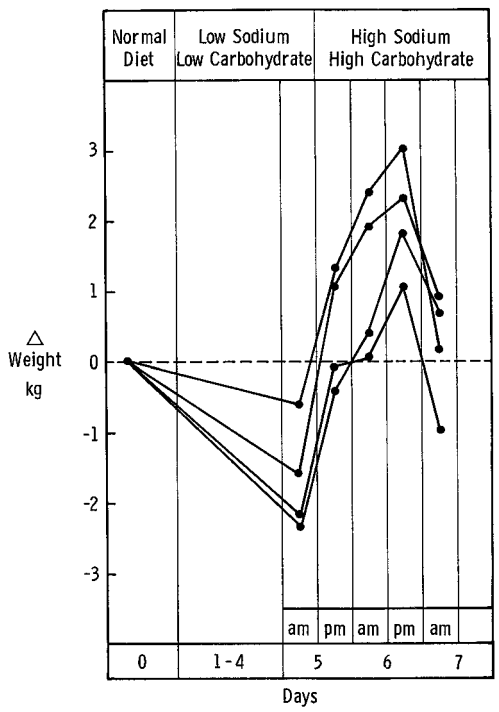

FIGURE 82-5. Changes in weight in four normal young women after changing from their normal diet to a low-sodium, low-carbohydrate diet, and then to a high-sodium, high-carbohydrate diet. (Reprinted from: MacGregor GA, et al. Is "idiopathic" oedema idiopathic? *Lancet* 1979;1:397, with permission.)

retention, especially when the patient receives a large sodium or water load, particularly on standing (7), could also be related to the prior administration of diuretics. There is no doubt that many patients with idiopathic edema show all the characteristics of chronic volume depletion (35). It is likely that the prolonged administration of diuretics causes persistent stimulation of the renin–angiotensin system and juxtaglomerular hypertrophy and may well cause other long-standing changes in mechanisms regulating sodium and water balance, so that even when diuretics have been stopped for some time there may be abnormal responses in these patients. Pelosi et al. claimed that idiopathic edema was a psychosomatic disease that was unrelated to diuretics. However, of the 25 patients they studied, 23 admitted to having taken diuretics (36)!

Patients who have not had diuretics often find that the fasting they subject themselves to leads to constipation. To seek relief from constipation, they start using laxatives and then find that when used to excess, laxatives are helpful in relieving them of the large volume of sodium and water they retain when they eat excessively. The incidence of laxative abuse in these patients is probably greatly underestimated, and all patients should have routine tests to make sure they are not abusing laxatives. Clearly, laxatives can induce the same syndrome as diuretics (37).

Indeed, a study in which chronic laxative abusers had laxatives withdrawn showed rebound retention of sodium and water followed by resolution in seven of the nine patients studied (38). Other patients find that after binge eating, surreptitious vomiting stops their abdominal bloating and sodium and water retention (39). In our experience, surreptitious vomiting can be very difficult to exclude because most patients are ashamed of their habit and are very adept at hiding their vomiting from both their physicians and families.

In our view, therefore, idiopathic edema is related to the patient's habit of binge eating and fasting and then using some method of obtaining relief from the excess sodium and water retained. This usually involves the use of diuretics, laxatives, or surreptitious vomiting. All of these cause long-term stimulation of the renin–angiotensin–aldosterone system and subsequent worsening of sodium and water retention, which is compounded by continued fluctuations in sodium and carbohydrate intake.

MANAGEMENT

The management of idiopathic edema is not satisfactory. It is necessary first to obtain a full history of exactly what has precipitated the edema—diuretics, laxatives, surreptitious vomiting, or persistent fasting and binge eating. Sympathetic understanding and handling of the patient can help in elucidating a full history. Many patients feel rejected by their doctors, and this rejection compounds their feelings of resentment and leads to exaggeration of symptoms to try to get the doctor to take notice of them. Clearly, other causes of sodium and water retention need to be excluded, but this is usually not difficult. All patients should have the investigations detailed in Table 82-1, and further investigations should be done as appropriate and when indicated. Management largely depends on whether the patient is using diuretics.

Patients on Diuretics

All patients taking diuretics should stop taking them; however, patients develop edema if diuretics are stopped suddenly. Therefore, it is better to withdraw diuretics gradually and, at the same time, advise patients to restrict salt intake. Our experience indicates that most patients do not experience any severe rebound retention of sodium and water and therefore do not become edematous if they are able to restrict salt intake to approximately 50 mmol/day (40). It is helpful if patients continue to restrict salt intake; this usually controls edema satisfactorily if they maintain moderate sodium restriction without excessive fluctuations in salt intake. Some patients soon find that they can return to a normal salt intake without developing edema, particularly if they had been taking small doses of diuretics previously. Others find they are unable to maintain a constant moderate sodium restriction, and consequently their sodium and carbohydrate intake continues to fluctuate. In these patients, sympathetic understanding and careful work by a dietitian may be helpful in an attempt to regulate sodium and carbohydrate intake to regular amounts compatible with the patient's usually overwhelming desire to lose weight.

TABLE 82-1

INVESTIGATIONS FOR IDIOPATHIC EDEMA

Blood	Full blood count, urea, electrolytes, creatinine, liver function tests, total plasma albumin, fasting blood sugar, **thyroxine,** thyroid-stimulating hormone, renin activity
Urine tests	Urinary protein, 24-hr urinary sodium excretion, test for phenolphthalein
Other tests	Chest x-ray, electrocardiogram, glucose tolerance test. In selected cases only; venography, lymphangiogram

Patients Not on Diuretics

In this group of patients it is very important to exclude laxative abuse, covert use of diuretics, or surreptitious vomiting. Measurement of plasma renin activity and/or aldosterone while on a normal sodium intake is a good indicator of whether patients are depleting themselves of sodium. For patients who are not depleting themselves of sodium and are definitely not taking diuretics, who still have edema, we have found that moderate restriction of sodium intake to about 50 mmol/day usually relieves the symptoms, provided there is no fluctuation in food intake. Some studies have claimed that ß-blockers or an angiotensin-converting enzyme inhibitor such as captopril (41) may be helpful in treating unusual patients with persistent edema despite moderate sodium restriction, but there are no adequate randomized control trials. Many patients find that lying flat during the day for a period may be helpful, particularly during a bad episode of edema, but this is not practical for most active women. Ephedrine has been reported to reduce edema formation in patients with diabetes and possibly may be helpful in some patients with idiopathic edema (42); however, there are no well-controlled studies. Even amphetamines such as dexamphetamine have been used (43), but the dangers of addiction to these controlled drugs are far too great to contemplate their use without a well-designed randomized double-blind control trial.

Occasional patients are encountered in whom edema persists. The use of a long-acting diuretic at the lowest effective dose may be justified, often combined with distally acting drugs. Loop diuretics should be avoided because they cause large fluctuations in sodium balance. It is interesting that there are very few women older than the age of 60 who complain of the symptoms of idiopathic edema; apparently, the syndrome eventually resolves with increasing age.

Women who continue to fast and binge intermittently, surreptitiously take diuretics, vomit, or abuse laxatives are much more challenging to treat and usually have the most severe associated psychological disorders, often merging into the same type of psychological disorder found in anorexia nervosa. Such patients can be extremely difficult to help and usually drift on a tide of discontent from one doctor to another.

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CHAPTER 83 ■ CARDIAC FAILURE AND THE KIDNEY

WILLIAM T. ABRAHAM AND ROBERT W. SCHRIER

Like liver disease and the nephrotic syndrome, the kidney plays a central role in the sodium and water retention and edema formation associated with cardiac failure. Therefore, heart failure represents another edematous state in which renal sodium and water retention is observed despite an excess of total body sodium and water. This finding of continued renal sodium and water retention despite total body sodium and water excess, in part, defines the clinical syndrome of heart failure. In this regard, the pathophysiology of heart failure has been described as a cardiorenal syndrome, where left ventricular systolic and/or diastolic dysfunction leads to renal sodium and water retention that in turn produces the clinical syndrome of heart failure. Clinically, manifestations of cardiac failure are almost always associated with fluid volume retention, resulting in elevated ventricular filling pressures and congestive signs and symptoms. That is, most of the cardinal signs and symptoms of heart failure are attributable to fluid retention rather than to an abnormal cardiac output (Table 83-1).

Moreover, worsening fluid retention is the proximate cause of heart failure hospitalization (i.e., morbidity) in nearly 90% of cases (1,2). As will be discussed in subsequent text of this chapter, renal dysfunction as measured simply by elevated blood urea nitrogen (BUN) and/or serum creatinine portends a very poor prognosis (i.e., mortality) in both acutely decompensated patients and patients with chronic heart failure (3–6). Consequently, the kidney provides a sensitive bioassay for prognosis in patients with heart failure. This observation underscores the importance of cardiorenal interactions in the natural history of heart failure. This chapter reviews the mechanisms of edema formation and sodium and water retention associated with cardiac failure, discusses the clinical implications of cardiorenal interactions in heart failure, and reviews current and future treatment options.

MECHANISM OF EDEMA FORMATION

Edema is a clinical sign that indicates an increase in the volume of sodium and water in the interstitial space. This increase in interstitial-space volume is caused by an alteration of the Starling forces that govern the transfer of fluid from the vascular compartment into the surrounding tissue spaces (7). Edema may result from local factors such as obstruction of lymphatic or venous flow. However, the type of edema considered in this chapter reflects a generalized disturbance of sodium and water balance and is associated with a net increase in extracellular fluid (ECF) volume, a situation that is usually not present when edema results from a local disruption of normal capillary mechanisms. Generalized edema results when altered Starling forces affect all capillary beds. The development of generalized edema thus indicates a widespread disturbance in the normal balance between tissue capillary and interstitial hydrostatic and colloid osmotic pressures, which control the distribution of ECF between the vascular and extravascular (interstitial) compartments. In edematous disorders such as cardiac failure, sodium and water retention by the kidney leads to the progressive expansion of the ECF volume and alteration of the Starling forces that subsequently result in edema formation.

Transcapillary solute and fluid transport consists of two types of flow: convective and diffusive. Bulk water movement occurs via convective transport induced by the imbalance between transcapillary hydraulic pressure and colloid osmotic pressure (7). Transcapillary hydraulic pressure is influenced by a number of factors, including systemic arterial and venous blood pressures, regional blood flow, and the resistances imposed by the precapillary and postcapillary sphincters. Systemic arterial blood pressure, in turn, is determined by cardiac output, intravascular volume, and systemic vascular resistance. Systemic venous pressure is determined by right atrial pressure, intravascular volume, and venous capacitance. These latter hemodynamic parameters are largely determined by sodium and water balance and by various neurohormonal factors. For example, right atrial pressure or right ventricular preload is modulated both by changes in the intravascular volume, which are largely determined by the kidney, and alterations in venous capacitance, which are governed in part by neuroendocrine mechanisms such as the sympathetic nervous system, renin–angiotensin system, nonosmotic release of arginine vasopressin (AVP), and the natriuretic peptides. As discussed in this chapter, activation of these two mechanisms (i.e., renal sodium and water retention and neurohormonal activation), which may influence transcapillary hydraulic and oncotic pressures, is observed with cardiac failure.

Several mechanisms are capable of minimizing edema formation or diminishing the transudation of solute and water across the capillary bed. In several vascular beds, the local transcapillary hydraulic pressure gradient exceeds the opposing colloid osmotic pressure gradient throughout the length of the capillary bed, so that filtration occurs across its entire length (8). Filtered fluid consequently must return to the circulation via lymphatics. Increased lymphatic drainage and the ability of lymphatic flow to increase may thus be seen as one protective mechanism that minimizes edema formation. Other protective mechanisms that reduce interstitial fluid accumulation include precapillary vasoconstriction, increased net filtration with a resultant rise in intracapillary plasma protein concentration, and increased interstitial fluid volume with a resultant augmentation of tissue hydraulic pressure. For example, increased net filtration itself, such as that associated with hypoalbuminemia and the resultant decreased plasma oncotic pressure, leads to a dissipation of capillary hydraulic pressure, a dilution of interstitial fluid protein concentration, and a corresponding rise in intracapillary protein concentration, all of which alter the balance of Starling forces to mitigate further interstitial fluid accumulation.

These buffering factors directed against interstitial fluid accumulation may explain why, in patients with congenital

TABLE 83-1

COMMON SIGNS AND SYMPTOMS OF CONGESTIVE HEART FAILURE

Primarily related to fluid retention/increased ventricular filling pressures	Primarily related to abnormal cardiac output
Ascites	Cool extremities
Dyspnea	Fatigue
Hepatomegaly	Low blood pressure
(RUQ fullness, pain)	Poor capillary refill
Jugular venous distension	
Orthopnea	
Paroxysmal nocturnal dyspnea	
Peripheral edema	
Pleural effusions	
Pulmonary rales	

RUQ, Right upper quadrant.

analbuminemia, positive sodium and water balance and edema formation do not occur and sodium loads are excreted (9). Because the continued loss of intravascular fluid volume to the interstitial space without renal sodium and water retention may result in cessation of interstitial fluid formation, the presence of generalized edema, therefore, implies concomitant renal sodium and water retention. This is unquestionably the case in cardiac failure, as well as in liver disease and the nephrotic syndrome. The disturbances in microcirculatory hemodynamics associated with edema and expansion of the ECF volume are described in Table 83-2.

MECHANISMS OF FLUID RETENTION IN CARDIAC FAILURE

Cardiac failure may be defined as the inability of the heart to deliver enough blood to peripheral tissues to meet metabolic demands. In the case of low-output cardiac failure, a decrease in cardiac output initiates a complex set of compensatory mechanisms in an attempt to maintain circulatory integrity. The adjustments that serve to stabilize cardiac performance and arterial perfusion in such patients include increases in plasma volume, atrial and ventricular filling pressures, peripheral vasoconstriction, and cardiac contractility and heart rate. The retention of sodium and water is a major renal compensation for a failing myocardium, but it also accounts to a great extent for the familiar clinical syndrome of heart failure, which consists of pulmonary or peripheral edema, or both, and exercise intolerance. In fact, the ability to excrete a sodium load has been used as an index of the presence of heart failure (10), and a defect in water excretion is regularly encountered in such patients (11).

TABLE 83-2

DISTURBANCES IN MICROCIRCULATORY HEMODYNAMICS ASSOCIATED WITH EDEMA AND EXPANSION OF EXTRACELLULAR FLUID VOLUME

Increased venous pressure transmitted to the capillary
Adjustments in precapillary and postcapillary resistances to favor interstitial fluid accumulation
Inadequate lymphatic flow of drainage
Altered capillary permeability (K_f)

Classically, two theories have attempted to explain how the kidney becomes involved in the renal sodium and water retention of heart failure. According to the "backward failure" hypothesis advanced by Hope (12) and Starling (7), central venous pressure and then peripheral venous pressure rise as the cardiac pump fails. With this increase in peripheral venous pressure, the hydraulic pressure in the capillaries exceeds opposing forces and causes the transudation of fluid from the intravascular compartment to the interstitial space, and thus the development of edema. This loss of intravascular fluid volume then signals the kidney to retain sodium and water in an attempt to restore the circulating volume to normal. The "forward failure" theory states that as the heart fails, there is inadequate perfusion of the kidney, resulting in decreased sodium and water excretion (13). As will become apparent from the following discussion, both an increase in central venous pressure or "backward failure" and a decrease in cardiac output or "forward failure" may contribute to the sodium and water retention of low-output cardiac failure via systemic and renal hemodynamic effects and through activation of various vasoconstrictor and antinatriuretic neuroendocrine systems. According to our unifying hypothesis of body fluid–volume regulation (14–20), neurohormonal activation plays a central role in the efferent limb of the sodium and water retention in cardiac failure, liver disease, and the nephrotic syndrome, whereas the afferent limb of this volume regulatory system is initiated by altered systemic hemodynamics. The following discussion addresses this unifying hypothesis of body fluid–volume regulation and the afferent and efferent mechanisms for sodium and water retention in edematous disorders, particularly heart failure.

Afferent Mechanisms for Renal Sodium and Water Retention in Heart Failure

The kidney alters the amount of dietary sodium excreted in response to signals from volume receptors and chemoreceptors in the circulation. These receptors may affect kidney function by altering renal sympathetic nerve activity and changing levels of circulating hormones with vasoactive and nonvasoactive (e.g., direct sodium-retaining) effects on the kidney. Important "effector" hormones include angiotensin II (AT-II), aldosterone, AVP, endothelin, nitric oxide, prostaglandins (PGs), and the natriuretic peptides, especially atrial and brain natriuretic peptides (ANP and BNP, respectively). Both high- and low-pressure baroreceptors as well as cardiac and hepatic chemoreceptors have been implicated in the activation of these neurohormonal systems.

High-Pressure Baroreceptors

In humans, evidence for the presence of volume-sensitive receptors in the arterial circulation originated from observations in patients with traumatic arterial-venous (AV) fistulae (21). Closure of AV fistulae is associated with a decreased rate of emptying of the arterial blood into the venous circulation, as demonstrated by closure-induced increases in diastolic arterial pressure and decreases in cardiac output. This results in an immediate increase in renal sodium and water excretion without changes in either glomerular filtration rate (GFR) or renal blood flow (21). This observation implicates the "fullness" of the arterial vascular tree as a "sensor" in modulating renal sodium and water excretion. In fact, the fullness of the arterial vascular compartment or the so-called effective arterial blood volume (EABV) (22) has been proposed as a major determinant

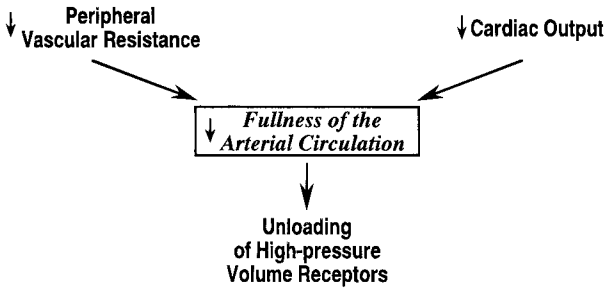

FIGURE 83-1. Peripheral vascular resistance and cardiac output as the determinants of arterial filling or the "effective arterial blood volume." Here, either a decrease in vascular resistance or diminished cardiac output results in decreased fullness of the arterial circulation with unloading of high-pressure volume receptors and activation of various neurohormonal responses (see text).

of renal sodium and water handling, according to the unifying hypothesis of body fluid volume regulation (14–20).

The EABV is a measure of the adequacy of arterial blood volume to "fill" the capacity of the arterial circulation. Normal EABV exists when the ratio of cardiac output to peripheral vascular resistance maintains venous return and cardiac output at normal levels. Arterial or high-pressure volume receptors, therefore, may be stimulated when either cardiac output falls or peripheral vascular resistance diminishes to such an extent that the arterial circulation is no longer effectively "full" (Fig. 83-1). Therefore, in the case of low-output cardiac failure, it is the diminution of cardiac output that is perceived by the arterial circulation as inadequate to maintain EABV. In high-output cardiac failure, decreased peripheral vascular resistance may serve as the signal for arterial underfilling (14–20). The concept of arterial underfilling in low- and high-output cardiac failure is discussed in the following.

Studies using one model of low-output cardiac failure—constriction of the vena cava in the dog—support the notion that a fall in cardiac output may be a primary stimulus for sodium and water retention by the kidney. Using this model, Lifshitz and Schrier, Schrier and Humphreys, and Schrier et al. (23–25) showed that constriction of the thoracic inferior vena cava (TIVC) is associated with a decrease in cardiac output, arterial pressure, and urinary sodium excretion, even when renal perfusion pressure and renal venous pressure were held constant. Of note, renal denervation and adrenalectomy did not abolish this antinatriuresis. Furthermore, sodium retention did not correlate with changes in GFR or renal vascular resistance. Constriction of the superior vena cava to cause a decrease in cardiac output similar to that observed in the TIVC studies resulted in a similar decrease in urinary sodium excretion, despite the absence of concomitant hepatic, renal, and abdominal venous congestion. These findings support the hypothesis that the kidney decreases sodium excretion in response to a decrease in cardiac output and the associated arterial underfilling.

The preceding proposal was questioned by Migdal et al. (26), who compared the renal response in three different models of experimental heart failure. Specifically, they compared models of TIVC constriction, pulmonary artery occlusion (which is similar to caval constriction except that right-sided heart pressures are increased rather than decreased), and acute left ventricular infarction, another model of low-output heart failure but with increased left-sided heart pressures. This investigation demonstrated that with comparable decrements in cardiac output in all three models, only the TIVC constriction animals exhibited an antinatriuresis. The authors concluded that low cardiac output *per se* is not the afferent signal for sodium retention in low-output heart failure. These authors and others (27) suggested that in some way, decreased right-sided heart pressure mediates the antinatriuresis.

An alternative interpretation of the findings of Migdal et al. (26) is that a decrease in cardiac output is a stimulus for renal sodium and water retention, but an acute rise in atrial or ventricular end-diastolic pressures, in animals with acute pulmonary hypertension or acute left ventricular infarction, with the release of the natriuretic peptides ANP and BNP, initially obscures this effect. Support for this interpretation may be found in a report from Lee et al. (28), who examined sodium excretion in two models of low-output heart failure in the dog, acute heart failure produced by rapid ventricular pacing, and a TIVC constriction model. Similar to the animals in the study of Migdal et al., the dogs with TIVC constriction demonstrated diminished cardiac outputs and arterial pressures without an increase in atrial pressures or plasma ANP level but with avid renal sodium retention. Of note, plasma renin activity (PRA) and plasma aldosterone concentrations were substantially elevated in these TIVC-constriction animals. In the case of pacing-induced heart failure, cardiac output and arterial pressure were similarly decreased, whereas atrial pressures and the plasma ANP concentration were significantly increased. In the animals with elevated rather than normal circulating ANP concentrations, urinary sodium excretion was maintained and PRA and plasma aldosterone concentration were not increased. Finally, dogs with TIVC constriction were given exogenous ANP to achieve circulating concentrations comparable to that seen in the pacing-induced heart failure animals. Exogenous administration of ANP to such levels prevented sodium retention, renal vasoconstriction, and activation of the renin–angiotensin–aldosterone system. These observations support the notion that decreased cardiac output is a stimulus for renal sodium retention in heart failure and suggest an important role for the natriuretic peptides in acutely attenuating this renal response. A further discussion of the role of ANP and BNP in heart failure is presented elsewhere in this chapter.

Other experimental evidence supports a role for diminished cardiac output as a determinant of the sodium and water retention of heart failure. Rats with small-to-moderate myocardial infarctions and decreased cardiac outputs exhibit decreased fractional sodium excretion despite normal right and left ventricular end-diastolic pressures (29). Using the model of TIVC constriction, Priebe et al. (30) demonstrated that the renal retention of sodium and water was reduced markedly when cardiac output was restored to normal by autologous blood transfusions. Moreover, reduction of pressure or stretch at the carotid sinus, like that produced by decreased cardiac output or arterial hypotension, activates the sympathetic nervous system and promotes renal sodium and water retention (31,32). Pharmacologic or surgical interruption of sympathetic afferent neural pathways emanating from high-pressure baroreceptor sites also inhibits the natriuretic response to volume expansion (24,25,33–37). High-pressure baroreceptors also appear to be important factors in regulating the nonosmotic release of AVP, thereby affecting renal water excretion (38,39). Finally, the juxtaglomerular apparatus, an arterial baroreceptor located in the afferent arterioles within the kidney, has been implicated in the modulation of renal renin release (31,40,41) and thus may stimulate increases in circulating AT-II and aldosterone, both of which promote sodium retention by the kidney.

Low cardiac output cannot be the only cause of sodium and water retention in heart failure, because diminished renal sodium and water excretion is also observed in states of high-output cardiac failure. In heart failure secondary to beriberi, anemia, thyrotoxicosis, or AV fistulae, cardiac output is increased as a consequence of a decrease in peripheral vascular resistance. This decrease in vascular resistance diminishes EABV (i.e., causes arterial underfilling) and serves as the stimulus for neurohormonal activation and renal sodium and water

retention in these instances of high-output heart failure (14–20). As noted already in humans (21) and dogs (42), closure of an AV fistula causes increased sodium excretion, whereas opening of an AV fistula decreases urinary sodium excretion. These changes in renal sodium excretion correlate with changes in arterial pressure and peripheral vascular resistance rather than GFR or renal blood flow, supporting the importance of arterial circulatory "fullness" as a determinant of the renal response to heart failure.

These observations of decreased sodium and water excretion in both low- and high-output cardiac failure support the theory that arterial underfilling initiates reflex stimuli for the kidneys to retain sodium and water. In this regard, high-pressure baroreceptors in the carotid sinus, aortic arch, left ventricle, or the juxtaglomerular apparatus may comprise an important part of this reflex loop. Although these data support a role for arterial underfilling as the primary stimulus of the renal sodium and water retention of heart failure, low-pressure baroreceptors also may play an important role.

Low-Pressure Baroreceptors

In addition to the high-pressure arterial baroreceptors, the venous side of the circulation seems to be a logical place for receptors sensitive to changes in blood volume to be found. In fact, 85% of blood volume may be found in the venous circulation, whereas just 15% of circulatory volume resides in the arterial circulation (43). Although the smaller arterial blood volume may result in a higher sensitivity to detect blood volume changes, the larger amount of venous blood volume also may constitute an important component of the body fluid–volume regulatory system.

The atria of the heart are highly distensible and densely populated with nerve endings that are sensitive to small changes in passive distention (44). Similar afferent low-pressure volume receptors may also be found in the pulmonary vasculature (45). Increased filling of the thoracic vascular and cardiac atria would be expected to signal the kidney to increase urinary sodium excretion in order to return the blood volume to normal. As expected, maneuvers that increase this thoracic or "central" blood volume such as weightlessness, negative-pressure breathing, head-out water immersion, recumbency, and exposure to cold all produce a natriuresis (46–51). Similarly, measures that decrease intrathoracic blood volume, including positive-pressure breathing, upright posture, and application of tourniquets to the lower extremities result in renal sodium retention (48,52,53). Therefore, effective "central" blood volume, in addition to EABV, may serve as the afferent stimulus for regulation of renal sodium and water excretion.

Considerable evidence implicates the left atrium as an important site of low-pressure receptors (54–56). It is believed that changes in pressure or distention within the left atrium modulate electrical activity of the atrial receptors, which in turn may regulate renal sympathetic nerve activity. Left atrial nerves, therefore, can alter blood volume through changes in sodium excretion (56–58) as well as solute-free water excretion by influencing AVP release (59–61). Acutely increasing left atrial volume by inflation of a balloon within the left atrium results in increased urinary volume excretion (55), whereas hypotensive hemorrhage (62,63) and atrial tamponade (64) cause decreased atrial volume and diminish urine volume. However, in the setting of chronic heart failure, renal sodium and water retention occur despite left atrial distention and, frequently, loading of the other central baroreceptors (pulmonary veins, right atrium). Therefore, in chronic heart failure, diminished cardiac output with arterial underfilling may exert the predominant effect via unloading of high-pressure arterial baroreceptors. Chronic studies in animals employing either experimental tricuspid insufficiency (65) or right atrial distention with an inflatable balloon (66) support this hypothesis. In these animal models, the increase in right atrial pressure was associated with avid renal sodium retention rather than the expected natriuresis. However, a concomitant fall in cardiac output could explain the sodium retention. Alternatively, alterations in cardiopulmonary baroreceptor function may occur in chronic but not acute heart failure.

Zucker et al. (67) demonstrated that the inhibition of renal sympathetic nerve activity seen during acute left atrial distention is lost during chronic heart failure in the dog. Moreover, a decrease in cardiac preload fails to produce the expected parasympathetic withdrawal and sympathetic activation in humans with heart failure (68–70). Nishian et al. (70) described paradoxical forearm vasodilation and hemodynamic improvement during acute unloading of cardiopulmonary baroreceptors in patients with severe chronic heart failure. This paradoxical response to lower body negative pressure was associated with static plasma norepinephrine levels (70), rather than the expected increase in plasma norepinephrine concentrations, further demonstrating this altered response to low-pressure baroreceptor unloading in heart failure. These observations confirm those made in heart failure patients during other forms of orthostatic stress (68,69). These findings are also consistent with the observation of a strong positive correlation between left atrial pressure and coronary sinus norepinephrine, a marker of cardiac adrenergic activity, in patients with chronic heart failure (71). Finally, Fonarow et al. (72) have shown that reduction in left ventricular filling pressure rather than an increase in cardiac output during tailored hemodynamic management of heart failure improves survival over a 2-year period of follow-up. Taken together, these findings suggest that the normal inhibitory control of sympathetic activation accompanying increased atrial pressures is lost in heart failure patients and somehow may be converted to a stimulatory signal.

Cardiac and Pulmonary Chemoreceptors

In the heart and lungs, both vagal and sympathetic afferent nerve endings respond to a variety of exogenous and endogenous chemical substances, including capsaicin, phenyldiguanidine, bradykinin, substance P, PGs. Baker et al. (73) demonstrated stimulation of sympathetic afferent nerve endings by bradykinin in the heart of the cat. In conscious dogs, the administration of PGE_2 and arachidonate inhibited the cardiac baroreflex (74). Moreover, Zucker et al. (75) showed that PGI_2 attenuates the baroreflex control of renal nerve activity via an afferent vagal mechanism. Because substances such as bradykinin and PGs may circulate at increased concentrations in subjects with heart failure (76), it is possible that altered central nervous system input from chemically sensitive cardiac or pulmonary afferents contributes to the neurohormonal activation and sodium retention of chronic heart failure. This possibility may have important implications for the treatment of heart failure, because commonly prescribed medications such as angiotensin-converting enzyme (ACE) inhibitors may alter circulating bradykinin and PG levels. At the present time, however, the exact roles of these hormones and cardiac and pulmonary chemoreceptors in heart failure are incompletely understood.

Hepatic Receptors

Theoretically, the liver should be in an ideal position to monitor dietary sodium intake and thus adjust urinary sodium excretion. Indeed, when compared with peripheral venous administration, infusion of saline solution into the portal circulation was reported to result in greater natriuresis (77,78). Similarly, the increment in urinary sodium excretion has been claimed to be greater when the sodium load is given orally than when given intravenously (79–81). In addition, the pathophysiologic

retention of sodium in patients with severe liver disease is also consistent with an important role for the liver in the control of sodium excretion. However, some investigators (82,83) were unable to demonstrate a difference in sodium excretion between animals infused with 5% sodium chloride systemically and animals receiving the same solution via the portal vein. Moreover, Obika et al. (84) found similar sodium excretions after sodium loads given intravenously or by gastric lavage. Therefore, the experimental evidence in favor of sodium or volume hepatic receptors remains controversial.

In summary, the afferent mechanisms for sodium and water retention in chronic heart failure may be preferentially localized on the arterial or high-pressure side of the circulation where EABV may serve as the primary determinant of the renal response. However, reflexes from the low-pressure cardiopulmonary receptor system also may be altered so as to influence renal sodium and water handling in heart failure. In this regard, increases in atrial and ventricular end-diastolic pressures also stimulate the release of the natriuretic peptides and inhibit AVP release, which may be important attenuating factors in renal sodium and water retention.

Efferent Mechanisms for Renal Sodium and Water Retention in Heart Failure

The Neurohormonal Response to Cardiac Failure

As mentioned, activation of various neurohormonal vasoconstrictor and antinatriuretic systems mediates to a large extent the renal sodium and water retention associated with the edematous disorders. Arterial underfilling secondary to a diminished cardiac output or peripheral vasodilation, perhaps in association with an alteration in low-pressure baroreceptor function, elicits these "compensatory" neuroendocrine responses in order to maintain the integrity of the arterial circulation by promoting peripheral vasoconstriction and expansion of the ECF volume through renal sodium and water retention (Fig. 83-2). The three major neurohormonal vasoconstrictor systems activated in response to arterial underfilling are the sympathetic nervous system, the renin–angiotensin–aldosterone system, and the nonosmotic release of AVP. Baroreceptor activation of the sympathetic nervous system appears to be the primary integrator of the hormonal vasoconstrictor systems involved in renal sodium and water retention. The nonosmotic release of AVP involves sympathetic stimulation of the supraoptic and paraventricular nuclei in the hypothalamus (85), whereas activation of the renin–angiotensin–aldosterone system involves renal β-adrenergic stimulation (86). However, this latter system may provide positive feedback stimulation of the sympathetic nervous system and nonosmotic AVP release. Various counterregulatory, vasodilatory, and natriuretic hormones, including the natriuretic peptides and PGs, are also activated in heart failure and the other edematous disorders, and may attenuate the renal effects of vasoconstrictor hormone activation. The effects of these neurohormonal systems, as well as the effects of alterations in systemic hemodynamics, on renal hemodynamics and tubular sodium and water reabsorption in heart failure are discussed in the following.

Glomerular Filtration Rate

The GFR is usually normal in mild heart failure and is reduced only as cardiac performance becomes more severely impaired. Until 1961, it was generally accepted that the rate of glomerular filtration was a major determinant of renal sodium excretion. In 1961, de Wardener et al. (87) published their classic paper indicating that acute expansion of ECF volume by saline loading was accompanied by a brisk natriuresis even when GFR was reduced. Moreover, in sodium-retaining heart failure patients, GFR is often normal and may even be elevated in states of high-output cardiac failure. These observations argue against an important role for diminished GFR in the sodium retention of heart failure *per se* (i.e., in the initiation of sodium retention), although a diminished GFR may be a contributing factor in patients with advanced heart failure or comorbid disorders that directly impair this aspect of renal function. It also should be emphasized that the contribution of GFR to sodium balance is difficult to evaluate because very minute changes in GFR are difficult to measure and may account for important changes in sodium excretion. For example, under normal conditions, with a GFR of 100 mL/minute, the filtered load of sodium amounts to approximately 20,000 mEq/day. This amount of filtered sodium is enormous compared to the normal urinary sodium excretion of approximately 200 mEq/day. In view of this considerable difference, it is apparent that very small changes in GFR can result in major alterations in sodium excretion if tubular reabsorption remains unaltered. In any event, although GFR may be diminished in patients with advanced heart failure, a reduction in GFR alone is probably not an important cause of fluid retention in these patients because sodium retention can be observed in heart failure patients who

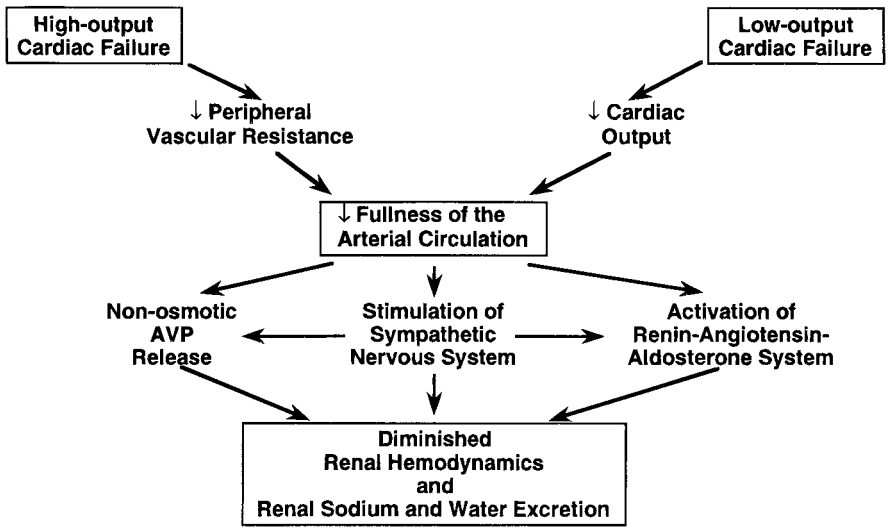

FIGURE 83-2. Mechanism explaining the defect in renal sodium and water excretion in both high- and low-output heart failure. *AVP,* arginine vasopressin.

have GFRs comparable to normal subjects who are capable of maintaining sodium balance.

Renal Blood Flow

Heart failure is commonly associated with an increase in renal vascular resistance and a decrease in renal blood flow (88). In general, renal blood flow decreases in proportion to the decrease in cardiac output. Some investigators also showed a redistribution of renal blood flow from the outer cortical nephron to juxtaglomerular nephrons during experimental heart failure (89,90). It was proposed that deeper nephrons with longer loops of Henle reabsorb sodium more avidly. Therefore, the redistribution of blood flow to these nephrons in patients with heart failure might account for or substantially contribute to the renal sodium retention observed. However, other investigators were not able to demonstrate such a redistribution of blood flow in other models of cardiac failure (91,92). At the present time, the role of redistribution of renal blood flow in the sodium retention of cardiac failure therefore remains uncertain.

The increased renal vascular resistance in heart failure could be caused by enhanced renal sympathetic activity or increased circulating concentrations of AT-II, norepinephrine, vasopressin, or other vasoconstricting substances. Alternatively or in addition, decreased synthesis of or the development of tachyphylaxis to known vasodilating substances such as the natriuretic peptides and PGE_2 and PGI_2 may contribute to the increased renal vascular resistance. Studies performed in rats demonstrated the ability of the adrenergic neurotransmitter norepinephrine and AT-II to promote efferent arteriolar constriction (93,94). In a rat model of low-output heart failure caused by myocardial infarction, the marked elevation in efferent arteriolar resistance was abolished after infusion of an ACE inhibitor (94), thereby implicating the renal vasoconstrictor properties of AT-II in heart failure. Clinical results from our laboratory also favor AT-II as a major renal vasoconstrictive substance in patients with heart failure (95). In patients with advanced heart failure, GFR was improved after 1 month of treatment with the ACE inhibitor captopril. However, similar patients receiving another vasodilating agent, prazosin, with identical improvement in cardiac output and left ventricular end-diastolic pressure but without any effect on the renin–angiotensin system had no improvement in GFR (95). Moreover, a published review of the literature on renal function alterations induced by ACE inhibition during heart failure concluded that the net effect of ACE inhibitors in patients with heart failure is to augment renal blood flow to a greater extent than cardiac output (96). This observation also supports an important role for AT-II in the renal hemodynamic alterations of heart failure. However, the renal response to ACE inhibition in patients with heart failure is variable; as a result it is acknowledged that volume status and the degree of neurohormonal activation may influence this response (see the following).

In heart failure, the interaction between norepinephrine or AT-II and PGs may also provide a means of preserving near-constancy of renal blood flow in response to arterial underfilling. Although inhibition of PG synthesis does not generally impair GFR in normovolemic animals (97,98) or humans (99), in states of high plasma concentrations of endogenous AT-II induced by volume depletion, blockade of PG synthesis may be associated with substantial declines in renal blood flow and GFR (97,98). Recent clinical results have underscored the importance of PGs in the maintenance of renal function in patients with heart failure (76,100). In patients with heart failure, PG activity is increased and correlates with the severity of disease as assessed by the degree of hyponatremia (76). In these 15 patients, plasma levels of the metabolites of vasodilator PGI_2 and PGE_2 were found to be elevated three to 10 times above those seen in normal subjects. Of note, plasma levels of both metabolites also correlated positively with PRA and plasma AT-II concentrations. Administration of the PG synthesis inhibitor indomethacin in three of the hyponatremic heart failure patients resulted in a marked increase in peripheral vascular resistance and a fall in cardiac output. Riegger et al. (100) recently evaluated the renal effects of another PG synthesis inhibitor, acetylsalicylic acid, in patients with moderate heart failure consuming a normal sodium diet. In these patients, acetylsalicylic acid in doses that decreased the synthesis of renal PGE_2 resulted in a significant reduction in urinary sodium excretion. Moreover, the administration of a cyclooxygenase inhibitor in heart failure patients occasionally may result in acute reversible renal failure, an effect proposed to be due in part to the inhibition of vasodilating renal PGs and the resultant renal vasoconstriction (101). It should be noted, however, that the extent to which the effects on renal function and sodium and water handling result from renal hemodynamic or the tubular actions of the PGs remains unclear.

As mentioned, norepinephrine may also contribute to the increased renal afferent arteriolar resistance in heart failure patients. In this regard, Oliver et al. (102) demonstrated that the venous to arterial norepinephrine concentration gradient across the kidney, a crude measure of renal nerve traffic, is increased in response to acute reduction of cardiac output. Moreover, Hasking et al. (103) showed that during a steady-state tritiated norepinephrine infusion, the spillover of norepinephrine to plasma from the kidney is significantly elevated in patients with heart failure. In these patients, the increased renal norepinephrine spillover substantially contributed to the increase in whole-body norepinephrine spillover. These findings demonstrate that renal adrenergic activity is increased in patients with heart failure, and thus contribute to the renal vasoconstriction. In support of this latter hypothesis, the administration of α-adrenergic receptor antagonists increased renal blood flow in edematous patients with heart failure (104). Ongoing renal denervation studies in patients with heart failure may shed further light on the role of renal sympathetic activation.

Filtration Fraction, Proximal Tubular Sodium and Water Reabsorption, and Factors Acting Beyond the Proximal Tubule

Because renal blood flow falls as cardiac output decreases and GFR is usually preserved, filtration fraction often is increased in early heart failure. An increase in filtration fraction results in increased protein concentration and oncotic pressure in the efferent arterioles and peritubular capillaries that surround the proximal tubule (94). Such an increase in peritubular oncotic pressure has been proposed to increase sodium and water reabsorption in the proximal tubule (105–109). Direct evidence for increased single-nephron filtration fraction was provided by micropuncture studies in rats with myocardial infarction induced by coronary ligation (94). In rats with large myocardial infarctions involving approximately 40% of the left ventricular circumference, the single-nephron filtration fraction was markedly elevated (0.38 ± 0.02 versus 0.25 ± 0.02, $p < 0.005$) when compared with that in sham-operated control rats. The measurement of preglomerular, glomerular, and postglomerular pressures and flows revealed that these reductions in glomerular plasma flow rate and elevations in filtration fraction were associated with a profound constriction of the efferent arterioles. The effect of the latter was to sustain glomerular capillary hydraulic pressure, thereby preventing a marked fall in GFR. Significantly, fractional proximal fluid reabsorption was elevated in this model. Of interest, in these animals with myocardial infarction, the intravenous infusion of the ACE inhibitor teprotide led to the return of glomerular plasma flow rate, single-nephron filtration fraction, single-nephron GFR,

efferent arteriolar resistance, and fractional proximal fluid reabsorption to, or toward, the levels found in the control rats (94). Consistent with these experiments, micropuncture studies performed in other models of heart failure such as acute TIVC constriction (110) and acute cardiac tamponade (111) in dogs showed that the proximal tubule was at least one major nephron site responsible for renal sodium retention or a blunted response to saline infusion.

Despite the convincing nature of many studies, not all investigators have been able to detect an effect of peritubular oncotic pressure on proximal tubular sodium and water reabsorption. Rumrich and Ullrich (112), Lowitz et al. (113), Bank et al. (114), and Holzgreve and Schrier (115) were unable to find changes in proximal reabsorption despite marked changes in peritubular oncotic pressures. Moreover, Conger et al. (116) directly perfused peritubular capillaries with either a protein-free or protein-rich solution and found that neither perfusate influences the rate of proximal reabsorption. Trying to reconcile these observations, Ott et al. (117) found that proximal reabsorption was different after changes in peritubular oncotic pressure in volume-expanded dogs compared with hydropenic animals. These authors suggested that expansion of ECF volume resulted in an increased passive backleak that could be reversed by raising the peritubular oncotic pressure. During hydropenia, however, when passive backleak was relatively less, raising the peritubular capillary oncotic pressure did not influence proximal reabsorption.

The effects of increased filtration fraction might be expected to be exerted primarily on proximal tubular sodium reabsorption. Nevertheless, although clearance and micropuncture studies in animals with heart failure have demonstrated increased sodium reabsorption in the proximal tubule, distal sodium reabsorption also seems to be involved. In this regard, clearance and micropuncture studies performed in dogs with AV fistulae (118), chronic pericarditis (111), and chronic partial thoracic vena caval obstruction (119) documented enhanced distal nephron sodium reabsorption. Levy (119) also showed that the inability of dogs with chronic vena caval obstruction to excrete a sodium load is a consequence of enhanced reabsorption of sodium at the loop of Henle. This nephron segment was similarly implicated in rats with AV fistulae (92). Physical factors also could be involved in the augmented reabsorption of sodium chloride by the loop of Henle in dogs with constriction of the vena cava (119).

Intrarenal mechanisms, specifically decreased delivery of tubular fluid to the distal diluting segment of the nephron, may also contribute to the impaired water excretion observed in heart failure. Evidence supporting this intrarenal mechanism of water retention in heart failure has been provided by studies involving the administration of mannitol (120) or the loop diuretic furosemide (121) to patients with heart failure and hyponatremia. Administration of either of these agents converted the cardiac patient's hypertonic urine to a dilute urine (120,121). Both mannitol and furosemide may diminish the tubular reabsorption of sodium and water in the more proximal portions of the nephron, thus increasing fluid delivery to the more distal nephron sites of urinary dilution. Other factors may, however, be implicated to explain these results: (i) the infusion of mannitol may produce volume expansion, thereby suppressing the baroreceptor-mediated release of AVP, and (ii) the furosemide-induced hypotonic urine was found not to be responsive to the administration of exogenous AVP, thus suggesting antagonism of AVP by furosemide (121). In support of this latter hypothesis, Szatalowicz et al. (122) provided further evidence that furosemide interferes with the renal action of AVP in humans.

In summary, the exact contribution of proximal versus distal nephron sites in the augmented sodium and water reabsorption seen in heart failure may depend on the severity of the heart failure and the concomitant degree of arterial underfilling. The fact that changes in filtration fraction have been observed in patients with heart failure before changes in sodium balance occur may question the dominance of peritubular factors and proximal reabsorption in the sodium retention characteristic of heart failure (123). This observation suggests that other factors, such as the direct tubular effects of neurohormonal activation, may play a significant role in the renal sodium and water retention of heart failure. The renal effects of these various neurohormonal systems are discussed in detail in the following, starting with activation of the vasoconstrictor mechanisms.

Vasoconstrictor Systems

Activation of the Sympathetic Nervous System in Heart Failure. The sympathetic nervous system is activated early in patients with heart failure. Numerous studies have documented elevated peripheral venous plasma norepinephrine concentrations in heart failure patients (103,124–127). In advanced heart failure, using tritiated norepinephrine to determine norepinephrine kinetics, Hasking et al. (103) and Davis et al. (126) demonstrated that both increased norepinephrine spillover and decreased norepinephrine clearance contribute to the elevated venous plasma norepinephrine levels seen in these patients, suggesting that increased sympathetic nerve activity is at least partially responsible for the high circulating norepinephrine levels. Our laboratory (127) has demonstrated that in earlier stages of heart failure, the rise in plasma norepinephrine in patients with heart failure was due solely to increased norepinephrine secretion (Fig. 83-3), supporting the notion that sympathetic nervous system activity is increased early in the course of heart failure. Significantly, in our heart failure patients with mild-to-moderate symptoms, plasma epinephrine, a marker of adrenal activation, was not substantially elevated, confirming the neuronal source of the increased norepinephrine.

The Studies of Left Ventricular Dysfunction (SOLVD) investigators (128) reported the presence of adrenergic activation in patients with asymptomatic left ventricular dysfunction. In this substudy of the SOLVD trials, neurohormonal activation was assessed in 56 control subjects, 151 patients with left ventricular dysfunction (ejection fractions ≤35%) but no overt heart failure, and 81 patients with overt heart failure, prior to randomization to receive placebo versus an ACE inhibitor. The plasma norepinephrine concentration was significantly increased by 35% in subjects with asymptomatic left ventricular

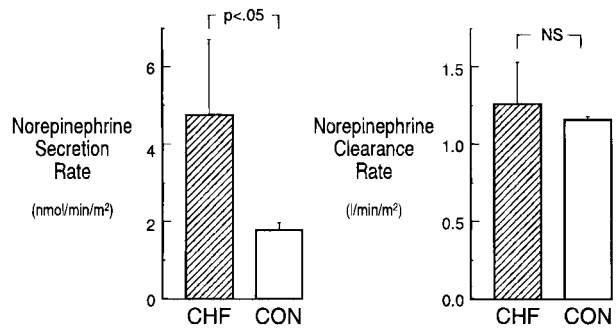

FIGURE 83-3. Plasma norepinephrine secretion and clearance rates in patients with mild-to-moderate heart failure (CHF) and in normal control subjects (CON). The findings of increased norepinephrine secretion and normal norepinephrine clearance in the CHF patients are consistent with early activation of the sympathetic nervous system in cardiac failure. *NS,* not significant. (From: Abraham WT, Hensen J, Schrier RW. Elevated plasma noradrenaline concentrations in patients with low-output cardiac failure: dependence on increased noradrenaline secretion rates. *Clin Sci* 1990;79:429, with permission.)

dysfunction compared to healthy control subjects, and by 65% greater than control values in the overt heart failure patients. These data also demonstrate that adrenergic activation occurs early in the course of heart failure or left ventricular dysfunction and are consistent with the observation that plasma norepinephrine concentrations or the degree of adrenergic activation are directly correlated with the degree of left ventricular dysfunction in patients with heart failure (124,125,129,130). Finally, studies employing peroneal nerve microneurography to directly assess sympathetic nerve activity to muscle (MSNA) confirmed increased adrenergic nerve traffic in patients with heart failure (131).

As mentioned, studies in human heart failure demonstrated the presence of renal adrenergic activation (103). In this study of whole-body and organ-specific norepinephrine kinetics in heart failure patients, cardiac and renal norepinephrine spillovers were increased 504% and 206%, respectively, whereas norepinephrine spillover from the lungs was normal. These findings demonstrate the presence of selective cardiorenal adrenergic activation in heart failure. A discussion of the cardiac effects of this adrenergic activation is beyond the scope of this chapter. However, a recent report supports the value of continuously assessing heart rate variability (a relative measure of cardiac sympathetic versus parasympathetic activity) as a predictor of hospitalization for worsening heart failure (132). Of note, in this report most hospitalizations for worsening heart failure were associated with fluid–volume overload and a reduction in heart rate variability (i.e., sympathetic activation). Therefore, measuring heart rate variability may provide insight into the systemic as well as cardiac effects of heightened adrenergic activity. Moreover, numerous adverse effects of increased cardiac adrenergic activity have been documented in humans (133), and positive experience with the use of β-adrenergic receptor antagonists in heart failure patients (133–139) supports the hypothesis that norepinephrine is harmful to the myocardium. In this regard, blocking the deleterious effects of norepinephrine on the heart results in reverse ventricular remodeling; that is, the dilated failing heart becomes smaller and stronger following chronic adrenergic blockade. Finally, it should be noted that a single resting venous plasma norepinephrine level provides a better guide to prognosis than do many other commonly measured indices of cardiac performance, in which high plasma norepinephrine levels are associated with a poor prognosis in patients with heart failure (140).

Renal Tubular Effects of Adrenergic Activation in Heart Failure. Renal nerves exert a direct influence on sodium reabsorption in the proximal tubule. Bello-Reuss et al. (57) demonstrated this direct effect of renal nerve activation to enhance proximal tubular sodium reabsorption in whole-kidney and nephron studies in the rat. In these animals, renal nerve stimulation produced an increase in the tubular fluid-to-plasma inulin concentration ratio in the late proximal tubule, a result of increased fractional sodium and water reabsorption in this segment of the nephron. On the basis of results of an elegant series of studies, DiBona et al. (141) implicated activation of the renal nerves in the sodium and water retention observed in the various edematous disorders. Experiments were conducted in conscious, chronically instrumented rats with either heart failure (myocardial infarction), cirrhosis (common bile duct ligation), or the nephrotic syndrome (doxorubicin injection). In each experimental model, renal sodium or water excretion of an acutely administered oral or intravenous isotonic saline load was significantly less than that in control rats. Bilateral renal denervation in the experimental rats restored their renal excretory response to normal. Moreover, in response to the acute administration of a standard intravenous isotonic saline load, the decrease in efferent renal adrenergic nerve activity was significantly less in all three experimental models than control animals. These results support an increased basal efferent renal sympathetic nerve activity in heart failure and the other edematous disorders that fails to suppress normally in response to the isotonic saline load. These findings also are consistent with the aforementioned alterations in low-pressure baroreceptor function observed in human heart failure, where adrenergic activation is seen despite chronic increased loading of these cardiopulmonary receptors.

In dogs (142) and humans (104) with heart failure, α-adrenergic receptor blockade induces a natriuresis. Moreover, adrenergic blockade with either phenoxybenzamine or hexamethonium abolishes the sodium retention seen in acute TIVC constriction (24). Moreover, the comprehensive adrenergic blocking agent carvedilol, but not metoprolol, increases renal blood flow and GFR in patients with chronic heart failure (143). Conversely, sodium retention persists in dogs with denervated transplanted kidneys and chronic vena caval constriction (144). In addition, in dogs with pacing-induced heart failure, no differences in renal hemodynamic or electrolyte excretion between innervated or denervated kidneys in compensated or decompensated animals were observed (145). These latter observations implicate factors in addition to renal nerves in the sodium retention of heart failure. However, in these renal denervation experiments and in human heart failure, other hormonal factors (e.g., AT-II, aldosterone, and AVP) may play an important role in the sodium and water retention.

Experience with the partial β_1-adrenergic receptor agonist xamoterol in heart failure suggests a role for the renal β-receptor in modulating proximal tubular sodium reabsorption (146). Botker et al. (146) examined the acute renal effects of xamoterol in 12 patients with mild-to-moderate heart failure. Each patient was given xamoterol (0.2 mg/kg) or placebo in random order separated by 2 weeks of a clinically stable drug washout period. Renal clearance and excretion measurements were made with the patient in the supine position at 30- to 60-minute intervals before, during, and up to 6 hours after infusion. Lithium clearance was used as a measure of proximal tubular sodium handling (147). Blood pressure, heart rate, renal plasma flow, GFR, and urinary flow rate remained unchanged, whereas xamoterol significantly decreased renal sodium excretion by 30%. This acute decrease in sodium excretion with xamoterol was associated with an increase in proximal tubular sodium reabsorption, as indicated by decreased lithium clearance. Of note, plasma concentrations of AT-II and aldosterone were unaffected by xamoterol. These observations suggest a direct effect of acute xamoterol to enhance proximal tubular sodium reabsorption in heart failure. In patients with heart failure, the endogenous adrenergic receptor agonist and neurotransmitter norepinephrine may exert a similar effect on the proximal renal tubule.

Finally, as noted, renal nerves have been implicated as a stimulus for renin release from the kidney (86). Therefore, with heart failure, adrenergic activation may lead to activation of the renin–angiotensin–aldosterone system. Conversely, β-adrenergic receptor blockade may decrease renin release and improve the neurohormonal milieu in heart failure patients. In this regard, Eichhorn et al. (148) showed that the third-generation β-adrenergic receptor blocker bucindolol lowers PRA in patients with mild-to-moderate heart failure. The renal tubular effects of AT-II and aldosterone are discussed in the following.

Activation of the Renin–Angiotensin–Aldosterone System in Heart Failure. The renin–angiotensin–aldosterone system is usually activated in patients with heart failure, as assessed by PRA and plasma aldosterone (129,149,150). In the substudy report from the SOLVD investigators (128), PRA was increased not only in the patients with established heart failure but also in the subjects with asymptomatic left ventricular

dysfunction. Of note, activation of the renin–angiotensin–aldosterone system is associated with hyponatremia and an unfavorable prognosis in patients with heart failure (76,151). Dzau et al. (76) first described the association of PRA and hyponatremia in a group of 15 heart failure patients. These data showed that normal or suppressed PRA is associated with a normal serum sodium level, whereas the highest PRA is associated with the lowest serum sodium concentrations. Lee and Packer (151) subsequently confirmed this association between PRA and hyponatremia in a larger cohort of heart failure patients. Moreover, these investigators demonstrated the association of this hyponatremic, hyperreninemic state with poor survival. Finally, the proven beneficial effects of ACE inhibition or AT-II receptor blockade (ARB) on symptoms, hemodynamics, exercise capacity, and survival in heart failure patients further underscore the deleterious effects of AT-II and aldosterone in these patients (152–157).

Recently, a positive feedback between the renin–angiotensin–aldosterone system and sympathetic activation was proposed (158). This interaction is based in part on the ability of AT-II to augment neuronal norepinephrine release at the presynaptic level (159). In humans, presynaptic facilitation of norepinephrine release by AT-II may play a role in the cardiorenal adrenergic activation of heart failure. Clemson et al. (160) demonstrated AT-II-mediated increases in norepinephrine spillover in the human forearm. In heart failure patients, we demonstrated increased neuronal norepinephrine release from the heart during AT-II infusion, whereas cardiac adrenergic activity was decreased by the bolus injection of the ACE inhibitor enalaprilat (161). In addition, Gilbert et al. (162) showed that chronic ACE inhibition with lisinopril lowers cardiac adrenergic activity in patients with chronic symptomatic heart failure. Thus, activation of renal nerves is a stimulus for renal renin release, thereby activating the renin–angiotensin–aldosterone system, whereas activation of the renin–angiotensin–aldosterone system may further stimulate adrenergic activity at the presynaptic level.

Renal Tubular Effects of Angiotensin II and Aldosterone in Heart Failure. In animal models, AT-II has a direct effect on enhancing proximal tubular sodium reabsorption (163). In these studies of the rat proximal tubule, the administration of AT-II resulted in a marked increase in the rate of sodium chloride reabsorption, whereas the infusion of the AT-II receptor antagonist saralasin significantly reduced proximal tubular sodium chloride reabsorption. Moreover, in a study from Abassi et al. (164), administration of the AT-II receptor antagonist losartan to decompensated sodium retaining rats with heart failure secondary to AV fistulae produced a marked natriuresis. Although proximal tubular sodium handling was not examined in this study, the observation that losartan restored renal responsiveness to ANP is consistent with a losartan-induced increase in the delivery of sodium to the distal tubular site of ANP action. The role of distal tubular sodium delivery in the renal sodium retention of heart failure is discussed in the following.

In humans with heart failure, the finding that urinary sodium excretion correlates inversely with PRA and urinary aldosterone excretion also supports a role for AT-II or aldosterone, or both, in renal sodium retention (165). However, the administration of ACE inhibitors to patients with heart failure results in inconsistent effects on renal sodium excretion, despite a consistent fall in plasma aldosterone concentration (166). A simultaneous fall in blood pressure or decline in renal hemodynamics owing to decreased circulating AT-II concentrations, however, could obscure the beneficial renal effects of lowered AT-II and aldosterone concentrations. Support for this hypothesis may be found in a report from Motwani et al. (167). These investigators examined the hemodynamic and hormonal correlates of the initial effect of ACE inhibition with captopril on blood pressure, GFR, and natriuresis in 36 patients with moderate heart failure. In these subjects, a captopril-induced fall in GFR was predicted by a decrease in renal plasma flow, low pretreatment GFR, *and* low absolute posttreatment serum AT-II concentration. A decrease in urinary sodium excretion was related to this fall in GFR. Conversely, Good et al. (168) showed in eight patients with chronic heart failure that long-term AT-II suppression with captopril enhances renal responsiveness to the loop diuretic furosemide. This observation also supports a role for AT-II in the renal sodium retention of heart failure.

The role of aldosterone in the renal sodium retention of heart failure has been debated for many years. In the presence of a high sodium intake, dogs with caval constriction retain sodium even after surgical removal of the adrenal source of aldosterone (169). Moreover, patients with heart failure do not always show increased urinary sodium excretion after the administration of the aldosterone antagonist spironolactone (170). In addition, Chonko et al. (171) showed that patients with heart failure may have edema without increased aldosterone secretion. However, a normal plasma aldosterone level in heart failure patients may be relatively high in the presence of excess total body sodium. A role for aldosterone in the renal sodium retention of human heart failure was demonstrated by our group (172). We examined the effect of spironolactone on urinary sodium excretion in patients with mild-to-moderate heart failure who were withdrawn from all medications prior to study. Sodium was retained in all subjects throughout the period prior to aldosterone antagonism (Fig. 83-4). With an average sodium intake of 97 ± 8 mmol/day, the average sodium excretion before spironolactone treatment was 76 ± 8 mmol/day. During therapy with spironolactone, all heart failure patients demonstrated a significant increase in urinary sodium excretion to 131 ± 13 mmol/day. Moreover, the urine sodium-to-potassium concentration ratio significantly increased during spironolactone administration, consistent with a decrease in aldosterone

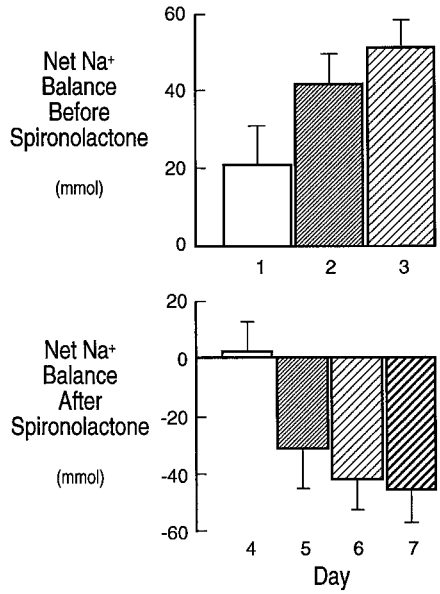

FIGURE 83-4. Reversal of sodium retention with aldosterone antagonism in patients with heart failure. Net positive cumulative sodium balance, by day, for the period before spironolactone therapy (*upper panel*) and net negative cumulative sodium balance after the initiation of spironolactone, 400 mg per day (*lower panel*) are shown. (From: Hensen J, et al. Aldosterone in congestive heart failure: analysis of determinants and role in sodium retention. *Am J Nephrol* 1991;11:441, with permission of S. Karger AG, Basel.)

action in the distal nephron. Of note, norepinephrine concentration and PRA increased and ANP decreased during spironolactone administration, suggesting a possible explanation for the attenuation of the natriuretic effect of spironolactone in long-term studies. Therefore, the combined use of an aldosterone antagonist with other neurohormonal antagonists (e.g., ACE inhibitors, ARBs, and β-blockers) may result in optimal long-term benefit. Several observations support this notion, including randomized controlled trials in patients with chronic heart failure or postmyocardial infarction (MI) left ventricular dysfunction (173–176). For example, the Randomized Aldactone Evaluation Study (RALES) demonstrated that the addition of spironolactone (25 mg/day) to ACE inhibition decreased both hospitalizations and mortality by more than 30% as compared to controls in patients with advanced heart failure (175). Another trial of the selective aldosterone antagonist, eplerenone, demonstrated a 15% reduction in all-cause mortality in patients with post-MI heart failure (176). Although blocking aldosterone-mediated cardiac fibrosis was proposed to explain these survival benefits of aldosterone antagonism, a contribution to improved survival attributable to the renal effects of these agents cannot be excluded.

Nonosmotic Release of Vasopressin in Heart Failure. Plasma AVP is usually elevated in patients with advanced heart failure and correlates with the clinical and hemodynamic severity of disease and the serum sodium concentration (177–182). Several clinical and experimental observations indicate that nonosmotic mechanisms are responsible for increased AVP release in heart failure. A study from our laboratory (182) found plasma AVP concentrations to be inappropriately elevated in 30 of 37 hyponatremic patients with heart failure. The 30 patients with detectable plasma AVP levels had higher levels of BUN and serum creatinine and higher ratios of BUN to serum creatinine than did the seven patients with undetectable plasma AVP levels. This latter finding could be dissociated from diuretic use because it was also observed in 14 patients who had never received diuretics. The presence of prerenal azotemia in these patients is consistent with diminished cardiac output as a mediator of the nonosmotic AVP release. Alternatively, this observation of prerenal azotemia in association with hyponatremia also supports an intrarenal component of the impaired water excretion.

Osmotically inappropriate elevations of plasma AVP in human heart failure were also reported by Riegger et al. (180), Rondeau et al. (181), and Goldsmith et al. (177). The study by Riegger et al. (180) demonstrated a decrease in the elevated plasma AVP levels after improvement in cardiac function by hemofiltration, whereas no change in plasma AVP was observed after decreasing left atrial pressure with prazosin. Moreover, the elevated plasma AVP levels seen in patients with heart failure often (179,183), but not always (11), failed to suppress normally in response to acute water loading. Taken together, these observations demonstrate that there is enhanced nonosmotic release of AVP in heart failure and support the hypothesis that diminished cardiac output, rather than alterations in atrial pressures, is responsible. As previously mentioned, baroreceptor activation of the sympathetic nervous system in response to arterial underfilling likely mediates this nonosmotic AVP release (85).

To shed further light on the mechanism of nonosmotic stimulation of AVP in heart failure patients and, more specifically, to determine the precise relationship between AVP release, cardiac hemodynamics, and the renin–angiotensin system, we studied 25 consecutive patients with severe heart failure (cardiac index 2.1 $\pm$ 0.1 L/minute/m^2 and pulmonary capillary wedge pressure 27.5 $\pm$ 1.5 mm Hg) (95). These patients received two water loads of 15 mL/kg of body weight, the first load without drugs on day 1 and the second on day 3 after receiving vasodilator therapy with either captopril or prazosin for 2 days. Baseline and hourly hemodynamic, renal, and hormonal measurements were obtained for 5 hours following the water load. Basal plasma AVP was detectable (mean 3.0 $\pm$ 0.4 pg/mL) in 17 of the 25 patients (group 1) despite a diminished plasma sodium concentration (P_{Na}, 133.5 mmol/L) and low effective plasma osmolality (E_{osm}, 262 $\pm$ 3 mOsm/kg H_2O). The remaining eight patients (group 2) had appropriately suppressed plasma AVP (<0.5 pg/mL, undetectable) for their P_{Na} (136.5 $\pm$ 0.9 mol/L) and E_{osm} (268 $\pm$ 2 mOsm/kg H_2O). Cardiac index (1.9 versus 2.6 L/minute/m^2, p <0.005) and the percentage of water load excreted (31.4% versus 57.1%, p <0.005) were lower in group 1 than in group 2 patients, but GFR was similar (55 versus 54 mL/minute per 1.73 m^2). The PRA and plasma aldosterone concentrations were higher in group 1 patients, suggesting arterial underfilling. In group 1 patients, vasodilators increased the cardiac index from 1.9 to 2.1 L/minute per m^2 and the percentage of water load excreted from 31% to 53% (both p <0.001). In these same patients, plasma AVP decreased from 3.0 to 1.8 pg/mL (p <0.01), platelet-associated AVP decreased from 8.6 to 5.1 pg/mL (p <0.005), and minimal urinary osmolality decreased from 375 to 208 mOsm/kg H_2O (p <0.001). There was no change in GFR. In group 1 patients in the control condition as well as after vasodilator therapy, plasma AVP decreased with plasma osmolality during the water load, suggesting some preservation of the osmoregulation of AVP, but with a lower osmotic threshold in these patients. Moreover, changes in the renin–angiotensin–aldosterone system were unrelated to changes in water excretion after vasodilator therapy. We consequently concluded that plasma and platelet AVP levels were the major determinants of the abnormal water excretion in many patients with heart failure. These results, therefore, favor a role of impaired cardiac function to cause arterial underfilling with resultant nonosmotic AVP release as a mediator of "resetting" the osmotic threshold for AVP in patients with heart failure. Improved cardiac function secondary to afterload reduction diminishes this resetting of the osmotic threshold. Of interest, our results are reminiscent of earlier studies that suggested that an occasional hyponatremic cardiac patient responds to a large water load by the prompt onset of a water diuresis (11). Also, more recently, AVP secretion was found to respond in exaggerated fashion to osmotic loading in patients with heart failure undergoing radiologic procedures with radiocontrast hyperosmolar agents (184). This latter finding also suggests a form of reset osmostat.

Renal Effects of Vasopressin in Heart Failure. Vasopressin, via stimulation of its renal V_2 receptor (185), induces insertion of the aquaporin-2 (AQP2) water channel into the collecting duct apical membrane with resultant water reabsorption. Elevations in plasma vasopressin concentration and AQP2 are believed to contribute to water retention in heart failure. In animal models of heart failure, the absence of a pituitary source of AVP is associated with normal or near-normal water excretion (186,187). For example, in intact dogs with diminished cardiac outputs owing to TIVC constriction, removal of the pituitary with glucocorticoid replacement results in normalization of the impaired water excretion (186). In these animals, acute constriction of the TIVC caused a significant fall in cardiac output associated with a marked increase in urinary osmolality and a decrease in solute-free water clearance. The effects of TIVC constriction were dissociated from renal hemodynamic changes and the presence or absence of renal sympathetic innervation. However, in hypophysectomized, steroid-replaced animals, both urinary osmolality and solute-free water clearance were maintained at basal levels during constriction of the TIVC. Impaired water excretion also occurs in rats with heart failure because of AV fistulae (187). Significantly, the impairment in water excretion seen in this high-output model of heart

failure was not demonstrable in Brattleboro rats with central diabetes insipidus (i.e., AVP deficiency), supporting a role for persistent AVP release in the abnormality in water excretion associated with high-output cardiac failure. Similar results were obtained by Riegger et al. (188).

Further evidence implicating a role for AVP in the water retention of heart failure comes from studies using selective peptide and nonpeptide V_2 receptor AVP antagonists in several animal models of heart failure (189–192). Ishikawa et al. (189) assessed the antidiuretic effect of AVP in a low-output model of acute heart failure secondary to TIVC constriction in the rat. In these animals, plasma AVP concentrations were increased and a peptide antagonist of the V_2 receptor of AVP reversed the defect in solute-free water excretion. Yared et al. (190) showed a similar reversal of water retention using another peptide antagonist to the antidiuretic effect of AVP in rats with cardiac failure owing to coronary artery ligation. An orally active nonpeptide V_2 receptor AVP antagonist, OPC-31260, was described (193). Intravenous administration of OPC-31260 during a dose-ranging study in normal human subjects increased urine output to a similar extent as 20 mg of furosemide given intravenously (194). In these healthy volunteers, urine osmolality was significantly lower after administration of the V_2 receptor antagonist, thus indicating an increase in solute-free water clearance. Moreover, this agent reversed the impairment in renal water excretion in rats with experimental heart failure owing to myocardial infarction (191) and in dogs with pacing-induced heart failure (192), further supporting a role for AVP in the renal water retention of heart failure. This effect of the non-osmotic release of AVP to cause water retention in cardiac failure was associated with increased transcription of messenger RNA (mRNA) for the AVP preprohormone in the rat hypothalamus (195).

The effects of V_2 receptor antagonists on water metabolism in heart failure have now been studied at the molecular level. Kidney AQP2 expression is increased in experimental heart failure. Rats with cardiac failure due to coronary ligation demonstrate an increase in renal AQP2 expression (196,197) that was reversed with nonpeptide V_2 receptor antagonism (182). V_2 receptor antagonism also reversed water retention in rats with heart failure. Recent studies have been undertaken in hyponatremic heart failure patients treated with various V_2 receptor antagonists (198–202). One investigational agent, lixivaptan, produced a dose-related increase in water excretion, correction of hyponatremia, and a decrease in urinary AQP2 excretion (198,201). It is known that 3% to 6% of AQP2 water channels that traffic to the luminal membrane are excreted in the urine (203). Therefore, AQP2 excretion serves as a bioassay of AVP effect and thus V_2 receptor antagonism *in vivo* in humans and experimental animals. Other investigational agents, such as tolvaptan and conivaptan, have demonstrated similar effects on diuresis (aquaresis) and correction of hyponatremia (199,200,202). Interestingly, preliminary data suggest that AVP antagonists, like inhibitors and antagonists of the sympathetic and renin–angiotensin–aldosterone systems, may prolong survival in heart failure patients (202). Large-scale randomized controlled trials addressing this possibility are now underway.

Endothelin in Heart Failure. Endothelin is a potent vasoconstrictor, and its concentration is increased in patients with heart failure (204). Results of a study from Teerlink et al. (205) suggest that endothelin plays an important role in the maintenance of arterial pressure in experimental heart failure, as shown by a significant decrease in blood pressure following the administration of the endothelin antagonist bosentan in rats with coronary artery ligation. In the kidney, mesangial cells, endothelial cells, epithelial glomerular cells, and inner-medullary collecting duct cells are capable of synthesizing endothelin (206). In this regard, recent studies in experimental heart failure demonstrate early activation of the cardiac and renal endothelin systems (207). Unfortunately, the role of increased endothelin in the pathogenesis of the renal sodium and water retention of heart failure is currently unknown. In this regard, however, endothelin may be a potent mediator of renal vasoconstriction via stimulation of endothelin A (ETA) receptors and thus influence renal sodium and water handling. In experimental cardiac failure, endothelin has been associated with an antinatriuresis (208–211). Conversely, experimental evidence suggests that the endothelin B (ETB) receptor may play a role in renal vasodilation and/or natriuresis (210,211). In this regard, the clinical effects of investigational nonselective endothelin antagonists in heart failure have been disappointing. The use of these agents has been associated with a greater likelihood of worsening heart failure—associated with fluid volume overload—and worse clinical outcomes (212). Consistent with the aforementioned postulated differences between ETA and ETB receptor functions, selective antagonism of ETA receptors may produce a more desirable effect on renal function and excretory capacity in heart failure.

In summary, activation of the three major neurohormonal vasoconstrictor systems—the sympathetic nervous system, the renin–angiotensin–aldosterone system, and the non-osmotic release of AVP—is implicated in the renal sodium and water retention of heart failure. The role of other vasoconstrictor systems (e.g., endothelin) is less well defined. These neuroendocrine systems exert direct (tubular) and indirect (hemodynamic) effects on the kidneys to promote retention of sodium and water. Furthermore, these observations provide the rationale for the use of neurohormonal antagonists in the treatment of heart failure (see the following). In this regard, endogenous counterregulatory vasodilatory and natriuretic hormones may play an important attenuating role in heart failure, and the exogenous administration of these agents may be important in the treatment of heart failure.

Vasodilator Systems

Natriuretic Peptides in Heart Failure. The natriuretic peptides, including but not limited to ANP and BNP, circulate at increased concentrations in patients with heart failure (213–219). These peptide hormones possess natriuretic, vasorelaxant, and renin, aldosterone, and possibly AVP and sympathetic-inhibiting properties (220–225). Both of these peptide hormones appear to be released primarily from the heart in response to increased atrial or ventricular end-diastolic pressure or to increased transmural cardiac pressure (226,227). In a study of ANP kinetics in patients with cardiac dysfunction, we demonstrated that increased ANP production rather than decreased metabolic clearance was the major factor contributing to the elevated plasma ANP concentrations in these patients (228). This finding is consistent with the observed increase in expression of both ANP and BNP mRNA in the cardiac ventricles of humans and animals with heart failure (229,230). However, given the peripheral vasoconstriction and sodium retention associated with heart failure, these elevated circulating natriuretic peptide levels must be inadequate to attenuate fully vasoconstrictor hormone activation. In this regard, volume expansion experiments performed in dogs with heart failure demonstrated a deficiency to further increase the elevated ANP levels (231). This relative deficiency of ANP secretion may contribute to the body's limited ability to maintain hemodynamic and renal function during the advanced stages of heart failure. In support of this notion, a preliminary report from a National Institutes of Health–sponsored clinical trial in advanced heart failure showed that 10% to 20% of patients admitted to the hospital with clinically and hemodynamically decompensated heart failure had low measured levels of the natriuretic peptide, BNP (Stevenson LW, personal

communication). In a coronary ligation model of heart failure in the rat, the infusion of a monoclonal antibody shown to specifically block endogenous ANP *in vivo* caused a significant rise in right atrial pressure, left ventricular end-diastolic pressure, and peripheral vascular resistance (232). Alternatively, a study by Colucci et al. found that a 6-hour infusion of the recombinant human BNP, nesiritide, significantly decreased pulmonary-capillary wedge pressure and improved symptoms in patients hospitalized with symptomatic heart failure (233). In a pivotal trial leading to Food and Drug Administration (FDA) approval of nesiritide, infused BNP was shown to improve both hemodynamics and symptoms of decompensated heart failure (234).

Renal Effects of the Natriuretic Peptides in Heart Failure. In normal humans, ANP and BNP increase GFR and urinary sodium excretion with no change or only a slight fall in renal blood flow (226,235). The changes in renal hemodynamics are likely mediated by afferent arteriolar vasodilation with constriction of the efferent arterioles, as indicated by micropuncture studies in rats (236,237). In addition to increasing GFR and filtered sodium load as a mechanism of their natriuretic effect, ANP and BNP are specific inhibitors of sodium reabsorption in the collecting tubule (238–240). An important role for endogenous ANP in the renal sodium balance of heart failure was demonstrated by the aforementioned study of Lee et al. (28). However, the administration of synthetic ANP to patients with low-output heart failure results in a much smaller increase in renal sodium excretion and less significant changes in renal hemodynamics as compared to normal subjects (226). Like ANP, the natriuretic effect of BNP is blunted in rats with high-output heart failure produced by AV fistulae (241). Nevertheless, in hypertensive patients with mild-to-moderate heart failure and normal renal sodium excretory capacity, the natriuretic effect of BNP appears comparable to that in control subjects (242). Because ANP and BNP appear to share the same receptor sites (243), it is possible that the natriuretic effect of BNP is also blunted in sodium-retaining patients with more advanced heart failure. Support for this hypothesis may be found in reports from our group (244) and from Gottlieb et al. (245). In our study, in 16 patients with advanced decompensated New York Heart Association (NYHA) class III heart failure due to either ischemic or idiopathic dilated cardiomyopathy (left ventricular ejection fraction 18 $\pm$ 2%, cardiac index 1.84 $\pm$ 0.15 L/minute per m^2, pulmonary capillary wedge pressure 27 $\pm$ 3 mm Hg), the administration of BNP at either 0.025 or 0.050 μg/kg per minute for 4 hours produced a natriuresis in only four patients. The effect of BNP on GFR and renal blood flow was inconsistent in these patients and did not predict the natriuretic response. It is noteworthy that the doses of BNP infused in this study were 2.5 to 5 times greater than that currently approved as an initiating dose for the treatment of heart failure. In any event, although the renal effects of BNP were blunted in some of these heart failure patients, BNP did produce a significant 50% decrease in pulmonary capillary wedge pressure. At the higher dose, BNP also significantly lowered peripheral vascular resistance and improved cardiac performance. Gottlieb's study demonstrated essentially the same lack of beneficial renal effects of infused BNP in a similarly small group of patients with heart failure (245).

Recently, concern has been raised regarding the renal effects of BNP in heart failure. A meta-analysis of five trials suggested a higher rate of worsening renal dysfunction (defined as an increase in serum creatinine of at least 0.5 mg/dL) in nesiritide-treated subjects compared to controls (246). However, this analysis had several limitations including the pooling of studies using different starting doses of nesiritide. Because it is likely that the renal hemodynamic effects of BNP in heart failure relate to the relative degree of renal versus peripheral vasodilation (i.e., the distribution of regional blood flow) induced by the drug, higher doses associated with more profound reductions in peripheral vascular resistance and systemic blood pressures may be expected to produce worsening renal function, whereas lower doses may actually preserve (or perhaps improve) renal hemodynamics. Support for this hypothesis may be found in a recent analysis of the effects of BNP on renal function that stratified patients on the basis of nesiritide starting dose (247).

In contrast to the previously mentioned findings of ANP and BNP resistance in heart failure, Elsner et al. (248) recently suggested that renal responsiveness to urodilatin (ANP_{95-126}), a slightly extended form of ANP_{99-126}, is preserved in heart failure. Urodilatin appears to be produced in the kidney by different posttranslational processing of the ANP prohormone ANP_{1-126} (249). Endogenous urodilatin appears to be confined to the kidney (250); that is, it is not a circulating hormone like ANP and BNP. In normal humans, exogenously administered urodilatin produces hemodynamic and renal effects similar to those of ANP (222). In the report from Elsner et al. (219), 12 patients with class II or III heart failure received urodilatin, 15 ng/kg per minute, or placebo ($N = 6$ in each group) for 10 hours. Although the urodilatin-treated patients did demonstrate a modest natriuresis during urodilatin infusion, it should be noted that (i) digoxin and furosemide were continued during the study, (ii) the patients were maintained on an 8-g/day sodium intake, and (iii) the patients received a 500-mL water load (300 mL orally and 200 mL intravenously) during the hour preceding study drug infusion. In the former instance, furosemide likely facilitated the delivery of sodium to the distal nephron. In the latter two cases, the high daily sodium intake and oral water load would be expected to diminish the degree of vasoconstrictor neurohormone activation. In fact, plasma vasoconstrictor hormone concentrations were, at most, mildly elevated in these patients. Therefore, these findings do not exclude the existence of renal resistance to urodilatin in patients with heart failure and more advanced degrees of neurohormonal activation. Ongoing studies of urodilatin in heart failure promise to clarify these issues.

The mechanism of the relative resistance to the natriuretic effect of ANP (and possibly BNP and urodilatin) in heart failure remains controversial. Possible mechanisms include: (i) downregulation of renal ANP receptors (252,253), (ii) secretion of inactive immunoreactive ANP (254), (iii) enhanced renal neutral endopeptidase activity limiting the delivery of ANP to receptor sites (255), (iv) hyperaldosteronism by an increased sodium reabsorption in the distal renal tubule (256), and (v) diminished delivery of sodium to the distal renal tubule site of ANP action (238–240). In sodium-retaining patients with heart failure, we found a strong positive correlation between plasma ANP and urinary cyclic guanosine monophosphate (cGMP, the second messenger for the natriuretic effect of ANP, BNP, and urodilatin *in vivo*) (257,258). This observation supports the active biologic responsiveness of renal ANP receptors in heart failure and thus suggests that diminished distal tubular sodium delivery may be involved in the natriuretic peptide resistance observed in patients with cardiac failure. Further support for this hypothesis is found in our experience with cirrhosis, another edematous disorder associated with renal ANP resistance, in which maneuvers that definitely increase distal tubular sodium delivery reversed the ANP resistance (259) (see the following). In addition, heart failure maneuvers that are expected to increase distal tubular sodium delivery, such as the administration of an AT-II receptor antagonist or furosemide, also improve the renal response to ANP (164,260). Finally, studies in rats with experimental heart failure demonstrated that renal denervation reverses the ANP resistance (261). Because proximal tubular sodium reabsorption is enhanced by adrenergic stimulation, this effect of renal denervation to

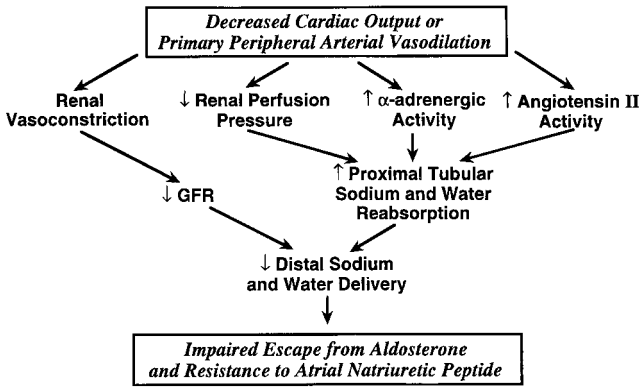

FIGURE 83-5. Proposed mechanism of natriuretic peptide resistance and impaired aldosterone escape in states of arterial underfilling. (From: Schrier RW, Better OS. Pathogenesis of ascites formation. *Eur J Gastroenterol* 1991;3:721, with permission.)

enhance ANP sensitivity in experimental cardiac failure is also compatible with a role of distal sodium delivery. The proposed role of diminished distal tubular sodium delivery in natriuretic peptide resistance and impaired aldosterone escape is shown in Figure 83-5.

Summary

As the heart begins to fail, the renal tubule reabsorbs sodium and water more avidly. The afferent stimuli for this "compensatory" volume retention may involve aspects of both the forward and backward theories of heart failure. An acute fall in cardiac output may inactivate high-pressure baroreceptors located in the aortic arch, carotid sinus, and juxtaglomerular apparatus and thus activate the adrenergic nervous system. Diminished renal perfusion and increased renal sympathetic tone enhance the release of renin and thus activate the renin–angiotensin–aldosterone system. In acute high-output heart failure, in which the cardiac output is insufficient to meet circulatory demands, the fall in peripheral vascular resistance provides the stimulus for arterial underfilling and deactivates high-pressure receptors. Although acute loading of the low-pressure receptors of the thorax may inhibit AVP release and stimulate the release of natriuretic peptides, this counterregulatory response to sodium and water retention may become ineffective because of progressive insensitivity of the cardiopulmonary receptors in the setting of chronic heart failure. Further cardiac compromise, resulting from either progression of the primary cardiac pathology or increased cardiac demand, results in further renal sodium and water retention, expansion of the ECF volume, and overt edema formation. The development of increased cardiac filling pressures with subsequent pulmonary or peripheral edema substantially contributes to the high morbidity and mortality of heart failure.

The efferent mechanisms for renal sodium and water retention in heart failure are multifactorial. Inactivation of receptors in the high-pressure circulation and blunting of receptors in the low-pressure system initiate reflexes in which renal sympathetic tone is augmented and renal vasoconstriction results. Renal blood flow decreases to a greater extent than GFR, and therefore the filtration fraction rises. This increase alters ultrafiltration of plasma and peritubular physical forces, which may in turn increase proximal tubular sodium reabsorption. Changes in cardiac output, ventricular filling pressures, and renal perfusion pressure also activate the renin–angiotensin–aldosterone system and non-osmotic stimulation of AVP, and increase the secretion or production, or both, of PGs and the natriuretic peptides. At some point in the natural history of cardiac failure, the vasoconstrictive forces overcome the vasodilating effects of PGs, natriuretic peptides, and other vasodilating substances, and peripheral vasoconstriction and renal sodium and water retention occur. Increases in ventricular preload and afterload ensue, resulting in a further deterioration in cardiac performance and further stimulation of neurohormonal vasoconstrictor systems.

CLINICAL SIGNIFICANCE OF THE CARDIORENAL SYNDROME

As mentioned in the introduction to this chapter, the kidney represents an important marker of heart failure clinical status and a sensitive predictor of clinical outcomes in both acutely decompensated and chronic heart failure. For example, in the PRIME II trial an estimated GFR less than 60 mL/minute was associated with a significantly worse mortality in 1,708 chronic heart failure patients followed for more than 2 years (3). Reduced GFR was a more potent predictor of mortality than many other common predictors of outcome such as the left ventricular ejection fraction, NYHA functional class ranking, hypotension, tachycardia, and the presence of comorbidity. Similarly, in 2,086 chronic heart failure patients followed in the Italian Network Project (IN-CHF) a serum creatinine level greater than 2.5 mg/dL was associated with a relative risk for 1-year mortality of 4.33 (95% confidence interval, 1.79–10.44) (4). In multivariable regression analysis, other independent clinical predictors of poor outcome included advanced NYHA class, advanced age, presence of a third heart sound, and no ACE-inhibitor therapy. However, none of these predictors was as strong as an elevated serum creatinine. Even modest elevations of serum creatinine have been associated with increased risk for morbidity and mortality in cardiac failure patients. A retrospective analysis of 6,797 heart failure patients enrolled in the SOLVD trial demonstrates this (5). The SOLVD trial excluded patients with baseline serum creatinine level greater than 2.0 mg/dL. Dries et al. (5) stratified patients on the basis of serum creatinine into two groups, those with a serum creatinine less than 1.5 mg/dL and those with a serum creatinine between 1.5 mg/dL and 2.0 mg/dL. Those in the elevated serum creatinine group demonstrated increased risk for all-cause mortality (relative risk, 1.41; 95% confidence interval, 1.25–1.59), mortality due to pump failure (relative risk, 1.5; 95% confidence interval, 1.25–1.8), and sudden cardiac death (relative risk, 1.28; 95% confidence interval, 0.99–1.63). Therefore, impairment in glomerular filtration as measured by serum markers represents a potent predictor of mortality in patients with chronic heart failure.

Similarly, renal dysfunction predicts in-hospital mortality in patients with acutely decompensated heart failure. Definitive observations come from the Acute Decompensated Heart Failure National Registry (ADHERE), which has enrolled more than 150,000 cases from approximately 275 community, tertiary, and academic hospitals in the United States (1,6). Using classification and regression tree (CART) analysis to define covariate adjusted odds ratios of death, a practical user-friendly bedside tool for risk stratification of patients hospitalized with acute decompensated heart failure was developed (6). Specifically, CART analysis of the ADHERE database was performed using the first 65,235 discharges enrolled. The first 33,046 hospitalizations (from October 2001 through February 2003) served as the derivation cohort and were analyzed to develop the risk-prediction model. Then, the validity of the model was prospectively tested using data from 32,229 subsequent hospitalizations (validation cohort) enrolled in ADHERE from March 2003 through July 2003. In-hospital mortality was similar in the derivation (4.2%) and validation (4.0%) cohorts. Recursive partitioning of the derivation cohort for 39

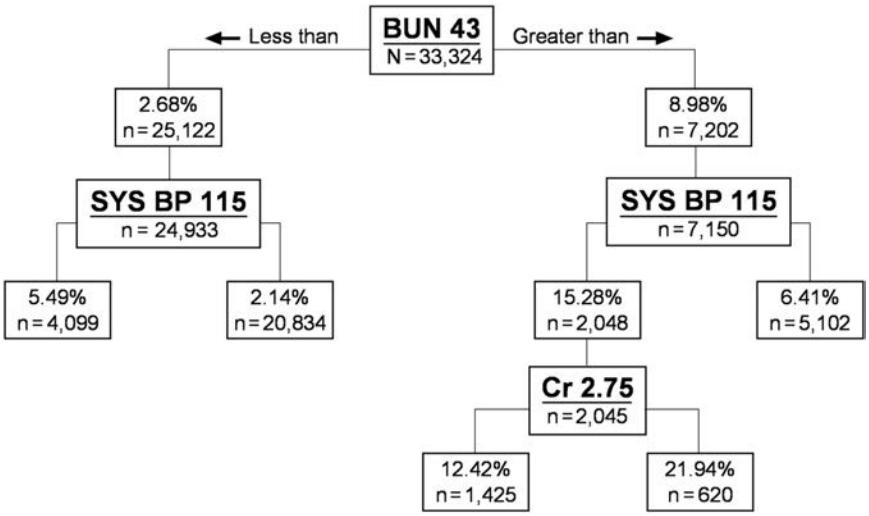

FIGURE 83-6. ADHERE risk assessment tree from CART analysis. Numbers and percentages come from the derivation dataset and have been confirmed in a separated validation dataset (not shown). Note that two of the three predictors are measures of renal function. (From: Fonarow GC, et al., for the ADHERE Scientific Advisory Committee, Study Group, and Investigators. Risk stratification for in-hospital mortality in acutely decompensated heart failure: classification and regression tree [CART] analysis. *JAMA* 2005;293:572, with permission.)

variables indicated that the best single predictor for mortality was high admission levels of BUN (≥43 mg/dL), followed by low admission systolic blood pressure (<115 mm Hg), and then by high levels of serum creatinine (≥2.75 mg/dL). A simple risk tree identified patient groups with mortality ranging from 2.1% to 21.9% (Fig. 83-6). The odds ratio for mortality between patients identified as high and low risk was 12.9 (95% confidence interval, 10.4–15.9) and similar results were seen when this risk stratification was applied prospectively to the validation cohort. These results suggest that acute decompensated heart failure patients at low, intermediate, and high risk for in-hospital mortality can be easily identified using vital sign and laboratory data obtained on hospital admission. In the context of the present chapter, it is noteworthy that two of the three most potent predictors of in-hospital mortality in ADHERE are measures of renal function.

PHYSIOLOGIC BASIS FOR THE TREATMENT OF SODIUM AND WATER RETENTION IN HEART FAILURE

In heart failure, as in all of clinical medicine, effective therapy should be dictated by an understanding of the pathophysiologic process involved. Depressed ventricular function is associated with a vicious cycle of maladaptive responses, including increased neurohormonal activation, systemic vasoconstriction and renal sodium and water retention, and increased ventricular preload and afterload (Fig. 83-7). Treatment of heart failure should be directed at modifying the afferent and efferent factors responsible for the salt and water retention. Therefore,

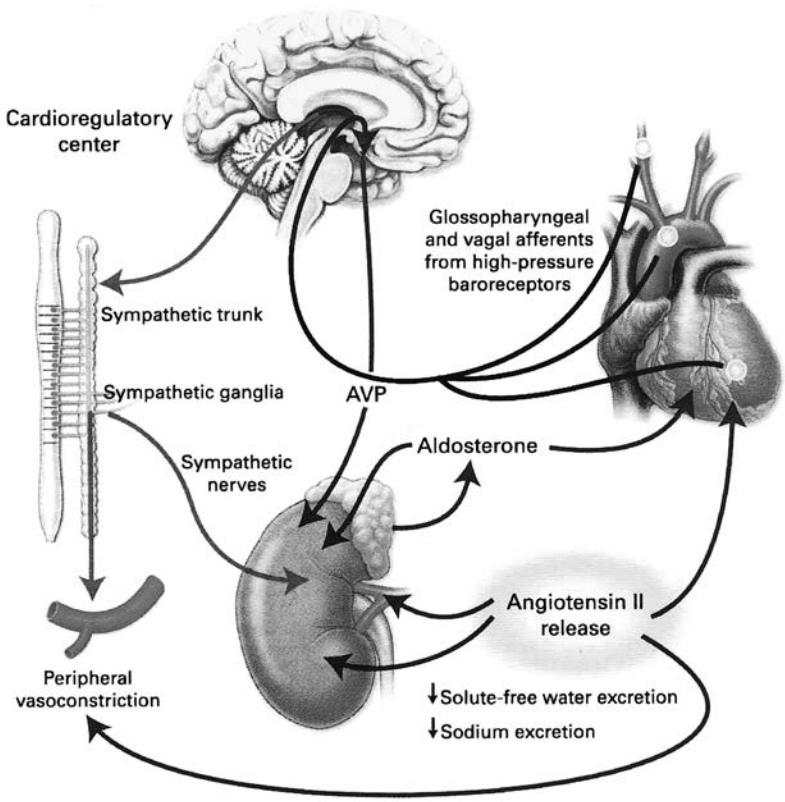

FIGURE 83-7. Pathophysiology of heart failure. Unloading of high-pressure baroreceptors (*blue circles*) in the left ventricle, carotid sinus, and aortic arch generates afferent signals (*black*) that stimulate cardioregulatory centers in the brain, resulting in the activation of efferent pathways in the sympathetic nervous system (*green*). The sympathetic nervous system appears to be the primary integrator of the neurohumoral vasoconstrictor response to arterial underfilling. Activation of renal sympathetic nerves stimulates the release of renin and angiotensin II, thereby activating the renin–angiotensin–aldosterone system. Concomitantly, sympathetic stimulation of the supraoptic and paraventricular nuclei in the hypothalamus results in the nonosmotic release of arginine vasopressin (AVP). Sympathetic activation also causes peripheral and renal vasoconstriction, as does angiotensin II. Angiotensin II constricts blood vessels and stimulates the release of aldosterone from the adrenal gland, and it also increases tubular sodium reabsorption and causes remodeling of cardiac myocytes. Aldosterone may have direct cardiac effects, in addition to increasing the reabsorption of sodium and the secretion of potassium and hydrogen ions in the collecting duct. The blue lines designate circulating hormones. (From: Schrier RW, Abraham WT. Hormones and hemodynamics in heart failure. *N Engl J Med* 1999;341:577, copyright © 2000, Massachusetts Medical Society. All rights reserved.)

the primary goal in the treatment of cardiac failure is to improve the function of the heart as a pump. This increases the integrity of the arterial circulation and decreases the venous hypertension, thereby interrupting two of the major afferent mechanisms leading to sodium and water retention. Unfortunately, this goal of improving the contractile state of the heart is often difficult to accomplish. In certain cases of heart failure, however, left ventricular function may be improved by surgical intervention. For example, some patients with coronary artery disease and ischemic cardiomyopathy may exhibit improved cardiac function and less severe heart failure after surgical or percutaneous transluminal revascularization of the ischemic myocardium. A more classic example of surgically correctable heart failure is that seen in the setting of severe aortic stenosis. Patients with critical aortic stenosis often exhibit a severe degree of low-output heart failure with very avid renal sodium and water retention that is usually completely reversible following replacement of the stenotic aortic valve.

In other instances of heart failure, cardiac function may be augmented by the cardiac glycosides, such as digoxin, which modestly improve cardiac contractility and may favorably influence baroreceptor function (262). However, digoxin does not improve survival in heart failure patients (263). Vasodilators, such as nitrates and hydralazine, and the ACE inhibitors may improve cardiac function by decreasing cardiac preload and afterload (264). Investigational nonglycoside inotropic agents may acutely improve cardiac output but longer-term use has been shown, thus far, to increase mortality (265–267). β-Adrenergic receptor antagonists, once thought to be contraindicated in patients with low-output heart failure, can exhibit a favorable effect on cardiac function and outcome in patients with chronic heart failure. In fact, these agents improve the left ventricular ejection fraction to a greater extent than does any other form of heart failure therapy (133). Carvedilol, a nonselective third-generation β-blocker/vasodilator with α_1-adrenergic receptor-blocking properties, produces a dose-related improvement in ejection fraction and reduction in mortality in patients with class II to IV heart failure (135). In the U.S. Carvedilol Heart Failure Trials Program, this agent reduced all-cause mortality by 65% compared to placebo in patients with mild-to-moderate heart failure (136). Likewise, the Second Cardiac Insufficiency Bisoprolol Study demonstrated a 34% reduction in all-cause mortality versus placebo during treatment of heart failure with this β_1 selective agent (137). In a randomized study of metoprolol CR/XL treatment of 3,991 patients with class II to IV heart failure, treatment with metoprolol CR/XL was associated with a 34% decrease in all-cause mortality, 38% decrease in cardiovascular mortality, 41% decrease in sudden death, and 49% decrease in death owing to progressive heart failure as compared to controls (138). β-Blockers have also been shown to improve outcome in post-MI left ventricular dysfunction with or without heart failure and in severe heart failure (129,139). However, these effects are not seen with all β-blockers (268).

Another recently-approved strategy for improving pump function and outcome in some heart failure patients (i.e., those with ventricular dyssynchrony) is the use of cardiac resynchronization therapy. This device-based treatment for heart failure works to optimize ventricular filling and improve contraction pattern via atrial-synchronized biventricular pacing. Resynchronization therapy has been shown to improve hemodynamics, quality of life, functional status, and exercise capacity while reducing the risks of heart failure hospitalization and all-cause mortality (269–272). Cardiac resynchronization has been associated with preservation of renal function (273) and, anecdotally, a reduction in diuretic dose in patients with chronic heart failure.

As an improvement in pump function is a primary goal in the treatment of heart failure, agents that might further impair cardiac contractility should be avoided in this setting. Unfortunately, many medications that have been demonstrated to produce a negative effect on cardiac inotropy are commonly prescribed in cardiac disease patients. For example, most antiarrhythmic drugs and the commonly prescribed first-generation calcium channel antagonists exhibit some degree of negative inotropy *in vivo* (274). However, newer vascular-selective calcium channel blockers may be well tolerated in patients with heart failure.

The neuroendocrine activation in patients with heart failure provides another target for therapy. In fact, recent experience with various neurohormonal antagonists suggests that inhibition or antagonism of neurohormonal vasoconstrictor systems may be more beneficial than nonspecific diuretic or vasodilator therapy. This is certainly the case with adrenergic blockade, as noted in the preceding text. AT-II is known to mediate myocardial hypertrophy, increase fibrosis and collagen deposition, and cause activation of the sympathetic nervous system. Therefore, administration of ACE inhibitors would be anticipated to decrease myocardial remodeling and hypertrophy and decrease activation of the sympathetic nervous system. ACE inhibition also decreases the degradation of bradykinin, which is a well-known vasodilator that can reduce cardiac afterload. The proven beneficial effects of ACE inhibition on symptoms, hemodynamics, exercise capacity, and survival in heart failure patients support this hypothesis (152,143,266). Moreover, in the patients of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS), all with class IV heart failure, significant reductions in mortality were consistently found in the patients treated with enalapril who had baseline hormone levels greater than median values (152). In the group of patients treated with the ACE inhibitor, there were significant reductions from baseline to 6 weeks in levels of AT-II, aldosterone, norepinephrine, and ANP, but not epinephrine. These results suggest that the effect of enalapril on mortality was related to diminished hormonal activation in general and to the renin–angiotensin system in particular (275). In the SOLVD studies of less severe heart failure, the addition of enalapril to conventional therapy also significantly reduced mortality and hospitalization rates (153). Studies support the use of ACE inhibition in post-MI left ventricular dysfunction with or without clinical heart failure, as well (154). Recent data support the noninferiority of ARBs in the treatment of post-MI ventricular dysfunction or chronic heart failure (155–157). Such studies have led to the perceived interchangeability of ACE inhibitors and ARBs. In fact, recent revision to hospital-based quality measures from the Centers for Medicare and Medicaid Services endorse the equivalency of ACE inhibitors and ARBs in the treatment of heart failure, as do the 2005 Update to the American College of Cardiology/American Heart Association Guidelines for the Evaluation and Management of Chronic Heart Failure in the Adult (276).

Diuretics are indicated to restore the ECF volume toward normal as heart failure becomes more advanced and when edema formation occurs. Diuretic therapy is discussed extensively in Chapter 81. Of note, although most patients with cardiac failure respond to a potent loop diuretic, for example, furosemide, and this agent can increase solute-free water clearance in patients with cardiac edema (121), cardiac output may actually decline during treatment (277). Volume depletion owing to overzealous diuretic treatment must be considered in any heart failure patient with worsening signs or symptoms of a low-output state. For example, diminished renal perfusion may occur in the setting of excessive diuretic treatment, resulting in elevations in BUN and serum creatinine concentrations. More challenging, however, is the common circumstance of worsening renal function despite persistence of ECF volume overload. That is, BUN and serum creatinine may rise in the face of continued fluid volume overload, during diuresis. This may relate to a further reduction in cardiac output. However, in many instances the worsening renal function appears to be

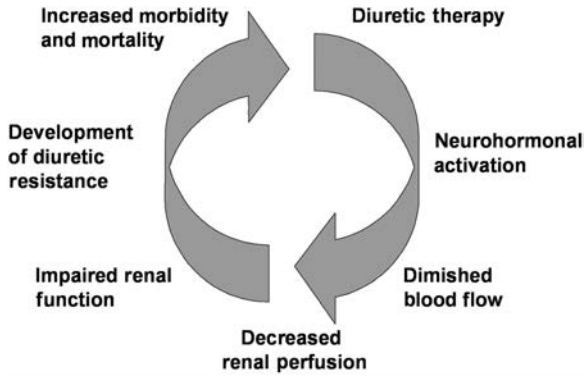

FIGURE 83-8. The "iatrogenic" cardiorenal syndrome of heart failure. Scheme by which diuretic therapy may worsen the neurohormonal and renal hemodynamic milieu of heart failure, leading to diuretic resistance and poor outcomes in heart failure patients.

due to the diuretic therapy *per se*. In this regard, loop diuretics have been shown to reduce GFR on average in heart failure patients (278).

This situation of worsening renal function despite ECF volume excess during diuresis may also be associated with diuretic resistance. Diuretic resistance is not an uncommon finding in patients with advanced heart failure. Because intraluminal delivery of loop diuretics via tubular secretion is necessary for these agents to inhibit sodium chloride reabsorption in the thick ascending limb of Henle, renal vasoconstriction may play an important role in diuretic resistance associated with heart failure (Fig. 83-8). Moreover, increased distal tubule sodium reabsorption further contributes to diuretic resistance in this condition. Therefore, the addition of a more distal acting diuretic, such as metolazone or hydrochlorothiazide, may reverse resistance to loop diuretics. In some cases, however, diuretic resistance is impossible to overcome. Such patients are often unable to be safely discharged from the hospital or are repetitively readmitted to the hospital, if discharged.

Fluid removal by intermittent or continuous ultrafiltration has been suggested to have several advantages over diuretic therapy (279–283). In addition to the reduction of excess ECF volume in heart failure patients, it has been suggested that ultrafiltration of cytokines, which suppress myocardial contractility, may improve cardiac function. This remains to be proven, however. As compared to diuretics, fluid/electrolyte and acid–base disturbances may be more easily corrected and avoided with ultrafiltration. Furthermore, for the same amount of fluid removal, more sodium is removed with ultrafiltration than with diuretics, because the sodium concentration in the ultrafiltrate is equivalent to plasma, whereas with diuretic therapy the urinary sodium concentration is virtually always less than plasma. Recently, a simple approach to ultrafiltration using peripherally inserted catheters has been introduced for the treatment of heart failure (283). Efficient removal of fluid has been demonstrated, using this technique. Small case series support the use of ultrafiltration in hospitalized patients and intermittently in outpatients with refractory heart failure. Although ultrafiltration provides an effective means of fluid removal, there are currently no data available to suggest that ultrafiltration prolongs life in patients with severe heart failure. A randomized controlled trial of ultrafiltration versus diuretics in hospitalized decompensated heart failure patients is underway.

Water restriction remains the mainstay of therapy in patients with heart failure who are hyponatremic. Studies also suggested that in hyponatremic patients with heart failure receiving furosemide and captopril, plasma sodium values tended to normalize, whereas they did not in patients receiving other vasodilators (284,285). These data support the concomitant use of ACE inhibitors and loop diuretics in hyponatremic heart failure patients. Alternatively, a selective V_2-receptor AVP antagonist hopefully will be available in the future to correct the hyponatremia of heart failure.

Finally, other measures, including sodium restriction and oxygen administration, contribute to the overall management of patients with heart failure. Special emphasis should be placed on the salutary influence of bed rest, which increases osmolar and solute-free water clearances, cardiac output, renal plasma flow, and GFR and decreases plasma catecholamines and PRA (286). Such considerations lay the foundation for the physiologic basis of therapy in heart failure.

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CHAPTER 84 ■ LIVER DISEASE AND THE KIDNEY

PERE GINÈS, ANDRÉS CÁRDENAS, AND ROBERT W. SCHRIER

The presence of abnormalities of kidney function in patients with liver diseases has been recognized for several decades (1). More than a century ago, Frerichs in Europe and Flint in the United States reported the association between liver diseases and kidney dysfunction (2,3). These reports described the development of oliguria in patients with chronic liver disease in the setting of normal kidney histology and proposed the first pathophysiologic interpretation of kidney dysfunction in liver disease by linking the abnormalities in kidney function to the disturbances in the systemic circulation. Since then, the relationship between the liver and kidney function has been the object of a considerable amount of research and substantial progress has been made in the last two decades with regard to the pathophysiology and management of renal dysfunction in liver diseases (Table 84-1).

Most derangements of renal function in liver diseases occur in patients with cirrhosis, and are pathophysiologically related to the presence of an expanded extracellular fluid volume, which leads to the development of ascites and/or edema. This chapter discusses the pathophysiology, clinical features, and treatment of ascites and renal function abnormalities in cirrhosis. The abnormalities in kidney function in relation to other liver disease are not discussed.

RENAL ABNORMALITIES IN CIRRHOSIS

Most abnormalities of kidney function in cirrhosis are of functional origin (i.e., they occur in the absence of significant alterations in kidney histology) (4–8). These abnormalities are usually referred to as functional renal abnormalities, as opposed to nonfunctional renal abnormalities, which may also develop in patients with cirrhosis (i.e., glomerulonephritis).

The most common functional renal abnormalities in cirrhotic patients are an impaired ability to excrete sodium and solute-free water and a reduction of renal blood flow (RBF) and glomerular filtration rate (GFR), the latter two being secondary to vasoconstriction of the renal circulation. Sodium retention is a key factor in the expansion of the extracellular fluid volume and ascites and edema formation, whereas solute-free water retention is responsible for the development of dilutional hyponatremia. Renal vasoconstriction, when severe, leads to hepatorenal syndrome (HRS). Chronologically, sodium retention is the earliest alteration of kidney function observed in patients with cirrhosis, whereas dilutional hyponatremia and HRS are later findings. In most patients, abnormalities of kidney function usually worsen with time as the liver disease progresses. However, in some patients a spontaneous improvement or even normalization of sodium and, less often, of solute-free water excretion may occur during the course of their disease (9–11). This improvement in renal function may be seen after alcohol abstinence in patients with alcoholic cirrhosis, but it may occur spontaneously in patients with nonalcoholic cirrhosis. The frequency and mechanism(s) of this improved renal function are not known. Improvement of renal function after the development of HRS is extremely unusual (12,13).

Sodium Retention and Ascites

Sodium retention is the most frequent abnormality of kidney function in patients with cirrhosis and ascites. The existence of sodium retention in cirrhosis was first documented more than 50 years ago when methods to measure electrolyte concentration in organic fluids became available (14–16). Since then, it has been well established that sodium retention plays a key role in the pathophysiology of ascites and edema formation in cirrhosis. The amount of sodium retained within the body is dependent on the balance between the sodium ingested in the diet and the sodium excreted in the urine. As long as the amount of sodium excreted is lower than that ingested, patients accumulate ascites and/or edema. The important role of sodium retention in the pathogenesis of ascites formation is supported by the fact that ascites can disappear just by reducing the dietary sodium content in some patients or by increasing the urinary sodium excretion with the administration of diuretics in others (16,17). Conversely, a high-sodium diet or diuretic withdrawal leads to the reaccumulation of ascites (15,16). The achievement of a negative sodium balance (i.e., excretion higher than intake) is the essence of pharmacologic therapy of ascites. Although no studies assessing the chronologic relationship between sodium retention and the formation of ascites have been performed in patients with cirrhosis, studies in experimental animals have provided conclusive evidence indicating that sodium retention precedes ascites formation, further emphasizing the important role of this abnormality of renal function in the pathogenesis of ascites in cirrhosis (18–22). This observation suggests that sodium retention is a cause and not a consequence of ascites formation in cirrhosis.

The severity of sodium retention in cirrhosis with ascites varies considerably from patient to patient. Some patients have relatively high urinary sodium excretion, whereas urine sodium concentrations are very low or even undetectable in others (Fig. 84-1). The proportion of patients with marked sodium retention depends on the population of cirrhotic patients considered. Most patients who require hospitalization because of severe ascites have marked sodium retention; they excrete less than 10 mEq per day. Among patients who require hospitalization, sodium retention is particularly intense in patients with ascites refractory to diuretic treatment (23,24). By contrast, in a population of cirrhotic patients with mild or moderate ascites, the proportion of patients with marked sodium retention is low, and most patients excrete more than 10 mEq per day spontaneously (without diuretic therapy). The response to diuretics is usually better in patients with moderate sodium retention than in those with marked sodium retention (17,25,26).

Nephron Sites of Sodium Retention. In most instances, sodium retention in cirrhosis is due to increased tubular reabsorption

TABLE 84-1

LANDMARKS IN ASCITES AND RENAL DYSFUNCTION IN LIVER DISEASE

1800s	First description of renal dysfunction in liver diseases
1940s	Demonstration of water retention in cirrhosis with ascites
1950s	Demonstration of sodium retention in cirrhosis with ascites and its relationship with increased mineralocorticoid activity Description of the hyperkinetic circulatory syndrome Clinical description of hepatorenal syndrome
1960s–1970s	Demonstration of renal vasoconstriction as the cause of hepatorenal syndrome Introduction of aldosterone antagonists in clinical practice Demonstration of increased activity of vasoconstrictor systems Demonstration of the role of prostaglandins in the maintenance of renal function Proposal of the overflow theory of ascites formation
1980s	Reintroduction of therapeutic paracentesis in clinical practice Proposal of the arterial vasodilation theory of ascites formation
1990s	Definition and diagnostic criteria of hepatorenal syndrome Description of functional renal failure after bacterial infections (i.e., ascites fluid infection) and its prevention by plasma volume expansion with albumin Use of splanchnic vasoconstrictors in hepatorenal syndrome
2000–present	Introduction of the MELD system as a prognostic index to estimate survival in cirrhotic patients. The scoring system includes creatinine as one of its variables. Use of transjugular portosystemic shunts (TIPS) as therapy for patients with refractory ascites, and selected cases of HRS.

HRS, hepatorenal syndrome; *MELD*, model for end-stage liver disease.

of sodium because it occurs in the presence of normal or only moderately reduced GFR (17,27). The exact contribution of the different segments of the nephron to this increased sodium reabsorption is not completely known. Micropuncture studies in rats with cirrhosis and ascites have demonstrated an enhanced reabsorption of sodium in the proximal tubule (18,28). Conversely, it has been shown that the development of a positive sodium balance and the formation of ascites in cirrhotic rats can be prevented by aldosterone antagonists, which suggests that the collecting ducts are important sites of the increased sodium reabsorption in experimental cirrhosis (21,29). Studies assessing the protein abundance of renal tubular sodium transporters in rats with CCL_4-induced cirrhosis showed an increased expression of the sodium chloride cotransporters of the distal tubule (NCC/TSC) and the epithelial sodium channel of the collecting duct (ENaC), both of which are regulated by aldosterone, consistent with a major role of hyperaldosteronism in sodium retention in this animal model (30). An increased abundance of the sodium-potassium-2-chloride cotransporter of the thick ascending limb (NKCC/BSC1) and a decreased

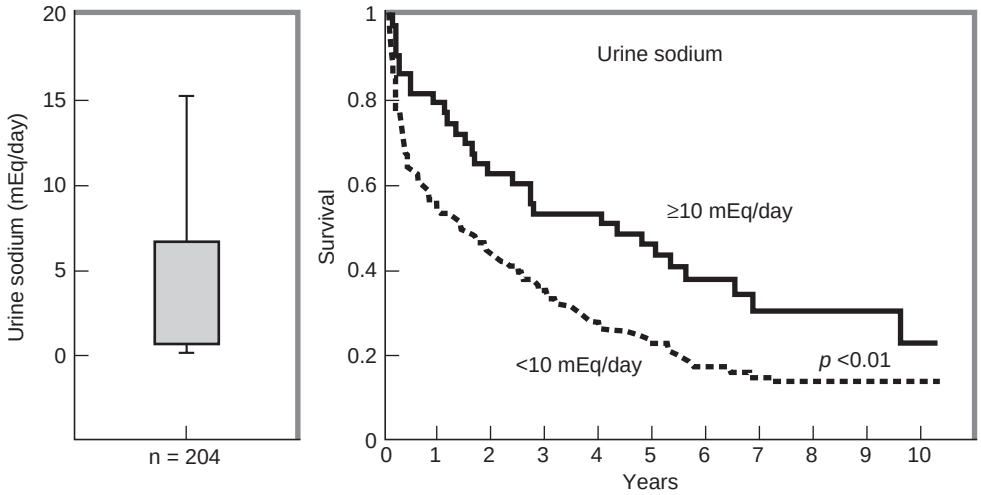

FIGURE 84-1. Box plot of urinary sodium excretion (in conditions of a low-sodium diet—50 mEq per day—and without diuretics) (**left panel**) and long-term survival according to sodium excretion (**right panel**) in a series of 204 patients with cirrhosis admitted to the hospital for the treatment of ascites.

abundance of the proximal sodium transporters (sodium hydrogen exchanger type 3-NHE-3, and sodium-phosphate cotransporter isoform, 2-NaPi-2) was also found, consistent with increased sodium reabsorption in the ascending limb of the loop of Henle and reduced reabsorption in the proximal tubule (30).

Investigations in patients with cirrhosis have also provided discrepant findings. Results from earlier studies using sodium, water, or phosphate clearances to estimate the tubular handling of sodium suggest that the distal nephron is the main site of sodium retention (31–34). Studies using lithium clearance, which estimates sodium reabsorption in the proximal tubule, suggest that cirrhotic patients with ascites show a marked increase in proximal sodium reabsorption (35,36). Nevertheless, distal sodium reabsorption is also increased, especially in patients with more avid sodium retention (36). Clinical studies using spironolactone to antagonize the mineralocorticoid receptor, indicate that this agent induces natriuresis in a large proportion of cirrhotic patients with ascites without renal failure, which supports a major role for increased sodium reabsorption in distal sites of the nephron in these patients (26,37–40). Taken together, these results suggest that in patients with cirrhosis without renal failure, an enhanced reabsorption of sodium in both proximal and distal tubules contributes to sodium retention. Potential mediators of this increased sodium reabsorption include changes in the hydrostatic and colloid osmotic pressures in the peritubular capillaries and increased activity of the sympathetic nervous system and the renin–angiotensin–aldosterone system (RAAS). Sodium retention is usually more marked in patients with renal failure than in those without renal failure due to both a reduction in filtered sodium load and a more marked activation of sodium-retaining systems.

Clinical Consequences. Because sodium is retained together with water isosmotically in the kidney, sodium retention is associated with fluid retention, leading to expansion of extracellular fluid volume and increased amount of fluid in the interstitial tissue. In some patients with cirrhosis, the total extracellular fluid volume may increase up to 40 L or even more (compared to the average 14 L in a 70-kg healthy adult), which represents an approximate cumulative gain of 3,400 mEq of sodium (26 L of excess extracellular fluid volume times 130 mEq/L). In most patients with advanced cirrhosis, sodium retention is manifested by the development of ascites. The most common clinical symptom of ascites is discomfort due to abdominal swelling. In cases with marked accumulation of fluid, physical activity and respiratory function may be impaired. Other clinical consequences related to the presence of ascites are the appearance of abdominal wall hernias and spontaneous infection of ascitic fluid (also known as spontaneous bacterial peritonitis) (41). Both complications, especially infection, contribute markedly to the increased morbidity and mortality associated with the presence of ascites.

Accumulation of fluid in the subcutaneous tissue, as edema, is also common in patients with cirrhosis and sodium retention and in most cases occurs concomitantly with the existence of ascites. Edema is most commonly observed in the lower extremities, but generalized edema may occur as well. Mild or moderate edema may decrease or even disappear during bed rest and reappear during the daytime, reflecting an increased natriuresis in the supine position as compared with the upright position (42,43). Both hypoalbuminemia and increased venous pressure in the inferior vena cava due either to constriction of the vena cava within the liver or increased intraabdominal pressure caused by ascites may contribute to the high incidence of edema in cirrhotic patients with ascites. Leg edema is common in patients with cirrhosis treated with either surgical portacaval shunts or transjugular intrahepatic portosystemic shunts (TIPS), presumably because of the increased pressure in the inferior vena cava secondary to these procedures.

Other clinical manifestations of sodium retention in cirrhosis include pleural and/or pericardial effusions. Hepatic hydrothorax is defined as a pleural effusion in patients with cirrhosis without associated cardiac and/or pulmonary disease. This complication occurs in approximately 10% of patients with cirrhosis (44,45). In most cases the effusion is mild or moderate, more frequent on the right side, and associated with the presence of ascites. Left-sided effusions are uncommon and usually occur in patients who have right-sided effusions as well. Occasionally, large right pleural effusions may exist in the absence of ascites and constitute the main manifestation of the disease (46,47). These cases usually recur after therapy and are due to the existence of anatomic defects in the diaphragm, which cause a communication between the peritoneal and pleural cavities. The gradient between the positive intraabdominal pressure and the negative intrathoracic pressure explains the passage of all fluid formed in the peritoneal cavity to the pleural cavity. Although less common than ascitic fluid, pleural fluid may also become infected spontaneously, a condition known as spontaneous bacterial empyema (47). Finally, between one- and two-thirds of cirrhotic patients with ascites also have mild or moderate pericardial effusions as demonstrated by echocardiography (48). These disappear after the elimination of ascites and are not associated with clinical symptoms.

Assessment of Sodium Excretion in Clinical Practice. The assessment of the urinary excretion of sodium is very useful in the clinical management of patients with cirrhosis and ascites because it allows the precise quantification of sodium retention. Urine must be collected under conditions of fixed and controlled sodium intake (usually a low-sodium diet of 70 to 90 mEq/day during the previous 5 to 7 days), as sodium intake may influence sodium excretion. The amount of sodium ingested does not affect the excretion of sodium in patients with marked sodium retention who have very low urine sodium regardless of the amount of sodium taken with the diet, but may affect sodium excretion in patients with mild or moderate sodium retention. Diuretics should not be given during the 5- to 7-day period prior to urine collection to avoid a pharmacologic increase in sodium excretion. This period of time is particularly important in patients receiving spironolactone or other aldosterone antagonists that have a very prolonged half-life (>3 days). Finally, although the measurement of sodium concentration in a spot of urine may provide a rough estimate of sodium excretion, the assessment of sodium excretion in a 24-hour period is preferable because it is more representative of sodium excretion throughout the day and takes into account the urine output.

In clinical practice, sodium excretion should be measured under the conditions stated above when patients with ascites are first seen or when there are signs suggestive of disease progression (e.g., marked increase in ascites or edema despite compliance with the sodium-restricted diet and diuretic therapy). Conversely, the measurement of sodium excretion in patients receiving diuretic therapy is very useful for monitoring the response to treatment.

Baseline sodium excretion is one of the best predictors of the response to diuretic treatment. Therefore, the measurement of urine sodium concentration and excretion is very helpful to establish the therapeutic schedule in cirrhotic patients with ascites. Patients with marked sodium retention in whom a positive sodium balance is anticipated despite a restriction in sodium intake should be started on moderately high doses of aldosterone antagonists (e.g., spironolactone 200 mg per day) alone or in association with loop diuretics (e.g., furosemide 40 mg per day). Conversely, patients with moderate sodium retention would likely respond to low doses of aldosterone antagonists (i.e., spironolactone 50 to 100 mg per day). Finally, the intensity of sodium retention also provides prognostic information in patients with ascites. Patients with baseline urine

TABLE 84-2

RENAL AND CIRCULATORY ABNORMALITIES IN PATIENTS WITH PREASCITIC CIRRHOSIS[a]

1. Increased cardiac output and blood volume
2. Portal hypertension and splanchnic arterial vasodilation
3. Inability to excrete an acute or chronic sodium load
4. Lack of escape from the sodium-retaining effect of mineralocorticoids
5. Reduced sodium excretion in upright posture and increased sodium excretion in recumbency
6. Increased atrial natriuretic peptide levels
7. Development of sodium retention and ascites and edema after treatment with vasodilators or nonsteroidal antiinflammatory drugs

[a]Patients with preascitic cirrhosis are those without past or current history of ascites or edema.

sodium lower than 10 mEq per day have a median survival time of only 1.5 years compared with 4.5 years in patients with urine sodium higher than 10 mEq per day (Fig. 84-1) (49–51).

Abnormalities of Renal Sodium Handling in Preascitic Cirrhosis. Patients with cirrhosis in the preascitic stage (without past or current history of ascites or edema) do not exhibit overt sodium retention but may have subtle abnormalities in renal sodium handling (Table 84-2) (42,52–67). The finding of increased blood volume in these patients strongly supports the existence of transient episodes of sodium retention sufficient to expand the intravascular volume but without causing ascites or edema (55–57). Preascitic cirrhotic patients come into sodium balance as long as their sodium intake is maintained within normal limits. However, some patients may be unable to handle a sodium load, and will develop ascites and/or edema in conditions of high-sodium intake (especially when they receive intravenous saline solutions) or when treated with nonsteroidal antiinflammatory drugs or vasodilators (54,58,59,68–71). This abnormal renal sodium handling of some preascitic patients is also evidenced by the lack of escape from the sodium-retaining effect of mineralocorticoids (52,53). Healthy subjects treated with mineralocorticoids for several days show an early phase characterized by sodium retention that results in increased extracellular fluid volume and plasma expansion, followed by increased sodium excretion with return of extracellular fluid volume and plasma volume toward normal values, despite the persistent administration of mineralocorticoids. This escape phenomenon is aimed at preventing a persistent sodium retention and subsequent development of edema and is due to the suppression of sodium-retaining mechanisms together with activation of natriuretic mechanisms. When mineralocorticoids are given to patients with preascitic cirrhosis, approximately 20% of patients do not show this escape phenomenon and develop marked sodium retention with formation of ascites and edema (53). Finally, it has been shown that patients in the preascitic stage receiving a normal sodium diet retain sodium while they are in upright posture, whereas they show an exaggerated natriuresis, as compared with healthy subjects, during bed rest (42,60). It has been suggested that this increased natriuresis during recumbency is responsible for the maintenance of sodium balance and may help prevent the formation of ascites or edema that would occur as a consequence of sodium retention that takes place during standing.

These subtle abnormalities in renal sodium handling present in compensated preascitic cirrhotic patients that are responsible for the increased blood volume are probably a homeostatic mechanism to compensate for the increased vascular capacitance of the splanchnic vascular bed in cirrhosis due to arterial vasodilation. This interpretation is supported by the observation that a pharmacologically induced vasodilation is followed by sodium retention and increased plasma volume and ascites and edema formation in most preascitic cirrhotic patients (69–72). It is also supported by the findings that patients who develop ascites or edema while on a high-sodium diet, or who fail to escape from the sodium-retaining effect of mineralocorticoids, are those with more marked abnormalities in systemic hemodynamics as indicated by higher cardiac output and lower total systemic vascular resistance (53). An alternative interpretation suggests that abnormal sodium handling in preascitic cirrhotic patients is related to the degree of liver dysfunction (59,62).

Water Retention and Dilutional Hyponatremia

Since the pioneer studies by Papper and Saxon (73) and Shear et al. (74), it is well known that a derangement in the renal capacity to regulate water balance occurs in advanced cirrhosis. Cirrhotic patients without ascites usually have normal or only slightly impaired renal water handling as compared with healthy subjects. Therefore, in these patients total body water, plasma osmolality, and serum sodium concentration are normal and hyponatremia does not develop, even in conditions of excessive water intake. By contrast, an impairment in the renal capacity to excrete solute-free water is common in patients with ascites and it occurs after the development of sodium retention (73–77). In patients with ascites there is a direct correlation between urinary sodium excretion and water excretion as estimated by urine flow after a water load (74,77). However, no correlation exists between these two parameters when only patients with marked sodium retention are considered. Therefore, sodium retention is necessary but not sufficient for the development of solute-free water retention in cirrhotic patients.

As with sodium retention, the impairment of water excretion is not uniform in all patients with ascites; rather, it varies markedly from patient to patient (Fig. 84-2). In some patients, water retention is moderate and can be detected only by measuring solute-free water excretion after a water load. These patients are able to eliminate water normally and maintain a normal serum sodium concentration as long as their water intake is kept within normal limits, but they may develop hyponatremia when water intake is increased. In other patients, the severity of the disorder is such that they retain most of their water intake causing hyponatremia and hypoosmolality. Therefore, hyponatremia in cirrhosis with ascites is almost always dilutional in origin, since it occurs in the setting of an increased total body water. Hyponatremia is paradoxical in that it is associated with sodium retention and a marked increase in total body exchangeable sodium. Spontaneous dilutional hyponatremia requires a profound impairment in solute-free water excretion, since it usually occurs with a solute-free water clearance after a water load of less than 1 mL/minute (75). Approximately one-third of hospitalized cirrhotic patients with ascites have spontaneous dilutional hyponatremia (75). The presence of dilutional hyponatremia in a cirrhotic patient is associated with a poor survival (51,75,78–84) (Fig. 84-3). The development of dilutional hyponatremia after a precipitating event such as hemorrhage or infection is associated with a better prognosis when compared to the spontaneous appearance of this complication (85). This is possibly related to a higher incidence of renal dysfunction and a more advanced stage of decompensated cirrhosis associated with spontaneous dilutional hyponatremia.

Several factors may aggravate the impairment of solute-free water excretion in cirrhotic patients with ascites and precipitate the appearance of hyponatremia. These include the administration of hypotonic fluids in excess of the capacity to excrete solute-free water, treatment with diuretics or nonsteroidal

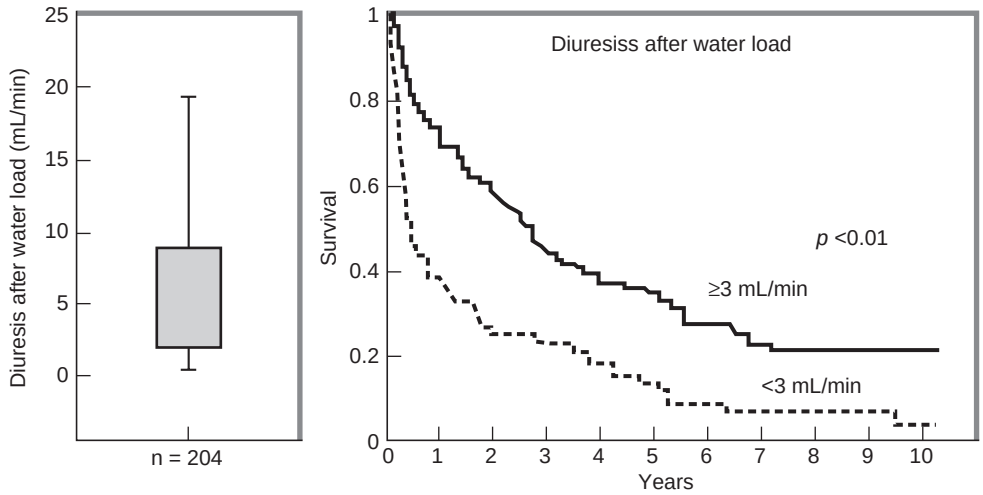

FIGURE 84-2. Box plot of diuresis after a water load (20 mL/kg body weight of 5% dextrose given i.v.) in conditions of a low-sodium diet—50 mEq per day—and without diuretics (**left panel**) and long-term survival according to diuresis after water load (**right panel**) in a series of 204 patients with cirrhosis admitted to the hospital for the treatment of ascites.

antiinflammatory drugs (NSAIDs), and large-volume paracentesis without plasma volume expansion (77,86–88).

Mechanisms of Impaired Renal Water Handling. The pathogenesis of water retention in cirrhosis is complex and probably involves several factors, including a reduced delivery of filtrate to the ascending limb of the loop of Henle, reduced renal synthesis of prostaglandins, and nonosmotic hypersecretion of arginine vasopressin (AVP) (89–91). Although definitive data are lacking about the relative importance of these factors in the pathogenesis of water retention in patients with cirrhosis, it is likely that AVP hypersecretion plays a major role in water retention. This contention is supported by recent studies in animals and patients with cirrhosis showing that the administration of drugs that antagonize the tubular effects of AVP (V2 receptor antagonists) improve solute-free water excretion and increase serum sodium concentration (92–98). In patients with renal failure it is likely that in addition to AVP, a reduced distal delivery of filtrate due to decreased filtered load and increased proximal sodium and water reabsorption plays an important role in water retention.

Clinical Consequences. The clinical consequence of impairment in solute-free water excretion is the development of dilutional hyponatremia. Dilutional hyponatremia in cirrhotic patients is defined as serum sodium <130 mEq/L in the presence of an expanded extracellular fluid volume, as indicated by the presence of ascites and/or edema (90). It is associated with sodium retention and increased total body sodium, and should be distinguished from hypovolemic hyponatremia that, although less common, may develop in cirrhotic patients with ascites and edema who are maintained on high doses of diuretics and sodium restriction after resolution of ascites and edema. In some patients, dilutional hyponatremia is asymptomatic, but in others it is probably associated with clinical symptoms similar to those found in dilutional hyponatremia of other etiologies, including anorexia, difficulty in mental concentration, sluggish thinking, lethargy, nausea, vomiting, and, occasionally, seizures. However, in many instances, it may be difficult to establish whether these symptoms are due to hyponatremia itself, hepatic encephalopathy and/or other associated conditions. The clinical consequences of dilutional hyponatremia in patients with cirrhosis are not completely understood but are currently being assessed. Because serum sodium concentration is the major determinant of the osmolality in the extracellular fluid, dilutional hyponatremia is associated with hypoosmolality of extracellular fluid. This hypoosmolality causes a shift of water from the extracellular to the intracellular compartment, which may probably affect cell function. One of the major effects of dilutional hyponatremia occurs in the brain.

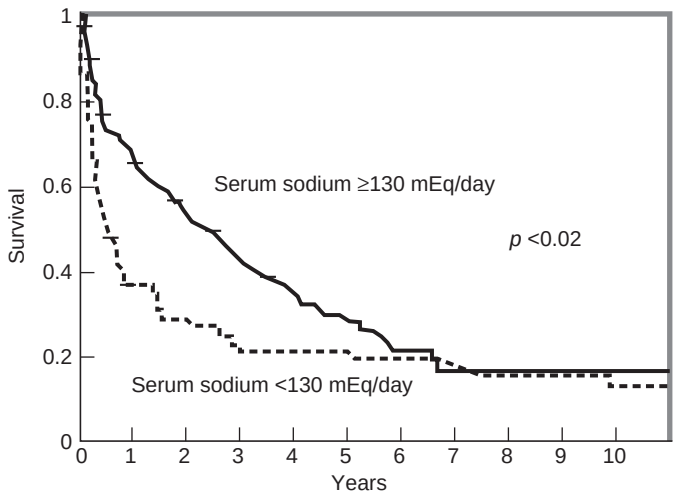

FIGURE 84-3. Long-term survival of a large group of cirrhotic patients with ascites with and without dilutional hyponatremia.

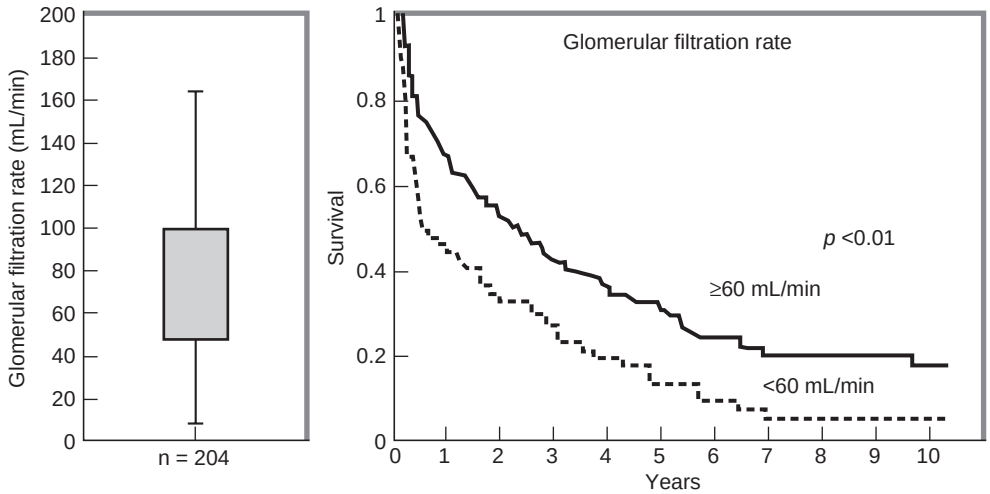

FIGURE 84-4. Box plot of glomerular filtration rate (in conditions of a low-sodium diet—50 mEq per day—and without diuretics) (**left panel**) and long-term survival according to glomerular filtration rate (**right panel**) in a series of 204 patients with cirrhosis admitted to the hospital for the treatment of ascites.

Brain cells of patients with cirrhosis and hyponatremia have reduced intracellular content of some organic osmolytes, particularly myoinositol, which may help prevent the development of brain cell edema as a consequence of passage of water from the extracellular to the intracellular compartment (99). These changes could affect cell function and predispose to the development of disturbances in neurologic function. Preliminary reports indicate that severe dilutional hyponatremia is a major predisposing factor to the development of hepatic encephalopathy in patients with cirrhosis (100). Nonetheless further studies in this area are required to establish this possible pathogenic relationship.

Assessment of Water Excretion in Clinical Practice. As stated previously, the complete assessment of the renal capacity to excrete solute-free water in cirrhotic patients with ascites should include not only the determination of serum sodium concentration but also the evaluation of the immediate response to the administration of water. The capacity to excrete solute-free water is a major prognostic factor of cirrhosis with ascites (78,81) and should be measured if possible when a thorough evaluation of the disease is required, especially in patients who are considered for liver transplantation (discussed in more detail later). Moreover, the assessment of solute-free water excretion is of importance in the clinical management of patients, as it allows for fluid intake to be adjusted to the renal capacity to eliminate water, thereby preventing the development of dilutional hyponatremia due to inappropriately high fluid intake.

Renal Vasoconstriction and Hepatorenal Syndrome

Investigations performed by Sherlock, Schroeder, and Epstein during the late 1960s and early 1970s provided conclusive evidence indicating that the renal failure of functional origin—the so-called hepatorenal syndrome (HRS)—was due to a marked vasoconstriction of the renal circulation (101–103). Later studies showed that, in addition to the striking renal vasoconstriction present in patients with HRS, mild-to-moderate degrees of vasoconstriction in the renal circulation are also very common in patients with cirrhosis and ascites (104–107). When renal perfusion is estimated by sensitive clearance techniques, such as *para*-aminohippurate or inulin clearances, in a population of hospitalized patients with ascites, normal values are found in only one-fifth of cases. In another 15% to 20%, renal hypoperfusion is very intense and meets the criteria of HRS. In the remaining patients, mild or moderate reductions in renal perfusion exist (Fig. 84-4). These latter patients show slightly increased serum creatinine and/or blood urea nitrogen (BUN) levels in baseline conditions (in the absence of diuretic therapy). This moderate renal vasoconstriction is clinically relevant for several reasons: first, it is often associated with marked sodium and water retention and the presence of refractory ascites (108); second, it predisposes to the development of HRS (13,109); and third, it is associated with an impaired survival (51,81).

Definition of Hepatorenal Syndrome. The definition of HRS proposed by the International Ascites Club, which is the most widely accepted, is as follows: "Hepatorenal syndrome is a clinical condition that occurs in patients with advanced chronic liver disease, liver failure, and portal hypertension characterized by impaired renal function and marked abnormalities in the arterial circulation and activity of the endogenous vasoactive systems. In the kidney there is marked vasoconstriction that results in low GFR, whereas in the extrarenal circulation there is predominance of arterial vasodilation, which results in reduction of total systemic vascular resistance and arterial hypotension" (108). Although HRS occurs predominantly in advanced cirrhosis, it may also develop in other chronic liver diseases associated with severe liver failure and portal hypertension, such as alcoholic hepatitis, or in acute liver failure (110).

Pathogenic Mechanisms. The pathophysiologic hallmark of HRS is a vasoconstriction of the renal circulation (102,108, 111,112). Studies of renal perfusion with renal arteriography, $_{133}$Xe washout technique, *para*-aminohippuric acid excretion and duplex Doppler ultrasonography, have demonstrated the existence of marked vasoconstriction in the kidneys of patients with HRS, with a characteristic reduction in renal cortical perfusion (102,112–118). The functional nature of HRS has been conclusively demonstrated by the lack of significant morphologic abnormalities in the kidney histology (5–8,119) and by the normalization of renal function after liver transplantation (120–124).

The mechanism of this vasoconstriction is likely multifactorial, involving changes in systemic hemodynamics, increased pressure in the portal venous system, activation of vasoconstrictor factors, and suppression of vasodilator factors acting on the renal circulation (discussed later). Contrary to the previous belief, other vascular beds in addition to the renal

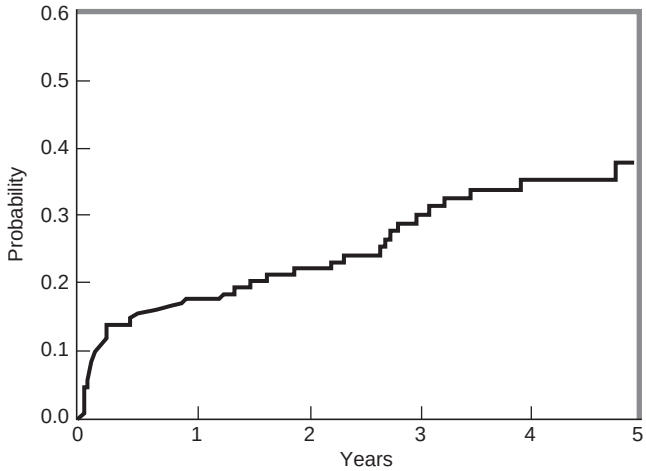

FIGURE 84-5. Probability of developing hepatorenal syndrome in a series of 234 nonazotemic cirrhotic patients with ascites. (From: Ginès A, Escorsell A, Ginès P, et al. Incidence, predictive factors, and prognosis of the hepatorenal syndrome in cirrhosis with ascites. *Gastroenterology* 1993;105:229, with permission.)

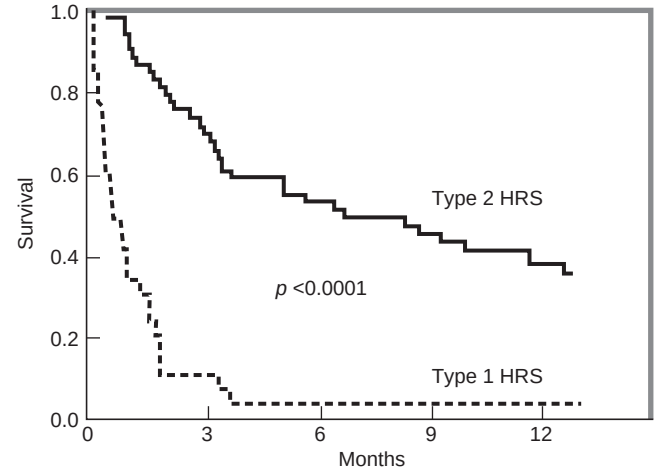

FIGURE 84-6. Survival of patients with cirrhosis according to the type of hepatorenal syndrome. (Reproduced from: Alessandría C, Ozdogan O, Guevara M, et al. MELD score and clinical type predict prognosis in hepatorenal syndrome: relevance to liver transplantation. *Hepatology.* 2005;41:1282, with permission.)

circulation are also vasoconstricted in patients with HRS, including the extremities and the cerebral circulation (125–127). This indicates the existence of a generalized arterial vasoconstriction in nonsplanchnic vascular beds of patients with HRS and suggests that the main vascular bed responsible for arterial vasodilation and reduced total peripheral vascular resistance in cirrhosis with HRS is the splanchnic circulation.

Clinical and Laboratory Findings. Hepatorenal syndrome is a common complication of patients with cirrhosis. In patients with ascites, the probability of developing HRS during the course of the disease is 18% at 1 year and increases up to 40% after 5 years of follow-up (13) (Fig. 84-5). The clinical manifestations include a combination of signs and symptoms related to renal, circulatory, and liver failure. Nonetheless there are no specific clinical findings in HRS.

Renal failure may have a rapid or insidious onset and is often associated with oliguria, intense urinary sodium retention (urine sodium <10 mEq/L), and spontaneous dilutional hyponatremia (serum sodium <130 mEq/L) (108,111). HRS may occur in two different clinical patterns, according to the intensity and form of the onset of renal failure (Table 84-3) (108,128). Type 1 HRS is the classic type of HRS and represents the end of the spectrum of changes in renal perfusion in cirrhosis. The dominant clinical features of type 1 HRS are those of severe renal failure with oliguria or anuria and increased serum levels of urea and creatinine. Type 1 HRS is characterized by a rapid and progressive impairment of renal function as defined by a doubling of the initial serum creatinine to a level greater than 2.5 mg/dL in less than 2 weeks. Despite an important reduction of GFR in these patients, serum creatinine levels are commonly lower than values observed in patients with acute renal failure of similar intensity with respect to the reduction in GFR, but without liver disease (111,118,129,130). This is probably due to the lower endogenous production of creatinine secondary to reduced muscle mass in patients with cirrhosis compared with patients without liver disease. Type 1 HRS is associated with a very low survival expectancy, the median survival time being only 2 weeks (Fig. 84-6) (128). Type 2 HRS is characterized by a more subtle course, with serum creatinine levels around 1.5 to 2.0 mg/dL. Patients are usually in a better clinical condition than those with type 1 HRS, and their survival expectancy is longer, approximately 6 months (Fig 84-6) (128). The dominant clinical feature of these patients is diuretic-resistant ascites due to the combination of intense sodium retention, reduced GFR, and marked stimulation of antinatriuretic systems (108). Severe spontaneous hyperkalemia is an uncommon feature of HRS. However, marked hyperkalemia may occur if patients are treated with aldosterone antagonists, especially patients with type 1 HRS. Severe metabolic acidosis and pulmonary edema, which are frequent complications of acute renal failure of patients without liver disease, are uncommon findings in patients with HRS. Because HRS is a form of functional renal failure, the characteristics of urine are those of prerenal azotemia, with oliguria, low urine sodium concentration, and increased urine osmolality and urine-to-plasma osmolality ratio (108,131). Nevertheless, there are non-oliguric forms of the syndrome, and in some cases urine sodium concentration is not extremely reduced (131,132). As shown in Table 84-4, urinary indices are not currently considered essential for the diagnosis of HRS (108).

Circulatory failure in patients with HRS is characterized by high cardiac output, arterial hypotension (most patients have a mean arterial pressure in the range of 60 to 80 mm Hg), and low total systemic vascular resistance, despite the existence of severe vasoconstriction in several vascular beds, as already discussed (108,126,128). In addition, patients may also have cirrhotic cardiomyopathy, a condition characterized by systolic and diastolic dysfunction of the left ventricle, which is clinically silent but may contribute to profound hemodynamic changes occurring in HRS, particularly when precipitated by spontaneous bacterial peritonitis (133–135).

TABLE 84-3

CLINICAL TYPES OF HEPATORENAL SYNDROME

Type 1	Rapid and progressive impairment of renal function as defined by a doubling of the initial serum creatinine to a level higher than 2.5 mg/dL or a 50% reduction of the initial 24-hour creatinine clearance to a level lower than 20 mL/minute in less than 2 weeks.
Type 2	Impairment in renal function (serum creatinine >1.5 mg/dL) that does not meet the criteria for type 1.

TABLE 84-4

DIAGNOSTIC CRITERIA FOR HEPATORENAL SYNDROME[a]

Major criteria
1. Low glomerular filtration rate, as indicated by serum creatinine greater than 1.5 mg/dL (133 μmol/L) or 24-hour creatinine clearance lower than 40 mL/minute.
2. Absence of shock, ongoing bacterial infection, fluid losses, and current treatment with nephrotoxic drugs.
3. No sustained improvement in renal function (decrease in serum creatinine to 1.5 mg/dL (133 μmol/L) or less, or increase in creatinine clearance to 40 mL/minute or more) following diuretic withdrawal and expansion of plasma volume with 1.5 L of a plasma expander.
4. Proteinuria lower than 500 mg/day and no ultrasonographic evidence of obstructive uropathy or parenchymal renal disease.
Additional criteria
1. Urine volume lower than 500 mL/day.
2. Urine sodium lower than 10 mEq/L.
3. Urine osmolality greater than plasma osmolality.
4. Urine red blood cells less than 50 per high power field.
5. Serum sodium concentration lower than 130 mEq/L.

[a] All major criteria must be present for the diagnosis of hepatorenal syndrome. Additional criteria are not necessary for the diagnosis, but provide supportive evidence.

Finally, the third type of clinical manifestations of HRS is related to the existence of liver failure. The majority of patients have features of advanced liver disease with hyperbilirubinemia, elevated prothrombin time, thrombocytopenia, hepatic encephalopathy, hypoalbuminemia, poor nutritional status, and a large amount of ascites, although some patients with HRS may show only moderate liver failure. In general, patients with type 1 HRS have more severe liver failure relative to patients with type 2 HRS (128).

Precipitating Factors. In some patients, HRS develops without any identifiable precipitating factor, whereas in others it occurs in close chronologic relationship with bacterial infections, particularly spontaneous bacterial peritonitis (108,136,137). Approximately one-third of patients with spontaneous bacterial peritonitis develop an impairment of renal function during or immediately after infection and in the absence of shock (136). This impairment in renal function is of functional origin and occurs in the setting of a further decrease in effective arterial blood volume of patients with ascites, as indicated by a marked activation of vasoconstrictor systems and increased serum and ascitic fluid levels of cytokines (137,138). In approximately one-third of patients developing renal failure after spontaneous bacterial peritonitis, the impairment in renal function is reversible after resolution of infection. However, in the remaining patients the impairment in renal function is not reversible after the resolution of the infection and meets the criteria of HRS (type 1 in most cases). Patients who develop type 1 HRS after spontaneous bacterial peritonitis have a dismal outcome, with an almost 100% hospital mortality if not treated appropriately (see subsequent text) (136,138). Similarly, large-volume paracentesis (>5 L) without albumin expansion may precipitate type 1 HRS in up to 15% of cases (88). This is one of the main factors that support the administration of intravenous albumin when large-volume paracentesis is performed (139). Gastrointestinal bleeding has been classically considered as a precipitating factor of HRS (131). However, the development of renal failure after this complication is uncommon in patients with cirrhosis (approximately 10%) and occurs mainly in patients with hypovolemic shock, in most cases associated with ischemic hepatitis, which suggests that renal failure in this setting is probably related to the development of acute tubular necrosis (ATN) and not of functional origin (140). Diuretic treatment has also been classically described as a precipitating factor of HRS, but there is no clear evidence to support such a relationship.

There are several predictive factors in patients with cirrhosis and ascites associated with a greater risk of developing HRS (13). For the most part these are related to circulatory and renal function and include severe urinary sodium retention, spontaneous dilutional hyponatremia, and low mean arterial blood pressure (<80 mm Hg). Interestingly, neither the degree of liver failure, as assessed by classic parameters of liver function (serum bilirubin, albumin, and prothrombin time) nor the Childs-Pugh classification, correlate with the risk of developing HRS (13).

Diagnosis. The diagnosis of HRS is currently based on several diagnostic criteria (Table 84-4) (108). The minimum level of serum creatinine required for the diagnosis of HRS is 1.5 mg/dL (133 μmol/L). Patients with cirrhosis with a serum creatinine greater than 1.5 mg/dL usually have a GFR of less than 40 mL/minute (111). In patients receiving diuretics, serum creatinine measurement should be repeated after diuretic withdrawal for 7 days because in some patients serum creatinine level may increase during diuretic therapy, even in the absence of excessive diuresis causing volume depletion.

Because no specific laboratory tests are available for the diagnosis of HRS and patients with advanced cirrhosis may develop renal failure of other etiologies (prerenal failure due to volume depletion, ATN, drug-induced nephrotoxicity, and glomerulonephritis in patients with hepatitis B or C), the most important step in the diagnosis of HRS is to rule out renal failure secondary to volume depletion or parenchymal diseases (108). Gastrointestinal fluid losses, due to vomiting or diarrhea, or renal fluid losses, due to excessive diuresis, should be sought in all patients with cirrhosis presenting with renal failure. If renal failure is secondary to volume depletion, renal function improves rapidly after volume repletion (1,500 mL of intravenous isotonic saline) and treatment of the precipitating factor. Shock is another common condition in patients with cirrhosis that may lead to renal failure due to ATN. Although hypovolemic shock related to gastrointestinal bleeding is easily recognized, the presence of septic shock may be more difficult to diagnose because of the paucity of symptoms of bacterial infections in some patients with cirrhosis. Moreover, arterial hypotension due to the infection may be erroneously attributed to the underlying liver disease. In some patients with septic shock oliguria is the first sign of infection. These patients may be misdiagnosed as having HRS if signs of infection (blood cell count, examination of ascitic fluid) are not intentionally examined. Conversely, as discussed previously, patients with cirrhosis and spontaneous bacterial peritonitis may develop renal failure during the course of the infection, in the absence of septic shock (136,138). Renal failure in these patients may either improve with the antibiotic therapy or evolve into HRS, even after the resolution of the infection has been achieved. The administration of NSAIDs is another common cause of acute renal failure in patients with cirrhosis and ascites, which is clinically indistinguishable from HRS (86,141,142). Therefore, treatment with these drugs should always be ruled out before the diagnosis of HRS is made. Recent studies in patients with cirrhosis and ascites indicate that drugs that inhibit selectively the cyclooxygenase 2 (COX-2) enzyme do not

cause renal failure, at least when administered for a short period (143,144). Patients with cirrhosis are also at high risk of developing renal failure due to ATN when treated with aminoglycosides (141,145,146). Because of this high risk of nephrotoxicity and the existence of other effective antibiotics (e.g., third-generation cephalosporins), treatment with aminoglycosides should be avoided in patients with chronic liver disease. Finally, patients with cirrhosis due to hepatitis B and C may also develop renal failure due to glomerulonephritis (147). In these cases, proteinuria and/or hematuria are almost constant and provide a clue for the diagnosis, which may be confirmed by renal biopsy in selected cases.

FACTORS INVOLVED IN FUNCTIONAL RENAL ABNORMALITIES IN CIRRHOSIS

Circulatory Abnormalities

Hepatic and Splanchnic Circulation

The existence of cirrhosis causes marked structural abnormalities in the liver that result in severe disturbance of intrahepatic circulation causing increased resistance to portal flow and subsequent hypertension in the portal venous system (148). Progressive collagen deposition and formation of nodules alter the normal vascular architecture of the liver. Moreover, selective deposition of collagen in the space of Disse, the space between sinusoidal cells and hepatocytes, may constrict the sinusoids, resulting in further mechanical obstruction to flow (149,150). In addition to this passive resistance to portal flow there is an active component of intrahepatic resistance, which is due to the contraction of hepatic stellate cells (myofibroblast like cells) present in sinusoids and terminal hepatic venules (151,152) and low levels of intrahepatic vasodilators. The contraction of these cells is affected by endogenous vasoconstrictors and can be modulated by vasodilators and drugs that antagonize the vasoconstrictor factors (153–155). Moreover, there is a strong body of evidence indicating that despite the overproduction of the vasodilator nitric oxide (NO) in the systemic circulation in cirrhosis, there is a reduced production of NO in the intrahepatic circulation of cirrhotic livers that contributes further to the increased intrahepatic resistance characteristic of portal hypertension (156–158).

Portal hypertension induces profound changes in the splanchnic circulation (159,160). Classically, portal hypertension was considered to cause only changes in the venous side of the splanchnic circulation. However, studies in experimental animals indicate that portal hypertension also causes marked changes in the arterial side of the splanchnic vascular bed. In the venous side, the main changes consist of increased pressure and formation of portocollateral circulation, which causes the shunting of blood from the portal venous system to the systemic circulation. In the arterial side, there is marked arterial vasodilation, which increases portal venous inflow (159–161). This high portal venous inflow plays an important role in the increased pressure in the portal circulation and may explain, at least in part, why portal pressure remains increased despite the development of collateral circulation. This arteriolar vasodilation is also responsible for marked changes in splanchnic microcirculation that may predispose to increased filtration of fluid. It has been shown that chronic portal hypertension causes a much greater increase in intestinal capillary pressure and lymph flow than does an acute increase in portal pressure of the same magnitude (162,163). This is probably due to a loss of the normal autoregulatory mechanism of the splanchnic microcirculation. The acute elevation of venous pressure in the intestine elicits a strong myogenic response, which leads to a reduction in blood flow. This phenomenon is thought to be a homeostatic response to protect the intestine against edema formation. This protective mechanism is not operative in chronic portal hypertension, and arteriolar resistance is reduced and not increased (163,164). The resultant increases in capillary pressure and filtration may be important factors in the formation of ascites in cirrhosis. The mechanism(s) by which portal hypertension induces splanchnic arteriolar vasodilation is not completely understood, although a number of vasoactive mediators have been proposed (and will be discussed subsequently) (159).

Several lines of evidence indicate that portal hypertension is a major factor in the pathogenesis of ascites. First, patients with early cirrhosis without portal hypertension do not develop ascites or edema. Moreover, a certain level of portal hypertension is required for ascites formation. Ascites rarely develops in patients with portal pressure, as assessed by the difference between wedge and free hepatic venous pressure, less than 12 mm Hg, (normal portal pressure: 5 mm Hg) (165–169). Second, cirrhotic patients treated with surgical portosystemic shunts for the management of bleeding gastroesophageal varices have a lower risk of developing ascites than do patients treated with procedures that obliterate gastroesophageal varices but do not affect portal pressure (i.e., sclerotherapy, esophageal band ligation) (170). Finally, reduction of portal pressure with side-to-side or end-to-side portacaval anastomosis or TIPS (placement of a stent between a hepatic vein and the intrahepatic portion of the portal vein using a transjugular approach) is associated with an improvement of ascites, renal function, and suppression of antinatriuretic systems (171–174) in cirrhotic patients with fluid retention. The mechanism(s) by which portal hypertension contributes to renal functional abnormalities and ascites and edema formation is not completely understood, yet three pathogenic mechanisms have been proposed: (i) alterations in the splanchnic and systemic circulation that result in activation of vasoconstrictor and antinatriuretic systems and subsequent renal sodium and water retention; (ii) hepatorenal reflex due to increased hepatic pressure that would cause sodium and water retention; and (iii) putative antinatriuretic substances escaping from the splanchnic area through portosystemic collaterals that would have a sodium-retaining effect in the kidney (as discussed later in this chapter).

Systemic Circulation

The development of portal hypertension is associated with marked hemodynamic changes not only in the hepatic and splanchnic circulation but also in the systemic circulation. These changes, which have been well characterized in human and experimental cirrhosis, consist of reduced systemic vascular resistance and arterial pressure, increased cardiac index, increased plasma volume, and activation of systemic vasoconstrictor and antinatriuretic factors. These changes in systemic hemodynamics appear before the formation of ascites and are more marked as the disease progresses (29,175–180). The hemodynamic profile of patients with cirrhosis in different stages of the disease is summarized in Table 84-5. The factor that appears to trigger all these hemodynamic changes of cirrhosis is an arterial vasodilation located mainly in the splanchnic circulation (159–161,180–182). The existence of splanchnic arterial vasodilation causes an abnormal distribution of blood volume, which results in a reduction of central blood volume (heart, lungs, and aorta) that is sensed by arterial and cardiopulmonary receptors. This central underfilling triggers a neurohormonal response, by activating the sympathetic nervous system (SNS), RAAS, and arginine vasopressin (AVP).

TABLE 84-5

HEMODYNAMIC PROFILE OF CIRRHOTIC PATIENTS IN DIFFERENT STAGES OF DISEASE

	Preascitic cirrhosis	Cirrhosis with ascites	Hepatorenal syndrome
Cardiac output	Normal or increased	Increased	Increased
Arterial pressure	Normal	Normal or reduced	Reduced
Systemic vascular resistance	Normal or reduced	Reduced	Reduced
Plasma volume	Normal or increased	Increased	Increased
Portal pressure	Normal or increased	Increased	Increased
Vasoconstrictor systems activity	Normal	Increased[a]	Increased
Renal vascular resistance	Normal	Normal or increased	Increased
Brachial or femoral vascular resistance	Normal or reduced	Normal or increased	Increased
Cerebral vascular resistance	Normal	Increased	Increased

[a]May be normal in 20% to 30% of patients.

This explains why systemic vasoconstrictor factors are activated despite an increased plasma volume that in normal conditions would suppress the activation of these systems. Investigations in patients with cirrhosis have assessed central blood volume by measuring the mean circulation time of an indicator or by magnetic resonance imaging (180,183–185). These studies have confirmed that central blood volume is reduced in patients with cirrhosis, particularly in those with ascites, and correlates directly with systemic vascular resistance and inversely with portal pressure, indicating that the greater the vasodilation and the pressure in the portal system, the lower the central blood volume. The crucial role played by the reduced central blood volume in the activation of vasoconstrictor systems has been further corroborated by studies showing that improvement of central blood volume by the combination of expansion of plasma volume or head-out water immersion and administration of vasoconstrictor agents, suppresses the activation of vasoconstrictor systems (186–189). Whether or not arterial vasodilation occurs also in nonsplanchnic territories is still controversial but most data indicate that the splanchnic circulation accounts for most, if not all, of the reduced arterial resistance in patients with cirrhosis (159,163, 180,190).

Despite extensive investigation, the mechanism(s) responsible for arterial vasodilation in cirrhosis is not completely understood. Several explanations have been proposed, including opening of arteriovenous fistulas, reduced sensitivity to vasoconstrictors, and increased circulating levels of vasodilator substances (159,163,180,190–193). This latter mechanism has been the most extensively studied. Increased production of release of nitric oxide (NO), carbon monoxide, glucagon, endocannabinoids, prostaglandins, vasoactive intestinal peptide, adenosine, bile salts, platelet-activating factor, substance P, natriuretic peptides, calcitonin gene-related peptide (CGRP), and adrenomedullin may participate in the development of splanchnic arterial vasodilation(159,163,180,190,194–204). At present, most available data, obtained mainly from experimental cirrhosis, indicate that NO is the main mediator of arterial vasodilation in cirrhosis (Table 84-6) (reviewed in reference 205). NO synthesis from cirrhotic arterial vessels is markedly increased compared to that of normal vascular tissue. This increased NO synthesis appears to be generalized, except for the intrahepatic circulation, but predominates in the splanchnic territory. Among the different isoforms of NO synthase, the constitutive form appears to be the one responsible for the increased NO synthesis. The normalization of NO synthesis

TABLE 84-6

EVIDENCE FOR A ROLE OF AN INCREASED VASCULAR PRODUCTION OF NITRIC OXIDE (NO) IN THE PATHOGENESIS OF ARTERIAL VASODILATION AND SUBSEQUENT SODIUM AND WATER RETENTION IN CIRRHOSIS

Experimental cirrhosis

1. Reversal of the impaired pressor response to vasoconstrictors of isolated aortic rings or splanchnic vascular preparations by NO synthase inhibition.
2. Enhanced vasodilator response to NO-dependent vasodilators.
3. Increased pressor effect of systemic NO synthase inhibition.
4. Increased NO synthesis in vascular tissue.
5. Normalization of the hyperdynamic circulation, activity of antinatriuretic systems, and sodium and water retention by chronic NO synthase inhibition.
6. Increased expression of NO synthase isoenzymes in vascular tissue.

Human cirrhosis

1. Correction of the arterial hyporesponsiveness to vasoconstrictors by NO synthase inhibition.
2. Enhanced vasodilatory response to NO-dependent vasodilators.
3. Increased plasma levels of NO and NO metabolites.
4. Increased NO in the exhaled air.
5. Increased NO synthase activity in polymorphonuclear cells and monocytes.

in experimental cirrhosis by the administration of inhibitors of NO synthesis is associated with a marked improvement of splanchnic and systemic hemodynamics, suppression of the increased activity of the RAAS and AVP concentration, increased sodium and water excretion, and reduction or disappearance of ascites (206). So far, only few studies have investigated the effect of acute NO synthesis inhibition in patients with cirrhosis on systemic hemodynamic and/or renal function, with discrepant findings (207–209). Unfortunately, no study has been reported investigating the effects of a prolonged inhibition of NO synthesis in human cirrhosis.

Neurohumoral Systems

The functional renal abnormalities that occur in cirrhosis are probably the result of a complex interrelationship between different systems and factors with effects on renal function. The relative contribution of a particular system in the pathogenesis of these abnormalities in cirrhosis has, therefore, been difficult to assess. This section reviews the different systems that may participate in renal dysfunction in cirrhosis. The evidence indicating the role of these systems in the pathogenesis of these abnormalities is discussed.

Renin–Angiotensin–Aldosterone System

Of all potential factors involved in pathogenesis of sodium retention in cirrhosis, aldosterone has been the most extensively studied. Plasma aldosterone levels are increased in most cirrhotic patients with ascites and marked sodium retention (33,57,106,177,210–215). In ascitic patients with moderate sodium retention, plasma aldosterone is either slightly elevated or normal. It should be pointed out, however, that these "normal" concentrations occur in the presence of an increase in total body sodium of a degree that generally suppresses aldosterone concentration in normal subjects. Three lines of evidence indicate that aldosterone plays an important role in the pathogenesis of sodium retention in cirrhosis: (i) there is an inverse correlation between urinary sodium excretion and plasma aldosterone levels (33,57,106,177,215,216); (ii) studies in animals with experimental cirrhosis have shown the existence of a chronologic relationship between hyperaldosteronism and sodium retention (21); and (iii) the administration of spironolactone, a specific aldosterone antagonist, is able to reverse sodium retention in the great majority of patients with ascites without renal failure (37,38,217–219). The observation that sodium retention may occur in cirrhotic patients in the absence of increased plasma aldosterone levels has raised the suggestion that other factors in addition to aldosterone may contribute to the increased sodium retention in cirrhosis (220). Nevertheless, it has also been suggested that cirrhotic patients may have an increased tubular sensitivity to aldosterone (33,57). This may explain the natriuretic response to spironolactone (38,219). Therefore, the possibility exists that aldosterone may participate in renal sodium retention in cirrhosis, even in the presence of normal plasma concentrations of the hormone. In addition to aldosterone, increased intrarenal levels of angiotensin II may also contribute to sodium retention in patients with cirrhosis by a direct effect on tubular sodium reabsorption (221).

The increased plasma aldosterone concentration in cirrhotic patients with ascites are due to a stimulation of aldosterone secretion and not due to impaired degradation, as the hepatic clearance of aldosterone is normal or only slightly reduced in these patients (55,210,220). Among the different mechanisms that regulate aldosterone secretion, an increased activity of RAAS is the most likely to be responsible for hyperaldosteronism in cirrhosis (Fig. 84-7). In fact, plasma renin activity (PRA), which estimates the activity of the RAAS, is increased in most patients with ascites and correlates closely with plasma aldosterone concentration (106, 177,211,212,222–224). Investigations using pharmacologic agents that interrupt RAAS have provided evidence suggesting that this system is activated as a result of a profound disturbance in systemic hemodynamics. The administration of angiotensin II receptor antagonists, or converting enzyme inhibitors, to cirrhotic patients with ascites and increased PRA induces a marked reduction in arterial pressure and systemic vascular resistance, which suggests that the activation of RAAS is a homeostatic response to maintain arterial pressure in these patients (225–228).

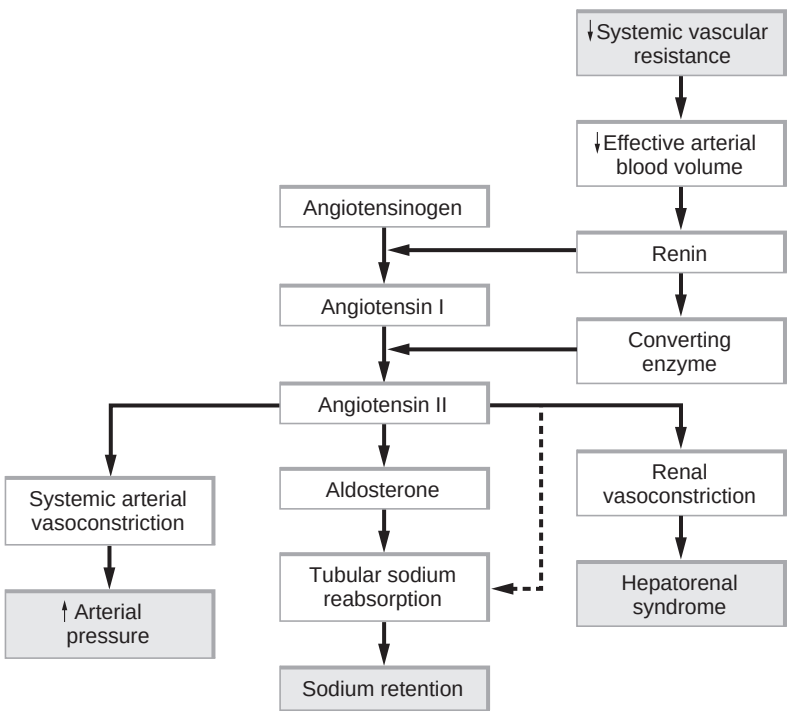

FIGURE 84-7. Proposed mechanism of activation and renal and systemic effects of renin–angiotensin–aldosterone system in cirrhosis with ascites.

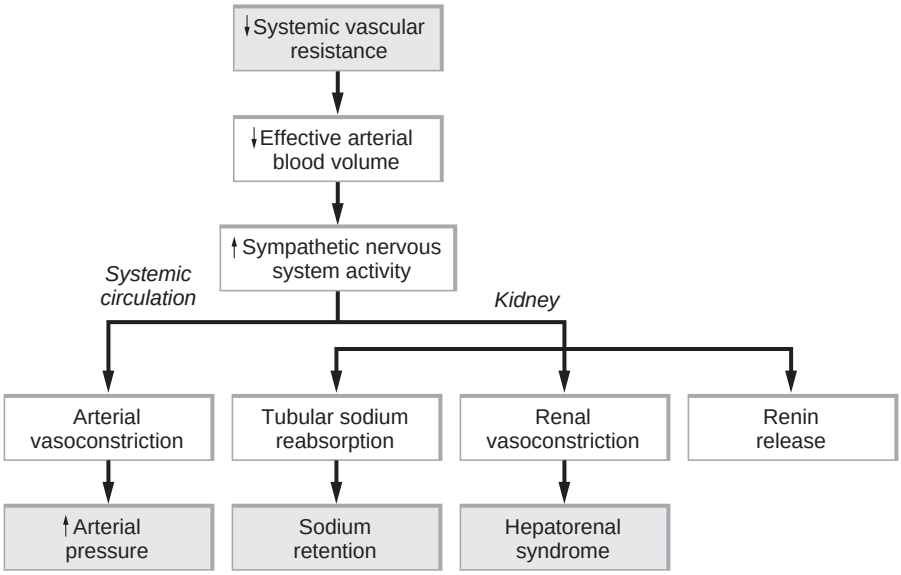

FIGURE 84-8. Proposed mechanism of activation and renal and systemic effects of sympathetic nervous system in cirrhosis with ascites.

The activation of RAAS is particularly intense in patients with HRS, suggesting a role for angiotensin II in the pathogenesis of renal vasoconstriction in HRS (229–234). This role is further supported by studies showing that the improvement of renal function in patients with HRS achieved by the administration of the vasopressin analogs ornipressin or terlipressin associated with albumin or the insertion of a TIPS is associated with a marked suppression of the activity of the RAAS (188,189,235,236). However, since the interruption of RAAS is associated with arterial hypotension in patients with high PRA, the effects of RAAS on renal function independent of those on systemic hemodynamics have been difficult to assess. Although administration of angiotensin II blockers such as losartan may induce natriuresis when given at low doses to patients with preascitic cirrhosis (221), the use of these drugs should be avoided in patients with ascites because of a high risk of hypotension and renal failure.

Sympathetic Nervous System

Numerous studies have presented evidence indicating an increased activity of the SNS in cirrhosis. The plasma concentration of norepinephrine (NE) in the systemic circulation, an index of the activation of the SNS, is increased in most patients with ascites and normal or only slightly elevated in patients without ascites (230,237–243). This "normal" plasma NE concentration, however, is relatively increased in the presence of plasma volume expansion, which occurs in early cirrhosis. Investigations using titrated NE, to provide a more accurate assessment of the SNS activity, have confirmed that the high plasma NE levels are caused by an increased activity of the SNS and not to an impaired elimination of NE, as the total spillover of NE to plasma is markedly increased in cirrhotic patients with ascites, whereas the plasma clearance of NE is normal (243–248). Measurements of NE release and spillover in specific vascular beds have shown that the activity of the SNS is increased in many vascular territories, including kidneys, splanchnic organs, heart, and muscle and skin, supporting the concept of a generalized activation of the SNS (245–249). Direct evidence of the overactivity of the SNS in cirrhosis has been provided by measuring the sympathetic nerve discharge rates from a peripheral muscular nerve. Muscular sympathetic nerve activity is markedly increased in patients with ascites and normal in patients without ascites, and correlates directly with plasma NE concentration (250).

Because the SNS has profound effects on renal function (251), it is reasonable to presume that the increased renal sympathetic nervous activity in cirrhosis may play a role in the pathogenesis of functional renal abnormalities (Fig. 84-8). In fact, evidence suggests that the SNS is involved in sodium and water retention in cirrhosis. The activity of the SNS, either estimated by plasma NE or total NE spillover to plasma, or measured from intraneural recordings, correlates inversely with sodium and water retention (238,245,250). Furthermore, bilateral renal denervation increases urine volume and sodium excretion in animals with experimental cirrhosis and ascites (252,253). Similarly, anesthetic blockade of the lumbar SNS, a maneuver that reduces the activity of the kidney SNS, improves sodium excretion in patients with cirrhosis and ascites (254). The acute inhibition of the renal sympathetic outflow with clonidine in patients with cirrhosis is associated with a reduction in renal vascular resistance and an increase in GFR and filtration fraction, suggesting that the activation of the SNS causes renal vasoconstriction by increasing arterial tone in the afferent arteriole (248). Finally, patients with HRS have significantly higher plasma levels of NE than do patients without renal failure, and arterial and renal venous NE correlate inversely with RBF, suggesting that the SNS may participate in the renal vasoconstriction observed in patients with HRS (230,214,255). Moreover, the circulating levels of neuropeptide Y, a neurotransmitter with a very potent vasoconstrictor action in the renal circulation released in the setting of a marked activation of the SNS, are increased in patients with HRS but not in those with ascites without renal failure (256).

The cause of the increased activity of the SNS in cirrhosis with ascites is not completely understood. Two major explanations have been proposed: either a baroreceptor-mediated response to a decrease in effective arterial blood volume due to arterial vasodilation (180,243,257) or a hepatorenal reflex resulting from activation of hepatic baroreceptors due to sinusoidal hypertension (258–260). The first explanation seems more likely, since the estimated central blood volume (i.e., the blood volume in the heart cavities, lungs, and central arterial tree) is reduced in cirrhotic patients and correlates inversely with SNS activity (183,184). The activity of the SNS can be suppressed by maneuvers that increase effective arterial blood volume, such as the administration of vasopressin analogs and albumin or the insertion of a peritoneovenous shunt (188,189,244,261).

Prostaglandins and Other Eicosanoids

Prostaglandins are known to have a protective effect on renal circulation in pathophysiologic situations associated with increased activity of renal vasoconstrictor systems (262). According to this formulation, prostaglandins appear to play a key role in the homeostasis of renal circulation and water excretion in cirrhotic patients with ascites. The urinary excretion of prostaglandin E2 (PGE2) and 6-keto-prostaglandin F1α, which estimate the renal synthesis of PGE2 and PGI2, respectively, are increased in patients with cirrhosis and ascites without renal failure as compared to healthy subjects and patients without ascites (107,229,230,263,264). Further evidence supporting a role for renal prostaglandins in the maintenance of RBF and GFR in cirrhosis with ascites derive from studies using NSAIDs to inhibit prostaglandin synthesis. The administration of NSAIDs, even in single doses, to cirrhotic patients with ascites causes a profound decrease in RBF and GFR in those who have a marked activation of vasoconstrictor systems, but has little or no effect in patients without activation of these systems (86,142,230,264,265). An increased renal production of PGE2 also contributes to the maintenance of solute-free water excretion in nonazotemic cirrhotic patients with ascites, as the inhibition of prostaglandin synthesis by NSAIDs in these patients impairs solute-free water excretion independently of changes in renal hemodynamics (87).

The relationship between the renal prostaglandin system and HRS is controversial. Several studies have reported that patients with HRS have lower urinary excretion of PGE2 and 6-keto-PGF1α than do patients with ascites without renal failure, which suggests that a reduced renal synthesis of vasodilator prostaglandins may play a role in the pathogenesis of HRS (107,229,266–268). The finding of low renal content of prostaglandin endoperoxide (PGH2) synthase (medullary cyclooxygenase) in patients with HRS is consistent with this hypothesis (269). Other studies, however, did not find reduced urinary excretion of vasodilator prostaglandins in patients with HRS (270,271). Nevertheless, "normal" synthesis of prostaglandins may be low relative to the increased activity of vasoconstrictor systems in cirrhosis. Because patients with HRS have the greatest activation of renal vasoconstrictor systems, an imbalance between vasoconstrictor systems and the renal production of vasodilator prostaglandins has been proposed to explain the marked reduction of RBF and GFR that occurs in this condition (229). It has also been suggested that HRS could be the consequence of an imbalance between the renal synthesis of vasodilator and vasoconstrictor prostaglandins based on the observation of reduced urinary excretion of PGE2 and 6-keto-PGF1α and increased urinary excretion of TXB2 in patients with HRS (268,271). These findings, however, were not confirmed by subsequent investigations (107,267,272). Moreover, the administration of inhibitors of TXA2 synthesis does not improve renal function in these patients (273).

Prostaglandin synthesis in cirrhosis is also increased in extrarenal organs. Patients with cirrhosis have high urinary excretion of 2-3-dinor-6-keto-PGF1α, a metabolite of PGI2 considered to be an index of systemic PGI2 production (194,270). Because prostaglandins are potent vasodilators in the systemic circulation, these observations raise the possibility that an increased prostaglandin synthesis may contribute to arterial vasodilation in cirrhosis. This suggestion is consistent with the observation that the NSAID indomethacin increases systemic vascular resistance and ameliorates the hyperdynamic circulation in cirrhotic patients (274).

Studies in rats with experimental cirrhosis and ascites have investigated the metabolic pathways leading to the increased synthesis of prostaglandins. Increased activity and expression of cytosolic phospholipase A2 (cPLA2) (the first enzyme of the metabolic cascade of eicosanoid synthesis) and cyclooxygenase-1 (COX-1) have been found in arterial and renal tissue of rats with cirrhosis and ascites compared with normal rats (275–277).

The possible role of eicosanoids other than prostaglandins in the pathogenesis of functional renal abnormalities in cirrhosis is not completely known. Nevertheless, the urinary excretion of leukotriene E4 and *N*-acetyl-leukotriene E4, compounds with a vasoconstrictor effect in the renal circulation, is increased in cirrhotic patients with HRS as compared to healthy subjects and patients without ascites, suggesting that leukotrienes may participate in the pathogenesis of this syndrome (278,279). Likewise, the urinary excretion of the vasoconstrictor compound 20-hydroxyeicosatetraenoic acid (20-HETE) is also increased in patients with cirrhosis as compared to healthy subjects (280).

Arginine Vasopressin

Studies in humans and experimental animals have provided several pieces of evidence indicating that AVP plays a key role in the pathogenesis of water retention in cirrhosis with ascites. These include: (i) plasma AVP levels are often increased in cirrhotic patients and correlate closely with the reduction in solute-free water excretion, patients with higher plasma AVP levels being those with the more severe impairment in water metabolism (87,90,91,281–285); (ii) a chronologic relationship between AVP hypersecretion and impairment in water excretion can be found in rats with cirrhosis and ascites (286,287); (iii) Brattleboro rats (rats with a congenital deficiency of AVP) with cirrhosis do not develop an impairment in water excretion (288); (iv) kidneys from cirrhotic rats with ascites show increased gene expression or redistribution to plasma membrane of aquaporin-2, the AVP-regulated water channel (289,290); (vi) the administration of specific antagonists of the tubular effect of AVP (V2 antagonists) restore the renal ability to excrete solute-free water in animal as well as in human cirrhosis (92–98).

The increased plasma AVP concentrations in cirrhosis are due to an increased hypothalamic synthesis and not to a reduced systemic clearance of the peptide (91,291–293). The hemodynamic changes occurring in cirrhosis (low arterial blood pressure, high cardiac output and low total systemic vascular resistance) cause arterial hypotension, which unloads the high pressure baroreceptors and stimulate a non-osmotic release of AVP with the subsequent increase in water reabsorption (87,281). The mechanism of this non-osmotic hypersecretion is hemodynamic, as plasma AVP levels correlate with PRA and plasma NE concentration (87,238) and are suppressed by maneuvers that increase effective arterial blood volume, such as head-out water immersion or peritoneovenous shunting in human cirrhosis (282,294) or inhibition of NO synthesis in experimental animal cirrhosis (206). This hemodynamic mechanism of AVP release in cirrhosis is also supported by the observation that the administration of a specific antagonist of the vascular effect of AVP (V1 antagonist) induces arterial hypotension in rats with experimental cirrhosis and ascites and water retention but not in control rats (295). This finding suggests that AVP hypersecretion in cirrhosis contributes not only to water retention but also to the maintenance of arterial pressure (Fig. 84-9).

Natriuretic Peptides

The natriuretic hormones, represented by the atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are increased in patients with cirrhosis and ascites (296–304). In patients without ascites, plasma ANP levels may be either normal or increased. The high plasma levels of ANP in cirrhosis with ascites are due to increased cardiac secretion of the

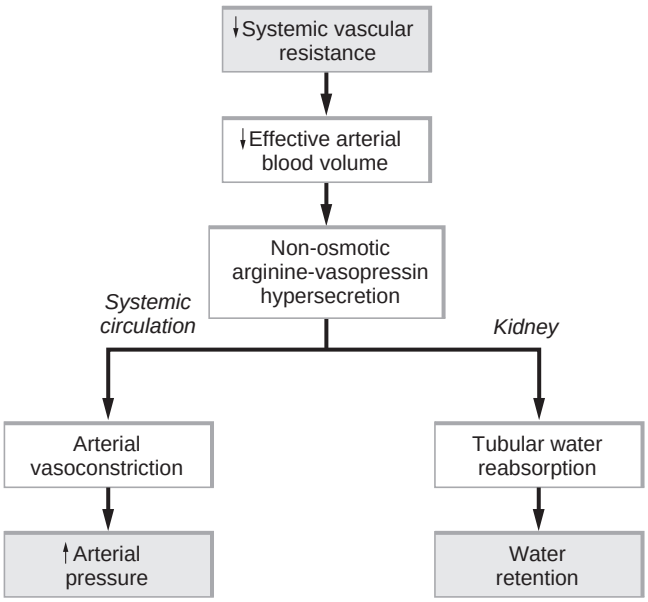

FIGURE 84-9. Proposed mechanism of hypersecretion and renal and systemic effects of arginine vasopressin in cirrhosis with ascites.

peptide and not reduced hepatic or systemic catabolism, as cardiac production of ANP is increased in cirrhotic patients with ascites but splanchnic and peripheral extraction are normal (296,305). Consistent with these observations is the finding of increased messenger RNA (mRNA) expression for ANP in ventricles from cirrhotic rats with ascites (306). In contrast to other diseases showing increased cardiac ANP secretion, in cirrhosis with ascites this increased secretion occurs in the presence of normal atrial pressure and reduced estimated central blood volume (183,296). The mechanism(s) responsible for this increased cardiac secretion of ANP is not known. The existence of increased plasma levels of ANP in cirrhosis with ascites sufficient to have a natriuretic effect in healthy subjects, together with the presence of renal sodium retention, indicates a renal resistance to the effects of ANP. This renal resistance has been confirmed in studies in human and experimental cirrhosis in which pharmacologic doses of natriuretic peptides (ANP or BNP) were administered (307–312). In these investigations patients with activation of antinatriuretic systems (RAAS and SNS) had a blunted or no natriuretic response after ANP infusion. This blunted response can be reversed by maneuvers that increase distal sodium delivery in human cirrhosis or by bilateral renal denervation in experimental cirrhosis, suggesting that the renal resistance to ANP in cirrhosis is related to the increased activity of antinatriuretic systems (253,313). Limited information exists on other peptides of the natriuretic peptide family. As with ANP, the plasma concentration of BNP is increased in cirrhotic patients with ascites as compared to healthy subjects (314). Finally, the urinary excretion of urodilatin, a member of the natriuretic peptide family exclusively synthesized in the kidney, which probably reflects the renal production of the peptide, is normal in patients with cirrhosis and ascites (315).

The role of natriuretic peptides in cirrhosis is not entirely clear. Because most of these peptides have vasodilator properties, a role in the pathogenesis of arterial vasodilation in cirrhosis has been proposed but not proved. By contrast, data from experimental studies suggest that these peptides play an important role in the maintenance of renal perfusion and modulation of RAAS activity, as the selective blockade of the natriuretic peptide A and B receptors causes renal vasoconstriction and increased PRA and aldosterone levels in experimental cirrhosis (316). It could be speculated, therefore, that the cardiac synthesis of ANP and BNP is increased in an attempt to maintain renal perfusion within normal levels and limit the activation of the RAAS. The mechanism(s) leading to this increased synthesis of natriuretic peptides remains unknown.

Endothelins

Endothelins comprise three homologous peptides (ET-1, ET-2, and ET-3) with a very potent vasoconstrictor action (317). Increased plasma levels of ET-1 and ET-3 have been found in patients with cirrhosis and ascites and in patients without ascites, albeit to a lesser extent (318–325). The increased plasma levels of ET-1 found in cirrhosis derive either from an increased production in the splanchnic circulation and/or an increased intrahepatic production. Increased levels of ET-1 and its precursor Big-ET-1 have been found in plasma samples obtained from the portal and hepatic veins of patients with cirrhosis (326). Moreover, increased levels of ET-1 have been demonstrated in hepatic tissue in human and experimental cirrhosis (327–330). In human cirrhosis, the increased hepatic ET-1 levels correlate with portal hypertension and the severity of ascites and liver failure (329,330). As opposed to other vasoconstrictor factors (e.g., angiotensin II or norepinephrine), it is unlikely that high endothelin levels in cirrhosis are a compensatory mechanism triggered by effective arterial hypovolemia. Endothelin levels are not suppressed by maneuvers that improve circulatory function, such as plasma volume expansion with or without concomitant administration of splanchnic vasoconstrictors (188,321,323). A role for endotoxemia in the increased endothelin levels in cirrhosis has also been proposed (318) but plasma endothelin concentration does not parallel endotoxin levels in cirrhotic patients (323).

The role that these increased circulating ET-1 levels play in the pathogenesis of abnormalities in renal, systemic, and hepatic circulation in cirrhosis is not known. A role for ET-1 in the pathogenesis of renal vasoconstriction in HRS has been proposed on the basis of markedly increased plasma endothelin levels in patients with HRS as compared with patients with ascites without HRS (128,320) and improvement of renal function after the administration of a selective antagonist of ET_A receptors in a small group of patients (331). A contribution of the increased endothelin levels to the maintenance of arterial pressure in cirrhosis is unlikely because most studies in experimental models of cirrhosis and portal hypertension have found no changes in arterial pressure after chronic endothelin receptor blockade (327,332–334). Because of the well-known vasoconstrictor effect of ET-1 in the intrahepatic circulation when infused through the portal vein, ET-1 has been postulated as a mediator of the increased intrahepatic resistance characteristic of diseases associated with portal hypertension. The results of these studies are conflicting and the role of endothelin in these abnormalities is unclear (335). Finally, recent studies suggest an important role for ET-1 in hepatic fibrogenesis by increasing collagen synthesis from hepatic stellate cells (328). In support of this hypothesis, a marked reduction in liver fibrosis has been demonstrated in bile duct-ligated rats chronically treated with an oral ET_A receptor antagonist (333). Despite the great efforts aimed at elucidating the role of ET in cirrhosis, its relevance in circulatory homeostasis in cirrhosis is still unclear. Further studies are needed to understand and characterize the role of endothelin in advanced cirrhosis.

Nitric Oxide

In addition to its effects in the regulation of systemic hemodynamics and arterial pressure, NO also participates in the regulation of renal function (336). Constitutive NO synthase has been found in several cell types in the kidney, including

endothelial cells, mesangial cells, and some tubular epithelial cells. Inducible NO synthase has also been demonstrated in mesangial cells and epithelial cells. Under normal circumstances, NO participates in the regulation of glomerular microcirculation by modulating arteriolar tone and mesangial cell contractility. Moreover, NO facilitates natriuresis in response to changes in renal perfusion pressure, and regulates renin release (336).

Three lines of evidence indicate that the renal production of NO is increased in experimental cirrhosis. First, kidneys from cirrhotic rats show enhanced endothelium-dependent vasodilator response as compared to control animals (337). Second, infusion of L-arginine, the precursor of NO, causes a greater increase in renal perfusion in cirrhotic rats as compared to control rats (338). Finally, increased expression of NO synthase in kidney tissue from cirrhotic rats has been found in two studies (338,339). However, both studies showed discrepant findings with respect to the NO synthase isoform responsible for the increased NO synthesis.

The inhibition of NO synthesis in rats with cirrhosis and ascites does not result in renal hypoperfusion because of a marked rise in prostaglandin synthesis (340). However, the simultaneous inhibition of NO and prostaglandin synthesis in experimental cirrhosis results in a marked renal vasoconstriction suggesting that NO probably interacts with prostaglandins to maintain renal hemodynamics (341).

PATHOPHYSIOLOGY OF FUNCTIONAL RENAL ABNORMALITIES IN CIRRHOSIS

Ascites as Primary Edema: The Overflow Theory

The existence of a primary renal sodium retention in cirrhosis with ascites was proposed in an attempt to explain the paradox of coexistence of sodium retention and increased plasma volume in patients with ascites (342,343). According to this theory, the expansion of plasma volume would result in increased cardiac index and reduced systemic vascular resistance as adaptive circulatory mechanisms to the excess of intravascular volume. The existence of portal hypertension and circulating hypervolemia would lead to "overflow" of fluid within the peritoneal cavity. It has been proposed that the primary signal for sodium retention would arise from the liver, either as a consequence of intrahepatic portal hypertension, by means of hepatic low-pressure baroreceptors, or liver failure, by means of decreased hepatic clearance of a sodium-retaining factor or reduced hepatic synthesis of a natriuretic factor (54,258–260,344–346). However, the hemodynamic pattern of cirrhotic patients with ascites does not correspond to that predicted by the overflow theory because the arterial vascular compartment is not overfilled, as arterial pressure is low in most patients despite the increased plasma volume and cardiac index (Table 84-5). Moreover, there is marked overactivity of vasoconstrictor mechanisms, which would be suppressed if there were overfilling in the systemic circulation (180,190).

Because of the increasing evidence against the existence of vascular overfilling in cirrhosis with ascites, the overflow theory has been redefined recently to exclusively explain changes that occur in the preascitic stage of cirrhosis. Proponents of this theory suggest that in the preascitic stage of cirrhosis subtle sodium retention leading to plasma volume expansion would have two components: one related to the circulatory changes occurring in the splanchnic circulation aimed at maintaining the effective arterial blood volume (EABV) and one related to the existence of intrahepatic portal hypertension (347,348). Recent studies in patients with cirrhosis without ascites indicate that the existence of arterial vasodilation is of crucial importance in the development of sodium retention and ascites formation. In fact, preascitic cirrhotic patients with sinusoidal portal hypertension treated with mineralocorticoids do not show mineralocorticoid escape and develop ascites only when marked arterial vasodilation is present (53). Moreover, pharmacologically induced vasodilation in preascitic cirrhotic patients by means of the administration of prazosin, an *a*-adrenergic blocker, is associated with the development of ascites and/or edema in a significant proportion of patients (69). It is important to note that the development of sodium retention in these two studies was neither related to the degree of portal hypertension nor to the intensity of liver failure. In fact, in patients receiving prazosin, sodium retention occurred despite a marked reduction of portal pressure and improvement of liver perfusion.

Ascites as Secondary Edema: From the Traditional Theory to the Arterial Vasodilation Theory

The traditional concept of ascites formation in cirrhosis (349,350) considers that the key event in ascites formation is a "backward" increase in hydrostatic pressure in the hepatic and splanchnic circulation due to the increased resistance to portal flow. This would cause a disruption of the Starling equilibrium and an increased filtration of fluid into the interstitial space. Initially, this capillary hyperfiltration is compensated by an increased lymphatic flow, which returns the fluid to the systemic circulation via the thoracic duct. However, as portal hypertension increases, the lymphatic system is not able to drain the excess of interstitial fluid, which then accumulates in the peritoneal cavity as ascites. Loss of fluid from the intravascular compartment results in true hypovolemia, which is then sensed by cardiopulmonary and arterial receptors resulting in a compensatory renal sodium retention. The retained fluid cannot adequately fill the intravascular compartment and suppress the sodium-retaining signals to the kidney because fluid is continuously leaking in the peritoneal cavity, thereby creating a vicious cycle. In cases with extreme hypovolemia, renal vasoconstriction develops, leading to HRS. This hypothesis is similar to the "backward" theory of edema formation in heart failure, which suggests that sodium retention and formation of edema is secondary to the disruption of the Starling equilibrium in the microcirculation due to the backward increase in capillary hydrostatic pressure (351). The "classic underfilling" theory of ascites formation, however, does not correspond with the systemic hemodynamic abnormalities associated with cirrhosis (Table 84-5). If this theory were correct, changes in systemic circulation would consist of reduced plasma volume and cardiac index and increased systemic vascular resistance. However, findings in patients with cirrhosis and ascites are exactly the opposite, with increased plasma volume and cardiac index and reduced systemic vascular resistance (179,180,190).

These traditional backward theories of edema formation in cirrhosis and heart failure have been substituted by new theories that fit more precisely with the modern concepts of regulation of extracellular fluid volume, which consider that a reduction in EABV is the main determinant of sodium retention in major edematous states (352–354). Arterial vasodilation would be the triggering factor for sodium retention in cirrhosis, whereas a reduction in cardiac output would be the triggering factor in heart failure. The "arterial vasodilation" theory considers that the reduction in EABV in cirrhosis with ascites is not due to true hypovolemia, as proposed by the "traditional" theory, but rather to a disproportionate enlargement of the

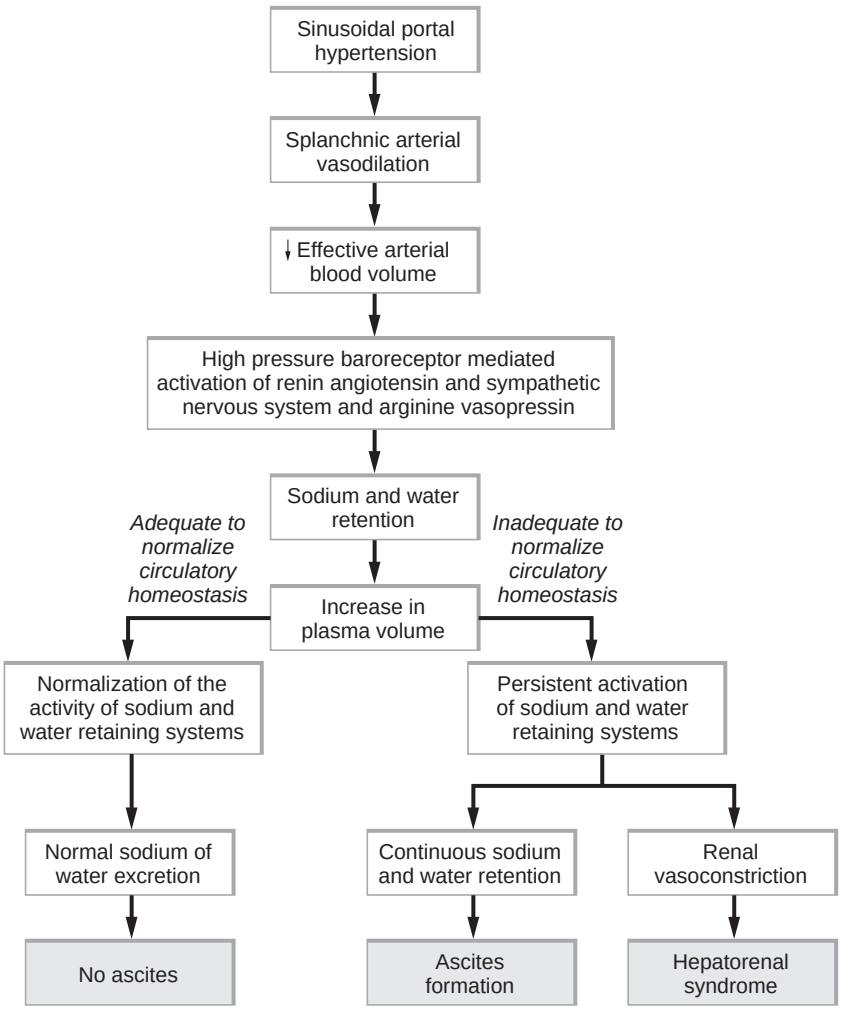

FIGURE 84-10. Pathogenesis of functional renal abnormalities and ascites formation in cirrhosis according to the arterial vasodilation hypothesis.

arterial tree secondary to arterial vasodilation (Fig. 84-10) (190,355,356). According to this theory, portal hypertension is the initial event, with resultant splanchnic arteriolar vasodilation causing underfilling of the arterial circulation. The arterial receptors then sense the arterial underfilling and stimulate the SNS and the RAAS and cause non-osmotic hypersecretion of AVP. Renal sodium and water retention are the final consequence of this compensatory response to a reduction in EABV. In early stages of cirrhosis, when splanchnic arteriolar vasodilation is moderate and the lymphatic system is able to return the increased lymph production to the systemic circulation, the EABV is preserved by transient periods of sodium retention. The fluid retained by the kidneys increases plasma volume and suppresses the signals stimulating the antinatriuretic systems, and sodium retention terminates. Therefore, no ascites or edema is formed at this stage and the relationship between EABV and extracellular fluid volume is maintained. As liver disease progresses, splanchnic arterial vasodilation increases, thereby resulting in a more intense arterial underfilling and more marked sodium and water retention. At this time, the EABV can no longer be maintained by the increased plasma volume, probably because the retained fluid leaks from the splanchnic circulation into the peritoneal cavity as ascites and/or from the systemic circulation to the interstitial tissue as edema. A persistent stimulation of vasoconstrictor systems occurs in an attempt to maintain EABV. The activation of these systems perpetuates renal sodium and water retention, which accumulates as ascites. The correlation between EABV and extracellular fluid volume is no longer maintained, as EABV remains contracted despite progressive expansion of extracellular fluid volume. HRS probably represents the most extreme manifestation of the reduction in EABV. Studies in experimental models of portal hypertension aimed at carefully investigating the chronologic relationship between abnormalities in the systemic circulation and sodium retention indicate that arterial vasodilation with reduced systemic vascular resistance precedes sodium retention and subsequent plasma volume expansion (357,358).

The arterial vasodilation theory not only provides a reasonable explanation for the circulatory changes and activation of antinatriuretic systems observed in cirrhosis with ascites, but also for the preferential location of retained fluid in the peritoneal cavity. The existence of splanchnic arterial vasodilation causes a "forward" increase in splanchnic capillary pressure that enhances the effects of portal hypertension on the filtration coefficient in splanchnic capillaries, which facilitates the formation of ascites (160,162,163).

MANAGEMENT OF ASCITES AND FUNCTIONAL RENAL ABNORMALITIES IN CIRRHOSIS

In this section, the different methods used in the management of ascites and renal dysfunction in cirrhosis are discussed. A practical approach to the management of ascites in clinical practice is summarized in Tables 84-7 through 84-9. For practical

TABLE 84-7

THERAPEUTIC APPROACH TO MANAGEMENT OF PATIENTS WITH CIRRHOSIS AND GRADE 2 MODERATE ASCITES

Initial therapy

Start with low-sodium diet (~80 mEq/day) and spironolactone (50–200 mg/day as a single dose). Monitor body weight daily and urine sodium weekly. Ideal weight loss should be 300–500 g/day in patients without peripheral edema and 800–1,000 g/day in patients with peripheral edema. Outpatients should be instructed to reduce the diuretic dosage in case of greater weight loss.

Low doses of loop diuretics (furosemide, 20–40 mg/day) may be used in combination with spironolactone to increase the natriuretic effect. Patients should be monitored closely to prevent excessive diuresis.

If no response is seen, check compliance with treatment and low-sodium diet. Increase the dose of diuretics stepwise every 7–10 days up to 400 mg/day of spironolactone and 160 mg/day of furosemide.

Maintenance therapy

Maintain sodium restriction and reduce the diuretic dosage approximately in half.

If ascites or edema does not recur, increase sodium intake progressively and maintain a low dose of diuretics.

purposes ascites has been graded by the International Ascites Club recommendations into three groups: in grade 1 ascites fluid is detected only by ultrasound; in grade 2, ascites is moderate with symmetrical distention of the abdomen; and in grade 3 ascites is large or tense with marked abdominal distention (359).

TABLE 84-8

THERAPEUTIC APPROACH TO MANAGEMENT OF PATIENTS WITH CIRRHOSIS AND GRADE 3 LARGE ASCITES

Initial therapy

Large-volume paracentesis plus intravenous albumin (8 g/L of ascites removed).

Maintenance therapy

Low-sodium diet (~80 mEq/day) associated with diuretic therapy.

If the patient was not taking diuretics before the development of severe ascites, start with spironolactone (100–200 mg/day as a single dose) with or without loop diuretics (furosemide, 40 mg/day) and then adjust the dose to maintain the patient with mild or no ascites or edema. Check body weight daily and urine sodium weekly. Closely monitor the patient during the first weeks of therapy.

If the patient was taking diuretics before the development of severe ascites, start with a dose slightly higher than the dose taken before paracentesis.

If ascites or edema increases, check compliance with treatment and the low-sodium diet. Increase the dose of diuretics stepwise every 7–10 days up to 400 mg/day of spironolactone and 160 mg/day of furosemide. Patients should be asked to reduce their physical activity.

If ascites or edema does not recur, a balance should be maintained between sodium intake and diuretic therapy.

TABLE 84-9

THERAPEUTIC APPROACH TO MANAGEMENT OF PATIENTS WITH CIRRHOSIS AND REFRACTORY ASCITES

Initial therapy

Total paracentesis plus intravenous albumin (6–8 g/L of ascites removed)

Maintenance therapy

Maintain a low-sodium diet (70–90 mmol/day) constantly.

In patients taking the highest doses of diuretics, check urinary sodium. If less than 30 mEq/day, stop diuretic therapy.

Total paracentesis plus intravenous albumin when necessary (approximately every 2–3 weeks).

Consider use of TIPS in patients with preserved hepatic function, no hepatic encephalopathy, either with loculated fluid, or unwilling to have repeated paracentesis.

TIPS, transjugular intrahepatic portosystemic shunts.

Sodium Restriction

In all diseases associated with generalized edema (cirrhosis, heart failure, renal failure), the amount of exogenous fluid retained depends on the balance between sodium intake and the renal excretion of sodium. Because sodium is retained isosmotically in the kidney, 1 L of extracellular fluid is gained for every 130 to 140 mEq of sodium retained. If sodium excretion remains constant, the gain of extracellular fluid volume (and the consequent increase in weight) depends exclusively on sodium intake and increases proportionally to the amount of sodium taken with the diet. Nevertheless, because sodium excretion may be increased pharmacologically by the administration of diuretics, the sodium balance depends not only on sodium intake but also on the natriuretic response achieved by diuretics.

With this background, it seems reasonable that a reduction in sodium intake (70 to 90 mmol/day (approximately 1.5 to 2 g/day of salt) will favor a negative sodium balance and facilitate the disappearance of ascites and edema. Although medical therapy is not recommended for grade 1, it is advisable that these patients refrain from excessive salt intake, which can lead to fluid retention. This contention was demonstrated in earlier studies (14,16) and is supported by the common clinical observation that the management of ascites is more difficult in patients with no compliance to the low-sodium diet compared with compliant patients. Noncompliant patients usually require higher doses of diuretics to achieve resolution of ascites and are more often readmitted to the hospital for recurrence of ascites. Surprisingly, however, several randomized comparative studies have not demonstrated an advantage of low-sodium diet as compared with an unrestricted sodium diet in the management of ascites (26,360,361). Nevertheless, it should be pointed out that in these studies most patients had mild sodium retention (urine sodium in the absence of diuretic therapy was close to sodium intake) and showed an excellent response to diuretic therapy (only less than 5% of patients did not respond to diuretics).

Therefore, on the basis of available data, it can be concluded that in patients with mild sodium retention a restriction of dietary sodium is probably not necessary because the hypothetical benefit of low-salt diet in the achievement of a negative sodium balance is overridden by the marked natriuretic effect of diuretics. By contrast, in patients with marked

sodium retention, who usually have a less intense natriuretic response to diuretics compared with patients with moderate sodium retention, dietary sodium restriction may facilitate the elimination of ascites and delay the reaccumulation of fluid after ascites has been removed. A more severe restriction of sodium (less than 50 mEq/day) is not recommended because it is not well accepted by patients and may impair their nutritional status.

Diuretics

Diuretics eliminate the excess of extracellular fluid present as ascites and edema by increasing renal sodium excretion, thereby achieving a negative sodium balance (40,362). Diuretics most often administered to patients with cirrhosis and ascites are aldosterone antagonists, mainly spironolactone and amiloride, that selectively antagonize the sodium-retaining effects of aldosterone in the renal collecting tubules, and loop diuretics, especially furosemide, that inhibit the Na^+–K^+–$2Cl^-$ cotransporter in the loop of Henle (40,362).

Despite the use of diuretics in clinical practice for more than 30 years, only a few randomized studies have been reported that compare the efficacy of different diuretic agents in the treatment of ascites (38,40,362). In patients without renal failure, aldosterone antagonists are more effective than loop diuretics (38). This higher efficacy of aldosterone antagonists has also been demonstrated in several prospective, yet not comparative, investigations (26,37,218,219). Based on these findings, aldosterone antagonists are considered the diuretics of choice in the management of cirrhotic ascites (359,363,364).

Diuretics are indicated for all those patients with grade 2 ascites (Table 84-7). In clinical practice, aldosterone antagonists are often given in combination with loop diuretics. Theoretical advantages of this combination include greater natriuretic potency, earlier onset of diuresis, and less tendency to induce hyperkalemia. Two different schedules of combined administration have been proposed. First, the dose of spironolactone is increased progressively (start with 50 to 100 mg per day usually up to 400 mg per day) and loop diuretics (furosemide start with 20 to 40 mg up to 160 mg per day) are added only if no response is achieved with the highest dose of aldosterone antagonists. Second, both drugs are given in combination from the start of therapy (start with ratio of 40 mg of furosemide and 100 mg spironolactone per day up to 160 mg/day of furosemide and spironolactone up to 400 mg/day).

Diuretic therapy is effective in the elimination of ascites in 80% to 90% of the whole population of patients with ascites, a percentage that may increase up to 95% when only patients without renal failure are considered (26,37–40,218,363–369). About 10% of patients with ascites are refractory to treatment with diuretics because either do not respond to diuretic therapy or develop diuretic-induced complications that prevent the use of high doses of these drugs. This condition is known as refractory ascites (108). Diagnostic criteria of refractory ascites are shown in Table 84-10. Complications of diuretic therapy in patients with cirrhosis include hepatic encephalopathy, hyponatremia, renal impairment, potassium disturbances, gynecomastia, and muscle cramps (40,362,370). The incidence of renal and electrolyte disorders and encephalopathy varies depending on the population of patients studied, being higher in patients with marked sodium retention and renal failure (who require higher doses of diuretics) and lower in patients with moderate sodium retention and without renal failure. Although some of these complications may be unrelated to diuretic therapy and due to the existence of an advanced liver disease (371), no doubt diuretics play a major pathogenic role in these complications because their frequency is markedly lower if ascites is removed by large-volume paracentesis (365–369).

Large-volume paracentesis with albumin infusion has substituted diuretics as the initial treatment of choice for hospitalized cirrhotic patients with grade 3 ascites in most centers (372,373). Furthermore, most authors consider large-volume paracentesis as the initial therapy for patients with grade 3 ascites (359,364). On this basis, current indications for use of diuretics in cirrhosis include: (i) treatment of patients with mild or moderate ascites or those with large ascites in whom paracentesis is not effective because of compartmentalization of ascitic fluid due to peritoneal adhesions; (ii) treatment of patients with edema without ascites; and (iii) prevention of ascites recurrence after therapeutic paracentesis.

Large-Volume Paracentesis

As stated previously, large-volume paracentesis has progressively replaced diuretics as the treatment of choice in the management of patients with cirrhosis and grade 3 ascites (359,364). This change in treatment strategy is based on the results of several randomized studies comparing paracentesis (either removal of all ascitic fluid in a single tap or repeated taps of 4 to 6 L per day) associated with plasma volume expansion versus diuretics (365–369). Because paracentesis does not modify renal sodium retention, patients should receive diuretics after paracentesis to avoid reformation of ascites (372).

TABLE 84-10

DEFINITION AND DIAGNOSTIC CRITERIA OF REFRACTORY ASCITES

Diuretic-resistant ascites	Ascites that cannot be mobilized or the early recurrence of which cannot be prevented because of a lack of response to sodium restriction and diuretic treatment.
Diuretic-intractable ascites	Ascites that cannot be mobilized or the early recurrence of which cannot be prevented because of the development of diuretic-induced complications that preclude the use of an effective diuretic dosage.
Requisites	Treatment duration: Patients must be on intensive diuretic therapy (spironolactone 400 mg/day and furosemide 160 mg/day) for at least 1 week and on a salt-restricted diet of less than 80 mmol/day.
	Lack of response: Mean weight loss of <0.8 kg over 4 days and urinary sodium output less than the sodium intake.
	Early ascites recurrence: Reappearance of grade 2 or 3 ascites within 4 weeks of initial mobilization.
	Diuretic-induced complications: Diuretic-induced hepatic encephalopathy is the development of encephalopathy in the absence of any other precipitating factor. Diuretic-induced renal impairment is an increase of serum creatinine by >100% to a value >2 mg/dL in patients with ascites responding to treatment.

Two aspects concerning the use of large-volume paracentesis in patients with cirrhosis and ascites deserve specific discussion: (i) the population of patients with cirrhosis in whom large-volume paracentesis should be used, and (ii) the use of plasma expanders to prevent disturbances in circulatory function after large-volume paracentesis. Although most physicians consider that therapeutic paracentesis is the treatment of choice for all patients with grade 3 ascites (359,364,372,373), others believe that large-volume paracentesis should be used only in patients with refractory ascites (375). Results obtained in randomized, comparative studies indicate that large-volume paracentesis is faster and associated with lower incidence of side effects compared with diuretics (365–369). Moreover, large-volume paracentesis has a better cost-effectiveness profile compared with diuretic treatment, which requires prolonged hospital stays. Therefore, on the basis of available data, it seems clear that the use of therapeutic paracentesis should not be restricted to patients failing to respond to diuretics and should be considered the treatment of choice for all patients with grade 3 ascites.

The removal of large volumes of ascitic fluid is associated with a circulatory dysfunction characterized by a reduction of effective blood volume (88,376–382). Four lines of evidence indicate that this circulatory dysfunction and/or the mechanisms activated to maintain circulatory homeostasis have detrimental effects in cirrhotic patients. First, circulatory dysfunction after large-volume paracentesis is associated with rapid reaccumulation of ascites (382). Second, approximately 20% of these patients develop irreversible renal failure and/or water retention leading to dilutional hyponatremia (88). Third, portal pressure increases in patients developing circulatory dysfunction after paracentesis, probably owing to an increased intrahepatic resistance due to the action of vasoconstrictor systems on the hepatic vascular bed (380). Finally, the development of circulatory dysfunction is associated with a shortened survival (382).

At present, the only effective method to prevent circulatory dysfunction is the administration of plasma expanders. Albumin is more effective than other plasma expanders (dextran-70, polygeline) probably owing to its longer persistence in the intravascular compartment (382). When less than 5 L of ascites are removed, dextran-70, polygeline, or saline show a similar efficacy when compared with albumin. However, albumin is more effective than these artificial plasma expanders when more than 5 L of ascites are removed (382). Despite this greater efficacy, randomized comparative studies have not shown differences in survival of patients treated with albumin compared with those treated with other plasma expanders (382–385). Very large trials would be required to demonstrate that the protective effect of albumin on circulatory function results in a survival benefit. Taken together, the currently available data indicate that circulatory dysfunction after large-volume paracentesis is potentially harmful to patients with cirrhosis and should be prevented. Albumin appears to be the plasma expander of choice when more than 5 L of ascites is removed.

Peritoneovenous Shunt

Peritoneovenous shunting causes the passage of ascitic fluid from the peritoneal cavity to the systemic circulation, which results in the improvement of effective arterial blood volume with subsequent reduction in the activity of vasoconstrictor and antinatriuretic systems (23). These favorable hemodynamic effects result in an increase in sodium excretion and, to a lesser extent RBF and GFR, which facilitate the management of ascites in most patients (386). Comparative studies of peritoneovenous shunting and large-volume paracentesis plus intravenous albumin in patients with refractory ascites have shown no differences in survival, but the probability of readmission to the hospital for ascites as well as the number of readmissions for ascites recurrence are markedly lower in patients treated by peritoneovenous shunting compared with those treated by large-volume paracentesis (24,387). Unfortunately, the use of the shunt is associated with a number of important complications, including coagulopathy, bacterial infections, peritoneal fibrosis, or shunt obstruction, which limit its clinical applicability (386,387). Obstruction is the most common complication and may be located either within the valve or due to thrombosis of the superior vena cava. Obstruction is associated with recurrence of ascites and requires surgical intervention with replacement of the clotted shunt (388–390). Although peritoneovenous shunting is an effective therapy for refractory ascites, the high incidence of side effects and the existence of an alternative therapy (e.g., therapeutic paracentesis) have resulted in its abandonment in most centers. It is currently not recommended in the management of cirrhosis with ascites (359,364).

Transjugular Intrahepatic Portosystemic Shunts

The reduction in portal pressure by surgical portosystemic shunts, especially side-to-side portacaval shunts, is associated with increased sodium excretion, suppression of antinatriuretic systems, and elimination of ascites in patients with cirrhosis and refractory ascites (171,172). Nevertheless, despite these favorable effects, surgical portosystemic shunts have not become a standard therapy of refractory ascites or HRS because of high operative mortality and an exceedingly high risk of chronic encephalopathy.

The usefulness of portosystemic shunting in the management of refractory ascites has been reevaluated with the use of the transjugular intrahepatic portosystemic shunts (TIPS), a nonsurgical method of portal decompression that acts as a side-to-side portacaval shunt and has the advantage over surgical shunts of an extremely low operative mortality (391). The most frequent complication of TIPS is hepatic encephalopathy and obstruction of the stent (70% in 1 year). The available information regarding the use of TIPS in patients with refractory ascites derives from several uncontrolled studies and five randomized comparative studies (173,174,392–399). The results of the randomized trials comparing TIPS to repeated paracentesis in patients with refractory ascites show that TIPS is associated with a lower rate of ascites recurrence, but a higher rate of hepatic encephalopathy compared with those of patients treated with paracentesis and albumin. There is also conflicting evidence in regards to survival because some studies have shown survival benefit with TIPS, whereas others have not shown any difference in survival. Two meta-analysis have concluded that there are no significant differences in survival (400,401). In addition, cost of therapy appears to be lower with repeated large-volume paracentesis plus albumin than that of TIPS (397). Therefore, large-volume paracentesis appears to be the treatment of choice because of its wider applicability, lower cost, and fewer side effects compared to TIPS.

Recent studies indicate that TIPS may also be of value in the management of HRS, yet the information available is still limited (235,236,402). TIPS improves renal perfusion and GFR and reduces the activity of vasoconstrictor systems in type 1 HRS (235,236,402). Because the use of TIPS is often associated with significant side effects, particularly hepatic encephalopathy and impairment of liver function, its role in the management of HRS needs to be established by prospective controlled investigations.

Vasoconstrictor Drugs

The administration of vasoconstrictors and plasma expansion are the best pharmacologic therapy for HRS. These drugs have been used in an attempt to improve renal perfusion by increasing systemic vascular resistance and suppressing the activity of endogenous vasoconstrictors. Vasoconstrictors used for HRS include vasopressin analogs (ornipressin and terlipressin), somatostatin analogs (octreotide), and α-adrenergic agonists (midodrine and noradrenaline). In most studies vasoconstrictors have been given in combination with albumin in an attempt to improve the efficacy of treatment. The administration of V1 receptor agonists, such as ornipressin or terlipressin, is associated with suppression of the activity of endogenous vasoconstrictor systems and marked improvement of renal perfusion and GFR and normalization of serum creatinine levels in most patients (188,189,403–405). The most studied vasopressin analog in HRS is terlipressin; ornipressin was abandoned due to severe side effects of ischemia. Use of intravenous terlipressin along with albumin as a plasma expander is associated with a significant improvement of renal function and reduction of serum creatinine to less than 1.5 mg/dL in approximately 50% to 75% of patients treated for at least 5 days (406). Unfortunately, terlipressin is not available in many countries, including the United States, and, therefore, other options such as α-adrenergic agonists are a reasonable alternative given that they are widely available. Midodrine, an α-adrenergic agonist in association with octreotide, a glucagon inhibitor, and albumin has also proved efficacious in HRS (236,407). Noradrenaline, in combination with albumin expansion also improves renal function in HRS (408).

Until more experience is obtained through randomized controlled trials, the recommendations for using vasoconstrictors in HRS are as follows: (i) treat for a maximum of 15 days; (ii) end-point of treatment is reduction of serum creatinine level to less than 1.5 mg/dL; (iii) recommended medications and doses are (a) terlipressin 0.5 to 1 mg intravenously every 4 hours and increase dose every 2 to 3 days up to 2 mg per 4 hours when there is no decrease in creatinine, (b) midodrine 7.5 mg orally three times daily with an increase to 12.5 mg three times daily and octreotide 100 μg subcutaneously three times daily with an increase to 200 μg three times daily if creatinine not decreasing, and (c) noradrelanine 0.5 to 3 mg per hour continuous intravenous infusion (iv) intravenous albumin infusion (20 to 40 g daily); (v) monitor for side effects such as chest pain, distal ischemia, and abdominal pain; and (vi) avoid in patients with cardiac diseases, peripheral vascular disease, and/or cerebrovascular disease, due to the potential risk of ischemic events.

Liver Transplantation

Ascites is one of the most frequent reasons for referral for evaluation of liver transplantation in the United States and Europe. Although randomized studies comparing liver transplantation with conventional medical therapy in patients with ascites are obviously not available, the 70% to 80% 5-year probability of survival obtained in adult cirrhotic patients undergoing liver transplantation in most centers is markedly greater than the expected 20% survival probability for patients with cirrhosis and ascites who do not undergo transplantation (80,81,122–124). Early referral is recommended due to the short survival some patients have once they develop ascites. Although there are no established prognostic models for patients with cirrhosis and ascites, predictive factors related to renal and circulatory function may be useful in identifying candidates for liver transplantation. These factors include dilutional hyponatremia, low arterial blood pressure, serum creatinine >1.2 mg/dL or 106 μmol/ L, and intense sodium retention (urine sodium less than 10 mEq/L).

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CHAPTER 85 ■ THE NEPHROTIC SYNDROME

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In contrast to heart failure and cirrhosis of the liver, in which the kidneys are structurally normal, the nephrotic state is characterized by intrinsic renal damage often, but not always, with a substantial decrease in renal function. This parenchymal damage may be responsible for the elevated arterial blood pressure observed in some patients with the nephrotic syndrome. Other contrasting features among these three classic edematous states are described in Table 85-1, in which nonexcretor edematous patients with cardiac failure, liver disease, and nephrotic syndrome are compared. As shown in Table 85-1, nephrotic patients were characterized by a relatively higher arterial blood pressure, a higher glomerular filtration rate (GFR), and less impairment in sodium and water excretion.

The state of arterial filling and the pathogenesis of the renal sodium and water retention in patients with the nephrotic syndrome remains controversial (1). Two contrasting hypotheses have been proposed to explain the mechanism of the abnormal sodium and water reabsorption observed in nephrotic syndrome. In nephrotic syndrome—usually defined as the combination of a 24-hour urinary protein excretion in excess of 3.5 g/1.73 m^2 of body surface area, hypoalbuminemia, hyperlipidemia, and edema—the underfill theory of edema formation is as follows. Proteinuria results in hypoalbuminemia with decreased oncotic pressure causing fluid loss from the intravascular space and reduced plasma and blood volume with resultant activation of homeostatic responses involving the sympathetic nervous system and the renin–angiotensin–aldosterone axis. This hypovolemia may also contribute to a reduction in GFR.

In contrast to this classic theory of edema formation in nephrotic syndrome, it has also been reported (2–4) that many patients with the nephrotic syndrome who retain sodium and water show elevated plasma volume and arterial blood pressure, whereas plasma renin activity (PRA) and plasma aldosterone concentration are not elevated or are even decreased. In these patients, the overfill theory of edema formation predominates, and the abnormal sodium and water retention is related to an intrinsic renal abnormality associated with the renal disease. Of interest, it was originally thought that this hypervolemic state was a characteristic of patients with nephrotic syndrome and abnormal renal histology (5,6), but it has been proposed that this hypervolemic state may also occur in patients with minimal change nephrotic syndrome (2–4,7–9). It is important to note, however, that the response to upright posture may be necessary to detect differences in plasma volume (see the following).

In the remainder of this chapter, we describe the potential afferent and efferent mechanisms of sodium and water reabsorption in the nephrotic syndrome. The arguments in favor of the underfill and overfill theories of edema formation in nephrotic syndrome are reviewed. In fact, both the underfill and overfill theories may be implicated, depending on the subset of nephrotic syndrome patients studied (i.e., hypovolemic versus hypervolemic). In the hypovolemic subset, as in arterial underfilling with heart failure and cirrhosis, activation of neurohormonal vasoconstrictor systems may in part mediate the renal sodium and water retention, whereas in the hypervolemic subset, intrarenal mechanisms also may be implicated. Finally, specific therapeutic modalities are discussed.

Afferent Mechanisms for Renal Sodium and Water Retention in the Nephrotic Syndrome

The Underfill Theory

The evidence supporting the traditional underfill view of edema formation in nephrotic syndrome includes the following factors. Plasma volume may be decreased in some nephrotic patients in the absence of diuretic therapy (8–12). Moreover, systemic arterial hypotension and diminished cardiac output, sometimes correctable by plasma volume expansion, have been observed in some patients with nephrotic syndrome (13–15). In addition, some nephrotic patients have "humoral markers" of arterial underfilling such as elevated plasma levels of renin, aldosterone, and catecholamines, suggesting a volume-contracted state (9,16,17). Finally, head-out water immersion (HWI) and intravascular infusion of albumin, maneuvers that increase plasma volume, may result in substantial increases in GFR and fractional excretion of sodium chloride and water in these patients (8,12). Of interest, the natriuresis observed during HWI was found to be directly related to the patient's estimated plasma volume (18).

Usberti et al. (19) described two groups of nephrotic syndrome patients distinguished on the basis of their plasma albumin concentrations. Patients in group 1 had a plasma albumin concentration of less than 1.7 g/dL associated with low blood volumes and atrial natriuretic plasma (ANP) levels, elevated plasma angiotensin II (AT-II) concentrations, and increased proximal tubular reabsorption of sodium (determined by lithium clearance). In contrast, group 2 patients with a plasma albumin concentration greater than 1.7 g/dL exhibited normal blood volumes and plasma hormone concentrations. In all patients blood volume was positively correlated with the plasma albumin concentration, and PRA was inversely correlated with both blood volume and plasma albumin concentration. Of note, GFR was not different between group 1 and group 2 patients (100 ± 25 versus 101 ± 22 mL/minute, not significant), whereas urinary sodium excretion was substantially lower in group 1 patients (4.88 ± 5.53 versus 29.9 ± 9.3 mEq/4 hours, $p < 0.001$). Moreover, the acute expansion of blood volume in group 1 patients normalized PRA, plasma AT-II and aldosterone concentrations, fractional sodium excretion, and lithium clearance, while increasing circulating ANP concentrations. Taken together, all of these observations (8–19) support the traditional underfill view of the pathogenesis

TABLE 85-1

DEMOGRAPHICS OF EDEMATOUS DISORDERS

	Cardiac failure patients ($n = 17$)	Cirrhotic patients ($n = 19$)	Nephrotic patients ($n = 16$)
Mean arterial blood pressure (mm Hg)	85.8 ± 2.7	86.3 ± 1.9	97.0 ± 3.0
Glomerular filtration rate (mL/minute/1.73 m^2)	55.4 ± 4.9	70.0 ± 7.3	72.6 ± 7.0
Percentage of water load excreted	31.4 ± 3.2	29.0 ± 3.4	43.3 ± 6.0
Urinary sodium excretion (mEq/5 hr)	5.5 ± 1.4	2.5 ± 0.7	13.5 ± 3.4
Plasma sodium (mEq/L)	133.5 ± 1.0	132.6 ± 1.4	139.8 ± 1.2
Minimal urinary osmolality during the water load (mOsmol/kg H_2O)	371 ± 36	243 ± 37	205 ± 45

Patients were usually given a 50-mEq sodium diet for 3 days to 1 week prior to the water-load procedure. Cardiac patients received a water load of 15 mL/kg of body weight; cirrhotic and nephrotic patients received a water load of 20 mL/kg of body weight. Most of the cirrhotic patients and all the nephrotic patients were *ad libitum* water intake; cardiac failure patients were limited to 800 mL of solute-free water intake per 24 hours.
(Modified from: Bichet DG, van Putten VJ, Schrier RW. Potential role of increased sympathetic activity in impaired sodium and water excretion in cirrhosis. *N Engl J Med* 1982;307:1552; Bichet D, Manzini C. Role of vasopressin (AVP) in the abnormal water excretion in nephrotic patients. *Kidney Int* 1984;25:160A, with permission.)

of edema formation in some patients with nephrotic syndrome.

The Overfill Theory

Dorhout-Mees et al. (2–4,7) and Meltzer et al. (6) challenged this traditional theory. Dorhout-Mees et al. (7) initially studied a group of 10 adult patients with 13 episodes of minimal change nephrotic syndrome. The patients were selected for the study because of increased blood volume and blood pressure. Each patient was studied prior to and following prednisone-induced remission. After remission, blood pressure fell in 12 cases, plasma volume fell in 10 cases, and PRA increased in eight cases. If total plasma volume and PRA are reliable indices of arterial circulatory integrity, these data constitute powerful arguments for the overfill theory of edema formation in some patients with nephrotic syndrome. In contrast to this study, in which all patients with increased blood volume had minimal lesion nephrotic syndrome, Meltzer et al. (6) found that their hypervolemic patients tended to have more severe glomerular involvement, lower GFR, and hypertension.

The potential problems associated with plasma volume measurements in nephrotic syndrome patients have been studied extensively (2). Most investigators now normalize their plasma and blood volume values for lean body mass, and the dry weight of the patients is measured by diuresing them to an edema-free state shortly after plasma volume and blood volume are measured (2–7,20). No correction is needed for an increased albumin disappearance rate from the vascular tree (21). In addition to these measurements, hypertension and low PRA, two indices suggesting volume expansion, have been frequently observed in patients with nephrotic syndrome (6,7,17). As described, hypoalbuminemia in animal studies as well as in patients with analbuminemia does not consistently lead to edema formation (22). Finally, it is important to note that a low filtration fraction is often observed in nephrotic patients, either with minimal lesions or with histologically defined glomerular lesions (3). This is of interest because arterial underfilling usually leads to renal hypoperfusion with a relatively well-maintained GFR and thus to an increase in filtration fraction. Taken together, these observations are compatible with the overfill view of edema formation in some patients with nephrotic syndrome.

Some explanations appear tenable in trying to reconcile the underfill and overfill theories of nephrotic edema formation (4). The method of plasma volume measurement must be considered. An analysis of 217 nephrotic patients from 10 studies found that only 25% had expanded plasma volumes, whereas 33% had reduced plasma volumes and 42% had normal plasma volumes (2). The variability of current techniques for plasma volume measurement is ±10%; therefore, plasma volume measurement may not be sufficiently sensitive to distinguish between underfill and overfill states. Moreover, it is important to recognize that the afferent stimulus for edema formation is likely to be a dynamic process (23). Most plasma and blood volume studies have been performed during the maintenance phase of edema after the patient has retained significant sodium and water and reached a new steady state of sodium balance. Therefore, these normal values do not preclude a role for a contracted plasma or blood volume in the initiating phase of edema formation. As early as 1963, Eisenberg (24) compared plasma volume measured during the recumbent and standing positions in nephrotic patients and normal individuals. In the standing position, there was a 7% fall in plasma volume in control subjects and a 15% to 20% decrease in nephrotic patients, thereby demonstrating a dynamic process even in the maintenance phase of edema formation. Results of studies indeed favor the dynamic nature of edema formation in the nephrotic syndrome. Serial measurements of plasma volume, PRA, and plasma aldosterone were made during the edema-forming stage, the stage of diuresis, and the stage of remission in 11 patients with steroid-responsive minimal change nephrotic syndrome (9). Of interest, plasma volume was decreased significantly at the edema-forming stage and increased during the stage of diuresis. In addition, an inverse correlation was obtained between plasma volume and PRA. It is therefore possible that the renin–angiotensin–aldosterone system may be a compensatory mechanism for hypovolemia in the edema-forming stage but has less importance in the chronic compensated stage of edema.

In summary, it is clear that the precise afferent stimulus of edema formation in nephrotic syndrome cannot be stated with certainty. However, it seems that edema in these patients cannot always be explained entirely by intravascular hypovolemia. Furthermore, a "nephritic" or intrarenal mechanism may be important in sodium retention not only in nephrotic syndrome with histologic glomerular lesions but also in minimal change nephrotic syndrome (3,4). The nephrotic patients with arterial underfilling are more likely to have very low plasma oncotic pressure secondary to severe hypoalbuminuria <2.0 g/dL). In Table 85-2 are shown factors that may give guidance as to whether a patient has overfill or underfill edema.

TABLE 85-2

FACTORS THAT MAY GIVE GUIDANCE AS TO WHETHER IN AN INDIVIDUAL PATIENT WITH THE NEPHROTIC SYNDROME HAS OVERFILL OR UNDERFILL EDEMA

	Overfill	Underfill
GFR <50% of normal	+	−
GFR >75% of normal	−	+
Serum albumin >2 g/dL	+	−
Serum albumin <2 g/dL	−	+
Histology minimal change	−	+
Hypertension	+	−
Postural hypotension	−	+

GFR, glomerular filtration rate.
(Reprinted from: Schrier RW, Fassett RG. A critique of the overfill hypothesis of sodium and water retention in the nephrotic syndrome. *Kidney Int* 1998;53:1111, with permission.)

Efferent Mechanisms for the Renal Sodium and Water Retention of the Nephrotic Syndrome

Nephron Sites of Sodium and Water Retention

Micropuncture Studies. The nephron site of enhanced renal sodium and water retention in the nephrotic syndrome has been studied predominantly in animal models of glomerulonephritis. Bernard et al. (25) used micropuncture and clearance methodology to study the site of sodium retention in saline-loaded rats with autologous immune complex nephritis. These rats developed heavy proteinuria, hypoalbuminemia, and hypercholesterolemia. Histopathologic examination of kidneys from these animals revealed slight thickening of the basement membranes; uniform finely granular deposits of immunoglobulin, IgG, and complement distributed along the basement membranes of all glomeruli; and electron-dense subepithelial deposits. These findings are similar to those observed in human idiopathic membranous nephropathy. Arterial blood pressure, hematocrit, GFR, and renal plasma flow were comparable in control and experimental animals. Proximal sodium reabsorption was actually decreased in the nephrotic rats (35% versus 44%). Absolute sodium reabsorption along the loop of Henle and in the distal convoluted tubule was comparable in nephrotic and control animals. Despite comparable or greater sites of sodium delivery beyond the late distal convoluted tubule, the fractional excretion of sodium was significantly lower in nephrotic (2.2%) than control (4.0%) animals. From these results, the authors concluded that nephron sites beyond the late distal convoluted tubule were responsible for the enhanced sodium reabsorption observed in this model of nephrotic syndrome. In this regard, Sonnenberg suggested that the collecting duct may be the nephron site concerned with final adjustments in renal sodium excretion (26). Alternatively, it remains possible that enhanced sodium reabsorption by deep nephrons (not accessible to micropuncture) also could contribute to the diminished sodium excretion. It should also be noted that in the experiments of Bernard et al. (25) the rats studied were markedly volume expanded; their conclusions thus may not clarify the mechanism of sodium retention in the hydropenic state.

Results different from those reported by Bernard et al. (25) were reported by Kuroda et al., which used a rat nephrotoxic serum model of nephrotic syndrome (27). In this model, proteinuria, hypoalbuminemia, and hypercholesterolemia also occurred. Histologic examination of the kidneys revealed mild glomerular hypercellularity, widely dilated proximal tubules, diffuse uniform glomerular linear immunofluorescence, and electron-dense subepithelial deposits. In contrast to the previous study (25), the animals were more actively retaining sodium. In micropuncture studies, single-nephron GFR was decreased, and the percentage of filtered water reabsorbed prior to late proximal and distal tubular convolutions was increased in the nephrotic rats. In addition, high proximal intratubular pressures were measured. From this study (27) and the study of Bernard et al. (25), it is clear that the nephron site of enhanced sodium reabsorption in nephrotic syndrome may vary, depending on the nature of the renal lesion and the stage of edema formation.

Ichikawa et al. (28) studied a unilateral version of the model of aminonucleoside-induced nephrosis in the rat and showed that proteinuria and diminished urinary sodium excretion were confined to the kidney treated with the puromycin aminonucleoside. A reduction of the glomerular capillary ultrafiltration coefficient was the major determinant of diminished single-nephron GFR in the superficial nephrons studied. In addition, the absolute rate of reabsorption of sodium in the superficial proximal tubule was decreased on the affected side. Assuming that superficial nephrons are representative of the entire nephron population, it was concluded that renal sodium retention in this model of nephrotic syndrome was mainly the result of intrarenal factor(s) acting beyond the proximal convoluted tubule. Similar results were obtained when kidneys were taken from nephrotic rats (puromycin aminonucleoside) and studied over a range of perfusion pressures using the isolated perfused kidney technique (29). When perfused with medium containing 6.7 g of albumin per deciliter, the nephrotic kidneys performed differently from control kidneys, with a reduction of sodium excretion at all pressures. Renal vascular resistance, inulin clearance, fractional sodium excretion, and fractional lithium excretion were also reduced. When kidneys were perfused without oncotic agents, the differences between nephrotic and control kidneys remained. In addition, captopril had no effect on the sodium retention or vascular resistance of nephrotic kidneys (29). Finally, the exact site within the distal nephron where increased sodium reabsorption takes place in nephrotic rats is not known, as puromycin-injected rats with a structurally altered inner renal medulla induced by bromoethylamine hydrobromide still demonstrated impaired sodium excretion (30).

Clearance Studies. Clearance studies have been undertaken in nephrotic patients in an attempt to clarify the nephron site of enhanced sodium and water reabsorption (4,31,32). These studies were undertaken in patients with a wide variety of primary renal diseases and GFRs. In 1979, Usberti et al. (33) measured tubular reabsorption of glucose in 21 patients with glomerulonephritis. Tubular glucose reabsorption was used as a marker of proximal tubular sodium reabsorption. The threshold for glucose reabsorption was reduced in the 10 nephrotic patients with edema, suggesting diminished proximal tubular reabsorption. In studies undertaken in five nephrotic patients, a similar conclusion was reached by Grausz et al. (31). In these clearance studies, blockade of sodium reabsorption in the distal nephron with ethacrynic acid and chlorothiazide was used to assess proximal sodium reabsorption. Proximal sodium reabsorption was lower in the nephrotic patients than in normal and in cirrhotic patients. Conversely, the more recent study of Usberti et al. (19), using the more precise technique of lithium clearance, demonstrated increased proximal tubular sodium reabsorption in nephrotic syndrome patients with low serum albumin concentrations and blood volumes and elevated PRA.

Gur et al. (32) used maximal solute-free water clearance as a marker of distal sodium chloride reabsorption in pediatric patients. Their data are inconclusive, however, because secretion

of arginine vasopressin (AVP) was not maximally suppressed during their experiments. More recently, Koomans et al. (4) also performed clearance studies in 10 patients with nephrotic syndrome before and after a single infusion of 75 g of albumin. Proximal fractional sodium chloride reabsorption was elevated before (95 ± 1.5%) and decreased after (93 ± 1.7%) the infusion. Distal fractional sodium chloride reabsorption was also elevated before (93 ± 6.4%) but was unaltered after (93 ± 5.6%) the albumin infusion.

In summary, it appears from experimental and clinical studies that distal nephron sites are involved in avid sodium and water retention in the nephrotic state. However, it is likely that increased proximal tubular sodium reabsorption also may be operative in selected cases, depending on the nature of the underlying renal disease and the phase of sodium retention. In some instances distal sodium reabsorption may be secondary to an intrinsic defect independent of intravascular hypovolemia, as suggested by the results from the unilateral aminonucleoside model. Nevertheless, in some nephrotic syndrome patients the distal, and possibly proximal, sodium and water reabsorption may result from neurohormonal activation, that is, the renin–angiotensin–aldosterone system.

Mechanisms of Enhanced Tubular Sodium and Water Reabsorption. Several studies have been undertaken to identify the mechanism underlying enhanced renal tubular sodium and water reabsorption in the nephrotic syndrome. Although a reduced GFR is frequently observed in nephrotic patients, many nephrotic patients with a normal GFR avidly retain sodium and water. Therefore, factors in addition to a reduced filtered load of sodium are involved in most patients. However, the presence of a marked reduction in GFR often is associated with the most profound sodium retention in patients with nephrotic syndrome (6,34).

Peritubular physical forces (oncotic and hydrostatic pressures) are believed to exert a modulating influence on renal sodium and water reabsorption in the proximal tubule. Conversely, the high renal plasma flow, normal renal vascular resistance, and diminished filtration fraction frequently observed in patients with nephrotic syndrome (3) suggest that factors other than peritubular physical forces are responsible for enhanced tubular sodium reabsorption. Several lines of evidence indicate a role for neurohormonal activation.

Vasoconstrictor Systems

The Sympathetic Nervous System in the Nephrotic Syndrome. Many patients with nephrotic syndrome exhibit elevated peripheral venous plasma norepinephrine concentrations (17), a finding consistent with activation of the sympathetic nervous system and arterial underfilling in these patients. Confirmatory evidence of adrenergic activation and arterial underfilling in nephrotic syndrome patients may be found in a report from our laboratory (35). We assessed sympathetic nervous system activity by determining plasma norepinephrine spillover and clearance rates using the whole body steady-state radionuclide tracer method in six edematous patients with the nephrotic syndrome and in six normal control subjects in the supine position. Mean creatinine clearances and serum creatinine concentrations were normal in both nephrotic syndrome patients and control subjects. However, the nephrotic syndrome patients exhibited significant hypoalbuminemia (2.0 ± 0.4 versus 3.8 ± 0.1 g/dL, $p < 0.01$). The supine plasma norepinephrine level was elevated in the patients with the nephrotic syndrome as compared with the control subjects; in nephrotic syndrome patients, the spillover rate of norepinephrine was markedly increased (0.30 ± 0.07 versus 0.13 ± 0.02 μg/minute per m^2, $p < 0.05$), whereas the norepinephrine clearance rate was comparable to that in the normal subjects (2.60 ± 0.29 versus 2.26 ± 0.27 L/minute, not significant). Of note, PRA and plasma aldosterone, AVP, and ANP concentrations were not different in the nephrotic syndrome patients compared with control subjects. These findings indicate that the sympathetic nervous system is activated in patients with the nephrotic syndrome prior to a significant fall in GFR or a marked activation of either the renin–angiotensin–aldosterone system or the non-osmotic release of AVP. Furthermore, these data support the presence of arterial underfilling in these patients with the nephrotic syndrome. Although the role of the adrenergic nervous system in the sodium and water retention of nephrosis remains to be defined, the observation that renal denervation ameliorates sodium and water retention in experimental nephrotic syndrome supports the potential influence of renal nerves on proximal tubular sodium reabsorption in the nephrotic syndrome (36) (see the following).

The Renin–Angiotensin–Aldosterone System in the Nephrotic Syndrome. A role for the renin–angiotensin–aldosterone humoral axis in the pathogenesis of nephrotic sodium retention remains controversial. Early work in this area strongly suggested a pathogenetic role for aldosterone in nephrotic edema (37,38). In rats made nephrotic with aminonucleoside, Tobian et al. (38) found an increase in juxtaglomerular cell granularity during sodium retention. Moreover, Kalant et al. (37) found that adrenalectomy prevented the sodium retention of aminonucleoside nephrosis. However, marked saline loading of these adrenalectomized nephrotic animals did result in edema formation (37). In addition, as described earlier in this chapter, sodium retention in aminonucleoside-induced nephrotic syndrome in the rat can occur secondary to intrarenal factors (28). Taken together, these experimental observations suggest a pathogenetic role for aldosterone as well as aldosterone-independent factors in experimental nephrotic edema.

Numerous studies have measured components of the renin–angiotensin–aldosterone system in nephrotic humans (5,6,7,9,23). These studies were undertaken in heterogeneous patient populations at a variety of stages of nephrotic syndrome, and a wide range of plasma hormone values varying from very high to very low were observed. Of interest, in 70 observations made in patients with nephrotic syndrome on a low-sodium diet there was a striking absence of correlation between the plasma albumin level and PRA or between blood volume and PRA. However, there was a significant negative correlation between plasma aldosterone concentration and sodium excretion ($r = 0.52$, $p < 0.001$) (3). In the aforementioned study from Usberti et al. (12), however, PRA and plasma aldosterone concentrations were correlated with plasma albumin concentrations, blood volume, and urinary sodium excretion in the hypovolemic subjects, whereas changes in the urinary excretion of aldosterone predicted the renal response in the nephrotic syndrome patients with normal blood volumes. The determinant of the stimulation of PRA in nephrotic patients is unclear. Similar conclusions were also obtained by other investigators; despite similar plasma volumes, PRA and plasma aldosterone were found to be significantly greater in nephrotic patients with minimal change disease than those in patients with other renal histopathology (16). It is therefore possible that a specific intrarenal factor independent of circulating blood volume, may stimulate the renin–angiotensin–aldosterone system in some nephrotic patients.

Brown et al. (39,40) and others (41) questioned the importance of aldosterone in the sodium retention of the nephrotic syndrome. This assertion is supported by studies involving the administration to patients with the nephrotic syndrome of either the angiotensin receptor antagonist saralasin or the angiotensin-converting enzyme (ACE) inhibitor captopril. In the nephrotic syndrome patients studied by Brown et al. (39,40), plasma aldosterone concentrations were pharmacologically suppressed by means of the ACE inhibitor, with no

associated increase in urinary sodium excretion. However, because blood pressure fell in these patients, the ACE inhibition must have reduced plasma AT-II concentrations, resulting in a decrease in renal perfusion pressure. This effect could have obscured the benefit of a decrease in plasma aldosterone concentration on urinary sodium excretion. A study by Shapiro et al. (42) supported this interpretation because the more specific inhibitor of aldosterone, spironolactone, allowed sodium-retaining patients with the nephrotic syndrome to attain sodium balance.

Arginine Vasopressin in the Nephrotic Syndrome. As described in Table 85-1 and in contrast to the two previously described clinical edematous entities (i.e., heart failure and decompensated cirrhosis), the nephrotic syndrome is less frequently associated with hyponatremia. In fact, serum sodium concentration is usually normal unless it is influenced by vigorous diuretic measures or an acute water load (7,31,43,44). Furthermore, in nephrotic patients, high serum lipid levels may induce pseudohyponatremia unless serum sodium concentration is measured by a direct ion-specific electrode (45). Nevertheless, abnormal water excretion was clearly demonstrated by Gur et al. (32) in six nephrotic children; their solute-free water clearance during water loading was only 0.9 ± 0.8 mL/minute compared with 3.6 ± 0.6 mL/minute after remission of their disease. Because HWI induces an increase in solute-free water clearance in patients with nephrotic syndrome (8,18), this improvement may be secondary to the suppression of the non-osmotic release of AVP. Alternatively, HWI might improve intrarenal hemodynamics and increase the amount of solute-free water delivered to the distal site of the nephron, thereby improving water excretion. Usberti et al. (12) studied 16 nephrotic patients and 13 normal control subjects in basal conditions and following a water load or isosmotic blood volume expansion with a 20% albumin infusion. The nephrotic patients had a delayed water excretion, and their plasma AVP levels were not suppressed by the water load procedure. Of interest, plasma AVP was inversely correlated with blood volume in nephrotic patients but not in normal control subjects. Furthermore, in the nephrotic patients the expansion of blood volume with 20% albumin, or HWI, was effective in reducing plasma levels of AVP and in promoting a water diuresis (12,46). Pedersen et al. (44) administered an oral water load of 20 mL/kg of body weight to 17 patients with nephrotic syndrome and 15 healthy control subjects. Creatinine clearance and the maximum increase in solute-free water clearance were significantly correlated in patients with nephrotic syndrome as well as in control subjects. Plasma AVP was reduced in both groups during water loading, but AVP was clearly elevated in nephrotic syndrome patients when compared with the control subjects both before and during the water load. The study by Shapiro et al. (34) also showed a close correlation between decrements in GFR and water excretion during a water load in nephrotic patients.

Bichet and Manzini also studied 24 consecutive stable adult patients with nephrotic syndrome (43). Of these 24 patients who received a 20 mL/kg water load, 16 displayed abnormal water excretion (nonexcretors, group I), since they excreted 43% ± 6% of the water load in 5 hours and had a minimal urinary osmolality of 205 ± 49 mOsm/kg H_2O. Eight patients (excretors, group II) had normal water excretion as they excreted 97 ± 5% of the water load in 5 hours and had a minimum urinary osmolality of 61 ± 4 mOsm/kg H_2O. Basal plasma sodium concentration and effective plasma osmolality were comparable in groups I and II (139.8 ± 1.2 versus 139.7 ± 0.5 mEq/L and 274 ± 2 versus 273 ± 1.2 mOsm/kg H_2O, respectively). Basal supine, upright, and mean plasma AVP values after the water load were significantly higher in group I than in group II (4.4 ± 1.0 versus 1.4 ± 0.3, $p < 0.01$; 9.3 ± 2.9 versus 1.8 ± 0.5, $p < 0.025$; and 2.9 ± 0.5 versus 1.25 ± 0.2 pg/mL, $p < 0.025$, respectively). Nonexcretor patients also had more urinary protein loss (12.4 versus 6.5 g/24 hours, $p < 0.05$) and a lower GFR (72.6 ± 7 versus 106 ± 5 mL/minute per 1.73 m^2, $p < 0.005$). Group I patients were also characterized by a lower fractional excretion of sodium (0.5 ± 0.1 versus 0.8 ± 0.1, $p < 0.05$) and higher PRA (3.4 versus 1.9 ng/mL per hours, $p < 0.025$), plasma aldosterone (15.5 versus 8.8 ng/dL, $p < 0.005$), plasma free norepinephrine (288 ± 48 versus 181 ± 50 pg/mL, $p < 0.005$), and plasma conjugated norepinephrine levels (2038 ± 564 versus 510 ± 93 pg/mL, $p < 0.02$).

Analysis of these studies, therefore, indicates that the impaired water excretion in nephrotic patients may be related both to intrarenal factors, involving a fall in GFR and diminished distal fluid delivery, and to extrarenal factors, which primarily involve the non-osmotic release of AVP. In this regard, Pyo et al. (47) reported elevated circulating AVP concentrations and increased hypothalamic AVP mRNA levels in rats with puromycin aminonucleoside-induced nephrotic syndrome, thereby supporting the notion that increased hypothalamic AVP biosynthesis and release occur in this model of the nephrotic syndrome.

Aquaporins in Nephrotic Syndrome. In contrast to heart failure and cirrhosis, aquaporin-2 expression appears to be decreased in experimental models of nephrotic syndrome. This occurs despite elevated plasma vasopressin concentrations and clinical edema. In rats with puromycin aminonucleoside-induced nephrotic syndrome, there was an 87% decrease in inner medulla aquaporin-2 expression and a 70% decrease in collecting duct basolateral aquaporin-3 expression as compared to control rats (48). Consistent with the finding of aquaporin-2 and -3 downregulation, rats with nephrotic syndrome also demonstrated decreased maximal urinary osmolality. Similar findings were also noted in an Adriamycin-induced model of nephrotic syndrome (49). Little data exist regarding the role of aquaporin-2 in water metabolism in humans with nephrotic syndrome. The mechanism by which Adriamycin and puromycin aminonucleoside induce nephrotic syndrome is unknown, but several accompanying tubular defects have been demonstrated (49). Therefore, extrapolation of the results of these studies to human nephrotic syndrome must be performed with caution. Studies of urinary aquaporin-2 excretion in humans with nephrotic syndrome are needed.

Vasodilator Systems

Natriuretic Peptides in the Nephrotic Syndrome. Plasma ANP and brain natriuretic peptide (BNP) concentrations may be elevated in animals and humans with the nephrotic syndrome (50,51); however, the hemodynamic and renal responses to exogenous ANP or BNP are blunted in experimental nephrosis (51) and in patients with the nephrotic syndrome (50). Recently, Perico and Remuzzi (52) proposed tubular insensitivity to ANP as an initiating factor in the formation of edema in the nephrotic syndrome. According to their hypothesis, renal unresponsiveness to ANP results in distal tubular sodium and water retention with subsequent edema formation; this renal ANP resistance may be receptor-independent, because urinary cGMP responds appropriately to ANP infusion in nephrotic animals (53). There is, however, experimental evidence of increased phosphodiesterase-mediated increased cyclic GNP degradation as a mediator of ANP resistance in experimental nephrosis (54). Alternatively, this blunted natriuretic response to ANP and BNP may be a secondary phenomenon owing to neurohormonal vasoconstrictor activation and decreased sodium delivery to the collecting duct site of natriuretic peptide action. Our aforementioned observations of sympathetic activation and normal plasma ANP concentrations in edematous

patients with the nephrotic syndrome support this latter hypothesis (35). Moreover, Koepke and DiBona (55) showed that renal denervation reversed the blunted diuretic and natriuretic responses to ANP in a rat model of the nephrotic syndrome. The exact role of the natriuretic peptides in the pathogenesis of the renal sodium and water retention observed in the nephrotic syndrome remains to be defined.

Other humoral factors such as kinins and prostaglandins may modulate renal sodium reabsorption in nephrotic patients. For example, inhibitors of prostaglandin synthesis reduce the GFR in patients with the nephrotic syndrome and may precipitate renal insufficiency (56). The role of these different humoral mechanisms in the sodium retention in nephrotic syndrome patients remains unanswered.

In summary, it appears that the effector mechanisms for sodium and water retention in the nephrotic syndrome may involve a fall in GFR and activation of the sympathetic nervous system, the renin–angiotensin–aldosterone system, and the non-osmotic release of AVP. Other still undefined factors may, however, also be involved in the enhanced renal tubular sodium and water reabsorption observed in nephrotic patients; some results suggest that a diminution in ANP sensitivity in nephrotic syndrome also may be involved in the sodium retention.

Physiologic Basis for Therapy of Sodium and Water Retention in the Nephrotic Syndrome

The treatment modalities in nephrotic patients have been reviewed (57,58). The first principle of therapy of nephrotic syndrome is to treat the primary disease process. Several histologic lesions that produce nephrotic syndrome appear to be favorably influenced by corticosteroid therapy (57). Obviously, if the lesion proves to be steroid responsive, the edematous state will improve and further modalities of treatment may be unnecessary. There is some evidence that nephrotic patients with minimal change disease who have severe hypoalbuminemia and anasarca may not respond to corticosteroid therapy until some diuretic therapy is instituted to diminish the edema. The mechanism for this observation is unclear but may be related to intrarenal edema with associated increased interstitial pressure in the kidney associated with anasarca.

In patients with hypoalbuminemia, it is reasonable to recommend the ingestion of 2 to 3 g of protein per kilogram of body weight provided the GFR is well maintained. However, there is no evidence that dietary protein supplementation consistently increases serum albumin concentration or lessens the edema (59).

When symptomatic edema develops, dietary salt restriction should be instituted. A sodium intake of between 2 and 3 g per day is generally a reasonable compromise between effectiveness and palatability. Water restriction need not be implemented unless the patient is hyponatremic with hyposmolality. Although impaired water excretion often occurs in nephrotic patients, clinically significant hyponatremia occurs primarily in association with concomitant diuretic usage.

Diuretic agents, the mainstay of therapy in edematous states, should be utilized only if symptomatic edema persists despite salt restriction. A few prospective controlled trials compared the efficacy and frequency of adverse effects of different diuretic agents used in nephrotic patients. These studies focused on the use of furosemide, metolazone, and piretanide in the edematous nephrotic patient (60–62). In view of the experimental data demonstrating enhanced renal sodium reabsorption beyond the proximal tubule in nephrotic syndrome, diuretics that act in distal tubular segments would be expected to be most efficacious, that is, spironolactone. Many nephrotic patients with well-maintained GFRs respond to thiazide diuretics alone.

In adult nephrotic patients, furosemide is usually an effective diuretic agent, and one study in children found furosemide to be safe and efficacious in the treatment of nephrotic edema (60). However, some children with low GFRs become resistant to as much as 5 mg/kg of furosemide (60). In this setting, both in children and in adults, the addition of a second distal blocker such as metolazone often produces a diuresis (61); severe hypokalemia, however, may be seen with this combination of diuretics.

The pharmacokinetic patterns of the "loop diuretics" have been evaluated extensively in nephrotic patients (63–65). Although the presence of nephrotic syndrome decreases plasma protein binding of furosemide, it does not appear to induce major changes in furosemide disposition when renal function is well maintained (63–65). The efficacy of high doses of spironolactone, the specific aldosterone antagonist, in nephrotic patients has been demonstrated and correlates with the activation of the renin–angiotensin–aldosterone system. Spironolactone may also be effective as an additive diuretic effect when given with other agents (66).

Diuretic therapy for nephrotic syndrome often can be instituted in the outpatient setting. Patients should be instructed to weigh themselves daily and to diminish or discontinue the diuretic if weight loss exceeds 1 pound per day or when edema no longer becomes a source of discomfort. Patients should also reduce or discontinue the diuretic when orthostatic lightheadedness develops and when only a small amount of edema remains. One practical way to follow these patients' proteinuria is with single voided urine samples to estimate quantitative urinary protein loss; a protein–creatinine ratio of more than 3 to 5 can be taken to represent "nephrotic" proteinuria (67). Hospitalization may be required to initiate and monitor diuresis in patients with either severe edema or marked hypoalbuminemia, especially when a significant decrease in GFR is present.

Albumin infusions have been utilized in the treatment of edema in nephrotic patients since the 1950s (68). This form of therapy increases plasma volume and may increase GFR, urine flow, sodium excretion, and solute-free water clearance (12,41,66,68). Leutscher et al. (68) treated 13 adult nephrotics with 50 g per day of salt-poor albumin (200 mL of 25% albumin solution) for 4 consecutive days. A diuresis was observed in all patients, and five of the 13 patients became edema free. More recently, Davison et al. (66) used albumin in the management of diuretic-resistant nephrotic patients. Twelve nephrotic patients, referred for a diuretic-resistant state, were studied. Diverse glomerular diseases were present in these patients, and nine of 12 patients had a creatinine clearance rate of less than 40 mL/minute. All patients were treated with furosemide in increasing doses to 500 mg per day. Spironolactone with increasing doses to 200 mg per day was added if a diuresis did not occur with furosemide. Six of the 12 patients could be satisfactorily diuresed with the preceding regimen. In the remaining six patients, diuresis either was unsuccessful (two patients) or resulted in serious complications, including increasing blood urea nitrogen in three patients and hyponatremia in one patient. In the six responsive patients, 300 mL of a 15% solution of salt-poor albumin led to a significant diuresis and resolution of edema without worsening renal function.

Results of studies both in nephrotic patients (69) and in an experimental model of nephrotic syndrome in the rat (70) indicate that rather than compensating for urinary protein losses, ingestion of a high-protein diet results in an augmentation of urinary albumin excretion and a net decrease in serum albumin concentration and body albumin pools. In addition, treatment with converting enzyme inhibitors reduced the rate of urinary protein loss (71,72) and retarded the progression of renal dysfunction (72) in nephrotic syndrome patients. Don

et al. (73) studied the effects of dietary protein restriction or treatment with an ACE inhibitor on protein metabolism in 12 patients with the nephrotic syndrome. Protein metabolism was assessed by plasma amino acid levels and nitrogen balance. Reducing dietary protein intake decreased, whereas treatment with enalapril increased nitrogen balance, although neither of these changes achieved a level of statistical significance. Moreover, Gansevoort et al. (74) recently showed comparable decreases in urinary protein excretion induced by either the AT-II receptor antagonist losartan or the ACE inhibitor enalapril in 11 nondiabetic nephrotic syndrome patients. This observation supports the notion that the antiproteinuric effect of ACE inhibition is mediated by the inhibition of AT-II rather than an ACE inhibitor-induced increase in bradykinin or prostaglandin levels. Further studies will be necessary to assess the potential value of changes in protein intake, converting enzyme inhibition, and AT-II receptor antagonism in the long-term treatment of nephrotic patients. As well, the potential beneficial effect of lipid-lowering agents on the progression and expression of nephrotic syndrome needs to be addressed (75,76).

Although albumin infusion continues to be an attractive therapeutic tool for the temporary treatment of patients with diuretic-resistant nephrotic syndrome, this form of therapy remains relatively expensive, and the diuretic effects of albumin infusion are usually short-lived (4). For example, in the study of Koomans et al. (4), 10 patients with the nephrotic syndrome received a 75-g hyperoncotic albumin infusion, and their urinary sodium excretion increased only minimally from 10 to 45 μEq/minute. This rate would account for a loss of less than 1 kg of extracellular fluid volume in 24 hours. Nevertheless, albumin infusions may be beneficial in some diuretic-resistant nephrotic patients. Water immersion has also been used to treat nephrotic patients, with weight loss ranging from 0.5 to 2.0 kg observed after 4 hours of HWI (77). This form of therapy, although extremely interesting from a physiopathologic viewpoint, remains impractical.

Vasopressin V_2 receptor antagonists have been shown to be useful aquaretic agents in animals and humans with heart failure and cirrhosis (78). Similar studies have not been performed in nephrotic syndrome. In contrast to diuretics, these agents have the theoretical advantage of inducing solute-free water clearance without electrolyte losses. Further study in this area is indicated.

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CHAPTER 86 ■ THE SYNDROME OF INAPPROPRIATE ANTIDIURETIC HORMONE SECRETION AND OTHER HYPOOSMOLAR DISORDERS

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The *syndrome of inappropriate antidiuretic hormone secretion (SIADH)* is produced when plasma levels of arginine vasopressin (AVP), the only known antidiuretic hormone, are elevated at times when physiologic AVP secretion from the posterior pituitary would normally be suppressed. Because the only clinical abnormality known to result from increased secretion of AVP is a decrease in the osmotic pressure of body fluids, the hallmark of SIADH is *hypoosmolality*. This clinical finding led to the identification of the first well-described cases of this disorder in 1957 (1) and the subsequent clinical investigations that resulted in the delineation of the essential characteristics of the syndrome (2). It is, therefore, appropriate to begin this chapter with a brief summary of some general issues concerning hypoosmolality and hyponatremia before discussing details that are specific to SIADH and related disorders associated with dilutional hypoosmolality of body fluids. Although much has been learned over the last four decades about the pathophysiology of SIADH and hyponatremia, it remains surprising how rudimentary our understanding is of some of the most basic aspects of this disorder (3,4). One particularly striking example of this is the controversy in the last decade concerning the most appropriate rate of correction of hyponatremic patients (5). Nonetheless, recent and ongoing clinical and basic studies have continued to shed new light on many heretofore incompletely understood aspects of hypoosmolar disorders. In addition, we are on the verge of an exciting new era with regard to the therapy of these disorders using antagonists of specific AVP receptors (6). Although some of the specific information contained in this chapter may well become outdated in the future, the basic concepts underlying the pathophysiology, diagnosis, and therapy of hypoosmolar disorders have withstood the tests of time and clinical utility, and likely will remain valid for some time to come.

HYPOOSMOLALITY AND HYPONATREMIA

Incidence

Hypoosmolality is one of the most common disorders of fluid and electrolyte balance encountered in hospitalized patients. The incidence and prevalence of hypoosmolar disorders depend on the nature of the patient population being studied as well as on the laboratory methods and diagnostic criteria used to ascertain hyponatremia. Most investigators have used the serum sodium concentration ([Na^+]) to determine the clinical incidence of hypoosmolality. When hyponatremia is defined as a serum [Na^+] of less than 135 mEq/L, incidences as high as 15% to 30% have been observed in studies of both acutely (7,8) and chronically (9,10) hospitalized patients. These high incidences in hospitalized patients are corroborated by frequency analysis of a large population of hospitalized patients, which demonstrated that serum [Na^+] and chloride concentrations were approximately 5 mEq/L lower than those in a control group of healthy, nonhospitalized subjects (11). However, incidences decrease to the range of 1% to 4% when only patients with serum [Na^+] less than 130 to 131 mEq/L are included (8,12–15), which represents a more appropriate level to define the occurrence of clinically significant cases of this disorder. Even when one uses these more stringent criteria to define hypoosmolality, incidences from 7% to 53% have been reported in institutionalized geriatric patients (16–18). Perhaps most importantly, reports of all studies to date have noted a high proportion of iatrogenic or hospital-acquired hyponatremia, which has accounted for as much as 40% to 75% of all patients studied (12,14). Therefore, although hyponatremia and hypoosmolality are exceedingly common, most cases are relatively mild and become manifest during the course of hospitalization.

These considerations could be interpreted to indicate that hypoosmolality is of relatively little clinical significance, but this conclusion is unwarranted for several reasons. First, severe hypoosmolality (serum [Na^+] levels <120 mEq/L), although relatively uncommon, is associated with substantial morbidity and mortality (19,20). Second, even relatively mild hypoosmolality can quickly progress to more dangerous levels during the course of therapeutic management of other disorders. Third, overly rapid correction of hyponatremia can itself cause severe neurologic morbidity and mortality (21). Finally, it has been observed that mortality rates are much higher, from 3- (13,15) to 60-fold (14), in patients with even asymptomatic degrees of hypoosmolality compared to normonatremic patients. Remarkably, hyponatremia has been found to represent an independent predictor of worsened outcomes in a variety of diseases, including myocardial infarction (22), congestive heart failure (23,24), and cirrhosis (25). This is probably because hypoosmolality is more an indicator of the severity of many underlying illnesses than it is an independent contributing factor to mortality, but this presumption may not be true of all cases. These considerations emphasize the importance of a careful evaluation of all hyponatremic patients, regardless of the clinical setting in which they present.

Osmolality, Tonicity, and Serum [Na^+]

As discussed in Chapter 3, the osmolality of body fluid normally is maintained within narrow limits by osmotically regulated AVP secretion and thirst. Although basal plasma

osmolality can vary appreciably among individuals, the range in the general population under conditions of normal hydration lies between 275 and 295 mOsmol/kg H_2O. Plasma osmolality can be determined *directly* by measuring the freezing-point depression or the vapor pressure of plasma. Alternatively, it can be calculated *indirectly* from the concentrations of the three major solutes in plasma:

$$\begin{aligned}\text{Posm (mOsm/kg } H_2O) = {} & 2 \times [Na^+]\,(\text{mEq/L}) \\ & + \text{glucose (mg/dL)}/18 \\ & + \text{blood urea nitrogen (mg/dL)}/2.8\end{aligned} \quad [1]$$

Both methods produce comparable results under most conditions. However, although either of these methods produces valid measures of total osmolality, this is not always equivalent to the *effective osmolality*, which is commonly referred to as the *tonicity* of the plasma. Only cell membrane impermeable solutes such as Na^+ and Cl^- that remain relatively compartmentalized within the extracellular fluid (ECF) space are "effective" solutes, because these solutes create osmotic gradients across cell membranes and thus generate osmotic movement of water from the intracellular fluid (ICF) compartment into the ECF compartment. By contrast, solutes that readily permeate cell membranes (e.g., urea, ethanol, and methanol) are not effective solutes, because they do not create osmotic gradients across cell membranes and thus do not generate water movement between body fluid compartments. Only the concentrations of effective solutes in plasma should be used to ascertain whether clinically significant hyperosmolality or hypoosmolality is present, since these are the only solutes that directly affect body fluid distribution (26).

Sodium and its accompanying anions represent the bulk of the major effective plasma solutes, so hyponatremia and hypoosmolality are usually synonymous. However, there are two important situations in which hyponatremia will not reflect true hypoosmolality. The first is *pseudohyponatremia*, which is produced by marked elevations of either lipids or proteins in plasma. In such cases the concentration of Na^+ per liter of plasma water is unchanged, but the concentration of Na^+ per liter of plasma is artifactually decreased because of the larger relative proportion of plasma volume that is occupied by the excess lipids or proteins (27,28). However, the increased protein or lipid will not appreciably increase the total number of solute particles in solution, so the directly measured plasma osmolality will not be significantly affected under these conditions. Measurement of serum $[Na^+]$ by ion-specific electrodes, which is now commonly employed by most clinical laboratories, is less influenced by high concentrations of lipids or proteins than is measurement of serum $[Na^+]$ by flame photometry (29), although recent reports have demonstrated that such errors can nonetheless still occur when using autoanalyzers that require a dilution of the plasma sample (30–32).

The second situation in which hyponatremia does not reflect true plasma hypoosmolality occurs when high concentrations of effective solutes other than Na^+ are present in the plasma. The initial hyperosmolality produced by the additional solute causes an osmotic shift of water from the ICF to the ECF, which in turn produces a dilutional decrease in the serum $[Na^+]$. Once equilibrium between both fluid compartments is achieved, the total effective osmolality remains relatively unchanged. This situation most commonly occurs with hyperglycemia and represents a frequent cause of hyponatremia in hospitalized patients, accounting for up to 10% to 20% of all cases (14). Misdiagnosis of true hypoosmolality in such cases can be avoided by measuring plasma osmolality directly or, alternatively, by correcting the measured serum $[Na^+]$ by 1.6 mEq/L for each 100 mg/dL increase in serum glucose concentration above normal levels (33). Recent studies have shown a more complex relation between hyperglycemia and serum $[Na^+]$, and have suggested that a more accurate correction factor may be closer to 2.4 mEq/L (34). However, when the plasma contains significant amounts of unmeasured solutes, such as osmotic diuretics, radiographic contrast agents, and some toxins (ethanol, methanol, and ethylene glycol), plasma osmolality cannot be calculated accurately. In these situations, osmolality must be ascertained by direct measurement, although even this method does not yield an accurate measure of the true effective osmolality if the unmeasured solutes are noneffective solutes that freely permeate cell membranes (e.g., ethanol).

Because of the previously noted considerations, it should be apparent that the determination of whether true hypoosmolality is present can sometimes be difficult. Nevertheless, a straightforward and relatively simple approach will suffice in most cases:

1. The effective plasma osmolality should be calculated from the measured serum $[Na^+]$ and glucose concentration ($2 \times [Na^+]$ + glucose/18); alternatively, the measured serum $[Na^+]$ can simply be corrected by 1.6 to 2.4 mEq/L for each 100 mg/dL increase in serum glucose concentration greater than normal levels (100 mg/dL).
2. If the calculated effective plasma osmolality is <275 mOsmol/kg H_2O, or if the corrected serum $[Na^+]$ is <135 mEq/L, then significant hypoosmolality exists, providing that large concentrations of unmeasured solutes or pseudohyponatremia secondary to hyperlipidemia or hyperproteinemia are not present.
3. To eliminate the latter possibilities, plasma osmolality should also be measured directly in all cases in which the hyponatremia cannot be accounted for by elevated serum glucose levels. Absence of a discrepancy between the calculated and measured total plasma osmolality (<10 mOsmol/kg H_2O) will confirm the absence of significant amounts of unmeasured solutes, such as osmotic diuretics, radiocontrast agents, or alcohol; if a significant discrepancy between these measures is found (called an "osmolal gap" [35]), appropriate tests must then be conducted to rule out pseudohyponatremia or to identify possible unmeasured plasma solutes (26,36,37). Whether significant hypoosmolality exists in the latter case will depend upon the nature of the unmeasured solutes; although this determination will not always be possible, the clinician will at least be alerted to uncertainty about the diagnosis of true hypoosmolality.

Pathogenesis of Hypoosmolality

Because water moves freely between the ICF and ECF across most cell membranes, osmolality will always be equivalent in both of these fluid compartments, since water distributes between them in response to osmotic gradients. Consequently, total body osmolality must always be the same as both ECF and ICF osmolality. Because the bulk of body solute comprises electrolytes, namely the exchangeable Na^+ (Na^+_E) in the ECF and the exchangeable K^+ (K^+_E) in the ICF along with their associated anions, total body osmolality will largely be a function of these parameters (38,39):

$$\begin{aligned}OSM_{ECF} &= OSM_{ICF} \\ &= \text{total body osmolality} \\ &= (\text{ECF solute} + \text{ICF solute})/\text{body water} \\ &= (2 \times Na^+_E + 2 \times K^+_E + \text{nonelectrolyte solute})/ \\ &\quad \text{body water}\end{aligned} \quad [2]$$

Although the above calculations represent an oversimplification of complex factors that determine the relative distribution of intracellular and extracellular solutes (see [40] for a

TABLE 86-1

PATHOGENESIS OF HYPOOSMOLAR DISORDERS

- **Depletion (primary decreases in total body solute and secondary water retention):**
  - Renal solute loss
    - Diuretic use
    - Solute diuresis (glucose, mannitol)
    - Salt-wasting nephropathy
    - Mineralocorticoid deficiency or resistance
  - Nonrenal solute loss
    - Gastrointestinal (diarrhea, vomiting, pancreatitis, bowel obstruction)
    - Cutaneous (sweating, burns)
    - Blood loss
- **Dilution (primary increases in total body water and secondary solute depletion):**
  - Impaired renal free water excretion
    - Increased proximal reabsorption
      - Hypothyroidism
    - Impaired Distal Dilution
      - Syndrome of inappropriate antidiuretic hormone secretion
      - Glucocorticoid deficiency
    - Combined increased proximal reabsorption and impaired distal dilution
      - Congestive heart failure
      - Cirrhosis
      - Nephrotic syndrome
    - Decreased urinary solute excretion
      - Beer potomania
      - Very-low protein diet
  - Excess water intake
    - Primary polydipsia
    - Dilute infant formula
    - Fresh-water drowning

revision of the original *Edelman equation* for predicting plasma [Na^+] based on exchangeable body Na^+ and K^+), nonetheless they are sufficiently accurate for the purpose of predicting changes in serum [Na^+]. By definition, the presence of plasma hypoosmolality indicates a relative excess of water to solute in the ECF. From the preceding equations, it should be apparent that this can be produced either by an excess of body water, resulting in a *dilution* of remaining body solute, or alternatively by a *depletion* of body solute, either Na^+ or K^+, relative to the remaining body water. Table 86-1 summarizes the potential causes of hyponatremia categorized according to whether the *initiating* event is dilution or depletion of body solute. It should be recognized that such a classification represents an obvious oversimplification, because most clinical hypoosmolar states involve significant components of both solute depletion and water retention. Nonetheless, it is conceptually useful as a starting point for understanding the mechanisms underlying the pathogenesis of hypoosmolality and as a framework for discussions of therapy of hypoosmolar disorders.

Solute Depletion

Depletion of body solute can result from any significant losses of ECF. Whether via renal or nonrenal routes, body fluid losses by themselves rarely cause hypoosmolality because excreted or secreted body fluids are usually isotonic or hypotonic relative to plasma and therefore tend to increase plasma osmolality. Consequently, when hypoosmolality accompanies ECF losses it is generally the result of replacement of body fluid losses by more hypotonic solutions, thereby diluting the remaining body solutes. This often occurs when patients drink water or other hypotonic fluids in response to ongoing solute and water losses, and also when hypotonic intravenous fluids are administered to hospitalized patients (41). When the solute losses are marked, these patients can show all of the obvious signs of volume depletion (e.g., Addisonian crisis). However, such patients often have a more deceptive clinical presentation because their volume deficits may be partially replaced by subsequently ingested or infused fluids. Moreover, they may not manifest signs or symptoms of cellular dehydration because osmotic gradients will draw water into the relatively hypertonic ICF. Therefore, clinical evidence of hypovolemia strongly supports solute depletion as the cause of plasma hypoosmolality, but absence of clinically evident hypovolemia never completely eliminates this as a possibility. Although ECF solute losses are responsible for most cases of depletion-induced hypoosmolality, ICF solute loss can also cause hypoosmolality as a result of osmotic water shifts from the ICF into the ECF (38). This mechanism likely contributes to some cases of diuretic-induced hypoosmolality in which depletion of total body K^+ often occurs (42–44).

Water Retention

Despite the obvious importance of solute depletion in some patients, most cases of clinically significant hypoosmolality are caused by increases in total body water rather than by primary loss of extracellular solute. This can occur because of either impaired renal free water excretion or excessive free water intake. However, the former accounts for most hypoosmolar disorders because normal kidneys have sufficient diluting capacity to allow excretion of up to 20 to 30 L/day of free water (see Chapter 3). Intakes of this magnitude are occasionally seen in a subset of psychiatric patients (45,46) but not in most patients, including patients with SIADH in whom fluid intakes average 2 to 3 L/day (47). Consequently, dilutional hypoosmolality usually is the result of an abnormality of renal free water excretion. The renal mechanisms responsible for impairments in free water excretion can be subgrouped according to whether the *major* impairment in free water excretion occurs in proximal or distal parts of the nephron, or both (see Table 86-1).

Any disorder that leads to a decrease in glomerular filtration rate (GFR) causes increased reabsorption of both Na^+ and water in the proximal tubule. As a result, the ability to excrete free water is limited because of decreased delivery of tubular fluid to the distal nephron. Disorders causing solute depletion through nonrenal mechanisms (e.g., gastrointestinal fluid losses) also produce this effect. Disorders that cause a decreased GFR in the absence of significant ECF fluid losses are, for the most part, edema-forming states associated with decreased *effective arterial blood volume (EABV)* and secondary hyperaldosteronism (48–50). Although these conditions are typified by increased proximal reabsorption of both Na^+ and fluid, it is now clear that in most cases water retention also results from increased distal reabsorption caused by non-osmotic baroreceptor-mediated stimulated increases in plasma AVP levels (51,52), with the possible exception of hypothyroidism.

Distal nephron impairments in free water excretion are characterized by an inability to dilute tubular fluid maximally. These disorders are usually associated with abnormalities in the secretion of AVP from the posterior pituitary. However, just as depletion-induced hypoosmolar disorders usually include an important component of secondary impairments of free water excretion, so do most dilution-induced hypoosmolar disorders involve significant degrees of secondary solute depletion. This was recognized even before the first clinical description of SIADH from studies of the effects of posterior pituitary extracts on water retention, which demonstrated that renal salt-wasting

was predominantly a result of the ECF volume expansion produced by the retained water (53). Therefore, after sustained increases in total body water secondary to inappropriately elevated AVP levels, sufficient secondary solute losses, predominantly as Na^+, occur and sometimes result in further lowering of plasma osmolality. The actual contribution of Na^+ losses to the hypoosmolality of SIADH is variable and depends in part on both the rate and volume of water retention (54). The major factor responsible for secondary Na^+ losses appears to be renal hemodynamic effects, and specifically the phenomenon of *pressure natriuresis and diuresis* induced by the volume expansion (55). However, volume-stimulated hormones such as atrial natriuretic peptide (ANP) are also elevated in response to the water retention of patients with SIADH (56,57), and it seems likely that these factors also contribute to the secondary natriuresis, possibly via interactions with intrarenal hemodynamic effects (58). Regardless of the actual mechanisms involved, the solute losses that occur secondary to water retention can be best understood in the context of volume regulation of the ICF and ECF fluid compartments in response to induced hypoosmolality, which is discussed in the next section.

Some dilutional disorders do not fit well into either category. Chief among these is the hyponatremia that sometimes occurs in patients who ingest large volumes of beer with little food intake for prolonged periods, frequently called "*beer potomania*" (59,60). Although the volume of fluid ingested may not seem sufficiently excessive to overwhelm renal diluting mechanisms, in these cases free water excretion is limited by very low urinary solute excretion, thereby causing water retention and dilutional hyponatremia. A recent case in which hyponatremia occurred in an ovolactovegetarian with a very low protein intake but no beer ingestion is consistent with this pathophysiologic mechanism (61). However, because most such patients have very low salt intakes as well, it is likely that relative depletion of body Na^+ stores also is a contributing factor to the hypoosmolality in at least some cases (62).

Adaptation to Hyponatremia: Intracellular Fluid and Extracellular Fluid Volume Regulation

Many studies have indicated that the combined effects of water retention plus urinary solute excretion cannot adequately explain the degree of plasma hypoosmolality observed in patients (2,63,64). This observation originally led to the theory of "*cellular inactivation of solute*" (2). Simply stated, this theory suggested that as ECF osmolality falls, water moves into cells along osmotic gradients, thereby causing the cells to swell. At some point during this volume expansion, the cells osmotically "inactivate" some of their intracellular solutes as a defense mechanism to prevent continued cellular swelling with subsequent detrimental effects on cell function and survival. As a result of this decrease in intracellular osmolality, water then shifts back out of the ICF into the ECF, but at the expense of further worsening the dilution-induced hypoosmolality. Despite the appeal of this theory, its validity has never been demonstrated conclusively in either human or animal studies.

An appealing alternative theory has been suggested by studies of cellular volume regulation, in which cell volume is maintained under hypoosmolar conditions by extrusion of potassium rather than by osmotic inactivation of cellular solute (65,66). Whole brain volume regulation via similar types of electrolyte losses was first described by Yannet in 1940 (67), and has long been recognized as the mechanism by which the brain was able to adapt to hyponatremia and limit brain edema to sublethal levels (68–70). Following the recognition that low-molecular-weight organic compounds, called *organic osmolytes*, also constituted a significant osmotic component of a wide variety of cell types, studies demonstrated the accumulation of these compounds in response to hyperosmolality in

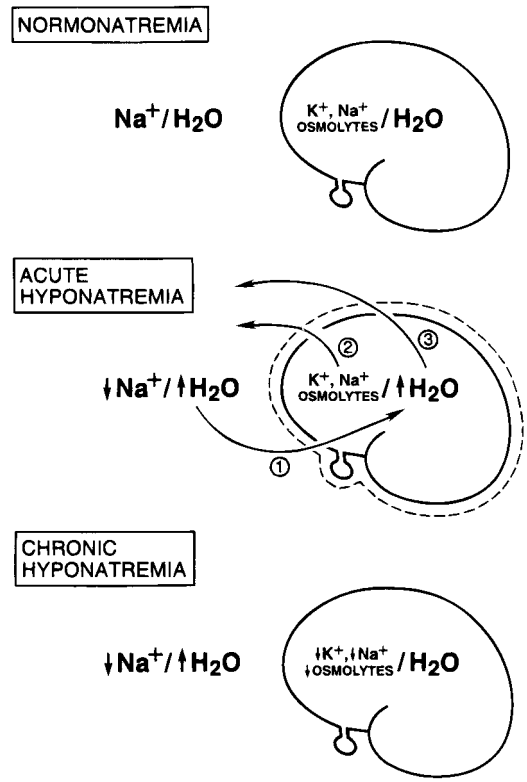

FIGURE 86-1. Schematic diagram of brain volume adaptation to hyponatremia. Under normal conditions brain osmolality and extracellular fluid (ECF) osmolality are in equilibrium (**top**; for simplicity the predominant intracellular solutes are depicted as K^+ and organic osmolytes, and the extracellular solute as Na^+). Following the induction of ECF hypoosmolality, water moves into the brain in response to osmotic gradients producing brain edema (*dotted line*, **middle, #1**). However, in response to the induced swelling the brain rapidly loses both extracellular and intracellular solutes (**middle, #2**). As water losses accompany the losses of brain solute, the expanded brain volume then decreases back toward normal (**middle, #3**). If hypoosmolality is sustained, brain volume eventually normalizes completely and the brain becomes fully adapted to the ECF hyponatremia (**bottom**).

both kidney (71) and brain (72) tissue. Multiple groups have now shown that the brain loses organic osmolytes in addition to electrolytes during the process of volume regulation to hypoosmolar conditions in experimental animals (73–76) and human patients (77). These losses occur relatively quickly (within 24 to 48 hours in rats) and can account for as much as one-third of the brain solute losses during hyponatremia (78). Such coordinate losses of both electrolytes and organic osmolytes from brain tissue enables very effective regulation of brain volume during chronic hyponatremia (Fig. 86-1). Consequently, it is now clear that cellular volume regulation *in vivo* in brain tissue occurs predominantly through depletion, rather than intracellular osmotic "inactivation," of a variety of intracellular solutes. Ongoing experimental studies are better defining the complex cellular and molecular mechanisms that underlie this profound adaptation to hypoosmolality (79–84).

Most recent studies have focused on volume regulation in the brain during hyponatremia, but all cells volume regulate to varying degrees (65), and there is little question that this process occurs throughout the body as whole organisms adapt to hypoosmolar conditions. Unexplained components of hyponatremia that led to previous speculation about cellular inactivation of solute are now better explained by cellular losses of both electrolyte and organic solutes as cells throughout the

body undergo volume regulation during hypoosmolar conditions. However, volume regulatory processes are not limited to cells. Although most cases of hyponatremia clearly result from initial water retention induced by stimulated antidiuresis, it has always seemed likely that the resulting natriuresis served the purpose of regulating the volumes of the ECF and intravascular spaces. Many experimental and clinical observations are consistent with ECF volume regulation via secondary solute losses. First, dilutional decreases in concentrations of most blood constituents other than $Na^+$and Cl^-do not occur in patients with SIADH (85), suggesting that their plasma volume is not nearly as expanded as would be predicted simply by the measured decreases in serum [Na^+]. Second, an increased incidence of hypertension has never been observed in patients with SIADH (86), again arguing against significant expansion of the arterial blood volume. Third, results of animal studies in both dogs (87) and rats (88) have clearly indicated that a significant component of chronic hyponatremia is attributable to secondary Na^+ losses rather than water retention. Furthermore, the relative contributions from water retention versus sodium loss vary with the duration and severity of the hyponatremia: water retention was found to be the major cause of decreased serum [Na^+] in the first 24 hours of induced hyponatremia in rats, but Na^+ depletion then became the predominant etiologic factor after longer periods (7 to 14 days) of sustained hyponatremia, particularly at very low (<115 mEq/L) serum [Na^+] levels (88). Finally, multiple studies have attempted to measure body fluid compartment volumes in hyponatremic patients, but without consistent results that indicate either plasma or ECF expansion (1,64,89,90). In particular, a report of body fluid space measurements using isotope dilution techniques in hyponatremic and normonatremic patients with small cell lung carcinoma showed no differences between the two groups with regard to exchangeable sodium space, ECF volume by $^{35}SO_4$ distribution, or total body water (91). Such results have traditionally been explained by the relative insensitivity of isotope dilution techniques for measurement of body fluid compartment spaces, but an equally plausible possibility is that in the chronically adapted hyponatremic state, body fluid compartments have regulated their volumes back toward normal via a combination of extracellular (predominantly electrolyte) and intracellular (electrolyte and organic osmolyte) solute losses (92). Figure 86-2 schematically illustrates some of the volume regulatory processes that likely occur in response to water retention induced by inappropriate antidiuresis. The degree to which solute losses versus water retention contribute to the resulting hyponatremia will vary in association with many different factors, including the etiology of the hyponatremia, the rapidity of development of the hyponatremia, the chronicity of the hyponatremia, the volume of daily water loading and subsequent volume expansion, and undoubtedly some degree of individual variability as well. It, therefore, hardly seems surprising that studies of hyponatremic patients have failed to yield uniform results regarding the pathogenesis of hyponatremia in view of the marked diversity of hyponatremic patients and their presentation at different times during the process of adaptation to hypoosmolality via volume regulatory processes.

Differential Diagnosis of Hyponatremia and Hypoosmolality

Because of the multiplicity of disorders causing hypoosmolality and the fact that many involve more than one pathologic mechanism, a definitive diagnosis is not always possible at the time of initial presentation. Nonetheless, a relatively straightforward approach based on the commonly used parameters of ECF volume status and urine sodium concentration generally allows a sufficient categorization of the underlying etiology to allow appropriate decisions regarding initial therapy and further evaluation in most cases (Table 86-2).

Decreased Extracellular Fluid Volume

The presence of clinically detectable hypovolemia always signifies total body solute depletion. A low urinary [Na^+] indicates a nonrenal cause of solute depletion. If the urinary [Na^+] is high despite hypoosmolality, renal causes of solute depletion are likely responsible. Therapy with thiazide diuretics is the most common cause of renal solute losses (44), particularly in the elderly (93,94), but mineralocorticoid deficiency as a result of adrenal insufficiency (95) or mineralocorticoid resistance (96) must always be considered as well. Less commonly, renal solute losses may be the result of a salt-wasting nephropathy, for example, polycystic kidney disease (97), interstitial nephritis (98), or chemotherapy (99).

Increased Extracellular Fluid Volume

The presence of clinically detectable hypervolemia always signifies total body Na^+ excess. In these patients hypoosmolality results from an even greater expansion of total body water caused by a marked reduction in the rate of water excretion (and sometimes an increased rate of water ingestion). The impairment in water excretion is secondary to a decreased EABV (48–50), which increases the reabsorption of glomerular filtrate not only in the proximal nephron but also in the distal and collecting tubules by stimulating AVP secretion (51,52). These patients generally have a low urinary [Na^+] because of secondary hyperaldosteronism, which is also a product of decreased EABV. However, under certain conditions urinary [Na^+] may be elevated, usually secondary to concurrent diuretic therapy but also sometimes because of a solute diuresis (e.g., glucosuria in diabetics) or after successful treatment of the underlying disease (e.g., inotropic therapy in patients with congestive heart failure). An additional disorder that can produce hypoosmolality and hypervolemia is acute or chronic renal failure with fluid overload (14) (although in early stages of renal failure polyuria from vasopressin resistance is more likely [100]). Urinary [Na^+] in these cases is usually elevated, but it can be variable depending on the stage of renal failure. It is important to remember that primary polydipsia will not be accompanied by signs of hypervolemia because water ingestion alone, in the absence of Na^+retention, does not produce *clinically* apparent degrees of ECF volume expansion.

Normal Extracellular Fluid Volume

Many different hypoosmolar disorders can potentially present clinically with euvolemia, in large part because it is difficult to detect modest changes in volume status using standard methods of clinical assessment; in such cases measurement of urinary [Na^+] is an especially important first step (101). A high urinary [Na^+] in euvolemic patients usually implies a distally mediated, dilution-induced hypoosmolality such as SIADH. However, glucocorticoid deficiency can mimic SIADH so closely that these two disorders are often indistinguishable in terms of water balance (102,103). Hyponatremia from diuretic use also can present without clinically evident hypovolemia, and the urinary [Na^+] will often be elevated in such cases because of the renal tubular effects of the diuretics (44). A low urinary [Na^+] suggests a depletion-induced hypoosmolality from ECF losses with subsequent volume replacement by water or other hypotonic fluids. The solute loss often is generally nonrenal in origin, but an important exception is recent cessation of diuretic therapy, because urinary [Na^+] can quickly decrease to low values within 12 to 24 hours after discontinuation of the drug. The presence of a low serum [K^+] is an important clue

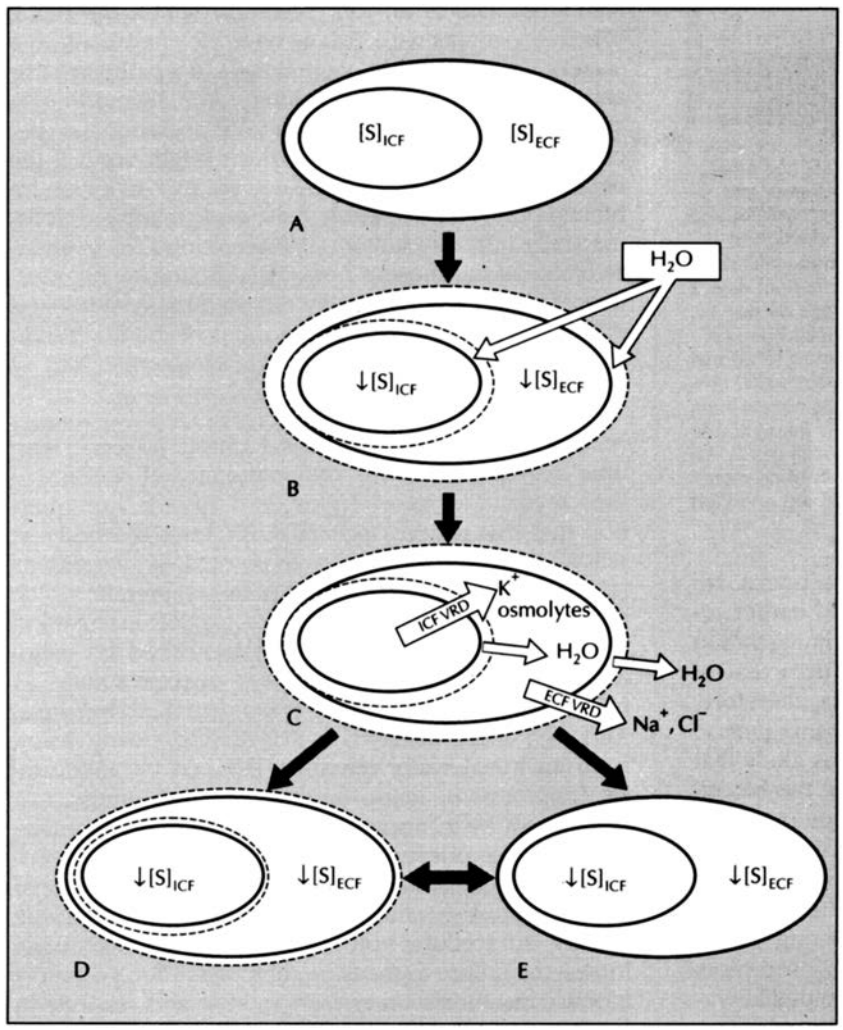

FIGURE 86-2. Schematic illustration of potential changes in whole body fluid compartment volumes at various times during adaptation to hyponatremia. Under basal conditions the concentration of effective solutes in the extracellular fluid ($[S]_{ECF}$) and the intracellular fluid ($[S]_{ICF}$) are in osmotic balance (**A**). During the first phase of water retention resulting from inappropriate antidiuresis the excess water distributes across total body water, causing expansion of both ECF and intracellular fluid (ICF) volumes (*dotted lines*) with equivalent dilutional decreases in $[S]_{ICF}$ and $[S]_{ECF}$ (**B**). In response to the volume expansion, compensatory volume regulatory decreases (VRDs) occur to reduce the effective solute content of both the ICF (via increased electrolyte and osmolyte extrusion mediated by stretch activated channels and down-regulation of synthesis of osmolytes and osmolyte uptake transporters) and the ECF (via pressure diuresis and natriuretic factors) (**C**). If both processes go to completion, such as under conditions of fluid restriction, a final steady state can be reached in which ICF and ECF volumes have returned to normal levels but $[S]_{ICF}$ and $[S]_{ECF}$ remain low (**E**). In most cases this final steady state is not reached, and moderate degrees of ECF and ICF expansion persist, but significantly less than would be predicted from the decrease in body osmolality (**D**). Consequently, the degree to which hyponatremia is due to dilution from water retention versus solute depletion from volume regulatory processes can vary markedly depending on which phase of adaptation the patient is in, and also on the relative rates at which the different compensatory processes occur (e.g., delayed ICF VRD can worsen hyponatremia due to shifts of intracellular water into the extracellular fluid as intracellular organic osmolytes are extruded and subsequently metabolized, likely accounting for some component of the hyponatremia unexplained by the combination of water retention and sodium excretion in previous clinical studies). (From: Verbalis JG. Hyponatremia: epidemiology, pathophysiology, and therapy. *Curr Opin Nephrol Hypertens* 1993;2:636, with permission.)

to diuretic use, because few of the other disorders that cause hypoosmolality are associated with significant hypokalemia. However, even in the absence of hypokalemia, any hypoosmolar, clinically euvolemic patient taking diuretics should be assumed to have solute depletion and treated accordingly; subsequent failure to correct the hypoosmolality with isotonic saline administration and persistence of an elevated urinary $[Na^+]$ after discontinuation of diuretics then requires reconsideration of a diagnosis of dilution-induced hypoosmolality. A low urinary $[Na^+]$ also can also be seen in some cases of hypothyroidism, in the early stages of decreased EABV before the development of clinically apparent salt retention and fluid overload, or during the recovery phase from SIADH. Hence, a low urinary Na^+ is less meaningful diagnostically than is a high value.

Because euvolemic causes of hypoosmolality represent the most challenging etiologies of this disease, both in terms of differential diagnosis as well as with regard to the underlying pathophysiology, the subsequent sections will discuss the

TABLE 86-2

DIFFERENTIAL DIAGNOSIS OF HYPONATREMIA

Extracellular fluid volume	Urinary [Na^+][a]	Presumptive diagnosis
↓	Low	Depletion (nonrenal): GI, cutaneous, or blood ECF loss
	High	Depletion (renal): diuretics, mineralocorticoid insufficiency (Addison's disease), salt-losing nephropathy
→	Low	Depletion (nonrenal): any cause + hypotonic fluid replacement Dilution (proximal): hypothyroidism, early decreased effective arterial blood volume Dilution(distal): siadh + fluid restriction
	High	Dilution (distal): siadh, glucocorticoid insufficiency Depletion (renal): any cause + hypotonic fluid replacement (especially diuretic treatment)
↑	Low	Dilution (proximal): decreased, effective arterial blood volume (CHF, cirrhosis, nephrosis)
	High	Dilution (proximal): any cause + diuretics or improvement in underlying disease, renal failure

[a]Urinary [Na^+] values <30 mEq/L are generally considered to be low and values ≥30 mEq/L to be high, based on studies of responses of hyponatremic patients to infusions of isotonic saline (101).
GI, gastrointestinal; *ECF*, extracellular fluid; *SIADH*, syndrome of inappropriate antidiuretic hormone secretion; *CHF*, congestive heart failure.

major causes of euvolemic hypoosmolality and hyponatremia in greater detail.

SYNDROME OF INAPPROPRIATE ANTIDIURETIC HORMONE SECRETION

SIADH is the most common cause of euvolemic hypoosmolality. It is also the single most prevalent cause of hypoosmolality of all etiologies encountered in clinical practice, with prevalence rates ranging from 20% to 40% among all hypoosmolar patients (9,14,47,104). The clinical criteria necessary to diagnose SIADH remain basically as set forth by Bartter and Schwartz in 1967 (2). A modified summary of these criteria is presented in Table 86-3 along with several other clinical findings that support this diagnosis. Several points about each of these criteria deserve emphasis and/or qualification:

1. True hypoosmolality must be present and hyponatremia secondary to pseudohyponatremia or hyperglycemia alone must be excluded.
2. Urinary concentration (osmolality) must be inappropriate for plasma hypoosmolality. This does not mean that urine osmolality must be greater than plasma osmolality (a common misinterpretation of this criterion), but simply that the urine must be less than maximally dilute (i.e., urine osmolality >100 mOsmol/kg H_2O). It also should be remembered that urine osmolality need not be elevated inappropriately at all levels of plasma osmolality, because in the *reset osmostat* variant of SIADH, AVP secretion can be suppressed with resultant maximal urinary dilution and free water excretion if plasma osmolality is decreased to sufficiently low levels (105,106). Hence, to satisfy the classical criteria for the diagnosis of SIADH, it is necessary only that urine osmolality be inadequately suppressed at *some* level of plasma osmolality less than 275 mOsmol/kg H_2O.
3. Clinical euvolemia must be present to establish a diagnosis of SIADH, because both hypovolemia and hypervolemia strongly suggest different causes of hypoosmolality. This does not mean that patients with SIADH cannot become hypovolemic or hypervolemic for other reasons, but in such cases it is impossible to diagnose the underlying inappropriate antidiuresis until the patient is rendered euvolemic and found to have persistent hypoosmolality.
4. The criterion of renal "salt-wasting" has probably caused the most confusion regarding diagnosis of SIADH. This criterion is included because of its utility in differentiating

TABLE 86-3

CRITERIA FOR THE DIAGNOSIS OF SYNDROME OF INAPPROPRIATE ANTIDIURETIC HORMONE SECRETION

ESSENTIAL

Decreased effective osmolality of the extracellular fluid (P_{osm} <275 mOsmol/kg H_2O).

Inappropriate urinary concentration (U_{osm} >100 mOsmol/kg H_2O with normal renal function) at some level of hypoosmolality.

Clinical euvolemia, as defined by the absence of signs of hypovolemia (orthostasis, tachycardia, decreased skin turgor, dry mucous membranes) or hypervolemia (subcutaneous edema, ascites).

Elevated urinary sodium excretion while on normal salt and water intake.

Absence of other potential causes of euvolemic hypoosmolality: hypothyroidism, hypocortisolism (Addison's disease or pituitary adrenocorticotropic hormone [ACTH] insufficiency) and diuretic use.

SUPPLEMENTAL

Abnormal water load test (inability to excrete at least 90% of a 20 mL/kg water load in 4 hours and/or failure to dilute U_{osm} to <100 mOsmol/kg H_2O).

Plasma AVP level inappropriately elevated relative to plasma osmolality.

No significant correction of serum [Na^+] with volume expansion but improvement after fluid restriction.

between hypoosmolality caused by a decreased EABV, in which case renal Na^+ conservation occurs, and distal dilution-induced disorders, in which urinary Na^+ excretion is normal or increased secondary to ECF volume expansion. However, two important qualifications limit the utility of urinary [Na^+] measurement in the hypoosmolar patient: urinary [Na^+] also is high when solute depletion is of renal origin, as seen with diuretic use or Addison's disease, and patients with SIADH can have low urinary Na^+ excretion if they subsequently become hypovolemic or solute depleted, conditions that sometimes follow severe sodium and water restriction. Consequently, although a high urinary Na^+ excretion is the rule in most patients with SIADH, its presence does not guarantee this diagnosis, and, conversely, its absence does not rule out the diagnosis.

5. The final criterion emphasizes that SIADH remains a diagnosis of exclusion. Therefore, the presence of other potential causes of euvolemic hypoosmolality must always be excluded. This includes not only thyroid and adrenal dysfunction, but also diuretic use, because this can also sometimes present as euvolemic hypoosmolality.

Table 86-3 also lists several other criteria that support, but are not essential, for a diagnosis of SIADH. The first of these, the water-loading test, is of value when there is uncertainty regarding the etiology of modest degrees of hypoosmolality in euvolemic patients, but it does not add useful information if the plasma osmolality is <275 mOsmol/kg H_2O. Inability to excrete a standard water load normally (with normal excretion defined as a cumulative urine output of at least 90% of the administered water load within 4 hours and suppression of urine osmolality to <100 mOsmol/kg H_2O [107]) confirms the presence of an underlying defect in free water excretion. Unfortunately, water loading is abnormal in almost all disorders that cause hypoosmolality, whether dilutional or depletion-induced with secondary impairments in free water excretion. Two exceptions are primary polydipsia, in which hypoosmolality can rarely be secondary to excessive water intake alone, and the reset osmostat variant of SIADH, in which normal excretion of a water load can occur once plasma osmolality falls below the new set-point for AVP secretion. The water load test may also be used to assess water excretion after treatment of an underlying disorder thought to be causing SIADH. For example, after discontinuation of a drug associated with SIADH in a patient who has already achieved a normal plasma osmolality by fluid restriction, a normal water load test can confirm the absence of persistent inappropriate antidiuresis much more quickly than can simple monitoring of the serum [Na^+] during a period of *ad libitum* fluid intake. Despite these limitations as a diagnostic clinical test, water-loading remains an extremely useful tool in clinical research for quantitating changes in free water excretion in response to physiologic or pharmacologic manipulations.

The second supportive criterion for a diagnosis of SIADH is an inappropriately elevated plasma AVP level in relation to plasma osmolality. At the time that SIADH was originally described, inappropriately elevated plasma levels of AVP (or "*antidiuretic hormone, ADH*") were merely postulated because the measurement of plasma levels of AVP was limited to relatively insensitive bioassays. With the development of sensitive AVP radioimmunoassays capable of detecting the small physiologic concentrations of this peptide that circulate in plasma (108), there was hope that measurement of plasma AVP levels might supplant the classic criteria and become the definitive test for diagnosing SIADH, as is the case for many syndromes of hormone hypersecretion. This has not occurred for several reasons. First, although plasma AVP levels are elevated in most patients with this syndrome, the elevations generally remain within the normal physiologic range and are abnormal only in relation to plasma osmolality (Fig. 86-3). Therefore,

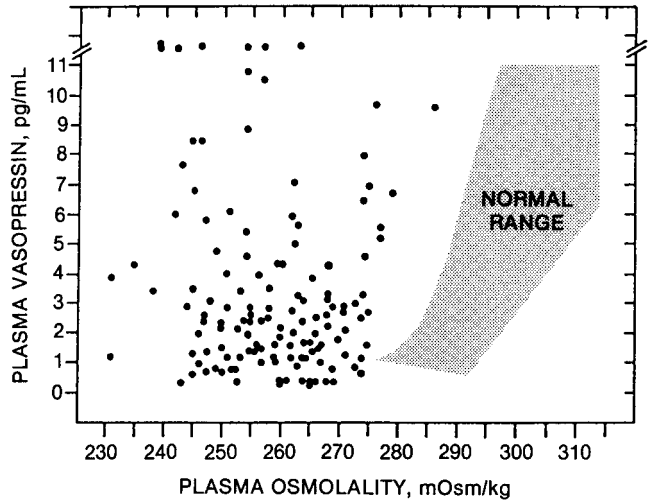

FIGURE 86-3. Plasma arginine vasopressin (AVP) levels in patients with SIADH as a function of plasma osmolality. Each point depicts one patient at a single point in time. The *shaded area* represents AVP levels in normal subjects over physiologic ranges of plasma osmolality. The lowest measurable plasma AVP levels using this radioimmunoassay was 0.5 pg/mL. (From: Robertson GL, Aycinena P, Zerbe RL. Neurogenic disorders of osmoregulation. *Am J Med* 1982;72:339, with permission.)

AVP levels can be interpreted only in conjunction with a simultaneous plasma osmolality and knowledge of the relation between AVP levels and plasma osmolality in normal subjects (see Chapter 3). Second, 10% to 20% of patients with SIADH do not have measurably elevated plasma AVP levels; as shown in Figure 86-3, many such patients have AVP levels that are at, or even below, the limits of detection by radioimmunoassay. Whether these cases are true examples of inappropriate antidiuresis in the absence of circulating AVP, or whether they simply represent inappropriate AVP levels that fall below the limits of detection by radioimmunoassay is not clear. For this reason, Zerbe et al. have proposed using the term "SIAD" (*syndrome of inappropriate antidiuresis*) rather than SIADH to describe this entire group of disorders (109). Recent studies of hyponatremic children have discovered two genetic mutations of the vasopressin V2 receptor (V2R) that were responsible for constitutive activation of antidiuresis in the absence of AVP-V2R ligand binding (110). The true incidence of these, and similar V2R mutations, as well as how often they are responsible for patients with SIADH but low or unmeasurable plasma AVP levels, remains to be determined. Third, just as water-loading fails to distinguish among various causes of hypoosmolality, so do plasma AVP levels. Many disorders causing solute and volume depletion are associated with elevations of plasma AVP levels secondary to hemodynamic stimuli. For similar reasons, patients with disorders that cause decreased EABV, such as congestive heart failure and cirrhosis, also have elevated AVP levels (see Chapters 83 and 84). Even glucocorticoid insufficiency has been associated with inappropriately elevated AVP levels, as will be discussed in the following section (111). Therefore, multiple different disorders cause stimulation of AVP secretion via *non-osmotic* mechanisms, rendering this measurement of relatively limited differential diagnostic value.

Finally, an improvement in plasma osmolality with fluid restriction but not with volume expansion can sometimes be helpful in differentiating between disorders causing solute depletion and those associated with dilution-induced hypoosmolality. Infusion of isotonic NaCl in patients with SIADH provokes a natriuresis with little correction of osmolality, whereas fluid restriction allows such patients to achieve solute and water balance gradually through insensible free water losses (1). By contrast, isotonic saline is the treatment of choice in disorders

of solute depletion, because once volume deficits are corrected the stimulus to continued AVP secretion and free water retention is eliminated. The diagnostic value of this therapeutic response is limited somewhat by the fact that patients with proximal types of dilution-induced disorders may show a response similar to that found in patients with SIADH. However, the major drawback is that this represents a retrospective test in a situation in which it would be preferable to establish a diagnosis before making a decision regarding treatment options. Nonetheless, in difficult cases of euvolemic hypoosmolality, an appropriate therapeutic response can sometimes be helpful in confirming a diagnosis of SIADH.

Etiology

Although the list of disorders associated with SIADH is long, they can be divided into several major etiologic groups (Table 86-4).

TABLE 86-4

COMMON ETIOLOGIES OF SYNDROME OF INAPPROPRIATE ANTIDIURETIC HORMONE SECRETION

Tumors
Pulmonary/mediastinal (bronchogenic carcinoma, mesothelioma, thymoma)
Nonchest (duodenal carcinoma, pancreatic carcinoma, ureteral/prostate carcinoma, uterine carcinoma, nasopharyngeal carcinoma, leukemia)

Central Nervous System Disorders
Mass lesions (tumors, brain abscesses, subdural hematoma)
Inflammatory diseases (encephalitis, meningitis, systemic lupus, acute intermittent porphyria, multiple sclerosis)
Degenerative/demyelinative diseases (Guillain-Barré; spinal cord lesions)
Miscellaneous (subarachnoid hemorrhage, head trauma, acute psychosis, delirium tremens, pituitary stalk section, transphenoidal adenomectomy, hydrocephalus)

Drug Induced
Stimulated AVP release (nicotine, phenothiazines, tricyclics)
Direct renal effects and/or potentiation of AVP antidiuretic effects (dDAVP, oxytocin, prostaglandin synthesis inhibitors)
Mixed or uncertain actions (amiodarone, angiotensin-converting enzyme inhibitors, carbamazepine and oxcarbazepine, chlorpropamide, clofibrate, clozapine, cyclophosphamide, 3,4-methylenedioxymethamphetamine [“Ecstasy”], omeprazole, serotonin reuptake inhibitors, vincristine)

Pulmonary Diseases
Infections (tuberculosis, acute bacterial and viral pneumonia, aspergillosis, empyema)
Mechanical/ventilatory (acute respiratory failure, chronic obstructive pulmonary disease [COPD], positive pressure ventilation)

Other
Acquired immunodeficiency syndrome (AIDS) and AIDS-related complex
Prolonged strenuous exercise (marathon, triathlon, ultramarathon, hot-weather hiking)
Chronic inflammation (interleukin 6)
Senile atrophy
Idiopathic

Tumors

The most common association of SIADH remains with tumors. Although many different types of tumors have been associated with SIADH (Table 86-4), bronchogenic carcinoma of the lung has been uniquely associated with SIADH, since the first description of this disorder in 1957 (1). In virtually all cases, the bronchogenic carcinomas causing this syndrome have been of the small cell (or oat cell) variety; a few squamous cell types have been described, but these are rare. Incidences of hyponatremia as high as 11% of all patients with small cell carcinoma (112), or 33% of cases with more extensive disease (113), have been reported. The unusually high incidence of small cell carcinoma of the lung in patients with SIADH, together with the relatively favorable therapeutic response of this type of tumor, makes it imperative that all adult patients presenting with an otherwise unexplained SIADH be investigated thoroughly and aggressively for a possible tumor. The evaluation should include a chest computed tomographic (CT) or magnetic resonance imaging (MRI) scan. In cases with a high degree of suspicion (e.g., hyponatremia in a young smoker), bronchoscopy with cytologic analysis of bronchial washings should be considered even if the results of routine chest radiography are normal, since several studies have reported hypoosmolality that predated any radiographically evident abnormality in patients who then were found to harbor bronchogenic carcinomas 3 to 12 months later (114,115). Head and neck cancers account for another group of malignancies associated with relatively higher incidences of SIADH (116,117), and some of these tumors have clearly been shown to be capable of synthesizing AVP ectopically (118). A recent report from a large cancer hospital showed an incidence of hyponatremia for all malignancies combined of 3.7%, with approximately one-third of these due to SIADH (15).

Central Nervous System Disorders

The second major etiologic group of disorders causing SIADH has its origins in the central nervous system (CNS). Despite the large number of different CNS disorders associated with SIADH, there is no obvious common denominator linking them. However, this is actually not surprising when one considers the neuroanatomy of neurohypophysial innervation. The magnocellular AVP neurons receive excitatory inputs from *osmoreceptor cells* located in the anterior hypothalamus, but also have a major innervation from brainstem cardiovascular regulatory and emetic centers (Fig. 86-4). Although various components of these pathways have yet to be elucidated fully, many of them appear to have inhibitory as well as excitatory components (119). Consequently, any diffuse CNS disorder can potentially cause AVP hypersecretion, either by nonspecifically exciting these pathways via irritative foci, or alternatively by disrupting them and thereby decreasing the level of inhibition impinging upon the AVP neurons in the neurohypophysis. The wide variety of diverse CNS processes that can potentially cause SIADH stands in contrast to CNS causes of diabetes insipidus, which are for the most part limited to lesions localized to hypothalamus and/or posterior pituitary that destroy the magnocellular vasopressin neurons (see Chapter 87).

Drugs

Drug-induced hyponatremia is one of the most common causes of hypoosmolality (120), and may soon replace tumors as the most common cause of SIADH. Table 86-4 lists some of the agents that have been associated with SIADH, and new drugs are being continually added to this list. In general, pharmacologic agents cause this syndrome by stimulating AVP secretion, by activating AVP renal receptors to cause antidiuresis, or by potentiating the antidiuretic effect of AVP on the kidney.

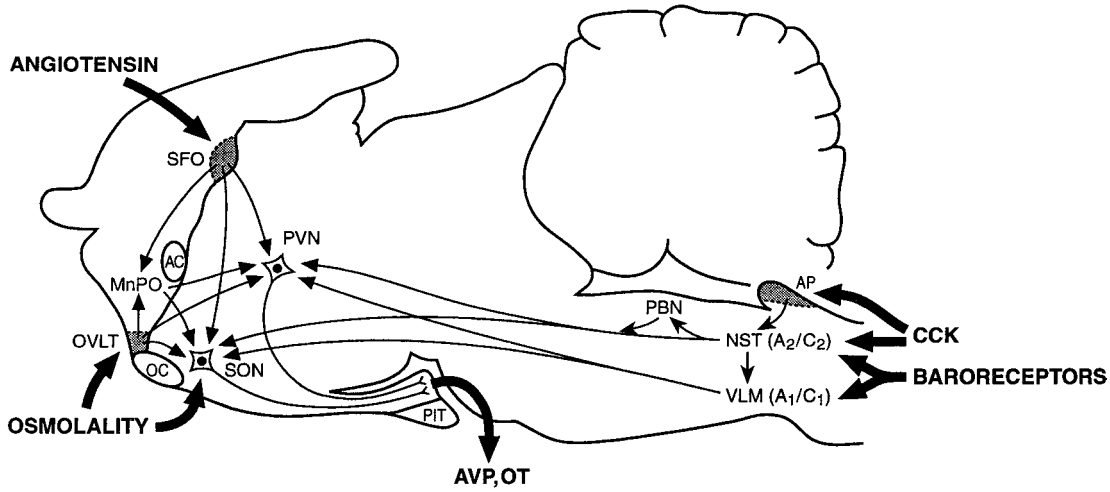

FIGURE 86-4. Diagrammatic summary of the primary brain pathways mediating AVP secretion in response to the major factors that stimulate pituitary AVP secretion. Osmolality activates neurons throughout the anterior hypothalamus, including the SFO and MnPO, but the OVLT appears to be uniquely sensitive to osmotic stimulation and is essential for osmotically stimulated AVP and OT secretion; in addition, osmotic stimulation can act directly on magnocellular neurons, which themselves are intrinsically osmosensitive. Similarly, circulating angiotensin II activates cells throughout the OVLT and MnPO, but the SFO appears to be its major and essential site of action. For both of these stimuli, projections from the SFO and OVLT to the MnPO activate both excitatory and inhibitory interneurons that project to the SON and PVN and modulate the direct circumventricular inputs to these areas. Emetic stimuli act both on gastric vagal afferents which terminate in the NST and in some cases also act directly at the AP. Most of the AVP secretion appears to be a result of monosynaptic projections from catecholaminergic A2/C2 cells in the NST. Baroreceptor-mediated stimuli such as hypovolemia and hypotension are considerably more complex. Although they also arise from cranial nerves (IX and X) which terminate in the NST, most experimental data suggest that the major projection to magnocellular AVP neurons arises from catecholaminergic A1 cells of the VLM that are activated by excitatory interneurons from the NST, although some component might also arise from multisynaptic projections through other areas such as the PBN. Abbreviations: *AC*, anterior commissure; *AP*, area postrema; *AVP*, arginine vasopressin; *MnPO*, median preoptic nucleus; *NST*, nucleus of the solitary tract; *OC*, optic chiasm; *OT*, oxytocin; *OVLT*, organum vasculosum of the lamina terminalis; *PBN*, parabrachial nucleus; *PIT*, anterior pituitary; *PVN*, paraventricular nucleus; *SFO*, subfornical organ; *SON*, supraoptic nucleus; *VLM*, ventrolateral medulla.

However, not all of the drug effects associated with inappropriate antidiuresis are fully understood; indeed, many agents may work by means of a combination of mechanisms. For example, chlorpropamide appears to have both a direct pituitary as well as a renal stimulatory effect, since it has been reported to increase urine osmolality, even in some patients with complete central diabetes insipidus (121,122). Agents that cause AVP secretion through solute depletion, such as thiazide diuretics, are not listed here, since these are generally considered to cause depletion-induced hypoosmolality rather than true SIADH. However, some studies have suggested that in some elderly patients the precipitous hyponatremia occasionally seen after administration of thiazide diuretics is caused by polydipsia and water retention more so than by stimulated Na^+ excretion (123). Whether this represents true SIADH independently of prior ECF volume contraction, as well as whether such cases are typical of a significant portion of patients with diuretic-induced hyponatremia, remains to be determined. A particularly interesting, and clinically important, class of agents associated with SIADH is the selective serotonin reuptake inhibitor (SSRI). Serotonergic agents have been found to increase AVP secretion in rats in some experimental studies (124), but most animal studies have suggested more direct effects on oxytocin rather than AVP secretion (125,126). Some studies of SSRIs in humans have failed to show significant effects on AVP secretion (127), although others support this mechanism (128,129). However, hyponatremia following SSRI administration has been reported almost exclusively in the elderly, at rates as high as 22% to 28% in some studies (130–132), although larger series have suggested an incidence closer to 1 in 200 (133). This therefore suggests the possibility that elderly patients are uniquely hypersensitive to serotonin stimulation of AVP secretion. A similar effect is likely also responsible for the recent reports of severe fatal hyponatremia caused by use of the recreational drug 3,4-methylenedioxymethamphetamine, "Ecstasy" (134–137), since this agent also possesses substantial serotonergic activity (138). Studies of cFos expression in rats indicate that Ecstasy appears to activate hypothalamic magnocellular neurons (139), suggesting direct effects on AVP secretion as the etiology of the SIADH, and recent studies in humans support this mechanism (140).

Pulmonary Disorders

Pulmonary disorders represent a relatively common but frequently misunderstood cause of SIADH. A variety of pulmonary disorders have been associated with this syndrome, but other than tuberculosis (141–143), acute pneumonia (144–147), and advanced chronic obstructive lung disease (148), the occurrence of hypoosmolality has been noted mainly in sporadic case reports. Some bacterial infections appear to be associated with a higher incidence of hyponatremia, particularly *Legionella pneumoniae* (149,150). Although one case of pulmonary tuberculosis has been reported that suggested the possibility that tuberculous lung tissue might synthesize AVP ectopically (151), several other studies have reported that advanced pulmonary tuberculosis is associated with the reset osmostat form of SIADH (106,141), presumably from non-osmotic stimulation of posterior pituitary AVP secretion. Most cases of pulmonary SIADH not associated with either

tuberculosis or pneumonitis have occurred in the setting of respiratory failure. Although hypoxia has clearly been shown to stimulate AVP secretion in animals (152,153), it appears to be less effective as a stimulus in humans (154), in whom the stimulus to abnormal water retention appears to be hypercarbia more so than hypoxia (155–157). When such patients were evaluated serially, the inappropriate AVP secretion was found to be limited to the initial days of hospitalization, when respiratory failure was most marked (145). Even cases of tubercular SIADH generally have occurred in patients with far advanced, active, pulmonary tuberculosis, although interestingly hyponatremia was also found in 74% of a series of patients with miliary tuberculosis (158). Therefore, SIADH in non–tumor-related pulmonary disease generally conforms to the following characteristics: (i) the pulmonary disease will always be obvious as a result of severe dyspnea or extensive radiographically evident infiltrates, and (ii) the inappropriate antidiuresis will usually be limited to the period of respiratory failure; once clinical improvement has begun, free water excretion generally improves rapidly. Mechanical ventilation can cause inappropriate AVP secretion, or it can worsen any SIADH caused by other factors. This phenomenon has been associated most often with continuous positive pressure ventilation (159), but it can also occur to a lesser degree with the use of positive end-expiratory pressure.

Other Causes

One of the most recently described causes of hypoosmolality is the acquired immunodeficiency syndrome (AIDS) or AIDS-related complex (ARC), in patients with human immunodeficiency virus (HIV) infection, with incidences of hyponatremia reported as high as 30% to 38% in adults (160–162) and children (163). Although there are many potential etiologies for hyponatremia in patients with AIDS/ARC, including dehydration, adrenal insufficiency, and pneumonitis, from 12% to 68% of AIDS patients who develop hyponatremia appear to meet criteria for a diagnosis of SIADH (160–162). Not unexpectedly, recent reports have implicated some of the medications used to treat these patients as the cause of the hyponatremia, either via direct renal tubular toxicity or SIADH (164,165).

A recent series of reports have documented a surprisingly high incidence of hyponatremia during endurance exercise events such as marathon (166) and ultramarathon (167) racing, triathlons (168), forced marching (169), and hiking (170). Occasionally this has caused fatal outcomes associated with hyponatremic encephalopathy from acute brain edema (171,172). Most studies support excess drinking during exercise as the major cause of the induced hyponatremia (173,174), but whether the water retention under such conditions is also contributed to by a degree of SIADH as a result of multiple potential non-osmotic stimuli (e.g., volume depletion, nausea, increased cytokine levels) has not been definitively ascertained and remains a subject of ongoing study.

Unexplained or idiopathic causes account for a relatively small proportion of all cases of SIADH. Although the etiology of the syndrome may not be diagnosed initially in many cases, the numbers of patients in whom an apparent cause cannot be established after consistent follow-up over time are relatively few. One exception to this appears to be elderly patients who sometimes develop SIADH without any apparent underlying etiology (175–177). Coupled with the significantly increased incidence of hyponatremia in geriatric patients (9,16,18,104,178), this suggests that the normal aging process may be accompanied by abnormalities of regulation of AVP secretion that predispose to SIADH. Such an effect could potentially account for the fact that virtually all causes of drug-induced hyponatremia occur much more frequently in elderly patients (94,179,180). In several recent series of elderly patients meeting criteria for SIADH, 40% to 60% remained idiopathic despite rigorous evaluation (181–183), leading some to conclude that extensive diagnostic procedures were not warranted in such elderly patients if routine history, physical examination, and laboratory evaluation failed to suggest a diagnosis (181).

Some well-known stimuli of AVP secretion are notable primarily because of their exclusion from Table 86-4. Despite unequivocal stimulation of AVP secretion by nicotine (184), cigarette smoking has only rarely been associated with SIADH, and primarily in psychiatric patients who have several other potential causes of inappropriate AVP secretion (45,185,186). This is in part because of chronic adaptation to the effects of nicotine, but also because the short half-life of AVP in plasma (approximately 15 minutes in humans [187]) limits the duration of antidiuresis produced by relatively short-lived stimuli such as smoking. Although nausea remains the most potent stimulus to AVP secretion known in man (188), chronic nausea is rarely associated with hypoosmolality unless accompanied by vomiting with subsequent ECF solute depletion followed by ingestion of hypotonic fluids (189). Similar to smoking, this is probably attributable the short half-life of AVP, but also to the fact that most such patients are not inclined to drink fluids under such circumstances. However, hyponatremia can occur when such patients are infused with high volumes of hypotonic fluids. This is likely a factor contributing to the hyponatremia that often occurs in cancer patients who are receiving chemotherapy (112). Finally, a causal relation between stress and SIADH has often been suggested, but never conclusively established. This underscores the fact that stress—independent of associated nausea, dehydration, or hypotension—is not a major stimulus causing sustained elevations of AVP levels in humans (190).

Pathophysiology

Sources of Arginine Vasopressin Secretion

Disorders that cause inappropriate antidiuresis secondary to elevated plasma AVP levels can be subdivided into those associated with either paraneoplastic ("*ectopic*") or pituitary AVP hypersecretion. Most ectopic production is from tumors, and there is conclusive, cumulative evidence that tumor tissue can, in fact, synthesize AVP: (i) tumor extracts have been found to possess antidiuretic hormone bioactivity and immunologically recognizable AVP and neurophysin, which is synthesized with AVP as part of a common precursor (191–193); (ii) electron microscopy has revealed that many tumors possess secretory granules; and (iii) cultured tumor tissue has been shown to synthesize not only AVP (194) but also the entire AVP prohormone (provasopressin [195,196]). Although it is thus clear that some tumors can produce AVP, it is not certain that all tumors associated with SIADH do so, because only about half of small cell carcinomas have been found to contain AVP immunoreactivity (197) and many of the tumors listed in Table 86-4 have not been studied as extensively as have bronchogenic carcinomas. The only nonneoplastic disorder that has been reported to possibly cause SIADH by means of ectopic AVP production is tuberculosis. However, this is based on studies of a single patient in whom extracts of tuberculous lung tissue were shown by bioassay to possess antidiuretic activity (151).

Pituitary Arginine Vasopressin Secretion: Inappropriate versus Appropriate

In the majority of cases of SIADH, the AVP secretion originates from the posterior pituitary. However, this is also true of greater than 90% of all cases of hyponatremia, including patients with hypovolemic and hypervolemic hyponatremia (14).

This raises the question of what exactly is "*inappropriate*" AVP secretion (92,198). It is well known that AVP secretion is most sensitively stimulated by increases in osmolality, but also occurs in response to a wide variety of non-osmotic stimuli, including hypotension, hypovolemia, nausea, hypoglycemia, angiotensin, and probably other stimuli yet to be discovered (199) (see Chapter 3). Consequently, AVP secretion in response to a hypovolemic stimulus such as hemorrhage is clearly physiologically "appropriate," but when it leads to symptomatic hyponatremia from secondary water retention it could be considered to be "inappropriate" for osmotic homeostasis. Despite such semantic difficulties, it is important that the criteria for diagnosing SIADH remain as originally described, specifically excluding other clinical conditions that cause known impairments in free water excretion, *even when* these are mediated by a secondary stimulation of AVP secretion via known physiologic mechanisms (e.g., hypovolemia, hypotension, hypocortisolism, edema-forming states, hypothyroidism, etc.). Without maintaining these distinctions, arguable as some of them may be, the definition of SIADH would become too broad to retain any degree of practical clinical usefulness.

Although measurable plasma AVP levels are found in most patients with SIADH, they are rarely elevated into pathologic ranges in most cases, even those associated with ectopic AVP production from tumors. Rather, in the majority of cases of SIADH, plasma AVP levels remain in "normal" physiologic ranges, which only become abnormal under hypoosmolar conditions when plasma AVP levels should be suppressed into unmeasurable ranges (Fig. 86-3). This is important for several reasons. First, the well known vasoconstrictive effects of AVP do not come into play until much higher plasma levels are achieved (30 to 80 pg/mL [200]), whereas maximal antidiuresis is achieved with much lower levels (5 to 10 pg/mL). Consequently, it is unlikely that any of the clinical manifestations of hyponatremia can be ascribed to vasopressor effects of AVP. In this regard, it is particularly worrisome that most animal models of induced hyponatremia have employed pharmacologic doses of AVP, which generally elevate plasma AVP levels well into vasopressor ranges, raising the possibility that some results of previous studies of experimental hyponatremia were due to activation of AVP V_1 vascular and hepatic receptors. Recent results that demonstrate the absence of mortality when hyponatremia is induced in animals using the V_2-selective agonist desmopressin (dDAVP) (70), or using vasopressin infusions that maintain plasma AVP levels at lower ranges (201), emphasize the need to take potential vasopressor effects of vasopressin into consideration in the interpretation of past and future studies. Second, the presence of "normal" plasma AVP levels, or of only mildly elevated urine osmolalities, cannot be used as arguments against SIADH as an etiology for hyponatremia. Low but nonsuppressible levels of AVP can clearly cause sufficient impairment of free water excretion to produce hypoosmolality when exogenous fluid intakes are high, as in psychiatric patients with polydipsia (202). Recent studies of patients with SIADH and hypopituitarism have measured high nonsuppressible levels of urinary aquaporin-2 excretion that correlated with their impaired water excretion, supporting persistent activation of AVP V_2 receptors as the cause of the water retention (203).

Patterns of Arginine Vasopressin Secretion

Studies of plasma AVP levels in patients with SIADH during graded increases in plasma osmolality produced by hypertonic saline administration have suggested four patterns of secretion (Fig. 86-5): (i) random hypersecretion of AVP; (ii) a "reset osmostat" system, whereby AVP is secreted at an abnormally low threshold of plasma osmolality but otherwise displays a normal response to relative changes in osmolality; (iii) inappropriate hypersecretion below the normal threshold for AVP release, but normal secretion in response to osmolar changes within normal ranges of plasma osmolality; and (iv) low or undetectable plasma AVP levels despite classic clinical characteristics of SIADH (109,204). The first pattern simply represents unregulated AVP secretion, which is often, but not always, observed in patients exhibiting paraneoplastic AVP production. Resetting of the osmotic threshold for AVP secretion has been well described with volume depletion (205,206) and also has been shown to occur in various edema-forming states, presumably as a result of decreases in EABV (48,52,207). However, most patients with a reset osmostat are clinically euvolemic (105,106). It has been suggested that chronic hypoosmolality itself may reset the intracellular threshold for osmoreceptor firing, but studies in animals have not supported a major role for this mechanism, since chronic hyponatremia does not appear to significantly alter the osmotic threshold for AVP secretion (208,209). Perhaps the best-known physiologic example of a reset osmostat for AVP secretion is the hypoosmolality and hyponatremia that occurs during late pregnancy. Despite intensive studies over many years to identify potential hormonal factors that might be responsible for this resetting, a single factor has not yet been identified (210), although recent studies have indicated that the placental hormone relaxin causes a stimulation of AVP and oxytocin secretion that closely resembles the reset osmostat pattern of AVP secretion (211,212). Perhaps the most perplexing aspect of the reset osmostat pattern is its occurrence in patients with tumors, which suggests that some of these cases represent tumor-stimulated pituitary AVP secretion rather than paraneoplastic AVP secretion (109,204,213). The pattern of SIADH that occurs without measurable AVP secretion is not yet well understood. This form of the syndrome may be attributable to the secretion of AVP with some bioactivity but altered immunoreactivity, to the presence of other circulating antidiuretic factors, to increased renal sensitivity to very low circulating levels of AVP, or possibly to constitutively activating mutations of the AVP V_2 receptor (110). A sufficient number of patients with this form of the disorder has not been studied to form any basis for discrimination among these possibilities, but the positive response of one such patient to a

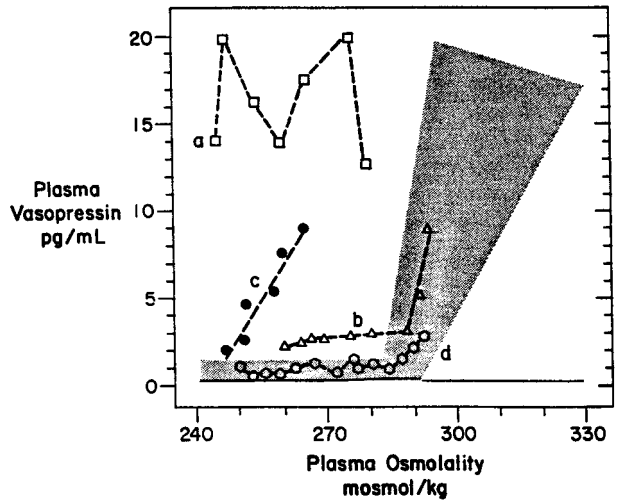

FIGURE 86-5. Schematic summary of different patterns of AVP secretion in patients with SIADH. Each line (*a–d*) represents the relation between plasma AVP and plasma osmolality of individual patients in whom osmolality was increased by infusion of hypertonic NaCl. The *shaded area* represents plasma AVP levels in normal subjects over physiologic ranges of plasma osmolality. (Reprinted from: Robertson GL. Thirst and vasopressin function in normal and disordered states of water balance. *J Lab Clin Med* 1983;101:351, with permission.)

vasopressin V_2-receptor antagonist suggests that at least some of these cases may represent increased renal sensitivity to low circulating levels of AVP (214). Despite these well-described patterns of abnormal AVP secretion in SIADH, it is surprising that no correlation has been found between any of these four patterns and the various etiologies of the syndrome (109).

Stimuli to Arginine Vasopressin Secretion in Patients with SIADH

Regardless of the pattern of pituitary AVP secretion, and whether this represents an "inappropriate" or physiologically "appropriate" secretion, it is important to try to identify the cause(s) of the continued AVP secretion in patients with this disorder. Because of the variety of stimuli that can stimulate AVP secretion independent of osmolality, it seems logical to hypothesize that SIADH can be caused by continued non-osmotic stimulation of AVP secretion despite the presence of plasma hypoosmolality. The effect of hypovolemia to lower the threshold and increase the sensitivity of osmotically stimulated AVP secretion is well known, and this mechanism almost certainly accounts for the elevated plasma AVP levels in patents with edema-forming disorders in whom a decreased EABV activates baroreceptor-mediated AVP secretion (49,50). Tumor interference with vagal pathways to brainstem baroreceptive centers could conceivably mimic or exaggerate such hypovolemic conditions, potentially accounting for the occurrence of a reset osmostat pattern of AVP secretion found in some patients with cancers. Recent reports of a 3% to 4% incidence of SIADH in patients with advanced head and neck malignancies represents a group in which some, although clearly not all (215), of the hyponatremia might also be secondary to interference with vagal baroreceptor pathways (116). However, not all cases of SIADH can be comfortably ascribed to nonosmotic stimuli, since it is difficult to identify any such possible stimuli in many patients. Another possibility is that brain pathways conveying afferent signals that actively inhibit AVP secretion from hypothalamic magnocellular neurons may be impaired in some patients. Substantial data support the likelihood that hypoosmolality does not simply lead to decreased AVP secretion by virtue of absence of excitatory osmoreceptor inputs, but rather represents a state of active inhibition of the AVP-secreting neurons (216), possibly via endogenous opioid (217) or γ-aminobutyric acid (GABA) pathways (119,218). In this case, it would be easy to imagine that impairments or alterations in the activity of these inhibitory pathways might allow continued AVP secretion despite hypoosmolality. Although such abnormalities have not yet been identified, there is one clinical situation in which a decreased inhibitory tone to AVP neurons does clearly lead to enhanced AVP secretion: Elderly patients have decreased AVP responses to orthostasis but exaggerated responses to osmotic stimuli (219,220). The latter is presumably due to a diminution of inhibitory, as well as excitatory, inputs from brainstem baroreceptive centers to the hypothalamus, thereby producing an unopposed stimulation by osmotic stimuli from the anterior hypothalamus (Fig. 86-4). This phenomena could contribute to the unusually high frequency of SIADH seen in elderly individuals (9,16–18,178). Despite our lack of precise information about the mechanisms responsible for osmotically inappropriate AVP secretion, it seems certain that this will prove to be a heterogeneous group of processes rather a single dominant cause (221).

Contribution of Natriuresis to the Hyponatremia of SIADH

Since the original cases studied by Schwartz and Bartter, increased renal Na^+ excretion has been viewed as one of the cardinal manifestations of SIADH, indeed one which later became embedded in the requirements for its diagnosis (2). However, next to the use of the term "inappropriate," probably no other aspect of SIADH has been so widely misinterpreted. Demonstration that the natriuresis accompanying administration of antidiuretic hormone is not due to AVP itself but rather to the volume expansion produced as a result of water retention was unequivocally shown by Leaf, even before the description of the clinical occurrence of this disorder (53). Subsequent metabolic balance studies demonstrated that excess urinary Na^+ excretion and a negative Na^+ balance occurred during the development of hyponatremia in patients with SIADH, but eventually urinary sodium excretion simply reflected daily sodium intake (1). Therefore, patients appear to exhibit "renal sodium wasting" because they continue to excrete sodium despite being hyponatremic, but in reality they have simply achieved a new steady-state in which they are in neutral sodium balance, albeit at a lower serum [Na^+]. Although this interpretation is now supported by abundant clinical and experimental evidence, several important questions remain unanswered regarding natriuresis and hyponatremia: What physiologic and/or pathophysiologic mechanisms underlie the natriuresis? Is natriuresis in SIADH always secondary to AVP-induced water retention or is hyponatremia sometimes caused primarily by Na^+ losses? Even when natriuresis is secondary to water retention, can the natriuresis further aggravate the hyponatremia?

As described previously, studies of long-term antidiuretic-induced hyponatremia in both dogs and rats have indicated that a larger proportion of the hyponatremia is attributable to secondary Na^+ losses rather than to water retention (87,88). However, it is important to appreciate that in these models the natriuresis actually did not worsen the hyponatremia, but rather allowed volume regulation of blood and ECF volumes to occur. Therefore, over long periods, what begins as a "purely" dilutional hyponatremia from water retention becomes a mixed hyponatremia in which urinary solute losses allow maintenance of equivalent levels of hyponatremia but with lesser degrees of volume expansion due to water retention. Much of the past difficulty in consistently demonstrating expanded plasma or ECF volumes in patients with SIADH using tracer dilution techniques (89–91) can probably be ascribed to this process. It has become clear that intrinsic renal mechanisms are capable of producing both diuresis and natriuresis in response to increases in renal perfusion pressures (so-called "*pressure diuresis*"); this mechanism has been shown to underlie the renal escape from antidiuresis produced when AVP-infused animals are continually fluid loaded (55). However, it has not yet been proved whether this mechanism is sufficiently sensitive to detect the relatively mild degrees of volume expansion that accompany dilutional hyponatremias. Another, not mutually exclusive, possibility is that the natriuresis is mediated via increases in circulating natriuretic peptides such as atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP). Most cases of SIADH have been shown to have elevated levels of these peptides into ranges that are capable of promoting renal sodium excretion (56,57,222).

The degree to which hyponatremia occurs primarily as a result of natriuresis has remained controversial over many years. *Cerebral salt wasting* syndrome was first proposed by Peters in 1950 (223) as an explanation for the natriuresis and hyponatremia that sometimes accompanies intracranial disease, particularly subarachnoid hemorrhage (SAH), in which up to one-third of patients often develop hyponatremia. Following the first clinical description of SIADH in 1957, such patients were generally assumed to have hyponatremia secondary to AVP hypersecretion with a secondary natriuresis (224). However, over the last decade, clinical and experimental data have suggested that some patients with SAH and other intracranial diseases may actually have a primary natriuresis leading to volume contraction rather than SIADH (225–228), in which case the elevated measured plasma AVP levels may actually be

physiologically appropriate for the degree of volume contraction present. The major clinical question as to the frequency of cerebral salt wasting syndrome as a cause of hyponatremia is dependent on the criteria used to assess the ECF volume status of these patients; opponents argue that there is insufficient evidence of true hypovolemia despite ongoing natriuresis (229), whereas proponents argue that the combined measures that have traditionally been used to estimate ECF volume do in fact support the presence of hypovolemia in many cases (230,231). With regard to the potential mechanisms underlying the natriuresis, both plasma and cerebrospinal fluid (CSF), ANP and BNP levels are clearly elevated in many patients with SAH (228,232–234), and have been found to correlate variably with hyponatremia in patients with intracranial diseases (228,234,235). However, because SIADH also is frequently associated with elevated plasma ANP and BNP levels, this finding alone does not prove causality. Ample precedent certainly exists for hyponatremia due to Na^+ wasting with secondary antidiuresis in Addison's disease, as well as diuretic-induced hyponatremia. Characteristic of these disorders, normalization of ECF volume with isotonic NaCl infusions restores plasma tonicity to normal ranges by virtue of shutting off secondary AVP secretion. If hyponatremia in patients with SAH occurred via a similar mechanism, it should also respond to this therapy. However, recent studies indicate that it does not. Nineteen patients with SAH were treated with large volumes of isotonic saline sufficient to maintain plasma volume at normal or slightly elevated levels, but despite removal of any volemic stimulus to AVP secretion, 32% still developed hyponatremia in association with nonsuppressed plasma AVP levels, an incidence equivalent to that found in previous studies of SAH (236). In contrast, other studies have demonstrated that mineralocorticoid therapy to inhibit natriuresis can reduce the incidence of hyponatremia in patients with subarachnoid hemorrhage (237); such results are not unique to patients with intracranial diseases, since a subset of elderly patients with SIADH have also been shown to respond favorably to mineralocorticoid therapy (238). Although seemingly disparate, these types of results support the existence of disordered AVP secretion as well as a coexisting stimulus to natriuresis in many such patients. It seems most likely that SAH and other intracranial diseases represent a mixed disorder in which some patients have *both* exaggerated natriuresis and inappropriate AVP secretion; which effect predominates in terms of the clinical presentation will depend on their relative intensities as well as the effects of concomitant therapy. The possibility of ANP- or BNP-induced natriuresis aggravating hyponatremia is not confined to intracranial diseases, and it has been suggested that ectopic ANP production might contribute to, or even cause, the hyponatremia accompanying some small cell lung cancers (239). In support of this, several recent studies have analyzed tumor cell lines from patients with hyponatremia and small cell lung carcinoma and found that many produced ANP or ANP mRNA in addition to, or in some cases instead of, AVP (240–242). These data allow the possibility that some patients with tumors may also develop hyponatremia as a result of ectopic ANP secretion. However, in clinical studies of such patients, the hyponatremia appears to correlate more with the plasma AVP levels than the plasma ANP levels (243). Consequently, it seems likely that such cases represent a mixture of inappropriate secretion of both hormones, analogous to patients with cerebral salt wasting, in which case the ANP and BNP could act to further exacerbate the secondary natriuresis produced primarily by AVP-induced water retention.

ADRENAL INSUFFICIENCY

The frequent occurrence of hyponatremia in patients with adrenal insufficiency was appreciated well before the discovery of the role of AVP in hypoosmolar disorders (244). Incidences as high as 88% have been reported in patients with primary adrenal insufficiency, particularly during episodes of "*Addisonian crisis*" (245,246). This section will summarize the factors related to the development of hyponatremia in patients with adrenal insufficiency; a more complete description of many of the studies upon which these conclusions are based can be found in an earlier edition of this textbook (247).

Etiology

The adrenal cortex produces many different types of corticosteroids, which can be broadly divided into three categories: glucocorticoids, mineralocorticoids, and androgens. Only the first two of these have been found to have significant effects on body fluid homeostasis. Disorders of impaired adrenal function can be divided into those in which the adrenal gland itself is damaged or destroyed, or *primary adrenal insufficiency*, and those in which the adrenal does not receive appropriate adrenocorticotropic hormone (ACTH) stimulation from the pituitary, or *secondary adrenal insufficiency*. Addison's disease is the major cause of primary adrenal insufficiency, and hypopituitarism is the best example of secondary adrenal insufficiency. The clinical presentation of these two types of adrenocortical insufficiency varies significantly, because adrenal destruction causes loss of both mineralocorticoids and glucocorticoids, whereas pituitary insufficiency causes only glucocorticoid insufficiency; this is because pituitary ACTH is not necessary for mineralocorticoid secretion, which is controlled primarily via the renin–angiotensin system. To understand the fluid and electrolyte abnormalities that accompany these disorders, the pathophysiology of hyponatremia due to mineralocorticoid and glucocorticoid deficiency must be considered separately.

Pathophysiology

Mineralocorticoid Deficiency

The absence of aldosterone impairs Na^+-K^+ exchange in the distal tubule. Because this defect occurs distally in the nephron, it cannot be completely compensated for by later Na^+ reabsorption, leading to the continued renal Na^+ excretion that is the hallmark of adrenal insufficiency (248). As long as sodium intake is sufficient to replace the ongoing renal losses, patients with mineralocorticoid insufficiency remain relatively stable. However, when sodium intakes are not sufficient, adrenally insufficient patients develop progressive hypovolemia, hyponatremia, and hyperkalemia, the classic fluid and electrolyte manifestations of Addisonian crisis (245,246). Proof that these effects were indeed caused primarily by the renal Na^+ losses was documented long ago by studies in animals (248,249) and Addisonian patients (250), which demonstrated that all of these abnormalities could be prevented by volume repletion with NaCl. However, the water retention of mineralocorticoid deficiency has multiple potential causes: (i) loss of aldosterone-mediated Na^+ reabsorption in the distal tubule impairs urinary dilution, similar to the use of thiazide diuretics, (ii) ECF volume contraction as a result of the Na^+ losses causes increased fluid reabsorption in the proximal tubule with decreased delivery to the distal diluting segments of the nephron, and (iii) ECF volume contraction also stimulates baroreceptor-mediated (i.e., non-osmotic) AVP secretion with resultant antidiuresis.

Numerous experimental studies have documented elevated plasma AVP levels despite hypoosmolality in adrenalectomized animals with mineralocorticoid insufficiency (251–253), and the elevated AVP levels generally return to normal ranges following volume replacement with NaCl (251). Proof that the elevations in plasma AVP levels were causally related to the

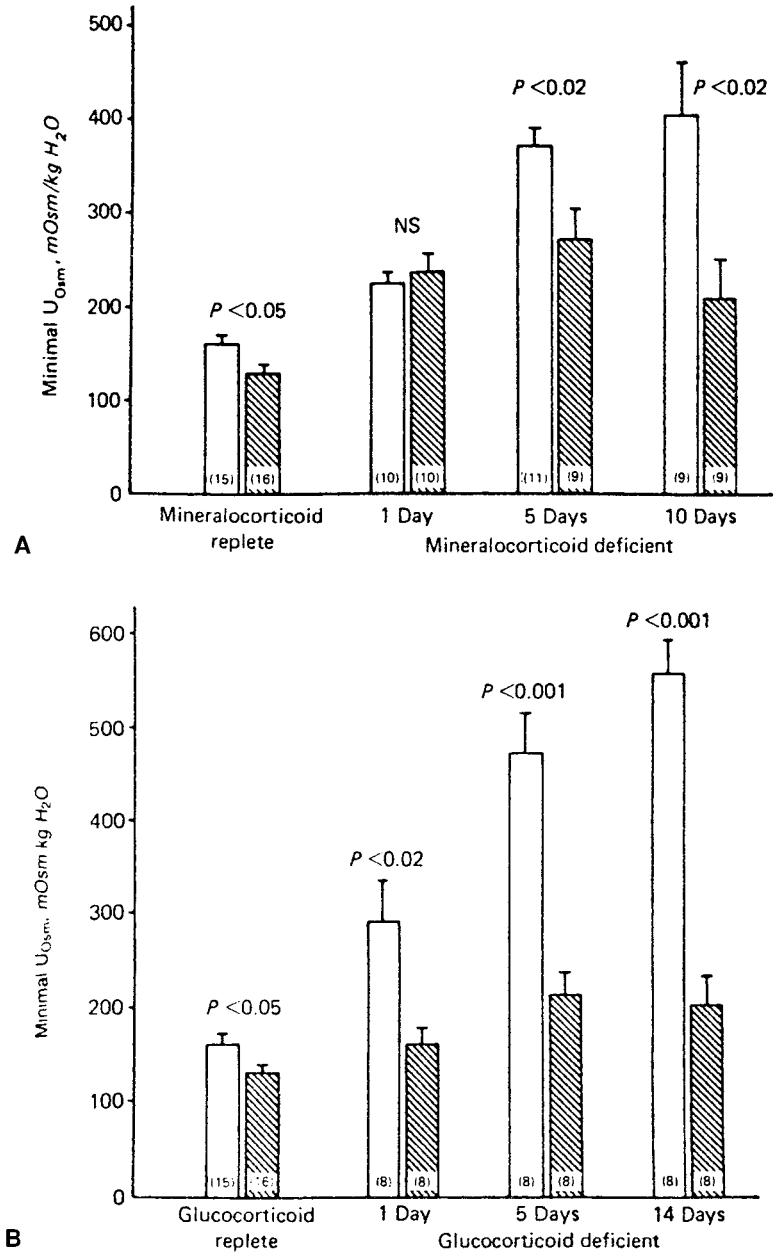

FIGURE 86-6. Effect of an AVP V_2 receptor antagonist on urine osmolality following an acute water load in adrenalectomized rats selectively replaced with either mineralocorticoids or glucocorticoids. The *open bars* depict vehicle-treated rats and the *hatched bars* the AVP antagonist-treated rats. The mineralocorticoid-deficient rats (**A**) demonstrated an impaired urinary dilution that became progressively worse over the 10 days of study; treatment with the AVP receptor antagonist significantly reduced the minimum urine osmolality, but not until 5 days after the adrenalectomy, consistent with a hypovolemia-mediated stimulation of AVP secretion by this time. The glucocorticoid-deficient rats (**B**) demonstrated impaired urinary dilution, which also became progressively worse over the 14 days of study, but in contrast to the mineralocorticoid-deficient rats the minimum urine osmolality returned toward normal ranges after treatment with the AVP antagonist as early as 1 day after the adrenalectomy, indicating a different type of non-osmotic stimulation of AVP secretion in these rats. (Reprinted from: Ishikawa S, Schrier RW. Effect of arginine vasopressin antagonist on renal water excretion in glucocorticoid and mineralocorticoid deficient rats. *Kidney Int* 1982;22:587, with permission.)

water retention was provided by studies in which adrenalectomized rats replaced with only glucocorticoids were given a vasopressin-V_2 receptor antagonist (254) (Fig. 86-6); the antagonist significantly reduced urine osmolality in chronically, but not acutely, mineralocorticoid-deficient rats, consistent with hypovolemia-mediated stimulation of AVP secretion as a result of progressive Na^+ depletion over time. Conversely, AVP-independent effects appear to play some role in the water retention as well. Studies in adrenalectomized homozygous Brattleboro rats, which cannot synthesize AVP, have demonstrated normalization of urine dilution, free water clearance, and solute clearance following physiologic aldosterone, but not glucocorticoid, replacement (255). These results demonstrate the contribution of multiple factors such as impaired urinary dilution due to the loss of aldosterone-mediated Na^+ reabsorption in the distal tubule, and increased proximal tubular fluid reabsorption as a result of hypovolemia, to the impaired water excretion of mineralocorticoid deficiency. The latter factor would be predicted to be reversed by volume repletion but not the former, possibly accounting for the observation that in some studies human patients with primary adrenal insufficiency still maintained higher urine osmolalities even under conditions of volume expansion (256), although other studies in humans (250) and animals (257) have shown complete normalization of water excretion following volume expansion. Whatever the contribution of these additional factors, it nonetheless seems appropriate to conclude that the major mechanism responsible for the impaired water excretion of mineralocorticoid deficiency is hypovolemia-stimulated AVP secretion.

Glucocorticoid Deficiency

As described previously, isolated glucocorticoid deficiency generally occurs with pituitary disorders that impair normal adrenocorticotropic hormone (ACTH) secretion but leave other stimuli to aldosterone secretion intact. That glucocorticoid deficiency alone could also impair water excretion was recognized based on longstanding clinical observations that anterior pituitary insufficiency ameliorates, and sometimes even completely masks, the polyuria of patients with coexistent

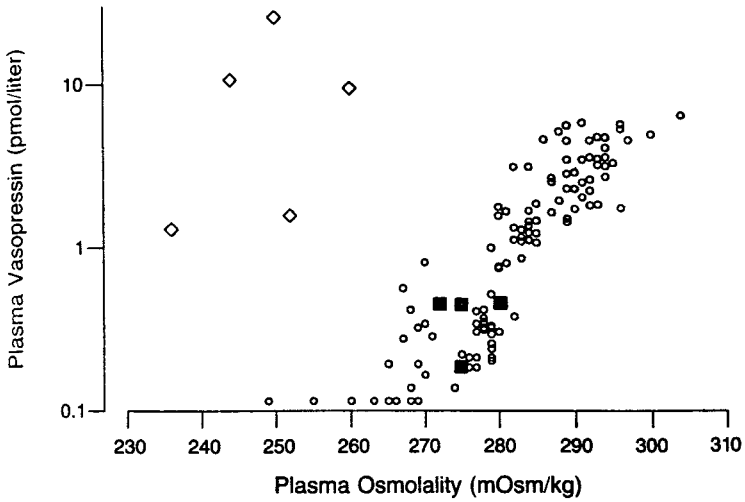

FIGURE 86-7. Plasma AVP levels as a function of plasma osmolality in patients with hypopituitarism and adrenocorticotropic hormone (ACTH) insufficiency. The *diamonds* show patients with untreated hypopituitarism and the *solid squares* the same patients after hydrocortisone therapy. The *open circles* depict AVP levels in normal subjects over physiologic ranges of plasma osmolality. In comparison with Figure 85-3, it is apparent that these patients would be indistinguishable from those with SIADH based on their plasma AVP-osmolality relation. (Reprinted from: Oelkers W. Hyponatremia and inappropriate secretion of vasopressin [antidiuretic hormone] in patients with hypopituitarism. *N Engl J Med* 1989;321:492, with permission.)

central diabetes insipidus (258,259). It is not surprising, therefore, that hyponatremia occurs relatively frequently in hypopituitary patients without diabetes insipidus (103,260–262). However, hypopituitary patients generally do not develop ECF volume contraction, since they maintain adequate aldosterone secretion to prevent renal Na^+ wasting. Consequently, volume replacement with NaCl does not reverse the impaired water excretion of patients with secondary adrenal insufficiency (256) as it does in primary adrenal insufficiency.

Despite the lack of an apparent hypovolemia-mediated stimulus to AVP secretion, nonetheless non-osmotic AVP secretion has been strongly implicated in the impaired water excretion of glucocorticoid insufficiency. Elevated plasma AVP levels have clearly been documented in animals (263) and patients (111) with hypopituitarism (Fig. 86-7). Similarly, since primary adrenal insufficiency has components of both mineralocorticoid and glucocorticoid deficiency, adrenalectomized animals maintained only on physiologic replacement doses of mineralocorticoids also have been found to have inappropriately elevated plasma AVP levels (264,265). That these elevated AVP levels were causally related to the impaired water excretion was again proved by studies using an AVP V_2 receptor antagonist, which demonstrated near normalization of urinary dilution in adrenalectomized mineralocorticoid-replaced rats (254) (Fig. 86-6). However, as with mineralocorticoid deficiency, AVP-independent mechanisms have also been suggested to play a role in the impaired water excretion of glucocorticoid deficiency, since Brattleboro rats maintained on aldosterone had somewhat decreased urine flow, which increased following glucocorticoid replacement (255). Because ECF volume depletion is generally not a manifestation of glucocorticoid deficiency, other factors must therefore be responsible for the AVP-independent aspects of the water retention. The possibility that glucocorticoids exerted direct effects on renal tubular epithelium, such that glucocorticoid insufficiency causes increased water permeability in the collecting tubules even in the absence of AVP has been suggested (256). However studies on isolated collecting tubules have failed to demonstrate any significant influence of glucocorticoids on water permeability of this tissue (266). Consequently, the AVP-independent effects of glucocorticoid insufficiency remain poorly defined at the present time.

Regardless of the etiology of the AVP-independent defect in water excretion, the major mechanism responsible for the impaired water excretion of glucocorticoid deficiency appears to be non-osmotically stimulated AVP secretion. However, the stimulus to AVP secretion under these conditions also remains unclear. Studies of prolonged glucocorticoid insufficiency in dogs have shown an increased pulse pressure and decreased cardiac stroke volume (264), and similar studies in rats have suggested decreases in cardiac index along with increased systemic vascular resistance (265). Although these findings differ somewhat, in both cases they raise the possibility of hemodynamically mediated effects on AVP secretion. Alternatively, glucocorticoid deficiency might directly stimulate AVP secretion via two possible mechanisms. First, both clinical (267) and experimental (268) studies have shown a modest but significant effect of glucocorticoids to inhibit pituitary AVP secretion. Presumably this is mediated via glucocorticoid receptors that have been localized in magnocellular neurons (269); interestingly, recent studies have shown that these receptors are increased during induced hypoosmolality, suggesting that glucocorticoids may play a role in the inhibition of AVP secretion under hypoosmolar conditions (270). Second, in the absence of glucocorticoid feedback inhibition of the parvocellular AVP neurons that project to the median eminence rather than to the posterior pituitary, AVP content increases markedly in this area (271,272). This presumably reflects increased secretion of AVP into the pituitary portal blood system in order to stimulate pituitary ACTH secretion (273–276). Because the pituitary portal blood eventually drains into the systemic circulation, increased levels of AVP released from the median eminence could therefore increase plasma AVP levels sufficiently to produce inappropriate antidiuresis; it is important to remember that such levels need not be very high, but simply inappropriate for the plasma osmolality, as shown in Figure 86-7.

HYPOTHYROIDISM

Although hypothyroidism is considerably more common than adrenal insufficiency, hyponatremia secondary to hypothyroidism occurs much less frequently than hyponatremia from adrenal insufficiency. The infrequent occurrence of hyponatremia with hypothyroidism has led some investigators to question whether hypothyroidism is in fact causally related to hyponatremia (277), but this is likely a manifestation of the fact that impaired water excretion is only seen in more severely hypothyroid patients. Typically such patients are elderly and meet criteria for *"myxedema coma"* as a result of their altered mental status (278–280). This section will summarize the factors related to the development of hyponatremia in patients with hypothyroidism; a more complete description of many of the studies upon which these conclusions are based can be found in an earlier edition of this textbook (247).

Etiology

Similar to adrenal insufficiency, hypothyroidism can result from either dysfunction or damage to the thyroid gland itself, or *primary hypothyroidism*, or from inadequate thyrotropin-stimulating hormone (TSH) stimulation from the pituitary, or *secondary hypothyroidism*. In addition, like adrenal insufficiency, there can be significant differences in the presentation of these two disorders. However, since the only biologically active products of the thyroid gland are the hormones thyroxine (T_4) and triiodothyronine (T_3), in this case the clinical variations are due mainly to quantitative differences in the severity of the thyroid hormone deficiency rather than to qualitative differences in the nature of the hormone deficits. With moderate degrees of hypothyroidism, patients with both primary and secondary disease have similar signs and symptoms of thyroid hormone deficiency (e.g., cold intolerance, increased fatigue, dry skin, constipation, etc.), but generally only patients with primary hypothyroidism progress to more severe degrees of myxedema, including the life-threatening metabolic and neurologic abnormalities of myxedema coma, whereas these extreme manifestations are virtually never seen with secondary hypothyroidism. This is because severe myxedema occurs only after plasma T_4 and T_3 levels have fallen to very low levels, often <1 μg/dL. This scenario can easily occur with primary hypothyroidism, since in the absence of thyroid tissue there is no alternative source of thyroid hormone production. However, T_4 and T_3 levels never decrease as severely in hypopituitary patients who simply lack TSH, and frequently plasma levels remain just at or slightly below the lower limits of normal (281). This likely reflects either some degree of constitutive thyroid hormone synthesis by the thyroid gland, or possibly low grade stimulation of TSH receptors by other circulating substances, analogous to the thyrotoxicosis produced by thyroid stimulating immunoglobulins in patients with Graves' disease. Because hyponatremia is seen only in hypothyroid patients who have progressed to severe degrees of myxedema, it follows that this manifestation generally occurs in patients with primary hypothyroidism. When hyponatremia accompanies hypopituitarism it is usually a manifestation of secondary adrenal insufficiency from glucocorticoid deficiency rather than coexisting hypothyroidism (102,282).

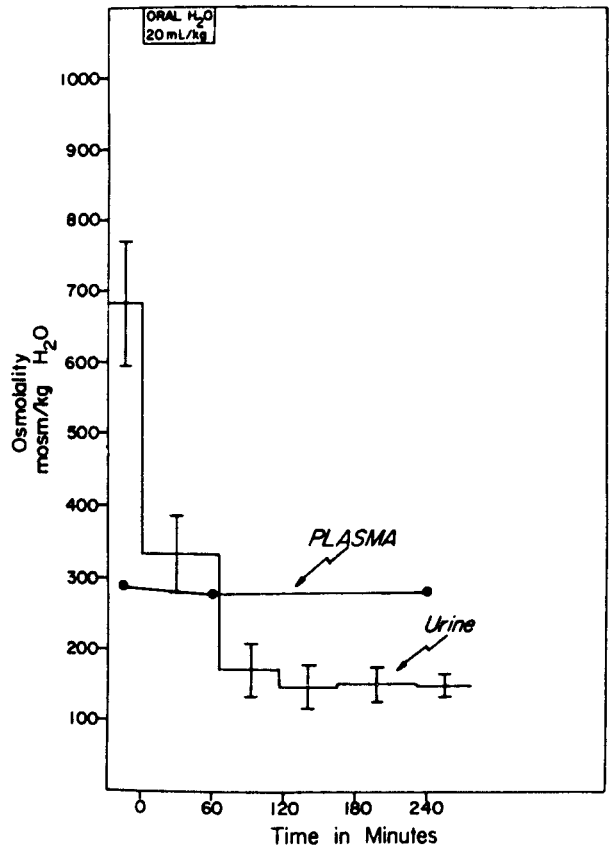

FIGURE 86-8. Mean plasma and urine osmolalities in 16 patients with untreated myxedema for 6 hours following an oral water load (20 mL/kg body weight). Urine osmolalities decreased significantly to less than 200 mOsmol/kg H_2O by 4 hours after the water load, indicating fairly intact renal diluting mechanisms in these patients. (Reprinted from: Derubertis FR, Jr, Michelis MF, Bloom ME, et al. Impaired water excretion in myxedema. *Am J Med* 1971;51:41, with permission.)

Pathophysiology

Several studies have clearly confirmed abnormalities of water excretion in hypothyroid patients. However, in almost all cases the abnormality was found to consist of a delayed excretion of water rather than major impairments in urinary dilution (283–285). This was best shown in the studies of DeRubertis et al., in which near normal urinary dilution occurred following water loading in hypothyroid patients (Fig. 86-8), even though cumulative excretion of the water load in the hypothyroid patients lagged far behind that of euthyroid controls (39.8 ± 5.1% versus 78.7 ± 5.7%) after 2 hours (284). Similar results have been found in studies of hypothyroid rats (286,287). Experimental studies in hypothyroid animals have implicated decreases in renal blood flow and GFR as the primary factors responsible for the delayed water excretion. In particular, the relation between free water clearance and distal tubular Na^+ delivery was found to be identical in hypothyroid and euthyroid rats, suggesting that the observed impairments in water excretion were likely secondary to reduced delivery of glomerular filtrate to the distal nephron in the hypothyroid rats (286). These results are consistent with findings of a decreased GFR in severely hypothyroid patients (284,285,288–290), which is most likely due to decreased renal blood flow as a result of the compromised cardiac output and increased peripheral vascular resistance known to occur in severely hypothyroid patients (291–293). Experimental studies have also supported this hypothesis, since a variety of maneuvers that increase distal tubular fluid delivery, for example, carbonic hydrase inhibition, isotonic saline infusion, and unilateral nephrectomy, all markedly increase free water clearance in hypothyroid rats (286,294,295). Therefore, similar to patients with edema-forming states, hypothyroid patients have increased proximal Na^+ and water absorption as a result of decreased EABV with subsequent decreased delivery of tubular fluid to the distal diluting sites of the nephron, thereby accounting for much of their impaired rate of water excretion.

As noted earlier, patients with edema-forming states also have baroreceptor-mediated stimulation of AVP secretion that leads to further impairment of free water excretion by preventing maximal urinary dilution (48). The results of some studies have supported a similar dual effect in hypothyroid patients as well. Fifteen of twenty patients studied by Skowsky and Kikuchi had elevated plasma AVP levels even after water loading, which then suppressed normally after the patients were made euthyroid (285). Similarly, other investigators have found frankly elevated plasma AVP levels (296,297), inappropriately normal levels despite plasma hypoosmolality (297), or a decreased osmotic threshold for AVP secretion in hypothyroid patients (298). Conversely, equal numbers of studies have failed to find evidence of inappropriately elevated plasma AVP levels, urine AVP secretion, or significantly altered osmotic

thresholds for AVP secretion or urinary dilution in hypothyroid patients (284,299–303). Consistent with these findings are several reported cases in which treatment with demeclocycline to antagonize renal AVP effects failed to increase serum [Na^+] or decrease urine osmolality in hyponatremic hypothyroid patients (299,304). Experimental studies have also shown variable results. Hypothyroid rats have been reported to manifest higher plasma AVP levels than euthyroid rats after water loading (287). However, hypothyroid Brattleboro rats appear to have similar defects in water excretion as rats with intact AVP secretion (286), supporting a major role for AVP-independent mechanisms of impaired free water excretion in hypothyroid animals. Recent studies of hypothalamic AVP gene expression have failed to demonstrate upregulation of AVP synthesis in hypothyroid rats (305), again arguing against a major stimulation of AVP secretion under these conditions, although the sensitivity of these methods for ascertaining small increases in hormone secretion and synthesis is limited. Perhaps the strongest argument against a major role for AVP-stimulated water retention in hypothyroidism has been the failure of any animal model of hypothyroidism to date to reproduce the degrees of hyponatremia commonly found in animal models of SIADH, adrenal insufficiency, and cardiac failure.

In light of the clinical and experimental observations to date, it has to be concluded that the major cause of impaired water excretion in hypothyroidism is an alteration in renal perfusion and GFR secondary to systemic effects of thyroid hormone deficiency on cardiac output and peripheral vascular resistance. Yet it must be recognized that severe hypothyroidism is a multisystem disease, and just as the presentation of patients with SIADH will vary depending on the degree of volume adaptation that has occurred, it is hardly surprising that different results have been reported regarding the potential role of AVP in hypothyroidism depending on the individual characteristics of the cases studied. Therefore, in uncomplicated hypothyroidism there appears to be little elevation of plasma AVP levels, and any defects in water excretion are due primarily to effects on renal hemodynamics. As the hypothyroidism becomes more severe, EABV can decease sufficiently to stimulate AVP secretion secondarily via baroreceptor mechanisms. However, even in this case the elevated AVP levels may not be causally related to the impaired water excretion, since several studies have suggested that hypothyroid animals are resistant to the effects of AVP based on decreased medullary cyclic AMP generation in response to AVP (287,306). However, when cardiac function becomes severely compromised, as often occurs with advanced myxedema, plasma AVP can become elevated sufficiently to override any renal resistance and cause antidiuresis, which then contributes to the hemodynamic impairments of water excretion. Whether hyponatremia develops at any stage of disease progression depends on the relative balance between water intake and excretory capacity; since maximal free water clearance decreases as these defects become more pronounced, this accounts for the increased incidence of hyponatremia as the severity of the underlying hypothyroidism worsens.

PRIMARY POLYDIPSIA

As discussed previously, excessive water intake is only rarely of sufficient magnitude to produce hyponatremia in the presence of normal renal function. However, it is often a significant factor contributing to hyponatremia in polydipsic patients, particularly those with underlying defects in free water excretion. In addition, because a positive water balance is required for the production of hyponatremia even under conditions of maximal antidiuresis in man and animals, an appreciation of the control mechanisms regulating water ingestion is important for understanding the development of hyponatremia in patients with SIADH and other hypoosmolar disorders.

Etiology

The most dramatic cases of primary polydipsia are seen in psychiatric patients, particularly with acute psychosis secondary to schizophrenia (307–314). The prevalence of this disorder based on hospital admissions for acute symptomatic hyponatremia may have been underestimated, since studies of polydipsic psychiatric patients have shown a marked diurnal variation in serum [Na^+] (from 141 mEq/L at 7 AM to 130 mEq/L at 4 PM), suggesting that many such patients drink excessively during the daytime but then correct themselves via a water diuresis at night (315). This and other considerations have led to defining this disorder as the "*psychosis-intermittent hyponatremia-polydipsia*" *(PIP) syndrome*. Polydipsia has been observed in up to 20% of psychiatric inpatients (313), with incidences of intermittent hyponatremia ranging from 5% to 10% (313,316,317). Despite the frequent occurrence of polydipsia in psychiatric patients, it is important to recognize that *not all polydipsia is caused by psychiatric disease*; infiltrative diseases such as CNS sarcoidosis (318) or critically placed brain tumors can also be associated with increased thirst and fluid ingestion. Consequently, polydipsic patients should be evaluated with a CT or MRI scan of the brain before concluding that excessive water intake is due to a psychiatric cause.

Pathophysiology

There is little question that excessive water intake alone can sometimes be sufficient to override renal excretory capacity and produce severe hyponatremia (46,319). Although the water excretion rate of normal adult kidneys can exceed 20 L per day, maximum hourly rates rarely exceed 800–1,000 mL/hour. Recent studies of water loading in exercising athletes have indicated a similar peak urine excretion rate of 778 ± 39 mL/hour (320). Because many psychiatric patients drink predominantly during the day or during intense drinking binges (309,315,321, 322), they can transiently achieve symptomatic levels of hyponatremia with total daily volumes of water intake of less than 20 L if it is ingested sufficiently rapidly. This likely accounts for many of the cases in which such patients present with maximally dilute urine, accounting for as many as 50% of patients in some studies (323), and correct quickly via a free water diuresis (324). However, other cases have been found to meet the criteria for SIADH (310,323,325–327), suggesting non-osmotically stimulated AVP secretion. As might be expected, in the face of much higher than normal water intakes, virtually any impairment of urinary dilution and water excretion can exacerbate the development of a positive water balance and thereby produce hypoosmolality. Therefore, hyponatremia has been reported in polydipsic patients taking thiazide diuretics (328,329) or drugs known to be associated with SIADH (185,313,316,330–334), in association with smoking and presumed nicotine-stimulated AVP secretion (335–337) (although a consistent relation with smoking has not been found [186]), and adrenal insufficiency (338). Acute psychosis itself can also cause AVP secretion (308,339), which often appears to take the form of a reset osmostat (202,309,326). It is therefore apparent that no single mechanism can completely explain the occurrence of hyponatremia in polydipsic psychiatric patients, but the combination of higher than normal water intakes plus even modest elevations of plasma AVP levels from a variety of potential sources appears to account for a significant portion of such cases.

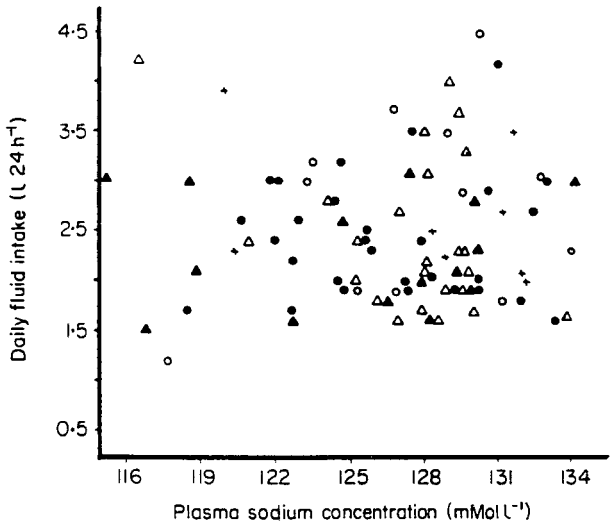

FIGURE 86-9. Daily fluid intakes of 91 hospitalized patients with hyponatremia of varying degrees and etiologies. Each point represents a single patient: *open circles*, SIADH; *open triangles*, cardiac failure; *closed circles*, volume contraction; *closed triangles*, cirrhosis; *pluses*, undiagnosed. Despite widely different etiologies for the hyponatremia, mean fluid intakes were equivalent in all groups of patients. (Reprinted from: Gross PA, Pehrisch H, Rascher W, et al. Pathogenesis of clinical hyponatremia: observations of vasopressin and fluid intake in 100 hyponatremic medical patients. *Eur J Clin Invest* 1987;17:123, with permission.)

Although patients with SIADH do not in general manifest the water intakes of patients with primary polydipsia, nonetheless continued water intake in the face of plasma hypoosmolality is inappropriate for maintenance of osmotic homeostasis. Analysis of daily fluid intakes of 91 hyponatremic patients showed an average fluid intake of 2.4 ± 0.2 liters per 24 hours (47) (Fig. 86-9), which does not differ appreciably from earlier measured intakes of medical students or hospitalized cardiac patients (mean fluid intakes of 2.4 and 2.8 L per 24 hours, respectively [340]), or studies of middle-aged subjects (mean fluid intake of 2.1 L per 24 hours [341]). This consistent pattern of continued water intake in hyponatremic patients raises important questions as to its cause. Most, although not all, patients treated with DDAVP do not become hyponatremic because they limit their water intakes in the absence of stimulated thirst. This observation has suggested the possibility that patients with SIADH and other hypoosmolar disorders might have a coexisting defect in thirst regulation; recent studies have supported this possibility by showing a 20 mOsmol/kg H_2O downward resetting of the thirst threshold in a group of patients with SIADH (342). A potential underlying mechanism could be stimulation of thirst by central AVP hypersecretion, but to date only relatively small effects of AVP to stimulate thirst have been seen in a single species (343). Alternatively, other animal studies have suggested that osmotic inhibition of thirst is a relatively weak phenomenon and easily overcome by a variety of nonhomeostatic stimuli causing drinking. Not only will rats increase intakes when fluids are made more palatable (344), but rats made antidiuretic with DDAVP will continue to ingest such fluids to the point of extreme hypoosmolality, and the degree of hypoosmolality achieved is proportional to the palatability of the fluid (345). Analogous results have been obtained with schedule-induced polydipsia in rats treated with AVP (346). In these examples, drinking continued despite the production of both osmotic dilution and volume expansion, and despite drinking behavior sufficient to activate both oropharyngeal and gastrointestinal inhibitory factors that modulate fluid ingestion (347). Obviously drinking will not continue indefinitely in the absence of renal excretion until some factor causes inhibition of further intake, but before this happens it is possible to achieve plasma dilutions of 20% to 30%. In man, similar to animals, there are many nonhomeostatic stimuli to drink fluids, including meal-associated drinking, oral habituation to various beverages, pleasurable sensations from palatable fluids, social interactions promoting fluid ingestion, and mouth dryness as a result of local factors, and these actually account for the major part of human fluid ingestion (341). By themselves such stimuli are benign and simply lead to more frequent urination of dilute urine to excrete the increased fluids ingested. However, in the presence of pathologic conditions that impair renal water excretion they can lead to hyponatremia. Therefore, although direct inhibitory physiologic stimuli to thirst and fluid ingestion clearly exist, they appear to be relatively weak in comparison to excitatory stimuli and can be overridden by a variety of nonhomeostatic stimuli that cause continued fluid ingestion despite plasma hypoosmolality (347). The extent to which such nonhomeostatic drinking versus disordered thirst regulation is responsible for the continued fluid ingestion in hypoosmolar disorders remains to be evaluated by more extensive clinical and experimental studies.

EXERCISE-ASSOCIATED HYPONATREMIA

Over the last two decades, exercise-associated hyponatremia (EAH) has emerged as an important complication of prolonged endurance physical activities. EAH is defined as the occurrence of hyponatremia in individuals engaged in prolonged physical activity who develop a serum or plasma sodium concentration ($[Na^+]$) below the normal reference range of the laboratory performing the test, generally less than 135 mmol/L (348).

Etiology

The first cases of hyponatremia in association with prolonged physical activity were reported in 1985. Noakes et al. published a series of four case reports of athletes who developed hyponatremia (serum $[Na^+]$ ranging from 115 to 125 mmol/L accompanied by neurologic symptoms including fluctuating levels of consciousness, seizures, and pulmonary edema) during marathon footraces in South Africa (167). In the same year, Hiller et al. reported that 28% of a prospectively studied cohort of the race finishers at the 1985 Hawaiian Ironman triathlon developed hyponatremia (349). Since that time, well over 100 cases of EAH have been reported in the literature from physical exercise activities as diverse as forced military marches, prolonged hiking, and marathon, ultramarathon and triathlon races, with at last five documented fatalities attributed to the hyponatremia (348). Several prospective studies have been performed on subsets of runners participating in organized endurance activities, and have documented incidences of hyponatremia from 13% to 29% (168,350–352). Interestingly, for all studies in which sex differences have been examined, the incidence of hyponatremia has been found to be substantially higher in female athletes, ranging from 22% to 45% (168,351,352). From the reported studies to date, it is clear that EAH can occur either during physical activity or within the 24 hour period after the activity, and most commonly occurs with prolonged physical activity generally lasting longer than 4 hours (although several cases have been reported with physical activity of shorter durations) (353,354).

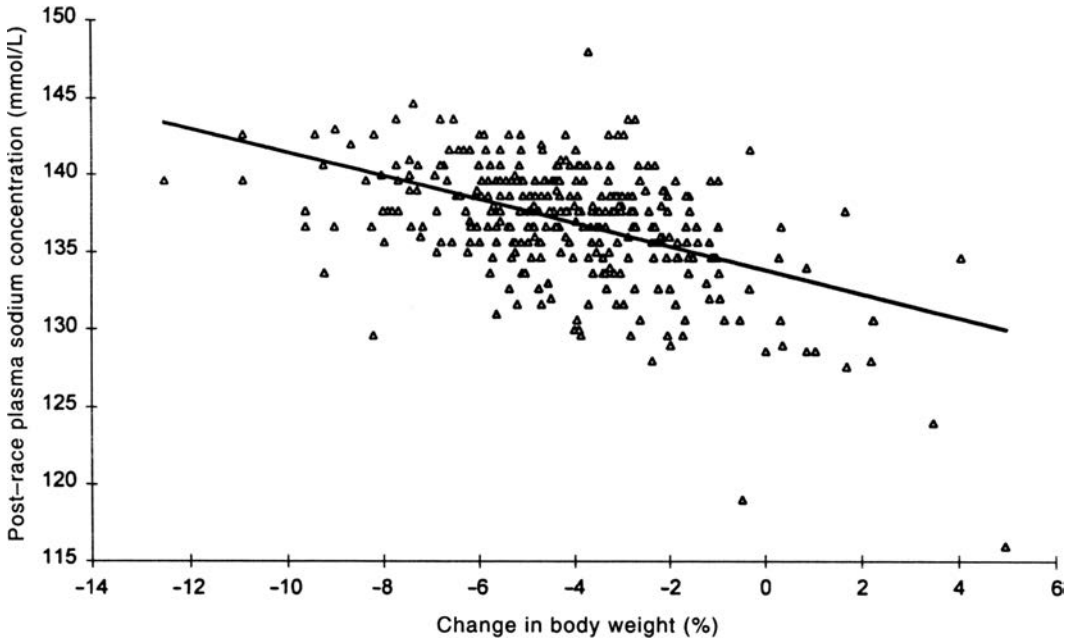

FIGURE 86-10. Inverse relation between the post race plasma sodium concentrations in 350 athletes at the finish of the 1997 New Zealand Ironman triathlon as a function of changes in body weight during the race. (From: Speedy DB, Noakes TD, Rogers IR, et al. Hyponatremia in ultradistance triathletes. *Med Sci Sports Exerc* 1999;31:809, with permission.)

Pathophysiology

From the very first reports of EAH there has been a divergence of opinion regarding the underlying pathophysiology. Noakes has proposed that most cases of EAH represent a dilutional hyponatremia secondary to excess fluid ingestion during physical activity, similar to primary polydipsia (173), whereas Hiller has implicated a depletional hyponatremia secondary to massive sodium losses from sweating during prolonged physical activity, especially in hot climates (355). Multiple lines of evidence now strongly support the development of a dilutional hyponatremia from excess water retention as the primary cause of most of these cases. First, in virtually all studies where body weight has been recorded before and after exercise, there has been a consistent inverse relation between weight and serum [Na^+], indicative of fluid retention. This is best illustrated in the data from the New Zealand Ironman triathlon in 1997 (Fig. 86-10), in which the most severe hyponatremia occurred in athletes who actually gained weight during this event (168). Second, high levels of fluid intake have been recorded in many of the athletes who develop EAH, often far in excess of the maximal renal excretory capacity of 800 to 1,000 mL per hour, and studies quantifying fluid intake have shown a significant negative correlation between ingested volumes and serum [Na^+] (351). Although fluid loses from sweating can be substantial during intense physical activity, this pattern of weight change suggests that fluid ingestion often exceeds the sum of fluid losses from renal excretion and sweating in EAH. Third, clinical evidence of volume depletion is not characteristic of most individuals who develop EAH, for example, hyponatremic runners from the Houston marathon in 2000 manifested lower levels of BUN rather than developing a prerenal azotemia (351). Fourth, two balance studies have been done on runners who developed EAH following ultramarathon races in comparison to a subset of normonatremic runners in these races (356,357). Both of these studies showed that over a 9 to 24 hour period after the race, the hyponatremic runners corrected their serum [Na^+] via a free water diuresis of 1.3 to 3.0 L, indicating the development of water retention during the race, whereas the normonatremic runners retained 0.5 to 2.7 L, indicating the development of dehydration during the race. Both groups of runners had moderate positive sodium balances ranging from 88 to 153 mmol, indicating net sodium losses during the race, but the levels of sodium retention were not different between the hyponatremic and normonatremic runners.

Although the above data clearly implicate fluid retention as the major cause of EAH, additional data do not fully support the concept that this is solely due to excess drinking. A retrospective review the U.S. Army inpatient data system from 1996 to 1997 revealed 17 cases of hyponatremia with a mean serum [Na^+] of 122 ± 5 mmol/L (169). Virtually all of the cases occurred in the south during hot summer months, and most occurred in first 4 weeks of military training. The majority presented with neurologic symptoms and had documented water intakes greater than 2 quarts per hour. One recruit died from cerebral and pulmonary edema. Although these cases were all classified as caused by overhydration on clinical grounds, detailed studies as to causation were not done. However, a more informative controlled study under similar conditions was performed earlier in Israel. Seventeen young males were studied during a 24-hour endurance march with *ad libitum* fluid ingestion. Serum [Na^+] levels decreased and were inversely related to total fluid intake, strongly suggesting a dilutional hyponatremia (358). Despite the hyponatremia and a measured expanded plasma volume (+16%), maximal urine outputs were only 4 mL/minute with urine osmolalities of approximately 200 mOsmol/kg H_2O, indicative of an inappropriate antidiuresis. Similarly instructive is a retrospective review of 44 hikers in the Grand Canyon who required medical treatment and had electrolytes measured in 1993 (170). Seven (16%) of the cases had hyponatremia, with serum [Na^+] ranging from 109 to 127 mmol/L; 5 of 7 had serious neurologic symptoms (three seizures, two disoriented) and had documented fluid intakes greater than the normonatremic patients (7.4 L versus 3.6 L), including sports drinks (4.3 L versus 0.5 L). Of particular significance, urine measurements showed osmolalities of 476 to 609 mOsmol/kg H_2O and sodium levels of 36 to 120 mmol/L.

A related study in Israel reported 7 patients who developed hyponatremia (serum [Na^+] = 115 to 123) after moderate exercise with urine sodium levels of 74 to 122 mmol/L (359). Most of these patients therefore met criteria for a diagnosis of SIADH, assuming the absence of thyroid and adrenal deficiencies, which are unlikely in young healthy individuals. Although SIADH had been dismissed in some reports because measured AVP levels were not "high," in this and other studies it is clear that the plasma AVP levels were not suppressed and consequently were inappropriate for the plasma osmolality. It therefore seems likely that EAH is caused by a combination of increased fluid intake in the setting of impaired renal excretory capacity during exercise. To what degree the decreased renal excretory capacity is secondary to renal hemodynamic changes, which can be marked during prolonged exercise versus AVP-induced antidiuresis, remains to be ascertained. Also unknown is the stimulus of AVP secretion during exercise. Although exercise itself is a mild stimulus of AVP secretion (360), it is likely that hypovolemia from sweat sodium losses as well as nonhomeostatic stimuli (e.g., nausea, hypoglycemia, hypoxia, stress, increased cytokines form rhabdomyolysis) also are contributory. The degree to which AVP secretion is stimulated, and whether it can be suppressed with sufficient fluid ingestion, will determine each individual's susceptibility to EAH as a result of fluid ingestion both before and after physical activity. Therefore, similar to the hyponatremia of schizophrenic patients, no single mechanism likely can completely explain the occurrence of hyponatremia in EAH, but the combination of higher-than-normal fluid intakes plus even modest elevations of plasma AVP levels from a variety of potential sources during prolonged physical activity appears to account for the majority of such cases.

It is interesting to consider why EAH was not recognized prior to 1985. Noakes has drawn attention to the close temporal relationship between the recent reports of hyponatremia and changes in the conduct of endurance races (173). First, these events became increasingly popular in the 1980s with large numbers of competitors, including competitors with lower fitness levels and hence longer running times. Second, multiple support stations to provide athletes with fluid at more frequent intervals during the race were introduced; prior to this time, fluid ingestion during prolonged exercise was considered unnecessary and possibly detrimental to performance. The convergence of these two developments is that athletes have more opportunity to drink during the events, and particularly the slower (i.e., novice) athletes. In support of this hypothesis, multiple studies have identified duration of time during races along with increased fluid consumption and weight gain as risk factors for the development of EAH (351,352). Although female sex has also been frequently identified as a risk factor, a recent report from the 2002 Boston marathon has suggested that this variable might be largely explained by the lower BMI of female athletes, thereby rendering them more susceptible to increased dilutional effects of excess fluid retention because of a smaller total body water volume (352).

CLINICAL MANIFESTATIONS OF HYPOOSMOLAR DISORDERS

Regardless of the etiology of hypoosmolality, the clinical manifestations are similar. Non-neurologic symptoms are relatively uncommon, but a number of cases of rhabdomyolysis have been reported (361–365), presumably secondary to osmotically-induced swelling of muscle fibers. Hypoosmolality is primarily associated with a broad spectrum of neurological manifestations, ranging from mild nonspecific symptoms (e.g., headache, nausea) to more significant disorders (e.g., disorientation, confusion, obtundation, focal neurologic deficits, and seizures) (366–368). This neurologic symptom complex has been termed *hyponatremic encephalopathy* (369) and primarily reflects brain edema resulting from osmotic water shifts into the brain because of decreased effective plasma osmolality (370). Significant neurologic symptoms generally do not occur until serum [Na^+] falls to less than 125 mEq/L, and the severity of symptoms can be roughly correlated with the degree of hypoosmolality (366,367). However, individual variability is marked, and, for any single patient, the level of serum [Na^+] at which symptoms appear cannot be predicted with great accuracy. Much of this variability can be understood within the framework provided by the process of *brain volume regulation* (Fig. 86-1), as discussed previously (78). Although most of the neurologic symptoms associated with acute hyponatremia are caused by brain edema as a result of osmotic water movement into the CNS, a potential exception is the development of seizure activity, which may possibly be caused or aggravated by increased brain ECF concentrations of the excitatory amino acids glutamate and aspartate as a result of cellular extrusion of these osmolytes during the process of brain volume regulation to hyponatremia (75). Once the brain has volume-adapted via solute losses, thereby reducing brain edema, neurologic symptoms are not as prominent and may even be virtually absent. This accounts for the fairly common finding of relatively asymptomatic patients despite severe levels of hyponatremia (19,367); however, recent studies of apparently asymptomatic patients with mild hyponatremia have suggested that many such patients in fact manifest subtle cognitive defects as well as gait instability, raising important questions about possible neurologic manifestations of even mild degrees of hyponatremia (371). It is also well known from animal studies that the rate of fall of serum [Na^+] is often more strongly correlated with morbidity and mortality than is the actual magnitude of the decrease (366). This is due to the fact that the volume-adaptation process takes a finite period to complete; the more rapid the fall in serum [Na^+], the more brain edema will be accumulated before the brain is able to lose solute and along with it part of the increased water content. These effects are responsible for the much higher incidence of neurologic symptoms, as well as the higher mortality rates, in patients with acute hyponatremia than in those with chronic hyponatremia (366,372). This phenomenon also likely underlies the observation that the most dramatic cases of death due to hyponatremic encephalopathy have generally been reported in postoperative patients in whom hyponatremia often develops rapidly as a result of intravenous infusion of hypotonic fluids (20,373), or in exercise-induced hyponatremia during endurance races or forced marches as a result of excess water ingestion (169,374,375). In such cases nausea and vomiting are frequently overlooked as potential early signs of increased intracranial pressure in acutely hypoosmolar patients. Because hypoosmolality does not cause any known direct effects on the gastrointestinal tract, the presence of unexplained nausea or vomiting in a hypoosmolar patient should be assumed to be of CNS origin and the patient treated for symptomatic hypoosmolality as described in the subsequent text. A recent study of runners in a marathon race found that vomiting was the only symptom that differentiated hyponatremia from other causes of exercise-associated collapse in this group (351). Similarly, critically ill patients with unexplained seizures should be rapidly evaluated for possible hyponatremia, since as many as one-third of such patients have been found to have serum [Na^+] of less than 125 mEq/L as the cause of the seizure activity (376). Underlying neurologic disease also affects the level of hypoosmolality at which CNS symptoms appear; moderate hypoosmolality is of little concern in an otherwise healthy patient, but can cause morbidity in a patient with an underlying seizure disorder. Non-neurologic metabolic disorders (e.g., hy-

poxia [377], acidosis, hypercalcemia) can similarly affect the level of plasma osmolality at which CNS symptoms occur.

In the most severe cases of hyponatremic encephalopathy, death results from respiratory failure after tentorial cerebral herniation and brainstem compression. Studies of patients with severe postoperative hyponatremic encephalopathy have indicated a high incidence of hypoxia, and one-fourth of these patients manifested hypercapnic respiratory failure, the expected result of brainstem compression, but three-fourths had pulmonary edema as the apparent cause of the hypoxia (378). Recent studies of acute hyponatremia after endurance races have similarly shown hypoxia and pulmonary edema in association with brain edema (166,172). These results, therefore, suggest the possibility that hypoxia from noncardiogenic pulmonary edema may represent an early sign of developing cerebral edema, even before the swelling progresses to the point of brainstem compression and tentorial herniation. Recent clinical studies have also suggested that menstruating women (373) and young children (379) may be particularly susceptible to the development of neurologic morbidity and mortality during hyponatremia, especially in the acute postoperative setting (369). However, other studies have failed to corroborate these findings (380,381). Consequently, the true clinical incidence as well as the underlying mechanisms responsible for these sometime catastrophic cases remains to be determined.

THERAPY OF HYPOOSMOLAR DISORDERS

Despite some areas of continuing controversy concerning correction of osmolality in hypoosmolar patients, a relative consensus has evolved regarding the most appropriate treatment of this disorder. The following recommendations are summarized in the diagnostic and therapeutic flow diagram shown in Figure 86-11.

Initial Evaluation

Once true hypoosmolality is verified, the ECF volume status of the patient should be assessed by careful clinical examination. If fluid retention is present, the treatment of the underlying disease should take precedence over correction of plasma osmolality. Often this involves treatment with diuretics, which should simultaneously improve plasma tonicity by virtue of stimulating excretion of hypotonic urine (see Chapter 81). If hypovolemia is present, the patient must be considered to have depletion-induced hypoosmolality, in which case volume repletion with isotonic saline (0.9% NaCl) at a rate appropriate for the estimated fluid deficit should be initiated. If diuretic use is known or suspected, the isotonic saline should be supplemented with potassium (30 to 40 mEq/L) even if serum [K^+] is not low, because of the propensity of such patients to develop total body potassium depletion. Most often, the hypoosmolar patient is clinically euvolemic, in which case the evaluation should then proceed to the measurement of urine osmolality and [Na^+]. However, several situations will dictate a reconsideration of solute depletion as a potential diagnosis, even in the patient without clinically apparent hypovolemia. These include: (i) a urine [Na^+] <30 mEq/L (101), (ii) a history of recent diuretic use, and (iii) any suggestion of primary adrenal insufficiency. Whenever a possibility of depletion-induced, rather than dilution-induced, hypoosmolality exists, it is most appropriate to treat the patient initially with isotonic saline, regardless of whether signs of hypovolemia are present. An improvement in, and eventual correction of, the hyponatremia verifies solute and volume depletion. Conversely, if the patient has SIADH rather than solute depletion, no harm will be done by administration of a limited volume (e.g., 1 to 2 L) of isotonic saline, because patients with SIADH simply excrete excess infused or ingested NaCl without significantly changing their plasma osmolality (1). However, in the absence of an initial positive response, continued infusion of isotonic saline should be avoided, since over longer periods sufficient free water can be retained to further decrease the serum [Na^+] (382).

The approach to patients with euvolemic hypoosmolality will vary according to the clinical situation. A patient who meets all the essential criteria for SIADH but has a low urine osmolality should be observed on a trial of modest fluid restriction. If the hypoosmolality is attributable to transient SIADH or severe polydipsia, the urine will remain dilute and the plasma osmolality will be fully corrected as free water is excreted. If, however, the patient has the reset osmostat form of the disorder, then the urine will become concentrated at some point before the plasma osmolality and serum [Na^+] return to normal ranges. If either primary or secondary adrenal insufficiency is suspected, glucocorticoid replacement should be initiated immediately after the completion of a rapid ACTH stimulation test (383,384). A prompt water diuresis after initiation of glucocorticoid treatment supports a diagnosis of glucocorticoid deficiency (261). However, the absence of a quick response does not necessarily negate this diagnosis because several days of glucocorticoid replacement are sometimes required for normalization of plasma osmolality (102). If hypothyroidism is suspected, thyroid function tests should be conducted and a plasma thyroid-stimulating hormone level should be obtained; usually, however, replacement therapy is withheld pending these results unless the patient is obviously myxedematous. If renal failure is present in a patient with hypoosmolality, a more extensive evaluation of renal function will be necessary before deciding what course of treatment is most appropriate (see Chapter 96).

Acute Treatment

In any significantly hyponatremic patient, one must decide how quickly the plasma osmolality should be increased, and to what level. This decision in turn depends upon knowledge about two basic questions: first, what are the risks of uncorrected hyponatremia, and second, what are the risks of the correction itself? As described in the previous section, hyponatremia is associated with a broad spectrum of neurologic symptoms (366), sometimes leading to death in severe cases (373,379). However, over the last two decades it has become clear that correcting severe hyponatremia too rapidly is also dangerous, since this can sometimes be associated with pontine and extrapontine myelinolysis, a brain demyelinating disease that also causes severe neurologic morbidity and mortality (21,385). Consequently, appreciation of the appropriate therapy for this disorder requires understanding this disease as well as the pathophysiologic underlying hyponatremic encephalopathy,

Pontine and Extrapontine Myelinolysis

Despite the obvious survival advantages afforded by brain volume regulation in response to hyponatremia, every adaptation made by the body in response to a perturbation of homeostasis bears within it the potential to create a new set of problems, and this is true for brain volume regulation as well. Over the last decade it has become apparent that the demyelinating disease of central pontine myelinolysis (CPM) occurs with a significantly higher incidence in patients with hyponatremia (386–388), and in both animal (389–393) and human studies (21,385,394) brain demyelination has clearly been shown

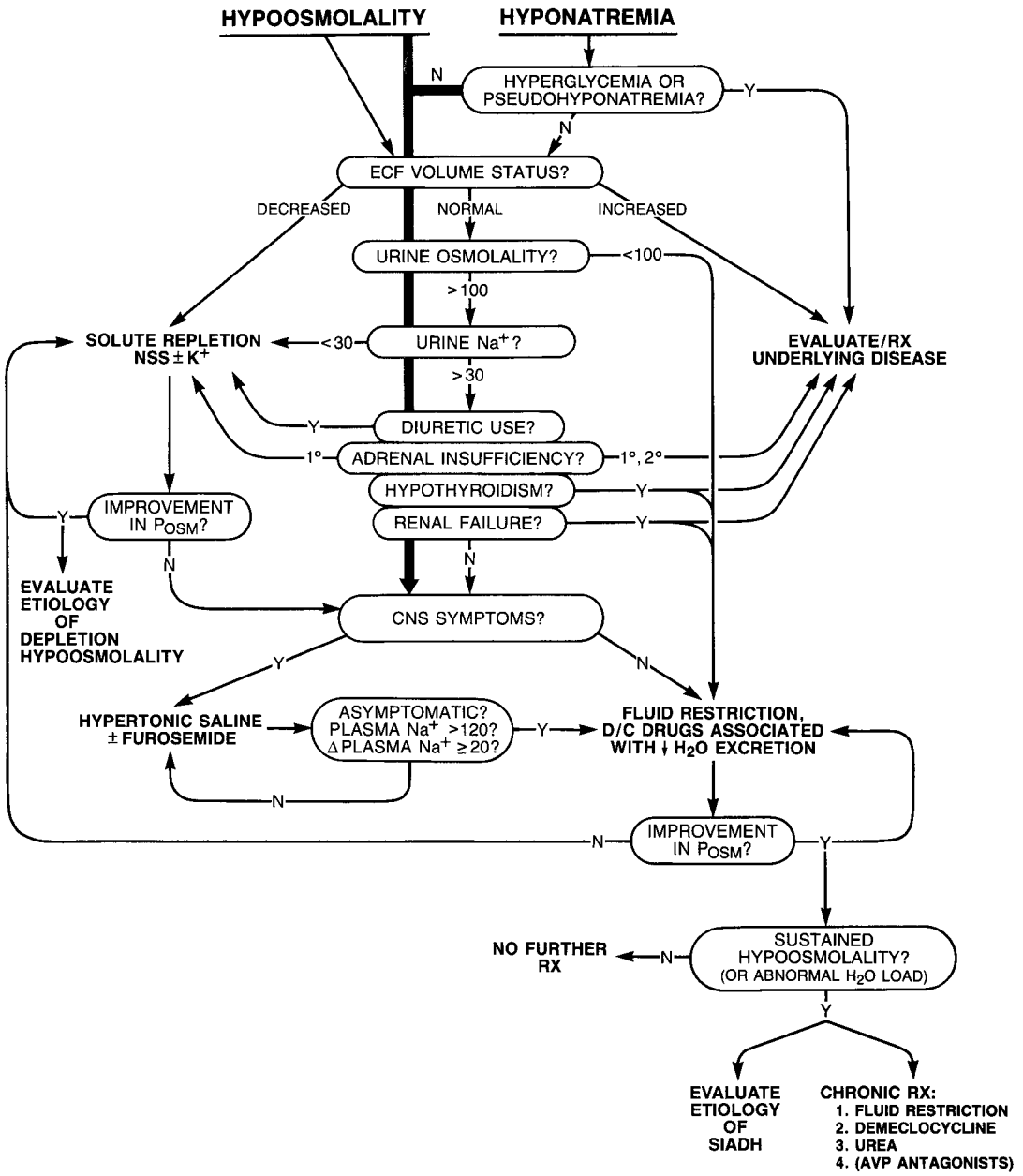

FIGURE 86-11. Schematic summary of the evaluation and therapy of hypoosmolar patients. The *dark arrow* in the center emphasizes that the presence of central nervous system dysfunction due to hyponatremia should always be assessed immediately, so that appropriate therapy can be started as soon as possible in symptomatic patients while the outlined diagnostic evaluation is proceeding. (Reprinted from: Verbalis JG. Inappropriate antidiuresis and other hypoosmolar states. In: Becker KL, ed. *Principles and Practice of Endocrinology and Metabolism,* Philadelphia: Lippincott; 1995:265, with permission.)

to be associated with the correction of existing hyponatremia rather than simply with the presence of severe hyponatremia itself. Although the mechanism(s) by which correction of hyponatremia leads to brain demyelination remain under investigation, this pathologic disorder likely is precipitated by the brain dehydration that has been demonstrated to occur following correction of serum [Na^+] toward normal ranges in animal models of chronic hyponatremia. Because the degree of osmotic brain shrinkage is greater in animals that are maintained chronically hyponatremic than in normonatremic animals undergoing similar increases in plasma osmolality (208,391,395), by analogy the brains of human patients adapted to hyponatremia are likely to be particularly susceptible to dehydration following subsequent increases in osmolality, which in turn lead to pathologic demyelination in some patients. Recent magnetic resonance studies in animals have shown that chronic hypoosmolality predisposes rats to opening of the blood–brain barrier following rapid correction of hyponatremia (396), and that the disruption of the blood–brain barrier is highly correlated with subsequent demyelination (397); a potential mechanism by which blood–brain barrier disruption might lead to subsequent myelinolysis is via an influx of complement, which is toxic to the oligodendrocytes that manufacture and maintain myelin sheaths of neurons, into the brain (398).

Although there has been considerable debate in the literature regarding the parameters of correction of hyponatremia

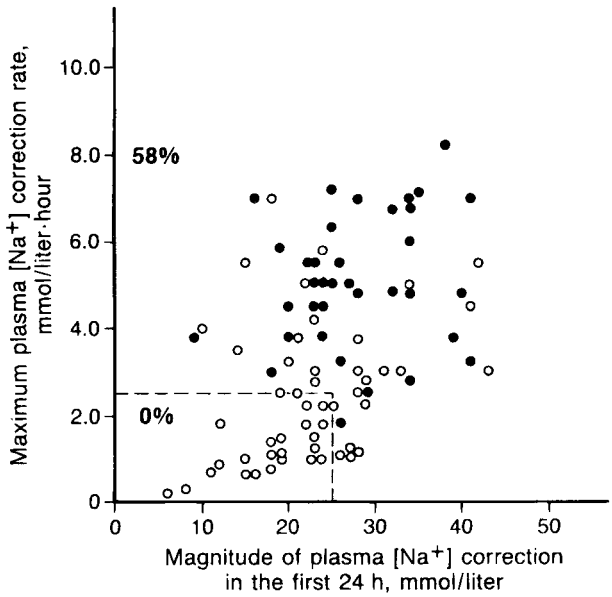

FIGURE 86-12. Relation between the production of brain myelinolysis and the maximal rate and 24-h magnitude of correction of hyponatremia in 87 rats with DDAVP-induced hyponatremia: *closed circles*, rats with no brain lesions; *open circles*, rats with demyelinative brain lesions. Different methods were used to correct the hyponatremia in order to produce varying initial rates and magnitudes of serum [Na^+] correction. The *dashed lines* enclose the area within which no rats developed demonstrable neuropathological lesions, whereas 58% developed lesions when their correction parameters were outside these ranges (From: Verbalis JG, Martinez AJ. Determinants of brain myelinolysis following correction of chronic hyponatremia in rats. In: Jamison RL, Jard S, eds. *Vasopressin*. Paris: John Libbey; 1991:539, with permission.)

associated with an increased risk of myelinolysis, studies in both patients (399–401) and experimental animals (391–393) support the notion that both the rate of correction of hyponatremia and the total magnitude of the correction over the first few days likely represent significant factors that increase the risk of demyelination (Fig. 86-12). Recent studies in rats have shown that the initial rate of correction of hyponatremia may not be important for the development of demyelinative lesions as long as the total magnitude of the correction remains less than 20 mEq/L in 24 hours (402), which supports clinical data indicating that magnitude of correction represents the major risk factor related to subsequent neurologic morbidity and mortality. Although there is still some disagreement as to the actual level at which patients are at risk for CPM; initial reports implicated increases in serum [Na^+] greater than 25 mEq/L over the first 24 to 48 hours of treatment (399), whereas later studies have suggested occurrence of CPM with even lesser increases in serum [Na^+] of greater than 12 mEq/L in 24 hours or greater than 18 mEq/hours in 48 hours (403). Although overcorrection of hyponatremia to supranormal levels is also clearly a risk factor for neurologic deterioration, it is important to note that both clinical and experimental studies have found that demyelination occurred following corrections to serum [Na^+] levels still below normal ranges. Regardless of the level of increase in serum [Na^+] at which CPM occurs, the methods used to correct hyponatremia do not appear to have any significant bearing on the production of brain demyelination, since both experimental studies (393) and clinical reports (21,404–406) have demonstrated that demyelination occurs independent of the method used to correct the hyponatremia.

Other factors also can clearly influence the susceptibility to demyelination following correction of hyponatremia. Perhaps most importantly are the severity and the duration of the preexisting hyponatremia. Both of these risk factors likely relate to the degree of brain volume regulation that has occurred prior to the correction: The more severe the hyponatremia and the longer it has been maintained, the greater the degree of solute loss that will have occurred during the process of brain volume regulation. As larger amounts of solute are lost, the ability of the brain to buffer subsequent increases in plasma osmolality is impaired, resulting in greater degrees of brain dehydration as serum [Na^+] is later raised, which in turn can lead to brain demyelination via mechanisms discussed previously. Clinical implications of this pathophysiologic mechanism are that CPM should not occur in cases of either mild or very acute hyponatremia. Both of these findings have been found to be true. CPM has only rarely been reported in patients with a starting serum [Na^+] of greater than 120 mmol/L (21,403,407), and also does not appear to occur in most patients with psychogenic polydipsia who are well known to develop hyponatremia acutely from episodes of massive water ingestion followed by rapid correction as they diurese the excess fluid (324). There are also some independent risk factors for the occurrence of CPM, particularly chronic alcoholism and malnutrition, which led to the original description of this disorder in 1959 (408). Although no studies to date have clearly documented interactive effects between these risk factors and CPM, it seems likely that the threshold for increases in serum [Na^+] that increase the risk for CPM will be lower in alcoholic and malnourished patients, and recent case reports of myelinolysis in patients with chronic alcoholism in whom the rate of correction stayed within the recommended guidelines supports this likelihood (409,410). Interestingly, one factor that appears to protect hyponatremic patients from myelinolysis following rapid correction of hyponatremia is uremia. Although uremic patients on dialysis frequently have large swings of serum [Na^+], only rare cases of osmotically induced demyelination have been reported in this group (411). A recent study in rats showed that azotemic rats were able to sustain large increases in serum [Na^+] without brain damage, purportedly because the urea acts as an intracellular osmolyte to stabilize intracellular volume and thereby reduces the degree of brain dehydration produced following rapid correction of hyponatremia (412).

Additional aspects of this unique disease deserve emphasis. First, demyelination following correction of hyponatremia frequently occurs in white matter areas of the brain other than the pons; this has led to proposals that this occurrence of the disorder in hyponatremic patients be called the *osmotic demyelination syndrome (ODS)* (21). Alternatively, the term *pontine and extrapontine myelinolysis (PEM)* would be more accurate than CPM (388), which is historically correct but anatomically too limited. Second, apropos the widespread nature of the neuropathologic lesions, a much broader range of neurologic disorders is now being reported in patients following correction of hyponatremia, including cognitive, behavioral, and neuropsychiatric disorders, presumably as a result of demyelination in subcortical, corpus callosal, and hippocampal white matter (413,414), and movement disorders, as a result of demyelination in the basal ganglia (415–417). Third, MRI scans often fail to demonstrate the characteristic demyelinative lesions in many cases because scans are usually negative until sufficient time has passed (generally 3 to 4 weeks) after the correction of hyponatremia and the onset of neurologic symptoms (418–420). Consequently, the presence of positive MRI findings strongly (though not unequivocally [421]) supports a diagnosis of PEM, but the absence of radiologic findings can never eliminate the possibility of this disorder. Fourth, although most cases of osmotically-induced PEM have been associated with rapid correction of hyponatremia, the disorder has also been reported with severe hypernatremia in both animal models (422) and patients (423). This is consistent with the

hypothesis that brain dehydration with subsequent disruption of the blood–brain barrier is related to the pathogenesis of the demyelinative process (397,398). Finally, it is clear that given our present knowledge, we cannot predict with any degree of certainty which patients will develop demyelination, regardless of the parameters used to correct hyponatremia. Many patients undergo very rapid and large corrections of their serum [Na^+] without subsequent neurologic complications (424), as is true of experimental animals as well (393,402) (see Fig. 86-12). Consequently, overly rapid correction of hyponatremia should be viewed as a factor that puts patients *at risk* for PEM, but does not inevitably precipitate this disorder.

Individualization of Therapy

Based on the previous discussions of hyponatremic encephalopathy and pontine and extrapontine myelinolysis, it follows that optimal treatment of hyponatremic patients must entail balancing the risks of hyponatremia against the risks of correction for each patient individually (Fig. 86-13). Although individual variability in response is great, and consequently one cannot always accurately predict which patients will develop neurologic complications from either hyponatremia or its correction, consensus guidelines for treating hypoosmolar patients allow a rational approach to minimizing the risks of both these complications. Implicit in these guidelines is the realization that treatment must be tailored to each patient's clinical presentation: *appropriate therapy for one hyponatremic patient may be inappropriate for another despite equivalent degrees of hypoosmolality* (5,425). To accomplish this, three factors should be taken into consideration when making a treatment decision for a hypoosmolar patient: (i) the severity of the hyponatremia, (ii) the duration of the hyponatremia, and (iii) the patient's neurologic symptomatology. The severity of the hypoosmolality is an important consideration because neither sequelae from hyponatremia itself nor myelinolysis after therapy are likely in patients whose serum [Na^+] is 120 mEq/L or greater, although occasionally significant symptoms of hyponatremic encephalopathy can develop even at higher serum [Na^+] levels if the rate of decrease of plasma osmolality is particularly rapid. The importance of duration and symptomatology both relate to how well the brain has adapted to the hyponatremia and consequently its degree of risk for subsequent demyelination with rapid correction.

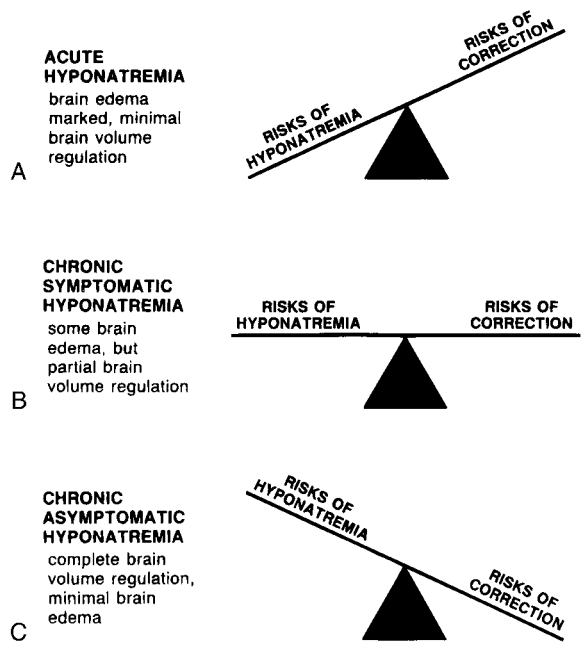

FIGURE 86-13. Schematic summary of the balance between the relative risks of hyponatremia versus the risks of rapidly correcting the hyponatremia in patients with hyponatremia of varying durations and symptomatology. Treatment indications are clear for both acute (≤48 hours' duration) symptomatic hyponatremia (**A**) and chronic (>48 hours' duration) asymptomatic hyponatremia (**C**). However, cases of chronic symptomatic hyponatremia (**B**) represent the greatest clinical challenge, since some brain volume adaptation has occurred but the presence of symptoms indicates some residual degree of brain edema as well; this renders the balance between the risks of the hyponatremia and the risks of the correction difficult to ascertain in individual patients. Such patients are optimally treated using limited and carefully controlled corrections regardless of the methods by which the increases in serum [Na^+] occur. (Reprinted from: Verbalis JG. Hyponatremia. Endocrinologic causes and consequences of therapy. *Trends Endocrinol Metab* 1992;3:1, with permission.)

Acute Hyponatremia. Cases of acute hyponatremia (arbitrarily defined as ≤48 hours in duration) are usually symptomatic if the hyponatremia is severe (i.e., ≤120 mEq/L). These patients are at greatest risk for neurologic complications from the hyponatremia, but rarely develop demyelination (324), presumably because sufficient brain volume regulation has not yet occurred to increase brain susceptibility to osmotic dehydration with the correction. Consequently, serum [Na^+] in such patients should be corrected to higher levels relatively quickly. Postoperative patients (20,426,427), and particularly young women and children in some studies (373,379,428), appear to be at greater risk for rapidly progressing hyponatremic encephalopathy and therefore should be treated especially promptly. Administration of hypotonic fluids should be avoided in such patients postoperatively (41,429–431), although this will not necessarily prevent the occurrence of postoperative hyponatremia in all surgical patients (382). The dark black arrow in Figure 86-11 emphasizes that hypoosmolar patients should always be evaluated quickly for the presence of neurologic symptoms so that appropriate therapy can be initiated, if indicated, even while other results of the diagnostic evaluation are still pending.

Chronic Asymptomatic Hyponatremia. Conversely, patients with more chronic hyponatremia (arbitrarily defined as >48 hours in duration) who have minimal neurologic symptomatology are at little risk from complications of hyponatremia itself but can develop demyelination after rapid correction because of greater degrees of brain volume regulation through electrolyte and osmolyte losses (78,208, 391). There is no indication to correct these patients rapidly, regardless of the initial serum [Na^+], and they should be treated using slower-acting therapies, such as fluid restriction.

Chronic Symptomatic Hyponatremia. Although the first two extremes have clear treatment indications, most hypoosmolar patients have hyponatremia of indeterminate duration and varying degrees of neurologic symptomatology. This group represents the most challenging treatment decision because the hyponatremia will have been present for sufficient duration to allow some degree of brain volume regulation, but not enough to prevent some brain edema and neurologic symptomatology (Fig. 86-13). Such patients should be treated promptly because of their symptoms, but using methods that allow a *controlled and limited increase* of their hypoosmolality (425,432). Some studies have suggested that correction parameters should consist of a maximal rate of correction of serum [Na^+] in the range of 1 to 2 mEq/L per hour so long as the total magnitude of correction does not exceed 25 mEq/L over the first 48 hours (399), whereas others recommend that these

parameters should be even more conservative, with maximal correction rates of 0.5 mEq/L per hour or less and magnitudes of correction that do not exceed 12 mEq/L in the first 24 hours and 18 in the first 48 hours (403). A reasonable approach for treatment of individual patients would therefore entail choosing correction parameters within these limits depending on their symptomatology: In patients who are only minimally symptomatic, one should proceed at the lower recommended limits of 0.5 mEq/L per hour or less, whereas in those who manifest more severe neurologic symptoms, an initial correction at a rate of 1 to 2 mEq/L per hour, or even 3 to 5 mEq/L per hour in comatose or seizing patients who are at risk for imminent tentorial herniation and respiratory arrest, would be more appropriate. Regardless of the initial rate of correction chosen, acute treatment should be interrupted once any of three end points is reached: (i) the patient's symptoms are abolished, (ii) a safe serum [Na^+] (generally >120 mEq/L) is achieved, or (iii) a total magnitude of correction of 20 mEq/L is achieved. Once any of these endpoints is reached, the active correction should be stopped and the patient treated with slower-acting therapies, such as oral rehydration or fluid restriction, depending on the etiology of the hypoosmolality. It follows from these recommendations that serum [Na^+] levels must be carefully monitored at frequent intervals (at least every 4 hours) during the active phases of treatment to adjust therapy to keep the correction within these guidelines. A recent retrospective study of chronic symptomatic hyponatremia in 53 postmenopausal women has confirmed the safety and efficacy of using a controlled and limited correction of the hyponatremia with intravenous NaCl. Patients whose serum [Na^+] was corrected by 22 ± 10 mEq/L over 35 hours had significantly better neurologic outcomes than those corrected much more slowly, 3 ± 2 mEq/L over 41 hours, via fluid restriction (433).

It must be emphasized that the preceding recommendations do not preclude prompt treatment of hyponatremia when it is severe and symptomatic, and particularly when it is acute and of short duration. Such patients are indeed at significant risk of complications from hyponatremic encephalopathy (369). However, even for this group of high risk patients it has been argued that only very limited corrections are actually necessary (434). The reasoning upon which this is based is the fact that the most brain edema that can be tolerated before tentorial herniation occurs is an approximately 8% to 10% increase in brain water. Therefore, decreasing brain water by this amount should have maximal therapeutic benefit, whereas exceeding this amount is more likely to produce brain dehydration with an increased risk of precipitating demyelination. By this reasoning, a 10% increase in plasma osmolality (i.e., a 10 mmol/L increase of serum [Na^+] in a patient with a starting serum [Na^+] of 100 mmol/L) should maximize potential benefit while minimizing potential risk. Although brain water content will not bear an exact relation to changes in plasma osmolality because of the complex nature of secondary adaptive processes, over short periods there will be a relatively good relation between these parameters and, therefore, this argument has considerable merit. Anecdotal reports have been cited as evidence supporting the contention that small increases in serum [Na^+] are sufficient to reverse hyponatremic seizures and other aspects of hyponatremic encephalopathy, but as for many other treatment recommendations no controlled studies yet exist to confirm the safety and efficacy of this approach.

Choice of Interventional (Active) Therapies for Acute Corrections

Controlled limited corrections can be accomplished with either isotonic or hypertonic saline infusions, depending on the etiology of the hypoosmolality. Patients with volume depletion hypoosmolality (e.g., clinical hypovolemia, diuretic use, or urine [Na^+] <30 mEq/L) usually respond well to isotonic (0.9%) NaCl (101). However, patients with diuretic-induced hyponatremia are especially prone to rapid corrections, since: (i) such patients are usually are only minimally volume depleted, (ii) they are often smaller elderly women with correspondingly small plasma volumes, and (iii) with cessation of diuretic therapy they often develop a free water diuresis as their urinary diluting defect dissipates (435). Furthermore, the hypokalemia that frequently accompanies the hyponatremia in such patients appears to be an additional risk factor for demyelination after correction (407), even in patients in whom the correction stays within currently accepted guidelines (406). Consequently, in the absence of marked neurologic symptomatology such patients should simply be treated by institution of a regular sodium diet (4 to 8 g/day) and discontinuing the diuretics; if isotonic saline is infused it should be done so judiciously (e.g., 50 to 75 mL/hour), with K^+ replacement, while the serum [Na^+] is monitored frequently to prevent overly rapid correction. Patients with euvolemic hypoosmolality (including patients with SIADH) generally will not respond to isotonic NaCl (1,101) and are best treated with hypertonic (3%) NaCl solution given by continuous infusion. An initial infusion rate can be estimated by multiplying the patient's body weight in kilograms by the desired rate of increase in serum [Na^+] in milliequivalents per liter per hour (e.g., in a 70-kg patient an infusion of 3% NaCl at 70 mL/hour will increase serum [Na^+] by approximately 1 mEq/L/hour, whereas an infusion of 35 mL/hour will increase serum [Na^+] by about 0.5 mEq/L per hour). Furosemide should be used to treat volume overload, in some cases anticipatorily in patients with known cardiovascular disease (436). Regardless of the therapy or rate initially chosen, it cannot be emphasized too strongly that it is necessary only to correct the plasma osmolality acutely to a safe range rather than completely to normonatremia.

Spontaneous (Passive) Correction

In some cases patients may spontaneously correct their hyponatremia by means of a water diuresis (400,404). If the hyponatremia is acute, such as water intoxication from psychogenic polydipsia, these patients appear to be at little risk for subsequent demyelination (324). However, in cases in which the hyponatremia has been chronic, such patients are at risk for demyelination (404,437,438), and intervention (e.g., administration of desmopressin or intravenous infusion of hypotonic fluid) should be considered to limit the rate and magnitude of correction of serum [Na^+], using the same end points as described previously for active corrections. A recent report demonstrates the utility of interrupting a spontaneous free-water diuresis with DDAVP (439). However, in some cases an overcorrection unfortunately occurs before it is noticed. Animal models of this situation have suggested that lowering the serum [Na^+] after overcorrection can prevent subsequent brain damage from occurring (440,441), and this would be consistent with the occurrence of a delayed immunologic demyelination as a result of complement influx into the brain following a sustained blood–brain barrier disruption (398). A recent clinical case in which delayed lowering of serum [Na^+] was associated with a reversal of symptoms suggestive of early myelinolysis supports this as a potential therapy in similar cases (442). Recent animal studies have shown that administration of high-dose glucocorticoids can prevent the development of osmotic demyelination after rapid correction of hyponatremia in rats (443), again likely via stabilization of the blood–brain barrier to prevent or minimize disruption. Although controlled clinical studies with glucocorticoids have not yet been done in humans, it would seem prudent to employ this relatively benign intervention in cases where a correction of hyponatremia in excess of current guidelines has already occurred.

Chronic Treatment

Fluid Restriction

If hyponatremia persists, then investigation of other potential causes of SIADH should be pursued (see Table 86-4), and chronic therapy should be instituted. The treatment of chronic SIADH entails a choice among several suboptimal therapeutic regimens. Continued fluid restriction represents the least toxic treatment choice and is currently the preferred treatment for most cases of mild-to-moderate SIADH. Several points should be remembered when using this approach: (i) all fluids, not only water, must be included in the restriction; (ii) the degree of restriction required depends on urine output plus insensible fluid loss (generally, discretionary, that is, nonfood, fluids should be limited to 500 mL a day below the average daily urine volume (107)); (iii) several days of restriction are usually necessary before a significant increase in plasma osmolality occurs, and (iv) only fluid, not sodium, should be restricted. Because of the ongoing natriuresis, patients with chronic SIADH often have a negative total body [Na^+] balance and, therefore, should be maintained on relatively high NaCl intakes unless otherwise contraindicated. However, just as failure to correct a presumed depletion-induced hyponatremia with isotonic saline should lead one to consider the possibility of a dilution-induced hypoosmolality, so should the failure of significant fluid restriction after several days of confirmed negative fluid balance prompt reconsideration of other possible causes, including solute depletion and clinically inapparent hypovolemia (see Fig. 86-11). At the time that fluid restriction is first initiated, any drugs known to be associated with SIADH should be discontinued or changed (e.g., newer generation oral hypoglycemic agents, which in general have not been associated with hyponatremia, should be substituted for chlorpropamide).

Treatment of Polydipsia

Patients whose hyponatremia is caused primarily by polydipsia should ideally have therapy directed at reducing fluid intakes into normal ranges. Unfortunately, this has proved difficult to accomplish in many cases. Patients with a reset thirst threshold will be resistant to fluid restriction because of the resulting thirst from stimulation of brain thirst centers at higher plasma osmolalities (444). In some cases the use of alternative methods to ameliorate the sensation of thirst (e.g., wetting the mouth with ice chips or using sour candies to increase salivary flow) can help to reduce fluid intakes. Fluid intake in patients with psychogenic causes of polydipsia is driven by psychiatric factors that have responded variably to behavioral modification and pharmacologic therapy. Several reports have suggested the efficacy of the antipsychotic drug clozapine as a promising agent to reduce polydipsia and prevent recurrent hyponatremia in at least a subset of these patients (445–449); this appears to be a specific property of this agent, since similar results have not been observed with other antipsychotic agents (450).

Pharmacological Therapy

Fluid restriction should always be tried as the initial therapy for patients with chronic SIADH, with pharmacologic intervention reserved for refractory cases in which the degree of fluid restriction required to avoid hypoosmolality is so severe that the patient is unable, or unwilling, to maintain it. In such cases reasonable efforts should be made to ameliorate thirst, such as substituting hard candy or ice chips for drinking fluids. Pharmacologic intervention should also be avoided initially in patients with SIADH that is secondary to tumors, because successful treatment of the underlying malignant lesion often eliminates or reduces the inappropriate AVP secretion (112). However, alternative pharmacologic management is often necessary. In such cases the currently preferred drug is the tetracycline derivative *demeclocycline* (451,452). This agent causes a nephrogenic form of diabetes insipidus (453), thereby decreasing urine concentration even in the presence of high plasma AVP levels. Appropriate doses of demeclocycline range from 600 to 1200 mg per day administered in divided doses. Treatment must be continued for several days to achieve maximal diuretic effects; consequently, one should wait 3 to 4 days before deciding to increase the dose. Demeclocycline can cause reversible azotemia, and sometimes nephrotoxicity, especially in patients with cirrhosis (454,455). Renal function should therefore be monitored on a regular basis in patients treated with demeclocycline and the medication discontinued if increasing azotemia is noted.

Other agents, such as lithium, have similar renal effects, but are less desirable because of inconsistent results and significant side effects (456). *Urea* has also been described as an alternative mode of treatment for SIADH, as well as for other hyponatremic disorders (457–460). Although it has long been recognized that any osmotic diuretic can be used to treat hypoosmolality by virtue of increasing free water excretion, such therapeutic modalities have generally proved impractical for chronic ambulatory use. Urea is an exception because it can be administered orally; furthermore, it corrects hypoosmolality not only by increasing free water excretion but also by decreasing urinary sodium excretion. Dosages of 30 g per day are generally effective; it is advisable to dissolve the urea in orange juice or some other strongly flavored liquid to camouflage the taste. Even if completely normal water balance is not achieved, it is often possible to allow the patient to maintain a less strict regimen of fluid restriction while receiving urea. The disadvantages associated with the use of urea include poor palatability, the development of azotemia at higher doses, and the unavailability of a convenient form of the agent. Several other drugs that have been described appear to decrease AVP hypersecretion in some cases (e.g., diphenylhydantoin, opiates, ethanol), but responses are erratic and unpredictable (204). One potential exception is the recent development of agonists selective for kappa opioid receptors, which appear to be more specific for inhibition of AVP hypersecretion in animal studies (461,462), and in clinical trials successfully produced an aquaresis in patients with cirrhosis (463).

Vasopressin Antagonists

Diseases caused by excess or inappropriate circulating levels of hormones can be treated by interfering with secretion of the hormone, interfering with the effects of the hormone at its target tissue, or indirectly counteracting the physiologic and pathophysiologic effects produced by the effects of the hormone. As described in the preceding text, all of these methods have been used with limited degrees of success in treating patients with SIADH. However, all of these therapies have significant limitations, and it has long been recognized that an *antagonist of the kidney AVP V_2 receptors* would be the ideal agent for treatment of patients with dilutional hyponatremia (464). Previous attempts at synthesizing peptide AVP receptor antagonists were frustrated by species variability with regard to partial agonistic effects of such compounds (465). Recently several nonpeptide AVP selective V_2 and combined V_{1a}/V_2 receptor antagonists have been described that appear to overcome these problems (6). At the time this chapter was written, the first vasopressin receptor antagonist, conivaptan, was approved by the FDA for the treatment of euvolemic hyponatremia, and several additional compounds were already in the late phases of clinical trials, with very promising results. All agents tested in humans to date have successfully induced a dose-related increase in free-water excretion, with subsequent

increases in serum [Na^+] in patients with euvolemic (i.e., SIADH) and hypervolemic (i.e., congestive heart failure [CHF] and cirrhosis) hyponatremia (466–469). Unlike the effects produced by standard diuretic agents, vasopressin receptor agonists do not cause increased urine solute excretion, particularly of sodium and potassium, which renders them particularly effective at correcting hyponatremia. To differentiate this unique selective effect on urine water excretion from the effects of standard diuretic agents that typically cause natriuresis and kaliuresis in association with the induced diuresis, they are called *aquaretic* rather than diuretic agents. (470)

It therefore appears that we are poised to begin a new era in both the evaluation and treatment of patients with all forms of dilutional hyponatremia. When eventually approved for clinical use, clinical trials using AVP receptor antagonists specific for the various subtypes of the AVP receptor, V_{1a}, V_{1b} and V_2, will enable investigators to address some long-standing questions about the role of AVP receptor activation in producing antidiuresis, as well as other potential effects in many different disease states (e.g., the cause of hyponatremia patients without measurable AVP levels [214], the potential role of V_{1a} receptor activation in the progression of heart failure [471], and the participation of V_{1b} receptor activation in psychiatric diseases [472]). Even more promising, therapy of patients with chronic hyponatremia will very likely be much easier and much more effective than at present. Caution will, of course, still be necessary in some situations. Such agents should not be used in cases of hypovolemic hyponatremia from solute depletion, since they will worsen the volume depletion with potential resulting hypotension. In addition, because demyelination appears to be independent of the method used to correct hyponatremia, patients corrected too rapidly with AVP receptor antagonists will also be at risk for pontine and extrapontine myelinolysis, which has already been documented with the use of peptide AVP receptor antagonists to correct hyponatremia in animals (473). Nonetheless, appropriate dosing and monitoring should allow successful adherence to the same guidelines for limited controlled correction that apply to other correction methods. Furthermore, it seems reasonable to predict that single doses of such agents may prove to be the ideal method for achieving prompt but limited controlled increases of serum [Na^+] that will satisfactorily reverse hyponatremic encephalopathy without producing dangerously large corrections that increase the risk of pontine and extrapontine myelinolysis, but controlled clinical trials will be necessary to evaluate this possibility. It, therefore, seems likely that AVP receptor antagonists will represent a significant addition to our therapeutic armamentarium for the treatment of both acute and chronic forms of hyponatremia.

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CHAPTER 87 ■ NEPHROGENIC AND CENTRAL DIABETES INSIPIDUS

DANIEL G. BICHET

Diabetes insipidus is a disorder characterized by the excretion of abnormally large volumes (greater than 30 mL/kg body weight per day for an adult patient) of dilute urine (less than 250 mmol/kg). This definition excludes osmotic diuresis, which occurs when excess solute is being excreted, for example, glucose in the polyuria of diabetes mellitus. Other agents that produce osmotic diuresis are mannitol, urea, glycerol, contrast media, and loop diuretics. Osmotic diuresis should be considered when solute excretion exceeds 60 mmol/hour. Four basic defects can be involved. The most common, a deficient secretion of the antidiuretic hormone (ADH) arginine vasopressin (AVP), is referred to as neurogenic (or central, neurohypophyseal, cranial, or hypothalamic) diabetes insipidus. Diabetes insipidus can also result from renal insensitivity to the antidiuretic effect of AVP, which is referred to as nephrogenic diabetes insipidus. Excessive water intake can result in polyuria, which is referred to as primary polydipsia; it can be due to an abnormality in the thirst mechanism, referred to as dipsogenic diabetes insipidus, or it can be associated with a severe emotional cognitive dysfunction, referred to as psychogenic polydipsia. Finally, increased metabolism of vasopressin during pregnancy is referred to as gestational diabetes insipidus.

Arginine Vasopressin

Synthesis

The regulation of the release of AVP from the posterior pituitary is primarily dependent, under normal circumstances, on tonicity information relayed by osmoreceptor cells in the anterior hypothalamus (1). AVP and its corresponding carrier, neurophysin II, are synthesized as a composite precursor by the magnocellular neurons of the supraoptic and paraventricular nuclei of the hypothalamus (for review see [2]). The precursor is packaged into neurosecretory granules and transported axonally in the stalk of the posterior pituitary. En route to the neurohypophysis, the precursor is processed into the active hormone. Pre-provasopressin has 164 amino acids and is encoded by the 2.5 kb *AVP* gene located in chromosome region 20p13 (3,4). The *AVP* gene (coding for AVP and neurophysin II) and the *OXT* gene (coding for oxytocin and neurophysin I) are located in the same chromosome region, at a very short distance from each other (12 kb in humans) in head-to-head orientation. Data from transgenic mouse studies indicate that the intergenic region between the *OXT* and the *AVP* genes contains the critical enhancer sites for cell-specific expression in the magnocellular neurons (2). It is phylogenetically interesting to note that *cis* and *trans* components of this specific cellular expression have been conserved between the *Fugu* isotocin (the homolog of mammalian oxytocin) and rat oxytocin genes (5). Exon 1 of the *AVP* gene encodes the signal peptide, AVP, and the NH_2-terminal region of neurophysin II. Exon 2 encodes the central region of neurophysin II, and exon 3 encodes the COOH-terminal region of neurophysin II and the glycopeptide. Provasopressin is generated by the removal of the signal peptide from pre-provasopressin and from the addition of a carbohydrate chain to the glycopeptide (Fig. 87-1). Additional posttranslation processing occurs within neurosecretory vesicles during transport of the precursor protein to axon terminals in the posterior pituitary, yielding AVP, neurophysin II, and the glycopeptide. The AVP-neurophysin II complex forms tetramers that can self-associate to form higher oligomers (6) (Fig. 87-2). Neurophysins should be seen as chaperone-like molecules facilitating intracellular transport in magnocellular cells. In the posterior pituitary, AVP is stored in vesicles. Exocytotic release is stimulated by minute increases in serum osmolality (hypernatremia, osmotic regulation) and by more pronounced decreases in extracellular fluid (hypovolemia, non-osmotic regulation). Oxytocin and neurophysin I are released from the posterior pituitary by the suckling response in lactating females.

Immunocytochemical and radioimmunologic studies have demonstrated that oxytocin and vasopressin are synthesized in separate populations of the supraoptic nuclei and the paraventricular nuclei neurons (7,8), the central and vascular projections of which have been described in great detail (9). Some cells express the *AVP* gene and other cells express the *OXT* gene. Immunohistochemical studies have revealed a second vasopressin neurosecretory pathway that transports high concentrations of the hormone to the anterior pituitary gland from parvocellular neurons to the hypophyseal portal system. In the portal system, the high concentration of AVP acts synergistically with corticotropin-releasing hormone (CRH) to stimulate adrenocorticotropic hormone (ACTH) release from the anterior pituitary. More than half of parvocellular neurons coexpress both *CRH* and *AVP*. In addition, while passing through the median eminence and the hypophyseal stalk, magnocellular axons can also release AVP into the long portal system. Furthermore, a number of neuroanatomic studies have shown the existence of short portal vessels that allow communication between the posterior and anterior pituitary. Therefore, in addition to parvocellular vasopressin, magnocellular vasopressin is able to influence ACTH secretion (10,11).

Osmotic and Non-osmotic Stimulation

The regulation of ADH release from the posterior pituitary is dependent primarily on two mechanisms involving the osmotic and non-osmotic pathways (12) (Fig. 87-3). Vasopressin release can be regulated by changes in either osmolality or cerebrospinal fluid (CSF) Na^+ concentration (13,14).

Although magnocellular neurons are themselves osmosensitive, they require input from the lamina terminalis to respond fully to osmotic challenges. Neurons in the lamina terminalis are also osmosensitive and because the subfornical organ (SFO) and the organum vasculosum of the lamina terminalis (OVLT) lie outside the blood–brain barrier, they can integrate this information with endocrine signals borne of circulating hormones, such as angiotensin II (AT-II), relaxin, and atrial natriuretic

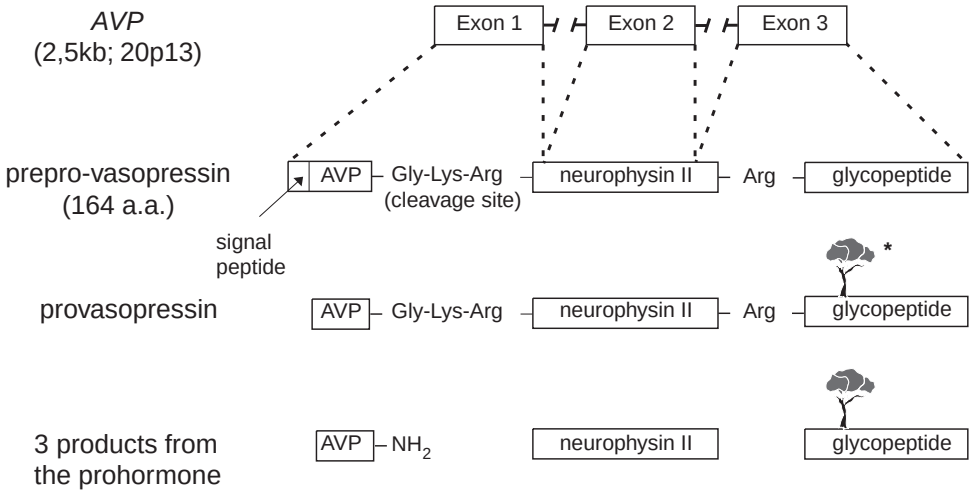

FIGURE 87-1. Structure of the human vasopressin (*AVP*) gene and prohormone.

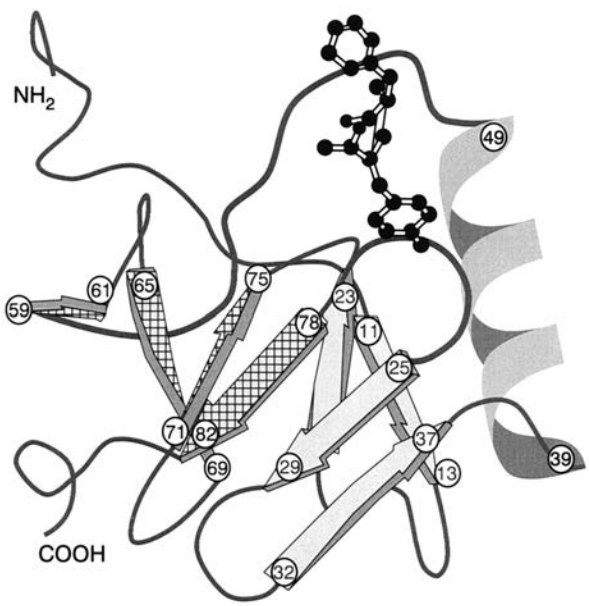

FIGURE 87-2. Three-dimensional structure of a bovine peptide-neurophysin monomer complex. The structure of each chain is 12% helix and 40% β-sheet. The chain is folded into two domains as predicted by disulfide-pairing studies. The amino-terminal domain begins in a long loop (residues 1-10), then enters a four-stranded (residues 11-13, 19-23, 25-29, and 32-37) antiparallel β-sheet (sheet I; *four shaded arrows*), followed by a 3-turn 3_{10}-helix (residues 39-49) and another loop (residues 50-58). The carboxyl-terminal domain is shorter, consisting of only a four-stranded (residues 59-61, 65-69, 71-75, and 78-82) antiparallel β-sheet (sheet II; *four cross-hatched arrows*) (6). The arginine vasopressin molecule (balls and sticks model) is shown in the peptide-binding pocket of the neurophysin monomer. The strongest interactions in this binding pocket are salt-bridge interactions between the $\alpha NH3^+$ group of the peptide, the γ-COO^- group of Glu^{NP47} (residue number 47 of the neurophysin molecule) and the side chain of Arg^{NP8}. The γ-COO^- group of Glu^{NP47} plays a bifunctional role in the peptide-binding pocket: (i) it directly interacts with the hormone; (ii) it interacts with other neurophysin residues to establish the correct, local structure of the peptide-neurophysin complex. Arg^{NP8} and Glu^{NP47} are conserved in all neurophysin sequences from mammals to invertebrates.

peptide (ANP). Although circulating AT-II and relaxin excite both oxytocin and vasopressin magnocellular neurons, ANP inhibits vasopressin neurons. In addition to an angiotensinergic path from the SFO, the OVLT and the median preoptic nucleus provide direct glutaminergic and GABAergic projections to the hypothalamoneurohypophyseal system. Nitric oxide may also modulate neurohormone release (2). A newly discovered neuropeptide, apelin, is co-localized with AVP in supra-optic nucleus (SON) magnocellular neurons and physiological experiments indicate that AVP and apelin are conversely regulated to facilitate systemic AVP release and suppress antidiuresis (15).

The cellular basis for osmoreceptor potentials has been characterized using patch-clamp recordings and morphometric analysis in magnocellular cells isolated from the supraoptic nucleus of the adult rat. In these cells, stretch-inactivating cationic channels transduce osmotically evoked changes in cell volume into functionally relevant changes in membrane potential. In addition, magnocellular neurons also operate as intrinsic Na^+ detectors. A transient receptor potential channel variant of Trpv1 is an osmotically activated channel expressed in AVP-releasing neurons in the SON (16).

Vasopressin release can also be caused by the non-osmotic stimulation of AVP. Large decrements in blood volume or blood pressure (greater than 10%) stimulate ADH release (Fig. 87-3).

The osmotic stimulation of AVP release by dehydration, hypertonic saline infusion, or both, is regularly used to determine the vasopressin secretory capacity of the posterior pituitary. This secretory capacity can be assessed *directly* by comparing the plasma AVP concentrations measured sequentially during the dehydration procedure with the normal values (17) and then correlating the plasma AVP values with the urine osmolality measurements obtained simultaneously (Fig. 87-4).

The AVP release can also be assessed *indirectly* by measuring plasma and urine osmolalities at regular intervals during the dehydration test (18). The maximal urine osmolality obtained during dehydration is compared with the maximal urine osmolality obtained after the administration of vasopressin (Pitressin, 5 U subcutaneously in adults, 1 U subcutaneously in children) or 1-desamino-8-D-arginine vasopressin (desmopressin [dDAVP], 1 to 4 μg i.v. over 5 to 10 minutes).

The non-osmotic stimulation of AVP release can be used to assess the vasopressin secretory capacity of the posterior pituitary in a rare group of patients with the essential hyponatremia and hypodipsia syndrome (19). Although some of these patients may have partial central diabetes insipidus, they respond

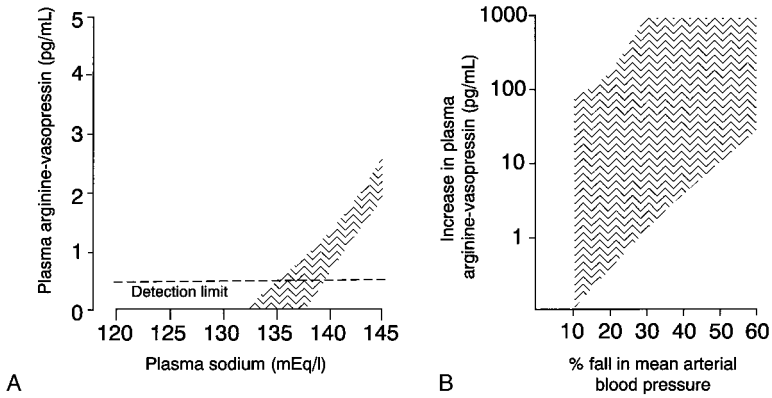

FIGURE 87-3. Osmotic and non-osmotic stimulation of arginine vasopressin (AVP). **A:** The relationship between plasma AVP (P_{AVP}) and plasma sodium (P_{Na}) in 19 normal subjects is described by the area with *vertical lines*, which includes the 99% confidence limits of the regression line P_{Na}/P_{AVP}. The osmotic threshold for AVP release is approximately 280 to 285 mmol/kg or 136 mEq of Na/L. AVP secretion should be abolished when plasma sodium is less than 135 mEq/L (180). (From: Vokes T, Robertson GL. Physiology of secretion of vasopressin. In: Czernichow P, Robinson AG, eds. *Diabetes insipidus in man*. Basel: S. Karger, 1985:127, with permission.) **B:** Increase in plasma AVP during hypotension (*vertical lines*). Note that a large diminution in blood pressure in normal humans induces large increments in AVP. (From: Zerbe RL, Henry DP, Robertson GL. Vasopressin response to orthostatic hypotension: etiological and clinical implications. *Am J Med* 1983;74:265, with permission.)

normally to non-osmolar AVP release signals such as hypotension, emesis, and hypoglycemia (19). In all other cases of suspected central diabetes insipidus, these non-osmotic stimulation tests will not give additional clinical information (20).

Clinically Important Hormonal Influences on the Secretion of Vasopressin

Angiotensin is a well-known dipsogen and has been shown to increase thirst in all the species tested (21). AT-II receptors have been described in the SFO and OVLT (for review see [22]). However, knockout models for angiotensinogen (23) or for angiotensin-1A (AT1A) receptor (24,25) did not alter thirst or water balance. Disruption of the AT2 receptor induced only mild abnormalities of thirst postdehydration (26). Earlier reports suggested that the intravenous administration of atrial peptides inhibits the release of vasopressin (27), but this was not confirmed by Goetz et al. (28). Furthermore, Ogawa et al. (29) found no evidence that ANP, administered centrally or peripherally, was important in the physiologic regulation of plasma AVP release in conscious rats. A very rapid and robust release of AVP is seen in humans after cholecystokinin (CCK) injection (30). Nitric oxide is an inhibitory modulator of the hypothalamoneurohypophyseal system in response to osmotic stimuli (31–34). Vasopressin secretion is under the influence of a glucocorticoid-negative feedback system (35), and the vasopressin responses to a variety of stimuli (hemorrhage, hypoxia, hypertonic saline) in normal humans and animals appear to be attenuated or eliminated by pretreatment with glucocorticoids. Finally, nausea and emesis are potent stimuli of AVP release in humans and seem to involve dopaminergic neurotransmission (36).

Cellular Actions of Vasopressin

The neurohypophyseal hormone AVP has multiple actions, including the inhibition of diuresis, contraction of smooth muscle, platelet aggregation, stimulation of liver glycogenolysis, modulation of ACTH release from the pituitary, and central regulation of somatic functions (thermoregulation, blood pressure) (37). These multiple actions of AVP can be explained by the interaction of AVP with at least three types of G protein-coupled receptors; the V_{1a} (vascular hepatic) and V_{1b} (anterior pituitary) receptors act through phosphatidylinositol hydrolysis to mobilize calcium (38), and the V_2 (kidney) receptor is coupled to adenylate cyclase (37). Oxytocin and vasopressin receptors are widely expressed in the brain and it is now well recognized that oxytocin and vasopressin are involved in adult–adult attachment (39) and in the modulation of autonomic fear responses (40).

The first step in the action of AVP on water excretion is its binding to arginine vasopressin type 2 receptors (hereafter referred to as V_2 receptors) on the basolateral membrane of the collecting duct cells (Fig. 87-5). The human gene that codes for the V_2 receptor (*AVPR2*) is located in chromosome region Xq28 and has three exons and two small introns (41,42). The sequence of the cDNA predicts a polypeptide of 371 amino acids with seven transmembrane, four extracellular, and four cytoplasmic domains (Fig. 87-6). The V_2 receptor is one of 701 members of the rhodopsin family within the superfamily of guanine-nucleotide (G) protein-coupled receptors ([43]; see also perspective of Perez [44]). The activation of the V_2 receptor on renal collecting tubules stimulates adenylyl cyclase via the stimulatory G protein (Gs) and promotes the cyclic adenosine monophosphate (cAMP)-mediated incorporation of water pores into the luminal surface of these cells. This process

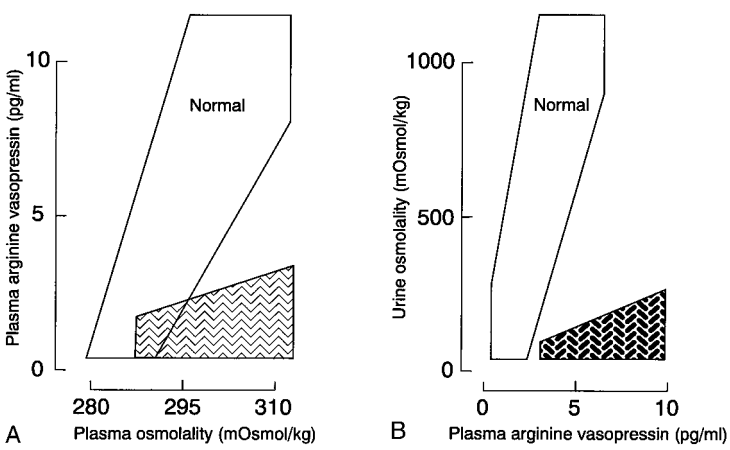

FIGURE 87-4. **A:** Relationship between plasma arginine vasopressin (AVP) and plasma osmolality during infusion of hypertonic saline solution. Patients with primary polydipsia and nephrogenic diabetes insipidus have values within the normal range (*open area*) in contrast to patients with neurogenic diabetes insipidus, who show subnormal plasma antidiuretic hormone (ADH) responses (*cross-hatched area*). **B:** Relationship between urine osmolality and plasma ADH during dehydration and water loading. Patients with neurogenic diabetes insipidus and primary polydipsia have values within the normal range (*open area*) in contrast to patients with nephrogenic diabetes insipidus, who have hypotonic urine despite high plasma ADH (*stippled area*). (From: Zerbe RL, Robertson GL. Disorders of ADH. *Med North Am* 1984;13:1570, with permission.)

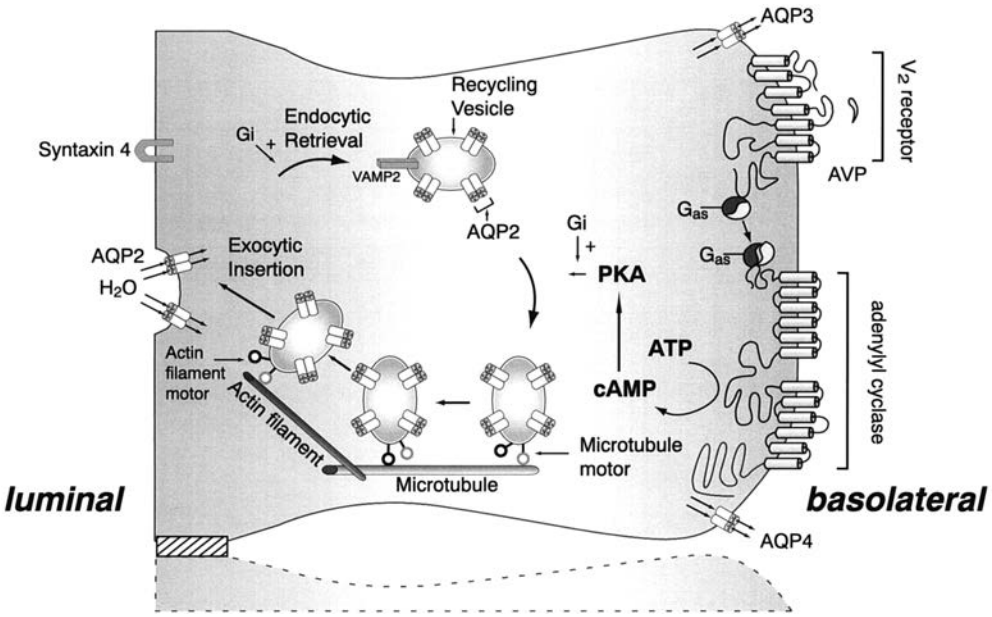

FIGURE 87-5. Schematic representation of the effect of arginine vasopressin (AVP) to increase water permeability in the principal cells of the collecting duct. AVP is bound to the V_2 receptor (a G protein-linked receptor) on the basolateral membrane. The basic process of G protein-coupled receptor signaling consists of three steps: a hepta-helical receptor that detects a ligand (in this case, AVP) in the extracellular milieu, a G protein that dissociates into α-subunits bound to GTP and $\beta\gamma$-subunits after interaction with the ligand-bound receptor, and an effector (in this case, adenylyl cyclase) that interacts with dissociated G protein subunits to generate small-molecule second messengers. AVP activates adenylyl cyclase increasing the intracellular concentration of cyclic adenosine monophosphate (cAMP). The topology of adenylyl cyclase is characterized by 2 tandem repeats of 6 hydrophobic transmembrane domains separated by a large cytoplasmic loop and terminates in a large intracellular tail. Generation of cAMP follows receptor-linked activation of the heteromeric G protein (G_s) and interaction of the free $G_{\alpha s}$-chain with the adenylyl cyclase catalyst. Protein kinase A (PKA) is the target of the generated cAMP. Cytoplasmic vesicles carrying the water channel proteins (represented as homotetrameric complexes) are fused to the luminal membrane in response to AVP, thereby increasing the water permeability of this membrane. Microtubules and actin filaments are necessary for vesicle movement toward the membrane. The mechanisms underlying docking and fusion of aquaporin-2 (AQP2)-bearing vesicles are not known. The detection of the small GTP-binding protein Rab3a, synaptobrevin 2, and syntaxin 4 in principal cells suggests that these proteins are involved in AQP2 trafficking (197). When AVP is not available, water channels are retrieved by an endocytic process, and water permeability returns to its original low rate. AQP3 and AQP4 water channels are expressed on the basolateral membrane.

is the molecular basis of the vasopressin-induced increase in the osmotic water permeability of the apical membrane of the collecting tubule (45).

The gene that codes for the water channel of the apical membrane of the kidney-collecting tubule has been designated aquaporin 2 (*AQP2*) and was cloned by homology to the rat aquaporin of the collecting duct (46–48). The human *AQP2* gene is located in chromosome region 12q13 and has four exons and three introns (47–49). It is predicted to code for a polypeptide of 271 amino acids that is organized into two repeats oriented at 180° to each other and that has six membrane-spanning domains, with both terminal ends located intracellularly, and conserved asparagine-proline-alanine (NPA) boxes (Figs. 87-7 and 87-8). It is now well recognized that aquaporins are transmembrane channels found in cell membranes of all life forms that efficiently transport water while excluding protons. Molecular dynamic simulations suggest that a global orientation control mechanism facilitates efficient movement of a single column of water molecules while preventing proton transport in the channel (50). Data from mouse models suggest that AQP3 and AQP4 may play an important role in urinary concentration at the basolateral membrane (51). New roles for aquaporins are being discovered including a possible role of AQP1 facilitated water permeability for cell migration related to angiogenesis (52).

AQP2 is detectable in urine, and changes in urinary excretion of this protein can be used as an index of the action of vasopressin on the kidney (53–55).

AVP also increases the water reabsorptive capacity of the kidney by regulating the urea transporter UT-A1, which is present in the inner medullary collecting duct, predominantly in its terminal part (56,57). AVP also increases the permeability of principal collecting duct cells to sodium (58).

In summary, as stated elegantly by Ward et al. (58), in the absence of AVP stimulation, collecting duct epithelia exhibit very low permeabilities to sodium, urea, and water. These specialized permeability properties permit the excretion of large volumes of hypotonic urine formed during intervals of water diuresis. In contrast, AVP stimulation of the principal cells of the collecting ducts leads to selective increases in the permeability of the apical membrane to water (P_f), urea (P_{urea}), and Na (P_{Na}).

These actions of vasopressin in the distal nephron are possibly modulated by prostaglandins E_2 and by the luminal calcium concentration. High levels of E-prostanoid (EP_3) receptors are expressed in the kidney (59). However, mice lacking EP_3 receptors for prostaglandin E_2 were found to have quasinormal regulation of urine volume and osmolality in response to various physiologic stimuli (59). An apical calcium/polycationic receptor protein expressed in the terminal portion of the inner

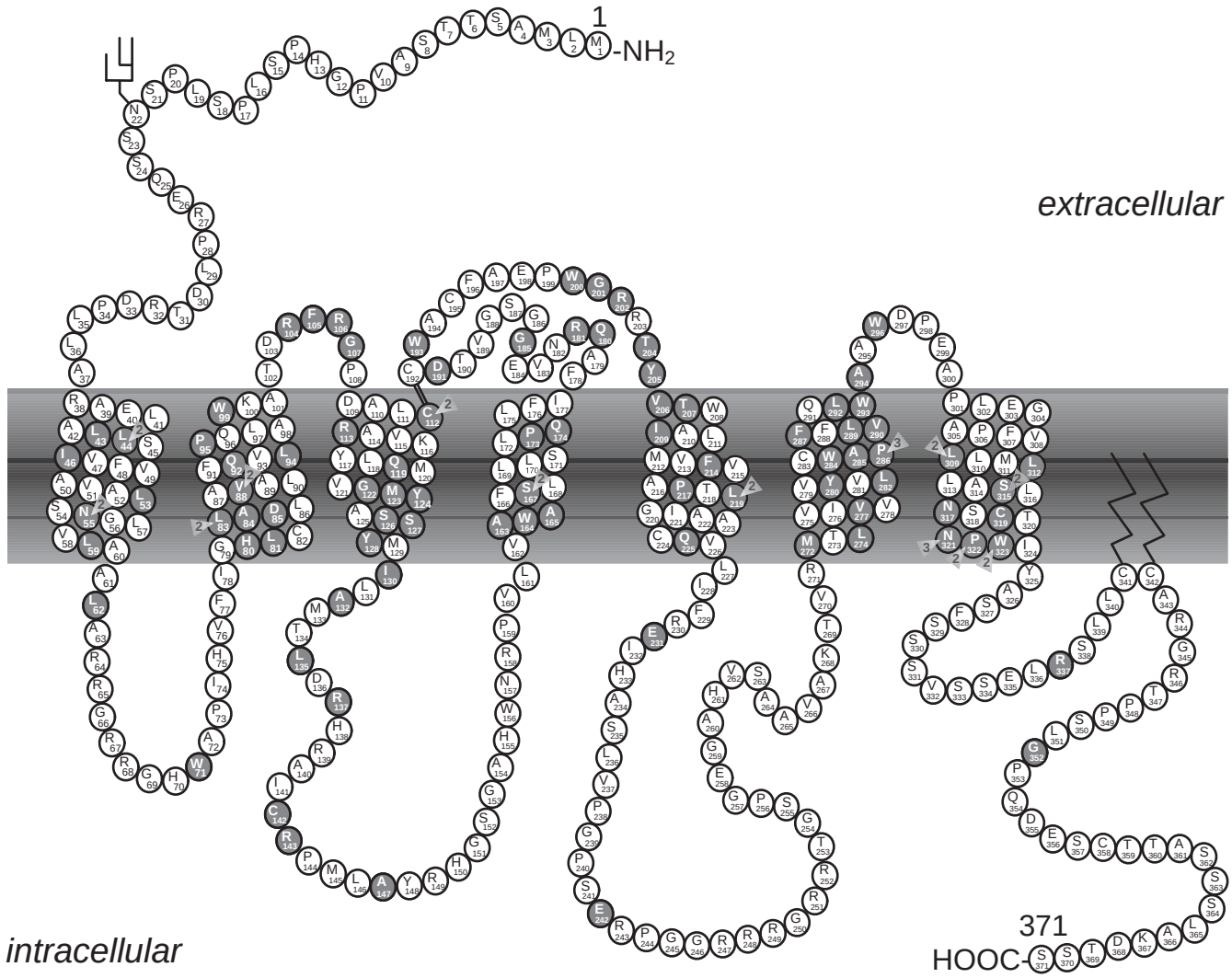

FIGURE 87-6. Schematic representation of the V_2 receptor and identification of 183 putative disease-causing *AVPR2* mutations. Predicted amino acids are given as the one-letter amino acid code. Solid symbols indicate missense or nonsense mutations; a number indicates more than one mutation in the same codon; other types of mutations are not indicated on the figure. The extracellular, transmembrane, and cytoplasmic domains are defined according to Mouillac et al. (198). There are 89 missense, 18 nonsense, 45 frameshift deletion or insertion, 7 in-frame deletion or insertion, 4 splice-site, and 19 large deletion mutations, and one complex mutation. See http://www.medicine.mcgill.ca/nephros for a list of mutations. (Reprinted from: Fujiwara TM, Bichet DG. Molecular biology of hereditary diabetes insipidus. *J Am Soc Nephrol* 2005;16:2836 [Fig. 1], with permission.)

medullary collecting duct of the rat has been shown to reduce AVP-elicited osmotic water permeability when luminal calcium concentration rises (60). This possible link between calcium and water metabolism may play a role in the pathogenesis of renal stone formation (60).

Knockout Mice with Urinary Concentration Defects

A useful strategy to establish the physiologic function of a protein is to determine the phenotype produced by pharmacologic inhibition of protein function or by gene disruption. Transgenic knockout mice deficient in AQP1, AQP2, AQP3, AQP4, AQP3 and AQP4, CLCNK1, NKCC2, AVPR2, or AGT have been engineered (61–69). Angiotensinogen (AGT)-deficient mice are characterized by both concentrating and diluting defects secondary to a defective renal papillary architecture (69).

As reviewed by Rao and Verkman (70) extrapolation of data in mice to humans must be made with caution. For example, the maximum osmolality of mouse (greater than 3,000 mOsmol/kg H_2O) is much greater than that of human urine (1,000 mOsmol/kg H_2O), and normal serum osmolality in mice is 330 to 345 mOsmol/kg H_2O, substantially greater than that in humans (280 to 290 mOsmol/kg H_2O). Protein expression patterns and thus the interpretation of phenotype studies may also be species-dependent. For example, AQP4 is expressed in both proximal tubule and collecting duct in mouse but only in collecting duct in rat and human (70).

The *Aqp3*, *Aqp4*, *Clcnk-1,* and *Agt* knockout mice have no identified human counterparts. Of interest, *AQP1*-null individuals have no obvious symptoms (71). Yang et al. (68) have generated an *AQP2-T126M* knock-in mutant mice to

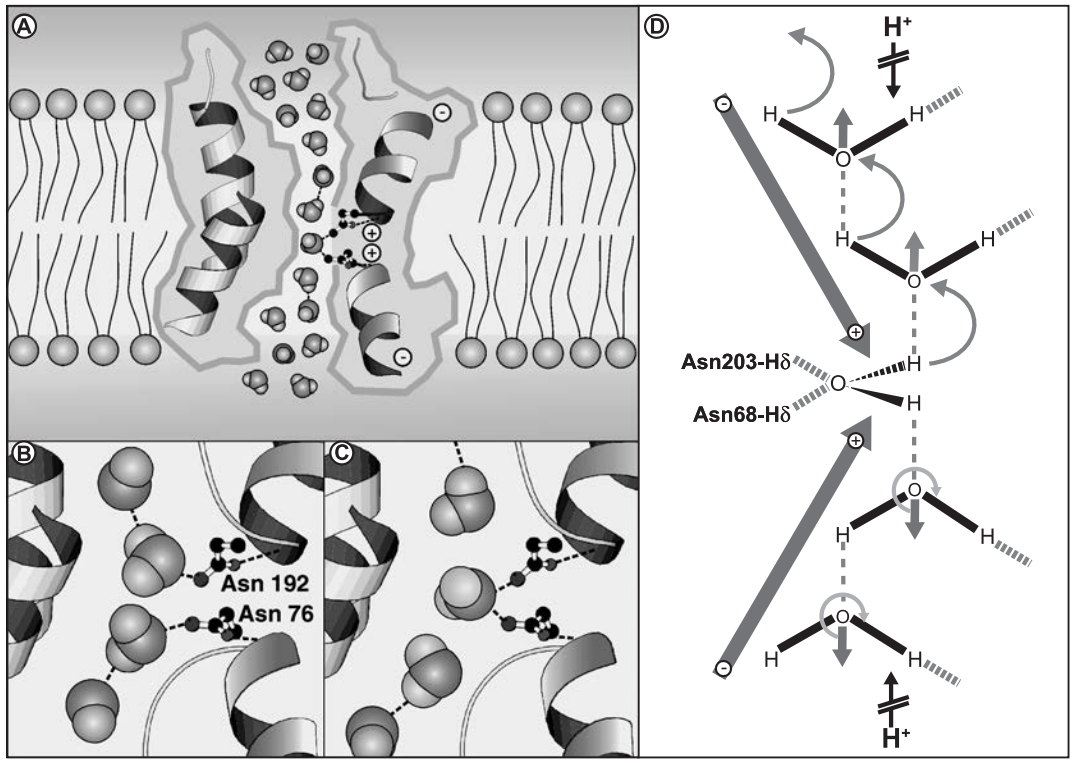

FIGURE 87-7. Schematic representations explaining the mechanism for blocking proton permeation of aquaporin 1 (AQP1). **A:** Diagram illustrating how partial charges from the helix dipoles restrict the orientation of the water molecules passing through the constriction of the pore. **B, C:** Diagrams illustrating hydrogen bonding of a water molecule with asparagines (Asn) 76 and/or Asn 192, which extend their amido groups into the constriction of the pore. The ribbon and ball-stick models were prepared with MOLSCRIPT (199). **D:** The two conserved asparagines in the aquaglyceroporin force a central water molecule to serve strictly as a hydrogen bond donor to its neighboring water molecules. Water reorientation is indicated by green arrows. (Reprinted from: Murata K, Mitsuoka K, Hirai T, et al. Structural determinants of water permeation through aquaporin-1. *Nature* 2000;407:599 and Tajkhorshid E, Nollert P, Jensen MO, et al. Control of the selectivity of the aquaporin water channel family by global orientational tuning. *Science* 2002;296:525, with permission.)

recapitulate the clinical features of the naturally occurring human *AQP2* mutation T126M (72). The mutant mice appeared normal at 2 to 3 days after birth, but failed to thrive and generally died by day 6 if not given supplemental fluid. These mice were characterized by an extremely severe concentrating defect with urine osmolality of 225 ± 9 mOsmol/kg H_2O with concomitant serum osmolality of 450 ± 50 mOsmol/kg H_2O and renal failure. Ethylnitrosourea-mutagenized mice heterozygous for the *F204V* mutation in the *Aqp2* gene have recently been described (73). The homozygous mice are viable because of a moderate phenotype with a possibility to concentrate urine from 161 to 470 mOsmol/kg H_2O in response to dDAVP. Cell biology experiments performed on renal tissue from $Aqp2^{F204V/+}$ animals suggest that the mutant protein is being rescued by the wildtype protein. Mice lacking the AVPR2 receptor (67) also failed to thrive and died within the first week after birth due to hypernatremic dehydration (67). The absence of the gene coding for the NaK2Cl cotransport (NKCC2) in the luminal membrane of the thick ascending loop of Henle in the mouse also caused polyuria that was not compensated elsewhere in the nephron and recapitulated many features of the human classical Bartter's syndrome (66). The absence of transcellular NaCl transport via NKCC2 probably abolished the lumen positive transepithelial voltage that enables paracellular reabsorption of Na and K across the wall of the thick ascending tubule. The combined absence of transcellular and paracellular transport of salt across the thick ascending limb cells prevents the establishment of the normal osmotic gradient necessary for urine concentration.

The Brattleboro Rat with Autosomal Recessive Neurogenic Diabetes Insipidus

The classical animal model for studying diabetes insipidus has been the Brattleboro rat with autosomal recessive neurogenic diabetes insipidus. These *di/di* rats are homozygous for a 1-bp deletion (G) in the second exon that results in a frameshift mutation in the coding sequence of the carrier neurophysin II (NPII) (74) (Fig. 87-9). The polyuric symptoms are also observed in heterozygous *di/n* rats. Homozygous Brattleboro rats may still demonstrate some V_2 antidiuretic effect, since the administration of a selective nonpeptide V_2 antagonist (SR121463A, 10 mg/kg i.p.) induced a further increase in urine flow rate (200 to 354 ± 42 mL per 24 hours) and a decline in urinary osmolality (170 to 92 ± 8 mmol/kg) (75). Oxytocin, which is present at enhanced plasma concentrations in Brattleboro rats, may be responsible for the antidiuretic activity observed (76,77). Oxytocin is not stimulated by increased plasma osmolality in humans. The Brattleboro rat model is, therefore, not strictly comparable with the rarely observed human cases of autosomal recessive neurogenic diabetes insipidus (78,79).

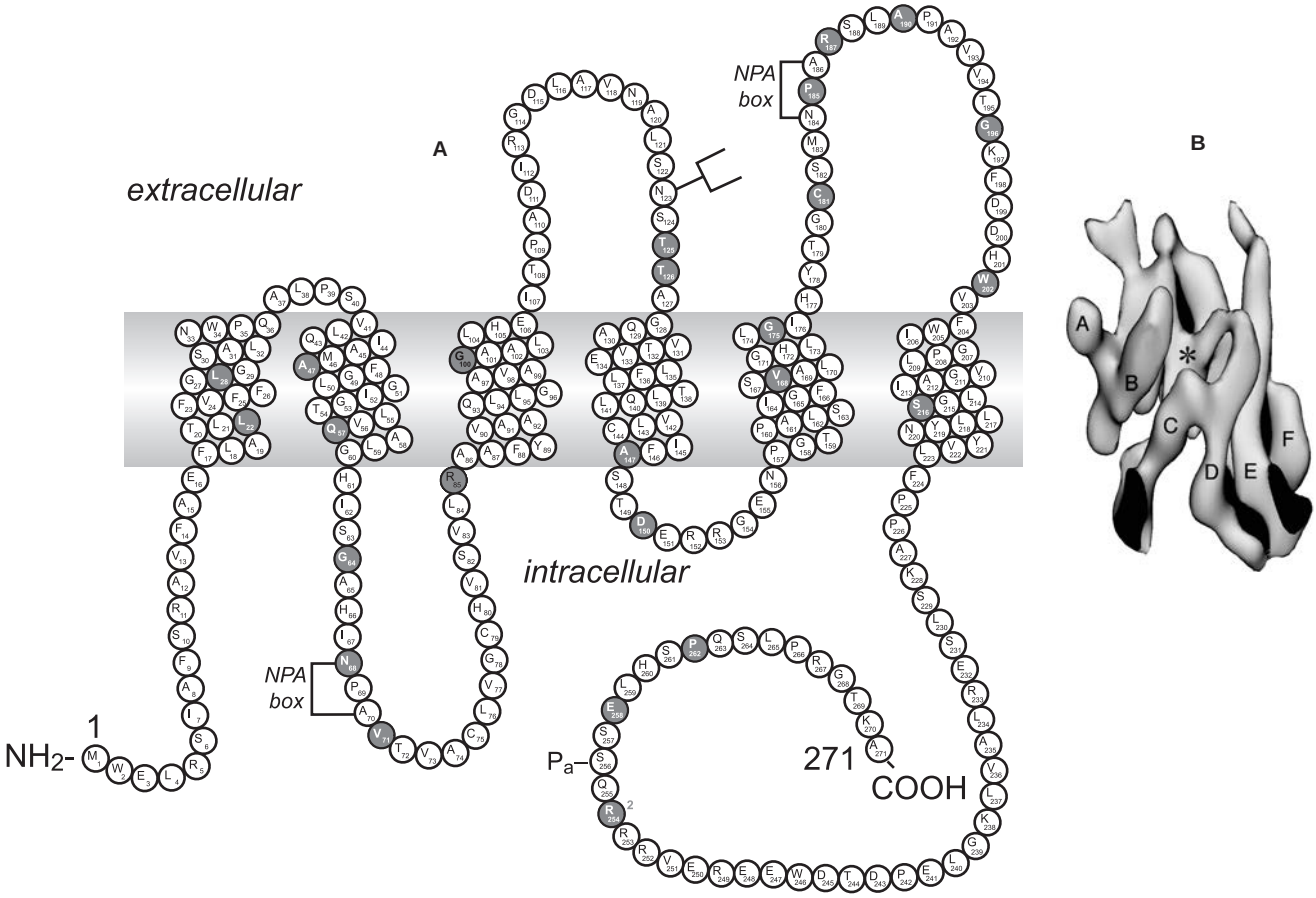

FIGURE 87-8. A: Schematic representation of aquaporin 2 (AQP2) and identification of 35 putative disease-causing *AQP2* mutations. See Figure 87-1 legend for a description of the symbols. The locations of the NPA boxes and the PKA phosphorylation site (Pa) are indicated. The extracellular, transmembrane, and cytoplasmic domains are defined according to Deen PM, Verdijk MA, Knoers NV, et al. Requirement of human renal water channel aquaporin-2 for vasopressin-dependent concentration of urine. *Science* 1994;264:92. *Solid symbols* indicate the location of the missense or nonsense mutations. There are 25 missense, 2 nonsense, 6 frameshift deletion or insertion, and 2 splice-site mutations. **B:** A monomer is represented with six transmembrane helices, A–F. The *asterisk* indicates where the molecular pseudo-2-fold symmetry is strongest (201). (Reprinted from: Fujiwara TM, Bichet DG. Molecular biology of hereditary diabetes insipidus. *J Am Soc Nephrol* 2005;16:283 [Fig. 2], with permission.)

Clinical Characteristics of Diabetes Insipidus Disorders

Central Diabetes Insipidus

Common Forms. Failure to synthesize or secrete vasopressin normally limits maximal urinary concentration and, depending on the severity of the disease, causes varying degrees of polyuria and polydipsia. Experimental destruction of the vasopressin-synthesizing areas of the hypothalamus (supraoptic and paraventricular nuclei) causes a permanent form of the disease. Similar results are obtained by sectioning the hypophyseal-hypothalamic tract above the median eminence. Sections below the median eminence, however, produce only transient diabetes insipidus. Lesions to the hypothalamic-pituitary tract are often associated with a three-stage response both in experimental animals and in humans (80), which consists of:

1. An initial diuretic phase lasting from a few hours to 5 to 6 days.
2. A period of antidiuresis unresponsive to fluid administration. This antidiuresis is probably due to vasopressin release from injured axons and may last from a few hours to several days. Because urinary dilution is impaired during this phase, continued water administration can cause severe hyponatremia.
3. A final period of diabetes insipidus. The extent of the injury determines the completeness of the diabetes insipidus, and as already discussed, the site of the lesion determines whether the disease will or will not be permanent.

A detailed assessment of water balance following transsphenoidal surgery has been reported (81). One hundred and one

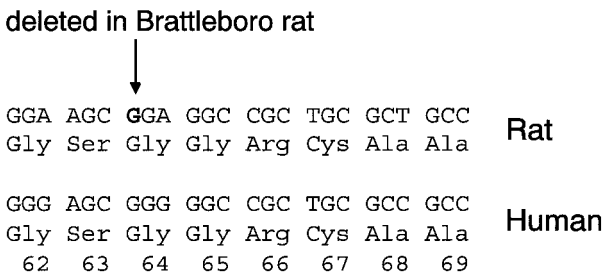

FIGURE 87-9. Neurophysin II genomic and amino acid sequence showing the 1 bp (G) deleted in the Brattleboro rat. The human sequence (GenBank entry M11166) is also shown. It is almost identical to the rat pre-pro sequence. In the Brattleboro rat, G1880 is deleted with a resultant frameshift after 63 amino acids (amino acid 1 is the first amino acid of neurophysin II).

TABLE 87-1

ETIOLOGY OF HYPOTHALAMIC DIABETES INSIPIDUS IN CHILDREN AND ADULTS

	Children (%)	Children and young adults (%)	Adults (%)
Primary brain tumor[a]	49.5	22	30
Before surgery	33.5		13
After surgery	16		17
Idiopathic (isolated or familial)	29	58	25
Histiocytosis	16	12	—
Metastatic cancer[b]	—		8
Trauma[c]	2.2	2.0	17
Postinfectious disease	2.2	6.0	—

[a]Primary malignancy: craniopharyngioma, dysgerminoma, meningioma, adenoma, glioma, astrocytoma.
[b]Secondary: metastatic from lung or breast, lymphoma, leukemia, dysplastic pancytopenia.
[c]Trauma could be severe or mild.
(Data from: Czernichow P, Pomarede R, Brauner R, et al. Neurogenic diabetes insipidus in children. In: Czernichow P, Robinson AG, eds. *Frontiers of Hormone Research*, vol 13, "Diabetes insipidus in man." Basel, Switzerland: S. Karger; 1985:190; Greger NG, Kirkland RT, Clayton GW, et al. Central diabetes insipidus. 22 years' experience. *Am J Dis Child* 1986;140:551; Moses AM, Blumenthal SA, Streeten DH. Acid–base and electrolyte disorders associated with endocrine disease: pituitary and thyroid. In: Arieff AI, de Fronzo RA, eds. *Fluid, Electrolyte, and Acid–base Disorders*. New York: Churchill Livingstone; 1985: 851; Maghnie M, Cosi G, Genovese E, et al. Central diabetes insipidus in children and young adults. *N Engl J Med* 2000;343:998.)

patients who underwent transsphenoidal pituitary surgery at the National Institutes of Health Clinical Center were studied. Twenty-five percent of the patients developed spontaneous isolated hyponatremia, 20% developed diabetes insipidus, and 46% remained normonatremic. Normonatremia, hyponatremia, and diabetes insipidus were associated with increasing degrees of surgical manipulation of the posterior lobe and pituitary stalk during surgery.

The etiologies of central diabetes insipidus in adults and in children are listed in Table 87-1 (82–84). Rare causes of central diabetes insipidus include leukemia, thrombotic thrombocytopenic purpura, pituitary apoplexy, sarcoidosis and Wegener's granulomatosis (85). A distinctive syndrome characterized by early diabetes insipidus with subsequent progressive spastic cerebellar ataxia has also been described (86). Five patients who all presented with central diabetes insipidus and hypogonadism as first manifestations of neurosarcoidosis have been reported (87). Finally, circulating antibodies to vasopressin do not play a role in the development of diabetes insipidus (88). Antibodies to vasopressin occasionally develop during treatment with ADH and, when they do, almost always result in secondary resistance to its antidiuretic effect (88,89). Maghnie et al. (84) studied 79 patients with central diabetes insipidus who were seen at four pediatric endocrinology units between 1970 and 1996. There were 37 male and 42 female patients whose median age at diagnosis was 7 years (range, 0.1 to 24.8 years). In 10 patients, central diabetes insipidus developed during an infectious illness or less than 2 months afterward (varicella in 5 patients, mumps in 3 patients, and measles, toxoplasmosis, and hepatitis B in 1 patient each). Deficits in anterior pituitary hormones were documented in 48 patients (61%) a median of 0.6 year (range, 0.1 to 18.0 years) after the onset of diabetes insipidus. The most frequent abnormality was growth hormone deficiency (59%), followed by hypothyroidism (28%), hypogonadism (24%), and adrenal insufficiency (22%). Seventy-five percent of the patients with histiocytosis of the Langerhans' cells had an anterior pituitary hormone deficiency that was first detected at a median of 3.5 years after the onset of diabetes insipidus. The frequency and progression of histiocytosis of the Langerhans' cells related to anterior pituitary and other nonendocrine hypothalamic dysfunction, and their response to treatment in 12 adult patients has also been recently reviewed (90). None of the patients with central diabetes insipidus secondary to *AVP* mutations developed anterior pituitary hormone deficiencies.

Rare Forms. Inherited neurohypophyseal diabetes insipidus (OMIM 125700) (91) due to mutations in the *AVP* gene (OMIM 192340) (91) and Wolfram syndrome 1 (OMIM 222300) (91) due to mutations in the *WFS1* gene. **Historically,** Lacombe (92) and Weil (93) described a familial non–X-linked form of diabetes insipidus without any associated mental retardation. The descendants of the family described by Weil were later found to have autosomal dominant neurogenic diabetes insipidus (94–96).

Patients with autosomal dominant neurohypophyseal diabetes insipidus retain some limited capacity to secrete AVP during severe dehydration, and the polyuria-polydipsic symptoms usually appear after the first year of life (97), when the infant's demand for water is more likely to be understood by adults. More than 50 *AVP* mutations segregating with autosomal dominant or autosomal recessive neurohypophyseal diabetes insipidus have been described (for a list of mutations see http://www.medicine.mcgill.ca/nephros). The mechanisms by which a mutant allele causes neurohypophyseal diabetes insipidus could involve the induction of magnocellular cell death as a result of the accumulation of AVP precursors within the endoplasmic reticulum (98–100). This hypothesis could account for the delayed onset of the disease. In addition to the cytotoxicity caused by mutant AVP precursors, the interaction between the wild-type and the mutant precursors suggests that a dominant-negative mechanism may also contribute to the pathogenesis of autosomal dominant diabetes insipidus (101). The absence of symptoms in infancy in autosomal dominant neurohypophyseal diabetes insipidus is in sharp contrast with nephrogenic diabetes insipidus (NDI) secondary to mutations in *AVPR2* or in *AQP2,* in which the polyuria-polydipsic symptoms are present during the first week of life.

Of interest, errors in protein folding represent the underlying basis for many inherited diseases (102–104) and are also pathogenic mechanisms for *AVP*, *AVPR2*, and *AQP2* mutants. Why AVP misfolded mutants are cytotoxic to AVP-producing neurons is an unresolved issue. Protein misfolding, an "unfolded protein response" in cells, and the accumulation of excess misfolded protein leading to apoptotic cell death are well documented for autosomal dominant retinitis pigmentosa (105).

Three families with autosomal recessive neurohypophyseal diabetes insipidus have been identified in which the patients were homozygous or compound heterozygotes for *AVP* mutations (78,79). Two of these families are characterized phenotypically by severe and early onset in the first three months of life with polyuria, polydipsia, and dehydration. As a consequence, early hereditary diabetes insipidus can be neurogenic or nephrogenic.

Wolfram syndrome, also known as DIDMOAD, is an autosomal recessive neurodegenerative disorder accompanied by insulin-dependent diabetes mellitus and progressive optic atrophy. The acronym DIDMOAD describes the following clinical features of the syndrome: *d*iabetes *i*nsipidus, *d*iabetes *m*ellitus, *o*ptic *a*trophy, and sensorineural *d*eafness. An unusual incidence of psychiatric symptoms has also been described in patients with this syndrome. These included paranoid delusions, auditory or visual hallucinations, psychotic behavior, violent behavior, organic brain syndrome typically in the late or preterminal stages of their illness, progressive dementia, and severe learning disabilities or mental retardation or both. Patients with Wolfram syndrome develop diabetes mellitus and bilateral optical atrophy, mainly in the first decade of life; the diabetes insipidus is usually partial and of gradual onset, and the polyuria can be wrongly attributed to poor glycemic control. Furthermore, a severe hyperosmolar state can occur if untreated diabetes mellitus is associated with an unrecognized posterior pituitary deficiency. The dilation of the urinary tract observed in the DIDMOAD syndrome may be secondary to chronic high urine flow rates and, perhaps, to some degenerative aspects of the innervation of the urinary tract. The gene responsible for Wolfram syndrome located in chromosome region 4p16.1, encodes a putative 890 amino acid transmembrane protein referred as wolframin. Wolframin is an endoglycosidase H-sensitive glycoprotein, which localizes primarily in the endoplasmic reticulum of a variety of neurons including neurons in the supraoptic nucleus and neurons in the lateral magnocellular division of the paraventricular nucleus (106,107).

The Syndrome of Hypernatremia and Hypodipsia. Some patients with the hypernatremia and hypodipsia syndrome may have partial central diabetes insipidus (108). These patients also have persistent hypernatremia, which is not due to any apparent extracellular volume loss; absence or attenuation of thirst; and a normal renal response to AVP. In almost all the patients studied to date, the hypodipsia has been associated with cerebral lesions in the vicinity of the hypothalamus. It has been proposed that there is a "resetting" of the osmoreceptor in these patients because their urine tends to become concentrated or diluted at inappropriately high levels of plasma osmolality. However, by using the regression analysis of plasma AVP concentration versus plasma osmolality, it has been possible to show that in some of these patients the tendency to concentrate and dilute urine at inappropriately high levels of plasma osmolality is due solely to a marked reduction in sensitivity or a gain in the osmoregulatory mechanism. This finding is compatible with the diagnosis of partial central diabetes insipidus. In other patients, however, plasma AVP concentrations fluctuate randomly, bearing no apparent relationship to changes in plasma osmolality. Such patients frequently display large swings in serum sodium concentrations and often exhibit hypodipsia. It appears that most patients with essential hypernatremia fit one of these two patterns (Fig. 87-10). Both of these groups of patients consistently respond normally to non-osmolar AVP release signals, such as hypotension, emesis, hypoglycemia, or all three of these signals. These observations suggest that the osmoreceptor may be anatomically as well as functionally separate from the non-osmotic efferent pathways and neurosecretory neurons for vasopressin. Furthermore, a hypothalamic lesion may impair the osmotic release of AVP, whereas the non-osmotic release of AVP remains intact, and the osmoreceptor neurons that regulate vasopressin secretion are not totally synonymous with those that regulate thirst, although they appear to be anatomically close if not overlapping.

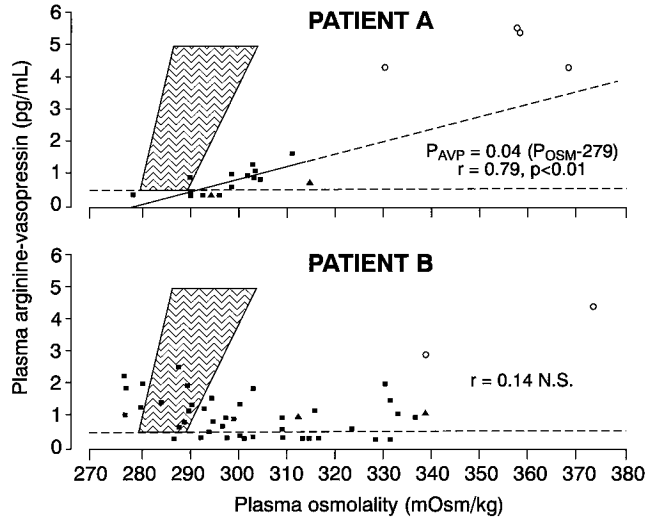

FIGURE 87-10. Plasma vasopressin (P_{AVP}) as a function of "effective" plasma osmolality (P_{OSM}) in two patients with adipsic hypernatremia. *Open circles* indicate values obtained on admission; *filled squares* indicate those obtained during forced hydration; *filled triangles* indicate those obtained after 1 to 2 weeks of *ad libitum* water intake; *shaded areas* indicate range of normal values. (From: Robertson GL. The physiopathology of ADH secretion. In: Tolis G, Labrie F, Martin JB, et al., eds. *Clinical Neuroendocrinology: A Pathophysiological Approach*. New York: Raven Press; 1979:247, with permission.)

Nephrogenic Diabetes Insipidus

It is clinically useful to distinguish two types of hereditary nephrogenic diabetes insipidus (NDI): a "pure" type characterized by loss of water only; and a complex type characterized by loss of water and ions. Patients with congenital NDI bearing mutations in the *AVPR2* or *AQP2* genes have a pure NDI phenotype with loss of water but normal conservation of sodium, potassium, chloride, and calcium. Patients bearing inactivating mutations in one of the five genes (*SLC12A1*, *KCNJ1*, *CLCNKB*, *CLCNKA* and *CLCNKB* in combination, or *BSND*) that encode the membrane proteins of the thick ascending limb of the loop of Henle have a complex polyuria-polydipsic syndrome with loss of water, sodium, chloride, calcium, magnesium, and potassium.

X-Linked Nephrogenic Diabetes Insipidus (OMIM 304800) (91) due to Mutations in the *AVPR2* Gene. X-linked NDI is generally a rare disease in which the affected male patients do not concentrate their urine after administration of AVP (109). Because this form is a rare, recessive X-linked disease, females are unlikely to be affected, but heterozygous females can exhibit variable degrees of polyuria and polydipsia because of skewed X chromosome inactivation. In Quebec, the incidence of this disease among males was estimated to be approximately 8.8 in 1,000,000 male live births (110). A founder effect of two particular *AVPR2* mutations (111), one in Ulster Scot

immigrants (the "Hopewell" mutation, *W71X*) and one in a large Utah kindred (the "Cannon" pedigree) result in an elevated prevalence of X-linked NDI in their descendants in certain communities in Nova Scotia, Canada, and in Utah, USA (111). These founder mutations have now spread all over the North American continent. To date, we have identified the *W71X* mutation in 42 affected males who reside predominantly in the Maritime Provinces of Nova Scotia and New Brunswick, and the *L312X* mutation in eight affected males who reside in the central United States. We know of 98 living affected males of the Hopewell kindred and 18 living affected males of the Cannon pedigree. To date, 183 putative disease-causing *AVPR2* mutations have been published in 287 NDI families (Fig. 87-6).

We propose that all families with hereditary diabetes insipidus should have their molecular defects identified. The molecular identification underlying X-linked NDI is of immediate clinical significance because early diagnosis and treatment of affected infants can avert the physical and mental retardation resulting from repeated episodes of dehydration. Diagnosis of X-linked NDI was accomplished by mutation testing of chorionic villous samples ($n = 7$), cultured amniotic cells ($n = 6$), or cord blood ($n = 31$). Three infants who had mutation testing done on amniotic cells ($n = 1$) or chorionic villous samples ($n = 2$) also had their diagnosis confirmed by cord blood testing. Of the 44 offspring tested, 21 were found to be affected males, 16 were unaffected males, and five were noncarriers (M.-F. Arthus, M. Lonergan, and D.G. Bichet, unpublished data). The affected males were immediately treated with abundant water intake, a low sodium diet, and hydrochlorothiazide. They have not experienced severe episodes of dehydration and their physical and mental development remain normal. Affected premature males may experience less severe polyuric symptoms and may need increased hydration only during their first week without a need for hydrochlorothiazide treatment. Water should be offered every 2 hours day and night, and temperature, appetite, and growth should be monitored. Admission to hospital may be necessary for continuous gastric feeding. The voluminous amounts of water kept in patients' stomachs will exacerbate physiologic gastrointestinal reflux in infants and toddlers, and many affected boys frequently vomit and have a strong positive "Tuttle test" (esophageal pH testing). These young patients often improve with the absorption of an H_2 blocker and with metoclopramide (which could induce extrapyramidal symptoms) or with domperidone, which seems to be better tolerated and efficacious. All polyuric states (whether neurogenic, nephrogenic, or psychogenic) can induce large dilations of the urinary tract and bladder (112–114), and bladder function impairment has been well documented in patients bearing *AVPR2* or *AQP2* mutations (115,116). Chronic renal failure secondary to bilateral hydronephrosis has been observed as a long-term complication in these patients. Renal and abdominal ultrasonography should be done annually and simple recommendations including frequent urination and "double voiding" could be important to prevent these consequences.

Classification of the defects of naturally occurring mutant human V_2 receptors can be based on a similar scheme to that used for the low-density lipoprotein receptor. Mutations have been grouped according to the function and subcellular localization of the mutant protein, the cDNA of which has been transiently transfected in a heterologous expression system (117). According to this classification, type 1 mutant V_2 receptors reach the cell surface but display impaired ligand binding and are consequently unable to induce normal cAMP production. The presence of mutant V_2 receptors on the surface of transfected cells can be determined pharmacologically. By carrying out saturation binding experiments using tritiated AVP, the number of cell-surface mutant V_2 receptors and their apparent binding affinity can be compared with that of the wild-type receptor. In addition, the presence of cell surface receptors can be assessed directly by using immunodetection strategies to visualize epitope-tagged receptors in whole-cell immunofluorescence assays.

Type 2 mutant receptors have defective intracellular transport. This phenotype is confirmed by carrying out, in parallel, immunofluorescence experiments on cells that are intact (to demonstrate the absence of cell surface receptors) or permeabilized (to confirm the presence of intracellular receptor pools). In addition, protein expression is confirmed by western blot analysis of membrane preparations from transfected cells. It is likely that these mutant type 2 receptors accumulate in a pre-Golgi compartment, because they are initially glycosylated but fail to undergo glycosyl-trimming maturation.

Type 3 mutant receptors are ineffectively transcribed and lead to unstable mRNAs, which are rapidly degraded. This subgroup seems to be rare, since northern blot analysis of cells expressing mutant AVPR2 receptors showed mRNAs of normal quantity and molecular size.

Most of the *AVPR2* mutants that we and other investigators have tested are type 2 mutant receptors. They did not reach the cell membrane and were trapped in the interior of the cell (118–121). Other mutant G protein–coupled receptors (122) and gene products causing genetic disorders are also characterized by protein misfolding. Mutations that affect the folding of secretory proteins, integral plasma membrane proteins, or enzymes destined to the endoplasmic reticulum, Golgi complex, and lysosomes result in loss-of-function phenotypes, irrespective of their direct impact on protein function, because these mutant proteins are prevented from reaching their final destination (123). Folding in the endoplasmic reticulum is the limiting step: mutant proteins that fail to fold correctly are initially retained in the endoplasmic reticulum and subsequently often degraded. Key proteins involved in the urine countercurrent mechanisms are good examples of this basic mechanism of misfolding. *AQP2* mutations responsible for autosomal recessive NDI are characterized by misrouting of the misfolded mutant proteins and are trapped in the endoplasmic reticulum (124). Mutants encoding other renal membrane proteins that are responsible for Gitelman syndrome (125), Bartter's syndrome (126,127), and cystinuria (128) are also retained in the endoplasmic reticulum.

The *AVPR2* missense mutations are likely to impair folding and to lead to rapid degradation of the misfolded polypeptide and not to the accumulation of toxic aggregates (as is the case for AVP mutants), because the other important functions of the principal cells of the collecting duct (where *AVPR2* is expressed) are entirely normal. These cells express the epithelial sodium channel (ENac). Decreased function of this channel results in a sodium-losing state (129). This has not been observed in patients with *AVPR2* mutations. By contrast, another type of conformational disease is characterized by the toxic retention of the misfolded protein. The relatively common Z mutation in α1-antitrypsin deficiency not only causes retention of the mutant protein in the endoplasmic reticulum but also affects the secondary structure by insertion of the reactive center loop of one molecule into a destabilized β sheet of a second molecule (130). These polymers clog up the endoplasmic reticulum in hepatocytes and lead to cell death and juvenile hepatitis, cirrhosis, and hepatocarcinomas in these patients (131).

If the misfolded protein/traffic problem responsible for so many human genetic diseases can be overcome and the mutant protein transported from the endoplasmic reticulum to its final destination, these mutant proteins could be sufficiently functional (103). Therefore, using pharmacologic chaperones or pharmacochaperones to promote escape from the endoplasmic reticulum is a possible therapeutic approach (104,123,132). We used selective nonpeptide V_2 and V_1 receptor antagonists to rescue the cell surface expression and function of naturally

occurring misfolded human V_2 receptors (118). Because the beneficial effect of nonpeptide V_2 antagonists could be secondary to prevention and interference with endocytosis, we studied the *R137H* mutant previously reported to lead to constitutive endocytosis (133). Of note, the gain-of-functions mutations *R137C* and *R137L* have recently been described in two patients with a "nephrogenic syndrome of inappropriate antidiuresis" (134). We found that the antagonist did not prevent the constitutive β-arresting-promoted endocytosis (119). These results indicate that as for other *AVPR2* mutants, the beneficial effects of the treatment result from the action of the pharmacologic chaperones. In clinical studies, we administered a nonpeptide vasopressin antagonist SR49059 to five adult patients with NDI bearing the *del62-64*, *R137H*, and *W164S* mutations. SR49059 significantly decreased urine volume and water intake and increased urine osmolality, whereas sodium, potassium, and creatinine excretions and plasma sodium were constant throughout the study (135). This new therapeutic approach could be applied to the treatment of several hereditary diseases resulting from errors in protein folding and kinesis (103,104).

Because most human gene-therapy experiments using viruses to deliver and integrate DNA into host cells are potentially dangerous (136), other treatments are being actively pursued. Sangkuhl et al. (137) used aminoglycoside antibiotics because of their ability to suppress premature termination codons (138). They demonstrated that geneticin, a potent aminoglycoside antibiotic, increased AVP-stimulated cAMP in cultured collecting duct cells prepared from *E242X* mutant mice. The urine concentrating ability of heterozygous mutant mice was also improved.

Autosomal Recessive (OMIM 222000) (91) and Dominant (OMIM 125800) (91) Nephrogenic Diabetes Insipidus due to Mutations in the *AQP2* Gene (OMIM 107777) (91). On the basis of desmopressin infusion studies and phenotypic characteristics of both males and females affected with NDI, a non-X-linked form of NDI with a postreceptor (post-cAMP) defect was suggested (139–141). A patient who presented shortly after birth with typical features of NDI but who exhibited normal coagulation and normal fibrinolytic and vasodilatory responses to desmopressin was shown to be a compound heterozygote for two missense mutations (R187C and S217P) in the *AQP2* gene (47). To date, 35 putative disease-causing *AQP2* mutations have been identified in 40 NDI families (Fig. 87-8). The oocytes of the African clawed frog (*Xenopus laevis*) have provided a most useful experimental system for studying the function of many channel proteins. This convenient expression system was key to the discovery of AQP1 by Peter Agre et al., since frog oocytes have very low permeability and survive even in fresh water ponds (142). Control oocytes are injected with water alone; test oocytes are injected with various quantities of synthetic transcripts from AQP1 or AQP2 DNA (cRNA). When subjected to a 20 mOsmol osmotic shock, control oocytes have exceedingly low water permeability but test oocytes become highly permeable to water. These osmotic water permeability assays demonstrated an absence or very low water transport for all the cRNAs with *AQP2* mutations. Immunofluorescence and immunoblot studies demonstrated that these recessive mutants were retained in the endoplasmic reticulum.

AQP2 mutations in autosomal recessive NDI, which are located throughout the gene, result in misfolded proteins that are retained in the endoplasmic reticulum. In contrast, the dominant mutations reported to date are located in the region that codes for the carboxyl terminus of AQP2 (143–145). Dominant AQP2 mutants form heterotetramers with wt-AQP2 and are misrouted. Investigation of *P262L*, the only recessive mutation in the carboxyl terminus, provided new insights into the loss-of-function and oligomerization of AQP2 proteins. Functional analysis in oocytes of *P262L* cRNA indicated that, unlike other AQP2 mutants in recessive NDI, it is a functional water channel, and that trafficking to the plasma membrane was not impaired (146). Furthermore, unlike other AQP2 recessive mutants, *P262L* cRNA forms heterotetramers with wt-AQP2 and is routed to the apical membrane, and thus in cellular experimental systems has most of the features of AQP2 mutants in dominant NDI. Carriers of the *P262L* mutation appear to be asymptomatic, but more precise measurements are needed to determine if there is a partial NDI phenotype.

Complex Polyuria-Polydipsic Syndrome. In contrast to a pure NDI phenotype, with loss of water but normal conservation of sodium, potassium, chloride, and calcium, in patients with Bartter's syndrome renal wasting starts prenatally and polyhydramnios often leads to prematurity. Bartter's syndrome (OMIM 601678, 241200, 607364, and 602522) (91) refers to a group of autosomal recessive disorders caused by inactivating mutations in one of five genes (*SLC12A1*, *KCNJ1*, *CLCNKB*, *CLCNKA* and *CLCNKB* in combination, or *BSND*) that encode membrane proteins of the thick ascending limb of the loop of Henle (for review see [147] and [148]).

Thirty percent of the filtered sodium chloride is reabsorbed in the thick ascending limb of the loop of Henle through the apically expressed sodium-potassium-chloride cotransporter NKCC2 (encoded by the *SLC12A1* gene), which uses the sodium gradient across the membrane to transport chloride and potassium into the cell. The potassium ions must be recycled through the apical membrane by the potassium channel, ROMK (encoded by the *KCNJ1* gene). In the large experience of Hanns Seyberth et al., who studied 85 patients with a hypokalemic salt-losing tubulopathy, all 20 patients with *KCNJ1* mutations (except one), and all 12 patients with *SLC12A1* mutations were born as preterm infants following severe polyhydramnios (149). Of note, polyhydramnios is never seen during the pregnancy leading to infants bearing *AVPR2* or *AQP2* mutations. The most common causes of increased amniotic fluid include maternal diabetes mellitus, fetal malformations and chromosomal aberrations, twin-to-twin transfusion syndrome, rhesus incompatibility, and congenital infections (150). Postnatally, polyuria was the leading symptom in 19 of the 32 patients. Renal ultrasonography revealed nephrocalcinosis in 31 of these patients. These patients with complex polyuro-polydipsic disorders are often poorly recognized and may be confused with "pure" NDI. As a consequence, congenital polyuria is not implying automatically *AVPR2* or *AQP2* mutations, and polyhydramnios, salt wasting, hypokalemia, and nephrocalcinosis are important clinical and laboratory characteristics that should be assessed. In patients with Bartter's syndrome, the dDAVP test (Fig. 87-11) will indicate only a partial type of NDI. The algorithm proposed by Peters et al. (149) is useful, since most mutations in *SLC12A1* and *KCNJ1* are found in the carboxyl terminus or in the last exon, and, as a consequence, are amenable to rapid DNA sequencing.

Acquired Nephrogenic Diabetes Insipidus. The acquired form of NDI is much more common than the congenital form of the disease, but it is rarely severe. The ability to elaborate a hypertonic urine is usually preserved despite the impairment of the maximal concentrating ability of the nephrons. Polyuria and polydipsia are therefore moderate (3 to 4 L per day). The more common causes of acquired NDI are listed in Table 87-2.

Lithium administration has become the most common cause of NDI. Boton et al. (151) reported that this abnormality was estimated to be present in at least 54% of 1,105 unselected patients receiving chronic lithium therapy. Nineteen percent of these patients had polyuria, as defined by a 24-hour urine output exceeding 3 L. Renal biopsy revealed a chronic tubulointerstitial nephropathy in all 24 patients with biopsy-proven

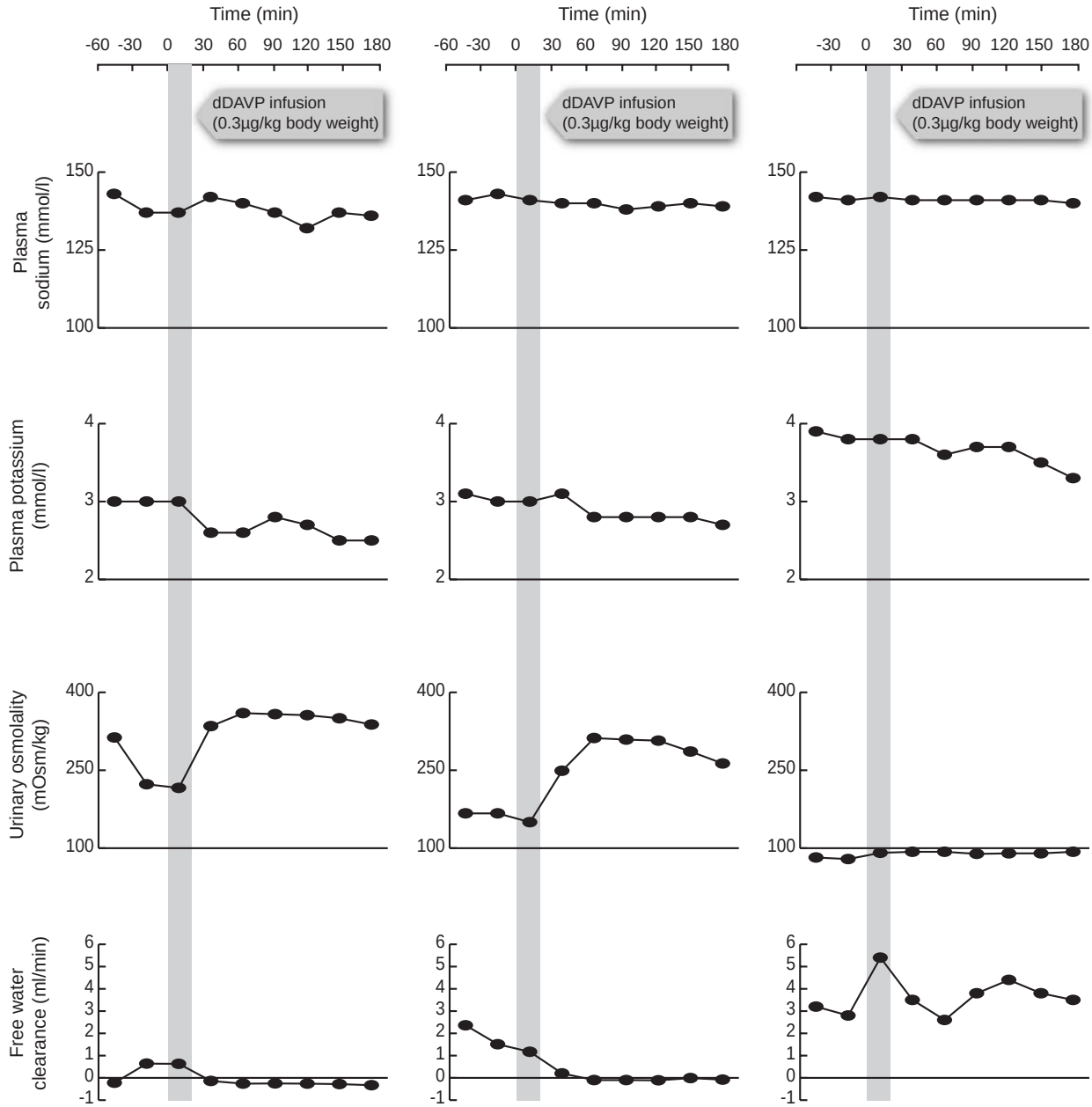

FIGURE 87-11. Measurements on two sisters with Bartter syndrome compared to a patient with NDI. The two sisters with polyuria, polydipsia, hypokalemia, hypocalcemia, and nephrocalcinosis are homozygous for the *A177T* mutation in the *KCNJ1* gene (126). Both sisters were born as preterm infants following severe polyhydramnios. The dDAVP infusion tests (203) were done at 9 (patient 1, *left panel*) and 11 (patient 2, *middle panel*) years of age. Indomethacin treatment was discontinued 1 week prior to testing; water was not restricted during the test. Plasma vasopressin was very low (<0.5 pg/mL) during the test but plasma renin activity was elevated (20 ng/mL per hour in patient 1, 10 ng/mL per hour in patient 2). By contrast, patients with *AVPR2* (4 year-old NDI patient with the *AVPR2* A132D mutation (111), *right panel*) or *AQP2* mutations generally have low urine osmolality unresponsive to dDAVP, normal plasma potassium, high vasopressin levels, and normal plasma renin activity. (Reprinted from: Fujiwara TM, Bichet DG. Molecular biology of hereditary diabetes insipidus. *J Am Soc Nephrol* 2005;16:2836 [Fig. 4], with permission.)

lithium toxicity (152). The mechanism whereby lithium causes polyuria has been studied extensively. Lithium has been shown to inhibit adenylate cyclase in a number of cell types, including renal epithelia (153,154). The concentration of lithium in the urine of patients on well-controlled lithium therapy (i.e., 10 to 40 mmol/L) is sufficient to inhibit adenylate cyclase. Measurements of adenylate cyclase activity in membranes isolated from a cultured pig kidney cell line (LLC-PK_1) revealed that lithium in the concentration area of 10 mmol/L interfered with the hormone-stimulated guanyl nucleotide regulatory unit (G_s) (155). The effect of chronic lithium therapy has been studied in rat kidney membranes prepared from the inner medulla. It caused a marked downregulation of AQP2 and AQP3 (156), only partially reversed by cessation of therapy, dehydration, or dDAVP treatment, consistent with clinical observations of slow recovery from lithium-induced urinary concentrating

TABLE 87-2

ACQUIRED CAUSES OF NEPHROGENIC DIABETES INSIPIDUS

Chronic renal disease
Polycystic disease
Medullary cystic disease
Pyelonephritis
Ureteral obstruction
Far-advanced renal failure
Electrolyte disorders
Hypokalemia
Hypercalcemia
Drugs
Alcohol
Phenytoin
Lithium
Demeclocycline
Acetohexamide
Tolazamide
Glyburide
Propoxyphene
Amphotericin
Foscarnet
Methoxyflurane
Norepinephrine
Vinblastine
Colchicine
Gentamicin
Methicillin
Isophosphamide
Angiographic dyes
Osmotic diuretics
Furosemide and ethacrynic acid
Sickle cell disease
Dietary abnormalities
Excessive water intake
Decreased sodium chloride intake
Decreased protein intake
Miscellaneous
Multiple myeloma
Amyloidosis
Sjögren's disease
Sarcoidosis

defects (157). Downregulation of AQP2 has also been shown to be associated with the development of severe polyuria due to other causes of acquired NDI (hypokalemia [158,159], release of bilateral ureteral obstruction [160, and hypercalcemia [161]). Therefore, AQP2 expression is severely downregulated in both congenital (53) and acquired NDI. More studies will be needed to determine whether nonpeptide vasopressin agonists, permeable cAMP-like compounds, or other signaling molecules will be able to restore AQP2 expression and function. In patients receiving long-term lithium therapy, amiloride has been proposed to prevent the uptake of lithium in the collecting ducts. Amiloride may thus prevent the inhibitory effect of intracellular lithium on water transport (162).

Primary Polydipsia

Primary polydipsia is a state of hypotonic polyuria secondary to excessive fluid intake. Primary polydipsia was extensively studied by Barlow and de Wardener in 1959 (163); however, the understanding of the pathophysiology of this disease has made little progress over the past 30 years. Barlow and de Wardener (163) described seven women and two men who were compulsive water drinkers; their ages ranged from 48 to 59 years except for one patient who was 24. Eight of these patients had histories of previous psychological disorders, which ranged from delusions, depression, and agitation to frank hysterical behavior. The other patient appeared normal. The consumption of water fluctuated irregularly from hour to hour or from day to day; in some patients, there were remissions and relapses lasting several months or longer. In eight of the patients, the mean plasma osmolality was significantly lower than normal. Vasopressin tannate in oil made most of these patients feel ill; in one, it caused overhydration. In four patients, the fluid intake returned to normal after electroconvulsive therapy or a period of continuous narcosis; the improvement in three was transient, but in the fourth it lasted 2 years. Polyuric female subjects might be heterozygous for *de novo* or previously unrecognized *AVPR2* mutations or autosomal dominant *AQP2* mutations (164) and may be classified as compulsive water drinkers (165). Therefore, the diagnosis of compulsive water drinking must be made with care and may represent our ignorance of yet undescribed pathophysiologic mechanisms. Robertson (165) has described under the term *dipsogenic diabetes insipidus* a selective defect in the osmoregulation of thirst. Three studied patients had under basal conditions of *ad libitum* water intake, thirst, polydipsia, polyuria, and high-normal plasma osmolality. They had a normal secretion of AVP, but osmotic threshold for thirst was abnormally low. Such cases of dipsogenic diabetes insipidus might represent up to 10% of all patients with diabetes insipidus (165). Primary polydipsic rats had low serum sodium, suppressed AVP, low urine osmolality, and decreased AQP2 protein abundance in their inner medulla (166).

Diabetes Insipidus and Pregnancy

Pregnancy in a Patient Known to Have Diabetes Insipidus. An isolated deficiency of vasopressin without a concomitant loss of hormones in the anterior pituitary does not result in altered fertility, and with the exception of polyuria and polydipsia, gestation, delivery, and lactation are uncomplicated (167). Treated patients may require increasing dosages of desmopressin. The increased thirst may be due to a resetting of the thirst osmostat (168). Increased polyuria also occurs during pregnancy in patients with partial NDI (169). These patients may be obligatory carriers of the NDI gene (170).

Syndromes of Diabetes Insipidus that Begin During Gestation and Remit After Delivery. Barron et al. (171) described three pregnant women in whom transient diabetes insipidus developed late in gestation and subsequently remitted postpartum. In one of these patients, dilute urine was present despite high plasma concentrations of AVP. Hyposthenuria in all three patients was resistant to administered aqueous vasopressin. Because excessive vasopressinase activity was not excluded as a cause of this disorder, the Barron group labeled the disease vasopressin resistant rather than NDI.

A well-documented case of enhanced activity of vasopressinase has been described in a woman in the third trimester of a previously uncomplicated pregnancy (172). She had massive polyuria and markedly elevated plasma vasopressinase activity. The polyuria did not respond to large intravenous doses of AVP but responded promptly to desmopressin, a vasopressinase-resistant analog of AVP. The polyuria disappeared with the disappearance of the vasopressinase. The incidence of vasopressinase-mediated, desmopressin-responsive diabetes insipidus is not known. However, another case of transient desmopressin-resistant diabetes insipidus in a pregnant woman has been described recently (173). It is suggested

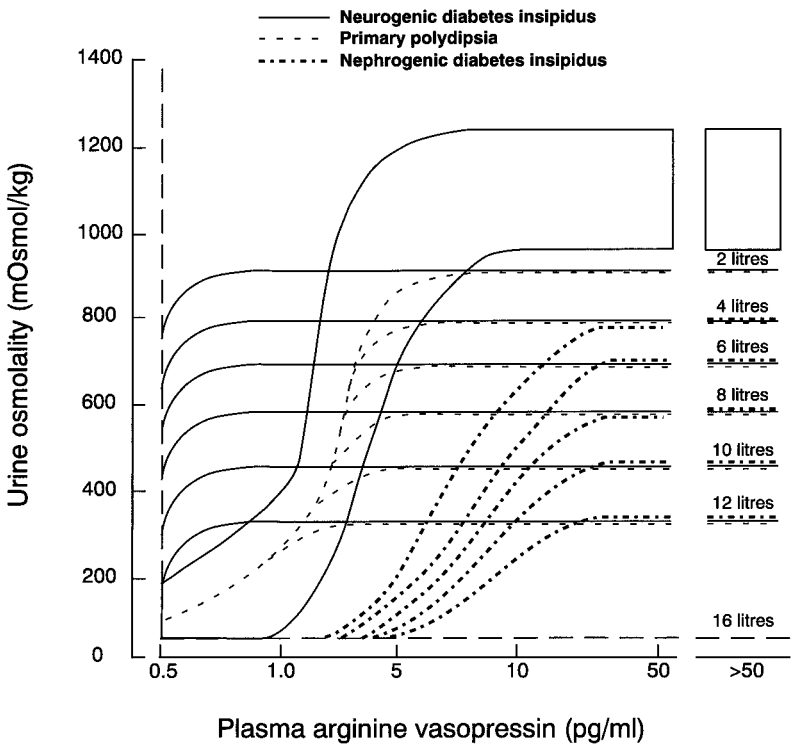

FIGURE 87-12. The relationship between urine osmolality and plasma vasopressin in patients with polyuria of diverse etiology and severity. Note that for each of the three categories of polyuria (neurogenic diabetes insipidus, nephrogenic diabetes insipidus, and primary polydipsia), the relationship is described by a family of sigmoid curves that differ in height. These differences in height reflect differences in maximum concentrating capacity due to "washout" of the medullary concentration gradient. They are proportional to the severity of the underlying polyuria (indicated in liters per day at the right end of each plateau) and are largely independent of the etiology. Therefore, the three categories of diabetes insipidus differ principally in the submaximal or ascending portion of the dose-response curve. In patients with partial neurogenic diabetes insipidus, this part of the curve lies to the left of normal, reflecting increased sensitivity to the antidiuretic effects of very low concentrations of plasma AVP. In contrast, in patients with partial nephrogenic diabetes insipidus, this part of the curve lies to the right of normal, reflecting decreased sensitivity to the antidiuretic effects of normal concentrations of plasma arginine vasopressin (AVP). In primary polydipsia, this relationship is relatively normal. (From: Robertson GL. Diagnosis of diabetes insipidus. In: Czernichow P, Robinson AG, eds. *Frontiers of Hormone Research,* vol 13, "Diabetes insipidus in man." Basel: S. Karger; 1985:176, with permission.)

that pregnancy may be associated with several different forms of diabetes insipidus, including central-, nephrogenic-, and vasopressinase-mediated (169).

Differential Diagnosis of Polyuric States

Plasma sodium and osmolality are maintained within normal limits (136 to 143 mmol/L for plasma sodium, 275 to 290 mmol/kg for plasma osmolality) by a thirst-ADH-renal axis. Thirst and ADH, both stimulated by increased osmolality, have been termed a "double-negative" feedback system (174). Therefore, even when the ADH limb of this double-negative regulatory feedback system is lost, the thirst mechanism still preserves the plasma sodium and osmolality within the normal range but at the expense of pronounced polydipsia and polyuria. Consequently, the plasma sodium concentration or osmolality of an untreated patient with diabetes insipidus may be slightly higher than the mean normal value, but since the values usually remain within the normal range, these small increases have no diagnostic significance.

Theoretically, it should be relatively easy to differentiate between central diabetes insipidus, NDI, and primary polydipsia. A comparison of the osmolality of urine obtained during dehydration from patients with central diabetes insipidus or NDI with that of urine obtained after the administration of AVP should reveal a rapid increase in osmolality only in the patients with central diabetes insipidus. Urine osmolality should increase normally in response to moderate dehydration in primary polydipsia patients.

However, these distinctions may not be as clear as one might expect because of several factors (175). First, chronic polyuria of any etiology interferes with the maintenance of the medullary concentration gradient, and this "washout" effect diminishes the maximum concentrating ability of the kidney. The extent of the blunting varies in direct proportion to the severity of the polyuria and is independent of its cause. Hence, for any given level of basal urine output, the maximum urine osmolality achieved in the presence of saturating concentrations of AVP is depressed to the same extent in patients with primary polydipsia, central diabetes insipidus, and NDI (Fig. 87-12). Second, most patients with central diabetes insipidus maintain a small, but detectable capacity to secrete AVP during severe dehydration, and urine osmolality may then rise above plasma osmolality. Third, many patients with acquired NDI have an incomplete deficit in AVP action, and concentrated urine could again be obtained during dehydration testing. Finally, all polyuric states (whether central, nephrogenic, or psychogenic) can induce large dilations of the urinary tract and bladder (113,114,176). As a consequence, the urinary bladder of these patients may contain an increased residual capacity, and changes in urine osmolalities induced by diagnostic maneuvers might be difficult to demonstrate.

Indirect Test

The measurements of urine osmolality after dehydration followed by vasopressin administration are usually referred to as "indirect testing" because vasopressin secretion is indirectly assessed through changes in urine osmolalities.

The patient is maintained on a complete fluid restriction regimen until urine osmolality reaches a plateau, as indicated by an hourly increase of less than 30 mmol/kg for at least 3 successive hours. After the plasma osmolality is measured, 5 U of aqueous vasopressin is administered subcutaneously. Urine osmolality is measured 30 and 60 minutes later. The last urine osmolality value obtained before the vasopressin injection and the highest value obtained after the injection are compared. The patients are then separated into five categories according to previously published criteria (18) (Table 87-3).

Direct Test

The two approaches of Zerbe and Robertson (17) are used. First, during the dehydration test, plasma is collected and assayed for vasopressin. The results are plotted on a nomogram depicting the normal relationship between plasma sodium or osmolality and plasma AVP in normal subjects (Fig. 87-4). If the relationship between plasma vasopressin and osmolality falls

TABLE 87-3

URINARY RESPONSES TO FLUID DEPRIVATION AND EXOGENOUS VASOPRESSIN IN RECOGNITION OF PARTIAL DEFECTS IN ANTIDIURETIC HORMONE SECRETION

	No. cases	Maximum U_{osm} with dehydration (mmol/kg)	U_{osm} after vasopressin (mmol/kg)	% Change (U_{osm})	U_{osm} increase after vasopressin (%)
Normal subjects	9	1068 ± 69	979 ± 79	−9 ± 3	<9
Complete central diabetes insipidus	18	168 ± 13	445 ± 52	183 ± 41	>50
Partial central diabetes insipidus	11	438 ± 34	549 ± 28	28 ± 5	>9 <50
Nephrogenic diabetes insipidus	2	123.5	174.5	42	<50
Compulsive water drinking	7	738 ± 53	780 ± 73	5.0 ± 2.2	<9

(Data from: Miller M, Dalakos T, Moses AM, et al. Recognition of partial defects in antidiuretic hormone secretion. *Ann Intern Med* 1970;73:721.)

below the normal range, the disorder is diagnosed as central diabetes insipidus.

Second, partial NDI and primary polydipsia can be differentiated by analyzing the relationship between plasma AVP and urine osmolality at the end of the dehydration period (Figs. 87-4 and 87-12). However, a definitive differentiation between these two disorders might be impossible because a normal or even supranormal AVP response to increased plasma osmolality occurs in polydipsic patients. None of the patients with psychogenic or other forms of severe polydipsia studied by Robertson (175) have ever shown any evidence of pituitary suppression. Zerbe and Robertson (17) found that in the differential diagnosis of polyuria, all seven of the cases of severe neurogenic diabetes insipidus diagnosed by the standard indirect test were confirmed when diagnosed by the plasma vasopressin assay. However, two of six patients diagnosed by the indirect test as having partial neurogenic diabetes insipidus had normal vasopressin secretion as measured by the direct assay; one was found to have primary polydipsia and the other NDI. Moreover, three of 10 patients diagnosed as having primary polydipsia by the indirect test had clear evidence of partial vasopressin deficiency by the direct assay (17). These patients were thus wrongly diagnosed as primary polydipsic! A *combined* direct and indirect testing of the AVP function is described in Table 87-4.

Therapeutic Trial

In selected patients with an uncertain diagnosis, a closely monitored therapeutic trial of desmopressin (10 μg intranasally twice a day) may be used to distinguish partial NDI from partial neurogenic diabetes insipidus and primary polydipsia. If desmopressin at this dosage causes a significant antidiuretic effect, NDI is effectively excluded. If polydipsia as well as polyuria is abolished and plasma sodium does not fall below the normal range, the patient probably has central diabetes insipidus. Conversely if desmopressin causes a reduction in urine output without a reduction in water intake and hyponatremia appears, the patient probably has primary polydipsia. Because fatal water intoxication is a remote possibility, the desmopressin trial should be carried out with closed monitoring.

TABLE 87-4

DIRECT AND INDIRECT TESTS OF AVP FUNCTION IN PATIENTS WITH POLYURIA

Measurements of AVP cannot be used in isolation but must be interpreted in light of four other factors:
- Clinical history
- Concurrent measurements of plasma osmolality, urine osmolality, and maximal urinary response to exogenous vasopressin in reference to the basal urine flow

AVP, arginine vasopressin.
(Data from: Stern P, Valtin H. Verney was right, but (editorial). *N Engl J Med* 1981;305:1581.)

Recommendations

Table 87-5 lists recommendations for obtaining a differential diagnosis of diabetes insipidus (177).

Radioimmunoassay of AVP and Other Laboratory Determinations

Radioimmunoassay of AVP

Three developments were basic to the elaboration of a clinically useful radioimmunoassay for plasma AVP (178,179): (i) the extraction of AVP from plasma with petrol–ether and acetone and the subsequent elimination of nonspecific immunoreactivity, (ii) the use of highly specific and sensitive rabbit antiserum, and (iii) the use of a tracer (^{125}I-AVP) with high specific activity. More than 25 years later, the same extraction procedures are widely used (180–183), and commercial tracers (^{125}I-AVP) and antibodies are available. AVP can also be extracted from plasma by using Sep-Pak C18 cartridges (184–186).

Blood samples collected in chilled 7-mL lavender-stoppered tubes containing ethylenediaminetetraacetic acid (EDTA) are centrifuged at 4°C, 1,000*g* (3,000 rpm in a usual lab centrifuge), for 20 minutes. This 20-minute centrifugation is mandatory for obtaining platelet-poor plasma samples because a large fraction of the circulating vasopressin is associated with the platelets in humans (181,187). The tubes may be kept for 2 hours on slushed ice prior to centrifugation. Plasma is then separated, frozen at −20°C, and extracted within 6 weeks of sampling. Details for sample preparation (Table 87-6) and assay procedure (Table 87-7) can be found in writings by Bichet et al. (180,181). An AVP radioimmunoassay should be validated by demonstrating (i) a good correlation between plasma sodium or osmolality and plasma AVP during dehydration and infusion of hypertonic saline solution (Fig. 87-4) and (ii) the inability to obtain detectable values of AVP in patients with severe central diabetes insipidus. Plasma AVP immunoreactivity may be elevated in patients with diabetes insipidus following hypothalamic surgery (188).

In pregnant patients, the blood contains high concentrations of cystine aminopeptidase, which can (*in vitro*) inactivate

TABLE 87-5

DIFFERENTIAL DIAGNOSIS OF DIABETES INSIPIDUS

1. Measure plasma osmolality and/or sodium concentration under conditions of *ad libitum* fluid intake. If they are greater 295 mmol/kg and 143 mmol/L, the diagnosis of primary polydipsia is excluded, and the workup should proceed directly to step 5 and/or 6 to distinguish between neurogenic and nephrogenic diabetes insipidus. Otherwise,
2. Perform a dehydration test. If urinary concentration does not occur before plasma osmolality and/or sodium reaches 295 mmol/kg or 143 mmol/L, the diagnosis of primary polydipsia is again excluded, and the workup should proceed to step 5 and/or 6. Otherwise,
3. Determine the ratio of urine to plasma osmolality at the end of the dehydration test. If it is <1.5, the diagnosis of primary polydipsia is again excluded, and the workup should proceed to step 5 and/or 6. Otherwise,
4. Perform a hypertonic saline infusion with measurements of plasma vasopressin and osmolality at intervals during the procedure. If the relationship between these two variables is subnormal, the diagnosis of diabetes insipidus is established. Otherwise,
5. Perform a vasopressin infusion test. If urine osmolality rises by more than 150 mOsmol/kg greater than the value obtained at the end of the dehydration test, nephrogenic diabetes insipidus is excluded. Alternately,
6. Measure urine osmolality and plasma vasopressin at the end of the dehydration test. If the relationship is normal, the diagnosis of nephrogenic diabetes insipidus is excluded.

(Data from: Robertson GL. Diseases of the posterior pituitary. In: Felig D, Baxter JD, Broadus AE, et al., eds. *Endocrinology and Metabolism*. New York: McGraw-Hill; 1981:251.)

enormous quantities (ng X mL^{-1} X min^{-1}) of AVP. However, phenanthrolene effectively inhibits these cystine aminopeptidases (Table 87-8).

Aquaporin-2 Measurements

Urinary AQP2 excretion could be measured by radioimmunoassay (53) or quantitative Western analysis (54) and could provide an additional indication of the responsiveness of the collecting duct to AVP (54,55).

Plasma Sodium and Plasma and Urine Osmolality Measurements

Measurements of plasma sodium and plasma and urine osmolality should be immediately available at various intervals during dehydration procedures. Plasma sodium is easily measured by flame photometry or with a sodium-specific electrode (189). Plasma and urine osmolalities are also reliably measured by freezing-point depression instruments with a coefficient of variation at 290 mmol/kg of less than 1%.

In our clinical research unit, plasma sodium and plasma and urine osmolalities are measured at the beginning of each dehydration procedure and at regular intervals (usually hourly) thereafter, depending on the severity of the polyuric syndrome explored.

In one case, an 8-year-old patient (31 kg body weight) with a clinical diagnosis of congenital NDI (later found to bear the *de novo* AVPR2 mutant 274insG [190]) continued to excrete large volumes of urine (300 mL/hour) during a short 4-hour dehydration test. During this time, the patient was had severe thirst, his plasma sodium was 155 mEq/L, his plasma osmolality was 310 mmol/kg, and his urine osmolality was 85 mmol/kg. The patient received 1 μg of desmopressin intravenously and was allowed to drink water. Repeated urine osmolality measurements demonstrated a complete urinary resistance to desmopressin.

It would have been dangerous and unnecessary to prolong the dehydration further in this young patient. Therefore, the usual prescription of overnight dehydration should not be used in patients, and especially children, with severe polyuria and polydipsia (more than 4 L/day). Great care should be taken

TABLE 87-6

AVP MEASUREMENTS: SAMPLE PREPARATION

4°C—blood in EDTA tubes
Centrifugation 1,000 $g \times 20$ minutes
Plasma frozen −20°C
Extraction:
- 2 mL acetone + 1 mL plasma
- 1,000 $g \times 30$ minutes 4°C
- Supernatant + 5 mL of petrol-ether
- 1,000 $g \times 20$ minutes 4°C
- Freeze −80°C
- Throw nonfrozen upper phase
- Evaporate lower phase to dryness
- Store desiccated samples at −20°C

AVP, arginine vasopressin; *EDTA*, ethylenediamine tetraacetic acid.

TABLE 87-7

AVP MEASUREMENTS: ASSAY PROCEDURE

Day 1 Assay setup
- 400 μL/tube (200 μL sample or standard + 200 μL of antiserum or buffer)
- Incubation 80 hours, 4°C

Day 4 ^{125}I-AVP
- 100 μL/tube

1,000 cpm/tube
- Incubation 72 hours, 4°C

Day 7 Separation dextran + charcoal

AVP, arginine vasopressin.

TABLE 87-8

MEASUREMENTS OF AVP LEVELS IN PREGNANT PATIENTS

1,10-Phenanthrolene monohydrate (Sigma) 60 mg/mL—solubilized with several drops of glacial acetic acid 0.1 ml/10 mL of blood
AVP, arginine vasopressin. (Data from: Davison JM, Gilmore EA, Durr J, et al. Altered osmotic thresholds for vasopressin secretion and thirst in human pregnancy. *Am J Physiol* 1984;246:F105.)

to avoid any severe hypertonic state arbitrarily defined as a plasma sodium greater than 155 mEq/L.

At variance with published data (17,181), we have found that plasma and serum osmolalities are equivalent (i.e., similar values are obtained). Blood taken in heparinized tubes is easier to handle because the plasma can be more readily removed after centrifugation. The tube used (green-stoppered tube) contains a minuscule concentration of lithium and sodium, which does not interfere with plasma sodium or osmolality measurements. Frozen plasma or urine samples can be kept for further analysis of their osmolalities because the results obtained are similar to those obtained immediately after blood sampling, except in patients with severe renal failure. In the latter patients, plasma osmolality measurements are increased after freezing and thawing, but the plasma sodium values remain unchanged.

Plasma osmolality measurements can be used to demonstrate the absence of unusual osmotically active substances (e.g., glucose and urea in high concentrations, mannitol, and ethanol) (191). With this information, plasma or serum sodium measurements are sufficient to assess the degree of dehydration and its relationship to plasma AVP. Nomograms describing the normal plasma sodium–plasma AVP relationship (Fig. 87-3) are equally as valuable as "classical" nomograms describing the relationship between plasma osmolality and effective osmolality (i.e., plasma osmolality minus the contribution of "ineffective" solutes: glucose and urea).

Magnetic Resonance Imaging in Patients with Diabetes Insipidus

Magnetic resonance imaging (MRI) permits visualization of the anterior and posterior pituitary glands and the pituitary stalk. The pituitary stalk is permeated by numerous capillary loops of the hypophyseal-portal blood system. This vascular structure also provides the principal blood supply to the anterior pituitary lobe, as there is no direct arterial supply to this organ. In contrast, the posterior pituitary lobe has a direct vascular supply. Therefore, the posterior lobe can be more rapidly visualized in a dynamic mode after administration of a gadolinium (gadopentetate dimeglumine) as contrast material during MRI. The posterior pituitary lobe is easily distinguished by a round, high-intensity signal (the posterior pituitary "bright spot") in the posterior part of the sella turcica on T1-weighted images. This round, high-intensity signal is usually absent in patients with central diabetes insipidus (84). MRI is reported to be "the best technique" with which to evaluate the pituitary stalk and infundibulum in patients with idiopathic polyuria. Therefore, the absence of posterior pituitary hyperintensity, although nonspecific, is a cardinal feature of central diabetes insipidus. In the five patients who did have posterior pituitary hyperintensity at diagnosis, this feature invariably disappeared during follow-up (84). Thickening of either the entire pituitary stalk or just the proximal portion was the second most common abnormality on MRI scans (84).

Treatment

In most patients with complete hypothalamic diabetes insipidus, the thirst mechanism remains intact. Therefore, these patients do not develop hypernatremia and suffer only from the inconvenience associated with marked polyuria and polydipsia. If hypodipsia develops or access to water is limited, then severe hypernatremia can supervene. The treatment of choice for patients with severe hypothalamic diabetes insipidus is desmopressin, a synthetic, long-acting vasopressin analog with minimal vasopressor activity but a large antidiuretic potency. The usual intranasal daily dose is between 5 and 20 μg. To avoid the potential complication of dilutional hyponatremia, which is exceptional in these patients due to an intact thirst mechanism, desmopressin can be withdrawn at regular intervals to allow the patients to become polyuric. Aqueous vasopressin (Pitressin) or desmopressin (4.0 μg/1-mL ampule) can be used intravenously in acute situations such as after hypophysectomy or for the treatment of diabetes insipidus in the brain-dead organ donor. Pitressin tannate in oil and nonhormonal antidiuretic drugs are somewhat obsolete and now rarely used. For example, chlorpropamide (250 to 500 mg daily) appears to potentiate the antidiuretic action of circulating AVP, but troublesome side effects of hypoglycemia and hyponatremia do occur. The treatment of congenital NDI has been reviewed (176,192,193) and is included earlier in this chapter in the description of X-linked NDI.

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CHAPTER 88 ■ DISORDERS OF POTASSIUM AND ACID–BASE METABOLISM IN ASSOCIATION WITH RENAL DISEASE

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In this chapter, we review disturbances in potassium and acid–base homeostasis seen in patients with renal disease. Our discussion is, however, limited to disorders of potassium and acid–base homeostasis seen in (a) patients with progressive chronic kidney disease (CKD) and (b) patients with renal insufficiency and defects in the renin–aldosterone axis or in the tubular response to aldosterone. To provide a background, we briefly review potassium and acid–base homeostasis in normal humans before focusing on patients with underlying renal disease. We do not, however, discuss normal renal handling of potassium and only briefly review renal handling of hydrogen ion. These two topics are extensively reviewed in Chapters 5 and 6.

POTASSIUM HOMEOSTASIS

Potassium is the most abundant cation in the body. The distribution of potassium is such that 98% of total body potassium is intracellular, whereas only 2% is extracellular (1). The high intracellular to extracellular potassium ratio (K_i/K_o) is crucial to the normal cell function, as it is the major determinant of the resting membrane potential. The body is able to maintain this distribution in a highly regulated and efficient fashion through hormonal modulation of Na-K-ATPase pump activity. This enzyme is composed of two subunits and is found in highest density in skeletal muscle (2,3). Functional activity of the Na-K-ATPase pump is restricted to the catalytic α subunit, whereas the β (glycoprotein) subunit acts to ensure α subunit assembly and maintain its stability. The α subunit contains binding sites for sodium, potassium, magnesium, ATP, Pi, and the inhibitor ouabain. Several hormones acutely modulate Na-K-ATPase pump activity by altering the phosphorylation status of the catalytic subunit and/or transporting pumps from subcellular locations to the cellular membrane where sodium and potassium transport occurs (2,3). For example, insulin and catecholamines regulate the phosphorylation status of the Na-K-ATPase pump through modulation of certain phosphatases and kinases. Rapid recruitment of inactive pump units from a preformed pool of pumps is also stimulated by both insulin and aldosterone. These Na-K-ATPase pumps are then deposited in the cell membrane and converted to an activated state (2,3). In contrast, chronic and sustained changes in Na-K-ATPase enzyme activity are achieved through regulation of the total number of active pump units available in the cell membrane. Chronic control occurs at the transcriptional and posttranscriptional levels, regulated through the effects of hormones such as aldosterone and insulin. Hence, the overall abundance of pump subunits is controlled at the levels of transcription, transcript stability, translation, and protein stability (2,3).

Large potassium loads continuously challenge humans, who are carnivorous intermittent eaters. On a long-term basis, this challenge is met primarily by the renal excretion of potassium load; however, on a short-term basis, a significant amount of potassium is shifted intracellularly (4). This shift temporarily buffers the expected change in K_i/K_o ratio until potassium intake is balanced by comparable output.

Internal Potassium Homeostasis

The kidney is able to excrete only about 50% of the administered potassium during the first 4 hours after intravenous or oral intake of potassium. Approximately 80% of the retained potassium is shifted intracellularly, and only 20% (or 10% of the total intake) remains in the extracellular space (5–7). The retained potassium will be excreted completely over the next 24 hours (8). The major regulators of this internal redistribution are: (a) insulin, (b) catecholamines and (c) mineralocorticoids. Although several studies in rats postulate an independent role for parathyroid hormone as well, the role of this hormone if any is of minor importance and will not be discussed in this chapter. In addition to these physiologic regulators, serum potassium is also regulated by acid–base status as well as plasma osmolality. These will be discussed later.

Insulin

The ability of insulin to shift potassium intracellularly has been known for over 70 years (9) and has been used therapeutically for the treatment of hyperkalemia. Several investigators have shown that pancreatectomized dogs tolerate exogenous potassium loads poorly (10). This is reversed by exogenous replacement of insulin (11,12). To study the role of endogenous insulin, DeFronzo et al. (6) examined potassium tolerance in dogs by intravenous potassium chloride infusion before and after inhibition of endogenous insulin secretion with somatostatin. Inhibition of endogenous insulin by 50% results in a twofold rise in serum potassium compared to controls (Fig. 88-1). If physiologic doses of insulin were added to the somatostatin infusion, potassium tolerance returned to normal. In healthy volunteers, somatostatin infusion in the postabsorptive state led to a 50% decline in the plasma insulin concentration and a 0.5 to 0.7 mEq/L rise in serum potassium that was reversed by a physiologic infusion of exogenous insulin (6). A similar phenomenon was observed in maturity-onset diabetic patients, who have normal or increased fasting plasma insulin levels, but not insulin-deficient juvenile diabetic patients (13).

The effect of hyperkalemia on endogenous insulin concentration is more complex. A 1.0- to 1.5-mEq rise in serum potassium is uniformly associated with a severalfold rise in serum insulin level (10,12,14,15), whereas a more physiologic rise of less than 1.0 mEq has no effect. Hiatt et al. (14) noted a significant rise in portal insulin level with a more modest rise of 0.3 to 1.0 mEq in serum potassium; however, in a more recent

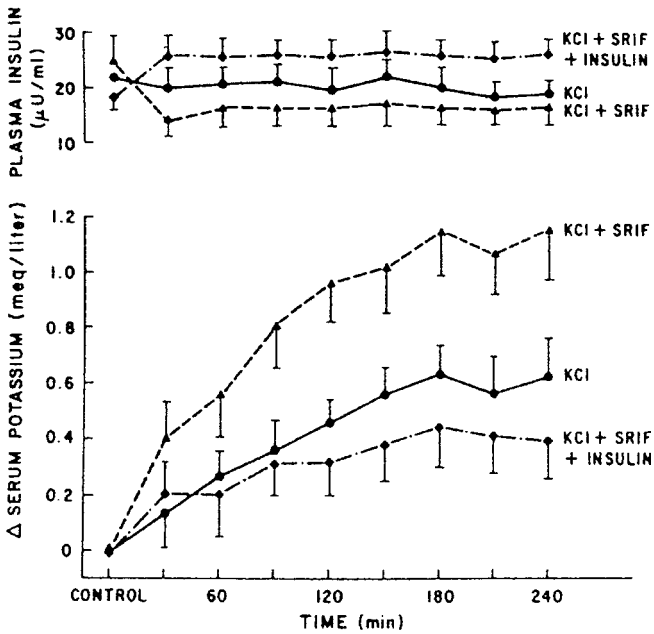

FIGURE 88-1. The effect of potassium chloride infusion on the plasma potassium concentration in the control state, with somatostatin (STS), which inhibits insulin secretion, or with somatostatin plus insulin. (From: DeFronzo RA, et al. Influence of basal insulin and glucagon secretion on potassium and sodium metabolism. Studies with somatostatin in normal dogs and in normal and diabetic human beings. *J Clin Invest* 1978;61:472, with permission.)

report, the rise in portal insulin could only be shown if serum potassium was increased by 2.0 mEq/L (16); therefore, the presence of a classic negative feedback loop at physiologic potassium concentration remains controversial. The primary sites of insulin-mediated potassium uptake include muscle and liver, although adipose tissue uptake of potassium is also enhanced (17–21). DeFronzo et al. (21), using variable doses of insulin in normal volunteers, have shown that during the first hour, liver is the primary site of potassium uptake. However, during the second hour, despite continued drop in serum potassium, there is net release of potassium from the portal and splanchnic bed, indicating a shift of potassium uptake to the peripheral tissue, especially muscle.

At the cellular level insulin reacts with specific receptors on the plasma membrane (22), increasing the activity of the Na-K-ATPase pump in the skeletal and heart muscle, epithelial cells of the kidney and bladder, as well as liver and fat cells (23). This augmentation is blocked by ouabain, which binds to the α subunit. As shown in animals (opossum and rat), it appears that insulin induces phosphorylation of the α-1 subunit of the Na-K-ATPase pump at the level of tyrosine 10 (24). Phosphorylation of this enzyme increases the activity of pumps, thereby facilitating sodium and potassium transport (2,24). Additionally, insulin also acutely triggers the translocation of previously unavailable pumps from intracellular pools to the plasma membrane in skeletal muscle, liver cells, and adipocytes (2,24). Ultimately, insulin's effect on the Na-K-ATPase pump stimulates a series of intracellular events leading to hyperpolarization of cell membranes (19,20,23). The time course for this interaction is consistent with both an increase in enzyme activity as well as the rapid recruitment of Na-K-ATPase pumps to the cellular membrane. In contrast, chronic stimulation by insulin probably increases the total number of available pump sites. This occurs through regulation of the Na-K-ATPase pump at the transcriptional and posttranscriptional levels by inducing the synthesis of new α and β subunits (2). Several *in vitro* studies have shown that insulin-driven potassium uptake by both muscle and liver is independent of glucose uptake (19,21). In addition, Cohen et al. (25), using euglycemic clamp in humans, have provided strong evidence that effect of insulin on potassium uptake is independent of its effect on glucose entry into the cell.

Catecholamines

D'Silva, beginning in 1934, first observed a biphasic response of plasma potassium to epinephrine injection (26,27). Plasma potassium rose during the first 1 to 3 minutes, but with continued infusion fell and remained lower than baseline. Other investigators have shown increased potassium tolerance in animals infused with pharmacologic doses of epinephrine (28,29) despite pancreatectomy (11) or nephrectomy (30). Brown et al. (31) have shown that infusion of stress-level doses of epinephrine resulted in a decrease in serum potassium by 0.4 to 0.6 mEq/L. Because epinephrine inhibits the renal excretion of potassium (32,33), the decline in potassium concentration is entirely accounted for by enhanced cellular potassium uptake.

Specific receptors are involved in the cellular disposal of potassium by catecholamines. α-receptor stimulation in humans by phenylephrine (41) significantly impairs cellular potassium tolerance, which is reversed by the α-antagonist phentolamine. This phenomenon may explain the initial rise in serum potassium after infusion of catecholamine (32,33). β_2-blockade impairs the catecholamine-induced shift of potassium into extrarenal tissues (42–45) and causes hyperkalemia despite increase in renal excretion of this ion (Fig. 88-2). In normal volunteers who exercise while taking β-adrenergic blocking agents, the serum potassium level is raised 2- to 2.5-fold higher than during similar exercise performed without β blockade (4,46,47). The effect of nonspecific β-blockers such as propranolol on serum potassium is mimicked by specific β_2-blockers (38,46,48,49) but not β_1-blockers (34). Although an important role for catecholamine-stimulated uptake of potassium by muscle has been demonstrated (43,50–52), the role of the liver remains controversial (34,53). The effect of potassium on catecholamine levels is less clear. In humans, physiologic changes in plasma potassium did not affect plasma epinephrine or norepinephrine levels (54). However, because the majority of catecholamines are locally released and rapidly retaken up by nerve endings, it is possible that changes in catecholamine

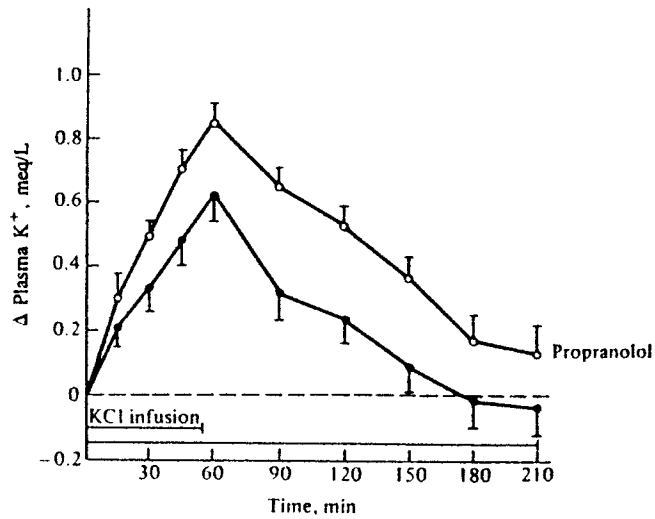

FIGURE 88-2. Changes in plasma potassium concentration after a potassium chloride infusion in the presence and absence of the nonselective β-adrenergic blocker propranolol. (From: Rosa RM, et al. Adrenergic modulation of extrarenal potassium disposal. *N Engl J Med* 1980;302:431, with permission.)

secretion are not reflected in the serum concentration of these mediators.

At the cellular level epinephrine binds to the β_2-receptor resulting in the stimulation of adenyl cyclase, and conversion of adenosine triphosphate to cyclic 3′,5′-adenosine monophosphate (cAMP). It is postulated that cAMP then activates protein kinase A, which then phosphorylates the Na-K-ATPase pump, increasing its activity and promoting potassium influx into the cell and Na^+ efflux (1,43,46,47). Binding of catecholamines to the α receptor decreases cellular potassium uptake through inhibiting adenylate cyclase activity and decreasing Na-K-ATPase pump activity (48). In addition, activation of the α-1 receptor alters cytoplasmic calcium, thereby increasing intracellular calcium concentration and opening calcium-activated potassium channels, which allow potassium to exit the cell (48). Interestingly, the effect of insulin and epinephrine on plasma potassium is additive, which confirms separate mechanisms of action (47). In insulin-induced hypoglycemia, hypokalemia is therefore due to the combined effect of both insulin and the hypoglycemia-induced rise in catecholamines (49).

Mineralocorticoids

The role of mineralocorticoids in external potassium homeostasis is of critical importance because aldosterone influences potassium excretion by the kidney (50), colon (51), salivary (52), and sweat glands (53). However, aldosterone's role in internal potassium homeostasis is unclear (54,55). Anephric rats adapted to high potassium intake handle an acute potassium load more efficiently than do nonadapted rats. This adaptation is lost by prior adrenalectomy and restored by exogenous mineralocorticoid replacement (56). However, Spital and Stern (57,58) observed that during the 20 hours of fasting before nephrectomy and acute potassium loading, these rats became potassium depleted owing to marked kaliuresis resulting from high-serum potassium coupled with a high aldosterone level. In adrenalectomized dogs, Young and Jackson (59) have shown that plasma potassium concentration at any exchangeable potassium level was a function of aldosterone replacement dose. High-dose aldosterone in anephric rabbits delays death due to hyperkalemia (60). Similarly, baseline potassium was significantly higher in hormonally deficient adrenalectomized rats, despite negative potassium balance, compared to exogenously replaced controls, supporting a defect in cellular uptake of the potassium (6,61). This impairment was corrected by either aldosterone or epinephrine replacement. In rat studies, aldosterone has been shown to increase Na-K-ATPase pump activity by inducing the synthesis of new α- and β-subunits in heart and vascular smooth muscle (2). This effect presumably represents the action of aldosterone on Na-K-ATPase pump gene expression and supports a role for aldosterone in cellular potassium homeostasis. In anephric humans treated with deoxycorticosterone acetate (DOCA), spironolactone, or placebo for 3 days, the baseline potassium was similar; however, the DOCA-treated subjects showed greater tolerance to acute potassium load than did the other two groups (62). In a recent study of 15 patients on hemodialysis that were treated with 0.05 to 2.0 mg/day of fludrocortisone acetate, the serum K^+ decreased significantly (63). Interestingly, the effect of exogenous mineralocorticoid was more pronounced in patients with low compared to high plasma aldosterone concentration. Low dose spironolactone (25 mg/day) was associated with an increase in mean serum K^+ concentration of 0.3 mEq/L over 4 weeks of therapy in 15 chronic hemodialysis patients (64). Very low dose spironolactone (25 mg thrice weekly) did not increase serum K^+ in 14 similar hemodialysis patients (65). High-dose spironolactone (300 mg/day) induced a significant rise in plasma potassium (0.5 mEq/L) and caused hyperkalemia after 3 weeks of therapy in nine chronically hemodialyzed end-stage renal disease (ESRD) patients (three were anephric) (66). In summary these studies support a small but important role for aldosterone in internal potassium homeostasis in anephric animals and humans.

Acid–Base Balance

The role of acid–base balance on the internal distribution of potassium (67–69) is based on the concept that during the development of acute acidemia hydrogen ion enters the cell in exchange for potassium and that the reverse occurs during the development of alkalemia (69–73). This dynamic interrelationship has been simplified clinically to a general rule that for each 0.1 U change in serum pH, the serum potassium changes in the opposite direction by 0.6 mEq/L. However, the relationship between serum potassium and serum pH is much more complex and depends on the type and severity of the acid–base disorder, the anion accompanying hydrogen, the duration of acidosis, changes in plasma bicarbonate concentration independent of changes in pH and the extent of intracellular buffering, and renal adaptation as well as hormonal changes in response to the disorder (74). In addition, in clinical settings, there are often other physiologic and pathophysiologic procecees that may be present, which would affect both transcellular as well as renal and extrarenal handling of potassium. The following generalizations should therefore be used with caution.

1. On the whole, acidosis is accompanied by a greater change in serum potassium than is alkalosis (54,75,76).
2. Mineral acidosis (Fig. 88-3) causes the greatest shift (0.24–1.7 mEq/L for each 0.1 U pH change), whereas organic acidosis has a much smaller effect (73,77–80). Mild mineral acidosis (decrease in serum bicarbonate by 5 mEq/L and and an increase in hydrogen ion concentration by 0.45 nmol/L) however does not result in significant change in serum potassium (81).
3. Acute respiratory alkalosis paradoxically results in a small but significant rise in serum potassium (+0.30 mEq/L with a drop in pCO2 of 16 to 22.5 mmHg). The rise was primarily due to stimulation of *alpha*-adrenergic receptors by

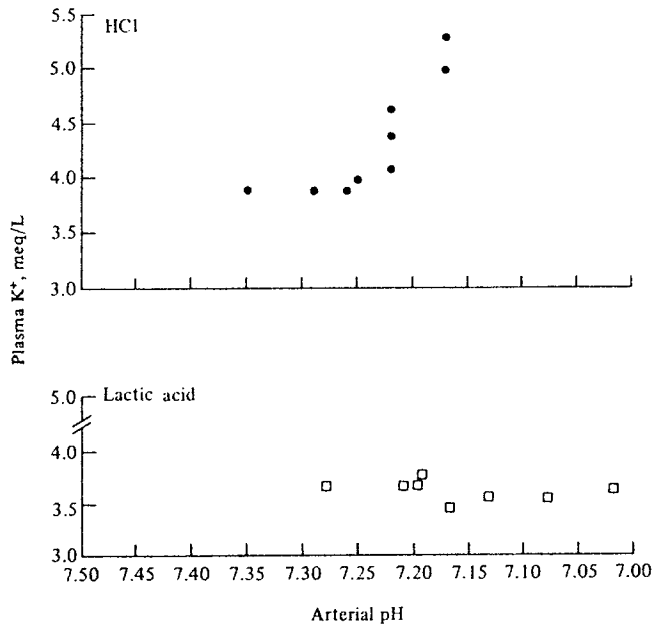

FIGURE 88-3. The effect of arterial pH on plasma potassium concentration in experimentally induced mineral acidosis (hydrochloric acid-HCl) and lactic acidosis in dogs. (From: Perez GO, Oster JR, Vaamonde CA. Serum potassium concentration in acidemic states. *Nephron* 1981;27:233, with permission.)

catecholamine (82). Chronic respiratory alkalosis however results in sustained hypokalemia.

4. The amounts of potassium shifted into the cell in metabolic and chronic respiratory alkalosis are approximately similar (0.1–0.4 mEq/L for each 0.1 U pH change).
5. Acute respiratory acidosis resulting in a decrease in pH to 7.24 had no effect on serum K^+ (83).
6. Changes in serum bicarbonate, independent of serum pH, have an inverse effect on the serum potassium concentration.
7. In chronic acidosis and alkalosis, the final serum K^+ is a function of the effect of acid–base disturbance on the renal handling of potassium, as well as on the transcellular distribution of this ion. In dogs with ammonium chloride-induced acidosis, Magner et al. (84) noted a fall in serum potassium below baseline by days 3 to 5, owing to severe kaliuresis (84).

Osmolality

The acute hyperkalemic effect of a sudden rise in plasma osmolality is probably caused by the shift of potassium-rich intracellular fluid by solvent drag (85). Clinically, this phenomenon is most commonly observed in hyperosmolar diabetic patients (Fig. 88-4), with or without ketoacidosis (86–89) when insulin deficiency augments the rise in potassium. Although, chronic hyperkalemia in diabetic patients is multifactorial, a sudden rise in plasma osmolality seems to play a contributory role. Infusion of hypertonic mannitol in normal humans (90) or hypertonic saline in patients with chronic kidney disease (91) results in a modest rise in serum potassium (0.4 to 0.6 mEq/L). These clinical observations support an independent role of sudden osmolar shifts in the regulation of serum potassium.

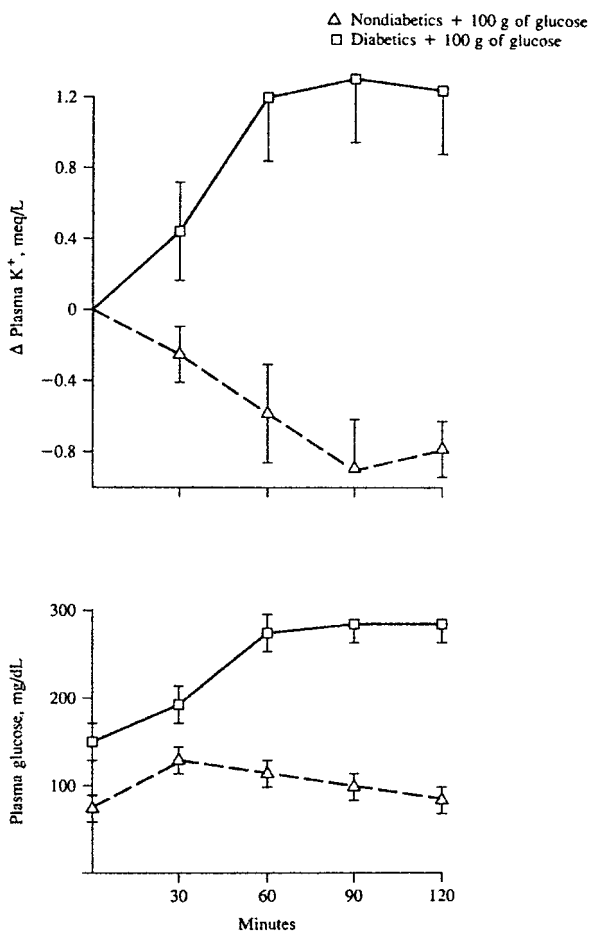

FIGURE 88-4. Effect of glucose infusion on plasma potassium and glucose concentrations in diabetics (*squares*) and normal subjects (*triangles*). The plasma potassium rises in diabetics owing to the development of hyperosmolality (hyperglycemia) but falls in normal subjects as a result of the glucose-induced release of endogenous insulin. (From: Nicolis GL, et al. Glucose-induced hyperkalemia in diabetic subjects. *Arch Intern Med* 1981;141:49, with permission.)

TABLE 88-1

ETIOLOGIES OF HYPERKALEMIA IN PATIENTS WITH RENAL INSUFFICIENCY

GFR <20 mL/minute
Defects in the renin–angiotensin–aldosterone axis
Tubular defects in potassium secretion
High potassium input (e.g., rhabdomyolysis, hemolysis, severe catabolic states, gastrointestinal bleeding, exogenous potassium administration)
Shift of potassium from intracellular compartment
Drugs that interfere with renal and extrarenal potassium homeostasis

POTASSIUM HOMEOSTASIS IN RENAL FAILURE

Patients with renal failure are able to maintain a near-normal serum potassium concentration despite a marked decrease in glomerular filtration rate (GFR) (92,93). However, under certain conditions, hyperkalemia may occur in patients with mild to moderate renal failure (Table 88-1). Although hyperkalemia could be due to increased potassium intake and/or rapid shifts of potassium from the cell, renal failure is the most important cause of hyperkalemia accounting for 77% of the cases reported by Acker et al. (94). In a random sample of 300 CKD patients (serum Cr 1.5–6.0 mg/dl) not receiving drugs that interfered with potassium homeostasis, 55% were noted to have hyperkalemia ($K^+ \geq 5.0$ mEq/L) (95). Treatment with drugs that interfere with potassium handling would be expected to further increase the development of hyperkalemia (see the following).

In this section, we initially discuss total body potassium content in patients with renal failure before treatment with dialysis and then review internal and external potassium homeostasis in these patients. In the subsequent section, we discuss hyperkalemia seen in patients with renal insufficiency with a defect in the renin–angiotensin–aldosterone axis or in the tubular responsiveness to aldosterone.

Total Body and Cellular Potassium Content in Renal Failure

Total body potassium content is a reflection of the balance between potassium intake and potassium output, whereas the cellular content reflects the distribution of potassium between the intracellular and the extracellular compartments. Exchangeable potassium (K_e) in pre-end stage renal disease (ESRD) patients has been generally reported as lower than normal (96). However, Berlyne et al. (97), after excluding patients with intercurrent problems (such as vomiting, diarrhea, or malnutrition), reported a normal value. As Patrick has pointed out (98), the normal range for K_e is not well defined and depends on age, sex, and the reference points used (e.g., total body weight, lean

body weight, or intracellular water). These reference points may be distorted in patients with CKD. Measurement of total body potassium by the use of a naturally occurring isotope (^{40}K) also has given normal values (99).

Cellular potassium content has been estimated by the use of muscle biopsy (100–105). Bergstrom et al. (100) extensively studied 102 patients with serum creatinine levels ranging from 4.8 to 25.0 mg/dL before therapy. In this and other studies, the intracellular potassium concentration was low owing to an increase in intracellular water, despite normal intracellular potassium content (100,103). However, Bilbrey et al. (106) and Montanari et al. (105) have reported normal intracellular potassium concentrations. Importantly, the intracellular potassium content was either low or normal (but not increased) in all four studies (100,103,105,106). The low intracellular potassium (and high intracellular sodium content) has also been reported in erythrocytes (102,107,108) and leukocytes (96,109) from these patients. This bespeaks a decrease in the number and/or the activity of the Na-K-ATPase pumps in the cell membrane. In chronic dialysis patients, the pump transport rate is higher immediately after fluid removal (110), and the abnormal levels of intracellular sodium and potassium in uremic patients return to normal following several weeks of dialysis (108,111). Because the number of pump sites inversely correlates with intracellular sodium, and a change in their number requires the production of new cells with lower intracellular sodium, the acute effect of fluid removal by dialysis may result from the removal of a volume-sensitive pump inhibitor (112). In contrast, the long-term effect of dialysis reflects the production of new cells with lower intracellular sodium and a higher number of pump sites. For a detailed discussion, refer to the article by Kaji and Kahn (112).

Internal Potassium Homeostasis in Chronic Kidney Disease

The role of cellular uptake of potassium in renal failure has been studied in both humans (92,93,113) and animals (114–117). Schon et al. (116) have shown that the cellular uptake of potassium in rats with remnant kidney is similar to that in normal rats maintained on a comparable diet but is lower than normal when both groups consume a high potassium diet. In contrast, in two different models of renal failure in rats, Bia and DeFronzo (114) showed impairment in the cellular disposal of an acute potassium load. Bourgoignie et al. (115) challenged chronically uremic dogs (remnant kidney model) that were adapted to different potassium intakes with an acute potassium load. Whereas the percentage of retained potassium that was shifted into the intracellular compartment was greater in normal dogs (90%), the absolute amount was significantly less than that in dogs with remnant kidney (9.0 versus 20.5 mEq, respectively). They concluded that extrarenal cellular uptake was normal in the dogs with renal failure. Gonick et al. (92) challenged patients with moderate renal failure with an oral potassium load. Whereas serum potassium 5 hours postchallenge was slightly higher in patients than in controls (5.2 versus 4.7 mEq/L), this result was entirely because of a lower urinary excretion. Perez et al. (113) studied patients with tubulointerstitial disease and showed that, whereas the absolute amount of potassium shifted into the cell was greater in patients compared with controls, the relative amount (expressed as percentage of total potassium retained) was similar. In contrast, Kahn et al. (93) observed a significantly greater rise in serum potassium in patients compared with controls when dietary potassium was increased by 50 mEq/day. The study by Kahn et al. cannot be strictly compared with others because they relied on 24-hour urinary potassium measurements and their study reflects long-term adaptation to a high-potassium diet in patients with CKD. In hemodialysis patients, serum potassium rose significantly more in patients than in controls challenged with acute potassium load (1.06 versus 0.39 mEq/L). However, the baseline potassium was significantly higher in patients than controls (5.17 versus 3.59 mEq/L), making the interpretation of this study difficult (118). More recently, Allon et al. (119) noted similar response in these patients with lower baseline potassium. In summary, extrarenal cellular uptake of an acute potassium load in CKD patients is near normal; however, it may be impaired in patients on hemodialysis with higher baseline plasma potassium.

As discussed previously, internal potassium homeostasis is regulated by insulin, catecholamines, and, to a lesser extent, aldosterone. Although the serum insulin level is increased in renal failure (120,121), three studies provide strong support for normal insulin-stimulated potassium uptake (121,122) by the splanchnic as well as by peripheral tissues (122). Alvestrand et al. (122), using the euglycemic insulin clamp technique, demonstrated a similar uptake of potassium by both splanchnic and leg tissues in patients with CKD. Inhibition of endogenous insulin by somatostatin results in a significantly greater rise in serum potassium in uremic rats than in controls (1.0 versus 0.2 mEq/L at 60 minutes) (123). Administration of glucose with potassium stimulates insulin secretion and attenuates the rise in potassium in patients on dialysis as well as normal controls (119).

Serum catecholamine levels have been reported to be high in chronic kidney disease (124,125). Yang et al. (126) noted higher mean potassium in patients on a nonspecific β-blocker, propranolol. Infusion of epinephrine resulted in two different responses: In four of 10 patients, serum potassium did not fall; in the remaining six, an exaggerated response was noted. The authors felt that the latter group of patients are those who have a propensity to develop hyperkalemia while on propranolol. Gifford et al. (127), using a much lower epinephrine dose, could not show a hypokalemic response in patients with ESRD. Plasma aldosterone is normal or high in most CKD patients (93,128–131). As noted, patients with ESRD who are taking DOCA, spironolactone, or placebo have similar baseline potassium levels; however, patients on DOCA can dispose an acute potassium load more promptly than the other groups (62). In addition, high dose spironolactone for 3 weeks in ESRD patients can result in a significant rise in serum potassium (63). These two studies would support a minor role for aldosterone in internal potassium homeostasis in ESRD patients. In summary, the major cellular defect in potassium disposal seems to be an abnormal response to catecholamines seen in a subgroup of patients on dialysis. The role of hyperparathyroidism, a common accompaniment of renal failure, while intriguing, is still speculative in humans and requires more studies.

External Potassium Homeostasis in Severe Renal Failure

Renal Adaptation

Patients with a marked decrease in GFR are able to excrete the ingested dietary potassium load and maintain near normal potassium balance. This adaptive process is reflected by an increase in the fractional excretion of potassium (FE_K) modulated by an increase in secretory rate per functioning nephron. However, this adaptive response is limited and a sudden increase in potassium intake may result in life-threatening hyperkalemia. The quantitative aspects as well as the anatomic and functional characteristics of this adaptive response are briefly reviewed herein.

In conscious dogs with a 10% remnant kidney, Schultze et al. (132) showed that potassium excretion by the remnant

kidney increased fourfold by 18 hours and approached 85% of the control value by seventh day. Kunau and Whinnery (133) and Wilson and Sonnenberg (117) reported similar data in rats. In experiments by Schultze et al. (132) and Finkelstein and Hayslett (134), animals with remnant kidney manifested an exaggerated kaliuresis following a potassium load. In contrast to these data and independent of previous potassium intake, dogs with 25% remnant kidney were only able to excrete 30% to 37% of the load in 5 hours compared with 70% to 90% in the control animals (115). There is no easy resolution to the differences in these two studies (115,132).

Gonick et al. (92) documented that human subjects with CKD were able to excrete only 20% of an oral potassium load in 6 hours compared with 46% in normal controls. Similar data have been reported by Perez et al. (113) in patients with tubulointerstitial disease. Kahn et al. (93), in 10 patients with stable chronic kidney disease, showed that renal adaptation to increased dietary potassium occurs in these patients. In summary, it can be concluded that residual renal tissue is able to maintain external potassium homeostasis in the postabsorptive state. However, the initial phase of this adaptation is impaired when an acute potassium load is administered.

The nephron sites involved in this adaptation have been studied utilizing a variety of techniques in both rats and rabbits and appear to include both the distal convoluted tubule and the collecting duct (117,133–137). The discrepancies reported in the literature are most likely owing to interspecies and intraspecies differences as well as the anatomic definition of different distal tubular segments.

The mechanisms involved in this renal adaptation have been partially defined. In both humans (128) and rodents (138), aldosterone has been shown to play an important role in the adaptive ability of the diseased kidney to maintain a normal rate of potassium excretion. This renal adaptation has been shown to be independent of dietary sodium intake (139). Schultze et al. (132) argued that aldosterone is not important in the renal potassium adaptation that occurs following a reduction in renal mass, since uremic dogs maintained on constant aldosterone replacement maintained normal rates of potassium excretion (132). However, the replacement dose of aldosterone in this study was in the high pharmacologic range. Serum potassium concentration itself plays an important role in augmenting urinary potassium excretion (95). Bourgoignie et al. (115) found a direct relationship between serum potassium and both the absolute and fractional potassium excretion. The slope of the curve relating serum potassium to the absolute rate of urinary potassium excretion was much steeper in normal dogs than in dogs with a remnant kidney. However, the slope of the curve relating serum potassium to the FE_K was similar in the control and uremic dogs.

Microperfusion studies by Fine et al. (136) indicate that adaptation is an inherent characteristic of the renal tubular cells of uremic animals and once learned can be retained *in vitro,* at least for short periods of time. Schon et al. (116) showed that augmented potassium excretion is associated with an increase in Na-K-ATPase in the outer medulla in animals subjected to three-quarter nephrectomy. This increase is quite specific to this enzyme and occurs only in the kidney (116) and colon (140). Muto et al. demonstrated that an increase in peritubular [K^+] increased renal potassium excretion by also enhancing K^+ conducatance (ROMK) and Na^+ conductance (ENaC) in principal cells (Fig. 88-5) (141). Other mechanisms may include a higher rate of potassium delivery and an increase in tubular flow rate in the distal nephron (133).

Intestinal Potassium Excretion in Renal Failure

Patients with renal failure secrete more potassium in the stool than do normal controls (128,142,143). Net colonic secretion of potassium is increased significantly above control levels in rats with renal insufficiency (142). This increase is associated with an increase in Na-K-ATPase activity in colonic mucosa and is functionally similar to the increase seen with the administration of DOCA, glucocorticoids, or high dietary potassium (144). Although the rise in fecal potassium concentration is significant, the absolute amount of K^+ lost through this route in patients with mild to moderate CKD is small and contributes only minimally to the external K^+ homeostasis. In patients with advanced renal insufficiency (GFR <5 to 10 mL/minute); however, up to 30% to 40% of the ingested potassium load may be excreted in the stool (143).

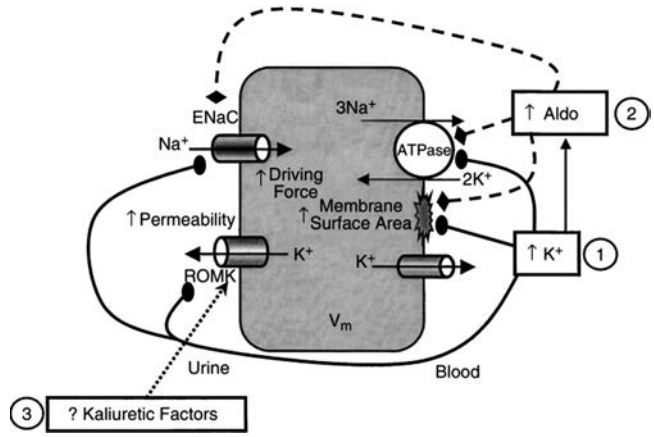

FIGURE 88-5. Major factors that regulate potassium secretion in principal cells. Sodium is reabsorbed across the luminal membrane through ENaC (epithelial sodium channels) with resultant cellular depolarization increasing the electrical driving force for potassium secretion through ROMK (potassium channels). The effects of aldosterone (Aldo) and hyperkalemia (↑K^+) on potassium secretion are noted. (From: Gennari FJ, Segal AS. Hyperkalemia: an adaptive response in chronic renal insufficiency. *Kidney Int* 2002;62:1.)

Acid–Base Homeostasis

The kidney through its ability to excrete nonvolatile (fixed) acid produced by combustion of foodstuff, chiefly proteins, plays a major role in hydrogen ion homeostasis. This is done by reclamation of filtered buffers (primarily bicarbonate) and regeneration of buffers lost in daily titration of ingested acids. Although the total fixed acid generated from the combustion of foodstuff is relatively small, estimated at 1 mEq/kg body weight/day, unless this is excreted it can deplete the total buffer reserve, estimated at 600 to 700 mEq, in days to weeks.

Bicarbonate reclamation occurs by hydrogen secretion through Na-H antiporter in the proximal tubules as well as H-ATPase pump in both proximal and distal tubules. This process accounts for reabsorption of approximately 4,000 to 5,000 mEq of base per day, resulting in acid urine devoid of bicarbonate. The bicarbonate regeneration occurs primarily in the distal tubules through protonation of urinary buffers made up of titratable acids (primarily phosphates) and ammonia. The proximal tubule is therefore responsible for 85% to 90% of bicarbonate reclamation, whereas the distal tubule is responsible for the remaining 10% to 15%. In addition, the distal tubule is also responsible for regeneration of 60 to 80 mEq of new bicarbonate. When faced with an additional acid load, the kidney increases H^+ secretion (and bicarbonate regeneration) primarily through an increase in ammonium production by the deamination of circulating glutamine. The ability of the

kidney to increase ammonium secretion is limited and is estimated at three to five times baseline production (144,145) (see Chapter 6).

Acid–Base Homeostasis in Renal Failure

The ability of the kidney to excrete hydrogen ion is progressively diminished with the diminution of GFR. Although it is commonly stated that a significant decrease in serum bicarbonate does not occur until GFR falls below 25 to 30 mL/minute, this is not strictly true. Widmer et al. (146) noted a serum bicarbonate reduction from 28 to 22 mEq/L in patients with a creatinine of 2 to 4 mg/dL and further reduction to 19 mEq/L in patients with a creatinine of 4 to 14 mg/dL (146). The anion gap remained unchanged in the first group and rose significantly with a further decrease in GFR. The concept of orderly progression of metabolic acidosis of renal failure from hyperchloremic to anion gap acidosis, however, occurs in the minority of patients. Wallia et al. (147) studied the electrolyte pattern in 70 patients with ESRD just before dialytic therapy was begun. Five patterns were found: 14 patients with normal electrolytes; 14 with anion gap metabolic acidosis; 21 with hyperchloremic acidosis; 11 with mixed hyperchloremic and anion gap acidosis; and 10 with normal serum chloride, low serum bicarbonate, and normal anion gap. This last group, however, had the lowest serum sodium and therefore were relatively hyperchloremic. Therefore, among these 70 patients with ESRD, 31 (44%) had hyperchloremic acidosis, only 14 (20%) had classic anion gap acidosis, and interestingly another 14 (20%) had normal electrolytes. Patients with increased anion gap, however, had a slight but significantly higher serum creatinine than patients with pure hyperchloremic acidosis or with normal electrolytes (13.2 versus 10.0 versus 9.0 mg/dL, respectively). In addition, these two studies did not support the common impression that hyperchloremic acidosis occurs more often in patients with tubulointerstitial than glomerular disease (146,147). Interestingly, diabetic patients with moderately severe renal failure (GFR <30 mL/minute) have recently been reported to have milder metabolic acidosis than nondiabetic patients with similar renal function (148).

Renal tubular acidosis (RTA) defines a group of disorders characterized by the presence of metabolic acidosis out of proportion to the decrease in GFR. The hallmark of these disorders is the presence of significant metabolic acidosis with hyperchloremia and normal anion gap. Renal tubular acidosis in patients with mild to moderate renal insufficiency is often associated with significant hyperkalemia and is discussed later in this chapter.

Pathophysiology of Metabolic Acidosis in Chronic Kidney Disease

Many studies have shown that acid production in renal failure is normal, and therefore uremic acidosis reflects a decrease in net acid excretion, defined as the difference between proton excretion in the form of titratable acid and ammonium ion (NH_4^+) and bicarbonate excretion (149–151). Careful metabolic studies by Goodman et al. (151) documented that patients with chronic renal failure have a daily bicarbonate deficit of approximately 13 to 19 mEq. It is notable that despite this persistent deficit, serum bicarbonate in patients with chronic renal failure, after an initial drop, remains stable over long periods of time (152,153). This is due chiefly to the buffering of excess hydrogen ion by bone buffers including calcium carbonate (152).

Renal Excretion of Bicarbonate

Several studies in humans have shown that some patients with severe kidney disease have significant bicarbonate wasting (149,154–158). In an early study by Schwartz et al. (149), three out of four patients with renal failure had significant bicarbonaturia, which disappeared only after the fall of serum bicarbonate to below 20 mEq/L. In a more detailed study in 17 uremic patients (serum creatinine of 5.6 to 18.9 mg/dL), the majority had significant bicarbonate wasting (fractional excretion of HCO_3 of 0% to 17.56%) despite the presence of metabolic acidosis (serum HCO_3 of 16 to 23 mEq/L). After NH_4Cl loading, serum bicarbonate decreased to below 14 mEq/L, and bicarbonaturia disappeared in all but four patients (158). Interestingly, the bicarbonate wasting in these four patients also disappeared with institution of a low-sodium diet. These two studies support the presence of a diminished maximal tubular reabsorption (T_m) for bicarbonate in the majority of patients with renal failure. Further, they demonstrate that the low T_m is partly responsive to volume status.

Arruda et al. (157) and Wong et al. (159), working with a remnant kidney model in dogs with variable levels of volume expansion and serum bicarbonate, noted that the ratio of absolute bicarbonate to sodium reabsorption was increased in chronic renal failure. In addition Wong et al. (159), using micropuncture method, showed that this ratio was also higher at the beginning of the distal tubule, indicating avid bicarbonate absorption by the proximal tubule of the remnant kidney. Although absolute absorption was higher, the absolute amount of bicarbonate delivered to the distal tubule was also higher, reflecting the marked increase in filtered load per nephron owing to an increase in single nephron GFR (159). In summary, the whole-kidney T_m for bicarbonate is in general diminished in chronic renal failure despite an absolute increase in bicarbonate resorption at the single nephron. The discrepancy in these findings may reflect the variation in the experimental designs and the role of nonvolume regulators in bicarbonate handling by the kidney.

Renal Excretion of Titratable Acid

Excretion of titratable acids chiefly reflects the amount of urinary phosphate and the urinary pH. Most CKD patients are able to maximally acidify their urine (149,160), and urine-serum P_{CO_2}, as a measure of hydrogen pump activity in the distal tubule, is normal (161). The amount of titratable acids in these patients is normal (151,152–164). This is primarily owing to an increase in fractional excretion of phosphate initiated by secondary hyperparathyroidism. It should be noted, however, that urinary phosphate does decrease with severe renal failure. This reflects both a decrease in dietary phosphate as well as the effect of phosphate binders commonly used in these patients.

Renal Excretion of Ammonium

Although bicarbonaturia may contribute to metabolic acidosis, the major abnormality is a decrease in renal excretion of ammonium. Ammonium is primarily produced by the deamination of amino acids, chiefly glutamine, in the proximal tubule and to a much lesser extent in the loop of Henle and distal convoluted tubule (144,145) (see Chapter 6). The production of ammonium in the proximal tubule is increased significantly by acidosis and inhibited by alkalosis (165). This pathway is regulated by two key enzymes, glutaminase and phosphoenolpyruvate carboxylase. In metabolic acidosis there is an increase activity of both enzymes in association to increase in their respective messenger RNA (166). The majority of ammonium produced is reabsorbed in the ascending thick limb of the loop of Henle

(TALH) by both active and passive processes and is added to the medullary interstitium. The active transport involves substitution of ammonium instead of potassium on Na^+-2Cl-K^+ transporter in the TALH, which is inhibited by hyperkalemia (167). In the medullary interstitium, ammonium ion exists in dynamic equilibrium with ammonia gas. The latter diffuses chiefly into the collecting duct, where it is protonated by hydrogen secreted by the H-ATPase pump, located in the luminal membrane of the intercalated cells, and is trapped intraluminally and finally excreted. However, some ammonia gas enters into the descending limb of the loop of Henle, creating a countercurrent mechanism for ammonium. The final urinary ammonium is therefore a reflection of production of ammonium in the proximal tubule, its transport into the loop of Henle, accumulation in the renal interstitium, and finally secretion of ammonia and its entrapment in the collecting duct (144,145).

In chronic kidney disease, fractional renal ammonium excretion initially increases by severalfold, thereby resulting in the maintenance of a normal absolute excretion rate (168). However, as the GFR decreases below 20 mL/minute, despite a maximal increase in fractional excretion of ammonium, the absolute excretory rate decreases significantly. Thus, progressive metabolic acidosis results. This decrease in the rate of ammonium excretion also reflects a decreased ability of the kidney to trap ammonia in the collecting duct (160). Warnock (153) has suggested that the decrease in ammonia trapping in the remnant kidney model may be secondary to excess delivery of bicarbonate to the collecting duct, thereby resulting in an unfavorable environment for the diffusion and trapping of ammonia.

The role of aldosterone in ammonium excretion is complex. Aldosterone increases the rate of Na^+-dependent and Na^+-independent H^+ secretion in the cortical and medullary collecting duct (169,170). Hypoaldosteronism is associated with a decrease in the rate of H^+ secretion, while the ability to maintain a steep H^+ gradient between urine and plasma, as measured by urinary pH and urine minus blood P_{CO_2} in alkaline urine, is not affected (171,172). The decrease in the rate of H^+ secretion is associated with a decrease in the availability of ammonium buffer in the urine that is not augmented appropriately in response to sodium sulfate infusion (173,174). Hypoaldosteronism is universally associated with a decreased potassium excretion and hyperkalemia. Hyperkalemia decreases renal ammonium excretion significantly. This is owing to a decrease in accumulation of ammonium in the renal interstitium despite normal production by the proximal tubule (175). In the syndrome of hyperkalemic renal tubular acidosis, this mechanism probably plays the major role in the production of hyperchloremic acidosis seen early in the course of renal failure. Reversal of hyperkalemia with sodium binding resin (176), mineralocorticoids (177), or low-potassium diet (178) ameliorates the metabolic acidosis by increasing ammonium secretion.

HYPERKALEMIC RENAL TUBULAR ACIDOSIS OWING TO A DEFECT IN RENIN–ANGIOTENSIN–ALDOSTERONE AXIS OR TUBULAR UNRESPONSIVENESS TO ALDOSTERONE

Although a decrease in GFR may be associated with the development of significant hyperkalemia and hyperchloremic (HCA) or anion gap metabolic acidosis, this usually occurs only with severe reductions in GFR, below 15 to 20 mL/minute. However, some patients with underlying renal disease and mild to moderate azotemia present with striking hyperkalemia with or without HCA. The elevated serum potassium in these patients is primarily owing to a disturbance in the renin–angiotensin–aldosterone axis or to renal tubular responsiveness to aldosterone (Fig. 88-5; Table 88-2). Since the report by Hudson et al. (179), numerous cases have been described in which hyperkalemia with or without HCA developed in the presence of only mild to moderate renal insufficiency (180–191). In the majority of these cases, the underlying renal disease has been diabetic or hypertensive nephropathy or chronic interstitial nephritis (181). In 1972, Schambelan et al. (192) presented evidence

TABLE 88-2

ETIOLOGY OF CHRONIC HYPERKALEMIA DUE TO DISTURBANCES IN RENAL POTASSIUM EXCRETION

I.	Decrease in GFR
	Acute renal failure
	Chronic kidney disease (GFR <15 to 20 mL/minute)
II.	Defect in renal tubular secretion of potassium
	Disturbance in the renin–angiotensin–aldosterone axis
	Hyporeninism: associated with renal insufficiency (diabetes mellitus, interstitial nephritis)
	Disturbance in angiotensin II activation or function (captopril, saralasin)
	Hypoaldosteronism
	With glucocorticoid deficiency (Addison disease, enzyme deficiency)
	Block in aldosterone synthesis (heparin, 18-methyloxidase deficiency)
	Primary hypoaldosteronism
	Tubular resistance to the action of aldosterone (renal tubular hyperkalemia)
	Pseudohypoaldosteronism
	Hyperkalemia, hypertension, and normal renal function
	Hyperkalemia with mild to moderate renal insufficiency and variable plasma aldosterone levels (sickle cell disease, systemic lupus erythematosus, renal transplant, obstructive uropathy, miscellaneous)
	Pharmacologic inhibition of the tubular action of aldosterone (spironolactone, eplerenone, triamterene, amiloride, pentamidine, trimethoprim) in distal nephron

GFR, glomerular filtration rate.

linking hypoaldosteronism with hyporeninism in six patients with this syndrome. This association was verified in subsequent reports (193–196), and the entity became known as hyporeninemic hypoaldosteronism (HHA). However, it became quickly clear that a significant minority of these patients had normal renin levels. DeFronzo (197) in 1980, after reviewing 81 published cases, came to the conclusion that in 20% of cases the low plasma aldosterone levels could not be explained by renin deficiency, and therefore a primary abnormality in aldosterone synthesis had to be postulated. At the same time, some patients with sickle cell disease (7,198,199), systemic lupus erythematosus (200,201), and renal transplantation (202,203) have a renal tubular secretory defect resulting in hyperkalemia despite a normal renin–aldosterone axis. Therefore, at the present time these patients can be divided into two large categories: (i) hyperkalemia resulting from hypoaldosteronism with or without hyporeninism; and (ii) hyperkalemia resulting from a primary renal tubular potassium secretory defect. One could consider this entity as a spectrum ranging from pure aldosterone deficiency with normal tubular responsiveness to severe tubular resistance with normal aldosterone secretion. Between these two extremes there are many overlapping presentations in which either the defect in the hormonal axis or the tubular responsiveness dominates. Although Table 88-2 summarizes all the hormonal or tubular defects that can lead to hyperkalemia, often with HCA our discussion is limited to the disturbances associated with renal insufficiency.

Hyperkalemic Renal Tubular Acidosis Owing to a Defect in Renin–Angiotensin–Aldosterone Axis

This group comprises approximately 80% of the patients with renal insufficiency and hyperkalemia (197,204–206). The hallmark of this group is a low plasma aldosterone concentration. The majority (80%) of this group also has low plasma renin activity (PRA) and therefore represents the classic syndrome of HHA. However, 20% have a normal PRA. Clinically and physiologically, these patients present with fairly uniform features. Several large (192,207) as well as smaller series (252,255–260), have defined the characteristics of these patients first summarized in a review by DeFronzo (197). These include: (a) mean age of about 60 years, (b) presence of diabetes mellitus in about 50%, (c) presence of mild to moderate renal failure in the majority, and (d) lack of symptoms referable to hyperkalemia in 75%. Physiologic features include: (a) low or low-normal baseline and/or stimulated aldosterone levels, (b) normal plasma cortisol, (c) low baseline and/or stimulated renin values in 80%, (d) normal aldosterone response to angiotensin or ACTH stimulation in the minority, (e) presence of hyperchloremic acidosis in well over 50%, and (f) lack of significant salt wasting.

To gain an understanding of the physiologic basis of this syndrome, we initially review the defect in renin secretion and then summarize our present understanding of aldosterone deficiency in this syndrome.

Hyporeninism

At present no single abnormality can explain the low PRA seen in 80% of these patients (197,205,206). Evidence has been presented in support of a defect in one or more physiologic regulators of renin secretion including volume, autonomic nervous system, serum potassium concentration, and prostaglandins.

Oh et al. (195), Perez et al. (212), and others (213,214) have demonstrated that long-term sodium and volume depletion in these patients is associated with a significant increase in the PRA. However, comparable data in normal controls with the same degree of volume depletion were not provided. In the report of Oh et al. (195), after 3 to 6 weeks of salt depletion, the PRA rose into the normal range, but plasma aldosterone remained subnormal. In the study by Chan et al, eight of the 12 patients with hyporeninism responded to 2 weeks of furosemide with increase in PRA without similar response in plasma aldosterone (214). In a study of four patients with acute postinfectious glomerulonephritis (181), plasma renin and aldosterone concentrations were low during the acute phase, but returned to normal following recovery from acute nephritis. Interestingly, in two patients, the renin and aldosterone levels remained low during the acute phase despite an excellent response to diuretics. These two patients, however, responded appropriately to physiologic doses of fludrocortisone. This study (181), coupled with previous studies of acute glomerulonephritis (215,216), supports the concept that although physiologic suppression of the renin–aldosterone axis by volume expansion may play a significant role in certain patients with glomerular disease, hypertension, and edema, other factors such as decreased GFR and damage to the juxtaglomerular apparatus play an important contributory role. Gordon et al. (217) have described a patient with hypertension, acidosis, hyperkalemia, and normal renal function associated with HHA. Prolonged sodium restriction resulted in correction of these abnormalities. A similar pathophysiologic mechanism has been postulated in hypertensive patients with hyperkalemia and renal insufficiency (218).

The autonomic nervous system plays an important physiologic role in the regulation of renin secretion. Sympathetic nerve terminals are known to innervate the juxtaglomerular apparatus, and renin secretion is stimulated by epinephrine (219,220). Therefore, autonomic insufficiency could result in a state of hyporeninemia. This hypothesis has been investigated primarily in diabetic patients, in whom autonomic neuropathy is common and circulating catecholamine levels are often low (221). In five diabetic patients with autonomic neuropathy, Tuck et al. (222) reported low basal PRA as well as diminished plasma aldosterone and norepinephrine concentrations. In addition, infusion of isoproterenol, a β-adrenergic agonist, did not increase PRA, indicating a possible block at or beyond the receptor level. In contrast, normal circulating catecholamine levels have previously been reported in diabetic patients with the syndrome of hypoaldosteronism (180,193,223,224). Fernandez-Cruz et al. (225) compared stimulated PRA in 16 normotensive diabetic patients without overt nephropathy and nine age-matched controls. The stimulated PRA was significantly lower in these patients and correlated directly with the degree of autonomic dysfunction as measured by the velocity of esophageal peristalsis. De Chatel et al. (226), however, were unable to demonstrate in a large group of diabetic individuals any correlation between the plasma epinephrine concentration and abnormalities in the renin–aldosterone axis. Therefore, although autonomic neuropathy may play a role in the development of hypoaldosteronism in some diabetic patients, it is not a uniform finding and certainly cannot explain the occurrence of this syndrome in nondiabetic patients.

Hyperkalemia is known to inhibit PRA (227); consequently, one could hypothesize that hyporeninemia is not a primary defect but is secondary to hyperkalemia. In two studies (192,196), short-term normalization of serum potassium did not increase PRA significantly; however, long-term studies have not been undertaken to examine this very important question.

Prostaglandins E_2, I_2, and D_2 are known stimulators of renin release (228,229), whereas prostaglandins E_1 and E_2 directly increase aldosterone biosynthesis *in vitro* (230). Furthermore, hyperkalemia has been reported following treatment with indomethacin, a potent prostaglandin inhibitor (231). These observations raise the possibility that a defect in prostaglandin

synthesis may play a role in the development of HHA in some hyperkalemic patients. Consistent with this possibility, Tan et al. (232) found a strong correlation between urinary PGE_2 level and the ratio of active to inactive renin in normal controls and in patients with the syndrome of hypoaldosteronism. In four of the nine patients, low urinary PGE_2 was associated with a low ratio of active to inactive renin. In normal controls, inhibition of prostaglandin synthesis with indomethacin resulted in a similar decrease in this ratio. These authors postulated that prostaglandins may play a critical role in the activation of renin, and therefore hypoaldosteronism in these patients may be secondary to a prostaglandin deficiency. In two patients with diabetes mellitus and hypoaldosteronism, the total renin concentration was normal, whereas PRA was low (233). Fractionation of the plasma yielded an inactive renin precursor (prorenin or "big renin"); unfortunately, prostaglandin levels were not measured in these diabetic patients. It should be noted, however, that other investigators have failed to find an association between prostaglandin deficiency and the development of HHA (234).

Another hypothesis that links CKD with hyporeninism is fibrosis of the juxtaglomerular apparatus owing to intrinsic renal disease. Although occasional reports of juxtaglomerular apparatus fibrosis have appeared (213), this is a rare finding. Besides, the presence of juxtaglomerular apparatus damage alone does not explain the development of hypoaldosteronism.

Hyperfiltration hypothesis has been linked to the development of HHA in both diabetic and nondiabetic CKD patients (235). According to this hypothesis, as the number of nephrons is reduced, there is an adaptive increase in the renal plasma flow and GFR by the remaining functioning glomeruli. These alterations in renal hemodynamics serve to inhibit renin synthesis and release, leading secondarily to the development of hypoaldosteronism.

Hypoaldosteronism

The hallmark of the syndrome of HHA is a low basal or low stimulated plasma aldosterone level in spite of normal levels of glucocorticoids and other ACTH-dependent steroids such as DOCA or corticosterone. Aldosterone secretion is primarily stimulated by the renin–angiotensin system. However, ACTH and serum potassium, as well as other regulators, play independent roles.

As stated previously, hyporeninemia is present in 80% of patients with hypoaldosteronism (197,205,206), and therefore it is logical to consider that the primary defect in these patients lies in renin synthesis or release. Schambelan et al. (206) showed that stimulation of renin by volume contraction resulted in a rise in plasma aldosterone that was appropriate for the increase in PRA. The slope of the curve relating plasma renin and aldosterone was similar in patients with HHA and normal controls. Surprisingly, for any given level of PRA, the plasma aldosterone concentration was disproportionately elevated, probably because of the independent stimulatory effect of plasma potassium on aldosterone secretion. Nevertheless, the highest levels of renin and aldosterone achieved in these patients were comparable only to the basal levels in control subjects. In contrast, as indicated, other investigators have found a clear disconnect between renin and aldosterone level after stimulation with volume depletion (212–214) and captopril (214). In all studies, however, the response of aldosterone was significantly blunted despite increase renin and persistent hyperkalemia. In addition, most investigators have reported a marked impairment in the ability of angiotensin II to stimulate aldosterone secretion (197,206). This finding, coupled with a subnormal aldosterone response to ACTH stimulation (197,206), and the failure of hyperkalemia to stimulate aldosterone secretion, has strengthened the possibility of a primary adrenal defect in some patients with hypoaldosteronism. This is further supported by the observation that 20% of patients with hypoaldosteronism have normal PRA (197,205,206). One should also consider the possibility that the poor response of aldosterone to ACTH, angiotensin II, and hyperkalemia may be secondary to long-term atrophy of the zona glomerulosa of the adrenal gland rather than to a specific enzymatic defect in aldosterone production. Consistent with this possibility, Fredlund et al. (236) provided evidence in isolated adrenal glomerulosa cells that the aldosterone response to hyperkalemia is dependent on the circulating angiotensin level. However, no study so far has evaluated response of adrenal gland to prolonged stimulation by angiotensin II in patients.

The serum potassium concentration is an important regulator of the plasma aldosterone level (237–239). In nephrectomized patients, a significant correlation between serum potassium and plasma aldosterone exists (240), and this relationship is independent of renin or ACTH. Therefore, in interpreting a given plasma aldosterone level, the effect of serum potassium must be considered. Schambelan et al. (206) categorized 31 patients into two groups based on the ratio of urinary aldosterone excretion to serum potassium concentration. Group A (23 patients) had a low ratio and was considered to have hypoaldosteronism. Group B (eight patients) had a normal ratio and was considered to have a primary tubular defect in potassium secretion. Twenty percent of group A had a normal PRA. Therefore, hypoaldosteronism in this group, in spite of normal PRA and high plasma potassium, is probably owing to a defect in aldo- sterone synthesis.

Another regulator of aldosterone secretion and plasma volume is atrial natriuretic factor (ANF). Atrial natriuretic factor has been shown to be a strong inhibitor of baseline as well as stimulated aldosterone in humans (241,242). In normal humans, ANF also prevents potassium-stimulated rise in aldosterone level (243). In addition, ANF level is markedly elevated 10- to 50-fold in patients with hypoaldosteronism (243). Although the rise in ANF (and suppression of aldosterone) could be secondary to volume expansion, ANF also suppresses potassium, angiotensin and ACTH-stimulated aldosterone secretion supporting presence of a common cellular mechanism for its action possibly through stimulation of cGMP (244).

Several investigators have explored the possibility of an enzymatic defect in aldosterone biosynthesis (212,224,227), and an enzymatic block involving the conversion of 18-hydroxycorticosterone to aldosterone has been postulated, but these findings have not been supported by other studies (192,245, 246).

As indicated, diabetic patients constitute a large percentage of patients with HHA. To explain this high incidence, two other postulates have been presented. Insulin is an important regulator of potassium uptake by a variety of tissues, and chronic hypoinsulinemia (absolute or relative) might be expected to result in a state of intracellular potassium deficiency. Furthermore, it is known that the intracellular potassium concentration is an important regulator of aldosterone synthesis (197). Potassium-deficient cultured zona glomerulosa cells have a blunted aldosterone response to angiotensin II and ACTH (236,247). Insulinopenia, by decreasing intracellular potassium, may lead to a defect in aldosterone synthesis and the syndrome of hypoaldosteronism (13). A second hypothesis is offered by Smith and DeFronzo (248) and involves the concept of tubuloglomerular feedback. Normal tubuloglomerular balance is disrupted in the presence of osmotic agents in the renal tubule (249,250), including glucose (251–253). It is postulated that, in diabetic patients with a high filtered glucose load, sodium chloride delivery out of the proximal tubule is enhanced, leading to increased delivery of solute to the loop

of Henle. Enhanced chloride reabsorption by the TALH may inhibit renin secretion (254), which secondarily leads to the development of hypoaldosteronism.

In summary, at present a unified etiologic hypothesis cannot be formulated to explain the occurrence of the syndrome of HHA in different patients. It is likely that this syndrome is quite heterogeneous and can be explained only by multiple etiologic abnormalities. In a given patient, the role of different regulatory systems (i.e., volume status, prostaglandins, ANF, autonomic nervous system, structural damage to the juxtaglomerular apparatus, enzymatic defects in aldosterone and renin biosynthesis, and intracellular adrenal potassium deficiency) should be considered and evaluated.

Hyperkalemic Renal Tubular Acidosis Owing to a Renal Tubular Secretory Defect

This group of disorders (Table 88-2 and Fig. 88-6) includes patients who have hyperkalemia out of proportion to the degree of renal failure or hypoaldosteronism. The primary defect is a partial resistance to the physiologic effect of aldosterone to promote potassium secretion. Perez et al. (255) named this syndrome renal tubular hyperkalemia and divided it into three groups: group I, patients with pseudohypoaldosteronism; group II, patients with hyperkalemia, hypertension, and normal renal function; and group III, patients with hyperkalemia, mild to moderate renal insufficiency, and normal-plasma aldosterone (group IIIa), low-plasma aldosterone (group IIIb), or high-plasma aldosterone (IIIc) (Table 88-2).

Groups I and II represent examples of a pure tubular secretory defect without renal insufficiency and is not discussed here. In this section we deal only with group III patients, who present with mild to moderate renal insufficiency, hyperkalemia, hyperchloremic acidosis, variable plasma renin and aldosterone levels, and resistance to physiologic doses of mineralocorticoids. This clinical entity has been described in patients with sickle cell disease, systemic lupus erythematosus, renal transplant, obstructive uropathy, acquired immunodeficiency syndrome (AIDS), and a group of miscellaneous diseases including lead nephropathy and chronic interstitial nephritis.

Sickle Cell Disease

A renal tubular potassium secretory defect in sickle cell disease was first reported in patients with normal renal function and normal serum electrolyte concentrations (7) and later in patients with sickle cell nephropathy (197,256), sickle cell trait (198), and sickle C disease (199). Although basal and stimulated aldosterone levels were normal in all subjects, these patients were unable to excrete a potassium load normally. Infusion of potassium chloride, sodium sulfate, and furosemide failed to augment potassium secretion normally. This defect is thought to result from ischemic damage to the collecting tubules and medullary area by sickle cells. An immunologic reaction against a renal tubular antigen also has been suggested. It should be noted that the syndrome of HHA also occurs in sickle cell disease (197,256).

Systemic Lupus Erythematosus

A defect in potassium secretion, similar to the defect in sickle cell disease, has been reported in several patients with systemic lupus erythematosus (SLE) (200,201). The defect is often accompanied by a defect in hydrogen ion secretion (200,201,257). In two patients, an increase in sodium and bicarbonate delivery (by furosemide, acetazolamide, and bicarbonate) to the distal sites of potassium exchange did not augment potassium secretion. High-dose fludrocortisone therapy also failed to increase potassium excretion in these two patients (200). Recently, a single patient has been reported in whom fludrocortisone was successful in controlling hyperkalemia (258). In a study of two patients with SLE and hyperkalemic RTA, Bastani et al. showed the presence of autoantibodies to collecting duct cells in one patient. The serum from

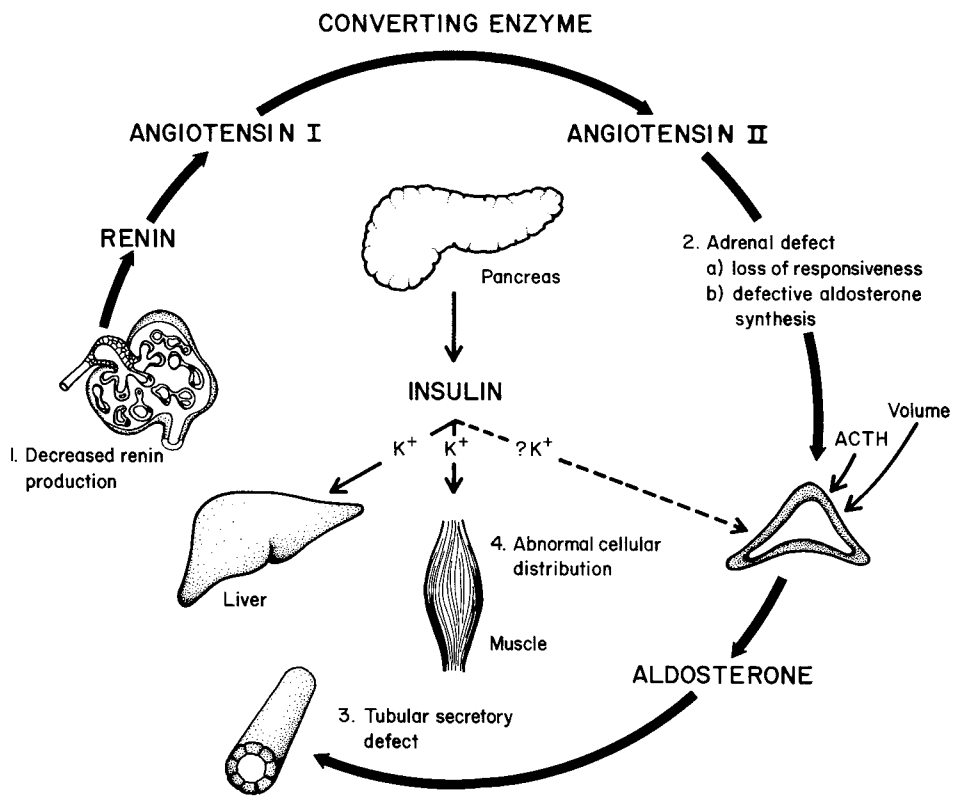

FIGURE 88-6. Schematic representation of potential hormonal, renal, and extrarenal defects resulting in hyperkalemia. Hyperkalemia may result from one of the following conditions: (a) decreased renin production, (b) decreased aldosterone production despite normal renin secretion (adrenal defect), (c) a renal tubular secretory defect, or (d) an abnormal distribution of potassium between intracellular and extracellular fluid compartments. (From: DeFronzo RA, et al. Nonuremic hypokalemia: a possible role for insulin deficiency. *Arch Intern Med* 1979;137:842, with permission.)

the patient with autoantibodies labeled the intercalated cell in rat kidney section. However, the serum from both patients did not react with the affinity-purified bovine H^+ATPase or human H^+ATPase beta subunit (259). This is in contrast to the finding in a single patient with Sjögren's syndrome who had absence of vacuolar H^+ATPase in intercalated cells (260). These findings support the concept that cellular and molecular mechanisms in these patients probably are heterogeneous in nature.

Obstructive Uropathy

Hyperkalemic RTA, as a complication of obstructive uropathy, is common and best described in a report of 13 patients by Batlle et al. (204). Two patterns were noted: (a) Five patients had normal plasma aldosterone levels but failed to increase urinary potassium excretion after administration of acetazolamide, fludrocortisone, and sodium sulfate. The primary defect in this group is renal tubular unresponsiveness to aldosterone. (b) Eight patients had low plasma aldosterone levels but failed to augment renal potassium excretion with mineralocorticoid administration. As noted, this reflects a combined defect in this group. Furthermore, urinary acidification in response to systemic acidosis and sodium sulfate infusion was abnormal in eight of 13 patients. In a rat model of acute ureteral obstruction, no change in the number or tubular distribution of vacuolar H^+ATPase was noted; however, the intracellular distribution was changed with a significant decrease in plasma membrane bound pumps in intercalated cells (261). This finding may explain HMA commonly noted in these patients.

Renal Transplantation

In the precyclosporine era, hyperkalemia was a relatively unusual phenomenon following successful renal transplantation (202,203,262). However, two large series from Australia and the United States (202,203) and a small series from Israel (263) have reported the occurrence of renal tubular hyperkalemia in this group. In the largest series, 23 of 75 patients with a successful kidney transplant had hyperkalemia unrelated to rejection episodes, renal failure, oliguria, or acidosis (202). The renin–angiotensin–aldosterone axis was normal in these patients, and hyperkalemia did not respond to furosemide. The hyperkalemia was transient, disappearing spontaneously, and did not correlate with clinical or laboratory evidence of rejection. In contrast, in two patients studied by Battle et al. (262), hyperkalemia was associated with very low levels of aldosterone, which did not respond to volume contraction. Urinary potassium was low and did not respond to infusion of sodium sulfate or acetazolamide. The etiology of this disorder is not clear, but immunologic damage to the renal tubular cells is postulated (202). In the cyclosporine era, hyperkalemia is more common in kidney transplant recipients (264,265). The role of cyclosporine and tacrolimus (FK-506) in the development of hyperkalemia is discussed in a following section, which outlines drugs that exacerbate hyperkalemia.

Hyperkalemic Renal Tubular Acidosis Associated with Acquired Immunodeficiency Syndrome

Acid–base and electrolyte disturbances, with or without renal failure, are common in patients with AIDS. As reviewed by Perazella and Brown, the incidence varies from 5% to 53% and is owing to a variety of causes including adrenal insufficiency, renal failure, type IV RTA, and finally as a complication of drugs used in these patients (266,267). The syndrome of hyporenin-hypoaldosteronism is relatively uncommon and usually is associated with AIDS nephropathy. Patients with AIDS are exposed to a variety of drugs that could result in hyperkalemia, which is often associated with HCA and/or renal insufficiency. These culprit drugs are discussed further in the section that outlines those medications that most often exacerbate hyperkalemia in patients with impaired potassium handling.

Miscellaneous Conditions

Renal tubular hyperkalemia has been reported in a variety of other renal diseases. These include chronic interstitial nephritis of unknown etiology (268), nephrosclerosis (207), diabetes mellitus (206), postinfectious glomerulonephritis (181,215, 216), lead nephropathy (269), and drug-induced acute interstitial nephritis (270). Although in our experience this entity seems to be relatively common in nonspecific interstitial nephritis, no incidence or prevalence data are available.

Drugs Associated with Hyperkalemia in Patients with Kidney Disease

In patients with underlying kidney disease, prescribed drugs or over the counter medications and supplements play an increasingly dominant role in development of hyperkalemia. It is therefore important to recognize that a variety of products are capable of elevating serum potassium concentration through multiple mechanisms (Table 88-3). Hyperkalemia, depending

TABLE 88-3

COMMON DRUGS THAT CAUSE HYPERKALEMIA AND MECHANISM OF ACTION

Medication	Mechanism of action
Potassium supplement	Increase intake
Salt substitutes	Increase intake
Nutritional/herbal supplements	Increase intake
Beta$_2$-blocking agents	Decrease potassium movement into cells, decrease renin/aldosterone
Digoxin intoxication	Decrease Na^+-K^+ ATPase activity
Lysine, arginine and epsilon-aminocaproic acid	Shift of potassium out of cells
Succinylcholine	Shift of potassium out of cells
Potassium-sparing diuretics	
Spironolactone, eplerenone	Aldosterone antagonism
Triamterene	Block Na^+ channels in principal cells
Amiloride	Block Na^+ channels in principal cells
NSAIDs, COX-2 selective inhibitors	Decrease renin/aldosterone Decrease RBF and GFR
ACE inhibitors and AII receptor antagonists	Decrease aldosterone synthesis Decrease RBF and GFR
Heparin	Decrease aldosterone synthesis
Trimethoprim and pentamidine	Block Na^+ channels in principal cells
Cyclosporine and tacrolimus	Decrease aldosterone synthesis Decrease Na^+-K^+ ATPase activity Decrease K^+ channel activity

NSAIDs, nonsteroidal antiinflammatory drugs; *ACE,* angiotensin converting enzyme; *AII,* angiotensin II; *COX-2*, cyclooxygenase-2.

on the criteria used, has been reported to develop in anywhere from 1.3% to 10% of patients and is often multifactorial. Of the many factors involved, culprit medications, either alone or in association with other disturbances in potassium homeostasis, were a contributing cause of hyperkalemia in 35% to 75% of hospitalized patients (271–275). Of note, kidney disease and older age (>60 years) were important predisposing risk factors in many studies (271–273).

Increased Potassium Input

Enteral and parenteral inputs of potassium are very common causes of hyperkalemia in hospitalized patients. Nonetheless, chronic hyperkalemia does not occur with these products unless an underlying defect in potassium homeostasis also is present. Deliberate potassium intake often lies at the root of hyperkalemia, although unsuspected potassium delivery also occurs. A 3.6% incidence of hyperkalemia among 4,921 patients taking physician-prescribed potassium supplements was documented in the Boston Collaborative Drug Surveillance Program (271). The mean peak K^+ concentration in these patients was 6.0 mEq/L, whereas a level greater than 7.5 mEq/L was noted in 13 of the 179 patients (7.3%). Azotemia and older age were more frequent among those with hyperkalemia (7). In addition, several other studies reveal that potassium supplements cause or contribute to hyperkalemia in 15% to 40% of hospitalized patients (272–275).

Salt substitutes and salt alternatives, recommended for patients with hypertension and edematous disorders, provide yet another rich source of potassium (276,277). Some "no-salt" salt substitutes contain 10 to 13 mEq of potassium per gram (277). A number of nutritional supplements contain as much as 49 to 54 mEq of potassium per liter, whereas foods prepared as "low sodium" contain greater amounts of potassium (because potassium replaces sodium in these foods). As a result, enteral feeds employing these products and some herbal remedies, such as noni juice (K^+, 56.3 mEq/L) can deliver excessive amounts of potassium to patients with impaired potassium homeostasis (278).

Another unsuspected source of potassium excess in the hospital includes the antibiotic penicillin G potassium (1.7 mEq of K^+ per 1 million units), which can cause hyperkalemia if administered in sufficiently high doses (279). The urinary alkalinizing agent potassium citrate (2 mEq of potassium per 1 mL), and packed red blood cells transfused after 10 or more days of storage (7.5 to 13 mEq of K^+ per L) can precipitate hyperkalemia in at risk patients (280,281). Potassium-containing cardioplegia solution employed during cardiac surgery may also cause hyperkalemia in patients with a defect in potassium handling.

Impaired Cellular Potassium Homeostasis

As discussed previously, cellular uptake of a potassium load is the primary mechanism by which the body acutely prevents the development of hyperkalemia. Several commonly prescribed drugs can impair this protective cellular response. β-Adrenergic blocking drugs through inhibition of renin secretion as well as cellular uptake of potassium have been associated with the development of mild and, on rare occasions, life-threatening hyperkalemia (38,45,282,283). Hyperkalemia often develops rapidly, as one would expect with disruption of cellular potassium homeostasis, but rarely develops in the absence of heavy exercise or other risk factors for hyperkalemia (4,284). As an example, three renal transplant recipients developed severe hyperkalemia (K^+ range 6.0 to 8.3 mEq/L) within hours of treatment with intravenous labetalol (285). Most studies evaluating hyperkalemia in hospitalized patients have shown that β-adrenergic blockers have caused or at least contributed to hyperkalemia in anywhere from 4% to 17% of patients (94,275,286–288). Not unexpectedly, the hyperkalemic potential of β-adrenergic blockers is increased by underlying renal insufficiency, the coexistence of diabetes mellitus or hypoaldosteronism, and concurrent therapy with other medications that reduce renal potassium secretion (45,282,283).

Digoxin by blocking Na-K-ATPase pump function has also been demonstrated to disrupt potassium homeostasis (289). As a result of this effect, impaired cellular uptake of potassium as well as reduced renal potassium excretion occurs. In general, therapeutic digoxin levels do not lead to hyperkalemia but in rare circumstances can be a contributing factor (289). Nonetheless, digoxin intoxication will result in hyperkalemia, which at times is fatal (289,290).

Both natural (lysine, arginine) and synthetic (ε-aminocaproic acid) amino acids have been associated with hyperkalemia (291–295). This is owing to the shift of potassium out of cells (291–295). Levinsky et al. demonstrated lysine uptake into isolated rat muscle within 1 hour in an amount equivalent to the potassium lost from the muscle tissue (291). In intact animals, infusion of lysine was associated with hyperkalemia, with a 1.0- to 1.5-mEq/L rise in plasma K^+ concentration noted for every 10-mEq/L increase in plasma lysine concentration (292). Hyperkalemia has also been described with intravenous arginine administration (293–295). In normal humans, serum potassium increased by approximately 1 mEq/L following infusion of 30 to 60 g of arginine, whereas patients with ESRD developed a mean increase in serum K^+ of 1.5 mEq/L at 2 hours after 30 g of intravenous arginine (293,294). In two patients with mild renal insufficiency and liver disease, K^+ concentrations were 7.5 and 7.1 mmol/L, respectively, after infusion of arginine (295). Serum potassium concentrations increased as early as 45 minutes after arginine infusion and peaked between 2 to 6 hours following injection, bespeaking a disturbance in cellular potassium homeostasis (294,295). Hyperkalemia can also develop in subjects treated with the synthetic amino acid, ε-aminocaproic acid, which is structurally similar to both lysine and arginine (296). A study in nephrectomized dogs demonstrated a significant rise in serum K^+ in animals administered intravenous ε-aminocaproic acid as either a constant infusion (2 or 4 g/hour) or a bolus injection of 2.5 g (296). Clinical relevance in humans was demonstrated in a case report where hyperkalemia (potassium 6.7 mEq/L) developed acutely in a patient with chronic renal insufficiency treated with ε-aminocaproic acid (three boluses of 10 g) to reduce perioperative blood loss during cardiac surgery (297). The rapid onset of hyperkalemia following ε-aminocaproic acid therapy in this patient suggested that cellular release of potassium was the cause of this electrolyte disturbance. In addition, Perazella et al. in a retrospective study in patients undergoing cardiac surgery noted higher intraoperative serum potassium concentrations (K^+, 5.9 mEq/L) in 232 patients treated with intravenous ε-aminocaproic acid as compared with 371 well-matched controls (K^+, 5.5 mEq/L) who did not receive this medication (298). Other possible confounding factors did not explain the rapid development of hyperkalemia in these patients. It is therefore likely that intravenous ε-aminocaproic acid causes hyperkalemia through the cellular release of potassium in exchange for this synthetic amino acid.

The anesthetic agent succinylcholine by depolarization of cell membrane can cause hyperkalemia (299–301). A rapid cellular potassium leak induced by these agents, resulting in the abrupt onset of hyperkalemia, has been demonstrated in muscle preparations in intact animals and humans (299–301). Plasma K^+ increased by 0.5 mEq/L within 3 to 5 minutes in patients with normal muscle, whereas increases as high as 3.0 mEq/L occurred in patients afflicted by trauma or nervous system disease (4,300). In 12 patients with renal insufficiency, plasma K^+ concentration rose by 1.2 mEq/L in one patient and up to 0.7 mEq/L in the rest (301).

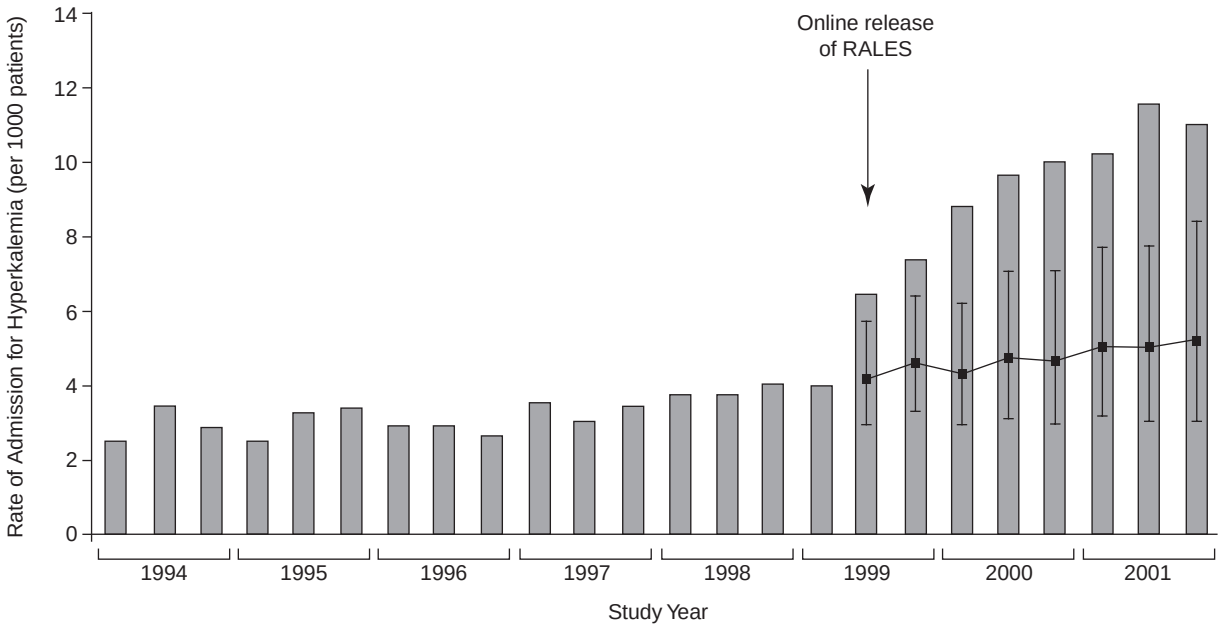

FIGURE 88-7. Rate of hospital admission for hyperkalemia among patients recently hospitalized for heart failure who wee receiving ACE inhibitors. Each bar demonstrates the rate of hospital admission for hyperkalemia per 1,000 patients during one 4-month interval. (From Juurlink DN, et al. Rates of hyperkalemia after publication of the Randomized Aldactone Evaluation Study. *New Engl J Med* 2004;351:543, with permission.)

Impaired Renal Potassium Excretion

Although an increase in K^+ intake can contribute to hyperkalemia, impaired renal excretion almost always plays the dominant role in this process. Potassium-sparing diuretics are used to enhance renal sodium losses and diminish potassium excretion in patients with hypertension and edematous states (302,303). Two basic mechanisms underlie the pharmacologic actions of these diuretics, which act to modulate principal cells residing in the distal nephron (304). The aldosterone antagonists, spironolactone and eplerenone, compete with aldosterone binding to cytoplasmic aldosterone receptors, thereby preventing nuclear uptake of the receptor and blunting aldosterone's effects on the principal cell (305,306). Amiloride and triamterene directly block sodium channel activity in the luminal membrane of the principal cell, effectively inhibiting sodium reabsorption through the epithelium and decreasing the driving force for potassium secretion (307,308). Moderate to severe hyperkalemia has been reported in 4% to 19% of patients treated with these medications (94,287,305–317). In one small study, treatment with the combination of triamterene and hydrochlorothiazide resulted in hyperkalemia in 26% of the patients (308). In a retrospective chart review, five patients were noted to develop severe hyperkalemia (K^+ concentrations in the 9.4 to 11 mEq/L range) within 8 to 18 days of combination therapy with amiloride/hydrochlorothiazide and an ACE inhibitor (311). All of these patients had diabetes and three had underlying CKD. The combination of spironolactone and losartan increased plasma K^+ by 0.8 mEq/L (up to 5.0 mEq/L) and decreased urinary potassium excretion (from 108 to 87 mEq/L) in eight normal subjects studied (313). Hyperkalemia occurred most frequently in patients with preexisting renal insufficiency or diabetes mellitus, and those taking K^+ supplements or another medication that also impairs potassium excretion (310,311,314–317). Several studies have demonstrated a brisk increase in the incidence of hyperkalemia from the use of either spironolactone or eplerenone in patients with heart failure following publication of the Randomized Aldactone Evaluation Study (RALES) and Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS) trials (318,319). For example, the spironolactone prescription rate increased from 34 per 1000 patients in 1994 to 149 per 1,000 patients in 2001 following publication of RALES (319). This was associated with an increase in the rate of hospitalization for hyperkalemia (2.4 per 1,000 patients in 1994; 11.0 per 1,000 patients in 2001) (Fig. 88-7) and mortality (0.3 per 1,000 patients in 1994; 2.0 per 1,000 patients in 2001) in heart failure patients treated with ACE inhibitors (319). In the EPHESUS trial, significant hyperkalemia ($K^+ > 6.0$ mEq/L) developed in 5.5% of treated patients versus 3.9% in placebo-treated patients (320). Hyperkalemia ($K^+ > 6.0$ mEq/L) was most prevalent in patients with impaired kidney function (Creatinine Clearance <50 mL/min) as 10.1% of eplerenone-treated patients developed this complication as compared with 5.9% of placebo-treated patients (320).

Nonsteroidal antiinflammatory drugs (NSAIDs) are widely prescribed for a variety of inflammatory diseases and pain syndromes. Hyperkalemia is one of the many renal complications associated with NSAID therapy and over the counter availability of these agents further increases the risk of drug toxicity (321). Nonsteroidal antiinflammatory drugs disturb potassium homeostasis via inhibition of renal prostaglandin synthesis, especially PGE2 and PGI2 (322). Inhibition of prostaglandin synthesis decreases potassium secretion through (a) lack of activation of renin–angiotensin system, (b) direct inhibition of potassium channels in principal cells, and (c) decreased renal blood flow and diminished delivery of sodium to the distal nephron (231,321–324). Several reports have confirmed the hyperkalemic complication of NSAIDs prescribed to normal subjects, diabetic patients, and patients with underlying renal insufficiency (277,323–328). This is especially problematic in patients with reduced effective renal perfusion such as those with intravascular fluid depletion, congestive heart failure (CHF), and third-spacing of intravascular fluid (321–323). Predictably NSAID-induced hyperkalemia occurs more often in patients with preexisting hyporeninemic hypoaldosteronism,

renal insufficiency, and concomitant therapy with potassium-sparing diuretics and ACE inhibitors (321–327). As with the traditional NSAIDs, the selective COX-2 inhibitors (celecoxib, rofecoxib, valdecoxib) cause hyperkalemia in at risk patients (328). Induction of hyporeninemic hypoaldosteronsim, reduced sodium delivery to the cortical collecting duct, and renal insufficiency are the mechanisms by which these drugs promote hyperkalemia (328).

Angiotensin-converting enzyme inhibitors indirectly reduce renal potassium excretion by inducing a state of hypoaldosteronism (277,329,330). These drugs may additionally impair renal potassium excretion by reducing effective glomerular filtration rate (GFR) in patients with volume depletion, renal artery stenosis, and/or moderate to severe chronic renal insufficiency. In these conditions, ACE inhibitors interfere with angiotensin II production and blunt the postglomerular arteriolar constriction induced by this hormone, thereby lowering effective filtration pressure and glomerular filtration rate. Ultimately, a reduction in distal nephron delivery of sodium and water results, together with decreased aldosterone production, may precipitate hyperkalemia (329). In hospitalized patients, ACE inhibitors have been noted to be the culprit drug in 9% to 38% of patients who developed hyperkalemia (94,287,288,331). In outpatients treated with an ACE inhibitor for 1 year, 10% developed a serum potassium concentration greater than 6.0 mEq/L (332). In this study, patients with renal impairment and age over 70 years were at highest risk. Most studies suggest that the risk of ACE inhibitor-induced hyperkalemia is directly proportional to the existing degree of renal insufficiency (94,287,288,329–331). However, serum potassium concentrations can rise significantly in patients with only modest renal insufficiency (329,330,333). For example, a rise in serum K^+ concentration, a positive cumulative potassium balance, and a reduction in both plasma and urinary aldosterone were demonstrated in 22 of 23 patients treated with high-dose captopril for 10 days despite a creatinine clearance greater than 50 mL/minute (330). In addition, another study noted a fall in aldosterone excretion and a rise in serum K^+ concentration (mean rise 0.8 mEq/L) in 23 of 33 hypertensive patients after 1 week of captopril therapy (329). In this study, all but three of the patients had a creatinine clearance above 60 mL/minute and peak serum K^+ concentration was not predicted by the pretherapy serum creatinine concentration (329). In contrast, Memon et al. demonstrated a significant positive correlation of hyperkalemia with serum creatinine and negative correlation with creatinine clearance, emphasizing the importance of the underlying level of renal function (331). In patients with renal impairment, reducing the dose of ACE inhibitor and initiating a low-potassium diet has been shown to decrease the development of hyperkalemia in a significant percentage of patients (331,333). Unfortunately, as many as one-third of patients still require discontinuation of this medication because of ongoing hyperkalemia (331). Predictably, combination therapy with an ACE inhibitor and other medications capable of altering potassium homeostasis can increase plasma potassium and precipitate hyperkalemia in patients with only modest renal impairment (277,329,330,334–339). As an example, elderly patients on an ACE inhibitor who were hospitalized for hyperkalemia were 27 times more likely to have been prescribed a potassium-sparing diuretic in the week prior to hospital admission (340). Other notable risk factors include hypoaldosteronism and states of effective arterial volume depletion, such as CHF and cirrhosis (277,329,330, 341,342).

Angiotensin-II receptor blockers (ARBs) are a relatively new class of drugs marketed for the treatment of hypertension. Their action to block binding of angiotensin-II (A-II) to its receptor ultimately decreases A-II-driven adrenal synthesis of aldosterone, causing hyperkalemia through the induction of hypoaldosteronemia in a manner similar to ACE inhibitors. Current data are conflicting with regard to the effect of this class of drugs on the development of hyperkalemia. In healthy patients with essential hypertension, the ARB, losartan (100 mg), and the ACE inhibitor, enalapril (20 mg) similarly depressed plasma aldosterone levels (50% decrease) and 24-hour urinary aldosterone excretion (343). The effect of these two drugs on the renin-angiotensin-aldosterone systerm (RAAS) did not include evaluation of serum K^+ concentrations in these patients (343). Data pooled from 16 double-blind clinical trials evaluating the safety of therapy with losartan as compared with ACE inhibitors in healthy patients with hypertension demonstrated no significant difference in the development of hyperkalemia ($K^+ > 5.5$ mEq/L) between the two drug classes (1.3% versus 1.5%) (344). It is important to remember that the patients evaluated in these studies were healthy and at very low risk of developing hyperkalemia (344). Evaluation of the effect of losartan in elderly patients demonstrated a significant rise in serum potassium (>0.5 mEq/L) in 19% of patients, whereas hyperkalemia actually developed in 7% of patients (345). A clinical history of diabetic nephropathy and a serum creatinine greater than 1.3 mg/dL were predictors of a significant increase in serum potassium. Bakris et al. compared the effects of the ACE inhibitor, lisinopril, to the ARB, valsartan, on serum potassium concentration, urinary potassium excretion, and plasma aldosterone in 35 subjects with a mean GFR of approximately 71 mL/minute per 1.73 m^2 (346). After 4 weeks of therapy with lisinopril, serum K^+ increased (0.2 mEq/L), whereas plasma aldosterone and urinary potassium excretion also decreased. In contrast, serum potassium, plasma aldosterone, and urinary potassium excretion were essentially unchanged in the valsartan group (346).

Combination therapy with ACE-inhibitors and ARBs raises concerns that patients may experience an increase in the development of hyperkalemia from more complete blockade of the RAS. The combined decline in GFR and more pronounced suppression of aldosterone synthesis may promote serious hyperkalemia. A multicenter randomized active-controlled parallel group trial studied patients with renal insufficiency (average creatinine clearance 20 to 45 cc/minute) (347). Patients were randomized to either valsartan alone or in combination with benazepril. Dual therapy, however, was associated with a very low risk of hyperkalemia. Serum K^+ concentration rose in each group ranging from 0.28 mEq/L to 0.48 mEq/L. An identical percentage (4.5%) of patients on monotherapy and dual blockade developed a serum K^+ concentration greater than 6.0 mEq/L. Other studies note similar rates of hyperkalemia, although small numbers of patients developed serum K^+ levels greater than 6.0 mEq/L (348). Despite these generally reassuring data, a risk remains for the development of hyperkalemia when these drugs are used alone or in combination. Clinicians should therefore monitor follow-up serum K^+ levels within 1 to 2 weeks once therapy has been initiated.

Trimethoprim and pentamidine are antimicrobial agents employed to treat infections in both HIV-infected patients as well as other hosts. Hyperkalemia evolves through a reduction in renal potassium secretion, the result of competitive inhibition of sodium transport channels in the luminal membranes of the distal nephron by these drugs (349). Blockade of epithelial sodium channel transport indirectly inhibits potassium secretion (Fig. 88-8) (349), because potassium movement into the distal nephron lumen is electrogenically linked to the movement of sodium out of the lumen (303,304,349). This action is identical to that exhibited by amiloride, which has a molecular structure very similar to both trimethoprim and pentamidine (348). Hyperkalemia was first described in a patient treated with "high-dose" trimethoprim (20 mg/kg per day) for *Pneumocystis carinii* pneumonia (350). Subsequently, a 50% incidence of mild hyperkalemia ($K^+ > 5.0$ mEq/L) and 10% to 12% incidence of severe hyperkalemia ($K^+ > 6.0$ mEq/L) were

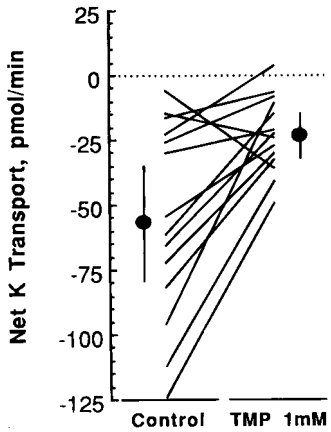

FIGURE 88-8. Net potassium transport during perfusion of 14 distal tubules with control and trimethoprim (TMP) solutions. Lines connect measurements in the same tubules. Black circles and vertical lines indicate means and confidence intervals. Positive values indicate absorption; negative values indicate secretion. (From: Velazquez H, et al. Renal mechanism of trimethoprim-induced hyperkalemia. *Ann Intern Med* 1993;119:296, with permission.)

observed in HIV-infected patients receiving high-dose trimethoprim (349). Shortly thereafter, 21% of hospitalized non-HIV patients treated with standard dose trimethoprim (360 mg/day) developed hyperkalemia ($K^+ > 5.5$ mEq/L) (351). Mild renal impairment (serum creatinine ≥ 1.2 mg/dL) was significantly associated with the development of a higher serum potassium concentration (351). More recently, a prospective, randomized controlled study in healthy outpatients treated with standard-dose trimethoprim revealed that 18% (9/51) and 6% (3/51) of trimethoprim-treated patients developed serum K^+ concentrations greater than 5.0 and 5.5 mEq/L, respectively (352). Older age, diabetes mellitus, and higher serum creatinine level appeared to predispose to more severe hyperkalemia. Additionally, therapy with pentamidine also has been complicated by hyperkalemia (353). A retrospective study in 32 patients with AIDS noted a significant increase in mean serum K^+ from 4.2 to 4.7 mEq/L, with 24% of the patients developing severe hyperkalemia (353). All cases of hyperkalemia were associated with renal insufficiency, providing an underlying risk factor in these patients.

Heparin and its congeners have been shown to inhibit adrenal aldosterone production and precipitate hyperkalemia in approximately 8% of patients treated with at least 10,000 U per day (354). This drug reduces both the number and affinity of angiotensin II receptors in the adrenal zona glomerulosa, thus decreasing the principal stimulus for aldosterone synthesis (354). Heparin also directly inhibits the final enzymatic steps of aldosterone formation (18-hydroxylation) and promotes atrophy of the zona glomerulosa in rats following prolonged administration, further reducing aldosterone production (354). Finally, excess anticoagulation with heparin may rarely precipitate adrenal hemorrhage and induce frank adrenal insufficiency (354). Although heparin-associated hyperkalemia has been reported in normal subjects, patients with preexisting hypoaldosteronism, kidney disease, or diabetes mellitus, and patients treated with other medications that disrupt K^+ homeostasis more commonly develop hyperkalemia (354).

Cyclosporine and tacrolimus (FK506) have been associated with the development of hyperkalemia in organ transplant recipients. In the precyclosporine era, 31% (23/75) of renal transplant patients were noted to develop transient hyperkalemia because of an underlying disturbance in potassium excretion (355). Not unexpectedly, therapy with cyclosporine and tacrolimus increases risk of this disorder in these patients (356). Heering and Grabensee (264) documented the presence of incomplete RTA in eight of 35 recipients on cyclosporine compared with none of the 15 on azathioprine. Four of the former group also had HHA syndrome. In a detailed study of 12 cadaveric recipients with hyperkalemia on cyclosporine, Kamel et al. (265) documented the presence of low urinary potassium excretion that did not respond to 0.2 mg of fludrocortisone. Renal K^+excretion, however, responded to bicarbonaturia initiated by acetazolamide, suggesting a defect in generating a favorable electrochemical gradient in the distal tubule, leading to hyperkalemia and varying degrees of hyperchloremic acidosis. Recently, Yu et al. demonstrated higher serum potassium concentrations and lower transtubular potassium gradients (TTKGs) in 35 renal transplant recipients receiving cyclosporine as compared with matched normal controls, supporting a disturbance in renal potassium excretion (357). Tacrolimus has similarly caused hyperkalemia in solid organ transplant patients. Hyperkalemia was noted in 26 of 49 (53%) pediatric heart transplant recipients treated with tacrolimus (358). Of note, the majority of subjects who developed hyperkalemia had impaired renal function. The reduction in renal potassium excretion that occurs with these two drugs is likely owing to a dose-dependent decrease in the activity of the basolateral Na-K-ATPase pumps in principal cells in the distal nephron (359,360). Calcineurin, which modulates sodium pump function through its regulation of phosphatase activity, is inhibited by both cyclosporine and tacrolimus (360). *In vitro* inhibition of calcineurin by these two drugs has been shown to decrease Na-K-ATPase pump activity and probably explains the observed reduction in renal potassium excretion. Ling and Eaton have also demonstrated the inhibition of apical secretory potassium channels by cyclosporine, providing yet another possible mechanism of decreased renal potassium excretion and hyperkalemia (361). Cyclosporine also impairs cellular potassium homeostasis and cause transient hyperkalemia by acutely increasing potassium efflux from cells (362). Although the mechanism is currently unknown, cyclosporine may cause hyperkalemia through the impairment of Na-K-ATPase pumps in muscle and liver cell membranes.

Acute Treatment of Serious Hyperkalemia

Severe hyperkalemia is a potentially life-threatening disorder because of its toxic effect on cardiac and other excitable neuromuscular tissues. Importantly, patients with underlying renal disease and disturbances in potassium homeostasis can develop serious hyperkalemia. It is therefore imperative that this electrolyte disturbance is rapidly recognized and aggressively treated. Symptoms of hyperkalemia are sometimes impressive and quite obvious; however, serious hyperkalemia also may present with only very subtle symptoms or signs. Rarely, patients may have absolutely no clinical evidence of this disorder, the presence of renal impairment or other disturbances in potassium homeostasis providing the only clues to hyperkalemia. Nonspecific muscle weakness and generalized malaise are common, but severe muscle weakness, paresthesias, and ascending paralysis may rarely be seen in these patients with extreme elevations in serum potassium levels (363). The cardiac toxicity of hyperkalemia may manifest as weakness or dizziness from arrhythmias that induce hypotension and cerebral hypoperfusion (363). Cardiac monitoring or a 12-lead electrocardiogram (ECG) may reveal a rhythm suspicious of hyperkalemia. These include tenting of the T waves (K^+, 5.5 to 6.0 mEq/L), lengthening of the P-R interval and widening of the QRS complex (K^+, 6.0 to 7.0 mEq/L), disappearance of the P waves (K^+, 7.0 to 7.5 mEq/L), and finally the sine wave pattern (K^+, 8.0 mEq/L or greater). These ECG changes may occur at different concentrations (higher or lower) of potassium,

TABLE 88-4

ACUTE TREATMENT OF SERIOUS HYPERKALEMIA

Stabilize excitable tissues (cardiac and neuromuscular)
Calcium gluconate (10% solution) given as a 10-mL to 20-mL intravenous bolus. Calcium chloride (10% solution) given as a 5-mL intravenous bolus. Each may be repeated every 5 minutes, if the ECG appearance does not improve. Calcium gluconate should be mixed in 100 mL of 5% and infused over 10 to 20 minutes if the patient has been treated with digoxin.
Shift potassium into cells
Regular insulin, 10 to 20 units plus 50% dextrose (50 mL), given as an intravenous bolus, followed by 10% dextrose at 50 mL/minutes until definitive therapy. Check glucose levels at 1-hour to 2-hour intervals. Albuterol (5 mg/mL), 10 to 20 mg, nebulized over approximately 10 minutes. Combination therapy of insulin/dextrose and nebulized albuterol.
Remove potassium from the body
Acute hemodialysis (low potassium dialysate) to remove potassium in patients with severe renal insufficiency. Sodium polystyrene sulfonate (15 to 30 g) plus sorbital (15 to 30 mL), oral ingestion or rectal administration (without sorbital).

depending on underlying heart disease and acuity of hyperkalemia (363). The presence of hypocalcemia, hypomagnesemia, and hyponatremia potentiate the toxic effects of hyperkalemia on the cardiac conduction system and potassium concentrations in the 6.0 to 6.5 mEq/L range can precipitate life-threatening arrhythmias (363). Additionally, patients with underlying cardiac disease may deteriorate directly to a ventricular arrhythmia in the absence of other ECG changes (363).

Once the clinician judges that hyperkalemia warrants treatment (plasma $K^+ > 6.0$ to 6.5 mEq/L, clinical manifestations, or ECG changes), immediate therapy should be commenced. Stabilization of excitable cell membranes, in particular cardiac tissue, is the most urgent priority in the treatment of hyperkalemia. Intravenous calcium, as either calcium gluconate (10% solution, calcium ion at 3 mEq/mL) or calcium chloride (10% solution, calcium ion at 13 mEq/mL), is the treatment of first choice and should be administered in a monitored setting (Table 88-4). Calcium acts within 1 to 3 minutes, and the effect persists for approximately one-half hour (363). If no effect is noted within 5 minutes following the first dose, repeated administration may provide benefit. Patients who have been treated with digoxin should receive a slower infusion of calcium (calcium mixed in 100 mL of 5% dextrose) over 10 to 20 minutes (363).

Intravenous administration of regular insulin as a 10-unit bolus followed by 50 mL of intravenous 50% dextrose (Table 88-4) should be the next therapeutic choice (364,365). Twenty units of intravenous insulin may promote an even greater reduction in plasma K^+ (366). The beneficial effect of insulin is observed within 15 minutes and lasts approximately 3 to 6 hours (364–366). Dextrose is given to prevent hypoglycemia in nondiabetic patients. However, because a high incidence of hypoglycemia occurs even with this regimen, it is prudent to monitor blood glucose levels and redose dextrose based on levels (364–366). Dextrose should not be infused before insulin because an acute worsening of hyperkalemia can occur with hyperglycemia, through a shift of potassium out of cells (364–366). Glucose levels should be checked prior to administration of dextrose to diabetic patients (364–366).

High-dose nebulized albuterol (10 to 20 mg), which is fourfold to eightfold higher than used to treat asthma, also effectively lowers potassium concentrations in patients with hyperkalemia (Table 88-4) (367). However, the potassium-lowering effect of albuterol is less reliable in ESRD patients, and as many as 40% of these patients are resistant to the potassium-lowering effect of this β agonist (367). In general, the plasma potassium concentration declines significantly at 30 minutes following albuterol inhalation and remains depressed for approximately 2 hours (367). To date, no adverse cardiovascular effects from albuterol have been documented in ESRD patients (367). Therefore, nebulized albuterol is useful to acutely lower plasma potassium concentration in most hyperkalemic patients; however, it should not replace insulin as the most important therapy to move potassium into cells.

Combined therapy with intravenous insulin and nebulized albuterol has been shown to be additive in the reduction of plasma K^+ concentrations (367). Plasma K^+ decreases approximately 0.6 mEq/L with 10 units of insulin, whereas 20 mg of nebulized albuterol lowers plasma K^+ to a similar degree (367); however, the combination of these agents lowers plasma K^+ by approximately 1.2 mEq/L (274). As a result it is worthwhile to combine these two agents to treat severe hyperkalemia (Table 88-4). Combined therapy with sodium bicarbonate and insulin reduced plasma K^+ more effectively, whereas sodium bicarbonate plus nebulized albuterol was no better than monotherapy (368).

Although sodium bicarbonate is listed as a useful treatment for hyperkalemia, critical evaluation of the literature suggests that this agent is ineffective as an isolated therapy to acutely lower plasma potassium (368,369). In studies where bicarbonate infusion successfully lowered plasma potassium concentrations in ESRD patients, the effect was not observed until at least 4 hours after treatment. Similarly, other studies have confirmed the utility of sodium bicarbonate therapy in the chronic (not acute) lowering of plasma K^+ concentrations (368,369). In contrast, patients with severe metabolic acidosis and concurrent hyperkalemia should receive bicarbonate to correct pH and stabilize cardiac tissue. In this setting, sodium bicarbonate (50 mEq) may be given intravenously to correct pH and serum bicarbonate levels in patients who are normocalcemic and can tolerate the sodium load (368,369).

WORKUP AND MANAGEMENT OF CHRONIC HYPERKALEMIC RENAL TUBULAR ACIDOSIS

Although acute hyperkalemia with or without significant HCA requiring immediate treatment occurs in patients with impaired potassium handling, the major challenge is the workup and treatment of chronic hyperkalemia seen in this setting. Given the frequency of this syndrome and lack of individualized treatment for specific subgroups, most patients can be adequately

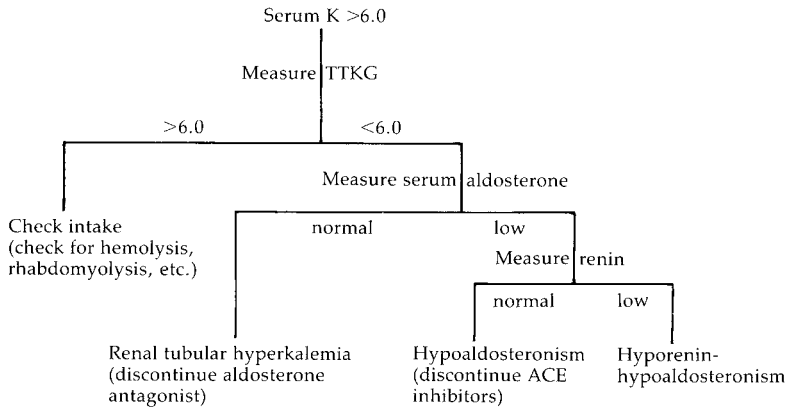

FIGURE 88-9. Pathophysiologic approach to chronic hyperkalemia. *ACE,* angiotensin converting enzyme; *TTKG,* transtubular potassium gradient.

managed without complex workups. However, in certain patients it may be important to make a more specific pathophysiologic diagnosis. Although HCA is the dominant finding in some patients, hyperkalemia is the prominent presentation requiring workup and treatment.

Figure 88-9 summarizes a simple pathophysiologic approach to chronic hyperkalemia in these patients. The first question to be answered is, "Is the hyperkalemia owing to an increase in intake or a decrease in output?" Although dietary history and pertinent clinical data may be helpful, a specific laboratory test that would answer this question could simplify the workup. Urinary potassium concentration and the urinary to serum potassium ratio do not account for the variability in the urinary potassium concentration as a function of water reabsorption in the collecting duct. The fractional excretion of potassium ($FE_K{}^+$) normalizes potassium excretion for GFR; however, as potassium is primarily secreted (and therefore, less dependent on filtration), its clinical utility is questionable.

Halperin et al. (81,370–372) have suggested correcting the urinary (U_K) to serum potassium (S_K) concentration by the ratio of urine (U_{Osm}) to serum osmolality (S_{Osm}) to normalize the data for water reabsorption. This ratio ($U_K{}^+/S_K{}^+ X\ S_{Osm}U_{Osm}$), called the *transtubular potassium gradient* (TTKG), attempts to approximate the gradient across potassium-secreting cells in the distal nephron. Despite several pitfalls (urine more diluted than the plasma or very low urinary sodium), a value less than 6 in patients with hyperkalemia suggests a lack of aldosterone or response to aldosterone; a value above 6 is in favor of an increase in potassium intake, with or without renal abnormality in potassium handling. It should, however, be noted that the published clinical experience with the use of TTKG is still very limited and therefore the values given here may be modified as further information becomes available. If the TTKG is normal, one should search for excessive potassium intake, either externally (e.g., potassium supplements or salt substitutes) or internally (e.g., severe hemolysis, rhabdomyolysis, or acidosis). In general, given the renal ability to handle a large oral potassium load (e.g., serum potassium rising by less than 1.0 mEq/L on a 400-mEq diet), a significant increase in serum potassium is indicative of either a major internal shift of potassium or a decrease in urinary excretion output. If the TTKG is low in the face of hyperkalemia, the aldosterone level should be measured to separate the group with tubular unresponsiveness from that with low aldosterone. Patients also can be challenged with exogenous mineralocorticoids (0.05 mg of fludrocortisone). If the TTKG increases to 7 or above, hypoaldosteronism is probably the major factor in the development of hyperkalemia (372). The role of renin–angiotensin in patients with hypoaldosteronism can be evaluated by measuring the renin level. A low renin associated with low aldosterone is the hallmark of the most common subgroup (i.e., hyporenin-hypoaldosteronism). If the renin level is normal, then either generation of angiotensin II is abnormal (e.g., in patients on ACE inhibitors) or the synthesis and secretion of aldosterone are abnormal. The adrenal response to angiotensin II infusion would provide appropriate answers to this question.

In practice, this type of workup should be reserved for unusual patients who do not represent the commonly recognized groups with this syndrome (e.g., diabetic, hypertensive patients), or as part of a research protocol. In addition, it should be noted that this approach does not lead to an etiologic diagnosis, but only a pathophysiologic one. The etiologic diagnosis (as discussed elsewhere in this chapter) should depend on other diagnostic evaluations.

Some patients with type IV RTA present primarily with HCA. In these patients, the diagnostic workup should focus on the pathogenesis and etiology of this abnormality. The major defect leading to HCA is either loss of bicarbonate, often through the gastrointestinal tract, or a decrease in regeneration of bicarbonate by the kidney through stimulation of ammoniogenesis. Urinary ammonium should be high in the former and low in the latter group. However, urinary ammonium is not commonly measured in clinical laboratories. Clinicians are forced to rely on measurements of surrogates for urinary ammonium excretion. The most commonly used surrogate is urinary anion gap, which is the difference between major urinary cations (Na+K) and urinary anions ($Cl+HCO_3$). As the amount of bicarbonate is very small in acid urine (urine pH <6.5), the difference between urinary Na^+, K^+ and Cl^- reflects the major missing ion (i.e., ammonium). Using this formula, one can demonstrate an inverse relationship between the urinary anion gap and the amount of ammonium in the urine (373,374) (Fig. 88-10). In the presence of extrarenal acidosis, the urinary ammonium excretion should increase severalfold, resulting in a very negative anion gap value. In contrast, in distal RTA, the urinary ammonium will remain low, resulting in a positive anion gap. The amount of ammonium in the urine also can be deduced from a modified urinary osmolar gap using the following formula:

$$\text{urinary ammonium} = 1/2(\text{urine osmolality} - 2(\text{Na} + \text{K}) + \text{urea nitrogen}/2.8 + \text{glucose}/18) \quad [1]$$

This is based on the concept that $NH_4{}^+$ with its accompanying anion is the major missing osmole accounting for the osmolar gap (375). It should be noted that neither calculation predicts the exact amount of ammonium in the urine but rather provides a qualitative estimate of it. This is still helpful if utilized to answer the appropriate question in a patient with HCA.

The major utility of urinary anion or osmolar gap is to differentiate renal from extrarenal causes of hyperchloremic acidosis such as diarrhea or ingestion of hydrochloric acid or its

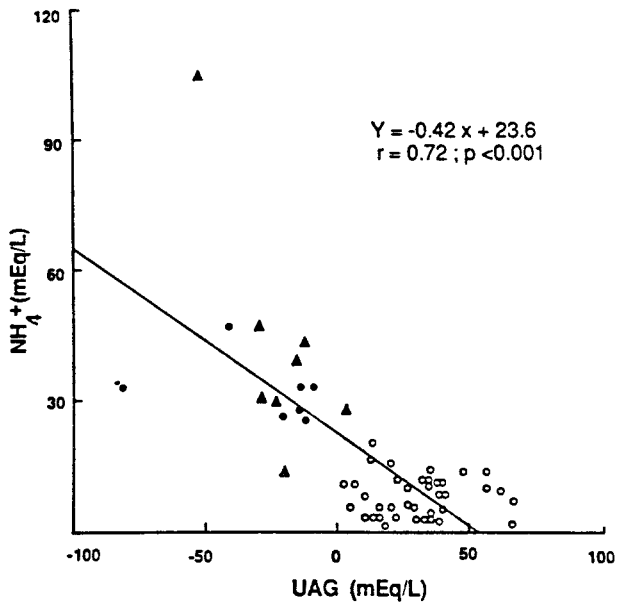

FIGURE 88-10. Urinary ammonium (NH_4^+) in relation to the urinary anion gap (UAG). The 38 patients with altered distal urinary acidification are represented by open circles; the seven normal subjects receiving ammonium chloride, by closed circles; and the eight patients with hyperchloremic metabolic acidosis associated with diarrhea, by triangles. (From: Battle DC, et al. The use of urinary anion gap in the diagnosis of hyperchloremic metabolic acidosis. *N Engl J Med* 1988;318:594, with permission.)

equivalent where the gap is negative. However, a low or negative anion gap in itself does not establish the diagnosis of type IV RTA, as this is also seen in classic RTA as well as uremic acidosis. Batlle et al. (374) studied a group of patients with classic RTA, hyperkalemic RTA, and selective aldosterone deficiency and compared the results to controls with a serum pH 7.30 to 7.35. These investigators noted a urinary anion gap of -20 ± 5.7 in controls and $+23 \pm 4.1$, $+30 \pm 4.2$, and $+39 \pm 4.2$ mEq/L in patients, respectively. The major pitfall in using urinary anion gap is the presence of a significant amount of bicarbonate or an unexpected charged molecule, such as penicillin or ketoacids, in the urine. In summary, urinary anion gap is a physiologic concept that indirectly assesses the amount of urinary ammonium. This measurement in conjunction with other data is helpful in establishing the pathogenesis of HCA in selected patients (376).

In patients with hyperkalemic RTA, treatment of chronic hyperkalemia should be instituted only when absolutely necessary (i.e., when clinical signs of hyperkalemia are present or plasma K^+ is over 6.0 mEq/L). If therapy is deemed necessary, simple modalities should be tried first before more complex therapies with their associated side effects are instituted.

Discontinuation of Drugs that Cause Hyperkalemia

As these patients have an intrinsic difficulty in the excretion of potassium, any drugs that can cause hyperkalemia should be immediately discontinued. The list of drugs that should be stopped includes those discussed in the previous section.

Dietary Intervention

The next step in patients with mild to moderate hyperkalemia is to decrease K^+ intake to less than 60 mEq/day. This can be done by the elimination of potassium-rich foods. This may be difficult if the patient is on a low-sodium diet because such a diet, by definition, contains foods that are high in potassium content.

Treatment of Acidemia

Because HCA is commonly associated with hyperkalemia, correction of the acidosis by sodium bicarbonate decreases the serum potassium concentration. The effect of bicarbonate is partly related to a change in H^+ concentration and is partly independent of pH change. As acidemia is corrected, H^+ moves out of cells in exchange for potassium. The inhibitory effect of acidemia on renal K^+ secretion also is removed. In addition, sodium bicarbonate, through volume expansion and delivery of both sodium and bicarbonate to the distal potassium exchange site, may also increase renal excretion of potassium.

In some patients with significant metabolic acidosis (HCO_3 <16 mEq/L and/or pH <7.30), it is important to treat acidosis with base replacement to prevent mobilization of bone calcium and protein catabolism. Bone provides a buffer sink for hydrogen ion, resulting in release of calcium and its loss in the urine (151,377). This phenomenon is independent of Vitamin D, parathyroid hormone, and calcitonin (378,379). In addition, there is increasing evidence for a catabolic role for metabolic acidosis independent of uremia in patients with chronic renal failure (380). Both effects can be reversed by alkali therapy. The bicarbonate needed in these patients is close to 0.5 to 0.75 mEq/kg per day and can be easily supplied as citric acid-sodium citrate (Shohl's) solution, which contains 1 mEq of bicarbonate equivalent per milliliter.

Volume Expansion

Volume expansion may enhance potassium excretion by increasing distal fluid and sodium delivery. This therapy is especially effective in patients with chronic volume depletion owing to mild sodium wastage.

Diuretic Therapy

Use of most diuretics, especially loop blockers and thiazides, results in hypokalemic, hypochloremic metabolic alkalosis. In patients with hyperkalemia, the previously mentioned side effects may ameliorate hyperkalemia and, when present, metabolic acidosis. To prevent volume depletion with its resultant decrease in distal tubular sodium and fluid delivery, a high salt intake can be added to the diuretic regimen. Thiazide diuretics have proved effective in some patients with renal tubular hyperkalemia despite the failure of loop blockers such as furosemide.

Mineralocorticoids

Mineralocorticoid replacement represents the most logical approach to therapy in these patients. DeFronzo (197) reported an 84% success rate with this therapy; however, the effective dose of fludrocortisone (up to 0.4 to 1.0 mg per day) was much higher than the true physiologic dose. This observation suggests that most of these patients possess some degree of tubular resistance to the potassium stimulatory effect of mineralocorticoids. Surprisingly, although such high doses were needed to augment renal potassium excretion and normalize serum potassium levels, the sodium-retaining effects of aldosterone remained intact in some patients, resulting in marked edema formation,

hypertension, and congestive heart failure. In general, if the dose of fludrocortisone required to maintain normokalemia exceeds 0.2 mg per day, side effects are common, and these drugs probably should be combined with diuretics or not employed at all. Use of mineralocorticoids should be limited to patients who have not responded to other maneuvers and continue to have clinically significant hyperkalemia.

Sodium-Potassium Exchange Resins

Sodium-potassium exchange resins are quite effective in reducing the plasma potassium concentration but have a low patient acceptability. This therapy should be used orally with an osmotic cathartic. The dose should be titrated against the decline in serum potassium level.

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CHAPTER 89 ■ DISORDERS OF PHOSPHORUS, CALCIUM, AND MAGNESIUM METABOLISM

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PHOSPHORUS

Phosphorus is a common anion ubiquitously distributed throughout the body. Approximately 80% to 85% of the phosphorus is present in the skeleton. The rest is widely distributed in the form of organic phosphate compounds that play fundamental roles in several aspects of cellular metabolism. The energy required for many cellular reactions including biosynthesis derives from hydrolysis of adenosine triphosphate (ATP). Organic phosphates are important components of cell membrane phospholipids. Changes in serum phosphorus influence the dissociation of oxygen from hemoglobin through regulation of 2,3-diphosphoglycerate concentrations. The concentration of phosphorus influences the activity of several metabolic pathways such as ammoniagenesis, glycolysis, gluconeogenesis, parathyroid hormone (PTH) secretion, and phosphate reabsorption, as well as the formation of 1,25-dihydroxycholecalciferol [$1,25(OH)_2D_3$] from 25-hydroxycholecalciferol [$25(OH)D_3$]. In the extracellular fluid (ECF), phosphorus is present predominantly in the inorganic form (P_i). The physiologic concentration of serum phosphorus ranges from 2.5 to 4.5 mg/dL (0.9 to 1.45 mmol/L) in adults (1). In serum, phosphorus exists mainly as the free ion, and only a small fraction (less than 15%) is protein bound (2,3). There is a diurnal variation in serum phosphorus of 0.6 to 1.0 mg/dL, with the nadir occurring between 8 AM and 11 AM. Ingestion of meals rich in carbohydrates decreases serum phosphorus concentrations as a result of the movement of phosphorus from the extracellular to the intracellular space.

Gastrointestinal Absorption of Phosphorus

Approximately 1 g of phosphorus is ingested daily in an average diet in the United States. About 300 mg is excreted in the stool, and 700 mg is absorbed (Fig. 89-1). Most of the phosphorus is absorbed in the duodenum and jejunum with minimal absorption occurring in the ileum (4). Phosphorus transport in proximal segments of the small intestine appears to involve both passive and active components and to be under the influence of vitamin D. The movement of phosphorus from the intestinal lumen to the blood requires (a) transport across the luminal brush-border membrane of the intestine; (b) transport through the cytoplasm; and (c) transport across the basolateral plasma membrane of the epithelium. The rate-limiting step and the main driving force of absorption is the luminal membrane step (1).

Luminal Membrane Transport

The mechanism of transport across the intestinal brush border epithelial membrane involves a sodium–phosphate (NaPi) cotransport system, as suggested by Berner et al. (5) and confirmed by the identification of the responsible molecular mechanism, a type IIb sodium–phosphate (NaPi) cotransporter, NaPi-IIb (6). The NaPi cotransporters are a secondary active form of ion transport using the energy of the Na gradient from outside to inside the cell to move phosphate ion uphill against an electrochemical gradient (Fig. 89-2).

The intestinal NaPi-IIb transporter is upregulated by a low phosphate diet (7,8). 1,25-dihydroxyvitamin D3 is also an important regulator of intestinal phosphate transport (7–12). Interestingly, a low phosphate diet results in upregulation of 1,25-dihydroxyvitamin D3; however, a recent study in vitamin D–receptor (VDR) null mice has indicated that the intestinal NaPi cotransport adaptation to a low phosphate diet occurs independent of vitamin D (13).

Intestinal NaPi cotransport activity and NaPi-IIb protein is also regulated by several other factors including the aging process (14), glucocorticoids (15) and epidermal growth factor (EGF) (16) that causes decreased intestinal NaPi transport, and estrogen (17) and metabolic acidosis (18) that causes increased intestinal NaPi transport.

Studies of phosphorus accumulation by rat intestinal brush-border vesicles have demonstrated that at physiologic pH levels, phosphorus uptake is dependent on luminal sodium, and that it is affected by the transmembrane potential, indicating that like the renal type IIa cotransporter, NaPi-2a, the intestinal type IIb cotransporter, NaPi-2b, is electrogenic (6). The $K_m(P_i)$ of NaPi-2b is approximately 50 μm, a finding that is consistent with the renal transport protein. In contrast to the renal NaPi-2a isoform, the intestinal NaPi-2b cotransporter is less dependent on the pH level and is slightly more active at acid pH values.

Transcellular Movement of Phosphorus

The second component of transcellular intestinal phosphorus transport involves the movement of phosphorus from the luminal to the basolateral membrane. Although little is known about the cellular events that mediate this transcellular process, evidence suggests a role for the microtubular microfilament system of intestinal cells (4). Microfilaments in the cell may be important in conveying phosphorus from the brush-border membrane to the basolateral membrane and may be involved in the extrusion of phosphorus at the basolateral membrane from the epithelial cell.

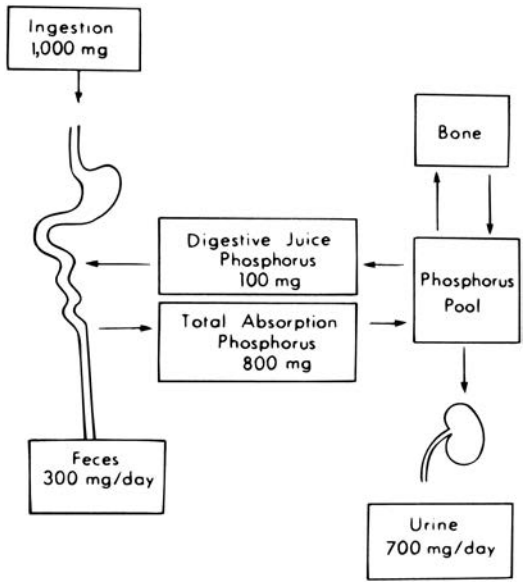

FIGURE 89-1. Summary of phosphorus metabolism in humans. Approximately 1 g of phosphorus is ingested daily, of which 300 mg is excreted in the stool and 700 mg in the urine. The gastrointestinal tract, bone, and kidney are the major organs involved in phosphorus homeostasis.

Phosphate Exit at Basolateral Membrane

Little is known about the mechanisms of phosphorus extrusion at the basolateral membrane of intestinal epithelial cells. The electrochemical gradient for phosphorus favors movement from the intracellular to the extracellular compartment because the interior of the cell is electrically negative compared with the basolateral external surface (10). Thus, it has been presumed that the exit of phosphorus across the basolateral membrane represents a mode of passive transport (19).

Renal Reabsorption of Phosphorus

Most of the inorganic phosphorus in serum (90% to 95%) is ultrafilterable at the level of the glomerulus. At physiologic levels of serum phosphorus, approximately 7 g of phosphorus is filtered daily by the kidney, of which 80% to 90% is reabsorbed by the renal tubules and the remainder is excreted in the urine (approximately 700 mg on a 1-g phosphorus diet) equal to interstial absorption (20). Thus, at steady-state, adults are in a state of balance between intake and excretion of phosphorus (Fig. 89-1). Micropuncture studies have demonstrated that 60% to 70% of the filtered phosphorus is reabsorbed in the proximal tubule. However, there is also evidence that a significant amount of filtered phosphorus is reabsorbed in distal segments of the nephron (21). When serum phosphorus levels increase and the filtered load of phosphorus increases, the capacity to reabsorb phosphorus also increases. However, a maximum rate of transport (Tm) for phosphorus reabsorption is obtained usually at serum phosphorus concentrations of 6 mg/dL. There is a direct correlation between Tm phosphorus values and glomerular filtration rate (GFR) even when the GFR is varied over a broad range. Micropuncture studies suggest two different mechanisms responsible for phosphorus reabsorption in the proximal tubule. In the first third of the proximal tubule, in which only 10% to 15% of the filtered sodium and fluid is reabsorbed, the ratio of tubular fluid (TF) phosphorus to plasma ultrafilterable (UF) phosphorus falls to values of approximately 0.6. This indicates that the first third of the proximal tubule accounts for approximately 50% of the total amount of phosphorus reabsorbed in this segment of the nephron. In the last two-thirds of the proximal tubule, the reabsorption of phosphorus parallels the movement of salt and water. In the remaining 70% of the pars convoluta, the TF: UF phosphorous ratio remains at a value of 0.6 to 0.7, whereas fluid reabsorption increases to approximately 60% to 70% of the filtered load. Thus, in the last two-thirds of proximal tubule, the TF: UF phosphorus reabsorption ratio is directly proportional to sodium and fluid reabsorption. A significant amount of phosphorus, perhaps on the order of 20% to 30%, is reabsorbed beyond the portion of the proximal tubule that is accessible to micropuncture. There is little phosphorus transport within the loop of Henle, with most transport distal to micropuncture accessibility occurring in the distal convoluted tubule. In this location, Pastoriza-Munoz et al. (21) found that approximately 15% of filtered phosphorus is reabsorbed under baseline conditions in animals subjected to parathyroidectomy, but that the value falls to about 6% after administration of large doses of PTH. The collecting duct is a potential site for distal nephron reabsorption of phosphorus (22–24). Transport in this nephron segment may explain the discrepancy between the amount of phosphorus delivered to the late distal tubule in micropuncture studies and the considerably smaller amount of phosphorus that appears in the final urine of the same kidney. Phosphorus transport in the cortical collecting tubule is independent of regulation by PTH. This is in agreement with the absence of PTH-dependent adenylate cyclase in the cortical collecting tubule (24).

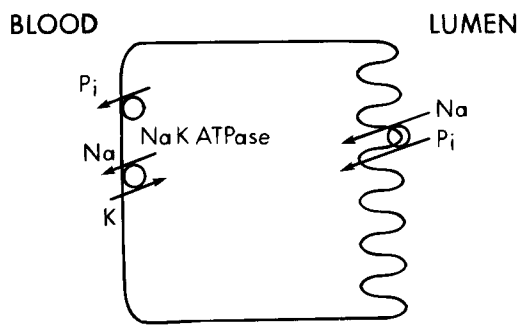

FIGURE 89-2. The apical membrane Na^+–inorganic phosphate (P_i) cotransport proteins utilize the electrochemical driving force for Na^+ to move P_i into the cell. The electrochemical Na^+ gradient is maintained by active Na^+ extrusion across the basolateral membrane through the action of Na^+-K^+-ATPase.

Comparison of Superficial and Deep Nephron Transport

The contribution of superficial nephrons and deep nephrons of the kidney to phosphorus homeostasis differs. Nephron heterogeneity in phosphorus handling has been evaluated under a number of conditions by puncture of the papillary tip and the superficial early distal tubule, with the recorded fractional delivery representing deep and superficial nephron function, respectively. Using this technique, Haramati et al. (25,26) demonstrated that in thyroparathyroidectomized (TPTX) rats fed a normal phosphorus diet, phosphorus reabsorption was greater in deep nephrons than in superficial nephrons, and this heterogeneous handling of phosphorus can be mitigated by both PTH infusion (26) and a low-phosphorus diet (25). Studies in TPTX rats fed a normal phosphorus diet have

presented *in vivo* measurements of maximum tubular reabsorptive capacities for phosphorus of deep and superficial nephrons. Deep nephron values (5.05 pmol/mL/minute) were significantly greater than the values obtained in superficial nephrons (3.38 pmol/mL/minute). These results suggest that the juxtamedullary nephrons are more responsive to body phosphorus requirements than the superficial nephrons. Also, microinjection of phosphorus tracer into thin ascending and descending limbs of loops of Henle reveals that only 80% of phosphorus was recovered in the urine, whereas 88% to 100% of phosphorus was recovered when the tracer was injected into the late superficial distal tubule. It was concluded that a significant amount of phosphorus must be reabsorbed by juxtamedullary distal tubules or by segments connecting the juxtamedullary distal tubules to the collecting ducts to account for the discrepancy between the results of superficial nephron injection and injection into the juxtamedullary ascending limb of loops of Henle. These data seem to support an increased reabsorptive capacity for phosphorus in deep as opposed to superficial nephrons.

In summary, phosphorus transport occurs in the distal nephron, particularly in the distal convoluted tubule and cortical collecting tubular system. This transport may be considerable under certain experimental conditions, but the importance of the terminal nephron system in day-to-day phosphorus homeostasis remains to be defined. It is also evident from data obtained from various micropuncture and microinjection studies that juxtamedullary and superficial nephrons have different capacities for phosphorus transport. The increased responsiveness of the deep nephrons to phosphorus intake suggests a key regulatory role for this system in phosphorus homeostasis.

Cellular Mechanisms of Phosphate Reabsorption in the Kidney

The apical membrane of renal tubular cells is the initial barrier across which phosphorus and other solutes present in the tubular fluid must pass to be transported into the peritubular capillary network. Because the electrical charge of the cell interior is negative to the exterior, and phosphorus concentrations are higher in the cytosol, phosphorus must move against an electrochemical gradient into the cell interior, whereas at the antiluminal membrane, the transport of phosphorus into the peritubular capillary is favored by the high intracellular phosphorus concentration and the electronegativity of the cell interior. Studies with apical membrane vesicles have demonstrated cotransport of Na^+ with phosphate across the brush-border membrane, whereas the transport of phosphorus across the basolateral membrane is independent of that of Na^+ (27). The apical membrane Na^+-phosphate cotransport protein (NaPi-2a) energizes the uphill transport of phosphate across the brush-border membrane (BBM) by the movement of Na^+ down its electrochemical gradient. The latter gradient is established and maintained by active extrusion of Na^+ across the basolateral cell membrane into the peritubular capillary through the action of Na^+-K^+-ATPase (Fig. 89-2) (28).

Three families of NaPi cotransport proteins of the proximal tubule (types I, II, and III) have been cloned using Xenopus laevis oocyte RNA expression strategies (29–34). The DNA clones encode 80- to 95-kd proteins that reconstitute Na^+-dependent concentrative, or "uphill," transport of phosphate upon chromosomal RNA injection in oocytes (30,31) or transfection of sf9 cells (35). The type I cotransporter, Npt1/SLC17, is expressed predominantly in the renal proximal tubule, and it accounts for about 13% of the known NaPi cotransporter mRNA in the mouse kidney (36). Npt1 is not regulated by dietary P_i, and studies in Npt1-cRNA–injected oocytes revealed that it may function not only as a NaPi cotransporter but also as a chloride and organic anion channel (37).

The type II cotransporter Npt2/SLC34 proteins are similar between several species including humans (32–34). In addition to Npt2a (NaPi-2a), the predominant isoform in the renal proximal tubule, recently another isoform Npt2c (NaPi-2c) has also been discovered (38–41). Nephron localization of Npt2 proteins has been limited to the proximal tubule of superficial and deep nephrons (greatest in the latter, concordant with physiologic studies) (32–34). Immunolocalization studies in renal epithelial cells demonstrated apical membrane and subapical membrane vesicle staining (32–34), suggesting that a functional pool of transporters is available for insertion into or retrieval from the brush-border membrane itself. This has been postulated to be a major mechanism of P_i transport regulation in response to acute changes in phosphorus and PTH levels (32–34,42). The Npt2 family is upregulated at message and protein levels by chronic feeding of low-P_i diets (43,44) and regulated at message and protein levels by PTH (43–45).

The type III NaPi cotransporters SLC20 were originally identified as retroviral receptors for gibbon ape leukemia virus (Glvr1) and rat amphotropic virus (Ram1) (46). They are ubiquitously expressed, and they comprise about 1% of the known NaPi cotransporter mRNAs in the mouse kidney (36). Their levels and activity adapt to dietary phosphate changes, but they are felt to represent mainly "housekeeping" NaPi cotransporters.

Studies of phosphorus exit across the basolateral membrane suggest that it is accompanied by the net transfer of a negative charge and occurs down a favorable electrochemical gradient via sodium-independent mechanisms (47).

Proteins that Interact with the Type IIa Na/Pi Cotransporter Protein

Several proteins with PDZ domains have been identified that interact with the NaPi-IIa protein and are localized in the BBM or the subapical compartment (Fig. 89-3). PDZ domains are modular protein interaction domains that often occur in scaffolding proteins and bind in a sequence-specific fashion to the C-terminal peptide sequence or at times the internal peptide sequences of target proteins. These domains of approximately 90 amino acids are known by an acronym of the first three PDZ-containing proteins identified including the postsynaptic protein PSD-95/SAP90, the Drosophila septate junction protein Discs-large, and the tight junction protein ZO-1 (48–51).

PDZ domain containing proteins including NHERF-1, NHERF-2, PDZK1, CAL, and ZO-1 play an important role in: (a) the regulation of the expression and activity of renal proximal tubular BBM transport proteins including NHE-3 (48,52–55), and NaPi IIa (42,56), basolateral membrane transport proteins including Na-K-ATPase (56) and Na-$HC0_3$cotransporter (NBC) (57), (b) the regulation of the expression and activity of cystic fibrosis transmembrane conductance regulator (CFTR), a cAMP-regulated chloride channel and channel regulator (58–62), (c) parathyroid hormone 1 receptor signaling (63) and endocytic sorting of the β2-adrenergic receptor (64) and platelet-derived growth factor receptor (PDGFR) (65,66), (d) epithelial cell polarity and formation of tight junctions (67,68), and (e) maintaining the integrity of the glomerular barrier to proteins through interactions with podocalyxin, negatively charged sialoprotein expressed on the surface of podocytes, the glomerular visceral epithelial cells (69–74).

In addition to their interaction with membrane proteins and receptors, the PDZ domain-containing proteins also interact with the F-actin cytoskeleton through their interactions with the ezrin- radixin-moesin (ERM) proteins (75) (Fig. 89-3)

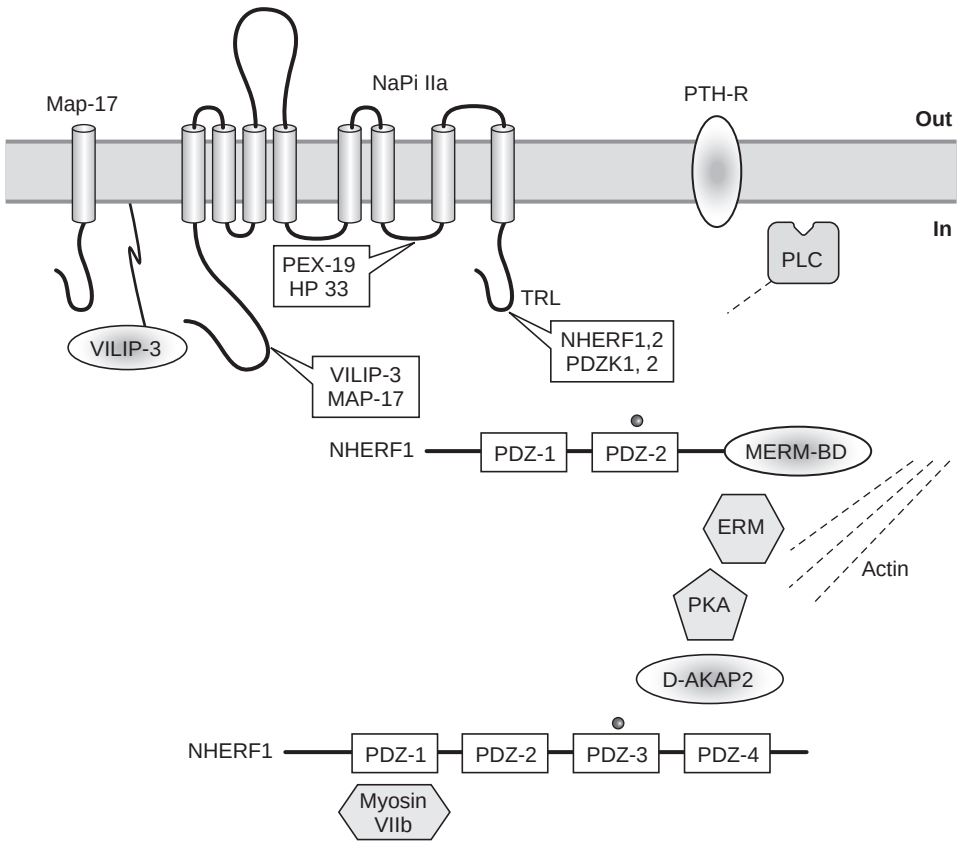

FIGURE 89-3. Interactions of NaPi IIa with several proteins expressed in the renal proximal tubule. MAP-17 is involved in the apical location of PDZK1 (via PDZ-4) in OK cells. (From: Biber J, Gisler SM, Hernando N, et al. PDZ interaction and proximal tabular phosphate reabsorption. *Am J Physiol Renal Physiol* 2004;287:F871.)

(76,77). ERM proteins are typically located peripherally in the membrane and link the cytoplasmic tails of membrane proteins and receptors to the cortical actin cytoskeleton. The ERM proteins play an important role in the formation of microvilli, cell-cell junctions and membrane ruffles and also participate in signal transduction pathways. The ERM proteins contain an F-actin binding site within their carboxy-terminal 30 residues. In addition, the ERM proteins have FERM (**f**our-point one, **e**zrin, **r**adixin, **m**oesin) domains, which are generally located at or near the amino terminal, and act as multifunctional protein and lipid binding sites. The FERM domain of ezrin interacts strongly with NHERF-1 and NHERF-2. NHERF-1 and NHERF-2 have 2 PDZ domains and have a carboxy-terminal sequence of 30 amino acids that bind ezrin.

Using the molecular approach (yeast two-hybrid) several proteins with PDZ domains that interact with the C terminus of NaPi IIa have been identified including: (i) NHERF-1/EBP50, (ii) NHERF-2/E3KARP, (iii) PDZK1/NaPi-Cap1, (iv) PDZK2/NaPi-Cap2, and (v) CAL, a CFTR-associated ligand (78–86).

Different studies suggest that apical expression of NaPi IIa depends on the presence of NHERF-1. Expression of NaPi IIa was reduced upon introduction of dominant-negative form of NHERF-1 in OK cells (78–81). The *in vivo* importance of the NaPi IIa interaction with NHERF-1 was also shown in a study where targeted disruption of the mouse NHERF-1 gene was associated with decreased BBM expression and increased intracellular localization of NaPi IIa resulting in decreased renal phosphate reabsorption and renal phosphate wasting (87). On the other hand targeted disruption of NHERF-1 did not modulate the BBM expression and localization of NHE3, however there was impaired regulation of NHE3 in response to PKA (87).

In contrast to NHERF-1, targeted disruption of the PDZK1 gene failed to modulate the BBM expression of NaPi IIa (88,89). NHERF-1, therefore, plays a critical and unique role in the renal proximal tubular apical membrane targeting of NaPi IIa protein and maintenance of phosphate homeostasis.

Recent studies have identified at least three additional proteins that may be important in the regulation of NaPi IIa targeting and trafficking: i) the peroxismal protein PEX 19, a farnesylated protein that confers PTH responsiveness to NaPi IIa (90), ii) the calcium binding protein Vilip-3, a myristoylated protein that may be important in calcium dependent regulation of NaPi IIa (91), and iii) MAP 17 which may be important for apical expression of PDZK1 (83) (Fig. 89-3).

Factors that Affect the Urinary Excretion of Phosphorus

Several factors are known to affect the urinary excretion of phosphorus. Of the multiplicity of factors that regulate phosphate transport in the kidney, the most important are dietary phosphate and PTH.

Alterations in Dietary Phosphorus Intake

The mechanism by which the kidney modulates phosphorus excretion when dietary phosphorus is reduced or increased continues to be intriguing. Earlier micropuncture studies suggested that the most striking adaptive increase in phosphorus transport occurs in the proximal tubule. Later studies (92) suggested that the entire nephron participates in the reduction of phosphorus excretion during dietary phosphorus deprivation. It has been shown that isolated perfused tubules obtained from rabbits that were fed a normal or low-phosphorus diet differ in their capacity to reabsorb phosphate. In normal animals, the proximal convoluted tubule (PCT) is capable of reabsorbing 7.2 ± 0.8 pmol/mL per minute, whereas tubules obtained from

phosphorus-deprived animals reabsorb 11.1 ± 1.3 pmol/mL per minute. Conversely, animals that are fed a high phosphorus diet show reduced phosphorus reabsorption when the proximal tubules are perfused *in vitro* (2.7 ± 2.6 pmol/mL per minute).

Based on renal BBM preparations, it has been suggested that the effect of reduced dietary phosphorus to stimulate renal phosphorus transport is intrinsic to the renal tubular epithelium and occurs specifically at the BBM Na^+-phosphate cotransporter. Considerable evidence has accrued from studies performed in cell lines isolated from mammalian kidneys, indicating that the adaptation to phosphate supply by the sodium–phosphate cotransporter is biphasic (93–95). These studies demonstrated that incubation of cells in a low-phosphate medium result in a twofold increase in Na^+-independent phosphate cotransport. The first phase of adaptation is observed rapidly (within 10 minutes) and is characterized by an increase in the V_{max} of the transporter. This initial phase is independent of new protein synthesis. A slower phase resulting in a doubling of the phosphate transport rate, again through an increase in V_{max}, occurs over several hours (maximum of about 15 hours) and is inhibited by blocking new protein synthesis. These studies have been interpreted to indicate that the immediate response to reduced phosphate availability is the insertion of new transport units into the brush-border membrane from an intracellular store. Secondly, through gene transcription and increased NaPi protein synthesis, additional units are produced and inserted into the brush border.

The cloning of the Npt2 NaPi cotransport proteins has enabled further confirmation of results of the earlier-mentioned P_i transport studies. As discussed already, chronic feeding of low-P_i diet increases steady-state mRNA levels of Npt2 in rats (96). Acute P_i deprivation does not affect Npt2 mRNA levels, compatible with a protein synthesis–independent action related to insertion of new transport proteins from subapical vesicles into the brush-border membrane (43,44,97). Indeed the adaptation to acute Pi deprivation occurs independent of de novo transcription and protein synthesis and is mediated by apical insertion of cytoplasmic NaPi II a transporters by a microtubule dependent mechanism (98) (Fig. 89-4).

Effects of PTH on Phosphorus Reabsorption by the Kidney

Parathyroidectomy decreases urinary phosphorus excretion, whereas administration of PTH rapidly increases phosphorus excretion (99,100). Micropuncture studies indicate that PTH inhibits phosphorus transport in the proximal tubule (101) and probably in segments of the nephron located beyond the proximal tubule (21). TF: UF phosphorus ratio reaches a value of 0.6 by the S_2 segment of the proximal tubule, and once achieved, this equilibrium ratio is maintained along the accessible portion of the proximal tubule. Within 6 to 24 hours after parathyroidectomy, the proximal TF: UF phosphorus ratio falls to a value of 0.2 to 0.4, indicating an increase in phosphorus reabsorption (92–103). TF phosphorus falls progressively with continuous fluid absorption along the length of the tubule, so by the end of the proximal tubule, the reabsorption of phosphorus is 70% to 85% of the filtered load, resulting in decreased phosphorus delivery to distal segments of the nephron. Because decreased delivery of phosphorus out of the proximal tubule complicates the evaluation of any distal effects of PTH on phosphorus excretion, maneuvers have been designed to increase phosphorus delivery to the distal nephron to study distal effects of parathyroidectomy on phosphorus reabsorption [e.g., phosphorus loading by intravenous infusion (104,105)]. In the nonphosphorus-loaded, acutely parathyroidectomized animal, virtually all the distal load of phosphorus is reabsorbed by the distal nephron, reducing urinary phosphorus excretion to very low levels (106,107). In the phosphorus-loaded animal, the distal reabsorption of phosphorus increases until saturation is approached and urinary phosphorus excretion begins to rise. Acute administration of PTH to phosphorus-loaded parathyroidectomized dogs sharply lowers the distal reabsorption. These experiments indicate that PTH inhibits reabsorption of phosphorus in the distal and the proximal nephron.

Administration of PTH *in vivo* results in decreased rates of Na^+-dependent phosphorus transport in brush-border membrane vesicles isolated from the kidneys of treated rats (108,109). The uptakes of d-glucose and Na^+ were not affected by administration of PTH. Intravenous infusion of dibutyryl cyclic adenosine monophosphate (cAMP) also decreased Na^+-dependent phosphorus uptake in isolated brush-border vesicles, but neither PTH nor dibutyryl cAMP decreased phosphate transport when added directly to membrane vesicles (108). These observations suggest that the effects of PTH on renal phosphate transport were mediated through altered functional characteristics (decreased V_{max}) of the renal brush-border membrane Na^+-dependent phosphate transporter (109).

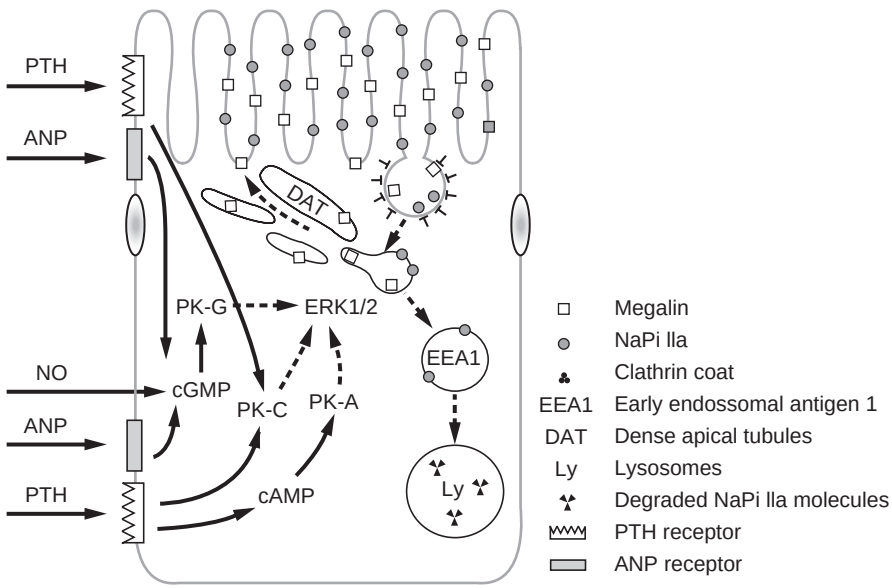

FIGURE 89-4. Mechanisms of NaPi IIa traffic in the apical membranes of proximal tubular cells. Microvillar NaPi IIa is retrieved in megalin containing clathrin coated vesicles. NaPi IIa moves from the vesicles into an endosomal pool marked by EEA-1, while megalin is recycled through dense apical tubules back to the microvilli. The endosomal NaPi IIa is targeted for lysosomal degradation. The process of NaPi IIa retrieval and lysosomal degradation is stimulated by several factors (PTH, ANP, NO) whose mechanisms of signal transduction (PK-A, PK-C, PK-G) merge in activation ERK1/2 that modulates the process. (From: Bacic D, Wagner CA, Hernando N, et al. Novel aspects in regulated expression of the renal type IIa Na/Pi-cotransporter. *Kidney Int* 2004;66:S5, with permission.)

PTH stimulates two signaling pathways in proximal tubule cells: adenylate cyclase and phospholipase C (PLC), resulting in activation of protein kinase A (PKA) and protein kinase C (PKC) (110). The first pathway, activation of the adenylate cyclase, differs from that of PKC. Studies in OK cells show that PKA activation by PTH decreases the expression of NaPi-2a cotransporter likely due to internalization and degradation of the transporter. Binding of PTH to its receptor leads to activation of PLC with the subsequent hydrolysis of phosphatidylinositol 4,5-bisphosphate to inositol 1,4,5-trisphosphate (IP_3) and 1,2-diacylglycerol (DAG). IP_3 generation leads to the release of intracellular calcium stores. DAG activates PKC (110). In addition to its direct effect on NaPi-2a, PTH inhibits NaPi transport indirectly by inhibiting the Na^+-K^+-ATPase by decreasing the favorable Na^+ gradient for Pi entry into the cell (111). Recent studies indicate that the activation of PKA and PKC signaling pathways by PTH also activates mitogen activated protein (MAP) kinase (MAPK) or extracellular receptor kinase (ERK1/2) which also induces inhibition of NaPi transport (112,113).

Measurement studies of *in vivo* renal reabsorption of phosphorus and calculations of kinetic parameters of Na^+-dependent phosphorus transport in membrane vesicles isolated from the renal brush-border membranes of normal dogs, parathyroidectomized dogs, dogs fed a low-phosphorus diet, and dogs receiving human growth hormone were performed (109,114). The latter three groups of dogs had greater baseline values for absolute tubular reabsorption of phosphorus compared with normal dogs. Na^+-dependent phosphate transport in brush-border membrane vesicles isolated from kidneys of these dogs was significantly increased compared with transport in brush-border vesicles from kidneys of normal dogs. Administration of PTH decreased significantly the apparent V_{max} for Na^+-dependent phosphorus transport in brush-border membrane vesicles isolated from kidneys of each of the four groups of dogs. The apparent K_m (intrinsic binding affinity) for Na^+-dependent phosphorus transport was not significantly changed by experimental maneuvers. Absolute tubular reabsorption of phosphorus measured *in vivo* was decreased by administration of PTH in each group of dogs with the exception of the dogs fed a low-phosphorus diet (109,114). Thus, alterations in phosphorus reabsorption measured *in vivo* were paralleled by alterations in Na^+-dependent phosphorus transport in isolated membrane vesicles, and the administration of PTH *in vivo* resulted in altered transport characteristics of the isolated brush-border membranes.

The cloning of the NaPi IIa cotransport proteins has not completely elucidated the mechanisms of PTH action on phosphate transport. Because the phosphaturic effect of PTH can be reproduced by analogs of cAMP, the intracellular mechanism of phosphate transport regulation is thought to involve the cAMP/PKA signal pathway. However, the NaPi IIa transport proteins are not characterized by a PKA–mediated phosphorylation site (115). Phosphorylation of brush-border membrane proteins *in vitro* occurs in parallel with inhibition with NaPi IIa cotransport (109). Parathyroidectomy of rats causes a twofold to threefold increase in the NaPi IIa protein content of brush-border membrane vesicles (45). Immunocytochemistry reveals the increase in protein exclusively in apical brush-border membranes of proximal tubules. PTH treatment of parathyroidectomized rats for 2 hours decreased protein levels and decreased the abundance of NaPi IIa-specific messenger RNA (mRNA) by 31% (45). Parathyroidectomy did not affect NaPi IIa mRNA levels. The effects of PTH were apparent within 2 hours of administration and indicate that PTH regulation of NaPi IIa is determined by changes in the expression of NaPi IIa protein in the renal brush-border membranes (45).

Recent studies indicate that PTH decreases the NaPi IIa protein content of the apical membrane by endocytic retrieval pathway which is megalin dependent, as this process is impaired in megalin deficient mice (116) (Fig. 89-4). In megalin intact mice or rats, following treatment with PTH NaPi IIa in internalized via clathrin-coated pits, and NaPi IIa is then delivered to early endosomes and eventually to the lysosomes where the protein is degraded. At the present time unlike some of the other proximal tubular transport proteins or receptors, there is no evidence that the NaPi IIa protein is present at the recycling endosomes.

Vitamin D

Controversy still surrounds the regulatory role of vitamin D in renal phosphorus handling. Several studies have demonstrated that the chronic administration of vitamin D to parathyroidectomized animals is phosphaturic (117–119). Conversely, other investigators reported that vitamin D acutely stimulates proximal tubular phosphorus transport in both parathyroidectomized and vitamin D–depleted rats (120). A unifying interpretation of these studies was hampered by the fact that the dosages of vitamin D administered and the status of the serum calcium, phosphorus, and PTH varied considerably from study to study.

Liang et al. (121) administered 1,25-dihydroxycholecalciferol to vitamin D–deficient chicks and subsequently examined the transport characteristics of isolated renal tubule cells. Three hours after the *in vivo* administration of vitamin D, phosphorus uptake by the cells was significantly increased, whereas 17 hours after the administration of vitamin D, phosphorus uptake was reduced. The serum phosphorus concentration, however, was significantly increased at 17 hours after administration, and administration of phosphorus to vitamin D–depleted animals so their serum phosphorus levels were comparable to those of the 17-hour vitamin D–replenished group resulted in a similar decrease in phosphorus uptake (121). In response to *in vitro* preincubation with as little as 0.01 pm of 1,25-dihydroxycholecalciferol, renal cells isolated from vitamin D–deficient chicks demonstrated a specific increase in sodium-dependent phosphorus uptake, which was blocked by pretreatment with actinomycin D. The stimulatory effect was relatively specific for 1,25-dihydroxycholecalciferol, and kinetic analysis indicated that the V_{max} of the phosphorus transport system was increased, whereas the affinity of the system for phosphorus was unaffected (121).

Kurnik and Hruska (122) also examined the relationship between vitamin D and renal phosphorus excretion in a normocalcemic, normophosphatemic weanling rat model fed a vitamin D–deficient diet. The animals were mildly vitamin D deficient (92 pg/mL of 1,25-dihydroxycholecalciferol versus 169 pg/mL in controls) but had no evidence of secondary hyperparathyroidism. Clearance studies performed in the basal partially vitamin D–deficient state showed an increase in both absolute and fractional phosphorus excretion compared with controls. Animals that were replenished with 1,25-dihydroxycholecalciferol and maintained on diets designed to protect against the development of hyperphosphatemia demonstrated a significant decrease in urinary phosphorus excretion. Other animals were similarly replenished with vitamin D but did not receive dietary adjustment; and in this group, both the serum phosphorus and the urinary phosphorus excretion level increased significantly. A third group was fed a normal diet and received smaller doses of 1,25-dihydroxycholecalciferol (15 pmol/g of body weight) for shorter periods, and although this dose had no effect on the serum phosphorus concentration, the phosphaturia was completely resolved.

Studies on brush-border membrane vesicles prepared from these animals revealed that in the partially vitamin D–deficient state, sodium-dependent phosphorus uptake was significantly reduced compared with control animals. Animals that were

replenished with vitamin D and fed a controlled diet had a greater sodium-dependent phosphorus uptake than both vitamin D–depleted and vitamin D–replenished animals not maintained on controlled diets.

The results of this series of studies suggest that the primary action of 1,25-dihydroxycholecalciferol is to increase tubular phosphorus reabsorption. Long-term administration of vitamin D, however, represents a more complex situation, where phosphaturia may occur secondary to changes in the filtered load of phosphorus, in the body distribution of phosphorus, or in intracellular phosphorus activity.

Effects of Changes in Acid–Base Balance on Phosphate Excretion

The effect of acid–base status on the renal excretion and transport of phosphate is complex. Acute respiratory acidosis increases and acute respiratory alkalosis decreases phosphate excretion (123). These effects occurs independent of PTH and plasma or luminal bicarbonate levels (123). However, other studies suggest that the effects of respiratory acid–base changes may be mediated by changes in plasma phosphate (123).

Acute metabolic acidosis has minimal effects on phosphate excretion; however, the phosphaturic effect of PTH is blunted (102). Acute metabolic alkalosis causes an increase in phosphate excretion independently of PTH (124–128). This effect is due, in part, to volume expansion produced by the infusion of bicarbonate (125,126).

Chronic acidosis increases phosphate excretion, again independent of PTH or changes in ionized Ca^{2+} (128–131). The effect appears to be directly on the sodium-dependent phosphate transport mechanism (132). Chronic alkalosis decreases phosphate excretion, probably by the same mechanism as acidosis, operating in the opposite direction (124,133).

Acute and chronic acidosis in rats decreases the proximal tubule cell luminal membrane expression of the NaPi IIa cotransporter (134). In acute acidosis, there is rapid internalization of the transporter and the total cortical homogenate cotransporter expression is unchanged. In chronic acidosis, there are parallel changes in NaPi IIa protein and mRNA abundance. The effects of acid–base perturbations are complex and depend on antecedent dietary intake, the chronicity of the change, and whether the change affects luminal or intracellular pH, or both.

Adrenal Hormones

Administration of pharmacologic amounts of cortisol leads to phosphaturia. Acute adrenalectomy reduces the GFR and increases the reabsorption of phosphorus in the proximal tubule. Frick and Durasene (135) concluded that glucocorticoid hormones could play an important role in the regulation of fractional reabsorption of phosphorus. Indeed administration of glucocorticoids to animals have been shown to decrease proximal tubular NaPi cotransport activity and induce phosphaturia by causing parallel decreases in NaPi IIa protein and mRNA levels (136). The inhibitory effects of glucocorticoids in NaPi transport is in part mediated by a the concomitant alterations in renal proximal tubular apical BBM glycosphingolipid composition, as inhibition of glycosphingolipid synthesis prevents in part the decrease in renal NaPi cotransport activity. In contrast to glucocorticoid administration, adrenalectomy with mineralocorticoid administration, resulting in selective glucocorticoid deficiency is associated with increased renal proximal tubular NaPi IIa protein expression (137).

Growth Hormone (GH)

An increase in serum phosphorus and a rise in renal phosphorus transport are characteristics of growth hormone excess during the period of rapid growth in the child, during acromegaly, or during exogenous growth hormone administration to experimental animals. Hammerman et al. reexamined this phenomenon in the brush-border membrane vesicle preparation in the dog (109) and demonstrated that growth hormone treatment resulted in an increased sodium-dependent phosphorus transport. These data reassert the importance of brush-border membrane phosphorus uptake in regulating overall renal phosphorus reabsorptive capacity. The action of growth hormone is likely mediated by insulin-like growth factor-1 (138).

Recent studies have further identified the nephron sites and mechanisms by which GH regulates renal Pi uptake (139). Micropuncture experiments were performed after acute thyroparathyroidectomy in the presence and absence of parathyroid hormone (PTH) in adult (14- to 17-week-old), juvenile (4-week-old), and GH-suppressed juvenile male rats. While the phosphaturic effect of PTH was blunted in the juvenile rat compared with the adult, suppression of GH in the juvenile restored fractional Pi excretion to adult levels. In the presence or absence of PTH, GH suppression in the juvenile rat caused a significant increase in the fractional Pi delivery to the late proximal convoluted (PCT) and early distal tubule, so that delivery was not different from that in adults. These data were confirmed by Pi uptake studies into brush-border membrane (BBM) vesicles. Immunofluorescence studies indicate increased BBM type IIa NaPi cotransporter (NaPi-2) expression in the juvenile compared with adult rat, and GH suppression reduced NaPi-2 expression to levels observed in the adult. GH replacement in the [N-acetyl-Tyr(1)-d-Arg(2)]-GRF-(1-29)-NH(2)-treated juveniles restored high NaPi-2 expression and Pi uptake. Together, these novel results demonstrate that the presence of GH in the juvenile animal is crucial for the early developmental upregulation of BBM NaPi-2 and, most importantly, describe the enhanced Pi reabsorption along the PCT and proximal straight nephron segments in the juvenile rat (139).

Thyroid Hormone

Because P_i is intensively used in general metabolism, P_i homeostasis should be regulated by factors controlling the rate of metabolism itself. One such factor is thyroid hormone, and its role in P_i reabsorption regulation has been extensively analyzed (140–142). Pharmacological doses of T_3 have been shown to increase Na/P_i cotransport in BBM vesicles from rat proximal tubules (140,141). In addition, T_3 concentrations approximating the association constant (K_m) of the thyroid hormone nuclear receptor also elicited a similar increase in P_i transport in opossum kidney (OK) cells (142). In both cases, the increase in transport rate was caused by an increase in the capacity of the transport system, whereas the affinity was not modified. Euzet et al. (143,144) have shown an important role for T_3 in the maturation of the renal Na/P_i cotransporter, which was associated with changes in both K_m and V_{max}, as well as in the type II Na/P_i cotransporter (NaPi-2) protein and mRNA abundance.

Recently Sorribas et al. determined the role of physiological concentration of thyroid hormone in renal phosphate transport *in vivo*; more exactly, the molecular mechanisms of enhancement of phosphate reabsorption by thyroid hormone. In addition, they also determined the potential role of thyroid hormone in impairment of phosphate reabsorption that accompanies the aging kidney (145). Their results show that chronically treated hypothyroid rats, using a physiological dose of T_3, exhibit increases in serum Pi levels, NaPi-2 mRNA and protein content, and Na/P_i cotransport activity in superficial and juxtamedullary renal cortex, all these effects by means of enhanced transcription of the corresponding NaPi-2 gene. The stimulatory effect of the hormone was less evident in the aging kidney, which shows a lower level of basal phosphate reabsorption. In this study, only pharmacological hyperthyroidism was able

to restore partially the level of serum Pi observed in young animals.

Epidermal Growth Hormone

Epidermal growth factor (EGF) is a 53-amino acid polypeptide. The kidney is a major site of synthesis of the EGF precursor, prepro-EGF. In renal epithelial cells grown in culture, EGF has been shown to modulate sodium gradient-dependent phosphate transport (Na-P_i cotransport) activity. In LLC-PK_1 cells, a tubular epithelial cell line derived from pig kidney cortex, EGF caused stimulation of Na-P_i cotransport (146). EGF also stimulated Na-P_i cotransport in isolated perfused proximal tubule derived from rabbit kidney (147). In contrast, in opossum kidney (OK) cells, a tubular epithelial cell line derived from the opossum kidney, EGF was shown to cause inhibition of Na-P_i cotransport (148,149). The *in vivo* effect of EGF on Na-P_i cotransport activity, however, is unknown. A recent study also determined whether EGF regulates Na-P_i cotransport activity *in vivo* and whether the effect of EGF to regulate Na-P_i cotransport is dependent on the developmental stage of the animal (i.e., suckling [12-day-old] vs. weaned [24-day-old] rats). Weaned rats had higher proximal tubular BBM vesicle (BBMV) Na-Pi cotransport activity than suckling rats. Chronic treatment with EGF induced inhibition of BBMV Na-Pi cotransport in both suckling and weaned rats. The inhibitory effect was selective for Na-Pi cotransport as there was no inhibition of Na-glucose cotransport. Weaned rats had a higher abundance of BBMV NaPi-2 protein than suckling rats and a twofold increase in NaPi-2 mRNA. The EGF-induced inhibition of Na-Pi transport was paralleled by decreases in NaPi-2 protein abundance in both weaned and suckling animals. In contrast, there were no changes in NaPi-2 mRNA abundance. This study, therefore, demonstrated that proximal tubule BBMV Na-Pi cotransport activity, NaPi-2 protein abundance, and NaPi-2 mRNA abundance are higher in weaned than in suckling rats. EGF inhibits Na-Pi cotransport activity in BBMV isolated from suckling and weaned rats, and this inhibition is mediated via a decrease in NaPi-2 protein abundance, in the absence of a change in NaPi-2 mRNA (150).

Aging

The aging process in humans and in the rat is associated with impairment in renal tubular reabsorption of Pi and renal tubular adaptation to a low Pi diet. A recent study determined whether changes in the abundance of type II Na-Pi contransporter (NaPi-2) protein and/or mRNA play a role in the age-related decrease in Na-Pi cotransport activity. In experiments using 3- to 4-month-old young adult rats and 12- to 16-month-old aged rats, it was found that there was an age-related twofold decrease in proximal tubular apical BBM Na-Pi cotransport activity, which was associated with similar decreases in BBM NaPi-2 protein abundance and renal cortical NaPi-2 mRNA level. Immunohistochemistry showed lower NaPi-2 protein expression in the BBM of proximal tubules of superficial, midcortical, and juxtamedullary nephrons. This study also found that in response to chronic (7 days) and/or acute (4 hour) feeding of a low Pi diet, there were similar adaptive increases in BBM Na-Pi cotransport activity and BBM NaPi-2 protein abundance in both young and aged rats. However, BBM Na-Pi cotransport activity and BBM NaPi-2 protein abundance were still significantly lower in aged rats, in spite of a significantly lower serum Pi concentration in aged rats. The results indicate that impaired expression of the type II renal Na-Pi cotransporter protein at the level of the apical BBM plays an important role in the age-related impairment in renal tubular reabsorption of Pi and renal tubular adaptation to a low Pi diet (151).

Stanniocalcin

Stanniocalcin is a recently identified calcium and phosphate regulating hormone found in serum and the kidney. It is produced in the Corpuscles of Stannius, which are specialized endocrine organs that are closely associated with the kidneys of telest fish. It is a glycohormone that plays a major role in the calcium and phosphate homeostasis of fish. Stanniocalcin inhibits calcium uptake by the gills and gut and stimulates phosphate reabsorption of the kidney (152–159).

Two mammalian homologues of Stanniocalcin have been identified. Stanniocalcin 1 and Stanniocalcin 2, with seemingly opposing effects on renal phosphate transport. Stanniocalcin I induces increased gastrointestinal and renal Pi transport (160). Stanniocalcin 2 on the other hand causes inhibition of renal Pi transport by transcriptional mechanisms (160).

Diuretics

Acetazolamide inhibits phosphate reabsorption by its effects on proximal tubule decreases in Na^+-dependent bicarbonate transport, essential for the maintenance of the Na^+ gradient. Furosemide inhibits carbonic anhydrase activity and thus decreases phosphate transport. Similar effects have been demonstrated with the administration of large doses of thiazide diuretics (161).

Hypophosphatemia

Hypophosphatemia refers to serum phosphorus concentrations of less than 2.5 mg/dL. Hypophosphatemia usually results from one or a combination of the following factors (162) (Fig. 89-5) (163): (a) increased excretion of phosphorus in the urine; (b) decreased gastrointestinal absorption of phosphorus; or (c) translocation of phosphorus from the extracellular to the intracellular space. The major causes of hypophosphatemia are listed in Table 89-1.

Increased Excretion of Phosphorus in the Urine

Several pathophysiologic conditions may increase excretion of phosphorus in the urine. Some of these conditions are characterized by elevated levels of circulating PTH. Because PTH decreases phosphorus reabsorption by the kidney, modest to marked elevations of this hormone may increase urinary excretion of this anion (Table 89-1). Decreased tubular reabsorption of phosphorus may also occur without increased levels of PTH and may be due to changes in the reabsorption of salt and water or to renal tubular defects specific for the reabsorption

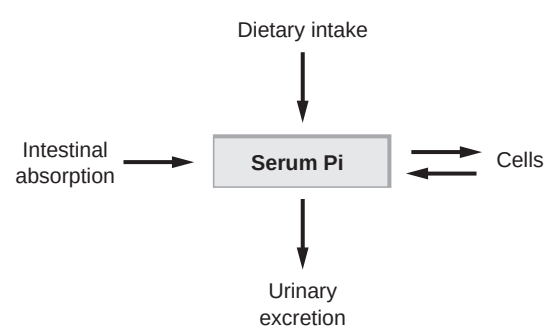

FIGURE 89-5. The major determinants of serum inorganic phosphate (Pi) concentration.

TABLE 89-1

CAUSES OF HYPOPHOSPHATEMIA

I. Increased excretion of phosphorus in the urine
   A. Primary hyperparathyroidism
   B. Secondary hyperparathyroidism
   C. Renal tubular defects (Fanconi's)
   D. Diuretic phase of acute tubular necrosis
   E. Postobstructive diuresis
   F. Extracellular fluid volume expansion
   G. Familial
      1. X-linked hypophosphatemia
      2. ADHR
      3. Oncogenic hypophosphatemic osteomalacia
      4. McCune-Albright syndrome/Fibrous dysplasia
      5. Mutations in NaPiIIa
      6. Hereditary hypophosphatemic rickets with hypercalciuria (HHRH)
   H. Post-transplant hypophosphatemia

II. Decrease in gastrointestinal absorption of phosphorus
   A. Abnormalities of Vitamin D metabolism
      1. Vitamin D–dependent rickets
      2. Familial
         a. Vitamin D–dependent rickets
         b. X-linked hypophosphatemia
   B. Malabsorption
   C. Malnutrition-starvation

III. Miscellaneous causes/translocation of phosphorus
   A. Diabetes mellitus: during treatment for ketoacidosis
   B. Severe respiratory alkalosis
   C. Recovery phase of malnutrition
   D. Alcohol withdrawal
   E. Toxic shock syndrome
   F. Leukemia, lymphoma
   G. Severe burns

of certain solutes or phosphorus. Hypophosphatemia may also occur in the diuretic phase of acute tubular necrosis or in postobstructive diuresis, presumably due to a combination of high levels of PTH and decreased tubular reabsorption of salt and water.

Primary Hyperparathyroidism

Primary hyperparathyroidism is a common entity in clinical medicine (164). PTH is secreted in excess of the physiologic needs for mineral homeostasis due to either adenomas or hyperplasia of the parathyroid glands (165). This results in decreased phosphorus reabsorption by the kidney. The losses of phosphorus in the urine result in hypophosphatemia. The degree of hypophosphatemia may vary considerably among patients, because mobilization of phosphorus from bone will in part mitigate the hypophosphatemia. Moreover, if the patient ingests large amounts of dietary phosphorus, the degree of hypophosphatemia observed may be mild. Because these patients also have elevated levels of serum calcium, the diagnosis is made relatively easy in most cases by the finding of elevated levels of immunoreactive PTH.

Secondary Hyperparathyroidism

Although secondary hyperparathyroidism is present in most patients with chronic renal disease, hyperphosphatemia rather than hypophosphatemia occurs in such patients because of decreased phosphorus excretion in the urine resulting from the fall in GFR. However, certain conditions characterized by malabsorption of calcium from the gastrointestinal tract may produce hypocalcemia, leading to development of secondary hyperparathyroidism (166). The elevated levels of PTH will decrease phosphorus reabsorption by the kidney, resulting in hypophosphatemia. Thus, patients with gastrointestinal tract abnormalities resulting in calcium malabsorption and secondary hyperparathyroidism will have low levels of serum calcium and phosphorus. In these patients, the hypocalcemia is responsible for the increased release of PTH. In addition, decreased intestinal absorption of phosphorus as a result of the primary gastrointestinal tract disease may also contribute to the decrement in the levels of serum phosphorus. In general, these patients have urinary losses of phosphorus that are out of proportion to the hypophosphatemia in contrast to patients with predominant phosphorus malabsorption and no secondary hyperparathyroidism in whom urinary excretion of phosphorus is low.

Renal Tubular Defects

Several conditions characterized by single or multiple tubular defects have been described in which phosphorus reabsorption is decreased. In the Fanconi syndrome (167), patients excrete not only increased amounts of phosphorus in the urine but also increased quantities of amino acids, uric acid, and glucose, resulting in hypouricemia and hypophosphatemia. Rare familial forms of hypercalciuria are often associated with one or more of the components of the Fanconi syndrome including hypophosphatemia or hyperphosphaturia (168–170). Interestingly, these familial syndromes, Dent's disease, and its variants have been found to be caused by a mutation in the CLCN5 chloride channel (168,171), which is an intracellular vesicular channel, perhaps related to the vesicles that harbor the NaPi cotransport proteins (172–175). There are other conditions in which an isolated defect in the renal tubular transport of phosphorus has been found, for example, fructose intolerance, which is an autosomal-recessive disorder (176). After renal transplantation, an acquired renal tubular defect may be responsible for the persistence of hypophosphatemia in some patients (177,178).

Diuretic Phase of Acute Tubular Necrosis

Most patients with acute renal failure develop secondary hyperparathyroidism and hyperphosphatemia during the oliguric phase. During the recovery phase of acute renal failure, the combined occurrence of a profound diuresis, secondary hyperparathyroidism, and continued use of phosphate binders may lead to severe hypophosphatemia. This hypophosphatemia is usually short lived, and serum phosphorus levels return to within the normal range as the diuretic phase of acute tubular necrosis subsides.

Postobstructive Diuresis

A marked phosphaturia may develop in some patients after relief of urinary tract obstruction. This phosphaturia may be severe enough in a few patients to lead to hypophosphatemia (178).

Extracellular Fluid Volume Expansion

Expansion of the ECF volume by the administration of solutions containing sodium increases the urinary excretion of phosphorus. An important mechanism by which ECF volume expansion produces phosphaturia consists of a fall in ionized calcium and subsequent release of PTH (179). This condition is probably of minor importance in clinical medicine, and restoration of the ECF volume to within the normal range results in the return of phosphorus reabsorption to physiologic levels.

Familial

Studies of two hereditary, X-linked hypophosphatemia (XLH) and autosomal dominant hypophosphatemic rickets (ADHR), and two acquired, oncogenic hypophosphatemic osteomalacia (OHO) and McCune-Albright's syndrome, renal phosphate wasting disorders have led to the identification of novel genes involved in the regulation of renal Pi transport and also vitamin D synthesis (180–186).

X-linked Hypophosphatemia (XLH)

XLH is a common cause of rickets with a prevalence of approximately 1 in 20,000. It is inherited in an X-linked dominant manner. Manifestations of XLH include short stature, bone pain, tooth abscesses, calcification of tendon insertions, ligaments, and joint capsules, and lower extremity deformities. XLH is characterized by hypophosphatemia, decreased reabsorption of phosphorus by the renal tubule, decreased absorption of calcium and phosphorus from the gastrointestinal tract, and varying degrees of rickets or osteomalacia. Patients with this disorder exhibit normal levels of 1,25-dihydroxycholecalciferol and reduced Na-phosphate transport in the proximal tubule in the face of severe hypophosphatemia.

The roles of alterations in vitamin D sensitivity or synthesis in the defective transport of phosphorus observed in both the gut and the kidney have been investigated in the murine homolog of X-linked hypophosphatemia (Hyp) (187). Tenenhouse and Scriver found that administration of small doses of 1,25-dihydroxycholecalciferol to healthy animals significantly increased plasma calcium, plasma phosphorus, and fractional calcium excretion without a change in fractional phosphorus excretion or phosphorus transport as measured in proximal tubular brush-border membrane vesicles (188). However, there was no response to this dose of 1,25-dihydroxycholecalciferol in the Hyp mice. At a fivefold higher dose of vitamin D, the familial hypophosphatemic mice did show increased plasma calcium and fractional calcium excretion, as well as increased plasma phosphorus levels, but again there was no change in fractional phosphorus excretion or in sodium-dependent phosphorus transport in brush-border membrane vesicles. Although 1,25-dihydroxycholecalciferol increased phosphorus transport in the intestine, there was no defect of phosphorus transport in the intestine of untreated hypophosphatemic mice. The conclusion from these studies is that vitamin D influences phosphorus homeostasis in Hyp mice by stimulation of phosphorus absorption from the gastrointestinal tract. The defect in renal phosphorus reabsorption was unchanged despite high levels of vitamin D. These observations are consistent with those of studies performed in humans with familial hypophosphatemia, in whom the renal reabsorptive defect persists despite correction of growth by administration of vitamin D and oral phosphate supplements (189,190). There is, however, evidence of defective or altered metabolism of vitamin D_3 in Hyp mice. Meyer et al. found that on a normal diet, plasma 1,25-dihydroxycholecalciferol levels were the same in Hyp and healthy mice (191), although 25-hydroxycholecalciferol levels were reduced in Hyp mice. Because hypophosphatemia increases plasma 1,25-dihydroxycholecalciferol levels, the hypophosphatemic mice were resistant to the stimulatory effect of hypophosphatemia. To test this possibility, these authors fed the animals a low-phosphorus diet and found a paradoxical reduction in 1,25-dihydroxycholecalciferol levels in hypophosphatemic mice. They concluded that Hyp mice have a defective control system for plasma 1,25-dihydroxycholecalciferol that is unresponsive to a low-phosphate diet stimulus. The issue is further complicated by studies by Beamer et al. (192), who studied the effect of various preparations of vitamin D_3 on intestinal transport of phosphorus. The Hyp mice responded to 1α-hydroxycholecalciferol but not to 1,25-dihydroxycholecalciferol, suggesting that intestinal phosphorus transport responsiveness is not genetically absent in this model but does not respond to normal endogenous levels of 1,25-dihydroxycholecalciferol. Thus, mice with familial hypophosphatemia appear to have impaired metabolism of vitamin D, but this impairment does not cause the renal phosphate transport defect. There is also evidence suggesting that the decreased tubular reabsorption of phosphorus is not due to increased PTH levels (192). The component of renal phosphorus transport, which is PTH-independent, is abnormal and responsible for the increased phosphaturia. Tenenhouse et al. (193) have shown that tissue phosphate levels were normal in Hyp mice, whereas these levels tended to be low in animals that have hypophosphatemia secondary to reduction in dietary phosphorus. It would seem, therefore, that X-linked hypophosphatemia is a selective disorder of the transepithelial transport of phosphate. However, there is no correlation between the degree of hypophosphatemia and the severity of bone disease. Moreover, alterations in vitamin D metabolism cannot be explained solely by the presence of hypophosphatemia.

Tenenhouse et al. (194) have extended their studies in Hyp mice to demonstrate that the message levels for the NPT2 cotransport protein are reduced by 50% in the renal cortex, similar to the reduction in apical membrane vesicle NPT2 protein levels. However, the mapping of NPT2 and NPT1 to chromosomes 5 and 6, respectfully (195), indicated that these genes were not candidates for the genetic defects leading to X-linked hypophosphatemia or Hyp.

X-linked hypophosphatemia and Hyp are due to abnormal levels of a circulating hormonal factor. Results of the cross-perfusion studies of Meyer et al. (196) and the cross-transplantation studies of Nesbitt et al. (197) were interpreted to indicate the presence of a circulating factor. Furthermore, Nesbitt et al. (197) were unable to demonstrate transmission of the Hyp renal defect by transplanting a Hyp kidney into a healthy recipient. Likewise, transplantation of a normal kidney into a Hyp recipient failed to correct the Hyp phenotypic abnormalities. These studies suggest that the presence of a circulating humoral factor could be capable of producing the Hyp and X-linked hypophosphatemia phenotype.

An interesting aspect of the Hyp phenotype is the presence of a normal Na-dependent phosphate cotransport in osteoblasts (198). However, the osteoblasts of Hyp have been shown to be defective in models of endochondral bone formation (199,200). Defective mineralization is an important aspect of osteomalacia, and mineralization is controlled by calcium, phosphate, and bone matrix proteins. Hruska et al demonstrated defective phosphorylation of a key bone matrix protein, osteopontin, in Hyp mice and abnormal low activity of a protein kinase (casein kinase II–like activity) responsible for osteopontin phosphorylation (201,202). Thus, multiple proteins appear to be defective in the Hyp phenotype.

From a therapeutic point of view, the combination of neutral phosphate and 1,25-dihydroxycholecalciferol has lead to an improvement in the bone disease of some patients with X-linked hypophosphatemia and in the Hyp mice (203,204). The administration of phosphorus in X-linked hypophosphatemia is usually divided into four doses, with the total amount ranging between 1 to 4 g per day. Pharmacologic doses of 1,25-dihydroxycholecalciferol on the order of 1 to 3 μg per day may be necessary to correct the skeletal alterations. 1,25-dihydroxycholecalciferol does not correct the increased fractional excretion of phosphate. The enthusiasm for this regimen is tempered by a high incidence of nephrocalcinosis and occasional renal failure (203–205).

The gene responsible for XLH has been identified by positional cloning and has been designated **PHEX** for **PH**osphate

regulating gene with homology to Endopeptidases on the X chromosome (206). PHEX is a member of the M13 family of type II cell surface zinc-dependent metallopeptidases which includes neprilysis, endothelin-converting enzymes 1 and 2, KELL, and DINE/X-converting enzyme. The mouse PHEX DNA sequence is highly homologous to that of humans and the inactivating mutations of PHEX have been identified in the mouse homologues of XLH including the *Hyp* and *Gy* mice. The PHEX database (http://www.phexdb.mcgill.ca) indicates at least 179 mutations in the PHEX gene that have been identified that results in loss of PHEX function. PHEX is expressed predominantly in osteoblasts, octeocytes, and odontoblasts, but not in the kidney. The pattern of PHEX expression is consistent with the skeletal and dental abnormalities seen subjects with XLH and the *Hyp* mouse. The current thinking is that PHEX is involved in the inactivation of a phosphaturic hormone or phosphatonin (see the subsequent text) or the activation of a phosphate conserving hormone. Thus PHEX mutation or loss of PHEX function results in excess of a phosphaturic hormone or deficiency of a phosphate conserving hormone (180–186).

As will be discussed in more detail in the ADHR section (later) fibroblast growth factor 23 (FGF23) is one of the most likely phosphaturic hormones in XLH (207). FGF23 serum levels are elevated in some patients with XLH and serum Pi concentrations are negatively correlated with circulating FGF23 levels in patients with XLH (208,209). Therefore, it has been speculated and initially shown that FGF23 may be a PHEX substrate (210). However several recent studies indicate that FGF23 is not a direct PHEX substrate (211–214). In fact, FGF23 has recently been shown to be cleaved by subtilisin-like protein convertases (SPC). SPCs are a seven-member family of calcium-dependent serine proteases responsible for the processing of peptide hormones, neuropeptides, adhesion molecules, receptors, growth factors, cell surface glycoproteins, and enzymes. Instead PHEX most likely through the actions of unidentified PHEX substrates or other downstream effectors regulates FGF23 expression as part of a potential hormonal axis between bone and kidney that controls systemic phosphate homeostasis and mineralization (214).

Autosomal Dominant Hypophosphatemic Rickets (ADHR)

The clinical presentation of ADHR is similar to XLH. However ADHR exhibits male to male transmission, consistent with autosomal dominant inheritance, and is characterized by incomplete penetrance and variable age of onset. Adults typically complain of bone pain, fatigue, and/or weakness, and can present with stress fractures or pseudofractures. Renal phosphate wasting and inappropriately normal serum calcitriol levels are the most typical laboratory findings.

The gene responsible for ADHR was identified by positional cloning as a new member of the fibroblast growth factor (FGF) family, FGF-23 (207). FGF-23 is a 251 amino acid peptide that is secreted and processes to amino- and carboxy-terminal peptides at a consensus pro-protein convertase (furin) site. In four unrelated ADHR families missense mutations have been identified in FGF-23 in the proteolytic cleavage site (R176Q, R179W, and R179Q) that interfere with peptide processing and result in gain of function of FGF-23 (215–217).

Administration of wild-type FGF-23 or FGF-23 expressing the ADHR mutations in the furin cleavage site (R176Q, R179W, or R179Q) to rats and mice has been shown to induce hypophosphatemia, increased urinary phosphate excretion via inhibition of the type IIa NaPi cotransport protein, and decreased 1,25 $(OH)_2D_3$ levels (40,218–220). Chronic administration of FGF-23 and increased expression of FGF-23 (FGF-23 transgenic mice) has also been shown to induce osteomalacia and decreased 1,25 $(OH)_2D_3$ levels via decrease in 25-hydroxyvitamin D 1α-hydroxylase mRNA (221–223). *In vitro* studies in OK cells have demonstrated that FGF-23 inhibits Na/Pi cotransport activity and type IIa NaPi cotransport protein abundance by MAPK signaling dependent pathway (224).

In contrast, targeted ablation of FGF23 in mice results in significantly increased serum Pi levels with increased renal Pi absorption. These mice also have increased serum 1,25 $(OH)_2D_3$ levels that is due to increased expression of renal 25-hydroxyvitamin D 1α-hydroxylase (1α-OHase) (225). Another study with homozygous ablation of FGF23 in mice revealed that these mice have marked hyperphosphatemia, increased serum 1,25 $(OH)_2D_3$ levels, growth retardation, increased total body bone mineral content but decreased bone mineral density of the limbs, and excessive mineralization in soft tissues, including in the heart and kidneys (226). Altogether these studies indeed indicate that FGF-23 is an important regulator of Pi homeostasis and vitamin D metabolism, although additional research is needed to elucidate the effects of FGF23 on bone and vascular tissue function and dysfunction.

Interestingly when the FGF-23 null mice were crossed with the *Hyp* mice, the murine equivalent of XLH, the resultant *Hyp* males lacking both FGF-23 alleles were indistinguishable from FGF-23 null mice, both in terms of serum Pi levels and skeletal changes, suggesting that that FGF-23 is upstream of PHEX (226).

Oncogenic Hypophosphatemic Osteomalacia (OHO) or Tumor Induced Osteomalacia (TIO)

This is an acquired disorder of renal phosphate wasting with clinical and biochemical features similar to XLH and ADHR. This disorder is characterized by hypophosphatemia associated with tumors. It was described initially in association with benign mesenchymal tumors; however, recent reports emphasized the association of this syndrome with malignant tumors (227,228). The other characteristics of this syndrome are increased phosphate excretion, low plasma 1,25-dihydroxycholecalciferol concentrations, and osteomalacia. All of the biochemical and pathologic abnormalities disappear when the tumor is resected.

The tumors associated with this syndrome are thought to secrete a substance that inhibits the renal tubular reabsorption of phosphate and suppresses 25-hydroxycholecalciferol 1α-hydroxylase activity. Whether this factor interacts directly with renal tubular cells is unknown. Studies by Cai et al. (229) have investigated the ability of media in which sclerosing hemangioma cells from a patient with osteogenic osteomalacia were cultured to affect Na-dependent phosphate transport. They found that the medium inhibited sodium-dependent phosphate transport, without increasing cellular concentrations of cAMP. The medium had PTH-like immunoreactivity but no PTH-related protein immunoreactivity. And the action of the tumor medium was not blocked by a PTH antagonist. The plasma 1,25-dihydroxycholecalciferol concentrations are low in patients with oncogenic osteomalacia despite the presence of hypophosphatemia (230–232), which usually increases plasma 1,25-dihydroxycholecalciferol concentrations by stimulating the renal 25-hydroxycholecalciferol 1α-hydroxylase in a PTH-independent manner (233). Besides the hypophosphatemia, deficient production of 1,25-dihydroxycholecalciferol is a factor contributing to the pathogenesis of the osteomalacia in these patients. Miyauchi et al. (234) have reported that 25-hydroxycholecalciferol 1α-hydroxylase activity of cultured renal tubular cells was decreased by incubating the cells with tumor extracts. This supports the concept that the tumor extracts contain a substance that inhibits the formation of 1,25-dihydroxycholecalciferol in the proximal tubule.

FGF-23 has been cloned from the tumors of patients who have presented with TIO and has established FGF-23 as the leading factor causing both the impaired renal Pi reabsorption resulting in hypophosphatemia and also the decreased serum 1,25 $(OH)_2D_3$ levels (235–237). Several studies have shown increased serum levels of FGF-23 and/or immunohistochemical detection of FGF-23 in patients who present with TIO and the serum levels to normalize after the resection of the tumors (238–242).

Serial analysis of gene expression (SAGE) in tumors associated with renal Pi wasting has identified additional genes including secreted frizzled-related protein 4 (sFRP-4) and matrix extracellular phosphoglycoprotein (MEPE) (243).

sFRP-4 has been detected in patients with TIO and it has been shown to inhibit Pi transport in OK cells and also in normal rats by PTH-independent mechanisms (243). sFRP-4 antagonizes Wnt signaling but at this time the role of the Wnt pathway in regulation of renal Pi transport or 25-hydroxyvitamin D 1α-hydroxylase has not been established.

MEPE is exclusively expressed in osteoblasts, osteocytes and odontoblasts and is markedly upregulated in murine XLH (*Hyp*) osteoblasts and TIO tumors (183,244–247). The recombinant human-MEPE has been shown to result in dose-dependent inhibition of renal Pi reabsorption, phosphaturia and hypophosphatemia (248). In addition, human-MEPE dose dependently inhibited BMP-2 mediated mineralization of a murine osteoblast cell line (2T3) *in vitro* (249).

A protease-resistant carboxy-terminal MEPE peptide containing the acidic serine-aspartate rich motif (ASARM) peptide has been shown to play a role in the inhibition of the mineralization (**minhibin**). PHEX prevents proteolysis of MEPE and release of ASARM. In XLH mutated PHEX may, therefore, contribute to the increased ASARM peptide seen in that disorder (249). Recent studies using surface plasmon resonance (SPR) indicates that MEPE binds to PHEX via the MEPE-ASARM motif which can provide a molecular basis for the inhibition of bone mineralization in XLH subjects and *Hyp* mice (249). The potential role of ASARM in regulation of renal Pi transport or 25-hydroxyvitamin D 1α-hydroxylase however, remains to be determined.

In contrast to the potential role of MEPE and ASARM to inhibit bone mineralization, disruption of MEPE gene in mice results in increased bone mass, resistance to age-associated trabecular bone loss, increased mineralization apposition rate and accelerated mineralization in *ex vivo* osteoblast cultures (250). These mice however have normal serum Pi levels, perhaps due to normal FGF-23 and PHEX expression.

McCune-Albright's Syndrome (MAS) and Fibrous Dysplasia (FD)

McCune-Albright's syndrome is characterized by the clinical triad of polyostotic fibrous dysplasia, café au lait skin pigmentation, and endocrine/metabolic disorders. The endocrine disorders include autonomous secretion of various hormones such as growth hormone, thyroid hormone, cortisol, estradiol, and testosterone. Rickets and osteomalacia due to hyperphosphaturic hypophosphatemia are prominent components of the syndrome (251–262).

The disorders of the syndrome share in common excessive function of cells whose actions are normally regulated by hormones that induce cAMP generation. The molecular basis for the phenotype is an activating mutation of GNAS1 which encodes the $G_{S\alpha}$ protein (α component of the stimulatory heterotrimeric guanosine triphosphate binding protein, G_S) in cells from affected tissues from patients with the syndrome (251–262). Kidney tissue, presumably proximal tubule, from patients has been reported to contain cells with the mutation.

A recent study using a combination of RT-PCR, in situ hybridization, ELISA of media conditioned by normal and FD stromal cells and trabecular bone cells, and measurements of FGF-23 in the serum, has determined that FGF-23 is expressed in FD tissues and osteogenic cells and that high levels of circulating FGF-23 correlate with renal Pi wasting in FD/MAS patients (263).

Mutations in the Type IIa NaPi Cotransporter

Epidemiologic studies suggest that genetic factors confer a predisposition to the formation of renal calcium stones or bone demineralization. Low serum phosphate concentrations due to a decrease in renal phosphate reabsorption have been reported in some patients with these conditions, suggesting that genetic factors leading to a decrease in renal phosphate reabsorption may contribute to them. A recent study by Prie and colleagues investigated if mutations in the gene coding for the main renal sodium-phosphate cotransporter (NPT2a) may be present in patients with these disorders. Twenty patients with urolithiasis or bone demineralization and persistent idiopathic hypophosphatemia associated with a decrease in maximal renal phosphate reabsorption were studied. The coding region of the gene for NPT2a was sequenced in all patients. The functional consequences of the mutations identified were analyzed by expressing the mutated RNA in Xenopus laevis oocytes. Two patients, one with recurrent urolithiasis and one with bone demineralization, were found to be heterozygous for two distinct mutations. One mutation resulted in the substitution of phenylalanine for alanine at position 48, and the other in a substitution of methionine for valine at position 147. Phosphate-induced current and sodium-dependent phosphate uptake were impaired in oocytes expressing the mutant NPT2a. Coinjection of oocytes with wild-type and mutant RNA indicated that the mutant protein had altered function. This study, therefore, concluded that heterozygous mutations in the NPT2a gene may be responsible for hypophosphatemia and urinary phosphate loss in persons with urolithiasis or bone demineralization (264).

A follow-up study by Virkki and colleagues recreated the two mutants, expressed them in Xenopus oocytes, and analyzed their kinetic behavior by two-electrode voltage clamp. They also performed coexpression experiments where we injected mRNA for wild-type (WT) and mutants containing an additional S462C mutation were injected, enabling complete inhibition of cotransport function with cysteine-modifying reagents. Finally, WT and mutant NaPi-IIa as C-terminal fusions to green fluorescent protein (GFP) in opossum kidney (OK) cells was expressed. They found in oocyte expression experiments that Pi-induced currents were reduced in both mutants, whereas Pi and Na affinities and other transport characteristics were not affected. The amount of cotransport activity remaining after cysteine modification, corresponding to WT activity, was not affected by coexpression of either mutant. Finally, GFP-tagged WT and mutants were expressed at the apical membrane in OK cells, showing that both mutants are correctly targeted in a mammalian cell (265). This, therefore, suggests that the heterozygous A48F and V 147M mutations cannot explain the pathological phenotype observed by Prie and colleagues and that there may be additional defects including interactions with PDZ domain containing proteins, including NHERF-1 that may explain the hypophosphatemia in these subjects (266,267).

Hereditary Hypophosphatemic Rickets with Hypercalciuria (HHRH). Hereditary hypophosphatemic rickets with hypercalciuria (HHRH) is a new autosomal form of hypophosphatemic rickets (268). This disease is characterized, and differs from other forms of hereditary hypophosphatemic rickets

and/or osteomalacia, by increased serum levels of 1,25-dihydroxyvitamin D, hypercalciuria, and complete remission of the disease on phosphate therapy alone.

Two recent studies attempting to identify the molecular defects in this genetic disease determined that mutations in the type IIa renal Na/Pi cotransporter gene are not the cause of this disease (269,270). Therefore, this raises the possibility of other molecular defects such as mutations in the type IIc renal Na/Pi cotransporter gene or mutations in NHERF-1 or other PDZ domain containing proteins.

Posttransplant Hypophosphatemia

Posttransplant hypophosphatemia, a common disorder, is well described in the literature. Although described mainly in patients following renal transplantation (271–282), posttransplant hypophosphatemia also occurs in patients undergoing bone marrow transplantation (283,284). In all reports, the decrease in serum inorganic phosphate (Pi) concentration was associated with an increase in urinary phosphate excretion and a significant decrease in the measured or derived ratio of maximal rate of renal tubular transport of phosphate to glomerular filtration rate (TmPi/GFR) (285). In addition to the impairment in renal tubular phosphate reabsorption, evidence indicates that intestinal phosphate absorption is impaired in transplant patients (286–289).

Thus far, the mechanism for posttransplant hypophosphatemia has not been fully elucidated. It has been thought, however, that this syndrome is somehow linked to disordered regulation of renal tubular reabsorption of Pi. As discussed earlier, parathyroid hormone (PTH) leads to a reduction in the expression of type II Na/Pi cotransport at the BBMs, which accounts for the phosphaturic action of PTH. Given this property of PTH, it has been postulated that increased PTH activity during chronic renal failure (CRF) may be the major mechanism responsible for maintaining Pi balance during CRF. According to this hypothesis, posttransplant hypophosphatemia has been attributed to persistent hyperparathyroidism (HPT), that is, incomplete involution of hyperplastic glands produced by renal failure prior to transplantation would cause hypophosphatemia and increased phosphaturia during the early posttransplant period (290). Several studies, however, have documented that protracted HPT cannot account for the phenomenon of posttransplant hypophosphatemia since it can be seen in transplant patients with normal PTH levels. Moreover, transplant recipients failed to decrease Pi excretion in the urine even when PTH was suppressed by calcium infusion (278). In addition, the phosphaturia following kidney transplantation could not be ascribed to the effects of nephrectomy or to the influence of immunosuppressive drugs (278).

A recent study determined whether an alternative non-PTH humoral mechanism could account for the entity of posttransplant hypophosphatemia (291). This study tested the hypothesis that a circulating serum factor [non-parathyroid hormone (non-PTH)], which operates during chronic renal failure (CRF) to maintain phosphate (Pi) homeostasis, can increase fractional excretion of Pi (FE(PO4)) in normal functioning kidney grafts during the early posttransplant period, thereby causing phosphaturia and hypophosphatemia. In this study, five groups of patients were studied: control subjects (group 1, N = 16); "early" (2 weeks to 1 month) posttransplant patients (group 2, N = 22); "late" (9 to 12 months) posttransplant patients (group 3, N = 14); patients with advanced CRF (glomerular filtration rate = 30 to 40 mL/minute; group 4, N = 8); and patients who suffered from end-stage renal failure and were treated by chronic hemodialysis (group 5, N = 14). Group 2 manifested significant hypophosphatemia and phosphaturia when compared with groups 1 and 3 (Pi = 0.9 ± 0.003 mg/dL, FEPi = 68 ± 5%, P <0.0005 vs. groups 1 and 3). Sera were taken from each of the five subject groups and applied to the proximal tubular opossum kidney (OK) cells. The activity of Na/Pi-type 4 (that is, OK-specific type II transporter) was evaluated by measuring Na-dependent Pi flux. The expression of Na/Pi type II mRNA and the abundance of Na/Pi protein were determined by Northern and Western blot assays, respectively. When compared with sera from groups 1 and 3, 10% sera taken from groups 2, 4, and 5 (incubated overnight with OK cells) inhibited Na/Pi transport activity by 25 to 30% (P <0.0003). Both Na/Pi mRNA and the expression of Na/Pi protein were markedly augmented under the same conditions (P <0.05 groups 2, 4, and 5 vs. groups 1 and 3). Time-course analysis revealed that the up-regulation of Na/Pi protein by sera from groups 2, 4, and 5 was observed as early as four hours of incubation, whereas augmented abundance of Na/Pi mRNA was only seen after eight hours of incubation. The addition of PTH (1-34) to sera from groups 2, 4, and 5 abolished the augmented expression of NaPi protein. OK cell surface membrane proteins were labeled with N-hydroxysuccinimide bound to biotin (NHS-SS-biotin). Biotinylated transporters incubated with the different sera were precipitated by strepavidin and identified by Western blot analysis. Cells incubated in sera from group 2 showed increased membrane bound transporter when compared with control sera, whereas the intracellular pool of the transporter was comparable between the two groups.

This study, therefore, determined that a non-PTH circulating serum factor that increases FEPi during CRF is also responsible for phosphaturia and hypophosphatemia in the early period following successful kidney transplantation. This factor however has different characteristics than FGF-23, sFRP-4, and MEPE discussed earlier as regulation is mediated by novel posttranslational mechanism that is characterized by inhibition of Na/Pi transport activity along with inhibition of the Na/Pi transport protein trafficking from the cell membrane into the cytosol (291).

Decrease in Gastrointestinal Absorption of Phosphorus

Abnormalities of Vitamin D Metabolism

Vitamin D and its metabolites play an important role in phosphorus homeostasis (292). Vitamin D promotes the intestinal absorption of calcium and phosphorus and is necessary to maintain the normal mineralization of bone. Dietary deficiencies of vitamin D increase the amount of osteoid tissue in the skeleton and decrease normal mineralization. Bone mineralization is a complex process that is not completely understood. Normally, the osteoblast is responsible for laying down normal collagen that is well organized and distributed in a lamellar fashion. Between the recently deposited collagen and the old bone, there is an area called the mineralization front. Initially, amorphous calcium phosphate is deposited in the mineralization front and eventually matures into hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$]. Thus, the osteoid tissue changes into bone. Optimal mineralization requires the following: (a) normal bone cell activity; (b) normal supply of minerals; (c) the appropriate pH level (7.4 to 7.6); (d) normal synthesis and composition of the matrix; and (e) control of inhibitors of calcification.

The appositional growth rate in normal bone is about 1 μm per day and complete mineralization of the osteoid requires 13 to 21 days. Thus, the thickness of the osteoid usually does not exceed 20 μm. Less than 20% of the surface of the bone is normally covered by osteoid. When a biopsy is performed in a healthy subject who has previously ingested two doses of

tetracycline separately and 3 weeks apart, one usually detects two fluorescent rings or bands, indicating the locations of the mineralization front. In a patient with osteomalacia, usually a single band, no band, or an irregular and spotty uptake of tetracycline is seen. In rickets or osteomalacia, there is a quantitative and qualitative defect in bone mineralization.

Vitamin D–Deficient Rickets. Diets deficient in vitamin D lead to the metabolic disorder known as rickets when it occurs in children or osteomalacia when it appears in adults (293). Vitamin D deficiency in childhood results in severe deformities of bone because of rapid growth. These deformities are characterized by soft loose areas in the skull known as craniotabes and costochondral swelling or bending (known as rachitic rosary). The chest usually becomes flattened, and the sternum may be pushed forward to form the so-called pigeon chest. Thoracic expansion may be greatly reduced with impairment of respiratory function. Kyphosis is a common finding. There is remarkable swelling of the joints, particularly the wrists and ankles, with characteristic anterior bowing of the legs, and fractures of the "greenstick" variety may also be seen. In adults, the symptoms are not as striking and are usually characterized by bone pain, weakness, radiolucent areas, and pseudofractures. Pseudofractures represent stretch fractures in which the normal process of healing is impaired because of a mineralization defect. Mild hypocalcemia may be present; however, hypophosphatemia is the most frequent biochemical alteration. This metabolic abnormality responds well to administration of small amounts of vitamin D.

Vitamin D–Dependent Rickets. These are recessively inherited forms of vitamin D–refractory rickets. The conditions are characterized by hypophosphatemia, hypocalcemia, elevated levels of serum alkaline phosphatase, and sometimes, generalized aminoaciduria and severe bone lesions. Currently, two main forms of vitamin D–dependent rickets have been characterized. The serum concentrations of 1,25-dihydroxycholecalciferol serves to differentiate the two types of vitamin D–dependent rickets. Type I is caused by a mutation in the gene converting 25(OH)D to 1,25-dihydroxycholecalciferol, the renal 1α-hydroxylase enzyme (294,295). This condition responds to very large doses of vitamin D_2 and D_3 (100 to 300 times the normal requirement of physiologic doses), however, 0.5 to 1.0 μg per day of 1,25-dihydroxycholecalciferol. Type II is characterized by an end-organ resistance to 1,25-dihydroxycholecalciferol. Plasma levels of 1,25-dihydroxycholecalciferol are elevated. This finding, in association with radiographic and biochemical signs of rickets, implies resistance to the target tissue to 1,25-dihydroxycholecalciferol. Cellular defects found in patients with vitamin D–resistant rickets type II are heterogeneous, providing in part an explanation for the different clinical manifestations of this disorder. Among the cellular defects are (a) decreased number of cytosolic receptors, (b) deficient maximal hormonal binding, (c) deficient hormone binding affinity, (d) normal hormonal binding but undetectable nuclear localization, and (e) abnormal DNA binding domain for the 1,25-dihydroxycholecalciferol receptor (296).

Numerous studies (297–304) have demonstrated that hereditary type II vitamin D–resistant rickets is a genetic disease affecting the vitamin D receptor (VDR). Defects in the hormone binding domain (297,298) and the DNA binding domain (299,300) have been defined. In addition, several cases of human vitamin D–resistant rickets have been studied and no abnormality in the coding region of the VDR has been found (301), suggesting a defect elsewhere in the hormone action pathway. An unexplained feature of this disease in adolescents is the tendency for calcium levels to normalize and for the radiographic abnormalities of rickets to improve, thus giving the appearance that they outgrow the disease. Human vitamin D–resistant rickets as a genetic defect in the VDR varies significantly from other genetic diseases of steroid hormone receptors caused by resistance to thyroid hormone, androgens, and estrogens (302–304). For example, individuals heterozygous for VDR mutations are apparently completely healthy. Secondly, no dominant negative mutations, which are prominent in thyroid hormone resistance, have been identified as a cause of human vitamin D–resistant rickets. Thus, much remains to be learned from the genetic analysis of this disease. The treatment of this condition requires large pharmacologic doses of calcium, which overcome the receptor defects and maintain bone remodeling (300). Studies in mice with targeted disruption of the *VDR* gene, an animal model of vitamin D–dependent rickets type II, confirm that many aspects of the clinical phenotype are due to decreased intestinal ion transport and can be overcome by adjustments of dietary intake (305).

Malabsorption

Because most of the absorption of phosphorus from the gastrointestinal tract occurs in the duodenum and jejunum, gastrointestinal tract disorders such as celiac disease, tropical and nontropical sprue, and regional enteritis may decrease the absorption of phosphorus (166). Phosphorus malabsorption has also been described in patients who have undergone surgical bypass procedures for morbid obesity. The degree of hypophosphatemia varies among patients with intestinal malabsorption, being extremely mild in some and severe in others.

Malnutrition

Most of the phosphorus ingested in the diet is present in protein, particularly meat, cheese, milk, and eggs. In many parts of the world where protein consumption is extremely low, hypophosphatemia occurs predominantly in children. Overall growth is retarded and a series of metabolic abnormalities are present (306).

Administration of Phosphate Binders

Certain compounds, mainly aluminum salts (aluminum hydroxide, aluminum carbonate gel) and calcium carbonate are used in the treatment of hyperphosphatemia (307). However, when these compounds are given in excess, they may produce profound hypophosphatemia. These gels trap phosphorus in the small intestine and increase the amount of phosphorus in the stool. Patients ingesting large amounts of phosphate binders and not followed closely may develop phosphate depletion. With time, such individuals may develop severe weakness, bone pain, and osteomalacia.

Miscellaneous Causes of Hypophosphatemia

Major reviews of the causes of hypophosphatemia in hospitalized patients (308,309) attributed most instances to *intravenous administration of carbohydrate*. However, many other causes were found, including *diuretic usage, hyperalimentation, alcoholism, respiratory alkalosis, and use of phosphate binders* (310). A 31% incidence of hypophosphatemia was seen in patients admitted to a general medical ward, and a further fall in serum concentrations occurred in all patients with *acute alcoholism* between the second and fifth day after admission

to a medical ward (311). Hypophosphatemia is also seen frequently during treatment of *diabetic ketoacidosis* (312). When diabetic patients develop ketoacidosis, they usually have an increase in phosphate excretion in the urine; however, the serum phosphate level may be slightly elevated due to acidosis. During the administration of insulin, there is a rapid decrease in the level of glucose with translocation of phosphate from the extracellular to the intracellular space, resulting in hypophosphatemia.

Acute respiratory alkalosis decreases urinary phosphate excretion but produces marked hypophosphatemia (313). In contrast, patients who receive sodium bicarbonate excrete large amounts of phosphate in the urine; however, the hypophosphatemia that may develop is only moderate in nature. It has been postulated that in respiratory alkalosis, there is an increase in the intracellular pH level with activation of glycolysis and increased formation of phosphate-containing sugars, leading to a precipitous fall in the concentration of serum phosphorus. The mild hypophosphatemia that may be seen during administration of sodium bicarbonate is probably secondary to increased renal phosphate excretion due to a decrease in ionized calcium and release of PTH, as well as to the consequences of ECF volume expansion.

In addition, new clinical disorders have been identified in which hypophosphatemia is an important aspect of the pathologic condition. Marked hypophosphatemia has been associated with acute leukemia or with *lymphomas in the leukemic phase* (314–316). These individuals typically present with hypophosphatemia, normocalcemia, and no evidence of excess PTH activity. Urinary phosphate concentration is typically extremely low. Although kinetic studies have not been performed in this setting, the facts that serum phosphate concentration correlates with a growth phase of the tumors and that hyperphosphatemia is seen when cells are destroyed by chemotherapy or radiotherapy strongly suggest that serum phosphorus was initially used in the rapid growth of new cells. Because these patients are often severely ill and under treatment with glucose infusions, as well as antacids and other drugs known to induce hypophosphatemia, they may be at great risk of developing severe acute phosphorus depletion.

Another clinical condition in which hypophosphatemia has been a prominent feature is the *toxic shock syndrome*. Chesney et al. (317) described 22 women with this disorder who showed hypocalcemia and hypophosphatemia as prominent manifestations. Whether respiratory alkalosis or staphylococcal sepsis induced release of substances that were responsible for acute phosphorus shifts into cells is unknown. Lindquist et al. (318) studied in a prospective fashion the importance of hypophosphatemia in patients with *severe burns*. In 33 patients studied for 2 weeks after injury, transient hypophosphatemia was seen in the second to tenth day in all these individuals. Five of seven patients who died from complications of the terminal injury had severe hypophosphatemia. Because urinary phosphorus excretion was not increased, tissue uptake seems to be the predominant mechanism responsible for the hypophosphatemia. Levy (319) reported the occurrence of severe hypophosphatemia during the *rewarming phase in a profoundly hypothermic patient*. In this individual, urinary excretion of phosphorus was minimal, suggesting that a shift of phosphate into the cells occurred as a result of rewarming. Finally, the development of hypophosphatemia resulting from refeeding clinically starved patients has been emphasized. Silvis et al. (320) showed that the classic phosphorus-depletion syndrome, consisting of paresthesias, weakness, seizures, and hypophosphatemia, can occur in individuals who receive oral caloric supplements after a prolonged period of starvation. To further evaluate this issue, they performed studies in normal dogs that had been starved or had received normal diets and found that the infusion of calories through an intragastric catheter to previously starved animals resulted in a fall in serum phosphorus concentration from an average of 4.8 mg/dL to 1.6 mg/dL. Nearly 50% of starved animals developed clinical signs of phosphate depletion after oral refeeding. Weinsier and Krumdiek reported two patients who developed the phosphorus-depletion syndrome in association with cardiopulmonary decompensation following overzealous hyperalimentation after prolonged caloric deprivation (321).

Clinical and Biochemical Manifestations of Hypophosphatemia

The manifestations of hypophosphatemia are presented in Table 89-2. It has been suggested that the clinical manifestations of hypophosphatemia and severe phosphorus depletion are related to disturbances in cellular energy and metabolism. Studies have examined the effects of phosphate depletion on cellular energetics and other components of cell function. A study of glycolytic intermediates and adenine nucleotides during insulin treatment of patients with diabetic ketoacidosis emphasized the important effects of insulin-induced cellular phosphate depletion on cell metabolism (322). These results demonstrated that the reduced level of 2,3-diphosphoglycerate (2,3-DPG) seen during insulin treatment of diabetes is due to intracellular phosphorus depletion, producing a decrease in glyceraldehyde 3-phosphate dehydrogenase activity rather than inhibition of the phosphofructokinase enzyme system. Ditzel (323) has suggested that repeated transient decreases in red cell oxygen delivery due to reduced 2,3-DPG with insulin-induced hypophosphatemia could contribute over many years to the microvascular disease seen in diabetic patients. Patients with mild degrees of hypophosphatemia are usually asymptomatic. However, if hypophosphatemia is severe—that is, if serum phosphorus levels are less than 1.5 mg/dL—a series of hematologic, neurologic, and metabolic disorders may develop. In general, the patients become anorectic and weak, and mild bone pain may be present if the hypophosphatemia persists for several months (Table 89-2).

Cardiovascular and Skeletal Muscle Manifestations

Severe cardiomyopathy with decreased cardiac output has been described in patients and animals with severe hypophosphatemia (324,325). Studies revealed that the resting muscle membrane potential fell, sodium chloride and water content of the tissue increased, and potassium content decreased in severe hypophosphatemia (326). These values returned to within the normal range after phosphate was administered. Skeletal muscle weakness and electromyographic abnormalities are associated with chronic hypophosphatemia and phosphate depletion. Dogs that were fed low-phosphate diets for several months developed changes in muscle, rhabdomyolysis, and characteristic increases in their levels of creatinine kinase and aldolase in blood (327). Rhabdomyolysis has been observed in alcoholic patients with hypophosphatemia (328). Knochel et al. (327) showed that myopathy associated with phosphate depletion in dogs did lead to changes in cell water content, sodium concentration, and transmembrane potential difference. Kretz et al. (329) examined the possibility that changes in calcium transport in the sarcoplasmic reticulum of muscle were responsible for the clinical myopathy seen in acute phosphate depletion. Despite significant hypophosphatemia and a reduction in muscle phosphorus concentration, they found no significant

TABLE 89-2

CLINICAL AND BIOCHEMICAL MANIFESTATIONS OF MARKED HYPOPHOSPHATEMIA

I. Cardiovascular and skeletal muscle
   A. Decreased cardiac output
   B. Muscle weakness
   C. Decreased transmembrane resting potential
   D. Rhabdomyolysis

II. Carbohydrate metabolism
   A. Hyperinsulinemia
   B. Decreased glucose metabolism

III. Hematologic alterations
   A. Red blood cells
      1. Decreased adenosine triphosphate (ATP) content
      2. Decreased 2,3-DPG
      3. Decreased P_{50}
      4. Increased oxygen affinity
      5. Decreased lifespan
      6. Hemolysis
      7. Spherocytosis
   B. Leukocytes
      1. Decreased phagocytosis
      2. Decreased chemotaxis
      3. Decreased bactericidal activity
   C. Platelets
      1. Impaired clot retraction
      2. Thrombocytopenia
      3. Decreased ATP content
      4. Megakaryocytosis
      5. Decreased lifespan

IV. Neurologic manifestations
   A. Anorexia
   B. Irritability
   C. Confusion
   D. Paresthesias
   E. Dysarthria
   F. Ataxia
   G. Seizures
   H. Coma

V. Skeletal abnormalities
   A. Bone pain
   B. Radiolucent areas (x-ray)
   C. Pseudofractures
   D. Rickets or osteomalacia

VI. Biochemical and renal manifestations
   A. Low parathyroid hormone levels
   B. Increased $1,25(OH)_2D_3$
   C. Hypercalciuria
   D. Hypomagnesemia
   E. Hypermagnesuria
   F. Hypophosphaturia
   G. Decreased glomerular filtration rate
   H. Decreased T_m for bicarbonate
   I. Decreased renal gluconeogenesis
   J. Decreased titratable acid excretion
   K. Increased creatinine phosphokinase
   L. Increased aldolase

(From: Slatopolsky E. Pathophysiology of calcium, magnesium, and phosphorus. In: Klahr S, ed. *The Kidney and Body Fluids in Health and Disease*. New York: Plenum Press; 1983:269, with permission.)

changes in the rate of calcium uptake of calcium-concentrating ability in vesicles prepared from muscle sarcoplasmic reticulum of phosphate-depleted rats. Thus, the role of altered transcellular calcium movements in phosphate-depleted tissues is yet to be completely resolved.

Effects on Carbohydrate Metabolism

Hyperinsulinemia and abnormal glucose metabolism suggesting insulin resistance have been described in phosphate depletion. DeFronzo and Lange (330) have used the glucose and insulin clamp technique to study the kinetics of glucose metabolism in patients with various chronic hypophosphatemic conditions including vitamin D–resistant rickets. When glucose was infused to maintain constant glycemia at 125 mg/dL, hypophosphatemic individuals required 36% less glucose to maintain these glycemic levels than controls. Also when euglycemic was achieved by combined insulin and glucose infusion, the hypophosphatemic individuals required 40% less glucose to maintain euglycemia than controls. Insulin catabolism was apparently unaffected in these hypophosphatemic individuals. These data indicate that hypophosphatemia is associated with impaired glucose metabolism in both hyperglycemic and euglycemic patients.

Hematologic Manifestations

Hematologic abnormalities of hypophosphatemia are a major manifestation of this syndrome (331–333). In addition to defects in affinity of oxyhemoglobin leading to generalized tissue hypoxia, there may be increased hemolysis (334,335). Quantitative and functional defects have also been described in platelets and leukocytes (336). These defects lead to diminished platelet aggregation and abnormalities in chemotaxis and phagocytosis of white blood cells. The latter may contribute to the increased risk of Gram-negative sepsis reported in hypophosphatemic patients (337). This is of particular concern in immunosuppressed patients receiving phosphate-poor alimentation through a central venous line.

Neurologic Manifestations

Manifestations at the level of the central nervous system, resulting in generalized anorexia and malaise or more severe disturbances such as ataxia, seizures, and coma, have been described in hypophosphatemia (338–340). Neuromuscular abnormalities include paresthesias and weakness, the result of both myopathic changes and diminished nerve conduction (341).

Skeletal Abnormalities

The skeletal abnormalities associated with hypophosphatemia, particularly in vitamin D–resistant rickets, may be quite marked. In addition, bony abnormalities, including osteomalacia and pathologic fractures, have been described in antacid-induced phosphate depletion (342,343), as well as in hypophosphatemic patients undergoing hemodialysis who did not receive phosphate binding gels (344). A rheumatic syndrome resembling ankylosing spondylitis also has been reported in hypophosphatemic patients (345).

Gastrointestinal Disturbances

These manifestations include anorexia, nausea, and vomiting (339). It has been speculated that hypophosphatemia in the alcoholic patient may further impair hepatic function through hypoxic insult.

Renal Manifestations

There is decreased phosphorus excretion and decreased tubular reabsorption of calcium, magnesium, bicarbonate, and glucose (346–351). The renal conservation of phosphorus occurs early in the syndrome and is the result of a primary increase in the tubular reabsorption of the anion and a decrease in the GFR and consequently in the filtered load of phosphorus (351,352). This mechanism results in complete renal conservation of phosphorus, with net losses representing only a small fraction of total body phosphorus stores (339). The increase in phosphorus reabsorption seen with phosphorus depletion is independent of several hormones known to influence phosphorus transport under other circumstances, including PTH, vitamin D, calcitonin, and thyroxine (353). The possibility that serum phosphorus concentration per se (or intracellular phosphorus) may in some manner regulate its absorption along the nephron seems plausible. Hypercalciuria of enough magnitude to produce a negative calcium balance is seen commonly in hypophosphatemic patients. Several factors contribute to this increase in calcium excretion including increased calcium mobilization from bone, enhanced gastrointestinal tract calcium absorption, and inhibition of renal tubular calcium reabsorption (346–351). These effects appear to be independent of PTH activity and may be the result of a direct effect of phosphate on these transport processes.

Acid–Base Disturbances

Renal bicarbonate wasting, diminished titratable acid excretion, and decreased ammoniagenesis have been reported in hypophosphatemia (347,354). However, these defects are counterbalanced to some extent by the mobilization of alkali from bone. Thus, steady-state pH may be near normal at the expense of skeletal buffers (348).

Differential Diagnosis of Hypophosphatemia

In general, the cause of hypophosphatemia can be determined either from the medical history or from the clinical setting in which it occurs. When the cause is in doubt, measurement of the urinary phosphorus excretion level may be helpful. If the urinary phosphorus concentration is less than 4 mg/dL when the serum phosphorus level is less than 2 mg/dL, renal losses may be excluded (355). Of the three major extrarenal causes including diminished phosphorus intake, increased extrarenal losses (gastrointestinal tract), and translocation into the intracellular space, the last is the most common, particularly in the hospitalized patient (308,356). When the urinary phosphorus excretion level is high, the differential diagnosis includes hyperparathyroidism, a primary renal tubular abnormality, or vitamin D–dependent or –resistant renal rickets. Measurements of serum calcium, PTH, and vitamin D and its metabolites, as well as urinary excretion of other solutes (glucose, amino acids, and bicarbonate) will usually elucidate the underlying disturbance that is responsible for the hypophosphatemia.

Treatment of Hypophosphatemia

There are several general principles that apply to the treatment of hypophosphatemic patients. As with any predominantly intracellular ion (e.g., potassium), the state of total body phosphorus stores, as well as the magnitude of phosphorus losses, cannot be readily assessed by measurement of the concentrations in serum. In fact, under conditions in which a rapid shift of phosphorus has resulted from glucose infusion or hyperalimentation, total body stores of phosphorus may be normal, although with diminished intake and renal losses, there may be severe phosphorus depletion. Furthermore, the volume of distribution of phosphorus may vary widely, reflecting in part the intensity and duration of the underlying cause (356).

In clinical situations in which hypophosphatemia is to be expected (e.g., glucose infusion or hyperalimentation in the alcoholic or nutritionally compromised patient during treatment of diabetic ketoacidosis), careful monitoring of the concentration of serum phosphorus is crucial. In these situations, addition of phosphorus supplementation to prevent the development of severe hypophosphatemia may prove very helpful. Certainly, other contributing causes of hypophosphatemia in this setting should be identified and treated. This is particularly true of the use of phosphate binding antacids (aluminum and magnesium hydroxide) for peptic ulcer disease, which may be replaced by aluminum phosphate antacids (Phosphagel) or cimetidine (Tagamet). It is now generally recommended that hyperalimentation solutions contain a phosphorus concentration of 12 to 15 mmol/L or 37 to 46.5 mg/dL, in order to provide an appropriate amount of phosphorus in the patient in whom renal impairment is absent (356). Phosphorus supplementation during glucose infusion or during the treatment of ketoacidosis is usually withheld until the serum phosphorus levels decrease to less than 1 mg/dL. Phosphorus may be given orally to these patients and others with mild asymptomatic hypophosphatemia in the form of skim milk, which contains 0.9 mg/mL, Neutraphos (3.3 mg/mL), or phosphorus soda (129 mg/mL). However, intestinal absorption is quite variable, and diarrhea often complicates the oral administration of phosphate-containing compounds. For these reasons, parenteral administration is usually recommended in the hospitalized patient. If oral therapy is permissible, Fleet Phospho-soda may be given at a dosage of 60 mmol daily in three doses (21 mmoL/5 mL or 643 mg/5 mL). A convenient method is to provide the phosphorus together with potassium replacement in these patients. Addition of 5 mL of potassium phosphate (K phosphate) into 1 L of intravenous fluid provides 22 mEq of potassium and 15 mmol (466 mg) of phosphorus (356). However, because potassium losses may greatly exceed the phosphorus deficit, the repletion of potassium should not be totally linked to phosphorus therapy. In patients with severe phosphate depletion, it is difficult to determine the magnitude of the total deficit of phosphorus and to calculate a precise initial dose. It is usually prudent to proceed with caution and repair the deficit slowly. The most frequently recommended regimen is 0.08 mmol/kg of body weight (2.5 mg/kg body weight) given over 6 hours for severe but uncomplicated hypophosphatemia and 0.016 mmol/kg of body weight (5 mg/kg of body weight) in symptomatic patients (356). Parenteral administration should be discontinued when the serum phosphorus concentration is greater than 2 mg/dL.

Calcium administration may be needed during phosphate repletion to prevent severe hypocalcemia. Calcium must not be added to bicarbonate- or phosphate-containing solutions because of the potential precipitation of calcium salts. Intravenous infusion of calcium gluconate or calcium chloride may be given until tetany abates. In addition to hypocalcemia,

metastatic calcification, hypotension, hyperkalemia, and hypernatremia are potential side effects of parenteral infusion of phosphorus. These problems can be prevented by judicious use of therapy and frequent monitoring of serum electrolyte concentrations.

Hyperphosphatemia

Hyperphosphatemia is said to occur when the serum phosphorus concentration exceeds 5 mg/dL in adults. It should be remembered that in children and adolescents, serum levels of phosphorus of up to 6 mg/dL may be physiologic. The most frequent cause of hyperphosphatemia is decreased excretion of phosphorus in the urine as a result of a fall in the GFR. However, increases in serum phosphorus concentration can also occur as a result of increased entry into the ECF due to excessive intake of phosphorus or increased release of phosphorus from tissue breakdown. The major causes of hyperphosphatemia are listed in Table 89-3.

Decreased Excretion of Phosphorus in Urine

Decreased Renal Function

In progressive renal failure, phosphorus homeostasis is maintained by a progressive increase in phosphorus excretion per nephron (357,358). As a result of this increased phosphorus excretion, it is unusual to see marked hyperphosphatemia until GFRs decrease to less than 25 mL per minute (357,358). Under physiologic conditions with a GFR of 120 mL per minute, a fractional excretion of 5% to 15% of the filtered load of phosphorus is adequate to maintain phosphorus homeostasis. However, as renal insufficiency progresses and the number of nephrons decreases, fractional excretion of phosphorus may increase to as high as 60% to 80% of the filtered load. This progressive phosphaturia per nephron, as renal disease progresses, serves to maintain the concentration of phosphorus within normal limits in plasma. However, when the number of nephrons is greatly diminished, if the dietary intake of phosphorus remains constant, phosphorus homeostasis can no longer be maintained and hyperphosphatemia develops. This usually occurs when the GFR falls to less than 25 mL per minute. As hyperphosphatemia develops the filtered load of phosphorus per nephron increases, phosphorus excretion rises and phosphorus balance is reestablished but at higher concentrations of serum phosphorus. Hyperphosphatemia is a usual finding in patients with far-advanced renal insufficiency unless phosphorus intake in the diet has decreased through dietary manipulations or the patient is receiving phosphate binders such as calcium carbonate or aluminum-containing salts that will decrease the absorption of phosphate from the gastrointestinal tract (359). In patients with acute renal failure, hyperphosphatemia is a usual finding (360). The degree of hyperphosphatemia in patients with acute renal failure varies considerably. It is quite marked in patients with renal insufficiency secondary to severe trauma or non-traumatic rhabdomyolysis, as frequently occurs in patients ingesting large amounts of alcohol or in heroin addicts (361). The degree of hyperphosphatemia depends on the amount of phosphorus released from damaged tissue because phosphorus intake and decreased GFR, to less than 2 mL per minute, are constant across different forms of oliguric acute renal failure. In most patients with acute renal failure, hyperphosphatemia is transitory, and serum phosphorus values return toward the normal range as renal function improves. However, in some of these patients, infection or tissue destruction resulting from many causes may maintain relatively high serum phosphorus values even during the recovery phase of renal function.

TABLE 89-3

CAUSES OF HYPERPHOSPHATEMIA

I. Decreased renal excretion of phosphate
   A. Renal insufficiency
      1. Chronic
      2. Acute
   B. Hypoparathyroidism
   C. Pseudohypoparathyroidism
      1. Type I
      2. Type II
   D. Abnormal circulating parathyroid hormone
   E. Acromegaly
   F. Tumoral calcinosis
   G. Administration of bisphosphonates

II. Increased entrance of phosphorus into the extracellular fluid
   A. Neoplastic diseases
      1. Leukemia
      2. Lymphoma
   B. Increased catabolism
   C. Respiratory acidosis

III. Increased intake and GT absorption of phosphorus
   A. Pharmacologic administration of Vitamin D metabolites
   B. Ingestion and/or administration of phosphate salts

IV. Miscellaneous
   A. Cortical hyperostosis
   B. Intermittent hyperphosphatemia
   C. Artifacts

(From: Slatopolsky E. Pathophysiology of calcium, magnesium, and phosphorus. In: Klahr S, ed. *The Kidney and Body Fluids and Disease*. New York: Plenum Press; 1983:269, with permission.)

Decreased or Absent Levels of Circulating PTH

Hypoparathyroidism is characterized by low or absent levels of PTH, low levels of serum calcium, and hyperphosphatemia (362). The most common causes of hypoparathyroidism results from injury to the parathyroid glands, or their blood supply during thyroid, parathyroid, or radical neck surgery. Idiopathic hypoparathyroidism is a rare disease. Because PTH normally inhibits the renal reabsorption of phosphorus, its absence leads to an elevation in the Tm for phosphorus and a decrease in the excretion of the anion in the urine. Balance is reestablished when the serum phosphorus concentration rises to 6 to 8 mg/dL. At this concentration of serum phosphorus, the filtered load of phosphate is increased, exceeding the Tm for phosphorus reabsorption, and a new steady-state is reestablished. Patients with hypoparathyroidism are easily diagnosed by the findings of a low level of serum calcium, hyperphosphatemia, and undetectable levels of circulating immunoreactive PTH. After several years of hypoparathyroidism, other signs may become manifest such as cataracts and bilateral symmetrical calcification of the basal ganglia on x-ray films of the skull. The most striking symptoms in patients presenting with hypoparathyroidism are related to an increase in neuromuscular

excitability resulting from a decrease in the levels of ionized calcium in serum. Some patients may not develop hypocalcemia and severe tetany, but increased neuromuscular excitability may be demonstrated by contraction of facial muscles in response to stimulus over the facial nerve (Chvostek's sign) or by carpal spasm (Trousseau's sign) occurring 2 or 3 minutes after inflating a blood pressure cuff around the arm above systolic blood pressure. In other patients, psychiatric disturbances, paresthesias, numbness, muscle cramps, and dysphagia may be presenting symptoms.

Pseudohypoparathyroidism

This is a relatively rare condition characterized by end-organ resistance to the action of PTH (363). Characteristically, the kidney and skeleton do not respond appropriately to the action of PTH. Some patients with pseudohypoparathyroidism may have specific somatic characteristics such as short stature, round face, short metacarpal bones and phalanges, and some degree of mental retardation. Biochemically, these patients, like those with hypoparathyroidism, have low concentrations of serum calcium and hyperphosphatemia. However, there are two important points in the differential diagnosis. First, in most patients with pseudohypoparathyroidism, the circulating levels of immunoreactive PTH are elevated, whereas in patients with true hypoparathyroidism PTH levels are low or absent. Second, patients with pseudohypoparathyroidism do not respond to the administration of exogenous PTH with phosphaturia. Patients with true hypoparathyroidism demonstrate a heightened phosphaturic response to administration of exogenous PTH. Two major types of pseudohypoparathyroidism have been described. In type I, patients fail to increase the excretion of cAMP or phosphate in the urine in response to the administration of exogenous PTH. This abnormal response seems to be related, at least in some of these patients, to a defect in the guanosine triphosphate (GTP) binding protein, G_S, of the adenylate cyclase complex (364,365). In other patients, there is an increase in cAMP in response to the administration of exogenous PTH but no phosphaturic response. This condition has been termed *pseudohypoparathyroidism* type II (366).

Abnormal Circulating PTH

This syndrome is characterized by hyperphosphatemia, hypocalcemia, chronic tetany, and cataracts. These manifestations, as described previously, are those observed in patients with hypoparathyroidism, but these patients have normal or high serum levels of PTH. However, in contrast to patients with pseudohypoparathyroidism, they do respond to the exogenous administration of PTH, with an increase in the excretion of cAMP and phosphaturia. It has been postulated that the defect in these patients relates to an abnormal form of endogenous PTH that is devoid of physiologic effects (367). However, this postulate has not been substantiated by characterization and analysis of the circulating PTH in these patients.

Acromegaly

Growth hormone decreases the urinary excretion of phosphorus and increases the Tm for phosphorus (368). Hypersecretion of growth hormone may lead to development of gigantism if the increased secretion occurs before the closure of the epiphysis or to acromegaly if the excessive secretion occurs after puberty. Hyperphosphatemia has been described in patients with acromegaly. It is known that serum phosphorus concentrations are higher in children (5 to 8 mg/dL) than in adults. This may be related in part to increased levels of circulating growth hormone in children.

Tumoral Calcinosis

The pathogenesis of this disease is probably related to a primary increase in phosphorus reabsorption by the kidney (369). This condition, which is seen more frequently in young African Americans, is characterized by hyperphosphatemia, ectopic calcification around large joints, normal levels of circulating immunoreactive PTH, and a normal response to administration of exogenous PTH (370,371). The extensive calcification of soft tissues observed in patients with this condition is most likely due to an elevated phosphorus–calcium product in blood. Despite the development of hyperphosphatemia, patients with tumoral calcinosis do not develop secondary hyperparathyroidism. This may be due to the fact that circulating levels of 1,25-dihydroxycholecalciferol remain within the normal range in these patients despite hyperphosphatemia. These normal levels of 1,25-dihydroxycholecalciferol maintain a normal gastrointestinal tract absorption of calcium. This, combined with the decreased urinary calcium observed in these patients, may serve to maintain normal serum calcium values and prevent the development of secondary hyperparathyroidism.

Familial Tumoral Calcinosis (FTC)

FTC is inherited in an autosomal recessive mode, but autosomal dominant inheritance has also been reported (372–378). The disease most commonly appears before the second decade of life. The subjects present with periarticular calcified masses often localized in the hip, elbow and shoulder. This disorder is associated with hyperphosphatemia and increased renal tubular Pi reabsorption, but with normal serum levels of calcium and parathyroid hormone. Serum levels of 1,25-dihydroxyvitamin D may be normal or elevated.

Recently bialleic mutations in the UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetylgalactosaminyltransferase 3 (*GALNT3*) gene have been identified in two large families as a cause of FTC (379). *GALNT3* encodes a glycosyltransferase responsible for initiating mucin-type O-glycosylation. Since the FTC phenotype is regarded as the metabolic mirror image of XLH, ADHR, and TIO involving abnormal FGF-23 and PHEX expression, recent studies have tried to identify disorders in these genes in subjects with FTC. In fact two recent studies have identified FGF-23 missense mutations in FTC. While wild-type FGF-23 is secreted as intact protein and then processed as N-terminal and C-terminal fragments, the mutated FGF-23 protein is retained in the Golgi complex and only the inactive C-terminal protein is secreted (380,381). The resultant phenotype, therefore, resembles the FGF-23 null mice described earlier.

Administration of Bisphosphonates

Administration of bisphosphonates, which are used in the treatment of Paget's disease and osteoporosis, may result in the development of hyperphosphatemia (382). The mechanisms by which bisphosphonates increase serum phosphorus are not completely clear but may involve an alteration in phosphate distribution between different cellular compartments and a decrease in renal phosphorus excretion. It appears that the levels of both circulating PTH and the urinary excretion of cAMP after administration of exogenous PTH are within the normal range in patients receiving bisphosphonates.

Redistribution of Phosphorus between Intracellular and Extracellular Pools

Tumor Lysis Syndrome

Various syndromes of tissue breakdown may result in the development of hyperphosphatemia and subsequent hypocalcemia. Hyperphosphatemia has been described in patients with several types of lymphomas. Patients receiving treatment for lymphoblastic leukemia may develop hyperphosphatemia with a concomitant decrease in serum calcium concentration (383). The phosphorus load originates primarily from the destruction of lymphoblasts, which have about four times the concentration of organic and inorganic phosphorus present in mature lymphocytes.

Similar findings have been described during treatment of Burkitt's lymphoma. Cohen et al. (384) reviewed the acute tumor lysis syndrome associated with the treatment of Burkitt's lymphoma. In 37 patients with American Burkitt's lymphoma, azotemia occurred in 14 patients and preceded chemotherapy in 8. Pretreatment of azotemia was associated with elevated levels of lactate dehydrogenase (LDH) and uric acid and sometimes extrinsic ureteral obstruction by the tumor. After chemotherapy, major metabolic complications related to tumor lysis were associated with large tumors and high LDH levels and were manifested by hyperkalemia, hyperphosphatemia, and hyperuricemia. Elevated phosphorus levels were seen in 31% of nonazotemic patients and in all azotemic patients. Hemodialysis was required in three patients for control of azotemia, hyperuricemia, hyperphosphatemia, or hyperkalemia.

Tsokos et al. (385) studied the renal metabolic complications of other undifferentiated lymphomas and lymphoblastic lymphomas. These workers found that serum LDH concentration before chemotherapy correlated well with the stage of disease and predicted the serum levels of creatinine, uric acid, and phosphorus in the posttreatment period. Patients with LDH values of more than 2,000 IU were likely to develop severe hyperphosphatemia. When azotemia developed in the postchemotherapy period, it was attributed to hyperuricemia or hyperphosphatemia. Some of these patients had elevated serum phosphorus levels in the range of 20 to 30 mg/dL, which may contribute to the development of renal insufficiency due to calcium deposition in the kidney and other tissues.

Thus, there is a *great* risk of hyperphosphatemia in patients undergoing chemotherapy for rapidly growing malignant lymphomas. The best method of prevention of this complication, as well as the best therapeutic intervention, has not been well defined. Initially, it appears useful to attempt to increase the renal excretion of phosphate during the induction of remission by chemotherapy in these patients. This requires infusion of large amounts of saline and possibly bicarbonate, which has been shown to increase renal phosphorus excretion above and beyond the mere effects of volume expansion. Acetazolamide, a potent phosphaturic agent, might also be beneficial in these individuals. The general recommendation of hemodialysis as the prime therapeutic modality for hyperphosphatemia and acute renal insufficiency resulting from tumor lysis is not based on experimental data. Although hemodialysis no doubt rapidly lowers serum phosphorus levels, the mass of phosphorus continually presented to the extracellular space from ongoing tissue breakdown is not continuously treated by this modality. Thus, it is possible that combined hemodialysis and peritoneal dialysis, or even peritoneal dialysis alone, might be as, if not more, beneficial and safer in individuals with tumor lysis syndrome.

Increased Catabolism

Conditions characterized by increased protein breakdown (e.g., severe tissue muscle damage and severe infections) may sometimes be accompanied by hyperphosphatemia. Although the hyperphosphatemia may be related simply to translocation of phosphorus into the extracellular space, other factors seem to play a role. Hyperphosphatemia has been described in patients with ketoacidosis before treatment. After administration of intravenous fluids and insulin therapy, the entrance of glucose into the cells is usually followed by movement of phosphorus back into the intracellular space, and some patients now may develop hypophosphatemia. Thus, the combination of dehydration, acidosis, and tissue breakdown in different catabolic states may lead to hyperphosphatemia.

Respiratory Acidosis

Acute respiratory acidosis may lead to a marked increase in serum phosphorus concentration (386). By contrast, chronic respiratory acidosis is usually not manifested by sustained elevated levels of serum phosphorous. Acute rises in P_{CO_2} in experimental animals have been shown to lead to increased serum phosphorus levels. The modest degree of hyperphosphatemia seen in chronic respiratory acidosis is probably related to renal compensation and increased phosphorus excretion via the kidney to maintain phosphorus homeostasis.

Increased Intake and GI Absorption of Phosphorus

Administration of Phosphate Salts or Vitamin D or Its Metabolites

Administration of vitamin D_3 or its metabolites, particularly 1,25-dihydroxycholecalciferol, may result in increases in serum phosphorus, particularly in uremic patients. These compounds very likely may result in hyperphosphatemia in uremic individuals by increasing phosphorus absorption from the gut and perhaps by potentiating the effect of PTH on the skeleton with increased release of phosphorus from bone. Decreased renal function limits the compensatory mechanism of the kidney to excrete the increased load of phosphate entering the extracellular space. In addition to elevating serum phosphorus levels, vitamin D metabolites may result in hypercalcemia. An increase in the phosphorus–calcium product may result in tissue deposition of calcium, particularly in the kidney, leading to further renal functional deterioration.

Ingestion or Administration of Salts Containing Phosphate

Hyperphosphatemia has been observed in adults ingesting laxative-containing phosphate salts or after administration of enemas containing large amounts of phosphate (387,388). Intravenous phosphate administration has been used in the treatment of hypercalcemia of malignancy. The administration of 1 to 2 g of phosphate intravenously decreases the concentration of serum calcium. Unfortunately, the severe hyperphosphatemia induced by administration of large amounts of phosphorus intravenously may lead to calcium-phosphate precipitation in important organs such as the heart and kidney, and several deaths resulting from this form of therapy have been reported. Hyperphosphatemia may develop in newborn infants who are fed cow's milk, which is higher in phosphorus content than human milk. This may be an important factor in the genesis of neonatal tetany.

Clinical Manifestations of Hyperphosphatemia

Most of the clinical effects of hyperphosphatemia are related to secondary changes of calcium metabolism. Hyperphosphatemia produces hypocalcemia by several mechanisms (Fig. 89-6), including decreased production of 1,25-dihydroxycholecalciferol, precipitation of calcium, and decreased absorption of calcium from the gastrointestinal tract, presumably due to a direct effect of phosphorus on calcium absorption (389). In addition to the manifestations by hypocalcemia, which are described elsewhere in this chapter, ectopic calcification is one of the important manifestations of hyperphosphatemia. The association of hyperphosphatemia and ectopic calcification has been observed in several clinical settings including in patients with chronic renal failure, hypoparathyroidism, and tumoral calcinosis. It appears that when the calcium–phosphorus product exceeds 70, the likelihood for calcium precipitation is greatly increased. In addition to the calcium–phosphorus product, local tissue factors may play an important role in calcium deposition. For example, regional changes in pH (local alkalosis) may favor calcification in tissue such as cornea and lungs. In patients with severe calcification (calciphylaxis), it appears that high levels of circulating PTH may also aggravate this condition. Hyperphosphatemia plays a key role in the development of secondary hyperparathyroidism in patients with renal insufficiency. It has been observed that when phosphate ingestion is decreased and hyperphosphatemia is prevented in experimental animals with induced renal insufficiency, hyperparathyroidism can be prevented (390). The mechanisms presumably relate to maintenance of serum calcium levels with prevention of hyperphosphatemia and, at the same time, continued synthesis of 1,25-dihydroxycholecalciferol, the circulating levels of which may directly influence the secretion of PTH (391,392). Several investigators (393–396) have demonstrated that dietary phosphate markedly influences the rate of parathyroid cell proliferation and PTH synthesis and secretion independent of changes in ionized calcium or 1,25-dihydroxycholecalciferol. It seems that the mechanism by which phosphorus increases PTH synthesis and secretion is posttranscriptional. Moreover, in experimental uremic rats, it has been shown that phosphate restriction suppresses parathyroid cell growth by inducing p21, a repressor of the cell cycle. On the other hand a high-phosphate intake rapidly (3 to 5 days) induces significant parathyroid cell hyperplasia by inducing an increase in transforming growth factor α (TGFα) (397). TGFα, which is known to promote growth not only in malignant transformation but also in normal tissues (398,399), is enhanced in hyperplastic and adenomatous human parathyroid glands (400). In patients on chronic hemodialysis, the degree of hyperparathyroidism correlates well with the concentration of serum phosphorus. Patients who do not adhere to their therapeutic prescriptions requiring ingestion of phosphate binders seem to develop more severe and persistent hyperphosphatemia with marked secondary hyperparathyroidism and bone disease than patients who adhere carefully to dietary and therapeutic prescriptions. Vascular calcification has been observed in some patients with chronic renal insufficiency and severe calcification, hyperphosphatemia, and hyperparathyroidism, leading to necrosis and gangrene of extremities. Slit-lamp examination may show ocular calcification, and some patients may develop acute conjunctivitis, the so-called red eye syndrome of uremia. Precipitation of calcium in the skin may be in part responsible for pruritus, a symptom that is usually seen in patients with far-advanced uremia. It has been reported that parathyroidectomy in such patients may alleviate the symptoms. From the therapeutic point of view, the most efficacious way of controlling hyperphosphatemia is through the use of phosphate binders that decrease the absorption of phosphorus from the gastrointestinal tract. In patients with adequate renal function, expansion of the ECF with saline will greatly increase phosphorus excretion in the urine and contribute to correction of the hyperphosphatemia.

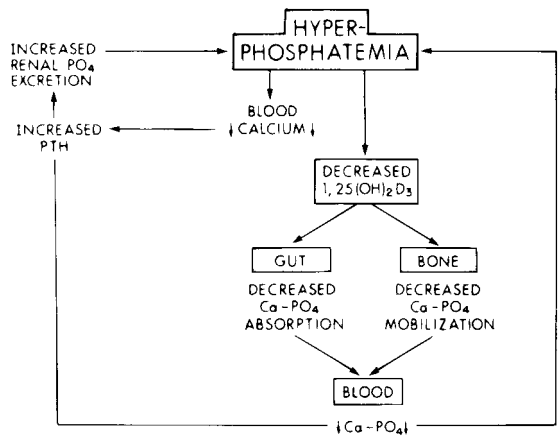

FIGURE 89-6. Pathophysiologic changes occurring during the development of hyperphosphatemia. These changes tend to increase the urinary excretion of phosphorus and to correct the hyperphosphatemia.

Treatment of Hyperphosphatemia

Decreased absorption of phosphate from the gastrointestinal tract is a cornerstone of treatment of hyperphosphatemia. Phosphate absorption from the gastrointestinal tract can be markedly decreased by decreasing the amount of phosphorus in the diet, by administering phosphate binding agents capable of decreasing absorption of phosphorus, or both. Because protein requirements limit the amount of phosphorus restriction that can be achieved through dietary manipulation, from a practical point of view, administration of agents capable of decreasing phosphorus absorption from the gastrointestinal tract is the mainstay of treatment. Administration of calcium salts has replaced aluminum salts as the traditional treatment to control hyperphosphatemia. Most of these preparations require the administration of two to four tablets or capsules three or four times daily. If the patient develops constipation, one of the complications of such medications, magnesium salts may be incorporated into these preparations. However, if the patient has hyperphosphatemia secondary to severe renal insufficiency, magnesium should not be given because of the likelihood of producing severe hypermagnesemia, which may lead to magnesium intoxication, muscle paralysis, and death.

The elucidation of aluminum toxicity, which results from prolonged administration of aluminum-containing salts, as phosphate binders to patients with chronic renal insufficiency, has led to diminished use of these agents or their elimination (401,402). Several studies indicate that calcium carbonate (403–405) is an effective agent for control of hyperphosphatemia in chronic renal failure. However, numerous investigators have demonstrated an increase in the number of aortic and mitral valve calcifications in patients on dialysis when compared with the general population. Cardiovascular events are responsible for a 40% to 60% mortality rate of patients on dialysis (406–409). Morbidity and mortality rates increase as the Ca–PO_4 product raises to more than 60. Braun et al. (410), with the use of the electron beam computed tomography, demonstrated a significant deposition of calcium in the coronary arteries of patients on dialysis. Although coronary artery calcifications worsen with age, this abnormality has been

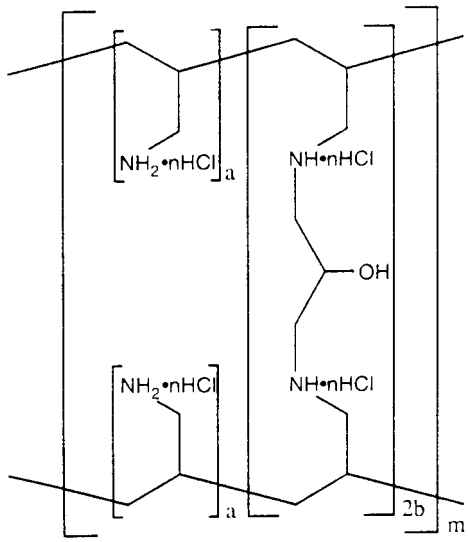

FIGURE 89-7. Structure of sevelamer hydrochloride (Renagel), cross-linked poly(allylamine hydrochloride).

demonstrated in young patients (411). In fact, postmortem examination of children with renal failure demonstrated that 60% to 70% had calcification of the heart, lungs, and blood vessels (412). Positive calcium balances of 500 to 900 mg daily were demonstrated in uremic patients receiving large doses of calcium carbonate (405). Thus, it is critical not only to reduce the Ca–PO_4 product to less than 60, but also to significantly decrease the calcium load that patients receive to control serum phosphorus.

To avoid these deleterious side effects, a well-tolerated calcium albumin–free phosphate binder has been developed, sevelamer hydrochloride (Renagel). This phosphate binder is a hydrogel of cross-linked poly(allylamine hydrochloride) that is resistant to digestive degradation and is not absorbed from the gastrointestinal tract (Fig. 89-7). Its mechanism of action relates to the presence of partially protonated amines spaced one carbon from the polymer backbone, which interact with phosphate ions by ionic and hydrogen bonding. Several short-term clinical studies in patients with end-stage renal disease have established that sevelamer hydrochloride is an effective phosphate binder without increasing the calcium load to the patients (413,414) (Fig. 89-8). In addition, sevelamer hydrochloride decreases low-density lipoprotein cholesterol by 30% to 40%, and in long-term studies increases high-density lipoprotein cholesterol by 20% to 30%; it does not affect triglycerides (415). Studies in progress will provide critical information on its potential efficacy in reducing morbidity and mortality rates in patients on dialysis.

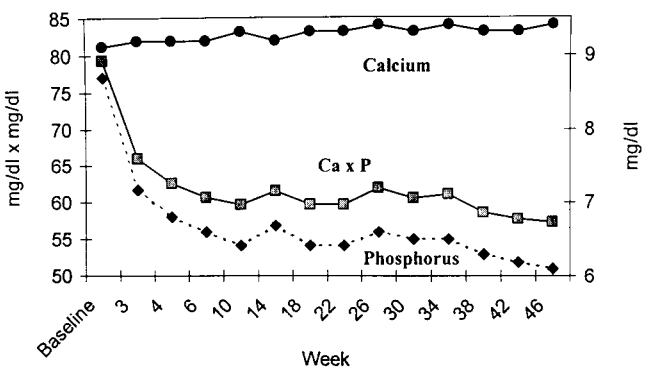

FIGURE 89-8. Effects of sevelamer hydrochloride (Renagel) on serum calcium, phosphorus, and calcium–phosphate product in a group of 192 patients on hemodialysis. (From: Chertow GM, Burke SK, Dillon MA, et al. Long-term effects of sevelamer hydrochloride on the calcium × phosphate product and lipid profile of haemodialysis patients. *Nephrol Dial Transplantation* 1999;14:2907, with permission.)

A recent study has shown that nicotinamide may also be an affective agent to decrease serum phosphorus levels. Because nicotinamide is an inhibitor of sodium-dependent phosphate cotransport in rat renal tubule and small intestine (416,417), the authors examined whether nicotinamide reduces serum levels of phosphorus and intact parathyroid hormone (iPTH) in patients undergoing hemodialysis. Sixty-five hemodialysis patients with a serum phosphorus level of more than 6.0 mg/dL after a 2-week washout of calcium carbonate were enrolled in this study. Nicotinamide was administered for 12 weeks. The starting dose was 500 mg/day, and the dose was increased by 250 mg/day every 2 weeks until serum phosphorus levels were well controlled at less than 6.0 mg/dL. A 2-week posttreatment washout period followed the cessation of nicotinamide. Blood samples were collected every week for measurement of serum calcium, phosphorus, lipids, iPTH, and blood nicotinamide adenine dinucleotide (NAD). The mean dose of nicotinamide was 1080 mg/day. The mean blood NAD concentration increased from 9.3 ± 1.9 nmol/105 erythrocytes before treatment to 13.2± 5.3 nmol/105 erythrocytes after treatment. The serum phosphorus concentration increased from 5.4 ± 1.5 mg/dL to 6.9 ± 1.5 mg/dL with the pretreatment washout, then decreased to 5.4 ± 1.3 mg/dL after the 12-week nicotinamide treatment, and rose again to 6.7 ± 1.6 mg/dL after the posttreatment washout. Serum calcium levels decreased during the pretreatment washout from 9.1 ± 0.8 mg/dL to 8.7 ± 0.7 mg/dL with the cessation of calcium carbonate. No significant changes in serum calcium levels were observed during nicotinamide treatment. Median serum iPTH levels increased with pretreatment washout from 130.0 (32.8 to 394.0) pg/mL to 200.0 (92.5 to 535.0) pg/mL and then decreased from the maximum 230.0 (90.8 to 582.0) pg/mL to 150.0 (57.6 to 518.0) pg/mL after the 12-week nicotinamide treatment. With nicotinamide, serum high-density lipoprotein (HDL) cholesterol concentrations increased from 47.4 ± 14.9 mg/dL to 67.2 ± 22.3 mg/dL and serum low-density lipoprotein (LDL) cholesterol concentrations decreased from 78.9 ± 18.8 mg/dL to 70.1 ± 25.3 mg/dL; serum triglyceride levels did not change significantly (418). This study, therefore, demonstrates that nicotinamide may provide an alternative for controlling hyperphosphatemia and hyperparathyroidism without inducing hypercalcemia in hemodialysis patients.

Although decreased gastrointestinal absorption of phosphorus is an effective way to control hyperphosphatemia in patients with renal insufficiency, excretion of phosphorus through the kidney is also an important mechanism. Thus expansion of the ECF volume may markedly increase phosphorus excretion by the kidney. This result is presumably related both to direct effects of volume expansion on the kidney, which decreases salt and water reabsorption and hence phosphorus reabsorption, and to increased PTH release, particularly as a consequence of decreased ionized calcium during volume expansion. In patients with marked renal insufficiency or with marked degrees of hyperphosphatemia due to tumor lysis or chemotherapy, peritoneal dialysis or hemodialysis may be used to remove large quantities of phosphorus from the extracellular space. Redistribution of phosphorus from the intracellular to the extracellular space can sometimes be rapidly corrected by the administration of glucose and insulin. In general, mild degrees of hyperphosphatemia can be tolerated, particularly if calcium levels are not markedly elevated. The goal in patients with chronic renal insufficiency is to keep phosphorus levels at less

than 4.5 mg/dL to avoid falls in serum ionized calcium and marked development of severe hyperparathyroidism.

CALCIUM

Calcium, the most abundant cation of the body and the principal mineral of the human skeleton, is essential to the integrity and function of cell membranes, neuromuscular excitability, transmission of nerve impulses, multiple enzymatic reactions, and regulation of hormones such as PTH, calcitonin, and 1,25-dihydroxycholecalciferol. A complex homeostatic system involving the interplay of the bones, the kidneys, and the intestine has evolved to maintain calcium concentrations within a narrow range.

Distribution of Calcium

The total amount of calcium in the human body ranges from 1,000 to 1,200 g or 20 to 25 g/kg of fat-free body tissue. Approximately 99% of body calcium resides in the skeleton; the other 1% is present in the extracellular and intracellular spaces. About 1% of the calcium in the skeleton is freely exchangeable with calcium in the ECF. Together, these two fractions are known as the miscible pool of calcium and account for 2% of total body calcium. Calcium in bone is found primarily in the form of small crystals similar to hydroxyapatite, although some calcium exists as amorphous crystals in combination with phosphate. The normal calcium:phosphate ratio in bone is 1.5:1.

Extracellular Calcium

In humans, the serum calcium concentration is kept remarkably constant, between 9.0 and 10.4 mg/dL, or 4.5 to 5.2 mEq/L, or 2.25 to 2.6 mmol/L. About 50% of serum calcium is ionized and 10% is complexed with citrate, phosphate, bicarbonate, and lactate. These two fractions, ionized plus complexed calcium (*ultrafilterable calcium*), make up approximately 60% of the total serum calcium. The rest, 40%, is protein bound, mainly to albumin. In hypoproteinemic states, such as the nephrotic syndrome or cirrhosis, although total serum calcium may be low, the ionized fraction may be within the normal range. Five percent to 10% of the calcium is bound to globulins. It is unusual for total serum calcium concentrations to change because of alterations in the levels of serum globulins. However, in severe hyperglobulinemia, such as may occur in patients with multiple myeloma or other dysproteinemias, elevations of total serum calcium concentrations may be observed.

One gram of albumin binds approximately 0.8 mg of calcium; thus, at the normal plasma albumin concentration of 4.0 to 4.5 g/dL, only 10% to 15% of the binding sites for calcium are occupied. Consequently, when excess calcium is added to blood *in vitro* or *in vivo,* all the fractions increase in the same proportion, and the ultrafilterable fraction as a percentage of the total calcium concentration does not change. The binding of calcium by albumin, therefore, acts as a buffer that reduces by about half the potential changes in ionized calcium that may result from acute gains or losses of calcium by the blood. The most important factor modifying the binding of calcium to albumin is the pH of plasma. Alkalosis increases the binding of free calcium, resulting in a fall in ionized calcium concentration; acidosis has the opposite effect. This is due not only to competition between H^+ and Ca^{2+} for binding sites on albumin but also to changes in the conformation of the albumin molecule. Changes in P_{CO_2} do not affect calcium binding other than through changes in pH. In the past, ionized calcium was difficult to measure. This difficulty has been overcome by the use of sensitive flow-through electrodes, which can measure changes in ionized calcium of as small as 0.1 mg/dL.

Intracellular Calcium

Calcium is the major intracellular ionic messenger for the activation of many biologic processes (419). The intracellular concentration of calcium is approximately 0.15 μM (Fig. 89-9). Cells extrude calcium via pumps or exchangers, sequester it in intracellular organelles, or use low-affinity binding sites with large capacities to maintain free calcium, Ca^{2+}, at the 0.15 μM level (420,421). Intracellular calcium is complexed with ions such as orthophosphate or pyrophosphate and is bound to organic molecules such as ATP and proteins. Three major cellular calcium pools exist: (a) bound to multiple diverse sites, (b) sequestered in intracellular organelles, and (c) bound or free within the cytosol (419).

Extrusion of Ca^{2+} from the cell and sequestration in intracellular organelles are transport functions generally carried out by two mechanisms, Na^+-Ca^{2+} exchange and Ca-ATPase (Fig. 89-9) (420–426). In cardiac muscle, nerve, brain, and kidney, calcium extrusion is directly coupled to sodium transport (422,427). The Na^+-Ca^{2+} transport system depends on the asymmetric distribution of Na^+ across the plasma membrane. The Na-K-ATPase of the plasma membrane is involved in the metabolic process necessary to maintain the Na^+ gradient. Thus, the movement of Na^+ into the cell is coupled to the flux of Ca^{2+} out of the cell. This Na^+-Ca^{2+} antiport system is electrogenic with a stoichiometry of three Na^+ per Ca^{2+} (427,428). A second and more ubiquitous mechanism of calcium efflux energizes uphill transport of calcium by the hydrolysis of high-energy–yielding phosphate bonds of ATP (425,426,429).

In the kidney, considerable progress has been made in clarifying the physiologic role of these transporters responsible in Ca^{2+} efflux from renal epithelial cells (426,430–432). In addition to involvement in regulating cytosolic Ca^{2+}, these exchangers participate in the extrusion of Ca^{2+} from basolateral plasma membranes into the blood upon stimulation of transcellular Ca^{2+} absorption. Such an action is likely in cortical thick ascending limbs (TALs), distal convoluted tubules, or (rabbit) connecting tubules, that is, those segments in which transcellular Ca^{2+} absorption is found. The cloning of cardiac, renal, and other forms of the Na^+-Ca^{2+} exchanger, NCX (422,427,433), has led to improved information regarding the role of this transporter. Three genes encoding NCX, designated *NCX1, NCX2,* and *NCX3,* have been identified in mammals (434). Only *NCX1* is found in the kidney, where it is mainly localized to the distal nephron along the basolateral membrane (433). This is in concordance with evidence for ATP-dependent Ca^{2+} extrusion, but not for Na^+-Ca^{2+} exchange in rabbit cortical TALs (435,436). In contrast, removal of basolateral Na^+ inhibited Ca^{2+} absorption and increased cytosolic Ca^{2+} in rabbit distal convoluted tubules and connecting tubules, consistent with the presence of Na^+-Ca^{2+} exchange in these nephron segments (437–440). Such observations are compatible with the view that basolateral membrane Ca^{2+}-ATPase may mediate cellular Ca^{2+} extrusion in cortical TALs, whereas Na^+-Ca^{2+} exchange and Ca^{2+}-ATPase may be responsible for Ca^{2+} efflux in distal convoluted tubules and connecting tubules. Na^+-Ca^{2+} exchanger transport activity increases in response to PTH (441–443). The development of specific NCX inhibitors may enable utilization of compounds to prevent the role of NCX in pathologic cell Ca^{2+} overload such as in ischemia/reperfusion injury (434).

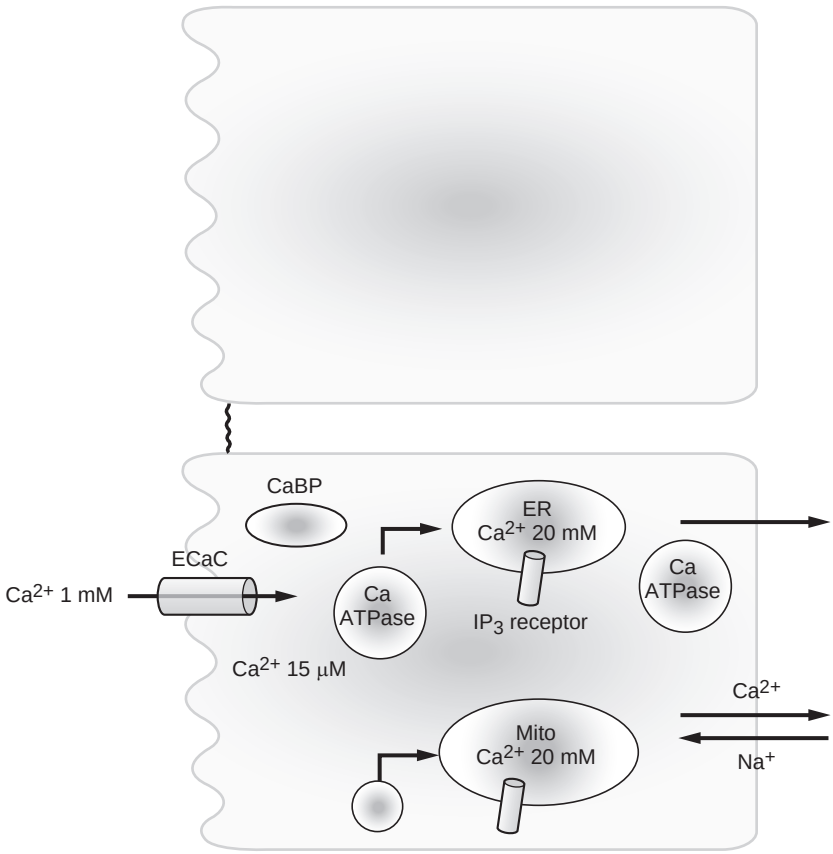

FIGURE 89-9. Control of intracellular calcium. The distal tubule epithelial cell is portrayed as an example of cellular control of cytosolic Ca^{2+} concentrations. Cells extrude Ca by energy (ATP) dependent pumps to maintain cytosolic levels at the 0.15 μM range. Intracellular stores in the endoplasmic reticulum (ER) and the mitochondria (Mito) have pumps to load in Ca and release channels, the IP_3 receptor and the Ryanodine receptor for the ER. Entry of calcium is controlled by entry channels, TRPV5 (which was originally called ECaC) in the case of the distal tubule epithelium and Na/Ca exchange transporters. Ca entering the cell is sequestered by vesicles enriched in Calbindin 8K (CaBP) or 25K in the kidney and intestine respectively.

The plasma membrane Ca^{2+}-ATPase (PMCA) is a P-type ATPase (444,445,446). In human kidneys, all four known isoforms (447) have been found with the highest staining for immunoreactive Ca^{2+}-ATPase along basolateral membranes of the distal convoluted tubules (448). These observations and others demonstrating PMCA1B transcripts in rabbit connecting tubule and cortical collecting duct suggest that human distal convoluted tubules express the Ca^{2+}-ATPase immunologically similar to that of human erythrocyte membranes. Immunologically distinct epitopes of the Ca^{2+}-ATPase are probably expressed on proximal tubule membranes and in other nephron segments. Magocsi et al. (449) have studied the localization of mRNAs encoding isozymes of plasma membrane Ca^{2+}-ATPases in rat kidney. Message for the first isoform of the rat plasma membrane Ca^{2+}-ATPase (RPMCA1) was found in the cortex and outer and inner medulla, whereas, RPMCA2 was abundant in the cortex and outer medulla. Transcripts for the third isoform, RPMCA3, were conspicuous in the outer medulla. mRNA for RPMCA2 was detected in the distal convoluted tubules and in the cortical TALs by *in situ* reverse transcription polymerase chain reaction. This is discordant with the rabbit, but it is possible that RPMCA2 may be specifically related to epithelial cells that are engaged in active Ca^{2+} absorption (449).

The cytosolic calcium concentration is also maintained by an active transport into mitochondria and the endoplasmic reticulum (Fig. 89-9). It has been shown that mitochondria accumulate Ca^{2+} through a Ca-uniporter, with Ca^{2+} moving down an electrochemical gradient. The K_m for Ca^{2+} of the uniporter is about 1 μmmol/L. Mitochondria also contain an Na^+-Ca^{2+} exchange mechanism. In the mitochondria, calcium and phosphate ions form insoluble amorphous tricalcium phosphate, a reaction that releases hydrogen ions into the cytosol. Cell injury may lead to a rise in intracellular calcium sufficient for Ca^{2+} to be sequestered in the mitochondria (450). Ca^{2+} is sequestered in the endoplasmic reticulum by the action of a Ca-ATPase, which differs in properties from that found on the plasma membrane and Golgi apparatus (451,452). During the early response of cells to certain stimuli, production of IP_3 and cyclic adenosine diphosphate ribose stimulates the opening of Ca^{2+} channels in the endoplasmic reticulum, serving to transiently increase cytosolic Ca^{2+} and allow the ion to act as an intracellular signal. Recently, polycystin-2 has been identified as an IP_3 sensitive ER Ca release channel (453). Polycystin-2 is the product of the gene mutated in type 2 autosomal dominant polycystic kidney disease (ADPKD) (453). This identifies polycystin-2 as a critical regulator of renal tubular epithelial cell Ca signaling, and suggests that disordered Ca signaling during development leads to polycystic kidney disease.

Skeletal Calcium

More than 99% of the total body calcium is found in the skeleton. Bone consists of approximately 40% mineral, 30% organic matrix, and 30% water. Bone mineral exists in two physical forms, the amorphous and the crystalline. The amorphous form consists mainly of brushite and tricalcium phosphate; the crystalline form is composed mainly of hydroxyapatite. More than 90% of the organic material of the bone matrix is in the form of collagen fibers that are arranged in bundles with specific interaction with hydroxyapatite. Crystalline skeletal Ca is the huge Ca depot that is slowly exchangeable with blood and interstitial fluid pools of extracellular Ca. A large rapidly exchangeable Ca pool is also found in the skeleton (Fig. 89-10). The nature of the freely exchangeable calcium pool in bone is unknown, but it is unlikely to be collagen-associated hydroxyapatite. The Ca pool is likely amorphous and found associated with areas of active bone formation (mineralization

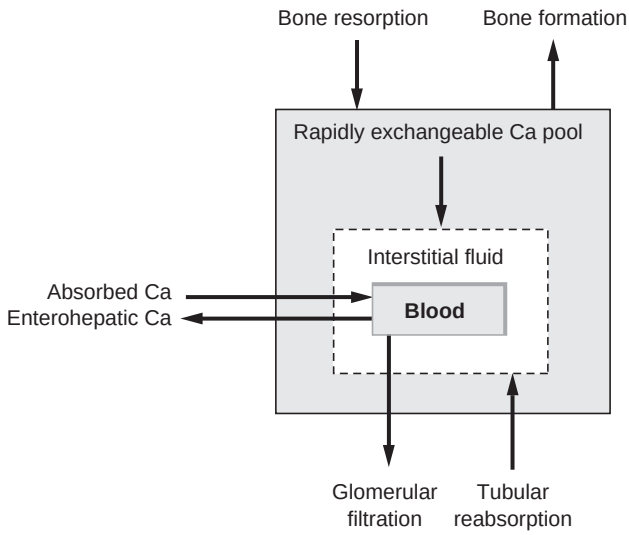

FIGURE 89-10. The exchangeable Ca pool. Absorbed Ca from the intestine enters the interstitial fluid and blood compartments. These compartments are in equilibrium with a larger complexed Ca compartment located in the mineralization fronts at sites of skeletal remodeling and bone formation. Ca leaves the exchangeable pool by the enterohepatic circulation, glomerular filtration and bone formation. Besides intestinal absorption, Ca enters the exchangeable pool by bone resorption and tubular reabsorption.

fronts) where Ca is being deposited into the crystalline (poorly exchangeable) pool of bone.

A coupled process of bone resorption and formation (remodeling) is responsible for exit of calcium from the exchangeable pool (bone formation) and release of skeletal calcium (bone resorption) into the exchangeable pool (bone resorption), but, bone remodeling probably contributes little to minute-to-minute control of serum calcium. Pathologic states in which bone resorption is greatly increased (i.e., when bone resorption is greater than bone formation) produce profound changes in calcium homeostasis. In states wherein bone formation is decreased and bone resorption continues in excess, (i.e., the renal adynamic bone disorder), hypercalcemia is often observed (454). Bone remodeling is a coupled process because the activation of a remodeling unit sets two cell differentiation programs into operation–that of the osteoblast and that of the osteoclast. Bone marrow stromal cells, the osteoprogenitors that will become osteoblasts, harbor the receptors that are recognized by the factors capable of activating bone remodeling. Their stimulation results in the synthesis of a cell-attached ligand for RANK (receptor for activation of nuclear factor kappa B) on osteoclast progenitors, known as RANK ligand (RANKL) (455–457). RANKL and macrophage colony-stimulating factor (MCSF-1) are the critical osteoclast differentiation factors, and these local bone marrow factors are sufficient to direct osteoclast formation. Thus, stimulation of osteoblastic cells leads to stimulation of osteoclasts, and the process of skeletal remodeling represents bone formation and bone resorption.

The osteoclasts responsible for bone resorption are multinucleated giant cells lying in irregular indentations of the bone surface known as Howship's lacunae. Bone resorption depends on the number and activity of osteoclasts. The process of bone resorption performed by the osteoclasts includes the production of an acidic environment by proton secretion and matrix degradation by cathepsin K. The osteoblasts, on the other hand, are the cells responsible for the repair process after bone resorption (bone formation). Differentiation of the cells in the osteoblast lineage begins with specification of mesenchymal stromal cells to the lineage by expression of osteoblast-specific transcription factors—Cbfa1/Osf2 (458) and Osterix (459). Cbfa1 expression is stimulated by the bone morphogenetic protein subfamily of the TGFβ superfamily responsible for the direction of osteoblast differentiation and bone formation. Cells early in the process of osteoblast differentiation initiate bone matrix production by the biosynthesis of collagen. Thereafter, the matrix is mineralized by the deposition of calcium and phosphate, with formation of amorphous material initially and then development of hydroxyapatite. The deposition of mineral occurs along a well-defined front ("mineralization front"), outside of which there is an osteoid border or seam. The osteoid begins to calcify about 10 days after deposition. From the architectural point of view, the skeleton is composed of two types of bone: (a) compact cortical bone, which surrounds the marrow cavity and forms the shaft of the long bones, and (b) cancellous or trabecular bone, which is the main component of flat bones, such as ribs, and vertebra.

A differentiation between two other general types of bone is critical in the diagnosis of metabolic bone disease. The first, called *woven bone* (immature bone) (460), is a loosely organized, highly mineralized bone in which the collagen fibers are coarsely arranged and the osteocytes are large and irregular in size and shape. Woven bone is formed by simultaneous and unorganized actions of many cells. The calcification of the tissue is patchy, occurring in a speckled pattern and independent of the presence of vitamin D activity. Woven bone is present in the fetus, but after age 14 is no longer found in the human skeleton, except with pathologic conditions such as chronic kidney disease, Paget's disease, hyperparathyroidism and during rapid bone turnover, as in the presence of healing fractures (460–462). The second general type of bone is lamellar bone (mature bone), which is the major component of the normal adult skeleton. It is a highly organized tissue in which the collagen bundles are arranged in successive layers, between which are cells called *osteocytes*. Lamellar bone is the product of synchronized activity by the osteoblast depositing collagen materials at a specific cell surface.

Another difference between woven bone and lamellar bone relates to the relation of mineral to collagen. In lamellar bone, the relative amounts of collagen and minerals are closely related, making hypermineralization in these bones difficult. Mineralization of woven bone is disorderly, and the degree of mineralization varies enormously; thus, hypermineralization (osteosclerosis) may occur in this type of bone (460,461,463).

PTH, in conjunction with PTH-related peptide (PTHrP), other locally produced cytokines, and vitamin D, play key roles in bone turnover. At physiologic doses, PTH has an anabolic effect, increasing bone formation. Thus, PTH, by increasing calcium reabsorption by the kidney and gut and through stimulation of the osteoblast, affects the rate of bone formation. However, in pathologic conditions (e.g., hyperparathyroidism), the concentration of PTH in serum may be increased 10- to 50-fold. At this high concentration, PTH increases the activity and number of osteoclasts; thus, bone resorption predominates over bone formation, and minerals and organic matrix are removed from bone and enter the ECF. Not only PTH but also other hormones such as PTH-related proteins, thyroxine, interleukin-1 (IL-1), and tumor necrosis factor can produce severe hypercalcemia by increasing the activity of osteoclasts. The bone remodeling in these conditions, such as renal failure, is characterized by increased woven bone (463–465).

Calcium Balance

Approximately 700 to 2,000 mg of calcium is ingested daily in the diet. However, this amount may vary depending on the amount of milk consumed. Milk and cheese are the major

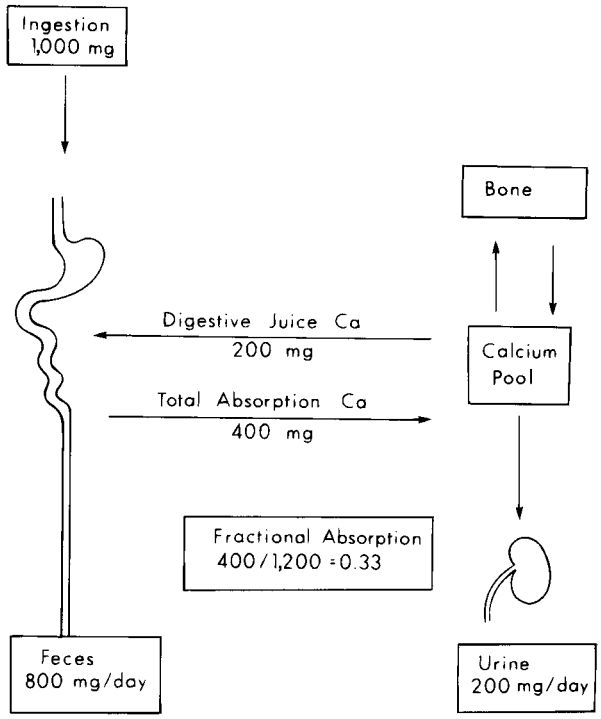

FIGURE 89-11. Diagrammatic representation of calcium metabolism in humans showing the contribution of the gastrointestinal tract, the kidney, and bone to the maintenance of the calcium pool.

sources of calcium, contributing 50% to 70% of the total amount ingested in the diet. In the United States, 1 L of milk contains approximately 800 to 900 mg of calcium. About 10 to 15 mg of calcium per kilogram of body weight is the recommended daily intake. Ca needs may vary widely, for instance during the last trimester of pregnancy, there is an increased requirement for calcium because approximately 20 to 30 g of calcium enters the fetus. Transfer of calcium from the mother to milk during lactation is another instance of increased Ca demand (466). In these states, intestinal absorption may not be sufficient and regulation of extracellular Ca by the calcium sensor, PTH and PTHrP, regulate the intestine and the skeleton to supply calcitriol, the needed Ca. With age, active intestinal calcium absorption declines; thus, an increase in calcium intake may be necessary to maintain calcium homeostasis. When 1 g of calcium is ingested in the diet, approximately 800 mg is excreted in the feces and 200 mg in the urine (Fig. 89-11). With a normal calcium intake (700 to 1,000 mg per day), approximately 30% to 40% of ingested calcium is absorbed in the intestine. However, on lower calcium diets, the percentage of calcium absorbed increases, and the percentage of calcium absorbed decreases when the diet has a high calcium content (more than 1,500 mg per day). The mechanisms responsible for this adaptation have been partially characterized and require the participation of PTH, vitamin D, and perhaps calcitonin. With low-calcium diet feeding, mild hypocalcemia activates the parathyroid gland chief cell Ca sensor and the release of PTH, which increases the conversion of 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol in the renal cortex. 1,25-Dihydroxycholecalciferol is the hormonal metabolite of vitamin D, and it increases the intestinal absorption of calcium and mobilizes calcium from bone, synergistically with PTH. Thus, serum calcium levels return to normal. On the other hand, if the patient is fed a high-calcium diet, the mild hypercalcemia inhibits the chief cell Ca sensor, suppressing PTH, and stimulates the release of calcitonin from the C cells of the thyroid. In the absence of PTH, the activity of the 1α-hydroxylase is diminished and the 24-hydroxylase is activated; thus, the kidney makes preferentially 24,25-dihydroxycholecalciferol [24,25$(OH)_2D_3$], which is less efficient than 1,25-dihydroxycholecalciferol in promoting calcium absorption from the gastrointestinal tract and mobilizing calcium from the skeleton. Fecal calcium consists of the fraction of ingested calcium that is not absorbed plus 100 to 200 mg of calcium secreted by the intestine daily. The secreted digestive juice calcium is known as *endogenous fecal calcium*. The amount of calcium secreted by the intestine is fairly constant and is not greatly influenced by hypercalcemia.

Intestinal Calcium Absorption

The mechanisms of calcium transport across the intestinal mucosa are complex, but our understanding of the physiology is rapidly improving. Intestinal calcium absorption occurs by two general mechanisms: active and passive transport (467,468) (Fig. 89-12). The passive process involves paracellular movement of calcium in some intestinal segments, and active transport involves movement through mucosal epithelial cells. When the intestine is perfused *in vitro* with increasing calcium concentrations, the rate of movement of calcium from the mucosa to the serosa increases without evidence of saturation or a maximum transport rate. An active transport process would be expected to be saturable. It has been estimated that at luminal calcium concentrations of more than 7.0 mmol/L, calcium is transported primarily by a diffusional process (469). This suggests that in regions of the intestine such as the ileum, where the calcium concentration is high, the passive transport process predominates. In the duodenum and jejunum, where the luminal calcium concentration is lower than 6.0 mmol/L, the active transport process assumes a predominant role (470).

Active intestinal calcium transport involves three steps: (a) the transport of calcium from the lumen into the cell; (b) the movement of calcium within the cell; and (c) the movement of calcium from the cell into the interstitial fluid (Fig. 89-12). Insulation of the cell interior from the millimolar Ca concentrations of plasma suggests that a brush-border component is instrumental in the transfer of calcium into the epithelial cell. The transfer of calcium across the intestinal brush-border surface is modulated by vitamin D (471–473). The early effects of 1,25-dihydroxycholecalciferol on calcium transport are mediated by changes in the structure of the luminal membrane of the intestine (474). Administration of 1,25-dihydroxycholecalciferol leads to an increase in *de novo* synthesis and total content of phosphatidylcholine of the brush-border membrane. These changes in lipid structure precede or occur simultaneously with the change in calcium transport rate (474). However, the major mechanism of calcium entry across the intestinal enterocyte brush border of the duodenum, proximal jejunum, and cecum is through a channel, CaT1 (475,476), which shares high homology (75%) with the renal tubular epithelial calcium channel (ECaC). These channels have been redesignated as TRPV6 for CaT1 and TRPV5 for ECaC (436,477). The TRPV acronym stands for the Transient Release Potential (TRP), family of Ca channels of the Vanilloid (V) receptor type, TRPV (477). TRPV6 is voltage-dependent and permeant to Sr and Ba but not Mg. It is inhibited by the trivalent cations Gd and La, and the divalent Cd and Co (432,475,476).

Less is known about the movement of calcium within the intestinal cell. When calcium enters the cell, it either diffuses or is carried across the cell to the basolateral membrane, where it is pumped out into the serosal medium. Studies suggest that calcium entering through the apical membrane is accumulated in subcellular organelles within the terminal web of the microvillus. This process is stimulated by 1,25-dihydroxycholecalciferol through nongenomic mechanisms

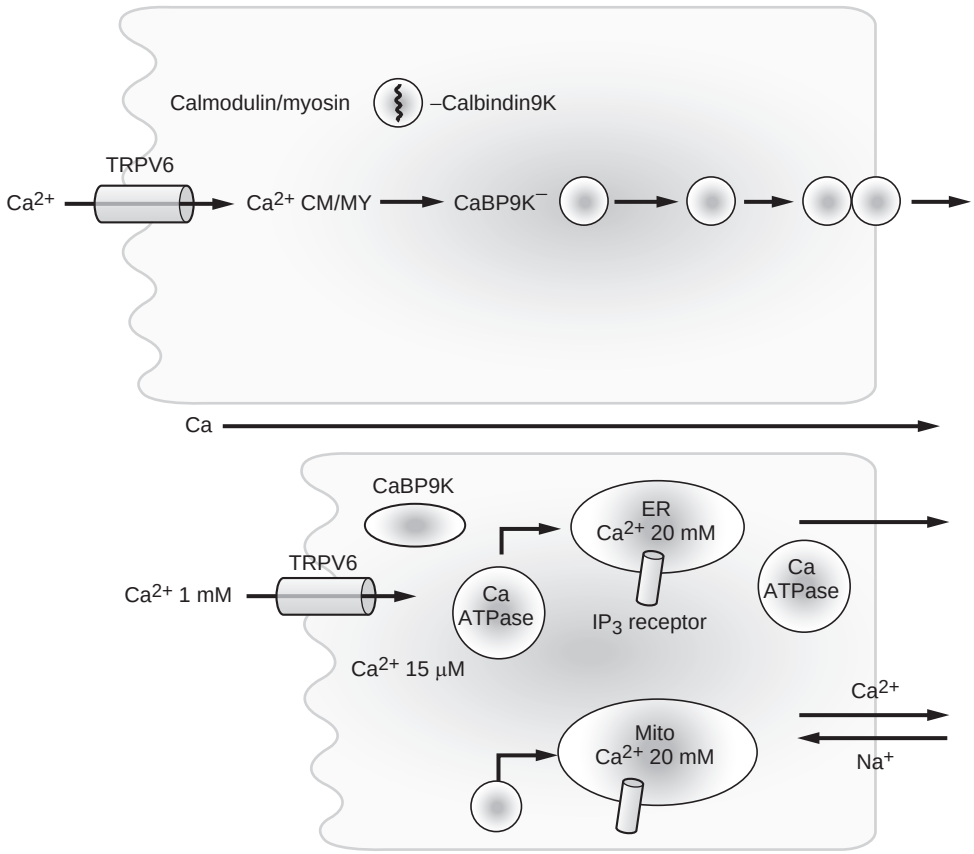

FIGURE 89-12. Intestinal Ca transport in jejunal enterocytes. Ca transport may be through the paracellular space or by an active process through the cell. Transport of Ca from the jejunal lumen through the microvillar membrane is through the TRPV6 channel. Ca is shuttled down the microvillar stalk by calmodulin/myosin (CM/MY) to the glycocalyx where it is bound to calbindin 9K and vesicle sequestered, Ca vesicles deliver Ca to the endoplasmic reticulum and to the efflux pathway, a Ca ATPase in the basolateral membrane.

(478). Calmodulin is the major calcium binding protein in the microvillus (479,480). Its concentration in the microvillus is increased by 1,25-dihydroxycholecalciferol by redistribution from the cytosol. No new calmodulin synthesis is required or observed after 1,25-dihydroxycholecalciferol administration (480). Calmodulin is thought to play a major role in calcium transport within the microvillus, whereas calbindin is thought to be the dominant calcium binding protein in the cytoplasm. The hypothesis put forth by Bikle et al. (479,480) is that calmodulin and myosin 1 regulate calcium movement within the microvillus to where calcium accumulates within intracellular organelles through the action of calbindin. Movement in the intracellular organelle provides calcium access to the efflux mechanisms. Thus, calcium is transported across the cell without affecting cytoplasmic calcium levels. Specific calcium binding proteins have been demonstrated in the mucosal cells of the intestine of many species (481–484). Their molecular weights are 8,000 to 25,000 and they are referred to as calbindins. Calbindins are transcriptionally regulated by vitamin D, and calbindin-$_{9K}$ is present in intestinal mucosal cells, whereas calbindin-$_{25K}$ is present in distal renal tubular cells involved in active transepithelial Ca^{2+} transport and the brain, but not in bone or other cells. The time course of the calbindins' appearance after vitamin D treatment is similar to the time course of changes in calcium transport; and they are localized in the glycocalyx surface of the brush border of the mucosal intestinal cells. The exact role of these proteins in calcium transport by mucosal cells is still unknown, but it appears to be related to movement of calcium from the entry channel to a shuttle mechanism delivering it to the cell exit mechanism (Fig. 89-12). Increased intestinal calcium absorption is accompanied by an increase in calbindin levels without changes in their intrinsic binding affinity (K_m) for calcium.

Calcium movement from the mucosa to the serosal surface of the intestinal epithelia occurs against a concentration gradient. This suggests that the intestinal cells contain a "pump" capable of moving calcium against an electrochemical gradient (420). The basolateral membrane of intestinal cells has a calcium-dependent ATPase that serves this pump function and whose activity is increased by vitamin D (429). The increase in calcium ATPase parallels the change in calcium transport after vitamin D repletion. Delivery of intracellular calcium to the exit pump is a process largely unknown but appears to involve calbindins.

Many factors regulate intestinal calcium absorption including (a) dietary calcium intake; (b) vitamin D intake; (c) age of the patient; (d) the general state of calcium balance; and (e) circulating levels of PTH, which all affect active transport. In addition to PTH and vitamin D, other factors such as phosphate influence calcium absorption. High-phosphate diets decrease calcium absorption, possibly due to the formation of relatively insoluble calcium–phosphate complexes that decrease the availability of calcium for transepithelial uptake and to decreased 1,25-dihydroxycholecalciferol synthesis secondary to hyperphosphatemia. Experimentally, large concentrations of lactose or other sugars (mannose, xylose) or certain amino acids (lysine, arginine) inhibit intestinal calcium absorption. The physiologic significance of these observations is unknown. The decreased calcium absorption produced by glucocorticoids has therapeutic implications in the management of hypercalcemic disorders associated with excessive intake or increased sensitivity to vitamin D.

Renal Handling of Calcium

In humans who have a GFR of 170 L per 24 hours and serum ultrafilterable calcium concentrations of 6 mg/dL, roughly 10 g of calcium is filtered per day. The amount of calcium excreted in the urine usually ranges from 100 to 200 mg per 24 hours;

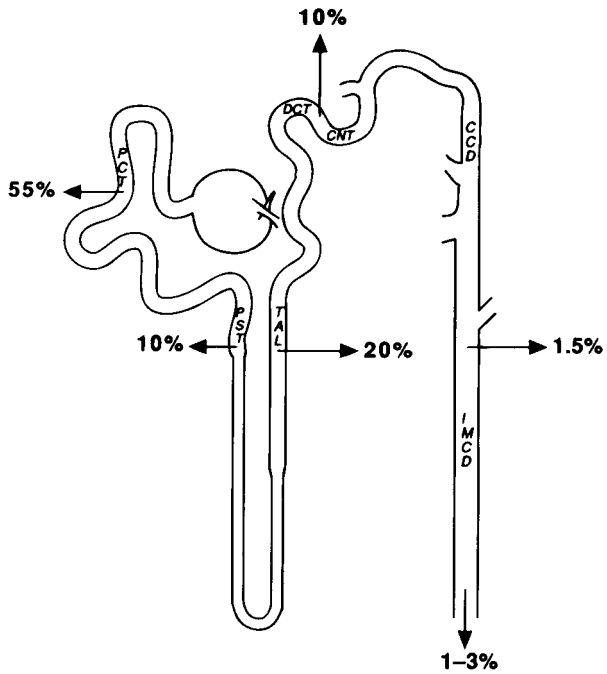

FIGURE 89-13. Schematic illustration of the reabsorption of calcium by different segments of the nephron. *CCD*, cortical collecting duct; *CNT*, connecting tubule; *DCT*, distal convoluted tubule; *IMCD*, intermedullary collecting duct; *PCT*, proximal convoluted tubule; *PST*, proximal straight tubule; *TAL*, thick ascending limb. (From: Friedman P, Gesek F. Calcium transport in renal epithelial cells. *Am J Physiol* 1993;264:F181, with permission.)

hence, 98% to 99% of the filtered load of calcium is reabsorbed by the renal tubular epithelium (Fig. 89-13). There are remarkable similarities in the handling of calcium and sodium by the kidney. Less than 2% of their filtered load is excreted normally, and there is no evidence of tubular secretion of either calcium or sodium in the mammalian nephron. Urinary excretion of either sodium or calcium is controlled by adjustments in tubular reabsorption. Approximately 60% of the filtered calcium is reabsorbed in the PCT (Fig. 89-13), 20% to 30% in the loop of Henle, 10% by the distal convoluted tubule, and 1% by the collecting system. The terminal nephron (connecting segment, distal tubule, and collecting duct), although responsible for the reabsorption of only 10% of the filtered calcium load, is the major site for regulation of calcium excretion.

Calcium in the Glomerular Filtrate

Micropuncture studies of the kidney in the Munich-Wistar rat with surface glomeruli have demonstrated that the ratio of calcium in fluid of Bowman's space to plasma (TF/P calcium) is 0.6, indicating that only the serum calcium not bound to protein is filterable (355). Thus, approximately 60% of the total calcium, which is the ultrafilterable calcium, is filtered across the glomerulus.

Proximal Convoluted Tubule

Potential factors regulating calcium reabsorption in the PCT include convection (solvent drag), concentration (increased calcium concentration in tubular fluid due to absorption of sodium and water), and transepithelial potential difference. Microperfusion studies of the rabbit PCT *in vitro* (485,486) and micropuncture of the rat *in vivo* (487) indicate that fluid absorption and solvent drag, as well as diffusion along an electrochemical gradient, contribute to net calcium flux. The reabsorption of calcium in the PCT parallels that of sodium and water: The ratio of tubular fluid to plasma ultrafilterable calcium in the earliest portion of the PCT rises to 1.1 and remains at this value along the rest of the PCT. This is compatible with passive calcium reabsorption secondary to sodium and water reabsorption along most of the PCT. The transepithelial movement occurs through the paracellular pathway across the tight junction. Although the passive movement of calcium through a paracellular pathway accounts for most of the calcium transport across the proximal tubule, there is evidence of an active transport component in this segment of the nephron (Fig. 89-14) (485,487–490). During stop-flow microperfusion experiments measuring net PCT efflux, Ullrich et al. demonstrated that the tubular fluid calcium concentration was lower than that in the capillary (487). They calculated the active transport rate as 3.4×10^{-13} mol/cm per second, which is in the range of 20% to 30% of the total reabsorptive rate for this segment.

The reabsorption of calcium transcellularly rather than through intercellular channels is a multistep process in which calcium enters the cell across apical membrane and exits across basolateral plasma membranes. Calcium-permeable channels in PCT cells have been described (491–494). However, these are activated by membrane stretch and, therefore, are thought to participate in cell volume regulation (494). Basolateral efflux of calcium PCTs may be mediated in whole or in part by Na^+-Ca^{2+} exchange (487,495–498).

Proximal Straight Tubule (Pars Recta)

Calcium is transported in the pars recta by a process that is not inhibited by ouabain (499). Because ouabain abolishes water and sodium transport, this suggests that the sodium–calcium exchange is not the major mechanism for calcium extrusion across the basolateral membrane in this segment of the nephron. Approximately one-third of the calcium transported can be attributed to sodium and water, and thus, it would seem that an active transport component plays an important role in the reabsorption of calcium in the proximal straight tubule.

Loop of Henle

Neither the thin descending limb nor the thin ascending limb of Henle's loop plays an important role in calcium reabsorption (499). In contrast, *in vitro* studies have shown that the TAL of Henle's loop reabsorbs calcium from lumen to bath in the absence of water movement. About 20% of the filtered calcium is reabsorbed in this segment of the nephron. The transepithelial flux of calcium is proportional to the positive transepithelial potential gradient generated by sodium chloride transport mechanisms (500–502). Much of this flux is probably paracellular (Fig. 89-14). Studies of the TAL suggest that the flux ratio for calcium may be greater than can be accounted for by the positive intraluminal potential; thus, an additional active transport process for calcium is present in this segment (503–505), and it accounts for up to 50% of the total calcium transported (Fig. 89-14). This transcellular component is regulated by PTH (490,504) and calcitonin in the cortical and medullary TALs, respectively (490,505).

Under resting conditions, Ca^{2+} transport is passive in the TAL. Changes in the electrochemical drive for Ca^{2+} determine the magnitude of passive, paracellular absorption. Under these circumstances, the transepithelial voltage is the primary determinant of the driving force, and the magnitude of the voltage, oriented electropositive in the lumen, is set by the rate of Na^+ absorption. As Na^+ absorption increases, transepithelial

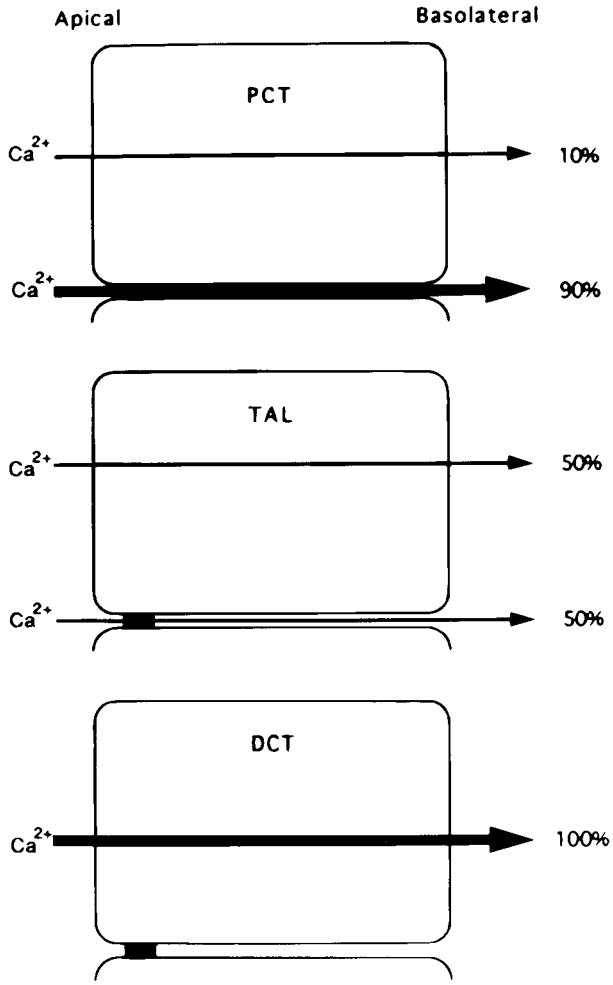

FIGURE 89-14. Cellular and paracellular calcium transport pathways along the nephron. Relative percentage of calcium absorbed by cellular or paracellular pathways in the proximal convoluted tubule (*PCT*), thick ascending limb (*TAL*), and distal convoluted tubule (*DCT*). (From: Friedman P, Gesek F. Calcium transport in renal epithelial cells. *Am J Physiol* 1993;264:F181, with permission.)

voltage increases (506,507) and Ca^{2+} flux increases. Peptide hormones that enhance Na^+ transport and thereby increase the transepithelial voltage in medullary TALs would be expected to stimulate passive Ca^{2+} absorption. Extensive evidence consistent with this model has been provided (508–510). Inhibition of Na^+ absorption reduces the transepithelial voltage and would be expected to decrease passive calcium absorption. Furosemide, bumetanide, and ethacrynic acid, which block sodium transport in the TAL of Henle's loop, also block calcium transport. The tight junction of the TAL has a specific permeability for calcium that participates in the voltage-dependent paracellular flux of the cation. This was proven by the discovery that mutations in the gene "PCLN1" which encodes for the protein Paracellin-1 (PCLN1), a member of the claudin family of epithelial tight junction proteins (511), causes a syndrome of renal magnesium wasting, hypercalciuria, and nephrocalcinosis (512).

Distal Convoluted Tubule, Connecting Tubule, and Collecting Tubule

Calcium transport in the distal convoluted tubule, connecting tubule and collecting ducts is an active process. It occurs against an electrochemical gradient. The epithelium is considered "tight," meaning there is very little fluid or electrolyte flux through the paracellular route (Fig. 89-14) (513). Free flow micropuncture studies in the rat demonstrate that TF_{Ca2+}/UF_{Ca2+} falls from a value of 0.6 in the early PCT to 0.3 by the early portion of the cortical collecting duct. This is consistent with active transcellular calcium movement.

Active transcellular Ca^{2+} absorption in the distal convoluted tubule and connecting tubule is a three-step process. Ca^{2+} enters the cell across apical plasma membranes, diffuses across the cytosol bound to calcium binding proteins, and is actively extruded from the cell across basolateral membranes (Fig. 89-15). The mechanism of Ca^{2+} entry into the cells was defined with the expression cloning of the ECaC (now TRPV5) (475) (Fig. 89-15). Apical influx is considered the rate-limiting step in transcellular calcium transport and, therefore, the regulatory target of stimulatory and inhibitory hormones (490,514) (Fig. 89-16). Evidence suggests that hormonal stimuli of Ca^{2+} transport produce an insertion of calcium channels into the apical membrane (436,514,515) (Fig. 89-16). Furthermore, thiazide diuretics stimulate Ca^{2+} transport in this segment (516,517). Thiazides produce their diuretic action by inhibiting a, Na^+-Cl^- cotransport protein of the apical membrane (517,518). How this is translated into a stimulation of Ca^{2+} transport was elucidated by Shimizu et al. (516) and Bordeau and Lau (437) (Fig. 89-17). In the presence of inhibited apical Na^+ entry, there is increased Na^+ flux into the cell across the basolateral membranes, which is coupled to Ca^{2+} extrusion, in other words actuation of a basolateral Na^+-Ca^{2+} exchanger. This nephron segment is also characterized by a hormonally regulated isoform of the Ca-ATPase, PMCA1b, found only in epithelia involved in active Ca^{2+} transport (448) (Figs. 89-15–89-19). In addition, the vitamin D–regulated calcium binding protein, calbindin-$_{28K}$, associated with Ca^{2+} transport is also localized in the distal tubule (513,519) (Figs. 89-15–89-19).

The apical Na-Cl cotransport, which is thiazide sensitive, appears mainly in the distal tubule (Figs. 89-18–89-19). In the connecting tubule and the collecting duct, Na^+ entry occurs through an apical, amiloride-sensitive Na^+ channel (513,517,520). Amiloride also stimulates Ca^{2+} reabsorption in these segments. The mechanism appears to be, as for thiazides, a limitation of apical Na^+ entry stimulating basolateral Na^+ entry through a Na^+-Ca^{2+} exchange, activating Ca^{2+} efflux (Figs. 89-18 and 89-19).

Factors that Regulate Calcium Transport

Maneuvers such as administration of PTH (24,430,521–523), cAMP (502), and calcitonin (524), ECF volume expansion (525), insulin administration (526,527), and phosphate depletion (4,528,529) have all been shown to inhibit proximal tubular reabsorption of calcium and increase the delivery of calcium to the more distal nephron segments. The relationship of these maneuvers on urinary calcium excretion, however, may be a decrease, no change, or an increase in calcium excretion, emphasizing again the critical role of the distal tubule in the final regulation of calcium excretion. Both metabolic acidosis (530) and phosphate depletion (103,528,529) are accompanied by increased calcium excretion in the urine. Experimental studies in animals suggest that the "defect" in calcium reabsorption in metabolic acidosis (530) and phosphate depletion (529) is located in the distal tubule and probably through an effect on the TRPV5 (432), although phosphate depletion may also affect calcium transport in the proximal tubule. The administration of sodium bicarbonate, which rapidly corrects acidosis, increases calcium reabsorption in the

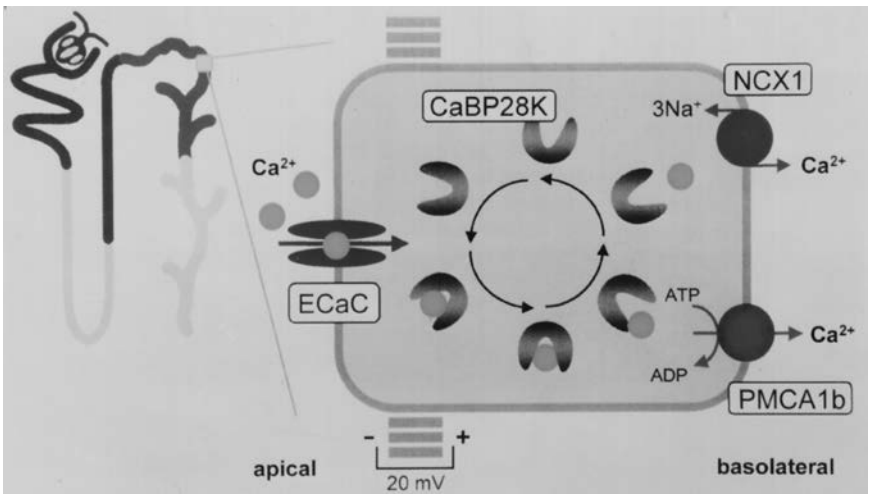

FIGURE 89-15. Model of transcellular Ca^{2+} transport by cells lining the distal part of the nephron. Entry of Ca^{2+} is facilitated by the apical epithelial Ca^{2+} channel (ECaC). Subsequently, the ion binds to calbindin-D_{28K} (CaBP28K) and diffuses through the cytosol to the basolateral membrane. Here, Ca^{2+} ions are extruded by a Na^+-Ca^{2+} exchanger (NCX1) and a Ca^{2+}-ATPase (PMCA1b). (From: Hoenderop JG, Willems PH, Bindels RJ. Toward a comprehensive molecular model of active calcium reabsorption. *Am J Physiol Renal* 2000;278:F352, with permission.) (See Color Plate.)

late distal tubule. Similar results are found when phosphate is given to an animal that has been previously phosphate depleted.

PTH plays an important role in the regulation of calcium transport and reduces urinary calcium excretion (Fig. 89-16). In humans, the status of the parathyroid gland greatly influences the amount of calcium excreted in the urine. At equal filtered loads of calcium, patients with high levels of circulating PTH have less calcium in the urine than those in whom the levels of PTH in serum are low. Experimental evidence indicates the main effect of PTH is in the distal tubule and connecting tubule (430,440,516,531,523) and is mediated through the adenylate cyclase system. Although PTH inhibits proximal tubular reabsorption of sodium and calcium, the main action of PTH is localized in more distal segments of the nephron.

Studies in rabbits have shown a PTH-sensitive calcium transport mechanism in the cortical TAL (521,522). Little is known about how PTH affects the paracellular pathway for Na^+ and Ca^{2+} reabsorption of the PCT. Studies in isolated renal cortical brush-border membrane vesicles indicate that PTH mimics the effect of membrane phosphorylation on calcium binding and translocation (514,532). Phosphorylation of brush-border membrane vesicles produces an increase in membrane-bound calcium due to production of negatively charged phospholipids (533). Aminoglycosides compete for the binding of calcium to phospholipids. In the presence of a chemical potential for calcium, PTH also stimulates calcium binding that is aminoglycoside inhibitable, as well as an increase in the brush-border membrane content of the acidic phospholipids produced by phosphorylation (533). The control of calcium efflux across the basolateral membrane of the PCT by PTH involves the Na^+-Ca^{2+} exchange. Scoble et al. (498) and Jayakumar et al. (534) demonstrated sodium gradient (outside >inside) dependent of calcium efflux stimulated by PTH in basolateral membrane vesicles from dog and rat renal cortex. These studies were thought to use membranes from the proximal tubule. However, more recent studies suggested that the Na^+-Ca^{2+} exchange activity is the highest in the distal tubule and the earlier studies may have been affected by contaminants from these segments (535).

PTH stimulation of Ca^{2+} transport in the TAL of Henle's loop is localized to the cortical portion, whereas the calcitonin effect is exerted in the medullary portion. Because PTH does not stimulate Na^+ or Cl^- transport, it is unlikely that it works to increase the lumen-positive transepithelial electrical driving force. Rather, its main action has been suggested to be at the level of the permeability to Ca^{2+} of the paracellular pathway (536,537) and to be related to the function of PCLN1. Recent thought indicates that an effect on transcellular transport may be involved (538,430).

Significant progress has been made in the understanding of PTH actions on Ca^{2+} transport in the connecting tubule and the cortical collecting duct. Here, Ca^{2+} reabsorption is

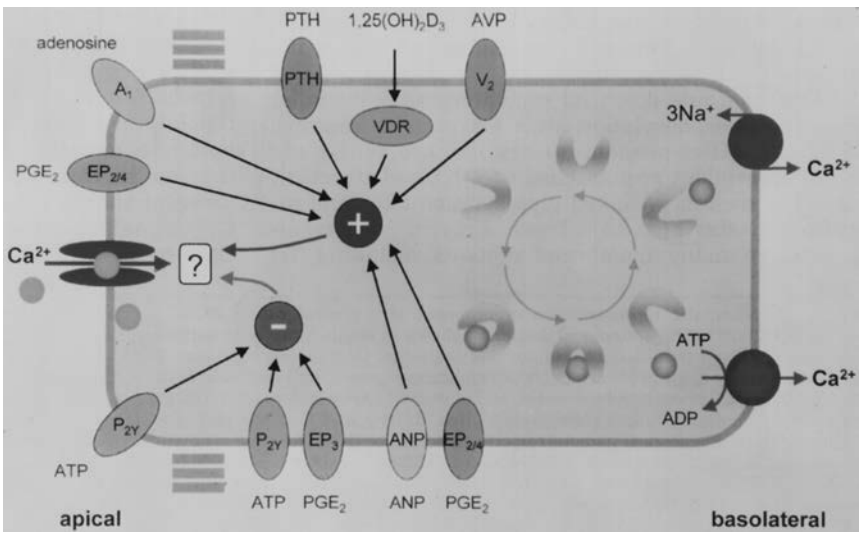

FIGURE 89-16. Schematic model for hormonal regulation of transcellular Ca^{2+} transport in distal nephron. Parathyroid hormone (PTH), V_2, atrial natriuretic peptide (ANP), and EP_3 receptors are localized in the basolateral membrane, whereas A_1 is present in the apical membrane. $EP_{2/4}$ and P_{2Y} are present in both membranes 1,25-dihydroxycholecalciferol passes plasma membranes and binds to the intracellular vitamin D receptor (VDR). Hormones can be divided into stimulatory hormones, including PTH, arginine vasopressin, ANP, prostaglandin E_2 (PGE_2) (via $EP_{2/4}$), and adenosine, and inhibitory hormones such as adenosine triphosphate and PGE_2 (via EP_3). (From: Hoenderop JG, Willems PH, Bindels RJ. Toward a comprehensive molecular model of active calcium reabsorption. *Am J Physiol Renal* 2000;278:F352, with permission.) (See Color Plate.)

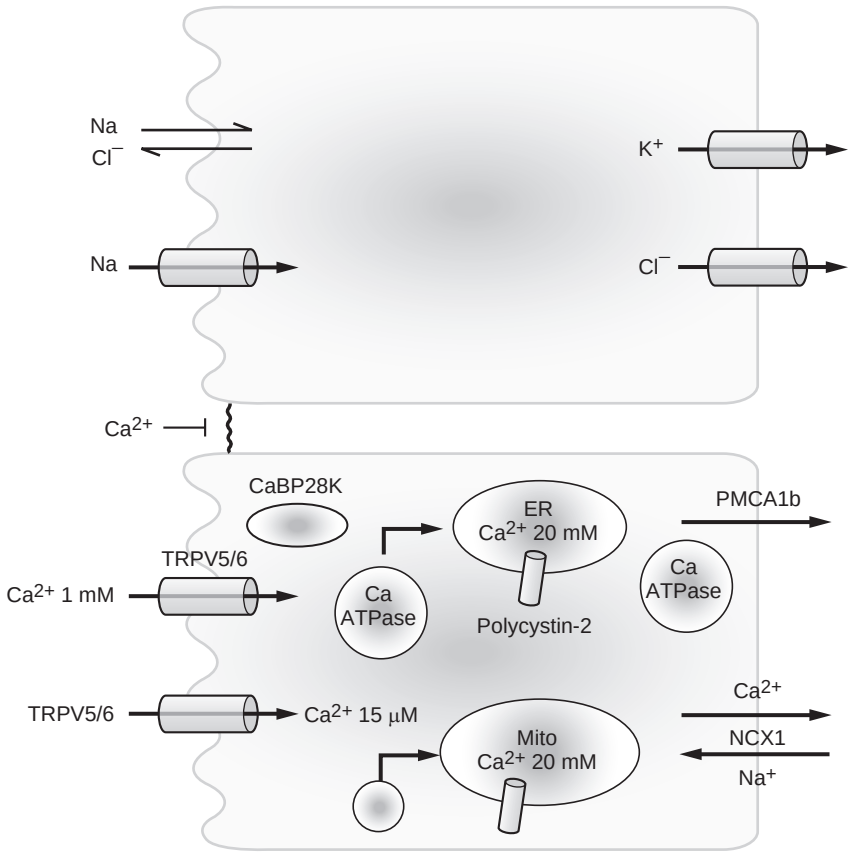

FIGURE 89-17. Calcium transport in distal convoluted tubule and connecting tubule. Transport mechanisms involved in transcellular Ca flux are depicted in the lower cell, while other transport proteins whose actions impinges on Ca absorption (apical NaCl cotransport, Na channels, basolateral chloride channels, and basolateral potassium channels) are shown in the top cell. There is no paracellular flux. The apical NaCl cotransport is inhibited by thiazide diuretics whose action in Ca transport is thought to be a limitation of Na availability leading to increased activity in the basolateral Na/Ca exchange, NCX1, causing increased Ca efflux. Likewise, the epithelial Na channel is inhibited by amiloride diuretics whose action also stimulates Ca transport similar to thiazide diuretics. Ca entry is facilitated by the apical epithelial Ca channel TRPV6/5 which assembles as homo- or heterotetrimers. Subsequently, the ion binds to CABP28K and maybe delivered to the endoplasmic reticulum. The ER may come into close association with PMCA1b resulting in delivery of Ca to the efflux pathways.

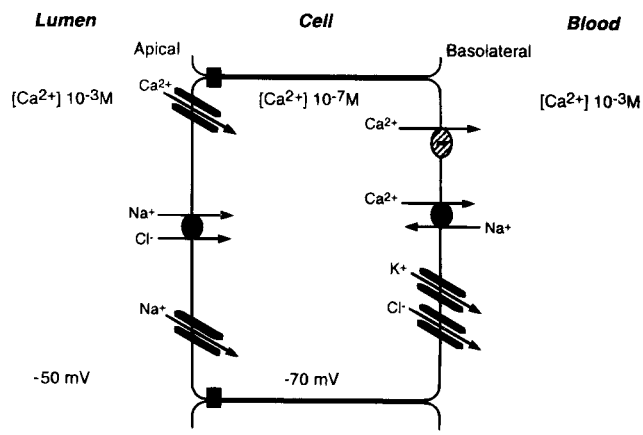

FIGURE 89-18. Model of Ca^{2+} transport in distal convoluted tubule, connecting tubule, and cortical collecting duct cells. Transport mechanisms involved in apical Ca^{2+} entry (channels) and basolateral efflux (Ca^{2+}-ATPase and Na^+-Ca^{2+} exchange) are depicted. Other transort proteins whose action impinges on Ca^{2+} absorption (apical Na^+-Cl cotransport, Na^+ channels, basolateral Cl channels, and basolateral K^+ channels) are shown. The apical Na^+-Cl cotransport is inhibited by thiazide diuretics whose action on Ca^{2+} transport is thought to be a limitation of Na^+ availability leading to increased activity of the basolateral Na^+-Ca^{2+} exchange cuasing increased Ca^{2+} efflux. This would assume basal activity of Ca^{2+} channel activity. The dependency of the thiazide effect on the presence of parathyroid hormone (PTH) would be expected because PTH stimulates insertion of Ca^{2+} channels into the apical membrane. (From: Friedman P, Gesek F. Calcium transport in renal epithelial cells. *Am J Physiol* 1993;264:F181.)

transcellular and an active energy consuming process. Studies (430,523,538,539) suggest that PTH hyperpolarizes the epithelium and produces insertion of voltage-operated Ca^{2+} channels in the apical membrane (515,538). Patch clamp studies of PTH-stimulated distal convoluted tubule cells (538,540) demonstrated an increase in open time of apical membrane channels with increasing membrane voltage (539). However, these findings remain controversial (538–540), and the mechanism of anomalous function of the apical calcium entry channel remains to be elucidated. Voltage-operated epithelial calcium channels are complex heterotetramers consisting TRPV5 and TRPV6 subunits (541) (Fig. 89-17). Identification of the distal nephron calcium entry channel, TRPV5 (432,475,542) (Fig. 89-15), which is insensitive to membrane potential, failed to shed light on the mechanism of calcium entry associated with hyperpolarization (543), and while the discovery of heterotetramers (Figs. 89-17 and 89-19) may explain the divergent electrophysiologic results (541), other Ca entry mechanisms probably await discovery.

Plasma Calcium Concentrations

Hypocalcemia

Hypocalcemia decreases the renal excretion of calcium, secondary to a decrease in the filtered load of calcium and enhanced tubular reabsorption of calcium. Hypocalcemia triggers the release of PTH, which increases calcium reabsorption in the TAL and distal tubule. The effects on the TAL could also be observed in the TPTX setting (544). Recent studies have indicated that the calcium sensor receptor (CaSR) inactivation plays an important role in the enhancement of calcium transport in the TAL during hypocalcemia (523,545,546).

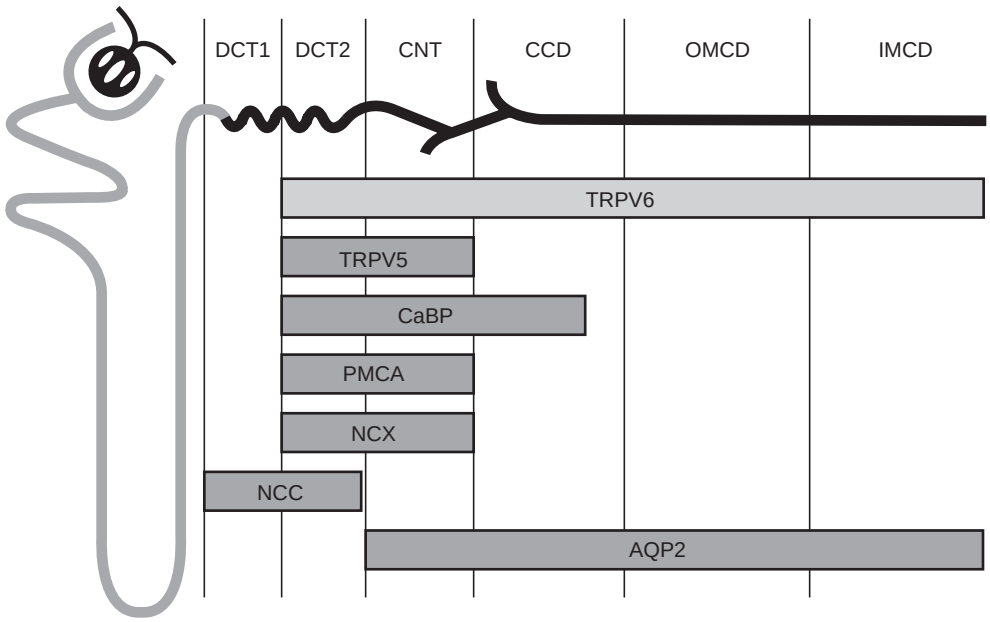

FIGURE 89-19. Renal distribution of TRPV6 in mouse kidney. Summary of the renal distribution of TRPV6 as determined by immunohistochemistry. *DCT*, distal convoluted tubule; *CNT*, connecting tubule; *CCD*, cortical collecting duct; *OMCD*, outer medullary collecting duct; *IMCD*, inner medullary collecting duct; *PMCA*, plasma membrane Ca^{2+}-ATPase; *NCX*, Na^+/Ca^{2+} exchanger; *NCC*, Na^+-Cl^- cotransporter; *AQP2*, aquaporin-2. (From: Nijenhuis T, Hoenderop JG, vander Kamp AW, et al. Localization and regulation of the epithelial Ca^{2+} channel TRPV6 in the kidney. *J Am Soc Nephrol* 2003;14:2731, with permission.)

Hypercalcemia

In general, patients with hypercalcemia have increased amounts of calcium in the urine, partly due to an increase in the filtered load of calcium and partly to suppression of PTH secretion. Activation of the CaSR also may increase the excretion of calcium by decreasing the activity of the apical K^+ channel and decreasing the positive potential difference (PD). Thus, less calcium and magnesium are reabsorbed via the paracellular pathway in the TAL (523,547).

Volume Status

Volume contraction decreases and volume expansion increases the renal excretion of sodium and calcium. Volume expansion decreases tubular reabsorption of both sodium and calcium even if the filtered load is reduced (548), clearly demonstrating that the regulation of these ions is primarily by changes in tubular reabsorption.

Diuretics

Furosemide produces a significant increase in Na^+ and Ca^{2+} excretion by inhibiting the reabsorption of both ions in the TAL (549). Furosemide decreases the PD in the TAL. Because the reabsorption of calcium in this segment of the nephron is passive, a decrease in the positive voltage of the lumen diminishes the movement of calcium through the paracellular pathway. Thiazide, on the other hand, produces dissociation between sodium and calcium excretion. A mild natriuresis is usually accompanied by a decrease in calcium excretion. Micropuncture studies have shown that this mechanism occurs in the distal portion of the nephron. Thiazide stimulates calcium entry through the apical Ca^{2+} channel by activating Cl channels (Fig. 89-14) (550). These effects are independent of PTH, although PTH is important for the presence of TRPV5 in the apical membrane of the connecting tubule. The chronic administration of thiazide produces significant decrease in calcium excretion secondary to volume contraction, since this effect can be reversed by the administration of NaCl.

Vitamin D

The acute administration of 1,25-dihydroxycholecalciferol increases renal transepithelial calcium transport by its effects on the distal, connecting, and collecting duct system. In these segments of the nephron, the transport of calcium is mainly active. 1,25-Dihydroxycholecalciferol has a positive transcriptional effect on TRPV5 gene transcription. In addition, an increase in calbindin-28K and on the calcium ATPase in the basolateral side of the cell is also transcriptionally regulated by 1,25-dihydroxycholecalciferol (Fig. 89-16) (432).

The chronic administration of 1,25-dihydroxycholecalciferol increases the excretion of calcium secondary to an increase in the filtered load of calcium. This is due to an increase in calcium absorption in the gut and calcium resorption in the skeleton.

Hypocalcemia

The clinical manifestations of hypocalcemia vary greatly among patients (551). Patients who suddenly become hypocalcemic, such as those with postsurgical hypoparathyroidism, may develop profound symptomatology, including tetany, even after a moderate decrease in serum calcium levels. On the other hand, patients with chronic renal insufficiency adjust well to low levels of serum calcium and seldom become symptomatic. Before the pathophysiologic mechanisms responsible for the hypocalcemia can be correlated with the clinical symptomatology, it is critical to determine whether both total and ionized

calcium levels are low. In conditions such as the nephrotic syndrome and cirrhosis with severe hypoalbuminemia, total serum calcium may be decreased, but ionized calcium levels may be within the normal range or only slightly decreased, and the patient remains asymptomatic.

Clinical Symptoms

Patients with significant hypocalcemia have increased neuromuscular irritability (552). The hallmark of hypocalcemia is tetany. Latent tetany may be detected by tapping over the facial nerves, which results in contraction of the facial muscles (Chvostek's sign), or by occluding the arterial blood supply to the forearm, which produces carpal spasm (Trousseau's sign). The symptomatology depends on the rapidity of onset of hypocalcemia. Patients with chronic renal failure occasionally have marked hypocalcemia; however, tetany is extremely rare. This may be due in part to the presence of metabolic acidosis. However, the changes in ionized calcium produced by metabolic acidosis in the majority of patients with profound hypocalcemia are not sufficient to bring the ionized calcium level back too normal. On the other hand, respiratory alkalosis due to hyperventilation can precipitate tetany. Clinically, the patient may complain of tingling in the tips of the fingers, stiff muscles, and cramps and may develop convulsions or impaired mental function. Children may develop mental retardation, and dementia may occur in adults. Extrapyramidal disorders also have been found in some patients. Psychiatric manifestations are characterized by confusion and hallucinations. Proximal muscle weakness is more frequently seen when the hypocalcemia is secondary to vitamin D deficiency.

Severe complications of hypocalcemia include development of cataracts (553), papilledema, and rarely, optic neuritis. In general, the skin may be dry and puffy, and the patient may develop dermatitis. Hypocalcemia may produce hypotension and a delay in ventricular repolarization, thus increasing the QT interval and ST segment. Ventricular arrhythmias and atrial fibrillation refractory to digoxin (554) have been seen in patients with hypocalcemia. Because calcium, as mentioned previously, has an inotropic effect, hypocalcemia may be responsible in part for a decrease in cardiac output (555–557).

The pathogenetic mechanisms responsible for the development of hypocalcemia are described in Table 89-4 and include; (I) absence of PTH; (II) decreased calcium mobilization from bone; (III) reduced calcium absorption in the gastrointestinal tract; (IV) translocation of calcium between different compartments of the body; (V) miscellaneous conditions.

Hypocalcemia Secondary to Low or Absent Levels of PTH in Blood

A decrease or absence of PTH will have significant effects on calcium metabolism (1,558,559). Because PTH plays a key role in the regulation of osteoclasts, which are the cells responsible for bone resorption, through the production of RANKL, a decrease in the activity or in the number of osteoclasts will eventually reduce the efflux of calcium from bone. In the absence of PTH, the capacity of the ascending portion of the loop of Henle and distal nephron to transport calcium is decreased; thus, at any filtered load of calcium, a greater amount of calcium will be excreted in the urine. Moreover, the absence of PTH decreases the activity of 1α-hydroxylase in the kidney and leads to decreased formation of 1,25-dihydroxycholecalciferol and a reduction in calcium absorption from the gastrointestinal tract. Thus, decreased mobilization of calcium from bone, excretion of larger amounts of calcium in the urine, and decreased absorption of calcium from the gut lead to profound hypocalcemia. The most common cause of hypoparathyroidism is excision or damage to the parathyroid glands at surgery. This may be secondary to thyroid or parathyroid surgery or to radical neck dissection performed for the treatment of cancer (560,561). Some patients might develop transient hypocalcemia. This phenomenon is observed in patients who have one adenoma of the parathyroid gland. The hypercalcemia

TABLE 89-4

CAUSES OF HYPOCALCEMIA

I. Hypocalcemia secondary to low or absent levels of parathyroid hormone in blood
   A. Hypoparathyroidism
      1. Congenital
      2. Idiopathic
      3. DiGeorge's syndrome
      4. Postsurgical
      5. Infiltration of parathyroid glands by malignancy or amyloidosis
   B. Transient hypoparathyroidism
      1. Neonatal
      2. Postsurgical (for parathyroid adenoma)

II. Hypocalcemia secondary to a decrease in calcium mobilization from bone
   A. Vitamin D deficiency
      1. Decreased ingestion
      2. Decreased absorption (gastrointestinal disorders)
         a. Partial gastrectomy
         b. Intestinal bypass
         c. Sprue
         d. Pancreatic insufficiency
   B. $25(OH)D_3$ deficiency
      1. Severe liver disease
         a. Biliary cirrhosis
         b. Amyloidosis
      2. Ingestion of anticonvulsant medication
      3. Nephrotic syndrome
   C. $1,25(OH)_2D_3$ deficiency
      1. Advanced renal failure
      2. Severe hyperphosphatemia
      3. Hypoparathyroidism
   D. Pseudohypoparathyroidism types I and II
   E. Magnesium deficiency

III. Hypocalcemia secondary to reduced calcium absorption in the gastrointestinal tract
   A. Deficiency of vitamin D or its metabolites

IV. Hypocalcemia secondary to translocation of calcium into different compartments
   A. Hyperphosphatemia
   B. Administration of citrate
   C. Administration of ethylenediaminetetraacetic acid
   D. Urinary excretion

V. Miscellaneous conditions
   A. Ca receptor gene mutations
   B. Pancreatitis
   C. Colchicine intoxication
   D. Pharmacologic dose of calcitonin
   E. Administration of mithramycin
   F. "Hungry Bone Syndrome"

(From: Slatopolsky E. Pathophysiology of calcium, magnesium, and phosphorus. In: Klahr S, ed. *The Kidney and Body Fluids in Health and Disease*. New York: Plenum Press; 1983:269, with permission.)

produced by the excessive secretion of PTH by the adenoma usually suppresses secretion from the other glands, and the removal of the adenoma may produce a transient period of hypoparathyroidism and hypocalcemia. However, the remaining glands, if they are intact, will respond to the hypocalcemia, and this abnormality will be reversible in a relatively short period of time.

Idiopathic hypoparathyroidism is a rare disease, and tetany may occur soon after birth. Idiopathic hypoparathyroidism may be associated with congenital absence of the thymus (DiGeorge's syndrome) (562–564). These patients have depressed cell immunity and many other malformations; they frequently have mucosal candidiasis and usually die in early childhood of severe hypocalcemia or severe infections (564). The parathyroid gland may be transiently suppressed at birth as a result of maternal hypercalcemia; thus, neonatal tetany should be looked for in the presence of hypercalcemia of any cause in the mother. The fetal parathyroid glands are suppressed by maternal hypercalcemia and when the hypocalemic infant is stressed; for example, with a phosphate load (cow's milk), tetany may result. Another factor that plays a key role in the secretion of PTH is magnesium (565–569). As will be discussed in a subsequent section, profound hypomagnesemia may decrease the release of PTH. In this syndrome, administration of magnesium to correct the hypomagnesemia increases the release of PTH within minutes.

Hypocalcemia Secondary to Decreased Calcium Mobilization from Bone

Vitamin D has a synergistic effect with PTH that increases the mobilization of calcium from bone. The mechanism by which vitamin D and its metabolites increase bone resorption is not fully understood. Both hormones are key factors in the differentiation of osteoclasts (570,571), and both factors regulate the osteoblast through differentiation of osteoblast precursors and direct regulation of bone matrix protein gene transcription (570,571). Many disorders can alter the metabolism of vitamin D, and different vitamin D metabolites could be responsible for decreased mobilization of calcium from bone. In vitamin D–deficient rickets, a nutritional condition observed in children, the lack of vitamin D is responsible for hypocalcemia, hypophosphatemia, and mild secondary hyperparathyroidism (572,573). Disorders of the gastrointestinal tract such as partial gastrectomy, intestinal bypass, tropical and nontropical sprue, and Crohn's disease may impair the absorption of vitamin D from the diet. Pathologic processes that involve the liver, such as hepatobiliary cirrhosis, may decrease the production of 25-hydroxycholecalciferol (574). The lack of this metabolite greatly diminishes the mineralization front, and adults with low levels of 25-hydroxycholecalciferol may develop osteomalacia. Although there may be an increase in the level of PTH, osteoclasts are unable to remove calcium because the osteoid material lacks minerals; therefore, there is decreased mobilization of calcium from bone. Administration of anticonvulsant medication may also result in low serum levels of 25-hydroxycholecalciferol, possibly due to the enhanced microsomal activity in the liver with increased catabolism of 25-hydroxycholecalciferol (575–577).

Chronic Renal Failure/1,25$(OH)_2D_3$ Deficiency

Chronic renal failure is characterized by moderate hypocalcemia. Serum calcium seldom falls to less than 7.0 mg/dL. The pathogenesis of hypocalcemia in chronic renal failure is multifactorial. However, phosphate retention and low levels of 1,25-dihydroxycholecalciferol play a key role in its genesis (578). In patients with profound hypomagnesemia, the skeleton becomes resistant to the action of PTH, and there is decreased calcium mobilization from bone (567,569).

Pseudohypoparathyroidism

Another important condition causing hypocalcemia is pseudohypoparathyroidism, which is a genetic disorder characterized by skeletal and somatic defects including short stature, rounded face, brachydactyly, subcutaneous calcification, and subnormal intelligence (579–582). The secretion of PTH is increased as assessed by elevated levels of immunoreactive PTH; thus, the hypocalcemia in pseudohypoparathyroidism is felt to represent a bone resistance to the effects of PTH (583,584). This syndrome is collectively referred to as Albright's hereditary osteodystrophy (AHO) (585,586). Many patients also have renal resistance to the action of the hormone because administration of exogenous PTH does not lead to increased urinary excretion of cAMP and phosphate. The syndrome has been subclassified as pseudohypoparathyroidism type 1a (PHP-1a), in which there is neither a cAMP nor a phosphaturic response to exogenous PTH. Maternally inherited mutations in one of the 13 GNAS exons encoding $G_{S\alpha}$ cause PHP-1a (583,587,588). The mRNA for G_S is also reduced about 50% in these patients (588). Heterozygous mutations of the $G_{S\alpha}$ gene have been identified in families of subjects with AHO, providing molecular confirmation that transmission of the $G_{S\alpha}$ gene defects accounts for the autosomal-dominant inheritance of AHO (589,590). When the same $G_{S\alpha}$ mutations are inherited paternally, affected individuals develop AHO in the absence of hormone resistance, and this condition is referred to as pseudopseudohypoparathyroidism (PPH) (583). Thus, the development of hormone resistance in a patient with a $G_{S\alpha}$ mutation is subject to paternal imprinting, i.e. it develops only after maternal transmission (9142,9140,9144). Genomic imprinting is a process by which specific genes undergo allele-specific epigenetic changes that lead to allele-specific differences in gene expression. One or more of the epigenetic changes is established in the male or female germline during gametogenesis. Often, imprinting is associated with allele-specific differences in DNA methylation at CpG dinucleotides during gametogenesis and maintained throughout development. Imprinting may be incomplete, as in tissue specific, as is the case for GNAS. $G_{S\alpha}$ is biallelically expressed in most tissues, but in the renal proximal tubule and some endocrine organs, it is expressed primarily from the maternal allele. As a result of this tissue-specific imprinting, patients who inherit $G_{S\alpha}$ null mutations from their mother develop multihormone resistance of PHP-1a (591).

Some subjects with pseudohypoparathyroidism type I lack features of AHO. Patients with this subtype, termed pseudohypoparathyroidism type Ib (PHP-1b), typically show hormone resistance that is limited to PTH target organs and have normal $G_{S\alpha}$ activity (592). This variant has been genetically linked to the GNAS locus on chromosome 20q13-3, and the pattern of inheritance indicates paternal imprinting (maternal transmission). Furthermore, affected individuals with autosomal dominant PHP-1b show loss of GNAS exon A/B methylation and a heterozygous ~3kb microdeletion located within STX1b approximately 220kb centiomeric of GNAS exon A/B (583). Hormone resistance in PHP-1b is limited to the PTH dependent actions in the renal proximal tubule and a few other tissues in which $G_{S\alpha}$ is paternally imprinted such as the thyroid (583).

Pseudohypoparathyroidism type II, in which there is no phosphaturia despite a normal cAMP excretion rate in response to PTH (2,366,593–596), is a heterogeneous disorder. Some of these patients have low levels of 1,25-dihydroxycholecalciferol,

perhaps representing renal resistance to PTH-stimulated 1α-hydroxylase activity. Thus, the high levels of PTH and the lack of response to the exogenous administration of PTH differentiate this syndrome from true hypoparathyroidism. The hyperphosphatemia that is present in this syndrome also may be partly responsible for the low levels of 1,25-dihydroxycholecalciferol. Thus, pseudohypoparathyroidism is a heterogeneous disorder; some patients have resistance to PTH at the renal level only, others at the skeletal level, and still others in both organs.

Hypocalcemia Secondary to Reduced Intestinal Calcium Absorption

A healthy individual who ingests a low-calcium diet usually does not develop hypocalcemia or develops it to a minimal degree because compensatory secondary hyperparathyroidism will correct mild hypocalcemia. Hypocalcemia is usually associated with pathologic processes of the gastrointestinal tract that affect the absorption of vitamin D. Under these circumstances, the low absorption of calcium, plus abnormalities in vitamin D metabolism, greatly affects calcium homeostasis, and the patient may develop profound hypocalcemia. Growing animals fed a low-calcium diet develop severe hypocalcemia.

Hypocalcemia Secondary to Translocation of Calcium into Different Compartments

Precipitation of ionized calcium is seen in disorders in which there is retention of phosphorus. Patients with advanced renal insufficiency, malignancies (316), or severe rhabdomyolysis and hyperphosphatemia (361) may precipitate calcium rapidly and may develop symptoms characterized by tremors, muscular irritability, and tetany. In the neonate, administration of cow's milk, which is high in phosphorus content compared with human milk, may produce severe hyperphosphatemia and hypocalcemia. Neonatal parathyroid function is not adequate to cope with this challenge, and the neonate may develop severe symptoms secondary to hypocalcemia. When large amounts of blood containing citrate are given to patients (open heart surgery, exchange transfusions for neonatal hyperbilirubinemia), the ionized calcium is complexed by citrate and hypocalcemia leading to tetany may develop.

Hypocalcemia secondary to increased urinary excretion of calcium is rare and self limited. The expansion of the ECF compartment produces a remarkable decrease in the reabsorption of sodium and calcium, and large amounts of these cations may be excreted in the urine. However, these are transitory mechanisms that are rapidly corrected by the release of PTH. Thus, if the PTH, vitamin D, and skeletal axis are intact, an increase in urinary calcium excretion should not result in significant hypocalcemia. Diuretics such as furosemide or ethacrynic acid, which block the reabsorption of calcium in the thick ascending portion of Henle's loop, are effective drugs in the treatment of hypercalcemia. However, very seldom do patients ingesting these drugs develop hypocalcemia.

Miscellaneous Conditions

Defects in the human CaSRs have been shown to cause familial hypocalciuria, hypercalcemia, and neonatal severe hyperparathyroidism (597–599). Recently, Pollak et al. (600) demonstrated that a missense mutation (GLU128 Ala) in this gene causes familial hypocalcemia in affected membranes of one family. In this syndrome, an alteration in the CaSR shifts the "set point" for calcium to the left, and the parathyroid glands are hyperresponsive to extracellular calcium.

Approximately 10% to 20% of patients with acute pancreatitis develop some degree of hypocalcemia. The hypocalcemia is related to deposition of calcium salts in areas of lipolysis and tissue necrosis (601). Some investigators have postulated that proteolytic digestion of PTH may explain the lack of elevated levels of PTH in the serum of patients with acute pancreatitis.

Some drugs such as calcitonin, mithramycin (used for testicular carcinoma), and colchicine (used in gout) can produce profound hypocalcemia by decreasing bone resorption. A series of disorders are characterized by increased bone formation in which the uptake of calcium by the skeleton is greatly increased. Such patients may develop profound hypocalcemia. A disorder known as "hungry bone syndrome" is seen in patients with chronic renal insufficiency and severe secondary hyperparathyroidism. The removal of the parathyroid glands in these uremic patients produces profound hypocalcemia, which sometimes is difficult to correct even with pharmacologic doses of 1,25-dihydroxycholecalciferol. Under these conditions, when the factors producing bone resorption have been removed and the osteoblastic activity is greatly increased, there is a remarkable flux of Ca into bone due to bone formation. The great uptake of minerals by the skeleton may produce profound hypocalcemia.

Hypercalcemia

Hypercalcemia is an elevation of total serum calcium levels to more than 10.5 mg/dL (when serum protein values are within the normal range). The manifestations of hypercalcemia differ among patients (602). Mild hypercalcemia may be totally asymptomatic and may be detected during routine blood chemistry tests; however, hypercalcemia may be severe enough to produce lethargy, disorientation, coma, and death.

Clinical Symptoms of Hypercalcemia

Patients with mild hypercalcemia may be totally asymptomatic (603); however, as serum calcium increases, usually more than 11.5 mg/dL, numerous symptoms may be present and practically every organ of the body is affected. The most common symptoms are nausea, vomiting, polyuria, polydipsia, lack of concentration, fatigue, somnolence, mental confusion, and even death (Table 89-5).

TABLE 89-5

CLINICAL MANIFESTATIONS OF HYPERCALCEMIA

I. General: Apathy, lethargy, weakness
II. Cardiovascular: Cardiac arrhythmias, hypertension, vascular calcification
III. Renal: Polyuria, hypercalciuria, stones, nephrocalcinosis-impaired concentration of urine renal insufficiency
IV. Gastrointestinal: Anorexia, nausea, vomiting, polydipsia, constipation, abdominal pain, gastric ulcer, pancreatitis
V. Neuropsychiatric and muscular: Headache, impaired concentration, loss of memory, confusion, hallucination, coma, myalgia, muscle weakness, arthralgia
VI. Heterotopic calcification: Band keratopathy, conjunctival irritation, vascular calcification, periarticular calcification

(From: Slatopolsky E. Pathophysiology of calcium, magnesium, and phosphorus. In: Klahr S, ed. *The Kidney and Body Fluids in Health and Disease*. New York: Plenum Press; 1983:269, with permission.)

Renal Effects

Hypercalcemia may cause either an acute and reversible decrement in the GFR or a chronic nephropathy. There are numerous mechanisms by which hypercalcemia decreases the GFR (604–606). Hypercalcemia may lead to vasoconstriction of the afferent arterioles and decreased renal blood flow. It can decrease ultrafiltration across glomerular capillaries. In addition, acute hypercalcemia may produce natriuresis and ECF volume contraction. In chronic hypercalcemic nephropathy, there is a fall in the GFR and a decrease in the maximum urinary concentrating capacity, and the urine is free of cells or casts, although mild proteinuria may be observed. The findings are similar to those seen in patients with interstitial nephritis. The characteristic abnormality of hypercalcemic nephropathy is an inability to concentrate the urine (607–609). This abnormality persists even after the administration of antidiuretic hormone (ADH) (610). The mechanisms by which hypercalcemia impairs concentration of the urine are multiple (611–613). The osmotic gradient of the medulla is decreased, partly because of decreased sodium transport in the thick ascending portion of the loop of Henle. Moreover, hypercalcemia decreases the permeability of the collecting duct to water by inhibiting the adenylate cyclase activity and generation of cAMP in response to ADH. There is some evidence to suggest that increased prostaglandin synthesis may mediate part of this effect. Prostaglandin E_2 (PGE_2) enhances medullary blood flow, inhibits sodium chloride transport in the loop of Henle, and antagonizes the effect of ADH on the collecting duct (614). It is possible that several of the effects of hypercalcemia on the concentrating mechanism are related to increased prostaglandin synthesis in the medulla. Thus, a salt-wasting nephropathy and the inability to concentrate the urine may explain some of the symptoms such as polyuria and polydipsia seen in patients with hypercalcemia. Chronic persistent hypercalcemia eventually leads to the development of nephrocalcinosis, most commonly localized to the medulla of the kidney.

Gastrointestinal Manifestations

Anorexia, nausea, and vomiting are frequently seen in patients with hypercalcemia. Occasionally, abdominal pain, distention, and ileus may be present (615). There is an increased incidence of peptic ulcer in patients with primary hyperparathyroidism, and it has been shown that calcium increases the release of gastrin and hydrochloric acid in the stomach. Moreover, the incidence of pancreatitis is also greatly increased. Several mechanisms have been implicated in the development of pancreatitis. Usually, hypercalcemia increases pancreatic enzyme secretion, and intraductal proteins may cause obstruction of the pancreatic duct. Enhanced conversion of trypsinogen to trypsin due to elevated calcium levels may contribute to the inflammatory process.

Cardiovascular Effects

Calcium has an inotropic effect on the cardiovascular system. Calcium increases peripheral resistance, and hypertension occurs in 20% to 30% of patients with chronic hypercalcemia (616–618). Renal parenchymal damage with elevated levels of renin, increased cardiac output, and severe vasoconstriction may participate in the development of hypertension. The most significant change in the electrocardiogram is a shortening of the QT interval. Because the positive inotropic effect of digitalis is enhanced by calcium, digitalis toxicity may be aggravated by hypercalcemia.

Neurologic and Psychiatric Effects of Hypercalcemia

Patients with hypercalcemia are frequently admitted to psychiatric wards because of nonspecific complaints characterized by lethargy, apathy, depression, and decreased memory. Patients with hypercalcemia secondary to increased PTH levels have electroencephalographic changes that are reversible after removal of the parathyroid adenoma. Moreover, the administration of large doses of PTH to dogs results in increased brain calcium and changes in the electroencephalogram (619).

Heterotopic Calcification

Patients with hypercalcemia may develop band keratopathy, which is the appearance of corneal calcification. The changes in the cornea are usually permanent. However, conjunctival irritation disappears after correction of the hypercalcemia. Arterial and periarticular calcifications are observed more frequently in patients who have some degree of renal insufficiency (620), especially those who also have hyperphosphatemia. Vascular calcification has become a critical complication of chronic kidney disease (621–625). Vascular calcification in CKD associated with the adynamic bone disorder is often associated with hypercalcemia (620,626,627).

Hypercalcemia

Pathologically, three general mechanisms may lead to the development of hypercalcemia (Table 89-6): (a) increased mobilization of calcium from bone, by far the most common and important mechanism, (b) increased absorption of calcium from the gastrointestinal tract, and (c) decreased urinary excretion of calcium (of minor importance). In some clinical disorders, although one or more of these mechanisms may be operative, compensatory adaptations develop and hypercalcemia may not occur. For example, in idiopathic hypercalciuria due to increased calcium absorption from the gastrointestinal tract, increased urinary excretion of calcium may prevent the development of hypercalcemia. On the other hand, in hyperparathyroidism, all three mechanisms (increased bone resorption, augmented gastrointestinal absorption of calcium, and decreased urinary calcium excretion) lead to the development of hypercalcemia.

Hypercalcemia Secondary to Increased Calcium Mobilization from Bone

Hypercalcemia Secondary to Malignancies/Humoral Hypercalcemia of Malignancy. Malignancy is the most common cause of hypercalcemia. Multiple mechanisms underlie the development of hypercalcemia of malignancy, but in general, it is due to a combined disorder of increased mobilization of calcium from the skeleton secondary to increased bone resorption by osteoclasts and variable decreases in the renal excretion of calcium (628,629). This increased resorption could be due to the action of malignant cells that have metastasized to bone from tumors of such organs as the breast, prostate, kidney, lung, and thyroid (628). The tumor cells in the bone metastasis produce RANKL and macrophage colony stimulating factor (MCSF) that stimulate production of active osteoclasts and local osteolysis. However, on some occasions, hypercalcemia occurs with no evidence of bone metastasis, and generally, the removal of the tumor results in correction of the hypercalcemia. In these cases, humoral agents are involved. The major humoral osteoclast-stimulating

TABLE 89-6

CAUSES OF HYPERCALCEMIA

I. Hypercalcemia secondary to increased calcium mobilization from bone
   A. Malignancy
      1. Metastatic
      2. Nonmetastatic
         a. Osteoclastic-activating factor
         b. Prostaglandin E_2
         c. Ectopic hyperparathyroidism
   B. Hyperparathyroidism
      1. Primary
         a. Adenoma
         b. Hyperplasia
         c. Neoplastic
      2. Secondary
      3. Multiple endocrine neoplasias
         a. Type I with pituitary and pancreatic tumors
         b. Type II with medullary carcinoma of thyroid and pheochromocytoma
   C. Immobilization
   D. Hyperthyroidism
   E. Vitamin D intoxication
   F. Renal disease
      1. Chronic renal failure
      2. After renal transplantation
      3. Diuretic phase of acute renal failure
   G. Vitamin A intoxication

II. Hypercalcemia secondary to an increase in calcium absorption from the gastrointestinal tract
   A. Sarcoidosis
   B. Vitamin D intoxication
   C. Milk-alkali syndrome

III. Hypercalcemia secondary to a decrease in urinary calcium excretion
   A. Thiazide diuretics
   B. Familial hypocalciuric hypercalcemia

IV. Miscellaneous
   A. Adrenal insufficiency
   B. Tuberculosis
   C. Berylliosis
   D. Dysproteinemias
   E. Hemoconcentration
   F. Hyperalimentation regimens

(From: Slatopolsky E. Pathophysiology of calcium, magnesium, and phosphorus. In: Klahr S. *The Kidney and Body Fluids in Health and Disease.* New York: Plenum Press; 1983:269, with permission.)

factor is PTH-related peptide (PTHrP) (628,630,631). This factor, which is a endochondral bone morphogen and an endogenous paracrine of the adult mammary glands, oviduct, fibroblasts, and vascular smooth muscle, has sufficient homology with PTH in its amino terminal region to act through PTH receptors on PTH target cells such as the osteoblast and the distal connecting tubule (466,632,633). Thus, its production by tumors mimics the action of high PTH levels in stimulating bone resorption and renal calcium retention. PTHrP accounts for about 80% of the hypercalcemia associated with malignancies. Other mechanisms of hypercalcemia due to tumors include secretion of PGE_2 (634–637), especially by solid tumors, the production of tumor necrosis factor α, as in patients with multiple myeloma and lymphosarcoma (638), and the secretion of IL-1 and TGFβ. The latter factors are often produced in association with PTHrP. TGFα works through epidermal growth factor receptors and stimulates bone resorption. Its production is highly prevalent (40%) in tumors associated with hypercalcemia, and it may play a secondary role in association with many cases in which PTHrP is being produced. In metastatic bone disease, there are usually two effects: (a) an increase in bone resorption and (b) an increase in woven bone formation. If the osteoblastic process (bone formation) predominates, hypercalcemia may not develop. However, if the osteolytic process predominates, the patient develops severe hypercalciuria and hypercalcemia. In contrast to tumors that produce a "parathyroid-like material," the serum phosphorus level or the tubular reabsorption of phosphate usually is not decreased in metastatic bone disease. However, the patient may develop hypophosphatemia when the disease progresses and malnutrition becomes evident. Although many tumors may secrete PTHrP (59 of 72 cases with hypercalcemia) (639), the two most important ones are the epidermoid squamous cell carcinoma of the lung and renal cell carcinoma. Rarely of patients with hypercalcemia of malignancy have elevated levels of circulating immunoreactive PTH (628,639). Most often this is due to coexistent parathyroid disease and malignancy (628,639).

Primary Hyperparathyroidism. Primary hyperparathyroidism is the most common endocrine disorder causing hypercalcemia (640). It is probably the major cause of asymptomatic hypercalcemia in young people and older women. A single adenoma of the parathyroid gland is the most common cause of primary hyperparathyroidism. In contrast, chief cell hyperplasia is commonly observed in patients with secondary hyperparathyroidism caused by renal insufficiency. The incidence of primary hyperparathyroidism increases substantially in both men and women older than 50 years but is two to four times more common in women (640). The mechanism of hypercalcemia is due to the action of PTH in the skeleton and kidney. The levels of PTH measurable by radioimmunoassay are elevated in primary hyperparathyroidism. The percentage of positive results to confirm the diagnosis depends on the type of antibody used and the sensitivity of each particular radioimmunoassay for PTH. Sensitive assays, using a carboxyterminal antibody, demonstrate elevated levels of PTH in 90% to 95% of patients with primary hyperparathyroidism, but these assays were sensitive to retention of fragments in kidney failure and have been replaced by assays measuring the intact hormone without loss of sensitivity (640,641). As PTH increases the activity and number of osteoclasts, bone resorption is seen on bone histology. X-ray films of the phalanges show subperiosteal bone resorption. The increased calcium mobilization from bone raises the filtered load of calcium and leads to the development of hypercalciuria despite the effect of PTH in increasing the reabsorption of calcium in the distal nephron. Moreover, the hypercalcemia is aggravated by increased calcium absorption from the gut secondary to high levels of 1,25-dihydroxycholecalciferol in response to high levels of PTH in blood. PTH decreases the renal reabsorption of phosphorus in the proximal and distal tubules, resulting in hypophosphatemia. Patients with primary hyperparathyroidism have a high incidence of peptic ulcer, renal stones, soft tissue calcification, neuromuscular disease, and psychiatric disorders. Because hypercalcemia interferes with the renal countercurrent mechanism responsible for the concentration of the urine, the patient develops polyuria and polydipsia. The treatment of this condition is surgery (parathyroidectomy), except in mild cases of asymptomatic hypercalcemia (plasma calcium

concentration of less than 11 mg/dL), especially in older adults (603,642–644).

Immobilization. Patients who are immobilized for several days develop some degree of hypercalciuria. However, some of these patients may develop hypercalcemia (616,645). This occurs in diseases involving increased bone turnover such as Paget's disease. Hypercalcemia is seen frequently in immobilized patients with multiple fractures. It seems that prolonged periods of immobilization disrupt the balance between bone resorption and formation. The resorptive process predominates because of the depression of osteoblastic activity, and calcium mobilization occurs.

Hyperthyroidism. Serum calcium concentration may increase in thyrotoxicosis. Usually the increment is mild and does not produce severe symptoms. Bone histology reveals an increase in osteoclastic bone resorption and fibroblastic proliferation resembling osteitis fibrosa cystica. Moreover, patients with thyrotoxicosis who present with hypercalcemia usually have low or undetectable levels of PTH in blood.

Vitamin D Intoxication. Vitamin D increases both calcium absorption from the gastrointestinal tract and bone resorption. Metabolites of vitamin D have been shown to increase the efflux of calcium from bone *in vitro*. Moreover, administration of 1,25-dihydroxycholecalciferol to dogs fed low-calcium diets leads to hypercalcemia, suggesting that the effect on bone was responsible for the rise in extracellular calcium. The manifestations of vitamin D intoxication include high levels of 25-hydroxycholecalciferol in blood, while the circulating levels of 1,25-dihydroxycholecalciferol may remain within the normal range. Of course, if a patient receives pharmacologic doses of 1,25-dihydroxycholecalciferol, the hormonal derivative of vitamin D produces toxic effects and the characteristic hypercalcemia.

Renal Disease. Hypercalcemia is rare in patients with *chronic kidney failure* (646) prior to dialysis. Most patients with renal disease have hypocalcemia, which leads to the development of secondary hyperparathyroidism. Hypercalcemia in patients with endstage kidney failure receiving treatment is commonly associated with the administration of vitamin D analogs and the adynamic bone disorder (454). The mechanism of hypercalcemia is both intestinal resorption and excess bone resorption over formation (464). Extreme hyperplasia of the parathyroid glands, may also develop in these patients, and progress to a point at which they no longer respond to normal feedback mechanisms due to the development of clonal adenomatous change (647). Thus, a greater degree of hypercalcemia may be necessary to suppress PTH secretion by such enlarged glands. In some patients, hypercalcemia may be seen after significant reductions of serum phosphorus levels or after ingestion of large amounts of calcium carbonate usually associated with vitamin D analog treatment.

A mineralization defect described in a substantial number of patients maintained on chronic hemodialysis (3,646) has been shown to be due to aluminum deposition in the interface between osteoid and mineralized bone is responsible for this defect. Many of these patients have mild hypercalcemia and relatively low levels of PTH. This finding has lead to the elimination of aluminum from the chronic dialysis environment.

Hypercalcemia occurs more frequently *after a successful renal transplantation* than in patients with chronic renal insufficiency (646). Because patients receiving kidney transplants usually have severe secondary hyperparathyroidism, the amount of 1,25-dihydroxycholecalciferol produced by the new kidney, if the graft is successful, is greatly increased due to both high levels of PTH and decreased serum phosphate levels due to the marked phosphaturia after the transplantation. Synergistically, 1,25-dihydroxycholecalciferol and PTH would increase calcium mobilization from bone, leading to hypercalcemia. Obviously, 1,25-dihydroxycholecalciferol also increases calcium absorption from the gastrointestinal tract. In most patients, the hypercalcemia does not require specific treatment and subsides after 3 to 4 weeks. However, in some patients, specific measures should be taken to prevent nephrocalcinosis, and if the hypercalcemia is severe and persists for several months or years, the patient may require surgical parathyroidectomy.

Vitamin A Intoxication. This is a very rare cause of hypercalcemia and is seen more frequently in children than adults (648–650).

Hypercalcemia Secondary to Increased Calcium Absorption from the Gastrointestinal Tract

There are several clinical entities such as sarcoidosis, vitamin D intoxication, and milk alkali syndrome that are characterized by increased calcium absorption from the gut and positive calcium balance. Some patients also have widespread soft tissue calcification and nephrocalcinosis. Most of these patients have low or undetectable levels of PTH.

Sarcoidosis. About 10% to 20% of patients with sarcoidosis have mild hypercalcemia (651). This abnormality is secondary to an increase in calcium absorption, and the mechanism responsible for the increased calcium absorption is unregulated production of calcitriol in macrophages of the sarcoid lesions. Serum levels of 1,25-dihydroxycholecalciferol may be elevated in sarcoidosis, but this is not uniform (652,653). Recent studies in an anephric patient with sarcoidosis with high levels of 1,25-dihydroxycholecalciferol (652) clearly indicate an extrarenal production of 1,25-dihydroxycholecalciferol in sarcoidosis. Adams et al. (654) first demonstrated that alveolar macrophages obtained by bronchial lavage from a patient with sarcoidosis and hypercalcemia converted 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol. In general, patients with sarcoidosis are sensitive to small doses of vitamin D and exposure to ultraviolet radiation of the skin. The administration of corticosteroids in these patients decreases intestinal calcium absorption and corrects the hypercalcemia. Hypercalcemia also has been demonstrated in patients with histoplasmosis (655), tuberculosis (656), disseminated coccidioidomycosis (657), and berylliosis (658) all due to unregulated calcitriol production by macrophages associated with disease lesions.

Vitamin D Intoxication. Vitamin D and its metabolites, especially 1,25-dihydroxycholecalciferol, increase intestinal calcium absorption. Thus, high concentrations of vitamin D metabolites (mainly 25-hydroxycholecalciferol) in blood are responsible for the hypercalcemia observed in vitamin D intoxication. As described already, vitamin D metabolites may also have a direct effect on bone resorption, which contributes to the development of hypercalcemia.

Milk Alkali Syndrome. This syndrome was seen frequently in patients with peptic ulcer disease who ingested large amounts

of sodium and calcium bicarbonate (659,660). Calcium carbonate contains 40% of elemental calcium. Some patients who ingested up to 20 g of calcium carbonate in 24 hours developed severe hypercalcemia. Moreover, alkalosis increases renal calcium reabsorption in the distal tubule and reduces bone turnover, thus decreasing calcium uptake by bone.

Hypercalcemia Secondary to Decreased Urinary Calcium Excretion. The decrease in urinary excretion of calcium may be secondary to a fall in the filtered load of calcium or to an increase in the tubular reabsorption of calcium. The fall in the filtered load of calcium may be secondary to a decrease in serum calcium level or the GFR. By definition, if the patient has a disorder that produces hypocalcemia with a decrease in urinary calcium excretion, he or she cannot be at the same time hypercalcemic; thus, such disorders can be excluded. A fall in the GFR may decrease calcium delivery to the distal tubule, and less calcium may be excreted in the urine. This situation, which may occur in profound dehydration, is self limited and the hypercalcemia does not persist for a prolonged time. Moreover, dehydration or other conditions that decrease GFR may also modify the transport of sodium and water and affect the reabsorption of calcium by the nephron.

Thiazide Diuretics. Patients taking thiazide diuretics may develop moderate hypercalcemia (661–664). The mechanisms for the hypercalcemia are not fully understood, and a number of factors are involved. Thiazides decrease urinary excretion of calcium by increasing calcium resorption in the distal tubule. This reduction in urinary calcium seems to require some degree of ECF volume contraction and the presence of PTH, because patients with hypoparathyroidism do not greatly reduce the amount of calcium in the urine after the administration of thiazides. Thus, in patients with increased calcium mobilization from bone, the administration of thiazides may blunt the expected hypercalciuria and potentially raise serum calcium. However, thiazides also have a direct effect on the skeleton (662). Administration of thiazides intravenously produces a mild change in ionized calcium. This effect is apparently potentiated by PTH because the effect is greater in patients with hyperparathyroidism than in healthy subjects (664). Finally, there is controversy about whether thiazides per se increase the release of PTH. Most of the evidence indicates that this is not the case.

Familial Hypocalciuric Hypercalcemia and Neonatal Severe Hyperparathyroidism. Marx et al. (665,666) described a syndrome characterized by hypocalciuria and mild hypercalcemia. Usually several members of the same family are affected. Familial hypocalciuric hypercalcemia is characterized by autosomal dominant transmission and generally follows a benign course. Some patients may have a mild degree of hyperparathyroidism; however, the hypercalcemia persists after subtotal parathyroidectomy. The main characteristic of this syndrome is a decrease in urinary calcium. Thus, the calcium:creatinine ratio provides an important diagnostic tool to differentiate familial hypocalciuric hypercalcemia from primary hyperparathyroidism. The development of hypercalcemia in young members of the family also favors this diagnosis. The pathogenesis of this syndrome is due to mutations in the calcium sensor of the parathyroid gland chief cells and the TAL/distal nephron epithelia or to auto antibodies (667–669). As a result, these cells do not downregulate PTH secretion and calcium transport with the correct sensitivity to the plasma calcium. Mutations in the calcium sensor are also responsible for neonatal primary hyperparathyroidism. Two abnormal alleles for calcium sensor mutations produce the primary hyperparathyroidism, whereas single abnormal alleles produce familial hypocalciuric hypercalcemia (670).

Treatment of Disorders of Calcium Metabolism

Hypocalcemia

The treatment of severe hypocalcemia and tetany is a medical emergency. Administration of calcium intravenously is mandatory to prevent severe complications and even death in these patients. If the patient has severe hypocalcemia and tetany in the absence of hypomagnesemia, the symptoms can be easily relieved by administration of 1 or 2 ampules of calcium gluconate given intravenously over 10 minutes (1 ampule of calcium gluconate has approximately 100 mg of elemental calcium). This initial treatment can be followed by administration of 1.0 g of elemental calcium dissolved in 500 mL of dextrose in water and given intravenously over 4 to 6 hours. If the condition responsible for the hypocalcemia cannot be corrected (e.g., hypoparathyroidism), a program for the chronic treatment of hypocalcemia should be instituted. The amount of calcium in the diet should be supplemented by 1 to 3 g of elemental calcium. Calcium carbonate has roughly 40% of elemental calcium; commercial preparations such as 3M Titralac or Os-Cal can be used in this situation. However, in many circumstances, administration of large amounts of calcium may not be sufficient to increase absorption by the intestine; therefore, different metabolites of vitamin D should be used. In chronic situations, 1,25-dihydroxycholecalciferol could be used. The dosage used ranges from 0.5 to 2 μg per 24 hours. Most patients eventually require 0.5 μg per day. If this metabolite of vitamin D is not available, vitamin D_2 or D_3, about 50,000 U three times a week, could be used instead. The dosage can be gradually increased up to 50,000 to 100,000 units daily. The serum calcium should be carefully monitored to prevent severe hypercalcemia, nephrocalcinosis, and potentially irreversible renal disease.

Hypercalcemia

A useful maneuver to correct hypercalcemia is to increase urinary calcium excretion (Table 89-7) (671). As discussed previously, only 1% to 2% of the filtered load of calcium is excreted by the kidney. This percentage can be greatly increased, and the kidney may thus become an excellent excretory organ for calcium. Because most patients with hypercalcemia develop dehydration and volume contraction with a consequent decrease in GFR, one of the first therapeutic maneuvers is the expansion of the ECF space. Expansion with saline requires several liters per day; therefore, it is mandatory that strict records be kept to maintain accurate determination of the intake and output of fluids. In most patients, it is convenient to determine the central venous pressure (CVP), which will allow volume expansion and prevent the potential risk of overexpansion and heart failure. Thus, after a CVP line is inserted, volume expansion with saline should be instituted until the venous pressure increases to 10 to 14 mm Hg. This maneuver alone will increase the GFR and decrease the reabsorption of calcium in the proximal tubule and in the ascending portion of the loop of Henle. Thus, fractional excretion of calcium will be greatly increased. This effect can be enhanced by administration of diuretics such as furosemide, bumetanide, or ethacrynic acid. The administration of 40 to 120 mg of furosemide every 4 hours is recommended in most patients. Using these maneuvers, fractional excretion of calcium can be increased to 10% of the filtered load. Thus, 1 g of calcium can be easily excreted in the

TABLE 89-7

TREATMENT OF HYPERCALCEMIA

Agent	Dosage	Route of administration	Effect	Mechanism of action	Side effects
I. Measures directed to enhance renal excretion of calcium					
Saline	1 to 3 L	IV	4 to 8 hours	GFR; tubular reabsorption of Ca	Heart failure; electrolyte imbalance
Furosemide	40 to 20 mg every 2 to 4 hour	IV	2 to 4 hours	Tubular reabsorption of Ca	Hypokalemia
II. Measures directed at decreasing bone resorption					
Biphosphonates					
Paminodrate	60 to 90 mg	IV	24 to 72 hours	DBR, effect on osteoclasts	Hypocalcemia. Mandibular osteonecrosis
Alendronate	5 to 10 mg daily	PO	2 to 3 days	DBR effect on osteoclasts	Gastrointestinal disorders
Risedronate	30 mg daily	PO	2 to 3 days	DBR effect on osteoclasts	Gastrointestinal disorders
Zoledronic Acid	4 mg once/year	IV	24 to 72 hours	DBR effect on osteoclasts	Renal failure
Cinacalcet	15 to 30 mg daily	PO	24 to 72 hours	Decreases PTH levels	Hypocalcemia
Calcitonin	2 to 5 MRC/kg every 4 to 8 hour	IM	4 to 12 hours	DBR effect on osteoclasts	Allergic reaction, nausea, flushing
Mithramycin	25 μg/kg in 500 mL saline	IV	24 to 72 hours	DBR (marked)	Thrombocytopenia, bleeding
Indomethacin	75 mg q12h	PO	2 to 4 days	DBR secondary to prostaglandins	Gastrointestinal disorders
Aspirin	1 g q6h	PO	2 to 4 days	DBR secondary to prostaglandins	Gastrointestinal disorders, allergic
III. Measures directed to decrease calcium absorption in gastrointestinal tract					
Prednisone	20 to 30 mg q12h	PO	2 to 4 days	Decreased gastrointestinal absorption	Acute toxic steroid effects
IV. Measures directed to decrease serum calcium					
Hemodialysis	Low dialysate calcium		1/2 hour	Direct removal from serum	

IV, intravenous; *IM*, intramuscular; *PO*, by mouth; *MRC*, Medical Research Council; *DBR*, decreased bone resorption; *GFR*, glomerular filtration rate.

urine in 24 hours. The administration of large amounts of saline and diuretics usually increases the excretion of potassium. To prevent arrhythmias, serum potassium should be maintained between 3.5 and 5.0 mEq/L. This can be achieved by adding 10 to 30 mEq of potassium to each liter of saline.

Although expansion of the ECF may control the hypercalcemia, this effect is temporary, and because in most circumstances, hypercalcemia is secondary to increased mobilization of calcium from bone, the physician may be forced to add a second line of medications to decrease the efflux of calcium from bone. In addition, many patients with hypercalcemia have renal failure and are not responsive to diuretics.

A derivative of the bisphosphonates, disodium dichloromethylene diphosphonate (672), was originally used with success on an experimental basis in patients with tumors and bone metastases and severe hypercalcemia (673). Pamidronate, ibandronate, and zoledronic acid, second- and third-generation bisphosphonates, respectively, have become the standard of therapy for hypercalcemia of malignancy (674–678) and other causes of hypercalcemia requiring inhibition of bone resorption. One or two doses of 30 to 60 mg of Pamidronate or a single dose of zoledronic acid intravenously are usually effective (677). Potent bisphosphonates, including alendronate and risedronate, have become available and are effective as oral agents for hypercalcemia. Renal toxicity has to be considered when using these agents (679).

Cinacalcet

Cinacalcet maintains long-term normocalcemia in patients with mild or asymptomatic primary hyperparathyroidism (644). Cinacalcet is an orally bioavailable calcimimetic that increases the sensitivity of calcium sensing receptors to extracellular calcium. Cinacalcet in doses of 30 to 50 mg twice daily is sufficient to normalize serum calcium in hyperparathyroidism. Cinacalcet is also used in the treatment of secondary hyperparathyroidism seen in chronic kidney disease (680).

Calcitonin produces hypocalcemia by decreasing the activity of osteoclasts. The dose commonly used ranges from 2 to 5 MRC U/kg of body weight every 6 to 12 hours. The degree of hypocalcemia produced by this drug is mild, and the decrease is usually 1 to 3 mg/dL (681). Calcitonin can be given either intramuscularly or intravenously in a concentration of 5 MRC U/kg dissolved in 500 mL of 5% dextrose in water to be given over 6 hours. Calcitonin is also available as a nasal spray. Unfortunately, in most patients, there is an escape from the hypocalcemic effect of calcitonin after 6 to 10 days of administration.

Mithramycin is an antibiotic originally introduced for the treatment of testicular tumors. Mithramycin blocks the activity of osteoclasts and may result in severe hypocalcemia (682,683). It is usually given intravenously over 3 to 4 hours in a dose of 25 μg/kg of body weight dissolved in 500 mL of 5% dextrose in water or saline. Mithramycin is an effective drug. However, the effects may be seen only after 48 to 72 hours. One of the toxic effects of the drug is severe thrombocytopenia and bleeding. In general, the drug should not be given more than once every 4 or 5 days, and its use has been almost eliminated by development of effective less toxic agents such as the bisphosphonates (673).

There are rare tumors that produce prostaglandins that have resulted in the development of hypercalcemia. The use of aspirin in a dosage of 1 g four times daily or indomethacin 75 mg twice daily has ameliorated the hypercalcemia (684–686).

If hypercalcemia is mainly due to increased absorption of calcium from the gastrointestinal tract, such as in sarcoidosis, it is obviously important to decrease the amount of calcium in the diet and to administer corticosteroids, which will result in decreased absorption (687,688). Usually prednisone (20 mg twice daily) has been effective in conditions such as sarcoidosis, which is characterized by increased 1,25-dihydroxycholecalciferol production by macrophages in the granulomas. The dose of corticosteroid should be titrated down to the lowest dose required to maintain normocalcemia.

Phosphate has been used in the treatment of hypercalcemia (689). However, the presence of a normal or slightly elevated serum phosphorus level or decreased renal function precludes the use of this medication. Phosphorus should be given only when the serum phosphorus level is low, and its use is generally discouraged. If an elevation of the serum phosphorus level is achieved and the serum calcium level decreases, the patient may deposit calcium phosphate in soft tissues.

There are some general measures that are important in the treatment of hypercalcemia. Immobilization should be avoided as much as possible, especially in patients with rapid bone turnover such as those with Paget's disease. Because most patients with hypercalcemia have an underlying tumor that is causing the hypercalcemia, physicians should be aware of this pathogenetic mechanism and join efforts with oncologists in the diagnosis and treatment of the malignancy. Finally, when the hypercalcemia is very severe and the patient has advanced renal insufficiency, acute hemodialysis is an effective method of correcting the hypercalcemia (Table 89-7).

MAGNESIUM

General Considerations

Magnesium is the second most abundant intracellular cation (after potassium) and the fourth most abundant cation of the body (after calcium, potassium, sodium). Magnesium (Mg^{2+}) is divalent, has an atomic weight of 24. Mg^{2+} has an essential role as a cofactor for various enzymes, most of which use ATP. Mg^{2+} increases the stimulus threshold in nerve fibers and in pharmacologic doses has a curare-like action on neuromuscular function, probably inhibiting the release of acetylcholine at the neuromuscular junction. Mg^{2+} decreases peripheral resistance and lowers blood pressure. Like Ca^{2+}, Mg^{2+} plays a role in the regulation of PTH secretion. Hypermagnesemia suppresses the release of PTH. Acute hypomagnesemia has the opposite effect; however, profound magnesium depletion decreases the release of PTH. *In vitro,* magnesium increases the solubility of both calcium and phosphorus.

Body Stores of Magnesium

The total body magnesium concentration is approximately 2,000 mEq, or 25 g. As with calcium, only a small fraction (about 1%) of the body magnesium is present in the ECF compartment. Approximately 60% of the total body magnesium is found in bone. Most of the magnesium in bone is associated with apatite crystals, and a significant amount is present as a surface-limited ion on the bone crystal and is freely exchangeable. Approximately 20% of the total body magnesium is localized in the muscle. The remaining 20% is localized in other tissues of the body; the liver has a high magnesium content. The concentration of magnesium in blood is maintained within narrow limits, ranging between 1.5 and 1.9 mEq/L. Approximately 75% to 80% of the magnesium in serum is ultrafilterable, and the rest is protein bound (690,691). Most of the ultrafilterable magnesium is present in the ionized form. Red cell magnesium concentration is approximately 5 mEq/L.

Intracellular free Mg^{2+} levels in renal tubular cells are in the range of 500 μmol/L (692). High-performance liquid chromatography and fluorescent methods have been used to ascertain intracellular Mg^{2+} levels. Mitochondrial inhibitors that deplete intracellular ATP produce modest increases in intracellular Mg^{2+} and Ca^{2+}. The effects of these inhibitors are due to the changes in ATP levels (692). Another agent, antimycin, diminishes ATP levels and decreases intracellular Mg^{2+} to 430 μmol/L but increases cytosolic Ca^{2+}, indicating that Mg^{2+} movements can be distinguished from those of Ca^{2+} by fluorescent techniques. Also, these studies indicate that intracellular regulation of Mg^{2+} is distinctive from that of Ca^{2+}. The role of intracellular Mg^{2+} in the control of cell function remains poorly understood (693). However, intracellular Mg^{2+} levels are rapidly changed through a number of different influences that have important effects on cell function.

Magnesium Balance

Approximately 300 mg, or 25 mEq, of magnesium is ingested daily in the diet (1 mEq = 12 mg). A large portion of dietary magnesium is provided by the ingestion of green vegetables. A minimal magnesium intake of 0.3 mEq/kg of body weight is apparently necessary to maintain magnesium balance in the average person. Of the total amount of magnesium ingested in the diet, about one-third is eliminated in urine and the rest in feces (Fig. 89-20). Thus, on a normal diet containing approximately 300 mg of magnesium, 30% to 40% of the ingested magnesium is absorbed (Fig. 89-20). Small amounts of magnesium, on the order of 15 to 30 mg per day, are secreted by the gastrointestinal tract. Many studies have shown that animals fed low-magnesium diets can excrete urine that is very low in magnesium (690,693,694). However, the gastrointestinal tract continues to secrete small amounts of magnesium, and the animal becomes magnesium depleted. Most of the magnesium is absorbed in the upper gastrointestinal tract. Magnesium shares with calcium similar pathways for absorption in the intestine, but whereas most of the evidence suggests that calcium is actively absorbed from the gastrointestinal tract, magnesium is absorbed mainly by ionic diffusion and "solvent drag" resulting from the bulk flow of water. A carrier mechanism also may be involved in this process (695,696). Intestinal magnesium absorption occurs via two different pathways: a nonsaturable paracellular passive transport and a saturable active transport. At low intraluminal concentrations, magnesium is absorbed primarily via the active cellular route, and with increasingly concentrations, via the paracellular pathway. In hypomagnesemia with secondary hypocalcemia, all magnesium is absorbed via the paracellular pathway. This is evidenced by mutations in TRPM6 which leads to disruption of transcellular magnesium

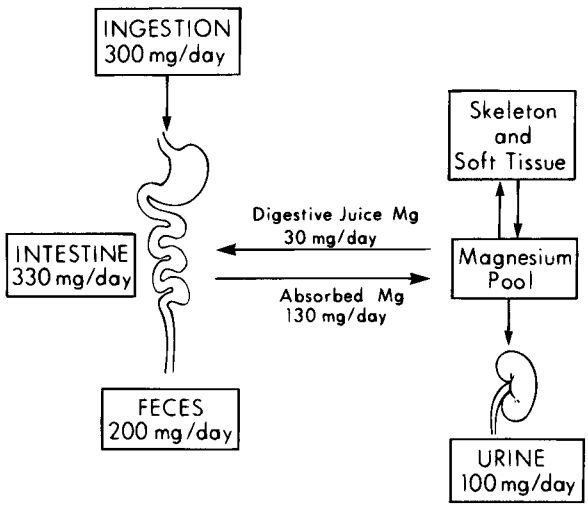

FIGURE 89-20. Diagrammatic representation of magnesium metabolism in humans showing the contribution of the gastrointestinal tract, the kidney, bone, and soft tissues to the magnesium pool. (From: Slatopolsky E, Rosenbaum R, Mennes P, et al. The hypocalcemia of magnesium depletion. In: Massry S, Ritz E, Rapado A, eds. *Homeostasis of Phosphate and Other Minerals*. New York: Plenum; 1978:263.)

absorption in the intestine and kidney (697). The factors controlling the absorption of magnesium from the bowel are not fully understood. Although there is some evidence to suggest that vitamin D may influence the absorption of magnesium, this role seems to be less important for magnesium than for the absorption of calcium (698,699). It is known that patients with severe renal insufficiency and low levels of 1,25-dihydroxycholecalciferol may develop profound hypermagnesemia by slightly increasing the amount of magnesium in the diet without modifying the metabolites of vitamin D in serum. The sigmoid colon has the capability of absorbing magnesium, and there are several reports in the literature of patients who developed magnesium toxicity after receiving enemas containing magnesium; most of these patients also had renal insufficiency. Experimental evidence in different species suggests an interrelationship between magnesium and calcium absorption from the gastrointestinal tract. Diets high in calcium decrease the absorption of magnesium, and diets low in magnesium increase the absorption of calcium.

Renal Handling of Magnesium

Approximately 2 g of magnesium is filtered daily by the kidney, and about 100 mg appears in the urine. Thus, 95% to 97% of the filtered load of magnesium is reabsorbed, and 1% to 3% is excreted in the urine (Fig. 89-21) (693,700). In states of magnesium deficiency, the kidney can reduce the amount of magnesium excreted in the urine to less than 0.5% of the filtered load. On the other hand, during magnesium infusion (Fig. 89-21) or in patients with far-advanced renal insufficiency, as is commonly seen, the kidney can excrete 40% to 80% of the filtered load of magnesium (701). The proximal tubule is poorly permeable to magnesium (690,700–704), and probably no more than 15% to 20% is reabsorbed in this segment. This is in contrast to the amount of sodium and calcium (60%) reabsorbed in this segment of the nephron. The tubular fluid magnesium is usually 1.5-fold greater than the plasma magnesium. Studies by Quamme and Dirks (705) using microperfusion techniques in the proximal tubule indicated that the absolute magnesium concentration increased along the perfused tubule in a linear manner, with net water reabsorption. This study (705) confirmed the low permeability of the superficial proximal tubule to magnesium. Further studies indicated a low level of backflux from peritubular membrane into the lumen (706,707). In the descending limb of the loop of Henle, the magnesium concentration is raised severalfold over the ultrafilterable serum concentration due to water removal. The TAL of the loop of Henle seems to play a critical role in the reabsorption of magnesium. Early studies by LeGrimellec et al. (708) and by Morel et al. (709) demonstrated that the loop of Henle was the major site for magnesium reabsorption. Approximately 60% to 70% of the filtered magnesium was reabsorbed between the last accessible portion of the proximal tubule and the early distal

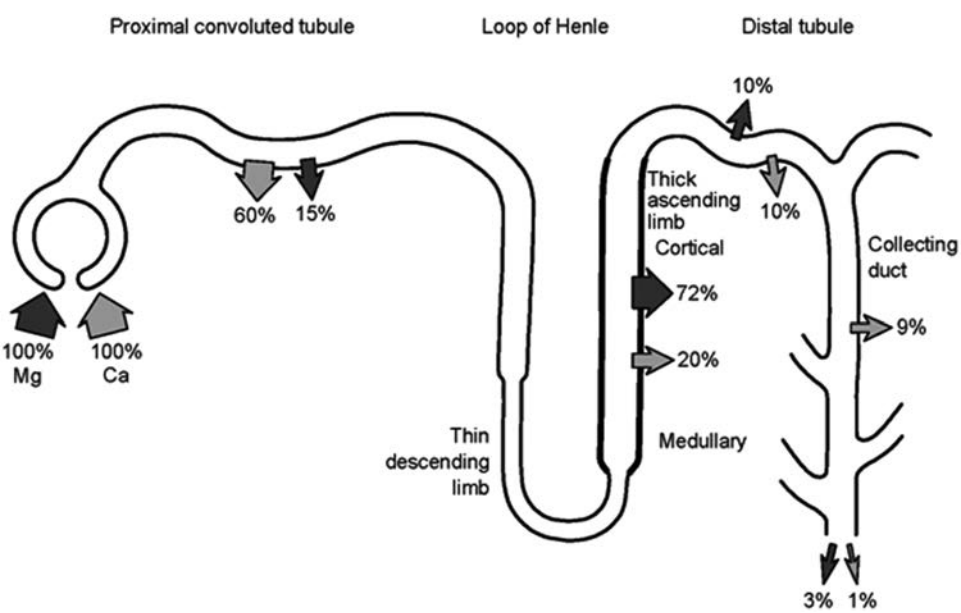

FIGURE 89-21. Summary of segmental magnesium absorption along the nephron relative to sodium and calcium reabsorption. (From: Quamme GA, De Rouffignac C. Renal magnesium handling. In Selding D, Giebisch G, eds. *The Kidney*. New York: Lippincott Williams & Wilkins; 2004:1711.)

tubule. Studies by Quamme and Dirks (705) further characterized the behavior of the loop of Henle. These investigators demonstrated that in the presence of a normal plasma magnesium concentration, magnesium absorption increased with intraluminal magnesium concentration. There was no indication of a T_{max} for magnesium when the luminal magnesium concentration increased from 0 to 5 mmol/L. On the other hand, an increase in plasma magnesium concentration (i.e., on the basolateral membrane) resulted in a significant depression of magnesium absorption, suggesting that hypermagnesemia decreases magnesium absorption in the loop of Henle by inhibiting magnesium transport at the basolateral membrane. Thus, the permeability of the TAL to magnesium is quite different from that of the proximal tubule. Two mechanisms have been proposed to explain magnesium transport in the TAL of the loop of Henle: (a) passive, secondary to the potential difference generated by the active transport of sodium chloride, which facilitates paracellular movement of Mg^{2+} (500); and (b) active (502–504), because the chemical concentration of magnesium in the cells is higher than that in the lumen, and the potential gradient may not be great enough to explain the entry of magnesium into cells. Diets deficient in magnesium or the administration of PTH enhances the reabsorption of magnesium in the TAL of the loop of Henle. On the other hand, diets containing large amounts of magnesium or factors that decrease the reabsorption of sodium chloride in this portion of the nephron (ECF volume expansion, administration of diuretics such as furosemide, bumetanide, or ethacrynic acid) also decrease the reabsorption of magnesium.

Hypomagnesemia with Hypercalciuria and Nephrocalcinosis

Recently a protein, paracellin 1 (PCLN1) was detected in the TAL and in the distal tubule (512). PCLN1 a member of the claudin family of high-junction proteins. PCLN1 is a highly negative-charged protein, with 10 negatively charged residues and a net charge of –5 (512) (Fig. 89-22). Mutations in the protein can induce renal Mg^{2+} wasting, hypercalciuria, nephrocalcinosis, and renal failure. PCLN1 plays an important role in the conductance of the TAL. The negative charges may contribute to the cationic selectivity of the paracellular pathway for the reabsorption of calcium and magnesium (512).

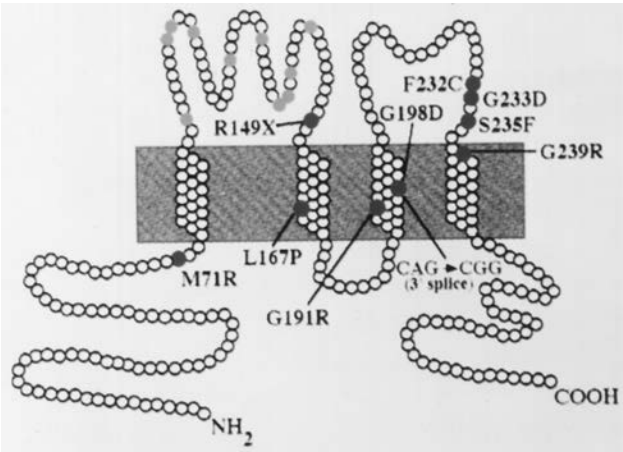

FIGURE 89-22. Structure of the PCLN-1 human gene. The red dots indicate mutations in PCLN-1 in patients with recessive renal hypomagnesemia. (From: Simon DB, Lu Y, Cahote KA, et al. Paracellin-1, a renal tight junction protein required for paracellular Mg^{2+} resorption. *Science* 1999;285:103.) (See Color Plate.)

The terminal segment of the nephron (late distal tubule and collecting duct) appears to play a minor role in the reabsorption of magnesium under normal conditions (705,710,711). However, more recent studies by the same investigators indicate that the distal tubule also plays an important role in magnesium conservation (712). The distal tubule normally reabsorbs about 10% of the filtered load of magnesium. Because there is little reabsorption of magnesium in the collecting duct, the distal tubule plays a key role in determining the final urinary excretion of magnesium.

Recent studies with immortalized DCT cell lines have shown that magnesium uptake is specific and regulated by factors shown to influence distal magnesium reabsorption. Quamme and DeRouffignac (713) speculated that Mg^{++} entry is through a channel, and transport is dependent on the transmembrane voltage. The active step is at the basolateral membrane where Mg^{++} leaves the cell against both electrical and concentration gradient. Na^{+} - Mg^{++} exchange may occur. Na^{+} moving back into the cell, coupled with Mg^{++} exiting from the cell into the interstitium. A large number of hormones: PTH, calcitonin, glucagon and vasopressin stimulate Mg^{++} reabsorption in the thick ascending loop and distal tubule, however they have no effect in the proximal tubule.

Chronic administration of mineralocorticoids increases magnesium excretion. Several interrelationships between calcium and magnesium reabsorption have been demonstrated. The administration of one of these two elements decreases the reabsorption of the other. When large amounts of magnesium are given intravenously, there is a remarkable decrease in the renal reabsorption of calcium and vice versa. Alcohol also affects the handling of magnesium by the kidney. A remarkable short-lived hypermagnesuria is seen after alcohol is given to experimental animals or humans. The intravenous administration of glucose has a similar effect.

In summary, in contrast to calcium, it seems that the thick ascending portion of the loop of Henle is the most important portion of the nephron in the regulation of magnesium reabsorption. Magnesium reabsorption in the loop occurs within the cortical TAL primarily by passive means driven by the transepithelial voltage through the paracellular pathway. On the other hand, magnesium reabsorption in the distal tubule is transcellular and active in nature. Moreover, the reabsorption of magnesium in the proximal tubule, in contrast to that of sodium, calcium, and phosphate, is rather limited.

Hypermagnesemia

By far the most common cause of hypermagnesemia is chronic renal insufficiency (714). The kidney can excrete large amounts of magnesium in the urine. Thus, hypermagnesemia is seldom seen in patients with normal renal function, even if the patient ingests large amounts of magnesium such as antacids containing magnesium or laxatives such as milk of magnesia. Mild hypermagnesemia may be seen in patients with GFRs of approximately 10 mL per minute. However, moderate hypermagnesemia is usually seen in patients with GFRs of less than 5 mL per minute. As renal insufficiency progresses, the fractional excretion of magnesium in the urine significantly increases. Patients with a GFR of 120 mL per minute excrete approximately 5% of the filtered load of magnesium. However, patients with far-advanced renal failure (GFR of less than 10 mL per minute) may excrete up to 40% to 80% of the filtered load of magnesium (714). Thus, patients with chronic renal failure may not be able to increase magnesium excretion further after ingestion of

large amounts of magnesium. Therefore, if magnesium ingestion is increased (after administration of laxatives or antacids containing magnesium) in patients with advanced renal failure, profound hypermagnesemia and death may occur. In obstetric wards, magnesium is still used for the treatment of eclampsia (715). In some of these patients, the GFR is decreased, and the administration of large amounts of magnesium sulfate may result in hypermagnesemia. Although most of the magnesium is absorbed in the small intestine, the sigmoid colon can also absorb magnesium. Healthy subjects receiving large amounts of magnesium sulfate per rectum have been found to have serum magnesium levels of more than 10 mEq/L (716).

Symptoms and Signs of Hypermagnesemia

Profound hypermagnesemia blocks neuromuscular transmission and depresses the conduction system of the heart. The neuromuscular effects of magnesium are antagonized by the administration of calcium. Mild hypermagnesemia is well tolerated. However, if serum magnesium levels increase to 5 to 6 mg/dL, there may be a decrease in tendon reflexes (717) and some degree of mental confusion. If the serum magnesium level increases to 7 to 9 mg/dL, the respiratory rate slows and the blood pressure falls. If serum magnesium levels increase to about 10 to 13 mg/dL, there is usually profound hypotension and severe mental depression. When the levels increase further to about 15 mg/dL, death may occur (716–718). In uremic patients, the adverse effect of hypermagnesemia may be worsened by the presence of hypocalcemia. Acute hypermagnesemia may also produce mild hypocalcemia. This may be due to (a) suppression of the release of PTH and (b) competition for tubular reabsorption between calcium and magnesium, leading to decreased calcium reabsorption and hypercalciuria, which aggravates the hypocalcemia produced by decreased release of PTH. In chronic renal insufficiency, there is probably an increase in red cell magnesium and muscle magnesium, but the results are controversial. The amount of magnesium in bone is apparently increased in cortical and trabecular bone (718).

TABLE 89-8

CAUSES OF HYPOMAGNESEMIA

I. Decreased intestinal absorption
   A. Severe diarrhea
   B. Intestinal bypass
   C. Surgical resection
   D. Tropical and nontropical sprue
   E. Celiac disease
   F. Invasive and infiltrative process; lymphomas
   G. Prolonged gastrointestinal suction
II. Decrease intake
   A. Starvation
   B. Protein energy malnutrition
   C. Chronic alcoholism
   D. Prolonged therapy with intravenous fluids lacking magnesium
III. Excessive urinary losses
   A. Diuretic phase of acute tubular necrosis
   B. Postobstructive diuresis
   C. Diuretic therapy
   D. Diabetic ketoacidosis (during treatment)
   E. Chronic alcoholism
   F. Hypercalcemic states
   G. Primary aldosteronism
   H. Inappropriate antidiuretic hormone secretion
   I. Aminoglycoside toxicity
   J. Idiopathic renal magnesium wasting
   K. Cisplatinum
   L. Cyclosporin
   M. Gitelman's syndrome

(From: Slatopolsky E. Pathophysiology of calcium, magnesiuum, and phosphorus. In: Klahr S, ed. *The Kidney and Body Fluids in Health and Disease*. New York: Plenum Press; 1983:269, with permission.)

Hypomagnesemia

Hypomagnesemia is defined as a decrease in serum magnesium to levels less than 1.5 mg/dL. Diseases involving the small intestine may decrease magnesium absorption and are the most common cause of hypomagnesemia (Table 89-8). It is difficult to predict the degree of total body magnesium deficiency by determining only serum magnesium concentration. Because only 1% of magnesium is present in the ECF compartment, changes in intracellular magnesium and skeletal magnesium can modify the concentration of serum magnesium, and it may not be possible to assess precisely the degree of magnesium deficiency by determining serum magnesium level. Probably the determination of skeletal or muscle magnesium may provide a better index of magnesium deficiency. However, these determinations are not practical in clinical medicine. In patients with magnesium deficiency, the administration of 50 to 100 mEq of magnesium per day usually corrects the hypomagnesemia after a short time. Magnesium depletion can also produce changes in other electrolytes. Usually there is an increase in potassium excretion in the urine, and patients may develop hypokalemia (719). In several experimental studies, it has been shown in humans (720) and animals (721) that magnesium depletion is accompanied by urinary potassium losses. Potassium alone did not increase muscle potassium unless magnesium replacement was given as well to patients receiving diuretics (722). It has been suggested that the effect of magnesium on intracellular potassium is a result of magnesium stimulating Na-K-ATPase activity, allowing the cell to maintain a potassium gradient (723). However, the most important manifestation of hypomagnesemia is the development of hypocalcemia and tetany. Experimental animals fed a low-magnesium diet develop hypocalcemia (724–727). However, the rat becomes hypercalcemic. The pathogenesis of hypocalcemia in magnesium depletion is multifactorial. Hypomagnesemia has profound effects on PTH metabolism and bone physiology. It is known that mild hypomagnesemia increases acutely the levels of PTH *in vivo* (728) or *in vitro* (729); on the other hand, profound hypomagnesemia decreases the levels of PTH in blood (565). It seems that neither the biosynthesis nor the conversion of pro-PTH to PTH is greatly affected by the concentration of magnesium (730,731). However, the release of PTH is influenced by the serum magnesium concentration (732). Several investigators have demonstrated that the administration of magnesium to patients with severe hypomagnesemia who have low levels of immunoreactive PTH in serum increases the release of PTH a few minutes after magnesium administration. Also, there is evidence to indicate that during hypomagnesemia, the skeleton is resistant to the action of PTH (733,734), and in general, the administration of mildly pharmacologic doses of PTH does not elicit a normal calcemic response in patients with magnesium depletion. Studies by Freitag et al. (735) have further clarified this abnormality.

The uptake of PTH by bones obtained from dogs with experimental magnesium depletion was greatly diminished, and the release of cAMP by bone was also blunted in hypomagnesemia. In addition to a decrease in the release of PTH and skeletal resistance to this hormone, in magnesium depletion, there is evidence that the ionic exchange from the hydration shell of bone between calcium and magnesium also is decreased (736); thus, on a physicochemical basis, less calcium is mobilized from bone in hypomagnesemia. Thus, the decrease in the release of PTH, the low uptake of PTH by bone, and the decreased heteroionic exchange of calcium for magnesium in bone are all pathogenetic factors responsible for the hypocalcemia observed in patients with profound magnesium depletion.

Clinical Manifestations of Hypomagnesemia

Patients with severe hypomagnesemia usually develop some degree of anorexia, mental confusion, and vomiting. In general, there is increased neuromuscular irritability, and tremors and seizures are usually observed in these patients. Muscle fasciculation and positive Trousseau's and Chvostek's signs can be observed. Nodal or sinus tachycardia and premature atrial or ventricular contractions may occur. The electrocardiogram may show prolongation of the QT interval and broadening and flattening or even inversion of the T waves. Magnesium deficiency potentiates the action of digitalis, and there is an enhanced sensitivity to the toxic effects of digitalis. Because magnesium plays a key role in regulating the activity of Na-K-ATPase, which is the enzyme responsible for the maintenance of intracellular potassium concentration, severe alterations in skeletal muscle and myocardial function are observed. Sometimes it is difficult to decide whether the changes in the electrocardiogram are related to magnesium or potassium depletion. Most of these patients also have profound hypocalcemia, and sometimes it is difficult to determine whether the symptoms are due to magnesium deficiency or to the concomitant hypocalcemia. Other neurologic manifestations may include vertigo, ataxia, nystagmus, and dysarthria. Changes in personality, depression, and sometimes hallucinations and psychosis have been observed. Patients also may show some degree of hypophosphatemia. In the rat, it has been shown that magnesium deficiency promotes renal phosphate excretion (737).

Mechanisms Responsible for the Development of Hypomagnesemia

From the pathogenetic point of view, three main mechanisms are responsible for the development of hypomagnesemia: (a) decreased intestinal absorption, (b) decreased intake, and (c) excessive urinary losses.

Hypomagnesemia Secondary to Decreased Intestinal Absorption of Magnesium

By far the most common causes responsible for the development of hypomagnesemia are pathologic entities affecting the small bowel. In these conditions, the kidney adapts to the hypomagnesemia and decreases the urinary excretion of magnesium. However, the amount of magnesium in the stool does not decrease appropriately (probably the secretion of magnesium is not greatly reduced), and the patient develops hypomagnesemia. Severe magnesium depletion is associated with steatorrheic syndromes (738). Pathologic processes such as celiac disease, tropical and nontropical sprue, malignancies (characteristically lymphoma), surgical resection, intestinal bypass, and profound diarrhea have all been considered responsible for the development of hypomagnesemia. From 1960 to 1975, when the number of surgical bypass procedures for the treatment of obesity increased greatly, it was noted that many of these patients developed profound hypomagnesemia and tetany. Hypomagnesemia is especially prominent in patients with idiopathic steatorrhea and diseases affecting the terminal ileum.

As described before, the characterization of TRPM6 mutations demonstrated a key role in hypomagnesemia and hypocalcemia secondary to a decrease in intestinal absorption and renal reabsorption of magnesium.

Hypomagnesemia Secondary to Decreased Magnesium Intake

Magnesium depletion has been described in children with protein-calorie malnutrition (739). The hypomagnesemia results from a combination of decreased intake and gastrointestinal losses due to diarrhea or severe vomiting. In a hospital setting, perhaps the most common cause of hypomagnesemia is prolonged therapy with intravenous fluids lacking magnesium. Often, when surgical patients require intestinal suction, they are given intravenous fluid, sometimes for several weeks, and seldom is magnesium added to the intravenous fluids. Alcoholism is probably the most common cause of hypomagnesemia in the United States (740–742). The chronic ingestion of alcohol produces hypomagnesemia. The mechanisms are multifactorial. Usually patients with chronic alcoholism ingest diets poor in magnesium. Alcohol increases the urinary excretion of magnesium (743). From the point of view of differential diagnosis, the clinical history, evidence of malnutrition, the presence of diarrhea and vomiting, or a history of surgery may help to differentiate individuals with decreased absorption of magnesium due either to a primary gastrointestinal disease or to decreased intake from individuals with increased urinary excretion of magnesium. As mentioned previously, when there is decreased intake or absorption of magnesium from the gastrointestinal tract, the amount of magnesium excreted in the urine is greatly reduced, on the order of 10 to 15 mg per day.

Hypomagnesemia Secondary to Increased Urinary Losses of Magnesium

Because 60% to 70% of magnesium is absorbed in the TAL of the loop of Henle, any factor that blocks the reabsorption of sodium chloride in this part of the nephron will also promote the urinary excretion of magnesium. In conditions in which the ECF volume is increased and in entities characterized by profound diuresis (diuretic phase of acute tubular necrosis, postobstructive diuresis), the patient may excrete 20% to 30% of the filtered load of magnesium and may develop profound hypomagnesemia. Administration of large amounts of diuretics such as ethacrynic acid, bumetanide, or furosemide has a significant effect on renal magnesium excretion. Patients with ketoacidosis may develop hypomagnesemia. Serum magnesium, phosphorus, and potassium concentrations may be elevated during periods of ketoacidosis; however, the levels usually fall after the administration of insulin and fluid replacement. Increased excretion of magnesium has been seen after the treatment of diabetic ketoacidosis (744) and in metabolic conditions characterized by an excess of mineralocorticoids (745)

such as primary aldosteronism. A specific defect has been described in patients receiving aminoglycosides (746) or cisplatin (an antitumoral agent) (747). The usual lesions produced by aminoglycosides are acute tubular necrosis, renal insufficiency, and hypermagnesemia; however, several patients have developed a specific tubular defect characterized by profound hypermagnesuria and hypomagnesemia that may persist for several weeks after the drug is discontinued. Some of these patients also developed hypokalemia.

Gitelman Syndrome

Patients with chronic hypokalemia and a phenotype other than that of Bartter's syndrome, who have hypomagnesemia and excess urinary magnesium, are described as having Gitelman's syndrome (748). Gitelman's syndrome is actually more common than Bartter's syndrome and is characterized as follows. The patients may be children or adults with primary renal tubular hypokalemic metabolic alkalosis with magnesium deficiency, hypocalciuria, and skin lesions. Hyperreninemic hyperaldosteronism is present, as are the other features of Bartter's syndrome. The inheritance is autosomal recessive, and linkage analysis to the locus encoding the renal thiazide–sensitive Na-Cl cotransporter is uniform (749). Thus, reduced sodium chloride reabsorption in the diluting segment is the pathogenesis of the disease, and it further leads to abnormalities in magnesium transport.

Treatment of Alterations in Magnesium Metabolism

Hypermagnesemia

Hypermagnesemia is seen very seldom in clinical medicine. In general, it is observed in patients with far-advanced renal insufficiency, usually with a GFR of less than 10 mL per minute. The treatment is similar to that for hypercalcemia (i.e., volume expansion with saline and administration of furosemide). However, care is required because this therapeutic regimen will also increase the excretion of calcium in the urine and potentiate the toxic effects of hypermagnesemia. Thus, if expansion with saline and furosemide is used, calcium should be added to the solutions, approximately 1 to 3 ampules of calcium gluconate per liter of saline, to prevent hypocalcemia. If the patient's GFR is extremely low, and volume expansion with saline and diuretics is not effective, dialysis with a low or zero magnesium dialysate should be instituted. Hypermagnesemia is also seen clinically when large amounts of magnesium are given intravenously to patients. A decrease in the dose administered will rapidly correct the condition.

Hypomagnesemia

Profound magnesium depletion may be accompanied by hypocalcemia and tetany. Thus, the treatment of severe hypomagnesemia may constitute a medical emergency. Profound hypomagnesemia can be easily corrected by administration of magnesium intravenously, provided that the patient has fairly normal renal function. In patients with compromised renal function, magnesium should be given cautiously, and serum magnesium should be closely monitored. Fifty to 75 mEq of magnesium sulfate or magnesium chloride should be mixed in 500 mL of dextrose in water and given intravenously over 6 to 8 hours. The next morning, serum magnesium should be measured, and if hypomagnesemia persists, the amount of magnesium should be increased to 100 mEq dissolved in the same type of solution and given over 8 hours. In some circumstances, this procedure should be repeated two or three times until the serum magnesium level increases to 2.5 mg/dL. If the patient requires magnesium orally over a prolonged period, magnesium salts can be given to these patients. One gram of magnesium oxide has roughly 50 mEq, or 600 mg, of magnesium. Thus, magnesium oxide in a dose of 250 to 500 mg can be given to patients two to four times daily. Larger doses are not well tolerated, and most patients will develop diarrhea. It is important to emphasize that a normal diet provides approximately 25 mEq, or 300 mg, of magnesium.

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CHAPTER 90 ■ FLUID–ELECTROLYTE AND ACID–BASE DISORDERS COMPLICATING DIABETES MELLITUS

HORACIO J. ADROGUÉ

Diabetes mellitus, the most prevalent endocrine illness, is a most challenging condition responsible for the development of severe abnormalities in whole body composition and target damage of critical functions. The deranged metabolic pathways lead to defects in the normal fluid–electrolyte and acid–base homeostasis, which are reviewed in this chapter. We first examine the basic defects of diabetes mellitus, and then describe each of the various disturbances of water, electrolyte, and acid–base composition observed in association with this disease.

Ketosis and Ketoacidosis

Ketosis is an abnormal state of nutrient metabolism that develops when the rate of production of ketones exceeds their removal; as a result, ketones accumulate in body fluids as reflected by high blood and urine levels (1–4). Because ketones are largely organic acids (e.g., β-hydroxybutyric and acetoacetic acid) that dissociate almost completely at the pH of the body fluids of the body fluidsHhhh, their production generates H^+ ions, which consume HCO_3^- and leads to metabolic acidosis. Hence, the designation of ketoacidosis is given. The term *ketosis* is used to describe a mild form of the disturbance, reserving the term *ketoacidosis* for the full-blown condition that features substantial metabolic acidosis (5).

The mechanisms underlying ketoacidosis are essentially the same whether it develops as an acute complication of diabetes mellitus or in nondiabetic subjects (e.g., starvation ketosis, alchoholic ketoacidosis). Abnormal levels or action of insulin and glucagon are required for the development of ketosis (5–10). Insulin deficiency or resistance impairs glucose utilization in skeletal muscle and increases adipose tissue and muscle breakdown, thereby augmenting delivery of glycerol and alanine (gluconeogenic substrates) to the liver. Hepatic gluconeogenesis, in turn, is stimulated by insulin deficiency and, more importantly, by glucagon excess. The fatty acids released from the enhanced lipolysis are converted to ketones by the hepatocytes under the influence of glucagon excess. Pancreatic β-cell destruction is largely responsible for the hormonal imbalance observed in most cases of diabetic ketoacidosis. Conversely, insulin deficiency in the presence of normal β-cells plays a major role in ketosis associated with fasting, starvation, ethanol ingestion, and some liver diseases.

Diabetic ketoacidosis (DKA) is a disease state characterized by the presence of hyperglycemia and hyperosmolality, metabolic acidosis due to ketoacid accumulation, extracellular and intracellular fluid depletion, and varying degrees of electrolyte deficiency, particularly of potassium and phosphate (9–11).

Roles of Insulin and Glucagon

In normal fasting individuals, the major source of glucose (approximately 90%) is from the liver, through glycogenolysis and gluconeogenesis. The kidney contributes the remaining 10% through synthesis of glucose from three-carbon precursors (gluconeogenesis). After a meal, glucose absorption increases the plasma glucose level. The resultant hyperglycemia-induced stimulation of insulin secretion suppresses hepatic glucose production, largely through inhibition of glycogenolysis, and stimulates glucose uptake by the liver, the gut, and peripheral tissues, including skeletal muscle.

The abnormal metabolism of carbohydrates and lipids observed in DKA is largely caused by a rise in the molar ratio of glucagon/insulin in plasma. The two hormones are metabolic antagonists with respect to fuel production and utilization but their primary effects occur on different tissues. Insulin acts on muscle and adipose tissue augmenting glucose transport and inhibiting lipolysis. Conversely, glucagon primarily acts on the liver increasing glycogenolysis, gluconeogenesis, and ketogenesis. Insulin's action on the hepatocyte is essentially that of an antiglucagon hormone, as it has minimal hepatic effects in the absence of glucagon-induced metabolic changes. Insulin decreases glucagon release from α cells in the pancreatic islets and inhibits a glucagon-activated, cAMP-dependent protein kinase in the hepatocyte (5). Beyond the critical role of glucagon in ketone body production, other hormones, including catecholamines, cortisol, growth hormones, and thyroid hormones increase hepatic ketogenesis and may participate in the pathogenesis of diabetic ketoacidosis (12) (Fig. 90-1).

DISTURBANCES IN BODY COMPOSITION

The major fluid–electrolyte and acid–base disorders complicating diabetes mellitus are conveniently classified as single dominant disturbances and combinations of multiple disturbances (Table 90-1). Each of the single dominant disturbances, including defects in homeostasis of glucose, lipids, water, sodium, potassium, phosphate, and acid–base balance, is characterized by unique features that are reviewed in this section. A combination of multiple disturbances comprising the simultaneous presence of defects in the homeostasis of glucose, fluid, electrolyte, and acid–base balance, are responsible for the development of the full-blown clinical pictures of diabetic ketoacidosis, nonketotic hyperglycemia (NKH), and renal failure complicating diabetes mellitus. These combined disturbances are examined thereafter, with the exception of renal failure, which is discussed elsewhere.

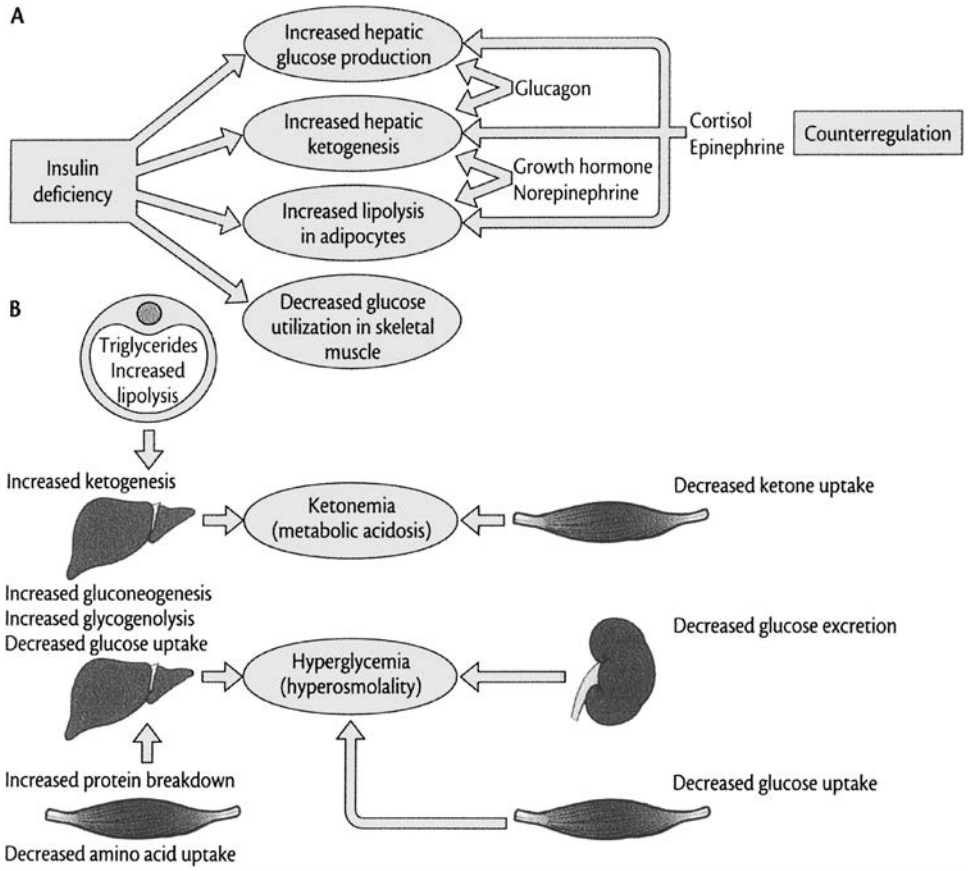

FIGURE 90-1. Role of insulin deficiency, counterregulatory hormones, and various tissues and organs in the pathogenesis of hyperglycemia and ketosis in diabetic ketoacidosis (DKA). **A:** Metabolic processes affected by insulin deficiency, on the one hand, and excess of glucagon, cortisol, epinephrine, norephrine, and growth hormone, on the other. **B:** The roles of the adipose tissue, liver, skeletal muscle, and kidney in the pathogenesis of hyperglycemia and ketonemia. Excessive hepatic production of glucose and impairment of glucose utilization are the main determinants of hyperglycemia. Increased hepatic production of ketones and their reduced utilization by peripheral tissues account for the ketonemia. (From: Adrogué HJ, Madias NE. Disorders of acid–base balance. In: Berl T, Bonventre JV, eds. *Atlas of Diseases of the kidney*. Boston: Blackwell Scientific; 1999.)

TABLE 90-1

MAJOR FLUID–ELECTROLYTE AND ACID–BASE DISORDERS COMPLICATING DIABETES MELLITUS

Condition	Defect in homeostasis of	Specific entity
Single dominant disturbance	Glucose	Hyponatremia (hypertonic or translocational)
		Hypernatremia
	Lipids	Pseudohyponatremia
	Water	Hyponatremia (hypotonic)
		Hypernatremia
	Sodium	Volume depletion
		Volume expansion
	Potassium	Hypokalemia, K^+ depletion
		Hyperkalemia
	Acid–base balance	Ketoacidosis
		Hyperchloremic acidosis
		Renal tubular acidosis
		Lactic acidosis
Combination of multiple major disturbances	Glucose, fluid, and acid–base balance	Diabetic ketoacidosis
		Hyperosmolar nonketotic syndrome
		Renal failure

Single Dominant Disturbances

Defect in Glucose Homeostasis

In uncontrolled diabetes, hyperglycemia is caused by increased hepatic and renal glucose production and decreased glucose utilization in muscle and adipose tissue. Decreased glucose utilization, once considered the major contributor to hyperglycemia in uncontrolled diabetes, is currently believed to play a smaller role than excessive glucose production. Figure 90-2 depicts the hepatic and renal contribution to endogenous glucose production in conscious normal and diabetic dogs (13). The rise in the glucagon/insulin ratio in plasma characteristic of uncontrolled diabetes activates key enzymes that accelerate the rates of both glycogenolysis and gluconeogenesis. The increased ratio also promotes glucose overproduction by modulating the effects of other hormones, availability of substrate, and rates of fatty acid oxidation (Fig. 90-1). Volume depletion secondary to hyperglycemia-induced osmotic diuresis reduces the urinary loss of glucose, thereby worsening hyperglycemia.

An important contributor to the development of hyperglycemia in uncontrolled diabetes may be the prevailing acidemia (14–18). In animals with acidemia induced by hypercapnia, for example, a substantially smaller glucose infusion rate maintains euglycemia as compared to dogs without respiratory acidosis during constant insulin infusion, reflecting less glucose entry into cells for a given insulin level (19) (Fig. 90-3). Although the sympathetic surge characteristic of acidemia undoubtedly contributes to glucose intolerance, adrenergic blockade during acute respiratory acidosis does not prevent the disturbed glucoregulation. Nor do plasma levels of insulin fall

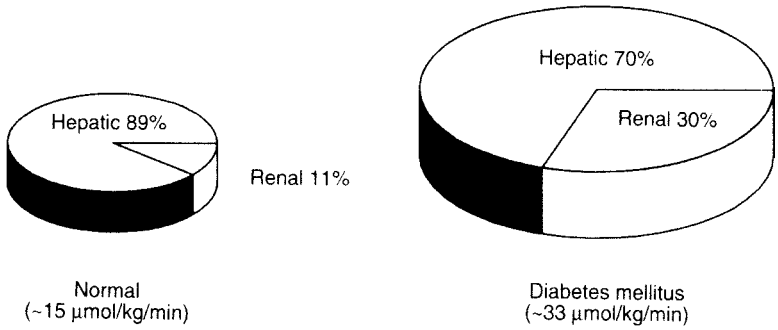

FIGURE 90-2. Contributions of the liver and the kidney to endogenous glucose production in conscious normal and diabetic dogs. Total glucose production is indicated in parentheses. (From: Adrogué HJ. Glucose homeostasis and the kidney. *Kidney Int* 1992;42:266.)

during acute respiratory acidosis. In fact, acidemia reduces tissue extraction of insulin and, more specifically, insulin uptake by the liver (19). Although plasma glucagon levels also increase during metabolic or respiratory acidosis, the glucagon/insulin ratio in the portal circulation remains unchanged, thereby reducing the possible role of glucagon in the hyperglycemia of acidemic states. The weight of the evidence suggests that the hyperglycemia of acidemia is mediated by reduction of insulin binding to its receptor and decreased tissue sensitivity to the hormone (19–22).

The defect in glucose homeostasis observed in uncontrolled diabetes might lead to either hyponatremia or hypernatremia (23–25). The hyperglycemia-induced increase in effective osmotic pressure of the extracellular fluid (ECF) triggers a shift of water out of cells, most prominently skeletal muscle, which reduces serum [Na^+]. An increase of 100 mg/dL (5.6 mmol/L) in the glucose concentration decreases serum [Na^+] by approximately 1.7 mEq/L, the end result being a rise in serum osmolality of approximately 2.0 mOsm/kg H_2O (25,26). The resulting expansion of the ECF compartment is, however, brief due to simultaneous renal and extrarenal loss of fluids. The hyperglycemia-induced increase in the filtered load of glucose exceeds the renal tubular reabsorptive capacity resulting in substantial glucosuria—one of the hallmarks of DKA. In turn, glucosuria causes osmotic diuresis that results in urinary losses of 75 to 150 mL/kg of water and 4 to 10 mEq/kg of Na^+ and Cl^- over an entire episode of DKA.

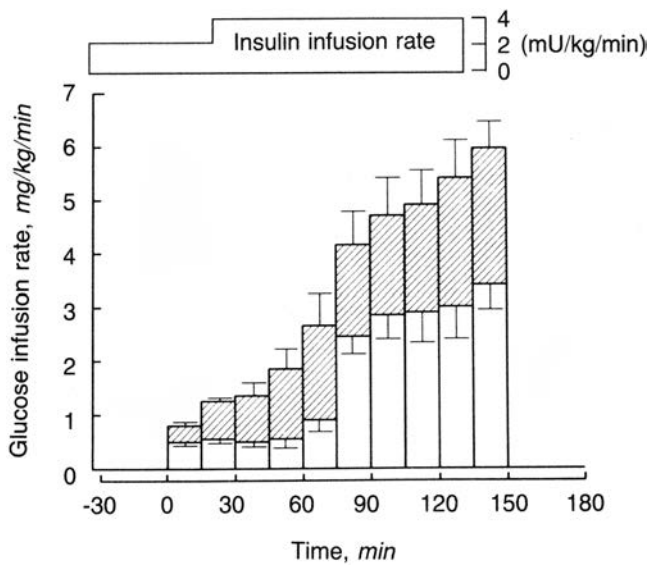

FIGURE 90-3. Rate of glucose infusion required to maintain euglycemia during insulin infusion studies in normal and acidemic dogs (respiratory acidosis, arterial pH = 7.18). Open area in each column represents the value in acidemic dogs; the entire column is the value in normal dogs. (From: Adrogué HJ. Glucose homeostasis and the kidney. *Kidney Int* 1992;42:1266.)

Because the total of the Na^+ and K^+ concentrations in the urine falls short of that in serum, osmotic diuresis elevates serum [Na^+] and [Cl^-]; moderation of hyponatremia or frank hypernatremia can ensue (23,27). However, other factors also act to modify the serum [Na^+] and [Cl^-] (27). Some Na^+ enters cells replacing cellular K^+ losses, thereby decreasing serum [Na^+]. Urinary losses of Na^+ as ketone salts tend to increase serum [Cl^-], whereas selective Cl^- depletion during vomiting tends to cause hypochloremia. Further, the intake of fluid and electrolytes (sodium, potassium, and chloride) influences serum [Na^+] and [Cl^-]. Differences in the magnitude of these phenomena from one patient to another account for the variability in serum electrolyte composition observed at presentation. In Table 90-2 are reviewed the admitting laboratory values of patients with DKA (8). Note that serum sodium concentration is usually depressed; the rare presence of hypernatremia is indicative of a profound water depletion, usually seen in the most critically ill patients.

Prerenal azotemia due to volume depletion is almost always present in uncontrolled diabetes and is usually reversible, but occasionally it can progress to acute tubular necrosis (28,29). The levels of plasma urea nitrogen, creatinine, total protein, uric acid, hematocrit, and hemoglobin can all be elevated on admission for DKA, a reflection of ECF volume contraction and/or renal dysfunction, but they normalize swiftly after volume repletion.

Hyperlipidemia

Diabetes mellitus is commonly associated with hyperlipidemia, which in turn may reduce measured serum sodium concentration, causing the so-called pseudohyponatremia or false hyponatremia. This electrolyte disorder is characterized by a normal [Na^+] in plasma water in spite of a diminished [Na^+] in a plasma or serum sample. The decreased [Na^+] arises from an increased solid phase of plasma owing to severe hyperlipidemia or hyperproteinemia (e.g., myeloma). If [Na^+] is measured directly (without dilution of the sample) with ion-sensitive electrodes instead of flame photometry (the latter being the classic method), a normal value will be found; thus, this type of hyponatremia is false because: (a) the [Na^+] in plasma water is normal, and (b) its detection is dependent on the method used for measurement of [Na^+]. It should be evident that pseudohyponatremia does not produce any of the symptoms that classically occur with hypotonic hyponatremia. Furthermore, measured plasma osmolality is normal in pseudohyponatremia, because the solute concentration in plasma water is not altered in this condition (23).

Let us compare the hyponatremia owing to hyperglycemia or hypertonic infusions (e.g., mannitol) with that caused by hyperlipidemia. The decreased [Na^+] owing to hyperglycemia or hypertonic infusions is not a form of pseudohyponatremia because [Na^+] in plasma water also is diminished. The ECF

TABLE 90-2

SALIENT LABORATORY ABNORMALITIES ON ADMISSION FOR DIABETIC KETOACIDOSIS (DKA)

Parameter	Value			Comments
Glucose	350 to 750 mg/dL			Values below 200 mg/dL ("euglycemic DKA") can be seen, especially in alcoholics or pregnant insulin-dependent diabetics; also, values above 1,000 mg/dl can be seen, especially in severe volume contraction leading to renal failure and interruption of glucosuria, glucose concentration not related to severity of DKA
Serum ketones	Positive in plasma diluted 1:1 or greater			Nitroprusside reagent (Ketostix, Acetest) does not react with β-hydroxybutyrate; color reaction is mostly (>80%) due to acetoacetate
Bicarbonate	<15 mmol/L			Always reduced in DKA unless complicated by co-existing metabolic alkalosis
pH	<7.30			Always reduced in DKA unless complicated by co-existing metabolic alkalosis or respiratory alkalosis
	Plasma concentration			
	Low	Normal	High[a]	
Sodium	67	26	7	Body stores depleted
Chloride	33	45	22	
Potassium	18	43	39	Body stores depleted
Magnesium	7	25	68	
Phosphate	11	18	71	Body stores depleted
Calcium	28	68	40	
BUN, creatinine	High			Because creatinine can be spuriously elevated (crossreaction with acetoacetate), BUN can better reflect renal function
White blood cell count	Usually high			Not necessarily indicative of infection; associated with lymphopenia and eosinopenia
Hemoglobin, hemacrit, total protein	Frequently increased			Due to intravascular volume depletion
SGOT,SGPT,LDH, CPK[b]	High 20% to 65%			Partially due to interference of acetoacetate with colorimetric assays; elevated CPK might be related to phosphate depletion and possible associated rhabdomyolysis
Amylase glands	Often increased			Isoenzyme evaluation reveals that site of origin is pancreas (50%), salivary (36%), or mixed (14%)

[a]Data from Kreisberg (Ref 2).
[b]*SGOT*, serum glutamic-oxaloacetic transaminase; *SGPT*, serum glutamic-pyruvic transaminase; *LDH*, lactate dehydrogenase; *CPK*, creatine phosphokinase.

accumulation of solutes of relatively small molecular size, observed with hyperglycemia or hypertonic infusions, increases extracellular tonicity, which in turn osmotically pulls water from the intracellular fluid (ICF), diluting the $[Na^+]$ in ECF. By contrast, high plasma levels of large-molecular-size solutes (e.g., hypertriglyceridemia) fail to alter extracellular tonicity; therefore, not causing a shift of water from the ICF to the ECF. Thus, pseudohyponatremia is a spurious form of isoosmolar and isotonic hyponatremia identified when severe hyperlipidemia or paraproteinemia increases substantially the solid phase of plasma and the sodium concentration is measured by means of flame photometry. The increasing availability of direct measurement of serum sodium with ion-specific electrode has all but eliminated this laboratory artifact (23).

Defect in Water Homeostasis

Examination of the defect in water homeostasis that accompanies diabetes mellitus requires a brief overview of this topic (23–25). The disorders of salt and water balance may be classified into three major categories: (a) abnormalities in the size of body fluid compartments, (b) disturbances in the tonicity of body fluids, and (c) a selective deficit or excess of chloride with respect to sodium (25). The first group of disorders comprises an enlargement ("volume expansion") and a reduction ("volume depletion or contraction") in the size of body fluid compartments, which are produced by a combined salt and water excess and a combined salt and water deficit, respectively. Disturbances in the tonicity of body fluids include increases (e.g., hypernatremia) and decreases (e.g., hypotonic hyponatremia) in the effective osmolality of body fluids. As opposed to disturbances in the size of body fluids in which salt and water excess or deficit develops in proportion to the relationship found in the normal state, a discordant abnormality in salt and water balance occurs in disorders of body fluid tonicity. Thus, a disproportion between salt and water content of body fluids explains the development of hypernatremia (water content in body fluids is relatively small for the concomitant salt content) and hypotonic hyponatremia (water content in body fluids is relatively large for the concomitant salt content). The third group of salt and water disorders is characterized by an abnormal relationship between the $[Na^+]_p$ and $[Cl^-]_p$ (chloride concentration in plasma). Although the $[Na^+]_p$ is generally maintained within normal limits in these disorders because it is dependent on overall water homeostasis, the $[Cl^-]_p$ is either abnormally low or high. The major representatives of a selective deficit or excess of chloride with respect to sodium are hypochloremic metabolic alkalosis and hyperchloremic metabolic acidosis, respectively.

These acid–base disorders are reviewed in the section dealing with such abnormalities.

An important concept that must be understood relates to the identification of the basic abnormality responsible for the disturbances in the volume of body fluids and that responsible for altered tonicity (25). Disturbances in salt balance are the primary causes of volume excess and depletion, whereas disorders in water balance are responsible for the development of the tonicity disorders, hypertonicity (hypernatremia) and hypotonicity (hyponatremia). Because sodium chloride (NaCl) excess only transiently increases tonicity, leading to augmented antidiuretic hormone (ADH) (i.e., arginine vasopressin secretion and secondary water retention), hypernatremia is not clinically observed. Expansion of the ECF volume, instead, is the hallmark of a primary salt excess. In a comparable fashion, NaCl deficit only transiently decreases tonicity, inhibiting ADH secretion with secondary increase in water excretion, so that hyponatremia is not observed, whereas volume depletion becomes the major manifestation of this electrolyte imbalance. A primary and exclusive disturbance in water balance, deficit and excess, causes hypertonicity (hypernatremia) and hypotonicity (hyponatremia), respectively, but does not produce a major alteration in the size of the fluid compartments because the latter are primarily determined by the osmolar content in the compartment (NaCl content in the ECF, which is unaltered in exclusive disturbances of water balance).

Dysnatremias in Diabetes Mellitus. A defect in water homeostasis in patients with diabetes mellitus might lead to either hypotonic hyponatremia or hypernatremia in response to positive or negative water balance, respectively (25). Water and electrolyte losses caused by vomiting or diarrhea are commonly encountered in uncontrolled diabetes and in patients with this disease experiencing target organ damage in the alimentary tract (e.g., gastroparesis, nocturnal diarrhea). In addition, excessive urinary fluid losses may develop as a result of osmotic diuresis, use of diuretics, adrenal insufficiency, or other causes. Whether hypotonic hyponatremia or hypernatremia develops is dependent on the concomitant water intake. Hypernatremia might be observed if water intake is insufficient, whereas a large salt-free fluid intake might lead to hyponatremia. Longstanding diabetes mellitus commonly predisposes or leads to heart failure or renal failure, or both, thereby impairing renal water excretion that may lead to hypotonic hyponatremia. Concomitant medication, including diuretics, might also play a role in the development of hyponatremia.

Abnormal $[Na^+]_p$ can produce signs and symptoms of disease owing to central nervous system dysfunction and the clinical manifestations elicited by opposite changes in tonicity are remarkably similar, except for seizures that are mostly caused by cerebral edema secondary to dilutional (hypotonic) hyponatremia. When the patient's osmoregulating mechanisms (thirst, changes in water intake and ADH levels, renal water retention, or excretion) fail, an increase in plasma tonicity and $[Na^+]_p$, or conversely a decrease in plasma tonicity and $[Na^+]_p$, develops. Plasma hypertonicity induces brain water loss and hypotonicity produces water gain in this organ, accompanied in both cases by parallel volume changes. As a defense mechanism to correct brain volume changes, the intracellular osmolytes of this organ increase in hypernatremia and decrease in hypotonic hyponatremia. The adaptive increase in brain osmolytes is owing to a modest increase in cellular K^+ and to the accumulation of organic solutes (e.g., glutamine, glutamate, and other organic metabolites). Conversely, the adaptive decrease in brain osmolytes is owing to a decrease in cellular K^+ accompanied by a diminished concentration of organic solutes. These secondary responses of the brain to altered extracellular tonicity can be demonstrated within a few hours of the initiation of abnormal tonicity and are complete within a few days. *Idiogenic osmoles* is the name given to the cerebral organic osmolytes detected in response to the adaptation of the brain to increases in extracellular tonicity.

Hypotonic or dilutional hyponatremia. It represents an excess of water in relation to existing sodium stores, which can be decreased, essentially normal, or increased. Retention of water most commonly reflects the presence of conditions that impair renal excretion of water; in a minority of cases, however, it is caused by excessive water intake, with a normal or nearly normal excretory capacity.

Conditions of impaired renal excretion of water are categorized according to the characteristics of the ECF volume, as determined by clinical assessment. Decreased ECF volume can result from renal sodium loss (e.g., glucosuria-induced osmotic diuresis) or extrarenal sodium loss (e.g., vomiting). Conditions with essentially normal ECF volume include thiazide diuretics, syndrome of inappropriate secretion of antidiuretic hormone, decreased intake of solutes, hypothyroidism, and glucocorticoid insufficiency. Increased ECF volume with hyponatremia can be observed in pregnancy, renal failure, congestive heart failure, cirrhosis, and nephrotic syndrome. With the exception of renal failure, these conditions are characterized by high plasma concentrations of arginine vasopressin despite the presence of hypotonicity because arterial underfilling induces baroreceptor-mediated nonosmotic release of ADH overriding the osmotic regulation of the hormone, thereby impairing urinary dilution and causing hyponatremia. Depletion of potassium accompanies many of these disorders and contributes to hyponatremia, since the sodium concentration is determined by the ratio of the "exchangeable" (i.e., osmotically active) portions of the body's sodium and potassium content to total body water. Patients with hyponatremia induced by thiazides can present with variable hypovolemia or apparent euvolemia, depending on the magnitude of the sodium loss and water retention.

Excessive water intake can cause hyponatremia by overwhelming normal water excretory capacity (e.g., 15 to 20 L/day). Frequently, however, psychiatric patients with excessive water intake have plasma arginine vasopressin concentrations that are not fully suppressed and urine that is not maximally dilute, thus contributing to water retention.

The optimal treatment of hypotonic hyponatremia requires balancing the risks of hypotonicity against those of therapy (23). The presence of symptoms and their severity largely determine the pace of correction. Patients with symptomatic hyponatremia and dilute urine (osmolality, <200 mOsm/kg water) but with less serious symptoms usually require only water restriction and close observation. Severe symptoms (e.g., seizures or coma) call for infusion of hypertonic saline. On the other hand, patients who have symptomatic hyponatremia with concentrated urine (osmolality = 200 mOsm/kg water) in association with a hypovolemic state are best treated with isotonic saline; those having clinical euvolemia or hypervolemia require infusion of hypertonic saline.

There is no consensus about the optimal treatment of symptomatic hyponatremia (23). Nevertheless, correction should be of a sufficient pace and magnitude to reverse the manifestations of hypotonicity but not be so rapid and large as to pose a risk of the development of central pontine myelinolysis. Osmotic demyelination is serious and can develop one to several days after aggressive treatment of hyponatremia by any method, including water restriction alone. Shrinkage of the brain triggers demyelination of pontine and extrapontine neurons that can cause neurologic dysfunction, including quadriplegia, pseudobulbar palsy, seizures, coma, and even death. Hepatic failure, potassium depletion, and malnutrition increase the risk of the complication. Physiologic considerations indicate that a relatively small increase in the serum sodium concentration, on

the order of 5%, should substantially reduce cerebral edema in patients with symptomatic hypotonic hyponatremia. Even seizures induced by hyponatremia can be stopped by rapid increases in the serum sodium concentration that average only 3 to 7 mEq/L. Most reported cases osmotic demyelination occurred after rates of correction that exceeded 12 mEq/L per day were used, but isolated cases occurred after corrections of only 9 to 10 mEq/L in 24 hours or 19 mEq/L in 48 hours. After weighing the available evidence and the all-too-real risk of overshooting the mark, we recommend a targeted rate of correction that does not exceed 8 mEq/L on any day of treatment. Remaining within this target, the initial rate of correction can still be 1 to 2 mEq/L per hour for several hours in patients with severe symptoms.

The rate of infusion of the selected solution can be derived expediently by applying the following equations.

$$\Delta[Na^+]_s = \frac{[Na^+]_{inf} - [Na^+]_s}{TBW + 1} \quad [1]$$

The equation above projects the impact of 1 L of any infusate on the patient's $[Na^+]_s$

$$\Delta[Na^+]_s = \frac{([Na^+]_{inf} + [K^+]_{inf}) - [Na^+]_s}{TBW + 1} \quad [2]$$

This equation is a simple derivative of Equation #1, which projects the impact of 1 L of any infusate containing sodium and potassium on the patient's $[Na^+]_s$

The preceding equations project the change in serum sodium elicited by the retention of 1 L of any infusate (30). Dividing the change in serum sodium targeted for a given treatment period by the output of this equation determines the volume of infusate required, and hence the rate of infusion. Although water restriction ameliorates all forms of hyponatremia, as explained, it is not the optimal therapy in all cases.

Corrective measures for nonhypotonic hyponatremia are directed at the underlying disorder rather than at the hyponatremia itself. Administration of insulin is the basis of treatment for uncontrolled diabetes, but deficits of water, sodium, and potassium also should be corrected.

Hypernatremia. Defined as a rise in the $[Na^+]_p$ to a value exceeding 145 mEq/L, it represents a deficit of water in relation to the body's sodium stores, which can result from a net water loss or a hypertonic sodium gain (24). Net water loss accounts for the majority of cases of hypernatremia. It can occur in the absence of a sodium deficit (pure water loss) or in its presence (hypotonic fluid loss). Net water loss can result from pure water (e.g., hypodipsia, diabetes insipidus) or hypotonic fluid loss, the latter secondary to renal, gastrointestinal, or cutaneous causes.

An equation (31) that allows projection of the expected $\Delta[Na^+]_s$ in response to losing 1 L of fluid (fl) of variable electrolyte content from the renal or extrarenal route is as follows:

$$\Delta[Na^+]_s = \frac{[Na^+]_s - [Na^+ + K^+]_{fl}}{TBW - 1\,L} \quad [3]$$

Multiplying the output of the equation by the volume of the fluid loss in L provides a quantitative estimate of the impact of the fluid loss on $[Na^+]_s$. Obviously, application of this equation has greater practical value in the presence of large fluid losses (e.g., large gastrointestinal drainage, polyuria).

Hypertonic sodium gain usually results from clinical interventions (e.g., sodium bicarbonate infusion, hypertonic enemas) or accidental sodium loading. Signs and symptoms of hypernatremia largely reflect central nervous system dysfunction and are prominent when the increase in the serum sodium concentration is large or occurs rapidly (i.e., over a period of hours). Most outpatients with hypernatremia are either very young or very old. Common symptoms in infants include hyperpnea, muscle weakness, restlessness, a characteristic high-pitched cry, insomnia, lethargy, and even coma. Convulsions are typically absent except in cases of inadvertent sodium loading or aggressive rehydration. Brain shrinkage induced by hypernatremia can cause vascular rupture, with cerebral bleeding, subarachnoid hemorrhage, and permanent neurologic damage or death. Brain shrinkage is countered by an adaptive response that is initiated promptly and consists of solute gain by the brain that tends to restore lost water.

Proper treatment of hypernatremia requires a two-pronged approach: addressing the underlying cause and correcting the prevailing hypertonicity (24). Managing the underlying cause may mean stopping gastrointestinal fluid losses; controlling pyrexia, hyperglycemia, and glucosuria; withholding lactulose and diuretics; treating hypercalcemia and hypokalemia; moderating lithium-induced polyuria; or correcting the feeding preparation. In patients with hypernatremia that has developed over a period of hours (e.g., those with accidental sodium loading) rapid correction improves the prognosis without increasing the risk of cerebral edema, because accumulated electrolytes are rapidly extruded from brain cells. In such patients reducing the $[Na^+]_p$ by 1 mEq/L per hour is appropriate. A slower pace of correction is prudent in patient with hypernatremia of longer or unknown duration, because the full dissipation of accumulated brain solutes occurs over a period of several days. In such patients, reducing the $[Na^+]_p$ at a maximal rate of 0.5 mEq/L per hour prevents cerebral edema and convulsions. Consequently, we recommend a targeted fall in the $[Na^+]_p$ of 10 mEq/L per day for all patients with hypernatremia except those in whom the disorder has developed over a period of hours. The goal of treatment is to reduce the $[Na^+]_p$ to 145 mEq/L. Because ongoing losses of hypotonic fluids, whether obligatory or incidental, aggravate the hypernatremia, allowance for these losses must also be made.

The preferred route for administering fluids is the oral route or a feeding tube; if neither is feasible, fluids should be given intravenously. Only hypotonic fluids are appropriate, including pure water, 5% dextrose, 0.2% sodium chloride (referred to as one-quarter isotonic saline), and 0.45% sodium chloride (one-half isotonic saline). The more hypotonic the infusate, the lower the infusion rate required. The volume should be restricted to that required to correct hypertonicity because the risk of cerebral edema increases with the volume of the infusate. Except in cases of frank circulatory compromise, 0.9% sodium chloride (isotonic saline) is unsuitable for managing hypernatremia.

After selecting the appropriate infusate, the physician must determine the rate of infusion. This can be easily calculated with the use of an equation that estimates the change in the serum sodium concentration caused by the retention of 1 L of any infusate.

The equations for use in managing hypernatremia (24,30) are identical to those previously presented for managing hyponatremia and include:

$$\Delta[Na^+]_s = \frac{[Na^+]_{inf} - [Na^+]_s}{TBW + 1} \quad [1]$$

The equation above projects the impact of 1 L of any infusate on the $[Na^+]_s$

$$\Delta[Na^+]_s = \frac{([Na^+]_{inf} + [K^+]_{inf}) - [Na^+]_s}{TBW + 1} \quad [2]$$

This equation is a simple derivative of Equation #1, which projects the impact of 1 L of any infusate containing sodium and potassium on the patient's $[Na^+]_s$

The required volume of infusate, and hence the infusion rate, is determined by dividing the change in the $[Na^+]_p$ targeted for a given treatment period by the value obtained from

the equation. The sole indication for administering isotonic saline to a patient with hypernatremia is a depletion of ECF volume sufficient to cause substantial hemodynamic compromise. Even in this case, after a limited amount of isotonic saline has been administered to stabilize the patient's circulatory status, a hypotonic fluid (i.e., 0.2% or 0.45% sodium chloride) should be substituted in order to restore normal hemodynamic values while correcting the hypernatremia. If a hypotonic fluid is not substituted for isotonic saline, the ECF volume may become seriously overloaded.

Defect in Sodium Homeostasis

The quantity of solutes in each of the main compartments determines its size, so that deficit or excess of solutes in a particular space will shrink or swell that space in comparison with the other compartments (25). The partition of water is determined by the osmotic activity of the solutes confined to each body compartment. One major solute is responsible for the size of each fluid compartment. These solutes are potassium, sodium, and proteins, for the intracellular, extracellular, and intravascular spaces, respectively. Because the hydraulic permeability of most cell membranes is very high, solute-free water freely and rapidly moves among all body compartments.

Body stores of salt (NaCl) are determined by the balance of its intake and excretion. Under normal circumstances, salt intake is derived from the diet and its excretion occurs by urinary loss. A positive NaCl balance (intake exceeds excretion) increases salt stores, whereas a negative one (excretion exceeds intake) decreases salt stores. The effect of increased NaCl stores is expansion of ECF volume, while decreased NaCl stores lead to a reduced ECF volume. Thus, a NaCl deficit in body fluids (e.g., vomiting, diarrhea) reduces ECF volume, including the intravascular compartment. By contrast, NaCl excess (e.g., congestive heart failure) expands ECF volume and produces overt peripheral edema and accumulation of fluid in major body cavities (pleural effusion, ascites). A major decrease in serum protein concentration (mostly albumin) diminishes intravascular volume and promotes expansion of the interstitial compartment (e.g., nephrotic syndrome, hepatic cirrhosis). Diabetes mellitus is a common cause of both volume depletion and volume expansion. The former disturbance is characteristically observed in the course of severe metabolic complications of this disease, namely, DKA and NKH. Conversely, volume expansion is observed in patients having long-term diabetic complications including congestive heart failure, nephrotic syndrome, and renal failure.

Volume Depletion. Volume depletion in diabetic patients can result from fluid loss (e.g., renal and/or extrarenal) or from fluid sequestered into a "third space" (e.g., acute pancreatitis). Renal losses may occur in the presence of normal intrinsic renal function (e.g., osmotic diuresis caused by glucosuria or urea diuresis, adrenal insufficiency, diuretics) or in acute and chronic renal disease (e.g., acute tubular necrosis, diabetic glomerulosclerosis). Osmotic diuresis owing to renal excretion of glucose can produce a large natriuresis, leading to volume depletion. Patients with significant hyperglycemia, including those with DKA or nonketotic coma, may have a fluid deficit of 10% or more of body weight. Extrarenal losses include those from the gastrointestinal tract (e.g., vomiting, diarrhea, gastrointestinal suction, fistulas) and those from the skin (sweat, burns, extensive skin lesions). Fluid sequestration into a third space occurs with abdominal accumulation (e.g., intestinal obstruction, pancreatitis, peritonitis), bleeding, skeletal fractures, and obstruction of a major venous system.

The patient's history, physical examination, and laboratory data are critical elements in the evaluation of volume depletion, allowing the physician to (a) assess the severity of the deficit, and (b) establish its cause. Immediate recognition of hypovolemic shock is of utmost importance, because rapid intravascular volume expansion might prevent tissue injury and death. Evaluation of its severity allows establishment of the rate of infusion and the total fluid requirements. Recognition of the factors responsible for fluid loss permits initiation of specific therapeutic measures to correct the volume depletion (25).

Patients with volume depletion have signs and symptoms related to (a) the process responsible for volume depletion, and (b) the hemodynamic consequences of fluid loss. Through the first group of manifestations it is possible to recognize the cause of volume depletion, such as loss or sequestration of fluid. The second group of signs and symptoms includes hypotension, decreased cardiac output, and tachycardia owing to intravascular volume depletion. In addition, diminished tissue perfusion produces altered mental status, generalized weakness, and occasionally severe organ damage (e.g., acute tubular necrosis, cerebral ischemia, myocardial infarction).

The severity of volume deficit may be estimated through evaluation of blood pressure, heart rate, neck veins and venous pressure, skin turgor, moistness of mucous membranes, changes in body weight, and blood and urine samples. If volume depletion results from mechanisms other than hemorrhage, the fluid loss produces hemoconcentration with increased hematocrit (Hct). The ECF volume deficit can be estimated in states of a primary extravascular fluid loss from the rise in Hct as follows:

$$\text{ECV volume deficit} = 0.25 \times \text{body weight (kg)} \times (\text{actual Hct/normal Hct} - 1) \quad [4]$$

where 0.25 represents the fraction of ECF per kg of body weight (250 mL/kg). Because the normal range of Hct is relatively wide (38% to 45%), the patient's baseline Hct usually is unknown, and blood loss may have occurred, the reliability of changes in Hct is only modest. Therefore, a precise estimation of volume deficit is difficult. The loss of body weight from its baseline level (body weight prior to the episode of volume depletion) is a clinically useful index to estimate volume deficit, as follows:

$$\Delta\text{body weight (kg)} = \text{fluid deficit (L)} \quad [5]$$

The change in body weight is unreliable for the estimation of fluid deficit in patients with "third space" sequestration. If $[Na^+]_p$ remains within normal limits, the weight loss in kg truly represents loss of isotonic fluid. Volume deficit accompanied by hypernatremia or hyponatremia indicates the existence of a disproportionate water loss compared to Na^+ loss. (The water loss is larger for the former and smaller for the latter, compared to Na^+ loss.)

Pertinent data obtained from a blood specimen that are most useful in the diagnosis and management of volume depletion include: (a) BUN and serum creatinine levels; (b) Hct, total plasma protein, and/or albumin values; and (c) levels of serum electrolytes including Na^+, K^+, Cl^-, and total carbon dioxide (almost identical to plasma $[HCO_3^-]$). In volume depletion, BUN and plasma creatinine increase because of an overall depression of renal function, manifested in oliguria, reduced glomerular filtration rate (GFR), and renal plasma flow. Increased plasma creatinine is caused by a reduced GFR (when muscle necrosis, which could release this substance into the circulation, is absent). Conversely, an elevated BUN, not accompanied by increased plasma creatinine and reduced GFR, reflects enhanced renal reabsorption of urea accompanied by increased salt and water reabsorption. Consequently, the ratio of BUN over plasma creatinine increases from its normal value of 10:1 to 15:1 or more. Hematocrit and concentration of plasma proteins also can increase in volume depletion, a process referred to as hemoconcentration. Alterations in serum electrolytes are commonly observed and they depend on

the composition of the fluid lost (e.g., vomiting produces hypokalemia and metabolic alkalosis) as well as the concomitant water and electrolyte intake.

With respect to fluid therapy in volume depletion, considering that oral intake is the physiologic pathway for the entry of fluids, this route should be always considered. In addition, oral replacement therapy is effective, relatively inexpensive, and noninvasive; does not require hospitalization; and saves several million patients (mostly children in developing nations) each year from death owing to volume depletion. Nevertheless, the presence of vomiting, ileus, or altered mental status precludes its use, mandating intravenous administration of fluid. Most frequently, however, volume repletion in hospitalized patients is performed by the parenteral (intravenous) route.

Volume repletion should be promptly secured because severe volume depletion frequently produces a major reduction in intravascular volume and hypovolemic shock. The type of fluid to be used depends on the cause of volume depletion. Hypovolemia caused by bleeding (e.g., peptic ulcer, rupture of aortic aneurysm) must be treated with blood products or plasma volume expanders (e.g., packed red cells, albumin, or dextran solutions), whereas the one resulting from renal or extrarenal losses and fluid sequestration in body cavities (e.g., ileus, ascites) must be treated with saline or dextrose in saline or Ringer's solution. Plasma volume expanders can be used in the initial phase of treatment to secure a more rapid restoration of hemodynamic status in all patients with shock.

Various intravenous solutions can be selected in fluid therapy (25). The most commonly used intravenous fluids consist of a NaCl-containing solution (NaCl 0.23%, 0.45%, and 0.9%, known as *1/4 normal saline, 1/2 normal saline,* and *normal saline,* respectively) with or without 5% dextrose. The term normal used in reference to intravenous solutions does not imply "normality" (chemical notation) but simply refers to the isotonicity of intravenous solutions with respect to body fluids. It is more proper to refer to these solutions as 1/4 isotonic saline, 1/2 isotonic saline, and isotonic saline. Although 5% dextrose in water is isotonic with body fluids (i.e., 50,000 mg/L glucose divided by its molar weight of 180 mg/mmol equals 278 mmol/L), the glucose is metabolized so that this solution provides solute-free water without effective long-lasting osmoles (yet providing some caloric intake). The NaCl added to intravenous solutions provides effective osmoles that are preferentially retained in ECF. The efficacy of the various solutions with respect to volume deficit correction is a function of their NaCl concentration with normal saline as the most effective one and dextrose in water without NaCl the least effective. The selection of intravenous solution is also determined by the patient's $[Na^+]_p$; hypernatremic patients are most frequently treated with NaCl-free solutions (e.g., 5% dextrose in water) whereas those with hyponatremia are usually given isotonic saline or hypertonic (e.g., NaCl 3.0%) saline solutions. It is important to realize the expected changes in the volume of ECF and intracellular (ICF) in response to an infusion of isotonic saline, 1/2 isotonic saline, and salt-free water. The infusion of a normal saline solution expands the ECF exclusively (ECF volume increment is identical to the volume infused); thus, ICF volume remains unaltered. The infusion of a 1/2 isotonic saline solution expands both the ECF and ICF, with the former receiving 40% and the latter 60% of the volume load. Finally, a salt-free water infusion (e.g., 5% dextrose in water) will also expand both the ECF and ICF, but in this case, the latter receives 60% of the volume load. In summary, a pure water infusion expands all body compartments but predominantly the ICF, whereas isotonic saline expands the ECF exclusively (23).

With respect to the rate of intravenous fluid infusion in the therapy of volume depletion, because patients in hypovolemic shock are at immediate risk of death or ischemic tissue injury, the initial fluid infusion should be at the maximal flow allowed by the intravenous catheter ("wide open"); once blood pressure and tissue perfusion return to acceptable levels, the rate must be diminished to approximately 100 mL/hour to minimize the risk of pulmonary edema, owing to rapid intravascular expansion, even when the state of volume depletion has not yet achieved full correction. Patients with acceptable hemodynamic parameters should receive fluid at the initial rates of 100 to 200 mL/hour, with subsequent reduction after 6 to 12 hours to rates of about 100 mL/hour, to secure gradual repletion of all fluid compartments without imposing undue stress on the circulation. Exceptions to these rules are patients with extreme volume depletion (e.g., DKA, NKH) or large ongoing fluid losses (e.g., continuous drainage of large volume of gastrointestinal secretions, postobstructive diuresis, and diabetes insipidus) who might require fluids at a higher rate of infusion as described in the corresponding section of this chapter.

Proper monitoring of fluid replacement therapy is accomplished by evaluation of arterial blood pressure, presence of collapsed or distended neck veins, and urine output, to establish the optimum rate of fluid replacement. Additional information might be necessary in critically ill patients, including monitoring of left- and right-sided heart filling pressures, blood pressure measurement through an intraarterial line, arterial and/or venous blood gas analysis, and sequential chest x-ray films to detect pulmonary venous congestion and interstitial edema.

Volume Expansion. A syndrome of volume expansion caused by overt salt and water retention is commonly observed in longstanding diabetes mellitus (32,33). Both forms of generalized edema, the so-called primary as well as the secondary types, are encountered. In primary edema, renal retention of salt and water is the initial event that leads to expansion of plasma and ECF volume (e.g., diabetic glomerulosclerosis with reduced GFR and avid tubular reabsorption of salt and water). In secondary edema, also called *underfill edema,* the presence of renal hypoperfusion, owing to decreased "effective arterial circulating blood volume" initiates salt and water retention by the kidney (e.g., diabetes mellitus with congestive heart failure). Thus, the kidney is always involved in the development of positive salt and water balance that leads to generalized edema (in patients with and those without diabetes mellitus), yet the renal dysfunction might result from abnormalities initiated within it (primary edema) or be a response to "effective" intravascular arterial volume depletion (secondary edema) (25). It must be recognized that salt and water retention, owing to primary renal disease and congestive heart failure, is the main cause of generalized edema and normal or near-normal serum albumin. Absence of proteinuria argues against renal disease as the primary cause of fluid retention. Patients with heart failure usually have either minimal or mild urinary protein excretion (1+ or 2+ on dipstick determination), whereas those with nephrotic syndrome have, as a rule, severe proteinuria (4+ dipstick). The fluid retention observed in nephrotic syndrome appears to occur as a combination of primary and secondary edema. It is common for physicians to incorrectly diagnose congestive heart failure on the basis of bilateral lower extremity edema, without realizing that a similar syndrome is observed in patients with salt and water retention owing to a primary renal disease.

The management of localized and generalized edema must be directed, if possible, at the primary cause of fluid accumulation. Effective treatment of the primary cause leads to resolution of the edema. Therapy of the primary process in congestive heart failure can involve the use of afterload-reducing agents, digoxin, and diuretics. Patients with generalized edema most frequently require treatment of the fluid overload in addition to that directed at the primary disease. Correction of fluid overload involves restriction of dietary NaCl, and if this is unsuccessful, the use of diuretic therapy. In addition, all forms

of edema, localized and generalized, inflammatory and noninflammatory, are ameliorated by bed rest and elevation of the edematous body area (e.g., placement of swollen body region above the heart level, such as the head for facial phlegmon following dental surgery, and the lower extremity for thrombophlebitis of the calf). The benefits derived from bed rest and elevation of the edematous body region derive from: (a) facilitation of fluid exit from the edematous region owing to the salutary effect on the Starling forces, as reduction in arterial and increment in venous blood flow accompany the decreased metabolic demands of resting tissues, and the hydrostatic effect of elevation of the edematous region; (b) bed rest diminishes venous blood pooling, increasing venous return to the heart, which augments the atrial natriuretic factor that promotes renal vasodilation and increased NaCl excretion; and (c) bed rest suppresses the renin–angiotensin–aldosterone system and α-adrenergic-mediated vasoconstrictor influences, which in turn increase renal excretion of salt. Bed rest, extended for several days or intermittently (a few hours of daytime bed rest in addition to nocturnal sleep), helps to mobilize the dependent edema. The management of generalized edema caused by congestive heart failure, nephrotic syndrome, and diabetic glomerulosclerosis is examined in detail in other chapters.

Defect in Potassium Homeostasis

The levels of total body K^+ stores are established by the external K^+ balance, which is in turn determined by the difference between K^+ intake and excretion. The internal K^+ balance refers to the control mechanisms for the distribution of total body K^+ stores between the ICF and the ECF. Thus, internal K^+ balance refers to the internal exchanges of K^+. On the other hand, external K^+ balance refers to the determinants of total body K^+ stores, without any consideration for the distribution of K^+ among body compartments. The major factors that alter internal K^+ balance include hormones (insulin, catecholamines), the acidity of body fluids, the levels of other electrolytes, the tonicity of body fluids, and drugs (34).

Insulin is a major modulator of extrarenal K^+ homeostasis and promotes K^+ uptake in many cell types, including those from skeletal muscle and liver. The hypokalemic action occurs at very low concentrations of insulin and is independent of the effect of insulin on glucose uptake. The precise mechanism of this action remains to be fully defined but appears to involve the activation of several transport proteins (35) (Table 90-3). The cellular mechanisms of insulin-mediated K^+ loading include: (a) stimulation of the Na^+-K^+-ATPase, (b) stimulation of the Na^+-H^+ exchanger, and (c) changes in ionic conductance of some K^+ channels. The hypokalemic effect of insulin resulting from stimulation of the Na^+-K^+-ATPase occurs as follows. Direct stimulation of the Na^+-K^+ pump by insulin induces the translocation of K^+ to the cell interior (entry of two K^+ and exit of three Na^+). In addition, substantial changes occur in the transport of electrolytes across the cell. The immediate result of insulin-mediated stimulation of Na^+-K^+-ATPase on the electrical properties of the cell membrane is hyperpolarization of the membrane potential (a more negative cell interior). The secondary effects promoted by insulin-induced hyperpolarization of the cell membrane are: (a) a new electrical gradient, which favors cellular K^+ entry, and (b) deactivation of K^+ channels, which inhibits cellular K^+ exit. Thus, the secondary effects of insulin on the membrane potential increase the hypokalemic action of this hormone.

The hypokalemic effect of insulin also results from the stimulation of the Na^+-H^+ exchanger in the cell membrane. Insulin promotes the cellular entry of Na^+ and the cellular exit of H^+ by stimulation of this exchanger. The entry of Na^+ increases the $[Na^+]_i$ (intracellular concentration of sodium), which further stimulates the Na^+-K^+-ATPase. The cellular exit of H^+ results in cytosolic alkalinization, which in turn: (a) increases the K^+-binding capacity of intracellular anions, and (b) stimulates the Na^+-K^+ pump, therefore favoring cellular K^+ loading.

A third mechanism for the hypokalemic effect of insulin is mediated from its action on K^+ channels. Insulin controls gating of the inward rectifier K^+ channel of skeletal muscle. This type of K^+ channel is of significant relevance because it is responsible for most of the K^+ conductance of the skeletal muscle in the resting state. This channel allows K^+ to flow into cells much more easily than it exits from them. Consequently, when the cell membrane is hyperpolarized, the high inward conductance facilitates cellular K^+ entry, whereas when the cell membrane is depolarized, the low outward conductance reduces K^+ exit from cells. Insulin exaggerates the inward rectifying properties of this class of K^+ channel by a dual effect of stimulation of K^+ entry and depression of K^+ exit.

Glucagon also has significant effects on internal K^+ balance and plasma potassium levels (36). Glucagon induces glycogen breakdown in the hepatocytes, releasing glucose and K^+; therefore, high glucagon levels can elicit a transient increase in $[K^+]_p$. An increase in plasma glucagon in acute metabolic acidosis has been described and this hormonal response might play a role in acidosis-induced hyperkalemia (35).

K^+ Depletion with Hyperkalemia. The development of uncontrolled diabetes, including DKA, is usually accompanied

TABLE 90-3

INSULIN-MEDIATED CELLULAR K^+ UPTAKE

Primary action	Immediate effect	Secondary effect
Stimulation of Na^+-K^+-ATPase	Hyperpolarization of cell membrane	New electrical gradient favors K^+ entry Deactivation of some K^+ channels may prevent K^+ exit
Stimulation of Na^+-H^+ exchanger	Cytosolic alkalinization	Increased K^+-binding capacity of intracellular proteins Stimulation of Na^+-K^+-ATPase
	Increased cell $[Na^+]$	Stimulation of Na^+-K^+-ATPase
K^+ channels (inward rectifier)	Increased K^+ conductance when cell membrane is hyperpolarized	Exaggeration of the inward properties

From: Adrogué HJ. Mechanisms of transcellular potassium shifts in acid–base disorders. In: Hatano M, ed., *Proceedings XIth International Congress of Nephrology.* Tokyo: Springer; 1991:259.

by varying degrees of total body potassium depletion, which results from multiple causes, including massive kaliuresis secondary to glucosuria, decreased intake, and frequent vomiting. However, plasma potassium levels are rarely low at the time of hospitalization, ranging in most instances from normal to high levels and occasionally attaining dangerously elevated values. This paradoxical relationship has been classically attributed to the concomitant changes in blood acidity that would affect a shift in potassium out of the cells in exchange for hydrogen ions moving intracellularly (37). However, several of the metabolic derangements observed in patients presenting with DKA are known to alter potassium metabolism and may contribute to the development of hyperkalemia. Endogenous ketoacidemia and hyperglycemia correlate with increased plasma potassium concentration on admission in patients with DKA (36). However, exogenous ketoacidemia and hyperglycemia in the otherwise normal experimental animal fails to increase plasma potassium levels (38,39), so it would seem that the insulin deficit per se is the major cause of the hyperkalemia that develops in DKA (36).

Serum pH and bicarbonate levels are known to alter plasma potassium levels. Whereas some studies indicated that the changes in plasma potassium concentration observed during acute acid–base disorders are consequent to the attendant changes in plasma pH, others showed that a low plasma bicarbonate concentration, under isohydric conditions, may induce hyperkalemia (40,41). Increased effective serum osmolality is another abnormality characteristic of DKA that may affect serum potassium; extracellular hypertonicity resulting from the infusion of saline, mannitol, or glucose results in the translocation of potassium-rich cell water to the extracellular compartment (42). Hyperglycemia of either endogenous or exogenous origin unaccompanied by ketoacidosis results in hyperkalemia in insulin-deficient diabetics, especially when hypoaldosteronism also is present (39).

As previously described, glucagon may also play a role in the hyperkalemia of DKA. This hormone may cause an increased potassium output from the liver, an effect that is usually transient because of the counterregulatory enhancement of insulin secretion. However, in the presence of an impaired insulin secretion, as in patients with DKA, increments in plasma glucagon levels may result in uncontrolled hyperkalemia (43).

An additional mechanism that may be involved in the deranged potassium homeostasis observed in diabetes mellitus is the sympathetic nervous system. Potassium tolerance has been found to be markedly impaired in chemically sympathectomized animals, but is improved in animals given a simultaneous infusion of epinephrine (44). The effects of the adrenergic agents on the internal potassium balance are mediated by their effect on the plasma levels of insulin and glucagon, and a direct cellular effect on K^+ transport. Therefore, any physiologic condition or pharmacologic maneuver that blocks the β-adrenergic system could result in hyperkalemia, particularly during states of increased potassium load. It is possible then that diabetic patients may have a suboptimal epinephrine response or altered peripheral sympathetic activity, resulting in potassium movement from the intracellular to the extracellular space as well as an impairment in cellular entry of potassium.

In summary, acidemia with a decrease in serum bicarbonate, plasma hyperosmolality, high glucagon levels, and sympathetic nervous system dysfunction might induce a hyperkalemic response in states of insulin deficiency (36), thereby explaining the development of hyperkalemia in DKA. Overall, insulin deficiency is probably the major protagonist of the complex hormonal disarray that is responsible for the hyperkalemia in decompensated diabetes.

K^+ Depletion with Hypokalemia. Hypokalemia can result from the redistribution or depletion of K^+ stores. The hypokalemia that results from redistribution is caused by cellular uptake of K^+ from the ECF; K^+ redistribution can occur simultaneously with K^+ depletion so that the two processes leading to hypokalemia can have additive effects. The hypokalemia observed with K^+ depletion is characterized by a reduction in the K^+ content of all body fluids (34).

Potassium depletion can occur with diabetes mellitus when dietary K^+ intake is very low and therefore fails to counterbalance the obligatory urinary K^+ losses associated with glucosuria. Potassium depletion also can occur if K^+ losses are abnormally high and might develop in association with a normal dietary K^+ intake. Potassium losses may be renal or extrarenal as diarrhea in origin, yet a combination of losses from both routes is commonly encountered. It is of interest to compare the alteration in K^+ content in the ICF and ECF in the presence of K^+ depletion. Total body K^+ deficit results in a greater absolute reduction of K^+ content in ICF than in ECF. Nevertheless, the percent deviation in K^+ content is considerably smaller in ICF than in ECF. In a similar fashion, the decrease in $[K^+]_i$ with K^+ depletion is significantly smaller than the decrease in $[K^+]_p$. With respect to the relationship between $[K^+]_p$ and the degree of K^+ deficit, a linear relationship having a slope of 0.3 mEq/L per 100 mEq of K^+ deficit [$\Delta[K^+]_p/\Delta K^+$ stores] has been described for patients with K^+ depletion in the absence of redistribution of K^+ stores. According to this relationship, a K^+ depletion of 10% of total body K^+ stores (350 mEq) produces a decrease in $[K^+]_p$ of approximately 1 mEq/L.

Diabetic gastroparesis is a common cause of vomiting leading to fluid and electrolyte losses. Protracted vomiting leads to hypokalemia that is largely caused by increased renal K^+ excretion. The increased kaliuresis is owing to HCO_3^- excretion consequent of HCl depletion (metabolic alkalosis) and to secondary hyperaldosteronism resulting from ECF volume depletion. The direct loss of K^+ as a result of vomiting is relatively small, considering that $[K^+]$ in gastric juice is about 15 mEq/L (mean value). Diuretic therapy for the management of hypertension and congestive heart failure in patients with diabetes mellitus is a common additional cause of potassium depletion. Within a week from the start of diuretic therapy, a mild decrease (0.3 to 0.6 mEq/L) in $[K^+]_p$ occurs, and this new $[K^+]_p$ remains constant thereafter unless an intercurrent illness that decreases K^+ intake (vomiting) or increases K^+ loss (diarrhea) develops. Hypokalemia is most commonly observed with thiazides (5% of patients) than with loop diuretics (1% of patients). The decrease in $[K^+]_p$ is directly proportional to the daily dosage and duration of action of the diuretic; thus, daily administration and high-dosage regimens of chlorthalidone, a long-acting thiazide, are more likely to produce severe K^+ depletion and hypokalemia. The antihypertensive effect of thiazides is achieved with small dosages (6.25 to 25.0 mg daily), which have a small effect on K^+ balance; consequently, high dosages of thiazides in the treatment of hypertension are not warranted because they will result in K^+ depletion without better blood pressure control. Insulin administration in the therapy of DKA, a condition in which K^+ depletion is usually present, can result in profound and symptomatic hypokalemia.

K^+ Overload with Hyperkalemia. Potassium overload leading to hyperkalemia can occur because of an increased K^+ intake or because of decreased renal K^+ excretion. The high K^+ intake-induced hyperkalemia occurs when the adaptive increase in renal K^+ excretion is insufficient to match the larger-than-normal K^+ intake. Salt substitutes are K^+ salts (KCl) that mimic the taste of NaCl and their use may lead to hyperkalemia. Whenever a patient is told to reduce his or her NaCl intake because of hypertension, heart failure, or another cause, these salt substitutes are often recommended. As these products are available over the counter, patients frequently use them whether they are recommended by physicians or not. A low NaCl intake

reduces the ability of the kidney to excrete K^+ to its maximum; simultaneous ingestion of salt substitutes (K^+ salts) can lead to hyperkalemia owing to the combination of increased K^+ intake and reduced renal K^+ excretion. In fact, a low NaCl intake is the single most commonly observed contributing factor in the development of hyperkalemia in clinical practice. Removing the salt restriction promotes increased kaliuresis, which might partially or fully correct the hyperkalemia.

In addition to the previously discussed impairment in internal K^+ balance leading to hyperkalemia, diabetes mellitus commonly damages the renal mechanisms of potassium excretion (34). Such abnormality that limits renal K^+ excretion might result from: (a) oliguria or anuria of any cause, (b) decreased GFR, (c) decreased tubular secretion of K^+, (d) hypoaldosteronism or pseudohypoaldosteronism, or (e) drugs. In the absence of generalized renal failure (normal GFR), a diminished renal K^+ excretion can be owing to either a defect in the renin–angiotensin–aldosterone axis or renal resistance to aldosterone.

Renin deficiency leads to a low plasma aldosterone level that might reduce renal K^+ excretion. Renin deficiency occurs in certain physiologic states (advanced age, expansion of ECF volume), with the use of various drugs (β-adrenergic blockers, inhibitors of prostaglandin synthesis, methyldopa), with certain toxins (lead), in some systemic diseases (diabetes mellitus), and in some renal diseases (obstructive uropathy, interstitial nephritis). A common cause of renin deficiency is the so-called type 4 renal tubular acidosis, which is characterized by impaired excretion of both K^+ and H^+. Perhaps its most common presentation is in elderly diabetic patients. Angiotensin converting enzyme (ACE) inhibitors lead to hypoaldosteronism, which reduces renal K^+ excretion. The widely used antihypertensive agents (e.g., captopril, enalapril, fosinopril, and lisinopril) that belong to this category of drugs can increase $[K^+]_p$ levels by this mechanism.

One or more of the various syndromes of diminished aldosterone activity may be observed in diabetes mellitus. A diminished aldosterone activity can occur as a result of:

i) A primary defect in the adrenal synthesis of aldosterone owing to a disease or defect in the adrenal cortex. This adrenal abnormality is the cause or initial event that might lead to the defect in K^+ homeostasis.
ii) A secondary defect in the adrenal synthesis of aldosterone owing to failure in the production, release, or action of the various components in the cascade leading to stimulation of the adrenal synthesis of aldosterone (e.g., renin deficiency).
iii) End-organ resistance to aldosterone, owing to either drugs acting on the kidney (e.g., spironolactone) or renal disease.

Drug-induced mechanisms of hypoaldosteronism leading to hyperkalemia are commonly encountered in diabetic patients. Hyperkalemia induced by hypoaldosteronism can be elicited or accentuated in these patients with the administration of prostaglandin synthetase inhibitors, cyclosporine, or heparin. Prostaglandin synthetase inhibitors such as the nonsteroidal antiinflammatory drugs (NSAIDs) (e.g., indomethacin) and cyclosporine inhibit renin secretion, producing hyporeninemic hypoaldosteronism. ACE inhibitors decrease plasma levels of angiotensin II. Finally, heparin acts directly on the adrenal gland, inhibiting aldosterone secretion. Increased $[K^+]_p$ can occur with heparin administration in approximately 5% of hospitalized patients. The mechanism of K^+ retention by NSAIDs includes their inhibitory effect on prostaglandins of renal origin, which in turn might diminish GFR and renin release (which leads to decreased aldosterone secretion). NSAIDs can also have a direct nephrotoxic effect leading to renal failure, thereby causing additional renal K^+ retention.

Diabetes mellitus is also associated with end-organ resistance to aldosterone, leading to hyperkalemia, a syndrome known as *pseudohypoaldosteronism*. This entity, characterized by a renal resistance to aldosterone effects, can develop as a result of drug administration or renal diseases. Hyperkalemia caused by spironolactone, triamterene, and amiloride, collectively known as *K^+-sparing diuretics,* exemplifies drug-induced pseudohypoaldosteronism. Renal diseases that primarily damage the renal tubules and spare the glomeruli (or compromise the GFR only mildly), collectively known as *tubulointerstitial renal diseases,* elicit this hyperkalemic syndrome. Diabetes mellitus is the single major cause of end-stage renal disease (ESRD), and therefore commonly leads to hyperkalemia because of decreased renal function (diminished GFR). It must be recognized that in the presence of renal insufficiency, potassium balance might be maintained within normal limits until the GFR decreases to less than 25% of normal. The ability of the kidney with decreased GFR to maintain K^+ balance depends on the development of compensatory mechanisms, collectively known as *K^+ adaptation,* that increase the fractional K^+ excretion (FE_K) by the kidney (34). The augmented FE_K in renal insufficiency allows these patients to remain normokalemic in spite of a major reduction in GFR. Because $[K^+]_p$ might remain within normal limits in patients with only 25% of overall renal function (GFR), whereas K^+ intake is unchanged, a fourfold increase in $FE_K{}^+$ must be present. As the calculated FE_K in normal individuals amounts to approximately 10%, the estimated FE_K in a patient with this degree of renal insufficiency is about 40%.

Management of Hyperkalemia. The management of K^+ retention should be initiated even in the presence of a mild degree of hyperkalemia (34). Several measures should be undertaken at once in patients who have high-normal $[K^+]_p$ (i.e., 5.0 mEq/L) and a disease that predisposes to hyperkalemia such as diabetes mellitus with renal dysfunction. Severe restriction of dietary NaCl intake should be avoided because it impairs renal K^+ excretion; dietary NaCl intake should be at least 4 g/day. Restriction of dietary K^+ must be enforced. Medications that impair the renal excretion of K^+, including ACE inhibitors and K^+-sparing diuretics (i.e., triamterene, amiloride, eplerenone, spironolactone) should be discontinued. Metabolic acidosis, if present, should be treated with alkali therapy.

Proper management of simultaneous retention of Na^+ and K^+ in patients with diabetes mellitus and a salt-retaining disease (hypertension, congestive heart failure, nephrotic syndrome, renal insufficiency) who have an elevated $[K^+]_p$ or even a high-normal $[K^+]_p$ (i.e., 5.0 mEq/L), is effectively achieved by avoiding severe restriction of dietary NaCl intake while concomitantly administering diuretics (e.g., furosemide, thiazides). This recommendation with respect to the dietary NaCl intake should be instituted once a large ECF volume excess is no longer present. A moderate dietary NaCl intake of about 4 g/day will not result in ECF volume expansion if increased urine excretion of NaCl is achieved with the use of diuretics. This strategy of enhancing salt (NaCl) intake and excretion secures adequate kaliuresis (delivery of salt to the distal nephron is reduced with a salt-restricted diet and predisposes to hyperkalemia). Thus, to correct hyperkalemia by promoting increased kaliuresis, all patients, except those with ESRD or physical signs of fluid overload or pulmonary edema, must have substantial NaCl intake via an oral or parenteral route. In addition, the renal excretion of K^+ can be increased by the administration of a single diuretic agent (e.g., furosemide, thiazides) or a combination of these agents. Diuretics should not be administered to patients with ESRD because a meaningful kaliuretic response is not expected. A negative external K^+ balance is achieved in all patients with

ESRD by: (a) the utilization of cation exchange resins (such as Kayexalate, a sodium polystyrene sulfonate) that promote the excretion of K^+ in the stools, and (b) dialysis (hemodialysis or peritoneal dialysis).

Hyperkalemia is the major threat to life in patients with type 4 renal tubular acidosis; therefore, the main focus of attention should be placed on correcting this electrolyte abnormality. That is the reason why dietary K^+ restriction, diuretics (furosemide, thiazides), and K^+-binding resins are so valuable in these patients. The intake of NaCl should be encouraged, because the availability of Na^+ salts in the collecting tubules is a major determinant of renal K^+ excretion. The administration of fludrocortisone (Florinef) in daily doses of 0.1 to 0.3 mg helps in the correction of hyperkalemia and acidosis (enhances distal acidification), yet the associated volume expansion may induce hypertension or increase its severity. Alkali therapy (1 to 2 mEq/kg per day) is useful to ensure correction of hyperkalemia and acidosis.

The following three strategies must be considered whenever severe hyperkalemia is present in any patient (34):

i. To counterbalance the effect of hyperkalemia on the excitability of myocardial and skeletal muscle with the administration of drugs. This modality is not aimed at reducing the increased $[K^+]_p$.
ii. To modify internal K^+ balance, promoting the translocation of K^+ from ECF to ICF. This modality will not alter the total body K^+ stores.
iii. To modify external K^+ balance, inducing a net K^+ loss from the body.

The treatment of hyperkalemia with agents that ameliorate the effects of hyperkalemia on myocardial and skeletal muscle excitability involves the administration of Ca^{2+} salts (chloride or gluconate), which diminish tissue excitability by widening the difference between resting and threshold potentials. When Ca^{2+} salts are provided, the resting membrane potential will remain depolarized by the hyperkalemia, whereas the threshold membrane potential will be depolarized by the Ca^{2+} infusate, enlarging the difference between resting and threshold membrane potentials, thereby reducing tissue excitability. Calcium gluconate (20 mL of a 10% solution) can be infused intravenously over a 10-minute period. The intravenous administration of Ca^{2+} salts is definitely indicated when $[K^+]_p$ reaches 7.0 mEq/L or when significant electrocardiographic abnormalities (absence of P waves, prolongation of QRS complexes, etc.) are present. The effects of Ca^{2+} infusion are short lasting, with peak effect noted about 5 minutes after infusion.

The most important therapeutic agent that promotes cellular K^+ entry is insulin. Insulin leads to tissue uptake of K^+ as well as glucose; therefore, the latter must be infused to prevent hypoglycemia in patients presenting without hyperglycemia. Considering that hyperglycemia of endogenous and exogenous origin can result in hyperkalemia (especially in diabetics), caution should be exercised as to the rate of glucose infusion. Consequently, a situation that mimics a euglycemic insulin clamp (providing enough exogenous glucose to maintain normal plasma glucose level during insulin administration) must be instituted. A less important strategy that might translocate K^+ from ECF to ICF is sodium bicarbonate ($NaHCO_3$) infusion.

Achievement of a net K^+ loss is the most effective therapeutic modality to reduce and sustain a normal $[K^+]_p$ in patients with severe and persistent hyperkalemia. The first two modalities in the therapy of hyperkalemia, namely, the use of Ca^{2+} salts (to ameliorate the effect of hyperkalemia on the excitability of myocardial and skeletal muscle) and the administration of insulin and glucose, $NaHCO_3$, and albuterol (to modify internal K^+ translocation from ECF to ICF), are only temporary measures that remove the immediate threat to life resulting from hyperkalemia. Consequently, treatment of severe and persistent hyperkalemia must combine all three modalities.

The presence of associated clinical and laboratory abnormalities can prevent use of one or more treatment modalities of hyperkalemia. The use of K^+ exchange resins by oral or rectal routes is contraindicated in patients with significant gastrointestinal symptoms. Calcium infusions are contraindicated in patients with hypercalcemia. Sodium bicarbonate infusions are contraindicated in patients with alkalemia, patients with high $[HCO_3^-]_p$, those with hypernatremia, or in patients at a significant risk of developing pulmonary edema or with significant ECF volume expansion. Severe hyperkalemia accompanied by preserved renal function usually can be corrected without dialysis. Potassium removal can be achieved in these patients by inducing an enhanced kaliuresis with the administration of fluids containing NaCl or $NaHCO_3$, or both, and with the use of diuretics (acting on the proximal tubule, loop of Henle, and distal tubule, such as acetazolamide, furosemide, and thiazides, respectively). The majority of patients who develop severe hyperkalemia, however, have renal failure, and dialysis is the treatment of choice for this condition.

Defect in Phosphate Homeostasis

The osmotic diuresis of hyperglycemia leads to decreased renal reabsorption of phosphate accounting for phosphate depletion in DKA (45). Nonetheless, serum phosphate levels are usually normal or increased at presentation (Table 90-2). The initial serum phosphate correlates positively with serum osmolality, glucose, and anion gap. Insulin deficiency and metabolic acidosis induce a shift of phosphate from cells to the extracellular compartment thereby masking phosphate depletion. Insulin therapy shifts phosphate back into cells, rapidly lowering the serum levels (46–48).

Defect in Acid–Base Balance

Ketoacidosis and Hyperchloremic Metabolic Acidosis. Although the metabolic acidosis of DKA is mostly caused by the overproduction of β-hydroxybutyric and acetoacetic acids, additional acids, including lactic acid, free fatty acids, and other organic acids, can contribute to the fall in plasma $[HCO_3^-]$ (5). Insulin deficiency, coupled with counterregulatory hormone excess (largely glucagon), activates cAMP, which in turn leads to phosphorylation and activation of lipase in adipocytes, thereby promoting lipolysis (49–52). Lipolysis of triglyceride stores in the adipocyte provides long-chain fatty acids, which are the principal substrate for hepatic ketogenesis (53,54). However, hepatic triglycerides might also serve as a source of fatty acids in the presence of fatty liver, a not uncommon condition in patients with diabetes (5).

Role of fatty acid oxidation. In addition to augmented substrate in the form of long-chain fatty acids, development of substantial ketogenesis by the hepatocyte mitochondria requires a major increase in fatty acid oxidation. A transport system, the carnitine shuttle, is needed for long-chain fatty acids to enter the mitochondrial matrix. This carrier system consists of two carnitine palmitoyl-transferases (CPT)—an outer CPT I and an inner CPT II—and carnitine/acyl-carnitine translocase. The key regulatory step for fatty acid oxidation takes place in a transesterification reaction catalyzed by CPT I (53,55). This enzyme controls the entry of acyl-coenzyme A (CoA) esters, which are derived from the long-chain fatty acids, from the cytosol into the mitochondria (Fig. 90-4) (5). In the normal fed state and in well-controlled diabetes, CPT I is inhibited such that fatty acids cannot enter the mitochondria for oxidation

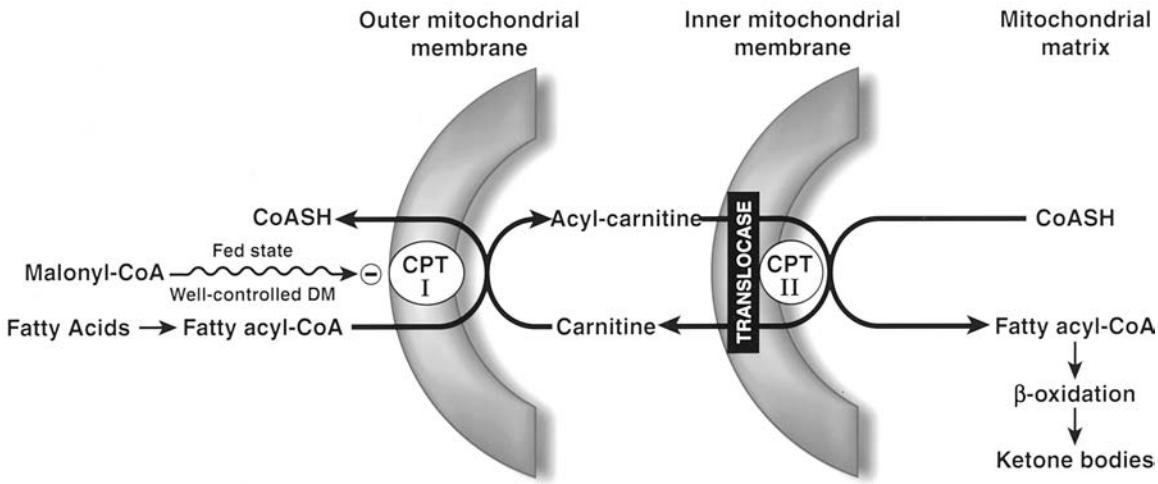

FIGURE 90-4. Regulation of hepatic ketogenesis. Synthesis of ketones in the liver depends on transfer of fatty acyl-CoA into the mitochondria by carnitine palmitoyl-transferase I (CPT I). In the fed state and in well-controlled diabetes, CPT I activity is inhibited by cytoplasmic malonyl CoA. During fasting and in patients with uncontrolled diabetes, the increase in the glucagon/insulin molar ratio suppresses malonyl CoA synthesis, allowing for increased transfer of fatty acyl-CoA into the mitochondria and increased ketogenesis. (From: Foster DW, McGarry JD. Acute complications of diabetes mellitus: ketoacidosis, hyperosmolar coma, and lactic acidosis. In: DeGroot LJ, Jameson JL. *Endocrinology*. 4th ed.; 2001.)

and ketoacid formation, but are re-esterified to triglycerides and transported out of the cytosol as very-low-density plasma lipoproteins. Inhibition of CPT I is provided by malonyl-CoA, a metabolite whose level is dependent on adequate glycolysis and activity of acetyl-CoA carboxylase (56–59). The concentration of malonyl-CoA is maximal in the fed state, but it sharply decreases with fasting and uncontrolled diabetes. In these conditions, an increase in the glucagon/insulin ratio blocks glycolysis and inhibits acetyl-CoA carboxylase, two processes that lead to a major drop in malonyl-CoA levels. Consequently, CPT I activity is enhanced, allowing conversion of the acyl-CoA esters of long-chain fatty acids to acyl-carnitines that can be transported toward the interior of mitochondria (60). The transesterification to acyl-carnitine is then reversed by CPT II, which works in conjuction with the translocase, allowing the release of fatty acyl-CoA in the mitochondrial matrix. The capacity of the hepatocytes for fatty acid oxidation is large and the most of the fatty acyl-CoA molecules entering the mitochondria are oxidized to ketone bodies.

The anion gap. During the development of DKA, ketoacids released into the body fluids are titrated by HCO_3^- and other body buffers (61–65). As a result of this buffering process, HCO_3^- ions are replaced by ketoanions in the extracellular fluid producing the characteristic increase in plasma unmeasured anions (the so-called "anion gap") that is seen in diabetic ketoacidosis. In uncomplicated DKA, the increment in the anion gap (AG) above its normal value should be approximately equal to the decrement in plasma [HCO_3^-] (61,64). Thus the ratio of excess AG (i.e., measured AG minus normal AG) in mEq/L to the decrement in [HCO_3^-] (i.e., normal plasma [HCO_3^-] minus measured plasma [HCO_3^-]) should be approximately 1.0. In fact, this pattern is seen in most patients with DKA (64,66–72). In Table 90-4 are presented admission data obtained or calculated from several published reports of patients with DKA. As can be seen in the table, the mean decrement in plasma [HCO_3^-] was essentially equal to the mean excess AG in most of these studies (in two studies, ΔAG actually exceeds Δ[HCO_3^-] by 6 to 8 mEq/L, most likely due to a preexisting metabolic alkalosis).

In Figure 90-5 (72) is charted the course of treatment in a patient with DKA presenting with a typical increased AG acidosis. In this patient, the decrement in plasma [HCO_3^-] was essentially equal to the increase in AG before treatment was begun. During the course of therapy, plasma [HCO_3^-] increased as the AG fell, and serum [Cl^-] rose concomitantly. By 16 hours after admission, serum [HCO_3^-] was 16 mEq/L, and blood pH was almost normal. Of note, the BUN was increased on admission, reflecting a pre-renal fall in renal function, which was corrected by treatment (see later).

Although most patients with DKA have an increased AG, one occasionally encounters a patient with pure hyperchloremic metabolic acidosis (32,73). The presenting data and course of treatment in one such patient are depicted in Figure 90-6 (72). Severe metabolic acidosis, accompanied by the appropriate respiratory response, was present on admission, but the decrement in plasma [HCO_3^-] was not associated with an increase in the AG (AG = 16 $\pm$ 4 mEq/L in this example, which includes [K^+] in the calculation). A notable additional feature is that the blood urea nitrogen concentration (BUN) is within normal limits. Despite standard treatment, 60 hours elapsed before the serum [HCO_3^-] rose to 17 mEq/L. Serum [Cl^-], although elevated on admission, increased further during treatment, and was associated with a reciprocal decrement in the AG. The fall in the AG, was due, at least in part, to a major reduction in the serum protein concentration. Thus, two differences are noteworthy in patients with DKA and a normal AG. The first is that there is less evidence of impairment of renal function, and the second is a slower recovery from metabolic acidosis compared with patients presenting with an increased AG. Considering that the DKA patient is unable to properly metabolize ketoacids, whether ketoanions are retained in the ECF or are wasted in the urine should have no major effect on the severity of the acid–base disorder.

The representative cases depicted in Figures 90-5 and 90-6 portray the extremes of the acid–base patterns observed on admission for uncomplicated DKA (72). In fact, most patients have elements of both increased AG and hyperchloremic acidosis with one element being dominant (Table 90-5). Although various factors could potentially alter the stoichiometric

TABLE 90-4

COMPARISON OF ADMISSION DATA IN DIABETIC KETOACIDOSIS

	Danowski et al. (N = 8)	Seldin et al. (N = 10)	Nabarro et al. (N = 7)	Shaw et al. (N = 30)	Assal et al. (N = 9)	Oh et al. (N = 35)	Adrogue et al. (N = 150)[a]
Blood pH					7.06 ± 0.03	7.07 ± 0	7.06 ± 0.0
$[Na]_p$, mEq/L	130 ± 3.2	129 ± 3.7	136 ± 0.5	131 ± 1.4	146 ± 3.0	136 ± 1.6	132 ± 0.6
$[K]_p$, mEq/L	4.9 ± 0.5	4.7 ± 0.2	5.7 ± 0.2	5.8 ± 0.3	5.6 ± 0.3		5.7 ± 0.1
$[Cl]_p$, mEq/L	94 ± 2.8	94 ± 3.5	93 ± 1.0	96 ± 1.3	106 ± 3.0	101 ± 1.4	98 ± 0.6
$[HCO_3]_p$, mEq/L	7.0 ± 0.8	7.4 ± 1.0	7.5 ± 1.1	5.0 ± 0.6[a]	5.6 ± 1.0	9.6 ± 0.3	6.2 ± 0.2[a]
Anion gap, mEq/L	34.3 ± 1.3	32.1 ± 1.5	40.8 ± 2.1	36.7 ± 1.7	40.0	25.0 ± 1.2[b]	33.5 ± 0.6
$\Delta[HCO_3]_p$, mEq/L[c]	17.0 ± 0.8	16.6 ± 1.0	16.5 ± 1.1	22.0 ± 0.6[a]	18.4	14.4 ± 0.3	20.8 ± 0.2
ΔAnion gap, mEq/L[d]	18.3 ± 1.3	16.1 ± 1.5	24.8 ± 2.1	20.7 ± 1.7	24.0	13.0 ± 0.9	17.5 ± 0.6

[a] TCO_2 was measured.
[b] The value was calculated with $[K]_p$.
[c] The values were derived using a baseline value of either 24 mEq/L or 27 mmol/L depending on whether $[HCO_3]_p$ or TCO_2 were measured, respectively.
[d] The values were derived using a baseline value of 16 mEq/L.

relationship between the increment in AG and the decrement in plasma bicarbonate, the overall level of renal function appears to be the major determinant of the type of metabolic acidosis encountered on admission for DKA.

Additional conditions might alter the ratio of excess AG/bicarbonate deficit in patients with DKA, including hyperproteinemia, vomiting or exogenous bicarbonate therapy, and hypocapnia. Differences in the apparent distribution volume of bicarbonate and ketone anions have also been proposed to explain the hyperchloremic acidosis of DKA; this hypothesis, however, has not been verified experimentally (64).

Role of the kidney in modulating the anion gap. Because renal reabsorption of filtered plasma ketoanions is limited and the production of ketoacids can reach levels as high as 1,000 to 2,000 mEq/day, the urinary excretion of the sodium and potassium salts of the ketoacid anions can be enormous (67,68,74–76). The renal "wasting" of ketone salts in association with

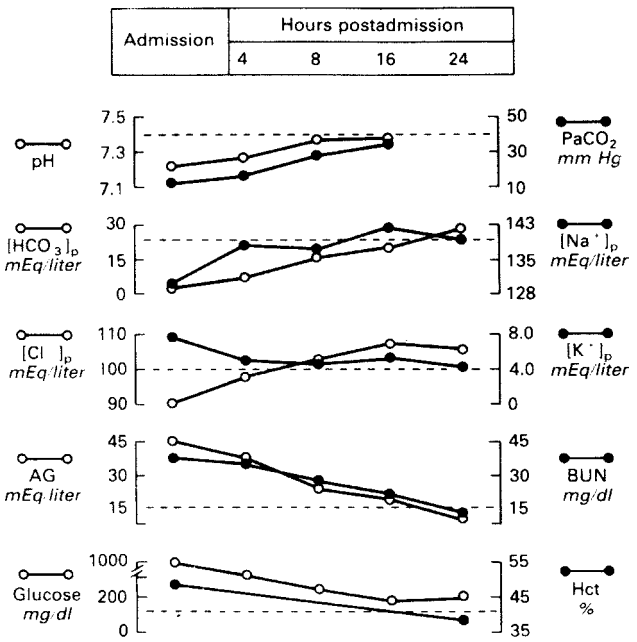

FIGURE 90-5. Laboratory data on admission and follow-up values in a patient admitted for diabetic ketoacidosis and featuring pure anion gap acidosis. Each symbol represents a single measurement. (From: Adrogué HJ, Eknoyan G, Suki WN, et al. Diabetic ketoacidosis: role of the kidney in the acid–base homeostasis re-evaluated. *Kidney Int* 1984;25:591.)

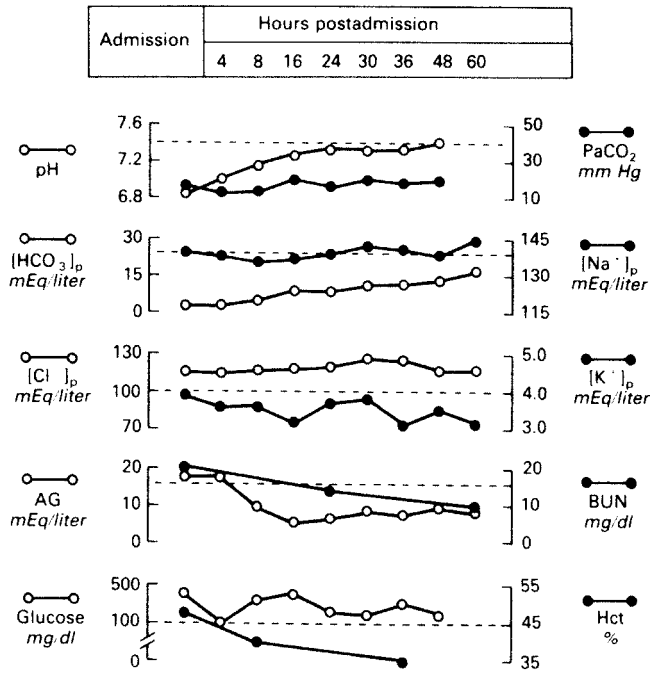

FIGURE 90-6. Laboratory data on admission and follow-up values in a patient admitted for diabetic ketoacidosis and featuring pure hyperchloremic acidosis. Each symbol represents a single measurement. (From: Adrogué HJ, Eknoyan G, Suki WN, et al. Diabetic ketoacidosis: role of the kidney in the acid–base homeostasis re-evaluated. *Kidney Int* 1984;25:591.)

TABLE 90-5

ACID–BASE PATTERNS OF PATIENTS WITH DIABETIC KETOACIDOSIS

	Pure high anion gap acidosis	Mixed forms	Pure hyperchloremic acidosis
Associated clinical features before start of therapy			
Fluid intake	Poor	⇔	Adequate
Extrarenal fluid loss	Present	⇔	Absent
ECF volume deficit	Severe	⇔	Mild
Impairment of renal function	Severe	⇔	Mild
Associated laboratory features before start of therapy			
Hematocrit, hemoglobin, serum proteins	Higher	⇔	Lower
BUN, creatinine, uric acid	Higher	⇔	Lower
Cause of changes in $\Delta AG/\Delta HCO_3^-$ after initiation of therapy[a]	Bicarbonate administration	⇔	Infusion of chloride-rich solutions

[a] AG = anion gap; $AG = [Na^+]_p - ([Cl^-]_p + [HCO_3^-]_p)$. Excess AG (mEq/L) equals measured AG minus normal AG, and bicarbonate deficit (mEq/L) equals normal plasma bicarbonate minus measured plasma bicarbonate. Expected value of $\Delta AG/\Delta HCO_3^-$ is 1.0 as the bicarbonate deficit is the result of its titration by ketoacids.

glucosuria-induced osmotic diuresis, poor fluid intake, and vomiting, result in ECF volume depletion and a resultant reduction in renal function. Renal blood flow and GFR both fall during DKA, with recovery to normal values following the episode (77,78). Increased urea production from enhanced catabolism of amino acids in patients with DKA also contributes to the elevation of blood urea nitrogen levels.

Patients with DKA who develop substantial volume depletion, will tend to present with an increased AG metabolic acidosis because of their limited ability to excrete ketone salts. Conversely, patients with DKA who are able to maintain sodium and water intake, thereby minimizing ECF volume depletion, will tend to present with variable degrees of hyperchloremic acidosis, due to urinary excretion of ketone salts and retention of chloride (64,72).

Effect of treatment on anion gap. Volume replacement with saline infusions during treatment of DKA causes dilution of both ketones and bicarbonate; thus, the AG decreases due to replacement of an unmeasured anion (ketones) with a measured anion (Cl^-). Additionally, correction of K^+ depletion with the chloride salt results in the cellular uptake of K^+ in exchange for H^+, while most of the chloride remains in the ECF. Because the H^+ extruded is titrated by HCO_3^-, the net effect is the development of hyperchloremic acidosis.

Renal response to ketoacidosis. Diabetic ketoacidosis stimulates renal acid excretion, leading to a several-fold increase in net acid excretion. It should be recalled that a voltage-dependent stimulus for H^+ secretion exists in the distal nephron whenever sodium is absorbed without an accompanying anion. A substantial electrical gradient favoring distal H^+ secretion is present in DKA as a result of increased distal sodium delivery (ketonuria and osmotic diuresis), avid sodium reabsorption (ECF volume contraction), and the presence of poorly reabsorbable anions (ketones). As a result, daily renal net acid excretion, as titratable acid and ammonium, can attain levels as high as 250 and 500 mEq, respectively (75). Although this vigorous response suggests that the kidney very effectively defends acid–base homeostasis in the course of DKA, such a conclusion is untenable. Maximal stimulation of renal acidification in DKA does not suffice to compensate for the large urinary loss of HCO_3^- precursors in the form of salts of ketoacids. In fact, experimental studies have shown that despite maximal stimulation of urinary acidification, for each mmol of β-hydroxybutyrate excreted, the kidney could salvage only about 0.5 mEq of potential base (79). Although ketones have been shown to inhibit the rate of renal ammoniagenesis in the experimental animal, this effect most probably plays only a minor role in humans, as a large increment in urinary ammonium excretion is present in DKA (80).

Effect of treatment on renal response. Balance studies carried out in the early period after admission for DKA revealed that correction of volume depletion resulted in massive urinary loss of bicarbonate precursors (salts of ketoacids) that exceeded the elevated levels of urinary titratable acidity and ammonium (Table 90-6) (72). Thus, in the initial period after admission, the kidneys behave in a maladaptive fashion relevant to the systemic acid–base composition (72). Only when the plasma ketones have fallen substantially, is stimulation of urinary excretion of titratable acidity and ammonium capable of generating sufficient new HCO_3^- to begin to correct the ketoacidosis.

Lactic Acidosis. Patients with diabetes mellitus may develop lactic acidosis, which represents a potentially serious condition (81). Its diagnosis is warranted in the presence of a high anion gap metabolic acidosis associated with blood lactate levels equal to or higher than 4 mEq/L. This acid–base disorder results from the imbalance between production and utilization of lactate; thus, lactic acidosis might result from increased production, decreased utilization, or a combination of these two mechanisms. The skeletal muscle and gut are the organs involved in the development of lactic acidosis because of overproduction. The liver and, to a lesser degree the kidney, are the organs playing the major role in lactate removal. Diabetes mellitus has been shown to be associated with type A as well as type B lactic acidosis. However, this classification has lost its appeal in clinical practice because patients frequently display features of type A as well as type B lactic acidosis simultaneously (82). Type A includes clinical conditions associated with impaired tissue oxygenation, which causes hyperlactatemia. Examples of type A lactic acidosis are clinical states with either reduced oxygen delivery (shock, cardiac arrest, severe hypoxemia, and sepsis) or those with increased oxygen demand (vigorous exercise, shivering, and generalized seizures). Type B includes clinical conditions in which there is no apparent oxygenation defect to explain the hyperlactatemia. Although patients with

TABLE 90-6

ROLE OF THE KIDNEY IN THE ACID–BASE DEFENSE IN NORMAL SUBJECTS AND DURING RECOVERY FROM DIABETIC KETOACIDOSIS

	Normal subjects[a] $TA + NH_4^+ - HCO_3^-$		Diabetic ketoacidosis[b] $TA + NH_4^+ - HCO_3^{-c}$	
	Balance	Cum. balance	Balance	Cum. balance
Post-admission	mEq		mEq	
0 to 4 hours	12.7	12.7	−45.0 ± 8.2	−45.0 ± 8.2
4 to 8 hours	12.7	25.4	−10.3 ± 13.5	−55.3 ± 20.8
8 to 12 hours	12.7	38.1	1.8 ± 6.8	−53.5 ± 27.1
12 to 16 hours	12.7	50.8	−16.5 ± 2.0	−37.0 ± 28.3
16 to 20 hours	12.7	63.5	−14.3 ± 2.3	−22.7 ± 28.9
20 to 24 hours	12.7	76.2	−21.2 ± 4.8	−1.5 ± 31.2

[a]Estimate based on net acid excretion equal to 1.25 mEq/kg body weight/day and a body weight equal to that of the diabetic patients (61 kg).
[b]The values presented are means ± 1 SE of four studies.
[c]Actual plus potential bicarbonate is shown; ketone salts other than ammonium represent potential bicarbonate.

type A acidosis develop clinical signs of tissue hypoxia or underperfusion, patients with type B lactic acidosis lack these manifestations. Examples of type B acidosis include congenital defects in glucose or lactate metabolism and many acquired conditions (e.g., diabetes mellitus, malignancies, toxins, and liver disease). Lactic acidosis caused by metabolic defects comprises: (a) disorders of glucoregulation, including hypoglycemia and diabetes mellitus; (b) major organ failure, involving hepatic, renal, or multiple-organ failure; and (c) neoplasias, especially lymphomas, leukemias, sarcomas, and lung carcinomas.

Several drugs and toxins might cause lactic acidosis in diabetic and nondiabetic patients. Ethanol abuse is probably the most common cause within this group. The oral hypoglycemic agents of the biguanides group (phenformin and metformin) used to be a major cause of lactic acidosis, particularly in patients with impaired renal function; the limited worldwide use of these drugs, at the present time, explains their diminishing importance in the etiology of lactic acidosis, particularly in patients with impaired renal function. Many other drugs, including salicylates, methanol, ethylene glycol, propylene glycol, nitroprusside, and isoniazid, might cause this condition.

Correction of the underlying cause of lactic acidosis is the cornerstone of treatment of this condition. The high mortality associated with lactic acidosis can be reduced by securing adequate support of vital functions. The hemodynamic status and tissue perfusion might improve with correction of volume deficit, enhancing cardiac output and avoiding vasoconstricting drugs (e.g., norepinephrine). Tissue oxygenation must be optimized by correcting anemia or providing a higher inspired oxygen mixture with or without mechanical ventilation. Energy stores must be replenished to prevent the development of hypoglycemia. If drugs or toxins are responsible for the lactic acidosis, it is mandatory to remove these agents promptly from the patient's tissues by whatever means available (i.e., hemodialysis or hemoperfusion, if necessary). Sepsis must be treated aggressively. Other measures in the management of lactic acidosis might include alkali therapy as well as the use of dichloroacetate (DCA), which enhances the oxidation of pyruvate, as described in the following.

The utilization of alkali therapy in the treatment of lactic acidosis is controversial because the rising pH associated with this treatment tends to further increase hyperlactatemia. It has been known for years that acidosis inhibits glycolysis, whereas alkalosis stimulates it and consequently results in elevated plasma lactate levels; this effect, however, is generally mild and usually results in an increase in plasma lactate of only 1 to 3 mEq/L. This pH feedback control is not unique to lactic acid but also occurs with other organic acids, including ketoacids, whose production is inhibited by acidosis and stimulated by alkalosis. Additionally, HCO_3^- therapy neither alters the natural course of the deranged metabolism leading to lactic acidosis nor diminishes the mortality of this condition. Yet, most experts advise HCO_3^- therapy in lactic acidosis in the presence of extreme acidemia (blood pH <7.20) or $[HCO_3^-]_p$ lower than 10 to 12 mEq/L. Bicarbonate administration, however, has several potential adverse effects that are described elsewhere in this chapter.

Other therapeutic measures include the administration of Carbicarb (0.33 mol/L sodium carbonate and 0.33 mol/L sodium bicarbonate) and DCA. The alkalinizing capacity of Carbicarb is identical to that of $NaHCO_3$, but Carbicarb produces less carbon dioxide (82). Thus, the potential risk of tissue acidosis owing to H_2CO_3 accumulation, associated with alkali therapy, appears to be lower, by comparison, when Carbicarb is used instead of pure $NaHCO_3$. However, the use of Carbicarb in clinical practice is not universally accepted. DCA limits lactate production by stimulating pyruvate dehydrogenase activity, resulting in oxidation of pyruvate to acetyl CoA. The usual dose in adults with lactic acidosis is 50 mg/kg of body weight, diluted in 50 mL of isotonic saline for intravenous infusion over a 30-minute period. This dose might be repeated if plasma lactate levels remain substantially elevated. Limited experience with DCA indicates that this therapy can be beneficial in some instances of lactic acidosis.

Renal Tubular Acidosis. A normal anion gap acidosis, also known as hyperchloremic acidosis, might develop either from a primary loss of HCO_3^- or from a failure to replenish HCO_3^- stores depleted by the daily production of fixed acids. These two defects are commonly encountered in patients with diabetes mellitus (61). A primary loss of HCO_3^- might result from intestinal (e.g., diarrhea) or urinary losses of alkali or its precursors (e.g., ketone salts of sodium and potassium). Hyperchloremic metabolic acidosis that results from failure to replace HCO_3^- stores that have been depleted by the daily production of fixed acids is observed in diabetics with distal tubular acidosis. In this condition, the daily net acid excretion by the kidney falls short of the daily acid production, leading to metabolic acidosis owing to depletion of HCO_3^- stores. Another example is adrenal insufficiency, in the form of diminished mineralocorticoid activity (selective hypoaldosteronism,

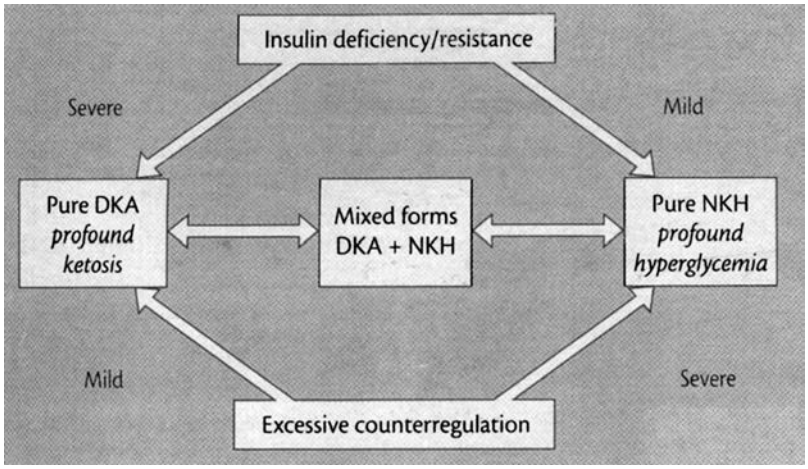

FIGURE 90-7. Diabetic ketoacidosis (DKA) and nonketotic hyperglycemia (NKH) are different forms of the same disease process.

aldosterone resistance, and the administration of the K^+-sparing diuretic spironolactone) as well as diminished glucocorticoid activity. The major causes of distal renal tubular acidosis accompanied with hyperkalemia (type 4 renal tubular acidosis) include diabetic renal disease, hypoaldosteronism, obstructive uropathy, sickle cell nephropathy, and renal transplant rejection (83).

Combination of Multiple Major Disturbances

The full-blown forms of DKA and NKH represent the most frequently observed and clinically relevant acute metabolic complications of uncontrolled diabetes mellitus (84,85). Clinicians generally consider that each of these two entities illustrates a distinct condition that develops in isolation from the other in the course of diabetes mellitus. In addition, major textbooks of internal medicine commonly describe DKA and NKH as different processes having little in common, thereby perpetuating such a notion. These conditions, however, are in fact closely interrelated and truly represent two different expressions of a remarkably similar pathophysiologic process (84–87). In fact, mixed forms having features of DKA and NKH are observed as or even more frequently than the pure forms of each of these disturbances. As depicted in Figure 90-7, insulin deficiency or resistance and excessive counterregulation are present in DKA as well as NKH, yet the severity of these abnormalities differs in the two clinical conditions. The profound ketosis characteristic of DKA is caused by severe insulin deficiency or resistance in association with a mild degree of excessive counterregulation. Conversely, the profound hyperglycemia observed in NKH is owing to mild insulin deficiency or resistance in association with a severe activation of counterregulatory hormones as well as superimposed renal failure.

The pathogenesis of ketosis and hyperglycemia in diabetes mellitus always involves the presence of either absolute or relative insulin deficiency. Such insulin deficit might occur because of augmented body requirements of insulin while receiving a fixed insulin dosage, withdrawal from insulin therapy, or failure of the pancreatic β-cells. Resistance to insulin action also participates in the ketosis and hyperglycemia. Factors that contribute to insulin resistance include counterregulatory hormones, deficiency of electrolytes (mostly potassium), increased tonicity of body fluids, and acidemia. Counterregulatory hormones play a major role in the development of hyperglycemia, as follows. Elevated levels of cortisol, growth hormone, and epinephrine depress insulin-mediated glucose uptake in peripheral tissues (e.g., skeletal muscle) and promote the release of gluconeogenic precursors from myocytes and adipocytes. The latter effects combined with augmented glucagon levels contribute to enhanced gluconeogenesis by the liver and kidney (less important role).

In Figure 90-1 are depicted the dominant derangements observed in the liver and peripheral tissues that are responsible for the ketonemia and hyperglycemia in decompensated diabetes. Triglycerides stored in adipocytes are decomposed into free fatty acids and glycerol by activation of a "hormone-sensitive" lipase within these cells. Whereas insulin inhibits this lipase, growth hormone, glucagon, and epinephrine stimulate its activity. Consequently, the hormonal disarray of uncontrolled diabetes augments the release of ketone precursors. The increased plasma levels of free fatty acids promote their uptake by hepatocytes where fatty acids are activated to their fatty acyl CoA derivative. The long-chain fatty acyl CoA may be consumed in the cytosol for the synthesis of fatty acids (including malonyl CoA), triglycerides, and phospholipids (dominant pathway in the normal state) or transported by the carnitine shuttle into the mitochondria where it undergoes β-oxidation producing acetyl CoA (dominant pathway in uncontrolled diabetes mellitus). Within the mitochondria of healthy individuals, the acetyl CoA condenses mostly with oxaloacetate for oxidation in Krebs tricarboxylic acid cycle forming carbon dioxide. The alternative pathway of acetyl CoA within the mitochondria is that two molecules of this compound combine with each other to form the "ketone bodies," acetoacetate and D-3-hydroxybutyrate (ketoacids). The latter route is greatly stimulated in uncontrolled diabetes mellitus because oxaloacetate is in short supply (being removed from the mitochondria for the augmented gluconeogenesis) and excessive acetyl CoA cannot be accommodated through the Krebs cycle so that it overflows through the ketogenic pathway (88). The carnitine shuttle is inhibited by cytosolic malonyl CoA, which is produced during fatty acid synthesis, ensuring that newly formed fatty acids are not immediately transported into the mitochondria and broken down. Because insulin augments, whereas glucagon and catecholamines diminish, the concentration of malonyl CoA, the hormonal imbalance of uncontrolled diabetes produces low cytosolic levels of malonyl CoA, enhancing fatty acid transport toward the mitochondria and thereby stimulating ketogenesis. In summary, synthesis of ketones in the liver largely depends on transfer of fatty acyl CoA into the mitochondria by CPT I. In the fed state of normal individuals, CPT activity is inhibited by cytoplasmic malonyl CoA. During fasting and in patients with ketoacidosis, malonyl CoA synthesis is suppressed, allowing for increased transfer of fatty acyl CoA into the mitochondria and increased ketogenesis. Decreased consumption of ketone bodies by peripheral tissues also contributes to the high levels of plasma ketones in DKA.

Clinical Manifestations and Diagnosis of Diabetic Ketoacidosis

Diabetic ketoacidosis is a medical emergency most often observed in type 1 diabetes. Less frequently, DKA is the presenting condition in obese patients with newly diagnosed type 2 diabetes. In patients with uncontrolled type 2 diabetes, DKA can be observed in combination with nonketotic hyperglycemia. The risk of developing DKA in type 1 diabetics is approximately 1% to 2% each year (90,91). The morbidity associated with DKA is dependent on the severity of the acid–base and electrolyte disturbances present. Despite advances in treatment, the mortality rate for DKA remains approximately 7% in the USA (91).

Precipitating events. Omission of insulin administration, various forms of stress, and dietary indiscretions (especially a large alcohol intake) represent the most common precipitating events of DKA (92–94). In young individuals with type 1 diabetes, emotional stress can trigger repeated episodes of DKA over short intervals. Infectious illnesses often precipitate DKA and must be aggressively treated if the ketoacidosis is to be controlled. Common such etiologies include seemingly trivial viral infections as well as pneumonia, pyelonephritis, and septicemia (73). Pregnancy, myocardial infarction, cerebrovascular accident, intra-abdominal catastrophes including pancreatitis, K^+ depletion, and drugs such as corticosteroids, can also precipitate DKA (84).

Symptoms and signs. Weakness, malaise, air hunger, thirst, polyuria, vomiting, and altered sensorium are commonly observed (1–3). Severe abdominal pain secondary to ketosis (possibly a hypertriglyceridemia-induced pancreatitis) can be observed, and these patients sometimes are mistakenly triaged to surgery (92). However, the abdominal pain can represent an independent process, such as acute appendicitis (that requires surgery) or pyelonephritis, which in turn may have precipitated DKA. Increased rate and depth of respirations, the so-called Kussmaul breathing, is an almost constant finding. Signs of volume depletion, including orthostatic hypotension, tachycardia, decreased skin turgor, and soft eyeballs, can be evident on physical examination (95,96). Although DKA is sometimes referred to as diabetic coma, only 10% of patients are unconscious at presentation and about 20% are alert (92). The majority of patients present, however, with a clouded sensorium. Severe obtundation, coma, or convulsions, rarely seen in pure DKA, are prominent manifestations of severe nonketotic hyperglycemia. The patient's temperature is often decreased but the presence of hypothermia does not rule out an infectious process (95). Conversely, if fever is present, the likelihood of infection is high. A search for tooth or skin infection, perirectal abscess, or other infectious precipitating event is most important. Patients might describe experiencing the distinct taste and smell of ketones, and the examiner might notice a fruity odor in the subject's breath.

Serum ketones. The clinical diagnosis of DKA depends on semiquantitative assessment of serum ketones with reagent sticks or tablets; reagent tablets should be powdered before use. A "large" reading (4+ reaction) for ketones in plasma diluted 1:1 or greater is diagnostic of DKA; such a reading in a urine sample is not diagnostic of DKA, however, as it can be observed in other conditions, including ordinary fasting, fasting induced by an illness that has precipitated lactic acidosis or nonketotic hyperglycemia, or nonketotic hyperglycemia itself (5). The low renal threshold for ketones accounts for the possible development of a sufficiently high urine ketone concentration in all ketotic states to be detected as a "large" test response (4+ reaction).

The semiquantitive test for ketones measures only acetoacetate and acetone (a product derived from nonenzymatic decarboxylation of acetoacetate); it does not detect β-hydroxybutyrate. The latter ketoacid is formed from the reduction of acetoacetate in a reaction utilizing NADH. Because the β-hydroxybutyrate concentration in all ketotic states is at least two to three times higher than that of acetoacetate, a relatively high plasma ketone level of at least 6 mmol/L is required to achieve a "large" test reading in an undiluted sample. Therefore, a "large" test reading in plasma diluted 1:1 or greater indicates that at least 12 mmol/L of ketones are present, a level found in DKA but rarely present in other ketotic states (97,98). Exceptions include a short-term fast in late pregnancy, lactating women, and some alcoholic patients (99–102).

The altered redox state of hepatocytes observed in tissue hypoxia, ethanol ingestion, or with high rates of fatty acid oxidation increases further the ratio of β-hydroxybutyrate to acetoacetate making the semi-quantitative essay less reliable for detection of ketosis. Sulfhydryl drugs, including captopril, can produce a false positive ketone test (103). An interaction of the colorimetric assay for creatinine with acetoacetate (chromogen) results in higher measured serum creatinine levels on admission for DKA, followed by large decreases after insulin's lowering effects on ketone levels. In Table 90-2 are presented a summary of salient abnormalities of blood measurements in patients presenting with DKA.

Diagnosis. The diagnosis is made by recognition of characteristic symptoms and signs, couple with biochemical features that include hyperglycemia and ketosis (84). A commonly used but more restrictive diagnostic criterion of DKA includes a triad of hyperglycemia (plasma glucose >250 mg/dL), ketosis (serum ketones 4+ positive by the nitroprusside reaction in a dilution 1:1 or greater), and metabolic acidosis (plasma bicarbonate <15 mEq/L, blood pH <7.30). However, the hypobicarbonatemia and acidemia might be absent in DKA because of the co-existence of additional acid–base disorders (104).

Differential diagnosis. The differential diagnosis of DKA includes conditions with symptoms and signs related to the neurologic, gastrointestinal, or respiratory systems. Neurologic disorders include metabolic diseases (e.g., hypoglycemia, uremia, nonketotic hyperglycemia, lactic acidosis), toxic encephalopathies (e.g., ethanol, methanol, ethylene glycol, opium derivatives, other narcotics), head trauma, cerebrovascular accident, meningitis, and encephalitis. Gastroenteritis (abdominal pain, nausea, vomiting) and pneumonia (dyspnea) can also resemble DKA.

Nonketotic Hyperglycemia. Nonketotic hyperglycemia (NKH) (84,85), is a syndrome characterized by the presence of severe hyperglycemia (usually >800 mg/dL) and the absence of clinically significant ketosis (see Table 90-7). DKA and NKH share the same general pathogenesis-insulin deficiency/resistance and excessive counter-regulation (the most important element being high glucagon levels), but the importance of each of these endocrine abnormalities appears to differ (86,87). Pure DKA, which features profound ketosis, might emanate from severe insulin deficiency/resistance, with milder abnormalities in counter-regulation (Fig. 90-7). By contrast, NKH, which features marked hyperglycemia without ketosis, might be caused by relatively mild insulin deficiency/resistance but more intense counter-regulation. The most important clinical distinction, however, is that the severity of the fluid deficit and secondary renal dysfunction is greater in NKH than in DKA. This finding in NKH plays a critical pathogenetic role in the profound hypertonicity observed (Table 90-8) (105–108). In some instances, a large exogenous source of glucose can be of great importance in the development of this condition (13) (Table 90-9).

TABLE 90-7

CONTRASTING BIOCHEMICAL FEATURES OF PURE DIABETIC KETOACIDOSIS (DKA) AND NONKETOTIC HYPERGLYCEMIA (NKH)[a]

Plasma levels	Pure DKA	Mixed forms	Pure NKH
Glucose	<800 mg/dL	⇔	>800 mg/dL
Ketone bodies	4+ in 1:1 dilution	⇔	Not 4+ in 1:1 dilution
Effective osmolality	<340 mOsm/kg	⇔	>340 mOsm/kg
pH	Decreased	⇔	Normal
$[HCO_3^-]$	Decreased	⇔	Normal
$[Na^+]$	Normal or low	⇔	Normal or high
$[K^+]$	Variable	⇔	Variable

[a]Mixed forms of DKA–NKH have intermediate features denoted by the symbol ⇔.

Hypertonicity and renal dysfunction. Renal dysfunction, most commonly caused by volume depletion, must be present to generate and sustain the extreme hyperglycemia observed in most patients with NKH (13). Fluid deficit can be caused by poor fluid intake (e.g., in patients who are frail and unable to perceive or respond to thirst because of sedation, stroke, or other causes) and/or abnormal fluid losses due to osmotic diuresis, vomiting, diarrhea, fever, or diuretics. A simple calculation demonstrates the virtual impossibility of maintaining plasma glucose levels substantially higher than 400 mg/dL in the presence of normal renal function, because of the magnitude of obligatory glucosuria (13). In a patient with a plasma glucose of 400 mg/dL and a normal GFR, for example, the amount of glucose that escapes reabsorption (difference between plasma glucose and the "renal threshold concentration," 180 mg/dL) is approximately 400 g/day. Glucose is also removed by cellular metabolism; whole-body glucose utilization is approximately 200 g/day under euglycemic conditions, and increases in proportion to the glucose concentration even in the absence of insulin. In patients with severe hyperglycemia and adequate renal function, therefore, substantial glucose removal, on the order of 600 to 1,000 g/day, would result both from glucosuria and internal disposal. An equal amount of new glucose must enter the circulation to satisfy these demands in steady-state, severe hyperglycemia. Thus, unless the patient has an exceptionally high level of endogenous glucose production (in excess of three times the normal value observed in the fasting state) or an extremely large exogenous source of glucose is present, severe hyperglycemia cannot be sustained in the absence of renal failure. Exogenous sources of glucose include tube-feeding solutions, parenteral hyperalimentation, or peritoneal dialysis with a high glucose concentration in the dialysate. In Table 90-9 are summarized the role of fluid deficit and glucose load as precipitating factors of NKH (13).

Absence of Ketosis

There are several theories to account for the absence of substantial ketosis in NKH (109–111). It has been proposed that in NKH there is sufficient insulin to inhibit lipolysis but not enough to stimulate peripheral glucose uptake. This explanation is based on the presumed less severe insulin deficit of patients with NKH, and the known inhibition of ketosis by relatively low insulin levels. Because hypertonicity has been shown to inhibit lipolysis in vitro, it may also be partly responsible for the absence of substantial ketoacidosis in NKH (112). A more recent explanation offered for the absence of ketosis is hepatic resistance to glucagon such that malonyl-CoA levels do not decrease as much as in DKA (113–115).

TABLE 90-8

CONTRASTING CLINICAL FEATURES OF PURE FORMS OF DIABETIC KETOACIDOSIS (DKA) AND NONKETOTIC HYPERGLYCEMIA (NKH)[a]

Feature	Pure DKA	Mixed forms	Pure NKH
Incidence	5 to 10 times higher	⇔	5 to 10 times lower
Mortality	5% to 10%	⇔	10% to 60%
Onset	Rapid (<2 days)	⇔	Slow (>5 days)
Age of patient	Usually <40 years	⇔	Usually >40 years
Type 1 diabetes	Common	⇔	Rare
Type 2 diabetes	Rare	⇔	Common
First indication of diabetes	Often	⇔	Rare
Volume depletion	Mild/moderate	⇔	Severe
Renal failure (most commonly of prerenal nature)	Mild	⇔	Severe
Subsequent therapy with insulin	Always	⇔	Occasional

[a]Mixed forms of DKA–NKH have intermediate features denoted by the symbol ⇔.

TABLE 90-9

FLUID DEFICIT AND GLUCOSE LOAD AS PRECIPITATING FACTORS OF NONKETOTIC HYPERGLYCEMIA (NKH) IN DIABETES MELLITUS

Condition	Physiologic derangement	Clinical entity
Fluid deficit	Poor water intake	
	Relatively preserved CNS function	Elderly/nursing home patients
	Major CNS abnormality	Cerebrovascular accident, subdural hemorrhage
	Increased urinary fluid loss	Large osmotic diuresis, diuretics
	Extrarenal fluid loss	
	Gastrointestinal	Gastroenteritis, peptic ulcer disease, gastrointestinal bleeding, pancreatitis
	Skin	Heat stroke, burns
Glucose load	Increased glucose intake	Highly sweetened drinks, enteral or tube-feeding; hyperalimentation, peritoneal dialysis
	Increased endogenous glucose production	
	Stress	Psychological/physical trauma
	Infection	Pneumonia, pyelonephritis, sepsis
	Major illness	Myocardial infarction
	Medication	Corticosteroids, phenytoin, calcium channel blockers[a]

[a]Phenytoin and calcium channel blockers may decrease insulin release and precipitate the HONKS.

Diagnosis. Typically, patients with this disorder are elderly with Type 2 diabetes, and present with a depressed sensorium, progressing to obtundation and finally coma. They may be oliguric or anuric. Patients with "pure" NKH have no acid–base abnormalities, no clinically significant ketonemia, a markedly elevated blood glucose concentration, and elevated BUN and creatinine concentrations. The diagnosis is based on the presence of severe hyperglycemia (glucose levels >800 mg/dL), hypertonicity (>340 mOsm/kg), absence of significant ketosis, and profound volume depletion (84). A comparison of the most salient clinical and biochemical features that distinguish pure forms of DKA from NKH are displayed in Tables 90-7 and 90-8. Notably, many patients exhibit a mixed pattern with clinical and biochemical features of both DKA and NKH. These patients should be diagnosed as having DKA-NKH (81).

Clinical manifestations. Depression of the sensorium, somnolence, obtundation, and coma are prominent manifestations of NKH, and the degree of CNS depression correlates with the severity of serum hypertonicity (105). Water loss from the central nervous system with brain shrinkage has been documented within 1 hour of severe hyperglycemia in experimental animals, but brain volume recovers at 4 to 6 hours. Neither ketosis nor metabolic acidosis, features that are consistently present in DKA but usually absent in NKH, produce extreme depression of the sensorium (105). In fact, a serum tonicity (i.e., effective osmolality) of 340 mOsm/kg H_2O or higher appears to be necessary for the development of coma, and such levels are commonly observed in NKH but not in DKA (84,105). Circulatory collapse secondary to profound volume depletion can be observed in NKH. All other symptoms and signs previously described for patients in DKA (except for dyspnea and Kussmaul respiration that arise from metabolic acidosis) may also be observed in NKH.

Assessment of effective osmolality. To estimate serum tonicity (effective osmolality), one should calculate its value, using only the sodium and glucose concentrations (effective osmolality $= 2 \times [Na^+] + \text{glucose}/18$). Not infrequently, a comatose diabetic patient is found to have, for example, a measured osmolality of 350 mOsm/kg H_2O but the calculated effective osmolality is only 310 mOsm/kg H_2O; in this case, the patient's coma is more likely to be due to conditions other than NKH (e.g., alcohol, uremia, cerebrovascular accident). Conversely, a comatose diabetic patient who is admitted with a calculated effective osmolality of 350 mOsm/kg H_2O most likely has NKH encephalopathy. Nonetheless, one should always exclude other causes for coma. Sometimes it is difficult to establish whether a neurologic finding is a cause or effect. For example, stroke can lead to NKH, and conversely NKH can cause a cerebrovascular accident (116–118). Rapid reversal of the neurologic syndrome with therapy for NKH indicates that the neurologic event was not the cause but the effect of this metabolic disorder.

Therapy of Diabetic Ketoacidosis and Nonketotic Hyperglycemia

The main therapeutic goals of the successful management of DKA and NKH include repletion of fluid deficit and securing an adequate circulation, reversal of the altered intermediary metabolism; correction of the electrolyte and acid–base imbalance, and treatment of the initiating event (119–125). To accomplish these objectives, a number of requirements must be fulfilled: continuous physician availability; 24-hour access to laboratory facilities; equipment and drugs for handling medical emergencies; and maintenance of a flowchart documenting evaluations of vital signs, mental condition, serum chemistries, urine tests, insulin intake, fluid administration (intravenous and oral), urine output, electrolyte intake, and other medications. A synopsis of the bedside and laboratory procedures for the diagnosis, management, and monitoring of DKA and NKH is presented in Table 90-10.

TABLE 90-10

PROCEDURES/STUDIES FOR DIAGNOSIS, MANAGEMENT, AND MONITORING OF DIABETIC KETOACIDOSIS (DKA) AND NONKETOTIC HYPERGLYCEMIA (NKH)[a]

Bedside	Laboratory procedures/studies
	Blood
1. Body weight	1. Glucose (hourly by test strip until <250 mg/dL, then every 2 hours; confirm in laboratory every 2 to 4 hours)
2. Blood glucose (test strips)	2. Electrolytes (every 2 to 4 hours)
3. Blood and urine ketone (test strips or tablets)	3. pH, $PaCO_2$, PaO_2 (every 2 to 4 hours)
4. Blood pressure and heart rate (every 30 minutes for 4 hours, hourly for next 4 hours, then every 2 to 4 hours)	4. Urea nitrogen, creatinine
5. Mental status (hourly)	5. Osmolality
6. Urine output (hourly for 6 hours every 4 hours thereafter)	6. Hb, WBC, and differential
7. CVP or peripheral venous pressure (hourly during the initial 4 to 8 hours of high-rate fluid infusion)	7. Cultures
8. Body temperature (every 2 hours for 6 hours and every 6 hours thereafter	**Urine**
9. Electrocardiogram	1. Microscopy and cultures
10. Chest x-ray	2. Glucose, ketones

[a]The frequency of repeat tests indicated above represents general guidelines that might require adjustments depending on the patient's presentation and response to treatment.

Fluids. Intravenous saline infusion should be started at once to correct the impaired hemodynamic status and the renal dysfunction; in addition, it lowers plasma glucose levels by enchancing glucosuria and decreasing counterregulatory hormone release (catecholamines) (126,127). Isotonic saline (i.e., 0.9%, NaCl) should be infused at the fastest rate possible in patients in circulatory shock. Patients who do not have an extreme volume deficit should receive about 500 mL/hour for the first four hours followed by 250 mL/hour for the next four hours. More rapid administration is not recommended, as it can delay correction of acidemia (see earlier) and increase the risk of cerebral edema (127). Some experts prefer lactated Ringer's solution to minimize the increase in serum [Cl^-], but there is no evidence that such a practice is of benefit (5); further, it has the potential of inducing rebound metabolic alkalosis, as it loads the patient with the HCO_3^- precursor, lactate. Once the patient is hemodynamically stable, the use of half-isotonic saline with the addition of 20 to 40 mEq of potassium per each L of infusate is appropriate to also secure potassium repletion.

Patients presenting with extreme volume depletion can require a fluid infusion of as much as 5 to 10 L within the first 24 hours (128–131). Fluid challenges of this magnitude demand that the patient receive careful monitoring for signs of pulmonary edema. Central venous or pulmonary artery catheterization may be required in some patients to accurately monitor intravascular volume.

It is unwise to allow oral fluid intake in the early phase of DKA and NKH because vomiting is common, especially if acute gastric distention is present. Although efforts should be made to avoid routine bladder catheterization in patients with uncontrolled diabetes to prevent the development or exacerbation of a urinary tract infection, a catheter might be required if the patient is stuporous or urine output cannot be monitored reliably.

Insulin. All patients with uncontrolled diabetes require insulin to reverse the ketoacidosis and correct hyperglycemia (132–134). These actions of insulin largely reflect suppression of ketone and glucose production in the liver because of insulin's antiglucagon effect. Of lesser importance is the insulin-induced enhancement of glucose utilization in muscle and adipose tissue as well as inhibition of lipolysis. Regular insulin should be given, if possible, intravenously (5,85).

Mild insulin resistance is virtually a constant finding, although occasionally it may be extreme (135,136). Consequently, insulin requirements in DKA are always several-fold higher than in normal persons. A loading dose of 15 to 30 units given as a bolus on arrival is recommended to secure binding of insulin to anti-insulin antibodies that might be present in patients who have been previously treated with animal insulin, and to assure saturation of insulin receptors (137). In patients who are markedly volume depleted, insulin therapy should be withheld for the initial 30 to 60 minutes to allow some repletion of the ECF volume with isotonic saline. In the absence of saline, insulin can exacerbate ECF volume depletion by translocating glucose into cells, thereby causing a fall in ECF tonicity and a shift of water to the ICF compartment. In fact, fluid repletion alone in the absence of insulin administration reduces plasma glucose concentration by 35 to 70 mg/dL per hour (138).

The initial insulin bolus should be followed by 10 to 20 units every hour (but only 3 to 8 units per hour if plasma glucose is between 150 and 300 mg/dL) until the patient is able to eat, at which time subcutaneous insulin administration is resumed (139–144). Although continuous intravenous insulin infusion is most commonly used, intramuscular or subcutaneous administration appears to be as effective in correcting hyperglycemia and ketoacidosis (46). Plasma glucose should fall at approximately 5% to 10% per hour. As the glucose level approaches 300 mg/dL, 5% dextrose in water at 50 mL/hour should be included in the intravenous fluids and the plasma glucose concentration should be followed closely.

A solution containing 100 units of insulin in 100 mL of isotonic saline is commonly used, adjusting the infusion rate to secure the insulin dosage desired. Because some patients are very resistant to insulin, much larger doses might be required. In all cases, the hourly dose should be doubled if the expected reduction in the plasma glucose is not observed within a few hours. Monitoring serum ketones during therapy is unnecessary since it does not help in the adjustment of insulin dosage (144).

Alkali. Bicarbonate administration should theoretically be unnecessary in DKA (at least in patients with predominantly

TABLE 90-11

POTENTIAL ADVANTAGES AND DISADVANTAGES OF BICARBONATE ADMINISTRATION IN THE MANAGEMENT OF DIABETIC KETOACIDOSIS (DKA)

Advantages
1. Improves hemodynamic status if shock persists after volume repletion and severe metabolic acidosis is present
2. Increases myocardial contractility and enhances cardiac and vascular responsiveness to catecholamines
3. Aids correction of hyperkalemia, especially in patients with prerenal azotemia
4. Prevents a rapid fall in CSF osmolality and therefore might decrease risk of cerebral edema
5. Aids correction of cell metabolism and function (including CNS), impaired by severe acidosis
6. Improves acidosis-induced glucose intolerance and insulin resistance

Disadvantages
1. Induces or worsens hypokalemia, leading to cardiac arrhythmias (especially in digitalized patients) and/or dysfunction of respiratory muscles (respiratory failure)
2. Produces ECF volume expansion that can cause pulmonary edema
3. Reduces cerebral blood flow (pH effect) and O_2 delivery to the brain
4. Worsens hypophosphatemia due to cellular uptake of phosphate and depresses O_2 delivery to tissues (increased affinity of Hb for O_2)
5. Produces hypernatremia and increased serum osmolality
6. Decreases further CSF pH, leading to worsening of CNS function
7. Induces overshoot (rebound) alkalosis once conversion of ketone salts to bicarbonate takes place
8. Aggravates ketogenesis and lactic acidosis
9. Predisposes to tetany resulting from hypocalcemia and alkalemia

increased AG acidosis) because ketones, when finally metabolized to CO_2 and H_2O, regenerate HCO_3^- (1–3). In support of this view, several studies have shown that HCO_3^- administration did nothing to improve the recovery of patients with DKA who presented with very severe acidemia (arterial pH of 6.90 to 7.10) (145,146). The administration of HCO_3^- in DKA may also have some potentially deleterious effects because it can augment hepatic ketogenesis and lead to worsening of the CNS acidosis, hypokalemia, and rebound metabolic alkalosis (145–147).

The potential advantages and disadvantages of HCO_3^- administration are summarized in Table 90-11. The controversy regarding its utility is based on variable assessment of the associated risks and benefits by different workers (84,147–151).

In Table 90-12 are summarized our recommendations in terms of indications, goals, and dose estimation for HCO_3^- administration in DKA (151,152). After weighing the arguments, it appears that judicious administration of HCO_3^- to severely acidemic patients, especially those with predominant hyperchloremic metabolic acidosis, confers net benefit; the goal is to support the arterial pH at 7.10 to 7.20 and serum $[HCO_3^-]$ at approximately 10 mEq/L. Bicarbonate should be added to an IV infusion, if possible, instead of administering by

TABLE 90-12

INDICATIONS, GOALS, AND DOSE ESTIMATION FOR BICARBONATE ADMINISTRATION IN THE MANAGEMENT OF DIABETIC KETOACIDOSIS (DKA)

INDICATION
1. Extreme metabolic acidosis (pH <7.00, TCO_2 <5 mmol/L), independent of prevailing hemodynamic status
2. Severe metabolic acidosis (pH <7.15, TCO_2 <10 mmol/L) in association with one or more of the following:
   - Shock unresponsive to volume repletion
   - Persistence or worsening of acidemia after several hours of therapy
   - Predominant hyperchloremic acidosis instead of the usual high anion gap acidosis
   - Worsening of mental status and CNS depression
3. Severe hyperkalemia ($[K^+]_p$ >7 mEq/L)

GOALS AND DOSE ESTIMATION
1. If TCO_2 <5 mmol/L (indication 1), it should be increased to no more than 8 to 10 mmol/L
2. If TCO_2 is 5 to 10 mmol/L (indication 2), it should be increased to no more than 13 to 15 mmol/L
3. Dose estimation: (desired plasma TCO_2 − current plasma TCO_2) × b.wt (kg) × 0.5[a]
4. Calculated bicarbonate dose can be added to IV infusion (1 to 2 ampules or 44 to 88 mEq $NaHCO_3$ per L) or given by IV bolus (50% of estimated dose immediately, and the rest within 2 to 4 hours provided that hypokalemia is not present or is simultaneously treated and that evidence of pulmonary edema is not found)
5. Monitor blood acid–base status every 30 to 60 min (for 2 to 4 hours) after initiation of bicarbonate therapy to adjust dose to patient's needs

[a]Derives from the "apparent space of distribution" of bicarbonate (retained HCO_3^- in mEq/kg divided by the $\Delta[HCO_3^-]_p$ from preinfusion) that is approximately 50% of body weight in normal subjects but higher in hypobicarbonatemic states. Thus, this formula purposefully underestimates bicarbonate requirements to avoid the risk of overcorrection.

TABLE 90-13

POTASSIUM SUPPLEMENTATION (KCl) IN THE MANAGEMENT OF DIABETIC KETOACIDOSIS (DKA) AND NONKETOTIC HYPERGLYCEMIA (NKH)

KCl should *not be* added to the initial 2 L of IV infusion to avoid hyperkalemia because
- Urinary output is initially unknown
- Initial $[K^+]_p$ is usually normal or high

Exception to the above rule on K^+ supplementation: add K^+ to the initial 2 L of IV fluids if
- Initial $[K^+]_p$ <4.0 mEq/L and
- Adequate diuresis is secured

KCl should *be* added to the third L of IV infusion and subsequently if
- Urinary output is adequate (should be at least 30 to 60 mL/hr) and
- $[K^+]_p$ <5.0 mEq/L

Rate of IV K^+ supplementation:
20 to 30 mEq/hour if $[K^+]_p$ <4.0 mEq/L
10 to 20 mEq/hour if $[K^+]_p$ >4.0 mEq/L

Concentration of K^+ in IV infusions:
20 mEq/L when IV supplementation is started and subsequently if $[K^+]_p$ <4.0 mEq/L, and IV infusion rate is = 1 L/hour
40 mEq/L (maximum) if $[K^+]_p$ <4.0 mEq/L and IV infusion rate is <1 L/hour

Monitoring of K^+ supplementation is accomplished by
$[K^+]_P$ every 2 to 4 hours during the initial 12 to 24 hours of therapy
ECG every 30 to 60 minutes during the initial 4 to 6 hours (i.e., only lead II)

intravenous bolus, unless hyperkalemia is present, because of the risk of severe hypokalemia.

Potassium. The typical patient with DKA has a potassium deficit of 4 to 8 mEq/kg at the time of admission, yet the initial serum $[K^+]$ will usually be normal or elevated (see earlier and Table 90-2). Serum $[K^+]$ decreases with therapy because of insulin-mediated uptake by cells, dilution due to volume repletion by intravenous fluids, correction of metabolic acidosis, and urinary K^+ losses. Thus, K^+ supplementation is required (1–3). Specifically, after the initial fluid challenge has restored the urinary output and assuming that serum $[K^+]$ is below 5.0 mEq/L, an intravenous infusion of 10 to 20 mEq/hour should be started and continued until the DKA is controlled and serum $[K^+]$ is 4.0 to 5.0 mEq/L. Serum $[K^+]$ should be monitored periodically and the K^+ infusion rate adjusted, as needed. Details about K^+ supplementation are provided in Table 90-13.

Phosphate. Because phosphate administration is of unproven clinical significance and potentially dangerous (i.e., it might result in hypocalcemia and hypomagnesemia), it should not be infused unless the serum phosphate is below 0.5 mg/dL; in those circumstances, 10 to 30 mmol of potassium phosphate might be added to the intravenous infusion and repeated if necessary to correct persistent hypophosphatemia. The hypophosphatemia of DKA may have serious consequences (e.g., impaired myocardial and/or skeletal muscle contractility) if it occurs in undernourished patients, such as chronic alcoholics. In this population, parenteral phosphate replacement of 60 to 120 mmol administered over a 24 hr period is recommended.

Cerebral Edema and Other Complications of Therapy

Patients with DKA may be admitted with a relatively normal mental status and subsequently become unconscious within the first 12 hours of therapy, in spite of a partial or complete correction of the hyperglycemia and ketoacidosis (153). These patients are typically, but not exclusively, children or young adults, and their morbidity and mortality is relatively high (154). Often, the fatal outcome is unexpected, as these patients do not have the underlying vascular, cardiac, and renal abnormalities found in older diabetics. At autopsy, cerebral edema is consistently present (154). Although cerebral edema leading to death or chronic sequelae (e.g. isolated growth hormone deficiency) is fortunately rare, milder forms can be regularly found in the course of standard treatment of DKA (5,155,156).

The pathogenesis of this condition remains poorly understood (116,157–159). Osmotic disequilibrium between brain cells and the CSF is often cited as the principal determinant of cerebral edema. In response to hyperglycemia, brain cells develop an increase in their osmolality to match that of the CSF within hours and thus defend themselves against shrinkage. Once this cellular adaptation to hypertonicity has occurred, a sudden decrease in CSF osmolality, due to hypotonic fluid infusions or a fall in plasma glucose, may cause swelling of these adapted cells and produce cerebral edema. Insulin administration also activates the plasma membrane Na^+/H^+ exchanger (NHE–1), which promotes sodium entry in brain cells, facilitating development of cerebral edema. The effects of rapid crystalloid volume loading in diabetic patients with DKA and the resulting dilutional hypoalbuminemia have been studied and some degree of brain swelling or increase in CSF pressure was found (160–165). Alterations in CSF pH and oxygen tension following HCO_3^- administration may also contribute to the development of cerebral edema.

These mechanisms are only hypotheses, however, and the pathogenesis of cerebral edema in DKA remains uncertain. Still, it seems prudent in the course of therapy of DKA (and that of nonketotic hyperglycemia) to avoid excessively aggressive volume replacement, sudden changes in the patient's serum glucose and $[Na^+]$, and excessive use of HCO_3^-.

A number of life-threatening complications may develop in the course of DKA and NKH in spite of adequate medical care (Table 90-14). Shock of cardiac origin or resulting from sepsis or volume depletion, as well as cerebral thrombosis and edema, are among the most prominent complications (92,166). Cerebral edema leading to death or responsible for chronic sequelae (118,155) fortunately is rare, yet milder forms may be regularly found with the standard treatment of DKA (160) and

TABLE 90-14

COMPLICATIONS OF DIABETIC KETOACIDOSIS (DKA) AND NONKETOTIC HYPERGLYCEMIA (NKH)

1. Hypoglycemia
2. Hypokalemia and hyperkalemia
3. Fluid overload (pulmonary edema)
4. Cerebral edema (with possible neurologic sequelae or death)
5. Acute gastric dilatation, erosive gastritis
6. Infection: urinary tract, pneumonia, mucormycosis
7. Venous and arterial thrombosis
8. Acute tubular necrosis (renal failure)

NKH (167). Mortality from DKA has declined from more than 40% in the 1930s to less than 5% in some institutions, as the result of improvements in medical technology and appreciation of the seriousness of the disease. Yet, the prognosis of patients with NKH is substantially worse, as evidenced by the higher mortality associated with this metabolic complication (85).

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SECTION XII
UREMIC SYNDROME

CHAPTER 91 ■ PATHOPHYSIOLOGY AND NEPHRON ADAPTATION IN CHRONIC KIDNEY DISEASE

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Two million functioning nephrons collectively ultrafilter some 180 L of fluid each day in the healthy human. Successive nephron segments modify the glomerular ultrafiltrate, so that the final urine volume and composition are appropriate for the daily ingested water and solute load. That we are endowed with a surfeit of nephrons, above that needed to maintain normal homeostasis, is regularly shown by the ability of a single kidney following uninephrectomy to carry out the functions previously performed by two. This surplus is even more apparent in the setting of progressive chronic kidney disease (CKD). Patients who have lost 75% of renal function are asymptomatic, and those whose glomerular filtration rate (GFR) is only 10% of the normal value retain the ability to excrete the daily water and solute load.

This chapter first reviews the compensatory changes in function and structure that occur when functioning nephron number is reduced, and that make possible the adaptations that maintain homeostasis. Alterations in handling of individual solutes and water are then described. We then describe the mechanisms that underlie adaptation, and then the transition stage in which these compensatory changes can prove maladaptive, and serve to accelerate loss of the remaining nephrons.

PRINCIPLES AND PATTERNS OF NEPHRON ADAPTATION: "INTACT NEPHRONS" AND GLOMERULOTUBULAR BALANCE

As nephrons fail during the course of CKD, each remaining nephron must increase its single nephron excretion of the filtered load of water and solutes. In 1952, Robert Platt (1) reasoned that the work of each remaining unit is increased, making an analogy with the remaining workers in a factory putting in overtime when their numbers are reduced by illness. Bricker and colleagues (2–4) later noted that the functional capacity of the residual nephrons determines the degree to which homeostasis is preserved, and that maintenance of homeostasis implies preservation (or even enhancement) of normal glomerular and tubular function in the remaining nephrons. Reasoning that globally hypofunctioning nephrons could not maintain homeostasis, these investigators coined the "intact nephron hypothesis" to explain the preserved ability of the remnant nephrons to excrete the required load of water and solutes (2–4). This hypothesis does not imply an all-or-nothing condition, whereby nephrons are either fully functional or dead. Rather, GFR may be reduced in some nephrons, while compensatory mechanisms elevate GFR and enlarge the tubules of the less damaged nephrons (5,6). As reviewed by Bricker and Fine (4), the original studies examining this question were performed in models of unilateral renal disease (7). GFR in the affected and unaffected contralateral kidneys was compared to values simultaneously determined for tubular transport functions. The ratio of glomerular to individual tubular functions was comparable in the diseased and normal kidneys. These data were interpreted to indicate that for any given volume of glomerular filtrate, diseased nephrons excrete the same amount of solute as do the nephrons of the contralateral kidney (4). Each remnant nephron presumably transports water and solutes in proportion to its individual GFR, whether reduced by disease processes or elevated by compensatory hyperfiltration and hypertrophy.

Another essential component of adaptation is maintenance of glomerulotubular balance. Glomerulotubular balance is maintained in both tubulointerstitial and glomerular diseases (5,8). In chronic experimental membranous glomerulonephritis, despite a wide range of single nephron GFR (SNGFR) values, proximal fluid reabsorption correlates closely with SNGFR in individual nephrons, so that fractional reabsorption is the same in hypofiltering and hyperfiltering nephrons (9). Presumably, structural changes in the proximal tubule (PT) and peritubular capillary network act together with alterations in Starling forces to perpetuate glomerulotubular balance. In those studies, the net hydraulic permeability of the peritubular capillary network surrounding severely damaged nephrons was reduced, suggesting loss of capillary surface area available for fluid reabsorption (9). Morphologic studies have further shown that nephrons whose glomeruli are severely damaged by immune injury exhibit PT cell atrophy, presumably associated with decreased reabsorptive capacity. In contrast, PT of less damaged nephrons increase in diameter and length (and presumably in reabsorptive capacity).

FUNCTIONAL ADAPTATIONS TO NEPHRON LOSS

Surgical ablation of renal tissue, which has been used since the late 1800s, is the simplest and most widely used model to mimic the more gradual loss of nephrons that occurs in CKD. Reduction of renal mass leads to structural and functional hypertrophy of the remaining nephrons. Hyperfiltration in remnant nephrons has been generally regarded as beneficial in the short term, since it minimizes the decrease in total GFR that would otherwise ensue. However, as described in the subsequent text, sustained activity of these forces is now recognized to contribute to loss of renal structure and function in the longterm. The border between adaptive and maladaptive responses is ill defined, but appears to relate more to the magnitude than to the character of the adaptive changes, since activation of the same growth promoting pathways occurs with modest or extreme ablation. The degree of nephron loss that represents a point of no return has not been identified. This

theoretical point is likely to vary depending on species, underlying cause of nephron loss, and a wide spectrum of risk factors discussed in the subsequent text. The exact scenario of events following the nephron loss still remains to be established, but it appears that functional adaptations precede changes in structure. Though discussed separately, it should be recognized that functional and structural adaptations are closely interrelated, often sharing common pathophysiological mediators. Furthermore, adaptive and maladaptive mechanisms will be discussed in parallel, with emphasis on putative mechanisms or factors underlying transition from adaptation to maladaptation.

FUNCTIONAL ADAPTATIONS

Renal Hemodynamic Adaptations and Maladaptations: Compensatory Hyperfiltration

Remaining nephrons compensate for nephron loss by increased perfusion and filtration rates. Studies of renal transplant donors (in whom the remaining nephrons are considered to be normal) indicate that within weeks after nephrectomy, GFR and renal plasma flow (RPF) rates in the remaining kidney increase by about 40%, so that the GFR is about 70% of the prenephrectomy value (10,11). GFR may be maintained reasonably well for up to 50 years after childhood nephrectomy (12) and for decades after nephrectomy in adulthood (13). The risk of reaching end-stage renal disease (ESRD) in living transplant donors has been estimated to be 0.2% to 0.5% (14). For those patients that do lose function after nephrectomy, the contribution of nephron ablation vs. extrarenal risk factor activity is unclear.

Studies in experimental models provide insight into the mechanisms of compensatory hyperfiltration. The SNGFR increases within 15 hours after nephrectomy in rats (15); by 2 weeks, the whole kidney and single nephron GFR values have increased by about 40% (16,17). Comparable changes are seen in the whole kidney and single nephron plasma flow rates. Vascular resistance is reduced in the afferent and efferent arterioles, allowing an increase in the glomerular capillary plasma flow rate (Q_A). Because the decrease in afferent arteriolar resistance (R_A) is proportionally greater than that in efferent arteriolar resistance (R_E), the hydraulic pressure in the glomerular capillary (P_{GC}) increases. Together, glomerular capillary hyperperfusion and hypertension account for the single nephron hyperfiltration in the remaining nephrons. The magnitude of these hemodynamic adaptations correlates with the amount of renal tissue excised. Thus, uninephrectomy leads to a 40% to 50% increase in the SNGFR (17); this rate more than doubles after removal of three-fourths of the renal mass (18–20). With uninephrectomy, the increase in SNGFR is largely due to a similar increase in Q_A. However, in more extensive renal ablation, substantial increases in P_{GC} occur. The magnitude of the adaptive increase in SNGFR is similar in superficial and juxtamedullary nephrons (21,22), and the tubuloglomerular feedback mechanism remains intact, with its setpoint altered in a way that permits hyperfiltration (23,24).

Of the glomerular hemodynamic determinants of adaptive hyperfiltration, glomerular capillary hypertension appears to be the crucial cause of eventual structural injury. The relative roles of hyperfiltration, hyperperfusion, and glomerular capillary hypertension were elucidated in a series of studies in the 1980s. Hostetter et al. (18) subjected rats to 85% to 90% nephrectomy. Untreated rats exhibited elevated SNGFR, due to elevations in both P_{GC} and Q_A. These hemodynamic adaptations were associated with proteinuria and extensive focal and segmental glomerular sclerosis (FSGS) (25). When dietary protein restriction was used to blunt the adaptive hyperfiltration, values for SNGFR, Q_A, and P_{GC} were nearly normalized. Limitation of Q_A and P_{GC} with dietary protein restriction is associated with slowing of the development of proteinuria and FSGS in this and a number of other models of renal disease (26).

Those studies did not separate out the relative roles of hyperfiltration and its specific determinants. Subsequent studies using angiotensin converting enzyme inhibitors (ACEI) were able to clarify this issue. ACEI reduce R_E and P_{GC}, without affecting Q_A or SNGFR. In rats with renal ablation, selective control of glomerular hypertension is protective, even in the absence of any changes in SNGFR or Q_A. Conversely, antihypertensive therapy that lowers systemic but not intraglomerular pressure may not protect the kidney at risk (19).

Uninephrectomy does not lead to increased blood pressure, whereas more extensive renal ablation is often accompanied by substantial systemic hypertension (18,19). Hypertension is both cause and consequence of CKD. Once present, hypertension is associated with faster loss of renal function in patients with acquired renal disease, as well as acceleration of the more moderate loss of renal function associated with normal aging (27,28). Studies in hypertensive renal disease models have helped to delineate the hemodynamic mechanisms of hypertensive injury. Specific patterns relate to changes in the arterial perfusion pressure and the accompanying patterns of glomerular arteriolar resistances. Afferent arteriolar vasodilation, and impaired ability to respond to changes in perfusion pressure, result in impaired autoregulation and enhanced transmission of systemic pressure into the glomerular capillary network, and thereby to glomerular capillary hypertension (29–31).

Alterations in Glomerular Permeability

Another index of glomerular function is the ability to restrict passage of macromolecules into the urinary space (32,33). The normal glomerular capillary wall imposes a formidable barrier to passage of plasma proteins. Permselectivity is characterized by examining the extent to which the glomerular capillary wall discriminates among molecules of different size, charge, and configuration. The most extensively used method for quantitation of glomerular permselectivity involves measurement of fractional clearances of test macromolecules, compared to clearance of inulin. Substances such as dextrans and Ficoll may be used in a neutral state, allowing assessment of size selectivity or, in a charged (anionic or cationic) state, allowing assessment of charge selectivity (33). The other major determinant of protein passage, molecular shape or configuration, has been less extensively studied. In the normal kidney, the fractional clearance of negatively charged dextran sulfate is lower than that for neutral dextran at any given molecular radius. Conversely, positively charged molecules pass through more freely (32,33).

Increased glomerular permselectivity to proteins is a component of adaptation to reduced nephron number. Uninephrectomy is associated with a modest, late development of proteinuria in the rat (34) and, in some cases, humans (35). However, in the setting of a predisposition to proteinuria, the course can be accelerated. For example, in a rat strain predisposed to spontaneous proteinuria, uninephrectomy induces a defect in charge selectivity (36). With more extensive renal ablation, even in previously normal kidneys, permselectivity studies indicate that proteinuria results from defects in both size selectivity and charge selectivity (25,37,38), with increased flux through the shunt pathway. As is discussed subsequently, this adaptation is associated with lesser capability of podocytes to adapt to the loss of nephron mass.

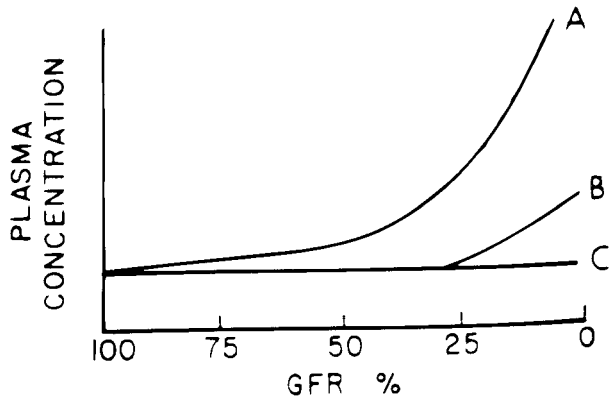

FIGURE 91-1. Patterns of adaptation for different solutes in chronic progressive renal disease. **A:** No regulation or adaptation. **B:** Regulation with limitation. **C:** Complete regulation. See text for discussion. (Reprinted from: Bricker NS, Fine LG. The renal response to progressive nephron loss. In: Brenner BM, Rector FC Jr, eds. *The Kidney.* Philadelphia: WB Saunders, 1981:1058, with permission.)

Functional Adaptations: Tubular

The adaptive changes in SNGFR and in proximal tubular reabsorptive capacity are accompanied by specific mechanisms that augment the ability to excrete a constant solute load in the face of a dwindling number of functioning nephrons. In general, the adaptive mechanisms are not unique to renal insufficiency; rather, they are the same mechanisms that allow the normal kidney to excrete an excessive load. There are limits to the adaptive capability, however; when that capability is exceeded, the complications of CKD ensue.

As elucidated by Bricker and Fine (4), there are three major patterns of adaptation to advancing CKD (Fig. 91-1). The pattern of adaptation is reflected by the change in serum concentration of specific solutes with the fall in GFR. If there is no regulation or adaptation, the plasma concentration increases (curve A of Fig. 91-1). Examples include urea and creatinine, in which the rate of excretion depends on the filtered load, and tubular reabsorption and secretion mechanisms fail to adapt sufficiently to prevent a rise in the serum concentration. Curve B represents "regulation with limitation" (i.e., maintenance of normal plasma concentration until the late stages of CKD); when GFR falls below a critical level, excretion can no longer keep up with intake, and plasma concentration rises. Solutes in this class, such as phosphate and urate, are excreted by filtration and either tubule reabsorption, tubule secretion, or a combination. The third pattern (curve C) is termed "complete regulation." Serum concentration is maintained in the normal range until terminal CKD stages; examples include sodium, potassium, and magnesium. For normal serum levels to be maintained, increased single nephron excretion results from altered tubular transport patterns, as discussed in the subsequent text. Changes in individual solute handling in CKD have been reviewed extensively elsewhere, and are briefly summarized here.

Sodium Excretion and the Regulation of Extracellular Fluid Volume

Extracellular fluid (ECF) volume is maintained remarkably close to normal in patients with CKD, until the very late stages (39). Although absolute sodium (Na) reabsorption in the PT increases in parallel with the rise in SNGFR (40,41), fractional excretion of Na and water increase (42–44). These changes result in a marked increase in Na delivery to the thick ascending limb of Henle and distal nephron (21).

Chronic adaptations enable maintenance of normal Na balance until the late stages, but the immediate natriuretic response to a large Na challenge is impaired (45,46). The natriuresis evoked by Na loading is generally reduced less than is the GFR (45–47), so acute volume expansion causes a greater increase in fractional Na excretion in uremic animals than in normal controls, a "magnification" phenomenon (45,48,49). In advanced CKD, ability to conserve Na is also compromised, such that most patients are unable to lower Na excretion below 20 to 30 mEq/day (a "salt floor") (4,50,51). With high Na intakes, the smaller number of functioning nephrons may not be able to increase Na excretion enough to maintain Na balance, and thus may be termed as having a low "salt ceiling." As CKD progresses, the distance between "floor" and "ceiling" increases, and maintenance of Na balance becomes more difficult (4). However, in some cases, slowly reducing Na intake may lead to more efficient reductions in Na excretion, or reversal of the "salt-losing" tendency (52).

With respect to mechanisms controlling adaptive changes in Na handling, early investigators postulated that decreased aldosterone formation or effect might play a role (8). However, observations that serum aldosterone levels are normal or high, that responsiveness to aldosterone antagonist therapy is present (53), and that exogenous mineralocorticoid therapy does not cause Na retention (42,54–56) in CKD patients have cast doubt on a major role for this mechanism. Later, the discovery of atrial natriuretic peptide (ANP) turned attention toward that hormone as a mediator of Na excretion in CKD. Rats with renal ablation exhibit increased ANP levels, which are related to dietary Na intake and fractional Na excretion (57,58). Increased ANP levels in CKD patients have been related to increased blood volume and to increased blood pressure (59,60). Interpretation of these studies may be complicated by the presence of heart failure and errors in the assay of plasma ANP caused by related peptides that are retained in CKD. However, studies with an ANP antagonist confirm that increased ANP levels make a major contribution to increased fractional Na excretion in experimental CKD (61), and giving a monoclonal anti-ANP antibody prevents the postnephrectomy diuresis in rats, by blunting both proximal and distal tubular Na reabsorption (62).

Hypertension may contribute to increased fractional Na excretion in CKD, as has been suggested by Guyton's group (63). According to this view, maintenance of constant Na intake in the setting of fewer nephrons leads to Na retention and expansion of ECF and blood volumes; the consequent increase in blood pressure in turn causes a higher fractional Na excretion. However, blood pressure is higher in CKD patients than in normal subjects whose blood volumes have been increased by salt loading, and reducing dietary salt intake does not consistently prevent hypertension in rats with renal ablation (64). Thus, blood pressure alone is not the sole factor regulating Na excretion in CKD.

The molecular basis for Na transport after reduction in nephron number has been explored. Using microdissected tubule segments of 5/6 nephrectomized rats, Terzi et al. (65) found increased Na-K-ATPase activity along the nephron when expressed per unit of nephron length. Enzymatic activity correlated with the degree of tubular hypertrophy. However, when expressed per tubule surface unit, no changes were found, suggesting that the density of pumps remained stable during compensatory tubular hypertrophy. Addressing this issue, Kwon et al. (66) explored protein expression of Na transporters and Na-K-ATPase along the nephron in rats 2 weeks after subtotal nephrectomy. They found a decrease in total kidney Na/H exchanger, Na-phosphate cotransporter, and basolateral Na-K-ATPase in remnant kidneys as compared to controls.

Densities of these transporters did not increase proportionally to the nephron hypertrophy and GFR. These findings reflected mainly changes in the PT and were associated with increased remnant nephron Na excretion. In contrast, expression of bumetanide-sensitive channels in thick ascending limb and thiazide-sensitive channels in distal tubules was increased, and Na-K-ATPase expression was maintained in these segments. These changes indicate compensatory increases in distal segments, partly because of elevated vasopressin and aldosterone levels associated with CKD. In contrast to rats with extensive renal mass reduction, more recent studies in uninephrectomized rats have shown upregulation and activation of NHE-3 (Na/H exchanger). Moreover, inhibition of NHE3 was associated with inhibition of compensatory growth in the remnant kidney (67).

Potassium Homeostasis

The failing kidney retains the ability to maintain potassium (K) homeostasis and normal serum K levels until the GFR reaches about 10% of normal. Potassium secretion per nephron must increase to maintain K balance; in fact, experimentally, fractional excretion of K may exceed 100% of the filtered load (7), and this adaptation occurs within a day of uninephrectomy (68). Similar to Na handling, animal studies show a significant inverse correlation between fractional excretion of K and GFR. The major factors responsible for increased K excretion per nephron appear to be elevation of plasma and intracellular K concentrations following K ingestion, particularly early in the course; later, an adaptive tubule process also augments K secretion (69–71). In uninephrectomized rats, K secretion occurs within hours after nephrectomy, and is mediated by amiloride-sensitive channels (72). In addition, reduced K reabsorption by the loop of Henle may facilitate excretion of acute potassium loads in renal failure (73).

Following ingestion of a K load, serum K increases by about the same increment in CKD patients and in normal subjects, inducing an increase in distal K secretion (74). When factored for GFR, the kaliuresis in patients with moderate CKD is the same as normal subjects (74,75). However, because the CKD patient excretes K more slowly than the normal subject, there is prolonged elevation of serum K following an oral load.

Later in the course, distal tubular adaptations, specifically, increased activity of Na-K-ATPase and basolateral surface area in principal cells of the cortical collecting duct, promote K excretion (76–80). As another adaptation, as CKD progresses, intestinal K excretion also increases in concert with increased colonic Na-K-ATPase activity (79,80). Administration of the aldosterone antagonist spironolactone to patients with CKD often results in dangerous hyperkalemia (81), and hyperkalemia is often observed when aldosterone synthesis is reduced by ACEI. Thus, it appears that adequate aldosterone levels are required to facilitate increased K secretion per nephron, and that hyperkalemia may occur earlier in CKD in patients who have low plasma aldosterone levels (82).

Water Homeostasis

The capacity to generate solute-free water (expressed as a fraction of the GFR) is remarkably well maintained when renal function is impaired (4,11,83–85). However, the GFR reduction impairs the water excretory capacity, as is reflected in a reduction in the minimum attainable urine osmolality. Reduction in the ability to excrete solute-free water puts these patients at risk for water intoxication when challenged with an excessive water load. In contrast to the somewhat preserved diluting mechanisms, the ability to concentrate the urine begins to fail relatively early in CKD, resulting in a marked decrease in fractional reabsorption of solute-free water. In CKD patients, severe dehydration is usually avoided because intact thirst mechanisms allow the patient to compensate for the urinary water losses. Accordingly, nocturia is the predominant symptom.

Urinary concentration requires maintenance of hypertonicity of the medullary interstitium and normal water transport across distal nephron segments in response to antidiuretic hormone (ADH). Maintenance of medullary interstitial hypertonicity in turn requires structural preservation of the countercurrent system. Part of the urinary concentrating defect in CKD may be attributed to the high solute load imposed on each nephron. However, diseases that profoundly disturb the medullary architecture (e.g., tubulointerstitial disease) may cause disproportionate impairment of concentrating ability (86,87). The presence of a concentration defect despite elevated ADH levels in plasma in CKD suggests a distal tubular defect. Limited ADH responsiveness (21,88,89) may be due to two factors. First, ADH-stimulated adenylate cyclase activity and water permeability in the distal nephron may be impaired (90). Also, increased tubular flow rates may limit the fraction of water that can be reabsorbed by the distal nephron in response to ADH (91). Several molecular mechanisms underlying the concentration defect in CKD have been suggested. Teitelbaum et al. (92) described the absence of ADH V2 receptor mRNA in the inner medulla of rats with CKD. Kwon et al. (93) studied protein expression of aquaporins (AQP) in rat remnant kidneys. Aquaporins are membrane proteins acting as water-selective channels. AQP1 is found mainly in proximal tubule and descending limb of the loop of Henle, whereas AQP2 and 3 localize in apical and basolateral membranes of collecting ducts, respectively. These authors reported marked reduction in all three channels with CKD. Furthermore, decreased AQP expression was resistant to treatment with ADH (93). Recently, downregulation of the Na cotransporter rBSCl has also been reported, with impaired responsiveness to water restriction (94).

Acid–Base Homeostasis

Metabolic acidosis is the predominant acid–base disorder in CKD, due to a reduced renal ability to excrete acid (90,91, 95–97). Early in the course, hydrogen balance is maintained by increased ammonium excretion per functioning nephron (91,97,98). However, later this adaptation proves insufficient, and acidosis is maintained primarily owing to reduced ammonia synthetic capacity. Ammonium excretion per total GFR rises to three to four times normal (99,100), but this increase is insufficient to counteract the profound reduction in functioning nephron number (91,98). Animal studies have shown that urinary ammonia excretion is impaired even before ammonia synthetic capacity is exhausted (100). Impaired excretion of ammonia was originally attributed to impairment of countercurrent mechanisms, which were thought to increase ammonium concentration in the medulla and facilitate "trapping" of ammonia by acidified luminal fluid in the collecting duct. However, this explanation may require modification in light of the discovery that ammonia enters tubule fluid by active secretion as well as by "trapping."

Renal acid excretion also requires reabsorption of filtered bicarbonate, and generation of a large hydrogen ion gradient in the distal nephron. Following uninephrectomy, stimulation of PT bicarbonate reabsorption occurs, as a result of a doubled transport rate. An increase in Na/H exchange contributes to this phenomenon (101). Some (43,102,103) but not all (100) micropuncture studies of rats with more radical nephron reduction have found slight increases in fractional reabsorption of bicarbonate, and studies of proximal tubule brush-border vesicles from rats with renal ablation have shown increases in V_{max} of the Na-H antiporter (44). However, clinically, Schwartz et al. (104,105) have presented evidence that a decrease in

bicarbonate reabsorptive ability develops, corresponding to more recent findings of reduced NHE-3 in proximal tubules of rat remnant kidneys (66). In severe CKD, the threshold for bicarbonate reabsorption may also be reduced (106), an effect enhanced by the actions of various stimuli including hyperkalemia, hyperparathyroidism, and ECF volume expansion (107,108).

In general, distal acidification is better maintained than proximal bicarbonate reabsorption in CKD, except in patients with distal renal tubular acidosis (109,110). However, CKD patients are unable to lower urinary pH as well as are normal subjects with experimental acidemia (91), and so a relative decrease in distal hydrogen ion pump capacity may also contribute to acidosis. Failure to attain normal minimal urinary pH prevents optimal titration of nonammonia buffers and thus reduces the excretion of the titratable acid. Restriction of dietary phosphate intake may further contribute to reduced excretion of titratable acid in uremic patients.

Phosphate Homeostasis

Abnormalities of phosphate and calcium metabolism and their contributions to renal osteodystrophy are discussed elsewhere in this book. The intrarenal adaptations that contribute to calcium and phosphate homeostasis in CKD are discussed here. A progressive increase in fractional phosphate excretion maintains phosphate balance early in the course of CKD (111,112). In more advanced disease, phosphate excretion is maintained by a further increase in the fractional excretion, along with an increase in serum phosphate levels. Studies by Slatopolsky et al. (112–114) suggested that increased parathyroid hormone (PTH) caused the increase in fractional phosphate excretion. In dogs subjected to renal ablation, fractional excretion of phosphorus increased, and the magnitude of the increased fractional excretion correlated with the magnitude of the increase in circulating PTH levels. Restricting phosphate intake prevented increases in both PTH levels and fractional phosphate excretion (114,115). These observations formed the basis for the Bricker's "trade-off hypothesis" (116), which postulated that some of the major stigmata of the uremic state may occur as indirect consequences of the adaptations in nephron function (4). According to this hypothesis, hyperparathyroidism represented the biologic price paid to maintain excretion of a constant dietary phosphate load when nephron number was reduced. With each decrement in GFR, a transient period of phosphate retention and decreased plasma calcium would stimulate PTH synthesis and secretion, and the increased PTH activity would act to partially restore phosphate balance by augmented phosphate excretion (117). This adverse "trade-off" could be avoided by reducing phosphate intake as renal function declined.

Although intellectually compelling, the details of this trade-off scenario have been questioned by more recent studies showing that the increased fractional phosphate excretion does not depend on an increase in PTH levels or tubule responsiveness to PTH (118,119). The data suggest that the increased fractional excretion is achieved via decreased phosphate reabsorption in the proximal nephron, as occurs in intact animals fed a high phosphate diet (120,121). Phosphate uptake per unit tubule mass is reduced in proximal nephron segments isolated from uremic rabbits, and sodium-phosphate cotransport activity is reduced in brush-border membrane vesicles from uremic dogs (122,123) and rats (66), but these reductions are not sufficient to fully account for the reduction in proximal phosphate reabsorption observed *in vivo*. Although reduced proximal reabsorption accounts for most of the increase in fractional phosphate excretion, there is also some evidence of altered distal phosphate transport (or reabsorption) (120,121).

Calcium Homeostasis

As CKD advances, production of active Vitamin D [$1,25(OH_2)D_3$] is impaired, leading to reduced intestinal calcium reabsorption and renal calcium excretion; the fractional calcium excretion then increases (124–126). In contrast to humans, animal studies have shown that urinary calcium excretion remains constant, whereas fractional excretion of calcium increases when the GFR is reduced to about one-third of normal (127,128). The mechanism responsible for the increased fractional calcium excretion in advanced CKD is unclear. Possible factors include acidosis, suppression of vitamin D production, increased distal nephron flow rates, and ECF volume expansion. Studies in rats with renal ablation suggest that the increase in remnant nephron calcium excretion associated with ECF expansion may be mediated by ANP (128).

STRUCTURAL ADAPTATIONS

The adaptive response to loss of renal mass includes early development of renal hypertrophy (129–131). The increased kidney size is due primarily to growth of the proximal convoluted tubules (132), resulting in disproportionate enlargement of cortex in comparison to medulla. An early hypothesis to explain compensatory hypertrophy was that of Addis (133), who reasoned that excretion of urea required energy, and that renal enlargement was a reflection of the need for added renal "work." Although Addis subsequently disproved his own hypothesis with regard to urea, the notion that the kidney grows in response to the need for some other form of work remains. Despite ingenious experimentation over the years, the question of whether hyperfiltration drives hypertrophy, or vice versa, remains incompletely answered. However, as discussed in the subsequent text, new understanding of the mechanisms and consequences of mechanotransduction are helping to elucidate this question.

Compensatory Renal Hypertrophy

"Dry" (desiccated) kidney weight increases in proportion to "wet" (fresh) kidney weight following nephrectomy, implying an increase in the mass of protein (134). The ratio of kidney protein to wet weight remains constant during renal hypertrophy, although the renal protein synthetic rate increases by 3 hours after uninephrectomy in the mouse (135). The quantity of RNA is notably increased within 12 hours after uninephrectomy, and RNA synthesis increases within 1 to 4 hours. The early increase in RNA synthesis reflects largely increased production of ribosomal RNA, due at least in part to an increase in transcription, with some contribution from decreased RNA degradation. The peak in the RNA content of individual cells is reached within 2 days after uninephrectomy (136–140).

DNA content begins to increase by 9 to 18 hours after nephrectomy, whereas a notable increase in mitotic figures, largely among proximal tubule cells, is apparent only after 1 to 2 days (135,141,142). The prevalence of tubule mitotic figures declines to normal levels over 1 to 2 weeks following uninephrectomy. The lag of DNA synthesis behind RNA synthesis may reflect stimulation of mitosis by increases in cell size (135). According to this scenario, the renal hypertrophic stimulus causes cell enlargement, and cells reaching the largest size are stimulated to divide. The ratio of increases in protein and DNA content has been used to estimate the relative contributions of cellular hypertrophy and hyperplasia to compensatory renal hypertrophy. In uninephrectomized adult animals, an increase in cell number of 10% to 25% accounts for up to half of the net increase in kidney size (135,143,144). In very young

animals and adult animals with more extensive renal ablation, remnant nephron enlargement is more pronounced, and cellular hyperplasia plays a proportionally greater role (145–148).

Structural Changes in Individual Renal Compartments

Glomerular Hypertrophy

Glomeruli also undergo progressive enlargement. Uninephrectomy in the rat increases glomerular tuft volume by 48%, 68%, and 75% at 3, 5, and 9 weeks, respectively (149,150). In more exuberant hypertrophic states, as occur with extensive ablation or mineralocorticoid-salt hypertension, the degree of glomerular enlargement is even greater (149, 151–157). Increased glomerular size does not necessarily parallel increased whole kidney size. For example, in young rats, one study found that remnant kidney growth and glomerular growth proceeded proportionally for 12 weeks; thereafter, kidney growth ceased, whereas glomerular size continued to increase (156). Serial structure-function studies in the rat have shown that glomerular volume and SNGFR increase in parallel after uninephrectomy (151,152). The contribution of constituent parts of the glomerulus to its expansion remain controversial and incompletely characterized. Morphometric studies demonstrate an increase in the total volume occupied by cellular constituents in the remnant glomeruli of nephrectomized rats (150,151,154,158–160). Overall, these studies indicate that the fractions of the glomerulus occupied by different structural components remain constant as the glomerulus enlarges, at least in the early phases of adaptation. However, long-term adaptation may follow a different pattern. Schwartz and Bidani (159) found no change in the relative volume of the mesangium and its components at 6 weeks after ablation, but at 26 weeks (about one-quarter of the life span) that the mesangial volume fraction, as well as the percentage of the mesangium occupied by cells and matrix, increased (154).

Several studies have examined to what extent different glomerular cell types increase in number and volume following renal ablation. An early study found a prominent increase in the number of glomerular endothelial, mesangial, and epithelial cells following uninephrectomy in very young rats (158). More recent studies have confirmed increased mesangial cellularity (161). In rats with 5/6 nephrectomy, a proliferative response involving glomerular endothelial cells was apparent 2 (162) and 12 (161) weeks after renal ablation. However, in the former study this response was followed by a decrease in endothelial proliferation below the level observed in sham operated rats. In addition, endothelial cell volume was also noted to be also increased in remnant kidneys (161). An endothelial proliferative response has also been noted in mice after uninephrectomy (163).

Increases in glomerular capillary length (153–156) and radius (64,131,150,164) occur, such that capillary surface area increases (153,154,160). Afferent and efferent arteriolar diameters also increase (160). Morphometric measurements have been used to estimate the filtering capacity of the enlarged remnant glomeruli. The glomerular capillary ultrafiltration coefficient (K_f) is the product of the surface area available for filtration (S) and the hydraulic permeability of the glomerular capillary wall (k). It is not clear which anatomic boundary constitutes the surface corresponding to S. As estimated by measuring the glomerular capillary area in direct apposition to epithelial foot processes, S increases following nephrectomy, albeit to a slightly lesser degree than total glomerular volume (150,151). Despite this potential increase in the filtration surface area, most functional studies have not found an increase in K_f of remnant glomeruli after extensive renal ablation (18,19). After uninephrectomy alone, some (165), but not all (17), studies have noted modest increases in K_f.

It is conceivable that k is reduced, because a decrease in S cannot be involved to explain the fall in K_f. In theory, an increase in average foot process width would cause a decrease in the length of filtration slit overlying each unit area of peripheral capillary surface and thereby could decrease k in remnant glomeruli. In fact, morphometric studies have revealed an increase in the average width of epithelial cell foot processes in rats subjected to extensive renal ablation (152). Alternatively, it may be that the filtering surface estimated by morphologic techniques in remnant glomeruli does not represent effective area available for filtration *in vivo*. Theoretical studies suggest that much of the glomerular capillary network is relatively underperfused in rats with extensive renal ablation (166). It is notable that no increase in S was found following uninephrectomy in rats when infusion of glomerular basement membrane antibody was used to estimate capillary surface area *in vivo* (167). Alternatively, it is possible that the decrease in K_f in remnant glomeruli is more functional than structural, as ACEI therapy routinely raises K_f to supranormal levels (19).

Compensatory glomerular hypertrophy has been proposed as a trigger of focal and segmental glomerular sclerosis (FSGS), as well. Studies in a number of models have noted a strong association between increased glomerular size and development of proteinuria and FSGS (64,149,151,168,169). Conversely, a protective effect of low glomerular volume could account for the finding that a strain of rats with unusually small glomeruli exhibits less glomerular injury following uninephrectomy than a strain with larger glomeruli (170,171). Miller et al. (172) compared the rate of development of proteinuria and FSGS in normal and uninephrectomized rats treated with a pressor dose of Ang II, which is known to increase P_{GC}. Despite the fact that the Ang II dose was halved in uninephrectomized rats, these rats demonstrated markedly faster development of renal injury. There were no differences in P_{GC} between those groups; however, glomerular volume was increased in uninephrectomized animals.

The combination of increases in both glomerular capillary intraluminal pressure and capillary radius is postulated to exert increased tension on the glomerular capillary wall (following Laplace's law), thus contributing to disruption of capillary wall integrity, activation of growth factors and FSGS. The important question of whether increases in glomerular pressure and/or flow stimulate glomerular growth requires further investigation. Available studies suggest that normalizing P_{GC} sometimes limits, but does not prevent, glomerular hypertrophy following renal ablation. Importantly, studies comparing two models of renal ablation showed differences in systemic and glomerular blood pressure, expression of prosclerotic factors, and the rate of development of renal injury, but similar degrees of glomerular hypertrophy (173). These studies indicate that the protective effect of reducing P_{GC} cannot be solely attributed to reduction in glomerular volume.

Podocyte Biology After Nephron Loss. Podocytes represent a crucial component of the filtration barrier and a major determinant of proteinuria. Therefore, particular attention has been given to changes in these cells in response to nephron loss. The potential additive deleterious effects of glomerular capillary hypertension and glomerular enlargement have also prompted speculation that injury may be mediated by detrimental effects on the glomerular visceral epithelial cells (151,156,168,174) and their lower ability to adapt. Adult podocytes possess diminished ability to divide in response to immune, metabolic, and hemodynamic (nephron loss) stimuli (175,176). Several groups have implicated the fact that podocytes undergo exaggerated stress as glomeruli enlarge, resulting in their dysfunction and

possibly destruction, in the pathogenesis of glomerulosclerosis (151,155,174).

Though unable to replicate, there is evidence that podocytes are able to undergo hypertrophy in response to loss of renal mass and glomerular enlargement (155,156). As recently reviewed by Kriz and LeHir (176), glomerular enlargement without podocyte replication leads to structural changes including foot process effacement, cell-body attenuation, pseudocyst formation, accumulation of absorption droplets, and finally detachment. The loss of podocytes then triggers the onset of FSGS, as is further discussed in the subsequent text.

Mechanisms of Renal Hypertrophy

General Principles: Hypertrophy vs. Hyperplasia

The term compensatory hypertrophy has been used to describe the aggregate changes in nephron structure and function, including both cellular hypertrophy and hyperplasia, which follow loss of renal mass. Extensive work in this area has been the subject of several reviews (177–181) and is briefly summarized here. Renal cells react to physical, biochemical, and humoral stimuli imposed by reduction of nephron number with coordinate activation of a variety of signaling pathways and changes in expression of their molecular components, which result in induction of genes encoding a large spectrum of proteins that are involved in structural adaptive and maladaptive responses.

Hyperplasia is a result of an increase in cell number associated with DNA replication and cell division, whereas hypertrophy is defined as cell enlargement due to increases in protein and RNA content without DNA replication (131). Both processes are involved in renal compensatory growth. During hyperplasia, cells progress through the whole cell cycle; hypertrophy occurs when the cells are engaged in the cycle but cannot progress to later stages. Interaction of the cell with a variety of growth factors that modulate activity of positive or negative regulators of the cell cycle then determines whether the cell will engage in hyperplasia or hypertrophy.

Role of Physical Forces

Cortes et al. (182,183) demonstrated that glomeruli can enlarge as perfusion pressure rises. In the remnant kidney, transmission of pressure fluctuations into the glomerular capillary tuft is not prevented by myogenic autoregulatory control. This results in glomerular distension and enlargement. Glomerular volume is determined by capillary wall tension, size of the glomerulus, and glomerular stiffness. Glomerular compliance is increased in remnant kidneys. This process is independent of humoral or biochemical factors, at least initially. Indeed, an increase in glomerular capillary radius is the earliest morphologic finding after uninephrectomy (155,156).

A number of studies have addressed the hypothesis that increased glomerular capillary pressures and/or plasma flow rates alter the growth and activity of glomerular component cells, inducing the elaboration or expression of cytokines and other mediators, which then stimulate mesangial matrix production and promote structural injury. Hemodynamic physical forces, such as shear stress or changes in blood flow, are well recognized to influence activity of endothelial and vascular smooth muscle cells (VSMC). Improved understanding of the cellular responses to mechanical stresses has recently helped to elucidate the close link between initial changes in renal hemodynamics and tubular flow on the one hand, and structural adaptation and damage in the remnant kidney on the other. Mechanical stress imposed on renal cells by enhanced capillary and tubular flows and pressures triggers a variety of physiological and pathophysiological responses. A key component of these responses, representing the actual link between mechanical stresses and growth in glomeruli and tubules, is stress-induced signaling or mechanotransduction. Intrarenal mechanotransduction has been demonstrated using *in vitro* systems capable of simulating stretch or pulsatile stresses imposed on mesangial, endothelial, tubular cells, or podocytes. Basic mechanisms are delineated in the subsequent text, followed by a review of findings relevant to nephron adaptation in CKD.

Principles of Mechanotransduction

Cells are subjected to physical forces originating either from tension created by cells themselves, or from the environment. Physical stimuli must be sensed by cells and transmitted through intracellular signal transduction pathways to the nucleus, resulting in physiological responses or pathological conditions. Complex systems work together to transduce these physical forces into molecular events, as has been recently reviewed (184–190). Via G-protein coupled receptors, G-proteins and receptor tyrosine kinases, mechanical stress itself may directly perturb the cell surface or alter receptor conformation, thereby initiating signaling pathways that lead to production of growth factors that may, in turn, evoke and amplify the response. Integrins and the cytoskeleton play a role, in that cells are in contact with basement membranes and extracellular matrix (ECM). The ECM protein organization is sensed by integrins, transmembrane receptors that mediate cell attachment to ECM proteins. Integrins act as mediators of cell adhesion, but can also transmit extracellular stimuli into intracellular signalling events. Mechanical stretch is also associated with activation of a small G-protein Rho and down-stream Rho kinase. Inhibition of Rho activation results in attenuation of stretch-induced MAPK activation in VSMC (194). With respect to ion channels, the altered stretch-activated channels in arterial VSMCs may contribute to the enhanced myogenic responses as well as the generation of hypertrophy and remodeling of arterial tissue in hypertension. Also, studies in endothelial cells have shown that new caveolae are formed in response to shear stress, and act as mechanosensors resulting in ras-raf-ERK and Akt kinase activation (191).

Signaling Pathways Involved in Renal Responses to Nephron Loss with a Special Emphasis on Mechanotransduction

In vascular and renal cells, mechanotransduction involves activation of the mitogen-activated protein kinase (MAPK) family (192). These serine/threonine kinases transduce signals in response to multiple agonists acting through growth factor receptors with intrinsic tyrosine kinases, G protein-coupled receptors, via nonreceptor tyrosine kinases, and cellular stress. ERKs have been implicated in mitogenic and other cellular responses, whereas JNK and p38 activation is associated with inflammatory cytokine action, cellular stress, and apoptosis. Activated MAPKs can translocate to the nucleus and lead to the phosphorylation and activation of transcriptional factors or phosphorylate their cytosolic substrates (193).

Protein kinase C (PKC) is another major family of serine/threonine kinases that are active in mechanotransduction. PKC are typically activated by lipid-derived second messengers, such as diacylglycerol or phospholipids. PKC has a number of downstream targets involved in hypertrophic signaling and production of vasoactive factors (206). PKC has also been shown to contribute to shear stress-induced MAPK activation.

Phosphatidylinositol-3 kinase (PI3K), another multifactional kinase activated by upstream G proteins can be

stimulated by shear stress (207) and contribute to shear stress-induced JNK activation in endothelial cells. Another line of evidence suggests that PI-3K is involved in endothelial mechanotransduction sensed by intergrins (208). Akt kinase (protein kinase B) is a major down-stream target of PI-3K. Akt is a potent survival kinase, transducing signals leading to inhibition of apoptosis, promoting cell proliferation, and cell cycle regulation. Akt kinase may also transduce potentially protective signals, such as shear-stress-induced generation of nitric oxide (209).

Rho-kinase, the effector of Rho, a family of small G-proteins that act as possible mechanosensors, has been implicated in mediating cell contraction, adhesion, migration, cytoskeleton organization, and proliferation, and has been studied in vascular injury (210,211). This is in general accomplished by Rho-induced inhibition of vasoprotective mechanisms, such as NO production, in parallel with facilitiation of prosclerotic mechanisms.

Mechanotransduction and Renal Hypertrophy

In support of the role of mechanotransduction in adaptive and maladaptive structural responses after nephron loss, earlier studies postulated that expansion of the glomerular capillaries and stretching of the mesangium in response to hypertension might be a force that translates high P_{GC} into increased mesangial matrix formation. Evidence for this mechanism comes from studies in microperfused rat glomeruli, in which increased P_{GC} was associated with increased glomerular volume; and in cultured mesangial cells, where cyclic stretching resulted in enhanced synthesis of protein, total collagen, collagen IV, collagen I, laminin, fibronectin, and TGF-β (200). Additionally, growing mesangial cells under pulsatile conditions has been reported to stimulate Ang II receptor and angiotensinogen mRNA levels (201) and protein kinase C, calcium influx, and protooncogene expression (202) and, as well as altered extracellular matrix protein processing enzymes (203,204). *In vivo,* Shankland et al. (205) observed that increases in P_{GC} induced by uninephrectomy in SHR were associated with glomerular expression of TGF-β and PDGF. Normalization of P_{GC} with an ACEI decreased glomerular TGF-β and PDGF. Similarly, Griffin et al. (173) compared P_{GC} in relation to glomerular TGF-β and PDGF expression between the excision and infarction models of renal ablation. The rats subjected to renal infarction demonstrated higher systemic and glomerular pressures, and markedly higher expression of TGF-β and PDGF mRNA in glomeruli.

The consequence of MAPK activation by mechanical stress can be illustrated by a large spectrum of genes encoding proteins involved in structural adaptation and maladaptation. These include genes coding for growth factors and cytokines, ECM proteins, cell cycle regulators, vasoactive factors and signaling molecules. More recent studies have linked these observations to stress induced signaling pathways. Scholey, Ingram and co-workers (192,206) demonstrated stretch-induced activation of p42/44, JNK and p38 in mesangial cells. These changes resulted in activation of the AP-1 family of transcription factors, and were in part Ca^{2+} and PKC-dependent. Stretch induced activation of p38 MAPK in mesangial cells leads to increased production of TGF-β (207). Of note, Ohashi et al. (208) found that inhibition of enhanced renal p38 MAPK activity in rats with renal ablation leads to acceleration of renal injury, with more proteinuria, FSGS, and interstitial fibrosis, but less tubular cell apoptosis. p38 inhibition was associated with ERK 1/2 activation, a possible factor explaining the worsening renal lesions. Thus, activation of p38 in remnant kidneys might play a protective role associated with inhibitory actions on ERKs signaling.

Several stretch-induced mechanisms have been recently reported in podocytes. Endlich et al. (209) found that mechanical stress induced a unique reorganization of the actin cytoskeleton in podocytes. The F-actin reorganization in response to mechanical stress depended on Ca^{2+} influx and Rho kinase. A dynamic cytoskeleton allows podocytes to withstand significant mechanical stress with elevation of P_{GC}. Martineau et al. (210) have demonstrated that stretch-induced p38 in podocytes is associated with enhanced prostanoid production via COX-2, increased expression of the EP4 prostanoid receptor and consequent alterations in podocyte cytoskeletal dynamics that could compromise filtration barrier function under conditions of increased P_{GC}. Importantly, in another study, long-term inhibition of Rho-kinase attenuated renal injury in nephrectomized spontaneously hypertensive rats (211).

Protooncogenes and Transcription

Expression of growth promoting systems is accompanied by upregulation of protooncogenes and transcription factors. AP-1, a transcription factor formed by heterodimerization of fos and jun proteins, is increased in remnant kidneys, and downregulated by nephroprotective treatment (212). Another transcription factor, nuclear factor-*k*B (NF-*k*B), is also upregulated in rats with renal ablation, and reduced with effective treatment (213). Mirza et al. (214) reported an important function of NF-*kB* in regulating of the enzyme transglutaminase, which is an activator of latent TGF-β. Transglutaminase also cross-links matrix proteins, possibly contributing to interstitial fibrosis in the remnant kidney model (215). The activation of NF-*k*B may have an important role in mediating cortical interstitial monocyte infiltration and tubular injury in nonimmune proteinuric tubulointerstitial inflammation (216,217).

The activation of early response genes or protooncogenes, whose protein products regulate transcriptional control of large numbers of other genes, is an early step in cell proliferation and differentiation evoked by stress-signaling, mitogens and growth factors. Thus far, studies of expression of early response genes in the remnant kidney following uninephrectomy have been inconsistent (137,138,218–222). For example, renal activity of protooncogenes, such as c-fos, c-myc, c-egr1, c-jun, and c-H-ras, following uninephrectomy has been found to increase in some studies, but not in others (139,220,221,223). Importantly, however, these studies suggest that protooncogene activation after uninephrectomy is modest or absent, consistent with the low risk of deterioration of renal function in this modest degree of nephron loss.

Cell Cycle Regulation as an Important Determinant of Structural Response to Nephron Loss

Genes induced by nephron loss include those coding for cell cycle regulatory proteins (224). Positive regulation (stimulation of transition from quiescent cell phase to ultimate cell division by mitosis) is carried out by cyclins and their partner molecules, cyclin-dependent kinases (CDK). Although CDK are constitutively expressed, cyclins are transcriptionally regulated and their levels are increased by specific mitogens such as growth factors. Negative regulation of the cell cycle is accomplished by CDK inhibitors, which bind to cyclin-CDK complexes and inhibit their activity. Two families of CDK inhibitors have been identified. The CIP/KIP family consists of inhibitors p21, p27, and p57; the INK4 family includes p15, p16, p18, p19, and p20. In addition to the preceding processes, the cells also engage in apoptosis. The total number of cells in a particular

organ reflects the balance between proliferation and apoptosis. Apoptotic cells exit from the cell cycle. Initiation of apoptosis also is regulated by cyclin-CDK complexes, and the progression of the cell into mitosis or apoptosis seems to be determined by the level of CDK inhibitor p27 (224).

Liu and Preisig (225) have provided detailed insight into renal tubular cell cycle regulation after uninephrectomy and resulting compensatory hypertrophy. The authors studied both rats and mice and confirmed that compensatory PT growth following uninephrectomy is hypertrophic, and associated with a cell cycle-dependent mechanism. The development of hypertrophy required that the cells enter G1 phase and initiate events of this phase, such as increased protein synthesis, increased cdk4/cyclin D kinase activity, and maintaining retinoblastoma protein in the hypophosphorylated state. However, the cells did not progress to S phase, where DNA synthesis occurs. Unlike the cdk4/cyclin D activation, there was insufficient or absent activation of cdk2/cyclin E preventing progression of cell cycle into S phase, resulting in hypertrophy instead of hyperplasia. This pattern of differential activation of cdk4/cyclin D and cdk2/cyclin E may reflect previously mentioned differences in expression and activities of p21 and p27, regulators of cdk2/cyclin E, but not p16, a regulator of cdk4/cyclin D kinase.

There is compelling evidence for the role of cell cycle regulating proteins in development of renal injury following nephron loss. Compensatory hypertrophy after renal ablation is associated with cyclin E and CDK2 expression coinciding with the early proliferative response (226). Petermann et al. (227) recently reported that mechanical stretch induces podocyte hypertrophy. Stretch reduced cell cycle progression in wild-type and p27 knockout mice, and induced hypertrophy, wheras hypertrophy was not induced in single p21 and double p21/p27 knockout podocytes. Stretch-induced hypertrophy was inhibited by blocking ERK 1/2 or Akt, but not p38.

It appears that CDK inhibitors also play a key role in subsequent development of renal injury. These molecules are regulated by factors that have been implicated in ablation nephropathy, including Ang II and TGF-β (228,229). Increased expression of CKD inhibitors has been found in other models, such as diabetic nephropathy (230) and ureteral obstruction (231). Megyesi et al. (232) found that p21 knockout mice subjected to renal ablation demonstrated striking resistance to development of FSGS, as compared to wild-type animals. In the absence of the p21 gene, the growth response in the remnant kidney was relatively more hyperplastic than hypertrophic. The authors alluded to a proposal, made by Goss (233) 40 years ago, suggesting that when an organ accommodates increases in work by hypertrophy rather than hyperplasia, it is at a serious physiologic disadvantage and is more likely to undergo regression of structure and function.

Tubular Structural Adaptations

Quantitatively, the proximal convoluted tubule enlarges by approximately 15% in luminal and outside diameter and by 35% in length after uninephrectomy in the rat (132). The increased PT size is proportional to the extent of nephron number reduction (234). Tabei et al. (235) found that an increase in tubule size follows the increase in fluid reabsorption by segments of isolated proximal straight tubule. Later in the course, the increased reabsorptive rate approximates the increase in PT size and protein content (40,41,236). Distal convoluted tubule enlarges by approximately 10% in luminal and outside diameter by 17% in length in the rat (132). In the distal convoluted tubules and collecting ducts, cross-sectional area of both lumen and epithelium is increased, but by lesser magnitude than occurs in the proximal tubule (132,237). The cross-sectional area of the medullary collecting duct is increased following renal ablation in the rabbit but not in the rat (238,239). Tubular cells do not enlarge symmetrically. Enlargement of basolateral portions of cells is more prominent as compared to luminal surface (240).

Tubular changes are accompanied with a transient increase in proliferation of peritubular endothelial cells after uninephrectomy in mice (163) and after more radical nephron reduction in rats (162). In the latter situation, this transient increase is followed by a decrease in peritubular capillary proliferation with a possible impact on progression of renal tubular and interstitial injury, as further discussed in the subsequent text. Similar to the role of hemodynamic responses in the development of glomerular hypertrophy, increased solute burden and luminal flow have been shown to contribute to tubular hypertrophy after renal ablation.

Tubular mechanotransduction. Compensatory hyperfiltration exposes tubular cells to increased flow and pressures of tubular fluid, and studies have linked tubular flow and growth. Sigmon et al. (241) demonstrated that prevention of NO-dependent hyperperfusion in uninephrectomized rats is also associated with lack of compensatory renal hypertrophy, further suggesting the link between the renal growth and hemodynamics. In another study, PT expression of angiotensinogen after uninephrectomy in mice correlated with hyperfiltration, suggesting a role for tubular flow in local regulation of RAS activity (242). Signalling events associated with stress-induced tubular hypertrophy are starting to be understood. Alexander et al. (243) found that cyclic stretch applied on rabbit PT cells triggered intensity-dependent ERK activation. Using specific inhibitors, phospholipase A2 was identified as a part of the up-stream mechanosensing process. A requirement for extracellular Ca^{2+} and stretch-activated Ca^{2+} channels was also documented. Cyclic stretch also caused rapid phosphorylation of the EGF receptor kinase and c-Src. Furthermore, arachidonic acid itself induced time- and dose-dependent phosphorylation of c-Src.

Mechanisms and Mediators of Functional and Structural Adaptations

Altered levels and/or activity of several endogenous mediators have been proposed to contribute to the adaptive changes that characterize nephron loss. It is important to note that many of the factors involved in hemodynamic adaptations are, in parallel, important determinants of renal growth responses; accordingly, the effects of each mediator system are discussed with respect to both pathways.

Renin–Angiotensin–Aldosterone System

Angiotensin II (Ang II), the major effector peptide of the renin–angiotensin–aldosterone system (RAAS), causes vasoconstriction of afferent and efferent arterioles, mesangial cell contraction, and a rise in P_{GC} (244,245). The ability of ACEI to reduce R_E and P_{GC} suggests that Ang II helps to sustain glomerular capillary hypertension in experimental CKD (19). Ang II may further elevate P_{GC} by promoting sodium retention and causing systemic hypertension, separate from a direct microcirculatory action (246). Several studies have sought to localize the source of the intrarenal RAAS components that contribute to glomerular hemodynamic adaptations. After uninephrectomy, renin mRNA increases in proximal tubules and glomeruli of the remnant kidney (247). In rats with ablation by partial renal infarction, renin activity is concentrated in areas adjacent to the infarcted tissue (248,249). Renin production by hypoperfused nephrons adjacent to the infarcted tissue could

explain why blood pressure is not increased when nephron number is halved by uninephrectomy (151) and why blood pressure tends to be higher when ablation is accomplished by infarction as compared with surgical removal of renal tissue (250,251). Generalized renin overexpression in tubular cells (252) and expression of interstitial Ang II (253) have been noted in rat remnant kidneys, further supporting the notion of the indirect effect of Ang II in mediating increased P_{GC}. In contrast, Ang II AT_1 receptor mRNA is decreased in remnant kidneys, likely as a result of increased tissue levels of Ang II (254), while there is a counterregulatory increase in expression of the AT_2 receptor (255).

Another effector molecule of the RAAS, aldosterone, has been implicated in the pathogenesis of hemodynamic and structural changes after renal ablation. Plasma aldosterone levels are high in this model (256), corresponding to levels in CKD patients (53). Adrenalectomy ameliorates renal injury in 5/6 nephrectomized rats (257). The nephroprotective effects of ACEI and ARBs, commonly associated with suppression of aldosterone secretion, are offset by concomitant infusion of the hormone (256). Arima et al (258) recently reported direct, nongenomic actions of aldosterone in the glomerular microcirculation, with dose-dependent vasoconstriction in afferent and efferent arterioles, but predominantly R_E, which would raise P_{GC}. Wistar-Furth rats, which are resistant to mineralocorticoid actions, are strikingly resistant to renal injury after renal ablation (259). The fact that P_{GC} is increased in mineralocorticoid salt models of hypertension (149) provides further evidence for a contribution of aldosterone to the progression of CKD.

Ang II can be viewed as a central molecule in several of the processes involved in CKD. In addition to its hemodynamic and tubular effects, Ang II exerts a number of actions that influence renal morphology. These effects have been a subject of excellent reviews (260–262), and are briefly reviewed here. Among the many growth effect of Ang II are stimulation of proto-oncogenes, stimulation of a plethora of growth factors (e.g., TGF-β, PDGF), activation of NF-kB, stimulation of MCP-1, and increasing ECM accumulation by increased synthesis (fibronectin, collagen, laminin, osteopontin) or diminished degradation (metalloproteinases, plasminogen activator inhibitor-1) of pro-fibrosis cytokines (260–262). Similar to other growth factors and systems, RAS components can be activated by shear stress and altered physical forces. Several years ago, it was reported that mechanical stretching of mesangial cells induces upregulation of Ang II receptor and angiotensinogen mRNA levels (201). More recently, Durvasula et al. (263) demonstrated mechanical strain-induced increased Ang II production, and up-regulation of the AT_1 angiotensin receptors and TGF-β mRNA expression in immortalized podocytes, associated with an increase in podocyte apoptosis. In accordance with *in vitro* observations, AT_1 receptor staining was increased in podocytes in rats with renal ablation (263).

Nitric Oxide, Oxidative Stress and Endothelial Dysfunction

In view of its renal hemodynamic actions (264), as well as evidence of shear-stress induced activation of nitric oxide (NO) generation (265), NO would be a good candidate for mediating hyperfiltration associated with reduced nephron mass. There are, however, striking differences between studies exploring renal NO activity in uninephrectomized models, and models with more radical nephron reduction. Uninephrectomized rats demonstrate greater renal vasoconstrictor responses to NO synthase (NOS) inhibition, and increased inducible (NOS) protein expression, as compared with sham-operated controls (266). More recently, Sigmon et al. (241) demonstrated that prevention of the NO-dependent increase in RPF in uninephrectomized rats is also associated with the lack of compensatory renal hypertrophy, further suggesting a link between the renal growth and hemodynamics.

In contrast, in rats with 5/6 nephrectomy, several groups found decreased renal expression and enzymatic activity of all three NOS isoforms (267–269). Furthermore, treatment with L-arginine, a NO precursor, or the NO donor molsidomine, ameliorated increases in P_{GC} or renal injury in the same model (270,271). A blunted response to nonspecific NOS inhibition has also been shown in 5/6 nephrectomized rats (272). Because chronic inhibition of NO production with L-arginine analogs causes severe glomerular injury associated with an increase in P_{GC} (273,274), suppression of NO synthesis in remnant glomeruli and vasculature could contribute to development of FSGS. As discussed in the subsequent text in more detail, the NO-deficient state of reduced renal mass may be associated with enhanced oxidative stress in remnant nephrons (275). Furthermore, the development of oxidative stress in conjunction with a shift from enhanced to reduced NO bioavailability may be one of the demarcation points characterizing transition from adaptive to maladaptive responses after renal mass reduction.

The delicate balance between production and activity of vasoprotective endothelium-derived NO affects endothelial function and ultimately vascular and renal pathophysiology. These processes seem to play an important role also in adaptive responses to nephron loss, and possibly in transition from adaptive to maladaptive structural responses. In a related area, the role of oxidative stress in remnant nephron pathophysiology has been well recognized (276). Reactive oxygen species (ROS) generated during this process not only alter integrity of a large spectrum of proteins and lipids, but also act as signaling molecules, for example in MAPK pathways.

Recent studies have elucidated several molecular mechanisms underlying oxidative stress and enhanced ROS production in the remnant kidney. Expression and, in particular, activities of antioxidant enzymes, such as catalase, copper/zinc superoxide dismutase, and glutathione peroxidase, increase during the course of CKD in the remnant kidney (277). Corresponding to clinical evidence suggesting endothelial dysfunction in CKD patients (278), Hasdan et al. (279) demonstrated impaired endothelium-dependent vasodilation of resistance arteries in partially nephrectomized rats. Treatment with the membrane-permeable superoxide dismutase (SOD)-mimetic tempol prevented the development of hypertension and restored vascular responsiveness to acetylcholine *in vitro*. Supporting the proposed pathophysiological roles of oxidative stress in the remnant kidney, further experimental studies suggested that antioxidant therapy with alpha-tocopherol attenuated the development of FSGS and interstitial fibrosis (280). However, it should be noted that there is as yet no persuasive clinical evidence that antioxidant treatment would significantly alter the clinical progression of CKD.

Enhanced ROS production is closely linked to reduced NO bioavailability in a given vascular bed or tissue. As demonstrated by Vaziri et al. (281) in rats with renal ablation, renal and vascular oxidative stress, which promote enhanced production of reactive carbonyl compounds and lipoperoxides and the accumulation of advanced glycation and lipoxidation end products, also lead to NO quenching, producing cytotoxic reactive nitrogen species capable of nitrating proteins and damaging other molecules. Renal and vascular expression of nitrotyrosine, a marker of tissue NO and ROS interaction, is markedly increased in rats with renal ablation. Furthermore, these abnormalities could be ameliorated by antioxidant treatment. In this context, the evidence suggests a protective role of NO in the development of injury in the remnant kidney. Chronic inhibition of NO with L-arginine analogues accelerates progression

of FSGS and interstitial fibrosis in the remnant kidney. This phenomenon is associated with more rapid loss of peritubular capillaries and altered tubular vascular endothelial growth factor (VEGF) expression (282). Furthermore, treatment with L-arginine, a NO precursor, or the NO donor molsidomine, ameliorates increases in P_{GC} and renal injury in the same model (270,271,283). As suggested by Katoh et al. (270), protective effects of L-arginine in the remnant in the kidney were observed even in the absence of changes in P_{GC}. Similar nephroprotective effects in rats with renal ablation rats were noted after long-term administration of tetrahydrobiopterin, a key co-factor for appropriate eNOS enzymatic activity (284).

In addition to other protective mechanisms mediated by endothelial NO, such as antithrombotic actions or antagonizing ROS, recent studies have identified new mechanisms of NO-mediated nephroprotection relevant to renal events after nephron loss. NO has been implicated as an important modulator of stress-induced signaling in renal cells. *In vitro* studies by Ingram and co-workers (285,286) have demonstrated that stretch-induced activation of p42/44, JNK, and p38 MAPK, their nuclear translocation, and stimulation of down-stream transcription factors, such as AP-1 and NF*k*B, in mesangial cells can be inhibited by the NO-cGMP pathway. Further studies suggested that these inhibitory effects are associated with NO-induced destabilization of the actin cytoskeleton (287), and mediated via inhibition of RhoA-Rho kinase signaling (288). These mechanisms may be responsible, at least in part, for the protective effect of NO in animal models with glomerular hypertension. In addition to eNOS-derived NO, studies have also suggested a protective role of nNOS-derived NO in the remnant kidney (289).

Prostaglandins

Clinical observations of increased susceptibility to acute renal failure with nonsteroidal antiinflammatory drugs (NSAIDs) suggest a strong dependence of hemodynamics on prostaglandins (PG) in CKD. Synthesis of PGE2, PGI2, and thromboxane A2 are increased in glomeruli isolated from subtotally nephrectomized rats, and per nephron excretion of PGE2 and thromboxane A2 is increased (290,291). Acute PG synthesis inhibition reduces Q_A and SNGFR but not P_{GC} in remnant kidney rats, whereas chronic thromboxane synthesis inhibition decreases vascular resistance and increases Q_A and SNGFR (292,293). PG synthesis inhibition reduces RPF and GFR in rabbits with renal ablation (294) and CKD patients (295). Zatz et al. (253,296) have reported mild reduction of P_{GC} and significant nephroprotection in remnant kidney rats after chronic treatment with nitroflurbiprofen, a compound combining nonselective cyclooxygenase (COX) inhibition and a nitric oxide-donating moiety, preventing critical depression of renal function. Additional studies confirm a nephroprotective effect of COX-2 inhibition alone (297, 298).

Cyclooxygenase is a key enzyme in the synthesis of PG and thromboxanes from arachidonic acid. Recent evidence suggests that COX-2–derived PG play a role in physiologic regulation in the normal kidney, being involved in modulation of afferent arteriolar vasoconstriction and myogenic afferent responses to increases in renal perfusion pressure (299), and stimulation of renin release (298). Expression of COX-2 is increased in macula densa of remnant kidneys (253,300), and increased activity of COX-2 is involved in the altered renal function and progression of CKD (297,298).

In addition to hemodynamic mechanisms, the nephroprotective effect of specific COX-2 inhibition may be mediated via suppression of TGF-β (297). Because thromboxane A2 is a product of the COX pathway, the authors hypothesized that thromboxanes contribute to the development of injury by stimulating TGF-β. However, vasoactive hormones, such as prostaglandin E2 (PGE2), may challenge the integrity of the actin cytoskeleton, alter podocyte morphology, and compromise glomerular permeability. PGE2 synthesis correlates with the onset of proteinuria and increased P_{GC} following reduced nephron mass (300).

Endothelin

Endothelin (ET) infusion increases P_{GC} in the normal kidney (301), and renal ET-1 activity rises after the first week following renal ablation (302). Long-term ET receptor blockade is associated with preservation of renal function and structure in remnant kidney rats (303). In the absence of micropuncture data, it is difficult to determine whether the nephroprotective effects of ET receptor blockers are due to inhibition of hemodynamic or growth actions, or both. However, in other models of progressive renal injury and glomerulosclerosis, ET receptor blockade reduces P_{GC} (304).

Importantly, ET-1 is a potent mitogen and fibrogenic molecule, which can contribute to interstitial fibrosis by causing ischemia due to its vasoconstrictor effects. However, ET-1 also has direct *in vitro* effects on matrix production (305), tubular cell proliferation (306), and upregulation of TGF-β (307). Further support for a role of ET-1 in renal structural injury was provided by studies in ET-1 transgenic mice, which develop renal interstitial fibrosis and cysts, and renal failure associated with normal blood pressure (308). It appears that the ETA receptor mediates the damage, while the ETB receptor is more likely protective (309).

Atrial Natriuretic Peptide

Another mediator of hyperfiltration appears to be atrial natriuretic peptide (ANP). In addition to its natriuretic actions, ANP is a potent vasodilator of the glomerular afferent arteriole. Plasma ANP levels are increased following renal ablation (57) and hyperfiltration is reversed with administration of an ANP receptor antagonist (61). Further supporting the role of this peptide in renal adaptations to nephron loss, ANP mRNA expression in the remnant kidney is significantly increased (310).

Uric Acid

Recently, hyperuricemia has been postulated as a novel risk factor for progression of cardiovascular and renal disease (311; see also Chapter 76). In addition to stimulation of vascular smooth muscle cell proliferation, induction of endothelial dysfunction, and growth effects, uric acid may contribute to progression via hemodynamic mechanisms. In rat models, mild hyperuricemia has been reported to induce glomerular capillary hypertension in normal (312,313) and remnant kidney rats (313).

Growth Factors

The broad area of growth factors in renal structural changes after nephron loss cannot be viewed separately from mechanotransduction, since both share common signaling pathways and target genes with often identical consequences to cells and tissues. The role of growth factors in initiating compensatory renal growth has been suspected for over a century. A circulating factor has been suggested since at least 1896, when Sacerdotti (314) infused blood from bilaterally nephrectomized dogs into normal dogs and induced renal growth in the recipients.

Evidence of growth of transplanted kidneys into anephric humans or animals further supports this notion (315,316). In the 1950s, Braun Menéndez (317) postulated the existence of a humoral renal growth factor, termed renotropin. Fractions of urine, serum, and liver from uninephrectomized animals, and urine and serum from humans, stimulate biochemical changes (such as incorporation of radiolabeled nucleotides into DNA) in isolated renal tissue preparations, and stimulate growth in cultures of kidney-derived cells. Such studies have been proposed as further evidence for the existence of renotropin (318). These properties do not establish that these fractions induce whole kidney growth, however, and no true "renotropic hormone" has been isolated.

Original evidence for the role of growth factors in structural adaptations to nephron loss was based on *in vitro* observations in cultured PT or mesangial cells. In general, however, these factors promote growth in many cell types, and therefore are likely to participate in compensatory renal hypertrophy in a nonspecific manner, after growth was triggered by some renal-specific signal. Moreover, factors that cause growth of kidney cells *in vitro* may not necessarily contribute importantly *in vivo* following nephron loss. For example, Ang II induces growth of PT and mesangial cells in culture, but Ang II blockade does not prevent compensatory hypertrophy in rats with renal ablation. Here we focus on those growth factors that have been implicated in hemodynamic and/or structural adaptation or maladaptation by using several experimental approaches, including *in vivo* studies. Of note, there is growing evidence that some of these growth factors, rather than being maladaptive, may prove protective in the setting of reduced renal mass.

Insulin-like growth factor (IGF-1). Evidence that IGF-1 increases renal perfusion and filtration in normal rats (319) and that levels of IGF-1 increase in the remnant kidney (320) suggest a potential role for that hormone. IGF-1 inhibition ameliorates hyperfiltration after uninephrectomy (321). Administration of IGF-1 increases GFR and kidney weight in intact rats, although it is not clear whether the kidneys grow disproportionately to body weight (322,323). Following renal ablation, renal IGF-1 levels increase, whereas IGF-1 receptor levels remain constant (322,324). There is disagreement concerning the time course of the increase in IGF-1, and some studies have found that renal IGF-1 levels start to increase only after compensatory hypertrophy is already detectable (322). Whether IGF-1 mRNA increases is also controversial. Early reports finding increased IGF-1 mRNA levels (325,326) were not confirmed by others showing no change in the mRNA for IGF-1 (322,327), IGF-1 receptor (327), and IGFBPs (324,327). Thus, the data suggest that IGF-1 participates in, but does not initiate, compensatory renal growth. On the other hand, Haylor et al. (328) found that administration of an IGF-1 receptor antagonist, started 3 days prior to uninephrectomy, ameliorated compensatory hypertrophy.

Some studies suggest that IGF may even be protective against FSGS. In contrast to growth hormone (GH) and growth hormone releasing factor (GHRF) overexpressing rats, IGF-1 transgenic mice do not develop glomerulosclerosis despite similar glomerular hypertrophy (329). Furthermore, IGFBP-1 transgenic mice, with decreased availability of IGF-1, develop FSGS without glomerular hypertrophy (330).

Epidermal growth factor (EGF). Though primarily studied in terms its growth-promoting effects (see the subsequent text), the related EGF also has hemodynamic actions, in that it has been shown to affect arteriolar resistances, and increase P_{GC} (331). Renal content of EGF, distribution of EGF, and receptor levels for EGF all remain constant over the first few days following uninephrectomy (332–334). An increase in EGF content and a reduction in EGF receptor levels have been observed only after compensatory renal hypertrophy is established (333,335). It is possible that EGF plays a pathophysiologic role later in the course of hypertrophic response to nephron loss, contributing to the development of interstitial fibrosis. As suggested by Terzi et al. (336), functional inactivation of the EGF receptor (EGFR) by targeted expression of a dominant-negative EGFR in renal PT cells reduced tubulointerstitial lesions in mice with 75% renal mass reduction.

Hepatocyte growth factor (HGF). Though hemodynamics have not been directly studied in CKD, it was recently reported that HGF is associated with a reduction in GFR, primarily mediated by ET-1 (337). There is a very early increase in remnant kidney HGF and HGF mRNA after uninephrectomy (335), though extrarenal increases are also found in distant organs (338). Recent work has examined whether HGF also modulates progression of injury after nephron loss. Liu et al. (339) observed increases in renal and systemic production of HGF in rats with renal ablation. With administration of an anti-HGF antibody, rats experienced a rapid decrease in GFR and increased renal fibrosis, in association with markedly increased ECM accumulation. Parallel *in vitro* data suggested that HGF preserves kidney structure by activating matrix degradation pathways via increased collagenase and matrix metalloproteinase-9 (MMP-9) expression, and suppression of tissue inhibitors of metalloproteinase (TIMPs), which are endogenous MMP inhibitors (339).

The link between HGF and renal matrix degradation via modulation of MMPs and TIMP in the remnant kidney model has been more recently confirmed in studies using treatment with recombinant HGF (340–342). For example, rats with renal ablation were administered recombinant HGF, an anti-HGF antibody, or with preimmune IgG for 2 weeks (342). HGF infusion halted proteinuria and decreased renal collagen accumulation. The treatment also attenuated renal inflammation in both glomeruli and tubulointerstitium, and reduced tubular expression of the chemokines macrophage chemoattractant protein-1 (MCP-1) and RANTES. In contrast, HGF neutralization worsened renal fibrosis, aggravated renal inflammation, and enhanced tubular expression of MCP-1 and RANTES. *In vitro*, HGF suppressed basal and TNF-alpha-induced expression of these chemokines at both the mRNA and protein levels in PT cells. HGF also blunted TNF-alpha-induced nuclear translocation and activation of NF-*k*B, a pivotal transcription factor that regulates chemokine expression. Immunohistochemistry showed that activated NF-*k*B was evident in tubules in remnant kidneys and increased remarkably with anti-HGF treatment. HGF infusion markedly suppressed expression of activated NF-*k*B in remnant kidneys. Thus, in addition to previously mentioned mechanisms, beneficial effects of HGF in chronic renal disease are attributable, at least in part, to a direct anti-inflammatory action, likely via NF-*k*B, on tubular epithelial cells.

HGF is also functionally linked to action of TGF-β. *In vitro* studies revealed that TGF-β1 significantly induced expression of c-met, a protoncogene encoding HGF, in renal tubular epithelial cells (343), suggesting that HGF may act as a natural TGF-β antagonist. In TGF-β1 transgenic mice with renal ablation, HGF suppressed expression of connective tissue growth factor (CTGF), a down-stream effector of TGF-β, in the remnant kidney, attenuating renal fibrosis and improving the survival rate (344).

Vascular endothelial growth factor (VEGF). There may also be a role for VEGF, a pluripotent angiogenic factor essential for kidney development. Administration of a neutralizing antibody to VEGF has been reported to partially attenuate renal hypertrophy, and fully prevent glomerular hypertrophy, after uninephrectomy in mice (345). However, other evidence

suggests a protective role for VEGF, particularly with respect to the tubulointerstitial changes in CKD. Studies by Kang et al. (162,346) demonstrated that loss of peritubular capillaries and impaired angiogenesis in renal microvasculature coincides with the progressive course of renal injury in remnant kidneys, and is associated with decreasing expression of VEGF. This decrease in VEGF expression may be a consequence of progressive macrophage infiltration, production of macrophage-associated cytokines, and the anti-angiogenic factor thrombospondin-1. These studies suggest that VEGF–induced preservation and neoformation of glomerular and peritubular capillaries limits the structural injury after severe nephron loss. In addition, differences in VEGF expression after nephron loss may underlie differences in susceptibility to progression of renal injury between male and female genders (346).

Platelet-derived growth factor (PDGF). PDGF is a potent mitogen acting on glomerular cells (347). Furthermore, its involvement in tubulointerstitial injury is likely because of its ability to transform fibroblasts to myofibroblasts (348). Similar to TGF-β, PDGF is stimulated by Ang II (349). TGF-β can be stimulated by PDGF resulting in a transition from cell proliferation to hypertrophy and fibroproduction (350). PDGF is upregulated in kidneys after 5/6 nephrectomy in the rat (351) and has been found in biopsies of diseased human kidneys (352). Its role in maladaptive rather than in adaptive processes after nephron reduction is underscored by the fact that overexpression of PDGF is detectable only from the second week after renal ablation (351). A pathophysiologic role of this factor in the development of FSGS is further supported by studies with PDGF transgenic rats (353).

Transforming growth factor-β (TGF-β). TGF-β, a multifunctional prosclerotic growth cytokine, is involved in a wide array of physiologic and pathophysiologic processes, and in many of the signaling and growth pathways which eventuate in CKD (reviews: 354–357). The trophic effects of Ang II are, at least in part, mediated by TGF-β, postulating the existence of a renin–angiotensin–TGF-β axis (358). Activation of TGF-β early after renal ablation has been described and implicated in development of structural damage (358). Furthermore, involvement of the RAAS–TGF-β axis has been documented by studies showing that amelioration of renal injury in rats with reduced renal mass, achieved by treatment with ACEI or AT_1 receptor blockers, is associated with a reduction of renal TGF-β expression (358,359).

The fibrogenic actions of TGF-β are at least in part mediated by additional down-stream molecules. It was demonstrated that connective tissue growth factor (CTGF) is expressed in PT epithelial cells that have been engulfed by interstitial fibrosis in the remnant kidneys of TGF-β transgenic mice (344). To further explore the role of CTGF in mediating TGF-β-induced interstitial renal fibrosis in the remnant kidney, Okada et al. (360) subtotally nephrectomized TGF-β1 transgenic mice. Treatment with CTGF antisense oligodeoxynucleotide significantly blocked CTGF expression in the PT cells in the remnant kidneys despite the sustained level of TGF-β1 mRNA. This reduction in CTGF mRNA level paralleled a reduction in mRNA levels of matrix molecules as well as proteinase inhibitors plasminogen activator inhibitor-1 and TIMP-1, suppressing renal interstitial fibrogenesis, and suggesting a nephroprotective potential for this new treatment.

Extracellular matrix (ECM) accumulation is a hallmark of FSGS and interstitial fibrosis, and there is a delicate balance between ECM production and degradation. ECM proteins are being constantly degraded by connective tissue proteases, such as cathepsins or MMPs, and activity is further controlled by their tissue inhibitors (361). The balance between proteinases and their tissue inhibitors is regulated by growth factors such as Ang II, TGF-β, PDGF, and others (362,363). Downregulation of MMP has been described in the remnant kidney (364). More information is available regarding the role of plasminogen activator inhibitor-1 (PAI-1) in CKD. In the radiation nephropathy model of FSGS, PAI-1 mRNA expression was colocalized with sites of glomerular injury (365). Mutant mice lacking PAI-1 do not develop FSGS after irradiation (363). The amelioration of FSGS in remnant kidney rats treated with ACEI or AT_1 receptor blockers is associated with marked inhibition of PAI-1 (363).

Modulators of Structural Adaptations After Nephron Loss

Hypertension

Probably the most widely accepted risk factor for progression of CKD is hypertension, which is covered in detail in section 8 of this book. Conclusive data indicate that suboptimal blood pressure control is associated with faster loss of GFR and that in most cases, reduction of blood pressure will slow the progression of CKD (27). As discussed earlier, one major mechanism of hypertensive renal injury is the associated transmission of high pressure into the glomerular capillary network, with induction and maintenance of glomerular capillary hypertension. This higher force is transmitted via mechanotransduction to the component cells of the glomerulus, with subsequent predisposition to development of FSGS.

Role of Gender

Male gender is a risk factor for progression of CKD in some clinical conditions and experimental models (366). It was originally suggested that the magnitude of compensatory hypertrophy is not influenced by androgens or gender (367–369). However, despite the generally benign course after uninephrectomy, some clinical studies reported increased risk for renal abnormalities in uninephrectomized males as compared to females (35,370). Gender influences renal compensatory growth following uninephrectomy. Although initial hypertrophy is comparable between the sexes, later in the course, kidney growth is greater in the males (371). Accelerated growth in males is accompanied by significant glomerular hypertrophy and glomerular and tubular lesions, and associated with the presence of testosterone. Further studies demonstrated that, in contrast to males, early renal growth in uninephrectomized female rats is not associated with enhanced GH secretion, although IGF-1 receptor mRNA is upregulated. Furthermore, females showed more hyperplastic response than males (372). Thus, uninephrectomy in the female rat theoretically can be viewed as a model of "true" adaptation with a minimal risk for development of renal injury. Further studies, better defining differences between the sexes in terms of renal responses to nephron loss, may be crucial for understanding of progressive nature of renal injury.

More recent studies have identified several mechanisms that could explain gender differences in progression of renal injury after nephron loss. *In vitro*, estradiol has been shown to modulate cellular activities via mechanisms generally consistent with a nephroprotective effect (373). Krepinsky et al. (374) reported that exposure of mesangial cells to mechanical strain caused increased 44/42 MAPK and JNK activities, as well as nuclear translocation of p44/42 MAPK and SAPK and nuclear protein binding to AP-1; the changes were inhibited by pretreatment with 17β-estradiol. Similar findings, (i.e., 17β-estradiol-induced inhibition of p42/44 activation, ROS signaling, and ET-1 production), were noted in endothelial cells exposed to

cyclical strain (375). *In vivo*, female gender and/or estrogen therapy has been associated with preservation of renal struction in a number of disease modes. For example, Gross et al. (376) studied uninephrectomized, sham-operated or ovariectomized SHR. Rats receiving estrogen therapy had significant reductions in albuminuria, glomerulosclerosis, and tubulointerstitial damage, in association with inhibitory effects on expression of TGF-β and ET-1 mRNA and protein expression. Estradiol also inhibits upregulation of PDGF-A mRNA in rats with renal ablation (377). Another potential mechanism relates to preservation of peritubular capillary architecture. Kang et al. (346) found that male gender was associated with downregulation of renal VEGF, and loss of peritubular capillaries, leading to tubulointerstitial fibrosis in remnant kidney rats, as compared with a more benign course in female rats.

Dietary Factors

Feeding a low protein diet to rats with renal ablation limits remnant kidney GFR and weight, whereas feeding a high-protein diet augments hypertrophy (34,378,379). These observations suggest that the stimuli to hyperfiltration and hypertrophy associated with nephrectomy and protein feeding are additive. Restriction of other dietary components also may influence the renal hypertrophic response. As compared to values for kidney weights in nephrectomized animals receiving normal diets, kidney weights are lower in nephrectomized rats ingesting diets that are restricted in sodium (64,380,381), phosphate (26), total calories (382), or carbohydrates (383), or are high in water (384). Though mechanisms are not yet fully understood, oral administration of a novel dietary adsorbent, AST-120, has also been shown to limit the progression of nephropathy in rats with renal ablation (213).

Age

Age at nephrectomy affects the magnitude of compensatory renal growth, with greater responses being seen in the younger kidneys (385,386). The increased magnitude of compensatory renal hypertrophy in youth may reflect generally greater responsiveness of young tissue to stimuli responsible for organ growth, as similar increases occur in compensatory growth of other organs.

Hyperlipidemia and Glomerular Lipid Deposition

It has been suggested that glomerular deposition of circulating lipids contributes to progressive glomerular injury in CKD (387,388). In animal models, feeding a high-cholesterol diet accelerates injury, whereas hypolipidemic therapy slows progression (387). Mechanisms of lipid-induced injury include a proliferative response of mesangial cells to low-density lipoproteins (LDL); synergistic interactions among LDL, ET-1, PDGF, and IGF-1; aggravation of glomerular macrophage influx and stimulation of monocyte chemoattractant protein (MCP-1); effects on mesangial type IV collagen synthesis; and increased TGF-β expression by infiltrating macrophages (387,388). Hypolipidemic therapy with lovastatin has been shown to influence mesangial cell metabolism, by inhibiting serum-induced proliferation (389), inhibiting PDGF-induced mesangial cell mitogenesis (390), and inhibiting MCP-1 and interleukin-6 mRNA expression and protein secretion (391). Furthermore, lovastatin decreases membrane-bound p21 ras in PT cells and inhibits serum-induced c-fos and c-jun protein and AP1 activity (392). As in atherosclerosis, hyperlipidemia may act synergistically with other risk factors, such as hypertension, in promoting glomerular injury.

Glomerular Capillary Thrombosis

It has been suggested that capillary thrombosis is precipitated by early endothelial cell injury in the remnant glomeruli, and that capillary microthrombi contribute to FSGS by direct occlusion of capillary lumina and by release of platelet-derived factors that aggravate glomerular injury (129,393). This hypothesis has prompted evaluation of anticoagulant drugs in experimental CKD. For example, heparin has proven protective in the remnant kidney model (394,395). The protective effect afforded by thromboxane synthesis inhibition has also been attributed to lessening thrombosis, whereas warfarin and aspirin have been shown to be less effective than heparin in preventing remnant glomerular injury (293,396,397). Heparin may also protect remnant glomeruli by other mechanisms, including reduction of blood pressure (394,395,398), suppression of ET-1 (399) and mesangial ECM accumulation (400), and inhibition of mesangial cell expression of basic fibroblast growth factor and PDGF, and of extracellular matrix proteins (401).

Altered Phosphate Metabolism and Renal Calcium Deposition

Studies by Alfrey and coworkers (402,403) provided the first evidence that intrarenal calcium deposition contributes to progressive loss of renal function in experimental CKD. These studies showed that restricting phosphate intake reduced renal calcium content, preserved renal function, and prolonged life span in rats with renal ablation or nephrotoxic serum nephritis. However, phosphate restriction may protect by additional mechanisms. Phosphorus restriction may protect remnant nephrons by lowering circulating lipid levels, reducing tubule energy consumption, altering remnant glomerular hemodynamic function, or reducing glomerular volume (404–406). Increased parathyroid hormone contributes to suppression of NO generation in the remnant kidney (268). Calcium citrate, which is used clinically to bind dietary phosphate, has been shown to slow the progression of CKD in rats with renal ablation, in association with improved metabolic acidosis, and reduction in proliferative activity of glomerular and tubular cells (407).

Tubule-Specific Mechanisms: Proteinuria of Glomerular Origin

The appearance of protein casts suggests that remnant nephron proteinuria causes tubule obstruction and dilatation. Proteins of glomerular origin may trigger tubular maladaptive processes by several mechanisms. First, tubular and interstitial cells can be activated by a number of growth-promoting factors generated by glomerular cells. These factors can be reabsorbed from the tubular fluid and further transported into interstitium (408), or they can reach the tubulointerstitial compartment via the postglomerular vasculature. Second, Remuzzi's group (409) has suggested that glomerular proteinuria per se can exert deleterious effects on the tubulointerstitial compartment. According to this hypothesis, as protein traffic across the glomerular barrier increases, the protein concentration in Bowman's capsule and tubules also increases. PT cells actively

reabsorb filtered proteins by phagocytosis. Increasing protein loads in tubular cells cause organelle congestion, lysosomal swelling and rupture, exposing the tubular cells and interstitium to lysosomal enzymes. Furthermore, protein may upregulate genes involved in tubulointerstitial infiltration and injury. For example, PT cells exposed to higher loads of proteins such as albumin or immunoglobulin may respond with an increase in ET-1 production. The release is primarily basolateral, suggesting the link with the development of interstitial injury (410). In addition to these injurious effects of glomerular protein leakage, primary tubulointerstitial processes may contribute to remnant nephron destruction following renal ablation.

Though intellectually compelling, the applicability of this mechanism to progression of CKD remains controversial. Clinical observations that interventions which reduce proteinuria also slow the loss of GFR show an association, but not a tight cause-and-effect relationship. A detailed discussion of this controversy can be found in the recent review of Kriz and LeHir (176).

Hypoxia Theory

This theory, pioneered by Fine and coworkers (411), introduced hypoxia of tubular and interstitial cells as a major trigger of events resulting in tubulointerstitial injury. It is assumed that the blood flow and oxygen delivery to the interstitial and peritubular capillary network is limited owing to glomerular injury, or that the tubulointerstitial capillary network suffers the same hemodynamic injury as glomeruli. Peritubular capillaries may be compressed by hypertrophic tubules (412). An alternative mechanism of tubular hypoxia relates to increased metabolic demands caused by enhanced reabsorptive work (276,413–415). Hypoxia leads to expression of growth factors, vasoactive molecules, cytokines, adhesion molecules and other mediators involved in renal injury (416–420). At the level of gene transcription, hypoxia response elements (HRE) have been identified in a number of genes forming a binding site for the hypoxia-inducible transcription factor (HIF-1) (421,422). Hypoxia-inducible transcription factor-1 acts in concert with other transcription factors, such as NF-*k*B and the fos and jun families, to induce gene expression (421). In cultured fibroblasts, hypoxia upregulates mRNA for TGF-β1 and decreases matrix turnover (423). *In vivo*, chronic hypoxia does not affect renal PDGF-A, PDGF-B, or VEGF mRNA levels, but VEGF is upregulated in the presence of ET receptor blockade (424).

Changes in Interstitial Osmolarity

Bouby and associates (384) demonstrated a nephroprotective effect of high water intake in rats with subtotal nephrectomy, and ascribed those effects to inhibition of the process of urinary concentration. *In vitro,* hypertonicity activates latent TGF-β into the biologically active form (425). *In vivo,* reduction of interstitial osmolality by high water intake in remnant kidney rats results in a decrease in TGF-β and fibronectin mRNA expression, and amelioration of predominantly tubulointerstitial, but also glomerular, injury (426).

Increased Ammoniagenesis

Increased ammonia production by remnant nephrons has been associated with intrarenal complement activation and interstitial inflammation (427). Administration of sodium bicarbonate, which reduces remnant nephron ammonia production, also limits tubulointerstitial injury in rats subjected to renal ablation (427).

THE PRICE OF ADAPTATION: PROGRESSIVE CHRONIC KIDNEY DISEASE

Once CKD begins, deterioration of renal function often continues, eventually leading to end-stage renal disease (ESRD). Progressive loss of function occurs after initial injury, which may arise from a number of initially dissimilar forms of renal injury. Whatever the initial cause, after enough nephrons are lost, the kidney will fail. These observations suggest that, after a certain point, reduction in functioning nephron number leads to failure of the remaining units, as adaptive mechanisms turn maladaptive (428). The following sections will briefly describe the glomerular and tubulointerstitial sequellae of loss of functioning nephrons. More detail can be found in several excellent reviews (129,176,428–430).

Glomerulosclerosis

Rennke (129) summarized the glomerular morphological changes associated with transition from glomerular hypertrophy to obsolescence (FSGS). Expansion of mesangial elements (i.e., disproportionate increase in fractional volume of the glomerulus occupied by the mesangium) is a typical feature in conditions associated with sustained hyperfiltration. Both mesangial hypercellularity and increased synthesis of ECM contribute to the expansion. In advanced stages, mesangial expansion leads to the obliteration of capillaries and glomerular obsolescence. The process is linked to the filtration of molecules into the mesangium due to increased P_{GC}, and local generation of growth factors by intrinsic renal (endothelial and mesangial) and blood-borne (platelets and leukocytes) cells. Subendothelial hyaline deposition occurs in association with increased P_{GC} and podocyte changes. A greatly hypertrophied or stretched podocyte may no longer maintain an efficient attachment to the underlying basement membrane and capillary loop, resulting in formation of areas with high hydraulic conductivity. Large macromolecules are filtered into these areas, forming aggregates that may ultimately occlude the capillary lumen. Formation of microthrombi is another feature often associated with hyperfiltering states. This process is most likely a consequence of glomerular endothelial injury and local production of proclotting factors. Finally, capillary microaneurysms may occur, in particular in conditions characterized by a rapid rise in P_{GC} (129).

The essential role of the podocyte was reiterated in a recent elegant description of pathways to nephron loss presented by Kriz and LeHir (176). In this formulation, the pattern of injury following nephron loss is considered to be degenerative. When podocytes are exposed to chronic stress (such as glomerular hypertension, and metabolic disturbances associated with abnormal activity of growth factors or vasoactive compounds), the consequences are twofold. Functionally, the size selectivity of the glomerular barrier is lost. Structurally, podocytes undergo a pattern of foot process effacement, followed by cell-body attenuation, pseudocyst formation, accumulation of absorption droplets, sometimes "microvillous transformation," and then detachment. The decreased density of these terminally differentiated cells leads to hypertrophy, but eventually the podocytes are unable to cover the tuft, parietal cells adhere to naked glomerular basement membrante (GBM), and

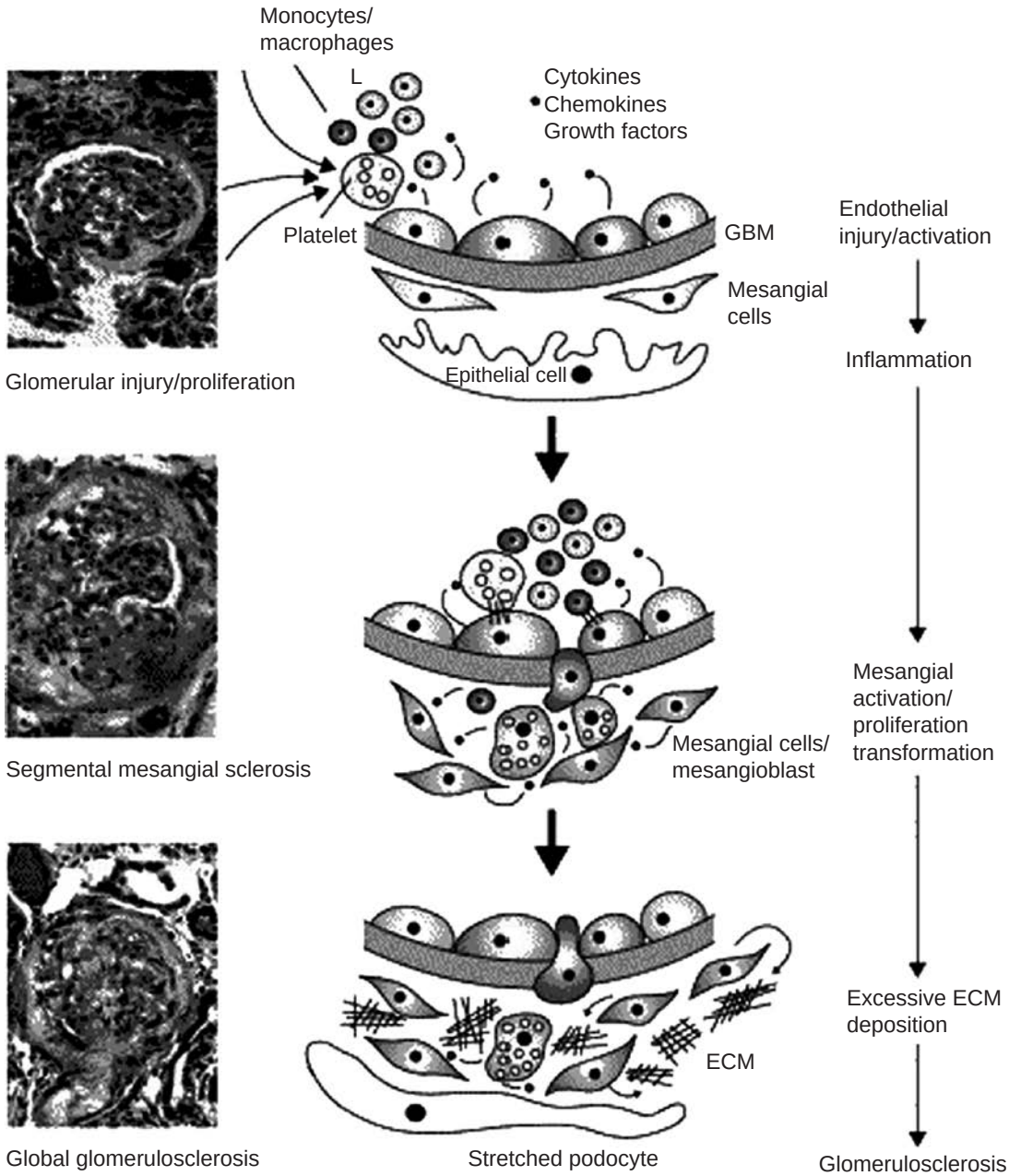

FIGURE 91-2. Diagrammatic representation of the stages of focal and segmental glomerular sclerosis (FSGS). *GBM*, glomerular basement membrane; *ECM*, extracellular matrix. (Reprinted from: El Nahas AM, et al. Chronic kidney disease: the global challenge. *Lancet* 2005;365:331, with permission.)

adhesion of the tuft to Bowman's capsule results. The adherent tuft portion allows capillaries to leak protein-rich filtrate into the interstitium instead of Bowman's space. Interstitial fibroblasts move in as a response, forming a crescent shaped space which eventually leads to formation of synechia and FSGS (176).

The role of those events, and the contributions of other intrinsic glomerular cells, was recently summarized by El Nahas and Bello (430). There is evidence that endothelial cells may initiate this process (431). Accordingly to this formulation, systemic factors such as hypertension, dyslipidemia, and smoking lead to endothelial damage and dysfunction, with proliferation of mesangial cells and injury to podocytes (Fig. 91-2). Hypertension-induced shear stress leads to injury and then activation and dysfunction of glomerular endothelial cells, which in turn initiates glomerular microinflammation leading to interactions between inflammatory cells (macrophages) and mesangial cells, with the activation, proliferation, and dysfunction of the latter. Under the influence of growth factors, particularly TGF-β1, mesangial cells regress to an embryonic mesenchymal phenotype (mesangioblasts) capable of producing excess extracellular matrix (ECM), leading to mesangial expansion. Simultaneously, podocytes are undergoing the effacement described earlier. The outcome of this glomerular "remodeling" depends on the balance of healing and scarring influences (430).

Recent evidence has provided details as to how the healing and scarring processes interact. After injury, the glomerulus

may either enter a phase in which its cellular constituents dedifferentiate into their mesenchymal embryonic precursors (reverse embryogenesis), or it may attract hematopoetic embryonic stem cells to recapitulate embryogenesis (432). The outcome will depend in part on the activity of the environmental (modulating) forces at hand, as well as potential influx of bone-marrow derived mesangial progenitor cells (433). Another aspect of the process is the degree of apoptosis. In the process of renal scarring, renal cells undergo enhanced apoptosis (434). Fragmented DNA in the glomeruli and tubules increases with progression of FSGS in the remnant kidney model. More recent studies found increased mRNA and protein expression of Bax, an apoptosis promoter, in tubules rats with remnant kidneys by 7 days after ablation. This was followed by a decrease in expression of the apoptosis protector Bcl-2, creating a sharp increase in the Bax/Bcl ratio in remnant kidneys (435). The role of apoptosis in the development of renal injury after nephron loss was further supported by studies in SHR with subtotal nephrectomy. In this model, control of blood pressure with various compounds led to slower progression of proteinuria and FSGS, and also induced marked decreases in apoptotic markers (436).

Tubulointerstitial Injury

Critical reduction of nephron number often leads to profound tubulointerstitial changes that involve tubule atrophy and loss, and interstitial expansion and fibrosis, also referred as tubulointerstitial scarring (361,378,429,437). The importance of this observation is highlighted by the fact that the degree of tubulointerstitial involvement has been suggested to be the best predictor of progression of CKD (438). As ECM production increases, tubules and peritubular capillaries disappear. Loss of the linkage between glomeruli and tubules leads to "atubular glomeruli," with open capillary loops but no attached tubules (429,439,440). Loss of these structures helps to explain the close association with the decline of renal function. Human studies have shown a negative correlation between renal interstitial and tubular renal function in various renal diseases (441). As summarized by Eddy (361), the interstitial "scar" is composed of normal interstitial ECM proteins (collagens, fibronectin, tenascin), basement membrane proteins (collagen IV, laminin), proteoglycans, and glycoproteins (hyaluronan, thrombospondin and SPARC). α-Integrins (442) may be involved in binding fibronectin in insoluble matrix. The various matrix proteins which accumulate in the interstitium are assembled into a complex three-dimensional scaffold supported by cross-linking of protein chains. Studies exploring the cellular origin of proteins involved in interstitial scarring identified tubular epithelial cells and interstitial fibroblasts. Importantly, later studies demonstrated ability of normal tubular and renal cells to transdifferentiate into other cell types with less predictable patterns, and atypical regulation of protein synthesis (443,444). In addition to resident renal cells, interstitial macrophages and infiltrating monocytes represent an important source of growth factors involved in fibroproduction, vasoactive molecules, and matrix proteins (445,446). The role of these cells is further supported by the finding that FSGS in remnant kidney rats is ameliorated by treatment with immunosuppressive agents such as mycophenolate mofetil (447,448).

PATHOPHYSIOLOGY OF PROGRESSIVE RENAL INJURY AFTER NEPHRON LOSS: UNIFYING SCENARIO

The different mechanisms proposed to account for remnant injury should not be regarded as mutually exclusive; indeed, there are most likely extensive interactions among them

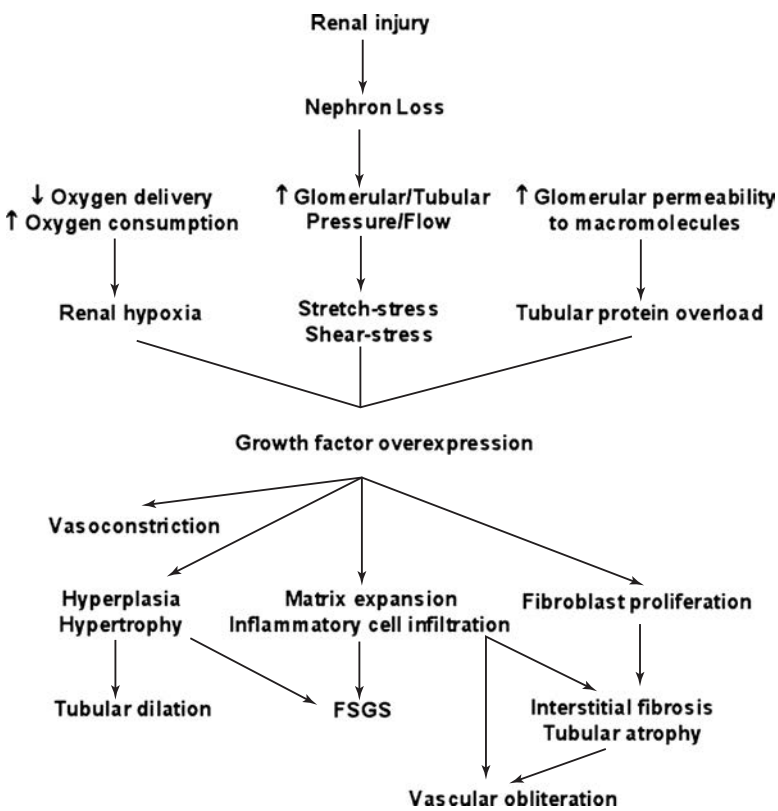

FIGURE 91-3. Hypothetical schema of pathways that link nephron reduction to the development of renal lesions via hemodynamic forces and growth factor overexpression. (Reprinted from: Terzi F, et al. Early molecular mechanisms in the progression of renal failure: role of growth factors and protooncogenes. *Kidney Int* 1998;53:S68, with permission.)

(129,130,176). Given the close apposition and functional interdependency of glomerular cell types, such interaction among mechanisms of glomerular injury should be expected. The foregoing studies suggest that the progression of renal injury associated with systemic hypertension is mediated by the resultant adaptive increase in P_{GC}, which then contributes to structural injury. The sequence of events whereby alterations in hemodynamics, oxygen delivery, and glomerular permeability initiate growth factor overexpression and subsequent cellular injury is schematized in Figure 91-3 (181). All glomerular cell types participate. Glomerular hypertension and hypertrophy also may cause FSGS by promoting movement of circulating macromolecules through the glomerular capillary wall. These permselective defects are associated with changes in podocyte structure and activity described earlier. Subendothelial deposition of large macromolecules in areas where macromolecule passage through the capillary wall is increased may result in hyalinosis, eventually proceeding to occlusion of capillary lumina. Together, damage to these cellular elements results in FSGS.

Meanwhile, tubulointerstitial injury develops due to a primary interstitial process or secondary to glomerular events. The cells in the tubulointerstitial compartment are injured by hypoxia, resulting from high metabolic demands imposed by the increased burden of molecules they process, impaired blood flow from obstruction in diseased glomeruli, and/or vascular injury of the interstitial capillary network. Increased protein trafficking secondary to leakage from affected glomeruli causes direct tubular toxicity, and includes humoral mediators of tubulointerstitial injury. Under these pathophysiologic stimuli, the cells in the tubulointerstitial compartment generate an array of prosclerotic and profibrotic mediators and transdifferentiate to more primitive cell types that further contribute to progressive injury. Tubulointerstitial injury then perpetuates nephron loss by creating atubular glomeruli (429,440). Progressive nephron destruction in turn contributes to systemic and glomerular hypertension, thus perpetuating the cycle.

THE PROGRESSION OF HUMAN RENAL INSUFFICIENCY: THERAPEUTIC IMPLICATIONS

Understanding of the pathways leading to nephron destruction has prompted a number of clinical trials exploring the potential of slowing the progression of clinical CKD. The wide spectrum of pathophysiologic mechanisms described in the preceding suggests that the problem can be approached from many different directions. This body of literature has been recently reviewed (27,430,449) and will not be reiterated here.

With respect to their role in the development of renal injury, systemic and glomerular hypertension represent the most attractive targets for intervention. Moreover, the same interventions can also ameliorate renal growth abnormalities, resulting in beneficial effects in the tubulointerstitial as well as glomerular compartments. Considering the variety of actions of Ang II, and its key position in the renal pathophysiology, inhibition of the RAAS still represents the major pharmacologic therapy of progressive CKD. ACE inhibitors and AT_1 receptor blockers are not only effective in reducing blood pressure. Based on experimental studies, these agents are effective in reducing glomerular pressure and growth abnormalities in the kidney. In clinical studies, ACEI are the class of antihypertensive agents most often associated with nephroprotection. However, it should be emphasized that control of hypertension *per se* seems to afford some protection in most nephropathies, regardless to the class of agent used (with some exceptions to the rule). This is not surprising with respect to the role of impaired autoregulation of renal blood flow in transmitting the systemic blood pressure into glomeruli.

Other therapeutic maneuvers also may prove helpful, as discussed elsewhere in this volume. One possible therapy is restriction of dietary protein intake, implemented early in the course of intrinsic renal disease. Anticoagulant agents have been shown in some studies to retard the progression of membranoproliferative glomerulonephritis. The protective effect of agents that lower serum lipid levels remains to be assessed. Finally, the efficacy of specific pharmaceutical blockers, such as those which limit the action of endothelin, TGF-β, and other mediators, is likely to undergo clinical testing in the coming years.

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CHAPTER 92 ■ ANEMIA IN RENAL DISEASE

ANATOLE BESARAB AND FATEN AYYOUB

The kidney has three major functions: (i) to produce urine and thereby excrete toxic waste, (ii) to produce and secrete hormones that regulate blood flow (renin) and vascular tone (prostaglandins), stimulate red blood cell production (erythropoietin), and to participate in calcium and bone metabolism (1,25-dihydroxycholecalciferol); and (iii) to perform a variety of metabolic processes (gluconeogenesis, reabsorption of amino acids, and conjugation of drugs). Chronic kidney disease (CKD) is associated with a variety of disorders resulting from the deleterious effects of solute retention, from the absence of renally produced hormones, particularly erythropoietin. The most characteristic hematologic abnormality in CKD is anemia, which results primarily from the failure of kidney endocrine function. Anemia persists as a significant problem in many patients receiving adequate dialysis prescriptions.

This chapter reviews the pathogenesis of the anemia associated with kidney disease and discusses its therapy. Throughout this chapter, erythropoietin, or EPO, refers to the endogenous circulating hormone. Epoetin or rHuEPO refers to a recombinant product that is produced in various systems and used therapeutically.

THE ANEMIA OF CHRONIC RENAL FAILURE: MAGNITUDE OF THE PROBLEM

For the purposes of further discussion, the five-stage disease severity classification of CKD the Kidney Disease Outcomes Quality Initiative (K/DOQI) will be used (1). K/DOQI uses a formula developed from and validated within the Modification of Diet in Renal Disease Study (2) to calculate the glomerular filtration rates (GFRs) per 1.73/m^2. It is estimated that up to 25 million people in the United States have CKD of variable degrees. Astor et al. (3) have estimated that up to 3 million individuals with CKD that does not require renal replacement therapy may have anemia, defined as a hemoglobin level of less than 12 g/dL in men and less than 11 g/dL in women. The prevalence of this condition among Mexican Americans and African Americans with CKD is increased compared to that among non-Hispanic caucasians.

CLINICAL AND LABORATORY FEATURES

Circulating Blood

Richard Bright (4) commented on the pallor of patients with renal disease in 1836. For the next 150 years, anemia remained an important clinical manifestation of progressive renal disease. The severity of anemia that is frequently associated with kidney disease (renal anemia) typically progresses in parallel to the deterioration of renal function (5), and profoundly influences morbidity and mortality, particularly in those with end-stage renal disease (ESRD) (6,7). As shown in Figure 92-1, in the early stages of CKD, the kidney produces erythropoietin in an expected fashion, and inadequate erythropoietin production is not the initial reason for renal anemia. Suppression of red blood cell synthesis by poorly defined toxins or from shortened red blood cell survival dominates mechanistically at this early stage. At more advanced stages of disease, erythropoietin production decreases and EPO levels become lower than those expected for the degree of anemia; true erythropoietin deficiency exists. As GFR approaches 20% of normal, the great majority of patients become anemic with hematocrit (Hct) less than 30% (7). In general, there is a direct, although imperfect, relation between the degree of renal insufficiency and the degree of anemia (5,8).

The uncomplicated anemia of patients with CKD is normocytic and normochromic (9). Echinocytes or burr cells are the most often observed morphologic red blood cell change, their frequency correlating with the severity of uremia (10), and considered to be characteristic of chronic renal failure. However, even normal cells can undergo a reversible transformation to spiculed, burr cell-like echinocytes when exposed to a glass surface or suspended in incubated plasma (11). Echinocytes seen in patients with advanced CKD are, at least in part, artifactual and do not circulate as such in blood. Grossly deformed cells, however, such as acanthocytes with a few large spicules or fragmented schistocytes, are undoubtedly formed in the microcirculation *in vivo* (12).

Bone Marrow

The bone marrow of patients with ESRD is usually "normally" cellular with a normal appearance and maturation sequence of all cellular elements, including the nucleated red cells (13). The "normality" of the marrow during anemia, however, is misleading, because a compensatory increase in erythroid activity is expected with anemia. The degree of erythropoiesis in the marrow is actually decreased relative to that expected for the severity of anemia. Transfusion of ESRD subjects to near-normal blood counts markedly reduces marrow erythropoiesis, thereby unmasking the hypoproduction. Following a drop in the hematocrit due to acute blood loss or following an episode of prolonged hypoxia, the amount of erythropoiesis in the marrow of CKD may increase to greater than normal level but not to the same extent as in comparably affected nonuremic patients. The marrow erythropoiesis can be described as predominantly effective (14,15), since the corrected reticulocyte count (percentage reticulocyte × [hematocrit/45]) correlates with the amount of erythroid activity in the bone marrow, and the measured plasma iron turnover rate is commensurate with the ^{59}Fe incorporated into red blood cells. Older studies suggest a modest decrease in the red blood cell lifespan in uremic patients (16). More recent studies in well-dialyzed patients indicate that the red blood cell lifespan can approach normal if blood losses associated with hemodialysis are avoided (17).

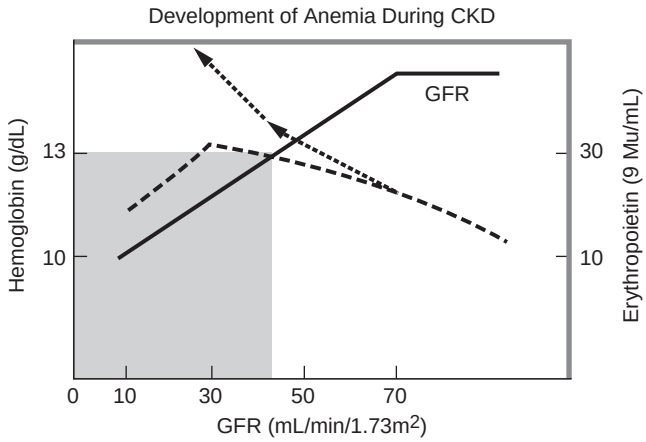

FIGURE 92-1. The mean hemoglobin levels at any level of glomerular filtration rate (*GFR*) is depicted by the *solid line*. The actual erythropoietin levels in chronic kidney disease (*CKD*) are depicted by the *dashed line*. The normal response is shown by the *dotted arrows*. In CKD, inability to produce sufficient erythropoietin for the degree of anemia occurs in fairly advanced disease corresponding to 60% to 75% of renal function. Erythropoietin (*EPO*) deficiency is thus relative. The need for anemia treatment can surface after patients enter the lower left quadrant of the graph (hematocrit less than 35% and GFR less than 50 mL/minute). Anemia in patients whose GFR is less than 30 mL/minute is caused by "true" EPO deficiency associated with a progressive reduction in kidney function.

Oxygen Transport

Changes in red blood cell oxygen-carrying capacity in ESRD subjects have been examined. When measured, the capacity of red blood cells to function as oxygen carriers is unimpaired (18). Changes in the oxygen affinity can occur as a result of changes in the concentration of crucial intraerythrocyte organic phosphates (19,20). The hemoglobin–oxygen affinity of uremic erythrocytes, however, is decreased to a greater extent than that of erythrocytes from comparably anemic nonuremic patients. In well-dialyzed patients, the intracellular concentration of 2,3-diphosphoglycerate (2,3-DPG) is appropriately increased in response to the level of anemia and the mild hyperphosphatemia, and the affinity of hemoglobin for oxygen is appropriately decreased (19). The effect of increased 2,3-DPG in erythrocytes from nonuremic anemic patients is offset by an increase in intracellular pH (mild respiratory alkalosis), but this pH shift does not occur in erythrocytes from uremic patients (20). Rather, systemic metabolic acidosis from renal failure (usually mild) further augments this decrease in oxygen affinity by shifting the oxygen dissociation curve to the right (Bohr effect). These combined effects favor the delivery of oxygen to peripheral tissues, even though acidosis also tends to decrease both glycolysis and the concentration of intracellular organic phosphates, both of which tend to increase the oxygen affinity of hemoglobin (19). Hemoglobin–oxygen affinity increases only slightly following hemodialysis as pH increases. Overall, the effect of decreased oxygen affinity on oxygen transport in hemodialysis patients is minimal, delivery of oxygen to peripheral tissues is slightly augmented, and the changes do not explain intra- or interdialytic symptoms.

Iron deficiency and hypophosphatemia are probably of more importance in oxygen delivery and the genesis of any intradialytic or postdialysis symptoms. Iron deficiency has a negative impact on exercise performance independent of the degree of anemia present (21). The frequent occurrence of iron deficiency in ESRD patients (22) probably contributes directly to the asthenia seen in the dialysis population. Other consequences of ESRD may contribute to altered oxygen delivery. Aggressive use of phosphate binders, defects in enteral phosphate absorption, and hyper alimentation, in concert with dialytic phosphate removal, can result in hypophosphatemia. This reduces the concentration of intracellular organic phosphate compounds (23), resulting in an increase in the oxygen affinity of hemoglobin and temporary decrease in tissue oxygenation.

PHYSIOLOGY OF ERYTHROPOIETIN AND ERYTHROPOIESIS

Historical Perspective

Bone marrow erythropoiesis in response to hypoxia was proposed in 1823 (24). In 1922, Brown and Roth (25) determined that the anemia of chronic nephritis resulted from reduced bone marrow production. The concept of a "hemopoietine" that stimulates marrow erythropoiesis dates from 1906 (26) and was quickly integrated into a hypoxia-induced feedback mechanism involving a hematopoietin (27) Reismann used parabiotic rats to demonstrate that hypoxia in one animal stimulated erythropoiesis in the normoxic partner (28). In 1953, Erslev described this "erythropoietic stimulating factor" that is now known as EPO when he transfused anemic rabbit plasma into normal recipient rabbits and demonstrated the unequivocal role of "intermediate humoral factor" in erythropoiesis (29). In 1957, Jacobson et al. (30) demonstrated that this factor was lacking in bilaterally nephrectomized animals. Subsequent studies showed that it was absent in anephric humans (31).

Understanding of erythropoiesis progressed before the erythropoietin molecule was biochemically characterized and before the intrarenal site of production was known. Goldwasser et al. first purified (32) then sequenced (33) the amino acids of erythropoietin, making it possible to construct a cDNA library, which allowed the identification and cloning of the erythropoietin gene (34,35). Transfection of the human gene into Chinese hamster ovary cells (35) or by gene activation (36) allowed mass production of recombinant erythropoietin for clinical use.

Similarly, physiologic studies were hampered until erythropoietin (EPO) could be measured. The first bioassay measured the utilization by polycythemic mouse of ^{59}Fe after infusion of a serum sample (37). In 1968, feedback control of erythropoiesis dependent on oxygen delivery to the EPO secretory site in the kidney was demonstrated for normal subjects. Urinary and plasma EPO increased logarithmically as hematocrit was lowered by phlebotomy, whereas these decreased with hypertransfusion (38). Erslev (39) in 1974 proved involvement of the kidney in erythropoiesis by showing that the perfusion of hypoxic rabbit kidneys *in vitro* with a serum-free solution resulted in the production of preformed EPO.

Extensive physiologic and clinical studies became possible after development of a radioimmunoassay (RIA) with a highly purified protein (40). The results of this assay correlate reasonably well with those of the bioassay. Commercially available radioimmunoassays are now widely available to clinicians and have been used widely to investigate the renal erythropoietin response of a variety of anemias. However, the clinician should be aware that the antisera currently used will measure all immunogenic erythropoietin fragments regardless of their biologic or physiologic activity (41,42).

Under normal circumstances plasma levels reflect predominantly renal synthesis of erythropoietin in response to tissue oxygen need. The kidneys produce more than 90% of EPO

(43). The major site of extrarenal erythropoietin production within the liver is also regulated primarily by the ratio of hepatic oxygen requirements to oxygen supply (44). In adult animals, the hepatocyte is the major cell producing erythropoietin (45,46). Extrarenal erythropoietin production is increased several-fold following various forms of hepatic injury such as subtotal hepatectomy (47) and carbon tetrachloride ingestion (48), effects not seen after nephrectomy or acute tubular necrosis. Extrarenal erythropoietin production declines immediately after the liver is injured, but then rises rapidly to supranormal levels during the period of liver regeneration. In acute renal failure, whether from tubular necrosis or acute crescenteric glomerulonephritis, deficient production persists; erythropoietin levels do not increase until excretory function recovers and the anemia recovers sluggishly (49,50).

Normal Erythropoiesis

Circulating erythropoietin originates predominantly from the kidney (90%), with a smaller contribution from the hepatic parenchyma (10%). EPO messenger RNA (mRNA) has also been detected in the spleen, lung, testis, and brain, but these sites do not secrete erythropoietin. *In situ* hybridization techniques have localized the mRNA to interstitial cells (also known as the type I interstitial cell (51,52), located near the base of proximal tubular cells, predominantly in the renal cortex (51). Interstitial cells positive for EPO mRNA are limited to the deep cortex and outer medulla under normal oxygen conditions. With increasing anemia and tissue hypoxia, the number of positive cells increases in number and spreads into the superficial cortex (52). However, there are some studies suggesting a major role for the proximal tubule cells in epoetin production (53). Appropriate compensation during severe anemia requires an approximately 100-fold increase in kidney EPO production. EPO-producing cells are recruited in an on–off fashion; increased production of EPO in response to hypoxia results from the recruitment of additional EPO-synthesizing cells. The number of EPO-producing cells increases exponentially as pO_2 decreases. Synthesis of EPO occurs *de novo,* secretion is rapid, and there is no significant intracellular accumulation (54). The short half-life of EPO mRNA provides a mechanism for rapidly modulating EPO production in response to variations in tissue oxygen tension. Maintenance of "normal" EPO plasma levels of 8 to 24 mU/mL in humans requires the daily continuous synthesis of approximately 2 to 3 U/kg body weight.

Erythropoietin has its primary effect in the bone marrow. The hematopoietic stem cell is capable of forming erythrocytes, leukocytes, and megakaryocytes (55,56). Under appropriate stimuli, these primitive cells have the capacity for both self-renewal and differentiation into committed progenitor cells. Renewal appears to occur by chance "stochastically" (57) and is initiated primarily by lineage, nonspecific cytokines such as interleukin-3 (IL-3), stem cell factor, insulin growth factor, and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Fig. 92-2).

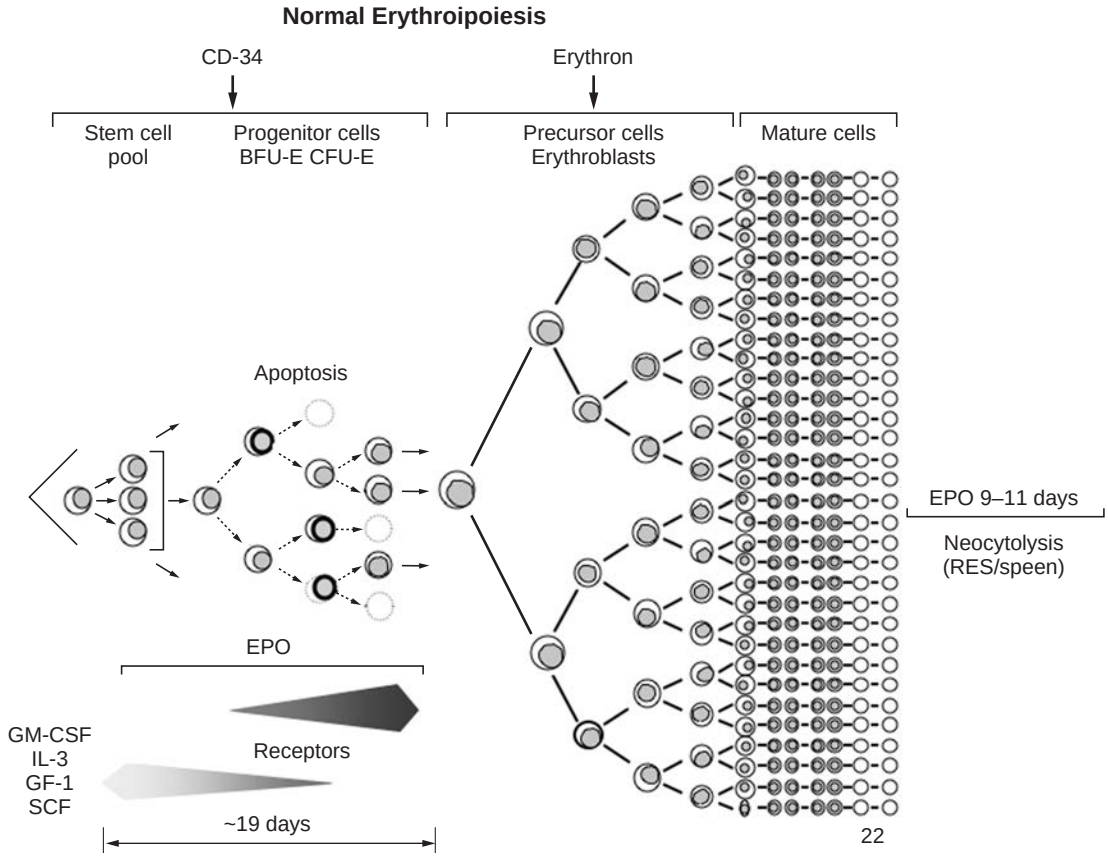

FIGURE 92-2. Normal erythropoiesis. Note that the interval from stem cell to erythroblast is about 19 to 26 days. Erythropoietin acts on the burst-forming unit-erythroid (BFU-E) and colony-forming unit-erythroid (CFU-E) for approximately 8 to 10 days. An EPO-independent phase lasts during rapid hemoglobin synthesis. Once the reticulocyte leaves the bone marrow, it is subject to neocytolysis as it transverse the spleen if EPO levels fall abruptly. (Adapted from: numerous sources including Schuster SJ, Caro J. Erythropoietin: physiologic basis for clinical applications. *Vos Sang* 1993;65:169; and Fried W, et al. Studies on extrarenal erythropoietin. *J Lab Clin Med* 1969;73:244.)

The transformation of a multipotential stem cell into a mature red blood cell occurs in two morphologically distinct stages as depicted in Figure 92-2 and is totally dependent on erythropoietin (58). The first stage begins with small mononuclear cells displaying CD34 on their surface (59). Thereafter and in sequence, the committed erythroid progenitor, the primitive and mature burst-forming unit-erythroid (BFU-E), and the colony-forming unit-erythroid (CFU-E) appear. In the second precursor stage, the cells appear as morphologically recognizable erythroblasts that mature into pronormoblasts and daughter erythrocytes.

Multipotential committed progenitor cells exist in the resting G_0 stage of the cell cycle (60). They are stimulated into the G_1 stage by IL-1 and IL-6, and granulocyte colony-stimulating factor (G-CSF). Under the influence of IL-3 and GM-CSF, they differentiate into primitive BFU-E. Peripheral demands for cellular production can be met only after this transformation of a multipotential stem cell to a unipotential progenitor cell. At this stage, the cells have lost their capacity for self-renewal, and have gained receptors for erythropoietin, which now becomes essential for the multiplication and differentiation of the BFU-E and CFU-E. There is an increasing dependence on, as well as sensitivity to, the effects of erythropoietin with the progressive maturation from primitive BFU-E into CFU-E (61–63). In fact, the CFU-E will only survive and differentiate into a pronormoblast in the presence of erythropoietin. In mice lacking the erythropoietin and EPO receptor genes, BFU-E and CFU-E are produced to normal levels, which indicates that erythropoietin stimulation is not necessary for commitment to erythroid progenitors but erythropoietin stimulation is essential for CFU-E survival and proliferation. Insulin or insulin-like growth factor 1 (IGF-1) is also required for CFU-E growth (64). In summary, constant presence of erythropoietin is critical to the sustenance, multiplication, and differentiation of the committed erythroid progenitors. This concept is key, as will be discussed later, with regard to clinical dosing practices and choosing the optimal route for recombinant erythropoietin administration.

Several other factors such as androgens, thyroid hormone, somatomedin, and catecholamines appear to augment the growth of CFU-E but are not essential (65). Other cytokines including IL-1α and IL-1β, IL-2, tumor necrosis factor alpha (TNF-α), and transforming growth factor beta (TGF-β) have a negative effect on erythropoiesis (66,67). These cytokines are significant mediators in the anemia of chronic diseases, may be activated by certain types of dialysis, and are invariably present during acute infection or inflammation. Their inhibitory effects produce resistance to exogenous erythropoietin in renal failure patients.

Erythropoietin exerts its signal through the erythropoietin receptor (p66), a 55 kDa transmembrane protein (68). Erythropoietin receptors are expressed at the CFU-E and proerythroblast stages and receptor number decreases during the final stages of erythroid differentiation such that reticulocytes and erythrocytes are devoid of erythropoietin receptors (58–61). Erythropoietic agents are being developed that are not at all epoetinlike, consisting of simple polypeptides that activate the receptor by molecular mimicry (69). Some of these peptides have only 14 amino acids. Once activated, the receptor dimerizes, and tyrose kinase activity via constitutively expressed JAK2 leads to phosphorylation of intracellular proteins (70), followed by activation of several signaling pathways, which include STAT, RAS, and phosphoinositol 3-kinase (71). The Signal Transducer and Activator of Transcription (STAT) pathway is the most important. STAT5 is a latent cytoplasmic transcription factor that, once activated via phosphorylation by JAK2, dimerizes, translocates into the nucleus, and binds to specific DNA sequences allowing transcription of the respective gene products. In addition, two tyrosine phosphatases, SHP-1 and SHP-2, play a role in erythropoietin-induced signaling and hence in stimulating cell proliferation, the former negatively and the latter positively (72,73). Erythropoietin also modulates calcium influx in erythroid cells through a transient receptor potential channel (74). Although, the exact signals or pathways are still poorly understood, it is unlikely that the ultimate signals are transcription factors for genes involved in the synthesis of globin or other mature erythroid proteins; it is more likely that these signals maintain the viability of progenitor cells (54). In the absence of erythropoietin, such cells undergo apoptosis and die before they reach the precursor cell stage. The anuclear cells in Figure 92-2 represent the process of apoptosis when erythropoietin becomes unavailable. In the presence of erythropoietin, they proliferate and eventually transform into precursor cells.

Erythroid progenitor cells exposed to optimal concentration of growth factors and erythropoietin proliferate luxuriously and produce a "burst" of colonies (75). At a certain level of maturation the CFU-E becomes activated and the cells are transformed into hemoglobin-synthesizing morphologically recognizable erythroblasts (73). Further proliferation and maturation of these cells appear to be unaffected by erythropoietin, proceeding at a fixed rate in the presence of adequate supplies of iron, folate, vitamin B_{12}, pyridoxine, ascorbic acid, and trace elements. Each cell that leaves the CFU-E stage ultimately produces 32 daughter cells that leave the marrow as reticulocytes.

Until recently erythropoietin was believed to have no effect on cells once they were released from the bone marrow. However, another erythropoietin-dependent mechanism affecting survival of circulating cells has been described. Hemolysis of recently formed red blood cells occurs when erythropoietin levels fall rapidly, as with descent from altitude, thereby permitting rapid adaptation when red blood cell mass is excessive for the new environment (76).

Although erythropoietin's main site of action is the bone marrow, several studies have now convincingly shown that epoetin acts on many tissues that express the erythropoietin receptor. Receptors for erythropoietin have been found in the retina, the central nervous system, vascular endothelial and smooth muscle cells, renal tubular cells, and even the heart myocyte. Erythropoietin markedly reduces the production and release of proinflammatory cytokines and chemokines (77), and dramatically reduces the influx of inflammatory cells into injured tissue in the brain and heart. Erythropoietin markedly prevents the apoptosis of cultured rat cardiomyocytes exposed to prolonged hypoxia, and erythropoietin reduces the size of myocardial infarction produced in rats and therefore helps to preserve myocardial function. Erythropoietin also is needed for normal differentiation of endothelial cells. In some tissues, erythropoietin may function in an autocrine manner (78). Ehrenreich et al. (79) has shown that erythropoietin improves the clinical outcome when given in acute strokes in humans, and it is likely extra bone marrow uses will become new applications for epoetin.

The human EPO gene, located on the long arm of chromosome 7, consists of five exons and four introns (80), but it is not directly regulated by molecular oxygen. Multiple transcription factors have been proposed most of which increase gene expression under conditions of hypoxia (81). GATA-2, a transcription factor that binds upstream to the erythropoietin promoter and represses gene transcription is unique as it is the primary candidate causing normoxic erythropoietin gene repression. In response to high pO_2, β-type cytochromes produce reactive oxygen species like H_2O_2. Human hepatoma cells decrease H_2O_2 production in response to decreased pO_2. It has been proposed that H_2O_2 inhibits erythropoietin gene expression via activation of GATA-2 (82). Other transcription factors have been implemented including HNF-4 (hepatocyte nuclear

factor-4), COUP-TF1 (chicken ovalbumin upstream promoter-transcription factor 1), TNF-α, IL-1β, and NFkβ.

However, the most important and best studied regulator is hypoxia-inducible factor, HIF-1, a 50-bp protein that acts on a hypoxia responsive element that is upstream of the gene (83,84). HIF-1 binds to the oxygen-sensitive enhancer to induce gene transcription of erythropoietin messenger RNA (mRNA) (83,85). The HIF-1α subunit is permanently present in all nuclei and hence regulation of HIF-1 is accomplished by abundance of the HIF-1α subunit. Hydroxylation of a proline residue within the HIF-1α domain by specific oxidases is a crucial step in the oxygen-sensing mechanism and eventual gene transcription of epoetin and other proteins (86). Abundance of HIF-1α is determined primarily by degradation via the ubiquitin-proteasome system in response to normoxia (87). Degradation requires the presence of a normal Von-Hippel-Lindau gene protein (pVHL) that binds to HIF-1α, permitting rapid proteasomic degradation in the presence of oxygen. Cells lacking pVHL are unable to degrade the factor in the presence of oxygen (hypoxia is mimicked) (88). Recently, superoxide (O_2^-) was found to block the accumulation of HIF-1β protein during hypoxia or $CoCl_2$ incubation (an inducer of erythropoiesis) by enhancing its degradation through ubiquitin-proteasome in renal medullary interstitial cells.

The EPO gene encodes for a 193-amino-acid prohormone. The first 27 amino acids are cleaved before secretion. Circulating EPO exists as a 166-amino-acid peptide containing two sulfide bridges and four sites of carbohydrate attachment (89). Four complex carbohydrate chains containing sialic acids are linked to the protein, and constitute 40% of its molecular weight of 34,000 daltons (34 kDa). The terminal arginine at position 166 is removed before secretion. The disulfide cross-links form two loops that are needed for biologic activity (89). Glycosylation is necessary for cellular secretion (90) and for the hormone's biologic activity *in vivo* because the sialic acid moieties allow EPO to circulate long enough to reach the bone marrow. Rapid receptor-mediated endocytosis when galactose residues are exposed reduces the half-time of desialated EPO to minutes (90). The fully sialated hormone has, in humans, a half-time varying from 4 to 12 hours (91). The carbohydrate sialic acid moieties are not essential for EPO action on bone marrow progenitor cells through receptor binding (92). The sialoglycoprotein released into the circulation is highly heat and pH resistant. Despite some differences in carbohydrate structure among available rHuEPO products, the biologic activity appears to be the same. The amino acid sequence has been modified to permit the addition of two additional sialic acid residues (93), producing a molecule (NESP) with a much longer pharmacologic half-life (94). The main site and mechanism of removal of native EPO, the recombinant epoetins, and NESP remain phenomenologic, although it seems most likely that all are degraded following EPO-receptor-mediated uptake into cells, chiefly erythrocytic progenitors in the bone marrow (95).

Erythropoietin Blood Levels

Under steady-state conditions in normal individuals, levels of erythropoietin in a given subject are remarkably constant. Diurnal variations do occur but are of relatively low amplitude (about 20% around a baseline of 10 to 17 mU/mL) with a peak at 1:00 AM and a nadir at 1:00 PM (96). Individuals with severe obstructive sleep apnea have higher baseline levels and larger amplitude swings, 46 mU/mL and 40%, respectively, resulting from prolonged nocturnal hypoxia.

In normal individuals, levels of EPO under steady-state conditions range from 6 to 25 mU/mL in both adults (27) and children (97). No differences in plasma immunoreactive EPO exist between the sexes in humans (98), although men produce more than woman because of their larger size. Prolonged nocturnal hypoxia increases (99), whereas significant protein deprivation decreases (100) baseline EPO levels. Under normal circumstances plasma levels reflect predominantly renal synthesis of erythropoietin in response to tissue oxygen need. Increased production of EPO results from the exponential recruitment of additional EPO-synthesizing cells (101) as the hypoxic stimulus increases (102). This exponential recruitment of EPO-producing cells produces the inverse relationship between circulating EPO levels and hematocrit.

Integration of the Erythropoiesis Control System

Erythropoietin regulates the number of committed erythroid precursors and causes them to mature into erythrocytes (103). The initial renal response to tissue hypoxia is similar whether the tissue hypoxia reflects hypoxemia low pO_2, reduced oxygen-carrying capacity (anemia), a hemoglobinopathy characterized by increased affinity for oxygen, or ischemia. The chief characteristic of the EPO–erythropoiesis system is that an error signal, reduced tissue oxygenation, defines the presence of an "inadequate erythron," leading to the production of EPO. The normal negative biofeedback system, induces an increase in EPO production that is reflected by plasma EPO concentrations. In the absence of renal disease, plasma EPO levels increase from normal values of less than 25 to approximately 100 mU/mL, as hematocrit decreases to mildly anemic levels of 27% to 33%. In severe anemia, EPO increases more than 100-fold. Levels in excess of 1,000 mU/mL are reached at hematocrits of less than 20% (Fig. 92-3) (50,104).

Changes in circulating erythropoietin levels depend almost entirely on hormone synthesis and release (105,106). The rate of erythropoietin production is determined primarily by the ratio of the oxygen requirements at or near the site of production to its oxygen supply (107,108). As a result, factors that decrease the supply of oxygen to the kidneys (such as decreased renal

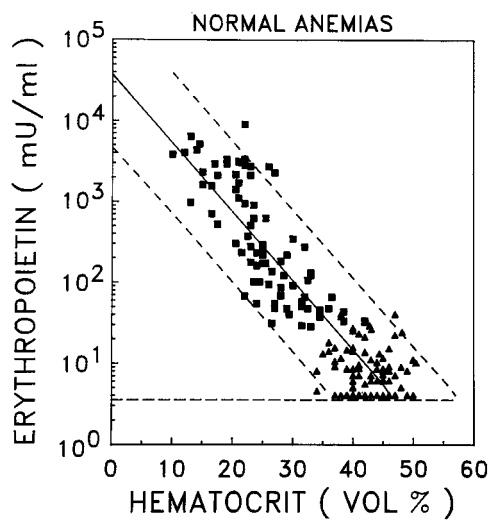

FIGURE 92-3. Inverse relationship of erythropoietin levels to hematocrit. Normal subjects are depicted by *triangles* and patients with various anemias (but excluding those with kidney disease, malignancy, or rheumatoid arthritis) by *squares*. *Dashed horizontal line* represents limits of detection of the radioimmunoassay for erythropoietin. (Adapted from: Erslev AJ. Erythropoietin. *N Engl J Med* 1991;324:1339, with permission.)

blood flow, decreased oxygen content of the arterial blood, or increased hemoglobin oxygen affinity) increase the production of erythropoietin and vice versa (the feedback control loop).

Oxygen transport is primarily dependant on the hemoglobin content of blood. Hemoglobin and hematocrit are related in a ratio of approximately 1:3. The kidney functions as a "critmeter," since it senses both oxygen tension and extracellular volume (109). Through erythropoietin it regulates red blood cell mass and through salt and water excretion it regulates plasma volume. The kidney thus regulates the numerator and denominator of the "crit." It is able to dissociate changes in blood flow from those in oxygenation. The normal hematocrit of 40% to 50% is not random, but rather one that maximizes delivery of oxygen to the tissues.

However, the ratio of tissue oxygen demand to supply, through its effects on EPO production, is only a "coarse" regulator of the rate of erythropoiesis. Other modulators must fine-tune the system because the red blood cell mass (total number of erythrocytes) would oscillate owing to the prolonged lifespan of the erythrocytes (120 days) and the lag time between exposure of CFU-E to elevated levels of EPO and the delivery of the mature reticulocyte to the bloodstream (~20–26 days).

These fine-tuning mechanisms are incompletely understood but may depend on various combinations of the following:

1. The maximum response of the bone marrow to markedly elevated EPO levels is limited. In severe anemias, EPO increases 100- to 1,000-fold, but the maximum red blood cell production rate increases only four- to sixfold above basal (106).
2. For any level of anemia, the negative correlation between the logarithm of EPO concentration and blood hemoglobin is shifted to higher levels in individuals with hypoplastic marrows compared to those without hyperplastic marrows (110).
3. A decrease in EPO levels is observed before a noticeable change in red blood cell mass occurs in chronically hypoxic rats (111). Similarly, EPO levels in normal humans peak within 24 hours after phlebotomy but rapidly decrease within the next 24 hours, although the red blood cell deficit remains uncorrected (112). This rapid return in EPO levels toward normal may result from intrarenal and systemic hemodynamic changes or in part from an expansion of progenitors that remove circulating EPO by receptor mediated binding and uptake (113).

The rapidity with which these changes in erythropoietin occur (1 to 2 days) suggests a direct effect on erythropoietin synthesis (114) and not merely on consumption by erythrocytic progenitors through the process of internalization and degradation (115). Furthermore, erythropoietin catabolism appears to be independent of marrow activity (116).

Nevertheless, in a responsive marrow the decrease in erythropoietin levels before an increase in red blood cell mass could result in part from an expansion of progenitors (particularly the CFU-E), and increased removal of erythropoietin by receptor binding. Modeling of the kinetics of hematopoietic stem cells during and after hypoxia indicates an up to a twofold increase in CFU-E by the fifth day of continuous hypoxia (117) and suggests that increased consumption could account, in part, for the decrease in levels. However, intermittent rather than continuous hypoxia maintains even greater levels, even though the increase in red blood cell mass (from marrow precursors) is about the same. Jelkmann (118) has suggested that this results from pH-dependent effects on the producing cells themselves or from the weight loss (and, therefore, the protein deficit) associated with prolonged continuous hypobaric hypoxia. In our studies in normal humans, there is a rapid (within 24 hours) decrease in erythropoietin levels from peak values following one and two-unit-phlebotomy (112). In these subjects there was no detectable weight loss. Changes in intrarenal oxygen tensions resulting from intrarenal and systemic hemodynamic changes may therefore explain this rapid return in erythropoietin levels toward normal.

PATHOPHYSIOLOGY OF THE ANEMIA OF RENAL DISEASE

Clinical Aspects

In progressive renal insufficiency, the degree of anemia is, in general, proportional to the severity of azotemia (5). Among patients, the correlation of hematocrit with glomerular filtration rate (GFR), blood urea nitrogen, or serum creatinine is imprecise (Fig. 92-4) (119). However, as renal function approaches that requiring replacement therapy, the hematocrit tends to plateau at a level that varies little thereafter in the absence of complications or institution of rHuEPO therapy. In the past, most patients initiating dialysis had hematocrit values between 15% and 25%. In nephric anemic patients with ESRD on dialysis, the mean baseline values of EPO are marginally higher than normal, 19 to 30 mU/mL (31) compared with 10 to 17 mU/mL in normal individuals (120,121). Therefore, although the end-stage kidney continues to produce EPO despite effective cessation of excretory function, it is incapable of augmenting EPO production *adequately* in response to an appropriate anemic hypoxic stimulus. Maintenance of "appropriate" plasma levels during severe anemia would require that the residual renal tissue have the capacity to increase erythropoietin production by about 5- to 20-fold if metabolic clearance rate were independent of plasma concentration. In anephric individuals, the levels are markedly decreased but still measurable by radioimmunoassay (RIA) (31,122). The absolute deficiency of erythropoietin in such anephric patients (96) made them blood transfusion-dependent prior to the advent of epoetin.

Anemia develops during acute renal failure from tubular necrosis (49), interstitial nephritis, and glomerulonephritis (50), with the hematocrit gradually falling to 25% to 30% in the absence of excessive blood losses. The anemia results from deficient EPO production (50) (which does not reverse

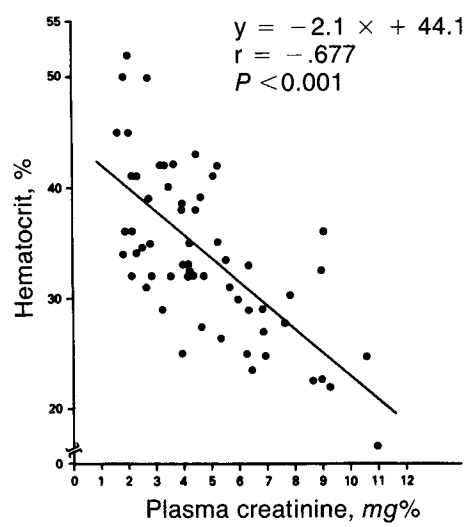

FIGURE 92-4. Relationship of hematocrit to creatinine. (Reprinted from: McGonigle RS, et al. Erythropoietin deficiency and erythropoiesis in renal insufficiency. *Kidney Int* 1984;25:437, with permission.)

until excretory function recovers), from shortened red blood cell survival (16), and from hemodilution (123). As renal function recovers, erythropoiesis gradually improves, with the red blood cell mass normalizing over several months.

Anemia may be more severe than anticipated in some forms of acute renal failure (Goodpasture's syndrome, Henoch-Schönlein glomerulonephritis). Multiple myeloma often presents with a disproportionately severe anemia because erythropoiesis is further compromised by plasma cell infiltration and marrow replacement. Erythropoietin production relative to renal function may be reduced more than usual in radiation nephritis (124). Hemolysis may be significant in systemic lupus erythematosus, scleroderma, and other vasculitides associated with acute renal failure. Hemolysis with grossly deformed cells (fragmented red blood cells on peripheral smear) formed in the microcirculation occurs in the hemolytic uremic syndrome. The hematologic features include a red blood cell production index that usually is greater than three times normal despite azotemia or acute renal failure.

The amount of erythropoietin that is produced per unit weight of remaining "normal" nonscarred kidney tissue may actually be normal. The importance of adequate renal mass is best illustrated by the correction of anemia following successful transplantation (125,126). Following such transplantation in ESRD patients, EPO levels increase and are sustained severalfold as excretory function recovers. With increased production of red blood cells, anemia corrects and erythropoietin levels progressively decrease into the normal range.

Inadequate Erythropoietin Production

The persistent observations of many investigators of "inappropriately" low EPO levels in virtually all cases of advanced kidney disease National Kidney Foundation Kidney Diaysis Outcomes Quality Iinitiative (NKF-KDOQI stages 4 and 5) indicates that the primary factor in the anemia of renal failure is the inadequate production of endogenous EPO by these diseased kidneys. Shortened red blood cell survival (hemolysis or chronic blood loss) also may play a role but probably is more instrumental in determining the severity of anemia. Renal disease therefore affects the red blood cell mass by interfering with both red blood cell production and red blood cell lifespan through disruption of the kidney's endocrine and exocrine functions. Other factors such as iron and other nutritional deficiencies and the putative effects of uremic inhibitors, if left unattended, will influence the severity of anemia and the outcome of rHuEPO treatment in any patient with progressive renal failure or ESRD. Specific and sensitive RIA can measure even subnormal serum EPO levels. Besarab et al. (112) and others (127) have shown that ESRD patients with acute blood loss or hemolysis can increase plasma RIA-EPO levels two- to five-fold, but the levels seldom reach those achieved by individuals without renal disease. Diseased kidneys appear incapable of augmenting EPO production chronically in response to an appropriate anemic hypoxic stimulus (128). Patients with autosomal dominant polycystic kidney disease are the exception and typically have higher EPO levels with less severe anemia (129). High EPO concentrations have been found in the fluid of cysts originating from proximal tubules with EPO mRNA identified in their interstitial cells (130).

The steady-state relationship between plasma RIA EPO levels and hematocrit (Fig. 92-4) reflects the operation of a negative feedback system and the homeostatic attempt of normal kidneys to correct the anemia (122). In patients with ESRD, this biofeedback mechanism is impaired. In stable patients on dialysis, hematocrit correlates directly rather than inversely with EPO levels (Fig. 92-5). Plasma EPO levels among different patients with ESRD vary 5- to 10-fold; within a given patient, EPO levels tend to be similar over time. Patients with ESRD

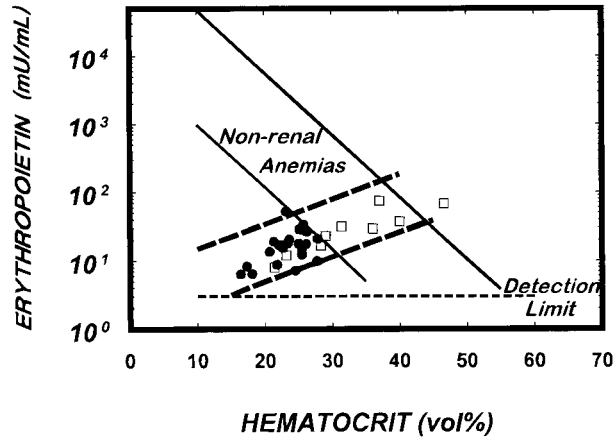

FIGURE 92-5. Relationship of erythropoietin levels to hematocrit in end-stage renal disease (ESRD). The 95% confidence range for erythropoietin values of patients without renal disease is given by the *solid lines*. The two *heavy dashed lines* represent 95% confidence range of patients just before renal transplantation. The *solid circles* and *open squares* represent erythropoietin levels of two cohorts of hemodialysis patients. Note the direct relationship between erythropoietin levels and hematocrit in patients with ESRD.

with acute blood loss or hemolysis can increase their plasma RIA EPO levels two to fivefold (112), but normal subjects sustaining equivalent degrees of blood loss increase their levels 10- to 100-fold. These observations indicate that basal EPO production is disordered in a variable way, but that the feedback loop between EPO and tissue oxygen delivery is still present. EPO production per unit weight of remaining "unscarred" kidney tissue may be above normal but limited by functional renal mass.

Shortened Erythrocyte Survival

The lifespan of red blood cells in patients with chronic renal disease usually is shorter than normal (131,132) (Fig. 92-6), typically decreasing from a normal value of 120 days to average values of 70 to 80 days. However, as first pointed out by Chaplin and Mollison in 1953 (131), a normal marrow should easily compensate for such mild decreases in red blood cell survival with sufficient circulating EPO. Uremic red blood cells survive normally when injected into healthy recipients, and normal red blood cells may have a shortened lifespan in uremic

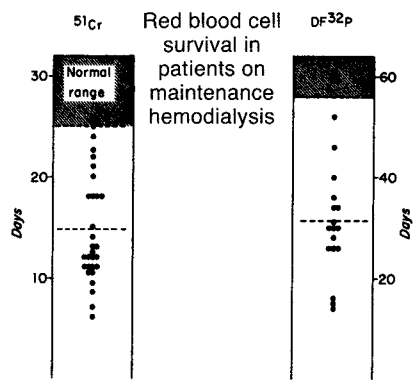

FIGURE 92-6. Red blood cell survival in patients on maintenance dialysis as assessed by 51Cr or DF 32P half-lives. (Reprinted from: Eschbach JW Jr, Funk D, Adamson J, et al. Erythropoiesis in patients with renal failure undergoing chronic dialysis. *N Engl J Med* 1967;276:653, with permission.)

recipients (130,133). In renal failure, both metabolic and mechanical factors may shorten survival of red blood cells. The presence of a metabolic factor is suggested by the normalization of red blood cell lifespan after intensive dialysis (134). However, most red blood cell enzymes in uremic red blood cells are normal or have increased activity, and ATP levels are high (135). Only the activities of transketolase, active in the hexose monophosphate shunt (136) and ATPase, which supports the Na^+,K^+ membrane pumps (137), are decreased. The decreased activity of the Na^+,K^+ pumps influences changes in red blood cell shape and rigidity, which in turn affects red blood cell lifespan. This defect has been attributed to either uremic impairment of the enzymatic activity (138) or to decreased synthesis of Na^+,K^+ pump units by uremic reticulocytes (139). The toxic substances responsible for these metabolic impairments have not been fully identified but are presumably dialyzable, since intensive dialysis corrects the defect. We have noted that red blood cell lifespan can approach normal in well-dialyzed patients, with particular attention to minimizing blood sampling for studies (17). Measurement of red blood cell lifespan measures both normal removal of senescent cells by the reticuloendothelial system and extracorporeal premature exit of erythrocytes from the circulation. The latter results from blood losses due to vascular access puncture, residual blood left in dialyzers, occasional blood leaks, phlebotomy for laboratory testing, and clotted dialyzers. Retention of red blood cells on the dialysis membranes accounts for a loss of 0.5 to 11 mL of red blood cells per dialysis, depending on the type of dialysis membrane used and how carefully the dialyzer is rinsed after dialysis. Such "routine" blood losses associated with hemodialysis, typically approximately 60 mL of whole blood per week (140,141) and contribute significantly to the iron deficiency of these patients, resulting in effective iron losses of between 1 to 3 g of iron per year. Such losses are obviously diminished in peritoneal dialysis.

Blood Loss

Blood loss may contribute to anemia in patients with advanced renal failure before dialysis. As high as 25% of such patients may be iron deficient because of chronic blood loss (15). Bleeding associated with renal insufficiency (142,143) has been known for decades. Common manifestations include telangiectasia and gastrointestinal angiodysplastic lesions (144). Functional abnormalities of platelets characterized by prolonged bleeding times, abnormal platelet aggregation and adhesiveness, and reduced platelet factor 3 release are well recognized (145). A reversible abnormality in the activation-dependent binding activity of glycoprotein IIb-IIIa occurs in uremia (143,146). Other defects include abnormalities in the multimeric structure of von Willebrand factor (147) and acquired platelet storage pool deficiencies of adenosine diphosphate and serotonin (148). Uremic plasma also induces nitric oxide synthesis by cultured endothelial cells, which inhibits platelet function (149). This inhibitor has been shown to be a guanidine derivative (150).

All these notwithstanding, anemia itself appears to be a major factor in sustaining the bleeding tendency, as prolonged bleeding times are corrected by both red blood cell transfusions (151,152) and the rise in hematocrit that occurs with therapeutic use of rHuEPO (153,154).

Iatrogenic Hemolysis

In dialysis patients, decreased activity of the hexose monophosphate shunt renders hemoglobin and the red blood cell membrane sensitive to oxidant drugs or chemicals (155,156). Approximately 25% of all uremic patients have defective red blood cell pentose-phosphate shunt activity, as detected by an abnormal ascorbate-cyanide screening test (156). This reduces NADPH production with decreasing available reduced glutathione. Reduced glutathione prevents the formation of unstable oxidized hemoglobin compounds that precipitate to form Heinz bodies. The presence of substances with strong oxidizing potential such as primaquine, quinidine, sulfones, Furadantin, and nitrofurantoin should be avoided. Hemolysis with formation of Heinz bodies (157) has been reported in association with inadequate removal of chloramine (158) coming from city tap water used for dialysate. Acute hemolysis must also be avoided by removal of copper (159) zinc (160), aluminum (161), and nitrates (162) in the water supply, and formaldehyde (163) from reprocessed dialyzer equipment. Vigorous treatment with nonabsorbable aluminum gels produces hypophosphatemia, which increases the intracellular calcium, in turn resulting in the polymerization of spectrin, making the red blood cell membrane rigid and susceptible to hemolysis (164).

Splenic Dysfunction

A significant number of patients on chronic hemodialysis have splenomegaly (165,166). These spleens contain hyperplastic lymphoid follicles and increased numbers of macrophages and plasma cells, changes consistent with reaction to chronic antigenic stimulation (163). One report has described macrophages laden with silicon particles (167), the silicon perhaps arising from dialysis blood line tubing stressed by roller pumps. Rosenmund et al. (154) reported that red blood cells from dialysis patients with hypersplenism are less filterable than those of other uremic subjects and are more susceptible to oxidative stress. Cells with unstable oxidized hemoglobin components may be sequestered in the splenic macrophages, which results in hyperplasia of splenic macrophages and splenomegaly. Splenectomy has been performed as a means of prolonging red blood cell lifespan in a few patients with splenomegaly and excessive red blood cell destruction documented by radioisotope studies. The results are rarely spectacular and the decision to operate should be carefully weighed and reserved for those with either coexistent neutropenia or thrombocytopenia, or in those with resistance to exogenous epoetin, particularly since the dose of epoetin can be increased to increase red blood cell production to match the rate of destruction.

Mechanical Fragmentation

Despite data supporting a metabolic basis for hemolysis, some investigators have failed to find a clearcut correlation between red blood cell lifespan and degree of renal failure (5). An alternative mechanism is that red blood cells are injured and prematurely destroyed by intravascular mechanical trauma rather than by metabolic alterations (168). Even normal erythrocytes become deformed when exposed to strong shearing stress and are vulnerable to monocyte-macrophage sequestration, especially at a fibrin interphase (168). This occurs in some cases of malignant hypertension (169) and in the hemolytic-uremic syndrome, in which the anemia is typically out of proportion to the degree of renal insufficiency in the early stages of the disease (170).

Inhibition of Erythropoiesis

In the absence of significant overt blood loss, decreases in red blood cell survival of 30% to 40% alone cannot fully account for the degree of anemia in renal failure. Whereas EPO levels are comparable, red blood cell production in patients with renal failure is only one-half of normal (Fig 92-7). If one examines the relationship of EPO levels to hemoglobin/hematocrit levels in the earliest stages of kidney disease, EPO levels are appropriately increased for the degree of anemia until renal function drops to less than 40% of normal (Fig. 92-1). Thereafter, EPO levels cannot be sustained and decrease progressively. Since the

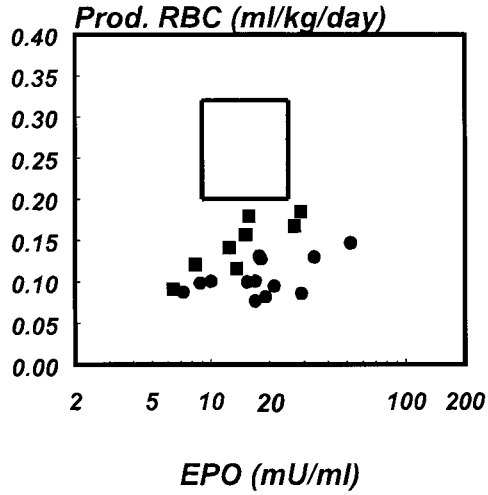

FIGURE 92-7. Red blood cell production (*Prod. RBC*) in normal subjects and patients on maintenance hemodialysis. Normal range is shown by the *area within the box*. Dialysis patients are segregated into those with red blood cell survival greater than 100 days (*circles*) and less than 100 days (*squares*). The erythropoietic (*EPO*) response is shifted down and to the right in patients on dialysis.

late 1960s, numerous *in vitro* studies have implicated an inhibitory effect of uremic serum on growth of erythroid precursors or on heme synthesis (171–174). Intensive dialysis can increase iron utilization and hematocrit without altering the level of circulating EPO (175). Older studies tried to identify polar lipids, arsenic, vitamin A, spermine, spermidine, and parathyroid hormone (171,172,176,177) as specific uremic inhibitors. More recent studies indicate that their role in the genesis of the anemia of renal failure is of very minor importance (178–182). In humans, acute ferrokinetic responses to rHuEPO do not differ among patients on hemodialysis, normal subjects, or patients with chronic renal failure restored to normal by transplantation (180). The improvement of anemia demonstrated after parathyroidectomy is due to the resolution of marrow fibrosis (182) rather than the removal of erythropoietic inhibition (181).

Despite the clinical experience showing that exogenous EPO overcomes the effects of such putative inhibitors, studies on uremic inhibitors continue because control of such factors could reduce the amount of epoetin needed. Recent studies have focused on the effects of albumin-bound furancarboxylic acid (183), activated monocytes, and polymorphonuclear leukocyte products (184) and cytokines on erythropoiesis. Quinolinic acid levels correlate positively with creatinine concentration and negatively with endogenous EPO levels in uremic rats. Urea-derived cyanate carbamylates endogenous erythropoietin and may decrease its biologic activity (185). Cobalt-stimulated erythropoiesis was suppressed dose-dependently in chronic quinolinic acid-treated rats. Moreover, quinolinic acid had a dose-dependent inhibitory effect on hypoxia or cobalt-induced EPO release and EPO gene expression suggesting disruption of EPO gene activation by HIF-1 (186).

T cells from uremic subjects may be unable to release general growth cytokines needed for optimal erythropoiesis (187). In addition, inflammation-associated cytokines inhibit erythropoiesis. CFU-colony formation is suppressed by soluble factors in uremic sera that lead to the production of interferon-γ and TNF-α (188). IL-6, a pro-inflammatory cytokine, is present at 8- to 10-fold higher levels in hemodialysis patients, being higher in patients treated with less-biocompatible membranes, and inhibits EPO-induced bone marrow proliferation (189). More intensive dialysis increases response to exogenous epoetin. Ifudu et al. (190) found a direct relationship between hematocrit and urea reduction ratio (URR) after adjustment for other variables. Analysis of Medicare data also shows a direct relationship between higher URR and higher hematocrit at lower epoetin doses (191). The role of high-flux membranes compared to low-flux membranes is unclear (192). The role of on-line treatment in which dialysate is free of toxins or pyrogens has produced conflicting results (193).

Nutritional Factors Contributing to Anemia

The patient with chronic renal failure or on maintenance dialysis is prone to anorexia, intercurrent illnesses, and dietary restrictions. Dialysis can also produce dialysate nutrient losses. All patients should be observed for malnutrition and vitamin deficiency syndromes. Folate deficiency in dialysis patients is uncommon (194) because routine use of supplements replaces dialysate losses. Most centers supplement their patients with 1 mg per day of folic acid. This is generally safe, as vitamin B_{12} deficiency is uncommon in dialysis patients. More recently higher dose folate has been used to unsuccessfully reduce homocysteine levels. Because of the water solubility of thiamine, pyridoxine, and vitamin B_{12}, deficiencies in one or more of these vitamins could develop from dialytic removal, but no cases have been reported in dialysis patients. Currently, only pyridoxine supplementation is recommended; 5 mg per day for those with progressive renal failure and 10 mg per day for dialysis patients (195).

Borderline or frank iron deficiency is the most common "nutritional deficiency." It occurs to a lesser extent in conservatively treated patients (pre-ESRD), in those on continuous ambulatory peritoneal dialysis (CAPD), and is a major impediment to the cost-effective use of epoetin. Three factors have been implicated in the pathogenesis of iron deficiency in renal failure patients: (i) blood loss caused by retained erythrocytes in dialyzers and blood tubing, and on dressings, and by frequent phlebotomies for diagnostic testing; (ii) the bleeding diathesis caused by uremia; and (iii) in the era of aluminum-containing phosphate binders, malabsorption of iron due to such aluminum binders (196).

Although iron absorption appears to be unimpaired (197,198,199) and iron turnover appears normal in severe renal impairment, iron utilization is regularly decreased, particularly in inflammatory renal disorders. In rare cases of nephrotic syndrome, urinary losses of transferrin can cause low iron-binding capacity, with impairment in the metabolic cycling of iron (200). Although iron absorption may be decreased in some patients, Eschbach et al. (197) observed that, as expected, the percentage of iron absorbed from the gastrointestinal tract of uremic subjects varies inversely with the iron stores. Although they concluded that uremia *per se* does not interfere with the physiologic regulation of iron absorption, the amount of iron taken orally may not be sufficient to meet the needs of active erythropoiesis, particularly after epoetin administration, and parenteral iron may be necessary. The nontransfused patient on hemodialysis is prone to iron deficiency because of such repetitive blood losses. At a target hematocrit of 30% to 36%, such losses of red blood cells equate to an additional 6- to 7-mg iron loss per dialysis above normal obligatory daily iron losses of 1 to 2 mg per day. With the additional losses from periodic laboratory tests, the yearly iron losses can exceed normal total body stores of iron of approximately 1,200 mg. Most regular hemodialysis programs require iron supplementation, averaging 2 g per year, usually parenterally, to prevent iron deficiency.

Decreased serum levocarnitine levels are associated with epoetin hyporesponsiveness, and replacement therapy has improved the response to epoetin therapy. Improved red blood cell survival and decreased Na/K-ATPase activity have been proposed as potential mechanisms (201,202).

DIAGNOSTIC WORK-UP AND MONITORING OF ANEMIA IN CHRONIC KIDNEY DISEASE

The treatment of the anemia of chronic renal failure has assumed greater clinical importance as the age of the CKD and ESRD population has increased. Such patients have more frequent and greater degrees of ischemic heart and peripheral vascular disease and are increasingly composed of diabetic patients with both microvascular and macrovascular disease. Many symptoms previously attributed to "uremia," such as fatigue, cold intolerance, and mental sluggishness, respond to correction of anemia. With the advent of epoetin therapy, nonspecific therapies such as transfusions and anabolic steroids are of historic interest only. Because epoetin therapy takes weeks to months to correct significant degrees of anemia, symptomatic patients with ischemic heart or cerebrovascular disease should receive transfusions. Similarly, treatment of symptomatic acute blood loss requires transfusions. If major surgery is anticipated, autologous blood donation for intraoperative use is possible if enough epoetin is used (203), thereby avoiding the need for homologous red blood cell transfusions. Patients with sickle cell disease remain transfusion-dependent despite very high doses of epoetins, and continue to experience iron overload.

The initial evaluation of the anemic patient with CKD should include a complete blood count (CBC) including red blood cell indices, a reticulocyte count, and determination of the serum iron, total iron binding capacity (TIBC), and ferritin (collectively referred to as *iron indexes*). Recently, the reticulocyte hemoglobin content (CHr) (204) and the percentage of hypochromic cells (205) have received attention as more reliable predictors of iron sufficiency in hemodialysis patients. Their utility in patients with CKD stages 3 to 5 is unknown. If the anemia is normochromic and normocytic with a normal or low reticulocyte index, the peripheral smear shows no abnormalities, and the iron-indexes are normal, then no further work-up of the anemia is necessary.

Assessment of Iron Status

The time-honored tests have been transferrin saturation (TSAT) that reflects the carrying capacity in blood and serum ferritin, reflecting tissue stores of iron. In non-CKD individuals, a ferritin value of less than 30 mg/L has been used as an indication for iron supplementation. Studies correlating serum ferritin, TSAT, and stainable bone-marrow iron have indicated that ferritin levels as high as 100 to 125 mg/L may indicate iron deficiency in ESRD patients (206,207), and even these higher levels are unable to detect "functional iron" (208–212). Transferrin saturation and ferritin levels should exceed 25% and 200 ng/mL, respectively, in the iron-replete hemodialysis patient to optimize erythropoiesis (213). These parameters likely apply to the patient with progressive renal failure as well. Periodic monitoring of the iron indices is mandatory to detect functional iron deficiency. Anemic patients with iron indices minimally greater than those enumerated become iron deficient during epoetin therapy unless supplemented with iron. The most common etiology for microcytosis remains iron deficiency. Low serum iron with elevated ferritin suggests systemic infection, inflammatory disease, or occult malignancy. Normal or high serum iron with normal ferritin but microcytic indices points to the presence of thalassemia, or now less frequently lead or aluminum toxicity. Macrocytosis is unusual in the well-dialyzed patient receiving a vitamin supplement and consuming a recommended protein diet of 1.0 to 1.4 g/kg.

Periodic monitoring of the complete blood count and red blood cell and iron indices in patients on dialysis detects variance from the patient's steady-state values. The nomogram of VanWyck et al. (214) is helpful in estimating the amount of exogenous iron needed during induction therapy to attain the target hemoglobin of 11 to 12.5 g/dL. We prefer to calculate the amount on the basis of 1 mg of iron needed for each 1 mL of packed red blood cells formed. The increase in packed cells is estimated from the starting and target hematocrits and the estimated blood volume of the patient (70 to 80 mL/kg body weight). Patients receiving hemodialysis have significant ongoing weekly blood losses of approximately 40 to 50 mg, and, unlike patients on CAPD, usually require parenteral iron, both during the period of correction of anemia as well as during the long-term maintenance phases.

Prior to the year 2000, iron dextran was the only available agent used extensively in the United States. Now as in Europe other agents are available (208,215). Administration of sodium ferric gluconate complex, 1.0 g over eight consecutive dialysis treatments, significantly increased hemoglobin levels, serum ferritin, and iron saturation in 83 iron-deficient dialysis patients (216). Many physicians are concerned about the risks of using parenteral intravenous iron dextran. The incidence of severe life-threatening reactions to one or more doses in any patient is quite low, at less than 1% (217,218). The rate per number of injections is even lower at 0.1%. Decreasing the infusion rate to less than 10 mg/minute minimizes reactions (personal experience).

Sodium ferric gluconate complex in sucrose may have fewer fatal adverse reactions than iron dextran, and its efficacy as good as iron dextran (219). Similar results have been found with iron sucrose (220). Both sodium ferric gluconate complex (219) and iron sucrose (221) have been administered to iron-dextran allergic patients. Cost-effectiveness may be the only factor preventing these agents from being preferred over iron dextran, since they are one-third more expensive. They also can not yet be given as total dose infusion, a practice possible with iron dextran. A new iron preparation, ferumoxytol, can be given at doses of 510 mg in less than one minute. Efficacy and safety data will soon be available.

The complication of iron overload from multiple transfusions has disappeared since the widespread application of epoetin therapy. Some have even used the strategy of periodic phlebotomy to reduce iron deposition, previously acquired by multiple transfusion, in epoetin-treated patients (222).

Paradoxical Absence of Anemia

Absence of anemia or amelioration of preexisting anemia in patients with progressive renal failure or on dialysis requires a search for potential causes. Possible etiologies include (i) contracted predialysis plasma volume, (ii) decreased hemoglobin oxygen saturation from cardiac or pulmonary disease, (iii) hereditary or acquired cystic kidneys, and (iv) conditions that cause decreased blood flow to the kidneys or liver. Increased endogenous EPO production (can occur in the last three circumstances (223) indicating that even anuric diseased kidneys can increase their production of EPO under some circumstances. Hypoxemia or conditions producing reduced renal blood flow can increase EPO production, but hematocrit seldom increases to greater than normal levels. Development of acquired cystic kidney disease (ACKD) associated with increased erythropoietin levels can increase the hematocrit and accounts for the occasional spontaneous increase in hematocrit in some patients maintained for years on dialysis (221,224). Because ACKD is progressive (225) the associated increase in hematocrit and erythropoietin levels seen in many patients could result from the progressive development of more cysts over time with compression of remaining renal tissue. Two mechanisms have been proposed for the increased erythropoietin production in such cases: (i) increasing tissue pressure on remaining renal parenchyma by the cysts, producing worsening

local hypoxia and (ii) autonomous production of erythropoietin by proliferating epithelial cells within the cyst wall (221,222). However, some investigators have not noted a correlation between the presence or absence of cysts and hematocrit; rather, the hematocrit appeared to correlate best with age, duration of dialysis, and increased renal tissue volume (226). Our experience indicates no correlation between the initial presence or absence of cysts and RIA-EPO levels and none between the subsequent development of ACKD and follow-up RIA-EPO levels (227). Therefore, the role of cysts *per se* is still unclear and may depend not only on the number of cysts but the cell type as well (228).

Very rarely, patients with CKD develop polycythemia, either coincidentally due to polycythemia rubra vera or secondary to processes known to cause secondary polycythemia. Those states associated with hypoxemia or reduced renal blood flow may result in an increase in erythropoietin production and improvement in the anemia. Conditions associated with paraneoplastic erythropoietin production potentially can result in marked increases in the red blood cell mass. In most instances, high hematocrits (>48%) in uremic patients cannot be explained but occur in association with increased erythropoietin levels. Erslev et al. (229) studied two anephric patients whose hematocrits gradually rose to more than 30% after being on chronic hemodialysis for more than a year. In both patients, elevated plasma erythropoietin levels could not be suppressed even after transfusion to hematocrits in excess of 40%. This suggests that extrarenal erythropoietin production in some patients is regulated differently than renal erythropoietin production, an observation supported by experimental data in transfused uremic rats (230). Erythrocytosis of renal origin can be distinguished from polycythemia vera by EPO levels because EPO is below normal in the latter condition.

On occasion, hematocrit increases in an ESRD patient during hepatitis. Kolk-Veghter et al. (231) observed a transient increase of hematocrit in several patients on hemodialysis during episodes of active hepatitis. Others have also noted an increase in the hematocrit and erythropoietin titer of patients with hepatitis during the period of elevated serum transaminases (232).

THERAPY FOR RENAL ANEMIA

Transfusions

The introduction of epoetin for the therapy of the anemia of CKD has virtually eliminated the need for repeated transfusions and the risk of developing hemochromatosis. The major groups still at risk are patients with sickle cell disease (233) or thalassemia (234), who do not respond to "reasonable" doses of erythropoietin.

Transfusions should not be withheld from patients with symptomatic or refractory anemia, particularly those with underlying ischemic heart disease, because it takes weeks for epoetin to increase hematocrit. In situations of gastrointestinal bleeding, postoperative blood loss, or hemolysis, transfusions still remain the mainstay of therapy. Given the infection risks of transfusion and the sensitization of potential transplant recipients, transfusions should be used prudently.

Anabolic Steroids

Response to epoetin can be augmented by anabolic androgens and is used by health care systems with limited resources. This therapy consists of blood transfusions with or without the administration of androgenic-anabolic steroids (235,236). Androgenic steroids increase the hematocrit of uremic patients by increasing erythropoietin production and to a lesser extent, stimulating committed bone marrow stem cells. Fluoxymesterone and oxymetholone are given by mouth in doses of 10 to 20 mg per day and 1 to 4 mg/kg body weight per day, respectively. Parenteral preparations, such as nandrolone decanoate or testosterone propionate or enanthate, are presumed to be more effective (234). They are given in doses of 1 to 4 mg/kg body weight once a week.

Recent studies have explored the use of androgens along with epoetin. One found synergy between epoetin and nandrolone decanoate with few side effects (237); the other found no synergy and many side effects (238). A third study found that the use of androgens in men younger than 50 years was as effective as epoetin and less costly (239). In view of the known side effects of androgen therapy, particularly in women, we do not advocate its use alone or in combination with epoetin.The recent NKF-KDOQI Guidelines for anemia also reject the adjuvant use of anabolic steroids

Epoetin Administration

Clinical trials of rHuEPO were initiated in 1985, and replacement therapy with rHuEPO quickly became the most rational therapy for anemia of chronic renal failure (240–244). These initial clinical trials convincingly demonstrated that the hematocrit could be increased by up to 10 points or more and maintained at a level greater than 30% in more than 90% of patients. Dialysis-treated patients increased their hematocrit in a dose-dependent manner (240), as shown in Figure 92-8. Maintenance intravenous doses needed thrice weekly to maintain steady-state hematocrits greater than 31% vary significantly among study patients: 15% required more than 150 U/kg and 20% less than 40 U/kg (240). Similar results have been observed worldwide (241). Comparison of epoetin dose variation in different countries shows much greater average doses in the United States compared to the United Kingdom and Italy (245). This may result from differences in route of administration, but the significantly larger variation in doses in the United States was related to markers of inflammation and the use of tunneled catheters.

Significant improvement has been made in the management of the anemia of CKD/ESRD during the last decade. More than 92% of hemodialysis patients and about 60% of Medicare-eligible patients on continuous ambulatory peritoneal dialysis (CAPD) were receiving epoetin at the end of 1994 (246). Since then both epoetin dose and mean hemoglobin have

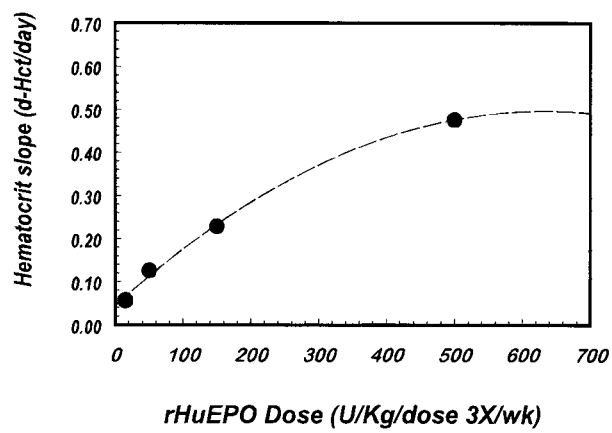

FIGURE 92-8. Dose–response curve to intravenous epoetin alfa (recombinant human erythropoietin [rHuEPO]) administered three times a week. The linear part of the response curve extends up to 150 U/kg/dose. (Adapted from: Eschbach JW, Egrie JC, Downing MR, et al. Correction of the anemia of end-stage renal disease with recombinant human erythropoietin: results of combined phase I and II clinical trials. *N Engl J Med* 1987;316:73.)

progressively increased, reaching the target range of 33% to 38% achieved in clinical trials. As of the last quarter of 2002, mean hemoglobin had reached 11.7 g/dL and epoetin dose exceeded 7,000 U/dose (ESRD CPM Project Report 2002) (247). In addition, there has been recognition of the need to optimize anemia management before patients reach the stage of kidney failure requiring dialysis (248,249).

In CKD patients not yet receiving renal replacement therapy (RRT), the subcutaneous route is effective and permits self-administration. A negative effect of epoetin on renal function has not been noted in clinical human trials or during longer term follow-up. No significant alteration in the progression of renal disease (as assessed by the slope of the reciprocal of plasma creatinine or by direct measurement of GFR) secondary to changes in systemic hemodynamics or blood volume (249–253) has been noted in carefully conducted clinical trials. In fact there is an increasing trend toward treating anemia early in CKD.

All investigators emphasize the importance of blood pressure control in preventing deterioration in renal function. If increases in blood pressure during rHuEPO therapy are controlled, correction of anemia does not alter renal hemodynamics or accelerate progression of renal insufficiency. Other studies have shown the safety of home subcutaneously injected epoetin once a week. With the development of epoetins with longer circulating half-times, dosing intervals in such patients are being extended out to every 4 to 6 weeks.

RESULTS OF EPOETIN THERAPY

Transfusion Avoidance

Before the availability of epoetin therapy, nearly 25% of hemodialysis patients were transfusion dependent (254), receiving up to 0.7 units of packed cells monthly. These patients were at risk for iron overload and organ dysfunction, although most patients with transfusional iron overload have hemosiderosis (255), a state with minimal organ dysfunction, and not hemochromatosis (256). Even livers that contain more than 1,000 μg iron/100 mg dry tissue (normal is <200 μg/100 g dry tissue) show little fibrosis or damage (257). However, iron overload may increase susceptibility of dialysis patients to infection (258). Iron-overloaded patients have been treated with higher dose epoetin to accelerate iron removal through periodic phlebotomies (222,259,260). Both magnetic resonance imaging (MRI) (261) and computed tomography (CT) (262) can be used to monitor hepatic iron during "phlebotomy" therapy. We have transitioned from an era in which iron overload was the problem to one in which the major problem for ESRD patients on maintenance epoetin therapy is the development of iron deficiency.

Studies have demonstrated that elimination of transfusions produces a marked reduction in the percentage panel reactive antibody (%PRA) (263) as well as in anti-HLA specific antibody titers (264). If blood transfusion cannot be avoided, irradiated packed red blood cells should be administered.

Quality of Life and Cognitive Functions

We now recognize that many of the symptoms attributed to uremia are really the result of anemia. Fatigue, cold intolerance, impotence, and mental sluggishness respond to correction of anemia. In patients with ESRD, correction of anemia with epoetin therapy improves quality of life (QOL) indices, including those assessing global well-being and depression in hemodialysis patients (265) and CAPD patients (266). CKD patients who do not yet require dialysis also show improvement in quality of life indices following rHuEPO therapy (267). Substantial improvements in subjective symptoms were noted in a large double-blind phase II study (268). Clinically important improvements were noted in fatigue, perceived strength, and global score of the Sickness Impact Profile. A phase IV trial of more than 1,000 patients receiving epoetin in clinical practice (269) confirmed these QOL effects even when only a modest mean hematocrit of 30% was achieved. The Canadian Erythropoietin Study Group (270) could not demonstrate an increase in QOL when hemoglobin was increased from 10.2 to 11.7 g/dL (hematocrit 31% to 35%), suggesting that there might be a plateau. By contrast, Moreno et al. (271) showed that both the Karnofsky functional scale and the Sickness Impact Profile correlate positively with the hematocrit between 29% and 35%. Whether QOL indicators can be improved further by raising hematocrit even higher is currently unclear.

Cognition

Correction of anemia also improves cognition when hematocrits are increased into the 32% to 36% range (272–274). Increasing the hematocrit to 42% with epoetin further improves brain and cognitive function (275), perhaps because maximum delivery of oxygen to the brain occurs within a hematocrit range of 40% to 45% (276). The reports that neuronal cells carry the EPO receptor (277) and that EPO can be produced within the brain in a paracrine fashion (278) predicted the possible clinical use of epoetin in stroke syndrome (279). Erythropoietin has effects in many tissues besides the bone marrow (Fig. 92-9) and may protect against hypoxia-induced neuronal

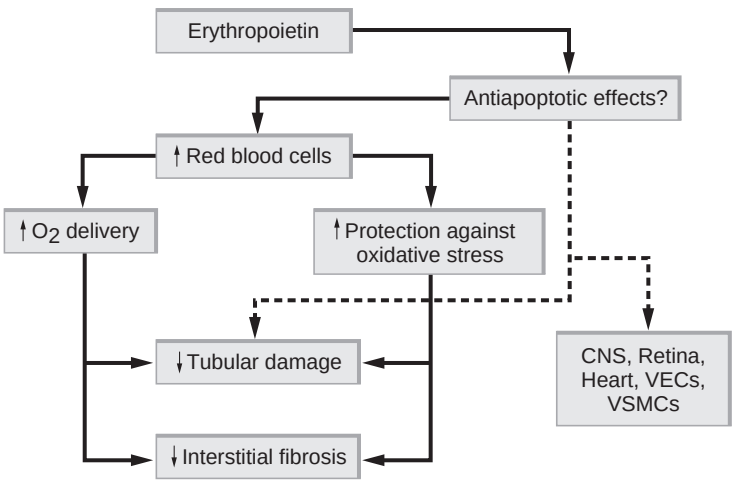

FIGURE 92-9. Erythropoietin-mechanisms which may modulate tissue injury or the progression of chronic kidney diseases. (Modified from: Rossert J, McClellan WM, Rogers SD, et al. Epoetin treatment: what are the arguments to expect a beneficial effect on renal disease progression? *Nephrol Dial Transplant* 2002;17:359.)

damage (280). Certainly the epoetin doses needed to fully correct anemia are higher than normal and may reach those permitting epoetin passage into the central nervous system (281).

Exercise Tolerance and Rehabilitation

Aerobic work capacity is improved following correction of anemia (282,283), accruing from an increase in oxygen carrying capacity as well as improvement in voluntary muscle function (284). The latter may result from possible treatment-induced improvement in muscle oxidative phosphorylation (285). Improvement in exercise capacity during epoetin therapy also results from increased erythrocyte 2,3 DPG levels (286) that permit improved oxygen delivery to tissues. A variety of muscle functions improve, including voluntary contractions, force generation, and duration of force contraction, as well as histologic improvements in architecture and fiber diameter (287). However, increases in maximal uptake of oxygen remain less than in normals and improvements in exercise or cardiopulmonary performances initially attained are not augmented further when tested 1 year later (288). The increase in oxygen carrying capacity produced by epoetin is accompanied by a significant reduction in peak blood flow to exercising muscle. This limits the gain in oxygen transport. Even after restoration of hemoglobin, O_2 conductance from the muscle capillary to the mitochondria remains considerably below normal (289).

Correction of anemia alone is unlikely to maximize exercise capacity and foster rehabilitation (290). Anemia management is only one component of effective rehabilitation, in conjunction with maintenance or improvement in exercise capacity, maintenance of adequate dialysis, and appropriate changes in socioeconomic and health policies (291). Other factors such as deconditioning, neuropathy, and cardiovascular disease probably contribute as well, and programs emphasizing exercise training are needed.

Effects on Coagulation

Renal insufficiency is associated with a bleeding tendency (292) attributed to platelet function abnormalities, characterized by prolonged bleeding time, abnormal platelet aggregation and adhesiveness, and decreased platelet factor 3 release (293,294). Many of the hemostatic abnormalities improve following correction of anemia with epoetin. This result from three effects: (i) a direct effect on megakaryocytes, which have erythropoietin receptors, and thus result in increased platelet count, an event noted in most large series; (ii) the migration of platelets radially (to the surface of the blood vessel) as hematocrit increases; and (iii) a change in platelet adhesiveness. Platelet adhesion and bleeding time are hematocrit-dependent. Both platelet adhesion and bleeding time, which correlates best with occurrence of clinical bleeding (295), are dependent on platelet number. Platelet counts are usually sufficient in CKD subjects. Even before the advent of rHuEPO, transfusion of washed filtered red blood cells devoid of plasma or other cellular components partially corrected bleeding time and platelet adhesiveness (296). The therapeutic use of epoetin confirmed that anemia *per se* was an important cause of the "bleeding abnormalities" in renal failure patients. The prolonged bleeding time corrects to normal as hematocrit increases to greater than 30%. However, the improvement in platelet function does not come without some consequences. Up to 11% of patients experience clotting of dialyzers or lines after epoetin therapy, and overall the heparin requirements at final target hematocrits of 30% to 38% have to be increased by 50% (297).

A controversial issue (298,299) is whether excessive thrombosis, particularly of vascular accesses, is or is not a major clinical and economic side effect of epoetin therapy. An increased propensity to clot vascular accesses has not been unequivocally documented (295,300). The thrombosis risk appears to be greater in patients with synthetic bridge grafts (301,302) and in patients with previously known access dysfunction (295,303). Native fistulas did not appear to be at risk.

Endocrine and Metabolic Changes

Younger uremic men manifest a variety of biochemical hormonal abnormalities as well as sexual dysfunction. Sexual function in younger uremic men treated with EPO improves (304); this improvement was believed initially to result from reduction in prolactin levels but the mechanism producing this improvement is unclear. Correction of anemia also improves responsiveness to thyroid-releasing hormone (TRH) and gonadotrophin-releasing hormone (GnRH) of the thyroidal and gonadal axes, and it is this correction that may be important rather than any EPO levels achieved by therapy (305). In elderly men, despite a significant erythropoietic response, no significant changes are seen in prolactin, testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), free thyroxine, triiodothyronine, and IGF-1 levels (306). Only a small decrease in serum growth hormone concentrations occurred. Advanced age may play a role in limiting hormonal responses.

A positive nitrogen balance occurs during the initial period of epoetin therapy (307), but this alone does not improve growth in children or alter the abnormalities in amino acid metabolism (403). Decreased total cholesterol with a fall in apoprotein B and serum triglycerides may occur following correction of anemia (308), an important observation in view of the risk of atherosclerotic complications in patients with ESRD. Diabetic retinopathy improves with correction of anemia (309). Raising or maintaining red blood cell mass in the 33% to 36% hematocrit range may be effective adjunctive therapy for correcting hypoxia at the level of the retina. In addition, exogenous erythropoietin may protect the retina from hypoxia or ischemia (310).

Dialysis Efficiency

After correction of anemia, serum potassium, phosphate, and creatinine increase (311–315) because of a decrease in water flow as red blood cell mass increases, but the changes are of small magnitude, and the major effect on urea kinetic modeling can easily be corrected by changing the dialysis prescription (316,317). Aside from a change in the patient's appetite, the major reason for the observed (but inconsistent) effects in chemistries is a decrease in solute clearance. Dialyzer reuse efficiency decreases, despite 15% to 50% increases in heparin dosing (313,315). A recent study of high-flux hemodialysis noted an increase in treatment time by 29 minutes from 140 minutes as hematocrit increased from 24% to 36% (318). With peritoneal dialysis, clearances of sodium, potassium, and urea, and changes in protein loss or glucose absorption do not change after recombinant human EPO therapy (319).

Protection of Kidney Function with Epoetin Therapy

Animal models show an effect of anemia and tissue hypoxia on the progression of kidney disease (320). Levin et al. (321,322) noted a strong influence of hemoglobin (Hb) on the relative risk of needing RRT at an initial estimated GFR of 10 to 60 mL/minute. In the RENAAL study, Keane et al. (323) found that the hazard ratio for the combined end point (doubling of SCr or reaching ESRD) increased progressively with each lower quartile of hemoglobin, reference being a hemoglobin of greater than 13.8 g/dL. In a Japanese study, Iseki (324) found that anemia was associated with an increased risk of ESRD more than threefold in women and almost twofold in men over an observation period of several years. Gouva at al. (325), in a randomized clinical trial, showed that early anemia treatment

of nondiabetic patients reduced adjusted hazard for a composite end point of doubling the serum creatinine, need for RRT, or death more than twofold. Another study showed that even mild anemia, hemoglobin less than 13 g/dL, increases the risk of progression of nephropathy to ESRD in type 2 diabetics (326).

The potential mechanisms of protection are probably multifactorial, as illustrated in Figure 92-9, but center around oxidative stress (OS) effects, and OS enhances both tubular damage and interstitial fibrosis. Remaining surviving nephrons increase their oxygen consumption, leading to increased production of reactive oxygen species, which predisposes the nephrons to additional injury (327). OS stimulates interstitial production of extracellular matrix as well as TGF-β production (328), drivers of fibrogenic processes. Reactive oxygen species also have proapoptotic effects (329) *In vivo*, treatment of rats with antioxidants protects against development of interstitial fibrosis (327), whereas deprivation of antioxidants seems to have opposite effects.

Renal protection following anemia correction may simply result from the increase in erythrocyte mass that increases the antioxidant capacity of blood to combat oxidative stress (330) and reduce effects of tissue hypoxia. Erythrocytes represent a major antioxidant component of the blood: red blood cells have a glutathione, superoxide dismutase, and catalase system, and cellular proteins that react with reactive oxygen species (low-molecular-weight proteins of the erythrocyte membrane, vitamin E, or coenzyme Q). In addition red blood cells can regenerate consumed redox equivalents through the pentose phosphate pathway, and through reduction of oxidized glutathione by glutathione reductase. The antioxidant capacity of blood becomes a function of the number of red blood cells.

However, recent studies indicate direct effects of erythropoietin on tubular cells, thereby preventing apoptosis Epoetin promotes survival of maturing precursor red blood cells by an inhibition of caspase activation (331) and by induction of the expression of antiapoptotic proteins such as Bcl-xL and Bcl-2 (332). With the demonstration that proximal tubular cells and medullary collecting duct cells express erythropoietin receptors (333), epoetin may have a direct effect on renal tubular cells to reduce or prevent apoptosis.

There are now some data that diminished oxygen content due to anemia may itself contribute to interstitial fibrosis and the progression of CKD and can be ameliorated or retarded by treating anemia (334). There is hope, therefore, that early treatment of anemia can retard progression of CKD through the elimination of tissue hypoxia (335) Certainly there have been no studies showing any adverse effects of partially correcting anemia in CKD patients (336,337), invalidating the animal study that demonstrated an adverse effect on kidney function during correction of renal anemia (338). The difference may be the result of better control of blood pressure in the human studies. All clinicians emphasize the importance of control of blood pressure as anemia is corrected.

Considerations in CKD and Congestive Heart Failure

Medicare data indicate that the most common cause of hospitalization in elderly patients (>67 years of age) with CKD prior to the need for dialysis or renal transplantation is congestive heart failure (CHF), which occurs in about 23% of all patients (339). Anemia worsens heart failure, which in turn compromises kidney function, producing the cardiorenal anemia process, a vicious cycle that can be broken by the appropriate management of anemia. Tissue hypoxia is central to the development of CKD and worsening cardiomyopathy as reflex changes in vascular tone and sympathetic activity occur. Anemia increases the likelihood for death before ESRD during a 2-year follow up of Medicare patients (AJ Collins, personal communication. See Samak et al. [339a] for published results.) Xue et al. (340) analyzed anemia management in more than 89,000 Medicare CKD patients who eventually progressed and required dialysis. Those who received no epoetin over 2 years compared to those who used epoetin regularly prior to renal replacement therapy had, during the pre-dialysis period, a 52% greater risk for cardiac disease, 38% greater risk for hospitalizations due to CHF, and 65% higher mortality rate within the first year after starting dialysis. Even an effect on the immune system was evident as there was a 41% increase in hospitalizations for infection after starting dialysis in the epoetin untreated group. Levin et al. (321,322) showed that the probability of remaining free of RRT was adversely influenced by the presence of cardiovascular disease.

Treatment of anemia with epoetin has been linked to positive cardiac outcomes at earlier stages of CKD (341). In patients with heart failure, Silverberg has shown in an open-label trial that treatment of anemia from a pretreatment hemoglobin average of 10.3 g/dL to greater than 12 g/dL improved cardiac function and New York Heart Association (NYHA) Class and markedly reduced hospitalization (342), despite a reduction in diuretic use in the anemia-treated group. In summary, anemia is associated with CHF; when anemia is treated, there is improved left ventricular ejection fraction (LVEF) and NYHA Class status, and decreased hospitalizations. An additional benefit of treating CHF with CKD is stabilization of kidney function (339).

Hemodynamic and Cardiovascular Effects of Epoetin

Uncorrected anemia produces a hyperdynamic state that contributes to the development of left ventricular hypertrophy (LVH) in renal failure. Anemia correction removes only this component but leaves unaffected any components arising from hypertension, hyperparathyroidism, or other structural abnormalities (343). Anemia also limits the myocardial oxygen supply, provoking angina pectoris or other ischemic events in those with coronary or peripheral vascular occlusive disease. Anemia and hypertension are, therefore, the major contributors to left ventricular dysfunction and congestive heart failure. Correction of anemia with epoetin is frequently associated with *de novo* or worsening of hypertension.

Hypertension

New or worsening arterial hypertension develops in a subset of 20% to 40% of treated patients, with the greatest increases affecting daytime systolic and night-time diastolic pressures (344,345). Increases in peripheral resistance range from 15% to 100%. Concomitant decreases in cardiac output vary from 10% to 35% (346,347). This variability may result from such factors as preexisting hypertension, compliance with antihypertensive medication, differences in neurohumoral characteristics, and degree of fluid control. Individuals who become hypertensive or have worsening hypertension are unable to adapt to the increase in peripheral resistance by reducing cardiac output. Hypertension following epoetin therapy is likely to be multifactorial in origin: preexisting hypertension, severe anemia at initiation, rapid increase in hematocrit, high epoetin doses given intravenously, and the presence of native kidneys.

Epoetin-induced hypertension usually develops within several weeks to months of starting epoetin therapy, as the hematocrit is rising (348); it appears to be a time-dependent consequence of long-term epoetin administration. However, increases in blood pressure do not correlate with the increase in hematocrit, either in humans clinically (349) or in animals (350), and EPO-induced hypertension can be dissociated from the rise in erythrocyte mass (351). In animals, EPO therapy does impair hypotensive response to nitric oxide (NO) donors, suggesting an impaired vasodilatory response to NO (345). Other studies show that the endogenous NO activity is

increased in epoetin-treated rats, perhaps a counterregulatory mechanism that limits the pressor effect (352).

Some patients will exhibit severe hypertension with epoetin administration but not with red blood cell transfusions that produce identical hematocrits (353). The recent demonstrations that erythropoietin can affect both vascular endothelial (354) and vascular smooth muscle (355) cells suggests that part of the hypertensive effect of epoetin results from direct vascular effects independent of changes in hematocrit. The high concentrations of erythropoietin achieved during intravenous injection (greater than 1,000 mU/mL) are capable of increasing endothelin-1 (ET-1) release (349).

Loss of hypoxic vasodilation (356) and changes in blood viscosity (343,357) following correction of anemia have also been postulated to be mechanisms increasing arterial pressure. A number of studies emphasize the important role of adequate control of body fluid and fluid gains in maintaining blood pressure control during epoetin therapy. Maintenance of normal blood volume requires equivalent decreases in plasma volume as red blood cell mass is increased to avoid changes in preload to the heart 358.

Whatever is the exact role of vasoactive substances in the genesis of EPO-induced hypertension, it appears to be specific to those with renal failure. It has been observed that a hypertensive response to epoetin does not occur when epoetin is used to treat other anemias, such as that associated with inflammatory disease (359), with cancer (360), or in nonrenal anemic pregnant patients (361) treated with epoetin for short or long periods. Experimental models indicate that epoetin produces hypertension in a remnant model of renal failure but not in sham control animals (362). In humans, advanced CKD must be present to see the hypertensive response to epoetin (363). Thus, advanced renal insufficiency appears to be a prerequisite for the development of hypertension during rHuEPO therapy.

Patients who become hypertensive or who have worsening hypertension during epoetin therapy appear to have an inadequate decrease in cardiac output in response to the EPO-induced increase in peripheral vascular resistance (364). Because of this hypertensive tendency and the inability to predict which patients might become significantly hypertensive, diastolic blood pressure must be controlled before initiating epoetin therapy. By contrast, patients with low blood pressure actually may benefit from the rise in diastolic blood pressure associated with the increase in red blood cell mass.

Left Ventricular Hypertrophy

Cardiovascular disease from hypertension and with LVH is a major risk factor for death. Anemia contributes to the development of LVH (365). Anemia produces a hyperdynamic state characterized by increased cardiac output and decreased peripheral vascular resistance. Both left ventricular mass as well as end-diastolic volume increase in response to these compensatory mechanisms in anemia (366). The increase in cardiac output during anemia correlates well with the degree of anemia and is reversed when the hematocrit is increased by more than 30% (367).

During partial sustained correction of anemia in hemodialysis patients with epoetin therapy, incomplete regression of LVH and volume occur (368,369). Mean decrease in left ventricular mass among 15 studies averaged 18% after a mean treatment time of 45 weeks as hematocrit was increased from an average of 20% to a range of 29% to 35% (370). Hypervolemia following correction of anemia can produce negative effects on left ventricular ejection fraction and function (371). To improve cardiac performance, blood volume expansion must be diligently prevented by appropriate changes in estimated dry weight (edema-free weight without hypotension) and by blood pressure control.

Anemia correction with epoetin improves exercise-induced ST-segment depression during exercise (372). Anemia correlates with mortality 30 days postmyocardial infarction in pre-ESRD patients (373) and correction of anemia is associated with improved NYHA functional class, improved GFR, and improved symptoms in NYHA Class III/IV heart failure patients already on "maximal" medical therapy (374). Foley demonstrated a hemoglobin of less than 8.8 mg/dL to be associated with higher mortality in ESRD patients (6). Although higher hematocrit (>44%) in cardiac patients with pre-ESRD is clearly associated with improved survival (375), once such patients progress to ESRD, treating cardiac patients with epoetin to higher hematocrit (>39%) is associated with worse outcomes (376).

Effects on Survival and Hospitalization in Dialysis Patients

Each decrease in hemoglobin of 1 g/dL below 10 g/dL increases mortality by 18% in adults (377). Yang et al. (378) found that each decrease in hematocrit of 1 point was associated with a 14% increase in mortality over a hematocrit range of 21% to 31%. Lowrie et al. (379) found a stepwise increase in mortality as hematocrit fell below 30%. Changes in cardiac morphometry and function require up to 1 year of therapy to achieve a maximum result (380). In a 15-year single-center longitudinal study, Avram et al. (381) found that hemoglobin at initiation of dialysis was a predictor of long-term survival in both hemo- and peritoneal dialysis patients.

In addition to beneficial effects on survival of adult dialysis patients, studies now show a reduction in overall hospitalization in EPO-treated patients (382–384). Total long-term costs of care for ESRD patients may be reduced through the use of epoetin (385).

Immune/Granulocyte Function

Since the early 1980s, the incidence of life-threatening infection in patients with ESRD has remained unchanged. Correction of anemia produces the following effects: (i) an increase in the number of natural killer cells and in helper/suppresser T-cell ratios (386); (ii) improvement in immune globulin production by peripheral mononuclear cells (387) and specifically in antibody titers after hepatitis B vaccine immunization (388); and (iii) correction of deficient phagocytic functions (164). Cytokine secretion is decreased in anemia and is corrected by increasing the hematocrit, whether by transfusion or epoetin treatment (389) Normalizing the hemoglobin decreased the prevalence of anergy and blunted the progressive increase in erythrocyte complement 1 receptor expression (390).

ADVERSE EVENTS OF RECOMBINANT HUMAN ERYTHROPOIETIN THERAPY

Side Effects

Adverse effects such as myalgias and seizures (usually resulting from uncontrolled hypertension) do occur but are manageable, and in the case of seizures totally preventable by close supervision of the patient. In the initial trials, development of encephalopathy or seizures was not uncommon (238,239) and resulted from less than optimal control of blood pressure. This is now much less often observed due to better appreciation of the need to control blood pressure during the ramp-up period. In most cases it is not related to the correction of anemia.

Flu-like reactions have been reported in a minority of patients (238,239,295,391). The onset is within hours of administration and usually subsides within 12 hours. Slowing the rate of administration (295) can decrease the symptoms. Therapy is discontinued in less than 1% of treated patients because of this side effect.

Pure Red Cell Aplasia

Development of pure red cell aplasia (PRCA) associated with the presence of neutralizing anti erythropoietin antibodies was initially reported in 13 patients from France treated with recombinant EPO (392). This complication, requiring transfusion, followed an initial response to epoetin and occurred after a successful duration or epoetin use of 3 to 67 months with a median of 7 months. Of nine evaluable patients, six recovered some erythropoietic function following discontinuation of recombinant epoetin, use of immunosuppressive therapy (immune globulin, plasmapheresis, corticosteroids) and/or renal transplantation. Cross-reactivity to other epoetins (epoetin beta and darbepoetin) was noted. Only four cases of PRCA associated with rHuEPO were reported before 1998 (393). Between January 1998 and April 2004, 175 cases were reported for Eprex, 11 for Neocormon, and 5 for Epogen. The estimated exposure-adjusted incidence was 18 cases per 100,000 patient-years for the Eprex (epoetin alfa) formulation without human-albumin, 6 cases per 100,000 patient-years for the Eprex formulation with human-albumin, 1 case per 100,000 patient-years for NeoRecormon (epoetin beta), and 0.2 cases per 100,000 patient-years for Epogen (epoetin alfa) (394). Subsequently the incidence decreased by 83%, although the incidence decreased more quickly than changes in procedures to ensure appropriate storage, handling, and administration could be implemented.

Although more than 150 patients with this complication have been noted in Europe, it is rare in the United States (395,396). The two most likely causes were the route of administration (subcutaneous in most of Europe versus intravenous in the United States) or an alteration in the immunogenicity of epoetin. Cournoyer et al. (397) investigated the incidence of antibody-positive PRCA and specific erythropoietin products. Global incidence of anti-EPO antibody-positive PRCA was low. Likelihood estimates for Epogen, Procrit, and Aransp did not differ and were independent of the formulation or route of administration. Eprex without human serum albumin administered subcutaneously (as opposed to intravenously) was associated with the greatest risk of PRCA. In Canada where the primary route of administration is subcutaneous, a screening program of 1,531 hemodialysis, peritoneal dialysis, and predialysis epoetin-treated patients found only one patient with a low-positive radioimmunoprecipitation test for anti-EPO antibody (398). This patient had previously had PRCA, had been treated with cyclosporine, and was being administered epoetin alfa with a good clinical result. Therefore, with the use of epoetin alfa with human serum albumin as in the United States, a large-scale screening program for anti-EPO antibodies is not justified.

Altered antigenicity of the European product (Eprex form of epoetin alfa) due to a difference in manufacturing has been suggested by epidemiologic data (399). A formulation change from albumin to another stabilizer may have induced the formation of micelles, thereby altering the epitopic presentation of sites on the molecule (400). A definite increase in cases followed the change in formulation in countries using this formulation but not in other countries using other preparations of epoetin alfa, beta, or omega. Most cases have been associated with the subcutaneous route of administration. In one patient, wheals developed at the site of subcutaneous injection. Skin responses were evoked at the same site following intravenous epoetin injection, indicating persistence of sensitized cells. The red blood cell aplasia gradually improved following prednisone treatment (401).

A new double-antigen enzyme-linked immunosorbent assay (ELISA) has been developed for detecting anti-EPO antibodies and is sensitive (detection limit 0.5 ng/mL) and specific (402). Anti-EPO antibodies could be detected in the sera of patients with PRCA. In contrast to previous reports, no anti-EPO antibodies could be detected in the sera of patients with systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Sjögren's syndrome (SS), or in the sera of dialysis patients.

Treatment of anti-EPO antibody PRCA has varied. Although at times the antibody cross-reacts with the newer epoetins, on occasion, treatment with Darbepoetin has been successful (403). In most cases, withdrawal of the epoetin is required (404) with immunosuppressive therapy with prednisone (405), cyclosporine (406), or rituximab to deplete CD20 cells (407).

TREATMENT OF ANEMIA

In the current U.S. health care environment, primary care physicians face a tremendous challenge in identifying, treating, and referring patients with CKD and CKD-related complications and comorbidities. As implied by the Arora study (408), early referral to a nephrologist increases the likelihood of appropriate anemia treatment but does not guarantee it. The proposed K/DOQI staging of CKD coupled with a national public-awareness initiative may promote a greater sensitivity by primary care physicians to be alert for CKD and its complications in patients with a GFR of less than 60 mL/minute/1.73 m^2 (stage 3) and to obtain guidance in managing anemia, hypertension, and renal osteodystrophy.

Target Hemoglobin/Hematocrit Levels

In both the United States and Europe, epoetin therapy is typically initiated when hemoglobin decreases to less than 11 g/dL. This value applies to more than 390,000 patients currently on dialysis in the United States and to millions of people with CKD who do not require dialysis (CKD stages 1 through 4). Currently the recommended upper limit for epoetin therapy is 12 g/dL for CKD and 12.5 g/dL for ESRD patients in the United States. No upper boundary has been set in Europe. An optimal "target" hemoglobin/hematocrit cannot be defined *a priori* for each patient because sedentary, older patients differ from younger, more active or working patients.

Prospective studies have failed to show much benefit and perhaps risk when normalizing hemoglobin. The Normal Hematocrit Trial (376) compared 1,233 patients with evidence of CHF or ischemic heart disease, whose anemia was then treated to an average hematocrit level of 30% versus 42%. The study was terminated because an interim intent-to-treat analysis showed an increased risk of vascular access thrombosis and a trend toward a higher rate of nonfatal myocardial infarction or death in the normal-hematocrit group (difference did not reach significance at the time of termination). Similarly, the Canadian Normal Hemoglobin Trial (409) showed that normalizing hemoglobin in hemodialysis patients with LVH produced no regression of established hypertrophy, but lower rates of progressive LV dilation if it was absent at entry. There was no difference between hemoglobin groups in access thrombosis, probably due to the use of native arteriovenous fistula (AVF) in Canada compared to the use of grafts in the United States, and no difference in mortality rates. These studies

indicate that the greater the degree of cardiac damage, the more difficult it is to reverse the damage or show an additional benefit, even with aggressive anemia treatment (410). It may be more important, even at a relatively advanced stage of renal dysfunction, to control blood pressure and volume, together with regulated metabolic and clinical indices to prevent or even reverse LV hypertrophy in a substantial proportion of patients (411).

Results differ if patients are enrolled at an earlier stage of CKD or not selected for preexisting cardiac disease. The Swedish study (412) randomized both CKD as well as ESRD to two levels of anemia treatment: 11 g/dL and 14 g/dL. After one year of follow-up, there was no evidence of increased mortality, rather the opposite. Within each group, mortality risk varied inversely with hemoglobin level, and QOL was found to be better at normal compared to subnormal hemoglobin levels, findings that were also found in the Normal Hematocrit Trial (376). This trial indicates that a normal hemoglobin is safe in CKD patients without heart disease with CKD stages 3 to 5. Epidemiologic evidence also suggests that higher hematocrit values may be less of a concern than previously considered (413). The most current data suggest that hematocrit values in the normal range should not be achieved with epoetins in patients with advanced cardiac disease. In others, hematocrits at least up to 39% appear to be safe and effective. Based on available evidence, a reasonable target hematocrit range may be 33% to 39%, which balances the risks and potential benefits.

The predialysis hemoglobin or hematocrit is not an accurate measure of red blood cell mass because it may increase postdialysis, owing to removal of large intradialytic weight gains. In addition, red blood cell swelling during transport of samples to central laboratories results in further errors in hematocrit but not hemoglobin. A recent study showed that the mean for time-averaged hemoglobin is approximately 1 g higher than the predialysis value (414). Hemodialysis patients with hematocrit greater than 38% or hemoglobin levels greater than 13 g/dL may be at risk from hemoconcentration effects, particularly if they have large interdialytic weight gains. Those with severe peripheral vascular disease may be most at risk. Samples can be collected at any time in peritoneal dialysis patients as the plasma volume is relatively constant during any given day; however, care should be taken to ensure the same posture.

Dosing, Pharmacokinetics, and Route of Administration

All epoetin-treated dialysis patients increase their hematocrit in a dose-dependent manner. There are two phases, the corrective phase and the maintenance phase. The appropriate dose in a given patient is one that permits attainment of the target hemoglobin/hematocrit over the lifespan of the erythrocyte using the same dose of erythropoietin as it satisfies both the initiation and maintenance requirements. The response to a given dose of epoetin among patients, however, is variable. The intravenous doses needed to attain a mean hematocrit of 32% to 38% show at least a 20-fold variation, from 12.5 to 500 U/kg thrice weekly (240).

Several principles govern the administration of epoetin: (i) the response to epoetin is dose-dependent, but with a variable subject response; (ii) the response depends on the route of administration—intravenous versus subcutaneous—and the frequency of administration—daily, twice weekly, three times weekly; and (iii) the response may be limited by inadequate iron stores, bone marrow fibrosis, and inflammation A pharmacodynamic model permits analysis of the effect of red blood cell lifespan and sensitivity to a given dose of epoetin (415). The ideal dose in a given patient is one that permits attainment of the target hematocrit over the lifespan of the erythrocyte because it satisfies both the initiation and maintenance requirements (416). During maintenance, new red blood cells should be formed at a rate comparable with the removal rate. Drug dosage should not be drastically lowered after reaching the target hematocrit because this produces a "yo-yo" or "ping-pong" effect.

In peritoneal dialysis patients, the response to rHuEPO is as good as in hemodialysis patients (417), and the dose frequency is frequently reduced to once a week for convenience (418,419). Most centers use the subcutaneous route permitting self-administration at home. CAPD patients respond better than hemodialysis patients to equivalent weekly doses, perhaps because of lower ongoing blood losses (418). Some centers dose only once a week and obtain good results (420,421).

For the first generation epoetins, subcutaneous injections are the most effective route of administration (422). Dosage requirements using the subcutaneous route are lower by approximately 30% than those using the intravenous route (93,423,424), providing greater cost efficiency (422). Current evidence indicates that efficient erythropoiesis requires maintenance of erythropoietin levels above a critical level, below which erythroid cells undergo apoptosis (425). With subcutaneous dosing, EPO levels are sustained in the interdialytic period (91), decreasing apoptosis and permitting more sustained erythropoiesis (Fig. 92-10).

Newer Epoetins

The current standard of care is to administer first-generation epoetin two to three times per week to patients with CKD. In practice, this schedule of administration is achieved mainly in patients receiving hemodialysis, because of the three-times-weekly dialysis schedule. In patients receiving peritoneal dialysis and in patients in the predialysis stage, it is generally given once weekly, due to the less frequent contact between patient and caregiver, and the inconvenience of frequent subcutaneous administration. The recommended dosage in all these patient groups is still three times weekly, however.

Frequent dosing, however, places a considerable burden on health care staff, as well as on patients. Over the last 5 years, a variety of new epoetins have been or are undergoing development. All of these have longer biologic half-lives, thereby permitting sustained blood levels and preventing apoptosis.

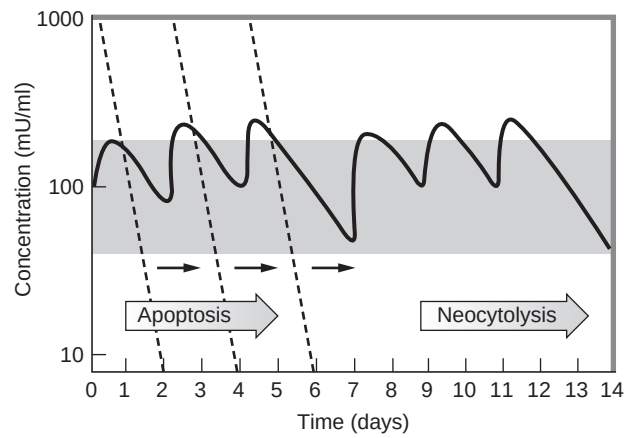

FIGURE 92-10. Epoetin beta concentration-time profiles: route of administration (120 U/kg/wk) in three divided doses. The *shaded area* represents the desired range of erythropoietin. Intravenous doses (*broken lines*) are unable to sustain levels in the interdialytic period resulting in both apoptosis and neocytolysis. Subcutaneous dosing (*solid curve*) provides levels within the appropriate erythropoietin range thus avoiding both processes. (Adapted from: Besarab A, Flaharty KK, Erslev AJ, et al. Clinical pharmacology and economics of recombinant human erythropoietin in end-stage renal disease: the case for subcutaneous administration. *J Am Soc Nephrol* 1992;2:1405.)

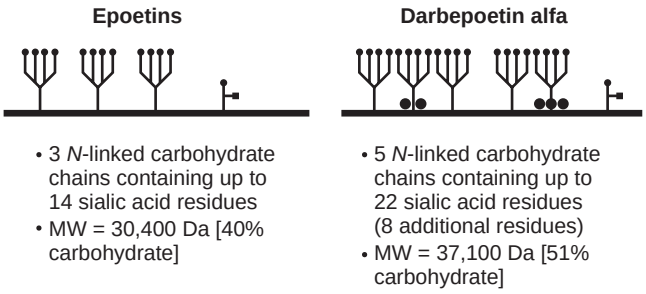

FIGURE 92-11. Structure of the first-generation epoetins (alfa, beta, and omega) compared with Darbepoetin alfa. The darbepoetin alfa molecule has a modified polypeptide backbone to allow it to carry additional carbohydrate side chains and increase the number of sialic acid residues from a maximum of 14 to 22 sialic acid residues. (Adapted from: Macdougall IC. Novel erythropoiesis stimulating protein. *Semin Nephrol* 2000;20:375; and Egrie JC, Browne JK. Development and characterization of novel erythropoiesis stimulating protein (NESP). *Nephrol Dial Transplant* 2001;16[Suppl 3]:3.)

Studies of erythropoietin carbohydrate isoforms have demonstrated a positive relationship between the sialated carbohydrate content of erythropoietin and its half-life and *in vivo* biologic activity (426). Darbepoetin alfa (Aranesp, Amgen, Thousand Oaks, CA), a novel erythropoiesis-stimulating protein [NESP, also known as Aranesp, Darbepoetin], was developed as a longer-acting erythropoietic agent using site-directed mutagenesis. It has two additional carbohydrate chains (Fig. 92-11). It has been shown to have a threefold longer terminal elimination half-life compared with epoetin [25 vs. 8.5 hours] when administered intravenously and approximately twice as long (49 hours) when administered subcutaneously (93,427). The molecule was approximately 3.6-fold more biologically active than epoetin when each molecule was administered three times per week and 13 to 14 times as active when injected once a week. Darbepoetin does not accumulate over time when given repeatedly. In early clinical trials, dosing frequency out to 3 weeks subcutaneously has been reached in CKD patients not on dialysis (428). An even longer pegylated epoetin beta preparation is undergoing clinical trials and has a half life of longer than 100 hours with the potential for true once monthly dosing (429).

Epoetin Hyporesponsiveness

Resistance to epoetin is defined by the requirement of either a large dose during initiation or by the development of refractiveness to a previous efficacious dose and dosage escalation to maintain a target hematocrit. In approximately 10% of subjects, a dose of greater than 450 U/kg per week will be needed in the absence of any "known" state producing resistance. Patients who require more than 450 U/kg per week are considered to be relatively resistant to erythropoietin. However, biologic heterogeneity—variation in intrinsic sensitivity and in red blood cell survival—accounts for many instances of patients who do require more than 450 U/kg per week to reach and then maintain a hematocrit greater than 30% (430).. However, in most patients, conditions known to produce epoetin resistance must be looked for. At least nine separate conditions can produce epoetin resistance (431,432):

Absolute and relative iron deficiency is the most common condition (241). Blood loss should always be suspected in those requiring increasing doses or large maintenance doses of intravenous iron. Oral iron is relatively ineffective and we favor intermittent parenteral iron administration.

Cofactor deficiency can develop in patients who were initially responsive to epoetin. Evaluation of folate and vitamin B_{12} is needed in select cases (433,434). Macrocytosis is an inadequate differentiating feature.

Infection and inflammation is the second most common condition, and responsiveness can usually be restored upon recovery. CKD is associated with the malnutrition, inflammation, atherosclerosis (MIA) syndrome. The MIA syndrome may underlie resistance to epoetin. Inflammatory mediators may arise from the underlying process (435,436) or from hemodialysis (437). Key agents are TNF-α and IL-1 (438,439). Patients with failed transplants returning to dialysis are frequently resistant to epoetin (440) until the transplant is removed. Peritonitis in CAPD patients usually produces temporary impairment, but on occasion prolonged refractiveness may be seen. Chronic infection may be difficult to diagnose, such as those associated with failed arteriovenous vascular access grafts (441). One approach may be to use agents that influence the MIA process. Macdougall (442) has advocated the use of pentoxifylline (400 mg daily) to reduce T-cell expression of TNF-α and interferon gamma (IFG-γ) and restore response to erythropoietin.

Hyperparathyroidism (182) is a less common cause, in which the relationship is between the degree of fibrosis and the amount of epoetin needed to maintain a target hematocrit. Serum levels of parathyroid hormone (PTH) do not correlate with epoetin dose.

Aluminum overload has become a much less common cause of epoetin refractiveness (443) as nonaluminum-containing phosphate binders have increasingly replaced aluminum-containing agents.

Hemoglobinopathies produce relative resistance. Experience in sickle cell disease has been disappointing (233,444). Therapy for thalassemia requires prolonged high-dose epoetin (445,446).

Malignancy: Patients with multiple myeloma on dialysis require higher dose epoetin (447). Larger doses of epoetin should be used in myeloma patients on RRT, however, because patients with myeloma but without renal insufficiency frequently require larger doses than those received by patients with progressive renal insufficiency (448).

Hemolysis shortens red blood cell lifespan, thereby producing epoetin refractiveness. It can be induced by residual *formaldehyde* in dialyzers (449) or shearing across heart valves. In the latter, transfusions may be needed despite use of large doses of epoetin (450).

Emergence of EPO antibodies (see section on PRCA).

Malnutrition is associated with low hematocrits among dialysis patients. Malnutrition can be quickly worsened by inflammation (451). Acute-phase reactants correlate with resistance to epoetin (452).

Miscellaneous Considerations

Angiotensin-Converting Enzyme Inhibitors. Although some reports suggest that ACE inhibitors decrease response to epoetin (453,454), others do not (455). In view of the importance of ACE-inhibitors for cardiac remodeling and antihypertensive treatment in hemodialysis patients, we believe that ACE-inhibitor therapy for blood pressure control or heart failure should not be withheld to minimize epoetin dosage.

Intraperitoneal Epoetin. Intraperitoneal epoetin can be effectively used in small children by infusion of epoetin in 50 mL of fluid into a "dry" abdomen (456,457).

Intercurrent Illness or Surgery. Erythropoietic response is reduced during intercurrent illness or surgery. Our policy is to continue the epoetin therapy throughout the intercurrent illness. We generally stop the therapy after renal transplantation. Because erythropoietin production may be delayed, particularly in the presence of delayed graft function, epoetin

can reduce the need for postoperative transfusion; however, the amount of epoetin needed to maintain hematocrit levels is twofold greater than the amount needed prior to transplantation (458).

Iron Therapy. Suboptimal response to epoetins commonly results from failure of an adequate delivery of iron to the erythron. Enhanced iron utilization due to erythropoietin-induced red blood cell formation can quickly deplete iron stores. Oral iron preparations frequently fail in hemodialysis patients (459). Because erythropoiesis has to be stimulated to a greater than normal degree to compensate for blood losses and or shortened red blood cell survival, functional iron deficiency can frequently develop. Although oral-nonheme iron is usually inadequate for maintenance of iron stores, there is interest in heme-iron peptide (HIP), which may overcome some of the limitations of traditional oral iron (460). Less parenteral iron was needed to maintain target hemoglobin.

The exact amount of iron administered to patients during initial therapy depends on whether the patient is iron replete (unusual in most patients starting dialysis) and unlikely to develop relative iron deficiency, using ferritin >800 ng/mL as cutoff value for the iron replete state. Iron-replete patients receive only the amount of iron needed to correct the red blood cell mass as 75 to 125 mg weekly doses until the desired amount is given. Regular monitoring of iron status by measurement of serum ferritin and transferrin saturation (TSAT) is mandatory in the cost-effective management of anemia with epoetin and should be performed monthly.

Once the maintenance period is reached, we prefer to use a maintenance protocol of 25 to 50 mg of iron intravenously weekly, the dose adjusted in response to sequential iron indices. CAPD patients require less iron due to smaller ongoing blood losses. In most cases, administration of 500 or 1,000 mg slowly over 4 to 6 hours is sufficient, and adequate response can be maintained for 6 months to 1 year.

In traditional intermittent dosing regimens, intravenous iron is given only when TSAT or ferritin fall to less than 20% and/or 100 ng/mL, respectively and then receive a pulse of 1,000 mg over 8 to 10 dialysis treatments. Many studies indicate that iron-replete patients receiving regular parenteral iron maintain better serum ferritin levels and require less recombinant human epoetin than patients receiving no parenteral iron supplementation or regular oral iron alone (209–211,461). Averaged over seven studies in iron-replete patients (TSAT >24% and ferritin from 200 to 600 ng/mL), administration of a prorated weekly dose of iron increased hematocrit by 14% with a concomitant decrease in epoetin dose of 38% (462). We compared a maintenance regimen of 25 to 100 mg every 1 to 2 weeks (to maintain a transferrin saturation between 30% and 50%) to a traditional intermittent dosing regimen (to maintain a transferrin saturation >20% or a ferritin level >200 ng/mL). The amount of recombinant erythropoietin required in the maintenance group was more than 50% lower than that utilized in the intermittent group to achieve the same target hemoglobin of 10 to 11 g/dL (463). An even more aggressive iron regimen was found to reduce epoetin even further (221) but this more aggressive regimen progressively elevated serum ferritin level to an average of 730 ng/mL (at month 6) with an average monthly iron administration of 400 mg/mo, twice that in the control group. Current KDOQI guidelines do not recommend the *routine* administration of iron to patients with ferritin levels greater than 500.

The frequency of administration of iron has been evaluated in a few studies only. Administration of 6.25 to 21.3 mg of sodium ferric gluconate complex at every treatment compared to 62.5 mg every 1 to 4 weeks was effective in increasing hemoglobin levels and prevented an increase in ferritin levels (464). Administration of iron during each dialysis was also studied using a novel delivery system. Dialysis with a solution containing soluble ferric pyrophosphate, a chelated iron that readily diffuses into the blood compartment, was found to be safe and effective in a preliminary 6-month study of stable hemodialysis patients (465).

Iron Overload. Occasionally, patients are clearly iron overloaded but respond poorly to and require large doses of epoetin. Several options exist. Ascorbic acid, 500 mg given intravenously after hemodialysis 1 to 3 times a week, can mobilize tissue stores (466). Because ascorbate is metabolized to oxalate which is removed poorly by dialysis, we recommend that no more than 300 mg be given thrice weekly. Although serum ferritin remained unchanged, transferrin saturation increased from 27% to 54% and hematocrit increased from 27% to 32% (466). A control group with normal iron status and without resistance to erythropoietin showed no changes in TSAT or hematocrit when challenged in the same manner with ascorbate (466). An alternative method uses desferrioxamine in patients with iron but without aluminum overload. Epoetin doses decreased dramatically from 400 to 25 U/kg while increasing hemoglobin levels to and then maintaining them at 11 g/dL (467).

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CHAPTER 93 ■ THE OSTEODYSTROPHY OF CHRONIC RENAL FAILURE

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Normal bone is a unique tissue in the body, involving a combination of organic and inorganic material under the control of two kinds of cells: osteoblasts and osteoclasts. These cells perform their contrasting but coordinated functions to maintain skeletal mass and modulate calcium balance. It is, in fact, alterations in calcium metabolism that commonly lead to metabolic bone disease. As calcium is removed from or prevented from entering bone, in order to maintain the circulating level, structural consequences result in failure of bone strength. In the conflict between bone's function as a calcium reservoir and its function as a structural support system, the reservoir function almost always predominates.

The organic matrix of bone is produced by the osteoblast and mainly comprises type I collagen (1). In the disorders in which bone formation is decreased, collagen (osteoid) production is also decreased, but it is not known to be abnormal qualitatively. Conversely, in the high bone formation states associated with secondary hyperparathyroidism, an abnormal-appearing "woven" osteoid may contribute to defective mineralization in the bone that is formed (1,2). Osteoblasts also synthesize several noncollagenous proteins that become constituents of the osteoid and may be important in the mineralization process. Two of these, alkaline phosphatase and osteocalcin (3), are secreted by osteoblasts and are elevated in serum during certain high turnover bone states, such as Paget's disease and hyperparathyroid bone disease. Although the exact role of these osteoblast-derived proteins in mineralization is unknown, they are useful clinically in the longitudinal assessment of bone turnover.

The inorganic material (largely calcium–phosphate salts) that deposits on the interlinking collagen strands of the osteoid forms apatite crystals of varying sizes. There is a link between osteoid maturation and crystal deposition in that the mineral does not deposit until the osteoid reaches a certain maturational state. The mineralization lag time is a commonly determined measurement that expresses the time it takes before newly formed osteoid will accept mineral deposition. Variations in this measurement reflect abnormalities in the process of bone mineralization (4).

Of the cells that control bone remodeling, relatively little is precisely known. The osteoblast is involved in the formation of the osteoid and presumably controls the entry of the mineral (5). At sites of bone formation, the cell assumes a cuboidal shape and begins to make the osteoid. After a delay of 2 to 3 weeks, the osteoid begins to mineralize and after a few months this bone-forming unit turns off, cells become quiescent, and bone formation moves to another site (6). Although the controls for osteoblast growth and differentiation are unknown, growth factors present in bone matrix may play a role (7–10). Perhaps more important are the cytokines and growth factors secreted by marrow precursor cells and osteoblasts. These include interleukin-1 (IL-1) and IL-6 (7), tumor necrosis factor-alpha (TNF-α), transforming-growth factor-beta (TGF-β) (11), fibroblast growth factor (FGF) (10), bone morphogenetic proteins (BMPs)(12), the insulin-like growth factors (IGFs) (13,14), and IGF-binding proteins (15,16), all of which stimulate either osteoblast proliferation or various differentiated cell functions (17,18). In addition to their probable autocrine function, they may be the primary mediators of hormonal stimulation of bone cell growth. This could be particularly important in the hyperparathyroidism of uremia, since parathyroid hormone (PTH) is known to stimulate osteoblast proliferation (19) as well as the osteoblast production of TGF-β (20), IL-6 (21), IGF-1 (22), and IGF-binding proteins (23). Moreover, the elevated circulating levels of IL-6 (24), IL-1 and TNF-α (25), and IGF-1 (26) in some dialysis patients may also significantly influence bone turnover.

Osteoclasts resorb bone. Derived from marrow macrophage precursors (27), it is not clear why osteoclasts choose to resorb bone at a particular site and time. Osteoclasts and osteoblasts are functionally linked such that when one is stimulated or suppressed, the other responds in the same direction and magnitude (28). This balanced response allows the bone to modulate calcium metabolism for a few weeks until counterregulatory processes come into play. At that point a new equilibrium is achieved between bone resorption and bone formation, protecting against major alterations in skeletal mass.

In the normal subject, bone is a dynamic organ, with approximately 400 mg of calcium entering and leaving the skeleton each day (29). Lesser amounts of other minerals as well as bicarbonate and phosphate accompany this movement of calcium. Deficiencies in calcium, phosphate, and bicarbonate may inhibit bone formation and enhance resorption. Other circulating factors that affect bone dynamics include PTH and vitamin D. Although calcitonin may also play a role it appears to be only a weak and transient inhibitor of bone resorption (effects lasting only a few days) (30). Other hormones, such as thyroid hormone and adrenal corticoids, may have permissive effects and/or lead to skeletal disorders when deficient or excessive, but are not usually primary offenders in metabolic bone disease and do not play major roles in renal osteodystrophy.

Although the hyperparathyroidism of renal failure is one of the most important biochemical derangements affecting bone remodeling, several other factors are also important in renal osteodystrophy. For example, uremia and its attendant acidosis inhibit some cellular processes that are important in bone remodeling (31,32), including impaired cartilage production (33,34) and decreased osteoblast function (35). Magnesium abnormalities may affect PTH metabolism (36,37) or directly impact on apatite formation (38). Heparin given in a continuous, high dose is well described as a cause of osteoporosis (39), but its role in causing bone loss during intermittent use in dialysis patients has not been determined.

TABLE 93-1

TYPES OF RENAL OSTEODYSTROPHY

I.	High turnover
	A. Mild disease (mild hyperparathyroidism)
	B. Osteitis fibrosa (severe hyperparathyroidism)
II.	Low turnover
	A. Aplastic (adynamic) disorder
	B. Osteomalacia
III.	Mixed osteodystrophy

Pathogenesis

High Turnover Bone Disease

Historically, high turnover bone disease is the predominant bone lesion in patients with renal failure. The lesion begins in a mild form and progresses to typical osteitis fibrosa (hyperparathyroid bone disease) (Table 93-1). This lesion is mediated by a steady rise in serum PTH (40). For many years it was thought that the trade-off hypothesis adequately explained PTH changes in renal failure (41–43). According to this hypothesis the rise in phosphate, which occurs as renal failure advances, lowers serum calcium levels, which stimulate PTH secretion. Although considerable clinical and experimental data seemed to support this theory, many patients did not follow this proposed pattern. Other investigators suggested that the "set point" for PTH inhibition by calcium was altered in patients with renal failure (44,45). Later it was proposed that this alteration in set point might be due to the lack of 1,25-dihydroxyvitamin D_3 (46,47). This seemed logical, since one function of PTH in normal subjects is to stimulate renal synthesis of 1,25-dihydroxyvitamin D_3; it would be appropriate physiologically for 1,25-dihydroxyvitamin D_3 to inhibit its trophic hormone (48–50). Data from patients with moderate renal failure show that serum 1,25-dihydroxyvitamin D_3 levels decline before serum calcium decreases and that serum 1,25-dihydroxyvitamin D_3 correlates inversely with the rise in PTH levels (51). The demonstration that intravenous calcitriol suppresses PTH independent of its effect on the calcium level appears to provide the final proof that calcitriol is a direct inhibitor of PTH secretion *in vivo* (52–54). Other important factors that likely contribute to hyperparathyroidism in renal failure include a decrease in 1,25-dihydroxyvitamin D receptors in parathyroid cells (55), the prolonged half-life of PTH in renal failure (56), the direct effect of high phosphate concentrations to stimulate PTH secretion and decreased activity of the calcium-sensing receptor (57) (Fig. 93-1).

Hyperphosphatemia had been suspected of playing more than an indirect role in promoting hyperparathyroidism (58), but convincing evidence for a direct stimulatory effect of phosphate on parathyroid metabolism has only recently been shown. High concentrations of phosphate in the media of parathyroid cultures stimulate PTH secretion (59,60) and phosphate administration to uremic rats increases PTH levels without changing serum calcium or 1,25-dihydroxyvitamin D (59,61). Moreover, serum phosphate is directly correlated with serum PTH in patients with mild-to-moderate chronic renal failure (62) (Fig. 93-2). The stimulatory effect of phosphate has been attributed to enhanced parathyroid cell proliferation (61) and to decreased calcium-sensing receptor expression (63). How these two pathways are linked remains to be established. It is clear, however, that the calcium-sensing receptor in the parathyroid glands of uremic patients is downregulated (64,65) and that the use of calcium receptor agonists (calcimimetics) is effective in suppressing PTH secretion in dialysis patients (66) (Fig. 93-3). Whether calcimimetics are capable of suppressing parathyroid gland hyperplasia in uremic patients (67) remains to be determined.

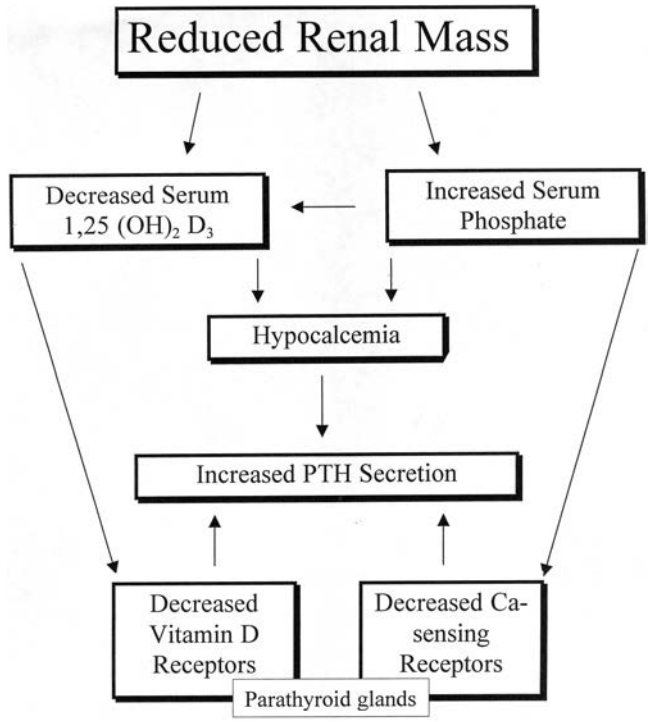


FIGURE 93-1. Pathogenesis of secondary hyperparathyroidism in chronic renal failure.

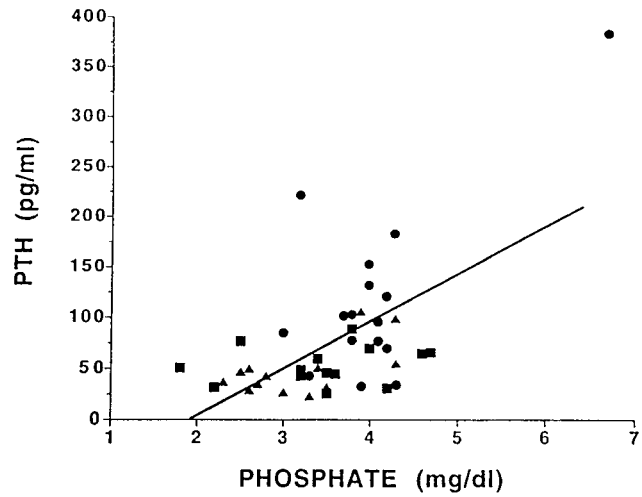


FIGURE 93-2. Relationship between serum parathyroid hormone (PTH) and serum phosphate in 44 patients with mild-to-moderate chronic renal failure (CRF) (serum creatinine <3.0 mg/dL); r = 0.62, p <0.01. (Reprinted from: Kates DM, Sherrard DJ, Andress DL. Evidence that serum phosphate is independently associated with serum PTH in patients with chronic renal failure. *Am J Kidney Dis* 1997;30:809, with permission.)

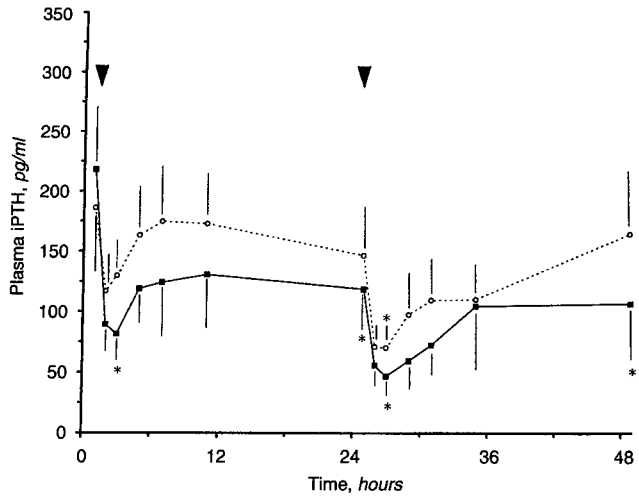


FIGURE 93-3. Plasma PTH levels in dialysis patients receiving the calcimimetic, NPS R-568. *Arrowheads* indicate the time of a single oral dose. *Open symbols* indicate low dose and *closed symbols* indicate high dose. (Reprinted from: Antonsen JE, Sherrard DJ, Andress DL. A calcimimetic agent acutely suppresses parathyroid hormone levels in patients with chronic renal failure. *Kidney Int* 1998;53:223, with permission.)

Low-Turnover Bone Disease

The recognition that fracturing osteodystrophy and dialysis dementia occurred in the same epidemiologic setting led to the identification of aluminum toxicity (68). In early reports it was evident that aluminum was entering the body through the dialysate. It has since become clear that aluminum also crosses the gut mucosa. In the normal subject, the small amount of aluminum that is absorbed (0.1% of the total) is rapidly eliminated by the kidney (69); only 10 to 30 mg is present in the body of healthy individuals (69). In renal failure, gastrointestinal aluminum absorption is enhanced (70) and accumulations become significant when high oral doses are administered chronically (69,71). Consequently, uremic patients not yet on dialysis may have elevated serum aluminum (72) and some of them develop aluminum bone disease (73). In other patients, residual renal function may offer protection against aluminum accumulation (74).

Aluminum deposition on bone surfaces is usually associated with a depressed rate of bone formation. However, this is not always true, and the fact that aluminum is often present deep within mineralized bone suggests that its mere presence does not inhibit bone formation (75–77). Probably the amount of aluminum on bone surfaces is in part an indication of the level and chronicity of exposure.

In addition to aluminum, two other metals, iron and strontium, have been implicated as a cause of low-turnover bone disease (78–81). Iron has been found at bone surfaces in patients with osteomalacia and aplastic bone disease. Although the prevalence of iron-related bone disease is unknown, it probably is low. It may be that iron, therefore, occasionally simulates the toxicity usually attributed to aluminum. When treatment of iron overload is necessary, deferoxamine is efficacious (82). Increased bone strontium levels have recently been identified in dialysis patients who have osteomalacia (81). Strontium is abundant in the earth's crust and is found in trace amounts in most foods, with grains and seafood containing the highest concentrations. In some regions, strontium levels in the water are quite high and can cause elevated serum levels in patients with moderate renal insufficiency and in patients receiving hemodialysis (83). The role of strontium in causing osteomalacia probably relates to its direct inhibition of the mineralization process (84) rather than by causing a decrease in osteoblast number, since the adynamic lesion cannot be induced by strontium in uremic rats (85). Adequate purification of the dialysate water and use of strontium-free concentrates (83) appear to be the best way to prevent strontium overload.

Whether there are other specific uremic toxins that cause low-turnover bone disease remains to be seen. We have now observed a large number of patients with the adynamic lesion in whom neither iron nor aluminum could be found (86). In some, diabetes likely has a role by inducing a relative hypoparathyroid state (87,88), possibly due to hyperglycemia-induced PTH suppression (89). In others who are not diabetic, low PTH levels are thought to result from excessive therapeutic parathyroid gland suppression (86,90). In others, an unidentified circulating low-molecular-weight inhibitor of osteoblast proliferation (35) may be important.

Aluminum seems to be capable of produce this low bone formation picture by itself and a marked reduction in PTH, as occurs with parathyroidectomy or aggressive medical therapy, accelerates its development (91). Therefore, it is extremely important to be certain of the need for parathyroidectomy, because such surgery, probably because of the marked slowing of bone formation that results, potentiates the accumulation of aluminum at bone surfaces (91–93). The mechanism by which aluminum leads to depressed bone formation is unclear, but there are several possibilities. Aluminum deposits in the parathyroid gland (94) and appears to suppress PTH secretion (95). It also enters the osteoblast and may inhibit its activity (96). Aluminum deposits in the mineral itself and may impair apatite crystal development (97) and collagen crosslinking (98). All these possibilities are discussed in greater detail subsequently. Interestingly, iron also apparently can lead to hypoparathyroidism in the hemochromatosis syndrome (99,100). Whether it deposits in the parathyroid gland in iron overload states associated with renal failure is unknown.

Mixed Uremic Osteodystrophy

The mixed lesion, as the name implies, combines features of both high- and low-turnover bone disease. This appearance can develop in at least two ways. First, if severe secondary hyperparathyroidism is associated with low calcium and/or low phosphate (as occurs in some poorly nourished patients), the high PTH will stimulate both fibrous proliferation and osteoid production. Lacking calcium, phosphate, or both, however, this osteoid will be slow to mineralize. Hypocalcemia was probably the major cause of this lesion in earlier reports. Second, if either hyperparathyroidism is being suppressed or osteomalacia is being treated by aluminum removal, a transitional state may occur in which a combination of both lesions is found.

Clinical Features

Clinical and laboratory features that distinguish high- and low-turnover disease are extraordinarily subtle. Pain, weakness, and muscle wasting may be present in both. Similarly, hypercalcemia, increased alkaline phosphatase, and high PTH levels can occur in either disorder. Finally, the pathognomonic radiographic feature of hyperparathyroidism, subperiosteal resorption, is commonly noted in patients who have severe osteomalacia histologically with no histologic findings at all of hyperparathyroidism (101). Therefore, as the clinical features of these entities are discussed, the focus will be to distinguish the two different processes.

Early Manifestations

As renal failure begins, few symptoms occur as a result of musculoskeletal problems. Most patients in the azotemic phase will have biochemical abnormalities and some may have early histologic changes, but only rarely will overt skeletal disease occur. An exception to this general rule can be seen in certain tubular disorders, particularly distal renal tubular acidosis (RTA type I) and proximal renal tubular acidosis (type II) associated with phosphate wasting. In these conditions, osteomalacia may occur even before the glomerular filtration rate declines (102). Correction of the phosphate depletion in type II RTA and the acidosis of type I RTA improve mineralization remarkably (102). In children, adults with very slow progression of renal failure, or anyone with marginal nutrition, musculoskeletal problems may occur of a severity not usually seen until several years of dialysis have elapsed. In children the superimposition of growth requirements may make the skeleton more sensitive to excessive PTH. The aluminum problem certainly appears commonly in children prior to dialysis (103), which may be due to better compliance with medications or to increased gut permeability.

In adults with advanced but stable renal failure, all the factors are in place that lead to osteodystrophy. Therefore, if there is enough time, it seems inevitable that bone disease will occur. Dietary innovations that may permit uremic patients to survive many years without dialysis will require careful monitoring for this problem (104).

High-Turnover Bone Disease

Almost every symptom of uremia has been ascribed to elevated PTH (105). Although this may yet prove to be true, controlling PTH does not seem to alleviate the entire uremic syndrome. Perhaps the earliest symptom related to high-turnover bone disease is itching. This is particularly difficult to manage as pruritus in the uremic patient has such a variety of causes, including dry skin, uremia itself, disordered porphyrin metabolism, and abnormal calcium metabolism. With respect to the calcium abnormalities, most pruritic patients have high calcium X phosphate products and elevated PTH levels (106,107).

Frequently associated with or following the appearance of pruritus, some patients develop metastatic calcifications. Usually, occurring in conjunction with high calcium X phosphate products, patients present with a variety of patterns. Tumorous masses may appear, particularly adjacent to joints, which can grow to 20 cm in diameter and weigh up to 10 kg (Fig. 93-4). The masses interfere with joint function and can become infected. Similar to those associated with foreign bodies, these infections are almost impossible to eliminate.

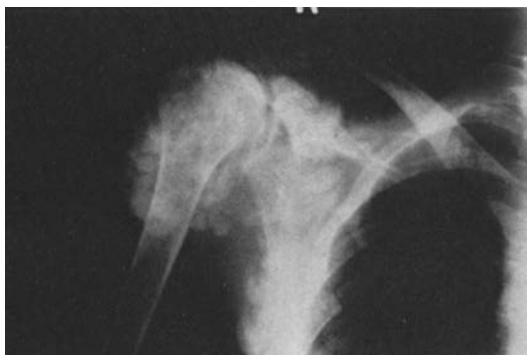


FIGURE 93-4. Periarticular soft tissue calcification in a patient receiving dialysis.

FIGURE 93-5. Ischemic necrosis from severe vascular calcifications in a patient with chronic renal failure.

Deposits in other sites may be even more problematic. Myocardial calcification (108) can result in refractory dysrhythmias or myocardial rupture. Diffuse pulmonary deposits in the alveolar–capillary basement membrane result in a restrictive lung disease with progressive pulmonary insufficiency (109). Vascular deposits likely contribute to the atherosclerosis to which these patients are prone, the most severe form of which is calciphylaxis (Fig. 93-5). The recent finding of enhanced mortality in dialysis patients who have a calcium X phosphate product greater than 65 or a phosphate concentration greater than 6.5 mg/dL (111) attests to the importance of fluctuating serum phosphate and the difficulty in ascribing a safe phosphate level for dialysis patients. More recently, Goodman et al. (111) have shown that coronary artery calcifications, as detected by electron-beam computed tomography, are detectable in young patients within the first few years of commencing dialysis (Fig. 93-6). The calcifications were noted to occur in those with higher calcium intakes and higher calcium X phosphate products.

Bone pain and proximal muscle weakness usually go hand in hand. It is often difficult to determine whether the muscle weakness is disuse atrophy (disuse because of skeletal pain and the patient's reluctance to move) or whether there is a specific muscle lesion. Occasionally, it is clear that proximal muscle atrophy and weakness, as occur in many other endocrine and metabolic disorders, develop before overt bone symptoms.

Bone symptoms are generally associated with weight-bearing activity. Pain occurs mostly in the lower extremities,

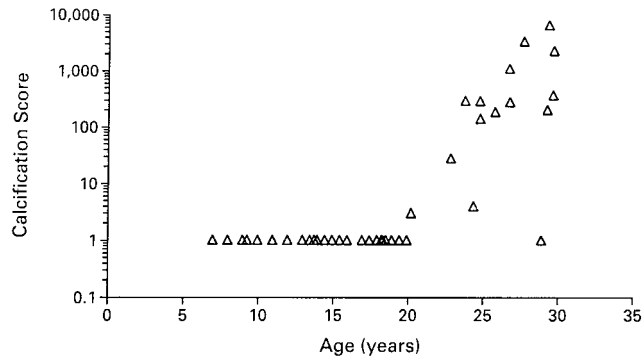


FIGURE 93-6. Coronary-artery calcification scores in 39 children and young adults with end-stage renal disease on dialysis, according to age. (Reprinted from: Goodman WG, et al. Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. *N Engl J Med* 2000;342:1478, with permission.)

particularly in the heels, knees, and hips. Less frequently the ankles and feet hurt as well. Fractures are uncommon and are usually of smaller bones, although a patient may fracture a large bone through a cystic lesion. In general, fractures heal rather quickly with brisk callous formation.

Enervation may occur with more severe hyperparathyroidism. Patients complain of a loss of enthusiasm, inability to "get started" with anything, and a vague depression. Obviously, all of these symptoms could be reactive depression, not uncommon in patients who feel overwhelmed by their disease. Changes in the electroencephalogram, brain calcium, and peripheral nerve conduction have been associated with the PTH elevation in uremia and may respond to its correction (112). The improvement with correction of the metabolic abnormality makes it seem likely that this is often a metabolic and not an emotional reaction.

A variety of arthropathies occur in patients with renal failure. Several may develop in conjunction with the bone disease. Pain with weight bearing in the hyperparathyroid patient is often felt in the joints, but there is usually little ancillary evidence of joint disease such as swelling, heat, tenderness, or limitation of motion. On the other hand, with high calcium X phosphate product, a painful crystal-induced arthritis can occur. This is particularly common in patients with large calcific deposits. In this setting, calcium pyrophosphate crystals may be seen in the joint. In the same patients, for unclear reasons, apatite crystals may form in the joint fluid and lead to a similar picture (113).

Low-Turnover Bone Disease

Many of the symptoms usually associated with high-turnover bone disease can be seen with low-turnover bone disease as well. Itching, metastatic calcification, and the crystal-induced arthropathies in general seem to correlate with the high phosphate levels. However, although these laboratory abnormalities are much more common in patients with secondary hyperparathyroidism, selective noncompliance in other patients can lead to similar laboratory abnormalities (see subsequent text) and hence similar symptoms.

Muscle weakness and proximal muscle wasting accompanying low-turnover bone disease are much more common and more severe (114). Although patients with hyperparathyroidism may complain of decreased strength and have modest quadriceps wasting, patients with low turnover bone disease often have severe weakness and atrophy and are unable to walk.

Similarly, bone pain, which is usually relatively mild and only minimally limiting with hyperparathyroidism, is much more severe with low-turnover bone disease. In addition, in contrast to high-turnover bone disease, patients very frequently experience fractures (115). A fracture with little or no trauma or involving the ribs should make one highly suspicious of this type of disease.

Mixed–Transitional Bone Disease

Depending more on the biochemistry and aluminum levels and the course the lesion is taking (e.g., whether the patient developing aluminum toxicity or PTH excess), the symptoms of this disorder may be any of the foregoing symptoms of either of the two more common lesions. Bone deformity, however, is much more common with this condition than with either of the other two. If deformity is present, then bone pain, muscle weakness (often severely incapacitating), and fractures commonly occur as well.

Radiographs and Magnetic Resonance Imaging

Conventional radiographic studies are of limited value in distinguishing the different bone lesions of renal osteodystrophy.

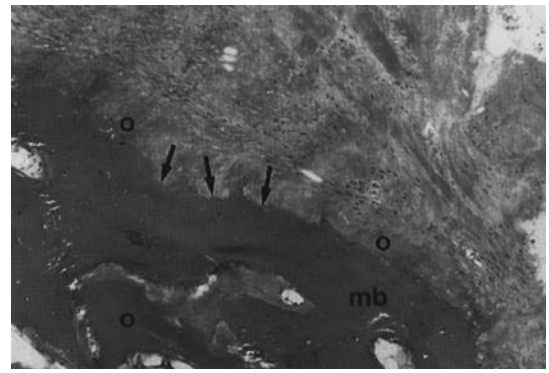


FIGURE 93-7. Subperiosteal resorption (*arrows*) of mineralized bone (*mb*) filled in with unmineralized osteoid (O) in a patient on dialysis with osteomalacia. (Goldner's stain, magnification ×115.) (See Color Plate.)

If x-rays reveal sclerotic bones of high density, the patient is more likely to have high-turnover bone disease. Conversely, the pseudofractures typical of osteomalacia–rickets in the patient without renal failure are also characteristic of osteomalacia with low bone turnover in patients with renal failure. Other than these two findings, x-rays are of little value and may even be misleading. This is especially true about subperiosteal resorption (101). Said to be pathognomonic of hyperparathyroidism, this picture may be seen very frequently in patients with osteomalacia. The reason for this is clarified in Figure 93-7. In the normal course of bone remodeling, bone is first resorbed by osteoclasts, and the eroded area is filled in by active osteoblasts that produce osteoid. Normally this osteoid is promptly mineralized, and x-rays do not detect the eroded area. In hyperparathyroidism, however, the high level of osteoclastic bone-resorbing activity makes this a common x-ray feature. In renal osteomalacia it is also common because the osteoblasts often fill in the resorption cavities with osteoid, but this osteoid takes up mineral slowly or not at all. The x-ray, then, being able to "see" only the calcified tissue, cannot distinguish the active resorption cavity of hyperparathyroidism from the inactive erosion of osteomalacia (101). Cystic bone lesions (Fig. 93-8), particularly in the long-term dialysis patient, would be found with beta-2-microglobulin (B_2M) amyloid deposition perhaps even more frequently than with hyperparathyroidism. Common sites for cystic changes are the femoral head, humerus, carpal bones, tibia, and pubic symphysis.

Although femoral fracture is a major complication of B_2M amyloid deposition, the more common complaint is shoulder pain. Secondary to deposition of B_2M amyloid in the synovium and tendon sheath around the humerus, the pain characteristically is continuous and worse with movement. Shoulder joint fluid can usually be detected early by magnetic resonance imaging (MRI), often in conjunction with other characteristic findings such as supraspinatus tendon thickening and cystic lesions of the humerus (116) (Fig. 93-9). The use of MRI in the early detection of B_2M amyloid lesions has made it easier for early referrals for renal transplantation.

Bone Mass Measurements

The most common radiographic finding in uremic patients is osteopenia (reduced bone density). Because this finding has no specificity, most physicians have discontinued the use of conventional x-rays to monitor bone disease in renal patients. Newer techniques, however, which evaluate bone mass or bone mineral density (BMD) directly may be beneficial for long-term monitoring. Quantitative computed tomography (QCT) and dual-energy x-ray absorptiometry (DEXA) are the two most

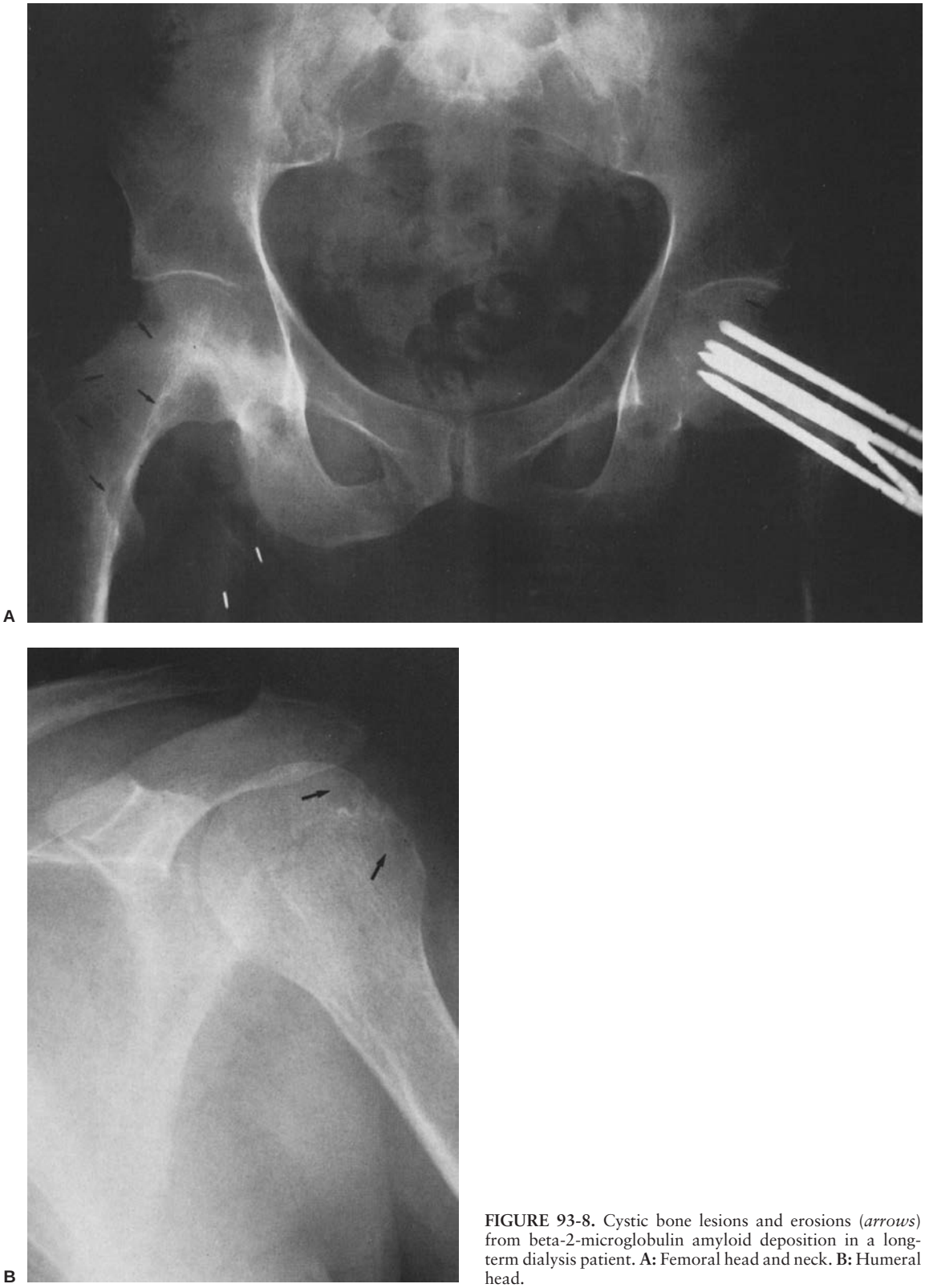


FIGURE 93-8. Cystic bone lesions and erosions (*arrows*) from beta-2-microglobulin amyloid deposition in a long-term dialysis patient. **A:** Femoral head and neck. **B:** Humeral head.

commonly used methods that quantify bone density. The former technique is less often used because of its higher radiation dosage and its inability to image the hip. DEXA scans, on the other hand, have good precision and low radiation exposure and are therefore good for longitudinal evaluation. Unfortunately, only a limited number of DEXA studies have been performed in patients with chronic renal failure, so it is unclear what role this type of imaging will have in the current management of renal osteodystrophy. Low values for femoral neck BMD have been observed in patients receiving peritoneal and hemodialysis (117,118), consistent with the high hip fracture rate recently documented in the dialysis population (119). Amenorrhea and prior transplantation were significant risk factors for reduced femoral neck BMD in one study of 250 dialysis patients (118). Femoral neck bone loss actually begins prior to starting dialysis. Rix et al. demonstrated that patients with

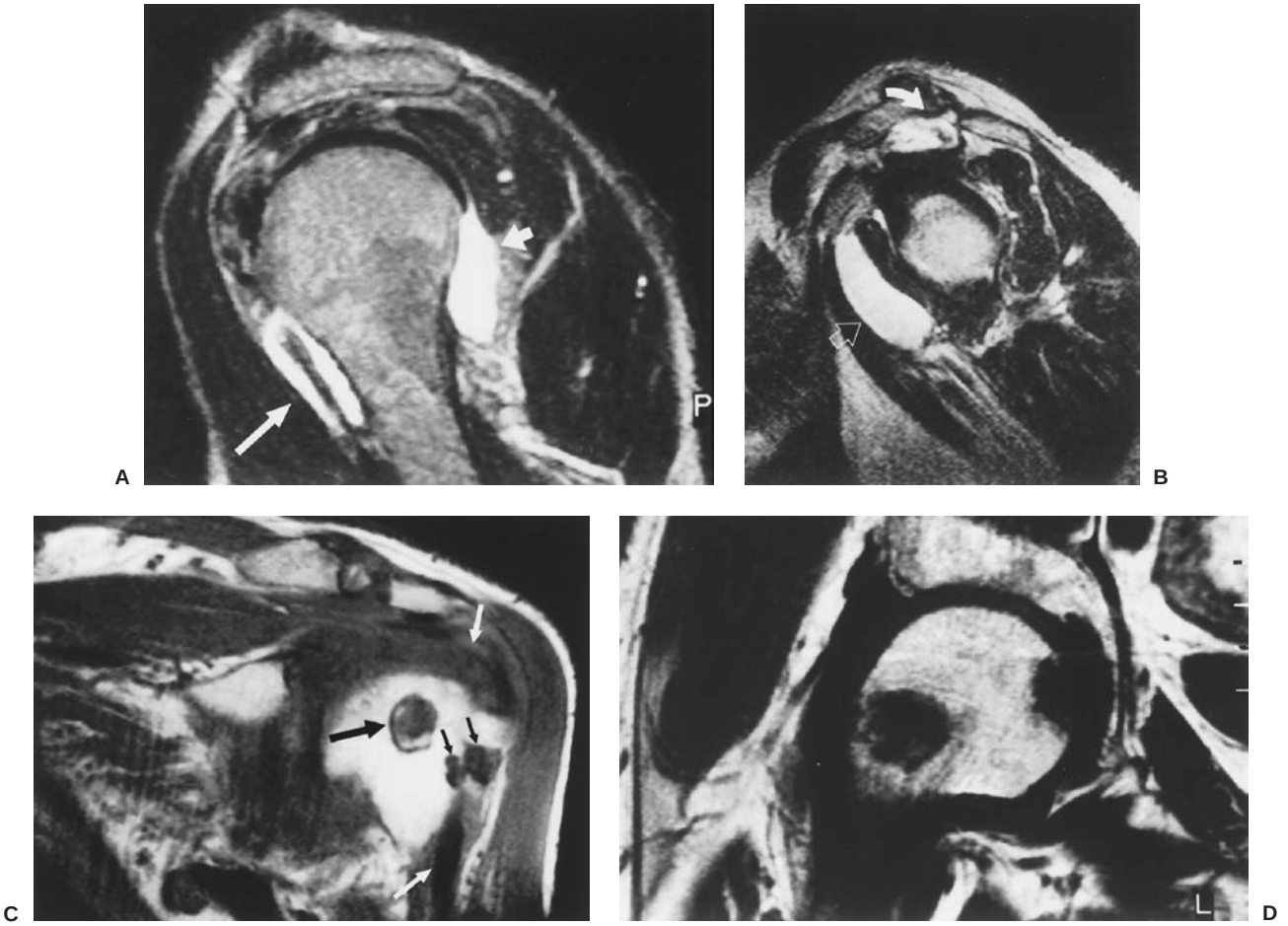


FIGURE 93-9. Magnetic resonance images (MRI) in dialysis patients with β-2 microglobulin amyloid deposition. **A:** Sagittal T2W1 image of the shoulder showing a large fluid collection in the biceps tendon (*long arrow*) with associated joint effusion (*short arrow*). **B:** Sagittal T2W1 image of the shoulder demonstrating a subcoracoid bursal effusion (*open arrow*) and subacromial bursal fluid (*curved arrow*). **C:** T1W1 image of the left shoulder demonstrating three focal lesions in the humeral head (*large black arrow*) and bicipital groove (*small black arrows*). Note thickening of the supraspinatus and biceps tendon (*white arrows*). **D:** Coronal T1W1 image showing two periarticular lesions in the femoral head. (Reprinted from: Escobedo EM, et al. Magnetic resonance imaging of dialysis-related amyloidosis of the shoulder and hip. *Skeletal Radiol* 1996;25:41, with permission.)

a mean GFR as high as 58 mL/minute have significantly reduced femoral neck BMD and a sixfold higher prevalence of osteoporosis compared to normal controls (120) (Fig. 93-10). Moreover, in the group with a mean GFR of 16 mL/minute, 42% had osteoporosis of the femoral neck (120). More studies with larger populations and longitudinal evaluations of hip BMD are needed to identify other potential risk factors and to answer questions regarding the role of PTH, androgens, estrogens, and vitamin D therapy in the preservation of bone mass.

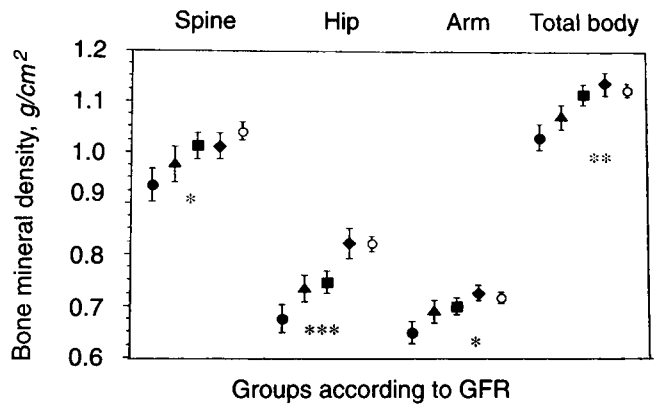


FIGURE 93-10. Bone mineral density (BMD) in patients with chronic renal failure. *Closed circles* indicate mean glomerular filtration rate (GFR) 16 mL/minute, *triangles* indicate mean GFR 39 mL/minute, *squares* indicate mean GFR 58 mL/minute, *diamonds* indicate mean GFR 83 mL/minute and *open circles* indicate normal controls with mean GFR 144 mL/minute. (Reprinted from: Rix M, et al. Bone mineral density and biochemical markers of bone turnover in patients with predialysis chronic renal failure. *Kidney Int* 1999;56:1084, with permission.)

Biochemical Studies

Serum Chemistries

Serum calcium and phosphate levels are not particularly helpful in distinguishing the various bone lesions. High values are possible in either low or high bone-turnover states. Low values for calcium or values of less than 45 for calcium X phosphate product would be more consistent with the mixed uremic osteodystrophy, although a wide range of values is seen in each group (121).

Although alkaline phosphatase levels are also nonspecific, they do show good correlations with bone histologic parameters of osteoblast function (122). Marked elevation (more than twice the upper limit of normal) would be very unlikely in low-turnover bone disease and would suggest either osteitis fibrosa or mixed uremic osteodystrophy. Normal values are unlikely with high-turnover bone disease. In low-turnover disorders values are usually normal. For unclear reasons, some dialysis patients have had a paradoxic increase in alkaline phosphatase levels following parathyroidectomy (123).

Serum immunoreactive osteocalcin may be a more specific parameter for bone cell activity than alkaline phosphatase. Osteocalcin levels are elevated in uremia and show good correlations with bone histologic parameters in dialysis patients (124). Serum osteocalcin becomes elevated early in renal failure and its rise mirrors the progressive elevation of PTH. To what extent the osteocalcin level reflects osteoblast production of this protein or its release from mineralized matrix during bone resorption is unclear.

Parathyroid Hormone

Immunoreactive PTH is varyingly elevated depending on the assay used. The physician must be familiar with the assays used to see which provides the best information (Table 93-2). Available assays are specific for carboxy-terminal, amino-terminal, or a midregion of the PTH molecule. Carboxy-terminal and midregion PTH assays are almost invariably elevated in renal failure, no matter what the actual size is of the patient's parathyroid gland. This is because these assays measure not only the active hormone but also many inactive metabolites that are normally excreted by the kidney (125). Therefore, carboxy-terminal or midregion assays in patients without parathyroid overactivity may be 10 times the upper limit of normal in some laboratories. Indeed, a normal value would imply decreased parathyroid activity (56).

Values for the amino-terminal or intact PTH assays seem somewhat more relevant to the true parathyroid status of the patients with renal failure (126,127). By using these assays one can more reliably predict what is occurring at the skeletal level. Values up to three times that of normal are fairly typical for the well-managed patients. Serum values greater than this raise concern about significant hyperparathyroid bone disease. Normal and particularly low-normal values in a patient with advanced renal failure should raise concern about low-turnover lesions (86,128) (Table 93-3).

Bioactive PTH measurements also correlate with some bone histologic parameters in dialysis patients (129). However, they do not appear to be more discriminatory than intact or N-terminal immunoreactive PTH assays in this patient group (129).

Whatever assay is used, low PTH values are of even greater concern if they do not increase with hypocalcemic stress. When a patient is given a calcium-free dialysate for the first hour of dialysis and the PTH fails to increase substantially, the diagnosis of low-turnover bone disease due to aluminum must be strongly suspected (130). Recently it was shown that patients with the aplastic lesion not due to aluminum respond with an increase in PTH and bone formation when challenged by hypocalcemia (86).

TABLE 93-2

PARATHYROID HORMONE (PTH) ASSAY CHARACTERISTICS

I. Carboxy-terminal PTH
   A. Oldest assay
   B. Measures intact and inactive metabolites
   C. Values unpredictably elevated in renal failure
II. Midregion PTH
   A. Measures inactive region
   B. Values unpredictably elevated in renal failure
III. Amino-terminal PTH
   A. Measures active end of molecule
   B. Values correlate with bone histology in renal failure
IV. Intact PTH
   A. Measures intact hormone and "7-84 fragments"
   B. Values correlate with bone histology in renal failure
V. Bio-intact (whole) PTH
   A. Measures only intact hormone
   B. Values correlate with bone histology in renal failure
   C. Ratio of intact to whole PTH is not predictive of bone histology.

Aluminum Assays

As with PTH measurements, many commercial laboratories seem to be promoting their aluminum assays. Although some of these are undoubtedly accurate, many are not. Until further information is available, one is best advised to be wary of many of the current assays. Assuming an assay is properly carried out (131), the basal serum aluminum value can be of considerable help in assessing the possibility of excess aluminum accumulation (see Table 93-3). Serum values less than 20 μg/L (approximately five times the level in normal subjects) essentially exclude aluminum toxicity, whereas values greater than 300 μg/L confirm the diagnosis (132).

Deferoxamine Challenge

In the wide range between these values, additional studies may be helpful. The deferoxamine (DFO) challenge test is often useful (133) (Table 93-4). In this test, 40 mg/kg of DFO is administered intravenously over a 2-hour period at the end of dialysis. The baseline aluminum level is compared to the post-DFO peak value 24 or 48 hours later. An increase greater than 150 μg/L confirms the presence of biologically significant aluminum accumulation (133). Because a negative DFO challenge test does not always exclude aluminum toxicity, we have found that combining this test with serum PTH levels improves the diagnostic accuracy (132) (see Table 93-4).

Beta-2-Microglobulin Assays

Serum B_2M is elevated in uremic patients but levels do not discriminate patients with B_2M amyloid deposition (134). B_2M measurements may be more helpful when evaluating B_2M clearance from blood during dialysis (135). Radionuclide scans utilizing ^{131}I-labeled B_2M have proved to be impractical owing to the high dose of radiation (136).

Bone Biopsy

Bone biopsy is sometimes necessary to confirm or exclude aluminum toxicity. For this procedure to be helpful, the physician will need to obtain careful instructions from the histology laboratory that will process the specimens. Few hospitals are capable of properly processing such specimens. In addition to proper handling, it is also important that time-spaced tetracycline labeling be given in the month prior to the biopsy. Normally, a patient would receive a single dose of tetracycline 3 weeks and 1 week before the biopsy (137). In special circumstances, the labeling interval or the interval between the last label and the biopsy can be altered, but this should be discussed with the laboratory that will handle the specimen. The

TABLE 93-3

DISTINCTIONS BETWEEN HYPERPARATHYROIDISM AND BONE ALUMINUM INTOXICATION IN PATIENTS WITH RENAL FAILURE

	Hyperparathyroidism	Aluminum toxicity
Bone pain	Mild Severe	
Weakness	Mild to moderate	Severe
Fracture	Rare, heal readily	Frequent, poor healing
X-ray	Osteopenia, erosions, patchy osteosclerosis rare	Osteopenia, erosions, pseudofractures rare
Calcium	10–12 mg/dL	10–12 mg/dL
Alkaline phosphatase	110%–400% of normal	
PTH intact[a]	>150 pg/mL	<100 pg/mL
Basal aluminum	<300 μg/L	>300 μg/L
Stimulated aluminum (DFO test)	Increases <150 μg/L	Increases >150 μg/L

PTH, parathyroid hormone; *DFO*, deferoxamine.
[a] Marked variation between assays.

TABLE 93-4

DIAGNOSIS OF ALUMINUM TOXICITY

I. Clinical features
  A. Bone pain/fracture
    1. Weight-bearing bones involved
    2. Fractures heal slowly
  B. Dementia
    1. Symptoms often appear first just during dialysis
    2. Sequential worsening
      a. Stuttering/halting speech
      b. Myoclonus
      c. Mutism
      d. Depressed consciousness
      e. Seizures
  C. Anemia
    1. Hypochromic, microcytic
    2. Normal iron levels
    3. May be erythropoietin resistant
II. Laboratory features
  A. Random serum aluminum >100 μg/L
    1. Most patients will have some evidence of toxicity
    2. At least 30% will not be toxic
  B. Deferoxamine challenge test
    1. A rise in aluminum level >300 μg/L
      a. Specificity—35%
      b. Sensitivity—90%
    2. A negative test—even with no increase—does not exclude aluminum toxicity
  C. Random PTH in the normal range.
III. Bone biopsy
  A. Gold standard for bone toxicity
  B. Should include tetracycline labeling
  C. Diagnosis positive if >25% surface aluminum, and bone formation rate < normal (possible if 15%–25% surface aluminum)
IV. EEG
  A. Specific pattern said to be "diagnostic" of aluminum-related dementia
  B. False-positive rate unknown

site of the biopsy is also important. The anterior superior iliac crest (between 2 and 5 cm posterior to the anterior superior iliac spine) is the preferred site, since most studies have utilized bone from this region. In addition, it is a particularly safe area to biopsy because there are no vital structures nearby and this bone is non-weight-bearing. The biopsy must contain trabecular bone and can usually be obtained with local anesthesia. There is minimal morbidity associated with such a procedure.

Bone Histology

Patients with chronic renal insufficiency invariably develop abnormalities in bone histology (138–148). The type of pathology observed is determined by a variety of factors, which relate to the duration of dialysis as well as to previous treatment. In general, the most common histologic findings are those associated with increased bone turnover, especially if the biopsy specimen is taken during the first few years of dialysis. Although a minority of patients beginning dialysis have low bone turnover, increasing numbers develop this type of bone lesion after long-term dialytic therapy.

High-Turnover Bone Disease

The earliest bone histologic change is an increase in the amount of unmineralized osteoid that covers the mineralized bone surface (Fig. 93-11). Although the osteoid width is usually not increased at this stage (149), the osteoid area (149) or osteoid volume exceeds normal values. In addition, the bone formation rate is either normal or increased (149,150). These changes in osteoid and bone formation parallel what has been described in early primary hyperparathyroidism (151,152) and in rats given excess PTH parenterally (153). Therefore, bone histologic evidence of mild hyperparathyroidism is usually apparent before dialysis commences (154) and is the predominant type of bone lesion in patients who have had renal disease for less than 5 years (149,155).

As serum levels of PTH continue to increase, a more severe form of hyperparathyroid bone disease, osteitis fibrosa, is observed (Fig. 93-12). Osteoclasts increase in number and size (156) and endosteal fibrosis becomes prominent. Osteoblasts lining the un-mineralized osteoid also increase in number and become cuboidal or "plump" in appearance. The direct

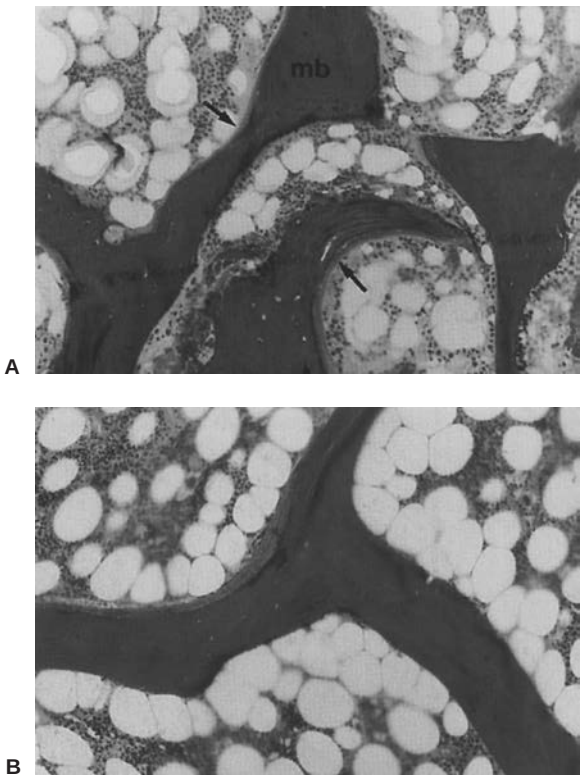


FIGURE 93-11. A: Histologic section of bone from a patient on dialysis with mild hyperparathyroidism. Length of unmineralized osteoid (*arrow*) is increased. Width of osteoid seam and volume of mineralized bone (*mb*) are normal. **B:** Bone section from a normal subject. (A and B, Goldner's stain, magnification ×115.) (See Color Plate.)

correlations between PTH levels and osteoblast number observed on bone histology (126) agree with the known stimulatory effect of PTH on osteoblast proliferation *in vitro* (19). Osteoid area as well as osteoid width increase substantially, reflecting increased osteoblast activity (149). In some cases the excess osteoid resembles osteomalacia. Without tetracycline labeling (tetracycline deposits in newly forming bone as a fluorescent line) it may be quite difficult to distinguish osteitis fibrosa from osteomalacia. Usually some of the osteoid has a normal lamellar pattern but this is often mixed with a large amount of woven osteoid, identified by its random arrangement of collagen fibers (Fig. 93-13). Woven osteoid is not found in normal bone but is characteristic of accelerated bone turnover states such as primary hyperthyroidism, Paget's disease, or fracture repair. Woven osteoid in uremic osteodystrophy is present during early renal failure and increases with declining renal function (154). It has been suggested that woven osteoid results when the osteoprogenitor cells revert to a more primitive form in the presence of excess PTH, leading to the deposition of irregularly oriented collagen fibers (29).

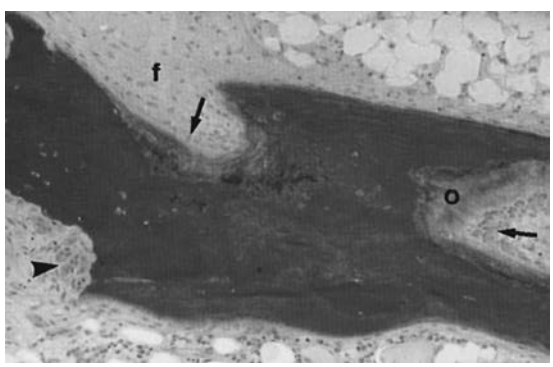


FIGURE 93-12. Osteitis fibrosa. Osteoid (*O*) is a mixture of woven and lamellar collagen. Plump osteoblasts (*arrows*) and multinucleated osteoclasts (*arrowhead*) are numerous. Fibrosis (*f*) is present. (Goldner's stain, magnification ×115.) (See Color Plate.)

Mineralization of osteoid in osteitis fibrosa is not impaired (149). This is determined by giving two time-spaced doses of tetracycline. In fact, the bone formation rate is markedly increased in the more severe forms of osteitis fibrosa (149) as evidenced by the increased uptake of tetracycline (Fig. 93-14). Earlier reports of low bone formation in chronic renal failure (157) are most likely attributable to the coexistent hypocalcemia that was present in the majority of those patients studied.

Despite the fact that osteoclastic bone resorption is increased in osteitis fibrosa, the total bone area is often greater than normal (149), implying that bone formation and resorption are no longer in balance and that the increase in bone formation is of a greater magnitude than the increase in resorption. The term osteosclerosis is reserved for the most severe examples of increased bone mass as a result of hyperparathyroidism (158). Histologically, all trabeculae appear thickened and the boundary between cortical and trabecular bone is obscured (Fig. 93-15). Usually, the other histologic features of osteitis fibrosa are also present in osteosclerosis, although the amount of marrow fibrosis does not parallel the large increase in mineralized bone.

As would be expected, parathyroid gland activity correlates with many of the parameters of bone histology in osteitis fibrosa. Uremic patients with osteitis fibrosa have more significant parathyroid hypertrophy than do patients without osteitis fibrosa (148,159) and positive correlations between serum PTH and bone histology have been clearly demonstrated (160–162). Studies in our laboratory comparing bone histology with different PTH assays indicated that an amino-terminal-specific PTH assay was a better predictor of osteitis fibrosa in dialysis patients (126) (Fig. 93-16). This presumably is due to its measurement of mainly intact PTH. Interestingly, in that study it was noted that the correlation coefficient between PTH and bone formation was substantially lower ($r = 0.46$) when patients with aluminum-related bone disease were excluded from analysis (126). Because this suggested that circulating factors other than PTH might be involved in regulating bone formation, we examined plasma IGFs in dialysis patients who did not have aluminum bone disease (163). The correlation of bone formation with plasma IGF-1 was slightly better than with plasma PTH. Moreover, all of the parameters of bone mineralization were better correlated with IGF-1, whereas the parameter for osteoblast number correlated better with PTH. This suggested that the mechanisms by which elevated IGF-1 and PTH enhance bone formation in uremia are probably different (163). A more recent study by Jehle et al. looked at circulating IGF and IGF-binding protein (IGFBP) levels in 319 patients having varying levels of chronic renal insufficiency, including half who were on chronic dialysis (164). They found that free IGF-1 levels were not elevated in the majority of dialysis patients, but that IGFBP-2, -3, -4 and -6 were markedly elevated, suggesting that these inhibitory IGFBPs may act to suppress IGF action at the osteoblast level. In addition, serum IGFBP-5 was uniformly low in dialysis patients and it was lower in the subgroup with low turnover bone disease compared to those with high bone turnover. These data suggest that a deficiency in IGFBP-5, an osteoblast stimulatory IGFBP, may contribute to the adynamic bone lesion (164), which is

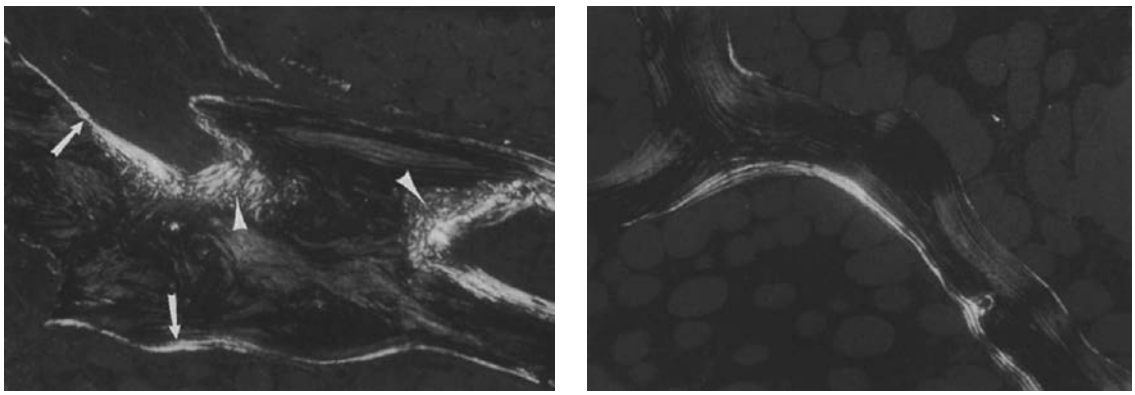


FIGURE 93-13. A: Polarized light section showing woven (*arrowheads*) and lamellar (*arrows*) collagen in osteitis fibrosa. **B:** Polarization of normal bone showing only lamellar collagen. (A and B, magnification ×115.) (See Color Plate.)

consistent with the known *in vitro* and *in vivo* effects of recombinant IGFBP-5 to stimulate osteoblast activity (16,165–167).

Low-Turnover Bone Disease

Osteomalacia, which is characterized by an excess accumulation of osteoid and an increase in osteoid seam width (Fig. 93-17), is the best-known type of low-turnover bone disease encountered in patients with chronic renal failure. Qualitatively, the osteoid is usually lamellar, although woven osteoid is occasionally present. Osteoblasts are typically flat or fusiform in appearance and decreased in number. Fibrosis is absent and the number of osteoclasts is normal or decreased. In general, osteoblasts appear to be inactive or absent. The dynamic assessment of bone function as determined by tetracycline uptake confirms this impression. Double labels of tetracycline are rarely found in osteomalacic bone. More often, no tetracycline is evident, although sometimes a single label can be discerned as a short thin line at the mineralization front (Fig. 93-18). Therefore, bone formation is either greatly reduced or absent.

Not all dialysis patients with low bone formation have excess osteoid accumulation typical of osteomalacia. Rather, a normal or reduced amount of unmineralized osteoid (Fig. 93-19) may be seen along with tetracycline evidence of decreased bone formation. Ott et al. used the term aplastic bone disease to differentiate this lesion from typical osteomalacia (168). Although some dialysis patients with aplastic (or adynamic) disease have no bone uptake of tetracycline, many have at least a single tetracycline label, whereas others show normally separated double labels that are markedly reduced in total length (Fig. 93-20). Therefore, even though the bone apposition rate (determined by the distance between the two labels) may be normal, the bone formation rate at the tissue level is reduced. When this situation occurs, it can be inferred that the total number of osteoblasts is decreased but that a normal rate of mineralization of osteoid continues in the few that remain.

The etiology of osteomalacia and aplastic bone disease was until recently an enigma. The fact that dialysis patients with osteomalacia failed to respond therapeutically to vitamin D treatment (114) was equally difficult to explain. It was not until researchers in the United Kingdom discovered that aluminum contamination of the water supply was linked to a severe form of fracturing osteomalacia (68,169) that the association of aluminum and bone disease in the United States was made. Subsequently, a retrospective analysis of bone biopsy specimens from patients with osteomalacia revealed that significant aluminum deposition was present in most of the specimens studied (168). In Figure 93-21 are shown the relative frequency of positive aluminum staining in bone biopsy specimens from patients with osteomalacia as well as those with other types of uremic bone disease. More than 80% of the specimens that had osteomalacia showed either heavy or moderate staining for aluminum (168), as evidenced by a precipitation line on the mineralized bone surface (77). Usually the aluminum was located at the interface of osteoid and mineralized bone,

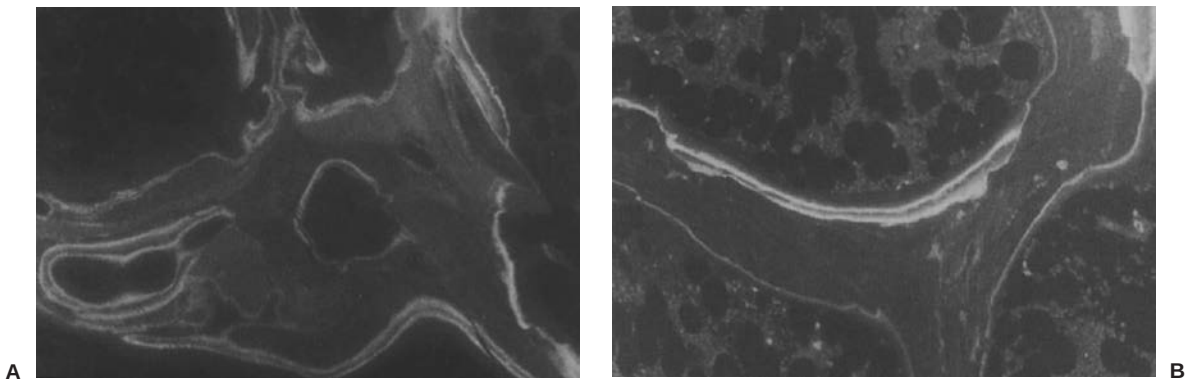


FIGURE 93-14. A: Double tetracycline labels in a patient with severe osteitis fibrosa. The distance between labels and the total length of the double labels is increased, demonstrating a high formation rate. **B:** Tetracycline labels from a normal subject. (A and B, unstained fluorescent micrographies, magnification ×115.) (See Color Plate.)

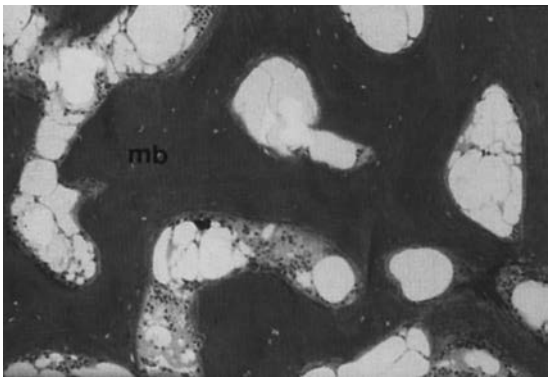


FIGURE 93-15. Osteosclerosis. The amount of mineralized bone (*mb*) is increased. (Goldner's stain, magnification ×115.) (See Color Plate.)

although it was also found on "neutral" surfaces of bone without overlying osteoid (Fig. 93-22). Although most of the positive stains for aluminum were seen in patients with osteomalacia, aluminum was detected in some patients with osteitis fibrosa and mild bone disease as well (168). Aluminum accumulation in osteitis fibrosa has since been shown to be highly variable (157,170,171).

Until 1981, it was thought that only a small minority of dialysis patients developed aluminum-associated bone disease (114). However, several studies have demonstrated a relatively high prevalence of bone aluminum accumulation in this patient population (172–174), many of whom develop symptomatic bone disease. In a study from our laboratory, prospective bone biopsy specimens from asymptomatic patients who had received dialysis for at least 8 years revealed a 37% prevalence of aluminum bone disease (172). In this study, aluminum bone disease was defined by an amount of aluminum on the bone surface that equaled at least 25% of the total mineralized bone surface in the presence of a bone formation rate below normal. This was done to distinguish patients with osteitis fibrosa who had small amounts of bone-surface aluminum from those patients with low turnover bone disease. Because both the tap water and dialysate were low in aluminum, it was inferred that

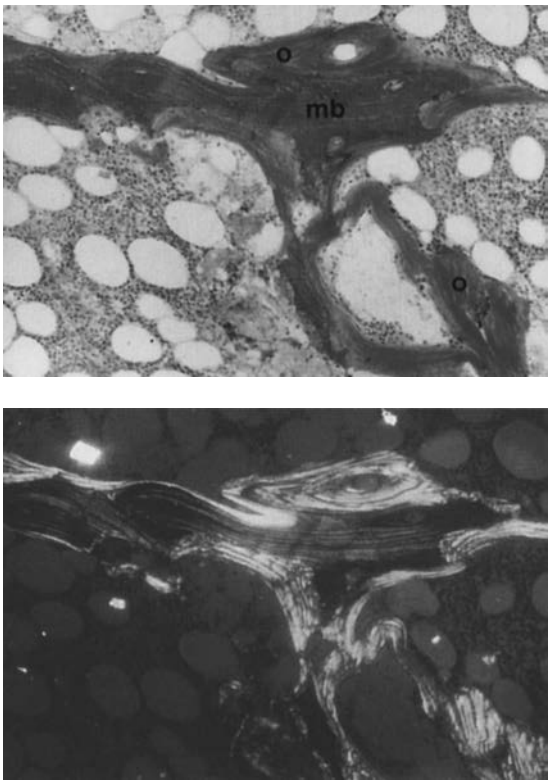


FIGURE 93-17. Osteomalacia. **A:** The unmineralized osteoid (O) is increased in width and total volume; *mb*, mineralized bone. **B:** Polarized section showing predominately lamellar orientation of collagen in osteomalacia. (A and B, magnification ×115.) (See Color Plate.)

the high prevalence of low bone formation was a result of the chronic ingestion of aluminum-containing phosphate binders (172). Subsequent studies showed that patients with the osteomalacic lesion had greater total body aluminum stores than did those with the aplastic lesion (175).

There is other evidence to support the role of oral aluminum loading in bone aluminum deposition. Bone aluminum

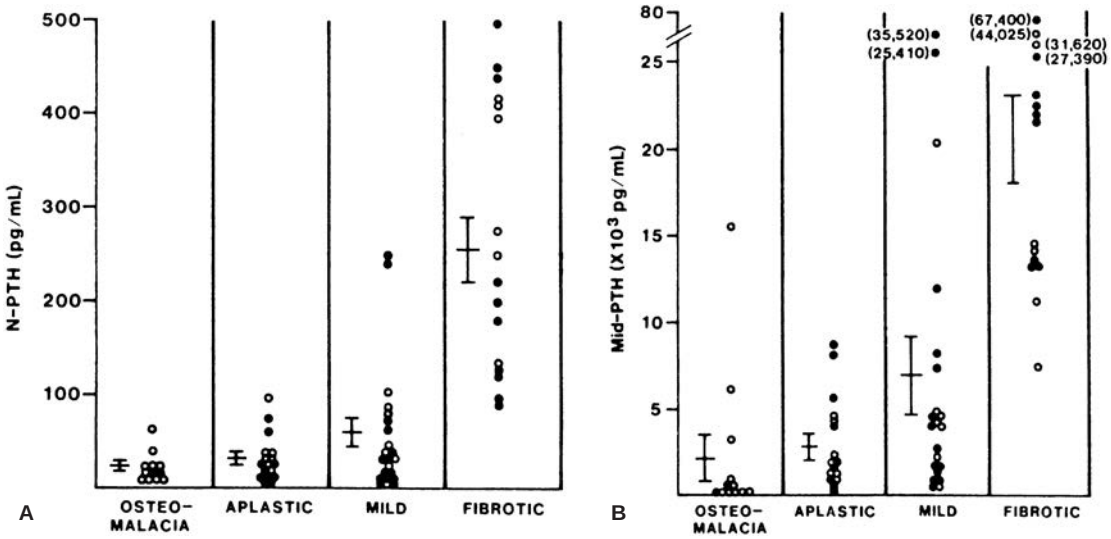


FIGURE 93-16. **A:** Serum amino (N)-terminal PTH values in four types of renal osteodystrophy. **B:** Serum midregion PTH values in the same patients. (Reprinted from: Andress DL, et al. Comparison of parathyroid hormone assays with bone histomorphometry in renal osteodystrophy. *J Clin Endocrinol Metab* 1986;63:1163, with permission.)

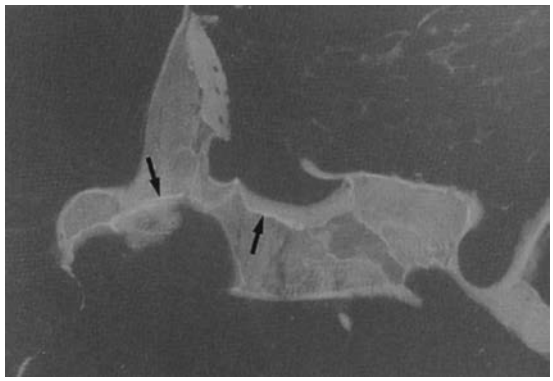


FIGURE 93-18. Single tetracycline label (*arrows*) in bone from a patient on dialysis with osteomalacia given two time-spaced doses of tetracycline. Absence of double labels indicates abnormally low bone formation. (Unstained fluorescent micrograph, magnification ×115.) (See Color Plate.)

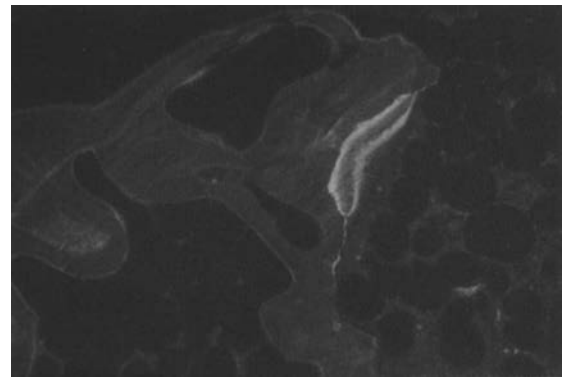


FIGURE 93-20. Aplastic (adynamic) bone disease. The total amount of tetracycline uptake is decreased, as is the number of double tetracycline labels. When present, the double label is shorter than normal, demonstrating reduced bone formation. (Unstained fluorescent micrography, magnification ×115.) (See Color Plate.)

accumulation (174) and aluminum-associated bone disease (73,103) can occur in uremic patients before dialysis has been initiated. Moreover, diabetic dialysis patients have been shown to have accelerated bone aluminum accumulation that is directly related to the cumulative oral intake of aluminum and to whole-body aluminum stores (176) (Fig. 93-23). Whether diabetic patients are also "hyper-absorbers" of aluminum remains to be evaluated.

The observation that serum PTH levels are often normal in dialysis patients with heavy bone aluminum deposition (114,128) suggests that functional hypoparathyroidism probably has an important role in the pathogenesis of aluminum bone disease. Aluminum in the parathyroid glands of humans, as measured by neutron activation analysis, can be related to the ingestion of aluminum (94). *In vitro* studies using parathyroid gland slices have shown that aluminum inhibits PTH secretion, but not production, in a dose-dependent manner (95,177). Moreover, correction of aluminum contamination of the dialysate results in increased PTH levels in some patients (178). Because dialysis patients with high levels of bone aluminum are more likely to have suppressed PTH levels during an acute hypocalcemic challenge than are patients with low levels of bone aluminum (130), a PTH-stimulation test has been advocated as one method to screen for aluminum bone disease. While dialyzing patients using a calcium-free dialysate, we found that the serum amino-terminal PTH levels after 1 or 2 hours accurately discriminated patients who had documented aluminum bone disease. In further support for the role of hypoparathyroidism in the development of aluminum bone disease is the observation that bone aluminum accumulation is markedly enhanced following parathyroidectomy (91) (Fig. 93-24). The decreased bone formation after parathyroidectomy was attributed to the decline in circulating levels of PTH. To what extent aluminum on the bone surface also contributed to the decreased bone formation could not be determined. It did appear, however, that the high rates of bone formation before parathyroidectomy protected against bone-surface aluminum accumulation (91). Functional hypoparathyroidism is also likely to be important in causing reduced bone formation and increasing bone aluminum in the diabetic uremic patient (87,176). In this situation, high glucose concentrations within parathyroid cells may decrease PTH secretion (89).

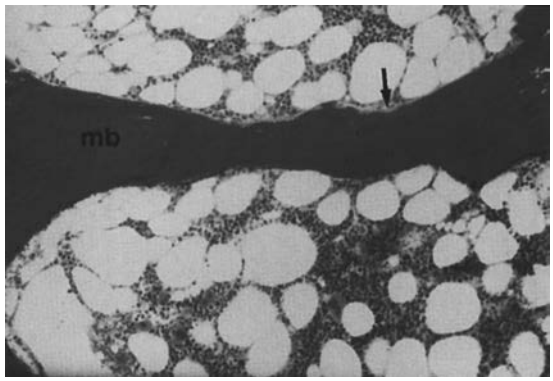


FIGURE 93-19. Aplastic (adynamic) bone disease. Marked decrease in amount of unmineralized osteoid (*arrows*); *mb*, mineralized bone. Osteoblasts are flat when present. (Goldner's stain, magnification ×115.) (See Color Plate.)

The exact mechanism by which aluminum inhibits bone mineralization in humans has not been identified. Studies in rats (179–184) and dogs (185) have clearly shown that osteomalacia can be produced by the parenteral administration of aluminum. Moreover, the mineralization defect is enhanced when chronic renal failure is also present (179,181,184,185), is made worse by parathyroidectomy (186), and is ameliorated by PTH infusions (187,188). Studies performed by Plachot et al. (96) support the idea that PTH may protect osteoblast function when aluminum is deposited on bone. Using electron microscopy and x-ray microanalysis, they discovered aluminum within the mitochondria of osteoblasts in patients with osteitis fibrosa. Because the number of osteoblasts was increased, the authors suggested that the high serum PTH was responsible for stimulating osteoblast growth as well as the cellular uptake and release of aluminum into the organic matrix (96). The fact that PTH administration is known to increase the bone aluminum content in rats (189) supports this hypothesis. However, most patients with osteitis fibrosa have a lower trabecular bone aluminum content than do patients with osteomalacia (190). Therefore, it is possible that differences in the distribution of aluminum within bone may partially explain these discrepant findings. For example, the location of much of the aluminum in osteomalacia or aplastic bone disease is on the surface of the mineralized bone interfaced with the overlying un-mineralized osteoid as determined by electron microprobe analysis (76). In contrast, very little aluminum is seen on the bone surface in osteitis fibrosa (166) but is more diffusely distributed throughout the mineralized compartment (170).

Whether aluminum within the osteoblast is responsible for inducing a mineralization defect characteristic of

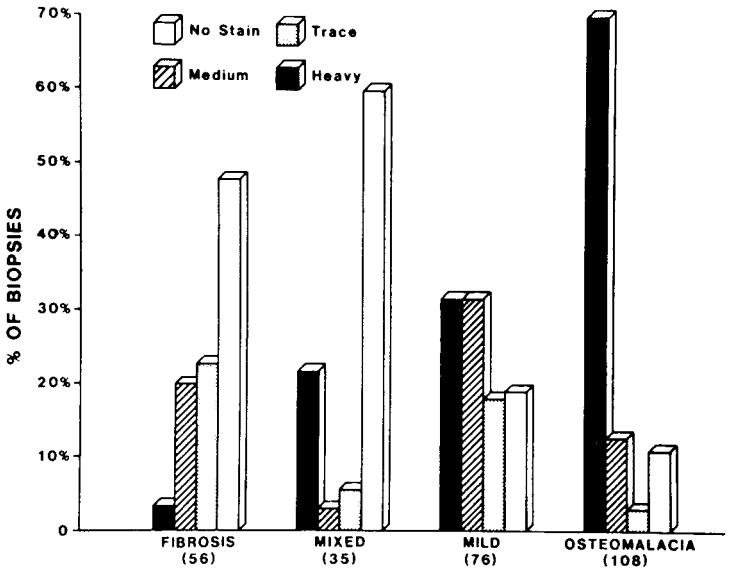


FIGURE 93-21. Distribution of aluminum stain in bone among the histologic groups of uremic bone disease. (Reprinted from: Ott SM, et al. The prevalence of bone aluminum deposition in renal osteodystrophy and its relation to calcitriol therapy. *N Engl J Med* 1982;307:709, with permission.)

osteomalacia or aplastic disease has not been decided. However, *in vitro* studies involving aluminum incubation with osteoblasts indicate that aluminum has an adverse effect on bone cell function. Lieberherr et al. (191) have shown that the bone phosphatase activity in rat bone cells is inhibited at high aluminum concentrations (6×10^{-6} M). In addition, aluminum decreased the stimulatory action of PTH on bone phosphatase activity (191). In other experiments aluminum has been shown to suppress osteoblast proliferation in vitro (192,193), supporting clinical studies that show an inverse relationship between osteoblast number and bone-surface aluminum (172,194). The adverse effect of aluminum on osteoblast function may also be mediated by alterations in osteoblast synthesis of 1,25-dihydroxyvitamin D (195) and bone PTH receptors (196).

It is possible of course that aluminum may also inhibit mineralization by a mechanism that is not osteoblast mediated. Aluminum chloride reduces amorphous calcium phosphate transformation and hydroxyapatite crystal growth *in vitro* at concentrations of 0.25 to 0.50 mM (87). Although these concentrations are generally higher than those found in the serum of dialysis patients, they approximate the levels found in mineralized bone in aluminum-intoxicated animals (179) and humans (76,190).

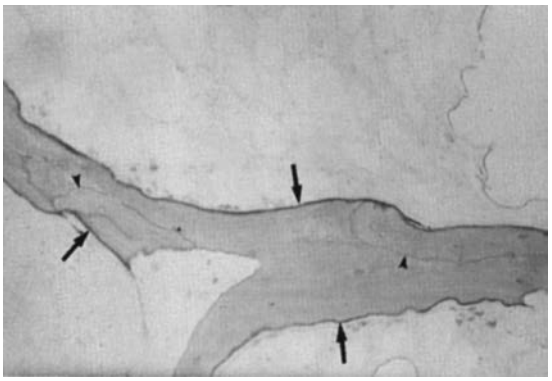


FIGURE 93-22. Histologic section showing a positive stain for aluminum on the surface (*arrows*) of mineralized bone and within cement lines (*arrowheads*). (Aurin-tricarboxylic acid, magnification ×115.) (See Color Plate.)

Aluminum has been the cause of low-turnover bone disease in the majority of patients with that lesion up to now (197). However, in an increasing number of patients being identified with aplastic bone disease, neither aluminum nor iron is the cause. Although diabetes (87,88) or low-molecular-weight inhibitors (35) may be involved in some, in many the lesion is associated with excessive PTH suppression (86). Whether this condition is a disease state is not, in fact, clear. Based on considerations mentioned in the preceding text, such excessive suppression of PTH may predispose to aluminum toxicity even if, by itself, it causes no problems. For these reasons it appears that bone in uremic patients requires a modest PTH elevation to maintain normal bone turnover. This form of low-turnover bone disease requires considerable additional investigative work before we will understand its place in the spectrum of renal osteodystrophy.

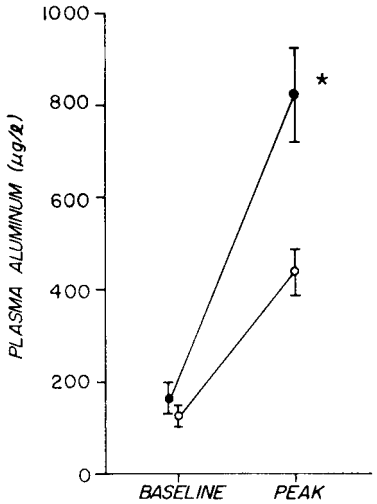


FIGURE 93-23. Comparison of plasma aluminum response to a single deferoxamine infusion in matched diabetic and nondiabetic dialysis patients. *Open circles* denote patients whose cumulative oral aluminum intake was more than 0.5 kg and *closed circles* denote patients whose cumulative oral aluminum intake was less than 0.5 kg. (Adapted from: Andress DL, et al. Effect of parathyroidectomy on bone aluminum accumulation in chronic renal failure. *N Engl J Med* 1987;316:292.)

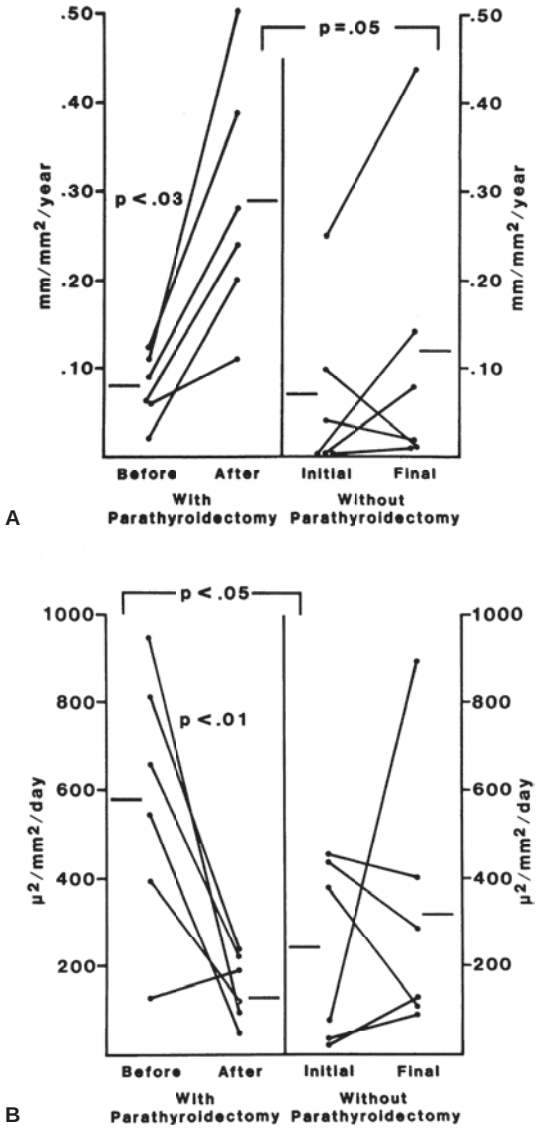


FIGURE 93-24. **A:** Rates of bone-surface aluminum accumulation in six dialysis patients before and after parathyroidectomy (*left panel*) and in six control dialysis patients (*right panel*). **B:** Rates of bone formation in the same patients. (Reprinted from: Andress DL, et al. Effect of parathyroidectomy on bone aluminum accumulation in chronic renal failure. *N Engl J Med* 1985;312:468, with permission.)

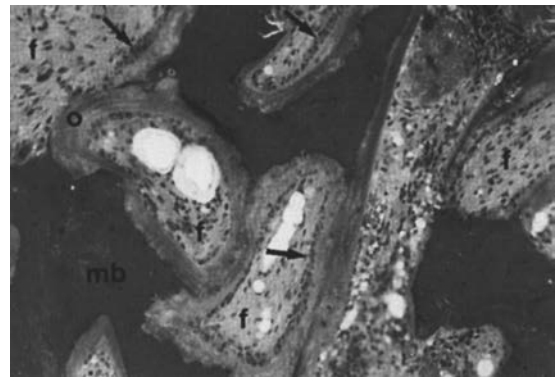


FIGURE 93-25. Mixed bone disease in a patient on dialysis. Width and total volume of unmineralized osteoid (O) are increased; *mb*, mineralized bone. The number of osteoblasts (*arrows*) also is increased, and fibrosis (*f*) is present in most of the marrow space. (Goldner's stain, magnification ×115.) (See Color Plate.)

Mixed and Transitional Bone Histology

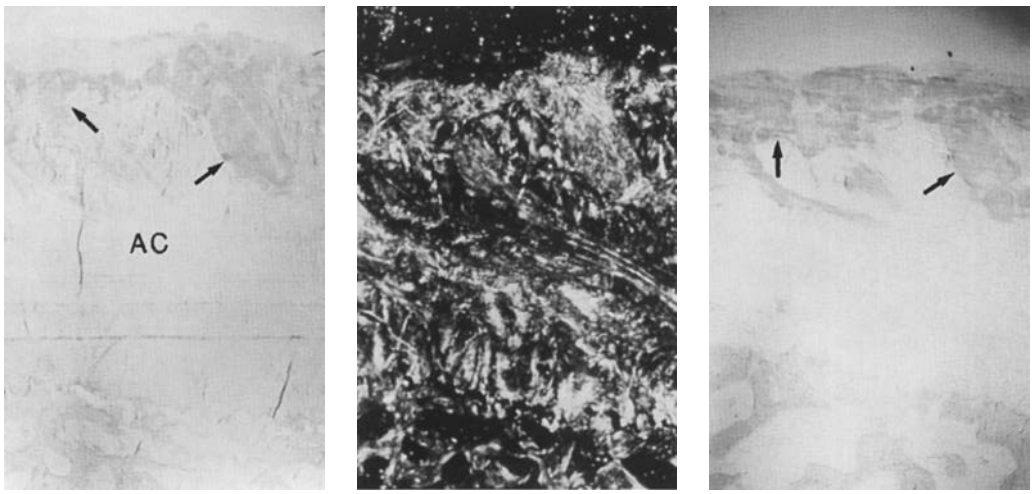
Although the majority of the bone histologic changes in uremic osteodystrophy can be classified as either mild disease, osteitis fibrosa, osteomalacia, or aplastic disease, a minority of patients do not fit into any of these categories. Rather, mixed histologic changes are sometimes seen in which aspects of both osteomalacia and osteitis fibrosa are present (198). Woven osteoid, marked fibrosis, and excess un-mineralized osteoid, with or without low bone formation, are the distinguishing features of mixed osteodystrophy (Fig. 93-25). The pathogenesis and evolution of this bone lesion are unclear but the lesion may represent a transitional state to a more clearcut form of either osteomalacia or osteitis fibrosa. Because serum PTH levels are often high and woven osteoids are invariably present, we regard osteoblast function in the mixed lesion to be enhanced with respect to osteoid production. The histologic finding of cuboidal or "plump," active-appearing osteoblasts supports this hypothesis. The mineralization defect, when present, can be attributed to coexistent hypocalcemia. Once the hypocalcemia is corrected, mineralization proceeds normally and the amount of un-mineralized osteoid diminishes while bone formation increases. Correction of the hypocalcemia also results in a lowering of the PTH levels, although serum PTH usually remains elevated. Therefore, the new bone histologic picture more often resembles osteitis fibrosa following correction of hypocalcemia.

Other transitional states in bone histology have also been noted but are relatively rare. Sometimes the bone histology shows a significant accumulation of bone-surface aluminum in the presence of fibrosis, normal osteoid volume, and low-normal bone formation, suggesting a transition from osteitis fibrosa to mild or aplastic bone disease. At other times, a lesser amount of bone aluminum is present while fibrosis and a slightly higher bone formation rate is noted, suggesting a transition to osteitis fibrosa. Because most bone histologic studies in uremic patients are cross-sectional in nature, it is difficult to know how a bone lesion is evolving without a previous biopsy.

Beta-2-Amyloid

The deposition of B_2M has been implicated as a cause of femoral fractures in long-term dialysis patients (108,199). Already known for its role in causing carpal tunnel syndrome (200), this circulating 11.8-kDa protein, which forms amyloid fibrils spontaneously (201), has been found in cystic lesions of long bones, particularly in the femoral and humeral heads (134,202). The cystic radiolucency of this bone lesion has been mistaken for the "brown tumor" of hyperparathyroidism.

In a retrospective analysis of bone specimens from 224 dialysis patients, we found that B_2M deposits were absent in patients on dialysis for less than 6 years but progressively increased with time, reaching a prevalence of 19% in those on dialysis for more than 10 years (203). In femoral head specimens, B_2M deposits were localized to synovium (Fig. 93-26), cartilage, and bone marrow. Its location in the iliac crest was predominantly in the periosteum (Fig. 93-27), although bone marrow deposition of B_2M occurred occasionally. In patients with B_2M localized to the iliac periosteum there was a 62% prevalence of femoral fracture, in contrast to only 4% in matched patients without B_2M at that location ($p < 0.001$). Although the pathogenesis of bone B_2M deposition could not be determined, B_2M deposits were not associated with aluminum or iron overload but were more common in patients with osteitis fibrosa (203). It is unknown whether B_2M has a role in promoting high-turnover bone disease. This is intriguing



A,B C

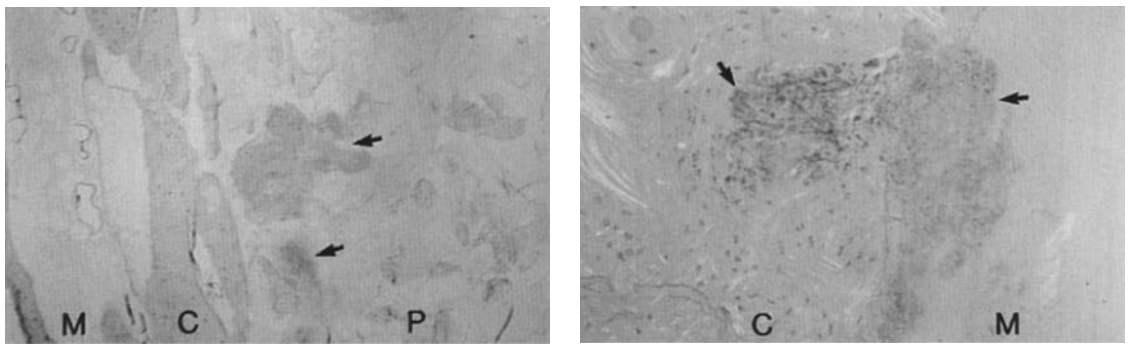
FIGURE 93-26. Serial sections of femoral bone from a patient on dialysis. **A:** Congo red-stained amyloid deposits (*arrows*) are seen in the superficial articular cartilage (*AC*). **B:** The same seen under polarized light. Congo red-stained amyloid deposits demonstrate green birefringence. **C:** Immunohistochemical staining with anti-β_2-microglobulin. Amyloid deposits (*arrows*) stain brown for β_2-microglobulin. (From: Onishi S, et al. Beta-2 microglobulin deposition in bone in chronic renal failure. *Kidney Int* 1991;39;990, with permission.) (See Color Plate.)

because B_2M can stimulate osteoblast proliferation *in vitro* (8). However, using an *in vitro* bone mineralization model, Kataoka et al. have shown that B_2M inhibits calcification (204). Moreover, Moe et al. have shown that B_2M stimulates osteoclastic bone resorption (205). Therefore, it may be that B_2M deposition in the femoral head stimulates bone turnover but inhibits bone mineralization, thereby causing predominant bone resorption and cyst formation.

In summary, the bone histology of uremic osteodystrophy is dependent on the state of parathyroid gland function, the presence or absence of hypocalcemia, and the extent of aluminum accumulation. Mild hyperparathyroid bone disease is the most common finding in uremic patients, particularly during moderate renal failure and shortly after beginning dialytic therapy. The severity of osteitis fibrosa and accelerated bone turnover increases with increasing parathyroid gland activity. In some patients enhanced bone formation is associated with elevated levels of IGF-1, and depressed bone formation is associated with the serum accumulation of inhibitory IGFBPs. In patients with mixed bone histology (osteitis fibrosa plus osteomalacia) hypocalcemia is usually present and, when corrected, results in normalization of the mineralization defect. Bone aluminum accumulation is associated with the low bone formation of osteomalacia and aplastic bone disease. Decreased PTH secretion, which is often present in aluminum-associated bone disease, probably plays a major role in the development of low bone formation. Although bone fractures are the major complications of low-turnover bone disease, femoral fractures may be more often caused by B_2M deposition, particularly in patients on dialysis for more than 10 years.

Unique Pediatric Features

Although much of the foregoing material is certainly true of children as well, there are some differences in the pediatric population. One of these relates to growth. Attention to maintaining appropriate levels of calcium and phosphate and judicious use of vitamin D appear to enhance bone growth and improve metabolic bone disease (206–211). Normalization of height is uncommon in uremic children due possibly to the serum accumulation of IGFBPs, which inhibit IGF-1 stimulation of cartilage (212), and to low-molecular-weight inhibitors of cartilage (33) and osteoblast (35) activity. Recent studies with recombinant growth hormone (rGH) have demonstrated its benefit in



A B

FIGURE 93-27. **A:** Iliac crest bone biopsy with anti-β_2-microglobulin and hematoxylin counterstain. β_2-Microglobulin deposits (*arrows*) are located in the iliac periosteum. **B:** Bone section from the tibia with anti-β_2-microglobulin and methylene blue counterstain. β_2-Microglobulin deposits (*arrows*) appear to invade the cortical bone (*C*) from the marrow (*M*). (From: Onishi S, et al. Beta-2-microglobulin deposition in bone in chronic renal failure. *Kidney Int* 1991;39:990, with permission.) (See Color Plate.)

stimulating growth in these patients (213,214). Interestingly, serum levels of free IGF-1, IGFBP-3, and IGFBP-5 were elevated after rGH therapy, whereas IGFBP-1 and IGFBP-2 levels were reduced (215), suggesting that GH treatment shifted the balance between potential growth promoters (IGF-1, IGFBP-3, and IGFBP-5) and growth inhibitors (IGFBP-1 and IGFBP-2).

A second problem relates to aluminum toxicity, which at one time was relatively common in children who were taking aluminum-containing phosphate binders (216). Children with serum creatinine levels as low as 2 mg/dL have been found with serious aluminum-related bone disease. Even dementia has been reported (217). Whether this is due to enhanced gastrointestinal absorption or better compliance with aluminum gels for phosphate control is not clear. One recent prospective study documented that even low doses of aluminum-containing phosphate binders result in substantial aluminum accumulation (218). It is clear that aluminum levels must be monitored in children and other approaches to phosphate control must be attempted (e.g., calcium carbonate, calcium acetate, or sevelamer).

Special Diagnostic Problems

Hypercalcemia

Elevated calcium can occur in a dialysis patient for all the same reasons that it occurs in a patient without renal disease. Too often the physician faced with this problem will ignore the possibility of these common disorders because the patient is on dialysis or has renal failure. Before attempting to assess the specific renal failure-dialysis-related causes, these better known etiologies should be considered.

In the chronic dialysis patient there are four major causes of hypercalcemia: hyperparathyroidism, aluminum intoxication, vitamin D intoxication, and bed rest. Hyperparathyroidism occurs for reasons described previously and causes hypercalcemia because of excessive osteoclastic bone resorption, usually in the setting of intact PTH levels greater than 1,000 pg/mL.

Vitamin D intoxication is common because of the frequency of vitamin D use in these patients and the fact that therapeutic doses are close to toxic ones. For this reason, a short-acting vitamin D preparation, such as calcitriol, is preferable when vitamin D deficiency is present. Calcitonin may be particularly useful in controlling transient hypercalcemia from vitamin D excess.

Aluminum excess causes hypercalcemia in part by blocking bone formation and perhaps by stimulating osteoclast-mediated bone resorption. When patients receive calcium orally or intravenously, it enters the extracellular fluid compartment. The calcium level in this compartment is normally buffered by the huge bone reservoir. If aluminum prevents entry of calcium into the bone, then administered calcium quickly leads to hypercalcemia (114). Because most dialysate calcium levels are high and result in calcium transfer to the patient, persistent hypercalcemia is a frequent finding. Lowering the calcium dialysate (e.g., 2.5 mEq/L) may help with this problem (219). Perhaps the most confusing element of aluminum-induced hypercalcemia is the accompanying finding of modestly elevated PTH levels. This is usually due to renal failure and retention of PTH metabolites. The physician must be very wary of this problem and be certain that PTH elevation is sufficiently elevated to make a diagnosis of "renal hyperparathyroidism." An incorrect decision to remove the parathyroids in such patients is an invitation to disaster, as this can worsen the bone disease (91,92,219,220), possibly as a result of accelerated bone-surface aluminum accumulation (91).

Bed rest, in some ways, is physiologically similar to aluminum from the bone's point of view because bone formation ceases and bone resorption continues. In normal subjects, bone resorption continues and the calcium lost from bone disappears into the urine. This "bed rest" hypercalciuria may be sufficiently severe enough to cause renal stones (221). Without kidney function there is no route for the excess calcium to escape, so it accumulates in the extracellular fluid. Temporarily, low-calcium dialysate may be used, but in the long run the patient should increase his or her activity as soon as possible because low-calcium dialysate (<2.5 mEq/L) will deplete total body calcium.

Bone Pain and Fracture

The differential diagnosis of bone pain and fracture is, in general, the distinction of hyperparathyroidism from aluminum intoxication (see Table 93-3). Clinically the pain is usually very severe, often immobilizing, with aluminum intoxication, whereas the hyperparathyroid patient complains, but carries on. Where data are available, the PTH level is normal to moderately elevated with aluminum excess, but very high with hyperparathyroidism (114,126,128). Aluminum levels, either basal or following deferoxamine challenge and hypocalcemic stimulation of PTH, as described previously, may be useful. If the diagnosis is not absolutely clear after these studies, then bone biopsy should be done before therapy is instituted. In long-term (>6 years) dialysis patients with hip fractures, the possibility of B_2M disease must be considered. X-ray or MRI evidence of bone cysts should not be assumed to be "brown tumors" of hyperparathyroidism. In the older dialysis population, osteoporosis may also play a role (119).

Preventive Therapy

Hyperphosphatemia

A major emphasis has been placed on normalizing serum phosphate levels (Table 93-5) because of the importance of phosphate retention in causing decreased 1,25-dihydroxyvitamin D synthesis, hypocalcemia (222), and secondary hyperparathyroidism (223,224). Although excellent control of serum phosphate helps prevent metastatic calcification, prevention of hyperphosphatemia during moderate renal failure will also enhance 1,25-dihydroxyvitamin D production (225) and will further assist in PTH suppression (226). Reduction of dietary phosphorus intake (227) and use of aluminum-containing phosphate binders during meals have been the most effective forms of treatment. The increasing concern about aluminum toxicity, however, has led to the use of calcium salts as preferred alternatives to aluminum hydroxide (228). Both calcium carbonate (229–231) and calcium acetate (232) are useful phosphate binders when taken with meals. Calcium citrate, while an excellent phosphate binder, must be avoided because it markedly enhances aluminum absorption (233,234), a complication that has not been observed with calcium acetate (235). Recent clinical practice guidelines (236) suggest that aluminum-containing phosphate binders not be used except for short durations when serum phosphate is unacceptably high. It also suggests that a maximum intake of 1,500 mg of elemental calcium per day not be exceeded as the phosphate binder (and not more than 2,000 mg/day for total intake) due to concerns about the effects of excessive calcium loading on arterial and soft-tissue calcification (111,236).

Because hypercalcemia has become more of a problem since calcium salts became the phosphate binder of choice, there has been a renewed interest in identifying calcium and aluminum-free binders. Magnesium, which has been available for more than a decade, effectively lowers phosphate levels

TABLE 93-5

SEQUENTIAL TREATMENT OF HYPERPARATHYROIDISM IN RENAL FAILURE

I. Prophylactic measures
  A. Phosphate control (goals)
    1. Stage 3 and 4 CKD: serum phosphate <4.5 mg/dL
    2. Stage 5 dialysis patients: serum phosphate <6.5 mg/dL
  B. Calcium supplementation (do not exceed 2,000 mg elemental calcium/day)
  C. Calcitriol (daily oral dosing to correct hypocalcemia)
  D. Dialysis patients: injectable vitamin D thrice weekly (paricalcitol preferred for survival advantage)

II. With bone pain or fractures
  A. Hypocalcemic patients
    1. Institute prophylactic measures
    2. Increase calcitriol dose rapidly (every 2 weeks) to achieve high normal calcium
    3. Monitor calcium weekly
  B. Normocalcemic patients
    1. Institute prophylactic measures
    2. Consider injectable vitamin D (dialysis patients)
      a. Paricalcitol (preferred for survival benefit)
      b. Calcitriol
      c. Doxercalciferol
  C. Hypercalcemic patients
    1. Reduce dose of intermittent intravenous paricalcitol
    2. Add cinacalcet (start at lowest dose)
    3. Parathyroidectomy if hypercalcemia persists

III. Hypercalcemia without bone symptoms (and receiving paricalcitol)
  A. If calcium X phosphate product >75: add cinacalcet
  B. Parathyroidectomy if the above measures fail to suppress intact PTH to 300 pg/mL

CKD, chronic kidney disease; *PTH*, parathyroid hormone.

(237). Delmez et al. showed that the combination of magnesium carbonate with calcium acetate was effective in controlling phosphate levels. It also allowed the use of higher intravenous calcitriol doses when compared to patients receiving calcium acetate alone (238). A potential limitation of magnesium as a binder is the need to lower the dialysate magnesium to prevent hypermagnesemia. A metal-free compound that was recently approved for binding dietary phosphate is sevelamer. This nonabsorbable, cross-linked poly allylamine gel not only effectively lowers serum phosphate in dialysis patients but also decreases serum LDL cholesterol (239). Lanthanum carbonate is another new calcium-free, aluminum-free phosphate binder that effectively lowers serum phosphate in dialysis patients (240). Future studies will be needed to confirm that its long-term use does not result in significant bone and soft-tissue accumulations.

Calcium Supplementation

The decreased intestinal calcium absorption of uremic patients can for the most part be attributed to the decreased production of calcitriol. When replaced in the diet, calcitriol improves calcium absorption and increases serum calcium levels. However, simply increasing the dietary intake of calcium can also improve calcium balance in uremia (241,242). Intestinal calcium absorption is especially enhanced when calcium salts are taken in combination with oral calcitriol. Therefore, the high-calcium dialysates formerly recommended to maintain calcium balance (243,244) are now inappropriate. Although a dialysate calcium of 2.5 mEq/L may be the preferred concentration (219), some flexibility (2.0 to 2.5 mEq/L) may be needed for individual patient variability when high doses of calcium are prescribed.

Calciferol (Vitamin D_3)

Treatment with vitamin D metabolites has been shown to ameliorate defects in calcium homeostasis in chronic renal failure. In 1943, Liu and Chu reported that physiologic doses of calciferol were ineffective in normalizing calcium homeostasis in uremic patients (245). Larger doses of calciferol, however, have generally been helpful in promoting positive calcium balance (142), resulting in a decrease in serum PTH (246). In addition, amelioration of osteitis fibrosa can be found after treatment with calciferol (246). The major limitation of calciferol is the prolonged hypercalcemia that sometimes occurs (247,248).

Calcifediol (25-Hydroxyvitamin D)

One of the natural metabolites of calciferol is calcifediol. Produced in the liver by 25-hydroxylation of calciferol, 25-hydroxyvitamin D has a pharmacologic half-life of approximately 30 days (249). Oral maintenance doses of calcifediol in the range of 200 μg three times a week are effective in increasing intestinal calcium absorption in uremic patients (250). Although high serum PTH levels are decreased in some patients after treatment with calcifediol (251,252), other studies have not shown this effect (253). In general, bone histology is improved after calcifediol treatment (161,251,253). Bordier et al. described decreases in osteoclast number, bone resorption, and fibrosis and increased calcification front activity in those patients who had a decrease in serum PTH (251). Teitelbaum et al. reported similar changes in five patients treated for 3 to 9 months (161). Most agree that the improvements in bone histology are related to the degree of suppression of the hyperparathyroidism. This in turn depends on the extent of increased calcium absorption and correction of hypocalcemia. Because most dialysis patients have normal circulating levels of 25-hydroxyvitamin D (45,250), treatment with this substance results in a marked elevation of serum 25-hydroxyvitamin D (250), and prolonged hypercalcemia is a potential hazard of this long-lived metabolite also.

Calcitriol (1,25-Dihydroxyvitamin D)

In 1970, Fraser and Kodicek discovered that the most potent form of vitamin D, 1,25-dihydroxyvitamin D, is synthesized in the kidney from 25-hydroxyvitamin D (254). Since that time numerous studies have been conducted exploring the use of calcitriol in the treatment of renal osteodystrophy (255–258). Oral calcitriol causes a marked increase in intestinal calcium absorption (239) that is more pronounced than with any of the other vitamin D metabolites. Because of the relatively short plasma half-life (12 to 16 hours), episodes of hypercalcemia can be quickly corrected, usually within 1 week of stopping treatment. The incidence of hypercalcemia seems to depend on the underlying bone pathology. In general, patients with osteitis fibrosa respond better to calcitriol and have fewer episodes of hypercalcemia initially than patients with pure osteomalacia (255,259,260). Amelioration of hyperparathyroidism is particularly apparent when hypocalcemia is present before treatment (261). Patients with mild hypercalcemia at the start of calcitriol therapy have a tendency to become hypercalcemic early during treatment when compared to patients with pretreatment serum calcium levels in the low-normal range.

Alphacalcidol (1-α-Hydroxyvitamin D_3)

A synthetic analog of calcitriol, 1-α-hydroxyvitamin D_3, has a half-life two to three times longer than calcitriol (262) and must

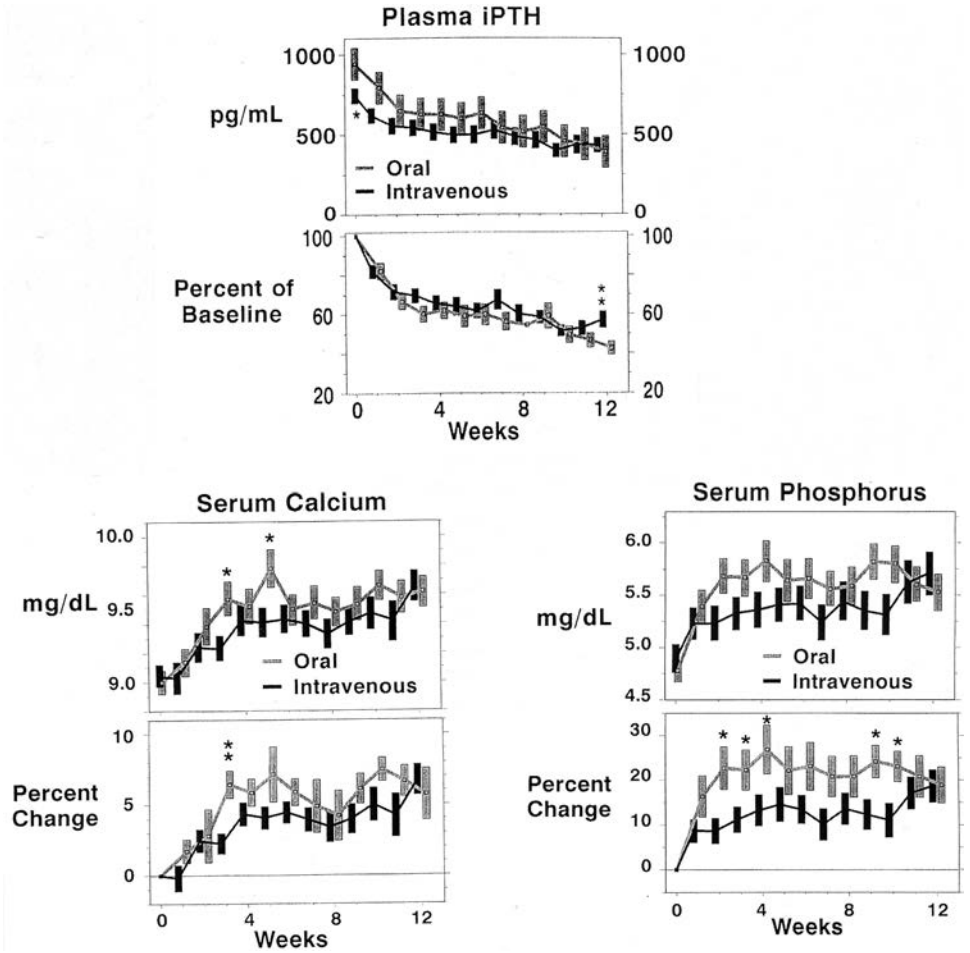


FIGURE 93-28. Changes in PTH, calcium and phosphate levels during treatment with intermittent intravenous and oral doxercalciferol in dialysis patients. (Reprinted from: M. Maung, et al. Efficacy and side-effects of intermittent intravenous and oral doxercalciferol (1alpha-hydroxyvitamin D_2) in dialysis patients with secondary hyperparathyroidism: A sequential comparison. *Am J Kidney Dis* 2001;37:532, with permission.)

undergo hepatic hydroxylation at the 25 position to become activated. Therefore, larger doses of 1-α-hydroxyvitamin D_3 are necessary for comparable effects and hypercalcemia, when it occurs, is more long lasting (263). Because of the need for hepatic hydroxylation, the effectiveness of 1-α-hydroxyvitamin D_3 can be negated by the concomitant use of anticonvulsants, since both compete for the same hydroxylation enzymes (264).

The biologic effects of alphacalcidol and calcitriol are indistinguishable (121,265). Most dialysis patients with mild-to-moderate osteitis fibrosa or mixed bone disease have shown improvement in bone histology after treatment with alphacalcidol (266–268). However, poor responses to treatment were observed in patients with severe osteitis fibrosa or osteomalacia (267,269). Therefore, treatment failures with alphacalcidol, like calcitriol, seem to occur in patients in whom hypercalcemia limits therapeutic dosing. The dose required to maintain serum calcium may be lower in those patients maintained on bicarbonate-supplemented dialysis (270).

Successful use of calcitriol or alphacalcidol in patients with moderate renal failure has been described. Both have caused declines in serum PTH and improvement in trabecular fibrosis (271–273). Massry et al. have shown healing of osteitis fibrosa and lowering of serum PTH levels at calcitriol doses of 0.5 to 1.0 μg per day for 1 year (274). More recently it was shown that lower doses are effective (275,276), although in some patients abnormally low rates of bone formation have resulted. Although a concern for premature declines in renal function during calcitriol or alphacalcidol therapy has been expressed (277,278), other studies have not shown this association (274,279,280).

Doxercalciferol (1-α-Hydroxyvitamin D_2)

Studies in vitamin D–deficient rats have shown that 1-α-hydroxyvitamin D_2 is equipotent to 1-α-hydroxyvitamin D_3 in stimulating intestinal calcium transport and in healing rickets (281). When used in dialysis patients, either orally or by intravenous administration, Maung et al. demonstrated effective PTH suppression (50%) over a 12-week treatment period, but with significant elevations in serum calcium (+7%) and phosphate (+19%) (282) (Fig. 93-28). Oral doxercalciferol was also shown to effectively suppress PTH (46%) in patients with pre-dialysis chronic kidney disease through 4% developed hypercalcemia and there was a 6% increase in serum phosphate ($p < 0.05$). Moreover, the mean urine calcium excretion increased by 42% ($p < 0.01$) (283).

Dihydrotachysterol

Dihydrotachysterol (DHT) is a synthetic compound that, after undergoing hepatic hydroxylation, resembles calcitriol structurally. Early studies with DHT confirmed its effectiveness in causing an increase in intestinal calcium absorption (245) as well as a decrease in serum PTH and alkaline phosphatase (142). In a more recent study of hemodialysis patients with osteitis fibrosa, Cordy and Mills demonstrated significant decreases in bone resorption and osteoid volume after a minimum of 12 months of treatment with DHT at a dosage of 0.25 to 1.00 mg per day (284). Unfortunately, tetracycline labeling was not performed to assess possible reductions in bone formation. It appears from limited studies of bone histology that DHT

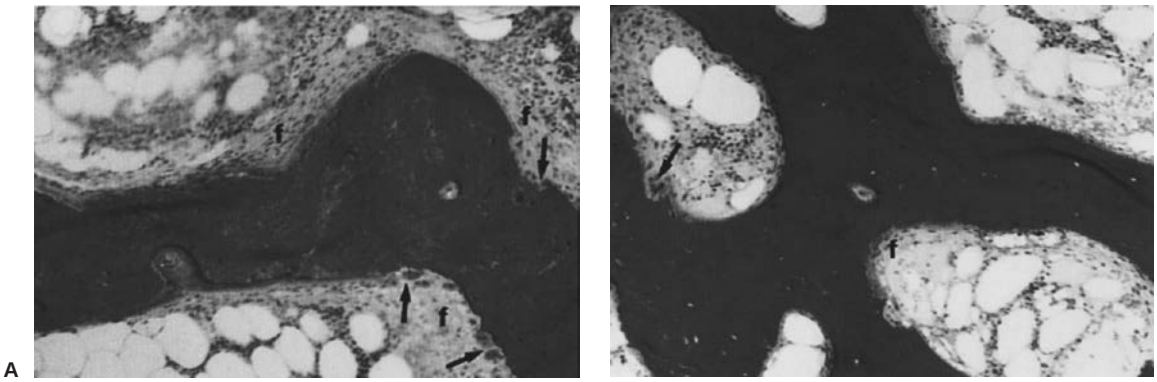


FIGURE 93-29. A: Ostitis fibrosa in a patient on dialysis refractory to treatment with oral calcitriol. **B:** Histologic section from the same patient after treatment with intravenous calcitriol. Amount of fibrosis (*f*) and number of osteoclasts (*arrows*) are decreased after treatment. (A and B, Goldner's stain, magnification ×115.) (See Color Plate.)

can cause healing of osteitis fibrosa. Whether similar effects in patients with pure osteomalacia also occur has not been determined. DHT has a potency about three times that of calciferol. It takes approximately 1 to 2 weeks to correct hypercalcemia after cessation of therapy.

In summary, treatment of osteitis fibrosa with vitamin D metabolites is best begun early in the disease process. Although oral calcifediol therapy seems sufficient in some patients, a favorable response is not universal. A prolonged half-life renders this therapy less useful than that with the 1-hydroxylated vitamin D metabolites. Dihydrotachysterol is inexpensive and effective at doses of 0.5 to 1.0 mg per day for the treatment of osteitis fibrosa but also has the hazard of a long half-life. Oral calcitriol in doses of 0.25 to 1.00 μg per day or alphacalcidol in doses of 1.0 to 2.0 μg per day is effective in healing osteitis fibrosa. However, patients with severe hyperparathyroidism or pure osteomalacia are at an increased risk for developing hypercalcemia. In patients where hypercalcemia limits the dosing of calcitriol, pulse intermittent therapy with doxercalciferol or paricalcitol may prove to be more effective in this setting. Rarely have these metabolites been helpful in the treatment of pure osteomalacia.

Treatment of Advanced Disease

Osteitis Fibrosa (Hyperparathyroid Bone Disease)

With severe hyperparathyroid bone disease, a sequential approach is useful. First, all of the preventive measures discussed in the preceding text should be introduced: phosphate control, calcium supplementation, and oral calcitriol or 1-α-hydroxyvitamin D therapy. Phosphate levels less than 6.5 mg/dL and calcium levels between 9.5 mg/dL and 10 mg/dL are desirable. If these measures fail, then intermittent intravenous infusions of calcitriol or paricalcitol may be tried.

Intravenous Calcitriol

The intravenous form of calcitriol has proved to be effective in the treatment of severe hyperparathyroidism refractory to oral calcitriol. In the initial studies, Slatopolsky et al. demonstrated that the infusion of calcitriol in doses of 1.75 to 4.00 μg thrice weekly at the end of each dialysis resulted in a decline of serum PTH by 35% over 3 weeks (54). Although serum calcium level eventually increased in all patients, the decline in serum PTH was noted before increases in serum calcium were evident. Subsequent studies evaluating bone histology indicated that long-term treatment with intravenous calcitriol ameliorates the osteitis fibrosa lesion in patients who are refractory to oral calcitriol (286) (Fig. 93-29). In dialysis, patients who had previously failed daily oral calcitriol treatment, intravenous calcitriol given thrice weekly effectively lowered serum PTH levels, significantly improved marrow fibrosis, and lowered bone turnover to more normal levels (Fig. 93-30,

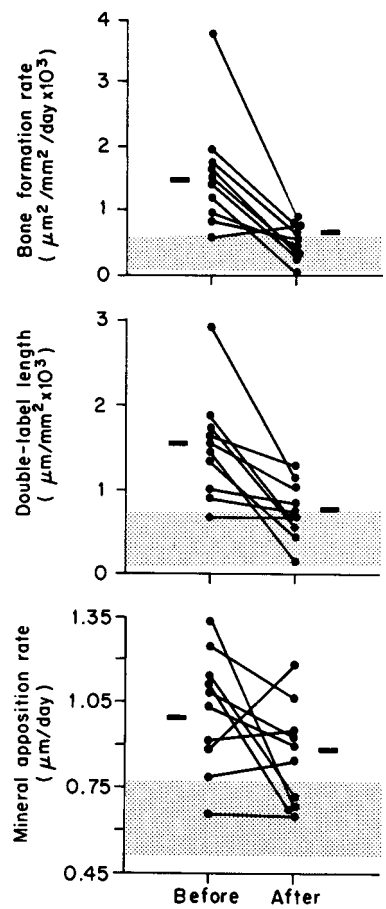


FIGURE 93-30. Response of bone formation rate, double tetracycline label length, and mineral apposition rate to intravenous calcitriol in 10 dialysis patients previously refractory to oral calcitriol. Shaded areas represent normal range for values. (Reprinted from: Andress DL, et al. Intravenous calcitriol in the treatment of refractory osteitis fibrosa of chronic renal failure. *N Engl J Med* 1989;321:274, with permission.)

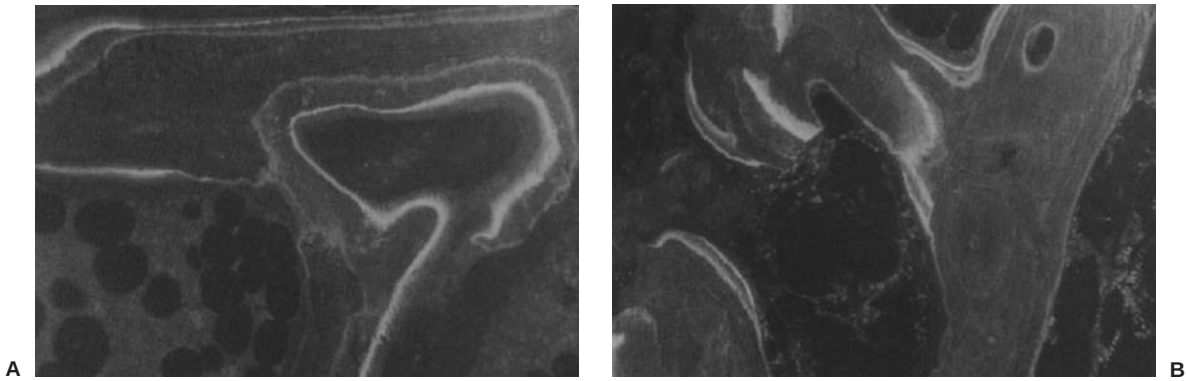


FIGURE 93-31. A: Tetracycline labels in a patient on dialysis with osteitis fibrosa and high bone formation. **B:** Tetracycline labels in the same patient after treatment with intravenous calcitriol. The reductions in the interlabel distance and the length of double labels after treatment indicate a decrease in bone formation to normal. (A and B, unstained fluorescent micrographs, magnification ×115.) (See Color Plate.)

Fig. 93-31). It was noted that transient episodes of asymptomatic hypercalcemia were quickly reversed by temporarily halting treatment or by decreasing the dosage (285). Since then a number of reports have documented that intravenous calcitriol effectively lowers PTH levels (286,287). Unfortunately, because of the exclusive use of calcium salts for phosphate binding, a higher frequency of hypercalcemia has also been reported (288).

Intravenous Paricalcitol

Because hypercalcemia frequently limits the use of higher calcitriol doses, newer vitamin D compounds have been developed to counteract this effect. Intravenous paricalcitol (19-nor-1,25-dihydroxyvitamin D_2) was shown in normal rats to be less calcemic than calcitriol and to suppress PTH in uremic rats without increasing ionized serum calcium (289). Martin et al then demonstrated that intravenous paricalcitol decreased PTH levels in dialysis patients by 60% over the 12-week treatment period with few episodes of hypercalcemia (290) (Fig. 93-32). More recently, Sprague et al. demonstrated in a double-blind, randomized study comparing intravenous paricalcitol to intravenous calcitriol, that paricalcitol was more effective in suppressing PTH. In addition, patients receiving paricalcitol had significantly fewer episodes of hypercalcemia or elevated calcium X phosphate product (291) (Fig. 93-33). Finally, in a retrospective comparison of intravenous paricalcitol to intravenous calcitriol in more than 60,000 dialysis patients, there was a 16% survival benefit in those who were on paricalcitol for a 3-year observation period (292) (Fig. 93-34).

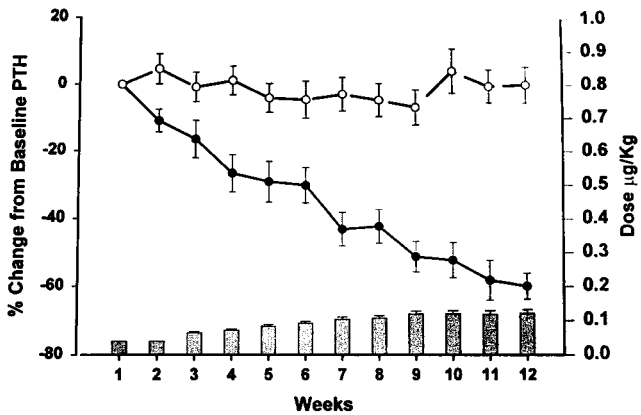


FIGURE 93-32. Changes in PTH levels during treatment with intermittent intravenous paricalcitol in dialysis patients. Patients were randomized to placebo (*open circles*) or paricalcitol (*closed circles*). *Bars* depict the doses of paricalcitol that increased according to the protocol. (Reprinted from: Martin KJ, et al. 19-nor-1α-25-dihydroxyvitamin D_2 (paricalcitol) safely and effectively reduces the levels of parathyroid hormone in patients on hemodialysis. *J Am Soc Nephrol* 1998;9:1427, with permission.)

Calcimimetics

A new form of PTH suppression involves activating the calcium-sensing receptors in parathyroid tissue to suppress PTH secretion (293). A phase I trial of one such calcium receptor agonist, NPS-R568, showed that it was effective in acutely suppressing serum PTH in dialysis patients (66) (Fig 93-3). Declines in PTH were usually noticeable within 1 to 2 hours after oral ingestion of the drug, with peak effects occurring 4 to 6 hours later. Ionized calcium levels decreased with only one treatment, suggesting that the acute changes in serum calcium may not be related to changes in bone turnover (66). Long-term studies show that the calcium receptor agonist, cinacalcet, achieved the PTH primary end point (intact PTH <250 pg/mL) in 60% of the patients, while lowering mean serum calcium levels by 5% (294). The majority of patients who received cinacalcet also required vitamin D treatment (usually calcitriol or paricalcitol) throughout the study. The group that received cinacalcet had a higher incidence of substantial gastrointestinal side-effects (nausea and vomiting) than the placebo control group (p <0.05) (294).

Parathyroidectomy

Parathyroidectomy is reserved for dialysis patients who have severe osteitis fibrosa that is unresponsive to vitamin D therapy. Other indications for parathyroidectomy have included persistent hypercalcemia, intractable pruritus, progressive extraskeletal calcifications, and calciphylaxis (110). In general, removal of hyperplastic parathyroid glands is effective in relieving symptoms (295–297), although a minority of patients require repeated neck explorations for displaced or supernumerary parathyroid glands that result in persistent elevations of PTH (296). In a small number of patients, it has been shown that parathyroidectomy results in bone demineralization and osteomalacia (92,161,219,220). Because the decline in bone formation that follows parathyroidectomy is associated with accelerated bone-surface aluminum accumulation (91), it is

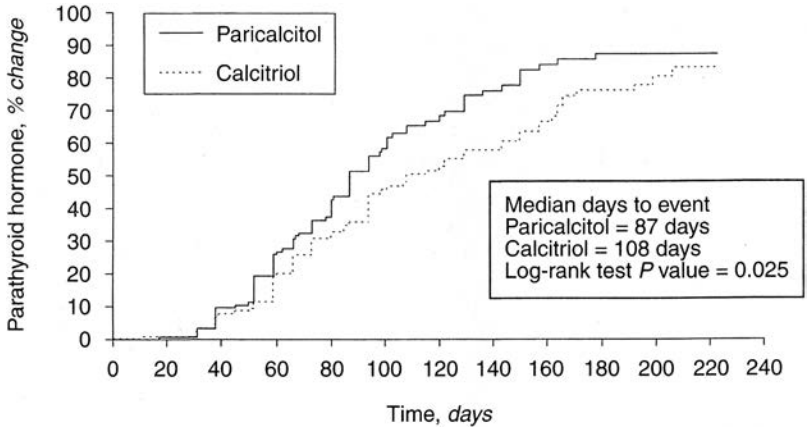


FIGURE 93-33. Changes in PTH levels during treatment with either intravenous paricalcitol or intravenous calcitriol in dialysis patients. Patients were randomized to paricalcitol (*open circles*) or calcitriol (*closed circles*). (Reprinted from: Sprague S, et al. Paricalcitol versus calcitriol in the treatment of secondary hyperparathyroidism. *Kidney Int* 2000;63:1483, with permission.)

suggested that all potential candidates for parathyroidectomy be evaluated for aluminum excess prior to surgery. This would be particularly important in those undergoing total parathyroidectomy, because this procedure results in markedly reduced rates of bone formation (298). It is possible that intravenous paricalcitol therapy, with or without cinacalcet, may replace the need for surgical parathyroidectomy in many patients. Failure to respond to these treatments likely indicates irreversible reductions in the receptors for vitamin D and calcium, which is characteristic of nodular parathyroid hyperplasia (299).

Aluminum-Associated Osteodystrophy

Treatment of aluminum bone disease involves taking preventive measures with respect to parenteral and oral aluminum intake as well as initiating chelation therapy once aluminum toxicity is

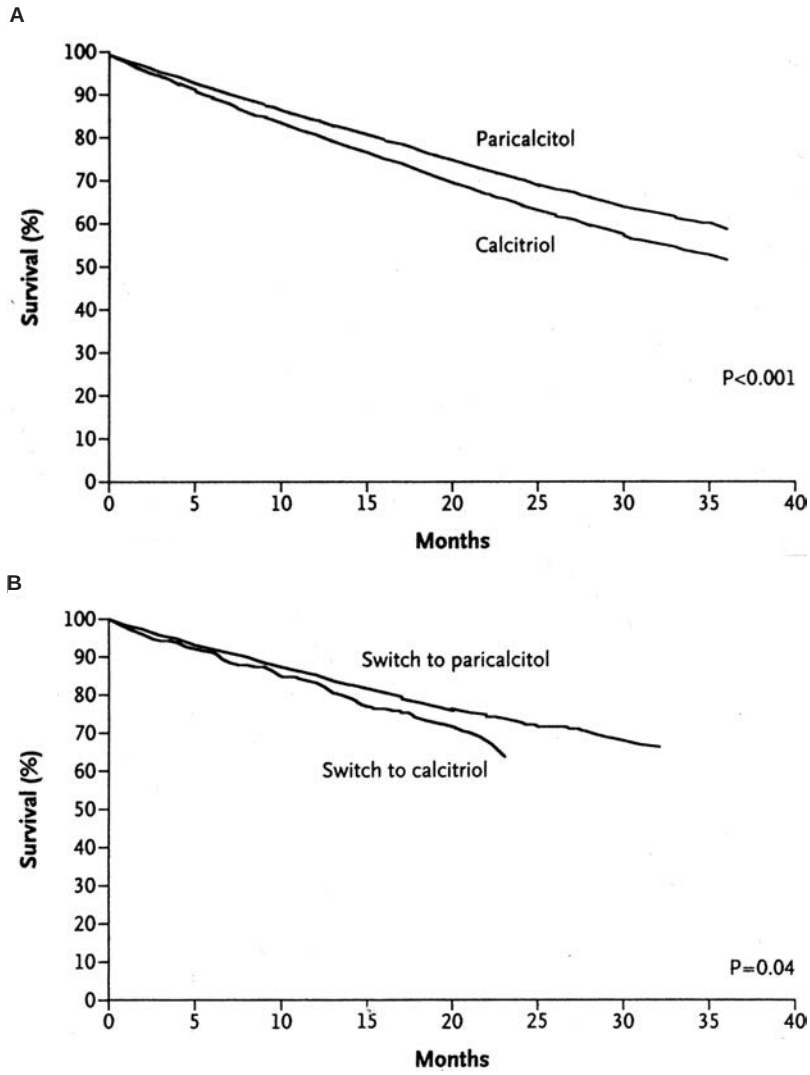


FIGURE 93-34. Survival curves of hemodialysis patients receiving either intravenous paricalcitol or intravenous calcitriol. (Reprinted from: Teng M, et al. survival of patients undergoing hemodialysis with paricalcitol or paricalcitol therapy. *N Engl J Med* 2003;349:446, with permission.)

TABLE 93-6

TREATMENT OF ALUMINUM OVERLOAD

I. Asymptomatic or minimal symptoms—eliminate all sources of aluminum
   A. Check and correct dialysate
   B. Check and eliminate all medication sources
   C. Use calcium carbonate or calcium acetate as phosphate binder
   D. Add or substitute sevelamer as the phosphate binder
II. Moderate to marked symptoms
   A. Eliminate all sources of aluminum
   B. Initiate deferoxamine therapy at 5–10 mg/kg intravenously during the last 2 hours of dialysis once a week
   C. Monitor closely for fungus infection
   D. Discontinue deferoxamine when symptoms abate
III. Symptoms worsen with chelation
   A. Administer half the dose of deferoxamine 4–6 hours before dialysis once a week
   B. After 1–2 months gradually increase the dose

diagnosed (Table 93-6). Water treatment with deionization or reverse osmosis is essential in maintaining dialysate aluminum concentrations at less than 10 μg/L (300,301). Restricting the oral intake of aluminum now seems warranted, owing to the association of aluminum bone disease with a high intake of aluminum-containing phosphate binders (172). Recent studies suggest that both calcium carbonate (223,302) and calcium acetate (232,303) are effective in binding intestinal phosphate when taken with meals.

Long-term aluminum chelation with deferoxamine is effective in the treatment of aluminum bone disease (160,304–308). Aluminum removal from the bone surface (Color Plate 17) is accompanied by improvements in bone formation (Color Plate 18) and bone symptoms. Some patients with a prior parathyroidectomy appear not to respond as readily to deferoxamine (305), suggesting that increased PTH secretion may be important in the healing of aluminum-related low-turnover bone disease. Because this poor response is in part related to the extent of bone-surface aluminum, it is suggested that patients with a prior parathyroidectomy have a quantitative assessment of bone-surface aluminum to determine whether more prolonged treatment with deferoxamine may be needed.

Only about 30% of the total circulating deferoxamine–aluminum chelate is removed by hemodialysis using cuprophane membranes (309). Until recently there had been few improvements in the removal efficiency of deferoxamine–aluminum complexes using the different membranes (310). Polysulfone dialysis, however, appears to provide more efficient removal than dialysis with the cuprophane membrane, a result that is only partly due to the larger surface area (311). Charcoal hemoperfusion may also accelerate removal (312,313).

Although aluminum chelation with deferoxamine appears promising in the treatment of aluminum bone disease (a major side effect), infection with the fungus mucormycosis markedly limits its use (314–317). Almost always fatal, this complication may occur in 10% of treated dialysis patients. Cataracts and retinal changes (318,319) and thrombocytopenia (320) have also been reported complications of chronic deferoxamine therapy. The development of dialysis dementia with the initiation of deferoxamine treatment has been reported in a few patients (321). Avoidance of aluminum is preferable and chelation therapy is recommended in only critically toxic patients. Because of these concerns it is often necessary to document aluminum toxicity by bone biopsy. When necessary, deferoxamine should be given at low doses (5 to 10 mg/kg per week), by intravenous, intramuscular (322), or intraperitoneal routes (323) for no more than 3 months at a time.

Renal Transplantation

Renal transplantation, in most instances, corrects the abnormal calcium and phosphorous metabolism of uremia. Improvements in bone pathology, however, can be delayed depending on the severity of the underlying lesion (324). For example, patients who have had renal failure for a short duration may have essentially normal skeletons at the time of transplantation. In contrast, those who have had renal disease for a long duration plus a prolonged course of dialysis are likely to have significant bone disease at the time of transplantation. Transplant recipients in the latter group generally have more long-term problems with bone disease.

Renal synthesis of calcitriol resumes normally following transplantation (325). Resolution of hyperparathyroidism is seen in the majority of patients within the first 4 months after transplantation (326). Those who continue to have persistent elevations of PTH are often hypercalcemic (327). In some, however, enhanced PTH secretion is present despite normal calcium levels (328). Hypophosphatemia or phosphate depletion can also elevate serum calcium levels (326). The prevalence of hypophosphatemia and hypercalcemia in transplant recipients is approximately 25% (327,328). The cause of the hypophosphatemia is probably multifactorial. Possible mechanisms include renal tubular phosphate leak (329), decreased phosphorus absorption secondary to corticosteroid therapy (330), and persistent hyperparathyroidism (331). Usually the hypercalcemia and hypophosphatemia are mild and can be controlled with oral phosphate supplements. Occasionally the hypophosphatemia results in osteomalacia, although more often it is not associated with significant bone histologic abnormalities (332).

Despite the fact that several factors may be involved in decreasing bone mineral content during the early posttransplantation period, the deleterious effects of corticosteroids are the predominant concern (333). The debilitation of osteonecrosis in renal transplant recipients has been well described (252,334–337). Total hip replacement is sometimes indicated. Although osteonecrosis has occurred in dialysis patients who have not received corticosteroids (338), the prevalence and severity of the disease in transplant patients appear to be related to the cumulative steroid intake (339,340). Fortunately, early detection of osteonecrosis during the asymptomatic stage may be possible by MRI (340).

Glucocorticoids affect bone by decreasing bone formation and increasing bone resorption (339,341). Osteoblast function is thought to be decreased by steroids because the synthesis of bone cell protein (342) and collagen (343) are inhibited and because osteoblasts are induced to undergo apoptosis (programmed cell death) (344). The increased osteoclast activity and bone resorption probably occur secondary to the enhanced PTH secretion (339,345,346) because parathyroidectomy in glucocorticoid-treated animals abolishes the response (347).

In addition to increased PTH levels, intestinal calcium absorption is decreased and serum levels of 25-hydroxyvitamin D are normal (339,348,349) or low (350) in renal transplant patients. Even though serum levels of 25-hydroxyvitamin D were normal in one group of patients studied by Hahn et al. who were receiving an average of 17.5 mg per day of prednisone or an equivalent, the investigators noted that intestinal calcium absorption increased and serum PTH levels decreased after treatment with calcifediol (339). In a study by Rickers et al., there was no significant difference in bone mineral content among steroid-treated transplant patients receiving sodium fluoride, vitamin D_2, and calcium phosphate compared to the

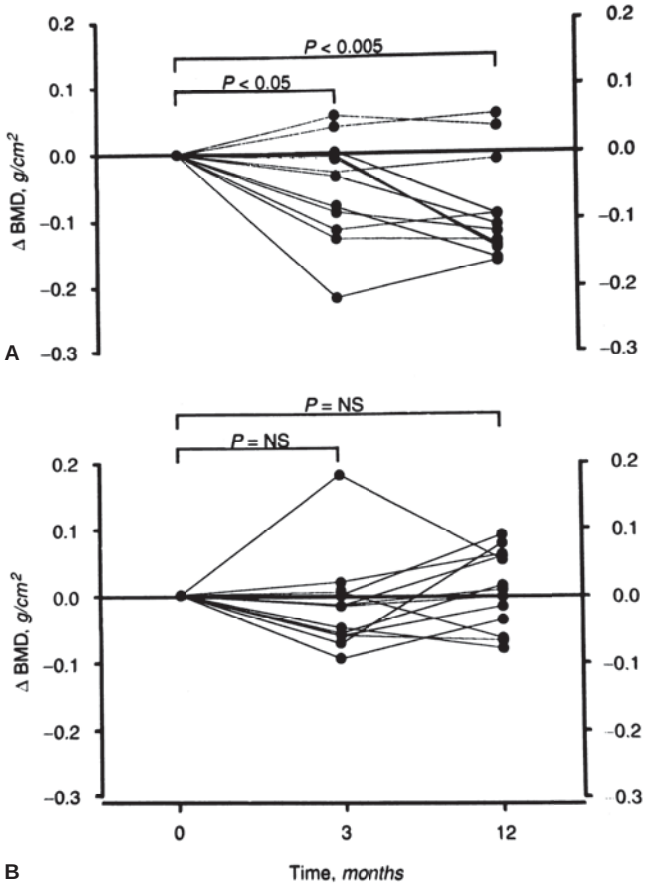


FIGURE 93-35. Effect of pamidronate on femoral neck bone mineral density (BMD) in renal transplant patients. **A:** Control patients. **B:** Pamidronate treated patients. (Reprinted from: Fan SS, et al. Pamidronate therapy as prevention of bone loss following renal transplantation. *Kidney Int* 2000;57:684, with permission.)

control group that received prednisone alone (351). Certainly, more controlled trials are necessary before specific recommendations about treatment of steroid bone disease can be given. However, correction of abnormally low serum levels of calcium, phosphorus, or 25-hydroxyvitamin D is certainly indicated.

The bisphosphonates, recently described as beneficial in postmenopausal osteoporosis (352,353) and in glucocorticoid-induced osteoporosis (354), may have potential in preventing osteoporosis in transplant patients. Fan et al. recently demonstrated that two doses of intravenous pamidronate, given at the time of transplantation and one month later, effectively prevented the rapid bone loss that characterized the first year posttransplantation (355) (Fig. 93-35).

In summary, the bone disease seen in patients with renal disease has a complicated pathogenesis and therapy. Increasing understanding of the factors involved has provided a rationale for management. A knowledgeable physician and compliant patient should be able to avoid most of the difficulties described.

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CHAPTER 94 ■ NERVOUS SYSTEM MANIFESTATIONS OF RENAL FAILURE

ALLEN I. ARIEFF

Advancements in dialysis therapy and renal transplantation and their medical management have resulted in improvement of both the duration and quality of life in patients with end-stage renal disease (ESRD). However, patients with renal failure continue to manifest a variety of neurologic disorders. Those with chronic renal failure who have not yet received dialysis therapy may develop a symptom complex progressing from mild sensorial clouding and tremor to delirium and coma. Even after the institution of otherwise adequate maintenance dialysis therapy, patients may continue to manifest more subtle nervous system dysfunction such as impaired mentation, generalized weakness, and peripheral neuropathy. The central nervous system (CNS) disorders of both untreated renal failure and that persisting despite dialysis are referred to as uremic encephalopathy. The treatment of ESRD with dialysis has itself been associated with the emergence of at least four distinct disorders of the CNS: Dialysis dysequilibrium, dialysis dementia, stroke, and sexual dysfunction. The dialysis disequilibrium syndrome is a consequence of the initiation of dialysis therapy in a minority of patients. Dialysis dementia is a progressive, generally fatal encephalopathy that can affect patients on chronic hemodialysis as well as children with chronic renal failure who have not been treated with dialysis. Cardiovascular disorders are the major cause of death in hemodialysis patients, accounting for 40% of deaths (1). These include myocardial infarction, cardiomyopathy, ischemic heart disease, and stroke. The factors associated with uremia that lead to an increased incidence and mortality from stroke are not well known but are beginning to be elucidated (1–3). In addition, parathyroid hormone (PTH) may be a major factor in the pathogenesis of stroke in patients with ESRD (4,5). In addition to the previously noted manifestations of neurologic dysfunction that are specifically related to uremia, dialysis, or both, a number of other neurologic disorders occur with increased frequency in patients who have ESRD and are being treated with chronic hemodialysis. Subdural hematoma, acute stroke, certain electrolyte disorders (hyponatremia, hypernatremia, phosphate depletion, and hypercalcemia), vitamin deficiencies, Wernicke's encephalopathy, drug intoxication, hypertensive encephalopathy, and acute trace element intoxication must be considered in patients with chronic renal failure who manifest an altered mental state. In the recent past renal transplantation was associated with a variety of nervous system infections and neoplasms, such as reticulum cell sarcoma and lymphoma, which were probably a direct result of immunosuppressive therapy. Immunosuppressive therapy for renal transplantation has undergone a major change over the last decade, so that older immunosuppressive agents (azathioprine, cyclophosphamide) are essentially no longer used (6). Therefore, reticulum cell sarcoma and lymphoma should become very rare in patients who have undergone renal transplantation.

Patients with renal failure are also at risk for developing organic brain disease and metabolic encephalopathy, which can afflict the general population. Therefore, when a patient with ESRD presents with altered mental status, a thorough and complete evaluation is necessary.

Uremic Encephalopathy

Uremic encephalopathy is an acute or subacute organic brain syndrome that regularly occurs in patients with acute or chronic renal failure when glomerular filtration rate declines to less than 10% of normal. As with other organic brain syndromes, patients with uremic encephalopathy display variable disorders of consciousness, psychomotor behavior, thinking, memory, speech, perception, and emotion (7–9). The term *uremic encephalopathy* is used to describe the early appearance and dialysis responsiveness of the nonspecific neurologic symptoms of uremia. Other systemic abnormalities observed in patients with chronic renal failure are separable from uremic encephalopathy on the grounds that they tend to appear late in the progressive clinical course, infrequently produce symptoms, are detected in tissues and organs rather than as integrated whole organism phenomena, and respond sluggishly and irregularly to dialysis procedures. The symptoms may include sluggishness and easy fatigue; daytime drowsiness and insomnia with a tendency toward sleep-inversion; itching; inability to focus or sustain attention or to perform mental (cognitive) tasks and manipulation; inability to manage ideas and abstractions; slurring of speech; anorexia, nausea, and vomiting probably of central origin; restlessness; imprecise memory; diminished sexual interest and performance; volatile emotionality and withdrawal; myoclonus and "restless legs;" "burning feet;" asterixis; hiccoughs; paranoid thought content; disorientation and confusion with bizarre behavior; hallucinosis, muttering and mumbling; meningeal signs, nystagmus; vertigo and ataxia; transient pareses and aphasic episodes; coma and convulsions.

Certain salient characteristics of the symptoms of uremic encephalopathy are especially noteworthy: They are due to dysfunction of the nervous system and are manifested as cognitive, neuromuscular, somatosensory, and autonomic impairments; their severity and overall rates of progression vary directly with the rate at which renal function develops. Uremic symptoms are generally more severe and progress more rapidly in patients with acute renal failure than in those with chronic renal failure. In slowly progressive renal failure the number and severity of symptoms also typically vary cyclically, with intervals of acceptable well-being in an otherwise inexorable downhill course toward increasing disability. The symptoms are readily ameliorated by dialysis procedures and suppressed by maintenance dialysis regimens. They are also usually relieved entirely following restoration of renal function (e.g. after successful renal transplantation). Therefore, the encephalopathy of renal failure is important to recognize precisely because it is promptly and decisively treatable by clinical methods that are generally available. The causes of uremic encephalopathy are doubtless

multiple and complex. Recent studies demonstrate that brain oxygen utilization is diminished in patients with ESRD (10). Most such individuals had anemia, and partial correction of the anemia did not improve the impaired brain oxygen utilization (10). However, since the widespread introduction of recombinant human erythropoietin (EPO) as a therapeutic agent in patients with various degrees of renal insufficiency, including those being treated with hemodialysis, it is now clear that brain function and quality of life are improved by correction of the anemia associated with ESRD with EPO (11–14).

Diagnosis of Uremic Encephalopathy

The diagnosis of uremic encephalopathy in most patients is suspected if there is an elevated plasma creatinine along with a constellation of clinical signs and symptoms that indicate that there is renal or urologic disease or injury. However the presenting symptoms of uremia are similar to those of many other encephalopathic states. Therefore, there is a risk of misdiagnosis and mistreatment. The differential diagnosis is even more complex, since patients with renal failure are subjected to other intercurrent illnesses that may also induce other encephalopathic effects. Moreover, if a drug or its metabolites with potential CNS toxicity is excreted or significantly metabolized by the kidney, the ensuing encephalopathic symptoms may not be entirely attributable to "uremia" but to the drug that has reached toxic levels at ordinary dose rates. When levels of azotemia are discovered that are sometimes associated with uremic encephalopathy in the absence of associated illness, differentiation of the effects of drug versus renal failure may be very difficult. One or more dialysis treatments may restore more normal body fluid composition and also reduce drug levels, so that the question remains moot while the patient recovers. Despite the possibilities that such multiple causes of encephalopathy might occur simultaneously, uremic encephalopathy may be successfully differentiated in most instances by means of the usual clinical methods.

In patients who have other medical problems such as advanced liver disease with hepatic insufficiency, it is often difficult to differentiate whether the encephalopathy is due to hepatic or renal causes. Under normal conditions, protein and amino acids in the gastrointestinal tract are metabolized by colonic bacteria and mucosal enzymes to form ammonia (15). Ammonia then enters the liver through the portal circulation where it participates in the urea cycle to form urea. More than 90% of the urea produced is excreted in the urine and the remainder enters the colon via hepatoportal recirculation. However, in patients with renal failure, the major route for elimination of urea is not available; therefore, there is an increase in blood urea. The amount of urea that enters the colon is increased because of the elevated plasma urea. Urea is then acted on by colonic bacteria and mucosal enzymes in a similar manner to their action on protein and amino acids. This leads to increased ammonia production in uremic subjects that may either increase plasma ammonia levels or lead to misinterpretation of this test.

If the patient with kidney failure also has cirrhosis or some other form of liver failure, this additional ammonia load may present a stress that cannot be adequately handled by the diseased liver. The result may be increased blood and CNS ammonia levels with development of encephalopathy (15). Therefore, patients with cirrhosis and end-stage kidney disease are at particular risk for developing encephalopathy, since both conditions act synergistically to increase both blood and CNS ammonia. It would, therefore, be prudent to institute early dialysis in patients with end-stage liver and kidney diseases in order to reduce the plasma urea and thus the production of ammonia from urea in the gut. Ammonia can also be removed from the blood by hemodialysis. It should also be noted that plasma urea and serum creatinine do not always adequately reflect renal function in patients with severe liver disease. Recent studies suggest that many patients who have cirrhosis, ascites, and normal plasma urea and creatinine may in fact have severe renal functional impairment (16–18). In such individuals, differentiation of hepatic from uremic encephalopathy on clinical grounds can be very difficult.

ACUTE RENAL FAILURE

The clinical manifestations of acute renal failure have been studied in several large series of patients (7,19,20). Abnormalities of mental status have been noted as early and sensitive indices of a neurologic disorder, which progressed rapidly into disorientation and confusion (21,22). Fixed attitudes, torpor, and other signs of toxic psychosis were common. When uremia is untreated and allowed to progress, coma often supervenes. Cranial nerve signs such as nystagmus and mild facial asymmetries are common, although usually transient. There can be visual field defects and papilledema of the optic fundi. About half of the patients have dysarthria, and many have diffuse weakness and fasciculations. Marked variation of deep tendon reflexes is noted in most patients, often in an asymmetrical pattern. Progression of hyperreflexia, with sustained clonus at the patella or ankle, is common (23).

Electroencephalograms (EEGs) in patients with acute renal failure (24) have generally been grossly abnormal when the diagnosis of renal failure was first established (Fig. 94-1). In most instances, the percentages of EEG power less than 5 Hz and less than 7 Hz, which are standard measurements of the percentage of EEG power devoted to abnormal (delta) slow wave activity, are more than 20 times the normal value. The percentage of electroencephalographic frequencies greater than 9 Hz and less than 5 Hz are not affected by dialysis for 6 to 8 weeks, but return to normal with recovery of renal function. Similar findings have been shown in experimental animals with renal failure (25). The EEG may worsen both during and for several hours after hemodialysis and up to 6 months after initiation of dialytic therapy (25–27). In patients with acute renal failure, the EEG is abnormal within 48 hours

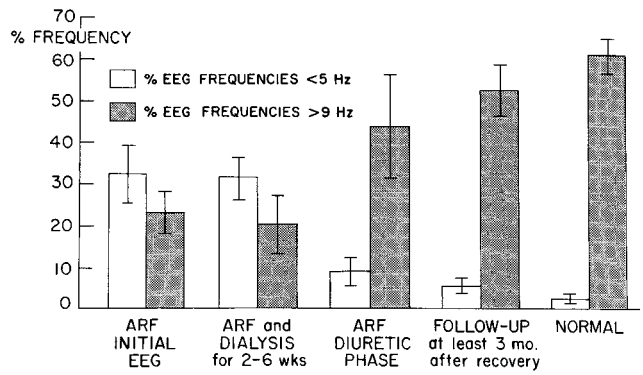

FIGURE 94-1. Percentage of electroencephalographic frequencies below 5 Hz and above 9 Hz. Normal values are on the *right*. Patients with acute renal failure (ARF) have significant changes in frequencies above 9 Hz and below 5 Hz. These changes are unaffected by dialysis but become normal with return of renal function. Data from 16 patients with ARF are illustrated. Data are mean ± SE. (From: Cooper JD, Lazarowitz VC, Arieff AI. Neurodiagnostic abnormalities in patients with acute renal failure: evidence for neurotoxicity of parathyroid hormone. *J Clin Invest* 1978;61:1449, with permission.)

of the onset of renal failure (24) and is generally not affected by dialysis within the first 3 weeks (Fig. 94-1). During this interval, patients with acute renal failure have been shown to have elevated levels of PTH in plasma (24,28). Several months after the return of renal function, plasma PTH levels also return to normal. Although there are doubtlessly many factors that contribute to uremic encephalopathy, many investigators have shown no correlation between encephalopathy and any of the commonly measured plasma indicators of renal failure (e.g., blood urea nitrogen (BUN), creatinine, bicarbonate, arterial pH, and potassium) (22,24,28). For this and other reasons, PTH has been postulated to be a major CNS "uremic toxin" (28,29).

CHRONIC RENAL FAILURE

The incidence of ESRD in the United States is 260 cases per million population (30) Among patients with ESRD who are treated with chronic hemodialysis, comorbid diseases, mainly infectious and cardiovascular, continue to increase. Among patients with ESRD who were treated with chronic hemodialysis in 2000, there were 13.9 hospital days per patient-year. Among such patients, the mortality in the United States is 182 deaths per 1,000 patient-years at risk, or 247 deaths per 1,000 patient-years at risk for hemodialysis patients (30). In the United States, the most frequent causes of ESRD are diabetes, hypertension, glomerulonephritis, and polycystic kidney disease (30). The neurologic manifestations reported with chronic renal failure (CRF) are numerous and varied (31–35). Many are not improved by chronic dialysis.

The electroencephalographic findings in patients with CRF are well described and generally less severe than those observed in patients with acute renal failure (9). Several investigations have shown a good correlation between the percentage of electroencephalographic frequencies and power below 7 Hz and the decline of renal function as estimated by serum creatinine (7–9). After the initiation of dialysis, there may be an initial period of clinical stabilization during which time the EEG deteriorates (up to 6 months), but it then approaches normal values (26). However, still more improvement is seen after renal transplantation (8,36). Cognitive functions have also been shown to be impaired in uremia. These include sustained attention, selective attention, speed of decision making, short-term memory, and mental manipulation of symbols (9).

The causes of the electroencephalographic abnormalities observed in uremic patients are probably multifactorial, but there is evidence that a very important element may be an effect of PTH on the brain. In experimental animals with either acute or chronic renal failure, many of the electroencephalographic abnormalities can be shown to be related to a direct effect of PTH on the brain, which leads to an elevated brain content of calcium (25). Studies in patients with either acute renal failure or CRF suggest a similar pathogenesis (37,38).

PSYCHOLOGICAL TESTING

Several different types of psychological tests have been applied to subjects with CRF. These have been designed to measure the effects of dialysis, renal transplantation or parathyroidectomy (28,39). The Trailmaking Test has been administered to a number of uremic subjects. In general, their performance was less effective than that of normal individuals; improvement with practice limits repeated use of this test. The Continuous Memory Test correlates quite well with the degree of renal failure, as did the Choice Reaction Time (CRT). Scores in both tests improved with treatment by dialysis or renal transplantation. Similar but less impressive results are obtained with the Continuous Performance Test. Of all these tests, it appeared that the CRT was best correlated with renal function and with improvement in the patient's condition as a result of dialysis or transplantation (9,40).

Patients who had CRF who were maintained on dialysis have been evaluated as to the possible effects of PTH on psychological function (28). After establishment of baseline values, patients with CRF underwent parathyroidectomy for other medical reasons (e.g., bone disease, soft tissue calcification, and persistent hypercalcemia, all of which were unresponsive to medical management). In these patients, parathyroidectomy resulted in a significant improvement in several areas of psychological testing. They showed significant improvement in Raven's Progressive Matrices percentile scores and visual motor index (VMI) raw and percentage scores. These are tests of general cognitive function, nonverbal problem solving, and visual-motor or visual spatial skills (9,40).

In addition, they manifested significantly fewer errors on the Trailmaking Test as well as significantly lower raw and T-score values on the Profile of Mood States Fatigue Scale postoperatively, in which they reported feeling significantly less fatigue, weariness, and inertia after undergoing surgery. Control subjects who underwent neck surgery for other reasons showed significant postoperative improvement in the Trailmaking Test but showed no change in any of the other tests (28). Other studies have shown that there is intellectual impairment in most patients with CRF being treated with dialysis, virtually all of whom have secondary hyperparathyroidism (33,35,41,42). In these studies the procedures included the full Wechsler Adult Intelligence Scale (WAIS), the Walton-Black Modified Word Learning Test (MWLT), and the Block Design Learning Test (BDLT).

In patients with CRF, the overall intellectual level, as measured by the WAIS full-scale IQ, did not differ significantly from normal. The patients' impaired performance was due mainly to the digit symbol, block design, and picture arrangement subtests, all of which produced scores significantly below normal. The impairment of intellectual level as represented by the Wechsler deterioration quotient was also outside the normal range. The data on verbal learning obtained with the MWLT and performance learning obtained with the BDLT did not indicate any gross learning abnormality. Cognitive data were compared with other information, such as age, sex, length of dialysis, and biochemical variables by a multiple regression technique. The analysis suggested that of the cognitive data, those obtained with the BDLT bore the strongest relation to duration of dialysis. Other studies have suggested that the WAIS full-scale IQ in dialysis patients is less than that of the general population (9). There appears to be a consensus, based on psychological testing, that CRF results in organic-like losses of intellectual function, particularly information-processing capacities (9,33,35,41,42).

Biochemical Changes in Brain

To determine the possible causes of the electroencephalographic abnormalities and clinical manifestations observed in patients with either acute renal failure or CRF, *in vivo* biochemical studies have been carried out in brain of both patients and laboratory animals. Measurements have included brain intracellular pH and concentrations of $Na^{+,}$ K^+, Cl^-, Al^{3+}, Ca^{2+}, Mg^{2+}, urea, adenine nucleotides (creatine phosphate, ATP, ADP, and AMP), lactate, and ($Na^+ + K^+$)-activated adenosine triphosphatase (ATPase) enzyme activity (22,24,37,43–48), In patients with acute renal failure, the brain content of water, K^+ and Mg^{2+} is normal, whereas Na^+ is modestly decreased and Al^{3+} is slightly elevated (24). However, cerebral cortex Ca^{2+} content is almost twice the normal value (24,37,45). Similar

findings have been observed in dogs with acute renal failure (25,49). Permeability of uremic rat brain to inert molecules, (e.g., insulin and sucrose) is increased. The permeability of brain to weak acids, such as sulfate, penicillin, and dimethadione, is normal to low (46,50,51).

Alterations of cerebral metabolism that might be related to the changes in permeability mentioned in the preceding text have also been studied in animals (45,42–54). There are at least four studies that have attempted to evaluate the effects of uremia on the CNS using subcellular analysis. The first two studies evaluated Na,K-ATPase enzyme activity in crude microcosmal fractions obtained from brains of acutely uremic rats (43,44). There was a significantly decreased Na,K-ATPase enzyme activity in the preparation and the authors suggested that the depressed enzyme activity was not due to acidosis but to the uremic state itself. Conversely, an earlier study by Van den Noort et al. (43) found no significant difference in cationic ATPase activity in normal and uremic rat brains. In the brain of rats with acute renal failure, creatine phosphate, ATP, and glucose were increased, but there were corresponding decreases in AMP, ADP, and lactate. Total brain adenine nucleotide content and (Na^+,K^+)-activated ATPase were normal to low. The uremic brain utilized less ATP and thus failed to produce ADP, AMP, and lactate at normal rates. The brain energy charge was normal, as was the redox state, and these findings were not altered by hypoxia (45). There was a corresponding decrease in brain metabolic rate, along with elevated glucose and low lactate levels (45). Other studies of uremic brain have shown a decrease in cerebral oxygen consumption (10), but such findings were not apparently related to the presence of anemia. Patients with chronic renal insufficiency (glomerular filtration rate less than 20 mL/minute) have decreased brain uptake of glutamine and increased ammonia uptake. The relevance of these findings, in terms of neurotransmitters or other brain function, is unknown (54).

Studies have been carried out in neurons and glia of uremic rat brain (55). Two different cytoskeletal proteins, both early indicators of brain injury, were examined. These included glial fibrillary acidic protein (GFAP), which is specific to astrocytes, and microtubule-associated protein-2 (MAP-2), which localizes to neuronal cell bodies and dendrites. MAP-2 loss provides one of the earliest indications of neuronal degeneration. In uremic brain (12 hours of acute renal failure), there was a diffuse increase in GFAP in cerebral cortex. Changes in MAP-2 immunoreactivity were observed in all regions of the cerebral cortex. These data suggest that there may be profound degenerative changes in neurons even with only moderate azotemia. These studies suggest subcellular anatomic changes in brain of animals with acute renal failure, but do not elucidate any metabolic abnormalities.

In animals with either acute or chronic renal failure, both urea concentration and osmolality are similar in brain, cerebrospinal fluid (CSF), and plasma. The solute content of brain in animals with acute renal failure is such that essentially all of the increase in brain osmolality is due to an increase of brain urea concentration. However, in animals with CRF, about half of the increase in brain osmolality is due to the presence of undetermined solute (idiogenic osmoles) with the other half due to an increase in urea concentration (47,48,53).

In dogs with CRF, brain content of Na^+, K^+, Cl^- and water are not different from control values. Similarly, the extracellular space was not different from control (53). Calcium content was measured in eight parts of the brain in dogs that had CRF for 4 months. Calcium content was found to be normal in the subcortical white matter, pons, medulla, cerebellum, thalamus, and caudate nucleus. However, as shown in Figure 94-2, calcium was approximately 60% greater than control values in both cortical gray matter and hypothalamus. Magnesium content was normal in all eight parts of the brain, as was water

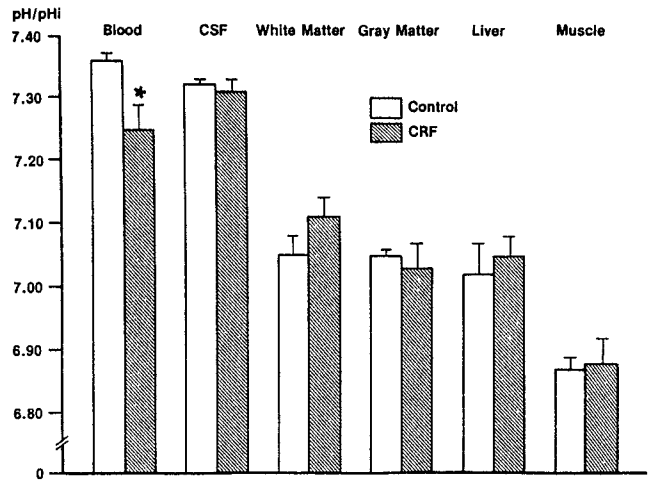

FIGURE 94-2. The effects of 4 months of chronic renal failure (CRF) on the intracellular pH (pHi). In dogs with CRF the arterial pH is significantly less than control values. However, pHi is normal in brain white and gray matter, the liver, and skeletal muscle (*p <0.01). *CSF*, cerebrospinal fluid. (From: Mahoney CA, Arieff Al. Central and PNS effects of chronic renal failure. *Kidney Int* 1983;24:170, with permission.)

content (53). Other investigators have also found an elevated cerebral cortex calcium content in dogs with CRF (56). In animals that have acute renal failure and metabolic acidosis, the intracellular pH (pHi) of brain and skeletal muscle is normal (47) (Fig. 94-2). In dogs with CRF, intracellular pH is normal in brain, liver, and skeletal muscle (53). In patients with renal failure, intracellular pH has been reported to be normal in both skeletal muscle and leukocytes, as well as in the "whole body" (57–59) (Fig. 94-2). The pH of CSF has also been shown to be normal in both patients and laboratory animals with renal failure (47,53). Therefore, despite the presence of extracellular metabolic acidemia in patients or laboratory animals with either acute renal failure or CRF, the intracellular pH is normal in brain, white blood cells, liver, and skeletal muscle.

In general then, studies of brain tissue from both intact animal models of uremia and humans with renal failure have revealed many different biochemical abnormalities associated with the uremic state. However, such investigations have not as yet revealed much about the fundamental mechanisms that might induce such abnormalities. Such studies probably will have to be done in isolated cell systems or subcellular systems from the brain. These systems have the advantage of permitting one to study isolated manifestations of the uremic state while removing the numerous potential confounding influences present in an *in vivo* model.

UREMIC NEUROTOXINS

Central Nervous System

Uremic neurotoxins would imply retention of solutes that have detrimental effects upon nervous system function, either peripheral nervous system or CNS (60–62).

There are at least three different types of uremic solutes that can be characterized (62–64). These include (i) small water-soluble compounds, such as urea and creatinine (60,61); (ii) middle molecules; and (iii) protein-bound compounds. Most of the small water-soluble compounds, such as urea and creatinine, are not particularly toxic and are easily removed with dialysis. Guanidine compounds have been postulated to

be "uremic toxins" for many years (65), based upon possible detrimental effects upon the CNS.

This is based in part on the effects of guanidine compounds on mitochondrial function (66,67). In the brain of uremic patients, guanidine compounds were measured in 28 different regions (68,69). Guanidine succinic acid was elevated by up to 100 fold in uremic brain versus control brain, and levels increased with increasing levels of uremia. The brain levels of guanidine succinic acid were similar to those observed in animal brain following injection to blood levels that cause convulsions (68). Guanidines inhibit neutrophil superoxide production, can induce seizures, and suppress natural killer cell response to interleukin-2 (70). Other guanidines, which are arginine analogs, are competitive inhibitors of nitric oxide (NO) synthetase, and can lead to vasoconstriction, hypertension, ischemic glomerular injury, immune dysfunction, and neurologic changes (70).

Middle molecules are large-molecular-weight compounds (300 to 12,000 Da) which have in the past been believed to be responsible for many of the manifestations of uremia. Despite the fact that at one time, dialysis membranes were designed with the specific intent of removing more middle molecules, evidence of their toxicity is generally lacking (71–73). There has recently been a renewed interest in these molecules (73,74), but evidence of their toxicity is still conjectural (70).

Advanced glycosylation end products (AGEs) can modify tissues, enzymes, and proteins and may play a role in the pathogenesis of dialysis-associated amyloidosis (64). Advanced glycosylation end products may also play a role in the pathogenesis of diabetic nephropathy (75). Advanced glycosylation end products are markedly elevated in plasma of patients with ESRD (76). The AGEs react with vascular cells to inactivate endothelial nitric oxide and may increase the propensity of ESRD patients to develop hypertension. Current dialysis therapy is relatively ineffective in removal of AGEs, so that there is accumulation of AGEs in patients with ESRD, particularly those with diabetes mellitus (76). The AGEs are "middle molecules" and have the potential to cause tissue damage and lead to hypertension. Therefore, at least some middle molecules may actually be deleterious in patients with ESRD, and they are poorly removed with conventional dialysis (76). PTH is a high-molecular-weight compound and may be an important CNS uremic toxin (24,25,28,29). Protein-bound compounds (toxins) are not substantially removed by dialysis, and almost all are lipophilic. Such compounds include polyamines such as spermine. Spermine is postulated to be a uremic toxin and appears to react with the N-methyl-D-aspartate (NMDA) receptor, which affects calcium and sodium permeability in brain cells (77). Stimulation of the NMDA receptor in brain is the final common pathway for brain cell death in a number of pathologic pathways (78–80). The uremic state is associated with increased oxidative stress, resulting in protein oxidation products in plasma and cell membranes. There is eventual alteration of proteins with formation of oxidized amino acids, including glutamine and glutamate (63). Such reactions may eventually lead to stimulation of the NMDA receptor in brain, with brain cell damage or death (2). In recent reviews, there are no new candidates for uremic toxins that impair the CNS (81). The anemia of renal failure had been suggested as a contributor to uremic toxicity, but this has been largely eliminated by the widespread use of erythropoietin (11,12).

Peripheral Nervous System

There are a number of solutes that are purported to impair peripheral nerve function. Several possible uremic toxins have been identified that appear to be correlated with depression of motor nerve conduction velocity (MNCV) in laboratory animals (65,82,83). However, these studies do not take into account the fact that (i) depressed MNCV is cyclical, with abnormal low values one day and normal values the next (19), (ii) there is a day-to-day variation in MNCV that approaches 20% (84); and (iii) the finding of depressed MNCV in laboratory animals associated with high plasma levels of potential uremic neurotoxins has generally not been confirmed in human subjects with renal failure (71,85,86). Although it is possible to relate impairment in MNCV with levels in blood of various substances, the best correlation was obtained between reduced MNCV versus a reduction in glomerular filtration rate.

Among the potential peripheral nerve uremic neurotoxins is PTH, based on a correlation between plasma PTH levels and MNCV in patients with CRF (87). Parathyroid hormone is of high molecular weight, and if neurotoxic could qualify as a high molecular weight uremic toxin (88). Some earlier studies suggested a possible effect of PTH on MNCV in the dog (89), but these suggestions have not been confirmed (53). In patients who have hyperparathyroidism without uremia, PTH has no observable effect on peripheral nerve function (90). In both patients and laboratory animals with acute renal failure, the MNCV has been found to be normal (24,82,91) (Fig. 94-3). In all studies of both patients and laboratory animals with CRF, the MNCV had not been shown to be affected by PTH (53). Therefore, in both patients and laboratory animals with either acute renal failure or CRF, or primary or secondary hyperparathyroidism, no effect of PTH on nerve function can be demonstrated. In patients with CRF, there is no change in MNCV as a result of either recovery of renal function or chronic hemodialysis; there was also no effect of parathyroidectomy (82,91). In addition, when patients begin dialysis therapy, MNCV either stabilizes or improves (92,93). However, most of these patients have elevated plasma PTH levels (94).

Animal studies suggest that in either acute renal failure or CRF, changes in MNCV take longer than 6 months to develop and are probably not related to an effect of PTH. Mahoney

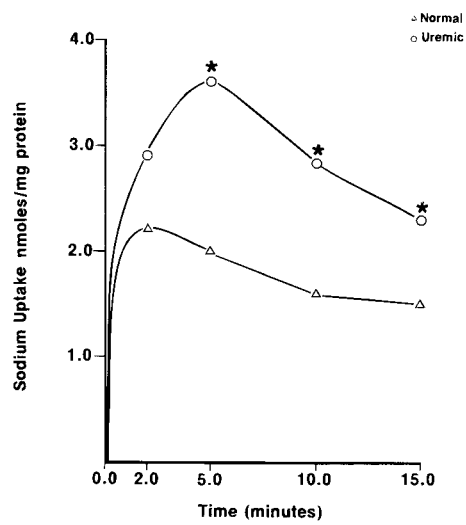

FIGURE 94-3. The difference between the veratridine-stimulated Na^+ uptake and the control uptake (without addition of neurotoxins) in both groups of synaptosomes is plotted in this graph. The *top curve* shows the veratridine-stimulated sodium uptake in uremic synaptosomes, whereas the uptake in normal synaptosomes is represented by the lower curve. The increased veratridine-stimulated sodium uptake in uremic rats is significantly less than in normal rats (*$p < 0.001$) at 5, 10, and 15 minutes. (From: Fraser CL, Sarnacki P, Arieff Al. Abnormal sodium transport in synaptosomes from brain of uremic rats. *J Clin Invest* 1985;75:2014, with permission.)

et al. studied dogs that had renal failure for periods of 3.5 days to 6 months (53). There was no change in the MNCV after any of the aforementioned intervals of renal failure, and the MNCV was normal, even after 6 months with a glomerular filtration rate of less than 22% of control.

SUBCELLULAR STUDIES

One system that appears to lend itself particularly well to studies of metabolic abnormalities in uremia is the synaptosome. When brain tissue is homogenized in isoosmotic media, the presynaptic nerve terminals are seared off and resealed to form intact membrane vesicles called synaptosomes. Like the intact nerve endings, synaptosomes contain mitochondria and synaptic vesicles and are metabolically active (95,96). The synaptosome can be studied as an intact system or be hypotonically lysed into its constituents and ghost synaptic plasma membrane (97,98). Although the synaptic junction was first isolated at the turn of the century and first described histologically by Cajal (99), it was not until the late 1950s that the synaptosome was finally isolated (100,101). Isolation of synaptosomes subsequently proved to be an important step in understanding the response of the brain to numerous metabolic insults. Synaptosomes have been used in the investigation of neurotransmitter substances, ion transport, drug actions, and effects of nervous system toxins on numerous metabolic processes (52,97,98). Synaptosomes are that portion of the nerve cell where neurotransmitter substances are synthesized, stored, and released. Therefore, any abnormalities present in the uremic state might be expected to affect synaptosomal function and information transfer in the CNS. There are at least two ways by which information transfer occurs between neurons in the CNS, both of which may be abnormal in the uremic state. Communication between cells is either by chemical or electrical transmission at the synaptic junction. Because of these methods of communication across the synaptic space, the nerve cells are able to communicate rapidly with each other over great distances. Conduction via the axon and transmission at the synaptic junction make possible this rapid and precise communication. Transmission at most synapses is by chemical means, although some synapses operate purely by electrical conduction. The type of transmission utilized at the synapses appears to be dependent on the type of synaptic junction in question. Transmission through the gap junction is primarily electrical, whereas transmission through the unbridged, larger synaptic junction is felt to be strictly a chemical phenomenon. Because of these intricate interrelationships at the synapses in the CNS, it is not surprising that dysfunction in information transfer at the synapses does occur in uremia (102,103).

Physiology of Neurotransmission

The neuron is the vehicle of communication and the center for the processing of information in the CNS. The neuron consists of three major portions: the axon, the dendrites, and the soma (cell body). Each neuron is contacted by hundreds of axons from other neurons that may either inhibit or excite the receptor area of the cell.

The synaptosome is usually about 0.6 to 1.2 μm in diameter. The two most important internal organelles in the synaptosomes are synaptic vesicles and mitochondria. The synaptic vesicles contain neurotransmitter substances that, when released into the synaptic space, either inhibit or excite the postsynaptic receptor area. Mitochondria provide the energy for the metabolic processes in the neuron. They are the source of ATP, which is required to synthesize transmitter substances and maintain ionic equilibrium at the nerve terminals. When an action potential reaches the presynaptic terminal of a neuron, the plasma membrane depolarizes, causing a rapid influx of calcium into the terminal. The accumulated calcium serves to aggregate the synaptic vesicles to reach other neurons and to the surface of contact with the opposing neuron. This facilitates the release of neurotransmitter substances from the synaptic vesicles into the synaptic space by exocytosis. The neurotransmitter substance acts on the postsynaptic terminal in such a way as to change the membrane permeability of the postsynaptic neuron. The effector neuron is then either excited or inhibited, depending on the type or receptor present on the affected cell.

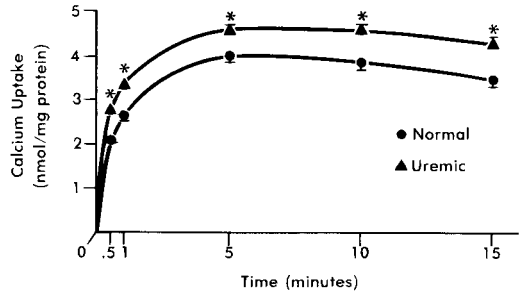

FIGURE 94-4. Sodium-calcium exchange in normal versus uremic synaptosomes. The *top curve* shows calcium uptake in uremic synaptosomes. The *lower curve* shows update in normal synaptosomes. This graph is a mean of eight experiments, and each time-point was done in triplicate. The result is expressed as mean +SE. The points indicated by the *asterisks* represent differences that are significant to $p < 0.005$. (From: Fraser CL, Sarnacki P, Arieff Al. Abnormal sodium transport in synaptosomes from brain of uremic rats. *J Clin Invest* 1985;76:1789, with permission.)

Studies by Fraser and Arieff (97) and Fraser et al. (98) in rat brain synaptosomes demonstrated abnormalities of both sodium and calcium transport and decreased Na-K-ATPase pump activity in the brains of rats with uremia (Fig. 94-3, 94-4). They suggested that these findings may affect neurotransmitter release in the uremic state (97,98). This defect did not appear to be due to the uremic environment at the time of study, since synaptosomes were washed several times and frozen before uptake studies were carried out. The defect observed in uremia appeared to be due to a physical alteration of the synaptosomal membrane in acute uremia. These workers also demonstrated alterations of calcium transport in uremic rat brain (105) (Fig. 94-4). Based on the relationship between extracellular calcium and the release of neurotransmitter substances in nerve terminals, the investigators concluded that this defect may affect neurotransmitter release and information processing in the uremic state.

In subsequent studies, the increase in calcium transport in uremia appeared to be PTH dependent (98,104,105). When calcium transport by both the Na-Ca exchanger and the ATP-dependent calcium pump was carried out in brain of parathyroidectomized uremic rats, calcium accumulation into these vesicles returned to normal values. In addition, parathyroidectomized uremic rats that were injected with parathyroid extract showed abnormalities of calcium transport similar to those described in uremia (105). These findings suggested that in uremic animals, PTH may be responsible for some of the alterations in synaptosomal calcium transport (105). It was also interesting to note that clinically, the uremic parathyroidectomized rats tolerated uremia better than control uremic rats and uremic parathyroidectomized rats treated with PTH. Therefore, there appear to be both clinical and physiologic changes in the uremic brain that may be PTH dependent.

Fraser et al. (98,104,105) also evaluated the effects of acute uremia on sodium and calcium transport in uremic rat brain. They demonstrated decreased Na-K-ATPase pump activity in uremia (98). There appeared to be to a physical alteration

of the synaptosomal membrane in uremic rat brain. These contentions were based on the observation that veratridine-stimulated sodium uptake was markedly greater in synaptosomes from uremic rats than from normal rats (98) (Fig. 94-3).

Fraser et al. also demonstrated increased calcium transport in uremic rat brain synaptosomes (104) (Fig. 94-4). This increase in calcium transport observed in uremia may be PTH dependent (105). When calcium transport was studied in synaptosomes from parathyroidectomized uremic rats, calcium accumulation into these vesicles returned to normal control values. In addition, parathyroidectomized uremic rats that were injected with intact bovine parathyroid extract showed abnormalities of calcium transport similar to those previously described in uremia (106). Therefore, in the presence of a uremic environment, PTH may be responsible for the alterations in calcium transport observed in uremic brain (104,106). As mentioned previously, when a neuron in the CNS is stimulated, an action potential is generated that results in a change in the membrane permeability to calcium of the synaptic nerve terminal. This results in calcium influx from the extracellular space into the cytosol of the nerve terminal (107). The mechanisms by which calcium enters cells include sodium channels, calcium channels, and the Na-Ca exchanger (108). As opposed to nonexcitable cells where the Na-Ca exchanger participates only in calcium efflux (108), in excitable cells such as neurons and myocardium the exchanger participates in both calcium influx and efflux (109,110). In order to maintain calcium balance, calcium that enters the cell from the extracellular space has to be pumped out, and this is achieved by the Na-Ca exchanger and the Ca-ATPase pump. These two mechanisms comprise the primary exporter of calcium from either excitable or nonexcitable tissues (111,112). The overall effect of these transporters is to maintain a calcium gradient of 10,000:1 (outside-inside cells), and in the steady state they maintain a net calcium flux across the plasma membrane of zero. The Na^+-Ca^{2+} exchanger that operates electrically (exchanging three Na^+ for one Ca^{2+}) is a high-capacity, low-affinity system, whereas the Ca^{2+}-ATPase is a low-capacity, high-affinity system that continuously pumps calcium from the cells. Therefore, in cellular regulation of calcium, the Ca^{2+}-ATPase pump modulates subtle changes in cytosolic calcium, whereas the Na^+-Ca^{2+} exchanger is important in regulating major changes in calcium flux (113).

Although calcium transport in synaptosomes in uremia appears to be influenced by PTH, not all transport processes are affected in this manner. Verkman and Fraser evaluated water and urea transport in synaptosomes by stopped-flow light-scattering technique and found no differences in either water or urea permeabilities in normal rats compared to those with uremia, or uremic animals subjected to parathyroidectomy (52). From these studies they were also able to show that synaptosomal water and urea transport occurred by a lipid diffusive pathway and was not affected by uremia (52).

CENTRAL NERVOUS SYSTEM PATHOLOGY WITH UREMIA

Pathologic studies of brain have been reported in more than 400 patients dying of CRF (114,115). It has been shown that there is some necrosis of the granular layer of the cerebral cortex. Small intracerebral hemorrhages and necrotic foci are seen in about 10% of uremic patients and focal glial proliferation is seen in about 2%. In general, changes in the brain of patients who died of CRF are probably nonspecific and related more to any of a number of concomitant underlying disease states (116). Subdural hemorrhage, once a common finding in autopsied uremic patients, is now quite unusual. Cerebral edema has not been observed in the brains of humans and laboratory animals with either acute renal failure or CRF (24,28).

The recent use of advanced neuroimaging techniques has led to substantial *in vivo* study of uremic brain in humans (117). Acute and subacute movement disorders have been observed in patients with ESRD. These have been associated with bilateral basal ganglia and internal capsule lesions (118–120). Cerebral atrophy has been observed in chronic hemodialysis patients, and it tends to worsen as dialysis therapy continues (121–123). Cerebral atrophy had previously been thought to be associated with dialysis dementia, but this is apparently not the case (124). ESRD has also been reported to lead to deterioration of vision. Some cases are associated with uremic pseudotumor cerebri, and, in these selected cases, surgical optic nerve fenestration may improve visual loss (125,126).

Pathophysiology

Although there are many factors that contribute to uremic encephalopathy, most investigators have shown no correlation between encephalopathy and any of the commonly measured indicators of renal failure. In recent years, there has been considerable discussion of the possible role of PTH as a uremic toxin. There is a substantial amount of evidence to suggest that PTH may exert an adverse effect on the CNS (24,25,28,37).

Role of Parathyroid Hormone

In patients dying with acute renal failure or CRF, the calcium content in brain cerebral cortex is significantly elevated (24,25,28) (Fig. 94-5). Dogs with acute or chronic renal failure show increases of brain gray matter calcium and electroencephalographic changes similar to those seen in humans with acute renal failure (45,48,53,127). In dogs, both the electrocardiographic and brain calcium abnormalities can be prevented by parathyroidectomy. Conversely, these abnormalities can be reproduced by administration of PTH to normal animals while maintaining serum calcium and phosphate in the normal range. Therefore, PTH is essential to produce some of the CNS manifestations in the canine model of uremia (25,56).

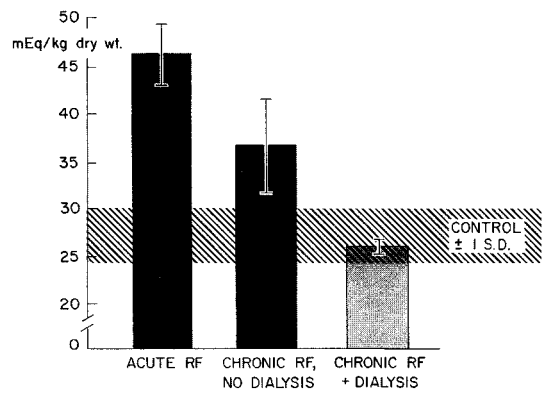

FIGURE 94-5. The brain (cerebral cortex) content of calcium (Ca^{2+}) in patients who had acute renal failure, those with chronic renal failure treated with dialysis, or patients with end-stage chronic renal failure treated for at least 2 years with hemodialysis. Brain Ca^{2+} content is significantly greater than control values in patients who had acute renal failure ($p < 0.001$) or chronic renal failure not treated with dialysis ($p < 0.03$). However, in patients treated with dialysis CA^{2+} content is normal. Data are mean +SE. *RF*, renal failure. (From: Arieff, AL. Neurological complications of uremia. In: Brenner BM, Rector FC, Jr, eds. *The Kidney*. Philadelphia: Saunders; 1986, with permission.)

PTH is known to have CNS effects in humans, even in the absence of impaired renal function. Neuropsychiatric symptoms have been reported to be among the most common manifestations of primary hyperparathyroidism (128,129). Patients with primary hyperparathyroidism also have electroencephalographic changes similar to those observed in patients with acute renal failure (24,130). The common denominator appears to be elevated plasma levels of PTH (24,25,28,37).

In patients with acute renal failure, the EEG is abnormal within l8 hours of the onset of renal failure and is generally not affected by dialysis for periods of up to 8 weeks (24). In patients with either primary or secondary hyperparathyroidism, parathyroidectomy results in an improvement of both EEG and psychological testing, suggesting a direct effect of PTH on the CNS. Similarly, dialysis results in a decrement of brain (cerebral cortex) calcium toward normal in both patients and laboratory animals with renal failure concomitant with improvement of the EEG (24,25,28). In uremic patients, both electroencephalographic changes and psychologic abnormalities are improved by parathyroidectomy or medical suppression of PTH. PTH, a high brain calcium content, or both are probably responsible, at least in part, for many of the encephalopathic manifestations of renal failure.

The mechanisms by which PTH might impair CNS function are only partially understood. The increased calcium content in such diverse tissues as skin, cornea, blood vessels, brain, and heart in patients with hyperparathyroidism suggests that PTH may somehow facilitate the entry of Ca^{+2} into such tissues. The finding of increased calcium in the brains of both dogs and humans with either acute or chronic renal disease and secondary hyperparathyroidism is consistent with the concept that part of the CNS dysfunction and electroencephalographic abnormalities found in acute renal failure or CRF may be due in part to a PTH-mediated increase in brain calcium (Fig. 94-5). Calcium is essential for the function of neurotransmission in the CNS and for a large number of intracellular enzyme systems. Therefore, an increased brain calcium content could disrupt cerebral function by interfering with either of these processes, and it is also possible that PTH itself may have a detrimental effect on the CNS.

NEUROLOGIC COMPLICATIONS OF END-STAGE RENAL DISEASE AND ITS THERAPY

Dialysis Disequilibrium Syndrome

In patients with ESRD, there are several CNS disorders that may occur as a consequence of dialytic therapy. Dialysis disequilibrium syndrome (DDS) is a clinical syndrome that occurs in patients being treated with hemodialysis. The syndrome was first described in l962 and may include symptoms such as headache, nausea, emesis, blurring of vision, muscular twitching, disorientation, hypertension, tremors, and seizures (53,131–133). The syndrome of DDS has been expanded to include milder symptoms, such as muscle cramps, anorexia, restlessness, and dizziness (134–136). Although DDS has been reported among all age groups, it is more common among younger patients, particularly the pediatric age group (137). The syndrome is most often associated with rapid hemodialysis of patients with acute renal failure, but it also has occasionally been reported following maintenance hemodialysis of patients with CRF (138,139). The pathogenesis of DDS has been extensively investigated and the findings are summarized elsewhere (131,140). The symptoms are usually self-limited but recovery may take several days. It appears that present methods of dialysis have altered the clinical picture of DDS. Most reports of seizures, coma, and death were reported prior to l970. The symptoms of DDS as reported in the last 25 years (l975 to 2000) have generally been mild, consisting of nausea, weakness, headache, fatigue, and muscle cramps. Almost all cases have occurred in patients undergoing their initial four hemodialyses. It is also unclear whether any patient ever actually died from DDS or in fact from other neurologic complications associated with dialysis, such as acute stroke, subdural hematoma, subarachnoid hemorrhage, or hyponatremia (136). A differential diagnosis of patients presenting with these symptoms is shown in Table 94-1. Recently, the diagnosis of DDS has become a "wastebasket" for a number of disorders that can occur in patients with renal failure and that may affect the CNS (136). It is important to recognize that the diagnosis of DDS should be one of exclusion.

TABLE 94-1

DIFFERENTIAL DIAGNOSIS OF DIALYSIS DISEQUILIBRIUM SYNDROME

Subdural hematoma
Uremia, *per se*
Acute cerebrovascular accident
Dialysis dementia
Cardiac dysthymia
Malfunction of fluid-proportioning system
Hypoglycemia
Hypercalcemia
Hyponatremia

Dialysis disequilibrium syndrome has been treated either by addition of osmotically active solute (glucose, glycerol, albumin, urea, fructose, NaCl, or mannitol) to the dialysate, or by intravenous infusion of mannitol or glycerol. With the technique of *pure ultrafiltration* the patient is subjected to ultrafiltration without dialysis. The net result is loss of fluid without the patient undergoing dialysis. Ultrafiltration followed by dialysis does not appear to be associated with DDS (141). In addition, DDS can be prevented by decreasing the time on dialysis and increasing the frequency of dialysis at the initiation of hemodialysis in patients. Mannitol infusion accompanying the initial three hemodialyses has been successful in prevention of DDS (142). Administration of 50 mL of 50% mannitol both at the initiation of dialysis and after 2 hours of dialysis has generally been successful in preventing symptoms of DDS. The same effects can be obtained by addition of glycerol to the dialysate, but technical considerations render this option less popular. Chronic peritoneal dialysis is currently in use worldwide. Different types of peritoneal dialysis are carried out either in-center, at home, or combinations of ambulatory plus home. Patients undergo continuous low-volume peritoneal dialysis for as long as 24 hours per day. Symptoms of DDS have not been presently reported in patients utilizing this mode of dialysis.

CHRONIC DIALYSIS DEPENDENT ENCEPHALOPATHY

Radiologic and Pathologic Examination of Uremic Brain

There have been no large prospective studies undertaken of the radiology of uremic brain in humans. Pathologic studies of brain of patients who died of CRF are old, and there are no

extensive studies of uremic brain that have utilized more modern pathologic methodology (115,143). Prior to 1974, subdural hemorrhages were believed to be very common in dialyzed subjects, and were reported in about 1% to 3% of such autopsies. In addition, intracerebral hemorrhages were said to be present in approximately 6% of dialysis patients who died. Cerebral edema is not found in brain of patients or laboratory animals with CRF, either according to biochemical or histologic criteria. Generalized but variable neuronal degeneration is often present but its anatomic location is variable. Among patients who have died with uremia, there is some evidence of necrosis of the granular layer of the cerebral cortex. Small intracerebral hemorrhages and necrotic foci are seen in approximately 10% of uremic patients, and focal glial proliferation is found in about 2%.

Neuroimaging studies demonstrate bilateral basal ganglia lesions in diabetic patients with ESRD (122). Positron-emission tomographic scanning demonstrates decreased glucose utilization in these lesions. A few cases of optic neuropathy have been reported in uremic individuals (144).

Uremic patients are prone to several risk factors that have a tendency to increase the incidence of stroke. These risk factors include diabetes mellitus, a smoking history, hypertension, chronic infection (often catheter related), chronic inflammation, elevated cholesterol and triglycerides, and advanced atherosclerosis (145). When uremic patients maintained with chronic dialysis sustain a possible stroke, brain neuroimaging using CT or MRI is likely to be carried out for diagnostic purposes. However, as yet, a series of such neuroimaging cases has not been assembled for research purposes. The few available studies suggest that brains of patients with uremia have a very high incidence of cerebral atrophy, which is disproportionately high for the age of the individuals studied (122,123). Earlier studies had also found a high incidence of cerebral atrophy among chronic hemodialysis patients (146). There appears to be subtle brain damage, not detectable by standard neuroimaging techniques or the EEG, but often manifested by deterioration of intellectual capability (43).

It is currently not unusual for patients to survive on hemodialysis for 25 years (147). However, among patients who have been on hemodialysis for more than a decade, there is often mental deterioration, with markedly decreased intellectual capability, even without medical evidence of actual stroke (33–35). The collective syndrome of *chronic dialysis dependant encephalopathy* is a combination of probable organic mental disorders plus psychiatric disorders commonly associated with hemodialysis (148,149). The clinical manifestations include impaired intellectual capability and cognition, decreased exercise capability, sexual dysfunction, and psychiatric disorders ranging from depression to psychosis and suicidal behavior (33–35).The recent use of advanced neuroimaging techniques has led to an increase in our understanding of changes in uremic brain in humans (117). Acute and subacute movement disorders have been observed in patients with ESRD (118). These have been associated with bilateral basal ganglia and internal capsule lesions (118,127). Cerebral atrophy has been observed in chronic hemodialysis patients (123) and it tends to worsen as dialysis therapy continues (117,122). Cerebral atrophy had previously been thought to be associated with dialysis dementia, but this is apparently not the case (124). ESRD has also been reported to lead to deterioration of vision (126). Some cases are associated with uremic pseudotumor cerebri (125).

UREMIC NEUROTOXINS

A large number of compounds are retained by the body in patients with renal failure, either singly or in combination, is substantial (150). Multiple studies have been carried out over the years in order to attempt to identify which of the many compounds that are elevated in uremic subjects functions as a uremic toxin. Bergstrom and Furst have established criteria for the identification of uremic toxins as follows (151): (i) The compound should be chemically identified and quantifiable in biologic fluids; (ii) The concentration of the substance in plasma from uremic subjects should be higher than that found in subjects who do not have renal insufficiency; (iii) The concentration of the substance in plasma should somehow correlate with specific uremic symptoms, and these should be alleviated with reduction of the substance to normal; (iv) The toxic effects of the substance should be demonstrable at concentrations comparable to those found in plasma of uremic patients.

The term uremic neurotoxin implies retention of solutes that have specific detrimental effects upon nervous system function, whether the peripheral nervous system or the CNS (60,61). There are at least three different types of uremic solutes that are potentially toxic and that can be characterized (62–64). These include (i) small water-soluble compounds, such as urea and creatinine (70,152); (ii) middle molecules; (iii) protein-bound compounds. Low-molecular-weight proteins may also be considered a distinct class of uremic toxins (153). Most of the small water-soluble compounds, such as urea and creatinine, are not particularly toxic and are easily removed with dialysis. In addition, since acute renal failure is present in a very substantial portion of patients with sepsis, the clinical manifestations of sepsis may be confused with uremic toxicity (154).

Guanidine Compounds

Guanidine compounds have been postulated to be "uremic toxins" for many years (65), based upon possible detrimental effects upon the CNS. Recent studies have demonstrated that several guanidine compounds are present in uremic brain (68) and may be important in the etiology of uremic encephalopathy (69). There are at least four guanidine compounds that are experimental convulsants. These guanidine compounds appear to work by activation of NMDA receptors by guanidine succinic acid. Activation of the NMDA receptor is a major pathologic mechanism in the etiology of several types of brain damage, including head trauma (155) and stroke (78,79).

In addition, guanidine compounds can have a depressant effect on mitochondrial function (66). In the brain of uremic patients, guanidine compounds were measured in 28 different regions (68). Guanidine succinic acid levels were elevated by up to 100-fold in uremic brain versus control brain, and levels increased with increasing extent of uremia. The brain levels of guanidine succinic acid in uremic brain were similar to those observed in normal animal brain following injection to blood levels that cause convulsions (68). Guanidines inhibit neutrophil superoxide production, can induce seizures, and suppress natural killer cell response to interleukin-2 (70).

Other guanidines, which are arginine analogs, are competitive inhibitors of nitric oxide (NO) synthetase, which impairs removal of AGEs (156) and can lead to vasoconstriction, hypertension, ischemic glomerular injury, immune dysfunction, and neurologic changes (70,152).

Middle molecules are large-molecular-weight compounds (300 to 12,000 Da), which have in the past been implicated in some of the manifestations of uremia (71). Despite the fact that at one time dialysis membranes were designed with the specific intent of removing more middle molecules, evidence of their toxicity is generally lacking (62,71). There has recently been a renewed interest in these molecules, but evidence of their toxicity is still conjectural (70).

With established renal insufficiency, guanidines, which are competitive inhibitors of NO synthetase, will rapidly accumulate in blood, and their presence will impair removal of AGEs (156) and can lead to worsening hypertension, immune dysfunction (157), and neurologic changes such as stroke (70).

Advanced Glycation End Products

Advanced glycosylation (glycation) end products (AGEs) can modify tissues, enzymes, and proteins and may play a role in the pathogenesis of dialysis-associated amyloidosis (63,64). Advanced glycosylation end products may also play a role in the pathogenesis of diabetic nephropathy (75). Advanced glycosylation end products are markedly elevated in plasma of patients with ESRD (76). The AGEs react with vascular cells to inactivate endothelial nitric oxide and may increase the propensity of ESRD patients to develop hypertension. Current dialysis therapy is relatively ineffective in the removal of AGEs, so that there is accumulation of AGEs in patients with ESRD, particularly in those with diabetes mellitus (76). The AGEs are "middle molecules" and have the potential to cause tissue damage and lead to hypertension. Therefore, at least some middle molecules may actually be deleterious in patients with ESRD, and they are poorly removed with conventional dialysis (76). There is evidence that angiotensin-converting enzyme antagonists decrease the formation of AGEs (158). Protein-bound compounds (toxins) are not substantially removed by dialysis, and almost all are lipophilic. Such compounds include polyamines such as spermine (77). Spermine is postulated to be a uremic toxin and appears to react with the NMDA receptor, which affects calcium and sodium permeability in brain cells (77). Stimulation of the NMDA receptor in brain is the final common pathway for brain cell death in a number of pathologic pathways (78–80). The uremic state is associated with increased oxidative stress, resulting in protein oxidation products in plasma and cell membranes. There is eventual alteration of proteins with formation of oxidized amino acids, including glutamine and glutamate (63,64). Such reactions may eventually lead to stimulation of the NMDA receptor in brain, with brain cell damage or death (2).

DIALYSIS DEMENTIA

Dialysis dementia (also called dialysis encephalopathy) is a progressive, frequently fatal neurologic disease, which was initially described in several reports from 1970 to 1973 (159–161). Existence of the syndrome was then independently confirmed worldwide by several different groups in the early to mid-1970s (162–166). In adults, the disease is seen almost exclusively in patients being treated with chronic hemodialysis. The early literature focused on the distinctive neurologic findings (159–163,166,167). However, other reports from both Europe and the United States suggest that some forms of dialysis dementia may be a part of a multisystem disease that may include encephalopathy, osteomalacic bone disease, proximal myopathy, and anemia (116,167–169).

The etiology of this syndrome remains controversial (166,170). Although an increase in brain aluminum content has been strongly implicated in some cases of dialysis dementia, the evidence is far less convincing in others. At this stage of our knowledge it seems useful to subdivide dialysis dementia into three categories (Table 94-2): (i) an epidemic form that is related to contamination of the dialysate, often with aluminum; (ii) sporadic cases in which aluminum intoxication is less likely to be a contributory factor; and (iii) dementia associated with congenital or early childhood renal disease. This latter entity has been reported in several children who were never dialyzed or exposed to aluminum compounds. These early childhood cases may represent developmental neurologic defects resulting from exposure of the growing brain to a uremic environment (171).

TABLE 94-2

SUBGROUPS OF DIALYSIS DEMENTIA

1. Sporadic endemic
   A. No clear relation to aluminum intake
   B. Worldwide distribution
   C. No known therapy
2. Epidemic
   A. Geographic clusters
   B. Often related to aluminum (Al^{+3}) in dialysis water
   C. Epidemic usually stops with treatment of water supply
   D. Probably often related to other trace elements in water, such as tin, manganese, cobalt, magnesium and iron
3. Childhood
   A. May be secondary to effects of uremia on the immature brain
   B. No clear association with aluminum administration

The initial reports of dialysis dementia in the 1970s were soon followed by reports throughout the world (21). These patients all had the endemic form and typically had been on chronic hemodialysis for more than 2 years before the onset of symptoms. Early manifestations consisted of a mixed dysarthria-apraxia of speech with slurring, stuttering, and hesitancy. Personality changes, including psychoses, led to dementia, myoclonus, and seizures. Symptoms initially were intermittent and were often worse during dialysis, but generally became constant. In most cases, the disease progressed to death within 6 months. Speech disturbances were found in 90% of patients, affective disorders culminating in dementia in 80%, motor disturbances in 75%, and convulsions in 60% to 90%. In contrast to this fairly distinct clinical picture, brain histology has generally been normal or nonspecific.

Early in the disease, the EEG shows multifocal bursts of high amplitude delta activity with spikes and sharp waves, intermixed with runs of more normal appearing background activity. These electroencephalographic abnormalities may precede overt clinical symptoms by 6 months. As the disease progresses, the normal background activity also deteriorates to slow frequencies (139). The EEG has been said to be pathognomic, but a similar pattern may also be seen in other metabolic encephalopathies. The diagnosis depends on the presence of the typical clinical picture and is confirmed by the characteristic electroencephalographic pattern (163). Magnetoencephalography (MEG) has only recently been used in the evaluation of uremic patients (172) and has not yet been used in the evaluation of patients with dialysis dementia.

Aluminum intoxication was first implicated in this disorder by Alfrey et al. (165). Aluminum content of brain gray matter was elevated to 11 times the normal value in patients with dialysis dementia, versus an increase of three times normal in patients receiving chronic hemodialysis without dialysis dementia. Aluminum content was also increased in bone and other soft tissue. Oral phosphate binders containing aluminum [$Al(OH)_3$ and $Al_2(CO_3)_2$] were originally suspected as the source of the aluminum.

Most of the aluminum in blood is bound to transferrin, so that there is very little free aluminum in the blood (173). The brain contains few transferrin receptors, so that normally aluminum uptake into brain is negligible. Any free aluminum in blood, usually in the form of aluminum-citrate, can readily

enter the CNS. Normally, there is an excess of gallium-binding sites in plasma, so that even in situations where blood aluminum is increased, there is still almost no free aluminum in blood. Aluminum binding can be studied with the aluminum analog gallium (174). In studies of gallium-transferrin binding in blood of patients having either Alzheimer's disease, Down syndrome or renal failure treated with chronic hemodialysis, gallium binding to transferrin was significantly reduced in patients with either Down syndrome or Alzheimer's disease (173). However, gallium binding to transferrin was normal in patients with CRF treated with hemodialysis. In such patients, there was accumulation of aluminum in those brain regions with high densities of transferrin receptors (173).

The aforementioned findings involve studies in only nine patients with dialysis dementia (173,175) and five with CRF treated with hemodialysis. More such studies are needed before it can be conclusively stated that the distribution of aluminum in brain of patients with dialysis dementia is not similar to that in patients with Alzheimer's disease. In patients with CRF without dialysis dementia, neurofibrillary changes have not been found.

Recent studies have further added to our knowledge of the possible effects of aluminum on the CNS in patients with CRF. A possible pathophysiologic basis for detrimental effects of aluminum on the CNS has been described by Altmann et al. (176). Dihydropteridine reductase is an important enzyme in the synthesis of several important neurotransmitters, such as tyrosine and acetylcholine. They found that erythrocyte levels of dihydropteridine reductase activity were less than predicted values and correlated with plasma aluminum levels (176). After treatment with desferrioxamine, red blood cell dihydropteridine reductase activity levels doubled. Although brain levels of dihydropteridine reductase activity were not evaluated, it was suggested that high brain aluminum levels might lead to decreased availability of dihydropteridine reductase in the brain. It has been suggested that merely the presence of an increased body aluminum burden has an adverse effect on overall mortality (177). More specifically, an increased body aluminum burden (estimated by the desferrioxamine infusion test) has been associated with memory impairment and increased severity of myoclonus with decreased motor strength (178).

Altmann et al. evaluated patients with CRF and apparently normal cerebral function (179). They found that when compared to a control group with similar IQ, the patients with CRF had abnormalities in six tests of psychomotor function. Plasma aluminum levels were only mildly elevated (59 ± 9 μg/mL). When 15 of these patients were treated for 3 months with desferrioxamine, anemia improved and the erythrocyte activity of dihydropteridine reductase rose significantly. Changes in erythrocyte dihydropteridine reductase activity correlated significantly with changes in psychomotor performance (176,179). Even at high blood aluminum levels, most aluminum is bound to transferrin (173) and thus cannot bind to the cerebral transferrin receptors. It may be that patients who develop dialysis dementia have less transferrin binding capacity, less transferrin, or a greater density of transferrin receptors in the brain.

Therefore, aluminum may be potentially toxic in patients with CRF, possibly leading to both dialysis dementia and osteomalacia (162,169,180). Most nephrologists would agree that the potential hazards of poor control of plasma phosphate are worse than the potential toxicity of aluminum accumulation from oral aluminum containing antacids. However, progress in the control of hyperphosphatemia in hemodialysis patients by other means has almost eliminated the use of aluminum containing compounds (in 2006). Calcium carbonate (or acetate) may be more effective for the control of hyperphosphatemia than is aluminum hydroxide (181). More recently, sevelamer (Renagel, Genzyme Corp, Cambridge, MA), a polymeric phosphate binder, has come into wide use for control of phosphate in chronic dialysis patients (182). Renagel has been found to be more effective than either calcium carbonate, calcium acetate, or aluminum hydroxide for the treatment of hyperphosphatemia in dialysis patients (182), and it does not introduce aluminum into the body. Lanthanum carbonate (Fosrenol) has only recently been introduced in the United States for the control of hyperphosphatemia in patients with ESRD (183,184). Its place in the management of dialysis patients with hyperphosphatemia remains to be determined.

However, aluminum is still the second most prevalent element in the earth's crust, and a substantial quantity will enter the body, even without administration of aluminum-containing antacids (185–187).

Deionization of the water used to prepare dialysate has been employed as a preventive measure (188). However, deionization may be beneficial by removing any number of other agents. Other trace elements may be present in water that can result in CNS toxicity. Such elements include cadmium, mercury, lead, manganese, copper, nickel, thallium, boron, and tin (Fig. 94-5). Among these potentially neurotoxic elements brain content of cadmium, mercury, nickel, thallium, vanadium, or boron has not been measured. Manganese has been found to be increased in cortical white matter in the eight encephalopathic patients in whom it was measured (189). These patients also had elevated aluminum levels in gray matter.

Most of the controversy over the etiology of dialysis dementia has involved those cases that occur sporadically. As noted previously, dialysate aluminum levels are not always elevated. The use of aluminum-containing antacids is no different in sporadic patients with dialysis dementia than in unaffected patients, and brain aluminum levels in patients with dialysis dementia may overlap with those of unaffected patients (170). The largest group of "sporadic cases" has been reported from Nashville, Tennessee (180). The reported incidence of dialysis dementia in the area is 5%, despite the use of deionized water for dialysate with aluminum levels below 5 μg/L. Osteomalacic bone disease was not clinically apparent in this group. Serum aluminum levels in the encephalopathic group were three to four times higher than other dialyzed patients, despite equivalent prescribed doses of aluminum-containing phosphate binders. These results suggest greater absorption and/or retention of aluminum or other trace metal contamination in this group of encephalopathic patients. No other metals were measured in the Nashville study.

The evidence available thus far indicates that aluminum is elevated in the brain (cortical gray matter) of patients with dialysis dementia. However, the actual contribution of aluminum to the encephalopathy remains unclear. Aluminum content has been reported to be elevated in the brain of patients with other disorders, including senile dementia and Alzheimer's syndrome, and might actually be a nonspecific finding associated with dementia. Aluminum is also elevated in the brains of patients who have other disorders associated with altered blood–brain barrier. Such disorders include renal failure, hepatic encephalopathy, and metastatic cancer (Fig. 94-5). Other evidence suggests that brain aluminum content may also increase as a function of the aging process. Therefore, blood–brain barrier abnormalities can result in increased brain aluminum content (190).

Despite these unresolved questions, most outbreaks of the epidemic form of dialysis dementia have been associated with high levels of aluminum in the dialysate (162,191,192). Lowering the dialysate aluminum to below 20 μg/L, usually by deionization, appears to prevent the onset of the disease in patients who are beginning dialysis. New cases may continue to appear in those patients who were previously exposed to the high aluminum dialysate, although the course is milder and mortality is somewhat decreased. In patients with overt disease, eliminating the source of aluminum has resulted in improvement in some but not all patients. Renal transplantation has

TABLE 94-3

RELATION BETWEEN NERVE FUNCTION AND VARIOUS "UREMIC NEUROTOXINS"

Reference	Putative "uremic neurotoxin"	Correlation coefficient with MNCV	Other
(1) Giulio SD, et al. Parathormone as a nerve poison in uremia. *N Engl J Med* 1978;299:1134.	Parathyroid hormone	0.09	No effect of PTH on motor nerve function
(2) Avram MM, et al. Search for the uremic toxin. *N Engl J Med* 1978;298:1000.	Parathyroid hormone	0.45	
(3) Nielsen VK. The peripheral nerve function in chronic renal failure. VI. *Acta Med Scand* 1973;194:455.	Urea Creatinine Glomerular filtration rate	0.41 0.51 0.68–0.84	
(4) Blagg CR, et al. Nerve conduction in relationship to the severity of renal disease. *Nephron* 1968;5:290.	Urea Creatinine	0.51 0.57	
(5) Blumberg A, et al. Myoinositol-uremic neurotoxin? *Nephron* 1978;21:186.	Myoinositol	0.03	
(6) Reznek RH, et al. Plasma myoinositol concentrations in uraemic neuropathy. *Lancet* 1977;1:675.	Myoinositol	0.67	
(7) Clements RS, et al. Raised plasma-myoinositol levels in uraemia and experimental neuropathy. *Lancet* 1973;1:1137.	Myoinositol	Not available	Detrimental effect of myoinositol on nerve function
(8) Man NK, et al. An approach to "middle molecules" identification in artificial kidney dialysate, with reference to neuropathy prevention. *TASAIO* 1973;19:320.	Middle molecules	Not available	No *in vivo* evidence of MNCV impairment with renal failure
(9) Scribner BH, Babb AL. Evidence for toxins of "middle" molecular weight. *Kidney Int* 1975;7(Suppl 3):S349.	Middle molecules	Not available	As above
(10) Kjellstrand CM, et al. Considerations of the middle molecule hypothesis II. *TASAIO* 1973;19:325.	Middle molecules	Not available	As above
(11) Giovannetti S, et al. Methylguanidine in uremia. *Arch Intern Med* 1973;131:709.	Methylguanidine	Not available	Chronic injection depressed MNCV in patients
(12) Lonergan ET, et al. Erythrocyte transketolase activity in dialyzed patients. *N Engl J Med* 1971;284:1399.	Transketolase deficiency	Not available	Deficiency related to impaired MNCV in patients
(13) Mahoney CA, et al. Central and peripheral nervous system effects of chronic renal failure. *Kidney Int* 1983;24:170.	Parathyroid hormone	0.05	No effect of PTH on motor nerve function
(14) Kanda F, et al. Somatosensory evoked potentials in acute renal failure. *Kidney Int* 1990;38:1085.	Parathyroid hormone	Not available	No effect of PTH on sensory nerve function

MNCV, motor nerve conduction velocity.

generally not been helpful in patients with established dialysis dementia. Diazepam or clonazepam is useful in controlling seizure activity associated with the disease, but becomes ineffective later on and does not alter the final outcome (193). Treatment of sporadic cases in which the etiology is not clear is more difficult. Every effort should be made to identify a treatable cause. Dialysis dementia must be differentiated from other metabolic encephalopathies, such as hypercalcemia and hypophosphatemia, hyperparathyroidism, acute heavy metal intoxications, and structural neurologic lesions, such as subdural hematoma (170) (Table 94-3). Because of the low incidence, the uncertain etiology, and the poor correlation of plasma with tissue aluminum levels, screening tests have not generally been employed.

The source of excess Al^{+3} in the brain is not entirely clear. Some Al^{+3} apparently is absorbed after oral administration of aluminum-containing antacids (170). Significant absorption of oral aluminum can occur in patients with CRF, but the weight of evidence is against oral aluminum as the major source. The retention of Al^{+3} after oral administration of Al^{+3} salts is greater in patients with renal failure than in normal subjects (194,195). The typical daily dietary Al^{+3} intake is 10 to 100 mg (196), although absorption is normally minimal. This quantity of dietary Al^{+3} is more than enough to account for the entire increase of brain Al^{+3} observed in patients with dialysis dementia. Among 22 such patients, the mean brain Al^{+3} content (116) was 22 mg/kg dry weight. The normal human brain weighs about 1,500 g and is about 80% water, or 300 g dry weight. Therefore, the total increase in Al^{+3} content for the whole brain is less than 7 mg in patients with dialysis dementia. and the entire increase of brain Al^{+3} in such patients can theoretically be accounted for by dietary aluminum. The increase in body aluminum stores may also be, in part, the result of Al^{+3} contamination from other sources, such as Al^{+3} in dialysate water, dialysis system aluminum pipes, or aluminum leaked from anodes (196).

There are a large number of children who have renal insufficiency and also require hospitalization with intravenous

therapy. Such children may receive large quantities of intravenous aluminum (Al^{+3}) from contamination of intravenous solutions with aluminum salts (197–202). Therefore, even in the absence of hemodialysis therapy, children with CRF may receive large quantities of intravenous aluminum, which may explain the development of dialysis dementia even in the absence of dialysis (202). The location of the aluminum in brain of patients with dialysis dementia has not been well established. In Alzheimer's disease, it initially appeared that the aluminum was localized only to the nuclear regions of neurofibrillary tangles (203). More recent investigations reveal that in Alzheimer's disease, aluminum accumulates in at least four different sites: DNA-containing structures of the nucleus; protein moieties of neurofibrillary tangles; amyloid cores of senile plaques; cerebral ferritin (204–206).

Senile plaques and neurofibrillary tangles are of course diagnostic features of Alzheimer's disease (207). It is not generally appreciated that in dialysis dementia, the brain also contains senile plaques and neurofibrillary tangles in the majority of cases (175). However, in dialysis dementia, aluminum was not located in the neurons but rather in glial cells and the walls of blood vessels (188). The aforementioned findings involve studies in small numbers of patients (CRF treated with hemodialysis, 5; dialysis dementia, 9) (173,175). More such studies are needed before it can be conclusively stated that the distribution of aluminum in brain of patients with dialysis dementia is not similar to that in patients with Alzheimer's disease.

The source of the increased Al^{+3} in brain of patients with dialysis encephalopathy can theoretically be accounted for on the basis of increased Al^{+3} intake. However, it is unclear as to how the Al^{+3} enters the brain in increased quantities. It may be that the increased body aluminum burdens present in uremic subjects may contribute to increased Al^{+3} content in the brain of such individuals. To clarify the role of oral ingestion of aluminum salts in the causation of increased brain Al^{+3} content, it would be instructive to examine brain tissue from patients without renal failure who had ingested large quantities of Al(OH)3, that is, patients with chronic peptic ulcer disease. However, since such material is not likely to be available, but studies in laboratory animals given large quantities of aluminum salts should provide similar information. In both rats and dogs receiving oral aluminum salts, there is a significant increment in brain Al^{+3} content (208–210). Administration of PTH to rats receiving aluminum salts results in an additional increment of brain Al^{+3} content (195,208). Therefore, in laboratory animals, both a chronic increase in oral Al^{+3} ingestion, or PTH excess, can lead to an increase of cerebral cortex Al^{+3}, even in the absence of renal failure.

ALTERNATIVE ETIOLOGIES

Many other possible causes of dialysis dementia have been proposed. These include other trace element contaminants, normal pressure hydrocephalus, slow virus infection of the CNS, and regional alterations in cerebral blood flow (72,170,211). Some patients with dialysis dementia may have altered CSF dynamics, which are at least suggestive of normal pressure hydrocephalus (212). Six patients with dialysis dementia were found to have normal CSF dynamics, but only mild dilation of the cerebral ventricles (212). In this study, however, control subjects (uremic patients treated with hemodialysis but who did not have dialysis dementia) were not evaluated, and results of recent studies suggest that many patients who have ESRD without dialysis encephalopathy may also have ventricular dilation with cerebral atrophy (121). Furthermore, there is a generally poor correlation between ventricular dilation, cerebral atrophy, and the presence of dementia (213).

Slow virus infection of the nervous system is a possible etiology for dialysis dementia. The clinical manifestations resemble those of other slow virus infections, such as Kuru or Creutzfeldt-Jakob disease (214,215). In at least once instance, a slow virus (foamy virus) has been isolated from the brain of patients who died of dialysis encephalopathy (214). In summary, dialysis dementia probably represents end point in a disease of multiple etiology. There are at least three subgroups and in two of them the etiology of dialysis encephalopathy must be regarded as unknown. The possible role of aluminum, or other trace element abnormalities, is unclear. At this time, there is no known satisfactory treatment for patients with dialysis encephalopathy. Most patients reported in the literature thus far have not survived, usually dying within 18 months of the time of diagnosis. The syndrome is not alleviated by increased frequency of dialysis, and usually not by renal transplantation (116,170). Definitive therapy must await a better understanding of the pathogenesis of this disorder. There have been preliminary reports of improvement in patients with dialysis dementia treated with deferoxamine (212). Deferoxamine can be used to remove aluminum from the body, but this approach has not been shown to improve the clinical status of patients with dialysis dementia.

OTHER CENTRAL NERVOUS SYSTEM COMPLICATIONS OF DIALYSIS

In addition to dialysis dementia and DDS, there are several other neurologic disorders that have been reported in patients being treated with dialysis. In most instances, patients have presented initially with headache, nausea, emesis, or hypotension, whereas some have had seizures. Most such patients have initially been diagnosed as having DDS, whereas others, particularly those with chronic subdural hematoma, have been suspected of having dialysis dementia. The disorders include copper intoxications, subdural hematoma, muscle cramps, nonketotic hyperosmolar coma with hyperglycemia, cerebral embolus secondary equipment malfunction, acute cerebrovascular accident, depletion syndrome, malfunction of fluid proportioning system, excessive ultrafiltration with hypotension and seizures, hypoglycemia, and Wernicke's encephalopathy (21,36). Subdural hematoma is not an infrequent cause of death in patients maintained with chronic hemodialysis (216–218). This condition may initially present with headache, drowsiness, nausea and vomiting. If the patient loses consciousness or develops signs of increased intracranial pressure, a diagnosis of subarachnoid bleeding should be considered. Such episodes in uremic patients are usually fatal unless treated surgically. If the previously noted symptoms persist between hemodialysis, or progressively worsen, subdural hematoma is likely, particularly if the patient is taking anticoagulants. On physical examination, there is often evidence of localized neurologic disease; there may be signs of meningeal irritation, and somnolence and focal seizures may be observed. The diagnosis can usually be made by modern neuroimaging techniques (computed tomography [CT] scan or MRI) (117,219), Subarachnoid bleeding in hemodialysis patients is often related to anticoagulant excess (220). The initial symptom when intracranial hemorrhage occurs is usually depression of sensorium: Convulsions may follow, and the patients may lapse into coma.

Improper proportioning of dialysate, due to either human or mechanical error, is still an important cause of neurologic abnormality in dialysis patients (221). The usual effect of such dialysate abnormalities is either hypo- or hypernatremia. Both of these abnormalities of body fluid osmolality can lead to seizures and coma, although different mechanisms

are involved. In acute hypernatremia, there will be excessive thirst, lethargy, irritability, seizures, and coma, with spasticity and muscle rigidity. In acute hyponatremia there is weakness, fatigue, and dulled sensorium, which may also progress to seizures and coma, respiratory arrest, and death. Such symptoms developing soon after initiation of hemodialysis should alert the physician to the possibility of such an error with the dialysate. A check of the dialysate osmolality or sodium concentration is the most rapid means of detecting this problem. Death has been reported as a consequence of either hyper- or hyponatremia (222,223).

About one liter of fluid per hour can be removed by ultrafiltration hemodialysis and about 300 mL per hour by peritoneal dialysis using hypertonic dialysate. Such a rate of fluid removal from the intravascular space may be faster than the rate at which fluid can be replaced from the interstitial compartment, and hypotension may develop. Symptoms of hypotension may include seizures which, although actually due to cerebrovascular insufficiency, may be mistaken for DDS, particularly in diabetic subjects.

Most of the neurologic complications of renal transplantation relate to secondary afflictions, such as infection and neoplasia (224). As already discussed, most of the neurologic complications of the uremic state tend to improve following renal transplantation. These include the neuropathy, encephalopathy, and electroencephalographic changes (224–226).

STROKE IN PATIENTS TREATED WITH CHRONIC HEMODIALYSIS

Cerebrovascular disease is a common cause of death in chronic hemodialysis patients, and stroke represents the second most frequent cause of death (227–229). The three most frequent causes of death are heart attack, stroke, and infection (230). In the United States and western Europe (including Israel), cardiovascular disease is far more common in dialysis patients than in the rest of the population (3,231). Part of the reason may be that the major cause of ESRD in the United States is diabetes mellitus, which is more common than in Europe and Japan (3,231). Among the factors that undoubtedly contribute to the high incidence of stroke in patients with ESRD treated with hemodialysis are the high incidence of hypertension, the large number of such patients who have diabetes mellitus, and the accelerated arteriosclerosis in such patients. Uremic patients tend to have high cholesterol levels and a high incidence of obesity, and they tend to smoke cigarettes excessively (232). There is a high incidence of chronic infection in dialysis patients, which leads to elevated blood levels of atherogenic risk factors, such as cytokines, which appear to contribute to the increased incidence of stroke in such patients (233,234). The elevated cytokines are largely a result of the high incidence of chronic inflammatory conditions in chronic hemodialysis patients (233,234).

There is substantial evidence that chronic inflammation plays a role in the pathogenesis of cardiovascular disease (92,235). Cytokines released from involved tissues stimulate the liver to synthesize acute phase proteins, including C-reactive protein (CRP). Elevated levels of CRP and other cytokines constitute an independent risk factor for cardiovascular disease (236). AGEs can modify tissues and enzymes (63,64,237). As noted previously, AGEs are markedly elevated in the plasma of patients with ESRD, particularly if they also have diabetes mellitus (75,76,238). These AGEs react with vascular cells to inactivate endothelial nitric oxide and may increase the propensity of patients with ESRD to develop arteriosclerosis and hypertension (76). Patients with uremia have an accumulation of proinflammatory compounds, including AGEs, and these probably impair defense mechanisms against oxidative injury (239). There is evidence for increased cytokine production secondary to blood interaction with bioincompatible dialysis components. In particular, blood-dialyzer interaction can activate mononuclear cells, leading to production of inflammatory cytokines (149). Synthetic high-flux dialyzer membranes are permeable to the proinflammatory cytokines, and are capable of removing interleukin-1B (IL-1B), tumor necrosis factor alpha, and IL-6, thus offering a potential therapeutic approach (240). It is unclear, however, whether cytokine removal by continuous renal replacement therapy will decrease the incidence of stroke (241). The use of sorbents with continuous plasma filtration offers another possibility for a novel therapeutic approach (242,243). Some of the cytokines, such as IL-1B, tumor necrosis factor alpha, and IL-6, may induce an inflammatory state, and are believed to play an important role in dialysis-related mortality (234,244). Recent prospective studies have demonstrated that patients with ESRD and higher blood levels of certain cytokines have a greater mortality and have a larger number of cardiovascular events (239). Contaminated dialysate water can result in pyogenic substances of bacterial origin being absorbed into the dialysis membrane (240). The consequence could be induction of an inflammatory response in certain dialysis patients. Substances of bacterial origin activate circulating mononuclear cells to produce proinflammatory cytokines. IL-1B, tumor necrosis factor alpha, and IL-6 mediate the acute phase response resulting in elevated levels of acute phase proteins, including CRP (240). The effects of dialysis reuse on cytokine production has not been evaluated, but may be important, as reuse could theoretically lead to more contamination of dialysate (233,240). Reactive carbonyl compounds and AGEs, which tend to modify proteins in a deleterious manner (64), can be decreased by use of a peritoneal dialysate containing icodextrin and amino acids instead of glucose (245).

The cardiovascular disease and cerebrovascular disease can lead to cerebral ischemia. Cerebral ischemia initiates a number of processes that can lead to progressive brain damage (Fig. 94-6) (246). Cerebral ischemia can lead to activation in the brain of free radicals, NMDA, and apoptosis, all potential mechanisms of brain damage in patients with hypoperfusion or stroke (247–249) (Fig. 94-6). Anoxic injury to brain endothelial cells can increase production of nitric oxide, which can lead to free radical formation (250,251). Apoptosis, or programmed cell death, is another mode of destruction of brain cells in stroke (249). Glutamate activates the NMDA receptor complex and can also lead to later activation of apoptosis (250,251). In general, the ischemic event of a stroke only serves to initiate the biochemical events that may lead to brain damage. Interventions that counter these biochemical events may decrease the brain damage associated with acute stroke (252,253).

Recent knowledge of the pathogenesis of stroke has led to a major expansion in the opportunities for prevention of stroke. High grade carotid stenosis can lead to stroke, although the exact numbers of patients who will suffer stroke when they have carotid stenosis is not known (254). Screening patients who have renal failure for the presence of carotid stenosis could diagnose a substantial number of such patients, although at considerable cost. However, because of noninvasive diagnostic techniques such as duplex Doppler ultrasonography, screening for carotid stenosis involves essentially no morbidity (253).

Studies of the aortic arch for the presence of large atherosclerotic plaques (more than 4 mm thick) is an important predictor for the possibility of stroke in the future (255), as is the presence of atrial fibrillation (256). Other common preventive measures include treatment of hypertension, cessation of smoking, lowering of plasma cholesterol, control of plasma glucose in diabetic patients, weight loss, increased exercise and

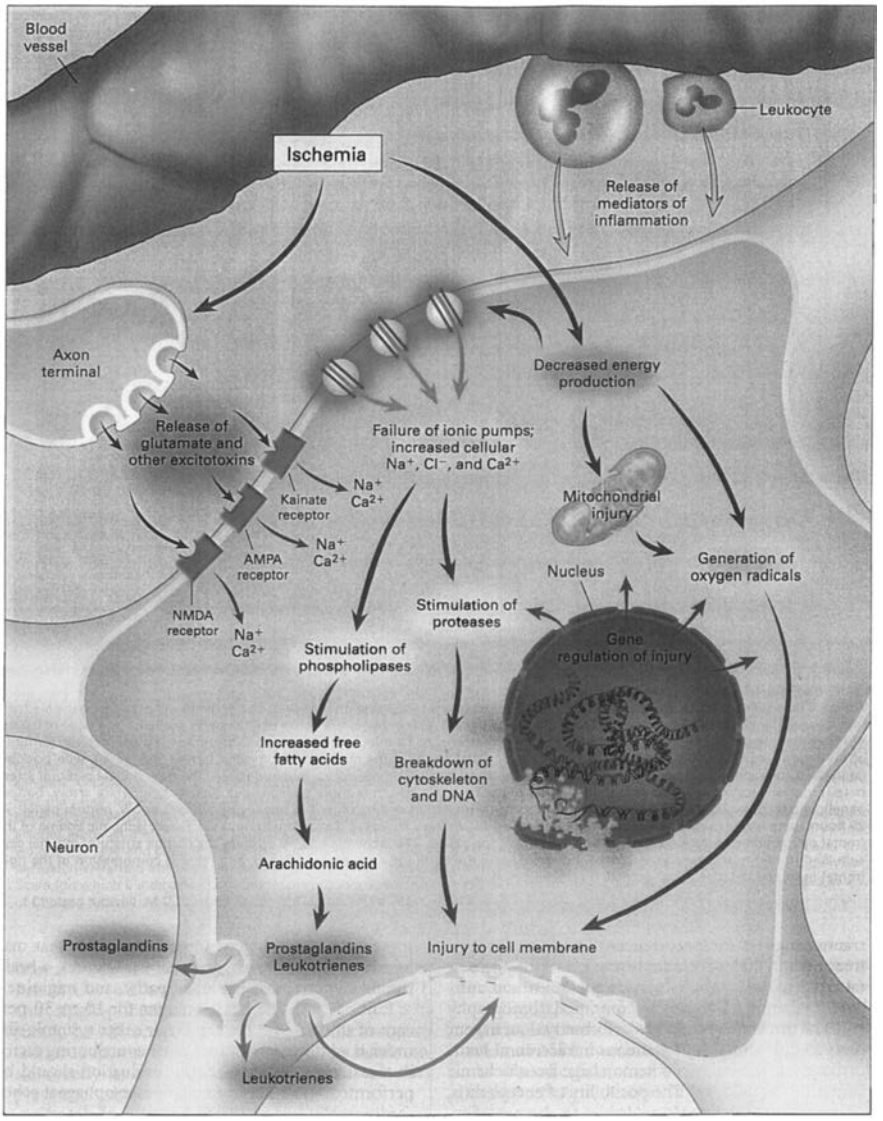

FIGURE 94-6. The molecular events initiated in brain tissue by acute cerebral ischemia. Interruption of cerebral blood flow results in decreased energy production, which in turn causes failure of ionic pumps, mitochondrial injury, activation of leukocytes (with release of mediators of inflammation), generation of oxygen radicals, and release of excitotoxins. Increased cellular levels of sodium, chloride, and calcium ions result in stimulation of phospholipases and proteases, followed by generation and release of prostaglandins and leukotrienes, breakdown of DNA and the cytoskeleton, and, ultimately, breakdown of the cell membrane. Alteration of genetic components regulates elements of the cascade to alter the degree of injury. *AMPA*, alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; *NMDA*, N-methyl-D-aspartate. (Reprinted from: Brott T, Bogousslavsky J. Treatment of acute ischemic stroke. *N Engl J Med* 2000;343:710, with permission.)

decreased alcohol consumption (232). Other possible preventive measures include dietary antioxidants, low-dose aspirin and a decreased intake of saturated fatty acids (257). Although treatment of hypertension is known to decrease the incidence of stroke, not all antihypertensive agents are of equal efficacy. In general, only β-blockers, thiazide diuretics, and angiotensin-converting enzyme inhibitors have been shown to reduce the incidence of stroke, whereas, α-adrenergic blocking agents and calcium channel blockers may not (258). Given that the aforementioned are likely mechanisms of brain damage in stroke, a whole new field has opened in the area of potential therapeutic agents for decreasing brain damage associated with stroke. Such agents include calcium channel blockers (259,60), inhibitors of NMDA receptors (261), and agents that scavenge free radicals (262). It is now clear that in many cases acute stroke can be successfully treated, but only if physicians realize that stroke should now be considered a medical emergency where timely therapy can make the difference in functional survival of the brain (263). Therapies for acute stroke are now being administered in teaching hospitals in the United States and most start with acute neuroimaging in the emergency room. An initial CT scan will usually reveal acute stroke, and, if present, the scan with differentiate occlusive from hemorrhagic stroke. If a nonhemorrhagic stroke is present, treatment prospects can be examined with magnetic resonance angiography (MRA), which is noninvasive. Contrast should not be administered to patients with impaired renal function, but can be given to dialysis patients (264). When acute stroke is diagnosed within the appropriate time window (within 3 hours of the onset of symptoms), current therapies may include intravenous thrombolytic therapy (265), intraarterial thrombolytic therapy, antithrombotic and antiplatelet drugs, defibrinogenating agents and neuroprotective drugs (241,266–268). Administration of the defibrinogenating agent ancrod to patients with acute ischemic stroke resulted in a better functional status after 3 months follow-up (268). Nizofenone can scavenge free radicals and inhibit glutamate release, and may prove useful as a cerebroprotective agent (269).

Some cases of acute stroke will be caused by dissection of the carotid or vertebral artery systems. These patients have lesions that are not amenable to dissolution of clot, as the obstructing lesions is in fact a hemorrhage in the arterial wall (270–272). Dissection of the carotid or vertebral artery system can be initiated by chiropractic manipulation of the cervical spine, so that such maneuvers should probably be avoided in dialysis patients (270). In addition, some cases of apparent acute stroke in

dialysis patients will be due to subdural hematoma, which must always be considered in the differential diagnosis of stroke in dialysis patients (273).

SEXUAL DYSFUNCTION IN UREMIA

Disturbances in sexual function are a common complication of CRF (31,276). These complications occur in both genders and may include erectile dysfunction, decreased libido, and decreased frequency of intercourse (31,277). Studies in uremic rats showed that erectile impairment is associated with a disturbance in nitric oxide synthetase gene expression (274). Sexual dysfunction in men with ESRD treated with maintenance hemodialysis is common, and impotence has been observed in at least 50% of such patients (275). A number of abnormalities associated with renal failure appear to be important in the genesis of impotence. There are abnormalities in autonomic nervous system function (277), impairment in arterial and venous systems of the penis (along with vascular pathology in other vascular beds), hypertension (many drugs used to treat hypertension cause secondary impotence), and other associated endocrine abnormalities (278). There are also the associated effects of aging, with impotence observed in more than 50% of men older than 60 years old who do not have renal failure (279). There is a variety of approaches to the evaluation of impotency in uremic men (280). Patients with ESRD have a high incidence of cardiovascular disease (1), which impairs penile vessels along with those of the rest of the body (281). The incidence of hypertension is also higher in ESRD patients than the rest of the population, and hypertension is a major contributor to vascular disease (1,282). Many drugs used to treat hypertension can lead to impotence (calcium channel blockers, thiazides, guanethidine). The incidence of depression is high in patients with ESRD, and many drugs used to treat depression can lead to impotence (phenothiazines, tricyclics, fluoxetine). Although appreciation of the aforementioned abnormalities may increase our understanding, until very recently, there was little that could be done other than to discontinue certain drugs used to treat hypertension or depression (31). There are now a number of drugs that can successfully treat impotence (283). Alprostadil was successful, but had to be delivered transurethrally (284). In particular, sildenafil can be administered orally and is highly effective, even in men who have cardiovascular disease (285) or uremia (286,276). Other treatments for impotence among men with ESRD include penile prostheses, direct injection of alpha blocking agents or other vasodilators (papaverine, phentolamine, alprostadil) into the penis, and vacuum constrictive devices (283,287).

UREMIC NEUROPATHY

Clinical Manifestations

Peripheral neuropathy in patients with renal failure has been recognized for more than 100 years. This disorder, however, was not fully appreciated until the early 1960s (288,289). Prior to the institution of chronic dialysis therapy, approximately 65% of patients with ESRD probably did not live long enough to develop clinically apparent neuropathy. Although existing data are difficult to evaluate, neuropathy is probably present in about 65% of patients with ESRD at the time of the institution of dialysis (290). Uremic neuropathy may involve all modalities of nerve function—motor, sensory, and autonomic, including both sympathetic and parasympathetic pathways (288,291).

Many patients with CRF who are neurologically asymptomatic may exhibit abnormalities on physical examination. They may also have evidence of autonomic neuropathy such as impotence and postural hypotension. Moreover, in patients who have renal insufficiency, abnormal nerve conduction may be present in the absence of symptoms or abnormal findings on physical examination. In addition, alternations in nerve conduction do not necessarily indicate structural changes in the peripheral nerves.

The motor nerve conduction velocity (MNCV) is a test that is frequently used to assess peripheral neuropathy. The test, however, is somewhat unreliable, since there is a large normal variation in MNCV (up to 20% on a day-to-day basis) (84) and the test has very limited utility in detecting moderate impairment of peripheral nerve function. Sensory nerve conduction velocity (SNCV) is more sensitive than MNCV, but the test is quite painful and most patients do not permit repeated tests.

In general, there are two broad categories of peripheral neuropathy. These are described in terms of the pattern of involvement of the peripheral nervous system. First, there are processes that result in a bilaterally symmetric disturbance of function that can be designated as polyneuropathies. Polyneuropathy tends to be associated with agents such as toxic substances, metabolic disorders (uremia, diabetes, deficiency states), and certain examples of immune reaction that act diffusely on the peripheral nervous system. The second category comprises isolated lesions of peripheral nerves (mononeuropathy) or multiple isolated lesions (multiple mononeuropathy). In severe symmetric polyneuropathies, a generalized loss of peripheral nerve function may occur, and the impairment is usually maximal distally in the limbs. A mixed motor and sensory polyneuropathy with a distal distribution results in weakness and wasting that is most frequently observed peripherally in the arms and legs. There are also distal sensory changes of "glove and stocking" distribution. In those neuropathies that involve "dying back" of the axons from the periphery (289), it is possible that the neurons that have the longest axons to maintain appear to be the first to be affected.

Uremic neuropathy is a distal, symmetric, mixed polyneuropathy. Motor and sensory modalities are both generally affected, and the lower extremities are more severely involved than are the upper extremities. Clinically, uremic polyneuropathy cannot be distinguished from the neuropathies associated with certain other metabolic disorders such as diabetes mellitus, chronic alcoholism, and various deficiency states. The occurrence of neuropathy bears no relationship to the type of underlying disease process (i.e., glomerulonephritis or pyelonephritis). However, certain diseases that can lead to renal failure may simultaneously affect peripheral nerve function in a manner separate from the manifestations of uremia. Such diseases include amyloidosis, multiple myeloma, systemic lupus erythematosus, polyarteritis, nodosa, diabetes mellitus, and hepatic failure (288,289). The clinical manifestations of uremic neuropathy are characterized by several different stages. It appears that when glomerular filtration rate exceeds 12 mL per minute, clinical evidence of neuropathy is generally absent (7).

Peripheral Nerves

The restless leg syndrome is a common early manifestation of CRF. Clinically, patients experience sensations in lower extremities such as crawling, prickling, and pruritus. The sensations are worse distally than proximally and are generally more prominent in the evening. The restless leg syndrome may initially be present in up to 40% of patients with CRF (92,292). Another symptom experienced by patients with early uremic neuropathy is the burning foot syndrome, which is present in less than 10% of patients with CRF (92). Rather than

"burning," the actual symptoms consist of swelling sensations, constriction, and tenderness of the distal lower extremities.

The physical signs of peripheral nerve dysfunction often begin with loss of deep tendon reflexes, particularly knee and ankle jerks (293). Impaired vibratory sensation is also an early sign of uremic neuropathy. Loss of sensation in the lower leg is common and often takes the form of "stocking glove" anesthesia of the lower leg. The sensory loss includes pain, light touch, vibration, and pressure.

Metabolic Neuropathy

Uremic neuropathy is one of a group of central-peripheral axonopathies, also known as dying-back polyneuropathies, which have been described by Spencer and Schaumberg (295).

The causes of such central-peripheral axonopathies include many types of toxic compounds. These include neuropathies associated with diabetes, multiple myeloma, certain hereditary polyneuropathies, and uremia (294). There is also an associated degeneration of the spinal cord, particularly involving posterior columns, as well as other portions of the CNS. Such findings are usually attributed either to local CNS disease or to damage of spinal ganglion cells secondary to ascending peripheral nervous system damage. It is likely, however, that the CNS is involved in distal axonopathies. The clinical characteristics of such distal axonopathies as described by Spencer and Schaumberg include the following (295).

1. *Insidious onset*. In most human toxic neuropathies, there is a steady low-level exposure. Because only the distal portion of selected, scattered fibers is affected, the patient may still function well despite the axonal degeneration.
2. *Onset in legs*. Large and long axons are affected early, and fibers of the sciatic nerve are especially vulnerable.
3. *Stocking-glove sensory loss*. Degeneration in the distal axon proceeds toward the cell body, resulting in clinical signs in the feet and hands initially.
4. *Early loss of Achilles reflex*. Fibers to the calf muscles are of large diameter and among the first affected by many toxins, even when longer, smaller-diameter axons in the feet are spared.
5. *Moderate slowing of motor nerve conduction*. In demyelinating neuropathies, motor nerves or roots are diffusely affected; in axonal neuropathies, scattered motor fibers are often intact and MNCV may appear normal or only slightly slow despite severe paresis.
6. *Normal CSF protein content*. Pathologic changes are usually distal and nerve roots are spared.
7. *Slow recovery*. Axonal regeneration (in contrast to remyelination) is slow—about 1 mm per day. Thus, after institution of dialysis or renal transplantation, recovery of nerve function may take months or years.
8. *Residual disability*. Most toxic axonopathies are characterized by tract degeneration of long, large-diameter fibers in the CNS concomitant with changes in the peripheral nervous system. Signs of lesions in the corticospinal and spinocerebellar pathways may not be clinically apparent if there is severe peripheral neuropathy. However, on recovery from the neuropathy, there may be spasticity or ataxia.

It can readily be recognized that the previously noted features are similar to many descriptions of uremic neuropathy (226,288,295). The cellular basis for distal axonopathies, however, remains unclear. Spencer et al. (297) have emphasized that a number of chemically unrelated neurotoxic compounds and several types of metabolic abnormalities can cause strikingly similar patterns of distal symmetric polyneuropathy in humans and animals.

These authors suggest a possible common metabolic basis for many distal axonopathies. Neurotoxic compounds may deplete energy supplies in the axon by inhibiting nerve fiber enzymes required for the maintenance of energy synthesis. Resupply of enzymes from the neuronal soma may fail to meet the increased demand for enzyme replacement in the axon, causing the concentration of enzymes to decrease in distal regions. This could lead to a local blockade of energy-dependent axonal transport, which could then produce a series of pathologic changes culminating distal nerve fiber degeneration.

TABLE 94-4

CLINICAL MANIFESTATIONS OF CHRONIC DIALYSIS DEPENDENT ENCEPHALOPATHY[a]

Cerebral atrophy (on neuroimaging study)
Abnormal electroencephalogram (EEG)
Impaired intellectual capability
Impaired cognition
Decreased exercise capability
Acute and subacute movement disorders
Sexual dysfunction
Psychiatric disorders
Depression
Psychosis
Suicidal behavior
Deterioration of vision
Increased fatigue

[a]Data from references 7–9,21,33,35,117,118,120–26.

Uremic Toxins and Nerve Conduction

Several possible uremic toxins have been identified, which appear to be correlated with depression of MNCV in laboratory animals (149). However, these studies do not take into account the fact that: (i) depressed MNCV is cyclical, with abnormal low values one day and normal values the next; (ii) there is a day-to-day variation in MNCV that approaches 20% (84); (iii) the finding of depressed MNCV in laboratory animals associated with high plasma levels of potential uremic neurotoxins has generally not been confirmed in human subjects with renal failure. Although it is possible to relate impairment in MNCV with levels in blood of various substances, the best correlation was obtained between reduced MNCV versus a reduction in glomerular filtration rate (Table 94-4).

Parathyroid Hormone

Among the potential uremic neurotoxins is PTH, based on a possible correlation between plasma PTH levels and MNCV in patients with CRF (87). Although some earlier studies suggested a possible effect of PTH on MNCV in the dog (89), these impressions have not been confirmed (53,297). In patients who have hyperparathyroidism without uremia, PTH has no observable effect on peripheral nerve function (53,82,297). In both patients and laboratory animals with acute renal failure, the MNCV has been found to be normal (22,24) (Fig. 94-7). In all studies of both patients and laboratory animals with CRF, the MNCV had not been shown to be affected by PTH (22) (Fig. 94-7). Therefore, in both patients and laboratory animals with either acute or chronic renal failure, or primary or secondary hyperparathyroidism, there is no demonstrable effect of PTH on nerve function. In patients with CRF, there is no change in MNCV as a result of either recovery of renal function, chronic hemodialysis, or parathyroidectomy (32,82). In addition, when patients begin dialysis therapy, MNCV either

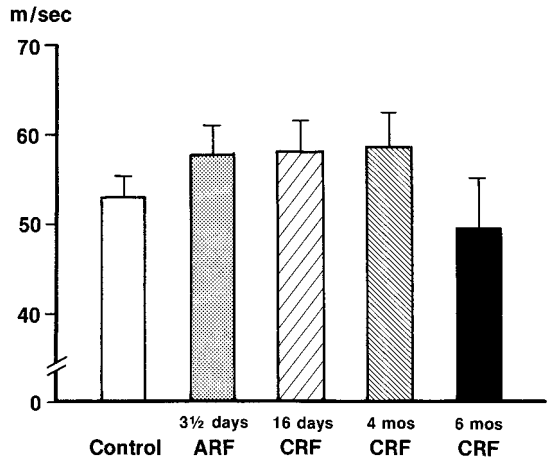

FIGURE 94-7. The motor nerve conduction velocity (MNCV) in the peroneal nerve in control dogs ($N = 12$) and in dogs who had renal failure for the following periods: acute renal failure (ARF) 3.5 days ($N = 10$), chronic renal failure (CRF) 16 days ($N = 11$), CRF 4 months ($N = 10$), and CRF 6 months ($N = 5$). None of the values of MNCV in dogs with renal failure is significantly different from the control value. (From: Mahoney CA, Arieff AL. Central and peripheral nervous system effects of chronic renal failure. *Kidney Int* 1983;24:170, with permission.)

stabilizes or improves (32,93). However, virtually all of these patients have elevated plasma PTH levels (94).

Animal studies suggest that in either acute renal failure or CRF, changes in MNCV take longer than 6 months to develop and are probably not related to an effect of PTH (Fig. 94-7). Mahoney et al. studied dogs that had renal failure for periods of 3.5 days to 6 months (53). There was no change in the MNCV after any of the aforementioned intervals of renal failure, and the MNCV was normal even after 6 months, with glomerular filtration rate less than 22% that of control.

It has also been suggested that either PTH or acute renal failure somehow resulted in an increase of nerve calcium content and that this might be related to impaired MNCV (89). However, this has never been confirmed. In dogs with renal failure for periods of 3.5 days to 4 months, there were no observed increases in nerve calcium content (53) (Fig. 94-8). Nerve calcium values in dogs with acute renal failure with or without parathyroidectomy also were not different and in fact actually fell significantly (versus control) in dogs with CRF (Fig. 94-8).

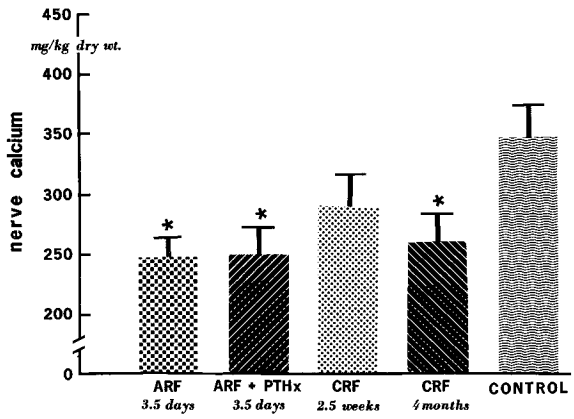

FIGURE 94-8. Nerve calcium content in peripheral (sciatic) never of dogs with renal failure for intervals indicated. Renal failure for all intervals shows caused significant decrement in nerve calcium content compared to control values. *ARF*, acute renal failure; *CRF*, chronic renal failure; *PTHx*, parathyroidectomy. (Data from: Mahoney CA, Arieff AL. Central and peripheral nervous system effects of chronic renal failure. *Kidney Int* 1983;24:170, with permission.)

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CHAPTER 95 ■ CARDIAC DISEASE IN CHRONIC RENAL DISEASE

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End-stage renal disease (ESRD) and cardiovascular disease have been inextricably linked since the earliest days of chronic dialysis. Clyde Shields, the first patient on long-term dialysis, died of myocardial infarction (MI) in 1970, at the age of 50 years, 11 years after starting hemodialysis (1). Several statistics accrued since that time attest to the impact of cardiovascular disease in renal patients. Approximately one-half of all deaths in patients with ESRD are attributable to cardiovascular disease, a proportion that is remarkably similar throughout the world (2–10). The annual incidence of MI or angina requiring hospitalization among patients on hemodialysis is 8%, and that of heart failure requiring hospitalization or treatment with ultrafiltration is 10% (11). Among patients starting maintenance dialysis, approximately 80% exhibit left ventricular hypertrophy (LVH) or systolic dysfunction disorders that are predictive of congestive heart failure (CHF), ischemic heart disease (IHD), and death (12). This high prevalence of left ventricular (LV) abnormalities pertains to patients receiving renal transplants as well (13). Furthermore, this rate of cardiovascular death in patients on dialysis is substantially higher than that of the general population in all age groups, particularly in the younger group (Fig. 95-1). In recent years there has been increasing awareness that chronic kidney disease (CKD) at all stages—not just dialysis—has an impact on the patient's prognosis for cardiac disease (14–20).

Several factors are thought to contribute to this high burden of cardiac disease in CKD. The prevalence of many traditional risk factors for cardiovascular disease and death, such as diabetes and hypertension, is clearly higher among patients with renal disease than in the general population. Whether renal failure itself is a risk factor for cardiac disease is still not known for certain, but several metabolic and hemodynamic disturbances that occur and progress in relation to declining renal function may modify cardiovascular risk (21). There is growing evidence that these uremia-related risk factors (e.g., anemia, hyperhomocysteinemia, hypoalbuminemia, divalent ion abnormalities, inflammation, and oxidative stress) contribute to the excess burden of cardiovascular disease in CKD patients. Finally, it has become clear that cardiovascular disorders arise early in CKD, progress during dialysis, and may partially regress subsequent to transplantation, only to progress again as the allograft fails. The burden and relative importance of traditional and uremia-related cardiac risk factors likely evolve parallel to these phases of renal disease as well.

In this chapter, we discuss the pathogenesis, risk factors, and treatment of IHD and disorders of LV geometry and function. As demonstrated in Figure 95-2, IHD may result from critical coronary artery disease (CAD) or nonatherosclerotic disease, and is associated with cardiomyopathy; heart failure (the major manifestation of pump dysfunction) results from ischemic disease or cardiomyopathy, and cardiomyopathy may be the result of concentric LVH, LV dilation, or systolic dysfunction. Emphasis is placed where possible on direct evidence obtained in epidemiologic studies of clinically relevant populations. Where the data permit, differences in clinical profile, risk factors, and treatment during the chronic phase of illness, dialysis, and transplantation are highlighted. The topic of cardiovascular disease in renal failure is in ferment and many questions, particularly those regarding treatment, cannot be answered definitively because adequate studies are lacking. Where reasonable, these gaps are filled by extrapolation from non-CKD populations.

ISCHEMIC HEART DISEASE

Pathogenesis

Symptomatic myocardial ischemia usually results from flow-limiting critical coronary atherosclerotic disease, but may be present in the absence of significant angiographic CAD in approximately 25% of patients (22–24) (Fig. 95-3). In the latter case, small-vessel disease or a decrease in myocardial capillary density consequent to LVH or fibrosis may be the culprits (25–28). LVH itself may predispose to ischemia.

In non-CKD populations, the initiating event of atherosclerosis appears to be endothelial injury caused by mechanical stress (e.g., hypertension) or endothelial toxins (nicotine, oxidative stress, hyperlipidemia, and inflammation) that alter the endothelial phenotype to a more permeable, procoagulant state (29). Endothelial denudation or, more commonly, alterations in cell surface receptor expression permit access of lipoproteins and macrophages into the subintimal space (29,30). Oxidative modification of lipoproteins, particularly of low-density lipoprotein (ox-LDL), is chemotactic for macrophages and facilitates uptake of oxidized lipids by macrophages, resulting in formation of foam cells (31). Ox-LDL stimulates elaboration of growth factors that are mitogenic for smooth muscle and profibrotic (32). These processes result in the accumulation of oxidatively modified lipids and inflammatory cells at the center of a fibrous "cap" of variable thickness. This cap may rupture, causing subocclusive thromboses that may be minimally symptomatic or associated with acute coronary syndromes (e.g., unstable angina, MI). These thrombi eventually become organized in the wall of the vessel. Calcific deposits may develop. The mature atheroma thus may contain, in addition to lipids, inflammatory cells, collagen, organized thrombus, and calcium (33). The propensity for rupture varies with the composition of the atheroma. Lipid and inflammatory cell–rich atheromas with thin fibrous caps are thought to be more unstable than those with a greater degree of fibrosis and less lipid (34).

Theoretically, renal failure can modify this process at multiple levels. Hypertension and flow overload may increase stresses on the vascular wall. In many patients on dialysis, a pro-oxidant and chronic inflammatory state pertains, and both may contribute to endothelial dysfunction (35). Hyperhomocysteinemia may promote endothelial activation and

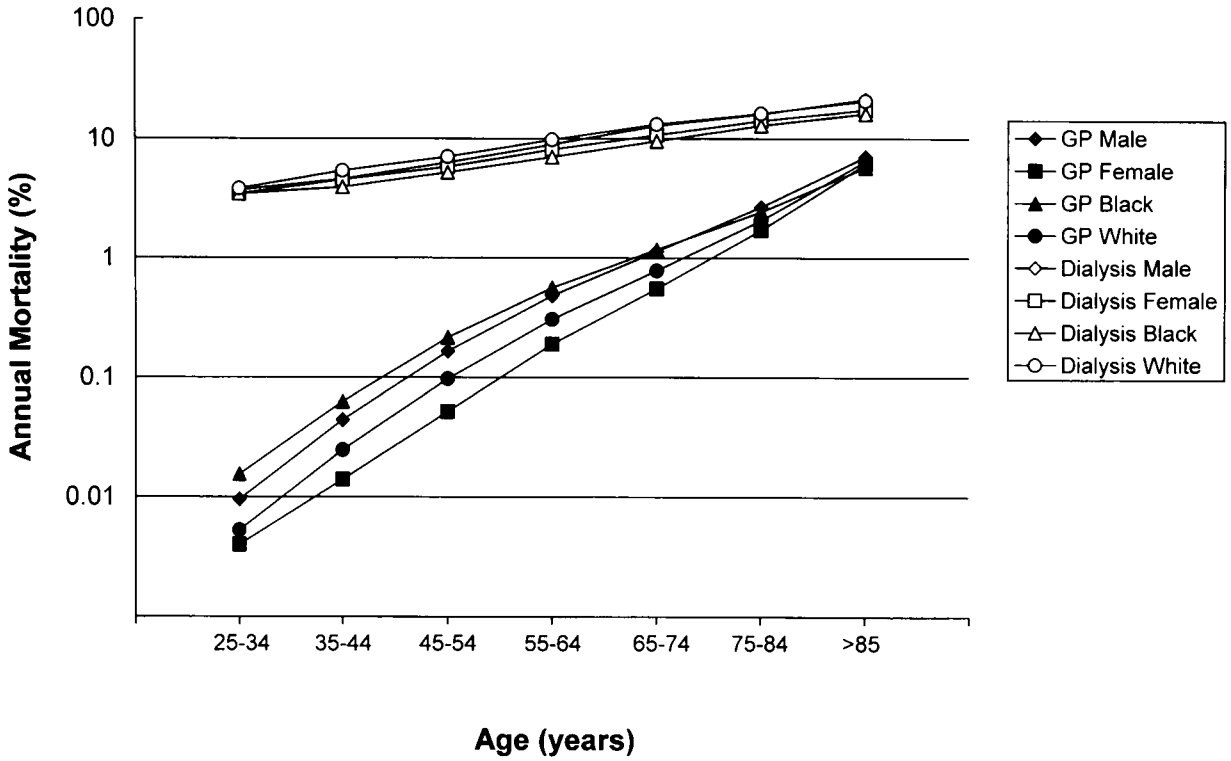


FIGURE 95-1. Annual cardiovascular mortality by age group in the general population and in patients on dialysis. (From: Foley RN, Parfrey PS, Sarnak MJ. Epidemiology of cardiovascular disease in chronic renal disease. *J Am Soc Nephrol* 1998;9:S16, with permission.)

thrombosis by mechanisms that are yet unclear (36,37). Hyperparathyroidism may promote vascular calcification and medial hypertrophy (35). Postmortem data from patients with CKD show increased vascular medial thickness and smaller lumen area relative to control subjects matched for age and gender (34). The presence of calcium by electron beam tomography correlates with advanced atherosclerosis and appears to be more prevalent in patients with ESRD (38–40).

In the absence of flow-limiting CAD, ischemic symptoms may result from a reduction in coronary vasodilator reserve and altered myocardial oxygen delivery. Intracoronary ultrasonography has shown that angiographically normal vessel segments may contain areas of nonencroaching atheroma. Endothelial function is impaired in these vessels and may reduce coronary vasodilator reserve (i.e., the ability of the vessel to dilate above baseline) in response to increased myocardial oxygen demand (41). Vasodilator reserve is clearly impaired in vessels with lumen-encroaching disease (42). The resulting mismatch of supply and demand may give rise to symptomatic ischemia.

Small vessel disease may occur in LVH and diabetes, and because of uremia *per se*. In LVH, small vessel smooth muscle hypertrophy and endothelial abnormalities can diminish coronary reserve. Diabetes may be associated with microvascular disease characterized by endothelial proliferation, subendothelial fibrosis, and exudative deposits of hyaline in the intima

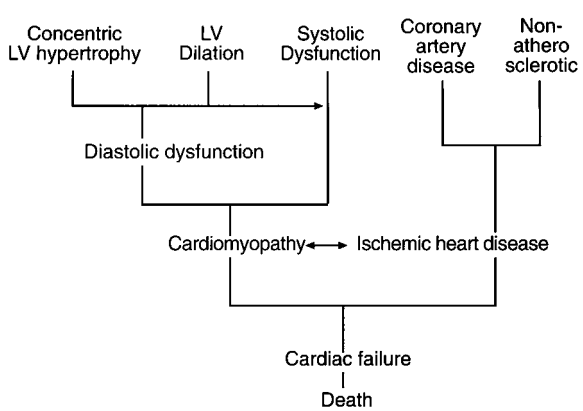


FIGURE 95-2. Cardiomyopathy and ischemic heart disease in chronic uremia.

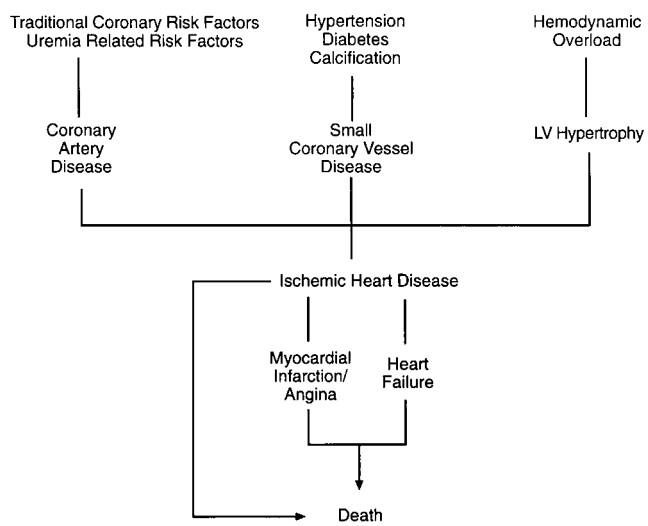


FIGURE 95-3. The etiology of ischemic heart disease in chronic uremia.

(43). In uremic rats, LVH is associated with a severe reduction in myocardial capillary density compared with hypertensive rats matched for weight and blood pressure (27). Small–vessel calcification may impair coronary reserve; an association between elevated $Ca \times PO_4$ product and CAD has been reported (44).

Dysregulation of energy metabolism in the myocyte may increase cellular susceptibility to ischemia. A reduced myocardial phosphocreatine–adenosine triphosphate ratio under stress has been observed in uremic animals (45). Hyperparathyroidism may play a critical role in the dysregulation of cellular energetics, and may thus exacerbate ischemic damage to the myocardium (46).

Diagnosis

Symptoms of myocardial ischemia in patients with CKD are in general similar to those in the nonuremic population. However, the prevalence of silent myocardial ischemia in this group of patients is very high (47,48). This has been best demonstrated in diabetic patients with ESRD because they often are subjected to screening coronary angiography before renal transplantation. In one series of 100 diabetic patients with ESRD, for example, 75% of the patients with angiographically demonstrated CAD had no typical angina symptoms (49). The prevalence of asymptomatic CAD in nondiabetic patients with ESRD is not as well studied. Exertional dyspnea is also less specific for cardiac disease in patients with CKD than in the general population. Such symptoms may be attributable to anemia, acidosis, heart failure, or fluid overload; therefore, careful interpretation within the clinical context is required.

Patients with CKD and symptomatic IHD should be investigated in a manner similar to patients with normal renal function, provided that revascularization will be considered should critical CAD be identified. In the general population, there is some suggestion that silent ischemia might lead to an increased mortality rate (50). There is no evidence, however, that revascularization of such patients prolongs their survival. Routine screening for CAD in asymptomatic patients with CKD therefore is not recommended (51). Several organizations have issued clinical practice guidelines for CAD screening of patients before noncardiac surgery (52–54), and it is appropriate to apply these to the CKD population as well. These groups generally recommend screening before surgery when the combination of risk factors and the nature of the operation place the patient at moderate or higher risk of a cardiovascular event. Patients being evaluated for renal transplantation are an exception to the aforementioned recommendations. The adverse prognostic implications of CAD, the desire to avoid allograft injury from posttransplantation invasive cardiac testing, and the need to ration transplantable organs are justification for screening all but the lowest-risk patients. The American Society of Transplant Physicians has published guidelines for the evaluation of renal transplantation candidates that include recommendations for the investigation of CAD (55).

Biomarkers for Ischemic Heart Disease

Cardiac troponin T and troponin I (cTnT and cTnI) are currently the standard biomarkers of myocardial injury. These tests are more specific for myocardial damage than creatinine kinase–myocardial band (CK-MB) but still are not always indicative of an ischemic mechanism of injury (56). Nevertheless, the definition and diagnosis of the acute coronary syndrome (ACS), including myocardial infarction, has changed radically based on the introduction of these markers in the last decade. The interpretation of these tests in patients with renal failure has been somewhat controversial. Studies using the first generation cTnT assays in ESRD patients without evidence of ischemia reported elevated levels in up to 71% of patients (57–59). Conversely, cTnI appears to be increased in approximately 7% of patients with renal failure (57,59,60). Although there is no difference between the diagnostic and prognostic accuracy of the two tests in the general population (61,62), the lower incidence of increased cTnI in the renal failure population has led some authors to suggest that this is the preferred test in this clinical setting (63,64).

Despite concerns about falsely positive tests, troponins are clearly very useful in the evaluation of suspected ACS. In one retrospective analysis, cTnI was shown to be the best performing marker in suspected ACS in patients with CKD or ESRD when compared to alternatives such as CK-MB or myoglobin (65). A study of 56 dialysis patients with CKD and suspected ACS demonstrated a sensitivity of 94% and specificity of 100% using cTnI to predict MI (63). The gold standard in this case was echocardiography, nuclear stress testing, or angiography. Although cTnT is more likely to be elevated at baseline in patients with renal impairment, a normal level has useful negative predictive value. A sequential rise in either of the serum troponins is consistent with new myocardial damage regardless of symptoms (66).

Noninvasive Testing for Coronary Artery Disease

Exercise electrocardiography has been the traditional method of noninvasive diagnosis of CAD. The sensitivity of this test is only 50% to 60% for single-vessel disease but is greater than 85% for triple-vessel CAD in the general population (67). These figures are based on the assumption that the patient reaches an adequate exercise level (i.e., 85% of the age-adjusted predicted maximal heart rate). A large proportion of patients with ESRD are unable to achieve this target because of poor exercise tolerance, anemia, poorly controlled hypertension, or the use of cardiac medications. One study of 85 diabetic uremic patients showed that only 6 achieved an adequate exercise level (68). In another study, only 12 of 60 diabetic patients with ESRD achieved target heart rates (69). Pharmacologic agents, therefore, often are used for noninvasive testing for CAD in these patients. The sensitivity of dipyridamole-thallium testing in patients with ESRD ranges from 37% to 86%, with a specificity of approximately 75% (70–73). Dobutamine stress echocardiography may be the method of choice where it is available because the reported sensitivity in patients with CKD is relatively high at 69% to 95%, with a specificity of approximately 95% (74–77).

Coronary Angiography

Cardiac catheterization and coronary angiography remain the gold standard for the diagnosis of CAD. A major disadvantage associated with this mode of investigation is the potential for renal toxicity from radiocontrast agents. CKD, especially in diabetic patients, is a major risk factor for contrast-induced acute renal failure (78). The risk of clinical nephrotoxicity is related to the severity of prior renal impairment. Although most patients who develop nephropathy eventually recover renal function, there is a risk that contrast administration may precipitate ESRD in patients with severe impairment in renal function at baseline. The risk of contrast nephropathy in high-risk patients may be reduced by using nonionic contrast media and by saline infusion (78). Many interventions intended to reduce the risk of nephropathy have been studied, but none has been shown to be consistently effective (78,79). A number of trials using N-acetylcysteine in CKD patients have demonstrated a reduction in the incidence of contrast nephropathy (80,81), whereas others have not (82,83). A recent meta-analysis of this topic indicated that the relative risk of nephropathy is reduced by N-acetylcysteine (relative risk 0.65, 95% confidence interval

[CI] 0.43–1.00, $p = 0.049$), but there has been significant heterogeneity in studies to date (84). Given its low cost and lack of adverse effects it is reasonable to use N-acetylcysteine in high risk patients prior to angiography. A large trial with clinically meaningful outcomes is still required before it can be universally recommended.

Burden of Disease

The prevalence and incidence of IHD and cardiovascular death among patients with mild-to-moderate renal failure are not well studied but may approach those seen in ESRD (85). In a small prospective study in France, age-adjusted annual incidence of MI or ischemic stroke was three times that in the general population (86). In the Modification of Diet in Renal Disease (MDRD) study of nondiabetic CKD, the annual incidence of first hospitalizations for cardiovascular disease was 3% (87). In a study of the effect of the angiotensin-converting enzyme (ACE) inhibitor benazepril on the progression of renal disease, the combined annual incidence of sudden cardiac death or fatal or nonfatal MI was 1% (88). Because these data are drawn from clinical trial inductees, they likely represent conservative estimates of the true event rates (89).

In Canada, by the time patients reach ESRD, the prevalence of CAD approaches 40%; approximately 22% have angina pectoris, whereas 18% have had a prior MI (4). The annual incidence of angina or MI among Canadian patients on hemodialysis is 10% (11). MI is the cause of death in approximately 14% of patients on dialysis in the United States (2).

Among renal transplant recipients (RTRs), the overall prevalence of CAD is approximately 15% (90). The annual incidence of MI, revascularization, or death from MI among RTRs is 1.5% (91).

Risk Factors

Although experimental studies in animal models and cross-sectional or case-control studies in small groups of patients can lend insight into the mechanisms of IHD in renal failure, only large, rigorous, prospective studies in relevant clinical populations can identify widely generalizable risk factors for cardiovascular disease. The Framingham Study (92) is the archetypal population-based cohort study in the field of cardiovascular disease, and the risk factors identified by it have been widely vindicated in subsequent intervention trials on blood pressure control and cholesterol reduction (93–95). Unfortunately, few large, adequately designed prospective cohort studies on cardiovascular outcomes have been performed in renal failure populations (20,96). An analysis of risk factors for cardiovascular disease in renal failure must of necessity include data from retrospective cohorts, national registries, and cross-sectional and case-control studies that, in declining order of reliability, provide less conclusive information.

Nonmodifiable Risk Factors

Age. Advancing age is associated with an increased risk of IHD in the general population (92,97). Older age has been independently associated with arteriographic CAD (24), *de novo* occurrence of angina pectoris, MI, or coronary revascularization in patients on dialysis (relative risk [RR] 1.6 per decade) (96), and increased risk of death among patients on dialysis (4). Age is also a significant predictor of the development or worsening of CVD in the CKD population (20,98). Among RTRs, age is an independent risk factor for MI or coronary revascularization or death from MI (RR 1.5 per decade) (91), and for all-cause death (RR 1.5 per decade) (99).

Diabetes Mellitus. Diabetes is an independent risk factor (RR 1.5 to 2.2) for the development of CAD in the general population (100–104). One recent cohort study suggests that diabetes confers a similar increased risk of CVD events for CKD patients (adjusted RR 2.01 to 4.67) (105), whereas another has shown slightly higher risk (OR 5.35, $p = 0.018$) (20). Among patients on dialysis, diabetes is independently associated with the development of *de novo* IHD and death (adjusted RR 3.98 and 3.86, respectively) (96,99). Among RTRs, diabetes is strongly associated with multiple cardiovascular outcomes (RR of 2.09 for MI, revascularization, or death from MI; RR of 2.98 for ischemic stroke; and RR of 25.7 for development of peripheral vascular disease) (91).

The impact of diabetes in all renal populations may well be underestimated. Angiographic studies in asymptomatic diabetic patients on dialysis show that approximately one-third have at least one coronary artery stenosis of 50% or greater (47). Among asymptomatic diabetic patients referred for renal transplantation, 88% of those older than 45 years had significant coronary stenosis (106).

Modifiable Risk Factors

Hypertension. Hypertension is a long-established risk factor for IHD in the general population (107,108). Pharmacologic therapy of hypertension reduces the risk of MI by 14% to 16% for each 6 mm Hg reduction in diastolic blood pressure (109,110).

The prevalence of hypertension in patients with CKD is approximately 80% and varies with the cause of renal disease (111). In the MDRD Study, although 91% of patients were treated with antihypertensive agents, only 54% had blood pressures of 140/90 mm Hg or less (112). Although hypertension is clearly a key risk factor in the progression of renal disease, few studies have directly assessed the role of hypertension in the evolution of IHD in CKD. In a cohort of approximately 800 patients, Muntner et al. reported that hypertension doubled the risk of coronary heart disease (RR 2.02, 95% CI 1.27–3.22)(105). In a smaller study, Levin et al. have shown that low diastolic blood pressure is protective with respect to CVD progression (odds ratio [OR] 0.72)(20).

A recent study has found that hypertension is present in 86% of U.S. dialysis patients, yet it is adequately controlled in only 30% of cases (113). The link between hypertension and IHD or mortality among patients on dialysis is complex. Increased pulse pressure, which is primarily due to systolic hypertension, is associated with increased cardiovascular mortality (114). Conversely, studies that have analyzed systolic and diastolic blood pressure separately have consistently demonstrated a paradoxical relationship in that hypertension predicts longer survival (112,115–118). However, the high prevalence of cardiac disease at the start of ESRD therapy is a confounding issue even for well-executed, prospective cohort studies. Because cardiac dysfunction can cause low blood pressure (so-called reverse causality) and is independently associated with death, even prospective studies cannot exclude the possibility that low blood pressure is simply a surrogate marker for poor pump function, unless patients with cardiac abnormalities at baseline are excluded from analysis. This is difficult to do in practice because most (75% to 80%) patients have echocardiographic abnormalities at the start of maintenance dialysis. In one prospective cohort study of 433 patients on dialysis, high blood pressure was positively associated with the development of IHD and LVH but negatively associated with mortality (118). In this same cohort, LVH was predictive of CHF, which in turn was associated both with mortality and with a drop in mean arterial pressure. Lower mean arterial pressure after an episode of CHF was independently associated with mortality.

TABLE 95-1

ESTIMATED PREVALENCE (%) OF LIPOPROTEIN ABNORMALITIES IN RENAL DISEASE

Category	TC >240 mg/dL	LDL-C >130	HDL-C <35	TG >200	Lp(a) >30
General population	20	40	15	15	15
Dialysis					
Hemodialysis	20	30	50	45	30
Peritoneal dialysis	25	45	20	50	50
Chronic renal insufficiency	90	85	50	60	60
Nephrotic					
Non-nephrotic	30	10	35	40	45
Renal transplant recipients	60	60	15	35	25

TC, total cholesterol; *LDL-C,* low-density lipoprotein cholesterol; *HDL-C,* high-density lipoprotein cholesterol; *TG,* triglycerides; *Lp(a),* lipoprotein (a). (Adapted from: Kasiske B. Hyperlipidemia in patients with chronic renal disease. *Am J Kidney Dis* 1998;32:S142.)

These observations suggest, but do not prove, the following causal sequence:

Hypertension ⟶ IHD and LVH ⟶
pump failure ⟶ hypotension and death

Although definitive proof for this chain of events still is lacking, the authors believe that hypertension is deleterious in patients on dialysis, as it is in the general population.

The prevalence of hypertension among RTRs is 70% to 80% (91). Elevated blood pressure has been associated with shortened graft and patient survival, as well as higher rates of CAD (2,119). However, retrospective analyses of large transplant cohorts have not found an association between hypertension and mortality after adjustment for age, diabetes, tobacco use, and time on dialysis before transplantation (91,120).

Clearly, further study is needed of the link between hypertension and IHD. This is particularly true in the CKD population, where the prevalence of IHD at baseline is lower and confounding by "reverse causality" less likely, and, thus, causal relationships more reliably discerned. Future studies in dialysis patients should ideally be based on inception cohorts of adequate size, consider relevant comorbidities, and incorporate standardized methods for accurate blood pressure measurement. In the interim, on the basis of strong biologic rationale, robust evidence in the general population, and strong evidence in renal disease populations for hypertension as a risk factor for LVH and IHD, hypertension should be considered a risk factor for IHD in patients with renal failure and treated appropriately.

Smoking. Smoking is a powerful risk factor for IHD in the general population, approximately doubling the risk of cardiovascular events in the Framingham cohort (121). Smoking cessation can reduce the risk of IHD by 50% even in long-time heavy smokers (122).

Approximately 25% of patients with CKD are current or former smokers(123). The impact of smoking in these patients has not been well studied, but smoking has been associated with progression of CKD, stroke, and the development of IHD (86,124). Approximately 30% to 40% of patients starting dialysis are smokers (11,96), and smoking has been associated with an excess mortality rate of 26% in incident patients on hemodialysis, even after extensive covariate adjustment (2).

Among RTRs, 25% to 40% smoke at the time of transplant assessment and, despite admonitions to quit, most continue to smoke after transplantation (125–127). A smoking exposure of 10 to 25 pack-years has been independently associated with ischemic stroke (RR 1.2 to 1.6), peripheral vascular disease (RR 1.2 to 1.6), and death (RR 1.4 to 2.0) (91,125,126).

Dyslipidemia. Elevated total cholesterol (TC), LDL-C, lipoprotein(a) (Lp[a]), and triglycerides (TGs) and low high-density lipoprotein (HDL-C) are associated with IHD in the general population, and the efficacy of targeted LDL-C lowering with HMG-CoA reductase inhibitors has been established by clinical trials (95,128–132). An atherogenic lipid profile is highly prevalent in patients with renal disease (Table 95-1), particularly in patients with nephrotic syndrome (133).

With respect to clinical outcomes, there are almost no data linking hypercholesterolemia to cardiovascular disease in CKD. One prospective study in nondiabetic subjects has suggested that low HDL is an independent risk factor for cardiovascular events (86). Among patients on dialysis, the data are conflicting. The highest mortality risk appears to be associated with low, not high, cholesterol (134). However, low TC is strongly correlated with poor nutritional status and low albumin levels, both of which are associated with increased mortality risk. In a prospective study of patients starting dialysis, Liu et al. recently demonstrated that high cholesterol reduces all-cause mortality risk in the presence of inflammation but increases risk in noninflamed individuals (hazard ratio [HR] 1.32 per 1.0-mmol/L increment in total cholesterol; 95% CI, 1.07–1.63) (135). This supports the notion that the inverse association of total cholesterol level with mortality in dialysis patients is likely due to the cholesterol-lowering effect of systemic inflammation and malnutrition, not to a protective effect of high cholesterol concentrations.

Lp(a) deserves special consideration because it is elevated in patients on dialysis and, unlike TC and LDL-C, has been prospectively and independently associated with IHD and death (136,137). However, in both renal and nonrenal disease populations, total experience with Lp(a) as a risk stratification tool is still limited and clinical intervention trials with IHD end points are nonexistent. Ongoing studies may address these concerns.

The prevalence of dyslipidemias in RTRs is high. Hypercholesterolemia (90,138) and low HDL level (91) have been linked to ischemic events in this population.

Homocysteine. Homocysteine (Hcy) is a byproduct of methionine metabolism, a sulfhydryl-containing essential amino acid,

and lies at the junction of two metabolic pathways, *trans*-sulfuration and remethylation. The rate of Hcy elimination by both pathways depends on an adequate supply of B vitamin cofactors, particularly B_{12} and folate. An association between Hcy and cardiovascular disease was first suggested by observations that patients with homocystinuria, a rare genetic disorder, have marked elevations in serum Hcy and markedly accelerated rates of atherosclerosis. More recently, several large observational studies have shown that variations in Hcy levels in the general population are independently associated with cardiovascular disease (139–141). Although the epidemiologic link is strong, there is yet no clinical trial evidence that reducing Hcy levels will reduce cardiovascular event rates in the general population.

Progressive renal failure is associated with increasing Hcy levels: 83% of patients have Hcy levels above the 90th percentile for the general population by the time they reach ESRD (142). Cross-sectional studies of the association between Hcy and cardiovascular disease in patients on dialysis have yielded conflicting results (142). Several prospective studies have found an association between Hcy and combined cardiovascular end points (143–145). Total numbers were small, limiting the generalizability of these results. Moreover, extensive multivariate adjustment was not possible, so the independence of the Hcy effect from other cardiovascular risk factors in patients on dialysis remains to be established. Limited prospective data in RTRs and CKD suggest that Hcy may be an independent risk factor in these populations as well (146,147).

High-dose folate supplementation (15 mg/day) reduces Hcy levels in ESRD by 25% to 30%, but cannot normalize levels in most patients (37,148,149). Thus far, no studies have demonstrated an improvement in clinical outcomes for dialysis patients with high dose folic acid relative to low dose (i.e., 1 mg/day) supplementation (150). In RTRs and CKD, therapy with supraphysiologic doses of vitamins B_6, B_{12}, and folic acid may reduce Hcy to normal levels (149,151). A large scale, randomized trial of Hcy lowering in RTRs is underway and it is hoped will elucidate the clinical benefit of this therapy.

Other Risk Factors

Hypocalcemia has been associated independently with excess mortality in cross-sectional analyses of patients on dialysis (134) and with *de novo* development of IHD in an inception cohort of Canadian patients on dialysis (152). The pathogenetic mechanism is unclear but may relate to an association with hyperparathyroidism, which itself is associated with lipid disturbances, LVH, myocardial fibrosis, and impaired cellular bioenergetics, all of which may cause or exacerbate ischemia or cardiomyocyte death (46,153,154). Conversely, uncontrolled hyperphosphatemia and high calcium-phosphate product are also associated with adverse cardiac outcomes (155,156). Ganesh et al. reported that hyperphosphatemic dialysis patients had a 41% higher risk of cardiovascular death and 20% higher risk of sudden death relative to those who did not (157).

Hyperphosphatemia and elevated calcium phosphate product are predictors of cardiovascular calcification. There has been increased interest in vascular calcification in recent years, a phenomenon that may be at least partly due to the advent of electron beam computed tomography (EBCT). This technique can be used to noninvasively and accurately measure coronary artery calcification. Data from the general population suggests that it correlates well with plaque burden and is predictive adverse cardiac events (158,159). Coronary artery calcification of scores as measured by EBCT are much higher in ESRD patients than age-matched controls, even when compared to patients with confirmed CAD (39,40,160). There are less data for CKD and RTR populations. A recent retrospective analysis of a cohort of veterans with nondialysis dependent CKD demonstrated that a serum phosphate level greater than 3.5 mg/dL was associated with a significantly increased risk for death (161). Recent data also indicate that stage 3 to 5 CKD is indeed associated with increased EBCT calcification scores, but this association may be stronger among those with diabetes (162).

The clinical significance of these findings is unclear. At least one study has shown no significant correlation between degree of coronary artery stenosis and EBCT calcification score for individual vessels in ESRD patients (163). At this time it appears reasonable to control hyperphosphatemia and hypercalcemia as a potential cardiac risk factor, but routine surveillance for coronary artery calcification is of unproven benefit.

Outcomes

Registry and retrospective cohort data show that the presence of IHD in patients starting dialysis is associated with excess short- and long-term mortality rates (2,96). In a Canadian prospective cohort study, patients with clinical IHD at the start of dialysis were more likely to have an admission for CHF (RR 1.7) or to die (RR 1.5) than patients free of IHD at baseline, after adjustment for age and diabetes (96). In these patients, most of the excess mortality associated with IHD seemed to be through the development of CHF. In diabetic RTR, presence of IHD is associated with a fourfold risk of future events and death (106).

The coexistence of CKD portends a worse prognosis if a cardiac event occurs. A study of 3,106 patients with MI reported an in-hospital mortality of 2% for patients with normal renal function, 6% for mild CKD, 14% for moderate CKD, 21% for severe CKD, and 30% for patients dependent on dialysis (14). In a large medicare cohort, one-year mortality after MI was 24% without CKD, 46% with mild CKD, and 66% with moderate CKD (19). Finally, a meta-analysis of trials of thrombolysis for MI indicated an inverse correlation between glomerular filtration rate (GFR) and mortality at one month (16).

LEFT VENTRICULAR HYPERTROPHY AND SYSTOLIC DYSFUNCTION

Diagnosis

Because echocardiography is widely available, simple, and reproducible, it has become the method of choice for assessment of LVH (164–166). Systolic dysfunction is defined as an ejection fraction of less than 40%, indicating impaired myocardial contractility. It often is associated with LV dilation (LV end-diastolic diameter $\geq$5.6 cm), defined echocardiographically as LV cavity volume index greater than 90 mL/m^2 (164). Concentric LVH is characterized by a thickened LV wall ($\geq$1.2 cm during diastole) with normal cavity volume. LV mass index is a calculated parameter that reflects the degree of muscular hypertrophy in the LV. Epidemiologic studies in nonrenal patients have established that the upper limits of LV mass index are 130 g/m^2 for adult men and 102 g/m^2 for adult women (167). Values above these limits indicate hypertrophy. The patterns of LVH on echocardiography are shown in Figure 95-4.

The calculation of LV mass and volume are not independent of volume status. As a result, the patient should be as close as possible to a "dry weight." In patients on hemodialysis, it is important to standardize the time and conditions of the study in relation to the dialysis session. Echocardiograms in these

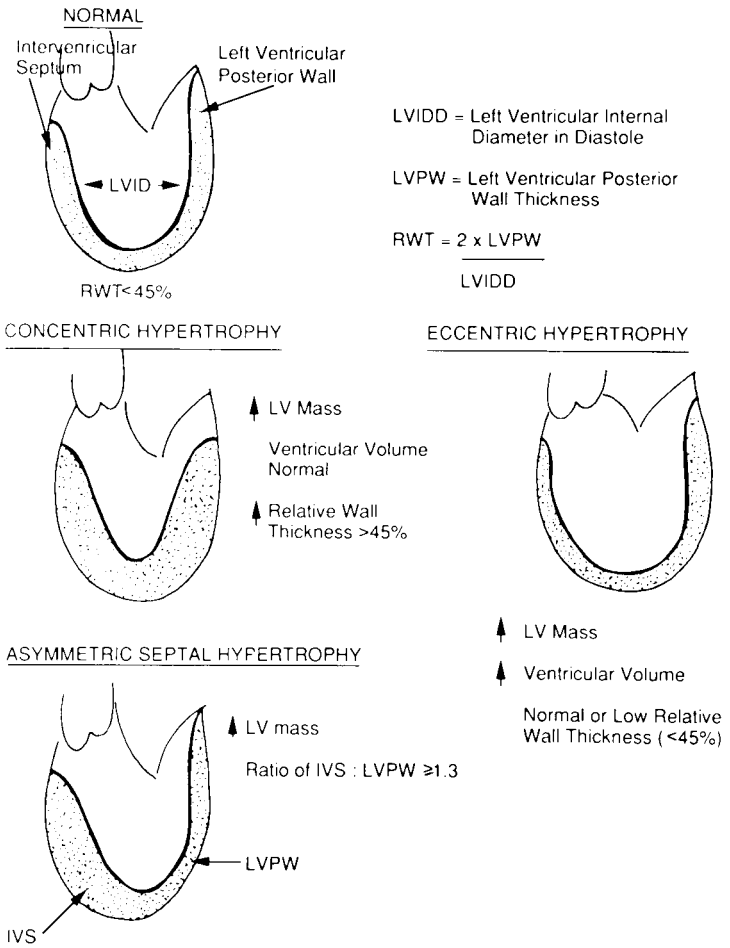


FIGURE 95-4. Patterns of left ventricular hypertrophy on echocardiography.

patients probably should be obtained the day after the dialysis session, with the patient at dry weight or at most 1 kg greater than it.

Pathogenesis

Ventricular growth occurs in response to mechanical stresses, primarily volume or pressure overload (Fig. 95-5). Volume overload results in addition of new sarcomeres in series, leading to increased cavity diameter (168,169). A larger diameter results in increased wall tension, a direct consequence of Laplace's law, which states that wall tension (T) is proportional to the product of intraventricular pressure (P) times the ventricular diameter (D): in symbols, $T = PD/4$. An increase in wall tension secondarily stimulates the addition of new sarcomeres in parallel. This remodeling thickens the ventricular wall, distributing the tension over a larger cross-sectional area of muscle and returning the tension in each individual fiber back to normal, thereby alleviating the stimulus to further hypertrophy. This combination of cavity enlargement and wall thickening is called *eccentric hypertrophy.* Pressure overload increases wall tension by increasing intraventricular pressure, resulting directly in the parallel addition of new sarcomeres and its functional consequences as described. Because sarcomeres are not added in series, isolated pressure overload does not lead to cavity enlargement (concentric hypertrophy).

Both eccentric and concentric hypertrophies are initially beneficial. Dilation permits an increase in stroke volume without an increase in the inotropic state of the myocardium and as such is an efficient adaptation to volume overload (168). It also

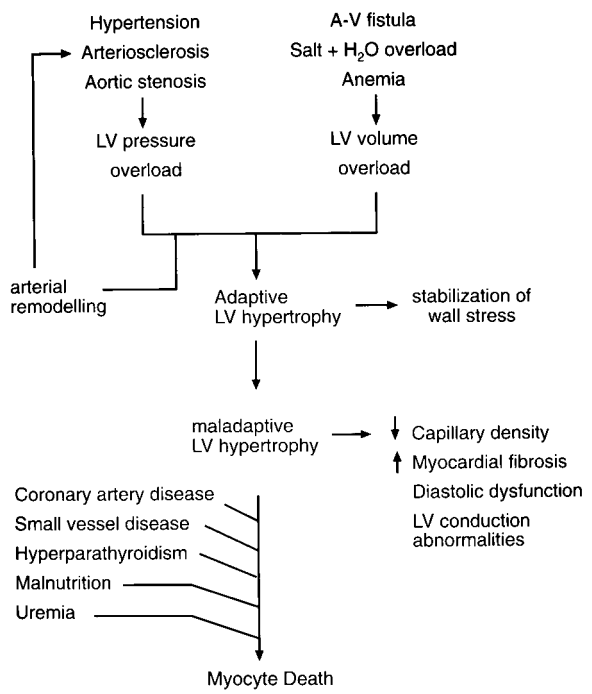


FIGURE 95-5. The etiology of myocyte death in chronic uremia.

permits the maintenance of a normal stroke volume and cardiac output in the presence of *decreased* contractility. Muscular hypertrophy returns the tension per unit muscle fiber back to normal, thereby decreasing ventricular stress.

Ultimately, however, LVH becomes maladaptive. Muscular hypertrophy is associated with several progressive, deleterious changes in cell function and tissue architecture. Early in the evolution of LVH, slow reuptake of calcium by the sarcoplasmic reticulum leads to abnormal ventricular relaxation. Combined with decreased passive compliance of a thickened ventricular wall, these changes may precipitate diastolic dysfunction (170). More advanced sarcoplasmic reticulum dysfunction is associated with calcium overload and cell death. Decreased capillary density, impaired coronary reserve, and abnormal relaxation may decrease subendocardial perfusion, promoting ischemia (171). Frequent coexistence of CAD may exacerbate ischemia and myocyte attrition (154). Fibrosis of the cardiac interstitium also occurs and appears to be more marked in pressure than volume overload (172). Myocyte apoptosis, ischemia, and neurohormonal activation (e.g., increased catecholamines, angiotensin II, and aldosterone) are thought to contribute to myocardial dysfunction (168,173,174). In the late phases of chronic and sustained overload, oxidative stress is prominent and contributes to cellular dysfunction and demise (175). Together, these various processes lead to progressive cellular attrition, fibrosis, pump failure, and, ultimately, death.

The uremic milieu of chronic renal disease can potentiate many of these processes. Anemia, salt and water excess, and arteriovenous fistulae in patients on dialysis are prevalent causes of volume overload, whereas hypertension is a major cause of pressure overload. These disturbances are probably the primary stimuli to ventricular remodeling in uremia. These same stimuli promote arterial remodeling in the large and resistance arteries, characterized by diffuse arterial thickening and stiffening (arteriosclerosis), which can increase the effective load on the left ventricle independently of mean arterial pressure (175,176). Secondary hyperparathyroidism may be associated with aortic valve calcification and, in some cases stenosis, a less frequent cause of pressure overload (177).

The attrition of myocytes in chronic uremia may be exacerbated by several factors. Underlying CAD promotes ischemia and infarction. Hyperparathyroidism increases susceptibility to ischemia through dysregulation of cellular energy metabolism (46), and appears to promote myocardial fibrosis directly (178). Clinical studies have shown that the extent of myocardial fibrosis in patients with ESRD is more marked than in patients with diabetes mellitus or essential hypertension with similar LV mass (179). Malnutrition, oxidative stress, and inadequate dialysis may all additionally promote myocyte death (35,154,180). Such cell death in the presence of LVH and continuing pressure and volume overload may be catastrophic, leading to a severe "overload cardiomyopathy" and, ultimately, death (181).

Burden of Disease

Disorders of LV geometry begin well before the initiation of dialysis. In a cross-sectional study conducted by Greaves et al., patients with CKD (serum creatinine >3.4 mg/dL) had a mean LV mass index of 120 g/m^2, which was intermediate between that of sex- and age-matched control subjects (79 g/m^2) and patients on dialysis (136 g/m^2) (182). An abnormal echocardiogram, primarily LVH, was observed in 63% of patients with CKD, versus 72% of patients on dialysis, suggesting a relationship between LV morphology and worsening renal function. Levin et al. (183) have reported a prevalence of LVH of 26.7% in patients with a creatinine clearance rate greater than 50 mL/minute, 30.8% in those with clearances of 25 to 49 mL/minute, and 45.2% in those with clearances less than 25 mL/minute. In the prospective arm of this study, an association between rising LV mass index and falling glomerular filtration rate was observed. Another cross-sectional study has yielded comparable results (184). The overall prevalence of LVH among patients beginning dialysis is 75% (185–188). In a large prospective cohort study, only 16% had normal echocardiograms at inception. Fifteen percent (15%) had systolic dysfunction, 28% had dilation with preserved contractility, and 41% had concentric LVH (96). In a subset of patients on dialysis who underwent yearly consecutive echocardiograms, LV mass index and LV cavity volume progressively increased, the biggest increase occurring between baseline and 1 year (189).

In patients about to undergo transplantation, the distribution of echocardiographic abnormalities is similar to those in patients on dialysis: normal 17%, concentric LVH 41%, dilation 32%, and systolic dysfunction 12% (13). In one longitudinal study, the proportion of patients with normal studies doubled (36%) and systolic function normalized in all patients with fractional shortening of less than 25% 1 year posttransplantation (13). In a more recent cohort study, LVH at the time of transplantation was an independent risk factor for death (RR 1.9, 95% CI 1.22–3.22) and CHF (RR 2.27, 95% CI 1.08–4.81) in the next 5 years (190).

Risk Factors

Nonmodifiable Risk Factors

Age. As with IHD, age is an important clinical marker of risk for LVH in the general, CKD, ESRD, and transplant populations (167,183,191,192).

Gender. The role of gender as a risk factor for LVH and systolic dysfunction is less clear. In a registry-based study, women were found to be more likely to have radiographic cardiomegaly or a history of CHF and less likely to exhibit electrocardiographic or echocardiographic evidence of LVH than men (193). In contrast to this result, a large prospective cohort study of patients beginning dialysis found no relationship between sex and development of heart failure. Moreover, female gender was associated with concentric LVH, whereas male gender was predictive of LV dilation and CHF (194). These contradictory findings may relate to differences in sample size, design, and criteria for LV geometry.

Diabetes Mellitus. There is evidence for a specific diabetic cardiomyopathy in diabetic patients without ESRD (195,196). LVH is a more frequent finding in hypertensive diabetic patients than in hypertensive nondiabetic patients, as is cardiac fibrosis. Diabetes has been identified as a predictor of hypertrophy in patients on dialysis (180,182).

Modifiable Risk Factors

Hypertension. The prevalence of hypertension in the various renal populations has already been discussed. Hypertension is associated with increased risk of progression of CKD and earlier onset of dialysis. A lower-than-usual target blood pressure (<125/75 mm Hg) appears more effective in delaying renal decline than the usual goal (<140/90 mm Hg) in patients with proteinuria (>1 g/day), suggesting a lower threshold for hypertensive damage among proteinuric renal patients. Hypertension has been shown to promote LV growth in a large cohort of patients with CKD (115) and its control may prevent or regress LVH (197).

In patients on dialysis, each 10 mm Hg increment in blood pressure is associated with a 48% higher risk for development

of LVH (118). Paradoxically, *low* blood pressure has been linked with increased mortality rates in cross-sectional and longitudinal studies (116,134). As was discussed earlier, the resolution of this paradox may lie in the observation that low blood pressure is more likely the result, not the cause, of pump failure, which is known to predispose to death.

In RTRs, elevated blood pressure has been associated with shortened graft and patient survival, as well as higher rates of CAD (2,119). However, a retrospective study of a large transplant cohort did not find an association between hypertension and death after adjustment for age, diabetes, tobacco use, and time on dialysis before transplantation (120). A retrospective study examining the natural history of cardiomyopathy after transplantation determined that hypertension was associated with progression to LVH in patients with normal hearts at transplantation (192). A prospective cohort study by the same group confirmed that diastolic BP was an independent risk factor for increasing LV mass between the first and fifth years after transplantation but systolic BP was the only predictor of *de novo* LVH at 5 years (190).

Anemia. Anemia has been associated with LV dilatation and LVH in patients with CKD and in patients on dialysis (RR for LVH progression, per 10 g/L hemoglobin drop, is 1.74 in CKD and 1.48 in dialysis) (115,180,189,198–200). Anemia also is a risk factor for the development of *de novo* cardiac failure and death in dialysis (200). Partial correction of anemia is associated with regression of hypertrophy in cohort studies (201,202).

In terms of anemia interventions, two questions have become prominent: does the correction of anemia reduce mortality, and what is the optimal hemoglobin target? Two trials have studied the impact of full correction of hemoglobin on cardiovascular endpoints in dialysis. A large American trial compared normalization of hemoglobin versus partial correction in patients on hemodialysis *with* preexisting IHD or cardiac failure (203). The primary outcome was death or MI. The trial was stopped early because an interim analysis precluded the possibility of demonstrating survival benefit in the normalization group by the end of the trial. Increased mortality and increased dialysis access loss were observed among patients randomized to normalization. In a Canadian trial, patients on hemodialysis without symptomatic cardiac disease were allocated to normalization of hemoglobin with erythropoietin or to partial correction of anemia (204). Normalization of hemoglobin seemed to prevent progressive LV dilation in those with normal cardiac volumes at baseline. These two studies suggest that full correction of anemia is not beneficial in patients with established cardiac disease, but may prevent development of cardiomyopathy in those without it.

Whether hemoglobin normalization at an earlier stage of renal disease (e.g., in CKD) may be beneficial is still unclear. Small, uncontrolled studies have suggested correction of anemia was associated with reductions in LVH (205,206). This has been contradicted by a trial in which 155 patients with CKD were randomized to a target hemoglobin of 120 to 130 g/L versus 90 to 100 g/L. At the end of follow-up (2 years or the initiation of dialysis) there was no significant difference in changes in LV mass index between the groups (207).

In RTRs, anemia is an independent risk factor for increasing LV mass after transplantation (190) and has been identified as a risk factor for cardiovascular outcomes (208).

Ischemic Heart Disease. The risk factors and outcome for IHD are discussed elsewhere in this chapter. It is worth repeating that CAD is an important cause of systolic and diastolic dysfunction in the general population and in patients on dialysis (180,209).

Mode and Quantity of End-Stage Renal Disease Therapy. Declining function of native kidneys has been associated with LV growth in patients with CKD (115) and patients on peritoneal dialysis (210). The impact of the dialysis dose on LVH is not definitively known. One randomized, crossover trial has suggested that more intensive dialysis tends to ameliorate echocardiographic abnormalities (211). Frequent dialysis, either with short daily hemodialysis or nocturnal hemodialysis, has been associated with regression of LVH (212).

Analyses of data from multiple large, prospective studies have shown that a dialysis dose threshold exists below which mortality rates increase sharply (11,213). These inflection points have been used to formulate targets for Kt/V_{urea} for both peritoneal dialysis (weekly Kt/V >2.0) and hemodialysis (per treatment Kt/V >1.2). The HEMO study, a large randomized trial comparing patients receiving standard versus high dose of dialysis and low-flux versus high-flux dialyzers, demonstrated no survival benefit from either intervention (214).

Whether one modality (peritoneal dialysis vs. hemodialysis) is less "cardiotoxic" than the other remains the subject of debate. Observational data suggest that the relative risk of death and hospitalization may be less for patients on peritoneal dialysis in the first 2 years, but these therapies are largely viewed as equivalent within this time period (215–219). This area has been controversial as study results are highly sensitive to the mode of analysis and to inclusion criteria (incident vs. prevalent patients). LVH has been found to be more severe in peritoneal dialysis relative to hemodialysis patients (220). In most observational studies, however, patients on peritoneal dialysis have higher comorbidity, including more severe hypertension.

Renal transplantation appears to induce normalization of systolic dysfunction and regression of concentric LVH and LV dilation, at least in patients without clinically evident IHD (13). This effect seems to persist for at least 3 years posttransplantation (192). It is not known which adverse risk factors characteristic of the uremic state have been corrected to produce the improvement in LV contractility, but blood pressure control likely plays a role (13,192).

Hypoalbuminemia. Several studies have shown that hypoalbuminemia is a powerful predictor of poor outcome in patients with ESRD. Hypoalbuminemia has been associated with LV dilation and predisposes to *de novo* cardiac failure and IHD (221). The mechanisms underlying this association are unknown. Hypoalbuminemia is associated with a hypercoagulable state and therefore may predispose to MI and ischemic cardiomyopathy. Alternatively, it may be a marker for malnutrition, inadequate dialysis, vitamin deficiency, or a chronic inflammatory state, all of which hypothetically could accelerate myocyte death and the development of cardiomyopathy, as discussed previously.

An inverse relationship between serum albumin and cardiovascular risk has been observed in the general population (222). The cardiovascular impact or implications of hypoalbuminemia among patients with CKD and RTR appear to be similar (105,223).

Aortic Stenosis. Acquired aortic stenosis may occur in a minority of patients (177) and may induce concentric LVH. Calcification of the aortic valve has been observed in 28% to 55% of patients on dialysis in various series, whereas hemodynamically important stenosis has been reported in 3% to 13%. Progression at times may be extremely rapid. The major factors predisposing to aortic valve calcification appear to be hyperparathyroidism, duration of dialysis, and degree of elevation of the calcium × phosphate product.

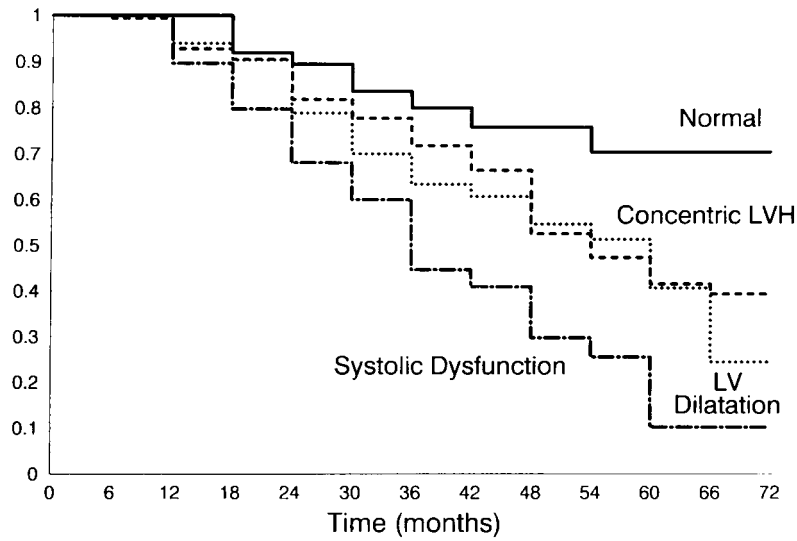


FIGURE 95-6. The cumulative survival of patients starting dialysis with a normal echocardiogram (*solid line*), concentric left ventricular (LV) hypertrophy (*dotted line*), LV dilatation (*short-dashed line*), and systolic dysfunction (*long-dashed line*). (From: Parfrey PS, Foley RN, Harnett JD, et al. The outcome and risk factors for left ventricular disorders in chronic uremia. *Nephrol Dial Transplant* 1996;11:1277, with permission.)

Salt and Water Overload. Salt and water overload is a persistent problem in patients on dialysis and is problematic to a lesser extent in patients with CKD and in RTRs. Blood volume correlates directly with LV diameter in patients on hemodialysis (224), as does the magnitude of interdialytic weight changes (225). LV diameter decreases with volume contraction during hemodialysis (166). Keeping the patient's dry weight optimal can minimize the degree of enlargement of the LV (198). Recent observational studies suggest that greater interdialytic weight gain and noncompliance with the prescribed dialysis regimen are independently associated with higher blood pressure (226,227). In a prospective study of peritoneal dialysis patients, sodium and fluid removal as well as hypertension were predictive of death within 3 years of starting dialysis (227).

Despite these associations, it is difficult to clearly discern cause and effect. Salt and water overload is by definition blood volume overload and hence probably plays a causal role in the development of LVH. However, it is possible that salt and water retention is induced by preexisting systolic or diastolic dysfunction in some patients.

Outcomes

The presence of concentric LVH, LV dilation with normal contractility, and systolic dysfunction at the time of ESRD therapy initiation has been associated with progressively worse survival, independent of age, sex, diabetes, and IHD (186) (Fig. 95-6). All three abnormalities are associated with increased risk for the development of CHF. Excessive hypertrophy in concentric LVH (high LV mass to volume ratio) and inadequate hypertrophy in LV dilation (low LV mass to volume ratio) were independently associated with late mortality in a Canadian dialysis cohort (12).

CONGESTIVE HEART FAILURE

Pathogenesis

Congestive heart failure may result from systolic dysfunction or diastolic dysfunction, the latter occurring because of concentric or eccentric hypertrophy (Fig. 95-2). IHD is an additional independent predictor (194).

Among patients with diastolic dysfunction, CHF results from impaired ventricular relaxation; this leads to an exaggerated increase in LV end-diastolic pressure for a given increase in end-diastolic volume. As a result, a small excess of salt and water can rapidly lead to a large increase in LV end-diastolic pressure, culminating in pulmonary edema.

In dilated cardiomyopathy, cardiac output is maintained at the expense of an increase in both end-diastolic fiber length and end-diastolic volume (i.e., through the Frank-Starling mechanism). As ventricular volume increases, failure to achieve adequate hypertrophy leads to an increase in wall stress and an increase in end-diastolic pressure, leading ultimately to pulmonary edema.

Diagnosis

The development of CHF, even in the presence of salt and water overload, suggests an underlying cardiac abnormality. Because the management of diastolic dysfunction differs from that of systolic dysfunction, an echocardiogram of the left ventricle is useful in planning management.

Burden of Disease

The relationship between CKD and CHF has received considerable attention in the last several years. Approximately one-half of patients with CHF have CKD, as defined by a GFR of less than or equal to 60 mL/minute (228,229). The observation that patients with worsening CHF often have a coincident decline in renal function has led some authors to speculate that this clinical scenario is in fact a "vicious circle" whereby CHF and CKD mutually aggravate each other (230).

On starting dialysis, 37% of patients have had a previous episode of CHF (6). A baseline history of CHF carries a twofold risk of death independent of age, diabetes, and heart disease (6,186,231). The risk for development of pulmonary edema requiring hospitalization or ultrafiltration after starting maintenance hemodialysis is 10% annually (11). Among patients free of CHF at initiation of dialysis, *de novo* CHF developed in 25% over 41 months of observation. In a cohort of 244 CKD patients, 20% developed new or worsening cardiac symptoms, including a change in their CHF symptoms (111). A recent study of RTRs who were free of cardiac disease one year after transplantation indicated that *de novo* CHF occurred as frequently as *de novo* IHD (1.26 versus 1.22 events/100 patient-years, respectively) (232).

Risk Factors

In a group of patients with ESRD followed prospectively from initiation of dialysis, two groups were identified: one without a previous history of CHF, and one with a history of CHF at initiation. In the absence of a history of CHF, the independent predictors of CHF at initiation were age, diabetes, IHD, systolic dysfunction on echocardiogram, and low serum albumin. In patients free of baseline CHF, the significant predictors of the *de novo* development of CHF, independent of age, diabetes, and systolic dysfunction at baseline, were hypertension, anemia, low albumin, and hypocalcemia (11,194). It appears that factors that predispose to volume or flow overload (e.g., anemia), pressure overload (e.g., hypertension), and cell death (e.g., malnutrition, hypocalcemia/hyperparathyroidism, and IHD) are associated with CHF in dialysis.

The risk factors for LVH in CKD have been discussed and include anemia and hypertension (20,115). Because LVH is a risk factor for CHF in the general and ESRD populations, it is likely that anemia and hypertension predispose to CHF in CKD. In a large cohort of RTRs without cardiac disease one year after transplantation, age, diabetes, gender, blood pressure, and anemia were identified as independent risk factors for *de novo* CHF (232).

Outcomes

CKD is an important predictor of mortality risk in patients with CHF (233–235). In a secondary analysis of the Digoxin Intervention Group trial, Shlipak et al. found that patients with stable CHF and an ejection fraction less than 45% had a higher risk of death with a GFR of less than or equal to 50 mL/minute (HR 2.6, 95% CI 1.69–2.51) (236). Patients with a GFR of 50 to 60 mL/minute had no increase in mortality relative to the patients with better renal function (236). For dialysis patients, CHF has a poor prognosis. In the cohort discussed in the previous section, the median survival of patients who had heart failure at or before initiation of ESRD therapy was 36 months, compared with 62 months in subjects without baseline CHF. This adverse prognosis was independent of age, diabetes, and IHD. Among patients who had heart failure at baseline, recurrent heart failure developed in 56% and the remaining 44% were failure-free during follow-up. Median survival in those with recurrent CHF was 29 months, significantly less than in those without recurrence (45 months) (194). For RTRs, the development of new-onset CHF carries a prognosis similar to that for new IHD (RR for death, 1.78, 95% CI 1.21–2.61) (for CHF versus 1.50, 95% CI 1.05–2.13 for IHD)(232).

MANAGEMENT

The goals of therapy for cardiac disease are to alleviate symptoms, minimize hospitalization, and improve mortality rates. Because relatively few trials have been conducted in the CKD and ESRD populations, many of the following recommendations are based on data from patients without renal impairment.

Ischemic Heart Disease

There are no randomized trials concerning the treatment of either the acute coronary syndrome (unstable angina and acute MI) or the nonacute presentations of CAD (stable angina and CHF) in patients with CKD. In the absence of such data, it is generally recommended that the treatment should be the same as in the nonuremic population (51,237). Unfortunately, observational data suggest that dialysis patients with an acute MI are far less likely to receive standard therapy (i.e., aspirin, β-blockers, and ACE inhibition) compared to other patients (238). Control of extracellular fluid volume by ultrafiltration and partial correction of anemia is an important therapeutic adjunct specific to CKD and patients on dialysis.

Medical Management

As in the general population, patients with CKD and stable angina pectoris who have not had an MI should be treated with antianginal agents for relief of symptoms. Coronary arteriography is recommended for patients with symptoms at rest or after minimal exertion, LV dysfunction, or signs of severe ischemia at low level of exercise during a stress test. For patients who have an MI, β-adrenergic blockade is recommended indefinitely, as is an ACE inhibitor for patients with LV dysfunction (239). In the general population, aspirin therapy is of proven benefit in the treatment of acute MI and after acute MI, as well as for long-term use in patients with a wide range of prior manifestations of cardiovascular disease (240). Although far fewer data are available for the CKD population, a recent study of 1,724 patients with an acute MI categorized patients into quartiles based on their renal function. In the group with the lowest GFR (<46.3 mL/min), aspirin and β-blocker use was the lowest among all groups of patients, yet the observed risk reduction was similar among all ranges of GFR. This suggests that aspirin and β-blocker are at least as effective in CKD and dialysis patients as in the nonrenal-failure populations (241). This, combined with the substantial improvement in cardiovascular disease outcomes in nonuremic patients with preexisting cardiovascular disease, is sufficient to recommend that patients with CKD or ESRD with ACS or established CAD should be treated with aspirin in a manner similar to patients with normal renal function. However, given that the complications from aspirin probably are more frequent in patients with CKD, the universal use of aspirin for the primary prevention of CAD in these patients is not recommended (51). Individual patient risks and benefits must be considered before beginning such therapy.

Although the general population appears to benefit from newer antiplatelet therapies such as aspirin plus clopidogrel (242,243), the relative safety and efficacy of such treatment in patients with CKD or ESRD is not yet known.

Dyslipidemia

There is overwhelming evidence that the treatment of dyslipidemia in the general population provides substantial survival benefit. Unfortunately, the evidence linking dyslipidemia to CAD in patients with CKD or ESRD is weak and data regarding the efficacy and safety of cholesterol lowering therapy are just starting to emerge. This may explain why ESRD patients appear to be significantly undertreated; although the majority of dialysis patients have dyslipidemia, a Canadian study indicated that lipid lowering therapy was used in only 9.5% of patients without and 29.4% of patients with evidence of atherosclerotic disease (244). United States Renal Data System (USRDS) data suggest that less than 10% of hemodialysis patients and 15% of peritoneal dialysis patients are receiving such treatment (245,246).

Despite the low utilization of lipid lowering therapy, observational evidence suggests that it is effective. Tonelli et al. (247), for example, performed a secondary analysis of a subset of patients with a GFR of 30 to 60 mL/minute from three randomized trials of pravastatin versus placebo. Among the 4,491 subjects identified, pravastatin significantly reduced the incidence of MI, coronary death, or coronary revascularization

(HR 0.77, 95% CI 0.68–0.86), similar to the effect of pravastatin on the primary outcome in subjects with normal kidney function (HR 0.78, 95% CI 0.65–0.94) (247). Thus far no sufficiently large randomized trials of lipid lowering therapy have been reported for the CKD population, but one such study is currently underway (248). The only trial of cholesterol lowering therapy in RTRs to date has been the ALERT study. This randomized, placebo-controlled trial of fluvastatin (40 to 80 mg/day) in 2,102 RTRs failed to find a benefit with respect to primary composite cardiac end point (myocardial infarction, cardiac death, and cardiac interventions). *Post hoc* analysis using alternative outcomes suggested that fluvastatin reduced the incidence of cardiac death or definite myocardial infarction from 104 to 70 events (RR 0.65; 95% CI 0.48–0.88; $p = 0.005$) (249).

For dialysis patients, an analysis of USRDS Morbidity and Mortality Wave 2 study data indicted that the use of HMG-CoA reductase inhibitors was associated with a reduction in cardiovascular death and total mortality (RR of death 0.64, 95% CI 0.45–0.91, and RR 0.68, 95% CI 0.54–0.87, respectively) (246). The 4-D (Die Deutsche Diabetes Dialyse) Study, a randomized trial of atorvastatin versus placebo in type 2 diabetics who have been on hemodialysis for no more than 2 years, will determine if there is a reduction in the rate of cardiovascular mortality and of nonfatal myocardial infarction (250). The results of this trial will be published in the near future. At this point, there is still equipoise concerning the benefit of lipid lowering therapy in this group of patients, and further trials are necessary.

Despite the paucity of data for renal failure populations, the clear benefit observed in the general population makes it reasonable to treat uremic patients in a similar manner. The NKF-Dialysis Outcomes Quality Initiative (K/DOQI) guidelines for managing dyslipidemias in CKD (251) are similar to the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP III) guidelines (252). Patients with CKD should be considered the highest-risk group, however, and thus LDL cholesterol levels of 100 mg/dL (2.56 mmol/L) or more and 130 mg/dL (3.33 mmol/L) or more are treatment thresholds for diet and drug therapy, respectively. Unlike the ATP III guidelines, drug therapy is recommended if LDL cholesterol level remains in the range of 100 to 129 mg/dL despite 3 months of lifestyle changes. The target LDL level is 100 mg/dL (2.56 mmol/L) or less. A cholesterol-lowering diet should be part of the treatment program, but most patients require pharmacologic therapy. For patients with high LDL levels, HMG-CoA reductase inhibitors are recommended as first-line therapy. These agents are safe and effective in uremic patients, but screening for myositis should be undertaken. Fibrates are also effective in CKD patients, but dose reduction appropriate to the level of renal failure is important. The combination of an HMG-CoA reductase inhibitor and a fibrate is associated with a high risk of muscle toxicity and in general should be avoided. According to the ATP III guidelines, fibrates are contraindicated in ESRD, but the K/DOQI guidelines state that these drugs may be used either for patients with triglyceride levels greater than 500 mg/dL or statin-intolerant patients who have triglycerides of greater than 200 mg/dL with non-HDL cholesterol of greater than 130 mg/dL. Similar guidelines have been published for the RTR population (253).

Revascularization

The potential risks and benefits of coronary revascularization procedures in patients with CKD or ESRD are different from those in the general population. The reported perioperative mortality rate of coronary artery bypass grafting (CABG) surgery in patients on dialysis has ranged from 0% to 20%, significantly higher than in the general population, but the studies on which these figures are based are mostly small and retrospective, and do not make adjustment for comorbid factors (254–265). When the results of these studies are combined, the perioperative mortality rate is approximately 8% to 9%, roughly three times the expected rate for patients without ESRD. The perioperative morbidity rate of CABG surgery also is greater, both in patients on dialysis and non–dialysis-dependent patients with CKD than in matched control patients (261,262,266,267). Unadjusted 2-year survival rates for patients on dialysis undergoing CABG have been reported to range from 45% to 92% (254,257,261–263). The few studies that have been of sufficient duration to determine the 5-year cumulative survival rate of patients on dialysis after CABG indicate that it is approximately 50% (259,260,263). These survival rates are comparable with those seen in the overall ESRD population (2), but are considerably lower then the overall 5-year survival rate of 85% after CABG observed in the Coronary Artery Surgery Study (268). There are few data as to the outcome of revascularization procedures in patients who have had renal transplantation. However, case reports and small series of patients suggest that transplanted patients with near-normal renal function have a perioperative mortality rate and long-term survival rate close to those observed in the non-ESRD population (264,269–273).

There have been no randomized trials of CABG versus medical management in patients with CKD and symptomatic CAD, but one retrospective study has suggested that patients with severe CAD do benefit from the procedure (261). The only randomized trial of revascularization versus medical therapy in patients with ESRD to date enrolled 26 asymptomatic diabetic patients who were found to have CAD on screening coronary angiography before renal transplantation (274). Ten of 13 medically managed and two of 13 revascularized patients had a cardiovascular end point (unstable angina, MI, or cardiac death), and the trial was stopped early by an external review committee. This provides some evidence that revascularization may improve the prognosis of asymptomatic CAD in this select population, but medical therapy in this instance consisted only of a calcium channel blocking drug and aspirin. Regardless of any survival benefit, CABG usually offers good relief from angina (254,260,262,264).

The role of percutaneous transluminal angioplasty (PTCA) in the CKD or ESRD patients is controversial. Despite a high rate of initial technical success in patients with ESRD, PTCA seems to be associated with frequent recurrence of symptoms, usually resulting from restenosis (259, 263, 275–278). Early results from studies using PTCA with stenting of dilated vessels in the dialysis population suggest a lower recurrence rate with this procedure (279,280). CABG appears to have better outcomes in dialysis patients than PTCA with or without stenting (263,281–283). Most retrospective and uncontrolled comparisons of patients on dialysis who have undergone PTCA and patients who had CABG have demonstrated no overall survival difference in the two groups, although patients treated with PTCA do have a significantly higher long-term risk of MI or recurrence of ischemic symptoms (259,263). Herzog et al., however, analyzed USRDS data from dialysis patients undergoing their first revascularization procedure. After comorbidity adjustment, the relative risk for CABG (versus PTCA) patients was 0.80 (95% CI 0.76–0.84, $p < 0.0001$) for all-cause death and 0.72 (95% CI 0.67–0.77, $p < 0.0001$) for cardiac death. For stent (versus PTCA) patients, the RR was 0.94 (95% CI 0.88–0.99, $p = 0.03$) for all-cause death and 0.92 (95% CI 0.85–0.99, $p = 0.04$) for cardiac death (282). To date, there have been no studies comparing PTCA with medical management of patients with ESRD or CKD and CAD.

In summary, the indications for coronary revascularization in patients with CKD or ESRD are in general the same as those in the nonuremic population. Revascularization appears

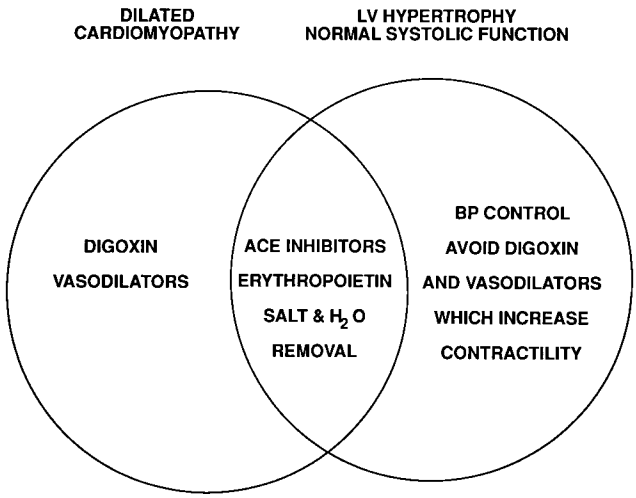


FIGURE 95-7. The management of cardiac failure in chronic uremia. See text for discussion of β-adrenergic receptor blockers.

to be beneficial in high-risk patients and those with persistent symptoms of myocardial ischemia, despite maximal medical therapy, provided that their life expectancy is otherwise reasonable. Based on the existing evidence, CABG appears to be the revascularization procedure of choice. PTCA with stenting seems to be a reasonable alternative in single-vessel disease or multiple-vessel disease with culprit lesions.

Congestive Heart Failure

For all patients with symptoms of heart failure, potentially reversible precipitating and aggravating factors (e.g., ischemia, tachycardia, dysrhythmias, or hypertension) should be sought and appropriately managed. The treatment of heart failure differs for those with systolic and diastolic dysfunction (Fig. 95-7).

Angiotensin-Converting Enzyme Inhibitors

The utility of this class of drugs for the management of heart failure has been well demonstrated in the general population. ACE inhibitors have been found to improve symptoms, reduce morbidity, and improve survival (284,285), making them an important component of CHF therapy. Although trials in the CKD or ESRD population have not been conducted, the high degree of benefit shown in other patients makes it very reasonable to treat these patients in a similar manner. ACE inhibitor therapy therefore is recommended for patients with symptomatic heart failure, post-MI patients with an LV ejection fraction less than 40%, and asymptomatic patients with an LV ejection fraction less than 35% (284,286).

Digoxin

The benefit of digoxin in the setting of symptomatic LV systolic dysfunction and atrial fibrillation is widely accepted. The use of digoxin in similar patients with normal sinus rhythm has been more controversial, but a substantial body of evidence supporting its use does exist (287–290). Nonuremic patients with symptomatic LV systolic dysfunction have less morbidity and improved exercise tolerance when given digoxin in conjunction with standard therapy (i.e., diuretics and ACE inhibitors) (290), but digoxin does not appear to improve survival (287). The only data from a randomized trial concerning patients with CKD are the aforementioned *post hoc* analyses of data from the Digoxin Intervention Groups (DIG) trial. In this case the efficacy of digoxin did not differ by level of GFR, which ranged from patients with normal renal function to those on dialysis (236). Digoxin therefore should be considered for patients with CKD and systolic dysfunction, with a reduction in dose appropriate to their level of renal impairment. Digoxin should not be given to patients whose heart failure is due primarily to diastolic dysfunction because the increased contractility could worsen diastolic impairment.

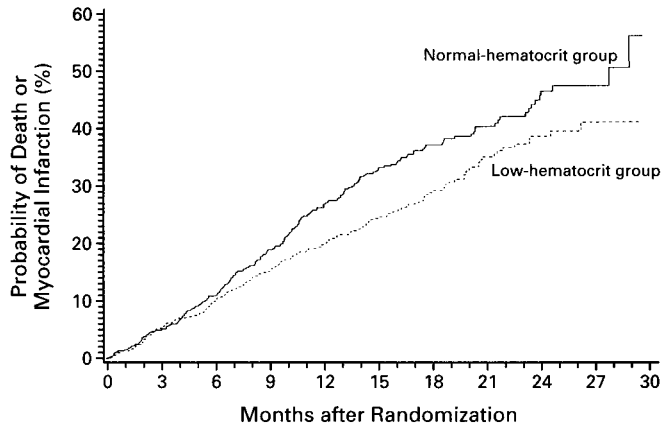
β-Adrenergic Receptor Blockers

A large amount of data from recent, well designed trials supports the use of β-adrenergic receptor antagonists in the management of LV systolic dysfunction. Improvements in mortality or hospitalization rates have been shown in patients with mild-to-moderate symptomatic heart failure treated with carvedilol, bisoprolol, or controlled-release metoprolol (291–294). The use of β-receptor antagonists with intrinsic sympathomimetic activity appears to be detrimental and these agents should not be used in patients with heart failure. Current guidelines for the general population suggest the routine use of β-receptor antagonists in clinically stable patients with an LV ejection fraction less than 40% and mild-to-moderate heart failure symptoms who are on standard therapy (i.e., diuretics, an ACE inhibitor, and digoxin) (286,295). Such therapy also should be considered for asymptomatic patients with an LV ejection fraction less than 40%, but the evidence supporting its use in this setting is not as strong. In one of the few randomized trials performed in dialysis patients, Cice et al. (296,297) have demonstrated that treatment of patients dilated cardiomyopathy with carvedilol reduced two years mortality (51.7% in the carvedilol group, compared with 73.2% in the placebo group [$p < 0.01$]). There were fewer cardiovascular deaths (29.3%) and hospital admissions (34.5%) among patients receiving carvedilol than among those receiving a placebo (67.9% and 58.9%, respectively; $p < 0.00001$) (296,297). This supports the notion that β-receptor antagonists may be safely used in the CKD and ESRD populations in the same manner recommended for the general heart failure population. As in the nonrenal-failure population, however, the agents should be started in low doses with careful clinical reevaluation during the titration phase.

Other Drugs

Diuretics remain an essential component of the symptomatic treatment for heart failure in non–dialysis-dependent patients with CKD, although multiple agents at high doses may be required in patients with more advanced renal impairment. Loop diuretics are widely used to maintain euvolemia in most patients with CHF, but their effect may be negligible in patients requiring dialysis. Thiazide diuretics usually become ineffective with a GFR less than 30 mL/minute and are therefore not useful in patients with severe renal impairment. Aldosterone antagonists are similarly ineffective in patients with ESRD, but hyperkalemia can result when these drugs are combined with renin-angiotensin system (RAS) blockade and β-receptor antagonists. They should be avoided in such patients. Long-acting nitrates may be useful in some patients, particularly those with diastolic dysfunction. Direct vasodilators are considered inadvisable in this setting (284).

Data regarding the use of angiotensin II type 1 (AT_1) receptor blockers in patients with heart failure are beginning to accumulate. Although the ELITE I study suggested a survival advantage in heart failure patients treated with the AT_1 blocker losartan versus placebo, this was not confirmed in ELITE II (298,299). More recently, the CHARM studies indicated that candesartan is a good alternative for patients with chronic heart failure who cannot tolerate ACE-inhibitors (300). Although it is possible that the AT_1 receptor blockers will prove to have an efficacy in heart failure similar to that of ACE inhibitors,



No. at Risk										
Normal hematocrit	618	540	476	415	353	259	186	124	69	26
Low hematocrit	615	537	485	434	391	292	216	131	80	20

FIGURE 95-8. Cumulative survival of patients on hemodialysis with preexisting cardiac disease, treated with erythropoietin, randomly allocated to partial correction of hematocrit or to normal hematocrit. (From: Besarab A, Bolton WK, Brown JK, et al. The effects of normal as compared with low hematocrit values in patients with cardiac disease who are receiving hemodialysis and epoetin. *N Engl J Med* 1998;339:584, with permission.)

the latter remain the agents of choice for the treatment of LV dysfunction, with or without symptomatic heart failure (295).

The add-on effects of an AT_1 blocker on top of an ACE-inhibitor are less clear. This approach was questioned when the ValHeFT study suggested that in patients with CHF already treated with both an ACE inhibitor and a β-blocker, adding an AT_1 blocker was associated with an increased risk for death (301). The CHARM-Added trial, however, demonstrated a moderate reduction in cardiovascular mortality and hospitalization with concomitant use of an ACE-inhibitor and an AT_1 blocker (302), suggesting that the ValHeFT results in this particular subset were due to chance.

Uremia-Related Interventions

Anemia

The optimal target hematocrit for improving outcomes in patients with CAD or LV dysfunction is not known. At least one trial has shown that maintaining a normal hematocrit, compared with the usual target level of 33% to 36%, in patients with established cardiac disease is not of benefit and may be detrimental (203) (Fig. 95-8). Partial correction of anemia consistently has been shown to induce some regression of LVH (303–307), and normalization of hemoglobin may prevent progressive LV dilation in those with normal cardiac volumes at baseline (204). Until further data become available, however, it is reasonable to follow the current NKF-Dialysis Outcomes Quality Initiative guidelines for treatment of anemia for all patients, including those with cardiac disease. These guidelines recommend a target hematocrit of 33% to 36%, based on improvement in quality of life and exercise tolerance (308).

Hypertension

As in the general population, there is evidence that treatment of hypertension leads to regression of LVH in patients with renal disease (309–311). It is not known whether the regression of LVH in patients with CKD leads to greater improvement in outcomes than control of hypertension alone. It is likely that a number of classes of antihypertensive agents lead to regression of LVH, although there is increasing evidence that ACE inhibitors are more effective than other classes of drugs (312). For the general population, the Joint National Committee recommendations for hypertension treatment do not differ for patients with and without LVH. CHF is, however, considered a "compelling indication" for the use of ACE inhibitors (or AT_1 blockers in ACE inhibitor intolerant individuals) (313). Comprehensive guidelines for the management of hypertension in patients with CKD have recently been published by K/DOQI. These guidelines also do not identify LVH as a criterion for selecting antihypertensive agents, but make similar recommendations for the treatment of CKD patients with hypertension and CHF as discussed in the preceding text (314).

Mode and Quantity of Dialysis

The recommended target Kt/V_{urea} is more than 2.0 per week for peritoneal dialysis and more than 1.2 per treatment for hemodialysis (213,315). Whether higher doses of dialysis improve cardiac outcomes is not known. The establishment and maintenance of an accurate dry weight is extremely important. Symptoms of heart failure clearly can be worsened by salt and water retention. Myocardial ischemia may be aggravated by increased LV end-diastolic pressure associated with extracellular fluid volume overload, and by the fluid shifts accompanying rapid ultrafiltration during hemodialysis. Evidence that either dialysis modality is associated with improved outcomes for patients with cardiac disease is scarce. Despite the conventional wisdom that some patients who are unable to tolerate the intradialytic volume expansion associated with intermittent hemodialysis may be more easily managed with peritoneal dialysis, observational evidence suggests otherwise. In an analysis of registry data from more than 100,000 incident dialysis patients, Stack et. al. reported that patients with CHF treated with peritoneal dialysis had higher mortality over 2 years compared to hemodialysis patients (RR 1.24, 95% CI 1.14–1.35) (316). More studies in this area are clearly warranted.

CONCLUSIONS

The burden of cardiac disease in all phases of renal failure is high, and its clinical impact is grave. The most frequent clinical manifestations are IHD and CHF. IHD may be atherosclerotic or nonatherosclerotic in origin. CHF results from IHD, cardiomyopathy, or both. The manifestations of uremic cardiomyopathy include concentric and eccentric LVH and systolic dysfunction. Risk factors for cardiac disease in uremia include age, diabetes, hypertension, anemia, volume overload, hyperparathyroidism, dyslipidemia, and perhaps uremia itself.

Although studies have traditionally focused on the dialysis population, it has become evident that risk factors for cardiovascular disease and their initial consequences to the heart already are present during the predialysis phase and persist

despite significant amelioration in the transplantation phase. Even so, our knowledge of these risks and consequences remains far from definitive, particularly in the CKD and transplantation phases. The dearth of experimental evidence is particularly acute with regard to therapy. Although many data exist on the impact of risk factor modification and therapy of CHF for the general population, corresponding trials in the renal population have not been done. In the interim, it is useful to remember that many of the risks for cardiac dysfunction are common to both renal and nonrenal patients, that many of these risks are higher in the renal failure population, and that the absolute benefit of any intervention tends to increase with increasing risk. It is likely, therefore, that the validity of extrapolations from the general population will be vindicated in future trials. The most logical approach at present seems to be to treat according to recommendations for the nonrenal population unless there is a compelling contraindication, or there are convincing contrary data from studies in the renal failure population. Ultimately, this approach will have to be validated by clinical trials of adequate statistical power.

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CHAPTER 96 ■ METABOLIC AND ENDOCRINE DYSFUNCTIONS IN UREMIA

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PATHOMECHANISMS UNDERLYING ENDOCRINE DISORDERS IN PATIENTS WITH CHRONIC KIDNEY DISEASE

Disturbances of endocrine function in patients with chronic kidney disease may arise from a number of different causes, which are summarized in Table 96-1.

The clinician has at his or her disposal measurements of hormone concentration, which may or may not be abnormal. However, endocrinological assessment is more than looking at plasma hormone concentrations. Hormone concentrations per se fail to provide proper assessment of the adequacy of the hormonal state, e.g., hormone concentrations may be inappropriate to the stimulating or suppressing signal, the test may detect inactive hormone isoforms, or the response of the target organ may be abnormal. It is, therefore, indispensable to interpret hormone levels in the appropriate context, e.g., insulin concentration relative to glucose concentration, parathyroid hormone (PTH) in relation to serum ionized calcium concentration and plasma 1,25-dihydroxycholecalciferol ($1,25[OH]_2D_3$) concentration.

DISORDERS OF CARBOHYDRATE METABOLISM IN PATIENTS WITH CHRONIC KIDNEY DISEASE

In patients with chronic kidney disease (CKD) abnormalities in carbohydrate metabolism are encountered at different levels of the insulin-glucose cascade (Table 96-2). Patients with chronic kidney disease almost always display resistance to the peripheral action of insulin, although the half-life of insulin is prolonged, because insulin removal by the damaged kidney and by extrarenal organs is impaired so that plasma insulin concentrations tend to be higher (1,2). The normal response of the β cell to insulin resistance is to increase secretion of insulin (3,4) and, therefore, hyperglycemia as a pointer to glucose intolerance is seen only when this adaptive response of β cells fails. In CKD patients glucose intolerance is seen only in patients who have both insulin resistance and impaired insulin secretion (1,5).

Peripheral Resistance to Insulin Action

Using the forearm perfusion technique, Westervelt and Schreiner (6) showed that peripheral glucose uptake is reduced in uremic patients. This observation was confirmed with the methodological gold standard, the euglycemic insulin clamp, which allows to quantitate the amount of glucose metabolized per unit of insulin (7,8). Peripheral resistance to insulin is seen even in patients with early stages of CKD. This abnormality is clinically important, because it is tightly correlated to the enhanced cardiovascular risk (9) and to the rate of progression of CKD (10).

Liver and skeletal muscles are the major sites of peripheral glucose uptake. Liver and kidney are the major sites of glucose production in the fasting state (11,12). The available data indicate that glucose metabolism by the liver is usually not impaired in CKD. Hepatic glucose production (2,8), as well as its suppression by insulin (2,8), are not altered and glucose uptake by the liver, small to begin with, is not affected in CKD (8).

The skeletal muscles are the primary sites of decreased insulin sensitivity. The defect is not at the level of the insulin receptor; the number and affinity of receptors are normal (13–19) as was insulin receptor kinase activity in skeletal muscles of chronic uremic rats (19). The defect is presumably at the postreceptor level. Impaired phosphatidylinositol 3-kinase activity (PI3-K), probably due to excess of p85, was recently found (20). Consequently higher levels of insulin will be required to increase glucose uptake by skeletal muscle in uremia and this has, indeed, been documented by Smith and DeFronzo (13).

When the beta chain of the receptor has been phosphorylated, a complex series of interactions ensues with insulin receptor substrate as well as with numerous cofactors (the reader is referred to two recent reviews, namely, 21,22). The more distal effects are mediated via the MAP kinase pathway (e.g., proproliferative actions) and the inositriphosphate (IP3) pathway. The latter is responsible for the upregulation of the insulin-regulated glucose transporter (GLUT-4). The PI3-K complex is heterodimer composed of an 85-kDa (p85) regulatory subunit and 110-kDa (p110) catalytic subunit. Subunits and p85-p110 complexes are differentially regulated by glucocorticoids (23,24). Stimulation of p85 expression by glucocorticoid treatment leads to reduction of PI3-K activity (25). This may be relevant, because glucocorticoid synthesis is increased in uremia (26), perhaps explaining the increase of p85 and the suppression of PI3-K activity (20) in the muscle of uremic rats.

The insulin-regulated glucose transporter (GLUT-4) is unique to muscle and adipose tissue. The abundance of GLUT-4 was similar in muscles of chronic kidney disease patients and healthy subjects (27). In the heart of uremic rats, however, we observed (in unpublished studies) diminished insulin-dependent glucose uptake and unchanged total GLUT-4, but reduced GLUT-4 incorporation into the plasma membrane. Carbamylation of proteins is common in uremia. Carbamylation of proteins involved in signal transduction and translocation of GLUT-4 has been shown to modify their structure and function. It is, therefore, of interest that N-carbamoyl-L-asparginine reduces insulin sensitive glucose uptake by interfering with GLUT-4 activity (28).

Peripheral resistance to insulin action is often found even early in the course of renal disease and is present in the majority of patients with advanced CKD. The resistance to the

TABLE 96-1

DIFFERENT PATHOMECHANISMS UNDERLYING ENDOCRINE DISORDERS IN CHRONIC KIDNEY DISEASE

Type of defect	Example
Abnormalities of hormone production/catabolism	
Reduced production of hormone in the kidney	Diminished or inappropriate concentrations of $1,25(OH)_2D_3$ and erythropoietin
Reduced production of hormone in extrarenal production sites (testis, ovary)	Diminished concentrations of testosterone and estrogen(s)
Abnormal secretion pattern (pulsatility; circadian rhythm)	PTH; GH, LH
Reactive hypersecretion of hormone to reestablish homeostasis	PTH, FGF 23
Inappropriate hypersecretion due to disturbed feedback	LH, prolactin, corticotropin
Decreased metabolic clearance	Particularly peptide hormones, e.g. PTH, insulin, gastrin, MSH, ghrelin, leptin, adiponectin
Abnormalities of hormone action	
Disturbed activation of prohormones	Proinsulin/insulin, proinsulin-like growth factor 1A/IGF-1, thyroxine (T_4)/triiodothyronine (T_3)
Increased isoforms with potentially less bioactivity (from glycosylation, sialysation)	LH
Increased hormone binding proteins reducing availability of free hormone	IGF
Decreased hormone binding proteins increasing availability of free hormone	Leptin
Abnormal target organ response	
Inhibitory factors	$PTH_{1,84}$ versus $PTH_{7,84}$ (?)
Changed receptor number, structure, modification	Low parathyroid vitamin D receptor
Disturbed postreceptor steps, e.g., signaling receptor	Insulin, GH

peripheral action of insulin is markedly improved after several weeks of hemodialysis (29–31) and of peritoneal dialysis (30–32). Presumably, a dialyzable uremic toxin, possibly a protein breakdown product, is involved in the genesis of deranged insulin action. Sera of uremic patients contain a compound that inhibits glucose metabolism by normal rat adipocytes (33). This compound with a molecular weight of 1 to 2 kDa is specific for uremia, because it is not found in nonuremic patients with insulin resistance. Hippurate accumulates in the blood of patients with chronic kidney disease and inhibits glucose utilization by rat diaphragm, brain, kidney cortex, and erythrocytes. It, therefore, may contribute to insulin resistance of patients with CKD (34). In hemodialyzed patients, insulin resistance is ameliorated by treatment with erythropoietin (35,36) or $1,25(OH)_2D_3$ (37,38) and in predialysis patients by restriction of dietary protein (39–41).

TABLE 96-2

GLUCOSE AND INSULIN METABOLISM IN PATIENTS WITH CHRONIC KIDNEY DISEASE

Usually normal fasting blood glucose, but tendency to spontaneous hypoglycemia
Fasting hyperinsulinemia with prolonged insulin half-life and elevated blood levels of proinsulin and C peptide
Decreased requirement for insulin by diabetic patients
Usually decreased early, but exaggerated late-insulin reponse to hyperglycemia induced by oral or intravenous glucose administration
Elevated plasma immunoreactive glucagon concentration
Impaired glucose tolerance(decreased peripheral sensitivity to insulin action, but normal suppression of hepatic glucose production by insulin)

Plasma concentrations of the insulin antagonists, glucagon (42,43) and growth hormone (44,45), are frequently elevated in CKD and it has been proposed that they contribute to insulin resistance. This is less likely in view of the demonstration that dialysis improves insulin sensitivity despite no change in the concentrations of growth hormone and glucagon (46,47). Tumor necrosis factor-α (TNF-α) (48,49) and angiotensin II (Ang II) (50,51) also interfere with insulin sensitivity, but whether they contribute to insulin resistance in CKD has not been explored.

Recently, it has been postulated that the adipose tissue-derived hormone, resistin, is involved in insulin resistance (52). Plasma resistin concentrations are high in CKD patients, probably because of diminished renal clearance (53), but no relationship was found in CKD between insulin sensitivity and plasma resistin (53).

A sedentary lifestyle causes insulin resistance (54). Conversely, exercise training improves insulin sensitivity, which is true for CKD patients as well (55,56).

Insulin Secretion and Pancreatic Islet Metabolism

Insulin secretion by β cells of the pancreatic islets is a complex process. The β cells are stimulated by nutrient (glucose,

amino acids, or fatty acids) or nonnutrient agents, such as hormones and neurotransmitters. The islets may use different mechanisms to detect nutrient and nonnutrient stimuli; insulin secretion, however, uses the same intracellular processes whether the β cells are activated by nutrient or nonnutrient secretagogs.

Glucose-induced insulin secretion begins with the uptake of glucose by the β cells, followed by its metabolism to produce adenine triphosphate (ATP). The latter facilitates the closure of ATP-dependent potassium channels, followed by cell depolarization, and subsequent activation of voltage-sensitive calcium channels. As a consequence, calcium enters the islets causing an acute rise in cytosolic calcium concentration and secretion of insulin.

Why is insulin secretion impaired in CKD? One factor is PTH. Insulin secretion assessed by the hyperglycemic clamp technique is improved when the parathyroid gland is suppressed (57,58). Glucose-induced insulin secretion by pancreatic islets is impaired in parathyroid intact uremic rats, but is normal in parathyroidectomized uremic rats (59,60). Conversely glucose-induced insulin secretion was impaired when rats with normal renal function received daily injections of PTH (59,61).

Apart from glucose, amino acids, most potently L-leucine (62), and potassium (63) stimulate insulin secretion as well. In CKD both L-leucine-induced (64) and potassium-induced (65) insulin secretion is impaired. The generalized failure of insulin secretion in response to secretagogs is, in major part, the result of the chronic excess of PTH (64,65).

$1,25(OH)_2D_3$ affects insulin secretion as well. Islet cells express the vitamin D receptor (66,67) and the vitamin D-dependent calcium-binding protein (68) and when one administers labeled $1,25(OH)_2D_3$, it is retrieved in the β cells (69). Insulin secretion is impaired in vitamin D-deficient rats with normal renal function (70); the defect is reversed by vitamin D (71). This may be relevant for CKD, because acute intravenous administration of $1,25(OH)_2D_3$ to dialysis patients improved the early and late phases of insulin secretion (72).

Insulin Clearance

The kidney plays an important role in insulin metabolism and clearance. Insulin is filtered by the glomeruli and reabsorbed in the proximal tubule (73). In normal subjects the renal clearance of insulin is about 200 mL/minute (74), the value exceeding glomerular filtration rate and indicating, in addition, peritubular uptake of insulin (75). It is estimated that daily 6 to 8 U of insulin are removed by the kidney, accounting for 25% to 40% of the removal of insulin.

The decrease in the metabolic clearance rate of insulin becomes apparent in patients with a glomerular filtration rate (GFR) less than 40 mL/minute and significant prolongation of the insulin half-life is observed when GFR falls below 20 mL/minute (76). When dialysis treatment is started, insulin clearance remarkably increases and, presumably, as a result, insulin removal by liver and muscle is increased.

In patients with CKD, diminished renal (76) and extrarenal (liver and muscles) (77) insulin clearance accounts for fasting hyperinsulinemia and higher insulin concentrations, after administration of glucose. Similarly, it accounts for decreased insulin requirements in diabetic patients with impaired renal function.

Hypoglycemia

Episodes of spontaneous hypoglycemia may occur in diabetic, but also in nondiabetic patients with CKD. In the diabetic patients, decreased degradation of the administered insulin may result in higher than expected blood insulin levels, which may precipitate hypoglycemia. In diabetic patients repeated episodes of hypoglycemia are occasionally the first clinical sign drawing attention to the presence impaired renal function. Careful adjustment of the insulin dose is then needed.

When diabetic patients with impaired renal function are treated with sulfonylurea compounds, it is important that many sulfonylurea compounds or their active metabolites are cleared via the kidney and accumulate in CKD patients (78,79). Appropriate dose adjustment is again necessary, but it is even better to switch the patients to insulin. The sulfonylurea gliquidone is eliminated predominantly by liver and does not accumulate, but long-term safety data are not yet available (80).

Spontaneous hypoglycemia has also occasionally been encountered in nondiabetic patients with CKD (81–83). The underlying mechanism is not clear. In one such patient, a reduction in hepatic glucose output, due to diminished availability of substrate for gluconeogenesis, was found (84). In another patient, spontaneous hypoglycemia occurred after parathyroidectomy, which was followed by a significant increase in insulin secretion (82). In a study of 21 nondiabetic hemodialysis patients treated with glucose-free dialyzate, nine developed hypoglycemia (84). Poor nutritional status, diminished gluconeogenesis, impaired glycogenolysis, and impaired degradation in insulin may all contribute to the spontaneous hypoglycemia (83). Hypoglycemia during hemodialysis with bicarbonate dialyzate has also been ascribed to diffusion of glucose into erythrocytes, due to greater consumption of glucose when changes in cytoplasmic pH accelerate anaerobic metabolism (85).

Clinical Consequences

Why is the impairment of glucose metabolism in renal failure clinically relevant? There are good arguments that hyperglycemia and insulin resistance contribute to accelerated atherogenesis in renal failure. Shinohara et al. (9) followed 183 nondiabetic hemodialysis patients for more than 5 years. Cumulative cardiovascular deaths by Kaplan–Meier estimation were significantly more frequent in subjects of the top tertile of insulin resistance assessed by the HOMA technique (HOMA IR higher than 1.4) compared to the lower tertiles (Fig. 96-1). The adverse effect of insulin resistance on mortality was independent on body mass, hypertension, and dyslipidemia. Moreover, hyperinsulinemia and insulin-resistance contribute to hypertension (86) and lipid abnormalities.

Insulin is also an important regulator of lipoprotein lipase activity and insulin deficiency or resistance to its action is associated with reduced activity of this enzyme (87). Lipoprotein lipase plays a major role in triglyceride removal. In patients with CKD lipoprotein lipase activity is impaired and this is the major cause of hypertriglyceridemia in these patients (88). Further support for the role of insulin in this regard is provided by the observation that administration of insulin to uremic rats corrected the defect in lipoprotein lipase activity as well as the hypertriglyceridemia (89).

Insulin resistance may also participate in pathogenesis of malnutrition, which is commonly found in CKD patients. In rats with acute renal failure protein degradation in perfused or incubated muscle is increased. This defect is closely related to abnormalities in insulin-dependent carbohydrate metabolism (90). Insulin deficiency stimulates breakdown of muscle and activates a common proteolytic pathway via the ubiquitin–proteasome system (91,92). Therefore, insulin resistance may contribute to increased protein catabolism and malnutrition in CKD patients.

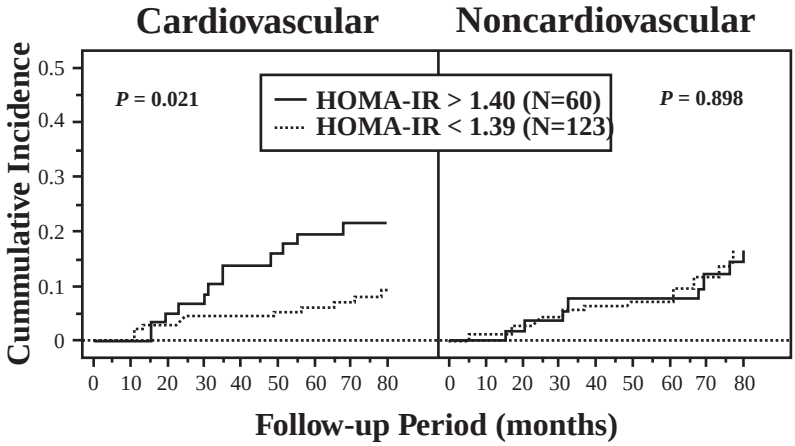

FIGURE 96-1. Cumulative incidence of cardiovascular and noncardiovascular deaths by Kaplan–Meier estimation in 183 hemodialysis patients of top tertile of HOMA IR (HOMA IR higher than 1.4), and those in the lower tertiles (From: Shinohara K, Shoji T, Emoto M, et al. Insulin resistance as an independent predictor of cardiovascular mortality in patients with end-stage renal disease. *J Am Soc Nephrol* 2002;13:1894, with permission).

DISORDERS OF LIPID METABOLISM IN CHRONIC KIDNEY DISEASE

Richard Bright commented on the milky aspect of the serum of patients suffering from kidney diseases (93). Since this early observation, dyslipidemia and hyperlipidemia of renal patients had been well known, but only recently did it attract more general interest after it had been recognized that atherosclerotic complications are extremely frequent in patients with impaired renal function, raising suspicion that this was the result of dyslipidemia.

It had long been underappreciated how severe dyslipidemia actually is, because measurements were usually restricted to the determination of total cholesterol and triglycerides in plasma. Only today's more sophisticated analyses of lipid subfractions, postprandial lipid changes, apolipoprotein concentrations and modification by oxidation, glycation, and carbamylation have fully disclosed the profound and highly atherogenic character of the lipid changes in uremia.

Lipids, Lipoproteins, and Lipid Transport and Metabolism

Some background information is useful to understand the changes of lipoprotein metabolism in renal disease. Lipids circulating in plasma are incorporated into spherical, water-soluble particles (lipoproteins) that contain a core of nonpolar lipids (cholesterol, cholesterol esters, and triglycerides) surrounded by a monolayer composed of specific proteins (apoproteins), polar lipids, and esterified cholesterol and phospholipids. This outer monolayer permits the lipoprotein to remain dissolved in plasma. Lipoproteins are an efficient vehicle for the transport of triglycerides and cholesterol in plasma (94–96).

Lipoproteins can be separated by electrophoresis and ultracentrifugation in salt solutions (Fig. 26-2). Lipoprotein fractions are characterized on the basis of their separation by ultracentrifugation, according to their density. The density of lipoproteins differs widely. Four main classes of lipoproteins are present in the plasma of fasting individuals: very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL). Chylomicrons (large triglyceride-rich particles of intestinal origin) are the fifth class of lipoproteins. These particles are usually present in the plasma only transiently during the postprandial state. Each of these lipoproteins is heterogeneous, however, with respect to size, lipid composition, and apoprotein content. Subfractions of the major lipoprotein classes can be isolated by electrophoresis, gradient ultracentrifugation, or affinity column chromatography. A sixth lipoprotein subfraction, Lp(a), has a hydrated density between 1.06 and 1.08 g/mL. Lp(a) lipoprotein consists of an LDL particle to which an additional protein, apoprotein (a), is covalently bound (97).

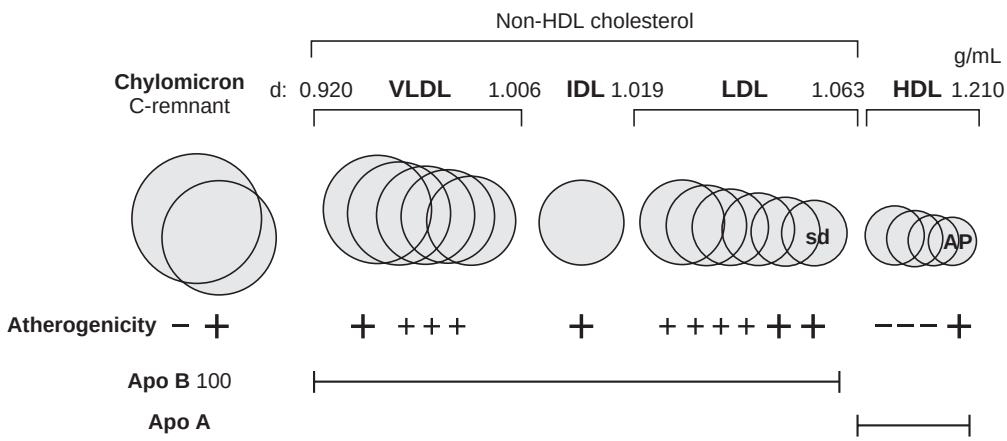

FIGURE 96-2. Atherogenicity of major lipoprotein classes (From: Otvos J. Measurement of triglyceride-rich lipoproteins by nuclear magnetic resonance spectroscopy. *Clin Cardiol* 1999;22:21, with permission).

TABLE 96-3

METABOLIC FUNCTIONS OF PLASMA APOPROTEINS[a]

Apoprotein	Molecular weight	Metabolic role
A-I	28,000	Activates LCAT
A-II	17,500	Activates hepatic lipase; may inhibit LCAT
A-IV	46,000	Unknown, possibly involved in lipid transfer between lipoproteins
B-48	264,000	Transport of lipids from the gut as chylomicrons
B-100	512,000	Transport of lipids from the liver as VLDL and LDL; recognized by cellular LDL receptors
C-I	7,000	Activates LCAT
C-II	9,000	Activates lipoprotein lipase
C-III	9,000	May inhibit activation of lipoprotein lipase by apo C-II
D	22,000	May be involved in lipid transfer between lipoproteins
E	34,000	Recognized by hepatic apo E receptors and cellular LDL receptors; recognition facilitates hepatic uptake of chylomicron and VLDL remnants
Cholesterol ester transfer protein (CETP)	66,000	Facilitates the exchange of cholesterol esters and triglycerides between HDL and VLDL and LDL

(Adapted from Illingworth DR. Lipoprotein metabolism. *Am J Kidney Dis* 1993;22:90, with permission.)
[a] *LCAT,* lecithin–cholesterol acyltransferase; *LDL,* low-density lipoprotein; *VLDL,* very-low-density lipoprotein.

The protein components of lipoproteins (apoproteins) are classified by letters of the alphabet and Roman numeral suffixes (Table 96-3). Some of these apoproteins, such as apoprotein A-I in HDL, apoprotein B-100 in LDL, and apoprotein B-48 in chylomicrons, play a structural role. Some apolipoproteins are ligands for receptors such as apoprotein B-100 for the LDL receptor and apoprotein E for the chylomicron remnant receptor. The potential functions of some of these apoproteins are listed in Table 96-3.

Four enzymes play a pivotal role in lipid metabolism: lipoprotein lipase (98), hepatic triglyceride lipase (HTGL) (99), lecithin–cholesterol acyltransferase (LCAT) (100), and a specific transfer protein, cholesteryl ester-transfer protein (CETP) (101,102).

The major physiologic action of *lipoprotein lipase* is to degrade triglyceride-rich particles in the postprandial state. The activity of the enzyme is dependent on the presence of a cofactor, apoprotein C-II. The enzyme apparently exerts its action at the level of the endothelial surface. It releases fatty acids that can be utilized to satisfy energy needs or can be stored in adipose tissue, depending on the metabolic conditions. As a consequence of hydrolysis, the core size of VLDL particles decreases. They lose a portion of the surface components, such as phospholipids and proteins, and become smaller and more dense (98).

Hepatic lipase has two major actions: (a) hydrolysis of triglycerides and phospholipids of HDL_2, and (b) hydrolysis of triglycerides of VLDL and IDL (99).

The enzyme *LCAT,* which is synthesized in the liver, catalyzes the formation of cholesterol esters in the plasma by transferring a fatty acyl chain from phospholipids to the hydroxy group of unesterified cholesterol. This action, which occurs in the circulation, accounts for the balance between cholesterol on the surface and in the core of lipoprotein particles (100).

In addition, a specific transfer protein, *CETP*, facilitates the exchange of cholesterol esters and triglycerides between HDL and VLDL and LDL (101,102).

Metabolism of Very-Low-Density Lipoproteins

The liver is the main site of VLDL synthesis. VLDL particles transport endogenous triglycerides from the liver to peripheral tissues. The intravascular hydrolysis of VLDL triglycerides depends on the activity of lipoprotein lipase. Remnant particles that result from VLDL lipolysis are either taken up by the liver via apo E-mediated metabolism or converted to LDL (Fig. 96-3). LDL is the major product of VLDL metabolism in human plasma (Figure 96-4). Several factors, including diet, plasma concentrations of free fatty acids as well as plasma concentrations of insulin, glucagon, and epinephrine modulate the secretion of hepatic VLDL, a major determinant of VLDL concentrations in plasma. In subjects with normal triglyceride concentrations, all the apo B that enters plasma as a component of VLDL is preserved when the particle is metabolized to IDL and to LDL. In contrast, in patients with severe hypertriglyceridemia, most of the VLDL particles are removed before they can be converted to LDL. As a result the plasma concentration of LDL is then low. Moderate hypertriglyceridemia, that is, plasma triglycerides of 300 to 800 mg/dL, usually reflects the accumulation of endogenous VLDL particles in plasma. Such an accumulation may result from enhanced rates of VLDL synthesis, genetic or acquired defects in triglyceride hydrolysis, or combinations of both (103).

Metabolism of Low-Density Lipoproteins

LDL is the major cholesterol-carrying particle present in human plasma and is the end product of VLDL catabolism. The liver accounts for 70% of the catabolism of LDL and peripheral tissues account for the remaining 30%. Both receptor-mediated and nonreceptor-mediated pathways facilitate LDL catabolism. In normal subjects, receptor-mediated pathways account for about 75% of the clearance of plasma LDL. The uptake of LDL by receptor-mediated endocytosis results in the suppression of endogenous cholesterol biosynthesis,

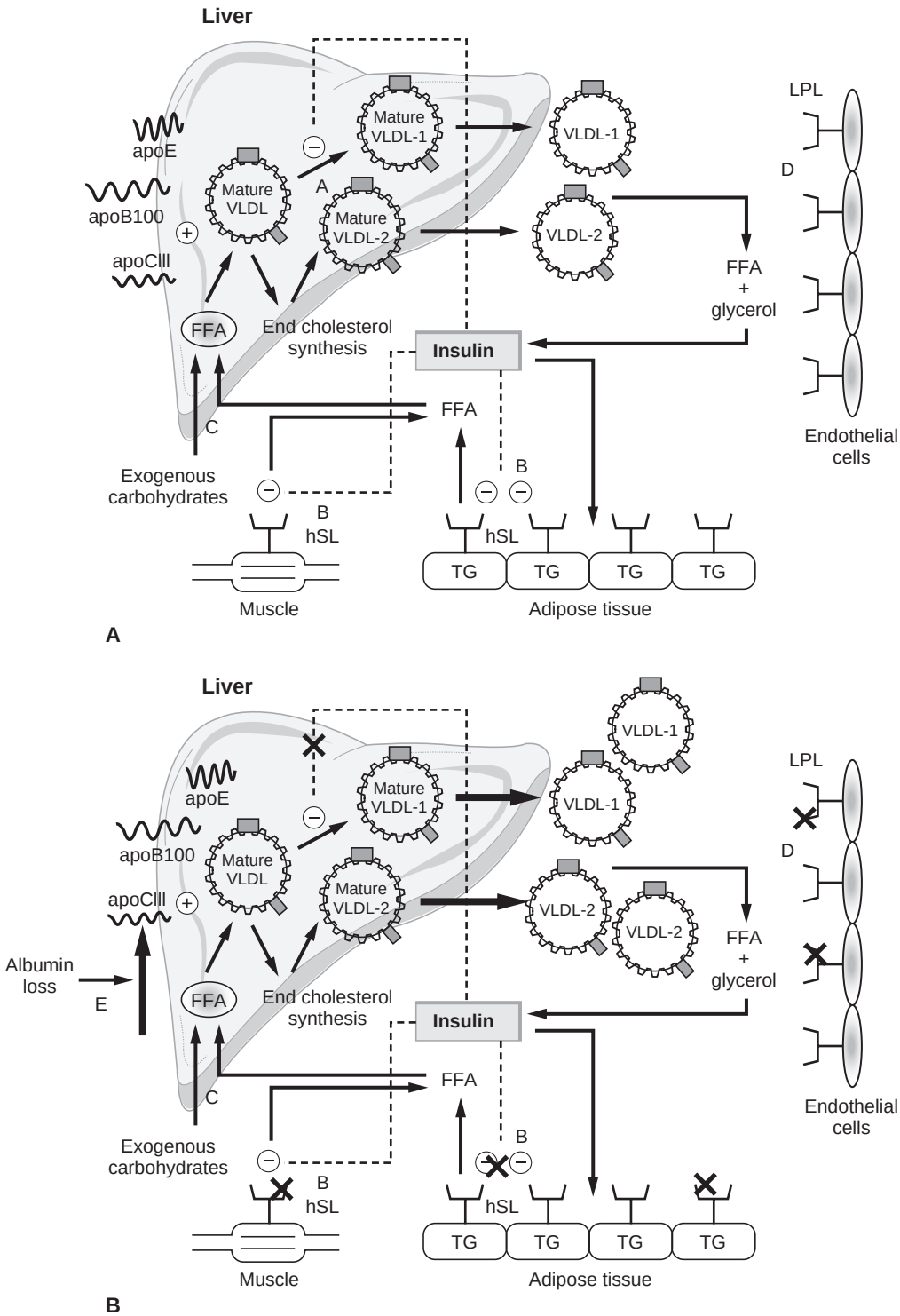

FIGURE 96-3. Triglycerides metabolism in normal subjects (**A**) and in chronic kidney disease patients (**B**). *FFA,* free fatty acids; *hSL*, lipase; *TG*, triglycerides; *LPL*, lipoprotein lipase; *VLDL*, very-low-density lipoproteins (From: Prinsen BH, de Sain-van der Velden MG, de Koning EJ, et al. Hypertriglyceridemia in patients with chronic renal failure: possible mechanisms. *Kidney Int* 2003;63:S121, with permission).

an enhanced rate of intracellular cholesterol esterification, and a reduction in the number of high-affinity LDL receptors expressed on the cell surface (95). Hypercholesterolemia, with increased plasma concentrations of LDL cholesterol, may be the consequence of genetic and acquired disorders the underlying mechanisms comprising: (a) an enhanced rate of VLDL and LDL apo B synthesis, (b) defects (genetic or acquired) of LDL catabolism (e.g. impaired expression of high-affinity LDL receptors or modifications in the receptor binding domain of LDL), or (c) a combination of both resulting in enhanced synthesis plus impaired catabolism (94,95).

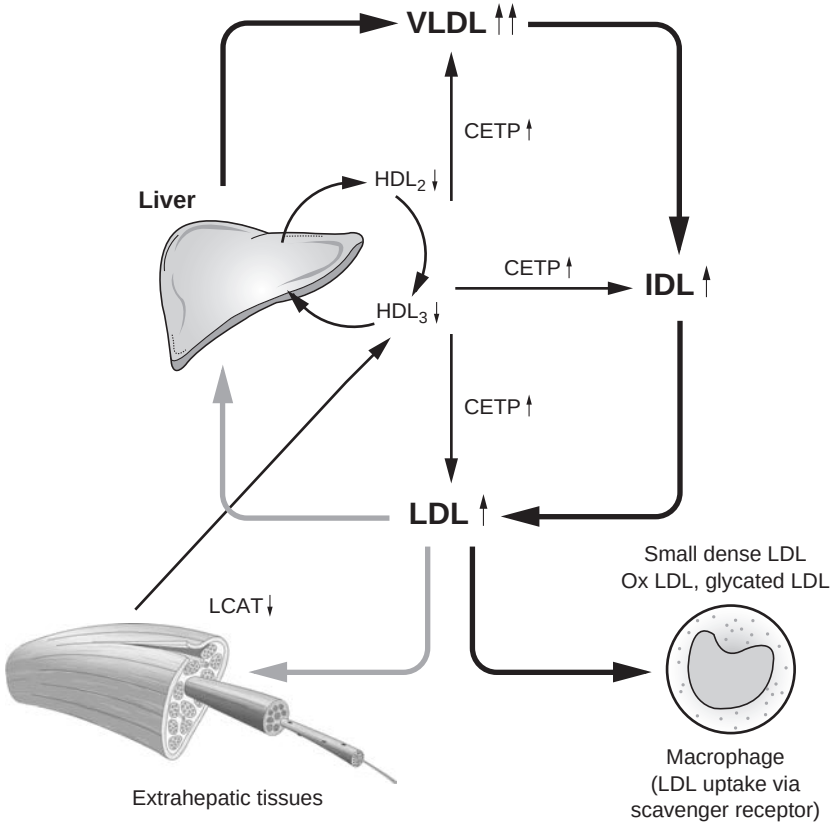

FIGURE 96-4. Lipoprotein metabolism in chronic kidney disease patients. *LCAT*, lecithin-cholesterol acyltransferase; *CETP*, cholesterol ester transfer protein; *LPL*, lipoprotein lipase, *VLDL*, very-low-density lipoproteins; *IDL*, intermediate density lipoproteins, *LDL*, low-density lipoproteins.

Metabolism of High-Density Lipoproteins

HDL is generated in the liver and from the intravascular lipolysis of VLDL particles and chylomicrons. The newly synthesized HDL contains predominantly protein, free cholesterol, and phospholipids, but when exposed to LCAT becomes enriched in cholesterol esters (106). The apoprotein content of individual HDL particles differs. Some HDL particles contain apo A-I and apo A-II (HDL_3), whereas others contain only apo A-I (HDL_2). The half-life of HDL is from 4 to 6 days and is influenced by diet, as well as by several drugs. Epidemiologic studies in the general population documented an inverse relation between plasma HDL cholesterol concentration and risk of coronary artery disease (94,104).

In summary, the general mechanisms of transport and metabolism of lipoproteins in humans are relatively well understood. Numerous factors, however, both genetic and acquired, may modify this transport system and result in abnormal concentrations of lipoproteins in plasma. Increased plasma concentrations of cholesterol-rich VLDL, of chylomicron remnants, of small, dense LDL and of Lp(a) are highly atherogenic. Increased concentrations of HDL cholesterol protect against atherosclerosis. Covalent modification of lipoproteins, particularly oxidation, glycosylation and carbamylation of LDL, has a profound impact on their metabolism and causes particularly enhanced uptake by scavenger receptors of tissue macrophages, thus causing foam cell formation (105–107).

Abnormalities of Lipoprotein Metabolism in Chronic Kidney Disease

The mechanisms by which loss of renal function affects lipoprotein metabolism are not completely understood. The dyslipoproteinemia found in CKD patients is usually secondary to their renal disease (108,109), but, of course, may also be the result of genetic abnormalities (or a combination of both). The family history or examination of family members may provide useful hints for a potential role of genetic factors. Comorbid conditions can further alter the lipid pattern and should also be considered, e.g., diabetes mellitus, steroid treatment, and nephrotic proteinuria.

Some lipid abnormalities are demonstrable in patients with renal disease even when GFR is still normal (110–113), but disorders of lipoprotein metabolism increase in frequency and severity as renal insufficiency advances. The typical profile of patients with chronic kidney disease, that is, the constellation of moderate elevation of plasma triglyceride concentrations, combined with low plasma HDL-cholesterol, corresponds to the pattern of dyslipidemia type IV according to Frederickson et al. (114). In CKD, total cholesterol is usually normal or even low. This may be the result of an additional microinflammatory state and/or malnutrition (115) (Table 96-4).

The prevalence of dyslipidemia in patients with chronic kidney disease is greater than in the general population (116). The characteristic features of dyslipidemia remain essentially unchanged during long-term conventional hemodialysis. (117,118). Studies on the influence of high-flux dialysis modalities have yielded conflicting results (119–123). However, in the majority of studies improvement of lipid profile after treatment with high-flux dialysis was found (119,122,123). Recently, Wanner et al. (123) showed in a randomized crossover study that 6 weeks of treatments with high-flux polysulfone and modified cellulose membrane significantly lowered serum triglycerides concentration when compared with low-flux dialysis with polysulfone membrane. Significant improvement of lipid profile was also observed when patients were switched from conventional hemodialysis to home-based nocturnal hemodialysis (124). In patients treated by CAPD, the concentrations of total plasma cholesterol, LDL cholesterol, and triglycerides are

TABLE 96-4

ABNORMALITIES OF LIPID METABOLISM IN PATIENTS WITH CHRONIC KIDNEY DISEASE

Quantitative changes in plasma lipid profile
Moderate elevation of plasma triglyceride concentrations
Low plasma HDL-cholesterol concentration
High plasma VLDL and IDL-cholesterol
Normal plasma LDL cholesterol
High ratio total cholesterol/HDL cholesterol
High ratio LDL cholesterol/HDL cholesterol
Quantitative changes in plasma lipoproteins
Decreased plasma concentrations of apo A-I and A-II
Normal or elevated plasma concentration of apo B
High plasma concentrations of apo C-I, C-II, C-III
Postprandial changes in plasma lipoproteins
Prolonged persistence of chylomicrons in the circulation postprandially
Qualitative lipoprotein changes
Postribosomal modification of apolipoproteins by oxidation, glycation and carbamylation
Alteration in HDL component (changed it from antioxidant to pro-oxidant lipoprotein)
Accumulation of small dense LDL
Atherogenic apo(a) phenotype (low molecular weight)

[a] *LDL,* low-density lipoprotein; *VLDL,* very-low-density lipoprotein; *IDL,* intermediate-density lipoprotein; *HDL,* high-density lipoprotein

even higher than in hemodialysis patients (125–128). Such aggravation is most likely due to two additional factors: loss of protein (7 to 14 g/day) with peritoneal dialyzate and absorption of glucose (150 to 200 mg/day) from the dialysis fluid (129,130). The protein loss may concern not only albumin, but also apolipoproteins, and possibly further lipoprotein- regulating substances, as occurs in the nephrotic syndrome. The glucose load increases the availability of free fatty acids and stimulates the synthesis of triglycerides and lipoproteins by the liver (131). This hypothesis is supported by the observation that conversion of patients from conventional glucose containing dialysis fluids to icodextrin containing dialysis fluids in the overnight dwell reduced plasma cholesterol concentrations (132).

The most frequent constellation of lipid abnormalities in CKD comprises a decrease in HDL-cholesterol, an increase in VLDL- and IDL-cholesterol, as well as normal or even low LDL cholesterol (reflects a redistribution of cholesterol from HDL to VLDL and IDL) (133–135). The ratios of total cholesterol/HDL cholesterol and LDL cholesterol/HDL cholesterol are usually increased. The reduced levels of HDL reflect reductions of both HDL_2 and HDL_3 subfractions (136). In CKD, a defect in cholesterol transport (137), probably due to diminished LCAT activity (138,139), and increased activity of CETP, have been documented (140). The recently proposed index of non-HDL-cholesterol, reflecting the sum of LDL and VLDL particles appears to be more sensitive and is a superior predictor of cardiovascular risk (141,142) (Table 96-4).

Hypertrigliceridemia is commonly found in CKD (116) and is the result of both increased synthesis of triglycerides and VLDL apo B100 apoprotein in the liver and decreased catabolism of VLDL (143,144). Increased triglyceride and VLDL apo B100 synthesis in the liver were documented in animal experiments and in CKD patients (Fig. 96-3) (144–147). Triglyceride-rich lipoproteins are sequentially broken down by lipoprotein lipase, followed by further catabolism via hepatic triglyceride lipase, and finally taken up by apo B and apo E receptors in the liver (98,99,103,143). The activity of lipoprotein lipase in plasma and in adipose tissue is decreased in animal experiments and in patients with CKD, even when the plasma triglyceride concentration is normal (148–152). Insulin resistance, which is usually present in patients with chronic kidney disease, also contributes to diminished lipoprotein lipase activity (88). The activity of lipoprotein lipase is differentially influenced by the apolipoprotein C isoforms: apolipoprotein C-II activates and apolipoprotein C-III inhibits lipoprotein lipase activity (153). A decreased ratio of apo C-II/C-III is commonly found in uremia (113). This abnormality is observed even in the early stage of CKD (153). However, not only the activity of lipoprotein lipase, but also that of hepatic triglyceride lipase is decreased in animal models and patients with CKD (149,150,154,155). The decreased activities of both lipases cause a major defect in the catabolism of triglyceride-rich lipoproteins. Reduced lipoprotein lipase activity explains the disturbed first step in the breakdown of both chylomicrons (circulating after absorption of fat from the gut) and of VLDL (synthesized and secreted by the liver). Because of the reduced activity of the hepatic triglyceride lipase, the second step—i.e., the clearance of partially metabolized lipoproteins and chylomicrons is disturbed as well. The VLDL receptor is expressed in skeletal muscle, heart, brain, and adipose tissue, which use fatty acids for energy production or storage. The receptor binds and internalizes VLDL particles. The expression of the VLDL receptor was reduced in experimental uremia (156).

Disorders of lipid metabolism in chronic kidney disease are not adequately reflected by the conventionally measured parameters, that is, plasma concentrations of total cholesterol, LDL-cholesterol, and triglycerides. The above parameters do not provide information on further lipid abnormalities, which almost certainly impact on the atherogenic risk: (a) abnormal concentrations of apolipoproteins (low apo A1 and apo A2; high apo B, apo C-II and apo E serum concentrations), (b) postribosomal modification of apolipoproteins by oxidation, glycation, and carbamylation, (c) inflammation-induced alterations of HDL (transforming HDL from an antioxidant to a pro-oxidant lipoprotein), (d) accumulation of IDL and small, dense LDL, (e) prolonged postprandial persistence of chylomicrons in the circulation, and (f) atherogenic apo(a) genotypes (Table 96-4).

There are also abnormalities of apolipoproteins in CKD. The concentrations of apo A-I and A-II are decreased. Significantly reduced levels of apo A-I or apo A-II are present in CKD patients even when plasma lipid concentrations are in the normal range (117). In hemodialysis patients it has recently been documented that decreased apo A-I levels are due to an increased rate of catabolism, whereas reduced apo A-II levels are primarily due to a decreased rate of production (157). These apolipoprotein abnormalities are more marked in patients with advanced renal failure and in patients with hypertriglyceridemia (158,159). The apo B concentrations are usually normal in early stages of CKD and are minimally elevated, at best, in patients with advanced renal insufficiency (112,127,158,159). In addition, the concentration of apo C-III, and less pronounced also the concentrations of apo C-I and C-II, are usually increased (159–161). Increased concentrations of apo C-III tend to correlate with the plasma triglycerides concentration, but may be found even in patients with normal triglycerides (159,162). Elevated plasma concentrations of apo E are indirect evidence of accumulation of remnants (113). The ratios of apo A-I/C-III and apo A-I/B are reduced, and the ratio of apo C-III/apo E is increased (112,134,159).

We reemphasize that, in early stages of CKD, disturbances of apoprotein metabolism may be found even in the presence of normal plasma lipid concentrations. In patients with GFR >15 mL/minute and normal plasma lipid concentrations, a

significant decrease in the concentrations of apo A-I and A-II and increase in the concentrations of apo C-III were found (112,113). Similar abnormalities of apo B, C-III, and E concentrations can also be found, however, in patients with incipient to moderately advanced CKD (111,113). Even in CKD patients with normal GFR increased apo A-IV concentrations were found (110).

In addition to quantitative changes in lipoprotein particles several qualitative lipoprotein changes have been demonstrated to occur in CKD. These include postribosomal modification of apolipoproteins by oxidation (163), glycation (164), and carbamylation (165). Modified lipoproteins are not recognized by their respective receptors (166). Their half-life in the circulation is increased. The prolonged residence time in the circulation permits their uptake by the nonsaturable scavenger receptor pathway. Oxidation does not reduce the affinity of oxidized LDL to the scavenger receptor (105) and oxidized LDL uptake by the macrophage scavenger receptor is increased favoring the formation of foam cells (105). In addition to its pivotal role in foam cell formation, oxidized LDL exhibits additional atherogenic properties, including cytoxicity and stimulation of thrombotic as well as inflammatory events (167). LDL oxidation is currently considered as a key early event in the pathogenesis of atherosclerosis. HDL protects against oxidation of LDL (168,169). In hemodialysis patients, the capacity of HDL to prevent LDL oxidation is reduced, however (170). The acute phase response changes the HDL composition, transforming HDL from an antioxidant to a pro-oxidant lipoprotein (171). The reported alterations include an increase in apolipoprotein J and serum amyloid A and a decrease paroxonase activity and platelet-activating factor (PAF) (171). Paroxonase, a HDL-associated enzyme, reduces the susceptibility of LDL to oxidation (172). Serum paroxynase activity is reduced in patients with CKD (173–175) with potential repercussions on HDL and LDL structure and function.

IDL, an intermediate of VLDL catabolism, accumulates in the plasma of CKD patients (154,176,177). The IDL concentration is a predictor of the severity or progression of atherosclerosis (178). In a study of 280 hemodialysis patients, Shoji et al. (179) demonstrated that plasma IDL concentration is an independent risk factor for aortic atherosclerosis as determined by pulse-wave Doppler sonography.

LDL particles are heterogeneous in size and density (180). In 1988, Austin et al. (180) found that small, dense LDL particles increased the risk of myocardial infarction threefold. They also found that small, dense LDL were associated with increased plasma triglyceride and decreased HDL cholesterol concentrations. No correlation was found between LDL-cholesterol concentration and LDL size (181). Small, dense LDL accumulate even in early stages of CKD, when total plasma cholesterol and triglyceride concentrations are still normal (183). In hemodialysis patients the increase in small dense LDL is particulary pronounced in diabetic and hypertriglyceridemic patients (152). In hemodialysis patients, a strong correlation is found between the concentrations of plasma triglycerides and small dense LDL. A plasma triglyceride concentration >2 mmol/L had a sensitivity of 86%, and a specificity of 79% to identify hemodialysis patients with atherogenic plasma concentrations of small, dense LDL (183). Several theories have been proposed regarding the link between small, dense LDL and atherogenicity: (a). their decreased affinity to the LDL receptor (184,185) combined with their increased clearance via the scavenger receptor (185), (b). their increased susceptibility to oxidation and glycation (186), (c). their increased transcapillary escape because of smaller particle size (187), and (d). their greater affinity to extracellular matrix, such as arterial wall proteoglycans (188).

Chylomicrons, that is, large triglyceride-rich particles of intestinal origin, are only transiently present in plasma in the postprandial state under physiological conditions (189). It has been postulated that prolonged postprandial persistence of chylomicrons in the circulation causes endothelial damage and promotes atherosclerosis (189). In CKD patients, the clearance of chylomicrons is severely impaired (190). This abnormality may contribute to the hypertriglyceridemia in CKD.

Lp(a) lipoprotein is a plasma lipoprotein, which contains a protein component called apo(a). Apo(a) exhibits high homology to plasminogen (97) and extreme size polymorphism, depending on the number of repeats; the apo(a) isoproteins range in size from 420 to 840 kDa (97). The main modulator of apo(a) size is genetics. There are numerous apo(a) gene variants (97) and an inverse correlation is found between apo(a) size and plasma Lp(a) concentration (191). In the general population, high plasma concentration of Lp(a) are associated with increased cardiac risk (192). In patients with chronic renal failure, prospective studies on the influence of plasma Lp(a) concentration on atherosclerotic complications have yielded conflicting results (193–196). In cross-sectional studies, a higher frequency of apo(a) low-molecular-weight phenotypes [small apo(a)] was found in hemodialysis patients with atherosclerotic complications (197–199). In prospective studies, Kronenberg et al. (200) and Longenecker et al. (201) found in hemodialysis patients that the small apo(a) genotype predicted coronary events and total mortality (200,201). Hervio et al. (202) reported that small apo(a) isoforms bind more strongly to fibrin than large isoforms—a possible mechanism how apo(a) isoform size might accelerate atherogenesis.

Dyslipidemia and Outcome—An Example Of Reverse Epidemiology

Following the seminal report of Degoulet et al. (203), numerous investigators found a paradoxical inverse relationship between plasma cholesterol concentration and overall mortality, as well as cardiovascular mortality (204,205). Usually a U- or J-shaped relationship was noted between plasma cholesterol concentration and cardiovascular mortality—i.e., a higher mortality at low as well as high plasma cholesterol concentrations (204). The most plausible explanation for this paradox is that this represents an example of reverse epidemiology (206), i.e., a relationship, which is reversed by a confounding factor. The recent work of Liu et al. (207) is important in this respect (207). They identified microinflammation as a confounding factor. In dialysis patients with low hsCRP concentrations a direct positive relation was noted between LDL cholesterol and CV mortality as in individuals with no renal disease. In contrast, in patients with high hsCRP concentrations, the mortality was higher at low LDL cholesterol concentrations (Fig 96-5) (207). This finding is important, because in such circumstances serum cholesterol and LDL cholesterol concentrations may no longer be a valid guide to establish the indication for lipid-lowering therapy. This may also explain the recent negative outcome of an intervention trial in diabetic hemodialysis patients treated with atorvastatin (208).

Treatment of Dyslipidemia in Renal Failure

Because of the limited predictive power of abnormal lipid concentrations and the negative outcome of a recent intervention trial, the indications for lipid lowering in CKD patients are currently not well established (208). There is no doubt that in early stages of renal dysfunction lipid lowering by statins is effective. This is indicated by the reduction of cardiovascular events in the subgroup of patients with impaired renal function observed in several recent controlled trials, e.g., the ALERT study in renal graft recipients (209), the ASCOT study

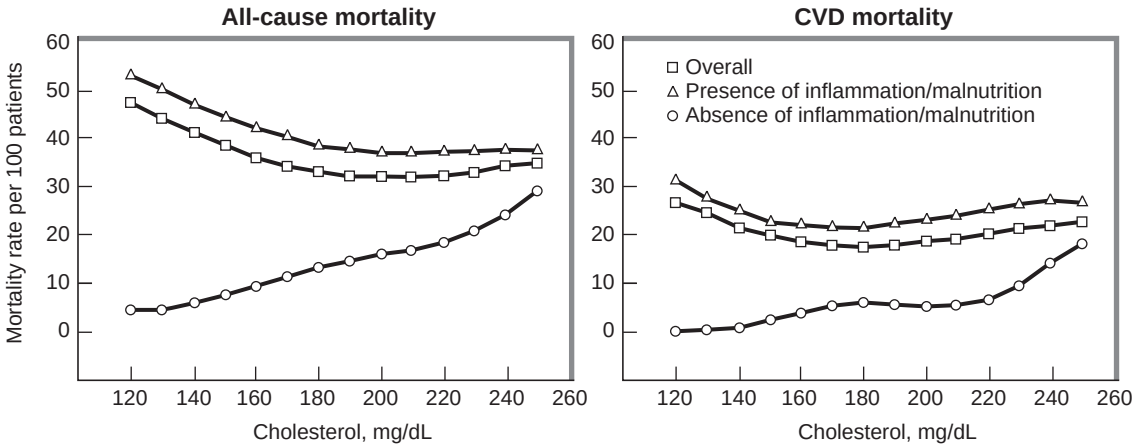

FIGURE 96-5. Estimated 3 years all-cause and cardiovascular mortality (CVD) in a study of 832 hemodialysis patients. In hemodialysis patients with low hsCRP concentrations, the anticipated direct relationship between LDL cholesterol and CV mortality was noted. In contrast, in patients with higher hsCRP concentrations the mortality was higher at low LDL cholesterol concentrations. (From: Liu Y, Coresh J, Eustace JA, et al. Association between cholesterol level and mortality in dialysis patients: role of inflammation and malnutrition. *JAMA* 2004;291:451, with permission.)

(210), and CARE study (211) in patients at high CV risk. A retrospective comparison of survival in hemodialysis patients with and without statin administration had also suggested a significant survival benefit in patients on statins (212). A note of caution was suggested, however, by the observation of Fathi et al. (213). They compared coronary artery disease (CAD) in patients without kidney disease and patients with advanced CKD. In both groups LDL cholesterol was lowered to values substantially below 100 mg/dL. The maximum intima media thickness of the carotid artery, however, decreased in patients with CAD, but failed to change in patients with chronic renal failure. This observation possibly indicates that alternative pathogenetic pathways are operative in advanced CKD and negate the beneficial effect of lipid lowering. This notion is supported by the results of the 4D study in hemodialyzed type 2 diabetic patients randomized to atorvastatin or placebo. Despite substantial lowering of LDL cholesterol, the primary endpoint of cardiovascular events was not significantly altered, although the combined cardiac events were lowered by 18% (208). At any rate, the efficacy of statins was significantly lower than in nonrenal patients with type 2 diabetes in whom cardiovascular events were lowered by 37% (214).

Which intervention strategies do we have? There is no doubt that dyslipidemia in patients with advanced CKD can be modified by dietary interventions, that is, reduction of saturated fatty acids and reduction of carbohydrate intake to reverse VLDL overproduction by the liver and lower plasma triglyceride levels (215–217). It is also possible to achieve weight loss in obese patients with advanced chronic kidney disease. These interventions have not gained universal acceptance. Such lifestyle changes are difficult to implement in chronic patients. Moreover, the safety of dietary interventions in patients with advanced chronic renal failure who are prone to catabolism and malnutrition is uncertain. A recent reanalysis of the participants in the MDRD trial (218) found clear evidence of malnutrition in patients randomized to a low-protein diet. Conversely, a high body mass index (BMI) is predictive of better survival in hemodialyzed patients and this is true even in the range of morbid obesity (219). As a result, these interventions are sensible in the early stages of CKD, but, in our view, are no longer safe and indicated in advanced stages. Another nonpharmacological approach is physical exercise, which has been shown to reduce insulin resistance and improve the lipid pattern in CKD patients as it does in nonrenal patients (55,56). Again, in our experience, adherence to such interventions is less than optimal.

In patients on hemodialysis, improvement of dyslipidemia has been shown in the studies comparing hemodialysis with high-flux membranes versus conventional dialysis (119,122,123) and more recently in patients on nocturnal hemodialysis (124).

What is the role of pharmacological treatment?

Probucol causes a modest reduction of LDL cholesterol concentrations. It also reduces the concentrations of HDL cholesterol. Its major benefit may be related to its antioxidant effects. This drug appears to be devoid of major side effects and in CKD patients experience with this drug, which is no longer available, is limited.

Nicotinic acid lowers plasma LDL cholesterol concentration, but is likely to cause side effects, such as flushing, worsening glucose tolerance, and hepatotoxicity. Studies investigating the effect of nicotinic acid in hemodialysis patients are sparse (220,221).

Bile acid sequestrants are poorly tolerated and cause gastrointestinal distress and constipation when not taken with considerable amount of fluids. Bile acid sequestrants are also contraindicated in hypertriglyceridemia and are likely to further increase plasma triglyceride concentration. Therefore, probucol, nicotinic acid, and bile acid sequestrants are not recommended in hemodialysis patients.

The pattern of hypertriglyceridemia with low-plasma HDL cholesterol concentration appears, at first sight, as an ideal indication for a PPAR-alpha agonist, that is, a fibric acid analog. These agents effectively lower triglyceride and raise HDL concentrations (222–225). The problem is that they accumulate in renal insufficiency. Therefore, except for gemfibrozil, the dose must be reduced in CKD patients as a function of GFR (224). Accumulation may cause myopathy and rhabdomyolysis. The use of fibrates has, therefore, not gained wide acceptance and their use requires caution (226).

Statins lower plasma total cholesterol and LDL cholesterol concentrations effectively and safely (227); atorvastatin also lowers triglyceride concentrations (228). There are also indications from experimental studies (229) and clinical observations (230) that statins reduce progression of renal failure, possibly as a consequence of their pleiotropic effects. The latter result from the fact that isoprenylation of small G proteins modulates signaling pathways independent of cholesterol. The clinical

relevance of this action is currently uncertain. As outlined above, statins are safe and effective in early stages of CKD. The efficacy of their use in advanced stages is less certain (208), but this issue requires further studies (231).

DISORDERS OF PROTEIN AND AMINO ACID METABOLISM IN CHRONIC KIDNEY DISEASE

Nutrients can be divided into six general classes: proteins, lipids, carbohydrates, minerals, vitamins, and water. The first three classes are organic compounds that serve as sources of energy required for carrying out the biochemical and functional activities of organs and cells (232). In addition to being an energy source, proteins in the diet provide the amino acids that are used to synthesize body proteins. Proteins and their constituent amino acids are essential to life.

The protein requirement of an individual is defined as the lowest level of dietary protein intake that will balance the losses of nitrogen from the body and maintain energy balance at modest levels of physical activity. The need for dietary protein largely arises because turnover of tissue and organ proteins is accompanied by an inefficient capture of their constituent amino acids to form new body proteins. The amino acids are lost via oxidative metabolism. Most estimates of protein and amino acid requirements in humans have been obtained directly or indirectly from measurements of nitrogen balance. In the course of carrying out their functional roles, proteins and amino acids turn over, and part of their nitrogen and carbon is lost via excretory pathways. This includes carbon dioxide in expired air and urea and ammonium in urine. Thus, to maintain an adequate protein and amino acid balance, these losses must be replaced by an appropriate dietary supply of a usable source of nitrogen and by indispensable and conditionally indispensable amino acids. These are required to replace amino acids that are lost during the course of metabolic processes or those that are deposited during growth and tissue replacement. Adults in stable conditions synthesize and degrade approximately 3.5 to 4.5 g protein/kg body weight (i.e., 245 g to 315 g protein in a 70 kg adult person) each day (233). The protein content of muscle is about 20%. Therefore, daily protein turn over is the equivalent of 1.2 to 2 kg muscle. Because protein turnover is so large, even a small increase in protein degradation or a decrease in protein synthesis rate, persisting for longer periods, can cause a marked loss of lean body mass.

The essential amino acids are valine, leucine, isoleucine, threonine, methionine, phenylalanine, lysine, tryptophan, and histidine. The nonessential amino acids are glycine, alanine, serine, cystine, aspartic acid, glutamic acid, and hydroxyproline. A third category, "conditionally indispensable," is based on the observation that under specific dietary conditions function is best maintained when these amino acids are part of nutrient intake. These conditionally indispensable amino acids are glycine, cystine, tyrosine, proline, arginine, citruline, glutamine, and taurine (232).

Abnormalities in Plasma and Intracellular Amino Acid Concentrations in Chronic Kidney Disease

Some disturbances in the amino acids plasma concentrations are observed in chronic kidney disease even before renal replacement therapy is started (234–237). The severity of amino acid abnormalities is related to the degree of chronic kidney disease and the presence of uremic symptoms (238). These disturbances are complex and not well understood.

The plasma concentrations of tryptophan, tyrosine, and the branched-chain amino acids, particularly valine, are low (236,237,239–241). Elevated plasma concentrations of citrulline, methylhistidine, and of the sulfur-containing amino acids, cystine and methionine, are found in CKD patients (237,239–242). In summary, plasma concentrations of essential amino acids, with some exceptions, tend to be decreased, whereas plasma concentrations of the nonessential amino acids tend to be increased. The pattern of the plasma amino acid concentrations does not accurately reflect the intracellular pattern (243). The intracellular concentrations of valine, threonine, tyrosine, and taurine in muscle are decreased (244,245). The concentrations of phenylalanine, alanine, arginine, and citruline are increased (241,244).

In CKD patients, low plasma amino acid concentrations and low intracellular amino acid concentrations may be due to: (a) anorexia, (b) decreased amino acid synthesis, (c) increased catabolism, (d) loss during the dialysis procedure, and (e) impaired binding to serum albumin caused by substances that accumulate in the blood in uremia.

As in nonuremic individuals, in CKD patients, poor dietary intake of protein and nutrients leads to decreased concentrations of such amino acids as histidine, isoleucine, leucine, valine, and tyrosine (245). In CKD patients, plasma concentration of several of amino acids are inversely correlated with protein intake (246).

In rats with experimental chronic renal disease, the principal cause of low plasma concentrations of branched-chain amino acids (valine, leucine, isoleucine) is increased catabolism (244). This is stimulated by acidosis and caused by increased activity of branched-chain keto acid dehydrogenase, a key enzyme in the amino acid degradation pathway (247). In patients undergoing hemodialysis, an inverse correlation is found between the levels of intracellular valine and plasma bicarbonate concentration (241). The correction of metabolic acidosis increases the concentration of the above three amino acids in muscle (248). Reduced binding by albumin probably accounts for the low total plasma tryptophan concentration (249,250).

Low intracellular levels of taurine with normal or slightly elevated concentrations of this amino acid in plasma are also found in CKD patients. Since the plasma concentration of precursors of taurine, such as cystine, methionine, and cystine sulfonic acid are elevated, a selective metabolic block at the level of cystine sulfonic acid decarboxylase has been proposed to explain the decrease in intracellular taurine (251). In addition, low intracellular levels of threonine and lysine and low ratios of essential to nonessential amino acids (valine–glycine and, phenylalanine–tyrosine) have been found in patients with CKD (252). It has been suggested that the insulin resistance present in uremia impairs the uptake of amino acids by cells (253).

Reduced renal excretion probably accounts for the increased plasma concentration of hydroxyproline (254). Collagen is the main reservoir for hydroxyproline and, in conditions of enhanced bone resorption, such as secondary hyperparthyroidismus, there may be increased release of hydroxyproline from bone (255).

Protein Metabolism in Chronic Kidney Disease

The detailed observations of renal patients by Richard Bright (256) pointed to an important role of inanition in kidney disease. More recently, a high prevalence of protein malnutrition has been reported in hemodialysis patients as well. Mild to moderate protein malnutrition occurs in approximately

33% of hemodialysis patients and severe malnutrition occurs in additional 6% to 8% of these individuals (257). The interpretation, however, of what constitutes malnutrition and how it relates to patient outcome remains controversial (258). Pure-energy protein malnutrition (kwashiorkor) is not associated with accelerated atherogenesis and cardiovascular events. These events, however, are commonly found in wasted dialysis patients and associated with markers of microinflammation, such as high hsCRP, low plasma concentrations of albumin and fetuin, as well as high plasma concentrations of interleukin-6, interleukin-18, and TNF-α (259). This constellation has been rephrased malnutrition, inflammation, and atherosclerosis (MIA) syndrome (260). It has remained uncertain, however, whether low muscle and body mass per se (261) or rather the process of active wasting have negative effects on outcome (260). The paradoxical finding that survival is best in dialysis patients with high BMI, even in the range of frank obesity (219), may indicate that obesity increases tolerance toward episodes of catabolism. Energy expenditure is increased in uremia (261). In addition, some factors common in uremic patients may trigger catabolism, such as fasting resulting from loss of appetite. Acidosis or insulin resistance (262) activate the ubiquitin proteasome system as the final common pathway of protein breakdown (262). There is also evidence that the dialysis procedure per se is a catabolic stimulus (263,264). A recent study of Pupim at al. (265) confirmed that dialysis causes whole body and muscle proteolysis, which can be overcome, at least acutely, by intravenous infusion of amino acids, glucose and lipids.

The rates of synthesis and degradation of proteins can be quantitated by the infusion of either radiolabeled or stable isotopes bearing amino acids. This allows the calculation of total body protein synthesis and total average proteolysis, as well as amino acid oxidation (266). Patients with stable CKD have been studied using this methodology when ingesting either of two different levels of dietary protein, 0.6 g/kg or 1.0 g/kg body weight. Studies were performed both after overnight fasting and in the fed state (267). No differences were found in either the rate of protein turnover or amino acid oxidation compared to control subjects. Thus, the dynamics of amino acid metabolism are apparently normal at the whole body level in stable, nonacidotic patients with chronic kidney disease (267).

In contrast to nondialyzed, stable CKD patients, nitrogen balance studies indicate that hemodialysis patients are unable to conserve nitrogen normally and have increased dietary requirements (268). The increased protein needs are higher than accounted for by loss of amino acids, peptides, and proteins into the dialyzate (269). These findings are consistent with the existence of a chronic, low-grade catabolic state in hemodialysis patients (269). Such a catabolic state may be due to presence of chronic inflammation, acidosis, insulin resistance, or a combination of these conditions (262).

When protein intake is restricted, supplemental calories may improve nitrogen balance (270,271). If calorie intake is inadequate in patients eating a low-protein diet, the risk of catabolizing body protein is increased (270,271). Maroni (271) examined the caloric needs of patients on low-protein diets as well as the nutritional adequacy and mechanisms of adaptation to a very low-protein diet containing 0.28 g/kg body weight and supplemented with either essential amino acids or a mixture of keto acids. When protein intake was reduced, the nitrogen balance improved when the caloric intake was increased.

Caloric Requirements in Patients with Chronic Kidney Disease

Although protein-calorie malnutrition is common in CKD patients, few studies have examined the caloric requirements of these patients. Inadequate caloric intake may be present when energy requirements are increased, when caloric intake is decreased, or when a combination of both is present.

In a study on 10 hemodialysis patients, Ikizler et al. (261) found 7% higher than expected energy expenditure during both dialysis and nondialysis days, suggesting that uremia per se increases energy expenditure. This conclusion is controversial, however. Monteon et al. (272) measured energy expenditure of CKD patients during rest and exercise; CKD patients did not differ from control subjects. This issue may be clinically important, since a prospective study showed a correlation between high resting energy expenditure and increased mortality or cardiovascular death in patients on continuous ambulatory peritoneal dialysis (273).

In CKD patients, the caloric intake tends to be decreased (274). CKD patients do not ingest prescribed amount of calories (275), even despite dietary counseling. This is exemplified by the MDRD study; the initial energy intake was below the recommended limit (30 to 35 kcal/kg/day) and, during the study (average duration 2.2 years), energy intake declined further despite intensive dietary counseling (276).

Nutrition in Hemodialysis Patients

Hypoalbuminemia, negative nitrogen balance, loss of muscle mass, and wasting are commonly seen in long-term dialysis patients (277). Several indices of malnutrition have been utilized, but they do not exactly measure the same abnormality (258). The procedures range from the well-known anthropometric measurements, such as skinfold thickness and midarm muscle circumference, BMI, waist–hip ratio, to subjective global assessment as an evaluation of components going beyond body composition (278). Low plasma albumin concentrations are closely related to mortality (279), while BMI and urine creatinine excretion as an index of muscle mass are not (280). Even small decrements in plasma albumin concentration (in the range of 3.5 to 3.9 g/dL) have been associated with increased mortality in hemodialysis patients. Plasma albumin concentration appears to be a late index of malnutrition. Because of its relatively long half-life (21 days) and the vast capacity of the liver to synthesize albumin, a decrease in serum albumin concentration lags behind the onset of malnutrition by several months (281). Prealbumin, with a half-life of 2 days, may be a more useful earlier indicator of malnutrition, at least in individuals without renal disease (258). Other indicators of malnutrition include plasma cholesterol concentrations below 150 mg/dL, decreased plasma concentration of transferring, and a decrease in body weight (258). Indices of malnutrition in CKD patients undergoing renal replacement therapy are shown in Table 96-5.

Although protein–calorie malnutrition is the most commonly recognized nutritional deficiency in patients with ESRD, other nutritional deficits may also occur (vitamin B_6, folic acid, vitamin C) (282).

Factors that Affect Nutritional Status in Chronic Kidney Disease

Several factors contribute to the high prevalence of protein–energy malnutrition in CKD patients (Table 96-6). Several catabolic factors may participate in pathogenesis of malnutrition in CKD patients, including: (a) metabolic acidosis, (b) inflammation, (c) insulin resistance, (d) dialyzate amino acids, protein, and glucose losses, and (e) bioincompatibility of dialysis membranes.

TABLE 96-5

INDICES OF MALNUTRITION IN PATIENTS WITH CHRONIC KIDNEY DISEASE

Body weight and anthropometric measures
Body weight <80% of ideal weight
Continuous, unintentional decline of "dry weight"
Reduction in anthropometric measurements (skinfold thickness, midarm circumference)
Biochemical parameters
Plasma albumin concentration <4.0 g/dL
Plasma prealbumin concentration <30 mg/dL
Plasma cholesterol concentration <150 mg/dL
Plasma transferrin concentration <200 mg/dL
Low plasma creatinine and urea concentrations in patients without residual renal function
Low plasma predialysis serum potassium and serum phosphorus concentrations

The results of experimental as well as clinical studies suggest that metabolic acidosis is an important factor causing excessive catabolism of amino acids and proteins in CKD patients (283). Metabolic acidosis activates the specific pathways involved in the degradation of branched-chain amino acids catalyzed by branched-chain keto acids dehydrogenase (247). It also activates the ubiquitin–proteasome system, the final common pathway of muscle protein degradation (284). Profound acidemia following ingestion of ammonium chloride causes cachexia in humans without CKD (285). Conversely, in CKD patients correction of metabolic acidosis decreases protein degradation considerably (286–289) and in the study of Reaich et al. (287) by 28%. Long-term therapy with higher concentration of lactate buffer in peritoneal dialyzate caused decreased expression of mRNA encoding ubiquitin in muscle (290). It also leads to weight gain and improvement of anthropomorphic indices (290,291). Correction of metabolic acidosis by oral sodium bicarbonate suplementation in hemodialysis and peritoneal dialysis patients increases plasma albumin concentration and muscle mass (292,293).

Low-grade inflammation is often present in chronic kidney disease patients. In hemodialyzed patients, high plasma concentration of proinflammatory cytokines are found (294). The cause of inflammation in CKD is not clear (294). In hemodialysis patients, an association is found between hypoalbuminemia and high CRP concentrations (295). From TNF-α experimental studies it is known that administration of TNF-α stimulates protein degradation in muscle (296,297). This and other observations suggest that there is a link between chronic inflammation and malnutrition.

Another catabolic factor, which is commonly found in CKD patients, is insulin resistance. Absence of, and potentially resistance to, insulin stimulate the ubiquitin–proteasome system, that is, the common proteolytic pathway, in muscle, (91,92).

The hemodialysis procedure may be also a catabolic factor. Whole-body protein breakdown is acutely stimulated during a dialysis session (263,264). This effect may be mediated, at least in part, via complement activation by contact between blood and bioincompatible membranes (291). It has, therefore, been proposed that the use of more biocompatible dialysis membranes may prevent malnutrition by reducing complement activation. Unfortunately, two long-term, prospective studies on this issue yielded conflicting results (298,299) and the issue of the relation between bioincompatibility and malnutrition remains a matter of debate (298,299).

During dialysis with cuprophane, 2 to 8 g of free amino acids, 2 to 5 g of peptides, and about 25 g of glucose are lost into the dialyzate (248,300), but losses are significantly higher with high-flux polysulfone membranes (300). These losses, although small, contribute to the catabolism of hemodialysis patients.

A further important factor contributing to malnutrition in CKD patients is loss of appetite. Changes in the motility and function of the gastrointestinal tract including gastroparesis, malabsorption, and constipation, as well as the long-term adherence to low-protein diets without adequate supplementation with keto acids or amino acids, are further contributory factors.

TABLE 96-6

FACTORS THAT AFFECT THE NUTRITIONAL STATUS OF PATIENTS WITH CHRONIC KIDNEY DISEASE[a]

Gastrointestinal disturbances
Anorexia
Gastroparesis and delayed gastric emptying
Malabsorption
Esophagitis, gastritis
Subjective feeling of fullness from dialyzate in the abdomen (in CAPD patients)
Biochemical derangements
Metabolic acidosis
Low-grade inflammation
Insulin resistance
Iatrogenic factors
Dialyzate amino acids, protein and glucose losses
Bioincompatibility of dialysis membranes
Multiple medications, particularly sedatives
Other factors
Long-term, low-protein intake
Low socioeconomic status
Depression
Underlying illness
Frequent hospitalizations

[a] *CAPD*, continuous ambulatory peritoneal dialysis.

Dietary and Energy Intake Recommended in Patients Undergoing Dialysis Replacement Therapy

In Table 96-7 are summarized the recommended nutrient intake for patients undergoing maintenance hemodialysis or peritoneal dialysis (301).

If severe malnutrition develops despite adequate dialysis and measures designed to ameliorate anorexia and increased catabolism, it may be necessary to initiate enteral or parenteral alimentation to supply adequate calories and nutrients (265). Hospitalization for such treatment may be necessary in severely malnourished patients. It is preferable to use enteral alimentation through a thin nasogastric tube rather than parenteral alimentation, which carries the risk of catheter-related infections and sepsis. Parenteral nutrition with amino acids has been used with some success to improve nutrition. A mixture of essential and nonessential amino acids should be used. Special amino acid solutions for use in CKD patients are available and have been used with some success (302–304).

TABLE 96-7

RECOMMENDED DIETARY PROTEIN AND ENERGY INTAKE FOR PATIENTS UNDERGOING MAINTENANCE HEMODIALYSIS OR PERITONEAL DIALYSIS

	Maintenance hemodialysis	Continuous ambulatory or cyclic peritoneal dialysis
Protein	1.2 g/kg/d ≥50% high-biologic-value protein	1.2–1.3 g/kg/d ≥50% high-biologic-value protein Unless a patient has demonstrated adequate protein nutritional status on 1.2 g/kg/d diet, 1.3 g/kg/d should be prescribed
Energy	≥35 kcal/kg/d 30 to 35 kcal/kg/d for patients 60 years or older	

(Based on: Kidney diseases outcomes quality initiative clinical practice guidelines for nutrition in chronic renal failure. *Am J Kidney Dis* 2000;35[suppl 2].)

ENDOCRINE DISORDERS IN CHRONIC KIDNEY DISEASE

Abnormalities in the Hormones of the Hypothalamic–Pituitary–Gonadal Axis

Both female and male CKD patients present a variety of derangements of the hypothalamic–pituitary–gonadal axis (Table 96-8). In males, these abnormalities are involved in the pathogenesis of impotence and gynecomastia. In females, these abnormalities account for anovulatory menstrual cycles and infertility.

The Hypothalamic–Pituitary–Gonadal Axis in Male Chronic Kidney Disease Patients

Luteinizing Hormone (LH)

Pulsatile secretion of the gonadotropin-releasing hormone (GnRH) with one pulse every 90 to 120 minutes is essential for effective secretion of hypophyseal gonadotropin, luteinizing hormone (LH), and accounts also for the corresponding pulsatile secretion of LH. In CKD patients, several studies found that the gonadotropin response to exogenous GnRH was delayed and blunted (305), but this finding was not replicated in other studies (306–309).

In the majority of CKD patients, basal plasma LH concentrations are higher by a factor of approximately 1.5 to 2 compared with healthy controls. Such concentrations approach the concentrations in patients with primary hypogonadism (306–312). The mechanisms underlying the increased plasma LH concentrations are not completely understood. The rate of catabolism of LH is decreased (313,314) and, conversely, its half-life of LH in CKD is increased by a factor of 2 to 4 compared to normal subjects (314). Apart from abnormal basal LH concentration, there is also an abnormality of pulsatile LH secretion. Schaefer et al. (315) found decreased amplitudes of the secretory bursts of bioactive and immunoreactive LH, but no change in the number of bursts. The proportion of LH released by pulsatile secretion was diminished and basal nonpulsatile immunoreactive LH was increased. This phenomenon may be secondary to abnormal pulsatile secretion of GnRH. To summarize, in CKD both LH secretion and its metabolic clearance rate are abnormal.

LH stimulates production of testosterone by the Leydig's cells of the testes. Testosterone, in turn, exerts negative feedback control on the secretion of GnRH and secondarily of LH. Low plasma testosterone concentration in CKD (313,316–320) may, therefore, contribute to, but does not fully account for, the elevation of LH concentration. Low plasma testosterone concentrations are not the only signal stimulating GnRH release. This consideration may explain why in CKD patients exogenous testosterone did not consistently suppress LH secretion (319–323).

TABLE 96-8

ABNORMALITIES OF HYPOTHALAMIC–PITUITARY–GONADAL AXIS IN PATIENTS WITH CHRONIC KIDNEY DISEASE[a]

	Male	Female
Basal prolactin	↑	↑
Prolactin response to TRH	↓	↓ and delayed
Prolactin suppression test	Impaired	Impaired
Basal FSH	↑	N
FSH response to GnRH	N but delayed	N
Basal LH	↑	↑
LH response to GnRH	N	N
Testosterone	↓	—
Estradiol	N	↓
Progesterone	—	↓

[a] *FSH,* follicle-stimulating hormone; *GnRH,* gonadotropin-releasing hormone; *LH,* luteinizing hormone; *N,* normal; *TRH,* thyrotropin-releasing hormone.

Follicle-Stimulating Hormone (FSH)

In CKD patients, the basal plasma concentrations of follicle-stimulating hormone (FSH) are in the upper normal range or elevated. The response to gonadotropin-releasing hormone (GnRH) is normal in magnitude, but delayed (310,318). The mechanisms responsible for these derangements have not been well elucidated. FSH is important for spermatogenesis. It stimulates testicular growth and increases the production of testosterone-binding protein by Sertoli cells. In testicular tubules, FSH accounts for the high local concentrations of testosterone required for sperm maturation (324). In patients with testicular damage, but no CKD, basal FSH concentrations

are usually elevated (325). In CKD patients, spermatogenesis is impaired despite elevated blood levels of FSH (313,318,326), a finding consistent with the following explanations: (a) resistance of the testis to the action of FSH causes testicular damage with a consequent increase in FSH concentrations and/or (b) testicular damage is the primary abnormality and the elevated FSH concentrations represent the normal response of the hypothalamic–pituitary axis. In either case, the negative feedback between testis and hypothalamic–pituitary axis appears to be normal in CKD.

Inhibin, a polypeptide secreted by Sertoli cells of the seminiferous tubules, inhibits FSH secretion and is probably important in mediating the negative feedback interaction between testis and hypothalamic–pituitary axis (327). It was suggestive to assume that abnormalities of production and/or secretion of inhibin in CKD patients explain elevated FSH concentrations, but studies of prepubertal boys with CKD showed that the concentrations of both FSH and immunoreactive inhibin were elevated and no correlation was found between the two parameters (328).

Prolactin

The basal plasma prolactin concentrations are elevated in the majority (40% to 70%) of male hemodialysis patients (329–334). As CKD progresses, elevated plasma prolactin concentrations correlate with plasma creatinine concentration (335). Apart from elevated basal prolactin concentrations, the circadian rhythm of prolactin secretion is also disturbed. Finally, the characteristic sleep-induced secretory bursts are not observed, although episodic secretion occurs during daytime (335).

The mechanisms explaining hyperprolactinemia in CKD are not well understood. It seems that both diminished prolactin clearance (336) and increased autonomous production rate contribute to hyperprolactinemia.

The response to stimulation or suppression of prolactin is diminished in CKD. This observation is consistent with the notion of increased autonomous production (330,331,336). The underlying mechanism is presumably inadequate dopaminergic inhibition of prolactin release from pituitary lactrotrophs (337).

In hemodialysis patients, the prolactin response to stimuli, such as thyrotropin-releasing hormone (TRH), chlorpromazine, metoclopramide, arginine, and insulin-induced hypoglycemia is blunted (330,338,339). Treatment with erythropoietin caused a decrease in serum prolactin and improvement of sexual dysfunction (340), suggesting that either anemia and/or deficiency of erythropoietin per se participate in the genesis of the hyperprolactinemia of CKD. It is of interest that in some patients correction of the hyperprolactinemia by bromocryptine also caused improvement of sexual dysfunction (341).

Testicular Hormones

In most male hemodialysis patients, plasma testosterone concentrations are low, although a wide overlap with age-corrected normal concentrations is found (312,313,316–319,342,343). Since testosterone binding capacity is normal, free testosterone concentration is also low (342). The normal circadian rhythm of plasma testosterone concentrations, with a peak at 4 to 8 AM and nadir at 8 to 12 PM is maintained in CKD patients (344). It is unknown whether the decreased plasma testosterone concentrations are due to reduced synthesis, increased catabolism, or a combination of both (345,346). LH stimulates testosterone secretion; however, despite numerous studies it is not clear whether the deranged LH metabolism accounts for the reduced testosterone concentrations. The reduced amplitude of pulsatile secretory LH bursts, found in CKD, may be more critical for testosterone secretion than the sustained elevation of LH concentration. Alternatively, LH resistance of testosterone-producing cells may lead to impaired testosterone production and/or secretion. In this context, it is possible that elevated prolactin concentrations interfere with the action of LH on the testes and contribute to LH resistance (347). The response to 4 days administration of human gonadotropin is sluggish and delayed; no increase of testosterone concentration was seen after 8 hours, but a two- to threefold increase was seen after 4 days (319). It has been argued that secondary hyperparathyroidism plays a role (348), but in placebo-controlled trials, $1,25(OH)_2D_3$ therapy failed to normalize low plasma testosterone concentrations (349,350). Malnutrition is also likely to participate in the reduction of plasma testosterone concentration in CKD male patients. In CKD patients on a low-protein diet, essential amino acids and keto analogs supplementation raised low testosterone plasma concentration (348).

Apart from testosterone, testosterone metabolites are also of interest. The reports on this issue are scarce. Increased plasma dihydrotestosterone and androstandiol concentrations (351,352) as well as decreased plasma concentration of androstenedione and dihydoepiandrosterone sulfate have been reported (353).

Abnormalities in the Hormones of the Hypothalamic–Pituitary–Gonadal Axis in Female Chronic Kidney Disease Patients

Luteinizing Hormone (LH)

Pulsatile secretion of GnRH at 90-minute intervals during the follicular phase of cycle is essential for effective hypophyseal gonadotropin secretion. In healthy premenopausal females, the secretion of LH is pulsatile. In female CKD patients, the spontaneous pulsatile LH secretion is disturbed (354). Plasma LH concentration is elevated in most of premenopausal CKD patients. The response to stimulation with GnRH is delayed, but normal or supranormal plasma concentrations of LH are finally reached (355). Diurnal pulsatile LH secretion and high preovulatory peaks of GnRH and LH plasma concentrations are absent in the most female CKD patients. In healthy females, estradiol lowers the amplitude of LH pulses. In females with CKD, estradiol fails to influence the LH surge, suggesting impaired positive feedback (355).

Follicle-Stimulating Hormone (FSH)

In contrast to the abnormal plasma LH concentration, the plasma FSH concentration is normal in most premenopausal CKD patients (356,357) and the FSH/LH ratio is decreased. The decreased FSH/LH ratio argues against primary ovarian failure and suggests hypothalamic–hypophyseal dysregulation in CKD females.

Prolactin

Plasma prolactin concentrations are often elevated in female hemodialysis patients (356,358), but the increase of plasma prolactin after administration of thyrotropin-releasing hormone (TRH) is blunted (356). In CKD females, amenorrhea is frequent in patients with high plasma prolactin concentrations and, conversely, in females with regular menstruation plasma prolactin, concentrations are lower (359).

Estrogens

In female CKD patients, the plasma estradiol concentrations are normal or low (354,360) and are consistently lower in CKD females with hyperprolactinemia (361). In the second one-half of the menstrual cycle, plasma progesterone concentrations are low because of defective luteinization of the follicles (360). The hormonal derangements of females with CKD are clearly the

consequence of abnormal regulation at the level of the hypothalamus (362).

A major consequence of the low plasma estrogen concentration concerns bone disease. Weisinger et al. (363) studied young female hemodialysis patients. Amenorrhoic patients had not only significantly lower plasma estrogen concentrations, but also significantly lower bone mineral density compared normally menstruating female dialysis patients. Furthermore, in amennorheic patients, a significant positive correlation was found between bone mineral density and plasma estradiol concentration (363). A causal role is suggested by the observation of Matuszkiewicz-Rowinska et al. (364). In a small group of postmenopausal women on dialysis, they showed that treatment with transdermal estradiol and cyclic addition of norethisterone acetate for 1 year increased lumbar spine bone mineral density significantly. Similarly, in a placebo-controlled, randomized trial 1 year of treatment with raloxifene, a selective estrogen receptor modulator (SERM), significantly increased bone mineral density of the lumbar spine in hemodialyzed postmenopausal females (365). In view of current concern about potential adverse cardiovascular effects of hormonal replacement therapy, it must be emphasized that currently long-term studies on the effectiveness and safety of hormone replacement or SERM therapy in CKD female are not available.

Abnormalities in the Growth Hormone–Insulinlike Growth Factor (Somatotrophic) Axis

The somatotrophic axis comprises growth hormone (GH), insulinlike growth factor-1 and -2 (IGF-1 and -2), six IGF-binding proteins (IGFBP-1 to -6), and the IGFBP proteases (BP-Pr). All are involved in the modulation of somatic growth, cellular proliferation, metabolism, and numerous other processes (366). The somatotrophic axis regulates body growth in children. Poor growth and reduced final height are well-known complications of children with CKD. It is not surprising that several abnormalities (Table 96-9) in the somatotrophic axis have been reported in children and adults with CKD.

TABLE 96-9

ABNORMALITIES IN THE GROWTH HORMONE—INSULIN-LIKE GROWTH FACTOR (SOMATOTROPHIC) AXIS IN PATIENTS WITH CHRONIC KIDNEY DISEASE[a]

Growth hormone (GH)
Increased plasma GH concentration
Decreased plasma concentrations of high-affinity GH binding protein
Peripheral resistance to GH due to defect in GH intracellular signal transduction
Insulin-like growth factor (IGF)
Slightly decreased IGF-1 and increased IGF-2 plasma concentration
Reduced free IGF-1 plasma concentration
Increased IGFBPs (IGFBP-1, -2, -3, -4, and -6) plasma concentration
Presence of low molecular weight (1,000 Da) inhibitor of IGF-1 in plasma
Peripheral resistance to IGF-1 probably due to postreceptor defect in IGF-1 action

[a] *GH,* growth hormone; *IGF-1* and *-2*, insulin-like growth factor-1 and -2; *IGFBP*, IGF binding protein.

Growth Hormone (GH)

Growth hormone is produced and secreted by the somatotrophs of the pituitary glands. The secretion of GH is pulsatile. A diurnal rhythm also exists; the secretion is high before awakening and decreases toward the end of the day. The secretion of GH is mainly controlled by two opposing hypothalamic factors; growth hormone-releasing hormone (GHRH), which stimulates GH secretion, and somatostatin, which inhibits GH secretion. Hypoglycemia, amino acid infusion, and protein meals stimulate GH secretion, while hyperglycemia inhibits GH secretion. The monomeric form (22 kDa) of GH predominates (43% to 48%) in plasma. The rest consists of a 20 kDa form (7% to 8%), of dimers, of aggregates and of large fragments (367,368). The kidney is the main site of growth hormone degradation (369).

In children and adult patients with CKD, the plasma concentration of GH is usually elevated (370–374). The increase in plasma GH concentration is correlated to GFR (373,375). Plasma GH concentrations are higher in continuous ambulatory peritoneal dialysis (CAPD) than in hemodialysis patients (376). The increase in plasma GH concentration is caused by a reduction of the metabolic clearance rate and by an increase of GH secretion. In CKD the metabolic clearance rate of GH is reduced (377,378) and the GH secretion rate is elevated, as documented in adult hemodialysis patients (379). The latter finding is not consistent, however, and in pubertal patients with advanced CKD, the GH secretion rate was decreased (380).

The dysregulation of GH secretion is explained by several abnormalities of the central neuroendocrine control mechanisms. This issue has been investigated by suppressing and stimulating manuevers testing the hypothalamic–pituitary function in CKD patients. Hyperglycemia by glucose infusion suppresses GH secretion in normal individuals, but fails to do so in CKD patients (381). Conversely, in CKD patients, the response of GH secretion to the administration of GHRH (381) or L-dopa (382) is exaggerated. Exogenous thyrotropin-releasing hormone (TRH) does not affect GH release in normal subjects, but stimulates GH secretion in CKD (382) and a sustained exaggerated increase of GH secretion is also seen after stimulatory manuevers, such as arginine infusion and insulin-induced hypoglycemia (382–384).

Approximately 45% of plasma GH is bound to plasma proteins (367). Decreased plasma concentrations of the high affinity GH-binding protein have been found in CKD (385,386). The constellation of increased concentrations of plasma GH and decreased concentrations of the high-affinity GH-binding protein implies that the fraction of free hormone is increased to which target tissues are exposed. When chondrocytes are isolated from bones of uremic rats and exposed to growth hormone or IGF-1, the response was blunted, however, (387) suggesting that in CKD the high concentrations of free GH are counteracted by peripheral resistance to GH. The resistance appears to be at the postreceptor and not at the receptor level. In the liver, GH receptor binding is similar in uremic and pair-fed rats (388) and experiments of Schaefer et al, (389) suggest that resistance to GH is due to defective intracellular signal transduction. The authors found impaired phosphorylation and nuclear translocation of GH-activated STAT protein.

Insulinlike Growth Factors (IGFs)

GH promotes linear growth partially by stimulating systemic and local concentrations of insulinlike growth factors. The two most important IGFs are IGF-1 and IGF-2. These peptide growth factors are produced locally by the most tissues, including the growth plate, but the liver is the main source of circulating hormone. Synthesis of IGFs is stimulated by GH. Conversely, as part of a negative feedback loop, IGF-1 inhibits GH presumably through stimulation of somatostatin secretion by the hypothalamus (390). Plasma IGF-1 forms complexes

with six IGF-binding proteins (IGFBP-1 to -6). IGFBP-3 is the predominant IGFBP isoform in human plasma. Its main production site is the liver. IGFBP-3 binds IGF-1 and binding prolongs the half-life of IGF and serves as a reservoir of IGF-1 (366).

In advanced CKD the plasma concentration of total IGF-1 is slightly decreased and that of IGF-2 is increased (391–393). The plasma concentration of free IGF-1 is lower by 50% (394). Moreover, the so-called somatomedin bioactivity in blood, an index of IGF activity measured by sulfate incorporation into porcine costal cartilage, is reduced in uremia (395).

The discrepancy between normal or elevated total IGF plasma concentration and low bioactivity in CKD may be explained by one or a combination of the following: (a) increased plasma concentration of IGFBPs, (b) circulating IGF inhibitor, and (c) receptor or postreceptor defect. There is some evidence for all three possibilities.

Plasma concentrations of 5 of the 6 IGF binding proteins (IGFBP-1, -2, -3, -4, and -6) are markedly higher in CKD patients (396–398). The increased binding capacity of IGF-1 decreases the concentration of free IGF-1 (399). This imbalance between plasma IGF-1 and plasma IGFBPs concentrations seems to be relevant in the pathogenesis of growth failure in CKD. A significant negative correlation is found between plasma concentrations of IGFBP-1, -2, and -4, on the one hand, and standardized height in CKD children, on the other hand (393,399).

A low-molecular-weight (1,000 Da) inhibitor of IGF-1 has been identified in the plasma of CKD patients (400), but molecular details have not yet been characterized.

Finally, Ding et al. (401) characterized a postreceptor defect to the action of IGF-1 in the epitrochlearis muscle of uremic rats and this is in line with observations of Fouque et al. (402), who found resistance to the metabolic effects of recombinant human IGF-1 in patients with advanced CKD.

Clinical Consequences

Elevation of plasma GH concentration and low IGF bioactivity is compatible with the notion that growth failure in CKD is mainly due to end-organ hyporesponsiveness to growth hormone. The demonstration of the resistance to the action of GH and IGF-1 in CKD provides the rationale for the use of GH in the treatment of CKD children with retarded growth despite normal or elevated hormone concentrations. In a multicenter randomized double-blind placebo-controlled study, 2 years of administration of recombinant human GH in 125 prepubertal children with CKD caused an increase in growth rate and in standardized height without undue advancement of bone age or significant side effects (403).

Abnormalities in the Adrenocorticotropin–Cortisol Axis

The adrenocorticotropin–cortisol axis is only mildly affected in CKD. In CKD patients, plasma adrenocorticotropin (ACTH) concentrations are normal or elevated (404–409). In CKD patients, ACTH secretion following the administration of corticotropin-releasing hormone (CRH) occurs earlier but the magnitude of the response is blunted (408–410).

In CKD patients, the basal blood levels of cortisol are normal (409,411–414) or modestly elevated (410,415–419). No significant correlation was found between free cortisol concentrations and GFR (420). The circadian rhythm of cortisol secretion is not disturbed. The cortisol half-life is prolonged in CKD patients (411,415,421,422) and decreased catabolism may contribute to the mildly elevated basal levels of cortisol.

A reduced stimulated cortisol secretion to CRH despite prolonged elevation of ACTH has been observed in hemodialysis patients (423), but the results are not uniform. Zager et al. (405) found a normal cortisol response to exogenous ACTH.

In CKD patients, ACTH secretion can not be suppressed by standard oral doses of dexamethasone (415,424). This is probably due to reduced dexamethasone absorption in the gut (413), because higher doses of dexamethasone suppress ACTH secretion. Therefore, when Cushing's syndrome is suspected in CKD patients, a 2-day dexamethasone test is recommended. Overall, the adrenocorticotropin–cortisol axis is either normal or only mildly altered in CKD and the clinical significance of this finding is unknown.

Abnormalities in Vasopressin

In CKD patients, the plasma vasopressin concentration is elevated (425–429). The major cause is a decreased metabolic clearance rate (430,431).

The main physiological stimuli for vasopressin secretion are increased plasma osmolality and decreased cardiac output or arterial vasodilation. Most studies found intact osmotic regulation of vasopressin secretion in CKD (427,432–434). The vasopressin response to nonosmotic stimuli is apparently also normal. In hemodialyzed patients the plasma vasopressin concentration increases during ultrafiltration (435) and plasma volume contraction (436–439). Conversely, it decreases during central hypervolemia induced by water immersion (428). It was shown that in hemodialysis patients, the hierarchy of stimuli regulating vasopressin secretion is osmotic followed by nonosmotic factors (439).

The clinical significance of the elevated blood levels of vasopressin in CKD is uncertain and it is not clear whether this abnormality plays a role in the pathogenesis of hypertension and increased thirst in patients with CKD.

Abnormalities in the Thyroid Gland and Hypothalamo-Pituitary–Thyroid Axis

Abnormalities in the structure and function of the thyroid gland and in the metabolism and plasma concentrations of thyroid hormones are common in patients with CKD (440–442). These derangements may be due to the uremic state per se, to nonthyroid disorders (chronic disease), or to concomitant disorders of the thyroid, the pituitary, or the hypothalamus. A detailed profile of the prevalent indices of thyroid status in CKD as compared to primary hypothyroidism and chronic nonthyroidal illness is presented in Table 96-10.

Goiter, Thyroid Nodules, and Thyroid Carcinoma

Available data indicate that the prevalence of goiter is increased in CKD patients (440–443). High-resolution ultrasound scanning shows increase of thyroid volume in about 50% of hemodialysis patients (443). Kaptein et al. (440) studied 306 CKD patients and compared them to 139 hospitalized control patients without renal disease. Goiter was present in 40% of the CKD patients and in 43% of those treated with dialysis, compared to 6.5% in the control group. The frequency of goiter was higher in patients treated for more than 1 year with hemodialysis (50%) than in those treated for a shorter time (39%). The female/male ratio was 1.4:1 in CKD patients with goiter compared with 2.8:1 in control group, suggesting that uremia-related factors may be relatively more important in hemodialyzed patients (440). The frequency of goiter in CKD patients was not related to age, race, diabetes mellitus, plasma TSH and PTH concentrations, or antimicrosomal

TABLE 96-10

ABNORMALITIES OF HYPOTHALAMIC–PITUITARY–THYROID AXIS IN PATIENTS WITH CHRONIC KIDNEY DISEASE, CHRONIC NONTHYROIDAL NONKIDNEY ILLNESS AND PRIMARY HYPOTHYROIDISM[a]

	Total T_4	Free T_4	Total T_3	Free T_3	rT_3	TSH	TSH response to TRH
Chronic kidney disease	↓	N, ↓	↓	↓	N	N	N, ↓
Chronic nonthyroidal nonkidney illness	↓	N, ↓	↓	↓	↑	N	N, ↓
Primary hypothyroidism	↓	↓	↓	↓	N	↑	↑

[a] ↑, increased; ↓, decreased; *N*, normal; *TRH*, thyrotropin-releasing hormone; *TSH*, thyroid-stimulating hormone; T_4, thyroxine; T_3, triiodothyronine; rT_3, reverse triiodothyronine.

antibody titers (440). It has been suggested that in CKD, goiter formation is the result of increased plasma iodide concentrations (441,444–446).

Thyroid nodules are more common in CKD patients than in the general population and were found in 55% of female hemodialysis patients compared with 21% of normal females (447). We emphasize that it is necessary to exclude malignancy in CKD patients with solitary nodules.

The prevalence of thyroid carcinoma is increased in CKD patients. In a large sample of patients in the U.S., the relative risk of thyroid malignancy was increased 2.9 times in females but not in males (1.2 times) (448). In the Europe (EDTA-ERA registry), the frequency of thyroid cancer was increased by a factor of 4 to 8 in young female dialysis patients and by a factor of 2 in older dialysis patients (449).

Thyroid Hormones

The plasma concentrations of both total thyroxine (T_4) and total triiodothyronine (T_3) are reduced in CKD patients (440,441). Plasma total T_3 is more markedly decreased than T_4. In series of 287 euthyroid patients with advanced CKD, decreased plasma total T_4 concentrations were found in 21% patients and total plasma T_3 lower than 100 ng/dL in 76% (440).

In plasma, the thyroid hormones are bound to thyroid hormone-binding globulin, albumin, and prealbumin. Thyroid hormone-binding globulin concentrations are usually normal in hemodialysis patients (450,451), or low to normal in CAPD patients. Thyroid hormone-binding globulin is lost into the peritoneal dialyzate (451,452).

Low total T_4 in CKD is mainly related to the impaired binding of this hormone to plasma protein carriers. In the plasma of CKD patients, several inhibitors of T_4 binding have been identified, including 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF), indoxyl sulfate, and hippuric acid (453,454).

Using equilibrium dialysis, normal free T_4 plasma concentration were found in CKD patients (455,456). This method seems to be the most appropriate one for patients with a significant reduction of serum T_4 binding capacity (441). Measurement of free T_4 with analog radioimmunoassay or with label antibody assay yields lower values than those determined with the equilibrium dialysis assay because the high concentrations of indoxyl sulfate and hippuric acid in uremic patients interfere with the assay (457). The normal *in vivo* production of T_4 (456,458) and of rT_3 from T_4 (456,458) in euthyroid CKD patients indicates that the plasma concentration of free T_4 is normal and that the low plasma free T_4 concentrations estimated by some assays are spurious.

In CKD patients, the plasma concentration of free T_3 is frequently low. The reduced plasma T_3 concentration is the result of decreased peripheral conversion of T_4 to T_3 in several tissues. In contrast, the production of T_3 in the thyroid gland is normal. T_3 clearance rates are normal or decreased (456,459). Impaired conversion T_4 to T_3 may also be the result of malnutrition, because in CKD patients a significant positive correlation was found between total plasma T_3 concentration and plasma albumin, as well as transferin concentrations (440).

In contrast to the other chronic nonthyroideal diseases, rT_3 plasma concentration is normal in CKD patients (455,459).

Although T_3 is the most active thyroid hormone, CKD patients with low plasma T_3 concentrations appear clinically euthyroid (460). The expression of messenger RNA of c-erb-A α and β T_3 receptors by mononuclear cells is increased in CKD patients compared with normal subjects (461). This response of the receptor may help to maintain a euthyroid state despite low free T_3 concentrations.

In CKD, abnormal thyroid hormone indices do not indicate a state of hypothyroidism, but are a reflection of the state of chronic illness and/or malnutrition. Therefore, abnormal thyroid hormone indices (low T_3 and T_4 plasma concentrations) do not require therapy, which even carries a hazard. In CKD patients with low plasma T_3 concentration, it has been shown that triiodothyronine supplementation causes a negative protein balance (462). The low T_3 state of CKD can be viewed as being protective, promoting conservation of protein.

Hypothalamo-Pituitary–Thyroid Axis

Despite low plasma concentrations of total and free T_4 and T_3, the plasma concentration of thyroid-stimulating hormone (TSH) is usually normal in CKD patients (463–465). The normal plasma TSH concentration despite a low plasma concentration of the thyroid hormones suggests an abnormal regulation of the hypothalamo-pituitary–thyroid axis. The TSH response to TRH is usually blunted (440,466–468). In CKD patients, the normal diurnal rhythm of TSH with a peak in the late evening or early morning is blunted (469,470) and the nocturnal TSH surge is reduced (469). The pattern of pulsatile TSH secretion is altered by the appearance of low-amplitude high-frequency pulses (470). In the liver of rats with chronic uremia, the T_3 content was reduced (471), but in the pituitary the T_3 content was normal (471). This dissociation between the local generation of T_3 in the liver and pituitary, respectively, explains why TSH fails to increase in CKD patients. Obviously the central thyreostat has been reset toward lower concentrations of thyroid hormones in the circulation.

Primary Hypothyroidism and Hyperthyroidism

Primary hypothyroidism is two to three times more frequent in CKD patients than in the general population (440). Risk factors are female sex, age greater than 50 years (440), and increased iodine intake (472). Hypothyroidism may also be observed in patients with extensive hemosiderosis (473), diabetes type 1 (440), and children with cystinosis (474).

It is very difficult to make the clinical diagnosis of hypothyroidism in CKD patients. The signs and symptoms of hypothyroidism, such as pallor, hypothermia, and asthenia are also found in patients with advanced CKD and no hypothyroidism (475). The only reliable procedure to diagnose hypothyroidism in renal failure is the finding of an elevated plasma concentration of TSH associated with clearly low plasma concentrations of total and free T_4 in the presence of a normal concentration of thyroid hormone-binding globulin. Because heparin competes with T_4 at the binding site of the hormone binding protein, causing an increase of total and free plasma T_4 concentrations for at least 24 hours, blood for the determination of thyroid hormones should be sampled before heparin administration at the beginning of a dialysis session (476).

Primary hyperthyroidism is extremely rare in CKD patients (441).

Abnormalities in the Parathyroid Glands

CKD patients almost always have hyperplasia of the parathyroid glands, but the volume and mass of the glands vary among patients and among the four glands in the same patients. The size of the gland may reach 10 to 50 times the normal value, but in some patients the size of the glands may even be normal (477–479). In the enlarged glands, one finds chief cell hyperplasia with or without hyperplasia of oxyphil cells. Apart from diffuse hyperplasia, as time goes by, one finds with increasing frequency nodular or adenomalike growths within the hyperplastic glands (477–479). The nodules are usually well demarcated and surrounded by a fibrous capsule (480,481). The cells in the nodular hyperplasia express less vitamin D (VDRs) (482) and calcium-sensing receptors (CaR) (483). The proliferative potential is markedly higher than that of the cells in diffuse hyperplasia (484,485). The first change in the parathyroid glands is polyclonal diffuse hyperplasia, but, as time goes by, monoclonal nodules form. If several monoclonal nodules of different size develop (nodular hyperplasia), the cells of one nodule may gain a growth advantage, proliferate faster and more vigorously, resulting in a large nodule occupying the entire gland (single nodular gland). Arnold et al. (486) were the first to document that the majority of adenomatous growths are monoclonal. Several studies have shown abnormalities in chromosome 11 (where the suppressor gene MEN-1 is located), allelic loss of 11q 13, and loss of several gene markers located on chromosome 11p (487–489). These molecular changes are implicated in the tumori genesis of the parathyroid gland nodules in CKD.

Available data indicate that in CKD phosphate retention (490,491), skeletal resistance to the calcemic action of PTH (492–495), and altered vitamin D metabolism (496,497), possibly also high FGF23 concentrations (498,499), interact to cause hyperplasia of the parathyroid glands.

It appears that phosphate retention in early stages of renal insufficiency interferes with the ability of patients to augment renal production of $1,25(OH)_2D_3$. This notion is supported by the studies of Llach and Massry (496) in adults and of Portale et al. (500) in children. These patients had dysfunction of organs that were targets for vitamin D action, such as impaired intestinal absorption of calcium and skeletal resistance to the calcemic action of PTH (496) despite normal blood levels of $1,25(OH)_2D_3$. Dietary phosphate restriction was followed by a significant increment in blood levels of $1,25(OH)_2D_3$ (386,388) and normalization of the abnormalities cited above (496). Thus, it appears that a state of relative $1,25(OH)_2D_3$ deficiency is present in early renal failure, leading to defective absorption of calcium and impaired calcemic response to PTH (496). These two abnormalities produce hypocalcemia and, subsequently, secondary hyperparathyroidism. Hypocalcemia stimulates PTH secretion (501) and causes parathyroid cell hypertrophy (502). Dietary phosphate restriction in these patients restored the blood levels of PTH to normal (496,500).

It should be mentioned that with more advanced CKD when hyperphosphatemia develops, the elevated blood levels of phosphorus may suppress blood levels of calcium and contribute to the hypocalcemia of advanced CKD. In addition, experimental evidence indicates that the very high blood levels of phosphorus may directly affect the function of the parathyroid glands (503); such high phosphorus may induce hyperplasia of the parathyroid glands independent of calcium or $1,25(OH)_2D_3$, and by posttranscriptional mechanism, increase PTH synthesis and secretion.

The relative deficiency of $1,25(OH)_2D_3$ in patients with early and moderate renal failure may, directly and independent of the hypocalcemia, affect parathyroid gland activity. Several lines of evidence indicate an interaction between $1,25(OH)_2D_3$ and parathyroid glands. First, the parathyroid cells have intracellular receptors for $1,25(OH)_2D_3$ and contain the mRNA for this receptor (504,505). Second, $1,25(OH)_2D_3$ inhibits prepro-PTH mRNA in a dose-dependent manner (506,507), and a deficiency of $1,25(OH)_2D_3$ is associated with an increase in prepro-PTH mRNA and in the weight of the parathyroid glands in experimental renal failure (502–510). Third, $1,25(OH)_2D_3$ inhibits PTH secretion (511), as well as parathyroid cell proliferation (512). Fourth, Oldham et al. (513) and Madsen et al. (514) report that $1,25(OH)_2D_3$ renders the parathyroid glands more susceptible to the suppressive action of increased ionized calcium.

The relationship between blood levels of PTH and calcium is best described by a sigmoidal curve (515) and the value of the set point for calcium, defined as the calcium concentration that produces half-maximal inhibition of PTH—i.e. the midpoint between the maximal and minimal PTH secretions (515). Alterations in the set point for calcium, with a shift to the right (i.e., 50% inhibition of PTH secretion occurs at higher calcium concentration), were observed in parathyroid glands of patients with primary (516) or secondary hyperparathyroidism (515,517).

The cells of the parathyroid glands possess calcium receptor (CaR) (518). This receptor protein is located in the membrane of these cells. The levels of serum calcium and $1,25(OH)_2D_3$ as well as dietary phosphate do not appear to regulate the synthesis of CaR. In CKD, there is a reduction in the density of CaR of the parathyroid gland cells (483).

The CaR interacts with calcium as well as with other divalent-, trivalent-, and polycations (519). This receptor protein plays an important role in the ability of parathyroid glands to recognize changes in the concentration of calcium ion in the blood; and as such, CaR mediates the effect of calcium on the secretion of PTH from the parathyroid glands. A decrease in the concentration of ionized calcium in blood is recognized by the G protein-coupled CaR and subsequently leads to PTH release. An increase in the concentration of calcium in the blood activates the CaR, an event that leads to an increase in the concentration of cytosolic calcium secondary to mobilization of calcium from intracellular calcium stores. This rise in cytosolic calcium results in inhibition of PTH release from the parathyroid glands.

Changes in intracellular cAMP affect PTH secretion. Any factor that increases cAMP (hypocalcemia, β-adrenergic agonists, dopaminergic agents, secretin, prostaglandin E, and cholera toxin) enhances PTH secretion. On the other hand, factors that reduce intracellular cAMP (hypercalcemia, hypermagnesemia, α-adrenergic agonists, prostaglandins of the F series, and nitroprusside) inhibit PTH release. Calcium is the most important regulator of PTH secretion and the effect is mediated by changes in the intracellular concentration of calcium. An increase in the concentration of intracellular calcium influences

PTH secretion through several mechanisms: (a) inhibition of cAMP accumulation, (b) inhibition of cAMP action, and (c) stimulation of intracellular degradation of preformed PTH.

The available data strongly support the proposition that deficiency of $1,25(OH)_2D_3$ may initiate secondary hyperparathyroidism, even in the absence of overt hypocalcemia; this has been demonstrated in dogs with a 70% decrease in GFR (520). Since patients with CKD have both hypocalcemia and deficiency of $1,25(OH)_2D_3$, they are in double jeopardy regarding the development of secondary hyperparathyroidism. They may develop very large glands due to hyperplasia or hypertrophy of the parathyroid cells.

The pathogenesis of secondary hyperparathyroidism in CKD, discussed previously, has important clinical implications. It is evident that dietary phosphate restriction in proportion to the fall in GFR in patients with early CKD failure is adequate to reverse and correct secondary hyperparathyroidism and other abnormalities in divalent ion metabolism. However, achieving the proper and adequate dietary phosphate restriction and successful patient compliance with the dietary regimen is difficult. Since the available data indicate that dietary phosphate restriction exerts its effect through the increased production of $1,25(OH)_2D_3$ and since this vitamin D metabolite also exerts a direct effect on the parathyroid glands, an alternative therapeutic approach would be supplementation of $1,25(OH)_2D_3$. Indeed, treatment of patients with moderate CKD with $1,25(OH)_2D_3$ for 6 to 12 months was associated with improvement or normalization of the disturbances in divalent ion metabolism, including secondary hyperparathyroidism and bone disease (521,522).

The hyperplasia of the parathyroid glands in CKD causes marked elevation in blood levels of PTH (523,524), which becomes evident early in the course of renal failure (523,524). There is marked variation of the serum concentrations of PTH in patients with advanced CKD and in those treated with hemodialysis; the levels may be 2 to 200 times normal. Low or undetectable levels of PTH may be encountered in patients with advanced renal failure who are hypomagnesemic and magnesium deficient (525), those who have low-turnover osteomalacia with (526) and without (527,528) increased body burden of aluminum, and those who have primary hypoparathyroidism. Patients with diabetes mellitus and renal failure may have lower levels of PTH than those with renal failure due to other causes (529). The serum levels of PTH in the patients on dialysis may be affected by the concentration of calcium in the dialyzate; patients treated with high calcium dialyzate may have lower serum levels of PTH than those treated with lower calcium dialyzate.

The PTH in the serum of CKD patients is made up of many fragments, but mostly of the midregion and C-terminal fragments. Therefore, assays using an antibody directed toward these moieties of PTH yield spuriously high values; in contrast, the use of an antibody to the N-terminal fragment may reveal modest elevation in the serum levels of PTH. A two-site immunoradiometric assay (IRMA), which measures intact PTH molecule, has been developed (530). It is precise and provides reliable values of the serum levels of intact PTH in CKD patients (531).

A large body of evidence has accumulated, indicating that the excess blood levels of PTH in CKD patients are deleterious to the function of many organs. Excess PTH has been implicated in the pathogenesis of the abnormalities of the central (531–541), peripheral (542,543), and autonomic (544–546) nervous systems; myocardiopathy (547–551); skeletal myopathy (552,553); glucose intolerance and impaired insulin secretion (64,65); disorders of red blood cells (554–558), polymorphonuclear leukocytes (559–564) and T-cell (565,566) and B-cell (567,568) function; and abnormalities of the immune system (569).

This widespread effect of PTH on so many cells indicates that these cells must have receptors for PTH. Both Urena et al. (570) and Tian et al. (571) have demonstrated that the mRNA for the PTH-parathyroid hormone-related protein (PTH-PTHrP) receptor is present in many cells including kidney, bone, heart, brain, liver, spleen, aorta, ileum, skeletal muscle, lung, and testes. Thus, both pathophysiologic and molecular evidence exist supporting the notion that almost all body organs are targets for PTH actions and, therefore, it is not surprising that chronic excess of PTH in CKD may exert widespread deleterious effects on body function in uremia. Hence, PTH is being considered a major uremic toxin.

In CKD, the mRNA of the PTH-PTHrP receptor is downregulated in both the traditional (kidney) and nontraditional (liver and heart) target tissues for PTH action (572–574). The mechanism underlying this phenomenon is not fully elucidated. However, since in CKD PTH causes a rise in $[Ca^{2+}]i$ in many cells, and since the elevation in $[Ca^{2+}]i$ is a major contributor to cell dysfunction in CRF (46), it is plausible that the rise in $[Ca^{2+}]i$ provides a negative feedback-control mechanism through which the mRNA of the PTH-PTHrP is downregulated so that the increment in $[Ca^{2+}]i$ of cells does not progress unabated as the blood levels of PTH continue to rise with CKD progression. Tian et al. (573) and Smogorzewski et al. (574) provide evidence supporting this notion. They report that normalization of the $[Ca^{2+}]i$ of the kidney and heart in animals with CRF by verapamil treatment prevents the downregulation of the mRNA of the PTH-PTHrP receptor despite significant elevation in the blood levels of PTH.

Abnormalities in the Vitamin D Metabolites

The parent vitamin D is first hydroxylated in the liver to 25-hydroxyvitamin D (25[OH]D) (575,576). The turnover of vitamin D may be accelerated by infection or drugs stimulating microsomal liver enzymes, resulting in lower plasma levels of 25(OH)D (577). This vitamin D metabolite binds to a α-globulin (vitamin D–binding protein [DBP]) (578). In patients with nephrotic syndrome, the DBP is lost in the urine and with it 25(OH)D (579). Therefore, these patients have low blood levels of 25(OH)D. Similarly, DBP and 25(OH)D are lost in the peritoneal fluid in patients treated with CAPD. These patients may also have low blood levels of 25(OH)D (580,581). The normal blood level of 25(OH)D in adults living in the United States is 15 to 30 ng/mL (582). These levels display seasonal fluctuation related to sun exposure; they are higher in the summer and lower in the winter (583). The half-life of 25(OH)D is about 12 days (584).

In CKD patients with normal function of the gastrointestinal tract and liver, plasma 25(OH)D levels are usually normal (585–587), but are increased (588) in patients treated with vitamin D. Excessive proteinuria (579,586) and concomitant severe extrarenal diseases (586,589) are associated with low blood levels of 25(OH)D. In CKD patients treated by CAPD or hemofiltration (590), plasma levels of 25(OH)D are usually low normal, while they are normal in hemodialysis patients. Low plasma 25(OH)D levels in CKD patients are usually due to reduced exposure of the skin to ultraviolet light, reduced intake of vitamin D, intestinal malabsorption, or impaired hepatic 25-hydroxylation.

The 25(OH)D is transported to the kidneys for further hydroxylation, resulting in the production of the active metabolite $1,25(OH)_2D_3$ (591–593). This metabolite also binds to DBP, but its binding efficiency to DBP is less than that of 25(OH)D. $1,25(OH)_2D_3$ may also be produced by other cells, such as normal alveolar (594) and peritoneal (595) macrophages, peripheral blood macrophages (596), and macrophages of granulomas (597).

The main factors that regulate the function of renal 1 α-hydroxylase enzyme and, hence, the production of $1,25(OH)_2D_3$ are PTH, calcium, and phosphorus (598,599). Plasma levels of $1,25(OH)_2D_3$ are dependent on several factors, including biosynthesis of vitamin D in the skin, intake of vitamin D or its metabolites, absorption of vitamin D by the intestinal tract, intensity of 25-hydroxylation in the liver, and of 1-α-hydroxylation of 25(OH)D in the kidneys (600), and loss of vitamin D metabolites in urine of patients with nephrotic syndrome (579) or in the dialyzate of CAPD patients (580,581). The normal blood levels of $1,25(OH)_2D_3$ range between 25 and 33 pg/mL in adults and between 49 and 66 pg/mL in children (601). The half-life of $1,25(OH)_2D_3$ is about 20 hours (602), which is considerably shorter than that of 25(OH)D.

The blood levels of $1,25(OH)_2D_3$ are usually normal in patients with moderate CKD (448,450). Despite these normal levels, these patients display abnormalities in the function of the target organs for vitamin D action (448). These observations indicate that these patients have a vitamin D–resistant state. As CKD progresses, an absolute vitamin D–deficient state develops, with the blood levels of $1,25(OH)_2D_3$ being reduced when GFR falls below 50 mL/minute in children (450,603) and below 30 mL/minute in adults (448,604). In anephric patients and in those treated with dialysis, the blood levels of $1,25(OH)_2D_3$ are usually very low or undetectable (605–607). The finding of low blood levels of $1,25(OH)_2D_3$ in anephric patients and especially in those receiving vitamin D indicates that $1,25(OH)_2D_3$ is produced by extrarenal sites in these patients. Patients with CKD and granulomatous diseases, such as sarcoidosis, may have elevated blood levels of $1,25(OH)_2D_3$ (608) caused by the extrarenal production of this metabolite by the macrophages of the granulomatous tissue (597).

The metabolic clearance rate (MCR) of $1,25(OH)_2D_3$ in uremic dogs (609) and humans (610) is not different from normal values. In contrast, Hsu et al. (611,612) report that the MCR of $1,25(OH)_2D_3$ in uremic rats is lower than in normal rats. These authors attribute this phenomenon to suppression of the activity of 24-hydroxylase activity (an enzyme that is involved in the degradation of $1,25[OH]_2D_3$) by an unidentified factor present in the uremic serum (613). In contrast, Taylor et al. (614) report that the MCR of $1,25(OH)_2D_3$ is normal in rats after unilateral nephrectomy.

The deficiency of $1,25(OH)_2D_3$ plays a paramount role in the genesis of many of the disturbances of divalent ions observed in patients with CKD. These abnormalities include secondary hyperparathyroidism, defective intestinal absorption of calcium, skeletal resistance to the calcemic action of PTH, defective mineralization of bone, growth retardation in children, and proximal myopathy.

$1,25(OH)_2D_3$ exerts its action by binding to an intracellular vitamin D receptor (VDR), which is located predominantly in the nucleus (615). The hormone–receptor complex interacts with DNA-responsive elements in target genes with the synthesis of bioactive proteins (616), which, in turn, mediate the actions of $1,25(OH)_2D_3$. Patients with CKD display end-organ resistance to the action of $1,25(OH)_2D_3$. This phenomenon is due to impairment in the cascade of the cellular events culminating in the biologic actions of $1,25(OH)_2D_3$. First, there is a decrease in the concentration of $1,25(OH)_2D_3$ receptors in rats (617,618), dogs (619), and humans (620) with renal failure. Second, certain as yet unidentified uremic toxin inhibits the binding of the $1,25(OH)_2D_3$–receptor complex with DNA-responsive elements in the target gene (618). This defect would, therefore, interfere with the production of the bioactive protein required for the action of $1,25(OH)_2D_3$. In advanced CKD, the number of VDRs is reduced (482,619), leading to vitamin D resistance. Thus, in CKD patients, there is vitamin D deficiency and vitamin D resistance, as well.

The factors responsible for the decrease in the number of VDRs in uremia are not fully elucidated but may include:

1. Reduced levels of $1,25(OH)_2D_3$, since this metabolite affects the production of VDR (504); specifically, low levels of $1,25(OH)_2D_3$ downregulate the mRNA of VDR.
2. Hyperparathyroidism of renal failure may play a role, since high levels of PTH interfere with the $1,25(OH)_2D_3$-induced upregulation of VDR (621).
3. Uremic toxins may decrease the stability of the mRNA of VDRs, resulting in transcriptional reduction of VDR protein (622).

Alterations of the Renin–Angiotensin–Aldosterone System in Chronic Kidney Disease

The renin-angiotensin-aldosterone system (RAS) is both an endocrine and a paracrine system, which plays a major role under physiologic and pathophysiologic conditions. The RAS is involved in the regulation of blood pressure, control of volume, and sodium balance, as well as growth and remodeling of cardiovascular and renal tissues under pathologic conditions, to name only a few. Space does not permit to give an exhaustive overview and we restrict the discussion to problems where measurement of the components of the system provides guidance to the clinician.

Plasma Renin Activity

In CKD patients suffering from primary renal disease the activity of the renin-angiotensin system is inappropriately high. Weidmann et al. (623) documented that in hemodialysis patients, with or without hypertension, plasma renin activity is higher at any given level of exchangeable body sodium than in normal subjects. These observations demonstrate that sodium retention and hypervolemia do not adequately suppress renin secretion, indicating disruption of the negative feedback between volume state and renin secretion. In renal disease an important mechanism, accounting for increased and unregulated renin secretion, is luminal narrowing of preglomerular vessels because of vascular sclerosis. Consequently, the "baroreceptor" in the juxtaglomerular apparatus will measure falsely low perfusion pressures—analogous to the kidney with a Goldblatt clip of the renal artery. Renin will, therefore, be secreted even when blood pressure is high and exchangeable sodium increased. Normally plasma renin activity (PRA) decreases asymptotically with increasing blood pressure. In contrast, in patients with renal disease, renin secretion is not adequately suppressed by high blood pressure values and PRA remains inappropriately high.

In CKD patients the basal values of PRA, measured in peripheral blood, vary. Low, normal, and elevated PRA values have been reported (425,623–626). These variations are most likely due to variable degrees of renal ischemia in different renal diseases, to variable disruption of the negative feedback-control system between body fluid volume and renin secretion, nonstandardized conditions of examination, and others. Weidmann and Maxwell (627) reported that the PRA is the highest in patients with nephrotic syndrome, elevated in those with a glomerulonephritis, and normal in patients with tubulointerstitial disease. Meltzer et al. (628) showed that PRA is high in patients with nephrotic syndrome due to minimal change disease, but low or normal in other forms of nephrotic syndrome. On the other hand, in some patients with renal disease, particularly diabetic nephropathy, obstructive uropathy, or interstitial nephritis, hyporeninemic hypoaldosteronism with low PRA values is seen rather frequently. (629–633). It

should also be mentioned that hypertensive CKD patients are treated with medications that may affect renin secretion, contributing to the variability of PRA. After hemodialysis, PRA may increase dramatically as a result of ultrafiltration and hypovolemia (634–636). In patients on CAPD, PRA is usually higher than in patients on hemodialysis, possibly as a result in the latter patients of volume expansion (414,429). These arbitrary examples illustrate that the values of PRA must be interpreted, taking into consideration numerous confounding factors, such as type of renal disease, volume state, blood pressure, medications, etc.

It should be mentioned that PRA in the circulation does not adequately reflect the activity of local tissue RAS systems. The paradox that drugs, which block the RAS, are highly effective and renoprotective, e.g., in patients with diabetic nephropathy as documented by the Lewis trial (636) and the IDNT (637) or RENAAL trials (638), is explained by the activation of local renin systems in proximal tubular epithelial cells, in podocytes, in mesangial cells, etc., despite low PRA in the circulation (639–641).

The importance of the RAS in CKD is illustrated by the fact that in CKD patients, blockade of the system reduces progression (636–638,642,643), lowers elevated blood pressure (644), and induces partial regression of cardiovascular structural abnormalities, such as left ventricular hypertrophy (LVH) (645–647). In CKD patients, treatment blockade of the RAS carries an increased risk of hyperkalemia (648).

Aldosterone

Plasma aldosterone concentrations are strikingly elevated in subtotally nephrectomized rats (649). After adrenalectomy and substitution of glucocorticoids, the animals have lower blood pressures, less proteinuria, and less structural lesions (650). Aldosterone influences several factors, which are relevant in the progression of kidney disease, exemplified, for instance, by increased collagen IV synthesis by mesangial cells (651), elevated glomerular pressures by direct actions on glomerular microcirculation (652), and many others. The pathogenetic role of stimulation of the mineralocorticoid receptor is suggested by the observation that mineralocorticoid receptor blockade reduces renal injury in spontaneous hypertensive rats (SHR–sp). It reduces proteinuria, as well as glomerulosclerosis (653).

Plasma aldosterone concentrations are variable among CKD patients (654). Normal (655,656), decreased (626,628, 632,657–662), and elevated (663–667) basal levels of plasma aldosterone have been reported. In a study in which plasma aldosterone levels were measured in patients with various degrees of renal insufficiency, the levels were normal in those with GFR greater than 50 mL/minute and increased progressively with the decline in GFR (654).

In some CKD patients, usually those with interstitial disease, hyporeninemia, and hyperkalemia, plasma aldosterone concentrations are low—the constellation typical for the syndrome of hyporeninemic hypoaldosteronism (632,657–659). In such patients, ACTH and Ang II administration fails to stimulate aldosterone secretion (658,659).

In CKD patients, before starting dialysis with normal PRA and normokalemia, plasma aldosterone levels are normal (655–657) or increased (632,657–661). In these patients, the response of plasma aldosterone to ACTH or Ang II administration is intact (656,663,669,671).

The plasma concentrations of aldosterone in hemodialysis patients are variable (655,663,667,668) and correlate well with the plasma concentrations of potassium and PRA. In these patients, plasma aldosterone increases with upright posture and after administration of ACTH (656,667). The changes in plasma aldosterone concentrations, following the dialysis procedure, are also dictated by the changes in body volume and in the concentrations of plasma potassium. Thus, during ultrafiltration, without changes in plasma potassium, plasma aldosterone increases (667–670). A decline in plasma potassium concentration during hemodialysis would blunt the rise in plasma aldosterone concentrations induced by fluid removal (667–670). In anephric hemodialysis patients, plasma aldosterone concentrations are usually reduced (655,656,667–673), but also normal plasma concentrations may be encountered (427,428). These patients may have impaired responses to Ang II (655,674), ACTH (655,667,674), upright posture (655,667), or changes in volume status (625,673,674). Patients undergoing CAPD have higher plasma aldosterone concentrations than those treated by hemodialysis (414,424).

A correlation was found between plasma aldosterone concentration and the rate of CKD progression in diabetic patients (674). In type II diabetic patients, Sato et al. (675) found that if aldosterone escape occurred under angiotensin-converting enzyme inhibitor therapy, that is, a secondary rise of plasma aldosterone after initial decrease, spironolactone, independent of blood pressure, reduced albuminuria (675). A similar observation with eplerenone was made in the preliminary study of Epstein et al. (676). The importance of aldosterone escape on progression is supported by observational study by Schjoedt et al. (677) in type 1 diabetic patients with nephropathy. The decline in GFR was significantly higher in patients with an aldosterone escape than in patients without one (677).

It was shown that in patients with severe heart failure inhibition of aldosterone with low dose of spironolactone is associated with morbidity and mortality reduction (678). Aldosterone is a potent mediator of myocardial and vascular fibrosis (679–681) and reduced markers of cardiac damage were measured after aldosterone antagonist treatment (682). Aldosterone is a potent kaliuretic hormone. Therefore, usage of aldosterone blocker may increase the risk of hyperkalemia in CKD patients, particularly when used in combination with angiotensin-converting enzyme inhibitors or Ang II receptor blocker (683). However, the results of small, nonrandomized, nonblinded studies suggest that treatment with a low dose of spironolactone (25 mg/day or 25 mg three times per week) was not associated with an increased frequency of hyperkalemia (684,685). Therefore spironolactone may also be considered in hemodialysis patients as a treatment option for heart disease. However, plasma potassium concentrations should be monitored carefully during aldosterone antagonist therapy.

Abnormalities in the Cardiac Natriuretic Peptides

Cardiomyocytes produce and secrete a family of related peptide hormones named cardiac natriuretic hormones, which have potent diuretic, natriuretic, and vascular smooth muscle relaxing effects (686,687). Cardiac natriuretic hormones include atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and their related peptides. ANP is released by atrial myocytes in response to stretch associated with increased atrial pressure, while ventricular production and release of this peptide are triggered only in the presence of ventricular hypertrophy (688). BNP is produced by ventricular myocytes and its generation rate is increased in heart failure and left ventricular hypertrophy (689). Therefore, plasma concentration of cardiac natriuretic hormones is increased in diseases characterized by expanded fluid volume, including renal failure, liver cirrhosis, and heart failure (690).

In CKD patients, plasma concentrations of ANP and BNP are elevated (685–702). In the predialysis phase of CKD, there is a significant correlation between plasma ANP and serum creatinine concentrations (695). The causes of high

plasma concentrations of ANP and BNP in advanced CKD are multifactorial and depend on: (a) an increase in intravascular filling and to atrial distension (437,700), (b) concomitant heart failure (703,704), and (c) diminished renal clearance (705,706). Indeed, removal of fluid by ultrafiltration during dialysis therapy is associated with a decrease in the plasma ANP (707–709) and BNP concentrations (709).

The measurement of ANP and BNP plasma concentration was used as a biochemical marker of volume overload in the CKD patient for improved identification of "dry weight" (710,711). The use of ANP and BNP to improve the definition of dry weight has yielded variable results, however (710–714). The weight of evidence indicates that measurements of ANP and BNP plasma concentration add little to the clinical examination (710,712). This is because of: (a) wide variability in results, (b) lack of correlation with measures of extracellular volume, (c) inability to detect volume depletion (no differences between normovolemia and hypovolemia), and (d) often the presence of cardiac dysfunction as a confounder (715).

Results of recent studies suggest that, in general, estimation of plasma concentrations of cardiac natriuretic hormones could be useful for differential diagnosis of heart failure (703,704). Moreover, a number of studies in heart failure showed a prognostic relevance of cardiac natriuretic hormones plasma concentrations (703,704). Among patients with heart failure, those with high plasma concentrations of cardiac natriuretic hormones are characterized by a worse prognosis (716,717).

Left ventricular hypertrophy and left ventricular dysfunction are considered as a predictor of cardiovascular and total mortality in dialysis patients. Mallamaci et al. (715) found that measuring of cardiac natriuretic hormones plasma concentrations, particularly BNP, may be useful for the identification of dialysis patients with left ventricular hypertrophy or for excluding systolic dysfunction. Moreover, in a prospective study in a cohort of dialysis patients without overt heart failure Zoccali et al. (718) found that high plasma concentrations of cardiac natriuretic peptides, particularly BNP, were strong predictors of cardiovascular mortality. The prognostic value of concentrations of cardiac natriuretic peptides in hemodialysis patients was confirmed by several recent studies (719–721).

Abnormalities in the Gastrointestinal Hormones

Elevated plasma gastrin concentrations have been reported in CKD patients (722–728). Hypergastrinemia in CKD is due predominantly to "big" gastrin (G34). Plasma concentrations of "little" gastrin (G17) are normal in CKD patients (725). "Big" gastrin is biologically six to eight times less active than "little" gastrin. This may explain the fact that the incidence of peptic ulcer in CKD patients is comparable with the general population in the most of studies (727).

Postprandial gastrin release in CKD patients in the first 30 minutes was similar to that in normal subjects, but the peak values were attained later, and the response was more prolonged (726). Hemodialysis treatment does not affect the elevated plasma gastrin concentration (725,728,729), although, in some studies, a decline in gastrinemia was observed (726). A significant decline of gastrinemia after treatment with erythropoietin was found (730).

Since the kidney is the main site of gastrin biodegradation (731), hypergastrinemia in uremic patients is regarded mainly as the consequence of reduced renal degradation of this hormone.

The plasma concentrations of other gastrointestinal hormones, such as cholecystokinin (732–734), gastric inhibitory peptide (729), pancreatic polypeptide (725,730,735,736), secretin (732), gastrin releasing peptide (737), ghrelin (738–740), and motilin (724) are elevated in CKD patients. Both normal (725) and markedly elevated (741) plasma concentrations of vasoactive intestinal polypeptide (VIP) were found in CKD patients. The pathophysiologic importance of these findings remains to be elucidated.

Abnormalities in the β-Lipotropin and Opioid Peptides

Plasma β-lipotropin concentrations, like most of the other pituitary-derived polypeptide hormones, are moderately or markedly elevated in CKD patients (742,743), due to decreased renal clearance (743). Similarly elevated plasma concentrations of β-endorphin (744–746) and met-enkephalin (744,745) have been found in CKD patients. Plasma met-enkephalin levels were directly correlated with plasma creatinine and urea concentrations (747). In contrast, plasma leu-enkephalin levels are significantly lower in CKD patients than in healthy subjects (747). After treatment with a low-phosphorus, low-protein diet supplemented with essential amino acids and keto analogs, a significant decline of plasma α-endorphin concentrations was reported (745).

The elevated plasma levels of the opioid peptides suggest a state of hyperendorphinism, which may play a role in many of the endocrine abnormalities in CKD. Support for this notion is provided by studies by Kokot et al. (748,749), who demonstrated that the administration of naloxone, which causes blockade of opioid receptors, is followed by amelioration of many of the endocrine derangements in CKD.

Abnormalities in the Hormones of Adipose Tissue

The adipose tissue is an important endocrine organ producing biologically active substances with local and/or systemic action. An incomplete list comprises PAI-1, transforming growth factor β (TGF-β), tissue factor (TF), complement factors (e.g., adipsin), adipocyte complement-related protein, TNF-α, acylation stimulating protein (ASP), angiotensinogen, prostaglandin ($PGI^{-}2\alpha$), IGF-1, macrophage inhibitory factor (MIF), sex hormones, glucocorticoids, Ang II, leptin, adiponectin, and resistin (750,751).

Leptin

Leptin is a protein that is predominantly produced by adipocytes. It is encoded by the ob gene. It is presumed that leptin is involved in the regulation of appetite, food intake and energy expenditure, sexual maturation and fertility, hematopoiesis, and activity of the hypothalamic-pituitary-gonadal axis. Obese individuals have high plasma leptin concentrations.

Patients with advanced CKD have elevated plasma leptin concentration compared to BMI and sex-matched healthy individuals (752–755). Leptin concentrations are normalized by successful kidney transplantation (756). Interestingly, the influence of impaired kidney function on plasma leptin concentration is less pronounced in noninflammatory acute renal failure than in CKD, suggesting participation of the other factors influencing leptin secretion in this state (757).

It was shown that the decreased leptin clearance by the insufficient kidneys leads to its' accumulation in circulation (758,759). However, the cascade of events responsible for leptin catabolism by the kidneys is unknown. Some published data suggests that, in part, leptin elimination is independent from

glomerular filtration rate (759–761). Some experimental and clinical studies have shown no correlation between GFR (measured by creatinine clearance) and fractional renal extraction of leptin (760–762). Results of kinetic studies suggest that renal metabolism of leptin involves active uptake of leptin by the renal tissue (763). Cumin et al. (764) studied changes of plasma leptin concentration in Zucker obese rats subjected to bilateral nephrectomy or bilateral ureteral ligation. Bilateral ureteral ligation reduced glomerular filtration by increasing tubular pressure. Following the bilateral nephrectomy, in these experiments, plasma leptin concentration increased by 300%, a value much higher than only 50% increase after bilateral ureteral ligation. Results of this elegant experimental study suggest that leptin elimination is only in part dependent on glomerular filtration (764). Therefore, renal elimination is not necessarily affected by the disease in direct proportion to changes in glomerular filtration.

Increased plasma leptin concentration in CKD is not due to oversecretion of this protein. It was shown that leptin gene expression in adipocytes in CKD patients is lower than in healthy individuals (762). Leptin plasma concentrations are not reduced by low-flux dialysis membranes, whereas high-flux dialysis membrane decreases leptin levels (765–767). CAPD patient are characterized by higher plasma leptin concentration than hemodialysis patients (768–770).

There is growing evidence that leptin, originally considered exclusively as an anorexigenic hormone, exerts actions in the periphery outside of the central nervous system. Indeed, leptin receptors are found in many tissues. It was initially thought that hyperleptinemia was an adequate explanation of anorexia in CKD patients (771,772). However, subsequent studies addressing the relation between nutritional status and plasma leptin concentration in CKD yielded conflicting results (773–777). Therefore, the role of high plasma leptin concentration in anorexia and malnutrition in CKD patients is not proved. Leptin stimulates proliferation and differentiation of hemopoietic stem cells (778). It is likely that the effects of leptin and of erythropoietin are synergistic. It deserves consideration whether high plasma leptin concentrations in CKD patients counteract the development of anemia when a plasma erythropoietin concentration is relatively decreased.

Leptin may also participate in CKD progression. It was shown in rat that leptin infusion lead to the increase of glomerular TGF-β and collagen IV expression and to the enhancement of proteinuria (779). Moreover, leptin stimulates proliferation of cultured glomerular rat endothelial cells (780).

Adiponectin

Adiponectin is one of the protein hormones secreted by adipocytes, circulating in the blood stream in relatively high concentrations (almost 0.01% of total plasma protein) (780). It shows structural homology with collagen VIII, X, and complement factor C1q (781). It was shown that adiponectin is characterized by antiatherogenic and insulin-sensitizing properties (782). Adiponectin receptors were found in skeletal muscles (783,784), liver (783), and endothelial cells (785). It was suggested, that adiponectin might inhibit formation of initial atherosclerotic lesions by decreasing the expression of adhesion molecules (VCAM-1; ICAM-1, E-selectin) in endothelial cells in response to inflammatory stimuli, such as TNF-α (786) and suppressing cytokine (also TNF-α) production by macrophages (787). Moreover, adiponectin suppresses lipid accumulation in human monocyte-derived macrophages and inhibits macrophage-to-foam cell transformation (788). It was also shown that adiponectin inhibits oxidized LDL-stimulated cell proliferation and suppresses cellular superoxide generation (785). Finally, adiponectin protects Apo E-deficient mice (well known animal model of atherosclerosis) from atherosclerosis (789,790). Numerous experimental studies showed that adiponectin also improves insulin sensitivity (782). This effect is due to stimulation of glucose utilization and fatty acid oxidation in skeletal muscles and in liver and enhanced suppression of glucose liver production (782). Recently, it was shown that adiponectin-deficient mice were more prone to renal injury after induction of inflammatory process by anti-GBM serum (791). Results of above cited study showed that adiponectin directly modulates the inflammatory process by modifying the expression of inflammatory cytokines in anti-GBM glomerulonephritis experimental model (791). Plasma adiponectin concentrations in humans are lower in obese than in nonobese subjects, in males than in females, and in patients with coronary artery disease, diabetes mellitus type II, and essential hypertension than in healthy subjects (792–796).

The plasma adiponectin concentration was markedly elevated in hemodialysis patients with CKD (797–799). Shoji et al. (800) showed that plasma of CKD patients contains intact adiponectin. It was recently shown that successful kidney transplantation was accompanied by prompt reduction of plasma adiponectin concentration, but it remains still higher than in healthy controls (801). It is thought that kidneys are important organs participating in the adiponectin biodegradation and elimination. An inverse relationship between GFR and plasma adiponectin concentration was found in patients with essential hypertension and in apparently healthy individuals (802,803). Recently, measurements of plasma adiponectin concentrations in renal veins and aorta in human subjects confirmed that kidneys play an important role in adiponectin elimination (Adamczak et al. personal unpublished data) and that the increased plasma adiponectin concentration in CKD is not due to oversecretion. Marchlewska et al. (804) has found that expression of the adiponectin gene (ApM1) in adipocytes of patients with end-stage renal failure is decreased. Low adiponectin gene expression may be associated with the state of low-grade inflammation in CKD patients. In the general population, an inverse correlation was found between adiponectinaemia and serum C-reactive protein (CRP) concentration (805,806). Such relationships were also found in hemodialysis patients (798,807,808). This relationship may be of clinical relevance, because it was found that TNF-α and interleukin-6 exert an inhibitory effect on adiponectin gene expression in cultured adipocytes (809–811).

Zoccali et al. (797) demonstrated in a large cohort of hemodialysis patients that a relatively low plasma adiponectin concentration is an independent predictor of fatal and nonfatal cardiovascular complications in these patients. This observation was confirmed by Ignacy et al. (808), who observed that hemodialysis patients within the lowest plasma adiponectin tertile were characterized by shorter survival time.

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SECTION XIII: MANAGEMENT OF END-STAGE RENAL DISEASE

CHAPTER 97 ■ IMMUNOBIOLOGY AND IMMUNOPHARMACOLOGY OF RENAL ALLOGRAFT REJECTION

MANIKKAM SUTHANTHIRAN, CHOLI HARTONO, AND TERRY B. STROM

Renal transplantation is the treatment of choice for a significant number of patients with irreversible renal failure (1). An improved understanding of the antiallograft repertoire, better preservation of donor kidneys, judicious usage of immunosuppressive drugs and antibodies, and the clinical application of infection prophylaxis protocols have all contributed to the steady improvements in outcome following renal transplantation.

IMMUNOBIOLOGY OF RENAL TRANSPLANTATION

The Antiallograft Response

Allograft rejection is contingent on the coordinated activation of alloreactive T cells and antigen-presenting cells (APCs). Through the intermediacy of cytokines and cell-to-cell interactions, a heterogeneous contingent of lymphocytes, including CD4+ helper T cells, CD8+ cytotoxic T cells, antibody-forming B cells, and other proinflammatory leukocytes are recruited into the antiallograft response (Fig. 97-1 and Table 97-1).

T Cell Activation and the Immunologic Synapse: Signal One

The immunologic synapse consists of a multiplicity of T cell-surface protein forms and clusters, thereby creating a platform for antigen recognition and generation of various crucial T cell activation-related signals (2). The synapse begins to form when the initial adhesions between certain T cell (e.g., CD2, LFA-1) and APC surface proteins (e.g., CD58, ICAM-1) are formed (Table 97-2). These adhesions create intimate contact between T cells and APCs and thereby provide an opportunity for T cells to recognize antigen. Antigen-driven T cell activation, a tightly regulated, preprogrammed process, begins when T cells recognize intracellularly processed fragments of foreign proteins (approximately 8 to 16 amino acids) embedded within the groove of the major histocompatibility complex (MHC) proteins expressed on the surface of APCs (3–5). Some recipient T cells directly recognize the allograft (i.e., donor antigen[s] presented on the surface of donor APCs), while other T cells recognize the donor antigen after it is processed and presented by self-APCs (6) (Fig. 97-1).

The T cell antigen receptor (TCR)–CD3 complex is composed of clonally distinct TCR a and β peptide chains that recognize the antigenic peptide in the context of MHC proteins and clonally invariant CD3 chains that propagate intracellular signals originating from antigenic recognition (2,7,8) (Fig. 97-2). The TCR variable, diversity, junction, and constant region genes (i.e., genes for regions of the clone-specific antigen receptors) are spliced together in a cassettelike fashion during T cell maturation (7). A small population of T cells expresses TCR γ and δ chains instead of the TCR α and β chains.

CD4 and CD8 proteins, expressed on reciprocal T cell subsets, bind to nonpolymorphic domains of human leukocyte antigen (HLA) class II (DR, DP, DQ) and class I (A, B, C) molecules, respectively (2,7) (Fig. 97-1; Table 97-2). A threshold of TCR to MHC-peptide engagements is necessary to stabilize the immunologic synapse stimulating a redistribution of cell-surface proteins and coclustering of the TCR/CD3 complex with the T cell-surface proteins (8–10). CD5 proteins (9,10) join the synapse. The TCR cluster already includes integrins (e.g., LFA-1) and nonintegrins, e.g., CD2 (2,8,9) that have created T cell–APC adhesions. Hence, antigen recognition stimulates a redistribution of cell-surface proteins and coclustering of the TCR/CD3 complex with the T cell-surface proteins (2,7–9) and signaling molecules. This multimeric complex functions as a unit in initiating T cell activation.

Following activation by antigen, the TCR–CD3 complex and coclustered CD4 and CD8 proteins are physically associated with intracellular protein–tyrosine kinases (PTKs) of two different families, the src (including $p59^{fyn}$ and $p56^{lck}$) and ZAP 70 families (2). The CD45 protein, a tyrosine phosphatase, contributes to the activation process by dephosphorylating an autoinhibitory site on the $p56^{lck}$ PTK. Intracellular domains of several TCR/CD3 proteins contain activation motifs that are crucial for antigen-stimulated signaling. Certain tyrosine residues within these motifs serve as targets for the catalytic activity of src family PTKs. Subsequently, these phosphorylated tyrosines serve as docking stations for the SH2 domains (recognition structures for select phosphotyrosine-containing motifs) of the ZAP-70 PTK. Following antigenic engagement of the TCR/CD3 complex, select serine residues of the TCR and CD3 chains are also phosphorylated (2,5).

The wave of tyrosine phosphorylation triggered by antigen recognition encompasses other intracellular proteins and is a cardinal event in initiating T cell activation. Tyrosine phosphorylation of the phospholipase $C\gamma_1$ activates this coenzyme and triggers a cascade of events that lead to full expression of T cell programs: hydrolysis of phosphatidylinositol 4,5-biphosphate (PIP_2) and generation of two intracellular messengers, inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (11) (Fig. 97-2). IP_3, in turn, mobilizes ionized calcium from intracellular stores, while diacylglycerol, in the presence of increased cytosolic free Ca^{2+}, binds to and translocates protein kinase C (PKC)—a phospholipid/Ca^{2+}-sensitive protein serine/threonine kinase—to the membrane in its enzymatically active form (5,11). Sustained activation of PKC is dependent on diacylglycerol generation from hydrolysis of additional lipids, such as phosphatidylcholine.

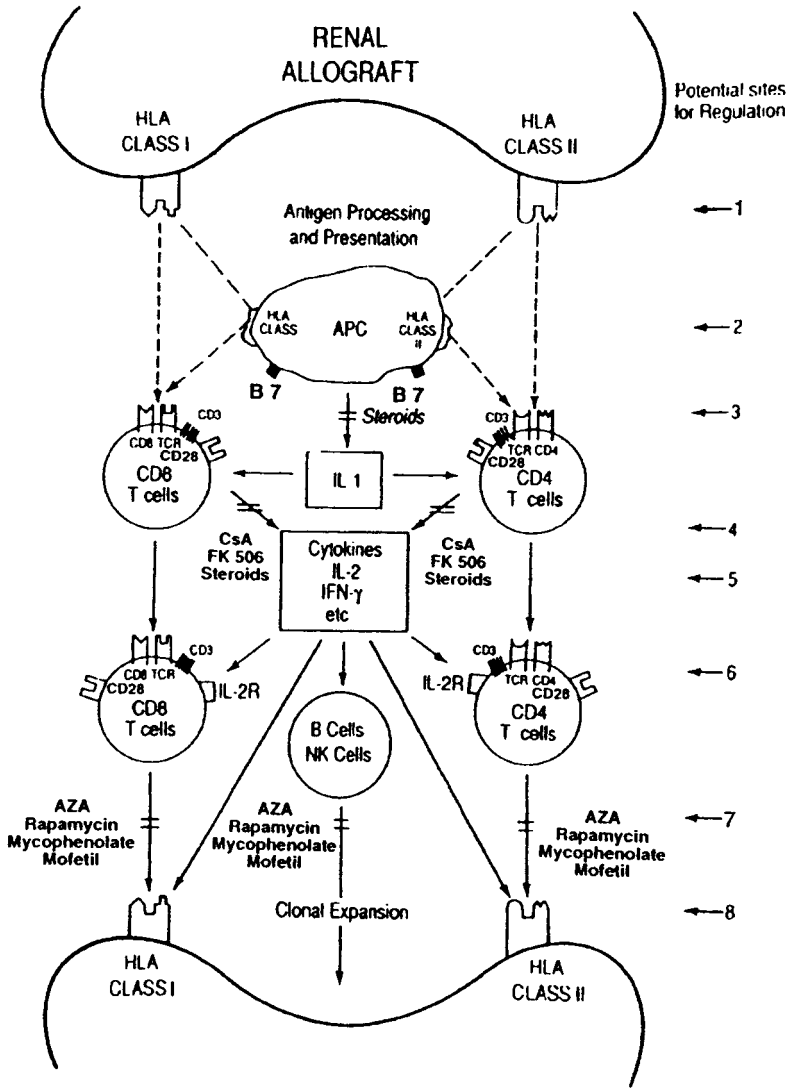

FIGURE 97-1. The antiallograft response. Schematic representation of human leukocyte antigens (HLA), the primary stimuli for the initiation of the antiallograft response, cell-surface proteins participating in antigenic recognition and signal transduction, contribution of the cytokines and multiple cell types to the immune response, and the potential sites for the regulation of the antiallograft response. *Site 1*: Minimizing histoincompatibility between the recipients and the donor (e.g., HLA matching). *Site 2*: Prevention of monokine production by antigen-presenting cells (e.g., corticosteroids). *Site 3*: Blockade of antigen recognition (e.g., OKT3 mAbs). *Site 4*: Inhibition of T cell cytokine production (e.g., cyclosporin A [CsA]). *Site 5*: Inhibition of cytokine activity (e.g., anti-interleukin-2 [IL-2] antibody). *Site 6*: Inhibition of cell cycle progression (e.g., anti-IL-2 receptor antibody). *Site 7*: Inhibition of clonal expansion (e.g., azathioprine [AZA]). *Site 8*: Prevention of allograft damage by masking target antigen molecules (e.g., antibodies directed at adhesion molecules). HLA class I: HLA-A, B, and C antigens; HLA class II: HLA-DR, DP, and DQ antigens. *IFN-γ*, interferon-γ; *NK cells*, natural killer cells.

The increase in intracellular free Ca^{2+} and sustained PKC activation promote the expression of several nuclear regulatory proteins (e.g., nuclear factor of activated T cells [NF-AT], nuclear factor kappa B [NF-κB], activator protein 1 [AP-1]) and the transcriptional activation and expression of genes central to T cell growth (e.g., interleukin-2 [IL-2] and receptors for IL-2 and IL-15) (2,5,12).

Calcineurin, a Ca^{2+}- and calmodulin-dependent serine/threonine phosphatase, is crucial to Ca^{2+}-dependent, TCR-initiated signal transduction (13,14). Inhibition by cyclosporine and tacrolimus (FK-506) of the phosphatase activity of calcineurin is considered central to their immunosuppressive activity (15).

Costimulatory Signals: Signal Two

Signaling of T cells via the TCR/CD3 complex (signal one) is necessary, albeit insufficient, to induce T cell proliferation; full activation is dependent on both the antigenic signals and the costimulatory signals (signal two) engendered by the contactual interactions between cell-surface proteins expressed on antigen-specific T cells and APCs (16,17) (Fig. 97-3; Table 97-2). The interaction of the CD2 protein on the T cell surface with the CD58 (leukocyte function-associated antigen 3 [LFA-3]) protein on the surface of APCs, and that of the CD11a/CD18 (LFA-1) proteins with the CD54 (intercellular adhesion molecule 1 [ICAM-1]) proteins (18), and/or the interaction of the CD5 with the CD72 proteins (10) aids in imparting such a costimulatory signal.

Recognition of the B7-1 (CD80) and B7-2 (CD86) proteins expressed upon CD4+ T cells generates a very powerful T cell costimulus (19). Monocytes and dendritic cells constitutively express CD86. Cytokines (e.g., granulocyte–macrophage colony-stimulating factor [GMCSF] or interferon-γ [IFN-γ]) stimulate expression of CD80 on monocytes, B cells, and dendritic cells (19). Many T cells express B7-binding proteins (i.e., CD28 proteins that are constitutively expressed on the surface of CD4+ T cells and CTLA-4 [CD152]), a protein whose ectodomain is closely related to that of CD28, and is expressed upon activated CD4+ and CD8+ T cells. CD28 binding of B7 molecules stimulates a Ca^{2+}-independent activation pathway that leads to stable transcription of the IL-2, IL-2 receptors, and other activation genes resulting in vigorous T cell proliferation (19). For some time, the terms CD28 and the costimulatory receptor were considered synonymous by some, but the demonstration that robust T cell activation occurs in CD28-deficient mice indicated that other receptor ligand systems contribute to signal two (20). In particular, the interaction between CD40 expressed upon APCs and CD40 ligand (CD154) expressed by

TABLE 97-1

CELLULAR ELEMENTS CONTRIBUTING TO THE ANTIALLOGRAFT RESPONSE[a]

Cell type	Functional attributes
T cells	The CD4+ T cells and the CD8+ T cells participate in the antiallograft response. CD4+ T cells recognize antigens presented by HLA class II proteins and CD8+ T cells recognize antigens presented by HLA class I proteins. The CD3/TCR complex is responsible for recognition of antigen and generates and transduces the antigenic signal.
CD4+ T cells	CD4+ T cells function mostly as helper T cells and secrete cytokines such as IL-2, a T cell growth/death factor, and IFN-γ, a proinflammatory polypeptide that can upregulate the expression of HLA proteins as well as augment cytotoxic activity of T cells and NK cells. Recently, two main types of CD4+ T cells have been recognized: CD4+ TH1, CD4+ TH2. IL-2 and IFN-γ are produced by CD4+ TH1 type cells and IL-4 and IL-5 are secreted by CD4+ TH2 type cells. Each cell type regulates the secretion of the other and the regulated secretion is important in the expression of host immunity.
CD8+ T cells	CD8+ T cells function mainly as cytotoxic T cells. A subset of CD8+ T cells expresses suppressor cell function. CD8+ T cells can secrete cytokines, such as IL-2 and IFN-γ and can express molecules such as perforin, granzymes that function as effectors of cytotoxicity.
APCs	Monocytes/macrophages and dendritic cells function as potent APCs. Donor's APCs can process and present donor antigens to recipient's T cells (direct recognition) or recipient's APCs can process and present donor antigens to recipient's T cells (indirect recognition). The relative contribution of direct recognition and indirect recognition to the antiallograft response has not been resolved. Direct recognition and indirect recognition might also have differential susceptibility to inhibition by immunosuppressive drugs.
B cells	B cells require T cell help for the differentiation and production of antibodies directed at donor antigens. The alloantibodies can damage the graft by binding and activating complement components (complement-dependent cytotoxicity) and/or binding the Fc receptor of cells capable of mediating cytotoxicity (antibody-dependent, cell-mediated cytotoxicity).
NK cells	The precise role of NK cells in the antiallograft response is not known. Increased NK cell activity has been correlated with rejection. NK cell function might also be important in immune surveillance mechanisms pertinent to the prevention of infection and malignancy.

[a] *APCs,* antigen presenting cells; *IFN,* interferon; *IL,* interleukin; *NK,* natural killer; *TCR,* T cell antigen receptor.
(Reproduced from Suthanthiran M, Morris RE, Strom TB. Transplantation immunobiology. In: Walsh PC, Retik AB, Vaughn ED Jr, et al., eds. *Campbell's Urology.* 7th ed. Philadelphia: Saunders; 1997:491, with permission.)

TABLE 97-2

CELL-SURFACE PROTEINS IMPORTANT FOR T CELL ACTIVATION[a,b]

T cell surface	APC surface	Functional response	Consequence of blockade
LFA-1 (CD11a, CD18)	ICAM (CD54)	Adhesion	Immunosuppression
ICAM1 (CD54)	LFA-1 (CD11a, CD18)		
CD8, TCR, CD3	MHCI	Antigen recognition	Immunosuppression
CD4, TCR, CD3	MHCII		
CD2	LFA3 (CD58)	Costimulation	Immunosuppression
CD40L (CD154)	CD40		
CD5	CD72		
CD28	B7-1 (CD80)	Costimulation	Anergy
CD28	B7-2 (CD86)		
CTLA4 (CD152)	B7-1 (CD80)	Inhibition	Immunostimulation
CTLA4 (CD152)	B7-2 (CD86)		

[a] Receptor/counterreceptor pairs that mediate interactions between T cells and APCs are shown in this table. Inhibition of each protein-to-protein interaction, except the CTLA4–B7.1/B7.2 interaction, results in an abortive *in vitro* immune response. Initial contact between T cells and APCs requires an antigen-independent adhesive interaction. Next, the T cell antigen-receptor complex engages processed antigen presented within the antigen-presenting groove of MHC molecules. Finally, costimulatory signals are required for full T cell activation. An especially important signal is generated by B7-mediated activation of CD28 on T cells. Activation of CD28 by B7.2 may provide a more potent signal than activation by B7.1. CTLA4, present on activated but not resting T cells, imparts a negative signal.
[b] *APC,* antigen-presenting cell; *ICAM,* intercellular adhesion molecule; *LFA,* leukocyte function-associated; *MHC,* major histocompatibility complex.
(Reproduced from Suthanthiran M, Morris RE, Strom TB. Transplantation immunobiology. In: Walsh PC, Retik AB, Vaughn ED Jr, et al., eds. *Campbell's Urology*. 7th ed. Philadelphia: Saunders; 1997:491, with permission.)

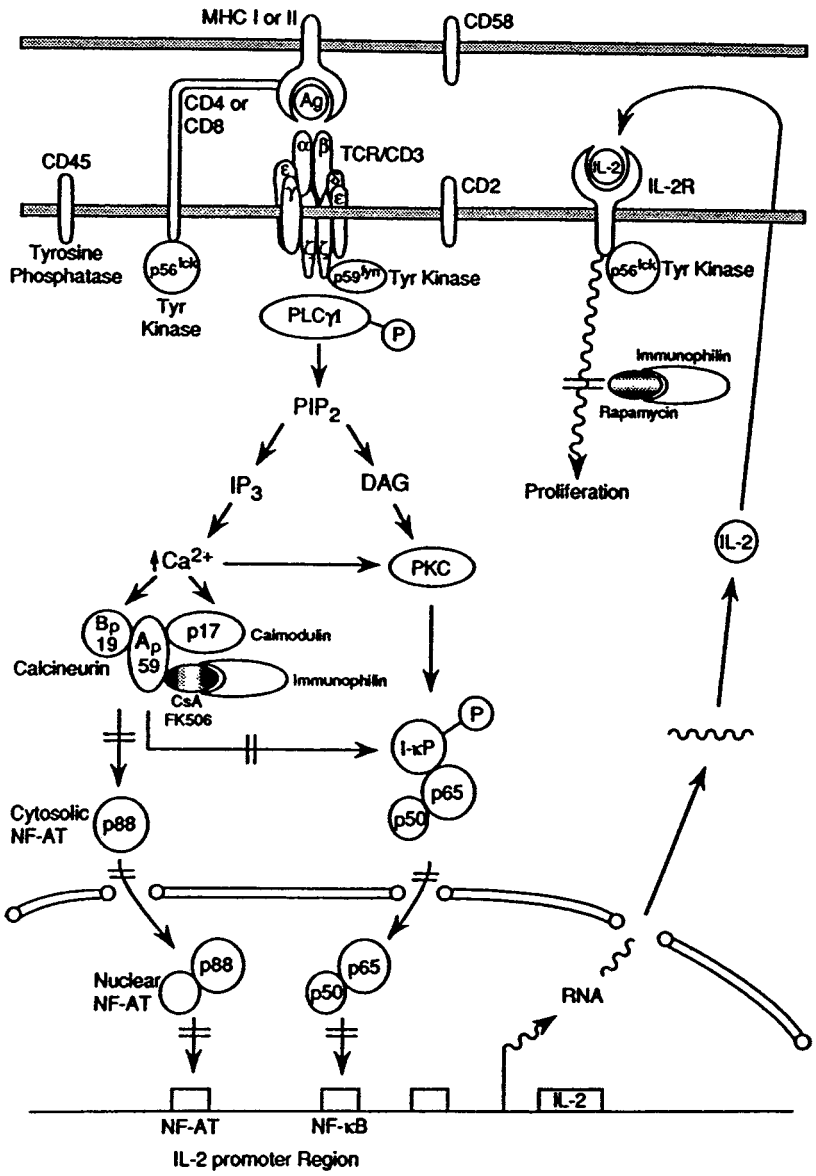

FIGURE 97-2. Signal transduction in T cells and mechanisms of action of cyclosporine A (CsA), FK-506, or rapamycin. Signaling molecules and transmembrane signaling events participating in the transduction of antigenic signals from the plasma membrane of the T cells to the nucleus are schematically shown. The sites of action of the drug (CsA/FK-506/rapamycin)–immunophilin complex are also shown. *Ag,* antigen; *Ap59* and *Bp19,* subunits of calcineurin; *DAG,* diacylglycerol; *I-κB,* inhibitory factor kappa B; *IL-2,* interleukin-2; *immunophilin,* cyclophilin or FK-binding protein; *IP_3,* inositol 1,4,5-triphosphate; *MHC,* major histocompatibility complex; *NF-AT,* nuclear factor of activated T cells; *NF-κB,* nuclear factor kappa B; *P,* phosphotyrosine; *PIP_2,* phosphatidylinositol 4,5-biphosphate; *PKC,* protein kinase C; *PLCγ1,* phospholipase C gamma-1; *Tyr kinase,* tyrosine kinase. (Adapted from: Schreier MH, Baumann G, Zenke G. Inhibition of T-cell signaling pathways by immunophilin drug complexes: are side effects inherent to immunosuppressive properties? *Transplant Proc* 1993;25:502.)

antigen-activated CD4+ T cells has received great attention as a potent second signal (21). The delivery of the antigenic first signal and the costimulatory second signal leads to stable transcription of the IL-2, several T cell growth-factor receptors, and other pivotal T cell activation genes (Table 97-2). The Ca^{2+}-independent costimulatory CD28 pathway is resistant to inhibition by cyclosporine or FK-506 as compared to the calcium-dependent pathway of T cell activation. In contrast, recognition of B7 proteins by CTLA-4, a protein primarily expressed on activated T cells, stimulates a negative signal to T cells. This signal is a prerequisite for peripheral T cell tolerance (22).

The formulation that full T cell activation is dependent on the costimulatory signal, as well as the antigenic signal is most significant, as T cell molecules responsible for costimulation and their cognate receptors on the surface of APCs then represent target molecules for the regulation of the antiallograft response. Indeed, transplantation tolerance has been induced in experimental models by targeting a variety of cell-surface molecules that contribute to the generation of costimulatory signals.

Interleukin-2/Interleukin-15 Stimulated T Cell Proliferation

Autocrine type of T cell proliferation occurs as a consequence of the T cell activation-dependent production of IL-2 and the expression of multimeric high affinity IL-2 receptors on T cells (Fig. 97-2) formed by the noncovalent association of three IL-2-binding peptides (a, β, γ) (12,23–25). IL-15 is a paracrine-type T cell-growth factor family member with very similar overall structural and identical T cell stimulatory qualities to IL-2 (12). The IL-2 and IL-15 receptor complexes share β and γ chains that are expressed in low abundance upon resting T cells; expression of these genes is amplified in activated T cells. The α-chain receptor components of the IL-2 and IL-15 receptor complexes are distinct and expressed upon activated, but not resting, T cells. The intracytoplasmic domains of the IL-2 receptor β and γ chains are required for intracellular signal transduction. The ligand-activated, but not resting, IL-2/IL-15 receptors are associated with intracellular PTKs (12,26–28). Raf-1, a protein serine/threonine kinase that

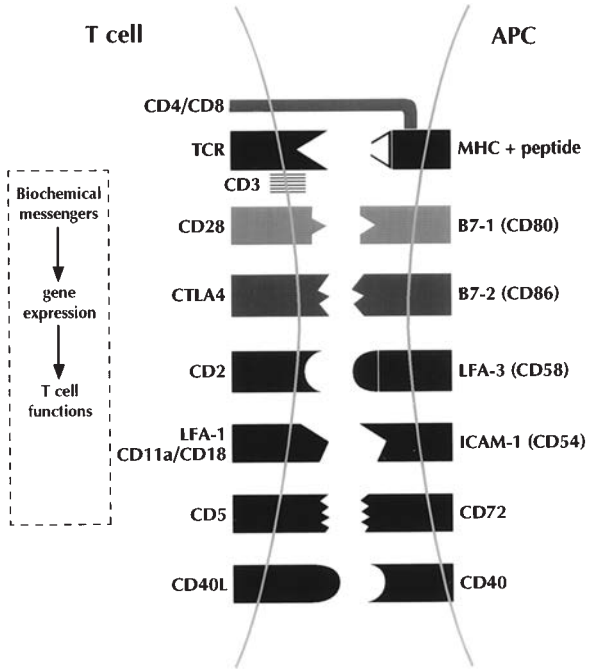

FIGURE 97-3. T cell/antigen-presenting cell contact sites. In this schema of T cell activation, the antigenic signal is initiated by the physical interaction between the clonally variant T cell antigen receptor (TCR) α-, β-heterodimer and the antigenic peptide displayed by MHC on antigen-presenting cells (APCs). The antigenic signal is transduced into the cell by the CD3 proteins. The CD4 and the CD8 antigens function as associative recognition structures, and restrict TCR recognition to class II and class I antigens of MHC, respectively. Additional T cell- surface receptors generate the obligatory costimulatory signals by interacting with their counterreceptors expressed on the surface of the APCs. The simultaneous delivery to the T cells of the antigenic signal and the costimulatory signal results in the optimum generation of second messengers (such as calcium), expression of transcription factors (such as nuclear factor of activated T cells), and T cell growth-promoting genes (such as IL-2). The CD28 antigen as well as the CTLA4 antigen can interact with both the B7-1 and B7-2 antigens. The CD28 antigen generates a stimulatory signal, and the recent studies of CTLA4-deficient mice suggest that CTLA4, unlike CD28, generates a negative signal. *CD,* cluster designation; *ICAM-1,* intercellular adhesion molecule-1; *LFA-1,* leukocyte function-associated antigen-1; *MHC,* major histocompatibility complex. (From: Suthanthiran M. Transplantation tolerance: fooling mother nature. *Proc Natl Acad Sci USA* 1996;93:12072.)

is prerequisite to IL-2/IL-15-triggered cell proliferation, associates with the intracellular domain of the shared β chain (29). Translocation of IL-2 receptor-bound Raf-1 serine/threonine kinase into the cytosol requires IL-2/IL-15-stimulated PTK activity. The ligand-activated common γ chain recruits a member of the Janus kinase family, Jak 3, to the receptor complex that leads to activation of a member of the STAT family. Activation of this particular Jak–STAT pathway is prerequisite for proliferation of antigen-activated T cells. The subsequent events leading to IL-2/IL-15-dependent proliferation are not fully resolved; however, IL-2/IL-15-stimulated expression of several DNA binding proteins including bcl-2, c-jun, c-fos, and c-myc contributes to cell cycle progression (30,31). It is interesting and probably significant that IL-2, but not IL-15, triggers apoptosis of many antigen-activation T cells. In this way, IL-15–triggered events are more detrimental to the allograft response than IL-2. As IL-15 is not produced by T cells, IL-15 expression is not regulated by cyclosporine or tacrolimus.

Immunobiology and Molecular Diagnosis of Rejection

The net consequence of cytokine production and acquisition of cell-surface receptors for these transcellular molecules is the emergence of antigen-specific and graft-destructive T cells (Fig. 97-1). Cytokines also facilitate the humoral arm of immunity by promoting the production of cytopathic antibodies. Moreover, IFN-γ and tumor necrosis factor-α (TNF-α) can amplify the ongoing immune response by upregulating the expression of HLA molecules as well as costimulatory molecules (e.g., B7) on graft parenchymal cells and APCs (Fig. 97-1). We and others have demonstrated the presence of antigen-specific cytotoxic T lymphocytes (CTL) and anti-HLA antibodies during or preceding a clinical rejection episode (32,33). We have detected messenger RNA (mRNA) encoding the CTL-selective serine protease (granzyme B), perforin, Fas-ligand attack molecules, and immunoregulatory cytokines, such as IL-10 and IL-15, in human renal allografts undergoing acute rejection (reviewed in reference 34). Indeed, these gene-expression events can anticipate clinically apparent rejection. More recent efforts to develop a noninvasive method for the molecular diagnosis of rejection have proved rewarding. Using either peripheral blood (35) or urinary leukocytes (36) rejection-related, gene-expression events evident in renal biopsy specimens are also detected in peripheral blood or urinary sediment specimens. We suspect that a noninvasive, molecular diagnostic-approach rejection may prove pivotal toward detection of insidious, clinically silent rejection episodes that, although rarely detected through standard measures, are steroid-sensitive but usually lead to chronic rejection (37).

Immunopharmacology of Allograft Rejection

Glucocorticosteroids

Glucocorticosteroids inhibit T cell proliferation, T cell-dependent immunity, and cytokine gene transcription (including IL-1, IL-2, IL-6, IFN-γ, and TNF-α gene) (38-40). While no single cytokine can reverse the inhibitory effects of corticosteroids on mitogen-stimulated T cell proliferation, a combination of cytokines is effective (41). The glucocorticoid and glucocorticoid–receptor bimolecular complex block IL-2 gene transcription via impairment of the cooperative effect of several DNA-binding proteins (42). Corticosteroids also inhibit formation of free NF-κB, a DNA-binding protein required for cytokine and other T cell- activation gene expression events (43) (Fig. 97-1 and Table 97-3).

Azathioprine

A thioguanine derivative of 6-mercaptopurine (44), azathioprine is a purine analog that acts as a purine antagonist and functions as an effective antiproliferative agent (Fig. 97-1 and Table 97-3) (44,45).

The Calcineurin Inhibitors: Cyclosporine and Tacrolimus (FK-506)

Cyclosporine, a small cyclic fungal peptide, and FK-506, a macrolide antibiotic, block the Ca^{2+}-dependent antigen triggered T cell activation (signal one) (46) (Fig. 97-2). The immunosuppressive effects of cyclosporine and FK-506 are dependent on the formation of a heterodimeric complex that consists of the native compound (cyclosporine or FK-506) and its respective cytoplasmic receptor "immunophilin" proteins,

TABLE 97-3

MECHANISMS OF ACTION OF SMALL MOLECULE IMMUNOSUPPRESSANTS[a]

Immunosuppressant	Subcellular site(s) of action
Azathioprine	Inhibits purine synthesis
Corticosteroids	Blocks cytokine gene expression
CsA/tacrolimus	Blocks Ca^{2+}-dependent T cell activation pathway via binding to calcineurin
Mycophenolate mofetil	Inhibits inosine monophosphate dehydrogenase and prevents *de novo* guanosine and deoxyguanosine synthesis in lymphocytes
Sirolimus/Everolimus	Blocks IL-2 and other growth-factor signal transduction; blocks CD28-mediated costimulatory signals
Leflunomide/FK778	Inhibits dihydroorotate dehydrogenase—a key enzyme for *de novo* pyramidine biosynthesis
FTY 720	Phosphorylated FTY720 binds sphingolipid 1-phosphate receptor and prevents S1P signaling of cells; sequestration of lympocytes within the lymph nodes and prevention cell egress into the peripheral circulation

[a] *CsA,* cyclosporine A; *IL,* interleukin.

cyclophilin and FK-binding protein (FKBP). Cyclosporine–cyclophilin and FK-506–FKBP complexes bind to calcineurin and inhibit its phosphatase activity (Table 97-3). The inhibition of the enzymatic activity of calcineurin is central to the immunosuppressive effects of cyclosporine and FK-506.

Since the phosphorylation status of transcription factors may alter their intracellular localization, it is interesting that cyclosporine–FK-506 inhibition of phosphatase activity of calcineurin apparently interferes with the dephosphorylation of cytoplasmic NF-AT and its subsequent import into the nucleus. The phosphorylation status of transcription factors can also affect their DNA binding ability and interaction with the rest of the transcriptional machinery. For example, the DNA binding activities of c-jun increase upon dephosphorylation. In addition to inhibiting the expression of NF-AT, cyclosporine also inhibits other DNA-binding proteins, such as NF-κB and AP-1 (47).

Blockade of cytokine gene activation does not totally account for the antiproliferative effect of cyclosporine and FK-506. It is significant that cyclosporine, in striking contrast to its inhibitory activity on the induced expression of IL-2, enhances the expression of transforming growth factor-β (TGF-β) (48). Because TGF-β is a potent inhibitor of T cell proliferation and generation of antigen-specific CTL (49), heightened expression of TGF-β must contribute to the antiproliferative/immunosuppressive activity of cyclosporine. This novel effect of cyclosporinee also suggests a mechanism for some of the complications (e.g., renal fibrosis and tumor metastasis) of therapy with cyclosporine, because TGF-β is a fibrogenic and proangiogenic cytokine.

Mycophenolate Mofetil

Mycophenolate mofetil (MMF) is a semisynthetic derivative of mycophenolic acid. It inhibits allograft rejection in rodents, diminishes proliferation of T and B lymphocytes, decreases generation of cytotoxic T cells, and suppresses antibody formation (50–52). MMF inhibits inosine monophosphate dehydrogenase (IMPDH), an enzyme in the *de novo* pathway of purine synthesis. Lymphocytes are dependent upon this biosynthetic pathway to satisfy their guanosine requirements (Table 97-3) (reviewed in reference 52). Early clinical trials have utilized MMF to replace azathioprine in the cyclosporine- and steroid-based immunosuppressive regimen. These controlled, prospective trials have shown a diminished incidence of early acute rejection episodes (52–54). Although follow-up studies over a 3-year period have indicated an advantage for MMF over azathioprine (54), a recent randomized trial comparing MMF with azathioprine in recipients of a first kidney transplant from a diseased donor found similar levels of acute rejection in the first 6-months of transplantation (55).

Sirolimus (Rapamycin)

Rapamycin (56–58) is a macrocyclic lactone isolated from *Streptomyces hygroscopicus* that, like FK-506, binds to FKBP. However, rapamycin and FK-506 affect different and distinctive sites in the signal transduction pathway (Fig. 97-2 and Table 97-3). Whereas rapamycin blocks IL-2 and other growth factor-mediated signal transduction, FK-506 (or cyclosporine) has no such capacity. Also, the rapamycin–FKBP complex, unlike the FK-506–FKBP complex, does not bind calcineurin. The antiproliferative activity of the rapamycin–FKBP complex is linked to blockade of the activation of the 70-kDa S6 protein kinases and blockade of expression of the bcl-2 proto-oncogene. Rapamycin also blocks the Ca^{2+}-independent CD28-induced costimulatory pathway. Substitution of rapamycin for azathioprine in a triple-therapy regimen reduced the frequency and severity of acute rejection (59).

Everolimus (RAD)

Everolimus is a derivative of rapamycin. The use of everolimus in Phase II clinical trials involving cyclosporine, steroids, and basiliximab induction resulted in excellent graft survival in 36 months (60). In a short-term Phase III trial, everolimus was comparable to MMF with cyclosporine and steroids in preventing acute rejection (61).

Leflunomide

Leflunomide is a synthetic isoxazole derivative that inhibits dihydroorotate dehydrogenase—a key enzyme for *de novo* pyrimidine synthesis. It belongs to the family of drugs known as malonitrilamides and is currently approved for the treatment of rheumatoid arthritis. Leflunomide has antiviral effects against cytomegalovirus, herpes simplex virus type 1, and polyomavirus (BK virus) (62–64). A short-term, open-label, prospective crossover trial of leflunomide comprised of 22 patients with chronic renal allograft dysfunction found 100% patient survival and 91% graft survival at 6-months

posttransplantation, and was well tolerated, with anemia being the most common adverse effect (65).

FK778

FK778 is an analog of the active metabolite (A771726) of leflunomide. A Phase II European multicenter randomized, double-blind, and FK778 dose-controlled trial was performed in 149 renal transplant recipients divided into three groups: Group 1: high-level FK778/tacrolimus/steroids, Group 2: low- level FK778/tacrolimus/steroids, and Group 3: placebo/tacrolimus/steroids (66). The incidence of acute rejection in groups 1, 2, and 3 were 28.6%, 25.9%, and 34.8%, respectively, and patients who reached target levels had lower incidence of acute rejection. Anemia was a commonly reported complication and was observed 43% in group 1, 31% in group 2, and 20% in group 3.

Muromonab–CD3 (OKT3)

OKT3 is a murine monoclonal antibody directed against the CD3 component of the T cell receptor complex. It is utilized in renal transplantation as an induction agent and for treatment of severe steroid-resistant acute rejection. The first dose reaction as a result of cytokine release may be severe and include fever, chills, respiratory symptoms, and headaches. A humanized preparation (HuM291) that potentially reduces cytokine release reaction is being investigated (67).

Antithymocyte Globulin (ATG)

Immunizing either rabbits or equines with human thymocytes produces antithymocyte globulin preparations. The antibodies generated are polyclonal in nature and are directed against several cell-surface antigens including: CD2, CD3, CD4, CD8, CD11a, CD18, CD25, CD44, CD45, HLA-DR, and HLA class I heavy chain (68). ATG preparations are used both as an induction agent, especially in high-risk renal transplant recipients, and for the treatment of acute rejection. In a steroid rapid-withdrawal protocol using calcineurin inhibitor and MMF or sirolimus, rabbit ATG was selected as the induction agent for low-risk, mostly Caucasian, renal transplant recipients and the actuarial acute rejection-free graft survival was 92% at 3 years (69).

Interleukin (IL)-2 Receptor Antagonists: Basiliximab and Daclizumab

IL-2 receptor antagonists (IL-2Ra) inhibit allograft rejection by competitively binding CD25 antigen (IL-2 receptor α chain or Tac subunit) on activated T lymphocytes. Both basiliximab (chimeric human/murine monoclonal $IgG_{1\kappa}$) and daclizumab (humanized monoclonal IgG1) are commonly utilized as induction agents for renal transplantation. A meta-analysis of clinical trials involving monoclonal antibodies directed at CD25 showed that when combined with standard double or triple immunosuppressive regimens, the use of these antibodies reduced the incident of acute rejection by 34% and by 49% the incidence of steroid-resistant rejection (70). The meta-analysis also showed that the efficacy of anti-CD25 monoclonal antibodies in preventing acute rejection was similar to that of OKT3 and that of polyclonal antibody preparations and, importantly, with fewer side effects.

Alemtuzumab

Alemtuzumab is a humanized monoclonal $IgG_{1\kappa}$ directed against CD52, which is a glycoprotein expressed on B and T lymphocytes, natural killer (NK) cells, monocytes, and macrophages. It is approved for the treatment of B cell chronic lymphocytic leukemia. In a pilot study of 29 primary renal transplant recipients treated with alemtuzumab and sirolimus monotherapy, profound and sustained depletion of lymphocytes was observed and eight of the patients developed acute rejection requiring conversion to standard triple therapy (71). In an investigation of 44 renal allograft recipients treated with alemtuzumab, tacrolimus (trough levels of 5 to 7 ng/mL), and MMF (500 mg twice a day), and with a median follow up of 9-months, four patients developed acute rejection and four developed infection and the patient and graft survival rates were 100% (72).

Rituximab

Rituximab is a chimeric murine/human monoclonal $IgG_{1\kappa}$ directed against the CD20 antigen expressed on the surface of B lymphocytes. It is approved for the treatment of CD20-positive, B cell non-Hodgkin's lymphoma. Initial experience with rituximab has shown promising results in the treatment of steroid-resistant acute renal allograft rejection (73). Rituximab has also been used as a component of a preconditioning regimen to prepare patients for renal transplantation from ABO incompatible donors (74).

Intravenous Immune Globulins (IVIG)

Due to its immunomodulatory effects, IVIG is used to treat a variety of autoimmune diseases (75). In the renal transplantation arena, IVIG is being utilized to reduce humoral immunity in two distinct settings: (a) to reduce the level of preexisting anti-HLA antibodies and convert a positive crossmatch to a negative crossmatch (76) and (b) to treat humoral rejection (77).

FTY720

FTY720 is a synthetic analog derived from the ascomycete *Isaria sinclairii*. The phosphorylated metabolite, FTY720-phosphate, is the biologically active compound. FTY720 affects the normal trafficking of lymphocytes and prevents their transmigration from lymph nodes to the allograft by binding lymphocytic sphingolipid 1-phosphate $(S1P)_1$ receptors. This process prevents the signaling of lymphocytes by serum S1P and the egress into the periphery in response to systemic inflammation (78). In the first human trial of FTY720 in stable renal allograft recipients, transient but asymptomatic bradycardia was noted following 10 of 24 doses examined (79). In a randomized, multicenter, double-blind, and placebo-controlled, Phase I study of stable renal allograft recipients, a dose-dependent decrease in peripheral blood lymphocytes was observed (80). A Phase IIA trial comparing FTY720 to MMF in combination with cyclosporine and steroids in *de novo* renal transplant recipients showed equivalent efficacy and safety with regard to prevention of acute rejection (81).

LEA29Y

LEA29Y (BMS-224818) is a fusion protein related to cytotoxic T lymphocyte-associated antigen 4 immune globulin (CTLA4Ig) designed to block the B7/CD28 costimulatory pathway. A Phase II trial comparing LEA29Y against cyclosporine (in a regimen consisting of basiliximab, MMF, and corticosteroids) has yielded promising results in preventing acute rejection of renal allografts (82).

Immunosuppressive Regimens

Immunologic considerations, including antirejection therapy, are organized around a few general principles. The first consideration is careful patient preparation and, in the circumstance of living donor renal transplantation, selection of the best available ABO-compatible HLA match in the event that several

potential living related donors are available for organ donation. Second is a multitiered approach to immunosuppressive therapy similar, in principle, to that used in chemotherapy; several agents are used simultaneously, each of which is directed at a different molecular target within the allograft response (Fig. 97-1 and Table 97-3). Additive/synergistic effects are achieved through application of each agent at a relatively low dose, thereby limiting the toxicity of each individual agent while increasing the total immunosuppressive effect. Third is the principle that higher immunosuppressive drug doses and/or more individual immunosuppressive drugs are required to gain early engraftment and to treat established rejection than are needed to maintain immunosuppression in the long term. Hence, intensive induction and lower-dose maintenance drug protocols are used. Fourth is careful investigation of each episode of posttransplant graft dysfunction, with the realization that most of the common causes of graft dysfunction, including rejection, can (and often do) coexist. Successful therapy, therefore, often involves several simultaneous therapeutic maneuvers. Fifth is the appropriate reduction or withdrawal of an immunosuppressive drug when that drug's toxicity exceeds its therapeutic benefit.

The basic immunosuppressive protocol used in most transplant centers involves the use of at least two and often three drugs, each directed at a discrete site in the T cell activation cascade (Fig. 97-1) and each with distinct side effects. While a calcineurin inhibitor plus MMF plus glucocorticoids is the most widely used regimen, there are concerns regarding nephrotoxicity associated with long-term use of calcineurin inhibitors (83) and several popular variations of the "triple" drug protocol are being explored in the clinic. A calcineurin-free regimen consisting of sirolimus, MMF, and glucocorticosteroids has been reported to result in better renal function (84). In a randomized controlled trial of cyclosporine withdrawal in recipients of first cadaver renal transplants, a 3-month course of cyclosporine followed by azathioprine and steroid maintenance therapy was superior to continuous cyclosporine-alone protocol (85). Early cyclosporine withdrawal from a regimen of cyclosporine, sirolimus, and steroids has been associated with a better renal function and renal allograft histology compared to patients maintained on the three-drug regimen (86).

Many centers employ "induction therapy" with antilymphocyte preparations. Monoclonal anti–T cell (OKT3) or polyclonal antilymphocyte antibodies are used as induction therapy for 5 to 14 days in the immediate posttransplant period, thereby establishing an immunosuppressive umbrella that enables early engraftment without immediate use of calcineurin inhibitors during the early posttransplant period. During this critical period, the graft is particularly vulnerable to CsA–FK-506-induced nephrotoxic effects. The incidence of early rejection episodes is reduced by the prophylactic use of antilymphocyte antibodies. This protocol is particularly beneficial for patients at high risk for immunologic graft failure (e.g., broadly presensitized or retransplant patients). The efficacy of the polyclonal antilymphocyte antibody preparation (e.g., thymoglobulin) or mAbs (e.g., OKT3) in preventing rejection is impressive, but profound lymphopenia and an increase in the incidence of opportunistic infections and lymphoma results. Because of selective targeting of IL-2R+ T cells, anti-CD25 mAb treatment appears to be safer than treatment with thymoglobulin or anti-T cell mAbs (70). Insofar as activated but not resting T cells express the IL-2 receptor α chain, anti-CD25 mAbs are employed as humanized (87,88) or chimeric (89,90) mAbs to selectively target and destroy alloreactive T cells. These efforts are based on successful exploration and application of IL-2–receptor targeted therapy in preclinical models (91). Low-dose tacrolimus and sirolimus protocol (92) may prove to rival the current sequential immune therapy regimens for use in patients at high risk to reject an allograft. Induction therapy protocols using either basiliximab or daclizumab have also been utilized to enable successful early steroid withdrawal in the first week of renal transplantation (93,94).

HLA and Renal Transplantation

The genes that code for the HLA antigens are lodged within the short arm of chromosome 6 (95,96). The class I proteins, HLA-A, B, and C antigens, are composed of a 41-kDa polymorphic chain linked noncovalently to a 12-kDa β_2-microglobulin chain that is encoded in chromosome 15. The class I molecules are expressed by all nucleated cells and platelets. The class II molecules, HLA-DR, DP, and DQ, are composed of a chain of 34 kDa and a β chain of 29 kDa. MHC class II molecules are constitutively expressed on the surface of B cells, monocytes/macrophages, and dendritic cells. Additional lymphoid cells, such as T cells and many nonlymphoid cells, such as renal tubular epithelial cells express class II proteins only on stimulation with cytokines.

The clinical benefits of HLA matching are readily appreciable in the recipients of renal grafts from living related donors. An analysis of the United Network for Organ Sharing (UNOS) scientific renal transplant registry data has revealed that the 1-year graft survival rate is 94% in recipients of two haplotype-matched, HLA-identical kidneys. It is 89% and 90%, respectively, when a one haplotype-matched parent or sibling is the donor (97) (Fig. (97-4A). The Collaborative Transplant Study, an international study that draws on 305 transplant centers located in 47 countries for data, has also demonstrated that the survival rate of HLA-identical transplants is superior to that of one-haplotype-matched grafts, even in the cyclosporine era (98).

The advantage of HLA-matching is maintained beyond the first year of transplantation. UNOS registry data (97) show estimated half-lives (the time needed for 50% of the grafts functioning at 1 year posttransplantation to fail) of 26.9 years for HLA-identical grafts and 12.2 years and 10.8 years for one-haplotype-matched sibling grafts and parental grafts, respectively. Data from the Collaborative Transplant Study, comprising 22,414 living related grafts, have also revealed a substantial long-term benefit (98) of HLA matching in recipients of living related grafts.

The effect of matching for HLA in cadaveric graft recipients has been examined in a prospective U.S. study (99) in which kidneys were shared nationally on the basis of matching for HLA-A, B, and DR antigens. All transplantation centers in the United States participated in this study. The 1-year graft survival rate was 88% for HLA-matched kidneys and 79% for HLA-mismatched kidneys (Fig. 97-4B). Moreover, the benefit of HLA matching persisted beyond the first year posttransplantation; the estimated half-life of the HLA-matched renal graft was 17.3 years and that of HLA-mismatched renal allografts, 7.8 years.

Since the inception of the U.S. national kidney-sharing program in 1987, more than 7,500 cadaveric kidneys have been distributed to transplantation centers located in 48 states, and a recent analysis confirmed and extended the observation that HLA-matched transplants have a superior outcome compared to HLA-mismatched transplants (100). The estimated 10-year rate of cadaveric graft survival was 52% for HLA-matched transplants and was 37% for HLA-mismatched transplants. Furthermore, the incidence of rejection was lower in HLA-matched transplants compared to mismatched ones. Interestingly, the mean duration of cold-ischemia time of nationally shared kidneys was not that different from locally transplanted kidneys; it was 23 hours compared to 22 hours for nonshared kidneys (100).

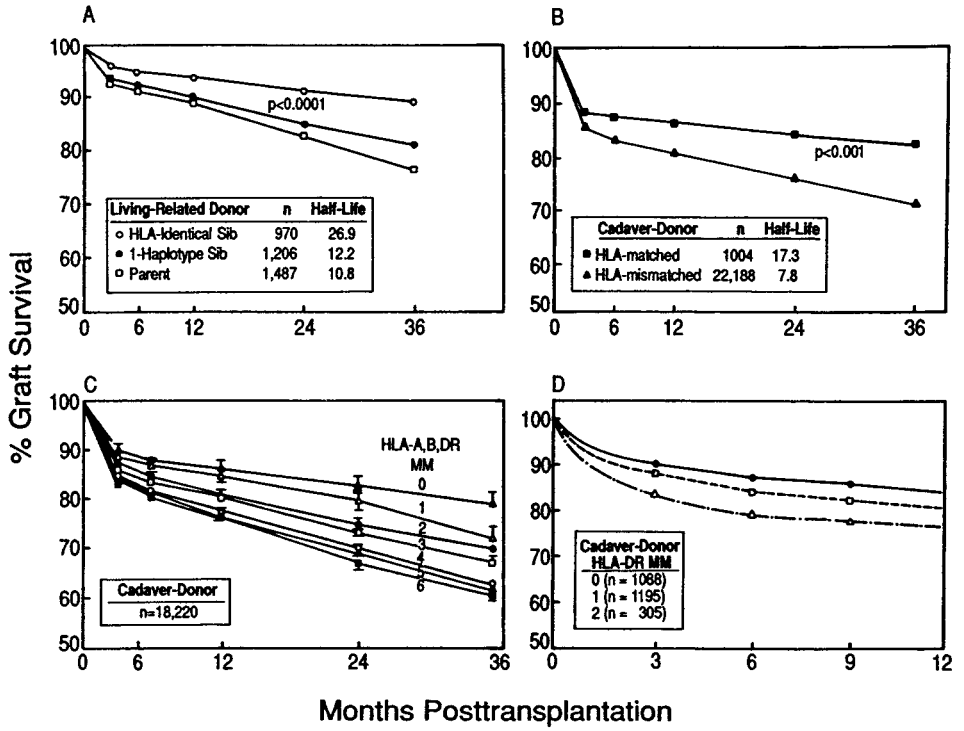

FIGURE 97-4. Impact of human leukocyte antigen (HLA) matching on renal allograft survival rates. **A:** The effect of haplotype matching in living related renal transplantation (68). **B:** The superior results found with HLA-matched (A, B, and DR antigens) cadaveric renal grafts compared with HLA-mismatched cadaveric renal grafts (70). **C:** The impact of different levels of HLA-A, B, and DR mismatching on the survival of cadaveric grafts (72). **D:** The stepwise improvement in the survival of cadaveric grafts following matching for the HLA-DR antigens identified by DNA typing (number of DR mismatches: •—•, 0;—, 1; •—Δ—•,2) (75).

A stepwise increase in the survival rate of cadaveric renal allografts has also been documented with increasing levels of HLA-A, B, and DR antigen matching (Fig. 97-4C). The improvement in the graft survival rate following HLA matching is more apparent when matching is based on better resolved HLA antigens (HLA split antigens) than when based on broad HLA antigens; the improvement in the graft survival rate between the best-matched and the worst-matched grafts increases with time (101). In the UNOS registry data, the difference in the graft survival rate between the best-matched and worst-matched recipient was 10% at 1-year posttransplantation and this difference increased to 18% by 3 years posttransplantation. The Collaborative Transplant Study of more than 67,000 primary cadaver grafts has also demonstrated a significant correlation between the number of HLA mismatches and graft loss (98).

A threshold level of HLA matching might exist: Allografts that are matched for four or more HLA antigens (or two or less HLA mismatches) have a superior short- as well as long-term outcome compared to less than four HLA antigen matches (or greater than two antigen mismatches) (101). It is noteworthy that the beneficial effect of different degrees of matching/mismatching for the HLA-A, B, and DR antigens (102), with the exception of phenotypically identical HLA transplants, is more evident in white recipients as compared to black recipients of cadaveric renal allografts (97,101).

The impact of each of the HLA loci, HLA-A locus, HLA-B locus, and HAL-DR locus, on renal allograft outcome has been investigated. Each locus impacts graft outcome. In the Collaborative Transplant Study, the influence of HLA-DR mismatches was greater than that of HLA-A or HLA-B mismatches in the first year following transplantation; with increased posttransplantation time, mismatches at any of the three loci impacted adversely on graft survival rates (98).

Molecular techniques are currently in place for the finer resolution of the HLA system (103). The clinical advantage of molecular matching was suggested originally by the observation that the 1-year cadaveric renal graft survival rate is 87% in patients who receive kidneys that are HLA-DR identical, not only by the serologic methods but also by molecular methods (DNA-RFLP method). This figure drops to 69% for patients who receive kidneys that are not HLA-DR identical by the molecular methodology (104). Application of molecular techniques for the identification of HLA-DR antigens has also resulted in the appreciation of a stepwise increase in the survival of cadaveric renal allografts matched for zero, one, or two HLA-DR antigens (Fig. 97-4D) (98,103). Molecular typing has also been used to detect mismatches at the HLA-A or HLA-B locus. Mismatches that were missed by conventional serologic techniques, but identified by molecular techniques, were found to adversely impact graft survival (98).

Current data suggest minimal impact of matching for HLA-C locus antigens. Matching for the HLA-DP antigen, on the other hand, appears to be important in repeat but not primary grafts (98).

Crossmatch

Crossmatches, testing of recipient's serum for antibodies reacting with the donor's HLA antigens, must be performed prior to renal transplantation. The standard crossmatch test consists of incubating the serum from the recipient with the donor's lymphocytes in the presence of rabbit serum as a source of complement.

The presence in the recipient's serum of cytotoxic antibodies directed at the donor's class I antigen (positive T cell crossmatch) is an absolute contraindication to transplantation because 80% to 90% of transplants performed in the presence of a positive crossmatch are subject to hyperacute rejection (105). The sensitivity of the standard crossmatch test has been increased by the addition of sublytic concentrations of antihuman globulin (AHG) to the test system. The graft survival rate is about 5% lower in recipients with a positive AHG test compared to recipients with a negative AHG test (106).

The significance of antibodies reacting with the donor's class II antigens (positive B cell crossmatch) is not fully resolved. A survival disadvantage, 7% in primary transplants and 15% in

TABLE 97-4

CLASSIFICATION OF TOLERANCE

A. Based on the major mechanism involved
  1. Clonal deletion
  2. Clonal energy
  3. Suppression
B. Based on the period of induction
  1. Fetal
  2. Neonatal
  3. Adult
C. Based on the cell tolerized
  1. T cell
  2. B cell
D. Based on the extent of tolerance
  1. Complete
  2. Partial, including split
E. Based on the main site of induction
  1. Central
  2. Peripheral

repeat transplants, however, has been noted in recipients with a positive B cell crossmatch (106).

A number of centers are currently using flow cytometry-based methodology to detect donor-specific antibodies. Flow cytometry crossmatches permit detection of low, sublytic concentrations of complement fixing as well as noncomplement-fixing antibodies. In the UNOS kidney transplant registry data (107), a positive flow cytometry crossmatch was associated with an increased incidence of early graft dysfunction requiring dialytic support, primary nonfunction of the allograft, prolonged hospitalization, and a greater incidence of allograft rejection. The negative impact of a positive flow cytometry crossmatch was greater in repeat transplants compared to primary transplants. Whereas a positive flow crossmatch was associated with a 5% decrease in the 3-year survival rate of primary grafts, a 19% decrease was observed in the 3-year survival of repeat grafts. In primary transplants, a T+B+ flow cytometry crossmatch and a T–B+ crossmatch had a similar outcome (76% versus 74% at 3-years posttransplantation), and in repeat transplants a T+B+ flow cytometry crossmatch has a much inferior outcome compared to a T–B+ crossmatch (60% versus 73% at 3-years posttransplantation).

TRANSPLANTATION TOLERANCE

Transplantation tolerance can be defined as an inability of the organ graft recipient to express a graft destructive immune response. While this statement does not restrict either the mechanistic basis or the quantitative aspects of immune unresponsiveness of the host, true tolerance is antigen-specific, induced as a consequence of prior exposure to the specific antigen, and is not dependent on the continuous administration of exogenous nonspecific immunosuppressants.

A classification of tolerance on the basis of the mechanisms involved, site of induction, extent of tolerance, and the cell primarily tolerized is provided in Table 97-4. Induction strategies for the creation of peripheral tolerance are listed in Table 97-5.

Several hypotheses, not necessarily mutually exclusive and, at times, even complementary, have been proposed for the cellular basis of tolerance. Data from several laboratories support the following mechanistic possibilities for the creation of a tolerant state: clonal deletion, clonal anergy, and immunoregulation.

TABLE 97-5

POTENTIAL APPROACHES FOR THE CREATION OF TOLERANCE

A. Cell depletion protocols
  1. Whole body irradiation
  2. Total lymphoid irradiation
  3. Panel of monoclonal antibodies
B. Reconstitution protocols
  1. Allogeneic bone marrow cells with or without T cell depletion
  2. Syngeneic bone marrow cells
C. Combination of strategies A and B
D. Cell-surface molecule targeted therapy
  1. Anti-CD4 mAbs
  2. Anti-ICAM-1 + anti-LFA-1 mAbs
  3. Anti-CD3 mAbs
  4. Anti-CD2 mAbs
  5. Anti-IL-2 receptor α (CD25) mAbs
  6. CTLA4Ig fusion protein
  7. Anti-CD40L mAbs
E. Drugs
  1. Azathioprine
  2. Cyclosporine
  3. Rapamycin
F. Additional approaches
  1. Donor-specific blood transfusions with concomitant mAb or drug therapy
  2. Intrathymic inoculation of cells/antigens
  3. Oral administration of cells/antigens

Clonal Deletion

Clonal deletion is a process by which self–antigen-reactive cells (especially those with high affinity for the self-antigens), are eliminated from the organism's immune repertoire. This process is called central tolerance. In the case of T cells, this process takes place in the thymus, and the death of immature T cells is considered to be the ultimate result of high-affinity interactions between a T cell with productively rearranged TCR and the thymic nonlymphoid cells, including dendritic cells that express the self-MHC antigen. This purging of the immune repertoire of self-reactive T cells is termed negative selection and is distinguished from the positive selection process responsible for the generation of the T cell repertoire involved in the recognition of foreign antigens in the context of self-MHC molecules. Clonal deletion or at least marked depletion of mature T cells as a consequence of apoptosis can also occur in the periphery (reviewed in reference 108). The form of graft tolerance, occurring as a consequence of mixed hematopoietic chimerism, entails massive deletion of alloreactive clones (109). Tolerance to renal allografts has been achieved in patients that have accepted a bone marrow graft from the same donor (110,111). It is interesting that IL-2, the only T cell-growth factor that triggers T cell proliferation as well as apoptosis, is an absolute prerequisite for the acquisition of organ graft tolerance through use of nonlymphoablative treatment regimens (112,113). Tolerance achieved under these circumstances also involves additional mechanisms, including clonal anergy and suppressor mechanisms (114–116).

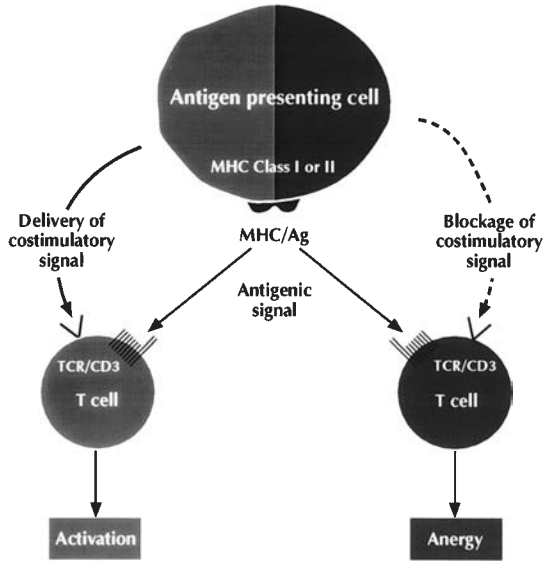

FIGURE 97-5. T cell activation/anergy decision points. Several potential sites for the regulation of T cell signaling are shown. The antigenic peptide displayed by major histocompatibility complex (MHC) (*site 1*), costimulatory signals (*site 2*), T cell antigen receptor (TCR) (*site 3*), and cytokine signaling (*site 4*) can influence the eventual outcome. Altered peptide ligands, blockade of costimulatory signals, downregulation of TCR, and interleukin (IL)-10 favor anergy induction, whereas fully immunogenic peptides, delivery of costimulatory signals, appropriate number of TCRs, and IL-12 prevent anergy induction and facilitate full activation of T cells. (From: Suthanthiran M. Transplantation tolerance: fooling mother nature. *Proc Natl Acad Sci USA* 1996;93:12072.)

Clonal Anergy

Clonal anergy refers to a process in which the antigen-reactive cells are functionally silenced. The cellular basis for the hyporesponsiveness resides in the anergic cell itself and the current data suggest that the anergic T cells fail to express the T cell-growth factor, IL-2, and other crucial T cell-activation genes because of defects in the antigen-stimulated signaling pathway.

T cell clonal anergy can result from suboptimal antigen-driven signaling of T cells, as mentioned earlier. The full activation of T cells requires at least two signals, one signal generated via the TCR–CD3 complex, and the second (costimulatory) signal initiated/delivered by the APCs. Stimulation of T cells via the TCR–CD3 complex alone—provision of signal 1 without signal 2—can result in T cell anergy/paralysis (Fig. 97-5 and Table 97-2).

B cell activation, in a fashion analogous to T cell activation, requires at least two signals. The first signal is initiated via the B cell antigen receptor immunoglobulin and a second costimulatory signal is provided by cytokines or cell-surface proteins of T cell origin. Thus, delivery of the antigenic signal alone to the B cells without the instructive cytokines or T cell help can lead to B cell anergy and tolerance.

Immunoregulatory (Suppressor) Mechanisms

Antigen-specific T or B cells are physically present and are functionally competent in tolerant states resulting from suppressor mechanisms. The cytopathic and antigen-specific cells are restrained by the suppressor cells or factors or express noncytopathic cellular programs. Each of the major subsets of T cells, the CD4 T cells and the CD8 T cells, has been implicated in mediating suppression. Indeed, a cascade involving MHC antigen-restricted T cells, MHC antigen-unrestricted T cells, and their secretory products have been reported to collaborate to mediate suppression. Recently, a subset of CD4+ T cells, the CD4+ CD25+ cells that express FOXP3, has been identified to mediate potent suppressive activity (117,118).

At least four distinct mechanisms have been advanced to explain the cellular basis for suppression:

1. An antiidiotypic regulatory mechanism in which the idiotype of the TCR of the original antigen-responsive T cells functions as an immunogen and elicits an antiidiotypic response. The elicited antiidiotypic regulatory cells, in turn, prevent the further responses of the idiotype-bearing cells to the original sensitizing stimulus.
2. The veto process by which recognition by alloreactive T cells of alloantigen-expressing veto cells results in the targeted killing (veto process) of the original alloreactive T cells by the veto cells.
3. Immune deviation, a shift in CD4+ T cell programs away from Th1-type (IL-2, IFN-γ expressing) toward the Th2-type (IL-4, IL-10 expressing) program.
4. The production of suppressor factors or cytokines. (e.g., the production of TGF-β by myelin basic protein-specific CD8 T cells or other cytokines with antiproliferative properties.) The process leading to full tolerance is infectious. Tolerant T cells recruit nontolerant T cells into the tolerant state (115). The tolerant state also establishes a condition in which foreign tissues housed in the same microenvironment as the specific antigen to which the host has been tolerized are protected from rejection (115). Tolerance is a multistep process (114–116).

Clearly more than one mechanism is operative in the induction of tolerance (Fig. 97-5). The tolerant state is not an all-or-nothing phenomenon, but is one that has several gradations. Of the mechanisms proposed for tolerance, clonal deletion might be of greater importance in the creation of self-tolerance, and clonal anergy and immunoregulatory mechanisms might be more applicable to transplantation tolerance. More recent data suggest both clonal depletion and immunoregulatory mechanisms are needed to create and sustain central or peripheral tolerance. From a practical viewpoint, a nonimmunogenic allograft (e.g., located in an immunologically privileged site or physically isolated from the immune system) might also be "tolerated" by an immunocompetent organ-graft recipient.

Authentic tolerance has been difficult to identify in human renal allograft recipients. Nevertheless, the clinical examples, albeit infrequent, of grafts functioning without any exogenous immunosuppressive drugs (either due to noncompliance of the patient or due to discontinuation of drugs for other medical reasons) does suggest that some long-term recipients of allografts develop tolerance to the transplanted organ and accept the allografts. The recent progress in our understanding of the immunobiology of graft rejection and tolerance and the potential to apply molecular approaches to the bedside hold significant promise for the creation of a clinically relevant tolerant state and transplantation without exogenous immunosuppressants—the ultimate goal of the transplant physician.

CONCLUSION

Successful organ transplantation represents the fruition of the dedicated efforts of basic scientists, clinicians, and allied personnel. An excellent paradigm for the effective application of knowledge gained by basic research to the alleviation of life-threatening illness, renal transplantation also affords marvelous opportunities for the investigation of the systemic basis for renal disease independent of organ-specific mechanisms.

Synergistic therapeutic protocols that target discrete steps in antigen recognition, signal transduction, and effector immunity are being explored in the clinic. The ultimate prize of transplantation would be that the basic principles learned would facilitate the prevention of the disease that necessitated transplantation in the first place.

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CHAPTER 98 ■ OUTCOMES AND COMPLICATIONS OF RENAL TRANSPLANTATION

LAURENCE CHAN, ALEX WISEMAN, WEI WANG, ALKESH JANI, AND IGAL KAM

Treatment options for patients with end-stage renal disease (ESRD) fall into three broad categories: hemodialysis, peritoneal dialysis, and transplantation. In most developed countries, there is a choice for each patient as to the modality of treatment that best suits the individual. The availability of these different methods of treatment has allowed flexibility in the management of the individual patient. Since the first successful renal transplant over 50 years ago (1), more than 500,000 patients with renal failure have had their lives prolonged with renal allografts (2–4). Renal transplantation has now become a preferred treatment modality for patients with ESRD. However, the number of patients with functioning grafts is still small when compared with the number of patients on maintenance dialysis (Fig. 98-1).

With improved transplant outcomes and widespread expectation that renal transplantation will be available, the growth in the number of patients wanting or waiting for a transplant has outpaced the supply of available organs (Fig. 98-2). The reported average waiting time for a cadaveric kidney transplant is more than 3 years. More patients are now being considered for living kidney donor transplantation (Fig. 98-3).

In the United States, Medicare funding was meant to achieve equal access to transplantation and dialysis for all ESRD patients in the Social Security System by removing financial barrier to care. There is no doubt that the access to dialysis care is reasonably equal demographic distribution of dialysis reflect the population at large and also seem to match the population in need. However, there are relatively large racial and gender difference with regard to kidney transplantation. In a retrospective analysis of the effect of patient and dialysis unit characteristics on access to kidney transplantation, Held et al. (3) concluded that white, male, young, nondiabetic, high-income patients treated in a small dialysis unit are more likely to receive a cadaver kidney than are other kidney patients. This is in agreement with a previous report by Kjellstrand (4).

The outcomes of renal transplantation are affected by a large number of variables (Table 98.1). These factors include: age, sex, and race of the recipient and donor; tissue compatibility; prior sensitization to HLA antigens; original renal disease, pretransplant health status and concomitant extrarenal disease of the recipient; compliance of the recipient; donor factors, such as cold ischemia time and nephron dosing effect; and, finally, the experience of the transplant center and the nature and extent of immunosuppresive therapy. Each of these factors contributes to some extent to the ultimate outcome of renal transplantation, as assessed by the survival of both the patient and the functioning graft. The short-term outcome has improved substantially in the past 15 years. One-year graft survival for cadaveric donor transplants now approaches 90% in experienced centers, but improvement in long-term graft survival has been more difficult to achieve. It has been empirically observed that after the first year, fractional graft survival over time is linear when plotted logarithmically. The half-lives ($T_{1/2}$) can be a useful method of analyzing long-term survival. Despite significant improvement in 1-year graft survival between 1972 and 1990, the $T_{1/2}$ has shown little improvement. However, in a recent analysis by Hariharan et al. (5) on graft survival for all 93,934 renal transplantations performed in the United States between 1988 and 1996, the 1-year survival rate for grafts from living donors increased from 88.8% versus 93.9%, and the rate for deceased donor grafts increased from 75.7% to 87.7%. The half-life for grafts from living donors increased steadily from 12.7 to 21.6 years and that for cadaveric grafts increased from 7.9 to 13.8 years. The improvement is not attributable to any of the newer immunosuppressive drugs, because it took place in the era of treatment with cyclosporine, azathioprine, and prednisone. These data, while quite promising, should be viewed in the context that graft survival was calculated upon projected, not actual, graft survival. These data from 1988 to 1995 were recently reanalyzed with findings that suggest that the half-life of deceased donor grafts improved from 6.0 to 8.0 years, but that the graft survival rate of first transplants did not appreciably improve (6). With the availability of better therapeutic protocols, we may expect even better outcome in renal transplantation with better long-term graft and patient survival. In the subsequent sections of this chapter, we will discuss each of the factors influencing outcome of renal transplantation, the recipient and donor evaluation prior to transplantation, immunosuppressive drugs, posttransplantation management, and complications.

PATIENT SELECTION AND PRETRANSPLANT EVALUATION

General Philosophy in Recipient Selection

Each transplant center has its own criteria in selecting patients for transplantation. The decision to place a patient on the waiting list for a transplant should be made jointly by the nephrologist and transplant surgeon. Most patients are transplanted after having been established on maintenance hemodiaysis or, to a lesser extent, peritoneal dialysis. However, many patients are being transplanted before they require dialysis. Indeed, if the supply of kidneys were to increase, this shortcut would become an increasingly common practice. In general, patients with ESRD should be given the option to choose between dialysis and transplantation. Patients who have expressed an interest in undergoing kidney transplantation should be fully evaluated by the transplant team as an outpatient during a clinic visit, or inpatient basis in a few centers.

Criteria for acceptance were more stringent in the past. Today there are few absolute contraindications to a kidney

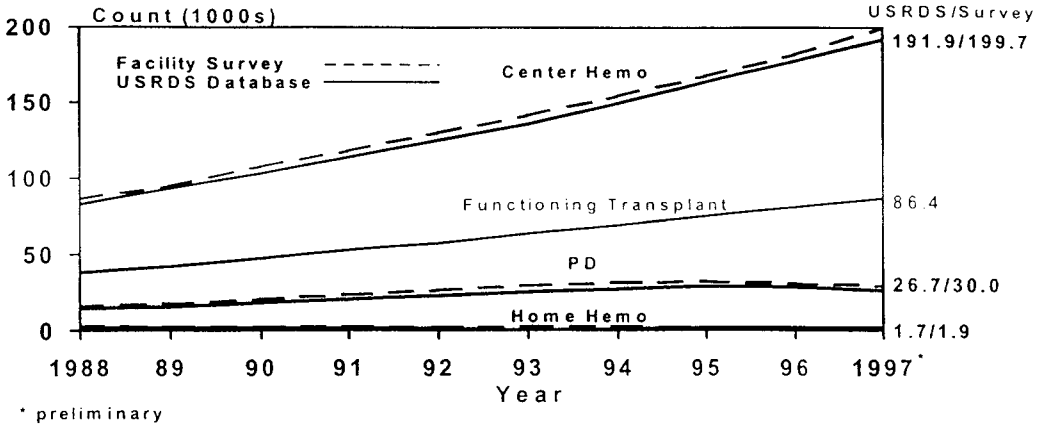

FIGURE 98-1. Point prevalence counts of patients with end-stage renal disease by treatment modality, data source, and year, 1988–1997. Percentages include Puerto Rico and U.S. territories. (From: United States Renal Data System. *USRDS 1999 Annual Data Report*. Bethesda, MD: National Institutes of Health and National Institute of Diabetes and Digestive and Kidney Diseases; 1999, with permission.)

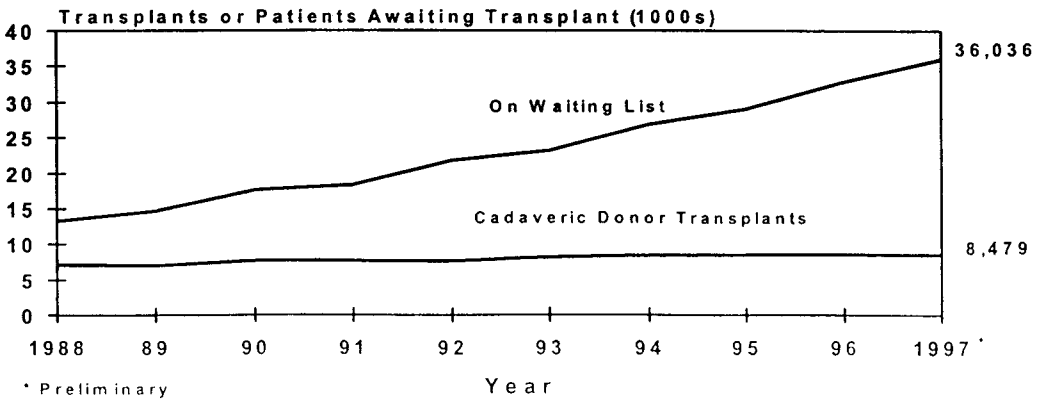

FIGURE 98-2. Counts of patients on cadaveric renal transplant waiting list and counts of renal transplants from a cadaveric donor by year, 1989–1997. (From: United States Renal Data System. *USRDS 1999 Annual Data Report*. Bethesda, MD: National Institutes of Health and National Institute of Diabetes and Digestive and Kidney Diseases; 1999, with permission.)

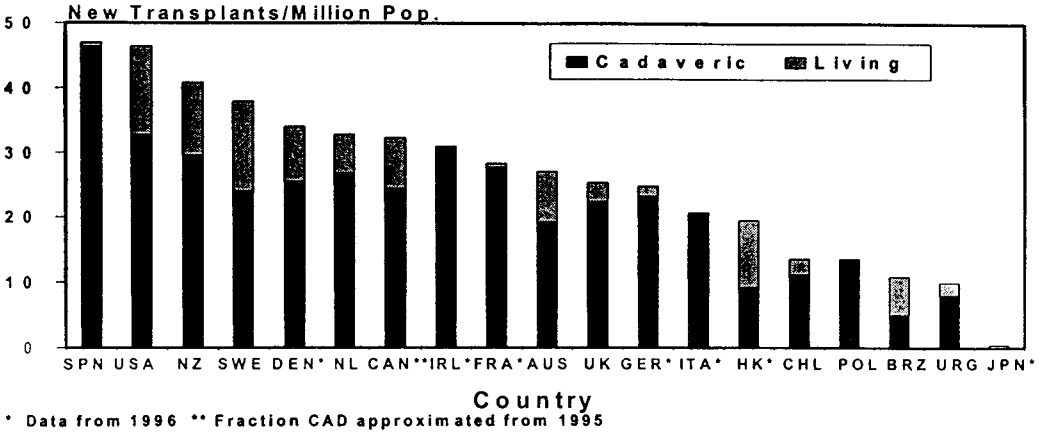

FIGURE 98-3. Transplantation rates (count of new renal transplants per million total population) in Australia, New Zealand, Canada, selected European countries, Japan, Hong Kong, Chile, Brazil, Uruguay, and the United States during 1997, by donor type (cadaveric or living donor). (Data from 1996: Germany, Denmark, Ireland, France, Hong Kong, and Japan.)

TABLE 98-1

FACTORS INFLUENCING THE OUTCOME OF CADAVERIC RENAL TRANSPLANTATION

Immunologic	Nonimmunologic
Immunosuppressive protocol	Delayed graft function/ischemic time
Matching for HLA	Compliance
Sensitization	Cardiovascular disease
Rejection	Recipient age
	Center effect/clinical care
	Nephron dose/donor and recipient sex

transplantation (7,8) and many of these contraindications are relative (Table 98-2). Successful renal transplantation in Jehovah's Witnesses has also been reported (9). Several medical and immunologic factors of the recipient, however, have been identified with a higher risk of death and graft loss. These include older age, diabetes, and heart disease. These factors are also associated with a higher risk of death in the general population and in patients with ESRD treated by dialysis.

Age

The very young patient (younger than 5 years of age) and the elderly recipient do have a poorer patient and graft survival than patients of ages between these two extremes (10–12). This is due to complications of immunosuppressive therapy leading to death or to nontransplant-related complications, in particular, cardiovascular disease in the elderly leading to death (13,14).

Until relatively recently, patients generally were not considered for transplantation over the age of 65 if significant cardiovascular disease was present (15–17). However, the ESRD population is rapidly "graying" (18). There has been a striking change over the past 10 years or so in the type of patient considered suitable for transplantation. A reluctance to accept transplantation in older patients was previously due to the belief that the peri- and postoperative complication rates outweighed the advantages. However, with the improvements in perioperative management and immunosuppressive strategies, advanced age itself is no longer a contraindication to renal transplantation. Based on a retrospective analysis of patients from the United Network for Organ Sharing (UNOS), it appears that older patients may have better immunologic survival despite the higher mortality from cardiovascular disease (19,20). One explanation may be an age-related change in immunologic function that confers less alloreactivity with aging. For this reason, many centers advocate the use of lower immunosuppression in elderly patients (21). Transplantation can now be safely and successfully performed in the elderly patient and will become much more widely practiced in this group of patients with end-stage renal failure (22–24).

TABLE 98-2

CONTRAINDICATIONS TO TRANSPLANTATION

Absolute	Relative
Active infection	Renal disease with high recurrence rate
Disseminated malignancy	Urologic abnormalities
Extensive vascular disease	Active systemic illness
High risk for perioperative mortality	Ongoing substance abuse
Persistent coagulation abnormality	Uncontrolled psychosis
Informed patient refusal	Refractory noncompliance

Obesity

Obesity alone is rarely an absolute contraindication to transplantation, yet it is a well-defined risk factor. Lower graft survival rates and higher postoperative mortalities and complications have been demonstrated in patients with a body mass index (BMI) greater than 35 kg/m^2 (25). The large body size is also a risk factor for progression and subsequent premature failure due to the physiologic changes that have been linked to nephron hyperfiltration (26,27). Thus, weight reduction is important for obese dialysis patient before proceeding to transplantation.

Prior Kidney Transplantation

Renal allograft failure is now one of the most common causes of ESRD, accounting for about 30% of patients awaiting renal transplantation. Graft survival of a second and third kidney transplant has been reported to be inferior to that of the first. Evaluation of a potential recipient for a second or third allograft requires careful attention to the reason for the graft failure (28–31), such as noncompliance with immunosuppressive medications, loss of the graft in association with recurrent renal disease, or high alloreactivity with high-panel reactive antibody titers (PRAs). These patients may also manifest complications of prior immunosuppressive therapy and, as such, should be screened for complications associated with these medications, such as infection and malignancy. No controlled, prospective studies have been performed to determine the best method for tapering or withdrawal of immunosuppression following renal allograft failure (32). Most centers have adopted a policy of immediate withdrawal of immunosuppression combined with preemptive nephrectomy for patients with early allograft failure. However, this practice is less common for patients with late graft failure. A longer taper of immunosuppression may permit the maintenance of some residual renal function while on dialysis. Several small studies have noted that patients who have undergone transplant nephrectomy have higher PRAs than those undergoing dialysis with allograft still in place. An unresolved issue is whether there might be some benefit for retransplantation from leaving the failed transplant kidney in place (33). However, there appears to be no significant difference in outcome after retransplantation among those with or without nephrectomy. Further studies are needed to determine if slower taper of calcineurin inhibitors or other immunosuppression can reduce the incidence of nephrectomy without untoward side effects in these patients while on dialysis.

Underlying Renal Diseases

It is most important to assess the cause of the potential recipient's renal failure. The primary pathologies leading to renal failure are expected to influence outcome, depending on the etiologic mechanisms, propensity for recurrence, and status of the immune system.

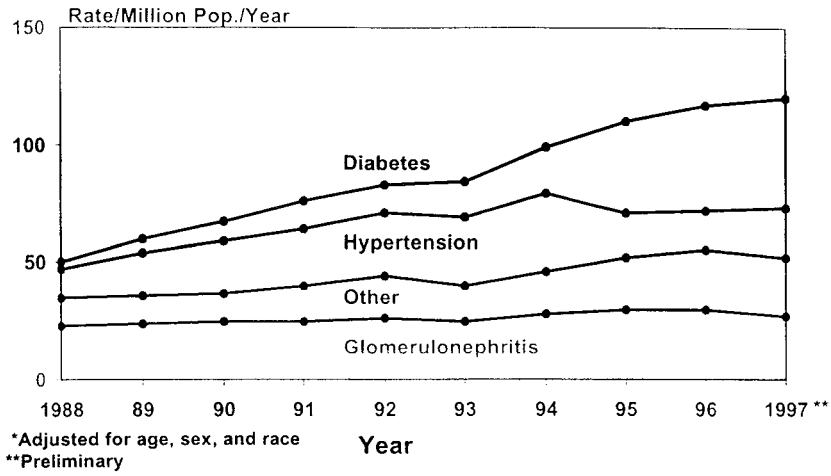

FIGURE 98-4. End-stage renal disease incidence rates per year by primary diagnoses, 1988–1997. Rates by diagnoses adjusted for age, sex, and race. Rates do not include patients from Puerto Rico or the U.S. territories. (From: United States Renal Data System. *USRDS 1999 annual data report*. Bethesda, MD: National Institutes of Health and National Institute of Diabetes and Digestive and Kidney Diseases, 1999, with permission.)

Diabetes Mellitus

Patients with diabetes mellitus are at increased risk with all forms of therapy for ESRD. The most dramatic change in patient selection has been the increase in diabetic patients with renal failure who are being offered transplantation as the treatment of choice (Fig. 98-4). For example, in the United States, diabetic patients now comprise over 40% of all patients accepted for treatment on ESRD programs; 12% classified as type I and 28% as type II. The number is much lower in Europe, but is steadily growing and is now around 20% overall. Although the diabetic is a high-risk patient for transplantation, it is now generally accepted that transplantation is the treatment of choice for many of these patients (34,35).

There is an increasing use of combined kidney and pancreas transplantation in selected patients with ESRD due to diabetes (36–39). Pancreas transplantation is performed mostly with a simultaneous kidney from the same donor. Benefits of pancreas transplantation include better glycemic control and improvement in some of the secondary complications of diabetes. As of 2003, more than 21,000 pancreas transplants were reported to the International Pancreas Transplant Registry. Patient survival and pancreas graft survival rates continue to improve, with data from U.S. centers demonstrating 95% and 86% 1-year patient and graft survival, and 85% and 70% 5-year patient and graft survival, respectively (40).

With the increase in utilization of living donors for kidney transplantation, solitary pancreas transplant after kidney transplant (PAK) has grown in popularity, comprising over 25% of pancreas transplants performed in 2002. While this offers the benefit of timely kidney transplant, ideally prior to the need for hemodialysis, this strategy requires two separate survival procedures and the risks inherent to surgery. Debate has arisen regarding the benefit derived from this strategy, as one study suggests an increase in mortality in patients who undergo PAK when compared to patients who remain on the waiting list for pancreas transplantation (41).

Another treatment option in development for the patient with type I diabetes is pancreatic islet cell transplantation. Once a theoretical concept, islet cell transplantation has now advanced to clinical trials as a potential therapeutic option for patients with type I diabetes. In 2000, researchers from the University of Alberta in Edmonton (42) reported that an approach, which utilizes immunosuppression using daclizumab (DZB), low-dose tacrolimus, and sirolimus have achieved insulin-free survival in seven consecutive patients. This novel and innovative approach utilized a steroid-free immunosuppression regimen with cells obtained from multiple donor pancreata and infused into the portal vein of the liver on several occasions. Follow-up results from this institution reveal a success rate (measured by on 1- and 2-year graft survival permitting insulin independence) comparable to whole-organ pancreas transplant (43), with ~80% insulin independence at 1 year and 60% to 70% graft survival at 2 years. In the $3^1/_2$ years following Edmonton's initial publication in July 2000, over 300 patients have undergone islet cell transplantation, with some centers demonstrating comparable graft survival compared to Edmonton (44–47). At present, this therapy should still be considered experimental, as the long-term graft survival is unknown and the risks of immunosuppression must be weighed against the benefits of normalization of blood glucose.

Recurrence of the diabetic nephropathy in type 1 diabetic recipients is a late and slowly developing complication. In a detailed follow-up of 100 type I diabetic recipients who were alive with a functioning graft 10 years after transplantation, only two patients went on to lose the graft due to recurrence. No patient from the 265 diabetic recipients had graft loss due to recurrence in the first 10 years (48).

Examination of biopsy specimens early after transplantation indicates that there are few glomerular pathologic abnormalities other than frequent afferent and efferent arteriosclerosis. Glomerular basement IgG deposition is seen in less than 2 years after transplantation, but the onset and progression of glomerular basement membrane thickening and mesangial expansion only occurs after 2 years. However, the typical nodular glomerular hyalinosis is rarely seen in these patients. Long-term follow-up has shown that recurrent nephropathy progresses to ESRD with the same time course as primary type I diabetic nephropathy. The mean time to recurrent ESRD is estimated to be 15 to 20 years. Therefore, recurrence of the lesion is not a barrier to long-term renal graft survival in diabetic recipients. The frequency and natural history of recurrence in type II diabetic recipients remain to be elucidated (49–52).

Metabolic and Congenital Disorders

Results of renal transplantation in the metabolic and congenital disorders causing end-stage renal failure such as Alport's syndrome, amyloidosis, cystinosis, familial nephritis, gout, and cystic disease are, in general, similar to those of the more common cause of end-stage renal failure with the exception of oxalosis, sickle cell, and Fabry's disease (53,54).

Oxalosis

The early transplant experience was disappointing because of early graft failure due to recurrent urolithiasis (Fig. 98-5), nephrocalcinosis, renal failure, and systemic oxalate

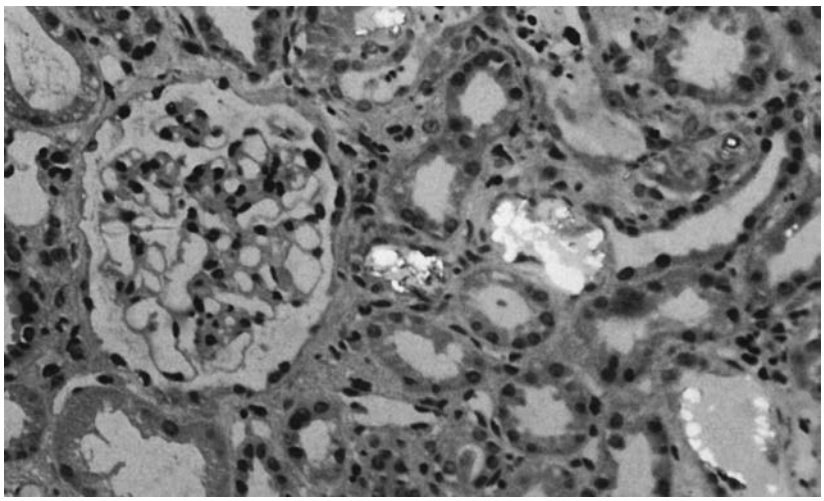

FIGURE 98-5. Renal biopsy specimen from a transplanted kidney showing recurrence of oxalosis.

deposition (55). Earlier recommendations to consider primary oxalosis a contraindication to transplantation are being challenged by recent reports of successfully prolonged graft function despite persistent hyperoxalosis (56–58). Those grafts with good long-term function have usually passed urine promptly after the operation and have had little rejection. To reduce the chance of oxalate accumulation, dialysis treatment or kidney transplantation should be considered when the glomerular filtration rate (GFR) approaches 20 mL/min. Aggressive dialysis schedules should be implemented before transplantation to deplete the oxalate metabolic pool. Medical therapy with pyridoxine, neutral phosphate, and magnesium should be given after transplantation to reduce oxalate deposition and recurrence. Combined renal and hepatic transplantation has also been recommended as a more definitive approach, and early results have been encouraging (59,60).

Unlike primary oxalosis, which is a congenital condition with enzymatic defects in oxalate metabolism, secondary oxalosis is due to excessive intake or absorption of oxalates from the diet. Secondary oxalosis is seen primarily in fat malabsorption, short bowel syndromes after gastrointestinal surgery, and high-oxalate diets. For these patients, consideration should be given to reanastomosis of gastric bypass, hydration, and dietary restriction of oxalates. Good allograft function can be achieved when attention is paid to reduce the oxalate excretion load (61).

Cystinosis

Cystine stones recur after transplantation, but have little effect on graft function (62). Renal transplantation has been recommended as a preferred therapy in children with ESRD due to cystinosis. However, the systemic effects of cystine accumulation, including corneal crystallization and retinal degeneration, leading to blindness, progress after renal transplantation. Despite recurrence of the disease, long-term survival has been reported (63,64).

Sickle Cell Disease

The autosomal recessive conditions of sickle cell disease and sickle cell trait may be complicated by a variety of renal abnormalities, which may eventually lead to ESRD. Glomerular lesions are relatively rare and, in some instances, may be traced to an acquired chronic hepatitis B infection due to multiple transfusions. There is little experience with renal transplantation in patients with sickle cell nephropathy (65). The overall results of the report by Barber et al. (66) from the University of Alabama were disappointing—with a 25% 1-year survival rate in eight patients. Others have claimed a more reasonable outcome. The importance of recurrence after transplantation is difficult to determine because of the relative nonspecific nature of sickle cell nephropathy. The North American Pediatric Renal transplant Cooperative Study reports a more favorable outcome in pediatric patients with patient survival of 89%, and graft survival at 12 and 24 months posttransplant of 89% and 71%, respectively (67).

Fabry's Disease

Fabry's disease is an X-linked disorder of glycosphingolipid metabolism due to a ceramide trihexosidase. Renal transplantation results have been disappointing with poor graft and patient survival. This poor outcome mostly has been due to continued morbidity from the underlying disease, which appears to be unaffected by successful restoration of normal renal function (68). However, European data concerning transplantation in Fabry's disease disagree with what has been previously stated (69). Despite recurrence of the disease, long-term survival has recently been reported (70). Recently, it has been postulated that genetic correction of bone marrow cells derived from patients with Fabry's disease may have utility for phenotypic correction of patients with this disorder (71,72).

Amyloidosis

Recurrent nephrotic syndrome and graft failure can occur in primary and secondary amyloidosis, but there is some indication that patients with amyloid-induced renal disease do better after renal transplantation than with dialysis as replacement therapy. The graft survival of patients with amyloid-induced ESRD who receive transplantation now appears to be equal to the survival of non-amyloid-induced ESRD patients who receive transplants (73). Familial Mediterranean Fever (FMF), rheumatoid arthritis, and osteomyelitis are the most common causes of secondary amyloidosis. Familial Mediterranean Fever is an autosomal recessive disorder which occurs in Sephardic Jews, Armenians, Turks, and Arabs of the Levant. In Israel, amyloidosis constitutes 6% of all patients on dialysis, compared to 0.6% in Europe. Although there has been a higher early mortality rate in the transplanted patients in the past, the incidence of rejection episodes is lower than in patients without amyloidosis. Reduced immunosuppression has decreased postoperative mortality and morbidity. Colchicine at 1 to 2 mg/day dramatically relieves the symptoms and reduces the incidence of attacks in FMF.

Alport Syndrome

Dialysis and transplantation pose no particular problems for patients with Alport syndrome. Recurrent disease has not been well documented. Improvement or stabilization of deafness after renal transplantation has occasionally been reported. There is a remote risk of developing *de novo* anti-glomerular basement membrane (anti-GBM) nephritis after transplantation (74–76).

Polycystic Kidney Disease

Autosomal dominant polycystic kidney disease is responsible for approximately 4% to 12% of ESRD in the United States and Europe. Early studies suggested that renal allograft survival and patient survival were better in those who had bilateral nephrectomy (77), but, in more recent studies, the results of renal transplantation without bilateral nephrectomy are excellent (78). The polycystic kidneys will shrink considerably in size following successful transplantation. Thus removal is only required if the kidneys are massive due to polycystic disease or there is associated persistent infection or severe hypertension. However, nephrectomy is indicated if cyst-related complications occur repeatedly (79). There is no increased risk of renal cell carcinoma in patients with autosomal dominant polycystic kidney disease. Polycystic patients on dialysis should be followed for cardiac valve abnormalities and cerebral aneurysms (80).

Glomerulonephritis

Glomerulonephritis remains the second most common cause of ESRD. Because of the more frequent use of renal biopsy early in the course of most glomerular diseases, the pathologic diagnosis has often been well established for potential transplant recipients. Almost all types of glomerulonephritis have been reported to recur after transplantation. There is, however, much variation between the various types of glomerulonephritis with regard to the frequency of recurrence, the clinical course, and the prognosis (81–83). The overall incidence of recurrence is less than 10 to 20% and recurrent disease accounts for less than 2% to 4% of all graft failures.

Focal Segmental Glomerulosclerosis (FSG)

Recurrent focal sclerosis may be seen early after transplantation, presenting with nephrotic–range proteinuria and a rapid decline in renal function (84). Histologically, the features on light microscopy that permit categorization are focal and segmental sclerosis, affecting a small number of glomeruli, often those in the deep juxtamedullary cortex. The development of foot-process fusion can be immediate after transplantation and precede glomerular segmental sclerosis by weeks to months (Fig. 98-6). The frequency of recurrence is about 20% in adults and may be as high as 40% in children. It is likely that some of these patients had secondary FSG due to nephron loss in reflux nephropathy, which would not be expected to recur in the transplant. Thus, the recurrence rate in primary FSG may be substantially higher than the reported values. Patients presented with rapid progression of renal disease from the time of diagnosis of nephrotic syndrome to ESRD have higher risk for recurrence. If a transplant patient lost his graft because of recurrent FSG, there is a 50% risk of subsequent allograft failure within 5 years of a second transplantation.

Treatment for recurrent FSG remains disappointing. Heavy proteinuria and nephrotic syndrome are usually resistant to steroids (85). Cyclosporine (CsA) has not proved effective in preventing recurrence. Because of the high risk of recurrence and rapid progression to ESRD, living donors generally are not used for the first allograft (86,87). Use of a cadaver kidney, however, is not precluded, since the disease will not recur in all cases and not all patients with recurrence will lose the graft. Some centers have also suggested that, if a first graft is lost to recurrent disease, a second transplant should be delayed for 1 to 2 years.

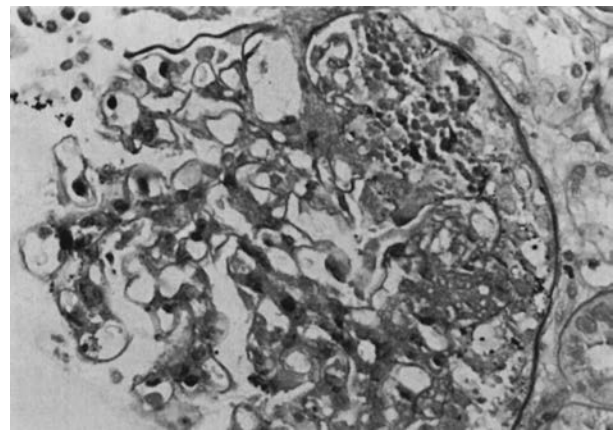

FIGURE 98-6. Renal biopsy specimen of a transplanted kidney showing recurrence of focal segmental glomerulosclerosis. (Periodic acid–Schiff stain, magnification ×250.)

The rapidity of recurrence strongly suggests the presence of a circulating factor in primary FSG that is toxic to the capillary wall. It has been shown that serum from some patients with FSG increases the permeability of isolated glomeruli to albumin. Testing of pretransplant sera with this approach can be used to predict recurrence after transplantation. Use of a regenerating protein adsorption column or plasma exchange can reduce protein excretion in patients with recurrent FSG in the transplant. More prolonged remissions have been achieved using plasma exchange that is initiated promptly after onset of proteinuria or the combination of plasma exchange and cyclophosphamide. These prolonged beneficial results have also been reported in children treated with plasma exchange and cyclophosphamide (88–92).

Antiglomerular Basement Membrane (Anti-GBM) Disease

Based on histology and fluorescence study, anti-GBM disease is associated with over 50% recurrence rate in the allograft. However, only 25% of patients with biopsy-proved IgG staining along the capillary wall have evidence for clinical disease activity. Furthermore, graft failure due to recurrence disease is less common. Although engraftment during the presence of anti-GBM antibodies has been reported to be successful (93), many transplant centers still prefer serologic quiescence of anti-GBM antibody production for 6 to 12 months before proceeding with transplantation to reduce the risk for recurrent anti-GBM disease. Despite delaying transplantation to allow anti-GBM antibody to fall, recurrence has been reported (94,95).

Hemolytic Uremic Syndrome (HUS)

HUS has a recurrence rate of 20% to 50% (96,97). It has pathologic features common to the small vessel findings in acute vascular rejection, CsA toxicity, and malignant hypertension. The recurrence rate is higher in recipients of living-related transplants. Live kidney donation should proceed with caution in view of the possibility of a familial tendency to an abnormality of prostacyclin synthesis. In 1998, a meta-analysis of 159 grafts in 126 patients by Ducloux et al. (98) found that

recurrent HUS was significantly associated with older age onset of HUS, short duration between disease onset and ESRD or transplantation, use of living related donors, and, to a lesser degree, the administration of calcineurin inhibitors (CsA or tacrolimus). In high-risk patients with history of HUS, prevention by the administration of low-dose aspirin and dipyridamole should be used. Calcineurin inhibitors and antilymphocyte serum should be used with caution in these patients. A review of 114 patients with both recurrent and *de novo* HUS after organ transplantation found that two-thirds of patients had been treated with CsA. There is no successful treatment for recurrent HUS. Salicylates, dipyridamole, plasma infusion, and plasma exchange have been shown to be of limited benefit. Once established, HUS often does not remit in the allograft and typically results in rapid graft loss within days. CsA and FK506 have both been associated with altered coagulation mechanisms and the development of *de novo* HUS in renal transplant recipients (99). These agents should, therefore, be used with caution in patients whose original kidney disease was due to HUS. If CsA or FK506 has been associated with recurrent HUS in the first transplant, it should be subsequently avoided. Preliminary evidence suggests that patients with allograft loss due to recurrent HUS associated with CsA or FK506 may be successfully retransplanted without the use of calcineurin inhibitors. An immunosuppressive protocol without calcineurin inhibitors, consisting of mycophenolate mofetil (MMF), corticosteroids, and antilymphocyte antibody (ALG) induction therapy was administered to six patients. No patient experienced disease recurrence, allograft loss, and or episode of acute rejection. Rapamycin has also been used in combination with MMF.

IgA Nephropathy/Henoch–Schönlein Purpura (HSP)

In many parts of the world, IgA nephropathy is the most common type of glomerulonephritis. The true incidence of recurrence is best seen in Berger's series where all 32 patients had routine follow-up biopsies and 17 (50%) showed recurrent IgA nephropathy (100). However, there is a low incidence of allograft dysfunction that leads to allograft loss (101). Of interest is the fact that IgA nephropathy with mesangial IgA deposit tends to disappear after transplantation to a non-IgA nephropathy recipient. Recent studies demonstrate that patients with IgA nephropathy had a highly significant survival rate compared to those with other diseases (102,103).

The closely related HSP has been reported to recur with mesangial deposits of IgA occurring in 17 of 20 patients with minimal clinical manifestations in one series (100). Clinically, recurrent HSP can be severe with crescentic glomerulonephritis, nephrotic syndrome, graft failure, and variable recurrence of purpura (104). To reduce recurrence, delay of engraftment is recommended for at least 6 to 12 months after the skin lesions of HSP have resolved (105,106).

Type I Membranoproliferative Glomerulonephritis (MPGN)

Type I MPGN can resemble transplant rejection glomerulopathy on light microscopy (107). Crescents, C3 deposition in the capillary wall, and dense subendothelial deposits on electron microscopy are common to both. The early development of nephrotic syndrome and persistent microscopic hematuria from the time of transplantation are clinical markers suggesting recurrence rather than rejection. The frequency of recurrence was estimated to be 20% to 30%. Approximately 30% to 40% of patients with recurrent type I MPGN will lose their allograft (108). Graft rejection is often a confounding factor. Reduced C3 levels before transplantation usually return to normal range after transplantation and do not correlate with disease activity.

Type II MPGN (Dense Deposit Disease)

The recurrence rate is reported to be 50% to 100%, with graft failure in 20% to 50% (109). Proteinuria with or without hematuria is the usual clinical presentation. Decrement in serum C3 levels and appearance of C3 nephritic factor may be present in some cases. Recurrence is usually evident within the first year after engraftment. The unique ultrastructural appearance of extensive deposit within the basement membrane allows this diagnosis to be made with certainty both before and after transplantation (110).

Membranous Nephropathy

An accurate rate of recurrence of membranous nephropathy is difficult to establish because of the relatively high frequency of primary *de novo* occurrence in allograft recipients (111). Recurrent membranous nephropathy with nephrotic syndrome generally occurs earlier, at an average of 10 months compared with *de novo* membranous nephropathy, which is usually seen about 18 to 20 months after transplantation (Fig. 98-7).

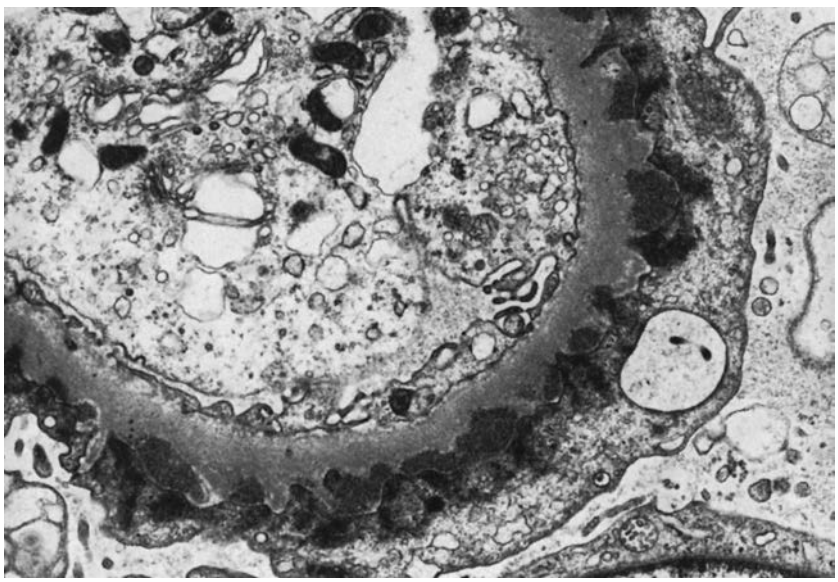

FIGURE 98-7. Electron micrograph of *de novo* membranous glomerulonephritis.

It seems more likely to recur when the primary disease has been aggressive, but the overall incidence is only about 10%. *De novo* membranous nephropathy is not reported to lead to graft loss. Recurrent membranous nephropathy, however, can have early and heavy proteinuria and an increased chance of graft loss.

Systemic Lupus Erythematosus (SLE)

Recurrence is relatively rare in SLE. Similarly, reactivation of SLE after transplantation is extremely infrequent and is often controlled by the immunosuppressive medications when it occurs (112,113). Recurrence is not predictable with serologic monitoring. However, there should be no systemic disease activity prior to transplantation (114,115). Recurrences can be successfully treated with steroids, mycophenolate mofetil, or chlorambucil.

Wegener's Granulomatosis

There are few data on transplantation in patients with Wegener's granulomatosis. Recurrence is generally rare after transplantation (116–118). Recurrence can be treated successfully by adding cyclophosphamide and by increasing the steroid dose.

Progressive Systemic Sclerosis (Scleroderma)

Recurrence in the graft can occur within the first few months after transplantation (119,120). Earlier reports have suggested that graft loss may be accelerated due to uncontrolled hypertension. Many of the reports on recurrence came from patients before the use of CsA and angiotensin-converting enzyme (ACE) inhibitors. Those patients with recurrence had malignant manifestations of scleroderma before transplantation, suggesting that it may be possible to clinically separate a subset of patients in whom early recurrence was more likely. The highest patient survival was noted in those individuals who have had bilateral native nephrectomies, usually to control severe hypertension. The current recommendation for transplantation is that the patient should be clinically stable with absence of visceral progressive systemic sclerosis activity prior to transplantation. Patients with early diffuse scleroderma should be closely monitored for new onset of hypertension and be treated continuous with ACE inhibitors. The majority of patients with scleroderma will improve generally after transplantation with loss of Raynaud's syndrome and improvement of the skin condition. Therefore, transplantation is justified if the patient has not been severely debilitated by the systemic effects of scleroderma.

Interstitial disease

Chronic Pyelonephritis

Chronic pyelonephritis is a diagnosis that has been frequently used for nonspecific interstitial nephritis, not necessarily caused by bacterial infection. The presence or history of significant urinary infection is important to identify. Because of the risk of residual foci of infection that may predispose to bacteremia or seed the urinary tract and transplant kidney, pretransplant nephrectomy may be indicated in these patients.

Analgesic Nephropathy

Patients with analgesic nephropathy need to be identified because cessation of the use of nephrotoxic analgesics is essential for these patients. Kidney function may improve after cessation of the use of analgesics and damage to the allograft is a significant risk if this use persists. There is an increase in the incidence of transitional cell carcinoma of the urinary tract in patients with analgesic nephropathy.

GENERAL EVALUATION

This assessment should include not only a complete medical evaluation and determination where possible of the underlying disease causing renal failure, but also a careful surveillance for problems that might arise following transplantation (121) (Table 98-3).

A careful physical examination should be performed to identify coexisting cardiovascular, gastrointestinal (GI), or genitourinary (GU) disease. Additional examinations should assess pulmonary reserve, define potential sources of infection, including dental caries, and assess the gynecologic risks for females.

The laboratory evaluation should include routine hematologic tests to detect leukopenia or thrombocytopenia, liver function tests to identify patients in whom the metabolism of cyclosporine may be abnormal, complete hepatitis and HIV profiles, viral titers, and throat and urine cultures.

Risk Factors

The major risk factors that have an impact on the recipient include age, the presence of diabetes mellitus, arteriosclerotic heart disease, chronic pulmonary disorders, and malignancy.

TABLE 98-3

PRETRANSPLANTATION RECIPIENT MEDICAL EVALUATION

1. History and physical examination
2. Social and psychiatric evaluation
3. Determine primary kidney disease activity and residual kidney function
4. Dental evaluation
5. Laboratory studies
   - Complete blood cell count and blood chemistry
   - HBsAg
   - HIV
   - Antibodies to cytomegalovirus and Epstein–Barr virus
   - HLA typing and antibodies screening
   - Urine analysis and urine culture
6. Chest x-ray
7. Electrocardiogram
8. Special procedures for selected patients
   - Abdominal ultrasound of gallbladder
   - Upper gastrointestinal study or endoscopy
   - Barium enema or colonoscopy
   - PPD skin test
   - Treadmill/exercise electrocardiogram
   - Thallium scan
   - Angiogram: coronary
   - Cystoureterography
9. Consults (optional)
   - Psychiatric
   - Gynecology evaluation and mammography (for female >40 y)
   - Urologic assessment (voiding cystoureterography, cystoscopy, or urodynamic studies in patients with vesicoureteric reflux, neurogenic bladder, bladder neck obstruction, or strictures)

Patient compliance has also been identified as an important cause of late graft failure (122). It is important to have social and psychiatric evaluation with a view to give support to these patients (123,124). Conditions excluding a patient from renal transplantation would probably be the presence of severe ischemic heart disease, although this might be approached by coronary artery bypass surgery, where appropriate, before transplantation (125), old age (perhaps over 70 years) although attitudes are still changing, and the presence of persistent infection or cancer. When a patient has had previous curative therapy for cancer, it is generally thought appropriate to wait at least 1 year, and possibly 2 years, with proved freedom from recurrence before going ahead with transplantation (126,127).

Cardiovascular Evaluation

Cardiovascular disease is a major cause of morbidity and mortality for the patient in ESRD, whether the patient remains on dialysis or chooses to have a kidney transplantation (128). It is, therefore, important to carefully screen the patient for any cardiovascular problem, especially in diabetic patients (129). Initial assessment of the severity of cardiovascular disease consists of careful clinical examination, an electrocardiogram (EKG) and x-ray of the chest and peripheral vessels for calcification. Evidence of moderate or severe myocardial ischemia is an indication for further investigation with a thallium stress test and/or coronary angiography (130). Coronary artery bypass grafting should be considered prior to transplantation in the presence of severe angina or double or triple vessel disease. Any patient who has had a recent myocardial infarction should be reassessed for a transplant 6 months after the incident. Approximately 20% to 30% of diabetic transplant candidates have significant coronary artery disease, which may be asymptomatic. Noninvasive testing and, if indicated, cardiac catheterization should be performed prior to renal transplantation, since active intervention may improve patient outcome. For the nondiabetic, asymptomatic patient extensive cardiac evaluation appears unnecessary, unless risk factors such as smoking, hypertension, hyperlipidemia, or family history of heart disease are present.

Gastrointestinal Evaluation

Although the incidence of peptic ulcer after renal transplantation is decreasing, complications of a peptic ulcer, such as perforation or hemorrhage, are associated with a high mortality in the transplant patient (131,132). For this reason, many centers actively screen patients for evidence of peptic ulceration before accepting them for transplantation and, in the past, have been quite aggressive about the management of these patients before transplantation. Similarly, in patients with symptomatic cholelithiasis or asymptomatic gall stones seen with ultrasonography, cholecystectomy should be performed to eliminate the risk of cholelithiasis and possible sepsis after transplantation. Patients with colonic disease, especially those with diverticulitis, should be evaluated with barium enema and colonoscopy and if appropriate should be treated with surgical resection prior to transplantation.

Gastrourinary Evaluation

Accurate evaluation of the lower urinary tract function prior to transplantation is important to minimize postoperative urologic complications. The original renal disease must be clearly defined. Any history of repeated urinary infections and current reports of urine cultures should be obtained. In the past, a voiding cystourethrogram has been performed on all patients to evaluate the urinary tract for evidence of outflow obstruction or vesicoureteral reflux. It is now considered necessary only if there is clinical evidence of a bladder or ureteric abnormality. Cystoscopy and urodynamic studies should be performed in patients with evidence of bladder dysfunction. Urologic operations are necessary either to correct or improve obstructive lesions or sometimes to provide a conduit in the presence of a neurogenic bladder or a previous cystectomy.

HBsAg Screening

Successful renal transplant in patients with positive HBsAg has been reported (133). However, when hepatitis B surface antigen-positive patients are retrospectively compared with an age matched group of hemodialysis patients known to be surface antigen-positive, the transplant patients have a higher frequency of chronic hepatitis and mortality due to hepatitis. The adverse effects are not apparent during the first 2 years after transplantation, but become evident over the long-term (134). Hemodialysis patients have a high rate of surface antigen persistence but rarely develop chronic hepatitis and the rate of seroconversion to surface antigen negativity is 15% to 20% per year. Because of this, some centers do not recommend renal transplantation in chronic hepatitis B surface antigen carriers. This decision however should be individualized. The better quality of life of the transplant patient should be weighed against the low, but definite, risk of development of chronic liver failure.

In a study by Pol et al. (135) from the Necker Hospital, patient and graft survival were similar between HBsAg-positive and -negative kidney recipients. Their data suggest that renal transplantation may be appropriate for patients with chronic hepatitis whatever their hepatitis virus status. However, no patient should undergo transplantation when he or she has evidence of active hepatitis. Before transplantation, potential recipients should have stable liver enzymes, preferably less than two or three times normal for several months.

Hepatitis C Virus (HCV) Screening

Transplant recipients are potentially at risk of developing hepatitis C virus infection due to reactivation of pretransplantation HCV infection or to infection acquired from blood products or from HCV-infected organ donors (136). In potential recipients with serologic evidence of HCV, a liver biopsy should be performed to assess the histologic severity of the disease (137).

HIV Screening

The HIV antibody status of all potential donors and recipients should be determined before transplantation. Potential recipients who are highly sensitized may have false-positive ELISA results because of a higher incidence of antibodies in their serum that react with HLA antigens on the target cell used in the serologic assay (138). The Western blot technique, which detects viral envelope protein, may be more accurate in these situations. Polymerase chain reaction (PCR) analysis to detect small amounts of HIV viral DNA in serum may further improve accuracy, but the assay is not widely available for routine screening (139).

Preparation for Transplantation

Nephrectomy

Nephrectomy before transplantation is now an uncommon procedure and may be associated with a significant morbidity (140,141). Thus, bilateral nephrectomy is only performed in the presence of persistent urinary tract infection (usually associated with vesicoureteral reflux), or in the presence of renal cancer, nephrolithiasis, or medically intractable hypertension (142). There is some evidence that

pretransplant bilateral nephrectomy improves renal graft survival (143).

Unilateral nephrectomy may be necessary in patients with large polycystic kidneys in order to provide room for a subsequent transplant on that side (78).

Transfusion

Prior to the use of CsA, the beneficial effect of blood transfusions on cadaveric graft survival was reported to be 10% to 20% better compared to nontransfused recipients (144–146). In the last few years, however, the role of blood transfusion as an adjunct to transplantation has been a controversial topic. With the availability of recombinant erythropoietin, there is also less need for blood transfusion in patients with ESRD (147). The two main disadvantages of blood transfusion are the induction of cytotoxic antibodies (148,149) and the risk of infections (150). In 1983, Kahan and his colleagues (151) reported that patients who received CsA immunosuppression showed diminished beneficial effects of pretransplant blood transfusion after cadaveric renal transplantation.

The Collaborative Transplant Study Data (152) demonstrated that the benefit of transfusion has diminished to a point at which the risks of infection and presensitization due to transfusion may outweigh any benefit on graft survival. The loss of transfusion effect is independent of the use of CsA and whether donor specific or random transfusions were given. Iwaki et al. (153) reexamined the UCLA database in 1989 to determine whether a beneficial blood transfusion was present since the introduction of CsA. Their data showed an increase in 1-year graft survival rate of 8% in first cadaver kidney transplants when one DR mismatch was present ($p < 0.01$), by 10% when two DR mismatches were present ($p < 0.01$), and 0% when no DR mismatches were present. The transfusion effect was greater in Black than Caucasian recipients. Their analysis suggests two to three deliberate pretransplant blood transfusions may still be helpful unless transplant candidates are to wait for a zero mismatched HLA-DR cadaver donor. Since the effect of transfusion is long-lasting, it is not necessary to give deliberate additional blood if the patient has received two to three transfusions during a lifetime.

In the case of donor-specific transfusions, where the recipient is given planned transfusions of blood from the potential donor before transplantation, there is a risk of a specific sensitization against the donor that could preclude subsequent transplantation from that donor (154). Sensitization occurs in approximately one-third of these patients. It is now customary to give either azathioprine or CsA with the donor-specific transfusions to lower the incidence of sensitization (155). This does appear to reduce the sensitization rate to about 5%. More recently, the concept of donor-specific transfusion has been challenged and evidence has been produced that random blood transfusions will result in equally good graft survival in this situation. Following transplantation, patients who have received donor-specific transfusions often have a fairly characteristic course with an acute cellular rejection episode occurring within the first 4 days of transplantation, but, in general, one that responds briskly to increased steroid therapy. Many transplant units now feel that donor-specific transfusion is no longer necessary since the advent of CsA (156).

Immunologic Evaluation

In addition to determining HLA antigens at the A, B, C, and DR loci, the potential recipient's serum should be screened regularly for HLA antibodies. Prophylactic measures are most important in the management of presensitization leading to hyperacute and accelerated rejection. The avoidance of both ABO incompatibility and positive T cell cross-matches has eliminated the major cause of hyperacute rejection seen in the early days of transplantation. The presence of lymphocyte cytotoxic antibodies in the patient's serum is due to sensitization to HLA antigens. It can occur after pregnancies, blood transfusion, and a renal transplantation. Autoantibodies, on the other hand, often occur spontaneously and are not related to any obvious antigenic challenge (157). One method of defining the highly sensitized patients is to include subjects who, at any time, have developed lymphocytotoxic antibodies, which react with 90% or greater of random panel cells. One approach to this is by removing the anti-HLA antibodies prior to transplantation (158,159). An alternative approach is to characterize the antibodies and to perform cross-match with different serological techniques. Recently, a technique using dithiothreitol (DTT) to eliminate IgM cytotoxic antibodies in sensitized recipients sera has allowed successful renal transplantation when the unmodified cross-match test is positive and the DTT-treated cross-match is negative (160), which indicates that the positive cross-match is due to irrelevant IgM antibodies.

The resurgence of transplantation across ABO incompatibility has been brought on by the lack of availability of suitable (ABO-compatible) cadaveric donor kidneys for type O recipients, the blood group with the largest waiting list, as well as the inability to transplant HLA identical but ABO-incompatible siblings (161). One approach is to transplant blood group A donors with A2 subtype into O recipients. The A antigen has been found to be weakly immunogenic and does not elicit an isohemagglutinin response. Several centers have found this to be successful, with 71% to 83% graft survival at around 1 year, providing a preformed anti-A2 isohemagglutinin titer is absent. Another experimental approach is to perform pretransplantation splenectomy (162), plasmapheresis on recipients to deplete circulating anti-A and anti-B antibodies (163), or infusion of intravenous immunoglobulin (164).

Patients who have rejected their first graft acutely represent a very high-risk group of patients for subsequent transplant (165). On the other hand, patients in whom the graft has functioned for at least a year have survival rates for second grafts that are no different to that of the first graft. Matching for both HLA-A and -B appears to exert a much greater influence on the survival of regrafts than in the case of first grafts (166,167) and, although it does not appear necessary to avoid previous HLA-A and -B incompatibilities with a second graft, it would appear logical to do so (167). In general, a regraft has a poorer graft survival than a first graft (Fig. 98-8) and this is

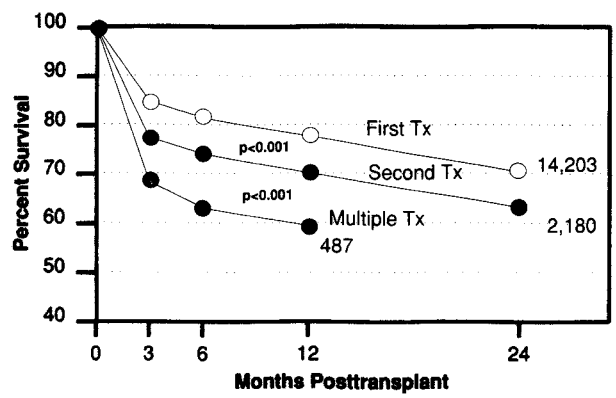

FIGURE 98-8. Graft survival rates of cadaveric kidney transplants. Graft survival rates for 14,203 first cadaveric donor transplants (tx) were 78% and 70% at 1 and 2 years, respectively. Graft survival rates for those receiving their second transplant were 70% and 63% at 1 and 2 years, respectively. Data are from the United Network for Organ Sharing Scientific Renal Transplant Registry, 1990. (From: Cecka J, Terasaki P, eds. *Clinical Transplants 1990*. Los Angeles, CA: UCLA Tissue Typing Laboratory; 1990:2, with permission.)

particularly evident if the first graft was lost from irreversible rejection during the first few months after transplantation (168). In addition to standard immunosuppressive regimen, the use of OKT3 or polyclonal antibodies as part of an induction therapy may be indicated. With appropriate immunosuppression, it is possible to have successful transplantation of highly sensitized patients without regard to HLA matching (169).

CADAVER VERSUS LIVING DONOR

Living related donor transplants comprise about 40% of all transplants performed in the United States (Fig. 98-9), whereas their proportion is much less (10% to 15%) in Europe and Australia (170). In addition, many programs will now accept living, non-blood-related or distantly related donors (spouses, cousins, uncles, aunts, stepchildren, stepparents, or even close friends).

Living related kidney donor transplantation is no longer controversial (171,172). There has been concern for many years about the long-term outcome of a healthy donor. In particular, the concerns regarding the possibility of long-term renal dysfunction resulting from hyperfiltration in the solitary kidney have prompted transplant centers to re-evaluate their living-related donor program. Several long-term follow-up studies have not revealed any adverse problems in living related donor with a single kidney (173,174). The donor mortality risk has shown to be less than 0.1%. Life expectancy in the donor remains unaffected. Long-term study in over 600 donors has shown no increased incidence of hypertension or impaired renal function (175). In one report, however, mild proteinuria and hypertension were found in male, but not in female, donors (176). Further studies and follow-up of kidney donors are necessary. In view of the shortage of cadaver kidneys, transplantation of a graft from a compatible living related donor should be considered if there is a suitable donor. Excellent results were also observed in kidney transplants from spousal and living unrelated donors (177,178). On the other hand, the proposal that kidneys might be purchased from living unrelated donors should be condemned. It is with these issues in mind that the Transplantation Society has issued guidelines for the practice of transplantation that avoid exploitation and commercialization of organ donation (179). Similarly, the U.S. Organ Transplantation Act of 1984 (HR5580, Title II) makes it a federal crime to engage in organ sale and commerce. Hopefully, the new legislation will help to strengthen the organ procurement program and increase the number of cadaver kidneys available for transplantations (180). Globally, barriers to wider development of effective cadaveric programs of renal transplantation were not due to religious objections. The main factors seemed to be a lack of public concern about the need for cadaver donor programs in patients with ESRD.

EXPANSION OF THE LIVING DONOR POOL

As patient and graft survival rates for kidney transplant recipients with living unrelated donors have been shown to be equivalent to living related donor transplant recipients, a greater willingness by society and the transplant community to consider the unrelated donor has emerged. An extension of living nonrelated donation is the "nondirected kidney donor," an individual who contacts transplant centers wishing to donate a kidney for purely altruistic reasons, to no specific recipient in particular. A pilot study by the University of Minnesota now provides a framework from which such donors may be evaluated and undergo nephrectomy in order to ensure the safety of this procedure from an ethical, medical, and societal perspective (181,182).

Unlike the nondirected kidney donor, two other circumstances have been specifically proposed to increase the number of potential living donors. The first, a "paired exchange program," attempts to identify two potential donors who wish to donate to a family or friend but are unable to due to blood group incompatibility or a positive cross-match. Two such donors and their prospective recipients are then paired, with donor A donating to recipient B and donor B donating to recipient A. An extension of this concept is the "mixed donor exchange" in which an incompatible donor donates to the cadaveric waiting list in exchange for their paired recipient moving to the top of the deceased donor list in their given blood type. These efforts are currently being tested for their equity and effect on transplantation rates in small pilot studies. The second circumstance is the "matched donor" in which a prospective recipient pays a monthly fee to a coordinating site, which presumably has access to a list of potential parties interested in donating their kidney. This strategy circumvents the UNOS waiting list and currently is under significant criticism from the American Society of Transplantation and UNOS.

Living Donor Evaluation

Live donors are usually first-degree relatives who are one or two haplotype matched. However, there is good evidence that zero haplotype-matched relatives can donate kidneys that provide excellent chance of short- and long-term graft survival. Similarly, good results have been reported with emotionally related living donors. Most living nonrelated donors are spouses

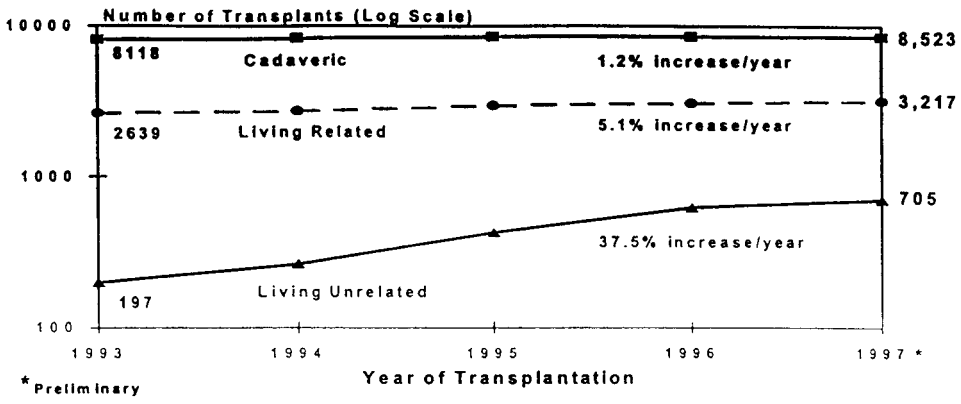

FIGURE 98-9. Total number of renal transplants by donor source and year, 1993–1997. Number of transplants shown on log scale. (From: United States Renal Data System. *USRDS 1999 Annual Data Report*. Bethesda, MD: National Institutes of Health and National Institute of Diabetes and Digestive and Kidney Diseases; 1999, with permission.)

or companions with long-standing emotional ties. This practice will likely become an important source of organ for transplantation. By 1995, about 10% of transplants were from living unrelated donors. This source of organs is expected to grow, especially in light of the fact that a high graft survival rate has been found associated with these donors. Initial screening should concentrate on related donors and tissue typing should be used to help choose the best potential donor among ABO-compatible candidates. The attitude toward the use of unrelated live donors varies considerably among centers. In general, live-donor transplantation is fraught with potential psychological problems and it is important to establish that the prospective donor has not been subject to family pressure (183). A very careful psychologic evaluation will be needed to determine that the motivation to donate the kidney is, indeed, genuine (184). HLA genotyping should be used to decide on the most suitable donor if there are several family members who are all keen to give a kidney. The initial series of tests which include ABO blood group and HLA tissue typing can be completed at a brief outpatient visit. Possible live-donor transplantation can then be considered with the individuals best matched to the recipient. The living donor not only needs a thorough medical evaluation, with particular attention to renal function and the urinary tract, but also a renal angiography or magnetic resonance angiography to identify vascular or anatomical variation of the kidneys or the collecting systems. It is important to ascertain that both kidneys are of normal size and configuration and that a donor kidney with a single renal artery can be obtained (Tables 98-4 and 98-5).

Living Donor Nephrectomy

Standard Open Nephrectomy

The standard method for removing a kidney from a living donor is through a flank incision by open nephrectomy. The approach to the kidney, which will usually be the left kidney since this has the longer renal vein, may be either below or through the bed of the 12th rib using a retroperitoneal approach or rarely via an anterior transperitoneal approach using a midline incision. Care must be given to retraction of the kidney during its removal to avoid traction injury of the renal artery and dissection in the hilum of the kidney, particularly between the ureter and the renal artery, which should be avoided to prevent damage to the ureteric blood supply. Furthermore, in removing the ureter down to the brim of the pelvis, care should be taken to leave an adequate amount of periureteric tissue.

TABLE 98-4

EXCLUSION CRITERIA FOR LIVE RELATED DONORS

Age <18 or >65–70 yr
Significant medical illness (e.g. cardiovascular/pulmonary diseases, recent malignancy)
History of recurrent kidney stones
History of thrombosis or thromboembolism
Psychiatric contraindications
Obesity (30% above ideal weight)
Hypertension (>140/90 mmHg or necessity for medication)
Proteinuria (>250 mg/24 hr)
Microscopic hematuria
Abnormal glomerular filtration rate (<80 mL/min)
Diabetes (abnormal glucose tolerance test or hemoglobulin A_{1c})
Urologic/vascular abnormalities in donor kidneys

TABLE 98-5

SUGGESTED EVALUATION PROCESS FOR POTENTIAL LIVE DONORS

Donor screening
Educate patient regarding cadaveric and live donation
Take family and social history and screen for potential donors
Review ABO compatibilities of potential donors
Tissue type and cross-match ABO-compatible potential donors
Choose primary potential donor with patient and family
Educate donor regarding process of evaluation and donation
Donor evaluation
Complete history and physical examination
Comprehensive laboratory screening to include complete blood count, chemistry panel, human immunodeficiency virus, very low-density lipoprotein, hepatitis B and C serology, cytomegalovirus, glucose tolerance test (for diabetic families)
Urinalysis, urine culture, pregnancy test
Protein, 24-hr urine collection
Creatinine, 24-hr urine collection
Chest radiogram, exercise treadmill for patients older than 50 years of age
Helical computed tomography urogram
Psychosocial evaluation
Repeat cross-match before transplantation

Laparoscopic Nephrectomy

Living-donor nephrectomy for transplantation can also be performed by laparoscopic approach. The techniques of endoscopically assisted nephrectomy are now well established. Over the last few years, there is an increased rate of donation with laparoscopic donor nephrectomy (185,186). This approach results in less postoperative surgical pain, a shorter hospital stay, and a quicker recovery than the standard open donor nephrectomy (Table 98-6).

Cadaver Donor Evaluation

The criteria for the diagnosis of brain death have been well defined in most western countries, although the requirements vary little from country to country (187) (Table 98-7). In the United

TABLE 98-6

ADVANTAGES AND DISADVANTAGES OF LAPAROSCOPIC NEPHRECTOMY

Advantages
Less postoperative pain
Minimal surgical scarring
Rapid return to fill activities and work (approx. 4 weeks)
Shorter hospital stay
Magnified view of renal vessels
Disadvantages
Impaired early graft function
Graft loss or damage during "learning curve"
Pneumoperitoneum may compromise renal blood flow
Longer operative time
Tendency to have shorter renal vessels and multiple arteries
Added expense of specialized instrumentation

TABLE 98-7

MEDICAL EVALUATION OF THE POTENTIAL CADAVER DONOR

I. Diagnosis of brain death
  A. Preconditions
    1. Comatose patient, on ventilator
    2. Positive diagnosis of cause of coma (irremediable structural brain damage)
  B. Exclusions
    1. Primary hypothermia (<33°C)
    2. Drugs
    3. Severe metabolic or endocrine disturbances
  C. Tests
    1. Absent brainstem reflexes
    2. Apnea (strictly define)
II. No preexisting renal disease
III. No active infection
  Tests:
    A. HBsAg; 5 antibodies to cytomegalovirus and hepatitis C virus
    B. HIV antibodies
    C. HIV antigen in high-risk patients

States, there are an estimated 20,000 brain-dead patients per year who would be acceptable donors, but fewer than 8,000 actually donate their organs for transplantation. The general acceptance of brain death (188) and improved preservation, in recent years, has led to the supply of better quality kidneys and establishment of organ sharing program to match donor and recipient on the basis of ABO blood group compatibility and HLA matching (189,190).

Although there is a slight influence of donor age on renal function in transplant recipients (191,192), acceptable donors are between age 3 to 65 years old and, in some centers, even younger and older donors are being considered. There should be no evidence of primary renal disease and no generalized viral or bacterial infection. A major consideration is the risk of transmitting infection with the allograft to an immunosuppressed recipient (193). Because of the possibilities of HIV transmission, HIV screening should be performed (194). All donors who are confirmed positive for HIV antibody should be excluded from donation. Those donors at high risk for HIV infection generally should not be accepted for donation because there is a period of seronegativity in early HIV infection before antibodies appear. HIV antigen testing should be performed in such donors.

In an effort to improve utilization of cadaveric organs, UNOS has defined and established guidelines for the use of organs that have traditionally resulted in excellent short-term function but diminished long-term function ("extended criteria donors, ECD"). These kidneys meet ECD criteria if they arise from donors over the age of 60 or if the donor is between the ages of 50 to 59 with two or three additional criteria: (1) Cerebrovacular accident as a cause of death, (2) prior diagnosis of hypertension or diabetes, or (3) serum creatinine greater than 1.5 mg/dL. These donor kidneys provide improved outcomes when compared to dialysis for a significant portion of the dialysis population, particularly elderly patients and patients with diabetes who generally have poorer outcomes on dialysis.

Additional attempts to increase the organ donor pool have addressed the use of donors who have died by cardiopulmonary arrest rather than brain death, termed "deceased by cardiac death donors (DCD)."

Cadaver Donor Nephrectomy

Currently, most kidneys will be removed as part of a multiple-organ harvesting procedure in which not only the kidneys are removed, but also the liver and heart and, occasionally, the lungs and pancreas. This necessitates coordination and careful cooperation between the interested parties (195). With experience and care, a donor may provide all the above organs, all of which can be satisfactorily transplanted (196). There are two basic approaches to cadaver donor nephrectomy. In one, each kidney is removed individually with a patch of aorta via an anterior approach, while in the other, which is the more satisfactory technique, both kidneys are removed en bloc with the appropriate segment of aorta and vena cava. The dissection of the vessels and the kidneys can then be completed after hypothermic perfusion and storage. *In situ* perfusion may be performed in both cases before and during removal.

Renal Preservation

Effective preservation of the kidney is an integral part of a kidney transplantation program and has evolved on the basis of known principles of preservation because of a need for longer storage of kidneys (197–199). The ability to preserve kidneys provides time for tissue typing and cross-matching and the selection of the most appropriate recipients for a particular donor on the basis of matching, as well as the preparation of the patients selected, who often may need dialysis before transplantation, and, finally, the transport of the kidneys to a center where an appropriately matched recipient may be awaiting a transplant.

There are two methods of preservation: simple cold storage in ice after flushing with a hypothermic solution to give a renal core temperature of O°C and a more complicated approach of continuous perfusion of the kidney with an oxygenated colloid solution (197). In general, preservation methods do not affect cadaver renal allograft outcome (200). The simple cold storage approach is now the most commonly used, for this provides adequate preservation for at least 24 hours, and even up to 48 hours with newer approaches to preservation.

The kidneys are initially flushed free of blood with a cold solution via the aorta and renal artery while the kidney is *in situ*. Many different flushing solutions have been used (Collins, citrate, University of Wisconsin solution) and the search for the optimal solution continues (Table 98-8). Drugs, metabolites, and other agents have been used to enhance the effects of cold preservation. The aim of these maneuvers is to reduce the incidence of posttransplant acute tubular necrosis. The University of Wisconsin solution has revolutionized the preservation of livers and pancreas, but whether it represents an improved method of preservation for kidneys has not yet been clearly established (197).

In the absence of any warm ischemia, which is generally the case with a brain-dead donor on a ventilator, immediate function can be obtained in most kidneys with up to 24 hours of preservation and even after 48 hours of preservation in some patients. However, from 24 hours onward, most kidneys will have a significant period of delayed function ranging from 1 to several weeks and there will be a significant incidence of permanent nonfunction (201). It has been suggested that for short-term outcome, local use of kidneys with poor HLA matching is as good as shared use with good matches (202).

Since 18 to 36 hours is an adequate time for most units and also allows time for transport of kidneys within a region or country, there has been widespread adoption of the simple cold-storage technique for preservation.

The second approach of machine preservation is more complex and expensive with limited benefits. With this system, a cold perfusate, either plasma protein fraction (PPF) or albumin

TABLE 98-8

COMPOSITION OF FLUSHING SOLUTIONS

	Collins'	Citrate	UW
Sodium (mM)	10	78	30
Potassium (mM)	108	84	120
Magnesium (mM)	—	40	5
Sulfate (mM)	—	40	5
Bicarbonate (mM)	10	—	—
Phosphate (mM)	60	—	25
Citrate (mM)	—	54	—
Glucose (mM)	180	—	—
Mannitol (mM)	—	120	—
Lactobionate (mM)	—	—	100
Raffinose (mM)	—	—	30
Adenosine (mM)	—	—	5
Allopurinol (mM)	—	—	1
Glutathione (mM)	—	—	3
Insulin (units/L)	—	—	100
Dexamethasone (mg/L)	—	—	8
Hydroxyethyl starch (g/L)	—	—	50

(203), is used to perfuse the kidney at low pressures using either pulsatile or continuous perfusion, the perfusate being oxygenated within the circuit. Both the temperature and the pressure of the perfusate are monitored and the flow is generally kept at 1 to 3 mL per gram of kidney per minute. Progressively rising resistance with a fall in flow rates and rise in pressure indicates inadequate preservation—but this is rarely seen within 3 days (197). However, normal perfusion characteristics are no guarantee of organ viability and function.

Prevention of Acute Tubular Necrosis (ATN)

Despite the advances made, many centers continue to report up to a 50% incidence of acute renal failure in the first few weeks posttransplant when cadaver kidneys are used. The problem is more apparent with the use of calcineurin inhibitors (CsA or tacrolimus). The implication of this acute renal failure may not be limited to simple delay of allograft function, but may affect eventual levels of function and probability of long-term graft survival (Fig. 98-10). A varying proportion of grafts never achieve, at any time, satisfactory function after transplantation and are removed ("primary nonfunction"). Rejection is, of course, more difficult to diagnose in such circumstances than in a functioning kidney. Therefore, the benefits of immediate function after transplantation, in terms of early recognition of rejection, is obvious. New approaches might be expected with better understanding of renal metabolism and its modification by hypothermia and other manipulations. Agents have been used to provide metabolic inhibition or substrate utilization for cellular energy requirements. Pretreatment with purine nucleotide precursors or addition of these precursors to the perfusate have given variable results (199). Recently, there is abundant data that intracellular calcium accumulation plays a significant role in organ dysfunction following ischemic injury (204). The demonstration of cellular and organ protection in several models of ischemic acute renal failure using calcium channel blockers led to the introduction of verapamil or other calcium-channel blockers as a component of preservation fluid.

THE TRANSPLANT OPERATION

The surgical technique of renal transplantation is standardized (205). In cadaver transplantation, the kidney must first be inspected to ensure that it is suitable for transplantation before undertaking the operation. This procedure should be carried out in operating room on a sterile back table. This procedure is to remove the unnecessary fatty tissue and to prepare the donor vessels. In small pediatric donors, both kidneys can be used en bloc for transplantation in adults.

The transplanted kidney is implanted in the retroperitoneal space in either the right or left iliac fossa through an oblique incision extending from suprapubic area to a point just above and medial to the anterior superior iliac crest. For transplantation, after failed transplants in both iliac fossae, a lower midline intraperitoneal approach should be used.

The iliac vessels should be carefully dissected (206) and the lymphatics ligated to prevent lymphocele formation (207). The donor renal vein is anastomosed end-to-side to the external iliac vein. The renal artery is anastomosed to the external or common iliac artery end to side using a cuff of aorta as a patch for the anastomosis, or it is anastomosed end to end to the internal iliac artery, which has been previously ligated and divided. The end-to-side anastomosis using a cuff of aorta, is the simpler anastomosis; it is the most appropriate one to use in cadaver transplantation when the renal artery is provided with a cuff of aorta. The end-to-end anastomosis to the internal iliac artery is technically more demanding and should only be used in living donor kidney transplantation.

Implantation of the ureter in the bladder is performed with one of two ways (208). The first is to anastomose the spatulated end of the ureter mucosa to dome of the bladder drawing muscle over the anastomosis to provide a tunnel (209). The second technique is to bring the ureter through the lateral wall of the bladder and down through a 2- to 3-cm submucosal tunnel and out in the vicinity of the patients own ureteric orifices at the trigone, where it is anastomosed mucosa to mucosa. The success of the first technique is greater than the second. Preventive antibiotics with appropriate broad-spectrum

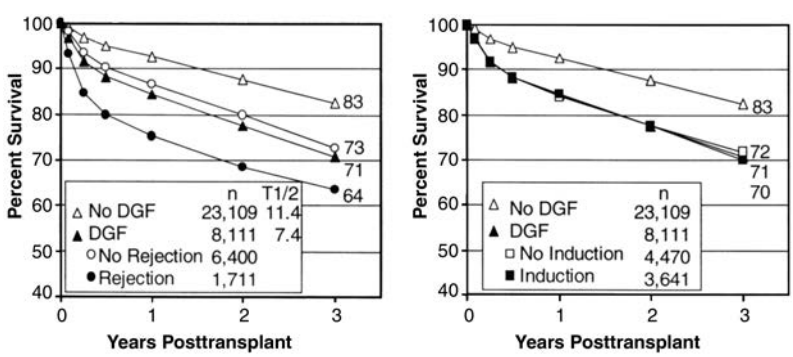

FIGURE 98-10. Effects of delayed graft function (DGF), early rejections, and induction immunosuppression. Data are from the United Network for Organ Sharing Scientific Renal Transplant Registry, 1999. (From: Cecka J, Terasaki P, eds. *Clinical Transplants 1999*. Los Angeles, CA: UCLA Immunogenetics Center; 2000:14, with permission.)

activity should be given with the premedication, in particular to protect against the possibility of infection being transmitted with the transplanted kidney.

General Postoperative Management and Follow-up

Routine postoperative observations should include monitoring of vital signs, fluid intake, and urine output. Postoperative hematuria is usually transient. The Foley catheter is generally left in these patients for 3 to 4 days because of high urine outflow rates that occur during this time in order to prevent overdistension of the bladder. This is particularly important in diabetic patients who frequently have neurogenic bladders and can have extremely larger bladder volumes before they develop an urge to micturate. Catheters should also be carefully monitored for obstruction and irrigated under sterile conditions if occluded by a clot.

Immediate function of the transplanted kidney makes postoperative management of the patient in the first few days much simpler than if the kidney is not functioning. The patient, particularly in the case of a living related transplant, may have a massive diuresis in the first 48 hours, and, for this reason, hourly monitoring of the urine output and a central venous line are essential to balance the fluid requirements appropriately. A very basic regimen, at least for the first few hours, is to replace fluid at the rate the last hour's output plus 50 mL per hour of IV fluid. This can then be modified according to the kidney function and the central venous pressure.

Within 48 hours, particularly with a functioning kidney, the patient's restored sense of well-being is quite remarkable and most patients can get out of bed on the second postoperative day. Provided that no complications ensue and that any early rejection episode can be dealt with satisfactorily with appropriate treatment, these patients are ready to leave the hospital by the fifth or sixth postoperative day.

After discharge from hospital, daily follow-up should be continued in the first week and the patient should then be seen two to three times a week in the first month after operation. Thereafter, the follow-up interval will depend on the patient's general condition and the development of additional problems. Routine biochemistry, hematology, and urine analysis should be obtained at each clinic visit (210).

IMMUNOSUPPRESSIVE THERAPY

Immunosuppressive options in kidney transplantation are rapidly expanding. Experience with newer immunosuppressive molecules is traditionally first obtained in kidney transplantation. Standard immunosuppressive therapy in renal transplant recipient consists of: (1) baseline therapy to prevent rejection and (2) short courses of antirejection therapy utilizing high-dose methylprednisolone, monoclonal antibodies or polyclonal antisera, such antilymphocyte globulin (ALG) and antithymocyte globulin (ATG). Most induction regimens incorporate elements of the maintenance immunosuppressive regimen. The basic approach involves the use of multiple drugs, each directed at a discrete site in the T cell activities cascade and each with distinct side effects. The immunosuppressants can be classified, on the basis of their primary site of action, as inhibitors of transcription (CsA, tacrolimus), inhibitors of nucleotide synthesis (azathioprine and mycophenolate, such as Cellcept or Myfortic), and inhibitors of growth factor signal transduction (sirolimus or everolimus). Current standard practice for chronic immunosuppression includes a calcineurin inhibitor, an antiproliferative agent, and steroids. The calcineurin inhibitors are either CsA or tacrolimus; the antiproliferative agent, typically a mycophenolate or sirolimus; and low-dose prednisone are used as basic immunosuppressive drugs for the induction as well as maintenance immunosuppressive therapy. Prednisone is started with a 20 to 30 mg daily dose for the first month and tapered by 2.5 to 5 mg every 2 weeks to a maintenance dose of 5 to 10 mg/day. Most centers do not routinely discontinue or switch to alternate-day steroids unless the patient is having problems with side effects (including worsening glucose control, hypercholesterolemia, or difficulties in blood pressure control) and has had stable renal allograft function with no episodes of acute rejection within the preceding 6 to 12 months. However, steroid-withdrawal strategies continue to be of significant interest both by transplant centers as well as potential transplant recipients. Single-center experiences with steroid withdrawal after antibody induction therapy have been increasingly successful, with multicenter trials in progress.

Polyclonal or monoclonal antibodies directed at cell surface proteins are also being used in the clinical setting as induction therapy and/or antirejection treatment. In the absence of delayed graft function, CsA or tacrolimus can be started as daily maintenance dose. The use of these immunosuppressive agents both to prevent and to treat rejection is associated with a number of side effects. The common medical problems in patients who have had transplantation are related to the use of these immunosuppressive agents. Overall, cumulative high-dose immunosuppression leads to increased infectious morbidity and mortality. The ideal of therapies in transplantation therefore, is to reduce the agent's dose to a level of immunosuppression that will prevent or suppress rejection, but minimize the risk of life-threatening infections and other problems related to the treatment (211,212).

Antibody Induction

Many centers use a prophylactic course of antilymphocyte agents for induction in the immediate posttransplant period. This strategy establishes an immunosuppressive cover that enables early engraftment without the use of potential nephrotoxic drugs, like CsA and tacrolimus, during the early posttransplant period. These antibody protocols have reduced the incidence of early rejection and are particularly useful for high-risk patients such as presensitized patients with high PRAs or patients undergoing retransplantation. Most clinical experience has been with the use of the pan-T–monoclonal antibody, OKT-3, or a polyclonal antilymphocyte antibody such as ATGAM (Pharmacia-UpJohn, Kalamazoo, MI) at 15 mg/kg/day or thymoglobulin (Genzyme, Cambridge, MA) at 1.5 mg/kg/day (or alternate day) for 4 to 10 days (213–216). The need for antibody induction is still controversial. However, a recent meta-analysis of seven randomized controlled trials of antibody induction therapy suggested a modest improvement in graft survival in those patients treated with antilymphocyte agent (217,218). In one recent study evaluating different induction therapies, acute rejection occurred in only 4% of those receiving thymoglobulin compared to 25% with ATGAM (215,216). Prophylactic OKT3 appears to be most effective in high-risk cadaver transplant recipients, including those who are sensitized or have two HLA-DR mismatches or a prolonged cold ischemia time (213,214). A report from The Collaborative Transplant Study clearly showed that sequential induction therapy with OKT3 followed by cyclosporine resulted in better 3-year graft survival rate in first and subsequent transplants (219). These benefits were not seen with simultaneous OKT3 and cyclosporine administration. Furthermore, the benefit of sequential therapy was greatest in high-risk patients with preformed panel reactive lymphocytotoxic antibodies above 50% and in Black and pediatric transplant recipients. In the highly

presensitized patients, for example, 3-year graft survival rates were higher in both first transplants (80% versus 63% in those not treated with OKT3) and in retransplants (73% versus 58%).

Anti-IL-2 receptor (CD25) monoclonal antibodies have been used for induction and can also reduce the incidence of early rejection episodes. Randomized prospective studies of two humanized antibodies against the α chain of the IL-2 receptor have recently shown a significant decrease in the incidence of acute rejection with few or no adverse events accompanying their use. In the first study, basiliximab (Simulect) was used in a double-blind trial, 376 patients undergoing a primary cadaveric renal transplant and receiving cyclosporine and corticosteroids were randomized to placebo or 20 mg of basiliximab administered on days 0 and 4 posttransplant (220). There was no evidence of a cytokine release syndrome with basiliximab and the incidence of infection and other adverse events was similar in the two groups. The benefits of this induction therapy include a lower incidence of biopsy-confirmed acute rejection at 6 months (30% versus 40%); a lower incidence of steroid-resistant first rejection episodes that required antibody therapy (10% versus 23%), and a lower mean daily dose of steroids at 2 and 4 weeks posttransplantation. Daclizumab (Zenepax) is another humanized anti-IL-2 receptor monoclonal antibody (221). As with basiliximab, daclizumab decreases the incidence of acute rejection. In one study of 153 renal transplant recipients receiving cyclosporine, azathioprine, and prednisone, the administration of daclizumab lowered the incidence of rejection (22% versus 35% with placebo). Both basiliximab and daclizumab have been approved by the FDA for use in renal transplantation. No trials have yet directly compared the efficacy of these two agents, but they differ in cost and frequency of administration. A total of five doses are recommended for daclizumab with each dose separated by 2-week intervals; only two doses are recommended for basiliximab with each separated by a 4-day interval. The lower incidence of acute rejection and the apparent safety and lack of side-effects associated with these antibodies suggest that these agents may eventually assume a significant role in induction immunosuppressive therapy in renal transplant recipients.

Treatment of Rejection

In the treatment of acute rejection, the most practical approach is to initially start a course of intravenous methylprednisolone and if there is not an early regression of the symptoms associated with the rejection, perform a needle biopsy of the kidney to establish the diagnosis and consider giving OKT3 or polyclonal anti-T cell antibodies. The use of these potent therapies should be confined to acute rejections with acute components that are potentially reversible, e.g., mononuclear interstitial cell infiltrate with tubulitis (see Fig. 98-16) or endovasculitis with acute inflammatory endothelial infiltrate (see Fig. 98-17).

Immunosuppressive Agents

Corticosteroids

Corticosteroids have been known for more than 40 years to have a suppressive effect on the immune system. Their first use in renal transplantation was in 1960, when cortisone was used to reverse a rejection episode in a living related donor transplant recipient who had been immunosuppressed by total-body irradiation. Since then steroids have been used for treatment of rejection episodes and as part of the standard immunosuppressive regimen for the prevention of rejection. The complications of steroid therapy are numerous and involve many organ systems. Acute side effects include fluid and salt retention, which may exacerbate hypertension, steroid-induced diabetes, which may result from impaired glucose tolerance, or preexisting diabetes, and, rarely, central nervous system (CNS) changes, such as steroid psychosis or pseudotumor cerebri. These changes occur when high doses of prednisone or methylprednisolonene are given during the initial posttransplant period or in the treatment of a rejection episode. Generally, these short-term effects lessen or disappear when the doses of steroids are tapered. The long-term side effects are more insidious in onset and are associated with Cushingnoid changes, poor wound healing, and increased frequency of infections. Other side effects include: cataracts, proximal myopathy, osteoporosis, and osteonecrosis.

In an effort to reduce the incidence of metabolic and infectious complications, the current trend is to use lower doses of steroids for maintenance (222–225) and IV pulses of methylprednisolone for treatment of rejection (226). Because of the growth-suppressive effects of corticosteroids, alternate-day steroids therapy was introduced for transplantation in children (227,228). However, this regimen has not been evaluated in randomized controlled trials in adults and concerns have been expressed that conversion to alternate-day therapy has been associated with an increase in incidence of rejection (229).

Steroid Withdrawal. Although steroid withdrawal after renal transplantation has definite advantages with reduction in weight, hypertension, and hypercholesterolemia and improved appearance, there is concern that withdrawal of steroids from cyclosporine-based protocols will be associated with a deterioration in renal function (229,230). However, several institutes have reported successful withdrawal of steroids in kidney (231–235), pancreas (236), as well as in liver transplantation (237–239). Data from uncontrolled studies of stable patients who have had no rejection episodes for at least 6 months have noted a successful outcome in 80% to 90% of cases maintained on cyclosporine and azathioprine. Cessation of steroid therapy is often associated with a fall in blood pressure and, in diabetic patients, better glycemic control; total cholesterol levels also fall, but there is an equivalent reduction in HDL cholesterol and, therefore, an uncertain effect on cardiovascular risk. Prevention of osteopenia and aseptic necrosis of bone are other potential benefits; there are, however, limited data at present to confirm this benefit. These beneficial metabolic effects must be balanced against the effect of steroid withdrawal on graft outcome. The Collaborative Transplant Study registry data suggests that graft survival is superior in those patients in whom steroids are withdrawn. A similar analysis from the Australian and New Zealand Dialysis and Transplantation Registry also found the patients with a functioning graft at 1 year who were not on steroids had a better patient and graft survival thereafter compared to patients who remained on steroids (240). However, these uncontrolled data have a possible defect in that patients may have remained on steroids because they had a more difficult early course, which might be associated with a poorer long-term graft survival course. Furthermore, a meta-analysis of prospective trials of steroid withdrawal in the first 3 months after transplantation suggested an increased incidence of acute rejection after withdrawal. Thus, early (within 3 months) cessation of steroids is associated with an increased incidence of acute rejection and a possible decrease in long-term graft survival. The effect of late withdrawal of steroids upon graft function was examined in a randomized prospective controlled trial of patients on triple immunosuppressive therapy. One hundred patients in whom renal transplantation had been performed 1 to 6 years prior to study entry were randomized to total prednisone withdrawal over 4 months or to continuation of triple therapy. Among the 49 patients randomized to

steroid withdrawal, 42 (86%) were able to achieve complete cessation. Withdrawal of steroids was associated with decreases in blood pressure (although not completely sustained) and in total cholesterol levels (a decrease on average of 38 mg/dL). Although no defined episodes of acute reaction were observed among these patients, a significant decrease in renal function and a significant increase in the percentage of patients with proteinuria were found compared to control patients. At 1 year, a higher number of steroid withdrawal patients had a plasma creatinine concentration that was more than 25% above baseline than control patients (53% versus 25%). Further increases were observed in the steroid withdrawal patients at 2 and 3 years. After correction for differences in baseline serum creatinine values, the steroid withdrawal patients had higher serum creatinine values at study exit and at 1 year than controls. This study confirmed the benefits of steroids of steroid withdrawal, namely, an improvement in weight, hypertension, and hypercholesterolemia. However, there was an associated 10% decline in renal function, which appears to be stable at 3 years. Furthermore, the study was able to define a group of patients in whom steroids could be withdrawn without risk (241). Thus, an element of caution is required before recommending routine use of steroid withdrawal. However, the introduction of the newer immunosuppressive drugs, such as tacrolimus, mycophenolate mofetil, and sirolimus or everolimus may allow steroids to be withdrawn at an earlier stage after transplantation.

Azathioprine

Despite newer immunosuppressive regimens, azathioprine will continue to be used for patients with successful transplants who are already receiving azathioprine, for patients who are intolerant of other agents, and for some patients because of economic reasons. Azathioprine was first used as an immunosuppressive agent for kidney transplantation in 1962 (242), and shortly afterward steroids were added for the prevention of rejection. For many years azathioprine at a dose of 1.75 to 2.5 mg/kg/day was used with high-dose steroids, which were responsible for most of the significant complications accompanying renal transplantation. Then, in 1970, McGeown and colleagues reported excellent graft survival with azathioprine and low-dose steroids (243,244). Their patients were not typical in that nearly all had bilateral nephrectomies and all had had large number of blood transfusions. For this reason, the Oxford group undertook a prospective randomized controlled trial of high- versus low-dose steroids in combination with azathioprine and showed not only that graft survival was identical, but that the reduction in steroid-related complications was striking (222,223). These data were subsequently confirmed in other prospective trials and low-dose steroids became the norm in combination with azathioprine (245). Before the 1980s, azathioprine and steroids were the mainstays of maintenance immunosuppression prior to the introduction of CsA (225,246,247). The side effects of azathioprine include bone marrow depression with granulocytopenia, hepatic dysfunction, pancreatitis, and an increased risk of infection and neoplasia. Macrocytic anemia with megaloblastic erythrocytosis (248), pure red cell aplasia (249), thrombocytopenia, and suppression of all marrow cell lines have been reported. Trimethoprim—sulfamethoxazole, when administered with azathioprine, may lead to neutropenia and thrombocytopenia (250), possibly because of the antibiotic's antifolate effect, resulting in enhanced 6-MP marrow toxicity.

Cyclosporine (CsA)

Cyclosporine is a fungal peptide with potent antilymphocyte properties. Cyclosporine was introduced to renal transplantation by Calne (251) and his colleagues in 1978. The initial use of CsA in Europe was based on the early Cambridge experience of using CsA alone whereas in North America CsA was used with steroids. There were a number of studies evaluating cyclosporine protocols (252–255). The initial recommended dose of 14 to 18 mg/kg/day was used in most clinical trials. There is a trend of using lower initial dose for renal transplantation in the ranges of 10 to 14 mg/kg/day. This dosage was then adjusted to achieve specific plasma/serum or whole blood levels. Although the need for steroids in patients treated with CsA remains unsolved (252,256), a majority of patients are likely to need high-dose methylprednisolone for the treatment of rejection episodes. For this reason, most centers use steroids with CsA from the time of transplantation. However, many patients can be managed without steroids or weaned off steroids during the first few months after transplantation. The Canadian Multicenter Study showed that steroids could be withdrawn successfully at 3 months and the results were comparable with the control patients that were maintained on steroids (255). The Australian Multicenter Study (257) achieved 83% 1-year graft survival using CsA alone, and over 60% of patients remained off maintenance steroids, which were only used for treatment of rejections. Salaman et al. (258) in a small study found no benefit of adding steroids compared with CsA alone, but commented that nephrotoxic side effect was more frequent in patients without steroids. Other groups have successfully used a combination of azathioprine and CsA to avoid steroids (257,259,260).

Side Effects. A number of side effects have been observed in patients receiving CsA (251,261–264). Nephrotoxicity is the most worrying side effect of cyclosporine. Dose-related, reversible acute nephrotoxicity is observed in about 20% of patients (263,264). Nephrotoxic episodes tend to occur after 1 month. In addition to nephrotoxicity, other side effects include hypertrichosis, neurasthesiae, convulsions, hepatic dysfunction, gingival hypertrophy, hyperkalemia, hypomagnesemia, hypertension, and fluid retention. CsA infrequently causes a microangiopathic hemolytic anemia in association with hemolytic uremic syndrome. An autoimmune hemolytic anemia has also been reported in patients receiving CsA when ABO blood group O kidneys are engrafted into blood group A or B recipients. It is postulated that passenger B lymphocytes cause graft-versus-host disease by secreting hemolytic antibodies; an autoimmune neutropenia and thrombocytopenia have also been reported in this setting of ABO incompatibility (265–267).

Pharmacokinetics. Oral absorption of CsA is slow and incomplete with peak blood concentrations occurring 2 to 4 hours after a dose. On the average, 37% of an orally administered dose will be absorbed. It then undergoes some first-pass metabolism, yielding an absolute bioavailability of 27% in the blood. It is primarily eliminated from the body via hepatic metabolism. Cyclosporine is administered either as a single daily dose or as two 12-hourly doses. The starting oral dosage is 8 to 12 mg/kg/day. The dose is further tapered to a maintenance dose of 3 to 6 mg/kg/day, depending on the graft function and trough levels. The rationale for the twice-daily dose is based on the pharmacokinetics observed that trough levels are reached between 12 and 16 hours after a single oral dose and on the assumption that nephrotoxicity might be related to a peak levels rather than trough levels (268,269).

Intravenous CsA, which may aggravate postoperative acute tubular necrosis, is to be avoided in the perioperative period. Patients with ileus or other gastrointestinal dysfunction, however, may require intravenous coverage of CsA. The IV dosage for CsA is 2 mg/kg or 1/3 of the oral dose and should be given in a slow infusion with 0.9% sodium chloride or 5% dextrose over 2 to 6 hours (270–272).

TABLE 98-9

CYCLOSPORINE OR TACROLIMUS DRUG INTERACTIONS

Increase level	Decrease level	Additive nephrotoxicity
Erythromycin	Barbiturate	Aminoglycosides
Diltiazem	Carbamazepine	Amphotericin B
Ketozonazole	Isoniazid	Cotrimoxazole
Metoclopramide	Phenytoin	Trimethoprim
Oral contraceptives	Rifampicin	Acyclovir
Nicardipine		

Drug Interactions. Cyclosporine is metabolized almost entirely in the liver, probably through the cytochrome P450 system. Most of the drug is excreted in the bile and liver dysfunction causes it to accumulate and serum levels to rise. Thus drugs, which induce hepatic enzymes, such as rifampicin, phenytoin, phenobarbitone, neflocin, and nefcillin will increase the rate of metabolism of cyclosporine and lower blood levels of the parent compound. Drugs that increase cyclosporine levels include: calcium entry blockers, such as diltiazem, verapamil and nicardipine, erythromycin, and ketoconazole. The well-known interactions are listed in Table 98-9. Impaired renal function does not affect plasma or whole blood levels, because only about 0.1% of the native drug is detected in the urine and only 10% of the metabolites of the parent compound are excreted in the urine. A number of drugs are now known to enhance nephrotoxicity of CsA. These are aminoglycosides, ketoconazole, amphotericin B, trimethoprim, and cotrimoxazole (273–275).

Monitoring of CsA Levels. Therapeutic monitoring CsA levels is an important part of the management because of the variation in the inter- and intrapatient metabolism. When Sandimmune was introduced, the trough level of cyclosporine, rather than the peak level, was measured because its timing was more consistent and appeared to correlate better with toxic complications. With the introduction of Neoral, this trend has continued. However, because of Neoral's consistent absorption, its peak levels (typically 2 hours after dosing) may correlate better with drug exposure and clinical events than trough levels (276–279).

Measurements to calculate the area-under-the-curve (AUC) will reflect the bioavailability of the drug and may allow a more precise and individualized patient management (280,281) (Table 98-10). Plasma or whole blood levels of the drug can be measured either by radioimmunoassay (RIA) using polyclonal or monoclonal antibodies, which detects the parent drug and its metabolites, or high-pressure liquid chromatography (HPLC), which selectively measures parent compound. Recently, a number of nonisotopic immunoassays have also been commercially developed. One of the most successful methods is the Abbot TDX System which uses fluorescence polarization immunoassay (FPIA) to monitor the reaction between the drug and the antibody. The results are comparable to RIA (Table 98-11). Values obtained by RIA are approximately 1.3 times higher than those obtained by HPLC. Forty to 60% of the drug concentration in whole blood is bound to red cells, lymphocytes, and granulocytes. Because drug binding to red cells is temperature-dependent, plasma must be separated from the red cells at 37°C to obtain reproducible results. There is considerable disagreement as to whether plasma or blood levels correlate with either CsA toxicity or immunosuppression. Nevertheless, most physicians would feel that CsA trough levels, along with all clinical, biochemical, and histological parameters, are helpful in distinction between nephrotoxicity and rejection (280–283).

TABLE 98-10

METHODS OF DETERMINING CYCLOSPORINE LEVELS

Assay	Specific for parent compound	Recommended trough levels (ng/mL)
RIA (monoclonal)	+	Serum 50–125 Blood 100–400
HPLC	+	Blood 100–400
RIA (polyclonal)	–	Serum 150–300 Blood 200–800
FPIA (TDX)	–	Blood 200–350

Cyclosporine Nephrotoxicity. Nephrotoxicity is the most frequently encountered and most important side effect of CsA therapy. CsA nephrotoxicity can occur acutely within days or weeks after renal transplantation. The pathogenesis of acute nephrotoxicity is due to a decline in renal blood flow with a consequent fall in glomerular filtration rate (GFR).

Acute CsA nephrotoxicity. Acute cyclosporine toxicity tends to occur about 3 or 4 weeks after transplantation, rather than within the first 2 weeks when the typical rejection episode might occur and is not associated with swelling or tenderness of the graft or with fever. A small increment in serum creatinine occurs that is frequently correlated with high serum CsA trough levels. Serum creatinine returns to baseline within 24 hours of reduction of CsA dosage (263,264).

Drugs which cause additive nephrotoxicity. Along with CsA-induced nephrotoxicity, other drugs may cause renal dysfunction including antibiotics, diuretics, and H2-receptor blockers. Trimethoprim–sulfamethoxazole, commonly used for infection prophylaxis, can cause an interstitial nephritis, or the trimethoprim moiety may interfere with tubule secretion of creatinine, leading to an elevation in the serum creatinine in the absence of a fall in GFR. Other antibiotics (aminoglycosides, amphotericin B) may be directly nephrotoxic. Diuretics may cause volume depletion or interstitial nephritis and should be avoided, if possible, in the early posttransplantation period. Cimetidine, an H2-receptor antagonist, used for peptic ulcer disease prophylaxis, may compete with creatinine for tubule secretion and also cause a "false" increase in the serum creatinine; this was not seen with ranitidine. Concomitant administration of CsA and sirolimus increased absorption of CsA. Lower doses of CsA are required to maintain a therapeutic trough level (284–286).

Chronic CsA nephrotoxicity. The debate about long-term nephrotoxicity of CsA remains unresolved. The pathogenesis of chronic CsA nephrotoxicity is currently unclear. Dose reduction does not reverse this form of chronic toxicity. There is evidence that renal prostaglandins and thromboxane production is stimulated in chronic CsA nephrotoxicity. Substitution of an oil base fluid with a fish oil leads to generation of vasodilatory arachidonate metabolites in the kidney. It has been suggested that CsA induces an arteriolar lesion in the kidney by reducing prostacyclin production secondary to decreased synthesis of prostacyclin-stimulating factor. The prostacyclin-stimulating factor may be a cytokine, produced by helper T cells, or interleukin 1, produced by macrophages. This may explain both immunosuppressive and nephrotoxic effects of

TABLE 98-11

PATIENT AND GRAFT SURVIVAL AND THE INCIDENCE OF ACUTE REJECTION IN TWO LARGE RANDOMIZED PROSPECTIVE MULTICENTER TRIALS OF TACROLIMUS AND CYCLOSPORINE IN EUROPE AND THE UNITED STATES

	Tacrolimus (%)	Cyclosporine (%)	*p*
European trial (*n* = 448)			
1-year patient survival	93.0	96.5	NS[a]
1-year graft survival	82.5	86.2	NS
Cumulative acute rejection	34.2	57.2	<0.001
Steroid-resistant rejection	11.3	21.6	0.001
U.S. trial (*n* = 412)			
1-year patient survival	95.6	96.6	NS
1-year graft survival	91.2	87.9	NS
Cumulative acute rejection	30.7	46.4	<0.001
Steroid-resistant rejection	10.7	25.1	<0.001

[a] NS, not significant.

CsA. Increased incidence of glomerular thrombosis in CsA treated renal and other organ transplant patients tend to support this hypothesis.

The stability of renal allograft function associated with long-term CsA therapy has been documented (259–261). In some cases, the deteriorating long-term renal function might be due to inadequate immunosuppression and chronic rejection rather then CsA nephrotoxicity. However, conversion from cyclosporine to azathioprine and prednisone is performed for documented nephrotoxicity or other worrying side effect of CsA. Several protocols have been proposed in the past (259,266,267). To avoid acute rejection after conversion, it was recommended that an overlap between discontinuation of CsA and the commencement of azathioprine and prednisone.

Differential diagnosis of CsA nephrotoxicity. In the early post-transplant period, functional CsA nephrotoxicity has clinical features similar to those of acute renal allograft rejection. In the CsA-treated patients, rejection may present with or without decline in urine output along with a rise in serum creatinine. Because CsA nephrotoxicity may present in a similar fashion, an allograft biopsy specimen should be obtained if the diagnosis is uncertain. Histologically, acute CsA nephrotoxicity can be distinguished from acute rejection chiefly by the absence of an extensive inflammatory infiltrate (287).

However, direct tubular toxicity has been described when CsA levels are elevated above 1,000 ng/mL in whole blood. Whereas morphologic evidence of tubular injury was commonly seen in transplant biopsies obtained in the early years with CsA therapy, the incidence of this form of nephrotoxicity has fallen. The glomeruli may be small because of chronic ischemia; they may have wrinkled or thickened basement membranes or increased mesangial matrix; or they may be completely hyalinized or sclerosed. The vascular changes may include intimal proliferation with luminal obliteration most striking in the interlobular and arcuate arteries and medial necrosis with degenerative changes of the internal elastic lamina. Tubular atrophy with luminal obliteration and interstitial fibrosis complete the picture (288,289). Needle-biopsy evaluation of class II MHC antigen expression has also been used for the differential diagnosis of CsA nephrotoxicity from kidney graft rejection (212). In the absence of the specimen, a clinical picture suggestive of CsA toxicity includes hypertension, tremor, elevated CsA trough levels, hyperkalemia, and a temporal relationship of improvement in allograft function after CsA dose reduction.

Neoral. The new microemulsion formulation of cyclosporine A is Neoral. The absorption of CsA from its classic formulation is slow and incomplete, and varies from day to day. The new microemulsion of CsA (Neoral) may overcome these shortcomings (274). Absorption is more rapid, complete, and consistent, and relatively independent of blood and bile flow. Maximum concentration is increased by 30% to 60%; mean time to reach maximum concentration is only 1.4 hours; and absorption, as measured by the area-under-the-time concentration curve, is 20% to 30% greater than with the classic CsA formulation. In addition, day-to-day variability is reduced by at least 50% for each of these parameters (282). Neoral has been approved by the Food and Drug Administration (FDA) in the United States. Initial trials have confirmed that Neoral is equivalent to cyclosporine A for prevention of graft rejection and no differences have been shown in graft or patient survival. The dose of the microemulsion is 10% to 20% lower than standard cyclosporine; however, the exact conversion dose must be carefully undertaken.

Generic Formulations of Cyclosporine. A number of generic formulations of cyclosporine have been developed. These include the capsule Gengraf (Abbots Labs), the capsule Cyclosporine USP (Eon Labs) and the liquid SangCya have been approved for use in the United States. Other generic formulations are also available outside the United States. SangCya (Sang-35) is an oral liquid formulation of cyclosporine that has pharmacokinetic properties similar to Neoral. As with Neoral, it does not appear to be dependent upon bile salts for absorption. Because of its bioequivalence, the impact of these generic formulation remains to be seen. Overall these generic formulations are about 20% less expensive than Neoral. No adverse events have been reported regarding their use (290–296).

Tacrolimus (FK506)

FK506 (tacrolimus or prograf) is a macrolide immunosuppressant and is similar to CsA in its mode of action (297,298). Both tacrolimus and CsA prevent the transcription of several T cell growth-promoting genes such as IL-2. While CsA binds to cyclophilin, tacrolimus binds to an immunophilin, FKBP (FK506 binding protein). The drug's immunosuppressive action is mediated via blockade of calcineurin-mediated T cell

receptor signal transduction and inhibition of IL-2. By inhibiting cytokine gene transcription, tacrolimus suppresses T cell and T cell-dependent B cell activation (299).

The initial experience with tacrolimus was mostly in liver transplantation since the drug was originally approved by FDA for use in liver transplantation. The drug has now been used in kidney transplantation (300–303). Although recent trials have shown similar graft survival outcomes with tacrolimus when compared to Sandimmune–CsA-based regimens in the setting of renal transplantation, the incidence of acute rejection and requirement for intensive immunosuppression may be less with tacrolimus. The multicenter FK506 Kidney Transplant Study Group trial (304) randomized 412 cadaveric renal transplant recipients to either tacrolimus or Sandimmune–CsA-based immunosuppressive regimens. Sandimmune- and tacrolimus-based therapy resulted in similar graft survival rates at 1 year. However, tacrolimus was associated with a significantly decreased incidence of biopsy proved acute rejection episodes (30.7% versus 46.4%) and requirement for antilymphocyte antibody therapy (10.7% versus 25.1%). The European Tacrolimus Multicenter Renal Study Group reported similar beneficial effect with tacrolimus in a 12-month follow-up (305) (Table 98-11). A meta-analysis of four studies involving over 1,000 patients also found that tacrolimus, compared with CsA, significantly reduced the incidence of acute rejection (306).

Tacrolimus may also be beneficial in renal transplantation as rescue therapy in patients with recurrent or resistant rejection episodes on CsA (307,308). Patients with biopsy-proved ongoing acute renal allograft rejection were switched from CsA to tacrolimus. The overall response rate was 74%. These beneficial results were confirmed in another trial which examined the use of tacrolimus among 73 patients in whom acute renal allograft rejection was refractory to therapy with corticosteroids (100%) and antilymphocyte antibodies (81%). Graft survival rate was 75% at 12 months after tacrolimus administration.

Tacrolimus has a toxicity profile slightly different from that of CsA. Both short- and long-term studies in liver and renal transplant recipients suggest that tacrolimus is at least as nephrotoxic as CsA (285,309). Tacrolimus can also cause hyperkalemia, hyperuricemia, and, rarely, the hemolytic–uremic syndrome (310–313). However, there are differences in toxicity with tacrolimus as compared to CsA. These include more prominent neurologic (314) and metabolic side effects: less frequent hirsutism, gingival hyperplasia, and hypertension, more frequent alopecia, and an increased proclivity for susceptibility to polyoma virus infection (315). Hypertrophic cardiomyopathy has also been reported in children treated with tacrolimus (316). The multicenter FK506 renal transplant study trial (304–306) found an important difference in the toxicity profile between the two drugs: a relatively high incidence of insulin-dependent diabetes mellitus in tacrolimus-treated patients as compared to cyclosporine-treated individuals (19.9% versus 4%, respectively).

The recommended starting dose for tacrolimus is 0.15 to 0.3 mg/kg/day, administered in a split dose each 12 hr. Doses are adjusted to maintain tacrolimus drug levels at between 8 to 16 ng/dL during the first few transplantation months and between 5 to 12 ng/dL thereafter. African-American patients were found to need a mean 37% mean higher dose than Caucasian patients to achieve comparable blood concentrations (317). Tacrolimus is absorbed primarily by small intestine and its oral bioavailability is about 25% with large inter- and intrapatient variability. Like CsA, it is primarily metabolized by hepatic cytochrome P450. Therefore, the drug level will be influenced by concomitant administration of medications that affect cytochrome P450. Two commercial kits are available for tacrolimus level determination. The microparticle immunoassay (Abbott Lab) is simpler and faster than enzyme-linked immunosorbent assay (ELISA) (318–322).

Mycophenolate Mofetil (MMF)

Mycophenolate mofetil (MMF) or Cellcept is a new immunosuppressive agent that has been recently approved by FDA for use in patients with kidney transplantation. The drug is converted *in vivo* to mycophenolic acid, a noncompetitive and reversible inhibitor of inosine monophosphate dehydrogenase (323) (Fig 98-11). This enzyme is responsible for the conversion of inosine monophosphate to guanosine monophosphate (GMP), which is required for the production of nucleic acids and other critical steps in cellular activation. Lymphocytes require the *de novo* synthesis of GMP, so that mycophenolic acid causes a profound inhibition of T and B cell function. Most other cells possess a salvage pathway that permits a resynthesis of guanine derivatives and are relatively resistant to mycophenolic acid.

Mycophenolate mofetil has been used in renal transplant recipients in dual, triple, or sequential therapy in combination with CsA. In each protocol, the incidence and severity of acute rejection and the requirement for antirejection therapy were decreased significantly in the patients receiving mycophenolate mofetil. Biopsy proved that rejection fell overall by 45% to 55%, the number of patients treated for rejection declined by 35% to 50%, and the use of OKT3 or ATG for refractory rejection fell by 40% to 66% compared with patients administered azathioprine (324,325). In the study comparing mycophenolate (2 or 3 g/day) to placebo in 491 recipients of a cadaveric renal transplant treated with cyclosporine and prednisone, at 6 months, the biopsy-proved incidence of acute rejection was 14% to 17% in the mycophenolate groups versus 46% with placebo. Placebo patients were also much more likely to require high doses of corticosteroids and antilymphocyte therapy to reverse the rejection episodes. Follow-up at 3 years revealed that the incidence of graft loss was significantly lower by 7.6% among those randomized to MMF. Another trial compared mycophenolate (2 to 3 g/day in divided doses) to azathioprine in 499 patients, who were also treated with CsA, prednisone, and antithymocyte globulin. Although 6- and 12-month patient and graft survival were similar in the two groups, there were fewer first rejection episodes (18% to 20% versus 38% with azathioprine). The other trial is a randomized clinical report by the Tricontinental Mycophenolate Mofetil Renal Transplantation Study Group (326,327). It also compared the effectiveness of MMF at two doses, 3 g/day (164 patients) and 2 g/day (173 patients), to azathioprine (100 to 150 mg/day, 166 patients). Patients were treated with equivalent doses of corticosteroids and CsA. Again, the MMF groups have a lower incidence of treatment failure at 6 months—35% and 38% versus 50% with azathioprine; a lower incidence of rejection—16% and 20% versus 36%; decreased use of antilymphocyte antibody for severe or steroid-resistant rejection episodes (4.9% and 8.8% versus 15.4%); and a nonsignificant trend toward improved graft survival at 1 year. At 3 years, both MMF groups continued to show a nonsignificant trend toward better graft survival and a lower rate of graft loss from rejection as compared to the azathioprine group (328,329). Despite the higher expense of mycophenolate, the lower incidence of rejection may make the use of this agent cost-effective. Mycophenolate has also been successfully utilized as the third agent in tacrolimus-based regimens (330). In one randomized study of 208 patients comparing tacrolimus and prednisone versus tacrolimus, prednisone, and mycophenolate, the incidence of rejection 1 year posttransplantation was lower among the group given mycophenolate (27% versus 36%).

The major side effects of MMF include mild neutropenia and gastrointestinal intolerance, such as diarrhea, esophagitis,

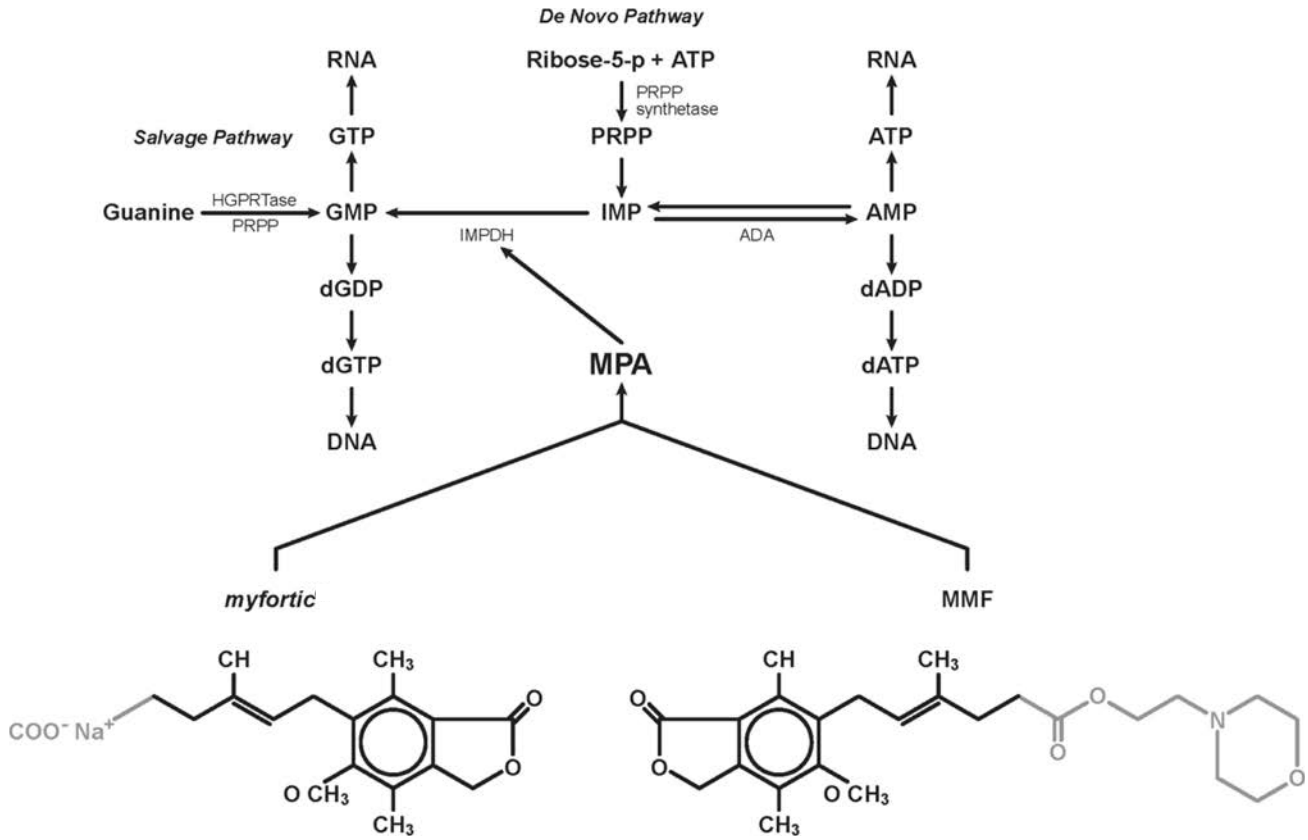

FIGURE 98-11. The site of inhibition by mycophenolic acid (MPA): MPA is the active moiety of MMF or Myfortic. It is a potent, selective, and reversible inhibitor of inosine monophosphate dehydrogenase (*IMPDH*). The *de novo* pathway for generation of guanosine monophosphate (*GMP*) is dependent upon the conversion of inosine monophosphate (IMP) by IMPDH.

and gastritis at high doses. The toxicity profile was greater with 3-g dosage compared to 2 g of MMF. The reason for leukopenia in transplant patients treated with MMF remains to be determined, since leukopenia was not predicted based on the mechanism of action of MMF and was not noted in patients with psoriasis or rheumatoid arthritis who were treated with this drug. The incidence of infection is not increased overall, although there may be a slight increase in tissue-invasive cytomegalovirus infection of the GI tract and liver (331,332). The use of MMF was associated with an increase in hepatitis C virus viremia (333).

A safe and effective dosage of MMF is 1 to 2 g per day. Therapeutic drug monitoring is still at a preliminary stage. HPLC methods have been established for the measurement of MMF, mycophenolic acid (MPA), and mycophenolic acid glucuronide (MPAG), the principal metabolite that is pharmacologically inactive. Orally administered MMF is rapidly absorbed and hydrolyzed to MPA in the liver and is then glucuronidated to an inactive form MPAG. Bioavailability of MMF is 90% with a half-life of 12 hr. There is no accumulation of MPA in hepatic or renal impairment (334–336). However, there are substantial intra- and interpatient variations in both adults and children. The maximum concentration of MPA and the AUC value determined immediately after transplantation were only 30% to 50% of those measured for patients 3 month after transplantation (337). In these patients, the free levels of MPA remained unchanged, suggesting that the increase in MPA levels was mainly due to increased levels of MPA bound to plasma proteins. Since the pharmacological effect of MMF is thought to be correlated with free and not bound MPA levels, a reduction in plasma protein levels may increase the free levels of MPA without affecting whole blood concentrations (326). There is evidence of pharmacologic interaction between MMF and tacrolimus. MPA levels increase when MMF is used with tacrolimus (338,339). Monitoring the pharmacodynamic activity of MMF, by monitoring the activity of the enzyme inosine monophosphate dyhydrogenase, which is inhibited by MPA, has also been suggested as a potential method to monitor the efficacy of MMF and adjust its dose (340). The variability in MPA levels suggests that measurements of plasma MPA levels could be beneficial. There appears to be a good correlation between the drug exposure and the probability of rejection (341,342).

Enteric-Coated Mycophenolate Sodium (Myfortic). *Myfortic* is an enteric-coated formulation of mycophenolate sodium that releases the active moiety mycophenolic acid MPA in the small intestine instead of stomach with the aim of improving MPA-related upper GI adverse events (343). It has been approved by FDA. Similar to Cellcept, it is an uncompetitive and reversible inhibitor of inosine monophosphate dehydrogenase and, therefore, inhibits the *de novo* pathway of guanosine nucleotide synthesis without incorporation to DNA. The recommended dose of Myfortic is 720 mg administered twice daily 1 hour before or 2 hours after food intake. Side effects are similar between Myfortic and mycophenolate mofetil in both *de novo* and maintenance patients (344–346). In the *de novo* study, despite the fact that the incidence of GI adverse events was comparable between the two treatment groups throughout the 12-month study period, the incidence of dose changes due to GI side effects was lower in the Myfortic group, indicating potentially less severe GI adverse events.

The "TOR Inhibitors:" Sirolimus and Everolimus

TOR (target of rapamycin) is a regulatory kinase in the process of cell division. The term "TOR inhibitor" refers to two similar immunosuppressant drugs whose mode of action is closely linked to the inhibition of this kinase. Sirolimus (Rapamune) also known as rapamycin, is a macrolide antibiotic compound that is structurally related to tacrolimus. Everolimus (Certican), also known as RAD, is a similar compound with shorter half-life (347–349).

Sirolimus formerly designated as rapamycin is a macrolide antibiotic produced by the fungus, *Streptomyces hygroscopicus*. It was first isolated from soil samples that had been collected on Easter Island (Rapa Nui) in 1969 (350,351). At that time, rapamycin was observed to arrest cell growth in *Candida* species and filamentous fungi. It was also shown to have tumoricidal activity, indicating that the antiproliferative effects seen in yeast could be extended to the mammalian system. Sirolimus activity in animal models of autoimmune disease was demonstrated in 1977 (352,353). The immunosuppressant activity of the drug was not documented in animal models until 1988 (354). Clinical trials were initiated 4 years later and it was not until 1999 that FDA approval was granted for the use of sirolimus for the prophylaxis of organ rejection in patients receiving renal transplants.

The immunosuppressive function of sirolimus ultimately results from the dampening of cytokine and growth-factor activity upon T, B, and nonimmune cells. Sirolimus binds to the immunophilins, a property shared with CsA and tacrolimus. While tacrolimus and CsA both inhibit calcineurin-dependent T cell receptor signal transduction, sirolimus inhibits a different pathway required for full T cell activation by blocking the phosphorylation of p70(s6) kinase and the eukaryotic initiation factor-4E-binding protein, PHAS-1. Like tacrolimus, sirolimus binds to FK-binding protein (FKBP). Therefore, sirolimus and tacrolimus compete for the same binding site on FKBP, but the resulting complexes, the functional unit for each, have different actions. The FKBP complex with tacrolimus block T cell activation and release of IL-2. The complex with sirolimus, on the other hand, blocks T cell proliferation in response to IL-2. Furthermore, the sirolimus–FKBP complex, unlike the tacrolimus–FKBP complex, does not bind calcineurin. The cellular receptors for the sirolimus–FKBP are being elucidated. Two proteins, designated as targets of rapamycin (TOR1 and TOR2) were identified as receptors for sirolimus. The antiproliferative activity of sirolimus appears to be a consequence in part of the sirolimus–FKBP complex blocking the activation of the protein kinases that are involved in cell proliferation. *In vitro*, sirolimus and tacrolimus compete for the same binding protein and are pharmacologically antagonists. However, both drugs interact *in vivo* to produce immunosuppression that is additive or synergistic (355–358).

Sirolimus is currently being developed for combination maintenance immunosuppressive therapy with cyclosporine. Results of a Phase I/II study suggest that combined therapy with CsA and sirolimus may permit the withdrawal of corticosteroids, in most patients, and reduce the incidence of acute rejection (359,360). In addition, another Phase II trial found that, among Caucasian recipients, a regimen consisting of rapamycin, corticosteroids, and CsA lowered the incidence of acute rejection and may be cyclosporine-sparing.

The major side effects of sirolimus are dyslipidemia and thrombocytopenia. Although the drug is minimally nephrotoxic when used alone, the combination of sirolimus and CsA has caused synergistic toxicity in animals (285,361). In healthy volunteers, concomitant administration of sirolimus and Neoral formulation of CsA increased the AUC for sirolimus by 230% compared with administration of sirolimus alone, while administration 4 hours after the CsA dose increased the AUC by 80%. For this reason, it is recommended that sirolimus be administered 4 hours after the morning CsA dose. The pharmacologic interaction between sirolimus and tacrolimus has not been rigorously explored. Careful surveillance for drug interactions with sirolimus will be required. Sirolimus-based immunosuppression has a different adverse effect profile among renal transplant recipients compared with a cyclosporine-based regimen. In this multicenter European trial, 83 patients were randomized to sirolimus or CsA, with all individuals receiving corticosteroids and azathioprine. At 12 months, no significant difference was observed in graft or patient survival, or the incidence of biopsy-confirmed acute rejection. However, compared with cyclosporine, sirolimus was associated with a higher incidence of hypertriglyceridemia (51% versus 12%), hypercholesterolemia (44% versus 14%), thrombocytopenia (37% versus 0%), and leukopenia (39% versus 14%) (362–365).

Sirolimus is given at a dose of 2 mg orally once daily 4 hours after the morning dose of either CsA or tacrolimus. Loading dose of 6 mg is given on the first day of treatment. African-American patients may benefit from a maintenance dose of 5 mg/day. The drug is rapidly absorbed, reaching peak concentrations in 1 to 2 hours. It has a long half-life, averaging 62 hours. The recommended target trough levels vary between 5 to 15 ng/dL. Blood levels of sirolimus can be determined by HPLC with UV light detection or HPLC–mass spectrometry. Drug levels of sirolimus exhibit considerable intra- and interpatient variability. Therapeutic drug monitoring by measuring trough blood levels could be expected to improve efficacy and reduce toxicity (366–368).

Overall, sirolimus is a new promising drug. Its potential role in slowing the progression of chronic transplant nephropathy has not yet been evaluated. In animal models, sirolimus has been shown to attenuate or prevent chronic rejection. In a preliminary study, the switch from calcineurin-dependent drugs to sirolimus in patients with chronic rejection lead to renal function improvement. However, these preliminary data need further randomized studies to clarify the role of sirolimus in chronic transplant nephropathy (369,370).

Polyclonal Antisera

Antilymphocyte globulin (ALG) or antithymocyte globulin (ATG) are polyclonal antisera derived from immunization of lymphocytes, lymphoblasts, or thymocytes into rabbits, goats, or horses have been used prophylactically as induction therapy during the early posttransplant period and for treatment of acute rejection (214,371–373).

The precise mechanism of action of the polyclonal antibodies is fully understood. The immunosuppressive product contains cytotoxic antibodies directed against a variety of T cell markers. After the administration, there is depletion of peripheral blood lymphocytes. The lymphocytes are either lysed or cleared by the reticuloendothelial system and their surface antigen may be masked by the antibody.

Until recently, ATGAM (Pharmacia–UpJohn, Kalamazoo, MI) was the only approved polyclonal agent available in the United States. ATGAM is a purified γ-globulin solution obtained by immunization of horses with human thymocytes. It contains antibodies to a wide variety of human T cell-surface antigens, including the major histocompatibility complex (MHC) antigens. Thymoglobulin (Genzyme, Cambridge, MA) is another polyclonal immunosuppressive agent derived from the rabbit (374,375). It recently gained approval for the treatment of rejection from the Federal Drug Administration. As with ATGAM, thymoglobulin contains antibodies to a wide variety of T cell antigens and MHC antigens. Both thymoglobulin and ATGAM reverse acute rejection, thereby reducing the risks of developing chronic allograft nephropathy. Data suggests that thymoglobulin may be superior to ATGAM for

the prevention and/or reversal of rejection. The ability of thymoglobulin (1.5 mg/kg/day) and ATGAM (15 mg/kg/day) to reverse rejection and prevent recurrent acute rejection was evaluated in a randomized study of 163 patients (376). Thymoglobulin was associated with a higher rejection reversal rate (88% versus 76%, $p = 0.027$) and a lower incidence of recurrent rejection 90 days after therapy (17% versus 36%). A third polyclonal preparation, the Minnesota antilymphocyte globulin (MALG), is no longer available. Its efficacy compared with other immunosuppressive agents has been extensively studied in multiple clinical trials (373,377,378).

Potential side effects of ALG include fever, chills, erythema, thrombocytopenia, local phlebitis, serum sickness, and anaphylaxis (379). The potential for development of host anti-ALG antibodies has not been a significant problem because of the use of less immunogenic preparations and probably because ALG suppresses the immune response to the foreign protein itself. To avoid allergic reactions, the patients receive intravenous medications consisting of methylprednisolone (30 mg) and diphenhydralammine hydrochloride (50 mg) 30 minutes before injection. Acetaminophen should be given before and 4 hours after commencement of infusion for fever control. Thrombocytopenia and leukopenia may necessitate reduction or curtailment of drug dosage.

OKT3 Monoclonal Antibodies

OKT3 is a mouse monoclonal antibody directed against the CD3 molecule of the T lymphocyte. OKT3 has been used either from the time of transplantation to prevent rejection or to treat an acute rejection episode (213,380–386). It has been shown in a randomized clinical trial (381) to reverse 95% of primary rejection episodes compared with 75% with high-dose steroids in patients who received azathioprine—prednisone immunosuppression. In patients receiving triple therapy (CsA—azathioprine—prednisone), 82% of primary rejection episodes were successfully reversed by OKT3 versus 63% with high-dose steroids. Like ALG, reduction of concomitant immunosuppression (discontinuation of CsA and reduction of azathioprine or Mofetil dose) decreases the incidence of infectious complications. Side effects include fever, rigors, diarrhea, myalgia, arthralgia, aseptic meningitis, dyspnea, and wheezing, but these rarely persist beyond the second day of therapy. Release of tumor necrosis factor (TNF), IL-2, and γ-interferon in serum are found after OKT3 injection. The acute pulmonary compromise due to a capillary-leak syndrome rarely has been seen because patients are brought to within 3% of dry weight before initiation of OKT3 treatment. Infectious complications, particularly infection with CMV, are increased after multiple courses of OKT3 (383–386). To avoid these potential complications during OKT3 treatment, a recommended protocol is listed in Table 98-12.

The development of host anti-OKT3 antibodies is a potential problem for the reuse of this drug in previously treated patients. About 33% to 100% of patients develop anti-mouse antibodies after the first exposure to OKT3, depending on concomitant immunosuppression (385). Anti-OKT3 titers of 1:10,000 or more usually correlate with lack of clinical response. If anti-OKT3 antibodies are of low titer, re-treatment with OKT3 is almost always successful. If re-treatment is attempted with antimouse titers of 1:100 or more, certain laboratory parameters, including the peripheral lymphocyte count, CD3 T cells, and trough-free circulating OKT3, should be monitored. If the absolute CD3 T-lymphocyte count is greater than 10/μL or free-circulating trough OKT3 level is not detected, it may be indicative of an inadequate dose of OKT3. The dose of OKT3 can be increased from 5 to 10 mg/day (384–386).

TABLE 98-12

RECOMMENDED PROTOCOL FOR OKT3 TREATMENT

I. Evaluation and treatment before administration
   A. Physical examination
   B. Laboratory tests including complete blood count
   C. Monitor intake and output, record weight changes
   D. Chest x-ray
   E. Hemodialysis or ultrafiltration for volume overload
   F. Premedication on day 0 and 1
      1. Methylprednisolone, 250–500 mg IV given 1 hr prior to dose
      2. Methylprednisolone or hydrocortisone sodium succinate, 250–500 mg IV given 30 min after the dose
   G. Diphenhydramine, 50 mg IV 30 min prior to dose daily
   H. Acetaminophen, 650 mg PO 30 min prior to dose
   I. Discontinue or decrease dose of CsA or tacrolimus
II. Administer OKT3 (Orthoclone), 5 mg/day IV, days 0–13
III. Monitor clinical course
   A. Check CD3 level on day 3
   B. Increase OKT3 dosage to 10 mg/day if either
      1. Anti-OKT3 antibody is high
      2. OKT3 level is low
      3. CD3 level is not low
IV. CMV chemoprophylaxis

Intravenous Immunoglobulin (IVIG)

Infusion of intravenous immunoglobulin (IVIG) may provide blocking or antiidiotypic antibodies that can reduce the production of anti-HLA antibodies in the pretransplant period (387–390). There is also limited experience in the use of IVIG in the treatment of allograft rejection. Successful prevention and treatment of acute humoral rejections were reported in a series of living donor kidney transplants (390).

Plasma Exchange

The role of plasmapheresis in the therapy of acute rejection is to remove circulating lymphocytotoxic antibodies that may be mediating allograft destruction. Given the small number of controlled studies on the therapy of acute rejection, plasmapheresis has not been shown to offer any advantage over steroids or antibodies in the reversal of acute vascular rejection, and has no long-term benefit on graft survival (391,392). Recently, a number of studies have reported favorable outcome of highly sensitized patients induced with pretransplant plasma exchange (393–395).

Total Lymphoid Irradiation (TLI)

Several groups have used total lymphoid irradiation (TLI) in renal transplantation. Myburgh and his colleagues (396–398) have reported 1- and 5- year graft survival of 86% and 60%, respectively. Because of the complexity and troublesome side-effects of TLI, it is unlikely that this method will be routinely adopted.

New Immunosuppressive Agents

New agents such as deoxyspergualin, brequinar sodium, myriocin, and its semisynthetic derivative FTY20, leflunomide, JAK 3 inhibitors, costimulatory blockade (primarily CTLA4-Ig and anti-CD154 mAb), and PGE1 analog are currently under experimental and clinical studies for maintenance immunosuppression as well as for the treatment of acute rejection.

Experimental antibody therapies are now being designed to directly target the CD4 molecule, the CD3 molecule by a humanized form of monoclonal anti-CD3, and adhesion molecules, such as ICAM-1 or LFA-1. A large multicenter study of induction with an anti-ICAM antibody failed to show any benefit in terms of the incidence of delayed graft function. A multicenter trial with anti-LFA-1 antibody, however, showed a nonsignificant trend in the incidence of delayed graft function in patients at risk (399–408).

FTY20 appears to be a very promising drug in experimental models. It reduces the number of T and B cells in the peripheral blood while increasing their numbers in lymph nodes and Peyer's patches. This redirected cell "homing" is thought to be due to modification by FTY20 of chemokine receptors on lymphocytes (409–413). The drug may potentiate the immunosuppressive effect of CsA. Phase III clinical trials are in progress.

Optimal and sustained T cell response after antigen recognition (signal 1) requires costimulatory signal (signal 2) delivered through accessory T cell-surface molecules (414–417). Recently, the central role of the B7-CD28 and CD40-CD40L costimulatory pathway in acute allograft rejection has been well studied. Blocking the B7-CD28 pathway with the fusion protein CTLA-4-Ig, blocking the CD40-CD40L pathway with anti-CD40L antibodies, or blocking both pathways simultaneously result in long-term allograft acceptance in rodents. This treatment with antibodies directed against molecules involved in the delivery of the second signal of T cell is promising and may offer future prospect for tolerance induction. Clinical trials will begin soon for anti-CD40 ligand and CTLA4-Ig in renal transplant recipients. Since T cell activation is a prerequisite for the induction of T cell anergy, the concurrent use of conventional immunosuppressive drugs may interfere with the mechanisms of action and tolerance induction by these antibodies.

PROBLEMS RELATED TO THE TRANSPLANTED KIDNEY

Acute Renal Failure in the Transplanted Kidney

In the immediate posttransplant period, there may be delayed graft function (DGF) or cessation of function after initial good function (418–423). The most likely cause of renal failure during this period is ischemic tubular damage, but a vascular accident, ureteric obstruction, a urine leak, or rejection are all possible etiologies (Table 98-13).

TABLE 98-13

CAUSES OF ACUTE RENAL FAILURE ASSOCIATED WITH RENAL TRANSPLANTATION

Prerenal	Hypovolemia
	Arterial stenosis or thrombosis
	Venous thrombosis
Renal	Acute tubular necrosis
	Hyperacute/accelerated rejection
	Acute rejection
	Nephrotoxicity
Postrenal	Ureteral obstruction
	Urinary leak
	Lymphocele
	Hematoma

Acute Tubular Necrosis

The pathogenesis of acute tubular necrosis (ATN) commonly begins with ischemic damage secondary to hypovolemia and hypotension in the donor (usually a cadaver), and prolonged warm ischemia and cold ischemia during preservation (424). There is some evidence to show that a delay in onset of function of the transplanted kidney is harmful in terms of long-term impairment to renal function (425–427). This may even be more important with the use of calcineurin inhibitors, such as CsA or tacrolimus, where there is a good deal of evidence suggesting that kidneys with delayed function have a much poorer graft survival than kidneys with immediate function. It should also be remembered that a not uncommon presentation of ATN is a reasonable urine output within the first 12 or 24 hours that gradually diminishes and is followed by oliguria. This pattern is seen more commonly with the use of calcineurin inhibitors and probably reflects some acute nephrotoxicity in an already damaged kidney.

Allograft Rejection

Rejection is the major cause of graft failure and if the injury to the tubules and glomeruli is sufficiently severe, the kidney may not recover. It is, therefore, important to diagnose acute rejection as soon as possible in order to promptly institute antirejection therapy.

Hyperacute Rejection. Hyperacute rejection is very rare and it is caused by antibodies against alloantigens that have appeared in response to previous exposure to antigens through prior transplantations, blood transfusions, or multiple pregnancies. The clinical manifestation of hyperacute rejection is a failure of the kidney to perfuse properly on release of the vascular clamps just after vascular anastomosis is completed. The kidney initially becomes firm and then rapidly becomes blue, spotted, and flabby. The presence of neutrophils in the glomeruli and peritubular capillaries in the kidney biopsy confirms the diagnosis. It can be prevented by careful testing of recipients for the presence of the preformed cytotoxic antibodies (387,428).

Accelerated Rejection. An accelerated rejection, which may start on the second or third day and tends to occur in the previously sensitized patient in whom preformed anti-HLA antibodies are present. It occurs in patients who have had a previous graft and presents with a decrease in renal function and has a similar clinical picture to that mentioned for hyperacute rejection (429,430).

Acute Rejection. Acute rejection episodes may occur as early as 5 to 7 days, but are generally seen between 1 and 4 weeks after transplantation. The classic acute rejection episode of the earlier era in a patient treated with azathioprine—prednisone, was accompanied by swelling and tenderness of the kidney and the onset of oliguria with an associated rise in serum creatinine; it was usually accompanied by a significant fever. However, in patients who have been treated with CsA, the clinical features of an acute rejection are really quite minimal in that there is perhaps some swelling of the kidney, usually no tenderness, and there may be a minimal to moderate degree of fever. Since such an acute rejection may occur at a time when there is a distinct possibility of acute CsA or tacrolimus toxicity, the differentiation between the two entities may be extremely difficult.

Differential Diagnosis. Acute renal failure following transplantation is a common and often treatable entity. The differential diagnosis of ATN, acute rejection, and CsA- or tacrolimus-induced nephrotoxicity may be difficult, especially in the early

TABLE 98-14

APPROACH TO THE PATIENT WITH ACUTE RENAL FAILURE FOLLOWING TRANSPLANTATION

Immediate Acute Renal Failure (48 hr)
- Rule out catheter obstruction
- Rule out hypovolemia
- Radioisotope scan to rule out vascular catastrophe
- Ultrasound to rule out urinary extravasation or obstruction
- Radiocontrast studies (if indicated by above)

Early or Late Acute Renal Failure (After 48 hr)
- History and physical examination to detect oliguria, tender swelling graft, fever
- Urine sodium, FENa (especially if baseline available)
- CsA or tacrolimus levels
- Radioisotope scan (to follow serially)
- Ultrasound
- Therapeutic trial of steroids/lowering cyclosporine dose
- Renal biopsy

posttransplant period when more than one cause of dysfunction can occur together. Knowledge of the natural history of several clinical entities is extremely helpful in limiting the differential diagnosis. Many nonspecific tests are available to aid in the differential diagnosis (Tables 98-12 and 98-14).

Noninvasive imaging of the transplanted kidney has been quite useful in differentiating the different causes of acute allograft dysfunction in the early posttransplant period. Local expertise, however, is a major factor in selection.

Radionuclide imaging. A renal flow study and scan are routinely performed on the first postoperative day in all transplant patients. Renal perfusion can be assessed by technetium diethylenetriamine pentaacetic acid (DTPA) or MAG3 and tubular function by iodohippurate (Hippuran) I^{131} uptake. A decline in initial renal blood flow and decrease in tubular function often occur during acute rejection (Fig. 98-11). Occasionally, scans can help diagnose uncommon posttransplant complications, such as venous thrombosis or urinary leak (431–433).

Ultrasound. Real-time and duplex Doppler sonography of the renal allograft are useful for evaluating recipients with surgical complications, including perinephric fluid collections, hydronephrosis (Fig. 98-12), and vascular complications (434–436). The sonography reveals variable findings in acute rejection, such as graft enlargement, enhanced echogenicity of the parenchyma, and increased resistive index (>70%) of the vessels. The studies will not confirm the diagnosis of rejection, but serve to exclude allograft thrombosis and urinary obstruction, and indicate that a percutaneous transplant kidney biopsy with pathologic examination should be performed.

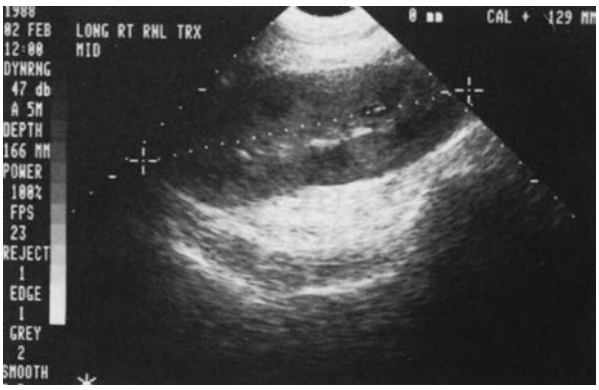

FIGURE 98-12. Normal renal ultrasound of a transplanted kidney.

Renal Transplant Biopsy. A biopsy will help to confirm the diagnosis of ATN by the exclusion of a histologic picture of severe rejection. Needle biopsy of the cadaver kidney just after implantation is helpful to detect renal disease or preservation injury that might confound interpretation of delayed renal allograft function. However, a poor understanding of the natural history of transplant histology makes it an imperfect gold standard.

Technique of renal transplant kidney biopsy. The use of percutaneous biopsy of the transplant kidney to diagnose rejection was first performed in 1967 (437). Since then, the technique has been well established as a useful tool in the differential diagnosis of allograft dysfunction (438–444). Transplant kidney biopsy can be more difficult than routine biopsy of native kidney. The position and alignment of the transplant kidney can vary from patient to patient (Fig. 98-13). In most instances, the transplant kidney is palpable and the orientation kidney can be estimated by reviewing an isotope scan of the transplant kidney (Fig. 98-14).

Biopsy can be obtained with a Vim–Silverman needle, a 14G–16G disposable Trucut biopsy needle, or an 18G automatic Biopty or Monopty needle (Fig. 98-15A, B, and C). It

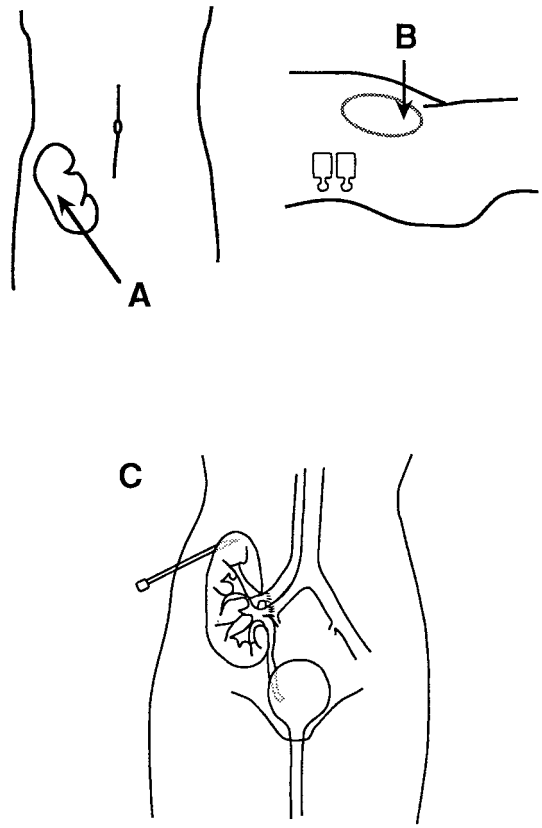

FIGURE 98-13. Site of biopsy for the transplanted kidney. The orientation of the transplanted kidney is localized by palpation and review of the operative record and renal scan. The kidney is approached either tangentially or vertically, (**A**) in a plane tangential to the lateral curvature of the allograft or (**B**) in a plane perpendicular to the kidney directed to the lower pole. (**C**) Tangential across the upper pole of the transplanted kidney.

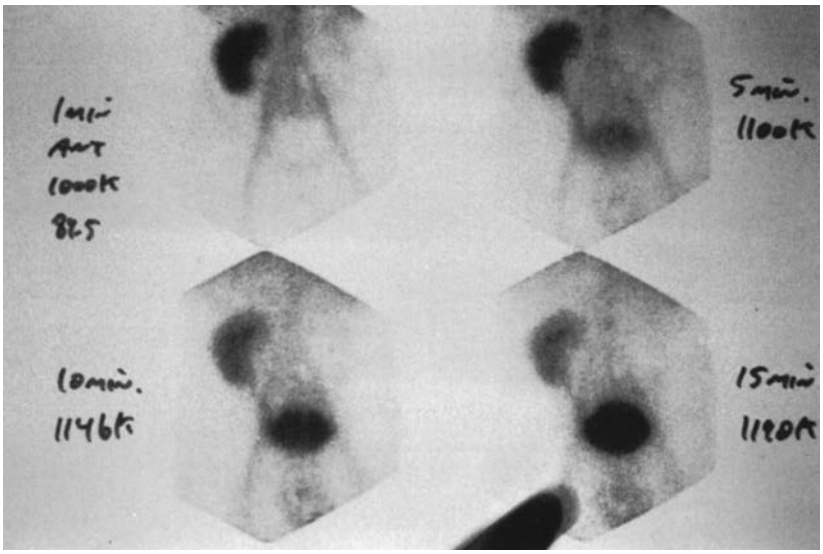

FIGURE 98-14. Diethylenetriamine pentaacetic acid scan showing good renal perfusion in a transplanted kidney.

is best performed under sonographic guidance. Relative contraindications to biopsy include abnormal coagulation studies or a low platelet count, active urinary tract infection, and significant renal allograft hydronephrosis. Hydronephrosis indicative of urinary tract obstruction should be further investigated. A small number of patients with urinary obstruction and rejection may coexist. It is interesting to note that significant macrophages infiltrate can occur with experimental urinary obstruction. In patients with prolonged bleeding time due to uremia, intravenous deamino-D-arginine vasopressin (dDAVP) (0.3 μg/kg) can be used to correct the coagulation defect prior to biopsy procedure. If the kidney has been transplanted via an intraabdominal approach or is difficult to localize, then consideration should be given to the biopsy being performed using computed tomographic (CT) direction, or, occasionally, via a limited open surgical approach in the operating room. The pathologic changes, which occur with acute cellular rejection, include interstitial infiltration with mononuclear cells and disruption of the tubular basement membranes (tubulitis) by the infiltrating cells (Fig. 98-16) (445). The presence of patchy mononuclear cell infiltrates without tubulitis is not uncommon in normal functioning renal allografts and is not sufficient to make the diagnosis of acute rejection. Vascular lesions are also common. The intrarenal arteries and arterioles show characteristic changes of intimal thickening and the presence of inflammatory cells within and adherent to the endothelium (Fig. 98-17). The glomerular changes are usually unremarkable in rejection. However, glomerular capillary and vascular intimal infiltrates can occur with acute vascular rejection (Fig. 98-18). Periodic examination of histologic features in the absence of changes in renal function may reveal silent allograft rejection (Tables 98-15 and 98-16). In a randomized control trial, Rush et al. (446,447) found that treatment of subclinical rejection detected by protocol biopsies lead to better graft function, compared with standard management without protocol biopsy. In addition to conventional histopathologic approach, a technique using monoclonal antibodies has been used to identify the different types of cellular infiltrate (448). The infiltrate in graft undergoing rejection comprises around 60% macrophages, 30% T lymphocytes, and less than 10% natural killer (NK) cells. This approach of identifying different T cell subsets has been used for immunologic monitoring and treatment of rejection. HLA antigen expression, which is increased on tubular cells during rejection, may be important in the rejection process. Immunohistologic evaluation of rejecting renal allografts shows an increased number of infiltrating MHC class II positive and IL-2 receptor positive mononuclear cells, when compared to controls.

Fine-Needle Aspiration Biopsy (FNAB). An alternative approach to needle biopsy is to use a fine-needle aspiration biopsy (FNAB) to sample parenchymal cells and infiltrating cells from the renal allograft. The technique allows serial sampling of cells for the determination of parenchymal cytology, nature of cellular infiltrate/T cell subsets, and molecular markers (438,449–452). Good correlation with needle biopsy has been reported with respect to cellular infiltrate, but not as good in showing vascular changes (453–455). Monoclonal antibody staining of the

TABLE 98-15

HISTOPATHOLOGY OF RENAL ALLOGRAFT USING NEEDLE BIOPSY

Changes Associated with Rejection
Glomerular
Swelling of endothelium
Endothelial/mesangial proliferation
Exudation of polymorphs, mononuclear cells
Interstitial
Edema
Infiltration of mononuclear cells
Macrophages
Eosinophils
Vascular
Endothelial edema
Mural infiltration
Necrosis, hemorrhage
Severe vasculitis (especially interstitial hemorrhage) predicts eventual graft failure
Changes Associated with Cyclosporine
Tubular changes
Giant mitochondria
Vacuolization
Microcalcification
Interstitium
Mononuclear infiltration
Vascular changes
Arteriolar necrosis

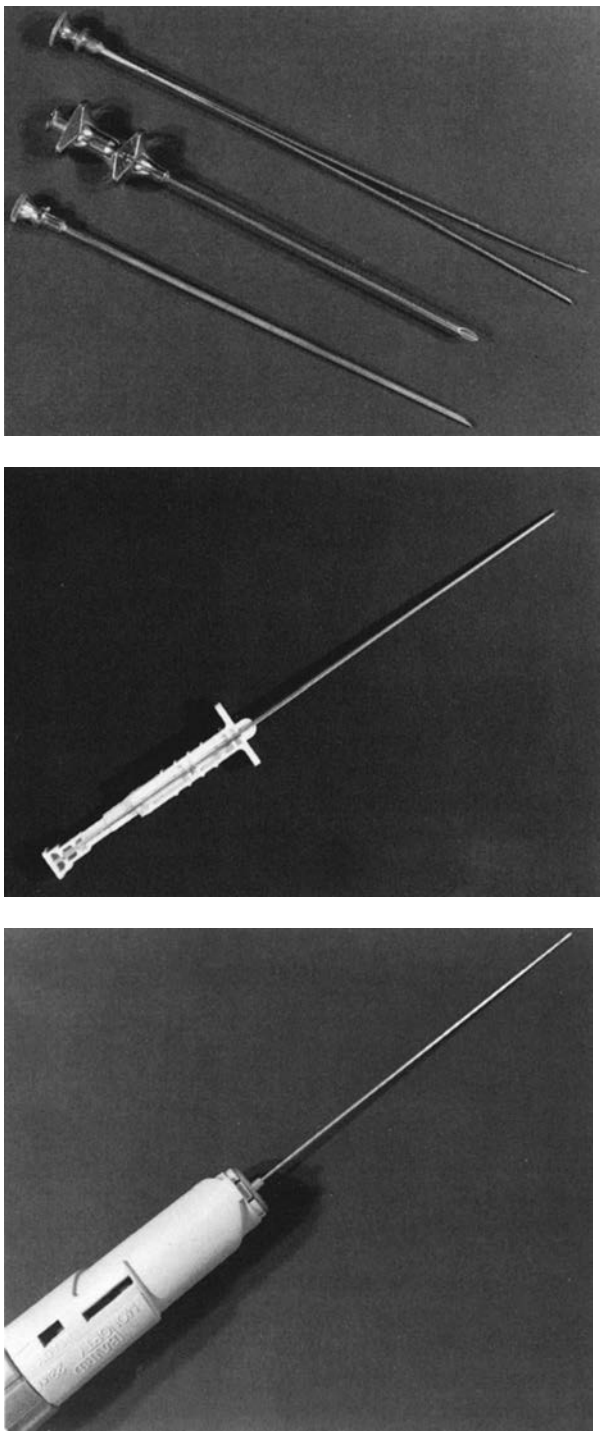

FIGURE 98-15. Different types of biopsy needles. **A:** Vim-Silverman. **B:** Tru-Cut 14 G (disposable needle). **C:** Biopty gun 18 G (disposable needle).

aspirate with antibodies to T cell subsets, activation markers, and MHC antigen expression has advanced the usefulness of this technique.

New Techniques. Monitoring of other products of inflammation, such as neopterin and lymphokines continues to be explored. It has been shown that acute rejection is associated with elevated plasma IL-1 in azathioprine-treated patients and IL-2 in cyclosporine-treated patients (456). Interleukin 6 is also increased in the serum and urine immediately after transplantation and during acute rejection episodes. The major problem, however, is that infection, particularly viral, can also elevate cytokine levels. PCR has also been used to detect mRNA for IL-2 in fine-needle aspirate of human transplant kidney. Using this approach, IL-2 could be detected 2 days before rejection was apparent by histologic or clinical criteria. Similar PCR approach can be used for quantitative analysis of intragraft gene expression, such as granzyme B, perforin, TGF-β, IL-10, and IL-15 (457–459).

Urinary sediment cytology coupled with immunologic as well as molecular techniques should complement FNAB in the diagnosis and monitoring of rejection processes (460–462).

Magnetic resonance spectroscopy and imaging techniques (463–466) are being evaluated for the diagnosis of rejection. Magnetic resonance imaging (MRI) can demonstrate normal kidney architecture in a noninvasive fashion without ionizing radiation. It can evaluate corticomedullary demarcation, size of collecting system, presence of focal parenchymal changes and shape of the kidney, and signal intensity of the sinus fat. Acute rejection can be diagnosed by the presence of a decreased or absent corticomedullary demarcation, with other signs of swelling, enlarged pyramids, and decreased signal intensity in the renal sinus fat (Fig. 98-19A and B). Changes for ATN and nephrotoxicity are not specific. Therefore, it has not yet been established as a primary imaging modality in renal transplant.

Chronic Rejection. Because chronic rejection is thought to be the end result of uncontrolled repetitive acute rejection episodes or a slowly progressive inflammatory process, its onset may be as early as the first few weeks after transplantation or any time thereafter. Progressive azotemia, proteinuria, and hypertension are the clinical hallmarks of chronic rejection (467–471). Immunologic and non-immunologic mechanisms are thought to play a role in the pathogenesis of this entity, including antibody-mediated tissue destruction possibly secondary to antibody-dependent cellular cytotoxicity leading to obliterative arteritis (Fig. 98-20), growth factors derived from macrophages and platelets leading to fibrotic degeneration, and glomerular hypertension with hyperfiltration injury due to reduced nephron mass leading to progressive glomerular sclerosis.

Nonimmunological causes can also contribute to the decline in renal function. Atheromatous renovascular disease of the transplant kidney may also be responsible for a significant number of cases of progressive graft failure (Fig 98-21). Although a smoldering subclinical inflammatory process may play a role in the pathogenesis of chronic rejection, the reduction in nephron mass (472,473), as a result of earlier immunologic injury, most likely contributes to further decline in function. In patients with chronic rejection on azathioprine–prednisone immunosuppression, a switch from azathioprine to CsA, about 5 mg/kg, was shown in two studies to slow the decline in allograft function compared with controls. On the other hand, adjustment of CsA dosage to avoid nephrotoxicity with addition of azathioprine for immunosuppression may preserve residual graft function.

Dietary protein restriction was also shown to reduce the rate of graft deterioration compared with controls after an 18-month follow-up period (474). Along with dietary protein restriction, aggressive control of hypertension may have a beneficial effect on hyperfiltration injury. Therefore, in the absence of a treatable rejection process or a surgically remediable cause, aggressive control of hypertension and dietary protein restriction may slow the inevitable decline in renal function. Clinical evidence suggests that control of hypertension with ACE inhibitor may preserve renal function in patients with (native) renal disease (475–479). Reduction of the urine protein

TABLE 98-16

BANFF 97 DIAGNOSTIC CATEGORIES FOR RENAL ALLOGRAFT BIOPSIES

1. Normal
   The presence of patchy mononuclear cell infiltrates without tubulitis is not uncommon in normally functioning renal allografts and when considered alone does not warrant the diagnosis of acute rejection
2. Antibody-mediated rejection
   Rejection demonstrated to be due, at least in part, to anti-donor antibody
   A. Immediate (hyperacute)
   B. Delayed (accelerated acute)
3. Borderline changes: "Suspicious" for acute rejection
   This category is used when no intimal arteritis is present, but there are foci of mild tubulitis (1 to 4 mononuclear cells/tubular cross section)
4. Acute/active rejection

Type (grade)	Histopathologic findings
IA	Cases with significant interstitial infiltration (>25% of parenchyma affected) and foci of moderate tubulitis (>4 mononuclear cells/tubular cross section or group of 10 tubular cells)
IB	Cases with significant interstitial infiltration (>25% of parenchyma affected) and foci of severe tubulitis (>10 mononuclear cells/tubular cross section or group of 10 tubular cells)
IIA	Cases with mild to moderate intimal arteritis
IIB	Cases with severe intimal arteritis comprising >25% of luminal area
III	Cases with "transmural" arteritis and/or arterial fibrinoid change and necrosis of medial smooth muscle cells

5. Chronic/sclerosing allograft nephropathy

Grade	Histopathologic findings
I (mild)	Mild interstitial fibrosis and tubular atrophy
II (moderate)	Moderate interstitial fibrosis and tubular atrophy
III (severe)	Severe interstitial fibrosis and tubular atrophy

6. Other Changes not considered to be due to rejection
   Posttransplant lymphoproliferative disorder
   Acute tubular necrosis
   Acute interstitial nephritis
   Cyclosporine or FK506-associated change

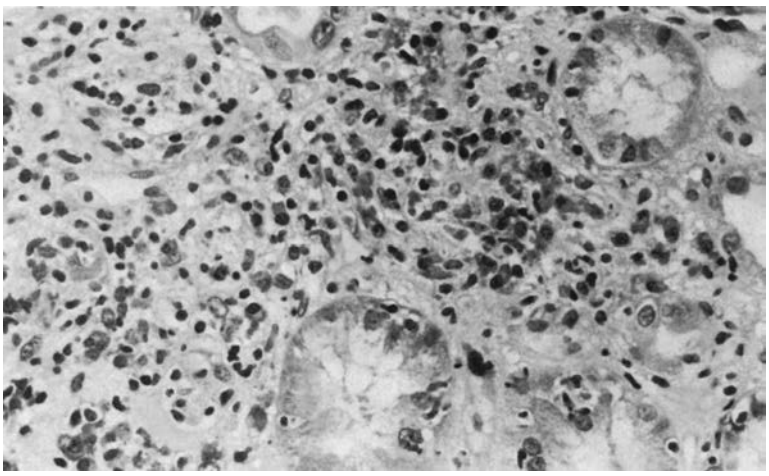

FIGURE 98-16. Acute cellular rejection. Percutaneous renal transplant biopsy specimen showing tubulitis with tubular epithelial infiltrates of lymphocytes and plasma cells.

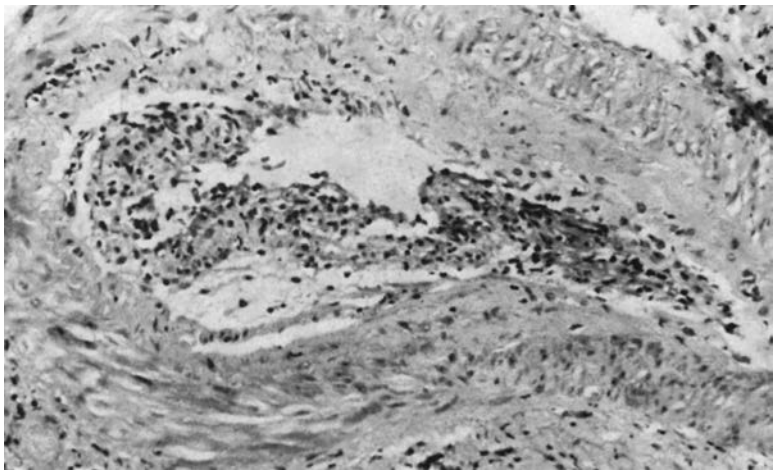

FIGURE 98-17. Acute rejection. Renal transplant biopsy specimen showing marked endovasculitis and acute inflammatory endothelial infiltrates.

excretion with ACE inhibitor or receptor antagonist may help to reduce lipid levels for patients with nephrotic-range proteinuria.

Patients with renal failure due to chronic allograft rejection should be evaluated early for initiation of dialysis treatment or retransplantation workup. Failed allografts, which were routinely removed in the past, usually are now left in place unless there are specific indications for surgery. Indications for transplant nephrectomy include infection, hemorrhage, signs of acute rejection with graft tenderness, swelling, and fever, and frank necrosis, usually occurring on cessation of immunosuppressive drugs. For patients who are on the transplant waiting list, it is preferable to remove failed graft before or at the time of stopping immunosuppressive therapy in the hope of minimizing the production of HLA antibodies.

Glomerulonephritis in Allografts

Four main types of glomerulonephritis may occur in allografts: (1) the donor's kidney may be the seat of nephritis before grafting; (2) recurrent glomerulonephritis may develop due to the persistence of the original stimulus and recurrence of the original disease in the recipient; (3) transplant glomerulopathy may occur as a result of host response to the graft; and (4) *de novo* glomerulonephritis may arise in a healthy graft due to changes taking place in the recipient often as a result of immunosuppression (83,111,480).

Nephritis of Donor Origin

Cadaver kidney donor may have unsuspected glomerulonephritis. It is, therefore, important to perform a graft biopsy during the transplant operation. Kidneys from patients with early undetected IgA nephropathy and patients with diabetes mellitus have been used for transplantation. Of particular interest is the fact that IgA nephropathy transplanted into a non-IgA nephropathy recipient tends to disappear after transplantation.

Recurrence of Primary Renal Disease

Essentially all glomerulonephritis have been described to recur in renal allograft. However, there is much variation between the various types of glomerulonephritis with regard to the frequency of recurrence, the clinical pattern, and the prognosis (Table 98-17). The clinical manifestations of recurrent glomerulonephritis include microscopic hematuria and proteinuria, which may progress to nephrotic syndrome. Recurrent glomerulonephritis is the most common cause of nephrotic syndrome following transplantation. Proteinuria may also be a manifestation of *de novo* glomerular disease or chronic rejection. While the documented overall incidence of graft failure from recurrent disease is less than 2%, this is an underestimate due to difficulty in firmly establishing this diagnosis and in defining the cause of primary ESRD and graft dysfunction or loss occurred because of the same pathologic process (481–483).

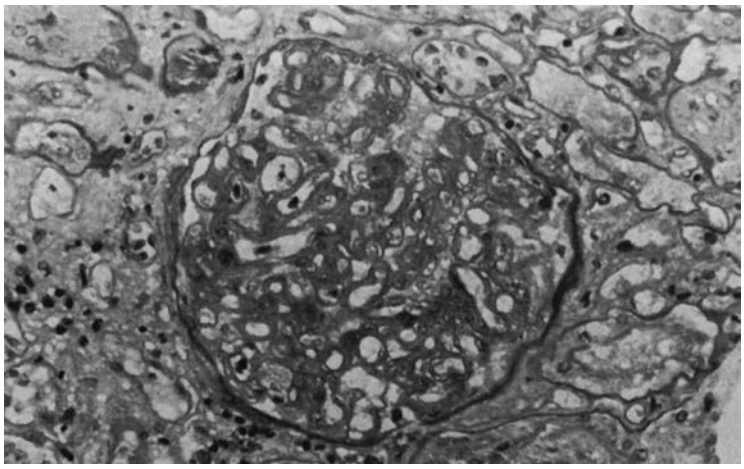

FIGURE 98-18. Acute vascular rejection. Glomerular and vascular endothelial infiltrates and swelling.

A

B

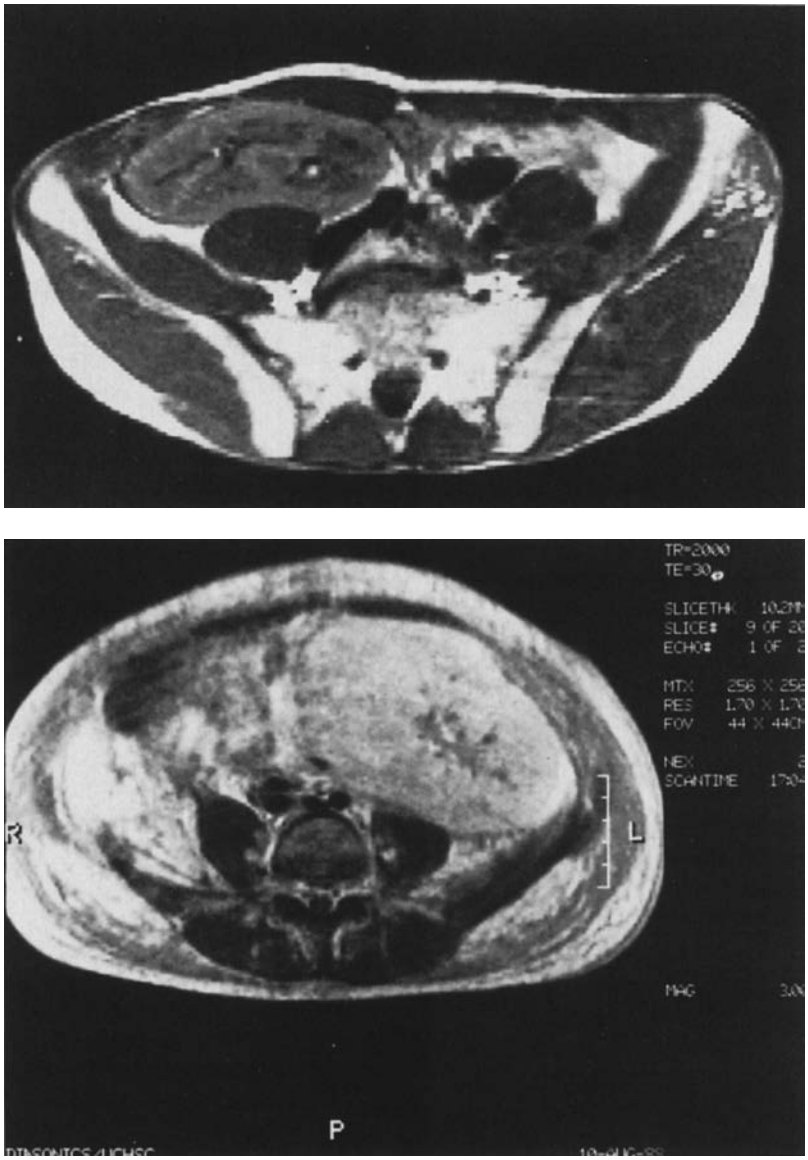

FIGURE 98-19. Magnetic resonance imaging of transplanted kidney. **A:** Normal transplanted kidney. **B:** A transplanted kidney showing edema and loss of corticomedullary demarcation of the kidney during acute rejection.

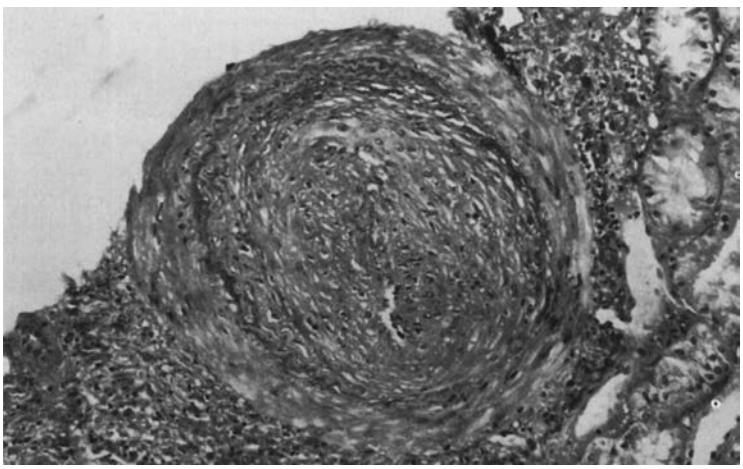

FIGURE 98-20. Chronic rejection. Renal transplant biopsy specimen showing obliterative arteriopathy and fibrointimal vascular narrowing in chronic rejection.

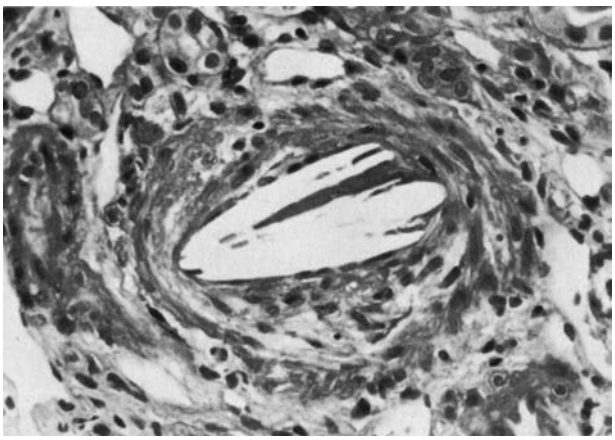

FIGURE 98-21. Percutaneous needle biopsy of a kidney 20 years posttransplantation showing atheroembolus in a small artery. (Trichrome stain, magnification ×400.)

Transplant Glomerulopathy

Transplant glomerulopathy is an entity that may be considered a special form of chronic rejection. It is believed to be related to rejection process because the frequency of glomerular lesions was found to be inversely related to HLA compatibility. Histologically, it may resemble membrano-proliferative glomerulonephritis (Type I MPGN) with mesangial proliferation and thickening or reduplication of the glomerular basement membrane. It is the most common cause of nephrotic syndrome in renal transplant patients. Along with proteinuria, the clinical presentations include microscopic hematuria and progressive graft dysfunction.

Cytomegalovirus (CMV) glomerulopathy is a controversial lesion (484). Cytomegalovirus infection of the allograft may produce a glomerulopathy, with impairment of function. It has been proposed that CMV infection, through elaboration of lymphokines and interferons, may cause upregulation of histocompatibility antigens on the allograft. This change results in induction of immune responses that lead histologically to glomerular endothelial changes and possibly vascular rejection and clinically to allograft dysfunction with decreased allograft survival (485).

De Novo Glomerulopathy

The development of glomerular lesions in patients with no history of glomerulonephritis suggests the presence of a *de novo* process in the allograft. *De novo* membranous nephropathy (Fig. 98-7) is reported to occur with an incidence of less than 1%. Nephrotic range proteinuria occurred at a mean time of 1 to 2 years after transplantation. In contrast to the indolent course of idiopathic membranous glomerulonephritis in nontransplant patients, *de novo* membranous nephropathy can lead to graft loss. This may be due to superimposed glomerular and interstitial lesions associated with chronic rejection.

Focal segmental glomerulosclerosis is not uncommon among transplant recipients whose original disease was not FSG (486). The mechanisms underlying *de novo* glomerulosclerosis are not clear. It may represent a nonspecific response to chronic rejection, glomerular ischemia, vesicoureteral reflux, or infections such as hepatitis B and HIV. The use of heterologous antilymphocyte globulin has been implicated in the development of *de novo* glomerulonephritis. Glomerulopathy may be related to antibodies cross-reacting with the glomerular basement membrane (81,487).

Circulating anti-GBM antibodies and anti-GBM disease can develop in some patients with Alport disease after renal transplantation (74,76). Patients with Alport disease lack a component of the GBM and do not bind anti-GBM antibodies isolated from patients with Goodpasture's syndrome. When the allograft, which contains these GBM proteins, is transplanted, the recipient may mount a humoral response against these proteins, which may lead to anti-GBM disease.

TABLE 98-17

RECURRENT DISEASE IN RENAL ALLOGRAFTS

Disease	Approximate recurrence rate (%)	Graft loss due to recurrence
Primary		
Glomerulonephritis		
FSGS	30–60	Common
HUS	20–50	Uncommon
Type I MPGN	20–30	Common
Type II MPGN	50–100	Uncommon
HSP	15–50	Uncommon
IgA nephrolopathy	50	Uncommon
Anti-GBM	25–50	Uncommon
Membraneous Glomerulonephritis	10	Uncommon
Systemic Disease		
Oxalosis	80–100	Common
Cystinosis	50–100	Uncommon
Fabry's disease	Rare	Common
Sickle cell disease	Rare	Common
Diabetes type I	100	Uncommon
SLE	<1	Uncommon

RESULTS OF KIDNEY TRANSPLANTATION

Patient Survival

In the 1960s and early 1970s, many patients were transplanted when there were no supportive facilities if the graft failed. In those days, the high mortality was related to uncontrolled infection when excessive immunosuppression was used for rejection processes (2,5,488,489). With improvement in clinical care and use of more specific immunosuppression, patient survival has been shown to improve both in the cadaveric and in the living related renal transplant recipients. Mortality at the end of the first year is currently less than 5% for living donor and under 10% for cadaver donor. Indeed, recent data indicate that patients currently receiving cadaver donor transplants generally survive longer than patients treated by dialysis. Infectious complications of immunosuppressive therapy and cardiovascular diseases are the most important cause of death (24,212,490–494). Based on UNOS registry data, the major causes of death among cadaver kidney transplant recipients were cardiovascular (26%) and infection (24%) during the first posttransplant year. Between 1 to 3 years, the percentage of deaths due to infection fell to 15% and malignancies accounted for 13% of patient death (418).

Graft Survival

Acute rejection is the most frequent cause of graft failure within the first year. Although there has been a progressive improvement in patient survival, rates of graft survival after cadaver transplant have remained virtually unchanged in the 1970s and early 1980s (2,5,212,418). The failure to improve these results is due to the lack of more specific forms of immunosuppressive therapy. In the years immediately following 1983, there were dramatic gains in cadaver graft survival probably related to the introduction of cyclosporine (319). The overall cadaver graft survival has increased from about 65% to about 80% to 85% at 1 year (Fig. 98-22A and B).

Based upon data reported to the UNOS Scientific Renal Transplant Registry regarding transplant performed between 1994 to 1998, the 1- and 3-year graft survival rates for 16,288 recipients of living-donor kidneys were 93% and 86%, respectively, with a half-life of 17 years. The overall results of 35,289 cadaver donor kidney transplants were 87% to 76% graft survival at 1 and 3 years with a half-life of 10 years (418). In addition to immunosuppressive agents, there are many other factors that may influence graft survival (2,418,495). The recipient's race also influenced graft survival rates (Fig. 98-22C). Asian recipients of cadaver kidneys had the highest graft survival rates of 91% and 85% at 1 and 3 years with a half-life of 18 years. The result of Caucasians and African-Americans were significantly lower. The graft half-life was 12 years for Caucasians and 7 years for African-Americans.

Living Related Transplantation

Monozygotic Twins. Provided that there are no technical mishaps as a result of the operation itself, one should expect twin kidney transplants to survive indefinitely without the need for immunosuppression (205). However, there has been a significant incidence of recurrent glomerulonephritis when this was the original disease in the recipients. In a series of 30 identical-twin transplants followed for up to 27 years, 9 developed recurrent nephritis, 1 as late as 16 years after transplantation (496). Of 41 renal transplants between monozygotic twins having recorded by the European Dialysis and Transplant Association, 36 were alive with functioning grafts from 1 to 14 years after transplantation (497). Two grafts failed from recurrent nephritis, two due to *de novo* glomerulonephritis and one died in a traffic accident. This has been considered an indication for continuous low-grade immunosuppression in those patients where there is a risk of recurrent disease, but perhaps of greater importance is the withholding of transplantation until the original disease is completely quiescent.

HLA Identical Siblings. The HLA identical sibling transplant is ideal and there have been recent reports of 3-year graft survival rates of 90% to 95% in such patients. Immunosuppression is still necessary since rejection does occur in a substantial number of patients and may even occasionally result in loss of a graft from rejection (498,499). These rejection episodes no doubt reflect recognition of, or sensitization to, minor histocompatibility antigens in the donor or to genetic recombination at the HLA-DR locus. As excellent results are obtained with azathioprine and prednisone, this would seem to still be the immunosuppressive therapy of choice at this time, in view of the nephrotoxicity associated with cyclosporine. However, some centers cover the recipient with cyclosporine for several months in case of unexpected rejection and then taper and discontinue the drug after 4 to 6 months. Steroids can usually be discontinued after 1 or 2 years if renal function is stable, although withdrawal of steroids should be done very cautiously over a period of at least 6 months.

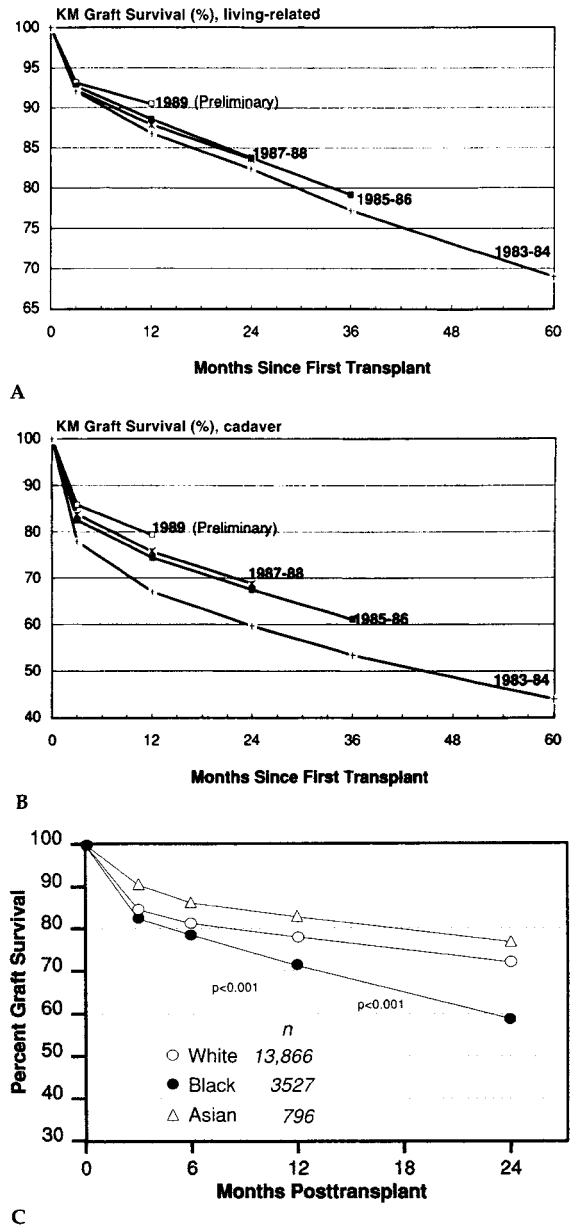

FIGURE 98-22. Graft survival. **A:** Kaplan–Meier (KM) graft survival for living related donor *(LRD)* transplantations performed between 1983 and 1988. **B:** KM graft survival for cadaveric donor transplantations performed between 1983 and 1988. Graft survival rates for cadaveric grafts are improving over time. (From: US Renal Data System. Annual data report, 1991. *Am J Kidney Dis* 1991;18(Suppl 2):61, with permission.) **C:** Effect of race on graft survival. The race of the recipients was a significant factor in the outcome of the first cadaveric transplants. Asian patients had the highest graft survival rates—83% and 77% at 1 and 2 years, respectively—whereas Blacks had the poorest survival rates—71% and 59% at 1 and 2 years, respectively. (From: Cecka J, Terasaki P, eds. *Clinical Transplants 1990*. Los Angeles, CA: UCLA Tissue Typing Laboratory; 1990:447, with permission.)

HLA Nonidentical Parent to Child or Siblings. A transplant may be performed between a patient and a child who will differ for one HLA haplotype or between the two siblings who differ either for one or both HLA haplotypes. The results of transplantation were related to the degree of HLA disparity, that is, two haplotype-disparate pairs were less successful than one haplotype-disparate pair and both were significantly worse than the results of transplantation between

HLA identical siblings (500). However, CsA treatment and donor-specific blood transfusion have substantially improved the results of transplantation between HLA nonidentical family members. Actuarial graft survival is now very close to that achieved for transplantation between HLA identical siblings (501–504).

Living Unrelated Transplantation

A case can be made for the use of emotionally related donors, such as a spouse or more distantly related members of the family, such as cousins, and perhaps even between very close friends, now that the expectation of a successful transplant is quite high (15,177,178). Despite greater histoincompatibility, the survival rates of these kidneys are greater than those of cadaveric kidneys. In a recent analysis by Terasaki and his colleagues (177), the 3-year survival rates were 85% for kidneys from 368 spouses, 81% for kidneys from 129 living unrelated donors who were not married to the recipients, 82% for kidneys from 3368 parents, and 70% for 43,341 cadaveric kidneys. The superior survival rate of grafts from unrelated donors could not be attributed to better HLA matching, white race, younger donor age, or shorter cold ischemia times, but might be explained by damage due to shock before removal in 10% of the cadaver kidneys. Living unrelated donors may thus become a major source of organ for kidney transplantation (505–507). However, living unrelated transplantation does give rise to a number of ethical and moral problems and should be considered only in exceptional circumstances, according to guidelines issued by The Transplantation Society (179, 508–510).

Cadaver Transplantation

The majority of kidneys used for transplantation have been from cadavers. Even though there may be variations from center to center (418), there has been a steady improvement in the results of cadaver transplantation in terms of both patient and graft survival over the last 10 years. Patient survival is now around 96% at 1 year and graft survival is approaching 80% (Fig. 98-22B); in selected groups of patients, such as those who have been transfused and who are receiving a first graft, graft survival is over 85% (418). This improvement in patient survival is due to use of less immunosuppression and, in particular, the use of low-dose steroid protocols. Cardiovascular disease has replaced infectious complication as a major cause of morbidity and mortality.

The improved graft survival, which obviously is due in part to improved patient survival, can be attributed to the recognition of the transfusion effect, HLA matching (Fig. 98-23), and, more recently, to better immunosuppression. However, controversy still exists as to whether matching is of any relevance because of the better results achieved with better immunosuppression (168,418). Although this question has not been resolved, data are gradually accumulating suggesting that matching, and, in particular, matching for HLA-DR, does exert the same influence on graft survival in patients treated with CsA as with those on conventional immunosuppressive therapy (511). The 6-year half-life of kidney transplants from cadaveric donors has been unchanged since the early 1970s (512). This is in comparison with the 20- to 25-year half-life for the same period in HLA-identical sibling transplants, emphasizing the effect of histocompatibility differences on graft survival (513).

With the improvement in patient and graft survival, renal transplantation has now become the treatment of choice by most patients with ESRD. However, there is a relatively lower percentage of black patients with a functioning renal graft, despite the fact that the incidence rates of ESRD in the black population in the United States are approximately three to four times as high as in the white population. It has been suggested previously that black recipients had significantly lower graft survival rates than white recipients in North America (Fig. 98-22C). More recent analysis have shown that factors other than race could explain the poorer survival of grafts in African-American recipients and of African-American kidneys in either race (418,514,515).

The results of cadaveric transplantation are now approaching a level at which, if there were an adequate supply of cadaver kidneys, there probably would be little justification for continuing living related transplantation, except when high sensitization of the recipient makes cadaver transplant unlikely or impossible. Most patients who have rejected a first graft will be sensitized to histocompatibility antigens and many of these patients are highly sensitized (516). This does make identification of a suitable donor much more difficult in that a negative cross-match with the donor must be found; this, in turn, implies the need for a better-matched graft. The patient who is highly sensitized and is awaiting a sequential graft provides one of the major reasons for the national and regional organ exchange network.

SURGICAL COMPLICATIONS OF RENAL TRANSPLANTATION

The surgical technique of renal transplantation is reasonably standardized and overall direct surgical complication rates are low, accounting for only a small percentage of graft losses. Nevertheless, the transplant physician must be familiar with the diagnosis and treatment of surgically related complications to minimize recipient morbidity and mortality. The allograft is placed extraperitoneally into the iliac fossa, in most cases. Thus, intraperitoneal bleeding, or bowel obstruction from adhesions or internal herniation, should not occur as a direct result of the surgery. In small children or in some recipients with a supravesical urinary diversion, the transplant is placed

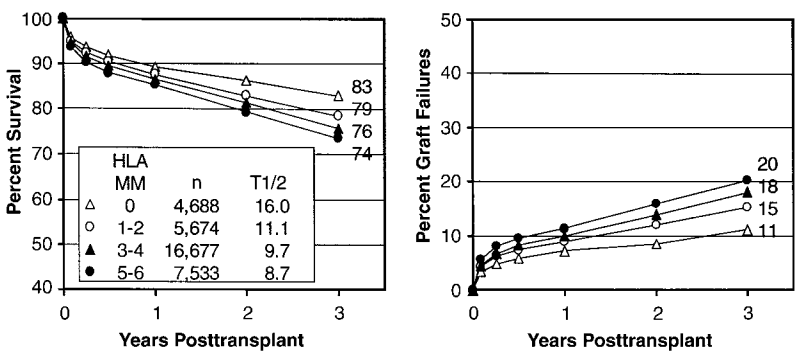

FIGURE 98-23. Effects of human leukocyte antigen (HLA) mismatches on survival of first cadaveric transplants. Graft survival rates at 1 and 2 years posttransplantation declined as the number of HLA-A, HLA-B, and HLA-DR mismatches increased. The difference between the best and the worst-matched grafts was 11% at 1 year and 14% at 2 years. (From: Cecka J, Terasaki P, eds. *Clinical Transplants 1999*. Los Angeles, CA: UCLA Immunogenetics Center; 2000:15, Figure 7, with permission.)

intraperitoneally and the potential surgical complications listed above must be considered.

Wound Complications

The most important causes of wound infection stem from the operative complications of hematoma, urine leak, and lymphocele. Transplant recipients are vulnerable to wound infection because of postoperative immunosuppressive medications and poorly controlled uremia. Wound infections following transplantation are extremely bothersome, because if they are deep-seated infections around the arterial anastomosis, secondary hemorrhage may occur. On presentation of a wound infection, adequate drainage should be provided immediately and appropriate antibiotic therapy introduced. However, prevention of contamination during donor nephrectomy and the use of preventive antibiotics before transplantation should ensure that the incidence of wound infection after transplantation is no more than 3% or 4%.

Patients with high fever, but benign-appearing incision sites, can harbor large purulent abscesses, emphasizing the effect of steroids on masking signs of inflammation. If unexplained fevers persist, ultrasonography or CT scanning of the wound may localize a fluid collection; needle aspiration of a fluid collection is indicated. Prophylaxis with the administration of intraoperative intravenous antibiotics has reduced both wound infection and sepsis.

Bleeding

Secondary hemorrhage following a renal transplant fortunately is an unusual event and is always secondary to infection, which usually has been introduced at the time of operation. During a fulminant rejection episode, acute swelling of the kidney may lead to rupture through its cortex, often originating at a previous biopsy site. Urgent surgical exploration is necessary in most cases of hemorrhage. The kidney and its surroundings should be examined, the source of bleeding identified if possible, and the wound evacuated to eliminate a potential nidus of infection. If a small cortical rupture is present without venous obstruction, it may be repaired by packing with autologous muscle or microfibrillar collagen. If the rupture is large or venous compromise is present, transplant nephrectomy is almost invariably indicated.

Vascular Complications

Arterial Thrombosis

Thrombosis of the renal artery is a rare complication in the early days after transplantation, probably due both to the high flow through the kidney and also to the associated anemia and coagulation defects present in most patients with end-stage renal failure (517). It occurs in less than 1% of renal transplants. Factors that may predispose to thrombosis include preexisting hypercoagulable state, technical difficulties with the anastomosis, heavy arteriosclerotic involvement of recipient or donor vessels, kidneys with multiple renal arteries, and hypotension (518). Thrombosis may also occur owing to CsA-associated arteriopathy or because of hyperacute humoral rejection. CsA has been associated with increased thromboembolic complications, possibly because of enhancement of platelet aggregation. Thrombosis due to an error in suture technique may occur in any case, but would be extraordinarily rare in an end-to-side anastomosis performed between a patch of donor aorta to an arteriotomy in the common or external iliac artery. In the later weeks after transplantation, renal artery thrombosis may be seen secondary to arteriolar thrombosis in an acutely rejecting kidney, but the major vessel thrombosis is not the primary event. A renogram will quickly establish the presence of an arterial blood supply to the kidney if this is in doubt.

Sudden cessation of urine flow in the setting of a previously working allograft and a patent urinary catheter should suggest the possibility of renal artery thrombosis. An emergency renal ultrasound study or radionuclide renal scan will confirm the presence or absence of parenchymal blood flow. A digital subtraction angiogram is reserved for the very few cases with a "no flow" renal scan. Attempts to remove the thrombus are usually unsuccessful because of extensive intrarenal clotting beyond the main arterial branches (519).

Venous Thrombosis

Thrombosis of the renal vein as an acute event in the transplanted kidney is unusual and is usually due to a technical mishap at the time of operation. Thrombosis of the renal vein at some later period after transplantation is probably more common than is realized. It may occur secondary to thrombosis of the common iliac vein or may occur occasionally as the primary event. Venous thrombosis may be related to CsA use, but it may also occur after placement of the allograft into a tight scarred, retroperitoneal pocket after removal of a previous graft.

In the absence of the clinical features of thrombosis of the common iliac vein, thrombosis of the renal vein itself may present with proteinuria and a marked increase in the size of the kidney, but may also occur without any notable features. Partial obstruction of the iliac vein by the pressure of the allograft can produce unilateral leg swelling on the side of the graft and rarely may lead to deep venous thrombosis. If the diagnosis of deep venous thrombosis is confirmed by Doppler plethysmography or venography, anticoagulation therapy should be initiated, unless there are absolute contraindications. Venography is the best test to confirm the diagnosis.

Treatment for well localized thrombosis involves thrombectomy and revascularization. Alternatively, it can be treated with systemic anticoagulation. Treatment often is successful if the condition was due to a transient hypercoagulable state, but rarely succeeds if the process was one manifestation of severe rejection and high renal vascular resistance with low flow. In practical terms, however, by the time the diagnosis is confirmed by angiography or radionuclide scanning, salvage of the kidney is unlikely and transplant nephrectomy is the usual outcome. Deep vein thrombosis occurs with a frequency of around 10% of transplant patients. Anticoagulation of these patients for several months is required because pulmonary embolism is not an uncommon cause of death in renal transplant patients.

Other Vascular Problems

In kidneys with multiple renal arteries, thrombosis of a polar branch can lead to ureteral necrosis or segmental parenchymal infarction with potential development of a calyceal cutaneous fistula. Careful attention to these tenuous, small-caliber polar branches has decreased the incidence of these complications. In cadaveric kidneys harvested en bloc, a small aortic cuff (Carrel patch) can be made surrounding the orifices of the renal arteries. The cuff can then be anastomosed end to side to the external iliac artery, preventing any possibility of anastomotic compromise. If no cuff is available, the polar branches can be anastomosed end to side to the main renal artery, followed by anastomosis of the main artery to the recipient.

Many male patients with ESRD have erectile dysfunction because of decreased penile arterial flow, neuropathy, or both. Repeat transplant recipients should have an end-to-side

reno–iliac arterial anastomosis if the contralateral hypogastric artery was used in the first transplant.

Urologic Complications

Accurate evaluation of lower urinary tract function prior to transplantation is important to minimize postoperative urologic complications. There are many approaches to the correction of these urologic complications, but, as a general rule, the approach should be early and aggressive rather than conservative (520). Many of the complications are preventable with careful attention to the technique of donor nephrectomy.

Urine Leak

Urine leak is an infrequent but serious problem and occurs in approximately 2% of renal transplant patients. It is seen early after transplantation and is usually secondary to necrosis of the entire or distal portion of the ureter or to infarction of the renal pelvis. This usually is due to the interruption or thrombosis of the ureteral artery—the main arterial supply to the donor ureter.

The source of the leak may be from the ureter, calyces, or the bladder. Upper urinary tract leakage is due to ischemia resulting from the loss of vascular supply during organ procurement. Preservation of hilar vessels and periureteral fat and adventitia is the key to prevention of this problem. Bladder urine leak may occur at the site of the ureteral reimplant or along the cystotomy closure.

The clinical presentation of a urine leak may be subtle unless a wound drain is in place. Leakage of urine from the lower end of the ureter is not usually evident until at least 1 week after transplantation and often much later. It will be associated with a decrease in urine output, fever, local tenderness, and swelling due to the localized collection of urine known as *urinoma*. Other clinical signs include unexplained fever and edema of the scrotum, labia, or thigh ipsilateral to the graft.

Ultrasonography is the preferred study for diagnosis of a suspected urine leak. Aspiration of the fluid mass and comparison of the fluid creatinine or urea content to serum values confirms the diagnosis of urine leak. The dynamic phase of a renal scan also may demonstrate urinary extravasation. Cystography with oblique and drainage films will confirm whether the leak is from the bladder. Confirmation of upper urinary tract leakage is more difficult, as attempts at retrograde pyelography in the early postoperative period often are unsuccessful.

Urine is a strong chemical irritant to tissues and predisposes the fresh vascular anastomoses to infection. Prolonged catheter drainage may be adequate treatment for a small bladder leak. Insertion of a percutaneous nephrostomy or ureteral stent can also be used to provide initial urinary drainage and stabilization of the patient. After function returns to baseline, surgical re-exploration and repair is usually attempted. If the distal ureter is necrotic or stenotic, the necrotic portion can be removed and the vascularized proximal ureter can be reimplanted into the bladder. If the ureter is too short or the renal pelvis necrotic, the ipsilateral native ureter can be detached from the native kidney near the pelvis and connected to the renal transplant by means of a ureteropyeloplasty. The anastomosis is protected by a temporary nephrostomy and ureteral stent. If a native ureter is not available or adequate, then the bladder can be mobilized and a Boari flap ureteronephrostomy constructed, or the bladder is anastomosed directly to the kidney pelvis, and a nephrostomy tube is left in place for several weeks (209).

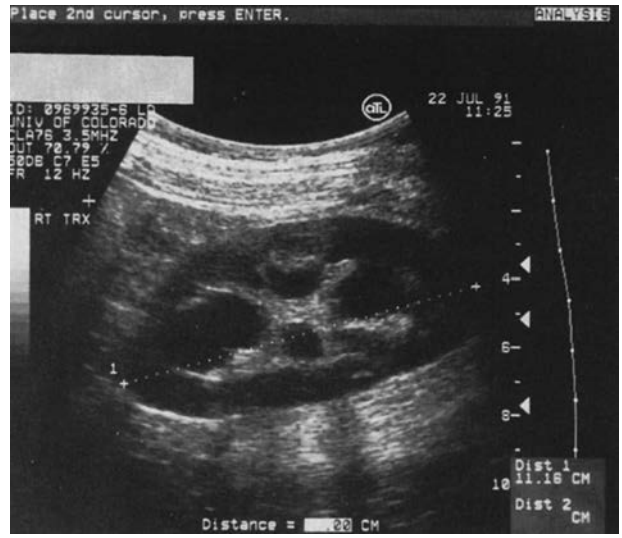

FIGURE 98-24. Ultrasound scan of renal transplant showing hydronephrosis due to ureteric obstruction.

Ureteral Obstruction

Acute ureteral obstruction in the early postoperative period may be due to distal ischemia, infarction, or rejection. Transient obstruction by clot in the ureter or bladder immediately postoperatively may cause erratic urine output and can usually be taken care of by bladder catheter irrigation. Technical error as an early cause of obstruction of the ureterovesical junction is rare. Oliguria or anuria in the immediate posttransplant period should make one suspect the diagnosis. A cystogram is usually performed first to rule out a bladder leak. The diagnosis is confirmed by the presence of hydronephrosis by ultrasonography (Fig. 98-24) or by deceased flow from ureter to bladder or evidence of extravasation on percutaneous nephrostogram (Fig. 98-25). The site of obstruction can be identified by an antegrade pyelogram. Occasionally obstruction of the ureter may be secondary to a hydrocele or hematoma, which can occur after a percutaneous needle biopsy.

Obstruction of the ureter may occur at some time remote from transplantation, often due to the development of a stricture, presumably as a result of previous ischemia. Progressive

FIGURE 98-25. Percutaneous nephrostogram of renal transplant. Nephrostomy tube placement with antegrade pyelogram to identify the site of obstruction.

stenosis of the distal transplant ureter secondary to fibrosis or chronic ischemia may present as progressive as azotemia over several months. Ultrasonography and an intravenous pyelogram or an antegrade pyelogram should confirm the diagnoses. Ureteric obstruction should always be considered in the patient with a gradual deterioration of renal function. Options for treatment include cystoscopic or percutaneous radiologic placement of an indwelling double-J ureteral stent, use of percutaneous nephrostomy, balloon dilatation, and surgical repair (521,522).

Reflux

Vesicoureteral reflux into the transplanted ureter has a reported incidence of 4% to 65%, depending on the technique of ureteral anastomosis; the creation of a distinct submucosal tunnel through the bladder wall has resulted in a lower incidence. The presence of vesicoureteral reflux in the transplanted allograft has not been found to increase the rate of urinary tract infections compared with nonrefluxing grafts (523). Mathew and co-workers (524) found an increased incidence of proteinuria, microhematuria, hypertension, and graft failure in the refluxing group, whereas Bootsma and co-workers (525) found no difference between refluxing and nonrefluxing groups with regard to proteinuria, graft function, and graft survival.

Lymphocele

The major complication associated with lymphatics is the occurrence of a lymphocele, which usually presents in the first 2 or 3 months after transplantation as a large cystic mass in the vicinity of the kidney (207,526). It is usually asymptomatic. Its presenting features are due to pressure on surrounding structures; it may cause deterioration in renal function due to pressure on the ureter, swelling of the leg, due to pressure on the iliac vein, urgency due to pressure on the bladder, and diarrhea and tenesmus due to pressure on the rectum. Ultrasonography can confirm the presence of a perinephric (lymph) collection. Studies with radiolabeled lymph reveal that the major source of fluid in lymphoceles is from the lymphatics along the recipient iliac vessels and not from the renal hilum. Therefore, meticulous ligation of the lymphatics during exposure of the vessels is the best prevention. Aspiration of the mass and measurement of fluid creatinine and potassium levels compared with the values in serum establishes the diagnosis of lymphocele and excludes urine leak, hematoma, or abscess. Sometimes, the lymphocele will not recur after two or three aspirations. However, if the lymphocele continues to recur, then it should be marsupialized into the peritoneal cavity, after checking the aspirate for urine products and bacterial growth.

SYSTEMIC COMPLICATIONS

Infection

The occurrence of infection is due primarily to the interplay between two factors: the degree of immunosuppression in the patient and the epidemiologic exposures that the patient encounters (527,528). Although the incidence of serious infection and the mortality from infection after transplantation has decreased dramatically during the last decade, infection remains a major hazard for the transplant patient, especially in the early months after the procedure (529,530). The fall in the incidence of infection is due to a number of factors, the main ones being the use of lower doses of immunosuppressive drugs and the general adoption of low-dose steroid protocols. The most common presentation of an infection in a transplant patient is fever; some guidelines to the approach to the patient with fever are given in Table 98-18. Some awareness of the times after transplantation when particular infections may occur is helpful in the diagnostic approach to a patient with a possible infection. During the first posttransplantation month, opportunistic infections are rare and the major infectious disease hazards are similar to those for patients undergoing major urologic surgery. The period between 1 and 6 months after transplantation is when most serious infections occur. This is because of the maximal effect of the immunosuppressive drugs on the host's defense system, as well as its coinciding with the period when attempts are made to reverse rejection episodes with potent antirejection therapy. As in other states of immune deficiencies, opportunistic infections derived from endogenous flora including *Cryptococcus*, *Candida Aspergillus*, *Pneumocystis carinii*, CMV, and herpes zoster are seen after transplantation. *Candida albicans*, a normal inhabitant of the oropharynx, intestine, and vagina in healthy individuals, may cause severe pharyngitis, esophagitis, vaginitis, and systemic infections in immunosuppressed patients (531). Wound infections and urinary infections are commonly due to bacterial infections. Septicemia is not uncommon after transplantation and is usually due to a Gram-negative organism with the primary focus in the urinary tract. However, *Staphylococcus aureus*, *Listeria*, and *Candida* may also cause septicemia. While awaiting the results of blood culture, appropriate broad-spectrum antimicrobial treatment should be commenced. A vigorous search for the focus of infection must be made and dealt with as appropriate.

TABLE 98-18

THE DIAGNOSTIC APPROACH TO THE TRANSPLANT PATIENT WITH AN UNEXPLAINED FEVER

Possible sites of infection
Chest: pulmonary infection, pericarditis, endocarditis
Mouth: *Candida*
Lower limb: deep venous thrombosis
Soft tissues: skin (e.g., fungi, *Nocardia*, mycobacteria), joints
Transplant wound: rejection, abscess, urine leak, hematoma
Peritoneal cavity: pancreas, colon, dialysis catheter
Urinary tract: bladder, prostate, native kidneys
Central nervous system (CNS): *Listeria*, *Cryptococcus*, aspergilli, Tuberculosis, *Nocardia*
Systemic: viral infection, tuberculosis
Investigations
Chest x-rays
Ultrasound of transplanted kidney
Cultures: mouth, sputum, urine, blood, stool, access sites
Serology: viral antibodies especially cytomegalovirus
Lumbar puncture and CT of head if CNS infection suspected

Viral Infections

Cytomegalovirus (CMV). CMV is the most important viral infection occurring in transplant recipients. The incidence and severity of CMV infection depend on the presence of latent infection in the donor, the immune status of the recipient, and the amount of immunosuppressants used (531–533). CMV infection also causes considerable morbidity and may be associated with decreased allograft survival rates. CMV infection takes two forms, namely, that of a primary infection of a secondary or reactivated infection. The primary infection is of most concern and occurs in patients who are seronegative at the time

of transplantation and received a kidney from a seropositive donor (534–536). Transfusions may also cause a primary infection in the seronegative patient, but this is far less common than the transmission of infection in the transplanted kidney (536).

The typical primary CMV infection commences as a fever that may be spiking or constant and usually occurs between 4 and 10 weeks after transplantation (537). It may be associated with a neutropenia and atypical lymphocytes may be identified in the blood smear. The leukopenia causes problems, especially in patients receiving azathioprine, where the dose will have to be reduced or often discontinued. The primary infection may lead to a CMV lung infection and less commonly to hepatitis, arthralgia, splenomegaly, myalgia, and gastrointestinal ulceration (538). A deterioration in function of the renal transplant may also be seen during the early stages of the infection (539), and a frank glomerulopathy may occur (540). In rare instances, chorioretinitis can occur 4 to 6 months after transplantation, occasionally without prior evidence of CMV activity. CMV encephalitis, transverse myelitis (541), and cutaneous vasculitis (542) also have been reported.

Patients with secondary infection or reactivation of latent CMV often are not symptomatic, whereas those with superinfection of a new viral strain have the acute symptoms of active CMV (543–549). Most patients have antibody against CMV at the time of transplantation. After transplantation, essentially all of these seropositive individuals shed virus in saliva, urine, and blood, partly as a result of reactivation of endogenous latent virus. Recipients who shed CMV after transplantation do so between the first and sixth months, whereas the peak incidence of clinical illness due to CMV occurs 2 to 3 months after grafting (549). The appearance of CMV-specific IgM or IgG in the primary infection, or a rise in titer in the secondary infection, can be used in determining infectivity. Use of DNA-restriction enzyme analytic methods to detect different CMV serotypes indicates that many of the clinical CMV infections in individuals seropositive for CMV before transplantation are due to superinfection with the donor virus strain (543,550,551).

During an active infection, CMV persists in the blood for several weeks. The presence of virus in the urine is only indicative of virus excretion. In the case of CMV pneumonia, virus can be isolated from bronchial lavage. A positive culture of the throat, urine, or blood (buffy coat) is generally sufficient evidence for the presence of active infection. Newer methods for determining the presence of viral infection include molecular hybridization using labeled viral DNA probes (552). They have been used successfully for the detection of viral DNA in blood during viremia, in urine, and in tissue biopsy specimens. The PCR technique for more rapid and sensitive diagnosis of CMV in urine and blood samples has also been used. This technique can detect much smaller amounts of virus than other methods and can be potentially done in 7 hours (542).

Prior vaccination with live, attenuated CMV vaccine did not reduce the incidence of CMV infection and disease after renal transplantation. However, there was evidence that the vaccine reduced the severity of CMV disease, especially among seronegative recipients of grafts from seropositive donors (553,554). Primary infection can be prevented by avoiding transplantation of a kidney from a seropositive patient into a patient who is seronegative (555). CMV immune globulin given after transplantation has been shown to reduce the incidence of CMV syndromes. Prophylactic administration of oral acyclovir to renal allograft recipients for 12 weeks after transplantation has been shown to reduce symptomatic active CMV infection (556). Other agents such as gancyclovir and valacyclovir have been studied. Valgancyclovir is the L-valine ester of gancyclovir and is administered orally at 450 to 900 mg per day for CMV prophylaxis. This dose produces similar AUC values to IV gancyclovir (5 mg/kg/day) and much higher values than oral gancyclovir (3 g/day). Both agents require dosage adjustment for decreased creatinine clearance. Treating all transplant recipients with CMV prophylaxis is costly and may not be necessary. Chemoprophylaxis for CMV, however, should be used according to the risk of the patient for CMV (548).

TABLE 98-19

DOSAGE ADJUSTMENT FOR INTRAVENOUS GANCICLOVIR IN THE TREATMENT OF CMV INFECTION

Serum creatinine (mg/dL)	Ganciclovir (IV)
<1.5	5 mg/kg q12hr
1.5–2.5	2.5 mg/kg q12hr
2.6–4.5	2.5 mg/kg q24hr
>4.5; No dialysis	1.25 mg/kg q48hr
Dialysis	1.25 mg/kg q24hr

The initial step in the treatment of overt CMV disease is to decrease the amount of immunosuppressive therapy being administered (557). Because of the association between CMV and *P. carinii* infection, trimethoprim–sulfamethoxazone prophylaxis is commonly given to the patients with clinical CMV disease (557). Specific treatment for CMV infection is indicated for those patients with life-threatening CMV pneumonitis or sight-threatening CMV chorioretinitis (533,558). Antiserum against CMV has been evaluated in the treatment of CMV pneumonia (559–561), both alone and in combination with ganciclovir, 9-(1,3-dihydroxy-2 propoxymethyl) guanine-DHPG, an antiviral agent that inhibits herpes virus replication (562–564). When used as single-agent treatment, CMV immunoglobulin did not provide optimal therapy for CMV pneumonia in transplant patients. The efficacy of ganciclovir has been shown in the treatment of pneumonia in bone marrow recipients. CMV pneumonitis and retinitis in renal transplants can also be ameliorated by ganciclovir. The major side effects of ganciclovir are bone marrow suppression, sterility, and potential nephrotoxicity (562–564). Dose adjustment is necessary for patients with renal impairment (Table 98-19).

Herpes Simplex Virus (HSV). Reactivation of latent herpes simplex virus infections is extremely common in transplant patients. The most common lesion is the orolabial HSV type 1 infection. Occasionally, anogenital lesion due to HSV type 2 infection may occur. Rarely, Kaposi's varicelliform eruption, due to disseminated HSV infection in the skin, may develop in transplant patients. Therapy of acute HSV infection with acyclovir will lead to clinical improvement (565,566).

Varicella Zoster. Varicella zoster is frequently seen in transplant patients occurring at any time after transplantation. It is commonly presented as localized zoster due to reactivation of latent virus present in dorsal root ganglion since childhood chickenpox. Intravenous acyclovir is the treatment of choice. Chickenpox is a rare but often extremely virulent infection. Should a patient without humoral immunity to varicellar zoster be exposed to chickenpox, varicella zoster immune globulin should be given within 72 hours of the exposure. If chicken pox develops, intravenous acyclovir needs to be instituted without delay (567).

Epstein–Barr Virus (EBV). In general, EBV is not a major problem in transplant patients, although occasionally EBV may be the cause of a glandular feverlike illness. There has been some concern that patients immunosuppressed with CsA may be

particularly prone to EBV infections because of the suppression of the generation of T cytotoxic cells against EBV-infected B lymphocytes. This, in turn, may cause an acute lymphoproliferative syndrome or even a polyclonal lymphoma (568,569). Using DNA hybridization technique, EBV has been identified in lymphoma and lymphoproliferative lesions of renal transplant patients (570).

Human Immunodeficiency Virus (HIV). The impact of HIV infection and acquired immunodeficiency disease (AIDS) on recipients of organ transplantation has not yet been fully realized. Immunosuppression may be associated with progression of the infection (571). As the population of asymptomatic HIV-seropositive individuals increases, it is of concern whether renal transplantation of these individuals with concomitant chemical immunosuppression hastens the course of HIV infection (572,573). Surprisingly, few cases of HIV infections or AIDS in transplant patients have been reported. In general, the same clinical spectrum of disease as found in the normal population (574,575) is seen. The transmission of HIV by blood transfusion and by the allograft itself has been well documented. In a case report of four individuals who received kidneys from donors with histories of intravenous drug use, HIV antibodies appeared 2 months after transplantation in two patients and were present in all four at 8 months. It is still unclear how patients who are HIV antibody positive, either before or after transplantation should optimally be managed. A retrospective survey of 1,043 transplant patients (all organs) at the University of Pittsburgh noted a 0.7% incidence of HIV seropositivity in recipients before transplantation. Of 860 individuals who were seronegative at transplantation, 11 converted at a mean time of 96 days after transplantation. One-half of the seropositive patients died within 6 months after surgery and the other one-half were alive a mean of 43 months after transplantation (576). Hence, the Pittsburgh experience contributed to the enigma as to whether or not HIV antibody-positive patients should be excluded as transplant candidate. This report serves to emphasize that a large and more complete database is essential before it will be possible to design optimal policies and clinical practices for managing transplant candidates who are HIV antibody positive and for treating transplant recipients who are either HIV antibody positive or have AIDS.

Polyomavirus. Both BK virus (Polyomavirus hominis) and JC virus (Polyomavirus hominis) belong to the human papovavirus family. They are nonenveloped, double-stranded DNA viruses. Both viruses have been cultured from the urine of transplant recipients. The excretion of BK virus is associated with ureteral stricture and interstitial nephritis and that of JC virus with progressive multifocal leukoencephalopathy. About 60% to 80% of adults are seropositive for BK virus. In immunocompetent individuals, the virus has no great clinical significance. The virus resides in a latent state in the kidney in healthy individual and can be activated without functional impairment or ill effects. Morphologic evidence of viral activation is the presence of polyomavirus-infected cells in the urine. In immunocompromised patients, polyomavirus can cause a morphologically manifested renal infection with cytopathic signs and functional impairment. In native and transplanted kidneys, BK virus is found in areas of interstitial nephritis. In addition, renal allograft recipients were reported to have BK virus associated with ureteral stenosis and bone marrow transplant recipients from hemorrhagic cystitis. BK virus infection in renal transplant recipients has recently become recognized more frequently. It presents clinically as an acute rejection that appears unresponsive to treatment (577–581). The biopsy specimens have changes of severe tubulointerstitial nephritis (582–584). The presence of polyomavirus infection is suggested by the finding of large basophilic intranuclear viral inclusion bodies in tubular epithelial cells along the entire nephron and also the transitional cell layer. The affected tubular cells were enlarged and often necrotic. Confirmation of the diagnosis can be made by special staining with polyomavirus monoclonal antibody. Urine samples from patients with persistent infection revealed positive PCR with abundant amplicons of 149 bp indicative of BK virus and urine microscopy showed decoy cells with ground glass-type intranuclear inclusions positive for BK virus by immunohistochemistry and electron microscopy. A major factor involved in the manifestation of BK virus disease is high-dose immunosuppression. No established therapeutic therapy for polyomavirus infection is available and reduction of immunosuppression offers the best therapeutic option. Tacrolimus as a possible risk factor has been suggested in the past. Most infected patients have been taking tacrolimus and alternative agents can be used if response is inadequate. Leflunomide, cidofovir, IVIG, and corticosteroids may be of clinical benefit and have been reported in uncontrolled studies. Leflunomide (orally 20 to 40 mg daily) has been recommended as the drug of first choice because of its combined antiviral and immunosuppressive properties and its lack of nephrotoxicity

Hepatitis

Chronic liver function impairment is not rare after renal transplantation (585–587). Viral hepatitis and drug-related hepatitis are the most common causes. Despite the exclusion of patients with hepatitis B surface antigen from organ and blood donation, the incidence of chronic liver disease after transplantation has remained high with abnormalities of liver function occurring in 7% to 24% of patients early in follow-up and death due to liver failure in 8% to 28% of the long-term survivors of renal transplantation. It is also important to rule out other causes of hepatitis. Drugs that may cause hepatic dysfunction include CsA, azathioprine, antihypertensives, and lipid-lowering agents. CsA-induced liver enzyme elevation usually resolves on dosage reduction. Azathioprine is also thought to be hepatotoxic (588). Reduction of azathioprine dose in patients with chronic hepatitis may be necessary (589). In patients with impaired liver function, cyclophosphamide at a dose of 1 mg/kg has been used in the past to replace azathioprine (590). In patients taking CsA or tacrolimus, azathioprine can be discontinued or substituted with MMF.

Hepatitis B Virus (HBV). Hepatitis B virus is a relatively uncommon viral infection after transplantation, but the main cause for concern is the outcome of transplantation in a patient who is a carrier of the hepatitis B antigen. Although graft survival in such hepatitis-B carrier patients is perhaps better than in other patients, reflecting some innate immunosuppressive defect in the patient, there is considerable concern about the possible progression of liver disease leading to liver failure. Furthermore, the incidence of hepatoma in those chronic carriers of hepatitis B is 15%, much higher than in the general population who contract hepatitis. It has suggested that immunosuppression enables persistent viral replication, leading to a greater frequency of hepatitis e antigen, viral DNA, and viral DNA polymerase in the sera of transplanted individuals.

The natural history of liver disease due to chronic hepatitis B in transplant patients differs from that in both the general population and hemodialysis patients (591,592). Transplant recipients who have hepatitis B typically remain surface antigen-positive for longer than 6 months and do not revert to seronegativity. Most episodes of hepatitis in the early post-transplantation period are relatively mild, but an unusually high rate of transformation from chronic persistent to chronic active hepatitis occurs in this patient population. Patients who have persistent hepatitis e antigenemia or concomitant delta

virus infection are at higher risk for chronic active hepatitis and more rapid deterioration.

Because of the poor conversion rate in patients with ESRD, hepatitis B vaccination of patients should be given early in the course of progressive renal failure. Previously vaccinated patients who are HBsAg-negative should be tested annually for anti-HBV antibodies and should receive booster vaccinations when the titer decreases to <10 mIU/mL. No known loss of graft function has occurred as a result of active vaccination with hepatitis B vaccine (593,594). HBsAg-positive transplant recipient should receive lamivudine (100 mg/day) starting at the time of transplantation and continued for at least 18 to 24 months. Two small studies demonstrated good responses to treatment with lamivudine for renal allograft recipients, with clearance of HBV DNA and normalization of transaminase levels (595,596).

Hepatitis C Virus. Anti-HCV antibodies have been reported for 10% to 40% of renal transplant recipients. HCV is the major cause of non-A/non-B hepatitis. Patients with ESRD are at increased risk for HCV infection because of their continued exposure to blood and blood products and horizontal transmission within hemodialysis units (150,592,597–601).

A national collaborative study of 3081 cadaver organ donors from eight organ procurement organizations in the United States found that the mean prevalence of positive ELISA-1 for anti-HCV antibodies was 5% and the prevalence of circulating HCV RNA as detected by PCR was 2.4%. In comparison, the prevalence of anti-HCV among healthy blood donors in the United States is only 0.6%. The higher prevalence among cadaver donors may reflect an increased incidence of risk factors associated with the spread of the viral infections. Stored sera from 716 consecutive cadaver donors in the New England organ Bank were screened for anti-HCV antibodies using ELISA-1 assay and 13 anti-HVC positive donors were identified. Twenty-nine recipients of organs from these anti-HCV donors were identified and studied. Fourteen (48%) of these recipients developed non-A and non-B hepatitis after transplantation; 12 had chronic liver disease. Posttransplantation sera tested positive for HCV RNA in all of the recipients from HCV RNA-positive donors, suggesting that almost all patients are at risk in this setting. However, standard clinical evaluation may not detect HCV infection as 52% of the patients did not have any evidence of hepatic disease and seroconversion occurred in only 62%. Following antibody titer will underestimate both the transmission of HCV and the role of HCV in posttransplantation liver disease (136,602,603). An increased incidence of acute renal allograft dysfunction is associated with α-interferon treatment among kidney transplant recipients (604,605). This is because interferon can induce cytokine gene expression, increase cell surface expression of HLA antigens, and enhance the function of natural killer cells, cytotoxic T cells, and monocytes. Hence, therapy with α-interferon carries the risk of inducing rejection in the allograft. Thus the risk of rejection must be weighed against the potential benefits of slowing or preventing the progression of chronic liver disease (606). Therefore, a better strategy might be to screen and treat HCV with interferon (and possibly ribavirin) before, rather than after, transplantation (607).

Pulmonary Infection

The overall incidence of pneumonia in renal transplant patients has decreased from 25% to 8% since 1980. The decrease in the incidence of pulmonary infections is related to the judicious use of immunosuppressive therapy and more aggressive treatment of infection (608). Nevertheless, infection of the lungs is still the most common form of life-threatening infections in renal transplant patient.

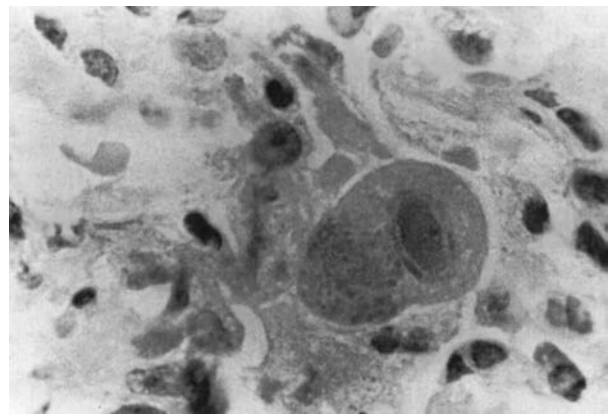

FIGURE 98-26. Typical cytomegalovirus-infected lung cell showing cytomegaly, large intracellular inclusions with peripheral chromatin clumping, and abundant intracytoplasmic inclusions. (Hematoxylin and eosin stain, magnification ×1,000.)

Opportunistic infections occur less commonly many years after transplantation, especially, in the patient who has good renal function and is on minimal immunosuppression. However, pneumococcal pneumonia can occur at any time and may have a very rapid progression, leading to death if not diagnosed and treated promptly. Finally, the definitive diagnosis can only be established by identification of a pathogen and it is important that an aggressive approach be adopted for obtaining specimens for microbiologic examination.

CMV Pneumonia. Lung infection is a serious complication of CMV infection. In the patient who presents with a fever and radiologic pulmonary changes between 1 and 4 months after transplantation and who was seronegative at the time of transplantation and had received a kidney from a seropositive donor, an initial diagnosis of CMV infection of the lung should be made once all other possible causes of the fever have been excluded. *Pneumocystis carinii* may also occur as a superinfecting organism during active CMV infection (557). If significant leukopenia develops, in association with the infection, then immunosuppressive drugs may have to be reduced. If possible, treatment should be withheld until a pathogen can be identified. If no indication of the diagnosis is available, bronchoscopy and bronchial alveolar lavage or open lung biopsy should be performed (Fig. 98-26).

Pneumocystis Carinii Pneumonia. This is a relatively common cause of pulmonary infection (609). The patient usually presents with a fever, often associated with some dyspnea, but with very few physical signs on examination. Chest x-ray shows diffuse shadowing that tends to be linear in distribution. Patients with suspected pneumocystis pneumonia should have blood gas and vital signs closely monitored. Cotrimoxazole in high doses is the antimicrobial of choice. Prophylactic cotrimoxazole is commonly used during the first few months after transplantation because of the high incidence of this infection (557).

Legionella Pneumonia. Legionella pneumonia has become much more common in recent years, or rather it is probably being identified more often. It proliferates in stagnant water and so may be spread by way of air conditioning, plants, and showers (610). The chest x-ray in the early stages shows irregular, nodular shadows that progress, often quite rapidly, to a lobar or diffuse consolidation. Identification of the organism is difficult with conventional staining techniques, but may be

identified with direct immunofluorescent staining and may be grown from appropriate sputum or biopsy samples on specialized media. A high level of suspicion for this infection is needed and erythromycin, in high doses, is the drug of choice.

Mycobacterial Infections

The incidence of tuberculosis in transplant recipients varies from region to region, but certainly is more common in transplant recipients than in the general population (611). The symptoms are frequently nonspecific and the site of infection is often in organs other than the lungs. Treatment of the established case should be the routine antituberculous therapy (e.g., rifampicin and isoniazid). It should be remembered that both these drugs are metabolized in the liver; rifampicin induces hepatic enzymes, therefore, if the patient is being immunosuppressed with CsA or FK506, the dose should be closely monitored. Chemoprophylaxis should be considered in patients with calcification on a chest roentgenogram and in the presence of a positive tuberculin skin test. Therapy for 6 to 9 months with isoniazid should be given to patients who have never received adequate treatment and who are PPD positive (612). Guidelines for targeted tuberculin testing and treatment of latent TB were recently developed jointly by the American Thoracic Society and the Centers for Disease Control and Prevention (613). The PPD (Mantoux) skin test involves an intradermal injection of 5 units of tuberculin PPD, with examination of the injection site 48 to 72 hours later. For individuals at very high risk, the minimal criterion for a positive skin test is a 5 mm diameter (including patients receiving immunosuppressive therapy).

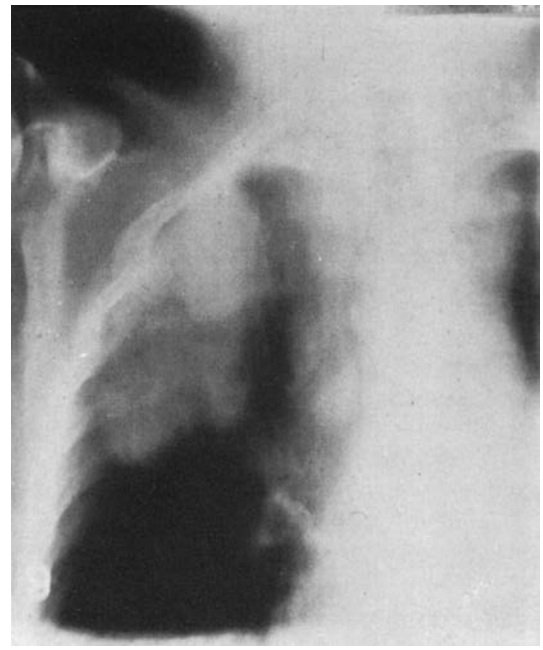

FIGURE 98-27. *Aspergillus* infection of the lung in a patient who underwent renal transplantation after several courses of antirejection therapy with high-dose intravenous methylprednisolone. (From: Morris PJ. *Kidney Transplantation: Principles and Practice*. 2nd ed. New York: Grune & Stratton; 1984, with permission.)

Fungal Infections

Fungal infections are relatively common in transplant patients and must always be considered as a possible cause of fever and pneumonia, especially in the presence of excessive immunosuppressive therapy (614). Pulmonary infiltrates due to fungal infection include *Aspergillus*, *Cryptococcus*, *Coccidiodes*, *Candida,* and histoplasmosis. *Aspergillus* is a hyphal saprophytic fungus in which infection is by inhalation of spores and the lungs are, therefore, the primary site of infection. In the lung, *Aspergillus* causes a patchy infiltration followed by a consolidation and abscess formation (Fig. 98-27). Histoplasmosis is another fungal pneumonia, which can occur in renal transplant recipients. This may also be acquired or result from reactivation and usually presents with fever, pulmonary infiltrates, and skin lesions at any time after transplantation. These infections require aggressive therapy with conventional amphotericin B, a lipid-based amphotericin B preparation (Abelcet, AmBisome, or Amphotec) (615), or an appropriate azole antifungal agent. Ketoconazole, fluconazole, and itraconazole are useful for treating mucocutaneous fungal infection and infection of the gentitourinary tract and gastrointestinal system, lungs and, under specific conditions, the central nervous system. All of the triazole antifungals impair calcineurin inhibitor metabolism and increase blood levels of CsA and tacrolimus. CsA or tacrolimus dose reduction, therefore, may be necessary while patients are on triazole treatment (616).

Central Nervous System (CNS) Infection

Infections of the CNS after renal transplantation typically present between 1 and 12 months posttransplant and are characterized by a subacute onset and the frequent lack of systemic signs. Organisms commonly associated with CNS infection in renal transplant recipients include *Listeria*, *Cryptococcus*, *Mycobacterium*, *Nocardia*, *Aspergillus*, mucormycosis, *Toxoplasma*, *Candida*, and *Strongyloides*. *Listeria* may cause an acute or focal brain infection. *Cryptococcus* and, less often, *Mycobacterium* and *Coccidioides* are important causes of subacute meningitis. Focal lesions are most common with *Aspergillus*, *Toxoplasma*, and *Nocardia*. HIV can cause a variety of CNS syndromes, most predominantly a global-dementing illness. JC virus infection can also cause dementia with progressive multifocal leukoencephalopathy (617).

In acute meningoencephalitis, nuchal rigidity may be absent. The development of fever and mild headache should be sufficient to alert the physician to the possibility of CNS infection. The aseptic meningitis that occurs during OKT3 administration is self-limited, but if severe or persistent, may require diagnostic workup to rule out infection. Focal findings on neurologic examination are not common except with well-developed focal brain infections. Because the early findings in these infections are often nonspecific, lumbar puncture and cranial CT scanning or MRI should not be delayed.

Aspergillus. *Aspergillus* may cause pneumonia in the immunocompromised host and may disseminate to the brain, skin, kidney, and gut. *Aspergillus*, which infiltrates the vasculature, is not found free in the cerebrospinal fluid (CSF) and is often impossible to diagnose before death. The organism may be suspected in patients with clinical evidence for meningitis and CSF cytology and chemistry determinations consistent with meningitis, especially in the absence of a positive culture, inflammatory foci, and culture or serologic findings consistent with cryptococcal infection. The treatment of choice is amphotericin B.

Cryptococcus. Although rare, *Crytococcus* is another cause of meningitis in the transplant patient. It tends to be seen relatively late in the transplantation course and has a rather nonspecific presentation, and, hence, the diagnosis is often delayed. Lung involvement is also common when this infection is present. Amphotericin B is again the indicated treatment.

Coccidioides. *Coccidioides* is quite rare in Europe but does occur commonly in parts of the United States. It may cause

destructive lesions of the lungs, liver, brain, and spleen and is sometimes due to reactivation of an existing latent infection. Amphotericin B is the appropriate treatment.

Listeria Monocytogenes. *Listeria monocytogenes* may present as meningitis, brain abscess, or as meningoencephalitis. It may occur at any time after transplantation, but is usually associated with increased or excessive immunosuppressive therapy for rejection. *Listeria* should be the primary suspect in a patient with meningoencephalitis because other causes of meningitis are rare in transplant recipients. CSF findings may not be striking. Treatment with ampicillin should be commenced as soon as cerebrospinal fluid (CSF) and other specimens for culture have been taken.

Nocardia. *Nocardia* is usually present as respiratory illness with an unproductive cough, fever, malaise, and a nodular infiltrate on the chest x-ray. Occasionally, the infection may spread to the brain presenting as a space-occupying lesion, but may also be seen as skin abscesses or joint infections. The treatment of choice is probably sulfonamide, which is given for at least 2 months, although some would advocate treatment for 12 months.

Urinary Tract Infection

Urinary tract infection is the most common bacterial infection following transplantation. The incidence however has improved in recent years, most likely due to increased attention to urologic techniques and to the use of antibiotic prophylaxis after transplantation (618–620). Urinary tract infections appearing within the first 3 or 4 months after transplantation are often associated with transplant pyelonephritis, septicemia, and relapse after standard antibiotic therapy. Patients with an anatomic abnormality requiring urinary diversion or stent placement and those with pyelonephritis should receive chronic suppressive antibiotics in addition to the 4- to 6-week course of primary treatment. Uncomplicated urinary tract infections that occur later after transplantation can be treated with a standard 1- or 2-week course of oral antibiotics.

Cancer in Transplant Patients

The incidence of cancer in transplant recipients varies considerably from region to region, ranging from a low incidence of 1.6% of patients developing cancer after transplantation in Europe to as high as 24% of patients in Australia (621–623). Much of this variation is due to the high incidence of skin cancer in those areas at risk for these cancers. In regions with limited exposure to the risk, there is a four- to sevenfold increase, but in areas with copious sunshine there is an almost 29-fold increase in incidence as compared with the control population. There is also a well recognized and highly significant increase in the risk of developing a malignant (non-Hodgkin's) lymphoma. Even with skin cancers and malignant lymphomas excluded from the analysis, there is an increased incidence in all forms of cancer in patients after transplantation (624–626). (Table 98-20). Careful physical examination to detect the common malignancies is essential in the long-term follow-up of renal transplant patients. The increased incidence of cervical cancer in females after transplantation implies that all female transplant patients should have an annual cervical smear.

A number of factors contribute to the increased risk of cancers in immunosuppressed recipients of a kidney transplant. These include depression of immune surveillance, chronic antigenic stimulation in the presence of immunosuppression, a directly neoplastic action of the immunosuppressive drugs themselves, and increased susceptibility to oncogenic viral infection. First, alterations in the immune surveillance due to immunosuppressive therapy may allow potentially malignant cell mutants to become established in the host, because they cannot be detected and killed in the usual fashion. The allograft with its foreign HLA may also stimulate the host lymphoreticular system, resulting in the development of lymphoid malignancies. It has been suggested that ALG or ATG may have an etiologic role in the production of neoplasia. These agents produce immunosuppression by eliminating certain types of lymphocytes involved in immune reactions. Recently, the incidence of lymphoma has increased with the use of monoclonal OKT3 antibodies for immunosuppression (627,628). Finally, latent oncogenic viruses may be activated in immunosuppressed hosts who are simultaneously experiencing stimulation immunologically by antigen. An association exists between papilloma virus and the development of squamous skin cancer, as well as condyloma acuminatum with cervical carcinoma. Epstein–Barr virus has also been implicated in polyclonal B cell lymphoproliferative disease. In addition to primary cancer developed *de novo* in patients after transplantation, cancer may be transferred in the transplanted kidney from a donor with cancer undetected at the time of donor nephrectomy.

Skin cancer is the most common neoplasia in transplant patients, with an incidence four to 21 times the population average (622,623,629). Squamous cell carcinoma predominates over basal cell skin cancer (630). Patients who live in warm climates should be carefully advised after transplantation to use sun-blocking creams and to wear appropriate clothing while in the sun. The appearance of neoplasia can be atypical and early biopsy of any suspicious lesion is indicated. The prognosis after resection of skin cancer is excellent, provided strict avoidance of sun exposure is followed. Reduction in immunosuppression may be considered if the malignancy is extensive or rapidly progressive.

Lymphoma occurs earlier than other tumors and accounts for 20% to 30% of posttransplant neoplasms. The incidence of this neoplasm is relatively higher in the last decade probably related to the use of monoclonal or polyclonal globulin and other immunosuppression. Two types of lymphoproliferative disease are seen in patients after transplantation. The first

TABLE 98-20

COMMON MALIGNANCIES ENCOUNTERED IN RENAL TRANSPLANT RECEIPTS

Cancers of Skin and Lips
Squamous cell carcinomas
Basal cell carcinomas
Malignant melanoma
Malignant Lymphomas
Non-Hodgkin's lymphoma
Reticulum cell sarcoma
B-cell lymphoproliferative syndrome (Epstein–Barr virus)
Kaposi's Sarcoma
Cutaneous form
Visceral and cutaneous form
Genitourinary Cancer
Carcinoma of native kidney (acquired cystic disease)
Carcinoma of transplanted kidney (hypernephroma)
Carcinoma of the urinary bladder (cyclophosphamide associated)
Uroepithelial tumors (associated with analgesic nephropathy)
Gynecologic Cancer
Carcinoma of cervix
Ovarian cancer

presents with an infectious mononucleosislike illness within the first year of transplantation with fever, sore throat, and lymphadenopathy. The clinical course is often short and can be fatal. Cessation of the immunosuppression will lead to regression in some patients. This type of lymphoproliferative disease is due to infection with EBV. With acyclovir treatment, remission can be achieved without cessation of immunosuppression. The second group of lymphoproliferative diseases presents as localized solid tumor masses and is confined to central nervous system in a high percentage of patients. Lymphoma, therefore, should be considered in the differential diagnosis of any CNS abnormality (631). These lymphomas are often more rapidly progressive than seen in the normal population and, although responding to conventional therapy for non-Hodgkin's lymphoma, remission tends to be short. In addition to standard established treatment for each malignancy, consideration must be given to reduction or cessation of immunosuppressive medications (632). Azathioprine usually is discontinued during chemotherapy. Many of the therapeutic agents are cytotoxic and additive suppression of the bone marrow can occur. In most cases, regression does not appear to occur with cessation of immunosuppression and the patients do not respond to acyclovir (633–637).

The incidence of Kaposi's sarcoma is 300 to 400 times that of the normal and accounts for 5% to 10% of post-transplant neoplasms. Those with Kaposi's sarcoma involving only the skin do better than those with visceral disease, with complete remission in 50% compared with 14%, respectively, after chemotherapy or cessation of immunosuppression. A third of the remissions in Kaposi's sarcoma confined to the skin occurred with discontinuation of immunosuppression as the sole therapy (638,639).

Gastrointestinal Complications

Gastrointestinal complications include peptic ulceration, esophagitis, intestinal or colonic perforation and hemorrhage, pseudomembranous colitis, necrotizing enterocolitis, and diverticulitis.

Complications of a peptic ulcer, either hemorrhage or perforation, are associated with a high mortality in the transplant patients. About 4% of deaths after transplantation were reported to be caused by gastrointestinal hemorrhage. Of these deaths, hematemesis or melena is seen in 75% of patients on presentation, whereas pain is the presenting symptom in 20%, and gastroduodenal perforation in about 10%. The most significant risk factor is a history of peptic ulcer disease (640,641). Whereas about 8% of patients with negative peptic ulcer histories before engraftment later develop gastroduodenal complications, 19% of those with previous episodes of uremic gastritis develop further complications after transplantation. For this reason, many centers actively screen patients for evidence of peptic ulceration before accepting for transplantation and, in the past, have been quite aggressive about the management of these patients, many being treated surgically before transplantation. Although this would still be the treatment of choice for active ulceration not responding to treatment or recurrent ulceration after treatment, most patients would now be given histamine antagonists prophylactically during the first few months after transplantation in the presence of a past history of ulceration but with no evidence of active ulceration at the time of transplantation. Both hemorrhage and perforation from a peptic ulcer after transplantation should be treated promptly and aggressively by surgery.

Infection of the gastrointestinal tract presents commonly as *Candida* stomatitis or esophagitis. This is particularly common in transplant patients who are debilitated from other complications or infections or in the presence of leukopenia or excess immunosuppressive therapy. Esophageal candidiasis is probably the most severe form of local infection due to this pathogen, but occasionally a septicemia may ensue. The epiglottitis and esophagitis respond to local nystatin, but more severe infections should be treated with amphotericin B or fluconazole. Classic enteric pathogens are not notably common after transplantation.

Spontaneous perforation of the small intestine is rare and the etiology is often not understood, although CMV infection, obstruction, intestinal ischemia, and the use of steroids have been implicated (642). Hemorrhage of the large bowel with ulceration and perforation occurs in 0.9% of such patients. Possible causes include uremia, the effects of immunosuppressive therapy, use of antibiotics, atherosclerosis, and the sequelae of irradiation. Administration of sodium polystyrene resin in sorbitol to treat patients with hyperkalemia can also be complicated by colonic perforation (643).

Pseudomembranous colitis is an antibiotic-associated diarrhea and thus may occur in transplant patients who are receiving broad-spectrum antibiotic therapy for a concomitant bacterial infection. However, it may also occur in transplant units where *Clostridium difficile* infection is endemic (644). This condition is highly infectious and should be treated as such to avoid spread within a transplant unit. Occasionally a necrotizing enterocolitis with gangrene of part or all of the colon, and even occasionally involving only the small bowel, is seen. This is inevitably fatal and the cause is uncertain, although it has been associated with CMV infection. Solitary ulcers, which may bleed or perforate, may also be encountered, especially in the cecum. Colonoscopy is a useful diagnostic tool in some of these colonic complications.

Diverticulitis is no more common in the transplant patient than the normal population except perhaps in patients with polycystic kidneys, but complicated diverticulitis does again present a very serious problem with a high mortality. For this reason, some surgeons believe that the presence of diverticulosis in patients before transplantation is an indication for colectomy in order to avoid complications arising after transplantation.

Pancreatitis

Although mild hyperamylasemia without pancreatitis is common in patients with poor graft function, due to decreased clearance of the enzyme, high serum amylase and lipase levels suggest active pancreatitis (645). Acute pancreatitis has been reported to occur in 2% to 12% of transplant recipients. Several causes have been considered. Inflammatory changes, possibly due to secondary hyperparathyroidism, may be seen in the glands of uremic patients. Microscopic examination occasionally has revealed changes consistent with the presence of CMV, but the role of this organism is unknown. Corticosteroids may produce pancreatitis both experimentally and clinically. Azathioprine is a rare cause of pancreatitis (646). Recently, there are case reports of pancreatic insufficiency and pancreatitis in a small fraction of patients on CsA (647). The pancreatic abnormalities due to CsA may be dose related since the abnormality improved with reduction of the CsA dose. Acute pancreatitis in renal transplant patients often follows a fulminating course with an acute abdomen, electrolyte disturbances, tetany, jaundice, and hypotension (647,648).

Cardiovascular Complications

Cardiovascular Disease

Cardiovascular disease is a major cause of morbidity and death after renal transplantation. With the increasing transplantation

of more elderly and diabetic patients and the longer survival of grafts, cardiovascular disease will take an increasingly prominent place in the problems of patents with successful renal transplants (649,650). Much of this risk is determined by and the cause of the underlying disease for renal failure, and, certainly, patients with primary diagnoses of hypertension or diabetes mellitus fare significantly worse in this respect than others. The cardiovascular disease can be attributed to the high prevalence of vascular pathology characteristic of both these diseases and which is independent of their end-stage renal failure. Nevertheless, once the patient has been transplanted, it is essential that rigorous advice be given to a correction of any risk factors that would increase the risks of cardiovascular disease, namely, hyperlipidemia, hypertension, cigarette smoking, obesity, and carbohydrate intolerance.

Hyperlipidemia

It has been known for some time that uremic patients frequently have type IV hyperlipidemia with marked hypertriglyceridemia. Total cholesterol is usually normal or low. In particular, high-density lipoprotein (HDL) levels are abnormally low (651–653). After transplantation, the hypertriglyceridemia of uremia shifts toward hypercholesterolemia. Very low-density lipoprotein and low-density lipoprotein cholesterol are elevated in transplant patients. Hypertriglyceridemia may persist, but triglyceride levels often decrease. Overall, the incidence of hyperlipidemia following transplantation is about 50% (654–659).

High dose prednisone may contribute to the development of hypercholesterolemia. Normalization of serum lipids, however, including HDL cholesterol, occurs after reduction of the initial steroid dose. HDL levels increase and become normal, with normal proportions of HDL3 and HDL2 (656). Studies imply that prompt tapering of prednisone dose after transplantation facilitates a return to normal lipid concentrations.

Dietary therapy should be initiated during the first 6 months after transplantation when hypercholesterolemia is most often marked. Patients should be advised to avoid high-calorie, high-carbohydrate and high-fat diet (657). Supplementation of the diet with omega-3 fatty acids may reduce triglyceride and cholesterol levels, and increase HDL levels. If hypercholesterolemia persists beyond 6 months on diet therapy and on maintenance steroid dose, drug therapy should be considered.

Potential pharmacologic agents include niacin, bile-binding resins, gemfibrozil, and lovastatin. Niacin lowers triglyceride and cholesterol levels. A slow-release preparation of niacin may reduce the side effects of flushing and gastrointestinal distress. Bile-binding resins can also be used, but may interfere with immunosuppressive drug absorption. Gemfibrozil primarily reduces triglyceride levels; but it can lower cholesterol when triglyceride levels are normal. Lovastatin inhibits 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis, and is effective in reducing cholesterol levels. Liver enzymes should be monitored in all patients receiving niacin, gemfibrozil, and lovastatin because hepatitis is a major adverse effect. Reports have been made of myositis and myalgia occurring at low frequency secondary to gemfibrozil and lovastatin. An increased risk of myositis has been described in those patients receiving CsA who also were treated with lovastatin (658–662).

Hyperhomocysteinemia

The association between fasting plasma total homocysteine levels and cardiovascular disease has been demonstrated by retrospective and prospective studies in the general population (663). A case control study demonstrated that renal transplant recipient with cardiovascular disease exhibited higher fasting total homocysteine levels (>10 μM) compared with renal transplant recipient without cardiovascular disease. Cross-sectional studies of renal transplant recipients also confirmed a similar association between total fasting homocysteine levels and cardiovascular disease. Since renal transplantation patients typically have fasting total homocysteine levels that are twofold higher than those of age- and gender-matched control subjects, the incidence is high enough to warrant screening (664,665). Hyperhomocyteinemia can be safely and effectively reduced by folate, vitamin B_6 and vitamin B_{12}. However, the lack of well-designed interventional trials make it difficult to recommend routine screening (666,667).

Hypertension

Hypertension is extremely common after renal transplantation. It is more common in recipients of cadaver grafts than those receiving living related grafts, suggesting that rejection is a significant factor in this high incidence of hypertension after transplantation (668–670). Hypertension before and after engraftment probably contributes to the accelerated vascular disease seen in patients with ESRD and is associated with reduced graft function (671). Hypertension in the transplant recipient may be due to the patient's diseased native kidney, rejection of the transplanted kidney, renal artery stenosis in the transplanted kidney, corticosteroids, and CsA therapy (672,673).

The relationship between hypertension and activity of the renin–angiotensin system in patients with a renal transplant is confusing (674,675). It is apparent that the patient's native kidneys may contribute to hyperreninemia, but conflicting reports exist concerning the role of the renin—angiotensin system in the transplanted kidney as a cause of hypertension. Native kidney disease and chronic rejection are the two most common causes of hypertension in the transplant population, whereas hemodynamically significant renal artery stenosis accounts for about 5%.

Steroid therapy certainly contributes to hypertension, although this is less likely now that low-dose steroid protocols are used by most centers. It is now apparent that the incidence of hypertension in patients being treated with CsA, either with or without steroids, is greater than that seen in patients treated with prednisone and azathioprine (676). The degree which CsA might increase blood pressure is dose dependent, as demonstrated by the fact that there is a generally decrease blood pressure following the reduction of the CsA dose to a maintenance therapy levels of 4 mg/kg/day.

Initial management of hypertension in patients with stable graft function includes salt restriction, weight reduction, elimination or reduction of medications that may contribute to hypertension, and the use of antihypertensive agents.

Most standard therapies have been demonstrated to be safe and effective after renal transplantation. There are, however, a number of management issues that are unique to transplant recipients. Transplant patients may be more prone to decreased renal function resulting from diuretic use than are hypertensive patients in the general population. Patients may occasionally develop decreased renal function after angiotensin-converting enzyme (ACE) inhibitor therapy, especially if the patients exhibit renal artery stenosis or chronic allograft nephropathy. Anemia and hyperkalemia may also be associated with the use of ACE inhibitors and angiotensin II (Ang II) receptor antagonists. Several studies however have shown that these drugs are generally safe, effective, and well tolerated. They may reduce proteinuria and stabilize the deterioration in renal function in chronic allograft failure, possibly by reducing TGF-β. They may also have additional benefit in reducing the incidence of cardiovascular events in high risk patients.

ACE inhibitors, such as captopril or enalapril, are useful in treating post-transplantation hypertension in patients who do not have transplant artery stenosis. Calcium channel blockers are being used more frequently in the treatment of hypertension. The hemodynamic effects of CsA include decreased effective renal plasma flow and increased renovascular resistance. Calcium channel blockers such as verapamil have been shown to affect glomerular filtration rate and PGE2 production (677). It has been suggested that the use of calcium channel blocker may have additional cytoprotective effect against ischemic and nephrotoxic injury in the transplanted kidney. Since calcium and its carrier protein, calmodulin, may be involved in various T cell functions, calcium channel blockers and CsA may be directly additive with regard to the calcium cellular immune mechanism (678,679). If the native kidneys are believed to be the source of hypertension, nephrectomy (680–682) should be considered. Overall, nephrectomy has been shown to substantially reduce blood pressure in these patients.

If the hypertension should persist, patients with hypertension associated with renal dysfunction should be evaluated to determine the cause of the dysfunction. Possibilities might include chronic rejection, CsA nephrotoxicity, or a recurrence of the original disease. A renal biopsy may be appropriate to rule out rejection. If hypertension is severe or associated with worsening renal function, with no evidence for rejection, transplant artery stenosis may be pursued by arteriography.

Renal Artery Stenosis

When hypertension can not be controlled, particularly if attempts to reduce blood pressure results in decreased renal function, the possibility of renal allograft artery stenosis should be considered. Transplant renal artery stenosis currently is diagnosed in approximately 2% of cases. In earlier reports, the incidence ranged from 1% to 25%. In the past, most stenoses have been described with the end-to-end anastomosis between the renal artery and the recipient hypogastric artery, presumably because of the discrepancy in size and quality of the two vessels, although rejection injury may be a predisposing factor (683,684). Occasionally, renal artery stenosis may occur in the early months after transplantation; at this time it is always due to a technical defect at the anastomosis. Since the use of end-to-side anastomosis of an aortic patch containing the renal artery origin onto the recipient external iliac artery, the incidence has become much lower (685,686). Currently, renal artery stenosis presents 1 to several years after transplantation with poorly controlled hypertension and deterioration of renal function. Other causes of arterial stenosis include arteriosclerosis, development of a fibrous plaque in the artery at the anastomotic site or constriction beyond it, technical narrowing of the anastomosis, perfusion injury, kinking of the vessels, and chronic microvascular rejection. Sudden occurrence or increase in severity of hypertension, the presence of a new bruit over the allograft, or a decline in renal function in the absence of rejection all suggest the possibility of renal artery stenosis. A rise in the serum creatinine level after treatment with an ACE inhibitor for hypertension is very suggestive of renal allograft arterial stenosis (1). On occasion, renal vein and peripheral renin levels may be of value. Renal artery stenosis is relatively common in the transplanted kidney as demonstrated angiographically, but this does not necessarily mean that it is the cause of hypertension. Along with immunologic rejection (687), abnormal hemodynamics and flow turbulence at or just distal to the anastomotic site have been suggested as possible causes of arterial stenosis. An acute-angle or kinking between donor and recipient arteries, or the excessive length of the hypogastric—renal artery system, may contribute to the flow turbulence.

The diagnosis of a functional renal artery stenosis is difficult, but in the presence of poorly controlled hypertension and deteriorating renal function, magnetic resonance angiography of the kidney or renal arteriography as well as renal biopsy should be considered. If the biopsy shows evidence of moderate-to-severe chronic rejection with intimal fibrosis of the arteries and arterioles, correction of the renal artery stenosis is unlikely to be very successful. Another more diagnostic sign of a functional stenosis is a loss of renal function following treatment with an ACE inhibitor, such as captopril or enalapril. This does imply a prominent role for the renin—angiotensin system in the etiology of the hypertension. Radionuclide scan may show a delay and decrease in allograft blood flow, but is a relatively insensitive tool for diagnosis of renal artery stenosis. Doppler ultrasonography is a moderately sensitive and noninvasive means of establishing the diagnosis, however, many false negative studies occur. The technique is easy to perform and interpret in cases with only one artery and an end-to-end anastomosis. Other screening tests include magnetic resonance angiography (688) and spiral CT (689). Angiography is, however, the most sensitive test for the diagnosis of renal artery stenosis in a transplanted kidney and is essential in therapy. However, establishing that a renal artery stenosis demonstrated angiographically is the cause of the hypertension, renal dysfunction, or both, is extremely difficult as both may be due to the associated vascular changes of chronic rejection. A renal biopsy may be helpful in making the diagnosis if it excludes significant chronic rejection. The results of surgical correction of the stenosis are good (690,691), but because of the difficulty of the surgery, there has been considerable interest in percutaneous transluminal angioplasty of renal artery stenoses (692–695).

Polycythemia

An increased erythrocyte mass has been demonstrated in some 17% of graft recipients (696). Erythrocytosis, defined as a hematocrit greater than 52%, most often occurs within the first year after transplantation and may be associated with good allograft function, chronic rejection, transplant renal artery stenosis (697), hydronephrosis, native kidney and hepatic erythropoietin production, and the use of androgenic steroids. In patients with good allograft function, it is postulated that correction of the uremic milieu allows overzealous red blood cell production, because of a reset marrow response to erythropoietin (EPO) (698,699). In patients with chronic rejection, renal artery stenosis, and hydronephrosis, intrarenal hypoxemia may stimulate erythropoietin production (700). In most cases, the precise etiology is uncertain, but studies on erythropoietin levels after transplantation indicate that graft function restores the hematopoietic response to normal. The phenomenon usually is self-limited, lasting 3 to 12 months. Low doses of an ACE inhibitor (beginning with 2.5 mg of enalapril per day or 12.5 mg of captopril twice a day) reduces the hematocrit to normal or near normal levels. The effect begins within 6 weeks and is complete in 3 to 6 months. An association between the ACE inhibitor-induced reduction in hematocrit and a fall in plasma EPO levels has been demonstrated in some studies. Also compatible with an EPO-independent mechanism is the observation that withdrawal of the ACE inhibitor results in a gradual rise in hematocrit without a concurrent elevation in EPO levels. ACE inhibitors can also induce anemia in some renal transplant recipients without erythrocytosis. The mechanism of action is unclear.

An alternative to ACE inhibition is theophylline. Theophylline appears to act as an adenosine antagonist in this setting, suggesting that adenosine facilitates both the release and perhaps the bone marrow response to EPO (701–704). In

severe cases (hematocrit >52%), phlebotomy is indicated to prevent thromboembolic complications, which may occur in as many as 20% of patients with erythrocytosis.

Bone Complications

The main types of renal osteodystrophy are secondary hyperparathyroidism and osteomalacia. After successful transplantation, the metabolic milieu of bone changes, with correction of acidosis, cessation of aluminum hydroxide gel therapy, and improved vitamin D metabolism (705). This leads to varying degrees of resolution of preexisting renal osteodystrophy and osteomalacia. A progressive resolution of the radiographic changes of hyperparathyroidism occurs as early as 3 months after transplantation, but abnormalities may persist for more than 12 months in some patients. However, many patients have sustained hyperparathyroidism. Indications for parathyroidectomy include progressive elevation of parathyroid hormone (PTH) and alkaline phosphatase levels, progressive or new metabolic bone disease, osteonecrosis, metastatic calcification, and severe symptoms of pruritus and proximal myopathy. Osteoporosis is related to steroid therapy. It is much less of a problem now that low-dose steroid protocols are used, but the postmenopausal woman, even on low-dose steroid therapy, is likely to develop significant osteoporosis.

The main bone disorder that can be directly attributed to transplantation is avascular necrosis or osteonecrosis, which most commonly affects the hips (Fig. 98-28) and tends to be bilateral, but may affect other joints, including the wrists, elbows, knees, ankles, and shoulders. Pain may be severe and is the most common presenting symptom, usually occurring between 1 and 3 years after transplantation. The mean time to onset was 12 months after transplantation (range, 6 to 21 months). The incidence of avascular necrosis has been as high as 15% after transplantation, but this was due almost entirely to high-dose steroid therapy used in conjunction with azathioprine. Since the general adoption of low-dose steroid protocols, the incidence of avascular necrosis has dropped dramatically to about 2% (222,706). Patients with preexisting secondary hyperparathyroidism at the time of transplantation may be at a higher risk for developing avascular necrosis of bone. Pain usually precedes any radiologic changes by several months. In well-established cases, the diagnosis can be made on plain radiographs, whereas CT scanning, MRI, and nuclear bone scanning may detect earlier changes. Percutaneous needle manometry of intramedullary pressures may demonstrate elevated pressures and venous outflow obstruction, which precede structural bone necrosis. If performed early, core decompression to relieve the intramedullary venous outflow obstruction can prevent osteonecrosis. With more severe disease, prosthetic total hip replacement has been used with excellent functional recovery. In general, surgery should be performed early in order to facilitate rehabilitation.

Prospective studies have demonstrated that bone loss occurs early and rapidly following renal transplantation (707–709). These patients lost 6.8% of their initial bone mass during the first 6 months after transplantation and developed a low-turnover bone disorder resembling that induced by glucocorticoids. By 18 months, bone loss had decreased 9.0% from baseline. The development of osteopenia places the patient at increased risk for pathologic fractures. The prevalence of atraumatic fractures in the renal transplant recipient may be as high as 22%; these fractures occur primarily at sites of high cancellous bone, such as the vertebrae and ribs. Posttransplant bone loss involves both preexisting risk factors, such as

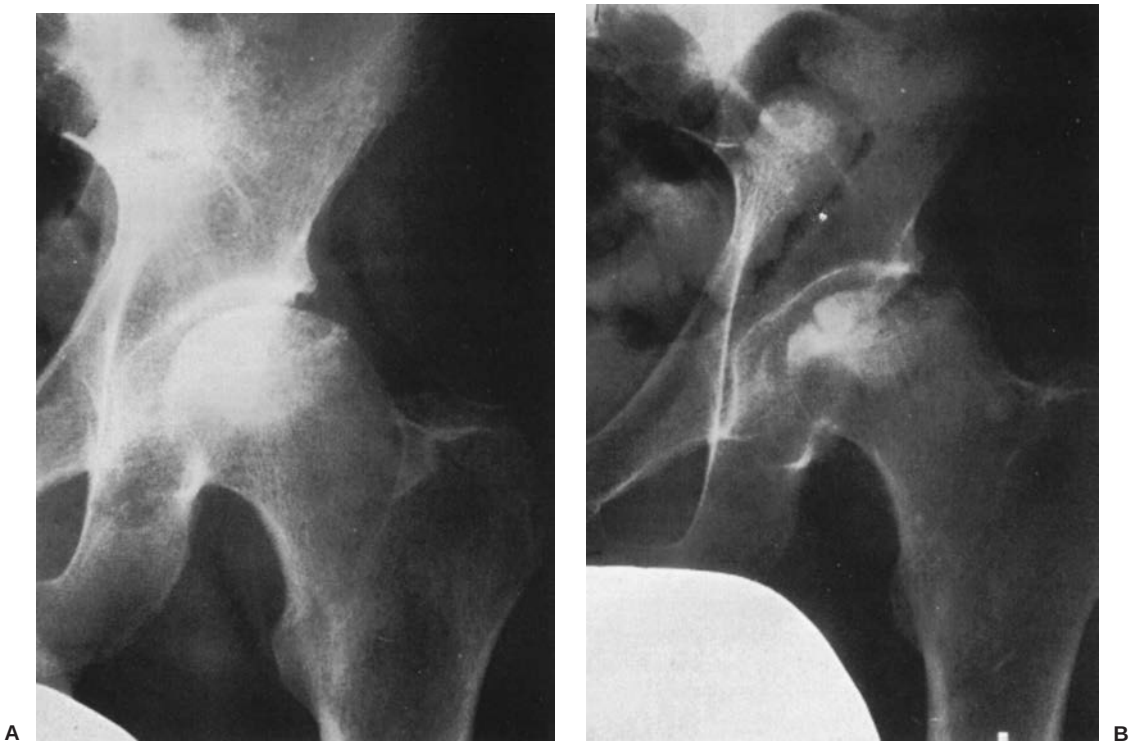

FIGURE 98-28. Avascular necrosis of the head of the femur after transplantation. **A:** Normal radiograph of the hip 10 months after transplantation, at which time, the patient was complaining of pain. **B:** The same hip 8 months later. This patient had received azathioprine and high-dose steroids and subsequently had a successful hip replacement. (From: Morris PJ. *Kidney Transplantation: Principles and Practice*. 2nd ed. New York: Grune & Stratton; 1984, with permission.)

hyperparathyroidism, and the adverse effects of immunosuppressive therapy. Glucocorticoid suppression of bone formation is the most important factor in the genesis of early bone loss. Steroids are directly toxic to osteoblasts and lead to increased osteoclast activity. They also have other effects that promote calcium loss and the development of osteopenia. These include decreased calcium absorption, reduced gonadal hormone production, diminished insulin-like growth factor-1 production, and decreased sensitivity to PTH. Cyclosporine, which induces a high turnover osteopenia in rodents, also may contribute to bone loss, especially in long-term survivors and in subjects treated only with cyclosporine. It is important to monitor bone mineral density in the renal transplant recipient using dual-energy x-ray absorptiometry (DEXA). It is recommended that lumbar spine and hip bone mineral densities should be measured at the time of transplant, after 6 months, and then every 12 months, if results are abnormal. Those subjects displaying rapid bone loss and/or a low initial bone density should be considered for treatment. Calcium supplementation (1 g/day) should be considered in nonhypercalcemic patients. The administration of vitamin D analogs (such as calcitriol) can further improve calcium absorption. If bone loss is severe and/or rapid, consideration should be given to the administration of calcitonin or other antiresorptive agents, such as the bisphosphonates (pamidronate) (710,711).

Renal Electrolyte and Tubular Disorders

Proximal bicarbonate wasting occurs most often in the early transplantation course and resolves gradually. Proximal renal tubular acidosis may be related to ischemic preservation injury, secondary hyperparathyroidism, malnutrition, acute tubular necrosis, and acute rejection (712–714). Distal renal tubular acidosis sometimes occurs either as a consequence of acute rejection or as a result of the interstitial nephropathy caused by chronic rejection. Hyperkalemia is common in patients on CsA and is readily reversible with lowering of the dose. The mechanism is unclear but the decreased potassium excretion may be due to diminished serum aldosterone levels or to a primary tubular defect.

Hypercalcemia

Acute hypercalcemia usually occurs in the setting of severe secondary hyperparathyroidism. Because of the improved management of secondary hyperparathyroidism preoperatively, this is less frequently seen with oral phosphate binders, calcium supplementation, and vitamin D administration. Still, 10% to 20% of patients may develop hypercalcemia within the first 1 to 2 years after transplantation (705). Most hypercalcemic patients have transient elevations in serum calcium levels, in the range of 11 to 12 mg/dL. The treatment of hypercalcemia includes dietary reduction of calcium and cessation of thiazide diuretics and vitamin D supplements, which may exacerbate hypercalcemia. Persistent mild hypercalcemia is generally managed conservatively with serial serum calcium determinations, unless there are indications for more aggressive intervention with parathyroidectomy. Serum intact PTH should be measured at 6 and 12 months and then annually posttransplant (715–717).

There are two major indications for parathyroidectomy in these patients: severe symptomatic hypercalcemia, usually occurring in the early posttransplant period, and persistent, marked hypercalcemia. Approximately 4% to 10% of patients remain hypercalcemic after 1 year. Elective parathyroidectomy should be considered if the plasma calcium concentration remains above 12.5 mg/dL (3.1 mmol/L) for more than 1 year, particularly if associated with radiologic evidence of increased bone resorption.

Hypophosphatemia

Hypophosphatemia (serum phosphorus levels of <2.6 mg/dL) is very common in the early weeks after transplantation. Newly transplanted kidney may waste phosphate under the influence of raised PTH level (718,719). Some transplant kidneys continue to lose excessive phosphate despite resolution of the secondary hyperparathyroidism (720). Hypophosphatemia is usually not symptomatic and typically resolves over 6 to 12 months. Hypophosphatemia is observed in 60% to 70% of patients within 1 year after transplantation. Hypophosphatemia may persist for more than 1 year in 20% to 25%, even in the absence of hyperparathyroidism. The most common cause is due to a renal phosphate-wasting syndrome in the absence of other evidence of proximal tubule dysfunction (721). The decrease in serum phosphorus levels is less among transplant recipients who receive corticosteroid-free immunosuppressive therapy. Plasma phosphate levels below 1.0 to 1.5 mg/dL (0.32 to 0.48 mmol/L) can cause muscle weakness. Severe and prolonged hypophosphatemia can lead to osteomalacia and fractures. Oral phosphate replacements are required if hypophosphatemia persists. One important exception is the patient with significant persistent hyperparathyroidism, as detected by elevated plasma intact PTH levels. In this setting, the administration of phosphate can exacerbate the hyperparathyroidism in part by complexing with calcium and lowering intestinal calcium absorption.

Hypomagnesemia

Hypomagnesemia (serum total magnesium levels <1.5 mg/dL) is common in the early weeks after transplantation. It can result from CsA- or tacrolimus-induced renal magnesium leaks. The use of thiazide diuretics is another common cause of renal magnesium loss. Up to 25% of long-term CsA-treated patients manifest hypomagnesemia (722–724). The prevalence decreases with time after transplantation, possibly because of decreasing CsA blood levels. The prevalence of CsA-induced hypomagnesemia may be higher among diabetic patients. Muscle weakness, hypokalemia, hypocalcemia, and, rarely, seizures may result from severe hypomagnesemia. Low magnesium levels may be associated with hypertension in CsA-treated patients. Low magnesium levels have been linked to hyperlipidemia in renal transplant recipients and magnesium replacement was demonstrated to reduce elevated total and LDL cholesterol levels in a small uncontrolled trial by Gupta et al. (725).

Hyperuricemia

Renal handling of uric acid is affected by the use of CsA leading to higher serum urate levels in CsA-treated patients (726–728). Asymptomatic hyperuricemia occurs in 55% of patients receiving CsA and in 25% of those taking azathioprine. There is no report of graft failure due to urate nephropathy in the transplanted kidney. Crystal-induced erosive arthritis can occur in these patients. Nonsteroidal anti-inflammatory agents should also be avoided because of potential negative influence on renal hemodynamics and the development of interstitial nephritis. Colchicine is the preferred treatment if symptoms persist. Allopurinol, a xanthine oxidase inhibitor, should be avoided in patients taking azathioprine. Concomitant administration of allopurinol and azathioprine results in marrow suppression and a fourfold increase in immunosuppression.

Hyperglycemia

Glycosuria occurs in about 60% of patients in the first 6 months after transplantation. Both FK506 and CsA may cause pancreatic toxicity, with hyperglycemia occurring when drug levels are high and in conjunction with prednisone administration (729–731). In large prospective and retrospective studies, the incidence varied from 4% to 18%. The incidence was reported to be higher among patients treated with CsA and prednisone, compared with azathioprine and prednisone. Tacrolimus has also been linked to a higher incidence of posttransplant diabetes mellitus. Older individuals and African-American or Hispanic patients are most susceptible. Glucose intolerance resolves with reduction of the steroid dose in most of these patients (230). Transpant recipients who develop diabetes are at greater risk of graft-related complications including infection, graft rejection, and graft loss.

Obesity

Many studies report the prevalence of obesity at the time of transplantation but few report its prevalence after transplantation. However, approximately 40% of renal transplant recipients are obese 1 year after transplantation. Obesity is defined as a body mass index (BMI) of more than 30% kg/m^2 or more than 130% ideal body weight (732,733). Increased calorie intake may occur after transplantation primarily, because of enhanced appetite associated with corticosteroid use. If obesity ensues, it may contribute to the development or exacerbation of hypertension, hyperlipidemia, cardiovascular disease, and steroid-induced diabetes. A consistent correlation between obesity and graft survival has not been found, although patient survival may be reduced, largely because of cardiac death. Weight loss is recommended to improve lipid profile, lower blood pressure, and improve glycemic control for patients with type II diabetes mellitus. In addition to limitation of calorie intake, management of posttransplantation obesity includes behavior modification, an exercise program, and early nutritional counseling (734).

Malnutrition

Up to 70% of the dialysis patients have some element of malnutrition and low serum albumin level is a predictor of mortality risk for ESRD patients on dialysis. Approximately 10% of patients exhibit hypoalbuminemia at 1 year and 20% at 10 years after transplantation. Low serum albumin levels may be the result of decreased production and/or increased catabolism (735,736). Increased urinary protein excretion, especially in patients with chronic transplant nephropathy, may also result in low serum albumin levels. Chronic allograft nephropathy with hypoalbuminemia is associated with decrease in muscle mass, which is reflected in decreases in urinary creatinine excretion. Corticosteroids accelerate the protein catabolic rate and frequently create negative nitrogen balance. Studies by Hoy et al. (737) have documented significant increases in the protein catabolic rate, accompanied by decreases in serum albumin levels, in the immediate posttransplant period. Even maintenance low-dose corticosteroid therapy increases protein catabolism and muscle wasting. Severe protein catabolism contributes to poor wound healing and an increased susceptibility to infection (738). Early nutritional support is indicated in high-risk patients. Assessment of nutritional status by a renal dietitian should be incorporated into the clinic visit. Serum albumin should be monitored annually. Serum prealbumin levels should be measured if albumin levels are low or if clinical findings suggest possible malnutrition. The degree of protein catabolism can be assessed by the measurement of urea nitrogen appearance. A daily protein intake ranging from 0.55 to 1.0 g/kg has been recommended for stable posttransplant patients.

Cataracts

Posterior lenticular cataracts appear in up to 10% of transplant patients receiving high-dose steroids. Usually the cataracts are small and do not present a severe handicap to the patient, although, in some instances, cataracts are large and require removal of the lens.

Growth in Children

Children with end-stage renal failure will have retarded growth and, after transplantation, one of the major problems of the use of steroids in immunosuppressive protocols is the continuing retardation of growth. Although the rate of growth usually improves after transplantation, catch-up growth is less common. Growth rate is related to the age of the child, the presence of near-normal renal function, and the use of alternate-day steroids (227,228,739). The use of cyclosporine, especially if used without steroids, in children appears to allow normal growth and, indeed, there is now some evidence that catch-up growth may occur in these patients (740,741).

Parenthood after Renal Transplant

Chronic renal failure is usually associated with a loss of libido, amenorrhea in women, and impotence in men. After successful transplantation, menstruation returns in young women, and men usually redevelop their libido and potency. Women have had successful pregnancies and men have fathered children. Of the first 697 transplant recipients at the University of Colorado (742), 50 male recipients were responsible for 67 pregnancies. There were 60 life births, 2 abortions, and 5 successful current pregnancies. Thirty-seven women recipients had 56 pregnancies resulting in 44 live births, 8 abortions, and 3 current pregnancies. Toxemia occurred in 27% of pregnancies, approximately four times the expected incidence in a nontransplant population. Further renal deterioration with preexisting renal impairment was considered a possible indication for termination of pregnancy. Irreversible deterioration of renal function attributable to pregnancy occurred in 7%. Forty-five percent of deliveries were premature and 30% of newborns had one or more complications. The occurrence of congenital anomalies was small. Since then, significant experience has accumulated enabling the physician to provide sound advice to women of childbearing age who have undergone transplantation (743). Before conception, immunosuppressive therapy should be stable and at low maintenance levels. Some recommend attempting conception between 2 and 5 years after transplantation when renal function is both normal and stable. Individuals with serum creatinine less than 2 mg/dL tend to do well with no decline in function. Proteinuria occurs in 40% of pregnant women with a kidney transplant, but it is usually insignificant and resolves after delivery. The rate of rejection is 9% late in pregnancy, which is not significantly different compared with nonpregnant transplant recipients (Table 98-21).

Although the incidence of spontaneous abortion may be higher, there is no evidence of an increased incidence of congenital abnormalities in pregnancies successfully carried through to term (426,744,745). Some 45% to 60% of the deliveries are preterm and low fetal birth weight for gestational age is present in about 20% of pregnancies. The pelvic location of

TABLE 98-21

CRITERIA FOR RENAL TRANSPLANTATION DESIRING PREGNANCY

1. At least $1^1/_2$–2 yr after transplantation
2. Stable graft function with minimal immunosuppression, serum creatinine <2.5 mg/dL
3. No evidence of graft rejection
4. No significant proteinuria
5. Good blood pressure control
6. No evidence of pelvicalyceal distortion

the renal allograft is not a contraindication to vaginal delivery. Cesarean section, performed in about one-fourth of the deliveries, should be reserved for obstetric indications only. Delivery should be performed with aseptic technique and prophylactic antibiotics administered for any surgical procedures. The steroid dose should be increased if there are signs of adrenal insufficiency.

There are no reported adverse effects of CsA or tacrolimus on human fetuses. Although rare, steroids may cause adrenal insufficiency in the neonate. Steroids and low concentrations of azathioprine and CsA are found in breast milk. Because there are few data on the effects of continued exposure to low doses of immunosuppressive agents perinatally, breast feeding is not recommended (746). Few data are available concerning the safety of pregnancy for patients receiving the newer immunosuppressive agents. Animal studies have raised the possibility of an increased risk of malformations in the newborn of females treated with MMF during pregnancy. It is recommended that MMF be discontinued 6 weeks before conception is attempted. However, outcomes of pregnancies among women exposed to MMF at conception or during pregnancy have been reported, no structural malformations have been noted among offsprings exposed to MMF, but exposure are limited (5 mothers, 29 fathers) (747).

CURRENT SUCCESS AND FUTURE CHALLENGE

Dramatic improvements have occurred in the outcome of renal transplantation over the past 40 years. Immunosuppressive drug regimens have become more sophisticated with better graft survival with less morbidity and mortality. Currently the 1-year graft and patient survival rates are 85% to 90% and over 95% in most transplant centers, despite the fact that an increasing number of high risk patients are undergoing transplantation as a replacement therapy for ESRD (2,5,418). The long-term issues confronting the patient and physician are both the relentless decline in allograft function resulting in poor graft survival beyond 5 years for cadaveric transplants (467,659) and the medical complications, particularly those resulting from the use of chronic immunosuppression (210,748). Renal allograft failure is now one of the most common causes of ESRD, accounting for 20% to 30% of patients awaiting renal transplantation. Efforts must also be directed to increase supply of donor organs. The limited availability of kidneys for transplantation raises ethical questions, such as patient selection and allocation of kidneys for transplantation (749).

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CHAPTER 99 ■ PERITONEAL DIALYSIS

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Since the first description of continuous ambulatory peritoneal dialysis (CAPD) in 1976, peritoneal dialysis (PD) has become the dominant modality for home dialysis across the globe. Over the last decade, the patterns of utilization rates for PD have changed. While the proportions of end-stage renal disease (ESRD) patients treated with PD has declined in many western countries, the utilization rates are increasing in several East European and Asian countries (1–3). In 2002, PD was used to treat 8% and 20% of the US and Canadian dialysis populations, respectively (1). A large number of single-center, multicenter, and registry-based comparisons of outcomes among patients treated with hemodialysis (HD) and PD have been made. Based on these studies, a few key observations can be made. First, patients commencing treatment with PD are younger and have a lower comorbidity burden than those that are treated with HD (4,5–7). Second, there appears to be a modality by time interaction, such that patients commencing PD have a higher probability of survival during the first 2 to 3 years of renal replacement therapy when compared to HD patients; this advantage diminishes over time (5,8–11). Third, the relative outcome of patients treated by HD and PD seem to be modified by their age and diabetic status and the presence or absence of comorbidities (5,8–10,12,13). Thus, among individuals with no baseline comorbidity, treatment with PD appears to be associated with a survival advantage among nondiabetic patients (all age groups) and young diabetic patients (age <45 years) (5). Among those with a baseline comorbidity, there was no significant differences in the outcomes of nondiabetic or young diabetic patients. However, treatment with PD was associated with a higher risk for death among older diabetic patients (age ≥45 years), with or without baseline comorbidity (5). It remains unclear if these differences in survival reflect a "modality effect" or from a selection bias undescribed by known comorbidities. Nonetheless, PD treatment is used by thousands of patients around the world and appears poised to remain an important modality for renal replacement therapy.

Space precludes us from describing an extensive physiologic basis for PD. We discuss the current major issues of concern for PD in this chapter—the definition of "adequate" therapy and the control or management of therapy-related complications.

PERITONEAL DIALYSIS MODALITIES

Technique variations for PD can be manual and/or automated and either continuous or intermittent. In most patients, the selection of the peritoneal dialysis modality depends upon which therapy suits the patient's lifestyle. However, in the absence of residual renal function, it is probably always desirable to use a continuous therapy.

Peritoneal Dialysis Techniques: Continuous Therapies

Continuous Ambulatory Peritoneal Dialysis (CAPD)

Until recently, CAPD was the most commonly used form of PD. Since its original description, there have been few changes in the basic therapy, although there have been many changes in the connection devices or "connectology" used to make the exchange. CAPD is a manual therapy and usually uses less dialysate than automated PD. The usual dialysis prescription for patients on this technique is four exchanges per day using 2.0- or 2.5 L of dialysate.

Continuous-Cycling Peritoneal Dialysis (CCPD)

The utilization of automated peritoneal dialysis (APD), of which CCPD is the most common, is rapidly increasing in many parts of the world, like the United States (1). Most often, patients undergoing CCPD use an automated cycler to perform exchanges while they sleep with a subsequent daytime dwell; therefore, this is a continuous therapy. Some patients also need a day-time "manual" exchange, either to maximize solute clearances or to enhance fluid removal.

Peritoneal Dialysis Techniques: Intermittent Therapies

Because intermittent therapies typically use multiple short dwells, they tend to be automated, but can be done manually. Intermittent peritoneal dialysis therapies are best suited for patients who are found to be high transporters based on peritoneal equilibration test (PET). However, they should rarely be used once the patient lose their residual renal function. These therapies also may be transiently indicated during peritonitis for some patients with problems with ultrafiltration.

Intermittent Peritoneal Dialysis (IPD)

By definition, IPD implies that therapy periods alternate with times when the peritoneum has been drained ("dry abdomen"). Typically the patient uses multiple short-dwell exchanges three or four times a week. Techniques include manual IPD, cycler IPD, reverse osmosis machine IPD, intermittent reciprocating dialysis with an extracorporeal reconstituting circuit, and others. In recognition of the importance of small and possibly, middle-molecule clearance, IPD is now rarely used.

Nonetheless, classic IPD therapies continue to have their uses. A report from Mexico suggested that cycler IPD may be more practical in countries where technical, social, and economic limitations restrict the use of CAPD (14). Other uses include treatment of refractory heart failure (15), as a transient therapy for immediately postoperative patients, or for those who are on CAPD and have developed hernias or leaks.

Nightly Intermittent Peritoneal Dialysis (NIPD)

NIPD utilizes nighttime cycling with a cycler and the patient does not have any intraperitoneal dialysate during the daytime. It is best utilized by patients with high or high-average transport, in the presence of residual renal function. Daytime ambulatory peritoneal dialysis (DAPD) is based on the same concept as NIPD, but DAPD is a manual technique, and the patient typically has a "dry time" during the night. The lower the peritoneal membrane transfer rates, the lower the 8-hour NIPD or DAPD clearances and, in some patients, time spent on NIPD or DAPD has to be prolonged by 10% to 40% to achieve minimal target clearances (16,17). Like IPD, NIPD can be used for management of patients with heart failure, as transient therapy for postoperative patients, or patients treated with CAPD or CCPD, who have developed hernias or leaks (18).

Tidal Peritoneal Dialysis (TPD)

TPD is best performed nightly by the use of an automated cycler. It involves the maintenance of an intraperitoneal reservoir of peritoneal dialysate, which is achieved by incomplete drainage of the fluid at the end of each dwell. Additional amounts of fluid are instilled with each exchange to maintain an optimal intraperitoneal volume. By maintaining an intraperitoneal reservoir of dialysate, it is assumed that tidal dialysis may maintain more continuous contact of dialysate with the peritoneal membrane. Furthermore, the more rapid cycling of dialysis may increase mixing and prevent formation of stagnant intraperitoneal fluid layers. Although preliminary studies suggested that small solute clearances are augmented (19,20), subsequent studies have been unable to confirm the ability of TPD to enhance clearances (21–23). It can, however, be used for patients who have infusion pain—the reservoir of dialysate minimized pain upon instillation of fresh dialysate (24). In prescribing tidal peritoneal dialysis, variables to be chosen include reserve volume, tidal outflow volume, tidal replacement volume, flow rates, and frequency of the exchanges. A major disadvantage of TPD is the cost of the extra dialysate (25).

DEFINING ADEQUACY OF DIALYSIS USING SMALL SOLUTE CLEARANCES

Minimal Versus Optimal Dialysis

The native kidneys perform excretory and endocrine functions and are key in order to maintain euvolemia. The loss of these functions in patients with progressive renal failure results in numerous metabolic and vascular abnormalities. In order to return the individual to complete health, some of the goals of optimal renal replacement therapy are summarized in Table 99-1. The concept of "optimal" renal replacement therapy, as applied to dialytic therapies, entails that the amount of dialysis delivered is not the rate-limiting step that determines patient outcome. In other words, an "optimal" dialysis prescription eliminates uremia as a potential variable, allows patients to achieve euvolemia and maximizes quality of life. Given the continuing high risk for morbidity and mortality and poor rehabilitation among the ESRD population, it is clear that the current renal replacement therapies are far from achieving the goal of "optimal" therapy (1,26). One of the reasons for this may be that, for a large number of solutes, the dialytic clearances typically replace <10% of the the native renal excretory function (Table 99-2) (27). Based on these considerations and the current state of knowledge relating small solute clearances to outcome, it is more reasonable to define clearance goals of dialytic therapy in terms of "minimal," rather than "optimal" dialysis.

TABLE 99-1

GOALS OF END-STAGE RENAL DISEASE REPLACEMENT THERAPY

Prolong life
Reverse uremic signs and symptoms
Optimize quality of life
Return to pre–end-stage renal disease functional status
Minimize patient inconvenience factors

Early studies among patients undergoing HD led to the recognition of the long-term, but often subtle, adverse effects of underdialysis on patient's health. This highlighted the importance of developing ways to proactively monitor the amount of dialysis delivered—the so-called dose of dialysis. Based upon the initial results and subsequent reanalysis of the National Cooperative Dialysis Study, the concept of urea kinetic modeling was developed to monitor the dose of HD (28,29). Shortly thereafter, the concept of monitoring the dose of dialysis using urea (and, subsequently, creatinine) kinetic modeling was extended to patients undergoing PD. Thus, over the last two decades, the adequacy of dialysis dose has been based upon an assessment of achieved clearances of small solutes.

However, as is clear from Table 99-2, small-solute clearance is substantially less for PD than HD. Yet, as discussed in the preceding section, the overall outcome is similar between HD and PD patients. It is also clear from Table 99-2, that solute clearances in CAPD exceed those of standard HD for all but the small-molecular-weight solutes. Is the reason that survival rates on CAPD and HD are similar because of comparable "middle-molecule" clearance? Should middle-molecule clearance be measured as the "PD yardstick?" At

TABLE 99-2

SOLUTE REMOVAL BY DIALYSIS AND THE NATURAL KIDNEY

Solute clearance	Natural kidney	HD[a] low flux	HD[a] high flux	CAPD[a]
Urea (L/wk)	750	130	130	70
Vitamin B_{12} (L/wk)	1,200	30	60	40
Inulin (L/wk)	1,200	10	40	20
B_2-Microglobulin (mg/wk)	1,000	0	300	250

[a] *HD,* hemodialysis; *CAPD,* continuous ambulatory peritoneal dialysis.
(Modified from: Keshaviah P. Adequacy of CAPD: a quantitative approach. *Kidney Int Suppl* 1992;38:S160.)

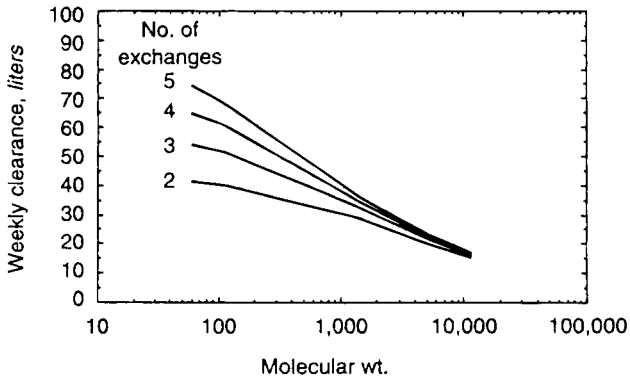

FIGURE 99-1. The influence of the number of exchanges on the weekly solute clearance for solutes with a range of molecular weights derived from a computerized model of peritoneal transport. (From: Keshaviah P. Adequacy of CAPD: a quantitative approach. *Kidney Int Suppl.* 1992;38:S160, with permission.)

this time, there are no interventional studies to support such a change in the "PD yardstick." However, it is important to note that strategies that maximize small-solute clearances do not necessarily enhance the clearance of larger-molecular-weight toxins (viz., "middle molecules") (30) (Fig. 99-1). The clearances of middle-molecular-weight substances are markedly time-dependent (number of hours of dwell time). Maneuvers that increase small-solute clearances while decreasing middle-molecule clearance may be associated with an increased relative risk of an adverse outcome (31).

Small-Solute Clearances and Mortality

The methods traditionally used to define the dose of dialysis among patients undergoing PD differ from among those undergoing HD in two regards. First, the clearance of two small-molecular-weight solutes have been used (urea, molecular weight 60, expressed as weekly Kt/V_{urea} and creatinine, molecular weight 113, expressed as creatinine clearance, liters/week/1.73 m^2 body surface area). Second, the clearances are expressed as a sum of renal and peritoneal (total) clearances. A large number of investigators have attempted to determine the relationship between the dose of dialysis (small-solute clearances) and outcome (Tables 99-3 and 99-4) (11,32–53). Most of the early observational studies did not evaluate the independent contribution of peritoneal clearances on patient outcome; virtually all these studies showed a consistent, positive relationship between higher total small-solute clearances and patient survival (32–39) (Table 99-3). In 1999, using the data base maintained at Fresenius Medical Care, Diaz-Buxo et al, (40) confirmed the association between total solute clearances as well as residual renal function with survival;

TABLE 99-3

CHRONOLOGICAL LISTING OF OBSERVATIONAL STUDIES THAT HAVE EVALUATED THE ASSOCIATION OF SMALL SOLUTE CLEARANCES AND SURVIVAL IN PATIENTS UNDERGOING CHRONIC PERITONEAL DIALYSIS

Author (ref)	Patient number	Patient type[a]	Follow-up, months[b]	Relationship between Kt/V and/or creatinine clearance and mortality		
				Total[c]	Peritoneal[c]	Renal[c]
Teehan, 1990 (32)	51	P	Up to 60	Yes	NE	NE
Blake, 1991 (33)	76	I	20	No	NE	NE
Brandes, 1992 (34)	18	P	Up to 12	Yes	NE	NE
Selgas, 1993 (35)	56	P	60	Yes	NE	NE
Genestier, 1995 (36)	201	I	24	Yes[d]	NE	NE
Maiorca, 1995 (37)	68	P	Up to 36	Yes	NE	Yes
CANUSA, 1996 (38)	680	I	15	Yes	NE	NE
Davies, 1998 (39)	210	I	Up to 48	Yes	NE	Yes
Diaz-Buxo, 1999 (40)	673	P	Up to 12	Yes	No	Yes
Jager, 1999 (41)	118	I	25	No[e]	No[e]	Yes[e]
Bhaskaran, 2000[f] (42)	122	P	20	—	No	—
Shemin, 2000 (43)	667	P	Up to 18	No	NE	Yes
Szeto, 2000 (44)	270	I + P	24	Yes	No	Yes
Rocco, 2000 (45)	873	P	7	Yes[d]	No	Yes
Lo, 2001 (46)	937	P	Up to 24	No	No	No
Szeto, 2001 (47)[f]	140	P	22	—	Yes	—
Bargman, 2001 (48)	601	I	Up to 24	NE	No	Yes
Brown, 2003[f] (49)	177	P	Up to 24	—	No	—
Termorshuizen, 2003 (11)	413	I	NR	NE	No	Yes
Szeto, 2004 (50)	270	I + P	35	NE	Yes	Yes

[a]Patient type: *I*, incident; *P*, prevalent; *I + P*, incident and prevalent.
[b]Mean follow-up interval, unless stated otherwise.
[c]*NE*, not examined; *NR*, not reported.
[d]In these studies, total creatinine clearance was related to outcome; there was no relationship between total Kt/V_{urea} and outcome.
[e]In this study, total, renal or peritoneal Kt/V_{urea} or creatinine clearance did not enter the final multivariate model; however, total and renal urea and creatinine appearances, as well as peritoneal creatinine appearance, was significantly related to survival. The relationship between peritoneal urea appearance and mortality did not reach statistical significance.
[f]Studies in anuric individuals.
(Modified adaptation from: Paniagua R, Amato D, Vonesh E, et al. Effects of increased peritoneal clearances on mortality rates in peritoneal dialysis: ADEMEX, a prospective, randomized, controlled trial. *J Am Soc Nephrol* 2002;13:1307.)

TABLE 99-4

SUMMARY OF RANDOMIZED, CONTROLLED CLINICAL TRIALS THAT HAVE EVALUATED THE EFFECT OF INCREASING DIALYTIC CLEARANCES ON OUTCOME AMONG PATIENTS UNDERGOING PERITONEAL DIALYSIS

Author (ref)	Patient number	Follow-up (months)	Patient type[a]	Mean achieved clearances[b]				Key results
				Peritoneal Kt/V_{urea}	Total Kt/V_{urea}	Peritoneal CrCl[d]	Total CrCl[d]	
Mak, 2000 (51)	66	12	I	1.56 1.92	1.67 2.02	48.4 56.6	54.6 61.9	Higher clearances associated with higher dietary protein intakes. No effect of increased clearances on serum albumin, hospitalization rates, or infectious complications
Paniagua, 2002 (52, 59)	965	22	I + P	1.62 2.13	1.80 2.27	46.1 56.9	54.1 62.9	No effect of increased clearances on patient or technique survival or serum albumin or hospitalizations or infectious complications or quality of life. More patients in the control group died from congestive heart failure or uremia/hyperkalemia/acidosis.
Lo, 2003 (53)	320	24	I[c]		1.5–1.7 1.7–.0 > 2.0			No effect of increased clearances on patient survival, nutritional status or hospitalizations. Higher incidence of anemia and higher Epo requirements in the group with the lowest clearances.

[a]Patient type: *I*, incident; *P*, prevalent; *I + P*, incident and prevalent.
[b]For each study, the first line refers to the clearances in the control group and the second line refers to the clearances in the intervention group.
[c]Only those incident patients with renal Kt/V_{urea} <1.0 were eligible to participate.
[d]*CrCl*, creatinine clearance.

however, they were unable to demonstrate any association between peritoneal clearances and outcome. Since this first report, several observational studies, including large prospective cohort studies like CANUSA, NECOSAD, and EAPOS, have been unable to demonstrate a survival benefit associated with higher peritoneal clearances (11,41,44,45,48,49). Furthermore, two randomized, controlled, clinical trials (ADEMEX and Lo et al.) have now provided the final confirmatory evidence that increases in peritoneal clearance within the range achieved in clinical practice, does not result in any significant improvement in patient morbidity or mortality (Table 99-4) (52,53). Even among anuric patients, two of the three observational studies have failed to demonstrate a statistically significant relationship between peritoneal clearances and patient survival (42,47,49). This accumulating body of data should not be taken to mean that peritoneal clearances are biologically irrelevant or that providing peritoneal clearances do not have a survival benefit—an anuric patient would die in the absence of peritoneal clearances. However, these data clearly suggest that within the range of clearances currently achieved in clinical practice, higher peritoneal clearances are unlikely to result in significant reductions in patient survival.

Small-Solute Clearances and Morbidity

ESRD patients suffer considerable morbidity, have impairments in the quality of life, and patients treated with PD continue to have a high rate of transfer off the therapy ("technique failure"). The relationship between the dose of dialysis and hospitalization has been studied in several single- and multicenter cohort studies. The preponderance of evidence suggests that higher total (peritoneal + renal) creatinine clearances are associated with a lower risk for hospitalization and technique failure; most studies have been unable to demonstrate any relationship of these morbid outcomes with total Kt/V_{urea} (33,35,36,44,47,54,55). Two of the three randomized, controlled trials were unable to demonstrate any beneficial effect of increasing peritoneal clearances on the risk for hospitalization or the number of hospital days or technique survival (Table 99-4) (52,53). In the study by Mak et al. (51), the intervention group had a higher hospitalization rate at the time of entry into the study when compared to the control group. Upon follow-up over 12 months, the hospitalization rate remained unchanged in the intervention group but increased in the control group, such that there was no significant differences in the hospitalization rates between the two groups over the study period (51).

Furthermore, observational studies have been unable to demonstrate any relationship between the small-solute clearances and the quality of life of PD patients (56–58). These findings have now been confirmed by ADEMEX—a randomized, controlled, clinical trial (59).

Thus, the existing body of evidence suggests that within the range of clearances currently achieved in clinical practice, higher peritoneal clearances are unlikely to result in significant improvements in hospitalization rate, technique failure, or quality of life of PD patients.

Small-Solute Clearances, Nutritional Status, and Patient Outcome

In order to determine the minimum dose of dialysis necessary for patient well-being, several investigators have attempted to determine the level of small-solute clearances that prevents nutritional decline and optimizes nutritional status. This approach is particularly relevant since there is a high prevalence of protein-energy malnutrition (PEM) among dialysis patients. Among PD patients, nutritional decline is evidenced by one or more of the following: low serum albumin, transferrin or prealbumin levels (38,60,–63), total body nitrogen and potassium (64–66), plasma amino acid profile and muscle protein content consistent with PEM (67–69), low body mass index (BMI) (62,70) and fat-free, edema-free mass (61,62,70), decreased skin-fold thickness (63,70,71) and hand grip strength (70), poor subjective global assessment (38,61,63,70,72–75), and composite nutritional index (46). Furthermore, various measures of nutritional status, viz., serum albumin (32,38,40,47,76–81), serum prealbumin (80), dietary protein intake (77), fat-free, edema-free mass (38,44,61,77), hand grip strength (82), total body nitrogen (64), subjective global assessment (38,61,77) and, composite nutritional index (46), are directly associated with survival of patients undergoing PD. Finally, not withstanding the evidence that the etiology of nutritional decline among ESRD patients is multifactorial (including an important role of inflammation), inadequate dietary intakes are probably important and independent contributors to the high prevalence of PEM among the dialysis population (83,84). It follows, then, if enchancing the dose of dialysis can result in an increase in dietary intakes, the higher dose would have the potential of improving their nutritional status; this, in turn, would be expected to have a salutary effect on patient outcome.

Cross-sectional studies have demonstrated a significant relationship between measures of small-solute removal (Kt/V_{urea}) and dietary protein intake (measured as normalized protein equivalent of nitrogen appearance, nPNA) in PD patients (55,85–91). It is now believed that part of this relationship is secondary to mathematical coupling (92,93). However, several lines of evidence suggest that enhancing solute clearances via dialytic therapies may be important in ameliorating anorexia, a key manifestation of the uremic state. Studies suggest a relationship between the dose of urea removed and protein intake, calculated from dietary records and interviews (85,92,94). The relationship is statistically significant, even when the dose of urea and creatinine removal is not normalized to body size (85,86,95). Moreover, in PD patients, dietary energy intakes also correlate with small-solute clearances (94,96,97). It appears that anorexia associated with renal failure is secondary to increased levels of anorexigens and decreased levels of orexigens. Recent data suggest that middle-molecule fractions, isolated from the dialyzer ultrafiltrate of plasma and from normal urine inhibit food intake in rats in a dose-dependent manner (98). Furthermore, reduced levels of appetite stimulants naturally present in the body, like neuropeptide Y and ghrelin, are associated with anorexia in CPD patients (99,100). Finally, addition of peritoneal clearances, as during commencement of CPD therapy or an increase in peritoneal clearances, are often associated with an increase in dietary protein intakes (51,77,96,101–104) (also, Table 99-4). All these data suggest that this relationship truly is physiologic.

Data indicate that the relationship between small-solute clearances and dietary protein intake may be curvilinear (105,106). The curve seems to plateau at dietary protein intakes of 1.0 to 1.2 g/kg/day. These data are consistent with N-balance studies that suggest that a dietary protein intake of 1.2 to 1.3 g/kg/day is necessary to maintain neutral or positive N balances in most patients undergoing chronic PD (85,107,108). Based upon these considerations, the point where the curve-relating dialysis dose and dietary protein intake plateaus may define the "optimal" dose of dialysis for PD patients. However, not withstanding the increase in dietary protein intake, recent randomized controlled trials have been unable to demonstrate an improvement in nutritional status with increasing peritoneal clearances (51,53) (Table 99-4).

TABLE 99-5

TARGETS FOR SMALL SOLUTE CLEARANCES RECOMMENDED BY VARIOUS ORGANIZATIONS FOR PATIENTS UNDERGOING CHRONIC PERITONEAL DIALYSIS

Committee	Nature of clearances	Kt/V	Creatinine clearance
United States—NKF-K/DOQI[a]	Renal + peritoneal	1.7	—
Eurpoean Best Practice Guidelines	Peritoneal	1.7	—
Canadian Society of Nephrology	Peritoneal	1.7[b]	—

[a]National Kidney Foundation's Kidney Disease Outcome Quality Initiative.
[b]For patients with residual renal function >4 mL/min, a peritoneal Kt/V between 1.0 and 1.7 is recommended.

Minimal Total Solute Clearance Goals

Several organizations around the world have developed clinical practice guidelines to define the target level of small-solute clearances required to optimize the health of patients undergoing PD. As would be expected, these guidelines have evolved with our understanding (as discussed above), particularly with the availability of the results of two large randomized controlled clinical trials (52,53). The updated guidelines by organizations in United States, Canada, and Europe are summarized in Table 99-5. When compared to guidelines published earlier, the current recommendations differ in several important respects. First, only one measure of adequacy is used to define the minimum dose of dialysis (Kt/V_{urea}). Early studies, including the CANUSA study, suggested that patient outcome was more dependent upon total (renal + peritoneal) creatinine clearances rather than total urea clearances (38). However, the contribution of renal creatinine clearance to total (renal + peritoneal) creatinine clearances is substantially greater than of native renal urea clearances. Since creatinine is secreted and urea is reabsorbed by renal tubules, renal creatinine clearance is always higher than renal urea clearance; on the other hand, peritoneal clearances are dependent upon the molecular weight of the solute in question. Thus, creatinine clearance (molecular weight, 113) is always lower than peritoneal urea clearance (molecular weight, 60). Consequently, the expected weekly creatinine clearance is different in a patient who is just starting PD with a residual renal Kt/V_{urea} of 2.0/week than in an anuric patient with a peritoneal Kt/V_{urea} of 2.0/week. Thus, although both markers of solute clearance may be predictors of outcome, the target or goal for creatinine clearance may have to change over time as residual renal function decreases and is replaced by peritoneal clearance. On the other hand, it appears from outcome studies that the Kt/V_{urea} target may not need to change. Furthermore, it is now recognized that the stronger relationship of creatinine clearances to patient outcome was a result of the effect of confounding effect of residual renal function. There is no evidence that peritoneal creatinine clearances are superior in predicting outcome, when compared to peritoneal urea clearance. In light of these considerations, the various expert groups recommend only the use of Kt/V_{urea} to determine the dose of dialysis (Table 99-5). Second, the targets for Kt/V_{urea} have been changed, such that Kt/V_{urea} of 1.7 is now considered to be the minimum dose necessary needed for patient well being. Based upon the results of the two recent randomized controlled trials, it is also recognized that some patients may require a higher dose of dialysis to manage uremic symptoms or to achieve euvolemia (52,53). Third, there are no differences in the definition of minimum dose of dialysis based upon the patients' transport type (see below). Fourth, some expert groups (Canada and Europe) have defined the adequacy of dialysis based only upon peritoneal clearances, while others (United States) define it based upon total clearances. Fifth, the targets are the same, irrespective of PD modality (CAPD or APD). Finally, volume control is recognized as an additional dimension to define adequate dialysis (see below).

MONITORING AND ADJUSTING SMALL-SOLUTE CLEARANCES

Determination of Peritoneal Transport

In its function as a dialysis membrane, the peritoneum has two important functions: diffusion (movement of solute across a concentration gradient) and convection (movement of solute along with water, ultrafiltration [UF]). There is interpatient variation in peritoneal membrane transport characteristics. A variety of methods have been suggested, standardized, and studied to assess the peritoneal membrane function (Table 99-6) (109–114). The most precise method to evaluate diffusive function of the peritoneum is to determine the mass transfer area coefficients (MTAC) of solutes like creatinine (115). These define transport independent of ultrafiltration (convection-related solute removal) and, consequently, are not influenced by dwell volume or glucose concentration. In order to determine the MTAC, additional laboratory measurements and computer models are necessary, but, once these are obtained, MTAC can be used easily in the clinical setting (112,115,116).

However, of these the peritoneal equilibration test (PET) is the most widely used test to characterize the peritoneal transport characteristics (109,110). All patients commencing PD therapy should undergo a PET. The first PET should probably be performed after at least 4 weeks of commencing peritoneal dialysis therapy (117). While some centers choose to repeat a PET only if clinically indicated, others perform the test periodically to monitor peritoneal membrane function.

In order to enhance the reproducibility of the test, several steps of the PET are standardized: (1) Long (8 to 12 hours) preceding exchange, (2) drain the preceding exchange as completely as possible over 20 minutes, (3) infuse 2 L of 2.5% dextrose dialysate over 10 minutes (time 0), (4) take samples of dialysate of times 0, 120, and 240 minutes, (5) in order to take samples, 200 ml of dialysate is drained into a bag, 10 ml is drawn for testing, and 190 ml is reinfused, (6) a blood sample is taken at 120 minutes, and (7) the dialysate is drained completely at 240 minutes and the drain volume is measured. Dialysate and serum urea, glucose, and creatinine are measured. For each dwell time (0, 120, and 240 minutes), dialysate

TABLE 99-6

TESTS TO EVALUATE PERITONEAL MEMBRANE FUNCTION

Test	Parameter used to evaluate solute removal function	Parameter used to evaluate fluid removal function
Peritoneal equilibration test (4-hour, PET)	D/P creatinine D/D_0 glucose	Drain volume
Dialysis adequacy and transport test (24-hour, DATT)	D/P creatinine	Drain volume
Standard peritoneal analysis (SPA)	MTAC creatinine	Drain volume, D/P sodium, and others
Personal dialysis capacity (PDC)	Area parameter	Ultrafiltration coefficient
Apex	Purification phosphate time	Apex time

to plasma ratios (D/P) of creatinine and urea are determined, as is the ratio of glucose at the drain time (120 and 240 minutes) to the initial dialysate glucose concentration (D/D_0). These results are plotted against time and compared to known standard curves (Fig. 99-2). Based upon the values of D/P creatinine or D/D_0 glucose, patients are classified into one of four categories: low, low average, high average, and high transporters. It should be noted that there is a significant discordance between the categorization of patients' transport type, based upon whether D/P creatinine or D/D_0 glucose is used (Fig. 99-3) (118). Recent studies suggest that abbreviating the preceding exchange to 2 to 3 hours does not significantly influence the values of D/P creatinine or D/D_0 glucose (119,120); thus, patients being treated with APD do not have to change their treatment schedule on the day prior to the PET.

As more has become known about ultrafiltration and water transport across the peritoneal membrane, it has been recommended that a 4.25% dextrose PET be used to characterize the ultrafiltration capacity of the peritoneum, including aquaporin-mediated water transport and solute transport (112). The 4.25% PET has been compared to the 2.5% PET in a cohort of chronic PD patients and no clinically relevant difference in classifying the patients into different transport types was noted, suggesting that the 4.25% PET may be as clinically useful in prescription management as is the 2.5% PET (121).

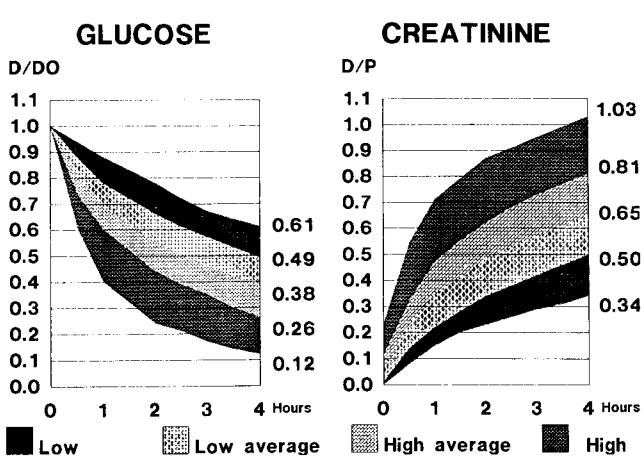

FIGURE 99-2. Dialysate to plasma ratios (D/P) for creatinine and drain time to initial dialysis concentration (D/D_0) ratios for glucose, generated from standard peritoneal equilibration testing. (From: Twardowski ZJ. *Blood Purif* 1989;7:95, with permission.)

Clinical Relevance of Characterizing Peritoneal Membrane Function

The PET is used specifically to characterize the patient's peritoneal membrane transport properties. Knowledge of the peritoneal transport allows a physician to choose an appropriate prescription for a patient; this is particularly useful when using computerized, kinetic modeling for prescription management.

In general, high (rapid) transporters of creatinine and urea also tend to be rapid absorbers of dialysate glucose (high D/P creatinine and low D/D_0 glucose). Therefore, although the D/P creatinine ratios for 4-hour dwell tend to be close to 1, drain volumes tend to be small. High transporters maximize their D/P ratios and intraperitoneal volumes early during the dwell. Once the osmotic gradient dissipates, UF ceases, followed thereafter by net fluid reabsorption. With standard CAPD, these patients may have drain volumes that are actually less than instilled volumes. Short dwell times often are needed to optimize clearance (122).

On the other hand, low (slow) transporters, peak UF occurs late during the dwell, and net UF can be obtained even after prolonged dwells because glucose absorption is slow (low D/P creatinine and high D/D_0 glucose). The D/P ratios for creatinine and urea increase almost linearly during the dwell. For these patients, dwell time is the crucial determinant of overall clearance. They do best with continuous therapies, such as standard CAPD or continuous-cycling PD (CCPD).

The PET also provides useful prognostic information. Early studies demonstrated that high transporters had greater peritoneal protein losses, lower ultrafiltration with CAPD and, hence, greater volume overload, higher symptom burden, greater morbidity, and higher technique failure (123–127). The reanalysis of the data from the CANUSA study indicated that a progressive increase in peritoneal permeability is associated with a higher risk for technique failure and/or death (Fig. 99-4) (128). These findings have been confirmed in several other observational studies (39,129–132). However, using peritoneal transport estimated either from the PET or 24-hour collections, other investigators have been unable to confirm the poor prognostic value associated with a high transport type (36,40,41,46,52,133). Furthermore, there was no apparent relationship between the transport type and outcome in patients undergoing APD (EAPOS study) (49). Not withstanding these discrepancies, the present state of knowledge would suggest a careful evaluation and aggressive management of nutritional and volume status and comorbidities among individuals with a higher transport type.

Finally, the PET cannot be used as a substitute to measure the dose of dialysis. While it is possible to estimate daily

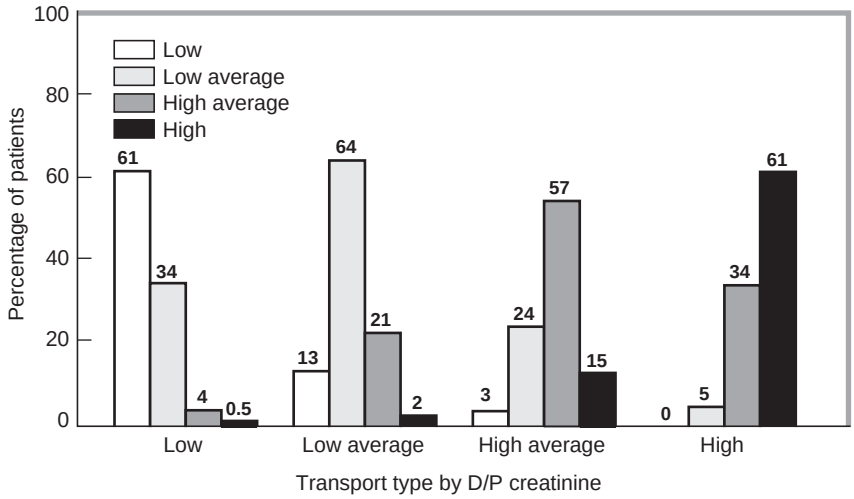

FIGURE 99-3. Discordance between categorization of patients' transport type by D/P creatinine or D/D_0 glucose. Thus, of the patients categorized low transporter by D/P creatinine, 61% of them will be classified as a low transporter by D/D_0 glucose; of the patients classified as low average transporter, 64% will be classified as low average transporter by D/D_0 glucose; of the patients classified as high average transporter, 57% will be classified as high average transporter by D/D_0 glucose; and of the patients classified as high transporter by D/P creatinine, 61% will be classified as high transporter by D/D_0 glucose. (Modified from: Mujais S, Vonesh E. Profiling of peritoneal ultrafiltration. *Kidney Int Suppl* 2002;81:S17, with permission.)

clearances from PET studies, these estimates can significantly over- or underestimate actual daily clearances (134).

Measurements to Monitor Dialysis Dose

It is recommended that monitoring should include both dialysis dose and nutritional parameters because outcomes correlate with both. As discussed above, over the last decade, the dose of PD has been monitored using both urea and creatinine kinetics (135). In light of emerging data, however, the consensus of the various expert groups seems to be to use only urea kinetics to monitor the dose of PD (Table 99-5). The only major difference appears to be with regard to defining the clearance targets based upon peritoneal or total (renal + peritoneal) Kt/V_{urea}. This is an important consideration, since for each 1 mL/minute of renal urea clearance, there is approximately 0.25 added to the total weekly Kt/V_{urea} for a 70-kg man.

Collections of dialysate and urine over 24 hours are relatively easy to obtain and can provide most of the clinically relevant data one needs to individualize a patient's prescription and monitor progress. These collections also can be used to calculate PNA, fat-free, edema-free mass (FFEFM), and other variables (Table 99-7). The data obtained from 24-hour collections is complementary to that obtained from PET and are routinely used together for developing a patient's dialysis prescription and problem solving.

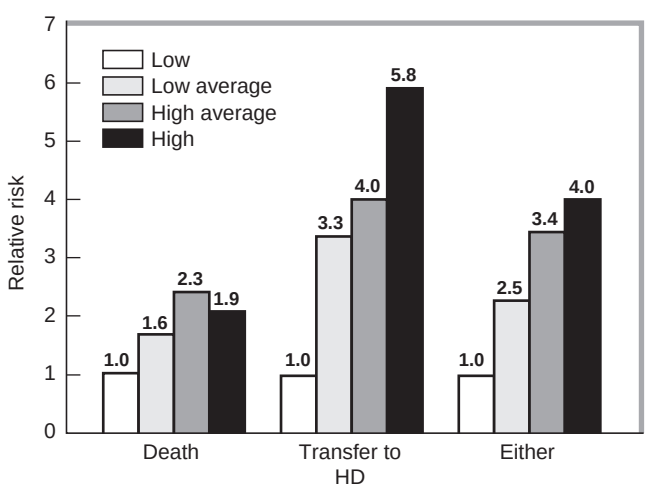

FIGURE 99-4. Relationship between transport type and patient outcome. With increasing permeability of the peritoneum, as defined by the peritoneal equilibration test, there is an increasing risk for death and/or technique failure. (Modified from: the reanalysis of the CANUSA study in Churchill DN, et al. Increased peritoneal transport is associated with poor patient and technique survival on continuous ambulatory peritoneal dialysis. *J Am Soc Nephrol* 1998;9:1285.)

Calculation of Dialysis Dose

To individualize dialysis dose and make comparisons of dose between patients, the solute clearances are typically normalized. If urea kinetics (Kt/V) are used, the sum of the daily dialysate and residual renal urea clearances are then divided

TABLE 99-7

USEFULNESS OF 24-HOUR DIALYSATE AND URINE COLLECTION

Urea kinetics
Renal urea clearance[a]
Peritoneal urea clearance[b]
Total urea clearance[a,b]
Protein equivalent of nitrogen appearance (PNA)[a,b]
Urea generation rate[a,b]
Creatinine kinetics
Renal creatinine clearance[a]
Peritoneal creatinine clearance[b]
Total creatinine clearance[a,b]
Creatinine production[a,b]
Fat-free, edema-free mass[a,b]
Dialysate to plasma creatinine[a]
Ratio of measure to predicted creatinine generation
Others
Estimated glomerular filtration rate (mean of renal urea and creatinine clearance)
24-Hour urine volume
Peritoneal drain volume—ultrafiltration rates

[a]Urine only.
[b]Dialysis only.
(Modified from: Burkart JM, et al. 24-hour dialysate collection versus peritoneal equilibrium test for determining adequacy of peritoneal dialysis. *Perit Dial Int* 1992;12:104.)

TABLE 99-8

COMMONLY USED FORMULAS FOR PROTEIN NITROGEN APPEARANCE[a]

PNA = 10.76 (G_{un} + 1.46) (Randerson)
PNA = 9.35 G_{un} + 0.294 V + protein losses (modified Borah)
PNA = 6.25 (UN_{loss} + 1.81 + 0.031 × body weight) (Teehan)
PNA = 6.25 × N loss (Kjeldahl)
PNA (g/24 h) = 15.1 + (6.95 × urea nitrogen appearance in g/24 h) + dialysate and urine protein in g/24 h (Bergstrom)[b]
In the absence of direct measurement of urinary and dialysate protein losses, this following less accurate formula may be used:
PNA (g/24 hours) = 20.1 + (7.50 × urea nitrogen appearance in g/24 h (Bergstrom)

[a] *PNA,* protein nitrogen appearance; G_{un}, urea nitrogen generation rate; *V,* volume of urea distribution; UN_{loss}, urea nitrogen loss; *N,* nitrogen.
[b] Bergstrom J, Heimburger O, Lindholm B. Calculation of the protein equivalent of total nitrogen appearance from urea appearance, which formulas should be used? *Perit Dial Int* 1998;18:467.
(Modified from: Keshaviah P, Nolph K. Protein catabolic rate calculations in CAPD patients. *Trans Am Soc Artif Intern Org* 1991;37:M400.)

by the volume of distribution for urea (V) (29). The urea V can be estimated to be 60% (males) or 55% (females) of the patient's weight in kilograms. More accurate estimations of V can be obtained using standardized nomograms, such as Watson (136) or Hume and Weyers (137). If creatinine clearance is used as a measure of the dose of dialysis, it is recommended that it be normalized to body surface area (BSA) using 1.73 m^2 as the standard (138).

A controversial issue is what weight should one use when calculating V and BSA (139). Weight has a different effect on normalization (V or BSA) for men or women and for Kt/V or creatinine clearance. The mathematical relationship between V and BSA is not fixed and is modified by gender and obesity. These differences are most marked when a patient's weight differs significantly from the norm in patients with the same height and frame size. The actual V is different in a patient with the same body weight if the increase in body weight from desirable is owing to overhydration or increase in adipose tissue. The same is true if loss of weight is due to malnutrition versus amputation. There are published recommendations for how to adjust for amputations when calculating V (140). Jones noted that when actual body weight was used for normalization, there was no difference in total solute clearances between nourished and malnourished patients (141). However, when V and BSA were calculated using desirable body weight, there was a significant difference. The NKF–DOQI guidelines suggest that in malnourished patients one must adjust the total solute clearance goals for that patient to promote anabolism. The guidelines suggest that the minimal weekly total solute clearance goal should be adjusted by the ratio of desired:actual body weight. In those cases, the minimal solute clearance in terms of urea kinetics would be higher than 1.7 (i.e., 2.0 × desired:actual body weight). Further studies are needed to confirm the appropriateness of this recommendation.

Calculation of Dietary Protein Intake

Dietary protein intake can be directly measured in metabolic wards, by dietary histories, or food recall records. An advantage of using food records is that they also evaluate total energy intake. Unfortunately, food records are time consuming and difficult to obtain because they require trained dietitians. Therefore, most reports relating dialysis dose to protein intake use estimations, based on urinary and dialysate nitrogen appearances and expressed as the protein equivalent of nitrogen appearance, PNA (142). The most commonly used formulas to estimate PNA are summarized in Table 99-8 (145–148).

The total PNA is then divided by the patient's body weight to determine the "normalized" PNA (nPNA), expressed in grams per kilogram of body weight per day. This term does not take into account differences in frame size and fat-free, edema-free mass (FFEFM). If a patient is markedly obese, the aforementioned calculations give a falsely low nPNA for the patient's actual FFEFM. Conversely, if a patient is malnourished and has a less than expected FFEFM, these equations give you a falsely elevated nPNA. Various attempts to avoid this problem are under investigation, but corrections have not been standardized. One modification uses actual measurements of V or data from nomograms that more accurately estimate V. This V is then "normalized" by dividing it by 0.58 kg/L to determine normalized body weight. The PNA is then divided by normalized body weight to get nPNA. An extension of these principles is utilized to determine FFEFM from creatinine kinetics (149).

Adjusting Dialysis Dose and Recognizing Pitfalls in Prescribing Peritoneal Dialysis

The initial PD prescription should be based upon a knowledge of patient's transport type (viz., determined using a PET), body size, and presence or absence of residual renal function. This can be done by using published algorithms (e.g., K/DOQI guidelines, data from EAPOS) or using computerized kinetic modeling (49,135,150,151). The clearances achieved with the initial prescription should be confirmed with 24-hour collections of urine and dialysate. If the patient is not at goal, the prescription should be adjusted. This adjustment can also be done either empirically or using computerized kinetic modeling programs. There are two general changes that can be made to maximize clearances in an individual patient—either increase the drain volume or increase the D/P ratio in the dialysate effluent. Increasing the instilled volume increases the total drain volume and thus, the convective clearance. By altering dwell time, one can change both the D/P ratio at the end of prescribed dwell and the drain volume. The strategies to maximize the drain volume in patients undergoing PD are summarized in Table 99-9 (152–163). If a patient does not have a continuously wet abdomen, providing 24-hour dialysis should be the first step to enhance clearances. In a patient with continuously wet abdomen, increasing the dwell volume should be the first

TABLE 99-9

STRATEGIES TO ENHANCE THE PERITONEAL SMALL SOLUTE CLEARANCES

Continuous ambulatory peritoneal dialysis	Automated peritoneal dialysis
Daytime exchanges Increase dwell volume (153) Increase ultrafiltration (tonicity of dialysate)	Daytime exchanges Add daytime dwell (if dry day) Add midday exchange (156) Increase dwell volume Increase ultrafiltration (tonicity of dialysate or alternative osmotic agents like icodextrin)
Nighttime exchanges Increase dwell volume (153) Increase number of exchanges (nighttime exchange device) Increase ultrafiltration (tonicity of dialysate or alternative osmotic agent like icodextrin)	Nighttime exchanges Volume of each dwell (153) Number of night time exchanges (162,163) Number of hours of cycling (8–10 hours) (156) Increase ultrafiltration (tonicity of dialysate)

step to enhance clearances. Most patients are able to tolerate the increase fill volumes without any discomfort and, if blinded to the fill volume, many are unable to correctly identify the amount of fluid instilled (154,155) In order to improve tolerance, the fill volumes may be increased when the patient is lying supine (i.e., for the night-time exchanges). Furthermore, cycler therapy allows a gradual increase in fill volumes and improves tolerance of increasing the volume of instilled dialysate.

Some issues to consider in patients on standard CAPD are: (a) inappropriate dwell times (a rapid transporter would do better with short dwells); (b) failure to increase dialysis dose to compensate for loss of residual renal function; (c) inappropriate instilled volume (patient may only infuse 2 L of a 2.5-L bag); (d) multiple rapid exchanges and one very long dwell (patient may do three exchanges between 9 AM and 5 PM, and a long dwell from 5 PM to 9 AM, limiting overall clearances); and (e) inappropriate selection of dialysate glucose for long dwells that may not maximize UF and clearance.

Other problems are specific for those patients on cycler therapy: (a) the drain time may be inappropriately long (more than 20 minutes), (b) inappropriately short dwell times may not maximize clearances, (c) failure to augment total dialysis dose with a daytime dwell ("wet" day versus "dry" day), and (d) inappropriate selection of dialysate glucose may not allow maximization of UF, resulting in less total clearance. One may be able to achieve weekly urea clearance targets, but not creatinine or middle-molecule clearance targets, with short dwell times, as in NIPD. A shortened time with fluid in the peritoneum is accompanied by decreased middle-molecule clearances and this may have an adverse effect on outcomes. It appears reasonable to say that when patients become anuric, they must maximize their "time" (most of day) on dialysis to maintain middle-molecular-weight clearances.

RESIDUAL RENAL FUNCTION AND PERITONEAL DIALYSIS

Over the last decade, the centrality of residual renal function (RRF) in maintaining health and welfare of patients undergoing PD has been established. Even though Maiorca et al. (37) were the first to demonstrate the survival benefit of RRF, the work by Diaz-Buxo et al. (40) first demonstrated the lack of equivalence of renal and peritoneal clearances for survival. This lack of equivalence of the two clearances for survival has now been established in numerous cohort studies (11,43,44,45,48). Over the same time period, data has accumulated that patients with significant RRF have a lower morbidity, have a lower severity of numerous complications associated with uremia, and have a higher quality of life (Table 99-10) (43,50,56,59,72,94,127,164–179). Thus, an understanding of the determinants of and strategies that retard the rate of loss of RRF are critical to the success of PD.

It is well documented that RRF is better preserved with PD than HD (Table 99-11) (180–186). Some studies have suggested patients undergoing automated peritoneal dialysis may have a faster decline of RRF than patients undergoing CAPD (187–190). Other investigators, however, have failed to confirm these

TABLE 99-10

REPORTED BENEFITS OF RESIDUAL RENAL FUNCTION IN PATIENTS UNDERGOING PERITONEAL DIALYSIS

Greater probability of survival (11,37,40,43,44,45,48,52)
Lower morbidity
- Hospitalizations (50,164)
- Peritonitis rates (164)
- Technique survival (50)

Cardiovascular
- Lower total body and extracellular water (127)
- Better control of blood pressure (165)
- Lower left ventricular mass index (166).

Nutritional status
- Higher dietary nutrient intakes (94,169).
- Higher serum albumin (43,166,168,170)
- Better nutritional status (72,168,170,172,173)

Anemia management
- Higher hemoglobin (166,170)

Divalent ion metabolism
- Better control of serum phosphorus (168,170,174,175)

Lower levels of circulating putative uremic toxins
- Low molecular weight like α1-microglobulin, alkaline RNAse (176)
- "Middle molecules" (178)
- Advanced glycosylation end-products like carboxymethyllysine (177)

Better quality of life (56,59,179)

TABLE 99-11

SUMMARY RESULTS OF SOME OF THE STUDIES THAT HAVE COMPARED THE RATE OF DECLINE OF RESIDUAL RENAL FUNCTION AMONG HEMODIALYSIS AND PERITONEAL DIALYSIS PATIENTS

Author (ref)	Patient no. HD[d]/PD	Study design	Baseline measure	Index of renal function	% Decline/ month (HD[d]/PD)	PD decline rate, % of HD[d] rate
Rottembourg, 1983 (180)	25/25	Prospective	Predialysis	C_{cr}[a]	6.0/1.2	80
Cancarini, 1986 (181)	75/86	Retrospective		C_{cr}[a]	5.8/2.9	50
Lysaght, 1991 (182)	57/48	Retrospective	Pre- and postdialysis	C_{cr}[a]	7.0/2.2	69
Misra, 2001 (183)	40/103	Retrospective	Postdialysis	Mean[b]	7.0/2.2	69
Lang, 2001 (184)	30/15	Prospective	Dialysis start	C_{cr}[a]	9.4/5.0	47
Jansen, 2002 (185)	279/243	Prospective	Predialysis	Mean[b]	10.7/8.1	24
McKane, 2002 (186)[d]	300/175	Retrospective	Pre- or postdialysis	Urea Cl[c]	Rate of decline similar in HD and PD	

[a] C_{cr}, timed creatinine clearance
[b] *Mean*, mean of timed urea and creatinine clearances.
[c] *Urea Cl*, timed urea clearance.
[d] All HD patients dialyzed with high flux dialyzers and ultrapure water.

findings (191–195) and, at this time, the importance of the PD modality on the rate of decline of RRF remains unresolved.

Notwithstanding the beneficial effect of treatment with PD on rate of decline of RRF, the renal function inexorably declines over time. The determinants that affect the rate of decline of RRF in PD patients have been studied in several cohorts (Table 99-12) (191,194,196–201). The differences in the result of the studies may reflect differences in sample size (and, hence, power), method of estimation of renal function, and different demographics of the various study populations. However, the preponderance of evidence suggests the presence of comorbidities, particularly cardiovascular comorbidities, hypertension, and proteinuria are the most important risk factors associated with the rate of decline of RRF. Consistent with the importance of hypertension and proteinuria in accelerating the rate of loss of RRF, randomized controlled trials have shown

TABLE 99-12

SUMMARY RESULTS OF THE MULTIVARIATE ANALYSES FROM COHORT STUDIES THAT HAVE EVALUATED THE RISK FACTORS ASSOCIATED WITH A MORE RAPID RATE OF DECLINE OF RESIDUAL FUNCTION AMONG PATIENTS UNDERGOING PERITONEAL DIALYSIS

Author (ref)	Patient number	Patient characteristics	Comorbidity	PD-related	Medications	Laboratory variables
Shemin, 1999 (196)					Aminoglycosides	
Shin, 1999 (197)	80			Higher peritonitis rate		
Moist, 2000 (194)	1032	Females non-Whites	CHF[a] Diabetes mellitus		ACE inhibitors[b] Ca channel blockers[b]	
Singhal, 2000 (191)	242	Large BMI	CHF[a] Diabetes mellitus	Higher peritonitis rate Larger dialysate vol.	No use of anti-HTN[d] Aminoglycosides	
Holley, 2001 (198)	184		Cardiac disease			
Caravaca, 2002 (199)	50	Females Baseline renal function[b]	CHF[a] Non-CIN[c] etiology of ESRD			Proteinuria
Jansen, 2002 (200)	243		Diastolic pressure	Episodes of dehydration		Proteinuria
Johnson, 2003 (201)	146	Large body surface area Baseline renal function High dietary protein intake	Diabetes mellitus	D/P creatinine		

[a] *CHF*, congestive heart failure.
[b] Patients with these characteristics or treatment with these medications associated with a ***slower*** decline in residual renal function.
[c] *CIN*, chronic interstitial nephritis.
[d] *Anti-HTN*, antihypertensives.

that treatment with angiotensin-converting enzyme inhibitors (ACEI, ramipril) or angiotensin-receptor blockers (ARB, valsartan) significantly slows the rate of decline of RRF (202,203). Finally, it appears that use of ACEI and ARB in patients undergoing PD is safe and dose does not result in significant elevations in serum potassium (204). This may be related to the use of PD fluids without any potassium, as currently practiced.

ULTRAFILTRATION

A certain minimal amount of daily UF is necessary to maintain water balance in patients with ESRD. This is achieved by an osmotic pressure gradient between blood and the dialysate using glucose as the effective osmotic force. During UF, retained solutes are swept along with the bulk solvent flow even in the absence of a concentration difference for diffusion. This contribution to net solute clearance has been termed solvent drag or convection; therefore, overall solute clearance is the sum of that owing to diffusion and convection.

Clinical Physiology

Simultaneous with UF of fluid from the bloodstream into the peritoneal cavity, there also occurs absorption of fluid from the peritoneal cavity, mainly via lymphatics (205). Intraperitoneal volume at any time is determined by the relative magnitudes of transcapillary UF and lymphatic reabsorption. Net UF at the end of any dwell is defined as the difference between drained volume and instilled volume. This definition assumes that the residual volume is constant, which is often not the case (206,207). This variation is insignificant for day-to-day clinical practice.

The driving force for UF decreases as the membrane becomes more permeable to solutes and the osmotic gradient diminishes. Ultrafiltration rates are highest at the beginning of the dwell. As glucose is absorbed, UF decreases as osmotic equilibrium is approached (208). Depending on the concentration of instilled glucose, osmotic equilibrium is reached at different times in the dwell cycle. For 2-L solutions containing 1.5% dextrose, osmotic equilibrium and maximal drain volume are reached after about 2 hours of dwell time in patients with average peritoneal membrane transport characteristics. For 4.25% dextrose solutions, peak intraperitoneal volumes are not likely to occur until after 3 or 4 hours (209). As osmotic equilibrium is approached, intraperitoneal volume and ultimate drain volume decrease owing to isosmotic absorption of fluids. In CAPD patients, this absorption rate ranges from 40 to 60 mL/hour and is attributable primarily to lymphatic drainage of the peritoneum (210).

Ultrafiltration Failure

Peritoneal UF is considered adequate when at least 5.5 mL of filtrate is generated per 1 g of absorbed glucose (211). Ultrafiltration failure can be defined as the failure of fluid removal by peritoneal dialysis to match the volume balance and blood pressure needs of the patient (i.e., there must be a balance of input and output). Ultrafiltration failure represents a failure to maintain volume homeostasis. This definition implies that clinically, UF failure can result from either inadequate fluid removal by PD or excessive salt and water intake. Furthermore, failure to remove adequate amounts of salt and water by the current PD prescription does not necessarily imply that there must be a pathologic alteration of the peritoneal membrane itself. Other possible causes include catheter malfunction, inadequate use of hypertonic fluids, inappropriately long dwell times, increased lymphatic reabsorption, fluid sequestration, including, as recently described, retroperitoneal leakage (212) and failure to match dwell time to peritoneal membrane transport status (213–215).

Ultrafiltration failure in CAPD may be defined as clinical evidence of fluid overload despite restriction of fluid intake and the use of three or more hypertonic (4.25% dextrose) exchanges per day (216). However, other definitions have been used in various publications; therefore, the exact incidence of UF failure is unknown. At one center, UF failure was observed in 6.2% of 227 CAPD patients over 10 years and the risk increased with time on PD (217). The prevalence was 2.6% after 1 year on PD and 30.9% after 6 years. Others claim that UF failure is responsible for up to 15% of PD technique discontinuation (218,219). If one considers a more rigid definition, such as one defined by the ability of a specified hypertonic dwell to remove a certain amount of fluid, the true incidence is rather low.

Clinical symptoms consistent with UF failure are not always caused by an actual loss of peritoneal UF capacity. Fluid overload, clinically mimicking UF failure, may occur when urine volume decreases and fluid intake is excessive or during noncompliance with the prescribed exchange regimen. Dialysate leaks from the intraabdominal cavity into extraabdominal tissue spaces may also result in loss of UF because of a decrease in dialysate contact with the peritoneal membrane. These causes are not associated with a change in peritoneal membrane transport characteristics. The first steps in the evaluation of a patient with suspected UF failure are to determine urine volume and to establish whether net effluent drain volume and/or peritoneal transport have changed. Apparent loss of UF is potentially reversible if caused by catheter malposition, dialysate leak, or recent peritonitis, but usually is permanent if kinetic studies suggest a reduction in UF capacity of the membrane.

An ad hoc committee has recently reviewed UF issues in PD and recommended a specific clinical definition for membrane failure while outlining a rational approach to fluid management and blood pressure control (220). This is based, in part, on prior classifications based on transport findings and has been revised for patients with irreversible UF failure (214,215). This group recommends using a 4-hour dwell with 2.0 L of 4.25% dextrose dialysate to diagnose and work-up UF failure. Using this definition, less than 400 mL of net ultrafiltration after a 4-hour dwell is diagnostic of true peritoneal membrane failure. A rational approach to the patient with suspected UF failure is found in Figure 99-5. If a patient presents with the clinical syndrome of volume overload, one (a) rules out dietary indiscretion; (b) assures that the patient is on the appropriate prescription for his or her peritoneal membrane transport type; and (c) rules out mechanical problems. If these do not confirm the cause, then a 4.25% PET is done, and UF volume and D/P creatinine ratios are noted. Further evaluation is based on D/P creatinine ratio.

Ultrafiltration Failure and High Solute Transport

Patients with loss of UF and current 4-hour PET ratios of D/D_0 glucose of less than 0.3 and D/P creatinine of greater than 0.81 are characterized as high solute transporters (see Fig. 99-2). These patients tend to have rapid small-molecular-weight solute transport and poor UF owing to high (rapid) glucose absorption and resultant rapid dissipation of the osmotic gradient. These patients are the largest group with true UF failure. Some patients have these transport characteristics at baseline

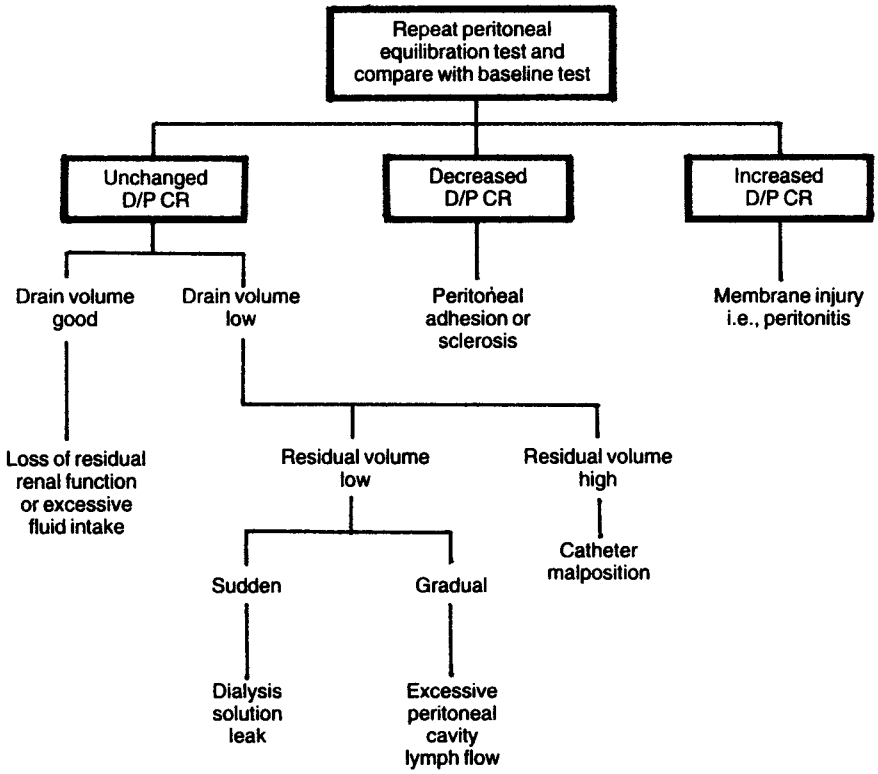

FIGURE 99-5. Algorithm for loss of ultrafiltration in continuous ambulatory peritoneal dialysis (CAPD) patients. *D/P CR,* dialysis:plasma creatinine ratio. (From: Khanna R, Nolph KD, Oreopoulos DG, eds. *Essentials of Peritoneal Dialysis.* Dordrecht, The Netherlands: Kluwer Academic; 1993:98, with permission.)

and, if their dwell times are mismatched for their membrane transport characteristics, they often appear to have UF failure as they lose residual renal function and no longer have urine flow to supplement dialysate daily fluid losses. In other patients, the loss of UF is owing to an increase in membrane transport. This increase is caused by either an acquired increase in transport (formerly called type I membrane failure) or membrane changes associated with a recent episode of peritonitis.

Recent Peritonitis

It is a common clinical experience for PD patients to experience fluid retention during peritonitis. Compared to baseline, a PET performed during peritonitis reveals an increase in the D/P ratio for creatinine and a decrease in the D/D_0 ratio for glucose. There is also an increase in dialysate protein losses and a significant decrease in net UF (221). These changes associated with peritonitis are usually reversible after recovery. Microscopic findings in patients with acute peritonitis have revealed denudation of the mesothelial surface (222,223). However, in some patients, remesothelialization never occurs even after recovery from peritonitis (224) leading to chronic UF loss. Di Paolo and co-workers (225) have demonstrated that autoimplantation of labeled cultured mesothelial cells in rabbits and CAPD patients with peritonitis resulted in reimplantation, improvement in UF parameters, and normalization of dialysate phospholipid concentrations. Further studies are needed before widespread clinical use of this technique is advocated. These patients often need a temporary change in their standard dialysis prescription (shorter dwell times or increased tonicity) to maintain UF. Several studies have indicated that UF during an episode of peritonitis can be maintained with alternative osmotic agents such as icodextrin (226).

Acquired Increase in Membrane Transport

Patients who develop an increase in peritoneal transport over time on PD (formally called type I membrane failure) are the most common cause of chronic UF failure in CAPD. Peritoneal equilibration testing confirms high or high average transport rates with resultant rapid glucose absorption, loss of the osmotic gradient, and a decrease in net transcapillary UF. In contrast to the situation seen with peritonitis, where transport changes and protein losses usually are transient (227), small-solute transport changes and protein losses are more permanent with type I membrane failure (216). There also tends to be less of a decline in dialysate sodium owing to the sodium sieving with convective transport as seen in controls. These changes are thought to result from an increase in effective surface area of the peritoneal membrane.

These changes were originally described with acetate-containing dialysis solutions (228) but have also been seen in patients who have used only lactate-containing dialysis (216). Reported microscopic findings are similar to those seen with peritonitis. Recurrent peritonitis and use of hypertonic dialysate have been implicated in some (229,230), but not all, studies (216,231). The incidence of an increase in membrane transport seems to increase with time on PD, implicating repeated exposure of the peritoneum to dialysate as a possible cause. Davies et al. (232) retrospectively analyzed glucose exposure in two patient cohorts: those whose transport characteristics were stable over 5 years and those who exhibited an increase in membrane transport over the same length of time. They found that the patients who were destined to exhibit increased transport over time had been exposed to a greater glucose load from the inception of peritoneal dialysis strongly suggesting a causal relationship (232).

Most cases can be managed by shortening dwell times. Because these patients have high (rapid) transport of small solutes, they have adequate urea and creatinine clearances even with short dwell exchanges. Occasionally, resting the peritoneum for at least 4 weeks through temporary transfer to HD has been associated with an improvement (233,234). Di Paolo and co-workers (235) found low phosphatidylcholine levels in patients with various types of UF failure. Increases in UF were observed after intraperitoneal phosphatidylcholine administration in CAPD patients without UF failure (236,237).

Possible explanations include direct membrane effects of phosphatidylcholine or decreased absorption via subdiaphragmatic lymphatics by: (a) an increase in cholinergic tone, causing contraction of subdiaphragmatic stomata (238); (b) neutralization of the anionic charges on the lymphatic endothelium by its cationic charge, which tends to keep stomas open; and (c) surface-acting properties of the molecule that tend to repel water and inhibit movement into the lymphatics. Despite these initial findings, routine use of intraperitoneal phosphatidylcholine cannot currently be recommended, because of concerns about safety and the possibility of adhesion formation. The effects of oral phosphatidylcholine are conflicting (239,240,241).

Patients with apparent ultrafiltration failure due to rapid solute transport should be strongly considered for treatment with icodextrin, a slowly metabolized glucose polymer which acts as a colloid osmotic agent. While the rate of ultrafiltration with icodextrin is slower than that with dextrose, the effect of icodextrin persists much longer, making it suitable for ultrafiltration during long dwells of up to 14 to 16 hours. In both CAPD and APD patients, icodextrin has been shown to provide ultrafiltration superior to that with either 2.5% or 4.25% dextrose (242,243). This is associated with decreases in total body and extracellular fluid water (242,244), lower blood pressure (244), and, possibly, regression of left ventricular hypertrophy (245). Available in Europe for over a decade, icodextrin has recently become available in the United States as well.

With the use of icodextrin it is usually possible to achieve adequate small-solute clearance in these patients; yet, a minority may require transfer to HD for volume and blood pressure control. There also is a concern that continued membrane damage may result in eventual progression to peritoneal sclerosis or type II membrane failure (246,247). If so, it would be important clinically to closely monitor patients with type I failure with PETs. If these patients continue on PD and their solute transport declines, it may be beneficial to temporarily switch them to HD to allow healing of the peritoneum (248).

Ultrafiltration Failure and No Change or Average Solute Transport

Loss of UF in patients with no change or average transport characteristics tends to result from catheter malfunction, fluid leaks, or excessive lymphatic reabsorption (formerly type III membrane failure), and aquaporin deficiency. If loss of UF is owing to catheter malfunction or fluid leaks, the patients do not have a functional change in their membrane and usually can be maintained on PD after the problem has been resolved.

Excessive Lymphatic Absorption

Excessive lymphatic absorption (historically called type III membrane failure) is a very uncommon cause of membrane failure related to excessive rates of lymphatic absorption of fluid (216). Although these patients may not have a significant change in D/P values when compared to baseline, they do have drain volumes after 4 hours of dwell that are less than baseline values or what would be expected based on standard therapy. A further diagnostic clue is that these patients tend to have higher dialysate sodium concentration during the dwell than controls (216,231) (Fig. 99-6).

Aquaporin Deficiency

This is a rare and only recently understood condition, which may become more prevalent as a more uniform diagnostic approach to UF failure is undertaken (249). Patients with suspected aquaporin deficiency have damage to, decreased number of, or no water channels (ultra-small pores) that can lead to deficient crystalloid-induced UF (250). These patients are diagnosed clinically by finding less than 400 mL of UF with a 4.25% PET and lack of sodium sieving early in the dwell. However, one must be careful to exclude patients who are very rapid transporters. Rapid transporters do exhibit sodium sieving, but it occurs so early in the dwell that if looked for after 60 to 90 minutes of dwell time it may be masked. These patients should respond clinically to use of colloid osmotic agents (such as icodextrin), which achieve ultrafiltration through a different mechanism and are not dependent on the water channels for UF.

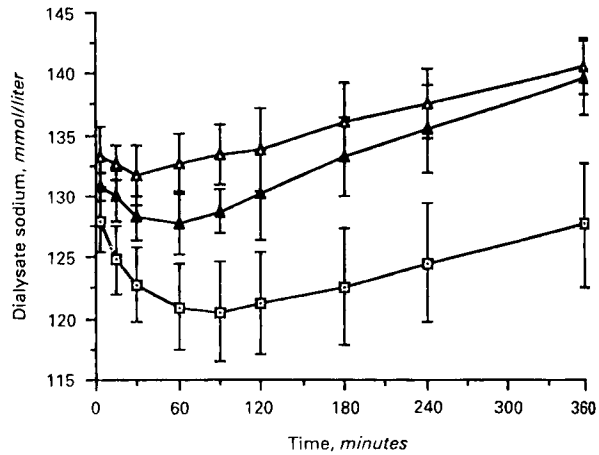

FIGURE 99-6. Dialysate sodium concentrations as a function of time in patients using 4.25% dextrose exchanges over 6-hour dwells. Results are compared in patients with normal ultrafiltration kinetics (*hollow squares*), those with high lymphatic absorption rates (*solid triangles*), and those with high glucose absorption rates (*hollow triangles*). (From: Heimburger O, et al. Peritoneal transport in CAPD patients with permanent loss of ultrafiltration capacity. *Kidney Int* 1990;38:495, with permission.)

Ultrafiltration Failure and Low Solute Transport

Patients with UF failure and low (slow) solute transport (D/D_0 glucose of more than 0.5 and a D/P creatinine of less than 0.5) tend also to have inadequate small-solute clearances. Poor UF occurs despite the maintenance of adequate osmotic gradients. These patients are found to have a loss of functional peritoneum and the differential should include: peritoneal sclerosis (formally called type II membrane failure) or multiple peritoneal adhesions and, at times, patients with high lymphatic absorption rates. These patients often require transfer to HD.

Peritoneal Sclerosis

An uncommon cause of UF failure is peritoneal sclerosis (type II membrane failure). Patients present with failure of both UF and small-solute transport. These patients have low net UF, normal glucose absorption, and high lymphokine levels in the dialysate associated with low prostaglandin E_2 levels when compared to individuals with normal or high peritoneal transport properties (251). Simple peritoneal sclerosis should not be confused with the rare syndrome of encapsulating peritoneal sclerosis (EPS) (368,369,370). With EPS, patients present with a thick-walled membrane "cocoon" entrapping loops of bowel with

associated anorexia, nausea, vomiting, malnutrition, and intestinal obstruction. As opposed to patients with EPS, patients with simple peritoneal sclerosis do not have the surgical findings of encapsulating fibrosis, but may have diffuse thickening and fibrosis of the parietal and visceral peritoneum (369,371). The etiology of EPS is uncertain. Peritoneal irritants, recurrent peritonitis, long-term use of PD, acetate-containing dialysis, chlorhexidine, beta-blockers, and high transporter status have all been implicated in the pathogenesis (252–254). Mortality is severe—as high as 60% within 4 months of diagnosis.

Treatment consists of corticosteroids, supportive care with parenteral nutrition, and, in extreme cases, surgical enterolysis. Recent experimental data supports a role for activation of the renin–angiotensin system in this disorder suggesting a possible therapeutic role for ACE inhibitors and/or ARBs (255,256). Tamoxifen, an antiestrogen agent that inhibits protein kinase C (PKC), has been used to treat retroperitoneal fibrosis, a process with pathologic findings similar to those of peritoneal sclerosis (257). It has also been used to stabilize the processes of both simple sclerosis (258) and EPS (252,259).

Multiple Abdominal Adhesions

Extensive intraabdominal adhesions may result after recurrent or severe peritonitis and after catastrophic intraabdominal events. These processes can cause a decrease in the peritoneal membrane surface area in contact with dialysate. Although normal transport may occur in the membrane that is in contact with dialysate, overall transport and net UF decrease simply from loss of surface area. Surgical lysis of adhesions may result in improvement and, if an adequate increase in surface area can be achieved, PD could continue.

CATHETERS

Types of Peritoneal Catheters

The Tenckhoff catheter originally designed by Palmer and modified by Tenckhoff continues to be used in the majority of PD patients (260,261–264). A number of variations are available. The straight or curved subcutaneous portion may have one or two cuffs. Double-cuffed catheters are used in the majority of patients (262). The intraabdominal portion of the catheter may be straight or curled. Curled catheters were designed to decrease outflow problems and infusion pain (264) but appear to have similar complication rates as straight Tenckhoff catheters (265,266).

The Toronto Western Hospital catheter has two silicone rubber disks 5 cm apart on the intraabdominal portion of the catheter (to prevent migration of the catheter tip) and a Dacron disk at the base of the second cuff (to decrease the risk of leaks) (267,268). The rates of complications are similar with the Toronto Western and the Tenckhoff catheters (266), although Flanigan and colleagues (269) found catheter survival to be superior with the Tenckhoff catheter.

To decrease migration of the intraabdominal portion and exit-site infections, Twardowski and associates (270) designed a catheter with a curved subcutaneous pathway in which both the internal and external exit sites are downward. Comparisons with the straight Tenckhoff catheter indicate similar 2-year catheter survival, peritonitis risk, and exit-site infection rates (271,272), although catheter migration may be decreased with the Swan neck catheter (272,273). Hwang and colleagues (274) found superior 3-year catheter survival rates with the Swan neck catheter compared to the Tenckhoff catheter, which was attributed to lower rates of cuff extrusion and pericatheter leakage. Downward-directed tunnels are associated with lower infection rates (275). A modification of the Swan neck catheter with a presternal exit site had excellent 2-year survival of 95% in the hands of an experienced team (276). Placement of a 5- to 10-gram weight at the tip of the intraabdominal portion of the catheter has also been shown to decrease catheter migration (277,278). In a recent multicenter study, use of this "self-locating" catheter was shown to result in significant decreases in catheter dislocation, peritonitis, tunnel infections, cuff extrusion, leakage, and obstruction with a concomitant improval in overall catheter survival (278). Self-locating catheters are not yet available in the United States.

The curled single-cuff Tenckhoff catheter is used in most children (279). Three lengths of catheters are available: neonatal (8 to 10 cm from cuff to tip), juvenile (16 cm cuff to tip), and adult, based on patient size (280).

Peritoneal Catheter Placement

The location of the exit site should be discussed with the patient prior to catheter placement to avoid the waistline. Preoperative laxatives are indicated for constipation, commonly present in patients because of phosphate binders, and an important cause of catheter malfunction (263). The patient should void prior to the procedure; if the patient has a neurogenic bladder, then urethral catheterization is performed. In many patients, placement can be accomplished using local anesthesia with sedation, although general anesthesia is occasionally necessary (281). The patient usually does not require admission. Prophylactic antibiotics (generally a cephalosporin) for catheter placement (282,283), given before the skin incision, decreases the risk of catheter-related peritonitis (284).

Most peritoneal dialysis catheters are inserted by a surgeon using a dissection technique (262). A small paramedian incision is made overlying the rectus sheath down through the muscle to the peritoneum (285,286). The catheter is inserted so that the deep cuff is within the rectus muscle and the tip is in the pelvis. A purse string of nonresorbable suture (to decrease the risk of subsequent leaks) is placed where the catheter enters the peritoneum. Catheter function is assessed by infusing and draining fluid (287). The subcutaneous tunnel is formed such that the superficial cuff is 3 cm from the skin surface (263) and directed downward. A small exit site wound formed by a tapered tunneling device of the same diameter as the catheter is best for minimizing trauma and decreasing the risk of subsequent exit-site infection and catheter-related peritonitis (288).

Placement via a peritoneoscope is used in a minority of catheters. This technique allows direct intraabdominal visualization (262,289,290). Adhesions can be avoided and the tip of the catheter placed to allow optimal catheter function. In a randomized comparison of laparoscopic versus conventional dialysis catheter insertion (both done by surgeons) outcomes were not different except that the conventional placement was faster (14 versus 22 minutes, $p < 0.0001$) (291). However, in another study comparing laparoscopic placement to historical controls in whom catheters were placed by the same experienced surgeons using conventional technique, use of the laparoscope resulted in a lower incidence of catheter dysfunction and superior three-year catheter survival (292). The peritoneoscope may be particularly useful in patients with previous surgery or when placement by dissection results in a nonfunctioning catheter (293,294).

Blind percutaneous catheter placement may be used for placement of a catheter for acute renal failure to be used for a short time (280). Percutaneous blind catheter insertion is the least desirable technique, as it can be associated with organ perforation and drain failure (295–297). Percutaneous catheter placement using fluoroscopy is not widely performed (298).

To decrease the risk of peritonitis via the tunnel, Moncrief and co-workers (299) developed a new insertion technique. At insertion, the entire external portion of the catheter is buried in abdominal wall subcutaneous tissue. Three to 5 weeks later, the catheter is externalized via a small incision, which becomes the exit site. Burying the external portion of the catheter for 6 weeks does not change technique survival (300); data regarding a possible decrease in infectious rates with this technique are conflicting (300,301). This technique has been employed for use with presternal as well as abdominal catheters (302).

Children require special consideration. In infants, the exit site is located above the diaper area to prevent contamination (303). Partial omentectomy is useful to prevent outflow problems. In boys, herniotomy and ligation of patent processus vaginalis at the time of catheter placement decrease the risk of subsequent inguinal hernia and hydrocele (303).

Perforation of the bladder or bowel or laceration of the spleen is an uncommon occurrence (304), but adhesions increase the risk (305). Perforation of a hollow viscus should be considered if the effluent is feculent or when watery diarrhea, polyuria, or watery vaginal discharge occurs with infusion of dialysate (305). Minor bleeding frequently occurs after catheter insertion, but generally stops quickly and spontaneously. Flushing the catheter with heparinized (500 U/L) dialysate is useful to clear the catheter and prevent blockage by clots (306).

Postoperative Management and Exit Site Care

If possible, initiation of PD is postponed to allow healing and prevent leaks (282). Under these circumstances, the catheter should be flushed several times with 1 L of dialysate or saline until the effluent is clear and then capped until training begins (307). The use of 2-L exchange volumes may begin immediately with no increased risk of leaks if bed rest is prescribed for the first 3 days (308). Alternatively, the patient may be maintained on HD until healing occurs. The risk of catheter complications is the same for patients placed on IPD versus those on temporary HD during the healing phase (307); therefore, training on the cycler (with the abdomen empty when erect) can be initiated immediately after catheter placement.

Postoperative sterile dressing changes until healing takes place may help reduce infection risk (283,309). The surgical dressing may be left intact for several days unless there is bleeding. The exit site should be kept dry until well healed; this may require up to 2 weeks. During this interval, patients should not shower or bathe in tubs; personal hygiene should be performed with sponge baths. Povidone-iodine solution is commonly used for postoperative cleaning of the exit site (283).

Once healed, many centers advise washing the exit site with bactericidal soap and water during routine bathing (283). Cleaning with povidone-iodine and the use of nonocclusive dressing results in fewer exit-site infections than use of nonbactericidal soap and water (310). The use of povidone-iodine ointment applied to sterile gauze used as a dressing to the exit site is effective to prevent early exit-site infections (311). Once the exit site is well healed, swimming in chlorinated pools or the ocean is permitted, but swimming in creeks or ponds or the use of hot tubs should be avoided, because this may result in infection (283).

Mechanical Complications

Early inadequate outflow occurs after 7% of catheter insertions, requiring replacement in one-half of these patients (281,312). Constipation may lead to shifting of the catheter position, drainage failure, and catheter loss. Ideally, the catheter tip should be in a pelvic gutter, because this location results in maximum outflow rate (313). Tip migration to the epigastric or hypochondrial regions is generally associated with dysfunction (281,312,314). Poor drainage owing to catheter malposition in the upper quadrants may be corrected by manipulation with a laparoscope or Fogarty catheter, fluoroscopically using a guide wire, or surgically (271,315–321).

There are other causes of catheter dysfunction in addition to catheter malposition. One- or two-way obstruction may result from clots or fibrin within the lumen (287,305). Forcibly flushing with heparinized saline may resolve this problem, but fibrinolytic agents may be effective if this fails (322–324). Omental obstruction may necessitate omentectomy, especially in children (281,287,303–305,325). Partial omentectomy at catheter placement improves catheter survival and is performed routinely in children (326).

Peritoneal dialysate leaks, which may occur at several different locations (285,327), develop in 5% to 10% of catheters in the immediate postoperative period (271,281,285,312) and in 2% to 4% (312) of catheters later in the course of CAPD. Dialysate leaking from the exit site presents as clear fluid that is strongly test-strip positive for glucose. Leaks at the internal cuff may present as abdominal wall edema. These leaks may result from the use of resorbable suture material at the deep cuff, placement in a median rather than paramedian site, early initiation of CAPD, or hernia formation (268,287,305,328). Computed tomography scan peritoneography (using Omnipaque, 50 mL/L of dialysate) is the best way to evaluate leaks and hernias (329). A dialysate leak may resolve with PD in the supine position or temporary cessation of PD (using HD) (285,305). Dialysate leaks from the exit site often are associated with infection; thus prophylactic antibiotics should be given (330). If a leak occurring more than 1 month after catheter insertion does not resolve within 4 days of reduced dialysate volumes, or if it recurs after full volumes are resumed, surgical correction generally is required (331).

INFECTIONS

Definitions of Peritoneal Dialysis-Related Infections

Peritonitis is present when the effluent is cloudy with a white blood cell count ≥100 cells/L with more than 50% polymorphonuclear cells (332). The patient usually has abdominal pain but often does not have a fever (332–335). The effluent white blood cell concentration is a less sensitive indicator of peritonitis if the patient is already on antibiotics or if the patient is on automated PD with either a dry abdominal cavity or a short dwell time when presenting with peritonitis. In these circumstances, the percentage of neutrophils (more than 50%) is more useful than is total white blood cell concentration (332).

Approximately 6% of patients with culture-positive effluent present with abdominal pain and clear effluent (336). A delayed effluent cell reaction occurs in two-thirds of these patients, but one-third never develop an appropriate cellular response to infection. When not experiencing peritonitis, such patients have a lower dialysate cell count (particularly macrophages and CD4 lymphocytes) and a delayed production of interleukin-6 and -8, compared to other patients. Any patient on PD, who presents with abdominal pain, should be considered to have peritonitis until proved otherwise.

An exit-site infection is defined by the presence of purulent drainage, with or without erythema, at the catheter exit site (337). Induration and tenderness at the exit site are abnormal and may indicate infection. In the absence of drainage, erythema of the exit site (which is normally flesh colored) does not necessarily indicate the presence of infection; erythema may

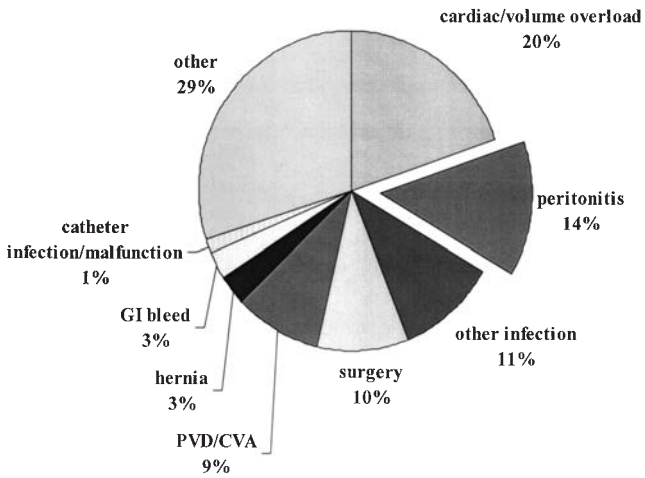

FIGURE 99-7. Causes of 274 hospitalization for 126 peritoneal dialysis patients, as percentages. (From: Fried L, et al. Hospitalization in peritoneal dialysis patients. *Am J Kidney Dis* 1999;33:927, with permission.)

result from irritation or trauma to the exit site (338), but is seldom associated with catheter loss unless drainage also is present (339). Nonpurulent drainage and crusting of the exit site do not necessarily represent infection, nor does a positive culture of a normal-appearing exit site (340).

An infection of the subcutaneous catheter (or "tunnel infection") is present when there is pain, tenderness, erythema, or induration over the subcutaneous pathway. Tunnel infections most often occur in the presence of an exit-site infection (340,341). Tunnel infections often are clinically occult. This has been shown by numerous studies using sonography of the subcutaneous tunnel in patients with exit-site infections (341–344). When peritonitis occurs in conjunction with an exit-site infection owing to the same microorganism (particularly *S. aureus* or *P. aeruginosa*), the presumption should be that there is a tunnel infection (345).

Peritoneal dialysis-related infections remain a major problem. Such infections are responsible for the majority of catheters lost (284,346) and contribute to transfer of the patient to HD (347–349). Peritonitis is a major cause of hospitalization (347,350,351) (Fig. 99-7). Peritonitis occasionally results in death, either directly from sepsis or indirectly from ensuing complications (284,352–354).

Dialysate Solutions and Peritoneal Immune Function

Peritoneal host defenses are of great importance in preventing peritonitis. Bacteria are cleared from the peritoneum by the processes of opsonization, chemotaxis, phagocytosis, and intracellular killing (355). Thus, bacterial contamination of the peritoneal cavity does not always result in peritonitis if the peritoneal immune defenses are intact. Phagocytosis by peritoneal macrophages decreases in the majority of patients 1 to 2 days prior to the onset of clinical peritonitis, suggesting that not only entry of bacteria into the peritoneal cavity but also malfunction of peritoneal immune function contributes to peritoneal infection (356).

Peritonitis decreases peritoneal ultrafiltration (357,358). During peritonitis there is a sharp rise in the dialysate to plasma ratios for low, middle, and high molecular weight solutes, which returns to baseline by 2 weeks. However, ultrafiltration does not completely return to the baseline values, probably because of impairment of transcellular water transport (358). Effluent levels of interleukin-1 and -6, as well as transforming growth factor-β and fibroblast growth factor (both of the latter being sclerogenic cytokines), remain elevated even 6 weeks after an episode of peritonitis (357), suggesting a mechanism for decreased ultrafiltration in some patients with increasing time on PD.

TABLE 99-13

DIALYSATE COMPOSITION

Dextrose, measured in g/dL (%) as the hydrous dextrose, available as 1.5%, 2.5%, and 4.25%
Sodium, measured as mEq/L, available at 132
Chloride, measured as mEq/L, available at 102, 96, and 95
Lactate, measured as mEq/L, available at 35 and 40
Calcium, measured as mEq/L, available at 2.5 and 3.5
Magnesium, measured as mEq/L, available at 0.5 and 1.5
Bag volumes, measured in L, available at 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 5.0, and 6

Dialysate interferes with peritoneal immune defenses. Dialysate contains sodium, chloride, lactate, calcium, magnesium, and dextrose (Table 99-13). The acidic pH, lactate content, and hypertonicity of dialysate are nonphysiologic and impair peritoneal immune function (359,360). The dilution effect of dialysate also increases peritonitis risk. The immunoglobulin and complement concentrations in dialysate are 1% to 2% of those in the peritoneal cavity of patients not on PD (355) and the concentration of peritoneal macrophages, important in peritoneal defense against infection, is low (359).

Lactate is the commonly used alkali, because dialysate containing both bicarbonate and glucose cannot undergo the routine heat sterilization procedures (361). Neutrophil function is better preserved with bicarbonate-containing dialysate compared to lactate-containing dialysate (362), although bicarbonate with a high glucose concentration remains cytotoxic (363). Lactate-containing dialysate with neutral pH is much less inhibitory of superoxide generation by neutrophils compared to standard lactate dialysate and is almost similar to bicarbonate-containing dialysate (364). Bicarbonate containing dialysate is feasible, in that the bicarbonate and dextrose can be kept in separate compartments and combined prior to infusion (361). Two-chambered bicarbonate lactate-buffered PD fluid confers better phagocytosis and is associated with lower glucose degradation products compared to standard dialysate (365). Use of bicarbonate-containing dialysate has been shown to improve peritoneal macrophage function (366,367). It remains to be seen whether this will result in lower peritonitis rates.

Polymers of glucose are an alternative to dextrose, particularly in high transporters (368,369). Dextrose is rapidly absorbed during a dwell, thus decreasing the osmotic gradient and leading to considerable caloric load. Glucose polymers are isosmolar; UF is obtained through colloid osmosis. In a randomized multicenter study, icodextrin (a large-molecular-weight glucose polymer), used for the overnight exchange, was safe and provided effective UF, compared to 4.25 g/dL dextrose dialysate (369). The glucose polymer solution is less suppressive, compared to 2.5 g/dL and 4.25 g/dL dextrose dialysate, on bacterial phagocytosis; this effect is predominantly owing to the lower osmolality (370,371). Peritonitis results in increased degradation of icodextrin, an increase in dialysate osmolality, and, therefore, increased ultrafiltration, in striking contrast to the changes seen with glucose dialysate in peritonitis (372).

Amino acid-containing dialysate has been proposed as an alternative to glucose-containing dialysate. Polymorphonuclear

cell function is not impaired by amino acid dialysate in contrast to dextrose-containing dialysate (373). Amino acid dialysate has similar small- and large-molecular-weight solute transport and UF to equimolar dextrose dialysate (374). The use of one exchange each day of a 1% amino acid dialysate for 6 months improved nitrogen balance, but did not result in a rise in the serum albumin (375). Disadvantages of amino acid dialysate include a rise in the blood urea nitrogen level and a fall in the bicarbonate (375); therefore, close attention must be paid to urea nitrogen clearance to prevent uremia and oral sodium bicarbonate often is necessary during use of amino acid dialysate. Amino acid-containing dialysate is not available in the United States.

Dialysate is available in 2.5 and 3.5 mEq/L calcium. Dialysate with 3.5 mEq/L calcium places the patient in positive calcium balance (376). Parathyroid hormone is suppressed, which may contribute to the high prevalence of aplastic bone disease in PD patients (377,378). The use of 3.5 mEq/L calcium is associated with a higher risk of hypercalcemia (379). Dialysate with 2.5 mEq/L calcium results in negative calcium balance, allowing increased doses of calcium salts to be used for phosphate control (376,379); however, the use of 2.5 mEq/L calcium dialysate, especially without calcitriol, may lead to an exacerbation of hyperparathyroidism (379,380).

Connection Devices

New connection techniques have resulted in a dramatic lowering of peritonitis rates, particularly those owing to organisms such as coagulase-negative *Staphylococcus*. For many years, the standard connection system was a straight line with an empty dialysate bag attached to the patient between dialysis exchanges (381). The exchange was performed manually. The straight-line system has been replaced with safer connection systems, such as the Y-set and double-bag system. With the Y-set, the patient connects the catheter to a Y-set of tubing attached to a full dialysate bag and an empty bag. The patient sequentially flushes dialysate through the line into the drain bag to clear air, then drains the effluent from the peritoneum, infuses the fresh dialysate, and disconnects the Y tubing, either capping the catheter or snapping off the tubing. This strategy is known as "flush before fill" and was initially brought into practice by Buoncristiani (382). The double-bag system is a further improvement in technology, because both the drain and fill bags are already attached to the Y tubing; therefore, the only possible site of contamination is during the connection the patient makes to the exchange tubing attached to the catheter. Peritonitis rates are significantly lower with the double-bag system compared to the Y-set in high-risk populations (383,384). The double-bag system offers little advantage over the Y-set in centers with low peritonitis rates (385). However, a close analysis indicates lower rates of Gram-positive peritonitis with the double-bag as compared to the Y-set, suggesting that this method further reduces the risk of contamination (Fig. 99-8). Therefore, the best connection device is that with the preattached bags (the double-bag system).

The data are conflicting on whether peritonitis rates are lower on the cycler compared to CAPD, although a number of studies suggest that is the case. deFijter and co-workers (386,387) randomly assigned 82 patients beginning peritoneal dialysis to cycler PD (type not identified) or CAPD using a Y-set. Peritonitis rates were lower (0.51 versus 0.94 per dialysis year, respectively) on the cycler. Similar differences, although accompanied by overall lower rates, were seen in a larger, nonrandomized study from Spain (388). Because this center has a protocol treating *S. aureus* nasal carriage, the rates of *S. aureus* peritonitis were extremely low in both groups (0.03 episodes per year at risk in both). Lower rates of coagulase-negative *Staphylococcus*, *Streptococcus* species, and Gram-negative bacteria reduced peritonitis in the cycler (Home Choice) group (Fig. 99-9). A strikingly high rate of Gram-negative peritonitis results from reuse of the cassettes (not recommended by the manufacturer) (389,390). A recent study suggests that NIPD may be associated with lower infection rates, perhaps due to enhanced peritoneal immune function as a consequence of the abdomen being kept dry for a portion of the day (391).

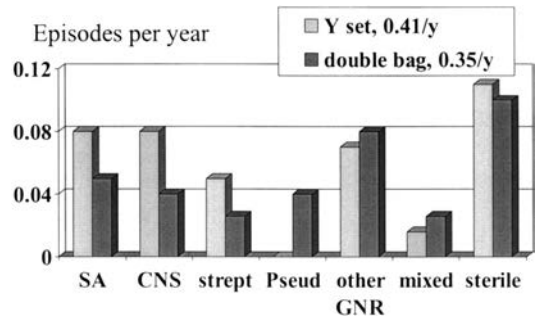

FIGURE 99-8. Episodes of peritonitis per dialysis year at risk in patients randomly assigned to the Y set or the double bag system for CAPD. (Modified from: Li PK, et al. Comparison of double bag and Y set disconnect systems in continuous ambulatory peritoneal dialysis: a randomized prospective multicenter study. *Am J Kidney Dis* 1999;33:535.)

Catheter Infections

The International Society for Peritoneal Dialysis has recently published a comprehensive review of the approach to infectious complications in the PD patient (337). The reader is referred to that article for detail; the following is a broad overview.

There is marked variation in reported rates of exit-site infections, in part because of differing definitions and because exit and tunnel infections are not always reported separately (392). The catheter infection rate is reported at about 0.6 per year in the National CAPD Registry and many single centers (339,392–395). The rate of clinically obvious tunnel infection is 0.19 per year (22) (396); however, when an exit-site infection is present, fluid collections along the subcutaneous pathway can be frequently demonstrated by ultrasound examination. Tunnel involvement is common when an exit-site infection is concurrent (341–344,397).

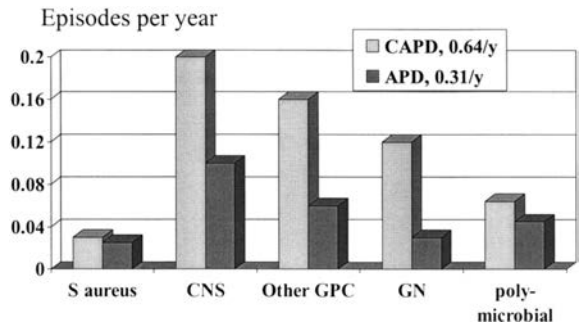

FIGURE 99-9. Episodes of peritonitis per dialysis year at risk in patients on CAPD versus APD The center uses prophylaxis for *S. aureus* nasal carriers; therefore, *S. aureus* peritonitis rates are very low in both groups. (Modified from: Rodriguez-Carmona A, et al. A comparative analysis on the incidence of peritonitis and exit-site infection in CAPD and automated peritoneal dialysis. *Perit Dial Int* 1999;19:253, with permission.)

TABLE 99-14

PATHOGENS CAUSING EXIT-SITE INFECTIONS

Pathogens	Episodes per year
S. aureus	0.3–0.4[a]
S. epidermidis	0.1
Gram-negative	0.1–0.2
Culture-negative	0.03–0.1
Total	0.6–0.7

[a] Much lower in programs using *S. aureus* prophylaxis.
(Modified from: Flanigan MJ, et al. Continuous ambulatory peritoneal dialysis catheter infections: diagnosis and management. *Perit Dial Int* 1994;14:248; Holley JL, Bernardini J, Piraino B. Infecting organisms in continuous ambulatory peritoneal dialysis patients on the Y-set. *Am J Kidney Dis* 1994;23:569.)

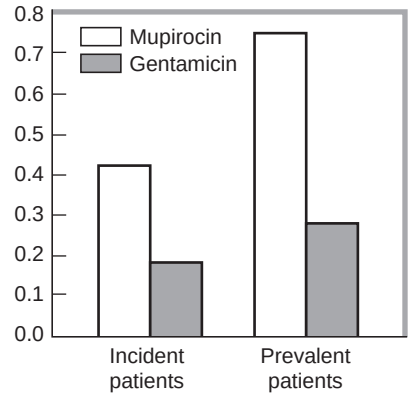

FIGURE 99-10. Rates of exit-site infections in incident (on ≤3 mo) and prevalent patients (on >3 mo). In both groups, those who were using gentamicin exit site cream had significantly lower ($p < 0.01$) rates than those who were using mupirocin.

Microorganisms causing exit-site infections are shown in Table 99-14. The most common organism causing exit-site and tunnel infections is *Staphylococcus aureus,* which may be difficult to resolve and can lead to peritonitis and catheter loss (347,345,393,394,396,398–401). *Pseudomonas aeruginosa* is the second most common cause of exit site and tunnel infections and frequently recurs or is refractory to antibiotic therapy and tunnel revision (402). Therefore, early catheter removal is appropriate if the patient does not respond to a course of antibiotics. *Staphylococcus epidermidis* and culture-negative exit-site infections are generally nonpurulent and, infrequently, cause peritonitis (393).

The peritonitis rate in patients who have catheter infections is more than twice that of patients who do not (341,393). Involvement of the tunnel, especially the inner cuff as demonstrated by ultrasound, predicts subsequent peritonitis (341). Even in the absence of a clinical tunnel infection and with resolution of exit-site infection with therapy, the deep cuff may harbor *S. aureus* or *P. aeruginosa,* resulting in recurrent peritonitis (395,403).

A number of studies have demonstrated the efficacy of local antibiotics applied to the exit site to prevent infections. Daily exit site mupirocin is highly effective in reducing *S. aureus* exit-site infections (404,405). It must be noted, however, that organisms with low-level mupirocin resistance have begun to emerge (406–408). While this is not yet a clinical concern, should the organisms acquire high-level resistance (MIC ≥512 μg/ml), increased infection and/or relapse rates may ensue. Ciprofloxacin otologic solution, 0.5 mL single-dose vial, applied daily as part of routine care, reduced both *S. aureus* and *P. aeruginosa* exit-site infections compared to historical controls (409). However, the use of ciprofloxacin may be prohibitively expensive for many patients. A recent double-blind study compared the effects of 0.1% gentamicin sulfate versus 2% mupirocin applied to the exit site daily (410) (Fig. 99-10). Use of gentamicin resulted in significantly decreased rates of exit-site infections and peritonitis. Gram-negative infections were markedly diminished by the use of gentamicin and there were no infections with *Pseudomonas aeruginosa*; the frequency of *Staphylococcus aureus* infections was unchanged. Gentamicin has the added advantage of being far less expensive than is mupirocin. This regimen is likely to become the preferred mode of prophylaxis for catheter-related infections.

Peritonitis

Peritonitis rates with the disconnect systems generally are 0.4 to 0.6 per year (382,398,411,412). The organisms that cause peritonitis are listed in Table 99-15 (398,413,414). Many other organisms in addition to those listed have been identified in episodes of peritonitis, including those caused by fungi, protozoans, algae, viruses, and mycobacteria (415–417). The outcome of peritonitis is highly organism-specific. Etiologies of peritonitis are shown in Figure 99-11.

Contamination at the time of an exchange, usually but not invariably resulting in coagulase-negative staphylococcal peritonitis, remains a leading cause of peritonitis (398,418). *Staphylococcus epidermidis* peritonitis is not usually caused by a catheter infection (348) or colonization of the skin, nose, or exit site (419,420); however, *S. epidermidis* can colonize the peritoneal catheter, producing a slime layer that can extend from the exit site through the cuff(s) into the peritoneal cavity (421). The rate of bacterial colonization of the catheter is related to the degree of bacterial contamination of the exit site at the time of catheter insertion, but, within 3 weeks of insertion, most catheters are colonized (421,422). The relationship of biofilm to peritonitis is unclear. Recurrent or

TABLE 99-15

PATHOGENS CAUSING PERITONITIS USING DISCONNECT SYSTEMS

Pathogens	Episodes/year
S. epidermidis	0.1–0.2
S. aureus	0.15[a]
Other Gram-positive	0.1–0.2
Gram-negative	0.1
Polymicrobial	0.01
Fungi	0.01
Culture-negative	0.01–0.1
Total	0.4–0.6

[a] Approximately one-third this in programs using *S. aureus* prophylaxis.
(Modified from: Holey JL, Bernardini J, Piraino B. Infecting organisms in continuous ambulatory peritoneal dialysis patients on the Y-set. *Am J Kidney Dis* 1994;23:569; Tofte-Jensen P, et al. PD-related infections of standard and different disconnect systems. *Adv Perit Dial* 1994;10:214; Lupo A, et al. Long-term outcome in continuous ambulatory peritoneal dialysis: a 10 year survey by the Italian cooperative peritoneal dialysis study group. *Am J Kidney Dis* 1994;24:826.)

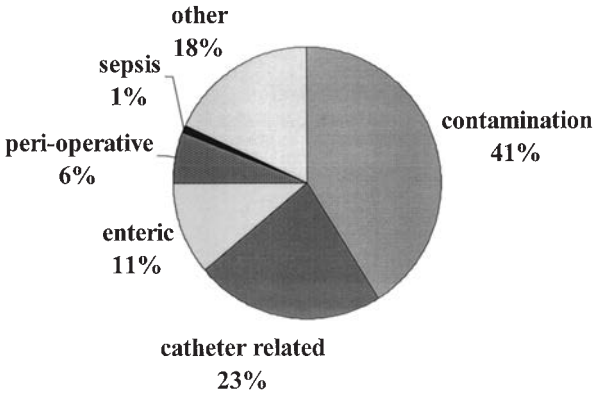

FIGURE 99-11. Etiologies of peritonitis. (Modified from: Harwell CM, et al. Abdominal catastrophe: visceral injury as a cause of peritonitis in patients treated by peritoneal dialysis. *Perit Dial Int* 1997;17:586, with permission.)

TABLE 99-16

FUNGAL PERITONITIS WITHOUT AND WITH PROPHYLAXIS

Reference	Prophylaxis	Incidence[a]
Zaruba (471)	Nystatin tid	0.20 vs. 0.03
Robitaille (472)	Nystatin or ketoconazole	0.14 vs. 0
Wadhwa (473)	Fluconazole qod	0.08 vs. 0.01
Lo (474)	Nystatin qid	0.02 vs. 0.01
Thodis (475)	Nystatin qid	0.02 vs. 0.02
Williams (476)	Nystatin qid	0.01 vs. 0.01

[a] Antibiotic associated fungal peritonitis, in episodes per year. Rate without prophylaxis given first.

relapsing peritonitis (defined as a second episode owing to the same organism within 2 to 4 weeks of stopping antibiotics) is generally caused by staphylococcus (423,424) and may be related to the presence of biofilm, which shields bacteria from antiobiotics. Some recurrent *S. epidermidis* peritonitis episodes have the same plasmid profile as the organisms causing the first infection (425,426). The coagulase-negative staphylococci isolated from patients with peritonitis are more likely to be producers of biofilm than are isolates not associated with peritonitis (427,428); however, biofilm formation does not invariably lead to peritonitis (425,429). The keys to preventing peritonitis caused by coagulase-negative staphylococcal peritonitis are avoidance of connection techniques requiring spiking of bags and extensive training of the patient in aseptic technique. Miller and Findon (430) have demonstrated that proper hand washing and drying prior to performance of an exchange sharply reduces bacterial numbers on the spike connection and in the peritoneal space after touch contamination (430).

Staphylococcus aureus carriage and catheter infections are another source of peritonitis. *S. aureus* in the nares, at the exit site, or on the skin is strongly associated with *S. aureus* catheter infection and peritonitis (431–436). Prevention of *S. aureus* peritonitis is critical, because the outcome is worse compared to that of other staphylococcal infections (437,438). Several antibiotic protocols have been shown to decrease the risk of *S. aureus* infection in PD patients (404,405,435,439–443). These predominantly use intranasal mupirocin cream, bid for 5 days monthly for carriers, or daily at the exit site. These protocols are uniformly effective in reducing exit-site infections. Exit-site mupirocin is also effective in reducing *S. aureus* peritonitis (404,405). As discussed above, low-level mupirocin resistance has been reported (406). Gentamicin is equally efficacious as mupirocin for prophylaxis against *S. aureus* and is superior for the prevention of Gram-negative infections (410).

Gram-negative peritonitis, which is associated with considerable morbidity (437), is not well understood. The bowel may be a source, through translocation of bacteria across the bowel wall or secondary to organ pathology (444,445). Constipation and enteritis have both been associated with peritonitis due to enteric organisms (446,447). Approximately one-third of peritonitis episodes owing to enteric organisms have underlying visceral injury (445). Peritonitis caused by intraabdominal pathology is associated with severe symptoms and commonly results in transfer of the patient to HD and death (352,444,445,448,449–451), especially if surgery is delayed. Causes of enteric peritonitis in PD patients include ischemic bowel, cholecystitis, appendicitis, perforated ulcers, colonic polypectomy, and diverticulae (445,451) (see section on intraabdominal catastrophes). An elevated amylase level in the dialysate effluent is a clue to the presence of enteric peritonitis (452).

Procedures, such as colonoscopy (453–455) endoscopy with sclerotherapy (456), dental manipulation, endometrial biopsy, liver biopsy, and laparoscopic cholecystectomy (457–459) can result in peritonitis; thus, antibiotic prophylaxis is indicated. Other unusual causes of peritonitis are vaginal leak of dialysate (460,461) and the use of intrauterine devices (462). It is recommended that the abdomen be emptied of fluid prior to procedures involving the abdomen or pelvis (337).

Fungal peritonitis accounts for 2% to 3% of all peritonitis episodes (463). Abdominal pain may be severe and associated with fever (450,464). Patients may be acutely ill and appear to have a surgical abdomen; death may result (465), particularly if catheter removal is delayed (466,467). Mortality with fungal peritonitis is 14% to 27% (463,468,469). Prior antibiotic therapy and frequent bacterial peritonitis are predisposing causes (463,468). Prophylaxis, mainly using nystatin during antibiotic therapy, appears to be most effective in programs with high fungal peritonitis rates (Table 99-16) (471–476). Programs with a low fungal peritonitis rate do not appear to benefit from prophylaxis.

The optimum technique for culture of peritoneal dialysate consists of centrifugation of 50 mL of peritoneal effluent at 3,000 *g* for 15 minutes followed by resuspension of the sediment in 3 to 5 mL of sterile saline and culture on both solid and liquid media (337). More commonly, however, 5 to 10 mL of dialysis effluent is injected directly into blood culture bottles. When processed in this fashion the culture is "sterile" in approximately 14% to 20% of episodes that meet the criteria for peritonitis based on cell count (398,477). A fastidious microorganism that has not grown in culture probably causes most of these episodes. When subsequently recultured, a microorganism is identified in one-third (477). Also, recent antibiotic exposure can render dialysate "sterile," despite active peritonitis (478). Mycobacteria always should be considered in peritonitis that is culture negative. Such patients have cloudy effluent, abdominal pain, and fever. Extraperitoneal TB is not necessarily present (479). Polymorphonuclear cells predominate in the effluent and, thus, do not distinguish *Mycobacterium* peritonitis from bacterial peritonitis. Acid-fast bacillus (AFB) smears of the effluent, even examining three concentrated specimens, are seldom positive; therefore, the diagnosis is generally made on culture, delaying treatment for weeks. Peritoneal tissue cultures are more optimal than culture of peritoneal fluid.

The differential diagnosis for truly sterile cloudy fluid is broad and is best approached by considering whether turbidity is due to cellular or acellular elements (480). Acellular

causes include fibrin and triglycerides; the latter may be due to lymphatic obstruction, superior vena cava syndrome, pancreatitis, or certain calcium channel blockers (480,481). Cellular elements may include polymorphonuclear leukocytes, eosinophils, red blood cells, or malignant cells (480). Intraperitoneal generic vancomycin (482) and amphotericin may cause chemical peritonitis (483). Icodextrin had been previously reported to cause sterile peritonitis with the number of cases peaking in 2002 (484–486). This was determined to be a consequence of contamination with a bacterial peptidoglycan that was introduced during the manufacturing process (487). Correction of the manufacturing process has virtually eliminated this problem (incidence now 0.01%).

A number of demographic features are associated with an increased risk for peritonitis. White nondiabetic patients aged 20 to 59 years have the lowest risk of peritonitis (392). The reason for the increased risk seen in Blacks is not understood (488–490). Conflicting data exist on whether diabetic patients have an increased risk of peritonitis (262,392,491,492). Age more than 60 years was a risk factor for peritonitis in the final report of the National CAPD Registry (392,493), but other studies indicate that elderly patients have similar peritonitis rates as younger patients (488,494,495). Peritonitis rates in children are higher than those of adults (279,496,497). Immunosuppressed patients are also at increased risk, especially for infections owing to *S. aureus* and fungi (498).

Other factors influencing peritonitis risk include connection technology; with the lowest rates occurring with the double-bag system for CAPD and a cycler system that does not involve spiking (discussed in the preceding). Peritonitis risk is increased after an episode of peritonitis (262,418). Single-cuff catheters are associated with a higher risk of peritonitis than double-cuff catheters (262,284). Neither catheter design nor insertion technique is associated with higher or lower peritonitis rates (262,284); however, downward directed tunnels decrease the risk of catheter-associated peritonitis (284).

Treatment of Peritoneal Dialysis-Related Infections

Exit-Site Infections

The initial antibiotic for an exit-site infection must be active against staphylococci, with subsequent therapy dependent on the specific organism identified. Oral antibiotics are as effective as intraperitoneal antibiotics with the exception of infections with MRSA (337,339); these will usually require treatment with vancomycin. Sonography of the tunnel is useful to determine length of therapy (Fig. 99-12). Those infections limited to the exit site require an average of 2 weeks of antibiotic therapy, whereas involvement of the superficial tunnel lengthens average therapy to 3 weeks or more. Involvement of the deep cuff requires 2 months or more of antibiotic therapy and may require removal of the catheter to prevent peritonitis (344). Local care of the exit site is generally intensified and, in mild or equivocal exit-site infection when the tunnel is not involved, this may suffice as therapy (283). If prolonged antibiotic therapy fails to resolve the exit-site infection, revision of the tunnel with removal of the external cuff (in two cuffed catheters) may help to prolong the life of the catheter (394,499), but often eventually results in peritonitis (394,500). An incision is made over the tunnel to expose the cuff, which is carefully shaved from the catheter. The area of granulation tissue and cellulitis may also be débrided (501). Cuff shaving and tunnel revision are never effective if catheter-related peritonitis is present (500).

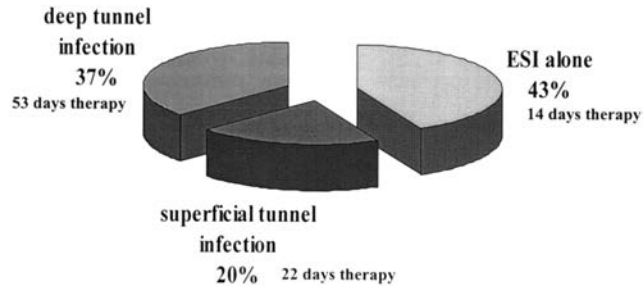

FIGURE 99-12. Extent of *S. aureus* catheter infection ($N = 49$) using sonography with mean days of therapy also shown. (From: Vychytil A, et al. Ultrasonography of the catheter tunnel in peritoneal dialysis patients: what are the indications? *Am J Kidney Dis* 1999;33:722, with permission.)

Pseudomonas exit-site infections are particularly prone to recurrence and often lead to peritonitis, which is a devastating complication (402,502). Therefore, if the patient has a history of prior exit-site infection with *Pseudomonas*, the antibiotic chosen for empiric therapy should be efficacious against that organism (e.g., oral quinolone). Dual therapy is sometimes needed and the duration of therapy may need to be extended to as long as 6 weeks. Recurrent and refractory exit-site infections might be best managed with catheter replacement. In such high-risk patients, to prevent recurrence in a new catheter, consideration should be given to using gentamicin or ciprofloxacin otic solution at the exit site, as previously described.

Peritonitis

As discussed above, not all patients who present with cloudy dialysate will prove to have peritonitis. Nevertheless, to avoid delay in treatment, empiric antibiotic therapy should be started upon presentation with cloudy dialysate (337). Treatment should be given quickly because shock and death can ensue rapidly, especially with streptococcal peritonitis (503,504). Initial therapy should include coverage for both Gram-positive and Gram-negative organisms (337). This should be guided by knowledge of both the patient's history and the individual program's pattern of microorganisms responsible for peritonitis and their antibiotic sensitivities.

A first-generation cephalosporin or vancomycin should be used to provide Gram-positive coverage. A single daily dose of cefazolin, 15 mg/kg, results in dialysate concentration levels above the minimum inhibitory concentration over 24 hours for sensitive organisms, allowing once a day dosing (for those without residual renal function) in CAPD patients (505). There are limited data on once daily dosing of cefazolin for CCPD patients. Therefore, these patients should be treated with continuous intraperitoneal cefazolin, until more data are available. Vancomycin is needed in patients with a cephalosporin allergy and should be used in centers with a high incidence of infection with methicillin-resistant organisms (506). Plasma vancomycin levels should be checked frequently, particularly in patients with residual renal function, and levels maintained $\geq 15\ \mu g/mL$.

Gram-negative coverage may be provided by a third-generation cephalosporin (e.g. cefepime or ceftazidime) or an aminoglycoside agent (Table 99-17). A single dose of ceftazidime, 15 mg/kg IP, results in serum and dialysate concentrations above the MIC (for susceptible organisms) for more than 24 hours, because the serum elimination half-life is 22 hours (507). Although long-term aminoglycoside therapy should be avoided when possible to preserve residual renal function (508) a short course of empiric therapy appears to be safe (509). Quinolones may be used by centers with documented local sensitivities of Gram-negative organisms to this class of drugs.

TABLE 99-17

ANTIBIOTIC DOSES FOR PERITONITIS

Antibiotic	Dose intraperitoneally
Cefazolin or cephalothin	15–20[a] mg/kg once daily
Vancomycin	30 mg/kg once, then 15 mg/kg every 5 d
Ceftazidime	15–20[a] mg/kg once daily
Gentamicin, tobramycin, or netilmycin	0.6 mg/kg once daily

[a]Higher dose for patients with residual renal function.
(Modified from: Piraino B, et al. Peritoneal dialysis related infections recommendations: 2005 update. *Perit Dial Int* 2005;25:107.)

Subsequent therapy after antibiotic loading depends on the organism isolated. *Staphylococcus aureus* or *S. epidermidis* may be treated with a first-generation cephalosporin alone, if methicillin-sensitive. Fifty percent or more of *S. epidermidis* causing peritonitis is resistant to cephalosporins (510,511). These patients should be treated with vancomycin, as should patients with methicillin-resistant *S. aureus* (MRSA) peritonitis (512). MRSA peritonitis has a failure rate of 60% when treated with vancomycin alone and, frequently, results not only in catheter loss but also in peritoneal adhesions precluding further PD (513). Therefore, rifampin should be added to vancomycin therapy. Peritonitis caused by vancomycin intermediate-resistant *S. aureus* has been reported and was successfully treated with rifampin and trimethoprim–sulfamethoxazole (514).

Streptococcal or enterococcal peritonitis is best treated with ampicillin; an aminoglycoside may be added for synergy in enterococcal infections. Vancomycin-resistant enterococcus as a cause of peritonitis in PD patients is still rare, accounting for 0.4% to 4% of episodes (511,515). This is probably because colonization of PD patients with vancomycin-resistant enterococci is infrequent (516–518). Vancomycin-resistant enterococci should be treated with linezolid or quinopristin/dalfopristin.

Infections due to *S. aureus* or enterococci require 3 weeks of therapy; 2 weeks is generally sufficient for other Gram-positive cocci. For any peritonitis, failure to achieve clear dialysis effluent after 5 days of appropriate antibiotic therapy defines refractory peritonitis and is an indication for catheter removal (337).

The subsequent therapy of Gram-negative organisms is dependent on sensitivities. Because of the need to preserve residual renal function, aminoglycoside therapy should be reserved for those infections in which sensitivities dictate the use of these drugs (508). Historically, aminoglycosides in PD patients have been given continuously in each PD exchange following a loading dose (519). However, continuous intraperitoneal dosing can lead to otovestibular toxicity, if courses exceed 2 weeks (520). Once a day dosing of intraperitoneal aminoglycoside, shown to be effective, provides high local levels of the antibiotic, while avoiding systemic toxicity (521,522). One-third of peritonitis episodes with enteric organisms have underlying organ pathology (445). This possibility can be further evaluated with an abdominal CT scan.

Pseudomonas aeruginosa peritonitis should always be treated with two drugs for a minimum of 3 weeks (337). Peritonitis caused by *P. aeruginosa* is difficult to treat (400,404) and can sometimes result in the death of the patient (523,524). Aminoglycosides are often ineffective therapy for these infections (400,404) and long courses may result in vestibular toxicity (404,520). Ceftazidime, cefepime, piperacillin, or oral quinolones are generally effective (337). Antibiotic therapy is much more likely to be effective if a catheter infection is not present (404), although long courses of therapy may be required to prevent relapse (525). If a *Pseudomonas* catheter infection is present in conjunction with the peritonitis, catheter removal is necessary.

Peritonitis owing to *Stenotrophomonas maltophilia* (formerly *Xanthomonas maltophilia*) is difficult to resolve as the organism displays very limited antimicrobial sensitivities (526,527). Despite treatment with multiple antibiotics, catheter removal may be necessary (528). Immunosuppression is a risk factor.

The growth of multiple Gram-negative or anaerobic organisms should lead to the strong suspicion of intraabdominal pathology. A surgical consult and an abdominal CT scan should be obtained, because delay in diagnosing an intraabdominal source can result in the patient's death. As mentioned previously, an increased amylase content of the dialysate is a clue to diagnosis (452). Metronidazole should be added to the therapy. Antibiotic therapy should be 21 days.

Antibiotic therapy of fungal peritonitis is not generally successful unless the catheter is removed. Amphotericin B has poor diffusion from blood into peritoneum (483), whereas intraperitoneal administration results in chemical peritonitis (483). Flucytosine, ketoconazole, and fluconazole diffuse readily from blood to peritoneum (483) and are more effective than amphotericin (464), although catheter removal still is often necessary (529–531). Fluconazole is particularly well tolerated when administered intraperitoneally. Chan and colleagues (469) found a cure rate of 9.5% using fluconazole therapy alone without catheter removal. Fluconazole plus catheter removal cured 67%, whereas 14% required addition of amphotericin. The ISPD recommends prompt catheter removal for fungal peritonitis (337).

Fibrinolytic agents have been used in the treatment of patients with recurrent or refractory peritonitis (532–534) or in relieving catheter obstruction associated with peritonitis (324). Approximately two-thirds of episodes of refractory or relapsing peritonitis (predominantly *S. epidermidis*) resolved with intraperitoneal urokinase compared to only 8% of patients given only antibiotics (534). In another randomized trial (535), recurrent peritonitis (predominantly coagulase-negative staphylococcus) resolved in 59% of patients given urokinase, compared to 95% of patients whose catheters were replaced. Urokinase is not currently available in the United States; data from a few small series suggest that tissue plasminogen activator (tPA) may be equally effective (536–538).

Temporary cessation of PD, which improves peritoneal immune function, has been successfully utilized to assist in resolving peritonitis, in conjunction with antibiotics (539–542). This approach has been useful in recurrent peritonitis episodes owing to coagulase-negative staphylococcus (543,544), but has also been helpful in resolving refractory *S. aureus* (543). It is effective only if catheter infection is absent.

Catheter removal is necessary to resolve the infection in some cases. Peritonitis owing to *S. aureus, P. aeruginosa,* or enteric peritonitis with an intraabdominal source often requires catheter removal (352,545,546). Catheter-related peritonitis accounts for approximately one-third of the catheters removed (312,398), whereas the proportion and rate of catheter removal for isolated peritonitis have decreased with use of improved connection systems (398). Simultaneous catheter removal and replacement are quite successful for recurring peritonitis and tunnel infections (535,547–552). This eliminates an interim period on hemodialysis. This approach should be used only when the effluent leukocyte cell count is under 100/μL (552). This approach is not recommended for fungal, mycobacterial, or *P. aeruginosa* peritonitis, nor when peritonitis is a consequence

of intraabdominal pathology; these episodes require that the patient spend a period of time off PD (548,549,552).

OTHER COMPLICATIONS

Pancreatitis

Pancreatic abnormalities including pancreatitis occur with a higher frequency in uremic patients (553–557). The highest incidence of pancreatitis among ESRD patients is in transplant recipients, but within dialysis populations, it is not clear that PD patients have a higher incidence than do HD patients (556–559). Reports from the mid-1980s suggested a greater incidence in PD patients (556,559), prompting several etiologic hypotheses. Pancreatitis was thought to be related to higher uremic solute concentrations in PD patients or even to the potentially direct toxic effects of dialysate, which bathes a portion of the pancreas. The dialysate dextrose concentration, hypertonicity, hypercalcemia, foreign particulate debris, bacteria, or antibiotics may induce inflammation in the sensitive pancreas (556,559–561). That the direct toxicity of dialysate may be causative is supported by the recurrence of pancreatitis after reinstitution of PD after initial resolution (562). Hyperlipidemia is both a risk factor for and complication of pancreatitis. The hyperlipidemia seen more frequently in PD patients is low-density lipoprotein hypercholesterolemia, which is not particularly toxic to the pancreas. On the other hand, HD patients are more likely to suffer from hypertriglyceridemia, which is a predisposing factor for pancreatitis when severe enough to be associated with hyperchylomicronemia. The high intake of simple carbohydrates in PD patients may be a factor in inducing hyperlipidemia. This is a major difference between HD and PD patients.

Even though pancreatitis is an infrequent complication of PD, it carries a high mortality rate of 20% to 60% (556,558). The typical clinical presentation for acute pancreatitis in a PD patient is characterized by abdominal pain with normal bowel sounds, nausea, vomiting, absence of fever, hyperamylasemia (more than three times normal), elevated effluent dialysate amylase concentration (more than 100 U/L), and a variable appearance of effluent dialysate, including being clear, hemorrhagic, tea-colored, or even cloudy (556–559,563). Hyperlipidemia and/or hypercalcemia is frequently present and may be predisposing metabolic abnormalities (556,558,563). Pancreatitis should be strongly considered if appropriately treated "peritonitis" does not resolve because this presentation is quite similar to PD-associated microbial peritonitis. The effluent in pancreatitis is usually sterile, even if hemorrhagic, cloudy or tea-colored. Burkart and associates (452) have suggested that dialysate effluent amylase concentration is low in bacterial peritonitis, even if slow to resolve, whereas it is more than 100 U/L with pancreatitis or other intraabdominal pathologies. If a diagnosis of pancreatitis is uncertain, computed tomography (CT) is the preferred imaging study over sonography because it is more sensitive (564). In addition to demonstration of an engorged pancreas, CT may be particularly useful to identify the ominous finding of a pseudocyst.

The principles of management do not differ from that in patients without ESRD. Offending agents should be discontinued and, if that includes dialysate, PD should be halted. However, peritoneal lavage can be helpful in removing inflammatory mediators, especially if the dialysate was not the culprit. There is no evidence to support a recommendation to halt PD in all patients with acute pancreatitis, and discontinuing PD probably does not alter the prognosis (558). Percutaneous pseudocyst drainage may be preferable to internal (jejunal) drainage and this may preclude continuation of PD. Hyperlipidemia and hypercalcemia should be corrected. The role of lower concentrations of calcium in dialysate is unknown.

Chyloperitoneum

There have been a few scattered case reports of chylous fluid leaking into the peritoneum and draining with effluent dialysate (565–568). The dialysate is cloudy but, on more careful examination, looks milky, reflecting the lipid-rich content of chyle. The most common cause is trauma to intraperitoneal lymph vessels, either catheter-induced or from external trauma. Rocklin and Teitelbaum (481) have recently reported a case of chyloperitoneum due to the superior vena cava syndrome. Certain dihydropyridine calcium channel blockers have also been associated with chyloperitoneum, perhaps due to impired lymphatic peristalsis (480). Patients are usually asymptomatic. Treatment initially is conservative, to decrease abdominal lymph production by a low-fat, high-calorie diet supplemented with medium-chain triglycerides. The next step is discontinuation of PD, because the presence of dialysate may retard closure of the leak. If this is unsuccessful, a trial of total parenteral alimentation may be considered. Should these steps fail to resolve the leak, catheter removal is indicated. Lymphangiography may identify the source of the leak should surgery be considered.

Hemoperitoneum

As little as 1 mL of blood in 2 L of dialysate results in readily evident visual hemoperitoneum (569), and 7 mL results in effluent dialysate that looks like red fruit juice. Fortunately, this is an uncommon occurrence, but, when it does occur, it is very disturbing to the patient. It is also fortunate that hemoperitoneum is almost always benign (570,571). In Table 99-18 are

TABLE 99-18

CAUSES OF HEMOPERITONEUM IN PERITONEAL DIALYSIS PATIENTS

Retrograde menstruation
Ovulation
Catheter-induced trauma (omental abrasion, repositioning, constipation)
Bowel disease (ischemic, inflammatory)
Peritonitis
Cysts (ovarian, polycystic kidney, acquired cystic kidney)
Abdominal trauma
Strenuous exercise (including sexual activity)
Systemic bleeding (thrombocytopenia, anticoagulants)
Hypertonic exchanges (hyperemia)
Pancreatitis
Vasculitis (systemic lupus erythematosus)
Sclerosing peritonitis
Adhesions
Granulosa cell tumor
Ectopic pregnancy
Cholecystitis
Colonoscopy
Dissection from adjacent sites (femoral hematoma, spleen, colon)
Previous hepatitis
Enema
Extracorporeal lithotripsy
Splenic infarction

listed causes of hemoperitoneum in PD patients. Hemoperitoneum occurs in 3.8% to 10% of PD patients and is twice as common in women as in men (570,571). When it occurs in women of childbearing age, 64% of the causes are related to ovulation or menses. In one series, this population experienced an almost 90% incidence rate (572). There does not appear to be a correlation with PD-associated peritonitis (570,571), nor does hemoperitoneum adversely impact long-term outcomes on PD (571).

Menstrual and surgical histories are informative (Table 99-18). If the patient is asymptomatic and the bleeding stops spontaneously, no evaluation is absolutely necessary. In the absence of active menses, bloody dialysate should be evaluated by effluent cell count, differential, Gram stain and culture, and effluent fluid amylase concentration. An abdominal ultrasound may be informative. Obviously, symptoms referable to the abdomen prompt further evaluation, which ultimately could include a laparotomy. Treatment is directed at the specific cause. However, because patients often are asymptomatic, precluding an extensive evaluation, treatment generally is supportive. Heparin administered intraperitoneally may protect from subsequent catheter occlusion from clots but may prolong bleeding. Three rapid flushes with room-temperature dialysate may induce peritoneal vasoconstriction and stop the bleeding (569). Dialysate that is significantly cooler than room temperature could precipitate cardiac dysrhythmias. Furthermore, cool dialysate should be avoided where mesenteric perfusion is compromised, because it could exacerbate ischemia of the bowel. This therapy is probably only effective in cases where the bleeding is secondary to a peritoneal membrane bleed. Gynecologic hormone therapy may be indicated in women who demonstrate hemoperitoneum during menses or ovulation (573).

Defects of the Peritoneal Cavity Boundary

Hernias and Genital and Abdominal Wall Edema

Intraabdominal pressure rises with increasing intraperitoneal volume, sitting, straining at stool, coughing, and strenuous physical activity. Combined with the extremes of age, debilitation of ESRD, and poor wound healing from uremia, it is no surprise that hernias are detected in 10% to 25% of PD patients (574,575). Over 13% of the hernias present are strangulated. Van Dijk and colleagues (576) recently reported the largest series of patients with defects of the peritoneal cavity boundary. The overall frequency of hernia in this population of nearly 1900 patients was 6.7%. The most common sites were: inguinal, 24.9% of total; umbilical, 18.9%, and ventral, 13.8%. They found hernias to be more common in men than women, although other studies have suggested the converse (414,577). Patients with cystic disease as the etiology of ESRD are at higher risk for the development of hernias (576,578). One-half of the hernias become clinically evident within the first year of PD (579), but many probably go undetected unless special scintigraphic studies are performed (580). Most of the scintigraphically diagnosed asymptomatic cases never progress to clinically appreciable disease. Many PD patients have hernias diagnosed prior to initiating PD and herniorrhaphies are performed at the time of catheter insertion (581). Patients can perform PD immediately after repair about one-half the time, and by 3 weeks, all can perform some type of PD. Usually exchange volume is decreased and supine positioning for PD is recommended (i.e., some form of cycler PD) (582). Another alternative is to utilize prosthetic overlay mesh within the herniorrhaphy and not interrupt the PD (583).

There probably is no benefit from routine screening scintigraphy in adults because clinical manifestations alone dictate the need to repair (584). In children, some programs routinely perform intraoperative peritoneograms (and herniorrhaphies if positive) at the time of catheter placement. To ensure prompt strength postoperatively, especially for large hernias, supporting prosthetic overlay mesh is inserted at herniorrhaphy (583). Placement of catheters through the rectus muscle in a paramedian approach probably reduces the incidence of subsequent incisional or catheter site hernias. Postinsertion leakage increases the likelihood of subsequent hernias. The incidence of hernias has been reported to be lower in PD patients undergoing intermittent treatments rather than continuous (574). This may reflect the lower intraabdominal pressures in the lying position, although Van Dijk and colleagues (576) found no difference between patients on CAPD versus APD.

Abdominal wall edema or genital edema is caused by either dialysate leakage through acquired peritoneal defects, such as the catheter insertion site, traumatic rents such as previous hernias or incisions, or congenital defects that go undetected until PD raises intraperitoneal pressures, opening them (patent processus vaginalis). Thus, the fluid could dissect between tissue layers or through natural pathways. In the study by Van Dijk and colleagues (576) pericatheter or subcutaneous leaks were present in 3% of the total population. Edema of the scrotum or perineal area is usually owing to a patent processus vaginalis (327,585). Scrotal edema may develop in up to 10% of men on CAPD (327,586), whereas labial edema occurs in only 1.4% of women (586). This can be managed temporarily by supine PD, but surgical correction is generally required, certainly if a hernia is present (285). Postoperative management may include hemodialysis for 1 week or more (285). Vaginal leakage of dialysate is rare but serious, because it can lead to recurrent peritonitis, often with fungus (460,461,587). This complication should be suspected in any woman with watery vaginal discharge that is positive for glucose. If the leak is through the fallopian tubes, then tubal ligation is corrective (588).

The site of a subcutaneous dialysate leak can be located with scintigraphy, ultrasonography, or contrast imaging (586,589–592). Surgical closure is recommended, hence the need for precise identification of the leak site. Although watchful waiting is tempting, the collective PD experience suggests that elective operative intervention is the best approach to these complications related to increased abdominal pressure.

Hydrothorax

Fluid migrates from the peritoneal to pleural space in 0.6% to 5% of patients undergoing PD either via transdiaphragmatic lymphatics or defects in the tendinous portion of the diaphragm (576,593–595). There is an increased incidence in women, patients with polycystic kidney disease (576,596) or hernias, those prone to peritonitis, and children. Almost 90% of the cases are isolated to the right pleural space (595). The heart or pericardium probably protects the tendinous portion of the left hemidiaphragm. The hydrothorax can occur abruptly and painfully following exercise or trauma and can be immediately life threatening. A more common presentation is that of gradual progression of orthopnea or dyspnea, usually without pain. One-half of the cases present within the first month of PD, and only one-fifth present 1 year or more after initiation (595). Resolution (i.e., being able to continue PD) is more likely in those cases where the presentation is within 1 year of initiating PD.

The simultaneous measurement of the concentrations of albumin, glucose, and lactate dehydrogenase in peritoneal effluent, pleural fluid, and blood may be helpful diagnostically. Peritoneal scintigraphy with radiolabeled albumin is a useful diagnostic maneuver (597,598); methylene blue should be avoided because of the pain it causes. Although helpful in localizing the defect, these diagnostic maneuvers probably do not alter management (599). If the origin of the hydrothorax is

dialysate, therapy is indicated regardless of whether there is a distinct leak versus lymphatic transport.

Initial attempts at conservative management should be made by decreasing volumes (decrease fill, decrease UF), performing PD supine, and periods of an empty abdomen. If conservative management fails, video-assisted thoracoscopic surgery is the preferred therapeutic modality (598,600). Chemical pleurodesis with tetracycline, blood, N-CWS (*Nocardia rubra* cell wall skeleton), triamcinolone, OK-432, talc, or fibrin adhesive (601) have each been successful; however, these procedures can be very painful and are associated with unpredictable results.

Hyperlipidemia

Compared to HD patients, PD patients demonstrate higher total cholesterol concentrations, triglycerides, Lp(a), apo A-I, and apo B and lower apo A-I: B ratios, serum albumin, and high-density lipoprotein (HDL) cholesterol concentrations (602–604). The cause of these abnormalities is multifactorial. Although total caloric intake is equal in PD and HD patients because of absorbed dextrose from peritoneal dialysate, oral caloric intake is actually less in PD patients (605,606). This absorbed simple carbohydrate may account for 25% of total caloric intake. The development of hypertriglyceridemia during the first year of PD has been correlated with glucose absorption (607). Patients who require frequent hypertonic exchanges do so because of increased fluid and/or food intake. Therefore, it is difficult to determine whether hyperlipidemia is secondary to diet or glucose-based dialysate. It should be noted, however, that use of icodextrin for the long dwell has been shown to decrease total cholesterol and LDL, with a trend ($p = 0.06$) toward decreased triglycerides as well (608). This supports a role for the caloric load from glucose in the pathogenesis of hyperlipidemia in PD patients. In addition to the above, there is loss into effluent dialysate of oncotic proteins (e.g., albumin) and liporegulatory species (e.g., HDL cholesterol, apoproteins) (609,610). This sets the stage for hyperlipidemia and atherosclerosis.

By virtue of their uremic condition and hypertension, patients with chronic renal failure are also at increased risk for vascular disease. This risk is even greater in diabetic patients with chronic renal failure (611). Many of the vascular risk markers described in patients with diabetes are even more abnormal with the addition of renal failure and dialysis (612). In dialysis patients treated with subcutaneous insulin, hyperlipidemia and hyperinsulinemia are considerably more frequent and severe than in nondiabetic dialysis patients. In addition to abnormally low levels of HDL cholesterol, dialysis patients also have significantly elevated levels of certain circulating markers of cardiovascular disease, such as Lp(a) lipoprotein, factor VII coagulant activity, plasminogen activator inhibitor, homocysteine, C-reactive protein, and fibrinogen (612–616).

The treatment of hyperlipidemia in PD patients must include an attempt to decrease the use of hypertonic exchanges. This should be done in conjunction with dietary restriction of fluids, fats, and simple carbohydrates. Lipid-lowering drugs of several classes have been utilized successfully. The major U.S. experience with fibric acid derivatives is with gemfibrozil, which increases lipoprotein lipase activity, the catabolism of very-low-density lipoproteins (VLDL), and the concentration of HDL_2 and HDL_3 (617). The dose should be reduced by initiation with 300 mg once daily and titrated gradually upward. Gemfibrozil can cause myositis, which may be manifested by increased serum potassium and/or creatine kinase concentrations. A less toxic but effective agent is clinofibrate (618). Hydroxymethylglutaryl-CoA reductase inhibitors, predominantly used to treat hypercholesterolemia, are safe and effective in PD patients (619–624).

Intraabdominal Pathology (IAP) in Peritoneal Dialysis Patients

Less than 6% of peritonitis episodes in PD patients are owing to intraabdominal pathology (IAP) (625). The culture isolation of multiple or a single unusual enteric pathogen(s), peripheral leukocytosis, an increasing PD cell count on antibiotic therapy, or an expanding pneumoperitoneum are important clues to IAP (281,450,625). Obvious signs of IAP, such as fecal or biliary material in the dialysate or diarrhea containing dialysate, are not commonly observed. Risk factors for the development of IAP include diverticulosis, constipation and its treatment (447), and unrepaired hernia. Death from IAP is linked to bowel gangrene, malnutrition, and comorbidities, such as liver failure, shock, bacteremia, pneumonia, and gastrointestinal or intracerebral hemorrhage, and delayed surgical intervention (626); therefore, by a broad consensus, early surgical intervention in suspected IAP is strongly recommended (445,627).

In general, slowly resolving peritonitis warrants close follow-up. Clear dialysate while on antibiotics is not an absolute sign of a benign process. Generalized abdominal peritonitis can mask localized signs and symptoms of IAP. Surgical consultation is urgently needed in the following conditions:

- Localized abdominal pain and tenderness
- Dilated loops of bowel on abdominal radiograph
- Progressive increase in intraperitoneal free air with continued peritonitis
- Mixed flora on dialysate gram stain or culture
- Hemoperitoneum with measurable dialysate hematocrit

Those perioperative interventions that best allow continuation or quick return to PD postoperatively include:

1. Tight wound closure for prevention of dialysate leakage, possibly using nonresorbable sutures.
2. Drain removal before resuming PD to allow adequate dialysis.
3. Preoperative extensive PD to increase platelet function and allow a few days without PD postoperatively for healing.
4. Elective repair of abdominal wall hernias (see earlier Abdominal Hernias in Continuous Peritoneal Dialysis) both for patient comfort as well as prevention of bowel incarceration.
5. Avoidance of constipation, because impacted stool often accompanies diverticulitis or perforated bowel.
6. Optimization of nutrition to counter the marked protein loss through an inflamed peritoneum.
7. Avoidance of PD with transfer to HD if extensive bowel wall repairs are made. A low threshold for transition to HD is generally a prudent decision.
8. Omentectomy at surgery if the omentum appears threatening to catheter flow function (628,629).

INTRAPERITONEAL INSULIN

Shortly after the advent of CAPD it was suggested that the intraperitoneal administration of insulin could improve glycemic control (630,631). While easily utilized in CAPD, the use of intraperitoneal insulin in patients performing APD, an increasing segment of the overall PD population, is more complex. Coupled with the trend toward use of longer-acting insulin preparations, this has resulted in a substantial decrease in the utilization of this route for insulin delivery. The interested reader is referred to reviews by Copley et al. or Tzamaloukas et al. (632,633).

HYPERTENSION

Volume control and sodium removal by PD are related to numerous factors, including dialysate composition (osmolality created by dextrose and sodium concentrations), peritoneal permeability and UF capacity, splanchnic circulation, and residual renal function (448,634). After many months of PD, the antihypertensive effect of PD may be due to other factors as well (635,636). At this time, weight may actually have risen, although this could reflect the increased caloric intake from the transperitoneal absorption of dextrose. Because the peritoneal membrane is associated with different transport properties than HD membranes, the more efficient removal of pressor substances by PD is speculated to play a role in this late hypertension control (635). These pressor compounds could include Na-K–ATPase inhibitors, norepinephrine, and endothelin. However, after a year or more of PD, hypertension is less effectively controlled than after PD initiation. This may be related to the development of peritoneal sclerosis (637), progressive obesity, dialysis prescription noncompliance (638), improved appetite and well being and dietary indiscretion, increased hematocrit, loss of residual renal function, or other as yet unidentified factors.

Recent studies have focused attention on the differences in blood pressure between patients performing CAPD or APD. Patients performing APD have higher blood pressure and left ventricular mass than do those on CAPD (639–641). This is likely due to decreased sodium removal and ultrafiltration in APD patients due to the shortened dwell times and high transport, respectively (639,640). It should be noted that the correlation between higher peritoneal transport status and blood pressure has been demonstrated within CAPD populations as well (642,643). Use of icodextrin for the long daytime dwell in APD has been reported to decrease both blood pressure (245) and left ventricular mass (246).

TRANSPLANTATION

Peritoneal dialysis patients may differ from their HD counterparts in several aspects that could influence transplant outcomes. Compared to HD patients, PD patients demonstrate a more normal immune response as characterized by T4:T8 lymphocyte ratios, T cell counts, T cell stimulation, cell-mediated immunity, and lymphocyte blastogenesis (644–648). Thus, it was not completely unexpected when two early small studies suggested that graft survival might be inferior in recipients previously treated by PD (644,649). It was speculated that the PD patients were more immunocompetent at the time of transplantation, thus decreasing graft tolerance. However, subsequent studies with larger population bases have shown that graft survival is essentially equal in recipients previously treated by HD or PD (650–654) and there is no difference in the rate of acute rejection in these populations (652–654).

Another difference between PD and HD patients potentially influencing transplantation is that the control of anemia, with or without erythropoietin, is easier with PD (655). Thus, PD patients are less likely to experience blood transfusions and subsequent enhanced graft tolerance. In fact, pretransplant blood transfusions appear to be of less benefit to PD patients (649). Furthermore, the decreased transfusion requirement of PD patients makes hepatitis less likely, which is important considering the adverse effects of viral hepatitis on graft survival (656) and the potential need for antiviral therapy prior to transplantation (657).

When compared to HD patients, PD patients have better blood pressure control and preserved residual renal function, which may affect care in the immediate posttransplant period. Patients receiving intraperitoneal insulin must be converted back to subcutaneous insulin as PD is terminated. Transperitoneal albumin losses with or without peritonitis contribute to protein malnourishment in PD patients. This may predispose them to wound infections or other steroid complications.

Peritoneal dialysis patients are prone to develop higher plasma fibrinogen and Lp(a) lipoprotein concentrations (658–660), which could contribute to posttransplant steroid-exacerbated atherosclerosis. PD patients have been reported to exhibit an increased frequency of early graft thrombosis (654,661,662). This may be reflective of severe atherosclerosis present at the time of engraftment. On the other hand, patients undergoing renal transplantation after performing PD have a significantly lower incidence of delayed graft function compared to those who have performed HD (652–654). Because of this low frequency of delayed graft function, and because of the location of a pancreatic allograft in adults or the renal allograft in children, it has become common to remove PD catheters at the time of transplantation. If desired, the PD catheter may be kept in place for up to 2 to 3 months after transplantation. In that case, frequent flushing is recommended to maintain catheter patency and to avoid unlubricated or unbuffered bowel contact which, especially in the patient on steroids, may result in abscess formation and potentially even erosion of the catheter through the bowel wall.

A tunnel infection or active peritonitis generally precludes transplantation at that time. A prudent policy is to observe the course of the peritonitis for at least 2 weeks following the discontinuation of antibiotics. If no relapse has occurred, the patient is then reactivated on the recipient list.

There is no difference in the frequency or types of nonperitonitis-related posttransplant infections in recipients previously dialyzed by PD or HD (650,651). If the PD catheter has been left in place and peritonitis develops, its course is not different from that seen in PD patients who are not receiving immunosuppressive medications. It requires essentially the same treatment with parenteral or intraperitoneal antibiotics, with the exception that allograft function may necessitate larger or more frequent doses. Posttransplant exit site or tunnel infections probably warrant catheter removal, especially if the infection is in proximity to the graft incision.

Posttransplant ascites may develop in PD patients, even with functioning grafts (663). It is probably related to a hyperemic peritoneum whose mesothelium has been altered by the previous presence of dialysate. It may take weeks, but this does subside spontaneously. The ascites should be drained only when dictated by patient comfort because the protein content is generally high and negative protein balance may ensue.

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CHAPTER 100 ■ CENTER AND HOME CHRONIC HEMODIALYSIS: OUTCOME AND COMPLICATIONS

ANNE MARIE MILES AND ELI A. FRIEDMAN

Although still "experimental" mechanical artificial hearts have sustained life in a few patients for a year or more, maintenance hemodialysis continues to be the only established long-term mechanical organ replacement therapy currently available, and has prolonged life for 30 or more years. Hemodialysis conceptualized as an "artificial kidney" in the laboratory of Abel, Rowntree, and Turner at Johns Hopkins University in 1913 (1) was first successfully implemented as a continuing renal replacement regimen by Scribner in 1960 (2). Growing successes in a limited population of young, relatively fit patients in renal failure prompted legislation in 1972 that added end stage renal disease (ESRD) to the list of the Medicare-funded disabilities in the United States. As a consequence, the number of federally funded ESRD patients has expanded continuously from fewer than 15,000 in 1972 to more than 431,000 in 2002 the United States Renal Data System (USRDS) Annual Data Report in 2004 (1).

Some of the seminal advances that have led to the current practice of chronic hemodialysis are listed in Table 100-1 (1–5). Because patients undergoing maintenance hemodialysis have the equivalent of less than 10% of normal kidney function, biochemical, endocrinologic and other clinical complications of uremia still may occur impacting significantly on their morbidity, life quality and ultimately mortality. This chapter encompasses hemodialysis techniques and prescription, multisystem complications that may contribute to morbidity during maintenance hemodialysis, as well as factors contributing to death on hemodialysis.

EPIDEMIOLOGY

The 2004 USRDS Annual Data Report noted that in 2002, of 431,000 prevalent ESRD patients, 65% were treated with hemodialysis, 5.8% with peritoneal dialysis, and 28.4% had a functioning kidney transplant (6). The cost of treating ESRD in America has, perforce, risen each year since 1972, and is projected to exceed $35 billion per year by 2010. A steady decline in the utilization of home hemodialysis in the United States seen since 1983 may be explained by a combination of factors: noncompetitive reimbursement rates compared to center hemodialysis, the growing popularity of chronic ambulatory peritoneal dialysis (CAPD) in the 1980s, changing ESRD demographics (a more elderly population with multisystem complications often related to diabetes mellitus, many unsuited for home dialysis), a lack of adequate physician endorsement of the self-care ideal, and physician bias (7). In some parts of the United States, home hemodialysis is used predominantly for elderly relatively affluent patients who may be too debilitated to travel back and forth to an in-center dialysis unit and who are willing to contribute copayments for each dialysis session.

Among center hemodialysis patients, the most common reported causes of renal failure are, in order of frequency: diabetes, hypertension, chronic glomerulonephritis, and polycystic kidney disease (Table 100-2) (6). A disproportionately high percentage of patients with polycystic kidney disease and chronic glomerulonephritis receive home hemodialysis, which is probably reflective of relatively slower disease progression in a younger and more self-reliant population, with medical disease usually free of extrarenal comorbidity.

Home versus Center Hemodialysis

Home hemodialysis was initially instituted in response to inadequate funding and shortage of hospital staff and space for the fledgling hemodialysis programs of the early 1960s. The first home hemodialysis program in the United States was started in Seattle, Washington, where in 1970, over 90% of hemodialysis patients were treated at home (8). Washington State still has the largest home hemodialysis population in the United States, followed by New York, Mississippi, New Jersey, and Tennessee (6,9). In 1973, 42% of approximately 10,000 U.S. ESRD patients were on home hemodialysis. Because of a combination of factors cited above, in 2002, there were only 12,930 patients on home hemodialysis in the United States (6). Any relatively stable patient who has a home and a partner, or who can afford payment outside of Medicare for a dialysis technician or nurse, is a candidate for home hemodialysis (Fig. 100-1). This option of renal replacement therapy should be presented to almost all new patients with chronic renal insufficiency. Home hemodialysis may also allow for the newer treatment regimens of slow nighttime or short daily dialysis (which provide Kt/v values well over 2.0 and which are currently under intensive study) to become more widely accessible (10,11).

Contraindications to home hemodialysis include severe, uncompensated cardiovascular disease, uncontrolled or very brittle diabetes, and significant loss of mental acuity. There is no age cutoff. Successful home hemodialysis has been accomplished in the eighth decade of life, in diabetic patients with limb amputations or limited vision, and in patients with severe arthritis or lower limb paralysis.

A multidisciplinary team, including a physician, nurse, and social worker, best effects initial assessment of candidates for home hemodialysis. Patient motivation, psychosocial and vocational factors, intelligence, home environment, and level of social support are assessed. A paid dialysis helper may be employed if there is no family member to assist. Medicare does not reimburse these workers, but some insurance companies and state-funded renal agencies provide coverage for home dialysis assistants.

TABLE 100-1

THE HISTORY OF HEMODIALYTIC THERAPY

1854	Scottish chemist Thomas Graham coins the term "dialysis" following work on solute movement across semipermeable membranes of ox bladder and vegetable parchment.
1913	John Jacob Abel, Leonard Rowntree, and BB Turner at Johns Hopkins University perform first dialysis on dogs using celloidin membranes and hirudin anticoagulation.
1942	George Haas in Germany performs first human hemodialysis, initially with hirudin, after 1928, with heparin.
1943	Willem Kolff in Holland constructs the rotating drum dialyzer using cellophane membranes from sausage casings.
1946	Nils Alwall in Sweden designs first ultrafiltration-controlled dialyzer.
1955	Twin coil dialyzer introduced by Watschinger and Kolff.
1960	Belding Scribner introduces the modified Kiil dialyzer, a low-volume, low-resistance, low-temperature continuous flow dialysis system, using a more permeable membrane, Cuprophane.
1960	Scribner, Quinton, and Dillard in Seattle, Washington introduce first means of chronic vascular access—the Teflon external shunt—and initiate the first patient on maintenance hemodialysis, Clyde Shields. He lived for 11 years.
1963	Albert Babb and others in Seattle design a central proportioning system for simultaneous supply of dialysis fluid to multiple patients.
1964	Home hemodialysis introduced in Boston, Seattle, and London.
1966	James Cimino and M. Brescia at the Bronx Veterans Administration Hospital, New York, construct the internal endogenous arteriovenous fistula.
1972	Medicare Act passed by Congress allowing the Federal Government to pay for the cost of dialysis.
1982	The National Cooperative Dialysis Study report affirms the importance of small molecular weight clearance as a measure of dialysis adequacy. Gotch and Sargent introduce the concept of urea kinetic modelling and Kt/V based on the results of the NCDS.
1989	Food and Drug Administration approves use of recombinant human erythropoietin in treatment of renal anemia.

Training for home hemodialysis within a free standing facility should take place in an area separate from the general hemodialysis treatment area. During initial training lasting 3 to 4 weeks, instruction in medical and dietary regimens, access cannulation, and operation of the dialysis machine are taught. Audiovisual aids, written material, and question-and-answer formats are useful. Subsequent training, usually completed in 3 to 6 weeks, covers problem solving in difficulty with the entire regimen, troubleshooting mechanical difficulties, and basic emergency intervention such as blood loss and severe hypotension. Arrangements for installation of equipment (dialysis machine, deionizer, and reverse osmosis machine), delivery of disposable supplies, servicing, and repair, are scheduled, permitting start of home hemodialysis usually within 3 to 4 months. A 24-hour on-call backup system involving a home hemodialysis nurse should be in place in case of emergencies.

TABLE 100-2

HEMODIALYSIS TREATMENT, 1997 (USRDS, 1999)[a]

Patient characteristic	Percent utilizing: Center hemodialysis	Percent utilizing: Home hemodialysis
All patients	62.3	0.6
Age		
0 to 19	17.3	0.4
20 to 24	41.5	0.4
45 to 64	58.4	0.6
65+	84.2	0.7
Male	60.1	0.6
Female	65.0	0.5
Native American	69.0	1.3
Asian/Pacific Islander	60.5	0.4
Black	76.8	0.5
White	54.7	0.6
Diabetes	72.2	0.5
Hypertension	74.9	0.5
Glomerulonephritis	44.8	0.6
Cystic kidney disease	39.8	0.7
All other	51.6	0.6

[a] By age, sex, race, and primary disease.

Monthly blood samples drawn at home are mailed or brought in to the affiliated center, and once yearly home follow-up visits by the training staff should be made.

Patient survival on home hemodialysis is equal to and often better than that on center hemodialysis (12); as with rehabilitation data, however, bias in assignment of healthier patients to home hemodialysis may be a factor. In some locales, an overrepresentation of older, sicker patients in home hemodialysis programs may modify these statistics. At our institution, the actuarial survival at 10 years for home hemodialysis patients (mean age 39.3 years) was 77%, and strikingly, every one of our 20-year dialysis survivors is on home hemodialysis (13). Other advantages of home hemodialysis are listed in Table 100-3. Home hemodialysis is the means of uremia therapy advocated by the authors when a renal transplant is declined or not possible.

HEMODIALYSIS EQUIPMENT AND TECHNIQUE

Hemodialysis is performed using a dialyzer (Fig. 100-2), a hemodialysis machine (essentially a system of pumps and sensors), and blood tubing. These components form two major circuits: the blood circuit and the dialysate circuit (Fig. 100-3). The blood circuit consists of an input arterial needle (inserted about 3 cm from the arteriovenous anastomotic site and pointing toward the anastomosis) and line that carry blood from the patient's vascular access to the dialyzer under the negative pressure of a roller type positive-displacement blood pump. Blood passes through the dialyzer blood compartment and is returned to the patient under positive pressure via an output venous blood tubing and needle; the latter is placed at least 3 to 5 cm proximal to the arterial needle, pointing in the opposite direction toward the heart. The dialysate circuit usually consists of a single-pass system in which concentrated dialysate is mixed with an appropriate amount of processed water (usually in a ratio of 1:34). Typical dialysate composition is: sodium

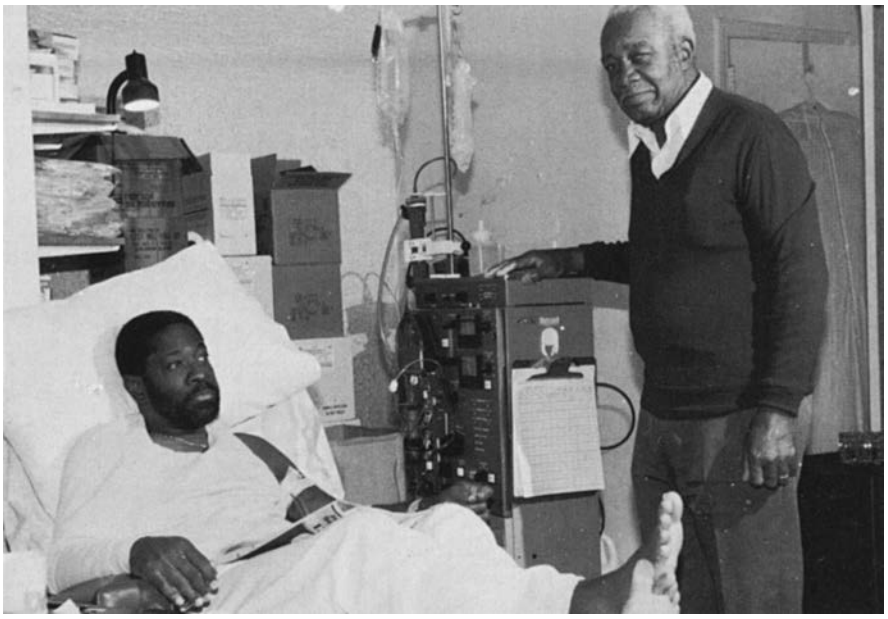

FIGURE 100-1. A 47-year-old Brooklyn man on home hemodialysis for 17 years shown with his helper, his 67-year-old father. He worked as a teacher for over 15 years, when complications related to vascular access led to retirement. Note storage shelves and boxes of dialysis equipment in background.

135 to 145 mEq/L, potassium 1.0 to 3.0 mEq/L, calcium 6 to 7 mg/dL, magnesium 0.5 to 1.0 mEq/L, chloride 100 to 124 mEq/L, bicarbonate 35 to 40 mEq/L, and glucose 100 to 200 mg/dL. Dialysate is then pumped through the dialyzer at a flow rate of 500 to 800 mL/minute and discarded into a drain. Blood and dialysate flow counter current through the dialyzer, blood through the some 300,000 capillary tubes of the commonly used hollow fiber dialyzers, and dialysate between the capillary tubes. Plasma solutes, including nitrogenous wastes in the blood diffuse across the dialyzer membrane down their concentration gradients. Convective loss or mass transfer of nitrogenous compounds occurs only at rates of high ultrafiltration. Most dialyzers used today are hollow fiber dialyzers developed in the mid-1970s. More than 70% of American dialysis centers (predominantly non-hospital-based, international corporate owned for-profit facilities) use dialyzers reprocessed with formaldehyde or peracetic acid (14). Parallel plate dialyzers, initially used by Scribner, are now used only infrequently, predominantly in patients who manifest anaphylactoid-type reactions thought to be related to the sterilizing agent ethylene oxide trapped within the potting compound in which the ends of the capillary tubes of hollow fiber dialyzers are embedded. Coil dialyzers and flat plate Kiil dialyzers are no longer employed in the United States. Membranes used to manufacture dialyzers and their ultrafiltration coefficients are shown in Table 100-4.

TABLE 100-3

ADVANTAGES OF HOME HEMODIALYSIS

Flexibility in scheduling allows for vocational and lifestyle requirements
Self-care ideal leads to more informed, independent patient
Better rehabilitation
Cheaper than center dialysis or continuous ambulatory peritoneal dialysis
Fewer vascular access complications related to single, experienced venepuncturist
Allows for high-intensity dialysis regimens of short daily or slow nighttime dialysis

Hemodialytic techniques may be varied depending on the need for fluid versus solute removal and the dose of dialysis required:

1. **Conventional Hemodialysis**
   Dialysis is performed using cellulosic membranes and blood flow rates of 200 to 350 mL/minute three times weekly for 3 to 4 hours at a time.
2. **Isolated Ultrafiltration or Sequential Hemodialysis**
   Isolated ultrafiltration or sequential hemodialysis is performed in patients with predominant or severe fluid overload who are hemodynamically unstable or prone to precipitous decreases in blood pressure during usual combined dialysis and ultrafiltration. Isolated ultrafiltration is usually performed prior to dialysis. Typically, blood is circulated through the dialyzer at 150 to 300 mL/minute, under negative pressure in the dialysate compartment such that an ultrafiltrate of plasma (usually 2,000 to 3,000 mL) is formed and discarded. Dialysate solution is not circulated, and the solute composition of blood is unchanged by the convective transport that occurs in isolated ultrafiltration. By use of bicarbonate-based, slightly cooled dialysate with sodium concentrations of 135 to 140 mEq/L, and volumetric instead of pressure-controlled dialysis machines, combined dialysis and fluid removal is possible in most patients and is ideal. However, in a small subset of patients who are intolerant of aggressive fluid removal with conventional dialysis, benefit may be had from an initial period of isolated ultrafiltration, with removal of up to 30 mL/kg per hour of fluid.
3. **Rapid Hemodialysis or High-Efficiency Hemodialysis**
   High-efficiency hemodialysis (HED) (15) is a reduced-time dialysis regimen in which solute and fluid removal is accomplished by: (a) increasing blood flow to more than 500 mL/minute; (b) increasing dialysate flow from 500 to 700 to 800 mL/minute; (c) use of dialyzers with a high mass transfer coefficient, low resistance to blood and dialysate flow, and a high ultrafiltration coefficient; (d) bicarbonate buffered dialysate because the capacity to metabolize acetate is exceeded at the high dialysate and blood flow rates; and (e) reliance on a mathematical model to calculate the length of each dialysis maintaining a midweek predialysis blood urea nitrogen (BUN) of about 80 10 mg/dL for a patient with a protein catabolic rate of 0.8 to 1.4 g/kg per day and a

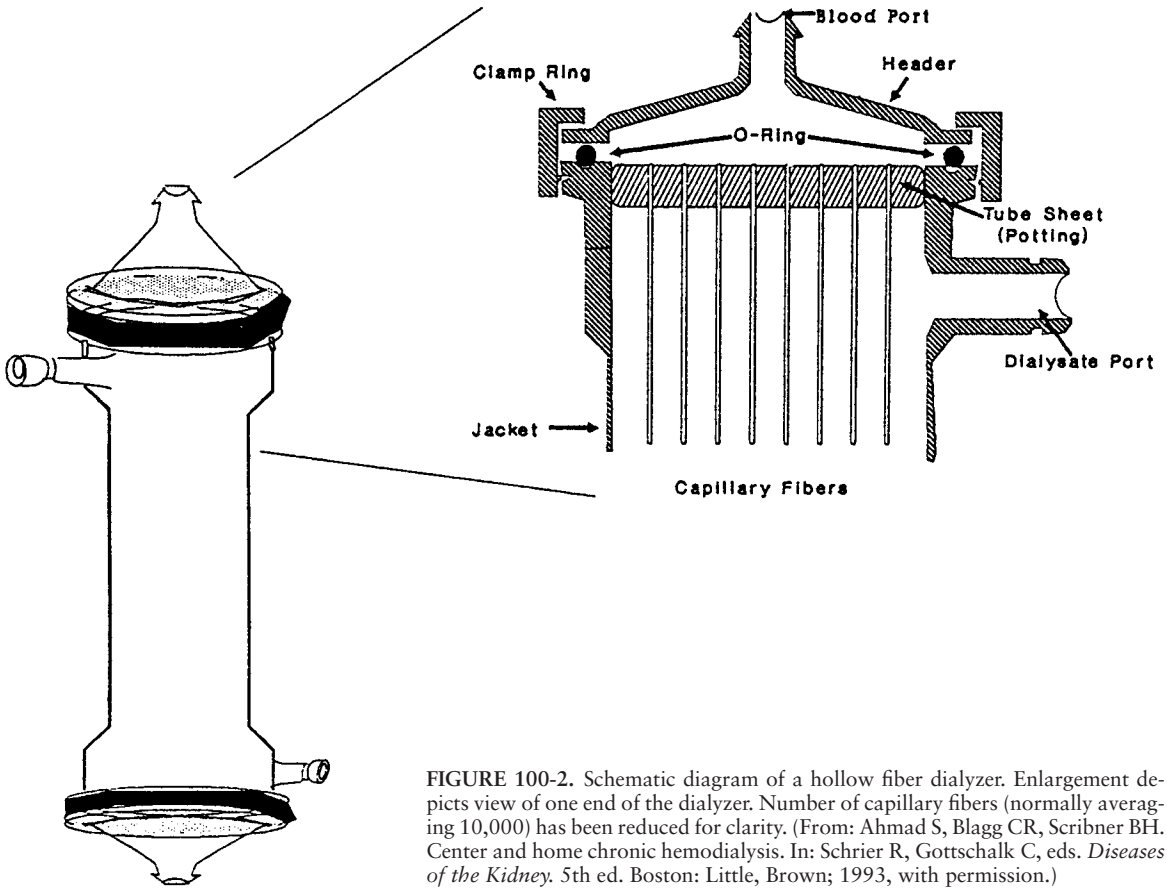

FIGURE 100-2. Schematic diagram of a hollow fiber dialyzer. Enlargement depicts view of one end of the dialyzer. Number of capillary fibers (normally averaging 10,000) has been reduced for clarity. (From: Ahmad S, Blagg CR, Scribner BH. Center and home chronic hemodialysis. In: Schrier R, Gottschalk C, eds. *Diseases of the Kidney.* 5th ed. Boston: Little, Brown; 1993, with permission.)

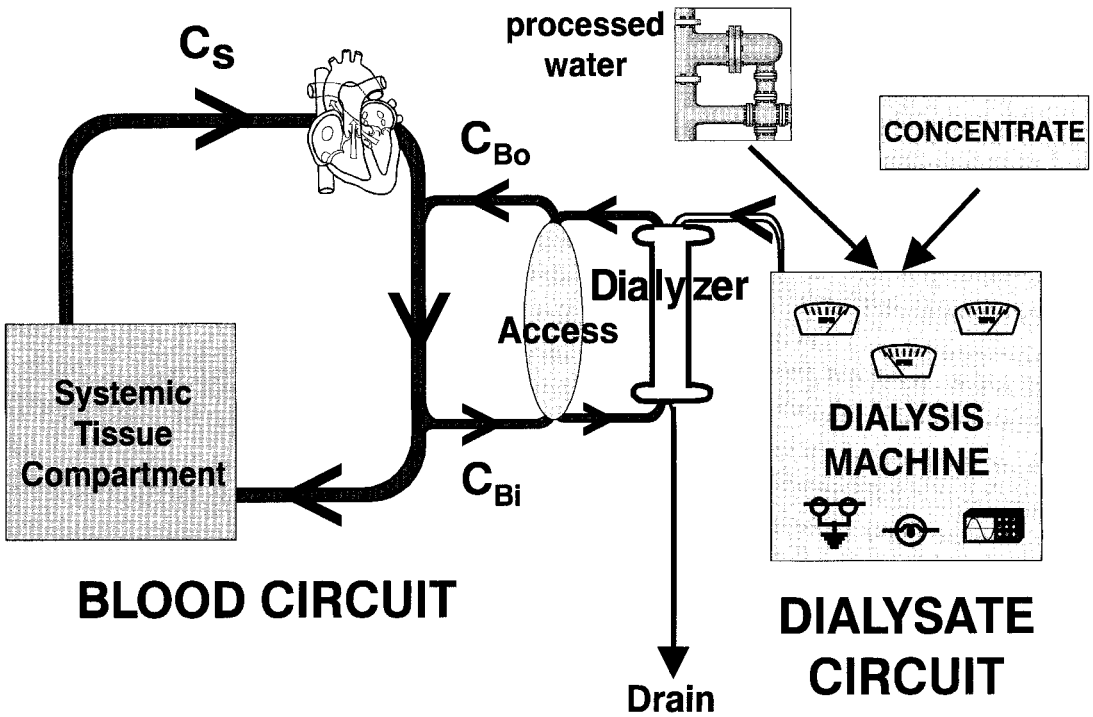

FIGURE 100-3. Schematic diagram of the blood and dialysate circuits and their components. C_{Bo}, outlet (venous line) solute concentration; C_{Bi}, blood inlet (arterial line) solute concentration; C_S, systemic solute concentration.

TABLE 100-4

TYPES OF DIALYZER MEMBRANES

Membrane material	Types	Tradename
Cellulose	Regenerated cellulose	
	Cuprammonium cellulose	Cuprophan
	Cuprammonium, Rayon	
	Saponified cellulose ester	SCE
Substituted cellulose	Cellulose acetate	Cellulate
	Cellulose triacetate	
Celluloso-synthetic (cellulose + tertiary ammonium compound)	Hemophan	
	Cellosyn	
Synthetic	Polyacrylonitrile	PAN
	PAN copolymerized with sodium methalyl sulfonate	AN69
	Polymethylmethacrylate	PMMA
	Polycarbamate	Gambrane
	Polyethylene vinyl alcohol	EVAL
	Polysulfone	

Kt/V of 1.0 to 1.3 (vide infra). Treatment times have been reduced to 2.5 to 3 hours.

4. **High-Flux Dialysis**
High-flux dialysis (HFD) is similar to HED in delivery of larger doses of dialysis and shorter treatment times, but the membranes employed and the dialyzer ultrafiltration coefficients are different. High-efficiency hemodialysis uses cellulosic membranes (ultrafiltration coefficient of 10 to 20 mL/hour per mm Hg), whereas HFD uses synthetic, open membranes, such as polysulfone (ultrafiltration coefficient of 20 to 60 mL/hour per mm Hg). High-efficiency hemodialysis involves the highly efficient removal of small molecules such as urea (molecular weight less than 300 daltons) with a prehemodialysis to posthemodialysis reduction in blood concentration of greater than or equal to 60%, whereas HFD efficiently removes larger, middle molecules (molecular weight 300 to 1,500 daltons) in addition.
5. **Hemodialysis in Parallel**
In large dialysis patients, who have inadequate dialysis by clinical or laboratory parameters despite use of large, synthetic dialyzers, high blood (greater than 500 mL/minute) and dialysate (greater than 800 mL/minute) flow rates, and dialysis times over 4 hours, use of two hemodialyzers in parallel (HDP) can provide increased clearance at a cost of approximately $14 extra per dialysis session (16).
6. **Slow Nighttime Dialysis and Short Daily Dialysis**
These new modalities are under intensive study in the United States and abroad (10,11). They may be performed in-center or, at lower cost, in the patient's home. Patients on a nocturnal regiment are instructed to dialyze for 8 to 12 hours overnight three times per week or for 2 to 3 hours 6 days a week to provide increased urea and middle molecule clearance. Aksys Ltd. developed and is presently field testing a modified computer-based dialysis system (The Personal Hemodialysis System dialysis machine) to facilitate these new dialysis modalities that is currently under review by the U.S. Food and Drug Administration. Preliminary reports from centers involved in small trials, in highly selected patients, of these two new dialysis regimes indicate reduced hospitalizations, 30% to 50% reductions in erythropoietin doses and use of antihypertensive medications, improvement in nutritional indices, and sharp reduction in the dose of phosphate binders, permitting improvement in well being and quality of life.

Assessing Adequacy of Dialysis: How Much is Enough?

The optimum dose of hemodialysis to maximize rehabilitation in ESRD patients has been a pivotal question since the earliest days of maintenance dialysis. The difficulty in precisely answering this question relates to the reality that the precise toxin(s) responsible for producing the uremic syndrome are still unidentified. Urea, measured as blood urea nitrogen (BUN) concentration, is a marker for products of protein catabolism, the source of most uremic toxins, and may be the best surrogate marker for actual uremic toxins despite its large volume of distribution and dependence on liver function. Nevertheless, because BUN levels are strongly altered by dietary protein intake and cardiovascular integrity, isolated BUN levels may prove misleading.

Optimum dialysis has been defined as that dose of dialysis above which no further improvement in the morbidity and mortality associated with dialysis can be expected (17). Objective parameters of dialysis adequacy include a midweek predialysis BUN level 75 to 85 mg/dL with consumption of a diet sufficient to generate a protein catabolic rate (PCR) greater than 0.8 g/kg per day; adequate muscle mass reflected in a creatinine generation rate of at least 125 micromol/kg per day; minimal or negligible acidosis, hyperkalemia, or hyperphosphatemia; hematocrit greater than 25%; minimal osteodystrophy; and no abnormalities on peripheral motor nerve conduction velocity studies or electroencephalography. National Kidney Foundation Dialysis Outcomes Quality Initiative (K/DOQI) recommendations for adequate hemodialysis are a urea reduction ratio of 70% and a dialyzer clearance (K) expressed as liters per minute times the time (t) of dialysis in minutes divided by the body volume of urea (V) expressed in liters using the formula Kt/V with a desired value of 1.3 or greater (5).

Attempts to quantify the amount of delivered dialysis originated in 1951, when Wolf (18) introduced the concept of dialysance (analogous to clearance) and relative dialysance (the ratio of a substance's dialysance relative to that of urea) and described the effects of dialysance, blood flow, bath volume, and volume of distribution on dialysis kinetics. Dialysis prescription remained largely empiric, however, until Babb and Scribner propounded the square meter per hour hypothesis in 1971 (19). Solute transfer across the dialysis membrane was proposed to be a function of dialyzer surface area and permeability, duration of dialysis, and to a lesser degree, blood and dialysate flow rates. From this then completely new concept evolved the Dialysis Index Model (20). This simple calculation allowed for residual glomerular filtration, and distinguished between small and middle molecule dialyzer clearance. The Dialysis Index relates a weekly creatinine clearance indexed to body size to a dialyzer surface area for a given amount of time. In two prospective studies (21,22) it was shown that a weekly B_{12} clearance of at least 30 L/week 1.73 m^2 was needed to prevent development of peripheral motor neuropathy in patients on hemodialysis. Based on the Dialysis Index, dialysis time was reduced to as little as 6 to 8 hours per week in patients with residual renal function, purportedly without ill effect (23), but the index was found to have limited correlative and predictive value overall. In 1974, Popovich and Moncrief (24) devised

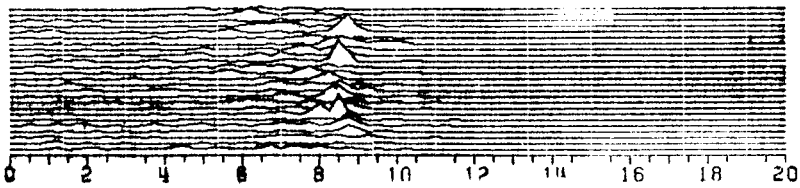

FIGURE 100-4. Electroencephalogram (EEG) power-spectrum analysis displayed in compressed spectral arrays. This tracing was obtained from a uremic patient complaining of drowsiness, insomnia, lassitude, anorexia, and nausea. Quantifying the EEG yielded a discriminant score of +0.24. (Courtesy of PE Teschan.)

an expression: the maximum (predialysis) metabolite concentration ratio (PMCR), which in a dialysis patient relates the serum concentration of nitrogenous compounds to a normalized value for the same patient with normal kidney function. These workers concluded that "chronic uremic patients receiving adequate dialysis therapy Y have predicted middle molecule PMCRs greatly in excess of 15 (times the normal concentration)." They reasoned that tolerance of high middle molecule concentrations (a usual circumstance in most dialysis patients) meant that "either middle molecules are not important neurotoxins or that the assumptions implicit in the calculation of the PMCR are decidedly invalid."

The late 1970s then saw a shift from emphasis on middle molecules as the culprits in uremic toxicity to consideration of small molecules such as urea. Ginn and Teschan reduced the hours of dialysis based on total urea clearance (a combination of dialyzer clearance and residual glomerular filtration) and presence of neurologic abnormalities (25,26). A total urea clearance of 3,000 mL/week per liter body water was proposed to be adequate dialysis because survival without symptoms in patients with chronic renal insufficiency and glomerular filtration rates of as little as 10% of normal had been observed. Reduced dialysis time, however, caused impaired cognitive function (Choice Reaction Time and Continuous Memory Task), slowing of EEG frequency, and decreased "sense of well being." As was true for four other studies, urea level appeared to proffer a better marker for dialysis adequacy than did middle molecules. The potential value of serial EEG tracings as a guide to uremia therapy was inferred from these trials of reduced dialysis time (Figs. 100-4 and 100-5).

In 1981, the importance of urea as a marker of uremic toxicity was affirmed in the landmark National Cooperative Dialysis Study (NCDS) (27). This multicenter clinical trial prospectively examined the clinical outcomes (death or hospitalization) in 165 hemodialysis patients over a 6-month period. Patients were randomly assigned to four dialysis regimens (Table 100-5) differing on the basis of dialysis time and the time averaged clearance of urea (TAC_{urea}) in mg/dL.

$$TAC_{urea} = [Td(C1 + C2) + 1d(C2 + C3)]/2(Td + Id)$$

where *Td* is dialysis time, *Id* is interdialytic interval, *C1* is predialysis BUN, *C2* is postdialysis BUN, and *C3* is BUN before the next dialysis.

All study subjects received conventional dialysis three times per week with cellulose-based dialysis membranes. Analysis of the study results revealed that TAC_{urea} (and not dialysis time) was the single most important factor determining outcome on dialysis. Later analysis and interpretation of the study data by Gotch and Sargent (28) led to the dissemination of urea kinetic modeling (UKM) that had been conceptualized a decade earlier (29). Reduced time dialysis (2.5 to 3 hours per session) was also widely adopted on the basis of the study results. Later, the importance of dialysis time in addition to urea levels in determining dialysis adequacy was reaffirmed (30), unfortunately not before thousands of hemodialysis patients on shortened treatment regimens had been subject to the effects of underdialysis. In 2002, the results of another landmark study, the multicenter HEMO study (31) were released and documented no significant difference in mortality between 1,846 hemodialysis patients randomized to standard dose (URR 66%) versus high dose (URR 73%) dialysis, or to use of low flux versus high flux dialyzers. The study did not address duration of dialysis sessions as a predictor of outcomes. When urea kinetic modeling is properly applied, it remains the best means of prescribing and assessing adequacy of dialysis. The urea product (Kt) (32) and the standardized Kt/v (eKt/v) are modifications that may have greater clinical accuracy and utility.

UREA KINETIC MODELING

Urea is assumed to be freely distributed throughout all tissues and circulating fluids in the body in a volume called the urea pool (or space). The urea pool is approximately equal to total body water (variable volume, single compartment model). During dialysis, the change in body urea content equals the difference between the urea generated and that removed.

$$\text{change in body urea} = \text{urea generation B urea removal}$$
$$d(V \times C)/dt = G\ B\ K \times C \qquad [1]$$

where *V* is volume of distribution of urea (L), *C* is urea concentration (mg/dL), *t* is dialysis time (minutes), *G* is urea generation rate (mg/minute), *K* is whole body urea clearance (mL/minute). Generation of urea during dialysis is assumed to negligible and solution of the differential equation [1] over time yields the formula:

$$K \times t/V = \log C_O/C \qquad [2]$$

where *Kt/V* is the normalized whole body urea clearance or dialysis dose, C_O is predialysis urea concentration, and *C* is postdialysis urea concentration. Values of K and V are calculated sequentially with the use of a computer or programmable calculator using the patient's height, weight, residual renal function, interdialytic weight gain, the dialyzer's expected urea clearance (manufacturer's data), calculated or

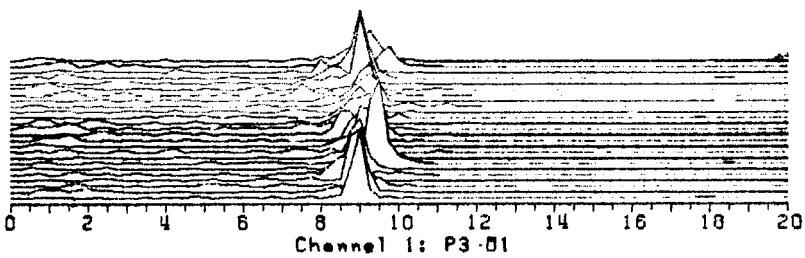

FIGURE 100-5. Following the initial series of hemodialyses, the same patient shown in Figure 100-4 improved clinically with disappearance of neurologic complaints. Normalized discriminant score corresponds to increased mean frequencies and power (amplitude) and decreased dispersion of wave frequencies. (Courtesy of PE Teschan.)

TABLE 100-5

FOUR GROUPS OF THE NATIONAL COOPERATIVE DIALYSIS STUDY

Groups	Td (h)	Predialysis BUN (mg/dL)	TAC_{urea} (mg/dL)	Patients (%)
Long Td, low BUN	4.5 to 5.0	60 to 80	50	86
Long Td, high BUN	4.5 to 5.0	110 to 130	100	46
Short Td, low BUN	2.5 to 3.5	60 to 80	50	69
Short Td, high BUN	2.5 to 3.5	110 to 130	100	31

Td, dialysis time; *BUN,* blood urea nitrogen; TAC_{urea}, time averaged concentration of BUN.

nomogram-derived V (V is commonly taken to be 58% of total body weight, or read from standard nomograms using the patient's age, sex, weight, and height), and midweek predialysis and postdialysis BUN levels. The modeled value of K is obtained using the formula derived value of V, and the modeled value of V is obtained using the expected dialyzer clearance (manufacturer's data). The interdialytic urea generation rate, TAC_{urea} (ideally 50 to 60 mg/dL), and normalized PCR in g/kg body weight per day (optimally greater than 0.8 g/kg per day) also can be derived (33). Protein catabolic rate is a measure of protein intake, muscle breakdown, and protein losses from the skin and gut. In stable dialysis patients in neutral nitrogen balance, PCR should equal daily protein intake and is defined by the following relationship.

$$PCR = 9.35 \times G + 0.29 \times V$$

where *G* is interdialytic urea generation rate in mg/minute, and *V* is volume of distribution of urea in liters. Proof of the long-held assumption that a well-dialyzed patient ingests more protein requires documentation of a direct correlation between PCR and Kt/V (34). The modeled value of K is compared to the expected value (manufacturer's data), and that of V to the formula- or nomogram-derived value in order to assess adequacy of delivered dialysis and detect blood-sampling errors (35). If the modeled value of V is much greater than the calculated value, errors in collection of postdialysis blood samples resulting in falsely low postdialysis BUN levels should be suspected. The Kt/V urea is calculated using modeled values of K, V, and dialysis time. The dialysis time needed to produce optimum values of PCR and K may also be calculated. TAC_{urea}, although not currently as popular as Kt/V as a measure of dialysis adequacy, is still favored by some and has the advantages of being unaffected by residual renal function, volume, and schedule changes, and errors inherent in urea modeling.

Controversy surrounds the optimum value of Kt/V urea. Initially thought to be 0.8 based on Gotch's discontinuous step function analysis of NCDS data (29), Keshaviah in a reanalysis of NCDS data has shown a continuous linear relationship between Kt/V and hospitalization or death, with the risk of adverse outcome being minimized at a Kt/V of approximately 1.3. This, in association with an increasing body of evidence confirming reduction in morbidity and mortality with Kt/V levels above 1.0 (36–40), has led to the current consensus of prescribing doses of dialysis of at least 1.3 with urea reduction ratio (URR) of 65%. A 15-year survival of 55% is reported in Tassin, France, with 24 hours of dialysis per week over the past 20 years in patients averaging 53 years of age and an average Kt/V of 1.67. Improved survival in this population is largely attributed to excellent blood pressure control without medication possible because of long, slow ultrafiltration (40). The Tassin population is younger and contains few diabetic patients compared to the U.S. hemodialysis population, however, thereby avoiding groups at greater risk of death. A large multicenter trial under the aegis of the National Institutes of Health examining morbidity and mortality in hemodialysis patients and focusing on adequacy of dialysis, membrane type, and dialyzer reuse is currently underway, and should provide great insight into the vexing question of dialysis adequacy.

Pitfalls in Urea Kinetic Modeling

Many inaccuracies may arise during measurement of all three components of Kt/V including:

Clearance Measurements (K)

Use of Manufacturers = Urea Clearance Data for Dialyzers. Use of manufacturers = urea clearance data for dialyzers (measured with aqueous solutions of urea) can overestimate *in vivo* clearance by 15% to 20% (41,42). Conventional arteriovenous sampling for measurement of dialyzer clearance may also result in overestimation of up to 23% if corrections for hematocrit and access recirculation are not made (43). Direct dialysis quantification (DDQ) is the most accurate method of measuring urea clearance but is tedious and time consuming. In DDQ, the value of dialyzer clearance is not assumed. Paired measurements of predialysis and postdialysis urea blood levels, coupled with measurement of urea in spent dialysate permits direct assessment of solute transfer, which, together with residual glomerular filtration rate, allows for application of single pool kinetics. Comparative application of urea kinetic modeling and DDQ techniques to the same group of 40 hemodialysis patients showed that kinetic modeling overestimated V, PCR, and K (44).

Loss of Dialyzer Surface Area and Volume. Dialyzer fiber clotting may occur (particularly toward the end of dialysis) with reduction in clearance. Dialyzer reuse may also contribute to reductions in dialyzer clearance, and may go unrecognized if monitoring of residual fiber bundle volume of the reused dialyzer is not frequent and scrupulous.

Measurement of Volume of Distribution of Urea (V)

Inaccurate Urea Assumption. A key inaccuracy arises from the assumption that urea is equally and freely distributed throughout the body. In fact, urea distributes in only 93% of plasma volume and in 86% of red cell volume.

Presence of Recirculation. Presence of recirculation, intra-access and cardiopulmonary (vide infra), especially at high blood flow rates, resulting in falsely low postdialysis BUN levels.

Improper Collection of Blood Samples. The predialysis BUN sample should be drawn from the arterial line before the blood

pump is started (serum BUN can fall significantly as soon as 1 minute after starting hemodialysis) and before administration of heparin or saline. The postdialysis BUN sample also should be drawn from the arterial line, at least 2 minutes after the blood pump is stopped in order to avoid recirculated blood being pumped through the access, and to reduce the effect of urea rebound. The arterial line is clamped or disconnected between the sampling port and the dialyzer, 5 to 30 cc of blood is withdrawn from the arterial needle (to flush venous blood that may have recirculated through the access), the sample for BUN is taken, and the 5 to 30 cc flush is returned to the patient.

Urea Rebound. Thirty minutes after termination of dialysis, urea levels often rise to 5 to 10 mg/dL above the level in samples drawn immediately after dialysis. This rebound of urea levels reflects equilibration of urea from the intracellular space, especially of relatively poorly perfused areas (skeletal muscle, bone, skin), and is most marked after short (2 to 2.5 hours) dialysis. Despite significant urea rebound within the first 30 minutes of dialysis termination, all clinical studies evaluating Kt/V have utilized values of postdialysis urea samples drawn 0 to 1 minutes after dialysis. Consequently, a Kt/V of 1.4, based on blood sampling 0 to 1 minute postdialysis often corresponds to Kt/V of only 1.1 to 1.2 if an equilibrated blood sample drawn 30 to 60 minutes after dialysis were drawn.

Shortened Dialysis Time (t)

Dialysis time may be lost owing to staff- or patient-related factors (Table 100-6). Because of these problems, and the relative complexity of the calculations involved, alternative, simpler methods of assessing the dose of dialysis measured by Kt/V have been formulated. The most popular is the urea reduction rate (URR) expressed as percent reduction in urea (PRU) (45,46).

$$\text{URR} = (\text{predialysis BUN B postdialysis BUN})/\text{predialysis BUN} \times 100$$

The URR is a measure of urea clearance during a single dialysis treatment and is a function of dialysis time, volume of distribution of urea, and dialyzer urea clearance. The URR has been graphically related to Kt/V (Fig. 100-6) and varies with the amount of ultrafiltrate removed. A URR of 55% corresponds to a KT/V of 1.0. The URR is also subject to drawbacks, however. Changes in K will not be detected because K is not directly assessed (falsely high value of K indicates either poor blood sampling technique or access recirculation); PCR values cannot be calculated; URR tends to underestimate Kt/V at high rates of ultrafiltration; and the URR is a less accurate measure of Kt/V at Kt/V values less than 0.8 or greater than 1.4.

TABLE 100-6

FORMULAE FOR CALCULATION OF Kt/V

$Kt/V = (4 \times URR) - 1.2$
$Kt/V = 1.18 \times -\ln(R)$
$Kt/V = 2.2–3.3 \times (R - 0.03 - UF/W)$
$Kt/V = -\ln(R - 0.03 - 0.75 \times OF/W)$
$Kt/V = -\ln(R - 0.03) + (4 - 3.5 \times R) \times UF/W$

URR, Pre-post BUN/pre BUN; *UF*, volume of fluid removed (L); *W*, postdialysis weight (kg); *R*, post/pre BUN ratio.
Accuracy of formulae increases from top to bottom.
(From: Daugirdas JT. Chronic hemodialysis prescription: a urea kinetic approach. In: Daugirdas JT, Ing TD, eds. *Handbook of dialysis*. 2nd ed. Boston: Little, Brown, and Company; 1994:92; with permission.)

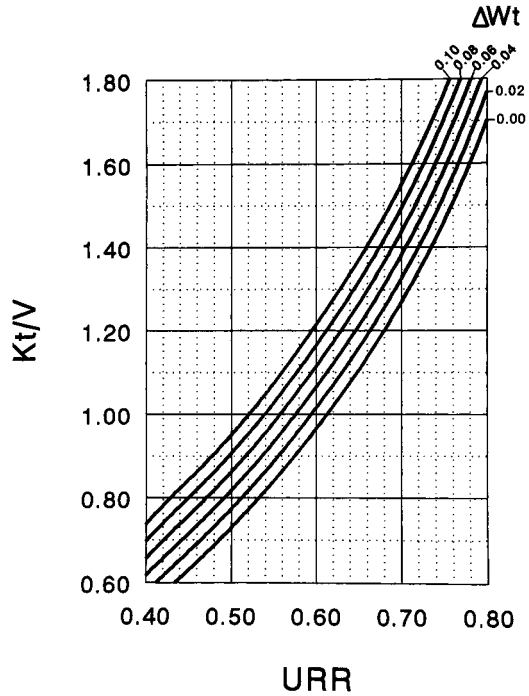

FIGURE 100-6. Graphic estimation of Kt/V from the urea reduction ratio at varying levels of dialytic weight loss. Curves are derived from formal urea modeling assuming a 3-hour dialysis, no residual renal function, and a volume of distribution of urea 58% of body weight. The nonlinear relationship between Kt/V and urea reduction ratio for treatments with net ultrafiltration losses from 0% to 10% of final (dry) body weight is shown. (From: Depner TA. Special feature estimation of Kt/V from the urea reduction ratio for varying levels of dialytic weight loss: a bedside graphic aid. *Semin Dial* 1993;6:242. Reprinted with permission from Blackwell Scientific Publications.)

Several formulae computing Kt/V from predialysis and postdialysis BUN levels also are in use (Table 100-6). The use of on-line blood and dialysate-based urea sensors for clearance measurements will allow accurate computation of whole body Kt/V in the near future.

HEMODIALYSIS OUTCOME

The quality of life during the extra years offered by hemodialytic therapy varies in patients depending on several factors including their age, renal diagnosis, comorbid conditions (diabetes, cardiovascular disease), nutritional status, presence of anemia, and dialysis dose (47). Some recipients of maintenance dialysis are nearly fully or significantly rehabilitated, whereas others (unfortunately the majority) experience varying degrees of chronic debility that renders them unable to fulfill the early expectations of the Medicare Act of 1972, which ensured financial support for treatment of all patients found suitable for chronic hemodialysis. Correction of the universal anemia of uremia by repetitive injections of recombinant erythropoietin (EPO) has improved rehabilitation in the large majority of patients (48), though sometimes unexplained resistance to the drug may blunt its potential beneficial effect in many patients.

Crude mortality rates in US maintenance hemodialysis patients are higher than in other industrialized nations, and have decreased minimally (10%) since 1988 (6). Hemodialysis patients in the United States have a gross yearly mortality rate of 22%, and their life expectancy is inferior to that of patients

TABLE 100-7

CAUSES OF DEATH IN END-STAGE RENAL DISEASE PATIENTS (USRDS, 1994)

Cause	Percent
Myocardial infarction	14.5
Other cardiac cause	29.9
Cerebrovascular disease	5.6
Septicemia	10.6
Withdrawal from dialysis	9.9
Malignancy	3.4
Pulmonary infection	2.4
Unknown cause	7.6
Other known cause	16.1

of the same age with prostate or colon cancer. A 49-year-old ESRD patient is estimated to have a life expectancy of 6.8 years and a 59-year-old ESRD patient, a life expectancy of 4.4 years. These values are 75% to 80% lower than those of the general population: an expected loss of life of 23 years for ESRD patients aged 49 and 17 years for those aged 59 years. Actuarial calculations reveal the average projected remaining lifetime of prevalently dialyzed patients with ESRD is one-fourth to one-sixth that of the general population through age 50. Mortality rates in American hemodialysis patients have improved over the past decade, however.

ESRD mortality rates are 15% to 30% higher in the United States as compared to Europe and Japan (49–51) and have been explained by: (a) greater comorbidity in U.S. patients because of advanced age and diabetes; (b) better death reporting in the United States; and (c) lower delivered dose of dialysis (related to shortened dialysis treatment times, use of smaller dialyzers ± widespread reuse of dialyzers) in U.S. patients compared to other developed countries (53). Hence, the continuing emphasis by some critics of the need for higher dialysis dose in the United States and the intense interest in reports of the salutary impact of nocturnal and daily hemodialysis. Causes of death in hemodialysis patients are shown in Table 100-7.

COMPLICATIONS OF CHRONIC HEMODIALYSIS

Underdialysis

Underdialysis remains one of the major contributors to dialysis morbidity and mortality. Causes of underdialysis are shown in Table 100-8. Recirculation of more than 15% to 20% of previously dialyzed venous blood into the arterial line is an important cause of underdialysis and may go unrecognized if not actively sought. This short-circuiting of blood (causing repeat dialysis of plasma) in an arteriovenous fistula or graft within an extremity, or in tubing leading to a single-needle (dependent on to and fro blood flow) dialysis circuit or a temporary double lumen catheter, reduces the efficiency of extracorporeal blood treatment. Although measured blood flow through the dialyzer may indicate attainment of a predetermined flow rate, dialysis of the same blood reduces effective solute extraction (clearance). Clues to the presence of blood recirculation during dialysis include the combination of a minimal decline in BUN levels as well as concentration of larger solutes (creatinine, Vitamin B_{12}), and signs of venous outflow stenosis or obstruction such as a swollen access limb, high venous pressures on dialysis, and a prominent, tapping arterial inflow pulse. As a simple test for recirculation, simultaneous measurements of BUN levels in arterial blood entering the dialyzer, and systemic blood should not differ by more than 0 ± 2 mg/dL. To quantify the amount of recirculation, simultaneous measurements of the same solute (urea, creatinine) in samples drawn from arterial and venous bloodlines during dialysis are compared with the level of the same solute in a simultaneous venous blood sample from the contralateral extremity (the three-needle method) (Fig. 100-3).

$$\text{recirculation}(\%) = \frac{C_s - C_{Bi}}{C_s - C_{Bo}} \times 100$$

where C_S is the systemic concentration, C_{Bi} is the blood inlet (arterial line) concentration, and C_{Bo} the outlet (venous line) concentration of an easily measured solute, such as urea, in mg/dL. It is important to distinguish the venous blood sample (C_{Bo}), also called a *postdialyzer* blood sample from a

TABLE 100-8

CAUSES OF UNDERDIALYSIS

Patient-related factors	Factors related to equipment malfunction or failure	Staff-related problems
Problems related to vascular access	Dialyzer deviation from manufacturer specifications	Incorrect dialysis prescription (time × surface area × blood flow rate)
Venous outflow stenosis resulting in recirculation	Reduction in clearance of reused dialyzers	Incorrect needle placement or reversal of blood lines with resultant recirculation
Poor access development or inflow stenosis resulting in low blood flow rates	Inadequate heparinization with dialyzer clotting and reduction in clearance	Shortening or improper tallying of dialysis time (time during which the blood pump is turned off or blood flow rate significantly reduced should not be included)
Missed or shortened dialysis sessions (depression/psychosocial problems)	Low blood flow rates secondary to improper blood pump calibration, collapse of pump line segment, or lack of total occlusion of blood pump	Improper collection of postdialysis blood samples for calculation of dialysis adequacy
Cardiovascular disease with hemodynamic instability, need for repeated interruptions in dialysis, and reduced dialysis time		
Unrecognized concurrent illness (malignancy, infection)		

postdialysis blood sample that is drawn from the arterial line at least 2 minutes after termination of dialysis for purposes of calculating the URR. If significant recirculation (greater than 15% to 20%) is detected, contrast angiography is indicated and a stenosis of the venous outflow tract of the access often is discovered.

A modification in the three-needle method usually employed for obtaining blood samples for the measurement of recirculation was introduced after recognition of the presence of cardiopulmonary recirculation (CPR) (52,53). The BUN of dialyzed blood returning to the heart is reduced by admixture with blood volume returning to the heart from other areas of the body, such that during dialysis, arterial BUN is always lower than venous BUN by 3 to 4 mg/dL. This CPR (also called arteriovenous dysequilibrium) accounts for about 7% of recirculation, and becomes especially significant in patients with low cardiac outputs and therefore more time for dilution of dialyzed blood. CPR in patients with congestive heart failure may approach 11% to 12%. It is therefore incorrect to assume that the BUN level in a peripheral vein of the nonaccess arm is equal to that at the arterial inlet line of the dialyzer when recirculation is absent; the peripheral vein BUN is always lower because of CPR. To eliminate the effect of CPR, a two-needle method utilizing a stop-flow or low flow technique is used to collect the C_S (peripheral) blood sample: The blood pump is stopped or slowed to 50 mL/minute, and a blood sample drawn from the arterial line no more than 30 seconds later. Collection of this C_S blood sample at minimal or no blood flow prevents collection of recirculated blood. Collection of the sample within 30 seconds of stopping or slowing dialysis minimizes the effect of urea rebound. Samples for C_{Bo} and C_{Bi} are collected in the usual manner at the set blood flow rate.

Cardiopulmonary recirculation does not occur with use of central venous catheters for dialysis access. If the standard three-needle technique is used for calculation of recirculation, samples should be drawn within the first 30 minutes of start of the hemodialysis treatment, as systemic blood venous BUN becomes lower than that of venous BUN in the nonaccess arm because of relatively poorer blood perfusion in the vasoconstricted extremity. The difference between nonaccess arm venous BUN and systemic venous BUN (veno-venous dysequilibrium) is most marked after 1 hour of initiation of hemodialysis, and can account for falsely high recirculation rates of up to 30%.

Underdialysis should be suspected in any patient who is deteriorating, without obvious cause, during maintenance hemodialysis. The integrity of the vascular access should be checked, the presence of blood recirculation excluded, and urea kinetic modeling performed and compared to previous analyses. To test the inference that underdialysis is responsible for the patient's worsening condition, the effect on clinical well being of an increase in effective dialysis should be determined. Substitution of a greater surface area dialyzer without change in dialysis time is an initial empiric step readily accepted by patient and staff. Should intradialytic hypotension, or prior selection of a large dialyzer preclude this approach, an extension of the duration of each dialysis is mandated as the next step. An increment of 1 hour per dialysis (for each dialysis), for 1 month, is usually sufficient to induce improvement in an underdialyzed patient. Improved work tolerance, and lessened pruritus, anorexia, and hiccoughs may be the first subjective indicators that the diagnosis of underdialysis was correct. A rising hematocrit and declining phosphorus and parathyroid hormones (PTH) levels over the ensuing 3 months afford further corroboration of the wisdom of having increased dialysis time. Changes in the amount of dialysis may require months to a year or longer to effect clinical well being.

Cardiovascular Disease

Hypertension

Hypertension is the second most serious threat (after diabetes) to longevity on maintenance hemodialysis. Hypertension requiring therapy was seen in 75% of ESRD patients in the HEMO Study (31), and its sequelae of left ventricular hypertrophy, ischemic heart disease, and cardiac failure account for the nearly 50% cardiovascular death rate in hemodialysis patients (54). Systolic hypertension with associated high pulse pressure is a greater risk factor for cardiovascular disease than is diastolic hypertension. In Tassin, France, where long, slow dialysis is practiced, fewer than 3% of patients need antihypertensive drugs, reflecting the better-tolerated and hence more adequate removal of fluid with slow dialysis (40).

By obtaining an ambulatory blood pressure (ABP) profile during 48 hours in 24 normotensive hemodialysis patients (4 hours, three times weekly), Battistella and co-workers provide insight into the response to worsening levels of uremia in the functionally anephric human (55). Ambulatory blood pressure is greater on the second postdialysis day (122/74 mm Hg) than on the day after dialysis (117/70 mm Hg), and nocturnal decrease in blood pressure is less than 5%. Surprisingly, increases in ABP on the second day do not correlate with the degree of interdialytic weight gain. The *white coat syndrome* continues to impact on blood pressure even after initiation of maintenance hemodialysis. No matter when it is taken, blood pressure before hemodialysis is continually higher than the diurnal ABP (138/74 mm Hg versus 121/73 mm Hg), even if the ABP is analyzed 1 hour prior to hemodialysis (129/77 mm Hg). Hypertension in renal failure, although multifactorial, usually responds to contraction of plasma volume induced by dietary salt and water restriction and ultrafiltration on dialysis. Loop diuretics may be used to augment water excretion in patients with residual renal function and creatinine clearances greater than approximately 8 mL/minute.

The management goal in balancing interdialytic weight gain against intradialytic fluid extraction is to attain the patient's "dry weight," the weight at which the patient is normotensive without use of antihypertensives and that permits interdialytic weight gain of 5 or more pounds without development of hypertension. Dry weight has also been defined as the lowest weight tolerated without development of symptomatic hypotension. Absence of clinical signs of volume overload such as peripheral edema and central vein distension are unreliable in assessing dry weight, as ultrasound scans document increased vena cava diameter indicating volume expansion in many dialysis patients without clinical signs of volume overload (56). Very often, the limiting factor in fluid removal (ultrafiltration) during hemodialysis is a fall in blood pressure to hypotensive levels accompanied by nausea, vomiting, leg and other muscle cramps, and syncope and altered mentation in those patients with compromised cerebrovascular or coronary function. A vicious cycle of need for antihypertensive drug use, hypotension on dialysis with interruption of dialysis and need for saline replacement, weight gain, exacerbation of volume-sensitive hypertension, and increased dose of antihypertensive medications may afflict some patients and contribute to underdialysis as a result of shortened dialysis time. Dry weight is much more easily acheived with short daily hemodialysis than with three times per week high flux dialysis.

Blood pressure lability during hemodialysis depends in part on the choice of dialysate buffer (acetate or bicarbonate) and the concentration of sodium in dialysate. Because sodium retention may aggravate underlying hypertension, the exact dialysis prescription seeks to minimize hypertension while protecting against intradialytic hypotension. Kramer and colleagues

studied six ESRD patients with sequential bicarbonate and acetate dialysate at four different dialysate sodium concentrations (135, 140, 145, and 150 mmol/L) (57). During hemodialysis, bicarbonate buffer protected against hypotensive episodes, as did a sodium dialysate sodium concentration of 140 mmol/L. There was no advantage in raising dialysate sodium concentration above 140 mmol/L. Zuccala and associates explored the question of whether one class of antihypertensive drugs was superior to others in eight hemodialysis patients (58) with a high plasma renin activity (greater than 2 ng/mL per hour). Patients were given 50 mg of captopril, clonidine, and nifedipine in random order. All three drugs produced an equivalent fall in mean blood pressure from a pretest value of 121 $\pm$ 8 mm Hg to 111 to 112 $\pm$ 4 mm Hg.

If hypertension is not significantly improved within a few weeks of beginning maintenance hemodialysis, low doses of an angiotensin converting enzyme inhibitor (ACEi) or angiotensin receptor blocker (ARB), calcium blocker, or β blocker may be added and progressively increased as needed. Most patients should not take their antihypertensive medications on the morning of dialysis so as to avoid intradialytic hypotension. So-called "tissue-specific" ACEis (ramipril, quinapril, perindopril, trandolopril) may be particularly beneficial in retarding macrovascular as well as cardiovascular disease, and we place almost all our diabetic hemodialysis patients on an ACEI. Another potential benefit of ACEi use is the retrospective observation that arteriovenous graft thromboses are decreased in ESRD patients on treatment with ACEis (59). The centrally acting alpha antagonist clonidine is efficacious in hypertensive ESRD patients but extraction of clonidine during hemodialysis can precipitate hypertension, agitation, and headache—"signs of withdrawal"—in patients on the drug. Minoxidil may be added in cases of recalcitrant hypertension, and its use has now long preempted the need for native binephrectomy, a previous last resort in difficult-to-control hypertensive patients. Side effects of minoxidil include hypertrichosis, tachycardia, and fluid retention in serous cavities. Concomitant prescription of a β blocker to counteract reflex tachycardia and increased ultrafiltration should be performed with initiation of minoxidil therapy. Active participation by the patient in drug dose modification is aided by provision of an inexpensive and reliable blood pressure monitor. We employ a simple aneroid, blinking light, beeping device that is widely available, under a variety of brand names, in surgical supply stores for about $80 (DIGI + PLUS DSP-80, Ueda Electronic Works, Ltd., Japan).

About 80% of American ESRD patients on maintenance hemodialysis are now treated with erythropoietin. One-third of previously normotensive hemodialysis patients show an increase in blood pressure during erythropoietin treatment. There is no correlation between the rise in blood pressure and patient age, sex, duration of ESRD, and previous levels of blood pressure. Return to baseline (pre-erythropoietin) blood pressure generally is easily accomplished either by increasing ultrafiltration or antihypertensive drugs during hemodialysis.

Coronary Artery Disease

In a study of the earliest patients started on hemodialysis, Lindner and associates found that 85% had died by the thirteenth year of dialysis of presumed cardiovascular causes, associated with accelerated atherosclerosis (60). The probability of myocardial infarction, angina, or pulmonary edema requiring hospitalization in hemodialysis patients is about 10% per year (61). The prevalence of congestive heart failure and complex ventricular ectopy are 10% and 18%, respectively (62,63). Cardiac dysfunction in the incident hemodialysis population is also common. Abnormalities of systolic function and/or left ventricular hypertrophy are found in over 70% of patients starting maintenance hemodialysis (72). Risk factors for atherosclerosis in dialysis patients include hypertension, cigarette smoking, diabetes mellitus, hyperlipidemia (30% to 50% of chronic dialysis patients are hypertriglyceridemic, with normal total cholesterol but unfavorable HDL:LDL ratios), abnormalities of calcium metabolism, hyperuricemia, and the presence in uremic serum of mitogenic factors that can stimulate smooth muscle proliferation, an early step in the genesis of atherosclerotic lesions. Investigators found that one such mitogenic substance, platelet-derived growth factor (PDGF), is increased in activity about threefold during dialysis with cuprophane membranes (64). Most dialysis prior to 1970 was performed with cuprophane membranes and they are still frequently used. An association between cuprophane and accelerated atherosclerosis is yet to be established, but the greater bioincompatibility of this material compared with newer membrane materials in use today mitigates against its continued widespread application in dialysis.

Cigarette smoking is a significant risk factor for coronary artery disease in dialysis patients. Of 20 patients who smoked cigarettes in a retrospective study of dialysis patients, 33% were dead by their seventh year of dialysis, and 75% by the eleventh year (55). Every patient who died of cardiovascular disease (5/5) was a cigarette smoker. By contrast, only 3/10 living patients were cigarette smokers. In dialysis patients assessed during transplantation for the presence and severity of atherosclerosis by Vincenti and associates (65), no correlation was found between preexisting metabolic and lipid abnormalities, and duration of hemodialysis. Correlation was found, however, between degree of hypertension, age, and severity of atherosclerosis, which caused the authors to conclude that atherosclerosis may not be accelerated by hemodialysis and may be prevented by more stringent control of hypertension in uremia.

The importance of calcium/phosphate balance in the genesis of obstructive coronary artery disease has been highlighted in recent studies utilizing magnetic resonance imaging or electron beam computed tomographic imaging of coronary artery plaque that documents increasing evidence of coronary plaque formation in hemodialysis patients with hyperphosphatemia or elevated calcium/phosphorus products (66,67). Metastatic or dystrophic deposits of calcium within coronary vessels and around cardiac valve rings can be significant contributors to cardiac mortality in dialysis patients.

Dialysis-Associated Pericarditis

Small asymptomatic pericardial effusions are common in dialysis patients who are volume overloaded. Fibrinous pericarditis with large effusions can produce cardiac tamponade, may occur in up to 8% to 10% of the chronic hemodialysis population, and is a reflection of underdialysis (68). Patients with dialysis-associated pericarditis may have chest pain, distended neck veins, a pericardial rub, or develop recurrent hypotension during the first 30 to 60 minutes of dialysis. Intensification of the dialysis regimen with daily heparin-free dialysis for 1 to 2 weeks brings about reduction in size of the effusion in 50% to 70% of patients. Surgical drainage of the pericardial space is indicated if the effusion is massive (greater than 250 mL), fails to respond to medical therapy, produces hemodynamic instability on dialysis, or if there are clinical or echocardiographic signs of early or imminent tamponade. Subxiphoid pericardiotomy is the procedure of choice; however, in emergent cases, needle aspiration of the pericardial space is first necessary.

Undernutrition

One-third of adult hemodialysis patients show evidence of moderate to severe protein calorie malnutrition (PCM). Factors

contributing to PCM in hemodialysis patients include anorexia associated with uremia, advanced age, medications, or intercurrent illness; depression; economic constraints; and the catabolic stress of the hemodialytic technique itself. Causes of dialysis-induced catabolism include: membrane bioincompatibility (37) hypothesized to be caused by interleukin-1 (IL-1) and tumor necrosis factor; the loss of up to 8 to 12 gm of amino acids per week on dialysis; and metabolic acidosis (69,70). The importance of PCM in contributing to dialysis mortality has been emphasized by Lowrie and Lew (71), who documented that mortality risk in chronic dialysis patients correlated best with hypoalbuminemia, followed by increasing age and serum creatinine. Hemodialysis patients with serum albumin levels between 3.5 and 4.0 g/dL have a two times greater mortality risk compared to those with values above 4 g/dL. With serum albumins of 2.5 to 3.0 g/dL, mortality risk is increased 16-fold. Low serum BUN and cholesterol were also associated with increased mortality risk. Hypoalbuminemia in dialysis patients also correlates with the prevalence of infections, congestive heart failure, and pericarditis.

Routine dietary assessment is mandated in hemodialysis patients. Indices of nutritional status such as transferrin levels, lymphocyte counts, in addition to serum albumin levels should be monitored. Protein catabolic rate by kinetic modeling and anthropometric measurements also are useful adjuncts. In order to maintain neutral nitrogen balance, it is recommended that patients on hemodialysis receive a total caloric intake of at least 35 kcal/kg per day with high-biological-value protein intake of 1.2 g/kg per day. About 35% of nonprotein calories should be from carbohydrates and the rest from fat. Correcting comorbid illnesses and increasing dialysis dose (72) may also improve nutritional status. Early use of enteral or parenteral nutrition during acute illnesses in hemodialysis patients is advocated. The role of intradialytic parenteral nutrition (IDPN) providing 500 to 600 calories/day is currently under debate (73). For the present, it is best limited to those patients who are unable to eat. Recombinant human growth hormone in doses of 5 to 10 mg subcutaneously three times weekly administered in conjunction with IDPN also has been shown to aid protein anabolism (74), but the cost of the hormone at $40/mg renders its widespread use prohibitive.

Vascular Access Complications

Problems related to vascular access account for one-fourth of all ESRD patient hospitalizations (75). A well-functioning vascular access is essential for delivery of dialysis and *adequate* dialysis. The economic and social cost of admissions for access declotting or repair is high, and morbidity is especially marked in the elderly. Long-term vascular access is best affected in the form of an endogenous fistula at the radial or brachial arteries. When inadequate veins or arteries are present (as documented by venography and arterial doppler imaging), a synthetic graft usually made of polytetrafluoroethylene (PTFE) may be used as an alternative, but is subject to higher complication rates. Use of composite grafts made partially of the usual expanded PTFE material along with the more resilient plasma PTFE, can allow early graft cannulation (within 24 hours) following construction or repair of a graft (80). Prospective trials using these and other composite grafts are underway.

Thrombosis and Infection

Failure of vascular access because of thrombosis with or without associated infection occurs in PTFE grafts more frequently than in endogenous fistulae. Polytetrafluoroethylene grafts have an average duration of patency of 1 to 3 years (2-year patency rate of 70%), whereas a well-constructed Brescia-Cimino fistula has a mean patency of 3 to 4 years or longer. The complication rate of PTFE grafts is four to five times that of fistulae. Access thrombosis usually is associated with an anatomic abnormality (venous stenosis, aneurysm formation, local scarring at a site of repeated needle puncture), extrinsic compression, or low flow state. Vascular accesses in diabetic patients have similar 1-year patency rates when compared to nondiabetic patients (76). In a series of 324 arteriovenous accesses created in 256 patients between June 1979 and October 1983, there were 34 diabetic patients in whom 22 Cimino fistulae and 27 PTFE grafts were placed (85). When compared to similar vascular accesses in nondiabetic patients, there was no difference in the patency rates at 1 year in diabetic and nondiabetic patients. Early failure of fistulae because of poor maturation or failure of maturation is more common in diabetic than nondiabetic patients, however.

The substrate for development of access thrombosis and occlusion is stenosis caused by myointimal hyperplasia and neovascularization of the outflow tract of the access just distal to the venous anastomosis where the jet of blood shunted across the access impinges (77,78). Impending thrombosis of the access related to venous outflow stenosis often can be detected by monitoring for high venous pressures (a venous pressure of greater than 150 mm Hg at a blood flow rate of 200 mL/minute is considered elevated); feeling a weakening access thrill; detecting an arterial pulse that is excessively prominent and tapping; and noting prolonged bleeding following removal of dialysis needles. Recirculation percentages also rise as a stenosis on the venous side of the access worsens. Noninvasive transcutaneous measurement of access blood flow rate (BFR) by doppler flow imaging, optical sensor systems or other methods has become widely used as adjunct to detect impending access thrombosis. If access blood flow falls by >25% over a 4-month period, or falls below 600 mL/minute, the patient should be preemptively referred for a fistulogram and possible angioplasty by the interventional radiologist or nephrologist (5) (Fig. 100-7). Angioplasty is associated with at least a 50% restenosis rate, however, and use of various types of stents in selected patients may retard this complication.

When the arterio-venous access thromboses, early surgical thrombectomy utilizing a Fogarty catheter with repair or bypass of stenotic areas (if present) will restore graft patency. Alternatively, thrombolysis using urokinase or tissue plasminogen

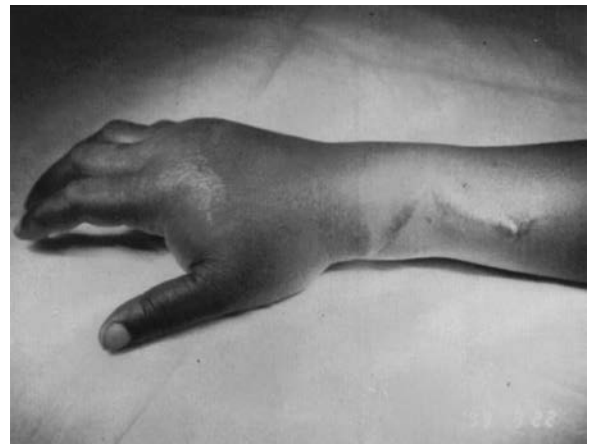

FIGURE 100-7. Chronic swelling of the access hand and arm in a patient with proximal axillary vein stenosis. (Courtesy of JH Hong.)

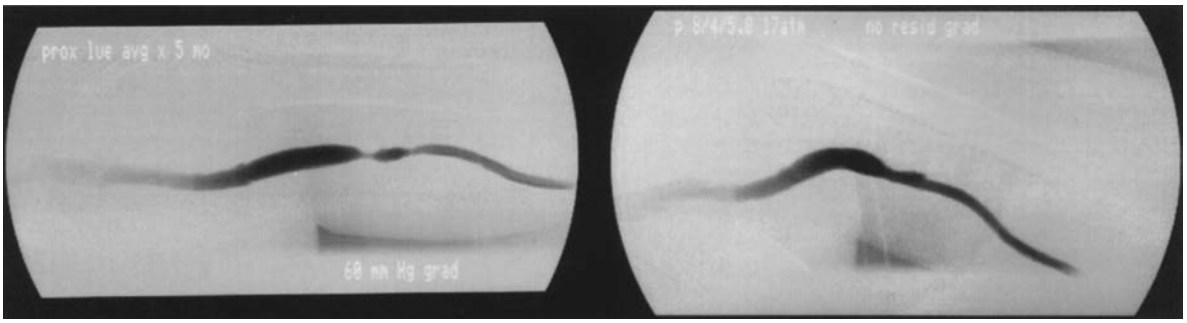

FIGURE 100-8. Contrast angiography showing stenotic area of the axillary vein before and after percutaneous balloon angioplasty.

activator can achieve graft patency in 60% to 90% of cases, but the risk of rethrombosis is high if the ubiquitously present venous outflow stenosis is not corrected.

Infection of arteriovenous grafts is more common than with fistulae, may occur in association with graft thrombosis, and may be present in the absence of obvious signs of local infection. Infection of a perigraft hematoma is a common initiating event. Strict attention to skin preparation before cannulation of the arteriovenous access is of great importance in reducing the incidence of graft infections.

Venous Hypertension

Another complication of placement of arteriovenous access is chronic swelling of the hand (Fig. 100-7), and especially the thumb ("sore thumb" syndrome), related to the presence of the distal segment of the vein used for creation of the access (81). Venous hypertension also may occur in association with venous stenosis of the access, or a more proximal stenosis at the level of the subclavian vein, which may have been previously catheterized for temporary vascular access. Ligature of the distal venous limb of the fistula or graft, or balloon dilatation of an area of stenosis usually corrects the problem.

Radial Steal Syndrome

The blood supply to the fingers may be compromised when the radial artery is used for construction of a side-to-side arteriovenous fistula with the cephalic vein. Radial arterial blood no longer supplies the fingers, whereas the fistula provides a low-pressure runoff system that short circuits the ulnar and interosseous arterial blood supply through the palmar arch, a system that may have been previously compromised because of medial arterial calcification, which is especially common in diabetic patients (82). Pain and numbness in the fingers may proceed to ischemia and gangrene (Figs. 100-8 and 100-9). A steal syndrome may be avoided or ameliorated by ligation of the distal radial artery segment to prevent retrograde flow of blood from the ulnar arterial system into the fistula, or by primary creation of an end-artery to side- or end-vein fistula.

Ischemic Monomelic Neuropathy

Multiple distal mononeuropathies may occur in the upper limb following placement of a proximal arteriovenous access (83). The condition is particularly common in diabetic patients and is characterized by acute painful weakness of the forearm and hand muscles, which occurs within hours of the access creation and is related to ischemia of the peripheral nerves owing to sudden diversion of the blood supply by placement of a proximal brachiocephalic fistula. Early closure of the fistula or removal of the graft may result in reversal of the syndrome.

FIGURE 100-9. Three cases of radial steal syndrome following placement of vascular access showing increasing degrees of severity of ischemia. The two most severe cases occurred in diabetics. (Courtesy of JH Hong.)

Hematologic Complications

Anemia

The introduction in 1989 of EPO for treatment of renal anemia was one of the most vital advances in hemodialytic therapy. Doses of 50 to 100 U/kg of EPO three times weekly usually are required to keep Hct above 30%. In 2001, the longer acting darbepoeitin alpha (an erythropoietin molecule with additional glycosylations) was approved for use (84). K/DOQI recommend a target hematocrit of 33% to 36% and hemoglobin 11 to 12 g/dL (5), but there is still active debate regarding the possible beneficial effect of even higher target Hcts (normalizing), particularly in patients with cardiac disease.

Iron deficiency should always be sought and treated first, even before initiation of EPO therapy because hemodialysis patients lose up to 1,000 mL/year of packed red blood cells equivalent to 1 g of iron. Transferrin saturation should be maintained between 20% and 50% while the serum ferritin is targeted as 100 to 800 mg/mL during EPO therapy (85).

Causes for failure of erythropoietin effect include iron deficiency, folate deficiency, infection or inflammation, malignancy, aluminum overload, red cell dyscrasia, and hyperparathyroidism with bone marrow fibrosis. Another cause of EPO failure/resistance was recognized in 1999 when cases of severe anemia associated with pure red cell aplasia due to anti-erythropoietin antibodies were reported in patients treated with a particular brand of subcutaneously administered epoietin alpha used outside of the United States (Eprex) (86). Antibody titers decrease with discontinuation of the drug, but immunosuppressive agents are usually required to decrease transfusion requirements.

Bleeding Diathesis

Uremic platelets show abnormal aggregation and adhesion, and there is decreased activity of von Willebrand factor and decreased levels of platelet factor III, such that the bleeding time may be greatly prolonged. In association with frequent heparinization during dialysis, this results in a bleeding tendency that may produce spontaneous retroperitoneal bleeds or subdural hematomas. Improvement may be effected by intensive dialysis, correction of anemia by erythropoietin, and the use of cryoprecipitate (87), desmopressin (dDAVP) (88), or conjugated estrogens (89).

Sepsis

Bacterial infections occur commonly in hemodialysis patients, and may present insidiously yet progress rapidly. Staphylococcal and Gram-negative organisms (*Escherichia coli* in particular) usually are implicated. Opportunistic pathogens are uncommon. Septicemia was responsible for 10.6% of deaths in Medicare ESRD patients between 1989 and 1991 (6). Impaired neutrophil chemotaxis caused by reduced ability to bind C5A and variable derangements in macrophage Fc function and IL-1 production have been reported in dialysis patients, resulting in defective antigen internalization and processing (90). Iron overload (if present) contributes to defective neutrophil function. In addition, dialysis patients show reduced T- and B-cell response to various mitogens, as well as to viral vaccines (influenza, hepatitis B). Initiation of dialysis therapy leads to significant improvement in *in vitro* T-cell proliferation as measured by T-cell IL-6 and IL-10 production (91). Despite these defects in immunity, the high rate of bacterial infections in hemodialysis patients is probably most strongly related to frequent breaches in the skin during vascular access cannulation, and the use of central line hemodialysis catheters (92). Use of mupirocin ointment at catheter exit sites, or instillation of antibiotic solutions (gentamicin or taurolidine) into the catheter lumen reduces catheter-related bacteremia (92). Infected PTFE grafts may lack obvious signs of infection, so that the index of suspicion in a febrile dialysis patient with a prosthetic graft vascular access must be high, particularly if there is associated thrombosis of the graft. Bacterial infections in hemodialysis patients generally appear more severe and more likely to progress to septicemia with attendant risks of endocarditis and seeding of a prosthetic graft if this was not already the original site of infection.

The urinary tract may be a source of infection in patients with polycystic kidney disease (PKD) and diabetic patients with neurogenic bladders. Lipid-soluble antibiotics such as ciprofloxacin or trimethoprim sulfamethoxazole that penetrate cyst walls well should be used to treat urinary infections in patients with PKD. Suspicion of previously unrecognized diverticulitis as the source of abdominal complaints and sepsis in patients with PKD should also be high because of an increased prevalence of colonic diverticula in PKD. Pneumonia is a significant cause of morbidity and mortality in dialysis patients and often presents with atypical pulmonary infiltrates. Tuberculosis is 10 times as common in hemodialysis patients as in the general population, and disease often is extrapulmonary, miliary, and atypical. Infections with Yersinia, Mucor (vide infra), Listeria, and Salmonella also are reported to be more common and/or severe in hemodialysis patients.

Diseases of Bone and Soft Tissue

Renal Osteodystrophy

Three predominant types of renal osteodystrophy occur, but combinations and transitional forms sometimes related to therapy commonly are seen.

High Turnover Renal Bone Disease. The consequence secondary hyperparathyroidism, high turnover renal bone disease is characterized by increased osteoblastic and osteoclastic activity with varying degrees of bone marrow fibrosis, the presence of woven osteoid, and normal or accelerated mineralization of bone. Pathogenetic factors include hyperphosphatemia, hypocalcemia and low levels of 1,25-dihydroxycholecalciferol. The hypocalcemia may be absolute or merely relative, as a shift of the set point of calcium (the serum calcium level needed to reduce the maximal PTH level by 50%) to the right on the PTH/calcium curve occurs in uremia so that a higher serum calcium than normal is required to inhibit PTH secretion. Serum calcium levels in uremic patients should be kept between 9.5 and 11.5 mg/dL. Radiologic changes develop late in hyperparathyroidism. In established disease, subperiosteal resorption is best seen on the radial borders of the middle phalanges of the middle fingers, erosion of the terminal tufts of the fingers, and subperiosteal erosions and cysts involving the lateral one-third of the clavicles are characteristic (Fig. 100-10). Multiple bone cysts first observed in the outer one-third of the clavicles and middle phalanges but involving mainly long bones are noted in advanced disease (osteititis fibrosa cystica). Osteosclerosis involving the axial skeleton and giving rise to the characteristic "rugger jersey" spine appearance also is a feature of high turnover renal bone disease. Intact or N-terminal parathyroid hormone (PTH) levels measured by immunoradiometric assay (iPTH) are greater than 150 to 200 pg/mL in patients with clinically significant hyperparathyroidism. Some 90% of patients with iPTH levels less than 900 pg/mL respond to treatment with intravenous or oral calcitriol. A new second generation immunoradiometric two-site sandwich assay which exclusively detects the biologically active PTH 1-84 moiety

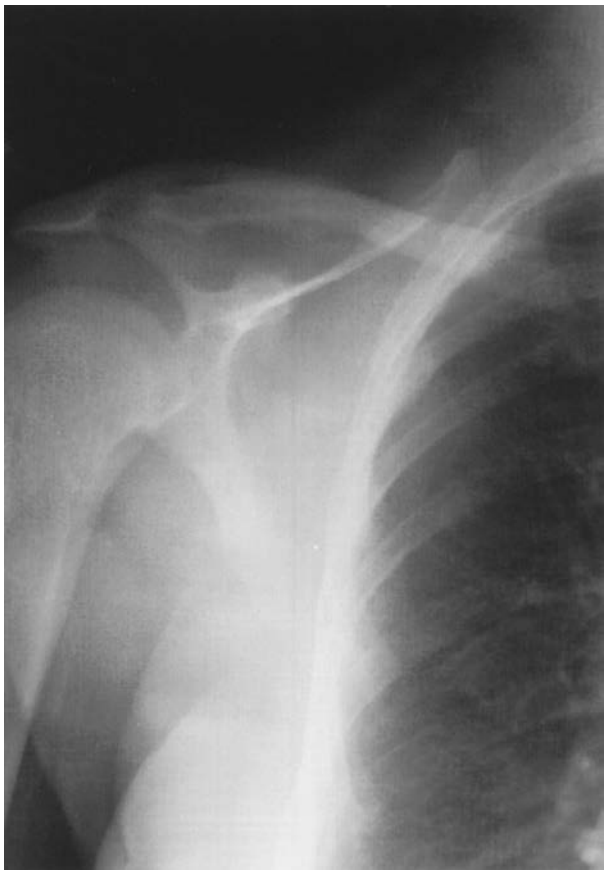

FIGURE 100-10. Gross erosion of the distal one-third of the clavicle (Nathan's sign) in a hemodialysis patient with secondary hyperparathyroidism.

(bio-intact PTH assay) and is roughly equivalent to 50% of intact PTH levels is now available but not yet in widespread use. The intravenous vitamin D analog, 19-nor-1α25-dihydroxyvitamin D2 (paricalcitol) is more potent and has less calcemic effect than 1,25 dihydroxyvitamin D) when used to treat high turnover renal bone disease, and is rapidly gaining popularity in the United States, as is the novel calcimimetic agent cinacalcet (Sensipar) which acts as an agonist of the calcium sensing receptors on parathyroid tissue hence decreasing PTH secretion (93).

In patients with severe hyperparathyroidism and iPTH levels greater than 900 pg/mL, however, medical therapy may be ineffective in over 60% of cases owing to autonomy of the parathyroid glands (tertiary hyperparathyroidism). These patients often have associated hypercalcemia and hyperphosphatemia with pruritus and calciphylaxis and benefit from surgical parathyroidectomy. Tertiary hyperparathyroidism may complicate renal transplantation, persisting after graft failure and resumption of maintenance hemodialysis.

Low Turnover Bone Disease. This multifactorial variant of renal osteodystrophy is characterized by low levels of parathyroid hormone (less than two times normal), a reduced number of osteoclasts and osteoblasts with low rates of bone turnover, and a tendency to develop hypercalcemia (particularly with treatment). Two types of low turnover bone disease are seen, aplastic bone disease and osteomalacia.

Aplastic Bone Disease. This disorder is characterized by decreased osteoclast and osteoblast activity, cessation of bone formation, and intact PTH levels less than 100 pg/mL. Etiologic factors include aluminum and iron overload, and the condition occurs more frequently in diabetic uremic patients, patients on CAPD, and uremic patients with previous parathyroidectomies. An increase in fractures and poor fracture healing may be features of aplastic bone disease. Aplastic bone disease accounted for 49% of bone lesions found in a population of 117 chronic hemodialysis patients and 142 CAPD patients followed for a 1-year period in three Toronto hospitals; therefore, it is the most common form of renal bone disease currently seen (94). In this study, only osteomalacia was associated with increased stainable aluminum on bone biopsy.

Osteomalacia. This form of renal bone disease is characterized by underactivity of bone matrix and is marked by excess deposition of unmineralized osteoid in large osteoid seams. There may or may not be associated aluminum (Al) excess; however, in dialysis patients with osteomalacia, Al is the most common etiologic factor (97). Conditions that predispose to the development of Al bone disease include factors that enhance gastrointestinal absorption and those that favor deposition in the bone. Situations that enhance the gastrointestinal absorption of Al

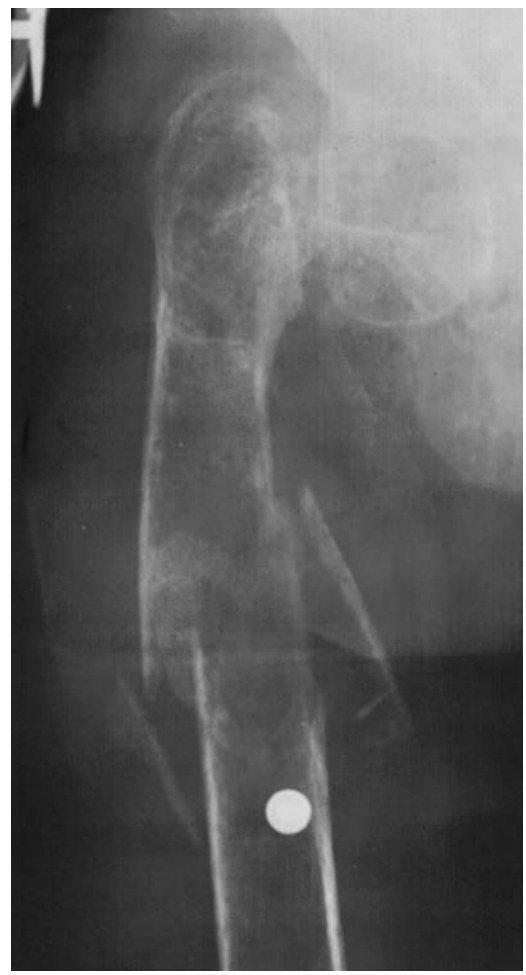

FIGURE 100-11. Anteroposterior radiograph of the proximal femur in a 33-year-old man on home hemodialysis for over a decade, with severe renal osteomalacia. There is nonunion of a previous femoral neck fracture as well as an acute comminuted fracture of the femoral shaft. The bones are severely osteopenic, demonstrating a medullary density equivalent to the soft tissues of the thigh. (Courtesy of TA Einhorn.)

include ingestion of phosphate binders on an empty, acid-filled stomach; children, young adults (95), and diabetic patients (96) with renal disease; and citrate (97), lactate (98), and possibly other organic anions. Cases of Al intoxication occurring in predialytic uremic patients as a result of aluminum hydroxide therapy, especially if associated with concomitant ingestion of citrate-containing compounds such as Shohl's solution or effervescent analgesic tablets, have been described in children and adults (99,100). Low-turnover bone such as that following a parathyroidectomy (101) or after chronic treatment with intravenous calcitriol (102) can also permit Al deposition in the bone with its detrimental effects. In cases of aluminum bone disease, deposition of stainable aluminum over more than 25% to 30% of trabecular bone surface is seen. Bone pain, proximal myopathy, pathological fractures or pseudofractures of the bones of the axial skeleton (ribs, vertebrae, pelvis, hips), and long bone fractures (Fig. 100-11) are common features.

Although bone disease is a dominant feature of chronic aluminum excess, neurologic symptoms are seen most commonly with acute exposure to aluminum, and may occur as an epidemic within a dialysis unit where the water supply has become contaminated with aluminum. Alfrey and associates in Denver in 1972 first described the syndrome of dialysis encephalopathy related to Al intoxication (103). Scanning dysarthria, myoclonus, progressive dementia, mutism, and seizures are typical features. Diagnosis is supported by an electroencephalogram (EEG) showing generalized slowing, with multifocal bursts of delta activity and spikes most prominent in the frontal lobes (Fig. 100-12). Electroencephalogram abnormalities may precede clinical signs by 6 to 8 months. The source of aluminum may be oral aluminum hydroxide used as a phosphate binder (before or after start of hemodialytic therapy), or aluminum from surface water treated with alum (to produce flocculation and aid in production of potable water for communities), or passing through aluminum containing soils.

Calcium and not aluminum, salts should be used as phosphate binders in dialysis patients *unless:* (a) The serum phosphate level is very high with a calcium phosphate product over 70 and risk of precipitating or worsening metastatic calcification if the serum calcium level is further raised by absorption of oral calcium. (b) Calcium salts have produced hypercalcemia. As many as one-third of hemodialysis patients (particularly those with aplastic bone disease) manifest hypercalcemia despite absorption of only 20% to 30% of ingested calcium carbonate. Before switching from calcium to an aluminum-based phosphate binder, however, dialysate calcium should be decreased to 1.5 or 2.0 mEq/L. Alternatively, the dose of 1,25-dihydroxycholecalciferol is reduced or withheld. In some patients, use of calcium acetate (2.3 g/day) instead of calcium carbonate may control hypercalcemia, as the acetate salt while binding twice as much phosphate as calcium carbonate is less well absorbed from the gut. A noncalcium containing binder such as sevelamer (Renagel) or Lanthanum carbonate (Fosrenol), (the latter currently only available in Europe) has become a good alternative to calcium based binders in patients who become hypercalcemic during therapy of renal bone disease. (c) The serum phosphate remains high despite use of calcium in adequate doses taken with meals (3 to 12 grams per day calcium carbonate), such that the largest dose of calcium is taken with the largest meal. Treatment of metabolic bone disease with or without secondary hyperparathyroidism has been benefited by introduction of cinacalcet (Sensipar), approved in 2004, as a means of increasing sensitivity of the calcium-sensing receptor to activation by extracellular calcium (94). Early reports indicate that weeks to months of oral administration of cinacalcet in patients undergoing maintenance hemodialysis will result in reduction of circulating levels of intact PTH without major change in laboratory parameters. The hope that treatment with cinacalcet will reduce need for subtotal parathyroidectomy may be realized in the near future.

Aluminum intoxication in dialysis patients should be suspected in the setting of compatible clinical features (encephalopathy, fracturing osteomalacia, microcytic anemia), when serum aluminum levels are persistently over 50 to 100 μg/L, or a desferrioxamine (DFO) infusion test produces increments in serum aluminum levels of greater than 300 μg/L. If diagnostic tests are inconclusive or contradictory, bone biopsy following ingestion of aurine tetrahydrochloride

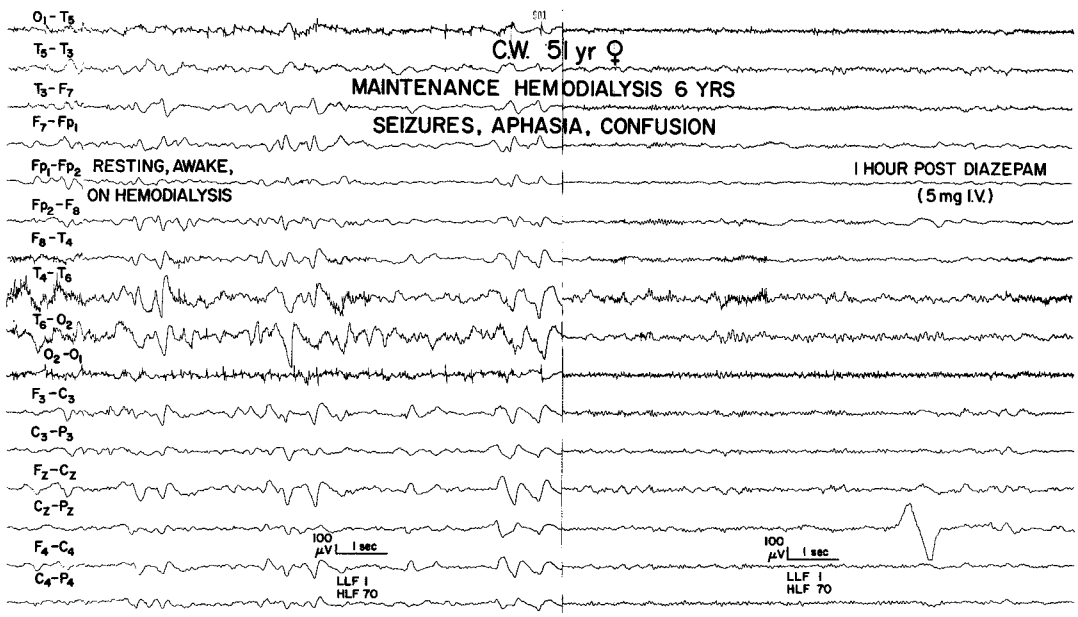

FIGURE 100-12. Electroencephalogram in dialysis dementia. Tracing on left obtained from dying 51-year-old woman after 6 years of dialysis with progressive neurologic signs including dysarthria, seizures, and aphasia. Improvement by diazepam, shown on right, is characteristic, although not pathognomonic of dialysis dementia.

demonstrates Al deposition on more than 25% to 30% of trabecular bone surfaces.

In mild Al intoxication, cessation of Al intake may suffice as therapy; in more severe cases, chelation therapy with intravenous DFO is used to mobilize Al from tissues and form stable diffusible complexes, which are dialyzable. Desferrioxamine is given in a dosage of 5 mg/kg once weekly (not exceeding 1 gm DFO per week) during the last hour of dialysis for 3 months. Serum Al increases and plateaus 12 to 24 hours after each dose of DFO. If levels rise above 350 to 400 μg/L, acute Al encephalopathy may be precipitated; therefore, the dose of DFO must be adjusted to keep serum Al below this level. Other side effects of DFO therapy include hypotension at infusion rates greater than 40 mg/kg per hour, anaphylaxis, skin rash, diarrhea, and visual and auditory abnormalities. Hyperparathyroidism may be unmasked and serum calcium levels fall as bone turnover and mineralization increases with therapy. Infections with certain species of fungi and bacteria (*Mucor* sp., *Yersinia* sp.) have been reported to be more common in patients treated with DFO; however, this risk has not been widely substantiated (104,105). Al removal at each dialysis session is between 15 and 20 mg per treatment, and clinical improvement is seen after several weeks.

Mixed Uremic Osteodystrophy. Here osteitis fibrosa is associated with evidence of defective bone mineralization.

Metastatic Calcification and Calciphylaxis

Soft tissue and vascular deposition of calcium phosphate aggregates may occur in dialysis patients with elevated calcium/phosphate products. Deposits are seen primarily in skin, peri-articular subcutaneous tissues and palpebral conjunctivae, but may also occur within parenchymal tissues (e.g., lung), and of course within blood vessel walls, although other factors may be operative here. K/DOQI recommendations are that the calcium/phosphate product be kept <55 in dialysis patients, with emphasis on phosphorus control between 2.5 to 5.5 mg/dL, and calcium levels towards the lower limit of normal, and definitely not above 10.2 mg/dL (5). Hence, the shift toward utilizing noncalcium containing phosphate binders, such as sevelamer, magnesium carbonate and the rare earth lanthanum. Short courses (3 to 4 weeks) of treatment with aluminum hydroxide remain quite efficacious in reducing high phosphorus levels. Metastatic calcific deposits must be differentiated from calcified tophaceous gout, tumoral calcinosis, myositis ossificans, and calcified tumors.

Calciphylaxis or calcific uremic arteriolopathy is characterized by ischemic necrosis of the soft tissues of the extremities associated with extensive vascular calcification in ESRD patients (106). Penetrating ulcers with eschar formation and secondary infection occur, death from sepsis is common, and pathogenesis and treatment remain uncertain. Aggressive control of calcium/phosphate product with urgent parathyroidectomy if necessary, substitution of heparin for coumadin (which has been associated with the condition) and meticulous wound care with a trial of hyperbaric oxygen therapy in selected patients are the mainstays of therapy.

Amyloidosis

Amyloidosis was first recognized as the cause of the ubiquitous carpal tunnel syndrome of chronic hemodialysis patients in Tassin, France in 1980 when amyloid tissue was noted in the synovial membranes of uremic patients undergoing surgery for carpal tunnel release (107). Polymerized β-2-microglobulin was then documented to be the constituent in deposits of amyloid found in the carpal tunnel and other musculoskeletal sites of long-term hemodialysis patients (108). β-2-Microglobulin amyloidosis is noted in up to 80% of hemodialysis patients on treatment for more than 15 years, and has been described in CAPD patients and patients with chronic renal failure before initiation of dialysis (109,110). The pathogenesis of amyloid formation is incompletely understood but may result from abnormal processing of a precursor protein under the influence of local tissue factors such as protease inhibitors or glycosaminoglycans, or from an amyloid enhancing factor (111). Levels of serum β-2-microglobulin in hemodialysis patients range between 30 and 50 mg/L, 15 to 20 times normal nonuremic levels, but levels in patients with and without amyloidosis do not differ. Part of the increased serum levels of β-2-microglobulin in dialysis patients is owing to accumulation in plasma caused by loss of normal glomerular filtration. Cuprophane membranes can also increase production of β-2-microglobulin. Although synthetic PAN membranes adsorb and filter β-2-microglobulin by diffusion and convection, removing up to 300 mg of the substance in a 4-hour hemodialysis session, they have not been demonstrated to reduce the risk or severity of amyloidosis in dialysis patients. Deposition of amyloid occurs predominantly in articular and periarticular areas and produces a number of well-described syndromes. The role of advanced glycosylated endproducts (AGEs) in the pathogenesis of dialysis related amyloidosis and associated syndromes has been reviewed by Kalousova et al (112). It is probable that AGE blockers such as aminoguanidine that have proven effective in animal models of diabetes and initial clinical trials may find near term application in amyloid-related complications of dialysis (113).

Carpal Tunnel Syndrome. This is the most common manifestation of dialysis amyloidosis. A history of paraesthesia and pain on the palmar surfaces of the thumb, index, middle finger, and radial aspect of the fourth finger is elicited. Pain often is worse at night and during hemodialysis sessions. Later, weakened opposition and abduction of the thumb with atrophy of the thenar eminence is seen. Physical examination, including testing for Tinel's sign (paresthesia or pain in the fingers reproduced by percussion over the volar surface of the wrist) and Phalen's sign (paresthesia or pain in the fingers reproduced by full flexion of the wrist for over 1 min) often is negative. Diagnosis is best confirmed by use of motor and sensory nerve conduction studies. Prolonged remission of symptoms may be obtained by wrist splints, which prevent flexion and extension, particularly during sleeping hours. Relief also can be obtained with the injection of 0.75 mL of 1% Xylocaine mixed with 0.75 mL triamcinolone (40 mg/mL) into the carpal tunnel. Ultimately, surgery is needed to transect the transverse carpal ligament, especially if there is evidence of motor or sensory loss; however, the condition often recurs after surgery.

Chronic Synovitis. Chronic synovitis of the shoulders, knees, wrists, and hips—with or without associated effusion, crepitation, deformity, and decreased range of motion—are seen with increasing frequency in long-term dialysis patients; it involves almost 100% of those at risk by the twentieth year of treatment. Amyloid deposits are found in the fluid of affected joints, and in surrounding tissues. Shoulder ultrasonography is useful and may show thick rotator cuffs and echogenic pads of amyloid tissue between the muscle groups. Chronic tenosynovitis of the flexor tendons of the fingers also is seen and may produce flexion contractures sometimes associated with subcutaneous deposits of amyloid in the tendon sheaths of the palms. Tenosynovitis of the Achilles and quadriceps tendons may result in their spontaneous rupture.

Destructive Spondyloarthropathy. This variant of dialysis amyloidosis predilects for the intervertebral discs of the upper cervical area, producing paravertebral erosions and cystic radiolucencies within and around the bones (114). These radiolucencies consist of abundant amyloid deposits invading

subchondral bone. A characteristic radiologic triad is described in destructive cervical spondyloarthropathy owing to amyloid: severe intervertebral disc space narrowing, erosion and geodes of the adjacent vertebral plates, and absence of osteophytosis. Localized neck pain may be the only symptom in early or mild cases, whereas vertebral collapse or subluxation may result in severe disease and can produce compressive myelopathy and even progress to paraplegia, particularly with neck manipulation (e.g., during induction of general anesthesia). Formation of periodontoid tumors of amyloid also may produce compressive myelopathy.

Pathologic Fractures of Long Bones. Juxtaarticular deposits of amyloid at sites of tendon insertions can produce large cystic lesions in the femoral heads, acetabula, humerus, and tibial plateaus that may fracture with minimal motion or injury. Total joint replacement may be required.

Systemic and visceral amyloid involvement is uncommon. Visceral amyloidosis in dialysis patients has been reported in a perivascular distribution in the gastrointestinal tract, heart, kidneys (with the formation of matrix stones) (115) and fatty tissue. Amyloidosis of the bowel wall may produce bleeding, intestinal obstruction, and deposits in the tongue odynophagia. Subcutaneous tumors in the buttocks, popliteal areas, elbows, and wrist also have been described.

There is no satisfactory treatment to prevent or arrest dialysis arthropathy. Renal transplantation is the only certain means of effecting improvement. Radiologic lesions take several years to heal following restoration of renal function by transplantation. Endoscopic resection of the coracoacromial joint may relieve shoulder pain (116). Prosthetic joint replacement of hip and knees may be necessary to preserve mobility, and spinal fusion with or without osteosynthetic grafts for cervical destructive arthropathy may prevent quadriplegia.

Muscle Cramps

Painful cramps involving the gastrocnemius, or small muscles of the feet, may occur in hemodialysis patients during dialysis or at night. Intradialytic or immediate postdialytic cramps probably reflect volume contraction and usually respond to fluid replacement with 0.9% saline, hypertonic saline, or hypertonic solutions of glucose or mannitol (117). Quinine sulfate 260 mg 2 hours before dialysis reduces the frequency of cramps from 17% to 6% of hemodialysis treatments (118). Carnitine supplementation (119) and Vitamin E 400 IU/day also are reported to be efficacious (120). Verapamil 120 mg at bedtime may help nocturnal cramps. Treatment is unsatisfactory in many patients, however, and patients learn to live with their annoying disability. Hypo- or hyperthyroidism, hypomagnesemia, hyponatremia, and hypocalcemia, as well as a variety of drugs also may produce muscle cramps; therefore, these factors should be ruled out as a cause of cramps in dialysis patients.

Machine- or Technique-Related Complications

Hemodialysis is extraordinarily safe despite the potential for mechanical failure and human error while processing blood and dialysate extracorporeally (121). Accidental death during hemodialysis is a rare event, occurring at a frequency of about 1 in 76,138 dialyses, whether performed by physicians, trained nurses and technicians, or the patient. Accidental injury to patients during hemodialysis results from overheated dialysate, contamination of dialysate with acid, sodium hypochlorite bleach (122) or formaldehyde (used to cleanse dialyzers for reuse), and chloramines used for water purification (123). An acute "new dialyzer" syndrome has been attributed to unidentified intoxicants leached from the dialyzer into the patient's blood (124). Disposable dialyzers and blood tubing are made of plastic, which may release organic intoxicants. Plasticizers such as diethylhexylphthalate, which are added to polyvinylchloride tubing to improve its low-temperature flexibility, may be leached out during hemodialysis. Plasticizers are highly toxic; exposure may cause necrotizing cutaneous vasculitis and a nonspecific, nonfatal, hepatitis. The risk of electrical shock during hemodialysis has been reduced to very little significance by attention to equipment design and periodic testing for current leakage (125). Rarely, when human intervention defeats (cancels) equipment safety monitors, fatal accidents have resulted from high or low dialysate temperatures, the exposure of blood to undiluted dialysate concentrate, high concentrations of chloramines, bleach, and formaldehyde or tap water lacking concentrate, all of which produce blood hemolysis. Air embolism should not occur if routine precautions are taken: ensuring tight tubing connections, intact tubing, proper clamping of the saline infusion port, and continuous arming of the air leak detector. In the event of this catastrophic and usually lethal complication, the venous bloodline should be clamped immediately and the blood pump stopped. Then the patient should be placed in a reversed Trendelenburg position with the left side down, and cardiopulmonary support initiated.

Neurologic Complications

In advanced uremia, a distal sensori-motor neuropathy due to axonal degeneration may develop, with symptoms compounded by associated diseases which may also produce neuropathy such as diabetes or myeloma. Electrophysiologic evidence of neuropathy is seen in up to 80% of uremic patients, though symptoms are apparent in only one half of these cases (128). Painful paraesthesiae of the feet are the initial symptoms, followed by progressive muscle weakness and atrophy, sometimes resulting in paraparesis. A syndrome of involuntary movement of the legs due to a sensation of severe discomfort (restless legs syndrome) may be prominent in some patients (129), and has also been associated with iron deficiency (130). Neuropathy reverses slowly with hemodialysis and faster with use of highly permeable synthetic membranes that remove the implicated middle molecules more efficiently. The incidence and severity of uremic polyneuropathy have decreased significantly with refinement in the technique and amount of hemodialysis. The condition is best treated by increased dialysis dose, and use of tricyclic antidepressants such as amitriptyline, or the newer gabapentin. Mega doses of Vitamin B_6 and evening primrose oil supplements may ameliorate sensory symptoms of uremic peripheral neuropathy.

Acquired Cystic Renal Disease

The kidneys, although scarred and shrunken, do not completely cease function after the start of dialysis. Epithelial cells, mostly of proximal tubular, continue to proliferate, leading to the formation of cysts. Cyst formation may start before dialysis initiation and is seen in up to 80% of patients on dialysis for over 10 years (131). Both kidneys are involved, with 4 or more cysts, and small to normal renal size (132). The stimulus for the proliferation and function of the cysts, is unknown. Grantham (133) proposed three possible causes for cyst formation in adult PKD that could be applicable to acquired cysts: (a) distal obstruction leading to high-pressure dilatation; (b) increased compliance of the tubular basement membrane with formation under normal pressure; or (c) proliferation of epithelial cells. The histologic nature of the proliferating cells points to the latter

as the most likely cause. Stimuli for the development of cystic lesions lie within the uremic milieu. This is inferred from their regression after restoration of renal function following a successful renal transplant (134). Suggested factors include endogenous substances such as growth factors responsible for renal hypertrophy; retained toxins such as polyamines, which can cause cellular proliferation; or androgens. The latter has been implicated by the observation that cystic formation appears sooner in male dialysis patients (135) and that 20 of 24 renal malignancies were found in cystic kidneys in men.

Most patients with acquired cystic kidney disease are asymptomatic, but hematuria, flank pain or shock due to retroperitoneal hematoma formation may occur. The feared complication of acquired cystic renal disease is development of renal adenocarcinoma. On morphologic examination, up to 80% of contracted cyst-bearing kidneys in dialysis patients contain adenomas. Their malignant potential is unknown. The prevalence of renal cell carcinoma in long-term dialysis patients is reported to be 1% to 2%, 40 times that in the general population (136,137). Cancer can be heralded by hematuria, flank pain, renal mass, hypercalcemia with suppressed parathyroid hormone, or sudden reversal of anemia. Death has resulted from metastatic spread in a few cases. Screening recommendations for acquired cystic renal disease are not uniform (138,139). We recommend ultrasonography for patients on dialysis for more than 5 to 10 years, with confirmatory CT scanning with contrast for positive cases.

Neoplasia in Chronic Hemodialysis

Several reports suggest an increased incidence of other malignancies as a result of persistent uremia in addition to malignant degeneration in cysts reported in long-term hemodialysis patients (vide supra) (140). Individual case reports by Oe and co-workers (141) of renal carcinoma development in hemodialyzed patients with polycystic kidneys, first suggested that cancer and dialysis are associated. *De novo* malignancy was noted in 15 patients on chronic hemodialysis (3% of the group at risk) and six kidney transplant recipients (4.9% of the risk group) leading Herr and associates to conclude that both uremia therapies impose a risk of neoplasia (142). Supporting this thesis is the finding in a nationwide survey of the Japanese dialysis population of an overall incidence of tumor death 2.3 times higher in males and 4.3 times higher in females than in the general population (143). Port and colleagues assessed the incidence of cancer in 4,161 ESRD patients on dialysis in Michigan compared to the calendar year-specific incidence rates of 4 million residents of the Detroit region (144). The tumor rate in dialysis patients was significantly increased for all *in situ* tumors combined, as well as for invasive tumors of the kidney, the corpus uteri, and the prostate. A four to five times excess risk was observed for renal and endometrial cancers. Echoing the concern of Port and associates, DeSala and co-workers observed that 14 of 324 long-term dialysis patients in a Spanish unit developed malignant tumors, four of which were cancer of the bladder (145).

Despite these reports, the consensus is that recipients of maintenance hemodialysis are not at significantly increased risk of malignancy. Support for this view is afforded by Kantor's study of 28,049 Medicare ESRD patients whose cancer rates were compared to the National Cancer Institutes cancer incidence rates for the United States. No overall increase in cancer risk was found in the hemodialysis population, although patients with chronic glomerulonephritis had a small excess of non-Hodgkin's lymphoma and biliary tract cancers (146). Bush and Gabriel also assessed the course of 834 uremic patients, noting that only five who contracted malignant disease, an incidence of 0.6% that was no greater than the risk in the general age-matched population (147).

Gastrointestinal Complications

Liver Disease

Hepatic disease was recognized as a complication of long-term hemodialysis shortly after its inception as uremia therapy (148). Initial regimens for maintenance hemodialysis required repeated blood transfusions to sustain a hematocrit of 25% to 35%, fostering continuing hemolysis; hepatic hemosiderosis; and the transmission of hepatitis non-A, non-B, and hepatitis B (149). Alarm over the risk to staff of the seemingly unavoidable risk of hepatitis B (as many as 20% of patients in some areas were hepatitis B antibody-positive) cast a pall over the growth of hemodialysis units during the early 1970s. Subsequently, elimination of pro forma transfusions and screening (i.e., exclusion of HBsAG-positive blood) reduced the prevalence of hepatitis in most dialysis units significantly, and after introduction of an effective hepatitis B vaccine (150), and institution of universal precautions, the incidence of new cases of hepatitis B within hemodialysis units has nearly disappeared. The prevalence of seropositivity for HBsAg in hemodialysis patients in the United States varied between 2.7% to 7.8% in the 1970s (151,152), but fell to 1.2% in 1992 (153). Hepatitis C (formerly non-A, non-B) remains the most common cause of transfusion-acquired hepatitis, and also is transmitted through contact within dialysis units because time on dialysis is an independent risk factor for development of infection with the virus. The prevalence of antibodies to hepatitis C virus on ELISA II assay in U.S. hemodialysis units varies between 25% and 36% (154,155). Problems with current antibody testing include the facts that 10% of HCV-infected dialysis patients never develop antibodies; that anti-HCV antibodies may first be detectable as much as 6 to 12 months postinfection; and that a positive anti-HCV test may indicate recent and/or remote infection, but does not necessarily mean infectivity, because only 17% to 27% of anti-HCV ELISA-positive blood transmits HCV (156,157). Third-generation ELISA tests are more sensitive, but currently are not as widely used. Detection of HCV RNA using molecular hybridization studies, therefore, will prove to be the most reliable means of testing for the level of virus in serum and liver (103); however, its cost and technical difficulty will prevent widespread use. In the meantime, hand washing and changing gloves between patients are still some of the most important ways to prevent the spread of hepatitis within dialysis units. Dialysis patients with chronic hepatitis B infection should be isolated and dialyzed on dedicated dialysis machines. All dialysis patients and staff should receive hepatitis B vaccination. A hepatitis C vaccine is not available presently. It is not currently recommended to isolate patients with hepatitis C antibody positivity.

Parfrey and co-workers studied the impact of hepatitis B disease prospectively in hemodialysis and kidney transplant patients in Canada (158). Although immunosuppressed renal transplant recipients developed chronic active hepatitis or cirrhosis, resulting in death from liver disease at a rate of 5% per patient-year, a coincident group of 10 HBsAg-positive hemodialysis patients fared better. Persistent liver dysfunction occurred in only one dialysis patient; none died of liver disease. Confirmation of the relative benignity of HBsAG positivity in hemodialysis patients was reported by Marchesini and associates, who noted in a prospective study of 38 positive carriers followed for 6 to 66 months that none died of liver disease (159). Death associated with persistent hepatitis B antigenemia is rare; after a mean retrospective follow-up of 52 patients for 5 months (some for 10 years), Harnett and co-workers

noted that only one of 49 hemodialysis patients died of liver disease (160).

The wide variation in reported prevalence of liver disease in dialysis patients reflects differences in time of reporting and population studied; it is highest where transfusions are routine. Thus, in the review by Toussaint and colleagues, 33% of hemodialysis patients were found to have liver dysfunction (161). Granulomatous liver diseases, including schistosomiasis and tuberculosis, and the late effects of viral infections, such as cytomegalovirus, should be considered, even in HbsAg-positive patients. Acquired immunodeficiency diseases syndrome (AIDS) in its agonal stages is frequently associated with multiple organ failure, including a hepatorenal syndrome.

Dialysis Ascites

Persistent ascitic fluid accumulation in a patient undergoing maintenance hemodialysis may be the consequence of disparate pathogenetic mechanisms, and the diagnosis of dialysis-associated ascites therefore is one of exclusion. Tuberculous peritonitis, whether miliary or a direct extension of intraabdominal tuberculosis, has not been infrequent in our urban dialysis patients (162). Fungal peritonitis like bacterial and tuberculous peritonitis causes an exudative (protein content greater than 3 g/dL) ascitic fluid. In approximately half of our cases of proven tuberculosis, the diagnosis was made by peritoneal biopsy when staining for acid-fast organisms in ascitic fluid was negative. Advanced liver disease, usually associated with hypoalbuminemia, is an important cause of abdominal swelling owing to ascitic fluid. Gluck and Nolph reviewed 138 patients with ascites and ESRD reported through 1986 and cited contributing mechanisms as: fluid overload, hypoproteinemia, lymphatic drainage disturbances, and peritoneal membrane changes (163). A specific underlying cause was discovered in 15% of cases. When evaluating the etiology of ascites in a dialysis patient, the diagnosis of liver failure, like that of heart failure with anasarca, is not difficult. Having excluded specific causes of ascites, there remains a group of hemodialysis patients suffering ascites without explanation, leading to the term dialysis ascites (164). Dialysis ascites is a serious sign of failure of the hemodialysis regimen, often accompanied by muscle wasting, anorexia, and worsening neuropathy, with 45% of the patients dead within 15 months of diagnosis. Treatment involves restoration of positive nitrogen balance where hypoalbuminemia is linked to inadequate dialysis, and a clinical trial of increased dialysis time. Improvement is induced by intraperitoneal steroid instillation (165), albumin infusion, or intensive ultrafiltration during hemodialysis only rarely (166). Reinfusion of ascitic fluid (167) and resort to peritoneal dialysis (168) with gradual reduction in ascitic fluid volume are complex solutions to the therapeutic dilemma (169).

Reproductive Dysfunction

Illness, especially uremia, mutes the sex drive. Hemodialysis patients remain azotemic, acidotic, and anemic with multiple endocrine abnormalities. Men on dialysis have low testosterone and high luteinizing hormone (LH) levels, which together with an elevated follicle stimulating hormone (FSH) level is associated with severe spermatogenic damage (170) and partial or complete impotence in 50% (171). Zinc deficiency is postulated to be one of 28 factors responsible for sexual malfunction in dialyzed men. A double-blind trial was conducted in hemodialyzed men given supplemental oral zinc using 50 mg of elemental zinc as zinc acetate (10 patients) with 10 control patients receiving placebo (172). Zinc treatment led to increased plasma zinc, serum testosterone, and sperm count, as well as an improvement in potency, libido, and frequency of intercourse. Sildenafil is highly (85%) effective and well tolerated in male hemodialysis patients with impotence (173), but is contraindicated in patients on nitrates or at risk for hypotension from cardiac or other reasons. The drug is nondialyzable and was not associated with increased risk of intra-dialytic hypotension in a series of 15 dialysis patients treated with a 50 mg dose either 2 hours before or after a dialysis session (174).

Fertility in women with renal insufficiency is reduced at serum creatinines as low as 1.5 mg/dL, and at a creatinine clearance of 10 to 15 mL/minute irregular menses develop and progress to amenorrhea and decreased libido as creatinine clearance falls below 4 mL/minute. Women on dialysis have low estrogen and high FSH and prolactin levels (175) that blunts sexual interest, causes anovulatory menstrual cycles and nearly always precludes successful pregnancy. A few successful pregnancies have been reported in women sustained by maintenance hemodialysis who were subjected to intensified dialysis (176), or who had some residual renal function. Almost 80% of the pregnancies that do occur end in spontaneous abortion (177).

In general, sexuality can be maximized by effective hemodialysis, proper nutrition, and awareness of the side effects (in men) of antihypertensive drugs that interfere with potency. Evaluation of the sexually incompetent man on dialysis requires assessment of historical performance (past ability to attain and maintain an erection), examination of a semen specimen, and, if all results are normal, sex hormone assay. Demonstration of nocturnal tumescence in impotent men desirous of intercourse indicates the patient's potential for development of an erection, while suggesting the possibility that sensitive counseling may improve sexual performance. Bromocriptine or its analog cabergoline (which produces less nausea) has been used with good effect in hyperprolactinemic men on dialysis. Even when testosterone levels are low, the administration of testosterone to uremic men usually fails to restore libido or potency, despite normalized serum testosterone and reduced release of LH and FSH. Progesterone therapy may be warranted in some female dialysis patients who have irregular menstrual bleeding with anovulatory cycles and estrogen excess (178). Therapy with erythropoietin can reverse hyperprolactinemia and produce normal menses in some women on hemodialysis (179).

Psychosocial Problems

Maladjustment to the rigorous regimen of maintenance hemodialysis is a transient although common experience for nearly all dialysis patients. Depression, a sense of futility and hopelessness, and an unfavorably altered body image are common expressions of anxiety and fear over an uncertain future. Although gentle reassurance and repeated explanation may minimize patient stress, acceptance of the reality of life dependent on a machine is a persistent enervating and unbearable burden for some patients. According to Stewart (180), approximately one-fourth of dialysis patients are depressed at any time because of concern over compliance with diet, medication, and sexual dysfunction. From 1% to 6% of dialysis patients overtly commit suicide (181,182). The most recent report of the USRDS lists withdrawal from dialysis as the cause of death in 21.6% of patients who died between 2000 and 2003, the third most common cause of death in hemodialysis patients (6).

New and Kjellstrand reviewed a series of 1,766 ESRD patients in Minnesota and noted that 155 patients (9%) discontinued dialysis accounting for 22% of all deaths (183). Dialysis was stopped more often in older than younger nondiabetic patients, and more often in young diabetic patients than in young nondiabetic patients. Half of the patients were mentally competent when the decision to terminate dialysis was made.

A derivative study of the same patients examined the course of 26 patients who withdrew from dialysis (4% of all deaths), although there was no evident medical or technical reason to do so (184). It was concluded that the competent patients who withdrew apparently die because they cannot stand the stress of the regimen. Approximately 10% of all deaths in a large Canadian dialysis program followed the elective decision to stop dialysis. Once dialysis was discontinued, patients lived a mean of 9.6 days and died for the most part without pain or prolonged suffering (185). In Brooklyn, very few of our predominantly Black patients elect to stop dialysis, a reflection of the lower rate in Blacks than Whites observed nationally. To eliminate these *avoidable deaths,* the authors advocate better support, particularly from physicians, as well as careful patient selection to exclude those who elect not to have life prolongation.

Family support, optimal hemodialysis, and empathetic counseling by members of the dialysis team are often helpful. Participation in patient group activities, such as programs sponsored by the American Association of Kidney Patients adds purpose and camaraderie to the dialysis patient's life. Use of selective serotonin reuptake inhibitors (SSRIs) may produce beneficial effects in up to 75% of all patients (186). They require 4 to 6 weeks for full effect and most do not require dose reduction for renal failure as do tricyclic anti-depressants. Sertraline and fluoexitine are commonly used. Unlike other SSRIs, trazodone produces drowsiness and weight gain; and may be of more benefit in patients with depression, insomnia, and anorexia.

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SECTION XIV: NUTRITION, DRUGS, AND THE KIDNEY

CHAPTER 101 ■ DIETARY FACTORS IN THE TREATMENT OF CHRONIC KIDNEY DISEASE

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NATURAL HISTORY OF CHRONIC KIDNEY DISEASE

In many normal adults, there is a decline in glomerular filtration rate (GFR) with age, but this abnormality differs from the progressive loss of renal function that occurs after kidney damage and aging-induced changes alone rarely require therapy for end-stage renal disease (ESRD). It is predicted that about two-thirds of normal adults over age 40 years, will experience a decrease in GFR, even when they have had no obvious kidney disease (1); in the remaining one-third of adults, GFR remains stable (2). The proportions of those with a declining GFR can vary, however, as Fliser et al. (3) noted, two-thirds of elderly subjects have GFR values that are within the range of younger adults. The cause of the decline in GFR that occurs with normal aging is obscure, but it may be linked to a decrease in dietary protein that is ingested by most elderly subjects (4) because variation in dietary protein accounts, in part, for the day-to-day variability in GFR (5). For this reason, the diet must be taken into account when evaluating the measured GFR or changes in GFR with time (i.e., those with progression).

In contrast to normal aging, patients who experience kidney damage from different diseases and develop manifestations of chronic renal failure insufficiency (CKD) continue to lose function (i.e., experience "progression"), even when the disease that initially damaged the kidneys is no longer active (e.g., obstructive uropathy [6] or cortical necrosis following obstetric accidents [7]). It should be noted, however, that there are patients (including those with diabetic nephropathy) who do not exhibit progressive loss of GFR over periods of observation for as long as 3 years, despite kidney damage (8,9). The investigators reporting this lack of progression concentrated on controlling blood sugar and blood pressure, respectively, and it seems likely that treating these abnormalities accounts, at least in part, for the lack of progression. Lack of progression was also found in the largest published series of changes in GFR (the Modification of Diet in Renal Disease [MDRD] Study [10]) as 15% of patients had no progression over the average of 2.2 years of study, even when they began the study with advanced renal insufficiency (GFR 13 to 23 mL/min/1.73 m^2). The reason(s) for the stability of renal function in these patients was not identified, while in those who had progressive loss of GFR, five factors were associated with faster rates of progression: (a) more proteinuria; (b) higher mean arterial pressure; (c) lower serum HDL cholesterol; (d) lower serum transferrin; and (e) polycystic kidney disease. Note that this list does not include dietary protein and this inconsistency is puzzling, based on review of published data.

Regarding the measurement of progression, there are two issues: (1) evaluating the accuracy of the method used to measure the remaining kidney function and (2) how accurately the method quantifies changes in renal function. These are distinct tasks, because the former assesses GFR at one point in time, while the latter examines how rapidly renal function is being lost.

ASSESSMENT OF GLOMERULAR FILTRATION RATE

The standard measure of GFR, the renal clearance of inulin, requires precise regulation of an intravenous infusion of inulin to achieve a steady-state plasma concentration, plus at least three accurately timed urine collections with complete emptying of the bladder (11). To minimize the influence of changes in posture, the patient should remain supine or be seated quietly, standing only to void (12,13). Patients are usually given a water load to improve the accuracy of collecting all the urine made and inulin is measured by chemical methods (14). Despite these precautions, the coefficient of variation of inulin clearance is at least 10% in patients with nearly "normal" GFR values and is higher in patients with CKD (11). To reduce the analytic error in measuring inulin, radiolabeled compounds cleared predominantly by glomerular filtration have been used; gamma-emitters usually are infused to avoid the error caused by variable quenching when beta-emitting compounds (e.g., ^{14}C-inulin) are used. In individual subjects, there is a close concordance between the clearances of inulin and ^{125}I-iothalamate (or other labeled compounds) over a wide range of GFR (12,15). With inulin, ^{125}I-iothalamate or other radiolabeled markers considerable supervision is needed to ensure complete emptying of the bladder and accurate timing of blood and urine collections (15,16).

To avoid urine collections, methods for calculating the plasma disappearance of an injected radiolabeled compound have been devised. The assumption underlying the use of a plasma clearance is that it is insignificantly higher than the renal clearance; before using any new plasma clearance marker, this assumption must be validated. To calculate GFR from a measured plasma clearance, at least three additional assumptions are made: (a) the extrarenal clearance of the radiolabeled compound is negligible, (b) the plasma clearance is constant during the period of sampling, and (c) the duration of blood sampling must be sufficiently prolonged to include the period that represents the terminal, steady-state period of monoexponential loss of plasma radioactivity with time. The most important factor is that a prolonged infusion is required for patients with advanced renal insufficiency before a stable plasma level is present. If these conditions are not satisfied, the plasma clearance will not represent GFR. In patients with severe CKD, there may be a small extrarenal clearance that is not detectable during short collection periods and, even more importantly, the time required to reach the terminal monoexponential phase of removal can be many hours (16). The plasma clearance technique is generally not as accurate as the renal clearance, but the error of a ^{51}Cr-ethylenediaminetetraacetic acid (EDTA) plasma

clearance is reportedly 4% to 5% in subjects with only mild impairment of renal function and approximately 12% for subjects with more advanced CKD (similar values have been reported after assessing the plasma clearance of nonradioactive iodinated compounds [17]). This degree of variation is similar to that obtained when the renal clearance of inulin is measured. Nonradioactive markers (e.g., iothalamate, iohexol, iodixanol) have been shown to be reasonable alternatives for measuring GFR, provided the dose is low enough to avoid the risk of nephrotoxicity (17–21).

Some investigators have advocated approximating the entire plasma disappearance curve by obtaining a single plasma sample only a few hours after an injection of a radiolabeled compound. This technique is less accurate compared to the multiple sampling techniques, especially when there is renal insufficiency (16,17,22–25).

Serum Creatinine

A single value of serum creatinine (S_{Cr}) is a crude and unreliable estimate of creatinine clearance (or GFR), because it is a function of both creatinine production and its clearance. Consequently, a muscular individual will have a higher serum creatinine than a patient who has lost muscle mass, even though they have the same creatinine clearance (C_{Cr}). This difference is due to the fact that creatinine production is directly proportional to lean body mass (LBM) (and, hence, muscle mass) (26). Moreover, rates of creatinine production are not constant, but depend on the amount of meat in the diet, while creatinine secretion by the kidney contributes a variable amount to total creatinine clearance (27–30). There have been attempts to estimate the GFR from serum creatinine (Table 101-1) and the results seem to be satisfactory as long as the patient is in the steady-state and the S_{Cr} exceeds 2 mg/dL (31). Overall, however, the practice of estimating GFR from S_{Cr} introduces an unacceptable degree of inaccuracy and should not be encouraged.

Creatinine Clearance

The 24-hour endogenous C_{Cr} usually exceeds inulin clearance, because of tubular secretion of creatinine, but with advanced CKD, values of C_{Cr} and GFR become numerically closer, even though the percentage difference between C_{Cr} and GFR will increase (11,27,32,33). There are other reasons for avoiding C_{Cr} as an estimate of GFR. These include the inconvenience of obtaining a 24-hour urine collection plus the uncertainty that the urine collection is complete. In addition, 24-hour C_{Cr} have a high coefficient of variation. In one study of 119 healthy ambulatory subjects, the coefficient of variation was 26% and other reports indicate it varies from 6% to 22% (27,30,34). A major cause of this variability is the day-to-day differences in creatinine excretion (28,34–36); among hospitalized patients, creatinine excretion varies by at least 10% (26,37,38). It is not surprising, therefore, that the slope of sequential values of C_{Cr} is poorly correlated with the slope of GFR (39,40). In fact, the relationship is so poor that some investigators recommend abandoning the 24-hour C_{Cr} as an index of CKD severity (41,42). The same caveats apply to calculating the average of the 24-hour creatinine and urea clearances to estimate GFR, even though this average value corresponds closely to inulin clearance when urine collections are accurate (32).

Another strategy is to measure C_{Cr} after giving cimetidine to inhibit creatinine secretion and increase the accuracy of the estimate of GFR. Generally, 1.2 g of cimetidine usually achieves complete suppression of creatinine secretion; a dose of only 0.4 g is often insufficient (43). Reportedly, with water loading and carefully timed urine collections, the cimetidine-adjusted C_{Cr} procedure yields clearance values that have a coefficient of variation of approximately 8% in patients who have GFR values ranging from 12 to 150 mL/minute (44). The technique

TABLE 101-1

EQUATIONS USED TO ESTIMATE CREATININE CLEARANCE OR GLOMERULAR FILTRATION RATE[a]

Name of equation (ref. no.)	Gender	Formula
Cockcroft–Gault C_{Cr}(37)	Men	$C_{Cr} = [(140 - \text{age}) \times \text{weight (kg)}]/S_{Cr} \times 72$
	Women	$C_{Cr} = ([(140 - \text{age}) \times \text{weight (kg)}]/S_{Cr} \times 72) \times 0.85$
Cockcroft–Gault GFR		GFR = 0.84 × C_{Cr} estimated from C_{Cr}
MDRD 1 (46)		$\text{GFR} = 170 \times [S_{Cr}]^{-0.999} \times [\text{age}]^{-0.176} \times [0.762 \text{ if patient is female}] \times [1.18 \text{ if patient is black}] \times [\text{BUN}]^{-0.170} \text{x } [\text{Alb}]^{0.318}$
MDRD 2 (47)		$\text{GFR} = 186 \times [S_{Cr}]^{-1.154} \times [\text{age}]^{-0.203} \times [0.742 \text{ if patient is female}] \times [1.212 \text{ if patient is black}]$
Jellife 1 (342)	Men	$(98 - [0.8 \times (\text{age} - 20)])/S_{Cr}$
	Women	$(98 - [0.8 \times (\text{age} - 20)])/S_{Cr} \text{x } 0.90$
Jellife 2 (343)	Men	$(100/S_{Cr}) - 12$
	Women	$(80/S_{Cr}) - 7$
Mawer (344)	Men	$\text{Weight} \times [29.3 - (0.203 \times \text{age})] \times [1 - (0.03 \times S_{Cr})] \times (14.4 \times S_{Cr}) \times (70/\text{weight})$
	Women	$\text{Weight} \times [25.3 - (0.175 \times \text{age})] \times [1 - (0.03 \times S_{Cr})] \times (14.4 \times S_{Cr}) \times (70/\text{weight})$
Bjornsson (345)	Men	$[27 - (0.173 \times \text{age})] \times \text{weight}/S_{Cr}$
	Women	$[25 - (0.175 \times \text{age})] \times \text{weight} \times 0.07/S_{Cr}$
Gates (346)	Men	$(89.4 \times S_{Cr}^{-1.2}) + (55 - \text{age}) \times (0.447 \times S_{Cr}^{-1.1})$
	Women	$(89.4 \times S_{Cr}^{-1.2}) + (55 - \text{age}) \times (0.447 \times S_{Cr}^{-1.1})$
Salazar–Corcoran (347)	Men	$[137 - \text{age}] \times [(0.285 \times \text{weight}) + (12.1 \times \text{height}^2)]/(51 \times S_{Cr})$
	Women	$[146 - \text{age}] \times [(0.287 \times \text{weight}) + (9.74 \times \text{height}^2)]/(60 \times S_{Cr})$

[a]These estimates are not corrected for body surface area. C_{Cr}, creatinine clearance; *GFR*, glomerular filtration rate; S_{Cr}, serum creatinine.

eliminates the use of radioactive material, but still requires trained personnel.

Cockcroft–Gault Formula

Frustration with the variability of the 24-hour C_{Cr} has led to the widespread practice of estimating C_{Cr} from serum creatinine and an estimated daily rate of creatinine excretion based on the age, weight, and sex of the patient (37,45). The widely used Cockcroft–Gault formula (Table 101-1), however, is derived from data of "normal, hospitalized" patients, and, hence, may not be appropriate for patients with CKD (37,45). For example, there is a decrease in creatinine excretion in CKD patients, which can be attributed to creatinine degradation. The amount of creatinine degraded can be estimated using an average extrarenal C_{Cr} of 0.04 L/kg/day and this value can be integrated with other factors affecting creatinine production to estimate the C_{Cr} of CKD patients. In men, the daily rate of creatinine production in milligrams per kilograms per day can be calculated as 28 − 0.2 A for men, and for women it is 23.8 − 0.17 A, where A is the age of the subject in years (37,45). Combining these formulas with the average extrarenal creatinine clearance yields a predicted renal creatinine clearance (C_{Cr}) in liters per kilogram per day: $C_{Cr} = (28 - 0.2\ A)\ S_{Cr}^{-1} - 0.04$ L/kg/day in men and $C_{Cr} = (23.8 - 0.17\ A)\ S_{Cr}^{-1} - 0.04$ L/kg/day in women, where S_{Cr}^{-1} is the reciprocal of the serum creatinine concentration (in milligrams per liter). Using these formulas, the measured 24-hour C_{Cr} of 95% of uremic adults with stable serum creatinine values above 8 mg/dL were estimated to within 1.5 mL/minute (45). Such calculated estimates incorporate several assumptions, including the deficiency of the Cockcroft–Gault equation, which is based on results from a homogenous cohort of predominantly Caucasian males. For this reason, the Cockcroft–Gault formula must be validated before using it in other populations. In addition, all C_{Cr} formulas systematically overestimate the GFR due to tubular secretion of creatinine.

In order to account for these defects, multiple correction factors have been proposed. Levey et al. (46) analyzed the performance of the various equations in predicting the actual GFR, which had been measured by iothalamate clearance. With a stepwise regression technique, they found a series of correction factors that improved the predictive accuracy of the various GFR formulas; their corrections for the Cockcroft–Gault equation were GFR = 0.84 × (Cockcroft–Gault formula for estimated C_{Cr}).

MDRD and Modified MDRD Formulas

The Modification of Diet in Renal Disease study was a multicenter, controlled trial that evaluated the effectiveness of dietary protein restriction plus strict blood pressure control on the progression of renal insuffiency as measured by changes in iothalamate clearance. Baseline values of GFR, serum creatinine, C_{Cr}, and several variables that affect the relation between them, such as albumin and protein intake were evaluated. From these baseline data, an equation to predict the measured GFR was developed (46). It was found to be superior to other equations, at least in this group of CKD patients.

The original MDRD equation is based on demographic assessments plus serum and urine variables (yielding a maximal R^2 of 91.2%):

$$\text{GFR} = 198 \times (S_{Cr})^{-0858} \times (\text{age})^{-0.167} \times (0.822 \text{ for female}) \times (1.1178 \text{ for black}) \times (\text{SUN})^{-0.293} \times (\text{UUN})^{0.249}$$

In this equation, SUN is serum urea nitrogen and UUN is urine urea nitrogen. A revised MDRD equation is based on demographic assessments and serum variables only (yielding a maximal R^2 of 90.3%):

$$\text{GFR} = 170 \times (S_{Cr})^{-0.999} \times (\text{age})^{-0.176} \times (0.762 \text{ for female}) \times (1.180 \text{ for black}) \times (\text{SUN})^{-0.170} \times (\text{Alb})^{0.318}$$

Finally, there is the modified MDRD equation, which was derived to avoid having to include serum albumin and urea nitrogen (46,47).

$$\text{GFR} = 214 \times (S_{Cr})^{-0.113} \times (\text{age})^{-0.174} \times (096 \text{ for female}) \times (0.92 \text{ for black}).$$

The modified MDRD equation has almost the same predictive ability as the other equations and its use is fully supported by the National Kidney Disease Education Program (NKDEP) of the NIH and the National Kidney Foundation and American Society of Nephrology. It is especially useful for estimating the GFR when staging the severity of kidney disease; an estimated GFR <60 mL/min/1.73 m^2 identifies patients at high risk for progressive CKD.

There are several caveats associated with use of the MDRD equation:

1. The equation was calculated using only two ethnic groups, Caucasian and African American. The performance of this equation in predicting GFR in other races has not been validated.
2. The MDRD equation was derived from a cohort of patients with CKD and a mean GFR of 39.8 mL/min/1.73 m^2. Multiple investigators (29,48–50) have identified serious deficiencies in the predictive value of these equations in persons with normal kidney function.

In a study of adults from the Netherlands, Vervoort et al. (48) compared the inulin clearance of 46 controls and 46 uncomplicated diabetic patients with the predicted GFRs based on the MDRD and Cockcroft–Gault equations. They found that the difference between predicted values and measured GFR were significant (+9.0 mL/min/1.73 m^2 for Cockcroft–Gault and +10.7 mL/min/1.73 m^2 for MDRD). Lin et al. (49) compared the predictive ability of the modified MDRD equation against GFR, measured as DTPA clearance. They concluded that the MDRD equation is decidedly less accurate in subjects without kidney disease, because it appears to underestimate the GFR systematically. In contrast, the Cockcroft–Gault formula overestimates GFR. They also reported that the iothalamate and DTPA techniques do not seem to be highly correlated, but other reports show an acceptable correlation (15). Other equations that have been used to estimate the GFR in patients with CKD are listed in Table 101-1.

Estimation of GFR from Cystatin C Concentration

Besides creatinine, cystatin C is another endogenous substance that has been used to estimate GFR. Cystatin C, is a low-molecular-weight protein that is an inhibitor of cysteine proteases and is produced by all nucleated cells. Cystatin C is filtered freely through the glomerulus and is almost completely reabsorbed and catabolized by the proximal tubule (51). Clearly, tubular reabsorption is a major potential problem with using cystatin C levels as an index of kidney function, because tubular function can vary as kidney function declines. It also is not known if the production of cystatin C is constant throughout the course of kidney failure. If it is not constant, variation in its blood level will be determined by loss of kidney function plus variations in cystatin C production. Despite these potential problems, several studies have shown that cystatin C provides a reasonable assessment of the level of GFR, especially in patients with early renal insufficiency (52). When used to

assess the rate of progression, several authors conclude that monitoring the reciprocal serum cystatin C concentration is more closely correlated with changes in GFR than is the reciprocal of serum creatinine concentration (53–64). Likewise, there are reports that there is no statistical difference between the analysis of changes in reciprocal serum creatinine and serum cystatin C (65) or that the reciprocal of serum cystatin C was inferior to that of the reciprocal of serum creatinine (66,67). Other discrepancies that are relevant to the use of cystatin C are that pregnancy alters the relationship between GFR and serum cystatin C level (68). In children, cystatin C levels may offer no improvement over serum creatinine levels in estimating GFR (69,70). Infants of less than 18 months of age also tend to exhibit higher cystatin C levels (71). Since the availability and cost of measuring cystatin C are likely to be much greater than serum creatinine, further study is needed to determine the utility of cystatin C in estimating renal function (67).

Estimation of GFR from Other Substances

β2-Microglobulin is filtered by the glomeruli and, like cystatin C, is reabsorbed by the proximal tubule, where it is metabolized. However, there is an additional problem in that the protein is degraded at acid pH so patients should be given bicarbonate to raise the urine pH above 7 when β2 microglobulin is used as a measure of kidney function. There are data indicating that its serum concentration may be more sensitive to early decline in GFR than is serum creatinine (72). Other candidates include beta-trace protein (73) and tryptophan glycoconjugate (74). These have not been widely studied.

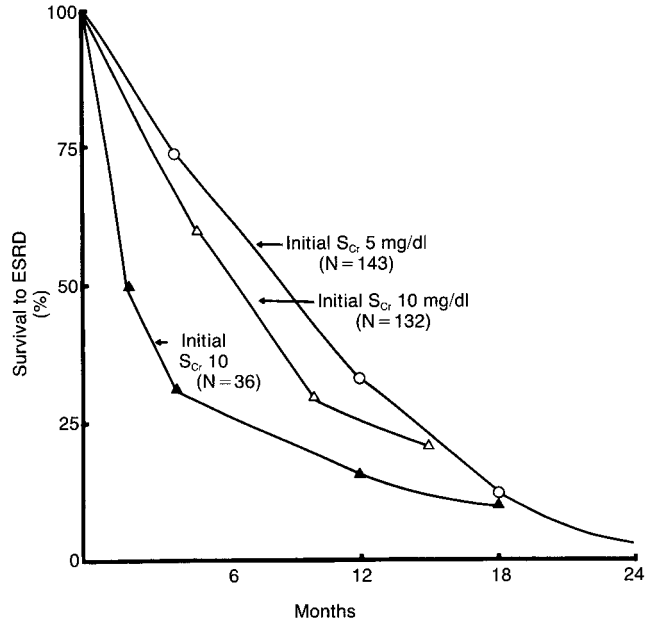

FIGURE 101-1. The survival to end-stage renal disease (*ESRD*) of patients with chronic renal failure. Three reports are examined: data of 143 patients with an initial serum creatinine (S_{Cr}) of 5 mg/dL (4), of 132 patients with an initial S_{Cr} of 10 mg/dL (5), and of 36 patients with an initial S_{Cr} of 10 mg/dL (3). The criteria for diagnosing ESRD were not given in these reports, but the wide variability in survival of different populations is apparent.

ASSESSMENT OF THE RATE OF PROGRESSION OF RENAL INSUFFICIENCY

Early studies suggested that the course of CRF was unpredictable and prognosis for the individual patient was assessed by estimating the average time before dialysis became necessary, or until a predetermined degree of renal insufficiency was reached (75–77) (Fig. 101-1). It is now recognized that most CKD patients experience a loss of renal function that is not chaotic, but, instead, the loss of function is constant and predictable (i.e., GFR declines linearly with time). Unfortunately, there have been very few examinations of the frequency of a linear decline in GFR in large numbers of patients with kidney disease, but, on average, a linear decline in GFR with time was observed in patients in the MDRD who were assigned a high-protein diet, plus several reports show that the reciprocal of serum creatinine (S_{Cr}^{-1}; see later) declines linearly with time in individual patients (27,78,79). As with other clinical evaluations, a linear decline in kidney function is not uniformly observed. Shah and Levey (80) reported that 32% of 77 patients had a "break-point" in the S_{Cr}^{-1} plot, suggesting that in those patients, the loss of renal function was not constant. Conversely, Coresh et al. (81) found significant breakpoints in only 19% of a series of 67 patients who were sequentially examined until ESRD was reached. It is unexplained why the rate of loss of renal function is so variable, even when CKD patients have the same underlying kidney disease (82).

The optimal protocol for assessing the rate of progression is to measure sequential changes in GFR with at least four measurements over 2 or more years (83,84). Like any measurement of a physiologic function, however, the methods must be strictly standardized to achieve accuracy. Unfortunately, measuring GFR is costly, because skilled personnel are required and reagents and measurements must be purchased. However, other methods (e.g., the time required for serum creatinine to double or repetitive measurements of C_{Cr}) provide less convincing evidence that the rate of progression has changed with therapy. The nephrologist is faced with deciding between the costs of personnel and supplies required to measure GFR or relying on a less accurate method for estimating the loss of renal function. Since it is no longer acceptable to estimate the rate of loss of GFR from serial measures of serum creatinine alone (33,85), the nephrologist needs a reliable means of estimating the rate of loss of renal function during medical management of a CKD patient.

For clinical purposes, the reciprocal of serum creatinine (S_{Cr}^{-1}) plot is a simple alternative to obtaining repetitive GFR measurements in treating CKD patients. There are two reasons for using this method. First, creatinine is simple to measure and reproducible (the day-to-day coefficient of variation in CKD patients with renal disease is only 6.5% [86]), and usually there are preexisting values for the assessment of changes in renal function. Second, S_{Cr}^{-1} declines linearly with time in most patients with progressive renal insufficiency (27,79,81).

Notably, a plot of changes in GFR in an individual patient calculated from the modified MDRD equation is essentially a plot of constant values multiplied by S_{Cr}^{-1}. Recognition that the decline in S_{Cr}^{-1}, with time, is linear in an individual patient, leads to the hypothesis that renal function is lost at a constant rate (87), and this seems to be true for most patients (27). For example, about 80% of the patients reported by Rutherford et al. (88) and Coresh et al. (81) had linear slopes for their S_{Cr}^{-1} plots. An important issue is whether there is spontaneous slowing of the rate of loss of renal function as GFR falls to low levels. Based on data from patients with different causes of CKD, Gretz et al. (89) estimated that changes in the decline of S_{Cr}^{-1} are more likely to accelerate at low GFR levels than to slow spontaneously. Oksa et al. (90) attributed this type of response to an improved control of hypertension. Rutherford et al. (88) found that approximately one-half of patients

with two slopes of S_{Cr}^{-1} (i.e., only five patients) had spontaneous slowing of progression, indicating that this is an unusual occurrence. A recent report, based on results from the AASK (African-American Study of Kidney Disease), suggested that the loss of GFR in patients with hypertensive nephrosclerosis accelerated when renal insufficiency was advanced (91). Close inspection, however, suggest that the loss of GFR accelerated in patients treated with amlodipine, but not with metoprolol. Moreover, accelerated loss of GFR did not occur in patients who were in the control group. These results raise the possibility that certain treatments or even the processes of nephrosclerosis can affect the course of kidney failure. The reason why these CKD patients, unlike those being observed while eating normal diets, experience acceleration of the loss of kidney function is unexplained (78).

Walser et al. (40) compared the rates of progression measured as changes in S_{Cr}^{-1} multiplied by an average value of creatinine excretion with rates of changes in GFR, estimated as the renal clearance of ^{99m}Tc-dithethylenetriamine pentaacetic acid (DTPA). They studied 17 patients and reported that four patients had a progressive loss of renal function, based on the estimated C_{Cr}, even though the GFR values remained stable. Two of these patients, however, had to begin dialysis, while changes in the GFR of the final patient, suggested an improvement in renal function while the estimated C_{Cr} remained stable. Moreover, when they examined progression during different treatment periods, there were 9 of 22 periods that exhibited a statistical difference between the slopes of estimated C_{Cr} and GFR. In two of these periods, GFR was stable, but changes in the C_{Cr} suggested progression, while, in two other periods, both methods were consistent with progression. The estimated C_{Cr} method, however, suggested faster progression and, in two other periods, GFR improved or worsened, but the C_{Cr} estimate was stable. These last two instances are the most worrisome in terms of the estimated C_{Cr} method giving misleading information. Overall, however, it appears that in only 4 of 22 periods did the estimate yield an inappropriate conclusion regarding whether kidney function worsened or improved.

There are other reasons why the estimated C_{Cr} method may not yield an accurate picture of the loss of residual renal function. First, it is well known that creatinine secretion accounts for differences between C_{Cr} and GFR and the estimated C_{Cr} method does not correct for it (33,90). Second, creatinine production is not equal to creatinine elimination, because there is extrarenal elimination of creatinine (presumably degradation by gastrointestinal bacteria [92]). As explained, this problem can be overcome by assuming a constant value of extrarenal clearance, 0.04 L/kg/day, and, using this extrarenal clearance value, it can be shown that the linear decline in C_{Cr} of patients with CRF is compatible with a linear decline in the S_{Cr}^{-1}(27,93). Unfortunately, it is not known whether extrarenal C_{Cr} does, indeed, remain constant throughout the course of CKD. Finally, there is the serious problem of dietary factors, especially variations in the amount of meat eaten. Meat, like other muscle, contains creatine, so varying meat in the diet varies the size of the creatine pool. Cooking meat converts a portion of the creatine to creatinine so the larger creatine pool will increase the amount of creatinine that is excreted; contrariwise, an abrupt reduction in meat intake decreases the creatine and creatinine pools, leading to a lower serum creatinine (as long as C_{Cr} is constant). When the protein (e.g., meat) content of the diet is relatively constant, any change induced by varying dietary creatine is short lived, since there is a slow rate of conversion of creatine to creatinine in muscle (and other cells); the turnover of the endogenous creatine pool averages only 1.7% per day (35,94–96). Thus, a new steady state of creatinine production after a sustained change in meat intake will occur in approximately 4 months (27,45). Another problem arising when protein intake is varied is that raising dietary protein acutely increases GFR and, hence, C_{Cr}(97). In summary, changes in serum creatinine alone should be discarded as a method of estimating the degree of kidney damage, but changes in the slope of S_{Cr}^{-1} can be used to screen and detect patients who are progressing or determine if there has been a sudden change in clinical status. If, however, a new or untried therapy is being investigated to determine if it slows progression, changes in GFR should be measured.

It is important to determine the rate of progression of each patient individually because it is well known that variation of rates of progression among patients is high. Jones et al. (82) reported that patients with diabetic nephropathy had rates varying as much as 20-fold and Walser (83) found that changes in ^{99m}Tc-DTPA clearance in 34 patients with different causes of CRF varied from –0.96 to +0.64 mL/minute/month. According to these results, about 15% of CKD patients may not show any progression (at least during the period of observation) (83,95). These results not only emphasize the importance of evaluating progression rates in individual patients but they also point out why clinical trials with no initial screening to identify patients that do have progressive loss of GFR will be at risk of studying an inadequate number of subjects leading to incorrect conclusions.

PROGRESSION OF EXPERIMENTAL RENAL DAMAGE AND DIETARY PROTEIN

The beneficial effects of restricting dietary protein to limit renal injury in models of experimental renal injury has been recognized for more than 65 years (98) (Table 101-2). Identification of the mechanisms and the separate contributions of dietary protein, phosphates, salt and/or unexcreted metabolic waste products, etc., that cause ongoing kidney damage would be useful not only to understand how protein intake affects the kidney, but also because it could lead to more specific therapies that would act to preserve kidney function. The mechanism by which the diet affects kidney function are probably not mutually exclusive, but they do involve complex mechanisms.

Hemodynamic Mechanisms of Progression

Systemic Hypertension

Arterial hypertension arises in the course of most cases of chronic renal insufficiency and generally increases as renal damage progresses. Some, but not all, clinical studies suggest that blood pressure control can reduce the rate of loss of renal function (99–102). A change in dietary protein alone

TABLE 101-2

EXPERIMENTAL RENAL DISEASES IMPROVED BY DIETARY PROTEIN RESTRICTION

Remnant kidney
Nephrotoxic serum nephritis
Doxorubicin nephrosis
DOCA salt hypertension
Spontaneous hypertension with reduced renal mass
Salt-sensitive hypertension with glomerulonephritis
Diabetes mellitus
Spontaneous glomerular sclerosis of aging
Antitubular basement membrane nephritis

probably does not increase blood pressure, but a high protein diet is often associated with a high salt diet (103). An increase in salt intake not only raises blood pressure in humans with mild CKD but also increases GFR (see below). Notably, increases in blood pressure and GFR do not occur when the salt intake of healthy subjects is increased (104).

In experimental kidney disease, there is an association between an increase in dietary protein and higher blood pressure and lowering dietary protein has been effective in reducing systemic arterial pressure in mineralocorticoid-induced hypertension in rats, so-called deoxycorticosterone (DOC) salt hypertension (105). In other forms of experimental hypertension, dietary protein restriction has not been so clearly associated with a reduced systemic pressure. The systemic hypertension that develops in rats following subtotal nephrectomy persists even when dietary protein is severely restricted. The two kidney, one-clip renovascular hypertension model is not influenced by lowering protein intake (106,107). In several models of CKD, dietary protein restriction diminishes renal injury, but there has been no consistent decrease in blood pressure, indicating that the beneficial side effects of protein restriction do not necessarily depend directly on reducing salt or eliminating some unidentified factor or compound that raises blood pressure.

In humans with essential hypertension, there is no clear cause–effect relationship between protein intake and arterial pressure (103). Regardless, hypertension frequently is the first sign of kidney damage, making it difficult to establish if the patient has essential hypertension or CKD. This is important because when adults with hypertension related to CKD are examined, systemic blood pressure has not been consistently reduced by restriction of dietary protein intake, similar to the results found in rats with CKD from subtotal nephrectomy (78,108–111). Still, it remains possible that in humans, dietary protein may influence the level of blood pressure in certain disorders, but this has not been established. The caveat is that a high protein diet is generally associated with a high salt intake, which will raise blood pressure in hypertensive CKD patients.

Intrarenal Hemodynamics

Although systemic arterial pressure is not consistently influenced by dietary protein, intrarenal hemodynamics regularly respond to changes in the level of dietary protein. In fact, dietary protein restriction appears almost unique in altering hemodynamics and protecting against experimental renal injury; other measures that successfully protect against progressive renal injury, including angiotensin-converting enzyme inhibitors, heparin, antiplatelet agents, and thromboxane synthetase inhibitors, also cause a fall in systemic arterial pressure and, therefore, a cause–effect relationship between these treatments and glomerular capillary hypertension can not be made. Regardless, even though dietary protein restriction does not routinely lower arterial pressure, it still could exert an important change in intrarenal hemodynamics.

Following loss of renal mass, animals ingesting unrestricted amounts of protein have structural enlargement of residual renal tissue and an increase in kidney function. Single-nephron GFR rises substantially and in direct proportion to the number of nephrons lost. Morphometric and biochemical indices of renal growth also increase rapidly; after unilateral nephrectomy, the RNA content of the kidney rises within 12 hours and radiolabeled choline is incorporated into renal membranes within the first minutes. The forces and blood flows closely linked to the augmentation in single-nephron GFR following loss of renal mass, include increases in glomerular capillary pressure and plasma flow related to reduced renal vascular resistance, leading to a compensatory increase in single-nephron filtration rate in remaining glomeruli (112,113).

Protein intake is a primary determinant of the degree of functional and structural growth in remaining nephrons of the remnant kidney; a high protein intake increases the weight of the kidney and stimulates cell growth (i.e., hypertrophy) while limiting dietary protein prevents an increase in kidney size. Similar events occur in rats with intact kidneys who are fed different levels of protein. In addition, functional changes can be produced in animals and humans by altering dietary protein intake; both GFR and renal blood flow increase following an acute or chronic increase in dietary protein.

The finding that a low-protein diet blunts the functional and structural changes that are induced by partial nephrectomy and the observation that dietary protein restriction diminishes renal injury in models of kidney disease have led to the hypothesis that the deleterious effects of increased or even normal levels of dietary protein can be explained by the adaptive increases in blood pressure and flow that occur in the remaining glomeruli. For example, rats with remnant kidneys that are fed a low-protein diet have lower intrarenal blood and hydrostatic pressures and less renal injury. There also is less proteinuria and abnormalities in glomerular permselectivity or histologic damage, especially glomerular sclerosis (106,112). The relationship between reduced glomerular capillary hypertension and glomerular injury and dietary protein restriction is also present in other rat models of renal injury, including mineralocorticoid hypertension, spontaneously hypertensive rats, experimental diabetic nephropathy, or modest reduction in renal mass (uninephrectomy) (114–116). Additional support for an adverse effect of dietary protein-mediated hemodynamic forces (e.g., increased glomerular capillary pressure) is from models with established compensatory renal hypertrophy and hyperfunction. Restricting dietary protein reduces kidney injury in these models (114). Results from nondietary methods of treating CKD also link an increase in glomerular capillary pressure to kidney damage (117,118). These results have led to the hypothesis that protein restriction diminishes progressive renal injury principally by reducing intraglomerular pressure.

The neurohumoral basis for the hemodynamic effects of dietary protein on normal and diseased nephrons is unknown, but there are several candidates. For example, infusion of amino acids causes vasodilatation; somatostatin blocks this response (119–121). However, somatostatin changes the secretion of many hormones, thus leading to several suggested mediators of renal hemodynamic responses. One prominent candidate is glucagons, because plasma glucagon levels rise with oral protein feeding or intravenous amino acids (119,122,123), and intravenous infusion of glucagon to achieve blood levels that are similar to those observed following an amino acid infusion does provoke a rise in GFR and renal blood flow in humans (123). This response to glucagon, however, appears to depend, at least in part, on prostaglandin (PG) synthesis, because the simultaneous administration of a cyclooxygenase inhibitor and either amino acids or glucagon blunts the expected increase in GFR and renal blood flow (123). The field of candidates is not narrowed much by this experiment, because several cyclooxygenase-dependent products could affect kidney vessels and urinary excretion of PGE and the prostacyclin metabolite, $PGF_{1\alpha}$, are quite variable following dietary protein loads, making it unlikely that these compounds are the sole mediators of renal vasodilatation (123). Another renal vasodilator system, the endothelium-derived relaxing factor or nitric oxide, could be involved, since blocking of the synthesis of nitric oxide does cause renal vasoconstriction in the rat (124–126). Moreover, the renal vasodilatation that occurs with amino acid infusion appears to be particularly susceptible to nitric oxide synthase blockade, suggesting that nitric oxide is a major factor affecting the renal hemodynamic response to dietary protein. In support of this proposal, a chronic increase in protein intake by normal rats

not only increases GFR, but also augments the excretion of metabolites of nitric oxide (126). Since both of these phenomena are blunted by the competitive inhibitor of nitric oxide synthase, L-nitro-arginine-methyl-ester (L-NAME), nitric oxide is a prime candidate for producing dietary protein-induced alterations in renal hemodynamics. The stimulus for nitric oxide production is uncertain, but some suggest it is arginine, the substrate for nitric acid production (127–135). On the other hand, signals besides nitric oxide are likely to contribute to the hemodynamic effect, because infusion of amino acids other than arginine will cause renal vasodilatation.

In rats with diseased kidneys, raising dietary protein increases GFR and renal blood flow, but to a limited extent, because excess dietary protein increases glomerular capillary pressure by increasing efferent compared to afferent vascular resistance (108). The major mediator of the rise in efferent resistance is renin secretion and the intrinsic, intrarenal angiotensin-converting enzyme activity. This raises angiotensin II (Ang II) to cause efferent arteriolar vasoconstriction. Notably, dietary protein does stimulate renin and angiotensin-converting enzyme expression in the kidney (108,116–118,136,137). Tonic stimulation of the efferent arteriole seems to be an insufficient explanation for glomerular hyperfiltration. The presence of a chronic and long-acting effector seems more likely to be the mediator, since acute pharmacologic blockade of the production or action of Ang II does not eliminate the increase in efferent vascular resistance or glomerular pressure in the rat remnant kidney model, even though chronic blockade of the renin system does reduce efferent anteriolar vasoconstriction (138,139). Alternatively, Ang II might increase the production of another intrarenal hemodynamic mediator, such as endothelin (108,140,141). As with hemodynamics, the mediator/mechanism of renal hypertrophy is unexplained. Ang II can stimulate renal cells to grow *in vitro*, but dietary protein-induced hypertrophy of kidney cells *in vivo* does not appear to depend on the renin–angiotensin system, since pharmacologic blockade of angiotensin does not block renal growth in rats fed a high-protein diet (142).

Manipulations of rats have been the basis for most of these reports, raising the question of whether similar events occur in other species. The answer is at least a partial yes: following subtotal nephrectomy, cats fed a high protein diet (51.7% protein) develop more serious kidney injury than cats fed a more restricted (27.6% protein) (143). Despite progression of the degree of injury, cats fed a high-protein diet, do not have a loss of GFR over the short term. Baboons subjected to a partial nephrectomy and fed 25% protein did lose GFR at a faster rate than animals fed 8% protein; the degree of histology damage was not reported (144). At least in these animals, the changes in GFR are not as striking as those observed in rats.

Besides raising the activity of the renin system, aldosterone levels increase with dietary protein (145). The observance is that aldosterone can induce a fibrotic response in organs, including the kidney (140). Hostetter and colleagues (146) treated subtotally nephrectomized rats with aldosterone inhibitors and compared results to those in untreated rats. The treated rats had less kidney damage and they concluded that aldosterone accelerates renal insufficiency (146). In recent studies, they have found that aldosterone treatment over only 3 days will stimulate TGF-β expression independently of the degree of hypertension. Thus, aldosterone should be added to the list of factors causing kidney damage in CKD.

The renal growth that is stimulated by excess protein intake can interact adversely with the renal hemodynamic responses induced by dietary protein. For example, glomerular enlargement may be a forerunner of glomerular sclerosis (113,147–149). When glomerular capillary pressure or the diameter of glomerular capillaries increase, the tension in the capillary walls will rise. In addition, efferent arteriole constriction will augment the increase in wall tension. Mechanical stress by these mechanisms could accelerate damage to the glomerulus and cause or at least predispose to sclerosis. Another mediator of damage augmented by dietary protein is an increase in proteinuria caused by accelerated growth leading to defects in glomerular epithelial cell structure or altered mesangial clearance of macromolecules (150,151).

Toxicity from proteinuria should also be considered, because high-protein diets potentiate proteinuria. It is well known that the degree of proteinuria is an index of the degree of kidney damage in several glomerular diseases (152), but recently it has been proposed that filtration and subsequent reabsorption of albumin by proximal tubule cells can excite inflammatory responses (153). For example, treatment of cultured proximal tubule cells with albumin increases the expression of NF-κB, a transcription factor that is heavily involved in mediating inflammatory responses (154). In this formulation, proteinuria accelerates progressive kidney failure and is not simply a marker of the degree of kidney damage (153,155). In fact, results from a multicenter trial (RENAAL) led to the conclusion that if the degree of proteinuria can be suppressed, there was a decrease in the risk of both kidney failure and major cardiovascular events, such as stroke, congestive heart failure, and death (156,157). These reports raise important questions about mechanisms of injury to the kidney and blood vessels and is mechanistically, as well as clinically, interesting. The relevance to dietary protein is that protein restriction suppresses proteinuria and will add to the effectiveness of the antiproteinuric response to angiotensin-converting enzyme inhibitors (158,159).

Nephrotoxic Compounds Derived from Dietary Protein

In 1905, Folin (160) pointed out that the principal metabolic response to an increase or a decrease in dietary protein content is a parallel change in urea excretion. Using somewhat more sophisticated techniques, the same relationship between protein intake and urea production has been demonstrated repeatedly in normal adults and patients with CKD (161–163). This occurs because dietary protein is metabolized to peptides and amino acids, which are further degraded to form a vast array of products that are excreted by the kidney. For these reasons, products arising from the metabolism of dietary protein will accumulate in patients in direct proportion to the amount of protein eaten and in inverse proportion to the degree of kidney failure. Accumulation of some of these compounds could prove injurious to the damaged kidney.

An example of a potentially toxic mechanism of progressive renal insufficiency is indoxyl sulfate. This compound arises from the metabolism of indoles, such as dietary tryptophan, and accumulates when kidney function is suppressed (164). Experimentally, administration of indoxyl sulfate (or indole compounds) accelerates kidney damage in models of glomerular sclerosis (165,166). The mechanism is complex because administration of indoxyl sulfate seems to increase the kidney expression of TGF-β and other signs of kidney damage, but how this occurs and whether other toxic mechanisms occur is unknown (167). Not surprisingly, the accumulation of indoxyl sulfate in uremic rats and CKD patients is reduced by restricting dietary protein (168). In conjunction with this, ingestion of a sorbent can reduce the accumulation of indoxyl sulfate; there are clinical trials assessing if this strategy can be a means of preventing uremic toxicity (168,169).

Uric acid is another potentially nephrotoxic compound that is related to protein intake. Based on results from a 12-year population study of 47,150 previously normal men, it was

concluded that diets with high levels of meat or seafood are associated with an increased risk of gout (170). Besides causing gout, uric acid could be nephrotoxic. Johnson et al (171,172) pointed out that humans lack the gene for urate oxidase and, hence, their plasma uric acid level is substantially higher than the level present in rodents. They also pointed out that hyperuricemia can predispose humans to hypertension of the salt-sensitive type, possibly via a renin-dependent mechanism. This hypothesis centering on uric acid is interesting for a number of reasons. First, Johnson et al. (172) have shown that there is an important association between uric acid and hypertension; in untreated otherwise normal adolescents with hypertension, serum uric acid levels were highly correlated with systolic blood pressure ($r = 0.8$). In a small group of these adolescents, their hypertension was largely corrected by treating them with allopurinol. Second, uric acid levels in patients with kidney disease do not rise to the level expected from the degree of lost kidney function, because there is extensive metabolism of uric acid, presumably by bacteria in the gastrointestinal tract (173). However, in CKD patients, the plasma uric acid levels are above the concentration that could induce hypertension and possibly kidney damage (172). The degree to which dietary protein restriction modifies the serum uric acid of CKD patients has not been established. Finally, potential mechanisms for the nephrotoxic effects of uric acid have been explored. In rats given an inhibitor of urate oxidase, serum uric acid rises slightly (~1–2 mg/dL) and a primary arteriolopathy develops, which is independent of hypertension; this abnormality is largely corrected by allopurinol treatment (117,174). These same rats also have glomerular hyperfiltration and this was prevented by allopurinol administration (117). Both normal rats and rats after subtotal nephrectomy exhibit evidence of collagen deposition, macrophage infiltration, and increase in juxtaglomerular renin content along with a decrease in expression of macula densa neuronal nitric oxide synthase (175). Potentially, uric acid could decrease nitric oxide synthesis in endothelial cells possibly through peroxynitrite production (125,172). In this case, oxidative damage is an inciting mechanism of progressive kidney damage.

Besides these compounds, there are other potentially toxic metabolites of dietary protein including those that could affect kidney function indirectly. For example, excess dietary protein can increase the levels of metabolites of phenylalanine in patients with renal insufficiency. In dialysis patients, there is accumulation of phenylacetic acid, which inhibits the expression of inducible nitric oxide synthase (176). This inhibition could increase atherosclerosis and, indirectly, the loss of kidney function. The metabolism of these compounds, like that of creatinine, becomes complex in patients with renal insufficiency (173,177). Metabolism of these (and other) compounds makes it difficult to predict how the plasma level of these compounds will change or how defects caused by their accumulation will respond to dietary protein restriction. Overall, there will be a decrease in their levels, but the magnitude of this decrease is unpredictable.

Inflammatory and Scarring Factors

Several of cytokines and growth factors are being incriminated as critical factors, causing the glomerulosclerosis and interstitial scarring that is characteristic of progressive renal disease (126). Dietary protein intake can also influence the expression of some of these factors and, hence, the progression of renal injury. TGF-β has received substantial support as a major factor in progressive renal disease, especially in diabetic nephropathy (178,179). The accumulation of TGF-β is also incriminated as the signal for the development of glomerulosclerosis and chronic tubular interstitial disease in other models of progressive injury (180). Sources of the TGF-β include resident renal parenchymal cells or infiltrating cells, particularly monocytes (178,181). Furthermore, both excess protein intake and an increase in Ang II are capable of stimulating the renal accumulation of TGF-β (perhaps dietary protein elicits expression of this growth factor by increasing Ang II levels) (182–185).

Another potential mediator that is affected by dietary protein is the renal synthesis of nitric oxide leading to toxic metabolites of nitric oxide, such as peroxynitrite. Accumulation of such compounds, because of a high-protein diet can damage cells and cell membranes (125,186). The possibility that excessive dietary protein promotes chronic kidney injury by stimulating other cytokines, such as interleukin-1 or platelet-derived growth factor, has been less fully examined, even though these compounds are associated with high-protein diets (187,188). In summary, there is abundant evidence that small molecules and other factors acting predominantly locally could participate in the initiation and perpetuation of chronic injury in the kidney. The generation of these or other mediators by high-protein diets may be the mechanism by which unrestricted diets cause kidney damage (98,189–192).

A close relationship between the severity of kidney dysfunction and tubulointerstitial infiltrate is present in most chronic renal diseases (193). Agus et al. (194) studied a model of interstitial nephritis induced by administration of an antitubular basement membrane antibody and found that the course kidney disease is improved by restricting dietary protein. Specifically, the degree of immune-mediated interstitial injury was diminished despite the presence of high titers of the tubular basement membrane antibody, leading to the hypothesis that the beneficial effect of the protein-restricted diet was due to a suppressive effect on effector T cell immunity. The level of dietary protein restriction employed in these studies was relatively severe, but whether less severe restriction might prove beneficial is unknown.

Deposition of complement component C3 and the terminal complement complex C5b-9 occurs around the renal tubules in most chronic progressive renal diseases, including those that are not caused by a primary immune etiology, including the rat remnant kidney model (195). The mechanism whereby complement components are deposited and their pathophysiologic significance are uncertain, because immunoglobulins are not localized in the same peritubular areas. Although complement deposition could just occur as a secondary effect of primary damage to renal cells, recent evidence supports a more primary role (195). As with adaptive increases in renal hemodynamics following nephron loss, adaptive increases in function of each remaining tubule particularly ammonia production, also occur. It is possible that intrarenal ammonia could be the trigger for activating the alternative complement pathway by a nucleophilic interaction of the free-base ammonia with the thiol ester of complement component C3 to form an amidated C3 (195). Amidated C3, in turn, can function as a convertase leading to chemotactic and membrane attack complexes. It also could interact with the leukocyte C3b receptor to stimulate an inflammatory response. Supporting this hypothetical mechanism are results demonstrating that suppression of ammonia production of remnant nephrons by chronic sodium bicarbonate loading does, indeed, reduce renal injury and the peritubular deposition of complement components (195). In experimental models of renal cystic disease, Torres et al. (196) produced evidence that renal ammoniagenesis promotes cyst formation as well as interstitial inflammation. Thus, dietary protein restriction may be advantageous, at least in part, because it lowers the amount of acid that must be excreted leading to reduced and, hence, ammonia production by remaining nephrons. A reduction in intrarenal ammonia could diminish activation of the complement system and its inflammatory, cystogenic, and cytotoxic consequences.

Lipid and Mineral Metabolism

Restriction of dietary protein changes lipid intake, but there are often alterations in the nature and quantity of lipids eaten when dietary protein is restricted. Hyperlipidemia and cardiovascular disease (i.e., large vessel damage) are common in patients with CKD. The role of lipids in small vessel diseases, including progressive glomerular capillary obliteration, was suggested by Keane and Guijarro (197). Employing the remnant kidney rat model, they demonstrated that clofibrate administration reduced the serum cholesterol level of rats ingesting a standard rat diet, leading to a decrease in proteinuria and fewer sclerotic glomeruli. Notably, these responses were achieved without altering the degree of hypertension or serum triglyceride levels so the mechanism is obscure. The role of protein intake per se in the hyperlipidemia of CRF is unknown, but a decrease in meat intake is generally associated with reduced ingestion of cholesterol and saturated fat. How hypercholesterolemia might contribute to glomerular pathology is not known, but it seems reasonable to speculate that mechanisms analogous to those causing large-vessel disease, including direct endothelial injury and the presence of cholesterol, lipoproteins, and other inflammatory agents in the capillary wall and mesangium, are probably involved.

Besides reducing nitrogen intake, a low-protein diet is generally associated with a reduction in phosphorus and sodium intake. The potential benefit of lowering salt intake in hypertensive CKD patients is obvious, but benefits of dietary phosphate restriction are less obvious. A low phosphate diet can mitigate the progressive renal disease that occurs after subtotal nephrectomy or experimental glomerulonephritis in the rat (198), but interpreting these results is complicated because there are studies in which dietary protein, but not phosphorus, was varied and it was concluded that dietary protein restriction without phosphate restriction can be beneficial (114,199). On the other hand, dietary phosphate restriction without simultaneous protein restriction is capable of reducing progressive experimental renal disease (198,200). The mechanism whereby phosphate restriction reduces renal injury is uncertain (201). Four principal possibilities have been suggested. First, phosphate restriction has been associated with reduced GFR so the advantages related to diminution of hyperperfusion in residual nephrons may be achieved by phosphate restriction. Second, with severe dietary phosphate restriction, defects in inflammatory responses due to dysfunction of leukocytes might be expected. Although suppression of inflammation could be beneficial, the severe degree of phosphate restriction needed to achieve the response is prohibitive clinically. Third, suppression of intrarenal deposition of calcium phosphate crystals, demonstrated histologically as far back as 1937, could be an important mechanism whereby dietary phosphate restriction slows the progression of chronic renal disease (202). Fourth, phosphate restriction could minimize nephron hypermetabolism in GFR (203). Clearly, these mechanisms are not mutually exclusive and all may contribute to the beneficial effects of phosphate restriction.

Metabolic Effects

Oxygen consumption by the remnant nephron rises sharply, suggesting another potential mechanism of kidney injury. The rise in oxygen consumption is related to the increased single-nephron filtration rate occurring in remnant nephrons, resulting in an increase in absolute sodium reabsorption per nephron. The mechanisms whereby enhanced sodium reabsorption occurs include adjustment of peritubular capillary forces, augmentation of apical membrane ion transporters, and increased Na-K-ATPase activity, factors that raise oxygen consumption by the normal kidney. Because hypertrophy, as well as hyperplasia, contribute to the compensatory growth of residual nephrons, oxygen consumption per cell is almost certainly enhanced in the remnant nephron. Increased oxygen consumption may be deleterious, because it generates potentially toxic oxygen free radicals. Reactive oxygen species can cause biologic damage by several mechanisms, including peroxidation of membrane fatty acids, depolarization of hyaluronic acid, sulfhydryl oxidation and cross-linking, and nucleic acid damage. Evidence from rats following experimental acute renal failure indicates that free radicals do cause several of these biochemical consequences and impair renal function. Moreover, using isolated perfused kidneys obtained from partially nephrectomized rats, Harris et al. (203) found there were increased rates of oxygen consumption by remnant nephrons compatible with heightened metabolic rates. Complementary results were obtained by Nath et al. (204) who made *in vivo* measurements in rats after subtotal nephrectomy. The latter report indicated that lipid peroxidation and oxygen consumption are augmented in diseased kidneys if animals ingest a higher-protein diet (204). Besides dietary protein restriction, phosphate restriction can diminish renal injury by suppressing the metabolic rate (205). In summary, dietary protein restriction exerts several beneficial effects: (1) it reduces proteinuria and, hence, the potentially toxic effects of albumin in renal tubule cells; (2) it reduces the accumulation of nephrotoxic compounds, such as indoxyl sulfate and uric acid; (3) it reduces hemodynamic forces in the kidney to ameliorate kidney damage; (4) there is a lower intake of phosphates (and potentially lipids); (5) dietary protein restriction (along with sodium and phosphate restriction) limits the production of potentially toxic oxygen metabolites; and (6) there are changes in hormones and circulating mediators of inflammation and metabolism. Clearly, the effects of modifying the diet are complex and involve more than simply limiting the accumulation of unexcreted metabolites of protein. At the same time, there is no single mechanism that explains why kidney function continues to decline following an initial injury and until the mechanism(s) for progression are identified, it seems unlikely that we will determine how dietary protein restriction does limit ongoing kidney damage in certain diseases.

THE INFLUENCE OF DIETARY THERAPY IN PATIENTS WITH CHRONIC KIDNEY DISEASE

It is well known that dietary counseling will improve uremic symptoms and there is evidence that dietary protein restriction can change the course of renal insufficiency, at least in some patients (206,207). There is, however, a concern, namely, that dietary protein restriction would increase the risk for developing malnutrition and, hence, compromise the patient's prognosis after dialysis therapy is initiated. This concern led some to conclude that dialysis therapy should be initiated "early" (e.g., creatinine clearance ~20 mL/min) in order to avoid the development of malnutrition (208,209). Before examining results from clinical trials of low-protein diets and progression, the possibility that low-protein diets cause loss of protein stores and jeopardize the clinical and metabolic well-being of CKD patients must be addressed. The requirements for protein, calories, and other nutrients are discussed in Chapter 104 (Dietary Considerations for Patients with Chronic Renal Failure and Transplantation). Regarding the ability of these diets to maintain nutritional status, early studies (210–214) demonstrated that neutral nitrogen balance was achieved during long-term therapy with low-protein diets and maintained normal values

of serum albumin. In fact, the use of supplemental essential amino acids (EAAs) and ketoacids is associated with an increase in the level of serum albumin in CRF patients who have low values (215). There also is evidence that restriction of dietary protein is safe for patients with the nephrotic syndrome and that such diets reduce the metabolic consequences of CKD (216–218). These results raise the important goal of identifying factors that cause loss of LBM and low values of serum proteins in CRF patients.

Abnormalities in Protein Turnover in Uremia

Concerns about the safety of low-protein diets have mainly arisen from results of cross-sectional evaluations of groups of dialysis patients. Specifically, body weights of these dialysis patients are low and there is anthropometric evidence for loss of muscle mass plus low values of serum proteins (219–221). Evidence that similar abnormalities are present in CKD patients who are not being treated by dialysis is much less striking. Coles (222) reported results of a cross-sectional evaluation of patients with advanced renal insufficiency; there was evidence of decreased body weight, reduced muscle mass, and decreased serum proteins. It is important to note that the diet of patients studied by Coles was unregulated, because lack of attention to the diet can cause serious problems. For example, Hakim and Lazarus (223) reported the biochemical status of 911 patients who had received minimal attention to their diet for up to 7 years (223). Patients with a serum creatinine >5 mg/dL commonly had serious acidosis (one-third had serum bicarbonate <15 mM), hyperphosphatemia (one-third had values >7 mg/dL), and severe azotemia (one-third had BUN values >120 mg/dL). Such abnormalities are rare, even when patients have advanced CKD, as long as attention is given to instructing a patient how to design and adhere to a low-protein diet that includes sufficient calories and providing a regular assessment of the impact of the diet on nutritional status (213,214,224–229). The consequences of ignoring the diet of CKD patients are unfortunate, because acidosis causes loss of muscle mass and it, like hyperphosphatemia, contributes to renal osteodystrophy, while low values of serum proteins are associated with an increased risk of death (230–232).

To what extent are dietary factors responsible for the abnormalities in protein stores of CKD patients? The constellation of hypoalbuminemia, loss of weight, and anthropometric evidence for loss of lean body mass has been ascribed to malnutrition or "uremic malnutrition." This is an incorrect assignment, because malnutrition is defined as abnormalities that arise because of an inadequate amount of food or an abnormal composition of the diet. In CKD, however, there are several other mechanisms that can cause these abnormalities and they provide persuasive reasons why there is loss of lean body mass and hypoalbuminemia (233,234). Distinguishing between these mechanisms and the label, malnutrition, is not simply pedantic or formalistic because a diagnosis of malnutrition implies that simply eating more protein can correct the abnormalities (234). On the contrary, it has been known for over 130 years that eating too much protein will aggravate the symptoms of CKD, leading to the constellation of findings that is termed "uremia" (206).

In 1934, the adverse effects of a high-protein diet were confirmed in rats with experimental CKD (190). More recently, it was shown that feeding a high-protein diet stunted both linear growth and weight gain and led to very high SUN values (235). In contrast, feeding CKD rats lower amounts of protein not only improved the utilization of dietary protein to promote growth, but also was associated with the lowest BUN values. The factor(s) that cause these abnormalities was not identified, but excess dietary protein is commonly associated with acidosis, hyperphosphatemia, and the accumulation of a wide variety of uremic toxins. From acidosis alone, results from studies of rats as well as CKD patients demonstrate that it stimulates the catabolism of protein and amino acids (230,236–238). These reports led Bergstrom to conclude that acidosis is the only factor that has been demonstrated to be a uremic toxin (220).

Another factor that could play a role in causing loss of LBM is the protein catabolism that is stimulated by inflammatory cytokines, because uremia is associated with evidence of inflammation (239). The signs of inflammation, including high circulating levels of acute-phase reactant proteins, are linked to subnormal levels of serum albumin in hemodialysis patients (240,241). In addition, there are high circulating levels of tumor necrosis factor and interleukins in hemodialysis and CKD patients (242–244). Finally, high levels of these factors can stimulate excessive protein catabolism and, hence, could cause the loss of LBM by stimulating excessive protein catabolism (245–247).

If metabolic acidosis and/or inflammatory cytokines cause loss of LBM, what is the mechanism that accounts for loss of protein stores? The answer to this question requires understanding two metabolic principles: (1) the daily turnover rate of protein is quite large; and (2) there are robust metabolic responses to a decrease in dietary protein that act to maintain protein balance. Regarding the first principle, protein turnover in a 70-kg adult, who is in neutral protein balance while eating 1 g protein/kg/day, is equal to the synthesis and degradation of approximately 280 g of protein each day (i.e., 4 times the protein intake of 1 g/kg/day) (139,248). Consequently, even a small, but sustained, decrease in protein synthesis or increase in protein degradation will cause a major loss of LBM when it is sustained.

Mechanisms that Regulate Body Protein Stores

Robust metabolic mechanisms act to maintain LBM following a change in protein intake, including changes in the rates of amino acid and protein metabolism. These responses are adjusted rapidly and precisely. Specifically, when dietary protein falls because of anorexia, or when the diet is restricted to treat uremic symptoms, the major metabolic response is to suppress the degradation of EAA throughout the body. This response will ensure that an adequate supply of EAA is available for protein synthesis. The ability to decrease EAA degradation, however, is limited and reaches a minimum level when the amount of protein being eaten is at the level that is just adequate for achieving nitrogen balance (i.e., ~0.6 g protein per kilogram ideal body weight per day). When dietary protein falls below this amount, there is a second adaptive response that suppresses protein degradation (protein synthesis may also increase, but this response is not consistently found). These responses limit loss of protein stores and are present in normal adults and CKD patients as long as there are no complications, such as acidosis or another catabolic illness (214,227,249). The same adaptive responses are also active in patients with the nephrotic syndrome (216). In nephrotic patients, however, the responses are especially interesting because EAA catabolism is suppressed when net protein intake changes (net protein intake is the difference between dietary protein and the amount of protein lost in the urine). When more protein is lost, the degradation of EAA is suppressed further (216), but how the body recognizes that net protein intake has decreased is unknown. When these metabolic responses are overcome, there is loss of protein stores. For example, metabolic acidosis or increased production of cytokines will activate catabolic mechanisms that degrade amino acids and protein. In this case, the

TABLE 101-3

EVIDENCE THAT METABOLIC ACIDOSIS INDUCES PROTEIN AND AMINO ACID CATABOLISM IN NORMAL INFANTS AND CHILDREN, AS WELL AS CKD PATIENTS

Subjects investigated (ref. no.)	Measurements of effectiveness	Outcome of trial
Infants (253)	Low birth weight, acidotic infants were given $NaHCO_3$ or NaCl	$NaHCO_3$ supplement improved growth
Children (251) with CKD	Children with CKD had protein degradation measured	Protein loss was ~twofold higher when HCO_3 was <16 mM compared to >22.6 mM
Normal adults (252)	Induced acidosis and measured amino acid and protein metabolism	Acidosis increased amino acid and protein degradation
Normal adults (282)	Induced acidosis and measured nitrogen balance and albumin synthesis	Acidosis induced negative nitrogen balance and suppressed albumin synthesis
Chronic renal failure (277)	Nitrogen balance before and after treatment of acidosis	$NaHCO_3$ improved nitrogen balance
Chronic renal failure (230)	Essential amino acid and protein degradation before and after treatment of acidosis	$NaHCO_3$ suppressed amino acid and protein degradation
Chronic renal failure (286)	Muscle protein degradation and degree of acidosis	Proteolysis was proportional to acidosis and blood cortisol
Chronic renal failure (278)	Nitrogen balance before and after treatment of acidosis	$NaHCO_3$ reduced urea production and nitrogen balance
Hemodialysis (238)	Protein degradation before and after treatment of acidosis	$NaHCO_3$ decreased protein degradation
Hemodialysis (283)	Serum albumin before and after treatment of acidosis	$NaHCO_3$ increased serum albumin
CAPD (237)	Protein degradation before and after treatment of acidosis	$NaHCO_3$ decreased protein degradation
CAPD (280)	Weight and muscle gain before and after treatment of acidosis	Raising dialysis buffer increased weight and muscle mass

CKD patient will be unable to respond to dietary protein restriction and will lose LBM (250).

There is abundant evidence (Table 103-3) that metabolic acidosis causes amino acid and protein catabolism in infants, children, normal adults, and CKD patients (230,249,251–255). For example, subnormal values of the essential, branched-chain amino acids (BCAAs) are common in CKD patients (220) and correction of acidosis raises plasma levels of BCAA (256,257). The mechanism for this response was worked out by Hara et al. (258). They found that plasma levels of valine, leucine, and isoleucine and the valine concentration in muscle water were lower in CKD rats with metabolic acidosis compared to values in pair-fed, sham-operated rats and showed that the abnormal levels were corrected simply by feeding sodium bicarbonate. The underlying mechanism causing abnormal BCAA metabolism involves stimulation of the activity of branched-chain ketoacid dehydrogenase (BCKAD), the rate-limiting enzyme for the irreversible oxidation of BCAAs. In fact, May et al. (259) reported that the V_{max} activity of BCKAD was increased in the muscle of rats with experimentally induced metabolic acidosis and England et al. (260) identified the biochemical mechanisms accounting for a higher V_{max} of BCKAD activity in the muscle of acidotic rats by showing that there is an increase in the fraction of the enzyme in the dephosphorylated, activated form. They also found there was an increase in the messenger RNAs (mRNAs) encoding E1a, E1b, and E2 subunits of the enzyme, implying that the expression of BCKAD is increased. Expression and activation of BCKAD depend on both acidification and glucocorticoids (261), leading to increased degradation of BCAA *in vivo* and lower levels of BCAA in plasma and muscle cells (236). In humans, as well, metabolic acidosis stimulates the degradation of BCAAs. When normal adults were fed NH_4Cl to induce acidosis, leucine oxidation increased by 25% (252) and when CKD patients were given sodium bicarbonate to raise their serum bicarbonate from 16 to 21 mM, the measured rate of leucine oxidation decreased by 29% (230). Lofberg et al. (257) reported that the free levels of BCAAs measured in the intracellular pool of a muscle biopsy specimen taken from patients just before a dialysis treatment were corrected to normal values after sodium bicarbonate was given to the patients. Finally, Mochizuki (256) reported that low levels of BCAAs and their nitrogen-free analogs or ketoacids in plasma of acidotic CKD patients increased substantially when acidosis was treated. Besides acidosis, unidentified metabolic abnormalities induced by renal failure can stimulate BCAA catabolism, as was demonstrated in a model of acute renal failure in rats (262).

Metabolic acidosis also stimulates the degradation of muscle protein. Accelerating muscle proteolysis will block the second adaptive response to dietary protein restriction, namely, the ability to suppress protein degradation. May et al. (263) studied rats with CKD and acidosis and found that their rate of nitrogen excretion was 22% higher, compared to the nitrogen losses by sham-operated, pair-fed rats. The mechanism causing nitrogen loss was a 90% increase in the rate of protein degradation in muscle. The critical finding was that the increase in muscle protein degradation was eliminated when acidosis in the rats was corrected by adding sodium bicarbonate to their diet. To identify mechanisms that could stimulate protein degradation, England et al. (264) studied protein turnover in BC_3H-1 myocytes and found that acidifying the incubation media by reducing the concentration of sodium bicarbonate stimulated protein degradation and that insulin did not block proteolysis. Isozaki et al. (265) extended these studies to show that the acidification-induced protein catabolism in BC_3H-1 myocytes requires glucocorticoids. They found no increase in protein degradation upon acidification of the media unless glucocorticoids were present and showed that stimulation by

glucocorticoids could be blocked by adding RU 486, the steroid receptor antagonist.

Mechanisms that Degrade Muscle Protein

Acidification of muscle cells activates the ATP–ubiquitin–proteasome system to degrade muscle protein (139). This proteolytic system utilizes energy to conjugate ubiquitin to proteins that are destined for degradation; ATP is also required to degrade protein in this system. Ubiquitin is a member of the heat-shock protein family and its conjugation to the substrate protein alters it so it is recognized by the 26S proteasome, which rapidly degrades the substrate protein to peptides and these are split to amino acids by cytoplasmic peptidases. Bailey and colleagues (266) proved that the acidosis of CKD is the major factor stimulating muscle protein catabolism by the ubiquitin–proteasome system. They showed that stimulation of the ATP–ubiquitin–proteasome system and increased transcription of genes encoding ubiquitin and subunits of the proteasome in muscle was blocked by preventing acidosis in CKD rats. Interestingly, the increase in mRNAs in muscle is reminiscent of the increase in mRNAs that encode subunits of BCKAD in muscle of rats with metabolic acidosis (260).

The mechanisms that activate the ATP–ubiquitin–proteasome-dependent proteolytic system are being hotly pursued, because the system is involved in the degradation of many types of proteins including transcription factors, regulatory proteins, and foreign molecules that become antigenic peptides in antigen-presenting cells, as well as the degradation of the structural proteins of muscle (139). Activation of this system in muscle is also responsible for the excessive protein degradation that occurs in response to diabetes, starvation, denervation, burns, sepsis, and cancer, as well as metabolic acidosis. Involvement of this proteolytic system in all of these catabolic conditions suggest that activation of the ubiquitin–proteasome system is part of a metabolic program that is activated to catabolize muscle protein (139,267). In rats with uremia, insulin deficiency, starvation, or cancer, the pattern of genes expressed in muscle is highly consistent and includes components of the ubiquitin–proteasome system (267). It is still unclear what signals trigger these responses in all of these different conditions. One possibility is that inflammatory mediators activate the system, but to date, interactions between inflammatory mediators and activation of the ATP–ubiquitin–proteasome pathway are complex and inconclusive. For example, NF-kB, the transcription factor that is activated by inflammatory cytokines, does not stimulate but instead, suppresses transcription of proteasome subunit genes in muscle cells (268). The mechanisms for this response involve glucocorticoids, because glucocorticoids block NF-kB from suppressing transcription of proteasome subunit genes (268). In contrast, the stimulation of ubiquitin gene transcription not only depends on the Sp1 transcription factor, but also glucocorticoids that act to stimulate Sp1 (269). These results indicate that the increase in expression of genes encoding components of the ubiquitin–proteasome system is not easily linked to a single transcription factor.

Recently, another protease has been added to the pathway that degrades muscle protein, the apoptosis protease, caspase-3. Caspase-3 plays a critical initial role, since, by itself, the ubiquitin–proteasome system is insufficient, because the system does not degrade actomyosin or myofibrils, but does degrade actin or myosin (270). The protease that initially cleaves actomyosin and myofibrils to produce the substrates for the ubiquitin system is caspase-3. Many catabolic conditions, including uremia, are associated with insulin resistance and/or increased circulating cytokine levels and both these factors regulate apoptosis (139,271,272). Caspase-3 not only cleaves actomyosin/myofibrils to substrate proteins, but also leaves a characteristic "footprint" of its activity, a 14-kDa actin fragment in muscle (273). The presence of this actin fragment indicates that activation of caspase-3 is in muscle of rats that are rapidly degrading muscle because of uremia or insulin deficiency. The cell-signaling mechanism that activates caspase-3 and the ubiquitin–proteasome system is decreased activity of phosphatidylinositol 3-kinase (P13K) and Akt (274). A low P13K activity stimulates Bax, causing release of cytochrome C from mitochondria, resulting in activation of caspase-3. Caspase-3 then cleaves actomyosin providing substrates for degradation in the ubiquitin–proteasome system. At the same time, decreased P13K activity stimulates the Forkhead transcription factor, FOXO, which, in turn, increases the expression of the key E3 conjugating enzyme, atrogin-1/MAFbx. These responses are associated with activation of the ubiquitin–proteasome system causing degradation of actin, myosin, and fragments of these proteins (266,275,276).

Abundant clinical evidence indicates that acidosis is an important cause of protein losses in uremia (Table 101-3). Papadoyannakis et al. (277) showed that treating CKD patients with sodium bicarbonate improved their nitrogen balance. Williams and colleagues (278) studied acidotic CKD patients who were eating a high-protein diet and reported that the rate of protein degradation did not decrease appropriately when the patients were switched to a low-protein diet. They restored the normal response of suppression of protein degradation when the same CKD patients were given sodium bicarbonate and raised their average serum bicarbonate level from 18 to 24 mM. Using a similar strategy, Reaich et al. (230) found that the rate of protein degradation in acidotic CKD patients is high, but when they were given sodium bicarbonate, protein degradation decreased by 28%; it increased again when the patients were given an equimolar amount of sodium chloride leading to acidosis. The stimulatory effect of metabolic acidosis on protein breakdown is also present in hemodialysis and CAPD patients (237,238,279) and in CAPD patients, increased muscle proteolysis is related to activation of the ubiquitin–proteasome system (280,281). Ballmer and co-workers (282) reported that induction of metabolic acidosis in normal adults not only caused negative nitrogen balance, but also reduced the rate of albumin synthesis while Movilli et al. (283) reported that correcting the metabolic acidosis of hemodialysis patients led to an increase in serum albumin concentration (283).

There is a key role for glucocorticoids in the stimulation of muscle protein degradation with acidosis (236,284,285). Garibotto et al. (286) measured the rate of protein degradation in the forearm muscle of CKD patients and found that it correlated inversely with the serum bicarbonate and directly correlated with the serum cortisol level. Despite these reports documenting the catabolic effects of metabolic acidosis, there are results from cross-sectional analysis of dialysis patients suggesting there is no relationship between the degree of acidosis and hypoalbuminemia, and weight loss (287). Besides the problems associated with establishing a cause–effect relationship based on results from cross-sectional studies (288), there are other problems with this conclusion: first, a single serum bicarbonate measurement is not sufficient to define the presence of acidosis; and, second, there is a major technical problem in measuring serum bicarbonate. The blood chemistry values of patients in many dialysis units are performed in laboratories that are at some distance from the unit, creating a delay in measuring the bicarbonate concentration. This delay changes the plasma bicarbonate, because carbon dioxide escapes (289). Metabolic changes induced by acidosis include variation in hormone responses. Krapf and colleagues (292) reported that induction of metabolic acidosis in normal adults impairs the function of growth hormone, thyroid hormone, and the conversion of vitamin D to its most active form, $1,25(OH)_2$ cholecalciferol

(290–292). Consequently, acidosis could interact to impair the ability of CKD patients to maintain protein stores by indirect mechanisms. Acidosis itself, however, is sufficient to reduce degradation of protein in isolated muscle cells if glucocorticoids are present (264,265).

Besides acidosis, insufficient action of insulin is another factor that is associated with loss of protein stores. Insulin deficiency is important, because the major role of insulin in regulating protein metabolism is to inhibit protein degradation (293–295). Second, insulin resistance is common in CKD (296,297), while metabolic acidosis alone causes insulin resistance (298). Third, insulin deficiency accelerates muscle protein degradation by activating the ubiquitin–proteasome system (275). Activation of muscle protein breakdown requires glucocorticoids. The glucocorticoids act in permissive fashion as they do not stimulate protein degradation unless there is also insulin deficiency (276). Finally, the pattern of gene expression in muscle occurring with insulin deficiency is quite similar to the pattern of changes that occurs with uremia (267). Thus, at least two conditions that are caused by renal insufficiency, metabolic acidosis or impaired insulin actin, can cause muscle atrophy.

In summary, the presence of metabolic acidosis and/or impaired insulin responses will block the adaptive metabolic responses that are required to maintain LBM, especially when dietary protein is restricted. Acidosis activates specific catabolic responses that degrade BCAAs and protein in muscle. For these reasons and because acidosis impairs bone metabolism and causes insulin insensitivity, acidosis should be corrected in all patients, especially those being treated with low-protein diets. When attention is given to ensuring an adequate intake of nutrients (Chapter 104, Dietary Considerations in Patients with Chronic Renal Failure and Transplantation) and avoiding metabolic abnormalities, such as acidosis, eating a low-protein diet will improve the nutritional state of CKD patients (218,228,229).

Assessment of Dietary Compliance

The classic report of Folin (160) pointed out that urea excretion is the principal change in urinary nitrogen that occurs when dietary protein changes. This has been repeatedly confirmed and provides a firm foundation for assessing compliance with low-protein diets (162,163,210,211,299). The rate of urea production exceeds the steady-state rate of urea excretion in both normal and uremic subjects, because there is an extrarenal clearance of urea. The extrarenal removal of urea is due to its degradation by bacterial ureases present in the gastrointestinal tract (300–303). However, urea degradation does not cause a net decrease in urea excreted or accumulating in body fluids. At one time, it was believed that urea degradation to ammonia would contribute substantially to amino acid synthesis in the liver and, hence, improve the nutritional status of uremic patients (304). This is incorrect; the ammonia nitrogen is simply used to synthesize more urea (302,303). Stated more precisely, the nitrogen arising from urea breakdown does not improve protein synthesis or nitrogen balance because it is reincorporated into urea. Fortunately, the rate of net urea production closely parallels dietary nitrogen (162,211,299) and net production or urea appearance is easily calculated because the concentration of urea is equal throughout body water (145,302). Since water represents ~60% of body weight in nonedematous patients, changes in the urea nitrogen pool can be calculated by multiplying 60% of body weight (in kilograms) by the serum urea nitrogen concentration (in grams per liter). The net production of urea, *urea appearance rate* (UNA), is the change in the urea nitrogen pool (positive or negative) plus urinary urea nitrogen excretion. If SUN and weight are stable, urea nitrogen accumulation is zero and UNA equals the urea excretion rate (Table 101-4).

TABLE 101-4

ESTIMATING COMPLIANCE WITH DIETARY PROTEIN USING UREA TURNOVER

1. A 70-year-old man with a urea clearance of 10 ml/min weighs 70 kg. He is taught a diet containing 0.8 g protein/kg/day in order to meet the daily allowance of protein recommended by the World Health Organization. His serum urea nitrogen (SUN) and weight are stable. A 24-hour urine collection contains 6 g of urea nitrogen.
   Since protein is 16% nitrogen, he is eating 9 g nitrogen daily. His nonurea nitrogen excretion is 70 kg × 0.031 g nitrogen/kg/day or 2.17 g nitrogen/day and his total nitrogen excretion is 6 + 2.2 = 8.8 indicating that he is compliant with the prescribed diet.
2. A 60-year-old woman with Stage 5 CKD is admitted to the hospital for plastic surgery. She has been taught a diet that contains 40 g protein/kg/day (~6.4 g nitrogen/day because protein is 16% nitrogen). She is excreting 4 g urea nitrogen per day but on the second day of admission, her SUN rises from 50 to 60 mg/dL.
   Her nonurea nitrogen excretion is estimated as 60 × 0.031 g nitrogen/kg/day or 1.9 g nitrogen/day. Thus, her total excretion is 9.5 g nitrogen/day (total nitrogen excretion = 4 g urinary urea nitrogen + 1.9 g nonurea nitrogen + the accumulation of 3.6 g urea nitrogen [36 liters estimated body water × 0.1 g urea nitrogen per liter of body water]). Her nitrogen excretion substantially exceeds her prescribed intake so a nutrition/dietician consult and a stool sample for blood are obtained.

Nonurea Nitrogen

Unlike urea nitrogen, nonurea nitrogen excretion (i.e., the nitrogen excreted in feces and in urinary uric acid, creatinine, and unmeasured nitrogenous products) varies minimally over a large range of dietary protein (162,163). The nonurea nitrogen excretion averages 0.031 g of nitrogen per kilogram ideal body weight per day (Fig. 101-2). This average value was derived from patients that have ≤5 g/day of proteinuria and if

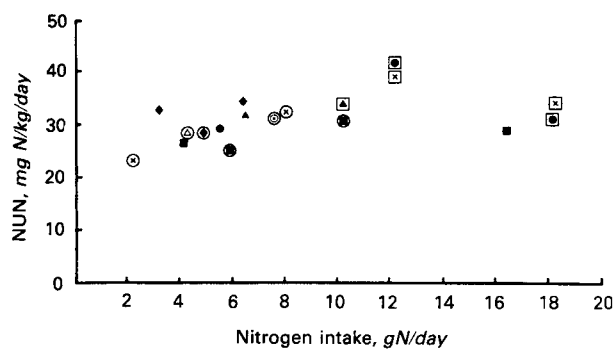

FIGURE 101-2. Calculated values of total nonurea nitrogen excretion (*NUN*) in normal subjects (*solid symbols*) and patients with chronic renal failure. The average value for patients not being treated by dialysis is 0.031 g of nitrogen per kilogram per day. (From: Maroni BJ, Steinman T, Mitch WE. A method for estimating nitrogen intake of patients with chronic renal failure. *Kidney Int* 1985;27:58, with permission.)

proteinuria exceeds 5 g per day, then an additional amount of nitrogen equivalent to the urinary loss of protein nitrogen should be added to nonurea nitrogen excretion. Recently, the relationship to nonurea nitrogen to the diet was reexamined by Kopple et al. (299a) and they concluded that fecal nitrogen of CRF patients varies with dietary protein. We examined results of over 70 nitrogen balance measurements and could find no significant correlation between dietary nitrogen and either fecal nitrogen or nonurea nitrogen excretion (163). This distinction is important, because determining that nonurea nitrogen excretion is independent of dietary protein, leads to a relatively simple method of assessing compliance with protein-restricted diets. Specifically, compliance can be assessed by converting the prescribed protein intake to its nitrogen equivalent by multiplying dietary protein by 0.16 (protein is 16% nitrogen). If nitrogen balance is assumed to be zero, then nitrogen intake will be equal to the sum of UNA (calculated as described) plus 0.031 g of nitrogen per kilogram per day (162). If intake equals output, then the patient is compliant. If intake is unequal to output, then assistance of a skilled dietician is needed. Clearly, this method does not apply to patients who are receiving total parenteral nutrition or eating completely digestible foods (e.g., astronauts). In summary, total nitrogen excretion is calculated from weight, SUN, and urea nitrogen excretion. It is compared to the prescribed protein intake and if the values are different by more than 20%, then reasons for noncompliance or reasons for nitrogen balance to be negative should be investigated (207). A potential use of the calculated dietary protein is to evaluate calorie intake. A patient's dietary history can be used to estimate the ratio of protein to calories in their diet. Since the amount of dietary protein is determined from nitrogen excretion, calorie intake can be easily checked. This type of analysis should be used regularly when monitoring CKD who are being treated by restricted diets. This is important for two reasons: first, calorie intake must be sufficient to utilize protein in the diet efficiently. If calories are insufficient, the dietician can recommend dietary changes. Second, there is accumulating evidence that patients frequently "under report" their calorie intake, especially if they are obese (305). If the physician and dietician estimate calorie intake, dietary planning is more efficient.

Other methods of assessing protein intake including dietary histories were exhaustively reviewed by Bingham (306). Interview methods are less accurate and their reliability during repeated interviews is questionable because patients quickly learn the appropriate responses to questions about dietary habits. An example of this problem was reported by Kloppenburg et al. and Avesani et al. (305,307). They evaluated food records and anthropometric and oxygen consumption data of dialysis patients (307) and CKD patients (305). They found that energy intake was grossly underestimated by many patients by diet records. Thus, dietary compliance should include the measured urea nitrogen and estimated nonurea nitrogen excretion rates to calculate actual intake.

DIETARY PROTEIN RESTRICTION AND PROGRESSION OF RENAL INSUFFICIENCY

Beneficial effects of dietary restriction on progression were reported in the late 1970s, but these and subsequent reports have been criticized, because they relied on changes in the serum creatinine or creatinine clearance to assess progression and there were problems with achieving compliance with low-protein diets, as well as difficulties in study design (including the lack of randomization and retrospective analysis). Moreover, the largest trial of dietary protein restriction and progression led to the conclusion that there was no benefit of protein-restricted diets on progression when changes in GFR were evaluated by an intention-to-treat analysis (78). As will be discussed, there are problems with this interpretation so the role of dietary manipulation in treating patients with CRF to slow progression of renal insufficiency is controversial.

Three types of low-protein dietary regimens have been used in attempts to delay the progression of CRF: a diet providing the minimum daily protein requirement (0.6 g of protein/kg/day of predominantly protein that is rich in EAAs); a diet providing approximately 0.3 g protein/kg/day, but supplemented with a mixture of EAAs; and the same diet supplemented with ketoacids (nitrogen-free analogs of EAA that are converted into EAA). One of the first trials was reported by Maschio et al. (308), who compared rates of progression in three groups of patients by assessing changes in serum creatinine and its reciprocal (308). The initial serum creatinine concentrations of patients in group I (25 patients) and group II (20 patients) were 1.5 to 2.7 and 2.9 to 5.4 mg/dL, respectively; both groups were prescribed a diet containing about 0.6 g of predominantly high-quality protein/kg/day, a phosphorus intake of less than 750 mg/day, and supplemental calcium to raise intake to 1.0 to 1.5 g/day. Thirty patients in group III served as a control group while eating an unrestricted diet (their initial serum creatinine values were between 1.6 and 4.7 mg/dL). The average intakes of protein, phosphorus, and calcium of the control group were 70 g, 900 mg, and 800 mg, respectively. It was concluded that patients eating the restricted diets (i.e., groups I to II) lost renal function far more slowly than did the control group. The presence of proteinuria and hypertension diminished the beneficial effects of dietary therapy in all groups. However, the groups were not randomized and the control group deteriorated more rapidly than did untreated patients from other series so these conclusions are tenuous. No adverse effects of the restricted diet were noted and the authors noted that protein nutrition was well maintained.

In 1989, the Verona group reported results from 390 patients who had been treated with a low-protein diet for an average of 54 months: 57% of the patients maintained stable serum creatinine values, 11% had slower deterioration (defined as a decrease in $1/S_{Cr}$ between −0.02 and −0.04 mg/dL/month), while 32% had rapid deterioration (i.e., >−0.04 mg/dL/month) compared to progression occurring before restricting the diet (309). Patients with interstitial nephritis fared better than did those with chronic glomerulonephritis or polycystic kidney disease. Independent factors that were related to a poorer prognosis included a higher initial serum creatinine, more proteinuria, or higher systolic and diastolic blood pressures before beginning therapy. No adverse effects of dietary therapy were reported and indices of protein nutrition were well maintained. Later, however, these investigators reported that there was a significant loss of muscle protein and a decrease in serum albumin and transferrin concentrations (anthropometric measurements, however, remained constant) in a subgroup of eight patients who continued the protein-restricted diet for an additional 5 years (310). Unfortunately, the energy intake of these eight patients was lower than prescribed (26 to 29 kcal/kg/day), so it is not clear that protein restriction alone causes loss of muscle.

In a prospective analysis of low-protein diets prescribed for at least 18 months, Rosman et al. reported results from 149 patients with creatinine clearances <60 mL/min/1.73 m^2 (311). The subjects were stratified according to age, sex, and renal function and then randomly assigned to a diet containing 0.4 to 0.6 g of protein per kilogram per day (predominantly high-biologic value protein) or an unrestricted diet. All patients received a multivitamin supplement including vitamin D; compliance was monitored by measuring the 24-hour urea nitrogen excretion every 3 months. Based on an analysis of changes in

serum creatinine and its reciprocal, the authors concluded that the diet reduced the average rate of progression by a factor of about 3. In contrast to the results of Maschio et al. (308), it appeared that older patients and those with chronic glomerulonephritis had a better response than did those with interstitial nephritis or polycystic kidney disease.

After 4 years of follow-up, Rosman et al. (311) examined responses from 153 of the 248 patients initially entering the study. There was smaller, but significant, benefit of dietary protein restriction on progression; it was most apparent in the group with more advanced renal insufficiency. Men but not women continued to show slowing of progression. However, the benefit was less than the response initially reported. Body weight and serum proteins were stable over 36 months of dietary therapy, indicating that the regimen preserved nutritional status. However, compliance with the diet (based on information) in the report and calculated by a standard method [162]) was not great; on average, the diet contained >0.7 g protein/kg/day. Parenthetically, the prescribed intake of 0.4 g protein/kg/day for patients with advanced CRF would be less than the minimum daily requirement of 0.6 g protein/kg/day (see Chapter 104, Dietary Considerations in Patients with Chronic Renal Failure and Transplantation) and, hence, cannot be recommended. The authors concluded that the most benefit occurred in patients with glomerulonephritis and that progression in patients with polycystic kidney disease appeared to be related entirely to blood pressure control. In the other diagnostic groups, blood pressure was not correlated with preservation of renal function.

In these reports as well as others, there is a recurring theme of a diminished response of patients with polycystic disease and as with other types of renal disease, hypertension and/or proteinuria are closely linked to progression (78,152,312). The mechanisms causing loss of renal function in polycystic kidney disease is unknown and the basis for the association between the severity of hypertension and/or proteinuria and progression is correlative. A low-protein diet will reduce the degree of proteinuria and can augment the antiproteinuric influence of angiotensin-converting enzyme (ACEi) (159,217,313–315). The influence of this effect of a low-protein diet on progression is unknown.

In Australia, Ihle et al. (315a) conducted a prospective randomized trial over an average of 18 months in 64 patients by comparing diets containing 0.4 g protein/kg/day to an unrestricted amount of dietary protein. The groups were initially well matched for blood pressure, serum creatinine (range, 4.0 to 11.0 mg/dL), serum calcium, and phosphorus concentrations. Changes in GFR were determined from measurements of the plasma disappearance of ^{51}Cr-EDTA (Fig.101-3). End-stage renal failure developed in 9 (27%) of 33 patients who followed the unrestricted diets compared to only 2 (6%) of 31 who were believed to be compliant with the protein-restricted diet (p <0.05). GFR decreased, on average, from about 15 to 6 mL per minute in patients eating an unrestricted diet, but did not change significantly in the protein-restricted group (e.g., the average change was about 2 mL/min). This trial was not based on an intention to treat design and the outcome of patients who did not comply with the restricted diet was not detailed. Based on urea excretion values, the average intake of the protein-restricted group who experienced slowed progression was more than 0.7 g/kg/day (162). On average, serum albumin and anthropometric measurements remained stable over the 18 months of follow-up, but weight, serum transferrin, and total lymphocyte count decreased significantly. Since the phosphorus content of the protein-restricted diet was approximately 30% to 40% less than that of the unrestricted diet, the relative importance of dietary protein versus phosphorus restriction on progression cannot be determined. The decline in some, but not all, nutritional indices raises concern about prescribing a diet with less than the minimum daily requirement of protein.

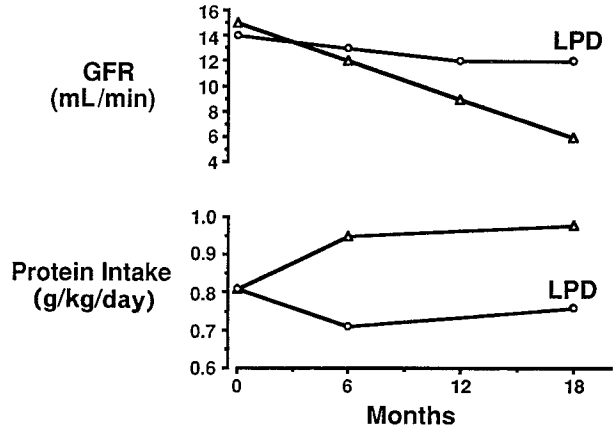

FIGURE 101-3. Changes in glomerular filtration rate (GFR) measured as the plasma clearance of ^{51}Cr-EDTA in patients prescribed a protein-restricted diet (*open circles*) or an unrestricted diet (*open triangles*). The calculated level of dietary protein based on urea excretion (120) is also shown. The low-protein regimen significantly reduced the decline in GFR. *LPD*, low-protein diet. (Figure was drawn from the results of Ihle et al. [109] and is reproduced with permission from Mitch WE, Klahr S. *Nutrition and the Kidney.* 2nd ed. Boston, MA: Little, Brown; 1993:254.)

In contrast to these reports indicating a beneficial effect of dietary protein restriction on progression, other investigators have not found a decrease in the loss of renal function. Williams et al. (316) randomly assigned 95 CRF patients to diets containing 0.6 g protein/kg/day or >0.8 g protein/kg/day; a third group was assigned to a diet with 0.8 g protein/kg/day plus a "low phosphorus intake" of 1 g/day. During a control period of 6 months, all patients were assigned to 0.8 g protein/kg/day, the diets were then begun, and the patients were followed for an average of 19 months while changes in creatinine clearance were measured. The decline in creatinine clearance of patients assigned to the 0.8 g protein diet was not different from that of the 0.6 g protein/kg/day group (Fig. 101-4). However, there was scarcely any difference in protein intake between

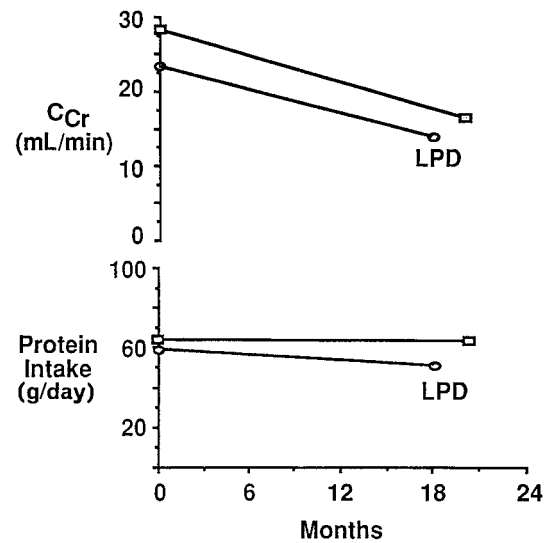

FIGURE 101-4. Changes in creatinine clearance (C_{Cr}) during therapy with a low-protein (*LPD; open circles*) and control diet (*open squares*) in the trial conducted by Williams et al. (173). Protein intake was calculated from urea excretion (120). There was no effect on the progression of renal failure. (From: Mitch WE. Dietary protein restriction in chronic renal failure. *J Am Soc Nephrol* 1991:2:823. © by American Society of Nephrology.)

the two groups; the calculated average difference was only 8 g protein/day (162). Since there was no difference in protein intake (see above), creatinine excretion did not differ between the groups nor did body weight change significantly (on average, there was a 1 to 2 kg decrease in weight in groups prescribed either the low-protein or the low-phosphorus diets). Since serum transferrin and immunoglobulins were normal, there was no evidence that prescribing the restricted diets adversely affected nutritional status.

A negative outcome was also reported following the completion of Northern Italian multicenter study (316a). In that trial, 456 patients were evaluated and 311 satisfied the criteria for enrollment (165 in the low-protein group and 146 in the control group). The rate of loss of renal function was evaluated by analyzing the time to dialysis or to doubling of serum creatinine level ("renal survival"). The outcome in terms of reaching end points was slightly, but not statistically, better in patients prescribed the low-protein diet (27 versus 42 in the control group). Assuming the patients weighed an average of 70 kg, the urea excretion values indicate that the protein intake was about 0.9 g/kg/day by the unrestricted diet and 0.78 g protein/kg/day by the low-protein diet group (overall, the difference in protein intake between the two groups was only about 9.5 g/day [162]). Clearly, simply assigning patients to a low-protein diet will not affect progression if the patients do not eat the prescribed low-protein diet. Regarding changes in nutritional status, only weights were recorded and, on average, they did not change.

Progression of Diabetic Renal Disease

Some, but not all, patients with insulin-dependent diabetes develop renal failure, but usually only after 15 to 20 years with the illness. Fortunately, the fraction of patients developing kidney failure seems to be decreasing, but it is still not known why only some patients are at greater risk. Factors that are associated with more rapid progression include hypertension and proteinuria and the importance of treating these problems with ACEi have been reviewed (317). Dietary protein restriction could exert an additional beneficial effect because short-term restriction of dietary protein reduces urinary protein in diabetic patients with microalbuminuria (318–321). This is relevant, because of the close association between proteinuria and the development of progression (322).

There are only a few publications examining whether a low-protein diet slows progression in diabetic nephropathy. In a 6-month evaluation of a diet set at 0.6 g protein/kg/day, Raal et al. (323) found that there was a decrease in proteinuria and slower loss of GFR compared to results from patients eating an unrestricted diet. The degree of compliance was not detailed and the short period of observation makes it unclear whether these responses can be assigned to beneficial effects of the diet. Using a different design, Walker et al. (318) changed the dietary protein of 19 insulin-dependent, proteinuric diabetic patients from an unrestricted protein intake (average, 1.13 g protein/kg/day) to a diet averaging 0.67 g protein/kg/day. They observed a significantly slower rate of loss of GFR (from 0.61 to 0.14 mL/min/month) and this benefit was independent of differences in blood pressure, energy intake, and glycosylated hemoglobin level. Zeller et al. (111) compared results achieved by a diet containing 0.6 g protein/kg/day with those measured in type 1 diabetic patients eating an unrestricted diet. Changes in renal function were evaluated from albuminuria, serum creatinine, and the renal clearances of creatinine and ^{125}I-iothalamate (i.e., GFR). Based on urea excretion values (162), the patients assigned to the low-protein diet ate an average of about 0.7 g protein/kg/day and no signs of malnutrition developed; the average decline in GFR and creatinine clearance was slowed by the protein-restricted diet. Note that some of the patients given the unrestricted diet did not exhibit progressive loss of GFR, emphasizing why it is important to ensure that patients in such trials have evidence of progression before beginning the trial (see Modification of Renal Disease subsequent discussion of the [MDRD] trial). The beneficial effects of the dietary regimen in the report by Zeller et al. could not be attributed to differences in blood pressure or glycemic control or to the frequency of examinations, leading to the conclusion that dietary restriction was the major factor. This conclusion is reinforced by meta-analysis of the influence of low protein diets on progression (324,325).

In the largest trial, Hansen et al. (326) studied 82 patients with type 1 diabetes and progressive diabetic nephropathy. The patients were randomly assigned to a diet of 0.6 g protein/kg/day or control diet and examined over 4 years. Fewer patients in the protein-restricted group developed ESRD or died and, when adjusted for cardiovascular disease at baseline, the relative risk of ESRD or death was reduced (0.07 to 0.72, $p < 0.01$). Dietary compliance was limited (0.89 in the treated group versus 1.02 g protein/kg/day in the control group). The loss of GFR was linear in both groups and did not differ statistically and nutritional status was not different. The authors conclude that modest dietary restriction can improve prognosis of patients with diabetic nephropathy.

Meta-analyses of Low-Protein Diets and Progression

Besides poor compliance, it is not clear why the outcome in these studies was so different. One factor could be that the number of patients was too small. To address this problem, several groups have analyzed published data about low-protein diets and progression using the meta-analysis technique (324–328). The meta-analysis technique is a method for pooling results from several studies to determine if there is a difference in the frequency of a unique event that can be associated with the intervention. In three meta-analysis, it was concluded that a low-protein diet exerted a beneficial effect on progression of renal failure (324,325,327). In each meta-analysis, the results were based on an intention to treat design. In this design, there must be randomized assignment to the intervention (i.e., diet level) and a unique outcome (e.g., progression to dialysis, a rise in serum creatinine.) must be discernible in each publication. In an intention to treat analysis, however, compliance with the diet is not considered and practicality of the intervention is examined. Fouque et al. (327) analyzed results from six studies of randomly assigned patients who were followed until they required dialysis or died and concluded that in five of the six trials there was a reduction in the number of renal "deaths" (61 in low-protein diet groups versus 95 in control groups). The authors calculated an odds ratio of the likelihood of progressing to ESRD and determined that dietary restriction significantly preserves renal function ($p < 0.002$). Pedrini et al. (324) examined results of 1413 patients participating in trials in which they were randomly assigned to a low protein or control diet. They concluded that assignment to a low-protein diet will reduce the risk of renal failure or death in nondiabetic patients by 33% ($p < 0.001$) and in diabetic patients, the risk of a decrease in GFR or creatinine clearance or an increase in proteinuria was reduced by 46% ($p < 0.01$). Fouque et al. (325) also examined the issue in even more patients by analyzing 7 trials and 1494 patients. Again, it was concluded that assignment to a low-protein diet was associated with slowing of the loss of kidney function. In contrast, Kasiske et al. (328) used many of the same data and concluded that the effect of dietary protein restriction was small.

Another factor that must be considered in assessing why there are contradictory conclusions about the influence of low protein diets on progression is compliance. This is important, because the hypothesis is that adherence to a low-protein diet will be associated with slowing of progression of renal insufficiency. Analysis of changes in renal function without taking compliance into account, does not test this hypothesis. For example, secondary analysis of the MDRD indicate that patients who adhered to the diet experienced slowing of the rate of loss of GFR (see below).

Low-Protein Diets Supplemented with Essential Amino Acids or Ketoacids

The results just discussed were derived from patients eating natural foods to meet the requirements for protein, calories, and other nutrients. Notably, dietary protein can not be reduced safely below 0.6 g protein/kg/day unless energy intake is sufficient and a supplement of EAA or a mixture of essential amino acids and their nitrogen-free analogs (ketoacids) are provided (see Chapter 104, Considerations for Patients with Chronic Renal Failure and Transplantation). In the United States, ketoacids are not commercially available, while EAA can be purchased as Aminess); both EAA and ketoacids are available in some other countries. During the 1980s, several reports provided evidence that a diet containing about 0.3 g protein/kg/day plus a supplement and sufficient calories was nutritionally sound in patients with advanced CRF, because they promoted neutral nitrogen balance and maintained normal nutritional indices (e.g., serum proteins) during long-term therapy (212–214,218,227). Regarding the influence of these regimens on progression of CRF, Alvestrand et al. (329) studied 17 patients with well-defined rates of decline in the reciprocal of serum creatinine despite treatment with a standard, low-protein diet. They prescribed an EAA-based regimen (0.3 g protein/kg/day plus an EAA supplement) and reported significant slowing of the decline in the reciprocal of serum creatinine in 14 of 17 patients (329). Subsequently, this group conducted a prospective, randomized trial and concluded that the diet was not a major factor in achieving a slower rate of progression (330). Instead, they concluded that slowing of progression was more closely linked to a small (-2 mm Hg) reduction in diastolic blood pressure and, possibly, more frequent visits to the clinic. Examination of their data, however, reveals that 10 (18%) of the 57 enrolled patients did not exhibit any loss of renal function during the baseline period while they were eating an "unrestricted" diet, so it was not possible to slow progression. Second, there was poor compliance with the dietary regimen (the average difference in protein intake between the groups (0.65 versus 0.86 g/kg/day) was small.

There is evidence that the very-low-protein diet–ketoacid regimen can slow progression of CRF. Barsotti et al. (331) measured the decline in creatinine clearance of 31 patients who were eating the standard, low protein diet of 0.6 g protein/kg/day. They found that the loss of creatinine clearance was constant (i.e., linear) in patients with nondiabetic kidney diseases and the decline was interrupted in 11 of 12 patients who were switched to a regimen containing about 0.2 g protein/kg/day plus a supplement of the calcium salts of ketoacids. In a subsequent report, Barsotti et al. (331) reported that 27 of 48 patients appeared to be compliant with the ketoacid-based regimen and, in this subgroup, the average loss of creatinine clearance was reversed from −0.65 to +0.15 mL/min/month ($p < 0.005$) (331). Other investigators have used basic amino acids (i.e., ornithine and lysine) salts of ketoacids rather than the calcium salts to test whether progression is slowed. This formulation used with a diet containing 0.3 g protein/kg/day, seemed to slow the loss of renal function. In the initial study of 17 patients with well-defined rates of decline in the reciprocal of serum creatinine, 10 (59%) had a significantly slower rise in serum creatinine (or a decline in creatinine clearance) during an average of 20 months of therapy. Patients with advanced CRF (serum creatinine >8 mg/dL) did not experience slowing of progression (332). Subsequently, Walser et al. (226) studied patients with advanced CKD (the initial GFR averaged 13 ml/min) and compared this ketoacid-based regimen with an EAA-based regimen using a cross-over design. The ketoacid regimen appeared to slow progression (measured as changes in GFR) more effectively than the EAA regimen, but only 12 patients were studied. As expected from earlier reports about metabolic effects of this regimen, there was no weight loss and serum albumin remained normal (213,332).

The efficacy of a ketoacid-based regimen has been compared to the standard, low-protein regimen containing 0.6 g protein/kg/day but no supplement. Di Landro et al. (333) measured changes in the reciprocal of serum creatinine found in 44 patients assigned to the standard, low-protein regimen and 46 patients given the ketoacid regimen while being followed more than 3 years. Progression was significantly slower and parathyroid hormone and serum cholesterol both decreased in patients given the ketoacid regimen. These studies suffer because of the relatively small number of patients and the inclusion of results from some patients who had no evidence of progression.

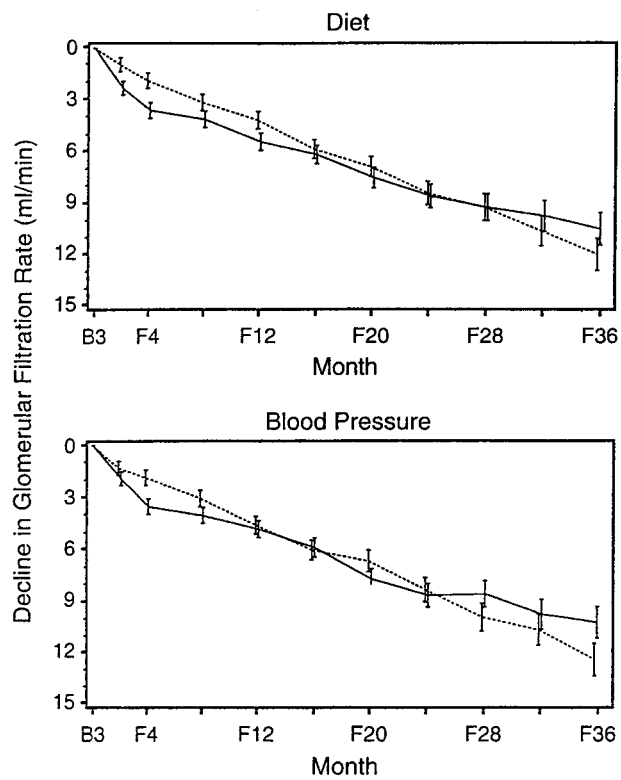

FIGURE 101-5. The decline in the mean (SEM) glomerular filtration rate from baseline values (B3) to the estimated value at 36 months (F36). **Upper panel,** comparison of results from those assigned to an unrestricted diet (about 1.3 g protein/kg/day, *dashed line*) and a low-protein diet (about 0.6 g protein/kg/day, *solid line*). **Lower panel,** comparison of results from those assigned to a "usual" blood pressure (mean arterial pressure <107 mm Hg, *dashed line*) and a "low" blood pressure (mean arterial pressure <92 mm Hg, *solid line*). (Reproduced from: Klahr S, Levey AS, Beck GJ, et al. The effects of dietary protein restriction and blood-pressure control on the progression of chronic renal disease. *N Engl J Med*. 1994;330:882, with permission.)

The Modification of Diet in Renal Disease Study

The problem of small numbers of patients in these and other studies prompted the National Institutes of Health to undertake a multicenter study, the Modification of Diet in Renal Disease (MDRD) trial. Initially, there was a feasibility trial in which 96 patients participated in order to test acceptance of the regimens; progression was not formally evaluated (334). Subsequently, two groups of patients were studied in the full-scale trial: Study A patients had GFR values of 25 to 55 mL/min/1.73 m^2, while study B patients had GFR values between 13 and 24 mL/min/1.73 m^2. Patients in study A were randomly assigned to one of two diets: at least 1 g protein/kg/day or 0.6 g protein/kg/day and to a mean blood pressure goal of either 105 or 92 mm Hg (78). Patients in study B were randomly assigned to the 0.6 g protein/kg/day or to a regimen containing 0.3 g protein/kg/day plus a ketoacid supplement and the same two blood pressure goals. In study B, there was no control group assigned to a high-protein or self-selected diet, because such a diet would be expected to worsen the uremic system. Average values of protein intake (estimated from urea appearance [162]) differed significantly among the groups in both studies A and B; differences in blood pressure were less impressive. The impact on progression was analyzed by measuring changes in ^{125}I-iothalamate renal clearance over an average follow-up period of 2.2 years.

During the first 4 months, GFR declined more rapidly in study A patients assigned to the low-protein diet and low blood pressure groups ($p = 0.004$ and $p < 0.01$, respectively). Thereafter, the rate of decline in GFR was 28% slower in patients assigned to the low-protein diet ($p = 0.009$) and 29% slower in the low blood pressure group ($p = 0.006$). In contrast, the loss of GFR of patients eating the unrestricted diet was remarkably linear with time. Based on an intention to treat analysis, however, neither prescribing the low-protein diet nor assigning patients to the lower blood-pressure goal slowed the loss of GFR (Fig. 101-5).

In analyzing results from patients with more advanced CKD (study B), the loss of GFR was 19% slower in the very-low-protein–ketoacid group compared to patients assigned to the standard, low-protein diet ($p = 0.065$). However, there was no statistical difference in the cumulative incidence of end-stage renal disease or death of patients. Patients with polycystic kidney disease did not benefit from either the diet or blood pressure control and African-American patients seemed to progress more rapidly than did other patients, but with a small number of subjects, the difference was not statistically significant. In fact, the sole positive effect of the MDRD Study in this report was that a lower blood pressure goal was associated with slowing of progression in patients who had >1 g proteinuria/day.

Do results from the MDRD settle whether a low-protein diet can slow progression? There are several reasons why the question is unsettled (78,207,335). First, the sample size was based on the assumption that GFR would decline at a rate of 6 mL/min/year in the control group (i.e., those prescribed an unrestricted diet and their usual blood pressure). However, progression was ~30% slower than expected overall and approximately 15% of patients in the study A control group did not progress. Obviously, it is impossible to determine if there is a beneficial influence of a low-protein diet on progression in patients who are not losing kidney function. Thus, the sample size may have been inadequate. In support of this concern, the meta-analysis results lead to the conclusion that low-protein diets slow the loss of renal function (see above). Second, there was a disproportionate number (~20%) of patients with polycystic disease and progression in these patients appears to be unaffected by either dietary manipulation or control of hypertension (336): inclusion of these patients may have obscured a benefit of a low-protein diet on progression in patients with other kidney diseases. Third, MDRD patients were give ACEi inhibitors in an unregulated fashion and use of this drug might have obscured the outcome (317). Fourth, the initial rapid

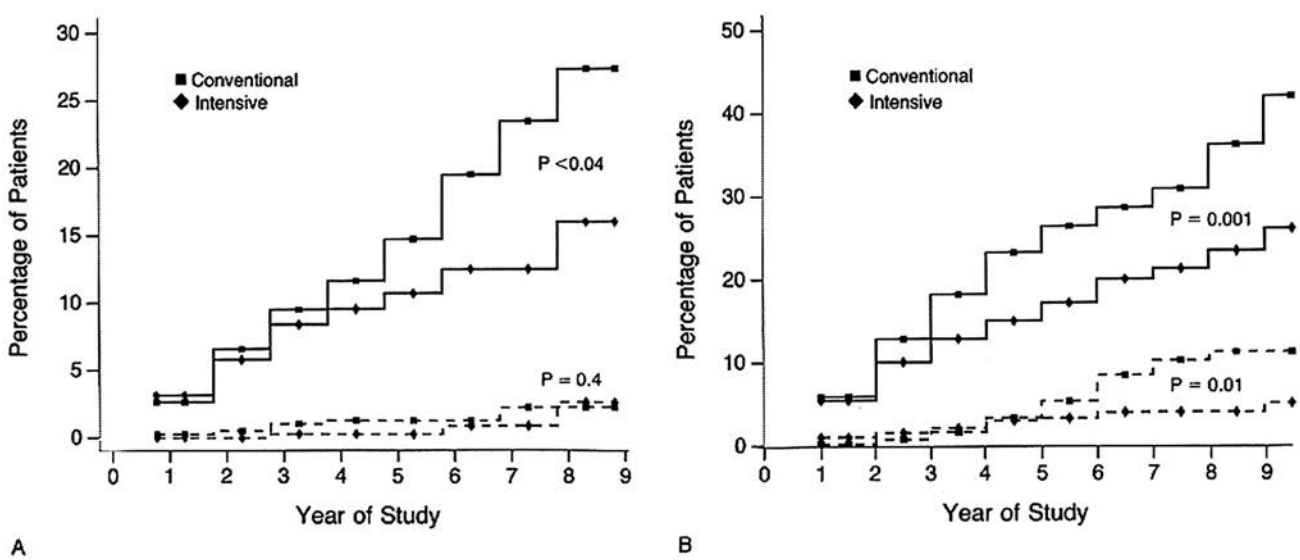

FIGURE 101-6. The cumulative incidence of urinary albumin excretion to a macroalbuminuria level (>300 mg/24 hr, *dashed line*) and a microalbuminuria level (<40 mg/24 hr, *solid line*). In the cohort without retinopathy (**A**), intensive insulin therapy reduced the risk of microalbuminuria by 34% ($p < 0.04$). In the cohort with early retinopathy (**B**) and an initial value of albuminuria 40 mg/24 hr or less, intensive insulin therapy reduced the risk of macroalbuminuria by 56% ($p = 0.01$) and microalbuminuria by 43% ($p = 0.001$) compared with conventional insulin therapy. (Reproduced from: the Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 1993;329:982, with permission.)

decline in GFR in patients assigned the low-protein diet and low blood pressure groups was unexpected and could have obscured a beneficial effect on progression, if the trial was more prolonged. For example, the evaluation of another metabolic intervention on kidney function (i.e., the DCCT Trial [337]) revealed no benefit of strict glycemic control for the initial 4 years and then the suppressing of proteinuria became obvious (Fig. 101-6). Finally, patients with more advanced CKD (study B) had no control group eating an unrestricted diet, so a comparison of benefit is limited. Moreover, the mixture of ketoacids was changed from the mixture that had shown a benefit in smaller trials conducted earlier (226,332,338). In fact, when changes in GFR of patients participating in the Feasibility Trial of the MDRD were analyzed, it was found that the loss of GFR was significantly slower ($p < 0.03$) in comparison to patients eating the standard, low-protein diet. More importantly, when MDRD results were analyzed according to the amount of protein actually consumed, a benefit of dietary protein restriction in study B patients was obvious: each 0.2 g/kg/day reduction in protein intake was associated with a 29% slower rate of loss of GFR and a 51% prolongation in the time to dialysis ($p < 0.01$) (95,339). No independent influence of the ketoacid regimen was detected in this secondary analysis, but, as noted above, the ketoacid mixture used in the MDRD Study differed from the mixture showing positive results in earlier studies (338). Thus, the benefit of a low-protein diet on the rate of loss of renal function remains controversial.

CONCLUSIONS

Results summarized in this and Chapter 103 (Considerations in Patients with Chronic Renal Failure, Acute Renal Failure and Transplantation) show that dietary manipulation reduces signs and symptoms of CRF and is nutritionally safe as long as there is no complicating factor, such as the existence of acidosis or infection. Thus, dietary manipulation in a treatment program that includes monitoring protein and calorie intake in consultation with a dietician (162,340) should be a mainstay of therapy for all patients. The observation that protein intake voluntarily decreases as renal insufficiency progresses is not a signal for increasing in dietary protein, because high protein diets will simply increase the degree of uremia (including hyperphosphatemia, aggravate hypertension, etc) and it will not raise serum proteins (218,235,341). On the contrary, a well-designed protein-restricted diet usually increases serum albumin (341). Regardless of an effect on progression, a low-protein diet could delay the need for dialysis by ameliorating uremic symptoms while maintaining nutritional status. Two publications provide strong evidence for the long-term safety of this approach (228,229). Patients in these studies were followed for an average of 1.5 to 2 years after GFR had decreased to 10 to 13 mL/min. Nutritional status was well maintained while they ate protein-restricted diets and dialysis was not required. Clearly, successful implementation of dietary therapy requires the motivation of both the patient and the physician. The principal, if not the only disadvantage of such therapy (assuming that it is successful in eliminating uremic symptoms), is the dietary restriction it entails, but it is reasonable to expect that dialysis therapy will be significantly delayed.

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CHAPTER 102 ■ PHOSPHATE, ALUMINUM, AND OTHER ELEMENTS IN CHRONIC RENAL FAILURE

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In the attempt to define uremic toxins, a great deal of attention has been directed at organic compounds. However, at least two inorganic compounds, phosphorus and aluminum, have been strongly implicated in the pathogenesis of a number of alterations that can occur in the uremic state. The metabolism and toxicity of phosphorus and aluminum in uremic patients and methods of preventing and treating disorders created by these two elements are reviewed in this chapter. Two other elements, magnesium and zinc, have also been implicated in the pathogenesis of some uremic symptomatology. The evidence of their involvement is, however, much weaker than that for phosphorus and aluminum, and, therefore, these latter two elements are only briefly reviewed herein. Finally, a variety of other disturbances in trace elements, both essential and nonessential, also have been recognized in uremic patients.

Phosphorus

Normally, 800 to 1,000 mg of phosphorus is ingested each day. The net absorption is approximately 60% of the ingested load, and this is readily excreted in the urine (1). In healthy persons, the serum phosphorus concentration is maintained in the normal range of 2.5 to 4.5 mg/dL. Even with renal failure, the serum phosphorus concentration is maintained within the normal range until the glomerular filtration rate falls to less than 25% of normal. The maintenance of phosphorus concentration in this context is accomplished initially by a reduction in renal tubular reabsorption of phosphorus and later by a combination of decreased renal tubular reabsorption of phosphorus and reduced gastrointestinal absorption of phosphorus. Renal tubular reabsorption of phosphorus is reduced as a consequence of increased secretion of parathyroid hormone (PTH) (2), as well as a non–PTH-dependent mechanism that occurs as a result of a reduction in renal functional mass (3). The most apparent reason for a decrease in the gastrointestinal absorption of phosphorus is decreased 1,25-dihydroxyvitamin D_3 (1,25$[OH]_2D_3$) (calcitriol), which occurs with more advanced chronic kidney disease (4). As glomerular filtration falls below 25 mL/minute, however, these alterations in phosphorus metabolism are no longer sufficient to maintain normal serum phosphorus concentration, and with further reductions in renal function there is a progressive rise in serum phosphorus concentration (3,4).

Consequences of Hyperphosphatemia

Hyperphosphatemia has been directly implicated in the pathogenesis of secondary hyperparathyroidism and its associated skeletal alterations (5), as well as metastatic calcifications (6), both of which occur with some frequency in uremic patients in whom phosphorus control is not maintained. In addition, phosphorus was implicated as a factor that may in part be responsible for accelerating functional deterioration in a diseased or damaged kidney (7–9).

Secondary Hyperparathyroidism. Hyperphosphatemia has been strongly implicated in the pathogenesis of the secondary hyperparathyroidism that is commonly seen in uremic patients (5,10). The suggested mechanism by which hyperphosphatemia induces hyperparathyroidism is as follows. As glomerular filtration rate decreases, there is a slight increase in serum phosphorus concentration. This causes the serum calcium concentration to fall, which in turn stimulates the secretion of PTH. The increased PTH concentration decreases renal tubular reabsorption of phosphorus, causing the concentration of serum phosphorus to return to normal. As glomerular filtration rate progressively decreases, increasing amounts of PTH are required to decrease tubular reabsorption of phosphorus and maintain serum phosphorus in, or close to, the normal range. This process has been termed the *trade-off hypothesis*. It is suggested that to maintain normal phosphorus concentration with progressive renal failure, the trade-off is the establishment of a hyperparathyroid state with its accompanying alterations.

Since it has been shown that there are receptors on the parathyroid glands for 1,25$(OH)_2D_3$ and that this hormone can suppress PTH synthesis, it was suggested that hypophosphatemia could suppress PTH concentration by stimulating 1,25$(OH)_2D_3$ production (11). Although early during the course of chronic kidney disease, phosphorus depletion stimulates 1,25$(OH)_2D_3$ production (12), later in the course phosphorus has no effect on 1,25$(OH)_2D_3$ concentration (13). In addition, the depletion of phosphorus suppresses PTH concentration in the absence of any change in serum calcium or 1,25$(OH)_2D_3$ (13,14). Phosphorus depletion also prevents the secondary hyperparathyroidism in vitamin D deficiency (15). These studies suggest that phosphorus *per se* has a direct effect on modulating PTH secretion.

Subsequent studies verified this conclusion. Fresh rat parathyroid glands were incubated in media with increasing phosphorus concentrations. A phosphorus concentration of 3 and 4 mM produced a three- to fourfold increase in parathyroid hormone secretion compared to 1 mM phosphorus. These authors also found that incubation in high phosphorus medium suppressed the ability of increasing concentrations of calcium to inhibit parathyroid hormone release (16). Nuclear transcript run-on experiments suggested that the effect of phosphorus is posttranscriptional (17). A protein, AUF1, binds to the 3′-untranslated region (3′-UTR) of the PTH transcript and stabilizes it (18). Binding of AUF1 to the PTH transcript is increased with hyperphosphatemia and hypocalcemia.

Dietary phosphorus also plays a role in the expression of the calcium sensing receptor. The calcium-sensing receptor (CaR) is a part of superfamily of G-protein–coupled transmembrane

receptors and is expressed on chief cells of the parathyroid gland and the luminal membrane of medullary collecting duct. Activation of the CaR by extracellular calcium ions engages the mitogen-activating protein kinase C cascade, through both G-protein-linked protein kinase C and a member of the Src tyrosine kinase family. The pathway eventually leads to activation of phospholipase A2 and the generation of arachidonic acid, which has a role in suppression of PTH. Therefore, PTH secretion is inhibited when the receptor is activated by elevated calcium concentration. Conversely, low serum calcium results in increased PTH secretion. Results from animal studies show that persistent hypocalcemia leads to a posttranscriptional increase in PTH mRNA and subsequently an increase in parathyroid cell proliferation (19).

In rats made uremic by 5/6 nephrectomy, those animals on a high-phosphorus diet (1.2% phosphorus) showed a 55% decrease in calcium-sensing receptor mRNA expression and an increase in parathyroid gland weight (2.77 ± 0.95 μg/g body weight versus 0.77 ± 0.16 μg/g body weight in controls). Immunofluorescence studies indicated that the receptor was downregulated in areas of proliferation. Dietary phosphorus restriction prevented both the decrease in calcium-sensing receptor transcript expression and parathyroid hyperplasia (20). This substantiates the view that abnormal control of parathyroid hormone secretion in uremia is due at least in part to the downregulation of the calcium-sensing receptor in hyperplastic parathyroid tissue.

Although hyperphosphatemia appears to be important in the causation of secondary hyperparathyroidism, control of serum phosphorus alone was not totally effective in the prevention of secondary hyperparathyroidism in uremia. In dogs, a reduction in dietary phosphorus intake that is proportional to the reduction in glomerular filtration rate initially prevents the expected rise in PTH concentration (21). Eventually, however, PTH concentration increases despite dietary phosphorus restriction and the maintenance of serum phosphorus concentration within the normal range. In addition, although it was routine practice for more than two decades to attempt to control serum phosphorus concentration in uremic patients, increased PTH concentration and histologic evidence of hyperparathyroid bone disease, which may be symptomatic, is still commonly seen in uremic patients (22–24). This is additional evidence that phosphorus restriction alone will not reverse the hyperparathyroid state.

Metastatic Calcification. Hyperphosphatemia was strongly implicated in the pathogenesis of the extraosseous calcification that occurs in uremic patients (6,25). The various types of extraosseous calcium phosphate deposited in uremic patients are listed in Table 102-1. The best documented types of extraosseous calcium-phosphate deposits that result from hyperphosphatemia are tumoral, periarticular and articular, and conjunctival calcifications (26–30).

TABLE 102-1

TYPES OF EXTRAOSSEOUS CALCIFICATIONS

Hyperphosphatemia related
Conjunctival
Calcific periarthritis
Tumoral
Other etiologies
Vascular calcification
Visceral calcification
Heart
Lung
Kidney

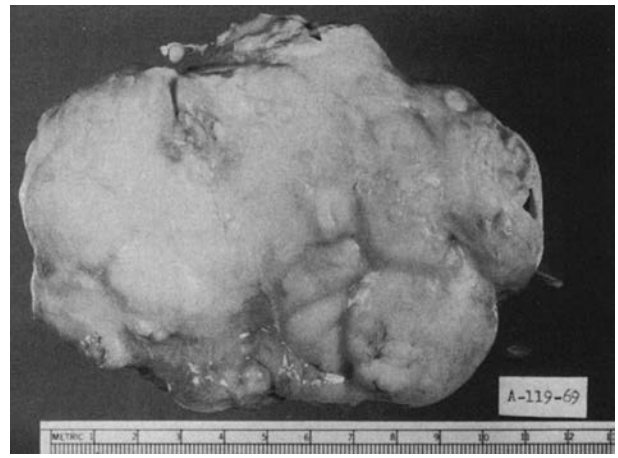

FIGURE 102-1. Excised tumoral calcification demonstrating fibrous enclosure of the calcium phosphate deposit. (From: Contiguglia SR, et al. Nature of soft tissue calcification in uremia. *Kidney Int* 1973; 4:229, Figure 2, with permission.)

Tumoral calcification represents calcium-phosphate deposits that are usually periarticular but do not involve the joints or their capsules. These deposits are encapsulated with a thick fibrous wall and are multiloculated (31) (Fig. 102-1). Calcium-phosphate deposits are present in a semisolid state as hydroxyapatite, and on examination the crystals are needle- and boat-shaped with a length of 30 to 40 nm and a width of 4.5 to 5.0 nm (25). In general, tumoral deposits cause little discomfort or other symptoms unless they are quite large, which may cause them to interfere with range of motion of the joint.

Conjunctival calcifications are characterized by an unpleasant gritty sensation in the eyes that is associated with a marked redness or inflammation of the conjunctiva (26,32). As with tumoral calcification, the calcium-phosphate deposit is hydroxyapatite. Local factors, such as minor tissue injury of the limboconjunctival epithelium secondary to a chronic decrease of tear fluid that occurs after each hemodialysis session, are considered as contributing factors to ocular calcification in dialysis patients (26).

Calcific periarthritis, like gout and pseudogout, is characterized by recurrent attacks of acute arthritis (26,31,32). In contrast to gout and pseudogout, however, the joint fluid contains neither uric acid nor calcium pyrophosphate crystals. Instead, there are extraarticular deposits of hydroxyapatite.

Tumoral, periarticular, and conjunctival calcifications share not only a similarity of the crystalline feature of the calcium-phosphate deposits but also the fact that their occurrence is closely associated with overt hyperphosphatemia. Not only can the occurrence of these deposits be prevented, but also even when present the deposits can be rapidly mobilized by lowering serum phosphorus concentration (33). Additional evidence that hyperphosphatemia is responsible for the production of these deposits is a study of a patient with extensive tumoral deposits. In this study, prior to renal transplantation and mobilization of these deposits, total body phosphorus was 969 g (normal 670 g), and calcium was 1,364 g (normal 1,185 g). Following transplantation, after the deposits had spontaneously mobilized (Figs. 102-2 and 102-3), total body phosphorus had fallen to 670 g and calcium to 1,282 g. The results of this study showed that total body phosphorus was increased much more than total body calcium, an increase that would be consistent with the fact that tumoral calcification is a condition of marked, total phosphorus excess.

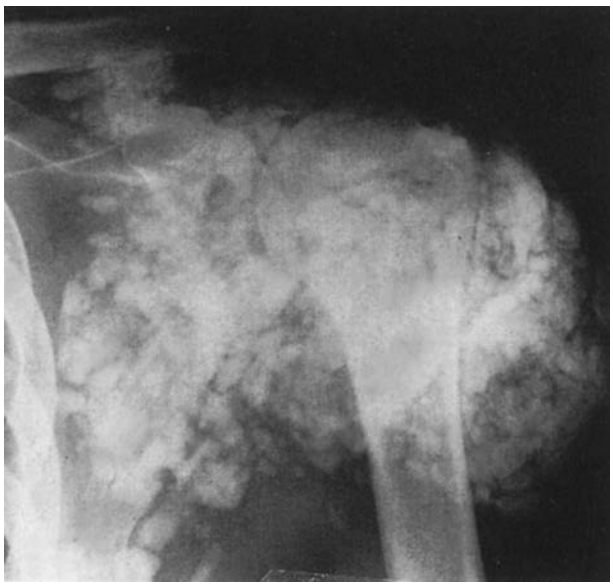

FIGURE 102-2. Tumoral calcification around the left shoulder prior to renal transplantation.

In the two other major types of extraosseous calcifications, visceral and arterial, the role of hyperphosphatemia in their pathogenesis is less clear. Visceral calcification occurs in a variety of tissues but exerts its major clinical consequences in the heart and lung, where it can cause pulmonary insufficiency, myocardial failure, or conduction disturbances (34,35). In older studies the reported incidence of visceral calcification was 40% to 45% in nondialyzed uremic patients and 75% in dialyzed uremic patients (36). Unfortunately, there are few recent studies reporting the frequency of visceral calcification in uremic patients. The available studies largely used indirect means of estimating visceral calcification (37–39). Because of the paucity of recent reports of this complication, it would appear that visceral calcification occurs much less frequently now than previously. The reason for this is unclear but it seems unlikely that phosphorus control is responsible based on evidence suggesting that hyperphosphatemia is not important in the pathogenesis of these types of deposits. First, the presence of visceral calcification seems to bear no relationship to the presence or absence of tumoral, periarticular, or conjunctival calcifications, which are known to result from hyperphosphatemia. In addition, it is not known whether prevention or correction of hyperphosphatemia has any effect on the formation or mobilization of visceral calcification. Finally, the calcium-phosphate deposit in visceral calcification is markedly different from that in tumoral, periarticular, and conjunctival deposits. Unlike these latter deposits, which are hydroxyapatite, visceral calcification is an amorphous compound high in magnesium, aluminum, and pyrophosphate and has the thermochemical properties of whitlockite (29,35). These unique features of visceral calcification suggest that factors other than hyperphosphatemia are important in the pathogenesis of these deposits.

FIGURE 102-3. Resolution of tumoral deposit about the left shoulder 3 months following successful renal transplantation.

Finally, although there is an impression that control of hyperphosphatemia may reduce the incidence of visceral calcification, there is no conclusive evidence supporting this supposition. In addition, other changes in dialytic techniques, such as more frequent dialysis, improved efficiency of dialysis, and water treatment for the preparation of the dialysate, were instituted about the time phosphorus control was initiated, thereby further confusing the issue of whether hyperphosphatemia is a major factor in the etiology of these deposits.

Vascular Calcification. There are some studies that suggest that hyperphosphatemia does not play an important role in the pathogenesis of vascular calcification (40–43). Vascular calcification is extremely common in uremic patients, being present in almost all uremic patients over the age of 50 (40–42). In some studies it did not correlate well with hyperphosphatemia (33,40–42). However, more recent evidence indicates that hyperphosphatemia may initiate phenotypic transformation of human vascular smooth muscle cells such that they express osteoblastic specific genes, as discussed further below. This suggests that abnormalities in phosphorus homeostasis may play a role in vascular calcification.

It was suggested that the calcification represents an accelerated aging of the vascular tree. This causes calcium to be deposited locally and the calcification may not be the result of hyperphosphatemia or some other alteration in the milieu (43). In uremia the predominant form of calcification is Mönckberg's medial sclerosis, at least in relatively young patients (44). Calcification of the media is associated with deposition of bone matrix proteins through an active cell-mediated process (45). Intimal calcification is also observed; however, typical risk factors for atherosclerosis including older age, smoking, and dyslipidemia are generally present in these patients (44). Age, duration of dialysis, and diabetes mellitus are associated with increased risk of vascular calcification. Extracellular inorganic phosphate regulates human aortic smooth muscle cell (HSMC) culture mineralization *in vitro*. HSMC cultured in media containing a phosphorus concentration comparable to that seen in hyperphosphatemic individuals, showed a dose-dependent increase in calcium deposition. In addition, HSMC expression of osteoblast-specific genes such as osteocalcin and cbfa-1 were increased (46).

A matrix gamma-carboxyglutamic acid (Gla) protein knockout mouse was generated (44). The mouse exhibited

extensive and lethal calcification of the media of all elastic arteries. Matrix Gla protein (MGP) is an inhibitor of calcification in the vessel wall and its activity is dependent on vitamin K–dependent gamma carboxylation. MGP mRNA is constitutively expressed by normal vascular smooth muscle cells and is upregulated in cells adjacent to medial and intimal calcification (45). Several Gla-containing proteins are expressed in human vasculature. Another inhibitor of ectopic calcification is serum protein α2-Heremans-Schmid glycoprotein (Ahsg, also known as fetuin-A). Ahsg-deficient mice are phenotypically normal, but develop severe calcification of microvasculature and tissues on a mineral and vitamin D–rich diet (46). Dysregulation of these proteins by the uremic state may play a role in the vascular calcification observed in end-stage renal disease (ESRD).

A final type of extraosseous calcification is known as calcific uremic arteriolopathy (*calciphylaxis)* (46–49). It is a condition in which the patient presents with a painful violaceous, mottled lesion of the extremities or trunk that progresses to skin and subcutaneous tissue necrosis, nonhealing ulcers, and gangrene. According to recent studies, calciphylaxis occurs more frequently than previously thought, with an incidence of 1% per year and a prevalence of 4% in dialysis patients (52–57). Female gender, hyperphosphatemia, high alkaline phosphatase, and low serum albumin concentration are risk factors for calciphylaxis (58). The histologic picture consists of calcifications of small subcutaneous arteries, and/or arterioles and infarctions of the adjacent subcutis and skin. These two changes are called primary and secondary lesions, respectively. Vascular calcification alone is usually insufficient to initiate infarction; other factors such as thrombosis or reduced perfusion may be involved (59). Recent studies emphasize the crucial role of a multidisciplinary therapeutic approach focusing on correction of the underlying abnormalities of calcium and phosphorus homeostasis (use of noncalcium-containing phosphate binders), local wound care with debridement of necrotic tissue, and aggressive treatment of infectious complications. The utility of parathyroidectomy, corticosteroid therapy, and hyperbaric oxygen remains controversial. Rapid improvement after intravenous pamidronate treatment in a patient with chronic renal failure was recently reported (60). Despite intensive combined treatments, the prognosis remains poor. Overall 1-year survival is 45% and 5-year survival is 35%. The relative risk of death is 8.5 compared with other dialysis patients (58).

Hyperphosphatemia was linked to hemodynamic alteration in patients with ESRD. These alterations are characterized by higher diastolic and mean blood pressures, and higher cardiac index caused by an increased stroke index and higher heart rate. Hyperphosphatemic patients tended to have a higher common carotid artery diameter, but similar carotid artery intima-media thickness, and lower carotid wall-to-lumen ratio than in patients with "normal" serum phosphorus concentration. As a result of lower wall-to-lumen ratio in the presence of higher mean blood pressure, the carotid tensile stress was higher in hyperphosphatemic ESRD patients. These findings suggest that, in stable ESRD patients, hyperphosphatemia is associated with increased blood pressure, hyperkinetic circulation, increased cardiac work, and high arterial tensile stress. These hemodynamic abnormalities could favor the development of cardiovascular complications and contribute to high cardiovascular morbidity and mortality (61). Another consequence of hyperphosphatemia in patients with ESRD is widespread calcification of the cardiovascular system, including the myocardium, conducting system, cardiac valves, and coronary arteries. Cardiovascular calcification was linked to an increased risk of cardiovascular events, including myocardial infarction, fatal dysrhythmia, congestive heart failure, and valvular heart disease (62).

Aluminum

Normal Aluminum Metabolism

Aluminum is the fifth most common element in the earth's crust, but because of its insoluble nature only trace amounts are actually ingested. Studies suggest that 2 to 8 mg of aluminum is ingested daily as a result of contamination in food (63,64). Because aluminum is used as a filler in some foods such as cheese and pickles and is a constituent of baking powder, eating habits can also affect the amount of aluminum ingested. An additional source of aluminum is municipal water supplies. Aluminum is commonly used to flocculate and remove turbidity from water sources. This can leave aluminum residues as high as 4 mg/L (65).

Most evidence suggests that the gastrointestinal tract represents a formidable barrier to aluminum absorption and little of the ingested aluminum is absorbed. This is based on the fact that total body aluminum is quite low, 35 to 40 mg, and does not increase with aging. In addition, urinary aluminum, which is believed to be the major pathway for the elimination of any systemic aluminum and reflects gastrointestinal absorption of aluminum, is approximately 10 μg/day (66).

The majority of studies showed that aluminum is absorbed passively by the gastrointestinal tract. This appears to occur largely if not entirely through the paracellular pathway (67–69). As of this time, only four factors are clearly shown to modulate aluminum absorption. These are the amount of aluminum compound ingested (58), the solubility of the aluminum compound (71), the integrity of the tight junctions of the small intestine (67–69), and the uremic state (72). By increasing aluminum intake from milligram quantities to gram quantities, aluminum absorption can be increased by 20 to 50 times normal to as high as 500 μg/day (70). More soluble aluminum compounds such as aluminum lactate, as compared to aluminum hydroxide, are also more readily absorbed (71). The factor that has the largest effect on aluminum absorption is the opening of the tight junction between intestinal cells. This is exemplified by giving aluminum compounds with citrate (67). Not only is aluminum citrate relatively soluble as compared to other aluminum compounds, but also citrate, probably as a result of chelating the calcium necessary for the maintenance of tight junctions, opens the paracellular pathways (67,68). This results in a very rapid and markedly enhanced aluminum absorption (67,68). Recent studies further showed that normal individuals chronically ingesting calcium citrate, given as a calcium supplement, also have enhanced aluminum absorption from normal dietary sources (73).

Although it was suggested that both vitamin D (74) and PTH (75) might enhance aluminum absorption from the gastrointestinal tract, this is not firmly established. In addition, it has been proposed that the status of body iron stores has an effect on aluminum absorption (76). Aluminum and iron metabolism may be closely interrelated, and there is a correlation between serum ferritin and plasma aluminum concentration in dialyzed uremic patients (76).

In the usual environmental exposure, the lungs represent major barriers to aluminum absorption. It seems that most inhaled aluminum is exhaled, and only a small amount of aluminum is retained in the pulmonary parenchyma. This supposition is based on the fact that pulmonary aluminum content increases with age but does not correlate with other tissue stores of this element (77,78). There is, however, evidence that industrial workers exposed to aluminum fumes absorb some of the aluminum as manifested by an increase in urinary aluminum excretion (79).

The skin is largely impervious to aluminum. Even with increased exposure, as can occur with antiperspirants; there is

little evidence that any aluminum can be absorbed through the skin.

The kidney is the major avenue for the elimination of any absorbed aluminum. Although renal excretion of aluminum was not fully evaluated, aluminum clearance seems to be 5% to 10% of the glomerular filtration rate (80,81). This is consistent with the finding that aluminum is approximately 90% to 95% protein bound in plasma (82). However, even the normal kidney has limited ability to excrete aluminum, and when large parenteral loads of aluminum are administered, varying amounts of the administered aluminum are retained (81).

Normally there is very little aluminum in bile, and even with acute aluminum loading biliary excretion of aluminum increases only modestly (83). Therefore, the biliary route of eliminating aluminum cannot replace that of the kidney, and loss of renal function markedly places the individual at risk of aluminum overload.

Effects of Uremia on Aluminum Metabolism

There are several reasons that an increased body burden of aluminum would be expected in uremic patients. First, the uremic state in experimental animals is associated with enhanced aluminum absorption from the gastrointestinal tract. The mechanism, however, remains unknown (72). The finding that tissue stores of aluminum were increased in more than 85% of a group of nondialyzed uremic patients who were studied during a period when aluminum-containing, phosphate-binding gels were not routinely administered is in keeping with the finding of enhanced absorption of aluminum in the uremic state (77, 78) (Fig. 102-4). In addition in the past, uremic patients sometimes receive large oral loads of aluminum hydroxide, which is given to reduce dietary phosphorus absorption.

In the dialyzed uremic patient natural barriers to aluminum absorption—gastrointestinal tract, lungs, and skin—can be circumvented by the parenteral administration of aluminum. This can occur by dialyzing the patient with dialysate prepared with aluminum-contaminated water (84). Aluminum is bound in plasma largely to transferrin (82,85). Transferrin has been suggested as an ideal aluminum carrier in plasma because most of the iron sites are normally unoccupied. Transferrin-binding capacity would normally be around 600 μg of aluminum per liter of plasma. Because of this binding capacity, any aluminum transferred from the dialysate to the patient is rapidly bound, maintaining a gradient from dialysate to patient and thus promoting additional aluminum uptake. Therefore, even a small amount of aluminum in the dialysate can result in the transfer of a large aluminum load to the patient during dialysis, appreciably enhancing the body burden of this element.

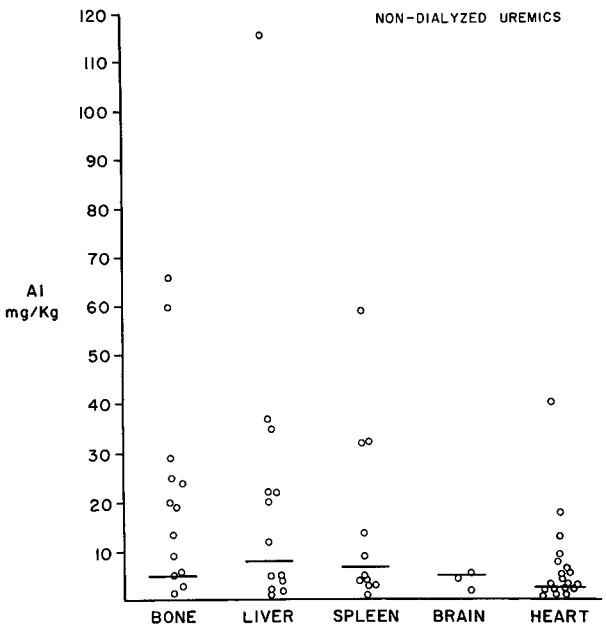

FIGURE 102-4. Tissue aluminum levels in nondialyzed uremic patients. The upper limits of values found in nonuremic control subjects are shown by the *horizontal line*.

Compromised renal function prevents the elimination of most of the absorbed or administered aluminum, further enhancing the body burden of this element. In addition to enhanced gastrointestinal absorption and impaired urinary excretion of aluminum, uremic animals also tend to have an altered compartmentalization of aluminum in the body. In uremic animals, bone aluminum uptake is enhanced, whereas liver aluminum uptake seems to be slightly decreased (86).

Another tissue in which aluminum uptake may be enhanced in uremia is brain (66). Although nonuremic patient populations, such as individuals on chronic total parenteral nutrition (TPN) and premature infants receiving large amounts of albumin or TPN solutions, receive large intravenous aluminum loads and have elevated bone and liver aluminum concentrations, aluminum neurotoxicity was not observed with these latter two conditions (81, 87). Similarly, studies showed that even animals given large parenteral loads of aluminum do not have nearly the high aluminum concentrations in brain as do uremic patients dying of aluminum neurotoxicity (88). This might suggest that the blood–brain barrier has to be compromised for brain aluminum to be markedly increased. Such an abnormality in the blood–brain barrier may be found in advanced uremia (89).

Aluminum Toxicity

Neurotoxicity. Aluminum neurotoxicity was initially described in 1886 (90) and was rediscovered in 1937 (91). These studies showed that local administration or application of aluminum to the brain caused animals to develop a seizure disorder. Subsequently it was found that certain species of animals, cats and rabbits, also developed neurofibrillary tangles following the application (92–96) or parenteral administration of aluminum (97). The finding that aluminum could induce neurofibrillary tangles in animals prompted the suggestion that aluminum was a possible cause of Alzheimer's disease. This theory was, however, disputed because not all patients with Alzheimer's disease have high brain aluminum concentrations (96). Moreover, the neurofibrillary tangles and biochemical alterations in Alzheimer's disease and those of aluminum intoxication are different. In addition, the senile plaques that are common in Alzheimer's disease are not seen in experimental aluminum toxicity (96).

In 1962 and 1975, two patients, one of whom had worked in an aluminum plant, developed a dementing neurologic disease in association with increased brain aluminum concentrations. These were the first reports to suggest that aluminum neurotoxicity could occur in humans (98,99).

In 1972 a new and distinct neurologic disease was first described in dialyzed uremic patients (100). The disease (dialysis encephalopathy or dialysis dementia) is characterized by an intermittent speech disturbance—a stuttering or stammering that is frequently first noted, and often intensified, during or immediately following the dialysis procedure. The speech disturbance is associated with personality changes, parietal lobe findings such as directional disorientation, seizures, myoclonus, and auditory and visual hallucinations. The findings are progressive, and over a 7- to 9-month period following the onset of symptoms the patients become totally mute and unable to

perform purposeful movements. Death rapidly ensues. Following the initial description of this disease, other dialysis patients from many different geographic areas have been found to have a similar syndrome (100–108).

Initially, the major considerations for the etiology of this disease were slow virus infection, communicating hydrocephalus, and environmental toxin (109). Slow virus infection was excluded because of the lack of histologic changes in brain and failure to transmit the disease into primates by inoculating brain tissue from patients who had died of encephalopathy. Similarly, communicating hydrocephalus was excluded by computed tomographic scans and lack of anatomic changes consistent with this condition. On the basis of the exclusion of these causes and the increasing awareness that dialysis encephalopathy was occurring in epidemic proportions in some dialysis units but rarely, if ever, in other units, strongly suggested that an environmental toxin was responsible for the condition.

In 1976, one trace element, aluminum, was initially shown to be consistently higher in brain gray matter in patients dying of dialysis encephalopathy than in dialysis patients dying of other causes and in nonuremic controls (110). This finding was confirmed by three other groups (112,114,116) and was followed by large epidemiologic studies in the United Kingdom that showed that in areas with a high incidence of dialysis encephalopathy, aluminum was present in high concentrations in water used to prepare the dialysate. In contrast, in areas where dialysis encephalopathy was rarely seen, aluminum content was low (111,112). This was subsequently supported by studies in France (102) and the United States (101,103,108,113), which also showed that centers with a high incidence of dialysis encephalopathy also had large amounts of dialysate aluminum. Further evidence incriminating aluminum as the toxin appeared when the number of cases of dialysis encephalopathy profoundly decreased when aluminum was removed from the water used to prepare the dialysate. The final evidence for neurotoxicity of aluminum comes from reports showing that aluminum toxicity can be reversed with chelation (114,115).

In contrast to dialysis encephalopathy that results from a chronic exposure to aluminum from aluminum-contaminated dialysate or less commonly orally administered aluminum compounds, an acute form of aluminum neurotoxicity has been observed in uremic patients (116,117). This occurs under three different conditions: dialysis performed with dialysate highly contaminated with aluminum (118), the oral administration of citrate compounds in association with aluminum compounds (116,117), and marked elevation of plasma aluminum concentrations resulting from deferoxamine therapy (119). It would appear that virtually all nondialyzed children (120,121) and adults with uremia (116,117) that developed aluminum neurotoxicity did so from the ingestion of the combination of citrate and aluminum compounds. Although patients may present with rather mild symptoms such as a speech disturbance similar to dialysis encephalopathy, more often there is an acute explosive onset of symptoms. In the adult patient, symptoms consist of agitation, confusion, myoclonic jerks, grand mal seizures, and obtundation, and can culminate in death within days of the onset of symptoms (116,117). In children aluminum neurotoxicity tends to be somewhat different in that it is usually manifested by regression of verbal and motor skills (120,121). This is exemplified by the finding that children that just started to walk and talk may lose these abilities.

If acute toxicity occurs as a consequence of high aluminum concentration in the dialysate, it usually develops during the dialysis procedure (118). Symptoms resulting from deferoxamine therapy occur during or immediately following a dialysis for which deferoxamine was administered (119). Although symptoms of acute aluminum intoxication can occur months after ingestion of the combination of citrate and aluminum compounds, it is not uncommon for symptoms to develop in a matter of weeks following the combined ingestion of these two compounds (116,117).

Standards for permissible concentrations of aluminum in water (<10 μg/L) used to prepare dialysate were established in 1981. By 1990 more than 99% of dialysis units in the United States were treating water to remove aluminum. This was associated with a reduction in the number of dialysis patients developing dialysis encephalopathy from 229 cases (0.4%) in 61,450 dialysis patients in 1980 to 129 cases (<0.1%) in 140,555 dialysis patients in 1990 (122).

Several case reports of acute aluminum encephalopathy following alum bladder irrigation were reported (123,124). Alum (aluminum ammonium sulfate) was introduced as a treatment for bladder hemorrhage in 1982 and is used as a second-line agent if bleeding does not stop with saline irrigation. In general, alum does not cause symptoms in patients with normal renal function even though increases in serum aluminum concentration do occur. Ten percent of alum by weight is aluminum; therefore, one liter of a 1% alum solution infused at a rate of one liter per hour exposes the bladder mucosa to 24 g of aluminum per day. Whether a damaged mucosa absorbs more aluminum than the mucosa of a normal bladder remains unclear. Acute encephalopathy was reported at serum concentrations as low as 17 μg/L. There are at least 12 case reports in the literature, and all but one had some degree of chronic kidney disease. Therefore, alum should not be used as a bladder irrigant in patients with acute or chronic renal failure.

The diagnosis of aluminum neurotoxicity is largely based on a history of either oral or parenteral exposure to aluminum in uremic patients that demonstrate the classic clinical features and had other neurologic disorders excluded by appropriate studies. The most useful laboratory test supporting the diagnosis of aluminum neurotoxicity is the electroencephalogram (EEG), which demonstrates early changes that precede clinical symptoms (125). Unlike most metabolic encephalopathies, including uremic encephalopathy, which exhibits a generalized slowing on the EEG, in aluminum intoxication the background rhythm is relatively normal with multifocal bursts of slow or delta waves frequently accompanied by spike activity. The CT scan may appear normal or only show mild cortical atrophy. There are no cerebrospinal fluid (CSF) abnormalities in aluminum neurotoxicity and aluminum concentration in the CSF is not increased. Histologic changes that occur in the brains of patients dying of dialysis encephalopathy are nonspecific and similar to changes seen in dialysis patients dying of other causes (109). Plasma aluminum concentrations in chronic intoxication are increased and frequently in the range of 100 to 200 μg/L. With acute aluminum intoxication plasma aluminum concentration is usually in excess of 500 μg/L.

Because anatomic changes are minimal and the disease is reversible with treatment, aluminum appears to cause neurologic impairment by interfering with the brain's biochemistry. Although aluminum inhibits hexokinase activity (82,126,127) and affects brain calmodulin function (128), the specific mechanism by which aluminum interferes with neurologic function remains to be elucidated.

Skeletal Toxicity. Aluminum skeletal toxicity is characterized by vitamin D–resistant osteomalacia, decreased or absent bone formation, low PTH concentration, mildly elevated serum calcium concentration, proximal myopathy, and failure to respond to vitamin D therapy (129–131). Unlike virtually all other osteomalacic states, serum phosphorus concentration is either normal or more commonly slightly elevated. The classic clinical feature of this disease is pathologic fractures (Fig. 102-5). Evidence for the skeletal toxicity of aluminum seems to be as convincing as it is for the neurotoxicity of aluminum. The first evidence that aluminum caused bone disease in

FIGURE 102-5. Pathologic fractures of the metatarsals found in a patient with aluminum-associated osteomalacia.

dialyzed uremic patients came from European epidemiologic studies that showed that fracturing osteomalacia tends to occur in association with dialysis encephalopathy in dialyzed uremic patients in areas with high aluminum concentration in the water used to prepare dialysate (111,112). As with encephalopathy, fracturing osteomalacia could be largely prevented or spontaneously healed by removing aluminum from dialysate (107,112,132). This abnormality can, however, also result from oral aluminum exposure, and this has been documented by two studies in which bone biopsies were randomly done in large dialysis populations with only oral aluminum exposure. These studies showed that 25% to 30% of the dialysis patients had aluminum-associated bone disease (22,23).

Besides the epidemiologic data, additional evidence for aluminum skeletal toxicity comes from the finding of markedly increased bone aluminum content in this condition compared to content found in other types of renal osteodystrophies (131). Aluminum also produced osteomalacia in rats, dogs, and pigs (88,133,134).

PTH seems to have a significant effect on the way aluminum manifests its skeletal toxicity. It was previously noted that patients with overt hyperparathyroid bone disease were protected from developing osteomalacia when they were dialyzed chronically with aluminum-contaminated dialysate (135). It was found that patients with hyperparathyroid bone disease that were treated with parathyroidectomy might subsequently develop aluminum-associated osteomalacia (136). In addition, uremic, parathyroidectomized animals given parenteral aluminum subsequently develop more severe osteomalacia with less bone aluminum than do nonparathyroidectomized uremic animals given a comparable parenteral aluminum load (86). Conversely, it was suggested that following chelation therapy for aluminum-induced osteomalacia, the patient might develop hyperparathyroid bone disease.

Diagnosis of aluminum-associated bone disease is based on the clinical features of bone pain; pathologic fractures, usually of the ribs and femoral neck; and proximal myopathy. Laboratory confirmation is based on bone biopsy specimens showing classic osteomalacia with aluminum, as determined by aluminum histochemical staining, present between the junction of calcified and noncalcified bone, and markedly increased quantitated bone aluminum (137). Plasma aluminum concentration is characteristically elevated, documenting aluminum exposure. In the past the majority of cases of osteomalacia in uremic patients were a result of aluminum (138). Although it was initially believed that adynamic bone disease in uremic patients was also a result of aluminum intoxication (139), evidence suggests that this is not the case (140).

Although the mechanism by which aluminum causes skeletal toxicity is not fully elucidated, aluminum has several effects that could cause skeletal injury. Aluminum was used in the tanning industry because of its ability to cross-link collagen fibrils (141). It was shown that aluminum cross-links collagen fibrils in bone matrix, destroying bone inductive properties (142). The presence of aluminum at the junction between calcified and noncalcified bone (137) could explain how it renders the osteoid noncalcifiable despite vitamin D replacement and an increased calcium × phosphorus product. In addition, aluminum, especially in association with citrate, is a potent inhibitor of crystal formation, and this could affect bone formation (143). Aluminum also can inhibit bone phosphatases (144). Finally, aluminum is deposited in the mitochondria of osteoblasts (145), which could injure the osteoblasts and prevent bone formation.

Hematologic Toxicity. Aluminum also is toxic to the hematopoietic system. In humans it produces a microcytic hypochromic anemia in the setting of adequate iron stores (146–148). Aluminum also blunts or prevents the response to erythropoietin (149). Evidence that this effect is a result of aluminum is the finding that anemia improves rapidly when aluminum exposure is eliminated or when aluminum is removed by chelation (147,148). Aluminum also can cause anemia in rats and rabbits (150,151).

The specific mechanism responsible for aluminum-associated anemia has not been clearly identified. It appears, however, that iron and aluminum metabolism is closely interrelated. As noted previously, like iron, aluminum is largely transported in plasma by transferrin. This suggests that the mechanism for the induction of aluminum-induced anemia is transferrin's deposition of aluminum at sites where iron is normally unloaded. Aluminum also inhibits ferroxidase and ceruloplasmin activity, which could also affect iron metabolism (152).

Aluminum Speciation and Toxicity. The speciation of aluminum may determine aluminum toxicity for the various organ systems. Aluminum, because of its high formation constant with transferrin, citrate, phosphate, and hydroxides, cannot exist in plasma or the body in a free form. Formation constants are greater for aluminum with transferrin and citrate than with hydroxides (153). Therefore, in plasma, aluminum exists primarily in association with transferrin and secondarily with citrate. In view of the association between acute aluminum neurotoxicity and high concentrations of small molecular species of aluminum complexed to citrate and deferoxamine, it is suggested that these species most readily cross the blood–brain barrier and are responsible for neurotoxicity of this element. The aluminum effect on erythropoiesis and its interference with iron utilization was studied in vitro (154). Whereas aluminum alone had no effect on human erythroid cultures, aluminum transferrin mixture caused a dose-dependent inhibition of erythroid colony growth. This is consistent with aluminum-transferrin

complexes being responsible for the hematopoietic toxicity of aluminum noted *in vivo*. Evidence was presented suggesting that aluminum forms coordinated complexes between carboxyl groups of collagen fibers in association with oxygen, cross-linking the collagen fibril and inhibiting bone induction properties (142). This type of aluminum complex in osteoid might explain the mechanism of aluminum-induced bone disease.

Management and Prevention of Hyperphosphatemia and Aluminum Intoxication

Because the treatment and prevention of hyperphosphatemia is one of the major causes of aluminum excess and toxicity in uremic patients, it seems logical to discuss the two conditions together. For reasons cited previously, it is essential to control serum phosphorus concentration in uremic patients. In the past, the most widely used agent for this purpose was aluminum hydroxide. However, other types of oral phosphate binders are now used to reduce aluminum exposure. Calcium carbonate is a fairly effective phosphate binder and is used to control serum phosphorus concentration in uremic patients (155). Calcium acetate and calcium citrate were used (156,157). In fact, calcium acetate was suggested to be a more effective phosphate binder than other calcium compounds (157). Major side effects of calcium-containing compounds include hypercalcemia and constipation. The risk of hypercalcemia can be reduced by administering the calcium compounds at meal times. Because of its effect on enhancing aluminum absorption and promoting acute toxicity, calcium citrate is contraindicated in uremic patients.

To avoid toxicity, patients with advanced chronic kidney disease should receive aluminum only in the short term (up to two weeks) to control severe hyperphosphatemia (serum phosphorus concentration >7.5 mg/dL). Alternative phosphate binders should be employed once the serum phosphorus concentration is less than 6.0 mg/dL. At no time should aluminum compounds be given in doses higher than 100 mg/kg per day. Such dosages can cause toxicity, especially in children (110,111,158). In fact, because of the risk of intoxication, aluminum compounds probably should not be used in uremic children.

Sevelamer hydrochloride is a calcium- and aluminum-free polymeric phosphate binder that is approved for use in humans. Short- and long-term studies in patients with ESRD show that it reduces serum phosphorus and low-density lipoprotein (LDL) concentrations without raising serum calcium concentration (159,160). The major problem with sevelamer hydrochloride remains its high cost. Other noncalcium and nonaluminum containing compounds that have been shown to reduce intestinal phosphate absorption include lanthanum chloride (161) and iron hydroxide complexes (162).

Another possible means of phosphorus control is more efficient removal of phosphorus during dialysis. This could possibly be accomplished by improved membranes or hemoperfusion of phosphate sorbents such as zirconium oxide. Until newer methods of control of serum phosphorus concentration are available, the major method will continue to be the use of phosphate-binding compounds. The choice of the optimal phosphate binder for any given patient remains problematic. Calcium-containing binders are low in cost but may contribute to vascular calcification and increase total body calcium burden. Sevelamer hydrochloride does not contain calcium and also lowers LDL cholesterol concentration but is expensive.

Ensuring that water used to prepare dialysate is virtually free of aluminum (<10 μg/L) is obviously of major importance in the prevention of aluminum toxicity. A well-functioning reverse osmosis unit or a deionizer polisher will eliminate aluminum from water used to prepare dialysate. In addition, aluminum tubing, pump headers, and other materials containing aluminum potentially leachable by treated water and dialysate should not be present in the dialysis system.

In dialyzed, as well as nondialyzed, uremic patients, plasma aluminum concentration should be monitored two to three times yearly to document that the dialysate is aluminum free and that patients ingesting oral aluminum compounds are not in danger of developing aluminum intoxication. Generally, uremic patients ingesting aluminum compounds have plasma aluminum concentrations of approximately 50 μg/L. A small subset of 10% to 15% of patients have aluminum concentrations in excess of 100 μg/L. These patients are either aluminum intoxicated or in danger of becoming aluminum intoxicated. Dialysis patients receiving treatment with aluminum-contaminated dialysate will have plasma aluminum concentrations greater than 100 to 200 μg/L (163). Because aluminum concentrations vary considerably from day to day and season to season, merely one or two determinations in water may be misleading. Plasma aluminum concentration remains high for weeks after a patient is exposed to aluminum-contaminated dialysate and are, therefore, a good indicator of a previous exposure.

In general, water treatment to remove aluminum from the dialysate and substitution of calcium-containing phosphate binders for aluminum binders was very effective in preventing aluminum intoxication. As stated already, water treatment alone largely eliminated dialysis encephalopathy. With the combination of water treatment and use of calcium-containing phosphate binders, the incidence of aluminum bone disease in a large series decreased from 43% in 1985 to 8% in 1993 (164). A more recent bone histomorphometric study showed that aluminum-associated osteomalacia in both hemodialysis and peritoneal dialysis patients accounts for less than 8% of all bone disease (24).

For treatment of aluminum intoxication, all aluminum exposure should be removed. The dialysate should be routinely checked to ensure that it is aluminum free, and alternative phosphate binders should be substituted for orally administered aluminum compounds. A number of patients with osteomalacia and far fewer with dialysis encephalopathy reportedly improved when they were no longer exposed to aluminum-contaminated dialysate (107,132). Moreover, a few patients with osteomalacia also improved following the discontinuation of oral aluminum compounds. The rate of improvement of osteomalacia can, however, be markedly increased by chelation with deferoxamine (165,166). Dialysis encephalopathy is much less likely to improve spontaneously following discontinuation of aluminum exposure than is osteomalacia (167). In addition, if chelation therapy is given late in the course of the disease, it may no longer be effective. Because of this observation, chelation seems to be indicated in all patients suspected of having dialysis encephalopathy.

A number of different approaches to chelation were recommended. It appears that 2 g of deferoxamine is as effective as 4 g (168,169). In addition, symptomatic improvement of bone disease can occur as rapidly in patients given 0.5 g as in patients given 6 g weekly of deferoxamine (170). At this time, for the management of aluminum neurotoxicity it is unclear whether 1 to 2 g of deferoxamine should be given with each dialysis or only once weekly for maximum efficiency with the smallest dose. At times, chelation therapy aggravates the symptoms in patients with aluminum neurotoxicity (119). It has been suggested that the deferoxamine dose be reduced if this occurs, possibly given intramuscularly the evening prior to dialysis, and maximally removed during the subsequent dialysis (170,171). Diazepam is effective in controlling deferoxamine-related seizures and myoclonic activity in the majority of patients.

The majority of patients with aluminum-associated bone disease experience improvement of clinical symptoms in 3 to

4 months, whereas patients with encephalopathy may require chelation for 10 to 12 months before there is any symptomatic improvement.

Therapy with deferoxamine is not without risk. As described previously, patients with marked aluminum overload may develop acute neurologic toxicity following deferoxamine therapy. Acute infections with *Yersinia* species and mucormycosis were reported in patients receiving deferoxamine (172,173). Feroxamine is considered to be an iron supplier for these non-siderophore-producing organisms, promoting their growth. Therefore, prior to treatment the clinical diagnosis of aluminum intoxication should be reasonably well established and evidence of aluminum overload demonstrated either by a rise of plasma aluminum concentration to greater than 150 to 200 μg/L 24 to 48 hours following deferoxamine infusion (174) or increased bone aluminum content in association with classic histologic findings of aluminum-associated bone disease.

Hydroxypyridones have undergone testing in rabbit and rat models of aluminum overload as alternatives to deferoxamine (175,176). These drugs considerably enhance excretion of aluminum in urine and reduce aluminum content in bone and brain. Advantages of these compounds over deferoxamine include lower cost and oral availability.

Other Elements

Magnesium

As renal function progressively falls, fractional magnesium clearance increases. As a result, even with advanced chronic kidney disease there is usually only a moderate rise in serum magnesium concentration. Although severe hypermagnesemia can occur if patients with advanced kidney disease are given large gastrointestinal loads of magnesium, it rarely, if ever, occurs with the usual exposure to magnesium.

Advanced uremia does represent a state of total magnesium excess. Magnesium concentration is consistently increased in serum and bone (177,178). Because magnesium is an inhibitor of crystal formation, it was once suggested that magnesium might be important in the deranged skeletal metabolism found in uremic patients (179). Recent evidence that aluminum is, however, intimately involved in this disturbance has cast some doubt on any important role for magnesium.

The possible role of magnesium in the pathogenesis of visceral calcification has not been clarified. In these deposits, magnesium is present in a constant molar ratio along with calcium and phosphorus. When incinerated, these deposits yield whitlockite $(CaMg)_3(PO_4)_2$, again suggesting that magnesium is an integral component of these deposits (31). At this time, however, the role of magnesium in the formation of visceral calcium phosphate deposits remains to be defined.

Zinc

Another element that has received some attention is zinc. Because serum zinc concentrations were consistently found to be subnormal in uremic patients, it was suggested that a number of symptoms, including loss of appetite, an altered sense of taste and smell, and impotence, which are found in uremic patients, might result from zinc deficiency. Although some studies showed that these symptoms could be improved with zinc supplementation, other investigations found no justification for zinc therapy (170–186). It should be noted that although serum zinc concentrations tend to be low, other tissue stores of zinc might be either unchanged or actually increased (187).

Minor Element Disturbances

The blood concentration of a number of essential trace elements were found to be decreased in both dialyzed and nondialyzed uremic patients. Blood selenium was decreased in most but not all studies (188–195), as were serum manganese (184) and nickel (196–198). The copper concentrations in the blood of uremic patients was usually reported as normal (199,200). Blood vanadium concentrations largely were reported to be increased (201–205). Two other essential trace elements were also found to be increased in the blood of uremic patients, namely, chromium (200) and silicon (206–209). Of the nonessential elements, in addition to aluminum, blood concentrations of lithium (210) were found to be increased, whereas blood rubidium and bromine concentrations were found to be decreased (211).

Multiple tissue trace element profiles were characterized in both dialyzed and nondialyzed uremic patients. As with blood, a number of trace elements in other tissues were found to be abnormal in uremic patients. These can be divided into three groups. The first group represents a similar disturbance in multiple tissues documenting an alteration in the total body burden of these elements. Six elements belong to this group. Total body tin, zinc, and strontium, like aluminum, are increased, whereas total body rubidium is decreased in dialyzed and nondialyzed uremic patients. Total body bromine is decreased only in dialyzed uremic patients (187).

The second group of disturbances represents elements that may be increased or reduced in some tissues, whereas they are either normal or actually affected in the opposite direction in other tissues. Examples of this alteration are the increased copper concentration in lungs associated with a reduced copper concentration in heart of both dialyzed and nondialyzed uremic patients (187).

A third disturbance in trace elements is a translocation of an element from one organ to another. Two elements that fall into this group are cadmium and molybdenum, both of which are reduced in diseased kidneys and increased in the liver (187).

Of the remaining essential trace elements, selenium occurs in normal amounts in tissues from uremic patients (187). Silicon is increased in the spleen and liver in patients with chronic kidney disease (212). Vanadium reportedly is increased in the bones of uremic patients (213). With regard to nonessential elements, tissue stores of lead (187,212,213) and mercury (187) appear to be normal in most uremic patients. Uranium may be increased in tissues from a limited number of dialysis patients. At this time, however, there is no evidence that any of these alterations, with the exception of aluminum, have any clinical consequences.

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CHAPTER 103 ■ DIETARY CONSIDERATIONS IN PATIENTS WITH CHRONIC RENAL FAILURE, ACUTE RENAL FAILURE, AND TRANSPLANTATION

JOEL D. KOPPLE

IMPACT OF CHRONIC RENAL FAILURE ON NUTRITIONAL, METABOLIC, AND INFLAMMATORY STATUS AND NUTRITIONAL REQUIREMENTS

In chronic renal failure (CRF) there are disorders in the intestinal absorption, urinary, intestinal and dermal excretion and/or metabolism of a wide array of nutrients. These abnormalities may change the dietary requirements for many nutrients. The alterations include retention of products of protein, peptide, nucleic acid and amino acid metabolism (1) and some products of carbohydrate metabolism (2–5), impaired urinary excretion of water, sodium, potassium, calcium, magnesium, phosphorus, trace elements, organic and inorganic acids, and other compounds (6–8), decreased intestinal absorption of calcium (9,10) and possibly also iron (11,12), riboflavin (13), folate (14), vitamin D3 (15), and certain amino acids (intestinal dipeptide absorption appears to be normal [16]), possibly increased plasma clearance of pyridoxine hydrochloride, antagonism to the actions of several vitamins (due in part to medicines ingested and possibly to uremic factors), and a high risk of developing certain vitamin deficiencies, especially for folic acid, vitamin B_6, vitamin C, and the most potent metabolite of vitamin D, 1,25-dihydroxycholecalciferol (17,18).

Patients with CRF also are prone to accumulate potential toxins that normally are eaten in small amounts and are readily excreted by the kidneys; notable among these are aluminum (19) and advanced glycoxylation end products; this latter class of compounds are also produced *in vivo* (20). There is mild carbohydrate intolerance that may partly improve with hemodialysis therapy (2–4). Nondialyzed CRF patients, maintenance hemodialysis (MHD) patients, and chronic peritoneal dialysis (CPD) patients display a high incidence of elevated serum triglycerides. Serum HDL-cholesterol is often reduced (21–26). Increased serum very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), lipoprotein (a) (Lp[a]), sometimes low density lipoproteins (LDL) occur, and CPD patients often have abnormally increased serum total cholesterol levels. In CRF, there are alterations in the concentrations and/or composition of certain lipoproteins, with an abnormal proportion of individual lipids and apolipoproteins in the various lipoproteins (21–23,25,26); serum LDL may be composed predominantly of small dense LDL (sd LDL) (27–31). It has been suggested that this serum lipoprotein and apolipoprotein pattern is associated with an increased risk of atherosclerosis and coronary artery disease (25,26). For example, high serum Lp(a) (i.e., >30 mg/dL is associated with a high risk for coronary artery atherosclerosis, cerebrovascular arteriosclerosis, and stenosis of saphenous vein bypass grafts (25,26). Production of triglycerides generally is normal, but the metabolic clearance is impaired (32). Plasma triglyceride, LDL cholesterol and apolipoprotein B concentrations tend to be higher in CPD patients than in those undergoing MHD (33–35).

Potentially toxic oxidants (36,37) and reactive carbonyl compounds [methylglyoxal, glyoxal (38), and 3-deoxyglucosone (39), which generate advanced glycation end products (AGEs) (40), and N^e-carboxymethyllysine, and pentosidine (41)] accumulate in plasma and tissues. Deficiencies of antioxidants (vitamins C and E), possibly selenium have been reported (18). This combination of alterations that have been referred to as oxidant and carbonyl stress, may also cause endothelial injury and predispose to cardiovascular, cerebrovascular and peripheral vascular disease. The frequent occurrence of inflammatory states in CRF patients adds to the oxidant and carbonyl stress of the patients and increases their risk for vascular disease (see the subsequent text).

Chronic kidney disease CKD has recently been categorized into five stages (42); the most advanced stage of chronic kidney disease, stage 5, includes people with GFR less than 15 mL/minute/1.73 m^2 or who are receiving chronic dialysis therapy. Recently, the classification has been modified to indicate whether the individual is a kidney transplant recipient or is receiving maintenance dialysis therapy (Table 103-1) (43). Virtually every survey of the nutritional status of stage 5 CKD patients and particularly individuals undergoing MHD or CPD (i.e., continuous ambulatory or automated peritoneal dialysis (CAPD or APD) indicates that they frequently suffer from wasting or protein-energy malnutrition (PEM) (43–69). The prevalence of PEM is reported to vary from about 18% to 75% in various studies. In our experience, approximately one-third of maintenance dialysis patients have mild to moderate malnutrition and 6% to 8% more have severe malnutrition. The evidence for protein-calorie malnutrition, summarized in Table 103-2, includes decreased relative body weight (the individual's body weight divided by the weight of normal people of the same age, height, sex, and frame size), or body mass index (body weight (kg) divided by the square of body height in meters^2), skinfold thickness (an estimate of body fat), arm muscle diameter or cross-sectional area (a reflection of total muscle protein mass), total body nitrogen, increased total body water and extracellular water, decreased serum concentrations of many proteins (including albumin, prealbumin, transferrin, and certain complement proteins), low muscle alkali-soluble protein, and low growth rates in children (44–74). The plasma amino acid pattern, which is unique to CRF, also shows many similarities to the amino acid pattern seen in PEM (75). Total body potassium is low in patients with CRF and normal in

TABLE 103-1

CLASSIFICATION OF CHRONIC KIDNEY DISEASE

		Classification by severity		
Stage	Description	GFR mL/minute/1.73 m^2	Related terms	Classification by treatment
1	Kidney damage with normal or increased GFR	≥90	Albuminuria, proteinuria, hematuria	
2	Kidney damage with mild decreased GFR	60 to 89	Albuminuria, proteinuria, hematuria	
3	Moderate decreased GFR	30 to 59	Chronic renal insufficiency, recipient early renal insufficiency	T if kidney transplant
4	Severe decreased GFR	15 to 29	Chronic renal insufficiency, early renal insufficiency	
5	Kidney failure	<15 (or dialysis)	Renal failure, uremia, end-stage renal disease	D if dialysis (MHD, CPD)

GFR, glomerular filtration rate; *ESRD*, end-stage renal disease.
Related terms for CKD stages 3 to 5 do not have specific definitions, except ESRD.
Chronic kidney disease is defined as kidney damage or a glomerular filtration rate (GFR) <60 mL/minute/1.72 m^2 for 3 months. Kidney damage is defined as pathological abnormalities or markers of damage, including abnormalities in blood or urine tests or imaging studies.
(Reprinted from: Levey AS, Eckardt KU, Tsukamoto Y, et al. Definition and classification of chronic kidney disease: a position statement from Kidney Disease: improving Global Outcomes (KDIGO). *Kidney Int* 2005;67:2089, with permission from the Editor of *Kidney International*.)

individuals undergoing MHD. The previously mentioned findings are also not uncommonly observed in nondialyzed stage 5 CKD patients.

The problem of maintaining adequate protein nutrition is potentially more difficult for the CPD patient, because the combined losses of proteins, amino acids, and peptides are greater than they are in patients undergoing hemodialysis, particularly since the losses with CPD occur every day, whereas hemodialysis is usually performed only three times weekly. Although CPD patients may gain weight, the weight gain is generally of fat and water, even in the absence of visible edema; the mean serum total protein, albumin and transferrin levels in CPD patients tend to be lower than normal, although in most patients the values do not decrease further during the course of treatment (51). One study reported extravascular albumin and total exchangeable albumin to be reduced in CAPD patients (70); whereas in another study, body albumin pools were normal (71). In a large series of CPD patients, there was no change in arm muscle circumference during CPD therapy (72). Some authors have reported a reduction in total body potassium in CAPD patients who sustained multiple episodes of peritonitis (74).

The propensity to develop PEM occurs rather early during the course of CKD. A cross-sectional study of the nutritional status of approximately 1,700 patients with chronic renal disease who were evaluated during the baseline period of the Modification of Diet in Renal Disease (MDRD) Study indicated that the dietary protein and energy intake and the nutritional status begin to decline when the glomerular filtration rate (GFR, ^{131}I-iothalamate clearance) is about 25 to 38 mL/minute/1.73 m^2 (76). This decline was noted not only for protein and energy intake, but also for serum transferrin, body weight, mid-arm muscle circumference and percent body fat. It should be emphasized that the patients were not malnourished—indeed frank malnutrition was a criterion for exclusion from the study—but there were clear statistically significant trends toward worsening nutritional status as the GFR decreased. Similar findings of progressively greater reduction in dietary protein intake and urinary creatinine excretion, an indicator of muscle mass, has been observed in other cross-sectional studies (77,78).

A decreased energy intake was also observed when the GFR was reduced to approximately 25 to 35 mL/minute/1.73 m^2. This was noted in both the pilot study and the main clinical trial of the MDRD Study, even though these patients met with a trained nephrology research dietitian once-monthly (77,79). These results may be particularly relevant because the patients in the MDRD were almost certainly a healthier subset of patients with chronic renal insufficiency because of the study's exclusionary criteria.

Many studies indicate that the median and average dietary protein and energy intake are low in maintenance dialysis patients (51,52,80–83). Dietary protein and energy often average 20% and 30%, respectively, below recommended intakes for MHD and CPD patients (51,52,80–83). This abnormally low nutrient intake has been observed in both the pilot study and the main clinical trial for the HEMO Study (81,83).

Although the nutritional disorder most commonly associated with CRF is protein-energy malnutrition, other nutritional deficiencies also frequently occur if the patient does not receive supplemental nutrients. Particularly common in patients with CRF who do not take nutritional supplements are deficiencies for dihydrotachysterol, 1,25-dihydroxycholecalciferol, vitamin B_6, folic acid, vitamin C, iron, and possibly carnitine and zinc (11,12,17,18,84–88).

The following are causes of wasting or malnutrition in CRF.

A. *Inadequate Nutrient Intake*
   i. Inadequate nutrient intake may be caused by anorexia due to uremic toxins, the debilitating effects of CRF and underlying illnesses (e.g., diabetes mellitus, lupus erythematosus), the impact of acute illness on the patient's ability to eat, and emotional disorders (51,52,80,89). Also, the fact that diets prescribed for renal failure patients may be marginally adequate in protein and other nutrients, less palatable to the patient, and difficult to prepare may contribute to an inadequate intake.
   ii. Serum leptin levels are elevated in uremia (90–92) and may induce anorexia. In rodents, leptin suppresses food intake via its actions on the hypothalamus and

TABLE 103-2

EVIDENCE FOR PROTEIN-ENERGY MALNUTRITION IN STAGE 4 TO 5 ADVANCED CHRONIC KIDNEY DISEASE*

Body composition	Biochemistry
Decreased	*Decreased*
Anthropometry	Serum
Body weight	Total protein
Height (children)	Albumin
Growth (children)	Transferrin
Body fat (skinfold thickness)	Transthyretin (prealbumin)
Fat-free solids	Cholinesterase
Intracellular water	Pseudocholinesterase
Muscle mass (midarm muscle area, diameter or circumference)	Retinol-binding protein
Total body potassium (nondialyzed)	Plasma
Total body nitrogen (CAPD patients)	Isoleucine
Total albumin mass, synthesis, and catabolism	Leucine
Valine pools (nondialyzed patients)	Total tryptophan
Kinetic processes	Valine
RNA:DNA ratio	Valine-glycine ratio
Creatinine appearance	Essential-nonessential ratio
	Muscle
	Alkali soluble protein
	Valine
	Tyrosine
	Normal or increased
	Plasma
	Total nonessential amino acids
	Glycine

The low body protein or muscle mass and/or total body fat mass have been determined by anthropometry, dual energy x-ray absorptiometry, bioelectrical impedance, near infrared interactance, and total body nitrogen and potassium measurements.
*Nondialyzed patients with stage 4 to 5 CKD and maintenance dialysis patients may have normal values for these parameters. However, statistical comparisons indicate that the levels are often abnormal in individual patients, and in group comparisons, stage 4 to 5 CKD patients usually have significantly altered values.

probably increases energy expenditure (93,94). Whether leptin suppresses appetite in humans and specifically in renal failure patients is less clear. Several studies describe an inverse relationship between serum leptin and dietary protein intake or a direct relation between serum leptin and weight loss in MHD or CPD patients (91,92). Serum leptin levels are probably elevated in CRF patients because of impaired degradation by the diseased kidney (95) and possibly also because insulin stimulates leptin synthesis (96) and CRF patients are often hyperinsulinemic. In addition, inflammation (see the subsequent text) and the acute phase response increase serum leptin levels (97).

iii. Inflammation, which is commonly present in maintenance dialysis (MD) patients (98–101), is associated with increased levels of the cytokines, interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α) (102,103). These cytokines may induce anorexia (104,105).

B. *Increased Losses of Nutrients*

i. The dialysis procedure itself may promote wasting by removing nutrients. During routine hemodialysis with low flux dialyzers there are losses of about 6 to 8 gm of free amino acids when patients are postabsorptive (fasting) and about 8 to 10 gm, when patients are postprandial (106–109). Approximately 2 to 3 gm of peptides or bound amino acids are also removed.

Hemodialysis with high flux membranes is reported to remove 8.0 ± 2.8 (SD) gm and 9.3 ± 2.7 gm of amino acids from patients, respectively (109,110). During the sixth reuse of high flux polysulfone hemodialyzers with bleach and formaldehyde reprocessing, amino acid losses are reported to be 12.2 ± 4.4 gm (109). About 2 to 3.5 gm per day of free amino acids are removed during CAPD (111–113) (Table 103-3). In normoglycemic individuals, about 15 to 25 gm of glucose may be removed when glucose-free dialysate is used (114). When the hemodialysate contains 200 mg/dL of glucose (180 mg/dL of anhydrous glucose), there is net absorption of approximately 10 to 12 gm of glucose with each dialysis.

Normally, little protein is lost during hemodialysis. Formerly, when some hemodialyzers containing polysulfone membranes were reused with bleach and formaldehyde reprocessing procedures, the membrane sieving coefficients for large molecules increased dramatically (109, 115); in one study, albumin losses increased to 10.78 ± 7.87 (SD) gm (range 1.1 to 25.6 gm) per hemodialysis as the dialyzer membrane reuses increased to over 24 (109). Polysulfone membranes are now chemically modified so that dramatic increases in permeability do not occur with multiple bleach and formaldehyde reprocessings.

In our experience, 8.8 ± 0.5 (SEM) gm per day of total protein and 5.7 ± 0.4 gm per day of albumin are lost into the dialysate with CAPD (Table 103-4) (116). Other investigators report similar results (117,118). During an acute peritoneal dialysis of about 36 hours duration, about 22 gm of total protein and 13 gm of albumin are removed (116). Much of this loss is from the washout of ascitic fluid. With mild peritonitis, the quantity of protein removed increases to an average of 15.1 ± 3.6 gm per day (116); protein losses can rise markedly with severe peritonitis, and we have observed peritoneal protein losses as high as 100 gm per day prior to initiating antibiotic therapy. Protein losses fall rapidly with antibiotic therapy but may remain elevated for many days to weeks (116).

Water-soluble vitamins and other bioactive compounds are removed by both hemodialysis and peritoneal dialysis (18,119–122). These vitamin losses can be easily replaced from the diet, but in patients with poor nutrient intake such losses may enhance vitamin malnutrition.

ii. Patients with renal failure often lose substantial quantities of blood secondary to occult gastrointestinal bleeding, frequent blood sampling for laboratory testing, and the sequestration of blood in the hemodialyzer (123,124). Since blood is rich in protein, these blood losses may contribute to protein wasting. For example, a person with a hemoglobin of 12.0 gm per dL and a serum total protein of 7.0 gm per dL will lose approximately 16.5 gm of protein in each 100 mL of blood removed.

C. *Increased Net Catabolism*

i. Patients with CRF frequently sustain superimposed illnesses (125–127). These comorbid conditions often induce a hypercatabolic state and also may physically

TABLE 103-3

PLASMA AMINO ACIDS AND AMINO ACID LOSSES INTO DIALYSATE IN NINE MEN UNDERGOING CAPD

	Plasma amino acids (μmol/L)		Dialysate amino acids (mg/24 hour)
	CAPD patients	Normal men	CAPD patients
Number of studies	14[a]	9	14[a]
Total essential[c]	963 ± 29[b]	1030 ± 40	1027 ± 96
Total nonessential[d]	2080 ± 115	2001 ± 135	1949 ± 226
Total[e]	3415 ± 134	3260 ± 145	3355 ± 334
Essential[c]-nonessential[d] ratio	0.48 ± 0.03	0.53 ± 0.03	0.59 ± .05

[a]Fourteen studies were carried out in 9 patients. Five of these patients were studied twice in a clinical research center, once while ingesting a diet providing 1.0 gm protein/kg body weight/day and once while eating a diet containing 1.4 gm protein/kg body weight/day. One patient was studied only with the lower protein diet, and one patient was studied with the higher protein diet. There were no differences in plasma essential, nonessential, or total amino acid concentrations between the two diets. The other two patients were ingesting diets providing about 1.1 to 1.2 gm protein/kg/day.
[b]Mean ± standard error.
[c]Calculated as the sum of concentrations of histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine.
[d]Calculated as the sum of the concentrations of alanine, arginine, asparagine, aspartate, glutamate, glutamine, glycine, ornithine, proline, and serine.
[e]Calculated as the sum of total essential amino acids, total nonessential amino acids, cystine, tyrosine, citrulline, and taurine.
(Adapted from: Kopple JK, Blumenkrantz MJ, Jones MR, et al. Plasma amino acid levels and amino acid losses during continuous ambulatory peritoneal dialysis. *Am J Clin Nutr* 1982;36:395. Reprinted from: Kopple JK, Blumenkrantz MJ. Nutrition in adult patients undergoing continuous ambulatory peritoneal dialysis. *Perspectives in peritoneal dialysis* 1984;2:1.)

prevent ingestion, gastrointestinal absorption or assimilation of foods (e.g., pancreatitis, gastrointestinal surgery).

ii. There is now abundant evidence that even in the absence of overt clinical illness, advanced CRF and MD patients may sustain inflammation with an acute phase response and elevation of serum acute phase proteins (APP). In addition, CRF per se is associated with increased serum levels of many proinflammatory cytokines and protein markers of inflammation. In maintenance dialysis patients, indicators of inflammation are correlated with measures of wasting or PEM (103–105,131,132). These findings, in association with the known catabolic effects of some proinflammatory cytokines (103–105), have bolstered the conclusion that inflammation promotes wasting.

Compounds that commonly are increased in maintenance dialysis patients and indicate an inflammatory state include C-reactive protein (CRP), serum amyloid A (SAA), C_3 complement, alpha-acid glycoprotein, fibrinogen, haptoglobin, and alpha-chymotrypsin (98–103,131–137). Serum concentrations of negative acute phase proteins decrease with inflammation; these proteins include albumin, transferrin, retinol binding protein, transthyretin (prealbumin) and certain lipropoteins (100,101,103,134,138). Epidemiological studies in MD patients indicate that increased serum CRP is at least as strong a predictor of mortality as is albumin (99,100,136,137). These elevations in serum acute phase proteins are associated with an increase in inflammatory cytokines including interleukin-1 (IL-1), TNF alpha, and IL-6 (139,140). It has been argued that these elevated cytokines may promote protein-energy malnutrition in patients with CRF both by inducing anorexia (i.e., IL-1, IL-6, TNF alpha) (104,105) and also by engendering protein catabolism (e.g., by activation of proteolytic enzymes released from granulocytes) or by suppressing protein synthesis (141,142). Indeed, evidence suggests that albumin synthesis is suppressed when serum CRP is elevated (142).

iii. In this regard, it has been argued that both biocompatible as well as bioincompatible hemodialyzer membranes, hemodialyzer tubing and filters, functioning as well as old thrombosed arteriovenous synthetic grafts, vascular access and peritoneal dialysis catheters, and

TABLE 103-4

SERUM PROTEINS AND DIALYSATE PROTEIN LOSSES IN EIGHT MEN UNDERGOING CAPD

	Serum proteins[a]	Protein losses[b] per 24 hour
Total protein	6.6 ± 0.1[c] gm/dL	8.8 ± 0.5 gm
Albumin	3.5 ± 0.1 gm/dL	5.7 ± 0.4 gm
Transferrin	228 ± 11 mg/dL	333 ± 22 mg
IgG	1.41 ± 0.12 gm/dL	1.25 ± 0.20 gm
IgA	220 ± 18 mg/dL	173 ± 21 mg
IgM	234 ± 36 mg/dL	71 ± 18 mg
C3	107 ± 6 mg/dL	70 ± 7 mg
C4	32 ± 2 mg/dL	21 ± 2 mg

[a]Grand mean of values from 13 metabolic balance studies in 8 men fed 1.0 or 1.4 gm protein/kg/day. Data were obtained periodically during the period of study with each diet.
[b]Losses of each protein into dialysate over 24 hours were measured on 110 occasions in the 8 patients.
[c]Mean ± standard error.
(Adapted from: Blumenkrantz MJ, Gahl GM, Kopple JD, et al. Protein losses during peritoneal dialysis. *Kidney Int* 1981;19:593. Reprinted from: Kopple JK, Blumenkrantz MJ. Nutrition in adult patients undergoing continuous ambulatory peritoneal dialysis. *Perspectives in peritoneal dialysis* 1984;2:1.)

hemodialysate and possibly peritoneal dialysate solutions (presumably due to low levels of endotoxins) and low grade infections, such as caused by chlamydia, can stimulate this inflammatory response (143–146). Exposure to these artificial materials, infectious agents, or thrombosed arteriovenous grafts may activate monocytes and/or macrophages (147). These inflammatory cells then release the cytokines that stimulate the acute phase response, IL-1, TNF-α and IL-6 (139,140). These cytokines, in turn, further augment the acute phase response with promotion of hepatic synthesis of the APP as indicated previously (139,140).

In some *in vivo* studies in patients, hemodialysis increases the urea nitrogen appearance (UNA or net urea generation) and, enhances net protein breakdown and promotes, negative nitrogen balance (148,149). There is a net release of amino acids from the leg, indicating enhanced net breakdown of skeletal muscle protein (149). Some research evidence indicates that more bioincompatible hemodialysis membranes, such as Cuprophan, are more likely to stimulate the release of interleukin and promote net protein degradation than are more biocompatible membranes (150,151). Several but not all studies provide evidence in support of the thesis that the artificial membranes, tubing, catheters or dialysate used in the dialysis procedure can promote the acute phase response (143–147,152–156).

iv. It would not be unexpected for the accumulation of endogenously formed uremic toxins to engender malnutrition. In renal failure, there are increased plasma or tissue concentrations of probably hundreds of metabolic products; over 120 such compounds have already been identified (1,157,158). Some of these compounds are bioactive (1,157), and it is not unlikely that may have catabolic or anti-anabolic actions. Acidemia enhances decarboxylation of branched chain amino acids and engenders protein catabolism in skeletal muscle, bone reabsorption and possibly inflammation (159–164). Studies in humans suggest that metabolic acidemia causes protein catabolism and negative nitrogen balance (161–164). Also, both oxidants and carbonyl compounds, as well as a deficiency of antioxidants, may cause tissue injury and inflammation and possibly cause a catabolic state.

Even modest increases in arterial blood hydrogen ion concentrations may cause loss of body proteins, Mehrotra et al has found that in CPD patients, an arterial blood pH of 7.36 to 7.38 appears to be associated with less positive protein balance than an arterial blood pH of 7.43 to 7.45 (165).

v. The endocrine disorders of uremia may also promote wasting or PEM. Resistance to the actions of insulin (168,169) and insulin-like growth factor-I (170–172), and hyperglucagonemia (173) may promote protein wasting. Parathyroid hormone increases hepatic gluconeogenesis (174,175), and secondary hyperparathyroidism in renal failure therefore may cause protein wasting. The findings that 1,25-dihydroxycholecalciferol has pervasive effects on calcium metabolism, that vitamin D deficiency may cause a proximal myopathy, and that 25-hydroxycholecalciferol stimulates muscle protein synthesis *in vitro* (176) suggest that deficiency of 1,25-dihydroxycholecalciferol may also cause muscle protein wasting. As indicated previously, serum leptin levels are elevated in renal failure and may contribute to anorexia (90–94).

Other possible, but less well-established causes of wasting include the following:

vi. Exogenously derived uremic toxins may cause debility (e.g., aluminum) and possibly wasting.

vii. Since the kidney synthesizes or degrades many biologically active compounds including certain amino acids, peptide hormones, other peptides, glucose, and fatty acids (177), it is possible that loss of these metabolic activities of the kidney in renal failure may disrupt the body's metabolism and promote protein-energy wasting. In this regard, some possible mechanisms by which failure of renal metabolic activity might promote protein-energy wasting or malnutrition are described earlier.

EFFECTS OF MALNUTRITION AND INFLAMMATION ON THE CLINICAL COURSE OF PATIENTS WITH CKD

Malnutrition

Many studies indicate that in patients undergoing MHD or CPD, nutritional status is a powerful predictor of morbidity and mortality. Indeed, many epidemiological studies indicate that the serum albumin is one of the strongest predictors of mortality after the age of the individuals (52,178–180). A direct relationship is commonly shown between morbidity or mortality rates in MHD patients and low dietary protein intake (as indicated by normalized protein nitrogen appearance i.e., protein nitrogen equivalent of total nitrogen appearance, normalized to the volume of distribution of urea or some other function of body mass, nPNA, also known as normalized protein catabolic rate, [nPCR]), decreased body weight for a given height, and low serum albumin, urea, creatinine, cholesterol, and potassium (63,131,132,178–183). With the exception of albumin, the foregoing measurements appear to relate to mortality in a "J" or "U" curve relationship where both low and very high values are negatively correlated with mortality (182,183). For CPD patients, in whom retrospective analyses, in general, have been based on smaller sample sizes, morbidity or mortality has been correlated with serum albumin and protein nitrogen appearance in some (178,184,185) but not all studies (186). Body weight-for-height and anthropometric parameters of skeletal muscle mass or fat mass (e.g., skinfold thickness), a potential indicator of cumulative energy balance, are also associated with morbidity or mortality (187–189). The correlation between low serum cholesterol and high mortality is consistent with such a relationship (63,178, 182,183).

As indicated previously, inflammation is frequently present in CRF and MD patients (131–133,135–137). Evidence for inflammation in these individuals includes increased serum levels of such acute phase proteins as C-reactive protein (CRP) and serum amyloid A and decreased serum levels of negative acute phase proteins, including albumin, transferrin, transthyretin (prealbumin) and cholesterol-carrying lipoproteins (131–133,135–138). Most surveys suggest serum CRP levels are increased in about 30% to 50% of American and European MHD patients and perhaps a lower percent of Asian MHD patients (132,133). Serum levels of oxidants and proinflammatory cytokines, including tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL-6), are commonly elevated in patients with advanced CRF (102,103,128,129,130,132,166,167). In advanced CRF and maintenance dialysis patients, there is also an accumulation of compounds that cause oxidant or carbonyl stress (41,130,166,167,190). Oxidant stress refers to cellular injury

caused by exposure of the cell to compounds that oxidize chemicals in the cell (166). Carbonyl stress refers to cellular injury caused by carbon containing compounds that react with compounds in the cell (167). Homocysteine is such a carbonyl-reactive compound that, as indicated previously, is increased in plasma in CRF and MD patients (191–193) and that, when elevated, exerts a number of adverse effects on the vascular endothelium (194).

The current high interest in PEM and inflammation relates to the findings that measures of either of these two conditions have been epidemiologically linked to increased risk of morbidity and mortality in maintenance dialysis patients (195). Markers of inflammation have been particularly linked to atherosclerosis and cardiovascular morbidity and mortality (132,133, 135–137). Moreover, laboratory research indicates that certain acute phase proteins, oxidants, reactive carbonyl compounds and proinflammatory cytokines can be directly toxic to the endothelium. These compounds may cause inflammation, cellular proliferation and increased matrix deposition in the endothelium with the formation of inflamed atherosclerotic plaques which are likely to rupture and increase the likelihood of myocardial infarction or stroke.

There is an overlap between the manifestations of PEM and inflammation. Thus, both PEM and inflammation may reduce serum levels of such negative acute phase proteins as albumin, transferrin, transthyretin and cholesterol-carrying lipoproteins (131–133,138,180,195). The lowest serum levels of these proteins are generally found with inflammation rather than PEM. Serum albumin is negatively correlated with serum CRP in MD patients, and serum CRP is at least as powerful a predictor of mortality as is serum albumin (100,102,134,196,197). Indeed, pure PEM usually does not reduce serum albumin below 3.0 g/dL. Inflammation may cause PEM by inducing anorexia—for example, TNF-α and IL-6 are anorexigens—and also by engendering a hypercatabolic state (103–105,133,198,199). Whether PEM may predispose a patient to inflammation, (e.g., by increasing the risk of infection or enhancing the inflammatory response to other stimuli), is not certain.

The occurrence of measures indicating the presence of either PEM or inflammation in CRF or maintenance dialysis patients has become a matter of great concern to nephrology researchers and clinicians because of the strong association between PEM or inflammation and an increased hazard ratio (HR) of morbidity and mortality (133,195,200). This issue is of particular importance because the unadjusted mortality rate for maintenance dialysis patients in the United States is very high—approximately 24% per year (127). Hence, in a population that is already at high risk for morbidity and mortality, the identification of clinical characteristics that demonstrate the presence of a subgroup of these individuals who are at even greater risk for these adverse outcomes is a cause for alarm, and, at the same time, it is a potential opportunity to develop interventions that may improve such poor prognoses.

The relative contributions of PEM and inflammation to the high morbidity and mortality of CRF patients are controversial, particularly because, the syndrome of PEM shares many clinical manifestations with inflammation. Because inflammatory processes may cause endothelial injury and/or predispose to atherosclerosis and vascular thrombosis, it is easy to perceive why there would be a causal connection between inflammation and morbidity and mortality from vascular disease.

These considerations have led some investigators to question whether PEM by itself is hazardous or whether it is only an important risk factor for morbidity and mortality when it occurs in association with inflammation (133). Intuitively, it seems that a nutrient intake that is inadequate to maintain a healthy quantity of body protein mass or that does not provide adequate energy must eventually place the patient at risk for increased morbidity and mortality. We suspect that PEM may, among its other adverse consequences, predispose to inflammation and vascular disease. This is a question that demands further investigation. In any case, PEM and inflammation occur together so commonly in ESRD patients that some investigators have described them as components of a single syndrome referred to as the malnutrition-inflammation complex syndrome (200). Will intervention to improve nutritional status or suppress inflammation reduce morbidity and mortality in MHD patients? There have been few publications on this matter. In several small-scale, randomized prospective clinical trials in MHD patients, nutritional interventions were shown to decrease or eradicate indicators of PEM, and particularly hypoalbuminemia (201,202). A recent small, uncontrolled clinical trial indicated that a nutritional preparation that contained both a protein supplement and an antioxidant increased serum albumin levels in MHD patients (203). Another clinical study reported that there was a reduced risk of progression of vascular disease in MHD patients given vitamin E (antioxidant) therapy (204).

Two retrospective analyses compared malnourished MHD patients receiving intradialytic parenteral nutrition (IDPN) to malnourished MHD patients not receiving this therapy (205,206). The data suggest that malnourished patients receiving IDPN had greater survival as compared to those who did not. In one study, when the serum albumin was 3.3 gm/dL or lower, the patients who received IDPN had significantly greater survival rates than those who were not given IDPN (206).

Altered Risk Factor Patterns and the Malnutrition-Inflammation Syndrome

A number of researchers have recently pointed out that relationships between traditional risk factors and mortality are markedly altered or even reversed in maintenance dialysis patients as compared to the public at large. These altered relationships, referred to as altered risk factor patterns, risk factor reversal, or reverse epidemiology, have been observed for body BMI or weight-for-a-given-height, predialysis serum total cholesterol, LDL-cholesterol and predialysis creatinine, urea nitrogen (SUN), urea nitrogen appearance (UNA—net urea production—see the subsequent text), blood pressure, magnitude of acidosis, and possibly serum homocysteine and parathyroid hormone levels (182,183,207–218).

Although several explanations have been advanced to explain these phenomena, the most likely mechanism is related to the malnutrition inflammation complex syndrome (182,183,212,216,217). Maintenance dialysis patients who eat inadequately, who have major comorbid conditions, or who have evidence for systemic inflammation are more likely to have both low BMI, serum cholesterol and homocysteine and also be at greater risk for mortality. Individuals who are healthier are more likely to have a greater appetite and to have a higher body weight, a greater protein intake and, hence, a higher UNA and greater metabolic acid production and metabolic acidosis. Serum total cholesterol, LDL-cholesterol, and homocysteine tend to be higher in healthy people who have greater food intakes. Because of their greater muscle mass and appetite, these individuals tend to have higher predialysis serum creatinine and urea concentrations. They are less likely either to suffer from cardiac pump failure or to have low blood pressures. Also, because they are healthier, they are more likely to live longer.

Survival bias may also account for some of the altered risk factor patterns (i.e., those individuals with increased levels of the normal risk factors may die earlier). These explanations cannot completely account for the altered risk factors in maintenance dialysis patients, because it turns out that markedly obese individuals; those with BMI of 45 kg/m^2 or greater have

greater unadjusted 2-year mortality rates than those patients who are overweight or mildly obese (210,211). Clearly, further research is indicated in this area.

DIETARY THERAPY FOR CHRONIC KIDNEY DISEASE AND CHRONIC KIDNEY FAILURE

General Approach to Dietary Management

The widespread nutritional and metabolic alterations, high incidence of malnutrition, and current evidence that dietary therapy may retard the rate of progression of CRF indicate that nutritional therapy is a critical aspect of the management of chronic kidney disease. There are four goals for dietary treatment:

a. To maintain good nutritional status.
b. To prevent or ameliorate uremic toxicity and the metabolic disorders of renal failure
c. To reduce the risk of cardiovascular, cerebrovascular and peripheral vascular disease.
d. To arrest or retard the progression of renal failure.

Adherence to specialized diets is often a difficult and frustrating endeavor for patients and their families. Patients usually must make fundamental changes in their behavior patterns and forsake some of their traditional sources of daily pleasure. Often, the patients must procure special foods, prepare special recipes, usually forego or severely limit their intake of favorite foods, or eat foods that they may not desire. Demands are made on the patient's time and daily activities and on the emotional support system of the family or close associates. Therefore, it is incumbent on the physician not to prescribe radical changes in the patient's diet unless there is good reason to believe that these modifications may be beneficial. To ensure successful dietary therapy, patients with renal disease must undergo extensive training concerning the principles of nutritional therapy and the design and preparation of diets, and they need to be continuously encouraged to adhere to the prescribed diet. They usually require repeated training regarding their nutritional therapy. Without careful monitoring of nutritional intake, retraining, encouragement, and sensitivity to the cultural background, psychosocial condition and lifestyle of the patients, they will be more likely to adhere poorly to their dietary prescription. They may eat too little rather than too much.

The recipes and meal plans should be designed specifically for the individual tastes of the patient. A team approach, including the physician, dietician, close family members, the nursing staff, and, when available, social workers, or psychiatrists may improve adherence. Since the prescribed diet for renal failure patients is complicated and obtaining an accurate diet history and maximizing dietary compliance for these individuals is a complex and subtle art, it is important for the physician to work with a dietician who is knowledgeable and experienced in nephrology dietetics. It is often advantageous to organize the dietetic program in a medical center so that the dieticians who treat nephrology patients work exclusively in this specialty. Data indicate that a systematic, problem-oriented approach to dietary compliance can substantially improve compliance (219,220).

The physician should strongly support the dietician's efforts to train and counsel the patient and to obtain dietary compliance. During each visit the physician should assess the patient's dietary intake (see the subsequent section) and should discuss the results with the patient. Generally, it is important for the spouse or other close relatives or friends to work closely with the patient to provide moral support and to assist, if needed, with the acquisition and preparation of foods. The entire medical team should assume an energetic, positive, and sympathetic approach in order to promote dietary adherence. A nurse, social worker, and psychiatrist can provide invaluable assistance by providing sympathetic counsel to the patient and informing other members of the team of problems that may impair the patient's adherence to the diet. The central issue is that if dietary therapy is prescribed to a patient, it requires a major commitment and effort by the patient and the medical staff to attain good results.

Monitoring Compliance

In general, to maintain good dietary compliance and to monitor closely the patient's clinical, fluid and electrolyte, and nutritional status, patients with advanced renal failure should be seen approximately monthly by the physician and the dietician. It is arguable as to whether patients with slowly progressive mild or moderate renal insufficiency who are clinically stable and adhere well to the diet may see a physician less frequently. However, to maximize compliance to the diet, most patients should continue to see the dietitian more frequently, often monthly. Bergstrom and co-workers emphasized the importance of close medical follow-up (221). These authors observed that frequent clinic visits, apparently independent of dietary prescription, were associated with a slowing in the rate of progression of renal insufficiency.

In many patients with progressive renal failure, the creatinine clearance decreases more or less linearly with time (222,223). Transposing the clearance equation, it can be predicted that the log of the serum creatinine or the 1.0/serum creatinine ratio also should decrease linearly with time. This has been confirmed in several studies (222,223); approximately 60% to 80% of the patients with chronic progressive kidney disease lose creatinine clearance in a roughly linear fashion. Thus, for patients with CRF, the creatinine clearance and/or the ratio of 1.0/serum creatinine ratio periodically should be plotted against time. The slope of 1.0/serum creatinine vs time has been shown to be a rather inaccurate measure of loss of GFR. If the rate of progression of renal failure slows, it may indicate that treatment is beneficial. On the other hand, acceleration of the rate of progression may indicate the development of new causes that are impairing renal function. Such causes include worsening hypertension, drug reactions, urinary tract obstruction, infection, intake of nonsteroidal antiinflammatory medicines, or increased activity of the underlying renal disease. It is well documented that the creatinine clearance is not as precise a measure of the GFR as is the clearance of insulin, iothalamate or several other solutes and may give misleading results (224,225). This is due primarily to variability in the renal tubular secretion of creatinine (226). Indeed, the creatinine clearance has been largely invalidated as a measure of GFR for clinical or laboratory research studies (225,226). However, the creatinine clearance or the mean of the creatinine and urea clearances (227) may be helpful for the clinical management of patients, particularly when serial measurements are taken, and may give a substantially more precise estimate of the actual GFR than does the serum creatinine. There are now several equations for estimating the GFR from the serum creatinine concentration (228–230). In the author's opinion, these equations are useful for screening purposes, for estimating the GFR in individuals for whom precise collection of a 24-hour urine collection is difficult or too inconvenient and for monitoring the GFR serially after the relationship between the individual's serum creatinine and GFR has been determined by more accurate measures of GFR.

Since the nutritional status may begin to deteriorate in patients with chronic progressive renal disease when the glomerular filtration rate (GFR) falls below 35 to 50 mL/minute/1.73 m^2 (76), careful attention to nutritional status should begin at this time or earlier. This is particularly important because CRF patients appear to be at greatest risk for malnutrition from the time that the GFR falls below 10 mL per minute until the patient is established on maintenance dialysis therapy (53,76). Also, although nutritional status often improves after commencing hemodialysis (231), nutritional status of patients at the onset of chronic dialysis treatment is a good predictor of their nutritional status 2 to 3 years later (53,232). Moreover, markers of poor nutritional status at the onset of maintenance dialysis therapy predict increased morbidity and mortality (179,189). Hence, particular effort should be given to preventing malnutrition as the patient approaches dialysis therapy and during the first few weeks of maintenance dialysis treatment. These efforts should be directed toward maintaining a good nutritional intake during this period, rapidly instituting therapy for superimposed illnesses, and maintaining good nutritional intake during such illnesses.

Monitoring Nutritional-Inflammatory Status

Since diets prescribed for renal insufficiency are often marginally low in some nutrients (e.g., protein) and high in others (e.g., calcium) and malnutrition is not infrequent, it is important to periodically evaluate the adequacy of the diet and the patient's nutritional status. The National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF K/DOQI) Clinical Practice Guidelines for Nutrition in Chronic Renal Failure has recommended that panels of nutritional measures should be used to assess protein-energy nutritional status in nondialyzed CRF and maintenance dialysis patients (233,233a). For nondialyzed CRF patients with a GFR less than 20 mL/minute, it is recommended that protein-energy nutritional status should be evaluated by serial measurements of a panel of markers including at least one measure from each of the following clusters: (i) serum albumin; (ii) edema-free actual body weight, percent standard (NHANES II) body weight, or subjective global assessment (SGA); and (iii) the normalized protein equivalent of total nitrogen appearance nPNA or dietary interviews and diaries (233). It is recommended that the standard body weight for normal individuals should be obtained from the second National Health and Nutrition Evaluation Survey (NHANES II) because Americans were less fat at that time than they are currently (234). For MHD or CPD patients, the recommended panel of measurements of protein-energy nutritional status is indicated in Table 103-5 and should routinely include predialysis or stabilized serum albumin, percent of usual body weight, percent of standard (NHANES II) body weight, subjective global assessment, dietary interviews and diaries, and nPNA (233). A predialysis serum measurement is obtained from an individual immediately before the initiation of a hemodialysis or intermittent peritoneal dialysis treatment. A stabilized serum measurement is obtained after the patient has stabilized on a given dose of continuous ambulatory peritoneal dialysis (CAPD).

There are other nutrition-related parameters that often will require monitoring; for example, urine sodium, phosphorus and albumin, serum potassium, phosphorus, calcium, parathyroid hormone, iron, ferritin, transferrin, vitamins, total LDL and HDL cholesterol and triglycerides, interdialytic weight gain, and, if relevant, bone densitometry or radiography (46). Measures of inflammation or oxidant or carbonyl stress are often valuable. Such measures can include serum total homocysteine levels, serum quantitative high sensitivity measurements of (CRP), and serum IL-6. Dieticians are often best qualified to perform anthropometric measurements of nutritional status

TABLE 103-5

NKF K/DOQI RECOMMENDATIONS FOR ROUTINE MONITORING OF NUTRITIONAL STATUS IN MAINTENANCE HEMODIALYSIS AND CHRONIC PERITONEAL DIALYSIS PATIENTS

Category	Measure	Minimum frequency of measurement
I. Measurements that should be performed routinely in all patients	• Predialysis or stabilized serum albumin	• Every 4 months
	• % of usual postdialysis (MHD) or post-drain (CPD) body weight	• Every 6 months
	• % of standard (NHANES II) body weight	• Every 6 months
	• Subjective global assessment (SGA)	• Every 6 months
	• Dietary interview and diary	• Monthly MHD; every 3 to 4 months CPD
II. Measures that can be useful to confirm or extend the data obtained from the measures in Category I.	• Predialysis or stabilized serum prealbumin	• As needed
	• Skinforld thickness	• As needed
	• Mid arm muscle area, circumference, or diameter	• As needed
	• Dual energy x-ray absorptiometry	• As needed
III. Clinically useful measures which, if low, might suggest the need for a more rigorous examination of protein-energy nutritional status.	Predialysis or stabilized serum	
	• Creatinine	• As needed
	• Urea nitrogen	• As needed
	• Cholesterol	• As needed
	• Creatinine Index	• As needed

MHD, maintenance hemodialysis; *CPD*, chronic peritoneal dialysis, *NHANES II*, Second National Health and Nutrition Evaluation Survey; *nPNA*, normalized protein equivalent of total nitrogen appearance (determined from the urea nitrogen appearance [233]).
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because of their experience and training, interest in nutritional therapy, and access to the patient.

UREA NITROGEN APPEARANCE (UNA) AND THE SERUM UREA NITROGEN-SERUM CREATININE RATIO

Since the control of protein intake is central to the nutritional management of patients with acute or CRF, it is important to accurately monitor nitrogen intake. Fortunately, for most patients this is possible. Patients who are in neutral nitrogen balance should have a total nitrogen output that is equal to nitrogen intake minus about 0.5 gm nitrogen per day from such unmeasured losses as sweat, respiration, flatus, blood drawing, and growth of skin, hair, and nails (235). Thus, nitrogen output should correlate closely with nitrogen intake in patients who are more or less in nitrogen balance. For the clinical management of patients, a slightly positive or negative balance will not substantially alter the use of the nitrogen output measurement to estimate nitrogen intake. In patients who are in very positive or negative nitrogen balance (e.g., from pregnancy or severe infection), nitrogen output may not reflect intake. However, when the patient is in very positive or negative balance, there is usually an accompanying alteration in the patient's clinical status that is readily apparent to the physician.

The measurement of total nitrogen outputs (i.e., the nitrogen losses in urine, feces, dialysate, or tube or fistula drainage) is too laborious and expensive to be widely applied for clinical uses. However, since urea is the major nitrogenous product of protein and amino acid degradation, the UNA can be used to estimate total nitrogen output and hence nitrogen intake (125,236,237). UNA is the amount of urea nitrogen that appears or accumulates in body fluids and all outputs (e.g., urine, dialysate, fistula drainage). The term *UNA* is employed rather than urea production or urea generation because some urea is degraded in the intestinal tract; the ammonia released from urea is largely transported to the liver and converted back to urea (238,239). Thus the enterohepatic urea cycle leads to an increase in absolute urea synthesis but has little effect on serum urea levels or total nitrogen economy, and this cycle can be ignored without compromising the accuracy of the UNA for estimating total nitrogen output or intake. This offers an important advantage because the recycling of urea cannot be measured without costly and time-consuming isotope studies.

UNA is calculated as follows:

$$\begin{aligned}\text{UNA (gm/day)} = {} & \text{urinary urea nitrogen (gm/day)} \\ & + \text{dialysate urea nitrogen (gm/day)} \\ & + \text{change in body urea nitrogen (gm/day)}\end{aligned} \quad [1]$$

$$\begin{aligned}&\text{Change in body urea nitrogen (gm/day)} \\ &= (\text{SUN}_f - \text{SUN}_i, \text{gm/L/day}) \times \text{BW}_i\,(\text{kg}) \\ &\times (0.60\ \text{L/kg}) + (\text{BW}_f - \text{BW}_i, \text{kg/day}) \\ &\times \text{SUN}_f\,(\text{gm/L}) \times (1.0\ \text{L/kg})\end{aligned} \quad [2]$$

where i and f are the initial and final values for the period of measurement; SUN is serum urea nitrogen (grams per L); BW is body weight (kilograms); 0.60 is an estimate of the fraction of body weight that is water; and 1.0 is the volume of distribution of urea in the weight that is gained or lost.

The estimated proportion of body weight that is water may be increased in patients who are edematous or lean and decreased in individuals who are obese or very young. Changes in body weight during the 1- to 3-day period of measurement of UNA are assumed to be due entirely to changes in body water. In patients undergoing hemodialysis, UNA may be calculated during the interdialytic interval and then normalized to 24 hours. Alternatively, UNA may be calculated by urea kinetic techniques (see Chapter 100). Two-pool models for urea kinetic measurements that take into account delays in equilibration of endogenous urea pools during hemodialysis will give more accurate results for both the UNA method described here and the urea kinetic technique (240). The 24-hour dialysate urea concentration can be readily measured in CAPD or APD (automated peritoneal dialysis) patients. Measurement of total protein as well as urea in peritoneal dialysate may give more accurate measurements of total nitrogen output and, hence, dietary protein intake (241).

In our experience, the relationship between UNA and total nitrogen appearance in chronically uremic patients not undergoing dialysis is as follows (180):

$$\text{TNA (gm/day)} = 1.19\ \text{UNA (gm/day)} + 1.27 \quad [3]$$

If the individual is approximately in neutral nitrogen balance, the UNA also will correlate closely with nitrogen intake. Equation 4 describes our observed relationship between UNA and dietary nitrogen intake in clinically stable, nondialyzed, chronic renal failure patients.

$$\begin{aligned}&\text{Dietary nitrogen intake (gm/day)} \\ &= 1.20\ \text{UNA (gm/day)} + 1.74\end{aligned} \quad [4]$$

When both nitrogen intake and UNA are known, nitrogen balance can be estimated from the difference between nitrogen intake and nitrogen output estimated from the UNA. If the patient is markedly anabolic, such as in pregnancy or in a young person with anorexia nervosa who is being refed, Equation 4 will underestimate nitrogen intake. In patients who have large protein losses (e.g., from the nephrotic syndrome or peritoneal dialysis) or who are acidemic and have sufficient kidney function to excrete large quantities of ammonia, Equations 3 and 4 will underestimate both nitrogen output and nitrogen intake. In most circumstances, however, these conditions are not present, and the UNA provides a powerful tool for monitoring nitrogen output and intake or for estimating balance. Other investigators have described similar approaches to monitoring nitrogen intake and output (242).

The ratio of the SUN to serum creatinine also correlates fairly closely with dietary protein or amino acid intake in nondialyzed patients with CRF (243). This relationship can be used to estimate the recent daily intake of these patients. Although the SUN/serum creatinine ratio is not as precise as the UNA and is influenced by a number of clinical factors, it is easy and inexpensive to measure. For clinically stable, nondialyzed, chronic renal failure patients, this ratio can also be used to estimate the level at which the SUN will stabilize for any given dietary protein intake and GFR (243).

PROTEIN, AMINO ACIDS, AND KETOACIDS—BACKGROUND INFORMATION

Low protein diets have been prescribed for many decades to reduce the accumulation of potentially toxic metabolites of protein metabolism in patients with CRF (244–251). There is considerable evidence that mixtures of essential amino acids can be utilized more efficiently than protein by CRF patients (252–259). Several investigators reported that in patients with CRF, low protein diets supplemented with essential amino acids promoted greater nitrogen retention as compared to unsupplemented low protein diets (e.g., about 0.3 g protein/kg/day) or diets providing very low quantities of essential amino acids

(e.g., about 20 gm/day) as the sole nitrogen source and was also effective at improving uremic symptoms (254,256,257). Substitution of α-ketoacid or α-hydroxyacid analogues for some essential amino acids may further reduce nitrogen intake while maintaining good nutrition (260–264). Alpha-ketoacids contain a keto group instead of an amino group on the alpha-carbon; alpha-hydroxyacids contain a hydroxyl group on this carbon.

Low protein diets have been reported to slow the rate of progression of chronic renal failure (265–269). Some studies suggest that the very low protein diets supplemented with ketoacids, hydroxyacids, and essential amino acids may be particularly effective at slowing the rate of loss of renal function (258,259,270–274). Not all studies confirmed these results (275). The nitrogen sparing effects of these essential amino acid or ketoacid supplemented diets are due, in part, to the branched-chain amino acids and ketoacids, particularly leucine and its ketoacid analogue, alpha-ketoisocaproic acid, that promote protein anabolism (276,277).

In recent years, there have been primarily three types of low nitrogen diets that have been used for the treatment of patients with CRF: (1) a low protein diet providing about 0.55 to 0.60 gm protein/kg body weight/day (219,251,259,265–270); (2) a very low protein diet providing approximately 16 to 20 gm per day of protein of miscellaneous quality (i.e., about 0.28 gm protein/kg/day) supplemented with about 10 to 20 gm per day of the nine L-essential amino acids (256,257,275); and (3) a similar 16 to 20 gm protein diet generally supplemented with four essential amino acids-histidine, lysine, threonine, and tryptophan-and the ketoacid or hydroxyacid analogues of the other five essential amino acids sometimes with a few other amino acids added (219,259,261,269,271–274).

There is strong evidence that histidine is an essential amino acid for both normal individuals and patients with CRF (278), and for this reason histidine is included in all essential amino acid and ketoacid preparations. The 0.55 to 0.60 gm protein/kg/day diet contains primarily high biological value protein (i.e., protein containing a high fraction of the essential amino acids proportioned roughly according to the daily dietary requirements for humans). Because the diets with very low protein intakes (0.28 gm/kg/day) are supplemented with amino acids or ketoacids, it is not important that the protein be of high biologic value.

The largest and most thorough examination of whether dietary control will retard the rate of progression of renal disease was the National Institutes of Health funded Modification of Diet in Renal Disease (MDRD) study (219). This project investigated, in an intention to treat analysis, the effects of three levels of dietary protein and phosphorus intakes and two blood pressure management goals on the progression of chronic renal disease. Patients with various types of renal disease (840 adults), except insulin dependent diabetes mellitus, were divided into two study groups according to their GFR.

Study A included 585 patients with a GFR, measured by ^{131}I-iothalamate clearances, of 25 to 55 mL/minute/1.73 m^2. They were randomly assigned to either a usual protein, usual phosphorus diet (1.3 g protein/kg standard body weight/day and 16 to 20 mg phosphorus/kg/day) or to a low protein, low phosphorus diet (0.58 g protein/kg/day and 5 to 10 mg phosphorus/kg/day), and also to either a moderate or strict blood pressure goal (mean arterial blood pressure 107 mm Hg [113 mm Hg for those 61 years of age or older] or 92 mm Hg [98 mm Hg for those 61 years of age or older]). In study B, 255 patients with a baseline GFR of 13 to 24 mL/minute/1.73 m^2 were randomly assigned to the low protein and low phosphorus diet or to a very low protein and phosphorus diet (0.28 g protein/kg/day and 4 to 9 mg/phosphorus/kg/day) with a keto acid-amino acid supplement (0.28 gm/kg/day). They were also randomly assigned to either the moderate or strict blood pressure control groups, as in Study A. The adherence to the dietary protein prescription in the different diet groups was excellent.

Among participants in Study A, those prescribed the low protein diet had significantly faster declines in GFR during the first four months than those on the usual protein diet. Thereafter, the rate of decline of the GFR in the low protein, low phosphorus group was significantly slower than in the group fed the usual protein and phosphorus diet. Over the course of the entire treatment period, there was no difference in the overall rate of progression of renal failure in the two diet groups. However, it is likely that the initial greater fall in GFR in the patients prescribed the low protein diet may reflect a hemodynamic response to the reduction in protein intake, rather than a greater rate of progression of the parenchymal renal disease. This might in fact be beneficial, reflecting a reduction of intrarenal hyperfiltration and intrarenal hypertension. If this explanation is correct, the subsequent slower rate of progression of disease after the first four months of dietary treatment is consistent with a beneficial effect of this intervention in renal disease. In Study B, the very low protein group had a marginally slower decline of GFR than the low protein group; the average rate of decline did not differ significantly between the two groups ($p = 0.066$).

In a secondary analysis of Study B in which the decrease in GFR was correlated with the actual quantity of protein ingested, there was no effect of ingesting the low protein diet versus the very low protein diet supplemented with ketoacids-amino acids on the progression of renal failure (279). However, if the two diet groups were analyzed together and the protein intake of the latter diet was considered to be the sum of the protein and ketoacid-amino acid supplement ingested, a significantly lower rate of decline in GFR was found in the patients who actually ingested lower protein diets (279). These findings suggest that a lower total protein intake, but not the ketoacid-amino acid preparation, itself, retarded the rate of progression of renal failure.

It should be emphasized that in the MDRD study, the very low protein ketoacid-amino acid supplemented diet was not compared to the usual protein intake. Also, it is possible that the lack of significant effect of the low protein diet on the progression of renal failure might reflect the rather short mean duration of treatment in the MDRD study, 2.2 years. Indeed, if the trend toward slower progression of renal failure in the low protein diet groups that was present at the termination of the MDRD study had persisted during a longer follow-up period, statistically significantly slower progression would have been observed with the 0.60 gm/kg protein diet in Study A and the very low protein, ketoacid-amino acid supplemented diet in Study B. This phenomenon is perhaps exemplified with the diabetes complication control trial (DCCT) of intensive versus more conventional serum glucose control. After 2 years of study, there was not even a trend toward less microalbuminuria in the more rigorously controlled glucose group (280). However, when the study was terminated after a mean of 6.5 years, a much lower incidence of microalbuminuria was found in these latter patients. The MDRD study also included a substantial proportion of patients assigned to each diet who did not show progression of renal failure during the course of the study; also, a large number of patients with polycystic kidney disease were enrolled in the study. The presence of both of these subgroups probably reduced the likelihood of showing a slowing effect of low protein diets on the progression of renal failure.

There was also no significant difference in the rate of decline in GFR between the moderate and low blood pressure groups in either Study A or Study B, although the difference in the mean arterial blood pressure during the follow-up period in the two groups was small, 4 mm Hg (219). But in Study A, patients assigned to the low blood pressure group who had a baseline urinary protein excretion $\geq$0.25 gm/day had a significantly slower rate of decline of GFR than those assigned to the

moderate blood pressure level. In Study B, patients with baseline urinary protein ≥1.0 gm/day had a slower decline in GFR when assigned to the low blood pressure group (281). Other analyses now confirm that strict blood pressure control (e.g., 130/185–125/75 mmHg or lower) is particularly beneficial for reducing the rate of progression of renal failure in hypertensive patients, particularly in those who have one or more grams of urinary protein per day or diabetes mellitus (282,283).

In general, the dietary and blood pressure treatments were well tolerated, safe, and acceptable to patients. There was a tendency for some patients to develop worsening parameters of protein-calorie nutritional status (79). This was particularly evident during the first four months of therapy in the patients prescribed the low protein and very low protein diets. It is possible that their lower dietary energy intakes may have contributed to this phenomenon (79), and that it may be more difficult to maintain an adequate energy intake with low protein diets. It should be emphasized that no treatment group developed evidence for frank protein-calorie malnutrition during the study.

RECOMMENDED DIETARY INTAKES

Note: Experimental studies are currently evaluating the potential benefits and safety of more frequent or daily hemodialysis. Uncontrolled reports indicate that patients treated with more frequent hemodialysis have greater appetites and food intake. It is anticipated that such individuals will probably have increased daily needs for certain nutrients than are discussed in this chapter and listed in Table 103-6. Such patients may also have greater tolerance for some nutrients than is indicated here. Since the benefits and safety of more frequent hemodialysis have not yet been established in randomized, prospective clinical trials; this treatment is still considered experimental for the general end-stage renal disease population, and the nutritional needs of patients treated with these techniques have not been systematically investigated, this chapter will not provide recommendations for nutritional intakes for this treatment.

Recommended Dietary Protein Intakes

Four meta-analyses have evaluated clinical trials of the effects of low protein diets, which are generally also low in phosphorus content, on the rate of progression of kidney failure. These meta-analyses each evaluated a somewhat different series of clinical trials, only some of which included the MDRD Study (284–287). In three of the meta-analyses, the key outcome was the onset of end-stage renal disease as determined by the chronic renal failure patient commencing treatment with MHD or CPD or receiving a kidney transplant (284–286). These meta-analyses reported that the relative risk of a CRF patient assigned to the low protein diets reaching this end point during the period of observation was significantly reduced to 0.54, about 0.67, and 0.61, respectively. One meta-analysis used the rate of decrease in GFR as the key outcome (287). This latter study described a slowing in the progression of renal failure of 6% that, although statistically significant, was of questionable clinical value.

The discrepancy between these two sets of findings may be explained in part by the fact that ingestion of low protein diets leads to a reduction in the generation of metabolic products of protein and amino acids, and that some of these metabolic products are toxic. Indeed, patients ingesting low protein diets are reported to be started on MHD or CPD at lower GFRs than individuals eating higher protein intakes. These meta-analyses also examined clinical trials that used an intention-to-treat type of analysis, whereby the data from individuals assigned to a given dietary intake were included in the results whether or not they adhered to the dietary prescription or even were available for follow-up testing.

One meta-analysis analyzed the results of five prospective clinical trials of the effects of such diets on progression of renal failure in patients with insulin dependent diabetes mellitus (285). This analysis indicated that low protein diets also retard progression in these individuals. However, the results were much less definitive because much smaller numbers of patients were analyzed; in two of the trials there was no randomized, concurrent control group, and the key endpoints were less definitive.

A recent 12-year follow-up analysis was conducted in the Study A MDRD patients concerning the hazard ratio, adjusted for baseline characteristics, of developing ESRD or of a combination of either ESRD or all-cause mortality (288). ESRD was defined as commencing maintenance dialysis therapy or receiving a kidney transplant. This study indicated that during the first 6 years after the onset of the dietary protein prescription, there was a statistically significant adjusted lower hazard ratio of incurring ESRD or the combination of either ESRD or mortality in those assigned to the 0.60 g protein/kg/per day diet vs. those assigned to the 1.3 g protein/kg per day diet (288). This difference tended to reverse itself in the second 6-year period of follow-up.

In Study B patients, those assigned to the keto-acid supplemented diet had a significantly greater hazard ratio, adjusted for baseline factors, for death after they developed ESRD during the 12 years after assignment to their diet prescription (289). These data are particularly intriguing because patients only were monitored, on average, for the first 2.2 years of follow-up and were then referred back to their usual physicians. Moreover, with few exceptions, keto-acid mixtures were not available in the United States except for those individuals participating in the MDRD Study during the time that they were assigned to this diet. Thus, it would have been very difficult for the Study B patients to continue taking ketoacids after the MDRD Study ended.

The Nurses' Health Study compared spontaneous protein intakes of individuals with different levels of GFR that were determined from their serum creatinine concentrations (290). In the study, 1624 women, ages 42 to 68 years, had their protein intake measured in 1990 and again in 1994 using a semi-quantitative food-frequency questionnaire. Those women with mildly reduced baseline estimated GFR levels that were greater than 55 mL/minute/1.73 m^2 but less than 80 mL/minute/1.73 m^2 showed a fall in GFR of –1.69 mL/minute/1.73 m^2 per 10-g increase in protein intake. However, after adjustment for measurement error, the change in estimated GFR was –7.72 mL/minute/1.73 m^2 per 10-g increase in protein intake. This association was of borderline statistical significance. A high intake of nondairy animal protein with mild renal insufficiency was associated with a significantly greater fall in estimated GFR (−1.21 mL/minute/1.73 m^2 per 10-g increase in nondairy animal protein intakes. A recent retrospective study in renal transplant recipients indicated that those recipients who spontaneously ingested higher protein diets experienced greater losses of GFR (291).

Taken together, the post hoc analysis of the MDRD Study data, the results of the approximately 12 years follow-up to the MDRD Study, the Nurses Health Study, the study in renal transplant recipients, and the four meta-analyses of CKD patients prescribed low protein diets all point to the probability that low protein diets will retard the rate of progression of renal failure in individuals with chronic kidney disease. Moreover, because these low protein diets often engender sufficiently lower uremic toxicity for a given level of reduced renal function, patients fed these diets may be able to avoid MHD or CPD therapy at GFR levels that would require

TABLE 103-6

RECOMMENDED DIETARY NUTRIENT INTAKE FOR PATIENTS WITH STAGE 3 TO 5 CHRONIC KIDNEY DISEASE NOT UNDERGOING DIALYSIS AND FOR PATIENTS UNDERGOING MAINTENANCE HEMODIALYSIS OR CHRONIC PERITONEAL DIALYSIS

	Stage 3 to 5 chronic kidney disease[a,b,c]	Maintenance hemodialysis (MHD)[c]	Continuous ambulatory peritoneal dialysis (CAPD) or automated peritoneal dialysis (APD)
Protein			
Low protein diet	0.60 gm/kg/day; protein may be increased up to 0.75 gm/kg/ adequate energy intake or if patient will not accept lower protein diets. 50% of protein is of high biologic value	1.2 gm/kg/day; 50 percent high biological value protein	1.2 to 1.3 gm/kg/day; 50% high biologic day if necessary to maintain value protein
Energy (kcal/kg/day)	Thirty-five kcal/kg/day for individuals less than 60-years-old and 30 to 35 kcal/kg/day for patients 60-years-old or greater. Patients with relative body weight greater than 120% or patients who gain or are afraid of gaining unwanted weight may be prescribed lower energy intakes. Energy intake includes energy obtained from diet and, if applicable, from hemodialysate or peritoneal dialysate.		
Fat (percent of total energy intake)[d,e]	25 to 35	25 to 35	25 to 35
Polyunsaturated fatty acids [d,e]	Up to 10% of total calories	Up to 10% of total calories	Up to 10% of total calories
Monounsaturated fatty acids[d,e]	Up to 20% of total calories	Up to 20% of total calories	Up to 20% of total calories
Saturated fatty acids[d,e]	Less than 7% of total calories	Less than 7% of total calories	Less than 7% of total calories
Cholesterol[d,e]	200 mg/day or lower	200 mg/day or lower	200 mg/day or lower
Carbohydrate[d,e,f]	50% to 60% of total calories	50% to 60% of total calories	50% to 60% of total calories
Total fiber intake (gm/day)[d,e]	20 to 30	20 to 30	20 to 30
Minerals (range of intake)			
Sodium (mg/day)	1,000 to 3,000[g]	1,000 to 2,000[g]	1,000 to 3,000[g]
Potassium (meq/day)	40 to 70	40 to 70	40 to 70
Phosphorus (mg/kg/day)	10 to 14[h]	12 to 17[h]	12 to 17[h]
Calcium (mg/day)	1,000 to 1,200[i]	1,000 to 1,200[i]	1,000 to 1,200[i]
Magnesium (mg/day)	200 to 300	200 to 300	200 to 300
Iron (mg/day)	≥10 to 18[j]	see text[j]	see text[j]
Zinc (mg/day)	15	15	15
Water (mL/day)	Up to 3,000 as tolerated[g]	Usually 750 to 1,500[g]	Usually 1,000 to 1,500[g]

Vitamins	Diets to be supplemented with these quantities		
Thiamin (mg/day)	1.5	1.5	1.5
Riboflavin (mg/day)	1.8	1.8	1.8
Pantothenic acid (mg/day)	5	5	5
Niacin (mg/day)	20	20	20
Pyridoxine HCl (mg/day)	5	10	10
Vitamin B_{12} (μg/day)	3	3	3
Vitamin C (mg/day)	60	60	60
Folic acid (mg/day)	1 to 15[k]	1 to 15[k]	1 to 15[k]
Vitamin A	No addition	No addition	No addition
Vitamin D	See text	See text	See text
Vitamin E (IU/day)	15	15	15
Vitamin K	None[l]	None[l]	None[l]

[a] GFR above 4 to 5 mL/minute/1.73 m^2 and less than 30 mL/minute/1.73 m^2 (see text for discussion of dietary intake for patients with less severe renal insufficiency). Note: Patients are to be informed that the ability of low protein diets to release progressive chronic renal disease in patients who are receiving angiotensin-converting enzyme inhibitors and/or angiotensin receptor blockers has not been definitely demonstrated (see text).
[b] The protein intake is increased by 1.0 gm per day of high biologic value protein for each gm per day of urinary protein loss.
[c] When recommended intake is expressed per kilogram body weight, this refers to the adjusted edema-free-body weight (see text).
[d] Refers to percent of total energy intake (diet plus dialysate); if triglyceride levels are very high, the percentage of fat in the diet may be increased to about 40% of total calories; otherwise, 30% or less of total calories is preferable. The recommendations regarding fat, carbohydrate, and fiber intake follow the NCEP TLC (Therapeutic Lifestyle Changes) Diet (346).
[e] These dietary recommendations are considered less crucial than the others. They are only emphasized if the patient has a specific disorder that may benefit from this modification or is complying well to more important aspects of the dietary treatment (see text).
[f] Should be primarily complex carbohydrates.
[g] Can be higher in CAPD patients or in hemodialysis patients or nondialyzed patients with chronic renal failure who have greater urinary losses.
[h] Phosphate binders (sevelamer HCl, lanthanum carbonate, aluminum carbonate, or hydroxide, or calcium carbonate, acetate, or citrate) often are needed to maintain normal serum phosphorus levels.
[i] Dietary intake usually must be supplemented to provide 1,000 mg calcium/day. The maximum calcium intake, including from binders, generally should not exceed about 2,000 mg/day.
[j] $\geq$10 mg/day for males and nonmenstruating females; $\geq$18 mg/day for menstruating females. For individuals receiving erythropoietin, large iron intakes, usually given intravenously, are usually necessary (see text).
[k] Folic acid, 1 mg/day, is adequate for CRF and MD patients. If hyperhomocysteinemia is present, folic acid, 5 to 15 mg/day, may be more effective at maximizing the reduction in plasma homocysteine concentrations (see text).
[l] Vitamin K supplements may be needed for patients who are not eating and who receive antibiotics.
Note: Patients undergoing hemodialysis more frequently than thrice-weekly may have increased tolerance and/or increased requirements for some nutrients.

individuals ingesting higher protein intakes to commence such therapy.

An interesting question is whether diets may promote or retard the development of renal failure in individuals with no underlying renal disease. At the present time, there are no clear answers to this question. Another unresolved issue is whether low protein diets will retard progression of renal failure in patients receiving ACEIs and/or ARBs. Since dietary protein restriction exerts many of the same hemodynamic and other physiological effects on the kidney as do ACEIs and ARBs (292–294), it is possible that the renal-protective effects of low protein diets, when combined with ACEI's and ARBs, are replicative, rather than additive. In one relatively small study, 82 patients with Type I diabetes mellitus were randomly assigned to a low protein (0.60 g protein/kg/day) diet or a more usual protein diet (295); most of the patients were receiving ACEIs. In this 4-year trial, the low-protein group experienced a 10% incidence of death or ESRD as compared to 27% in the patients eating the usual protein diet ($p < 0.042$). There was no difference in the rate of decline in GFR in the two groups (295).

Since proteinuria is associated with greater progression of renal failure and increased risk of cardiovascular disease, and since ACEIs and ARBs, even in combination, may reduce proteinuria but do not eradicate it, there may be a role for low protein diets at least in persistently proteinuric patients. More research in this area is clearly needed.

Recommended Protein Intake. GFR greater than 70 mL/minute/1.73 m^2. There is almost no information concerning the most desirable dietary protein and phosphorus prescription for patients with stage I or II CKD. Indeed, almost all of the studies of the effect of low protein diets on the rate of progression of renal failure have examined patients with moderately advanced to advanced CKD (i.e., stages 3, 4, and 5).

Currently we do not usually restrict protein (and phosphorus) intake for patients with GFR greater than 70 mL/minute/1.73 m^2 except possibly to 0.80 to 1.0 g protein and 1,200 to 1,400 mg phosphorus per kg/day unless there is evidence that renal function is continuing to decline. Under these circumstances, the patient is treated as indicated in the next paragraph.

GFR 30 to 70 mL/minute/1.73 m^2

At present, it is the author's policy to discuss with the patient the evidence that low protein, low phosphorus diets may retard progression and to indicate that the data, although not definitive, are sufficiently convincing to justify prescription of a protein and phosphorus restricted diet. If the patient agrees to dietary therapy, he is offered a diet providing about 0.60 to 0.75 gm protein/kg/day, of which at least 0.35 gm/kg/day is high biologic value protein to ensure a sufficient intake of the essential amino acids. This quantity of protein should maintain neutral or positive nitrogen balance, and for many patients should not be excessively burdensome. Ketoacid preparations are not available in the United States for therapeutic use. The effect of these preparations on retarding the progression of CKD is still open to question (219), and the intake of these compounds may be associated with increased mortality (see the earlier text) (289).

GFR Less Than 30 mL/minute/1.73 m^2 (Stage 4 and 5 CKD)

At this level of renal insufficiency, the potential advantages of using a low protein, low phosphorus diet become more compelling. First, at this degree of renal failure, potentially toxic products of nitrogen metabolism begin to accumulate in substantial quantities. The low protein diet will generate less nitrogenous compounds that are potentially toxic, both systemically and to the kidney itself. Second, because the low protein diet generally contains less phosphorus and potassium, the intake of these minerals can be lowered more readily with this diet (see later sections on recommended phosphorus and potassium intakes). Third, some patients with chronic renal insufficiency eat too little protein rather than too much. Specific training and encouragement to follow a prescribed diet may increase the likelihood that the patient will not ingest too little protein. Patients should be prescribed 0.60 gm protein/kg/day or, if this is not feasible, up to 0.75 gm protein/kg/day (Table 103-6). This diet will generally maintain neutral or positive nitrogen balance as long as energy intake is not deficient (Table 103-5), should generate a low UNA and reduce the development of uremic symptoms (236,237,251) and may be more effective at retarding the progression of renal failure.

The NKF K/DOQI Clinical Practice Guidelines in Nutrition recommend "for individuals with chronic renal failure (GFR less than 25 mL/minute) who are not undergoing maintenance dialysis, the institution of a planned low-protein diet providing 0.60 gm protein/kg/day should be considered. For individuals who will not accept such a diet or who are unable to maintain adequate dietary energy intake with such a diet, an intake of up to 0.75 gm protein/kg/day may be prescribed" (296). Allowing the dietary protein intake to increase to 0.75 gm/kg/day may enable the patient to increase dietary energy intake; this diet also is easier for most people to follow. Dietary protein intakes greater than 0.75 gm protein/kg/day may be associated with more rapid rates of progression of renal failure (279). At least 50% of the protein intake with these low protein diets should be of high biological value. The protein content of this diet should be increased by 1.0 gm per day of high biologic value protein for each gram of protein excreted in the urine each day. In the experience of the author, up to 15% of individuals with chronic renal disease are willing and able to adhere to diets providing 0.60 gm protein/kg/day or essential amino acid supplemented very low protein diets.

The clinical indications for cessation of low protein dietary therapy and inauguration of maintenance dialysis therapy or renal transplantation are still somewhat controversial. The NKF K/DOQI Clinical Practice Guidelines in Nutrition recommend a nutritional indicator for inaugurating renal replacement therapy as follows (297): "In patients with chronic renal failure, (e.g., GFR less than 15 to 20 mL/minute) who are not undergoing maintenance dialysis, if protein-energy malnutrition develops or persists despite vigorous attempts to optimize protein and energy intake and there is no apparent cause for malnutrition other than low nutrient intake, initiation of maintenance dialysis or a renal transplant is recommended." This guideline is based on the rationale that patients who have evidence for protein-energy malnutrition at the onset of maintenance dialysis therapy have an increased mortality rate, and that inaugurating maintenance dialysis therapy or performing a renal transplant will often improve nutritional status in malnourished individuals with advanced CRF (189,231,298,299).

Maintenance Hemodialysis (MHD)

There are few studies of dietary protein requirements in patients undergoing MHD. These investigations were all nitrogen balance studies; of the carefully controlled balance studies most were carried out using dialyzers and schedules of dialysis therapy that are no longer in use (148,300–302). It is apparent that maintenance dialysis patients have increased dietary protein

requirements because of the removal of amino acids and peptides by the dialysis procedure (106–110) and because hemodialysis appears to stimulate protein catabolism by engendering an inflammatory, catabolic response. (148,150,151). Nitrogen balance studies suggest that most maintenance hemodialysis patients require more than 1.0 gm protein/kg/day to maintain both protein balance and normal total body protein (148,301,302). The NKF K/DOQI Clinical Practice Guidelines on Nutrition in CRF recommend 1.2 gm protein/kg/body weight/day for clinically stable patients (303). To ensure adequate intake of essential amino acids, at least half of the dietary protein should be of high biologic value. This level of protein intake should maintain neutral or positive balance in almost all clinically stable patients undergoing MHD thrice weekly. There are currently no data concerning dietary protein requirements for patients undergoing MHD five or more times weekly. Most likely, the safe dietary protein intakes for these latter individuals also will be at least 1.2 gm protein/kg/day.

Chronic Peritoneal Dialysis (CPD)

Blumenkrantz and co-workers studied protein and mineral balances in eight clinically stable men who underwent 13 metabolic balance studies of 14 to 33 days' duration in a clinical research center (304). Patients were fed diets that provided an average of 0.98 gm protein/kg/day or 1.44 gm protein/kg/day. Total energy intake (diet plus dialysate) was 41.3 ± 1.9 and 42.1 ± 1.2 kcal/kg/day with the low and high protein diets, respectively. Nitrogen balance, adjusted for changes in body urea nitrogen but not for unmeasured losses, was +0.35 ± 0.83 gm per day with the 1.0 gm per kilogram protein diet and +2.94 ± 0.54 gm per day with the higher protein intake (p:NS). Only the latter balance was significantly positive, even if nitrogen balances were adjusted by about 1.0 gm per day for losses through skin, respiration, flatus, and blood sampling. There was a curvilinear relationship between dietary protein intake and nitrogen balance in the 13 studies (Fig. 103-1). Nitrogen balance rose as protein intake increased until protein intake was 1.09 gm/kg/day. At this level, balance was significantly positive. As dietary protein increased above this level, there was no further increment in nitrogen balance.

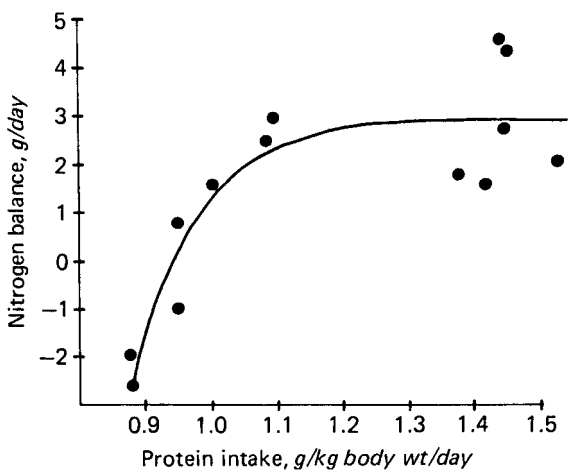

FIGURE 103-1. Relationship between dietary protein intake and nitrogen balance measured in 13 studies in 8 men undergoing CAPD. Each circle represents the mean balance data observed in an individual patient fed a constant diet for 14 to 33 days in a clinical research unit. The curved line represents the calculated relationship between nitrogen balance and protein intake. (From: Blumenkrantz MJ, Kopple, JD, Moran, JK, et al. Metabolic balance studies and dietary protein requirements in patients undergoing continuous ambulatory peritoneal dialysis. *Kidney Int* 1982;21:849, with permission.)

These findings are similar to those of Giordano and co-workers, who reported neutral to positive nitrogen balance in 7 of 8 CAPD patients who were fed about 1.2 gm protein/kg/day (305). Gahl and associates reported neutral or positive nitrogen balance in 5 outpatients undergoing CAPD who were ingesting lower protein diets that provided 0.71 to 0.96 gm protein/kg/day, but feces were not collected (306). Lindholm and co-workers carried out short-term (10- to 14-day) balance studies in 10 patients undergoing CAPD who were fed diets containing 0.76 to 1.07 gm protein/kg/day (307). Total energy intake was about 29 kcal/kg/day. Nitrogen balance, adjusted for unmeasured losses, was positive in all patients. However, there was a positive correlation between protein intake and nitrogen balance in these patients.

Based on the foregoing reports, it seems prudent to prescribe 1.2 to 1.3 gm protein/kg/day to CAPD patients. This is the recommendation of the NKF K/DOQI Clinical Practice Guidelines on Nutrition in CRF for clinically stable CPD patients (308). As with MHD patients, at least 50% of the dietary protein should be of high biological value (308). It is possible that CAPD patients who are protein depleted may become more anabolic when they ingest up to 1.5 gm protein/kg/day (see Fig. 103-1). In most CAPD patients, SUN levels and uremic toxicity should be adequately controlled with this protein intake, although there may be a need in some patients to increase the number of dialysate exchanges or at least the volume of dialysate outflow.

The author is unaware of any studies concerning dietary protein requirements of patients undergoing automated peritoneal dialysis. Since, the protein and amino acid losses with these latter peritoneal dialysis techniques should not be much different than with CAPD, the dietary requirements are probably similar to those of the patient receiving CAPD. It should be noted that there have been other nitrogen balance studies in MHD or CPD patients (309–311). However, either total nitrogen intake and/or outputs were not directly measured in these investigations or the duration of the study was too short to effectively interpret the results. Since measurement of nitrogen balances requires rigor and precision to obtain accurate data (312), the results of these latter studies are difficult to interpret.

It has been shown that patients classified as high peritoneal transporters by the peritoneal equilibration test lose more protein and amino acids into peritoneal dialysate than those classified as low transporters (e.g., typical 24-hour losses in high vs. low transporters: albumin, 4.9 ± 2 (SD) vs. 3.2 ± 1 gm/day, $p < 0.03$, total free amino acids, 15.4 ± 4 vs. 10 ± 4 mmol/day, $p = 0.002$) (193,194,194a). This trend is shown in all studies of high vs. low transporters. The high transporters also have, on average, lower serum albumin levels (313,314). The difference in protein and amino acid losses with high versus low transporters, however, are not great. Dietary protein intakes of 1.2 to 1.3 gm/kg/day should provide sufficient amounts of protein and amino acids to compensate for the increased peritoneal losses if the endogenous synthetic function for serum proteins is normal.

Some nephrologists describe MHD or CPD patients who have dietary protein intakes of about 0.9 to 1.0 gm/kg/day and who do not appear protein depleted and lead physically active, rehabilitated lives. These observations have raised questions as to whether the foregoing recommended dietary protein intake may be excessive for MD patients. Several comments may be pertinent in this regard. First, the number of MHD or CPD patients who have undergone careful nitrogen balance or other studies of their dietary protein requirements is small, and conclusions concerning their dietary protein needs therefore are somewhat imprecise; this is particularly true for MHD.

Moreover, some MD patients who appear to be doing well will, on close inspection, turn out to have evidence for protein depletion. Epidemiological studies have associated low protein intakes and protein depletion, even in mild forms, with increased mortality (131,132,207,209).

On the other hand, the concept of dietary allowances presupposes that in order to ensure a sufficient nutrient intake for virtually all individuals (i.e., about 97%) in a given population, the recommended allowance must be greater than the actual requirement for a large proportion of that population (315). This reasoning is similar to that used by the World Health Organization to determine the recommended dietary protein intakes for normal adults (316). Thus, if the recommended dietary protein allowance is 1.2 to 1.3 gm/kg/day, it is to be expected that many patients will tolerate lower protein intakes without developing protein depletion. At present, there is no known method that will identify in advance, under the conditions of clinical practice, which patients can safely ingest lower levels of protein. Hence, to be safe, unless the patient can be shown to maintain a healthy nutritional status on a low protein intake, he should be prescribed the recommended dietary allowance, an especially relevant consideration given the high incidence of protein malnutrition in MHD and CPD patients. Finally, subtle forms of malnutrition are particularly difficult to detect. The recommended protein intakes may provide some protection against mild forms of protein malnutrition. In this regard, more recent data suggest that a healthy serum albumin concentration may be about 4.0 to 5.0 gm per dL, which is higher than the not uncommonly used standard of 3.5 to 5.0 gm per dL (63).

ENERGY

In nondialyzed CRF patients and patients undergoing MHD or CAPD, energy expenditure, measured by indirect calorimetry appears to be normal during resting and sitting, following ingestion of a standard meal, and with defined exercise (302,317–323). In two studies of nondialyzed CRF or MHD and CPD patients, resting energy expenditure was increased (321,324). Resting energy expenditure also has been reported to be increased during the hemodialysis procedure in two studies (321,324); but this elevation in energy expenditure may have been due to the specific dynamic action from recently ingested foods. What is most impressive about the foregoing studies is that there is no report of decreased energy expenditure in CRF, MHD or CPD patients.

Nitrogen balance studies in nondialyzed stage 5 CKD patients ingesting 0.55 to 0.60 gm protein/kg/day indicate that the amount of energy intake necessary to ensure neutral or positive nitrogen balance is approximately 35 kcal/kg/day (302). In clinically stable patients undergoing MHD who ingested 1.13 ± 0.02 (SEM) gm protein/kg/day, nitrogen balance studies and anthropometric measurements indicate that close to 38 kcal/kg/day may be necessary to maintain body mass (325). On the other hand, virtually every study of the dietary habits of nondialyzed stage 4 and 5 CKD patients and MHD and CPD patients indicates that their mean energy intakes are lower than this level, usually about 24 to 27 kcal/kg/day (47,53,81,82,326). Children with CRF also have low energy intakes (51,327–329). Indeed, food preferences and eating habits are altered in patients with CRF (330,331). The finding that in nondialyzed patients with advanced CRF or patients undergoing MHD, decreased body fat is one of the more prominent alterations in nutritional status supports the contention that these patients require more energy than they usually ingest (47,49,53,208–211).

There are no nitrogen balance studies in CPD patients in which protein intake has been kept constant while energy intake was varied; although as indicated earlier, resting energy expenditure in CAPD patients is not different than normal (320). Many patients undergoing CPD tend to gain body fat and weight (52), probably due to the glucose uptake from dialysate exchanges. The surges in plasma insulin that accompany the peritoneal instillation of fresh dialysate and enhanced glucose absorption may also contribute to the increase in body fat of CPD patients. The quantity of glucose absorbed from CAPD is reported to vary between 78 and 316 gm per day (118,332–335). There is a close correlation for CAPD between the quantity of glucose instilled into the peritoneal cavity each day (X) and the quantity of glucose absorbed (334); (Y), viz., Y (gm/day) = 0.89X (gm/day) − 43, r = 0.91. In any individual patient, the daily rate of glucose absorption with the same dialysis regimen appears to be rather constant.

The NKF K/DOQI Clinical Practice Guidelines for Nutrition in CRF recommend that the energy intake for nondialyzed CRF patients (GFR less than 25 mL/minute) and for MHD and CPD patients should be 35 kcal/kg/day for individuals who are less than 60 years of age and 30 to 35 kcal/kg/day for those who are 60 years of age or older (Table 103-5) (336,337). This intake includes energy derived from the diet as well as from any fuels taken up from dialysate in MHD or CPD patients. Because individuals more than 60-years-old tend to be more sedentary, their recommended energy intake is somewhat lower. These recommendations are rather similar to those for normal individuals engaged in light to moderate activity as put forth in the Recommended Dietary Allowances by the Food and Nutrition Board, National Academy of Sciences (315). The same energy intakes are recommended for people with stage 3 or 4 CKD (i.e., GFR <60 mL/minute/1.73 m^2). Patients who are obese with an edema-free body weight greater than 120% of desirable body weight may be treated with lower calorie intakes. Some patients, particularly those with more mild renal insufficiency and young or middle-aged women, may become obese on this energy intake or may refuse to ingest the recommended calories out of fear of obesity. These individuals may require a lower energy prescription.

As indicated earlier, energy (and protein) intake seem to decrease when the GFR falls to about 35 to 50 mL/minute/1.73 m^2 (see the previous text) (76). Thus, to prevent malnutrition, it is important to monitor dietary intake and to treat inadequate intakes, even in clinically stable healthy appearing adults, when the GFR decreases to this level. This level of GFR may be associated with a serum creatinine as low as 1.4 to 2.5 mg/dL in some adult patients.

There are many commercially available high-calorie foodstuffs that are low in protein, sodium and potassium. The renal dietician can recommend these products as well as other low protein, high calorie foods that can be prepared easily at home.

TREATMENT OF RISK FACTORS FOR VASCULAR DISEASE

Lipids

There are a number of reviews of the causes for the abnormal serum lipids and lipoproteins in these patients (21–23,26). Stage 4 and 5 CKD patients and patients undergoing MHD and CPD have a high incidence of increased serum triglyceride levels, IDL, and very LDL (VLDL), and serum lipoprotein (a) Lp(a); serum high density lipoprotein (HDL) cholesterol is often low in CRF and MHD patients (21–26). CPD patients, often have higher serum total cholesterol, triglycerides, LDL cholesterol and apolipoprotein B levels than do MHD patients (33–35,338). Qualitative changes in the apolipoprotein

concentrations also occur; among these is an increase in small dense LDL (sd LDL) (27).

Elevated serum triglyceride levels in uremia appear to be caused primarily by impaired catabolism of triglyceride rich lipoproteins (32,339,340). This reduced catabolic rate leads to increased quantities of apoB-containing triglyceride-rich lipoproteins in IDL and VLDL and reduced concentrations of HDL. The key alteration in the apolipoprotein levels appears to be a decreased ratio of apoA-1 to apoC-III (341). Activities of plasma and hepatic lipoprotein lipase and lecithin cholesterol acyltransferase (LCAT) are reduced (21,22,26). Also, because diets for renal failure patients are usually restricted in protein, sodium, potassium, and water, it is often difficult to provide sufficient energy without resorting to a large intake of purified sugars that may increase triglyceride production. CPD, which provides a glucose load, appears to promote a further increase serum triglycerides and cholesterol. Patients with the nephrotic syndrome usually have hypertriglyceridemia with increased serum total, cholesterol, and LDL cholesterol. Low density lipoproteins, IDL, VLDL, and Lp(a) are increased (268), and serum HDL tends to be low. Serum phospholipids, and apoproteins B, C-II, C-III, and E are increased, whereas apoproteins A-I and A-II are normal (342). Elevated serum cholesterol is caused by increased hepatic synthesis of lipoproteins and cholesterol and reduction in LDL receptor activity, which plays an important role in the clearance of IDL. These changes are stimulated by urinary albumin losses. Decreased activity of lipoprotein lipase contributes to the elevated serum triglyceride levels. There is elevated plasma cholesterol ester transfer protein (CETP) and decreased catabolism of LDL apolipoprotein, at least by the more typical receptor pathway.

Renal transplant recipients may have high serum total and LDL cholesterol. LDL and IDL lipoproteins are increased. Increased serum total cholesterol, LDL-cholesterol and triglycerides are more likely to be present in transplant recipients with chronic rejection and to correlate with a decrease in kidney function (343–345). Medicinal therapy (glucocorticoids, cyclosporine A, sirolimus, tacrolimus, diuretics, and certain antihypertensives), renal failure, fasting hyperinsulinemia, and obesity, commonly present in renal transplant recipients, may add to the high incidence of serum lipid disorders.

Since alterations in lipid metabolism and serum lipids may contribute to the high incidence of atherosclerosis and cardiovascular cerebrovascular and peripheral vascular disease in CRF patients, attention has been directed toward reducing serum triglycerides and LDL cholesterol and increasing HDL cholesterol. Treatment of altered lipid levels and the risk of cardiac and vascular disease involves three components: nutrient intake, medicines, and exercise. It should be emphasized that publication of clinical trials comparing these three forms of therapy to outcome are just beginning to emerge, and most recommendations are derived from studies conducted in populations not selected to have renal disease.

We recommend a dietary plan that is based upon the National Cholesterol Education Program (NCEP) Therapeutic Lifestyle Changes (TLC) diet for all patients with CKD, including MHD and CPD patients, patients with the nephrotic syndrome and renal transplant recipients, especially if their serum LDL levels are over 100 mg/dL (346). Since all of these patients are at high risk for cardiovascular, cerebrovascular and peripheral vascular disease, we prefer to set a target LDL cholesterol of 70 mg/dL.

The TLC diet provides the following (346): No more than 25% to 35% of total calories from fat, polyunsaturated fatty acids providing up to 10% of total calories, monounsaturated fatty acids providing up to 20% of total calories, saturated fatty acids providing less than 7 percent of total calories, and a cholesterol content of 200 mg per day or lower. Carbohydrate intake should be 50% to 60% of total calories and derived predominantly from foods rich in complex carbohydrates. Fiber intake should be 20 to 30 g/day (346). Since the TLC diet may be less palatable to many patients, their energy intake should be monitored to ensure that it remains adequate (see the subsequent text). Patients are encouraged to control calorie intake to avoid becoming substantially overweight or frankly obese. (i.e., BMI greater than about 28 kg/m^2).

It is recognized that most individuals will not be able to adhere exactly to the TLC diet. However, there is reason to believe that even some modifications of dietary intake in the direction of lowering serum cholesterol may reduce the risk of adverse vascular events (347). Moreover, if CKD patients are not counseled on the characteristics and rationale underlying this diet, they may eat even a less healthy diet as they grow older.

Omega-3 fatty acids (e.g., eicosapentaenoic acid and docosahexaenoic acid, which are found in fish oil) lower serum triglycerides and have more variable effects on serum LDL cholesterol and HDL cholesterol (349). Fish oil also decreases platelet aggregation and appears to exert antiinflammatory effects, and omega-3 fatty acids may enhance immune function. Low-fat diets and lipid-lowering medicines retard the rate of progression of renal failure in animal models (350–352). In humans, some research suggests that omega-3 fatty acids may lower the progression of renal failure in renal transplant patients (353). A preponderance of studies suggest that omega-3 fatty acids given as fish oil may retard the rate of progression of IgA nephropathy (354–357).

3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) have been shown in the general population to reduce coronary artery morbidity and mortality in certain groups of high risk coronary heart disease patients (358). These findings have stimulated enormous interest in the use of these agents in persons with CKD. Interest has been further enhanced by the findings that, in addition to their LDL-cholesterol lowering effects, statins may slightly increase serum HDL-cholesterol and have antiinflammatory; antithrombotic, fibrinolytic effects, may inhibit NF-KB activation, increase nitric oxide biosynthesis and bioavailability, decrease synthesis of certain proinflammatory cytokines, improve impaired endothelial function and protect against progressive renal injury in animals (358,359). Statins clearly decrease LDL cholesterol in CKD patients including patients with the nephrotic syndrome, maintenance dialysis patients and renal transplant recipients (360–363). Statins appear to be safe (362–364). In the HOPE Trial, when those individuals with serum creatinine concentrations greater than 1.4 mg/dL were analyzed separately, those with either diabetes mellitus or hypertension who were given ramipril had a significantly lower incidence of the composite outcome (stroke, nonfatal myocardial infarction or all cause mortality) (365). On the other hand, in the 4D study of 1,255 MHD patients who were randomized to either atorvastatin or placebo, there was no reduction in cardiovascular or all-cause mortality in the atorvastatin-treated group (364).

A recent study examined 56 nondiabetic mild to moderate CKD patients receiving an angiotensin converting enzyme (ACE) inhibitor or an angiotensin AT_1 receptor antagonist (ARB) for 1 year who were then randomized to also receive atorvastatin or placebo for 1 year. In those given atorvastatin, proteinuria decreased significantly; whereas in the placebo treated group only, the creatinine clearance fell significantly (362). In a posthoc subset analysis of nondiabetic CKD patients from the CARE trial, those patients with a GFR less than 40 mL/minute who were treated with pravastatin as compared to placebo displayed a slower of loss of GFR, as determined from the MDRD GFR equation (361). In patients who had proteinuria, as determined by a positive dipstick, and who had initial GFR levels less than 50 mL/minute, pravastatin treatment was

associated with a slower loss of GFR (361). The fibric acid derivative, fenofibrate, also reduced progression to albuminuria in type 2 diabetic patients in the DAIS study (365a). It is important to keep in mind that both statins and fibric acid derivatives can induce a number of side effects including a myopathy (366) and that the combined use of a statin and a fibrate in kidney insufficiency are particularly likely to cause a severe myopathy and should be used together with great caution, if ever.

Abnormal carnitine metabolism (see the subsequent text) has been implicated as a cause of hypertriglyceridemia in CRF. However, the many studies of treatment of hypertriglyceridemia with carnitine in patients with CRF are divided between substantial numbers that show carnitine lowers serum triglycerides and substantial numbers that show no change or, rarely, a rise in serum triglycerides (367,368). Ingestion of activated charcoal may lower serum cholesterol and triglycerides in chronically azotemic rats (369). Sevelamer HCl, which binds phosphate in the gastrointestinal tract, also lowers serum LDL-cholesterol and increases serum HDL-cholesterol (370).

Other Potential Techniques for Reducing Cardiovascular Risk

Evidence for the protective nature of other methods for reducing cardiovascular risk is largely obtained from clinical trials in the general population. Antiplatelet therapy, such as aspirin, has been shown to reduce the risk of myocardial infarction in adult high risk patients, by 25%, (371) and also in CKD patients (371a) and maintenance dialysis patients (371b). In CRF patients, impaired hemostasis caused by aspirin may increase the risk of bleeding. A dose of 100 mg aspirin per day taken by CKD patients, maintenance dialysis patients or renal transplant recipients increased the risk of minor bleeding (e.g., epistaxis, ecchymoses, or bruising) by threefold ($p = 0.001$). However, it did not increase the risk of major bleeding episodes (e.g., leading to hospitalization or fatality) or of more rapid loss of renal function (363).

Multifactorial intervention may also reduce the risk of adverse cardiovascular events in patients with Type 2 diabetes mellitus (372). The intervention consisted of dietary treatment (fat intake less than 30% of daily energy intake, saturated fatty acids less than 10% of daily intake), 30 minutes of light to moderate exercise three to five times per week, smoking cessation classes for smokers, daily intake of ACE inhibitors or ARBs irrespective of blood pressure, daily vitamin-mineral supplements providing vitamin C 250 mg; D-alpha-tocopherol 100 mg, folic acid 400 μg, chromium picolinate 100 μg, aspirin (unless there were contraindicators), aggressive control of blood glucose, blood pressure, hypercholesterolemia and hypertriglyceridemia. These patients receiving this intensive therapy had a significantly lower risk of adverse cardiovascular events, renal disease (albuminuria greater than 300 mg/24 hours in two of three consecutive urine specimens), retinopathy and automatic neuropathy.

More intensive counseling of diabetic patients may significantly reduce the magnitude of albuminuria, the rate of progression of renal failure, adverse cardiovascular events and stroke (373). Other reports indicate that in individuals with borderline abnormal glucose tolerance tests, dietary counseling and exercise can delay the onset of diabetes mellitus (374).

Low serum HDL-cholesterol, a common phenomenon in CRF patients, appears to be an independent risk factor for adverse coronary artery events (375). A recent single blind, placebo controlled study indicated that an inhibitor of the cholesterol ester transfer protein, torcetrapib, can increase plasma HDL-cholesterol by an average of 46% ($p < 0.001$) to 106% ($p < 0.001$) depending on the dose of torcetrapib and whether atorvastatin was also taken (376). Treatment for hyperhomocysteinemia is discussed later.

At present, we recommend a TLC NCEP diet for nondialyzed patients with CRF, and maintenance dialysis patients and renal transplant recipients (see the previous text) (346). We treat hypertriglyceridemia by dietary modification or medicines only when fasting serum triglycerides are very high (i.e., 500 mg/dL or more). In this situation, dietary fat intake may be increased, but not above 40% of total calories. A high proportion of dietary carbohydrates should be complex. These modifications often lower the palatability of the diet; therefore, the patient's total energy intake must be monitored closely to ensure that it does not fall to levels that are inadequate to maintain desirable body and protein mass.

With high serum triglyceride values that are unresponsive to dietary therapy, a fibrate (e.g., fenofibrate) may be tried cautiously. For CPD patients, L-carnitine about 500 to 1,000 mg per day, or, for hemodialysis patients, L-carnitine, 10 to 20 mg/kg/day at the end of each dialysis three times weekly, may be tried if hypertriglyceridemia is severe and unresponsive to these treatments. The author's target for serum LDL-cholesterol is 70 mg/dL. This concentration may be attained with a 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitor in addition to the TLC diet. In the general population, higher doses of statins may be more protective against adverse cardiovascular events (348). Occasionally, it may be necessary to add the medicine ezetimibe, which reduces intestinal cholesterol absorption, to statins or other cholesterol-lowering medicines in order to reach these target serum concentrations of LDL-cholesterol (377).

Oxidant and Carbonyl Stress

Evidence suggests that end-stage renal disease is associated with oxidant and carbonyl stress and often with a state of chronic inflammation, and that these factors may promote atherosclerotic and proliferative vascular disease (36–41, see the earlier text). At present, there are no interventional trials evaluating the effects of reduction of these risk factors on morbidity or mortality of CRF or MD patients. Nevertheless, the high risk of cardiovascular disease and the evidence for oxidant and carbonyl stress and inflammation in CRF and MD patients has raised questions as to whether the following treatments may be beneficial for these individuals. Larger flux dialyzers that remove greater amounts of advanced glycation end products, and other reactive carbonyl compounds may be helpful. It is possible to ingest antioxidants or antioxidant precursors, such as vitamins E or C (see the subsequent vitamin section) or selenium. Supplemental selenium must be taken with caution because selenium is primarily excreted in the urine and may accumulate in renal failure (378). Also, it is difficult to measure the body selenium burden because it is protein bound in plasma and the possibility of altered concentrations or binding affinities for the binding protein may make it difficult to identify selenium excess. One glass/day of an alcoholic drink, perhaps particularly of red wine, HMG CoA reductase inhibitors, and regular exercise may also reduce oxidant stress (379).

Homocysteine

Plasma homocysteine is increased in nondialyzed chronic renal failure patients as well as in about 90% to 95% of MHD and CPD patients (216,217,380–387). The mechanism for this increase is unclear but may involve impaired remethylation of homocysteine back to methionine (381). In the general population, elevated plasma homocysteine is associated with a

high incidence of cardiovascular disease possibly through three biochemical mechanisms (380): homocysteine oxidation generating hydrogen peroxide, decreased methylation reaction due to accumulation of S-adenosylhomocysteine, and acylation of proteins by homocysteine thiolactone. These processes lead to damaged membrane proteins, endothelial injury, inhibition of endothelial cell growth as well as other effects. In renal failure patients, hyperhomocysteinemia has been shown to be a risk factor for cardiovascular disease in some (216,383–385), but not all (217,386), epidemiological studies. In addition to renal failure, low serum folate levels, and possibly protein-energy malnutrition (216), a common mutation in the methylenetetrahydrofolate reductase gene, C677T, is associated with higher plasma homocysteine concentrations (382).

Vitamin B_6 is a cofactor for cystathionine synthetase, which catalyzes the conversion of methionine to cystathionine, and for cystathionase, which converts cystathionine to cysteine. Pyridoxine HCl, a form of vitamin B_6, generally does not lower plasma homocysteine in CRF or MD patients. The folate metabolite, tetrahydrofolic acid, is necessary for the remethylation of homocysteine to reform methionine, and folic acid or folinic acid supplements will often decrease plasma homocysteine concentrations in nondialyzed CRF and MD patients, although usually not to normal values (380,386,387). The lowest dose of folic acid associated with the maximum lowering effect on plasma homocysteine appears to be about 5 to 15 mg/day (385–387). The U.S. Veterans Administration is currently conducting a multicenter randomized double blind placebo-controlled study (HOST Study) in nondialyzed CRF and maintenance dialysis patients to ascertain whether large doses of a combination of folic acid, pyridoxine HCl and vitamin B_{12} will reduce adverse cardiovascular events and all-cause mortality (see the vitamin section subsequently).

OTHER NUTRIENTS IN CHRONIC KIDNEY DISEASE—CARNITINE

Carnitine is a naturally occurring compound that is essential for life. It is both synthesized in the body and ingested. Carnitine facilitates the transfer of long chain (>10 carbon) fatty acids into muscle mitochondria (367,368). Since fatty acids are the major fuel source for skeletal and myocardial muscle at rest and during mild to moderate exercise, this activity is considered necessary for normal skeletal and cardiac muscle function. It is also argued that carnitine normally serves a detoxifying function by removing fatty acids from coenzyme A (i.e., from acylcoenzyme A), thereby forming acylcarnitine and free coenzyme A (388). Acylcoenzyme A is toxic or inhibitory for a number of biochemical reactions.

Patients undergoing maintenance dialysis, and particularly maintenance hemodialysis, but not patients with advanced CRF, display low serum free carnitine and, in some but not all studies, low skeletal muscle free and total carnitine levels (87,367,368,389–392). Also, in patients with stage 4 or 5 CKD, including those undergoing maintenance dialysis, serum and, in some reports, in MHD patients, muscle acylcarnitines (fatty acid-carnitine compounds) are increased, and serum total carnitine (i.e., acylcarnitines plus free carnitine) is normal or elevated (87,367,368,391,392). The low serum and skeletal muscle free carnitine levels have led some investigators to postulate that many maintenance dialysis patients are carnitine deficient. Such a deficiency could be due to impaired synthesis of carnitine *in vivo*, reduced dietary intake of carnitine, and removal of carnitine by dialysis (367,368). The weekly loss of free carnitine by dialysis is reported to be approximately equal to the normal weekly urinary excretion of carnitine. However, the finding that serum free carnitine is normal in nondialyzed patients with advanced stage 5 CKD and decreases after the onset of maintenance dialysis therapy is consistent with the thesis that dialysis of L-carnitine is the major cause of low serum carnitine in maintenance dialysis patients (393,394). Oral or intravenous administration of carnitine in maintenance dialysis patients often does not lead to rapid changes in the patient's clinical status, whereas in a number of studies longer term administration of L-carnitine is associated with clinical improvement. These findings have been interpreted to suggest that a simple deficiency of carnitine is not the sole cause of carnitine disorders in patients with CRF. One theory has been advanced that production of acylcarnitine is enhanced in CRF and MD patients due to alterations in lipid or carnitine metabolism (388). Another theory contends that increased concentrations of acylcarnitines interfere with normal carnitine physiology (368,395).

A number of clinical trials in patients with CRF, particularly those patients undergoing maintenance dialysis therapy, suggest that L-carnitine may provide clinical benefits. The types of improvement that have been described in randomized prospective studies include increased physical exercise capacity, reduced interdialytic symptoms of skeletal muscle cramps or hypertension, or improvement in overall global sense of well being or various symptoms often found in CRF patients (396,397). A decrease in predialysis serum urea, creatinine, and phosphorus and an increase in midarm muscle circumference have been reported (396); the mechanisms responsible for these effects are not clear. In this regard, one study suggests that L-carnitine added to peritoneal dialysate may increase nitrogen balance in CAPD patients who have protein-energy malnutrition (398). Other studies, usually nonrandomized, suggest that carnitine may increase hematocrit, reduce cardiac arrhythmias and improve ventricular function in maintenance dialysis patients (397,399–402).

Many nephrologists remain unconvinced by the published results of carnitine benefits. The reasons are that a number of the studies were not designed well (e.g., not prospective and randomized or not double-blinded), the results were not consistent in all studies, the outcome measures were often soft or difficult to quantify precisely, and it seems that patients may not respond to carnitine treatment for up to several months. Nonetheless, many published studies concerning carnitine in MD patients appear to show benefits. Also, L-carnitine appears to be a safe agent. The NKF K/DOQI Clinical Practice Guidelines for Nutrition (403) conclude that "there are insufficient data to support the routine use of L-carnitine for maintenance dialysis patients. Although the administration of L-carnitine may improve subjective symptoms such as malaise, muscle weakness, intradialytic cramps and hypotension, and quality of life in selected maintenance dialysis patients, the totality of evidence is insufficient to recommend its routine provision for any proposed clinical disorder without prior evaluation and attempts at standard therapy. The most promising of proposed applications is treatment of erythropoietin-resistant anemia."

Until more definitive studies are available, it is the author's policy to utilize L-carnitine for MHD or CPD patients who satisfy both of the following conditions: a) Disabling or very bothersome skeletal muscle weakness or cardiomyopathy, skeletal muscle cramps or hypotension during hemodialysis treatment, severe malaise, or anemia refractory to erythropoietin therapy for no apparent or correctable cause; and b) These conditions do not respond to more standard treatment. The patient is given a 3- to 6-month trial of L-carnitine (up to 9 months for refractory anemia). If there is no measurable improvement in symptoms by the end of the treatment period, carnitine therapy is discontinued. L-carnitine may be administered orally, intravenously or into dialysate. Oral L-carnitine is less expensive, but has been less well studied than intravenous carnitine

for MD patients. The optimal dose of carnitine is not well-defined. Some workers use an L-carnitine dose of 20 mg/kg at the end of each hemodialysis, three times weekly (392,397).

CARBOHYDRATES

Patients should be encouraged to eat complex rather than purified carbohydrates to reduce triglyceride synthesis and to improve glucose tolerance if it is abnormal.

SODIUM

Normally, the renal tubules reabsorb more than 99% of the filtered sodium (404). As renal insufficiency progresses, both the glomerular filtration and the fractional reabsorption of sodium fall progressively. Thus, many patients with renal failure are able to maintain sodium balance with a normal sodium intake. In both normal individuals and people with CRF, only about 1 to 3 mEq per day of sodium are excreted in the feces, and in the absence of visible sweating, only a few meq per day of sodium are lost through the skin. Despite the adaptive reduction in the renal tubular reabsorption of sodium, patients with advanced renal failure may be unable to excrete the quantity of sodium ingested, and they may develop edema, hypertension, and congestive heart failure. This syndrome is particularly likely to occur when the GFR is below 4 to 10 mL per minute. When congestive heart failure, the nephrotic syndrome, or advanced liver disease complicates renal insufficiency, the propensity for sodium retention is increased. In renal failure patients, hypertension often is more easily controlled when they are sodium restricted, and hypertension may be accentuated by an increased sodium intake, probably because of expanded extracellular fluid volume (405) and possibly due to altered intracellular electrolyte composition within arteriolar smooth muscle cells that increase contractility. With a decreased ability to excrete sodium or an expanded extracellular fluid volume, restriction of sodium and water intake and the use of diuretic medications may be necessary.

Nondialyzed patients with CRF often have an inability to conserve sodium normally (6,404). A low sodium intake may not be sufficient to replace urinary and extrarenal sodium losses, and the patient may develop sodium depletion, a decrease in extracellular fluid volume, blood volume, renal blood flow, and a further reduction in GFR (i.e., prerenal insufficiency superimposed on CRF). Volume depletion may be difficult to recognize. Unexplained weight loss or reduction in blood pressure may be signs of volume depletion. If the nondialyzed patient with CRF does not have evidence of fluid overload, hypertension, or heart failure, he may be cautiously given a greater sodium intake to determine whether the GFR can be improved slightly by extracellular volume expansion.

The antiproteinuric effects of ACE inhibitors and probably ARBs are substantially abrogated by high sodium diets; as urinary sodium excretion rises above approximately 100 mEq/day, the antiproteinuric effects of these agents abate (406). Treatment with a thiazide diuretic appears to almost completely reestablish the urinary protein lowering effects of the ACE inhibitors in the presence of these higher sodium intakes (406).

Usually, when sodium balance is well controlled, the thirst mechanism will regulate water balance adequately. However, when the GFR falls below 2 to 5 mL per minute, the risk of overhydration increases, and water intake should be controlled independently of sodium to prevent overhydration. In patients with diabetes mellitus, hyperglycemia may also increase thirst and enhance positive water balance. For patients with far advanced renal failure whose total body water is considered appropriate (as indicated by normal or near normal blood pressure, absence of edema, and normal serum sodium), urine volume may be a good guide to water intake; the daily water intake should equal the urine output plus approximately 500 mL to replace insensible losses.

In most nondialyzed patients with advanced renal failure, a daily intake of 1,000 to 3,000 mg (40 to 130 meq) of sodium and 1,500 to 3,000 mL of fluid will maintain sodium and water balance. The requirement for sodium and water varies markedly, and each patient must be managed individually. Patients undergoing MHD or CPD frequently are oliguric or anuric. For hemodialysis patients, sodium and total fluid intake generally should be restricted to 1,000 to 2,000 mg per day and 1,000 to 1,500 mL per day, respectively. Because sodium and water can be removed easily with CAPD or other forms of CPD, a more liberal salt and water intake is usually allowed. Indeed, by maintaining a larger dietary sodium and water intake, the quantity of fluid removed from the CPD patient and hence the daily dialysate outflow volume can be increased. This may be advantageous, since with CPD the daily clearance of small- and middle-sized molecules is directly related to the volume of dialysate outflow. Thus, for some CPD patients, a higher sodium and water intake (e.g., 6 to 8 gm per day of sodium and 3 L per day of water) may enable the patient to use more hypertonic or hyperoncotic dialysate to increase the dialysate outflow volume, thereby increasing dialysate clearances and energy uptake from dialysate, if hypertonic glucose is used.

This treatment may be undesirable for obese or severely hypertriglyceridemic patients, because the greater use of hypertonic glucose exchanges will increase their glucose load. Also, there is the potential disadvantage that some patients may become habituated to high salt and water intakes; if they change to hemodialysis therapy, they may have difficulty in curtailing their sodium and water intake.

In nondialyzed CKD patients or patients undergoing maintenance dialysis who are not anuric and who gain excessive sodium or water despite attempts at dietary restriction, a potent diuretic, such as furosemide or bumetanide, may be tried to increase urinary sodium and water excretion.

POTASSIUM

The kidney normally is the major vehicle for potassium excretion. In renal failure, potassium retention may occur and cause fatal hyperkalemia. Three factors may counteract this side effect of renal failure: First, when the urine volume is approximately 1,000 mL per day or greater, the tubular secretion of potassium per unit GFR tends to increase, and therefore the renal potassium clearance does not fall as markedly as the GFR. Second, in both nondialyzed patients with CRF and maintenance hemodialysis and CPD patients, the fecal excretion of potassium increases, probably owing to enhanced intestinal secretion (251,304). Third, potassium intake tends to fall as a result of anorexia and also in response to dietary counseling.

Thus patients with CRF usually do not become hyperkalemic unless there is (a) excessive intake of potassium; (b) acidemia, oliguria, hypoaldosteronism (e.g., secondary to decreased renin secretion by the diseased kidney or renal tubular resistance to the actions of aldosterone); (c) catabolic stress; or (d) hypoinsulinism or use of medicines such as ACE inhibitors, angiotensin receptor blockers, aldosterone receptor blockers (e.g., spironolactone, eplerenone) and nonsteroidal antiinflammatory drugs (407). Patients with stage 4 or stage 5 CKD (i.e., GFR less than 29 mL/minute), including those undergoing maintenance dialysis, should generally receive no more than 70 mEq of potassium per day.

MAGNESIUM

In nondialyzed patients with CRF and in maintenance dialysis patients, there is a net absorption of about 40% to 50% of ingested magnesium from the intestinal tract (net absorption is the difference between dietary intake and fecal excretion) (304,408). Since the absorbed magnesium is excreted primarily by the kidney, hypermagnesemia may occur in renal failure patients (409). Magnesium also commonly accrues in bone in CRF and may play a causal role in renal osteodystrophy (410). The restricted diets of stage 4 or 5 CKD patients are low in magnesium (usually about 100 to 300 mg per day for a 40 gm protein diet), and the patients' serum magnesium levels are therefore usually normal or only slightly elevated unless the patient takes substances that are high in magnesium content, such as magnesium-containing antacids and laxatives (408,409). Nondialyzed patients with stage 5 CKD require about 200 mg per day of magnesium to maintain neutral magnesium balance (408). The optimal dietary magnesium allowance for the maintenance dialysis patient has not been well defined. Experience suggests that when the magnesium content is about 1.0 meq per L in hemodialysate or 0.50 to 0.75 meq per L in peritoneal dialysate, a dietary magnesium intake of 200 to 300 mg per day will maintain the serum magnesium at normal or only slightly elevated levels.

PHOSPHORUS AND PHOSPHATE BINDERS

The rationale for controlling dietary phosphorus and the use of gastrointestinal binders of phosphate in order to prevent and treat hyperphosphatemia, a high serum calcium-phosphorus product, calcium phosphate deposition in soft tissue, and hyperparathyroidism are discussed in Chapters 89 and 96. This section will consider the prescription of dietary phosphorus intake and phosphate binders. In renal failure patients, a large dietary phosphorus intake can lead to a high plasma calcium-phosphorus product with increased risk of calcium and phosphate deposition in soft tissues including arteries. Moreover, hyperphosphatemia, by lowering serum calcium, provides a strong stimulus to the development of hyperparathyroidism. Also, animal and human studies suggest that a low phosphorus intake may reduce the rate of progression of renal failure in individuals with CKD (411–414).

There are little data concerning the optimal level of phosphorus restriction for retarding progressive renal failure or for minimizing or preventing hyperparathyroidism. For both stage 3 and 4 CKD patients, the serum phosphorus concentrations should be maintained at or above 2.7 mg/dL and no higher than 4.6 mg/dL (415). Since there is a rough correlation between the protein and phosphorus content of the diet, it is much easier to reduce phosphorus intake if a lower protein diet is used. One approach for patients with moderate to advanced CKD (stages 3, 4 and 5) is to maintain phosphorus intake at about 10 to 14 mg/kg/day. This quantity of phosphorus intake is not overly difficult to attain by patients who are ingesting a 0.60 to 0.70 gm protein/kg/day diet. For nondialyzed patients prescribed a 0.55 to 0.60 gm protein/kg/day diet, the phosphorus intake can often be decreased to as low as 8 to 9 mg/kg/day although this may increase the burdensomeness of the diet. It is usually difficult to comply with a dietary phosphorus prescription below 10 mg/kg/day, even when patients ingest 0.55 to 0.60 g protein/kg/day.

The National Kidney Foundation KDOQI Clinical Practice Guidelines for Bone Metabolism and Disease in Chronic Kidney Disease recommends for patients with stage 5 CKD patients (GFR below 15 mL/minute/1.73 m^2), including MHD and CPD patients, that serum phosphorus be maintained between 3.5 to 5.5 mg/dL (415). This often requires a low phosphorus intake of about 800 to 1,000 mg/day, (i.e., about 12 to 17 mg phosphorus/kg/day) (415), particularly when CKD patients have elevated serum phosphorus concentrations and especially if they have moderate or severe hyperparathyroidism (see the subsequent text). This higher upper limit was chosen because with their greater protein intakes, dialysis patients cannot readily ingest less phosphorus without making the diet too restrictive. Without phosphate binders, there is a net intestinal phosphate absorption (diet minus fecal phosphorus) of roughly 60% of the phosphorus intake (408). Therefore, this level of dietary phosphorus restriction usually will not maintain normal serum phosphorus levels in patients with stage 5 CKD (MHD and CPD patients), even with a substantial reduction in the renal tubular reabsorption of phosphorus. Hence, binders of gastrointestinal phosphate are also employed. Maintenance dialysis patients almost always require phosphate binders to prevent hyperphosphatemia. Serum phosphorus levels should be monitored monthly following the initiation of dietary phosphorus restriction to ensure that serum phosphorus remains within the normal range.

Traditionally, the two most commonly used phosphate binders have been aluminum carbonate and aluminum hydroxide. Usually, two to four 500 mg capsules taken three to four times per day are needed. Larger doses may be used if necessary. Evidence that aluminum-induced osteomalacia, anemia and dementia may be caused by the intake of aluminum phosphate binders has led nephrologists to use other phosphate binders, and to only prescribe aluminum binders when other binders are not safe, tolerated or sufficiently effective (416–419).

Several alkaline calcium salts bind phosphate. Calcium carbonate, calcium citrate, and calcium acetate have been used for this purpose (420–423). Of these three compounds, calcium acetate may have the greatest binding affinity to phosphate and may be more effective at lowering serum parathyroid hormone (423–425). Intestinal calcium absorption may be lower with calcium acetate than with calcium carbonate or citrate (423–425). However, calcium acetate may cause more symptoms such as nausea, diarrhea and constipation which may reduce compliance (425). Hypercalcemia is reported to be less common with calcium acetate as compared to calcium carbonate (423). Calcium citrate enhances the intestinal absorption of aluminum (422) and should not be given with aluminum salts.

In general, calcium chloride should not be given to chronically uremic patients because of its acidifying characteristics. Because calcium preparations may contain phosphorus and other ingredients, it is advisable to ascertain the composition of any new calcium preparation before it is taken by a patient with renal insufficiency. Treatment with 1,25-dihydroxycholecalciferol will decrease the daily calcium requirement by enhancing intestinal calcium absorption (9,426). Calcium binder doses should not provide more than about 600 to 1,500 mg of elemental calcium per day (total calcium intake [from diet plus binders] should not exceed 1,000 to 2,000 mg/day) to prevent excessive accumulation of calcium in soft tissues (427).

Calcium binders should be taken in divided doses with meals and should not be prescribed if the serum phosphorus level is very high in order to avoid precipitation of calcium and phosphate in soft tissues. Thus, hyperphosphatemic patients may be treated with other binders of phosphate (e.g., sevelamer HCl or lanthanum carbonate) until serum phosphorus falls to normal or near normal. At that time the regimen may be changed to a calcium binder. Calcium comprises 40% of calcium carbonate, 25% of calcium acetate, 21% of calcium citrate, and 9% of calcium gluconate.

Two other noncalcium-nonaluminum-and nonmagnesium-containing phosphate binders are currently available. These include sevelamer HCl (428–432) and lanthanum carbonate (433–435). Sevelamer HCl is often given in doses of two to six 800 mg capsules three or four times per day with meals. This drug is generally well tolerated, although some individuals develop discomfort or nausea and can become mildly acidemic because of the large hydrochloride content of this preparation. Sevelamer hydrochloride also has the benefit of lowering serum LDL-cholesterol, increasing serum HDL-cholesterol and attenuating progression of calcification of the coronary and arteries and aorta (428,432). Lanthanum carbonate appears to be roughly as effective a phosphate binder as sevelamer HCl and the calcium salts. Small quantities of lanthanum accumulate in tissues of maintenance dialysis patients with daily doses (434); it has not been shown that these are harmful to patients. If maintenance dialysis patients remain hypererphosphatemic (serum phosphorus greater than greater than 5.5 mg/dL); these latter two phosphate binders may need to be given in combination with each other or with calcium binders.

Interest in developing noncalcium containing phosphate binders has intensified because of the recognition that the use of calcium binders of phosphate results in soft tissue calcification and particularly of arteries, including the coronary artery (see the subsequent calcium discussion). Phosphate binders, in general, bind only up to 300 to 400 mg phosphorus per day, even when given in maximum doses. Thus, the use of phosphate binders does not substitute for the need to restrict dietary phosphorus.

CALCIUM, VITAMIN D, PARATHYROID HORMONE

Nondialyzed patients with CRF and patients undergoing maintenance dialysis therapy have an increased dietary requirement for calcium because they have vitamin D deficiency and resistance to the actions of vitamin D. These abnormalities impair the intestinal absorption of calcium. The risk of calcium deficiency in these patients is enhanced because the diets prescribed for CRF patients are almost always low in calcium. Foods high in calcium content are usually high in phosphorus (e.g., dairy products) and are therefore restricted for uremic patients. For example, a 40 gm protein diet generally provides only about 300 to 400 mg per day of calcium, whereas the recommended dietary allowances for healthy, nonpregnant, nonlactating adults is about 800 to 1,200 mg per day.

Balance studies indicate that nondialyzed stage 5 CKD patients not receiving calcitriol (1,25-dihydroxycholecalciferol) or other vitamin D compounds usually require about 1,200 to 1,600 mg per day of calcium for neutral or positive calcium balance (408). There is strong evidence that MHD and CPD patients often have extensive calcification of their arteries including the coronary arteries (436,437). In the general population, the extent of coronary artery calcification is directly related to the risk of myocardial infarction (438). Moreover, recent findings indicate that many nondiabetic and diabetic stage 3 and 4 CKD patients display calcification of their coronary arteries (439). Hence, it may be preferable to maintain total calcium intake closer to 1,000 mg/day in these stage 3 to 5 CKD patients including those undergoing MHD or CPD. This may particularly important when patients take calcitriol or other vitamin D analogues that increase intestinal calcium absorption. Frequent monitoring of serum calcium is important because hypercalcemia may develop with these treatments.

In summary, low protein diets need to be supplemented with approximately 600 to 800 mg of elemental calcium daily. To reduce the risk of calcium phosphate deposition in soft tissues, supplemental calcium should not be given in stage 3 to 5 CKD patients unless the serum phosphorus concentration is normal (e.g., 2.7 to 4.6 mg/dL and between 3.5 and 5.5 mg/dL in stage 3 to 4 CKD and stage 5 CKD, respectively) (415). Also, the serum calcium-phosphorus product in stage 3 to 5 CKD patients should be maintained below 55 mg^2/dL^2 (439). This product should be attained primarily by controlling serum levels of phosphorus within the target range as indicated previously. In addition, frequent monitoring of serum calcium is important because hypercalcemia may develop, particularly if serum phosphorus should fall to low-normal or low levels. This is especially likely to occur if the patient also has hyperparathyroidism, a common complication of chronic renal failure (436).

The DOQI Guidelines on Bone Metabolism and Disease in Chronic Renal Failure make the following recommendations (439): For stage 3 and 4 chronic kidney disease patients, serum levels of calcium, corrected for any change in albumin levels, should be maintained within the normal range for the laboratory used, but preferably toward the lower end of normal (8.4 to 9.5 mg/dL). As indicated earlier, it is the author's policy that total elemental calcium intake from the diet and calcium-based phosphate binders combined, should not exceed 1,000 to 2,000 mg/day. If the serum total corrected calcium is below the lower limit for the laboratory used (less than 8.4 mg/dL), the patient should ingest more calcium to increase serum calcium levels if there are clinical signs of hypocalcemia or if the plasma intact parathyroid hormone level is above the target range for chronic kidney disease.

Serum calcium corrected for serum albumin may be calculated as follows (440):

$$\begin{aligned}&\text{Corrected serum calcium (mg/dL)}\\&\quad = \text{Total serum calcium (mg/dL)}\\&\quad + 0.0704 \times [34 - \text{Serum albumin (g/L)}].\end{aligned} \qquad [5]$$

A simpler equation, which is about as accurate, is as follows:

$$\begin{aligned}&\text{Corrected total calcium (mg/dL)}\\&\quad = \text{Total calcium (mg/dL)} + 0.8\\&\quad \times [4 - \text{Serum albumin (g/dL)}].\end{aligned} \qquad [6]$$

As indicated earlier, treatment with vitamin D analogues will decrease the daily calcium requirement by enhancing intestinal calcium absorption. In order to reduce the total daily calcium load to the dialysis patient who is taking calcium binders of phosphate, the calcium content of dialysate is often reduced. Currently, this is a somewhat more effective method for controlling calcium balance when daily chronic peritoneal dialysis is employed than when thrice-weekly maintenance hemodialysis is used. The usual dialysate calcium concentration in MHD or CPD patients should be 2.5 mEq/L.

Secondary hyperparathyroidism can be treated in patients with chronic renal failure with the use of vitamin D analogues. In stage 5 CKD patients, serum intact parathyroid hormone should be maintained within a target range of 150 to 300 pg/mL.

If the serum level of 25-hydroxyvitamin D is less than 30 ng/mL, supplementation with vitamin D_2 (ergocalciferol) should be initiated. For patients with stage 5 chronic renal failure, therapy with an active vitamin D sterol (1,25-dihdroxycholecalciferol [calcitriol], alphacalcidol, paricalcitol, or doxercalciferol) should be provided if the plasma levels of intact parathyroid hormone are greater than 300 pg/mL (440).

Recently, a retrospective study in over 50,000 MHD patients indicated, that those patients treated with calcitriol or paricalcitriol had lower mortality than those individuals not receiving these medicines ($p < 0.001$) (441). Vitamin D has many other actions in addition to its effects on calcium absorption, parathyroid hormone secretion and bone metabolism. These effects include regulation of immune function and

antiproliferative, differentiating effects (442). Thus, it is not surprising if vitamin D and its congeners may affect survival rates.

Another recent retrospective study conducted in about 67,000 MHD patients indicated that MHD patients treated with the vitamin D analogue paricalcitrol had a lower mortality rate than MHD patients not receiving calcitriol (443). A recent addition to therapeutic treatment of secondary hyperparathyroidism in stage 4 and 5 CKD patients is the use of cinacalcet. This medicine increases the sensitivity of the calcium receptor in the parathyroid gland to calcium so that lower levels of serum calcium suppress parathyroid hormone secretion (444,445). This medicine is frequently effective at suppressing hyperparathyroidism in nondialyzed stage 4 and 5 CKD and maintenance dialysis patients who are hyperparathyroid and whose serum calcium or phosphorus levels and serum calcium—phosphorus product are at or above the acceptable range.

A syndrome called aplastic or hypoplastic bone disease has been described in chronic dialysis patients (440a,446–448). It is characterized by relatively low serum parathyroid hormone concentrations, decreased bone osteoblasts and marked reduction in bone turnover. The syndrome can be caused by aluminum toxicity, and probably excess iron, but also occurs in the absence of such toxicity (440a,446,447a). It has been postulated that treatment with large doses of calcium binders of phosphate or vitamin D with consequent suppression of parathyroid hormone is a cause of this disorder (440a,446,448).

In CAPD patients, the calcium uptake from dialysate is inversely correlated with the serum concentration of total calcium and ionized calcium (304,449,450). Net absorption of calcium from dialysate in CAPD patients averages about 84 mg per day (304). The calcium balance across dialysate varies according to the serum ionized calcium and is negative by roughly 80 mg per day in CAPD patients who have serum ionized calcium values greater than 5 mg/dL and is positive by roughly 40 mg per day in patients with serum ionized calcium levels below 4.4 mg/dL (450). Also, the more hypertonic the peritoneal dialysate, the greater the dialysate outflow volume and hence the larger the calcium losses; in one report, a net calcium uptake of roughly 10 mg per exchange occurs with 1.5% dextrose, and a net calcium loss of 21 mg per exchange occurs with 4.25% dextrose (304). In CAPD patients, calcium balance was usually neutral or positive when dietary calcium intake was 720 mg per day or greater (304). Based on these observations, CAPD patients should probably be given a total dietary calcium intake (diet plus dietary supplement) of about 800 to 1,000 mg per day.

The calcium content is 40% for calcium carbonate, 25% for calcium acetate, 21% for calcium citrate, 18% for calcium lactate, and 9% for calcium gluconate.

TRACE ELEMENTS

Trace element metabolism in CRF is discussed more extensively in Chapter 102, and this section will cover dietary requirements. A number of factors in CRF patients tend to either increase or decrease the body burden of certain trace elements. Many trace elements are excreted primarily in the urine, and with renal failure they may accumulate. Elements such as iron, zinc, and copper, which are protein bound, may be lost in excessive quantities when there are large urinary protein losses, e.g., the nephrotic syndrome (451). Excessive uptake or losses of trace elements may also occur during dialysis therapy depending on their relative concentrations in plasma and dialysate and the degree of binding to protein or red cells (452–456). Hemodialysance of copper, strontium, zinc, and lead, for example, which are largely bound to plasma proteins or red cells, should be minimal (452,453,457–459). Hemodialysis or hemodiafiltration may remove some trace elements if the dialysate concentrations are sufficiently low (e.g., bromide, iodine, lithium, rubidium, cesium, and zinc (460–462). Zinc may be taken up and copper lost from peritoneal dialysate. Because many trace elements are bound avidly to serum proteins, they may be taken up by blood against a concentration gradient when present in even small quantities in dialysate (452,453,458,463). These observations provided part of the justification for intensive purification of dialysate. Inhalation could result in increased intake. This may occur in people exposed to certain industrial processes, fertilizers, insecticides, herbicides, or burning of fossil fuels.

Protein-calorie malnutrition, by lowering serum concentrations of proteins that bind trace elements, may decrease the serum levels of a number of these elements including zinc, manganese, and nickel (464). Occupational exposure or pica may increase the burden of some trace elements. Therapeutic doses of trace elements may be administered through dialysis, as has been done for zinc (88). The effect of the altered dietary intake of the uremic patient on body pools of trace elements is unknown (464). Oral and possibly intravenous iron supplements are often given to patients who are iron deficient.

Assessment of trace element burden in renal failure patients is difficult because the binding protein concentrations may be decreased, thereby lowering serum trace element levels, and the binding characteristics of these proteins may be altered in renal failure as well. Also, red cell concentrations of trace elements may not reflect levels in other tissues. Trace element supplementation should be undertaken with caution, because impaired urinary excretion and poor dialysance of trace elements, due to protein binding, increase the risk of overdosage.

Dietary requirements for trace elements have not been well defined in uremic patients. Iron deficiency is common, particularly in hemodialysis patients, because intestinal iron absorption is sometimes impaired, there are often substantial blood losses; iron may bind to the dialyzer membrane, and the erythropoietin-induced rise in hemoglobin concentrations may deplete the body's iron supply (11,12). Not only do iron requirements increase during the time when erythropoietin therapy is initiated and hemoglobin concentrations rise, but higher serum iron levels and body iron burden are associated with a greater response to erythropoietin (465,466). Some researchers recommend that, in general, MHD or CPD patients should maintain serum transferrin saturation (TSAT) at 30% to 50% and serum ferritin at about 400 to 800 ng/mL (465–468).

Although some MD patients may maintain these iron values with oral iron supplements, many such individuals will not be able to do so unless they receive parenteral iron therapy (465–468). Oral ferrous sulfate, 300 mg three times per day one-half hour after meals, may be tried. Some patients develop anorexia, nausea, constipation, or abdominal pain with ferrous sulfate; these individuals sometimes will tolerate other iron compounds better, such as ferrous fumarate, gluconate, or lactate. Patients who are intolerant to oral iron supplements and the preponderance of MHD or CPD patients who will not maintain TSAT at 30% to 50% and serum ferritin levels of 400 to 800 ng/mL with oral iron may be treated with intravenous iron (467,468). Because of pain and risk or staining of skin with intramuscular iron injections, intravenous iron, given as ferrous gluconate, ferrous sucrose or iron dextran is generally the preferred method of administration. Because iron dextran has a slightly greater risk of anaphylactic reactions, many physicians prefer other iron salts.

As indicated earlier, in stage 5 CKD patients, including those receiving maintenance dialysis, increased body burden of aluminum has been implicated as a cause of a progressive dementia syndrome (particularly in MHD patients), osteomalacia, weakness of the muscles of the proximal limbs, impaired immune function, and anemia (436,446,447,469–471). Although

contamination of dialysate with aluminum previously was the major source of aluminum toxicity in many dialysis centers, current methods of water treatment have removed virtually all aluminum from dialysate. Today, ingestion of aluminum binders of phosphate is probably the major cause of the excess body burden of aluminum (469,470). Consequently, aluminum binders are now used sparingly, if at all, by nephrologists.

Increased body burden and toxicity of iron or aluminum in MHD or CPD patients may be reduced by reduction of intake or by infusion of deferoxamine, which is dialyzable (472). Care must be taken because deferoxamine may promote infection, particularly mucormycosis (473). Injections of erythropoietin with repeated phlebotomy is another method for removing excess body iron (474).

Although the zinc content of most tissues is normal in renal failure patients (475), usually serum and hair zinc are reported to be low and red cell zinc increased (85,88,454,459,476–479). In nondialyzed CRF patients, the fractional urinary excretion of zinc is increased; however, since the GFR is reduced, total urinary excretion of zinc may be decreased (479). Fecal zinc is increased (459) and a dietary zinc intake greater than the recommended dietary allowance (315) may be necessary to maintain normal body zinc pools. Further studies are needed to confirm this possibility. Some reports indicate that dysgeusia, poor food intake, and impaired sexual function, which are common problems of uremic patients, may be improved by giving patients zinc supplements (88,459,478,480). Other studies, however, have not confirmed these results (481). Intestinal absorption of zinc is not affected by administration of 1,25-dihydroxycholecalciferol (482).

The finding that serum selenium is low in dialysis patients has raised the question of whether selenium supplements are indicated (483,484). This question is of particular importance because selenium participates in the defense against oxidative damage of tissues, which may be increased in renal failure (485). Further research will be necessary to resolve this issue.

VITAMINS

Chronically uremic patients have a high incidence of vitamin deficiencies. These are due to several factors. First, in renal failure, 1,25-dihydroxycholecalciferol production by the diseased kidney is impaired (17). Second, vitamin intake is often decreased in uremic patients because of anorexia and reduced food intake. Intercurrent illnesses, which occur frequently in chronically uremic patients (125–127), also impair food intake (125,486–488). Many foods that are high in water-soluble vitamins are often restricted for renal failure patients owing to the elevated protein and potassium content of these foods; the diets prescribed for nondialyzed CRF and maintenance dialysis patients frequently contain less than the recommended daily allowances for certain water-soluble vitamins (18,489). Third, renal failure appears to alter the absorption, metabolism, or activity of some vitamins. Animal studies indicate that intestinal absorption of riboflavin, folate, and vitamin D_3 is impaired in renal failure (13–15). The metabolism of folate and pyridoxine appears to be abnormal in CRF (18,490). Fourth, certain medicines interfere with the intestinal absorption, metabolism, or actions of vitamins (18). Fifth, water-soluble vitamins are removed by dialysis (119–122,491,492) and high-flux/high efficiency hemodialysis may remove greater quantities of water-soluble vitamins (493).

On the other hand, vitamin B_{12} deficiency is uncommon in CRF (18,484–496) because the daily requirement for this vitamin is small (e.g., 3 μg/day for normal nonpregnant, nonlactating adults) (315), the body stores large quantities of this vitamin, and even though vitamin B_{12} is water soluble, it is protein bound in plasma and hence poorly dialyzed. There is a report of low serum vitamin B_{12} concentrations in 19 of 60 maintenance hemodialysis patients (121); the serum vitamin B_{12} concentrations tended to fall progressively over months of dialysis treatment, and serum vitamin B_{12} levels correlated directly with nerve conduction velocities, which improved with ingestion of large quantities of B_{12}. Another study reported low serum vitamin B_{12} levels in four hemodialysis patients, only one of which demonstrated an intestinal absorption defect for vitamin B_{12} (496). Their hematocrit improved after receiving vitamin B_{12} injections. The explanation for these two discrepant reports of low serum vitamin B_{12} levels in maintenance hemodialysis patients is not known.

Vitamin deficiencies have been observed with particular frequency for 1,25-dihydroxycholecalciferol, folic acid, vitamin B_6, (pyridoxine), vitamin C, and to a lesser extent, other water-soluble vitamins (18,119,120,122,489,494,497–500). Blood folate concentrations were reported to be decreased in MHD patients who did not take folic acid supplements (494,497,498). Several investigators found serum or red blood cell folate concentrations in the normal range in MHD patients (122,494,501). However, a substantial number of these patients had deficient levels (122,494,501). In addition, several investigators found hypersegmentation of polymorphonuclear leukocytes in such patients that decreased following administration of folate supplements (84,498).

Some investigators noted that the reticulocyte count and hematocrit rose when patients undergoing MHD were given folic acid supplements (84). We have observed normal serum folate concentrations in virtually all MHD patients who received 1.0 mg per day of supplemental folic acid (unpublished observations). Marumo reported higher folate levels in MHD patients as compared to controls, but the folate intake and type of dialysis were not indicated (502). Dietary folic acid requirements increase in stage 4 or 5 CKD patients during the time when they commence erythropoietin therapy and sustain a major rise in their hemoglobin levels.

There are substantial losses of vitamin C into dialysate (119,120,491,492). Low plasma and leukocyte ascorbic acid concentrations may occur in patients undergoing hemodialysis who were not receiving supplemental vitamin C (119,491,503). Clinical signs suggestive of mild scurvy have been described in several MHD patients with very low ascorbic acid concentrations (119). Administration of ascorbic acid orally or into the dialysate prevented negative vitamin C balance during hemodialysis.

Many but not all workers find evidence for low plasma or red blood cell vitamin B_6 concentrations in many subjects undergoing maintenance hemodialysis or CPD who do not take supplemental vitamin B_6 (84,86,504–507). Activation coefficients of erythrocyte transaminase enzymes (i.e., the activity of the enzyme divided into the activity of the same enzyme after the vitamin B_6 cofactor pyridoxal-5-phosphate is added to the assay) are not uncommonly elevated in MHD patients who do not receive supplemental vitamin B_6, indicating that these subjects are deficient in vitamin B_6 (86,504,505). Improvement in the activation coefficient during hemodialysis, has been reported, suggesting that there might be an inhibitor of vitamin B_6 in uremic sera that is removed by dialysis (504). Low stimulation ratios in mixed lymphocyte cultures from these patients have been reported (504). Pyridoxine hydrochloride supplements improve several parameters of immune function in hemodialysis patients including lymphoblast formation. These observations suggest that vitamin B_6 deficiency, which is known to cause abnormal immunologic function (504,508), may be one cause of the altered immune response in uremic patients. Interestingly, serum glutamate-oxaloacetate transaminase activity is reduced in renal failure. This effect appears to be related to vitamin B_6 activity, possibly due to a uremic inhibitor in serum (509–512).

Treatment with pyridoxine HCl has been attempted for the following two metabolic disorders associated with potential adverse clinical consequences in CRF patients: elevated plasma homocysteine levels and plasma oxalate concentrations. Plasma homocysteine is increased in nondialyzed CRF patients as well as MHD and CPD patients (see the previous text) and renal transplant recipients (see the subsequent text). Elevated plasma homocysteine is of great concern, because in the general population it is a risk factor for adverse cardiovascular events as well as thrombosis of vascular access sites including arteriovenous fistulae (see the previous text) (380–387,513–518). As indicated earlier, in maintenance dialysis patients, the relation of elevated plasma homocysteine to adverse outcome is less clear. Some studies support this relationship, whereas others show an altered risk factor pattern that is thought to be due to malnutrition and/or inflammation.

Either supplemental pyridoxine HCl or vitamin $B_{12,}$ taken alone, has not been shown to reduce plasma homocysteine in MHD patients (516). Similarly, it is not evident that adding these vitamins to folic acid therapy lowers plasma homocysteine more effectively than equal doses of folic acid alone. Disorders of folic acid may also contribute to elevated homocysteine levels because the folate metabolite, tetrahydrofolic acid, is necessary for the remethylation of homocysteine to reform methionine. Folic acid and the combination of folic acid and pyridoxine HCl have decreased plasma homocysteine levels in patients with CRF (513,516,519,520). Five to 10 mg/day of folic acid probably maximally lowers plasma homocysteine levels in most cases (386,387,520); occasionally, 15 mg folic acid/day may have an additional homocysteine lowering effect (519,521). However, homocysteine levels, on average, are not reduced to normal with this treatment (386,387,512,513,516,519–522). Intravenous folic acid given intravenously rather than orally may be more effective at lowering plasma homocysteine (516). High-flux/high efficiency hemodialysis significantly lowered plasma folate levels but did not alter the elevated plasma homocysteine (523).

It has been suggested that there may be an impairment in conversion of folic acid to 5-methylenetetrahydrofolate (MTHF) and also of the transmembrane transport of MTHF. To circumvent these possible disorders, 37 MHD patients were given intravenous folinic acid, a precursor of MTHF, 50 mg/week intravenously and large doses of pyridoxine HCl, 250 mg three times weekly (522). After a mean of 11.2 months treatment, there was a major reduction in plasma homocysteine levels and 78% achieved normal plasma homocysteine. Another study with large doses of these vitamins did not confirm such positive results (521).

Treatment with a combination of folic acid 5 mg, pyridoxine HC1 (vitamin B_6), 50 mg; and vitamin B_{12}, 0.7 mg given intravenously after each hemodialysis treatment not only significantly lowered predialysis serum homocysteine levels, although not to normal, but also significantly lowered predialysis serum methylmalonic acid and crystathionine concentrations (524). The Veterans Administration is currently conducting the HOST Study in over 2000 stage 4 and 5 CKD patients including individuals undergoing MHD or CPD to assess whether treatment of elevated serum homocysteine is associated with a reduction in adverse cardiovascular events and cardiovascular and all cause mortality (525). Patients are randomized in a double blind fashion to treatment with large doses of folic acid, pyridoxine HCl and vitamin B_{12} or to placebo.

Plasma oxalate levels are elevated in renal failure. Increased plasma oxalate appears to be largely due to impaired urinary excretion, although large doses of vitamin C (ascorbic acid), a precursor of oxalate, may increase oxalate production (see the subsequent text). Glyoxylate, a metabolic precursor of oxalate, may also be transaminated to form glycine. Vitamin B_6 is a cofactor for the enzyme that catalyzes this step. Indeed, vitamin B_6 deficiency increases urinary oxalate excretion in rats with experimental renal insufficiency as well as in normal individuals (526). In patients with CRF, some studies indicate that treatment with large doses of pyridoxine HCl decrease plasma oxalate levels, although not to normal values (527,528). Other clinical trials have not been able to confirm these results (529,530).

Five mg per day of pyridoxine hydrochloride produced a normal red cell erythrocyte glutamate-pyruvate transaminase activation index in nondialyzed, clinically stable, stage 5 CKD patients, and 10 mg per day of pyridoxine hydrochloride normalized this index in stable MHD patients and in nondialyzed CRF patients with superimposed infections (86).

Despite the water solubility of riboflavin, thiamine, pantothenic acid, and biotin, plasma concentrations of these vitamins are usually not decreased in patients undergoing MHD. It is possible that losses of these vitamins into hemodialysate are offset by their lack of urinary excretion. Low plasma niacin concentrations have been reported in some patients receiving maintenance dialysis therapy (494). Other investigators did not confirm these findings in patients receiving 7.5 mg per day of supplemental nicotinic acid (84). Low levels of thiamine and, in some patients, thiamine and pyridoxine when they were prescribed low protein diets have been described (499).

Serum retinol-binding protein and vitamin A are increased in CRF patients (531–533). Elevated liver vitamin A was described in two patients with CRF (534), although other investigators could not confirm elevated vitamin A levels in solid tissues (532). Moreover, even relatively small supplements of vitamin A, i.e., 7,500 to 15,000 IU/day (about 1,500 to 3,000 retinal equivalents per day), appear to cause bone toxicity and hypercalcemia in some individuals (535). Vitamin K deficiency is uncommon (18). Patients who do not eat (and hence do not ingest foods containing vitamin K) and who are receiving antibiotics that suppress intestinal bacteria for extended periods of time may require supplemental vitamin K to prevent deficiency of this vitamin (536).

Normal (537,538), low (485,539) and increased (540) plasma or red cell vitamin E (α-tocopherol) levels have been described in nondialyzed CRF or MHD patients. Vitamin E deficiency may cause oxidative injury to tissues (485). Several studies indicate that administration of 1,200 iu of vitamin E (all-racemic α-tocopherol acetate) to MHD patients may decrease oxidative stress induced by 100 mg of iron sucrose, and this antioxidant effect may be enhanced with the use of a combination of vitamin E and vitamin C (541). Treatment with vitamin E (α-tocopherol) is also reported to reduce progression of carotid artery stenosis (542). On the other hand, in the general population, randomized, prospective placebo controlled clinical trials indicate that vitamins E, C, beta carotene, or combinations of these compounds do not significantly reduce total adverse cardiovascular events or cancer (543,544). Indeed, in the HOPE and HOPE-TOO trial, a moderate dose of vitamin E (400 iu), as compared to placebo, was associated with an increased risk of heart failure and hospitalization for heart failure (544). The reason for these discrepant responses of advanced CKD patients, including those undergoing maintenance dialysis, and the general population is not known. It has been suggested that the greater oxidant stress of CRF patients may enable them to benefit from vitamin E therapy (543).

In CPD patients, dietary intake of several vitamins, including vitamin A, vitamin C, vitamin B_1(thiamine), vitamin B_6, vitamin $B_{12,}$ and nicotinamide, is often reduced below the recommended allowances for normal adults (489,506). In CPD patients not receiving supplements, investigators have found a high incidence of low (489,545) or normal (546) plasma folate levels, low vitamin B_1 (489,533), low vitamin B_6 (489,547) and low vitamin C (489,523). Reduced plasma vitamin E has been

reported in 13% of CPD patients (533), whereas others found increased plasma vitamin E levels (547).

Some authors have suggested that many MHD patients who do not receive vitamin supplementation may subsist for months without developing water-soluble vitamin deficiencies (122,507,548). These authors have therefore recommended that vitamin supplements should not be prescribed routinely to maintenance dialysis patients. However, in the studies indicating that maintenance dialysis patients may maintain normal plasma or blood cell levels of vitamins without supplements, patients were generally followed for less than 1 year; it is possible that with longer periods of time, the incidence of vitamin deficiency may increase. Moreover, in these studies, some water-soluble vitamins fell to borderline low levels in a number of patients. It is true that many of the studies that indicate a need for routine vitamin supplementation in nondialyzed CRF and maintenance dialysis patients were carried out in the 1960s and early 1970s when the incidence of poor nutritional intake of these individuals might have been greater than it is today (18). Nonetheless, a low intake of several vitamins is still common in renal failure patients (507,548), and many reports continue to show that substantial numbers of renal failure patients show evidence for vitamin deficiencies (485,499,506,507,547). Because the water-soluble vitamin supplements are generally safe, it would seem wise to use them routinely until these issues are more completely resolved.

The nutritional requirements for most vitamins are not well defined in renal failure patients, and it is likely that they will be modified in the future. There is evidence that, in addition to vitamin intake from foods, the following daily supplements of vitamins will prevent or correct vitamin deficiency (Table 103-6): pyridoxine hydrochloride, 5 mg in nondialyzed patients and 10 mg in maintenance hemodialysis or CPD patients; folic acid, 1.0 mg; and the recommended daily allowance for normal individuals for the other water-soluble vitamins (315). Many CRF patients may need less than 1.0 mg per day of folic acid to prevent abnormally low serum or erythrocyte folate levels. The many nondialyzed CRF patients and most MHD and CPD patients who have hyperhomocysteinemia may be treated with 5 to 15 mg/day of folic acid to reduce plasma homocysteine levels. For individuals with elevated plasma homocysteine, one may start treatment with 5 mg/day and then increase folic acid doses up to 15 mg/day, if necessary, depending on the response of plasma homocysteine levels. It is important to note that the reduction of plasma homocysteine has not been demonstrated to reduce the risk of cardiovascular disease. Moreover, no studies have systematically examined the safety of these large folate doses in CRF, MD or kidney transplant patients, a particularly important consideration because of the reduced ability of many of these individuals to excrete folate metabolites in the urine.

A supplement of only 60 mg per day of vitamin C (the recommended daily allowance (248)) is advised because ascorbic acid can be metabolized to oxalate. Large oral or intravenous doses of ascorbic acid have been associated with increased plasma oxalate levels in renal failure patients (549–551). Oxalate is highly insoluble, and there is substantial concern that high plasma oxalate concentrations can lead to precipitation in soft tissues, including the kidney, possibly causing further impairment in renal function in the nondialyzed patient with renal insufficiency.

Supplemental vitamin A is not recommended because serum levels are increased, and there is a high risk of vitamin A toxicity with even small supplements (534,535) (Table 103-6). Although some studies indicate that vitamin E supplements in MHD patients are beneficial (485,542), it is still not certain that vitamin E supplements are necessary, particularly because of negative or adverse results with vitamin E supplementation in the general population (543,544). Additional vitamin K is not needed unless the patient is not eating and receives antibiotics that suppress intestinal bacteria that synthesize vitamin K. Recommendations for vitamin D intake are given in Chapter 89.

ALKALINIZING AGENTS

Nondialyzed patients with advanced renal failure frequently develop metabolic acidosis. This is usually associated with an increased anion gap because there is impaired ability of the kidney to excrete acidic metabolites. In the earlier stages of CRF, and occasionally with advanced renal failure, hyperchloremic metabolic acidosis may also be caused by excessive renal losses of bicarbonate. Ingestion of low protein diets may prevent or decrease the severity of the acidosis because the endogenous generation of acidic products of protein metabolism will be reduced. Metabolic acidemia may engender oxidation of branched chain amino acids (valine, leucine, and isoleucine) (160), protein catabolism (158–162), impair albumin synthesis, increase beta-2 microglobulin turnover (552), cause bone loss, possibly predispose to inflammation and cause symptoms of weakness and lethargy (159–164). Conversely, correction of acidosis has been associated with decreased protein degradation rates (553) and increased plasma branched chain amino acids (554,555), serum albumin (555), body weight and midarm circumference (556).

A high serum anion gap is associated with greater survival in MHD patients (557,558). This relation is considered to be due to greater appetite and protein intake, signs of health in patients with higher anion gaps (558). On the other hand, after adjustment for measures indicating protein energy malnutrition or inflammation, it turns out that individuals with lower serum anion gaps (i.e., who are less acidotic) have greater survival (558).

Since the level of acidemia at which amino acid or protein loss or bone reabsorption is stimulated is not well defined, it would seem prudent to prevent any degree of chronic acidemia. Indeed, some evidence indicates that CPD patients are more anabolic or less catabolic when their arterial blood pH is 7.42–7.45 as compared to when it is 7.35 to 7.38 (165).

The National Kidney Foundation K/DOQI Clinical Practice Guidelines for Nutrition in Chronic Renal Failure (559) and the DOQI Clinical Practice Guidelines for Bone Metabolism and Disease in Chronic Kidney Disease (560) each recommend that the serum bicarbonate should be measured once-monthly in MHD and CPD patients and that the predialysis or stabilized serum bicarbonate should be maintained at or above 22 mmol/L in these individuals. Because of the safety of giving bicarbonate and the potential advantages of completely eradicating acidemia, the author currently recommends that serum bicarbonate be maintained in the normal range (i.e., about 25 mEq/L). A similar recommendation concerning the threshold for treating low serum bicarbonate would seem appropriate for nondialyzed patients with any level of kidney disease. Alkali therapy probably should be initiated if the arterial blood pH is below about 7.38 due to metabolic acidosis.

Because, in clinically stable patients with CRF, the rate of acid production is usually normal or below normal, alkalinizing medicines are usually very effective for preventing or treating acidemia. Sodium bicarbonate tablets, Shols solution or bicitra (1.0 mEq of alkali per mL of solution) may be given; usually doses of 20 to 60 mEq/day of bicarbonate are sufficient to neutralize most or all of the acidemia. Citrate salts may be given orally, although this may enhance the intestinal absorption of aluminum (422). Calcium carbonate, 5 gm per day, may correct mild acidosis, provide needed calcium, and reduce intestinal phosphate absorption. However, the risk of soft tissue and particularly arterial calcification limits the amount of

calcium that can be given to CKD patients (see the earlier text). If the acidosis is more severe, sodium bicarbonate or citrate may be administered intravenously. If the nondialyzed CKD patient is not oliguric and is not prone to develop edema, sodium is usually readily excreted when it is given as sodium bicarbonate or citrate. Since protein metabolism yields acidic products, a low protein diet (e.g., 0.60 to 0.80 gm protein/kg/day) will also reduce acid production and acidemia. Such a diet can be nutritious for nondialyzed stage 3, 4 and 5 CKD patients, but maintenance dialysis patients will require more dietary protein. If acidemia is severe and not controlled by the foregoing measures, hemodialysis or peritoneal dialysis may be employed.

Before implementing alkali therapy, it must be ascertained that the low serum bicarbonate is not a compensatory response to chronic respiratory alkalosis.

FIBER

Studies in normal individuals suggest that a high dietary fiber intake may lower the incidence of constipation, irritable bowel syndrome, diverticulitis, and neoplasia of the colon and possibly, improve glucose tolerance (559). In patients with CRF, a high dietary fiber intake also may reduce SUN by decreasing colonic bacterial ammonia generation and enhancing fecal nitrogen excretion (560). Because it seems reasonable that the benefits of a high dietary fiber intake in normal people would also occur in nondialyzed patients with CRF and in maintenance dialysis patients, a high dietary fiber intake of 20 to 30 gm per day is recommended.

PRIORITIZING DIETARY GOALS

The number and magnitude of the changes in the dietary intake for stage 3 to 5 CKD including MD patients are so great that if they were all presented to the patient at one time, the patient could become demoralized and lose his motivation to comply with the diet. We therefore prioritize goals for dietary treatment. Usually we emphasize the importance of controlling the protein, phosphorus, sodium, energy, potassium, and magnesium intake and the need to take calcium (for nondialyzed stage 4 to 5 CKD patients) and vitamin supplements. On the other hand, unless the patient has a lipid disorder or other risk factors that indicate there is a high odds ratio for adverse cardiovascular events, the recommended quantity and types of dietary carbohydrate, fat and fiber are discussed with the patient, but adherence to these dietary guidelines are not as strongly emphasized. Statins or fibric acid derivatives are usually better tolerated than dietary modifications, and prescription of these medicines, if lipid disorders are apparent, may enable patients to initially focus on other pressing aspects of dietary modification. If the patient has complied well with the other, more critical elements of dietary therapy, has a specific lipid disorder that may benefit from dietary therapy, or has expressed an interest in modifying fat, carbohydrate or fiber intake, then the modifications of the dietary intake of these latter nutrients are explored more intensively with the patient.

ADJUSTED EDEMA-FREE BODY WEIGHT (aBW_{ef})

Assessment of nutritional status and assessment and prescription of nutritional intake is often based on body weight. For individuals with renal disease, this represents a special problem because they not only often are obese or underweight, but they may be edematous. The National Kidney Foundation Clinical Practice Guidelines on Nutrition address this issue with the following statement (561): "The body weight to be used for assessing or prescribing protein or energy intake is the aBW_{ef} (sic, adjusted edema-free body weight). For hemodialysis patients, this should be obtained postdialysis. For peritoneal dialysis patients, this should be obtained after drainage of dialysate." The aBW_{ef} should be used for individuals with renal disease, including nondialyzed CRF patients and MD patients, who have an edema-free body weight that is less than 95% or greater than 115% of the median standard weight, as determined from the NHANES II data (562,563). For individuals with an edema-free body weight between 95% and 115% of the median standard weight, the actual edema-free body weight may be used. The guideline goes on to state: "For DXA measurements of total body fat and fat-free mass, the actual edema-free body weight obtained at the time of the DXA measurement should be used. For anthropometric calculations, the postdialysis (for MHD) or postdrain (for CPD) actual edema-free body weight should be used." Clinical judgment and, if desired, body composition measurements can be used to estimate the magnitude of the edema, if any. The adjusted edema-free body weight (aBW_{ef}) may be calculated as follows (240,561):

$$aBW_{ef} = BW_{ef} + [(SBW - BW_{ef}) \times 0.25] \quad [7]$$

where BW_{ef} is the actual edema-free body weight and SBW is the standard body weight as determined from the NHANES II data (562). The NHANES II weights are used for SBW because Americans as a group were less fat during the time that the NHANES II data were collected than they are today.

Note: When the recommended nutrient intake is given in terms of body weight, the latter refers to standard body weight as determined from the National Health and Nutrition Evaluation Survey (NHANES) data from 1976 to 1980 (562,563).

DIETARY THERAPY FOR THE NEPHROTIC SYNDROME PATIENT

This syndrome is characterized by proteinuria (more than 3.0 gm per day), lipiduria, hypoalbuminemia, hypercholesterolemia, and edema, which can be massive. Serum total proteins may decrease markedly, from 7 to 8 gm per dL to as low as 4 to 5 gm per dL, and the resultant fall in plasma oncotic pressure promotes extravascular movement of fluid, sodium retention, and edema formation. Two narrow transverse white bands (Muehrcke's lines) may occur in the fingernails in association with severe hypoproteinemia (564).

Serum triglycerides, phospholipids, and apoproteins B, CII, CIII, and E are increased, whereas apoproteins AI and AII are normal (565). Plasma lipoprotein (a) (Lp[a]) is elevated (566). LDL, IDL, and VLDL may be increased (567). There is increased serum cholesterol ester transfer protein (CETP) and decreased catabolism of LDL apolipoprotein, at least by the more typical receptor pathway. These metabolic changes contribute to the elevated LDL-cholesterol and to the frequently observed low HDL-cholesterol and HDL_2 levels (565,567–575). Hypercholesterolemia, hypertriglyceridemia, increased LDL-cholesterol and VLDL-cholesterol, increased CETP and lipoprotein(a) and low HDL-cholesterol all are risk factors for an increased incidence of atherosclerotic cardiovascular disease and cerebrovascular disease in nephrotic patients (565,567,576–578). It has been suggested that the altered serum lipid pattern in the nephrotic syndrome may accelerate the progression of renal failure (579). Altered membrane lipid composition and oxidant injury of the kidney (580) and also erythrocytes (581) have been described. Elevated serum lipids and lipoproteins may contribute to progressive renal injury in animal studies (582–586).

Low serum and tissue proteins are caused by enhanced urinary protein excretion, decreased protein synthesis and, possibly to a small degree, by degradation of the increased quantity of filtered protein in renal tubular cells (587–589). Also, albumin synthesis may be increased, normal, or decreased, depending at least partly on the adequacy of dietary protein intake. Massive proteinuria may occur (e.g., up to 40 gm or more of protein per day) and may be incapacitating or life-threatening (590). Serum levels of many biologically active proteins, including clotting factors and inhibitors, are reduced owing to enhanced excretion and renal degradation (591); the clinical importance of these effects are not well defined. However, low plasma concentrations of clotting inhibitors may contribute to the frequency of renal vein thrombosis in the nephrotic syndrome.

The vitamin D analogues, 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol, are bound to an alpha-like globulin (592) and may be lost in urine in the nephrotic syndrome (593,594). In experimental nephrosis, intestinal absorption of vitamin D appears to be normal (595). Evidence of vitamin D deficiency with low serum 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol, decreased ionized and total calcium, and bone disease has been found in patients with the nephrotic syndrome (593,596,597). Others have reported normal serum vitamin D levels and no bone disease (598). There is loss of organic iodide and thyroxine in the urine in the nephrotic syndrome, although hypothyroidism is probably not present (599,600). Also, there may be increased urinary losses of trace elements bound to proteins such as zinc, copper, and iron. These losses may cause nutritional deficiencies (451,601–603).

The severity of nutritional disorders in patients with the nephrotic syndrome varies greatly; in the author's experience, patients with the nephrotic syndrome are not infrequently malnourished and debilitated to a degree that is disproportionate to their renal insufficiency. This is particularly common in patients with more massive proteinuria. The causes of malnutrition and debility are large urinary protein losses, anorexia, the urinary losses of vitamins and trace elements that are protein bound in plasma, glucocorticoid and/or cytotoxic therapy, and the frequent incidence of infections that result from such treatment.

Protein-restricted diets providing less than 30% of calories from fat, less than 200 mg per day of cholesterol, and an abundant amount of polyunsaturated fatty acids, with 10% of calories from linoleic acid are reported to reduce serum total and LDL cholesterol and decrease proteinuria in nephrotic patients (604). Serum total LDL-cholesterol and Lp(a) in the nephrotic syndrome may be reduced by treatment with HMG-CoA reductase inhibitors, fibric acid derivatives, ACE inhibitors and ARBs. The effectiveness of ACE inhibitors and ARBs in reducing serum lipids appears to be due to decreases in proteinuria. (608,609). Although polyunsaturated fatty acids (PUFA) and fish oil, which is high in PUFA, have been reported to reduce serum lipids and to be renoprotective in some but not all animal studies of the nephrotic syndrome, (610–612) the results in nephrotic humans have been equivocal (357,613–615).

A high-protein diet providing up to 2.3 gm protein/kg/day has been reported to improve nutritional status, if advanced CRF is not present (616). High protein diets increase renal blood flow and GFR (617,618). The findings that low protein diets may retard progression of renal failure and reduce proteinuria (269,284,619) have prompted a reexamination of the use of low protein diets in this disorder. Kaysen and co-workers fed isocaloric diets providing 0.8 and 1.6 gm protein/kg/day to 9 nephrotic syndrome patients (620). They reported that with the lower protein intake, urine albumin, renal albumin clearance, and fractional urinary albumin excretion decreased. Serum albumin concentrations and plasma albumin mass were actually greater with the low protein diet, although total albumin mass did not differ with the two diets. A vegetarian soy-based low protein diet may reduce proteinuria and hyperlipidemia in nephrotic patients (621).

In rats and humans with proteinuria or the nephrotic syndrome, converting enzyme inhibitors and angiotensin receptor blockers may reduce but not abolish the proteinuria (622–626). The addition of converting enzyme inhibitor medicines seems to allow protein intakes to be increased modestly without increasing urinary protein losses, thereby increasing the likelihood of nutritional repletion. It is likely that angiotensin receptor blockers have similar effects. Reduction of proteinuria is an important goal because large urine protein losses are a risk factor for more rapid progression of renal failure (281). Long-term studies have not demonstrated whether these lower protein intakes will prevent or correct protein malnutrition; this problem is complicated by the difficulty of assessing protein nutrition in nephrotic patients.

At present, it is our policy to prescribe for patients with the nephrotic syndrome diets that provide 0.70 or 0.80 gm protein/kg/day. The higher value would be prescribed for patients with more severe proteinuria. At least 0.35 gm/kg/day of the protein should be of high biologic value. This diet should be supplemented with 1.0 gm of high biologic value protein for each gram of urine protein excreted each day. Intake of energy and minerals in general is prescribed as described for patients with chronic renal insufficiency. Angiotensin converting enzyme (ACE) inhibitors and/or angiotensin II receptor blockers are routinely added to reduce proteinuria.

Studies in rodents indicate that aldosterone receptor blockers may reduce renal fibrosis. Data from human studies indicate that these medicines may decrease proteinuria (627). There are not yet sufficient data from large scale clinical trials to ascertain the value of using aldosterone receptor blockers to safely reduce renal fibrosis and proteinuria. The use of these medicines poses a major risk of hyperkalemia, especially in people with renal insufficiency or diabetes mellitus who are also taking ACE inhibitors and/or ARBs.

Diuretics and sodium restriction (2 to 4 g sodium per day) are used to treat edema and the tendency to retain sodium. When sodium is restricted adequately, thirst will usually regulate water intake to prevent the development of substantial hyponatremia. To maximize the antiproteinuria effects of ACE inhibitors or angiotensin II receptor blockers, it is either necessary to maintain urinary sodium excretion at or less than 100 mEq/day by dietary sodium restriction or to use a diuretic (i.e., a thiazide [see the previous text]). An NCEP Therapeutic Life Style (TLC) diet (see the previous text) may benefit lipid metabolism (346), although, by itself, dietary therapy probably will not lower serum lipids adequately and successful treatment of hypercholesterolemia will usually require HMG CoA reductase inhibitors, possibly in association with ezetimibe which decreases intestinal cholesterol absorption (628). Phosphorus intake may need to be liberalized if the protein intake is much greater than 0.70 gm/kg/day. Vitamin supplements should provide the normal daily allowances for the fat-soluble vitamins, including vitamin D, as well as the water-soluble vitamins.

For patients with moderate or advanced renal insufficiency, 1,25-dihydroxycholecalciferol, probably should be substituted for vitamin D_3 or cholecalciferol or one of its analogues may need to be given. The potential benefits of administering trace elements have not been established, although for patients with large amounts of proteinuria, iron, zinc, copper and probably other trace element supplements should be given. Good blood pressure control is essential (281,623). More studies are needed to examine these issues as well as the optimal use of medicines and diet to treat hyperlipidemia and hypercholesterolemia in the nephrotic syndrome and to retard the progression of renal failure.

NUTRITIONAL THERAPY FOR ACUTE KIDNEY INJURY—ALTERED METABOLIC AND NUTRITIONAL STATUS IN ACUTE KIDNEY FAILURE

Patients with acute renal failure (ARF) have widely varying degrees of altered metabolic and nutritional status. Some patients maintain nitrogen balance and have normal water balance, plasma electrolyte concentrations, and acid–base status. In general, these patients do not have severely catabolic underlying illnesses. They are usually not oliguric, and the cause of their ARF is typically an isolated, noncatabolic event, such as administration of radiocontrast drugs or aminoglycoside nephrotoxicity. However, most patients with ARF for whom a nephrologist is consulted have some degree of increased net protein breakdown (synthesis minus degradation) and disordered fluid, electrolyte, or acid–base status. There is often excess total body water, azotemia, hyperkalemia, hyperphosphatemia, hypocalcemia, hyperuricemia, and a large anion gap metabolic acidosis.

Net protein degradation in ARF can be massive, with net losses as high as 200 to 250 gm per day (628–630); for comparison, the total noncollagen protein mass of a 70-kg man is about 6 kg (631). Patients are more likely to be catabolic when the ARF is caused by shock, sepsis, or rhabdomyolysis. In the study of Feinstein et al., mean UNA was 12.0 ± (S.D.) 7.9 gm per day in patients in whom ARF was caused by hypotension, sepsis, or both, and 12.3 ± 7.9 gm per day in those with rhabdomyolysis (629). These UNA rates were significantly greater ($p < 0.001$ and $p < 0.05$, respectively) than in patients with ARF from other causes (3.8 ± 2.4 gm/day).

In patients with ARF, marked net protein catabolism may accelerate the rate of rise in plasma concentrations of potassium, phosphorus, nitrogenous metabolites, and non-nitrogen containing acids. It is therefore not surprising that protein and energy wasting or protein-energy malnutrition is very prevalent in patients with ARF (629,632,633) and is associated with increased morbidity and mortality (633).

Animal studies suggest that acute uremia itself may cause disorders in amino acid and protein metabolism and promote breakdown. UNA is increased in rats with ARF compared with sham-operated controls. Livers from acutely uremic rats display increased uptake of several amino acids and enhanced urea production (634). Frohlich et al. studied glucose and urea output in perfused liver from rats with bilateral nephrectomy and reported increased hepatic release of glucose, urea, and the branched-chain amino acids valine, leucine, and isoleucine, which are not well metabolized by the liver (635). When a mixture of amino acids was added to the perfusate in concentrations that were approximately similar to those found in plasma, the increment in hepatic glucose and urea formation in the ARF rats was significantly greater than in acutely uremic rats not receiving amino acids (635). These observations suggest that in rats, acute uremia per se stimulates both gluconeogenesis and protein degradation in the liver. Although a number of these studies were conducted before the degree of inflammation in animals or patients with ARF could be readily characterized, their hypercatabolic state was almost certainly often associated with a severe systemic inflammatory response.

Some early studies suggest that protein synthesis may vary independently and according to the organ studied (636,637). However, these investigators did not examine the balance between both synthesis and degradation. Flugel-Link and coworkers found increased degradation and a trend toward impaired synthesis of protein in the perfused posterior hemicorpus of rats made acutely uremic by bilateral nephrectomy (638). Clark and Mitch also described reduced protein synthesis and enhanced protein degradation in skeletal muscle from rats with ARF (639). Insulin, added to the incubation media, enhanced protein synthesis and reduced protein degradation in muscle of these animals. The effect of insulin was less marked in the acutely uremic animals compared with the sham-operated controls, indicating that ARF causes insulin resistance in muscle. The acidemia that occurs in ARF contributes to the protein catabolism (640). Nonetheless, acute uremia per se seems to engender only a mild increase in catabolism, at least in the commonly studied rat model (638,639). It is when sepsis or hypoxia is superimposed that catabolism increases markedly (628–630,641).

The mechanisms responsible for increased gluconeogenesis and net protein degradation in rats with ARF are not well defined. In the liver of acutely uremic rats, increased activity has been reported for glutamate-oxaloacetate aminotransferase, which catalyzes the transamination of glutamate (642). Elevated activity of two urea cycle enzymes, ornithine transaminase, which catalyzes the conversion of glutamate to ornithine, and arginase, which catalyzes formation of urea and ornithine from arginine, has been observed. Increased activity of serine dehydratase, which oxidizes the gluconeogenic amino acid serine, has been described. In addition, in acutely uremic rats, there is normal hepatic activity of phenylalanine hydroxylase and increased activity of tyrosine aminotransferase, key enzymes in the degradation of phenylalanine and tyrosine (643).

Decreased muscle glycogen has been observed in acutely uremic patients who are catabolic (5) and in rats with bilateral nephrectomy (644). Increased activity of phosphorylase α, which catalyzes glycogenolysis, and decreased activity of glycogen synthetase 1, which catalyzes glycogen synthesis, has been observed in muscle of these rats. Supplementing a low-protein diet with serine increased glycogen synthetase I activity in these animals (644).

The catabolic effects of ARF may be induced by several causes. First, the many products of metabolism may be toxic in the high concentrations that occur in uremia. Second, alterations in plasma concentrations of catabolic hormones may promote wasting. Infusion of normal humans or dogs with cortisone, epinephrine, and glucagon in quantities sufficient to raise plasma concentrations to levels found in acutely catabolic patients causes a sustained increase in glucose production, increased protein turnover and protein degradation, increased energy expenditure, and negative nitrogen balance (645,646). These hormones may be increased in ARF due to associated illnesses (see the subsequent text). These illnesses are often associated with release of microbial toxins, elevated acute phase reactants, catabolic cytokines, increase in oxidant levels, and hormonal disorders associated with increased counterregulatory hormones.

A glucocorticoid receptor antagonist may block the catabolic response in acutely anephric rats (647). Moreover, in CRF patients there is evidence for increased sensitivity to the actions of glucagon (173); however, others were unable to demonstrate enhanced sensitivity to glucagon in rats with ARF (648). Experience in ARF patients infused with hypertonic glucose suggests that glucose intolerance is common (629). Parathyroid hormone, which may be increased in ARF (649,650), is another potentially catabolic agent (174,175).

Third, as indicated earlier, acidemia increases amino acid and protein catabolism (160,161,640). Fourth, proteolytic activity is increased in the sera of some patients with ARF who are very catabolic (651,652). This suggests that in the sera of hypercatabolic, acutely uremic patients, there may be either increased quantities of proteases, which degrade proteins, a reduction in protease inhibitors, or changes in other factors that lead to enhanced protease activity. In acutely uremic rats

that have increased muscle protein degradation, activities of muscle alkaline protease, cathepsin B, and cathepsin D, measured *in vitro*, were found not to be different from those of controls (638).

In addition to the potential catabolic effects of uremia per se, there are clearly other causes of wasting and malnutrition in ARF. These include the following:

A. Many patients are unable to eat adequately because of anorexia or vomiting. These symptoms may be caused by acute uremia, underlying illnesses, or the anorectic effects of dialysis treatment. Other causes of poor food intake are medical or surgical disorders that impair gastrointestinal function and the frequent diagnostic studies that require the patient to fast for several hours.
B. The patient's underlying medical disorders often cause a hypercatabolic state. Chief among these catabolic conditions in ARF are infection, hypotension, surgery, trauma, and rhabdomyolysis.
C. Losses of nutrients in draining fistulas and during dialysis therapy may lead to wasting and malnutrition. Moreover, hemodialysis itself may stimulate catabolism (see the previous text).
D. As indicated previously, blood drawing, gastrointestinal bleeding, which may be occult, and blood sequestered in the hemodialyzer are other causes of protein depletion.

EXPERIENCE WITH NUTRITIONAL THERAPY FOR ACUTE RENAL FAILURE

Prior to the mid-1960s, many clinicians recommended severe or total restriction of protein intake for patients with ARF (246,653,654). Small amounts of energy (e.g., 400 to 800 kcal/day) were provided from candy, butterballs, or intravenous infusions of hypertonic glucose to reduce the rate of protein degradation. This therapy was based on Gamble's studies of lifeboat rations for healthy young men. Gamble's observations indicated that administration of 100 gm per day of sugar could substantially reduce net protein breakdown in these starving but otherwise healthy volunteers (655).

Anabolic steroids were frequently administered because they could transiently decrease the UNA, the rate of rise of SUN or nonprotein nitrogen, and the development of acidemia (656,657). In the 1960s, the Giordano-Giovannetti diet was developed for patients with CRF (248,252). This diet, which contained about 20 gm per day of protein, most of which was usually supplied by two eggs, provided the recommended daily allowances for essential amino acids for young healthy adults. Some clinicians advocated this diet for the acutely uremic patient who was able to eat because they believed that the diet could be utilized efficiently and would minimize the degree of protein wasting while it reduced the UNA and the accumulation of nitrogenous metabolites (658). In the era before patients could be readily treated with dialysis or hemofiltration, it was thought that anabolic steroids or these low-nitrogen diets might reduce uremic toxicity and maintain life until renal function recovered. Maintaining good nutrition was a secondary aim. When dialysis first became available, it was usually employed only for specific sequelae of renal failure, such as uremic symptoms, congestive heart failure or hyperkalemia, and great efforts were often made to avoid the need for dialysis therapy. With the development of modern techniques for parenteral and enteral nutrition and the routine use of dialysis, the emphasis on nutritional therapy gradually changed so that the maintenance of good nutrition became a primary goal, and dialysis

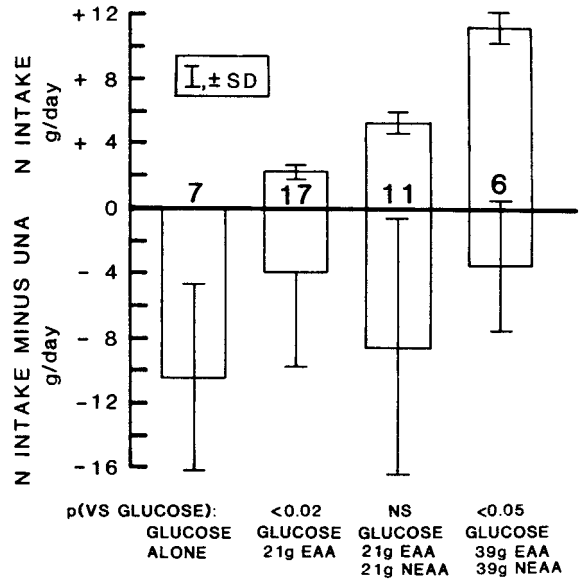

FIGURE 103-2. Mean nitrogen intake and approximated nitrogen balance in four groups of patients with acute renal failure who were receiving parenteral nutrition. Individuals were treated with glucose alone, glucose and 21 gm/day of essential amino acids (EAA), glucose and 21 gm/day of EAA and 21 gm/day nonessential amino acids (NEAA), and glucose and approximately 39 gm/day of EAA and 39 gm/day of NEAA. Nitrogen intake was calculated from the measured nitrogen content of the infused parenteral nutrition solutions and is plotted above the horizontal line. The nitrogen balance, estimated as the difference between the nitrogen intake and nitrogen output, calculated as the urea nitrogen appearance (UNA), is plotted below the horizontal line. Data for patients who received a given parenteral nutrition formulation represent the mean values of the average daily intake and balance data obtained from each of the patients during the parenteral nutrition treatment. The calculated nitrogen intake was not adjusted for the nitrogen received from transfusions of blood or blood products which contain proteins and small amounts of peptides, free amino acids and other nitrogen containing compounds. Nitrogen balance was not adjusted for nonurea nitrogen in urine, fecal nitrogen, and other unmeasured nitrogen losses (e.g., from respiration, wound drainage, nasogastric tube or fistula drainage, flatus, sweat, skin desquamation and replacement, and hair and nail growth). The numbers in the vertical bars indicate the sample size of each group. Data from two separate studies (629,630) are combined in this figure. (Reprinted from: Kopple JD. 1995 Jonathan E. Rhoads lecture: the nutrition management of the patient with acute renal failure. *J Parent Ent Nutr* 1996;20:3, with permission.)

was used as needed to control fluid and electrolyte balance and remove metabolic waste (659).

The essential amino acids were more efficiently utilized to decrease protein catabolism and, both per gram infused and for the degree of antianabolic effect, generated less urea and presumably other products of nitrogen metabolism (629,630,660) (Figs. 103-2 and 103-3). Infusion of small amounts of essential amino acids may also decrease serum potassium, phosphorus and magnesium and stabilize or decrease the SUN (661–668). However, infusion of larger quantities of both essential and nonessential amino acids appeared to have overall more anabolic or anticatabolic actions (629,630,660,669,670). These latter preparations also tended to generate more metabolic waste (629,630,660). Many of these observations were based on clear trends but, probably due to small sample sizes these differences were not statistically significant.

Today, there is a consensus that if the ARF is severe and the patient is catabolic, it is preferable to provide him with the nutritional support that will minimize his catabolic response and to dialyze and/or hemofilter him as needed. From the 1960s

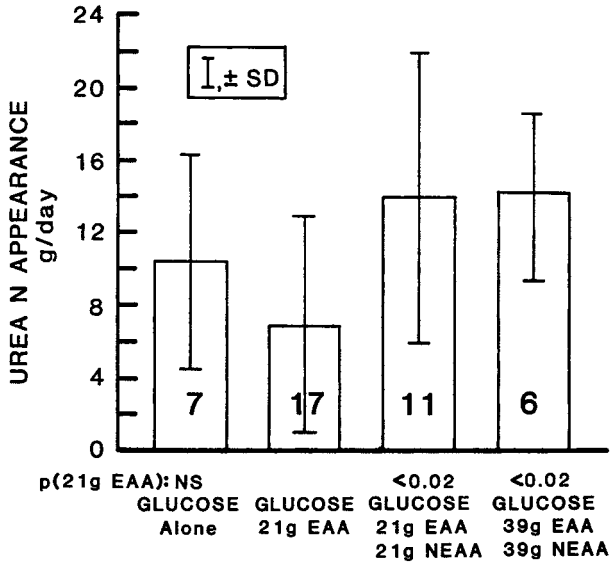

FIGURE 103-3. UNA in four groups of patients with acute renal failure who were receiving parenteral nutrition (629,630). Data for patients who received a given parenteral nutrition formulation represent the mean values of the daily average UNA for the patients in that treatment group. Symbols are explained in the legend to Figure 103-2 and the text. (Reprinted from: Kopple JD. 1995 Jonathan E. Rhoads lecture: the nutrition management of the patient with acute renal failure. *J Parent Ent Nutr* 1996,20:3, with permission.)

to the 1980s, a number of studies examined the use of mixtures of the essential amino acids or rather low quantities of essential and nonessential amino acids (i.e., usually less than 70 g/day) on the metabolic response or survival of patients with ARF or occasionally with CRF who needed intravenous nutrition (628–630,660–672). Only some of these studies were randomly controlled. In some studies comparisons were made to patients receiving intravenous glucose without amino acids. No randomized prospective study compared nutritional support to no nutritional support. In general, patients received intermittent hemodialysis therapy as needed, as determined by their clinical condition.

There was no effect of nutritional support with glucose and essential amino acids on overall survival (629,666), although in retrospect, these studies were markedly underpowered to assess a survival benefit, and the randomized control groups always received at least glucose, water and electrolytes. However, the data strongly indicated that adding amino acids to the intravenous infusions promoted anabolism or, at least, reduced the degree of negative protein balance.

Effect of CVVH/CVVHD on Nutritional Management of Patients with Acute Renal Failure

CVVH (continuous venovenous hemofiltration), CVVHD (CVVH with concurrent hemodialysis), or CVVHDF (continuous venovenous hemodiafiltration) are commonly used for the management of very ill patients with ARF, CRF or other causes of fluid or nitrogen intolerance (e.g., severe liver or congestive heart failure). CVVH/CVVHD/CVVHDF is performed with low blood and (for CVVHD) dialysate flow rates and is usually administered over 24 hours per day. CVVH/CVVHD/CVVHDF offer the following advantages to the patient: (a) Larger quantities of water, electrolytes and metabolites may be removed each day than with a standard 4-hour hemodialysis; (b) The rate of removal of water and electrolytes is slow and therefore generally does not cause or worsen hypotension; (c) Because of the high daily clearances of water and small molecules, including metabolic waste products, one may safely administer greater amounts of amino acids and other nutrients to the patient than is usually feasible with hemodialysis given three or four days per week; (d) Because of the high daily clearances of molecules by CVVH/CVVHD/CVVHDF, patients receiving CVVH/CVVHD/CVVHDF generally will not require hemodialysis therapy; and (e) CVVH/CVVHD/CVVHDF may be administered by nurses who are not specifically trained in hemodialysis (although some special training of nursing personnel is required). Thus, CVVH/CVVHD/CVVHDF may be safer, more effective, and more convenient to use, and the cost for this therapy may be no more or possibly even less expensive than for intermittent hemodialysis. Sustained low-efficiency dialysis (SLED), which is hemodialysis performed with low blood and dialysate flow rates and with consequent lower clearances for many hours each day, provides many of the advantages of CVVH/CVVHD/CVVHDF.

Amino acid losses during CVVH/CVVHD/SLED are influenced by the permeability characteristics of the filter membrane, the ultrafiltration and dialysis flow rates, and the rate of amino acid infusion, which will influence the plasma amino acid concentrations (673–675). Approximately 4 to 7 g per day of amino acids are removed with CVVH (673). In one study, amino acid losses were about 8.9 ± 1.2 (SEM)% and 12.1 ± 2.2% of the daily quantity of amino acids infused in patients with renal failure undergoing CAVHD with a dialysate flow rate of 1 and 2 L/hour, respectively (674). Amino acid losses with CVVHD generally average about 6% to 12% of the daily amino acids infused, but many increase to 16% to 17% of the infused amino acids with increasing amino acid intakes (i.e., up to 2.5 g/kg/day (675,676).

Calcium and magnesium losses during CVVH or CVVHD tend to be large, averaging roughly 2,800 and 600 mg/day, respectively (677,678). Dialysate or ultrafiltrate and urine zinc losses combined averaged 1.20 mg/day (677).

It is not clear that CVVH/CVVHD/CVVHDF/SLED reduces morbidity or mortality more than intermittent hemodialysis in patients with ARF (679). On the other hand, the larger quantities of amino acids that can be given with CVVH/CVVHD/CVVHDF/SLED have resulted in more positive nitrogen balance and more effective fluid and electrolyte control than can generally be achieved with thrice-weekly hemodialysis in catabolic ARF patients. Data from both nonrandomized and randomized prospective clinical trials in patients with ARF receiving enteral or parenteral nutrition indicate that the degree of positivity of nitrogen balance (or the degree to which nitrogen balance is less negative [680]) is directly related to the protein or amino acid intake (683). About 2.5 g of protein or amino acids/kg/day appeared necessary to maintain nitrogen balance (680,683). Moreover, the study of Scheinkestel et al indicated that nitrogen balance, after adjusting for age, sex and APACHE II score, was associated with a greater probability of survival in both the intensive care unit and also overall hospital survival (683). In contrast to nitrogen balance, there was no association between protein or amino acid intake and patient outcome. These intriguing findings suggest several possibilities: (a) that the catabolic state of the patient affects their prognosis; (b) that their nitrogen balance might affect their outcome; and (c) that it may be advantageous to monitor nitrogen balance in these ICU patients and to increase amino acid intake to maintain positive balance. These results must be interpreted with some caution because with very high protein intakes, the quantity of nitrogen lost through unmeasured routes (i.e., respiration, skin) may increase substantially, making nitrogen balance falsely more positive (312).

ENTERAL VERSUS PARENTERAL NUTRITION

Patients with ARF and superimposed illness should be given oral nutrition whenever feasible. If the patient will not eat adequately, he may be offered liquid formula diets, elemental diets, or tube or enterostomy feeding. Often enteral tube or gastrostomy feeding is the only possible method of nourishment through the alimentary tract (684) in critically ill patients. For patients who must be fed by an enteric tube or gastrostomy, there are many liquid protein or defined-formula (elemental) diets that may be prescribed (685). Some are specifically designed for renal failure patients. Dieticians usually can provide information concerning the composition of these preparations. There are a number of reviews of the techniques and complications associated with the use of the chemically defined diets and tube feeding (684,686,687). Most of the principles are applicable to the patient with renal failure. Enteral feeding has been used extensively in pediatric patients, and particularly infants, with CRF (688,689); enteral feeding is now becoming more widely used in adult patients with ARF or CRF (685,690).

Patients with ARF are more likely to have high gastric residual volumes and nasalgastric tube obstruction (685) if they receive nutrition, enternal, and they must be monitored carefully for these complications. However, for the majority of adult ARF patients, enteral feeding is a safe and effective way to provide nutrition. There is a tendency for intensive care patients with or without ARF prescribed enteral nutrition to not receive the prescribed nutrients (685,691). Careful attention to the rate of delivery of enteral nutrition and to potential disruptions due to medical and surgical care is necessary to ensure that the received nutrition is equal to prescribed nutrition.

Many patients with ARF do not have a sufficiently well-functioning gastrointestinal tract, and parenteral nutrition is the only technique that will provide adequate nutrient intake.This is particularly common in the more catabolic patients with ARF. Parenteral nutrition provides greater fluid loads and is more costly. Techniques for parenteral nutrition and the potential complications of this treatment have been reviewed elsewhere (692).

Peripheral parenteral nutrition has been suggested as an alternative to total parenteral nutrition (TPN) (693). The solutions are infused into a peripheral vein, and the risks of inserting a central catheter are avoided. A limitation of peripheral parenteral nutrition is that the osmolality of the infusate must be restricted to about 600 mosm or lower to prevent thrombophlebitis; even then, the needles or catheters must be changed frequently, usually every 18 to 48 hours. Thus, to provide adequate calories and amino acids, the patient must receive large fluid loads, which are not tolerated by most individuals with ARF who require parenteral nutrition. Moreover, the pharmaceutical costs of peripheral parenteral nutrition are usually similar to those of TPN because with the former technique, larger amounts of expensive lipid emulsions must be given to provide calories with a lower osmotic load.

Peripheral partial parenteral nutrition may be useful for patients with ARF who are able to receive only part of their daily nutritional requirements by oral or enteric feeding. The peripheral infusions may allow these patients to receive adequate nutrition without resorting to TPN through a large-flow vein. In these individuals, it is often preferable to infuse an 8.5% to 10% amino acid solution or a 20% lipid emulsion into a peripheral vein and administer as much as possible of the other essential nutrients through the enteral tract, including carbohydrates, which provide most of the osmotic load when given intravenously.

To avoid the risk of central vein catheterization, a peripheral vascular access that is used for hemodialysis can also be used for TPN (694). Because the blood flow through the vascular access is large, hypertonic solutions can be infused, and the water load to the patient can be reduced. However, in our experience, this technique increases the hazard of local infection, and it should not be used in patients who will need a hemodialysis access for an extended period of time.

EFFECT OF NUTRITIONAL INTAKE ON RECOVERY OF RENAL FUNCTION IN ACUTE RENAL FAILURE

Abel et al. observed that in patients with ARF who were treated with intravenous infusions of essential amino acids and glucose as compared with glucose alone, there was a tendency for the serum creatinine levels to decrease sooner and to lower levels (665). These findings raised the question of whether nutritional therapy may facilitate healing and enhance the rate of recovery of renal function in patients with ARF. In the proximal tubular cells of rats with acute tubular necrosis caused by injection of mercuric chloride, there is increased synthesis of protein, nucleic acids, and phospholipids and accelerated growth that is apparent as early as the second day after injection (695–697). Toback suggested that this regrowth should increase the requirements for nutrients and that this greater demand for nutrients occurs at a time when food intake is often decreased and uremic toxicity is developing (698). However, experiments in animals with ARF have given conflicting results as to whether nutritional support will enhance the rate of recovery of ARF (699–702), and no other studies in humans with ARF have shown an effect of nutritional support on recovery of renal function.

WHY BENEFITS OF NUTRITIONAL THERAPY HAVE NOT BEEN DEMONSTRATED UNEQUIVOCALLY

Notwithstanding the reports of Abel et al. (665) and Scheinkestel et al. (683), the foregoing studies, taken together, do not unequivocally demonstrate that treatment with amino acids and other nutrients will improve the rate or incidence of recovery of renal function or the nutritional status, overall clinical condition, or survival. Intuitively, it seems that nutritional therapy should benefit patients with ARF. For patients who have ARF for more than 2 to 3 weeks or who are convalescing from ARF but are still unable to eat, evidence is stronger that oral or parenteral nutritional support should improve nutritional status.

There are probably several reasons why it has been so difficult to demonstrate a beneficial effect of nutritional therapy. These may include the following: (a) The clinical course of patients with ARF is so variable and often so complex that it is necessary to study large numbers of patients in randomized prospective studies to show statistically significant advantages to nutritional therapy, if these benefits exist; (b) Many of these studies were retrospective or not randomly controlled, and this may have led to unintentional biases in the results; (c) The optimal composition of nutrients in the enteral or parenteral infusion solutions has not been defined, and the use of suboptimal formulations of nutrients may have made it more

difficult to demonstrate the clinical benefits of nutritional therapy; (d) The randomized prospective studies in patients with ARF did not examine whether nutritional support is beneficial. They compared only the response to different formulations of nutritional support. There has been no prospective controlled comparison between the clinical course of patients with ARF who were receiving nutritional therapy and those who were receiving no enteral or parenteral nutritional support; and (e) It is probable that catabolic patients or rats with ARF may need both good nutritional intake and metabolic intervention to suppress catabolic processes and promote anabolism in liver and other tissues. Because ARF patients who need nutritional therapy often suffer from underlying catabolic illnesses, they have undergone a metabolic reorganization so that they are more prone to degrade infused amino acids and energy substrates. Thus, the provision of nutrients without altering these metabolic processes may not benefit the nutritional status or clinical outcome. This may be particularly true during the initial days after the onset of ARF.

RECOMMENDED NUTRITIONAL THERAPY FOR ACUTE RENAL FAILURE

General Principles

From the current data, it is not possible to develop definitive protocols for the nutritional therapy of patients with ARF. The following therapeutic approach, summarized in Table 103-7, is

TABLE 103-7

COMPOSITION OF SOLUTIONS FOR TOTAL PARENTERAL NUTRITION IN PATIENTS WITH ACUTE RENAL FAILURE[a]

Volume	1.0	(liters)	Vitamins	
			Vitamin A[f]	2,000 IU/day
Essential and nonessential	42.5 to 50	gm/L	Vitamin D	see text
free crystalline amino			Vitamin K[g]	7.5 mg/week
acids (4.25% to 5.0%)[b]			Vitamin E[b]	10 IU/day
OR			Niacin	20 mg/day
Essential amino acids (5%)[b]	12.5 to 25	gm/L	Thiamine HCl (B_1)	2 mg/day
			Riboflavin (B_2)	2 mg/day
Dextrose (D-glucose)[c]	350	gm/L	Pantothenic acid (B_3)	10 mg/day
Lipid emulsion	10% or 20%	in 500 mL	Pyridoxine HCl	10 mg/day
Energy (approximately)[c]	1,140	kcal/L	Ascorbic Acid (C)	60 mg/day
			Biotin	200 mg/day
Electrolytes[d]			Folic Acid[g]	1 mg/day
Sodium[e]	40 to 50	mmol/L	Vitamin B_{12}[g]	3 μg/day
Chloride[e]	25 to 35	mmol/L		
Potassium	≤35	mmol/day		
Acetate	35 to 40	mmol/day		
Calcium	5	mmol/day		
Phosphorus	8	mmol/day		
Magnesium	4	mmol/day		
Iron	2	mmol/day		
Other trace elements	see text			

[a]The nutrients listed are present in each bottle containing 500 mL of 8.5% to 10% crystalline amino acids or 250 to 500 mL of 5% essential amino acids and 500 mL of 70% D-glucose. The vitamins and trace elements are an exception because they are added to only one bottle per day. For those doses of nutrients that are expressed as concentrations rather than as quantities per day, the dose refers to the quantity present in each liter of dextrose and amino acids, with or without lipids. The patient's fluid status and serum electrolytes and glucose values must be monitored closely. The composition and volume of the infusate may be modified according to the nutritional status of the patient (see text).
[b]For patients who are more catabolic (e.g., UNA ≥5 gm/day), who are undergoing regular dialysis treatments (particularly for 2 or more weeks), or who are very wasted, essential and nonessential amino acids should be infused; about 1.0 to 1.2 gm/kg/day for hemodialysis patients and 1.0 to 1.3 gm/kg/day for intermittent peritoneal dialysis, CAPD or APD patients (see text). 1.5 to 2.5 gm/kg/day of essential and nonessential amino acids may be given to patients undergoing CVVH/CVVHD/CVVHDF or SLED. For patients who are not very wasted, who are less catabolic, who are not undergoing regular dialysis therapy, and who will not be receiving TPN for more than 2 or 3 weeks, 21 to 40 gm per day of the nine essential amino acids may be infused. See text for discussion of the formulations of amino acids.
[c]Seventy percent D-glucose is added as necessary to obtain an energy intake of 30 to 35 kcal/kg/day (see text); lower energy intakes may be used in very obese patients. The D-glucose reported is actually dextrose monohydrate. For the higher levels of energy intake (i.e., 45 kcal/kg/day), additional 70% D-glucose may be added to the solutions. Generally lipids are infused each day to provide 20% to 30% of total calories in order to balance the sources of calories and to prevent essential fatty acid deficiency. For patients who are septic or at high risk for sepsis, about 10% to 20% of calories may be given as lipids. The lipids probably should be infused over 12 to 24 hours to reduce the hyperlipidemia that occurs with intravenous infusion of lipid emulsions and to avoid impairment of the reticuloendothelial system. The lipids may be infused through a separate line or mixed with the amino acid and dextrose solutions and infused soon after mixing (see text). Usually a 20% lipid emulsion (250 to 500 mL) is used to reduce the water load. The approximate calorie values are dextrose monohydrate, 3.4 kcal per gm; amino acids, 3.5 kcal per gm.
[d]When adding electrolytes, the amounts intrinsically present in the amino acid solution should be taken into account.
[e]Refers to the final concentrations of electrolytes after any additional 70% dextrose or other solutions have been added.
[f]About 600 μg/day of retinol activity equivalents (RAE), see text.
[g]Should be given orally or parenterally and not in the TPN solution because of antagonisms.
[h]May need to be increased with use of lipid emulsions.
CVVH, continuous venovenous hemofiltration; *CVVHD*, continuous venovenous hemofiltration with concurrent dialysis; *CVVHDF*, continuous venovenous hemodiafiltration; *SLED*, slow, low-efficiency dialysis; *TPN*, total parenteral nutrition.

based on the author's analysis of the literature and personal experience. In general, the policy is to administer sufficient quantities of nutrients to prevent or minimize the development of malnutrition. Fluid and mineral balance should be carefully monitored to avoid overhydration or electrolyte disorders.

Because the clinical status of patients with ARF is so diverse, the prescribed nutrient intake will vary greatly and should depend on the patient's nutritional status, catabolic rate, residual GFR, and the indications for initiating intermittent dialysis therapy, SLED or CVVH/CVVHD/CVVHDF. A patient who is malnourished or hypercatabolic might receive a surfeit of nutrients and be given intermittent hemodialysis or CVVH/CVVHD/CVVHDF/SLED as needed. Patients with a high residual GFR also may be given large quantities of nutrients, since there is less risk of developing fluid and electrolyte disorders or accumulating metabolic waste products.

For a patient who has little or no urine flow and is not very catabolic or uremic, a reduced intake of water and minerals and calories with small amounts of amino acids, often given as the essential amino acids, may decrease the need for dialysis; this may be particularly beneficial if the patient does not tolerate dialysis well because of myocardial ischemia, very low cardiac output or hemodynamic instability.

Similarly, a patient who is beginning to recover from ARF may be given small quantities of water, electrolytes, and amino acids in order to postpone the need for dialysis until renal function becomes adequate. In these latter patients, high-calorie diets or infusates providing small amounts of essential amino acids with little or no protein may be used for a limited amount of time. Such nutritional therapy may maintain near neutral nitrogen balance for short periods of time.

In general, the intake of water (including the water content from wet foods) should equal the output from urine and all other measured sources (for example, nasogastric aspirate, fistula drainage) plus about 400 mL per day. This regimen takes into account the contributions of endogenous water production from metabolism and insensible water losses (e.g., from respiration and skin) to water balance. On the other hand, if the patient is catabolic and in negative calorie and nitrogen balance, weight should be allowed to decrease by about 0.2 to 0.5 kg per day to avoid accumulation of water. The intake of sodium, potassium, phosphorus, magnesium, calcium, and trace elements should be restricted to prevent accumulation of these minerals. Sodium intake should equal output but should also be coordinated with the water balance to prevent hyponatremia or hypernatremia. Potassium and phosphorus intake should be designed to prevent abnormally high or low blood levels. By controlling the water and electrolyte intake and lowering the rate of accumulation of urea and other nitrogenous metabolites, it may be possible to reduce the need for dialysis treatments.

Insulin should be used to maintain normal plasma glucose concentrations. Glucose intolerance is very common in patients with ARF. With the large glucose loads frequently administered, hyperglycemia may occur in a large proportion of patients (629). Insulin is also a potent anabolic hormone. In nonuremic patients who have catabolic illnesses, insulin can reduce nitrogen output and improve negative nitrogen and protein balance (703–705). In surgical intensive care unit patients, intravenous insulin infusions to maintain blood glucose between 80 and 110 mg/dL may reduce mortality. (706). Recombinant human growth hormone (rhGH) may also promote nitrogen balance in patients with acute catabolic stress or chronic illness, including CRF (707,708). However, studies in critically ill patients indicate that rhGH may increase mortality in severely ill patients (709).

The following discussion of specific nutrient intakes for patients with ARF can be applied to individuals receiving oral, enteral or intravenous nutrition. TPN has been emphasized because this is frequently the route of choice for nutritional therapy in the sicker, more catabolic patients with ARF, and it is usually the most complicated of the techniques. However, clinical trials indicate that total enteral nutrition (TEN) is preferable to total parenteral nutrition (TPN) because the TEN is associated with a lower rate of morbid events (see the subsequent text) (111–114). This may be the result of better preservation of gut mass and host resistance in the intestinal tract and other organs with TEN as compared to TPN (115–121). Certain constituents of nutritional support solutions, such as arginine, RNA, and omega-3 fatty acids (122,123), or glutamine (88–90) appear to maintain more normal gut anatomy, enhance immune function, and possibly increase survival. The gut hormone, bombesin, also may improve intestinal structure and the immune function of the gut mucosa and upper-respiratory tract (124–126).

Nitrogen Intake

The physician is most likely to maximally benefit the patient's metabolic and clinical status if the nitrogen intake is tailored to the clinical condition of the patient. A low protein or amino acid intake, provided orally, enterally or intravenously, may be prescribed for patients who have a small UNA (i.e., equal to or less than 4 to 5 gm nitrogen per day), who have no evidence of severe protein malnutrition, and who will probably recover renal function within 1 to 2 weeks (Tables 103-7 and 103-8). A severely reduced GFR and the desire to avoid or reduce the frequency of dialysis therapy are other indications for low nitrogen intakes. One may give 0.30 to 0.50 gm/kg adjusted edema-free body weight (aBW_{ef}) per day (240,561) of essential amino acids with or without arginine. More than 40 gm per day of the nine essential amino acids are not prescribed because larger quantities may cause serious amino acid imbalances (see the subsequent text) (660,729,730). For patients who can eat, diets providing 0.10 to 0.30 gm/kg/day of miscellaneous protein and 10 to 20 gm per day of essential amino acids also may be used. These treatment protocols should promote a low rate of accumulation of nitrogenous metabolites, and unless the patient is very catabolic, they will usually maintain neutral or only mildly negative nitrogen balance. Hence the need for dialysis therapy may be minimized or avoided.

If the patient has substantial residual renal function (e.g., GFR of 5 to 10 mL/minute), is not very catabolic, and can eat well, he may be treated as a nondialyzed patient with stage 3 to 5 CKD, receiving 0.55 to 0.60 gm/kg aBW_{ef}/day (240,561) of primarily high biologic value protein or about 0.28 gm protein/kg/day supplemented with 6 to 10 gm per day of essential amino acids. If such a patient cannot be fed enterally, he may be given 0.55 to 0.60 gm/kg aBW_{ef}/day of essential and nonessential amino acids intravenously. For patients who are more catabolic and have a higher UNA (greater than 5 gm nitrogen/day), are severely wasted, and either have or are expected to have ARF for more than 2 weeks, a higher protein or amino acid intake is usually prescribed. This prescribed intake is generally 1.0 to 1.2 gm/kg/day for patients undergoing regular hemodialysis thrice weekly but is often greater, about 1.2 to 1.5 gm/kg/day for individuals receiving standard hemodialysis more frequently, and approximately 1.5 to 2.5 gm/kg/day, for individuals receiving SLED or CVVH/CVVHD/CVVHDF (681,682,731,732) (Table 103-8). Most patients with ARF receiving TEN or TPN will merit one of these latter two dosages.

In comparison with small quantities of essential amino acids, these larger nitrogen intakes may improve nitrogen balance, particularly after the first 1 or 2 weeks of dialysis treatments. However, the UNA will almost invariably rise, and the

TABLE 103-8

AMINO ACID COMPOSITION OF SOLUTIONS FOR TOTAL PARENTERAL NUTRITION FOR PATIENTS WITH ACUTE RENAL FAILURE[a,b,c]

	No dialysis or ultrafiltration	Intermittent hemodialysis	CVVH/CVVHD CVVHDF/SLED
Volume	About 1.2 to 1.7 L	About 1.7 to 2.0 L	Standard TPN volumes
Essential amino acids (g/kg/day) (usually 5% solutions)	0.30 to 0.50[d]	Not applicable	Not applicable
Essential and nonessential amino acids (g/kg/day) (usually 8.5% to 10% solutions)	0.60	1.0 to 1.5	1.5 to 2.5

CVVH, continuous venovenous hemofiltration; *CVVHD*, continuous venovenous hemofiltration with concurrent dialysis; *CVVHDF*, continuous venovenous hemodiafiltration; *SLED*, slow, low-efficiency dialysis.
[a]For patients who are more catabolic (e.g., urea nitrogen appearance ≥5 g/day), who are undergoing regular dialysis treatments (particularly for 2 or more weeks), or who are very wasted, essential and nonessential amino acids may be infused; about 1.0 to 1.2 g/kg/day for hemodialysis patients and 1.0 to 1.3 g/kg/day for patients undergoing intermittent peritoneal dialysis, continuous ambulatory peritoneal dialysis, or continuous cycling peritoneal dialysis. 1.5 to 2.5 g/kg/day of essential and nonessential amino acids may be given to patients undergoing CVVH, CVVHD, CVVHDF, or SLED. For patients who are not very wasted, who are less catabolic, who are not undergoing regular dialysis therapy, and who will not be receiving total parenteral nutrition for more than 2 or 3 weeks, 21 to 40 g/day of the nine purified essential amino acids may be infused. See text for discussion of the formulations of amino acids.
[b]Normally, formulations containing both essential and nonessential amino acids are constituted in about a 1.0 to 1.0 ratio. With administration of small quantities of amino acids, higher proportions of essential to nonessential amino acids sometimes have been advocated.
[c]Some authorities have recommended immune-enhancing nutritional formulations containing glutamine or the combination of arginine, RNA, and omega-3 fatty acids.
[d]No more than 30 to 40 g/day of essential amino acids if nonessential amino acids are not administered concomitantly (see text).

increased azotemia and water load (in patients treated with TEN or TPN) may increase the need for dialysis. Patients undergoing more frequent (e.g., daily) hemodialysis, SLED or CVVH/CVVHD/CVVHDF appear to tolerate increased amino acid and water loads more effectively because of the greater weekly clearances (Table 103-8). Peak SUN levels tend to be well controlled with these more intense hemodialysis regimens, even with larger nitrogen loads.

As indicated earlier, studies in patients with acute or CRF suggest that essential amino acids may be utilized more efficiently than mixtures of essential and nonessential amino adds (255,629,660). On the other hand, large doses (i.e., more than 40 gm/day) of essential amino adds alone may be hazardous. Several reports describe patients with ARF who were infused with, for their body weight, relatively large quantities of essential amino acids (660,729,730). Their plasma amino acid concentrations were bizarre; many plasma amino acids were markedly increased. Blood ammonia levels were high, and a metabolic acidosis occurred. Some patients became comatose. The plasma amino acid pattern improved markedly when patients were given an infusion of essential and nonessential amino acids provided in a 1.0:1.0 ratio. These considerations suggest that the inclusion of some nonessential amino acids in the mixture may enable the patient to tolerate a larger quantity of essential amino acids. Because solutions with a high ratio of essential to nonessential amino acids may be more anabolic than preparations with the more typical 1.0:1.0 ratio and may be safer than high doses (greater than 30 to 40 gm/day) of essential amino acids alone, the use of such formulations for patients with ARF should be examined (733).

Branched-chain amino acids, particularly leucine, may promote anabolism (276,277). Studies in catabolic patients without renal failure suggest that the intravenous infusion of amino acid solutions containing a large proportion of branched-chain amino acids (i.e., isoleucine, leucine, and valine) may have a modest anabolic effect (734,735); not all reports confirm these findings.

Ketoacid analogues of the branched chain amino acids also enhance anabolism *in vitro* tissue preparations and in clinical studies carried out in nonuremic individuals who were not hypercatabolic (277,736). In postoperative patients without ARF who were receiving TPN, intravenous infusion of the salt complex of α-ketoglutarate and ornithine appears to decrease UNA and improve nitrogen balance (737).

Energy

The energy expenditure and requirements for patients with ARF are primarily determined by the same factors that affect nonuremic individuals and include weight, age, sex, associated diseases, and physical activity, if any. Elevated energy expenditure appears to be largely but not entirely the result of sepsis or other catabolic illnesses (318,738,739). Whether ARF per se has an effect on energy expenditure or requirements is not known. CVVH/CVVHD may reduce energy expenditure in patients with ARF (740,741), presumably because of the cooling effect on the patient caused by the heat loss from the blood circulating through the apparatus. It is unclear whether this reduction in body temperature in severely ill patients is hazardous. In nonuremic individuals, the energy intake necessary to obtain neutral or positive nitrogen balance rises when nitrogen intake is low. Because patients with ARF are often given low quantities of nitrogen, this may indicate a need for greater energy intake. The fact that patients with ARF are often fluid intolerant sometimes influences the quantity of energy that may be given safely. Mault and co-workers reported that patients with ARF had about a 30% increase in resting energy expenditure as determined by indirect calorimetry; many, if not all, of these patients had associated catabolic illnesses (738). In a nonrandomized comparison, the authors found that patients who died had a more negative energy balance than those who survived (738). We have also observed that patients with ARF given higher energy intakes had greater survival (629). Although this might reflect a clinical benefit of high energy intakes, it is also possible that the patients with more negative energy balance were sicker and had medical disorders that made them more difficult to nourish (e.g., shock, pulmonary edema).

TABLE 103-9

ADJUSTMENT FACTORS FOR ESTIMATING ENERGY EXPENDITURE DURING ILLNESS

Type of stress	Fraction of normal basal energy expenditure[a]
Malnutrition (chronic, severe)	0.70
Nondialyzed chronic renal failure	1.00
Maintenance hemodialysis	1.00 to 1.05
Elective surgery	
Early (1 to 4 days)[b]	1.00
Late (18 to 21 days)	0.95
Peritonitis	1.15
Soft tissue trauma	1.15
Fractures	1.20 to 1.25
Infections	
Mild	1.00
Moderate	1.20 to 1.40
Severe	1.40 to 1.60
Burns (percent of body surface)	
0% to 20%	1.00 to 1.50
20% to 40%	1.50 to 1.85
40% to 100%	1.85 to 2.05

[a]The basal energy expenditure values during the normal healthy state may be multiplied by these approximate factors to estimate resting energy expenditure during acute or chronic illness.
[b]Associated with early starvation.
(Adapted from: Wilmore DW. In: Wilmore DW, ed. *The Metabolic Management of the Critically Ill*. New York: Plenum; 1977:34; Monteon FJ, et al. Energy expenditure in patients with CRF. *Kidney Int* 1986;30:741.)

One standard method for assessing energy needs is based on the Harris-Benedict equations, which estimate basal energy expenditure (BEE) from age, sex, body weight, and height. The Harris-Benedict equations are as follows (742):

$$\text{For men: BEE} = 66.5 + (13.8 \times \text{weight (kg)}) + (5.0 \times \text{height (cm)}) - (6.8 \times \text{age (years)}) \quad [8]$$

$$\text{For women: BEE} = 655.1 + (9.6 \times \text{weight (kg)}) + (1.8 \times \text{height (cm)}) - (4.7 \times \text{age (years)}) \quad [9]$$

The value calculated from these equations is then multiplied by an adjustment factor for the increase in energy expenditure caused by different clinical conditions. Some of these factors are shown in Table 103-9. Finally, the energy requirement is increased by 25% to adjust for individual variability, physical activity, and the potentially greater needs associated with a low nitrogen intake and ARF. Thus, the calculation of energy requirements in ARF is as follows:

$$\text{Energy requirements} = \text{estimated BEE} \times \text{adjustment for illness} \times 1.25 \quad [10]$$

In recent years, the Harris-Benedict equation has been reported to overestimate the resting metabolic rate by about 10% to 15% (744). This overestimate may be greater in patients with low resting metabolic rates. The World Health Organization (WHO) equations appear to give more accurate estimates of resting metabolic rates, at least in normal men and women (744). These equations, compiled from about 11,000 measurements of individuals of both genders, all ages, and a wide variety of ethnic groups and body mass indices, are as follows (745):

Age (years)	*Resting Metabolic Rate (RMR, kcal/day)*	
Men: 18 to 30:	$\text{RMR} = 15.4 \times \text{weight (kg)} - 27.0 \times \text{height (m)} + 717$	[11]
30 to 60 :	$\text{RMR} = 4.6 \times \text{weight (kg)} + 16.0 \times \text{height (m)} + 901$	[12]
Women: 18 to 30:	$\text{RMR} = 13.3 \times \text{weight (kg)} + 334.0 \times \text{height (m)} + 35$	[13]
30 to 60:	$\text{RMR} = 8.7 \times \text{weight (kg)} - 25.0 \times \text{height (m)} + 865$	[14]

Another method used to estimate energy requirements is to measure energy expenditure by indirect calorimetry and multiply this value by 1.25. In practice, the estimated energy requirements for patients with ARF requiring nutritional support usually fall between 30 and 35 kcal/kg aBW_{ef}/day.

The higher energy intakes (i.e., 35 kcal/kg/day) generally are prescribed for patients who have a higher UNA, who are severely ill, and who are not very obese. For example, if nitrogen balance, estimated from the difference between the patient's nitrogen intake and the nitrogen output calculated from the UNA (see the previous text), is negative, despite an appropriate amino acid intake, we may try to provide an energy intake close to 40 kcal/kg/day.

Larger energy intakes are not used because there appears to be little nutritional advantage to administering more kcal to catabolic patients. Indeed, because high-energy intakes generate more carbon dioxide from the infused carbohydrates and fat, they can promote hypercapnia if pulmonary function is impaired (746). Carbon dioxide retention is particularly likely to occur with very high carbohydrate loads, because, in comparison to fat, more carbon atoms are provided with carbohydrate to provide the same quantity of energy. Also, very high-energy intakes may cause obesity and fatty liver, and because of the large volume of fluids in the intravenous solutions, they may increase the water load to the patient (747).

Since most patients with ARF do not tolerate large water intakes, glucose is usually administered in a 70% solution. Glucose is added to intravenous solutions as glucose monohydrate, which is 90% anhydrous glucose. The energy available from glucose monohydrate is 3.4 kcal per gram or, for 70% dextrose, about 2.38 kcal per mL. Amino acids provide about 3.5 kcal per gram. The glucose and amino acid solutions are mixed so that the amino acids and calories are provided simultaneously (see Table 103-7).

Patients receiving TPN for more than about 5 days should receive lipid emulsions. Recent evidence underscores the importance of preventing hyperglycemia, and lipids are often given from the onset of TPN. The optimal amount of fat for TPN is somewhat controversial, especially because lipid clearance may be impaired in ARF (748). Twenty-five gm per day of lipids will prevent essential fatty acid deficiency. Some investigators recommend infusing up to 30 to 40% of calories as lipid emulsions to provide more normal amounts of fatty acids to organs that use lipids for energy and to more closely approximate the normal American dietary intake. Some findings suggest that infusion of lipid emulsions may lower host resistance. Several researchers report that infusions of large amounts of fat emulsions (e.g., 50 gm over 8 hours) may impair reticuloendothelial system function (749), presumably by overwhelming the phagocytic capacity of this system. Neonates receiving TPN with lipid emulsions are reported to have a higher incidence

of bacteremia than similar infants given TPN without lipids (750).

A prudent approach may be to infuse lipid emulsions over 12 to 24 hours to minimize increases in plasma lipids and to avoid overwhelming the reticuloendothelial system. Patients who are not septic may be given up to 20% to 30% of calories as lipid emulsions. Patients who are severely septic probably should not receive intravenous lipid emulsions for several days. Lipids are available as 10% and 20% fat emulsions that provide 1.1 and 2.0 kcal per mL, respectively. Traditionally, lipids have been infused separately from the glucose and amino acid mixtures. With careful attention to aseptic techniques, the lipid emulsions may be mixed with glucose and amino acids; the mixtures should be infused shortly after preparation (751). Several studies suggest that omega-3 fatty acids may enhance immune function and host resistance (752–754). Patients receiving enteral nutrition who have a diseased or shortened intestinal tract are often given lower quantities of lipids, often in the form of more readily absorbable triglycerides, to prevent malabsorption.

Minerals

Minerals can be added to parenteral nutrition solutions for ARF as shown in Table 103-7. Recommended intakes of minerals should be considered tentative and must be modified according to the clinical status of the patient. The patient must be monitored closely because the hormonal and metabolic changes that often occur in ARF may cause serum electrolytes to rise or fall dramatically. If the serum concentration of an electrolyte is increased, it may be advisable to reduce the quantity infused or to refrain from administering it at the onset of parenteral nutrition. However, parenteral nutrition may rapidly lower certain serum electrolytes, particularly potassium and phosphorus. On the other hand, a low serum concentration of a mineral may indicate that there is a need for a greater than usual intake of that element. Again, metabolic changes and the impaired GFR can lead to a rapid rise in the serum concentrations of electrolytes or glucose. Special attention should be paid to the increased calcium and magnesium needs with CVVH/CVVHD/CVVHDF (755).

With the exception of iron and possibly zinc, and selenium, trace elements are probably not necessary in parenteral nutrition solutions for ARF unless this is the sole source of nutritional support for at least 2 to 3 weeks. Patients treated with CVVH/CVVHD/CVVHDF may have increased nutritional needs for selenium and copper (756). Low plasma selenium levels are reported in patients with the multiple organ dysfunction syndrome (757). This might be related to reduced plasma concentrations or binding characteristics of the carrier protein. However, in a prospective open label study, patients with the severe systemic inflammatory response syndrome were randomized to receive, intravenously, different replacement doses of sodium selenite for 9 days (535, 285, and 155 μg/day for 3 days each, in order of descending dosage) followed by sodium selenite, 35 μg/day, throughout the treatment period (758). The patients given the larger dose of selenium experienced significantly reduced morbidity and need for CVVHD. The nutritional requirements for other trace elements are not well established for ARF patients receiving TEN or TPN. Many trace elements are routinely added to TEN preparations.

Vitamins

The vitamin requirements have not been well defined for patients with ARF, and there is a clear need for more research on this subject. Tentative recommendations for vitamin intake for patients receiving parenteral nutrition are shown in Table 103-7. Much of the recommended intake is based on information obtained from studies in stage 4 and 5 CKD patients, normal individuals, or acutely ill patients. There is a need to provide water-soluble vitamins to patients with ARF because they often eat inadequate quantities, lose vitamins in dialysate, may have increased vitamin needs, and receive medicines that antagonize the actions of many vitamins (see the previous text). CVVH/CVVHD/CVVHDF may increase losses of water soluble vitamins (678). It has been reported that serum concentrations of the fat soluble vitamins A, E, 25-OH vitamin D_3, and 1,25-$(OH)_2$ vitamin D_3 are decreased, parathyroid hormone and vitamin K are elevated, and retinol binding protein are normal in patients with ARF (759).

The quantities needed for the prevention or treatment of deficiencies of these vitamins are not known. Caution must be exhibited with vitamin A therapy because serum vitamin A levels are elevated in CRF, and rather small supplements of vitamin A are reported to cause toxicity in CRF patients (531,534,535,760). Gleghorn et al. reported biochemical and clinical evidence of vitamin A toxicity in three patients with acute or chronic renal failure who were receiving TPN (761). Each individual had been infused with a multivitamin additive that provided 1,500 μg/day of vitamin A (retinol). It is recommended that ARF patients should receive about 2,000 IU/day of vitamin A (i.e., about 600 μg/day of retinol activity equivalents (RAE) which is approximately two-thirds of the AMA-FDA adult formula). Some studies indicate that supplemental vitamin E is beneficial for CRF patients (542). Until more information is available, vitamin E 10 IU/day is recommended for ARF patients (see Table 103-7).

Vitamin E supplements, alone or in combination with selenium or pentoxifylline, have sometimes but not always been reported to protect against or enhance recovery of experimentally induced ARF in rodents, presumably because of the antioxidant properties of this vitamin (762,763). There have been no large-scale clinical trials of vitamin E supplementation in humans with ARF.

These recommendations are a modification of the 1975 recommendations of the Nutrition Advisory Group of the American Medical Association (AMA) for intravenous vitamin formulations for adult patients. These AMA recommendations were approved by the Food and Drug Administration in 1979 (764) and have been referred to as the AMA-FDA adult formula (692).

Vitamin K deficiency may occur in patients without renal failure who are not eating and are receiving antibiotics that may suppress vitamin K producing intestinal bacteria (536). Vitamin K supplements therefore should be given routinely to patients receiving parenteral nutrition (Table 103-7). Ten mg per day of pyridoxine hydrochloride (8.2 mg/day of pyridoxine) is recommended because studies in clinically stable or sick patients undergoing MHD indicate that this quantity may be necessary to prevent or correct vitamin B_6 deficiency (86). Patients should probably not receive more than 60 to 100 mg per day of ascorbic acid because of the risk of increased serum oxalate concentrations (549,550). Indeed, there is one report of a child with ARF from the hemolytic uremic syndrome who received parenteral nutrition providing 500 mg/day of ascorbic acid (765). The patient was found to have deposits of calcium oxalate in the kidneys and pancreas.

Although vitamin D is fat soluble and vitamin stores should not become depleted during the few days to weeks that most patients with ARF receive parenteral nutrition, the turnover of its most active analogue, 1,25-dihydroxycholecalciferol (calcitriol), is much faster (766). Deficiency of 1,25-dihydroxycholecalciferol has been described in the oliguric phase of ARF caused by rhabdomyolysis

(767). Also, in the adult general population, low serum levels of 25-hydroxyvitamin D, the metabolic precursor of calcitriol, are not uncommonly found (768,768a). Hence, there may be a need for calcitriol and/or 25 hydroxyvitamin D in patients with ARF. In MHD patients, intravenous 1,25-dihydroxycholecalciferol and its congeners can markedly suppress parathyroid hormone secretion (769). The finding that in ARF some vitamin D compounds are decreased and hyperparathyroidism may occur and have adverse effects (649,759), suggests a possible therapeutic role for calcitriol or for one of its analogues that has less calcemic effects (770).

It should be emphasized that the nutrient intake of patients with ARF must be carefully reevaluated, often each day and sometimes more frequently. This is particularly important because the clinical and metabolic condition of these patients may undergo rapid changes.

EFFECTS OF METABOLIC INTERVENTIONS TO ENHANCE RECOVERY OF RENAL FUNCTION

Other types of metabolic intervention have shown varying degrees of promise for preventing or ameliorating the severity of ARF or enhancing recovery of renal failure. These interventions include using calcium channel blockers to inhibit calcium flux into the cell, a preterminal event leading to cell death (771). Other studies addressed maintenance of the cellular energy supply. Siegel and co-workers infused magnesium chloride with adenosine triphosphate, diphosphate, or monophosphate into rats with ARF secondary to ischemia (772). The rats that received one of these adenine nucleotides with magnesium chloride had less impairment of insulin clearance, renal blood flow, and osmolar clearance, a lower fractional excretion of sodium, and less histologic evidence of renal injury as compared with rats that either received no infusion or that received adenosine triphosphate, adenosine or magnesium chloride alone. Rats with ARF caused by potassium dichromate injections who received thyroxine showed a greater rise in insulin clearance, a greater fall in fractional excretion of sodium and increase in urine osmolality, and a more rapid recovery of renal function as compared with control animals (773). However, a study in humans with ARF did not confirm that thyroxine enhanced recovery of renal function (774).

Recently growth factors have been employed in experimental models of ARF both to accelerate recovery of renal function and to enhance anabolism. Epidermal growth factor (775), hepatocyte growth factor (776) and insulin-like growth factor 1 (IGF-1) and IGF-1 analogues (777–779) appear to enhance the rate of recovery of renal function in rats with ARF.

Published studies are virtually uniform in describing that in rats with ARF, recombinant human IGF-1 (rhIGF-1) stimulates mitosis of the proximal tubular cells and possibly other cells in the kidney and increases the rate of recovery of both renal blood flow and glomerular filtration rate (777–779). This effect of IGF-1 is observed with ARF caused by ischemic injury (777–779), but not when it is caused by large doses of radiocontrast media (780). Recovery of renal function is enhanced regardless of whether rhIGF-1 is given concurrently with the renal insult or even several hours afterwards (778). Interestingly, rhIGF-1 also stimulates an anabolic response in these rats with ARF (778). Notwithstanding these promising animal studies, a prospective, randomized, double-blind clinical trial indicated that rhIGF-I treatment did not enhance the rate of recovery of renal function, reduce the need for dialysis treatment or improve survival in sick intensive care unit patients with established ARF (781). Another randomized double-blind clinical trial in patients undergoing vascular surgery indicated that there was a slightly lower reduction in creatinine clearance in the individuals randomized to receive preventive treatment with rhIGF-1 (782).

In a preliminary study in patients with ARF, atrial natriuretic peptide was reported to increase the creatinine or inulin clearances and reduce the dialysis requirement (783). Large scale randomized prospective clinical trials have been carried out to test the potential value of atrial natriuretic peptide for the treatment of patients with ARF. The data suggest that atrial natriuretic peptide has little or no effect on reducing the need for dialysis therapy and no effect on morbidity (784).

AMINO ACID AND PROTEIN INTAKE MAY PREDISPOSE TO ACUTE RENAL FAILURE

Several studies in rats suggest that a high amino acid or protein intake may increase the susceptibility to ARF caused by ischemia or nephrotoxicity (785–789). The nutrients seem to increase both the incidence and the severity of ARF induced by these agents. Several individual amino acids, particularly D-serine, DL-ethionine, and L-lysine, appear to be nephrotoxic (787,790). In contrast, glycine and alanine may protect against ischemic- or toxic-induced renal tubular cell damage (789), at least *in vitro* preparations. If amino acids or protein predispose to renal failure in humans, then patients who receive nephrotoxic medicines or who are at high risk for renal ischemia might benefit from low amino acid or protein intakes. On the other hand, if glycine and alanine are protective *in vivo*, it is possible that administration of these amino acids could reduce the likelihood of ARF in high risk patients. Clearly, more research is needed in this area.

SPECIAL TECHNIQUES FOR MANAGING CATABOLIC OR MALNOURISHED PATIENTS WITH ARF OR CRF—AGENTS THAT MAY ENHANCE MORE POSITIVE PROTEIN BALANCE

There have been a number of reports indicating the potential value of using growth factors to improve protein balance and of administering specific nutrients to enhance immunoreactivity and host resistance. In sick patients in the general population and particularly intensive care unit patients, insulin may decrease UNA, improve nitrogen balance, and insulin and/or more intense plasma glucose control may reduce morbidity and mortality (703–706). *In vitro* studies of skeletal muscle from rats with ARF indicate that insulin may increase protein synthesis and reduce protein degradation (69,350). Insulin has many anabolic effects. It promotes protein synthesis; inhibits lipolysis and lowers plasma glucose (790). In the setting of catabolic stress, insulin reduces elevated concentrations of plasma glucose which can be cytotoxic (791). In critically ill individuals insulin also may improve dyslipidemia, promote antiinflammatory effects, protect against dysfunction of the vascular endothelium and hypercoagulability and have antiapoptotic effects on the myocardium (791).

In a prospective randomized trial, 1548 adult surgical intensive care unit patients were randomly assigned to receive intensive insulin infusion therapy that maintained blood glucose between 80 and 110 mg/dL or conventional treatment in which insulin was infused only if the blood glucose exceeded

215 mg/dL and the target maintenance blood glucose was set between 180 and 200 mg/dL (706). Patients assigned to intensive insulin therapy had a significantly lower mortality rate of 4.6% vs. 8.0% with the conventional therapy ($p < 0.04$). The benefit of intensive insulin therapy could be attributed to decreased mortality in those patients who remained in the intensive care unit for more than five days. A retrospective analysis of these data indicated that there was a linear correlation between the blood glucose concentration and the risk of death in these patients (792). Anabolic steroidal compounds, many of which are androgenic and resemble testosterone, have been used in patients with ARF (656,657). These agents can reduce UNA and increase nitrogen balance; they also have been reported to decrease the need for dialysis treatments by lowering or slowing the rate of rise of SUN and presumably thereby decreasing uremic toxicity.

Experience with growth hormone in nonuremic individuals or animal models suggests that it may be useful for promoting more positive protein balance in patients with ARF or CRF and superimposed catabolic stress (707,708). Several studies indicate that recombinant rhIGF-1 and growth hormone (rhGH) may enhance protein retention in malnourished stage 5 CKD patients who are undergoing MHD or CPD (778, 793–797). People who are acutely stressed from infection or physical trauma or who receive low quantities of nutrients sometimes become refractory to rhGH, possibly because of down-regulation of growth hormone receptors with reduced ability to express IGF-I (798). Moreover, in very ill intensive care unit patients the use of rhGH has been associated with increased mortality rates (709). Therefore, for the present, growth hormone should not be given to patients who are very ill. Many studies demonstrate that growth hormone stimulates growth in children with CRF (799,800). Although rhIGF-1 has not been shown to enhance recovery from ARF in humans (781), it stimulates protein anabolism in rats with ARF and malnourished CPD patients (796). Because IGF-I appears to stimulate growth of dedifferentiated cells, neither growth hormone nor rhIGF-I should be given to patients with active malignancy.

Pentoxyphilline is reported to reduce negative protein balance, possibly by suppressing TNFα (801). L-carnitine appears to promote positive protein balance in malnourished CPD patients (802). The mechanism of action of this phenomenon is unknown, but may be related to suppression of catabolic cytokines. Ketoacid analogues of the branched chain amino acids also enhance anabolism in *in vitro* tissue preparations and in clinical studies carried out in nonuremic individuals who were not hypercatabolic (277,736). In postoperative patients without ARF who were receiving TPN, intravenous infusion of the salt complex of α-ketoglutarate and ornithine appears to decrease UNA and improve nitrogen balance (737).

Administration of large quantities of glutamine or arginine to acutely ill humans or animals is reported to improve immune function, reduce risk of infection and, for glutamine, to restore intracellular glutamine pools which fall early in acute stress, improve intestinal mucosal structure and physiology, and reduce the risk of bacterial translocation (803–809). The benefits of this therapy for critically ill patients have not yet been unequivocally demonstrated (810). There are no data as to whether large supplements of glutamine and arginine are beneficial for physically stressed patients with ARF or CRF. However, in rats with ischemic ARF, administration of L-arginine 300 mg/kg for 60 minutes is reported to acutely increase glomerular filtration rate, probably by increasing nitric oxide production (811).

Peptides in TPN solutions has been used to provide unstable or poorly soluble amino acids to the patient (i.e., dipeptides of glutamine, cysteine, and tyrosine (804)). Peptides also provide amino acids at a lower osmolality. Experimental evidence derived largely from animal or cell studies also indicates that omega-3 fatty acids and supplemental nucleotides may improve a number of parameters of immunologic function and host resistance (752–754,812). The omega-3 fatty acids appear to modify immune responses by altering the pattern of cytokines elaborated by inflammatory cells (752–754). Clinical trials with anticytokines or cytokine receptor antibodies have not yet been shown to be valuable for acutely ill patients.

Intravenous Nutrition Limited to Hemodialysis

For patients who have marginally adequate intakes and who will not ingest more nutrients through foods or food supplements, supplemental amino acids, glucose, or lipids may be infused intravenously during hemodialysis treatment (i.e., intradialytic parenteral nutrition [IDPN]) (205,206,813). The preparation is infused into the blood leaving the dialyzer; IDPN avoids the need for catheterization of a central or peripheral vein and allows the physician to remove excess water and minerals as these chemicals are infused. Evidence does not clearly show that IDPN is beneficial or that patients may not receive similar advantages from oral supplements or tube feeding (205,206,813). Regretfully, a large-scale prospective, randomized clinical trial with IDPN has never been performed.

IDPN is reported to be associated with increased protein synthesis and positive energy balance during hemodialysis (814). Improved serum albumin and edema-free body mass have been described in some studies, which were often not well controlled (813). Exercise on a stationary bicycle during hemodialysis in patients receiving IDPN promoted more positive protein balance (815).

A case control retrospective study (206) and a nonrandomized retrospective report (205) provide suggestive data that IDPN may increase survival in malnourished maintenance hemodialysis patients. In the case control study, those patients with a serum albumin of 3.3 gm/dL or lower who were given IDPN had greater survival than those who were not (206). In the retrospective comparison of nonrandomized maintenance hemodialysis patients, those individuals who received IDPN had a reduced mortality rate (205). The French Intradialytic Nutrition Evaluation Study involves at least 390 MHD patients who were randomized to receive either IDPN or no IDPN (816). The results of this prospective clinical trial should be available in the near future.

Some reports concerning indications for IDPN emphasize that IDPN is only indicated for malnourished MHD patients who ingest inadequate nutrients and for whom counseling, food supplements and tube feeding are not helpful or are contraindicated (817). IDPN is probably of value only for clinically stable patients who have a slightly or modestly suboptimal intake of nutrients. This technique is probably inadequate for physically stressed ARF patients because their oral or enteral intake is usually very low, their nutrient needs are high, and the nutritional supplements can be given only intermittently, when the patient undergoes hemodialysis.

Since most patients who need IDPN have decreased intake of energy and total nitrogen, we give 40 to 42 gm of essential and nonessential amino acids and 200 gm of D-glucose (150 gm of D-glucose if the dialysate contains glucose) and usually 250 mL of a 10% or 20% lipid solution (i.e., 25 to 50 gm of fat). The nutrients are administered at a constant rate throughout the dialysis procedure to minimize the decrease in glucose that occurs with the use of glucose-free hemodialysate and the fall in amino acid pools that normally occurs with a hemodialysis treatment. In the author's experience, about

85% to 90% of the amino acids and a large proportion of the glucose infused during hemodialysis are retained; the amino acid losses in dialysate rise by an average of only 4 to 5 gm (108). The nutrients may be utilized more efficiently because they are given continuously rather than as a bolus. Patients who have low serum concentrations of phosphorus or potassium at the start of the dialysis treatment may need supplements of these minerals during the amino acid and glucose infusions. If the dialysate is glucose free, the infusion is not stopped until the end of hemodialysis to prevent reactive hypoglycemia. Also, the patient should eat a source of carbohydrates 20 to 30 minutes before the end of the infusion. Otherwise, the infusion must be tapered or a peripheral infusion of glucose must be started to avoid hypoglycemia.

Nutritional Hemodialysis and Nutritional Peritoneal Dialysis

A number of investigators have examined the possibility of adding amino acids to the dialysate of patients undergoing CPD or maintenance hemodialysis (110,818–820). With hemodialysis, additional glucose also may be added. The nutrients diffuse into the body during dialysis. These techniques have the potential advantages of consolidating nutritional and dialysis treatment into one procedure, reducing the risk of fluid and electrolyte disturbances from intravenous nutrition, and decreasing the considerable costs of intravenous feeding. When the nutrients are added to the hemodialysate, some investigators have reduced the dialysis flow rate to increase the fractional extraction of amino acids and glucose from dialysate (818). This has the benefit of reducing the cost of the nutrients given to the patient, but it also decreases the efficiency of dialysis; hence, these patients may require more hours of dialysis therapy, which raises nursing costs. Also, nutritional hemodialysis would have to be performed daily if it were the only or the main source of nutrition for a patient. This procedure, if it were to have value, would probably be of benefit only for hospitalized patients, and it would have to be designed so that it was performed by intensive care unit personnel rather than by hemodialysis nurses in order to save costs. Under these conditions, it would be a variant of CVVHD.

Chazot et al added amino acids to the dialysate during a standard hemodialysis using a cellulose triacetate hemodialyzer (110). When about 46 gm of a mixture of 20 amino acids were added to the concentrate to provide a final dialysate amino acid concentration similar to fasting plasma levels, the amino acid losses into hemodialysate were prevented. When 139 gm of this amino acid mixture was added to the hemodialysate concentrate, there was a net transfer of about 39 gm of amino acids from the dialysate to the patient during the hemodialysis.

The possibility of adding amino acids to peritoneal dialysate for malnourished patients undergoing CPD has been investigated (819,820). By using amino acids; the glucose load from the dialysate can be reduced. Also, for malnourished patients who are ingesting low protein diets, the addition of amino acids appears to increase nitrogen balance, protein synthesis, and several serum proteins (819,820). In general, a mixture of both essential and nonessential amino acids are provided in about a 1.1% solution of standard peritoneal dialysate except that it is glucose free. One or two peritoneal dialysate exchanges of this solution are substituted each day for the patient's usual exchanges. Dwell times should last about 4 to 6 hours to ensure an uptake of about 80% of the total dialysate amino acid content and to prevent excessive reabsorption of other compounds in dialysate by an excessively long dwell time. It is to be emphasized that the calorie load from these solutions is very small. Hence, it is preferable that these exchanges are given during the major meals of the day. Again, it should be emphasized that dietary counseling and food supplements should be attempted and tube feeding should be considered before turning to these more expensive and incomplete nutritional supplements. Glucose and amino acids can be provided by peritoneal dialysis to children with ARF (821).

DIETARY THERAPY FOR RENAL TRANSPLANTATION

Patients who undergo successful renal transplantation often develop normal or even supranormal appetites; the latter is probably primarily a result of glucocorticoid therapy. Gain in body weight and fat is common. During the first year after transplantation, women may be particularly likely to increase dietary energy and protein intake and gain fat and lean body mass (822). Several other nutritional disorders appear to be related to nutritional problems associated with renal transplantation. These include obesity (823,824), insulin resistance and diabetes mellitus (825,826), impaired growth in children (827), protein wasting (828–833), altered serum lipid and homocysteine concentrations (834–837), and abnormalities in bone, mineral, and vitamin metabolism (478,838–841). Many of these complications are of particular concern because cardiovascular disease is the major cause of morbidity and mortality in renal transplant recipients (842). Also, preexisting obesity can increase the risk of postoperative morbidity and mortality in renal transplant recipients (843).

One of the most important causes of these disorders seems to be the administration of prednisone. Glucocorticoids, particularly in large doses, cause pervasive metabolic effects. These have been well reviewed elsewhere (844,845) and include enhanced gluconeogenesis, degradation of protein and nucleic acids, lipolysis, reduced protein synthesis and glucose uptake in many tissues. Glucocorticoids can increase serum cholesterol, inhibit intestinal calcium absorption (846,847) and reduce serum levels of 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol (847–850). Glucocorticoids also stimulate catabolism or inhibit anabolic processes in many tissues, including bone and connective tissues, and cause negative calcium balance and osteoporosis (851). Glucocorticoids may promote diabetes mellitus both by stimulating appetite and thereby predisposing to obesity and the metabolic syndrome and by directly causing insulin resistance.

Renal transplant patients who receive large doses of prednisone for antirejection therapy sustain negative nitrogen balance (828–833). Superimposed catabolic illnesses or the development of the nephrotic syndrome may also cause protein wasting in transplant patients. In one nonrandomized study, patients with cadaveric renal transplants were given prednisone starting at 120 mg per day and tapering to 70 to 90 mg per day over approximately 10 to 14 days (828). The authors reported that a protein and energy intake of 1.30 ± 0.06 (SEM) gm/kg/day and 33 ± 3 kcal/kg/day, respectively, maintained less negative estimated nitrogen balance (-0.02 ± 0.12 gm nitrogen/kg/day) than an intake of 0.73 ± 0.03 gm protein/kg/day and 20 ± 4 kcal/kg/day (estimated nitrogen balance, -0.72 ± 0.12 gm/kg/day). These investigators could not distinguish between the relative contributions of the higher nitrogen and calorie intakes to the more positive nitrogen balance. Nitrogen balance was estimated from the difference between nitrogen intake, and nitrogen output, calculated from the UNA, which underestimates nitrogen losses.

Whittier and associates reported that 12 nondiabetic postoperative renal transplant recipients were less cushingoid when they were given a high protein, low carbohydrate diet providing

(per kg/day) 2 ± 0.03(SD) gm protein, 1 ± 0.3 gm carbohydrate and 28 ± 2 kcal as compared to a diet providing (per kg/day) 1 gram protein, 3 gm carbohydrate and 31 kcal (830). Nitrogen balance was significantly more positive, and there was a tendency for potassium and sodium balance to be more positive in patients receiving the experimental diet. Chronic low doses of prednisone (i.e., 10 mg/day or lower) do not appear to alter body composition, as determined by anthropometry or dual energy x-ray absorptiometry, or resting energy expenditure (831).

Renal transplant patients often have increased serum triglyceride, VLDL triglyceride, and small dense and total LDL cholesterol concentrations. HDL cholesterol is often low, and the LDL/HDL cholesterol ratio may be increased (835,835,852). Several patterns of hyperlipoproteinemia may be present (853). Serum triglyceride levels correlate with the daily dose of prednisone, degree of obesity, and severity of renal insufficiency. These findings are of particular concern because several studies have found a correlation between increased serum lipids and the risk of cardiovascular disease, graft failure and fatality in renal transplant recipients (835,854–856). The causes of increased serum triglycerides and cholesterol include excessive fat and energy intake, obesity, glucocorticoids, diuretics, cyclosporine, tacrolimus, sirolimus, nephrotic range proteinuria and underlying diseases (e.g., diabetes mellitus) (857,858). In most but not all studies, dietary counseling of renal transplant recipients can reduce their energy intake, weight gain (859,860), and serum total cholesterol, LDL cholesterol and triglycerides, but without change in their serum HDL cholesterol concentrations (834,858–864).

A low cholesterol, high fiber diet with a polyunsaturated: saturated fatty acid ratio greater than 1.0 can lower serum total cholesterol and LDL cholesterol levels in renal transplant recipients (834,860,861,863). However, the altered lipoprotein pattern may not be affected. A combination of a similar diet with regular exercise may improve the plasma lipid pattern (865). Fish oil providing 3 gm per day of omega-3 fatty acids for three months decreased serum triglycerides and VLDL cholesterol in hyperlipidemic renal transplant recipients; however, there was no change in their serum total cholesterol or LDL cholesterol (866). However, the effects of diet on the improvement in the serum lipid pattern tend to be modest (837), and combining dietary therapy with serum HMG CoA reductase inhibitors is generally far more effective for reducing serum total and LDL cholesterol (858,867).

Plasma homocysteine concentrations are also elevated in renal transplant recipients. Some but not all evidence indicates that hyperhomocystenemia is a risk factor for cardiovascular complications and mortality in these patients (836,868–870). In maintenance dialysis patients who have had a successful kidney transplant, their plasma homocysteine decreases but remains greater than in nontransplanted individuals with similar levels of renal function (871). The causes of hyperhomocysteinemia include reduced glomerular filtration rate, which appears to be the most important determinant (872), low serum folate levels (873), and possibly cyclosporine A therapy. Reports differ as to whether cyclosporine A contributes to hyperhomocysteinemia (874–876). In one study in normal individuals, large doses of coffee, (e.g., about one L of paper-filtered coffee per day) increased plasma total homocysteine by 1.5 μmol/L or 18% (877). Folic acid decreases plasma homocysteine levels in renal transplant recipients, but not to normal (876). It has been suggested that the decrease in plasma homocysteine is greater when pyridoxine HCl, 50 mg/day, and vitamin B_{12}, 0.4 mg/day, are added to folic acid, 5 mg/day (878).

Serum folate levels are reported to be reduced in transplant patients but to increase slightly with time after transplantation (836). However, low serum folate levels were observed as long as 6 years after transplantation. Macrocytosis was observed in 52% of patients; it was only observed in patients with good renal graft function who were treated with azothioprine. The authors suggested that azathioprine rather than folate deficiency caused the macrocytosis. Serum thiamine and vitamin B_{12} levels are generally normal in renal transplant patients (879,880). After successful renal transplantation, serum vitamin A often remains elevated for extended periods of time and may not fall to normal levels in some patients for almost 2 years (881).

Mahajan and co-workers reported low plasma and hair zinc and hyperzincuria in 15 patients who had received a renal transplant within 12 months (478). These patients also had elevated taste detection and recognition thresholds. On the other hand, patients who had a functioning renal transplant more than 12 months after transplantation had normal plasma, hair, and urine zinc and taste detection and recognition thresholds. Patients more than 12 months posttransplantation who had advanced renal failure had low plasma and hair zinc and high taste detection and recognition thresholds.

Patients undergoing renal transplantation often have renal osteodystrophy with reduced bone mass. Bone mass may be further diminished after renal transplantation because of continuing suppression of bone formation, hyperparathyroidism and glucocorticoid induced bone wasting (851), inhibition of intestinal calcium absorption (846,847), and lowering of serum vitamin D levels (846,850). Calcium and vitamin D supplements may suppress serum parathyroid hormone and increase serum 1,25-dihydroxyvitamin D levels (846). Other immunosuppressive medicines may also affect the nutritional status of a renal transplant recipient. Cyclosporine A may reduce the glucocorticoid requirement. However, cyclosporine A itself may increase serum LDL-cholesterol and triglycerides (857), promote potassium retention with hyperkalemia (882), cause urinary magnesium wasting with hypomagnesemia (883), promote early satiety during eating. Tacrolimus and sirolimus also increase serum LDL-cholesterol, and cyclosporine and tacrolimus can cause glucose intolerance.

Recommended Nutrient Intake for Renal Transplant Recipients

Because there is little information concerning the nutritional requirements for renal transplant patients, the following recommendations should be considered tentative. Immediately after renal transplant surgery, during periods of catabolic stress or high prednisone dosage (e.g., 30 mg/day or greater), patients should probably receive about 1.3 to 1.5 gm protein/kg/day. If patients are receiving CVVH/CVVHD/CVVHDF or SLED or if they have normal or near normal renal function, protein intake may be increased to 2.0 gm/kg/day if the patient's UNA is very high. When the daily dose of prednisone is reduced below 30 mg per day, protein intake can be decreased.

The long-term management of protein intake should probably be directed toward preventing the progression of renal failure as well as maintaining good nutritional status. Some studies, not definitive, suggest that low protein diets may retard the progression of renal failure in renal transplant recipients (884). As indicated earlier, a retrospective analysis of renal transplant recipients indicated that those transplant recipients who spontaneously ate lower protein diets had lower losses of GFR (291). Also, it is not clear whether in renal transplant recipients who are receiving ACE inhibitors or ARBs, low protein diets will further retard the rate of progression of renal failure.

One approach is to discuss with the patient the lack of definitive information concerning the effect of protein restriction on progression of renal failure of the transplanted kidney. The apparent safety of dietary protein restriction may be described. If the patient agrees to a low protein diet, the following

treatment may be employed. No protein restriction if the GFR is 60 mL/minute or greater unless the patient demonstrates progressive loss of GFR. For patients with a GFR below about 60 mL/minute/1.73 m^2, who are on low doses of glucocorticoids (e.g., ≤15 mg prednisone/day), a protein intake of about 0.60 to 0.80 gm/kg/day may be prescribed. If higher glucocorticoid doses are given chronically, a greater protein intake is probably necessary (e.g., 0.80 to 1.2 gm/kg/day, depending on the glucocorticoid dose and the GFR). At least 0.35 gm/kg/day of the protein should be of high biologic value. Protein intake may be transiently adjusted upwards for acute increases in prednisone dosage or for large urinary protein losses as with nephrotic patients (see the previous text). Fish oil supplements are also reported to retard the progression of renal failure in renal transplant recipients (353).

The observation that a diet low in carbohydrate and modestly restricted in calories may reduce the cushingoid appearance suggests that such individuals may be given a low carbohydrate intake (1 gm/kg/day), which is limited to 28 to 30 kcal/kg/day (830). If such a low carbohydrate, moderately restricted energy intake is employed in renal transplant recipients, it should be limited to short periods of time when the prednisone dosage is very high (e.g., greater than 40 mg/day). This level of energy intake may not minimize the catabolic response during acute illness (see the previous text) and may lead to further wasting. However, the higher protein intake with such diets (e.g., 2 gm protein/kg/day) may reduce protein malnutrition. Also, given the abnormalities in lipid metabolism in renal transplant recipients, such a high-fat diet should not be continued for long periods of time. In general, renal transplant patients should be encouraged to ingest a National Cholesterol Education Program Therapeutic Lifestyle Changes (TLC) diet as described earlier for the nondialyzed patient with renal failure and the maintenance dialysis patient (346). Patients should be encouraged to exercise regularly and to maintain a normal (562,563) or desirable body weight (885). For transplant recipients with superimposed catabolic illnesses, 30 to 40 kcal/kg/day may be prescribed. Other maneuvers to correct abnormal serum lipids are as described for the nontransplant renal failure patient (see the previous text). For renal transplant recipients with serum LDL-cholesterol levels above about 100 mg/dL, the TLC diet should be offered. If dietary therapy does not reduce the serum LDL cholesterol to below this value or to 70 mg/dL or lower for high risk patients, HMG CoA reductase inhibitors should be considered (858).

After kidney transplantation, serum concentrations of calcium, phosphorus, total CO_2 and plasma intact PTH should be monitored as indicated in the National Kidney Foundation DOQI Clinical Practice Guidelines for Bone Metabolism and Disease in CKD (427). Bone mineral density should be obtained periodically (427).

Calcium intake should probably be maintained at about 1,400 to 1,600 mg per day to reduce bone wasting. Because high phosphorus intakes may promote hyperparathyroidism and possibly enhance the rate of progression of renal failure in experimental animals and possibly in humans, dietary phosphorus should be restricted proportionately to the reduction in protein intake, as described previously in this chapter. Hyperphosphatemia should be avoided. Treatment of abnormalities in bone and divalent ion metabolism will be influenced by the stage of CKD of the patient and the principles indicated in the previously mentioned guidelines (427). Serum bicarbonate should be maintained at or above 24 mEq/L.

The regulation of other macrominerals in the diet, except for the usual health enhancing guidelines, is usually unnecessary unless the patient has renal insufficiency, other diseases associated with sodium retention (e.g., congestive heart failure or liver failure), or hypertension. The data of Mahajan and coworkers suggest that patients with a well functioning kidney transplant might benefit from a small zinc supplement, possibly 15 mg per day of elemental zinc, for the first 12 months after the transplant (478).

Patients should receive a daily vitamin supplement containing the recommended dietary intake of water-soluble vitamins for patients with chronic renal insufficiency (see Table 103-5). Hyperhomocysteinemia may be treated with folic acid, probably 5 mg/day. It is not clear whether lowering serum homocysteine is beneficial for renal transplant recipients. It is not yet demonstrated whether large doses of pyridoxine HCl and vitamin B_{12} should be routinely given as well (878). Whether 1,25-dihydroxycholecalciferol or another vitamin D analogue should be routinely given to all renal transplant recipients is not yet established.

Diet-Drug Interactions

Grapefruit juice appears to inhibit activity of intestinal isoenzyme CYP3A4 and the carrier protein P-glycoprotein (885,886). This may lead to increased blood levels of orally ingested substrates including cyclosporine A, tacrolimus and certain calcium channel blockers. Orange juice might also affect drug levels by inhibiting P-glycoprotein (887). St. John's Wort may induce intestinal and hepatic CYP3A4 activities and intestinal P-glycoprotein (888). These actions of St. John's Wort might lower cyclosporin A levels and induce acute rejection of the renal transplant (889,890).

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CHAPTER 104 ■ USE OF DRUGS IN PATIENTS WITH RENAL FAILURE

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Renal impairment is associated with a great magnitude of morbidity and mortality in the United States. The recent data indicate that approximately 10 million Americans have renal insufficiency including 350,000 patients with end stage renal disease whom require scheduled dialysis several times per week. Infections, cardiovascular complications, and adverse drug reactions are the most common cause of mortality in patients with renal impairment despite the advances in the field of dialysis and management of comorbid conditions in these patients (1,2). A number of studies have documented the role of medication dosing errors in the overall increase in mortality of patients with renal failure. Although a number of algorithms and drug dosing recommendations have been proposed over the last two decades most are not up-to-date, not adequately studied, and have not kept pace with new advances in the field of dialysis (3). Acute or chronic renal insufficiency alters the pharmacokinetic and pharmacodynamic properties of most commonly used drugs significantly. Kidneys play an important role in the excretion of active drug and their pharmacologically active metabolites. Drug accumulation and adverse drug reactions can develop rapidly if drug dosages are not adjusted according to impaired renal function. Most drugs should be adjusted as renal function improves to ensure efficacy and dosage should be reduced if renal function continues to deteriorate. Even in drugs that are mostly metabolized through the liver, patients with renal failure are at greater risk of adverse drug reactions and toxicity. Drug interactions are also a common problem in this population since most patients with renal insufficiency often have serious comorbid conditions requiring pharmacologic intervention (4–16). In addition, a large part of the difficulty in prescribing drugs for the rapidly growing numbers of older patients is due to age-related declines in renal function (17). Finally, renal replacement therapies including hemodialysis are considered the treatment of choice in patients with end stage renal disease. The effects of dialysis on drug elimination and the need for supplemental dosing must also be considered in patients receiving renal replacement therapy (18–24).

In this chapter, the basic principles of pharmacokinetic modeling and drug dosing in patients with renal impairment are reviewed. The changes in drug pharmacokinetics and pharmacodynamics are highlighted, and practical guidelines for drug dosing in these patients are provided. However, dialysis patients also face the risk of drug-drug and drug-disease interactions, thus, no specific dosing guideline can be given confidently because individual patient factors such as age, gender, nutrition, body fluid volume, and disease states may influence pharmacokinetic and pharmacodynamic parameters significantly. To provide safe and effective pharmacotherapy, the clinician must utilize clinical judgments, knowledge of altered pharmacokinetic properties and the patient's specific physiologic status to administer drugs to a renal patient population. In order to optimize pharmacotherapy and avoid over and under medication, these factors should be taken into account and appropriate dosage adjustment should be considered.

PHARMACOKINETIC PRINCIPLES

The term *pharmacokinetics* refers to a mathematic model of the time course of drug concentration in the body. Pharmacokinetic properties of a drug define or predict plasma concentrations and therefore, drug activity or toxicity. Pharmacokinetics is the study of drug absorption, distribution, metabolism and elimination. Pharmacokinetics can be thought of as the body's effect on the drug over time. A simplified scheme of drug pharmacokinetics is illustrated in Figure 104-1. The pharmacologic effect of any drug depends on the concentration of the unbound active drug or an active metabolite at the receptor site of action. The blood and tissue levels of a drug are functions of the administered dose, rate of its absorption, concentration, rate of metabolism or biotransformation, and rate of elimination (5–9).

Drug Absorption

Following extravascular administration, drugs must be transported through a number of physiological barriers before reaching the systemic circulation. Drug absorption and bioavailability relate to the amount of drug that reaches the systemic circulation after oral administration. The fraction or percent of administrated drug that reaches systemic circulation is known as bioavailability (F). These parameters are often highly specific for a given compound and vary with the physical and chemical properties of the drug, its formulation, the integrity of the absorptive surface, and the presence of other agents and/or food in the gastrointestinal tract. Absorption rates of most therapeutic agents are slow and unpredictable. Uremia-induced vomiting or sluggish peristalsis secondary to enteropathy may further reduce the onset of action of most agents. In patients with diabetes mellitus, the drug absorption is more variable due to autonomic neuropathy. Both calcium- and aluminum-containing phosphate binders may form insoluble complexes with certain drugs such as antibiotics or ferrous sulfate, thereby impeding absorption. Acidic drugs prefer an acidic environment for optimal absorption while, weak basic drugs are better absorbed in a more alkalinized small intestine. Use of proton pump inhibitors or phosphate binders presumably reduces the rate of absorption of a number of acidic agents (10–14). The gastrointestinal tract edema in patients with hypoalbuminemia may also diminish drug absorption. Propranolol, morphine, verapamil are examples of drugs that undergo first pass metabolism (25,26). In first pass metabolism, a significant amount of the absorbed drug molecules are delivered to the liver via the portal vein. A drug is said to undergo significant first pass metabolism when it is metabolized in the liver so extensively upon absorption that no significant concentrations ever reach the systemic circulation (27,28). In addition many drugs may be metabolized via the cytochrome

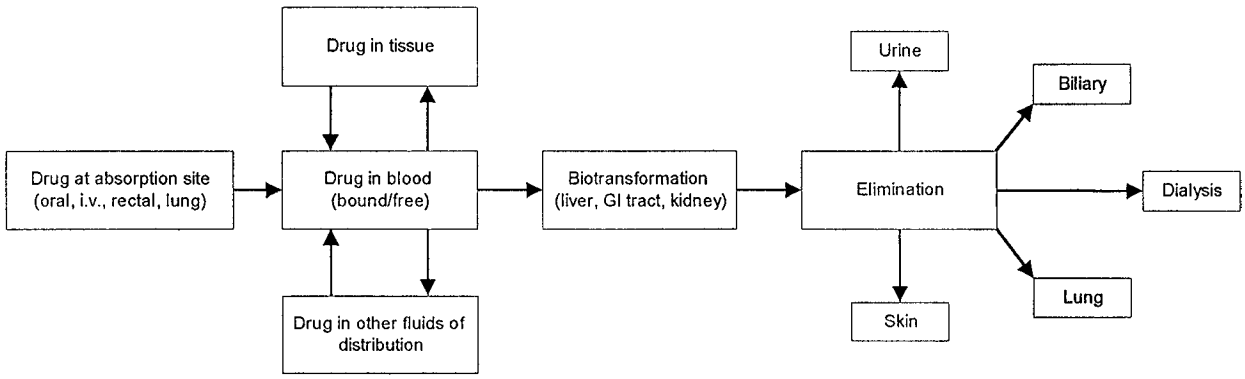

FIGURE 104-1. Pharmacokinetic factors involved in drug distribution.

P-450 system in the gastrointestinal tract before reaching systemic circulation. For example, it is well established that rifampin decreases and erythromycin increases the bioavailability of cyclosporine and calcium channel blockers by induction and inhibition of intestinal and hepatic cytochrome P-450 enzymes, respectively. Finally, patients with renal failure have a higher salivary urea concentration that increases the gastric ammonia levels and increases overall gastric pH. Drugs like iron and ketoconazole whose absorption is dependent on an acidic environment, may have reduced bioavailability in renal failure (26,29).

Volume of Distribution

The volume of distribution (Vd) for a specific drug is derived by dividing the fractional absorption of a dose by the plasma concentration.

$$\text{Volume of distribution (Vd)} = \frac{\text{Amount of drug in body}}{\text{Concentration of drug in plasma or blood (C)}} \quad [1]$$

It is important to emphasize that Vd does not signify the total body fluid. Rather, it is an apparent volume needed for equal distribution of drug throughout the body compartment. For example, the plasma volume of a normal 70 kg man is approximately 3 to 3.5 L, whereas the Vd of 0.25 mg of digoxin to obtain a 0.7 ng/dL plasma level is 350 L, which is 10 times greater than the plasma volume. Therefore, Vd does not refer to a specific anatomic compartment per se. Instead, it is the volume of fluid in which the drug would need to be dissolved to give the observed plasma concentration (30–32). A drug distributes in the body in a characteristic manner based on physiochemical properties of the drug and individual patient variables. Volume of distribution is used mathematically to determine the dose of a drug necessary to achieve a desired plasma concentration. While the Vd is relatively constant for a given drug, many factors such as obesity, extracellular fluid volume status, age, gender, thyroid function, renal function, and cardiac output influence drug distribution. Volume of distribution echoes the water solubility and protein and tissue-binding characteristics of an individual agent. Drugs with a small Vd (Vd less than ~0.7 L/kg) are usually considered more water soluble. Highly lipid-soluble drugs have a large Vd with little retention of drug in the plasma because the drug tends to stay in the lipophilic tissue compartment. Drugs that are highly tissue-bound, such as digoxin, will also have a large Vd. If tissue binding of drugs is decreased by azotemia, a decrease in Vd results. Digoxin is highly bound to cardiac and other tissue Na^+–K^+–ATPase transporters, accounting for its large Vd of 300 to 500 L and very low plasma concentrations. Waste products that accumulate in the azotemic patient serve to displace digoxin from its tissue-binding sites and thus reduce its Vd. Further, such waste products cross-react with the antidigoxin antibody used in drug monitoring assays, producing "therapeutic" digoxin levels in patients not even taking the drug. Insulin and methotrexate similarly have diminished Vd in the uremic state. As a general rule, plasma concentrations of a drug correlate inversely with its Vd (17,26,33).

Protein Binding

The third important pharmacokinetic concept is protein binding. Only unbound drug or unbound active drug metabolite are able to exert any pharmacologic effects. Disease states that effect total body proteins may significantly alter free drug concentration and increase the risk of drug toxicity. Quantity (binding site) and quality (affinity) of protein binding are substantially altered in patients with renal failure (34–36). Specifically, uremic toxins may decrease the affinity of albumin for a variety of drugs. Organic acids that accumulate in renal failure compete with acidic drugs for protein binding sites. This results in a larger fraction of acidic compounds existing in the unbound or active state. Conversely, basic drugs bind more readily to nonalbumin serum proteins such as α_1-acid glycoprotein and may demonstrate increased protein binding because this acute phase reactant is often elevated in patients with acute disease states including renal impairment. Malnutrition and proteinuria lower serum protein levels, which may increase the free fraction of a compound as well. Alterations in a drug's protein binding and subsequent effects on drug disposition may be difficult to predict. Drugs that are highly protein bounded (>80%) are not removed very effectively during dialysis. In general, drugs that are highly protein bound are largely confined to the vascular space and thus have a Vd of 0.2 L per kg or less. Generally, the Vd for a given agent increases as its protein binding decreases and diminishes as its protein-bound fraction increases (34–36).

Drug Metabolism or Biotransformation

The total body clearance of a drug is equal to the sum of renal clearance plus nonrenal clearance. Obviously, in patients with renal insufficiency, the contribution of renal clearance to total body clearance will be reduced. Nonrenal clearance, however, may be compensatorily increased, decreased, or unchanged in such patients. Specifically, hepatic pathways of drug metabolism or biotransformation including acetylation, oxidation, reduction, and hydrolysis may be slowed or accelerated

depending on the drug under consideration (37,38). Sulfisoxazole acetylation, propranolol oxidation, hydrocortisone reduction, and cephalosporin hydrolysis are all slowed in uremic patients. Most drugs undergo biotransformation to more polar but less pharmacologically active compounds that require intact renal function for elimination from the body (39–41). Active or toxic metabolites of parent compounds may accumulate in patients with renal failure. The antiarrhythmic agent procainamide is metabolized to *N*-acetylprocainamide, which is excreted by the kidney. Thus, the antiarrhythmic properties and toxicity of procainamide and its active metabolite are additive, particularly in patients with renal failure. Meperidine, a commonly used narcotic, is biotransformed to normeperidine, which undergoes renal excretion. Although normeperidine has little narcotic effect, it lowers the seizure threshold as it accumulates in uremic patients (42,43).

Renal Elimination

The most important route of drug elimination is the kidney. Specific processes involved in the renal handling and elimination of drugs include glomerular filtration, tubular secretion and reabsorption, and renal epithelial cell metabolism (44). All of these functions can be directly or indirectly influenced by renal impairment. Since plasma proteins are too large to pass through a normal glomerulus, only unbound compounds will be freely filtered across this barrier. When proteinuria exists, protein-bound molecules may move into the tubular fluid and be eliminated from the circulation. Changes in renal blood flow may affect both drug reabsorption and secretion. Drugs that are highly protein bound can be eliminated without exerting any pharmacologic effects. For example, binding of furosemide to intraluminal albumin in nephrotic states may contribute to the diuretic resistance characteristic of such conditions. When renal disease reduces nephron numbers, the kidney's ability to eliminate drugs declines in proportion to the decline in glomerular filtration rate (GFR). As patients progress toward dialysis dependency, drugs usually filtered and excreted begin to accumulate, leading to a high prevalence of adverse reactions unless dosage adjustments are instituted (45–48).

Drugs that are extensively bound to protein either have a low clearance or enter the filtrate by tubular secretion. Tubular handling of a drug is an energy-requiring, active transport process and involves two separate and distinct pathways in the proximal tubule that are used for the secretion and reabsorption of organic acids and bases (49). These processes are dependent on renal blood flow but not GFR. Accumulation of organic acids in the setting of renal failure competes with acidic drugs for tubular transport and secretion into the urinary space. This, in turn, may lead to drug accumulation and adverse reactions as serum concentrations of agents such as methotrexate, sulfonylureas, penicillins, and cephalosporins rise. Diuretics gain access to their intraluminal sites of action via organic acid secretory pumps. Competition for these secretory pathways by accumulated uremic wastes results in diuretic resistance and necessitates increased diuretic doses to elicit the desired natriuretic effect.

Drug metabolism occurs in the kidney due to a high parenchymal concentration of cytochrome P-450 enzymes. Endogenous Vitamin D metabolism and insulin catabolism are examples of processes that decline as renal failure progresses (32,36–38).

First-order pharmacokinetics describes the manner in which most drugs and their metabolites are eliminated from the body. Specifically, the amount of drug eliminated over time is a fixed proportion of the body stores. The half-life ($t_{1/2}$) of a given agent is most commonly used to express its elimination rate from the body and equals the time required for the drug's plasma concentration to fall by 50%. Half-life can be expressed mathematically as follows:

$$t_{1/2} = \frac{0.693}{\mathrm{Kr} + \mathrm{Knr}} \qquad [2]$$

where *Kr* represents the renal elimination rate constant and *Knr* represents the nonrenal elimination rate constant. As renal elimination declines with renal function, $t_{1/2}$ is prolonged.

DOSAGE ADJUSTMENT FOR THE PATIENT WITH RENAL FAILURE

The following outline provides a stepwise approach to prescribing drug therapy for patients with renal failure. Again, it must be emphasized that these steps simply provide a framework for dosage adjustments in patients with renal impairment and must be modified on a case-by-case basis.

Initial Assessment

A history and physical examination constitute the first step in assessing dosimetry in any patient but particularly in those with renal impairment. Renal dysfunction should be defined as acute or chronic and the cause ascertained if possible. In addition, a history of previous drug intolerance or toxicity should be determined. The patient's current medication list must be reviewed, including both prescription as well as nonprescription and herbal formulations to identify potential drug interactions and nephrotoxins. Calculation of ideal body weight will be based on physical examination findings. For men, the ideal body weight is 50 kg plus 2.3 kg for each 2.54 cm (1 inch) over 152 cm (5 feet). For women, the formula is 45.5 kg plus 2.3 kg per 2.54 cm over 152 cm. An assessment of extracellular fluid volume is also key because significant shifts can affect the Vd of many pharmacologic agents. The presence of hepatic dysfunction may also require additional dosage adjustments.

Calculating Creatinine Clearance

The rate of drug excretion by the kidney is proportional to the GFR. Serum creatinine per se is a crude marker of renal function. Though an overestimate of GFR, creatinine clearance (Ccr) more accurately approximates the GFR and can be estimated conveniently by the Cockcroft and Gault equation:

$$\mathrm{Ccr} = \frac{(140 - \mathrm{age})\,(\text{ideal body weight in kg})}{72 \times \text{serum creatinine in mg/dL}} \qquad [3]$$

For women, the calculated value is multiplied by 0.85. The use of this formula implies that the patient is in a steady-state with respect to serum creatinine. There is no accurate method to quantitate GFR when renal function is rapidly changing, and as such it is best to assume a GFR value of less than 10 mL/minute in acute renal failure to avoid drug accumulation and toxicity (50).

Choosing a Loading Dose

Loading doses are intended to achieve a therapeutic steady-state drug level within a short period of time. As such, the loading dose generally is not reduced in the setting of renal failure. Loading doses can be calculated if the Vd and desired peak level are known, as will be discussed. If extracellular volume depletion exists, the Vd may be reduced for certain pharmacologic agents, and slight reductions in the loading dose would

TABLE 104-1

THERAPEUTIC DRUG MONITORING

	Drug name	Therapeutic range	When to draw sample	When to check levels	Time to reach steady state	Other Considerations
Antibiotics	Aminoglycosides (conventional dosing) Gentamicin Tobramycin Amikacin	Gentamicin and Tobramycin: Peak: 5 to 8 mg/L Trough: <2mg/L (for moderate to severe non-UTI infections, or CF, peaks should be between 10 to 12) Amikacin: Peak: 20 to 30 mg/L Trough: <10 mg/L	Trough: Immediately prior to dose Peak: 30 minutes after a 30 minute infusion or 30 minutes after a 60 minute infusion CF Patients: 15 minutes after 30 minute infusion	With 3rd dose for initial therapy or after dose change. For therapy less than 72 hours, levels not necessary. Repeat drug levels if renal function changes.	Steady state is reached in 4 to 5 elimination half-lives; generally by the third dose.	Special considerations for renal patients and dialysis: 2 mg/kg then 1 mg/kg, redose if level less than 2 mg/L.
	Aminoglycosides (24 hour dosing) Gentamicin Tobramycin Amikacin	0.5 to 3 mg/L	Obtain random drug level 12 hours after dose	After initial dose. Repeat drug level in 1 week or if renal function changes.	Steady state is not reached in most cases due to insufficient drug accumulation between dosing intervals.	24 hour dosing not to be used for patients with burns, CrCl <20, cystic fibrosis, dialysis, endocarditis, neutropenia, pediatrics, pregnancy.
	Vancomycin	Peak: 20 to 50 mg/L Trough: 5 to 20 mg/L	Trough: Immediately prior to dose Peak: 60 minutes after a 60 minute infusion	With 3rd dose for initial therapy or after dose change. For therapy less than 72 hours, levels not necessary. Repeat drug levels if renal function changes.	Steady state is reached in 4 to 5 elimination half-lives; generally by the third dose.	Special considerations for renal patients—goal trough <15 mg/L.
Anticonvulsants	Carbamazepine	4 to 12 mcg/mL	Trough: Immediately prior to dosing	2 to 4 days after first dose or change in dose.	2 to 5 days	Monitor baseline LFT's, then 2 to 3 months after starting drug.
	Phenobarbital	15 to 40 mcg/mL	Trough: Immediately prior to next dose	2 weeks after first dose or change in dose. Follow-up level in 1 to 2 months.	14 to 21 days	
	Phenytoin Free phenytoin	10 to 20 mcg/mL 1 to 2 mcg/mL	Trough: Immediately prior to dosing	5 to 7 days after first dose or change in dose.	10 to 14 days	Adjustments necessary for albumin <3.5 g/dL or patients with renal impairment. For acutely ill patients, earlier levels should be considered.
	Valproic acid (includes divalproex sodium)	50 to 100 mcg/mL	Trough: Immediately prior to next dose	2 to 4 days after first dose or change in dose.	2 to 4 days	Monitor baseline LFT's, then 2 to 3 months after starting drug.

Cardiac	Digoxin	0.8 to 2.0 ng/mL	At least 8 hours after maintenance dose	5 to 7 days after first dose for patients with normal renal and hepatic function; 3 to 5 days in anephric patients to assess trend.	5 to 7 days	Levels should be drawn only if therapy is expected to last >72 hours and if there is a definite clinical indication. For acutely ill patients, earlier levels should be considered. After loading dose, levels may be checked earlier to assess trend.
	Lidocaine	1 to 5 mcg/mL	8 hours after IV infusion started or changed	After 24 hours from starting of infusion, especially if rate >2 mg/minute	6 to 12 hours	Causes seizures with toxic levels.
	Procainamide NAPA (n-acetyl procainamide) a procainamide metabolite	4 to 10 mcg/mL Peak: 8 mcg/mL Trough: 4 mcg/mL 10 to 20 mcg/mL Total: 5 to 30 mcg/mL	Trough: Immediately prior to next dose or 12 to 18 hours after starting or changing an infusion Draw with procainamide sample	In 3 to 7 days after initiating therapy in stable patient.	12 to 24 hours after first dose or change in dose 24 to 48 hours after first dose or change in dose	For acutely ill hospitalized patients with ventricular arrythmias earlier levels should be considered. Accumulates in renal failure.
Immunosuppressants	Cyclosporine	150 to 400 ng/mL	Trough: Immediately prior to next dose	Every 1 to 2 weeks	76 to 95 hours	May check levels more often in unstable patient. Therapeutic range may vary with type of transplant and elapsed time from date of transplant.
	Tacrolimus (FK-506)	5 to 15 mcg/mL	Trough: Immediately prior to next dose	Every 1 to 2 weeks	36 to 72 hours	May check levels more often in unstable patient. Therapeutic range may vary with type of transplant and elapsed time from date of transplant.
Other	Lithium	Acute: 0.8 to 1.5 mEq/L Chronic: 0.5 to 0.8 mEq/L	Trough: before AM dose at least 12 hours since last dose	4 days after first dose or change in dose	96 to 144 hours	
	Aminophylline IV or Theophylline PO	10 to 20 mcg/mL	Trough: Immediately prior to next dose	1 to 2 days after first dose and as patient condition warrants	32 to 40 hours	Aminophylline levels reported as theophylline.

TABLE 104-2

ANTIMICROBAL AGENTS IN RENAL FAILURE

Drugs	Normal dosage	% of renal excretion	Dosage adjustment in renal failure			Comments	HD	CAPD	CVVH
			GFR >50	GFR 10 to 50	GFR <10				
Aminoglycoside Antibiotics (63,84)						Nephrotoxic, ototoxic. Toxicity worse when hyperbilirubinemic. Measure serum levels for efficacy and toxicity. Peritoneal absorption increases with presence of inflammation. Vd increases with edema, obesity, and ascites.			
Streptomycin (63,84)	7.5 mg/kg q12h (1.0 g q24h for tuberculosis)	60%	q24h	q24 to 72h	q72 to 96h	For the treatment of TB. May be less nephrotoxic than other members of class.	1/2 normal dose after dialysis	20 to 40 mg/L/d	Dose for GFR 10 to 50 and measure levels
Kanamycin (85)	7.5 mg/kg q8h	50% to 90%	60% to 90% q12h or 100% q12 to 24h	30% to 70% q12 to 18h or 100% q24 to 48h	20% to 30% q24 to 48h or 100% q48 to 72h	Do not use once-daily dosing in patients with creatinine clearance less than 30 to 40 mL/minutes or in patients with acute renal failure or uncertain level of kidney function.	1/2 full dose after dialysis	15 to 20 mg/L/d	Dose for GFR 10 to 50 and measure levels
Gentamicin (86,87)	1.7 mg/kg q8h	95%	60% to 90% q8 to 12h or 100% q12 to 24h	30% to 70% q12h or 100% q24 to 48h	20% to 30% q24 to 48h or 100% q48 to 72h		1/2 full dose after dialysis	3 to 4 mg/L/d	Dose for GFR 10 to 50 and measure levels
Tobramicin (88,89)	1.7 mg/kg q8h	95%	60% to 90% q8 to 12h or 100% q12 to 24h	30% to 70% q12h or 100% q24 to 48h	20% to 30% q24 to 48h or 100% q48 to 72h		1/2 full dose after dialysis	3 to 4 mg/L/d	Dose for GFR 10 to 50 and measure levels
Netilmicin (89)	2 mg/kg q8h	95%	50% to 90% q8 to 12h or 100% q12 to 24h	20% to 60% q12h or 100% q24 to 48h	10% to 20% q24 to 48h or 100% q48 to 72	May be less ototoxic than other members of class. Peak 6 to 8 Trough <2	1/2 full dose after dialysis	3 to 4 mg/L/d	Dose for GFR 10 to 50 and measure levels
Amikacin (90,91)	7.5 mg/kg q12h	95%	60% to 90% q12h or 100% q12 to 24h	30% to 70% q12 to 18h or 100% q24 to 48h	20% to 30% q24 to 48h or 100% q48 to 72h	Monitor levels. Peak 20 to 30 Trough <5	1/2 full does after dialysis	15 to 20 mg/L/d	Dose for GFR 10 to 50 and measure levels

Cephalosporin						Coagulation abnormalities, transitory elevation of BUN, rash and serum sickness-like syndrome.			
Oral Cephalosporin (92)									
Cefaclor (93)	250 to 500 mg tid	70%	100%	100%	50%		250 mg bid after dialysis	250 mg q8 to 12h	N/A
Cefadroxil (94)	500 to 1 g bid	80%	100%	100%	50%		0.5 to 1.0 g after dialysis	0.5 g/d	N/A
Cefixime (95)	200 to 400 mg q12h	85%	100%	100%	50%		300 mg after dialysis	200 mg/d	Not recommended
Cefpodoxime (92)	200 mg q12h	30%	100%	100%	100%		200 mg after dialysis	Dose for GFR <10	N/A
Ceftibuten (92)	400 mg q24h	70%	100%	100%	50%		300 mg after dialysis	No data: Dose for GFR <10	Dose for GFR 10 to 50
Cefuroxime axetil (92)	250 to 500 mg tid	90%	100%	100%	100%	Malabsorbed in presence of H2 blockers. Absorbed better with food.	Dose after dialysis	Dose for GFR<10	N/A
Cephalexin (96)	250 to 500 mg tid	95%	100%	100%	100%	Rare allergic interstitial nephritis. Absorbed well when given intraperitoneally. May cause bleeding from impaired prothrombin biosynthesis.	Dose after dialysis	Dose for GFR<10	N/A
Cephradine (97)	250 to 500 mg tid	100%	100%	100%	50%		Dose after dialysis	Dose for GFR<10	N/A
IV Cephalosporin									
Cefamandole (98)	1 to 2 g IV q6 to 8h	100%	q6h	q8h	q12h		0.5 to 1.0 g after dialysis	0.5 to 1.0 g q12h	Dose for GFR 10 to 50
Cefazolin (62)	1 to 2 g IV q8h	80%	q8h	q12h	q12 to 24h		0.5 to 1.0 g after dialysis	0.5 g q12h	Dose for GFR 10 to 50
Cefepime (99)	1 to 2 g IV q8h	85%	q8 to 12h	q12h	q24h		1 g after dialysis	Dose for GFR<10	Not recommended
Cefmetazole (100,101)	1 to 2 g IV q8h	85%	q8h	q12h	q24h		Dose after dialysis	Dose for GFR<10	Dose for GFR 10 to 50
Cefoperazone (102,103)	1 to 2 g IV q12h	20%	No renal adjustment is required			Displaced from protein by bilirubin. Reduce dose by 50% for jaundice. May prolong prothrombin time.	1 g after dialysis	None	None

(*continued*)

TABLE 104-2

(CONTINUED)

Drugs	Normal dosage	% of renal excretion	Dosage adjustment in renal failure			Comments	HD	CAPD	CVVH
			GFR >50	GFR 10 to 50	GFR <10				
Cefotaxime (104,105)	1 to 2 g IV q6 to 8h	60%	q8h	q12h	q12 to 24h		1 g after dialysis	1 g/d	1g q12h
Cefotetan (106,107)	1 to 2 g IV q12h	75%	q12h	q12 to 24h	q24h		1 g after dialysis	1 g/d	750 mg q12h
Cefoxitin (108,109)	1 to 2 g IV q6h	80%	q6h	q8 to 12h	q12h	May produce false increase in serum creatinine by interference with assay.	1 g after dialysis	1 g/d	Dose for GFR 10 to 50
Ceftazidime (110,111)	1 to 2 g IV q8h	70%	q8h	q12h	q24h		1 g after dialysis	0.5 g/d	Dose for GFR 10 to 50
Ceftriaxone (112,113)	1 to 2 g IV q24h	50%	No renal adjustment is required				Dose after dialysis	750 mg q12h	Dose for GFR 10 to 50
Cefuroxime sodium (114,115)	0.75 to 1.5 g IV q8h	90%	q8h	q8 to 12h	q12 to 24h		Dose after dialysis	Dose for GFR <10	1.0 g q12h
Penicillin (116)						Bleeding abnormalities, hypersensitivity. Seizures.			
Oral Penicillin									
Amoxicillin (117,118)	500 mg po tid	60%	100%	100%	50% to 75%		Dose after dialysis	250 mg q12h	N/A
Ampicillin (119)	500 mg po q6h	60%	100%	100%	50% to 75%		Dose after dialysis	250 mg q12h	Dose for GFR 10 to 50
Dicloxacillin (120,121)	250 to 500 mg po q6h	50%	100%	100%	50% to 75%		None	None	N/A
Penicillin V (116)	250 to 500 mg po q6h	70%	100%	100%	50% to 75%		Dose after dialysis	Dose for GFR<10	N/A
IV Penicillin									
Ampicillin (122)	1 to 2 g IV q6h	60%	q6h	q8h	q12h		Dose after dialysis	250 mg q12h	Dose for GFR 10 to 50
Nafcillin (116)	1 to 2 g IV q4h	35%	No renal adjustment is required				None	None	Dose for GFR 10 to 50
Penicillin G (116)	2 to 3 million Units IV q4h	70%	q4 to 6h	q6h	q8h		Dose after dialysis	Dose for GFR<10	Dose for GFR 10 to 50
Piperacillin (123,124)	3 to 4 g IV q4 to 6h		No renal adjustment is required			Sodium, 1.9 mEq/g.	Dose after dialysis	Dose for GFR <10	Dose for GFR 10 to 50
Ticarcillin/clavulanate (125)	3.1 g IV q4 to 6h	85%	1 to 2 g q4h	1 to 2 g q8h	1 to 2 g q12h	Sodium, 5.2 mEq/g.	3.0 g after dialysis	Dose for GFR <10	Dose for GFR 10 to 50
Piperacillin/ tazobactam (123)	3.375 g IV q6 to 8h	75% to 90%	q4 to 6h	q6 to 8h	q8h	Sodium, 1.9 mEq/g.	Dose after dialysis	Dose for GFR <10	Dose for GFR 10 to 50

Quinolones (64,66)						Food, dairy products, tube feeding, and Al(OH)3 may decrease the absorption of quinolones.			
Cinoxacin (66)	500 mg q12h	55%	100%	50%	Avoid		Avoid	Avoid	Avoid
Fleroxacin (66,126)	400 mg q12h	70%	100%	50% to 75%	50%		Dose for GFR <10	400 mg/d	N/A
Ciprofloxacin (127,128)	200 to 400 mg IV q24h	60%	q12h	q12 to 24h	q24h	Poorly absorbed with antacids, sucralfate, and phosphate binders. Intravenous dose 1/3 of oral dose. Decreases phenytoin levels.	250 mg q12h (200 mg if IV)	250 mg q8h (200 mg if IV)	200 mg IV q12h
Lomefloxacin (64,66)	400 mg q24h	76%	100%	200 to 400 mg q48h	50%	Agents in this group are malabsorbed in the presence of magnesium, calcium, aluminum, and iron. Theophylline metabolism is impaired. Higher oral doses may be needed to treat CAPD peritonitis.	Dose for GFR <10	Dose for GFR <10	N/A
Levofloxacin (64,66)	500 mg po qd	70%	q12h	250 q12h	250 q12h	L-isomer of ofloxacin: appears to have similar pharmacokinetics and toxicities.	Dose for GFR <10	Dose for GFR <10	Dose for GFR 10 to 50
Moxifloxacin (64,66)	400 mg qd	20%	No renal adjustment is required				No data	No data	No data
Nalidixic acid (129)	1.0 g q6h	High	100%	Avoid	Avoid	Agents in this group are malabsorbed in the presence of magnesium, calcium, aluminum, and iron. Theophylline metabolism is impaired. Higher oral doses may be needed to treat CAPD peritonitis.	Avoid	Avoid	N/A
Norfloxacin (64,130)	400 mg po q12h	30%	q12h	q12 to 24h	q24h	See above.	Dose for GFR <10	Dose for GFR <10	N/A
Ofloxacin (64,131)	200 to 400 mg po q12h	70%	q12h	q12 to 24h	q24h	See above.	100 to 200 mg after dialysis	Dose for GFR <10	300 mg/d

(*continued*)

TABLE 104-2

(CONTINUED)

Drugs	Normal dosage	% of renal excretion	Dosage adjustment in renal failure			Comments	HD	CAPD	CVVH
			GFR >50	GFR 10 to 50	GFR <10				
Pefloxacin (64,66)	400 mg q24h	11%	100%	100%	100%	Excellent bidirectional transperitoneal movement.	None	None	Dose for GFR 10 to 50
Sparfloxacin (64,66)	400 mg q24h	10%	100%	50% to 75%	50% q48h		No data: Dose for GFR <10	No data	Dose for GFR 10 to 50
Trovafloxacin (64,66)	200 to 300 mg po q12h	10%	No renal adjustment is required			Liver toxicity.	No Data	No Data	No Data
Miscellaneous Agents									
Azithromycin (65)	250 to 500 mg po qd	6%	No renal adjustment is required			No drug-drug interaction with CSA/KF.	None	None	None
Clarithromycin (132)	500 mg po bid		No renal adjustment is required				None	None	None
Clindamycin (133)	150 to 450 mg po tid	10%	No renal adjustment is required			Increase CSA/FK level.	None	None	None
Dirithromycin (65)	500 mg po qd		No renal adjustment is required			Nonenzymatically hydrolyzed to active compound erythomycylamine.	None	No data: None	Dose for GFR 10 to 50
Erythromycin (134,135)	250 to 500 mg po qid	15%	No renal adjustment is required			Increase CSA/FK level, avoid in transplant patients.	None	None	None
Imipenem/Cilastatin (136,137)	250 to 500 mg IV q6h	50%	500 mg q8h	250 to 500 q8 to 12h	250 mg q12h	Seizures in ESRD. Nonrenal clearance in acute renal failure is less than in chronic renal failure. Administered with cilastin to prevent nephrotoxicity of renal metabolite.	Dose after dialysis	Dose for GFR <10	Dose for GFR 10 to 50
Meropenem (65)	1 g IV q8h	65%	1 g q8h	0.5 to 1g q12h	0.5 to 1g q24h	Less seizures compared to imipenem.	Dose after dialysis	Dose for GFR <10	Dose for GFR 10 to 50
Metronidazole (138,139)	500 mg IV q6h	20%	No renal adjustment is required			Peripheral neuropathy, increase LFTs, disulfiram reaction with alcoholic beverages.	Dose after dialysis	Dose for GFR <10	Dose for GFR 10 to 50

Pentamidine (140,141)	4 mg/kg/day	5%	q24h	q24h	q48h	Inhalation may cause bronchospasm, IV administration may cause hypotension, hypoglycemia, and nephrotoxicity.	None	None	None
Trimethoprim/ Sulfamethoxazole	800/160 mg po bid	70%	q12h	q18h	q24h	Increase serum creatinine. Can cause hyperkalemia.	Dose after dialysis	q24h	q18h
Vancomycin (127,142)	1 g IV q12h	90%	q12h	q24 to 36h	q48 to 72h	Nephrotoxic, ototoxic, may prolong the neuromuscular blockade effect of muscle relaxants. Peak 30 to 40. Trough 5 to 10.	500 mg q12 to 24h (high FLX)	1.0 gm q24 to 96h	500 mg q12hrs
Vancomycin (127,142)	125 to 250 mg po qid	0%	100%	100%	100%	Oral vancomycin is indicated only for the treatment of C. diff.	100%	100%	100%
Antituberculosis Antibiotics									
Rifampin (143)	300 to 600 mg po qd	20%	No renal adjustment is required			Decrease CSA/FK level. Many drug interactions.	None	Dose for GFR <10	Dose for GFR <10
Antifungal Agents (144,145)									
Amphotericin B (146,147)	0.5 mg to 1.5 mg/kg/day	<1%	No renal adjustment is required			Nephrotoxic, infusion related reactions, give 250 cc NS before each dose.	q24h	q24h	q24 to 36h
Amphotec (144,145)	4 to 6 mg/kg/day	<1%	No renal adjustment is required						
Abelcet (144,145)	5 mg/kg/day	<1%	No renal adjustment is required						
AmBisome (144,145)	3 to 5 mg/kg/day	<1%	No renal adjustment is required						
Azoles and other Antifungals (148,149)						Increase CSA/FK level.			

(*continued*)

TABLE 104-2

(CONTINUED)

Drugs	Normal dosage	% of renal excretion	Dosage adjustment in renal failure			Comments	HD	CAPD	CVVH
			GFR >50	GFR 10 to 50	GFR <10				
Fluconazole (150,151)	200 to 800 mg IV qd/bid	70%	100%	100%	50%		200 mg after dialysis	Dose for GFR <10	Dose for GFR 10 to 50
Flucytosine (152,153)	37.5 mg/kg	90%	q12h	q16h	q24h	Hepatic dysfunction. Marrow suppression more common in azotemic patients.	Dose after dialysis	0.5 to 1.0 g/d	Dose for GFR 10 to 50
Griseofulvin (144,145)	125 to 250 mg q6h	1%	100%	100%	100%		None	None	None
Itraconazole (154,155)	200 mg q12h	35%	100%	100%	50%	Poor oral absorption.	100 mg q12 to 24h	100 mg q12 to 24h	100 mg q12 to 24h
Ketoconazole (156)	200 to 400 mg po qd	15%	100%	100%	100%	Hepatotoxic	None	None	None
Miconazole (144,145)	1,200 to 3,600 mg/day	1%	100%	100%	100%		None	None	None
Terbinafine (144,145)	250 mg po qd	>1%	100%	100%	100%				
Antiviral Agents (157)									
Acyclovir (158,159)	200 to 800 mg po 5×/day	50%	100%	100%	50%	Poor absorption. Neurotoxicity in ESRD. Intravenous preparation can cause renal failure if injected rapidly.	Dose after dialysis	Dose for GFR <10	3.5 mg/kg/d
Amantadine (160,161)	100 to 200 mg q12h	90%	100%	50%	25%		None	None	Dose for GFR 10 to 50
Cidofovir (157)	5 mg/kg weekly ×2 (induction); 5 mg/kg every 2 weeks	90%	No data: Avoid	No data: Avoid	No data: Avoid	Dose-limiting nephrotoxicity with preteinuria, glycosuria, renal insufficiency; nephrotoxicity and renal clearance reduced with coadministration of probenecid.	No data	No data	Avoid
Delavirdine (162)	400 mg q8h	5%	No data: 100%	No data: 100%	No data: 100%		No data: None	No data	No data: Dose for GFR 10 to 50
Didanosine (162,163)	200 mg q12h (125 mg if <60 kg)	40% to 69%	q12h	q24h	50% q24h	Pancreatitis	Dose after dialysis	Dose for GFR <10	Dose for GFR <10

Famciclovir (157)	250 to 500 mg po bid to tid	60%	q8h	q12h	q24h	VZV: 500 mg po tid HSV: 250 po bid. Metabolized to active compound penciclovir.	Dose after dialysis	No data	No data: Dose for GFR 10 to 50
Foscarnet (164)	40 to 80 mg IV q8h	85%	40 to 20 mg q8 to 24 h according to ClCr			Nephrotoxic, neurotoxic, hypocalcemia, hypophosphatemia, hypomagnesemia, and hypokalemia.	Dose after dialysis	Dose for GFR <10	Dose for GFR 10 to 50
Ganciclovir IV (165)	5 mg/kg q12h	95%	q12h	q24h	2.5 mg/kg qd	Granulocytopenia and thrombocytopenia	Dose after dialysis	Dose for GFR <10	2.5 mg/kg q24h
Ganciclovir (157)	1,000 mg po tid	95%	1,000 mg tid	1,000 mg bid	1,000 mg qd	Oral ganciclovir should be used ONLY for prevention of CMV infection. Always use IV ganciclovir for the treatment of CMV infection.	No data: Dose after dialysis	No data: Dose for GFR <10	N/A
Indinavir (162)	800 mg q8h	10%	No data: 100%	No data: 100%	No data: 100%	Nephrolithiasis; acute renal failure due to crystalluria, tubulointerstitial nephritis.	No data: None	No data: Dose for GFR <10	No data
Lamivudine (162)	150 mg po bid	80%	q12h	q24h	50 mg q24h	For hepatitis B.	Dose after dialysis	No data: Dose for GFR <10	Dose for GFR 10 to 50
Nelfinavir (157)	750 mg q8h	No data	No data	No data	No data		No data	No data	No data
Nevirapine (162)	200 mg q24h × 14d	<3	No data: 100%	No data: 100%	No data: 100%	May be partially cleared by hemodialysis and peritoneal dialysis.	Dose after dialysis	No data: Dose for GFR <10	No data: Dose for GFR 10 to 50
Ribavirin (166)	500 to 600 mg q12h	30%	100%	100%	50%	Hemolytic uremic syndrome.	Dose after dialysis	Dose for GFR <10	Dose for GFR 10 to 50
Rifabutin (157)	300 mg q24h	5% to 10%	100%	100%	100%		None	None	No data: Dose for GFR 10 to 50
Rimantadine (157)	100 mg po bid	25%	100%	100%	50%				

(*continued*)

TABLE 104-2

(CONTINUED)

Drugs	Normal dosage	% of renal excretion	Dosage adjustment in renal failure			Comments	HD	CAPD	CVVH
			GFR >50	GFR 10 to 50	GFR <10				
Ritonavir (157)	600 mg q12h	3.50%	No data: 100%	No data: 100%	No data: 100%	Many drug interactions.	No data: None	No data: Dose for GFR <10	No data: Dose for GFR 10 to 50
Saquinavir (157)	600 mg q8h	<4%	No data: 100%	No data: 100%	No data: 100%		No data: None	No data: Dose for GFR <10	No data: Dose for GFR 10 to 50
Stavudine (162)	30 to 40 mg q12h	35% to 40%	100%	50% q12 to 24h	50% q24h		Dose for GFR <10 after dialysis	No data	No data: Dose for GFR 10 to 50
Valacyclovir (157)	500 to 1,000 mg q8h	50%	100%	50%	25%	Thrombotic thrombocytopenic purpura/hemolytic uremic syndrome.	Dose after dialysis	Dose for GFR <10	No data: Dose for GFR 10 to 50
Vidarabine (157)	15 mg/kg infusion q24h	50%	100%	100%	75%		Infuse after dialysis	Dose for GFR <10	Dose for GFR 10 to 50
Zanamivir (157)	2 puffs bid × 5 days	1%	100%	100%	100%	Bioavailability from inhalation and systemic exposure to drug is low.	None	None	No data
Zalcitabine (162)	0.75 mg q8h	75%	100%	q12h	q24h		No data: Dose after dialysis	No data	No data: Dose for GFR 10 to 50
Zidovudine (162,167,168)	200 mg q8h, 300 mg q12h	8 to 25%	100%	100%	100 mg q8h	Enormous interpatient variation. Metabolite renally excreted.	Dose for GFR <10	Dose for GFR <10	100 mg q8h

be prudent. Specifically, drugs with narrow therapeutic–toxic profiles such as digoxin and ototoxic aminoglycosides should be administered with a 10% to 25% reduction in their loading dose when volume contraction is present in patients with renal failure.

Choosing a Maintenance Dose

Maintenance doses of a drug ensure steady-state blood concentrations and lessens the likelihood of subtherapeutic regimens or overdosage. In the absence of a loading dose, maintenance doses will achieve 90% of their steady-state level in three to four half-lives. One of two methods can be used to adjust maintenance doses for patients with renal insufficiency. The "dosage reduction" method involves reducing the absolute amount of drug administered at each dosing interval proportional to the patient's degree of renal failure. The dosing interval remains unchanged, and more constant drug concentrations are achieved. The "interval extension" method involves lengthening the time period between individual doses of a drug, reflecting the extent of renal insufficiency. This method is particularly useful for drugs with a wide therapeutic range and long half-life.

Monitoring Drug Levels

Blood, serum, and plasma drug concentrations may not be equivalent. As a result, drug levels can only be interpreted if the dosage schedule is known including the dose administered, timing, and route of administration. A peak level is usually obtained 30 minutes following intravenous administration and 60 to 120 minutes after oral ingestion. It reflects the maximum level achieved after the rapid distribution phase and before significant elimination has occurred. A trough level is obtained just prior to the next dose, reflects total body clearance, and may be a marker of drug toxicity. If the concentration of a drug and its Vd are known, the dose required to achieve a desired therapeutic level can be calculated by the following formula where Vd in L per kilogram is multiplied by ideal body weight in kilograms (IBW) and the desired plasma concentration in milligrams per L (Cp):

$$\text{Dose} = \text{Vd} \times \text{IBW} \times \text{Cp} \qquad [4]$$

Drug-level monitoring is a clinically useful tool when used appropriately. Clinical judgment is paramount because drug failure or toxicity can occur within "therapeutic concentrations." For example, digitalis intoxication can occur in the presence of therapeutic serum levels if hypokalemia or metabolic alkalosis coexists. Phenytoin toxicity is a common problem in patients with renal failure and hypoalbuminemia because of an increase in the unbound or biologically active fraction of phenytoin despite a low total phenytoin plasma concentration. In this setting phenytoin levels should be adjusted for reduced protein binding and the effect of renal failure on phenytoin distributions (see TDM Table 104-1).

DIALYSIS AND DRUG DOSING

Patients undergoing renal replacement therapy (dialysis therapy) require special attention in terms of dosage adjustment because dialysis membranes significantly remove many therapeutic agents. An array of modalities including high efficiency, high flux, continuous, and conventional hemodialysis exist and differ from one another based on membrane porosity, surface area, and blood as well as dialysate flow rates. These differences, in turn, affect drug removal. In Table 104-2 are summarized drug properties and dialysis parameters that determine dialytic clearance of pharmacologic agents. In general, in thrice weekly intermittent hemodialysis (IHD), the drug removal is affected by blood and dialysate flow rate, molecular weight (MW) of the drug, fraction of protein binding and dialyzer surface area. Drugs with MW greater than 500 D, highly protein bound (80%), highly tissue bound and lipophilic are poorly dialyzed by conventional IHD. However, drugs with small MW, low protein binding, small volume of distribution and of a hydrophilic character are effectively removed by IHD (51–54).

Drug Properties Affecting Dialytic Clearance

A drug's molecular weight is a major determinant of its dialyzability. Specifically, drugs larger than 500 daltons are primarily cleared by convection as opposed to diffusion. If too large to pass through a given membrane, the drug will not be cleared from the circulation. An inverse semilogarithmic relationship exists between molecular weight and dialysis clearance.

Protein binding represents another major determinant of drug dialyzability. Compounds that are highly protein bound have a smaller fraction of unbound drug available for removal by dialysis. Since heparin stimulates lipoprotein lipase, free fatty acid levels may increase during dialysis. Free fatty acid levels may displace sulfonamides, salicylates, and phenytoin from their protein binding sites, resulting in increased free fractions of each drug. In contrast, free fatty acids can increase protein binding of certain cephalosporins. The free fraction of phenytoin is increased by free fatty acids.

As discussed earlier, drugs with a large Vd (greater than 2 L/kg) tend to have low concentrations in the intravascular space and are thus not readily dialyzable. The lower the Vd (less than 1 L/kg), the greater the drug's availability to the circulation and, similarly, to the dialyzer.

Larger molecular-weight compounds do not equilibrate rapidly between the extracellular and intracellular compartments during dialysis; little change is detected in intracellular concentrations while extracellular levels may fall significantly. As such, postdialysis rebound may occur in which pharmacologic agents move down their concentration gradients into the extracellular space. Rebound can be sizable as well as highly unpredictable in its time course, as demonstrated by vancomycin. Ultrafiltration raises the hematocrit, which can influence the dialytic clearance of drugs that partition into red blood cells. Drugs such as ethambutol, procainamide, and acetaminophen partition into red blood cells and demonstrate decreased dialytic clearance due to hemoconcentration following dialysis ultrafiltration.

TABLE 104-3

FACTORS AFFECTING DRUG REMOVAL DURING DIALYSIS

Drug properties	Dialysis system properties
Renal clearance	Filter properties
Volume of distribution	Blood flow, dialysate flow, and ultrafiltration rates
Water and lipid solubility	
Protein binding	
Drug charge	
Molecular weight	

TABLE 104-4

ANALGESIC DRUG DOSING IN RENAL FAILURE

Analgesics	Normal dosage	% of renal excretion	Dosage adjustment in renal failure			Comments	HD	CAPD	CVVH
			GFR >50	GFR 10 to 50	GFR <10				
Narcotics and Narcotic Antagonists (42)									
Alfentanil (42)	Anesthetic induction 8 to 40 mcg/kg	Hepatic	100%	100%	100%	Titrate the dose regimen.	N/A	N/A	N/A
Butorphanol (169)	2 mg q3 to 4h	Hepatic	100%	75%	50%		No data	No data	N/A
Codeine (170)	30 to 60 mg q4 to 6h	Hepatic	100%	75%	50%		No data	No data	Dose for GFR 10 to 50
Fentanyl (70)	Anesthetic induction (individualized)	Hepatic	100%	75%	50%	CRRT-titrate.	N/A	N/A	N/A
Meperidine (171)	50 to 100 mg q3 to 4h	Hepatic	100%	75%	50%	Normeperidine, an active metabolite, accumulates in ESRD and may cause seizures. Protein binding is reduced in ESRD. 20% to 25% excreted unchanged in acidic urine.	Avoid	Avoid	Avoid
Methadone (172)	2.5 to 5 mg q6 to 8h	Hepatic	100%	100%	50% to 75%		None	None	N/A
Morphine (173,174)	20 to 25 mg q4h	Hepatic	100%	75%	50%	Increased sensitivity to drug effect in ESRD.	None	No data	Dose for GFR 10 to 50
Naloxone (42)	0.4 to 2 mg IV	Hepatic	100%	100%	100%		N/A	N/A	Dose for GFR 10 to 50
Pentazocine (42)	50 mg q4h	Hepatic	100%	75%	75%		None	No data	Dose for GFR 10 to 50
Propoxyphene (175)	65 mg po q6 to 8h	Hepatic	100%	100%	Avoid	Active metabolite norpropoxyphene accumulates in ESRD.	Avoid	Avoid	N/A
Sufentanil (42)	Anesthetic induction	Hepatic	100%	100%	100%	CRRT-titrate.	N/A	N/A	N/A
Non-Narcotics									
Acetaminophen (176)	650 mg q4h	Hepatic	q4h	q6h	q8h	Overdose may be nephrotoxic. Drug is major metabolite of phenacetin.	None	None	Dose for GFR 10 to 50
Acetylsalicylic acid (177)	650 mg q4h	Hepatic (renal)	q4h	q4 to 6h	Avoid	Nephrotoxic in high doses. May decrease GFR when renal blood flow is prostaglandin dependent. May add to uremic GI and hematologic symptoms. Protein binding reduced in ESRD.	Dose after dialysis	None	Dose for GFR 10 to 50

Thus, parent compounds and their metabolites will be eliminated by dialysis to a greater extent if they possess a low molecular weight, limited Vd, and are water-soluble. An increase in drug clearance of 30% or greater by dialytic therapy is considered significant and may warrant supplemental dosing following dialysis.

Dialytic Factors Affecting Drug Clearance

Dialysis membranes, dialysate flow rates, and the dialytic technique used can significantly alter drug clearance (Table 104-3) (55). A wide variety of membranes have been developed including cellulose, cellulose acetate, polysulfone, polyamide, polyacrylonitrile (PAN; AN69), and polymethylmethacrylate (PMMA) in an effort to improve membrane permeability for larger uremic toxins. Similarly, albumin can cross polysulfone membranes to a limited extent (56). Vancomycin clearance is significantly increased when polysulfone or PAN membranes are used (52,53). Likewise, cuprammonium rayon membranes allow greater aminoglycoside removal compared to cellulose fibers (42). Two endogenous compounds that are poorly dialyzed, phosphate and β_2-microglobulin, undergo enhanced clearance when PAN, PMMA, and polysulfone membranes are used due to the increased surface area of these membranes (57). The electrical charge of a dialysis membrane as well as the drug may help or hinder clearance. Like charges will repel one another, while opposite charges between membrane and drug may lead to drug adsorption to the membrane, ultimately reducing clearance (58,59).

Drug clearance is achieved primarily by two processes: diffusion and convection. Diffusion of a compound increases as its molecular weight decreases and is negligible when standard membranes are used for substances larger than 1,000 daltons (18,60). Diffusion of a drug is enhanced when the concentration gradient between blood and dialysate is maximized by countercurrent flow and increased blood and dialysate flow rates. Flow rates have less impact on the diffusion of middle-sized and large molecules, but the surface area and hydraulic permeability of the membrane assume greater significance. Diffusion can be hindered, however, when high ultrafiltration rates lead to the mixing of dialysate and ultrafiltrate. This results in a decreased concentration gradient between blood and dialysate, reducing diffusive clearance (61). Convection refers to the movement of solute by way of ultrafiltration, which affects molecules of all sizes but particularly large molecular-weight substances, which diffuse poorly. To be removed by dialysis, compounds greater than 1,000 daltons require ultrafiltration when cellulose membranes are used, while those greater than 2,000 daltons demonstrate limited clearance. Ultrafiltration, and thus convection, can be reduced by protein binding to membrane surfaces during the dialytic procedure, which ultimately diminishes drug removal (18,19).

Continuous Renal Replacement Therapies and Drug Removal

Critically ill patients may require continuous renal replacement therapies (CRRT) such as hemofiltration or hemodialysis, and an awareness of drug handling by such procedures is crucial to the patient's outcome. Continuous hemofiltration removes solute by convection. The degree to which a solute can convectively cross a membrane can be quantitated by its sieving coefficient (S), the ratio of solute concentration in the ultrafiltrate to solute concentration in the retentate (returning to the patient's circulation). This can be approximated by the formula:

$$S = UF/A \qquad [5]$$

where UF = ultrafiltrate and A = arterial concentrations of solute, which will remain relatively constant during hemofiltration because blood flow does not affect sieving. Clearance of a solute (drug) is determined by multiplying the ultrafiltration rate by the S for that substance. The sieving coefficient for a given molecule can change, however, when comparing different dialysis membranes and is likely due to drug–membrane binding. Sieving can also be reduced by negatively charged solutes, although exceptions to this exist (20,22). Since inulin (5,200 Daltons) can readily cross polysulfone hemofiltration membranes, nearly all therapeutic agents would be expected to permeate such membranes given molecular weights less than that of inulin. The drug's degree of protein binding will be the major limiting factor to drug removal during hemofiltration.

In contrast to hemofiltration, drug removal during continuous hemodialysis occurs primarily via diffusion rather than convection. Protein binding again plays a central role whereby unbound drug diffuses more readily than protein-bound drug and molecular weight correlates inversely with diffusion. It should be noted that during continuous hemodialysis with venovenous access and average blood flow rates of 200 mL/minute, a GFR of 20 to 30 mL/minute can be achieved, which may provide greater drug clearance than expected. As previously discussed, when supplemental dosing is indicated, the amount of drug required to achieve a desired blood level can be calculated by multiplying the drug's Vd by the patient's ideal body weight and the difference between the desired drug concentration and the trough concentration.

Lastly, peritoneal dialysis generally provides minimal drug removal, as dialysate flow rates are significantly slower than other forms of dialytic therapy. Drugs that are dialyzable via peritoneal dialysis must be small in size and have a low Vd. Drugs that are highly protein bound, however, may undergo greater clearance with peritoneal dialysis versus hemodialysis given the large protein losses commonly seen with peritoneal therapy.

SPECIFIC CONSIDERATIONS FOR DRUG PRESCRIBING IN RENAL FAILURE

In this section, the effects of renal insufficiency on drug pharmacokinetics and pharmacodynamics are reviewed and dosage adjustment guidelines are provided. It is imperative to reiterate that the following recommendations provide a framework for dosage adjustments that must be applied to individual patients with caution. Continual monitoring and modification of drug therapy are required by concurrent illnesses, clinical response, and side effects present in the individual patient. Drugs are listed in tabular form (Tables 104-3 through 104-13) by generic name in alphabetical order and are grouped into categories based on therapeutic effect. General comments about each group precede the individual dosing table. For reference, the standard dose given to patients with normal renal function is included. Specific dosing guidelines are provided for each drug based on the patient's level of renal function in terms of GFR. It should be remembered that creatinine clearance always overestimates true GFR.

The maintenance dosage regimen may be modified by either extending the interval between doses, decreasing the individual dose while maintaining normal dosing intervals, or a combination of the two methods. As outlined previously, the variable interval method allows a more convenient and less costly dosing schedule but may result in periods of subtherapeutic drug levels. The variable dose method maintains more constant drug levels because the dosing interval remains unchanged but risks greater toxicity because the difference between peak and trough

TABLE 104-5

ANTIHYPERTENSIVE AND CARDIOVASCULAR AGENT DOSING IN RENAL FAILURE

Antihypertensive and cardiovascular agents	Normal doses		% of renal excretion	Dosage adjustment in renal failure			Comments	HD	CAPD	CVVH
	Starting dose	Maximum dose		GFR >50	GFR 10 to 50	GFR <10				
ACE-Inhibitors (178,179)							Hyperkalemia, acute renal failure, angioedema, rash, cough, anemia, and liver toxicity.			
Benazepril (180)	10 mg qd	80 mg qd	20%	100%	75%	25% to 50%		None	None	Dose for GFR 10 to 50
Captopril (181,182)	6.25 to 25 mg po tid	100 mg tid	35%	100%	75%	50%	Rare proteinuria, nephrotic syndrome, dysgeusia, granulocytopenia. Increases serum digoxin levels.	25% to 30%	None	Dose for GFR 10 to 50
Enalapril (183,184)	5 mg qd	20 mg bid	45%	100%	75%	50%	Enalaprilat, the active moiety formed in liver.	20% to 25%	None	Dose for GFR 10 to 50
Fosinopril (185,186)	10 mg po qd	40 mg bid	20%	100%	100%	75%	Fosinoprilat, the active moiety formed in liver. Drug less likely than other angiotensin-converting enzyme inhibitors to accumulate in renal failure.	None	None	Dose for GFR 10 to 50
Lisinopril (187,188)	2.5 mg qd	20 mg bid	80%	100%	50% to 75%	25% to 50%	Lysine analog of a pharmacologically active enalapril metabolite.	20%	None	Dose for GFR 10 to 50
Pentopril (178,179)	125 mg q24h		80% to 90%	100%	50% to 75%	50%		No data	No data	Dose for GFR 10 to 50
Perindopril (178,179)	2 mg q24h		<10%	100%	75%	50%	Active metabolite is perindoprilat. The clearance of perindoprilat and its metabolites is almost exclusively renal. Approximately 60% of circulating perindopril is bound to plasma proteins, and only 10% to 20% of perindoprilat is bound.	25% to 50%	No data	Dose for GFR 10 to 50
Quinapril (178,179)	10 mg qd	20 mg qd	30%	100%	75% to 100%	75%	Active metabolite is quinaprilat. 96% of quinaprilat is excreted renally.	25%	None	Dose for GFR 10 to 50
Ramipril (189,190)	2.5 mg qd	10 bid	15%	100%	50% to 75%	25% to 50%	Active metabolite is ramiprilat. Data are for ramiprilat.	20%	None	Dose for GFR 10 to 50
Trandolapril (178,179)	1 to 2 mg qd	4 mg qd	33%	100%	50% to 100%	50%		None	None	Dose for GFR 10 to 50

Angiotensin-II-Receptors Antagonists (76)							Hyperkalemia, angioedema (less common than ACE-inhibitors).			
Candesartan (76)	16 mg qd	32 mg qd	33%	100%	100%	50%	Candesartan cilexetil is rapidly and completely bioactivated by ester hydrolysis during absorption from the gastrointestinal tract to candesartan.	None	None	None
Eprosartan (76)	600 mg qd	400 to 800 mg qd	25%	100%	100%	100%	Eprosartan pharmacokinetics more variable ESRD. Decreased protein binding in uremia.	None	None	None
Irbesartan (76)	150 mg qd	300 mg qd	20%	100%	100%	100%		None	None	None
Losartan (76)	50 mg qd	100 mg qd	13%	100%	100%	100%		No data	No data	Dose for GFR 10 to 50
Telmisartan (76)	20 to 80 mg qd		<5%	100%	100%	100%		None	None	None
Valsartan (76)	80 mg qd	160 mg bid	7%	100%	100%	100%		None	None	None
Beta Blockers (191,192)							Decrease HDL, mask symptoms of hypoglycemia, bronchospasm, fatigue, insomnia, depression, and sexual dysfunction.			
Acebutolol (193,194)	400 mg q24h or bid	600 mg q24h or bid	55%	100%	50%	30% to 50%	Active metabolites with long half-life.	None	None	Dose for GFR 10 to 50
Atenolol (195,196)	25 mg qd	100 mg qd	90%	100%	75%	50%	Accumulates in ESRD.	25 to 50 mg	None	Dose for GFR 10 to 50
Betaxolol (197)	20 mg q24h	80% to 90%	100%	100%	50%	50%		None	Dose for GFR 10 to 50	Dose for GFR 10 to 50
Bopindolol (191,192)	1 mg q24h	4 mg q24h	<10%	100%	100%	100%		None	None	Dose for GFR 10 to 50
Carteolol (198,199)	0.5 mg q24h	10 mg q24h	<50%	100%	50%	25%		No data	None	Dose for GFR 10 to 50
Carvedilol (191,192)	3.125 mg po tid	25 mg tid	2%	100%	100%	100%	Kinetics are dose dependent. Plasma concentrations of carvedilol have been reported to be increased in patients with renal impairment.	None	None	Dose for GFR 10 to 50
Celiprolol (191,192)	200 mg q24h		10%	100%	100%	75%		No data	None	Dose for GFR 10 to 50
Dilevalol (200,201)	200 mg bid	400 mg bid	<5%	100%	100%	100%		None	None	No data
Esmolol (IV only) (202,203)	50 mcg/kg/min	300 mcg/kg/min	10%	100%	100%	100%	Active metabolite retained in renal failure.	None	None	No data

(*continued*)

TABLE 104-5

(CONTINUED)

Antihypertensive and cardiovascular agents	Normal doses		% of renal excretion	Dosage adjustment in renal failure			Comments	HD	CAPD	CVVH
	Starting dose	Maximum dose		GFR >50	GFR 10 to 50	GFR <10				
Labetalol (204,205)	50 mg po bid	400 mg bid	5%	100%	100%	100%	For IV use: 20 mg slow intravenous injection over a 2-minute period. Additional injections of 40 mg or 80 mg can be given at 10-minute intervals until a total of 300 mg. or continuous infusion of 2 mg/minute.	None	None	Dose for GFR 10 to 50
Metoprolol (206)	50 mg bid		100 mg bid	<5%	100%	100%		None	None	None
Nadolol (207,208)	80 mg qd	160 mg bid	90%	100%	50%	25%	Start with prolonged interval and titrate.	40 mg	None	Dose for GFR 10 to 50
Penbutolol (209)	10 mg q24h	40 mg q24h	<10	100%	100%	100%		None	None	Dose for GFR 10 to 50
Pindolol (210)	10 mg bid	40 mg bid	40%	100%	100%	100%		None	None	Dose for GFR 10 to 50
Propranolol (211,212)	40% to 160 mg tid	320 mg/day	<5%	100%	100%	100%	Bioavailability may increase in ESRD. Metabolites may cause increased bilirubin by assay interference in ESRD. Hypoglycemia reported in ESRD.	None	None	Dose for GFR 10 to 50
Sotalol (213,214)	80 bid	160 mg bid	70%	100%	50%	25% to 50%	Extreme caution should be exercised in the use of sotalol in patients with renal failure undergoing hemodialysis. To minimize the risk of induced arrhythmia, patients initiated or re-initiated on BETAPACE® should be placed for a minimum of three days (on their maintenance dose) in a facility that can provide cardiac resuscitation and continuous electrocardiographic monitoring.	80 mg	None	Dose for GFR 10 to 50
Timolol (191,192)	10 mg bid	20 mg bid	15%	100%	100%	100%		None	None	Dose for GFR 10 to 50

Calcium Channel Blockers (215)							Dihydropyridine: headache, ankle edema, gingival hyperplasia and flushing. Non-dihydropyridine: bradycardia, constipation, gingival hyperplasia and AV block.			
Amlodipine (216)	2.5 po qd	10 mg qd	10%	100%	100%	100%	May increase digoxin and cyclosporine levels.	None	None	Dose for GFR 10 to 50
Bepridil (215)	No data	<1%	No data	No data	No data	Weak vasodilator and antihy-pertensive.		None	No data	No data
Diltiazem (217,218)	30 mg tid	90 mg tid	10%	100%	100%	100%	Acute renal dysfunction. May exacerbate hyperkalemia. May increase digoxin and cyclosporine levels.	None	None	Dose for GFR 10 to 50
Felodipine (219,220)	5 mg po bid	20 mg qd	1%	100%	100%	100%	May increase digoxin levels.	None	None	Dose for GFR 10 to 50
Isradipine (221,222)	5 mg po bid	10 mg bid	<5%	100%	100%	100%	May increase digoxin levels.	None	None	Dose for GFR 10 to 50
Nicardipine (223)	20 mg po tid		30 mg po tid	<1%	100%	100%	Uremia inhibits hepatic metabolism. May increase digoxin levels.	None	None	None
Nifedipine XL (224,225)	30 qd	90 mg bid	10%	100%	100%	100%	Avoid short-acting nifedipine formulation.	None	None	None
Nimodipine (215)	30 mg q8h		10%	100%	100%	100%	May lower blood pressure.	None	None	Dose for GFR 10 to 50
Nisoldipine (215)	20 mg qd	30 mg bid	10%	100%	100%	100%	May increase digoxin levels	None	None	Dose for GFR 10 to 50
Verapamil (226,227)	40 mg tid	240 mg/day	10%	100%	100%	100%	Acute renal dysfunction. Active metabolites accumulate particularly with sustained-release forms.	None	None	Dose for GFR 10 to 50
Diuretics (228,229)							Hypokalemia/hyperkalemia (potassium sparing agents), hyperuricemia, hyperglycemia, hypomagnesemia, increase serum cholesterol.			

(*continued*)

TABLE 104-5

(CONTINUED)

Antihypertensive and cardiovascular agents	Normal doses		% of renal excretion	Dosage adjustment in renal failure			Comments	HD	CAPD	CVVH
	Starting dose	Maximum dose		GFR >50	GFR 10 to 50	GFR <10				
Acetazolamide (230)	125 mg po tid	500 mg po tid	90%	100%	50%	Avoid	May potentiate acidosis. Ineffective as diuretic in ESRD. May cause neurologic side effects in dialysis patients.	No data	No data	Avoid
Amiloride (231)	5 mg po qd	10 mg po qd	50%	100%	100%	Avoid	Hyperkalemia with GFR <30 mL/minute, especially in diabetics. Hyperchloremic metabolic acidosis.	N/A	N/A	N/A
Bumetanide (232,233)	1 to 2 mg po qd	2 to 4 mg po qd	35%	100%	100%	100%	Ototoxicity increased in ESRD in combination with aminoglycosides. High doses effective in ESRD. Muscle pain, gynecomastia.	None	None	N/A
Chlorthalidone (234)	25 mg q24h	50%	q24h	q24h	Avoid	Ineffective with low GFR.		N/A	N/A	N/A
Ethacrynic alcid (235)	50 mg po qd	100 mg po bid	20%	100%	100%	100%	Ototoxicity increased in ESRD in combination with aminoglycosides.	None	None	N/A
Furosemide (236,237)	40 to 80 mg po qd	120 mg po tid	70%	100%	100%	100%	Ototoxicity increased in ESRD, especially in combination with aminoglycosides. High doses effective in ESRD.	None	None	N/A
Indapamide (238)	2.5 mg q24h	<5%	100%	100%	Avoid	Ineffective in ESRD.		None	N/A	None
Metolazone (228,229)	2.5 mg po qd		10 mg po bid	70%	100%	100%	High doses effective in ESRD. Gynecomastia, impotence.	None	None	None
Piretanide (239,240)	6 mg q24h	12 mg q24h	40% to 60%	100%	100%	100%	High doses effective in ESRD. Ototoxicity.	None	None	N/A
Spironolactone (241)	100 mg po qd	300 mg po qd	25%	100%	100%	Avoid	Active metabolites with long half-life. Hyperkalemia common when GFR <30, especially in diabetics. Gynecomastia, hyperchloremic acidosis. Increases serum by immunoassay interference.	N/A	N/A	Avoid
Thiazides (242)	25 mg bid		50 mg bid	>95%	100%	100%	Avoid	Usually ineffective with GFR <30 mL/min. Effective at low GFR in combination with loop diuretic. Hyperuricemia.	N/A	N/A

Torasemide (228,229)	5 mg po bid	20 mg qd	25%	100%	100%	100%	High doses effective in ESRD. Ototoxicity.	None	None	N/A
Triamterene (243)	25 mg bid	50 mg bid	5% to 10%	q12h	q12h	Avoid	Hyperkalemia common when GFR <30, especially in diabetics. Active metabolite with long half-life in ESRD. Folic acid antagonist. Urolithiasis. Crystalluria in acid urine. May cause acute renal failure.	Avoid	Avoid	Avoid
Miscellaneous agents										
Amrinone (244,245)	5 mg/kg/ minute daily dose <10 mg/kg	10 mg/kg/ minute daily dose <10 mg/kg	10% to 40%	100%	100%	100%	Thrombocytopenia. Nausea, vomiting in ESRD.	No data	No data	Dose for GFR 10 to 50
Clonidine (246,247)	0.1 po bid/tid	1.2 mg/ day	45%	100%	100%	100%	Sexual dysfunction, dizziness, postal hypotension.	None	None	Dose for GFR 10 to 50
Digoxin (228,248)	0.125 mg qod/qd	0.25 mg po qd	25%	100%	100%	100%	Decrease loading dose by 50% in ESRD. Radioimmunoassay may overestimate serum levels in uremia. Clearance decreased by amiodarone, spironolactone, quinidine, verapamil. Hypokalemia, hypomagnesemia enhance toxicity. Vd and total body clearance decreased in ESRD. Serum level 12 hours after dose is best guide in ESRD. Digoxin immune antibodies can treat severe toxicity in ESRD.	None	None	Dose for GFR 10 to 50
Hydralazine (249)	10 mg po qid	100 mg po qid	25%	100%	100%	100%	Lupus-like reaction.	None	None	Dose for GFR 10 to 50
Midodrine (250)	No data	No data	75% to 80%	5 to 10 mg q8h	5 to 10 mg q8h	No data	Increased blood pressure.	5 mg q8h	No data	Dose for GFR 10 to 50
Minoxidil (251)	2.5 mg po bid	10 mg po bid	20%	100%	100%	100%	Pericardial effusion, fluid retention, hypertrichosis, and tachycardia.	None	None	Dose for GFR 10 to 50
Nitroprusside (252,253)	1 mcg/ kg/min	10 mcg/ kg/min	<10%	100%	100%	100%	Cyanide toxicity.	None	None	Dose for GFR 10 to 50
Amrinone (254)	5 mcg/ kg/min	10 mcg/ kg/min	25%	100%	100%	100%	Thrombocytopenia. Nausea, vomiting in ESRD.	No data	No data	Dose for GFR 10 to 50
Dobutamine (255,256)	2.5 mcg/ kg/min	15 mcg/ kg/min	10%	100%	100%	100%		No data	No data	Dose for GFR 10 to 50
Milrinone (257,258)	0.375 mcg/ kg/min	0.75 mcg/ kg/min		100%	100%	100%		No data	No data	Dose for GFR 10 to 50

TABLE 104-6

HYPOGLYCEMIC AGENTS DOSING IN RENAL FAILURE

Hypoglycemic agents (259)	Normal doses		% of renal excretion	Dosage adjustment in renal failure			Comments	HD	CAPD	CVVH
	Starting dose	Maximum dose		GFR >50	GFR 10 to 50	GFR <10				
							Avoid all oral hypoglycemic agents on CRRT.			
Acarbose (260)	25 mg tid	100 mg tid	35%	100%	50%	Avoid	Abdominal pain, N/V, and flatulence.	No data	No data	Avoid
Acetohexamide (259)	250 mg q24h	1,500 mg q24h	None	Avoid	Avoid	Avoid	Diuretic effect. May falsely elevate serum creatinine. Active metabolite has $T_{1/2}$ of 5 to 8 hours in healthy subjects and is eliminated by the kidney. Prolonged hypoglycemia in azotemic patients.	No data	None	Avoid
Chlorpropamide (259)	100 mg q24h	500 mg q24h	47%	50%	Avoid	Avoid	Impairs water excretion. Prolonged hypoglycemia in azotemic patients.	No data	None	Avoid
Glibornuride (259)	12.5 mg q24h	100 mg q14h	No data	No data	No data	No data		No data	No data	Avoid
Gliclazide (261)	80 mg q24h	320 mg q24h	<20%	50% to 100%	Avoid	Avoid		No data	No data	Avoid
Glipizide (262)	5 mg qd	20 mg bid	5%	100%	50%	50%		No data	No data	Avoid
Glyburide (263)	2.5 mg qd	10 mg bid	50%	100%	50%	Avoid		None	None	Avoid
Metformin (259)	500 mg bid	2,550 mg/day (bid or tid)	95%	100%	Avoid	Avoid	Lactic acidosis.	No data	No data	Avoid
Repaglinide (259)	0.5 to 1 mg	4 mg tid								
Tolazamide (259)	100 mg q24h	250 mg q24h	7%	100%	100%	100%	Diuretic effects.	Avoid	Avoid	Avoid
Tolbutamide (259)	1 g q24h	2 g q24h	None	100%	100%	100%	May impair water excretion.	None	None	Avoid
Piogkitazone (259)	15 mg qd	45 mg qd	3%	100%	100%	100%	Drug interactions.	None	None	NA
Rosiglitazone (259)	2 mg qd	8 mg qd	3%	100%	100%	100%	Increases LDL.	None	None	NA
Troglitazone (259)	200 mg qd	600 mg qd	3%	100%	Avoid	Avoid	Decrease CSA level, hepatotoxic.	None	None	Avoid
Parenteral Agents							Dosage guided by blood glucose levels.			
Insulin (259)	Variable		None	100%	75%	50%	Renal metabolism of insulin decreases with azotemia.	None	None	Dose for GFR 10 to 50
Lispro insulin (259)	Variable		No data	100%	75%	50%	Avoid all oral hypoglycemic agents on CRRT.	None	None	None

TABLE 104-7

HYPERLIPIDEMIC AGENTS DOSING IN RENAL FAILURE

Hypoglycemic agents (79)	Normal doses		% of renal excretion	Dosage adjustment in renal failure			Comments	HD	CAPD	CVVH
	Starting dose	Maximum dose		GFR >50	GFR 10 to 50	GFR <10				
Atorvastatin (79)	10 mg/day	80 mg/day	<2%	100%	100%	100%	Liver dysfunction, myalgia, and rhabdomyolysis with CSA/FK.	No data	No data	No data
Bezafibrate (79)	200 mg bid–qid 400 mg SR q24h		50%	50% to 100%	25% to 50%	Avoid		No data	No data	No data
Cholestyramine (79)	4 gm bid	24 gm/day	None	100%	100%	100%		No data	No data	No data
Clofibrate (264)	500 mg bid	1,000 mg bid	40% to 70%	q6 to 12h	q12 to 18h	Avoid		No data	No data	No data
Colestipol (79)	5 gm bid	30 gm/day	None	100%	100%	100%		No data	No data	No data
Fluvastatin (79)	20 mg daily	80 mg/day	<1%	100%	100%	100%		No data	No data	No data
Gemfibrozil (265)	600 bid	600 bid	None	100%	100%	100%		No data	No data	No data
Lovastatin (266,267)	5 mg daily	20 mg/day	None	100%	100%	100%		No data	No data	No data
Nicotinic acid (268)	1 g tid	2 g tid	None	100%	50%	25%		No data	No data	No data
Pravastatin (269)	10 to 40 mg daily	80 mg/day	<10%	100%	100%	100%		No data	No data	No data
Probucol (270)	500 mg bid		<2%	100%	100%	100%		No data	No data	No data
Simvastatin (271)	5 to 20 mg daily	20 mg/day	13%	100%	100%	100%		No data	No data	No data

TABLE 104-8

ANTITHYROID DOSING IN RENAL FAILURE

Antithyroid drugs (272)	Normal dosage	% of renal excretion	Dosage adjustment in renal failure			HD	CAPD	CVVH
			GFR >50	GFR 10 to 50	GFR <10			
Methimazole (273)	5 to 20 mg tid	7	100%	100%	100%	No data	No data	Dose for GFR 10 to 50
Propylthiouracil (272)	100 mg tid	<10	100%	100%	100%	No data	No data	Dose for GFR 10 to 50

levels is diminished. When the interval extension method (I) is used, the dosing interval length (in hours) is indicated. When the dosage reduction method (D) is used, the percentage of the standard dose normally used is indicated.

The requirement for supplemental dosing after hemodialysis and special dosing considerations for peritoneal dialysis and continuous renal replacement therapies are also included. For many drugs, specific data are not available on dialytic drug clearance, and the likelihood of dialytic removal is based on molecular weight, Vd, and protein binding.

Antimicrobial Agents

Infection is the leading cause of morbidity and mortality in patients with renal failure. Many antibiotics require dosage adjustment in the setting of renal insufficiency due to alterations in pharmacokinetic and pharmacodynamic parameters. An increased incidence of extrarenal toxicity is also observed in patients with renal insufficiency due, in part, to accumulation of drug and/or active metabolites. Specific dosing guidelines for individual antimicrobial agents are provided in Table 104-3. One or more pharmacokinetic parameters may be altered in the patient with renal failure (62,63). Number of oral antibiotics are now available for the treatment of infections in patients with renal impairments. However, a decreased absorption may occur for some agents such as tetracycline or ciprofloxacin (64,65). Most antibiotics should be administrated at least two to four hours after iron and phosphate binder therapy. Although the majority of antibiotics are excreted partially or completely by glomerular filtration, a number of antimicrobials such as trimethoprim–sulfamethoxazole or ciprofloxacin reach the urinary space by tubular secretion. This, in turn, achieves high urinary concentrations of such agents even though the GFR is diminished (66). This feature is used to therapeutic advantage for treatment of urinary tract infections in patients with renal insufficiency or cyst infections in patients with polycystic kidney disease.

For most drugs, the loading dose will be the same as that used in patients with normal renal function because rapid achievement of therapeutic antibiotic levels are critical for life-threatening infections. Recently, the postantibiotic effect has been observed with a number of antimicrobials including aminoglycosides, newer macrolides, and the penems (67,68). Clinically, the persistence of antibiotic activity beyond the time point at which blood levels fall below the minimum inhibitory concentration may be used to design extended and more convenient dosing intervals of antimicrobial agents without jeopardizing patient outcomes.

Peritoneal dialysis patients often use the intraperitoneal route of antibiotic administration for treatment of peritonitis. Detailed reviews of this therapeutic modality exist elsewhere, and intraperitoneal antibiotic dosing for peritonitis has been reviewed by Keane and colleagues. Patients with renal dysfunction have an increased risk of antimicrobial-induced nephrotoxicity whereby dosage adjustments and close monitoring are required to minimize further renal injury, particularly with aminoglycosides (89). Less predictably, acute interstitial nephritis often complicates courses of antimicrobial therapy, but no known risk factors or preventive measures exist. Spurious rises in serum creatinine may result when certain cephalosporins interfere with the creatinine assay or trimethoprim blocks tubular secretion of creatinine. Lastly, significant potassium and salt loads may accompany the administration of certain antibiotics, particularly penicillins.

Analgesics and Agents Used by Anesthesiologists

Opioid analgesics remain the primary pain modality for the treatment of pain in patients with renal failure (69). In Table 104-4 are summarized dosage recommendations for analgesics and agents used by anesthesiologists. Most analgesics are metabolized by the liver and thus require little dosage adjustment, but renal failure tends to increase the sensitivity to the pharmacologic effects of these drugs (70). Special attention needs to be paid when prescribing meperidine or propoxyphene for patients with reduced renal function. Normeperidine, a meperidine metabolite excreted by the kidneys, has central nervous system excitatory properties that can lower the seizure threshold in patients with renal failure. Similarly, the prophoxyphene metabolite has cardiovascular toxicity effects. Because of these serious adverse effects, both meperidine and propoxyphene should be avoided in patients with renal problems (69). Acetaminophen should be considered initially for mild or moderate pain. If not effective, opioids analgesics or nonselective cyclooxygenase-2 inhibitors should be considered. Use of selective cycloxygenase-2 inhibitors should be avoided in patients with ischemic heart disease because of its risk of cardiovascular complications (71–73). Finally, many of the neuromuscular blocking agents are excreted by the kidney and thus may display prolonged action and depolarization in patients with impaired renal function as the effects of the antagonist dissipate (23,74,75).

Antihypertensive and Cardiovascular Agents

In Table 104-5 are summarized dosage recommendations for antihypertensive and cardiovascular agents. The most common cause of death in the end-stage renal disease population is cardiovascular disease. A number of factors contribute to cardiovascular diseases in patients with renal failure. The risk of cardiovascular diseases increases with age, hypertension, hyperlipidemia, smoking, and anemia. Unfortunately, most of the cardiovascular interventional studies have been done in

TABLE 104-9

GASTROINTESTINAL AGENTS DOSING IN RENAL FAILURE

Gastrointestinal agents	Normal doses		% of renal excretion	Dosage adjustment in renal failure			Comments
	Starting dose	Maximum dose		GFR >50	GFR 10 to 50	GFR <10	
Anti-ulcer agents							
Cimetidine (274,275)	300 mg po tid	800 mg po bid	60%	100%	75%	25%	Multiple drug-drug interactions; beta blockers, sulfonylurea, theophylline, warfarin, etc
Famotidine (276,277)	20 mg po bid	40 mg po bid	70%	100%	75%	25%	Headache, fatigue, thrombocytopenia, alopecia
Lansoprazole (278)	15 mg po qd	30 mg bid	None	100%	100%	100%	Headache, diarrhea
Nizatidine (279,280)	150 mg po bid	300 mg po bid	20%	100%	75%	25%	Headache, fatigue, thrombocytopenia, alopecia
Omeprazole (281,282)	20 mg po qd	40 mg po bid	None	100%	100%	100%	Headache, diarrhea
Rabeprazole (283)	20 mg po qd	40 mg po bid	None	100%	100%	100%	Headache, diarrhea
Pantoprazole (284)	40 mg po qd	80 mg po bid	None	100%	100%	100%	Headache, diarrhea
Ranitidine (285,286)	150 mg po bid	300 mg po bid	80%	100%	75%	25%	Headache, fatigue, thrombocytopenia, alopecia
Cisapride (287)	10 mg po tid	20 mg qid	5%	100%	100%	50% to 75%	Avoid with azole antifungal, macrolide antibiotics and other P450 IIIA-4 inhibitors
Metoclopramide (287)	10 mg po tid	30 mg po qid	15%	100%	100%	50% to 75%	Increase cyclosporine/tacrolimus level. Neurotoxic
Misoprostol (288)	100 mcg po bid	200 mcg po qid		100%	100%	100%	Diarrhea, N/V, abortifacient agent
Sucralfate (289)	1 gm po qid	1 gm po qid	None	100%	100%	100%	Constipation, decrease absorption of MMF

TABLE 104-10

NEUROLOGIC/ANTICONVULSANT DOSING IN RENAL FAILURE

Anticonvulsants (290)	Normal doses		% of renal excretion	Dosage adjustment in renal failure			Comments	HD	CAPD	CVVH
	Starting dose	Maximum dose		GFR >50	GFR 10 to 50	GFR <10				
Carbamazapine (291,292)	2 to 8 mg/kg/day; adjust for side effect and TDM		2%	100%	100%	100%	Plasma concentration: 4 to 12, double vision, fluid retention, mylosuppression.	None	None	None
Clonazepam (290)	0.5 mg tid	2 mg tid	1%	100%	100%	100%	Although no dose reduction is recommended, the drug has not been studied in patients with renal impairment. Recommendations are based on known drug characteristics not clinical trials data.	None	No data	N/A
Ethosuximide (293)	5 mg/kg/day; adjust for side effect and TDM		20%	100%	100%	100%	Plasma concentration: 40 to 100, headache.	None	No data	No data
Felbamate (290)	400 mg/tid	1,200 mg/tid	90%	100%	50%	25%	Anorexia, vomiting, insomnia, nausea.	Dose after dialysis	Dose for GFR <10	Dose for GFR 10 to 50
Gabapentin (290)	150 mg tid	900 mg tid	77%	100%	50%	25%	Less CNS side effects compared to other agents.	300 mg load, then 200 to 300 after hemodialysis	300 mg qod	Dose for GFR 10 to 50
Lamotrigine (290)	25 to 50 mg/day	150 mg/day	1%	100%	100%	100%	Autoinduction, major drug-drug interaction with valproate.	No data	No data	Dose for GFR 10 to 50
Levetiracetam (290)	500 mg bid	1,500 mg bid	66%	100%	50%	50%		250 to 500 mg after dialysis	Dose for GFR <10	Dose for GFR 10 to 50
Oxcarbazepine (290)	300 mg bid	600 mg bid	1%	100%	100%	100%	Less effect on P450 compared to carbamazapine.	No data	No data	No data
Phenobarbital (290)	20 mg/kg/day; adjust for side effect and TDM		1%	100%	100%	100%	Plasma concentration: 15 to 40, insomnia.			
Phenytoin (294,295)	20 mg/kg/day; adjust for side effect and TDM		1%	Adjust for renal failure and low albumin			Plasma concentration: 10 to 20, nystagmus, check free phenytoin level.	None	None	None

Primidone (296)	50 mg	100 mg	1%	100%	100%	100%	Plasma concentration: 5 to 20.	1/3 dose	No data	No data
Sodium valproate (297,298)	7.5 to 15 mg/kg/day; adjust for side effect and TDM		1%	100%	100%	100%	Plasma concentration: 50 to 150, weight gain, hepatitis, check free valproate level.	None	None	None
Tiagabine (290)	4 mg qd, Increase 4mg/day, titrate weekly		2%	100%	100%	100%	Total daily dose may be increased by 4 to 8 mg at weekly intervals until clinical response is achieved or up to 32 mg/day. The total daily dose should be given in divided doses two to four times daily.	None	None	Dose for GFR 10 to 50
Topiramate (290)	50 mg/day	200 mg bid	70%	100%	50%	Avoid		No data	No data	Dose for GFR 10 to 50
Trimethadione (290)	300 mg tid-qid	600 mg tid-qid	None	q8h	q8 to 12h	q12 to 24h	Active metabolites with long half-life in ESRD. Nephrotic syndrome.	No data	No data	Dose for GFR 10 to 50
Vigabatrin (290)	1 g bid	2 g bid	70%	100%	50%	25%	Encephalopathy with drug accumulation.	No data	No data	Dose for GFR 10 to 50
Zonisamide (290)	100 mg qd	100 to 300 mg qd-bid	30%	100%	75%	50%	Manufacturer recommends that Zonisamide should not be used in patients with renal failure (estimated GFR <50 mL/minute) as there has been insufficient experience concerning drug dosing and toxicity. Zonisamide doses of 100 to 600 mg/day are effective for normal renal function. Dose recommendations for renal impairment based on clearance ratios.	Dose for GFR <10	Dose for GFR <10	Dose for GFR <10 to 50

TABLE 104-11

RHEUMATOLOGIC DOSING IN RENAL FAILURE

Arthritis and gout agents	Normal dosage	% of renal excretion	Dosage adjustment in renal failure			Comments	HD	CAPD	CVVH
			GFR >50	GFR 10 to 50	GFR <10				
Allopurinol (80,299)	300 mg q24h	30	75%	50%	25%	Interstitial nephritis. Rare xanthine stones. Renal excretion of active metabolite with $T_{1/2}$ of 25 hours in normal renal function; $T_{1/2}$ one week in patients with ESRD. Exfoliative dermatitis.	1/2 dose	No data	Dose for GFR 10 to 50
Auranofin (300)	6 mg q24h	50	50%	Avoid	Avoid	Proteinuria and nephritic syndrome.	None	None	None
Colchicine (301,302)	Acute: 2 mg then 0.5 mg q6h Chronic: 0.5 to 1.0 mg q24h	5 to 17	100%	50% to 100%	25%	Avoid prolonged use if GFR <50 mL/minute.	None	No data	Dose for GFR 10 to 50
Gold sodium (303)	25 to 50 mg	60 to 90	50%	Avoid	Avoid	Thiomalate proteinuria; nephritic syndrome; membranous nephritis.	None	None	Avoid
Penicillamine (304)	250 to 1,000 mg q24h	40	100%	Avoid	Avoid	Nephrotic syndrome.	1/3 dose	No data	Dose for GFR 10 to 50
Probenecid (305)	500 mg bid	<2	100%	Avoid	Avoid	Ineffective at decreased GFR.	Avoid	No data	Avoid
Nonsteroidal anti-inflammatory drugs (306,307)						May decrease renal function. Decrease platelet aggregation. Nephrotic syndrome. Interstitial nephritis. Hyperkalemia. Sodium retention.			
Diclofenac (308)	25 to 75 mg bid	<1	50% to 100%	25% to 50%	25%		None	None	Dose for GFR 10 to 50

Diflunisal (309)	250 to 500 mg bid	<3	100%	50%	50%		None	None	Dose for GFR 10 to 50
Etodolac (310)	200 mg bid	Negligible	100%	100%	100%		None	None	Dose for GFR 10 to 50
Fenoprofen (306,307)	300 to 600 mg qid	30	100%	100%	100%		None	None	Dose for GFR 10 to 50
Flurbiprofen (311)	100 mg bid-tid	20	100%	100%	100%		None	None	Dose for GFR 10 to 50
Ibuprofen (312)	800 mg tid	1	100%	100%	100%		None	None	Dose for GFR 10 to 50
Indomethacin (313,314)	25 to 50 mg tid	30	100%	100%	100%		None	None	Dose for GFR 10 to 50
Ketoprofen (315)	25 to 75 mg tid	<1	100%	100%	100%		None	None	Dose for GFR 10 to 50
Ketorolac (316)	30 to 60 mg load then 15 to 30 mg q6h	30 to 60	100%	50%	25% to 50%	Acute hearing loss in ESRD.	None	None	Dose for GFR 10 to 50
Meclofenamic acid (306,307)	50 to 100 tid-qid	2 to 4	100%	100%	100%		None	None	Dose for GFR 10 to 50
Mefanamic acid (306,307)	250 mg qid	<6	100%	100%	100%		None	None	Dose for GFR 10 to 50
Nabumetone (306,307)	1.0 to 2.0 g q24h	<1	100%	50% to 100%	50% to 100%		None	None	Dose for GFR 10 to 50
Naproxen (317)	500 mg bid	<1	100%	100%	100%		None	None	Dose for GFR 10 to 50
Oxaproxin (306,307)	1,200 mg q24h	<1	100%	100%	100%		None	None	Dose for GFR 10 to 50
Phenylbutazone (306,307)	100 mg tid-qid	1	100%	100%	100%		None	None	Dose for GFR 10 to 50
Piroxicam (318)	20 mg q24h	10	100%	100%	100%		None	None	Dose for GFR 10 to 50
Sulindac (319)	200 mg bid	7	100%	100%	100%	Active sulfide metabolite in ESRD.	None	None	Dose for GFR 10 to 50
Tolmetin (306,307)	400 mg tid	15	100%	100%	100%		None	None	Dose for GFR 10 to 50

TABLE 104-12

SEDATIVE DOSING IN RENAL FAILURE

Sedatives	Normal dosage	% of renal excretion	Dosage adjustment in renal failure			Comments	HD	CAPD	CVVH
			GFR >50	GFR 10 to 50	GFR <10				
Barbiturates (82)						May cause excessive sedation, increase osteomalacia in ESRD. Charcoal hemoperfusion and hemodialysis more effective than peritoneal dialysis for poisoning.			
Pentobarbital (82)	30 mg q6 to 8h	Hepatic	100%	100%	100%		None	No data	Dose for GFR 10 to 50
Phenobarbital (82)	50 to 100 mg q8 to 12h	Hepatic (renal)	q8 to 12h	q8 to 12h	q12 to 16h	Up to 50% unchanged drug excreted with urine with alkaline diuresis.	Dose after dialysis	1/2 normal dose	Dose for GFR 10 to 50
Secobarbital (82)	30 to 50 mg q6 to 8h	Hepatic	100%	100%	100%		None	None	N/A
Thiopental (320)	Anesthesia induction (individualized)	Hepatic	100%	100%	100%		N/A	N/A	N/A
Benzodiazepines (83)						May cause excessive sedation and encephalopathy in ESRD.			
Alprazolam (321)	0.25 to 5.0 mg q8h	Hepatic	100%	100%	100%		None	No data	N/A
Clorazepate (322)	15 to 60 mg q24h	Hepatic (renal)	100%	100%	100%		No data	No data	N/A
Chlordiazepoxide (323)	15 to 100 mg q24h	Hepatic	100%	100%	50%		None	No data	Dose for GFR 10 to 50
Clonazepam (82)	1.5 mg q24h	Hepatic	100%	100%	100%	Although no dose reduction is recommended, the drug has not been studied in patients with renal impairment. Recommendations are based on known drug charactersitcs not clinical trials data.	None	No data	N/A
Diazepam (82)	5 to 40 mg q24h	Hepatic	100%	100%	100%	Active metabolites, desmethyldiazepam, and oxazepam may accumulate in renal failure. Dose should be reduced if given longer than a few days. Protein binding decreases in uremia.	None	No data	None

Estazolam (82)	1 mg qhs	Hepatic	100%	100%	100%		No data	No data	N/A
Flurazepam (82)	15 to 30 mg qhs	Hepatic	100%	100%	100%		None	No data	N/A
Lorazepam (324)	1 to 2 mg q8 to 12h	Hepatic	100%	100%	100%		None	No data	Dose for GFR 10 to 50
Midazolam (325)	Individualized	Hepatic	100%	100%	50%		N/A	N/A	N/A
Oxazepam (326)	30 to 120 mg q24h	Hepatic	100%	100%	100%		None	No data	Dose for GFR 10 to 50
Quazepam (327)	15 mg qhs	Hepatic	No data	No data	No data		Unknown	No data	N/A
Temazepam (82)	30 mg qhs	Hepatic	100%	100%	100%		None	None	N/A
Triazolam (328,329)	0.25 to 0.50 mg qhs	Hepatic	100%	100%	100%	Protein binding correlates with alpha-1 acid glycoprotein concentration.	None	None	N/A
Benzodiazepines (82)						May cause excessive sedation and encephalopathy in ESRD.			
Benzodiazepine Antagonist									
Flumazenil (82)	0.2 mg IV over 15 sec	Hepatic	100%	100%	100%		None	No data	N/A
Miscellaneous Sedative Agents									
Buspirone (330)	5 mg q8h	Hepatic	100%	100%	100%		None	No data	N/A
Ethchlorvynol (331)	500 mg qhs	Hepatic	100%	Avoid	Avoid	Removed by hemoperfusion. Escessive sedation.	Avoid	Avoid	N/A
Haloperidol (82)	1 to 2 mg q8 to 12h	Hepatic	100%	100%	100%	Hypertension, excessive sedation.	None	None	Dose for GFR 10 to 50
Lithium carbonate (332)	0.9 to 1.2 g q24h	Renal	100%	50% to 75%	25% to 50%	Nephrotoxic. Nephrogenic diabetes insipidus. Nephrotic syndrome. Renal tubular acidosis. Interstitial fibrosis. Acute toxicity when serum levels >1.2 mEq/L. Serum levels should be measure periodically 12 hours after dose. $T_{1/2}$ does not reflect extensive tissue accumulation. Plasma levels rebound after dialysis. Toxicity enhanced by volume depletion, NSAIDs, and diuretics.	Dose after dialysis	None	Dose for GFR 10 to 50
Meprobamate (82)	1.2 to 1.6 g q24h	Hepatic (renal)	q6h	q9 to 12h	q12 to 18h	Excessive sedation. Excretion enhanced by forced diuresis.	None	No data	N/A

TABLE 104-13

ANTIPARKINSON DOSING IN RENAL FAILURE

Antiparkinson agents	Normal dosage	% of renal excretion	Dosage adjustment in renal failure			Comments	HD	CAPD	CVVH
			GFR >50	GFR 10 to 50	GFR <10				
Carbidopa (333)	1 tab tid to 6 tabs daily	30	100%	100%	100%	Require careful titration of dose according to clinical response.	No data	No data	No data
Levodopa (333)	25 to 500 mg bid to 8 g q24h	None	100%	50% to 100%	50% to 100%	Active and inactive metabolites excreted in urine. Active metabolites with long $T_{1/2}$ in ESRD.	No data	No data	Dose for GFR 10 to 50

patients with normal renal function and patients with serum creatinine greater than 2 mg/dL have been excluded. It is still strongly recommended to treat these risk factors aggressively. Hypertension complicates the management of most patients with renal insufficiency and impacts adversely on renal disease progression and increases the risk of cardiovascular events (76).

Patients with renal disease, in particular diabetic patients and patients with the metabolic syndrome, have high level of serum triglyceride, low-density lipoproteins (LDL) and total cholesterol. All patients with renal insufficiency should have an annual lipid panel and should be managed according new ATP III guideline (77,78).

Endocrine and Metabolic Agents

Dosage recommendations for endocrine and metabolic agents are provided in Tables 104-6, 104-7, and 104-8. Renal failure is associated with peripheral insulin resistance and decreased insulin metabolism by the kidney. In the presence of renal insufficiency, sulfonylureas that are excreted primarily by the kidney should be avoided, as prolonged hypoglycemia may result from the accumulation of such agents. Peritoneal dialysis allows for intraperitoneal insulin therapy, which has been shown to provide better overall control of plasma glucose when compared to standard subcutaneous injection therapy Hyperlipidemia also complicates renal failure and adds to the increased risk of atherosclerotic complications in this population. Lipid-lowering agents such as bile acids can add to fluid overload and worsen acidosis in patients with renal failure, while other agents, such as lovastatin and clofibrate, have been associated with rhabdomyolysis (79).

Gastrointestinal Drugs

In Table 104-9 are summarized dosage recommendations for gastrointestinal drugs. Patients with renal insufficiency experience gastrointestinal disorders more often than the general population, particularly peptic ulcer disease. Prior to the development of H_2 blockers, antacid therapy with compounds containing aluminum, calcium, magnesium, or bicarbonate was the mainstay. Excessive intake of calcium carbonate can result in the milk–alkali syndrome, characterized by hypercalcemia, metabolic alkalosis, and renal failure. Aluminum toxicity has been well described with chronic ingestion of aluminum-containing antacids in the setting of renal failure.

Neurologic Agents

Table 104-10 includes the dosage recommendations for neurologic agents. Unfortunately, seizure disorders complicate renal insufficiency and end-stage renal disease (490). Phenytoin is commonly used as an anticonvulsant, but in patients with renal impairment, its Vd increases while its degree of protein binding decreases. As a result, a low total plasma phenytoin level may not reflect subtherapeutic drug levels, as the "free phenytoin" level may be adequate. Following free or unbound phenytoin levels is prudent in patients with markedly reduced renal function.

Rheumatologic Agents

Summarized dosage recommendations for rheumatologic agents are in Table 104-11. Although dosage reductions of nonsteroidal antiinflammatory drugs (NSAIDs) are generally not required in renal failure, several precautions must be considered. When renal perfusion is reduced as in congestive heart failure or cirrhotic patients, vasodilatory prostaglandins may be key in maintaining renal blood flow. NSAIDs in such settings may cause reversible abrupt declines in renal function due to prostaglandin inhibition. Impaired potassium, sodium, and water excretion have also been attributed to NSAID use.

Patients with gout and renal insufficiency require reductions in allopurinol dosing because accumulation of its metabolite may underlie the complication of exfoliative dermatitis (80). Similarly, renal failure increases the risk of myopathy and polyneuropathy associated with colchicine use. Use of IV colchicines should be avoided in all patients with renal impairment (81).

Sedatives, Hypnotics, and Psychiatric Agents

In Tables 104-12, 104-13, and 104-14 are provided dosage recommendations for sedatives, hypnotics, and psychiatric agents. The majority of drugs in this category are lipid-soluble, highly protein bound, and excreted primarily by hepatic transformation to inactive metabolites, but increased sensitivity to the sedative side effects occurs in patients with renal impairment. Additionally, though dosage reduction generally is not required, increased sensitivity to the side effects of tricyclic antidepressants mandates a cautious approach in their use. Similarly, renal failure may exacerbate extrapyramidal side effects associated with phenothiazine therapy. In contrast, lithium is water-soluble and undergoes renal elimination. Renal

TABLE 104-14

ANTIPSYCHOTIC DOSING IN RENAL FAILURE

Antipsychotics (334,335)	Normal dosage	% of renal excretion	Dosage adjustment in renal failure			Comments	HD	CAPD	CVVH
			GFR >50	GFR 10 to 50	GFR <10				
Phenothiazines (334,335)						Orthostatic hypotension, extrapyramidal symptoms, and confusion can occur.			
Chlorpromazine (336)	300 to 800 mg q24h	Hepatic	100%	100%	100%		None	None	Dose for GFR 10 to 50
Promethazine (337)	20 to 100 mg q24h	Hepatic	100%	100%	100%	Excessive sedation may occur in ESRD.	No data	No data	Dose for GFR 10 to 50
Thioridazine (334,335)	50 to 100 mg po tid. Increase gradually. Maximum of 800 mg/day.								
Trifluoperazine (334,335)	1 to 2 mg bid. Increase to no more than 6 mg.								
Perphenazine (334,335)	8 to 16 mg po bid, tid, or qid. Increase to 64 mg daily								
Thiothixene (334,335)	2 mg po tid. Increase gradually to 15 mg daily								
Haloperidol (338)	1 to 2 mg q8 to 12h	Hepatic	100%	100%	100%	Hypotension, excessive sedation.	None	None	Dose for GFR 10 to 50
Loxapine (334,335)	12.5 to 50 mg IM q4 to 6h					Do not administer drug IV.			
Clozapine (339)	12.5 mg po. 25 to 50 daily to 300 to 450 by end of 2 weeks. Maximum: 900 mg daily	Metabolism nearly complete							
Risperidone (334,335)	1 mg po bid. Increase to 3 mg bid.								
Olanzapine (334,335)	5 to 10 mg	Hepatic				Potential hypotensive effects.			
Quetiapine (334,335)	25 mg po bid. Increase in increments of 25 to 50 bid or tid. 300 to 400 mg daily by day 4	Hepatic							
Ziprasideone (334,335)	20 to 100 mg q12h	Hepatic							

TABLE 104-15

MISCELLANEOUS DOSING IN RENAL FAILURE

Corticosteroids (340)	Normal dosage	% of renal excretion	Dosage adjustment in renal failure			Comments	HD	CAPD	CVVH
			GFR >50	GFR 10 to 50	GFR <10				
Betamethasone (340)	0.5 to 9.0 mg q24h	5	100%	100%	100%	May aggravate azotemia, Na^+ retention, glucose intolerance, and hypertension.	No data	No data	Dose for GFR 10 to 50
Budesonide (340)	No data	None	100%	100%	100%	Same as above.	No data	No data	Dose for GFR 10 to 50
Cortisone (340)	25 to 500 mg q24h	None	100%	100%	100%	Same as above.	None	No data	Dose for GFR 10 to 50
Dexamethasone (340)	0.75 to 9.0 mg q24h	8	100%	100%	100%	Same as above.	No data	No data	Dose for GFR 10 to 50
Hydrocortisone (340)	20 to 500 mg q24h	None	100%	100%	100%	Same as above.	No data	No data	Dose for GFR 10 to 50
Methylprednisolone (341)	4 to 48 mg q24h	<10	100%	100%	100%	Same as above.	Yes	No data	Dose for GFR 10 to 50
Prednisolone (342)	5 to 60 mg q24h	34	100%	100%	100%	same as above.	Yes	No data	Dose for GFR 10 to 50
Prednisone (342)	5 to 60 mg q24h	34	100%	100%	100%	Same as above.	None	No data	Dose for GFR 10 to 50
Triamcinolone (340)	4 to 48 mg q24h	No data	100%	100%	100%	Same as above.	No data	No data	Dose for GFR 10 to 50

TABLE 104-16

ANTICOAGULANT DOSING IN RENAL FAILURE

Anticoagulants	Normal doses		% of renal excretion	Dosage adjustment in renal failure			Comments	HD	CAPD	CVVH
	Starting dose	Maximum dose		GFR >50	GFR 10 to 50	GFR <10				
Alteplase (343)	60 mg over 1 hour then 20 mg/h for 2 hours		No data	100%	100%	100%	Tissue-type plasminogen activator [tPa].	No data	No data	Dose for GFR 10 to 50
Anistreplase (344)	30 U over 2 to 5 minures		No data	100%	100%	100%		No data	No data	Dose for GFR 10 to 50
Aspirin (177)	81 mg/day	325 mg/day	10%	100%	100%	100%	GI irritation and bleeding tendency.			
Clopidogrel (345)	75 mg/day	75 mg/day	50%	100%	100%	100%				
Dalteparin (346)	2,500 units Sq/day	5,000 units Sq/day	Unknown	100%	100%	100%				
Dipyridamole (347)	50 mg tid		No data	100%	100%	100%		No data	No data	N/A
Enoxaparin (348)	20 mg/day	30 mg bid	8%	100%	75% to 50%	50%	1 mg/kg q12h for treatment of DVT. Check anti-factor Xa activity 4 hours after 2nd dose in patients with renal dysfunction. Some evidence of drug accumulation in renal failure.			
Heparin (349)	75 U/kg load then 15 U/kg/hour		None	100%	100%	100%	Half-life increases with dose.	None	None	Dose for GFR 10 to 50
Iloprost (350)	0.5 to 2.0 ng/kg/min for 5 to 12 hours		No data	100%	100%	50%		No data	No data	Dose for GFR 10 to 50
Indobufen (351)	100 mg bid	200 mg bid	<15%	100%	50%	25%		No data	No data	N/A
Streptokinase (352)	25,0000 U load then 10,0000 U/hour		None	100%	100%	100%		N/A	N/A	Dose for GFR 10 to 50
Sulfinpyrazone (353)	200 mg bid		25% to 50%	100%	100%	Avoid	Acute renal failure. Uricosuric effect at low GFR.	None	None	Dose for GFR 10 to 50
Sulotroban	No data		52% to 62%	50%	30%	10%		No data	No data	No data
Ticlopidine (354)	250 mg bid	250 mg bid	2%	100%	100%	100%	Decrease CSA level and may cause severe neutropenia and thrombocytopenia	No data	No data	Dose for GFR 10 to 50
Tranexamic acid (355)	25 mg/kg tid-qid		90%	50%	25%	10%		No data	No data	No data
Urokinase (352)	4,400 U/kg load then 4,400 U/kg qh		No data	No data	No data	No data		No data	No data	Dose for GFR 10 to 50
Warfarin (356)	5 mg/day	Adjust per INR	<1%	100%	100%	100%	Monitor INR very closely. Start at 5 mg/day. 1 mg Vit. K IV over 30 minutes or 2.5 to 5 mg po can be used to normalize INR.	None	None	None

syndromes induced by lithium therapy include nephrogenic diabetes insipidus as well as acute and chronic renal failure. Lithium toxicity can be minimized by avoidance of volume depletion and concurrent diuretic therapy (82,83).

Miscellaneous Agents

In Tables 104-15 and 104-16 are provided dosage recommendations for a wide variety of miscellaneous agents.

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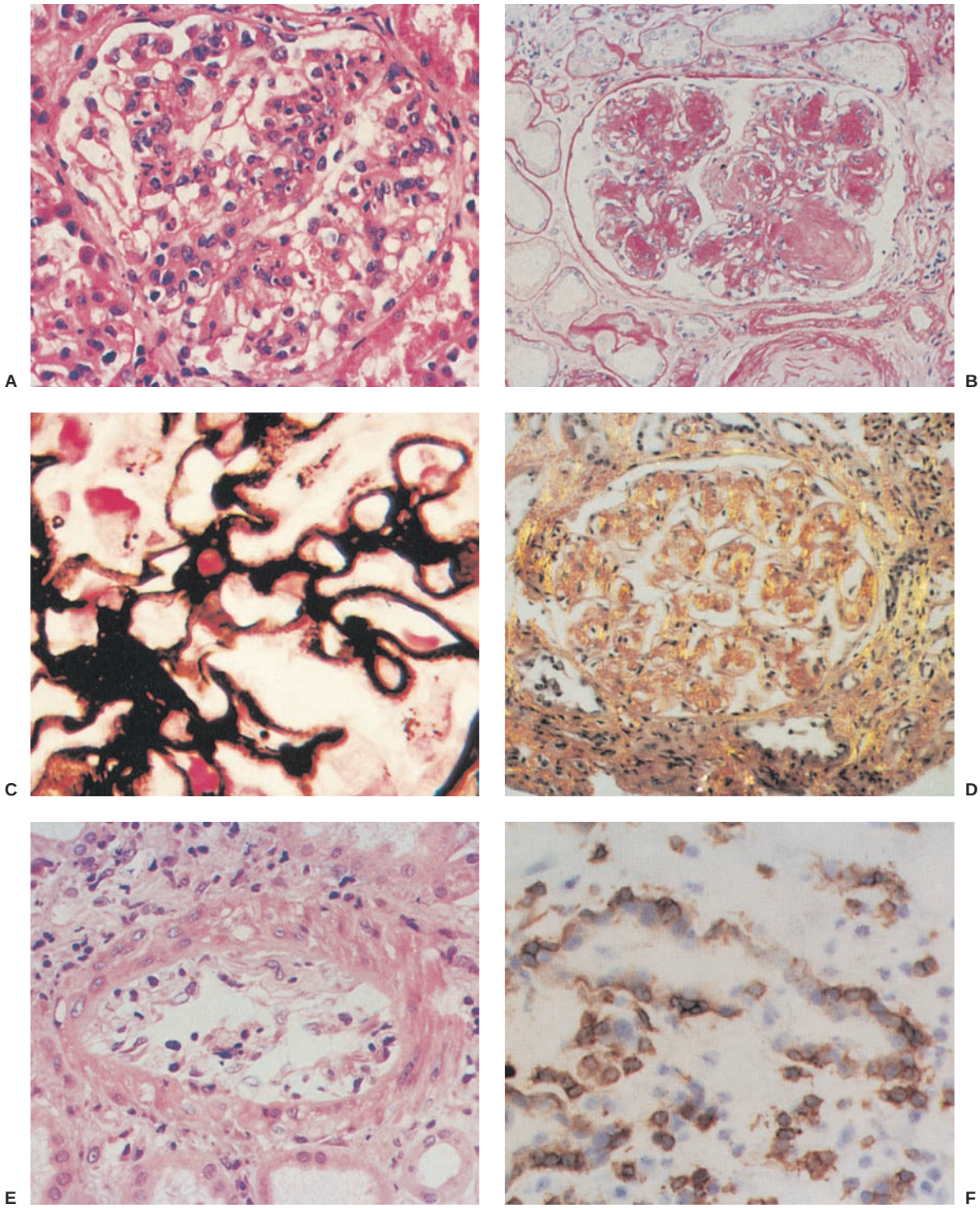
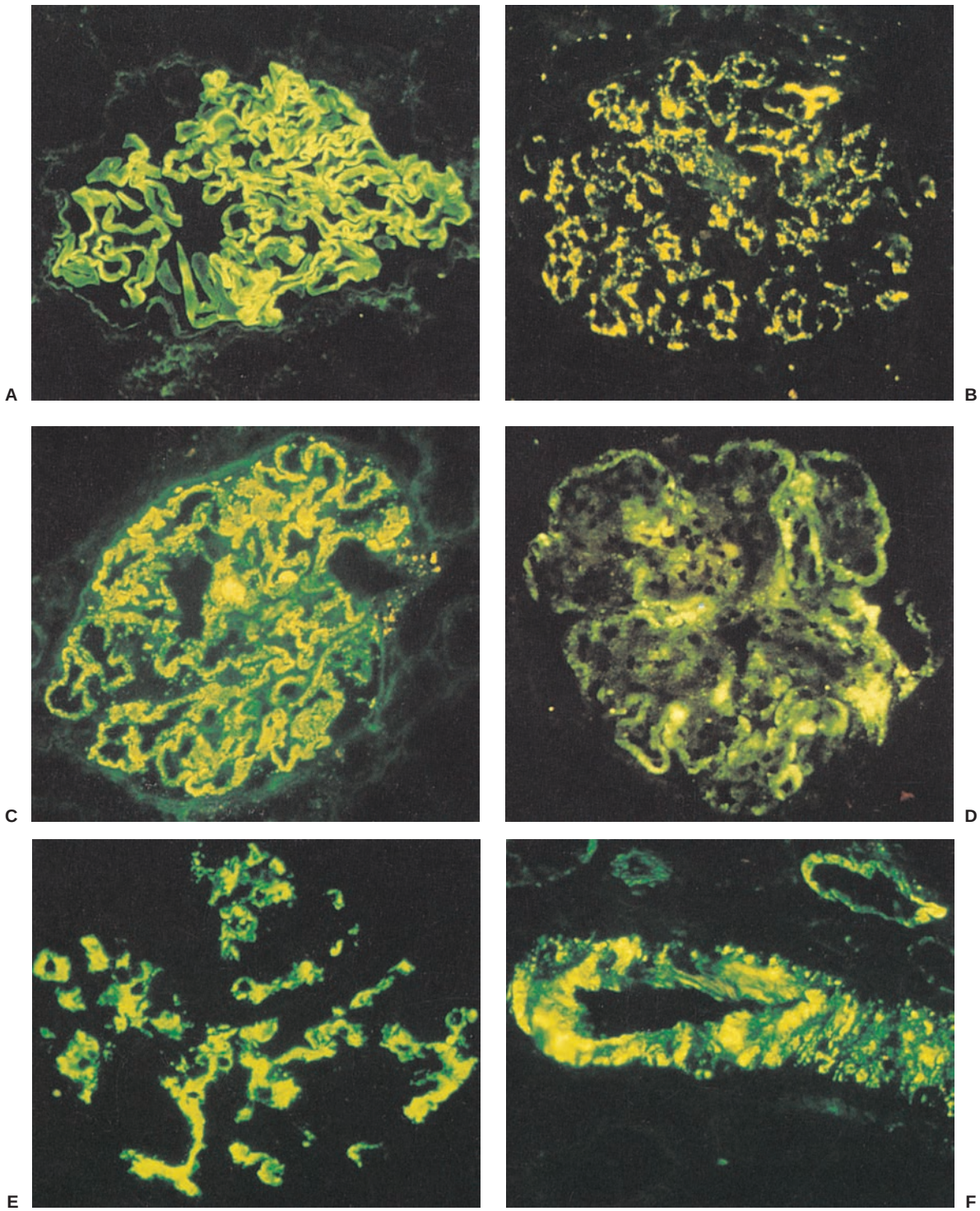
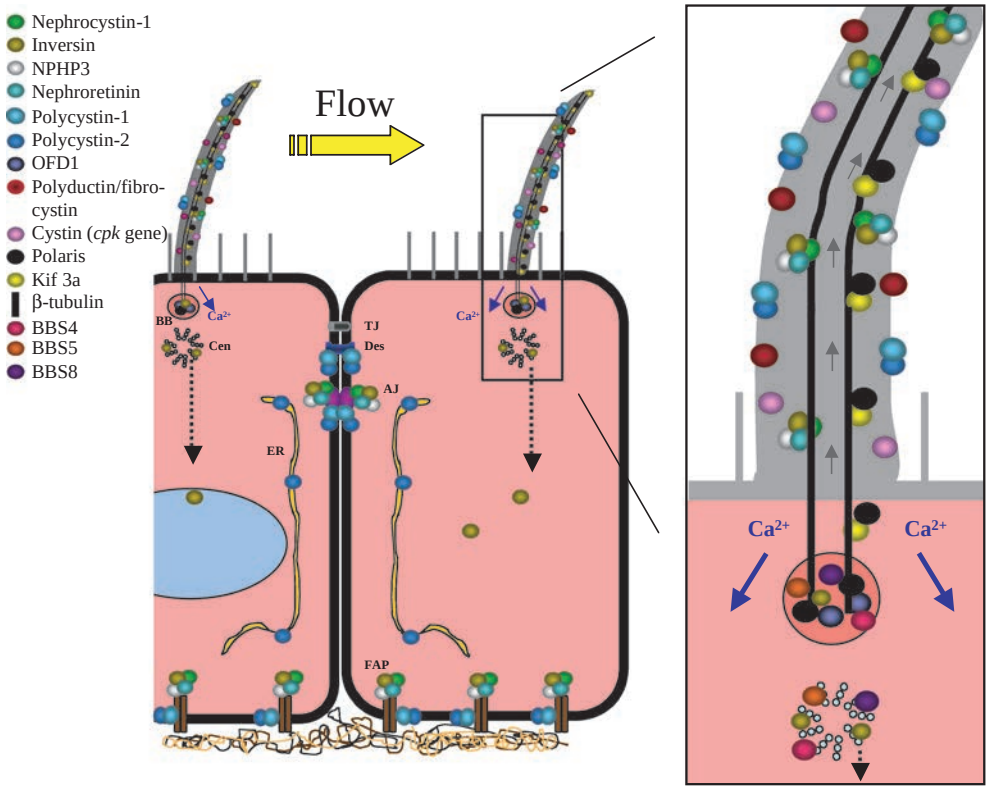


COLOR FIGURE 15-4. A: H&E stain of a glomerulus with moderate neutrophilic infiltrate in acute poststreptococcal glomerulonephritis. (Magnification ×200.) **B:** PAS stain of a glomerulus, illustrating early and late nodular mesangial expansion typical of nodular intercapillary glomerulosclerosis of diabetic nephropathy. (Magnification ×200.) **C:** PAMS stain depicting a portion of a capillary tuft with spikelike projections extending outward from the capillary basement membrane. This picture is characteristic of stage II idiopathic membranous glomerulonephritis. (Magnification ×1,000.) **D:** Depiction of the yellow-green birefringence of amyloid when stained with Congo red. Other tissue structures (particularly fibrous tissue) may rotate light and appear white when viewed with the polarizing microscope and should be distinguished from amyloid. (Polarization optics, magnification ×200.) **E:** This interlobular artery exhibits inflammation primarily in the intima, which is seen commonly in acute vascular rejection. Compare with the immunofluorescence pattern in Figure 15-22F, which demonstrates the transmural nature of the process. (H&E, magnification ×400.) **F:** Photomicrograph of immunoperoxidase preparation using LEU 2A antibody, an antibody to cytotoxic T cells, and diaminobenzidine. Several cross-sections of tubules and interstitium are shown from a typical example of acute cellular rejection. The T cells infiltrate both the interstitium and the tubular epithelial cells. (Magnification ×200.)

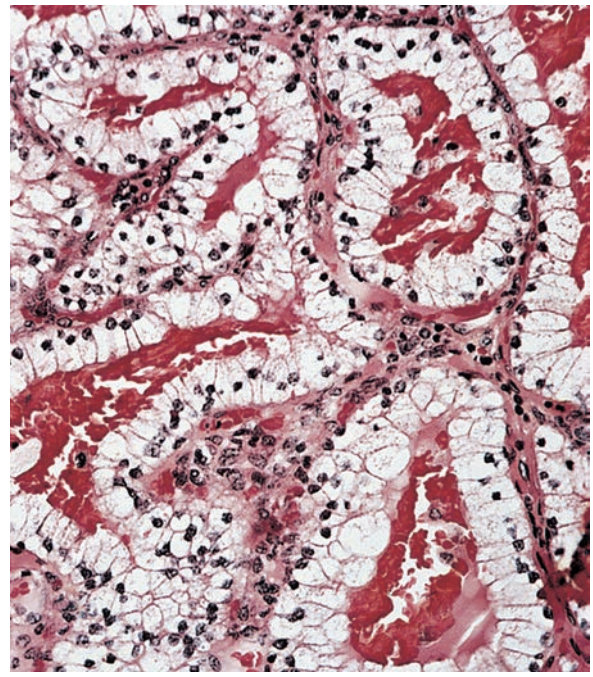


COLOR FIGURE 15-22. A: Glomerulus with fine, ribbonlike basement membrane staining with IgG indicative on anti-glomerular basement membrane disease. (Magnification ×200.) **B:** Glomerulus exhibiting coarse granular deposits of IgG distributed irregularly along the glomerular basement membranes. This pattern is typical of poststreptococcal glomerulonephritis. C3 is usually present in the same pattern. (Magnification ×200.) **C:** Glomerulus with fine IgG granules evenly deposited along the glomerular basement membrane. This pattern is typical of the subepithelial deposits of idiopathic membranous glomerulonephritis. (Magnification ×200.) **D:** Glomerulus demonstrating the typical pattern of subendothelial immune complex deposits. The outer edge of the deposits abuts the inner surface of the glomerular basement membrane and is relatively smooth. The inner aspect of the deposits is shaggy and irregular and may merge with the mesangium, which is expanded. This pattern is characteristic of membranoproliferative glomerulonephritis. (IgM, magnification ×200.) **E:** Glomerulus exhibiting the typical pattern of IgA mesangial immune deposits. Note the absence of basement membrane localization. The dark spaces in the midst of the deposits represent mesangial cells, not capillary lumina. This pattern is typical of IgA nephrology, Henoch-Schönlein purpura, and mesangiopathic lupus nephritis. (Magnification ×200.) **F:** Arteriole with transmural staining for IgM. This pattern is characteristic of relatively mild acute vascular lesions of vascular rejection. In more severe lesions there is greater disruption of the vessel wall, which also can be seen by light microscopy. This pattern should be distinguished from the subintimal glossy deposits of hyaline that are seen in chronic vascular disease. (Magnification ×400.)

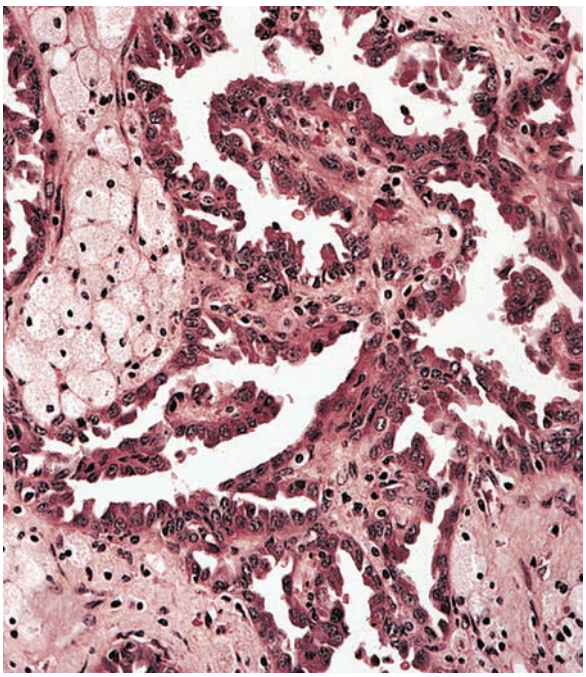


COLOR FIGURE 16-8. Subcellular localization of cystoproteins. Many cystoproteins have been localized to more than one intracellular domain. In this illustration, all known members of an interacting complex are shown colocalized to the same locations, even though this has not necessarily been shown for each unit. There are two exceptions: (i) polycystin-2 has been reported to have endoplasmic reticulum–specific functions as a calcium release channel that are independent of polycystin-1, and (ii) at least one form of inversin has been localized to the centriole and to the nucleus. The speckled arrow in the primary cilium indicates the direction of anterograde transport along the microtubule system mediated by kinesin-II, a heterotrimeric protein composed of two motor units (Kif3a, Kif3b) and one nonmotor unit (KAP3). *AJ*, adherens junction; *BB*, basal body; *Cen*, centriole; *ER*, endoplasmic reticulum; *FAP*, focal adhesion plaque; *TJ*, tight junction; *PC-1*, polycystin-1; *PC-2*, polycystin-2. (Adapted from: Watnick T, Germino G. From cilia to cyst. *Nat Genet* 2003;34:355, with permission.)

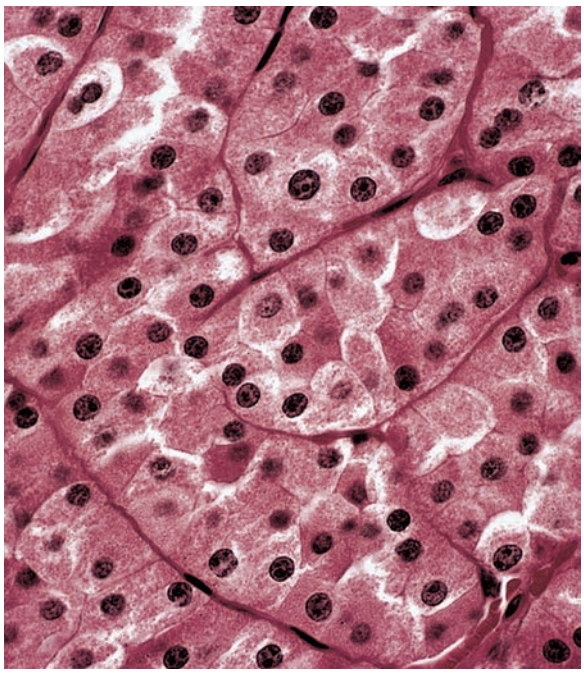
COLOR FIGURE 28-2. Renal cell carcinoma with metastatic spread to the forehead.



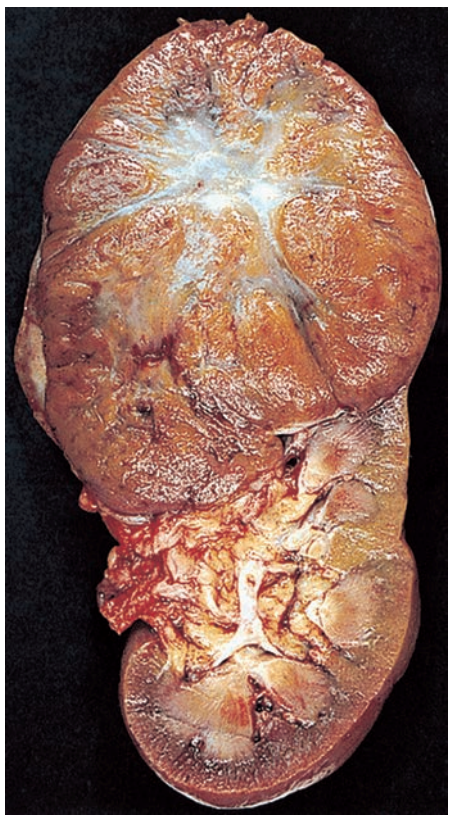
COLOR FIGURE 28-3. Renal cell carcinoma, clear-cell type: The cells are in an acinar arrangement and feature optically clear cytoplasm. (From: Murphy WM, Beckwith JB, Farrow GM. *Tumors of the kidney, bladder, and related urinary structures,* 3rd series. Washington, DC: The American Registry of Pathology, 1994, with permission.)



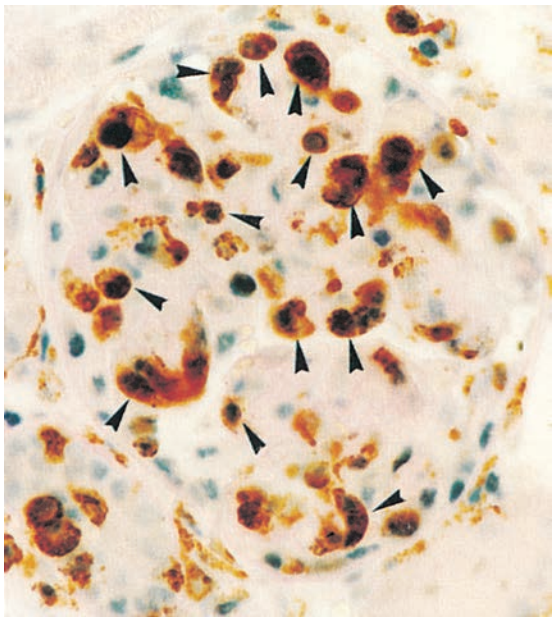
COLOR FIGURE 28-5. Renal cell carcinoma, papillary type: neoplastic cells over papillary structures with numerous stromal xanthoma cells. (From: Murphy WM, Beckwith JB, Farrow GM. *Tumors of the kidney, bladder, and related urinary structures,* 3rd series. Washington, DC: The American Registry of Pathology, 1994, with permission.)



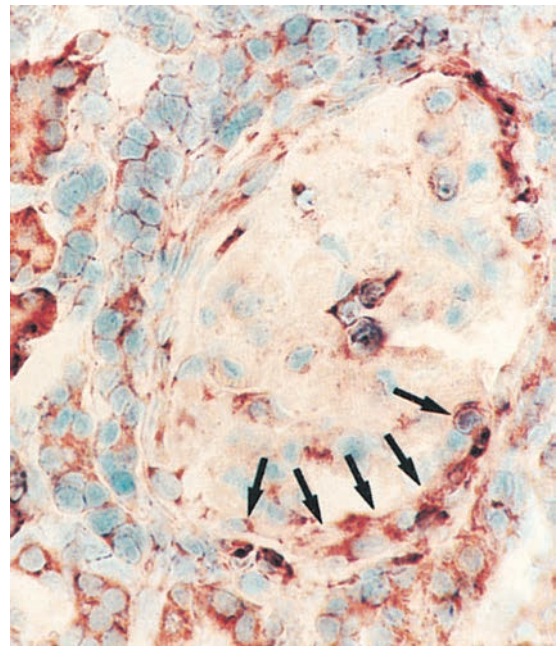
COLOR FIGURE 28-9. Renal oncocytoma. The cells are in tight alveolar arrangements and feature acidophilic cytoplasm. The nuclei are round and regular and exhibit no or single small nucleoli. (From: Murphy WM, Beckwith JB, Farrow GM. *Tumors of the kidney, bladder, and related urinary structures,* 3rd series. Washington, DC: The American Registry of Pathology, 1994, with permission.)



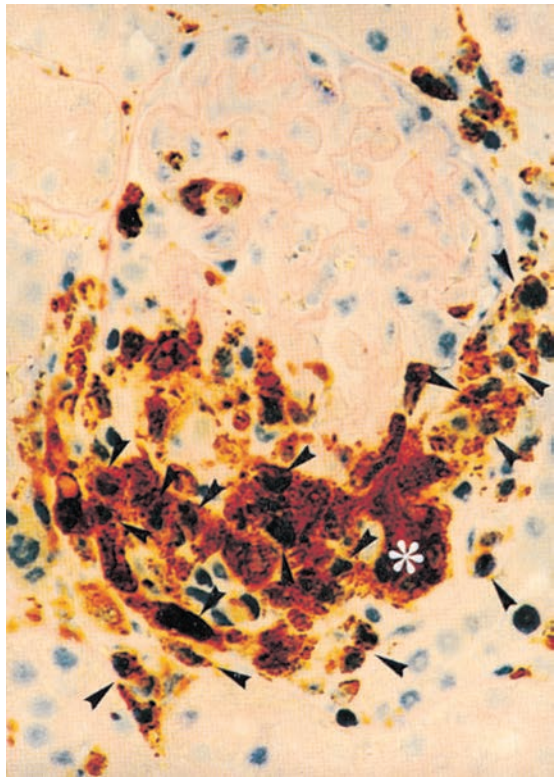
COLOR FIGURE 28-10. Renal oncocytoma. This mahogany-brown tumor exhibits a stellate central scar. Such scars can be visible on computed tomography (*CT*) images and suggest the diagnosis of oncocytoma. (From: Murphy WM, Beckwith JB, Farrow GM. *Tumors of the kidney, bladder, and related urinary structures,* 3rd series. Washington, DC: The American Registry of Pathology, 1994, with permission.)



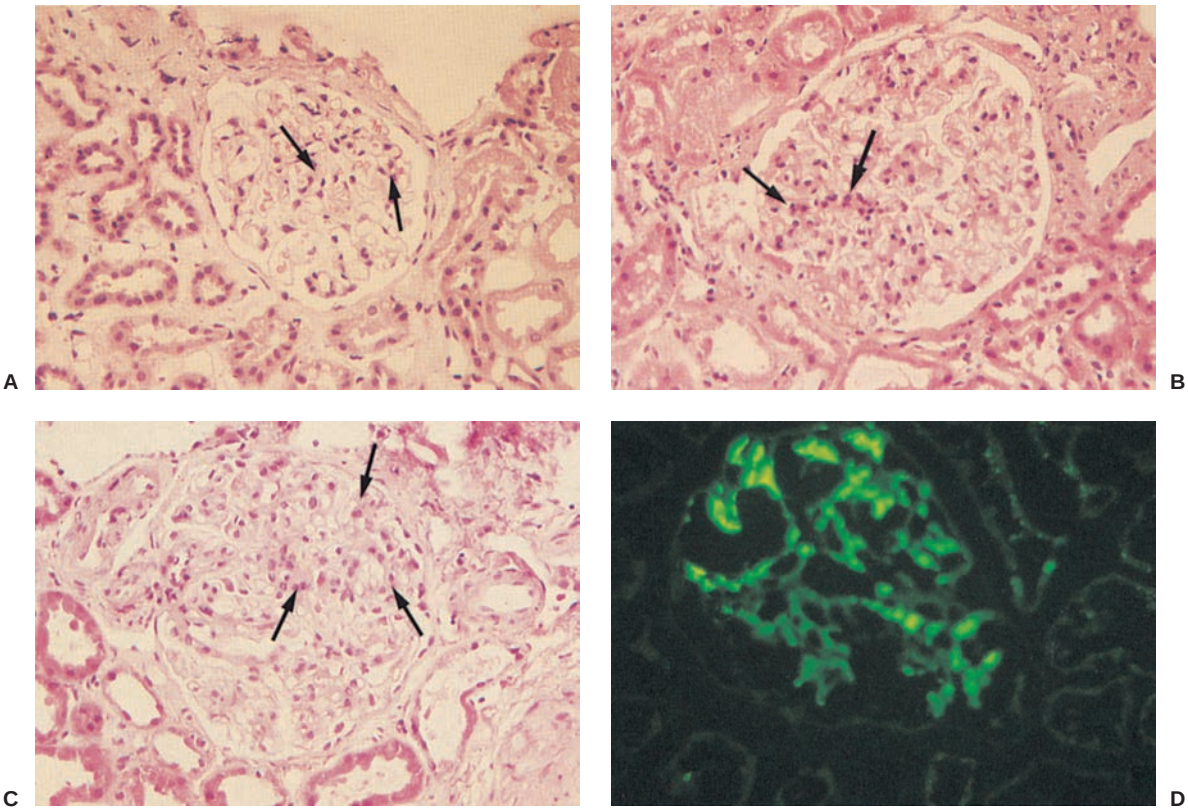
COLOR FIGURE 60-6. A rat glomerulus demonstrating hypercellularity from an animal with induced anti-glomerular basement membrane nephritis. Double immunostaining demonstrated that numerous glomerular macrophages (*arrows*; ED-1 positive [*brown*]) were proliferating (proliferating cell nuclear antigen positive [*blue/black*]) and contributing to the glomerular hypercellularity. (Periodic acid-Schiff, magnification ×400.)



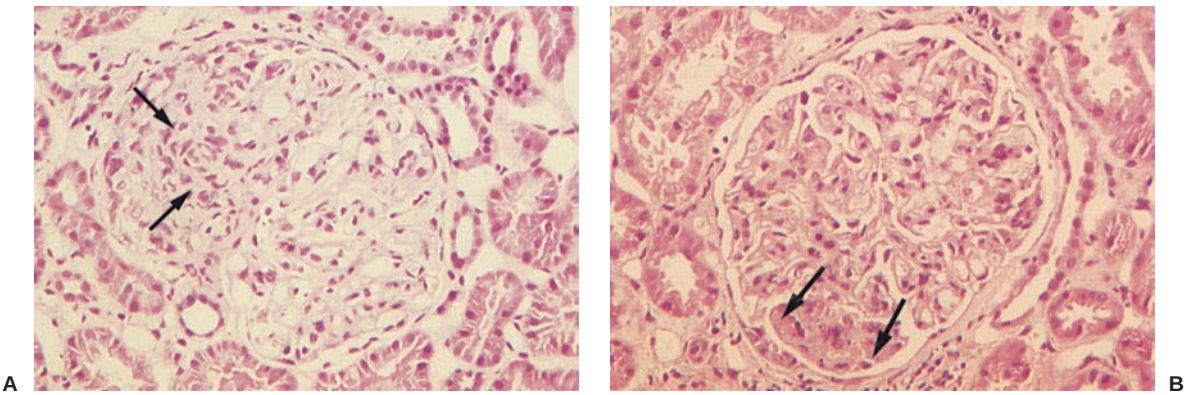
COLOR FIGURE 60-10. *In situ* hybridization demonstrating intercellular adhesion molecule-1 (ICAM-1) messenger RNA (mRNA) expression in a crescent from a rat with anti–glomerular basement membrane nephritis (*arrows*). The ICAM-1 mRNA has been depicted using *in situ* hybridization histochemistry. (Magnification ×400.)



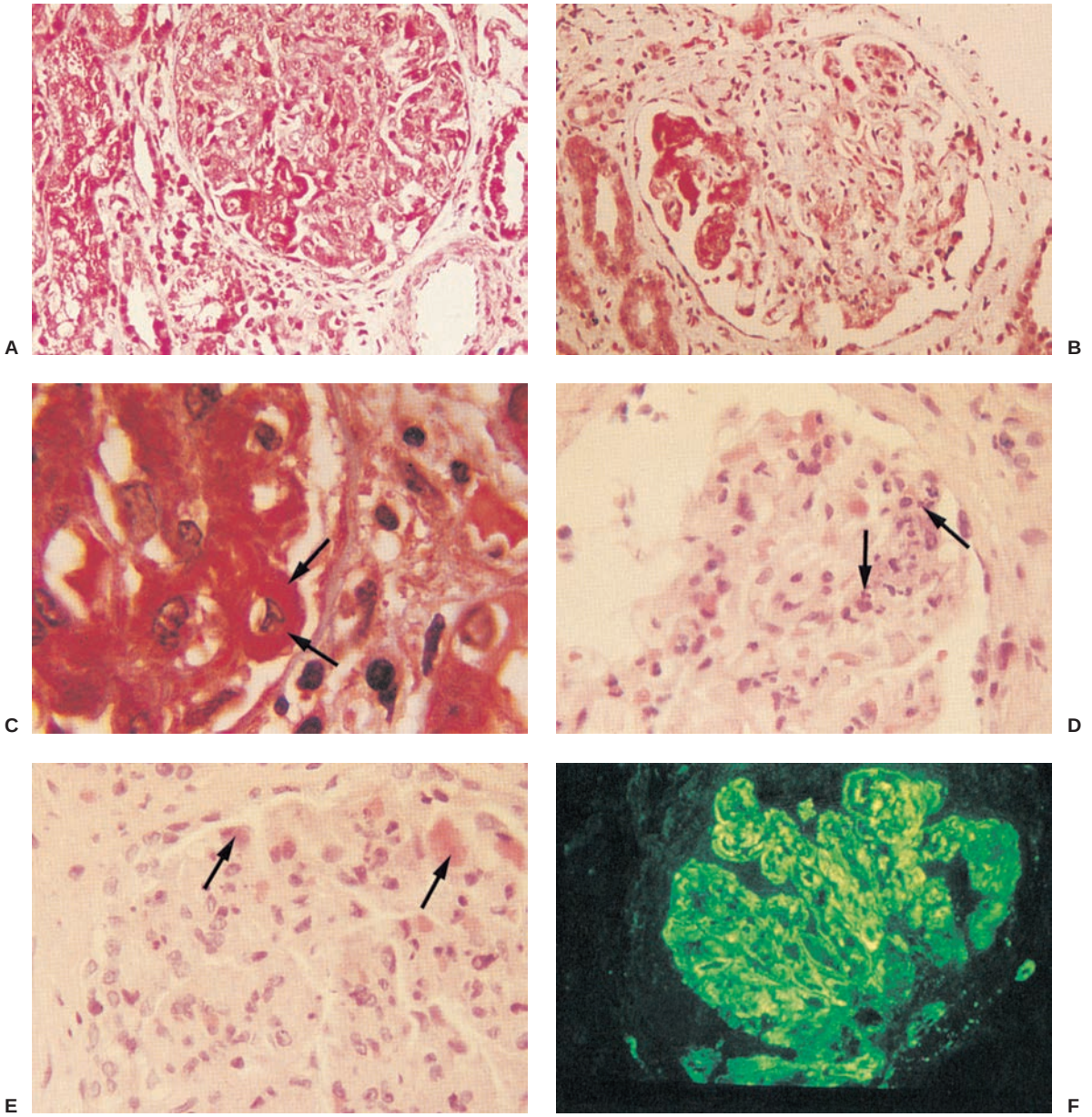
COLOR FIGURE 60-14. A rat glomerulus demonstrating an extensive crescent from an animal with anti-glomerular basement membrane nephritis. Macrophages are demonstrated using the ED-1 monoclonal antibody (*brown*), and the proliferating cell nuclear antigen antibody (*blue*) has been used to demonstrate proliferating cells. Proliferating macrophages are indicated by *arrowheads* and mononuclear giant cells by the *asterisk*. (Periodic acid-Schiff, magnification ×400.)



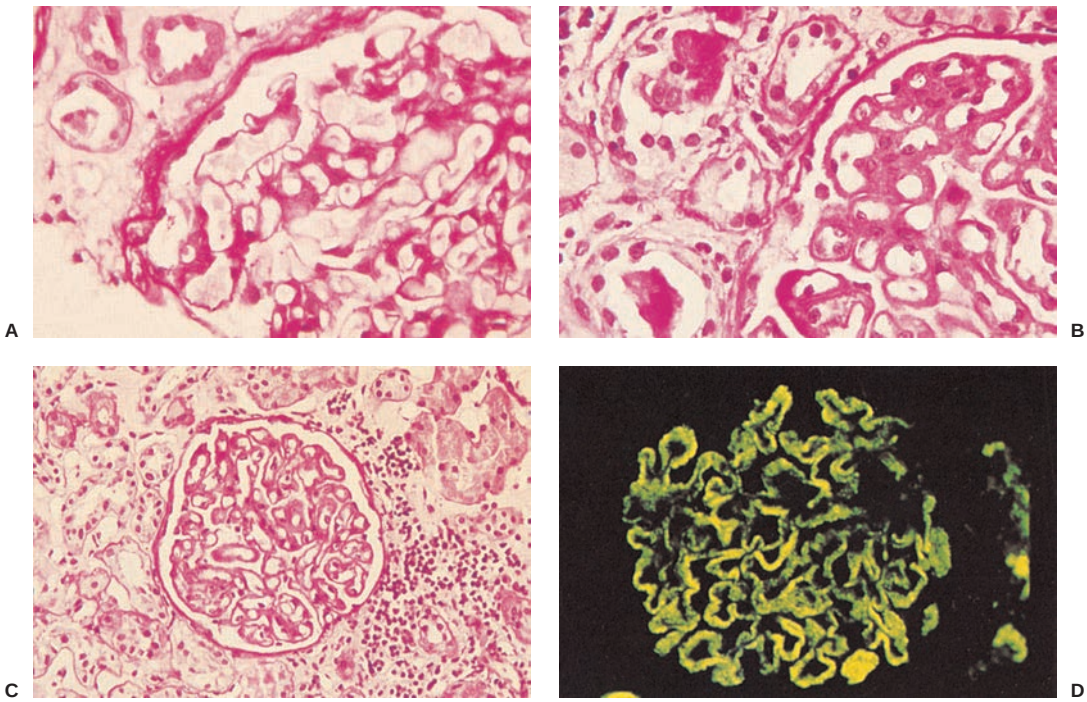
COLOR FIGURE 65-2. Histologic characteristics of mesangial glomerulonephropathy (World Health Organization Class II) in a patient with systemic lupus erythematosus. **A:** A normal glomerulus for comparison. Note the number and distribution of mesangial cells (*arrows*). (Hematoxylin and eosin, magnification ×200.) **B:** An early mesangial nephropathy displaying a subtle increase of mesangial cells (*arrows*). Compare with the normal glomerulus in **A**. (Hematoxylin and eosin, magnification ×200.) **C:** Another glomerulus from the same patient as in **B** but with a segmental accentuation of mesangial cells (*arrows*). This would be classified as a World Health Organization Class IIb lesion. (Hematoxylin and eosin, magnification ×200.) **D:** The mesangial matrix is accentuated by immunofluorescent deposits of IgG. This is an immunofluorescence analysis of the biopsy material shown in **B**. (Anti-IgG, magnification ×200.)



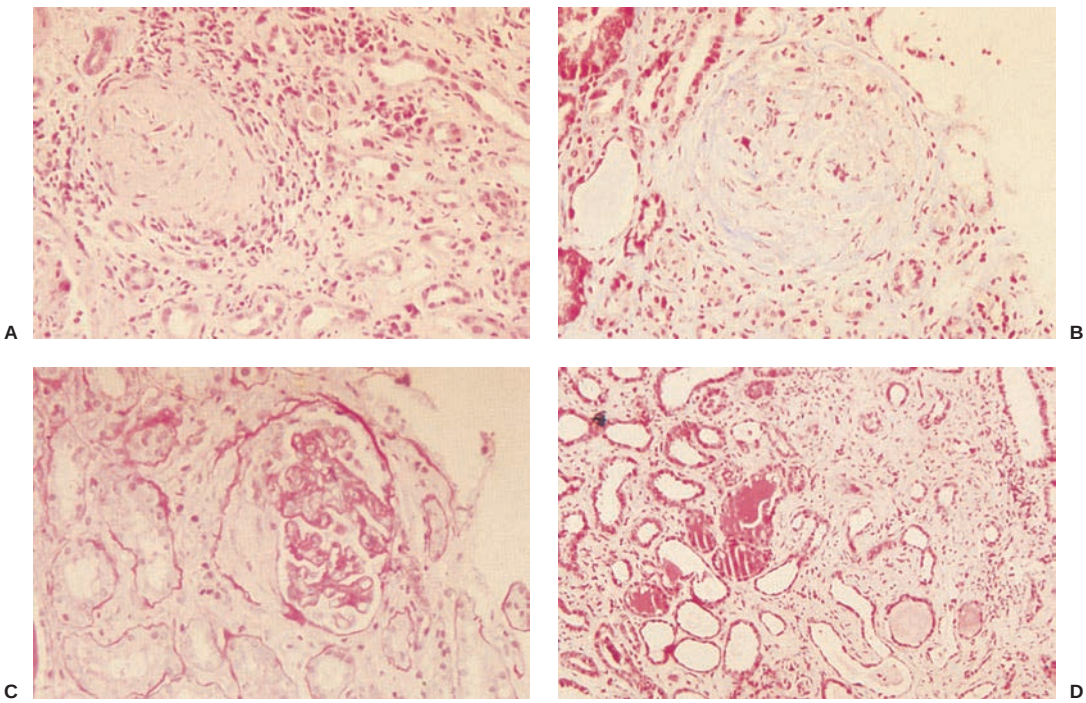
COLOR FIGURE 65-4. Histologic characteristics of focal proliferative glomerulonephritis (World Health Organization Class III) in a patient with systemic lupus erythematosus. **A:** Approximately one-half of this glomerular tuft is involved by mesangial proliferation (*arrows*), which at medium power is discernible as a segmental lesion. (Hematoxylin and eosin, magnification ×200.) **B:** Another glomerulus from the same patient. Here there is a mild global increase of mesangial cells and a segmental lesion with a suggestion of early tuft necrosis (*arrows*). (Hematoxylin and eosin, magnification ×200.) Less than half of the glomeruli in this biopsy demonstrated segmental lesions.



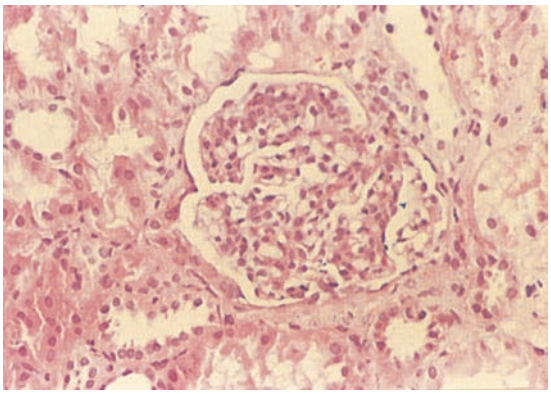
COLOR FIGURE 65-5. Histologic characteristics of diffuse proliferative glomerulonephritis (World Health Organization Class IV) in a patient with systemic lupus erythematosus. This plate also shows examples of the abnormalities used in the scoring of the activity index (Table 65-4). **A:** The majority of glomeruli in this case were involved by a global proliferation of mesangial cells and varying indicators of acute activity. (Trichrome, magnification ×200.) **B:** This trichrome stain accentuates the intracapillary thrombi present in the same biopsy as **A**. **C:** Subendothelial deposits are seen within the capillary loop (*arrows*). The resultant thickening of the membrane contour is the source of the descriptive term "wire loop." (Trichrome, magnification ×800.) **D:** Multiple neutrophils are apparent within the mesangium and capillary lumens (*arrows*). (Hematoxylin and eosin, magnification ×400.) **E:** Hematoxylin bodies (*arrows*) are not often seen in biopsy material. They are the result of altered nuclear DNA and are considered pathognomonic of lupus nephritis. Notice also the neutrophilic infiltrates and cellular proliferation, both features of an active lesion. (Hematoxylin and eosin, magnification ×400.) **F:** Extensive glomerular deposition of immunoglobulin demonstrated by immunofluorescent staining for IgG. Compare the distribution and intensity of staining with the immunofluorescence analysis of mesangial nephropathy shown in Figure 65-2D. (From: Kotzin BL, O'Dell JR. Systemic lupus erythematosus. In: Frank MM, et al, eds. *Samter's Immunologic Diseases,* 5th ed. Boston: Little, Brown; 1994, with permission.)



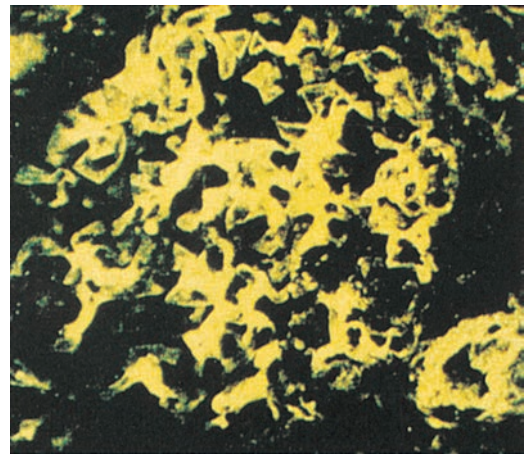
COLOR FIGURE 65-7. Histologic characteristics of membranous nephropathy (World Health Organization Class V) in a patient with systemic lupus erythematosus. **A:** Normal glomerulus for comparison. Notice the thin, delicate profile of the basement membranes. (Periodic acid–Schiff, magnification ×400.) **B:** Membranous nephropathy with thickened basement membranes. (Periodic acid–Schiff, magnification ×400.) Compare with the normal glomerulus in **A**. **C:** The thickened, rigid-appearing basement membranes of advanced membranous nephropathy are obvious even at medium power. (Periodic acid–Schiff, magnification ×200.) Lymphocytes are seen in the interstitium, and there is a hint of tubular dropout, an indicator that this is a chronic lesion. **D:** Immunofluorescence study of the same patient illustrated in **C**. The membranes are highlighted by fluorescent deposits of IgG. (Anti-IgG, magnification ×200.)



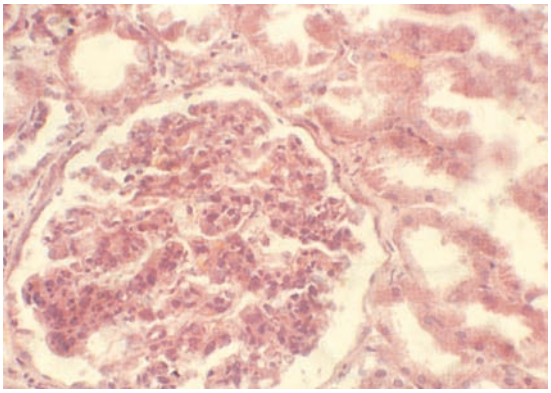
COLOR FIGURE 65-9. Examples of histologic features utilized in the chronicity index. **A:** An obsolescent glomerulus is surrounded by small strophic tubules. (Hematoxylin and eosin, magnification ×200.) **B:** The residuum of a glomerulus is barely visible in this example of a fibrous crescent. (Trichrome, magnification ×200.) **C:** A shrunken glomerular tuft is displaced by a fibrous crescent. **D:** Simplified, dilated tubules in a fibrotic stroma. (Hematoxylin and eosin, magnification ×200.)



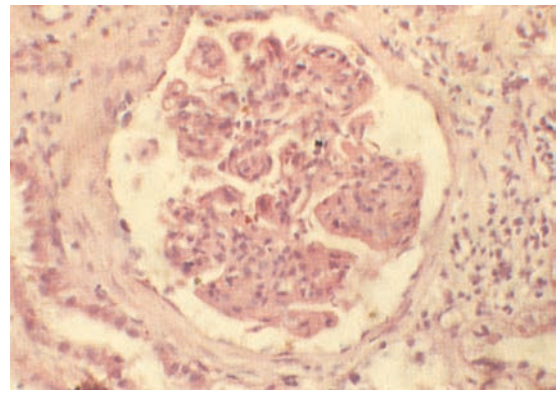
COLOR FIGURE 78-12. Axial mesangial proliferation in early schistosomal glomerulopathy. (Hematoxylin and eosin, magnification ×200.) (From: Barsoum RS. Schistosomal glomerulopathies. *Kidney Int* 1993;44:1, with permission.)



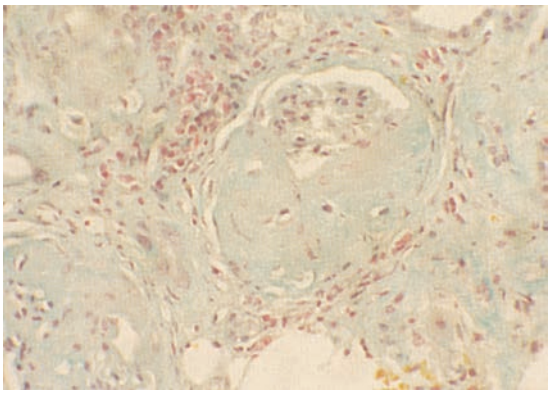
COLOR FIGURE 78-13. Mesangial deposits of schistosomal Gut antigens in early schistosomal glomerulopathy.



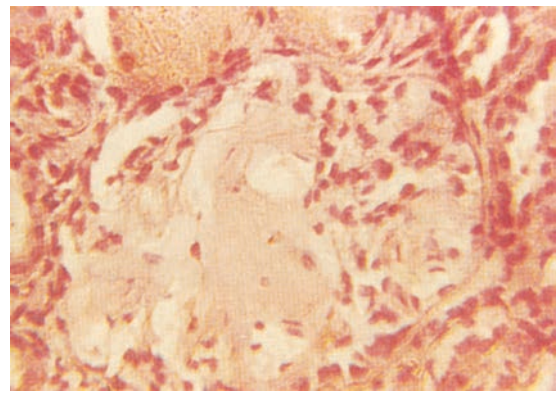
COLOR FIGURE 78-14. Exudative glomerulonephritis in the nephrotic syndrome associated with combined Schistosoma-Salmonella infection. Note the abundance of neutrophils and monocytes in the glomerular capillaries and mesangium. (Hematoxylin and eosin, magnification ×200.) (From: Barsoum RS. Schistosomal glomerulopathies. *Kidney Int* 1993;44:1, with permission.)



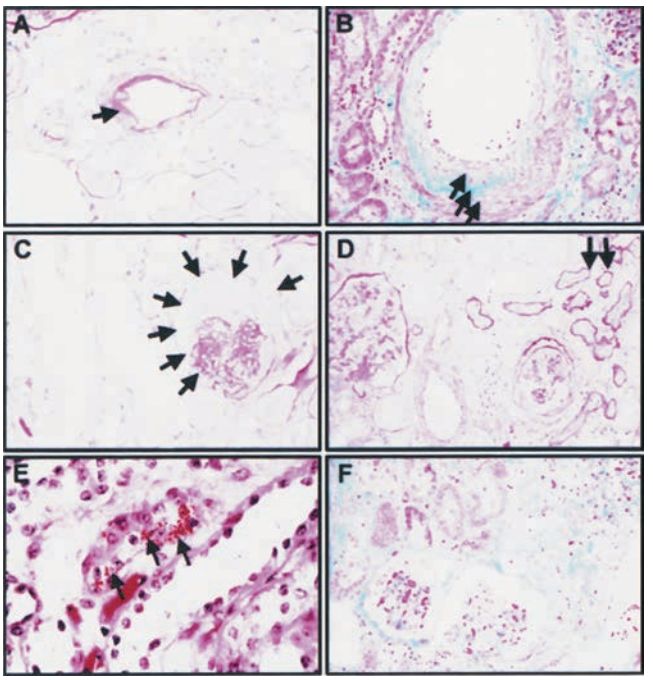
COLOR FIGURE 78-15. Mesangiocapillary glomerulonephritis in advanced schistosomal glomerulopathy. Note the mesangial matrix expansion and the thickening of the glomerular basement membrane. (Hematoxylin and eosin, magnification ×200.) (From Barsoum RS. Schistosomal glomerulopathies. *Kidney Int* 1993;44:1, with permission.)



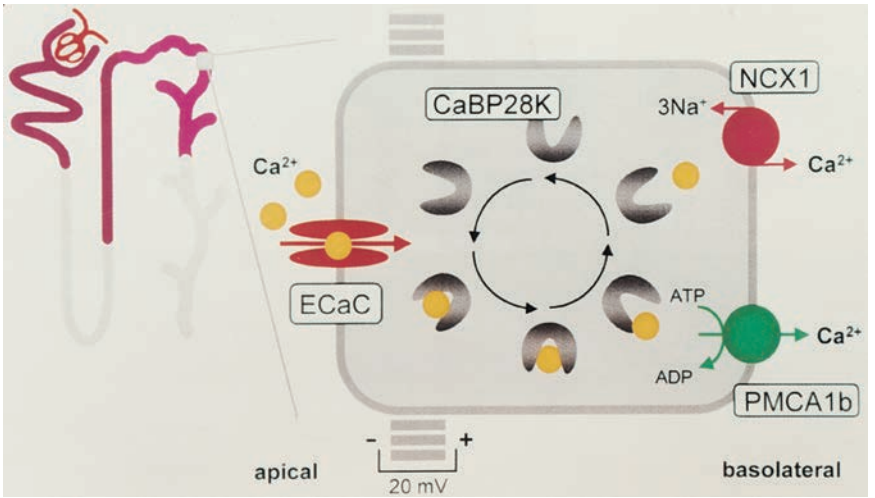
COLOR FIGURE 78-16. Focal segmental glomerulosclerosis in advanced schistosomal glomerulopathy. (Masson trichrome stain, magnification ×200.)



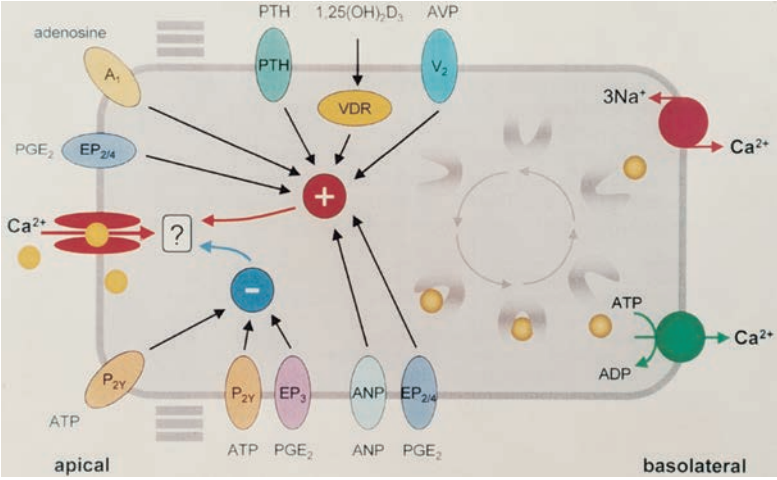
COLOR FIGURE 78-17. Glomerular amyloid deposition in mixed Schistosoma haematobium and Schistosoma mansoni infection. (Hematoxylin and eosin, magnification ×200.) (From: Barsoum RS, et al. Renal amyloidosis and schistosomiasis. *Trans R Soc Trop Med Hyg* 1979;73:367, with permission.)



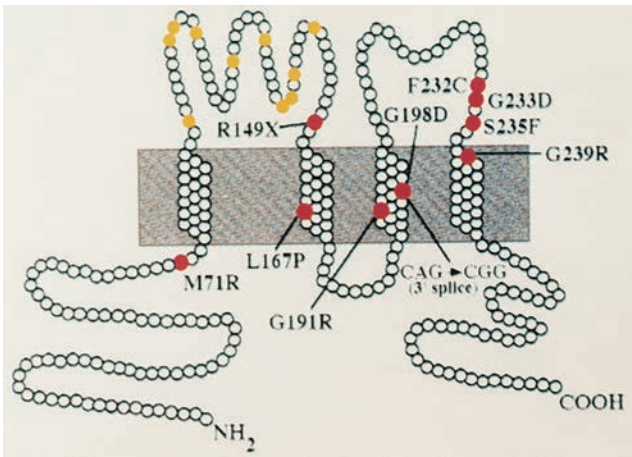
COLOR FIGURE 80-1. Histology of renal senescence: (**A**) arteriohyalinosis, (**B**) fibrous intimal thickening, (**C**) glomerulosclerosis, (**D**) tubular atrophy, (**E**) lipofuscin pigment, (**F**) interstitial fibrosis. (Reprinted from: Melk A, Halloran PF. Cell senescence and its implications for nephrology. *J Am Soc Nephrol* 2001;12:385.)



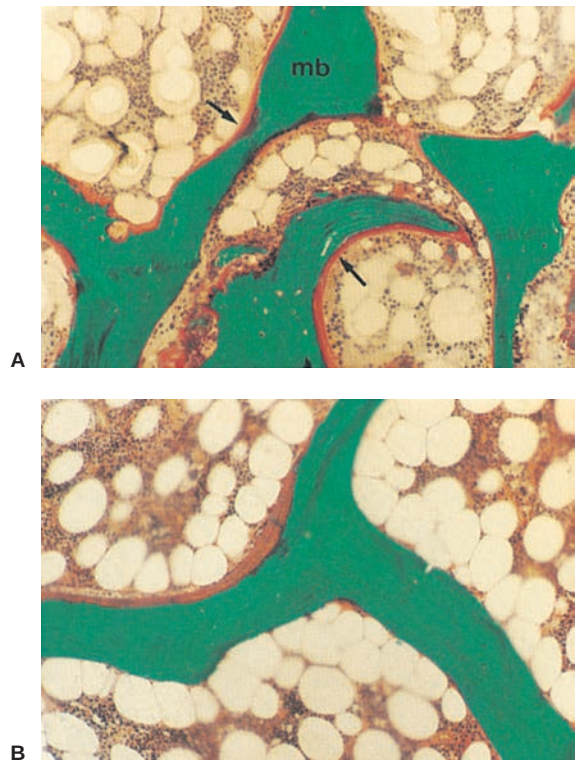
COLOR FIGURE 89-15. Model of transcellular Ca^{2+} transport by cells lining the distal part of the nephron. Entry of Ca^{2+} is facilitated by the apical epithelial Ca^{2+} channel (ECaC). Subsequently, the ion binds to calbindin-D_{28K} (CaBP28K) and diffuses through the cytosol to the basolateral membrane. Here, Ca^{2+} ions are extruded by a Na^{+}-Ca^{2+} exchanger (NCX1) and a Ca^{2+}-ATPase (PMCA1b). (From: Hoenderop JG, Willems PH, Bindels RJ. Toward a comprehensive molecular model of active calcium reabsorption. *Am J Physiol Renal* 2000;278:F352, with permission.)



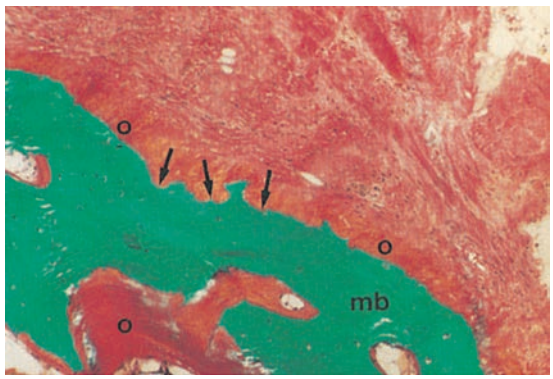
COLOR FIGURE 89-16. Schematic model for hormonal regulation of transcellular Ca^{2+} transport in distal nephron. Parathyroid hormone (PTH), V_2, atrial natriuretic peptide (ANP), and EP_3 receptors are localized in the basolateral membrane, whereas A_1 is present in the apical membrane. $EP_{2/4}$ and P_{2Y} are present in both membranes 1,25-dihydroxycholecalciferol passes plasma membranes and binds to the intracellular vitamin D receptor (VDR). Hormones can be divided into stimulatory hormones, including PTH, arginine vasopressin, ANP, prostaglandin E_2 (PGE_2) (via $EP_{2/4}$), and adenosine, and inhibitory hormones such as adenosine triphosphate and PGE_2 (via EP_3). (From: Hoenderop JG, Willems PH, Bindels RJ. Toward a comprehensive molecular model of active calcium reabsorption. *Am J Physiol Renal* 2000;278:F352, with permission.)



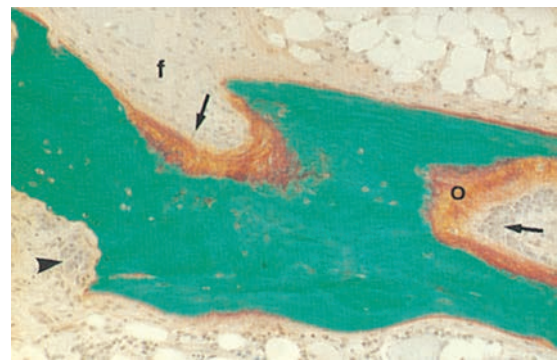
COLOR FIGURE 89-22. Structure of the PCLN-1 human gene. The red dots indicate mutations in PCLN-1 in patients with recessive renal hypomagnesemia. (From: Simon DB, Lu Y, Cahote KA, et al. Paracellin-1, a renal tight junction protein required for paracellular Mg^{2+} resorption. *Science* 1999;285:103.)



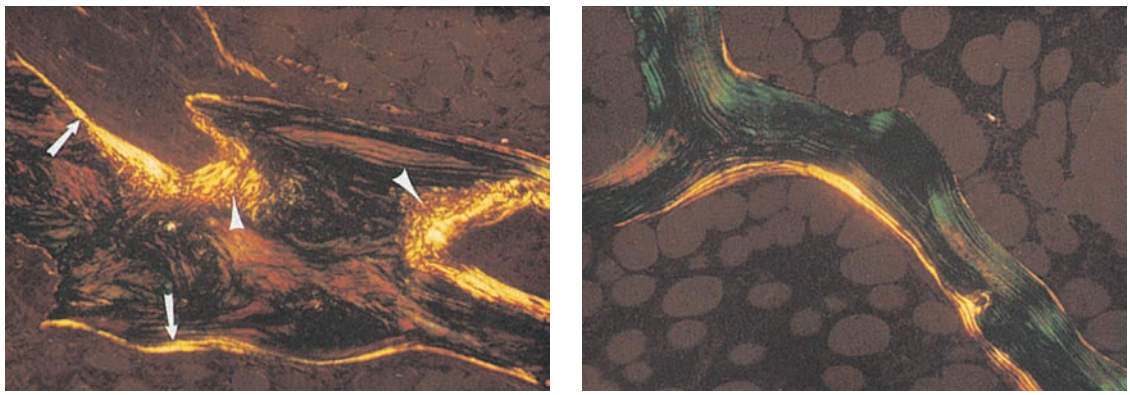
COLOR FIGURE 93-11. A: Histologic section of bone from a patient on dialysis with mild hyperparathyroidism. Length of unmineralized osteoid (*arrow*) is increased. Width of osteoid seam and volume of mineralized bone (*mb*) are normal. **B:** Bone section from a normal subject. (A and B, Goldner's stain, magnification ×115.)



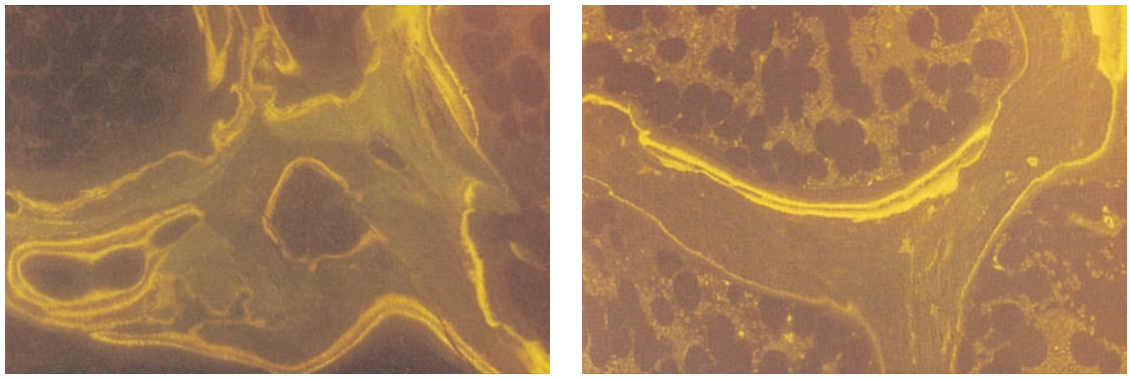
COLOR FIGURE 93-7. Subperiosteal resorption (*arrows*) of mineralized bone (*mb*) filled in with unmineralized osteoid (O) in a patient on dialysis with osteomalacia. (Goldner's stain, magnification ×115.)



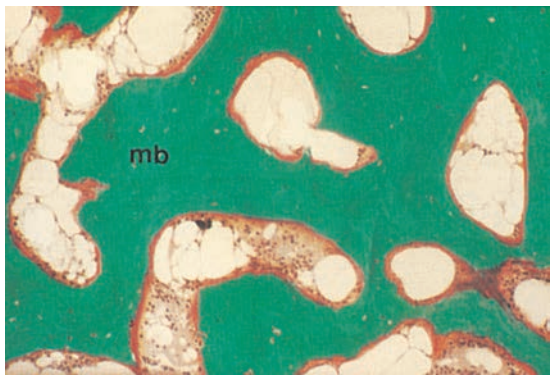
COLOR FIGURE 93-12. Osteitis fibrosa. Osteoid (O) is a mixture of woven and lamellar collagen. Plump osteoblasts (*arrows*) and multinucleated osteoclasts (*arrowhead*) are numerous. Fibrosis (*f*) is present. (Goldner's stain, magnification ×115.)



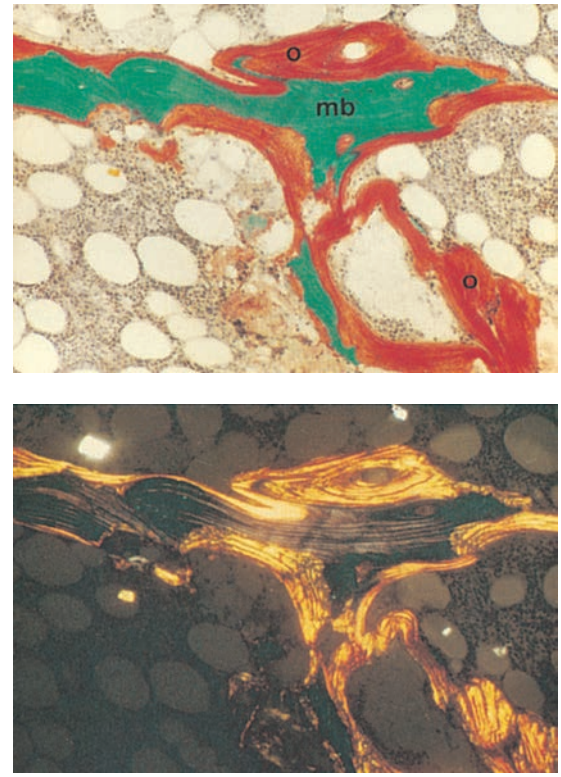
COLOR FIGURE 93-13. A: Polarized light section showing woven (*arrowheads*) and lamellar (*arrows*) collagen in osteitis fibrosa. **B:** Polarization of normal bone showing only lamellar collagen. (A and B, magnification ×115.)



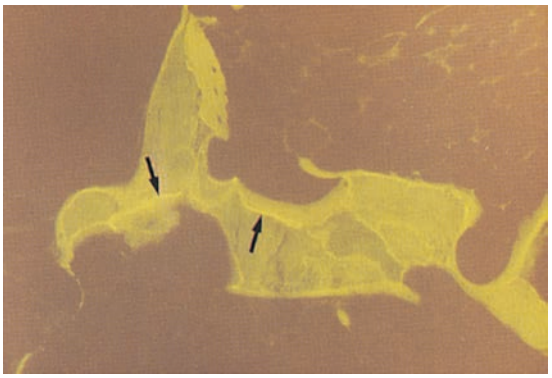
COLOR FIGURE 93-14. A: Double tetracycline labels in a patient with severe osteitis fibrosa. The distance between labels and the total length of the double labels is increased, demonstrating a high formation rate. **B:** Tetracycline labels from a normal subject. (A and B, unstained fluorescent micrographies, magnification ×115.)



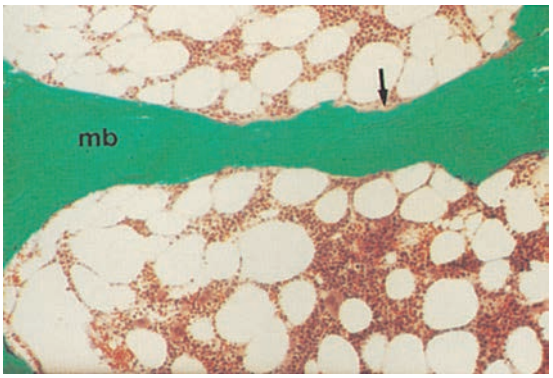
COLOR FIGURE 93-15. Osteosclerosis. The amount of mineralized bone (*mb*) is increased. (Goldner's stain, magnification ×115.)



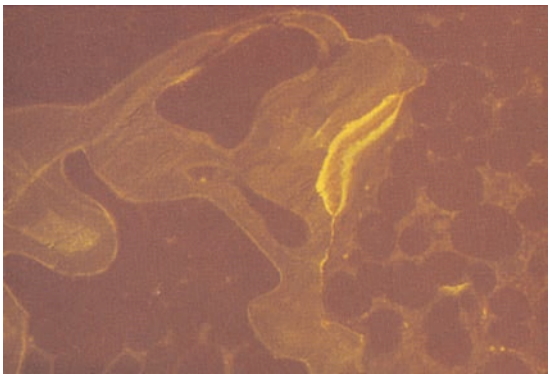
COLOR FIGURE 93-17. Osteomalacia. **A:** The unmineralized osteoid (O) is increased in width and total volume; *mb*, mineralized bone. **B:** Polarized section showing predominately lamellar orientation of collagen in osteomalacia. (A and B, magnification ×115.)



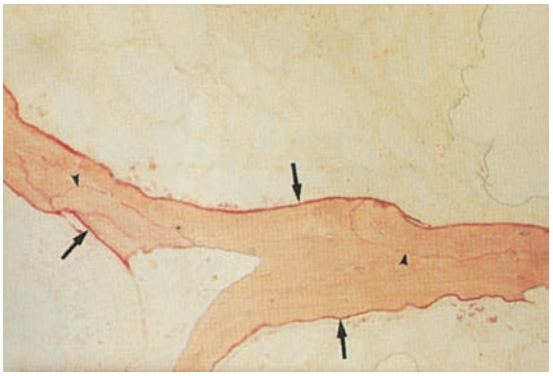
COLOR FIGURE 93-18. Single tetracycline label (*arrows*) in bone from a patient on dialysis with osteomalacia given two time-spaced doses of tetracycline. Absence of double labels indicates abnormally low bone formation. (Unstained fluorescent micrograph, magnification ×115.)



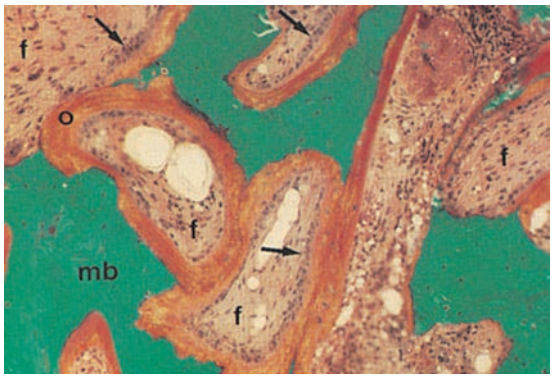
COLOR FIGURE 93-19. Aplastic (adynamic) bone disease. Marked decrease in amount of unmineralized osteoid (*arrows*); *mb*, mineralized bone. Osteoblasts are flat when present. (Goldner's stain, magnification ×115.)



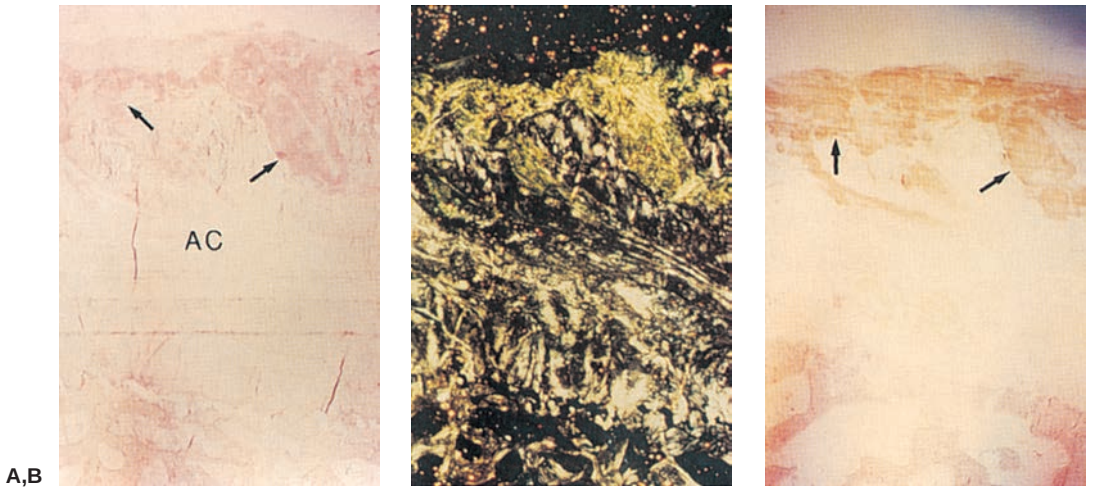
COLOR FIGURE 93-20. Aplastic (adynamic) bone disease. The total amount of tetracycline uptake is decreased, as is the number of double tetracycline labels. When present, the double label is shorter than normal, demonstrating reduced bone formation. (Unstained fluorescent micrography, magnification ×115.)



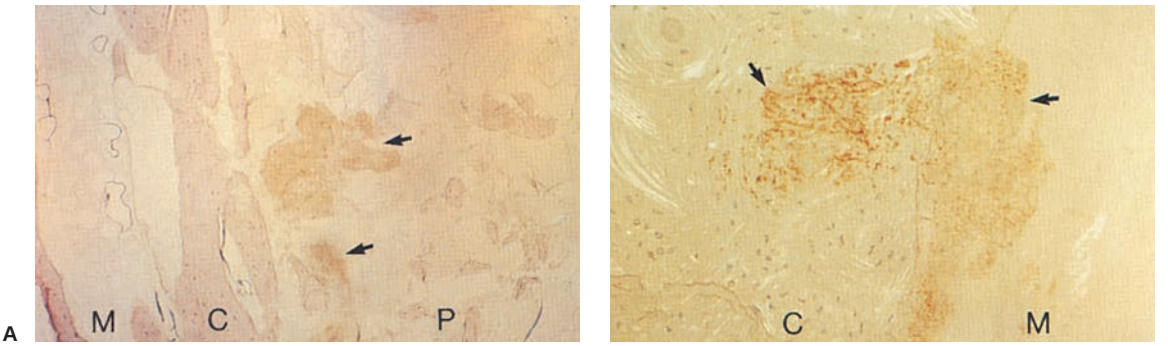
COLOR FIGURE 93-22. Histologic section showing a positive stain for aluminum on the surface (*arrows*) of mineralized bone and within cement lines (*arrowheads*). (Aurin-tricarboxylic acid, magnification ×115.)



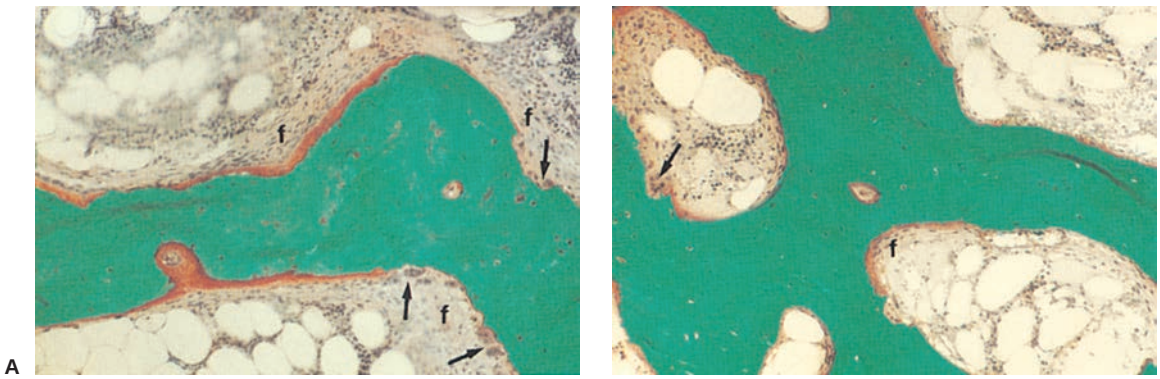
COLOR FIGURE 93-25. Mixed bone disease in a patient on dialysis. Width and total volume of unmineralized osteoid (*O*) are increased; *mb*, mineralized bone. The number of osteoblasts (*arrows*) also is increased, and fibrosis (*f*) is present in most of the marrow space. (Goldner's stain, magnification ×115.)



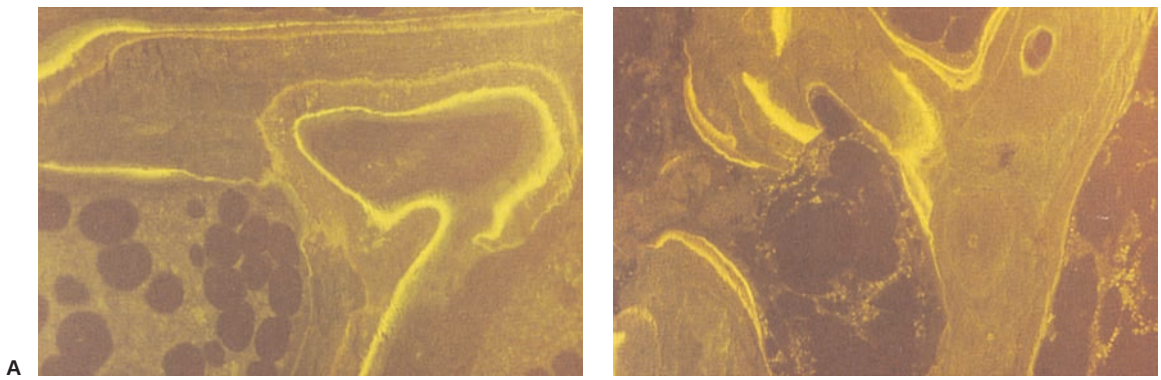
COLOR FIGURE 93-26. Serial sections of femoral bone from a patient on dialysis. **A:** Congo red-stained amyloid deposits (*arrows*) are seen in the superficial articular cartilage (*AC*). **B:** The same seen under polarized light. Congo red-stained amyloid deposits demonstrate green birefringence. **C:** Immunohistochemical staining with anti-β_2-microglobulin. Amyloid deposits (*arrows*) stain brown for β_2-microglobulin. (From: Onishi S, et al. Beta-2 microglobulin deposition in bone in chronic renal failure. *Kidney Int* 1991;39;990, with permission.)



COLOR FIGURE 93-27. **A:** Iliac crest bone biopsy with anti-β_2-microglobulin and hematoxylin counterstain. β_2-Microglobulin deposits (*arrows*) are located in the iliac periosteum. **B:** Bone section from the tibia with anti-β_2-microglobulin and methylene blue counterstain. β_2-Microglobulin deposits (*arrows*) appear to invade the cortical bone (*C*) from the marrow (*M*). (From: Onishi S, et al. Beta-2-microglobulin deposition in bone in chronic renal failure. *Kidney Int* 1991;39:990, with permission.)



COLOR FIGURE 93-29. A: Ostitis fibrosa in a patient on dialysis refractory to treatment with oral calcitriol. **B:** Histologic section from the same patient after treatment with intravenous calcitriol. Amount of fibrosis (*f*) and number of osteoclasts (*arrows*) are decreased after treatment. (A and B, Goldner's stain, magnification ×115.)



COLOR FIGURE 93-31. A: Tetracycline labels in a patient on dialysis with osteitis fibrosa and high bone formation. **B:** Tetracycline labels in the same patient after treatment with intravenous calcitriol. The reductions in the interlabel distance and the length of double labels after treatment indicate a decrease in bone formation to normal. (A and B, unstained fluorescent micrographs, magnification ×115.)